



Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

State of West Virginia
Request for Quotation
01 - Agricultural

Proc Folder: 265333

Doc Description: Triple Quad LC/MS/MS

Proc Type: Central Purchase Order

Date issued	Solicitation Closes	Solicitation No	Version
2016-10-24	2016-11-17 13:30:00	CRFQ 1400 AGR1700000005	1

BID RECEIVING LOCATION

BID CLERK

DEPARTMENT OF ADMINISTRATION

PURCHASING DIVISION

2019 WASHINGTON ST E

CHARLESTON

WV 25305

US

VENDOR

Vendor Name, Address and Telephone Number:

Shimadzu Scientific Instruments, Inc.
7102 Riverwood Drive
Columbia, MD 21046
410-381-1227

11/17/16 11:19:32
WV Purchasing Division

FOR INFORMATION CONTACT THE BUYER

Linda Harper

(304) 558-0468

linda.b.harper@wv.gov

Signature X

FEIN # 52-1035956

DATE 11/15/16

All offers subject to all terms and conditions contained in this solicitation

ADDITIONAL INFORMATION:

The West Virginia Purchasing Division for the Agency, The West Virginia Department of Agriculture is soliciting bids from qualified vendors to establish a "One-Time" contract for the purchase of a Liquid Chromatography/Mass Spectrometer (LC/MS) instrument per the Specifications, Terms & Conditions and bid requirements as attached.

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US OPTION 1: \$297,921.81 OPTION 1: \$266,544.92	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
1	LCMSMS, Workstation, software, printer	1.00000	EA	OPTION 2: \$333,439.20	OPTION 2: \$302,062.10

Comm Code	Manufacturer	Specification	Model #
41100000	Shimadzu	Please see enclosed information.	Nexera X2 with LCMS-8040 and LCMS-8045

Extended Description :

LCMSMS, Workstation, software, printer per specification 3.1.1 & 3.1.2

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Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
2	Shipping Charges and inside delivery	1.00000	EA	\$850.00	\$850.00

Comm Code	Manufacturer	Specification	Model #
78121603	N/A	N/A	N/A

Extended Description :

Shipping Charges and inside deliver per section 3.1.3

INVOICE TO		SHIP TO	
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Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
3	Installation/validation	1.00000	EA	\$0.00	\$0.00

Comm Code	Manufacturer	Specification	Model #
73171605	Shimadzu	N/A	N/A

Extended Description :

Installation/validation per section 3.1.3

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
4	Training/Warranty	1.00000	EA	\$8610.00	\$0.00

Comm Code	Manufacturer	Specification	Model #
73171605	Shimadzu	N/A	N/A

Extended Description :

Training/Warranty per section 3.1.3

INVOICE TO		SHIP TO	
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Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
5	Service	1.00000	EA	\$30,526.90	\$30,526.90

Comm Code	Manufacturer	Specification	Model #
73171605	Shimadzu	N/A	N/A

Extended Description :

Service per section 3.1.3

SCHEDULE OF EVENTS

Line	Event	Event Date
1	Question Deadline 3:00 p.m.	2016-11-04

INSTRUCTIONS TO VENDORS SUBMITTING BIDS

1. REVIEW DOCUMENTS THOROUGHLY: The attached documents contain a solicitation for bids. Please read these instructions and all documents attached in their entirety. These instructions provide critical information about requirements that if overlooked could lead to disqualification of a Vendor's bid. All bids must be submitted in accordance with the provisions contained in these instructions and the Solicitation. Failure to do so may result in disqualification of Vendor's bid.

2. MANDATORY TERMS: The Solicitation may contain mandatory provisions identified by the use of the words "must," "will," and "shall." Failure to comply with a mandatory term in the Solicitation will result in bid disqualification.

3. PREBID MEETING: The item identified below shall apply to this Solicitation.

☒ A pre-bid meeting will not be held prior to bid opening

☐ A NON-MANDATORY PRE-BID meeting will be held at the following place and time:

☐ A MANDATORY PRE-BID meeting will be held at the following place and time:

All Vendors submitting a bid must attend the mandatory pre-bid meeting. Failure to attend the mandatory pre-bid meeting shall result in disqualification of the Vendor's bid. No one person attending the pre-bid meeting may represent more than one Vendor.

An attendance sheet provided at the pre-bid meeting shall serve as the official document verifying attendance. The State will not accept any other form of proof or documentation to verify attendance. Any person attending the pre-bid meeting on behalf of a Vendor must list on the attendance sheet his or her name and the name of the Vendor he or she is representing.

Additionally, the person attending the pre-bid meeting should include the Vendor's E-Mail address, phone number, and Fax number on the attendance sheet. It is the Vendor's responsibility to locate the attendance sheet and provide the required information. Failure to complete the attendance sheet as required may result in disqualification of Vendor's bid.

All Vendors should arrive prior to the starting time for the pre-bid. Vendors who arrive after the starting time but prior to the end of the pre-bid will be permitted to sign in, but are charged with knowing all matters discussed at the pre-bid.

Questions submitted at least five business days prior to a scheduled pre-bid will be discussed at the pre-bid meeting if possible. Any discussions or answers to questions at the pre-bid meeting are preliminary in nature and are non-binding. Official and binding answers to questions will be published in a written addendum to the Solicitation prior to bid opening.

4. VENDOR QUESTION DEADLINE: Vendors may submit questions relating to this Solicitation to the Purchasing Division. Questions must be submitted in writing. All questions must be submitted on or before the date listed below and to the address listed below in order to be considered. A written response will be published in a Solicitation addendum if a response is possible and appropriate. Non-written discussions, conversations, or questions and answers regarding this Solicitation are preliminary in nature and are nonbinding.

Submitted e-mails should have solicitation number in the subject line.

Question Submission Deadline: Friday, November 4, 2016, 3:00 p.m.

Submit Questions to: Linda Harper, Senior Buyer
2019 Washington Street, East
Charleston, WV 25305
Fax: (304) 558-4115 (Vendors should not use this fax number for bid submission)
Email: Linda.B.Harper@wv.gov

5. VERBAL COMMUNICATION: Any verbal communication between the Vendor and any State personnel is not binding, including verbal communication at the mandatory pre-bid conference. Only information issued in writing and added to the Solicitation by an official written addendum by the Purchasing Division is binding.

6. BID SUBMISSION: All bids must be submitted electronically through wvOASIS or signed and delivered by the Vendor to the Purchasing Division at the address listed below on or before the date and time of the bid opening. Any bid received by the Purchasing Division staff is considered to be in the possession of the Purchasing Division and will not be returned for any reason. The Purchasing Division will not accept bids, modification of bids, or addendum acknowledgment forms via e-mail. Acceptable delivery methods include electronic submission via wvOASIS, hand delivery, delivery by courier, or facsimile.

The bid delivery address is:
Department of Administration, Purchasing Division
2019 Washington Street East
Charleston, WV 25305-0130

A bid that is not submitted electronically through wvOASIS should contain the information listed below on the face of the envelope or the bid may be rejected by the Purchasing Division.:

SEALED BID: Triple Quad LC/MS/MS
BUYER: Linda B. Harper
SOLICITATION NO.: CRFQ AGR1700000005
BID OPENING DATE: November 17, 2016
BID OPENING TIME: 1:30 p.m.
FAX NUMBER:

The Purchasing Division may prohibit the submission of bids electronically through wvOASIS at its sole discretion. Such a prohibition will be contained and communicated in the wvOASIS system resulting in the Vendor's inability to submit bids through wvOASIS. Submission of a response to an Expression of Interest or Request for Proposal is not permitted in wvOASIS.

For Request For Proposal ("RFP") Responses Only: In the event that Vendor is responding to a request for proposal, the Vendor shall submit one original technical and one original cost proposal plus N/A convenience copies of each to the Purchasing Division at the address shown above. Additionally, the Vendor should identify the bid type as either a technical or cost proposal on the face of each bid envelope submitted in response to a request for proposal as follows:

BID TYPE: (This only applies to CRFP)

☐ Technical

☐ Cost

7. BID OPENING: Bids submitted in response to this Solicitation will be opened at the location identified below on the date and time listed below. Delivery of a bid after the bid opening date and time will result in bid disqualification. For purposes of this Solicitation, a bid is considered delivered when confirmation of delivery is provided by wvOASIS (in the case of electronic submission) or when the bid is time stamped by the official Purchasing Division time clock (in the case of hand delivery).

Bid Opening Date and Time: November 17, 2016, 1:30 p.m.

Bid Opening Location: Department of Administration, Purchasing Division
2019 Washington Street East
Charleston, WV 25305-0130

8. ADDENDUM ACKNOWLEDGEMENT: Changes or revisions to this Solicitation will be made by an official written addendum issued by the Purchasing Division. Vendor should acknowledge receipt of all addenda issued with this Solicitation by completing an Addendum Acknowledgment Form, a copy of which is included herewith. Failure to acknowledge addenda may result in bid disqualification. The addendum acknowledgement should be submitted with the bid to expedite document processing.

9. BID FORMATTING: Vendor should type or electronically enter the information onto its bid to prevent errors in the evaluation. Failure to type or electronically enter the information may result in bid disqualification.

10. ALTERNATES: Any model, brand, or specification listed in this Solicitation establishes the acceptable level of quality only and is not intended to reflect a preference for, or in any way favor, a particular brand or vendor. Vendors may bid alternates to a listed model or brand provided that the alternate is at least equal to the model or brand and complies with the required specifications. The equality of any alternate being bid shall be determined by the State at its sole discretion. Any Vendor bidding an alternate model or brand should clearly identify the alternate items in its bid and should include manufacturer's specifications, industry literature, and/or any other relevant documentation demonstrating the equality of the alternate items. Failure to provide information for alternate items may be grounds for rejection of a Vendor's bid.

11. EXCEPTIONS AND CLARIFICATIONS: The Solicitation contains the specifications that shall form the basis of a contractual agreement. Vendor shall clearly mark any exceptions, clarifications, or other proposed modifications in its bid. Exceptions to, clarifications of, or modifications of a requirement or term and condition of the Solicitation may result in bid disqualification.

12. COMMUNICATION LIMITATIONS: In accordance with West Virginia Code of State Rules §148-1-6.6, communication with the State of West Virginia or any of its employees regarding this Solicitation during the solicitation, bid, evaluation or award periods, except through the Purchasing Division, is strictly prohibited without prior Purchasing Division approval. Purchasing Division approval for such communication is implied for all agency delegated and exempt purchases.

13. REGISTRATION: Prior to Contract award, the apparent successful Vendor must be properly registered with the West Virginia Purchasing Division and must have paid the \$125 fee, if applicable.

14. UNIT PRICE: Unit prices shall prevail in cases of a discrepancy in the Vendor's bid.

15. PREFERENCE: Vendor Preference may only be granted upon written request and only in accordance with the West Virginia Code § 5A-3-37 and the West Virginia Code of State Rules. A Vendor Preference Certificate form has been attached hereto to allow Vendor to apply for the preference. Vendor's failure to submit the Vendor Preference Certificate form with its bid will result in denial of Vendor Preference. Vendor Preference does not apply to construction projects.

16. SMALL, WOMEN-OWNED, OR MINORITY-OWNED BUSINESSES: For any solicitations publicly advertised for bid, in accordance with West Virginia Code §5A-3-37(a)(7) and W. Va. CSR § 148-22-9, any non-resident vendor certified as a small, women-owned, or minority-owned business under W. Va. CSR § 148-22-9 shall be provided the same preference made available to any resident vendor. Any non-resident small, women-owned, or minority-owned business must identify itself as such in writing, must submit that writing to the Purchasing Division with its bid, and must be properly certified under W. Va. CSR § 148-22-9 prior to contract award to receive the preferences made available to resident vendors. Preference for a non-resident small, women-owned, or minority owned business shall be applied in accordance with W. Va. CSR § 148-22-9.

17. WAIVER OF MINOR IRREGULARITIES: The Director reserves the right to waive minor irregularities in bids or specifications in accordance with West Virginia Code of State Rules § 148-1-4.6.

18. ELECTRONIC FILE ACCESS RESTRICTIONS: Vendor must ensure that its submission in wvOASIS can be accessed by the Purchasing Division staff immediately upon bid opening. The Purchasing Division will consider any file that cannot be immediately opened and/or viewed at the time of the bid opening (such as, encrypted files, password protected files, or incompatible files) to be blank or incomplete as context requires, and are therefore unacceptable. A vendor will not be permitted to unencrypt files, remove password protections, or resubmit documents after bid opening if those documents are required with the bid.

19. NON-RESPONSIBLE: The Purchasing Division Director reserves the right to reject the bid of any vendor as Non-Responsible in accordance with W. Va. Code of State Rules § 148-1-5.3, when the Director determines that the vendor submitting the bid does not have the capability to fully perform, or lacks the integrity and reliability to assure good-faith performance.”

20. ACCEPTANCE/REJECTION: The State may accept or reject any bid in whole, or in part in accordance with W. Va. Code of State Rules § 148-1-4.5. and § 148-1-6.4.b.”

21. YOUR SUBMISSION IS A PUBLIC DOCUMENT: Vendor’s entire response to the Solicitation and the resulting Contract are public documents. As public documents, they will be disclosed to the public following the bid/proposal opening or award of the contract, as required by the competitive bidding laws of West Virginia Code §§ 5A-3-1 et seq., 5-22-1 et seq., and 5G-1-1 et seq. and the Freedom of Information Act West Virginia Code §§ 29B-1-1 et seq.

DO NOT SUBMIT MATERIAL YOU CONSIDER TO BE CONFIDENTIAL, A TRADE SECRET, OR OTHERWISE NOT SUBJECT TO PUBLIC DISCLOSURE.

Submission of any bid, proposal, or other document to the Purchasing Division constitutes your explicit consent to the subsequent public disclosure of the bid, proposal, or document. The Purchasing Division will disclose any document labeled “confidential,” “proprietary,” “trade secret,” “private,” or labeled with any other claim against public disclosure of the documents, to include any “trade secrets” as defined by West Virginia Code § 47-22-1 et seq. All submissions are subject to public disclosure without notice.

GENERAL TERMS AND CONDITIONS:

1. CONTRACTUAL AGREEMENT: Issuance of a Award Document signed by the Purchasing Division Director, or his designee, and approved as to form by the Attorney General's office constitutes acceptance of this Contract made by and between the State of West Virginia and the Vendor. Vendor's signature on its bid signifies Vendor's agreement to be bound by and accept the terms and conditions contained in this Contract.

2. DEFINITIONS: As used in this Solicitation/Contract, the following terms shall have the meanings attributed to them below. Additional definitions may be found in the specifications included with this Solicitation/Contract.

2.1. "Agency" or "Agencies" means the agency, board, commission, or other entity of the State of West Virginia that is identified on the first page of the Solicitation or any other public entity seeking to procure goods or services under this Contract.

2.2. "Bid" or "Proposal" means the vendors submitted response to this solicitation.

2.3. "Contract" means the binding agreement that is entered into between the State and the Vendor to provide the goods or services requested in the Solicitation.

2.4. "Director" means the Director of the West Virginia Department of Administration, Purchasing Division.

2.5. "Purchasing Division" means the West Virginia Department of Administration, Purchasing Division.

2.6. "Award Document" means the document signed by the Agency and the Purchasing Division, and approved as to form by the Attorney General, that identifies the Vendor as the contract holder.

2.7. "Solicitation" means the official notice of an opportunity to supply the State with goods or services that is published by the Purchasing Division.

2.8. "State" means the State of West Virginia and/or any of its agencies, commissions, boards, etc. as context requires.

2.9. "Vendor" or "Vendors" means any entity submitting a bid in response to the Solicitation, the entity that has been selected as the lowest responsible bidder, or the entity that has been awarded the Contract as context requires.

3. CONTRACT TERM; RENEWAL; EXTENSION: The term of this Contract shall be determined in accordance with the category that has been identified as applicable to this Contract below:

☐ **Term Contract**

Initial Contract Term: This Contract becomes effective on _____ and extends for a period of _____ year(s).

Renewal Term: This Contract may be renewed upon the mutual written consent of the Agency, and the Vendor, with approval of the Purchasing Division and the Attorney General's office (Attorney General approval is as to form only). Any request for renewal should be submitted to the Purchasing Division thirty (30) days prior to the expiration date of the initial contract term or appropriate renewal term. A Contract renewal shall be in accordance with the terms and conditions of the original contract. Renewal of this Contract is limited to _____ successive one (1) year periods or multiple renewal periods of less than one year, provided that the multiple renewal periods do not exceed _____ months in total. Automatic renewal of this Contract is prohibited. Notwithstanding the foregoing, Purchasing Division approval is not required on agency delegated or exempt purchases. Attorney General approval may be required for vendor terms and conditions.

Delivery Order Limitations: In the event that this contract permits delivery orders, a delivery order may only be issued during the time this Contract is in effect. Any delivery order issued within one year of the expiration of this Contract shall be effective for one year from the date the delivery order is issued. No delivery order may be extended beyond one year after this Contract has expired.

☐ **Fixed Period Contract:** This Contract becomes effective upon Vendor's receipt of the notice to proceed and must be completed within _____ days.

☐ **Fixed Period Contract with Renewals:** This Contract becomes effective upon Vendor's receipt of the notice to proceed and part of the Contract more fully described in the attached specifications must be completed within _____ days.

Upon completion, the vendor agrees that maintenance, monitoring, or warranty services will be provided for one year thereafter with an additional _____ successive one year renewal periods or multiple renewal periods of less than one year provided that the multiple renewal periods do not exceed _____ months in total. Automatic renewal of this Contract is prohibited.

☒ **One Time Purchase:** The term of this Contract shall run from the issuance of the Award Document until all of the goods contracted for have been delivered, but in no event will this Contract extend for more than one fiscal year.

☐ **Other:** See attached.

4. NOTICE TO PROCEED: Vendor shall begin performance of this Contract immediately upon receiving notice to proceed unless otherwise instructed by the Agency. Unless otherwise specified, the fully executed Award Document will be considered notice to proceed.

5. QUANTITIES: The quantities required under this Contract shall be determined in accordance with the category that has been identified as applicable to this Contract below.

☐ **Open End Contract:** Quantities listed in this Solicitation are approximations only, based on estimates supplied by the Agency. It is understood and agreed that the Contract shall cover the quantities actually ordered for delivery during the term of the Contract, whether more or less than the quantities shown.

☐ **Service:** The scope of the service to be provided will be more clearly defined in the specifications included herewith.

☒ **Combined Service and Goods:** The scope of the service and deliverable goods to be provided will be more clearly defined in the specifications included herewith.

☐ **One Time Purchase:** This Contract is for the purchase of a set quantity of goods that are identified in the specifications included herewith. Once those items have been delivered, no additional goods may be procured under this Contract without an appropriate change order approved by the Vendor, Agency, Purchasing Division, and Attorney General's office.

6. EMERGENCY PURCHASES: The Purchasing Division Director may authorize the Agency to purchase goods or services in the open market that Vendor would otherwise provide under this Contract if those goods or services are for immediate or expedited delivery in an emergency. Emergencies shall include, but are not limited to, delays in transportation or an unanticipated increase in the volume of work. An emergency purchase in the open market, approved by the Purchasing Division Director, shall not constitute a breach of this Contract and shall not entitle the Vendor to any form of compensation or damages. This provision does not excuse the State from fulfilling its obligations under a One Time Purchase contract.

7. REQUIRED DOCUMENTS: All of the items checked below must be provided to the Purchasing Division by the Vendor as specified below.

☐ **BID BOND (Construction Only):** Pursuant to the requirements contained in W. Va. Code § 5-22-1(c), All Vendors submitting a bid on a construction project shall furnish a valid bid bond in the amount of five percent (5%) of the total amount of the bid protecting the State of West Virginia. The bid bond must be submitted with the bid.

☐ **PERFORMANCE BOND:** The apparent successful Vendor shall provide a performance bond in the amount of _____. The performance bond must be received by the Purchasing Division prior to Contract award. On construction contracts, the performance bond must be 100% of the Contract value.

☐ **LABOR/MATERIAL PAYMENT BOND:** The apparent successful Vendor shall provide a labor/material payment bond in the amount of 100% of the Contract value. The labor/material payment bond must be delivered to the Purchasing Division prior to Contract award.

In lieu of the Bid Bond, Performance Bond, and Labor/Material Payment Bond, the Vendor may provide certified checks, cashier's checks, or irrevocable letters of credit. Any certified check, cashier's check, or irrevocable letter of credit provided in lieu of a bond must be of the same amount and delivered on the same schedule as the bond it replaces. A letter of credit submitted in lieu of a performance and labor/material payment bond will only be allowed for projects under \$100,000. Personal or business checks are not acceptable.

☐ **MAINTENANCE BOND:** The apparent successful Vendor shall provide a two (2) year maintenance bond covering the roofing system. The maintenance bond must be issued and delivered to the Purchasing Division prior to Contract award.

☐ **INSURANCE:** The apparent successful Vendor shall furnish proof of the following insurance prior to Contract award and shall list the state as a certificate holder:

☐ **Commercial General Liability Insurance:** In the amount of _____ or more.

☐ **Builders Risk Insurance:** In an amount equal to 100% of the amount of the Contract.

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The apparent successful Vendor shall also furnish proof of any additional insurance requirements contained in the specifications prior to Contract award regardless of whether or not that insurance requirement is listed above.

☐ **LICENSE(S) / CERTIFICATIONS / PERMITS:** In addition to anything required under the Section entitled Licensing, of the General Terms and Conditions, the apparent successful Vendor shall furnish proof of the following licenses, certifications, and/or permits prior to Contract award, in a form acceptable to the Purchasing Division.

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The apparent successful Vendor shall also furnish proof of any additional licenses or certifications contained in the specifications prior to Contract award regardless of whether or not that requirement is listed above.

8. WORKERS' COMPENSATION INSURANCE: The apparent successful Vendor shall comply with laws relating to workers compensation, shall maintain workers' compensation insurance when required, and shall furnish proof of workers' compensation insurance upon request.

9. LITIGATION BOND: The Director reserves the right to require any Vendor that files a protest of an award to submit a litigation bond in the amount equal to one percent of the lowest bid submitted or \$5,000, whichever is greater. The entire amount of the bond shall be forfeited if the hearing officer determines that the protest was filed for frivolous or improper purpose, including but not limited to, the purpose of harassing, causing unnecessary delay, or needless expense for the Agency. All litigation bonds shall be made payable to the Purchasing Division. In lieu of a bond, the protester may submit a cashier's check or certified check payable to the Purchasing Division. Cashier's or certified checks will be deposited with and held by the State Treasurer's office. If it is determined that the protest has not been filed for frivolous or improper purpose, the bond or deposit shall be returned in its entirety.

10. LIQUIDATED DAMAGES: Vendor shall pay liquidated damages in the amount of

for _____

This clause shall in no way be considered exclusive and shall not limit the State or Agency's right to pursue any other available remedy.

11. ACCEPTANCE: Vendor's signature on its bid, or on the certification and signature page, constitutes an offer to the State that cannot be unilaterally withdrawn, signifies that the product or service proposed by vendor meets the mandatory requirements contained in the Solicitation for that product or service, unless otherwise indicated, and signifies acceptance of the terms and conditions contained in the Solicitation unless otherwise indicated.

12. PRICING: The pricing set forth herein is firm for the life of the Contract, unless specified elsewhere within this Solicitation/Contract by the State. A Vendor's inclusion of price adjustment provisions in its bid, without an express authorization from the State in the Solicitation to do so, may result in bid disqualification.

13. PAYMENT: Payment in advance is prohibited under this Contract. Payment may only be made after the delivery and acceptance of goods or services. The Vendor shall submit invoices, in arrears.

14. PURCHASING CARD ACCEPTANCE: The State of West Virginia currently utilizes a Purchasing Card program, administered under contract by a banking institution, to process payment for goods and services. The Vendor must accept the State of West Virginia's Purchasing Card for payment of all orders under this Contract unless the box below is checked.

☐ Vendor is not required to accept the State of West Virginia's Purchasing Card as payment for all goods and services.

15. TAXES: The Vendor shall pay any applicable sales, use, personal property or any other taxes arising out of this Contract and the transactions contemplated thereby. The State of West Virginia is exempt from federal and state taxes and will not pay or reimburse such taxes.

16. ADDITIONAL FEES: Vendor is not permitted to charge additional fees or assess additional charges that were not either expressly provided for in the solicitation published by the State of West Virginia or included in the unit price or lump sum bid amount that Vendor is required by the solicitation to provide. Including such fees or charges as notes to the solicitation may result in rejection of vendor's bid. Requesting such fees or charges be paid after the contract has been awarded may result in cancellation of the contract.

17. FUNDING: This Contract shall continue for the term stated herein, contingent upon funds being appropriated by the Legislature or otherwise being made available. In the event funds are not appropriated or otherwise made available, this Contract becomes void and of no effect beginning on July 1 of the fiscal year for which funding has not been appropriated or otherwise made available.

18. CANCELLATION: The Purchasing Division Director reserves the right to cancel this Contract immediately upon written notice to the vendor if the materials or workmanship supplied do not conform to the specifications contained in the Contract. The Purchasing Division Director may also cancel any purchase or Contract upon 30 days written notice to the Vendor in accordance with West Virginia Code of State Rules § 148-1-6.1.e.

19. TIME: Time is of the essence with regard to all matters of time and performance in this Contract.

20. APPLICABLE LAW: This Contract is governed by and interpreted under West Virginia law without giving effect to its choice of law principles. Any information provided in specification manuals, or any other source, verbal or written, which contradicts or violates the West Virginia Constitution, West Virginia Code or West Virginia Code of State Rules is void and of no effect.

21. COMPLIANCE: Vendor shall comply with all applicable federal, state, and local laws, regulations and ordinances. By submitting a bid, Vendor acknowledges that it has reviewed, understands, and will comply with all applicable laws, regulations, and ordinances.

22. ARBITRATION: Any references made to arbitration contained in this Contract, Vendor's bid, or in any American Institute of Architects documents pertaining to this Contract are hereby deleted, void, and of no effect.

23. MODIFICATIONS: This writing is the parties' final expression of intent. Notwithstanding anything contained in this Contract to the contrary no modification of this Contract shall be binding without mutual written consent of the Agency, and the Vendor, with approval of the Purchasing Division and the Attorney General's office (Attorney General approval is as to form only). Any change to existing contracts that adds work or changes contract cost, and were not included in the original contract, must be approved by the Purchasing Division and the Attorney General's Office (as to form) prior to the implementation of the change or commencement of work affected by the change.

24. WAIVER: The failure of either party to insist upon a strict performance of any of the terms or provision of this Contract, or to exercise any option, right, or remedy herein contained, shall not be construed as a waiver or a relinquishment for the future of such term, provision, option, right, or remedy, but the same shall continue in full force and effect. Any waiver must be expressly stated in writing and signed by the waiving party.

25. SUBSEQUENT FORMS: The terms and conditions contained in this Contract shall supersede any and all subsequent terms and conditions which may appear on any form documents submitted by Vendor to the Agency or Purchasing Division such as price lists, order forms, invoices, sales agreements, or maintenance agreements, and includes internet websites or other electronic documents. Acceptance or use of Vendor's forms does not constitute acceptance of the terms and conditions contained thereon.

26. ASSIGNMENT: Neither this Contract nor any monies due, or to become due hereunder, may be assigned by the Vendor without the express written consent of the Agency, the Purchasing Division, the Attorney General's office (as to form only), and any other government agency or office that may be required to approve such assignments. Notwithstanding the foregoing, Purchasing Division approval may or may not be required on certain agency delegated or exempt purchases.

27. WARRANTY: The Vendor expressly warrants that the goods and/or services covered by this Contract will: (a) conform to the specifications, drawings, samples, or other description furnished or specified by the Agency; (b) be merchantable and fit for the purpose intended; and (c) be free from defect in material and workmanship.

28. STATE EMPLOYEES: State employees are not permitted to utilize this Contract for personal use and the Vendor is prohibited from permitting or facilitating the same.

29. BANKRUPTCY: In the event the Vendor files for bankruptcy protection, the State of West Virginia may deem this Contract null and void, and terminate this Contract without notice.

30. PRIVACY, SECURITY, AND CONFIDENTIALITY: The Vendor agrees that it will not disclose to anyone, directly or indirectly, any such personally identifiable information or other confidential information gained from the Agency, unless the individual who is the subject of the information consents to the disclosure in writing or the disclosure is made pursuant to the Agency's policies, procedures, and rules. Vendor further agrees to comply with the Confidentiality Policies and Information Security Accountability Requirements, set forth in <http://www.state.wv.us/admin/purchase/privacy/default.html>.

31. YOUR SUBMISSION IS A PUBLIC DOCUMENT: Vendor's entire response to the Solicitation and the resulting Contract are public documents. As public documents, they will be disclosed to the public following the bid/proposal opening or award of the contract, as required by the competitive bidding laws of West Virginia Code §§ 5A-3-1 et seq., 5-22-1 et seq., and 5G-1-1 et seq. and the Freedom of Information Act West Virginia Code §§ 29B-1-1 et seq.

DO NOT SUBMIT MATERIAL YOU CONSIDER TO BE CONFIDENTIAL, A TRADE SECRET, OR OTHERWISE NOT SUBJECT TO PUBLIC DISCLOSURE.

Submission of any bid, proposal, or other document to the Purchasing Division constitutes your explicit consent to the subsequent public disclosure of the bid, proposal, or document. The Purchasing Division will disclose any document labeled "confidential," "proprietary," "trade secret," "private," or labeled with any other claim against public disclosure of the documents, to include any "trade secrets" as defined by West Virginia Code § 47-22-1 et seq. All submissions are subject to public disclosure without notice.

32. LICENSING: In accordance with West Virginia Code of State Rules § 148-1-6.1.e, Vendor must be licensed and in good standing in accordance with any and all state and local laws and requirements by any state or local agency of West Virginia, including, but not limited to, the West Virginia Secretary of State's Office, the West Virginia Tax Department, West Virginia Insurance Commission, or any other state agency or political subdivision. Upon request, the Vendor must provide all necessary releases to obtain information to enable the Purchasing Division Director or the Agency to verify that the Vendor is licensed and in good standing with the above entities.

33. ANTITRUST: In submitting a bid to, signing a contract with, or accepting a Award Document from any agency of the State of West Virginia, the Vendor agrees to convey, sell, assign, or transfer to the State of West Virginia all rights, title, and interest in and to all causes of action it may now or hereafter acquire under the antitrust laws of the United States and the State of West Virginia for price fixing and/or unreasonable restraints of trade relating to the particular commodities or services purchased or acquired by the State of West Virginia. Such assignment shall be made and become effective at the time the purchasing agency tenders the initial payment to Vendor.

34. VENDOR CERTIFICATIONS: By signing its bid or entering into this Contract, Vendor certifies (1) that its bid or offer was made without prior understanding, agreement, or connection with any corporation, firm, limited liability company, partnership, person or entity submitting a bid or offer for the same material, supplies, equipment or services; (2) that its bid or offer is in all respects fair and without collusion or fraud; (3) that this Contract is accepted or entered into without any prior understanding, agreement, or connection to any other entity that could be considered a violation of law; and (4) that it has reviewed this Solicitation in its entirety; understands the requirements, terms and conditions, and other information contained herein.

Vendor's signature on its bid or offer also affirms that neither it nor its representatives have any interest, nor shall acquire any interest, direct or indirect, which would compromise the performance of its services hereunder. Any such interests shall be promptly presented in detail to the Agency. The individual signing this bid or offer on behalf of Vendor certifies that he or she is authorized by the Vendor to execute this bid or offer or any documents related thereto on Vendor's behalf; that he or she is authorized to bind the Vendor in a contractual relationship; and that, to the best of his or her knowledge, the Vendor has properly registered with any State agency that may require registration.

35. VENDOR RELATIONSHIP: The relationship of the Vendor to the State shall be that of an independent contractor and no principal-agent relationship or employer-employee relationship is contemplated or created by this Contract. The Vendor as an independent contractor is solely liable for the acts and omissions of its employees and agents. Vendor shall be responsible for selecting, supervising, and compensating any and all individuals employed pursuant to the terms of this Solicitation and resulting contract. Neither the Vendor, nor any employees or subcontractors of the Vendor, shall be deemed to be employees of the State for any purpose whatsoever. Vendor shall be exclusively responsible for payment of employees and contractors for all wages and salaries, taxes, withholding payments, penalties, fees, fringe benefits, professional liability insurance premiums, contributions to insurance and pension, or other deferred compensation plans, including but not limited to, Workers' Compensation and Social Security obligations, licensing fees, etc. and the filing of all necessary documents, forms, and returns pertinent to all of the foregoing.

Vendor shall hold harmless the State, and shall provide the State and Agency with a defense against any and all claims including, but not limited to, the foregoing payments, withholdings, contributions, taxes, Social Security taxes, and employer income tax returns.

36. INDEMNIFICATION: The Vendor agrees to indemnify, defend, and hold harmless the State and the Agency, their officers, and employees from and against: (1) Any claims or losses for services rendered by any subcontractor, person, or firm performing or supplying services, materials, or supplies in connection with the performance of the Contract; (2) Any claims or losses resulting to any person or entity injured or damaged by the Vendor, its officers, employees, or subcontractors by the publication, translation, reproduction, delivery, performance, use, or disposition of any data used under the Contract in a manner not authorized by the Contract, or by Federal or State statutes or regulations; and (3) Any failure of the Vendor, its officers, employees, or subcontractors to observe State and Federal laws including, but not limited to, labor and wage and hour laws.

37. PURCHASING AFFIDAVIT: In accordance with West Virginia Code § 5A-3-10a, all Vendors are required to sign, notarize, and submit the Purchasing Affidavit stating that neither the Vendor nor a related party owe a debt to the State in excess of \$1,000. The affidavit must be submitted prior to award, but should be submitted with the Vendor's bid. A copy of the Purchasing Affidavit is included herewith.

38. ADDITIONAL AGENCY AND LOCAL GOVERNMENT USE: This Contract may be utilized by other agencies, spending units, and political subdivisions of the State of West Virginia; county, municipal, and other local government bodies; and school districts ("Other Government Entities"). Any extension of this Contract to the aforementioned Other Government Entities must be on the same prices, terms, and conditions as those offered and agreed to in this Contract, provided that such extension is in compliance with the applicable laws, rules, and ordinances of the Other Government Entity. If the Vendor does not wish to extend the prices, terms, and conditions of its bid and subsequent contract to the Other Government Entities, the Vendor must clearly indicate such refusal in its bid. A refusal to extend this Contract to the Other Government Entities shall not impact or influence the award of this Contract in any manner.

39. CONFLICT OF INTEREST: Vendor, its officers or members or employees, shall not presently have or acquire an interest, direct or indirect, which would conflict with or compromise the performance of its obligations hereunder. Vendor shall periodically inquire of its officers, members and employees to ensure that a conflict of interest does not arise. Any conflict of interest discovered shall be promptly presented in detail to the Agency.

40. REPORTS: Vendor shall provide the Agency and/or the Purchasing Division with the following reports identified by a checked box below:

☐ Such reports as the Agency and/or the Purchasing Division may request. Requested reports may include, but are not limited to, quantities purchased, agencies utilizing the contract, total contract expenditures by agency, etc.

☐ Quarterly reports detailing the total quantity of purchases in units and dollars, along with a listing of purchases by agency. Quarterly reports should be delivered to the Purchasing Division via email at purchasing.requisitions@wv.gov.

41. BACKGROUND CHECK: In accordance with W. Va. Code § 15-2D-3, the Director of the Division of Protective Services shall require any service provider whose employees are regularly employed on the grounds or in the buildings of the Capitol complex or who have access to sensitive or critical information to submit to a fingerprint-based state and federal background inquiry through the state repository. The service provider is responsible for any costs associated with the fingerprint-based state and federal background inquiry.

After the contract for such services has been approved, but before any such employees are permitted to be on the grounds or in the buildings of the Capitol complex or have access to sensitive or critical information, the service provider shall submit a list of all persons who will be physically present and working at the Capitol complex to the Director of the Division of Protective Services for purposes of verifying compliance with this provision. The State reserves the right to prohibit a service provider's employees from accessing sensitive or critical information or to be present at the Capitol complex based upon results addressed from a criminal background check.

Service providers should contact the West Virginia Division of Protective Services by phone at (304) 558-9911 for more information.

42. PREFERENCE FOR USE OF DOMESTIC STEEL PRODUCTS: Except when authorized by the Director of the Purchasing Division pursuant to W. Va. Code § 5A-3-56, no contractor may use or supply steel products for a State Contract Project other than those steel products made in the United States. A contractor who uses steel products in violation of this section may be subject to civil penalties pursuant to W. Va. Code § 5A-3-56. As used in this section:

- a. "State Contract Project" means any erection or construction of, or any addition to, alteration of or other improvement to any building or structure, including, but not limited to, roads or highways, or the installation of any heating or cooling or ventilating plants or other equipment, or the supply of and materials for such projects, pursuant to a contract with the State of West Virginia for which bids were solicited on or after June 6, 2001.
- b. "Steel Products" means products rolled, formed, shaped, drawn, extruded, forged, cast, fabricated or otherwise similarly processed, or processed by a combination of two or more or such operations, from steel made by the open hearth, basic oxygen, electric furnace, Bessemer or other steel making process. The Purchasing Division Director may, in writing, authorize the use of foreign steel products if:
- c. The cost for each contract item used does not exceed one tenth of one percent (.1%) of the total contract cost or two thousand five hundred dollars (\$2,500.00), whichever is greater. For the purposes of this section, the cost is the value of the steel product as delivered to the project; or
- d. The Director of the Purchasing Division determines that specified steel materials are not produced in the United States in sufficient quantity or otherwise are not reasonably available to meet contract requirements.

43. PREFERENCE FOR USE OF DOMESTIC ALUMINUM, GLASS, AND STEEL: In Accordance with W. Va. Code § 5-19-1 et seq., and W. Va. CSR § 148-10-1 et seq., for every contract or subcontract, subject to the limitations contained herein, for the construction, reconstruction, alteration, repair, improvement or maintenance of public works or for the purchase of any item of machinery or equipment to be used at sites of public works, only domestic aluminum, glass or steel products shall be supplied unless the spending officer determines, in writing, after the receipt of offers or bids, (1) that the cost of domestic aluminum, glass or steel products is unreasonable or inconsistent with the public interest of the State of West Virginia, (2) that domestic aluminum, glass or steel products are not produced in sufficient quantities to meet the contract requirements, or (3) the available domestic aluminum, glass, or steel do not meet the contract specifications. This provision only applies to public works contracts awarded in an amount more than fifty thousand dollars (\$50,000) or public works contracts that require more than ten thousand pounds of steel products.

The cost of domestic aluminum, glass, or steel products may be unreasonable if the cost is more than twenty percent (20%) of the bid or offered price for foreign made aluminum, glass, or steel products. If the domestic aluminum, glass or steel products to be supplied or produced in a "substantial labor surplus area", as defined by the United States Department of Labor, the cost of domestic aluminum, glass, or steel products may be unreasonable if the cost is more than thirty percent (30%) of the bid or offered price for foreign made aluminum, glass, or steel products. This preference shall be applied to an item of machinery or equipment, as indicated above, when the item is a single unit of equipment or machinery manufactured primarily of aluminum, glass or steel, is part of a public works contract and has the sole purpose or of being a permanent part of a single public works project. This provision does not apply to equipment or machinery purchased by a spending unit for use by that spending unit and not as part of a single public works project.

All bids and offers including domestic aluminum, glass or steel products that exceed bid or offer prices including foreign aluminum, glass or steel products after application of the preferences provided in this provision may be reduced to a price equal to or lower than the lowest bid or offer price for foreign aluminum, glass or steel products plus the applicable preference. If the reduced bid or offer prices are made in writing and supersede the prior bid or offer prices, all bids or offers, including the reduced bid or offer prices, will be reevaluated in accordance with this rule.

DESIGNATED CONTACT: Vendor appoints the individual identified in this Section as the Contract Administrator and the initial point of contact for matters relating to this Contract.

Heather Juzwa, Senior Field Sales Engineer

(Name, Title)

Heather, Juzwa, Senior Field Sales Engineer

(Printed Name and Title)

7102 Riverwood Drive, Columbia, MD 21046

(Address)

410-381-1227 x 1673, 410-290-9140

(Phone Number) / (Fax Number)

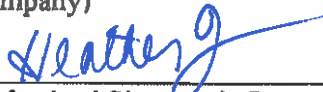
hljuzwa@shimadzu.com

(email address)

CERTIFICATION AND SIGNATURE: By signing below, or submitting documentation through wvOASIS, I certify that I have reviewed this Solicitation in its entirety; that I understand the requirements, terms and conditions, and other information contained herein; that this bid, offer or proposal constitutes an offer to the State that cannot be unilaterally withdrawn; that the product or service proposed meets the mandatory requirements contained in the Solicitation for that product or service, unless otherwise stated herein; that the Vendor accepts the terms and conditions contained in the Solicitation, unless otherwise stated herein; that I am submitting this bid, offer or proposal for review and consideration; that I am authorized by the vendor to execute and submit this bid, offer, or proposal, or any documents related thereto on vendor's behalf; that I am authorized to bind the vendor in a contractual relationship; and that to the best of my knowledge, the vendor has properly registered with any State agency that may require registration.

Shimadzu Scientific Instruments, Inc.

(Company)



Senior Field Sales Engineer

(Authorized Signature) (Representative Name, Title)

Heather Juzwa

(Printed Name and Title of Authorized Representative)

11/15/16

(Date)

410-381-1227 x 1673, 410-290-9140

(Phone Number) (Fax Number)

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

SPECIFICATIONS

1. **PURPOSE AND SCOPE:** The West Virginia Purchasing Division is soliciting bids on behalf of West Virginia Department of Agriculture to establish a contract for the one time purchase of a **Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS), workstation PC, software, printer, shipping, installation, validation, warranty, training, and service.**
2. **DEFINITIONS:** The terms listed below shall have the meanings assigned to them below. Additional definitions can be found in section 2 of the General Terms and Conditions.
 - 2.1 **"APCI"** means atmospheric pressure chemical ionization.
 - 2.2 **"Contract Services"** means the LC/MS/MS with inside delivery, installation, validation, warranty, and training.
 - 2.3 **"ESI"** means electrospray ionization.
 - 2.4 **"FG"** means femtogram.
 - 2.5 **"Installation"** means unpacking and setting instrumentation in place with all connections secured for the instrument(s) to be in working order including software installation on the computer connected to the instrument.
 - 2.6 **"LC/MS/MS"** means Liquid Chromatography Triple Quadrupole Mass Spectrometer.
 - 2.7 **"MRM"** means multiple reactions monitoring.
 - 2.8 **"MSMS"** means tandem mass spectrometry.
 - 2.9 **"Pricing Page"** means the pages, contained in wvOASIS or attached as Exhibit A, upon which Vendor should list its proposed price for the Contract Items.
 - 2.10 **"Service"** means performing routine maintenance work or repair to the instrument or software.
 - 2.11 **"SIM"** means selected ion monitoring
 - 2.12 **"S/N"** means signal noise.

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- 2.13 “Solicitation”** means the official notice of an opportunity to supply the State with goods or services that is published by the Purchasing Division.
- 2.14 “Training”** means teaching staff how to use and maintain the instrument and software.
- 2.15 “Validation”** means is the process used to confirm that the analytical procedure employed for a specific test or matrices is suitable for its intended use.
- 2.16 “Warranty”** means the written warranty of the manufacturer of a new instrument of its condition and fitness for use, including any terms or conditions precedent to the enforcement of obligations under that warranty.

3. GENERAL REQUIREMENTS:

- 3.1 Mandatory Contract Item Requirements:** Contract Item must meet or exceed the mandatory requirements listed below for the **Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS)**.

3.1.1 Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS)

- 3.1.1.1** Must be capable of detecting a variety of analytes including pesticides, herbicides, toxins, and drugs in matrices such as foods, soil, vegetation, animal feed, and water.
- 3.1.1.2** MSMS must have two ion sources that operate independently which can be set to electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The instrument must enable combinations such as ESI/APCI, ESI/ESI, APCI/APCI with the same or opposite polarities without having to remove the sources to switch modes.
- 3.1.1.3** Minimum sensitivity requirement for positive ion mode: signal/noise (S/N) 2000:1 25 femtograms (fg) of reserpine on column. Minimum sensitivity requirement for negative ion mode: signal/noise (S/N)>2000:1, 25 femtograms (fg) of chloramphenicol on column.
- 3.1.1.4** The source probes must be easy to remove without the use of tools.
- 3.1.1.5** Ion source must have flat response across flow rate up to 3 milliliters per minute without loss of sensitivity.

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Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

- 3.1.1.6** Capable of switching between rapidly between positive and negative ion detection without high voltage switching.
- 3.1.1.7** Acquisition modes: Q1 scan, Q2 scan, multiple reactions monitoring (MRM), selected ion monitoring (SIM), Neutral Loss scans, Product Ion, Precursor Ion, Time managed MRM
- 3.1.1.8** Minimum mass range requirement: 5-1500 mass to charge ratio (m/z)
- 3.1.1.9** Mass stability required: 0.05 atomic mass unit (amu) in 24 hours
- 3.1.1.10** Mass accuracy needed: minimum 0.1 unit across mass range
- 3.1.1.11** Scan speed: $\leq 30,000$ daltons per second (da/s)
- 3.1.1.12** Quad resolution: unit, low and high, minimal sensitivity loss at 0.1 Daltons resolutions
- 3.1.1.13** Polarity switching time: ≤ 15 milliseconds
- 3.1.1.14** Dynamic range: 6 orders
- 3.1.1.15** Dual source switching speed: < 20 milliseconds
- 3.1.1.16** Minimum multiple reactions monitoring (MRM) Dwell Time: 1 millisecond
- 3.1.1.17** MRM transitions: 450 per time segment $> 40,000$ ion transactions per method
- 3.1.1.18** Must have high selectivity mass filter at 0.3 Daltons. Signal loss must not be more than 10%.
- 3.1.1.19** For minimal tuning during method development and minimal maintenance of the ion path, the LC/MS/MS utilizes Hot Source Induced Desolvation interface to the mass analyzer by patented Laminar Flow Ion Guide using gas flow
- 3.1.1.20** High performance liquid chromatograph capable of solvent and column switching without user intervention
- 3.1.1.21** High performance liquid chromatograph capable of regulating column temperature of at least 2 different columns
- 3.1.1.22** High performance liquid chromatograph with an autosampler
- 3.1.1.23** The LC/MS/MS instrument must be fully automated for analysis with a system controller that is loaded with the necessary software.
- 3.1.1.24** The LC/MS/MS must include a maintenance kit.
- 3.1.1.25** Vendor must provide documentation for recommended environmental conditions, electrical requirements, gas requirements, or any other factor that would affect instrument performance.

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Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

3.1.2 Workstation and software

- 3.1.2.1** Data station with windows based operating system capable of multitasking allowing data processing and data acquisition simultaneously.
- 3.1.2.2** Operating system must be fully integrated to control LS/MS/MS.

3.1.3 Shipping, Installation, Validation, Warranty, Training and Service

- 3.1.3.1** Vendor must be on-site for delivery and perform the installation (labor and supplies included) of the LCMSMS.
- 3.1.3.2** The vendor must provide a written validation of the instrument's performance after installation.
- 3.1.3.3** Vendor will provide a full one-year parts and labor warranty on all items, including 2 preventative maintenances.
- 3.1.3.4** Vendor must be able to perform resolutions to service requests within 72 hours which includes on-site resolutions.
- 3.1.3.5** Vendor will provide on-site training (labor and non-consumable supplies included) for all instruments and software.
- 3.1.3.6** Vendor will provide copies of all system manuals (operations, training, technical, service, maintenance).

4. CONTRACT AWARD:

4.1 Contract Award: The Contract is intended to provide Agencies with a purchase price for the Contract Items. The Contract shall be awarded to the Vendor that provides the Contract Items meeting the required specifications for the lowest overall total cost as shown on the Pricing Pages.

4.2 Pricing Page: Vendor should complete the Pricing Page by placing all inclusive information in each column for item number, model/brand name, unit price and extended amount. There should be a price for the LCMSMS, workstation, software, printer, shipping/inside delivery, installation, validation, warranty, training and service. If there is no charge for any deliverable, indicate in the cell with "no charge". The bidder/vendor information must be completed and include an authorize signature. Vendor should complete the Pricing Page in full as failure to complete the Pricing Page in its entirety may result in Vendor's bid being disqualified.

Vendor should type or electronically enter the information into the Pricing Page to prevent errors in the evaluation.

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

5. PAYMENT:

5.1 Payment: Vendor shall accept payment in accordance with the payment procedures of the State of West Virginia.

6. DELIVERY AND RETURN:

6.1 Shipment and Delivery: Vendor should ship the Contract Items immediately after being awarded this Contract and receiving a purchase order or notice to proceed. Contract Items must be delivered to Agency at 313 Gus R. Douglass Lane, Charleston, WV 25312.

6.2 Late Delivery: The Agency placing the order under this Contract must be notified in writing if the shipment of the Contract Items will be delayed for any reason. Any delay in delivery that could cause harm to an Agency will be grounds for cancellation of the Contract, and/or obtaining the Contract Items from a third party.

Any Agency seeking to obtain the Contract Items from a third party under this provision must first obtain approval of the Purchasing Division.

6.3 Delivery Payment/Risk of Loss: Vendor shall deliver the Contract Items F.O.B. destination to the Agency's location.

6.4 Return of Unacceptable Items: If the Agency deems the Contract Items to be unacceptable, the Contract Items shall be returned to Vendor at Vendor's expense and with no restocking charge. Vendor shall either make arrangements for the return within five (5) days of being notified that items are unacceptable, or permit the Agency to arrange for the return and reimburse Agency for delivery expenses. If the original packaging cannot be utilized for the return, Vendor will supply the Agency with appropriate return packaging upon request. All returns of unacceptable items shall be F.O.B. the Agency's location. The returned product shall either be replaced, or the Agency shall receive a full credit or refund for the purchase price, at the Agency's discretion.

6.5 Return Due to Agency Error: Items ordered in error by the Agency will be returned for credit within 30 days of receipt, F.O.B. Vendor's location. Vendor shall not charge a restocking fee if returned products are in a resalable condition. Items shall be deemed to be in a resalable condition if they are unused and in the original packaging. Any restocking fee for items not in a resalable condition shall

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

be the lower of the Vendor's customary restocking fee or 5% of the total invoiced value of the returned items.

7 VENDOR DEFAULT:

7.1 The following shall be considered a vendor default under this Contract.

- 7.1.1** Failure to provide Contract Items in accordance with the requirements contained herein.
- 7.1.2** Failure to comply with other specifications and requirements contained herein.
- 7.1.3** Failure to comply with any laws, rules, and ordinances applicable to the Contract Services provided under this Contract.
- 7.1.4** Failure to remedy deficient performance upon request.

7.2 The following remedies shall be available to Agency upon default.

- 7.2.1** Immediate cancellation of the Contract.
- 7.2.2** Immediate cancellation of one or more release orders issued under this Contract.
- 7.2.3** Any other remedies available in law or equity.

8 FACILITIES ACCESS: Performance of Services will require access to the facility.

- 8.1** Vendor must identify principal service personnel who will be asked for identification upon entrance to the facility.
- 8.2** Anyone performing under this Contract will be subject to Agency's security protocol and procedures.
- 8.3** Vendor shall inform all staff of Agency's security protocol and procedures.

State of West Virginia
VENDOR PREFERENCE CERTIFICATE

Certification and application is hereby made for Preference in accordance with **West Virginia Code, §5A-3-37**. (Does not apply to construction contracts). **West Virginia Code, §5A-3-37**, provides an opportunity for qualifying vendors to request (at the time of bid) preference for their residency status. Such preference is an evaluation method only and will be applied only to the cost bid in accordance with the **West Virginia Code**. This certificate for application is to be used to request such preference. The Purchasing Division will make the determination of the Vendor Preference, if applicable.

1. Application is made for 2.5% vendor preference for the reason checked:

☐ Bidder is an individual resident vendor and has resided continuously in West Virginia for four (4) years immediately preceding the date of this certification; or,

☐ Bidder is a partnership, association or corporation resident vendor and has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or 80% of the ownership interest of Bidder is held by another individual, partnership, association or corporation resident vendor who has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or,

☐ Bidder is a nonresident vendor which has an affiliate or subsidiary which employs a minimum of one hundred state residents and which has maintained its headquarters or principal place of business within West Virginia continuously for the four (4) years immediately preceding the date of this certification; or,

2. Application is made for 2.5% vendor preference for the reason checked:

☐ Bidder is a resident vendor who certifies that, during the life of the contract, on average at least 75% of the employees working on the project being bid are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; or,

3. Application is made for 2.5% vendor preference for the reason checked:

☐ Bidder is a nonresident vendor employing a minimum of one hundred state residents or is a nonresident vendor with an affiliate or subsidiary which maintains its headquarters or principal place of business within West Virginia employing a minimum of one hundred state residents who certifies that, during the life of the contract, on average at least 75% of the employees or Bidder's affiliate's or subsidiary's employees are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; or,

4. Application is made for 5% vendor preference for the reason checked:

☐ Bidder meets either the requirement of both subdivisions (1) and (2) or subdivision (1) and (3) as stated above; or,

5. Application is made for 3.5% vendor preference who is a veteran for the reason checked:

☐ Bidder is an individual resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard and has resided in West Virginia continuously for the four years immediately preceding the date on which the bid is submitted; or,

6. Application is made for 3.5% vendor preference who is a veteran for the reason checked:

☐ Bidder is a resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard, if, for purposes of producing or distributing the commodities or completing the project which is the subject of the vendor's bid and continuously over the entire term of the project, on average at least seventy-five percent of the vendor's employees are residents of West Virginia who have resided in the state continuously for the two immediately preceding years.

7. Application is made for preference as a non-resident small, women- and minority-owned business, in accordance with West Virginia Code §5A-3-59 and West Virginia Code of State Rules.

☐ Bidder has been or expects to be approved prior to contract award by the Purchasing Division as a certified small, women- and minority-owned business.

Bidder understands if the Secretary of Revenue determines that a Bidder receiving preference has failed to continue to meet the requirements for such preference, the Secretary may order the Director of Purchasing to: (a) reject the bid; or (b) assess a penalty against such Bidder in an amount not to exceed 5% of the bid amount and that such penalty will be paid to the contracting agency or deducted from any unpaid balance on the contract or purchase order.

By submission of this certificate, Bidder agrees to disclose any reasonably requested information to the Purchasing Division and authorizes the Department of Revenue to disclose to the Director of Purchasing appropriate information verifying that Bidder has paid the required business taxes, provided that such information does not contain the amounts of taxes paid nor any other information deemed by the Tax Commissioner to be confidential.

Under penalty of law for false swearing (West Virginia Code, §61-5-3), Bidder hereby certifies that this certificate is true and accurate in all respects; and that if a contract is issued to Bidder and if anything contained within this certificate changes during the term of the contract, Bidder will notify the Purchasing Division in writing immediately.

Bidder: Shimadzu Scientific Instruments, Inc.

Signed:



Date: 11/15/16

Title: Senior Field Sales Engineer

OPTION 1: Nexera X2 UHPLC with LCMS-8040

PRICING PAGE					
Item No.	Description	Model No/Brand Name	Quantity	Unit Price	Extended Amount
3.1.1 & 3.1.2	LCMSMS, workstation, software, printer	Nexera X2 UHPLC and LCMS-8040	1	\$297,921.82	\$266,544.92
3.1.3	Shipping charges and inside delivery	N/A	1	\$850.00	\$850.00
3.1.3	Installation/validation	N/A	1	\$0.00	\$0.00
3.1.3	Training/warranty	N/A	1	\$8,610.00	\$0.00
3.1.3	Service	2 Premium Preventative Maintenance Visits	1	\$30,526.90	\$30,526.90
	Failure to use this form may result in disqualification			GRAND TOTAL	\$297,921.82
Bidder / Vendor Information					
Name:	Shimadzu Scientific Instruments, Inc.				
Address:	7102 Riverwood Drive				
	Columbia, MD 21046				
Phone:	410-381-1227				
Email Address:	customer.service@shimadzu.com, hljuzwa@shimadzu.com				
Signature:					

OPTION 2: Nexera X2 UHPLC with LCMS-8045

PRICING PAGE					
Item No.	Description	Model No/Brand Name	Quantity	Unit Price	Extended Amount
3.1.1 & 3.1.2	LCMSMS, workstation, software, printer	Nexera X2 UHPLC and LCMS-8045	1	\$333,439.20	\$302,062.10
3.1.3	Shipping charges and inside delivery	N/A	1	\$850.00	\$850.00
3.1.3	Installation/validation	N/A	1	\$0.00	\$0.00
3.1.3	Training/warranty	N/A	1	\$8,610.00	\$0.00
3.1.3	Service	2 Premium Preventative Maintenance Visits	1	\$30,526.90	\$30,526.90
	Failure to use this form may result in disqualification			GRAND TOTAL	\$333,439.20
	Bidder / Vendor Information				
Name:	Shimadzu Scientific Instruments, Inc.				
Address:	7102 Riverwood Drive				
	Columbia, MD 21046				
Phone:	410-381-1227				
Email Address:	customer.service@shimadzu.com, hljuzwa@shimadzu.com				
Signature:					

STATE OF WEST VIRGINIA
Purchasing Division

PURCHASING AFFIDAVIT

MANDATE: Under W. Va. Code §5A-3-10a, no contract or renewal of any contract may be awarded by the state or any of its political subdivisions to any vendor or prospective vendor when the vendor or prospective vendor or a related party to the vendor or prospective vendor is a debtor and: (1) the debt owed is an amount greater than one thousand dollars in the aggregate; or (2) the debtor is in employer default.

EXCEPTION: The prohibition listed above does not apply where a vendor has contested any tax administered pursuant to chapter eleven of the W. Va. Code, workers' compensation premium, permit fee or environmental fee or assessment and the matter has not become final or where the vendor has entered into a payment plan or agreement and the vendor is not in default of any of the provisions of such plan or agreement.

DEFINITIONS:

"Debt" means any assessment, premium, penalty, fine, tax or other amount of money owed to the state or any of its political subdivisions because of a judgment, fine, permit violation, license assessment, defaulted workers' compensation premium, penalty or other assessment presently delinquent or due and required to be paid to the state or any of its political subdivisions, including any interest or additional penalties accrued thereon.

"Employer default" means having an outstanding balance or liability to the old fund or to the uninsured employers' fund or being in policy default, as defined in W. Va. Code § 23-2c-2, failure to maintain mandatory workers' compensation coverage, or failure to fully meet its obligations as a workers' compensation self-insured employer. An employer is not in employer default if it has entered into a repayment agreement with the Insurance Commissioner and remains in compliance with the obligations under the repayment agreement.

"Related party" means a party, whether an individual, corporation, partnership, association, limited liability company or any other form or business association or other entity whatsoever, related to any vendor by blood, marriage, ownership or contract through which the party has a relationship of ownership or other interest with the vendor so that the party will actually or by effect receive or control a portion of the benefit, profit or other consideration from performance of a vendor contract with the party receiving an amount that meets or exceeds five percent of the total contract amount.

AFFIRMATION: By signing this form, the vendor's authorized signer affirms and acknowledges under penalty of law for false swearing (W. Va. Code §61-5-3) that neither vendor nor any related party owe a debt as defined above and that neither vendor nor any related party are in employer default as defined above, unless the debt or employer default is permitted under the exception above.

WITNESS THE FOLLOWING SIGNATURE:

Vendor's Name: Shimadzu Scientific Instruments, Inc.

Authorized Signature: [Signature] Date: 11/14/2016

State of PENNSYLVANIA

County of WASHINGTON, to-wit:

Taken, subscribed, and sworn to before me this 16th day of NOVEMBER, 2016

My Commission expires MAY 10, 2017.

AFFIX SEAL HERE

NOTARY PUBLIC

[Signature]
Purchasing Affidavit (Revised 08/01/2015)

COMMONWEALTH OF PENNSYLVANIA

Notarial Seal

Bradley J. Bozovich, Notary Public
North Strabane Twp., Washington County
My Commission Expires May 10, 2017

MEMBER, PENNSYLVANIA ASSOCIATION OF NOTARIES



Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

State of West Virginia
Request for Quotation
01 - Agricultural

Proc Folder: 265333

Doc Description: Addendum # 1 - Triple Quad LC/MS/MS

Proc Type: Central Purchase Order

Date Issued	Solicitation Closes	Solicitation No	Version
2016-11-09	2016-11-17 13:30:00	CRFQ 1400 AGR1700000005	2

BID RECEIVING LOCATION

BID CLERK

DEPARTMENT OF ADMINISTRATION

PURCHASING DIVISION

2019 WASHINGTON ST E

CHARLESTON

WV 25305

US

VENDOR

Vendor Name, Address and Telephone Number:

Shimadzu Scientific instruments, Inc.
7201 Riverwood Drive
Columbia, MD 21046
410-381-1227

FOR INFORMATION CONTACT THE BUYER

Linda Harper

(304) 558-0468

linda.b.harper@wv.gov

Signature X

FEIN # 52-1035956

DATE 11/15/16

All offers subject to all terms and conditions contained in this solicitation

ADDITIONAL INFORMATION:

Addendum 1 issued for the following reasons:

1. To modify Section 3.1.1.2 of the specifications based on Q.A./14. of vendor questions. Revised specifications attached.
2. To publish the vendor questions with responses.

No other changes.

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US OPTION 1: \$297,921.81 OPTION 1: \$266,544.92	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
1	LCMSMS, Workstation, software, printer	1.00000	EA	OPTION 2: \$333,439.20	OPTION 2: \$302,062.10

Comm Code	Manufacturer	Specification	Model #
41100000	Shimadzu	Please see enclosed documentation.	Nexera X2 UHPLC and LCMS-8040 and LCMS-8045

Extended Description :

LCMSMS, Workstation, software, printer per specification 3.1.1 & 3.1.2

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
2	Shipping Charges and inside delivery	1.00000	EA	\$850.00	\$850.00

Comm Code	Manufacturer	Specification	Model #
78121603	Shimadzu	N/A	N/A

Extended Description :

Shipping Charges and inside deliver per section 3.1.3

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
3	Installation/validation	1.00000	EA	\$0.00	\$0.00

Comm Code	Manufacturer	Specification	Model #
73171605	Shimadzu	N/A	N/A

Extended Description :
Installation/validation per section 3.1.3

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
4	Training/Warranty	1.00000	EA	\$8,610.00	\$0.00

Comm Code	Manufacturer	Specification	Model #
73171605	Shimadzu	N/A	N/A

Extended Description :
Training/Warranty per section 3.1.3

INVOICE TO		SHIP TO	
PROCUREMENT OFFICER 304-558-2221 AGRICULTURE DEPARTMENT OF ADMINISTRATIVE SERVICES 1900 KANAWHA BLVD E CHARLESTON WV25305-0173 US		AUTHORIZED RECEIVER 304-558-2227 AGRICULTURE DEPARTMENT OF REGULATORY PROTECTION DIVISION 313 GUS R DOUGLAS LN, BLDG 11 CHARLESTON WV 25312 US	

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Total Price
5	Service	1.00000	EA	\$30,526.90	\$30,526.90

Comm Code	Manufacturer	Specification	Model #
73171605	Shimadzu	N/A	Premium Preventative Maintenance

Extended Description :
Service per section 3.1.3

SCHEDULE OF EVENTS

Line	Event	Event Date
1	Question Deadline 3:00 p.m.	2016-11-04

SOLICITATION NUMBER: AGR1700000005

Addendum Number: 1

The purpose of this addendum is to modify the solicitation identified as ("Solicitation") to reflect the change(s) identified and described below.

Applicable Addendum Category:

- ☐ Modify bid opening date and time
- ☒ Modify specifications of product or service being sought
- ☒ Attachment of vendor questions and responses
- ☐ Attachment of pre-bid sign-in sheet
- ☐ Correction of error
- ☐ Other

Description of Modification to Solicitation:

Addendum 1 issued for the following reasons:

1. To modify Section 3.1.1.2 of the specifications based on Q.A./14. of vendor questions. Revised specifications attached.
2. To publish the vendor questions with responses.

No other changes.

Additional Documentation: Documentation related to this Addendum (if any) has been included herewith as Attachment A and is specifically incorporated herein by reference.

Terms and Conditions:

1. All provisions of the Solicitation and other addenda not modified herein shall remain in full force and effect.
2. Vendor should acknowledge receipt of all addenda issued for this Solicitation by completing an Addendum Acknowledgment, a copy of which is included herewith. Failure to acknowledge addenda may result in bid disqualification. The addendum acknowledgement should be submitted with the bid to expedite document processing.

ATTACHMENT A

CRFQ AGR1700000005

Vendor Questions

Q.1. Specification 3.1.1.20 says that the system should come with solvent and column selection. Can you please clarify how many solvents per pump (2, 3, 4, etc.) and how many columns (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12? Specification 3.1.1.21 mentioned 2 columns, but we want to be certain.

A.1. 4 solvents per pump preferred and 2 columns

Q.2. Specification 3.1.1.21 mentioned a column temperature, but no temperature range on the column oven is provided. We offer a heating oven and a heating (ambient up to 85 degrees) and cooling oven (10 degrees below ambient up to 85 degrees). Can you please clarify which you need?

A.2. 4-90°

Q.3. Specification 3.1.1.22 mentions a High Performance Liquid Chromatograph with an autosampler. This leads to a series of questions on the configuration:

a. I assume you want a binary high pressure pump? Or, did you want a quaternary low pressure pump?

3a. quaternary low pressure pump

b. What pressure range do you want on the system? We offer standard (6000 psi), mid (9000 psi) and UHPLC (19000 psi).

3b. since systems vary 15,000 to 19,000 psi is acceptable

c. Should the autosampler be ambient or Peltier-cooled?

3c. cooled

d. Will you be sampling from 2 mL vials, which is typically standard, or do you want to sample from microtitre plates, etc..?

3d. possibly both

e. How many vials do you want your autosampler to hold? Shimadzu offers 100 and 324 2 mL vial options.

3e. 48 vials or more

f. Do you want the HPLC to include a traditional HPLC detector (such as a photo diode array) inline to help with troubleshooting and method development?

g. 3f. yes

- Q.4. There is no mention of a Nitrogen or Air generator, and this will be necessary to run any LC/MS/MS. Should this be included on the bid, or should just the specifications be included as per 3.1.1.25?
- A.4. Do not include in bid
- Q.5. There is no mention of a UPS on the bid. This is highly recommended to protect the turbomolecular pump and deliver a stable source of power to the instrument. Should this be added?
- A.5. Do not include in bid
- Q.6. Section 3.1.3.4 refers to response time for service requests. Does this refer to requests under warranty or post warranty? Also, is there a requirement for post warranty contractual support pricing for this solicitation?
- A.6. This refers to any service when the instrument has failed and the laboratory cannot process samples. The best response time is critical to the laboratory to maintain production, therefore, a 72 hour resolution to service requests is critical.
- Q.7. Is the lab that is interested in purchasing an LCMS system interested or currently running any regulated EPA, FDA, or USDA LCMS Methods and if so which method numbers are being analyzed?
- A.7. Multiple methods from the EPA, FDA, USDA, FERN, AOAC are being ran or are intended to be ran.
- Q.8. Would they be able to outline which pesticides, herbicides, toxins, and drugs they would like analyze with the LCMS/MS system and what limits of detection and quantification they need for their work?
- A.8. The laboratory will test an array of substances that are of concern under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In addition, two specific methods are of current interest – USDA FSIS CLG.TOX 001 and TOX 002 which screen for drugs, pesticides and poisons.
- Q.9. Would they be able to outline a specific LCMS method that requires a flow rate of 3 mL/min and why this would be more advantageous than a lower flow rate method?
- A.9. No specific LCMS method is referenced. The specification was referring to a loss in sensitivity at different flow rates and anything that meets that requirement or is more sensitive is required.
- Q.10. Are they currently using any sample preparation for the analysis of these components and if they could optimize or potentially eliminate sample prep would that be something they would be interested in doing?
- A.10. It is dependent upon the official method. If the method dictates the sample preparation, then the laboratory will have to follow it. Some preparations can be eliminated if the laboratory validates

the method. Also changes is methods that are validated will have to be approved by our customers before a validation is performed.

Q.11. Would they be able to outline a specific LCMS method that requires a scan rate of 30,000 Da/sec and how is this advantageous?

A.11. No specific LCMS method is referenced, however the laboratory will be performing method development and will be comparing if the chromatographic resolution is better with the higher scan rates.

Q.12. How does size of the LCMS solution they are looking to obtain affect their decision? If they could get a small footprint LCMS system would that add value to the work they do?

A.12. If all the specifications are met in the RFQ, the size could be something that would determine an award of the bid.

Q.13. We would like to be able to propose suggestion exceptions to terms and conditions with a mutual negotiation and agreement. Will you allow this should we be awarded the bid or will you disqualify us? Bid states "may" result in disqualification but we would like to attempt to negotiate if possible.

A.13. Suggested exceptions should be submitted and will be reviewed for acceptance. Certain State of West Virginia terms and conditions are non-negotiable.

Q.14. Can you clarify the need and importance for the spec listed in 3.1.1.2? I don't see how it applies to the real world applications. We have canned methods for all of the applications in question and none require this functionality.

A.14. There is an error in the specification 3.1.1.2. It should state "dual ion sources that operate independently which can be set to electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). A copy of the revised specifications attached.

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

SPECIFICATIONS

1. **PURPOSE AND SCOPE:** The West Virginia Purchasing Division is soliciting bids on behalf of West Virginia Department of Agriculture to establish a contract for the one time purchase of a **Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS)**, workstation PC, software, printer, shipping, installation, validation, warranty, training, and service.
2. **DEFINITIONS:** The terms listed below shall have the meanings assigned to them below. Additional definitions can be found in section 2 of the General Terms and Conditions.
 - 2.1 “APCI” means atmospheric pressure chemical ionization.
 - 2.2 “Contract Services” means the LC/MS/MS with inside delivery, installation, validation, warranty, and training.
 - 2.3 “ESI” means electrospray ionization.
 - 2.4 “FG” means femtogram.
 - 2.5 “Installation” means unpacking and setting instrumentation in place with all connections secured for the instrument(s) to be in working order including software installation on the computer connected to the instrument.
 - 2.6 “LC/MS/MS” means Liquid Chromatography Triple Quadrupole Mass Spectrometer.
 - 2.7 “MRM” means multiple reactions monitoring.
 - 2.8 “MSMS” means tandem mass spectrometry.
 - 2.9 “Pricing Page” means the pages, contained in wvOASIS or attached as Exhibit A, upon which Vendor should list its proposed price for the Contract Items.
 - 2.10 “Service” means performing routine maintenance work or repair to the instrument or software.
 - 2.11 “SIM” means selected ion monitoring
 - 2.12 “S/N” means signal noise.

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

- 2.13 “Solicitation”** means the official notice of an opportunity to supply the State with goods or services that is published by the Purchasing Division.
- 2.14 “Training”** means teaching staff how to use and maintain the instrument and software.
- 2.15 “Validation”** means is the process used to confirm that the analytical procedure employed for a specific test or matrices is suitable for its intended use.
- 2.16 “Warranty”** means the written warranty of the manufacturer of a new instrument of its condition and fitness for use, including any terms or conditions precedent to the enforcement of obligations under that warranty.

3. GENERAL REQUIREMENTS:

- 3.1 Mandatory Contract Item Requirements:** Contract Item must meet or exceed the mandatory requirements listed below for the **Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS)**.

3.1.1 Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS)

- 3.1.1.1** Must be capable of detecting a variety of analytes including pesticides, herbicides, toxins, and drugs in matrices such as foods, soil, vegetation, animal feed, and water.
- 3.1.1.2** MSMS must have dual ion sources that operate independently which can be set to electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The instrument must enable combinations such as ESI/APCI, ESI/ESI, APCI/APCI with the same or opposite polarities without having to remove the sources to switch modes.
- 3.1.1.3** Minimum sensitivity requirement for positive ion mode: signal/noise (S/N) 2000:1 25 femtograms (fg) of reserpine on column. Minimum sensitivity requirement for negative ion mode: signal/noise (S/N)>2000:1, 25 femtograms (fg) of chloramphenicol on column.
- 3.1.1.4** The source probes must be easy to remove without the use of tools.
- 3.1.1.5** Ion source must have flat response across flow rate up to 3 milliliters per minute without loss of sensitivity.

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

- 3.1.1.6** Capable of switching between rapidly between positive and negative ion detection without high voltage switching.
- 3.1.1.7** Acquisition modes: Q1 scan, Q2 scan, multiple reactions monitoring (MRM), selected ion monitoring (SIM), Neutral Loss scans, Product Ion, Precursor Ion, Time managed MRM
- 3.1.1.8** Minimum mass range requirement: 5-1500 mass to charge ratio (m/z)
- 3.1.1.9** Mass stability required: 0.05 atomic mass unit (amu) in 24 hours
- 3.1.1.10** Mass accuracy needed: minimum 0.1 unit across mass range
- 3.1.1.11** Scan speed: $\leq 30,000$ daltons per second (da/s)
- 3.1.1.12** Quad resolution: unit, low and high, minimal sensitivity loss at 0.1 Daltons resolutions
- 3.1.1.13** Polarity switching time: ≤ 15 milliseconds
- 3.1.1.14** Dynamic range: 6 orders
- 3.1.1.15** Dual source switching speed: < 20 milliseconds
- 3.1.1.16** Minimum multiple reactions monitoring (MRM) Dwell Time: 1 millisecond
- 3.1.1.17** MRM transitions: 450 per time segment $> 40,000$ ion transactions per method
- 3.1.1.18** Must have high selectivity mass filter at 0.3 Daltons. Signal loss must not be more than 10%.
- 3.1.1.19** For minimal tuning during method development and minimal maintenance of the ion path, the LC/MS/MS utilizes Hot Source Induced Desolvation interface to the mass analyzer by patented Laminar Flow Ion Guide using gas flow
- 3.1.1.20** High performance liquid chromatograph capable of solvent and column switching without user intervention
- 3.1.1.21** High performance liquid chromatograph capable of regulating column temperature of at least 2 different columns
- 3.1.1.22** High performance liquid chromatograph with an autosampler
- 3.1.1.23** The LC/MS/MS instrument must be fully automated for analysis with a system controller that is loaded with the necessary software.
- 3.1.1.24** The LC/MS/MS must include a maintenance kit.
- 3.1.1.25** Vendor must provide documentation for recommended environmental conditions, electrical requirements, gas requirements, or any other factor that would affect instrument performance.

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

3.1.2 Workstation and software

3.1.2.1 Data station with windows based operating system capable of multitasking allowing data processing and data acquisition simultaneously.

3.1.2.2 Operating system must be fully integrated to control LS/MS/MS.

3.1.3 Shipping, Installation, Validation, Warranty, Training and Service

3.1.3.1 Vendor must be on-site for delivery and perform the installation (labor and supplies included) of the LCMSMS.

3.1.3.2 The vendor must provide a written validation of the instrument's performance after installation.

3.1.3.3 Vendor will provide a full one-year parts and labor warranty on all items, including 2 preventative maintenances.

3.1.3.4 Vendor must be able to perform resolutions to service requests within 72 hours which includes on-site resolutions.

3.1.3.5 Vendor will provide on-site training (labor and non-consumable supplies included) for all instruments and software.

3.1.3.6 Vendor will provide copies of all system manuals (operations, training, technical, service, maintenance).

4. CONTRACT AWARD:

4.1 Contract Award: The Contract is intended to provide Agencies with a purchase price for the Contract Items. The Contract shall be awarded to the Vendor that provides the Contract Items meeting the required specifications for the lowest overall total cost as shown on the Pricing Pages.

4.2 Pricing Page: Vendor should complete the Pricing Page by placing all inclusive information in each column for item number, model/brand name, unit price and extended amount. There should be a price for the LCMSMS, workstation, software, printer, shipping/inside delivery, installation, validation, warranty, training and service. If there is no charge for any deliverable, indicate in the cell with "no charge". The bidder/vendor information must be completed and include an authorize signature. Vendor should complete the Pricing Page in full as failure to complete the Pricing Page in its entirety may result in Vendor's bid being disqualified.

Vendor should type or electronically enter the information into the Pricing Page to prevent errors in the evaluation.

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

5. PAYMENT:

5.1 Payment: Vendor shall accept payment in accordance with the payment procedures of the State of West Virginia.

6. DELIVERY AND RETURN:

6.1 Shipment and Delivery: Vendor should ship the Contract Items immediately after being awarded this Contract and receiving a purchase order or notice to proceed. Contract Items must be delivered to Agency at 313 Gus R. Douglass Lane, Charleston, WV 25312.

6.2 Late Delivery: The Agency placing the order under this Contract must be notified in writing if the shipment of the Contract Items will be delayed for any reason. Any delay in delivery that could cause harm to an Agency will be grounds for cancellation of the Contract, and/or obtaining the Contract Items from a third party.

Any Agency seeking to obtain the Contract Items from a third party under this provision must first obtain approval of the Purchasing Division.

6.3 Delivery Payment/Risk of Loss: Vendor shall deliver the Contract Items F.O.B. destination to the Agency's location.

6.4 Return of Unacceptable Items: If the Agency deems the Contract Items to be unacceptable, the Contract Items shall be returned to Vendor at Vendor's expense and with no restocking charge. Vendor shall either make arrangements for the return within five (5) days of being notified that items are unacceptable, or permit the Agency to arrange for the return and reimburse Agency for delivery expenses. If the original packaging cannot be utilized for the return, Vendor will supply the Agency with appropriate return packaging upon request. All returns of unacceptable items shall be F.O.B. the Agency's location. The returned product shall either be replaced, or the Agency shall receive a full credit or refund for the purchase price, at the Agency's discretion.

6.5 Return Due to Agency Error: Items ordered in error by the Agency will be returned for credit within 30 days of receipt, F.O.B. Vendor's location. Vendor shall not charge a restocking fee if returned products are in a resalable condition. Items shall be deemed to be in a resalable condition if they are unused and in the original packaging. Any restocking fee for items not in a resalable condition shall

REQUEST FOR QUOTATION
Liquid Chromatography/Mass Spectrometer (LC/MS) Instrument

be the lower of the Vendor's customary restocking fee or 5% of the total invoiced value of the returned items.

7 VENDOR DEFAULT:

7.1 The following shall be considered a vendor default under this Contract.

7.1.1 Failure to provide Contract Items in accordance with the requirements contained herein.

7.1.2 Failure to comply with other specifications and requirements contained herein.

7.1.3 Failure to comply with any laws, rules, and ordinances applicable to the Contract Services provided under this Contract.

7.1.4 Failure to remedy deficient performance upon request.

7.2 The following remedies shall be available to Agency upon default.

7.2.1 Immediate cancellation of the Contract.

7.2.2 Immediate cancellation of one or more release orders issued under this Contract.

7.2.3 Any other remedies available in law or equity.

8 FACILITIES ACCESS: Performance of Services will require access to the facility.

8.1 Vendor must identify principal service personnel who will be asked for identification upon entrance to the facility.

8.2 Anyone performing under this Contract will be subject to Agency's security protocol and procedures.

8.3 Vendor shall inform all staff of Agency's security protocol and procedures.

ADDENDUM ACKNOWLEDGEMENT FORM
SOLICITATION NO.: AGR1700000005

Instructions: Please acknowledge receipt of all addenda issued with this solicitation by completing this addendum acknowledgment form. Check the box next to each addendum received and sign below. Failure to acknowledge addenda may result in bid disqualification.

Acknowledgment: I hereby acknowledge receipt of the following addenda and have made the necessary revisions to my proposal, plans and/or specification, etc.

Addendum Numbers Received:

(Check the box next to each addendum received)

☒ Addendum No. 1	☐ Addendum No. 6
☐ Addendum No. 2	☐ Addendum No. 7
☐ Addendum No. 3	☐ Addendum No. 8
☐ Addendum No. 4	☐ Addendum No. 9
☐ Addendum No. 5	☐ Addendum No. 10

I understand that failure to confirm the receipt of addenda may be cause for rejection of this bid. I further understand that any verbal representation made or assumed to be made during any oral discussion held between Vendor's representatives and any state personnel is not binding. Only the information issued in writing and added to the specifications by an official addendum is binding.

Shimadzu Scientific Instruments, Inc.

Company



Authorized Signature

11/15/16

Date

NOTE: This addendum acknowledgement should be submitted with the bid to expedite document processing.
Revised 6/8/2012

October 25, 2016

Ms. Linda Harper
State of West Virginia
PO Box 50130
Charleston, WV 25305
Re: **CRFQ 1400 AGR1700000005**

Dear Ms. Harper,

I am writing in regards to CRFQ 1400 AGR1700000005 for Triple Quad LC/MS/MS.

Shimadzu will be submitting two quotes. These quotes are for the first and second of our four LCMS instrument tiers, including our entry-level LCMS-8040, and our second-tier LCMS-8045.

As recently as 2011, Shimadzu sold the Ionics instrument that this bid is written upon. We called it the LCMS-8080, and Perkin Elmer now calls it the QSight. At the time, this instrument served as Shimadzu's high sensitivity instrument. Unfortunately, due to the system design, even our LCMS experts had a difficult time keeping the platform running, leaving new users little if any chance for successful use. Each time there was an issue, our customers had to send major part assemblies back to Canada, and lead times for the replacement parts were quite long. Shimadzu discontinued this instrument within 2 years because it was unreliable hardware and software, unproductive, difficult to use and repair, overly expensive to own, and ultimately a poor solution for our valued customers. Additionally, over that time Shimadzu released more capable and robust instruments, namely our LCMS-8040, LCMS-8045, LCMS-8050, and LCMS-8060 instruments.

Shimadzu LCMS platforms are uniquely designed for maximum uptime and performance. From its first LCMS forward to today, Shimadzu has set the standard for fastest scan speeds and polarity switching times, providing users the ability to analyze hundreds, even thousands of large analyte panels weekly. This should become apparent as you review our supporting documents, such as our newest pesticide method, showing quantitative measurement of 646 pesticides in 10.5 minutes.

As part of our commitment to you getting the most productivity from your instrument, included with your purchase, Shimadzu provides training from your regional Mass Spectrometry Technical Support Specialist to work onsite in front of *your* instrument with *your* users to familiarize them with using the instrument. Also included at no charge with your purchase are software and hardware training classes at our Customer Training and Education Center (CTEC) in Columbia, MD. Additionally, free technical support by phone is included for the life of the instrument, regardless of service coverage!

Shimadzu has a sizeable and growing installation base, and we take great pride in ensuring our customers' success. We have many happy references. Due to its historic issues with robustness, speed, and lack of service,



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Ionics/QSight has an extremely limited installation base. I encourage you to ask for names of happy customers; their installation base is extremely limited and possibly still zero in the US (other than placements).

Shimadzu also brings the advantage of a large, global LC/MS/MS footprint with many resources working on similar applications as the WVDA. This can be seen in the large application booklet included with this bid. This booklet is just a snapshot; it is by no means a comprehensive picture of all we have done on pesticides, herbicides, toxins and drugs in a variety of matrices. In committing to Shimadzu, you are committing to a market leader with vast experience so you can hit the ground running with your samples.



Shimadzu's instruments are proudly manufactured in the United States with locally manufactured parts. While all other HPLC and LC/MS/MS manufacturers have moved their manufacturing overseas to save money, Shimadzu has recently expanded the square footage and headcount of our Canby, Oregon manufacturing center and use locally sourced parts when possible.

Thank you for reviewing this proposal in its entirety. We are happy to answer any questions and look forward to the opportunity to help you enhance the quality of life for West Virginians by enforcing standards for wholesomeness and making quality inspections of agriculture.

Sincerely,

Jeremy Post
Life Science Account Manager

SPECIFIC BID RESPONSE

3.1.1 Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC/MS/MS)

3.1.1.1 Must be capable of detecting a variety of analytes including pesticides, herbicides, toxins, and drugs in matrices such as foods, soil, vegetation, animal feed, and water.

MEETS: Please note that Shimadzu has added a divert valve to its quote. Though this was not on the bid specifications, it is advisable to use because it keeps the MS clean. With a divert valve in place, you can divert unwanted flows from entering the MS and going into the waste. Shimadzu can provide application support specific to these analytes.

3.1.1.2 MSMS must have two ion sources that operate independently which can be set to electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The instrument must enable combinations such as ESI/APCI, ESI/ESI, APCI/APCI with the same or opposite polarities without having to remove the sources to switch modes.

EXCEEDS: This salient characteristic is essentially a sole source specification which could be construed as an attempt at a directive verdict towards a specific manufacture, Perkin Elmer. Other vendors utilize different instrument designs that serve the same purpose equally well, if not better. Our former LCMS-8080 and the Ionics MS (QSight), does have a dual-source mix and match ESI and APCI. The only realistic reason is for alternating and simultaneous ionization techniques of ESI and APCI, but Shimadzu offers a dual ionization source, the DUIS (DUal Ionization Source). Our DUIS is our standard ESI source with the APCI corona needle in place. There is absolutely no ESI-APCI switching time on our DUIS source; the voltages are applied simultaneously. That means ionization from both sources is continuous and simultaneous. Furthermore, we can also use the DUIS source with the fastest polarity switching on the market (5 ms).

Furthermore, in order to utilize the ESI-ESI and APCI-APCI modes on the QSight, one must employ a multiplexing HPLC to attached to the parallel streams. Perkin Elmer can do the sources, but they do not offer the front end hardware necessary to do multiplexing.

3.1.1.3 Minimum sensitivity requirement for positive ion mode: signal/noise (S/N) 2000:1 25 femtograms (fg) of reserpine on column. Minimum sensitivity requirement for negative ion mode: signal/noise (S/N)>2000:1, 25 femtograms (fg) of chloramphenicol on column.

EXCEEDS: Like most LCMS vendors in this industry, Shimadzu's LC/MS/MS specifications are based on 1 fg of reserpine (positive ESI) and 1 fg of chloramphenicol (negative ESI). The bid specification asks for 25 fg, so our platforms start out at 25 times better sensitivity. That said, the S/N values for each of our instruments at 1 fg (both positive and negative) are:

MODEL	SPECIFICATION
LCMS-8040	>10,000:1 (or 125 times better than the specification)
LCMS-8045	>100,000:1 (or 1,250 times better than the specification)

A benefit of additional sensitivity is that less material must be injected for the same detection levels, namely the reduced use of costly stable labeled internal standards. Injection of smaller sample amounts also leads to less buildup of matrix residue in the source and front end ion optics meaning more instrument up time and less instrument maintenance.

3.1.1.4 The source probes must be easy to remove without the use of tools.

MEETS: If the DUIS source is used, you have both ESI and APCI capability without having to make any hardware changes. Exchanging sources between ESI, APCI, or DUIS can all be easily accomplished in 30 seconds or less by a novice user without use of any tools.

3.1.1.5 Ion source must have flat response across flow rate up to 3 milliliters per minute without loss of sensitivity.

EXCEPTON: Shimadzu LCMS instruments can accommodate 2 mL/min. For many reasons, while sometimes flowing at rates as low as 0.0002 mL/min, essentially no LCMS method utilizes flow rates above 1.2 mL/min. To name a few these reasons include the excessive amount of solvent required for high flow rates which causes a dilution of the sample which reduces sensitivity; the size of the vacuum system and heaters needed to remove excessive solvent, the excessive amount of gas required to dry that amount of solvent meaning more dewars or larger gas generators. All of these factors indicate higher cost and diminished returns. One would be hard-pressed to find a method running 3 mL/min into the mass spectrometer. However, the Shimadzu LC front end is capable of

10 mL/min flow rates which can be beneficial for on-line SPE procedures or other processes where for instance the flow could be split between the LCMS and a fraction collector for simultaneous quantitation with semi-preparative separations.



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3.1.1.6 Capable of switching between rapidly between positive and negative ion detection without high voltage switching.

EXCEEDS: Since the release of our first LC/MS/MS in 2010, Shimadzu has set the benchmark for speed in polarity switching capability. 'Rapidly' is not really a specification; it is just marketing. Shimadzu engineered its own high voltage power supplies to make possible the fastest polarity switching of *any* LC/MS/MS commercially available at 5 m/s with both polarities being fully functional. Our polarity switching actually takes place in 1 millisecond. We allow the additional 4 milliseconds for the signal to fully stabilize. Most of our competitors do not allow for signal stabilization, resulting in poor quantitation data when performing polarity switching. Shimadzu does not have this issue as can be seen in our various application notes.

3.1.1.7 Acquisition modes: Q1 scan, Q2 scan, multiple reactions monitoring (MRM), selected ion monitoring (SIM), Neutral Loss scans, Product Ion, Precursor Ion, Time managed MRM

EXCEEDS: All of these acquisition modes are easily acquired on any Shimadzu LCMS. Since the release of our first LC/MS/MS in 2010, Shimadzu has set the benchmark for scan speeds initially at 15,000 amu/second with the LCMS-8040 and 30,000 amu/second with the LCMS-8045. Having a fast MS enables the user to do all these scan types simultaneously. One of the biggest weaknesses of competitors is that when attempting to scan at high speed, the instrument suffers from mass shifts which drive sensitivity down due to a speed induced mismatch of method specific versus actually detected mass values. This is a problem for competitors when attempting to measure too many MRMs simultaneously. The speed of the Shimadzu high voltage power supplies allows for these MRMs to be collected along with these additional scans, including data dependent product ion scans if desired to scan for unknowns *without* losing data points across the peak or sensitivity due to mass shifts.

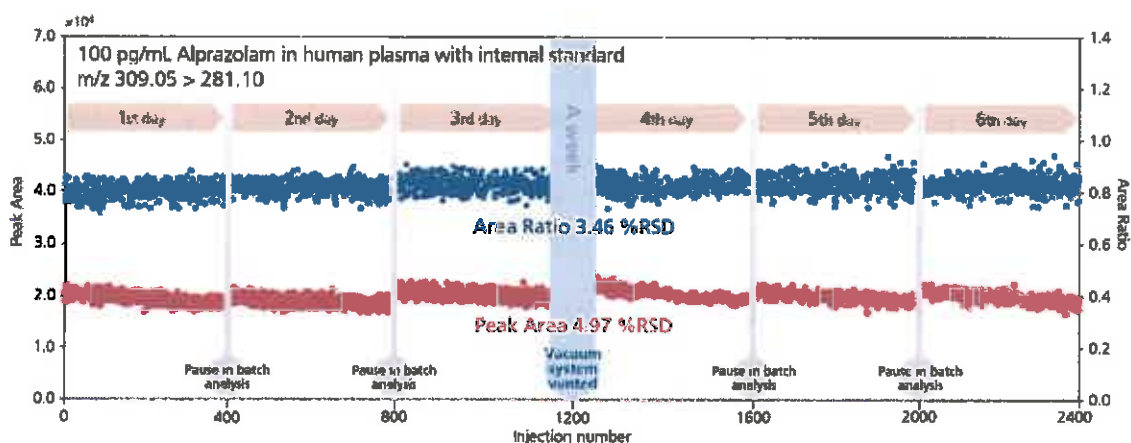
3.1.1.8 Minimum mass range requirement: 5-1500 mass to charge ratio (m/z)

EXCEEDS: Shimadzu's LC/MS/MS platforms have a mass range of 2-2000 m/z.

3.1.1.9 Mass stability required: 0.05 atomic mass unit (amu) in 24 hours

EXCEPTION: Shimadzu's LCMS platform has a mass stability of 0.05 amu in 12 hours. Please note

that Shimadzu's LC/MS/MS are built with unsurpassed robustness, and this is just a specification provided by the vendor. The graphic below shows a nasty plasma matrix over 2,400 injections in 6 days. The RSD's are very tight:



Intra-day and Inter-day Variations on LCMS-8060

Compound	%RSD							Inter-Day Variation		
	1st day	2nd day	3rd day	4th day	5th day	6th day	Average	Days 1-3	Days 4-6	6 Day Total
Alprazolam	5.04	4.94	5.06	5.38	4.55	4.83	4.97	3.19	1.63	2.74
Alprazolam-d5 (ISTD)	5.04	4.68	5.48	5.31	4.26	4.91	4.95	2.62	1.89	2.18
Area ratio	3.48	3.11	3.48	3.44	3.71	3.54	3.46	1.79	0.26	1.40

3.1.1.10 Mass accuracy needed: minimum 0.1 unit across mass range

MEETS: Shimadzu's LC/MS/MS records data in 0.05 unit intervals across the mass range.

3.1.1.11 Scan speed: $\leq 30,000$ daltons per second (da/s)

MEETS: As stated above, since the release of our first LC/MS/MS in 2010, Shimadzu has set the benchmark for speed. In fact, we are the only manufacturer whose MS can scan at maximum speed *without* losing sensitivity and *without* seeing any mass shifts. Competitors claiming similar scan speeds cannot **effectively** scan at this rate.

MODEL	SPECIFICATION
LCMS-8040	15,000 daltons/second
LCMS-8045	30,000 daltons/second

3.1.1.12 Quad resolution: unit, low and high, minimal sensitivity loss at 0.1 Daltons resolutions

EXCEPTION: We suggest that any quadrupole set to a resolution of 0.1 Da will lose sensitivity versus a more permissive setting of 0.4, 0.7 Da or more. We posit that no vendor could provide actual proof of superior performance on a real sample analyzed at 0.1 resolution. Shimadzu LCMS instruments are set by default at 0.7 Da resolution for Unit, 0.4 or 0.3 Da for High, and 1.5 Da for Low Resolution. All of our LC/MS/MS can be tuned to lower values if required. A provision is included in the tune process to select the resolution needed.

3.1.1.13 Polarity switching time: ≤ 15 milliseconds

EXCEEDS: As stated previously, since the release of our first LC/MS/MS in 2010, Shimadzu has set the benchmark for polarity switching capability. In fact, Shimadzu has the ability to collect a minimum of 30 data points across a UHPLC peak timeframe for co-eluting species switching between positive and negative modes. This is a powerful tool for method optimization and for quality quantitation from one injection. We are able to polarity switch at these speeds *without* losing sensitivity.

MODEL	SPECIFICATION
LCMS-8040	15 milliseconds
LCMS-8045	5 milliseconds

3.1.1.14 Dynamic range: 6 orders

EXCEEDS: Shimadzu's LC/MS/MS platforms utilize pulse counting detectors, which have dynamic range of 2×10^7 cps.

3.1.1.15 Dual source switching speed: < 20 milliseconds

EXCEEDS: As stated above, Shimadzu's DUIS source, there is absolutely no ESI-APCI switching time on our DUIS source; the voltages are applied simultaneously.

3.1.1.16 Minimum multiple reactions monitoring (MRM) Dwell Time: 1 millisecond

EXCEEDS: As repeatedly stated, Shimadzu's LC/MS/MS are the fastest on the market. And, even when we are going the fastest, we *do not* lose sensitivity or see a mass shift. Our MRM Dwell Time is 0.8 milliseconds.

3.1.1.17 MRM transitions: 450 per time segment > 40,000 ion transactions per method

EXCEEDS: Shimadzu's LC/MS/MS can do 555 MRM transitions/second. And, we can even do these using the DUIS (without having to worry about source switching) and whilst doing polarity switching! This capability for pesticides can be seen in one of our latest methods showing 646 pesticides in 10.5 minutes!

EXCEPTION: This salient characteristic is essentially a sole source specification which could be construed as an attempt at a directive verdict towards a specific manufacture, Perkin Elmer. Other

3.1.1.18 Must have high selectivity mass filter at 0.3 Daltons. Signal loss must not be more than 10%.

vendors utilize different instrument designs that serve the same purpose equally well, if not better. This specification is in no way indicative of how an instrument would be used in the real world. It is highly likely that no real data exists that shows a performance enhancement on a real sample analyzed at a 0.3 u resolution.

It is unclear in normal operation where a resolution of 0.3 u is important. If it is on Q1, then fewer ions would reach the collision cell, resulting in a large loss in sensitivity. On the other hand, if the 0.3 u resolution is on Q3, the MRM and voltage settings would have already eliminated nearly any possibility of a false identification of a fragment. Sacrificing sensitivity at Q3 is counterproductive. And, setting a 0.3 u resolution on *both* Q1 and Q3 simultaneously provides the worst of both worlds.

3.1.1.19 For minimal tuning during method development and minimal maintenance of the ion path, the LC/MS/MS utilizes Hot Source Induced Desolvation interface to the mass analyzer by patented Laminar Flow Ion Guide using gas flow

EXCEPTION: This salient characteristic is essentially a sole source specification which could be construed as an attempt at a directive verdict towards a specific manufacture, Perkin Elmer. Other vendors utilize different instrument designs that serve the same purpose equally well, if not better. In the LCMS-8045, the Shimadzu heated source is superior to other source designs in that it applies heat to the very outlet of the liquid stream, so there is no possibility of boiling or outgassing during the spray process. This insures better heat exchange, thus better quantitation than other approaches to heat the liquid prior to spraying. Also, Shimadzu uses a technique called flow injection analysis to do rapid method development. Your standard is put into the autosampler vial, and injections are made automatically without a column. Based on the resulting data, an MRM table and method is automatically built. This technology is one of the things that enables

Shimadzu to optimize these very large analyte panels in 1-2 days instead of months or never.

The LCMS-8040 does not have a heated source.

3.1.1.20 High performance liquid chromatograph capable of solvent and column switching without user intervention

MEETS: Shimadzu's HPLC front ends are modular, and we offer both column and solvent selection. Per Addendum 1 (Q1), Shimadzu offers LPGE components allowing for four solvents per pumps and additional valves in the column oven to accommodate column switching for two columns. Though not mentioned, Shimadzu has also included a vacuum degasser for all 8 requested lines and 2 for the autosampler. We have also included solvent bottles and a reservoir tray for the bottles.

3.1.1.21 High performance liquid chromatograph capable of regulating column temperature of at least 2 different columns

EXCEPTION: Shimadzu's CTO-20AC column oven can hold up to 12 columns inside, and is forced air instead of block. Forced air ovens offer the benefit of size as well as more uniform column temperature than block heaters. Temperatures span from 10 degrees below ambient to 85 degrees C (Addendum 1 Q2).

3.1.1.22 High performance liquid chromatograph with an autosampler

EXCEEDS: Shimadzu offers quaternary pumps, pumps with 19,000 psi capability, and cooled autosampler capable of utilizing vials or multiwell plates of many types among many additional options. Shimadzu's platforms also allow the user to add a traditional HPLC detector like a fluorescence or photodiode array in the future. And, as per Addendum 1 Q3, Shimadzu has included its new SPD-M30A Diode Array Detector. Other detectors can be added in the future, and control will be direct from within the same LCMS software package. Shimadzu's SIL-30AC can hold 105 2 mL vials and 2 microtiter plates.

3.1.1.23 The LC/MS/MS instrument must be fully automated for analysis with a system controller that is loaded with the necessary software.

MEETS: Shimadzu's LC/MS/MS come with high end workstations with software. The HPLC stack includes a system controller.

MEETS: Shimadzu's LC/MS/MS comes with all necessary tools for installation and future maintenance. In addition, it should be noted that Shimadzu's LC/MS/MS platforms were designed with uptime and ease of maintenance in mind. To that end, our platforms are designed with the ability to change the desolvation line without breaking vacuum! The desolvation line is the part of the MS that is meant to get dirty! This part is inexpensive (under \$500) and can be changed by the user in minutes and with no tools! This part is typically only changed during annual PM. Below are some pictures of this process:



MEETS: Shimadzu has provided site preparation guides for both the LCMS-8040 and the LCMS-8045.

3.1.2.1 Data station with windows based operating system capable of multitasking allowing data processing and data acquisition simultaneously.

3.1.2.2 Operating system must be fully integrated to control LS/MS/MS.

MEETS: Because Shimadzu released its first triple quadrupole just 6 years ago, all electronics and software were engineered with the user in mind and are the most modern on the market. The



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software is quite robust and based on our popular HPLC platform. There is no contract closure between the HPLC and the MS/MS; there is complete seamless control from a single vendor. In summary, Shimadzu's LC components and MS are *designed and manufactured* in the same factory and controlled with the same software package. Your lab will not have to run two software packages nor worry about contract closures, where the LC could potentially continually pump into a MS that is not ready, etc.

3.1.3 Shipping, Installation, Validation, Warranty, Training and Service

3.1.3.1 Vendor must be on-site for delivery and perform the installation (labor and supplies included) of the LCMSMS.

MEETS: Shimadzu has a team of direct employees that are factory-trained MS Specialists. The WVDA Engineer will come from VA and has many years of experience with MS and with Shimadzu.

3.1.3.2 The vendor must provide a written validation of the instrument's performance after installation.

MEETS: Shimadzu's factory-trained Service Engineers do a series of installation performance tests and can provide results to WVDA.

3.1.3.3 Vendor will provide a full one-year parts and labor warranty on all items, including 2 preventative maintenances.

MEETS: All Shimadzu instruments come with a full 1-year warranty with parts, labor, and travel. We have added two of our Premium Preventative Maintenance Visits as well. Please note that we adjusted our service agreement inclusions to more closely match our competitors. Our standard Preventative Maintenance Visit is more of a clean and inspect with a truncated list of replacements while our Premium Preventative Maintenance offers significantly more bang for your investment dollar. Please see below, from our included Service Agreement Programs Brochure:

AA/ICP • Balancing • Biotech/MALDI • EDX/XRF/XRD • Fluorescence • FTIR • GC • GC/MS
HPLC/UHPLC • LC/MS/MS • Software • Testing Machines • Thermal • TOC/TN/TP • UV-VIS-NIR
Boston • New Jersey • Washington, DC • Wilmington/Durham • Chicago • Houston • Kansas City • San Francisco • Carlsbad • Puerto Rico

	Premium Total Coverage	Extended Warranty Plus	Premium PM	PM	Extended Warranty
Service performed by certified Shimadzu Service Engineer	✓	✓	✓	✓	✓
Unlimited Hardware phone support	✓	✓	✓	✓	✓
Unlimited Software phone support	✓	✓	✓	✓	✓
Response time prioritized over billable call	✓	✓	✓	✓	✓
On-demand service visit	✓	✓	✓	✓	✓
On-site service includes labor, travel, parts	✓	✓	(for PM visit)	(for PM visit)	✓
Includes annual PM service visit	✓	✓	✓	✓	✓
Includes consumable parts needed during PM service visit	✓	✓	✓	✓	✓
Includes Turbo Pump coverage for MS	✓	✓	✓	✓	✓
Scheduled minor software & firmware upgrades	✓	✓	✓	✓	✓
Includes consumable parts used on service visit outside of PM	✓	✓	✓	✓	✓
Rebate provided	✓	✓	✓	✓	✓
Age premium is waived	✓	✓	✓	✓	✓

Service Agreement Option Descriptions

PM (Preventative Maintenance)

Preventative Maintenance is the most basic maintenance option using only the most integral consumable parts needed to bring the system back to a functioning unit. This may include necessary cleaning, adjustments, verification, lubrication, consumable parts replacement and testing to return the instrument to a functional unit.

Premium PM

Premium Preventative Maintenance is a higher-level PM than the previously listed. The Premium PM contains all the standard consumables found in the PM; however, it can contain additional consumable parts or testing deemed necessary to bring the system back to the manufacturer's operational specification.

3.1.3.4 Vendor must be able to perform resolutions to service requests within 72 hours which includes on-site resolutions.

MEETS: Shimadzu commits to resolving resolutions within 72 hours. Furthermore, we typically resolve issues within 48 hours. We can do this because we have a free telephone technical support line, regardless of warranty or service coverage, and this group is highly skilled at triaging the situation and understanding what parts need to be onsite for maximum efficiency.

3.1.3.5 Vendor will provide on-site training (labor and non-consumable supplies included) for all instruments and software.

EXCEEDS: Shimadzu's approach to training is unique in the industry. We provide free onsite training for as long as all users need to feel comfortable using the instrument. Customers can also request additional refresher or re-trainings at no charge if needs change, new projects arise, or new staff joins WVDA. In our commitment to WVDA, we will provide 6 training classes at our Customer Training and Education Center (CTEC) in Columbia, MD.



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3.1.3.6 Vendor will provide copies of all system manuals (operations, training, technical, service, maintenance).

MEETS: Shimadzu provides copies of all manuals with its instrumentation.

AA/ICP • Biobanchem • Biotech/MALDI • EDX/XRF/XRD • Fluorescence • FTIR • GC • GC/MS
HPLC • ICP/MS • LC/MS/MS • Software • Testing Machines • Thermal • TGC/TH/TP • UV-VIS-NIR

Boston • New Jersey • Washington, DC • Baltimore/Durham • Chicago • Houston • Kansas City • San Francisco • Cardiff • North Wales

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Sales Engineer:

Heather Juzwa
Shimadzu Scientific Instruments
7102 Riverwood Drive
Columbia, MD 21046
Regional Office: (410) 381-1227 Ext. 1673
Direct Dial: (800) 388-6996
E-mail: hljuzwa@SHIMADZU.com

Sales Proposal

Quotation: SSI-106429-W8P1

Expiration: 3/17/2017

Quote Description:

Proc Folder: 265333. CRFQ 1400 AGR1700000005 Triple Quad LC/MS/MS. OPTION 1: LCMS-8040 with Nexera X2 19,000 UHPLC System.

Proposed Ship Date: 30 Days/ARO

FOB: DESTINATION **Ship Method:** BEST WAY

Shipping Terms: PREPAID & ADD

Incoterms:

Additional Information:

For proposal questions or modifications, please contact your sales representative.

For Order Placement:

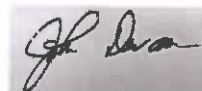
Reference Quotation Number on Purchase Order
If you are tax exempt, please send a copy of your exemption certificate with your purchase order.
Certificate may also be faxed to 410-381-1222.

Thank you for your interest in Shimadzu Scientific Instruments.

Shimadzu Scientific Instruments
7102 Riverwood Drive, Columbia, MD 21046
Toll Free: 800-477-1227
Local: 410-381-1227 Fax: 410-381-6781
E-mail: customer.service@shimadzu.com

International:
Fax: 410-309-6130
E-mail: icsc@shimadzu.com

Authorization Signature



John Dwan

Date: 11/15/2016

Sales Quotation - please reference the quotation when submitting purchase order.

LCMS-8040

Product #	Qty	Description	List Price	Ext'd Price
1 220-91588-01	1	LCMS-8040 Package	\$235,137.00	\$181,055.49
2 225-12229-58	1	LCMS-8030/8040 DUIS Kit without Socket Assembly	\$3,708.00	\$2,855.16
3 225-14232-41	1	LCMS-8030/8040 Socket Assembly, Common to APCI and DUIS	\$1,236.00	\$951.72
4 228-45015-42	1	FCV-20AH2	\$3,595.00	\$2,696.25
5 220-91600-32	1	LCMS-8030/8040 Installation Kit	\$1,625.00	\$1,251.25
6 I&F	1	INSTALLATION AND CUSTOMER FAMILIARIZATION	\$0.00	\$0.00
7 220-95367-01	1	Epson Workforce 30 Color Inkjet Printer (USE W/TOC-LCS)	\$134.00	\$113.90
8 220-97301-05	1	HIGH SPEED USB 2.0 PRINTER CABLE	\$29.00	\$24.65
9 228-16006-00	4	Plug, LC-6A/6AD	\$18.00	\$54.00
10 1YW	1	1 YEAR WARRANTY	\$0.00	\$0.00
11 220-99204-04	3	Introduction to the LCMS-8030/8040/8050/8060	\$1,700.00	\$0.00

HPLC

Product #	Qty	Description	List Price	Ext'd Price
12 220-91398-20	1	CBM-20A W/ Network Switch	\$3,245.00	\$2,433.75
13 228-45019-58	2	DGU-20A5R, ROHS-compliant	\$3,308.00	\$4,962.00
14 220-91413-02	1	1L Bottle/Cap/Res Tray PKG	\$628.00	\$471.00
15 228-45162-42	2	Nexera LC-30AD HPLC Pump	\$12,978.00	\$19,467.00
16 228-45180-58	2	Nexera X2 LPGE-30 No Mixer, ROHS Compliant	\$3,245.00	\$4,867.50
17 228-45168-41	1	Nexera LC-30AD Micro Mixer, 20 uL	\$1,195.00	\$896.25
18 220-91397-22	1	Nexera SIL-30AC Autosampler with Installation Kit	\$28,900.00	\$21,675.00
19 228-37546-92	1	Sample Rack for DWP, SIL-20A/AC, SIL-30AC	\$731.00	\$548.25
20 228-45010-42	1	CTO-20AC	\$4,550.00	\$3,412.50
21 228-45166-58	1	FCV-32AH 2-Position, 6-Port High Pressure Switching Valve, ROHS-Compliant	\$5,570.00	\$4,177.50
22 220-91522-02	1	Valve Mounting Kit for CTO-20/30A, 2-position	\$227.00	\$170.25
23 228-54450-91	1	Stainless Steel Manifold, Analytical	\$742.00	\$556.50
24 220-91403-02	1	SPD-M30A Photodiode Array Detector with Ethernet Switch	\$18,540.00	\$13,905.00
25 220-95315-05	1	HPLC Virtual Advisor USB for Prominence and Nexera Systems	\$1,250.00	\$0.00
26 I&F	1	INSTALLATION AND CUSTOMER FAMILIARIZATION	\$0.00	\$0.00
27 1YW	1	1 YEAR WARRANTY	\$0.00	\$0.00
28 220-99208-00	3	TRAINING COURSE, HPLC W/TROUBLE SHOOTING	\$1,170.00	\$0.00

Service



Company Name: WVDA
Quote Number: SSI-106429-W8P1
Activation Date: 10/25/2016
Expiration Date: 3/17/2017

	Product #	Yrs	Description	List Price	Ext'd Price
29	PREMIUM-PM-ONLY	2	Premium Preventive Maintenance Only Service Agreement	\$17,957.00	\$30,526.90

Total List Price:	\$394,700.00
Total Line Item Discount:	\$97,628.18
Quote Sub-Total:	\$297,071.82
Estimated Freight:	\$850.00
Total Amount:	\$297,921.82

Sales Quotation - please reference the quotation when submitting purchase order.
Optional Items

Optional Items:**LCMS-8040**

Product #		Qty	Description	List Price	Ext'd Price
1	225-14271-58	1	LCMS-8030/8040 APCI Kit without Socket Assembly, ROHS	\$8,240.00	\$8,240.00

Service Agreement Plans Available

As Quoted

Yrs of Coverage	Plan Name	Plan Cost	Products Covered

Descriptions of Service Coverage

Preventative Maintenance

Preventative Maintenance (PM) visits will be scheduled by SSI at the mutual convenience of the buyer and SSI, or performed during any other service visit. PM visits, when provided under this Agreement, may include necessary cleaning, adjustments, verification, lubrication and parts replacement according to the PM checklist. Labor, travel expenses, and selected consumable parts will be included during the PM visit at no additional cost to the buyer.

Premium Preventative Maintenance

Premium Preventative Maintenance visits will be scheduled by SSI at the mutual convenience of the customer and SSI, or performed during any other service visit. This Premium PM will be performed by SSI according to established SSI procedures. Premium PM visits, when performed under this Agreement, will include necessary cleaning, adjustments, verification, lubrication and parts replacement. Labor, travel expenses and selected consumables parts* (list of parts available upon customer request) will be included during the Premium PM visit at no additional cost to the customer. A travel zone fee is added to the agreement to cover travel costs. Purchase of the Premium PM option does not excuse Customer from performing normal daily, weekly or monthly maintenance that may be required.

Extended Warranty

Extended Warranty coverage includes all labor and parts (excluding consumables) necessary to restore the products to operating specifications.

This coverage level is not available for MS products and the associated turbo and roughing pumps. The MS products require a coverage level which includes a Premium PM.

Extended Warranty Plus

A combination of one scheduled Premium PM visit and Extended Warranty Coverage. Premium Preventative Maintenance visits will be scheduled by SSI at the mutual convenience of the customer and SSI, or performed during any other service visit. Premium PM visits, when performed under this Agreement, will include necessary cleaning, adjustments, verification, lubrication and parts replacement. Labor, travel expenses and selected consumables parts* (list of parts available upon customer request) will be included during the Premium PM visit at no additional cost to the customer. The extended Warranty Includes all labor and parts (excluding consumables outside of PM) necessary to restore the products to operating specifications. A travel zone charge is added to the contract to cover travel costs. In the event of a covered product failure, SSI will use its best effort to provide on-demand service.

Premium Total Coverage

This level of agreement provides the customer with a level of coverage includes all labor and non-consumable parts, a Premium PM visit, and in addition provides for the replacement of consumable parts outside of a PM visit at no additional cost to the buyer. Use of this level of Service will be monitored by SSI and in the event that the buyer's use does not exceed two visits during the effective dates of the agreement, the buyer will be entitled to an agreed upon rebate at the end of the agreement term. This rebate must be applied to the purchase of future service agreements or other services offered by SSI.

Sales Quotation - please reference the quotation when submitting purchase order.
Line Item Descriptions
Product # Description
PREMIUM-PM-ONLY

Premium Preventative Maintenance visits will be scheduled by SSI at the mutual convenience of the customer and SSI, or performed during any other service visit. This Premium PM will be performed by SSI according to established SSI procedures. Premium PM visits, when performed under this Agreement, will include necessary cleaning, adjustments, verification, lubrication and parts replacement. Labor, travel expenses and selected consumables parts* (list of parts available upon customer request) will be included during the Premium PM visit at no additional cost to the customer. A travel zone fee is added to the agreement to cover travel costs. Purchase of the Premium PM option does not excuse Customer from performing normal daily, weekly or monthly maintenance that may be required.

LCMS-8040
220-91588-01 LCMS-8040 Package

LCMS-8040 package includes LCMS-8040 Triple Quadrupole Detector, Advanced Data Station Dell PC, 24" LCD Monitor, LabSolutions Software and License, Startup Kit, Rotary Pump, Oil Return Kit, and accessories. The LCMS-8040 features ultra-fast multiple reaction monitoring (MRM) transitions, enabling data acquisition with up to 555 different channels per second. The electronics provide ultrafast mass spectrum measurement speeds of 15,000 u/sec without sacrificing sensitivity or resolution, and ultra-fast polarity switching (15msec) for the most information without signal deterioration. Patented UFSweeper II @ technology accelerates ions out of the collision cell by forming a pseudo-potential surface. The result is high-efficiency collision-induced dissociation (CID) and ultra-fast ion transport, maintaining high sensitivity and minimizing cross talk. In addition, higher radio frequency (RF) power capability minimizes pauses between each transition. The LCMS-8040 Software includes unified control of LC and MS components providing seamless operation of the entire LCMS system, unmatched qualitative and quantitative analysis, increased productivity, and accelerated workflows for high throughput data analysis. MRM Synchronization optimizes MRM cycle times for overlapping retention time windows and helps to simplify method development and routine analysis with multiple MRM experiments. In the Quantitation Browser, peak information, quantitative results, and statistical calculations of a series of data can be rapidly viewed in a single window. For Research Use Only. Not for use in diagnostic procedures.

Includes Dell Workstation PC with Windows 7 Pro 64bit, Quad Core Intel Xeon Processor, 8GB RAM, and 1 TB hard drive. LabSolutions software license controls one LCMS system and includes post-analysis processing capabilities. Single phase 230V +/-5% AC, 20 A (50/60 Hz) or Single phase 200V +/-5% AC, 20 A (50/60 Hz) power required for MS operation.

225-12229-58 LCMS-8030/8040 DUIS Kit without Socket Assembly

Shimadzu's DUIS Dual Ion Source adds APCI capability to the standard ESI configuration. By adding APCI capability for non-polar molecules, the user benefits from faster method development and the ability to analyze more compounds in a single method. The LCMS-8030/8040 DUIS Kit without socket assembly includes the DUIS needle assembly and the high voltage power supply for the DUIS system.

225-14232-41 LCMS-8030/8040 Socket Assembly, Common to APCI and DUIS

LCMS-8030/8040 Socket Assembly, for use with APCI and DUIS Options. Required for use with 225-12229-41 or 225-14271-91.

228-45015-42 FCV-20AH2

High pressure, 2-position, 6-port valve for column switching, online sample pretreatment, or flow line switching. Can be controlled by contact closure, local front panel control, or through the CTO-20A/AC. For contact closure control, requires one Event output from CBM, CBM-lite, or SCL-10Avp

220-91600-32 LCMS-8030/8040 Installation Kit

LCMS-8030/8040 Installation Kit. This kit contains 50' of 6mm OD PTFE nitrogen gas tubing, 50' of 1/8" cleaned copper argon gas tubing, 1 Nitrogen regulator, 1 Argon regulator, 1 1/8" Swagelok fitting, 1 6mm Swagelok union (6mm to 6mm), and 1 6mm Swagelok Reducer (6mm to 1/4" FNPT). This kit provides all of the necessary items to install the instrument to the facility's electrical supply provided they have installed either an L6-20R or L6-30R and to the facility's nitrogen system utilizing either a Dewar, nitrogen generator, or house gas system. Also included in this kit is the necessary items to connect the instrument to the Argon supply cylinder.

I&F

I&F Installation (setup/checkout) and operational familiarization of hardware and software.

220-95367-01 Epson Workforce 30 Color Inkjet Printer (USE W/TOC-LCS)

Requires USB Printer cable pn 220-97301-05

220-97301-05 HIGH SPEED USB 2.0 PRINTER CABLE
228-16006-00 Plug, LC-6A/6AD

Stainless steel plug for unused mixer or Valco manifold ports. Can also be used as a column plug.

1YW 1 YEAR WARRANTY

1 YEAR WARRANTY

220-99204-04 Introduction to the LCMS-8030/8040/8050/8060

Introduction to the LCMS Triple Quadrupole. A 3-Day course taught at Shimadzu Customer Training and Education Center. Suggested for those new to LCMS or those new to the LCMS-8030/8040/8050 hardware and software. The greatest successes are achieved by those who have used the instrument enough to bring their questions to class.

HPLC
220-91398-20 CBM-20A W/ Network Switch

Central communication and control module for the Prominence and Nexera Series. System components connect via fiber optic cables for easy "plug and play" configuration. Internal web server offers convenient control and monitoring of the HPLC system through a web browser.

Connections via Ethernet and RS-232C possible; Offers 8 Fiber Optic ports; 4 Events; 2 PC-55N A/D boards can be installed. Includes 4 port 10/100 Ethernet switch. VP and 10A Series modules with a fiber optic port can be controlled by the CBM-20A.

228-45019-58 DGU-20A5R, ROHS-compliant

Inline membrane degasser utilizing Teflon AF® for maintaining the degassed state of HPLC mobile phases. Use in conjunction with preliminary degassing by vacuum aspiration/sonication. A convenient 5th channel is included for degassing the autosampler rinse phase for optimum injection reproducibility performance.

Recommended for optimum gradient performance; connects to VP; Prominence (LC-20) and Nexera (LC-30) series pumps for power. 400 µL internal volume. REQUIRES power supply (228-45110-92) for use with 3rd party, non-VP, non-Prominence, or non-Nexera pumps. Many fluorinated solvents, strong acids (30+%) and peroxides will damage the membrane; check manual for listing. Maximum flow rate is 10mL/min per channel.

220-91413-02 1L Bottle/Cap/Res Tray PKG

Prominence LC-20A reservoir tray with a set of (5) 1-L solvent bottles and (5) 3-hole caps. Caps include a solid plug and a filter plug with a stainless steel frit.

Filter plug included with caps for use with He sparge.

228-45162-42 Nexera LC-30AD HPLC Pump

The LC-30AD utilizes micro-volume plungers (10 µL each) for pulse-free HPLC solvent delivery. The sapphire plunger has a specially engineered ultra-smooth surface which minimizes frictional heat for improved seal lifetime. An automatic rinse kit is included as a standard accessory. Flow rate range is 0.0001 to 5.000 mL, maximum pressure is 130 MPa (19,000 psi). The Nexera X2 LC-30AD is ideal for low flow rate (< 1 mL/min) and LCMS applications, and for analyses using small particle size columns (3 µm or less) that generate high back pressure.

Newly designed plunger holder ensures proper alignment with the seal; 3 nL/step motor control ensures excellent retention time reproducibility with short runs and steep gradients. Requires CBM/CBM-lite ROM version 1.30 for full control in LC-30 mode. 20A compatibility mode is available for 3rd party software control. Not compatible with the SCL-10Avp.

228-45180-58 Nexera X2 LPGE-30 No Mixer, ROHS Compliant

Four-position valve for the Nexera X2-LC-30AD pump that can be used as a solvent selector for gradient former. Requires an additional mixer; the 180 µL MIRC mixer 228-45168-46 is recommended.

Requires 4 available DGU channels. If a SIL-30AC is in the configuration, 7 total DGU channels are required. A DGU-20A3 and DGU-20A5 are recommended to accommodate both the LPGE and SIL-30AC.

228-45168-41 Nexera LC-30AD Micro Mixer, 20 µL

High efficiency mixer using micro reactor technology to thoroughly blend solvents in a small volume. Use for standard analytical (1-2 mL/min) and LCMS flow rates. For applications using TFA in the mobile phase, the 180 µL mixer 228-45168-42 is recommended.

Includes 2 mounting brackets for installation in either the CTO-30A or in the CTO-20A/AC or on the side of a pump.

220-91397-22 Nexera SIL-30AC Autosampler with Installation Kit

The SIL-30AC provides high speed injections (10 sec for 10 μ L) with ultra-low carryover achieved through advanced material technology and rinse options (if necessary) with up to 4 solvents. The standard configuration uses the needle in the flow path design for 0.1 to 50 μ L injection volumes; an optional fixed-loop configuration (5 or 20 μ L) is user-changeable to reduce delay volume and band broadening in isocratic separations. The sample compartment uses a Peltier cooler for temperature control from 4-40C. An external rinse pump is standard, and provides additional rinsing to the outer surface of the needle. Includes 105-position rack for 1.5 (2mL) vials and a control rack for 10 1.5 (2 mL) vials, 1.5 mL vial set, vial sample pack, and 2.1 x 50mm, 1.9 μ m C18 column and 4-component test mix. Sample capacity is 175 1 mL vials, 70 4 mL vials, 2 MTP (STD 96, DW 96, or STD 384). Maximum pressure is 130 MPa (19,000 psi).

Pretreatment functions include dilution and reagent addition as well as user-programmable sample prep routines. Three rinse solvents are available to clean the inside of the loop and needle, as well as for injection port rinsing. Requires CBM/CBM-lite ROM version 1.30 or higher for full control in LC-30 mode. 20A compatibility mode is available for 3rd party software control. Pretreatment is not available in compatibility mode, but all rinse functions can be programmed from the front panel.

228-37546-92 Sample Rack for DWP, SIL-20A/AC, SIL-30AC

Capacity of 2 Deep Well MTP plates, 96 well format.

Can be used in SIL-20A, SIL-20AC, and SIL-30AC

228-45010-42 CTO-20AC

Full-sized oven with forced air temperature control from 10C below ambient temperature to 85C. Can accommodate a manual injector, mixer, and 2 switching valves.

Linear temperature programming is possible; optional Column Management Device (228-37281-91) with CMD Cable (228-39991-01) can be used. FCV-12/14 Control Board (228-44291-91) required to install non-Prominence color FCV-12/14 in CTO-20A. Subcontroller is no longer needed unless the CTO-20 is used in VP mode.

228-45166-58 FCV-32AH 2-Position, 6-Port High Pressure Switching Valve, ROHS-Compliant

High pressure 6-port, 2-position valve for automatic 2-column switching, online sample pretreatment, or flow line switching. When installed in the CTO-30A column oven, dual preheater 228-52597-42 is recommended. Maximum pressure is 130 MPa (19,000 psi).

220-91522-02 Valve Mounting Kit for CTO-20/30A, 2-position

Mounting kit for installing the 10,18, or 25K psi 2-position valves in the CTO-20A/AC or CTO-30A. Includes bracket, wiring harness, and screws to secure the assembly to the CTO-20/30. One kit has enough parts for 2 valves.

228-54450-91 Stainless Steel Manifold, Analytical

Stainless steel manifold for connecting up to 6 column outlets in a method development or method scouting system. Mounting bracket included for installation inside the CTO-20A/AC column oven. Requires stainless steel plug 228-16006-00 or PEEK plug 228-28090-00 for unused ports. Internal volume is 0.44 μ L.

220-91403-02 SPD-M30A Photodiode Array Detector with Ethernet Switch

The SPD-M30A photodiode array detector utilizes a capillary flow cell for ultra-high sensitivity and minimal sample dispersion. Temperature controlled optics ensure a stable baseline in all laboratory environments with reduced startup time.

Includes Ethernet switch and crossover cable for PC connection.

220-95315-05 HPLC Virtual Advisor USB for Prominence and Nexera Systems

HPLC Virtual Advisor for Prominence and Nexera Systems. Includes two separate modules on a 8 GB USB drive. These modules cannot be run simultaneously. Installation Instructions are included on .zip files.

I&F

I&F Installation (setup/checkout) and operational familiarization of hardware and software.

1YW 1 YEAR WARRANTY

1 YEAR WARRANTY

220-99208-00 TRAINING COURSE,HPLC W/TROUBLE SHOOTING

HPLC maintenance class with emphasis on evaluating performance and preventing/solving problems with the LC-2010, the LC-10A / LC-10Avp Series and/or the LC-20 Series injectors, autosamplers, pumps, and detectors. Students should indicate at REGISTRATION what instrument series and modules are of interest.

Optional Item Descriptions

Product # Description

LCMS-8040

225-14271-58 LCMS-8030/8040 APCI Kit without Socket Assembly, ROHS

APCI Probe and Accessories for LCMS-8030/8040 provides Atmospheric Pressure Chemical Ionization for the analysis of non-polar molecules.

Requires 225-14232-41 LCMS-8030 Socket Assembly, Common to APCI and DUIS Options

Company Name: WVDA
Quote Number: SSI-106429-W8P1
Activation Date: 10/25/2016
Expiration Date: 3/17/2017

Terms and Conditions

SALES AGREEMENT

The sales agreement ("Sales Agreement"), as referenced herein below, shall mean and refer to these General Terms and Conditions of Sale ("General Terms and Conditions of Sale"), together with such other documents concerning the purchase of equipment and/or Products designed and/or manufactured by Shimadzu ("Products"), which documents have been or will be executed by and between Shimadzu Scientific Instruments, Inc. ("SSI") and the individual or entity identified therein as the purchaser of said Products ("Buyer"). The documents that comprise the Sales Agreement may include, but are not limited to: these General Terms and Conditions of Sale; price quote provided by SSI; purchase order issued by Buyer as modified and accepted by SSI; bill of sale for Products; bill of lading issued for the shipment of Products; and product invoice. **All Products except designated CL versions of HPLC and LCMS, shall be for research use only and not for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat or prevent disease or its sequelae ("Diagnostic Purposes").**

PRICES

The prices set forth in the Sales Agreement:

- (i) are SSI's domestic prices based upon manufacture of the quality and type of Product(s) ordered for shipment to and end use within the United States - all Products shipped for end use outside the United States shall be subject to SSI's international pricing;
- (ii) are subject to revision when interruption, engineering changes or changes in quantity or quality are caused or requested by Buyer; and
- (iii) unless otherwise specified, do not include warranty service or installation outside the United States. Errors in Product pricing or related terms by SSI which may, in SSI's sole discretion, be deemed clerical errors are subject to correction by SSI at any time.

SPECIFICATIONS

Weights and dimensions set forth in Product related sales literature ("Sales Literature") are not guaranteed unless previously certified in writing. SSI may, without affecting the obligations under the Sales Agreement, make what SSI regards in its sole discretion as minor changes to the specifications of the Product or Products delivered under the Sales Agreement from those contained in Sales Literature.

TERMS OF PRODUCT USE

Buyer represents and warrants that the Products have not been purchased for Diagnostic Purposes and that the Buyer will not at any time use the Products for Diagnostic Purposes, except designated CL versions of HPLC and LCMS Products. The Buyer acknowledges that use of the Product for Diagnostic Purposes is cause for Termination of this Agreement, as set forth in the "Contingencies; Force Majeure" section below.

TERMS OF PAYMENT

Subject to credit approval by SSI, terms of payment on the Sales Agreement are net thirty (30) days from date of the invoice unless otherwise specifically stated on the invoice, by check, wire. Payment by credit card is subject to 3% charge back at time of payment. Invoices are payable at par on date due at any place of collection designated by SSI in funds bankable at par. Payment made beyond terms will be subject to simple interest of 1 - 1/2% per month on the outstanding balance. All orders are accepted and SSI's obligation to make deliveries, are subject to SSI's right to either:

- (i) require Buyer to make payment of all or any part of the purchase price in advance of delivery of Product(s), or
 - (ii) make shipment of Product(s) to Buyer payable C.O.D.
- If Buyer fails to make advance payment when requested to do so by SSI or if Buyer is or becomes delinquent in the payment of any sum of any kind due SSI or refuses to accept C.O.D. shipments (whether or not arising out of the Sales Agreement), then SSI shall have the right, in addition to any other remedy to which it may be entitled in law or in equity, to cancel the Sales Agreement, refuse to make further deliveries, and declare immediately due and payable all unpaid amounts for Products and services previously delivered to or performed for Buyer. Each shipment shall be considered a separate and independent transaction and payment therefor shall be made accordingly. Buyer must notify SSI within fifteen (15) days from the invoice date if Buyer has not received the Product(s).

REPOSSESSION

In the event of nonpayment, either of the entire purchase price or a portion thereof, Buyer expressly consents to the right of SSI to reclaim by repossession of any and all Products delivered to Buyer pursuant to the Sales Agreement for which payment has not been made within (90) days of invoice. In the event that SSI elects to exercise its right to reclaim Products by repossessing the same, said election shall be deemed to be in the alternative and not to the exclusion of any other remedy to which SSI may be entitled, at law or in equity. To the extent that SSI successfully reclaims Products, it shall credit Buyer's account for the value of the same, as determined by SSI in its sole discretion. No Products may be reclaimed by repossession unless and until Buyer is provided with prior notice of SSI's intention to elect such remedy. Such notice shall state the date, time and location that Products shall be available for repossession. Such notice shall also provide Buyer with a final opportunity to cure the default of nonpayment within five (5) days of notice to avoid the execution of the remedy of repossession. The costs of repossession, including legal fees and costs of collection, shall be added to Buyer's account.

SECURITY INTEREST

In addition to any other remedies hereunder or otherwise provided at law or in equity, SSI shall have the right, and Buyer hereby grants SSI the right, without further notice to or consent from Buyer, to secure any and all payment obligations of Buyer by reclamation of a security interest in the Products that are the subject of the Sales Agreement. Said security interest shall be in the form of a UCC Financing Statement (UCC Form 1) or other form prescribed by contract or applicable law. No UCC Financing Statement recorded for the benefit of SSI will be released unless and until all financial obligations to SSI incurred by Buyer with respect to the Products have been fully satisfied.

SHIPMENTS

(i) The cost of packaging for domestic shipments is included in the quoted price unless otherwise provided. For international shipments or where special packaging is specified or necessary, a charge will be made to cover such expense.

(ii) For shipments to and from places within the United States, all shipments, unless otherwise agreed in writing, shall be FOB point of shipment and title and risk of loss or damage shall pass to Buyer at the point of shipment. The cost of transportation and insurance (if requested by Buyer) shall be borne by Buyer.

(iii) For shipments from the United States to ports and/or places outside the United States all shipments are, unless otherwise agreed in writing, FOB Columbia, MD. SSI's obligation to affect shipment of the Products purchased by Buyer shall be fully discharged, and beneficial ownership, legal title and all risk of loss or damage shall pass to Buyer when the Products are made available for shipment to a carrier at the designated FOB location. If shipped FOB Destination, upon arrival Buyer shall be entitled to conduct a reasonable investigation of the Products purchased by it, but all claims for losses due to loss or damage to Products while in transit shall be waived unless made immediately in writing by Buyer to SSI, but not more than (30) thirty days after arrival. If Buyer shall fail or refuse to accept delivery of any of the Products for unverifiable claims for loss or damage to Products occurring while in transit, all sums paid on deposit shall be retained by SSI as liquidated damages, provided, however, that SSI may recover in full its actual damage from Buyer in the event that actual damages exceed the amount retained as liquidated damages.

(iv) All claims for damage or loss of insured shipments shall be immediately communicated, when possible, to SSI at Shimadzu Scientific Instruments, Inc. 7102 Riverwood Drive, Columbia, Maryland 21046-2502 Attn: Customer Service, Phone: (410) 381-1227.

Buyer shall immediately notify delivering carrier of loss or damage to the shipment and SSI will cooperate with Buyer in the adjustment of all claims. Buyer agrees to permit SSI or SSI's representative to inspect damaged Products.

TERMINATION

Upon any termination or cancellation of the Sales Agreement by Buyer (if otherwise specifically permitted by the terms of the Sales Agreement), either in whole or in part, Buyer agrees to promptly pay appropriate termination or cancellation charges invoiced by SSI. At SSI's sole discretion, the termination charge shall be not less than twenty percent (20%) of the total amount of the Sales Agreement.

RETURNED PRODUCTS

All returns must be pre-authorized by SSI and a Return Goods Authorization ("RGA") number must appear on the face of the package. Returned Products will be subject to a restocking charge. If deemed appropriate at SSI's sole discretion, the restocking charge shall be not less than twenty percent (20%) of the total amount of the Sales Agreement.

ALLOCATION OF PRODUCTS

If SSI is unable for any reason to supply the total demands for Products specified in Buyer's order, SSI may allocate its viable supply among any or all buyers, including Buyer, on such basis as SSI may deem fair and practical, without liability for any failure of performance which may result therefrom.

DELIVERY

The scheduled shipping or delivery date shown on the face hereof is SSI's best estimate of the time the Product order will be shipped and SSI assumes no liability for loss, general damages, or special or consequential damages due to delays.

TAXES AND OTHER CHARGES

All taxes on Products, goods and/or services sold under the Sales Agreement, including but not limited to federal, state and local excise, sales or use taxes, shall be borne solely by Buyer. Buyer shall be obligated to pay all taxes set forth on invoices, in accordance with the terms of payment, provided that SSI shall not invoice taxes when within 30 days of placing the order, Buyer has furnished SSI with written proof of exemption from tax in the form of a certificate of exemption or an equivalent document which Buyer represents and warrants is properly completed and validly executed. If, notwithstanding the foregoing, any taxing authority attempts to assess taxes, Buyer shall indemnify and hold harmless SSI from any loss, damage, claim or cause of action, including, but not limited to, tax, interest, penalties and professional fees, related to such attempted assessment and shall make payment to SSI for any such costs paid by SSI and invoiced to Buyer in accordance with the terms of payment.

PATENTS

SSI shall defend any suit or proceeding brought against Buyer so far as based upon an assertion that any Product furnished under the Sales Agreement constitutes a direct infringement of any United States patent having a claim of claims covering solely the Product itself, if notified promptly in writing and given authority, information and assistance (at SSI's expense) for the defense of same, and SSI shall pay all damages and costs awarded therein against Buyer. In the event said Product in such suit is held to constitute infringement and the use of said Product is enjoined, SSI shall, at its own option and at its own expense, either:

- (i) procure for Buyer the right to continue using said Product, (ii) replace the same with a non-infringing Product, (iii) modify it so it becomes non-infringing, or (iv) remove said Product and refund the purchase price and transportation costs thereof.

The foregoing obligations of SSI shall not apply to any infringement claim based upon:

- (i) any use of any Product sold hereunder in any process or in conjunction with any other product, (ii) any Product manufactured to Buyer's design or any Product having a design arising from SSI's compliance with

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Buyer's specifications; or (iii) use of any Product sold hereunder, if the Product has been modified or customized by Buyer.

The foregoing sets forth the entire liability of SSI for patent infringement by said Product. If any suit or proceeding is brought against SSI based on claims that the goods manufactured by SSI in compliance with Buyer's specifications and supplied to Buyer directly infringe any fully issued United States patent, then the patent indemnity obligations herein stated with respect to SSI shall reciprocally apply with respect to Buyer.

WARRANTY

Subject to the exceptions and upon the conditions stated below, SSI warrants that the Products sold under the Sales Agreement shall be free from defects in workmanship and materials for one year after shipment of the Products to the original Buyer by SSI (the "Warranty"), and if any such Products should prove to be defective within such one year period, SSI's sole liability (and Buyer's sole and exclusive remedy) shall be, at its option, either (i) to correct by repair or, at SSI election, by replacement with equivalent product any such defective Product, provided that investigation and factory inspection discloses that such defect developed under normal and proper use, or (ii) to refund the purchase price. The exceptions and conditions mentioned above are as follows:

(i) Components or accessories manufactured by SSI which by their nature are not intended to and will not function for one year are warranted only to give reasonable service for a reasonable time; what constitutes reasonable time and reasonable service shall be determined solely by SSI. A complete list of such components and accessories is maintained at the factory;
(ii) SSI makes no warranty with respect to components or accessories not manufactured by it; in the event of defect in any such component or accessory SSI will give reasonable assistance to Buyer in obtaining from the respective manufacturer whatever adjustment is authorized by the manufacturer's own warranty;

(iii) any Product claimed to be defective must, if required by SSI, be returned to the factory, transportation charges prepaid, and will be returned to Buyer with transportation charges collect unless the Product is found to be defective, in which case SSI will pay all transportation charges;

(iv) if the Product is a reagent or the like, it is warranted only to conform to the quantity and content and for the period (but not in excess of one year) stated on the label at the time of delivery;

(v) SSI may from time to time produce a special printed warranty with respect to a certain Product, and where applicable, such warranty shall be deemed incorporated herein by reference;

(vi) SSI shall be released from all obligations under all warranties, whether expressed or implied, if any Product covered hereby is repaired or modified by persons other than its own authorized service personnel unless such repair by others is made with the written consent of SSI; and

(vii) SSI's obligations pursuant to all warranties, either express or implied, shall be expressly conditioned upon payment in full by Buyer for the Products covered by the warranties. In the event of non-payment, whether for the entire purchase price or a part thereof, SSI shall be released from all obligations under all warranties, either express or implied.

IT IS EXPRESSLY AGREED THAT THIS WARRANTY SHALL BE IN LIEU OF ALL WARRANTIES OR WARRANTY OF ANY NATURE WHATSOEVER, WHETHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION WARRANTIES OF FITNESS AND MERCHANTABILITY, AND THAT NEITHER BUYER NOR SSI SHALL HAVE ANY LIABILITY FOR SPECIAL OR CONSEQUENTIAL DAMAGES OF ANY KIND OR CAUSE WHATSOEVER, INCLUDING, BUT NOT LIMITED TO LOSS OF USE, LOSS OF DATA, LOSS OF PRODUCTIVITY, LOSS OF BUSINESS, LOSS OF PROFIT, LOSS OF PLANT, EQUIPMENT OR PRODUCTION. THE LIMITATION OF LIABILITY FOR SUCH DAMAGES SHALL BE APPLICABLE EVEN IF SSI HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, ARISING OUT OF THE MANUFACTURE, USE, SALE, HANDLING, REPAIR, MAINTENANCE OR REPLACEMENT OF ANY OF THE PRODUCTS SOLD UNDER THE SALES AGREEMENT.

If an SSI Special Warranty (covering a designated item or items) is contained in the manual or is otherwise shipped with such designated item or items, the terms and conditions specified therein are incorporated herein by reference and shall supplement the foregoing warranty. In the event of a conflict between the terms and conditions specified herein and those specified in such Special Warranty, the terms and conditions specified herein shall control. SSI's warranties made in connection with this sale shall not be effective if SSI has determined, in its sole discretion, that Buyer has misused the Products in any manner, has failed to use the Products in accordance with industry standards and practices, or has failed to use the Products in accordance with instructions, if any, furnished by SSI. Representations and warranties made by any person, including dealers and representatives of SSI, which are inconsistent or in conflict with the terms of this warranty, shall not be binding upon SSI unless expressly assumed in a writing that is approved and signed by an authorized officer of SSI. By signing the Sales Agreement, the Buyer specifically acknowledges that no representations or warranties were made by any person, including dealers and representatives of SSI, that the Product may be used for Diagnostic Purposes.

CONTINGENCIES; FORCE MAJEURE

SSI shall be entitled to cancel or rescind the Sales Agreement, without liability for loss or damage resulting therefrom, if:

(i) any Product covered by the Sales Agreement is purchased for end use outside the United States; (ii) the Buyer breaches any of its representations or warranties found in this Agreement; or (iii) the performance of SSI's obligations under the Sales Agreement is in any way adversely affected by the occurrence of any contingency beyond the control of either SSI or SSI's suppliers, including but not limited to:

- (a) war (whether an actual declaration thereof is made or not), sabotage, insurrection, riot, act of a public enemy, or other act of civil disobedience;
- (b) failure or delay in transportation;
- (c) act of any government, government agency or subdivision of any government, or any judicial action affecting the terms of the Sales Agreement;
- (d) labor dispute, shortage of labor, fuel, raw material or technical or yield failure where SSI has exercised ordinary care in the prevention thereof; and
- (e) accident, fire, explosion, flood, storm or other act of God.

U.S. GOVERNMENT CONTRACTS

If the Products to be furnished under the Sales Agreement are to be used in the performance of a U.S. Government contract or subcontract, and a U.S. Government contract number shall appear on Buyer's order, those clauses of the applicable U.S. Government procurement regulations that are required to be included in U.S. Government contracts and subcontracts shall be incorporated herein by reference, including, without limitation, the Federal Acquisition Regulations and the Fair Labor Standards Act of 1938, as amended.

INDEMNIFICATION

Buyer shall at its own expense apply for and obtain any permits and inspections required for the installation and/or use of the Products. SSI makes no promise or representation that the Products or related services will conform to any federal, state or local laws, ordinances, regulations, codes or standards, except as particularly specified and agreed upon in writing by an authorized representative of SSI. SSI's Products are not for use in or with any nuclear facility unless specifically so stated by SSI in writing. SSI's Products are not for use for Diagnostic Purposes. SSI shall not be responsible for any losses or damages sustained by Buyer or any other person as a result of improper installation or misapplication of the Products. Buyer shall defend, indemnify and hold harmless SSI and its agents and employees against any loss, damage, claim, suit, liability, judgment or expense (including, without limitation, attorney's fees) arising out of or in connection with any injury, disease or death of persons (including, without limitation, Buyer's employees and agents) or damage to or loss of any property or the environment, or violation of any applicable laws or regulations resulting from or in connection with the sale, transportation, installation, use, or repair of the Products by Buyer or of the information, designs, services or other work supplied to Buyer, whether caused by the concurrent and/or contributory negligence of Buyer, SSI, or any of their agents, employees or suppliers. The obligations, indemnities and covenants contained in this paragraph shall survive the consummation or termination of this transaction.

CONFIDENTIAL INFORMATION

Except as required by law, neither party shall use (except for purposes connected with the performance of its obligations hereunder), divulge or communicate to any third party any information of the other it reasonably knows to be confidential.

FDA REQUIREMENTS

If any Product sold by SSI to Buyer is subject to regulation by the Food and Drug Administration as a device, then as to any such Product, sale and delivery is contingent upon successful completion and processing of a 510(k) notice. If such a 510(k) notice cannot be obtained by SSI, SSI shall have no obligation to deliver the Product.

COMPLIANCE WITH LAWS

Buyer agrees to comply fully with all federal, state, county and local laws, rules and regulations concerning the purchase, sale and use of Products. Without limiting the foregoing, Buyer agrees to comply with any the Export Administration Regulations; Federal Food, Drug & Cosmetics Act; the International Traffic in Arms Regulations of the United States; and the Foreign Corrupt Practices Act of the United States in so far as they apply to the sale of Products. To the extent the Products require license for export, the Products are licensed by the United States for delivery to the ultimate destination as shown on the shipment/invoice address and any contrary diversion may be prohibited by law.

APPLICABLE LAW; JURISDICTION VENUE

The Sales Agreement is made and entered into, and shall be governed, enforced and interpreted in accordance with the laws of the State of Maryland. The Buyer hereby expressly consents to the jurisdiction of the courts of the State of Maryland with regard to all issues and questions of law or fact pertaining to the Sales Agreement. In the event that either party commences litigation to enforce the Sales Agreement, said litigation shall be brought in the courts of Howard County, Maryland. The prevailing party to any such action shall be entitled to an award of all costs and attorney's fees actually incurred.

GENERAL PROVISIONS

The rights and obligations under these General Terms and Conditions of Sale will inure to the benefit of, and be binding upon the parties hereto and their respective heirs, administrators, executors, personal representatives, successors and permitted assigns. No action, failure of action or delay by either party will constitute a waiver of any of its rights or remedies under these General Terms and Conditions of Sale. SSI and Buyer are not, and will not be, joint ventures, partners, agents, servants, or employees or fiduciaries of the other, and do not have the power to bind or obligate the other. The waiver of a breach of any provision does not constitute a waiver of a subsequent breach of the same or different provision. If any of the terms of these General Terms and Conditions of Sale or the Sales Agreement are subsequently or are now illegal, they will be severed without affecting the remaining terms. The section headings are for reference only and will not be considered controlling as to the content and/or interpretation of any section.

ENTIRE AGREEMENT

These General Terms and Conditions of Sale, together with the Sales Agreement, and any attachments, exhibits and supplements specifically referred to in the Sales Agreement, are intended by the parties as a complete and exclusive statement of the terms of their agreement, and supersede all prior agreements, written or oral. No course of prior dealings between the parties and no usage of the trade may be used by Buyer to supplement or explain any term used herein.

END GENERAL TERMS AND CONDITIONS OF SALE

This document contains the number of pages indicated, including all attachments. Authorized signature required on quotation pages

Customer:

Brenda Keavey

WVDA

4720 Brenda Lane

Charleston, WV 25312

Phone: (304) 558-2227

Fax:

E-mail: bkeavey@ag.state.wv.us

Sales Engineer:

Heather Juzwa

Shimadzu Scientific Instruments

7102 Riverwood Drive

Columbia, MD 21046

Regional Office: (410) 381-1227 Ext. 1673

Direct Dial: (800) 388-6996

E-mail: hjuzwa@SHIMADZU.com

Sales Proposal

Quotation: SSI-107233-H7V4

Expiration: 3/17/2017

Quote Description:

Proc Folder: 265333. CRFQ 1400 AGR1700000005 Triple Quad LC/MS/MS. OPTION 2: LCMS-8045 with Nexera X2 19,000 UHPLC System.

Proposed Ship Date: 30 Days/ARO

FOB: DESTINATION **Ship Method:** BEST WAY

Shipping Terms: PREPAID & ADD

Incoterms:

Additional Information:

For proposal questions or modifications, please contact your sales representative.

For Order Placement:

Reference Quotation Number on Purchase Order

If you are tax exempt, please send a copy of your exemption certificate with your purchase order.

Certificate may also be faxed to 410-381-1222.

Thank you for your interest in Shimadzu Scientific Instruments.

Shimadzu Scientific Instruments

7102 Riverwood Drive, Columbia, MD 21046

Toll Free: 800-477-1227

Local: 410-381-1227 Fax: 410-381-6781

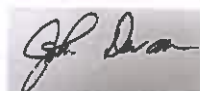
E-mail: customer.service@shimadzu.com

International:

Fax: 410-309-6130

E-mail: icsc@shimadzu.com

Authorization Signature



John Dwan

Date: 11/15/2016

Sales Quotation - please reference the quotation when submitting purchase order.

LCMS-8045

	Product #	Qty	Description	List Price	Ext'd Price
1	220-91645-01	1	LCMS-8045 Package	\$289,000.00	\$216,750.00
2	220-91545-01	1	LCMS SOLVENT KIT FOR INSTALLATIONS, B&J, DROP SHIP	\$0.00	\$0.00
3	228-45015-42	1	FCV-20AH2	\$3,595.00	\$2,696.25
4	220-91600-52	1	LCMS-8045/8050/8060 Installation Kit	\$2,100.00	\$2,100.00
5	225-19605-58	1	DUIS Dual Probe Set for LCMS-8045/8050/8060	\$3,708.00	\$2,781.00
6	228-16006-00	4	Plug, LC-6A/6AD	\$18.00	\$54.00
7	220-95367-01	1	Epson Workforce 30 Color Inkjet Printer (USE W/TOC-LCS)	\$134.00	\$113.90
8	220-97301-05	1	HIGH SPEED USB 2.0 PRINTER CABLE	\$29.00	\$24.65
9	I&F	1	INSTALLATION AND CUSTOMER FAMILIARIZATION	\$0.00	\$0.00
10	220-99204-04	3	Introduction to the LCMS-8030/8040/8050/8060	\$1,700.00	\$0.00
11	1YW	1	1 YEAR WARRANTY	\$0.00	\$0.00

HPLC

	Product #	Qty	Description	List Price	Ext'd Price
12	220-91398-20	1	CBM-20A W/ Network Switch	\$3,245.00	\$2,433.75
13	228-45019-58	2	DGU-20A5R, ROHS-compliant	\$3,308.00	\$4,962.00
14	220-91413-02	1	1L Bottle/Cap/Res Tray PKG	\$628.00	\$471.00
15	228-45162-42	2	Nexera LC-30AD HPLC Pump	\$12,978.00	\$19,467.00
16	228-45180-58	2	Nexera X2 LPGE-30 No Mixer, ROHS Compliant	\$3,245.00	\$4,867.50
17	228-45168-41	1	Nexera LC-30AD Micro Mixer, 20 uL	\$1,195.00	\$896.25
18	220-91397-22	1	Nexera SIL-30AC Autosampler with Installation Kit	\$28,900.00	\$21,675.00
19	228-37546-92	1	Sample Rack for DWP, SIL-20A/AC, SIL-30AC	\$731.00	\$548.25
20	228-45010-42	1	CTO-20AC	\$4,550.00	\$3,412.50
21	228-45166-58	1	FCV-32AH 2-Position, 6-Port High Pressure Switching Valve, ROHS-Compliant	\$5,570.00	\$4,177.50
22	220-91522-02	1	Valve Mounting Kit for CTO-20/30A, 2-position	\$227.00	\$170.25
23	220-91403-02	1	SPD-M30A Photodiode Array Detector with Ethernet Switch	\$18,540.00	\$13,905.00
24	228-54450-91	1	Stainless Steel Manifold, Analytical	\$742.00	\$556.50
25	220-95315-05	1	HPLC Virtual Advisor USB for Prominence and Nexera Systems	\$1,250.00	\$0.00
26	I&F	1	INSTALLATION AND CUSTOMER FAMILIARIZATION	\$0.00	\$0.00
27	1YW	1	1 YEAR WARRANTY	\$0.00	\$0.00
28	220-99208-00	3	TRAINING COURSE, HPLC W/TROUBLE SHOOTING	\$1,170.00	\$0.00

Expert Mode Kit



Company Name: WVDA
Quote Number: SSI-107233-H7V4
Activation Date: 11/14/2016
Expiration Date: 3/17/2017

	Product #	Qty	Description	List Price	Ext'd Price
29	PREMIUM-PM-ONLY	2	Premium Preventive Maintenance Only Service Agreement	\$15,263.45	\$30,526.90

Total List Price:	\$442,414.90
Total Line Item Discount:	\$109,825.70
Quote Sub-Total:	\$332,589.20
Estimated Freight:	\$850.00
Total Amount:	\$333,439.20

Sales Quotation - please reference the quotation when submitting purchase order.
Optional Items

Optional Items:**LCMS-8045**

Product #		Qty	Description	List Price	Ext'd Price
1	225-19601-58	1	APCI Dual Probe Set for LCMS-8045/8050/8060	\$11,021.00	\$8,265.75

Service Agreement Plans Available

As Quoted

Yrs of Coverage	Plan Name	Plan Cost	Products Covered

Descriptions of Service Coverage

Preventative Maintenance

Preventative Maintenance (PM) visits will be scheduled by SSI at the mutual convenience of the buyer and SSI, or performed during any other service visit. PM visits, when provided under this Agreement, may include necessary cleaning, adjustments, verification, lubrication and parts replacement according to the PM checklist. Labor, travel expenses, and selected consumable parts will be included during the PM visit at no additional cost to the buyer.

Premium Preventative Maintenance

Premium Preventative Maintenance visits will be scheduled by SSI at the mutual convenience of the customer and SSI, or performed during any other service visit. This Premium PM will be performed by SSI according to established SSI procedures. Premium PM visits, when performed under this Agreement, will include necessary cleaning, adjustments, verification, lubrication and parts replacement. Labor, travel expenses and selected consumables parts* (list of parts available upon customer request) will be included during the Premium PM visit at no additional cost to the customer. A travel zone fee is added to the agreement to cover travel costs. Purchase of the Premium PM option does not excuse Customer from performing normal daily, weekly or monthly maintenance that may be required.

Extended Warranty

Extended Warranty coverage includes all labor and parts (excluding consumables) necessary to restore the products to operating specifications.

This coverage level is not available for MS products and the associated turbo and roughing pumps. The MS products require a coverage level which includes a Premium PM.

Extended Warranty Plus

A combination of one scheduled Premium PM visit and Extended Warranty Coverage. Premium Preventative Maintenance visits will be scheduled by SSI at the mutual convenience of the customer and SSI, or performed during any other service visit. Premium PM visits, when performed under this Agreement, will include necessary cleaning, adjustments, verification, lubrication and parts replacement. Labor, travel expenses and selected consumables parts* (list of parts available upon customer request) will be included during the Premium PM visit at no additional cost to the customer. The extended Warranty Includes all labor and parts (excluding consumables outside of PM) necessary to restore the products to operating specifications. A travel zone charge is added to the contract to cover travel costs. In the event of a covered product failure, SSI will use its best effort to provide on-demand service.

Premium Total Coverage

This level of agreement provides the customer with a level of coverage includes all labor and non-consumable parts, a Premium PM visit, and in addition provides for the replacement of consumable parts outside of a PM visit at no additional cost to the buyer. Use of this level of Service will be monitored by SSI and in the event that the buyer's use does not exceed two visits during the effective dates of the agreement, the buyer will be entitled to an agreed upon rebate at the end of the agreement term. This rebate must be applied to the purchase of future service agreements or other services offered by SSI.

Sales Quotation - please reference the quotation when submitting purchase order.

Line Item Descriptions

Product #	Description
LCMS-8045	
220-91645-01	LCMS-8045 Package
	LCMS-8045 package includes LCMS-8045 Triple Quadrupole Detector, Advanced Data Station Dell PC, 24" LCD Monitor, LabSolutions Software and License, Startup Kit, Rotary Pump, Oil Return Kit, and accessories. The LCMS-8045 features a heated ESI probe for high sensitivity analysis and advanced electronics for ultra-fast multiple reaction monitoring (MRM) transitions, enabling data acquisition with up to 555 channels per second. The system is capable of ultrafast mass spectrum acquisition speeds up to 30,000 u/sec without sacrificing sensitivity or mass accuracy, and ultra-fast polarity switching (5msec) for the most information without signal deterioration. Patented UFSweeper III ® collision cell technology results in high-efficiency collision-induced dissociation (CID) and ultra-fast ion transport, yielding high sensitivity and low crosstalk. In addition, higher radio frequency (RF) power capability minimizes pauses between each transition. The LCMS-8045 Software includes unified control of LC and MS components providing seamless operation of the entire LCMS system, unmatched qualitative and quantitative analysis, increased productivity, and accelerated workflows for high throughput data analysis. MRM Synchronization optimizes MRM cycle times for overlapping retention time windows and helps to simplify method development and routine analysis with multiple MRM experiments. In the Quantitation Browser, peak information, quantitative results, and statistical calculations of a series of data can be rapidly viewed in a single window. For Research Use Only. Not for use in Diagnostic Procedures. Includes Dell Workstation PC with Windows 7 Pro 64bit, Quad Core Intel Xeon Processor, 8GB RAM, and 1 TB hard drive. LabSolutions software license controls one LCMS system and includes post-analysis processing capabilities. Single phase 230V +/-5% AC, 20 A (50/60 Hz) or Single phase 200V +/-5% AC, 20 A (50/60 Hz) power required for MS operation.
220-91545-01	LCMS SOLVENT KIT FOR INSTALLATIONS, B&J, DROP SHIP
228-45015-42	FCV-20AH2
	High pressure, 2-position, 6-port valve for column switching, online sample pretreatment, or flow line switching. Can be controlled by contact closure, local front panel control, or through the CTO-20A/AC. For contact closure control, requires one Event output from CBM, CBM-lite, or SCL-10Avp
220-91600-52	LCMS-8045/8050/8060 Installation Kit
	LCMS-8045/8050/8060 Installation Kit. This kit contains 100' of 6mm OD PTFE nitrogen gas tubing, 50' of 1/8" cleaned copper argon gas tubing, 1 Nitrogen regulator, 1 Argon regulator, 1 1/8" Swagelok fitting, 1 6mm Swagelok union (6mm to 6mm), 1 6mm Swagelok Reducer (6mm to 1/4" FNPT), and 1 6mm Swagelok union (6mm to 1/4"). This kit provides all of the necessary items to install the instrument to the facility's electrical supply provided they have installed either an L6-20R or L6-30R and to the facility's nitrogen system utilizing either a Dewar, nitrogen generator, or house gas system. Also included in this kit are the necessary items to connect the instrument to the Argon supply cylinder. The kit provides the necessary items to attach the system to the combined nitrogen/air generator or to terminate with a 1/4" Swagelok fitting allowing connection to either an air compressor or house gas system for delivery of air to the LCMS unit.
225-19605-58	DUIS Dual Probe Set for LCMS-8045/8050/8060
	Shimadzu's DUIS Dual Ion Source adds APCI capability to the standard ESI configuration. By adding APCI capability for non-polar molecules, the user benefits from faster method development and the ability to analyze more compounds in a single method. Includes Needle Unit, TQ-HVD Dual Assembly, Needle Socket Assembly. Separate socket assembly not required.
228-16006-00	Plug, LC-6A/6AD
	Stainless steel plug for unused mixer or Valco manifold ports. Can also be used as a column plug.
220-95367-01	Epson Workforce 30 Color Inkjet Printer (USE W/TOC-LCS)
	Requires USB Printer cable pn 220-97301-05
220-97301-05	HIGH SPEED USB 2.0 PRINTER CABLE
I&F	
	I&F Installation (setup/checkout) and operational familiarization of hardware and software.
220-99204-04	Introduction to the LCMS-8030/8040/8050/8060

Introduction to the LCMS Triple Quadrupole. A 3-Day course taught at Shimadzu Customer Training and Education Center. Suggested for those new to LCMS or those new to the LCMS-8030/8040/8050 hardware and software. The greatest successes are achieved by those who have used the instrument enough to bring their questions to class.

1YW

1 YEAR WARRANTY

1 YEAR WARRANTY

HPLC

220-91398-20 CBM-20A W/ Network Switch

Central communication and control module for the Prominence and Nexera Series. System components connect via fiber optic cables for easy "plug and play" configuration. Internal web server offers convenient control and monitoring of the HPLC system through a web browser.

Connections via Ethernet and RS-232C possible; Offers 8 Fiber Optic ports; 4 Events; 2 PC-55N A/D boards can be installed. Includes 4 port 10/100 Ethernet switch. VP and 10A Series modules with a fiber optic port can be controlled by the CBM-20A.

228-45019-58 DGU-20A5R, ROHS-compliant

Inline membrane degasser utilizing Teflon AF® for maintaining the degassed state of HPLC mobile phases. Use in conjunction with preliminary degassing by vacuum aspiration/sonication. A convenient 5th channel is included for degassing the autosampler rinse phase for optimum injection reproducibility performance.

Recommended for optimum gradient performance; connects to VP; Prominence (LC-20) and Nexera (LC-30) series pumps for power. 400 µL internal volume. REQUIRES power supply (228-45110-92) for use with 3rd party, non-VP, non-Prominence, or non-Nexera pumps. Many fluorinated solvents, strong acids (30+%) and peroxides will damage the membrane; check manual for listing. Maximum flow rate is 10mL/min per channel.

220-91413-02 1L Bottle/Cap/Res Tray PKG

Prominence LC-20A reservoir tray with a set of (5) 1-L solvent bottles and (5) 3-hole caps. Caps include a solid plug and a filter plug with a stainless steel frit.

Filter plug included with caps for use with He sparge.

228-45162-42 Nexera LC-30AD HPLC Pump

The LC-30AD utilizes micro-volume plungers (10 µL each) for pulse-free HPLC solvent delivery. The sapphire plunger has a specially engineered ultra-smooth surface which minimizes frictional heat for improved seal lifetime. An automatic rinse kit is included as a standard accessory. Flow rate range is 0.0001 to 5.000 mL, maximum pressure is 130 MPa (19,000 psi). The Nexera X2 LC-30AD is ideal for low flow rate (< 1 mL/min) and LCMS applications, and for analyses using small particle size columns (3 µm or less) that generate high back pressure.

Newly designed plunger holder ensures proper alignment with the seal; 3 nL/step motor control ensures excellent retention time reproducibility with short runs and steep gradients. Requires CBM/CBM-lite ROM version 1.30 for full control in LC-30 mode. 20A compatibility mode is available for 3rd party software control. Not compatible with the SCL-10Avp.

228-45180-58 Nexera X2 LPGE-30 No Mixer, ROHS Compliant

Four-position valve for the Nexera X2-LC-30AD pump that can be used as a solvent selector for gradient former. Requires an additional mixer; the 180 µL MIRC mixer 228-45168-46 is recommended.

Requires 4 available DGU channels. If a SIL-30AC is in the configuration, 7 total DGU channels are required. A DGU-20A3 and DGU-20A5 are recommended to accommodate both the LPGE and SIL-30AC.

228-45168-41 Nexera LC-30AD Micro Mixer, 20 µL

High efficiency mixer using micro reactor technology to thoroughly blend solvents in a small volume. Use for standard analytical (1-2 mL/min) and LCMS flow rates. For applications using TFA in the mobile phase, the 180 µL mixer 228-45168-42 is recommended.

Includes 2 mounting brackets for installation in either the CTO-30A or in the CTO-20A/AC or on the side of a pump.

220-91397-22 Nexera SIL-30AC Autosampler with Installation Kit

The SIL-30AC provides high speed injections (10 sec for 10 µL) with ultra-low carryover achieved through advanced material technology and rinse options (if necessary) with up to 4 solvents. The standard configuration uses the needle in the flow path design for 0.1 to 50 µL injection volumes; an optional fixed-loop configuration (5 or 20 µL) is user-changeable to reduce delay volume and band broadening in isocratic separations. The sample compartment uses a Peltier cooler for temperature control from 4-40C. An external rinse pump is standard, and provides additional rinsing to the outer surface of the needle. Includes 105-position rack for 1.5 (2mL) vials and a control rack for 10 1.5 (2 mL) vials, 1.5 mL vial set, vial sample pack, and 2.1 x 50mm, 1.9 µm C18 column and 4-component test mix. Sample capacity is 175 1 mL vials, 70 4 mL vials, 2 MTP (STD 96, DW 96, or STD 384). Maximum pressure is 130 MPa (19,000 psi).

Pretreatment functions include dilution and reagent addition as well as user-programmable sample prep routines. Three rinse solvents are available to clean the inside of the loop and needle, as well as for injection port rinsing. Requires CBM/CBM-lite ROM version 1.30 or higher for full control in LC-30 mode. 20A compatibility mode is available for 3rd party software control. Pretreatment is not available in compatibility mode, but all rinse functions can be programmed from the front panel.

228-37546-92 Sample Rack for DWP, SIL-20A/AC, SIL-30AC

Capacity of 2 Deep Well MTP plates, 96 well format.

Can be used in SIL-20A, SIL-20AC, and SIL-30AC

228-45010-42 CTO-20AC

Full-sized oven with forced air temperature control from 10C below ambient temperature to 85C. Can accommodate a manual injector, mixer, and 2 switching valves.

Linear temperature programming is possible; optional Column Management Device (228-37281-91) with CMD Cable (228-39991-01) can be used. FCV-12/14 Control Board (228-44291-91) required to install non-Prominence color FCV-12/14 in CTO-20A. Subcontroller is no longer needed unless the CTO-20 is used in VP mode.

228-45166-58 FCV-32AH 2-Position, 6-Port High Pressure Switching Valve, ROHS-Compliant

High pressure 6-port, 2-position valve for automatic 2-column switching, online sample pretreatment, or flow line switching. When installed in the CTO-30A column oven, dual preheater 228-52597-42 is recommended, Maximum pressure is 130 MPa (19,000 psi).

220-91522-02 Valve Mounting Kit for CTO-20/30A, 2-position

Mounting kit for installing the 10,18, or 25K psi 2-position valves in the CTO-20A/AC or CTO-30A. Includes bracket, wiring harness, and screws to secure the assembly to the CTO-20/30. One kit has enough parts for 2 valves.

220-91403-02 SPD-M30A Photodiode Array Detector with Ethernet Switch

The SPD-M30A photodiode array detector utilizes a capillary flow cell for ultra-high sensitivity and minimal sample dispersion. Temperature controlled optics ensure a stable baseline in all laboratory environments with reduced startup time.

Includes Ethernet switch and crossover cable for PC connection.

228-54450-91 Stainless Steel Manifold, Analytical

Stainless steel manifold for connecting up to 6 column outlets in a method development or method scouting system. Mounting bracket included for installation inside the CTO-20A/AC column oven. Requires stainless steel plug 228-16006-00 or PEEK plug 228-28090-00 for unused ports. Internal volume is 0.44 uL.

220-95315-05 HPLC Virtual Advisor USB for Prominence and Nexera Systems

HPLC Virtual Advisor for Prominence and Nexera Systems. Includes two separate modules on a 8 GB USB drive. These modules cannot be run simultaneously. Installation instructions are included on .zip files.

I&F

I&F Installation (setup/checkout) and operational familiarization of hardware and software.

1YW 1 YEAR WARRANTY

1 YEAR WARRANTY

220-99208-00 TRAINING COURSE,HPLC W/TROUBLE SHOOTING

HPLC maintenance class with emphasis on evaluating performance and preventing/solving problems with the LC-2010, the LC-10A / LC-10Avp Series and/or the LC-20 Series injectors, autosamplers, pumps, and detectors. Students should indicate at REGISTRATION what instrument series and modules are on interest.

Expert Mode Kit

PREMIUM-PM-ONLY

Premium Preventative Maintenance visits will be scheduled by SSI at the mutual convenience of the customer and SSI, or performed during any other service visit. This Premium PM will be performed by SSI according to established SSI procedures. Premium PM visits, when performed under this Agreement, will include necessary cleaning, adjustments, verification, lubrication and parts replacement. Labor, travel expenses and selected consumables parts* (list of parts available upon customer request) will be included during the Premium PM visit at no additional cost to the customer. A travel zone fee is added to the agreement to cover travel costs. Purchase of the Premium PM option does not excuse Customer from performing normal daily, weekly or monthly maintenance that may be required.

Optional Item Descriptions

Product #	Description

LCMS-8045	

225-19601-58	APCI Dual Probe Set for LCMS-8045/8050/8060

Shimadzu's APCI Probe easily replaces the standard ESI configuration. By employing APCI ionization for non-polar molecules, the user is able to analyze difficult to ionize compounds.

Includes APCI Unit, Needle Unit, APCI Pipe assembly and accessories.

Separate socket assembly NOT required.

Terms and Conditions

SALES AGREEMENT

The sales agreement ("Sales Agreement"), as referenced herein below, shall mean and refer to these General Terms and Conditions of Sale ("General Terms and Conditions of Sale"), together with such other documents concerning the purchase of equipment and/or Products designed and/or manufactured by Shimadzu ("Products"), which documents have been or will be executed by and between Shimadzu Scientific Instruments, Inc. ("SSI") and the individual or entity identified therein as the purchaser of said Products ("Buyer"). The documents that comprise the Sales Agreement may include, but are not limited to: these General Terms and Conditions of Sale; price quote provided by SSI; purchase order issued by Buyer as modified and accepted by SSI; bill of sale for Products; bill of lading issued for the shipment of Products; and product invoice. **All Products except designated CL versions of HPLC and LCMS, shall be for research use only and not for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat or prevent disease or its sequelae ("Diagnostic Purposes").**

PRICES

The prices set forth in the Sales Agreement:

- (i) are SSI's domestic prices based upon manufacture of the quality and type of Product(s) ordered for shipment to and end use within the United States - all Products shipped for end use outside the United States shall be subject to SSI's international pricing;
- (ii) are subject to revision when interruption, engineering changes or changes in quantity or quality are caused or requested by Buyer; and
- (iii) unless otherwise specified, do not include warranty service or installation outside the United States. Errors in Product pricing or related terms by SSI which may, in SSI's sole discretion, be deemed clerical errors are subject to correction by SSI at any time.

SPECIFICATIONS

Weights and dimensions set forth in Product related sales literature ("Sales Literature") are not guaranteed unless previously certified in writing. SSI may, without affecting the obligations under the Sales Agreement, make what SSI regards in its sole discretion as minor changes to the specifications of the Product or Products delivered under the Sales Agreement from those contained in Sales Literature.

TERMS OF PRODUCT USE

Buyer represents and warrants that the Products have not been purchased for Diagnostic Purposes and that the Buyer will not at any time use the Products for Diagnostic Purposes, except designated CL versions of HPLC and LCMS Products. The Buyer acknowledges that use of the Product for Diagnostic Purposes is cause for Termination of this Agreement, as set forth in the "Contingencies; Force Majeure" section below.

TERMS OF PAYMENT

Subject to credit approval by SSI, terms of payment on the Sales Agreement are net thirty (30) days from date of the invoice unless otherwise specifically stated on the invoice, by check, wire. Payment by credit card is subject to 3% charge back at time of payment. Invoices are payable at par on date due at any place of collection designated by SSI in funds bankable at par. Payment made beyond terms will be subject to simple interest of 1 -1/2% per month on the outstanding balance. All orders are accepted and SSI's obligation to make deliveries, are subject to SSI's right to either:

- (i) require Buyer to make payment of all or any part of the purchase price in advance of delivery of Product(s), or
- (ii) make shipment of Product(s) to Buyer payable C.O.D.

If Buyer fails to make advance payment when requested to do so by SSI or if Buyer is or becomes delinquent in the payment of any sum of any kind due SSI or refuses to accept C.O.D. shipments (whether or not arising out of the Sales Agreement), then SSI shall have the right, in addition to any other remedy to which it may be entitled in law or in equity, to cancel the Sales Agreement, refuse to make further deliveries, and declare immediately due and payable all unpaid amounts for Products and services previously delivered to or performed for Buyer. Each shipment shall be considered a separate and independent transaction and payment therefor shall be made accordingly. Buyer must notify SSI within fifteen (15) days from the invoice date if Buyer has not received the Product(s).

REPOSSESSION

In the event of nonpayment, either of the entire purchase price or a portion thereof, Buyer expressly consents to the right of SSI to reclaim by repossession of any and all Products delivered to Buyer pursuant to the Sales Agreement for which payment has not been made within (90) days of invoice. In the event that SSI elects to exercise its right to reclaim Products by repossessing the same, said election shall be deemed to be in the alternative and not to the exclusion of any other remedy to which SSI may be entitled, at law or in equity. To the extent that SSI successfully reclaims Products, it shall credit Buyer's account for the value of the same, as determined by SSI in its sole discretion. No Products may be reclaimed by repossession unless and until Buyer is provided with prior notice of SSI's intention to elect such remedy. Such notice shall state the date, time and location that Products shall be available for repossession. Such notice shall also provide Buyer with a final opportunity to cure the default of nonpayment within five (5) days of notice to avoid the execution of the remedy of repossession. The costs of repossession, including legal fees and costs of collection, shall be added to Buyer's account.

SECURITY INTEREST

In addition to any other remedies hereunder or otherwise provided at law or in equity, SSI shall have the right, and Buyer hereby grants SSI the right, without further notice to or consent from Buyer, to secure any and all payment obligations of Buyer by recordation of a security interest in the Products that are the subject of the Sales Agreement. Said security interest shall be in the form of a UCC Financing Statement (UCC Form 1) or other form prescribed by contract or applicable law. No UCC Financing Statement recorded for the benefit of SSI will be released unless and until all financial obligations to SSI incurred by Buyer with respect to the Products have been fully satisfied.

SHIPMENTS

(i) The cost of packaging for domestic shipments is included in the quoted price unless otherwise provided. For international shipments or where special packaging is specified or necessary, a charge will be made to cover such expense.

(ii) For shipments to and from places within the United States, all shipments, unless otherwise agreed in writing, shall be FOB point of shipment and title and risk of loss or damage shall pass to Buyer at the point of shipment. The cost of transportation and insurance (if requested by Buyer) shall be borne by Buyer.

(iii) For shipments from the United States to ports and/or places outside the United States all shipments are, unless otherwise agreed in writing, FOB Columbia, MD. SSI's obligation to affect shipment of the Products purchased by Buyer shall be fully discharged, and beneficial ownership, legal title and all risk of loss or damage shall pass to Buyer when the Products are made available for shipment to a carrier at the designated FOB location. If shipped FOB Destination, upon arrival Buyer shall be entitled to conduct a reasonable investigation of the Products purchased by it, but all claims for losses due to loss or damage to Products while in transit shall be waived unless made immediately in writing by Buyer to SSI, but not more than (30) thirty days after arrival. If Buyer shall fail or refuse to accept delivery of any of the Products for unverifiable claims for loss or damage to Products occurring while in transit, all sums paid on deposit shall be retained by SSI as liquidated damages, provided, however, that SSI may recover in full its actual damage from Buyer in the event that actual damages exceed the amount retained as liquidated damages.

(iv) All claims for damage or loss of insured shipments shall be immediately communicated, when possible, to SSI at Shimadzu Scientific Instruments, Inc. 7102 Riverwood Drive, Columbia, Maryland 21046-2502 Attn: Customer Service, Phone: (410) 381-1227.

Buyer shall immediately notify delivering carrier of loss or damage to the shipment and SSI will cooperate with Buyer in the adjustment of all claims. Buyer agrees to permit SSI or SSI's representative to inspect damaged Products.

TERMINATION

Upon any termination or cancellation of the Sales Agreement by Buyer (if otherwise specifically permitted by the terms of the Sales Agreement), either in whole or in part, Buyer agrees to promptly pay appropriate termination or cancellation charges invoiced by SSI. At SSI's sole discretion, the termination charge shall be not less than twenty percent (20%) of the total amount of the Sales Agreement.

RETURNED PRODUCTS

All returns must be pre-authorized by SSI and a Return Goods Authorization ("RGA") number must appear on the face of the package. Returned Products will be subject to a restocking charge. If deemed appropriate at SSI's sole discretion, the restocking charge shall be not less than twenty percent (20%) of the total amount of the Sales Agreement.

ALLOCATION OF PRODUCTS

If SSI is unable for any reason to supply the total demands for Products specified in Buyer's order, SSI may allocate its viable supply among any or all buyers, including Buyer, on such basis as SSI may deem fair and practical, without liability for any failure of performance which may result therefrom.

DELIVERY

The scheduled shipping or delivery date shown on the face hereof is SSI's best estimate of the time the Product order will be shipped and SSI assumes no liability for loss, general damages, or special or consequential damages due to delays.

TAXES AND OTHER CHARGES

All taxes on Products, goods and/or services sold under the Sales Agreement, including but not limited to federal, state and local excise, sales or use taxes, shall be borne solely by Buyer. Buyer shall be obligated to pay all taxes set forth on invoices, in accordance with the terms of payment, provided that SSI shall not invoice taxes when within 30 days of placing the order, Buyer has furnished SSI with written proof of exemption from tax in the form of a certificate of exemption or an equivalent document which Buyer represents and warrants is properly completed and validly executed. If, notwithstanding the foregoing, any taxing authority attempts to assess taxes, Buyer shall indemnify and hold harmless SSI from any loss, damage, claim or cause of action, including, but not limited to, tax, interest, penalties and professional fees, related to such attempted assessment and shall make payment to SSI for any such costs paid by SSI and invoiced to Buyer in accordance with the terms of payment.

PATENTS

SSI shall defend any suit or proceeding brought against Buyer so far as based upon an assertion that any Product furnished under the Sales Agreement constitutes a direct infringement of any United States patent having a claim of claims covering solely the Product itself, if notified promptly in writing and given authority, information and assistance (at SSI's expense) for the defense of same, and SSI shall pay all damages and costs awarded therein against Buyer. In the event said Product in such suit is held to constitute infringement and the use of said Product is enjoined, SSI shall, at its own option and at its own expense, either:

- (i) procure for Buyer the right to continue using said Product, (ii) replace the same with a non-infringing Product, (iii) modify it so it becomes non-infringing, or (iv) remove said Product and refund the purchase price and transportation costs thereof.

The foregoing obligations of SSI shall not apply to any infringement claim based upon:

- (i) any use of any Product sold hereunder in any process or in conjunction with any other product, (ii) any Product manufactured to Buyer's design or any Product having a design arising from SSI's compliance with

Buyer's specifications; or (II) use of any Product sold hereunder, if the Product has been modified or customized by Buyer.

The foregoing sets forth the entire liability of SSI for patent infringement by said Product. If any suit or proceeding is brought against SSI based on claims that the goods manufactured by SSI in compliance with Buyer's specifications and supplied to Buyer directly infringe any fully issued United States patent, then the patent indemnity obligations herein stated with respect to SSI shall reciprocally apply with respect to Buyer.

WARRANTY

Subject to the exceptions and upon the conditions stated below, SSI warrants that the Products sold under the Sales Agreement shall be free from defects in workmanship and materials for one year after shipment of the Products to the original Buyer by SSI (the "Warranty"), and if any such Products should prove to be defective within such one year period, SSI's sole liability (and Buyer's sole and exclusive remedy) shall be, at its option, either (i) to correct by repair or, at SSI election, by replacement with equivalent product any such defective Product, provided that investigation and factory inspection discloses that such defect developed under normal and proper use, or (ii) to refund the purchase price. The exceptions and conditions mentioned above are as follows:

- (i) Components or accessories manufactured by SSI which by their nature are not intended to and will not function for one year are warranted only to give reasonable service for a reasonable time; what constitutes reasonable time and reasonable service shall be determined solely by SSI. A complete list of such components and accessories is maintained at the factory;
- (ii) SSI makes no warranty with respect to components or accessories not manufactured by it, in the event of defect in any such component or accessory SSI will give reasonable assistance to Buyer in obtaining from the respective manufacturer whatever adjustment is authorized by the manufacturer's own warranty;
- (iii) any Product claimed to be defective must, if required by SSI, be returned to the factory, transportation charges prepaid, and will be returned to Buyer with transportation charges collect unless the Product is found to be defective, in which case SSI will pay all transportation charges;
- (iv) if the Product is a reagent or the like, it is warranted only to conform to the quantity and content and for the period (but not in excess of one year) stated on the label at the time of delivery;
- (v) SSI may from time to time produce a special printed warranty with respect to a certain Product, and where applicable, such warranty shall be deemed incorporated herein by reference;
- (vi) SSI shall be released from all obligations under all warranties, whether expressed or implied, if any Product covered hereby is repaired or modified by persons other than its own authorized service personnel unless such repair by others is made with the written consent of SSI; and
- (vii) SSI's obligations pursuant to all warranties, either express or implied, shall be expressly conditioned upon payment in full by Buyer for the Products covered by the warranties. In the event of non-payment, whether for the entire purchase price or a part thereof, SSI shall be released from all obligations under all warranties, either express or implied.

IT IS EXPRESSLY AGREED THAT THIS WARRANTY SHALL BE IN LIEU OF ALL WARRANTIES OR WARRANTY OF ANY NATURE WHATSOEVER, WHETHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION WARRANTIES OF FITNESS AND MERCHANTABILITY, AND THAT NEITHER BUYER NOR SSI SHALL HAVE ANY LIABILITY FOR SPECIAL OR CONSEQUENTIAL DAMAGES OF ANY KIND OR CAUSE WHATSOEVER, INCLUDING, BUT NOT LIMITED TO LOSS OF USE, LOSS OF DATA, LOSS OF PRODUCTIVITY, LOSS OF BUSINESS, LOSS OF PROFIT, LOSS OF PLANT, EQUIPMENT OR PRODUCTION. THE LIMITATION OF LIABILITY FOR SUCH DAMAGES SHALL BE APPLICABLE EVEN IF SSI HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, ARISING OUT OF THE MANUFACTURE, USE, SALE, HANDLING, REPAIR, MAINTENANCE OR REPLACEMENT OF ANY OF THE PRODUCTS SOLD UNDER THE SALES AGREEMENT.

If an SSI Special Warranty (covering a designated item or items) is contained in the manual or is otherwise shipped with such designated item or items, the terms and conditions specified therein are incorporated herein by reference and shall supplement the foregoing warranty. In the event of a conflict between the terms and conditions specified herein and those specified in such Special Warranty, the terms and conditions specified herein shall control. SSI's warranties made in connection with this sale shall not be effective if SSI has determined, in its sole discretion, that Buyer has misused the Products in any manner, has failed to use the Products in accordance with industry standards and practices, or has failed to use the Products in accordance with instructions, if any, furnished by SSI. Representations and warranties made by any person, including dealers and representatives of SSI, which are inconsistent or in conflict with the terms of this warranty, shall not be binding upon SSI unless expressly assumed in a writing that is approved and signed by an authorized officer of SSI. By signing the Sales Agreement, the Buyer specifically acknowledges that no representations or warranties were made by any person, including dealers and representatives of SSI, that the Product may be used for Diagnostic Purposes.

CONTINGENCIES; FORCE MAJEURE

SSI shall be entitled to cancel or rescind the Sales Agreement, without liability for loss or damage resulting therefrom, if:

- (i) any Product covered by the Sales Agreement is purchased for end use outside the United States; (ii) the Buyer breaches any of its representations or warranties found in this Agreement; or (iii) the performance of SSI's obligations under the Sales Agreement is in any way adversely affected by the occurrence of any contingency beyond the control of either SSI or SSI's suppliers, including but not limited to:

- (a) war (whether an actual declaration thereof is made or not), sabotage, insurrection, riot, act of a public enemy, or other act of civil disobedience;
- (b) failure or delay in transportation;
- (c) act of any government, government agency or subdivision of any government, or any judicial action affecting the terms of the Sales Agreement;
- (d) labor dispute, shortage of labor, fuel, raw material or technical or yield failure where SSI has exercised ordinary care in the prevention thereof; and
- (e) accident, fire, explosion, flood, storm or other act of God.

U.S. GOVERNMENT CONTRACTS

If the Products to be furnished under the Sales Agreement are to be used in the performance of a U.S. Government contract or subcontract, and a U.S. Government contract number shall appear on Buyer's order, those clauses of the applicable U.S. Government procurement regulations that are required to be included in U.S. Government contracts and subcontracts shall be incorporated herein by reference, including, without limitation, the Federal Acquisition Regulations and the Fair Labor Standards Act of 1938, as amended.

INDEMNIFICATION

Buyer shall at its own expense apply for and obtain any permits and inspections required for the installation and/or use of the Products. SSI makes no promise or representation that the Products or related services will conform to any federal, state or local laws, ordinances, regulations, codes or standards, except as particularly specified and agreed upon in writing by an authorized representative of SSI. SSI's Products are not for use in or with any nuclear facility unless specifically so stated by SSI in writing. SSI's Products are not for use for Diagnostic Purposes. SSI shall not be responsible for any losses or damages sustained by Buyer or any other person as a result of improper installation or misapplication of the Products. Buyer shall defend, indemnify and hold harmless SSI and its agents and employees against any loss, damage, claim, suit, liability, judgment or expense (including, without limitation, attorney's fees) arising out of or in connection with any injury, disease or death of persons (including, without limitation, Buyer's employees and agents) or damage to or loss of any property or the environment, or violation of any applicable laws or regulations resulting from or in connection with the sale, transportation, installation, use, or repair of the Products by Buyer or of the information, designs, services or other work supplied to Buyer, whether caused by the concurrent and/or contributory negligence of Buyer, SSI, or any of their agents, employees or suppliers. The obligations, indemnities and covenants contained in this paragraph shall survive the consummation or termination of this transaction.

CONFIDENTIAL INFORMATION

Except as required by law, neither party shall use (except for purposes connected with the performance of its obligations hereunder), divulge or communicate to any third party any information of the other it reasonably knows to be confidential.

FDA REQUIREMENTS

If any Product sold by SSI to Buyer is subject to regulation by the Food and Drug Administration as a device, then as to any such Product, sale and delivery is contingent upon successful completion and processing of a 510(k) notice. If such a 510(k) notice cannot be obtained by SSI, SSI shall have no obligation to deliver the Product.

COMPLIANCE WITH LAWS

Buyer agrees to comply fully with all federal, state, county and local laws, rules and regulations concerning the purchase, sale and use of Products. Without limiting the foregoing, Buyer agrees to comply with any the Export Administration Regulations; Federal Food, Drug & Cosmetics Act; the International Traffic in Arms Regulations of the United States; and the Foreign Corrupt Practices Act of the United States in so far as they apply to the sale of Products. To the extent the Products require license for export, the Products are licensed by the United States for delivery to the ultimate destination as shown on the shipment/invoice address and any contrary diversion may be prohibited by law.

APPLICABLE LAW, JURISDICTION VENUE

The Sales Agreement is made and entered into, and shall be governed, enforced and interpreted in accordance with the laws of the State of Maryland. The Buyer hereby expressly consents to the jurisdiction of the courts of the State of Maryland with regard to all issues and questions of law or fact pertaining to the Sales Agreement. In the event that either party commences litigation to enforce the Sales Agreement, said litigation shall be brought in the courts of Howard County, Maryland. The prevailing party to any such action shall be entitled to an award of all costs and attorney's fees actually incurred.

GENERAL PROVISIONS

The rights and obligations under these General Terms and Conditions of Sale will inure to the benefit of, and be binding upon the parties hereto and their respective heirs, administrators, executors, personal representatives, successors and permitted assigns. No action, failure of action or delay by either party will constitute a waiver of any of its rights or remedies under these General Terms and Conditions of Sale. SSI and Buyer are not, and will not be, joint ventures, partners, agents, servants, or employees or fiduciaries of the other, and do not have the power to bind or obligate the other. The waiver of a breach of any provision does not constitute a waiver of a subsequent breach of the same or different provision. If any of the terms of these General Terms and Conditions of Sale or the Sales Agreement are subsequently or are now illegal, they will be severed without affecting the remaining terms. The section headings are for reference only and will not be considered controlling as to the content and/or interpretation of any section.

ENTIRE AGREEMENT

These General Terms and Conditions of Sale, together with the Sales Agreement, and any attachments, exhibits and supplements specifically referred to in the Sales Agreement, are intended by the parties as a complete and exclusive statement of the terms of their agreement, and supersede all prior agreements, written or oral. No course of prior dealings between the parties and no usage of the trade may be used by Buyer to supplement or explain any term used herein.

END GENERAL TERMS AND CONDITIONS OF SALE

This document contains the number of pages indicated, including all attachments. Authorized signature required on quotation pages

LC/MS/MS Method Package for Residual Pesticides

For LabSolutions Version 5.41 SP1

UV-1800 UV-VIS Spectrophotometer

Available for purchase if the MRM list is applicable to WVDA!



Question:

Can Shimadzu provide pre-optimized MRM or HPLC conditions?

Solution:

Yes, by using a method package.

 SHIMADZU

Ready-to-Use Methods Provided

Shimadzu provides method files, including pre-registered MRM parameters with optimized quantitative and reference ions, LC separation parameters, and retention times and peak identification parameters for each compound, as well as report templates for outputting quantitation results, as a package. If retention times are adjusted when the system is introduced, based on the HPLC configuration delivered, the analysis process can be started as soon as the specified columns, mobile phases, and standard samples are supplied.

Methods Easily Customized

Since LabSolutions Version 5.41 SP1 enables the addition or deletion of components to be analyzed, methods can be easily customized. In addition, the package includes a compound parameter list created in Excel format, which enables the creation of new tables that list only the components needed.

Intuitive Operation Using LabSolutions Quantitation Browser

The quantitation browser provides an ideal environment for confirming the separation and shape of chromatogram peaks for multiple components at the same time, such as for the simultaneous multi-component analytical methods included in the method package. Since chromatogram and calibration curve displays are linked to component names, clicking on a component name automatically changes the displayed information, which allows correcting peak integration parameters and calibration curves directly.

The Quantitation Browser Enables Batch Editing and Processing of Measurement Data



HPLC Conditions

Analytical column: Shim-pack FC-ODS (150 mmL x 2.0 mmI.D., 3µm)
P/N 228-40511-95

Mobile phase A: 5 mmol/L Ammonium acetate - water

Mobile phase B: 5 mmol/L Ammonium acetate - methanol

Flow rate: 0.2 mL/min

Injection volume: 5 µL

Peak	Time (min)	Area	Height	Width	Time (min)	Area	Height
0	0	85	15	5	17.5	5	95
1	1	60	40	6	30	5	95
2	3.5	60	40	7	30.1	85	15
3	6	50	50	8	40	85	15
4	8	45	55				

LC/MS/MS Method Package for Residual Pesticides

For LabSolutions Version 5.41 SP1

Index of Compounds

1-Naphthaleneacetic Acid	Chlorimuron-ethyl	Fenpropimorph	Lufenuron	Pyrazolynate
2,4-D (2,4-PA)	Chloroxuron	(E)-Fenpyroximate	MCPA (MCP)	Pyrazophos
3-Hydroxycarbofuran	Chlorsulfuron	(Z)-Fenpyroximate	MCPB	Pyrazosulfuron-ethyl
4-Chlorophenoxyacetic acid	Chromafenozide	(E)-Ferimzone	Mecoprop (MCP)	Pyriftalid
Abamectin B1a	Cinosulfuron	(Z)-Ferimzone	Mefenpyr-diethyl	Pyrimethanil
Acibenzolar-S-methyl	Clodinafopacid	Flamprop-methyl	Mepanipyrim	Quizalofop-ethyl
Acifluorfen	Clodinafop-propargyl	Flazasulfuron	Mesosulfuron-methyl	Silafluofen
Aldicarb	Clofentezine	Florasuram	Methabenzthiazuron	Simeconazole
Aldoxyzcarb (Aldicarb Sulfone)	Cloprop	Fluazifop	Methiocarb	SpinosynA
Ametryn	Cloquintocet-mexyl	Fluazuron	Methomyl	SpinosynD
Amitraz	Cloransulam-methyl	Flufenacet	Methoxyfenozide	Sulfosulfuron
Anilofos	Clothianidin	Flufenoxuron	Metosulam	Tebufenozide
Azamethiphos	Cumyluron	Flumetsulam	Metsulfuron-methyl	Tebuthiuron
Azimsulfuron	Cyazofamid	Fluridone	Monolinuron	Teflubenzuron
Azinphos-methyl	Cycloate	Fluroxypyr	Naproanilide	Tetrachlorvinphos (CVMP)
Azoxystrobin	Cycloprothrin	Fomesafen	Naptalam	Thiabendazole
Benalaxyl	Cyclosulfamuron	Forchlorfenuron	Novalron	Thiacloprid
Bendiocarb	Cyflufenamid	Furametpyr	Oryzalin	Thiamethoxam
Benfuracarb	Cyprodinil	Furathiocarb	Oxabetrinil	Thidiazuron
Bensulfuron-methyl	Dichlorprop	Gibberellin	Oxamyl	Thifensulfuron-methyl
Benzofenap	Diclosulam	Halosulfuron-methyl	Oxaziclorfene	Thiodicarb
Boscalid	Diflubenzuron	Haloxypop	Oxycarboxin	Triasulfuron
Bromacil	Dimethirimol	Hexaflumuron	Pencycuron	Tribenuron-methyl
Bromoxynil	Dimetomorph	Hexythiazox	Penoxsulam	Triclopyr
Butafenacil	Diuron (DCMU)	Imazalil	Pentoxazone	Trifloxystrobin
Carbaryl (NAC)	Dymron (Daimuron)	Imazosulfuron	Phoxim	Trifloxysulfuron
Carbetamide	Epoxiconazole	Imidacloprid	Pirimicarb	Triflumizole
Carbofuran	Ethametsulfuron-methyl	Indanofan	Primisulfuron-methyl	Triflumizole Metabolite
Carbosulfan	Ethoxysulfuron	Indoxacarb	Prometryn	Triflurumuron
Carfentrazone-ethyl	Etiozazole	Iodosulfuron-methyl	Propaquizafop	Triflusulfuron-methyl
Carpropamid	Fenamidone	Ioxynil	Propoxycarbazone	Triticonazole
Chlorbufam	Fenhexamid	Iprovalicarb	Prosulfuron	
Chlorfluazuron	Fenoxaprop-ethyl	Isoxaflutole	Pymetrozine	
Chloridazon	Fenoxycarb	Linuron	Pyraclostrobin	



JQA-0376

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. We maintain a global network of sales, service, technical support and applications centers on six continents, and have established long-term relationships with a host of highly trained distributors located in over 100 countries. For information about Shimadzu, and to contact your local office, please visit our Web site at www.shimadzu.com



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URL <http://www.shimadzu.com>

Printed in Japan 3295-04108-30AIT

Remarks and Precautions

1. Shimadzu makes no warranty regarding the accuracy of information included in the database or the usefulness of information obtained from using the database.
2. Be sure to perform tests using standard samples to confirm qualitative and quantitative information obtained with the given system.
3. To reliably identify registered substances using this database, measure samples using the instrument parameters specified in the method template files included in the product.

The contents of this brochure are subject to change without notice.

Shimadzu
Liquid Chromatograph Mass Spectrometer

LCMS-8030/8040/8050
(8045)

Pre-Installation Requirements

 **SHIMADZU CORPORATION**
KYOTO JAPAN
ANALYTICAL & MEASURING INSTRUMENTS DIVISION

Notice

- 1) All rights are reserved, including those to reproduce this manual or parts thereof in any form without permission in writing from Shimadzu Corporation.
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 - 2) Information in this manual is subject to change without notice and does not represent a commitment on the part of the vendor.
 - 3) Any errors or omissions which may have occurred in this manual despite the utmost care taken in its production will be corrected as soon as possible, although not necessarily immediately after detection.
-

1. START HERE

These Pre-Installation Requirements describe prior preparation required at the customer's site in order to facilitate installation of the LCMS-8030/8040/8050.

Please make sure that the content of this document is carefully observed to ensure that the instrument functions stably, providing data acquisition with a high degree of reliability.

To assist in your preparation we have included a Check List on the next page. Every section has a reference to a detailed description of the requirement. If any point is unclear, please contact the Shimadzu representative that provided this document.

When you have completed the checklist, please return a hardcopy or electronic copy to the Shimadzu representative that provided this document. Completion and return of the checklist will generate a walk-through by a Shimadzu representative after which an installation date will be scheduled.

Company Name: _____

City & State: _____

Representative Name: _____

I certify that all preparations on the Pre-Install Check List are complete and our lab is ready for installation.

Representative Signature: _____

Representative Phone: _____

2. Pre-Installation Check List

Topic	Item	Requirement	Reference Section	Completed
Environment	Temperature	65 to 82F - See Important heat load Information	3.5	☐
	Humidity	20 to 70 % No condensation and discharge caused by static electricity	3.7	☐
	Bench Area	8' x 30" plus table for computer monitor	3.4	☐
	Bench Strength	1000 pound capacity	3.3	☐
	Wall	A minimum of 2 feet clearance behind the Mass Spec bench is required for the rotary pump and for maintenance.	3.2	☐
Power	Voltage	190-210V OR 218-241V "Single Phase" - See notes	4.1	☐
	Amperage	30 Amp	4.1	☐
	Receptacle	NEMA - L6-30R - ALSO Supply Plug L6-30P	4.1	☐
	Power Quality	UPS is STRONGLY RECOMMENDED	4.2	☐
	Nitrogen Generator	If an optional N2 Generator will be used see...	4.1 & 5.3	☐
Gas	Nitrogen	20 Liters/Min supplied by a Nitrogen Generator or Liquid Nitrogen Dewar - Cylinder not Recommended	5.1	☐
		99.5% Purity at 100 - 115 PSI		☐
		Regulator - If a Dewar is used, a dual stage regulator with a CGA-580 input and 1/4" Swagelok output is required		☐
	Argon	99.99% Purity at 90 - 115 PSI	5.1	☐
		Cylinder is recommended		☐
		Regulator - A dual stage regulator with a CGA-580 input and 1/4" Swagelok output is required		☐
	Air (LCMS-8050 ONLY)	20 Liters/Min supplied by a Generator - Cylinder not Recommended	5.1	☐
		Dry Air - Water-free and Oil-free at 100 - 115 PSI		☐
		Regulator - If a cylinder is used, a dual stage regulator with a CGA-580 input and 1/4" Swagelok output is required		☐
Vent	4" Exhaust Pipe X2	Two separate 4" (or larger) diameter pipes with a flow of 200 CFM (Cubic Feet per Minute) of flow to the outdoors is required.	5.6	☐
Drain	Solvent Waste	A 4 Liter chemically resistant waste bottle is required if not purchased from Shimadzu.	5.6	☐
	Water Waste	If using an optional Nitrogen Generator a 4 Liter waste container will be required for condensed water.	6.2	☐
IQOQ	Calibrated Balance	If an IQOQ document (optional) has been ordered, a 4 place calibrated balance will be needed for the required tests.	7	☐

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5.3. Using a Nitrogen Gas Generator (optional)

The optional Nitrogen gas generator is designed to use 1/4" O.D. tubing. The generator can work properly on a length of 1/4" O.D. tubing that is up to 33' long. If you need to place the Nitrogen gas generator further away than 33' you will need to run 3/8" O.D tubing. Please be sure to convert back to 1/4" O.D at the instrument.

If there is already a Nitrogen generator present on site, or one will be purchased from another vendor, please verify that it can supply a minimum of 20 Liters per minute of Nitrogen. Also ensure that it is supplied with 1/4" O.D. tubing.

Noise Level 54 dB(A) @ 1m

5.4. Supplying Nitrogen Gas from Centralized System

If the Nitrogen will be supplied by a centralized house system make sure that tubing of a sufficient diameter is used to produce the required pressure and flow. Generally 1/4" O.D. tubing is sufficient for runs up to 33' long. If the gas supply is further away than 33' you will need to run 3/8" O.D. tubing. Please be sure to convert back to 1/4" O.D. at the instrument.

5.5. Using a Gas Cylinder

When using and storing high pressure gas cylinders, a restraining device (either wall or floor) is required. Please make sure these arrive with or before the gas cylinder.

Operating time for Argon: $(7,000 \text{ (L)}) / (0.010 \text{ (L/min)}) / 60 \text{ (min)} = \text{approx. 486 (days)}$

Precautions When Using High Pressure Gas Cylinders

- Ideally gas cylinders should be placed outdoors, in a well-ventilated location out of direct sunlight. Deliver the gas indoors via gas pipes.
- Do not allow the gas cylinder to reach or exceed a temperature of 105 °F. Do not allow any open flame to come within 6 feet of the cylinder.
- **Secure cylinders with chain or by other means to prevent them from tipping or falling over.**
- Use a valve and regulator designated as oil-less.

6. Vent

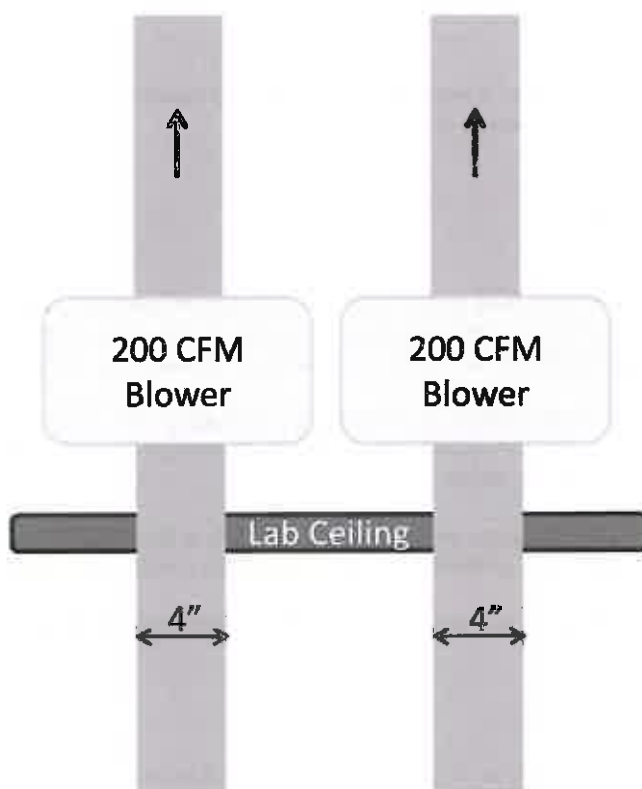
6.1. Exhaust System

To keep the room clean, the exhaust gases from the rotary pump and the vapors from solvents used for mobile phases must be vented from the room.

Two separate 4" (or larger) pipes with a flow of 200 CFM (Cubic Feet per Minute) of flow to the outdoors is required. – See diagram for example.

The system comes with 15 feet of exhaust hoses for venting the rotary pump and the drain containers. Please locate the vent pipe close enough for these lines to reach from the LCMS to the vent.

Exhaust System Example



The diagram above is offered for a basic exhaust system example ONLY. It is incumbent upon the purchaser to insure that all safety, environmental and other regulations are met.

6.2. Ventilation and Open Flame Precautions

Some solvents used in liquid chromatograph mass spectrometers are flammable and poisonous. Also, large quantities of nitrogen gas are used on this instrument. Be sure to ventilate inside the installation room.

Open flames inside the installation room are forbidden, in particular, when using flammable solvents. Also, avoid installing other instruments that are likely to emit open flames inside the same installation room.

7. Drain

7.1. Solvent Waste

A canister is required for solvent waste. If an optional waste container was not purchased from Shimadzu, a 4 Liter bottle may be used.

7.2. Water Waste

If using a Nitrogen Generator, a waste container will be needed for condensate. Alternatively the water could be routed to a house drain system

8. IQOQ

If you have purchased an IQOQ (Installation Qualification/Operation Qualification) document you will need to have a certified 4 place balance available for the tests required to complete your document. A 4 place balance is a balance capable of measuring .1 mg.

If you do not already have a balance, Shimadzu can supply one. You may wish to use "Shimadzu balance model 321-62900-68 (AUY220 Analytical Balance) or equivalent". Please contact your local Shimadzu office.

Important: Even a new balance **must be calibrated** by a local technician before your IQOQ is started. This service is NOT provided by your Shimadzu installation representative.

Please remember to review and approve your IQOQ document BEFORE the installation begins.

9. Lab Supplies

It is strongly recommended that you purchase lab supplies including three 600ml beakers (for performing maintenance) and an ultrasonic cleaner large enough to accommodate them.

10. Questions

If you have any questions about this document or any of the requirements, please contact the person that supplied you with this document.

Determined to Deliver Quality Service

Shimadzu Service Agreement Programs



At Shimadzu Scientific Instruments, our goal is to not only find the best solutions for research, development and applications to meet your specific disciplinary needs, but to ensure the products you purchase remain in optimum working condition. Shimadzu's premium levels of service are your best value. We now offer five levels of service coverage customized for your laboratory's needs.

	Premium Total Coverage	Extended Warranty Plus	Premium PM	PM	Extended Warranty
Service performed by certified Shimadzu Service Engineer	✓	✓	✓	✓	✓
Unlimited Hardware phone support	✓	✓	✓	✓	✓
Unlimited Software phone support	✓	✓	✓	✓	✓
Response time prioritized over billable call	✓	✓	✓	✓	✓
On-demand service visit	✓	✓			✓
On-site service includes labor, travel, parts	✓	✓	(for PM visit)	(for PM visit)	✓
Includes annual PM service visit	✓	✓	✓	✓	
Includes consumable parts needed during PM service visit	✓	✓	✓		
Includes Turbo Pump coverage for MS	✓	✓			
Scheduled minor software & firmware upgrades	✓	✓			
Includes consumable parts used on service visit outside of PM	✓				
Rebate provided	✓				
Age premium is waived	✓				

Service Agreement Option Descriptions

PM (Preventative Maintenance)

Preventative Maintenance is the most basic maintenance option using only the most integral consumable parts needed to bring the system back to a functioning unit. This may include necessary cleaning, adjustments, verification, lubrication, consumable parts replacement and testing to return the instrument to a functional unit.

Premium PM

Premium Preventative Maintenance is a higher-level PM than the previously listed. The Premium PM contains all the standard consumables found in the PM; however, it can contain additional consumable parts or testing deemed necessary to bring the system back to the manufacturer's operational specification.

Extended Warranty

Extended Warranty is a repair option. This coverage level DOES NOT include the replacement of any consumable parts. It is intended for the use of repair/replacement of any mechanical, electrical or electromechanical parts not due to customer misuse. All time and materials (excluding consumables) will be covered.

Extended Warranty Plus

Extended Warranty Plus coverage includes both the Premium PM and Extended Warranty. Customers will receive one (1) Premium PM per calendar year as well as the replacement of any mechanical, electrical or electromechanical parts due to hardware failure.

Premium Total Coverage

Premium Total Coverage is 100% full and total coverage of the customer's system. The customer's problems are covered no matter what the issue. Coverage level still guarantees one (1) Premium PM as well as the replacement of any additional (consumables, mechanical, electrical and electromechanical) parts at no additional charge to the customer. If at the end of the year the customer has only used their one Premium PM and two additional service repair visits, customer will be given a REBATE to be used towards the purchase of additional Shimadzu service products.

Below is a representative listing of Premium PM Procedures.

GCMS-2010

- Clean Ion Source Box, Repeller and Ceramics or replace with comparable part.
- Clean Lens stack and Ceramics.
- Clean Pre-Rods.
- Replace Filaments.
- Perform injector AFC leak check per service manual and repair any leaks.
- Replace Injection Port O-ring and Glass Insert.
- Replace Injection Port Septum.
- Replace carrier gas filters.
- Replace Column Ferrules if column has been removed.
- Perform Vacuum Leak Check per SOP. Repair any vacuum leaks due to consumable parts.
- Change Oil for Rotary Vacuum Pump.
- Check Run Time for Turbo Pump.
- Reset Consumable counters in the software.
- Check helium purifier: check the installation date and how many tanks have been used since the purifier was installed; if more than four tanks were used, Replace.
- Check response for Standard Sample per SOP.

LCMS-2020

- Replace Oil in Rough Pump
- Refurbish ESI Probe
- Clean Spray Chamber
- Replace DL pipe Assy.
- Inspect and clean Oil Mist Filter
- Refurbish APCI Probe
- Inspect and replace damaged O-rings
- Inspect oil mist filter, replace oil element
- Remove and clean Orifice
- Remove and clean Q-Array
- Remove and clean Skimmer
- Remove and clean Octapole assembly
- Adjust RF power supplies
- Adjust rough pump ballast
- Autotune and calibration
- Evaluate performance with standard sample

MALDI/AXIMA

- Lubrication of Stage lead screws
- Clean and rebuild Lens Assy
- Cleaning of Delay Plate
- Rough Pump oil change
- Activated Aluminum replacement
- Silica Gel exchange
- Inline filter replacement
- Camera Lamp replacement
- Replace 10Meg resistor (if applicable)
- Cooling fan inspection, lower unit
- Cooling fan inspection, Transient Recorder
- Cleaning and duct removal
- Tuning and calibration
- Performance evaluation with standard sample

Nexera UHPLC LC-30AD

- Clean pump heads
- Replace plunger seals
- Replace rinse diaphragms
- Inspect/replace plungers
- Replace line filter
- Replace suction filters
- Replace inlet and outlet check valves
- Inspect/replace drain valve assembly
- Lubricate oil pads, cams and cam followers
- Remove dust from fan and electronics
- Verify stored function parameters and ROM version
- Perform flow rate check

TOC-V & L Series

- Replace TC Combustion Tube and Catalyst
- Replace all O-Rings on TC and IC Injection Assemblies and Test
- Replace Syringe
- Inspect Syringe Drive Assembly
- Replace Eight Port Valve
- Replace Halogen Scrubber
- Replace TC and IC injection tubing
- Inspect all Tubing
- Inspect and Clean all Glassware
- Inspect and Clean Humidifier and Drain Vessel
- Replace IC Reaction Pump Head
- Check Flow Rate and Pressure
- Check Baseline Position
- Replace Dilution Water, Acid
- Perform Calibration Curve with Standards



GCMS Vacuum System Covered
(Turbo & Roughing Pump)

**Built-In Discount on Parts & Labor vs.
Time & Material Billable Calls**

**System Certification Can Be
Purchased with PPM**

**Priority Service for Service
Agreement Customers**

Travel Charge: Flat rate on maintenance plans.

Time & Material PM Travel is actual travel billed at \$280/hr. from the nearest service center.

Our commitment is to provide you with the **best** instrumentation and **highest quality service** in the industry. In line with that commitment, your instrumentation will be serviced by **certified factory-trained** Shimadzu employees utilizing N.I.S.T. certified test equipment where applicable.

Purchasing a Shimadzu Service Agreement will ensure you are receiving the best service for your Shimadzu instrumentation at the best price. For more information or to request a quote, please contact your regional office.

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Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

ASMS 2016 TP 223

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Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Introduction

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food. National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRL's) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL

enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This paper describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (typically 3 MRM transitions for each targeted pesticide resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Materials and Methods

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of

LCMS quality purchased from Sigma-Aldrich. A six point calibration curve from 0.002 – 0.1 mg/kg (2 – 100 pg/μL) were generated using an internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Table 1. LC and MS/MS acquisition parameters used to create the generic LC-MS/MS method.

Liquid chromatography																	
UHPLC	: Nexera LC system																
Analytical column	: Restek Raptor biphenyl (100 x 2.1 mm, 2.7 μ m)																
Column temperature	: 35°C																
Flow rate	: 0.4mL/minute																
Solvent A	: 2 mmol/L ammonium formate in 0.002% formic acid solution																
Solvent B	: 2 mmol/L ammonium formate in 0.002% formic acid solution																
Binary Gradient	: <table border="1"> <thead> <tr> <th>Time (mins)</th><th>%B</th></tr> </thead> <tbody> <tr><td>0</td><td>3</td></tr> <tr><td>1</td><td>10</td></tr> <tr><td>3</td><td>55</td></tr> <tr><td>10.5</td><td>100</td></tr> <tr><td>12</td><td>100</td></tr> <tr><td>12.01</td><td>3</td></tr> <tr><td>15</td><td>Stop</td></tr> </tbody> </table>	Time (mins)	%B	0	3	1	10	3	55	10.5	100	12	100	12.01	3	15	Stop
Time (mins)	%B																
0	3																
1	10																
3	55																
10.5	100																
12	100																
12.01	3																
15	Stop																
Injection volume	: 2 μ L sample (plus 40 μ L water)																
Mass spectrometry																	
LC/MS/MS	: LCMS-8060																
Ionisation mode	: Heated electrospray																
Polarity switching time	: 5 msec																
Pause time	: 1 msec																
Total MRM transitions	: 1919 (1819 positive; 100 negative)																
MRM Dwell	: 4msec (target ion); 1msec (reference ion)																
Interface temperature	: 350°C																
Heating block	: 300°C																
Desolvation line	: 150°C																
Heating gas	: 10 L/min																
Drying gas	: 10 L/min																
Nebulising gas	: 3 L/min																

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Results

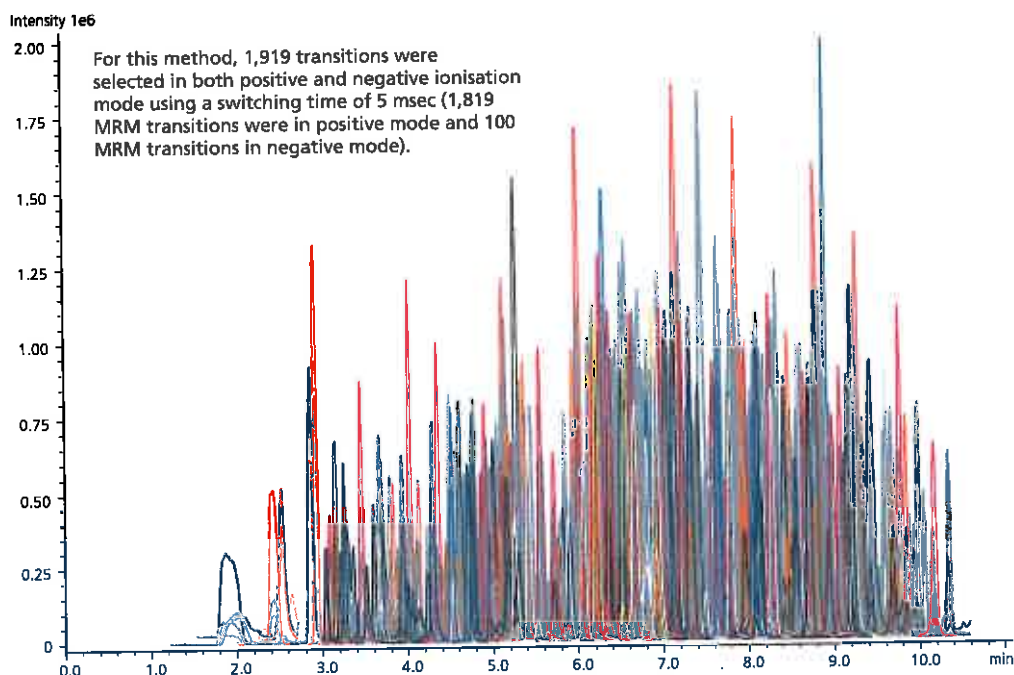


Figure 1. Chromatogram of 646 pesticides spiked into a mint extract at 0.010 mg/kg.
(Up to 3 MRMs per compound and 5 msec polarity switching time).

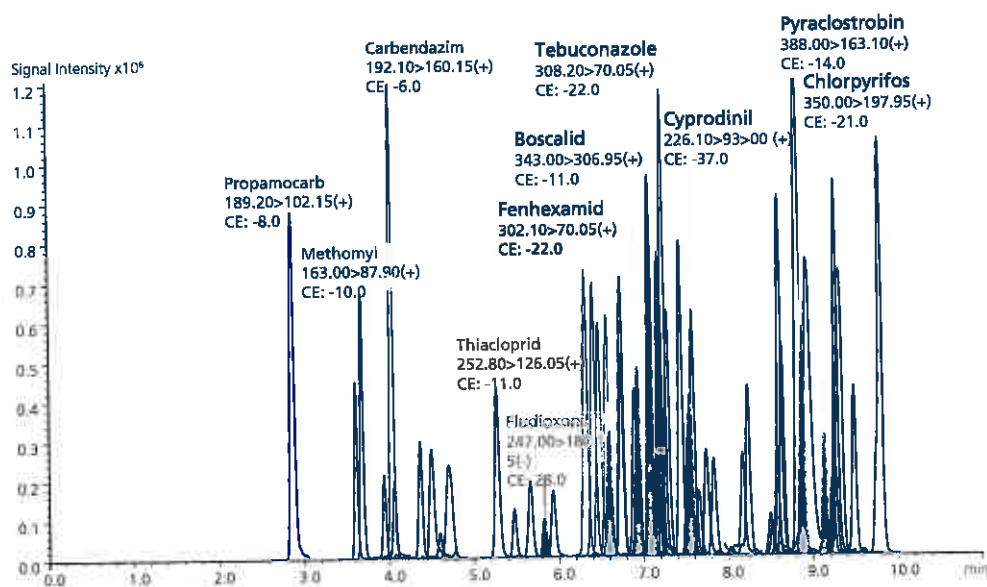


Figure 2. MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyrifos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4% of the samples analyzed. The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

High data density acquisitions

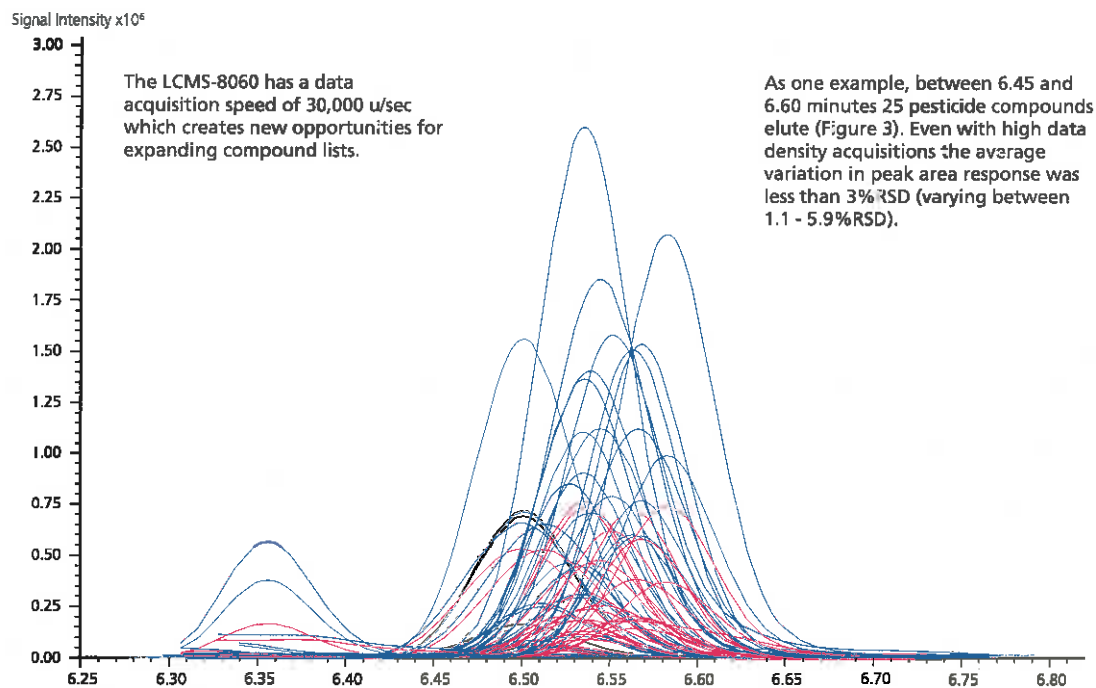


Figure 3. The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Table 2. The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density.
Between 6.45 and 6.60 minutes 25 compounds were monitored with a peak area variation less than 6% (%RSD; n=6).
(The sample is a mint matrix extract at the reporting limit of 0.01 mg/kg).

Compound Name Identifiers	CAS number	Formula	M	Polarity	MRM	RT (mins)	%RSD (n=6)
					Quantitation Ion		
Trinexapac-ethyl	95266-40-3	C ₁₃ H ₁₆ O ₅	252.0998	+	252.90>69.05	6.45	3.1
Iprovalicarb	140923-17-7	C ₁₈ H ₂₈ N ₂ O ₃	320.2100	+	321.20>119.15	6.46	2.8
Dodemorph	1593-77-7	C ₁₈ H ₃₅ NO	281.2719	+	282.30>116.15	6.47	4.2
Fluopyram	658066-35-4	C ₁₆ H ₁₁ ClF ₆ N ₂ O	396.0464	+	397.00>145.00	6.47	1.9
Flutolanil	66332-96-5	C ₁₇ H ₁₆ F ₃ NO ₂	323.1133	+	324.10>242.00	6.48	2.7
Trifloxysulfuron	145099-21-4	C ₁₄ H ₁₄ F ₃ N ₅ O ₆ S	437.0617	+	438.00>182.15	6.48	2.5
Azaconazole	60207-31-0	C ₁₂ H ₁₁ Cl ₂ N ₃ O ₂	299.0228	+	300.00>159.00	6.50	2.0
Terbutryn	886-50-0	C ₁₀ H ₁₉ N ₅ S	241.1361	+	242.10>157.95	6.50	3.4
Prometryn	7287-19-6	C ₁₀ H ₁₉ N ₅ S	241.1361	+	242.10>158.00	6.50	2.6
Azimsulfuron	120162-55-2	C ₁₃ H ₁₆ N ₁₀ O ₅ S	424.1026	+	425.10>182.10	6.50	1.8
Metominostrobin	133408-50-1	C ₁₆ H ₁₆ N ₂ O ₃	284.1161	+	285.10>193.95	6.51	1.7
Thiufuzamide	130000-40-7	C ₁₃ H ₆ Br ₂ F ₆ N ₂ O ₂ S	525.8421	+	528.60>148.05	6.51	5.9
Nicarbazin	330-95-0	C ₁₃ H ₁₀ N ₄ O ₅	302.0651	-	301.10>137.15	6.52	2.6
Bromobutide	74712-19-9	C ₁₅ H ₂₂ BrNO	311.0885	+	312.10>194.10	6.53	2.1
Saflufenacil	372137-35-4	C ₁₇ H ₁₇ ClF ₄ N ₄ O ₅ S	500.0544	+	501.00>198.00	6.53	2.3
Cyproconazole	94361-06-5	C ₁₅ H ₁₈ ClN ₃ O	291.1138	+	292.10>70.05	6.54	1.7
Clomazone	81777-89-1	C ₁₂ H ₁₄ ClNO ₂	239.0713	+	239.90>125.00	6.54	1.7
Fensulfothion	115-90-2	C ₁₁ H ₁₇ O ₄ PS ₂	308.0306	+	309.00>281.00	6.54	1.4
Oxasulfuron	144651-06-9	C ₁₇ H ₁₈ N ₄ O ₆ S	406.0947	+	407.10>150.15	6.54	1.1
Rimsulfuron	122931-48-0	C ₁₄ H ₁₇ N ₅ O ₇ S ₂	431.0569	+	432.00>182.00	6.55	1.8
Fenthion-oxon	6552-12-1	C ₁₀ H ₁₅ O ₄ PS	262.0429	+	263.10>231.00	6.55	1.4
Nitrothal-isopropyl	10552-74-6	C ₁₄ H ₁₆ NO ₆ Na	317.0875	+	295.10>230.95	6.56	3.0
Chlorantraniliprole	500008-45-7	C ₁₈ H ₁₄ BrCl ₂ N ₅ O ₂	480.9708	+	483.90>452.90	6.57	2.7
Fipronil-sulfone	120068-36-2	C ₁₂ H ₄ Cl ₂ F ₆ N ₄ O ₂ S	451.9336	-	451.00>414.90	6.57	2.0
Valifenalate	283159-90-0	C ₁₉ H ₂₇ ClN ₂ O ₅	398.1608	+	399.20>155.00	6.59	1.9

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Robustness

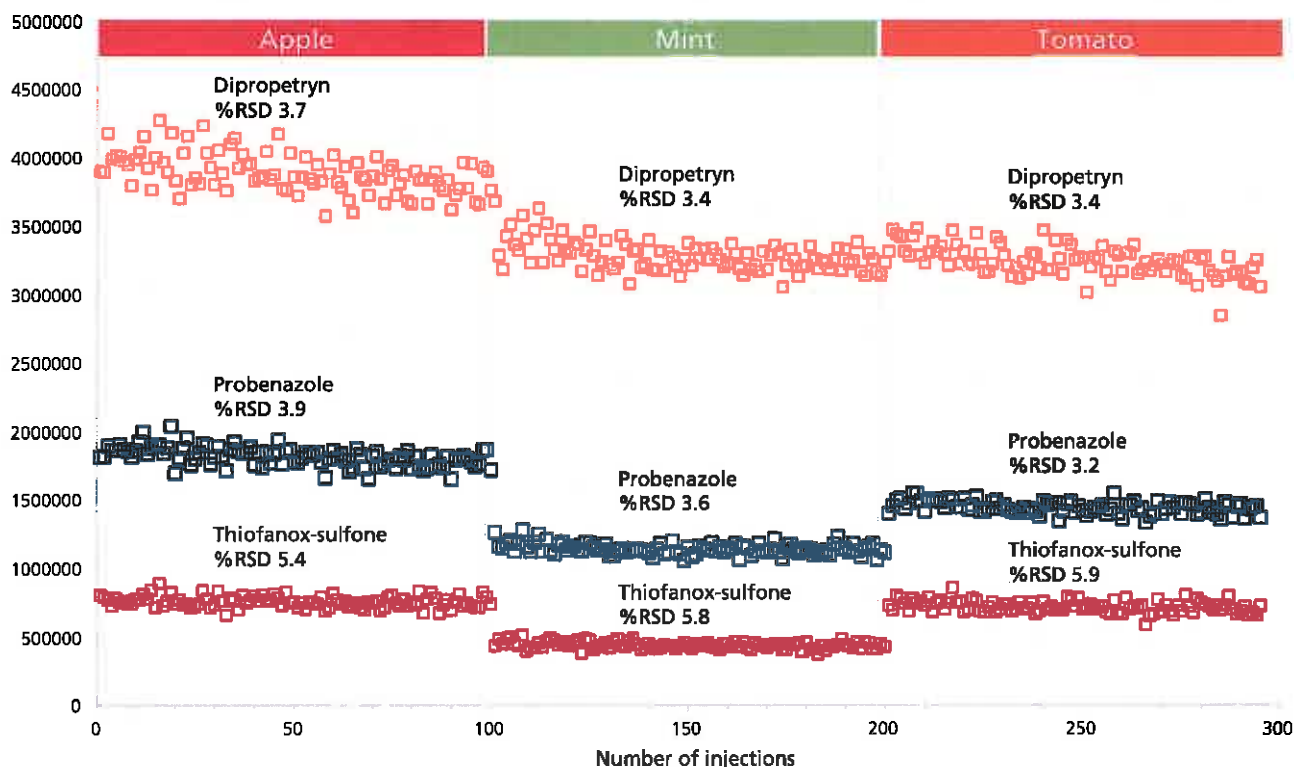


Figure 4. Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Figure 3, compounds were selected to reflect peak area response throughout the chromatographic run (Table 2).

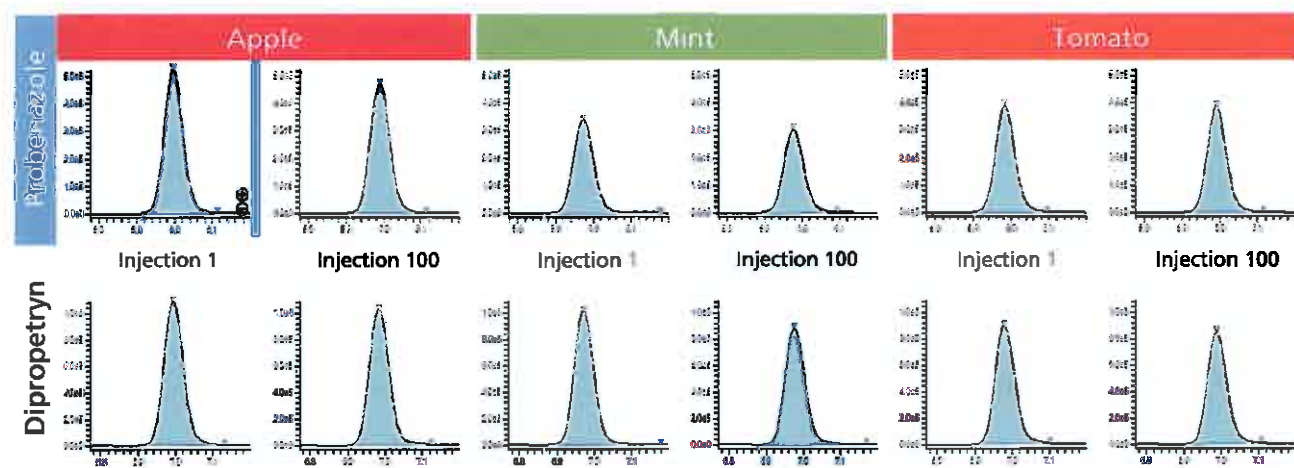


Figure 5. Peak area response for probenazole (RT 5.995 min) and dipropetryn (RT 6.999 min) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Conclusions

- A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.
- As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.
- The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120%.

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List of Publications to Help WVDA Hit the Ground Running

Publication Type	Instrument	Title
Application Note	LCMS-8030	Quantitation of Pesticides in Fruits and Vegetables using a Fast Scanning and MRM-LCMS Method
Application Note	LCMS-8030	Analysis of Pesticides in Foods Using a Triple Quadrupole LC/MS/MS [LCMS-8030]
Application Note	LCMS-8030	Steroids: Testosterone, Progesterone, Estradiol, Ethinylestradiol
Application Note	LCMS-8030	Perfluorocarbons
Application Note	LCMS-8030	Mycotoxins
Application Note	LCMS-8030	Quantitation by HPLC and Identification by LC/MS for Total Aflatoxins in Food
Application Note	LCMS-8050	Analysis of Residual Chloramphenicol in Shrimp by LC/MS/MS with QuEChERS Sample Pre-treatment
Application Note	LCMS-8050	Analysis of Glufosinate, Glyphosate, and AMPA in Drinking Water Using a Triple Quadrupole LC/MS/MS System
Application Note	LCMS-8050	Analysis of Cartap, Pyraclostrobin, and Ferimzone in Drinking Water Using the Triple Quadrupole LCMS-8050
Application Note	LCMS-8050	Simultaneous Analysis of 36 Veterinary Drugs using Triple Quadrupole LC/MS/MS
Application Note	LCMS-8050	Analysis of Diarrhetic Shellfish Toxin Using Triple Quadrupole LC/MS/MS (LCMS-8050)
Application Note	LCMS-8050	Highly Polar Pesticide Multi-Residue Analysis in Food Safety by LC-MS/MS
Application Note	LCMS-8050	Analysis of Nivalenol, Deoxynivalenol, 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol Using Triple Quadrupole LC/MS/MS (LCMS-8050)
Application Note	LCMS-8050	Quantitative Analysis of Pyrethroids in Soil and Sediment Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer
Application Note	LCMS-8050	Quantitative Analysis of Steroid Hormones Using Triple Quadrupole LC/MS/MS
Application Note	LCMS-8050	Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer
Application Note	LCMS-8050 LCMS-8060	Shimadzu Pesticide MRM Library Support for LC/MS/MS
Customer Publication	LCMS-8040	An automatic versatile system integrating solid-phase extraction with ultra-high performance liquid chromatography–tandem mass spectrometry using a dual-dilution strategy for direct analysis of auxins in plant extracts
Poster	LCMS-8030	High Speed Analysis of Mycotoxins
Poster	LCMS-8040	High Throughput LC-MS-MS Analysis of Carbendazim in Orange Juice
Poster	LCMS-8040	Fast LC-MS/MS Screening Method for Antipsychotics with Data Dependent Analysis for Untargeted Analytes
Poster	LCMS-8040	The application of LC-UFMSMS to the analysis of pesticide residues in negative ion ESI
Poster	LCMS-8050	LCMS-MS Method for Evaluation of PPCPs in Environmental Water
Poster	LCMS-8050	Development of MRM-based detection of the plant metabolome using liquid chromatography/tandem mass spectrometry
Poster	LCMS-8050	High sensitivity analysis of pesticides in dried hops cones and hops pellets by liquid chromatography-triple quadrupole mass spectrometry
Poster	LCMS-8050	Rapid determination of beta-agonist residues in animal tissues using UHPLC-tandem mass spectrometry including mass spectral library searching
Poster	LCMS-8060	Ultra-sensitive and rapid assay of neonicotinoids, fipronil and some metabolites in honey by UHPLC-MS/MS



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Poster	LCMS-8060	Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time
Poster	LCMS-8060	Simultaneous analysis of 23 mycotoxins using ultra-high speed LC-MS/MS with a Pentafluorophenyl column
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Talk	LCMS-8030	High Throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS
Talk	LCMS-8030	Rapid and Highly Sensitive Quantitative Analysis and Screening of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry
Talk	LCMS-8040	Multiresidue pesticide analysis from dried chili powder using LC/MS/MS
Talk	LCMS-8040	Micro Flow UHPLC-MS/MS in Pesticide Analysis of Infant Foods
Talk	LCMS-8040	An LCMS Method for the Detection of Cocoa Butter Substitutes, Replacers, and Equivalents in Commercial Chocolate-like Products
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Talk	LCMS-8040	Rapid simultaneous assay of 23 mycotoxins in a variety of food samples by UHPLC-MS/MS using fast polarity switching
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Talk	LCMS-8040	Rapid development of quantitative method for determination of plant growth regulators and streptomycin in fruits using LC/MS/MS
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Talk	LCMS-8050	A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

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Talk	LCMS-8060	A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples
Technical Report	LCMS-8030	Comprehensive Two-dimensional Liquid Chromatography for Determination of Polyphenols in Red Wines (LCxLC-PDA-MS/MS for polyphenol analysis in red wine)
Technical Report	LCMS-8030	Screening and Individual Quantitative Works of Regulated Substances in Food Using Triple Quadrupole Mass Spectrometer LCMS-8030

Application News

SSI-LCMS-013

Liquid Chromatography Mass Spectrometry

Quantitation of Pesticides in Fruits and Vegetables using a Fast Scanning and MRM-LCMS Method



LCMS-8030



Summary

Using the Shimadzu LCMS-8030 triple quadrupole mass spectrometer, efficient and accurate methods have been developed to identify and quantify trace amounts of 13 pesticides found in fruits and vegetables.

Background

Food safety is a topic of great interest globally. With recent contamination issues in a wide range of commodities, ensuring the quality of our food supply is becoming increasingly important. Recent regulations on food and environmental analysis require the screening for pesticides using confirmatory techniques, including LC/MS/MS.

Method

A series of five different standard samples were analyzed for this application: a. Pesticide mix of 10 standards (acetamiprid, abamectin B1a, carbendazim, chlorantraniliprole, etoxazole, imazalil, novaluron, spinosad A, spirotetramat and thianbendazole) in methanol. b. Abamectin B1a standard in methanol. c. Morpholine standard in methanol. d. Glyphosate standard in water. e. ethephon standard in

methanol. The chemical structures for these pesticides are shown in Figure 1. The real samples used in this application were acetonitrile extracts from green onion, strawberry, lemon and grape.

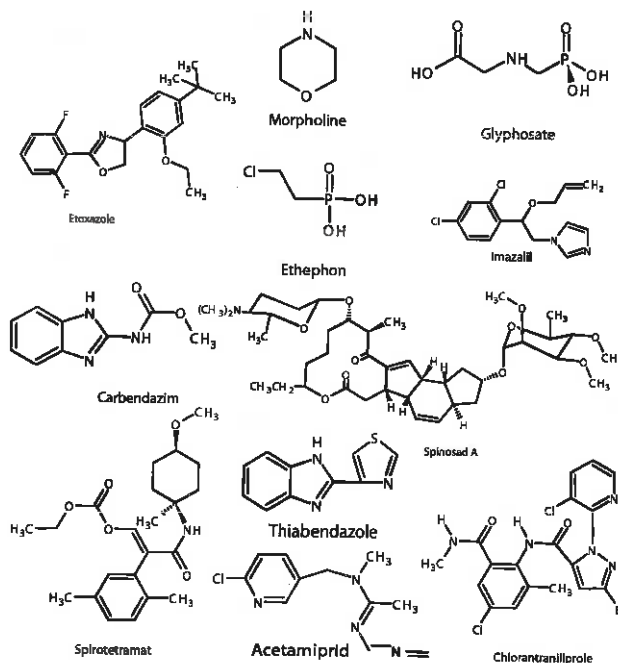


Figure 1: Chemical Structures of 11 pesticides.

Two different LCMS methods were used in this application. The MS conditions for each method are shown in Table 3. The pesticides were run in electrospray (ESI) positive mode in scan, multiple reaction monitoring (MRM), and data dependent product ion modes. The details of the MS parameters for each compound are summarized in Table 4. MRM optimization (collision energy, Q1 and Q3 prerod-bias) was done automatically by the LCMS-8030. An example of MRM optimization is shown in Figure 2.

A Shimadzu Nexera UHPLC and LCMS-8030 mass spectrometer were used for this application. The flow rate was 0.4 mL/min and the oven temperature was 40 degrees C.

Time (min)	% B	Time (min)	% B
0	30	0	0
5	100	2	0
8	100	5	90
8.1	30	7.5	90
10	30	7.6	0
		10	0

Table 1 (Left): Gradient LC method 1.

Table 2 (Right): Gradient LC method 2.

For quantitation of 10 pesticide standards and abamectin B1a standard a Shim-pack ODSII (2.2 mm x 2.0 mm x 150 mm) column was used with a binary gradient consisting of 5 mM ammonium formate in water and 5 mM ammonium formate in 95/5 acetonitrile/water mix. The gradient conditions for LC method 1 are shown in Table 1.

For quantitation of morpholine and ethephon, a Phenomenex Kinetex HILIC (2.6 mm x 2.1 mm x 50 mm) column was used with a binary gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. For quantitation of glyphosate an Agilent Eclipse Plus C18 RRHD (1.8 mm x 2.1 mm x 50 mm) column was used with same gradient conditions as morpholine and ethephon. The gradient conditions for the second LC method are shown in Table 2.

ESI - Positive Mode	
DL Temperature (°C)	250
Heat Block Temperature (°C)	400
Drying Gas Flow Rate (L/min)	15
Nebulizing Flow Rate (L/min)	2
Scan Rate (u/s)	15,000
Scan Range (m/z)	50-900

Table 3: MS conditions for both LC methods.

Type	+/-	MRM transition	Compound name	Dwell Time (msec)	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
MRM	+	88.0 > 70.0	Morpholine	10	-14	-22.5	-11
MRM	+	145.0 > 81.0	Ethephon	10	-14	-22.5	-16
MRM	+	170.0 > 88.0	Glyphosate	10	-11	-12.5	-16
MRM	+	192.2 > 160.0	Carbendazim	10	-13	-20	-15
MRM	+	202.1 > 175.0	Thiabendazole	10	-13	-30	-16
MRM	+	223.2 > 126.0	Acetamiprid	10	-15	-22.5	-25
MRM	+	297.0 > 159.0	Imazalil	10	-14	-22.5	-14
MRM	+	360.1 > 141.0	Etoxazole	10	-17	-30	-28
MRM	+	374.2 > 302.0	Spirotetramat	10	-18	-17.5	-20
MRM	+	484.1 > 285.8	Chlorantraniliprole	10	-17	-16	-18
MRM	+	493.0 > 158.0	Novaluron	10	-25	-19	-30
MRM	+	732.0 > 142.0	Spinosad A	10	-20	-35	-29
MRM	+	895.4 > 449.2	Abamectin B1a	10	-20	-52	-29

Table 4: MRM parameters for 13 pesticide compounds.

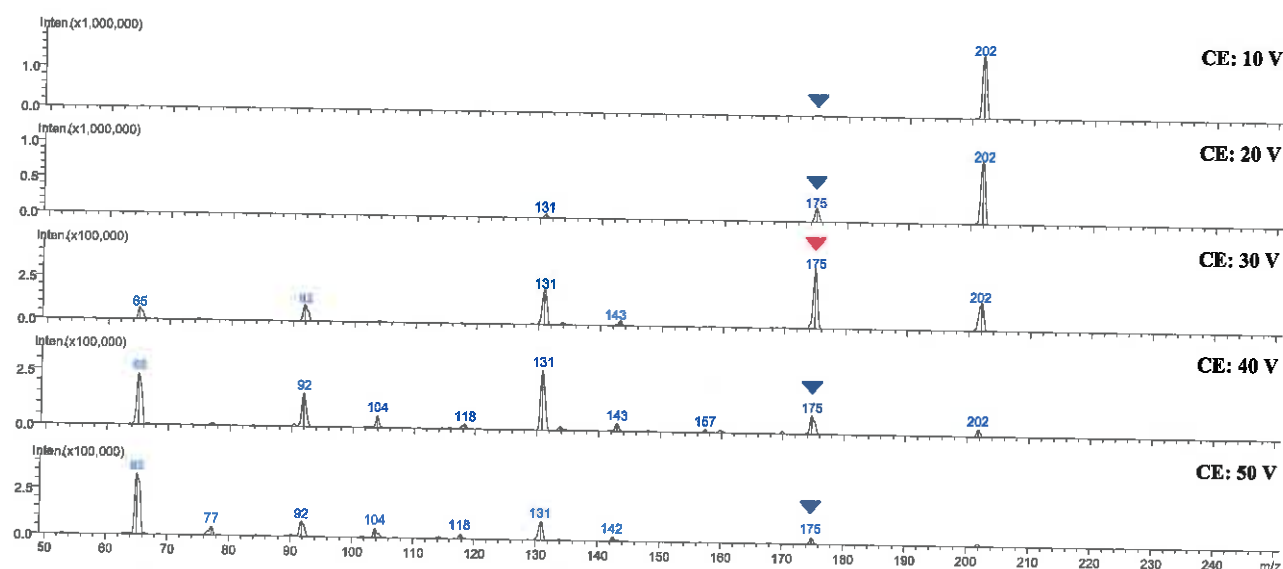


Figure 2: An example of MRM CE auto optimization for transition 202 > 175. Product ion scan spectra with different CE from 10V to 50V are shown. 30V was chosen as the optimized CE.

Results and Discussion

The 10 pesticide standards in the standard mix could be well differentiated and quantified with a single LC/MS/MS method. A different MRM method was applied to quantify morpholine, ethephon and glyphosate. The LC/MS/MS chromatograms for the MRM transitions for the 13 pesticide standards are shown in Figure 3. MRM triggered product ion scans provide compound structure information for the 13 pesticides monitored. Details of the product ion scans are shown in Figure 4.

The limit of quantitation (LOQ) for the 13 pesticide standards was 1 ppb for five of the analytes and 10 ppb for the other 8 analytes. The respective LC/MS/MS chromatograms for the 13 pesticide standards at their LOQs are shown in Figure 5. The linear ranges and correlation coefficients (r) of the quantitation of the 13 pesticide standards is shown in Table 5. The calibration curves for the 13 pesticide standards are shown in Figure 6. Once the calibration curves were determined for each pesticide, the quantitation of the target pesti-

cides in real samples was performed. A summary of the quantitation results of the target pesticide concentrations in green onion, strawberry, lemon and grape samples are shown in Table 6. The LC/MS/MS chromatograms of the quantified target pesticides in the real samples are shown in Figure 7.

Conclusion

An efficient, sensitive and accurate LC/MS/MS method has been developed to quantify 13 different pesticides with m/z from 80-895 in both standard mix and real fruit/vegetable samples. A 10 minute LC gradient was applied to isolate each target compound with only one injection needed to quantify all 10 compounds present in the pesticide standard mix and real samples. The LOQ of the 13 pesticides were between 1 and 10 ppb for all compounds with a wide linear range (4-5 magnitude). The MRM triggered product ion scans could provide direct compound structure information and the concentration of target compounds in the real samples was determined directly by the LCMS software.

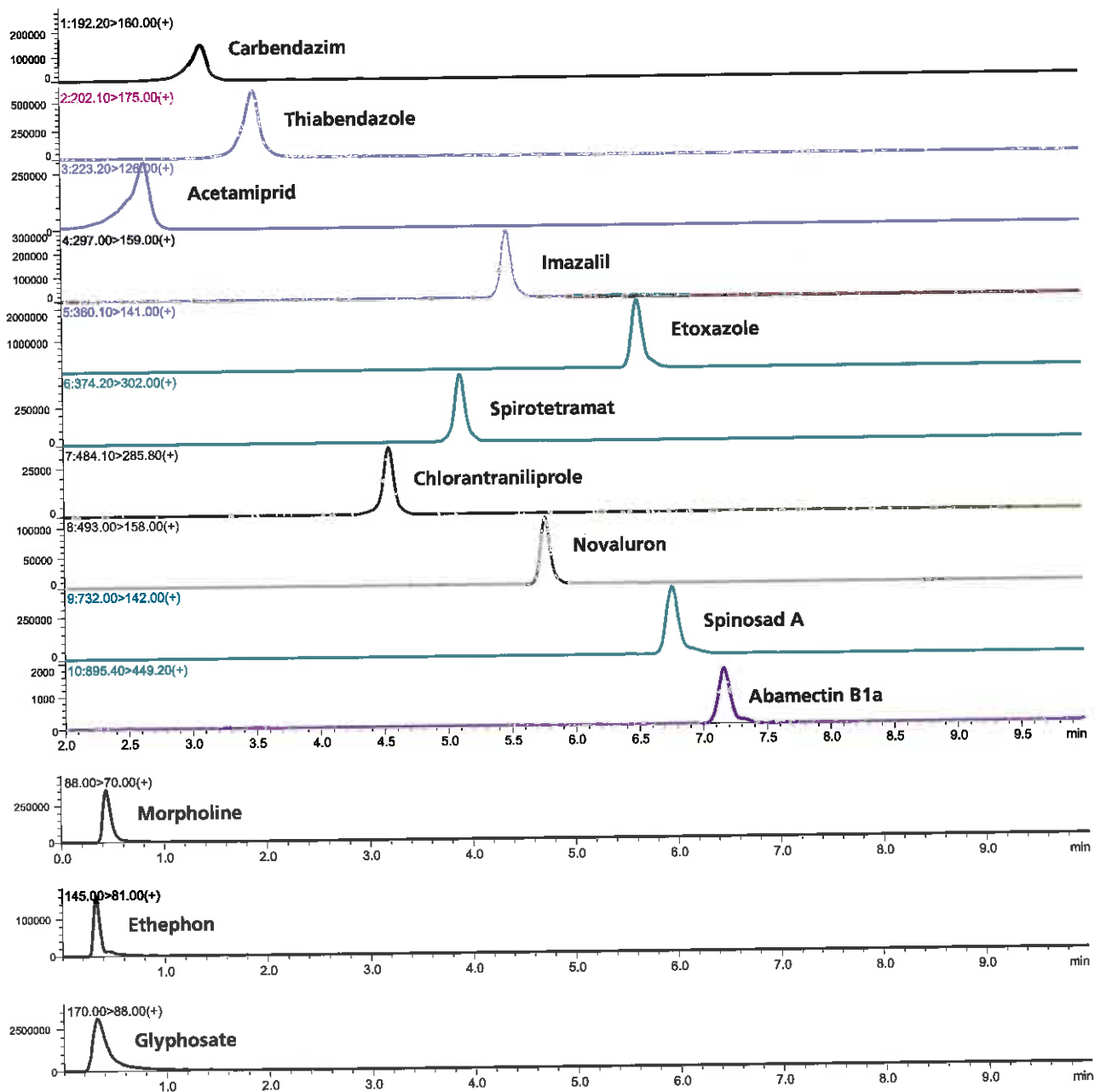


Figure 3: LC/MS/MS chromatogram of the MRM transitions of the 13 pesticide standards.

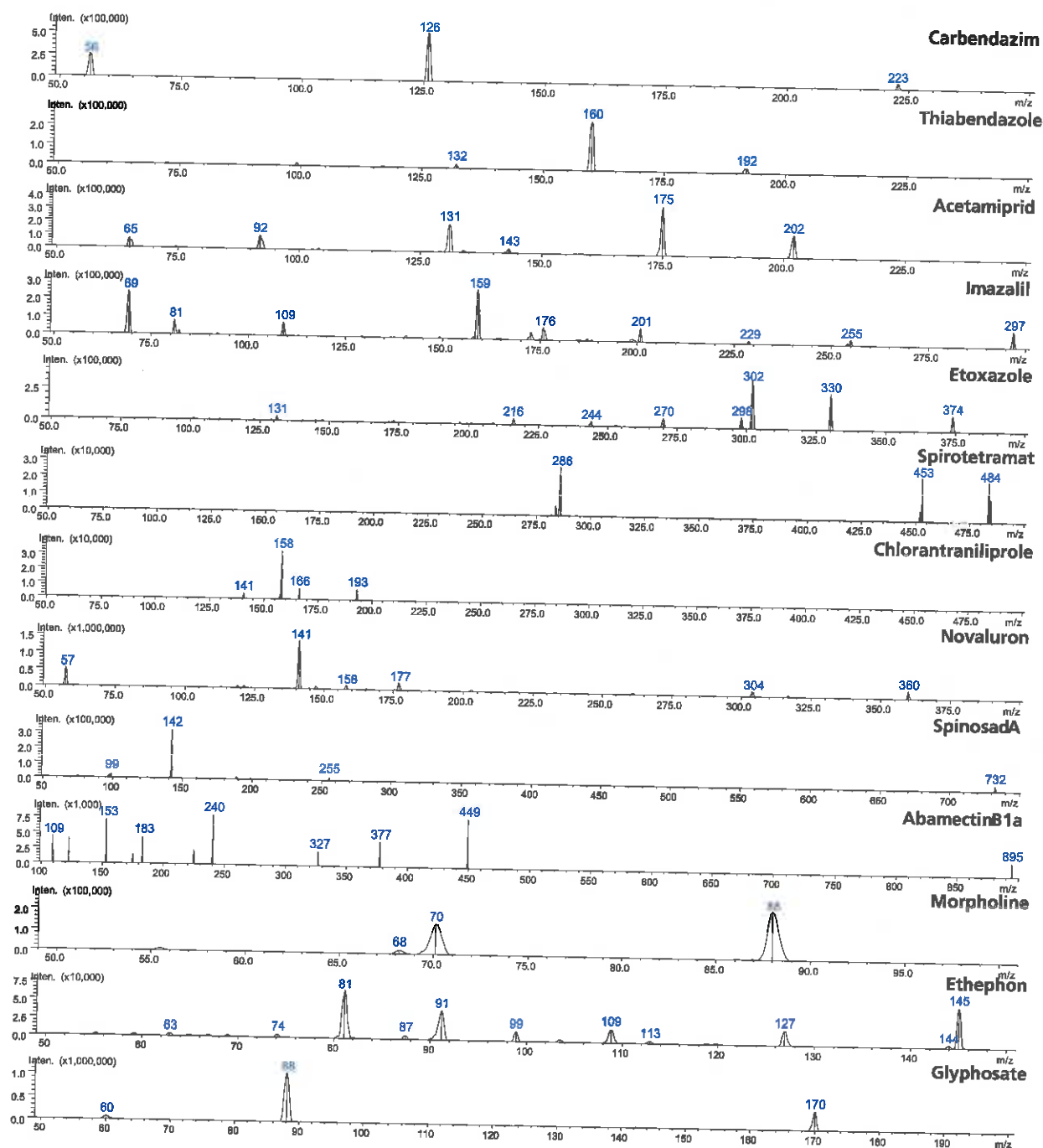


Figure 4: MRM triggered product ion scan spectra of the 13 pesticide standards.

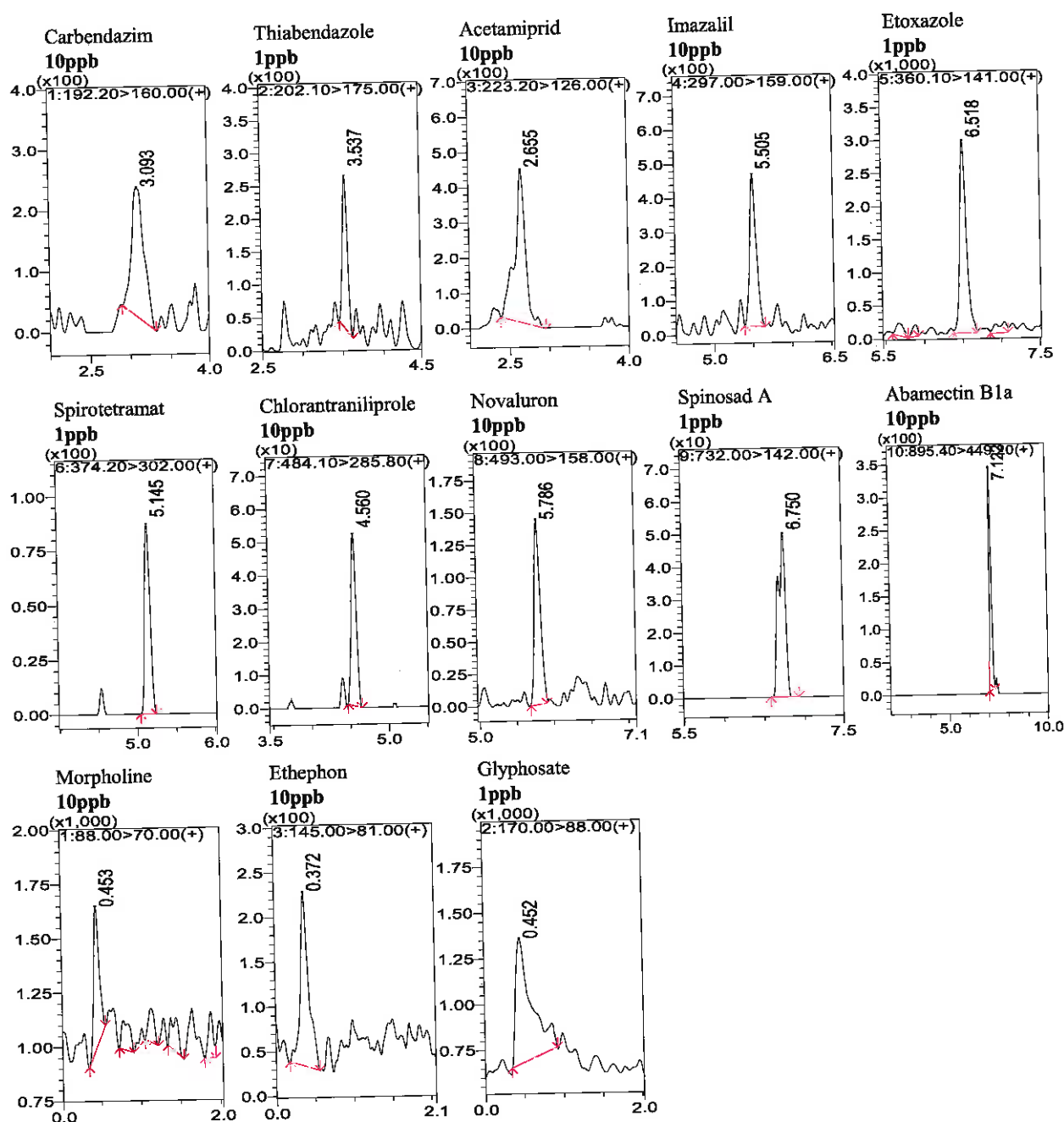


Figure 5: The LC/MS/MS chromatogram of the 13 pesticide standards at their LOQs.

In summary, the Shimadzu 8030 triple quadrupole mass spectrometer coupled with Shimadzu Nexera UHPLC is a powerful system to quantify trace amounts of pesticides in real food samples.

MRM transition	Compoundname	Range(ppb)	r^2
192.2 > 160.0	Carbendazim	10-10,000	0.9999
202.1 > 175.0	Thiabendazole	1-10,000	0.9998
223.2 > 126.0	Acetamiprid	10-10,000	1.0000
297.0 > 159.0	Imazalil	10-10,000	0.9997
360.1 > 141.0	Etoxazole	1-10,000	0.9994
374.2 > 302.0	Spirotetramat	1-10,000	0.9996
484.1 > 285.8	Chlorantraniliprole	10-10,000	1.0000
493.0 > 158.0	Novaluron	10-10,000	1.0000
732.0 > 142.0	Spinosad A	1-10,000	0.9999
895.4 > 449.2	Abamectin B1a	10-1,000	0.9989
88.0 > 70.0	Morpholine	10-10,000	0.9990
145.0 > 81.0	Ethephon	10-10,000	0.9991
170.0 > 88.0	Glyphosate	1-1,000	0.9993

Table 5: Linear ranges and correlation coefficients (r) of the quantitation of 13 pesticide standards.

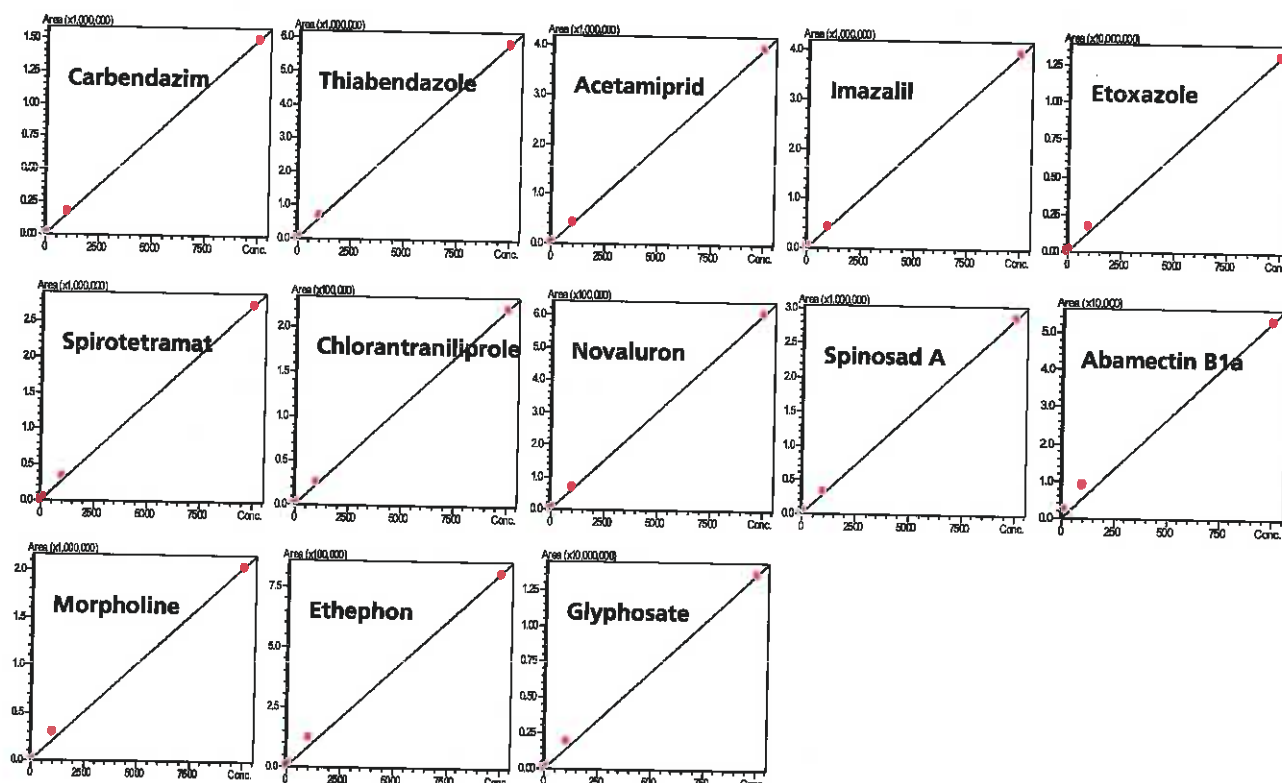


Figure 6: Calibration curves of the 13 pesticide standards

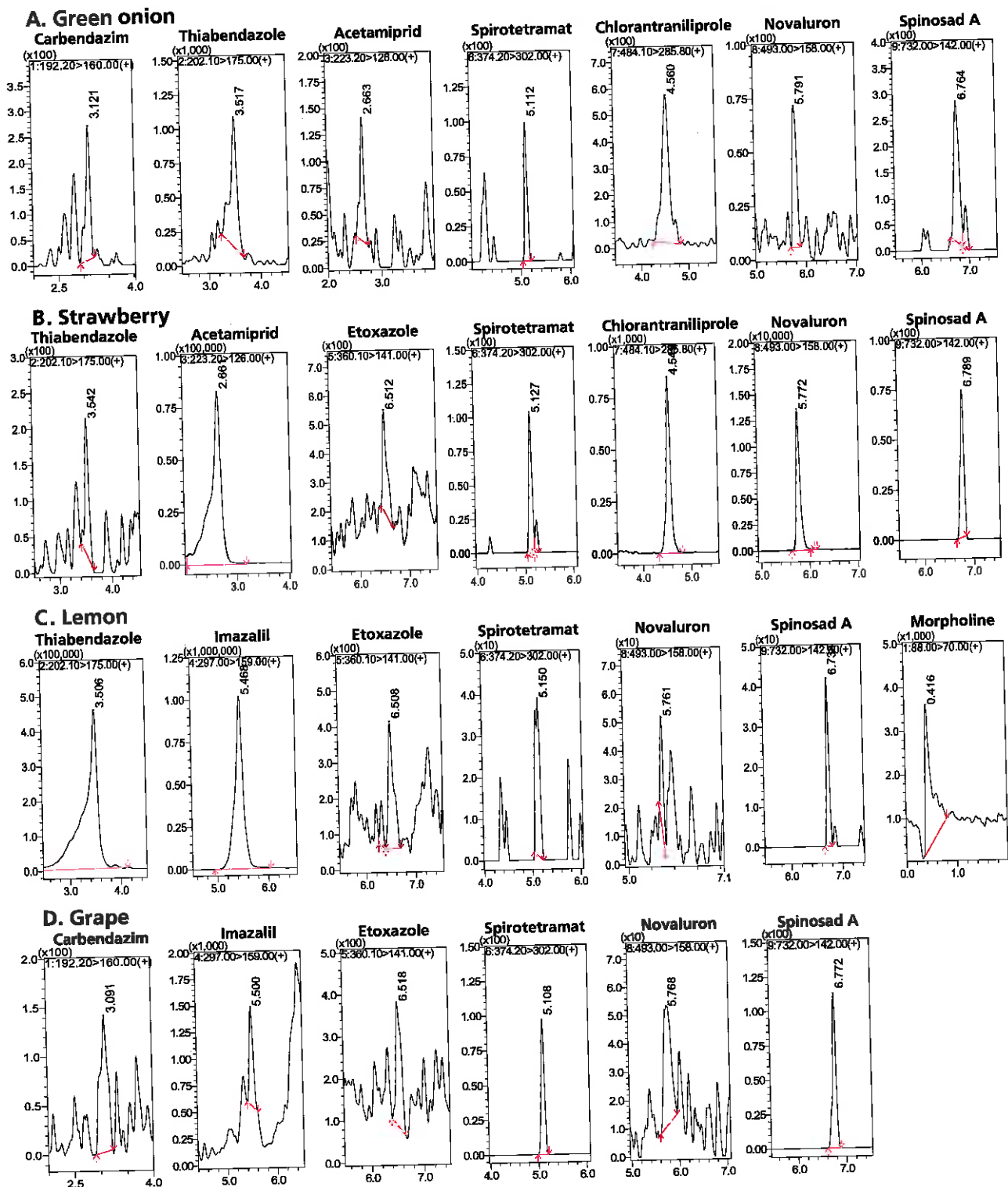


Figure 7: LC/MS/MS chromatograms of the quantified target pesticides in real samples, green onion, strawberry, lemon and orange.

UPLC-MS

ULTRA FAST MASS SPECTROMETRY



LCMS-8040



LCMS-8030



LCMS-8080



LCMS-2020



LCMS-IT-TOF

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Application News

No. C87

Liquid Chromatography Mass Spectrometry

Analysis of Pesticides in Foods Using a Triple Quadrupole LC/MS/MS [LCMS-8030]

Pesticides are widely used in agricultural products to protect crops from insect infestation during cultivation and product transport. With the heightened concern for food safety, "Positive List System" was introduced in Japan on May 29, 2006. These limits ban the sale of food containing pesticides that exceed specified concentrations to ensure the safety of food circulating in the market. Currently, residue standards and analytical methods have been established for about 800

kinds of pesticides and veterinary pharmaceuticals, and this number is expected to increase even further. Therefore, there is increasing need for simpler analytical methods capable of simultaneous multi-residue analysis.

Here, using the LCMS-8030, we optimized methods for the simultaneous multi-residue analysis of 43 pesticides, and report the results of the analysis of actual vegetable extracts (paprika and leek) in solution.

Pesticides Screening Using a Method Package

In LC/MS/MS analysis, the measurement conditions, including the precursor and product ion m/z values, as well as collision energy voltage, etc., must all be set beforehand. As the number of analytes increases, so too does the amount of time and effort required to optimize each analysis condition.

Here, we conducted measurements using a simultaneous analysis method that screens for 167 substances that are included in the "residual pesticide LC/MS/MS method package" for pesticide screening in actual samples. Through the use of the residual

pesticide method package, measurements can be conducted immediately without having to perform routine method such as optimization tasks.

A paprika solution, spiked with 10 $\mu\text{g/L}$ each of imidacloprid, lufenuron, and 8 other pesticides, was used for the analysis. Fig. 1 shows an example of the pesticide screening results using the method package. The MRM results illustrated detection, with excellent sensitivity, of the positive list pesticides in the sample solution at the standard criterion concentration of 10 $\mu\text{g/L}$.

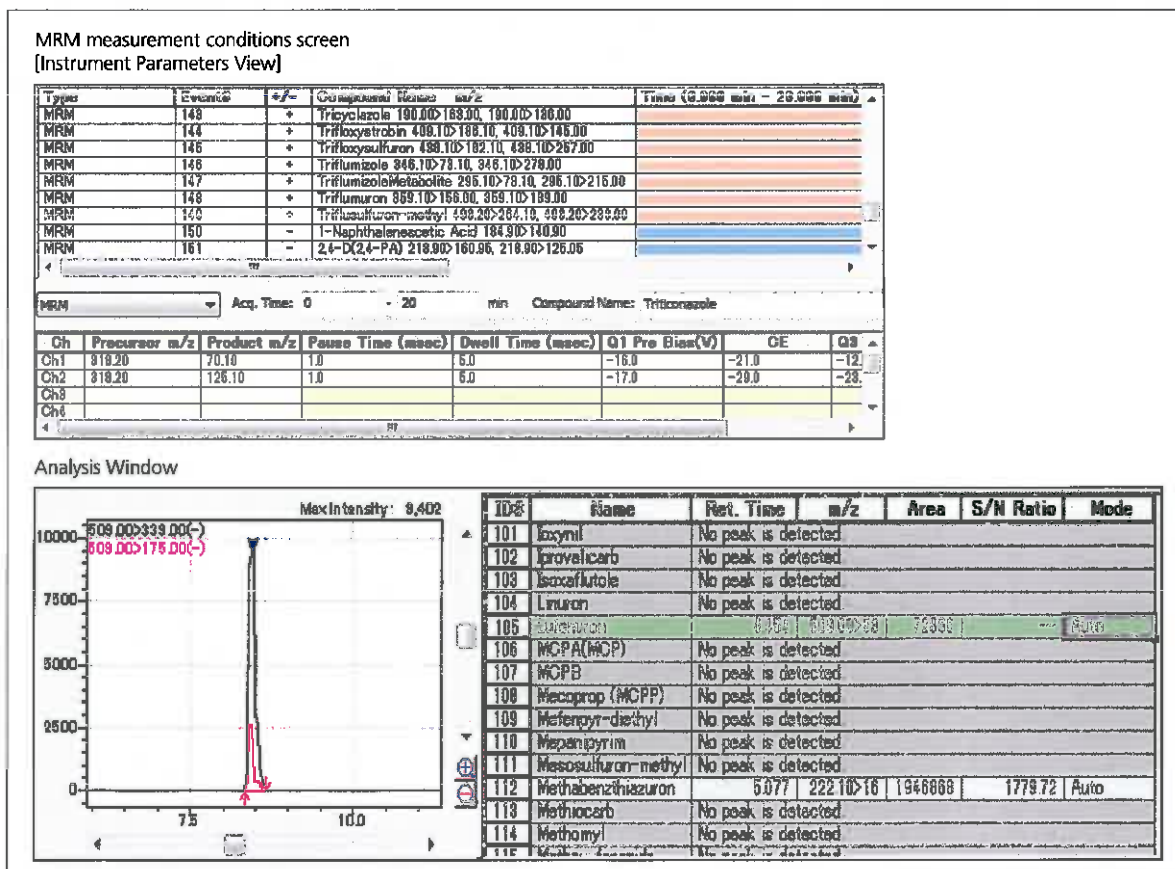


Fig. 1 Screening of Pesticides Using a Method Package

MRM Results of 43 Pesticides

We conducted MRM measurements of a standard solution of 43 pesticides using the MRM conditions incorporated in the method package. Fig. 2 shows the MRM chromatograms of the 43 pesticide substances in the mixed standard solution, and Table 1 shows the

MRM transitions for each pesticide, in addition to the calibration curves.

Excellent linearity was obtained for each substance over the entire concentration range which varied from 0.5 or 1-100 ppb.

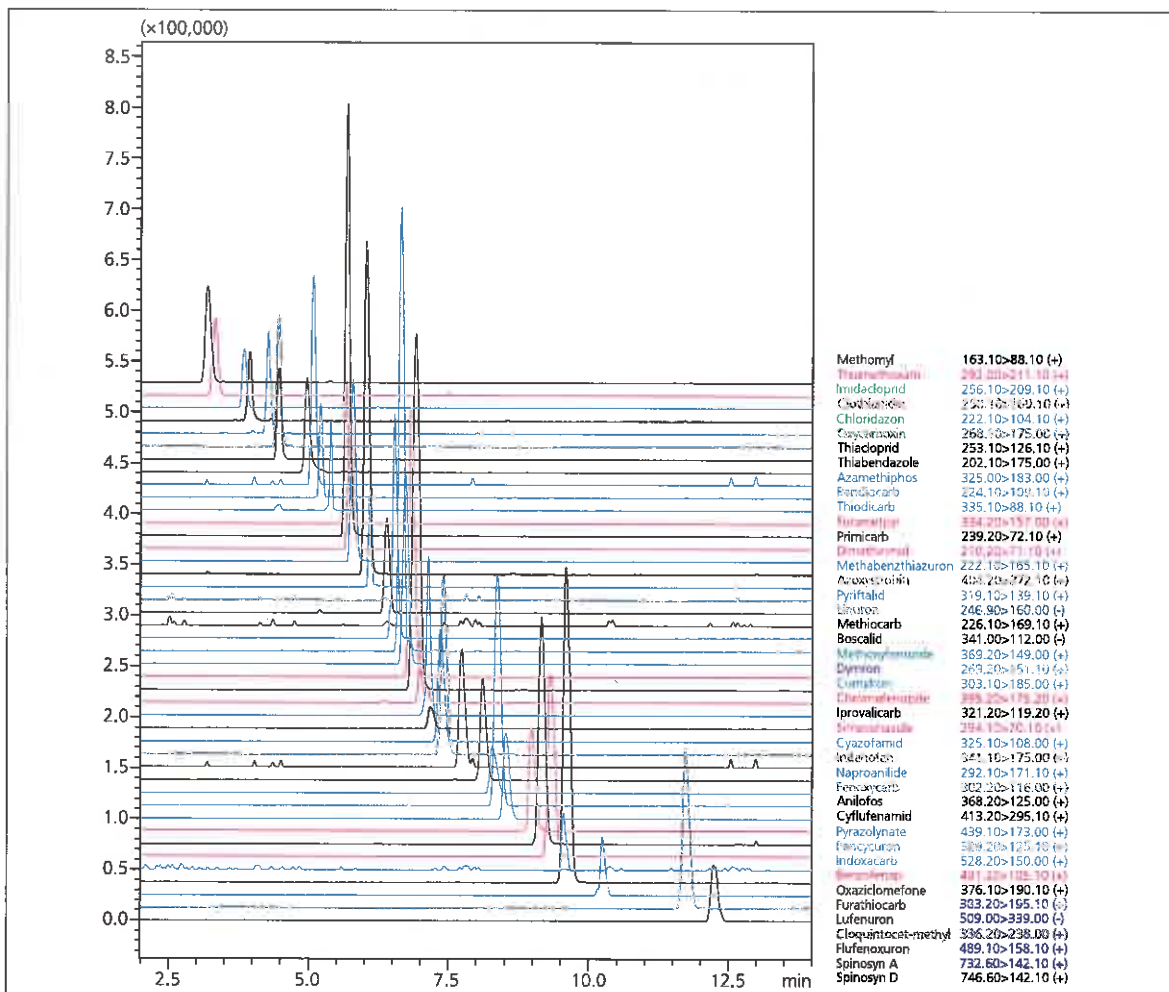


Fig. 2 MRM Chromatograms of 43 Pesticides in the Mixed Standard Solution

Table 1 MRM Transitions and Quantitative Results for 43 Pesticides

Compound	Polarity	Transition	Calibration curve(ppb)	r ²	Compound	Polarity	Transition	Calibration curve(ppb)	r ²
Methomyl	+	163.10>88.10	0.5-100	0.9995	Cumyluron	+	303.10>185.00	0.5-100	0.9999
Thiamethoxam	+	292.00>211.10	0.5-100	0.9998	Chromafenozide	+	395.20>175.20	0.5-100	0.9999
Imidacloprid	+	256.10>209.10	0.5-100	0.9992	Iprovalicarb	+	321.20>119.20	0.5-100	0.9997
Clothianidin	+	250.10>169.10	0.5-100	0.9992	Simeconazole	+	294.10>70.10	0.5-100	0.9991
Chloridazon	+	222.10>104.10	0.5-100	0.9994	Cyazofamid	+	325.10>108.00	0.5-100	0.9999
Oxycarboxin	+	268.10>175.00	0.5-100	0.9999	Indanofan	+	341.10>175.00	1-100	0.9999
Thiacloprid	+	253.10>126.10	0.5-100	0.9997	Naproanilide	+	292.10>171.10	0.5-100	0.9999
Thiabendazole	+	202.10>175.00	0.5-100	0.9990	Fenoxycarb	+	302.20>116.00	0.5-100	0.9999
Azamethiphos	+	325.00>183.00	0.5-100	0.9996	Anilofos	+	368.20>125.00	0.5-100	0.9999
Bendiocarb	+	224.10>109.10	0.5-100	0.9997	Cyflufenamid	+	413.20>295.10	0.5-100	0.9998
Thiodicarb	+	335.10>88.10	0.5-100	0.9999	Pyrazolynate	+	439.10>173.00	0.5-100	0.9999
Furametpyr	+	334.20>157.00	0.5-100	0.9997	Pencycuron	+	329.20>125.10	0.5-100	0.9998
Primicarb	+	239.20>72.10	0.5-100	0.9998	Indoxacarb	+	528.20>150.00	0.5-100	0.9995
Dimethirimol	+	210.20>71.10	0.5-100	0.9976	Benzofenap	+	431.20>105.10	0.5-100	0.9995
Methabenzthiazuron	+	222.10>165.10	0.5-100	0.9994	Oxazidomefone	+	376.10>190.10	0.5-100	0.9999
Azoxystrobin	+	404.20>372.10	0.5-100	0.9997	Furathiocarb	+	383.20>195.10	0.5-100	0.9999
Pyrifthalid	+	319.10>139.10	0.5-100	0.9998	Lufenuron	-	509.00>339.00	1-100	0.9997
Linuron	-	246.90>160.00	1-100	0.9998	Cloquintocet-mexyl	+	336.20>238.00	0.5-100	0.9998
Methiocarb	+	226.10>169.10	0.5-100	0.9999	Flufenoxuron	+	489.10>158.10	0.5-100	0.9992
Boscalid	-	341.00>112.00	0.5-100	0.9998	Spinosyn A	+	732.60>142.10	0.5-100	0.9997
Methoxyfenozide	+	369.20>149.00	0.5-100	0.9992	Spinosyn D	+	746.60>142.10	0.5-100	0.9999
Dymron (Daimuron)	+	269.20>151.10	0.5-100	0.9999					

Pesticide Analysis in Paprika and Leek Samples

MRM measurements were conducted for pesticides in actual sample solutions originating from paprika and leek. The LC/MS/MS analysis was conducted following preparation of each sample according to the method stipulated by Japan's Ministry of Health, Labour and

Welfare Notification, "Multi-residue analysis of pesticides by LC/MS - Method I."

Fig. 3 shows the MRM chromatograms obtained from analysis of the paprika and leek sample solutions.

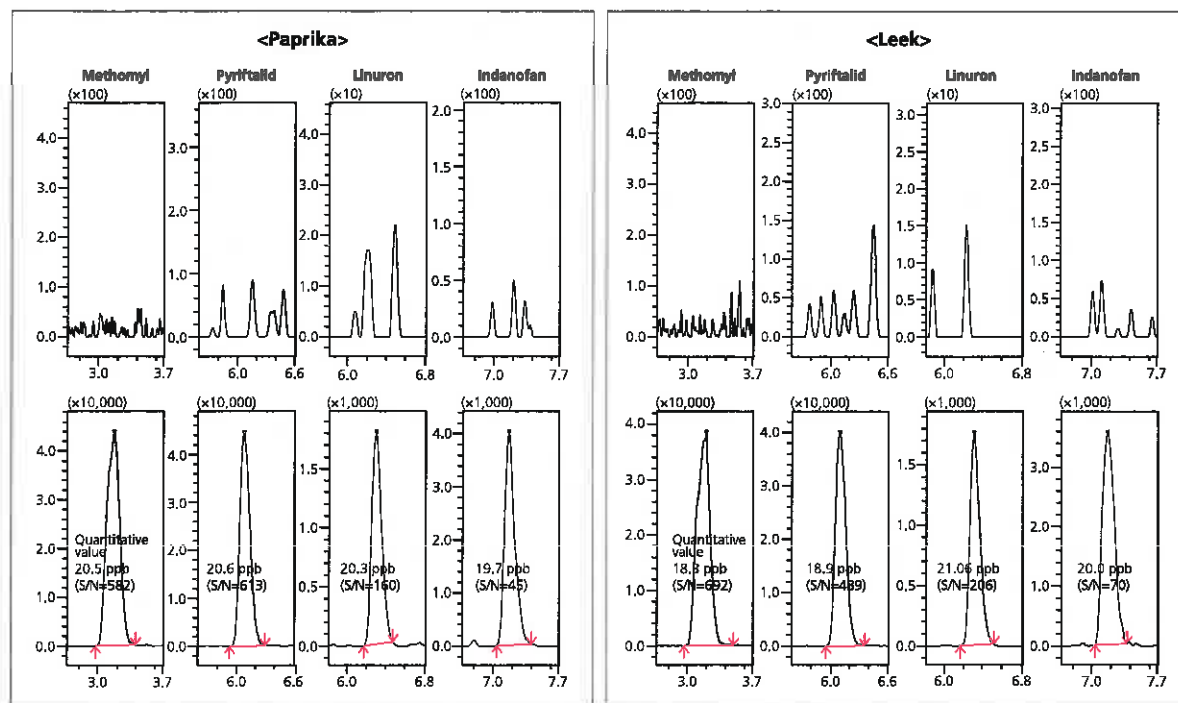


Fig. 3 MRM Results of Samples (Paprika/Leek) (upper: blank, lower: standards 20 ppb spiked)

The recoveries of the pesticides used to spike each sample are shown in Table 2. Good recovery results were obtained for the pesticides in the paprika, ranging from 96-111 %. The leek solution produced good recoveries, ranging from 70-110 % for nearly all of the

pesticides. The lower recoveries might be attributable to an interfering component of the sample matrix and further examination of the method (sample pretreatment, etc.) is warranted with the aim of reducing matrix effects.

Table 2 Recovery of Pesticides (%)

Compound	Paprika	Leek	Compound	Paprika	Leek
Methomyl	100.7	89.5	Cumyluron	104.3	102.9
Thiamethoxam	107.0	75.2	Chromafenozide	100.3	99.9
Imidacloprid	(658.8)	94.7	Iprovalicarb	100.5	95.6
Clothianidin	102.2	107.0	Simeconazole	98.6	92.9
Chloridazon	110.7	93.8	Cyazofamid	103.7	95.7
Oxycarboxin	103.4	64.6	Indanofan	99.7	101.2
Thiactoprid	101.7	57.5	Napronilide	100.8	99.1
Thiabendazole	96.3	53.4	Fenoxycarb	98.7	97.3
Azamethiphos	99.2	76.6	Anilofos	101.7	96.5
Bendiocarb	100.9	73.5	Cyflufenamid	99.5	98.8
Thiodicarb	102.0	84.4	Pyrazolynate	98.8	94.3
Furametpyr	101.7	75.7	Pencycuron	96.6	95.8
Pirimicarb	98.7	74.2	Indoxacarb	102.3	101.6
Dimethirimol	100.8	61.8	Benzofenap	98.5	98.4
Methabenzthiazuron	98.2	75.1	Oxaziclonofene	100.6	96.1
Azoxystrobin	(106.3)	(756.1)	Furathiocarb	102.6	95.7
Pyrifthalid	100.5	92.2	Lufenuron	110.5	107.7
Linuron	99.2	102.7	Cloquintocet-mexyl	101.0	94.8
Methiocarb	106.3	89.0	Flufenoxuron	98.2	85.1
Boscalid	102.6	102.5	Spinosyn A	99.5	86.2
Methoxyfenozide	96.6	100.4	Spinosyn D	96.3	73.8
Dymron (Daimuron)	103.0	99.9			

Note: Recoveries for some items (values in parentheses) were difficult to calculate given that they were also detected in the blank.

■ Comparison of Sensitivity due to Differences in the Ammonium Acetate Concentration in Mobile Phase

Here, the LOQ's for all 43 pesticides measured was 10 µg/L or lower, indicating that they were all within the standard criterion. However, there were cases which required some sort of remedial measure, such as sample dilution to reduce the influence of the matrix and improve sensitivity.

To further increase detection sensitivity, we also evaluated the effect of reducing the ammonium acetate

concentration of the aqueous and methanol mobile phases to 0.1 mmol/L.

Table 3 shows the LOQ of each pesticide measured using a salt concentration of 0.1 mmol/L, and the percent change in detection sensitivity with respect to the 5 mmol/L. It was confirmed that lowering the mobile phase salt concentration increased the S/N for almost all of the pesticides.

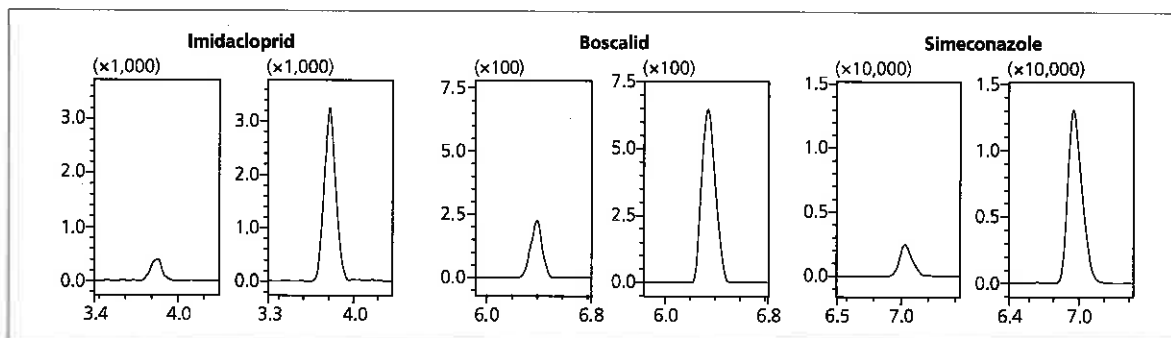


Fig. 4 Comparison of MRM Chromatograms (left: 5 mmol/L, right: 0.1 mmol/L)

Table 3 Comparison of Sensitivity at Different Salt Concentration

Compound	S/N (1 ppb)			Compound	S/N (1 ppb)		
	5 mmol/L	0.1 mmol/L	0.1 mmol/L / 5 mmol/L		5 mmol/L	0.1 mmol/L	0.1 mmol/L / 5 mmol/L
Methomyl	51	103	2.0	Cumyluron	175	533	3.0
Thiamethoxam	93	178	1.9	Chromafenozide	240	501	2.1
Imidacloprid	87	153	1.8	lprovalicarb	186	222	1.2
Clothianidin	41	40	1.0	Simeconazole	177	288	1.6
Chloridazon	146	390	2.7	Cyazofamid	147	460	3.1
Oxycarboxin	80	369	4.6	Indanofan	33	34	1.1
Thiacloprid	261	546	2.1	Naproanilide	81	123	1.5
Thiabendazole	70	119	1.7	Fenoxycarb	262	340	1.3
Azamethiphos	297	470	1.6	Anilofos	299	304	1.0
Bendiocarb	75	231	3.1	Cyflufenamid	80	117	1.5
Thiodicarb	226	217	1.0	Pyrazolynate	236	237	1.0
Furametpyr	201	354	1.8	Pencycuron	160	226	1.4
Pirimicarb	204	250	1.2	Indoxacarb	266	281	1.1
Dimethirimol	154	171	1.1	Benzofenap	115	153	1.3
Methabenzthiazuron	71	171	2.4	Oxazidomefene	257	479	1.9
Azoxystrobin	128	215	1.7	Furathiocarb	106	219	2.1
Pyrifthalid	84	111	1.3	Lufenuron	28	115	4.0
Linuron	19	44	2.3	Cloquintocet-mexyl	357	417	1.2
Methiocarb	222	223	1.0	Flufenoxuron	219	502	2.3
Boscalid	56	229	4.1	Spinosyn A	549	619	1.1
Methoxyfenozide	53	77	1.4	Spinosyn D	137	288	2.1
Dymron (Daimuron)	99	177	1.8				

Table 4 Analytical Conditions

[Condition 1]		Probe Voltage	
Mobile Phase A	: 5 mmol/L Ammonium acetate - water		: +4.5 kV (ESI-positive mode), -3.5 kV (ESI-negative mode)
Mobile Phase B	: 5 mmol/L Ammonium acetate - methanol	Nebulizing Gas Flow	: 3.0 L/min
Gradient Program	: 20 % B (0 min) - 70 % B (3 min) - 95 % B (10-15 min) - 20 % B (15.01-20 min)	Drying Gas Flow	: 10 L/min
		DL Temperature	: 300 °C
		BH Temperature	: 500 °C
		DL Voltage/Q-array Voltage	: Using default values
[Condition 2]			
Mobile Phase A	: 0.1 mmol/L Ammonium acetate - water		
Mobile Phase B	: 0.1 mmol/L Ammonium acetate - methanol		
Gradient Program	: 20 % B (0 min) - 70 % B (3 min) - 95 % B (10-15 min) - 20 % B (15.01-20 min)		
Column	: Shim-pack XR-ODS II (75 mmL, × 2.0 mmI.D., 2.2 µm)		
Flow Rate	: 0.2 mL/min		
Injection Volume	: 3 µL		
Column Temperature	: 40 °C		

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Steroids: Testosterone, Progesterone, Estradiol, Ethinylestradiol

Summary

Sensitivity was tested for four steroids on the LCMS-8030 triple quadrupole mass spectrometer coupled with a Nexera UHPLC.

Background

Steroids are powerful hormones that are needed for normal biological activity but may also be present in the environment. Water contaminated with steroids poses a serious environmental and public health threat. Sensitive and rapid methods are needed for accurate quantitative analysis. UHPLC-MS-MS methods for analysis of these compounds were developed and the sensitivity for each analyte was tested.

Method

Standards for four steroids were obtained at a concentration of 1 mg/mL in methanol. The standards were: Testosterone (T4), Progesterone (P4), Estradiol (E2), and Ethinylestradiol (EE2). Standards were diluted in 50/50 water/acetonitrile for optimization of mass spectrometry parameters.

Multiple Reaction Monitoring (MRM) was used for analysis. Collision energy, quadrupole prerod bias voltage, and other MS parameters were optimized by flow injection of the individual standards. T4 and P4 ionized most efficiently using a 0.1% formic acid/acetonitrile mobile phase using the DUIS dual ion

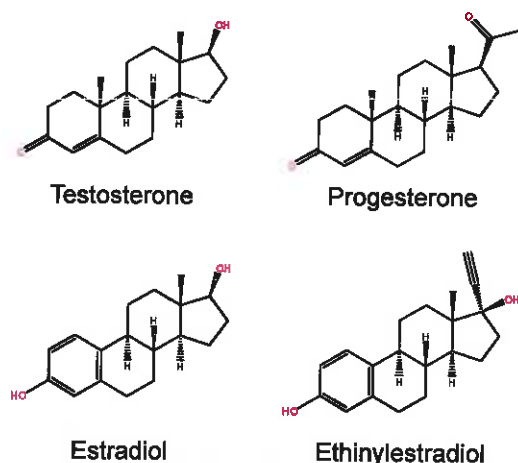


Figure 1: Structures of tested steroids

Level	T4	P4	E2	EE2
L1	370.3	370.3	10000	10000
L2	123.5	123.5	3333	3333
L3	41.17	41.17	1111	1111
L4	13.72	13.72	370.3	370.3
L5	4.57	4.57	123.5	123.5
L6	1.52	1.52	41.17	41.17
L7	0.582	0.582	13.72	13.72
L8			4.57	4.57

Table 1: Dilution levels (ng/mL)

Type	Event#	+/-	Compound Name (m/z)	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3 Pre Bias(V)	Measurement Time (min)
MRM	1	+	Testosterone 288.9>97	80	-14	-25	-10	0-2
MRM	2	+	Progesterone 315.4>109.1	80	-12	-30	-20	0-2
Type	Event#	+/-	Compound Name (m/z)	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3 Pre Bias(V)	Measurement Time (min)
MRM	1	-	Estradiol 271.4>183.1	50	13	41	30	0-2
MRM	2	-	Ethinylestradiol 295.4>145	50	14	47	28	0-2

Table 2: MRM parameters

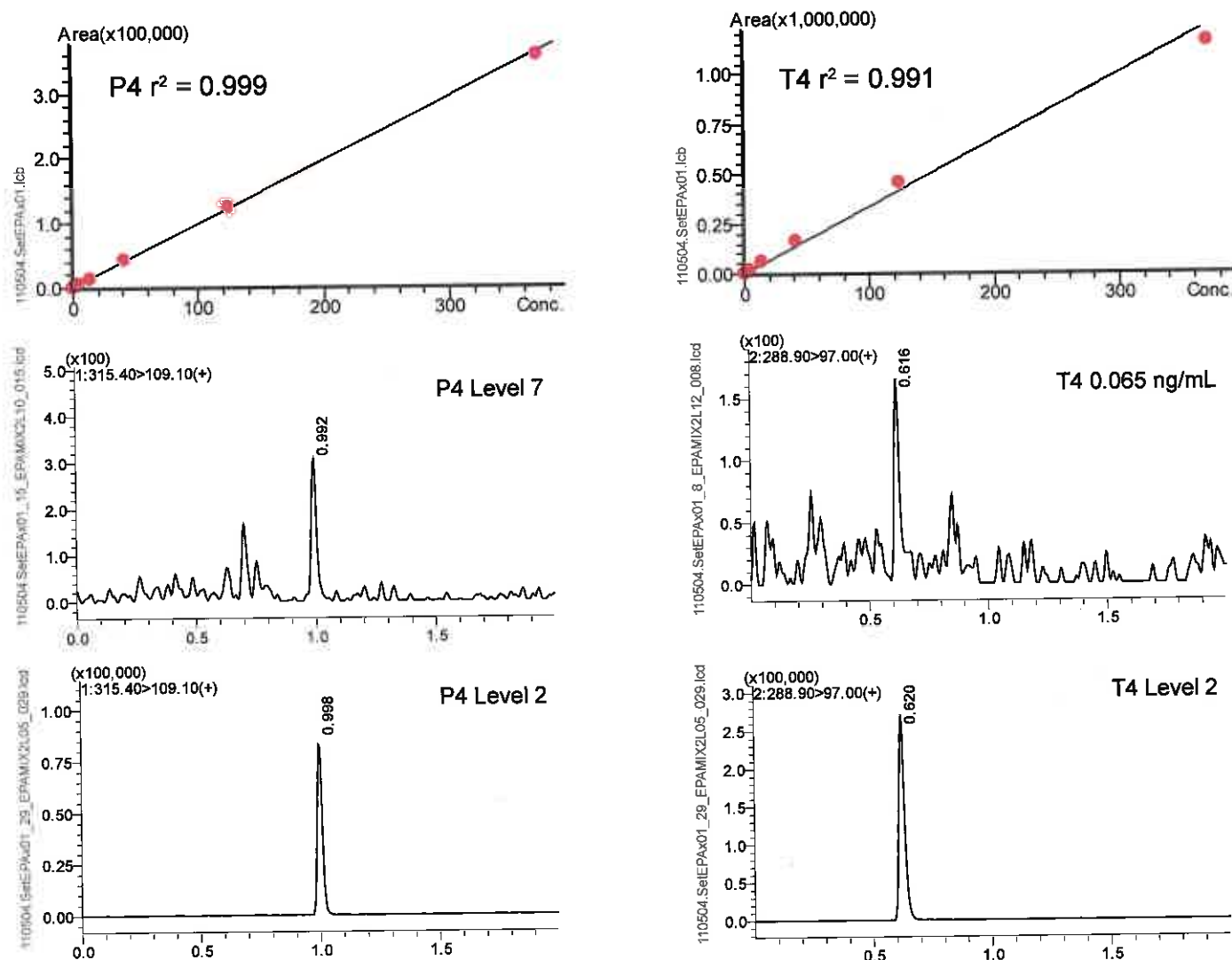


Figure 2: Calibration curves and selected mass chromatograms for P4 and T4

spray (ESI with corona discharge) source in positive mode. The MRM transition of m/z 288.9 $\rightarrow$ 97 was monitored for T4 and the transition of m/z 315.4 $\rightarrow$ 109.1 was used for P4.

E2 and EE2 ionized most efficiently using a water/acetonitrile mobile phase with atmospheric pressure chemical ionization (APCI) in negative mode. Therefore these compounds were analyzed in a separate method. The MRM transitions for E2 and EE2 were 271.4 $\rightarrow$ 183.1 and 295.4 $\rightarrow$ 145.0 respectively.

A Shimadzu Shimpack-XR ODS III column (1.6 μ m, 2 $\times$

50 mm) was used for analysis. A binary gradient of 0.1% formic acid and acetonitrile was used for T4 and P4 while a binary gradient of water and acetonitrile was used for E2 and EE2. The gradient began at 55% acetonitrile and increased to 95% over 1 minute. After a 6 second isocratic hold, the column was re-equilibrated for approximately 1 minute. The column temperature was 40 $^{\circ}$ C and the injection volume was 10 μ L.

Calibration curves were prepared by serial dilution of a mixed standard as shown in Table 1.

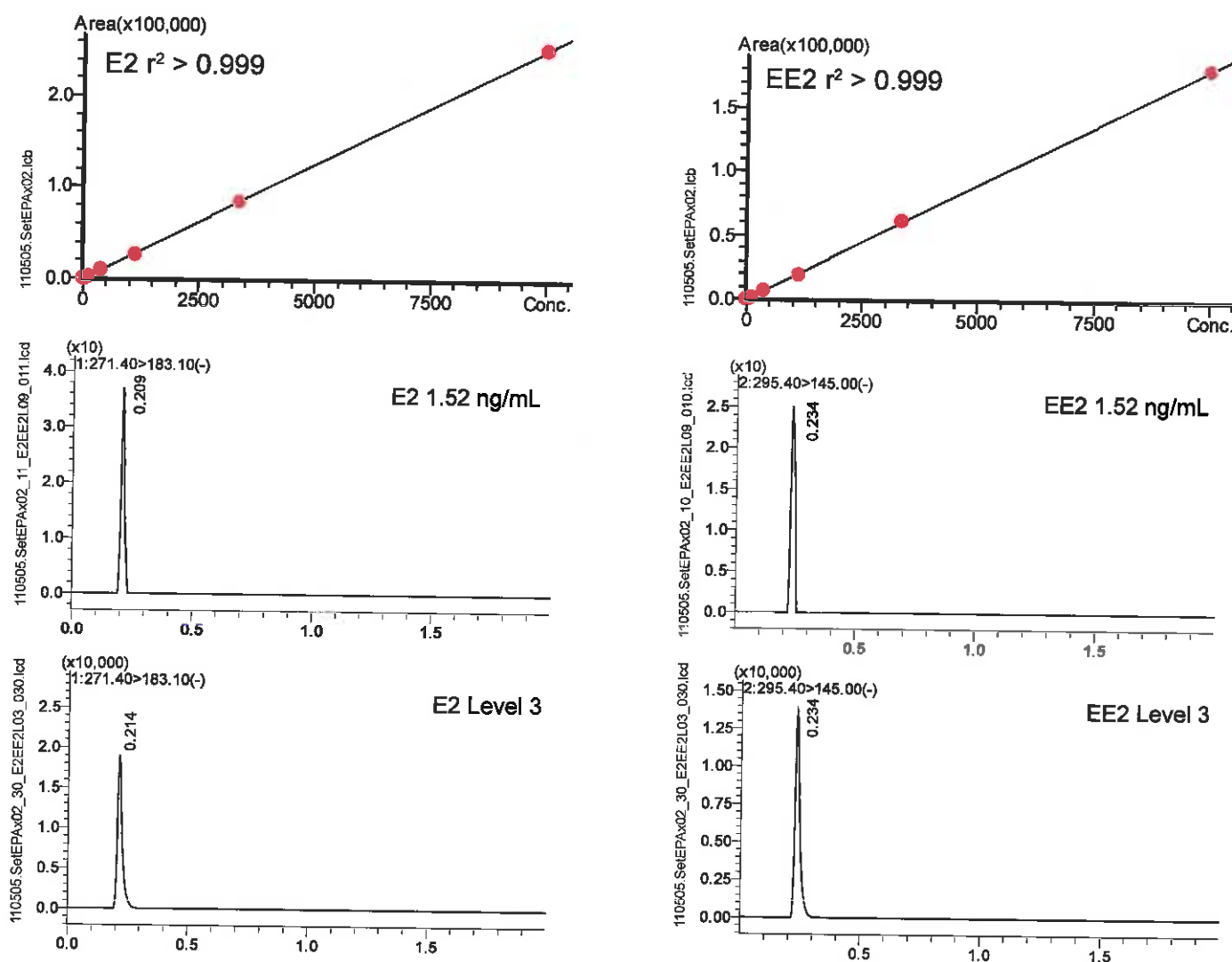


Figure 3: Calibration curves and selected mass chromatograms for E2 and EE2

Results and Discussion

Representative chromatograms of the T4/P4 and E2/EE2 methods are shown in **Figures 2 and 3**. The limit of detection (LOD, $S/N > 3$) for P4 was between 0.194 and 0.582 ng/mL using a 10 μ L injection, and the limit of quantitation (LOQ, $S/N > 10$) was approximately 0.5 ng/mL. For T4, the LOD was approximately 0.065 ng/mL and the LOQ was approximately 0.2 ng/mL. For T4 and P4, the calibration curve was constructed between 0.194 and 370 ng/mL. The curves were linear in the tested range ($r^2 > 0.99$).

For E2, the LOD was approximately 1.5 ng/mL and the LOQ was approximately 4.5 ng/mL. The LOD and LOQ for EE2 was similar. The calibration curves were constructed from 4.57 ng/mL up to 10 μ g/mL, and the curves were both linear ($r^2 > 0.99$).

Conclusion

Rapid and sensitive methods for determination of four steroids was developed using UHPLC-MS-MS.



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LCMS-8030 Application Note

Perfluorocarbons

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LCMS-8030

Triple Quadrupole Mass Spectrometer

Summary: The sensitivity of the LCMS-8030 was evaluated for ten perfluorocarbon compounds.

Background: Perfluorocarbons (PFCs) and their derivatives have many industrial uses but pose environmental and health concerns. Sensitive and selective methods for detection of perfluorocarbons by LC-MS-MS are needed.

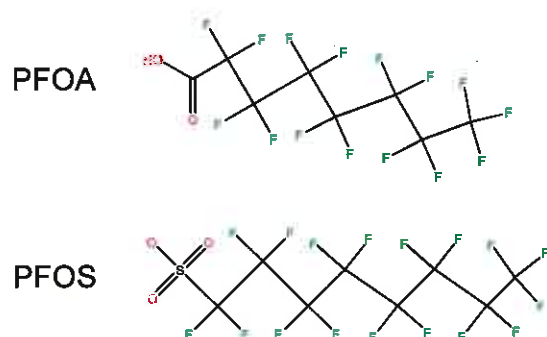
Method: A PFC standard mix in solution as well as a milk-spiked and extracted standard mix at 0.3 and 3 ppb were supplied by FDA for evaluation. Representative PFCs are shown in **Figure 1**.

Flow injections were used to optimize MRM conditions using an automated wizard. Negative ion electrospray was used in Multiple Reaction Moni-

toring (MRM) mode. Optimized parameters are shown in **Table 2**.

A ProteCol-G C-18 column (3 μ m, 2.1 x 150 mm, SGE Analytical Science, Melbourne, Australia) was used. A binary gradient of 2 mM ammonium acetate in methanol was used as shown in **Table 1**. The flow rate was 0.3 mL/min and the column temperature was 35 °C. The injection volume for the test samples was 10 μ L.

The ESI probe, gasses, and temperatures were adjusted to achieve best sensitivity for the analytes. The DL temperature was 300 °C, the nebulizing gas flow rate was 3 L/min, the heat block temperature was 200 °C, and the drying gas was 20 L/min. The spray voltage was 1 kV.



Time	% Methanol
0	60
12	95
13.5	95
13.6	60
22	60

Table 1: Gradient time program

Figure 1: Structures of typical PFCs

Type	Event#	+/-	Compound Name (m/z)	Dwell Time (msec)	Q1 Pre Bias(V)	CE (V)	Q3 Pre Bias(V)	Measurement Time
MRM	1	-	PFBS 299>80, 299>99	25, 5	22	37, 35	29, 16	0-22 min
MRM	3	-	PFHxS 399>80, 399>99	25, 5	14	41, 37	29, 16	0-22 min
MRM	5	-	PFOS 499>80, 499>99	25, 5	13	53, 43	12, 16	0-22 min
MRM	7	-	PFHxA 313>269, 313>119	25, 5	16	9, 19	16, 22	0-22 min
MRM	9	-	PFHpA 363>319, 363>169	25, 5	26	9, 19	20, 17	0-22 min
MRM	11	-	PFOA 413>369, 413>169	25, 5	15	13, 19	22, 30	0-22 min
MRM	13	-	PFNA 463>419, 463>219	25, 5	17	11, 19	27, 23	0-22 min
MRM	15	-	PFDA 513>469, 513>219	25, 5	20	11, 19	29, 21	0-22 min
MRM	17	-	PFUnDA 563>519, 563>269	25, 5	22	16, 20	32, 11	0-22 min
MRM	19	-	PFDoDA 613>569, 613>169	25, 5	22	10, 35	40, 19	0-22 min

Table 2: MRM parameters

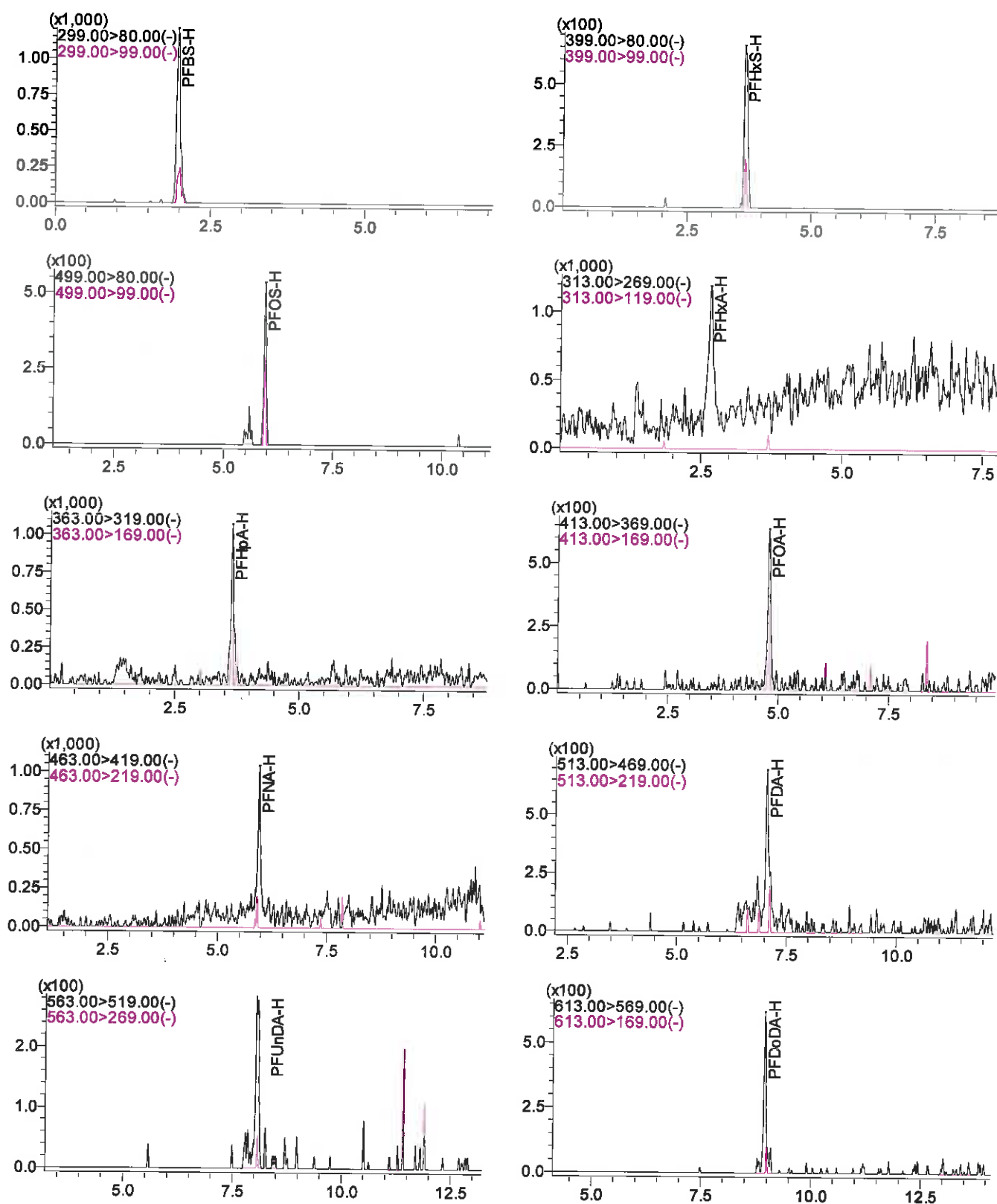


Figure 2: Mass chromatograms from sample PFC1A consisting of 0.3 ppb standards in solution.
Data File: 110518.SetPFCx01_5182011_3_003.lcd

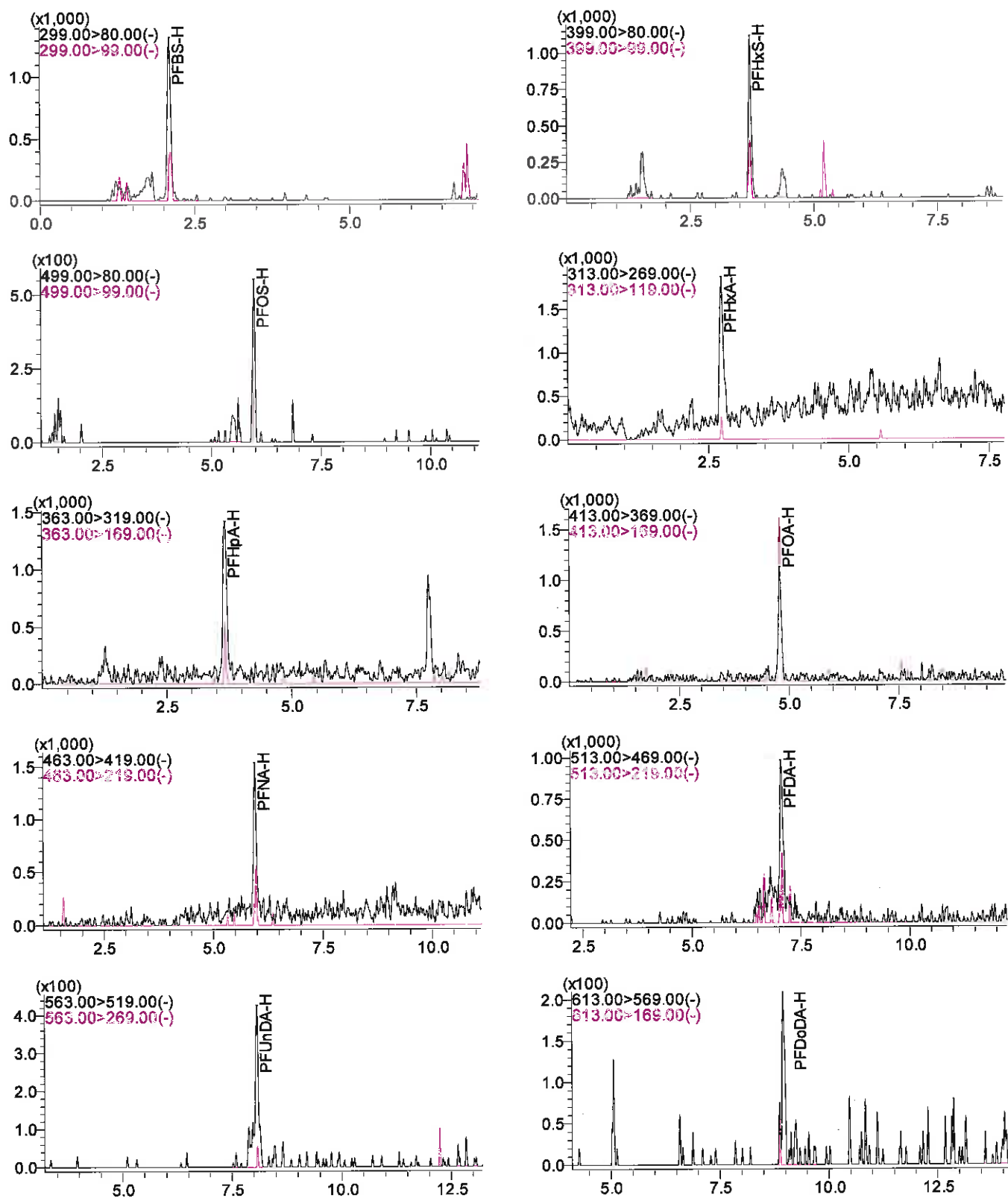


Figure 3: Mass chromatograms from sample PFC1B consisting of 0.3 ppb standards in milk extract. Data File: 110518.SetPFCx01_5182011_4_004.lcd

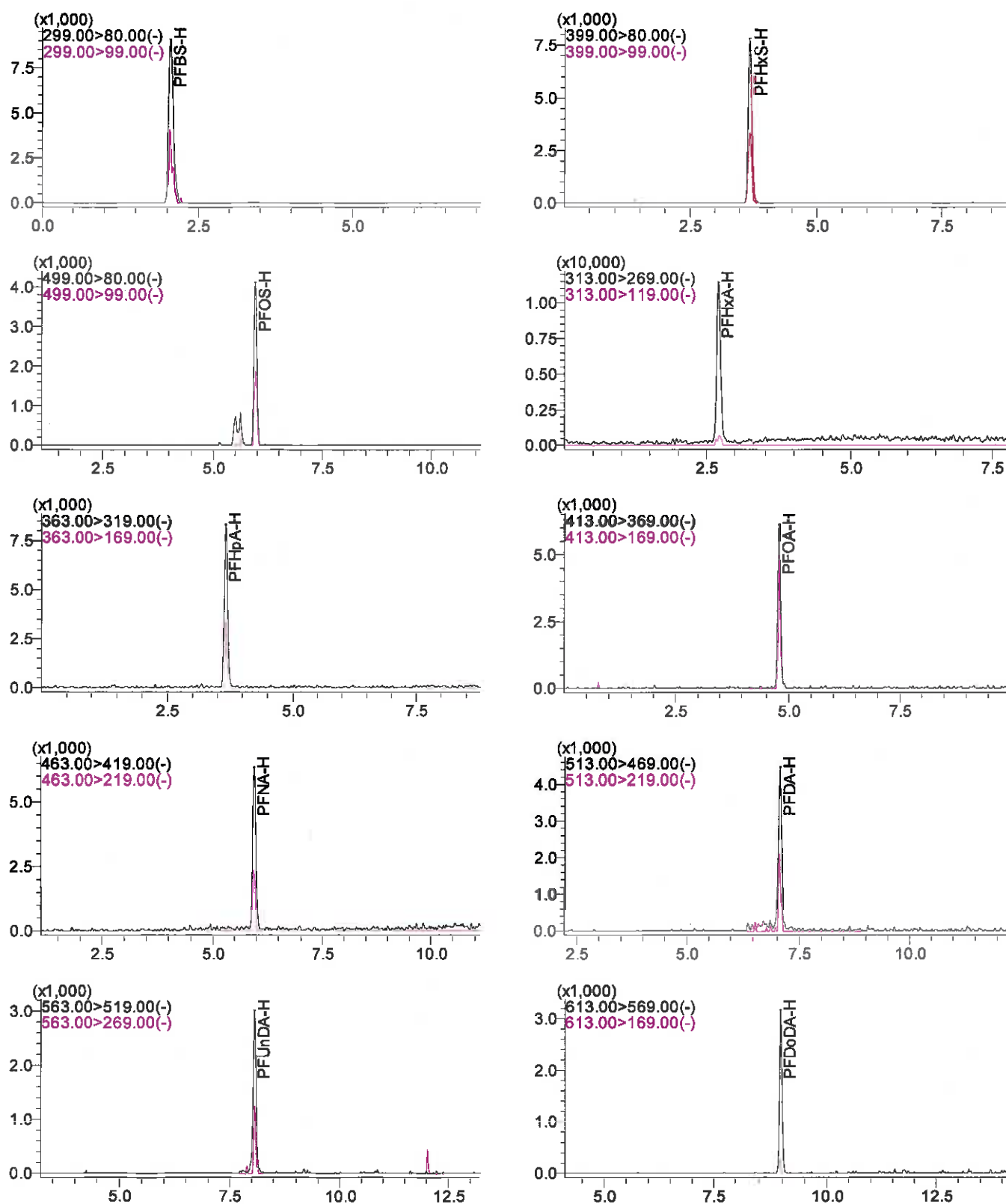


Figure 4: Mass chromatograms from sample PFC2A consisting of 3 ppb standards in solution.
Data File: 110518.SetPFCx01_5182011_6_006.lcd

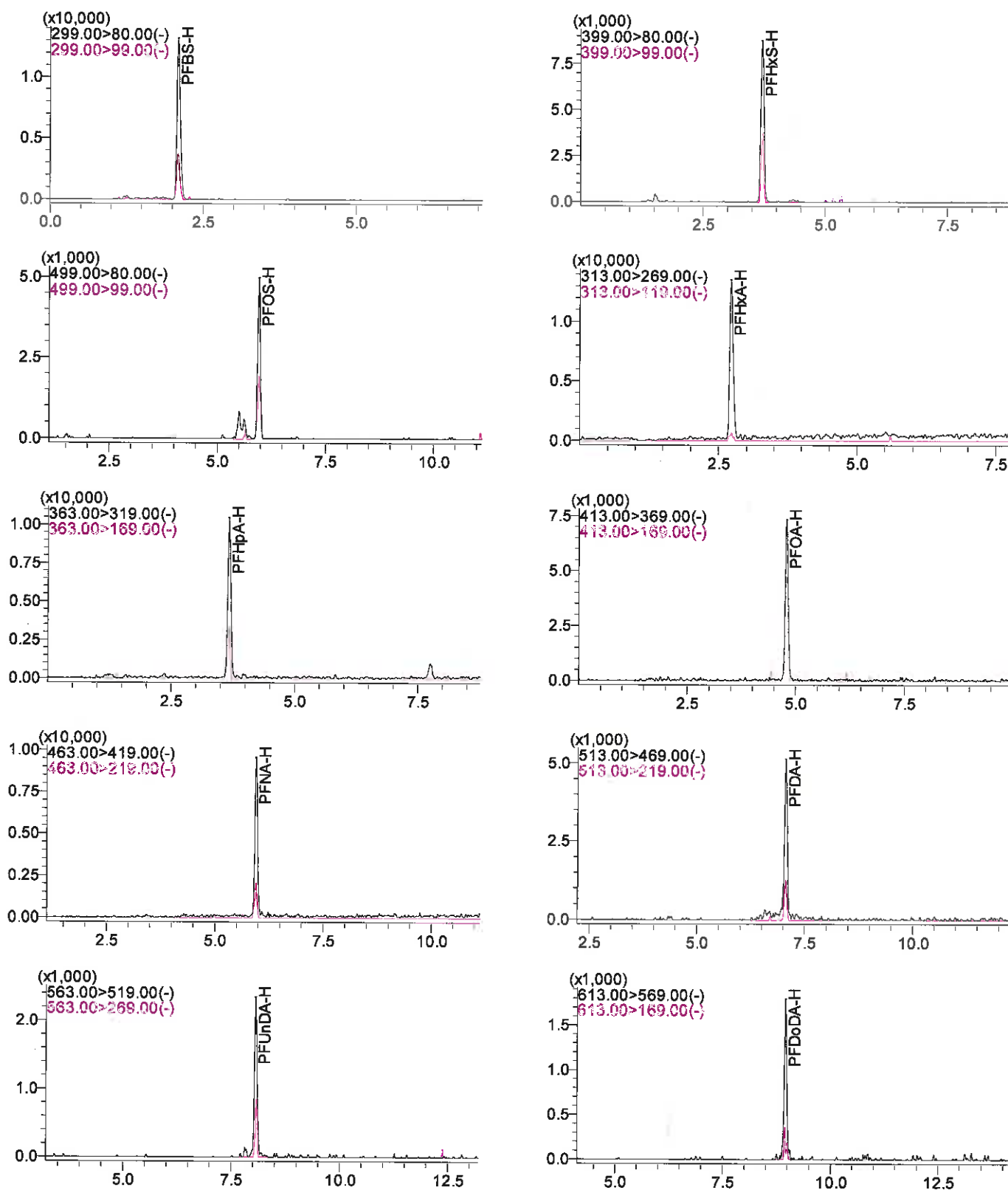


Figure 5: Mass chromatograms from sample PFC2B consisting of 3 ppb standards in milk extract.
Data File: 110518.SetPFCx01_5182011_7_007.lcd

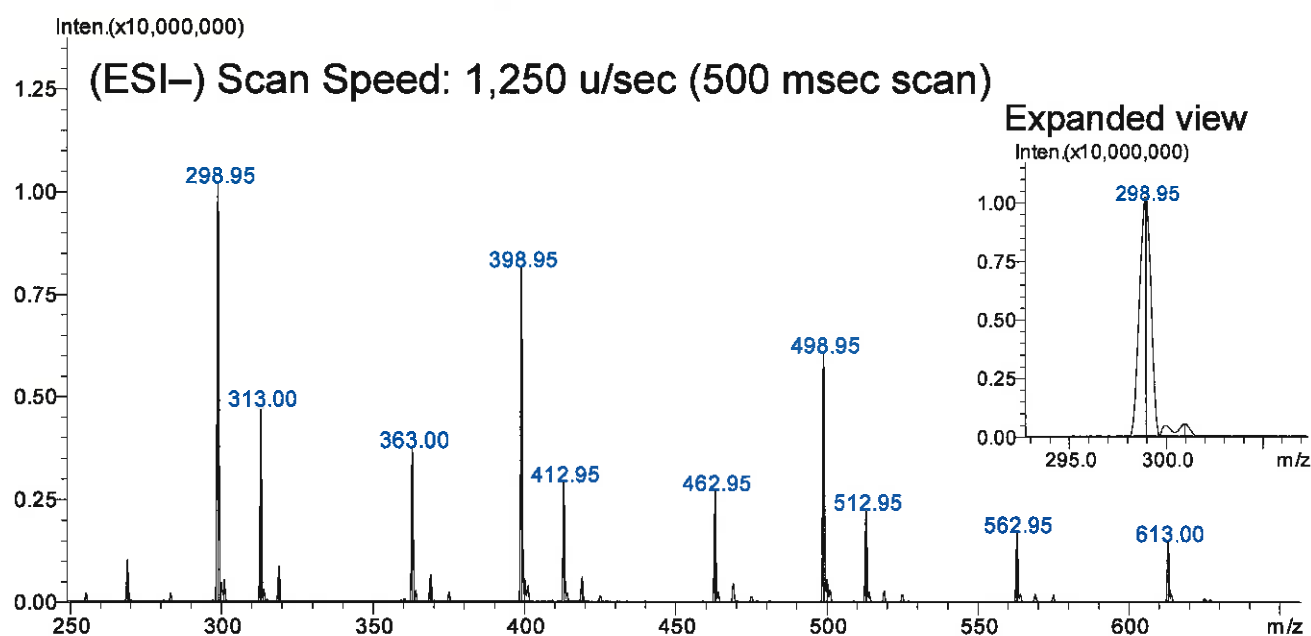
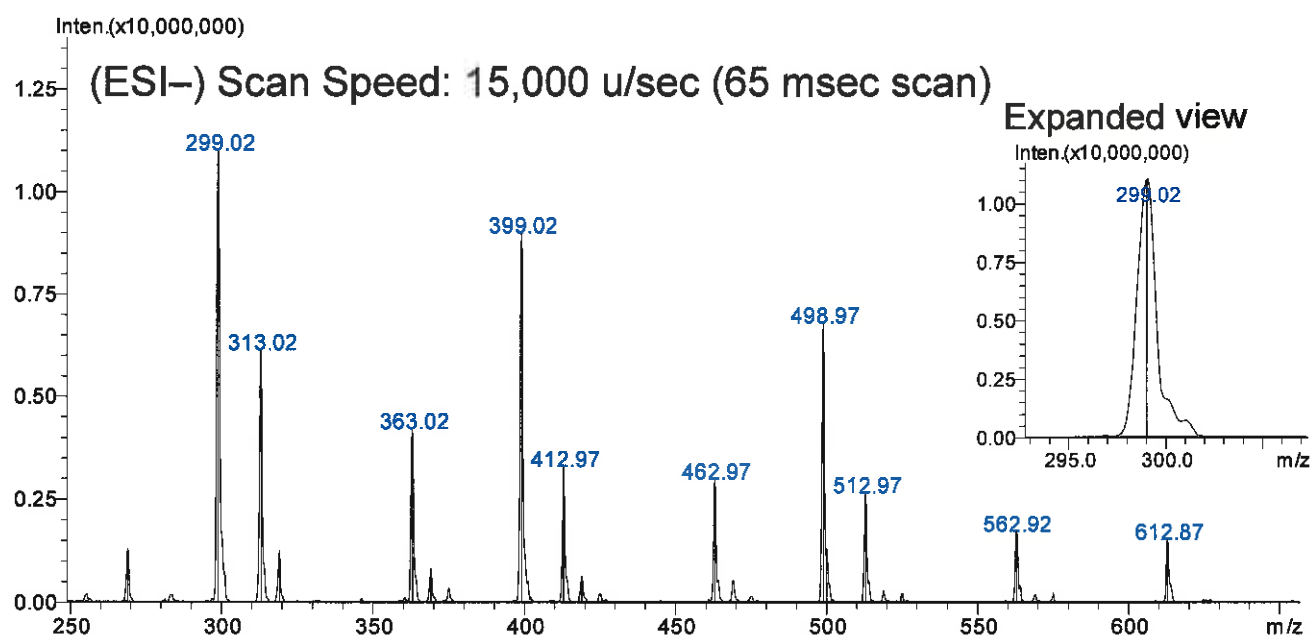
	Peak Area			
	PFC1A	PFC1B	PFC2A	PFC2B
PFBS	5244	5467	47163	50551
PFHxS	3244	4347	36668	38521
PFOS	2065	2097	17997	22155
PFHxA	4881	8078	54119	57968
PFHpA	3825	6468	38244	46530
PFOA	3189	5549	28138	35670
PFNA	3074	5709	31563	39509
PFDA	3097	4352	22318	21658
PFUnDA	1535	1807	14031	10239
PFDoDA	2405	1172	12436	7682

Table 3: Peak areas for each analyte in each sample

Results and Discussion: Mass chromatograms from the test samples PFC1A, 1B, 2A, and 2B are shown in **Figures 2–5**. Retention time is plotted on the X-axis in minutes and the signal intensity is plotted on the Y-axis in counts per second. In each chromatogram, the black trace corresponds to the quantifier ion and the pink trace corresponds to the qualifier ion. The transitions and compound names are reported in each chromatogram.

Each of the PFCs could be detected in both the pure solvent standards as well as the milk extracts at the 3 ppb and 0.3 ppb spiked levels. The areas for each peak are shown in **Table 3**.

Conclusion: The sensitivity of the LCMS-8030 was tested for a series of PFCs in solution and in milk extract matrix.



Mass spectra collected in the same flow injection at fast and slow scan speeds.

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Application News

SSI-LCMS-009

Liquid Chromatography Mass Spectrometry

Mycotoxins



LCMS-8030



Summary

An extremely fast LC-MS-MS method for the determination of mycotoxins and other untargeted chemical threats was developed.

Background

Mycotoxins are fungal poisons that threaten the world food supply. Regulatory agencies have imposed limits on levels of mycotoxins allowed in food. Food safety is ensured by testing for the presence of mycotoxins by methods such as LC-MS-MS.

In addition to mycotoxins, many other known and unknown chemicals threaten the food supply, however their presence might be missed by LC-MS-MS methods which rely exclusively on MRM settings for detection. Therefore we have developed an extremely fast and reliable method for the detection of mycotoxins in food which has the additional advantage of collecting survey scan and data dependent MS-MS for untargeted screening for other chemical threats.

Method

Reference standards were obtained for each mycotoxin. Each was diluted in an 80/20 mixture of 5 mM ammonium formate/methanol at the concentrations listed in **Table 1**. A Shimadzu Shim-pack XR-ODS III UHPLC column (2 x 50 mm, 1.6 μ m) was used at a flow rate of 0.4 mL/min and a temperature of 50 °C.

A binary gradient of 5 mM ammonium formate and methanol was used and is shown in **Figure 3**. Electrospray ionization was used with polarity switching. The MRM transitions and MS parameters for each compound were optimized and are listed in **Table 2**.

Reference samples of feed corn and wheat with independently determined levels of various mycotoxins were prepared by solid phase extraction and analyzed by LC-MS-MS. Alternatively, oatmeal spiked with mycotoxins was prepared by reconstitution and dilution before analysis.

2

	L2	L3	L4	L5	L6
Aflatoxin B1	67.2	22.4	7.5	2.5	0.8
Aflatoxin B2	20.3	6.8	2.3	0.8	0.3
Aflatoxin G1	67.2	22.4	7.5	2.5	0.8
Aflatoxin G2	20.3	6.8	2.3	0.8	0.3
Fumonisin B1	833.3	277.8	92.6	30.9	10.3
Fumonisin B2	833.3	277.8	92.6	30.9	10.3
Mycotoxin T2	1666.7	555.6	185.2	61.7	20.6
DON	8100.0	2700.0	900.0	300.0	100.0
Ochratoxin	81.0	27.0	9.0	3.0	1.0
ZON	2025.0	675.0	225.0	75.0	25.0

Table 1: Concentrations of standards (ppb)

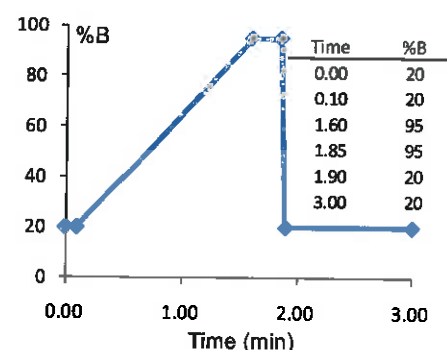


Figure 3: Binary gradient program

Type	Event#	+/-	Compound Name (m/z)	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3 Pre Bias(V)	Measurement Time
MRM	1	+	Aflatoxin B1 313>241	25	-25	-39	-25	1.25-1.75
MRM	2	+	Aflatoxin B2 315>259	25	-25	-33	-25	1.25-1.75
MRM	3	+	Aflatoxin G1 329>243	25	-25	-31	-25	1.25-1.75
MRM	4	+	Aflatoxin G2 331>245	25	-25	-33	-25	1.25-1.75
MRM	5	+	Fumonisin B1 722.4>334.4	25	-20	-40	-15	1.6-3.0
MRM	6	+	Fumonisin B2 706.4>336.2	25	-20	-40	-24	1.75-3.0
MRM	7	+	Mycotoxin T2 484.3>305.1	25	-24	-16	-21	1.75-3.0
MRM	8	-	DON 295>138	25	15	19	30	0-1.0
MRM	9	-	Ochratoxin 402.1>167	25	15	40	30	1.75-3.0
MRM	10	-	ZON 317>131	25	15	30	25	1.75-3.0

Table 2: MRM parameters

Additionally, a feed corn sample was spiked with the pesticide bromacil to demonstrate untargeted screening with data-dependent MS-MS and spectral pattern matching.

Mass spectra were collected at 15,000 u/sec in positive and negative mode continuously throughout the run. Data dependent MS-MS was triggered based on these survey scans at a product ion scan speed which was also set at 15,000 u/sec.

To help identify any untargeted compounds of potential interest, data dependent product ion scans were compared to a database containing authentic tandem mass spectra of hundreds of substances including pesticides.

Results and Discussion

ESI was found to give the best overall signal intensity for the mycotoxins. The protonated molecules and sodium adducts of each of the mycotoxins were observed by ESI, APCI, and DUIS (a combination ESI and APCI source). A formic acid buffered mobile phase was found to depress ionization of the protonated molecules, whereas ammonium formate increased their signal intensity dramatically. Sodium adducts of the mycotoxins had the same or lower signal intensity when ionized in the presence of ammonium formate. The four aflatoxins ionized well in positive mode, while some of the other mycotoxins ionized more efficiently in negative mode.

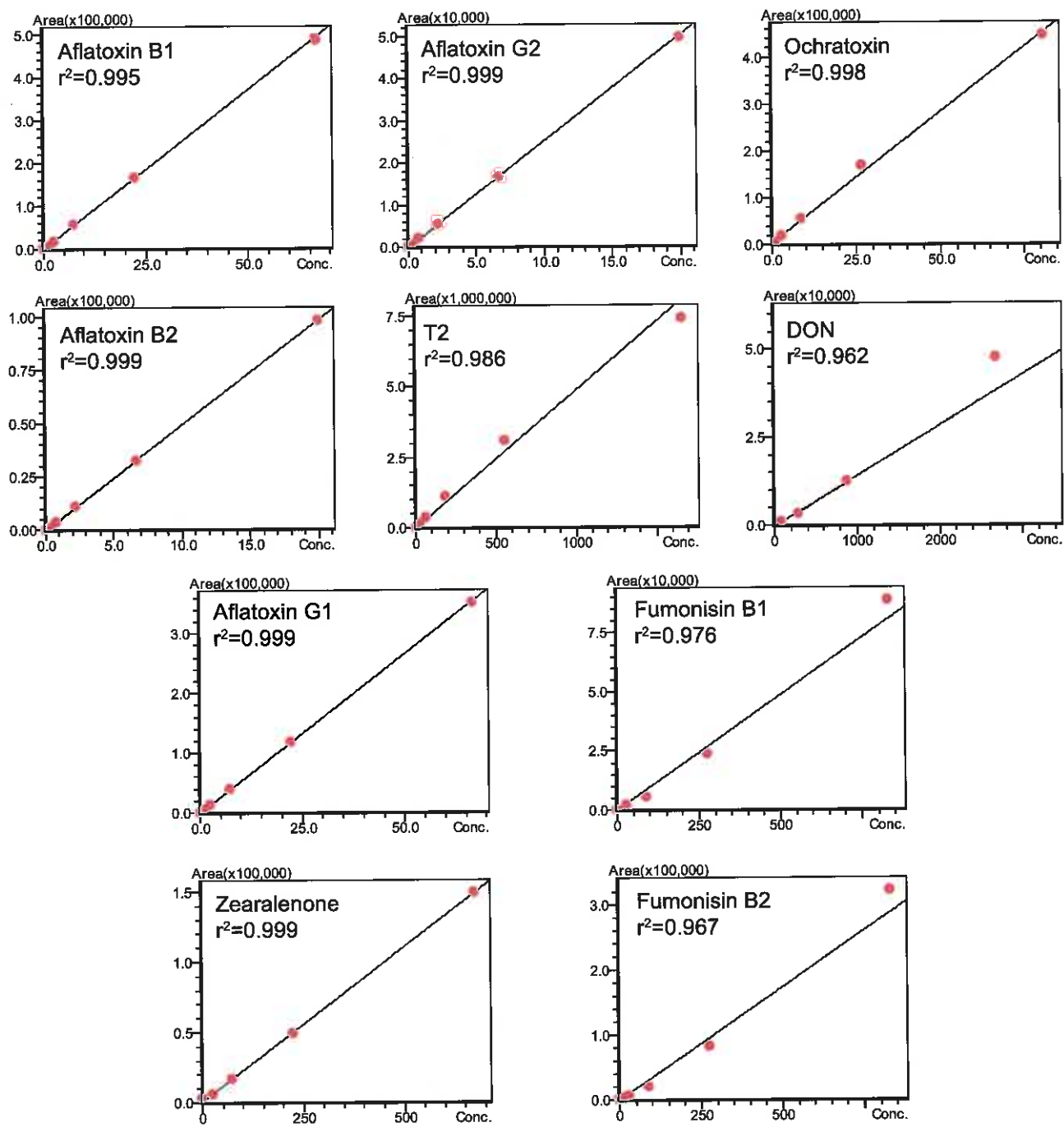


Figure 4: Calibration curves for each mycotoxin

	L2	L3	L4	L5	L6
Aflatoxin B1	99.48	100.52	99.72	100.40	99.90
Aflatoxin B2	100.86	101.20	102.90	93.68	102.72
Aflatoxin G1	97.94	100.54	101.70	100.26	99.72
Aflatoxin G2	101.58	100.88	103.90	91.36	104.60
DON	137.40	114.40	93.42	81.68	107.58
Ochratoxin	102.58	96.40	94.30	113.40	97.10
ZON	84.30	101.86	123.68	132.42	82.62

Table 3: Percent Accuracy of mycotoxin standards in solution ($n=5$)

Aflatoxin B1	Measured (ppb)	Expected (ppb)	Aflatoxin B2	Measured (ppb)	Expected (ppb)
Oatmeal	Measured (ppb)	Expected (ppb)	Oatmeal	Measured (ppb)	Expected (ppb)
Aflatoxin G1	Measured (ppb)	Expected (ppb)	Aflatoxin G2	Measured (ppb)	Expected (ppb)
Oatmeal	Measured (ppb)	Expected (ppb)	Oatmeal	Measured (ppb)	Expected (ppb)
Zearalenone (ZON)	Measured (ppb)	Expected (ppb)	Ochratoxin	Measured (ppb)	Expected (ppb)
Oatmeal	Measured (ppb)	Expected (ppb)	Oatmeal	Measured (ppb)	Expected (ppb)
Deoxynivalenol (DON)	Measured (ppb)	Expected (ppb)	Mycotoxin T-2	Measured (ppb)	Expected (ppb)
Wheat	Measured (ppb)	Expected (ppb)	Wheat	Measured (ppb)	Expected (ppb)
Fumonisin B1	Measured (ppb)	Expected (ppb)	Fumonisin B2	Measured (ppb)	Expected (ppb)
Corn	Measured (ppb)	Expected (ppb)	Corn	Measured (ppb)	Expected (ppb)

Table 4: Measured and expected amounts of mycotoxins in each grain sample. Expected values based on measurement by an independent method.

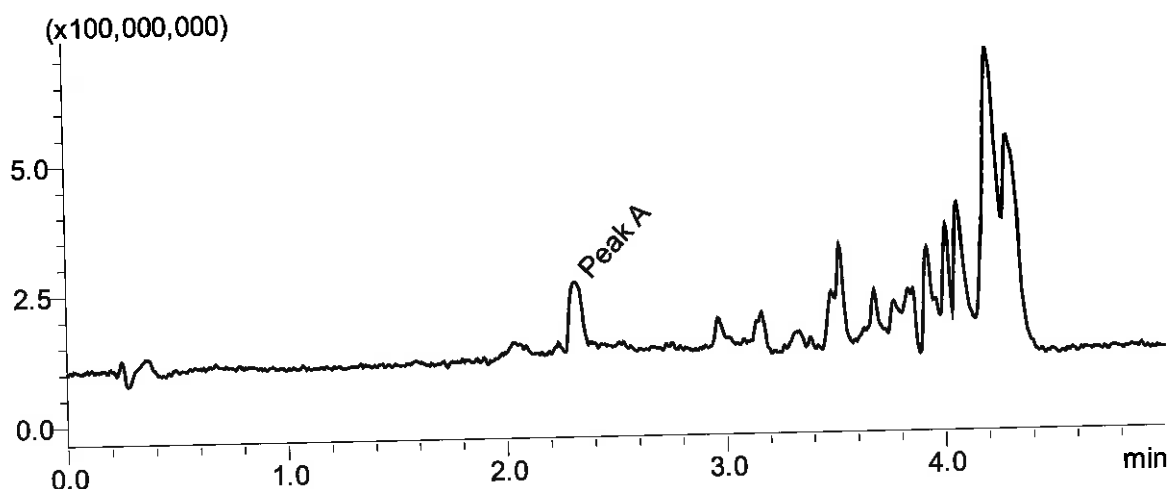
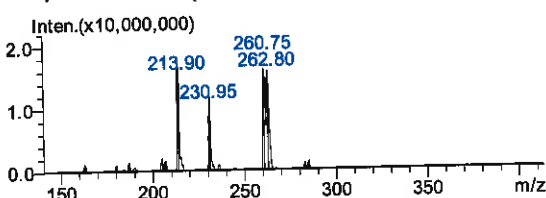


Figure 5: Total ion chromatogram from a feed-corn sample. The peak at 2.25 minutes was automatically selected for data-dependent MS-MS.

ESI+ Survey Scan for Peak A
15,000 u/sec (33 msec)



ESI- Survey Scan for Peak A
15,000 u/sec (33 msec)

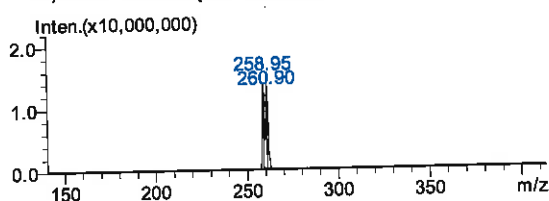


Figure 6: Survey scans of Peak A

Injection solvent was found to be critical to achieving proper peak shape. Therefore samples were diluted in mobile-phase matched solvent (80/20) 5 mM ammonium acetate/methanol).

Calibration curves were prepared for each mycotoxin with a weighting factor of $1/A^2$. The curves were linear ($r^2 > 0.95$) in the tested range. The calibration curves are shown in **Figure 4**. Five replicates of each standard were analyzed and the accuracy reported in **Table 3**.

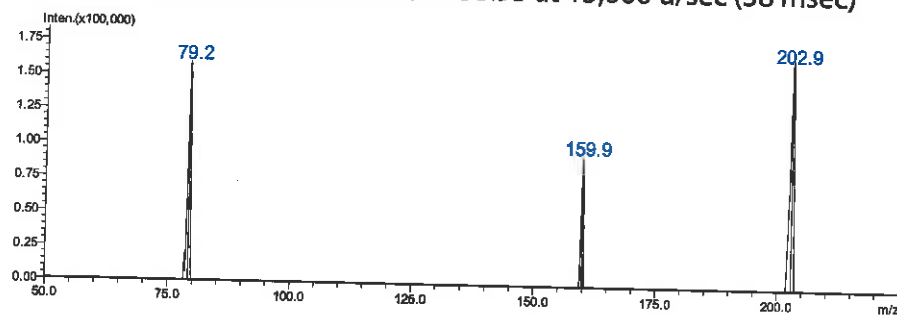
The lower limit of quantitation (LLOQ) for each mycotoxin standard in solution corresponds approximately to the most dilute level reported in **Table 1**. For Fumonisin B1 and B2, the LLOQ was lower than 10 ppb and for Mycotoxin T2 the LLOQ

was significantly lower the lowest tested concentration of 20.6 ppb.

Measured values for each mycotoxin are shown in **Tables 3** and **4**. The rapid chromatographic method enables fast analysis of many samples whether the sample preparation is SPE or a simple solvent extraction.

Survey scanning at extremely fast scan speeds of 15,000 u/sec enables qualitative data to be collected without compromising quantitative performance. Automatically triggered fast tandem mass spectra were collected and used to search a library of known contaminants for untargeted screening.

ESI — Triggered Product Ion Scan of m/z 258.95 at 15,000 u/sec (38 msec)



ESI — Product Ion Scan of Bromacil matched from Tandem MS Library

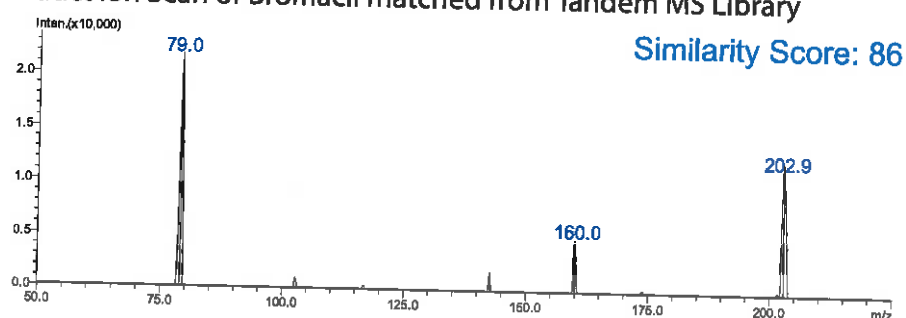


Figure 7: Data dependent product ion scan from Peak A matched to library spectrum of Bromacil.

The mass and tandem MS spectrum of Peak A matched the pesticide Bromacil as shown in **Figure 7**. Even though the transitions specifically corresponding to bromacil were not a part of the MRM method, the presence of bromacil could be confirmed.

Conclusion

A rapid, selective, and sensitive LC-MS-MS method for determination of mycotoxins as well as untargeted screening was developed.

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LCMS-8030



LCMS-8080



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LCMS-IT-TOF

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Application News

No.L435

High Performance Liquid Chromatography

Quantitation by HPLC and Identification by LC/MS for Total Aflatoxins in Food

Aflatoxins are mycotoxins that are extremely carcinogenic and acutely toxic. In Japan, foods in which the total aflatoxins (sum of B₁, B₂, G₁ and G₂ aflatoxins) are detected at levels exceeding 10 µg/kg are in violation of Article 6, Item 2 of the Food Sanitation Act¹⁾. Previously, in Application News articles L422, L428 and L430, we introduced examples of simultaneous analysis of these 4 substances (B₁, B₂, G₁ and G₂) using HPLC and UHPLC. In accordance with the test method²⁾ specified in the notification of August 16, 2011, after pretreatment using an immunoaffinity column, we conducted (1) quantitation using an HPLC system compliant with the test method, (2) identification using a LC/MS system compliant with the test method, and (3) rapid quantitation and identification using the Nexera UHPLC. Here, we present these analyses in addition to the results of the validation.

Total Aflatoxins Test Flow

Fig. 1 shows the complete workflow, including sample preparation, for total aflatoxins according to the specified test method (HPLC or LC/MS), and Fig. 2 shows the structures of those aflatoxins. For processed foods and spices, after extraction with a solution containing an organic solvent, the aflatoxins are purified using an immunoaffinity column (IAC). The fluorescence intensity of aflatoxins B₁ and G₁ is increased by following pretreatment procedure (A) with trifluoroacetic acid (TFA), and quantitative testing is carried out using an HPLC with an RF-20AXS fluorescence detector. If the aflatoxins are detected at a level greater than the reference value, it is re-dissolved in mobile phase without TFA derivatization after drying the purified solution eluted from IAC (pretreatment procedure (B)), and identification of the contained aflatoxins is carried out by LC/MS.

Among the properties of aflatoxins is their susceptibility to degradation when exposed to ultraviolet light, and their tendency in aqueous solution to adsorb to glass surfaces. Therefore, when conducting analysis, it is necessary to use silanized, amber glass vials or polypropylene vials to prevent reduced recoveries. Also, for analysis of actual samples, it is recommended to clean the column after each analysis with mobile phase containing a higher concentration of organic solvent than that used for the analysis.

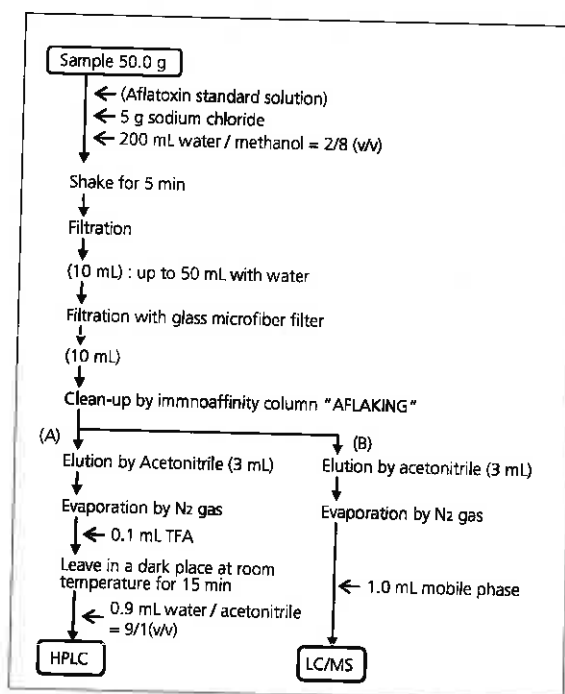


Fig. 1 Total Aflatoxin Test Workflow

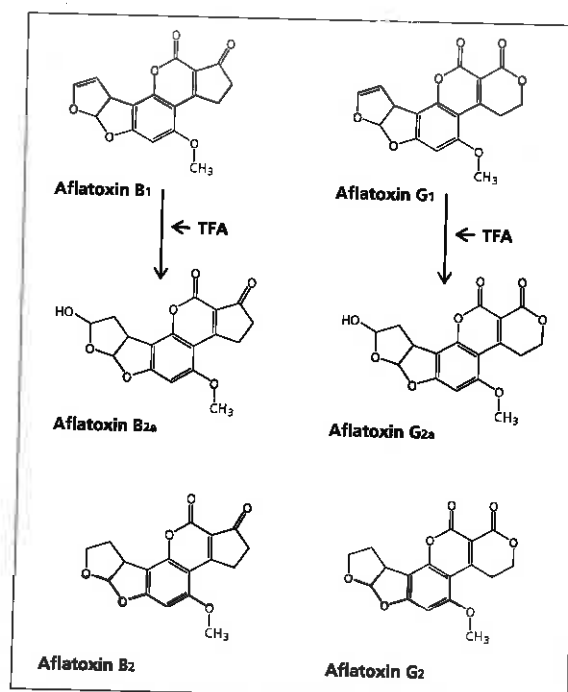


Fig. 2 Structures of Aflatoxins

(1) Quantitation by HPLC –Accuracy and Precision–

Aflatoxins in commercially available roasted peanuts were processed according to procedure (A) in Fig. 1, and analysis was conducted by HPLC using the test method to verify the recovery (accuracy) and repeatability (precision). The analytical conditions are shown in Table 1.

The roasted peanuts were spiked with aflatoxin B₁ and G₁ at a concentration of 0.8 µg/kg each, and aflatoxin B₂ and G₂ at a concentration of 0.2 µg/kg each (total aflatoxins: 2 µg/kg). The accuracy and precision with respect to each substance in repeat testing (n = 5) are shown in Table 2. Accuracy of 76–100 % was obtained that satisfied the criteria of the specified reference value in the guideline (70–110 %). In addition, the precision obtained was 5.7–9.6 %, which also met the specified reference value (less than 20 %).

Table 1 Analytical Conditions

Column	: Shim-pack FC-ODS (150 mmL, × 4.6 mmI.D., 3 µm)
Mobile Phase	: Water / Methanol / Acetonitrile = 6/3/1 (v/v/v)
Flow Rate	: 0.8 mL/min
Column Temp.	: 40 °C
Detection	: RF-20Axs, Ex at 365 nm, Em at 450 nm
RF Cell	: Conventional cell
Cell Temp.	: 25 °C
Injection Volume	: 20 µL

Table 2 Accuracy and Precision of Quantitation by HPLC

	Aflatoxin B ₁	Aflatoxin B ₂	Aflatoxin G ₁	Aflatoxin G ₂
Recovery (%)	79	90	76	100
%RSD	9.6	8.9	7.3	5.7

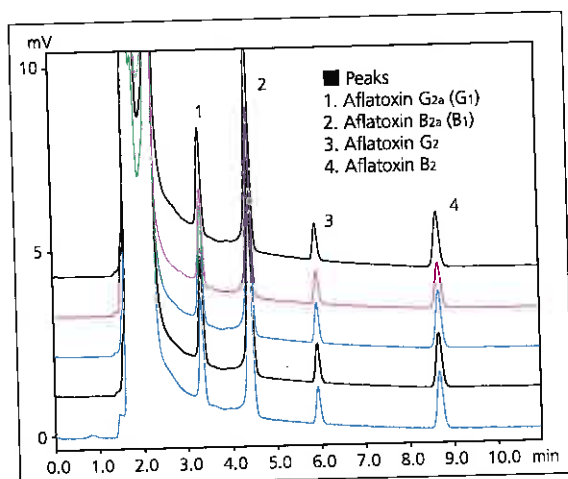


Fig. 3 Chromatograms of Roasted Peanuts Spiked with Aflatoxins (Quantitation by HPLC)

(2) Identification Testing by LC/MS

Commercially available roasted peanuts that were not spiked with the aflatoxin standard solution were subjected to dry processing according to procedure (B) in Fig. 1, and a matrix sample solution (blank) was prepared by dissolving the residue in 1 mL of 10 % acetonitrile solution. The matrix sample solution and aflatoxin standard solution were mixed 1:1, and standard addition matrix samples were prepared at 0.05, 0.25, 0.5, 2.5 and 5 µg/L, respectively (equivalent

to 0.2, 1, 2, 10, 20 µg/kg, respectively). Analyses (n = 6) were then conducted using the LCMS-8030 triple stage LC/MS/MS. Fig. 4 shows the MRM* chromatograms obtained from analysis of the 0.5 µg/L standard addition matrix sample, and Table 3 and Table 4 show the analytical conditions. The LCMS-8030 clearly demonstrated sufficient sensitivity to conduct identification testing.

*MRM...Multiple Reaction Monitoring

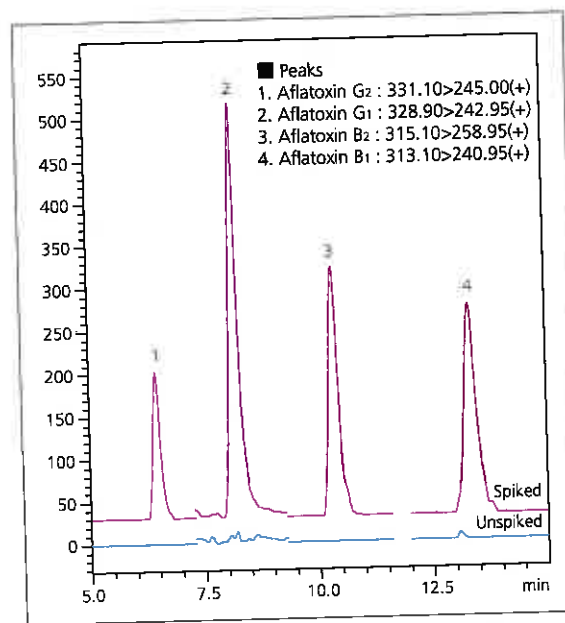


Fig. 4 MRM Chromatograms of Roasted Peanuts Matrix (Upper) Spiked, (Lower) Unspiked

Table 3 Analytical Conditions

[HPLC]	
Column	: Shim-pack FC-ODS (150 mmL, × 2.0 mmI.D., 3 µm)
Mobile Phase	: 10 mmol/L Ammonium acetate water / Methanol = 3/2 (v/v)
Flow Rate	: 0.2 mL/min
Column Temp.	: 40 °C
Injection Volume	: 6 µL
[MS]	
Probe Voltage	: +4.5 kV ESI-positive mode
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 15 L/min
DL Temp.	: 250 °C
Heat Block Temp.	: 400 °C

Table 4 MRM Mode Parameters

Compound	Transition	Dwell time (ms)	CE (V)	Resolution (Q1, Q3)
Aflatoxin B ₁	313.10 > 240.95	200	-40	Unit
Aflatoxin B ₂	315.10 > 258.95	200	-33	Unit
Aflatoxin G ₁	328.90 > 242.95	200	-30	Unit
Aflatoxin G ₂	331.10 > 245.00	200	-32	Unit

In addition, to evaluate the validity of the standard addition method for quantitation of aflatoxins, we checked the linearity of the calibration curves of spiked matrix samples, in addition to the repeatability. Fig. 5 shows the calibration curves, and Table 5 shows the average area and repeatability of the peaks corresponding to 0.25, 0.5 and 5 µg/L. Excellent linearity was obtained over the range of 0.05–5 µg/L, with a coefficient of determination greater than $R^2 = 0.999$, and good repeatability was also confirmed. As a result, identification testing was confirmed to be possible using the LCMS-8030, and quantitation using calibration curves of matrix samples spiked with standards was also confirmed to be effective.

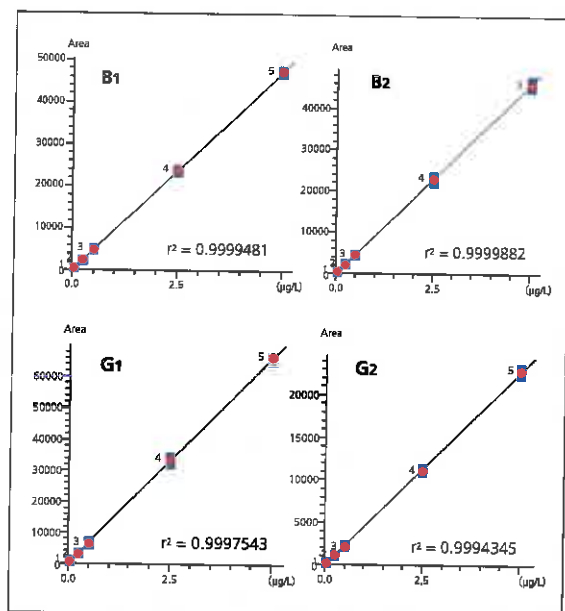


Fig. 5 Calibration Curves of Matrix Samples Spiked with Aflatoxins (Roasted Peanuts, 0.05–5 µg/L)

Table 5 Average and Repeatability of Peak Area (n = 6)

	0.25 µg/L		0.5 µg/L		5 µg/L	
	Area	%RSD	Area	%RSD	Area	%RSD
Aflatoxin B ₁	1010	6.37	2033	3.92	22936	1.49
Aflatoxin B ₂	3156	1.87	6481	5.09	66012	0.54
Aflatoxin G ₁	2209	6.07	4517	3.74	45841	1.45
Aflatoxin G ₂	2296	5.96	4749	4.74	47250	0.83

(3) Rapid Quantitation and Identification by Nexera UHPLC with LCMS-8030

Although TFA derivatization is specified in the quantitative test by HPLC according to the official test method, if the obtained value for total aflatoxins exceeds the reference value, follow-up analysis is required. The additional pretreatment is done without TFA derivatization (in Fig. 1 (B) for LC/MS analysis). However, if detection with sufficient sensitivity is achieved through direct detection (without TFA derivatization) as specified in the quantitative test, sample preparation, quantitative testing and identification can all be done using just the procedure of Fig. 1 (B). As a result, such a complicated pretreatment procedure does not need to be repeated. Furthermore, UHPLC what enables the high-throughput analysis brings even greater efficiency of the test for total aflatoxin.

Fig. 6 shows an example of the quantitative testing results obtained using the Shimadzu Nexera UHPLC after conducting pretreatment of aflatoxin-added roasted peanuts according to Fig. 1 (B), and Table 6 shows the analytical conditions. The roasted peanuts were spiked with aflatoxins B₁ and G₁ at 2.4 µg/kg each, and aflatoxins B₂ and G₂ at 0.6 µg/kg each. The accuracy and precision obtained for each substance in repeat analysis (n = 5) are shown in Table 7. Accuracy of 76–80 % and precision of 4.5–6.5 % were obtained, that satisfied the criteria of the specified reference value in the guideline.

In addition to the extremely good sensitivity of this analytical method as shown in Application News L422 and L430, it was confirmed that this method is effective for rapid quantitative testing of aflatoxins.

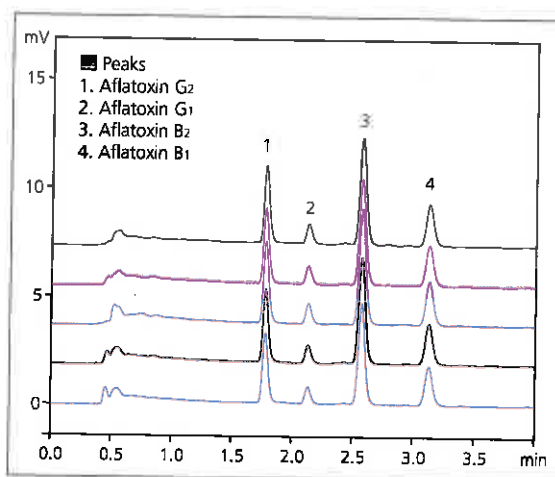


Fig. 6 Chromatograms of Roasted Peanuts Spiked with Aflatoxins (Direct Detection) (10 µL Injected)

Table 6 Analytical Conditions

Column	: Shim-pack XR-ODS II (100 mmL × 3.0 mmI.D., 2.2 µm)
Mobile Phase	: Water / Methanol / Acetonitrile = 6/3/1 (v/v/v)
Flow Rate	: 1.0 mL/min
Column Temp.	: 50 °C
Detection	: RF-20Axs, Ex. at 365 nm, Em. at 450 nm
RF Cell	: Conventional cell
Cell Temp.	: 25 °C
Injection Volume	: 10 µL

Table 7 Accuracy and Precision of Quantitation by UHPLC without Derivatization

	Aflatoxin B ₁	Aflatoxin B ₂	Aflatoxin G ₁	Aflatoxin G ₂
Recovery (%)	76	77	77	80
%RSD	4.5	5.1	6.1	6.5

Furthermore, since the LCMS-8030 is an LC/MS/MS that supports UHPLC, when used in combination with the high-speed analysis offered with the above-mentioned Nexera, rapid identification of aflatoxins becomes possible.

Fig. 7 shows the MRM chromatograms generated using high-speed analysis of the same sample of Fig. 4 using the analytical conditions of Table 8 and Table 9. All 4 aflatoxins eluted within 4 minutes, and identification was clearly obtained with sufficient sensitivity even under such high-speed conditions.

Fig. 8 shows the calibration curves generated using the same HPLC analytical conditions (Fig. 5) and matrix samples spiked with aflatoxins. Excellent linearity was obtained over the range of 0.05–25 µg/L, with a coefficient of determination greater than $R^2 = 0.999$. The peak area repeatability (%RSD, $n = 6$) obtained for each of the aflatoxin matrix samples at a concentration of 0.25 µg/L was B₁: 14.24 %, B₂: 5.33 %, G₁: 5.45 % and G₂: 18.9 %, demonstrating good repeatability even when using high-speed analysis.

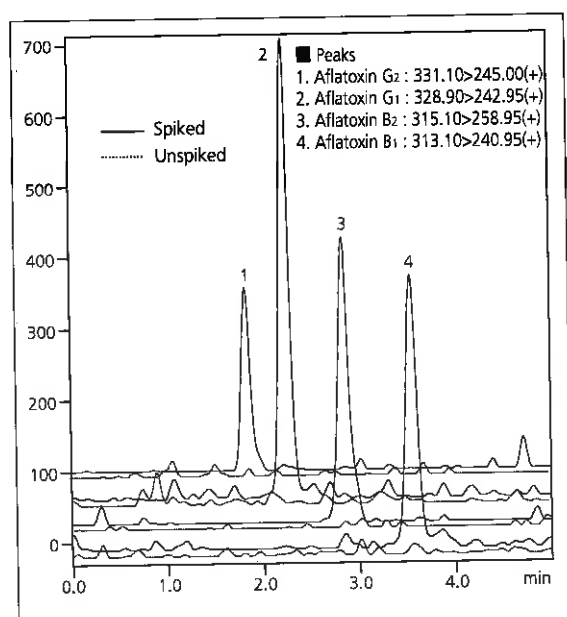


Fig. 7 MRM Chromatograms of Roasted Peanuts
(Upper, Solid Line) Spiked, (Lower, Broken Line) Unspiked

Table 8 Analytical Conditions

[HPLC]	
Column	: Shim-pack XR-ODS II (100 mmL × 2.0 mmID., 2.2 µm)
Mobile Phase	: 10 mmol/L Ammonium acetate water / Methanol = 3/2 (v/v)
Flow Rate	: 0.45 mL/min
Column Temp.	: 50 °C
Injection Volume	: 6 µL
[MS]	
Probe Voltage	: +4.5 kV ESI-Positive mode
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 15 L/min
DL Temp.	: 250 °C
Heat Block Temp.	: 400 °C

Table 9 MRM Mode Parameters

Compound	Transition	Dwell time (ms)	CE (V)	Resolution (Q1, Q3)
Aflatoxin B ₁	313.10 > 240.95	100	-40	Unit
Aflatoxin B ₂	315.10 > 258.95	100	-33	Unit
Aflatoxin G ₁	328.90 > 242.95	100	-30	Unit
Aflatoxin G ₂	331.10 > 245.00	100	-32	Unit

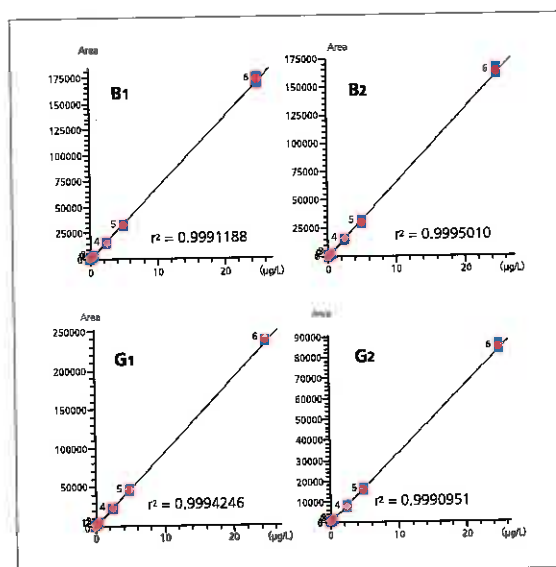


Fig. 8 Calibration Curves of Matrix Samples Spiked with Aflatoxins (Roasted Peanuts, 0.05–25 µg/L)

[References]

- 1) Handling of Foods Containing Aflatoxins (Japanese Ministry of Health, Labour and Welfare, Dept. of Food Safety Issue 0331 No. 5, March 31, 2011)
- 2) Test Method for Total Aflatoxins (Dept. of Food Safety Issue 0816 No. 1, August 16, 2011)

Application News

No. AD-0089

Food Safety Analysis / LCMS-8050

Analysis of Residual Chloramphenicol in Shrimp by LC/MS/MS with QuEChERS Sample Pre-treatment

□ Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic that is widely used in the growth of animals and marine culture [1]. However, CAP can cause aplastic anaemia and is a possible carcinogen in humans [2]. Its use in livestock farming including fish and shrimps has been banned in European Union and many other countries. However, illegal use of CAP is still present due to its low cost and ready availability. As there is not a safe maximum residue level (MRL) determined in food for CAP, a highly selective and sensitive analytical method to detect CAP residues in complex matrices such as food samples is required. Here, we present a LC/MS/MS method for accurate and reliable quantitation of CAP in shrimp samples on LCMS-8050 with a heated ESI interface. The shrimp samples were pre-treated with a modified QuEChERS procedure. The method performance was evaluated with spiked samples and exhibits good accuracy, reproducibility, linearity and specificity over the concentration range from 0.005-20 ng/mL. The quantitation limit of the method with 5 μ L injection volume are determined to be 0.01 ng/mL, which is equivalent to 0.2 μ g/kg of CAP in shrimp samples after incorporating a dilution factor of 20 from the sample pre-treatment procedure. This value satisfies the Minimum Required Performance Limit (MRPL) set at 0.3 μ g/kg by the European Union.

□ Experimental

Instrumental and analytical conditions

A high sensitivity LCMS-8050 (Shimadzu Corporation) was used in this work. The new heated ESI probe incorporated into LCMS-8050 improves desolvation efficiency, matrix removal and hence enhances the sensitivity of this instrument. The details of column, mobile phases and gradient program of LC separations and MS conditions are compiled into Table 1a and Table 1b.

Standards and samples

Chloramphenicol standard was purchased from Sigma Aldrich and QuEChERS sample pre-treatment kit was obtained from Restek. Fresh shrimp samples were purchased from the local supermarket. The procedure of sample preparation for shrimp samples using a modified QuEChERS procedure is illustrated in Figure 1. It includes spiking of standards into homogenized shrimp samples, extraction and clean-up with pre-weighed salts from QuEChERS kit. The supernatant collected after clean-up was diluted 10 times with ultrapure water, filtered and injected directly to the LC/MS/MS system.

Table 1a: LC/MS/MS analytical conditions (LC) for CAP analysis

LC Condition	
Column	Ascentis® Express C18, 100 x 2.1mm, 2.7 μ m
Mobile Phase	A: Water B: Acetonitrile
Elution Program	10% (0.0 to 1.0min) $\rightarrow$ 95% (1.0 to 6.0min) $\rightarrow$ 95% (6.0 to 10.0min) $\rightarrow$ 10% (10.0min to 11.0min) $\rightarrow$ 10% (11.0 to 13.0min)
Flow Rate	0.40 mL/min
Oven Temp	40 °C
Injection	5 μ L

Table 1b: LC/MS/MS analytical conditions (MS) for CAP analysis

MS Interface Condition	
MS Mode	ESI, Negative
Block Temp.	400°C
Interface Temp	300°C
DL Temp.	250°C
Nebulizing Gas Flow	N ₂ , 2.0L/min
Drying Gas Flow	N ₂ , 10.0L/min
Heating Gas Flow	Zero air, 10.0L/min
CID Gas	Ar (270kPa)

- [1] Weigh 10g homogenized sample in 50mL centrifuge tube
 - ↓ Add 4mL water, 16mL acetonitrile and 20μl formic acid
- [2] Add Q-sep Q100 Packet salt
 - ↓ Shake vigorously, vortex for 20 mins and centrifuge at 6000rpm for 10 mins
- [3] Transfer ACN supernatant into 20mL volumetric flask and wash with 4mL ACN
 - ↓ Combine washing into volumetric flask and top up to mark
- [4] Transfer ACN solution to 50mL centrifuge tube and add 20mL hexane, vortex for 5 mins
 - ↓ Centrifuge at 6000rpm for 10 mins and discard hexane (top layer)
- [5] Transfer 1mL ACN solution into 2mL QuEChERS dSPE tube, Cat. #26125
 - ↓ Vortex 2 min and centrifuge 10min at 13000rpm
- [6] Transfer 100μL extract to 1.5mL tube
 - ↓
- [7] Dilute with 900μL of ultrapure water
 - ↓ Filter through 0.2μm Nylon syringe filter
- [8] Analyze by Shimadzu LCMS-8050

Note :

- For pre-spiked recovery samples, spike 10μL of Chloramphenicol of known concentration in step 1.
- For post-spiked calibration curve, spike 10μL of Chloramphenicol of known concentration in step 6 and add 890μL of water instead in step 7.
- Total dilution factor: 20

Figure 1: Flow chart of shrimp sample pre-treatment

Results and Discussion

Development of LC/MS/MS method

A MRM quantitation method for chloramphenicol was developed on LCMS-8050 using an ESI interface. The MRM optimization was performed in negative mode using an automated MRM optimization program with LabSolutions workstation. Two optimized MRM transitions for chloramphenicol were selected (Table 2), the first transition was used for quantitation and the second transition for confirmation.

Table 2: MRM transitions and voltage parameters of Chloramphenicol

Name	RT (min)	MRM Transition (m/z)		Voltage (V)		
		Precursor [M-H] ⁻	Product	Q1 Pre Bias	CE	Q3 Pre Bias
CAP	3.326	321.0	152.2	21	16	30
			194.2	21	10	12

Calibration curve, linearity and accuracy

Two sets of standard samples were prepared by spiking in diluent and post-spiking in shrimp matrix obtained after sample clean-up. Each set included nine levels (triplicate) from 0.005 ng/mL to 20 ng/mL (Table 3). The processing of the calibration curves includes setting up a weighting method of 1/C. Linearity with R^2 greater than 0.999 were established as shown in Figure 2. The MRM chromatograms of standards spiked in diluent are shown in Figure 3. The accuracy of the MRM based quantitation method is between 95.3% and 112.7%.

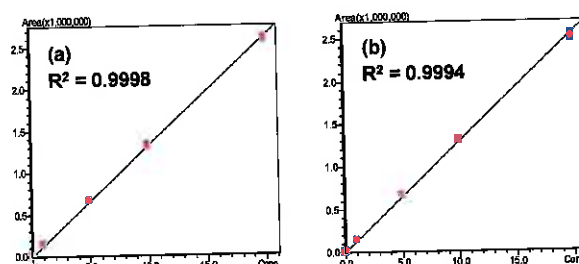


Figure 2: Calibration curves of chloramphenicol in diluent (a) and post-spiked shrimp matrix (b).

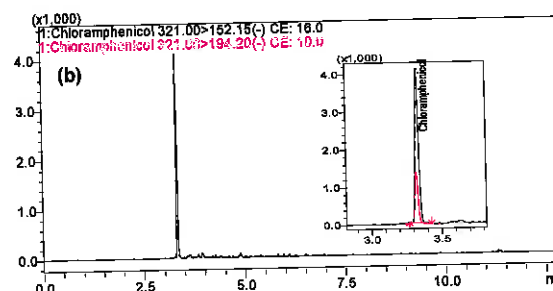
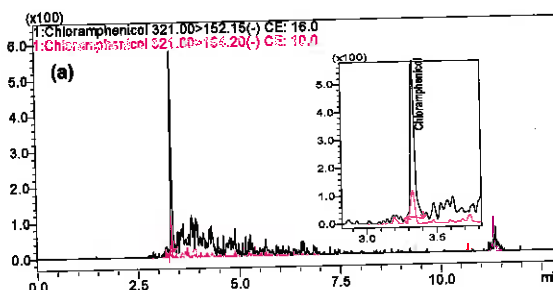


Figure 3: MRM chromatograms of chloramphenicol at 0.005 ng/mL (a) and 0.05 ng/mL (b) in diluent.

Matrix effect, recovery, repeatability and LOD/LOQ

Matrix effect of the MRM method was evaluated by comparison of peak areas obtained from calibration curve in diluent and in shrimp matrix. The calculation was based on the average area obtained from triplicate injections of every concentration from 0.01 ng/mL to 20 ng/mL.

It was noticed that the shrimp matrix blank contained an interference at the same retention time as chloramphenicol.

Table 3: Summary of performance evaluation for chloramphenicol

Concentration (ng/mL)	Area		Accuracy (%)		Matrix Effect (%)
	Neat	Post-spiked	Neat	Post-spiked	
0.010	1478	2506	112.7	95.3	169.5
0.025	3412	4445	104.1	98.7	130.2
0.050	7012	9010	107.0	117.5	128.6
0.100	14539	14428	110.9	103.0	99.2
1.000	136544	140032	104.1	108.5	102.6
5.000	670056	658068	102.2	102.7	98.2
10.00	1315274	1297491	100.3	101.3	98.6
20.00	2595906	2512621	99.0	98.2	96.8

Neuhaus et al. [3] reported also the observation of an interference MRM (m/z 321>152) peak which appeared at the retention time as chloramphenicol. By using high resolution LC-TOF, we confirmed that the interference was not from chloramphenicol. Hence, matrix effect as well as recovery of chloramphenicol in the shrimp matrix were calculated after compensating the area from the interference [3]. The matrix effect of the method obtained is between 169.5% and 96.8% for the spiked concentration between 0.01 ng/mL and 20 ng/mL (Table 3).

Recovery of the sample pre-treatment method was determined by comparing the peak area obtained from pre-spike and post-spike shrimp samples. Shrimp samples were pre-spiked with 0.5, 2 and 20 ng/mL (final analyzed concentration was 0.025, 0.1 and 1 ng/mL respectively) of chloramphenicol and sample clean-up was done using the modified QuEChERS procedure. Each concentration level was repeated in triplicates over three days, a total of 9 samples being prepared and analyzed. Each sample was injected for three times and the average area of the three injections was used for recovery calculation. The recovery results are between 81% and 110% (Table 4).

Repeatability of the method was evaluated with standard samples in diluent and in post-spiked shrimp matrix. The concentrations used for repeatability studies were 0.005, 0.01 and 0.025 ng/mL and the RSD results for seven consecutive injections are tabulated in Table 5. The RSD values of chloramphenicol in diluent and shrimp matrix are below 6.3% for all three concentrations.

The LOD and LOQ of the method were determined directly from the calibration curve of chloramphenicol post-spiked in shrimp matrix. The LOQ of the method was found to be 0.01 ng/mL. The LOD was estimated to be or better than 0.005 ng/mL.

Table 4: Summary of recovery results (inter-day, n=3)

Concentration (ng/mL)	Recovery % (triplicates)			Average Recovery % (n=3)	Recovery %RSD (n=3)
	Day 1	Day 2	Day 3		
0.025	89.6	114.6	125.5	109.9	16.7
0.10	85.4	77.3	80.3	81.0	5.0
1.00	100.0	83.8	96.1	93.3	9.1

Table 5: Summary of repeatability results (n=7)

Concentration (ng/mL)	RSD%	
	Neat	Post-spiked
0.005	6.3	3.8
0.010	5.3	1.7
0.025	6.1	2.0

□ Conclusions

A LC/MS/MS method with ESI had been developed and optimized for quantitative determination of chloramphenicol residue in shrimp samples using a modified QuEChERS procedure for sample pre-treatment. The QuEChERS procedure used for sample pre-treatment shows its advantages of being simpler, faster and very efficient. The detection and quantitation range for this analysis method was from 0.005 to 20 ng/mL with a 5µL injection volume. The LOQ of this method was determined to be 0.010 ng/mL, which is equivalent to 0.20 µg/kg of chloramphenicol in shrimp samples and satisfy the MRPL set at 0.3 µg/kg by the European Union. The performance of the method was evaluated thoroughly, including linearity, accuracy and repeatability. The results obtained from matrix effect and recovery indicated that the LC/MS/MS method with simple QuEChERS sample pre-treatment is highly sensitive and reliable in detection and quantitation of chloramphenicol in shrimp samples.

□ References

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2. Evangelos Gikas et al, "Development of a Rapid and Sensitive SPE-LC-ESI MS/MS Method for the Determination of Chloramphenicol in Seafood" *J. Agric. Food Chem.*, 52 (5) (2004): 1025-1030
3. B.K. Neuhaus, J.A. Hurlbut, and W. Hammack, "LC/MS/MS Analysis of Chloramphenicol in Shrimp" *U.S. FDA Laboratory Information Bulletin* No. 4290, Volume 18, No. 9, September 2002



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Application News

No.C120

Liquid Chromatography Mass Spectrometry

Analysis of Glufosinate, Glyphosate, and AMPA in Drinking Water Using a Triple Quadrupole LC/MS/MS System

Glufosinate is a popular amino acid-based herbicide and glyphosate a popular foliage treatment herbicide. Glyphosate metabolizes in soil or water to form aminomethylphosphonic acid (AMPA).

In March 2015, the Japan's Ministry of Health, Labour and Welfare, Health Service Bureau, Water Supply Division issued a notification (No. 0325 Item 3 - 6) specifying the use of LC/MS/MS for water quality control inspections of glufosinate, since it is one of the pesticides designated for monitoring based on the specified target values for drinking water quality control, but no method for inspecting them has been specified so far.

Sample Pretreatment

Method 22 involves first derivatizing samples with 9-fluorenylmethyl chloroformate (Fmoc-Cl) under basic conditions and then concentrating the samples with solid phase extraction. The structural formulas of derivatized glufosinate, glyphosate, and AMPA are shown in Fig. 1.

A flowchart of the pretreatment process is shown in Fig. 2.

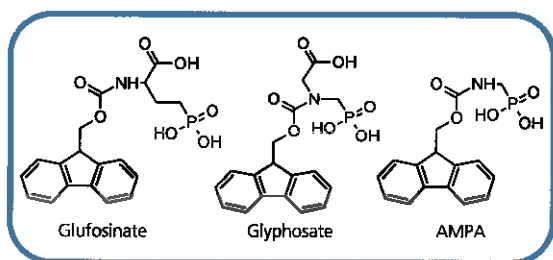
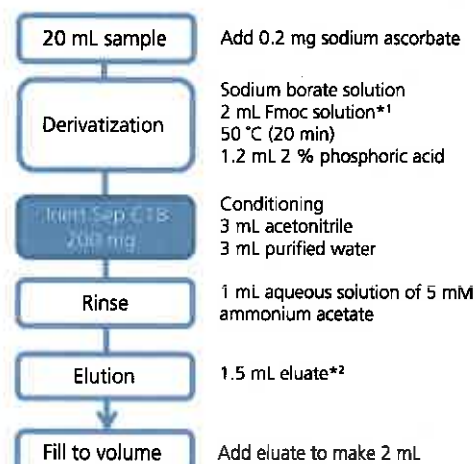


Fig. 1 Structure of Each Fmoc Derivative

The Appendix Method 22 specified for glyphosate inspection by simultaneous analysis using a "derivatization - solid phase extraction - liquid chromatograph-mass spectrometer" system, can analyze both glyphosate and AMPA at the same time, which were previously inspected using separate methods (Appendix Methods 12 and 15) involving high-performance liquid chromatography.

In this example, Appendix Method 22 is used to analyze glufosinate, glyphosate, and AMPA. In addition, by using the LCMS-8050, samples can be analyzed directly without the pretreatment process of concentrating samples by solid phase extraction.



*1 Fmoc 1 mg/mL acetonitrile solution

*2 Acetonitrile / 5 mmol/L ammonium acetate = 40/60

Fig. 2 Pretreatment Process

Analyzing Glufosinate, Glyphosate, AMPA Standard Solutions (with Solid Phase Extraction)

After derivatizing the glufosinate, glyphosate, and AMPA standard mixture solution (with 0.1 µg/L each), the solution was concentrated by ten times with solid

phase extraction, and measured. The resulting MRM chromatograms are shown in Fig. 3. Analytical conditions are indicated in Table 2.

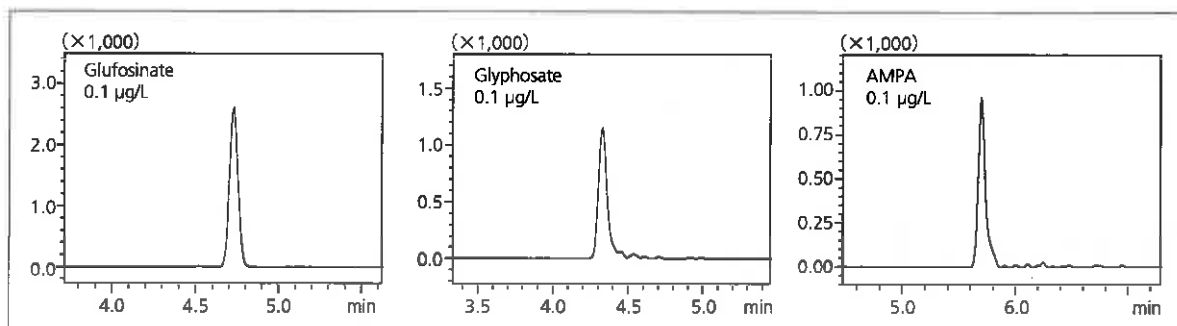


Fig. 3 MRM Chromatograms of Glufosinate, Glyphosate, AMPA Standard Solutions (with Solid Phase Extraction)

Analyzing Glufosinate, Glyphosate, AMPA Standard Solutions by Derivatization and Direct Analysis

The glufosinate, glyphosate, and AMPA standard mixture solutions (0.1 µg/L) were also analyzed without pretreatment by solid phase extraction, after only derivatization. The resulting MRM chromatograms and area value %RSD (n = 5) are shown in Fig. 4. Even for

concentrations lower than 1/100 of the target value, results easily satisfied the criteria of less than 30 %RSD and even if the sample concentration step is skipped, high-sensitivity analysis is possible using the LCMS-8050.

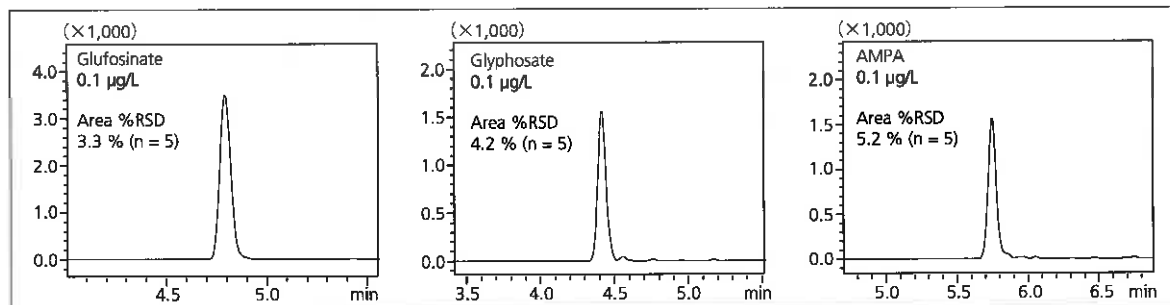


Fig. 4 MRM Chromatograms of Glufosinate, Glyphosate, AMPA Standard Solutions (Without Solid Phase Extraction)

Spike and Recovery Test Using Drinking Water (Derivatization - Direct Analysis)

Glufosinate, glyphosate, and AMPA were added to actual public drinking water and the recovery rate was evaluated by analyzing samples with only derivatization, without solid phase extraction pretreatment. MRM chromatograms for the blank drinking water, and water spiked respectively with 0.2 µg/L glufosinate, glyphosate, and AMPA are shown in Fig. 5. The corresponding recovery rates are indicated in Table 1. Good recovery rates (accuracy) were obtained within 70 to 120 %.

Table 1 Spike and Recovery Test Results (n = 5)

	Recovery Rate (%)	
	0.2 µg/L Added	2 µg/L Added
Glufosinate	96	99
Glyphosate	88	77
AMPA	96	104

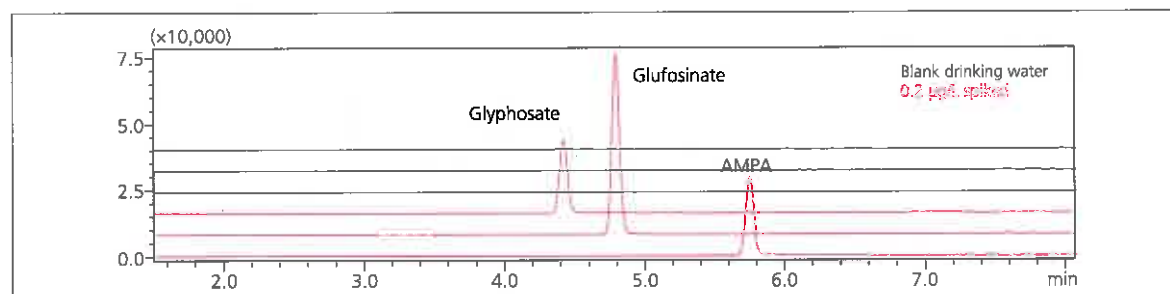


Fig. 5 MRM Chromatograms for Drinking Water (Blank) and Water Spiked with Glufosinate, Glyphosate, and AMPA (Without Solid Phase Extraction)

Table 2 Analytical Conditions

Column	: Mastro C18 (100 mm L. x 2.1 mm I.D., 3 µm, Shimadzu GLC)		
Mobile Phases	: A: 5 mmol/L Ammonium acetate-Water B: Acetonitrile		
Flowrate	: 0.25 mL/min		
Time Program	: B: conc 5 % (0 min) → 50 % (7 min) → 95 % (7.01 - 11 min) → 5 % (11.01 - 13 min)		
Column Temperature	: 40 °C		
Injection Volume	: 2 µL (With solid phase extraction), 20 µL (Without solid phase extraction)		
Probe Voltage	: - 3 kV (ESI - Negative)		
DL Temperature	: 150 °C		
Block Heater Temperature	: 400 °C		
Interface Temperature	: 300 °C		
Nebulizing Gas Flow	: 2 L/min		
Drying Gas Flow	: 10 L/min		
Heating Gas Flow	: 10 L/min		
MRM Transition	Glyphosate	m/z 390 > 168	Glyphosate m/z 402 > 180
	AMPA	m/z 332 > 110	

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Application News

No.C119

Liquid Chromatography Mass Spectrometry

Analysis of Cartap, Pyraclonil, and Ferimzone in Drinking Water Using the Triple Quadrupole LCMS-8050

Cartap, pyraclonil, and ferimzone are widely used agricultural insecticides, with pyraclonil and ferimzone intended for use in flooded rice fields. These pesticides are designated for monitoring based on target values for drinking water quality control. Though target values were specified, no method for inspecting them has been specified so far.

However, in March 2015, the Japan's Ministry of Health, Labour and Welfare, Health Service Bureau, Water Supply Division issued a notification (No. 0325 Item 3 - 6) specifying the use of LC/MS/MS for water quality

control inspections of cartap, pyraclonil, and ferimzone, for which an inspection method was not previously specified.

This article describes an example of inspecting samples for these three components by simultaneous analysis using a liquid chromatograph-mass spectrometer system, as specified in Appendix Method 20-2. Cartap decomposes to nereistoxin in water, so the Cartap is measured as nereistoxin. Ferimzone includes type E and Z isomers, the concentrations of which are totaled to determine the Ferimzone concentration.

Analyzing a Standard Mixture Solution of Nereistoxin (Cartap), Pyraclonil, and Ferimzone

A standard solution with 1/100 the target concentration of nereistoxin, pyraclonil, and ferimzone respectively was measured, with the resulting MRM chromatogram shown in Fig. 1.

Fig. 2 shows calibration curves for a concentration range that includes 1/100 of each target concentration value, as well as indicating the repeatability at the lowest calibration point concentration. The results showed good linearity and repeatability for all substances.

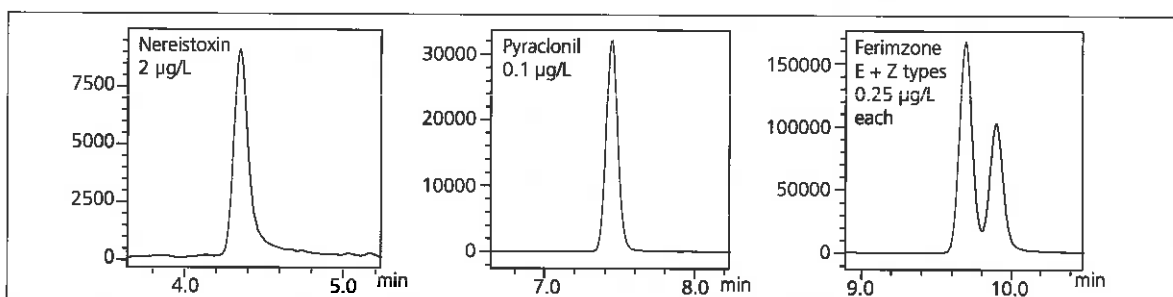


Fig. 1 MRM Chromatograms for Each Substance in a Standard Mixture Solution of Nereistoxin (Cartap), Pyraclonil, and Ferimzone

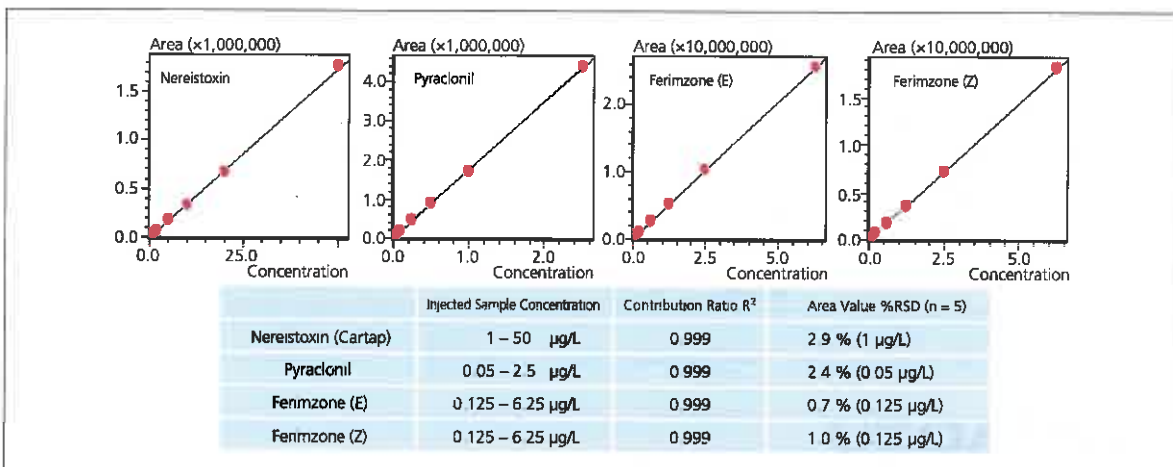


Fig. 2 Contribution Ratio and Area Repeatability

Spike and Recovery Test Using Drinking Water

Drinking water was spiked with nereistoxin, pyraclostrobin, and ferimzone to test the recovery rate. The public drinking water was treated to remove residual chlorine using sodium thiosulfate, rather than sodium ascorbate. (20 mg was added per liter of drinking water.)

Fig. 3 shows MRM chromatograms of blank pretreated drinking water and pretreated drinking water spiked with 1/100 the target value of each substance (2 µg/L nereistoxin, 0.1 µg/L pyraclostrobin, and 0.125 µg/L each of ferimzone types E and Z).

The recovery rate was calculated from the average of area values measured from five repetitions.

Good results were obtained for each substance, with 102 % recovery for nereistoxin, 95 % for ferimzone E, 100 % for ferimzone Z, and 95 % for pyraclostrobin.

Analytical conditions for these measurements are listed in Table 2.

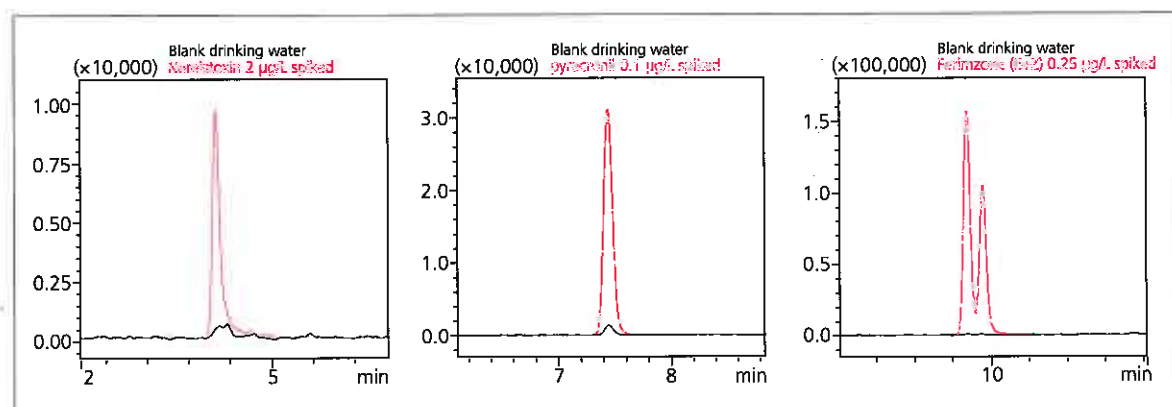


Fig. 3 MRM Chromatograms for Blank Drinking Water and Drinking Water Spiked with Nereistoxin, Pyraclostrobin, and Ferimzone

Table 1 Spike and Recovery Test Results (n = 5)

	Spike Concentration	Recovery Rate (%)
Nereistoxin (Cartap)	2 µg/L	102
Pyraclostrobin	0.1 µg/L	95
Ferimzone (E)	0.125 µg/L	95
Ferimzone (Z)	0.125 µg/L	100

Table 2 Analytical Conditions

Column	: L-Column2 ODS (75 mm L. × 2.1 mm I.D., 2 µm, CER)
Mobile Phases	: A 5 mmol/L Ammonium acetate-Water B 5 mmol/L Ammonium acetate-Methanol
Flowrate	: 0.2 mL/min
Time Program	: B. Conc 5 % (0 min) → 45 % (2 min) → 75 % (12 - 13.5 min) → 5 % (13.51 - 20 min)
Column Temperature	: 40 °C
Injection Volume	: 10 µL
Probe Voltage	: 4 kV (ESI-Positive)
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C
Interface Temperature	: 200 °C
Nebulizing Gas Flow	: 2 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
MRM Transition	: Nereistoxin (Cartap) m/z 150 > 105 Pyraclostrobin m/z 315 > 169 Ferimzone m/z 255 > 91

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Application Data Sheet

No.40

LC-MS

Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of 36 Veterinary Drugs using Triple Quadrupole LC/MS/MS

The Japanese Ministry of Health, Labor, and Welfare (MHLW) has established an analytical standard for each veterinary drug found in food. Instrumentation measuring these standards must have sensitivity exceeding 1 ng / mL per analyte in order to detect trace levels of these drugs in food. Additionally, because the MHLW has established many compounds as residue standards, instrumentation used for these analyses must have the capability to make simultaneous measurements with sensitivity, specificity, and speed.

This report illustrates the simultaneous analysis of 36 veterinary drugs measured in 15 minutes using the Shimadzu LCMS-8050 Ultra Fast Triple Quadrupole Mass Spectrometer, featuring ultrafast polarity switching. The polarity switching speed of the LCMS-8050 is just 5 milliseconds.

Combination of the Nexera X2 LC and the LCMS-8050 provides incomparably fast run times without compromising the quality of the results.

HPLC conditions (Nexera X2)

Column	: YMC-Triart C18 (Manufactured by YMC) (2.0 mm I.D. × 150 mm L., 1.9 μm)
Mobile phase A	: 0.1% formic acid in water
Mobile phase B	: Acetonitrile
Time program	: 1% B. (0.00 min) → 10% B. (0.10 min) → 80% B. (11.00 min-12.00 min) → 1% B. (12.01 -15.00min)
Flow rate	: 0.4 mL/min
Injection volume	: 5 μL
Column oven	: 40 °C

MS conditions (LCMS-8050)

Ionization	: ESI (Positive / Negative)
Nebulizing gas flow	: 2.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
Interface temperature	: 300 °C
DL temperature	: 200 °C
Heat block temperature	: 200 °C
Interface voltage	: +0.5, +1.5, +5.0kV (Positive) -1.0, -4.0kV (Negative)

* Interface voltage was optimized for each compound

Compound	Polarity	Precursor (m/z)	Product (m/z)	Area reproducibility (1 ng/mL)
Dexamethasone	+	393.30	337.30	10.3%
Clopidol	+	191.70	101.10	2.2%
Enrofloxacin	+	360.00	316.20	4.1%
Flubendazole	+	314.10	282.05	3.2%
Flumequine	+	282.10	244.05	0.9%
Mebendazole	+	296.10	264.00	2.1%
Nalidixic acid	+	233.10	215.05	1.5%
Orbifloxacin	+	396.00	352.20	6.8%
Oxolinic acid	+	261.90	160.00	4.2%
Sarafloxacin	+	386.00	299.10	9.3%
Tylosin	+	916.50	174.10	2.2%
Warfarin	+	309.05	163.00	0.9%
Ciprofloxacin	+	331.90	288.20	11.7%
Thiabendazole	+	202.10	175.00	2.5%
Levamisole	+	205.10	178.20	3.4%
Sulfachloropyridazine	+	285.10	156.10	1.2%
5-Hydroxythiabendazole	+	218.10	191.20	1.7%
Benzocaine	+	166.10	138.20	8.4%

Compound	Polarity	Precursor (m/z)	Product (m/z)	Area reproducibility (1 ng/mL)
Bromacil	+	261.00	205.00	0.6%
Diaveridine	+	261.15	123.10	0.9%
Famphur	+	326.00	93.10	0.9%
Josamycin	+	828.55	173.95	2.4%
Meloxicam	+	352.10	115.20	1.2%
Menbutone	+	259.25	241.10	2.8%
Oxibendazole	+	250.00	176.30	0.5%
Sulfaethoxypyridazine	+	295.15	156.05	1.6%
Valemline	+	565.40	263.20	3.7%
Cafopezzone	+	646.40	143.30	9.4%
Difloxacin	+	400.10	356.20	2.2%
Methylprednisolone	+	375.20	161.20	3.6%
Nafcillin	+	415.10	199.10	1.3%
Ofloxacin	+	362.10	148.30	12.4%
Phenoxyethylpenicillin	-	349.10	208.30	1.1%
Thiamphenicol	-	353.80	185.10	11.3%
Florfenicol	-	356.10	185.30	5.4%
Clorsulon	-	377.90	342.20	2.2%

Table 1: MRM transition and area reproducibility at 1 ng / mL

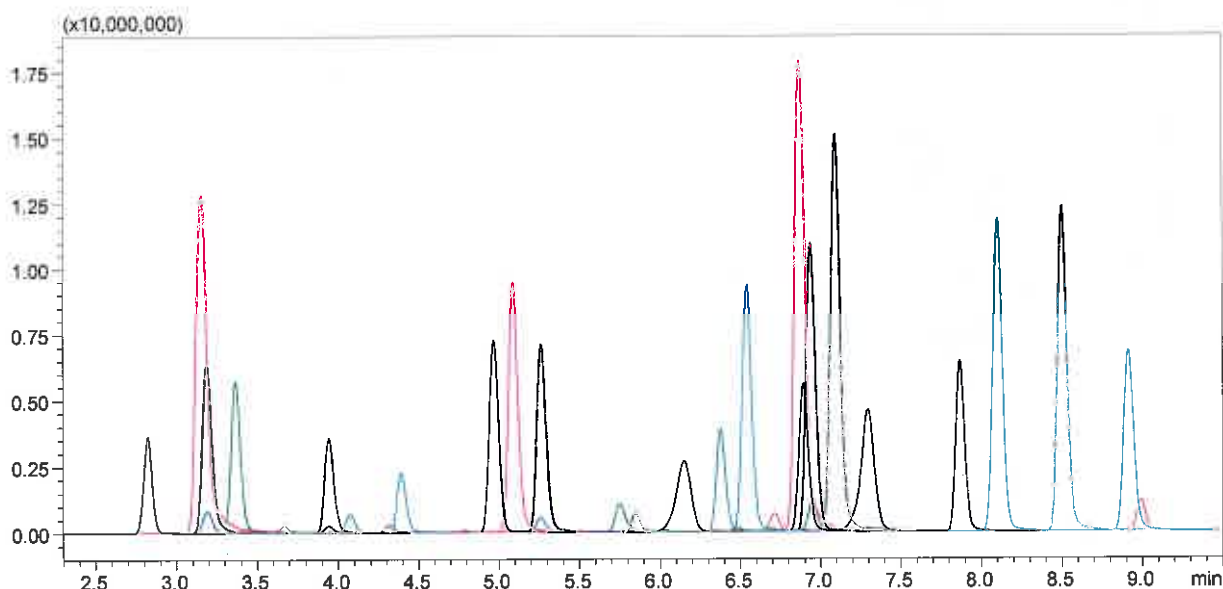


Fig. 1: Representative MRM chromatograms for 36 veterinary drugs at 0.5 µg / mL

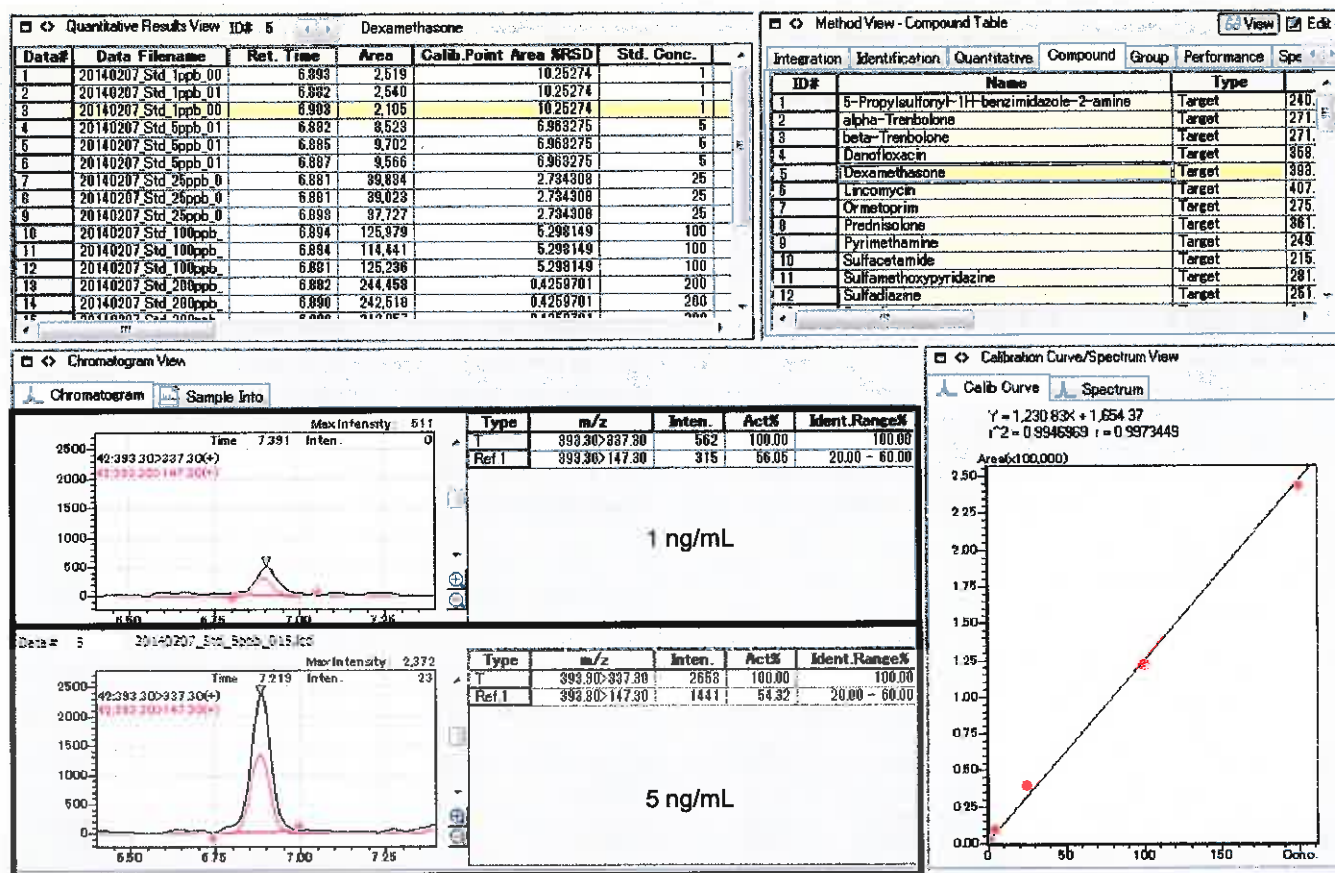


Fig. 2: Results of Dexamethasone

Area %RSDs of these 36 veterinary drugs are less than 20% at 1 ng / mL and all compounds show excellent linearity (R^2), greater than 0.99. Results above are from Dexamethasone.

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Application News

No.C104

Liquid Chromatography Mass Spectrometry

Analysis of Diarrhetic Shellfish Toxin Using Triple Quadrupole LC/MS/MS (LCMS-8050)

The Japanese Ministry of Health, Labour and Welfare (JMHLW) specified in July, 1980 that the mouse bioassay (MBA) be used as the official method for diarrhetic shellfish toxin, and that the permissible exposure limit be 0.05 MU per gram of edible shellfish*. Shellfish in which the toxin exceeds this limit are prohibited from being sold at market according to the Japanese Food Sanitation Law Article 6, Item 2.

Due to significant technological advances since 1980, the sensitivity and accuracy obtained using the MBA method are significantly inferior compared to the high-precision, high-sensitivity possible using liquid chromatography mass spectrometry analytical instrumentation, which is currently used for this application. A complete transition to instrumental analysis for lipophilic marine biotoxins is scheduled to be implemented by January 2015 throughout the EU.

Based on this international trend, the JMHLW is currently considering migration to an instrumental analysis assay and setting new reference values to be used with instrumental analysis, in addition to the introduction of the Codex standard for okadaic acids (OA, Reference 1).

Table 1 CODEX Standard 292-2008

Reference Value	
OA Adds (OA and DTX group)	Permissible ingestion limit of 0.16 mg OA per kg of edible shellfish

Fig. 1 shows examples of LC/MS/MS high-sensitivity analysis of okadaic acid (OA), dinophysistoxin 1 (DTX1) and pectenotoxins (PTX1, 2, 6) and yessotoxin 1 (YTX1). Thus, it is possible to conduct high-sensitivity, high-separation analysis of each component.

Fig. 2 and Fig. 3 show MRM chromatograms of standard samples of OA and DTX1, respectively.

* The amount of toxin resulting in the death of two out of three mice following intraperitoneal administration of the equivalent of 20 g per edible shellfish.

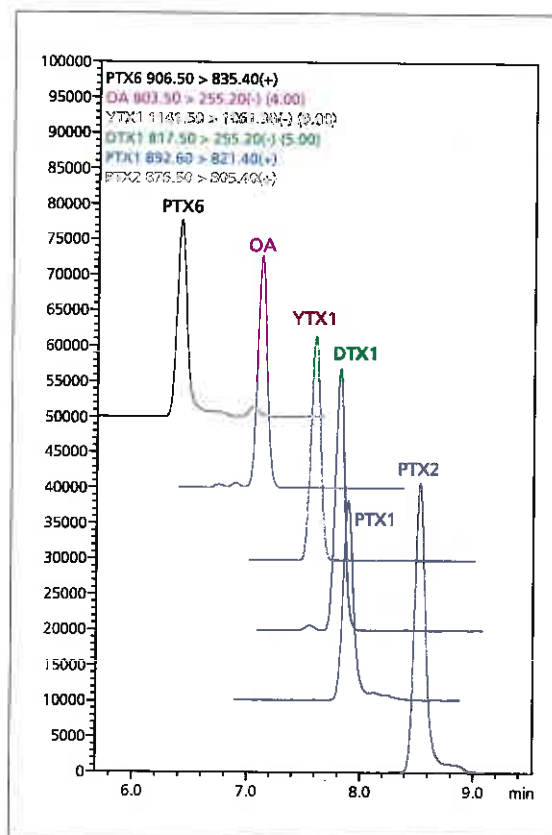


Fig. 1 MRM Chromatograms of Diarrhetic Shellfish Toxin (1 ng/mL)

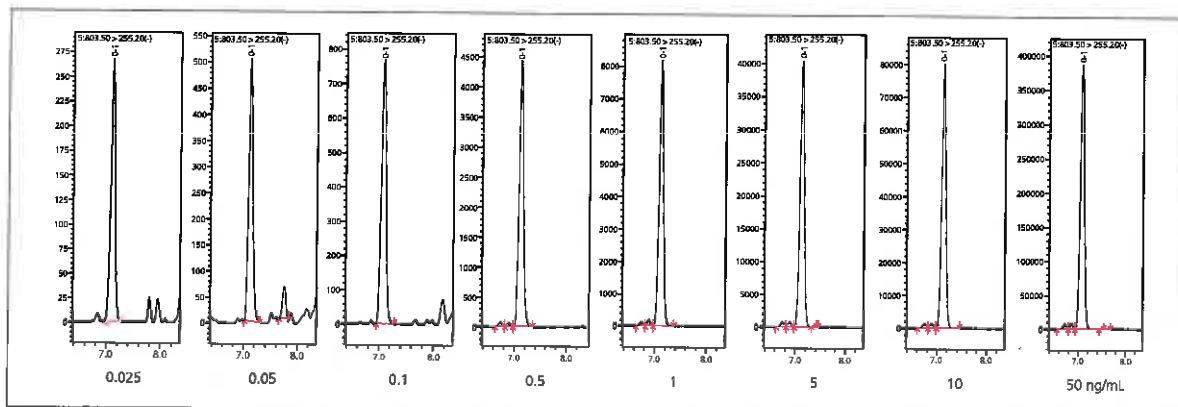


Fig. 2 MRM Chromatograms of Okadaic Acid (OA)

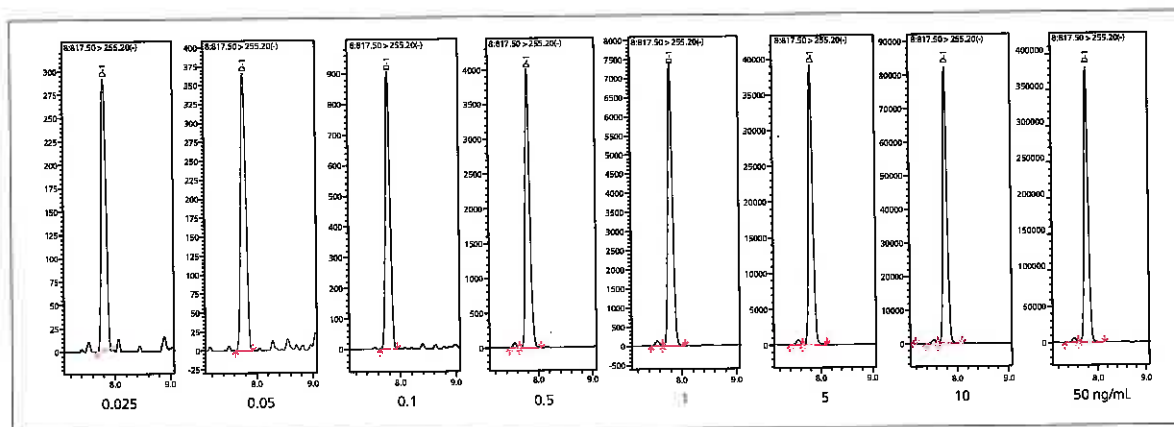


Fig. 3 MRM Chromatograms of Dinophysistoxin 1 (DTX1)

In addition, the calibration curves of OA and DTX1 are shown in Fig. 4. In both cases, the coefficient of determination R^2 was greater than 0.9999, indicating excellent linearity. Comparable linearity was also obtained for the other four substances.

Thus, instrumental analysis of shellfish by LC/MS/MS offers high sensitivity and accuracy, making it a highly effective analytical method. For this reason it is attracting attention as an alternative to the traditional MBA method.

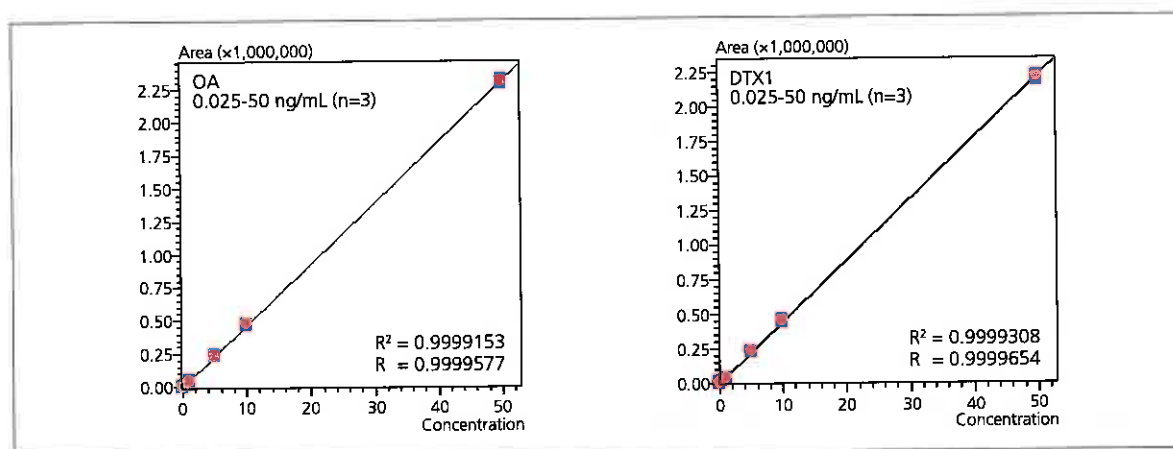


Fig. 4 Calibration Curves of OA and DTX1

Table 2 Analytical Conditions

Column	: InertSustain C8 (50 mm L. × 2.1 mm I.D., 3 μm)
Mobile Phases	: A 2 mmol/L Ammonium Formate – Water (pH adjusted to 8.5 with ammonia water) : B Methanol
Time Program	: 20 %B (0 min) – 100 %B (10 min) – 20 %B (10.01 min) – STOP (15 min)
Flowrate	: 0.2 mL/min
Column Temperature	: 40 °C
Injection Volume	: 10 μL
Probe Voltage	: +4.0 kV/-3.0 kV (ESI-positive / negative mode)
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C
Interface Temperature	: 350 °C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
MRM Transition	: (+) PTX6 906.50 > 835.40, PTX1 892.60 > 821.40, PTX2 876.50 > 805.40 : (-) OA 803.50 > 255.20, YTX1 1141.50 > 1061.30, DTX1 817.50 > 255.20

The diarrhetic shellfish toxin standards were provided courtesy of Dr. Toshiyuki Suzuki of the Japanese National Research Institute of Fisheries Science.

Reference 1: July, 2014, Food Safety Commission of Japan "Natural Poison Evaluation Report – Okadaic Acid Group Among Bivalves"
<http://www.fsc.go.jp/fscis/evaluationDocument/list?itemCategory=009>

First Edition: Apr. 2015



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Table 2. Method 1 MS acquisition parameters, retention time and internal standard

Compound	Ret. time (min)	Polarity	SRM transitions	Q1 (V)	CE	Q3 (V)	ISTD	MS1 Res.	MS2 Res.
Amitrole	3.1	Positive	85 > 43	-14	-25	-14	Paraquat d8	Unit	Unit
			85 > 57	-14	-20	-20		Unit	Unit
			85 > 58	-14	-23	-22		Unit	Unit
Chlormequat	4.1	Positive	122 > 58	-28	-27	-21	Chlormequat d4	Unit	Unit
			122 > 59	-28	-23	-21		Unit	Unit
			122 > 63	-28	-22	-23		Unit	Unit
Daminozide	2.2	Positive	161 > 143	-16	-14	-25	Chlormequat d4	Unit	Unit
			161 > 44	-16	-22	-16		Unit	Unit
			161 > 45	-16	-23	-16		Unit	Unit
Diquat	4.0	Positive	183 > 157	-12	-21	-27	Paraquat d8	Unit	Unit
			183 > 78	-12	-39	-12		Unit	Unit
			183 > 130	-12	-34	-22		Unit	Unit
Kasugamycin	7.8	Positive	380 > 112	-18	-20	-18	Chlormequat d4	Unit	Unit
			380 > 200	-18	-13	-20		Unit	Unit
								Unit	Unit
Mepiquat	4.5	Positive	114 > 98	-22	-29	-15	Mepiquat d3	Unit	Unit
			114 > 58	-22	-26	-21		Unit	Unit
			114 > 42	-22	-45	-14		Unit	Unit
Paraquat	9.0	Positive	186 > 171	-12	-20	-30	Paraquat d8	Unit	Unit
			186 > 77	-12	-45	-27		Unit	Unit
			186 > 169	-12	-35	-29		Unit	Unit
Trimesium	5.1	Positive	77 > 62	-13	-21	-22	Paraquat d8	Unit	Unit
			77 > 47	-13	-27	-17		Unit	Unit
			77 > 45	-13	-45	-16		Unit	Unit
Chlormequat d4	4.1	Positive	126 > 58	-21	-29	-21		Unit	Unit
Mepiquat d3	4.5	Positive	117 > 101	-20	-29	-18		Unit	Unit
Paraquat d8	9.0	Positive	193 > 178	-13	-21	-30		Unit	Unit

Table 3. Method 2 MS acquisition parameters, retention time and internal standard

Compound	Ret. time (min)	Polarity	SRM transitions	Q1 (V)	CE	Q3 (V)	ISTD	MS1 Res.	MS2 Res.
Glyphosate	3.7	Positive	170 > 88	-17	-9	-18	Glyphosate C13	Unit	Unit
			170 > 42	-17	-26	-17		Unit	Unit
			170 > 60	-17	-16	-24		Unit	Unit
Gluphosinate	2.9	Positive	182 > 136	-12	-11	-26	Maleic hydrazide d2	Unit	Unit
			182 > 56	-12	-24	-23		Unit	Unit
			182 > 119	-12	-19	-23		Unit	Unit
ETU	3.1	Positive	103 > 44	-19	-18	-15	ETU d4	Unit	Unit
			103 > 60	-19	-28	-23		Unit	Unit
			103 > 86	-19	-21	-28		Unit	Unit
Fosethyl	9.9	Negative	109 > 81	23	13	29	Fosethyl d15	Unit	Unit
			109 > 63	23	25	23		Unit	Unit
			109 > 79	23	24	28		Unit	Unit
Maleic hydrazide	13.7	Positive	113 > 40	-11	-27	-16	Maleic hydrazide d2	Unit	Unit
			113 > 67	-11	-19	-27		Unit	Unit
			113 > 85	-11	-17	-17		Unit	Unit
Nicotine	2.0	Positive	163 > 130	-16	-21	-22	Nicotine d3	Unit	Unit
			163 > 117	-16	-25	-20		Unit	Unit
			163 > 132	-16	-17	-23		Unit	Unit
Perchlorate	30.1	Negative	99 > 83	22	26	30	Perchlorate 18O4	Unit	Unit
			99 > 67	22	37	23		Unit	Unit
			101 > 85	22	26	30		Unit	Unit
PTU	3.1	Positive	117 > 58	-20	-16	-19	ETU d4	Unit	Unit
			117 > 60	-20	-29	-20		Unit	Unit
			117 > 72	-12	-22	-26		Unit	Unit
ETU d4	3.0	Positive	107 > 48	-18	-19	-16		Unit	Unit
Fosethyl d5	9.6	Negative	114 > 82	24	15	30		Unit	Unit
Maleic hydrazide d2	13.6	Positive	115 > 42	-11	-20	-17		Unit	Unit
Glyphosate 13C2 15N	3.6	Positive	173 > 91	-11	-8	-19		Unit	Unit
Nicotine d3	1.7	Positive	166 > 130	-30	-22	-21		Unit	Unit
Perchlorate 18O4	30.1	Negative	107 > 89	23	27	30		Unit	Unit

■ Results and Discussion

Following evaluation of several different analytical columns, mobile phases and mass spectrometer settings, two methods were developed for a range of highly polar pesticides that typically require single residue methods to analyse. A ZIC-HILIC column, a zwitterionic stationary phase covalently attached to porous silica, was used in method 1 to analyse the following; amitrole, chlormequat, daminozide, diquat, kasugamycin, mepiquat, paraquat and trimesium. While a Hypercarb PGC (porous graphitic carbon), which behaves as a strongly retentive alkyl-bonded silica gel, was used in method 2 to analyse the following; glufosinate, glyphosate, ethephon, fosetyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, and nicotine.

Three MRM transitions were acquired for each analyte, with exception of two transitions for kasugamycin. Linearity was evaluated for all compounds in the range 0.005 mg/kg – 0.2 mg/kg (5 – 200 ppb) in apple matrix. The concentration of each calibration level is listed in the experimental section. All seven target compounds achieved excellent correlation coefficients greater than $R^2 > 0.9975$, using internal standards for quantitation, linear fit and 1/C weighting. Calibration curves for several compounds are displayed in Figure 3 (using LC method 1) and Figure 5 (using LC method 2). The linearity results for all target compounds is listed in Table 4.

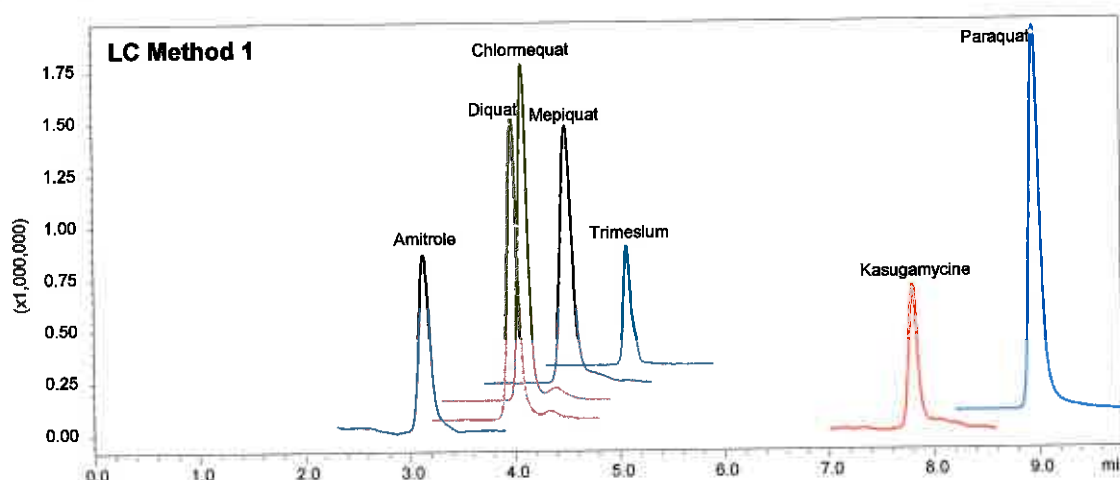


Figure 2 . Target analytes at 0.05mg/kg in apple matrix using a ZIC-HILIC based separation (LC Method 1)

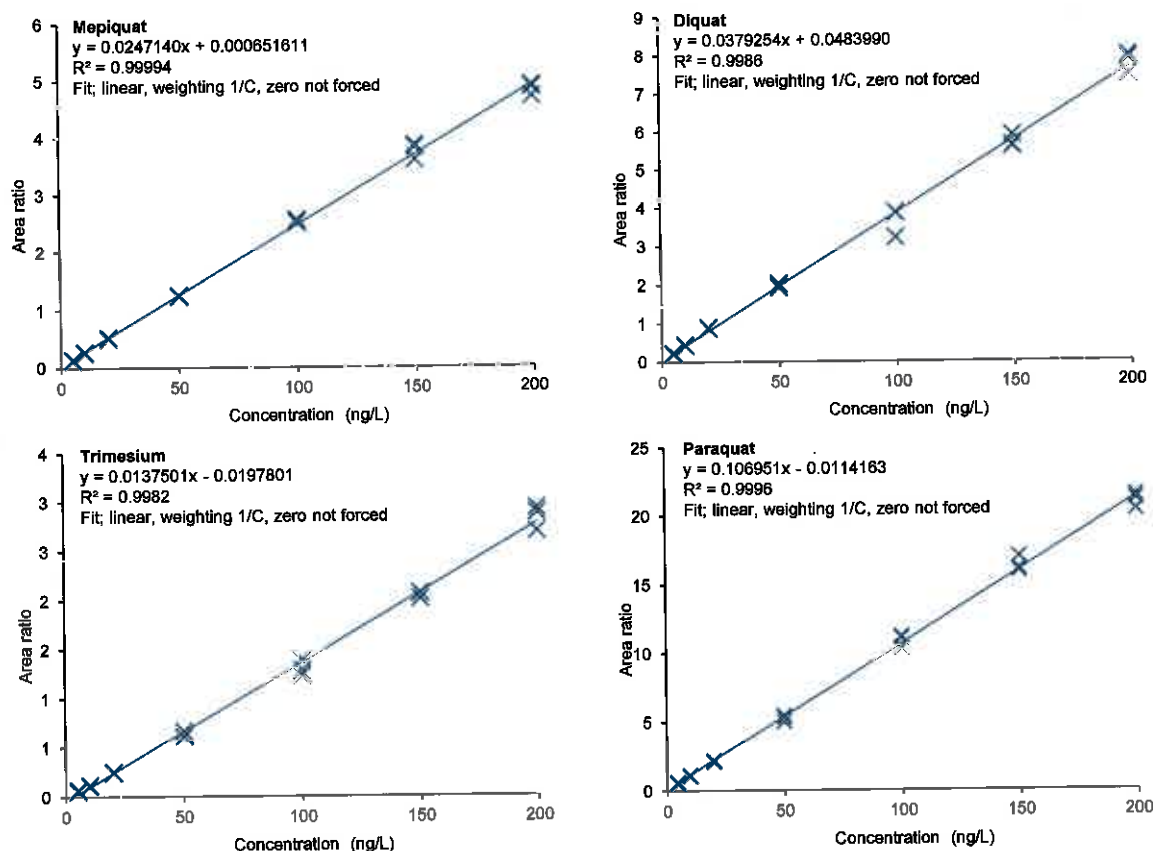


Figure 3. Calibration curves for paraquat, mepiquat, trimesium and diquat using a ZIC-HILIC based separation (LC Method 1)

Figure 2 displays a chromatogram of each compound at 0.05 mg/kg using a ZIC-HILIC based separation (LC method 1) and Figure 4 display a chromatogram using a Hypercarb PGC based separation (LC method 2). All target analytes were identified at 0.01 mg/kg. This concentration is below the European Union (EU) maximum residue limit (MRL) for all of the target analytes in this study. For example, the EU MRL for the following compounds in the majority of commodities is;

glyphosate 0.1 mg/kg, glufosinate 0.1 mg/kg, chlomequat 0.05 mg/kg, paraquat 0.02, mepiquat 0.05 mg/kg, daminozide 0.02 mg/kg and ethephon 0.05 mg/kg.² Consequently, the sensitivity achieved in these methods is far below what is required and therefore dilution of sample extracts is possible in order to reduce matrix effects.

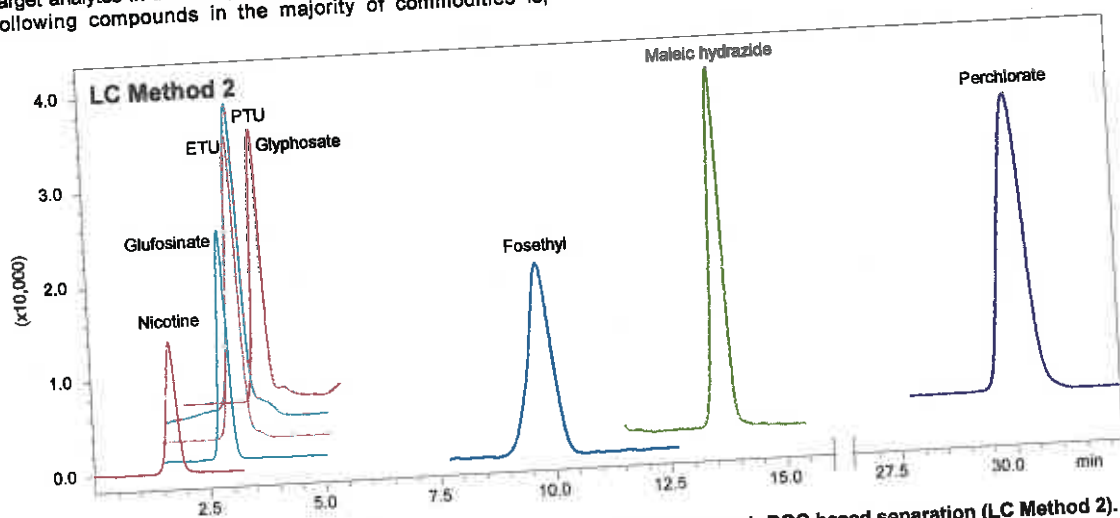


Figure 4. Target analytes at 0.05mg/kg in apple matrix using a Hypercarb PGC based separation (LC Method 2).

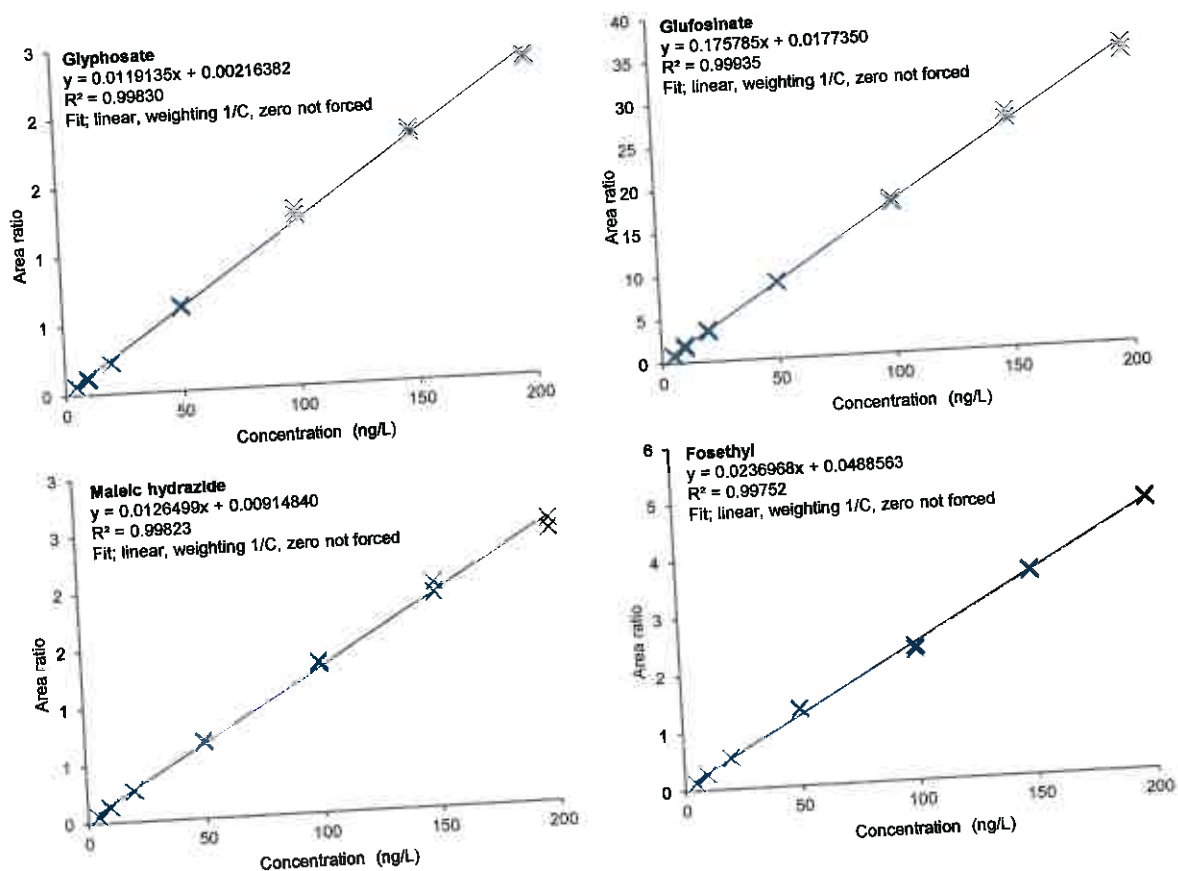


Figure 5. Calibration curves for glyphosate, glufosinate, maleic hydrazide and fosethyl using Hypercarb PGC based separation (LC Method 2)

Conclusion

Two LC-MS/MS methods were developed for the measurement of a range of highly polar pesticides in their underivatised state using the LCMS-8050 triple quadrupole mass spectrometer. The developed multi-residue methods offer significant time savings in comparison to single residue methods typically used for analysis of these analytes. All compounds were quantified in the range 0.005 – 0.2 mg/kg with correlation coefficients greater than 0.997. The excellent sensitivity achieved, which is most cases is far below the EU MRL, offers the opportunity to dilute sample extracts prior to LC-MS/MS injection in order to reduce matrix effects.

References

1. Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM). Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPPE-Method). 2012. Version 7
2. Commission Regulation (EC). 2005. No 396/2005 of the European Parliament and of the Council, maximum residue levels of pesticides in or on food and feed of plant and animal origin. Official Journal of the European Union, L 70: 1-16. http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=homepage&language=EN

Table 4. Target analytes linearity results using LC method 1 and LC method 2

Compound	R ²	Fit type	Weight	Method
Diquat	0.9988	Linear	1/C	Method 1
Chlormequat	0.9988	Linear	1/C	Method 1
Amitrole	0.9981	Quadratic	1/C	Method 1
Kasugamycin	0.9992	Linear	1/C	Method 1
Daminozide	0.9985	Quadratic	1/C	Method 1
Mepiquat	0.9993	Linear	1/C	Method 1
Paraquat	0.9995	Linear	1/C	Method 1
Trimesium	0.9981	Linear	1/C	Method 1
ETU	0.9998	Linear	1/C	Method 2
Fosetyl	0.9975	Linear	1/C	Method 2
Gluphosinate	0.9993	Linear	1/C	Method 2
Glyphosate	0.9983	Linear	1/C	Method 2
Maleic hydrazide	0.9982	Linear	1/C	Method 2
Nicotine	0.9984	Linear	1/C	Method 2
Perchlorate	0.9998	Linear	1/C	Method 2
PTU	0.9991	Linear	1/C	Method 2

Application News

No.C103

Liquid Chromatography Mass Spectrometry

Analysis of Nivalenol, Deoxynivalenol, 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol Using Triple Quadrupole LC/MS/MS (LCMS-8050)

Nivalenol and deoxynivalenol are mycotoxins which are produced by the fusarium fungi. A provisional reference value of 1.1 ppm was established in Japan for deoxynivalenol (Notification No. 0521001 issued by the Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on May 21, 2002). The test methods specified for deoxynivalenol are HPLC for both qualitative and quantitative analysis, and LC/MS for verification testing (Notification No.

0717001 issued by the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on July 17, 2003).

This paper describes an LC-MS/MS method for high-sensitivity simultaneous analysis of the four compounds, nivalenol, deoxynivalenol and the deoxynivalenol metabolites, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol.

■ Analysis of a Standard Mixture

Fig. 1 shows the chromatograms obtained using a 2 μ L injection of the four-component standard mixture (each 10 ppb), and Table 1 shows repeatability of retention time and peak areas for the four substances, respectively, using six repeat measurements.

Nivalenols are detected using the heated electrospray ionization (hESI) method in negative mode. Although water and acetonitrile alone can be used as the LC eluent for LC/MS analysis, higher sensitivity was obtained for each compound by adding low-concentration ammonium acetate (in this case, 0.5 mmol/L) to eluent A. Fig. 1 shows the mass chromatograms for the highest sensitivity MRM transitions for each compound. The analytical conditions are shown in Table 2.

Next, six repeat analyses of a 10 ppb standard solution were conducted, corresponding to approximately 1/100 the concentration of the provisional reference value. The relative standard deviations (%RSD) for the measured retention times and peak areas are shown in Table 1. Good repeatability was obtained for both retention time and peak area.

Table 1 Repeatability (10 ppb, n=6)

	R.T. %RSD	Area %RSD
Nivalenol	0.04	2.57
Deoxynivalenol	0.04	6.52
15-Acetyldeoxynivalenol	0.06	4.09
3-Acetyldeoxynivalenol	0.05	2.58

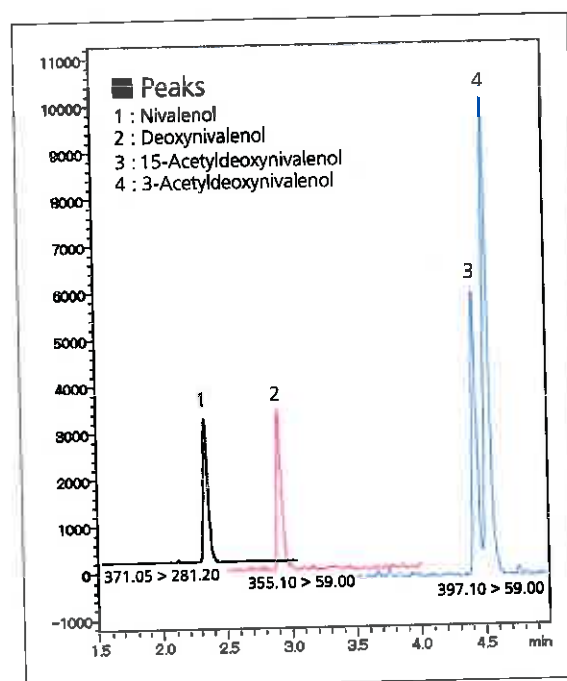


Fig. 1 MRM Chromatograms of a Standard Mixture (10 ppb each)

■ Linearity of Calibration Curves

Fig. 2 shows the calibration curves generated using the analytical conditions of Table 2. Excellent linearity with a coefficient of determination greater than $R^2 = 0.999$

was obtained for calibration curves using a concentration range from 1 to 250 ppb for each component.

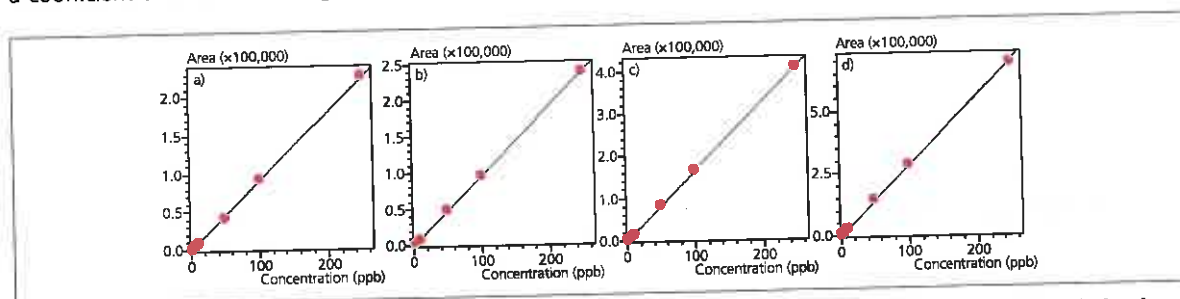


Fig. 2 Linearity of Calibration Curves: a) Nivalenol b) Deoxynivalenol c) 15-Acetyldeoxynivalenol d) 3-Acetyldeoxynivalenol

■ Analysis of Wheat

Fig. 3 describes the sample pretreatment procedure for wheat. The wheat extract solution was purified using either the MultiSep #227 multi-function column (Romer Labs) or the Autoprep MF-T column (Showa Denko K.K.). The chromatograms generated using the samples prepared using the MultiSep #227 (unspiked samples) and the standard-spiked samples, respectively, are shown in Fig. 4. The standard mixture was added to obtain a final concentration of 25 ppb for the four components (about 1/40 of the provisional reference

value), respectively. No large contaminant peaks were detected in the chromatograms of the pretreated samples. Furthermore, although deoxynivalenol was detected, it was at a level below that of the provisional reference value. The spike-and-recovery rates for the four components were excellent, from 101 to 107 %, without any particular matrix effects. Even in samples pretreated using Autoprep MF-T, comparable spike-and-recovery test results were obtained.

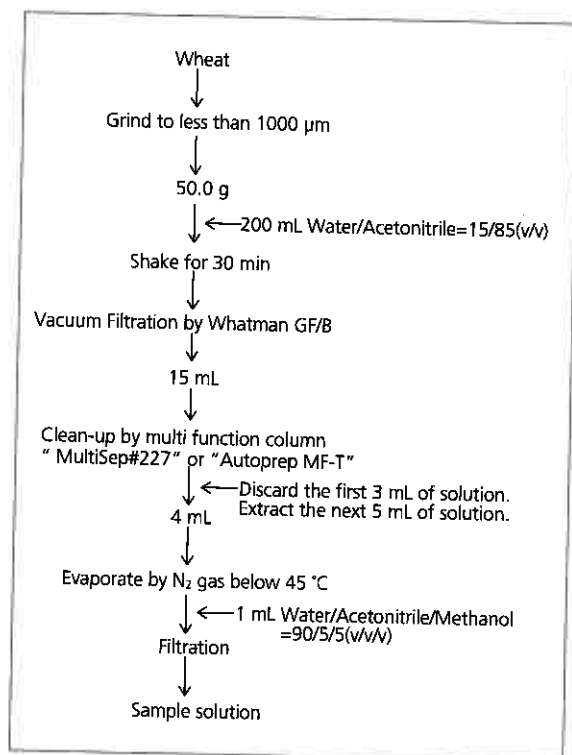


Fig. 3 Pretreatment

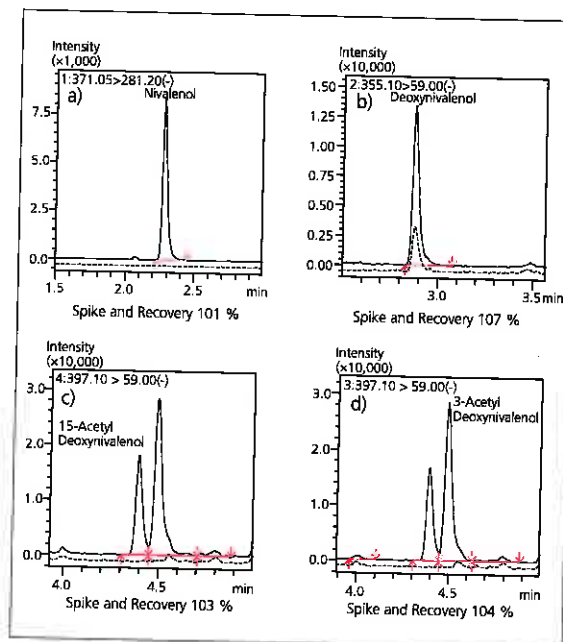


Fig. 4 Chromatograms of Wheat
(Dotted line: Unspiked Sample, Solid line: Spiked Sample, Spiked at 25 ppb each)
a) Nivalenol b) Deoxynivalenol
c) 15-Acetyldeoxynivalenol d) 3-Acetyldeoxynivalenol

Table 2 Analytical Conditions

Column	: Shim-pack XR-ODS III (150 mm L. x 2.0 mm I.D., 2.2 μm)		
Mobile Phases	: A 0.5 mmol/L Ammonium Acetate - Water		
	: B Acetonitrile		
Time Program	: 5 %B (0 min) → 45 %B (5.0 min) → 95 %B (5.01-7.0 min) → 5 %B (7.01 min) → STOP (12 min)		
Flowrate	: 0.3 mL/min		
Column Temperature	: 40 °C		
Injection Volume	: 2 μL		
Probe Voltage	: -3.0 kV (ESI-negative mode)		
DL Temperature	: 100 °C		
Block Heater Temperature	: 200 °C		
Interface Temperature	: 200 °C		
Nebulizing Gas Flow	: 2 L/min		
Drying Gas Flow	: 10 L/min		
Heating Gas Flow	: 10 L/min		
MRM Transition	Nivalenol	371.05 > 281.20	CE: 16.0 V
	Deoxynivalenol	355.10 > 59.00	CE: 22.0 V
	15-Acetyldeoxynivalenol	397.10 > 59.00	CE: 22.0 V
	3-Acetyldeoxynivalenol	397.10 > 59.00	CE: 26.0 V

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Application News

No.C100

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Pyrethroids in Soil and Sediment Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Pyrethroid pesticides are used widely around the world as agricultural and household insecticides. Synthetic pyrethroids are slightly soluble in water and easily adsorbed in soil. In recent years, pyrethroid residues have been confirmed in soil and sediment in both agricultural and urban areas. Pyrethroids, while posing little danger to humans, exhibit a high toxicity to aquatic organisms and insects, making their impact on the ecosystem a matter of concern. Therefore, there is

a need for a sensitive technique which can rapidly measure pyrethroid pesticides in soil and sediment. Due to their low polarity, pyrethroid pesticides are typically measured by GC and GC-MS, however this Application News demonstrates simultaneous positive- and negative-ion mode analysis of 14 pyrethroid pesticides using LC-MS/MS with electrospray ionization (ESI).

MRM Analysis of Standards and Generation of Calibration Curves

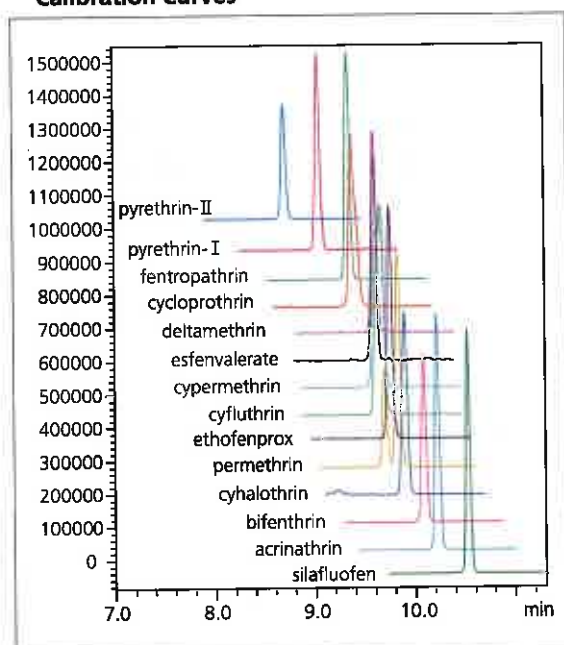


Fig. 1 MRM Chromatograms of Pyrethroids

Table 1 MRM Transitions and Calibration Curves of Pyrethroids

Compound Name	Polarity	Precursor (m/z)	Product (m/z)	Calibration Curve Range (μg/L)	r ²
Pyrethrin-I	+	329.20	161.10	0.5 - 500	0.9996
Pyrethrin-II	+	373.20	161.20	0.5 - 500	0.9997
Fenpropathrin	+	367.20	125.20	0.02 - 100	0.9993
Cycloprothrin	+	498.90	181.10	0.5 - 100	0.9991
Deltamethrin	+	522.80	280.90	0.05 - 100	0.9992
Esfenvalerate	+	437.10	167.30	0.5 - 100	0.9990
Cypermethrin	+	433.10	191.10	0.05 - 100	0.9986
Cyfluthrin	+	450.90	191.00	0.5 - 100	0.9976
Ethofenprox	+	394.20	177.30	0.01 - 100	0.9993
trans-Permethrin	+	408.10	183.30	0.02 - 100	0.9996
cis-Permethrin	+	408.10	183.30	0.02 - 100	0.9994
Cyhalothrin	+	467.10	225.10	0.1 - 100	0.9993
Bifenthrin	+	440.00	181.20	0.02 - 100	0.9995
Acrinathrin	-	540.10	372.20	0.1 - 100	0.9994
Silafluofen	+	426.20	287.10	0.01 - 100	0.9999

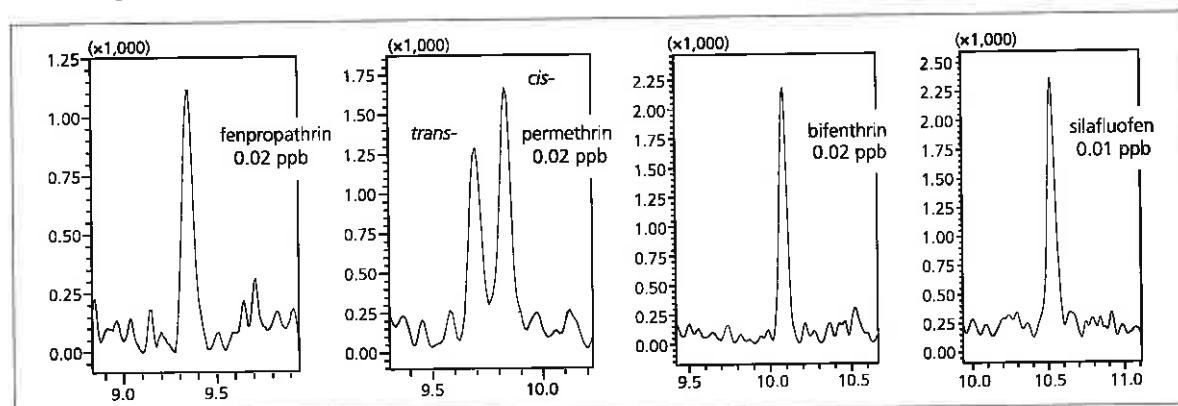


Fig. 2 MRM Chromatograms at Pyrethroid LOQs

Sample Preparation for Soil and Sediment Using QuEChERS Method

Soil samples are generally prepared using solid-phase extraction, however, this process can be both time-consuming and labor-intensive. In this application, the easy pretreatment method referred to as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), typically used for analysis of residual pesticides in food, was used for the pretreatment of soil and sediment samples. Fig. 3 shows the protocol employed to pretreat these soil and sediment samples. The combined acetonitrile extraction and cleanup process requires only 15 minutes per sample to complete.

Recoveries Using Actual Samples

A mixed pesticide standard solution was added to soil and sediment solutions respectively to obtain a concentration of 10 ppb before or after pretreatment was conducted by the QuEChERS method, and recovery tests were then conducted. Good recoveries of 70 to 120 % were obtained for both soil and sediment samples, as shown in Fig. 4.

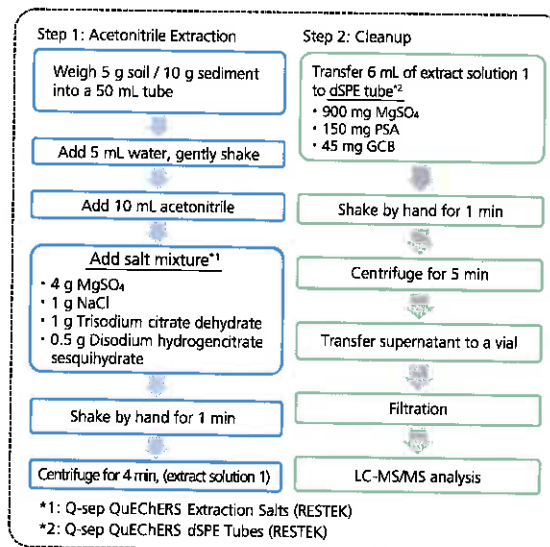


Fig. 3 Sample Preparation Using QuEChERS Method

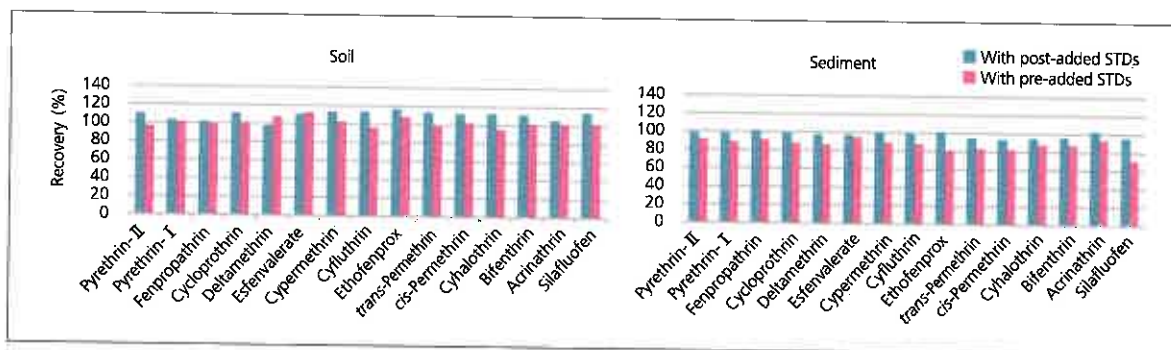


Fig. 4 Recoveries from Soil and Sediment

Table 2 Analytical Conditions

Column	: Phenomenex Kinetex 2.6 μm PFP 100Å (100 mm × 2.1 mm I.D.)
Mobile Phase A	: 5 mM Ammonium Acetate - Water
Mobile Phase B	: Methanol
Time Program	: 40 %B (0 min) → 100 %B (10 - 12 min) → 40 %B (12.01 - 15 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 1 μL
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +4.0 kV / -3.0 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 15.0 L/min.
Heating Gas Flow	: 15.0 L/min.
Interface Temperature	: 100 °C
DL Temperature	: 100 °C
Block Heater Temperature	: 400 °C

Application News

LCMS

No.C118

Liquid Chromatography Mass Spectrometry

Highly Polar Pesticide Multi-Residue Analysis in Food Safety by LC-MS/MS

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Abstract

The analysis of highly polar pesticides by a single LC-MS/MS method is extremely challenging as a consequence of diverse separation and detection behaviour. Conventional approaches in highly polar pesticide analysis often use single residue methods or small group specific methods which are time consuming and limit throughput. In this study, the panel of target analytes selected for analysis included a series of compounds that are typically addressed by multiple methods and workflows; glufosinate, glyphosate, ethephon, fosetyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, nicotine, amitrole, chlomequat, daminozide, diquat, kasugamycine, mepiquat, paraquat and trimesium.

To accelerate turnaround times and increase sample sizes for more complete testing programs two LC-MS/MS methods were developed for the measurement of a range of highly polar pesticides in their underivatised state using the LCMS-8050 triple quadrupole mass spectrometer. All target compounds were quantified at 0.01 mg/kg which is below the European Union maximum residue limit for all studied compounds delivering a measurable impact on sample cycle time and productivity.

Keywords: Highly polar pesticides, LCMS-8050, food safety, glyphosate, diquat, paraquat, perchlorate

Introduction

The use of pesticides in the environment is constantly under review and in recent years regulatory bodies have adopted a hazards-based approach to pesticide regulation leading to an increased use of highly polar pesticides which present lower persistence and toxicity. Enforcing pesticide limits within regulatory limits defined as the maximum residue levels (MRL's; the maximum concentration of pesticide residues permitted in food and feed) requires methods that provide results quickly and accurately for a broad spectrum of chemical structures in a diverse range of food samples.

Pesticide residue monitoring laboratories utilise multi-residue LC-MS/MS methods for the quantification of an ever increasing list of target pesticides. However, the measurement of highly polar pesticides by a single LC-MS/MS method is extremely challenging as a consequence of diverse separation and detection behaviour. For this reason, single residue methods or small group specific methods are often utilised to analyse these compounds, in some cases including the use of pre- or post-column derivatisation. Therefore, there is a clear need to reduce the number of separation methods applied to the analysis of highly polar pesticides to help accelerate sample throughput, reduce the cost platform, simplify analytical workflows and enhance data quality for regulatory reporting limits.

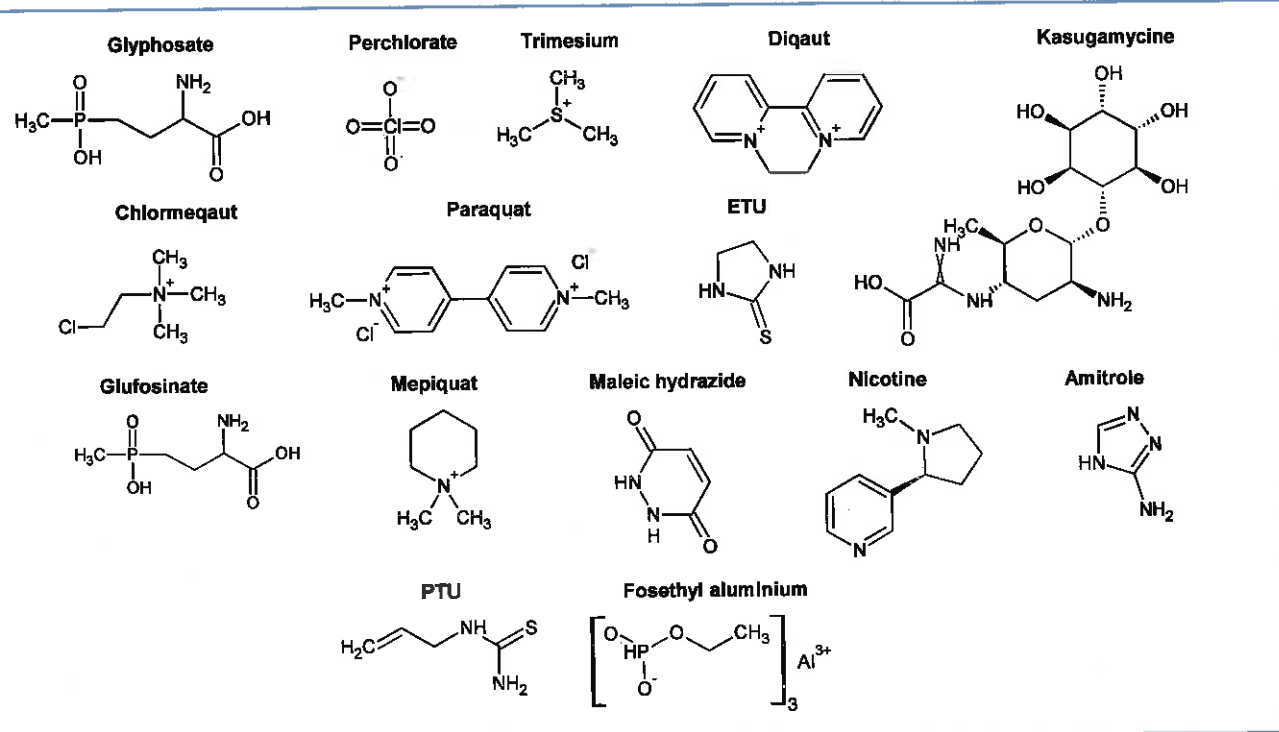


Figure 1. Target analyte structures

The highly polar pesticides targeted in this study included glufosinate, glyphosate, ethephon, fosetyl aluminium, maleic hydrazide, perchlorate, ETU, PTU, nicotine, amitrole, chlomequat, daminozide, diquat, kasugamycin, mepiquat, paraquat and trimesium. Structures for these compounds are displayed in Figure 1. All of the compounds included in this study were polar, characterised with LogKow < 1. The most polar compounds being the cationic quaternary ammonium herbicides diquat (LogKow -4.6) and paraquat (LogKow -4.5). Several of the compounds also have a low molecular mass, for example trimesium (77 g/mol), amitrole (84 g/mol) and ETU (102 g/mol).

The analysis of highly polar pesticides is extensively reported in literature but the methods have been limited to a small number of specific target compounds and not to a group with such a diverse chemical space. For example, a common approach for the analysis of one of the world's biggest selling herbicides glyphosate is typically achieved by FMOc derivatization. This derivatization step is specific for glyphosate, glufosinate and AMPA residues in water and food samples but it is relatively complex, limits throughput and repeatability and reproducibility can suffer due to the derivatisation step.

The aim of this study was to develop a fast, sensitive and simple methodology for a range of challenging highly polar pesticides that require single-residue methods, by as few multi-residue LC-MS/MS runs as possible and without the need for derivatisation. Several different analytical columns and mobile phases were evaluated in this study, in addition to assessing the MS/MS parameters. Isotopically labelled standards were used to compensate for matrix effects. Initial data was collected in food matrix using a triple quadrupole mass spectrometer in MRM mode.

Experimental

Individual reference standards for each compound were provided by Phytocontrol in methanol at a concentration of 10 ng/μL. Mobile phase solvents and additives were all LC-MS quality and purchased from Sigma-Aldrich. Apple extracts were provided by Phytocontrol and extracted according to the EURL-SRM QuPPe methodology.¹ Briefly, apple samples (10 g) were prepared by chopping up the sample, freezing, homogenizing with dry ice, adding 1% formic acid in methanol solution (10 mL) and centrifuging (4000 RPM). Linearity was evaluated by spiking sample extracts at the following levels: 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 mg/kg. Deuterated internal standards were used for calibration. All calibration points were analysed in duplicate. Plastic vials were used for analysis to prevent interaction of certain pesticides (e.g. paraquat, diquat and glyphosate) with glass surfaces.¹

SRM transitions and analyte specific MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimised automatically using the SRM optimisation feature available in LabSolutions software. SRM transitions are listed in Table 2 and Table 3.

Preliminary investigations involved the testing of several different analytical columns: SIELC Obselisc R (150 x 2.1mm, 5μm); Hypercarb PGC (100 x 2.1mm, 5μm); SeQuant ZIC-HILIC (100 x 2.1mm, 3.5μm), SeQuant ZIC-cHILIC (100 x 2.1mm, 3.5μm), Scherzo SM-C18 (50 x 2, 3 μm), Scherzo SW-C18 (50 x 2, 3 μm), Fortis Phenyl (100 x 2.1mm, 5μm), Luna Phenyl-Hexyl (100 x 2.1mm, 3μm), and Restek IBD (150 x 2.1mm, 3μm). These columns were tested with several different mobile phase additives including acetic acid, formic acid, ammonium formate, ammonium acetate and ammonium hydroxide (depending on appropriate conditions for each column and the progression of results). Reversed phase, HILIC, and mixed mode chromatography were tested depending on the column suitability for each mode. The final LCMS/MS method conditions are listed in Table 1.

Table 1. LC/MS/MS parameters for Method 1 and Method 2

Liquid chromatography		
	Method 1	Method 2
UHPLC	Nexera UHPLC system	Nexera UHPLC system
Analytical column	ZIC-HILIC (100 x 2.1mm, 3.5μm)	Hypercarb PGC (100mm x 2.1mm, 5μm)
Mobile phase	A = Water 20mM ammonium formate and 0.3% formic acid	A = Water 1% acetic acid
	B = Acetonitrile	B = Methanol 1% acetic acid
Gradient	Time (mins)	%B
	0	97
	5.8	88
	9	15
	10	15
	10	97
	18	Stop
Column temp.	35°C	35°C
Injection volume	6μL (40μL acetonitrile co-injected)	5μL
Flow rate	0.4mL/min	0.3mL/min
Mass spectrometry		
LCMS/MS	LCMS-8050	
Ionisation mode	Heated electrospray	
Polarity switching time	5 ms	
Pause time	1 ms	
Dwell times	5-50ms	
Interface temperature	350°C	
Heating block	300°C	
Desolvation line	200°C	
Gas	Heating gas 10 L/min; drying gas 10 L/min; Nebulising gas 3 L/min	

Application News

No.C116

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Steroid Hormones Using Triple Quadrupole LC/MS/MS

Steroid hormones are heavily involved with the control of metabolism, neurotransmission, and intracellular signaling, playing critical roles in the proper functioning of the body. Further, not only do steroids play roles in sedation and seizure prevention, they are known to be effective in cancer treatment and regenerative

medicine. Steroid quantitation in biological samples is therefore an important tool in clinical research. This Application News introduces the analysis of steroid hormones in human serum by LC/MS/MS following pretreatment using ISOLUTE® SLE+ (supported liquid extraction).

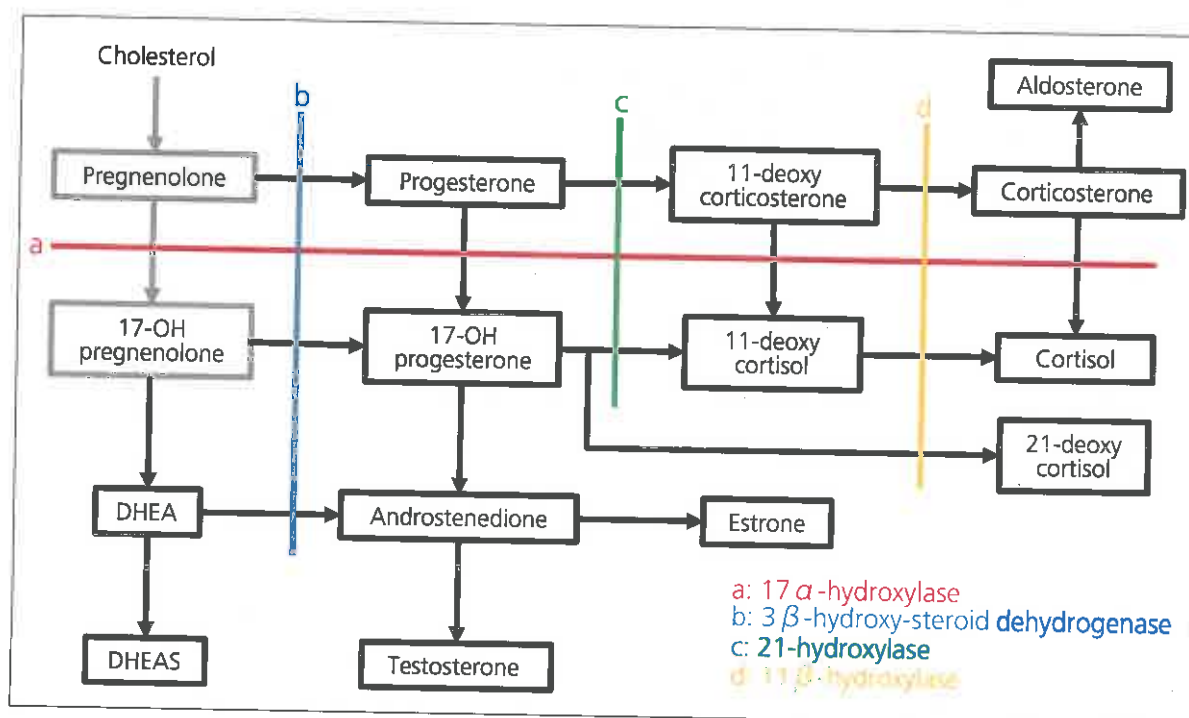


Fig. 1 Major Pathways of Steroid Biosynthesis

Table 1 Analytical Conditions

[LC] NexeraX2 System	
Column	: Shim-Pack FC-ODS (75 mm L. × 2 mm I.D., 3 μ m)
Column Oven	: 40 °C
Mobile Phase A	: 5 mM Ammonium Formate - Water
Mobile Phase B	: 5 mM Ammonium Formate - Methanol
Gradient	: 35 % B (0 - 2.5 min) → 35 % B → 45 % B (2.5 - 4 min) → 45 % B → 80 % B (4 - 12 min) 95 % B (12.01 - 15 min) → 35 % B (15.01 - 18 min)
Flowrate	: 0.3 mL/min
Injection Volume	: 10 μ L
[MS] LCMS-8050	
Ionization	: ESI
DL Temp.	: 110 °C
Heat Block Temp.	: 450 °C
Interface Temp.	: 370 °C
Nebulizer Gas	: 3 L/min
Drying Gas	: 7 L/min
Heating Gas	: 13 L/min

MRM Parameters

Compound Name	Group	Ret. Time (min)	Precursor m/z	Product m/z (1)	Product m/z (2)	Compound Name	Group	Ret. Time (min)	Precursor m/z	Product m/z (1)	Product m/z (2)
Aldosterone	1	5.52	361.20	315.20	343.00	Androstenedione	6	8.83	287.10	97.10	109.15
Aldosterone IS	1	5.47	367.20	349.25	331.10	Androstenedione IS	6	8.79	292.10	100.10	113.05
Cortisol	2	6.55	363.40	121.10	97.00	Testosterone	7	9.46	289.10	97.15	109.05
Cortisol IS	2	6.57	366.10	121.10	97.10	Testosterone IS	7	9.42	294.10	100.00	113.05
DHEAS	3	7.21	271.20	213.20	197.10	DHEA	8	9.03	271.20	213.20	253.15
DHEAS IS	3	7.18	277.10	219.20	203.10	Estrone	8	9.04	271.10	133.05	157.05
21-Deoxycortisol	4	7.46	347.20	311.20	121.05	11-Deoxycorticosterone	8	9.22	331.20	109.05	97.05
Corticosterone	4	7.84	347.20	121.15	97.15	17-OHP	8	9.72	331.10	97.00	109.00
Corticosterone IS	4	7.77	355.20	125.05	337.00	17-OHP IS	8	9.66	339.10	100.05	113.10
11-Deoxycortisol	5	8.04	347.20	109.10	97.05	Progesterone	9	11.34	315.20	97.05	109.10
11-Deoxycortisol IS	5	8.01	352.20	100.15	113.05	Progesterone IS	9	11.26	324.10	100.00	113.00
						DHEAS_neg	10	7.20	367.10	97.10	
						DHEAS_neg IS	10	7.17	373.10	98.00	

Type	Event#	m/z	Compound Name	m/z	Time (1.559 min - 12.991 min)
MRM	2	361.20>315.20	Aldosterone	361.20>315.20	
MRM	3	367.20>349.25	Aldosterone IS	367.20>349.25	
MRM	4	363.40>121.10	Cortisol	363.40>121.10	
MRM	5	366.10>121.10	Cortisol IS	366.10>121.10	
MRM	6	271.20>213.20	DHEAS	271.20>213.20	
MRM	7	277.10>219.20	DHEAS IS	277.10>219.20	
MRM	8	347.20>311.20	21-Deoxycortisol	347.20>311.20	
MRM	9	355.20>125.05	Corticosterone	355.20>125.05	
MRM	10	347.20>121.15	Corticosterone IS	347.20>121.15	
MRM	11	347.20>109.10	11-Deoxycortisol	347.20>109.10	
MRM	12	352.20>100.15	11-Deoxycortisol IS	352.20>100.15	
MRM	13	287.10>97.10	Androstenedione	287.10>97.10	
MRM	14	292.10>100.10	Androstenedione IS	292.10>100.10	
MRM	15	289.10>97.15	Testosterone	289.10>97.15	
MRM	16	294.10>100.00	Testosterone IS	294.10>100.00	
MRM	17	331.10>97.00	17-OHP	331.10>97.00	
MRM	18	339.10>100.05	17-OHP IS	339.10>100.05	
MRM	19	315.20>97.05	Progesterone	315.20>97.05	
MRM	20	324.10>100.00	Progesterone IS	324.10>100.00	
MRM	21	367.10>97.10	DHEAS_neg	367.10>97.10	
MRM	22	373.10>98.00	DHEAS_neg IS	373.10>98.00	

Calibration Curves

MRM measurement was conducted for 13 types of steroid hormones. Fig. 2 shows the calibration curves obtained using a mixed standard solution, in addition

the MRM chromatograms obtained using a concentration of each steroid in the vicinity of its LOQ.

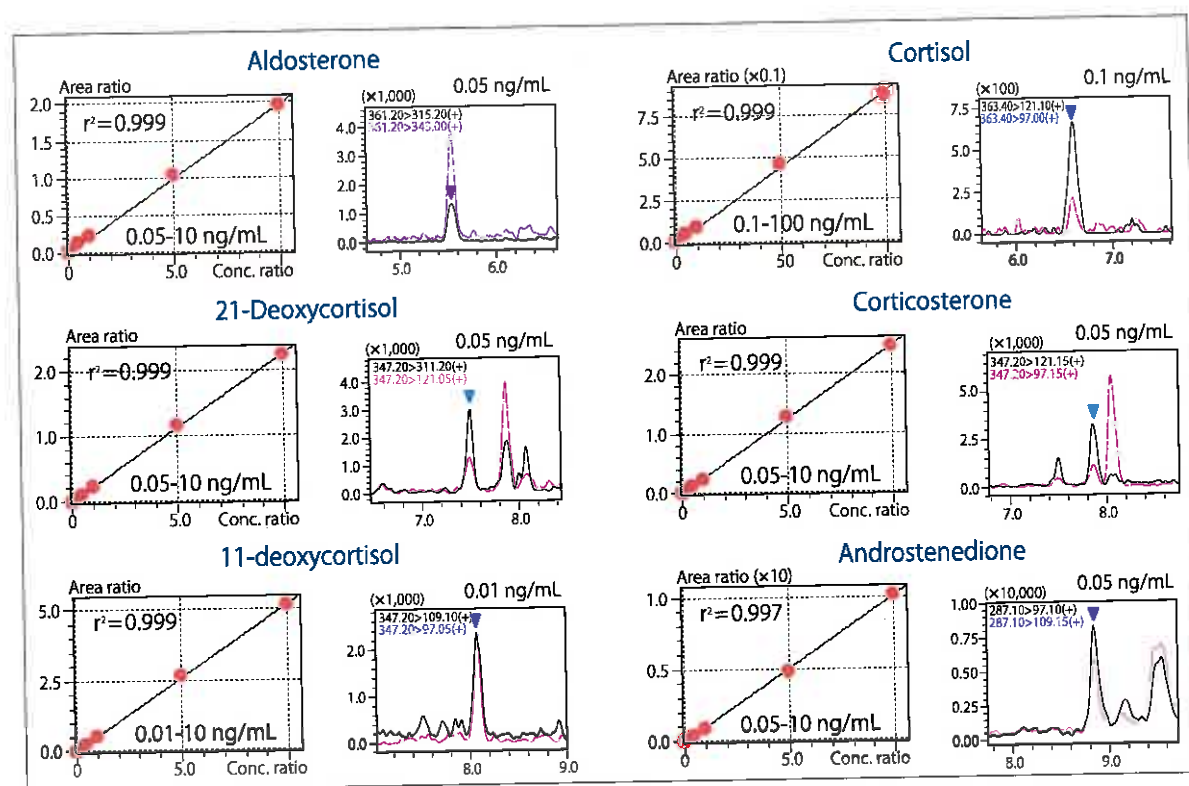
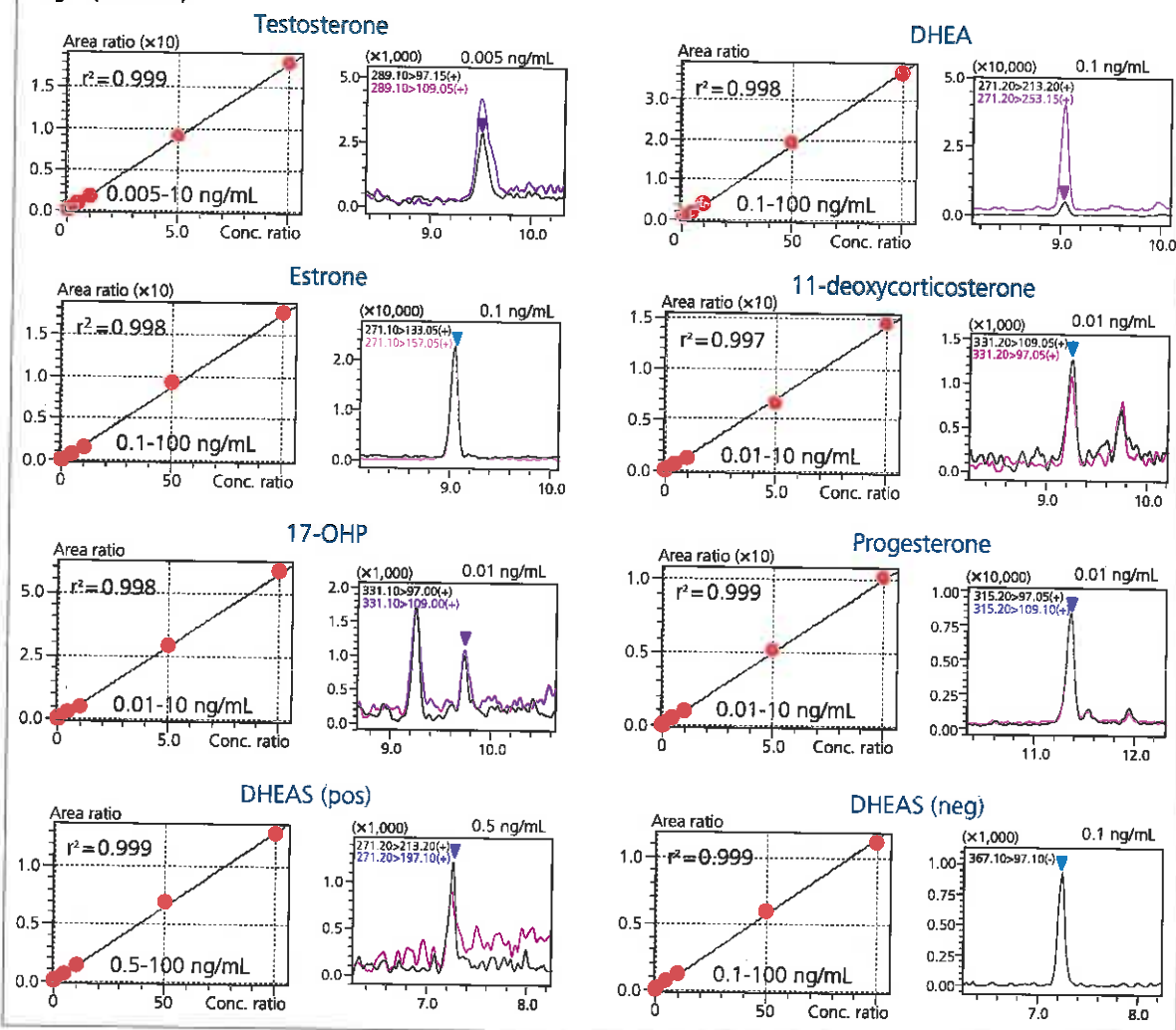


Fig. 2 Calibration Curves and MRM Chromatograms of 13 Steroid Hormones

Fig. 2 (continued)



Sample Preparation Using ISOLUTE® SLE+

Biological matrices are complex and require cleanup prior to LCMS analysis. ISOLUTE® SLE+ was used to eliminate the influence due to endogenous compounds such as proteins, phospholipids and salts. The reduction

of matrix interference allows for lower levels of quantitation, and higher confidence in analytical results. The method that was used for steroid hormone extraction is outlined below.

1. Sample Preparation

Dilute the serum sample (100 μ L) with ultrapure, HPLC grade water (300 μ L), and mix.

2. Sample Loading

Dispense the prepared sample (400 μ L) onto the plate and apply pressure. Wait 5 minutes for sample to completely absorb into diatomaceous earth.

3. Apply Extraction Solvent

Add dichloromethane (900 μ L $\times$ 2) to deep well plates to elute, and allow solvent to flow for 5 minutes under gravity. Then apply pressure for analyte elution.

4. Post Extraction Processing

Transfer eluate to glass vial, and heat to dryness at 40 $^{\circ}$ C. Re-dissolve in 100 μ L of solvent (mobile phase A 66 %, mobile phase B 35 %, v/v). Centrifuge and transfer supernatant onto a new plate.

Table 2 shows the concentration range added to the serum, the rates of recovery, and the matrix effect with respect to the 12 types of steroid hormones. The analysis recovery rates were determined by comparing the area values of Sample B, in which the serum was spiked with the steroids prior to pretreatment, and

Sample A, in which only the serum was subjected to pretreatment, after which it was spiked with the steroids. The matrix factor was determined by comparing the area values of Sample A with that of the standard solution S.

Table 2 Recovery and Matrix Factor of 12 Steroid Hormones (n=3)

Compound	Concentration Range Added to Serum (ng/mL)	Recovery %	Matrix Factor
Aldosterone	0.05-10	94.8 %	0.13
Cortisol	0.1-100	94.7 %	0.31
21-Deoxycortisol	0.05-10	94.6 %	0.13
Coricosterone	0.05-10	107.1 %	0.30
11-Deoxycortisol	0.01-10	93.7 %	0.21
Androstenedione	0.05-10	94.2 %	0.09
Testosterone	0.005-10	80.2 %	0.00
DHEA	0.1-100	89.8 %	0.19
Estrone	0.1-100	94.3 %	0.30
11-Deoxycorticosterone	0.01-10	96.2 %	0.26
17-OHP	0.01-10	94.6 %	0.19
Progesterone	0.01-10	89.8 %	0.19

The Recoveries and matrix factor were determined according to the following equations.

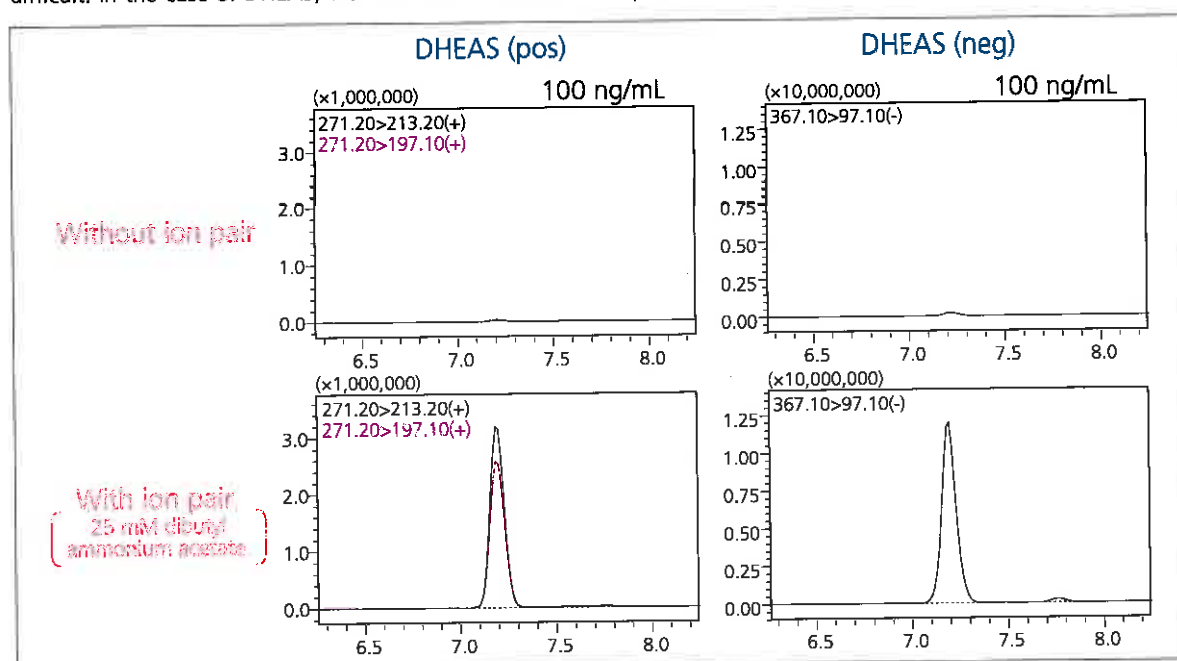
$$\text{Recovery} = [B]/[A] \times 100$$

$$\text{Matrix Factor} = 1 - [A]/[S]$$

■ Use of Ion Pair Reagents

The use of ion-pair reagents is a valid approach in analysis of ionic compounds where recovery is normally difficult. In the case of DHEAS, we were able to obtain

a high recovery rate by spiking the sample with an ion pair reagent (25 mM dibutyl ammonium acetate) during pretreatment.

**Fig. 3 Comparison of MRM Chromatograms of DHEAS With and Without Ion Pair Reagent****Table 3 Recovery and Matrix Factor of DHEAS (n=3)**

Compound	Concentration Range Added to Serum (ng/mL)	Recovery %	Matrix Factor
DHEAS (pos)	0.1-100	86.2 %	0.06
DHEAS (neg)	0.1-100	89.3 %	0.11

Note)

The published data was not acquired using an instrument registered by Japanese pharmaceutical affairs law.

[Acknowledgment]

We would like to express our deep gratitude for the cooperation of Biotage Japan Ltd. in the preparation of this Application News article.

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Application News

No. C99

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Veterinary Drugs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

Foods in which chemical residues, like pesticides, feed additives, and veterinary drugs found in excess of maximum residue levels have been banned from sale in many countries around the world. Compounds that are subject to residue standards vary widely and the list is expected to grow. Because of this, there is a need for a

highly sensitive and rapid analytical technique to analyze as many of these compounds as possible in a single run. This Application News introduces an example of the high-sensitivity analysis of 89 veterinary drugs in a crude extract of livestock and fishery products.

■ Sample Preparation

The typical samples used in the analysis of veterinary drugs contain large amounts of lipids because they are commonly meat and fish samples. Sample preparation is extremely important to ensure excellent sensitivity and repeatability. To avoid the typical time-consuming and laborious solid phase extraction sample preparation procedure, the QuEChERS method, which is typically used for the preparation of vegetables, was selected to simplify sample preparation.

The QuEChERS method normally consists of two steps, the first is an acetonitrile extraction and the second a cleanup step, but this time only the acetonitrile extraction step was used.

* QuEChERS Extraction Salts kit: Restek Q-sep™ AOAC2007.01

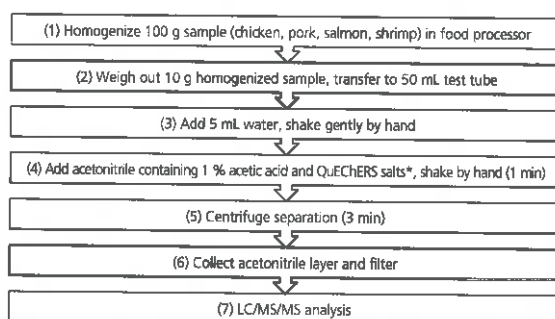


Fig. 1 Sample Preparation Procedure

■ Improved Peak Shape Using Sample / Water Co-Injection

When conducting reversed phase chromatography, the peaks of polar compounds may split or collapse depending on the relationship between the sample solvent and mobile phase. In cases where the sample solvent is rich in organic solvent, the elution strength must be lowered (by substitution or dilution) with the addition of water. As the pretreated sample solvent in this analysis consists of 100 % acetonitrile, injection in that state into the LC/MS will result in split peaks for some of the substances (Fig. 2 left).

To eliminate as much of the time and effort typically associated with sample preparation, the pretreatment features of the autosampler (SIL-30A) were utilized to conduct co-injection of sample and water, which resulted in improved peak shapes.

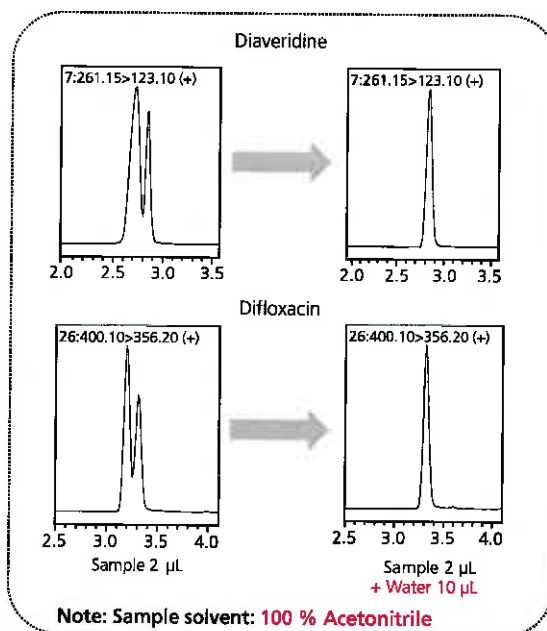
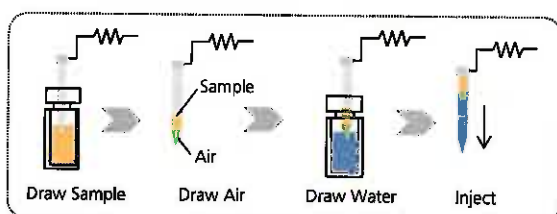


Fig. 2 Comparison of Peak Shape

MRM Analysis of Matrix Standards

Fig. 3 shows the MRM chromatogram of the matrix standard solution consisting of the sample solution with added standard solution (data obtained using pork extract solution). Table 1 shows the lower limits of quantitation for the standard solution without added matrix and with added matrix, respectively. In a crude extract obtained by acetonitrile extraction alone, sensitivity was comparable to that obtained for most of

the compounds using only standard solution. Although there were several compounds for which the lower limit of quantitation was different in the standard solution than the matrix-added solution, rather than attributing this to matrix effects, it is thought to be caused by elevated background due to ions derived from contaminating components (Refer to Fig. 5).

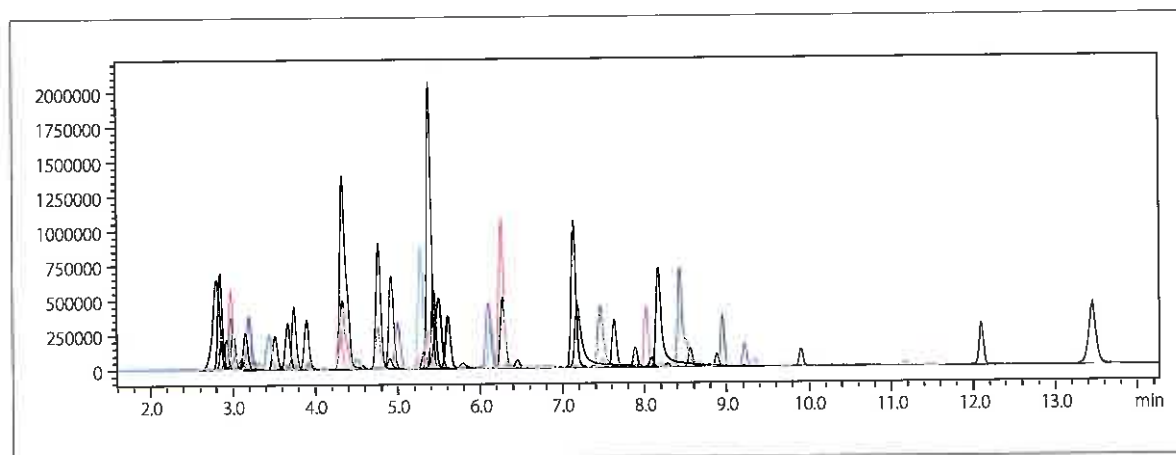


Fig. 3 MRM Chromatograms of 89 Veterinary Drugs (10 µg/L pork extract solution with added standard solution)

Table 1 LOQs of Veterinary Drugs in Neat Standards and Matrix Standards and Calibration Range of Veterinary Drugs in Matrix Standards

	Std. Solution		Matrix-Added Std. Solution			Std. Solution		Matrix-Added Std. Solution	
	Min. Conc.	Max. Conc.	Min. Conc.	Max. Conc.		Min. Conc.	Max. Conc.	Min. Conc.	Max. Conc.
Gentamicin	0.5	50	1	50	Sulfachloropyridazine	0.02	20	0.02	20
Sulfanilamide	1	50	1	50	Sulfadimethoxine	0.02	10	0.02	10
Levamisole	0.05	50	0.05	50	Tylosin	0.05	50	0.05	50
Lincomycin	0.01	10	0.01	10	Sulfamethoxazole	0.02	10	0.1	10
5-Propylsulfonyl-1-benzimidazole-2-amine	0.05	10	0.05	10	Sulfathoxypyridazine	0.02	10	0.02	10
Diaverdine	0.01	10	0.01	10	Tiamulin	0.01	50	0.01	50
Trimethoprim	0.02	20	0.02	20	Florfenicol	0.5	50	10	50
Meloxicam	0.01	50	0.01	50	2-Acetylamin-5-nitrothiazole	0.05	50	0.05	50
Sulfisomidine	0.02	20	0.02	20	Sulfatinoxazole	0.01	5	0.01	5
Norfloracin	0.5	50	0.5	50	Leucomycin	0.01	50	0.01	50
Ormetoprim	0.02	10	0.02	10	Sulfisoxazole	0.01	50	0.05	50
Thiabendazole	0.01	10	0.01	10	Oxolinic acid	0.01	50	0.1	50
Ciprofloxacin	0.05	10	0.5	10	Chloramphenicol	0.5	50	1	50
Neospiramycin	0.01	10	0.05	10	Clorsulon	0.5	50	1	50
Danofloxacin	0.1	10	0.1	10	Sulfabenzamide	0.01	10	0.01	10
Enrofloxacin	0.05	50	0.1	50	Ethopabate	0.01	10	0.01	10
Oxytetracycline	0.01	50	0.1	50	Sulfadoxine	0.02	20	0.02	20
Xylazine	0.01	10	0.01	10	Sulfaguanidine	0.02	10	0.02	10
Orbifloxacin	0.05	50	0.05	50	Prednisolone	0.1	20	0.05	20
Sulfacetamide	1	50	1	50	Orfloxacin	0.5	50	0.5	50
Clenbuterol	0.01	10	0.01	10	Flubendazole	0.01	50	0.01	50
Tetracycline	0.05	50	0.01	50	Methylprednisolone	0.5	50	0.5	50
Spiramycin I	0.01	50	0.01	50	Nalidixic acid	0.01	50	0.01	50
Sarafloxacin	0.5	50	0.5	50	Dexamethasone	0.5	50	0.5	50
Difloxacin	0.05	50	0.1	50	Flumequine	0.01	50	0.01	50
Sulfadiazine	0.02	20	0.1	20	Benzylpenicillin	0.5	50	0.5	50
Sulfathiazole	0.02	20	0.1	20	Sulfantran	0.2	50	0.2	50
Sulfapyridine	0.02	20	0.1	20	Sulfabromomethazine	0.01	50	0.01	50
Carbadox	0.05	10	0.05	10	beta-Trenbolone	0.02	50	0.1	50
Pyrimethamine	0.02	20	0.02	20	Emamectin B1a	0.01	50	0.01	50
Sulfamerazine	0.02	20	0.02	20	alpha-Trenbolone	0.02	50	0.1	50
Chlortetracycline	0.1	50	0.1	50	Promidic acid	0.01	50	0.05	50
Trimicosin	0.1	50	0.1	50	Zeranol	1	50	0.1	50
Thiamphenicol	1	50	1	50	Ketoprofen	0.01	50	0.05	50
Sulfadimidine	0.02	20	0.02	20	Testosterone	0.01	10	0.05	10
Sulfamethoxydiazine	0.01	10	0.02	10	Famphur	0.05	50	0.05	50
Sulfamethoxypyridazine	0.02	20	0.02	20	Fenobucarb (BPMC)	0.01	50	0.01	50
Sulfisoxazole	0.01	50	0.01	50	Clostebol	0.05	50	0.05	50
Trichlorfon (DEP)	0.05	50	0.05	50	Dichlofenac	0.01	50	0.01	50
Sulfamonomethoxine	0.02	20	0.02	20	Melengestrol Acetate	0.05	50	0.05	50
Furazolidone	1	50	1	50	Temephos (Abate)	0.01	50	0.5	50
Difurazone	0.05	50	0.05	50	Alledrin	0.1	50	1	50
Erythromycin A	0.01	50	0.01	50	Closantel	0.01	10	0.01	10
Cefazolin	0.5	50	0.5	50	Monensin	0.01	10	0.01	10

(Unit: µg/L)

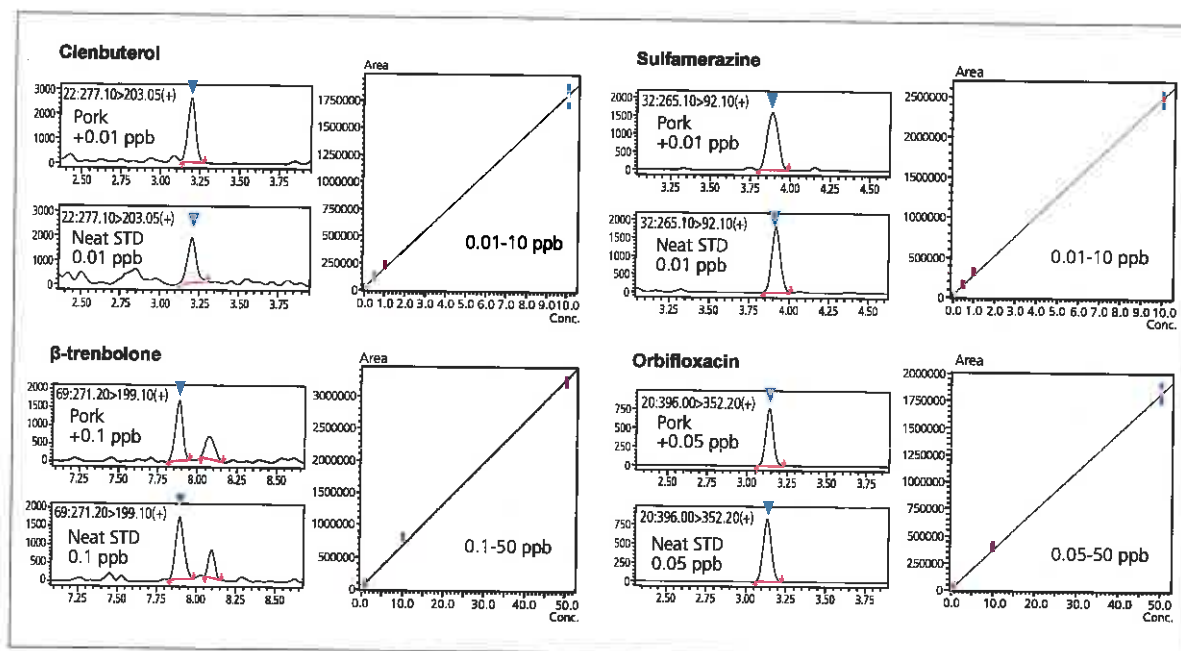


Fig. 4 MRM Chromatograms in the Vicinity of the LOQ and Calibration Curves of Typical Compounds

Recoveries of Veterinary Drugs in Crude Extracts from Livestock and Fishery Products (Matrix Effect Verification)

We examined whether or not the matrix affected measurement of actual samples. This time, four types of food product samples were used, including shrimp, chicken meat, pork, and salmon. Standard solution was added to the acetonitrile extraction solution of each of these to obtain a final concentration of 10 $\mu\text{g/L}$, after

which the rates of recovery were determined. The results indicated that 90 % of the compounds were recovered at rates of 70 to 120 % and measurement was accomplished without any adverse matrix effects even though the crude extract solution was subjected only to acetonitrile extraction.

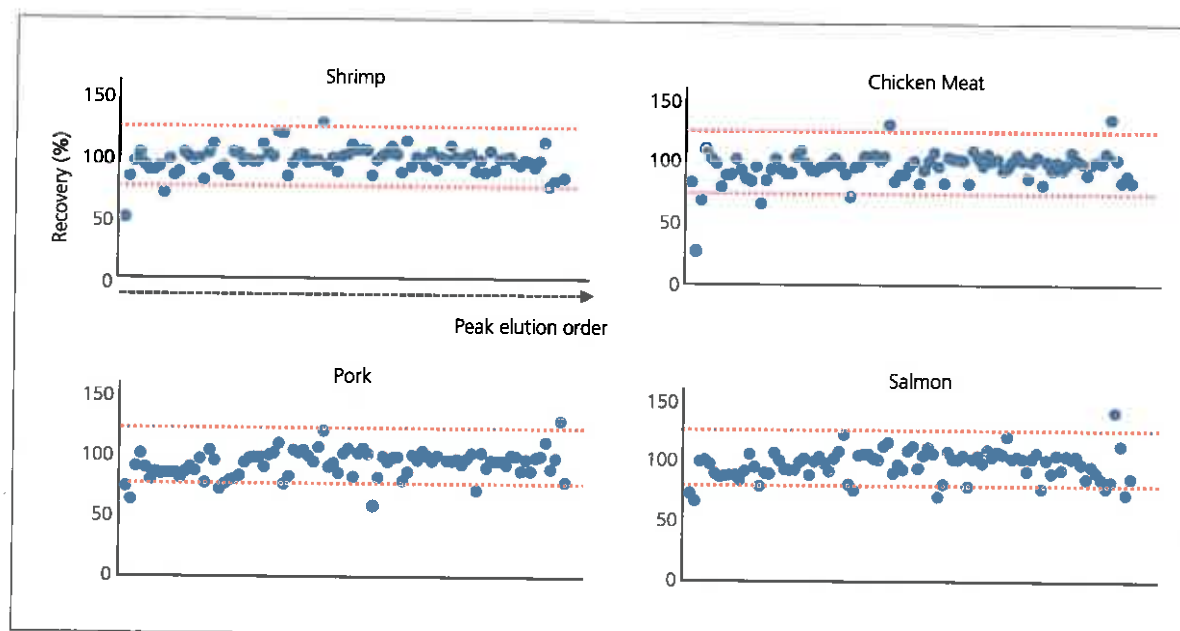


Fig. 5 Recoveries of Veterinary Drugs in Each of the Matrices

Acetonitrile Extraction Efficiency Using QuEChERS Method

To check the efficiency of acetonitrile extraction by the QuEChERS method, standard solution was added at stage (2) of Fig. 1 to obtain a concentration of 10 µg/L, and the recoveries were determined. Good recoveries of approximately 80 % were obtained in cases both

with and without the addition of matrix. However, relatively poor recoveries were seen for highly polar compounds such as tetracycline and quinolone. For these compounds, it is necessary to examine the use of a separate extraction solvent and extraction reagent.

Table 2 Recoveries (Pre-Spike)

Recovery	Without Matrix	With Matrix (Pork)	Compounds with Poor Recovery
< 50 %	17 (19 %)	13 (15 %)	Tetracyclines Quinolones
50 % - 70 %	1 (1 %)	8 (9 %)	
> 70 %	71 (80 %)	68 (76 %)	

Robustness

We checked the long-term stability of the instrument using a solution of pork crude extract (spiked with 10 µg/L standard solution). Even after continuous

measurement of an extremely complex matrix over a period of 3 days, we were able to obtain stable data.

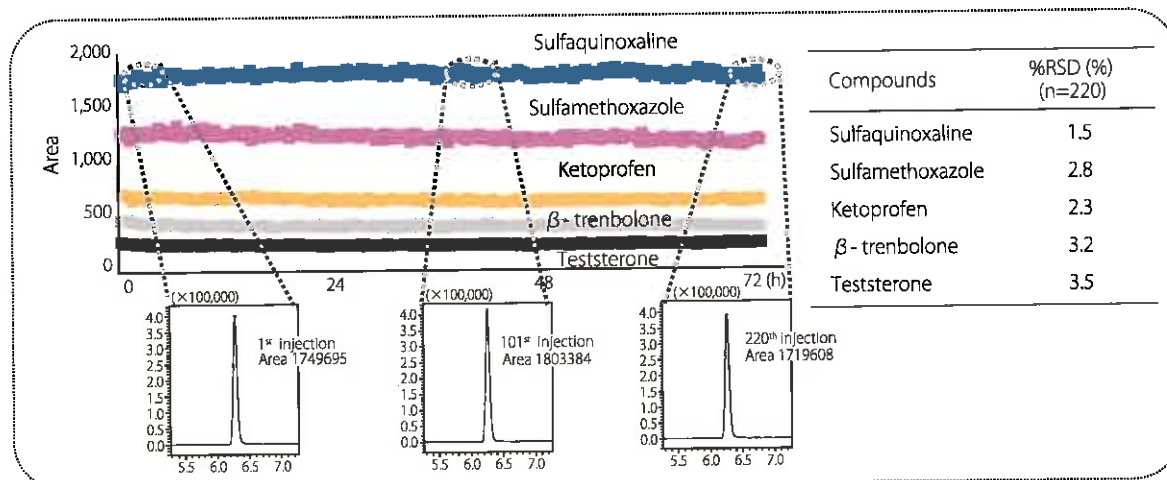


Fig. 6 Area Plot and %RSD of Typical Compounds with Continuous Analysis

Table 3 Analytical Conditions

Column	: Shim-pack XR-ODS II (75 mm × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: 0.1 % Formic Acid - Water
Mobile Phase B	: Acetonitrile
Time Program	: 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10-13 min) → 1 %B (13.01-16 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 2 µL (2 µL sample solution + 10 µL water)
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +2.0 kV / -1.0 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
Interface Temperature	: 400 °C
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C

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Application News

No.C135

Liquid Chromatography Mass Spectrometry

Shimadzu Pesticide MRM Library Support for LC/MS/MS

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■ Abstract

To help expand capabilities in LC/MS/MS pesticide monitoring programs we have created the Shimadzu Pesticide MRM Library. The Library has been created with 766 certified reference standards and has been verified for use with Shimadzu LCMS-8050 and 8060 systems.

The Library contains information that can be used to accelerate method development in LC/MS/MS pesticide analysis including:

An average of 8 MRM transitions for each reference standard (with optimized collision energies) are registered in the database including positive and negative ionization mode. In total, more than 6,000 MRM transitions are part of the Library.

Meta-data for each library entry such as CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (alanwood.net, PAN pesticide database, Chemical Book, ChemSpider). The meta-data is intended not only to set up new methods quickly but to help search for compound properties.

Key words: Pesticide MRM Library,
766 compound library

■ Using the Shimadzu Pesticide MRM Library

Expanding pesticide monitoring programmes (or creating focused methods) can be quickly set up using the Library data base (Table 1) and create fully optimized MRM methods for LC/MS/MS analysis.

Users select the target pesticides and corresponding transitions from the Library and simply copy the list into a Shimadzu LabSolutions analytical method. The method will include optimized MRM transitions. Once the acquisition method is created users can start to acquire data for screening or quantitative LC/MS/MS analysis.

Table 1 The Shimadzu Pesticide MRM Library supports a list of over 766 compounds. Designed to build extended LC/MS/MS methods quickly and to review pesticide information easily.

Library entries	
Compound information	Compound Name Synonyms Japanese name Chinese name CAS Chemical Formula Mono-isotopic mass Theoretical m/z ([M+H] ⁺ , [M+Na] ⁺ , [M+K] ⁺ , [M+NH ₄] ⁺ , [M-H] ⁻) Activity InChI InChIKey
MS/MS parameters	Ionization mode Q1 (m/z) Q3 (m/z) Q1 Pre Bias CE Q3 Pre Bias
Web links	Alanwood.net PAN Pesticide Database Chemical Book ChemSpider

Compound	CAS	Formula	M	[M+H] ⁺	[M-H] ⁻	Ionisation Mode	MSM Transitions
1 (E)-Fenpyroximate	134098-61-6	C24H27N3O4	421.2002	422.2075	420.1929	ESI+	6
2 (E)-Ferimzone	89269-64-7	C15H18N4	254.1531	255.1604	253.1458	ESI+	2
3 (Z)-Fenpyroximate	149054-53-5	C24H27N3O4	421.2002	422.2075	420.1929	ESI+	2
4 (Z)-Ferimzone	89269-64-7	C15H18N4	254.1531	255.1604	253.1458	ESI+	6
5 1-(3, 4-Dichlorophenyl)-3-methylurea	3567-62-2	C8H8Cl2N2O	218.0014	219.0087	216.9941	ESI+	19
6 1-(3, 4-Dichlorophenyl)urea	2327-02-8	C7H6Cl2N2O	203.9857	204.9930	202.9784	ESI+	17
7 1-(4-Isopropylphenyl)-3-methylurea	34123-57-4	C11H16N2O	192.1263	193.1336	191.1190	ESI+	6
8 1-(4-Isopropylphenyl)urea	56046-17-4	C10H14N2O	178.1106	179.1179	177.1033	ESI+	6
9 1-naphthaleneacetamide	86-86-2	C12H11NO	185.0841	186.0914	184.0768	ESI+	4
10 1-Naphthaleneacetic Acid	86-87-3	C12H10O2	186.0681	187.0754	185.0608	ESI-	1
11 2, 4, 5-T	93-76-5	C8H5Cl3O3	253.9304	254.9377	252.9231	ESI-	7
12 2, 4, 6-Tribromophenol	118-79-6	C6H3Br3O	327.7734	328.7807	326.7661	ESI+	10
13 2, 4, 6-Trichlorophenol	88-06-2	C6H3Cl3O	195.9249	196.9322	194.9176	ESI-	3
14 2, 4-D (2, 4-PA)	94-75-7	C8H6Cl2O3	219.9694	220.9767	218.9621	ESI-	7
15 2, 4-DB	94-82-6	C10H10Cl2O3	248.0007	249.0080	246.9934	ESI-	5
16 2, 4-dimethylaniline	95-68-1	C8H11N	121.0891	122.0964	120.0818	ESI+	5
17 2, 6-Dichlorobenzamide	2008-58-4	C7H5Cl2NO	188.9748	189.9821	187.9675	ESI+	13
18 2-Naphthoxy acetic acid	120-23-0	C12H10O3	202.0630	203.0703	201.0557	ESI-	2
19 2-Phenylphenol	90-43-7	C12H10O	170.0732	171.0805	169.0659	ESI-	2
20 3-(3-Indolyl)-propionic acid	830-96-6	C11H11NO2	189.0790	190.0863	188.0717	ESI+	6
21 3, 4, 5-Trimethacarb	2686-99-9	C11H15NO2	193.1103	194.1176	192.1030	ESI+	12
22 3-Indolyl-acetic acid	87-51-4	C10H9NO2	175.0633	176.0706	174.0560	ESI+	12
23 3-Methylphosphinopropionic acid	15090-23-0	C4H9O4P	152.0238	153.0311	151.0165	ESI+	12
24 4-(3-Indolyl)-butyric acid	133-32-4	C12H13NO2	203.0946	204.1019	202.0873	ESI+	14
25 4-Chlorophenoxyacetic acid	122-88-3	C8H7ClO3	186.0084	187.0157	185.0011	ESI-	4
26 6-chloro-3-phenylpyridazin-4-ol	40020-01-7	C10H7ClN2O	206.0247	207.0320	205.0174	ESI+	6
27 6-Furfurylamino-purine	525-79-1	C10H9N5O	215.0807	216.0880	214.0734	ESI+	9
28 Acephate	30560-19-1	C4H10NO3PS	183.0119	184.0192	182.0046	ESI+	6
29 Acequinocyl	57960-19-7	C24H32O4	384.2301	385.2374	383.2228	ESI+	6
30 Acetamiprid	135410-20-7	C10H11ClN4	222.0672	223.0745	221.0599	ESI+	10
31 Acibenzolar-S-methyl	135158-54-2	C8H6N2O5S2	209.9922	210.9995	208.9849	ESI+	6
32 Acifluorfen	50594-66-6	C14H7ClF3NO5	360.9965	362.0038	359.9892	ESI-	12
33 Aclonifen	74070-46-5	C12H9ClN2O3	264.0302	265.0375	263.0229	ESI+	2
34 Acrinathrin	101007-06-1	C26H21F6NO5	541.1324	542.1397	540.1251	ESI+	12
35 Alachlor	15972-60-8	C14H20ClNO2	269.1183	270.1256	268.1110	ESI+	12
36 Alanycarb	83130-01-2	C17H25N3O4S2	399.1286	400.1359	398.1213	ESI+	6
37 Aldicarb	116-06-3	C7H14N2O2S	190.0776	191.0849	189.0703	ESI+	5
38 Aldicarb-sulfone (Aldoxycarb)	1646-88-4	C7H14N2O4S	222.0674	223.0747	221.0601	ESI+	5
39 Aldicarb-sulfoxide	1646-87-3	C7H14N2O3S	206.0725	207.0798	205.0652	ESI+	8
40 Allethrin	584-79-2	C19H26O3	302.1882	303.1955	301.1809	ESI+	12
41 Allidochlor	93-71-0	C8H12ClNO	173.0607	174.0680	172.0534	ESI+	12
42 Ametoctradin	865318-97-4	C15H25N5	275.2110	276.2183	274.2037	ESI+	6
43 Ametryn	834-12-8	C9H17N5S	227.1205	228.1278	226.1132	ESI+	6
44 Amidosulfuron	120923-37-7	C9H15N5O7S2	369.0413	370.0486	368.0340	ESI+	8
45 Aminocarb	2032-59-9	C11H16N2O2	208.1212	209.1285	207.1139	ESI+	6
46 Aminopyralid	150114-71-9	C6H4Cl2N2O2	205.9650	206.9723	204.9577	ESI+	7
47 Amisulbrom	348635-87-0	C13H13BrFN5O4S2	464.9576	465.9649	463.9503	ESI+	10
48 Amitraz	33089-61-1	C19H23N3	293.1892	294.1965	292.1819	ESI+	2
49 Amitrole	61-82-5	C2H4N4	84.0436	85.0509	83.0363	ESI+	5
50 AMPA	1066-51-9	C6H6NO3P	111.0085	112.0158	110.0012	ESI-	3
51 Ancyridol	12771-68-5	C15H16N2O2	256.1212	257.1285	255.1139	ESI+	6
52 Anilazine	101-05-3	C9H5Cl3N4	273.9580	274.9653	272.9507	ESI+	12
53 Anilofos	64249-01-0	C13H19ClNO3PS2	367.0232	368.0305	366.0159	ESI+	12
54 Aramite	140-57-8	C15H23ClO4S	334.1006	335.1079	333.0933	ESI+	12
55 Asulam	3337-71-1	C8H10N2O4S	230.0361	231.0434	229.0288	ESI+	9
56 Atraton	1610-17-9	C9H17N5O	211.1433	212.1506	210.1360	ESI+	6
57 Atrazine	1912-24-9	C8H14ClN5	215.0938	216.1011	214.0865	ESI+	8
58 Atrazine-2-hydroxy	2163-68-0	C8H15N5O	197.1277	198.1350	196.1204	ESI+	6
59 Atrazine-desethyl	6190-65-4	C6H10ClN5	187.0625	188.0698	186.0552	ESI+	9
60 Atrazine-desethyl-2-hydroxy	19988-24-0	C6H11N5O	169.0964	170.1037	168.0891	ESI+	5
61 Atrazine-desisopropyl	1007-28-9	C5H8ClN5	173.0468	174.0541	172.0395	ESI+	10
62 Avermectin B1a	65195-55-3	C48H72O14	872.4922	873.4995	871.4849	ESI+	4
63 Avermectin B1b	65195-56-4	C47H70O14	858.4766	859.4839	857.4693	ESI+	3
64 Azaconazole	60207-31-0	C12H11Cl2N3O2	299.0228	300.0301	298.0155	ESI+	8
65 Azadirachtin	11141-17-6	C35H44O16	720.2629	721.2702	719.2556	ESI+	8
66 Azamethiphos	35575-96-3	C8H10Cl2N2O5PS	323.9737	324.9810	322.9664	ESI+	11
67 Azimsulfuron	120162-55-2	C13H16N10O5S	424.1026	425.1099	423.0953	ESI+	5
68 Azinphos-ethyl	2642-71-9	C12H16N3O3PS2	345.0371	346.0444	344.0298	ESI+	5
69 Azinphos-methyl	86-50-0	C10H12N3O3PS2	317.0058	318.0131	315.9985	ESI+	6
70 Aziprotryne	4658-28-0	C7H11N7S	225.0797	226.0870	224.0724	ESI+	4
71 Azobenzene	103-33-3	C12H10N2	182.0844	183.0917	181.0771	ESI+	2
72 Azoxystrobin	131860-33-8	C22H17N3O5	403.1168	404.1241	402.1095	ESI+	5
73 Barban	101-27-9	C11H9Cl2NO2	257.0010	258.0083	255.9937	ESI+	11
74 Beflubutamid	113614-08-7	C18H17F4NO2	355.1195	356.1268	354.1122	ESI+	10
75 Benalaxyl	71626-11-4	C20H23NO3	325.1678	326.1751	324.1605	ESI+	6

Compound	CAS	Formula	M	[M+H] ⁺	[M-H] ⁻	Ionization Mode	MRM Transition
76 Benazolin	3813-05-6	C ₉ H ₆ ClNO ₃ S	242.9757	243.9830	241.9684	ESI+	6
77 Benazolin-ethyl	25059-80-7	C ₁₁ H ₁₀ ClNO ₃ S	271.0070	272.0143	269.9997	ESI+	18
78 Bendiocarb	22781-23-3	C ₁₁ H ₁₃ NO ₄	223.0845	224.0918	222.0772	ESI+	6
79 Benfuracarb	82560-54-1	C ₂₀ H ₃₀ N ₂ O ₅ S	410.1875	411.1948	409.1802	ESI+	5
80 Benfuresate	68505-69-1	C ₁₂ H ₁₆ O ₄ S	256.0769	257.0842	255.0696	ESI+	2
81 Benodanil	15310-01-7	C ₁₃ H ₁₀ INO	322.9807	323.9880	321.9734	ESI+	6
82 Benoxacor	98730-04-2	C ₁₁ H ₁₁ Cl ₂ NO ₂	259.0167	260.0240	258.0094	ESI+	17
83 Bensulfuron-methyl	83055-99-6	C ₁₆ H ₁₈ N ₄ O ₇ S	410.0896	411.0969	409.0823	ESI+	6
84 Bensulide	741-58-2	C ₁₄ H ₂₄ N ₄ O ₄ PS ₃	397.0605	398.0678	396.0532	ESI+	9
85 Bentazone	25057-89-0	C ₁₀ H ₁₂ N ₂ O ₃ S	240.0569	241.0642	239.0496	ESI-	5
86 Benthiavalcarb-isopropyl	177406-68-7	C ₁₈ H ₂₄ FN ₃ O ₃ S	381.1522	382.1595	380.1449	ESI+	5
87 Benthiazole	21564-17-0	C ₉ H ₆ N ₂ S ₃	237.9693	238.9766	236.9620	ESI+	6
88 Benzanilide	93-98-1	C ₁₃ H ₁₁ NO	197.0841	198.0914	196.0768	ESI+	4
89 Benzofenap	82692-44-2	C ₂₂ H ₂₀ Cl ₂ N ₂ O ₃	430.0851	431.0924	429.0778	ESI+	2
90 Benzoximate	29104-30-1	C ₁₈ H ₁₈ ClNO ₅	363.0874	364.0947	362.0801	ESI+	12
91 Benzoylprop-ethyl	22212-55-1	C ₁₈ H ₁₇ Cl ₂ N ₂ O ₃	365.0585	366.0658	364.0512	ESI+	6
92 Benzthiazuron	1929-88-0	C ₉ H ₉ N ₃ O ₅	207.0466	208.0539	206.0393	ESI+	9
93 Benzyl dimethyl dodecyl ammonium	139-07-1	C ₂₁ H ₃₇ N	303.2926	304.2999	302.2853	ESI+	4
94 Benzyl dimethyl hexadecyl ammonium	122-18-9	C ₂₅ H ₄₅ N	359.3552	360.3625	358.3479	ESI+	3
95 Benzyl dimethyl tetradecyl ammonium	139-08-2	C ₂₃ H ₄₁ N	331.3239	332.3312	330.3166	ESI+	3
96 Bifenazate	149877-41-8	C ₁₇ H ₂₀ N ₂ O ₃	300.1474	301.1547	299.1401	ESI+	6
97 Bifenox	42576-02-3	C ₁₄ H ₉ Cl ₂ NO ₅	340.9858	341.9931	339.9785	ESI+	8
98 Bifenthrin	82657-04-3	C ₂₃ H ₂₂ Cl ₂ F ₃ O ₂	422.1260	423.1333	421.1187	ESI+	5
99 Bioresmethrin	28434-01-7	C ₂₂ H ₂₆ O ₃	338.1882	339.1955	337.1809	ESI+	6
100 Bispyribac-sodium	125401-92-5	C ₁₉ H ₁₇ N ₄ NaO ₈	452.0944	453.1017	451.0871	ESI+	8
101 Birtanitol	55179-31-2	C ₂₀ H ₂₃ N ₃ O ₂	337.1790	338.1863	336.1717	ESI+	6
102 Bixafen	581809-46-3	C ₁₈ H ₁₂ Cl ₂ F ₃ N ₃ O	413.0310	414.0383	412.0237	ESI+	12
103 Boscalid	188425-85-6	C ₁₈ H ₁₂ Cl ₂ N ₂ O	342.0327	343.0400	341.0254	ESI+	12
104 Brodifacoum	56073-10-0	C ₃₁ H ₂₃ BrO ₃	522.0831	523.0904	521.0758	ESI+	12
105 Bromacil	314-40-9	C ₉ H ₁₃ BrN ₂ O ₂	260.0160	261.0233	259.0087	ESI+	9
106 Bromadiolone	28772-56-7	C ₃₀ H ₂₃ BrO ₄	526.0780	527.0853	525.0707	ESI-	12
107 Bromfeninfos	33399-00-7	C ₁₂ H ₁₄ BrCl ₂ O ₄ P	401.9190	402.9263	400.9117	ESI+	17
108 Bromobutide	74712-19-9	C ₁₅ H ₂₂ BrNO	311.0885	312.0958	310.0812	ESI+	10
109 Bromophos-ethyl	4824-78-6	C ₁₀ H ₁₂ BrCl ₂ O ₃ PS	391.8805	392.8878	390.8732	ESI+	3
110 Bromophos-methyl	2104-96-3	C ₈ H ₈ BrCl ₂ O ₃ PS	363.8492	364.8565	362.8419	ESI+	6
111 Bromoxynil	1689-84-5	C ₇ H ₃ Br ₂ NO	274.8581	275.8654	273.8508	ESI-	11
112 Bromuconazole	116255-48-2	C ₁₃ H ₁₂ BrCl ₂ N ₃ O	374.9541	375.9614	373.9468	ESI+	11
113 Bupirimate	41483-43-6	C ₁₃ H ₂₄ N ₄ O ₃ S	316.1569	317.1642	315.1496	ESI+	6
114 Buprofezin	69327-76-0	C ₁₆ H ₂₃ N ₃ O ₅	305.1562	306.1635	304.1489	ESI+	6
115 Butachlor	23184-66-9	C ₁₇ H ₂₆ ClNO ₂	311.1652	312.1725	310.1579	ESI+	12
116 Butafenacil	134605-64-4	C ₂₀ H ₁₈ Cl ₂ F ₃ N ₂ O ₆	474.0805	475.0878	473.0732	ESI+	10
117 Butamifos	36335-67-8	C ₁₃ H ₂₁ N ₂ O ₄ PS	332.0960	333.1033	331.0887	ESI+	12
118 Butocarboxim	34681-10-2	C ₇ H ₁₄ N ₂ O ₂ S	190.0776	191.0849	189.0703	ESI+	3
119 Butocarboxim-sulfone	34681-23-7	C ₇ H ₁₄ N ₂ O ₄ S	222.0674	223.0747	221.0601	ESI+	14
120 Butocarboxim-sulfoxide	34681-24-8	C ₇ H ₁₄ N ₂ O ₃ S	206.0725	207.0798	205.0652	ESI+	6
121 Butralin	33629-47-9	C ₁₄ H ₂₁ N ₃ O ₄	295.1532	296.1605	294.1459	ESI+	6
122 Buturon	3766-60-7	C ₁₂ H ₁₃ ClN ₂ O	236.0716	237.0789	235.0643	ESI+	9
123 Butylate	2008-41-5	C ₁₁ H ₂₃ NOS	217.1500	218.1573	216.1427	ESI+	3
124 Cadusafos	95465-99-9	C ₁₀ H ₂₃ O ₂ PS ₂	270.0877	271.0950	269.0804	ESI+	5
125 Cafenstrole	125306-83-4	C ₁₆ H ₂₂ N ₄ O ₃ S	350.1413	351.1486	349.1340	ESI+	3
126 Captafol	2425-06-1	C ₁₀ H ₉ Cl ₄ NO ₂ S	346.9108	347.9181	345.9035	ESI+	1
127 Carbaryl (NAC)	63-25-2	C ₁₂ H ₁₁ NO ₂	201.0790	202.0863	200.0717	ESI+	6
128 Car bendazim	10605-21-7	C ₉ H ₉ N ₃ O ₂	191.0695	192.0768	190.0622	ESI+	5
129 Carbetamide	16118-49-3	C ₁₂ H ₁₆ N ₂ O ₃	236.1161	237.1234	235.1088	ESI+	6
130 Carbofuran	1563-66-2	C ₁₂ H ₁₅ N ₃ O	221.1052	222.1125	220.0979	ESI+	6
131 Carbofuran-3-hydroxy (3-Hydroxycarbofuran)	16655-82-6	C ₁₂ H ₁₅ NO ₄	237.1001	238.1074	236.0928	ESI+	12
132 Carbofuran-3-keto	16709-30-1	C ₁₂ H ₁₃ NO ₄	235.0845	236.0918	234.0772	ESI+	12
133 Carbophenothion	786-19-6	C ₁₁ H ₁₆ ClO ₂ PS ₃	341.9739	342.9812	340.9666	ESI+	9
134 Carbosulfan	55285-14-8	C ₂₀ H ₃₂ N ₂ O ₃ S	380.2134	381.2207	379.2061	ESI+	6
135 Carboxin	5234-68-4	C ₁₂ H ₁₃ NO ₂ S	235.0667	236.0740	234.0594	ESI+	6
136 Carfentrazone-ethyl	128639-02-1	C ₁₅ H ₁₄ Cl ₂ F ₃ N ₃ O ₃	411.0364	412.0437	410.0291	ESI+	5
137 Carpropamid	104030-54-8	C ₁₅ H ₁₈ Cl ₃ NO	333.0454	334.0527	332.0381	ESI+	18
138 Cartap	15263-53-3	C ₇ H ₁₅ N ₃ O ₂ S ₂	237.0606	238.0679	236.0533	ESI+	3
139 Chinomethionat	2439-01-2	C ₁₀ H ₆ N ₂ O ₂ S	233.9922	234.9995	232.9849	ESI+	6
140 Chloramphenicol	56-75-7	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₅	322.0123	323.0196	321.0050	ESI-	17
141 Chlorantraniliprole	500008-45-7	C ₁₈ H ₁₄ BrCl ₂ N ₂ O ₂	480.9708	481.9781	479.9635	ESI+	28
142 Chlorbromuron	13360-45-7	C ₉ H ₁₀ BrCl ₂ N ₂ O ₂	291.9614	292.9687	290.9541	ESI+	12
143 Chlorbufam	1967-16-4	C ₁₁ H ₁₀ ClNO ₂	223.0400	224.0473	222.0327	ESI+	4
144 Chlordimeform	6164-98-3	C ₁₀ H ₁₃ ClN ₂	196.0767	197.0840	195.0694	ESI+	12
145 Chlorfenvinphos	470-90-6	C ₁₂ H ₁₄ Cl ₃ O ₄ P	357.9695	358.9768	356.9622	ESI+	12
146 Chlorflazuron	71422-67-8	C ₂₀ H ₉ Cl ₃ F ₅ N ₃ O ₃	538.9630	539.9703	537.9557	ESI+	17
147 Chloridazon	1698-60-8	C ₁₀ H ₈ ClN ₃ O	221.0356	222.0429	220.0283	ESI+	11
148 Chlorimuron-ethyl	90982-32-4	C ₁₅ H ₁₅ ClN ₄ O ₆ S	414.0401	415.0474	413.0328	ESI+	12
149 Chloromequat-chloride	999-81-5	C ₅ H ₁₃ Cl ₂ N	157.0425	158.0498	156.0352	ESI+	6
150 Chlorophacinone	3691-35-8	C ₂₃ H ₁₅ ClO ₃	374.0710	375.0783	373.0637	ESI-	15

Compound	CAS	Formula	M	PM+H+	(M-H)	Ionization Mode	ABRIL Transitions
151 Chlorotoluron	15545-48-9	C10H13ClN2O	212.0716	213.0789	211.0643	ESI+	8
152 Chloroxuron	1982-47-4	C15H15ClN2O2	290.0822	291.0895	289.0749	ESI+	12
153 Chloroxynil	1891-95-8	C7H3Cl2NO	186.9592	187.9665	185.9519	ESI-	6
154 Chlorpropham	101-21-3	C10H12ClNO2	213.0557	214.0630	212.0484	ESI+	2
155 Chlorpyrifos	2921-88-2	C9H11Cl3NO3PS	348.9263	349.9336	347.9190	ESI+	16
156 Chlorpyrifos-methyl	5598-13-0	C7H7Cl3NO3PS	320.8950	321.9023	319.8877	ESI+	12
157 Chlorpyrifos-oxon	5598-15-2	C9H11Cl3NO4P	332.9491	333.9564	331.9418	ESI+	24
158 Chlorsulfuron	64902-72-3	C12H12ClN5O4S	357.0299	358.0372	356.0226	ESI+	18
159 Chlorthiamid	1918-13-4	C7H5Cl2NS	204.9520	205.9593	203.9447	ESI+	16
160 Chromafenozide	143807-66-3	C24H30N2O3	394.2256	395.2329	393.2183	ESI+	6
161 Cinidon-ethyl	142891-20-1	C19H17Cl2NO4	393.0535	394.0608	392.0462	ESI+	24
162 Cinosulfuron	94593-91-6	C15H19N5O7S	413.1005	414.1078	412.0932	ESI+	6
163 Clethodim	99129-21-2	C17H26ClNO3S	359.1322	360.1395	358.1249	ESI+	10
164 Climbazole	38083-17-9	C15H17ClN2O2	292.0979	293.1052	291.0906	ESI+	9
165 Clodinafop (free acid)	114420-56-3	C14H11ClFN4O4	311.0361	312.0434	310.0288	ESI+	8
166 Clodinafop-propargyl	105512-06-9	C17H13ClFN4O4	349.0517	350.0590	348.0444	ESI+	12
167 Clofentezine	74115-24-5	C14H8Cl2N4	302.0126	303.0199	301.0053	ESI+	10
168 Clomazone	81777-89-1	C12H14ClNO2	239.0713	240.0786	238.0640	ESI+	8
169 Clomeprop	84496-56-0	C16H15Cl2NO2	323.0480	324.0553	322.0407	ESI+	21
170 Cloprop	101-10-0	C9H9ClO3	200.0240	201.0313	199.0167	ESI-	2
171 Clopyralid	1702-17-6	C6H3Cl2NO2	190.9541	191.9614	189.9468	ESI-	2
172 Cloquintocet-mexyl	99607-70-2	C18H22ClNO3	335.1288	336.1361	334.1215	ESI+	9
173 Cloransulam-methyl	147150-35-4	C15H13ClFN5O5S	429.0310	430.0383	428.0237	ESI+	12
174 Clothianidin	210880-92-5	C6H8ClN5O2S	249.0087	250.0160	248.0014	ESI+	7
175 Coumachlor	81-82-3	C19H15ClO4	342.0659	343.0732	341.0586	ESI+	18
176 Coumaphos	56-72-4	C14H16ClO5PS	362.0145	363.0218	361.0072	ESI+	12
177 Coumatetralyl	5836-29-3	C19H16O3	292.1099	293.1172	291.1026	ESI+	6
178 Crimidine	535-89-7	C7H10ClN3	171.0563	172.0636	170.0490	ESI+	12
179 Crotoxyphos	7700-17-6	C14H19O6P	314.0919	315.0992	313.0846	ESI+	6
180 Crufomate	299-86-5	C12H19ClNO3P	291.0791	292.0864	290.0718	ESI+	12
181 Cumyluron	99485-76-4	C17H19ClN2O	302.1186	303.1259	301.1113	ESI+	2
182 Cyanazine	21725-46-2	C9H13ClN6	240.0890	241.0963	239.0817	ESI+	6
183 Cyanofenphos	13067-93-1	C15H14NO2PS	303.0483	304.0556	302.0410	ESI+	6
184 Cyazofamid	120116-88-3	C13H13ClN4O2S	324.0448	325.0521	323.0375	ESI+	5
185 Cyclanilide	113136-77-9	C11H9Cl2NO3	272.9959	274.0032	271.9886	ESI-	20
186 Cycloate	1134-23-2	C11H21NO5	215.1344	216.1417	214.1271	ESI+	5
187 Cycloheximide	66-81-9	C15H23NO4	281.1627	282.1700	280.1554	ESI+	12
188 Cycloprothrin	63935-38-6	C26H21Cl2NO4	481.0848	482.0921	480.0775	ESI+	2
189 Cyclosulfamuron	136849-15-5	C17H19N5O6S	421.1056	422.1129	420.0983	ESI+	6
190 Cycloxydim	101205-02-1	C17H27NO3S	325.1712	326.1785	324.1639	ESI+	10
191 Cycluron	2163-69-1	C11H22N2O	198.1732	199.1805	197.1659	ESI+	5
192 Cyflufenamid	180409-60-3	C20H17F5N2O2	412.1210	413.1283	411.1137	ESI+	6
193 Cyflumetofen	400882-07-7	C24H24F3NO4	447.1657	448.1730	446.1584	ESI+	8
194 Cyhalofop-butyl	122008-85-9	C20H20FNO4	357.1376	358.1449	356.1303	ESI+	3
195 Cymiazole	61676-87-7	C12H14N2S	218.0878	219.0951	217.0805	ESI+	6
196 Cymoxanil	57966-95-7	C7H10N4O3	198.0753	199.0826	197.0680	ESI+	4
197 Cypermethrin	52315-07-8	C22H19Cl2NO3	415.0742	416.0815	414.0669	ESI+	10
198 Cyphenothrin	39515-40-7	C24H25NO3	375.1834	376.1907	374.1761	ESI+	12
199 Cyproconazole	94361-06-5	C15H18ClN3O	291.1138	292.1211	290.1065	ESI+	10
200 Cyprodinil	121552-61-2	C14H15N3	225.1266	226.1339	224.1193	ESI+	6
201 Cyromazine	66215-27-8	C6H10N6	166.0967	167.1040	165.0894	ESI+	6
202 Daimuron (Dymron)	42609-52-9	C17H20N2O	268.1576	269.1649	267.1503	ESI+	6
203 Dalapon	75-99-0	C3H4Cl2O2	141.9588	142.9661	140.9515	ESI-	10
204 Daminozide	1596-84-5	C6H12N2O3	160.0848	161.0921	159.0775	ESI+	6
205 Dazomet	533-74-4	C5H10N2S2	162.0285	163.0358	161.0212	ESI+	6
206 Deet	134-62-3	C12H17NO	191.1310	192.1383	190.1237	ESI+	2
207 Deltamethrin	52918-63-5	C22H19Br2NO3	502.9732	503.9805	501.9659	ESI+	12
208 Demeton-Q	298-03-3	C8H19O3PS2	258.0513	259.0586	257.0440	ESI+	2
209 Demeton-S	126-75-0	C8H19O3PS2	258.0513	259.0586	257.0440	ESI+	3
210 Demeton-S-methyl	919-86-8	C6H15O3PS2	230.0200	231.0273	229.0127	ESI+	2
211 Demeton-S-methyl-sulfone	17040-19-6	C6H15O5PS2	262.0099	263.0172	261.0026	ESI+	6
212 Desmedipham	13684-56-5	C16H16N2O4	300.1110	301.1183	299.1037	ESI+	6
213 Desmetryn	1014-69-3	C8H15N5S	213.1048	214.1121	212.0975	ESI+	4
214 Diafenthiuron	80060-09-9	C23H32N2O5	384.2235	385.2308	383.2162	ESI+	12
215 Dialifos	10311-84-9	C14H17ClNO4PS2	393.0025	394.0098	391.9952	ESI+	12
216 Diallate	2303-16-4	C10H17Cl2NOS	269.0408	270.0481	268.0335	ESI+	12
217 Diazinon	333-41-5	C12H21N2O3PS	304.1010	305.1083	303.0937	ESI+	6
218 Dicamba	1918-00-9	C8H6Cl2O3	219.9694	220.9767	218.9621	ESI-	2
219 Dichlofenthiol	97-17-6	C10H13Cl2O3PS	313.9700	314.9773	312.9627	ESI+	8
220 Dichlofluanid	1085-98-9	C9H11Cl2FN2O2S2	331.9623	332.9696	330.9550	ESI+	11
221 Dichlorimid	37764-25-3	C8H11Cl2NO	207.0218	208.0291	206.0145	ESI+	19
222 Dichlorprop	120-36-5	C9H8Cl2O3	233.9850	234.9923	232.9777	ESI-	8
223 Dichlorvos	62-73-7	C4H7Cl2O4P	219.9459	220.9532	218.9386	ESI+	17
224 Diclobutrazol	75736-33-3	C15H19Cl2N3O	327.0905	328.0978	326.0832	ESI+	4
225 Diclofop	40843-25-2	C15H12Cl2O4	326.0113	327.0186	325.0040	ESI-	4

Compound	CAS	Formula	M	[M+H] ⁺	[M-H] ⁻	Ionisation Mode	MRM Transitions
226 Diclofop-methyl	51338-27-3	C16H14Cl2O4	340.0269	341.0342	339.0196	ESI+	12
227 Dicloran	99-30-9	C6H4Cl2N2O2	205.9650	206.9723	204.9577	ESI+	4
228 Diclosulam	145701-21-9	C13H10Cl2FN5O3S	404.9865	405.9938	403.9792	ESI+	9
229 Dicrotophos	141-66-2	C8H16NO5P	237.0766	238.0839	236.0693	ESI+	6
230 Dicyclanil	112636-83-6	C8H10N6	190.0967	191.1040	189.0894	ESI+	6
231 Didecyldimethylammonium	7173-51-5	C22H47N	325.3709	326.3782	324.3636	ESI+	6
232 Diethanolamine	111-42-2	C4H11NO2	105.0790	106.0863	104.0717	ESI+	6
233 Diethofencarb	87130-20-9	C14H21NO4	267.1471	268.1544	266.1398	ESI+	6
234 Difenacoum	56073-07-5	C31H24O3	444.1725	445.1798	443.1652	ESI+	12
235 Difenconazole	119446-68-3	C19H17Cl2N3O3	405.0647	406.0720	404.0574	ESI+	12
236 Difenoxyuron	14214-32-5	C16H18N2O3	286.1317	287.1390	285.1244	ESI+	6
237 Difenzoquat-methyl-sulfate	43222-48-6	C17H16N2	248.1313	249.1386	247.1240	ESI+	6
238 Diflubenzuron	35367-38-5	C14H9ClF2N2O2	310.0321	311.0394	309.0248	ESI+	9
239 Diflufenican	83164-33-4	C19H11F5N2O2	394.0741	395.0814	393.0668	ESI+	12
240 Dimefuron	34205-21-5	C15H19ClN4O3	338.1146	339.1219	337.1073	ESI+	5
241 Dimepiperate	61432-55-1	C15H21NOS	263.1344	264.1417	262.1271	ESI+	6
242 Dimethachion	24096-53-5	C10H7Cl2NO2	242.9854	243.9927	241.9781	ESI-	2
243 Dimethachlor	50563-36-5	C13H18ClNO2	255.1026	256.1099	254.0953	ESI+	12
244 Dimethametryn	22936-75-0	C11H21N5S	255.1518	256.1591	254.1445	ESI+	6
245 Dimethenamid	87674-68-8	C12H18ClNO2S	275.0747	276.0820	274.0674	ESI+	12
246 Dimethinmol	5221-53-4	C11H19N3O	209.1528	210.1601	208.1455	ESI+	3
247 Dimethoate	60-51-5	C5H12NO3PS2	228.9996	230.0069	227.9923	ESI+	6
248 Dimethomorph	110488-70-5	C21H22ClNO4	387.1237	388.1310	386.1164	ESI+	12
249 Dimetilan	644-64-4	C10H16N4O3	240.1222	241.1295	239.1149	ESI+	6
250 Dimoxystrobin	149961-52-4	C19H22N2O3	326.1630	327.1703	325.1557	ESI+	6
251 Diniconazole	83657-24-3	C15H17Cl2N3O	325.0749	326.0822	324.0676	ESI+	7
252 Dinocap	39300-45-3	C18H24N2O6	364.1634	365.1707	363.1561	ESI+	6
253 Dinoseb	88-85-7	C10H12N2O5	240.0746	241.0819	239.0673	ESI-	4
254 Dinotefuran	165252-70-0	C7H14N4O3	202.1066	203.1139	201.0993	ESI+	6
255 Dinoterb	1420-07-1	C10H12N2O5	240.0746	241.0819	239.0673	ESI-	4
256 Dioxacarb	6988-21-2	C11H13NO4	223.0845	224.0918	222.0772	ESI+	6
257 Dioxathion	78-34-2	C12H26O6P2S4	456.0087	457.0160	455.0014	ESI+	6
258 Diphenamid	957-51-7	C16H17NO	239.1310	240.1383	238.1237	ESI+	6
259 Diphenylamine	122-39-4	C12H11N	169.0891	170.0964	168.0818	ESI+	4
260 Dipropetryn	4147-51-7	C11H21N5S	255.1518	256.1591	254.1445	ESI+	6
261 Diquat	6385-62-2	C12H12N2	184.1000	185.1073	183.0927	ESI+	3
262 Disulfoton	298-04-4	C8H19O2PS3	274.0285	275.0358	273.0212	ESI+	3
263 Disulfoton-sulfone	2497-06-5	C8H19O4PS3	306.0183	307.0256	305.0110	ESI+	6
264 Disulfoton-sulfoxide	2497-07-6	C8H19O3PS3	290.0234	291.0307	289.0161	ESI+	6
265 Ditalimfos	5131-24-8	C12H14NO4P5	299.0381	300.0454	298.0308	ESI+	6
266 Dithianon	3347-22-6	C14H4N2O2S2	295.9714	296.9787	294.9641	ESI-	4
267 Dithiopyr	97886-45-8	C15H16F5NO2S2	401.0543	402.0616	400.0470	ESI+	6
268 Diuron (DCMU)	330-54-1	C9H10Cl2N2O	232.0170	233.0243	231.0097	ESI+	7
269 DMST	66840-71-9	C9H14N2O2S	214.0776	215.0849	213.0703	ESI+	4
270 DNOC	534-52-1	C7H6N2O5	198.0277	199.0350	197.0204	ESI-	6
271 Dodemorph	1593-77-7	C18H35NO	281.2719	282.2792	280.2646	ESI+	6
272 Dodine	2439-10-3	C15H33N3O2	287.2573	288.2646	286.2500	ESI+	6
273 Doramectin	117704-25-3	C50H74O14	898.5079	899.5152	897.5006	ESI+	10
274 Edifenphos	17109-49-8	C14H15O2PS2	310.0251	311.0324	309.0178	ESI+	6
275 Emamectin B1a	119791-41-2	C49H75NO13	885.5238	886.5311	884.5165	ESI+	5
276 Emamectin B1b	137335-79-6	C55H79NO15	871.5082	872.5155	870.5009	ESI+	3
277 Endosulfan-sulfate	1031-07-8	C9H6Cl6O4S	419.8118	420.8191	418.8045	ESI-	3
278 EPN	2104-64-5	C14H14NO4P5	323.0381	324.0454	322.0308	ESI+	6
279 Epoxiconazole	133855-98-8	C17H13ClFN3O	329.0731	330.0804	328.0658	ESI+	9
280 EPTC	759-94-4	C9H19NO3	189.1187	190.1260	188.1114	ESI+	5
281 Esfenvalerate	66230-04-4	C25H22ClNO3	419.1288	420.1361	418.1215	ESI+	2
282 Esprocarb	85785-20-2	C15H23NOS	265.1500	266.1573	264.1427	ESI+	5
283 Etaconazole	60207-93-4	C14H15Cl2N3O2	327.0541	328.0614	326.0468	ESI+	12
284 Ethametsulfuron-methyl	97780-06-8	C15H18N6O6S	410.1009	411.1082	409.0936	ESI+	6
285 Ethephon	16672-87-0	C2H6ClO3P	143.9743	144.9816	142.9670	ESI-	6
286 Ethidimuron	30043-49-3	C7H12N4O3S2	264.0351	265.0424	263.0278	ESI+	11
287 Ethiofencarb	29973-13-5	C11H15NO2S	225.0823	226.0896	224.0750	ESI+	10
288 Ethiofencarb-sulfone	53380-23-7	C11H15NO4S	257.0722	258.0795	256.0649	ESI+	8
289 Ethiofencarb-sulfoxide	53380-22-6	C11H15NO3S	241.0773	242.0846	240.0700	ESI+	4
290 Ethion	563-12-2	C9H22O4P2S4	383.9876	384.9949	382.9803	ESI+	6
291 Ethiprole	181587-01-9	C13H9Cl2F3N4O5	395.9826	396.9899	394.9753	ESI+	30
292 Ethinmol	23947-60-6	C11H19N3O	209.1528	210.1601	208.1455	ESI+	6
293 Ethofumesate	26225-79-6	C13H18O5S	286.0875	287.0948	285.0802	ESI+	11
294 Ethoprophos	13194-48-4	C8H19O2PS2	242.0564	243.0637	241.0491	ESI+	6
295 Ethoxyquin	91-53-2	C14H19NO	217.1467	218.1540	216.1394	ESI+	4
296 Ethoxysulfuron	126801-58-9	C15H18N4O7S	398.0896	399.0969	397.0823	ESI+	6
297 Ethylenethiourea	96-45-7	C3H6N2S	102.0252	103.0325	101.0179	ESI+	6
298 Etofenprox	80844-07-1	C25H28O3	376.2038	377.2111	375.1965	ESI+	6
299 Etoxazole	153233-91-1	C21H23F2NO2	359.1697	360.1770	358.1624	ESI+	6
300 Etrinfos	38260-54-7	C10H17N2O4PS	292.0647	293.0720	291.0574	ESI+	6

Compound	CAS	Formula	M	[M+H] ⁺	[M-H]	Ionization Mode	MRM Transitions
301 Famoxadone	131807-57-3	C22H18N2O4	374.1267	375.1340	373.1194	ESI+	6
302 Famphur	52-85-7	C10H16NO5PS2	325.0208	326.0281	324.0135	ESI+	12
303 Fenamidone	161326-34-7	C17H17N3O5	311.1092	312.1165	310.1019	ESI+	6
304 Fenaminosulf	140-56-7	C8H10N3NaO3S	251.0341	252.0414	250.0268	ESI+	2
305 Fenamiphos	22224-92-6	C13H22NO3PS	303.1058	304.1131	302.0985	ESI+	6
306 Fenamiphos-sulfone	31972-44-8	C13H22NO5PS	335.0956	336.1029	334.0883	ESI+	6
307 Fenamiphos-sulfoxide	31972-43-7	C13H22NO4PS	319.1007	320.1080	318.0934	ESI+	6
308 Fenarimol	60168-88-9	C17H12Cl2N2O	330.0327	331.0400	329.0254	ESI+	12
309 Fenazaquin	120928-09-8	C20H22N2O	306.1732	307.1805	305.1659	ESI+	6
310 Fenazox	495-48-7	C12H10N2O	198.0793	199.0866	197.0720	ESI+	6
311 Fenbuconazole	114369-43-6	C19H17ClN4	336.1142	337.1215	335.1069	ESI+	8
312 Fenbutatin-oxide	13356-08-6	C60H78O5Sn2	1054.4121	1055.4194	1053.4048	ESI+	11
313 Fenchlorazot-ethyl	103112-35-2	C12H8Cl5N3O2	400.9059	401.9132	399.8986	ESI+	118
314 Fenfuram	24691-80-3	C12H11NO2	201.0790	202.0863	200.0717	ESI+	6
315 Fenhexamid	126833-17-8	C14H17Cl2NO2	301.0636	302.0709	300.0563	ESI+	23
316 Fenitrothion	122-14-5	C9H12NO5PS	277.0174	278.0247	276.0101	ESI+	2
317 Fenobucarb	3766-81-2	C12H17NO2	207.1259	208.1332	206.1186	ESI+	6
318 Fenoprop	93-72-1	C9H7Cl3O3	267.9461	268.9534	266.9388	ESI+	8
319 Fenothiocarb	62850-32-2	C13H19NO2S	253.1136	254.1209	252.1063	ESI+	4
320 Fenoxanil	115852-48-7	C15H18Cl2N2O2	328.0745	329.0818	327.0672	ESI+	29
321 Fenoxaprop	95617-09-7	C16H12ClNO5	333.0404	334.0477	332.0331	ESI+	23
322 Fenoxaprop-ethyl	66441-23-4	C18H16ClNO5	361.0717	362.0790	360.0644	ESI+	12
323 Fenoxaprop-P-ethyl	71283-80-2	C18H16ClNO5	361.0717	362.0790	360.0644	ESI+	12
324 Fenoxycarb	79127-80-3	C17H19NO4	301.1314	302.1387	300.1241	ESI+	6
325 Fenpropathrin	64257-84-7	C22H23NO3	349.1678	350.1751	348.1605	ESI+	11
326 Fenpropidin	67306-00-7	C19H31N	273.2457	274.2530	272.2384	ESI+	6
327 Fenpropimorph	67564-91-4	C20H33NO	303.2562	304.2635	302.2489	ESI+	6
328 Fensulfothion	115-90-2	C11H17O4PS2	308.0306	309.0379	307.0233	ESI+	6
329 Fensulfothion-oxon	6552-21-2	C11H17O5PS	292.0534	293.0607	291.0461	ESI+	6
330 Fensulfothion-oxon-sulfone	6132-17-8	C11H17O6PS	308.0483	309.0556	307.0410	ESI+	4
331 Fensulfothion-sulfone	14255-72-2	C11H17O5PS2	324.0255	325.0328	323.0182	ESI+	6
332 Fenthion	55-38-9	C10H15O3PS2	278.0200	279.0273	277.0127	ESI+	6
333 Fenthion-oxon	6552-12-1	C10H15O4PS	262.0429	263.0502	261.0356	ESI+	6
334 Fenthion-oxon-sulfone	14086-35-2	C10H15O6PS	294.0327	295.0400	293.0254	ESI+	12
335 Fenthion-oxon-sulfoxide	6552-13-2	C10H15O5PS	278.0378	279.0451	277.0305	ESI+	3
336 Fenthion-sulfone	3761-42-0	C10H15O5PS2	310.0099	311.0172	309.0026	ESI+	4
337 Fenthion-sulfoxide	3761-41-9	C10H15O4PS2	294.0149	295.0222	293.0076	ESI+	6
338 Fenuron	101-42-8	C9H12N2O	164.0950	165.1023	163.0877	ESI+	6
339 Fenvalerate	51630-58-1	C25H22ClNO3	419.1288	420.1361	418.1215	ESI+	6
340 Fipronil	120068-37-3	C12H4Cl2F6N4O5	435.9387	436.9460	434.9314	ESI+	12
341 Fipronil-desulfinyl	205650-65-3	C12H4Cl2F6N4	387.9717	388.9790	386.9644	ESI+	12
342 Fipronil-sulfide	120067-83-6	C12H4Cl2F6N4S	419.9438	420.9511	418.9365	ESI+	12
343 Fipronil-sulfone	120068-36-2	C12H4Cl2F6N4O2S	451.9336	452.9409	450.9263	ESI+	12
344 Flamprop-isopropyl	52756-22-6	C19H19ClFNO3	363.1037	364.1110	362.0964	ESI+	10
345 Flamprop-methyl	52756-25-9	C17H15ClFNO3	335.0724	336.0797	334.0651	ESI+	4
346 Flamprop-M-isopropyl	63782-90-1	C19H19ClFNO3	363.1037	364.1110	362.0964	ESI+	10
347 Flazasulfuron	104040-78-0	C13H12F3N5O5S	407.0511	408.0584	406.0438	ESI+	6
348 Floccoumafen	90035-08-8	C33H25F3O4	542.1705	543.1778	541.1632	ESI+	12
349 Flonicamid	158062-67-0	C9H6F3N3O	229.0463	230.0536	228.0390	ESI+	8
350 Florasulam	145701-23-1	C12H8F3N5O3S	359.0300	360.0373	358.0227	ESI+	2
351 Fluacrypyrim	229977-93-9	C20H21F3N2O5	426.1403	427.1476	425.1330	ESI+	6
352 Fluazifop	69335-91-7	C15H12F3NO4	327.0718	328.0791	326.0645	ESI+	12
353 Fluazifop-butyl	69806-50-4	C19H20F3NO4	383.1344	384.1417	382.1271	ESI+	6
354 Fluazifop-P (free acid)	83066-88-0	C15H12F3NO4	327.0718	328.0791	326.0645	ESI+	12
355 Fluazifop-P-butyl	79241-46-6	C19H20F3NO4	383.1344	384.1417	382.1271	ESI+	6
356 Fluazinaf	79622-59-6	C13H4Cl2F6N4O4	463.9514	464.9587	462.9441	ESI+	12
357 Fluazuron	86811-58-7	C20H10Cl2F5N3O3	505.0019	506.0092	503.9946	ESI+	17
358 Flubendamide	272451-65-7	C23H22F7IN2O4S	682.0233	683.0306	681.0160	ESI+	5
359 Flucycloxuron	94050-52-9	C25H20ClF2N3O3	483.1161	484.1234	482.1088	ESI+	10
360 Flucythrinate	70124-77-5	C26H23F2NO4	451.1595	452.1668	450.1522	ESI+	4
361 Fludioxonil	131341-86-1	C12H6F2N2O2	248.0397	249.0470	247.0324	ESI+	6
362 Flufenacet	142459-58-3	C14H13F4N3O2S	363.0665	364.0738	362.0592	ESI+	6
363 Flufenoxuron	101463-69-8	C21H11ClF6N2O3	488.0362	489.0435	487.0289	ESI+	8
364 Flumetralin	62924-70-3	C16H12ClF4N3O4	421.0452	422.0525	420.0379	ESI+	3
365 Flumetsulam	98967-40-9	C12H9F2N5O2S	325.0445	326.0518	324.0372	ESI+	2
366 Flumioxazin	103361-09-7	C19H15F2N2O4	354.1016	355.1089	353.0943	ESI+	2
367 Fluometuron	2164-17-2	C10H11F3N2O	232.0823	233.0896	231.0750	ESI+	4
368 Fluopicolide	239110-15-7	C14H8Cl3F3N2O	381.9654	382.9727	380.9581	ESI+	11
369 Fluopyram	658066-35-4	C16H11ClF6N2O	396.0464	397.0537	395.0391	ESI+	12
370 Fluoroglycofen-ethyl	77501-90-7	C18H13ClF3NO7	447.0333	448.0406	446.0260	ESI+	12
371 Fluoxastrobin	361377-29-9	C21H16ClF4NO5	458.0793	459.0866	457.0720	ESI+	12
372 Flupyrsulfuron-methyl	144740-54-5	C15H14F3N5O7S	465.0566	466.0639	464.0493	ESI+	12
373 Fluquinconazole	136426-54-5	C16H8Cl2F2N5O	375.0090	376.0163	374.0017	ESI+	10
374 Fluridone	59756-60-4	C19H14F3NO	329.1027	330.1100	328.0954	ESI+	4
375 Flurochloridone	61213-25-0	C12H10Cl2F3NO	311.0092	312.0165	310.0019	ESI+	18

Compound	CAS	Formula	M	[M+H] ⁺	[M-H] ⁻	Ionisation Mode	LR/MS Transition
376 Fluroxypyr	69377-81-7	C7H5Cl2FN2O3	253.9661	254.9734	252.9588	ESI+	15
377 Fluroxypyr-1-methylheptyl ester	81406-37-3	C15H21Cl2FN2O3	366.0913	367.0986	365.0840	ESI+	12
378 Flurprimidol	56425-91-3	C15H15F3N2O2	312.1086	313.1159	311.1013	ESI+	6
379 Flurtamone	96525-23-4	C18H14F3NO2	333.0977	334.1050	332.0904	ESI+	6
380 Flusilazole	85509-19-9	C16H15F2N3S	315.1003	316.1076	314.0930	ESI+	6
381 Fluthiacet-methyl	117337-19-6	C15H15ClFN3O3S2	403.0227	404.0300	402.0154	ESI+	9
382 Flutolanil	66332-96-5	C17H16F3NO2	323.1133	324.1206	322.1060	ESI+	12
383 Flutriafol	76674-21-0	C16H13F2N3O	301.1027	302.1100	300.0954	ESI+	5
384 Fluxapyroxad	907204-31-3	C18H12F5N3O	381.0901	382.0974	380.0828	ESI+	11
385 Fomesafen	72178-02-0	C15H10ClF3N2O6S	437.9900	438.9973	436.9827	ESI+	21
386 Fonofos	944-22-9	C10H15O5P2	246.0302	247.0375	245.0229	ESI+	5
387 Foramsulfuron	173159-57-4	C17H20N6O7S	452.1114	453.1187	451.1041	ESI+	6
388 Forchlorfenuron	68157-60-8	C12H10ClN3O	247.0512	248.0585	246.0439	ESI+	12
389 Fosetyl-aluminium	39148-24-8	C2H7O3P	110.0133	111.0206	109.0060	ESI-	3
390 Fosthiazate	98886-44-3	C9H18NO3PS2	283.0466	284.0539	282.0393	ESI+	6
391 Fuberidazole	3878-19-1	C11H8N2O	184.0637	185.0710	183.0564	ESI+	6
392 Furalaxyl	57646-30-7	C17H19NO4	301.1314	302.1387	300.1241	ESI+	3
393 Furametpyr	123572-88-3	C17H20ClN3O2	333.1244	334.1317	332.1171	ESI+	12
394 Furathiocarb	65907-30-4	C18H26N2O5S	382.1562	383.1635	381.1489	ESI+	6
395 Furmecyclox	60568-05-0	C14H21NO3	251.1521	252.1594	250.1448	ESI+	6
396 Gibberellic acid (Gibberellin)	77-06-5	C19H22O6	346.1416	347.1489	345.1343	ESI-	11
397 Gluphosinate	77182-82-2	C5H12NO4P	181.0504	182.0577	180.0431	ESI+	10
398 Glyphosate	1071-83-6	C3H8NO5P	169.0140	170.0213	168.0067	ESI+	8
399 Halofenozide	112226-61-6	C18H19ClN2O2	330.1135	331.1208	329.1062	ESI+	12
400 Halosulfuron-methyl	100784-20-1	C13H15ClN6O7S	434.0411	435.0484	433.0338	ESI+	11
401 Haloxypfop	69806-34-4	C15H11ClF3NO4	361.0329	362.0402	360.0256	ESI+	9
402 Haloxypfop-2-ethoxyethyl	87237-48-7	C19H19ClF3NO5	433.0904	434.0977	432.0831	ESI+	12
403 Haloxypfop-methyl	69806-40-2	C16H13ClF3NO4	375.0485	376.0558	374.0412	ESI+	12
404 Haloxypfop-R-methyl	72619-32-0	C16H13ClF3NO4	375.0485	376.0558	374.0412	ESI+	12
405 Heptenophos	23560-59-0	C9H12ClO4P	250.0162	251.0235	249.0089	ESI+	9
406 Hexaconazole	79983-71-4	C14H17Cl2N3O	313.0749	314.0822	312.0676	ESI+	10
407 Hexaflumuron	86479-06-3	C16H8Cl2F6N2O3	459.9816	460.9889	458.9743	ESI-	12
408 Hexazinone	51235-04-2	C12H20N4O2	252.1586	253.1659	251.1513	ESI+	3
409 Hexythiazox	78587-05-0	C17H21ClN2O2S	352.1012	353.1085	351.0939	ESI+	11
410 Hydramethylnon	67485-29-4	C25H24F6N4	494.1905	495.1978	493.1832	ESI+	12
411 Hymexazol	10004-44-1	C4H5NO2	99.0320	100.0393	98.0247	ESI+	3
412 Imazalil	35554-44-0	C14H14Cl2N2O	296.0483	297.0556	295.0410	ESI+	12
413 Imazamethabenz-methyl	81405-85-8	C16H20N2O3	288.1474	289.1547	287.1401	ESI+	12
414 Imazamox	114311-32-9	C15H19N3O4	305.1376	306.1449	304.1303	ESI+	10
415 Imazapic	104098-48-8	C14H17N3O3	275.1270	276.1343	274.1197	ESI+	11
416 Imazapyr	81334-34-1	C13H15N3O3	261.1113	262.1186	260.1040	ESI+	11
417 Imazaquin	81335-37-7	C17H17N3O3	311.1270	312.1343	310.1197	ESI+	6
418 Imazethapyr	81335-77-5	C15H19N3O3	289.1426	290.1499	288.1353	ESI+	12
419 Imazosulfuron	122548-33-8	C14H13ClN6O5S	412.0357	413.0430	411.0284	ESI+	13
420 Imibenconazole	86598-92-7	C17H13Cl3N4S	409.9927	411.0000	408.9854	ESI+	22
421 Imidacloprid	138261-41-3	C9H10ClN5O2	255.0523	256.0596	254.0450	ESI+	8
422 Indanofan	133220-30-1	C20H17ClO3	340.0866	341.0939	339.0793	ESI+	6
423 Indoxacarb	173584-44-6	C22H17ClF3N3O7	527.0707	528.0780	526.0634	ESI+	12
424 Iodosulfuron-methyl	144550-36-7	C14H14IN5O6S	506.9710	507.9783	505.9637	ESI+	8
425 Ioxynil	1689-83-4	C7H3I2NO	370.8304	371.8377	369.8231	ESI-	4
426 Ipconazole	125225-28-7	C18H24ClN3O	333.1608	334.1681	332.1535	ESI+	5
427 Iprobenfos	26087-47-8	C13H21O3PS	288.0949	289.1022	287.0876	ESI+	3
428 Iprodione	36734-19-7	C13H13Cl2N3O3	329.0334	330.0407	328.0261	ESI+	4
429 Iprovalicarb	140923-17-7	C18H28N2O3	320.2100	321.2173	319.2027	ESI+	6
430 Irgarol 1051	28159-98-0	C11H19N5S	253.1361	254.1434	252.1288	ESI+	6
431 Isazofos	42509-80-8	C9H17ClN3O3PS	313.0417	314.0490	312.0344	ESI+	12
432 Isocarbamid	30979-48-7	C8H15N3O2	185.1164	186.1237	184.1091	ESI+	6
433 Isocarbofos	24353-61-5	C11H16NO4PS	289.0538	290.0611	288.0465	ESI+	6
434 Isofenphos	25311-71-1	C15H24NO4PS	345.1164	346.1237	344.1091	ESI+	6
435 Isofenphos-methyl	99675-03-3	C14H22NO4PS	331.1007	332.1080	330.0934	ESI+	6
436 Isofenphos-oxon	31120-85-1	C15H24NO5P	329.1392	330.1465	328.1319	ESI+	3
437 Isomethiozin	57052-04-7	C12H20N4O5S	268.1358	269.1431	267.1285	ESI+	6
438 Isonoruron	28805-78-9	C13H22N2O	222.1732	223.1805	221.1659	ESI+	6
439 Isoprocab	2631-40-5	C11H15NO2	193.1103	194.1176	192.1030	ESI+	3
440 Isopropalin	33820-53-0	C15H23N3O4	309.1689	310.1762	308.1616	ESI+	6
441 Isoprothiolane	50512-35-1	C12H18O4S2	290.0647	291.0720	289.0574	ESI+	6
442 Isoproturon	34123-59-6	C12H18N2O	206.1419	207.1492	205.1346	ESI+	6
443 Isoprazam	881685-58-1	C20H23F2N3O	359.1809	360.1882	358.1736	ESI+	9
444 Isoxaben	82558-50-7	C18H24N2O4	332.1736	333.1809	331.1663	ESI+	6
445 Isoxadifen-ethyl	163520-33-0	C18H17NO3	295.1208	296.1281	294.1135	ESI+	12
446 Isoxaflutole	141112-29-0	C15H12F3NO4S	359.0439	360.0512	358.0366	ESI+	5
447 Isoxathion	18854-01-8	C13H16NO4PS	313.0538	314.0611	312.0465	ESI+	6
448 Ivermectine	70288-86-7	C48H74O14	874.5079	875.5152	873.5006	ESI+	6
449 Karbutilate	4849-32-5	C14H21N3O3	279.1583	280.1656	278.1510	ESI+	16
450 Kasugamycin	6980-18-3	C14H25N3O9	379.1591	380.1664	378.1518	ESI+	3

Compound	CAS	Formula	M	[M+H] ⁺	[M-H]	Ionisation Mode	MRM Transitions
451 Kresoxim-methyl	143390-89-0	C18H19NO4	313.1314	314.1387	312.1241	ESI+	6
452 Lactofen	77501-63-4	C19H15ClF3NO7	461.0489	462.0562	460.0416	ESI+	12
453 Lambda-Cyhalothrin	91465-08-6	C23H19ClF3NO3	449.1006	450.1079	448.0933	ESI+	4
454 Lenacil	2164-08-1	C13H18N2O2	234.1368	235.1441	233.1295	ESI+	6
455 Linuron	330-55-2	C9H10Cl2N2O2	248.0119	249.0192	247.0046	ESI+	12
456 Lufenuron	103055-07-8	C17H8Cl2F8N2O3	509.9784	510.9857	508.9711	ESI-	10
457 Malaoxon	1634-78-2	C10H19O7P5	314.0589	315.0662	313.0516	ESI+	6
458 Malathion	121-75-5	C10H19O6PS2	330.0361	331.0434	329.0288	ESI+	12
459 Maleic-hydrazide	123-33-1	C4H4N2O2	112.0273	113.0346	111.0200	ESI+	3
460 Mandipropamid	374726-62-2	C23H22ClNO4	411.1237	412.1310	410.1164	ESI+	12
461 MCPA (MCP)	94-74-6	C9H9ClO3	200.0240	201.0313	199.0167	ESI-	3
462 MCPA-butoxyethyl ester	19480-43-4	C15H21ClO4	300.1128	301.1201	299.1055	ESI+	12
463 MCPB	94-81-5	C11H13ClO3	228.0553	229.0626	227.0480	ESI-	3
464 Mecarbam	2595-54-2	C10H20NO5PS2	329.0521	330.0594	328.0448	ESI+	6
465 Mecoprop (MCPP)	93-65-2	C10H11ClO3	214.0397	215.0470	213.0324	ESI-	2
466 Mecoprop-P	16484-77-8	C10H11ClO3	214.0397	215.0470	213.0324	ESI-	4
467 Mefenacet	73250-68-7	C16H14N2O2S	298.0776	299.0849	297.0703	ESI+	6
468 Mefenpyr-diethyl	135590-91-9	C16H18Cl2N2O4	372.0644	373.0717	371.0571	ESI+	24
469 Mefluidide	53780-34-0	C11H13F3N2O3S	310.0599	311.0672	309.0526	ESI+	10
470 Mepanipyrim	110235-47-7	C14H13N3	223.1109	224.1182	222.1036	ESI+	6
471 Mephosfolan	950-10-7	C8H16NO3PS2	269.0309	270.0382	268.0236	ESI+	6
472 Mepiquat	24307-26-4	C7H16N	114.1283	115.1356	113.1210	ESI+	6
473 Mepronil	55814-41-0	C17H19NO2	269.1416	270.1489	268.1343	ESI+	5
474 Meptyldinocap	6119-92-2	C18H24N2O6	364.1634	365.1707	363.1561	ESI-	6
475 Mesosulfuron-methyl	208465-21-8	C17H21N5O9S2	503.0781	504.0854	502.0708	ESI+	6
476 Mesotrione	104206-82-8	C14H13NO7S	339.0413	340.0486	338.0340	ESI+	6
477 Metaflumizone	139968-49-3	C24H16F6N4O2	506.1177	507.1250	505.1104	ESI+	6
478 Metalaxyl	57837-19-1	C15H21NO4	279.1471	280.1544	278.1398	ESI+	6
479 Metalaxyl-M	70630-17-0	C15H21NO4	279.1471	280.1544	278.1398	ESI+	6
480 Metamitron	41394-05-2	C10H10N4O	202.0855	203.0928	201.0782	ESI+	3
481 Metazachlor	67129-08-2	C14H16ClN3O	277.0982	278.1055	276.0909	ESI+	6
482 Metconazole	125116-23-6	C17H22ClN3O	319.1451	320.1524	318.1378	ESI+	4
483 Methabenzthiazuron	18691-97-9	C10H11N3OS	221.0623	222.0696	220.0550	ESI+	6
484 Methacrifos	62610-77-9	C7H13O5PS	240.0221	241.0294	239.0148	ESI+	12
485 Methamidophos	10265-92-6	C2H8NO2PS	141.0013	142.0086	139.9940	ESI+	6
486 Methfuroxam	28730-17-8	C14H15NO2	229.1103	230.1176	228.1030	ESI+	4
487 Methidathion	950-37-8	C6H11N2O4PS3	301.9619	302.9692	300.9546	ESI+	7
488 Methiocarb	2032-65-7	C11H15NO2S	225.0823	226.0896	224.0750	ESI+	6
489 Methiocarb-sulfone	2179-25-1	C11H15NO4S	257.0722	258.0795	256.0649	ESI+	9
490 Methiocarb-sulfoxide	2635-10-1	C11H15NO3S	241.0773	242.0846	240.0700	ESI+	6
491 Methomyl	16752-77-5	C5H10N2O2S	162.0463	163.0536	161.0390	ESI+	6
492 Methoprene	40596-69-8	C19H34O3	310.2508	311.2581	309.2435	ESI+	12
493 Methoprotrotyne	841-06-5	C11H21N5OS	271.1467	272.1540	270.1394	ESI+	6
494 Methoxyfenozide	161050-58-4	C22H28N2O3	368.2100	369.2173	367.2027	ESI+	6
495 Metobromuron	3060-89-7	C9H11BrN2O2	258.0004	259.0077	256.9931	ESI+	12
496 Metolachlor	51218-45-2	C15H22ClNO2	283.1339	284.1412	282.1266	ESI+	12
497 Metolcarb	1129-41-5	C9H11NO2	165.0790	166.0863	164.0717	ESI+	6
498 Metominostrobin	133408-50-1	C16H16N2O3	284.1161	285.1234	283.1088	ESI+	6
499 Metosulam	139528-85-1	C14H13Cl2N5O4S	417.0065	418.0138	415.9992	ESI+	24
500 Metoxuron	19937-59-8	C10H13ClN2O2	228.0666	229.0739	227.0593	ESI+	5
501 Metrafenone	220899-03-6	C19H21BrO5	408.0572	409.0645	407.0499	ESI+	12
502 Metribuzin	21087-64-9	C8H14N4O5	214.0888	215.0961	213.0815	ESI+	5
503 Metsulfuron-methyl	74223-64-6	C14H15N5O6S	381.0743	382.0816	380.0670	ESI+	6
504 Mevinphos	7786-34-7	C7H13O6P	224.0450	225.0523	223.0377	ESI+	5
505 Mexacarbate	315-18-4	C12H18N2O2	222.1368	223.1441	221.1295	ESI+	6
506 Molinate	2212-67-1	C9H17NO5	187.1031	188.1104	186.0958	ESI+	6
507 Monalide	7287-36-7	C13H18ClNO	239.1077	240.1150	238.1004	ESI+	20
508 Monocrotophos	6923-22-4	C7H14NO5P	223.0610	224.0683	222.0537	ESI+	12
509 Monolinuron	1746-81-2	C9H11ClN2O2	214.0509	215.0582	213.0436	ESI+	10
510 Monuron	150-68-5	C9H11ClN2O	198.0560	199.0633	197.0487	ESI+	10
511 Morpholine	110-91-8	C4H9NO	87.0684	88.0757	86.0611	ESI+	6
512 Moxidectin	113507-06-5	C37H53NO8	639.3771	640.3844	638.3698	ESI+	12
513 Myclobutanil	88671-89-0	C15H17ClN4	288.1142	289.1215	287.1069	ESI+	8
514 N-(2, 4-Dimethylphenyl) formamide	60397-77-5	C9H11NO	149.0841	150.0914	148.0768	ESI+	6
515 N-(2, 4-Dimethylphenyl) -N'-methylformamidine	33089-74-6	C10H14N2	162.1157	163.1230	161.1084	ESI+	6
516 N, N'-Diphenylurea	102-07-8	C13H12N2O	212.0950	213.1023	211.0877	ESI+	4
517 Naled	300-76-5	C4H7Br2Cl2O4P	377.7826	378.7899	376.7753	ESI+	6
518 Naproanilide	52570-16-8	C19H17NO2	291.1259	292.1332	290.1186	ESI+	2
519 Napropamide	15299-99-7	C17H21NO2	271.1572	272.1645	270.1499	ESI+	6
520 Naptalam	132-66-1	C18H13NO3	291.0895	292.0968	290.0822	ESI+	6
521 Neburon	555-37-3	C12H16Cl2N2O	274.0640	275.0713	273.0567	ESI+	9
522 Nicarbazin	330-95-0	C19H18N6O6	426.1288	427.1361	425.1215	ESI-	3
523 Nicosulfuron	111991-09-4	C15H18N6O6S	410.1009	411.1082	409.0936	ESI+	6
524 Nicotine	54-11-5	C10H14N2	162.1157	163.1230	161.1084	ESI+	6
525 Nitenpyram	150824-47-8	C11H15ClN4O2	270.0884	271.0957	269.0811	ESI+	6

Compound	CAS	Formula	M	(M+H) ⁺	(M-H) ⁻	Ionisation Mode	MMT Transitions
526 Nitratin	4726-14-1	C13H19N3O6S	345.0995	346.1068	344.0922	ESI+	12
527 Nitrothal-isopropyl	10552-74-6	C14H17NO6	295.1056	296.1129	294.0983	ESI+	6
528 Norflurazon	27314-13-2	C12H9ClF3N3O	303.0386	304.0459	302.0313	ESI+	16
529 Norflurazon-desmethyl	23576-24-1	C11H7ClF3N3O	289.0230	290.0303	288.0157	ESI+	12
530 Novaluron	116714-46-6	C17H9ClF8N2O4	492.0123	493.0196	491.0050	ESI+	18
531 Noviflumuron	121451-02-3	C17H7Cl2F9N2O3	527.9690	528.9763	526.9617	ESI-	6
532 Nuanmol	63284-71-9	C17H12ClF8N2O	314.0622	315.0695	313.0549	ESI+	11
533 Ofurace	58810-48-3	C14H16ClNO3	281.0819	282.0892	280.0746	ESI+	17
534 Omethoate	1113-02-6	C5H12NO4PS	213.0225	214.0298	212.0152	ESI+	4
535 Orbencarb	34622-58-7	C12H16ClNO5	257.0641	258.0714	256.0568	ESI+	12
536 Orthosulfamuron	213464-77-8	C16H20N6O6S	424.1165	425.1238	423.1092	ESI+	6
537 Oryzalin	19044-88-3	C12H18N4O6S	346.0947	347.1020	345.0874	ESI+	5
538 Oxabetnml	94593-79-0	C12H12N2O3	232.0848	233.0921	231.0775	ESI+	2
539 Oxadiargyl	39807-15-3	C15H14Cl2N2O3	340.0381	341.0454	339.0308	ESI+	14
540 Oxadiazon	19666-30-9	C15H18Cl2N2O3	344.0694	345.0767	343.0621	ESI+	6
541 Oxadixyl	77732-09-3	C14H18N2O4	278.1267	279.1340	277.1194	ESI+	12
542 Oxamyl	23135-22-0	C7H13N3O3S	219.0678	220.0751	218.0605	ESI+	3
543 Oxasulfuron	144651-06-9	C17H18N4O6S	406.0947	407.1020	405.0874	ESI+	6
544 Oxaziclofene	153197-14-9	C20H19Cl2N2O2	375.0793	376.0866	374.0720	ESI+	2
545 Oxycarboxin	5259-88-1	C12H13NO4S	267.0565	268.0638	266.0492	ESI+	3
546 Oxydemeton-methyl	301-12-2	C6H15O4PS2	246.0149	247.0222	245.0076	ESI+	6
547 Paclobutrazol	76738-62-0	C15H20ClN3O	293.1295	294.1368	292.1222	ESI+	8
548 Paraoxon-ethyl	311-45-5	C10H14NO6P	275.0559	276.0632	274.0486	ESI+	6
549 Paraoxon-methyl	950-35-6	C8H10NO6P	247.0246	248.0319	246.0173	ESI+	3
550 Paraquat	1910-42-5	C12H14Cl2N2	256.0534	257.0607	255.0461	ESI+	5
551 Parathion	56-38-2	C10H14NO5PS	291.0330	292.0403	290.0257	ESI+	3
552 Pebulate	1114-71-2	C10H21NO5	203.1344	204.1417	202.1271	ESI+	6
553 Penconazole	66246-88-6	C13H15Cl2N3	283.0643	284.0716	282.0570	ESI+	12
554 Pencycuron	66063-05-6	C19H21ClN2O	328.1342	329.1415	327.1269	ESI+	10
555 Pendimethalin	40487-42-1	C13H19N3O4	281.1376	282.1449	280.1303	ESI+	6
556 Penoxsulam	219714-96-2	C16H14F5N5O5S	483.0636	484.0709	482.0563	ESI+	6
557 Pentachlorophenol	87-86-5	C6HCl5O	263.8470	264.8543	262.8397	ESI-	3
558 Pentoxazone	110956-75-7	C17H17ClFNO4	353.0830	354.0903	352.0757	ESI+	2
559 Permethrin	52645-53-1	C21H20Cl2O3	390.0790	391.0863	389.0717	ESI+	12
560 Pethoxamid	106700-29-2	C16H22ClN2O2	295.1339	296.1412	294.1266	ESI+	7
561 Phenmedipham	13684-63-4	C16H16N2O4	300.1110	301.1183	299.1037	ESI+	6
562 Phenothrin	26002-80-2	C23H26O3	350.1882	351.1955	349.1809	ESI+	9
563 Phenthoate	2597-03-7	C12H17O4PS2	320.0306	321.0379	319.0233	ESI+	12
564 Phorate	298-02-2	C7H17O2PS3	260.0128	261.0201	259.0055	ESI+	6
565 Phorate-oxon	2600-69-3	C7H17O3PS2	244.0357	245.0430	243.0284	ESI+	6
566 Phorate-sulfone	2588-04-7	C7H17O4PS3	292.0027	293.0100	290.9954	ESI+	6
567 Phorate-sulfoxide	2588-03-6	C7H17O3PS3	276.0077	277.0150	275.0004	ESI+	6
568 Phosalone	2310-17-0	C12H15ClNO4PS2	366.9869	367.9942	365.9796	ESI+	12
569 Phosfolan	947-02-4	C7H14NO3PS2	255.0153	256.0226	254.0080	ESI+	6
570 Phosmet	732-11-6	C11H12NO4PS2	316.9945	318.0018	315.9872	ESI+	12
571 Phosphamidon	13171-21-6	C10H19ClNO5P	299.0689	300.0762	298.0616	ESI+	12
572 Phoxim	14816-18-3	C12H15N2O3PS	298.0541	299.0614	297.0468	ESI+	6
573 Picloram	1918-02-1	C6H3Cl3N2O2	239.9260	240.9333	238.9187	ESI+	9
574 Picolinafen	137641-05-5	C19H12F4N2O2	376.0835	377.0908	375.0762	ESI+	6
575 Picoxystrobin	117428-22-5	C18H16F3NO4	367.1031	368.1104	366.0958	ESI+	6
576 Pinoxaden	243973-20-8	C23H32N2O4	400.2362	401.2435	399.2289	ESI+	6
577 Piperonyl-butoxide	51-03-6	C19H30O5	338.2093	339.2166	337.2020	ESI+	12
578 Piperophos	24151-93-7	C14H28NO3PS2	353.1248	354.1321	352.1175	ESI+	6
579 Pirimicarb	23103-98-2	C11H18N4O2	238.1430	239.1503	237.1357	ESI+	3
580 Pirimicarb-desmethyl	30614-22-3	C10H16N4O2	224.1273	225.1346	223.1200	ESI+	6
581 Pirimicarb-desmethyl-formamido	27218-04-8	C11H16N4O3	252.1222	253.1295	251.1149	ESI+	2
582 Pirimiphos-ethyl	23505-41-1	C13H24N3O3PS	333.1276	334.1349	332.1203	ESI+	6
583 Pirimiphos-methyl	29232-93-7	C11H20N3O3PS	305.0963	306.1036	304.0890	ESI+	6
584 Prallethrin	23031-36-9	C19H24O3	300.1725	301.1798	299.1652	ESI+	6
585 Pretilachlor	51218-49-6	C17H26ClNO2	311.1652	312.1725	310.1579	ESI+	6
586 Primisulfuron-methyl	86209-51-0	C15H12F4N4O7S	468.0363	469.0436	467.0290	ESI+	9
587 Probenazole	27605-76-1	C10H9NO3S	223.0303	224.0376	222.0230	ESI+	4
588 Prochloraz	67747-09-5	C15H16Cl3N3O2	375.0308	376.0381	374.0235	ESI+	15
589 Profenofos	41198-08-7	C11H15BrClO3PS	371.9351	372.9424	370.9278	ESI+	12
590 Profoxydim	139001-49-3	C24H32ClNO4S	465.1741	466.1814	464.1668	ESI+	24
591 Promecarb	2631-37-0	C12H17NO2	207.1259	208.1332	206.1186	ESI+	6
592 Prometon	1610-18-0	C10H19N5O	225.1590	226.1663	224.1517	ESI+	6
593 Prometryn	7287-19-6	C10H19N5S	241.1361	242.1434	240.1288	ESI+	6
594 Propachlor	1918-16-7	C11H14ClNO	211.0764	212.0837	210.0691	ESI+	6
595 Propamocarb	24579-73-5	C9H20N2O2	188.1525	189.1598	187.1452	ESI+	6
596 Propanil	709-98-8	C9H9Cl2NO	217.0061	218.0134	215.9988	ESI+	9
597 Propaphos	7292-16-2	C13H21O4PS	304.0898	305.0971	303.0825	ESI+	10
598 Propaquizafop	111479-05-1	C22H22ClN3O5	443.1248	444.1321	442.1175	ESI+	12
599 Propargite	2312-35-8	C19H26O4S	350.1552	351.1625	349.1479	ESI+	6
600 Propazine	139-40-2	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	6

Compound	CAS	Formula	M	[M+H] ⁺	[M-H] ⁻	Ionization Mode	MRM Transitions
601 Propetamphos	31218-83-4	C10H20NO4PS	281.0851	282.0924	280.0778	ESI+	18
602 Propham	122-42-9	C10H13NO2	179.0946	180.1019	178.0873	ESI+	6
603 Propiconazole	60207-90-1	C15H17ClN3O2	341.0698	342.0771	340.0625	ESI+	12
604 Propisochlor	86763-47-5	C15H22ClNO2	283.1339	284.1412	282.1266	ESI+	12
605 Propoxur	114-26-1	C11H15NO3	209.1052	210.1125	208.0979	ESI+	6
606 Propoxycarbazone	181274-15-7	C15H18N4O7S	398.0896	399.0969	397.0823	ESI+	20
607 Propylene-thiourea	2122-19-2	C4H8N2S	116.0408	117.0481	115.0335	ESI+	6
608 Propyzamide	23950-58-5	C12H11Cl2NO	255.0218	256.0291	254.0145	ESI+	10
609 Proquinazid	189278-12-4	C14H17IN2O2	372.0335	373.0408	371.0262	ESI+	6
610 Prosulfocarb	52888-80-9	C14H21NO5	251.1344	252.1417	250.1271	ESI+	4
611 Prosulfuron	94125-34-5	C15H16F3N5O4S	419.0875	420.0948	418.0802	ESI+	7
612 Prothioconazole	178928-70-6	C14H15Cl2N3O5	343.0313	344.0386	342.0240	ESI+	10
613 Prothioconazole-desthio	120983-64-4	C14H15Cl2N3O	311.0592	312.0665	310.0519	ESI+	10
614 Prothiophos	34643-46-4	C11H15Cl2O2PS2	343.9628	344.9701	342.9555	ESI+	12
615 Prothoate	2275-18-5	C9H20NO3PS2	285.0622	286.0695	284.0549	ESI+	6
616 Pymetrozine	123312-89-0	C10H11N5O	217.0964	218.1037	216.0891	ESI+	4
617 Pyracarbolid	24691-76-7	C13H15NO2	217.1103	218.1176	216.1030	ESI+	3
618 Pyraclofos	89784-60-1	C14H18ClN2O3PS	360.0464	361.0537	359.0391	ESI+	12
619 Pyraclostrobin	175013-18-0	C19H18ClN3O4	387.0986	388.1059	386.0913	ESI+	11
620 Pyraflufen-ethyl	129630-19-9	C15H13Cl2F3N2O4	412.0204	413.0277	411.0131	ESI+	12
621 Pyrasulfotole	365400-11-9	C14H13F3N2O4S	362.0548	363.0621	361.0475	ESI+	9
622 Pyrazolynate	58011-68-0	C19H16Cl2N2O4S	438.0208	439.0281	437.0135	ESI+	2
623 Pyrazophos	13457-18-6	C14H20N3O5PS	373.0861	374.0934	372.0788	ESI+	12
624 Pyrazosulfuron-ethyl	93697-74-6	C14H18N6O7S	414.0958	415.1031	413.0885	ESI+	6
625 Pyrazoxyfen	71561-11-0	C20H16Cl2N2O3	402.0538	403.0611	401.0465	ESI+	12
626 Pyributicarb	88678-67-5	C18H22N2O2S	330.1402	331.1475	329.1329	ESI+	6
627 Pyridaben	96489-71-3	C19H25ClN2O5	364.1376	365.1449	363.1303	ESI+	12
628 Pyridalyl	179101-81-6	C18H14Cl4F3NO3	488.9680	489.9753	487.9607	ESI+	18
629 Pyridaphenthion	119-12-0	C14H17N2O4PS	340.0647	341.0720	339.0574	ESI+	6
630 Pyridate	55512-33-9	C19H23ClN2O2S	378.1169	379.1242	377.1096	ESI+	12
631 Pyrifeno	88283-41-4	C14H12Cl2N2O	294.0327	295.0400	293.0254	ESI+	8
632 Pyrifthalid	135186-78-6	C15H14N2O4S	318.0674	319.0747	317.0601	ESI+	2
633 Pyrimethanil	53112-28-0	C12H13N3	199.1109	200.1182	198.1036	ESI+	6
634 Pyrimidifen	105779-78-0	C20H28ClN3O2	377.1870	378.1943	376.1797	ESI+	12
635 Pyriminobac-methyl (E)	136191-64-5	C17H19N3O6	361.1274	362.1347	360.1201	ESI+	6
636 Pyrproxifen	95737-68-1	C20H19NO3	321.1365	322.1438	320.1292	ESI+	6
637 Pyroquilon	57369-32-1	C11H11NO	173.0841	174.0914	172.0768	ESI+	6
638 Pyroxulam	422556-08-9	C14H13F3N6O5S	434.0620	435.0693	433.0547	ESI+	6
639 Quinalphos	13593-03-8	C12H15N2O3PS	298.0541	299.0614	297.0468	ESI+	6
640 Quinclorac	84087-01-4	C10H5Cl2NO2	240.9697	241.9770	239.9624	ESI+	11
641 Quinmerac	90717-03-6	C11H8ClNO2	221.0244	222.0317	220.0171	ESI+	12
642 Quinoclamine	2797-51-5	C10H6ClNO2	207.0087	208.0160	206.0014	ESI+	19
643 Quinoxifen	124495-18-7	C15H8Cl2FNO	306.9967	308.0040	305.9894	ESI+	12
644 Quizalofop (free acid)	76578-12-6	C17H13ClN2O4	344.0564	345.0637	343.0491	ESI+	24
645 Quizalofop-ethyl	76578-14-8	C19H17ClN2O4	372.0877	373.0950	371.0804	ESI+	12
646 Quizalofop-methyl	76578-13-7	C18H15ClN2O4	358.0720	359.0793	357.0647	ESI+	12
647 Quizalofop-P	94051-08-8	C17H13ClN2O4	344.0564	345.0637	343.0491	ESI+	9
648 Quizalofop-P-ethyl	100646-51-3	C19H17ClN2O4	372.0877	373.0950	371.0804	ESI+	12
649 Rabenzazole	40341-04-6	C12H12N4	212.1062	213.1135	211.0989	ESI+	12
650 Resmethrin	10453-86-8	C22H26O3	338.1882	339.1955	337.1809	ESI+	6
651 Rimsulfuron	122931-48-0	C14H17N5O7S2	431.0569	432.0642	430.0496	ESI+	9
652 Rotenone	83-79-4	C23H22O6	394.1416	395.1489	393.1343	ESI+	6
653 Saflufenacil	372137-35-4	C17H17ClF4N4O5S	500.0544	501.0617	499.0471	ESI+	8
654 Sebuthylazine	7286-69-3	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	6
655 Sebuthylazine-desethyl	37019-18-4	C7H12ClN5	201.0781	202.0854	200.0708	ESI+	12
656 Secbumeton	26259-45-0	C10H19NO5	225.1590	226.1663	224.1517	ESI+	4
657 Sethoxydim	74051-80-2	C17H29NO3S	327.1868	328.1941	326.1795	ESI+	12
658 Siduron	1982-49-6	C14H20N2O	232.1576	233.1649	231.1503	ESI+	5
659 Silafluofen	105024-66-6	C25H29FO2Si	408.1921	409.1994	407.1848	ESI+	2
660 Silthiofam	175217-20-6	C13H21NO5Si	267.1113	268.1186	266.1040	ESI+	5
661 Simazine	122-34-9	C7H12ClN5	201.0781	202.0854	200.0708	ESI+	12
662 Simazine-2-hydroxy	2599-11-3	C7H13N5O	183.1120	184.1193	182.1047	ESI+	5
663 Simeconazole	149508-90-7	C14H20FN3OSi	293.1360	294.1433	292.1287	ESI+	6
664 Simetryn	1014-70-6	C8H15N5S	213.1048	214.1121	212.0975	ESI+	4
665 Spinetoram A	187166-40-1	C42H69NO10	747.4921	748.4994	746.4848	ESI+	2
666 Spinetoram B	187166-15-0	C43H69NO10	759.4921	760.4994	758.4848	ESI+	3
667 Spinosyn A	131929-60-7	C41H65NO10	731.4608	732.4681	730.4535	ESI+	4
668 Spinosyn D	131929-63-0	C42H67NO10	745.4765	746.4838	744.4692	ESI+	4
669 Spirodiclofen	148477-71-8	C21H24Cl2O4	410.1052	411.1125	409.0979	ESI+	11
670 Spiromesifen	283594-90-1	C23H30O4	370.2144	371.2217	369.2071	ESI+	4
671 Spirotetramat	203313-25-1	C21H27NO5	373.1889	374.1962	372.1816	ESI+	6
672 Spiroxamine	118134-30-8	C18H35NO2	297.2668	298.2741	296.2595	ESI+	6
673 Sulcotrione	99105-77-8	C14H13ClO5S	328.0172	329.0245	327.0099	ESI+	2
674 Sulfallate	95-06-7	C8H14ClNS2	223.0256	224.0329	222.0183	ESI+	9
675 Sulfiquinoxaline	59-40-5	C14H12N4O2S	300.0681	301.0754	299.0608	ESI+	6

Compound	CAS	Formula	M	IM+H ⁺	IM-H ⁻	Ionization Mode	MRM Transitions
676 Sulfometuron-methyl	74222-97-2	C15H16N4O5S	364.0841	365.0914	363.0768	ESI+	6
677 Sulfosulfuron	141776-32-1	C16H18N6O7S2	470.0678	471.0751	469.0605	ESI+	6
678 Sulfotep	3689-24-5	C8H20O5P2S2	322.0227	323.0300	321.0154	ESI+	6
679 Sulprofos	35400-43-2	C12H19O2PS3	322.0285	323.0358	321.0212	ESI+	6
680 Tau-Fluvalinate	102851-06-9	C26H22ClF3N2O3	502.1271	503.1344	501.1198	ESI+	15
681 Tebuconazole	107534-96-3	C16H22ClN3O	307.1451	308.1524	306.1378	ESI+	10
682 Tebufenozide	112410-23-8	C22H28N2O2	352.2151	353.2224	351.2078	ESI+	6
683 Tebufenpyrad	119168-77-3	C18H24ClN3O	333.1608	334.1681	332.1535	ESI+	12
684 Tebupirrifos	96182-53-5	C13H23N2O3PS	318.1167	319.1240	317.1094	ESI+	6
685 Tebutam	35256-85-0	C15H23NO	233.1780	234.1853	232.1707	ESI+	6
686 Tebuthiuron	34014-18-1	C9H16N4O5	228.1045	229.1118	227.0972	ESI+	6
687 Teflubenzuron	83121-18-0	C14H6Cl2F4N2O2	379.9742	380.9815	378.9669	ESI-	12
688 Tembotrione	335104-84-2	C17H16ClF3O6S	440.0308	441.0381	439.0235	ESI+	12
689 Ternephos	3383-96-8	C16H20O6P2S3	465.9897	466.9970	464.9824	ESI+	6
690 Tepaloxymid	149979-41-9	C17H24ClNO4	341.1394	342.1467	340.1321	ESI+	8
691 Terbacil	5902-51-2	C9H13ClN2O2	216.0666	217.0739	215.0593	ESI-	10
692 Terbutcarb	1918-11-2	C17H27NO2	277.2042	278.2115	276.1969	ESI+	12
693 Terbufos	13071-79-9	C9H21O2PS3	288.0441	289.0514	287.0368	ESI+	5
694 Terbufos-sulfone	56070-16-7	C9H21O4PS3	320.0340	321.0413	319.0267	ESI+	6
695 Terbufos-sulfoxide	10548-10-4	C9H21O3PS3	304.0390	305.0463	303.0317	ESI+	6
696 Terbumeton	33693-04-8	C10H19N5O	225.1590	226.1663	224.1517	ESI+	6
697 Terbumeton-desethyl	30125-64-5	C8H15N5O	197.1277	198.1350	196.1204	ESI+	3
698 Terbutylazine	5915-41-3	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	10
699 Terbutylazine-2-hydroxy	66753-07-9	C9H17N5O	211.1433	212.1506	210.1360	ESI+	6
700 Terbutylazine-desethyl	30125-63-4	C7H12ClN5	201.0781	202.0854	200.0708	ESI+	12
701 Terbutryn	886-50-0	C10H19N5S	241.1361	242.1434	240.1288	ESI+	6
702 Tetrachlorvinphos (CVMP)	22248-79-9	C10H9Cl4O4P	363.8993	364.9066	362.8920	ESI+	16
703 Tetraconazole	112281-77-3	C13H11Cl2F4N3O	371.0215	372.0288	370.0142	ESI+	7
704 Tetraethylpyrophosphate	107-49-3	C8H20O7P2	290.0684	291.0757	289.0611	ESI+	6
705 Tetramethrin	7696-12-0	C19H25NO4	331.1784	332.1857	330.1711	ESI+	12
706 Thenylchlor	96491-05-3	C16H18ClNO2S	323.0747	324.0820	322.0674	ESI+	12
707 Thiabendazole	148-79-8	C10H7N3S	201.0361	202.0434	200.0288	ESI+	6
708 Thiacloprid	111988-49-9	C10H9ClN4S	252.0236	253.0309	251.0163	ESI+	6
709 Thiamethoxam	153719-23-4	C8H10ClN5O3S	291.0193	292.0266	290.0120	ESI+	12
710 Thiazafuror	25366-23-8	C6H7F3N4O5	240.0293	241.0366	239.0220	ESI+	6
711 Thiazopyr	117718-60-2	C16H17F5N2O2S	396.0931	397.1004	395.0858	ESI+	6
712 Thidiazuron	51707-55-2	C9H8N4O5	220.0419	221.0492	219.0346	ESI+	6
713 Thienicarbazon-methyl	317815-83-1	C12H14N4O7S2	390.0304	391.0377	389.0231	ESI+	3
714 Thifensulfuron-methyl	79277-27-3	C12H13N5O6S2	387.0307	388.0380	386.0234	ESI+	6
715 Thifluzamide	130000-40-7	C13H6Br2F6N2O2S	525.8421	526.8494	524.8348	ESI+	29
716 Thiobencarb	28249-77-6	C12H16ClNOS	257.0641	258.0714	256.0568	ESI+	11
717 Thiodicarb	59669-26-0	C10H18N4O4S3	354.0490	355.0563	353.0417	ESI+	6
718 Thiofanox	39196-18-4	C9H18N2O2S	218.1089	219.1162	217.1016	ESI+	2
719 Thiofanox-sulfone	39184-59-3	C9H18N2O4S	250.0987	251.1060	249.0914	ESI+	9
720 Thiofanox-sulfoxide	39184-27-5	C9H18N2O3S	234.1038	235.1111	233.0965	ESI+	12
721 Thiometon	640-15-3	C6H15O2PS3	245.9972	247.0045	244.9899	ESI+	2
722 Thionazin	297-97-2	C8H13N2O3PS	248.0384	249.0457	247.0311	ESI+	6
723 Thiophanate-ethyl	23564-06-9	C14H18N4O4S2	370.0769	371.0842	369.0696	ESI+	6
724 Thiophanate-methyl	23564-05-8	C12H14N4O4S2	342.0456	343.0529	341.0383	ESI+	6
725 Thiram	137-26-8	C6H12N2S4	239.9883	240.9956	238.9810	ESI+	6
726 Tolclofos-methyl	57018-04-9	C9H11Cl2O3PS	299.9544	300.9617	298.9471	ESI+	12
727 Tolyfluanid	731-27-1	C10H13Cl2FN2O2S2	345.9780	346.9853	344.9707	ESI+	22
728 Topramezone	210631-68-8	C16H17N3O5S	363.0889	364.0962	362.0816	ESI+	12
729 Tralkoxydim	87820-88-0	C20H27NO3	329.1991	330.2064	328.1918	ESI+	6
730 Tralometrin	66841-25-6	C22H19Br4NO3	660.8098	661.8171	659.8025	ESI+	7
731 Triadimefon	43121-43-3	C14H16ClN3O2	293.0931	294.1004	292.0858	ESI+	12
732 Triadimenol	55219-65-3	C14H18ClN3O2	295.1088	296.1161	294.1015	ESI+	7
733 Tri-allate	2303-17-5	C10H16Cl3NOS	303.0018	304.0091	301.9945	ESI+	16
734 Triapenthenol	76608-88-3	C15H25N3O	263.1998	264.2071	262.1925	ESI+	12
735 Triasulfuron	82097-50-5	C14H16ClN5O5S	401.0561	402.0634	400.0488	ESI+	12
736 Triazamate	112143-82-5	C13H22N4O3S	314.1413	315.1486	313.1340	ESI+	4
737 Triazophos	24017-47-8	C12H16N3O3PS	313.0650	314.0723	312.0577	ESI+	6
738 Triazoxide	72459-58-6	C10H6ClN5O	247.0261	248.0334	246.0188	ESI+	11
739 Tribenuron-methyl	101200-48-0	C15H17N5O6S	395.0900	396.0973	394.0827	ESI+	5
740 Trichlorfon	52-68-6	C4H8Cl3O4P	255.9226	256.9299	254.9153	ESI+	10
741 Triclopyr	55335-06-3	C7H4Cl3NO3	254.9257	255.9330	253.9184	ESI-	2
742 Tricyclazole	41814-78-2	C9H7N3S	189.0361	190.0434	188.0288	ESI+	6
743 Tridemorph	81412-43-3	C19H39NO	297.3032	298.3105	296.2959	ESI+	6
744 Tinetazine	1912-26-1	C9H16ClN5	229.1094	230.1167	228.1021	ESI+	6
745 Triethanolamine	102-71-6	C6H15NO3	149.1052	150.1125	148.0979	ESI+	6
746 Trifloxystrobin	141517-21-7	C20H19F3N2O4	408.1297	409.1370	407.1224	ESI+	6
747 Trifloxysulfuron	145099-21-4	C14H14F3N5O6S	437.0617	438.0690	436.0544	ESI+	9
748 Triflumizole	68694-11-1	C15H15ClF3N3O	345.0856	346.0929	344.0783	ESI+	9
749 Triflumizole Metabolite	131549-75-2	C12H14ClF3N2O	294.0747	295.0820	293.0674	ESI+	2
750 Triflururon	64628-44-0	C15H10ClF3N2O3	358.0332	359.0405	357.0259	ESI+	8

Compound	CAS	Formula	M	TM+HM	TM+HL	Ionization Mode	MRM Transitions
751 Triflurosulfuron-methyl	126535-15-7	C ₁₇ H ₁₉ F ₃ N ₆ O ₆ S	492.1039	493.1112	491.0966	ESI+	8
752 Triforine	26644-46-2	C ₁₀ H ₁₄ Cl ₆ N ₄ O ₂	431.9248	432.9321	430.9175	ESI+	7
753 Trinexapac-ethyl	95266-40-3	C ₁₃ H ₁₆ O ₅	252.0998	253.1071	251.0925	ESI+	6
754 Triphenyl phosphate	115-86-6	C ₁₈ H ₁₅ O ₄ P	326.0708	327.0781	325.0635	ESI+	6
755 Tris (2-chloro-1-(chloromethyl)ethyl) phosphate	13674-87-8	C ₉ H ₁₅ Cl ₆ O ₄ P	427.8839	428.8912	426.8766	ESI+	26
756 Triticonazole	131983-72-7	C ₁₇ H ₂₀ ClN ₃ O	317.1295	318.1368	316.1222	ESI+	9
757 Tritosulfuron	142469-14-5	C ₁₃ H ₉ F ₆ N ₅ O ₄ S	445.0279	446.0352	444.0206	ESI+	4
758 Valifenalate	283159-90-0	C ₁₉ H ₂₇ ClN ₂ O ₅	398.1608	399.1681	397.1535	ESI+	16
759 Vamidothion	2275-23-2	C ₈ H ₁₈ NO ₄ PS ₂	287.0415	288.0488	286.0342	ESI+	6
760 Vamidothion-sulfone	70898-34-9	C ₈ H ₁₈ NO ₆ PS ₂	319.0313	320.0386	318.0240	ESI+	6
761 Vamidothion-sulfoxide	20300-00-9	C ₈ H ₁₈ NO ₅ PS ₂	303.0364	304.0437	302.0291	ESI+	6
762 Vernolate	1929-77-7	C ₁₀ H ₂₁ NO ₅	203.1344	204.1417	202.1271	ESI+	5
763 Warfarin	81-81-2	C ₁₉ H ₁₆ O ₄	308.1049	309.1122	307.0976	ESI+	6
764 XMC (3, 5-xylyl methylcarbamate)	2655-14-3	C ₁₀ H ₁₃ NO ₂	179.0946	180.1019	178.0873	ESI+	12
765 Ziram	137-30-4	C ₆ H ₁₂ N ₂ S ₄ Zn	303.9175	304.9248	302.9102	ESI+	2
766 Zoxamide	156052-68-5	C ₁₄ H ₁₆ Cl ₃ NO ₂	335.0247	336.0320	334.0174	ESI+	18

Further Information

Application News No.C136 describes the analysis of 646 pesticides in a single multi-residue method built using the Shimadzu Pesticide Library.

Scope and Legal Disclaimers

Whilst every effort has been made to ensure the accuracy of the Library, the method will need to be verified in a laboratory as conditions may differ marginally. The influence of sample matrices, extraction protocols, LC behaviour and technical experience may affect the performance of the LC/MS/MS analysis.

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An automatic versatile system integrating solid-phase extraction with ultra-high performance liquid chromatography–tandem mass spectrometry using a dual-dilution strategy for direct analysis of auxins in plant extracts



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ABSTRACT

An automatic versatile system which integrated solid phase extraction (SPE) with ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was developed. Diverse commercial SPE columns can be used under an ambient pressure in this online system realized by a dual-dilution strategy. The first dilution enabled the direct injection of complex samples with minimal pretreatment, and the second dilution realized direct introduction of large volume of strong eluent into the UHPLC column without causing peak broadening or distortion. In addition, a post-column compensation mode was also designed for the matrix-effects evaluation. The features of the online system were systematically investigated, including the dilution effect, the capture of desorption solution, the column-head stacking effect and the system recovery. Compared with the offline UHPLC system, this online system showed significant advantages such as larger injection volume, higher sensitivity, shorter analysis time and better repeatability. The feasibility of the system was demonstrated by the direct analysis of three auxins from different plant tissues, including leaves of *Dracaena sanderiana*, buds and petals of *Bauhinia*. Under the optimized conditions, the whole analysis procedure took only 7 min. All the correlation coefficients were greater than 0.9987, the limits of detection and the limits of quantitation were in the range of 0.560–0.800 ng/g and 1.80–2.60 ng/g, respectively. The recoveries of the real samples ranged from 61.0 to 117%. Finally, the post-column compensation mode was applied and no matrix-effects were observed under the analysis conditions. The automatic versatile system was rapid, sensitive and reliable. We expect this system could be extended to other target analytes in complex samples utilizing diverse SPE columns.

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1. Introduction

The development of hyphenated analysis systems is one of the predominant trends in modern analytical chemistry [1,2]. High-performance liquid chromatography (HPLC) is a mature technique commonly used in routine analysis [3]. However, despite the outstanding performance of modern chromatographic systems, sample preparation is still the most tedious and time-consuming

step, which is recognized as the main bottleneck of the analytical process [4,5]. Thus, special coupling of sample pretreatment methods to HPLC such as solid-phase extraction (SPE)–HPLC [6,7], solid phase microextraction (SPME)–HPLC [8–14] and so on have been developed, which provide a number of advantages including reduced analysis time, high sample throughput and great reproducibility.

SPE is an attractive approach to the preparation of environmental and biological samples. Compared with liquid–liquid extraction (LLE) technique and SPME, SPE provides higher enrichment factor and better reproducibility [15]. Most importantly, SPE offers countless diverse choices of sorbents, ranging from the traditional

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reversed-phase sorbents to some new materials such as carbon nanotubes [16], electrospun nanofibers [17], ionic liquid-modified materials [18,19], restricted access media [20,21] and so on. In addition, SPE could be performed on-line by directly connecting to the chromatographic system. Hyphenated SPE-HPLC on-line systems are designed to improve not only the sensitivity and selectivity but also the precision due to minimal human participation [22]. Up to now, many different types of SPE-HPLC online systems have been developed. Kantiani et al. [23] used a fully automated online SPE-LC/ESI-MS-MS system to detect β -lactam antibiotics in bovine milk samples, and the limits of quantification (LOQs) were between 0.09 ng/mL and 1.44 ng/mL. Liu et al. [24] established a polymer microchips integrating SPE with HPLC system using reversed-phase polymethacrylate monoliths for the separation and enrichment of peptides, free fluorescein and labeled proteins, where the sensitivity was significantly improved.

After the domination of separation science by HPLC for about half a century, ultra-high performance liquid chromatography (UHPLC) has recently emerged as a more powerful separation approach [25]. The main differences between UHPLC and HPLC are the column and the pressure of the system. UHPLC uses the columns packed with sub-2 μ m particles in ultra-high pressure conditions, while the columns used in HPLC are commonly packed with 5 μ m particles under a much lower pressure [26]. In addition, the reduction of particle size down to sub-2 μ m allows either speeding up of the analytical process while maintaining similar efficiency or a theoretical increase in efficiency for a shorter run time [27]. Furthermore, the combination of UHPLC with mass spectrometry (MS) detector appears to be a suitable approach that fulfills key requirements in terms of sensitivity, selectivity, and peak-assignment certainty for the rapid determination of analytes at low concentrations in complex matrices [26]. Thus, UHPLC has been well accepted as a powerful analytical tool for its fast separation, high sensitivity and improved throughput [28–30].

The on-line combination of SPE and UHPLC is expected to be an efficient method for the rapid and ultra-trace analysis of complex samples [31,32]. However, there are two bottlenecks during the achievement of SPE-UHPLC online system. One is the peak broadening and distortion caused by excessive volume of solvents with high elution strength. During SPE procedure, large volume of strong solvent is usually necessary for efficient SPE elution, which results in broad, flat-top shaped peaks with low plate counts in the following chromatographic analysis [30]. In fact, this volume overload problem is the main obstacle to online hyphenation of sample preparation techniques to HPLC, and the problem is much more serious for UHPLC due to its lower sample capacity which is limited by the narrower and shorter columns. Recently, an online analytical system integrating solid-phase-based extraction technique with UHPLC based on a fractionized sampling and stacking (FSS) strategy was established, which would address the volume overload problem of the online SPE-UHPLC system [33]. However, during FSS procedure, the valve needed to be manually switched 50 cycles. The other bottleneck is the high pressure of UHPLC system which normal SPE column could not stand. The pressure of UHPLC system which is much higher than that of HPLC system would cause serious damages to the SPE materials.

Auxin is one of the most crucial plant hormones, which regulates the growth and development of the plants [34]. The 3-indole acetic acid (IAA) is the first plant hormone used to stimulate rooting of cuttings. There are also several synthetic compounds with structure similar to that of IAA that elicits auxin-like physiological responses, such as 3-indole-propionic acid (IPA), 3-indole-butyric acid (IBA). Analysis of auxins has a great value for the study of biological process and its in-depth application, such as modern agriculture and biotechnology [35]. However, plant hormones are difficult to

analyze due to their lower concentrations in plant tissues which are very rich in interfering substances [36].

In this study, an automatic analysis online system coupling SPE with UHPLC-MS/MS using a dual-dilution strategy was developed. The dual-dilution strategy allowed the direct injection of complex samples and enabled direct introduction of large volume of strong eluent into the UHPLC column without causing peak broadening, which was the key ingredient for realizing this online system. Besides, a post-column compensation mode for the matrix-effects evaluation was designed with minor modification. In this dual-dilution SPE-UHPLC-MS/MS online system, common commercial SPE columns could be coupled with the UHPLC-MS/MS equipment, achieving automatic online analysis. To demonstrate the feasibility of the approach, this online system was applied to the direct analysis of three auxins in different plant tissues. And the features of the online system were investigated systematically.

2. Experimental

2.1. Reagents and samples

Standards of IAA, IPA and IBA were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All standard solutions were prepared in methanol and were stored at -20°C . Methanol, acetonitrile and formic acid of HPLC grade were purchased from CNW technologies (Shanghai, China). The other solvents of analytical grade were purchased from Guangzhou Chemical Reagents Factory (Guangzhou, China). Water used throughout the study was prepared with a Milli-Q Gradient A-10 water system (Millipore, Bedford, MA, USA).

Leaves of *Dracaena sanderiana*, buds and petals of *Bauhinia* were collected from Yuexiu Park in Guangzhou. After being cut into pieces, 1.00 g of each sample was added into 20 mL 80% (v/v) methanol aqueous solution. Then the mixture was homogenized by a homogenizer and extracted by ultrasonic wave, each for 10 min. The plant extracts were filtered with 0.45 μ m membrane before analysis.

2.2. Apparatus

The online system was converted from the Shimadzu LC-MS/MS system (Shimadzu, Kyoto, Japan). A LCMS-8040 tandem mass spectrometer (Shimadzu, Shimadzu, Kyoto, Japan) was equipped with an electrospray ionization (ESI) interface. The LC system consisted of a CBM-20A controller, two LC-30AD pumps (Pump A&B, which could stand ultra-high pressure up to 130 MPa), two LC-20AD pumps (Pump C&D, which could only stand normal pressure up to 40 MPa), two vacuum degassers, an autosampler, a column oven, and a SPD-M20A photodiode array (PDA) detector. Pump D was equipped with a solvent switching valve for the alternation between the loading solvent and the conditioning solvent. Two six-port valves, V1 and V2, which could respectively resist the maximum pressure of 34 MPa and 130 MPa, were mounted inside the column oven. Two mixers, Mixer 1 and Mixer 2 were used for the dilution of the injected solution and the desorption solvent, respectively. An in-line filter was installed between V1 and V2. The SPE column was mounted between two ports (NO. 1 and NO. 4) of V1 for extraction. A stainless steel capture loop of 500 μ L (Loop 1) was mounted between two ports (NO. 1 and NO. 4) of V2 for capturing of the target analytes. An in-line filter with 0.5 μ m mesh size was installed between V1 and V2 to filter the desorption solution. Another valve (V3) was installed after the UHPLC column for the post-column compensation mode and a capture loop of 1 mL (Loop 2) was mounted between two ports (NO. 1 and NO. 4) of V3 for loading of the standard solvent for matrix-effects evaluation.

Table 1
Time program of the analysis procedure.

Time (min)	Module	Action	Description
0.00	Pump B Autosampler	Pump B ratio = 10% Injection	LC gradient program (Pump A ratio = 90%). 50 μ L of sample was injected and online diluted by Mixer 1 and subsequently flow through the SPE column for extraction. 0.3 mL/min of the desorption solvent flow through the SPE column for desorption, and the desorption solvent was collected in Loop 1. Pump D changed the distribution from loading solvent to conditioning solvent. The desorption solvent inside Loop 1 was pushed by methanol from Pump B and then converged with water from Pump A in Mixer 2 for the second online dilution.
1.00	Valve 1	Switch to "inject"	
	Pump D	Switch from loading solvent to conditioning solvent	
2.50	Valve 2	Switch to "inject"	LC gradient program (Pump A ratio = 90%). Conditioning solvent flow through the SPE column. Fresh desorption solvent flow through Loop 1. LC gradient program (Pump A ratio = 30%). Pump D distributed from conditioning solvent back to loading solvent.
3.50	Pump B	Pump B ratio = 10%.	
4.50	Valve 1	Switch back to "load"	
4.70	Valve 2	Switch back to "load"	LC gradient program (Pump A ratio = 90%). Conditioning solvent flow through the SPE column. Fresh desorption solvent flow through Loop 1. LC gradient program (Pump A ratio = 30%). Pump D distributed from conditioning solvent back to loading solvent.
5.50	Pump B	Pump B ratio = 70%	
	Pump D	Switch from conditioning solvent to loading solvent	
6.98	Pump B	Pump B ratio = 70%	LC gradient program (Pump A ratio = 30%). LC gradient program (Pump A ratio = 90%). Analysis stopped.
6.99	Pump B	Pump B ratio = 10%	
7.00	Controller	Stop	

2.3. SPE and UHPLC–MS/MS conditions with the automatic analysis procedure

The MAYI-SAX column (10 $\times$ 4.6 mm) from Shimadzu, which is a restricted access media column with strongly-basic anion-exchange group modified inside, was used as the SPE column to selectively concentrate the anionic auxins while the macromolecular impurities in the plant extracts were excluded. The separation was carried out on a Shim-pack XR-ODS III column (2.0 mm i.d. $\times$ 50 mm, 1.8 μ m particle size) from Shimadzu. The mobile phase consisted of 0.1% (v/v) formic acid in water (A) and methanol (B), and the flow rate was 0.5 mL/min. The elution gradient was shown in Table 1. The desorption solvent of 0.5% (v/v) formic acid in methanol (C) was delivered by Pump C at 0.3 mL/min. Distributed by the solvent switching valve, the loading solvent of 80% (v/v) methanol aqueous solution and the conditioning solvent of 0.2% (v/v) formic acid in water were both delivered by Pump D alternatively at 3.0 mL/min.

All analytes were determined by multiple reaction monitoring (MRM) in positive ionization mode [37]. The parent ions, the product ions, and the compound-dependent mass spectrometric parameters were summarized in Table S1. The desolvation temperature, heat block temperature, nebulizing gas and drying gas were set at 250 °C, 400 °C, 3.0 L/min and 15.0 L/min, respectively. The dwell time and pause time for each ion channel were set at 50 ms and 3 ms, respectively.

The whole analysis procedure was automatically controlled by Shimadzu workstation LabSolutions (Version 5.60). The configuration of the system was shown in Fig. 1. The time program of the automatic analysis procedure including the valve switch, LC gradient and the solvent alternation of Pump D are shown in Table 1. The details of the analysis procedure were fully discussed in Section 3.

2.4. Offline SPE procedure

50 μ L sample solution was diluted into 3 mL with 80% (v/v) methanol aqueous solvent, and the solution was injected into the SPE column (MAYI-SAX, 10 $\times$ 4.6 mm) with a velocity of 3 mL/min. The target analytes were then eluted by 500 μ L desorption solvent of formic acid/methanol (0.5/99.5, v/v). The eluent was finally condensed into 50 μ L, and 5 μ L was injected for the UHPLC analysis.

2.5. System recovery test

The system recovery was evaluated by comparing the responses of the online system with that of the normal UHPLC system after the

directly injection of the standard solutions. With the same amount of auxins (5 μ L standard solutions of 1.00, 10.0, 20.0, 40.0, and 100 μ g/L for directly injected UHPLC, and the corresponding points for the online system are 50 μ L standard solutions of 0.100, 1.00, 2.00, 4.00, and 10.0 μ g/L, respectively), the linear equations of the online method and the offline method were built (Figure S8). The system recoveries were presented by the quotients of the slope of the directly injected UHPLC method divided by that of the online method.

3. Result and discussion

3.1. Analysis steps of the dual-dilution SPE-UHPLC–MS/MS system

The online system consisted of three analysis steps in sequence. The first step shown in Fig. 1-I was "the first dilution and extraction". In this step, an aliquot of sample solution was injected by autosampler and was transferred into Mixer 1 by Pump D. The sample solution was efficiently diluted in Mixer 1 and then went through the SPE column, on which the analytes of small molecular weight were adsorbed while the matrixes with larger molecular weight were excluded. The second step was "desorption and capture" (shown in Fig. 1-II). After Valve 1 was switched, the desorption solvent flowed through the SPE column and carried the analytes to Loop 1, at a certain time, the desorbed analytes was "captured" in Loop 1. The third step was "the second dilution, column-head stacking and UHPLC separation" (shown in Fig. 1-III). After Valve 2 was switched, the desorbed analytes in Loop 1 was driven into Mixer 2 by an organic phase from Pump A and mixed with the aqueous phase from Pump B. Because the ratio of the organic phase to the aqueous phase was set at 1:9 (v/v), the analytes were diluted in 90% (v/v) aqueous phase in Mixer 2 and introduced to the UHPLC column for the following separation.

3.2. The feature investigation of the dual-dilution SPE-UHPLC–MS/MS system

3.2.1. The first dilution: pre-SPE column online dilution

Usually, the analytes in biological samples combine firmly with the matrix. High concentration of matrix would cover the surface of SPE particles so that the adsorption capacity of SPE column would decrease [21]. Furthermore, the common used extraction solutions of the SPE column with reverse phase (RP) materials such as methanol and acetonitrile are of high elution strength. The

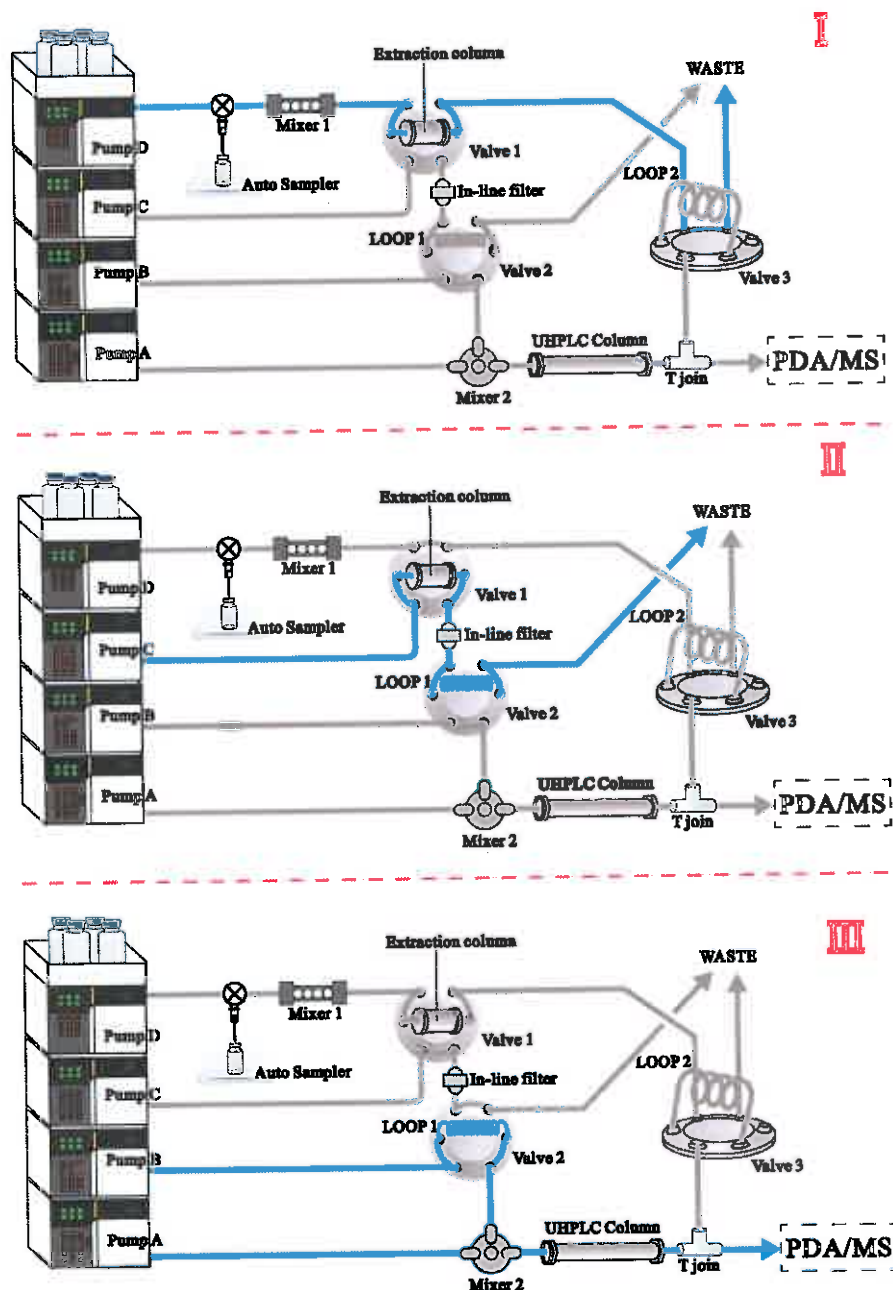


Fig. 1. Schematic diagram of the dual-dilution SPE-UHPLC-MS/MS system. I, the first dilution and extraction; II, desorption and capture; III, the second dilution, column-head stacking and UHPLC separation.

loading of high elution strength solvent directly would result in low recoveries [38]. Online dilution pre-SPE column not only effectively reduced the combination of analytes with proteins or other large molecules in matrix, but also converted the sample solution of high elution strength into weak loading solvent, which would increase the recoveries of SPE.

There were two kinds of normal online dilution modules: one T-joint with two pumps (shown in Figure S1-I) and two T-joints with one pump (shown in Figure S1-II), both of which were complicated and with poor reproducibility. In this study, a peculiar mixer (Mixer 1) with one pump was used as an online dilution module. The real picture and the inside structure of Mixer 1 were shown in Figure S2. There were 12 balls with inert surface inside the mixer, which could effectively diffuse the fluid. In order to evaluate the advantage of the pre-SPE column online dilution module, the dilution

effect of the mixer was compared with the other two dilution modules by injecting the same amount of sample solution. The dilution effect was measured with the peak width which was broadened. The results shown in Figure S3 illustrated that the mixer in this system had the largest peak width, which meant the best dilution effect.

In addition, the dilution ratio in this system could be adjusted by changing the flow rate. Figure S4 showed the dilution effects of the mixer at different flow velocities. As illustrated in Figure S4, the lower the flow rate was, the higher the dilution ratio was obtained, accordingly longer dilution time was acquired. Moreover, the reproducibility of the pre-SPE column online dilution module was studied and the results were shown in Figure S5. The results showed that the signals of 6 injections were perfectly overlapped, which meant the reproducibility of the module was excellent. The

advantage of the pre-SPE column online dilution module was based on the special dilution mechanism of the mixer. The analytes in the mixer is undergoing longitudinal diffusion. The longitudinal diffusion effect at a low flow rate is better than that at a high flow rate, and the change of flow rate will alter the dilution effect and the dilution time. Therefore, both the dilution effect and the dilution time should be taken into account during the method development. The dilution mechanism of the other two online dilution systems depended on the ratio of main flow rate and bypass flow rate, and the dilution effect was little influenced by the total flow rate. In conclusion, the simple pre-SPE column online dilution module which consisted of only one pump and one mixer had better dilution effect and adjustable dilution ratio.

3.2.2. The capture of the desorption solution

Common commercial SPE column could not stand high pressure. Normally, directly desorbing the analytes under HPLC pressure would cause irreversible damages to the SPE material [33]. In order to desorb the analytes from SPE column under a much lower pressure, the online system in this study used Pump C which was operated at 0.3 mL/min under 0.2 MPa to drive the desorption solvent to flow through the SPE column (shown in Fig. 1-II). After being desorbed from the SPE column, the target analytes in the desorption solution were further transported through Loop 1. At the very moment that the analytes were captured inside Loop 1, Valve 2 was switched for the second dilution for the column-head stacking. Besides, there is another advantage using an independent pump to deliver the desorption solution. Generally, the optimized desorption conditions including the solvent composition and the flow rate are not consist with the optimized LC conditions. Therefore, using a single Pump C to deliver the desorption solvent perfectly solves the problem.

The desorption conditions was also optimized and evaluated. Since the desorption solvent flowed constantly, the reproducibility of the capture and switching of the desorption solvent was very important to the method repeatability. The desorption repeatability shown in Figure S6 declared that the desorption of the analytes was effective, fast and steady under the optimized conditions.

3.2.3. The second dilution for the column-head stacking

Generally, the injection volume is inversely proportional to the elution strength of the sample solution. In order to keep the peak sharp, the injection volume should be small when strong elution solvent is used to dissolve sample [33]. On the other hand, when weak elution solvent is used, large volume of sample solution could be directly injected into the chromatographic column [39]. Theoretically, much larger volume of aqueous solution than that of organic solution could be injected into the reverse phase chromatographic column without peak broadening. The desorption solutions of RP-SPE such as methanol and acetonitrile are strong elution solvents, and the injection volume should be smaller than 20 μ L, otherwise condense or solvent switching should be conducted, which limited the realization of the online method [38].

In this system, the technique of column-head stacking was used. As shown in Fig. 1-III, strong elution solvent in Loop 1 was pushed by Pump B into Mixer 2 and mixed with the aqueous phase from Pump A. Mixer 2 is a patent product of Shimadzu Corporation, which is a high efficient solvent mixer with an inside volume of only 20 μ L. The real picture and the inside structure of Mixer 2 were shown in Figure S7. After being diluted in Mixer 2, the analytes were finally dissolved in 90% (v/v) aqueous phase and introduced to the UHPLC column. Since the solution with a high percentage of aqueous phase was a weak elution solvent, the analytes would be stacked on the head of the UHPLC column until the LC gradient changed for the following separation.

The column-head stacking effect of the online system was evaluated by comparing with the common UHPLC system. As shown in Fig. 2, in common UHPLC system, the peaks were sharp with 5 μ L injection volume of 10.0 μ g/L standards (Fig. 2A) but were broadened seriously with 50 μ L injection volume of 1.00 μ g/L standards (Fig. 2C) (Simulation of injecting large volume of strong elution solvent with low concentration of analytes), while in this online system, the peaks of three analytes were symmetric and the peak widths were all less than 10 seconds (Fig. 2B). The results illustrated that “the second dilution” in Mixer 2 for the column-head stacking significantly inhibited the solvent effect and increased the injection volume of strong elution solvents.

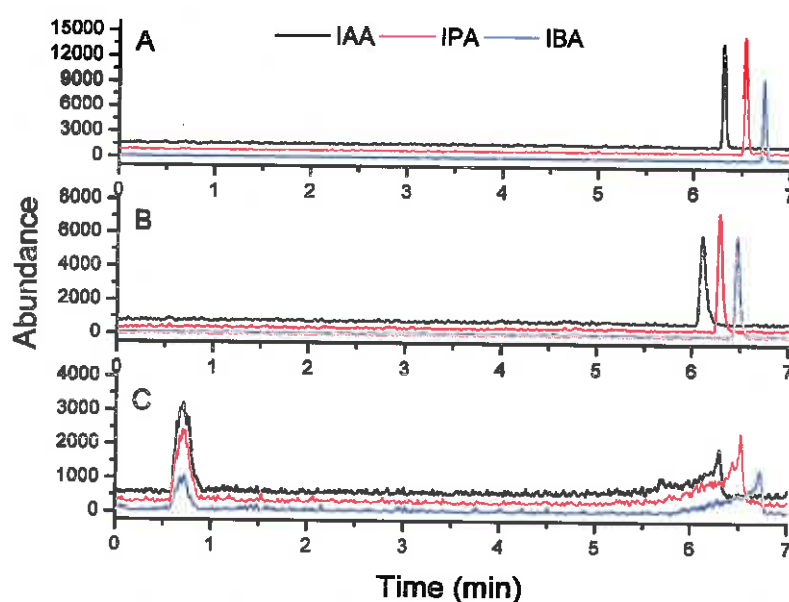


Fig. 2. Typical chromatograms obtained from: A, 5 μ L injection volume of 10.0 μ g/L standards in common UHPLC system; B, 50 μ L injection volume of 1.00 μ g/L standards in dual-dilution SPE-UHPLC-MS/MS system; C, 50 μ L injection volume of 1.00 μ g/L standards in common UHPLC system.

3.2.4. System recovery

The system recovery of the standard solution was evaluated by comparing the responses of the online system with that of the normal UHPLC system after the directly injection of the standard solutions. Linear regression coefficients for all six standard curves were greater than 0.9958, and the system recoveries presented by the quotients of the slope of the directly injected UHPLC method divided by that of the online method for IAA, IPA and IBA were 69.9%, 77.2% and 82.5%, respectively. The satisfactory system recovery indicated that the target analytes were extracted and desorbed effectively.

3.3. The optimization of the online analysis process

The influences of the main factors potentially affecting the online analysis process were evaluated systematically.

3.3.1. Loading solvent

The loading solvent which diluted the injected sample solution of high elution strength into a weak loading solvent could greatly affect the recoveries of SPE. The loading solvents tested in this study were: methanol/water (20/80, 40/60, 60/40, 80/20, v/v), ammonia/methanol/water (0.05/80/20, 0.1/80/20, 0.2/80/20, v/v), ammonia/water (0.1/99.9, v/v), acetonitrile/water (20/80, 40/60, 60/40, 80/20, v/v), 100% methanol, 100% acetonitrile and 100% water. Finally, methanol/water (80/20, v/v), a normal extraction solvent for the plant tissues, was chosen as the optimal loading solvent for it obtained the highest extraction amount (Figure S9).

As discussed in Section 3.2.1, the dilution ratio in this system could be adjusted by changing the flow rate. The flow rates of 1.0, 2.0, 3.0, 4.0 mL/min were investigated. Finally, 3.0 mL/min was chosen as the optimal flow rate (Figure S9).

3.3.2. Extraction time of SPE

For the extraction time of SPE was related to the flow rate. The extraction time was examined in the range of 0.5–4.0 min under different flow rates. By considering the extraction yield and the time consumption, 1.0 min of the extraction time under 3.0 mL/min of the flow rate was set for the following analysis.

3.3.3. Desorption solvent

The desorption efficiency was determined by the desorption solvent composition and its flow rate. The desorption solvents tested in this study were: formic acid/methanol (0.1/99.9, 0.3/99.7, 0.5/99.5, 0.7/99.3, v/v), formic acid/water/methanol (0.5/10/90, 0.5/30/70, 0.5/50/50, v/v), formic acid/acetonitrile (0.2/99.8, 0.5/99.5, v/v), 100% methanol and 100% acetonitrile. Finally, formic acid/methanol (0.5/99.5, v/v) was chosen as the optimal desorption solvent, which yielded the highest response and the shortest desorption time (Figure S9).

After formic acid/methanol (0.5/99.5, v/v) was chosen as the optimal desorption solvent, different flow rates ranged from 0.2–0.4 mL/min were tested, and 0.3 mL/min was finally chosen as the optimal flow rate of the desorption solvent for it got the best desorption and the finest repeatability (Figure S9).

3.3.4. Switching time for valve 2

The switching time for Valve 2 is crucial since it is directly related to the proportion of the target analytes introduced into UHPLC column. The later the switching time set, the greater the recovery of the system will be. However, the whole analysis time would be prolonged. Considering the total analysis time, 2.5 min was chosen as the optimal switching time.

3.3.5. Conditioning of SPE Column

SPE column needs to be conditioned before extraction, and the extraction performance of the anion exchange SPE column used in this study is related with pH. In order to ensure the reproducibility of the method, the pH of the SPE column should be the same before every extraction. Therefore, a conditioning procedure was set before extraction and the conditioning solvent was optimized. Formic acid/water (0.1/99.9, 0.2/99.8, 0.3/99.7, v/v), ammonia/water (0/100, 0.1/99.9, v/v) and formic acid/water/methanol (0/90/10, 0/80/20, 0.1/80/20, 0.1/90/10, v/v) were tested, and 0.2% (v/v) formic acid aqueous solution turned out to be the best for the system recovery and reproducibility.

3.4. Comparison with offline SPE-UHPLC method

3.4.1. Sensitivity

The system sensitivity was mainly determined by the injection volume when using the same detector. As shown in Fig. 2, the offline UHPLC system could only endure 5 μ L of strong solvent, while the online system enabled 50 μ L. The limits of detection (LODs) and limits of quantification (LOQs) of the offline UHPLC system, which were calculated at 3 and 10 times the signal to noise ratio (S/N), were in the range of 4.71–6.45 ng/g and 14.3–19.6 ng/g, respectively. By contrast, the LODs and LOQs of the online system were much lower, ranging from 0.560 to 0.800 ng/g and 1.85 to 2.64 ng/g, respectively (shown in Section 3.5.1).

3.4.2. Analysis time

The total analysis time of the online SPE-UHPLC-MS/MS system was 7 min, including 1 min (0–1.0 min) for SPE extraction, 1.5 min (1.0–2.5 min) for desorption, 2.2 min (2.5–4.7 min) for column-head stacking, and 2.3 min for UHPLC separation. By contrast, the offline analysis procedure was much slower, costing at least half an hour to finish all the procedure.

3.4.3. Repeatability

The repeatability of the offline method was estimated with 5 μ L standard solutions (10.0 μ g/L and 100 μ g/L, six repetitive standard solutions were processed on each level). The relative standard deviations (RSDs) of the peak areas at 10.0 μ g/L and 100 μ g/L obtained by the offline method were in the range of 4.9–10.5%, while the RSDs obtained by the online system were all less than 2.8% (Section 3.5.1), indicating that the repeatability of the offline method was much poorer than that of the online system.

3.5. Validation and application of the dual-dilution SPE-UHPLC-MS/MS system

3.5.1. Method validation

All three auxins determined by this dual-dilution SPE-UHPLC-MS/MS system were identified by their chromatograms and retention times compared with those of the standards. Linearity for all auxins was determined using standard solutions at the concentration levels ranging from 0.100 to 100 μ g/L. Linear regression coefficients for all auxins ranged from 0.9987 to 0.9999 (Table 2). The LODs and LOQs ranged from 0.560 to 0.800 ng/g and 1.85 to 2.64 ng/g, respectively. The reproducibility was estimated on two concentration levels (1.00 μ g/L and 10.0 μ g/L) and six repetitive standard solutions were processed on each level. The results showed that the RSDs of the retention times were less than 0.05% and the RSDs of the peak areas were less than 2.8%. The results indicated that the system had significant reproducibility, reflecting the perfect integration of the units in the system.

Table 2

Analytical performance of the dual-dilution SPE-UHPLC-MS/MS system for the determination of three auxins.

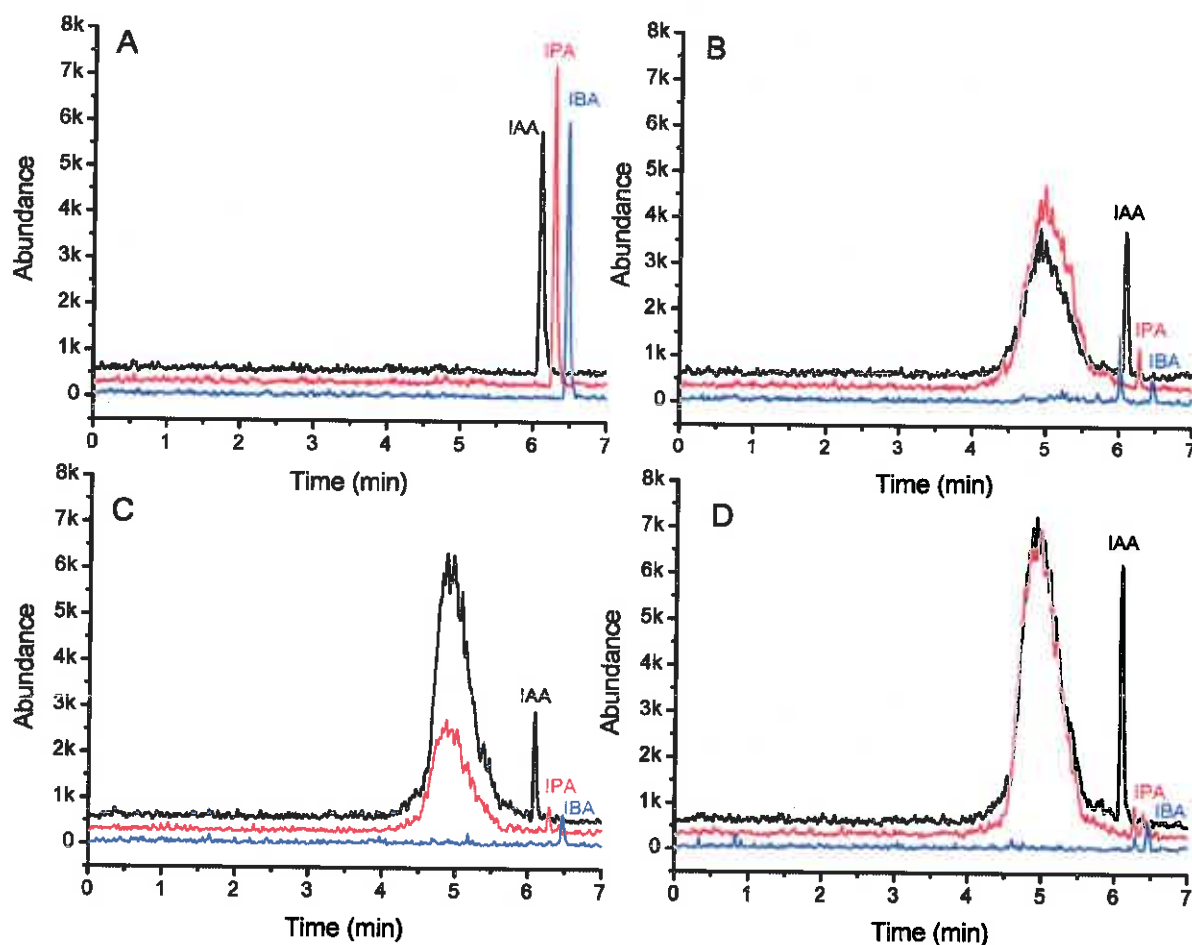
Compound	Linear equation	Correlation coefficient (r)	Linear range (μg/L)	LOD ^a (ng/g)	LOQ ^b (ng/g)
IAA	$Y = 2.60 \times 10^4 X - 5.65 \times 10^2$	0.9987	0.100–100	0.800	2.64
IPA	$Y = 2.23 \times 10^4 X + 6.05 \times 10^2$	0.9999	0.100–100	0.640	2.11
IBA	$Y = 1.83 \times 10^4 X + 4.99 \times 10^2$	0.9998	0.100–100	0.560	1.85

^a S/N = 3.^b S/N = 10.

Table 3

Detected amount and recoveries of the auxins in three plant tissue samples (n = 3).

Sample	Analyte	Real sample		Spiked sample of 2.00 ng/g		Spiked sample of 20.0 ng/g
		Detected amount (ng/g)	Detected amount (ng/g)	Average recovery (%)	Detected amount (ng/g)	Average recovery (%)
Leaves of <i>Dracaena sanderiana</i>	IAA	6.81 ± 0.21^a	8.11 ± 0.23	67.0	25.0 ± 0.3	90.4
	IPA	ND ^b	1.31 ± 0.15	65.0	16.5 ± 0.5	82.4
	IBA	ND	1.63 ± 0.21	83.0	20.0 ± 0.5	99.6
Buds of <i>Bauhinia</i>	IAA	2.83 ± 0.10	5.15 ± 0.11	117	18.3 ± 0.5	77.1
	IPA	ND	1.20 ± 0.08	61.3	14.2 ± 1.0	71.1
	IBA	ND	1.80 ± 0.04	90.7	20.0 ± 0.8	101
Petals of <i>Bauhinia</i>	IAA	13.1 ± 0.7	15.3 ± 0.2	109	24.9 ± 2.0	62.0
	IPA	ND	1.21 ± 0.03	61.0	12.7 ± 0.6	62.6
	IBA	ND	1.83 ± 0.07	92.3	19.3 ± 0.6	96.9

^a Mean $\pm$ SD, SD stands for standard deviation.^b ND = no detect.Fig. 3. Typical chromatograms of standards of 1.00 μg/L (A), the spiked samples at 2.00 ng/g of the leaves of *Dracaena sanderiana* (B), the petals of *Bauhinia variegata* (C) and buds of *Bauhinia variegata* (D).

3.5.2. Analysis of the plant tissue samples

The present online system was finally applied to the analysis of auxins in the leaves of *D. sanderiana*, the petals and buds of *Bauhinia variegata* collected from Guangzhou, China. No IPA or IBA were detected in the samples above the LODs. Only IAA was detected in all three kinds of samples, ranging from 2.84 to 13.8 ng/g. The spiked samples at two fortification levels (2.00 and 200 ng/g) were analyzed in 3 replicates. The recoveries of all auxins ranged from 61.0% to 117%, which are shown in Table 3. The typical chromatograms of blank samples and spiked samples processed by the dual-dilution SPE-UHPLC-MS/MS online system were shown in Figure S10 and Fig. 3, respectively. There was no significant interference from the plant samples observed near the retention times of the auxins, and all the peaks of the auxins were sharp and symmetrical. Compared with the other methods for determination of auxins in various samples [37,40–42], this online method showed significant advantages in terms of analytical speed, sensitivity and reproducibility (shown in Table S3).

3.6. Post-column compensation mode for the matrix-effects evaluation

Besides the online analysis, the dual-dilution SPE-UHPLC-MS/MS system can perform matrix-effects evaluation after minor modification into a post-column compensation mode. In this mode, the standard solution combined with the desorption solution containing matrix flowed into the mass spectrometer. If there were matrix-effects, the response of the mass spectrometer would change. The increase of the response meant the matrix enhanced effect while the decrease of the response meant the matrix weakened effect. If there was no change comparing with the pure standard solution, it meant there were no matrix-effects during the analysis. The schematic diagram and the analysis steps taken in this mode were shown in Figure S11. The time program was shown in Table S2, and the other analysis conditions were the same as the online analysis mode.

The typical MRM chromatograms of blank solvent and the extract of *D. sanderiana* leaves in the post-column compensation mode are shown in Figure S12. Comparing the two chromatograms, no significant difference was observed in the responses of the three auxins. The results indicated that there were no significant matrix-effects when analyzing the extract of *D. sanderiana* leaves after the purification of the online SPE procedure.

4. Conclusion

An automatic versatile SPE-UHPLC-MS/MS system using a dual-dilution strategy was originally developed in this study. With the dual-dilution process, this system allowed large volume injection of complex samples and the use of diverse commercial SPE columns for the online extraction without causing peak broadening or distortion. The feature investigation indicated that the online system had excellent dual-dilution effects, fast and steady desorption, and high system recovery. Compared with the offline UHPLC system, this online system provided larger injection volume, higher sensitivity, shorter analysis time and better repeatability. The feasibility of the system was demonstrated by the direct analysis of three auxins from different plant tissues. Excellent repeatability, low LODs and satisfactory recoveries were obtained under the optimized conditions. In addition, the post-column compensation mode has been applied and there were no matrix-effects during the analysis. In conclusion, the dual-dilution SPE-UHPLC-MS/MS online system is a simple and effective technique, which could be potentially extended to other target analytes in complex matrix by using diverse SPE columns.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2014.07.031>.

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Aflatoxins are low-molecular-weight natural products produced by fungi and are capable of causing disease and death. Aflatoxins are typically found in grain products and their occurrence is increased under certain heat and humidity conditions. Aflatoxin levels have been used as an index of aflatoxin production levels, and are dependent on the use and type of food products. Aflatoxin levels can vary widely within the same area, creating a potential need for increased testing to provide more accurate exposure estimates. A high-throughput, ELISA-based assay was developed that could provide separation of eight common mycotoxins in less than two hours at an affinity for increased testing. Details of the method will be presented.

Figure 1: Chemical structures of natural compounds.

Method: Reference standards were obtained for each mycotoxin. Each was diluted to an $8000 \mu\text{g/ml}$ solution of 3-methyl aminopropyl formate in the concentrations listed in Table 1. A Shimadzu Shim-pack XC-80 III HPLC column ($2 \times 50 \text{ mm}$, $5 \mu\text{m}$) was used at a flow rate of 0.6 ml/min and a temperature of 50°C .

A binary gradient of 3-methyl aminopropyl formate and methanol was used and is shown in Figure 5. Electrospray ionization was used with positive polarity. The BMR brushes and MS-310 brushes for each compound

	L2	L3	L4	L5	L6
Alfatekin G1	67.7	22.4	7.9	2.5	0.8
Alfatekin G2	20.3	6.8	2.3	0.8	0.3
Alfatekin G1	67.2	22.4	7.5	2.1	0.8
Alfatekin G2	20.3	6.8	2.3	0.8	0.3
Furmeselin G1	83.3	77.8	92.6	30.8	10.3
Furmeselin G2	82.3	77.8	92.6	30.8	10.3
Mycozantin T2	166.7	95.6	185.2	63.7	26.6
CON	810.0	270.0	800.0	300.0	100.0
Oxydezinim	81.0	27.0	9.0	3.0	1.0
DOF	202.0	69.0	22.0	7.0	2.0

Type	Exploit ID	Ref.	Component Name (url)	Disrupt Type (url)	CVE ID (url)	CV	Exploit (url)
Normal	1	-	Adaptive CS 3187-91	29	-25	-30	-35
Normal	2	-	Adaptive CS 3197-91	29	-39	-40	-35
Normal	3	-	Adaptive CS 3207-91	29	-20	-21	-20
Normal	4	-	Adaptive CS 3220-91	29	-25	-20	-20
Normal	5	-	Adaptive CS 3230-91	29	-20	-20	-20
Normal	6	-	Adaptive CS 3240-91	29	-20	-20	-20
Normal	7	-	Adaptive CS 3250-91	29	-20	-20	-20
Normal	8	-	Adaptive CS 3260-91	29	-20	-20	-20
Normal	9	-	Adaptive CS 3270-91	29	-20	-20	-20
Normal	10	-	Adaptive CS 3280-91	29	-20	-20	-20
Normal	11	-	Adaptive CS 3290-91	29	-20	-20	-20
Normal	12	-	Adaptive CS 3300-91	29	-20	-20	-20
Normal	13	-	Adaptive CS 3310-91	29	-20	-20	-20
Normal	14	-	Adaptive CS 3320-91	29	-20	-20	-20
Normal	15	-	Adaptive CS 3330-91	29	-20	-20	-20
Normal	16	-	Adaptive CS 3340-91	29	-20	-20	-20
Normal	17	-	Adaptive CS 3350-91	29	-20	-20	-20
Normal	18	-	Adaptive CS 3360-91	29	-20	-20	-20
Normal	19	-	Adaptive CS 3370-91	29	-20	-20	-20
Normal	20	-	Adaptive CS 3380-91	29	-20	-20	-20
Normal	21	-	Adaptive CS 3390-91	29	-20	-20	-20
Normal	22	-	Adaptive CS 3400-91	29	-20	-20	-20
Normal	23	-	Adaptive CS 3410-91	29	-20	-20	-20
Normal	24	-	Adaptive CS 3420-91	29	-20	-20	-20
Normal	25	-	Adaptive CS 3430-91	29	-20	-20	-20
Normal	26	-	Adaptive CS 3440-91	29	-20	-20	-20
Normal	27	-	Adaptive CS 3450-91	29	-20	-20	-20
Normal	28	-	Adaptive CS 3460-91	29	-20	-20	-20
Normal	29	-	Adaptive CS 3470-91	29	-20	-20	-20
Normal	30	-	Adaptive CS 3480-91	29	-20	-20	-20
Normal	31	-	Adaptive CS 3490-91	29	-20	-20	-20
Normal	32	-	Adaptive CS 3500-91	29	-20	-20	-20
Normal	33	-	Adaptive CS 3510-91	29	-20	-20	-20
Normal	34	-	Adaptive CS 3520-91	29	-20	-20	-20
Normal	35	-	Adaptive CS 3530-91	29	-20	-20	-20
Normal	36	-	Adaptive CS 3540-91	29	-20	-20	-20
Normal	37	-	Adaptive CS 3550-91	29	-20	-20	-20
Normal	38	-	Adaptive CS 3560-91	29	-20	-20	-20
Normal	39	-	Adaptive CS 3570-91	29	-20	-20	-20
Normal	40	-	Adaptive CS 3580-91	29	-20	-20	-20
Normal	41	-	Adaptive CS 3590-91	29	-20	-20	-20
Normal	42	-	Adaptive CS 3600-91	29	-20	-20	-20
Normal	43	-	Adaptive CS 3610-91	29	-20	-20	-20
Normal	44	-	Adaptive CS 3620-91	29	-20	-20	-20
Normal	45	-	Adaptive CS 3630-91	29	-20	-20	-20
Normal	46	-	Adaptive CS 3640-91	29	-20	-20	-20
Normal	47	-	Adaptive CS 3650-91	29	-20	-20	-20
Normal	48	-	Adaptive CS 3660-91	29	-20	-20	-20
Normal	49	-	Adaptive CS 3670-91	29	-20	-20	-20
Normal	50	-	Adaptive CS 3680-91	29	-20	-20	-20
Normal	51	-	Adaptive CS 3690-91	29	-20	-20	-20
Normal	52	-	Adaptive CS 3700-91	29	-20	-20	-20
Normal							

	1	2	3	4	5	6
Alfaterin B2	99.00	100.52	99.72	100.40	99.99	99.99
Alfaterin B2	100.00	101.20	100.80	99.90	100.77	100.77
Alfaterin G3	97.00	100.54	101.70	100.36	99.78	99.78
Alfaterin G3	100.00	100.00	100.00	99.86	100.00	100.00
OGN	137.40	116.40	112.42	123.40	102.20	102.20
Gothenburg	100.00	100.00	100.00	116.40	100.00	100.00
SDM	100.00	100.00	100.00	100.00	100.00	100.00

Mass spores were collected at 15,000 rpm in profile and require them continuously throughout the run. On this pendant 140-415 nm triggered based on the mass range scale at a resolution spread which they also act at 14,000 rpm.

[illegible]

Additionally, a feed corn sample was spiked with the peptide [Irrac] to demonstrate untargeted screening with site-dependent IRTMS and spectral pattern matching.

E31+ Survey Exam for Prep A

FDL: [FDL: Privacy from the Front](#)

Mixed conditions:
 10% ODS 2 x 50 min, 1.5 min
 isocratic, 7000 Hz, 50-50 ACN-MeOH
 1 ml/min, 80°C
 Aqueous mix 0.3 to 1 upl.
 Inj: 1 ul
 Reflow: 20-500 bar

[illegible]

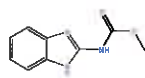
Abstract selective, and sensitive LC-MS-MS method for the determination of mycotoxins and xanthones screening was developed. A high throughput UHPLC method for aflatoxins B₁ and G₁ is also presented.

High Throughput LC-MS-MS Analysis of Carbendazim in Orange Juice

William Hedgepeth, Jeffrey H. Dahl, and Kenichiro Tanaka Shimadzu Scientific Instruments, Columbia, Maryland

Introduction

Highly sensitive analytical methods are necessary to prevent contaminated produce from entering the food supply. In order to help move fresh imports through customs without delay, methods must be rapid and cost effective. In order to test imports thoroughly, LC-MS offers the highly sensitive, robust measurement needed, while ultra high performance liquid chromatography enables high speed analysis. We developed an ultra fast UHPLC-MS-MS method for analysis of carbendazim in orange juice using a new high speed autosampler, the Nexera SIL-30ACMP. In tandem with a newly developed high sensitivity triple quadrupole mass spectrometer, the LCMS-8040.



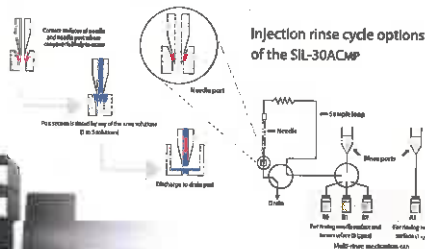
Carbendazim



Fungicides help protect oranges (*Citrus sinensis*) from disease, such as black spot mold

Method

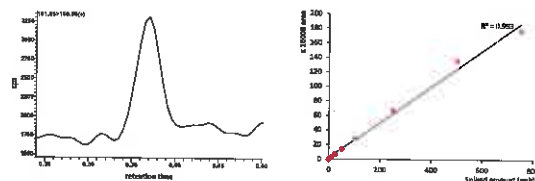
LC-MS-MS was performed using a Shimadzu Nexera UHPLC with a new high throughput autosampler, the Nexera SIL-30ACMP. A binary gradient of 5 mM ammonium acetate and methanol was used at a flow rate of 0.75 mL/min with a Phenomenex Kinetex XB-C18 (2.6 μ m, 2.1 $\times$ 30 mm) at 40 °C. Electrospray ionization in positive mode was used on a new fast-scanning, high sensitivity triple quadrupole mass spectrometer, the LCMS-8040. Various brands of orange juice were obtained and diluted 50-fold and filtered prior to injection; no other sample preparation was required. Due to the fast injection speed of the Nexera UHPLC system and the fast data acquisition of the LCMS-8040, the runtime was reduced to only 0.8 min.



The Nexera UHPLC combined with the increased sensitivity of the LCMS-8040 are perfect for higher throughput, higher sensitivity quantitation

Results and Discussion

Carbendazim was detected in several commercial brands of orange juice, all at levels below the FDA regulatory cutoff of 10 ng/mL and the health cutoffs established by the US EPA and European Union (80 and 200 ng/mL, respectively). The limit of detection of carbendazim in orange juice was approximately 0.4 ppb and the limit of quantitation was approximately 1 ppb. The sample preparation required only dilution and filtration, which enabled rapid sample analysis.



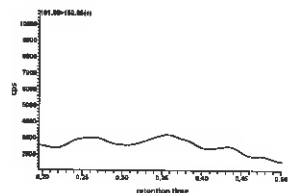
Chromatogram of orange juice spiked with 1 ppb carbendazim and calibration curve

Brand	Measured Carbendazim
HT *	ND
OV **	ND
GV	1.0 ppb
MM	8.1 ppb
T1	4.5 ppb
T2	1.1 ppb

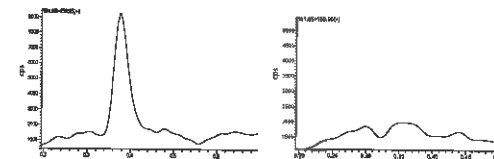
* Generic store brand
** Organic certified

Table of brands tested

Levels of carbendazim measured in different orange juice brands and an orange juice blank matrix.



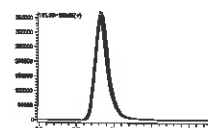
Orange juice matrix blank injected after 1 ppm standard



Representative chromatograms of two brands of orange juice. The MM brand contained 8.1 ppb carbendazim, while none was detected in the certified organic orange juice.

The Nexera UHPLC pumps enabled a fast gradient with a runtime of under 1 minute, while the SIL-30ACMP autosampler enabled injection cycle times of just 7 seconds. The improved ion optics of the LCMS-8040 made it possible to achieve better sensitivity with dilute-and-shoot sample preparation. Not only were the runs rapid, but the retention times and peak areas were extremely reproducible as well, with relative standard deviations of just 0.55 and 2.98% respectively.

File Name	Retention Time	Peak Area
CarbRSD-01.tst	0.361	861474
CarbRSD-02.tst	0.363	843203
CarbRSD-03.tst	0.363	843727
CarbRSD-04.tst	0.366	884182
CarbRSD-05.tst	0.365	833831
CarbRSD-06.tst	0.361	878459
Average	0.363	870174
%RSD	0.55%	2.98%



The retention times and peak areas were very reproducible even without using stable isotope labeled standards.

We found carbendazim levels remained consistent in orange juice samples even several months past their expiration dates under refrigerated storage conditions. This demonstrates the ability of carbendazim to persist in the food supply and underscores the need for thorough food safety screening.

Conclusion

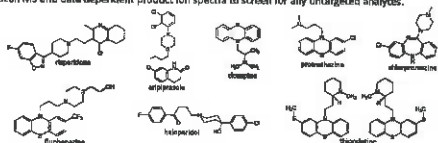
Carbendazim was detected in four commercial brands of orange juice at low ppb levels. Levels found were below FDA limits for a positive test. The calibration curve was linear up to 500 ppb, and the method was sensitive enough to detect carbendazim below the most stringent regulatory threshold. No carryover was detected and the method run time was under one minute, thanks to the extremely high speed autosampler which completed each injection cycle in just 7 seconds. Sample preparation only required dilution and filtration for rapid, efficient analysis.

Fast LC-MS/MS Screening Method for Antipsychotics with Data Dependent Analysis for Untargeted Analytes

Shannon L. Cook¹, Jeffrey H. Dahl¹, Christopher Gilles¹ Shimadzu Scientific Instruments, Inc., Columbia, MD

Introduction

Antipsychotic and atypical antipsychotics are used to treat a wide variety of psychiatric conditions such as schizophrenia, acute mania, bipolar disorder, and depression. Recently a high number of children are being treated with antipsychotics for emotional or behavioral disorders. Therapeutic drug monitoring is in demand due to the risk of toxicity, unwanted side effects, and potential pharmacokinetic interactions. Additionally, a co-administered drug can potentially inhibit or induce the metabolism of the antipsychotic drug such as antileptics, selective serotonin reuptake inhibitors, cigarette smoking, and barbiturates.¹ A fast screening method for a group of typical and atypical antipsychotics has been developed which incorporates the acquisition of full scan MS and data dependent product ion spectra to screen for any untargeted analytes.



Materials and Method

Antipsychotic standards were diluted in 0.35% formic acid at concentrations ranging from 4 ppm-1 ppm. Data was collected on a Shimadzu Hessea UHPLC with a Shimadzu LCMS-8040 mass spectrometer. An Accucore RP-Amide column was used with a binary gradient of 0.3% formic acid/acetonitrile at 0.4 mL/min. MRM transitions were optimized for the antipsychotics, thymosine and phenobarbital. Product ion scans were obtained for each analyte and a spectral library created. Dual purity data dependent product ion scans were triggered for precursor ions exceeding 1,000,000 cps. Synthetic serum (SeruSp) spiked samples were prepared by protein precipitation using 3:1 cold acetonitrile:serum. The samples were spun at 15,000 rpm for 5 min, the supernatant dried to completeness and reconstituted with 0.3% formic acid.

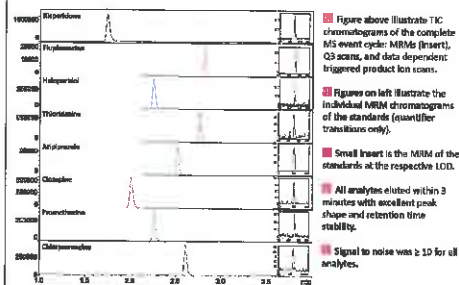
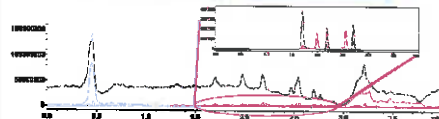
L&S Performance Thermal Stability		L&S Performance Thermal Stability	
C3000 Responder 40 °C		C3000 Responder 40 °C	
Time	% Solids Phase 0	Time	% Solids Phase 0
0:00	0	0:00	0
0:30	10	0:30	10
1:00	20	1:00	20
1:30	100	1:30	100
2:00	100	2:00	100
2:30	10	2:30	10
3:00	10	3:00	10
3:30	10	3:30	10
4:00	10	4:00	10

Qualitative Results



All antipsychotic standards were purchased from Ceflonit and MS/MS optimization was performed with CE and transitions (quantifier and qualifier ions) illustrated in the quantification section. Polarity switching was employed to excise data dependent product ion scans embedded within positive and negative Q3 scans to screen for unlabeled analogs.

MS Event Cycle		Loop Time: 0.4331	
Date	Event	Channel	Original Wave type (Avg 1-3 mts, 4.070 ms)
2007-07-01	1	1	1
2007-07-01	2	1	2
2007-07-01	3	1	3
2007-07-01	4	1	4
2007-07-01	5	1	5
2007-07-01	6	1	6
2007-07-01	7	1	7
2007-07-01	8	1	8
2007-07-01	9	1	9
2007-07-01	10	1	10
2007-07-01	11	1	11
2007-07-01	12	1	12
2007-07-01	13	1	13
2007-07-01	14	1	14
2007-07-01	15	1	15
2007-07-01	16	1	16
2007-07-01	17	1	17
2007-07-01	18	1	18
2007-07-01	19	1	19
2007-07-01	20	1	20
2007-07-01	21	1	21
2007-07-01	22	1	22
2007-07-01	23	1	23
2007-07-01	24	1	24
2007-07-01	25	1	25
2007-07-01	26	1	26
2007-07-01	27	1	27
2007-07-01	28	1	28
2007-07-01	29	1	29
2007-07-01	30	1	30
2007-07-01	31	1	31
2007-07-01	32	1	32
2007-07-01	33	1	33
2007-07-01	34	1	34
2007-07-01	35	1	35
2007-07-01	36	1	36
2007-07-01	37	1	37
2007-07-01	38	1	38
2007-07-01	39	1	39
2007-07-01	40	1	40
2007-07-01	41	1	41
2007-07-01	42	1	42
2007-07-01	43	1	43
2007-07-01	44	1	44
2007-07-01	45	1	45
2007-07-01	46	1	46
2007-07-01	47	1	47
2007-07-01	48	1	48
2007-07-01	49	1	49
2007-07-01	50	1	50
2007-07-01	51	1	51
2007-07-01	52	1	52
2007-07-01	53	1	53
2007-07-01	54	1	54
2007-07-01	55	1	55
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2007-07-01	83	1	83
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2007-07-01	89	1	89
2007-07-01	90	1	90
2007-07-01	91	1	91
2007-07-01	92	1	92
2007-07-01	93	1	93
2007-07-01	94	1	94
2007-07-01	95	1	95
2007-07-01	96	1	96
2007-07-01	97	1	97
2007-07-01	98	1	98
2007-07-01	99	1	99
2007-07-01	100	1	100



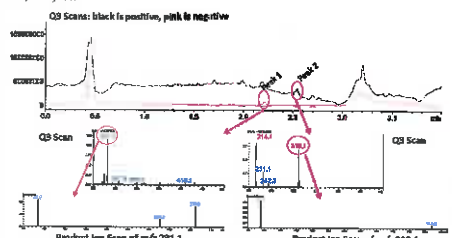
Quantitative Results

Standards were prepared in distilled synthetic urine and concentrations generated by serial dilution. All analytes produced linear responses with r^2 values between 0.9976–0.9990 and limits of detections (LODs) as low as 0.05 pg/mL.

[illegible]

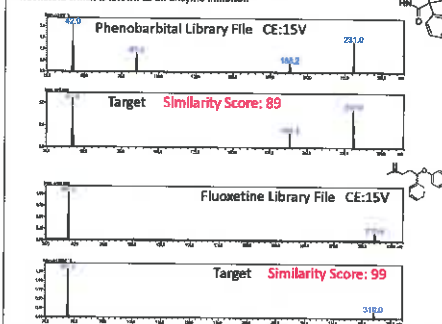
Data Dependent Analysis for Untargeted Analytes

Full mass spectra was collected at a scan rate of 10,000 u/sec throughout the entire run to screen for unknown analytes. Product ion scans were triggered on any ion that exceeded a set threshold level of 500,000 cps at a scan speed of 7,500 u/sec.



MS/MS Library Searching

The product ion spectra generated from the triggered data dependent events are then automatically compared to a MS/MS library database which contained 157 different analytes. The top hit for Peak A resulted in a similarity score of 89 with the analyte phenobarbital, a known enzyme inducer which ultimately leads to a reduction of certain antipsychotic uptake. The top hit for Peak B resulted in a similarity score of 99 which corresponds to the analyte fluoxetine which is known as an enzyme inhibitor.



Conclusion

A fast, robust, and sensitive LC/MS method for the analysis of antipsychotics has been developed using the Shimadzu LCMS-8040 mass spectrometer. Multiple reaction monitoring was used to screen and quantify the presence of known antipsychotic within synthetic serum. In addition, data dependent triggered product ion scans in both polarities enables the detection of unknown analytes, especially those which could interfere with the prescribed antipsychotic. The Shimadzu LCMS-8040 mass spectrometer achieved up to 3x the sensitivity when comparing the same data acquired on the Shimadzu LCMS-8030 mass spectrometer.

References

1. Sharif, Z.A.: Pharmacokinetics, Metabolism, and Drug-Drug Interactions of Atypical Antipsychotics in Special Populations. *Prim Care Companion J Clin Psychiatry*. 5, 22-25 (2003)

The application of LC-UFMSMS to the analysis of pesticide residues in negative ion ESI

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¹ Shimadzu Australasia, Rydalmere, NSW, AUSTRALIA; ² Metabolomics Australia, Murdoch University Node, Perth, AUSTRALIA; ³ Murdoch University, Murdoch, WA, AUSTRALIA

Introduction: Analysis using Nexera-LCMS-8040

Glyphosate is a widely used herbicide and residues in food and water for human consumption are of regulatory interest. The LCMS analysis of residual glyphosate and its metabolite AMPA in agricultural specimens is limited primarily by the impact of the polarity of these compounds on chromatography and sample preparation. We describe here the analysis of FMOG derivatised analytes using a reversed-phase separation and multiple ion MRM detection in negative ion ESI.

Method

Food samples were spiked with glyphosate and AMPA and extracted and reconstituted to give extracts equivalent to 0.001, 0.0025, 0.005, 0.01, 0.1 and 1 ppm. Extracts were derivatised with FMOG reagent using standard protocols (Figure 2, method details available upon request but not reported here). Derivatised extracts were delivered to the analyst as blind specimens. Analysis was completed on a Nexera LC-30-LCMS-8040 (Shimadzu, Kyoto, Japan) equipped with a Shimpack XR-ODS III (75mm x 2.1, 1.7µm particle) column. The sample was injected with a SIL-30AC autosampler and injection volume ranged in size from 1 to 10 µL. Analytical conditions were the same as those used for the LCMS-8030 analysis of herbicides (see right).

Figure 1: Organisations contributing to the blind analysis of FMOG samples

Discussion

The FMOG derivatives enable the use of a reversed-phase separation rather than a HILIC or other separation strategy (Figure 3). This approach allows the use of a more robust mobile phase to support negative ion ESI and the quantitation of glyphosate and AMPA in food extracts. The method sensitivity exceeded current regulatory requirements and so provided the capacity to incorporate miniaturisation of the sample preparation steps or the use of smaller specimens.

Conclusion

The UFSweeper™ equipped LCMS-8040 allowed detection of 1 pg of FMOG-glyphosate and 2.5 pg of FMOG-AMPA on column with signal to noise ratios of > 5:1 and > 6:1 respectively (Figure 4). The method was linear over the range of 1 – 1000 pg on column for both analytes with correlation coefficients of $r^2 = 0.9999$ for both the entire concentration range and also for the range 1-50 pg (Figure 5).

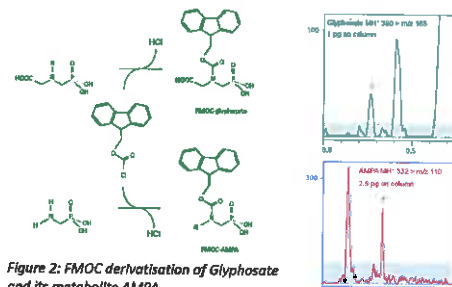


Figure 2: FMOG derivatisation of Glyphosate and its metabolite AMPA

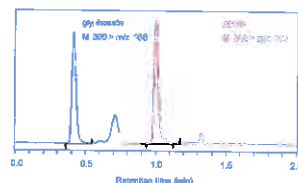


Figure 4 (above): S/N for AMPA and glyphosate. (* peaks from the matrix)

Figure 3 (left): The separation of FMOG derivatives of AMPA and glyphosate.

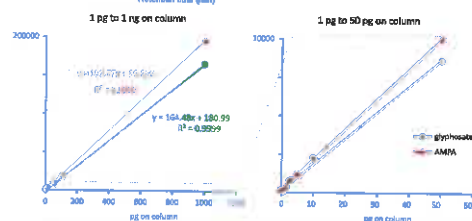


Figure 5: Linearity of the analysis shown over two concentration ranges.

Introduction : Analysis using Nexera-LCMS-8030

Phenoxy and carboxylate herbicides and related compounds are good targets for negative ion ESI. Sensitivity can be hindered by mixed chemistry (e.g. picloram) that supports zwitter-ionic character as well as early elution from reversed-phase columns. We describe the analysis of a range of herbicides by negative ion ESI using the Shimadzu LCMS-8030 ion source.

Method

Herbicide standards were spiked into food samples (grains or fruits), extracted using a Quechers regime and supplied as blind samples for analysis. Chromatography was carried out on a Nexera LC-30-LCMS-8030 (Shimadzu, Kyoto, Japan) equipped with a Shimpack XR-ODS III (75mm x 2.1, 1.7µm particle) column at a flow rate of 0.4mL/min and a mobile phase of 90% 10mM aqueous ammonium acetate in methanol (B). The initial solvent composition was held from 0 to 0.2 min then ramped to 95% solvent B at 10min (hold 2 min). MRM was optimised automatically and parameters (protonated molecular ion, selected fragment ion, Q1 pre-rod bias voltage, optimised collision potential difference and Q3 pre-rod bias) are shown in Table 1. The drying line was 280 °C, N₂ nebulising gas 3L/min, heater block 400 °C, the drying gas at 15L/min and the CID gas was argon at 230 kPa. Injections of 1 to 10 µL equivalent to 1 to 1000 pg on column were made.

	Parent	Fragment	Q1	CE (V)	Q3
1.	oboprolid	190.8	146.0	10	12
2.	picloram	238.8	184.9	25	10
3.	dicamba	218.8	175.0	22	8
4.	fluazifop	252.8	185.0	26	12
5.	bronnopol	275.8	185.9	13	34
6.	MCPA	168.9	141.1	21	14
7.	2,4-D	218.8	181.2	22	14
8.	triflurox	253.8	195.0	12	13
9.	2,4,5-T	252.8	184.8	20	12
10.	fluazifop	328.0	254.0	16	14
11.	quizalofop	342.9	271.0	17	14
12.	haloxyfop	350.9	287.9	17	14
13.	glyphosate	360.0	198.0	19	12
			150.0	19	12
14.	AMPA	332.0	110.0	18	7

Table 1: Negative ion MRM parameters for test compounds.

Discussion and Results

The LCMS-8030 allowed detection of herbicides extracted from food products (Figure 6). Linearity was demonstrable over three orders of magnitude and MRM ion ratios were also stable over this range. Negative ion sensitivity was < 2 pg on column for all compounds studied with signal to noise of 3:1 or greater (Figure 7). Sensitivity was improved by the inclusion of an in-line filter to suppress background contaminants from the water (data not shown). Sensitivity for pure standards was improved primarily because of the absence of matrix interferences.

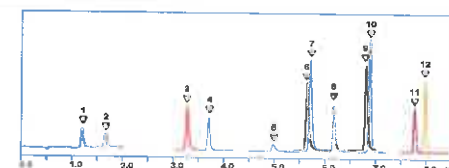


Figure 6: Chromatogram for phenoxy herbicides in food on LCMS-8030.

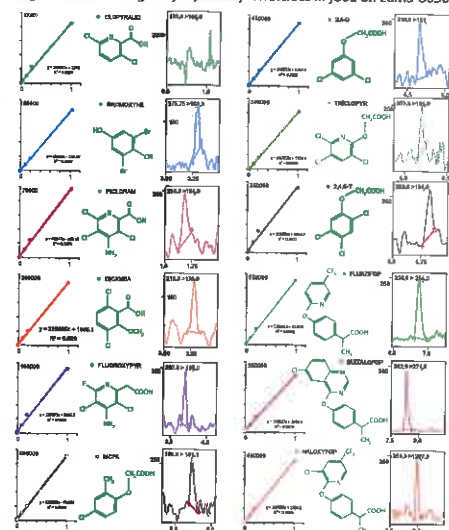


Figure 7: Linearity and uncorrected signal to noise for 1 pg on column.



LCMS-MS Method for Evaluation of PPCPs in Environmental Water

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¹Shimadzu Scientific Instruments, Columbia, MD

Novel Aspects

A rapid five minute method to analyze and quantify multiple pharmaceutical and personal care products in environmental water by LC-MS/MS.

Introduction

Pharmaceutical and personal care products (PPCPs) encompass a family of compounds used by individuals for health and cosmetic purposes, as well as compounds used by the agricultural industry to maintain health of livestock. The number of individuals who are using these products is increasing which has caused an increase in environmental detection. The effect on humans and the environment is still unknown causing the need for more research on the topic. These contaminants are introduced into local waterways via sewage plants and natural disposal (i.e. animal excrement and landfill waste). This poster demonstrates a method for evaluating PPCPs at parts per billion levels in local waterways using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods

Sixteen compounds and one internal standard were analyzed from various drug classes including broad-spectrum antibiotics, antifungals, stimulants, opiates, alkaloids, antihistamines, biguanides, and progestins. Heated Electrospray Ionization (HESI) was used in positive mode to ionize all of the compounds. Each analyte was optimized and separated using a binary gradient with reversed phase chromatography on a Restek Raptor C18 column, in a single 6.5 minute method.

Chromatography Parameters

Column: Raptor C18
Column Temp: 40 °C
Autosampler Temp: 15 °C
Injection Volume: 1 µl
Flow Rate: 0.5 mL/min

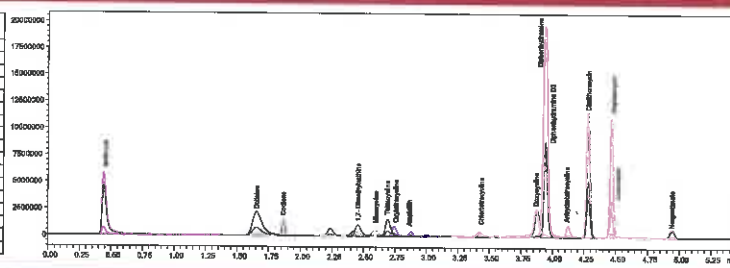
LCMS-8050 Parameters

Nebulizing Gas: 10 L/min
Interface Temp: 350 °C
CI Temp: 100 °C
Heat Block Temp: 150 °C
Drying Gas Flow: 4 L/min



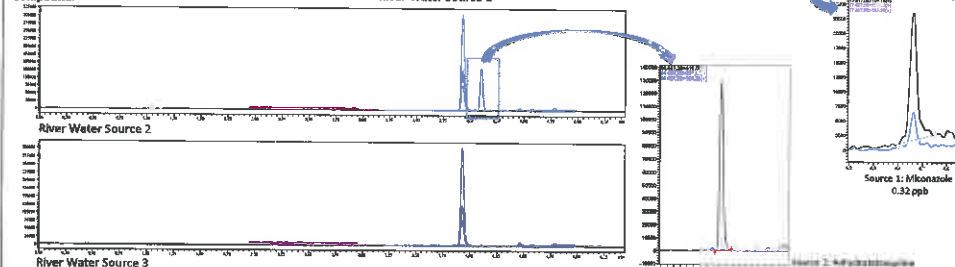
Results - Neat Sample

Name	Ret. Time	Target Transition	Reference Area
Metformin	0.415	132.05>95.03	71.29
Codeine	1.681	177.03>86.15	88.30
Cocaine	1.880	300.20>185.05	185.05
Minocycline	2.621	488.30>441.08	357.08
1,7-Dimethylxanthine	2.628	181.20>124.25	85.30
Tetracycline	2.789	445.10>412.11	427.00
Cloxytetracycline	2.829	481.25>426.05	443.00
Aspirin	2.843	335.15>106.10	162.35
Chloramphenicol	3.649	478.20>444.00	462.00
Doxycycline	3.987	446.25>409.00	419.00
Ciprotetracycline	4.012	354.32>167.20	165.20
Dihydrochloride DS	4.02	293.05>168.20	166.10
Amphotericin B	4.205	427.20>16.00	289.05
Clarithromycin	4.343	748.30>189.28	880.28
Miconazole	4.678	417.20>189.15	389.15
Virginiamycin	4.841	444.30>237.30	394.10
Norgestrel	5.007	376.30>95.18	316.18

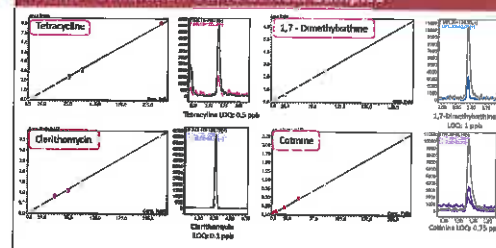


Results - River Water

Samples were obtained from three local water ways and prepared for analysis. Two samples were collected at each location. All of the samples were filtered with a 0.2µm syringe filter and injected. None of the samples contained any of the target analytes except for Sample 1. Miconazole was detected in Sample 1 at a concentration of 0.32 ppb which is below the lower limit of quantitation for this compound.



Results - Calibration Curve of Neat Samples



Name	LOQ (ppb)	Linear Range (ppb)	LOQ (ppb)
Metformin	0.1	0.1-100	2.80
Codeine	0.79	0.79-250	18.79
Cocaine	0.25	0.25-250	13.14
Minocycline	5	5-100	11.84
1,7-Dimethylxanthine	1	1-250	8.82
Tetracycline	0.5	0.5-250	9.81
Chloramphenicol	2.5	2.5-250	11.89
Aspirin	1	1-250	13.18
Dihydrochloride DS	1	1-250	1
Doxycycline	1	1-250	3.64
Chloramphenicol	0.25	0.25-250	3.14
Amphotericin B	5	5-250	4.64
Clarithromycin	0.1	0.1-250	14.3
Miconazole	0.5	0.5-250	27.26
Virginiamycin	0.1	0.1-250	16.86
Norgestrel	5	5-250	6.08

The calibration curve was made up in mobile phase starting conditions and spiked using a stock solution of the analytes.

All of the analytes had a %RSD of less than 20% at the LOQ and all of the limits of quantification were below 5 ppb.

Virginiamycin, Metformin, and Minocycline exhibited source saturation and required a quadratic line of fit. Virginiamycin's linear range is 0.1 - 50 ppb, while Metformin's linear range is 0.1 - 100 ppb, and Minocycline has a linear range of 5 - 100 ppb.

Conclusion

A robust and rapid 6.5 minute method for evaluating pharmaceuticals and personal care products was developed using ultra-fast liquid chromatography mass spectrometry. Most analytes had an lower limit of quantitation at a level between 0.1 ppb and 5 ppb. Miconazole was the only drug detected in the river water samples. The samples may have been too diluted from the excessive amount of rain in the area before sampling occurred. The method can be used to further test other river water samples when there is a more stable water table to test from.

For research purposes only. Not for use in diagnostic procedures.

Disclaimer: Shimadzu LCMS-8050 is Intended for Research Use Only (RUO). Not for Use in diagnostic procedures.

Introduction

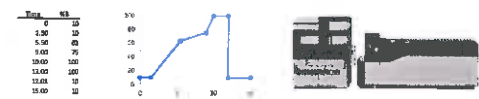
Hops (*Humulus lupulus*) are an essential ingredient in brewing beer but agricultural production of this plant is threatened by insects and other dangers. Pesticides and other chemicals may be used to protect hops and increase yield, but certain chemical residues may be harmful to consumers. Chemical residues may also have unintended negative effects on non-target insects such as honeybees. Sensitive and effective detection of chemical residues in hops is necessary to ensure protection of consumers and the environment. Methods using LC-MS provide efficient and effective detection of chemical residues in a complex sample matrix such as hops. We have developed an LC-MS method for detection of over 150 analytes in hops and carried out a market survey of over 50 different hops pellets samples.



Figure 1 Sample preparation procedure

Method

Test portions of hops pellets (1.5 grams) were homogenized with a SPEX Genogrinder and extracted using a modified QUeCHERS extraction with dispersive SPE cleanup (Restek cat. no. 25237 and 25243). Detection was carried out by LC-MS-MS using a Shimadzu Prominence HPLC with LCMS-8050 triple quadrupole mass spectrometer. Electrospray ionization was used with continuous polarity switching to measure analytes in both modes throughout the run. Pesticide recovery was determined using spiking experiments and matrix-matched calibration curves.



LC: Column	: Reversed ARC-18 (2.1×100 mm, 3 µm)	Interface Temp	: 400 °C
Mobile Phase A	: 5 mM NH ₄ OAC + 0.1% Formic Acid	Nebulizing Gas	: 3 L/min
Mobile Phase B	: Methanol	Drying Gas	: 10 L/min
LC: Flow Rate	: 0.5 mL/min	DI Temp	: 260 °C
Heater Gas	: 10 L/min	Host Block Temp	: 400 °C

Figure 1 Gradient time program (top left), LCMS-8050 (top right) and MS conditions

Results and Discussion

The method was performed with calibration curves and quality control samples in triplicate in three different representative sample matrices to verify recovery, check interferences, and matrix effects. Recovery was in the range of 70–120% for nearly all analytes. Quantitation limits were compound dependent but established at the 20, 50, 200 or 1000 ng/gram dried levels as appropriate. The calibration curve was established over the range of 16 to 4000 ng/gram; dried pellet level.

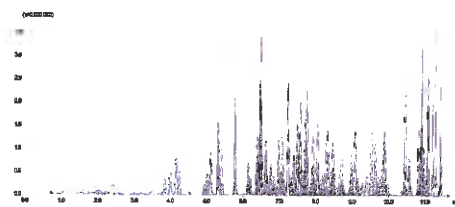


Figure 3 Representative chromatogram

Over fifty hops pellets samples were obtained from various manufacturers and represent growers from major hops-growing regions of the world. Pesticides were detected in most of the samples with the notable exception of Australia and New Zealand. A few pesticides such boscalid were detected in nearly every sample while some pesticides were found only infrequently. Certain pesticides such as acyzotrobin and myclobutanil were found with high frequency in European samples while not detected or infrequently detected in samples from North America.

[illegible]

Table 1 List of analytes measured in the method

[illegible]

Table 2 Pesticides detected in market survey samples. Levels reported in ng/gram hops pellets.

Conclusion

An LCMS method was developed for detection of chemical residues in hops. A market survey was carried out with more than 50 samples representing growers worldwide. Pesticides were efficiently and effectively detected with good sensitivity and selectivity.

Rapid determination of beta-agonist residues in animal tissues using UHPLC-tandem mass spectrometry including mass spectral library searching

David R. Baker¹, Simon Ashton¹, Neil Loftus¹, Simon Hird²

¹Shimadzu, Manchester, UK ²The Food and Environment Agency, York, UK

1. Introduction

- Beta-agonists are a group of veterinary drugs that can be added to animal feed to work as growth promoters due to their ability to increase muscle mass and decrease fat deposition. However, due to their adverse effects to human health, these compounds have been banned for growth promoting purposes in farm animals by the European Union (EU Council Directive 96/22/EC) and other regulatory agencies worldwide.
- To support regulatory compliance a simple and rapid UHPLC-MS/MS method was developed for the reliable quantification of beta-agonists in bovine urine and liver.
- To enhance data quality and reduce false positive reporting the method was optimized to acquire multiple SRM transitions for each compound and mass spectral library searching.

2. Materials and Methods

2.1. Sample preparation

Bovine liver samples were extracted by homogenisation in an aqueous buffer/methanol solution. Bovine liver extracts and urine were subjected to enzyme hydrolysis using β -Glucuronidase and placed in a water bath at 55°C overnight. The next day samples were prepared using mixed-mode SPE and the eluate evaporated to dryness under nitrogen at 40°C. Samples were re-constituted in water/methanol (95:5) prior to UHPLC-MS/MS analysis. Linearity was evaluated by spiking final extracts 0.125 to 2 times the screening target concentration (STC). Repeatability was assessed at low (0.5 x STC) and high (2 x STC) concentrations. Matrix effects were studied at 1 x STC.

Table 1. Calibration curve ranges for each compound.

Compound list	Calibration curve range
Bromobutanol, clenbuterol, hydroxymethylclenbuterol, mabuterol, mepenterol and tulobuterol	0.5 – 800 µg/L (0.0125 – 0.2 µg/kg)
Cimaterol, cimbuterol, clenpenterol, clenpropolol, isoxsuprine	0.125 – 2 µg/L (0.031 – 0.5 µg/kg)
Racetamine, salbutamol and zilpaterol	2.5 – 40 µg/L (0.063 – 1 µg/kg)
Terbutaline	7.5 – 120 µg/L (0.18 – 3 µg/kg)

2.2. UHPLC-MS/MS analysis

UHPLC	Nexera UHPLC system
Flow rate	0.4 mL/min
Mobile phase	A= Water with 10mM ammonium formate and 0.2% formic acid B= Methanol
Gradient	2% B – 100% B (5 min), 100% B (7 min), equilibration (9 min)
Analytical column	Kinetax XB-C18 (2.1 mm x100 mm, 1.7µm)
Column temperature	40°C
Injection volume	10 µL
MS	LCMS-8050 triple quadrupole mass spectrometer, ESI
Gas flows	3 L/min (nebulizing); 10 L/min (heating); 5 L/min (drying)
Source temperatures	Interface 200°C, Block 400°C, Desolvation 200°C

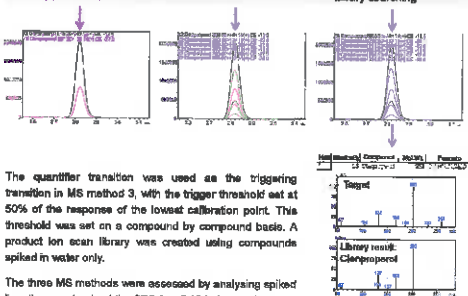
3. Results and discussion

3-1. Application of multiple SRMs and triggered product ion scanning

Conventional multi-residue LC-MS/MS methods typically use two SRM transitions (quantifier and qualifier ion), for example as described in Commission Decision 2002/657/EC for residue analysis in food safety. However, in complex matrices there is the potential for matrix interference to lead to false positive, as has been reported (Schumann et al, Rapid Comm. Mass Spectr. 23 (2009) 1186; Kaufmann et al, Anal. Chim. Acta. 1 (2010) 878). An advantage of high speed MS/MS data acquisitions lies in the ability to support multiple acquisition modes in a single analysis as shown below to reduce false positive reporting.

Table 2 – Overview of three MS methods compared in this study. The SRM chromatograms display the data obtained for clenpropolol in a bovine liver extract.

MS method 1	MS method 2	MS method 3
• 2 SRMs for each compound	• 2 SRMs for each compound	• 2 SRMs for each compound
	• Plus, additional SRMs (between 3-4) for each compound, 83 SRMs in total	• Plus, additional SRMs (between 3-6) for each compound
		• Plus, triggered product ion scans (15,000/scan) with library searching



The quantifier transition was used as the triggering transition in MS method 3, with the trigger threshold set at 50% of the response of the lowest calibration point. This threshold was set on a compound by compound basis. A product ion scan library was created using compounds spiked in water only.

The three MS methods were assessed by analysing spiked liver tissue extracts at the STC for all 16 beta-agonists. Peak area repeatability (n=7) was found to be similar in all three methods, as is listed in Table 3, therefore showing that extra information can be obtained without detriment to the data quality as when using 2 MRMs only. Figure 1 displays an overlay of repeatability injections (n=7) for four compounds (hydroxymethylclenbuterol, cimbuterol, tulobuterol and mabuterol) using the three different MS methods. These chromatograms show that the response, as well as the repeatability, is similar between all methods.

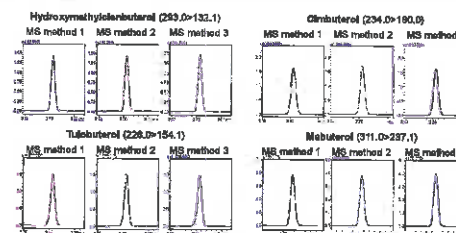


Figure 1 – Overlay of repeatability injections (n=7) for four example compounds using the three different MS methods. Repeatability and response were comparable in all three methods.

Table 3 – Peak area repeatability (%RSD, n=7) comparing the three different MS methods

Compound	MS method 1	MS method 2	MS method 3
Cimaterol	1.9	1.6	1.6
Terbutaline	0.8	0.8	1.4
Zilpaterol	0.9	1.4	0.8
Salbutamol	1.1	1.4	0.9
Clenbuterol	1.9	0.9	1.3
Hydroxymethylclenbuterol	1.1	0.7	1.2
Clenpropolol	0.6	1.9	2.6
Racetamine	1.0	1.8	2.1
Clenbuterol	0.9	1.7	1.8
Tulobuterol	1.3	1.4	1.0
Bromobutanol	2.8	1.7	1.8
Mabuterol	0.5	1.8	2.5
Isoxsuprine	2.7	1.2	0.7
Clenpenterol	1.1	1.3	2.7
Mepenterol	0.8	1.3	1.7

3-3. Final method performance

The LC-MS/MS method was optimized for the routine detection of 16 beta-agonists extracted from biological matrices. The sample cycle time was 9 minutes and all analytes were detected at the screening target concentration (STC). Matrix effects were assessed in bovine liver tissue and urine at the STC for each compound by spiking final extracts and comparing peak areas to those obtained in water standards. Matrix effects were determined to be in the range 21.1 – 75.4 % suppression and were typically higher in liver tissue extracts in comparison to urine extracts. Deuterated internal standards were used for quantification to adjust for matrix effects. Linearity (Linear, 1/x) was found to be excellent for all compounds with typical R² > 0.999. Repeatability was assessed at low (0.5 x STC) and high (2 x STC) concentrations and found to be typically < 3 %RSD. Method performance data is summarised in Table 4.

Table 4 – Final method performance data for studied compounds in bovine liver tissue and urine extracts

Compound	Response low conc	Response high conc	Linearity R ²	Repeatability %RSD	Matrix effect %	Matrix effect %
Cimaterol	2.0	0.8	1.1	0.9999	49.2	1.4
Terbutaline	2.0	2.1	1.1	0.9998	49.2	2.4
Zilpaterol	2.1	1.3	2.3	0.9995	38.3	3.3
Salbutamol	2.1	2.2	2.3	0.9995	39.3	1.2
Cimbuterol	2.3	1.4	1.4	0.9999	38.7	1.2
Hydroxymethylclenbuterol	2.8	2.2	1.8	0.9998	40.9	1.4
Clenpropolol	2.8	3.0	4.1	0.9995	44.0	3.8
Racetamine	2.9	2.6	4.2	0.9997	29.8	1.3
Clenbuterol	3.1	3.9	2.4	0.9994	73.7	2.8
Tulobuterol	3.3	1.7	1.3	0.9995	83.8	2.0
Bromobutanol	3.3	3.0	1.8	0.9988	58.8	0.8
Mabuterol	3.3	3.2	1.2	0.9988	83.8	1.1
Isoxsuprine	3.3	1.8	2.4	0.9993	58.3	1.1
Clenpenterol	3.4	2.3	0.8	0.9985	75.2	1.2
Mepenterol	3.8	2.0	0.8	0.9995	85.9	1.4

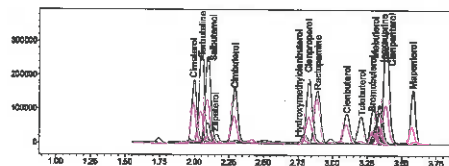


Figure 2 – SRM chromatograms of 16 beta-agonists extracted from bovine liver (0.5 x STC). All compounds were eluted within 3.7 minutes.

4. Conclusion

- In response to regulatory requirements to measure beta-agonists in animal tissue and urine, a simple and rapid LC/MS/MS method using multiple SRM and mass spectral library searching was developed for the reliable detection and quantification of 16 beta-agonists.
- This approach can markedly reduce false positive reporting without compromising sensitivity or reproducibility.
- 15 beta-agonists were detected below the required screening target concentration (STC) with R² > 0.999, low intraday %RSD, no observed carryover, high sensitivity at 0.5 x STC and an analysis cycle time of 9 minutes. Deuterated internal standards were used to compensate for matrix effects.

Ultra-sensitive and rapid assay of neonicotinoids, fipronil and some metabolites in honey by UHPLC-MS/MS.

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1. Introduction

Neonicotinoids are a class of insecticides widely used to protect fields as well as on fruits and vegetables.

Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybee colony collapse disorder. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. Following this the EFSA limited the use of thiamethoxam, clothianidin and imidacloprid. Fipronil, a pesticide from a different chemical class, has been also banned by EFSA for maize seed treatment due to its high risk for honeybee health.

In order to better understand the effect of these compounds on bees and their contamination in pollen and honey, a highly sensitive assay method was necessary.

2. Methods and Materials

Standards and Reagents

All analytical standards were provided by Sigma-Aldrich. Acetamiprid, clothianidin, thiamethoxam, imidacloprid, thiacloprid, dinotofuran, nitenpyram and fipronil were selected as target compounds. Acetamiprid-N-desmethyl and fipronil sulfone were selected as metabolites (see figure 1). Thiamethoxam-d3, imidacloprid-d4 and clothianidin-d3 were used as internal standards.

Solvents (including water and mobile phase additives) were of ULC/MS quality (Biosolve).

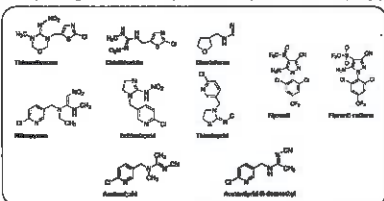


Figure 1 Structure of the target compounds

Sample Preparation

Compound extraction was performed using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with an additional dispersive Solid Phase Extraction (dSPE) step.

5 g of honey (±1%) were weighted in a 50 mL polypropylene tube. 5 µL of internal standard solution at 5 µg/mL of each compound in acetonitrile was added on honey and let dry for 10 minutes. 10 mL of ultra pure water were added and the samples were homogenized by vortex mixing for 1 minute. 10 mL of acetonitrile were then added followed by vortex mixing for 1 minute.

Salts mix (4g MgSO₄, 1g Sodium Citrate, 0.5g Sodium Citrate sesquihydrate, 1g NaCl; Biotage Q0020-15V) were added. After manual shaking, samples were centrifuged at 3000g for 5 minutes at 10°C.

Supernatant (8 mL) was transferred into a 15 mL tube containing 1200 mg of MgSO₄, 400 mg PSA and 400 mg C18 (Biotage Q0050-15V). After centrifugation at 3000 g and 10°C for 5 minutes the supernatant was transferred into inert glass vial for analysis (Shimadzu LabTotal 227-34001-01).

UHPLC-MS/MS Conditions

Analysis were performed using a Nexera X2 UHPLC system coupled with LCMS-8080 with Heated ESI in positive ionization.

Mobile phase composition was optimized to generate the highest sensitivity. Ion source parameters (gas flows, temperatures) were also optimized using the Interface Setting Support Software (Shimadzu Corp.). All parameters are shown in tables 1 and 2.

Table 1 UHPLC conditions

System	Shimadzu Nexera X2
Column	ACE Super C18 100 x 2.1 mm 2 µm
Column temperature	30°C
Mobile phases	A: Water + 0.05% ammoniac B: Methanol + 0.05% ammoniac
Flow rate	0.6 mL/min
Gradient	5% B to 100% B in 3 min. 100% B to 5% B in 1 min
Injection volume	2 µL (PCHS mode with 10 µL of vial)

Table 2 MS/MS conditions

System	Shimadzu LCMS-8080					
Ionization mode	Positive and negative ESI (polarity switching 5 msec)					
Acquisition mode	MRM					
MRM transitions	Name	Polarity	MRM Q1st	MRM Q2nd	MRM Q3rd	STD Group
	Acetamiprid	+	223.1 > 128.0	223.1 > 58.1	2	
	Acetamiprid-N-desmethyl	+	209.1 > 128.0	211.1 > 128.0	2	
	Clothianidin	+	250.1 > 169.1	250.1 > 122.0	2	
	Dinotofuran	+	203.0 > 114.0	203.0 > 87.0	2	
	Fipronil	+	435.0 > 322.0	435.0 > 260.0	3	
	Fipronil sulfone	+	451.0 > 338.0	451.0 > 262.0	3	
	Imidacloprid	+	256.1 > 175.1	256.1 > 211.1	2	
	Nitenpyram	+	271.0 > 128.0	271.0 > 225.0	3	
	Thiacloprid	+	283.1 > 125	283.1 > 95.1	1	
	Thiamethoxam	+	282.1 > 211.1	282.1 > 181.1	1	
	Thiamethoxam-d3	+	285.1 > 214.0	—	—	
	Imidacloprid-d4	+	280.1 > 178.1	—	—	
	Clothianidin-d3	+	253.1 > 122.0	—	—	

Dwell time 5 to 8 s (max) depending upon the number of compound injections to ensure to have at least 30 points per peak (min total loop time < 45 min)

Pause time 1 msec

Quadrupole resolution Q1: Unit Q2: Unit

Temperature HESI: 400°C DL: 100°C Heater Block: 400°C

Gas flow Interface: 10 L/min Nebulizer: 3 L/min Drying: 5 L/min

3. Results

Calibration

Calibration curves were prepared in acetonitrile to obtain final concentrations ranging from 0.5 µg/mL (1 µg on column) to 5 ng/mL. These concentrations corresponds to 1 ppt and 10 ppb in honey, respectively. For each compound, the lower limit of quantification was selected to give an accuracy between 80-120% (see table 3). A typical calibration curve is shown in figure 2.

Table 3 Limits of quantification

Compound	LOQ (µg/mL)	Accuracy (%)	Compound	LOQ (µg/mL)	Accuracy (%)
Acetamiprid	0.005 / 10	—	Fipronil sulfone	0.001 / 2	—
Acetamiprid-N-desmethyl	0.005 / 10	—	Imidacloprid	0.020 / 40	—
Clothianidin	0.020 / 10	—	Nitenpyram	0.020 / 40	—
Dinotofuran	0.010 / 10	—	Thiacloprid	0.005 / 10	—
Fipronil	0.001 / 2	—	Thiamethoxam	0.005 / 10	—

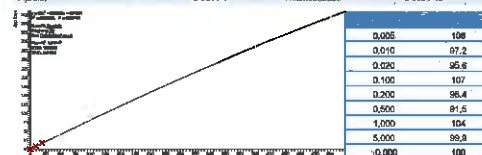


Figure 2 Calibration curve of Acetamiprid

Recovery

An "all-flowers" honey from the local supermarket was extracted with or without spike at 50 ppt. A blank extract (no honey) was prepared to evaluate losses or non specific interactions. Results are presented in Table 4.

No loss due to non specific interactions was observed. Calculated recoveries are within acceptance values 70-120% from EU SANTE/11945/2015.

Table 4 Recovery results

Compound	101%	79.8%	Compound	101%	74.2%
Acetamiprid	101%	79.8%	Fipronil sulfone	100%	83.2%
Acetamiprid-N-desmethyl	100%	83.4%	Imidacloprid	100%	83.2%
Clothianidin	98.5%	78.6%	Nitenpyram	102%	87.0%
Dinotofuran	100%	78.6%	Thiacloprid	99.2%	82.2%
Fipronil	98.0%	78.1%	Thiamethoxam	100%	75.6%

Real Sample Analysis

Nine honey samples purchased at the local supermarket were assayed as unknowns. Thanks to the very high sensitivity reached, even low concentrations of neonicotinoids were quantified. Results are presented in table 5.

Table 5 Honey sample results (concentrations in µg/kg)

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Acetamiprid	0.15	0.17	0.15	0.15	0.15
Acetamiprid-N-desmethyl	0.38	0.45	0.45	0.45	0.45
Clothianidin	0.27	0.47	0.47	0.47	0.47
Dinotofuran	1.7	1.5	1.5	1.5	1.5
Fipronil	1.2	1.2	1.2	1.2	1.2
Imidacloprid	0.14	0.05	0.05	0.05	0.05
Nitenpyram	0.34	0.11	0.11	0.11	0.11
Thiacloprid	0.052	0.005	0.005	0.005	0.005
Thiamethoxam	0.040	0.015	0.015	0.015	0.015
Thiamethoxam-d3	0.032	0.015	0.015	0.015	0.015
Imidacloprid-d4	0.028	0.016	0.016	0.016	0.016
Clothianidin-d3	0.008	0.008	0.008	0.008	0.008

Chromatograms

Calibration standard at the lowest limit of quantification (see table 3 for concentration values).

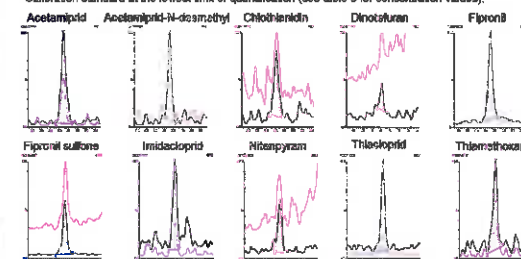


Figure 3 Standard chromatogram at the LOQ

4. Conclusions

A method for ultra sensitive assay of neonicotinoids in honey was set up. The sample preparation was simple but provided excellent recoveries, whatever is the honey type. The injection mode used prevented the use of tedious evaporation/reconstitution or dilution steps. The sensitivity obtained enabled assay in real samples at very low levels far under the regulated residue levels. This method can be a very efficient support tool to better understand the impact of neonicotinoids on honey bee colonies.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

Metabolomic profiling of beer types by widely-targeted LC/MS measurement

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1. Overview

Sensory tests have generally been performed on the flavor and taste of foods as an approach to quantitatively evaluate food quality. Recently, metabolome technique is also expected to reveal the components contributing significantly to food flavor and taste. Here, we report simultaneous analysis of metabolites for five types of beer by a triple quadrupole mass spectrometer followed by statistical analysis on the acquired data. As a result, five types of beer were categorized by the characteristic components.

2. Introduction

Metabolomics analysis in food science plays a key role for quantitative characterization of food quality. Particularly, it is expected that the development of value-added food production will be boosted thanks to the aid of food metabolomics technology. In this experiment, comprehensive analysis for 97 compounds was performed by a LC-MS/MS to classify five types of beer (two lager beers, an ale type beer, a beer-taste alcoholic beverage and a beer-taste non-alcoholic beverage). Targeted compounds in this analysis are amino acids, organic acids and nucleic acid-related compounds which are known to be the components closely relevant to the sweetness, the bitterness and the flavor in the food. The resulting data from LC-MS/MS analysis were evaluated by statistical analysis (PCA and HCA).

3. Methods

An aliquot of degassed-beer (0.2 mL) was transferred into 1.5 mL tube and then two internal standards were added so that the final concentration was 20 µM. After shaking for 30 sec, 0.2 mL solution was transferred into a centrifugal filter (MWCO 3kDa). Samples were centrifuged and then the filtrates were recovered. After shaking for 30 sec, the filtrate was diluted 200 folds in purified water. Diluted sample solution was measured by LC-MS/MS instrument including a Nexera UHPLC system and a LCMS-8060 triple quadrupole mass spectrometer (Shimadzu Corp.). For statistical analysis (principal component analysis and hierarchical clustering analysis), Traversal MS software (Reifycs Inc.) was used to analyze the MRM-based chromatographic data.

UHPLC conditions (Nexera system)

Column: Discovery HS F5 150 mm × 2.1 mm, 3.0 µm
Mobile phase A: 0.1% Formate/water, B: 0.1% Formate/acetonitrile
Flow rate: 0.25 mL/min
Time program: B conc. 0% (0–2.0 min)–25% (5.0 min)–35% (11.0 min)–95% (15.0–20.0 min)–0% (20.1–25.0 min)
Injection vol.: 3 µL
Column temperature: 40°C

MS conditions (LCMS-8060)

Ionization: Positive/Negative, MRM mode
DL Temp.: 250°C HB Temp.: 400°C Interface Temp.: 400°C
Drying Gas: 10 L/min Nebulizing Gas: 2.0 L/min Heating Gas: 10 L/min

4. Results

4-1. Simultaneous analysis of primary metabolites in five types of beer

Comprehensive analysis for 97 compounds (m/z) was performed to find out the different components between five types of beer (two lager beers, an ale type beer, a beer-taste alcoholic beverage and a beer-taste non-alcoholic beverage). Here, pentafluorophenylpropyl (PFPP) column was used to separate metabolites. The 58 peaks including amino acids, organic acids and nucleic acid-related compounds were detected in this experiment. Figure 1 shows the typical MRM chromatogram of three beers. As shown in the MRM chromatograms, the results depend from the type of beers and indicate the difference of the components in each beer. For example, Ile, Leu and Phe are abundant in beer-taste non-alcoholic beverage. The difference is observed in peaks of Arg, Phe and Trp between lager beer A and beer-taste alcoholic beverage.

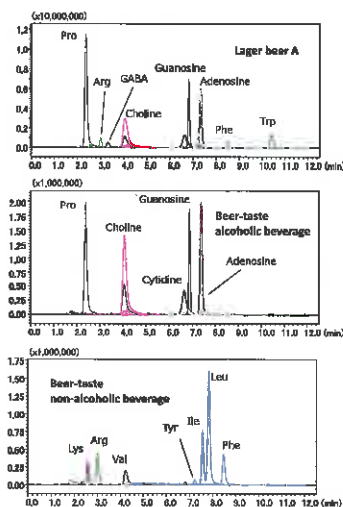


Figure 1 MRM chromatogram of three types of beer

4-2. Principal component analysis for 58 metabolites in five types of beer

Figure 2 shows multiple chromatograms displayed by MRM outlook function of Traversal MS software. This function enables to display the chromatograms for a single component from multiple samples side-by-side. First single reference data is loaded by Traversal MS software. Then peak identification for this data is automatically carried out by the characteristic algorithm. The parameter (ex. R.T., peak shape and reference ion etc.) used to identify the target peaks is applied to all data. If necessary, manual peak identification is also performed for some metabolite peaks. In this step, the MRM outlook function helps the operator to confirm peak identification from all data. Thus Traversal MS software identifies the peaks for the target components and calculates area or area ratio for IS. Then principal component analysis (PCA) is performed by using the area ratio calculated by Traversal MS software. As a result of PCA, five beers were successfully classified by the difference of components on the score plot. Figure 2 shows the result of score plot and loading plot. Furthermore, we could confirm some amino acids (proline and branched-chain amino acids) and nucleosides as components to characterize each beer on the loading plot.

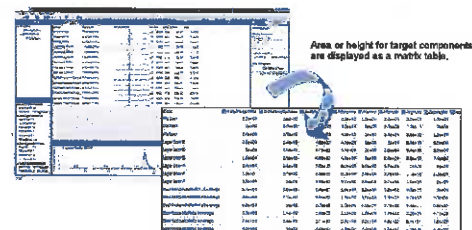


Figure 2 Multiple chromatograms displayed by MRM outlook function of Traversal MS

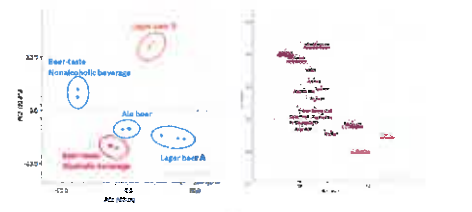


Figure 2 Principal component analysis for the detected 58 metabolites

4-3. Hierarchical clustering analysis for 58 metabolites in five types of beer

Finally we performed hierarchical clustering analysis (HCA) to evaluate the similarity between five types of beer. In this experiment, statistical analysis was carried out by using area ratio of 58 metabolites. Figure 4 shows the result of HCA. The result indicates that five types of beer were roughly classified into three groups. One was a group containing lager beer A and ale beer, second was lager beer B and finally the other was a group containing beer-taste alcoholic and non-alcoholic beverages. Furthermore, the components contributing to the characteristic of each beer were corresponding to the result of PCA (ex. proline, isoleucine, leucine, adenosine and guanosine).

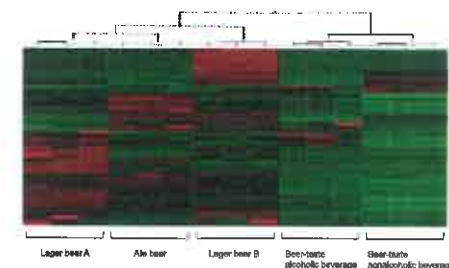


Figure 4 Hierarchical clustering analysis for five types of beer

In this experiment, we simultaneously analyzed 97 metabolites in five types of beer by LC-MS/MS and performed PCA and HCA for the detected 58 components in the beer. We could also classify five types of beer by either using PCA or HCA. Furthermore, we could detect some metabolites contributing to the characteristic of each beer. In the future, by combination with sensory test, correlation of metabolites with a flavor and a taste could be evaluated more in detail. Thus a combination of simultaneous analysis by LC-MS/MS with a statistical analysis could become a powerful tool to find out the components that greatly affects a taste in the food and to evaluate the quality of food.

4. Conclusions

- Primary metabolites (58 components) were detected by simultaneous analysis for five types of beer using pentafluorophenylpropyl column.
- Principal component analysis (PCA) for 58 metabolites successfully categorized five types of beer and highlighted some characteristic components.
- Hierarchical clustering analysis (HCA) for 58 components successfully classified five types of beer and determined the similarity between five beers.

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Simon Ashton¹, David Baker¹, Laetitia Fages¹, Eric Capodanno², Neil Loftus¹¹Shimadzu Corporation, Manchester, UK; ²Phytocontrol, Nîmes, France

1. Introduction

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food. National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRLs) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This paper describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (typically 3 MRM transitions for each targeted pesticide resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

2. Materials and Methods

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of LCMS quality purchased from Sigma-Aldrich. A six point calibration curve from 0.002 – 0.1 mg/kg (2 – 100 µg/L) were generated using an internal standard method. Two internal standards (Atrazine-d5 and Duron-d6) were spiked in during the auto-sampler sequence for quantitation.

LC/MS/MS parameters	LCMS-8060	LCMS-8060
UPLC	Niterra (LC-4000)	LCMS-8060
Analytical column	Reversed Phase (150 x 2.1 mm, 1.7 µm)	Reversed Phase (150 x 2.1 mm, 1.7 µm)
Column temperature	30°C	30°C
Flow rate	0.4 mL/min	0.4 mL/min
Solvent A	5 mMol/L ammonium formate (pH 0.025) (formic acid solution)	5 mMol/L ammonium formate (pH 0.025) (formic acid solution)
Solvent B	2 mMol/L ammonium formate (pH 0.025) (formic acid solution)	2 mMol/L ammonium formate (pH 0.025) (formic acid solution)
Binary Gradient	Time (min)	Time (min)
	0	3
	1	10
	5	55
	10.5	100
	12	100
	15.01	2
	15	Stop
Injection volume	2 µL sample (plus 40 µL water)	2 µL sample (plus 40 µL water)

Table 1. LC and MS/MS acquisition parameters used to create the generic LC-MS/MS method.

3. Results

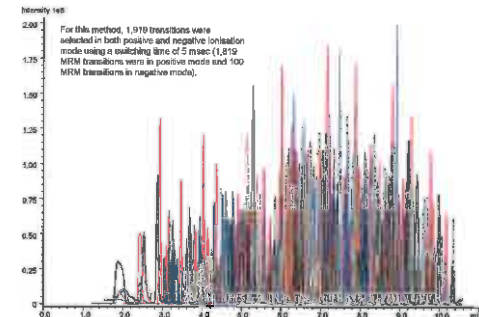


Figure 1. Chromatogram of 646 pesticides spiked into a mint extract at 0.010 mg/kg. (Up to 3 MRMs per compound and 5 msec polarity switching time).

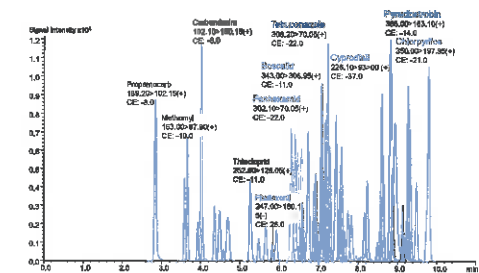


Figure 2. MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyrifos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4% of the samples analyzed. The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

3.1 High data density acquisitions

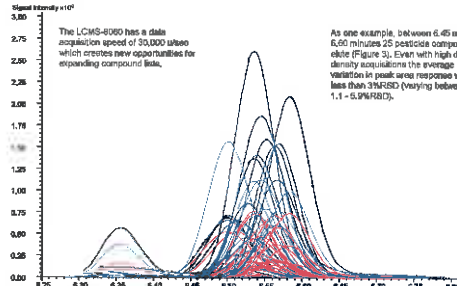


Figure 3. The LCMS-8060 can acquire MRM data at a high speed and enables precise quantitation even with high data density. Between 6.45 and 6.80 minutes 25 compounds were monitored (Table 2).

Compound	RT (min)	MRM1	MRM2	MRM3	MRM4	MRM5
Trifluoromethyl	6.45	312.00	116.15	8.46	8.1	
Propiconazole	6.46	312.00	116.15	8.46	8.2	
Diflufenican	6.47	312.00	116.15	8.47	8.2	
Flutolanil	6.47	312.00	116.15	8.47	8.2	
Trifluoromethyl	6.48	312.00	116.15	8.48	8.2	
Acetochlor	6.49	312.00	116.15	8.49	8.2	
Tebuconazole	6.50	312.00	116.15	8.50	8.2	
Trifluoromethyl	6.51	312.00	116.15	8.51	8.2	
Propiconazole	6.52	312.00	116.15	8.52	8.2	
Diflufenican	6.53	312.00	116.15	8.53	8.2	
Flutolanil	6.54	312.00	116.15	8.54	8.2	
Trifluoromethyl	6.55	312.00	116.15	8.55	8.2	
Acetochlor	6.56	312.00	116.15	8.56	8.2	
Tebuconazole	6.57	312.00	116.15	8.57	8.2	
Trifluoromethyl	6.58	312.00	116.15	8.58	8.2	
Propiconazole	6.59	312.00	116.15	8.59	8.2	
Diflufenican	6.60	312.00	116.15	8.60	8.2	
Flutolanil	6.61	312.00	116.15	8.61	8.2	
Trifluoromethyl	6.62	312.00	116.15	8.62	8.2	
Acetochlor	6.63	312.00	116.15	8.63	8.2	
Tebuconazole	6.64	312.00	116.15	8.64	8.2	
Trifluoromethyl	6.65	312.00	116.15	8.65	8.2	
Propiconazole	6.66	312.00	116.15	8.66	8.2	
Diflufenican	6.67	312.00	116.15	8.67	8.2	
Flutolanil	6.68	312.00	116.15	8.68	8.2	
Trifluoromethyl	6.69	312.00	116.15	8.69	8.2	
Acetochlor	6.70	312.00	116.15	8.70	8.2	
Tebuconazole	6.71	312.00	116.15	8.71	8.2	
Trifluoromethyl	6.72	312.00	116.15	8.72	8.2	
Propiconazole	6.73	312.00	116.15	8.73	8.2	
Diflufenican	6.74	312.00	116.15	8.74	8.2	
Flutolanil	6.75	312.00	116.15	8.75	8.2	
Trifluoromethyl	6.76	312.00	116.15	8.76	8.2	
Acetochlor	6.77	312.00	116.15	8.77	8.2	
Tebuconazole	6.78	312.00	116.15	8.78	8.2	
Trifluoromethyl	6.79	312.00	116.15	8.79	8.2	
Propiconazole	6.80	312.00	116.15	8.80	8.2	
Diflufenican	6.81	312.00	116.15	8.81	8.2	
Flutolanil	6.82	312.00	116.15	8.82	8.2	
Trifluoromethyl	6.83	312.00	116.15	8.83	8.2	
Acetochlor	6.84	312.00	116.15	8.84	8.2	
Tebuconazole	6.85	312.00	116.15	8.85	8.2	
Trifluoromethyl	6.86	312.00	116.15	8.86	8.2	
Propiconazole	6.87	312.00	116.15	8.87	8.2	
Diflufenican	6.88	312.00	116.15	8.88	8.2	
Flutolanil	6.89	312.00	116.15	8.89	8.2	
Trifluoromethyl	6.90	312.00	116.15	8.90	8.2	
Acetochlor	6.91	312.00	116.15	8.91	8.2	
Tebuconazole	6.92	312.00	116.15	8.92	8.2	
Trifluoromethyl	6.93	312.00	116.15	8.93	8.2	
Propiconazole	6.94	312.00	116.15	8.94	8.2	
Diflufenican	6.95	312.00	116.15	8.95	8.2	
Flutolanil	6.96	312.00	116.15	8.96	8.2	
Trifluoromethyl	6.97	312.00	116.15	8.97	8.2	
Acetochlor	6.98	312.00	116.15	8.98	8.2	
Tebuconazole	6.99	312.00	116.15	8.99	8.2	
Trifluoromethyl	7.00	312.00	116.15	9.00	8.2	

Table 2. The LCMS-8060 can acquire MRM data at a high speed and enables precise quantitation even with high data density. Between 6.45 and 6.80 minutes 25 compounds were monitored with a peak area validation less than 5% (MRSD; n=6). (The sample is a mint matrix extract at the reporting limit of 0.01 mg/kg).

3-2. Robustness

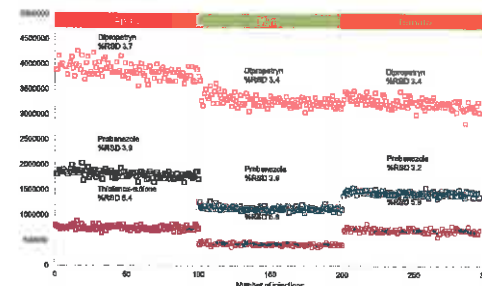


Figure 4. Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Figure 3, compounds were selected to reflect peak area response throughout the chromatographic run (Table 2).

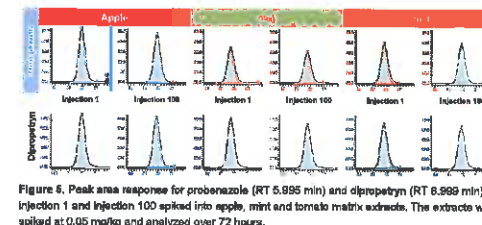


Figure 5. Peak area response for propiconazole (RT 5.995 min) and dipropetryn (RT 6.999 min) for Injection 1 and Injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.

4. Conclusions

- A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.
- As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.
- The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120%.

Simultaneous analysis of 23 mycotoxins using ultra-high speed LC-MS/MS with a Pentafluorophenyl column

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1. Introduction

Mycotoxins may contaminate food and feed and are known to give serious damages to health of human and animals. For food safety, manufacturers should monitor their products to avoid contamination based on analytical data. Several mycotoxins are regulated in US, EU and JP. The number of mycotoxins regulated continued to increase yearly. Current mycotoxin monitoring methodologies tend to focus on a specific mycotoxin, so it takes a long time to monitor various mycotoxins. In our previous study, we have developed the simultaneous analysis of 14 mycotoxins. This time, we tried to enhance the number of analytical mycotoxins (23 mycotoxins). As carryover of fumonisins (FMs) was observed in the high sensitivity analysis, the minimization of carryover also was attempted.

2. Materials and Methods

2-1. System

LC-MS/MS analysis was performed using a Nexera X2 UHPLC system coupled to an LCMS-8050 tandem quadrupole mass spectrometer, for Nexera X2, Nexera autosampler was equipped with multiple rinse modes with three rinse solvents.



Fig.1 LCMS-8050 triple quadrupole mass spectrometer

Ultra Fast Mass Spectrometer
• Polarity Switching: 5msac
• Scanning Speed: Max. 30000/scan

2-2. Sample and MS condition

Steridized of 23 mycotoxins (nivalenol, deoxynivalenol, 15-Acetyldeoxynivalenol, 3-Acetyldeoxynivalenol, diacetoxyscopolol, neosololol, fumonisin-K, 5 aflatoxins, T-2 toxin, HT-2 toxin, zearalenone, 2 zearalenol, 2 zearalenol, 3 fumonisins and ochratoxin A) were optimized on each compound-dependent parameter and MRM transition (Q1/Q3). Samples were applied to MS/MS with electrospray ion source, and then 15 mycotoxins were detected in positive mode while 8 mycotoxins were detected in negative mode. MRM transitions are showed in Table 1.

Table 1 MRM Transition of 23 mycotoxins

Mycotoxin	Isotope	MRM transition
15-Acetyldeoxynivalenol (15-ADON)	+	228.100-261.05
3-Acetyldeoxynivalenol (3-ADON)	+	228.100-261.05
Fumonisin B ₁ (FB1)	+	228.100-261.05
Fumonisin B ₂ (FB2)	+	228.100-261.05
Fumonisin B ₃ (FB3)	+	228.100-261.05
Fumonisin K (FK)	+	228.100-261.05
Neosololol (NEO)	+	228.100-261.05
Diacetoxyscopolol (DAS)	+	228.100-261.05
T-2 toxin (T-2)	+	228.100-261.05
HT-2 toxin (HT-2)	+	228.100-261.05
Zearalenone (ZEN)	+	228.100-261.05
2-Zearalenol (2-ZEN)	+	228.100-261.05
3-Zearalenol (3-ZEN)	+	228.100-261.05
Ochratoxin A (OTA)	+	228.100-261.05
Aflatoxin B ₁ (AFB1)	+	228.100-261.05
Aflatoxin B ₂ (AFB2)	+	228.100-261.05
Aflatoxin G ₁ (AFG1)	+	228.100-261.05
Aflatoxin G ₂ (AFG2)	+	228.100-261.05

2-3. Analytical Column

An analytical column, metal-free analytical column "Mastro"™ (Fig.2) were used. It is expected to inhibit adsorption of fumonisins onto metal ion of column body. Separation of 3-ADON and 15-ADON was examined using two Mastro Column, octadecylsilyl column "Mastro C18" and pentafluorophenyl column "Mastro PFP". As it is said that pentafluorophenyl column has unique separation selectivity by an electrostatic interaction between pentafluorophenyl group and target compound, Mastro PFP was used.



Fig.2 Metal-free column (Mastro™ Series)

2-4. Autosampler Rinse Condition

In order to eliminate carryover, both inner and outer needle were washed with multi rinse solution using autosampler SIL-30AC (Fig.3). As rinse solution, (1) 1% formic acid aq. / methanol / acetonitrile / iso-propanol = 1/1/1/1 and (2) 10mM sodium citrate aq. were used. It was thought that 1% formic acid aq. / methanol / acetonitrile / iso-propanol = 1/1/1/1 was able to effectively rinse compounds adsorbed with hydrophobic interaction. The other hand, it was expected that 10mM sodium citrate aq. could restrain adsorption of fumonisins onto metal ion of flow path.

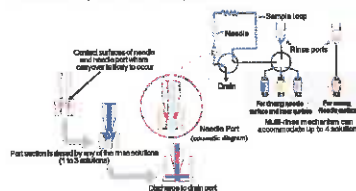


Fig.3 HPLC washing path inside SIL-30AC

3. Result

3-1. Optimization of LC-MS/MS condition

3-1-1. Separation of 3-ADON and 15-ADON

3-Acetyldeoxynivalenol (3-ADON) and 15-Acetyldeoxynivalenol (15-ADON) are regioisomer, and MRM transition is same. Therefore these must be separated by chromatography. It was known that sufficient separation of 3-ADON and 15-ADON in short time was difficult using typical ODS column. So separation of 3-ADON and 15-ADON was compared using ODS column "Mastro C18" and Pentafluorophenyl (PFP) column "Mastro PFP".



Fig.4 Structure of 3-ADON and 15-ADON

3-ADON and 15-ADON were not separated with ODS column. On the other hand, 3-ADON and 15-ADON could be completely separated with PFP column. There could be electrostatic interaction between fluorine atoms of PFP column and the hydroxyl group of ADON. It seems that differences in the hydroxyl group position was related to the separation of 3-ADON and 15-ADON.



Fig.5 Comparison of MRM chromatogram (3-ADON, 15-ADON)

3-1-2. Minimization carryover of Fumonisin

In our previous study, it was found that fumonisins (FMs) could be adsorbed with metal ion of analytical flow path and considerable carry of FMs (about 7%) was observed using a general rinse condition which involved an outer needle rinse using water/acetonitrile (1/1, v/v) as a rinse solvent¹.

It was assumed that FMs formed complexes with trace metals in the sample flow path when dispersed carboxyl groups in FMs with the metal ions were present (Fig.6).

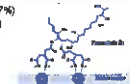


Fig.6 Possible coordination interaction with metal ions

Therefore, carryover of FMs was evaluated using our modified rinse method and a metal-free analytical column, Mastro PFP. First, the standard sample of 1000 µg/L fumonisins was analyzed, and then one blank was analyzed. As a result, carryover was reduced to less than 1 µg/L, by the needle's inner and outer rinses with 1% formic acid in water / methanol / acetonitrile / iso-propanol (1/1/1/1, v/v/v/v) and 10 mM sodium citrate aqueous solution. The metal-free column also contributed the elimination of carryover.

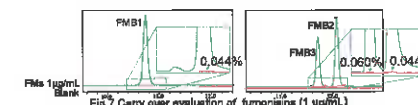


Fig.7 Carry over evaluation of fumonisins (1 µg/mL)

3-1-3. Simultaneous analysis using polarity switching

The separation of 23 mycotoxins is examined using a 10mM aqueous solution of ammonium acetate and a 2% acetic acid solution in methanol as mobile phase. 23 mycotoxins were successfully separated and analyzed within 13 minutes without carryover. Ultra fast polarity switching (series) enabled simultaneous measurement of the compounds in both polarity modes. The dilution series of these compounds were analyzed. Good linearity was observed in the calibration curves, and excellent sensitivity was achieved. (Table 2).

HPLC conditions (Nexera)
Column: Mastro PFP 100mm x 2.1mm, 3 µm
Mobile phase A: 10mM Ammonium acetate - Water B: 2% Acetic acid - Methanol
Flow rate: 0.4 mL/min (Gradient analysis)
Column temperature: 40 °C
Needle rinse
R1: 10mM Ammonium acetate aq. R2: 10mM Sodium citrate aq.
R3: 1% Formic acid aq. / Methanol / Acetonitrile / Iso-propanol = 1/1/1/1
Needle rinse program:
Inner needle rinse: R1 - R2 - R3 - R2 - R3
Outer needle rinse: R3 - R2

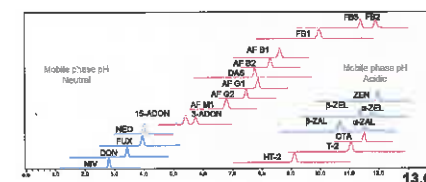


Fig.8 MRM chromatograms of 23 mycotoxins (10ng/mL)

Table 2 Linearity of 23 mycotoxins

Mycotoxin	Range (ng/mL)	Coefficient (R ²)	Mycotoxin	Range (ng/mL)	Coefficient (R ²)
NEV	0.1-1000	0.999	DAS	0.01-100	0.998
FK	0.1-1000	0.999	PM B1	0.1-1000	0.999
DOH	0.1-1000	0.999	PM B2	0.1-1000	0.999
3-ADON	0.1-1000	0.999	PM B3	0.1-1000	0.999
FK	0.1-1000	0.999	PM B4	0.1-1000	0.999
FK	0.1-1000	0.999	PM B5	0.1-1000	0.999
FK	0.1-1000	0.999	PM B6	0.1-1000	0.999
FK	0.1-1000	0.999	PM B7	0.1-1000	0.999
FK	0.1-1000	0.999	PM B8	0.1-1000	0.999
FK	0.1-1000	0.999	PM B9	0.1-1000	0.999
FK	0.1-1000	0.999	PM B10	0.1-1000	0.999
FK	0.1-1000	0.999	PM B11	0.1-1000	0.999
FK	0.1-1000	0.999	PM B12	0.1-1000	0.999
FK	0.1-1000	0.999	PM B13	0.1-1000	0.999
FK	0.1-1000	0.999	PM B14	0.1-1000	0.999
FK	0.1-1000	0.999	PM B15	0.1-1000	0.999
FK	0.1-1000	0.999	PM B16	0.1-1000	0.999
FK	0.1-1000	0.999	PM B17	0.1-1000	0.999
FK	0.1-1000	0.999	PM B18	0.1-1000	0.999
FK	0.1-1000	0.999	PM B19	0.1-1000	0.999
FK	0.1-1000	0.999	PM B20	0.1-1000	0.999
FK	0.1-1000	0.999	PM B21	0.1-1000	0.999
FK	0.1-1000	0.999	PM B22	0.1-1000	0.999
FK	0.1-1000	0.999	PM B23	0.1-1000	0.999

3-2. Analysis of Corn sample

This method was applied to the routine analysis of corn sample. Sample was prepared using a QuEChERS kit for extraction and a multistage cartridge for purification. Spiked corn sample (100ng/mL) could be detected with high sensitivity and good repeatability.

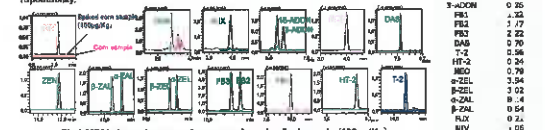


Fig.9 MRM chromatograms of corn sample and spiked sample (100ng/kg)

4. Conclusions

- Simultaneous analysis method of 23 mycotoxins was developed using a Pentafluorophenyl column. 3-ADON and 15-ADON could be completely separated with PFP column.
- The carryover of fumonisins could be reduced under the LOQs (1 µg/L) by using citrate in water and acidic organic solvent as needle rinse solvents and a metal-free column.

5. References

- M. Tanura, N. Mochizuki et al., J. Sep. Sci., Vol.37(13), 1552-1560(2014)
- N. Mochizuki, CHROMATOGRAPHY, Vol.53(5), 107-118(2012)
- M. Tanura, N. Mochizuki, K. Hayakawa et al., Toxins Vol.7, 1084-1092(2015)

Acknowledgement

We wish to express our sincere gratitude to Messyoshi Tanura (Asahi group Holdings) for his cooperation.

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Determination of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra High Performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry

ASMS 2013 TP-742

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Determination of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra High Performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry

1. Introduction

This poster describes a method by ultra high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) to analyze 4 derivatives of nitrofuran metabolites in marine products. Thanks for the high sensitivity and selectivity of UHPLC/MS/MS method, determination of these compounds was successful. The limit of quantitation (LOQ) of AMOZ was 0.4 µg/kg, which is lower than the guideline value (0.5 µg/kg)

proposed by the USDA.

The derivatives of nitrofuran metabolites used in breeding of marine products are known to have a carcinogenic effect. Quantities of these compounds in marine products were regulated by the USDA. This paper describes a UHPLC/ESI-MS/MS method to determine 4 derivatives of nitrofuran metabolites. The method is simple, rapid and highly sensitive and meets the requirements.

2. Methods and Materials

Sample Preparation

- (1) Weighing 2.0 g of the sample in a 50 mL centrifuge tube,
- (2) Add 10 mL of methanol - water (1:1) to the centrifuge tube, shaking for 10 minutes
- (3) Centrifuge at 4000 rpm for 5 minutes.
- (4) Discard the remaining solution in the centrifuge tube, add 10 mL of 0.2 mol/L hydrochloric acid, homogenize in a refiner.
- (5) Add 100 µL of o-nitrobenzaldehyde and the centrifuge tube and vortexed for 30 seconds, shake for 30 minutes, reaction for 16 hours at 37°C incubator,
- (6) Samples were removed by adding 2 mL of 0.3 mol/L potassium phosphate. The pH was adjusted at 7.4 using 2.0 mol/L sodium hydroxide.
- (7) Extract the sample with 10 mL of ethyl acetate. Shake for 10 minutes, centrifuged at 10000 rpm for 10 minutes for collecting the ethyl acetate solution. Repeat this process twice.
- (8) Drying of the ethyl acetate solution at 40°C with nitrogen. The residue was dissolved with 1.0 mL of 0.1% aqueous formic acid solution. Remove fat with n-hexane. Filtration of the lower water phase with a microporous membrane.

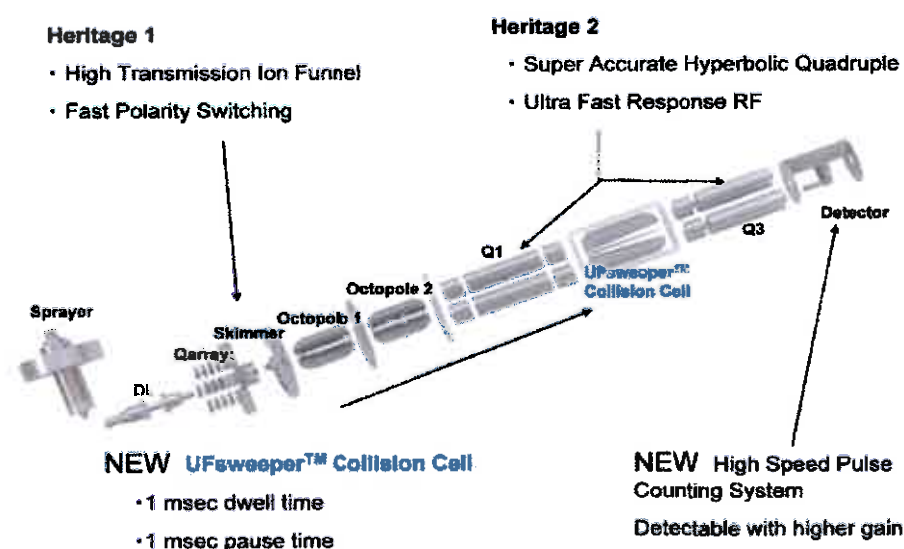
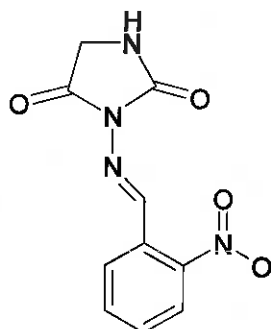
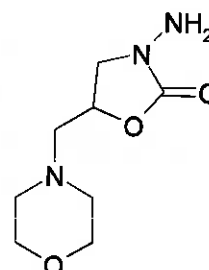


Fig. 1 Schematic diagram of LCMS-8030

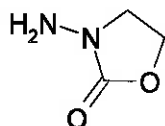
Isolation of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra-Performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry



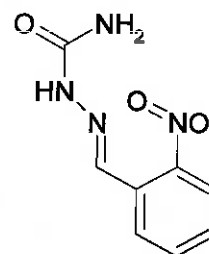
1-Amino-hydantoin (AHD)



3-Amino-5-morpholinomethyl-2-oxazolidinone (AMOZ)



3-Amino-2-oxazolidinone (AOZ)



Semicarbazide (SEM)

Fig. 2 Analytes

UHPLC/MS/MS Analysis

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A₃ degasser, and an SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-ODSIII column (150 mmL × 2.0 mm i.d., 2.2 μm) with the column temperature at 40°C. The mobile phase consisted of (A) 0.02% formic acid water and

(B) acetonitrile using a gradient elution of 40%B (0 min)-95%B (1.5 min)-40%B (1.51-4.0 min). The flow rate was 0.4 mL/min. A triple quadrupole mass spectrometer (Shimadzu LCMS-8030, Kyoto, Japan) was connected to the Shimadzu UHPLC instrument via an ESI interface. The MRM chromatograms were obtained in positive ion mode.

Analytical Conditions

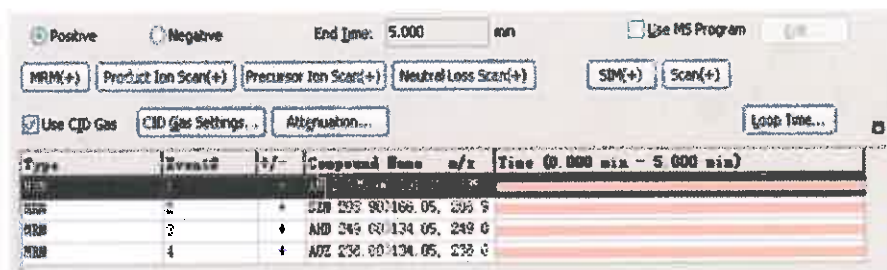
UHPLC (Nexera system)

Column	: Shim-pack XR-ODS III column (150 mm × 2.0 mmID, 2.2 μm)
Mobile Phase A	: 0.02% formic acid water
Mobile Phase B	: acetonitrile
Gradient Program	: 40%B (0 min)-95%B (1.5 min)-40%B (1.51-4.0 min)
Flow Rate	: 0.4 mL/min
Column Temperature	: 40°C
Injection Volume	: 20 μL

MS/MS (LCMS-8030 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: Positive
Probe Voltage	: +4.5 kV (ESI-Positive mode)
Nebulizing Gas Flow	: 3.0 L/min
Drying Gas Pressure	: 20 L/min
DL Temperature	: 300°C
BH Temperature	: 500°C

Determination of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra High Performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry



MRM parameter

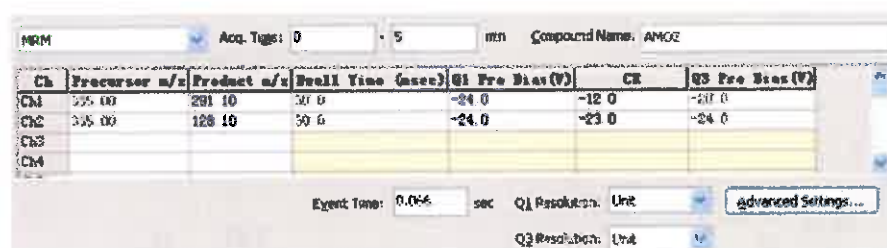


Fig. 3 User interface of MRM setting on LabSolutions software

Simple Quantitative Method

Based on the chromatograms obtained by injection of a fixed volume of individual reference standard solutions, the calibration curves of the 4 derivatives of nitrofuran

metabolites were prepared. The MRM parameters are shown in Table 1.

Table 1 MRM mode parameters of 4 marine toxins

No.	Compound	Precursor Ion (m/z)	Product Ion (m/z)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	AOZ	236	134.10* 104.05	-18.0 -18.0	-12.0 -24.0	-29.0 -22.0
2	AMOZ	335	291.00* 262.00	-13.0 -18.0	-11.0 -17.0	-22.0 -19.0
3	AHD	249	134.00* 103.95	-13.0 -13.0	-13.0 -22.0	-15.0 -11.0
4	SEM	209	166.10* 192.00	-11.0 -11.0	-11.0 -12.0	-18.0 -14.0

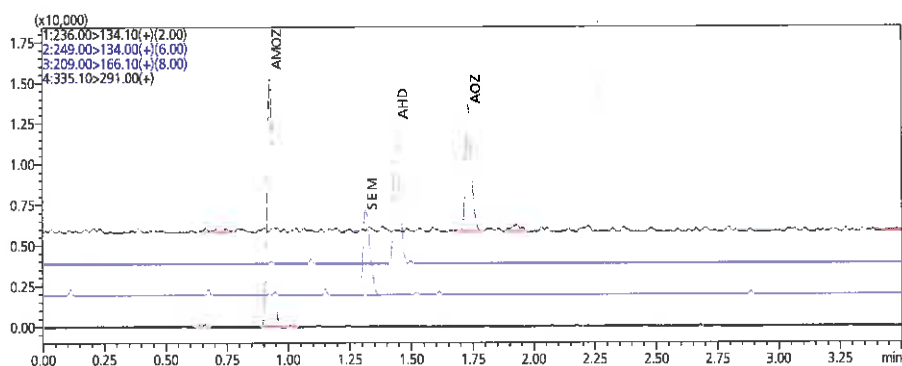


Fig. 4 MRM chromatograms of 4 derivatives of nitrofuran metabolites

ation of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry

Table 2 Repeatability of 4 marine toxins at different concentrations (n = 6)

No.	Compound	RSD% (1 µg/L)		RSD% (10 µg/L)		RSD% (50 µg/L)	
		R.T. (min)	Area	R.T. (min)	Area	R.T. (min)	Area
1	AOZ	0.13	3.84	0.15	2.36	0.09	2.16
2	AMOZ	0.11	4.24	0.09	2.13	0.07	1.69
3	AHD	0.14	3.56	0.27	3.49	0.12	2.24
4	SEM	0.27	4.76	0.28	4.66	0.12	1.68

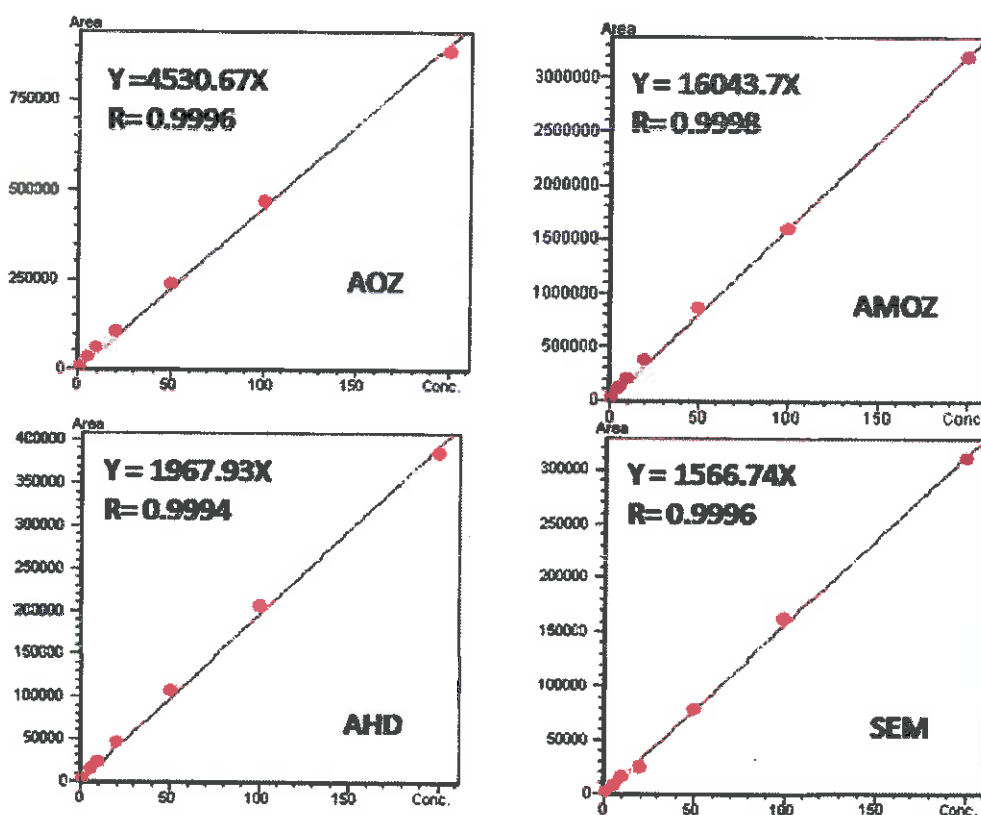


Fig. 5 Calibration curve of 4 derivatives of nitrofuran metabolites

3. Results and Discussion

The MRM chromatograms in the positive ion modes of 4 derivatives of nitrofuran metabolites are shown in Fig. 4. Fig. 5 shows the calibration curves of 4 derivatives of nitrofuran metabolites. Linearity was demonstrated in the range of 1.0 to 200 µg/L for AOZ, AMOZ, AHD and SEM,

with correlation coefficients greater than 0.999. The repeatability of those compounds in different concentration were investigated, and the RSDs of peak area were less than 4.8%, and RSDs of retention time were less than 0.3%, as shown in Table 3.

Determination of the Derivatives of Nitrofuran Metabolites in Marine Products by Ultra High Performance Liquid Chromatography / Triple Quadrupole Mass Spectrometry

4. Conclusions

A LC/MS/MS method has been developed for quantitative analysis of 4 derivatives of nitrofuran metabolites in marine products were detected by Shimadzu Nexera UHPLC and LCMS-8030 triple quadrupole mass spectrometer. All of them were separated in 4 minutes, and analyzed in positive

mode. The calibration curves were linear well between peak area of the selected MRM transitions and the concentration of target compounds with the correlation coefficient over 0.999. This method was established for fast quantitative determination of 4 derivatives of nitrofuran metabolites.

First Edition: June, 2013

High Throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS

ASMS 2012 WP27-580

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High Throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS

Introduction

Mycotoxins often exist as contaminants in grains. To ensure consumer food safety, manufactures of food and beverages have to strictly manage risks from such contaminants. To maintain the high-quality of food standards it is therefore essential to rapidly determine the concentrations of hazardous mycotoxins in foods or beverages.

UHPLC-MS/MS offers the best combination of selectivity, sensitivity, and speed for detection of these compounds in

complex matrices. In this study, a high throughput method for the quantification of 14 mycotoxins in beers was developed. Highest sensitivity of analysis is crucial to food safety, additionally, autosampler and system carry over need to be monitored to ensure these factors do not become a problem. In these experiments elimination of carry over was investigated through novel rinse condition cycles of the UHPLC autosampler.

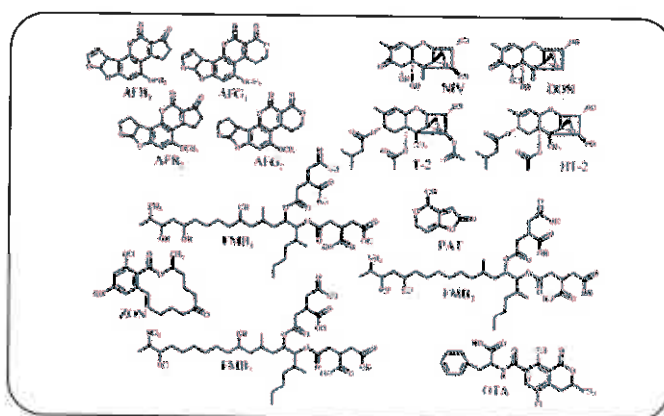


Fig. 1 Structure of mycotoxins

Methods and Materials

14 mycotoxins (patulin(PAT), nivalenol(NIV), deoxynivalenol (DON), aflatoxin(AF) B1, B2, G1, G2, T-2 toxin(T-2), HT-2 toxin(HT-2), zearalenone(ZON), fumonisin(FM) B1, B2, B3 and ochratoxin A(OTA)) were determined by LC-MS/MS using a UFLC HPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer.

The MRM method of 14 mycotoxins was optimized on

each compound-dependent parameter and MRM transition (Q1/Q3). As a result, all compounds were detected with high sensitivity by ESI. AFB1, B2, G1, G2, T-2, HT-2, FMB1, B2, B3 and OTA were detected in positive mode. While PAT, NIV, DON, ZON were detected in negative mode. Ultra Fast Polarity Switching of 15 msec enabled simultaneous determination of the compounds in both modes.



Fig. 2 LCMS-8030 triple quadrupole mass spectrometer

High Speed Mass Spectrometer

Polarity Switching

15 msec

Scanning Speed

Max. 15000 u/sec

Results

Method development for 14 mycotoxins

For UHPLC separation, various LC mobile phase conditions were examined. Tailing of fumonisins peaks were observed when only ammonium acetate was added in mobile phase. It was found that pH of a mobile phase effected peak shape of fumonisins. In order to reduce tailing of fumonisins, acetic acid was added in mobile phase B and

the gradient program was controlled to maintain high concentration of acetic acid when fumonisins were eluted. By controlling the concentration of acetic acid and ammonium acetate with gradient program, 14 mycotoxins were separated and detected excellently in 11 minutes (Fig. 3).

Analytical Conditions for LC-MS/MS

HPLC: UFLC system

Column: TriartC18 100 mm×2.0 mm, 1.9 μ m
Mobile phase A: 10 mM Ammonium acetate - Water,
B: 2% Acetic acid - Methanol
Flow rate: 0.4 mL/min
Gradient program: B conc.2%(0 min) - 55%(3 min) - 85%(7.0-8.0 min) - 2%(8.01-11 min)
Column temperature: Column temperature: 40 C

MS: LCMS-8030 triple quadrupole mass spectrometer

Ionization: ESI, Positive/Negative MRM mode Ion spray voltage: -3.5 kV

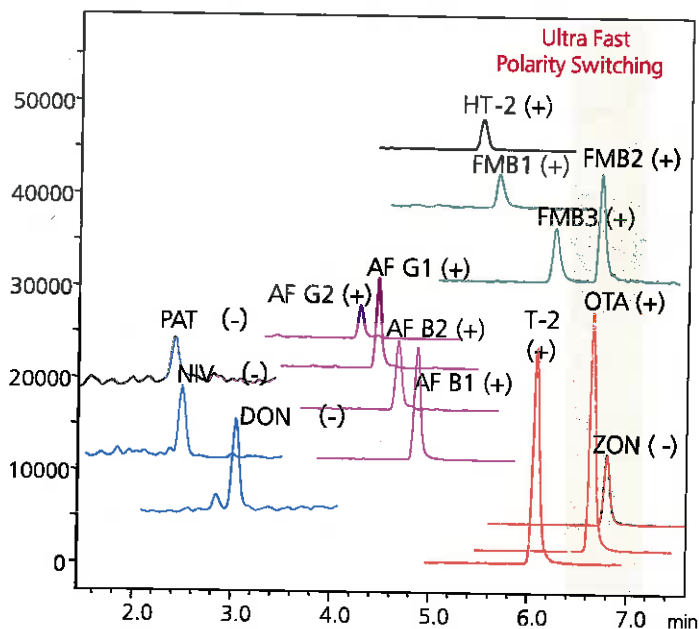


Fig. 3 14 mycotoxins analysis by LC-MS/MS
(PAT, NIV, DON, HT-2, T-2, OTA, ZON, FM B1/B2/B3 50ppb, AF B1/B2/G1/G2 10 ppb)

MRM Transition

Mycotoxin	MRM transition
AF G1 (+)	329.05 > 243.05
AF G2 (+)	331.00 > 245.00
AF B1 (+)	313.00 > 241.05
AF B2 (+)	315.00 > 259.00
HT-2 (+)	442.00 > 263.05 ([M+NH ₄] ⁺)
T-2 (+)	483.95 > 305.00 ([M+NH ₄] ⁺)
OTA (+)	404.10 > 238.90
ZON (-)	317.15 > 273.00
NIV (-)	371.10 > 281.25 ([M+CH ₃ COO] ⁻)
DON (-)	355.10 > 295.15 ([M+CH ₃ COO] ⁻)
PAT (-)	153.10 > 109.20
FM B1 (+)	722.45 > 334.30
FM B2 (+)	706.45 > 336.25
FM B3 (+)	706.45 > 336.25

Each mycotoxin standard was analyzed at six concentration levels. Good linearity was observed in the calibration curves, and excellent sensitivity was achieved.

High Throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS

Table 1 Linearity 14 mycotoxins

Mycotoxin	Range	Coefficient(r2)	Mycotoxin	Range	Coefficient(r2)
AF G1	0.4-20 ppb	0.999	ZON	2-100ppb	0.999
AF G2	0.4-20 ppb	0.999	NIV	2-100ppb	0.999
AF B1	0.4-20 ppb	0.999	DON	2-100ppb	0.997
AF B2	0.4-20 ppb	0.999	PAT	10-100ppb	0.999
HT-2	2-100 ppb	0.998	FM B1	2-100ppb	0.995
T-2	2-100 ppb	0.999	FM B2	2-100ppb	0.994
OTA	2-100 ppb	0.999	FM B3	2-100ppb	0.997

Rinse condition for eliminating carry over

Carry over of fumonisins was initially observed using the general rinse condition, because fumonisins formed complexes with trace metal ions in the sample's flow path.

Probably, several carboxyl groups of fumonisins coordinated with metal ion (Fig. 4).

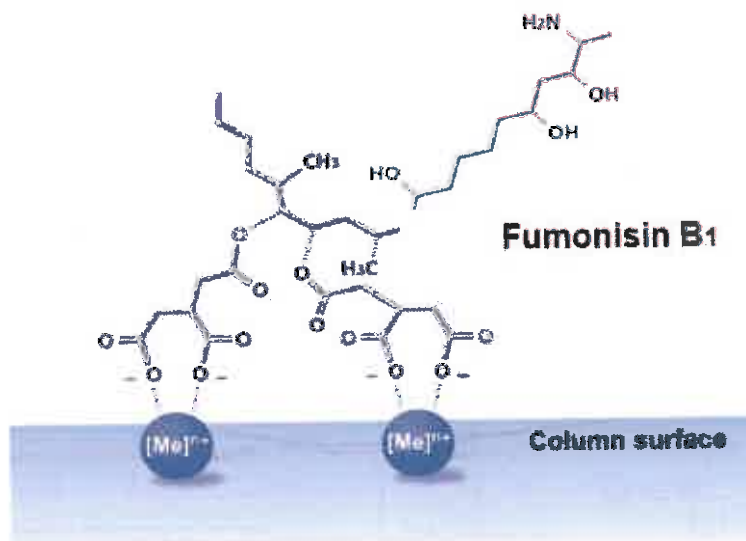


Fig. 4 Possible coordination interaction with metal ion

For eliminating carry over, rinse solvent and rinse method were examined. The performance of Nexera autosampler SIL-30AC, which can wash both inner and outer needle surfaces with 4 different solvents, was used.

It was thought that carboxyl groups of fumonisins may preferentially pair with hydrogen ions in the presence of low pH, therefore formic acid was added to rinse solvent. When investigating rinse methods, it was discovered the inner and outer rinse of needle reduced carry over more than the outer rinse of needle. Finally the modified rinse

solvent consisted of: 1% formic acid aq./methanol / acetonitrile / isopropanol (1/1/1).

To test the modified rinse cycle method, one injection of the 100ppb fumonisins standard solution was followed by one blank injection to check for carryover. Figure 5 shows chromatograms of the standards of FMB2 and B3, and the following blank injection. Low carry over was observed in the blank injection. It resulted from washing fumonisins adsorbed inside needle with the needle's inner and outer rinse method and the effective rinse solvent.

High throughput Quantitative Analysis of Multi-mycotoxin in Beer-based Drinks using UHPLC-MS/MS

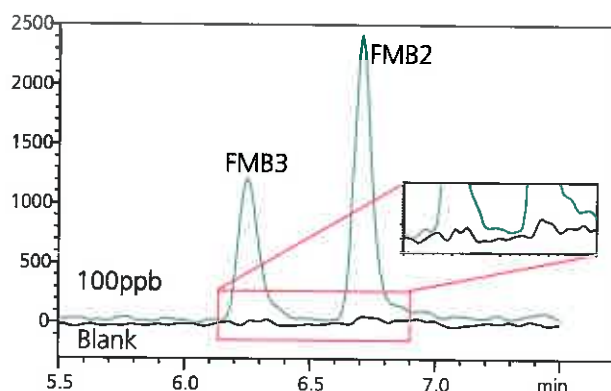


Fig. 5 Carry over evaluation of fumonisins

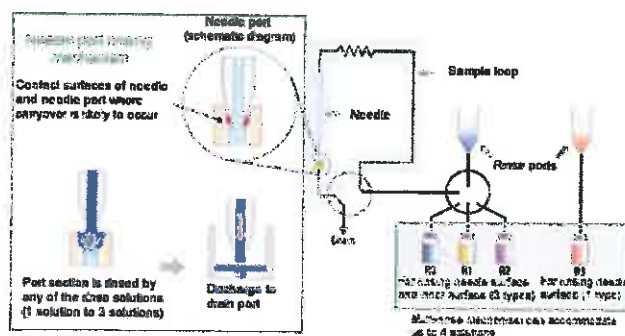


Fig. 6 HPLC path of SIL-30AC

Quantitative Analysis of 14 mycotoxins in beer-based drinks

Mycotoxins were extracted from samples and were purified with a solid phase extraction (SPE) cartridge.

20 commercial beers were analyzed by using this method.

The calibration curves were assessed using beer samples spiked with mycotoxins. PAT, AFB1, B2, G1, G2, NIV, T-2

and ZON were not detected in any of the beer samples.

Some of the tested samples were found to be contaminated with DON, HT-2, OTA, FMB1, B2, and B3 at concentrations of less than their respective LOQs (each 5 ppb).

Table 2 Mycotoxins detected in analyzed samples

Producing country	Concentration of mycotoxin/ppb (detected rate)					
	DON	HT-2	FMB1	FMB2	FMB3	OTA
Mexico (1sample)			< 5 (1/1)	< 5 (1/1)		
USA (1sample)	< 5 (1/1)					
China (1sample)	< 5 (1/1)					
Philippine (1sample)			< 5 (1/1)			
Australia (1sample)						
Japan (5sample)			< 5 (3/5)	< 5 (2/5)		

Producing country	Concentration of mycotoxin/ppb (detected rate)					
	DON	HT-2	FMB1	FMB2	FMB3	OTA
Hol I and (2samples)		< 5 (1/2)				< 5 (1/2)
Ireland (2sample)						
England (1sample)	< 5 (1/1)		< 5 (1/1)			< 5 (1/1)
Germany (1sample)						
Czech (1sample)						
Belguin (2samples)	6.7 (1/2)	< 5 (1/2)				
	< 5 (1/2)		< 5 (1/2)	< 5 (1/2)		

< 5 (less than 5ppb)

Conclusions

- High throughput LC-MS/MS method for 14 mycotoxins was developed, and could be applied to the quantification of these compounds in beers.
- Carry over of fumonisins was eliminated by using both the needle's inner and outer rinse method with effective rinse solvent.
- Results from these experiments indicate that the health risk to consumers posed by intake mycotoxins in commercial beers is relatively low.

Acknowledgemets

Reagents were provided from Wako Pure Chemical Ind., (Osaka, Japan) with substantial cooperation.



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Rapid and Highly Sensitive Quantitative Analysis and Screening of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

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Rapid and Highly Sensitive Quantitative Analysis and Screening of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

Introduction

Aflatoxins (AFs) are the most harmful mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and can contaminate foods such as cereals and nuts. To reduce the risk of the ingestion from foods, analyses of the AFs are carried out in many countries. It is necessary to quantitate the total aflatoxin (B1, B2, G1, G2) in foods by the regulation in JAPAN. The conventional LC/MS method proposed by the Ministry of Health, Labour and Welfare of Japan has a total analysis time of 30 minutes. In this study, we examined two alternative high-throughput LC-MS/MS methods. The first optimized for sensitivity & quantification; the second, a rapid screening method, using UHPLC for the purpose of increasing work flow.

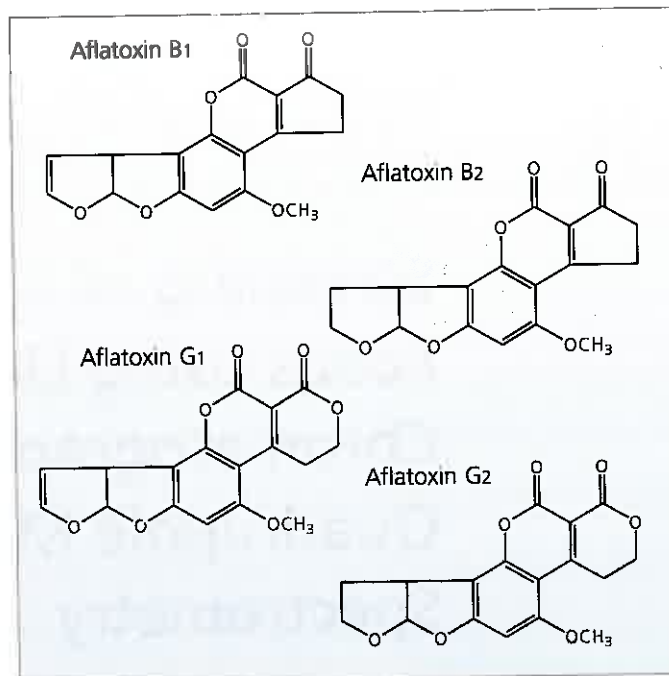


Fig. 1 Structure of Aflatoxins.

Materials and Methods

The system consisted of a SHIMADZU "Nexera" HPLC system, and "LCMS-8030" triple quadrupole mass spectrometer, equipped with an electrospray ionization (ESI) used in the positive ion MS/MS mode.

AFs standard solution were obtained from Biopure, MYCOTOXIN MIX5(AFLATOXINS) and Wako Chemicals, Aflatoxins Mixture Solution 1.

Sample preparation work flow (Fig. 2) shows how AFs in roast peanut was prepared by an immunoaffinity column (AFLAKING, HORIBA, JAPAN). Based on starting material 50g (roast peanut powder) spiked Aflatoxin B1 and G1(4 µg/Kg), B2 and G2 (1 µg/Kg) standard solution, final concentration of the sample solutions became: 2 µg/L B1 and G1, 0.5 µg/L B2 and G2.

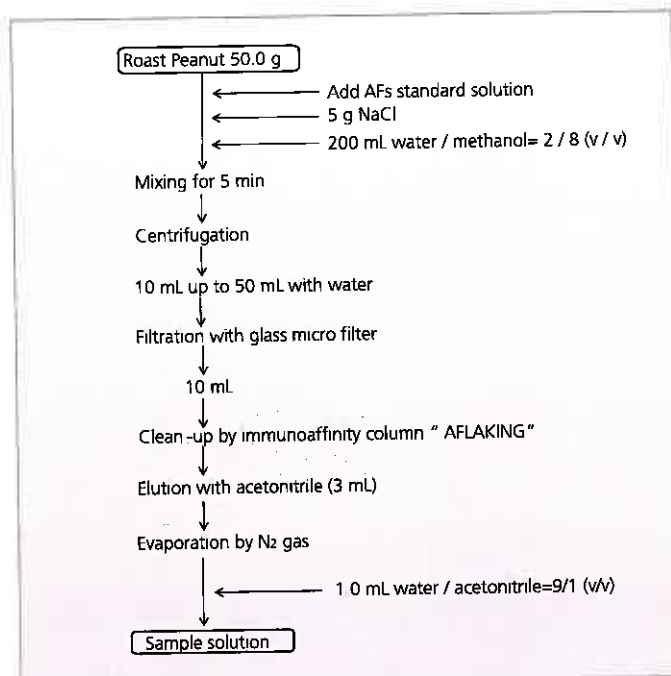


Fig. 2 Sample preparation work flow.

and Highly Sensitive Quantitative Analysis and ening of Aflatoxins in Foods Using Liquid Chromatography ple Quadrupole Mass Spectrometry

Experiment Analysis Standard Solution

The chromatogram and analytical conditions of the conventional method (Fig. 3, Table 1) were compared to and the high-speed method with using ultra-fast liquid chromatography (Fig. 4, Table 3). The flow rate was raised

to 0.45 mL/min accelerating AFs elution to 4 minutes (operating back pressure 44-50 Mpa). MRM transitions summarized in Table 2.

Table 1 Analytical conditions (typical 30 minute method).

HPLC

Column: Shim-pack FC-ODS
 (150 mm L. × 2.0 mm i.d., 3 μm)
 Mobile Phase A: 10 mmol/L Ammonium acetate-water
 Mobile Phase B: Methanol
 Gradient Program: 40%B(0-15 min) → 100%B
 (15.01-20 min) → 40%B (20.01-30 min)
 Flow Rate: 0.2 mL/min
 Column Temp.: 40°C
 Injection Vol.: 6 μL

MS

Probe Voltage: +4.5 kV ESI-Positive mode
 Nebulizing Gas Flow: 3 L/min
 Drying Gas Flow: 15 L/min
 DL Temp.: 250°C
 Heat Block Temp.: 400°C

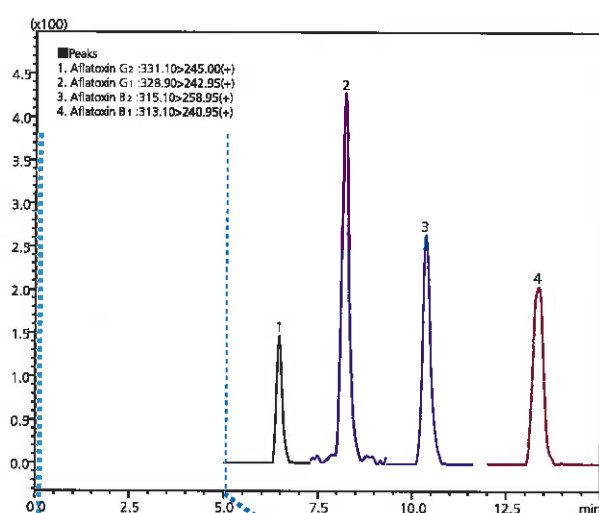


Fig. 3 Chromatograms of AFs (0.5 μg/L each).

Table 2 MRM Parameter.

Compound	Transition	Pause time (ms)	Dwell time (ms)	CE (V)	Resolution (Q1,Q3)
Aflatoxin B1	313.10 > 240.95	3	100	-40	Unit
Aflatoxin B2	315.10 > 258.95	3	100	-33	Unit
Aflatoxin G1	328.90 > 242.95	3	100	-30	Unit
Aflatoxin G2	331.10 > 245.00	3	100	-32	Unit

Table 3 Analytical conditions (optimized fast method).

HPLC

Column: Shim-pack XR-ODS II
 (100 mm L. × 2.0 mm i.d., 2.2 μm)
 Mobile Phase A: 10 mmol/L Ammonium acetate-water
 Mobile Phase B: Methanol
 Gradient Program: 40%B (0-4.5 min) → 100%B(4.51-6.5 min) → 40%B (6.51-12 min)
 Flow Rate: 0.45 mL/min
 Column Temp.: 50°C
 Injection Vol.: 6 μL

MS

Probe Voltage: +4.5 kV ESI-Positive mode
 Nebulizing Gas Flow: 3 L/min
 Drying Gas Flow: 15 L/min
 DL Temp.: 250°C
 Heat Block Temp.: 400°C

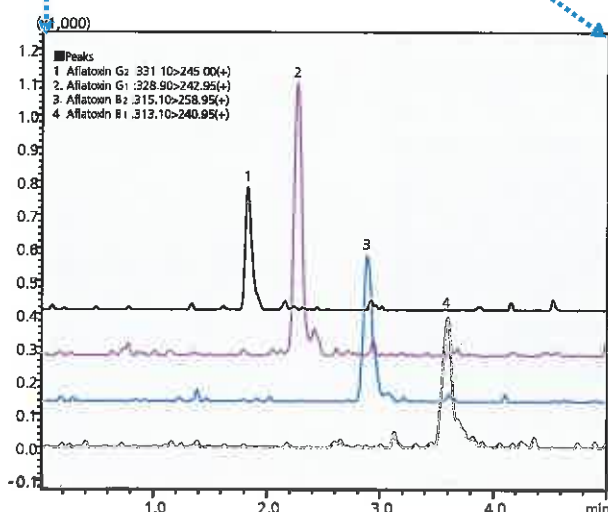


Fig. 4 Chromatograms of AFs (0.5 μg/L each).

Rapid and Highly Sensitive Quantitative Analysis and Screening of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

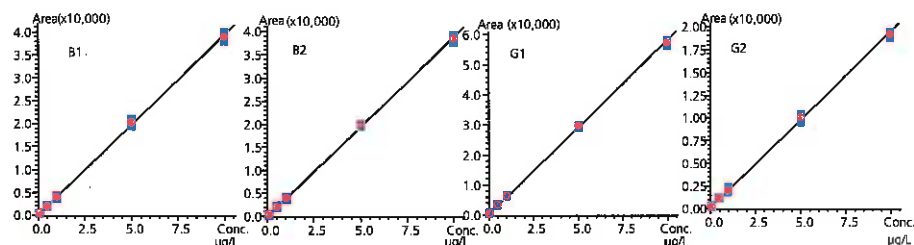


Fig. 5 Calibration curves AFs (linearity beyond $R^2=0.999$ was acquired).

Table 4 L.O.Q. and Linearity (n=6).

Compound	L.O.Q. (µg/L)	Linearity	R ²
Aflatoxin B1	0.1	0.1-10 µg/L	0.9995372
Aflatoxin B2	0.1	0.1-10 µg/L	0.9997556
Aflatoxin G1	0.05	0.05-10 µg/L	0.9994336
Aflatoxin G2	0.1	0.1-10 µg/L	0.9992275

Effect of Column Temperature

Column temperature was optimized in order to accelerate compound elution without compromising peak shape or intensity. Finally a column temperature of 50°C was chosen (Fig. 6).

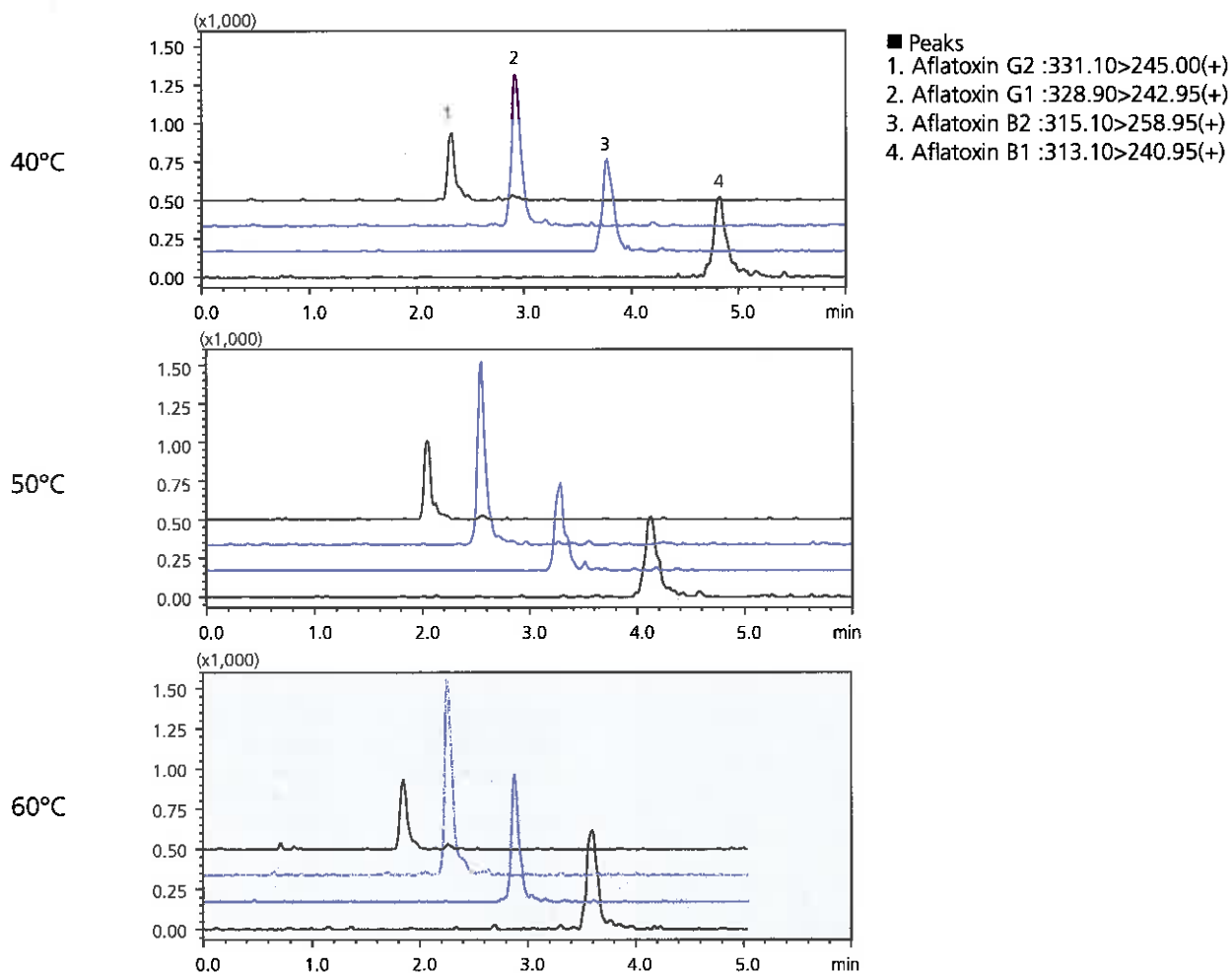


Fig. 6 Chromatographic optimization of column temperature.

And Highly Sensitive Quantitative Analysis and Determination of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

Effect of ESI Probe Position

Further optimisation was achieved through optimization of ESI probe position ranging from -2 mm to +3 mm from the central position (Fig. 7). Chromatographic comparison (Fig. 8) illustrates both peak intensity and level of noise are

influenced by probe position. Optimization required highest S/N and minimum noise at +1 mm (Fig. 9 & 10). [Noise was calculated by ASTM method with 3 blocks of 0.5 min around each

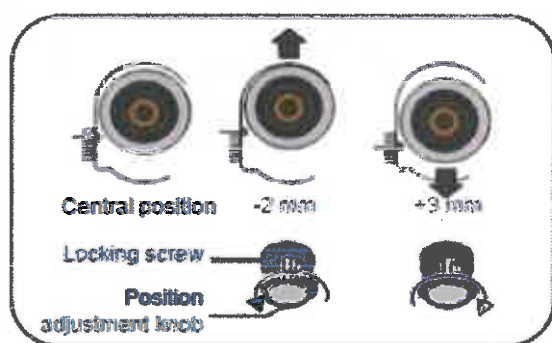


Fig. 7 ESI probe of LCMS-8030.

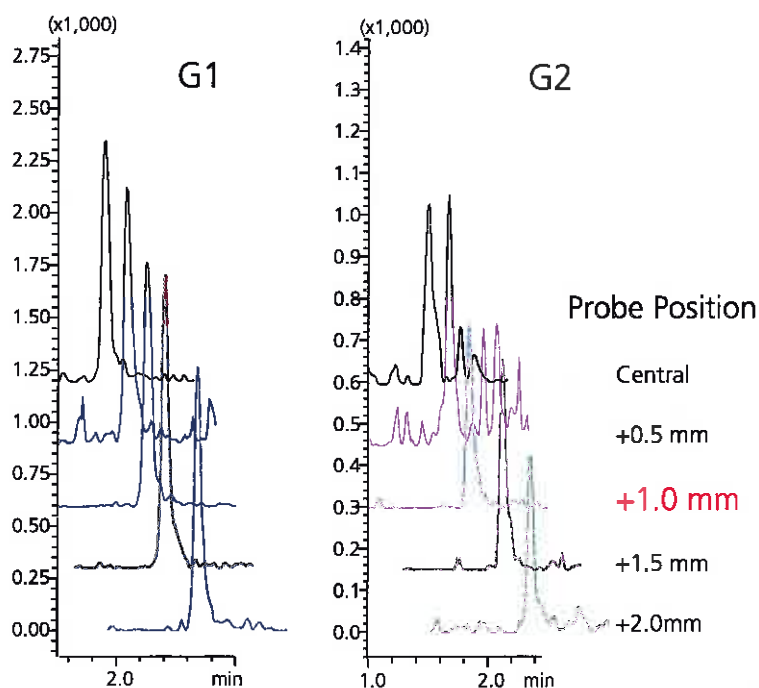


Fig. 8 Chromatograms comparison of a ESI probe position difference.

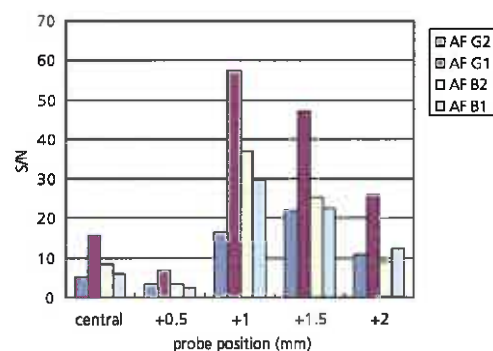


Fig. 9 S/N of probe position difference.

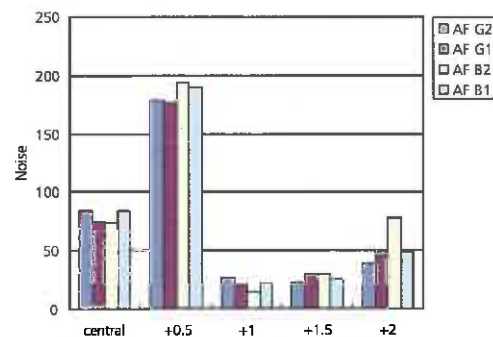


Fig. 10 Noise of probe position difference.

Rapid and Highly Sensitive Quantitative Analysis and Screening of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

Results

Analysis of Roast Peanut Matrix

The recovery test of AFs spiked into the roast peanut powder was performed in duplicate experiments. Overlaid chromatograms of spiked AFs in roast peanut matrix to un-spiked were shown (Fig. 11). Solid line - spiked compared to dotted line - un-spiked. Interference peaks were not detected in un-spiked samples.

Quantitation results using external standard method show the recovery rate was in the range of 69-86% (Table 5). This relatively low recovery rate is a known problem when extracting AFs with a solvent from the powder of a roast peanut. Further method development is underway to increase recovery rate.

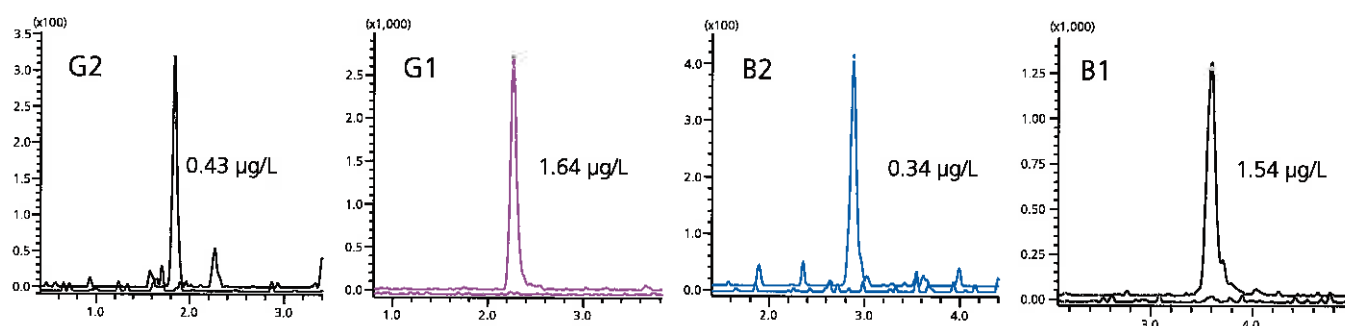


Fig. 11 Chromatograms of AFs in roast peanut matrix.

Table 5. Results of quantity analysis.

	Aflatoxin B1		Aflatoxin B2		Aflatoxin G1		Aflatoxin G2	
	Conc (µg/L)	Recovery (%)	Conc (µg/L)	Recovery (%)	Conc (µg/L)	Recovery (%)	Conc (µg/L)	Recovery (%)
Sample1	1.54	75	0.34	69	1.64	82	0.43	86
Sample2	1.44	70	0.34	69	1.64	82	0.37	74

And Highly Sensitive Quantitative Analysis and Monitoring of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

Ultra High-speed Method For Screening Analysis

Further improvements to speed of analysis were made using a reduced column particle size and length (1.6 μ m, Shim-pack XR-ODS III 50 mm $\times$ 2.0 mm). With these

conditions, AFs eluted within 2 minutes with L.O.Q. of 0.5 μ g/L. This ultra high-speed analysis could prove useful when screening many samples.

Table 6. Analytical conditions.

HPLC

Column: Shim-pack XR-ODS III (50 mm L. $\times$ 2.0 mm i.d., 1.6 μ m)
Mobile Phase A: 10 mmol/L Ammonium acetate-water
Mobile Phase B: Methanol
Gradient Program: 40%B(0-2.25 min) $\rightarrow$ 100%B(2.26-3.25 min) $\rightarrow$ 40%B(3.26-6.00 min)
Flow Rate: 0.45 mL/min
Column Temp.: 50°C
Injection Vol.: 6 μ L

MS

Probe Voltage: +4.5 kV ESI-Positive mode
Nebulizing Gas Flow: 3 L/min
Drying Gas Flow: 15 L/min
DL Temp.: 250°C
Heat Block Temp.: 400°C

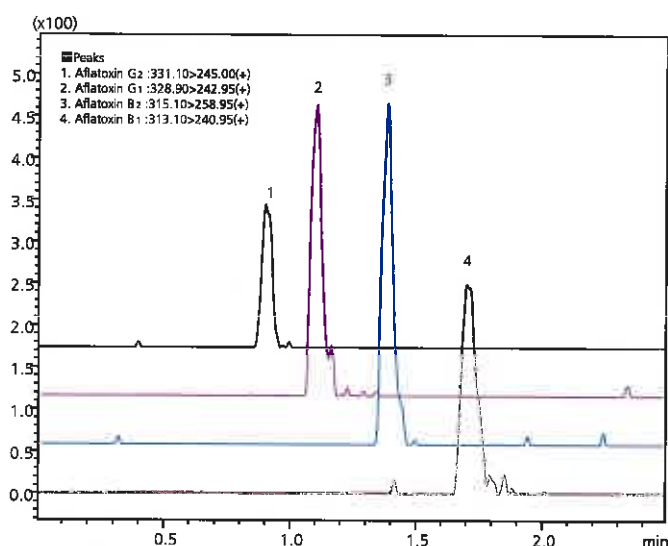


Fig. 12 Chromatograms of AFs (0.5 mg/L each).

Table 7 MRM Parameters.

Compound	Transition	Pause time (ms)	Dwell time (ms)	CE (V)	Resolution (Q1,Q3)
Aflatoxin B1	313.10 > 240.95	1	50	-40	Unit
Aflatoxin B2	315.10 > 258.95	1	50	-33	Unit
Aflatoxin G1	328.90 > 242.95	1	50	-30	Unit
Aflatoxin G2	331.10 > 245.00	1	50	-32	Unit

Conclusion

Analysis of AFs in the roast peanut was studied.

- Accelerated method was developed due to capacity of ultra high pressure liquid chromatography (Nexera).
- New choice of Shim-pack column enabled faster elution times.
- Two high-speed methods were developed eluting AFs within 4 and 2 min.
- Column temperature and ESI probe position were important conditions of AFs analysis.
- Immunoaffinity column was useful for cleanup from the roast peanut matrix.
- The results of recovery test was 69-86%.



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Multiresidue pesticide analysis from dried chili powder using LC/MS/MS

ASMS 2014 WP350

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Multiresidue pesticide analysis from dried chili powder using LC/MS/MS

Introduction

Pesticide residues in foodstuffs can cause serious health problems when consumed. LC/MS/MS methods have been increasingly employed in sensitive quantification of pesticide residues in foods and agriculture products. However, matrix effect is a phenomenon seen in Electro Spray Ionization (ESI) LC/MS/MS analysis that impacts the data quality of the pesticide analysis, especially for complex matrix like spice/herb. Chili powder is one such complex matrix that can exhibit matrix effect (either ion suppression or enhancement). A calibration curve based on matrix matched standards can demonstrate true sensitivity of analyte in presence of

matrix. Therefore, this approach was used to obtain more reliable and accurate data as compared to quantitation against neat (solvent) standards^[1]. Multiresidue, trace level analysis in complex matrices is challenging and tedious. Feature of automatic MRM optimization in LCMS-8040 makes method development process less tedious. In addition, the lowest dwell time and pause time along with ultra fast polarity switching (UFSwitching) enables accurate, reliable and high sensitive quantitation. UFSweeper™ II technology in the system ensures least crosstalk, which is very crucial for multiresidue pesticide analysis.

Method of Analysis

Sample Preparation

Commercially available red chili was powdered using mixer grinder. To 1 g of this chili powder, 20 mL water:methanol (1:1 v/v) was added and the mixture was sonicated for 10 mins. The mixture was centrifuged and supernatant was collected. This supernatant was used as diluent to prepare

pesticide matrix matched standards at concentration levels of 0.01 ppb, 0.02 ppb, 0.05 ppb, 0.1 ppb, 0.2 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb, 10 ppb and 20 ppb. Each concentration level was then filtered through 0.2 µ nylon filter and used for the analysis.

LC/MS/MS Analytical Conditions

Pesticides were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu

Corporation, Japan), shown in Figure 1. The details of analytical conditions are given in Table 1.

Table 1. LC/MS/MS analytical conditions

• Column	: Shim-pack XR-ODS (75 mm L x 3 mm I.D.; 2.2 µm)
• Guard column	: Phenomenex SecurityGuard ULTRA Cartridge
• Mobile phase	: A: 5 mM ammonium formate in water:methanol (80:20 v/v) B: 5 mM ammonium formate in water:methanol (10:90 v/v)
• Flow rate	: 0.2 mL/min
• Oven temperature	: 40 °C
• Gradient program (B%)	: 0.0–1.0 min → 45 (%); 1.0–13.0 min → 45-100 (%); 13.0–18.0 min → 100 (%); 18.0–19.0 min → 100-45 (%); 19.0–23.0 min → 45 (%)
• Injection volume	: 15 µL
• MS interface	: ESI
• Polarity	: Positive and negative
• Nitrogen gas flow	: Nebulizing gas 2 L/min; Drying gas 15 L/min
• MS temperature	: Desolvation line 250 °C; Heat block 400 °C
• MS analysis mode	: Staggered MRM

Residue pesticide analysis from dried chili powder LC/MS/MS



Figure 1. Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Results

LC/MS/MS method was developed for analysis of 80 pesticides belonging to different classes like carbamate, organophosphate, urea, triazines etc. in a single run^[2]. LOQ was determined for each pesticide based on the following criteria – (1) % RSD for area < 16 % (n=3), (2) % Accuracy between 80-120 % and (3) Signal to noise ratio (S/N) > 10.

LOQ achieved for 80 pesticides have been summarized in Table 2 and results for LOQ and linearity for each pesticide have been given in Table 3. Representative MRM chromatogram of pesticide mixture at 1 ppb level is shown in Figure 2. Representative MRM chromatograms at LOQ level for different classes of pesticides are shown in Figure 3.

Table 2: Summary of LOQ achieved

LOQ (ppb)	0.01	0.02	0.05	0.1	0.2	0.5	1
Number of pesticides	1	1	3	8	17	24	26

Table 3. Results of LOQ and linearity for pesticide analysis

Sr. No.	Name of compound	MRM Transition	Polarity	LOQ (ppb)	Linearity (R ²)
1	Spinosyn D	746.20>142.10	Positive	0.01	0.9987
2	Fenpyroximate	421.90>366.10	Positive	0.02	0.9915
3	Bifenazate	301.00>198.00	Positive	0.05	0.9947
4	Spinosyn A	732.20>142.10	Positive	0.05	0.9974
5	Spiromesifen	371.00>273.10	Positive	0.05	0.9957
6	Acetamiprid	222.90>126.00	Positive	0.1	0.9910
7	Carbofuran	221.70>123.00	Positive	0.1	0.9971
8	Dimethoate	229.80>198.90	Positive	0.1	0.9970
9	Dimethomorph I	387.90>301.00	Positive	0.1	0.9991
10	Dimethomorph II	387.90>301.00	Positive	0.1	0.9992
11	Isoprotruron	207.00>72.10	Positive	0.1	0.9984
12	Pirimiphos methyl	305.70>108.00	Positive	0.1	0.9997
13	Trifloxystrobin	408.90>186.00	Positive	0.1	0.9989

Multiresidue pesticide analysis from dried chili powder using LC/MS/MS

Sr. No.	Name of compound	MRM Transition	Polarity	LOQ (ppb)	Linearity (R ²)
14	Anilophos	367.70>198.85	Positive	0.2	0.9974
15	Atrazine	215.90>174.00	Positive	0.2	0.9985
16	Carboxin	235.90>143.00	Positive	0.2	0.9952
17	Cyazofamid	324.85>108.10	Positive	0.2	0.9971
18	Edifenphos	310.60>111.00	Positive	0.2	0.9997
19	Ethion	384.70>198.80	Positive	0.2	0.9957
20	Fipronil	434.70>330.00	Negative	0.2	0.9973
21	Linuron	248.80>159.90	Positive	0.2	0.9945
22	Metolachlor	283.90>252.00	Positive	0.2	0.9966
23	Oxycarboxin	267.90>174.90	Positive	0.2	0.9995
24	Phosalone	367.80>181.90	Positive	0.2	0.9987
25	Phosphamidon	299.90>173.90	Positive	0.2	0.9997
26	Thiacloprid	252.90>126.00	Positive	0.2	0.9976
27	Thiobencarb	257.90>125.10	Positive	0.2	0.9977
28	Thiodicarb	354.90>88.00	Positive	0.2	0.9906
29	Triadimefon	293.90>196.90	Positive	0.2	0.9994
30	Tricyclazole	189.90>162.90	Positive	0.2	0.9977
31	Aldicarb	208.10>116.05	Positive	0.5	0.9962
32	Benfuracarb	411.10>190.10	Positive	0.5	0.9981
33	Bitertanol	338.00>99.10	Positive	0.5	0.9935
34	Buprofezin	305.70>201.00	Positive	0.5	0.9933
35	Clodinafop propargyl	349.90>266.00	Positive	0.5	0.9978
36	Chlorantraniliprole	483.75>452.90	Positive	0.5	0.9994
37	Diclofop methyl	357.90>280.80	Positive	0.5	0.9976
38	Flufenacet	363.70>193.90	Positive	0.5	0.9997
39	Flusilazole	315.90>247.00	Positive	0.5	0.9983
40	Hexaconazole	313.90>70.10	Positive	0.5	0.9996
41	Hexythiazox	352.90>227.90	Positive	0.5	0.9909
42	Iodosulfuron methyl	507.70>167.00	Positive	0.5	0.9971
43	Iprobenfos	288.70>205.00	Positive	0.5	0.9981
44	Malaoxon	314.90>99.00	Positive	0.5	0.9996
45	Malathion	330.90>284.90	Positive	0.5	0.9997
46	Mandipropamid	411.90>356.20	Positive	0.5	0.9952
47	Metalaxyl	280.00>220.10	Positive	0.5	0.9996
48	Methabenzthiazuron	221.70>150.00	Positive	0.5	0.9957
49	Methomyl	162.90>88.00	Positive	0.5	0.9988
50	Oxadiazon	362.15>303.00	Positive	0.5	0.9963
51	Penconazole	283.90>70.10	Positive	0.5	0.9992
52	Phorate	260.80>75.00	Positive	0.5	0.9987
53	Phorate sulfoxide	276.80>96.90	Positive	0.5	0.9991
54	Thiophanate methyl	342.90>151.00	Positive	0.5	0.9996
55	Avermectin B1a	890.30>305.10	Positive	1	0.9990
56	Carpromamid	333.70>139.00	Positive	1	0.9985

Residue pesticide analysis from dried chili powder by LC/MS/MS

Sr. No.	Name of compound	MRM Transition	Polarity	LOQ (ppb)	Linearity (R ²)
57	Clomazone	241.90>127.00	Positive	1	0.9967
58	Clorimuron ethyl	415.30>186.00	Positive	1	0.9965
59	Cymoxanil	198.90>128.10	Positive	1	0.9949
60	Diafenthiuron	385.00>329.10	Positive	1	0.9961
61	Diflubenzuron	310.80>158.00	Positive	1	0.9982
62	Dodine	228.10>60.00	Positive	1	0.9980
63	Enamectin benzoate	886.30>158.10	Positive	1	0.9983
64	Fenamidone	311.90>236.10	Positive	1	0.9997
65	Fenarimol	330.70>268.00	Positive	1	0.9900
66	Fenazaquin	306.95>57.10	Positive	1	0.9992
67	Flonicamid	229.90>202.70	Positive	1	0.9971
68	Flubendiamide	680.90>254.05	Negative	1	0.9993
69	Forchlorfenuron	247.90>129.00	Positive	1	0.9956
70	Kresoxim methyl	331.00>116.00	Positive	1	0.9996
71	Paclobutrazol	293.90>70.10	Positive	1	0.9974
72	Pencycuron	328.90>125.00	Positive	1	0.9943
73	Pendimethalin	281.90>212.10	Positive	1	0.9932
74	Profenofos	372.70>302.70	Positive	1	0.9966
75	Propargite	368.00>231.10	Positive	1	0.9950
76	Propoxur	209.90>110.90	Positive	1	0.9987
77	Pyrazosulfuron ethyl	414.90>182.00	Positive	1	0.9992
78	Pyriproxyfen	321.90>96.10	Positive	1	0.9975
79	Simazine	201.90>103.90	Positive	1	0.9992
80	Thiomethon	246.80>89.10	Positive	1	0.9989

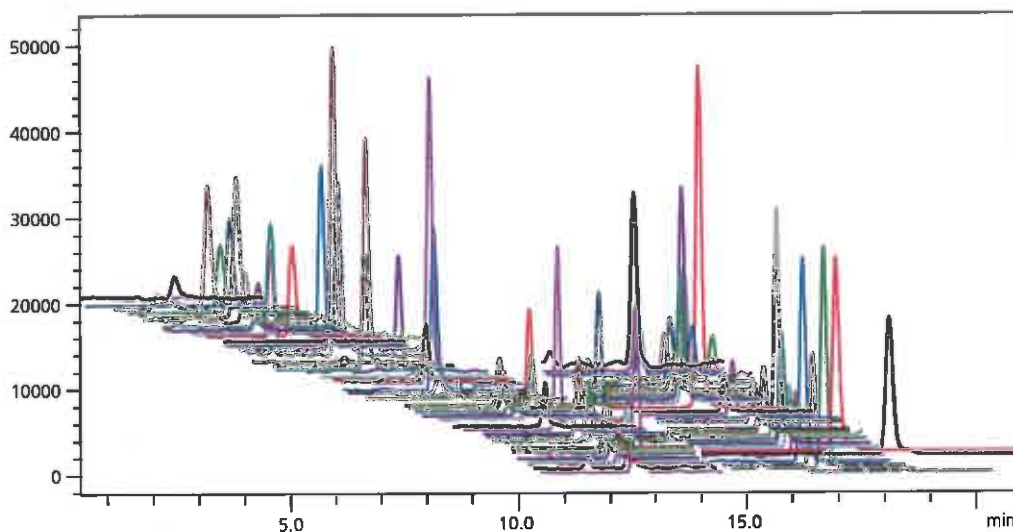


Figure 2. MRM chromatogram of pesticide mixture at 1 ppb level

Multiresidue pesticide analysis from dried chili powder using LC/MS/MS

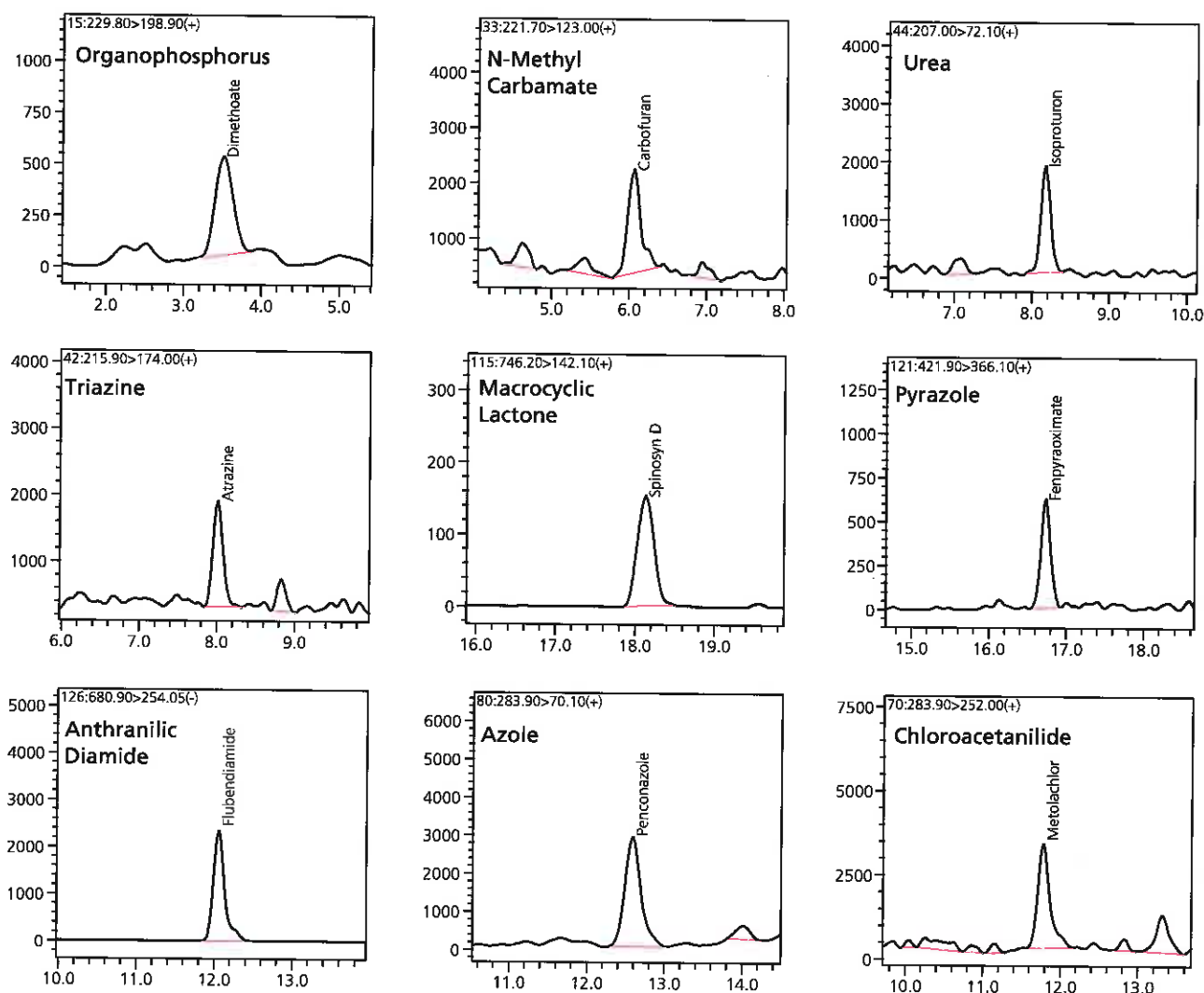


Figure 3. Representative MRM chromatograms at LOQ level from different classes of pesticides

Conclusion

- A highly sensitive method was developed for analysis of 80 pesticides belonging to different classes, from dried chili powder in a single run.
- Ultra high sensitivity, ultra fast polarity switching (UFSwitching), low pause time and dwell time along with UFSweeper™ II technology enabled sensitive, selective, accurate and reproducible multiresidue pesticide analysis from complex matrix like dried chili powder.

Residue pesticide analysis from dried chili powder by LC/MS/MS

References

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Micro Flow UHPLC-MS/MS in Pesticide Analysis of Infant Foods

ASMS 2013 ThP-540

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Micro Flow UHPLC-MS/MS in Pesticide Analysis of Infant Foods

1. Introduction

Food safety with regards to infant food is of the upmost importance; however, it is also recognised as a challenging matrix to analyse due to the low maximum residue limit (MRL) of 0.01 mg/kg required by European Directive 2006/141/EC for all pesticides. Furthermore, the European Directive prohibits the use of certain very toxic pesticides in the production of infant foods and establishes even lower MRLs for a few other very toxic pesticides. Additionally, the analysis of infant food is complicated by their wide range of fat content.

LC-MS/MS has been widely used for the quantitation of pesticides in infant food. The analytical methods typically

use conventional LC flow rates (approximately 0.5 mL/min). Micro flow LC uses significantly lower flow rates (10 to 100 μ L/min). With the same sample amount and identical LC peak width, the reduction in LC flow rate can result in an improved detection limit for concentration-dependent detection techniques such as electrospray ionization (ESI) mass spectrometry. Here, we utilise the improved response from micro flow LC to achieve the required low limits of detection for over one hundred pesticides in infant food. Initial validation results are presented for the micro flow LC method, in addition to robustness data.

2. Materials and Methods

2-1. Sample preparation

Samples were extracted using QuEChERS (quick, easy, cheap, effective, rugged and safe) methods developed by the Food and Environment Research Agency, UK. Sample

extracts in acetonitrile were spiked with 130 pesticides. Sample extracts were diluted five times with water before LCMS injection.

2-2. LC-MS/MS analysis

UHPLC	Nexera UHPLC system
Flow rate	90 μ L/min
Mobile phase	A= Water (95%) and methanol (5%) with 5 mM ammonium formate B= Methanol with 5 mM ammonium formate
Gradient	0% B - 100% B (12 min), 100% B (14 min), 10% B (17 min)
Analytical column	ACQUITY UPLC HSS T3; 1 mm $\times$ 100 mm, 1.8 μ m
Column temperature	35°C
Injection volume:	5 μ L
MS	LCMS-8040 triple quadrupole mass spectrometer
Ionisation	Electrospray, positive and negative mode
SRM	130 pesticides (260 SRMs) Pause 1 msec./Dwell 3 msec.
Desolvation line	200°C
Drying/Nebulising gas	15 L/min, 2 L/min
Heating block	400°C

3. Results and discussion

3-1. Micro flow LC method

Micro flow LC was utilised for the analysis of 130 pesticides using a 90 $\mu\text{L}/\text{min}$ flow rate and a 1.0 mm I.D. analytical column. The flow rate was reduced from conventional flow rates (approximately 500 $\mu\text{L}/\text{min}$) in order to increase analyte response and reduce costly solvent consumption. All 130 pesticides were eluted within 12.7 minutes with a

typical peak width of 7 seconds. An example chromatogram is displayed in Fig. 1. A high speed data acquisition mass spectrometer was utilised to acquire reliable data with a 1 msec. pause time and 3 msec. dwell time, which allowed the acquisition of a large number of overlapping MRM transitions.

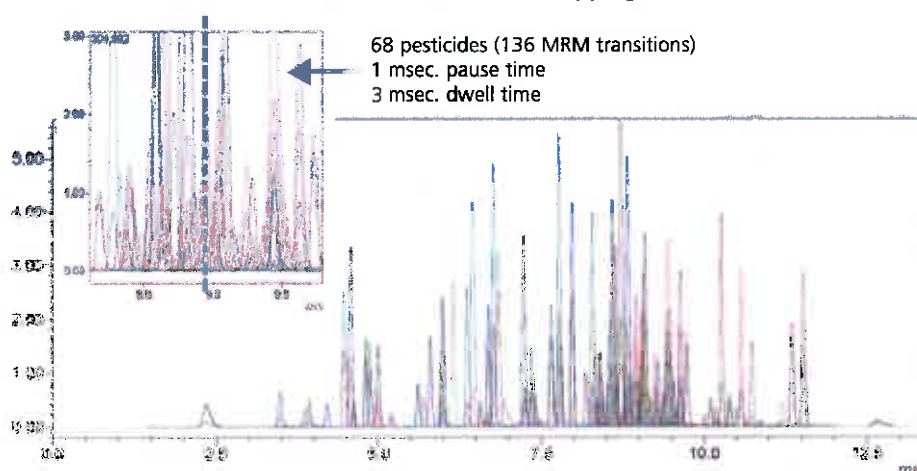


Fig. 1 Micro flow LC analysis of 130 pesticides in infant food (0.05 mg /kg)

3-2. Linearity

Calibration curves were prepared in the range 0.5xRL (reporting limit) to 20xRL. For the vast majority of pesticides this was in the range 0.005 - 0.2 mg/kg (0.5 – 200 ppb). The developed method achieved the necessary sensitivity to detect all 130 pesticides at the lowest required level. Fig. 2

displays calibration curves for nine example pesticides including the first eluting analyte (methamidophos) and latest eluting analyte (fenbutatin). All analytes displayed linearity with $R^2 > 0.996$, with typical R^2 of 0.9990.

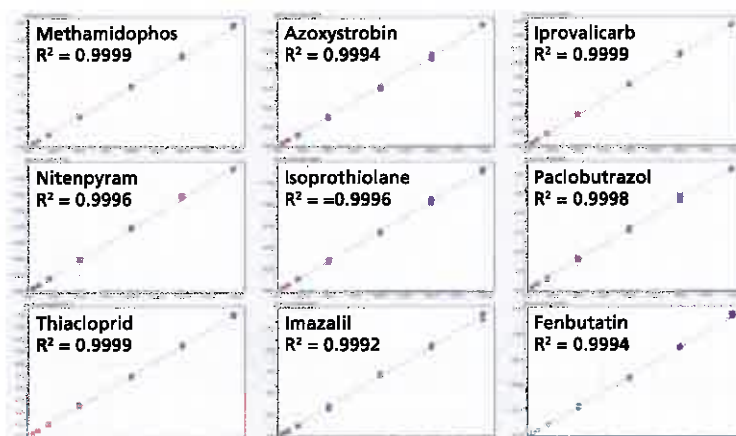


Fig. 2 Calibration curves (weighting 1/x), 0.005 0.2 mg/kg (0.5 200 ppb) of nine pesticides in infant food

Micro Flow UHPLC-MS/MS in Pesticide Analysis of Infant Foods

3-3. Intra- and inter-day precision

To assess the intra-day and inter-day variability of the LC-MS/MS method, QC samples were prepared in infant food at low (0.01 mg/kg) and high (0.2 mg/kg) concentrations. Intra- (n=6) and inter-day (n=3 over 3 days) precision were determined as %RSD of peaks areas. Table

2 lists precision data for compounds over the run time, including the first eluting analyte (methamidophos) and latest eluting analyte (fenbutatin). Intra-day precision was typically less than 4%, while inter-day precision was typically less than 7%.

Table 2 Intra- and inter-day precision of selected pesticides over the run time

Compound	Retention Time	Inter-day (%RSD)		Intra-day (%RSD)	
		Low	High	Low	High
Methamidophos	2.3	2.14	3.53	2.80	4.50
Acephate	3.4	1.56	2.55	3.54	3.85
Aldicarb sulfoxide	4.2	1.31	0.70	4.84	3.91
Methomyl	4.8	2.02	2.03	3.61	4.86
Imidacloprid	5.6	2.05	0.64	3.46	4.59
Carbendazim	6.5	1.70	0.72	2.69	3.80
Carbaryl	7.4	3.41	0.93	5.38	4.77
Fosfthiazate	7.6	1.21	1.19	2.55	4.50
Azoxystrobin	8.2	1.90	1.78	2.78	4.30
Dimethomorph	8.5	3.05	2.28	5.03	4.95
Triazophos	8.7	1.45	1.19	3.47	4.78
Iprovalicarb	8.8	2.40	1.71	3.08	4.47
Cyazofamid	8.9	2.60	2.16	2.89	5.74
Penconazole	9.3	1.58	1.61	4.55	4.15
Hexaconazole	9.4	2.37	1.77	4.16	4.35
Pencycuron	9.6	2.59	2.27	2.69	4.43
Pyriproxyfen	10.2	1.02	1.02	1.32	5.39
Fenpyroximate	10.7	2.96	1.29	12.8	2.97
Etofenprox	11.4	2.55	1.00	2.03	4.72
Fenbutatin	12.6	2.40	2.90	2.72	4.51

3-4. Carryover assessment

Carryover was assessed after the injection of six injections of the highest concentration calibration sample. No analytes were detected above the LOQ (Fig. 3).

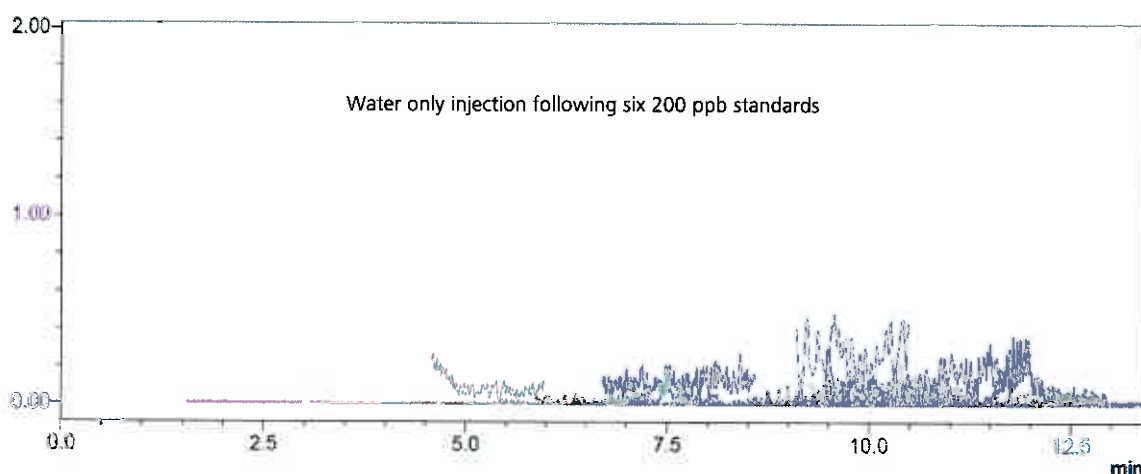


Fig. 3 Water only injection following the injection of six 200 ppb standards

3-5. System robustness

To investigate the robustness of the micro flow system infant food extract was repeatedly injected over 48 hours. In total, 162 injections were performed with infant food extract at 0.02 mg/kg. The stability of the system was

assessed in terms of peak area stability (Fig. 4) and retention time stability. The data showed excellent stability with peak area deviation typically 3-5% and retention time stability typically less than 0.12%RSD.

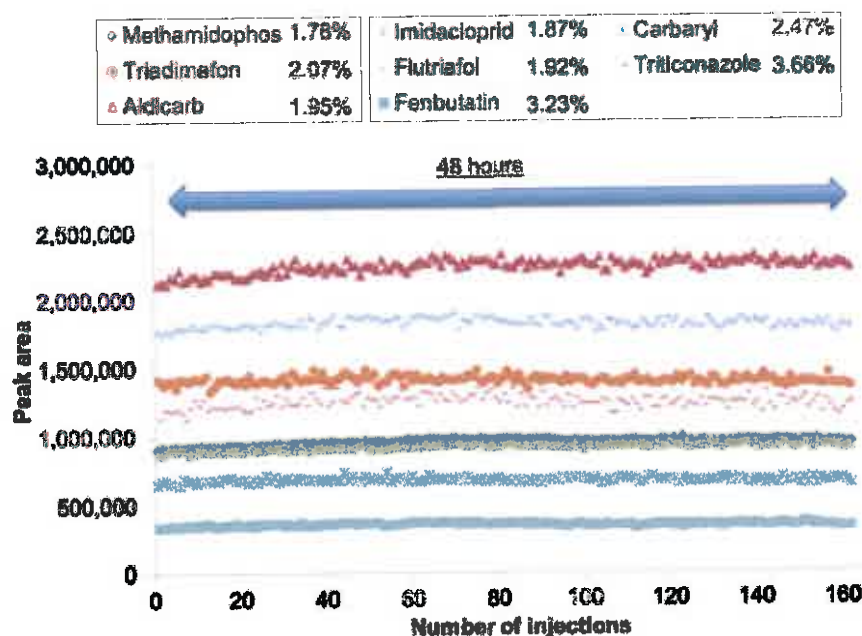


Fig. 4 Peak area stability (%RSD) of pesticides in infant food extract over 48 hours of repeat injection

4. Conclusion

- The developed micro flow LC methodology achieved the required MRL of 0.01 mg/kg for all 130 pesticides with all compounds eluted within 12.7 minutes.
- Initial validation data displayed excellent linearity for all compounds, low intra- and inter-day precision, no observed carryover, and good peak area and retention time stability over 48 hours.
- Precise data was acquired with the use of low pause and dwell times (1 msec. pause and 3msec. dwell times)
- Micro flow analysis was successfully carried out a UHPLC system capable of both conventional higher flow rates and lower micro flow rates.
- Micro flow LC is a possible alternative to conventional flow LC if extra sensitivity is needed or reduction in solvent consumption is required.

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An LCMS Method for the Detection of Cocoa Butter Substitutes, Replacers, and Equivalents in Commercial Chocolate-like Products

ASMS 2014 ThP632

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An LCMS Method for the Detection of Cocoa Butter Substitutes, Replacers, and Equivalents in Commercial Chocolate-like Products

Introduction

There is increasing demand for genuine cocoa butter (CB) in chocolate products in developed nations, however, this demand has created a shortage of CB and raised its costs. To overcome this, chocolate manufactures sometimes add vegetable-derived fats to some chocolate products to reduce costs while still maintaining desirable physical characteristics. It is of current interest to have a reliable method to detect, identify, and quantify the triacylglycerol (TAG) components of cocoa butter substitutes, replacers, and equivalents (CBEs) in chocolate products. Traditionally GC was used for this task, but due to the low volatility of triacylglycerides and their susceptibility to thermal decomposition, retention time is the only identifying factor

for the TAGs and typical GC analyses of this type can take 40 minutes. LCMS is able to not only provide faster throughput, but also has the additional advantage of allowing characterization of the TAG, including qualitative regiospecific analysis. We have developed a single, UHPLC column-based LCMS method to analyze the TAG components in commercial chocolate and chocolate-like products. This analysis has a runtime of 17minutes, making it suitable for relatively high throughput. Additionally, the method was very repeatable, with an interday variability of <7% for the absolute area counts of the three major TAGs in CB (POP,POS,SOS).

Materials and Method

A Shimadzu Nexera UHPLC coupled to a Shimadzu LCMS-8040 triple quadrupole mass spectrometer was utilized for this analysis. A pure CB standard was used as a

reference. Chocolate and chocolatey products were purchased in retail stores over a range of cocoa content.

Sample Preparation

For analysis, we slightly modified a sample preparation method originally used for algal oils. For analysis, 5mg of sample was weighed and then dissolved in a 3:1 Toluene-Isopropyl Alcohol solution. We then sonicated the

mixture for 5 minutes. The solution was filtered through a Thomson filter vial (P/N 35538-100) to remove sugars and other insoluble materials and diluted 5-fold using 3:1 Toluene-IPA and injected into the UHPLC-MS system.

Chromatography

Instrument	: Shimadzu Nexera UHPLC system
Column	: Shimadzu Shim-Pack XR-ODSIII (200x2.1mm,)
Mobile Phase A	: LC/MS Acetonitrile
Mobile Phase B	: 1:1 Dichloromethane-Isopropyl Alcohol
Gradient Program	: 48% B (initially) – gradient to 51% B (0-8.0 min) – gradient to 54% B (8.0 – 11.0 min) – gradient to 74% B (11.0-14.0 min) – hold at 74% B (14.0-15.0 min) – reequilibrate at 48% B (15.1-17 min)
Flow Rate	: 0.33 mL/min
Column Temperature	: 30°C
Injection Volume	: 1 µL

Mass Spectrometry

Instrument	: Shimadzu LCMS-8040 Triple Quadrupole Mass Spectrometer
Ionization	: APCI
Polarity	: Positive
Scan Mode	: Q3 Scan

Method for the Detection of Cocoa Butter Substitutes, Adulterants, and Equivalents in Commercial Chocolate-like Products

Results

Retail Chocolates from Hershey's, Lindt and Tcho, as well as a chocolatey candy - Charleston Chew - were compared against pure cocoa butter. The chocolates used were selected to cover a range of Cocoa content and purity. We specifically chose to use Hershey's Mr. Goodbar and Charleston Chews because they listed the use of vegetable oils in their ingredients list. As you can see in the chromatograms, the products that market themselves as pure chocolate have similar chromatograms in comparison to the pure CB.

We used an MS library that was provided to us by Dr. John Carney and Mona Koutchekina to identify the types of TAGs contained in the chocolates using the spectral information captured in the Q3 scans. A minimum similarity of 70 was required for a result to be considered a

match. In order to identify usage of CBEs, we applied the equation: $\%POP < 44.025 - 0.733 * \%SOS$, which was determined by the European Commission Joint Research Centre, which can detect around 2% CBE usage in CB content, or approximately 0.4% CBE content in chocolate. The chocolate products we tested all agreed with the expected results: All of the dark chocolate products we tested passed this specification, as well as Hershey's Milk Chocolate. The two products which had a higher %POP than is allowable, Mr. Goodbar and Charleston Chew, were selected specifically for the inclusion of vegetable oils. It may be informative to further test the accuracy of this testing method by adulterating cocoa butter with known quantities of CBEs. The data has been summarized in Table 1.

Table 1: Percentage of the major TAGs in CB in various chocolate products

Product	%POP	%POS	%SOS	%POP needs to be less than
Cocoa Butter	23.7%	46.9%	29.5%	43.8
Lindt 85% Cocoa	16.9%	46.4%	36.6%	43.8
TCHO 70% from Ghana	17.8%	46.1%	36.1%	43.8
TCHO 65% from Ecuador	20.9%	46.2%	32.9%	43.8
Hershey's Special Dark	20.0%	47.1%	32.9%	43.8
Hershey's Milk Chocolate	18.6%	46.6%	34.8%	43.8
Hershey's Mr Goodbar	44.8%	21.1%	34.1%	43.8
Charleston Chew	100.0%	0.0%	0.0%	44.0

An LCMS Method for the Detection of Cocoa Butter Substitutes, Replacers, and Equivalents in Commercial Chocolate-like Products

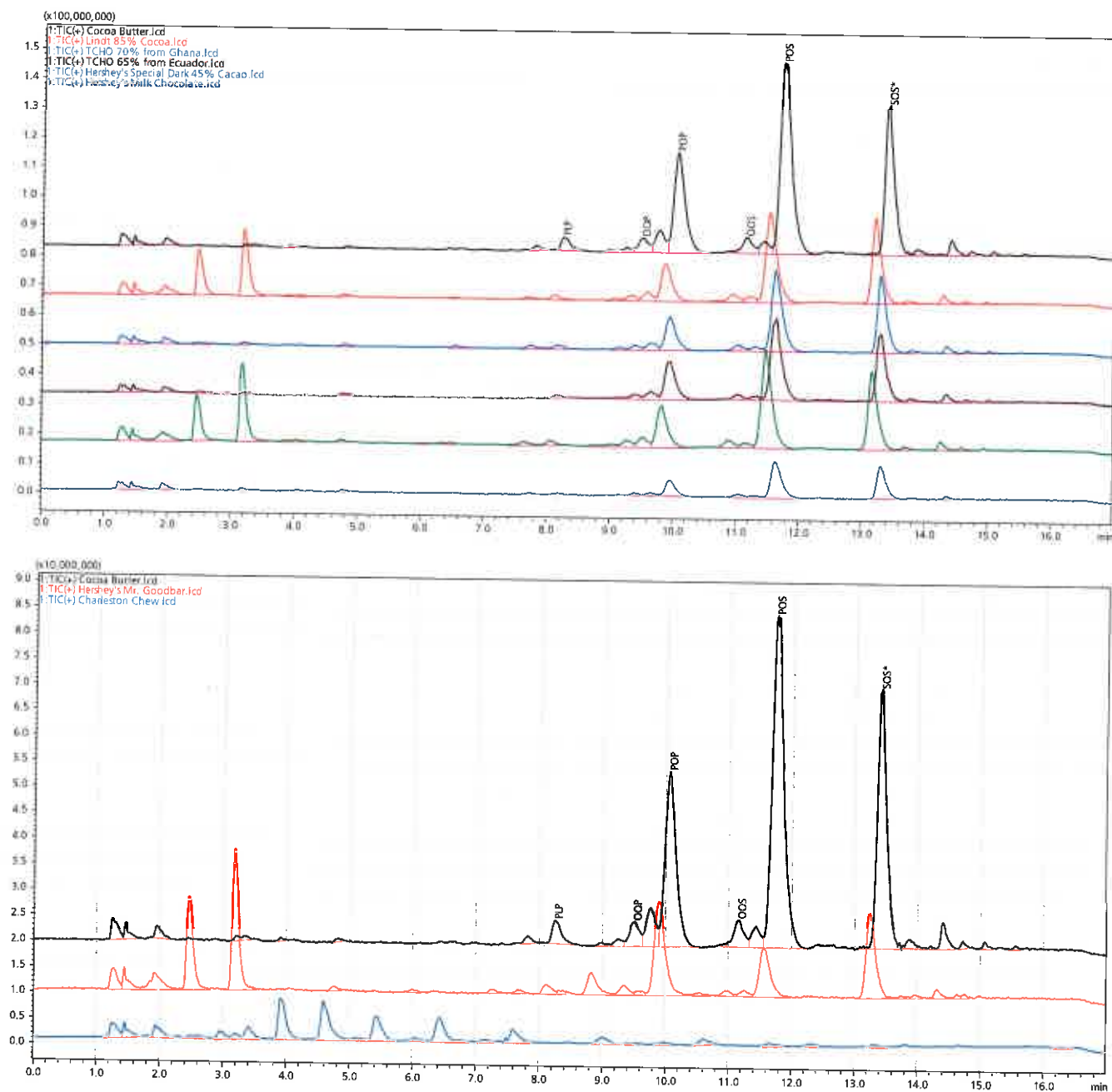


Figure 1. Chromatograms of the various chocolate products analyzed versus pure cocoa butter

Method for the Detection of Cocoa Butter Substitutes, Adulterants, and Equivalents in Commercial Chocolate-like Products

Conclusions

We have developed a 17 minute method for the rapid determination of CBE usage in chocolate products by using a UHPLC column and Q3 ion scans to analyze samples and then matching spectral information with an MS library of ion ratios for identifying TAGs.

Further studies could add a calibration curve to enable quantification of TAGs. This method should also provide a base method which can be modified to support TAG analysis in other product types.

References

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Acknowledgements

Dr. John Carney and Mona Koutchekinia for the invaluable information they provided.

Applying Micro Flow LC and High Speed Data Acquisition MS/MS to the Analysis of Pesticides Residues in Complex Spice Matrix

ASMS 2013 ThP-543

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Applying Micro Flow LC and High Speed Data Acquisition MS/MS to the Analysis of Pesticides Residues in Complex Spice Matrix

1. Introduction

In the UK, the Committee on Pesticide Residues in Food (PRiF) monitors levels of residues in food. The 2012 survey included the analysis of spices, which are recognised as a highly challenging matrix. Spices are known as "difficult" matrices, since they contain a bulk of extractable compounds (essential oils, pigments, etc.) causing significant problems during LC-MS/MS analysis including loss of chromatographic performance, system robustness, matrix effects and isobaric interference. Clean-up usually limits the scope of target analytes, thus, an extensive clean-up is not practical when faced with multi-residue analysis.

Here, we present the application of micro flow LC in comparison to conventional flow LC to minimise the challenges associated with the analysis of spice extracts. Lower flow rates can lead to increased sensitivity for concentration-dependent detection techniques such as electrospray ionization (ESI) mass spectrometry; which consequently permits the injection of smaller volumes of complex matrix into the LCMS system. Matrix effects were compared between conventional flow with the standard injection volume and micro flow employing a lower injection volume.

2. Methods

2-1. Sample preparation

Samples of spices were extracted using QuEChERS (quick, easy, cheap, effective, rugged and safe) methods developed by the Food and Environment Research Agency, UK. Sample extracts in acetonitrile were spiked with 210 pesticides. Sample extracts were directly injected or diluted 10 times with water; refer to main text.

2-2. LC-MS/MS analysis

Table 1 LCMS analysis conditions for the conventional flow and micro flow experiments

	Conventional flow	Micro flow
UHPLC	Nexera UHPLC system	
Flow rate	400 µL/min	90 µL/min
Mobile phase	A= Water with 5 mM ammonium formate B= Methanol with 5 mM ammonium formate	
Gradient	10% B - 100% B (12 min), 100% B (14 min), 10% B (17 min)	
Analytical column	ACQUITY UPLC HSS T3; 2.1 mm × 100 mm, 1.8 µm	ACQUITY UPLC HSS T3 1 mm × 100 mm, 1.8 µm
Column temperature	35°C	
Injection volume:	5 µL	
MS	LCMS-8040 triple quadrupole mass spectrometer	
Ionisation	Electrospray, positive and negative mode	
SRM	210 pesticides (469 SRMs) Dwell 3 msec. / Pause 1 msec.	
Desolvation line	250°C	200°C
Drying/Nebulising gas	15 L/min, 2 L/min	
Heating block	400°C	

3. Results and discussion

3-1. Conventional flow compared to micro flow

An established pesticide method using a 2.1mm ID × 100mm column and a 400 µL/min flow rate was compared to a micro flow method. Linear velocity was maintained during the micro flow analysis on a 1.0 mm ID × 100 mm column by using a 90 µL/min flow rate. A chromatogram of the micro flow LC is displayed in Fig. 1.

Both methods were utilised for the analysis of 210 pesticides in spice extracts. A comparison of the

conventional flow method and the micro flow method resulted, on average for all 210 pesticides, in an increase of 1.9 times peak height. Peak area was found to increase on average 2.5 times. Signal to noise ratios were not calculated as noise was typically extremely low or not visible in the scheduled MRM window. A comparison of selected compounds is displayed in Fig. 2.

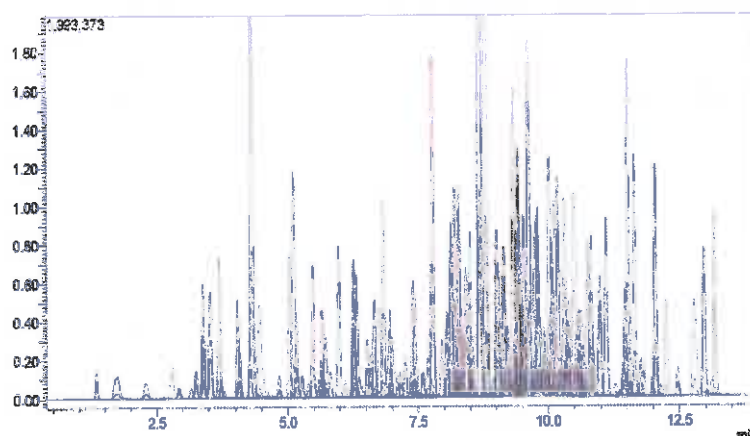


Fig. 1 Micro flow analysis (90 µL/min) of 210 pesticides in spice extract (0.05 mg kg⁻¹) using the Nexera UHPLC and the LCMS-8040

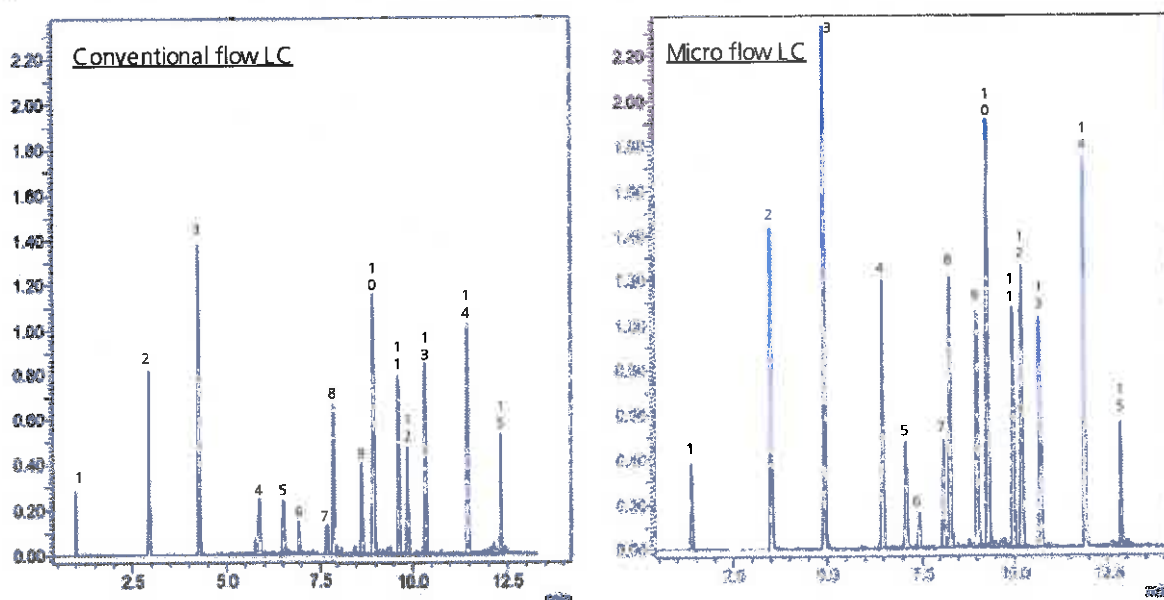


Fig. 2 Conventional flow analysis (left) at 400 µL/min and micro flow analysis (right) at 90 µL/min for selected pesticides over the run time. Injection volume 5 µL of spice extract (0.005 mg kg⁻¹) diluted 10 times with water.

Applying Micro Flow LC and High Speed Data Acquisition MS/MS to the Analysis of Pesticides Residues in Complex Spice Matrix

3-2. Linearity comparison

Linearity was investigated over a seven-point calibration with samples ranging from 0.005 mg kg⁻¹ - 0.2 mg kg⁻¹. Fig. 3 compares the calibration curves for nine pesticides analysed using conventional flow LC and micro flow LC. The same set of calibration samples was used in both

experiments. Linearity was excellent for both conventional flow and micro flow; typically $R^2 > 0.998$ (No weighting). Fig. 3 also highlights the increased peak area achieved by micro flow LC.

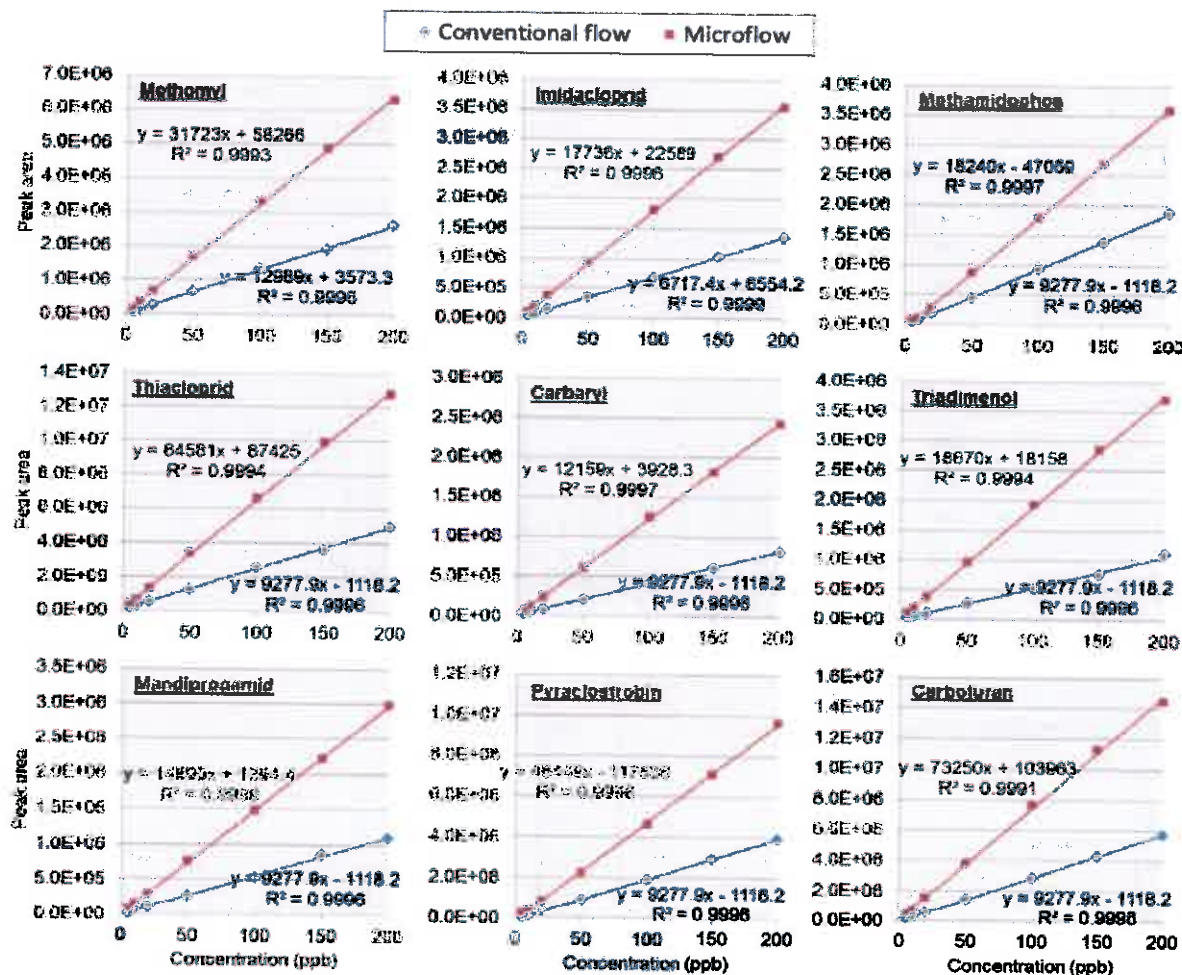


Fig. 3 Comparison of calibration curves using conventional flow LC and micro flow LC. Calibration samples prepared at 0.005 mg kg⁻¹ - 0.2 mg kg⁻¹ in acetonitrile extracts; samples subsequently diluted 10 times with water and injected

3-3. Matrix effects comparison

As a consequence of the superior sensitivity demonstrated by micro flow LC, injection volumes were reduced. Injection volumes were initially reduced by 40% from 5 μ L to 3 μ L (acetonitrile extract diluted 10 times with water), which achieved similar peak height responses for most pesticides to conventional flow with a 5 μ L injection. Given the

reduced injection volume, samples were analysed by micro flow LC without dilution; consequently a 0.3 μ L (not diluted) injection was performed. Fig. 4 displays a comparison of matrix effects using conventional flow with a 5 μ L injection (10x diluted sample) and micro flow with a 0.3 μ L injection (not diluted; acetonitrile extract).

g Micro Flow LC and High Speed Data Acquisition S to the Analysis of Pesticides Residues Complex Spice Matrix

Matrix effects were reduced for a large number of compounds with the smaller injection volume afforded by the micro flow method, typically by approximately 10-20%.

Furthermore, the ability to inject acetonitrile extracts directly saves on the time needed to dilute samples and improves compound stability.

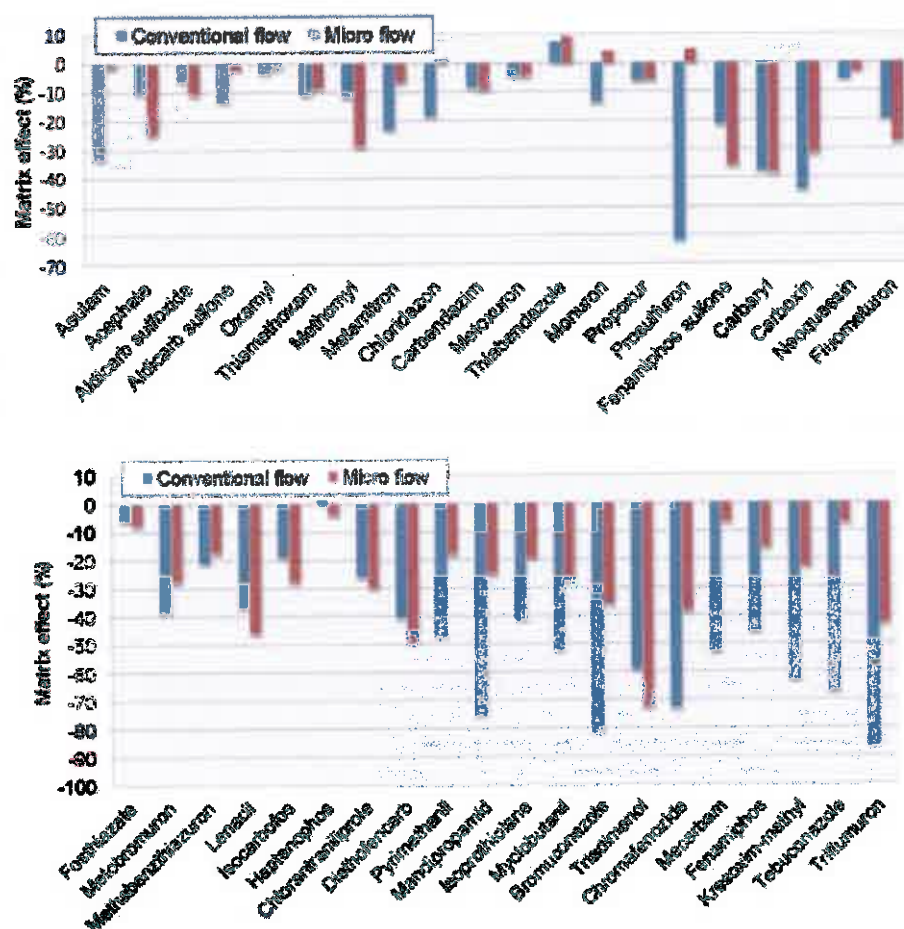


Fig. 4 Comparison of matrix effects in conventional flow LC (5 μ L injection of an acetonitrile extract diluted 10 times with water) and micro flow LC (0.3 μ L injection of an undiluted acetonitrile extract). Pesticides added at 0.005 mg kg⁻¹ before dilution. Errors were typically less than 10%RSD.

4. Conclusion

- Application of a micro flow LC method, in comparison to a conventional flow LC method, provided on average an increase in peak height of 1.9 times for 210 pesticides.
- The increased sensitivity afforded by micro flow LC was used to reduce injection volumes by 40%.
- Due to the reduced injection volume the direct injection of solvent extracts was possible without the need for dilution
- A combination of the reduced sample injection volume and the injection of solvent extracts reduced matrix effects by 10-20% for many pesticides in complex spice matrix.
- Micro flow LC is a possible alternative to conventional flow LC if improved sensitivity is required.

Applying Micro Flow LC and High Speed Data Acquisition MS/MS to the Analysis of Pesticides Residues in Complex Spice Matrix

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Characterization of Flavonoids and Phytoestrogens in an Extract of Pueraria Mirifica by UHPLC-MS-MS

ASMS 2013 MP-691

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Characterization of Flavonoids and Phytoestrogens in an Extract of Pueraria Mirifica by UHPLC-MS-MS

1. Introduction

Pueraria mirifica [Leguminosae] is a plant species native to Thailand whose tuberous roots are used medicinally for their anti-ageing properties. *P. mirifica* is a source of several phytoestrogens and flavonoids such as deoxymiroestrol, puerarin, genistein, and many others. Renewed interest in the herb as well as higher standards of identity and purity demand accurate and precise methods for detecting the various compounds in *P. mirifica* extracts both qualitatively and quantitatively. [1–3]

UHPLC-MS-MS is an effective strategy to characterize such extracts and accurately measure its key components. A selective and sensitive UHPLC-MS-MS method meeting these requirements was developed.



Fig. 1 Roots of Pueraria Mirifica

2. Methods

A Shimadzu Nexera UHPLC with an LCMS-8040 fast-scanning triple quadrupole mass spectrometer was used for analysis. A Shimpack XR-ODS III column (1.6 μ m, 2 $\times$ 50 mm) was used with a mobile phase of 0.1% formic acid and acetonitrile. The column temperature was 50°C

and the injection volume was 5 μ L. ESI or APCI ionization was used either independently, or in a combined dual ionization source (DUIS). Continuous polarity switching was used to enable measurement in both positive and negative mode throughout each run.

LC Column	: XR-ODS III (2 $\times$ 50 mm, 1.6 μ m)	Column Temp	: 50°C
Mobile Phase A	: 0.1% Formic Acid	Nebulizing Gas	: 3 L/min
Mobile Phase B	: Acetonitrile	Drying Gas	: 15 L/min
Flow Rate	: 0.5 mL/min	DL Temp	: 300°C
Probe Voltage	: 4.5 kV (+); 3.5 kV (–)	Heat Block Temp	: 200°C

	Ret. Time	Channel	Peak #	Area	Height	Conc (ng/mL)	
Puerarin	4.084	Ch1254nm	17	228007	90540	8250	*
Daidzin	4.427	Ch1254nm	19	55601	26514	2012	**
Genistin	4.847	Ch1254nm	23	16461	8938	335.6	***
Daidzein	5.526	Ch1254nm	28	23838	12704	486	*
Genistein	6.071	Ch1254nm	31	9341	4960	190	***
Deoxymiroestrol (total)	6.463	Ch1254nm	34	11470	4752	234	***

* Calculated by LCMS with external standards
 ** Calculated by puerarin relative UV response
 *** Calculated by daidzein relative UV response

Calculated results for quantitative analysis of selected flavonoids in Pueraria Mirifica

Characterization of Flavonoids and Phytoestrogens in Extract of *Pueraria Mirifica* by UHPLC-MS-MS

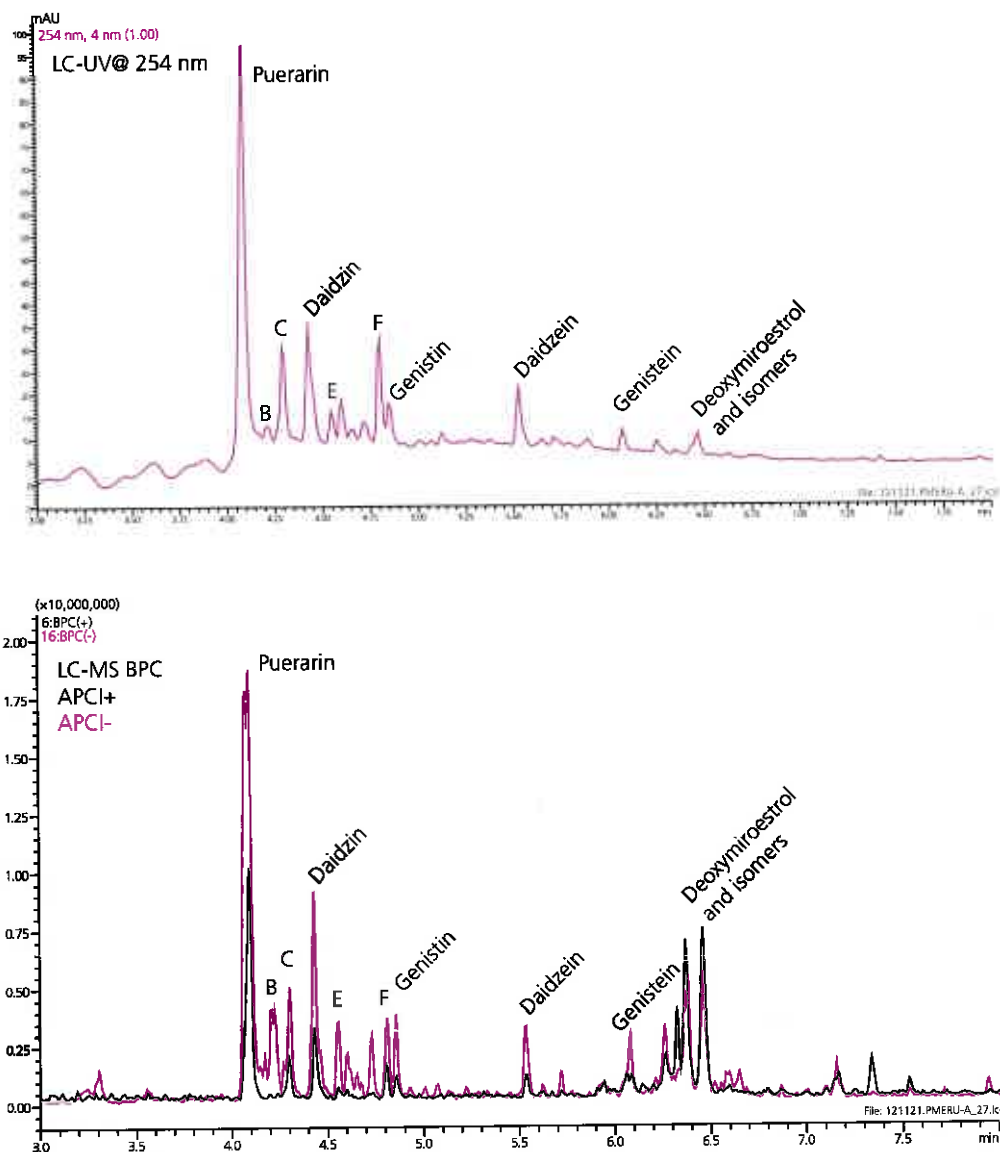


Fig. 2 LC-UV chromatogram (top) and LC-MS base peak chromatogram (bottom) of an injection of the *P. mirifica* extract.

3. Results and Discussion

MS measurement was carried out in several modes, including full scan, selected ion monitoring, multiple reaction monitoring, and product ion scan modes. Tandem mass spectra were compared with authentic standards or published spectra to propose identifications for each compound. In particular, the tandem mass spectra of

O-linked and C-linked flavonoid glycosides were examined and could be distinguished based on the fragmentation patterns of the glycoside ring. In addition, several isomers of deoxymiroestrol were detected and identified based upon their similar fragmentation patterns.

Characterization of Flavonoids and Phytoestrogens in an Extract of *Pueraria Mirifica* by UHPLC-MS-MS

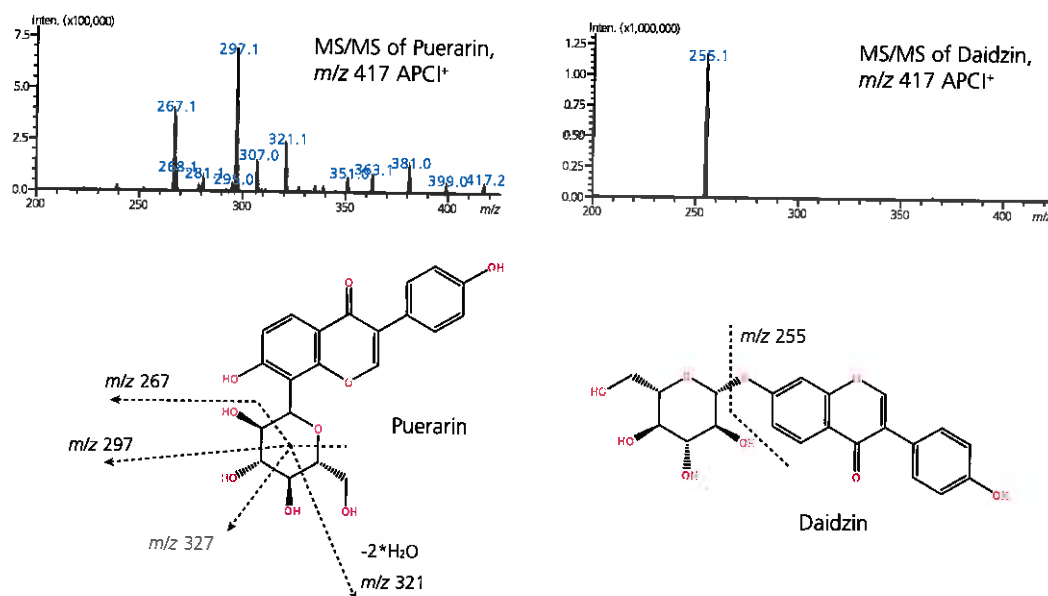


Fig. 3 Tandem mass spectra of puerarin and the peak identified as daidzin in positive mode APCI (Top left and right, respectively). Structure assignment for fragments of puerarin (bottom left) and daidzin (bottom right). The C-linked glycoside puerarin fragments differently than the O-linked glycoside daidzin.

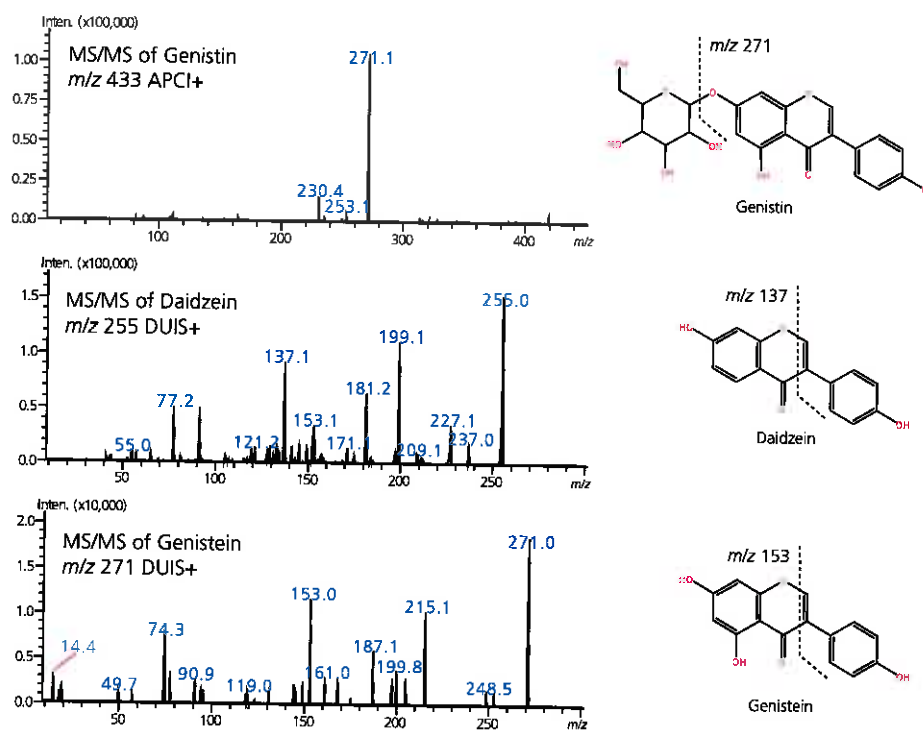


Fig. 4 Tandem mass spectrum and structure assignment of genistin, daidzein, and genistein.

Characterization of Flavonoids and Phytoestrogens in Extract of *Pueraria Mirifica* by UHPLC-MS-MS

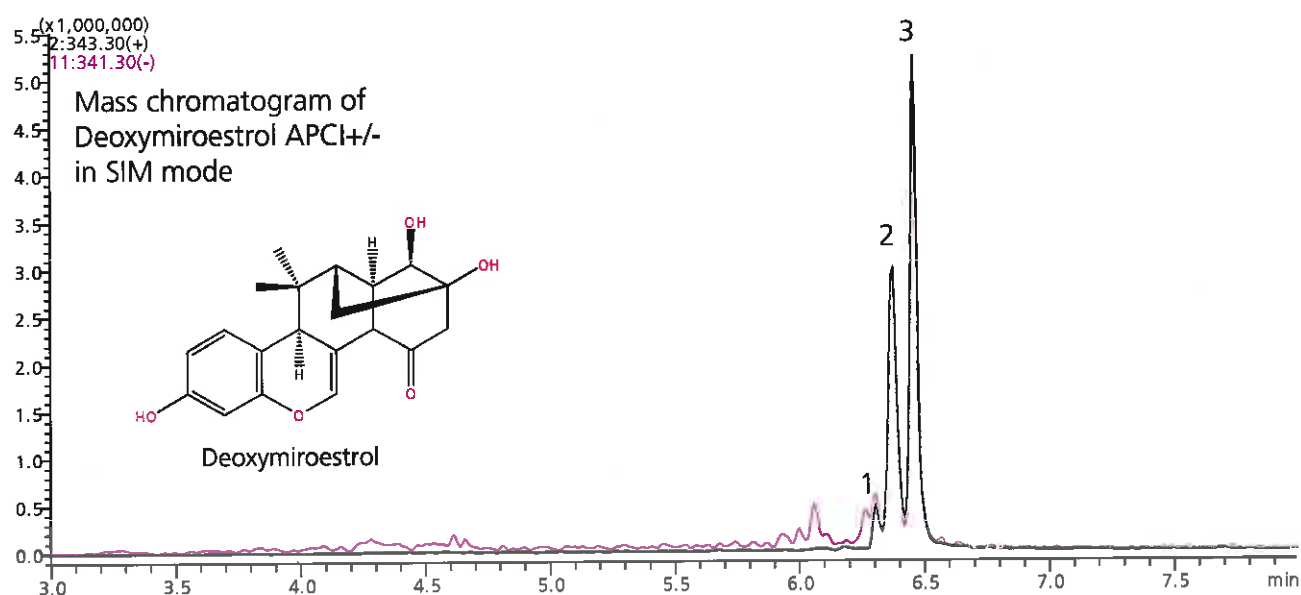


Fig. 5 Mass chromatogram of deoxymiroestrol in APCI with polarity switching. Several isomers of deoxymiroestrol, labeled 1–3, can be clearly distinguished

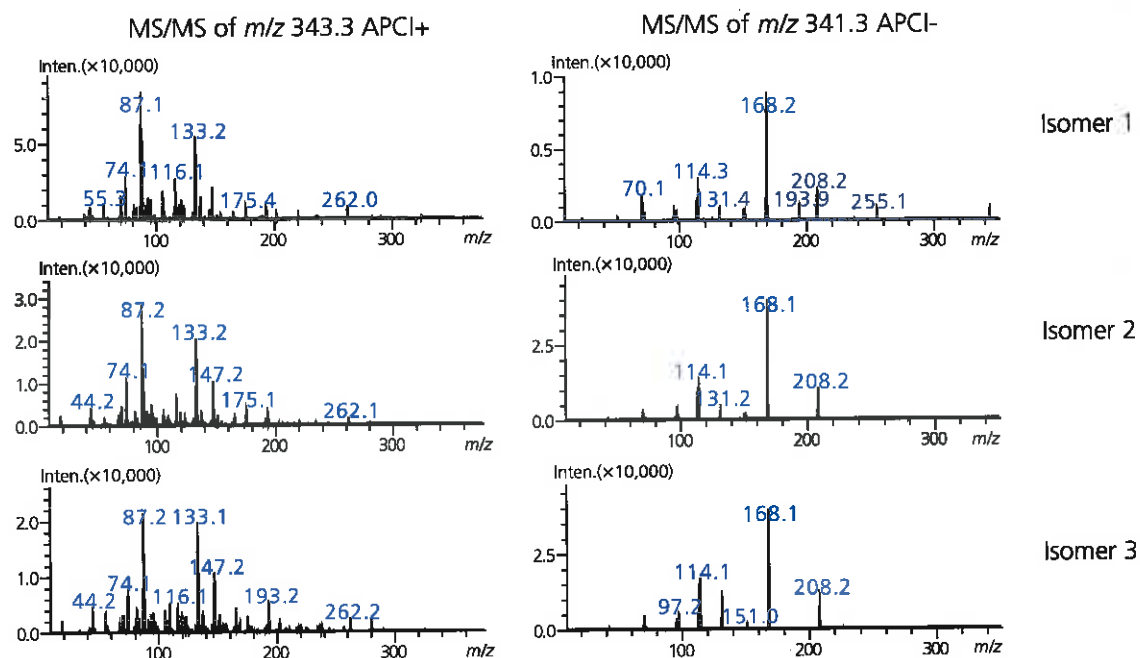


Fig. 6 Tandem mass spectra of three isomers of deoxymiroestrol. The left column are APCI+ spectra and the right column are APCI- spectra. Each row corresponds to one of the isomers of deoxymiroestrol.

Characterization of Flavonoids and Phytoestrogens in an Extract of Pueraria Mirifica by UHPLC-MS-MS

4. Conclusion

UHPLC-MS-MS was used to characterize an extract of *P. mirifica*, simultaneously revealing several O- and C-linked glycosides as well as several newly detected isomers of deoxymiroestrol. The fast scan speed and rapid polarity

switching of the LCMS-8040 triple quadrupole mass spectrometer allowed a full range of data to be acquired for each run with ESI and APCI ionization (DUI mode), enabling comprehensive characterization of the extract.

5. References

- [1] Ching-Che Lin et al. (2005) *J. Sep. Sci.* 28, 1785–1795
- [2] Raymond E. March et al. (2004) *Int. J. Mass Spectrom.* 232, 171–183
- [3] Satoko Shimokawa et al. (2012) *Nat. Prod. Res.* 27, 371–378

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Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

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Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

1. Introduction

Veterinary drugs are widely used in breeding of marine products. However, residual veterinary drugs could enter human body and harm to human health. Therefore, those veterinary drugs in marine products have been strictly regulated in the world. In recent years, the China government continues to strengthen supervision and is developing quicker and highly sensitive analytical method.

Usually, the qualitative method of LC/MS/MS is based on the ratio of intensities between qualitative ion and quantitative ion. This paper describes ultra high performance liquid chromatography-triple quadrupole mass spectrometry for rapid screening of 68 veterinary drugs which belong to 12 categories.

2. Methods and Materials

Sample Preparation

Samples of marine products were extracted with acetonitrile. After centrifugation, concentration and filtration, the final extract was injected to the LC-MS/MS instrument.

LC/MS/MS Analysis

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A₃ degasser, and an SIL-30AC autosampler. A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was

connected to the Shimadzu UHPLC instrument via an ESI interface. Samples were separated on a Shim-pack XR-ODSIII (50 mmL. × 2.0 mm i.d., 1.6 μm). A flow rate of 0.4 mL/min was used together with a gradient elution.

Analytical Conditions

UHPLC (Nexera system)

Column	: Shim-pack XR-ODSIII (50 mmL. × 2.0 mm i.d., 1.6 μm)
Mobile phase A	: 0.1% formic acid
Mobile phase B	: acetonitrile
Flow rate	: 0.4 mL/min
Elution mode	: gradient elution
Column temperature	: 40°C
Injection volume	: 10 μL

MS/MS (LCMS-8040 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: positive & negative
Probe voltage	: +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 10 L/min
DL temperature	: 250°C
BH temperature	: 400°C



Detection of 68 Veterinary Drugs in Marine Products by Ultra High Performance Chromatography/Triple Quadrupole Mass Spectrometry

Table 1 List of veterinary drugs

Sulfonamides [12]	1-Dehydrotestosterone Sulfate	Nitro imidazoles [5]
Sulfacetamide	Danazol	Rondazole
Sulfadiazine	Fluoxymesterone	2-methyl-5-nitroimidazole
Sulfathiazole	Testosterone	Metronidazole
Sulfapyridine	17-alpha-methyl/testosterone	4-Nitroimidazole
Sulfamerazine	Methadrostenolone	Iprnidazole
Sulfamethazine	Nandrolone	Nitrofuran metabolites [4]
Sulfamethoxypyridazine	19-nor-4-androstene-3,17-dione	Furazolidone
Sulfchloropyridazine	Trenbolone	Furaladone
Sulfamethoxazole	Megestrol-17-acetate	Nitrofurantion
Sulfisoxazole	Medroxyprogesterone	Fusadinium
Sulfadimethoxine	Medroxyprogesterone-17-acetate	
Sulfachinoxalin	Norgestrel	Tetracyclines [5]
	Chloramadinone 17-acetate	Tetracycline hydrochloride
	Norethindrone	Oxytetracycline
	Progesterone	Demeclocycline hydrochloride
		Chlorotetracydic hydrochloride
Quinolones [10]	Macrolides [5]	Doxycycline
Pipemidic acid	Spiramyon	Uncosamides [2]
Enoxacin sesquihydrate	Ileucornan hydrae	Uticorn sesquihydrate
Ofloxacin	Erythromycin	Uticorn sesquihydrate
Norfloxacin	Tilmicosin	
Ciprofloxacin hydrochloride	Acetylsaleryltylosin Tartrate	Other drug [5]
Lomefloxacin		Trimethoprim
Danofloxacin	Chloramphenicols [3]	Malachite green oxalate
Enrofloxacin	Thiamphenicol	Leucomalachue green
Sarafloxacin hydrochloride	Florfenicol	Basic violet 3
Cinoxacin	Chloramphenicol	Leucoprostal violet
Hormones [17]		
19-nor-4-androstene-3,17-dione		

Using a polarity switching speed of 15 msec and a scan speed of 15,000 u/sec, MRM spectra were generated in both positive and negative ionization. And fast polarity switching helps to provide information rich production spectra resulting in better detection and identification.

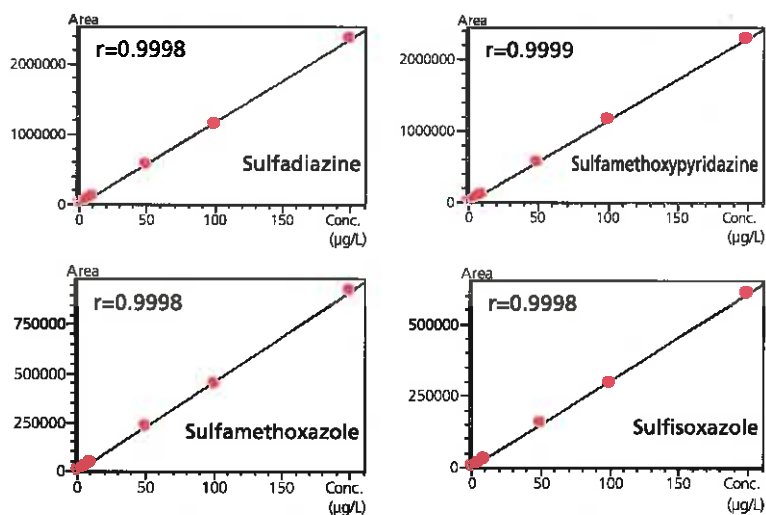


Fig. 1 Representative calibration curves of sulfonamides

Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

Table 2 Repeatability of 68 drugs (n=6)

No.	Compound	%RSD		No.	Compound	%RSD	
		R.T	Area			R.T	Area
1	Sulfacetamide	0.40	4.43	35	Medroxyprogesterone-17-acetate	0.14	3.33
2	Sulfadiazine	0.27	3.91	36	Norgestrel	0.21	4.13
3	Sulfathiazole	0.21	3.13	37	Chlormadinone-17-acetate	0.14	4.09
4	Sulfapyridine	0.32	2.85	38	Norethindrone	0.27	3.46
5	Sulfamerazine	0.28	3.86	39	Progesterone	0.17	3.28
6	Sulfamethazine	0.18	2.40	40	Spiramycin	0.67	1.37
7	Sulfamethoxypyridazine	0.21	4.02	41	leucomycin hydrate	0.11	0.85
8	Sulfchloropyridazine	0.11	3.47	42	Erythromycin	0.08	1.88
9	Sulfamethoxazole	0.08	4.46	43	Tilmicosin	0.05	2.01
10	Sulfisoxazole	0.07	4.41	44	Acetylisovaleryltylosin tartrate	0.07	1.28
11	Sulfadimethoxine	0.10	2.06	45	Thiamphenicol	0.15	3.34
12	Sulfachinoxalin	0.09	4.00	46	Florfenicol	0.09	2.39
13	Pipemidic acid	0.23	2.82	47	Chloramphenicol	0.16	1.68
14	Enoxacin sesquihydrate	0.50	1.68	48	Ronidazole	0.82	1.25
15	Ofloxacin	0.14	2.88	49	2-methyl-5-nitroimidazole	0.69	1.34
16	Norfloxacin	0.09	2.61	50	Metronidazole	0.76	1.37
17	Ciprofloxacin hydrochloride	0.08	2.18	51	4-Nitroimidazole	0.51	1.10
18	Lomefloxacin	0.04	2.14	52	Iprnidazole	0.07	0.91
19	Danofloxacin	0.02	2.42	53	Furazolidone	0.15	2.36
20	Enrofloxacin	0.04	2.04	54	Furaltadone	0.09	2.13
21	Sarafloxacin hydrochloride	0.03	2.27	55	Nitrofurantion	0.27	3.49
22	Cinoxacin	0.04	1.94	56	Furacilinum	0.28	4.66
23	19-nor-4-androstene-3,17-dione	0.19	3.71	57	Tetracycline hydrochloride	0.65	1.60
24	1-Dehydrotestosterone sulfate	0.19	2.57	58	Oxytetracycline	0.65	1.60
25	Danazol	0.23	3.51	59	Demeclocycline hydrochloride	0.64	1.80
26	Fluoxymesterone	0.21	4.78	60	Chlorotetrachlie hydrochloride	0.65	1.60
27	Testosterone	0.22	3.05	61	Doxycycline	0.65	2.00
28	17-alpha-methyltestosterone	0.12	3.67	62	Lincocin hydrochloride	0.11	2.37
29	Methadrolone	0.20	1.44	63	Clindamycin	0.19	3.26
30	Nandrolone	0.19	2.89	64	Trimethoprin	0.29	1.72
31	19-nor-4-androstene-3,17-dione	0.18	3.83	65	Malachite green oxalate	0.08	0.75
32	Trenbolone	0.23	2.94	66	Leucomalachite green	0.10	1.29
33	Megestrol-17-acetate	0.10	3.02	67	Basic violet 3	0.04	0.68
34	Medroxyprogesterone	0.25	3.17	68	Leucocrystal violet	0.10	1.83

3. Results and Discussion

The 68 veterinary drugs can be categorized into 12 groups. Fig.1 shows the representative calibration curves of sulfonamides. Excellent linearity was demonstrated in the range of 1 to 200 µg/L for sulfadiazine, sulfamethoxypyridazine, sulfamethoxazole and sulfisoxazole, with correlation

coefficients greater than 0.998. The repeatabilities of 68 drugs (1-10 µg/L) were investigated, and the %RSDs of peak area were less than 5%, and those for retention time were better than 0.9%, as shown in Table 2.

ation of 68 Veterinary Drugs in Marine Products by Ultra High Performance Chromatography/Triple Quadrupole Mass Spectrometry

Table 3 Recovery test of 52 compounds

	Compound	Recovery (%)		Compound	Recovery (%)
Sulfonamides	Sulfacetamide	88.6	Hormones	19-nor-4-androstene-3,17-dione	106.0
	Sulfadiazine	96.4		1-Dehydrotestosterone Sulfate	99.0
	Sulfathiazole	91.1		Danazol	93.0
	Sulfapyridine	106.3		Fluoxymesterone	113.5
	Sulfamerazine	94.0		Testosterone	106.0
	Sulfamethazine	97.2		17-alpha-methyltestosterone	94.7
	Sulfamethoxypyridazine	91.9		Methadrolone	90.5
	Sulfchloropyridazine	95.3		Nandrolone	95.0
	Sulfamethoxazole	103.1		19-nor-4-androstene-3,17-dione	80.0
	Sulfisoxazole	102.2		Trenbolone	96.1
	Sulfadimethoxine	94.1		Megestrol-17-acetate	95.0
	Sulfachinoxalin	93.9		Medroxyprogesterone	94.1
Quinolones	Pipemidic acid	81.0		Medroxyprogesterone-17-acetate	114.5
	Enoxacin sesquihydrate	68.0		Norgestrel	115.0
	Ofloxacin	88.0		Chloromadinone 17-acetate	110.5
	Norfloxacin	77.0		Norethindrone	111.6
	Ciprofloxacin hydrochloride	95.5		Progesterone	78.0
	Lomefloxacin	92.0	Macrolides	Spiramycin	89.0
	Danofloxacin	83.0		leucomycin hydrate	91.0
	Enrofloxacin	74.0		Erythromycin	84.0
	Sarafloxacin hydrochloride	76.0		Tilmicosin	104.0
Tetracyclines	Cinoxacin	82.5		Acetylisoaleryltylosin Tartrate	93.0
	Tetracycline hydrochloride	105.0	Chloramphenicols	Thiamphenicol	105.0
	Oxytetracycline	120.0		Florfenicol	81.5
	Demedocycline hydrochloride	110.0		Chloramphenicol	93.3
	Chlorotetrachdie hydrochloride	100.0			
	Doxycycline	115.0			

In this study, we studied the different marine products. The recoveries of drugs in fish samples (the concentration of spiked drugs ranged from 0.1 to 2 µg/kg) were summarized

in Table 3. The average recovery range of 52 compounds was from 74 to 120%.

4. Conclusions

A simultaneous and cost-effective method of identification and quantification of 68 veterinary drugs in marine products was developed.

Improved selectivity and sensitivity of the instrumental analysis was achieved by LC/MS/MS technique.

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Determination of 200 Residual Pesticides in Food by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

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Determination of 200 Residual Pesticides in Food by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

1. Introduction

Pesticides are widely used in crop planting and growth process. However, residual pesticides in food could enter human body and harm to human health. Therefore, the maximally allowed residual pesticides in food have strictly regulated in the world. In recent years, the China government continues to strengthen supervision and is developing quicker and highly sensitive analytical method. Usually, the qualitative LC/MS/MS is based on the ratio of intensities between targetions and reference ions.

But in some special occasions, especially for determination of low concentration samples, qualitative reliability is not high enough, which may cause false positive/negative results. So use of MS/MS library for screening and qualitative confirmation of the unknown pesticides is very meaningful. This paper describes UHPLC/MS/MS, a rapid and accurate screening method for determination of 200 residual pesticides in agricultural products.

2. Methods and Materials

Sample Preparation

The pesticides in food samples were extracted with acetonitrile. After centrifugation, solid-phase extraction with NH₂ sorbents was employed to purify the sample. After concentration and filtration, the final extract was injected to the LC-MS/MS instrument.

LC/MS/MS analysis

Analytical Conditions

UHPLC (Nexera, Shimadzu Corporation, Japan)

Column	: Shim-pack XR-ODSIII (150 mmL. × 2.0 mm i.d., 2.2 μm)
Mobile phase A	: 2 μmol/L ammonium acetate/0.1% formic acid water
Mobile phase B	: acetonitrile
Gradient program	: 10%B (0-1 min)-50%B (4 min)-75%B (20 min) -95%B (22-25 min)
Flow rate	: 0.4 μL/min
Oven temperature	: 40 °C
Injection volume	: 5 μL

MS/MS (LCMS-8040, Shimadzu Corporation, Japan)

Ionization	: ESI
Ionization voltage	: +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas flow	: 3.0 L/min
Drying gas pressure	: 20 L/min
DL temperature	: 250 °C
BH temperature	: 450 °C

ation of 200 Residual Pesticides in Food by Ultra High Performance Liquid atography/Triple Quadrupole Mass Spectrometry

3. Results and Discussion

Analytes' specific MS/MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimized using automated flow injection analysis. So, each compound was optimized in only a few minutes using the automated

software function provided in Labsolutions. The compound information and MS/MS parameters for 200 pesticides are listed in an excel document for using conveniently, as shown in Table 1.

Table 1 Representative compound information and MS/MS parameters of pesticides

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Serial	Q1 MS	Q3 MS	Target	Compound Name (ChE)	Compound Name (ChE)	Formula	MW	Isomer	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
173	173	288.1-47.6	Target	三唑酮	Triazophos	C ₁₂ H ₁₀ O ₃ P	272.2	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
174	174	32.6-6.4	Target	除虫菊	Pyrethrin	C ₂₁ H ₃₀ O ₆	370.4	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
175	175	408.1-16.2	Target	三唑酮	Triazophos	C ₁₂ H ₁₀ O ₃ P	272.2	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
176	176	288.1-47.6	Target	三唑酮	Triazophos	C ₁₂ H ₁₀ O ₃ P	272.2	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
177	177	141.0-21.7	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
178	178	89.0-16.6	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
179	179	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
180	180	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
181	181	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
182	182	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
183	183	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
184	184	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
185	185	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
186	186	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
187	187	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
188	188	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
189	189	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
190	190	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
191	191	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
192	192	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
193	193	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
194	194	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
195	195	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
196	196	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
197	197	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
198	198	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
199	199	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
200	200	84.0-14.9	Target	吡啶酮	Pyridinone	C ₆ H ₅ N	93.1	100-100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

For UHPLC/MS/MS analysis of residual pesticides which give positive and negative ions, the mobile phase was optimized. 200 pesticides were analyzed and the MRM chromatograms were obtained as shown in Fig. 1 The calibration curves, limits of quantitation (LOQs), repeatability, and recoveries were investigated. The calibration curves were given with good correlation coefficients greater than 0.999. LOQs for 93% of pesticides were better than 1 µg/kg.

The repeatability (%RSD) of standard pesticides at 10 µg/L was less than 5% for 95% of the target pesticides. Recoveries of pesticides spiked in a cucumber sample at 1 µg/kg were between 57.2% and 125.3%. Recoveries for 84% of all pesticides ranged from 80% to 120%. Representative quantitative results are shown in Table 2. The results indicate that this method is rapid and reliable.

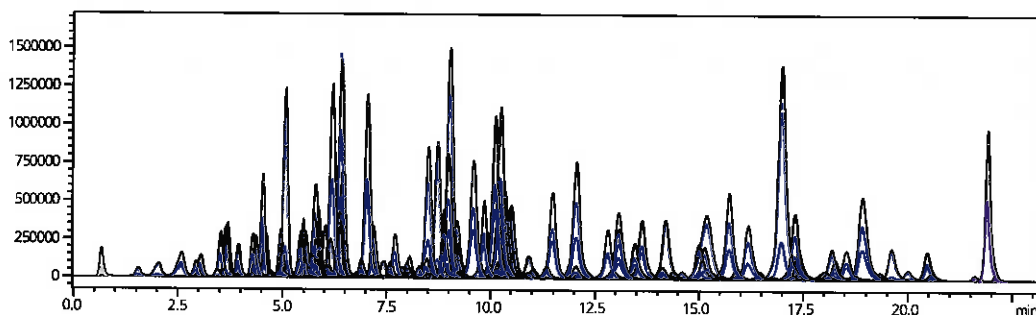


Fig. 1 MRM chromatograms of 200 standard pesticides (50 µg/L each)

Determination of 200 Residual Pesticides in Food by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

Table 2 Representative quantitative results of pesticides

No.	Name	Y=aX+b	Linearity Range (µg/L)	r	LQ (µg/kg)	%RSD (1 µg/L)	Recovery (1 µg/kg)
52	Diethofencarb	Y=(11607.9)X+(11387.8)	0.5~200	0.9997	0.22	3.21	94.20
53	Diethyltoluamide	Y=(79943.4)X+(63810.1)	0.5~200	0.9993	0.01	2.79	92.70
54	Dimepiperate	Y=(39447.4)X+(3718.42)	0.5~200	0.9997	0.02	2.67	89.60
55	Dimethirimol	Y=(30050.7)X+(21635.8)	0.5~200	0.9993	0.02	2.13	93.80
56	Dimethoate	Y=(11607.9)X+(26206.1)	0.5~200	0.9992	0.02	1.87	93.10
57	Dimethomorph	Y=(13116.6)X+(-2309.89)	0.5~200	0.9998	0.07	3.10	93.40
58	Diniconazole	Y=(4273.37)X+(-1549.44)	0.5~200	0.9991	0.23	3.39	110.70
59	Dinotefuran	Y=(14619.3)X+(3801.66)	0.5~200	0.9993	0.02	3.42	95.40
60	Diuron	Y=(7835.16)X+(1847.00)	0.5~100	0.9997	0.07	4.23	104.00
61	Dymron	Y=(246705)X+(158759)	0.5~200	0.9991	0.00	2.44	87.90
62	Epoxiconazole	Y=(8801.47)X+(4813.78)	0.5~200	0.9996	0.05	4.58	77.10
63	Esprocarb	Y=(34385.0)X+(-9941.46)	0.5~200	0.9999	0.07	3.68	79.00
64	Ethametsulfuron-methyl	Y=(13881.3)X+(-1589.33)	0.5~200	0.9994	0.02	4.46	102.10
65	Ethofencarb	Y=(41267.8)X+(15848.2)	0.5~100	0.9998	0.04	2.95	99.50
66	Ethion	Y=(6519.60)X+(-5245.53)	0.5~200	0.9995	0.06	1.90	90.70
67	Ethoprophos	Y=(14174.9)X+(15071.9)	0.5~200	0.9993	0.05	3.46	85.20
68	Fenamiphos	Y=(23409.3)X+(10202.8)	0.5~200	0.9993	0.02	2.65	99.50
69	Fenarimol	Y=(303.283)X+(728.986)	2~200	0.9997	2.64	8.84	57.20
70	Fenobucarb	Y=(4791.08)X+(6489.96)	0.5~200	0.9990	0.09	4.52	87.80
71	Fenoxycarb	Y=(2134.24)X+(-225.561)	0.5~200	0.9999	0.83	4.48	105.30

Nowadays, laboratories are expanding the number of target pesticides. Rapid and reliable method for determination of trace amount of residual pesticides is necessary. Usually, the qualitative LC/MS/MS is based on the ratio of intensities between target ions and reference ions.

But in some cases, especially for determination of pesticides at low concentration, qualitative reliability is not high enough, which may cause false positive/negative results. So use of the MS/MS spectra library for searching and qualitative confirmation of the pesticides is very meaningful.

Type	Event#	+/-	Compound Name	m/z	Time (0.350 min - 22.413 min)
Product Ion Scan	12	-	氧乐果	100.00 > 50.00, 250	
MRM	13	+	涕灭威亚砷	229.00 > 166.10	
Product Ion Scan	14	+	涕灭威亚砷	100.00 > 50.00	
MRM	15	+	呋虫胺	203.10 > 129.10, 20	
Product Ion Scan	16	+	呋虫胺	100.00 > 50.00, 250	
MRM	17	-	吡啶虫啉	244.10 > 100.10	
Product Ion Scan	18	+	涕灭威亚砷	100.00 > 50.00, 3	
MRM	19	-	吡啶虫啉	224.10 > 127.10, 22	

Acq. Time:	3.117	-	3.717	min	Compound Name:	涕灭威亚砷

Ch	Precursor m/z	Product m/z	Pause Time (msec)	Dwell Time (msec)	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
Ch1	245.10	166.10	1.0	1.0	-12.0	-15.0	-17.0
Ch2	245.10	100.10	1.0	1.0	-12.0	-20.0	-19.0
Ch3							

Fig. 2 Setting of product ion scan event triggered by MRM

ation of 200 Residual Pesticides in Food by Ultra High Performance Liquid atography/Triple Quadrupole Mass Spectrometry

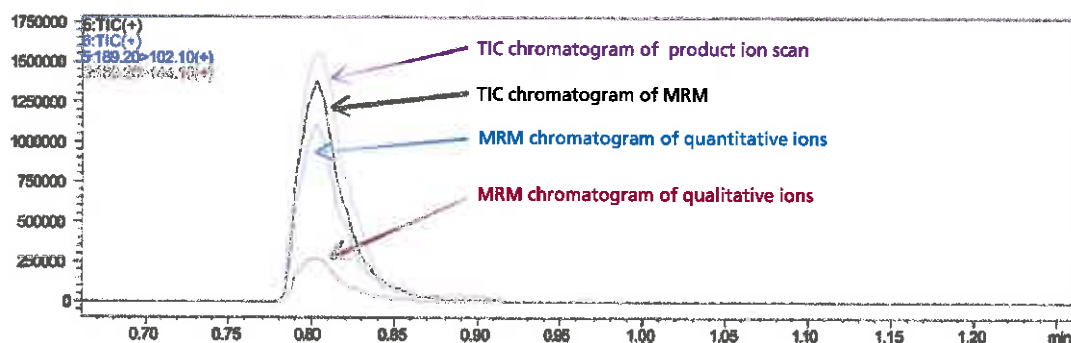


Fig. 3 Mass chromatograms of a target pesticide

A simultaneous quantitative and qualitative method file with a function of product ion scan event triggered by MRM is shown in Fig. 2 Both the TIC chromatogram of MRM and the TIC chromatogram of product ion scan were obtained as shown in Fig. 3 By searching the spectrum of product ion

scan using the pesticide spectra library, a pesticide (isoprocab) was found (Fig. 4). Here, 9 pesticides were detected, and their similarity indexes were over 70 when the concentrations of pesticides were larger than 1 $\mu\text{g/kg}$ (Table 3).

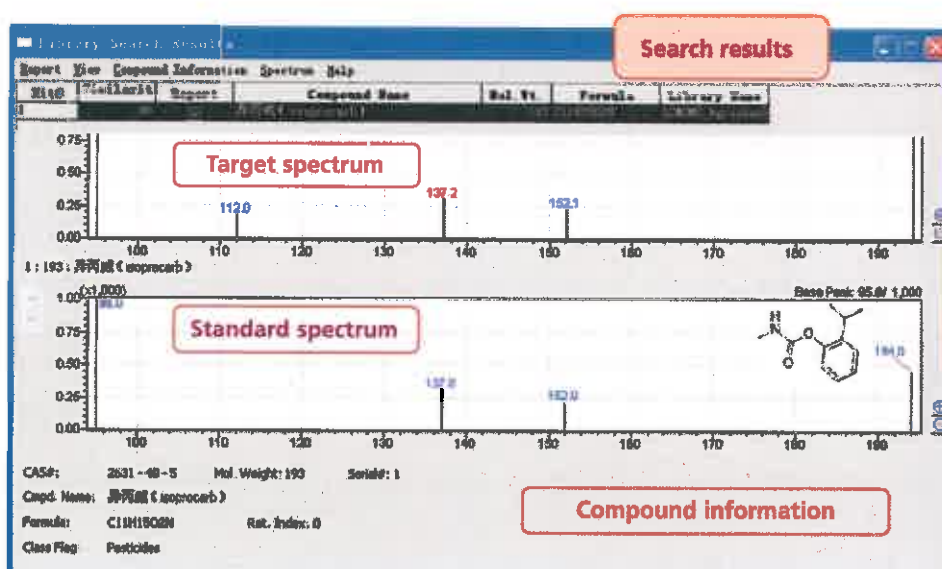


Fig. 4 Library search result of isoprocab detected in cucumber sample

Determination of 200 Residual Pesticides in Food by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

Table 3 The results of detected pesticides and their similarity score of library searching

No.	Compound	Concentration (µg/kg)	Similarity index
1	Aldicarb-sulfone	3.26	72
2	Carbaryl	2.54	91
3	Carbendazim	3.87	100
4	Carbofuran-3-hydroxy	11.90	89
5	Dimethomorph	0.19	67
6	Isoprocarb	3.31	88
7	Methomyl	4.95	99
8	Metolcarb	4.02	82
9	Propamocarb	0.77	88

4. Conclusions

An LC/MS/MS method has been developed for quantitative and qualitative analysis of 200 pesticides in food sample. Method validation was demonstrated to evaluate instrument capability. The results showed that this method

is rapid and reliable. For qualitative confirmation, the MS/MS spectra library search was performed, which made the qualitative results more reliable.

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Exploring the application of a universal method for pesticide screening in foods using a high data acquisition speed MS/MS

ASMS 2012 WP27-574

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Exploring the application of a universal method for pesticide screening in foods using a high data acquisition speed MS/MS

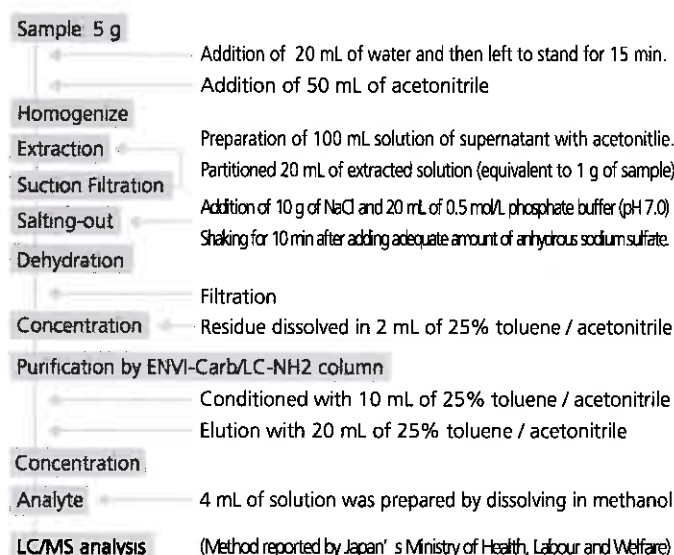
Introduction

Effective management, use, and disposal of agrochemicals, particularly pesticides, is an increasingly important health and environment issue in developing countries where economies may be heavily reliant on agriculture. The conventional approach to monitor these pesticides is to develop highly optimized triple quadrupole MRM methods to achieve the required levels of sensitivity, selectivity and

speed of analysis whilst still providing confidence in pesticide identification. In this study LCMS technology, developed for ultra-fast scanning MRM analysis, allows the possibility of a single generic 'universal' method. High speed MRM analysis and a generic parameters were used for screening 172 pesticides (344 MRM transitions) with 5 msec dwell and 1 msec pause times in food matrices.

Materials and Methods

Sample Preparation



Analytical Conditions

HPLC : Nexera UHPLC system

Column : Shim-pack XR-ODSII (75 mm x 2 mm I.D., 2.2 µm)
Mobile phase : A ; 5 mM ammonium acetate – water
B ; 5 mM ammonium acetate – methanol
Gradient program : 30% B (0 min.) → 80% B (4 min.) → 95% B (10-15 min.) → 30% B (15.01-20 min.)
Flow rate : 0.2 mL / min.
Column temperature : 40°C

MS : LCMS-8040 Triple quadrupole mass spectrometer

Ionization : ESI (Positive / Negative)
Ion spray voltage : +4.5 kV / -3.5 kV
MRM : 344 MRM transitions (2 MRMs / compound)

Features of LCMS-8040

- 5 times higher sensitivity compared to LCMS-8030
- An ultra fast scan speed of 15000 u / sec.
- An ultra fast polarity switching of 15 msec.
- An ultra fast MRM transition speed of 555 ch./ sec.

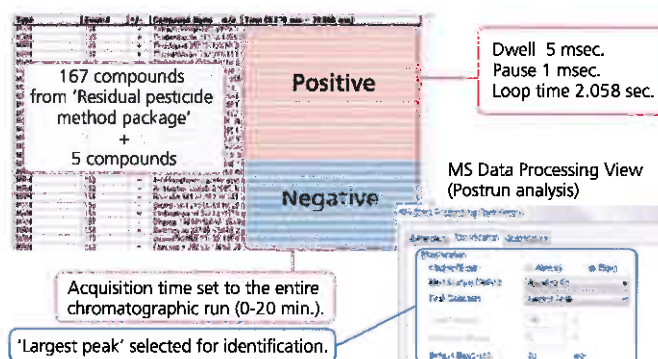


Fig. 1 LCMS-8040 Triple Quadrupole Mass Spectrometer

Setting of MRM analysis & integration parameter

- In this study, no scheduling of MRM transitions was applied; thereby creating a universal generic method.

FiltrInstrument Parameters View (Realtime analysis)



ing the application of a universal method for pesticide
ing in foods using a high data acquisition speed MS/MS

Results

Screening of 10 pesticides in food matrices

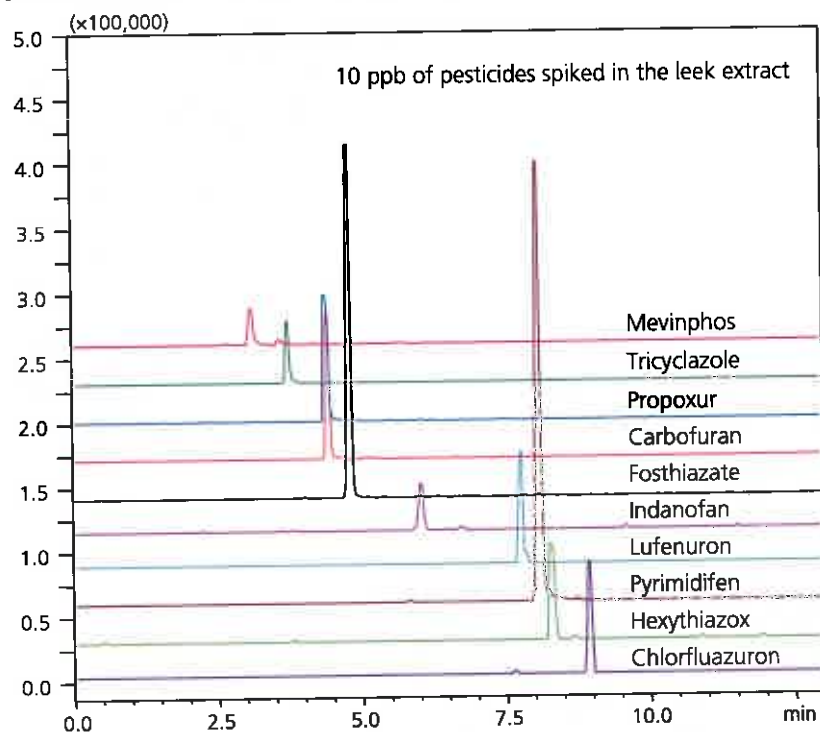


Fig. 2 MRM chromatogram of 10 pesticides

Table 2 Result of 10 pesticides screening (10 ppb spiked in each matrices)

Compounds	LeeK	Paprika	Green tea leaves
Carbofuran	✓	✓	✓
Chlorfluazuron	✓	✓	✓
Fosthiazate	✓	✓	✓
Hexythiazox	✓	✓	✓
Indanofan	✓	✓	✓
Lufenuron	✓	✓	✓
Mevinphos	✓	✓	✓
Propoxur	✓	✓	✓
Pyrimidifen	✓	✓	✓
Tricyclazole	✓	✓	✓
False positives*	8	7	10

• All peaks were automatically selected as the target compound to permit automatic identification of target analytes without retention time information.
(* Number of false positives out of 172 screened pesticides.)

Exploring the application of a universal method for pesticide screening in foods using a high data acquisition speed MS/MS

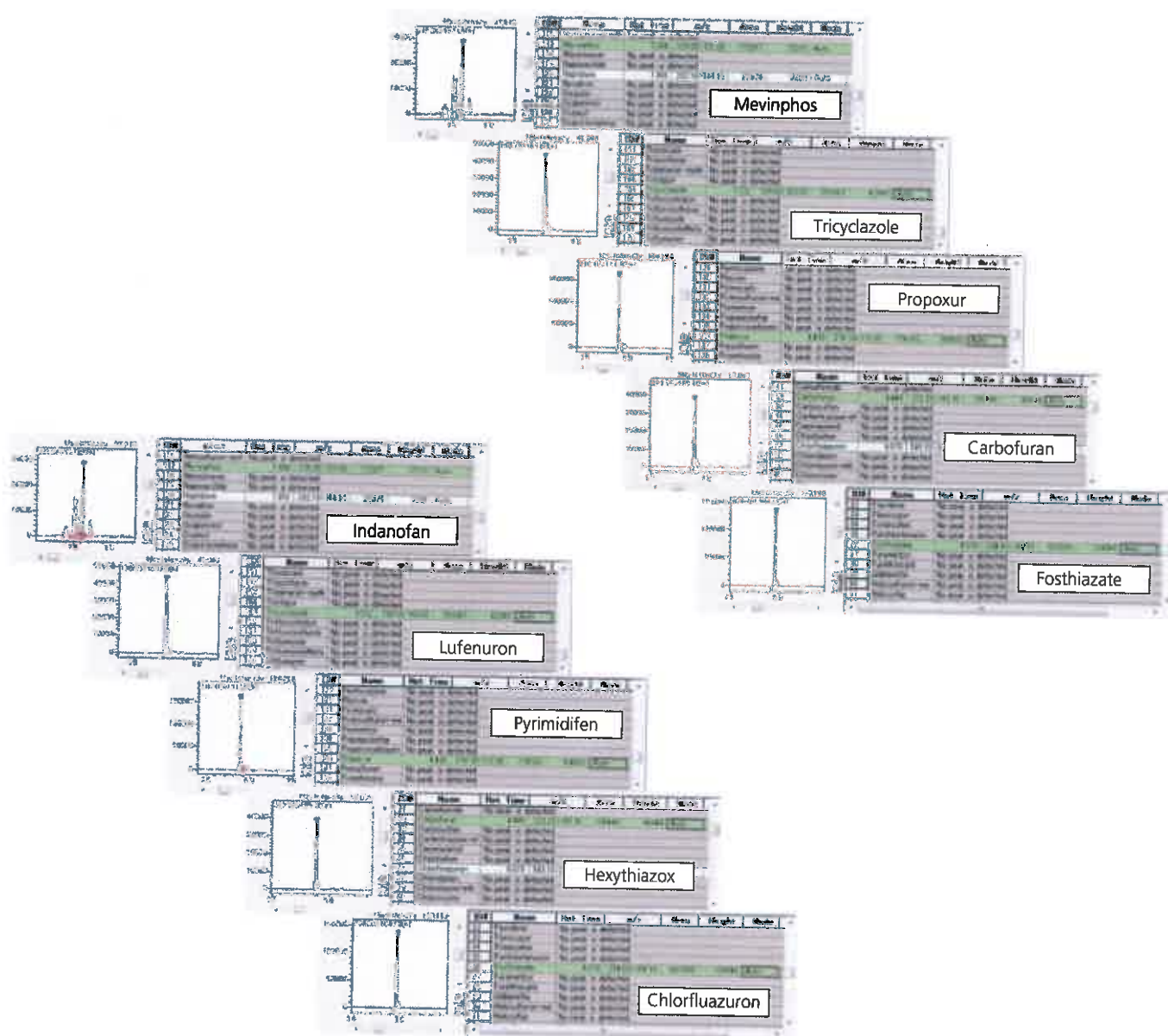


Fig. 3 Result of automatic identification (10 ppb spiked in the green tea leaves)

Conclusion

- Pesticides spiked in all matrices at 10 ppb (10 compounds) could be automatically detected using fast 5 msec MRM with 15 msec polarity switching without retention time information.



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Fast and sensitive assay of tobacco specific nitrosamines by UHPLC-MS/MS

ASMS 2013 TP-464

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Fast and sensitive assay of tobacco specific nitrosamines by UHPLC-MS/MS

1. Introduction

Tobacco specific nitrosamines (TSNA) are volatile compounds formed from nicotine and related alkaloids found in tobacco and tobacco products. The TSNA are created during fermentation, curing and burning of the

tobacco leaf. Due to their carcinogenic properties, the four major TSNA are assayed to control tobacco quality and to control cigarette smoke or public atmospheres.

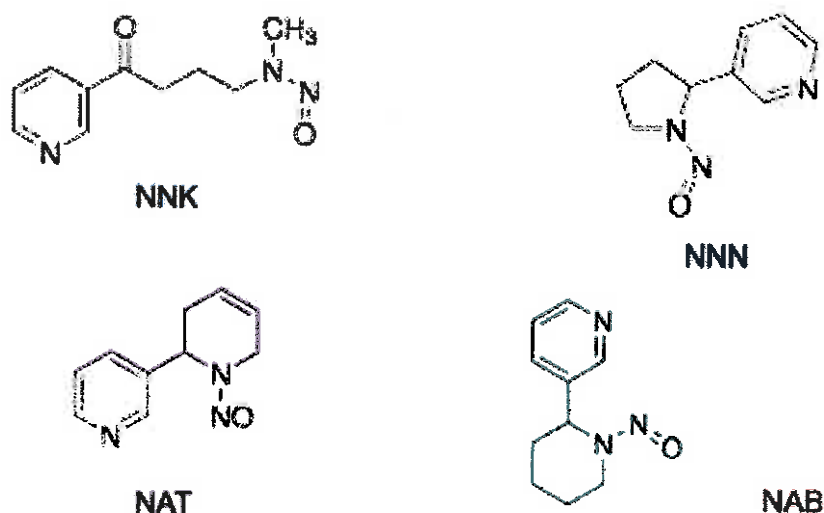


Fig. 1 Structure of the studied tobacco nitrosamines

2. Methods and Materials

The four major TSNA, i.e. N'-nitrosoanatabine (NNN), N'-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), were assayed by UHPLC-MS/MS. Specific solid phase

extraction was performed using selective molecularly imprinted polymers (Biotage). Deuterium labeled internal standards are used to improve assay ruggedness and accuracy.

Solid Phase Extraction with Molecularly Imprinted Polymers

Column: Biotage AFFINILUTE™ MIP – TSNAs 50 mg/3 mL

Sample preparation: Accurately weighted 250 mg of tobacco were transferred in a 30 mL glass tube with 10 mL of Ammonium acetate buffer (10 mM pH 5.5) and internal standards solution (NNN-D4, NNK-D4, NAT-D4, final concentration of 10 ng/mL). After 1 hr of sonication a 2 mL aliquot was centrifuged.

Extraction: Column was conditioned with 1 mL of Methanol and 1 mL of water. 1 mL of sample was applied to the column. Interferences were washed out with 1 mL of ammonium acetate (10 mM pH 5.5) and 1 mL of hexane. Elution was performed with 2 × 1 mL of methanol/dichloromethane 1/9. After complete evaporation, the dry residue was reconstituted with 1 mL of mobile phase mixture (A/B 9/1).

High sensitive assay of tobacco specific nitrosamines UHPLC-MS/MS

UHPLC conditions (Nexera MP system)

Column : Phenomenex Synergi Fusion-RP C18 50 x 2 mm 2.5 µm
 Mobile phase A : 5 mM Ammonium bicarbonate in water
 B : 5 mM Ammonium bicarbonate in methanol
 Flow rate : 0.4 mL/min
 Time program : B conc. 30%(0 min) -90%(0.35 min) - 30%(0.36-1.5 min)
 Injection vol. : 5 µL (with 1 µL air gaps)
 Needle wash : external wash only with methanol (rinse pump 2 sec)
 Column temperature: 30°C

MS conditions (LCMS-8040)

Ionization : ESI, Positive MRM mode
 Ion source temperatures: Desolvation line: 250°C
 Heater Block : 400°C

Gases: Nebulization : 2 L/min
 Drying : 15 L/min

MRM Transitions

Compound (msec)	MRM	Dwell time (msec)
NAB	192.10>162.30 (Quan)	12
	192.10>106.25 (Qual)	12
NAT	190.10>79.20 (Quan)	12
	190.10>160.25 (Qual)	12
NAT-D4	194.20>164.10	25
NNK	208.10>122.25 (Quan)	12
	208.10>79.20 (Qual)	12
NNK-D4	212.10>126.00	25
NNN	178.10>148.25 (Qual)	12
	178.10>120.25 (Quan)	12
NNN-D4	182.10>152.20	25

Pause time: : 3 msec

Loop time: : 0.2 sec (minimum 15 points per peak for each MRM)

Fast and sensitive assay of tobacco specific nitrosamines by UHPLC-MS/MS

3. Results

Mobile Phase Selection

Mobile phase solvent and additives were selected using a rational approach described elsewhere (60th ASMS, ThP512). Results are presented in Fig. 2.

Bicarbonate ammonium 5 mM and methanol were selected to obtain the best ionization efficiency.

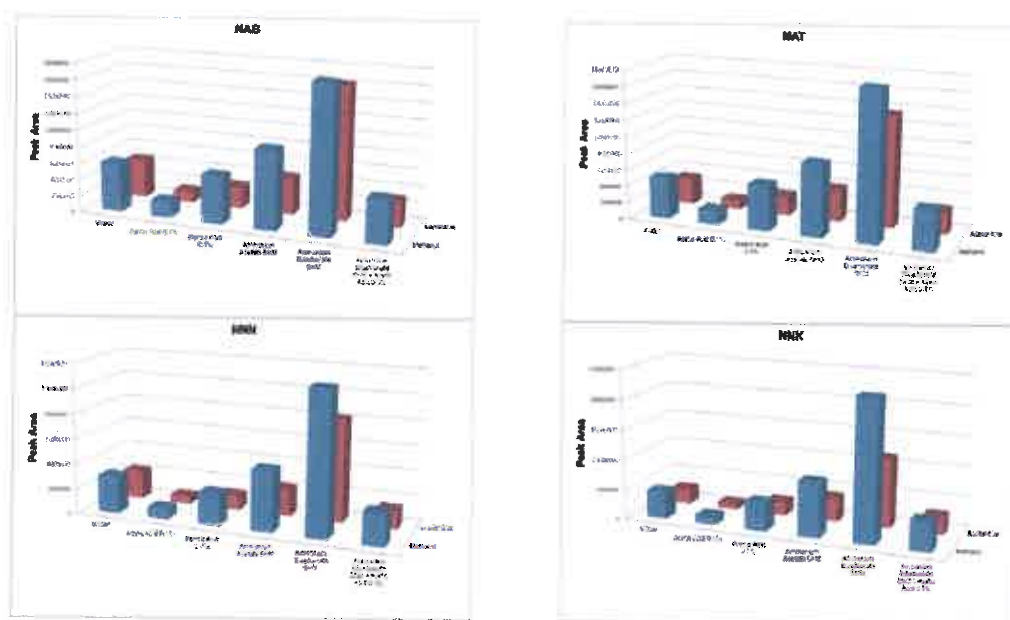


Fig. 2 Mobile phase optimisation results

Extraction Recovery and Matrix Effect

Due to their presence in all tobacco samples tested, tobacco extracts were spiked at 50 ng/mL with all TSNA's. Spiking was performed before and after extraction to measure extraction recovery. A neat solution at 50 ng/mL was compared to the extracts spiked after extraction to calculate matrix effect.

Sample	Extraction recovery	Ionization recovery
Cigarette tobacco brand A	NNN : 70% NNK : 93% NAT : 96% NAB : 72%	NNN : 99% NNK : 102% NAT : 97% NAB : 95%
Cigarette tobacco brand B	NNN : 75% NNK : 92% NAT : 93% NAB : 77%	NNN : 105% NNK : 93% NAT : 99% NAB : 99%
Cigarette tobacco brand C	NNN : 75% NNK : 96% NAT : 90% NAB : 74%	NNN : 100% NNK : 104% NAT : 101% NAB : 107%

A sensitive assay of tobacco specific nitrosamines iPLC-MS/MS

Calibration Curves

Calibration in neat solutions were prepared. For all TSNA, calibration range was of 0.1 ng/mL to 50 ng/mL. This corresponds to 500 fg to 250 pg of each TSNA injected; and this corresponds to a quantity of 0.004 µg/g to 2 µg/g in tobacco products. All calibration levels were injected in 5 replicates. Intra-level %RSD were inferior to 5% at all levels for all compounds. Fig. 3 presents the calculated calibration curves. Fig. 4 shows the LOQ chromatograms.

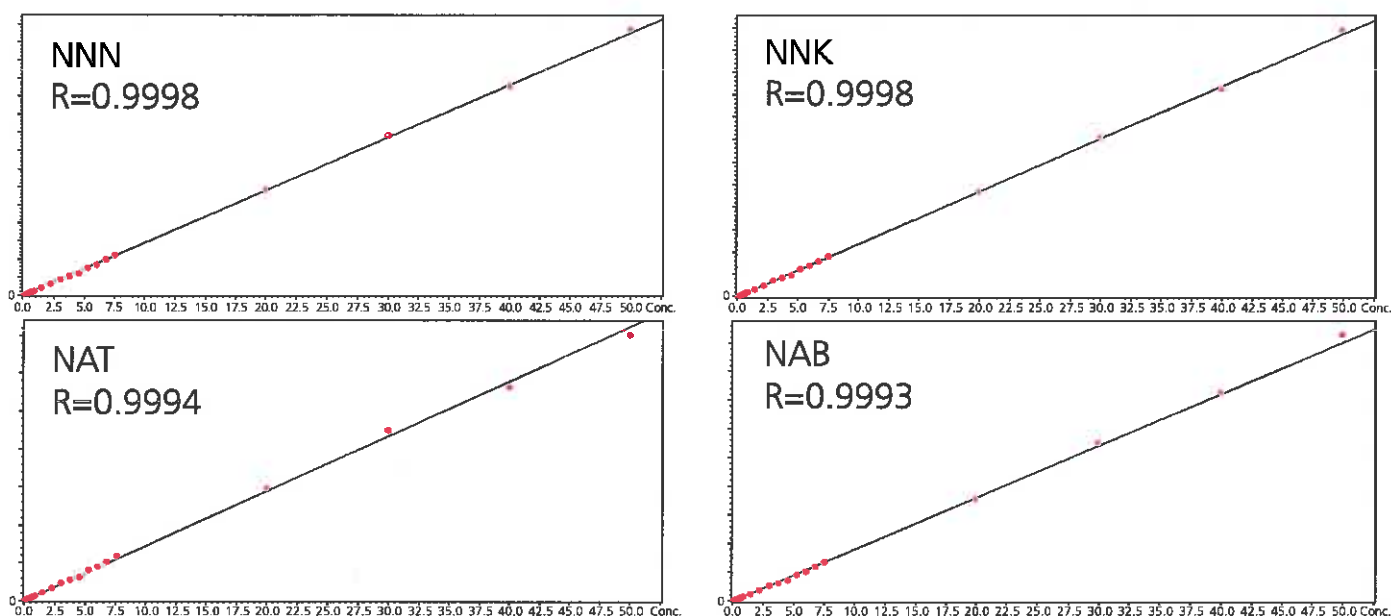


Fig. 3 Calibration curves

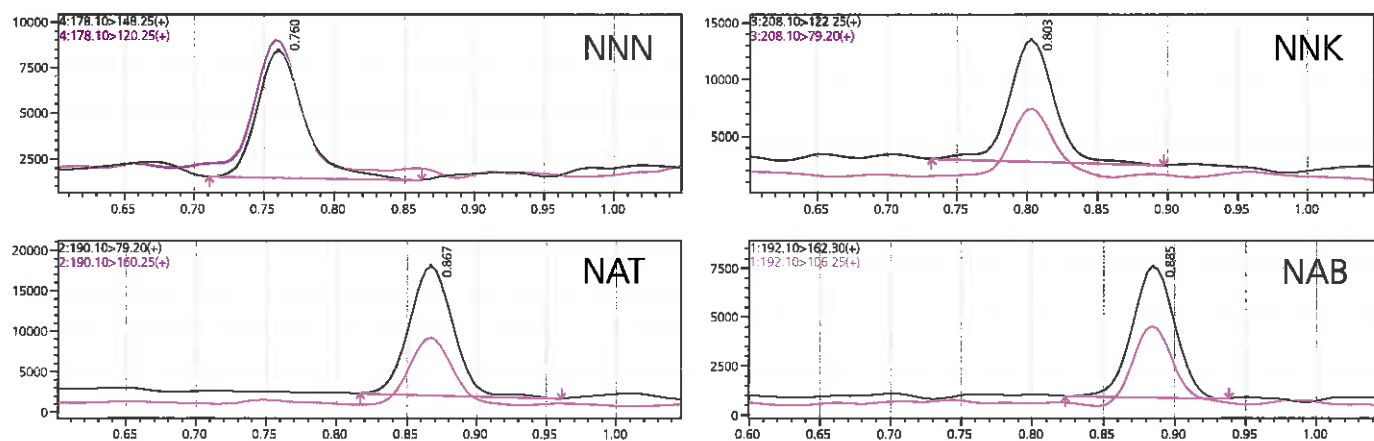


Fig. 4 Lowest calibration standard (100 pg/mL)

Fast and sensitive assay of tobacco specific nitrosamines by UHPLC-MS/MS

4. Conclusions

- Highly selective extraction sorbents enable fast LC-MS/MS without suffering from matrix effect (i.e. ion suppression),
- Therefore, multiple samples can be assayed and quantified against the same calibration curve made in neat solutions,
- Very fast and rugged LC-MS/MS analysis lead to high throughput result generation to test many tobacco samples in quality control,
- The method is sensitive enough to foresee analysis of tobacco smokes or new tobacco products.

First Edition: June, 2013

High Sensitive Detection and Quantification of Thirty-Two Synthetic PDE-5 Inhibitors and Analogues Adulterated in Health Supplements Using LC/MS/MS

ASMS 2015 TP325

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High Sensitive Detection and Quantification of Thirty-Two Synthetic PDE-5 Inhibitors and Analogues Adulterated in Health Supplements Using LC/MS/MS

Introduction

In recent years, synthetic phosphodiesterase type 5 enzyme (PDE-5) inhibitors like sildenafil (active component of VIAGRA) were found as adulterant in some health supplements described as plant or herbal products [1]. Only seven synthetic PDE-5 inhibitors, namely, sildenafil, tadalafil, vardenafil, avanafil, udenafil, mirodenafil and lodenafil carbonate are approved officially to be used for the treatment of erectile dysfunction (ED) in men. The adulteration is illegal and may be also dangerous for

consumers, because not only the above PDE-5 inhibitors, many newly appeared analogues that are synthesized with minor modification of the parent structures are also found used as adulterants. Analytical techniques like HPLC and LC/MS/MS have been applied for screening and quantitation of PDE-5 inhibitors and their analogues [1-3]. We describe here a high sensitivity LC/MS/MS method for detection and quantification of thirtytwo synthetic PDE-5 inhibitors and analogues spiked in health products.

Experimental

Thirty-two PDE-5 inhibitors and analogues were obtained from TLC PharmaChem (Canada), which include five approved ones (sildenafil, tadalafil, vardenafil, avanafil and udenafil) and twenty-seven analogues. These compounds were dissolved in MeOH at 100ppm or 10ppm as stocks which were used to prepare mixed standards calibrant series of ten levels (0.1, 0.5, 1, 2, 5, 10, 50, 100, 200 and 500 ppb) and spiked samples. Five health supplement products in capsules purchased locally were used as model samples for evaluation of method performance. These samples are named as M, C, PP, TA

and RK for convenience. The sample was extracted with MeOH at a ratio of 0.2 gram of the powders in 2mL of pure MeOH. The mixture was sonicated for 20 minutes, followed by filtration with 0.2µm PTFE filter before analysis. An LCMS-8040 triple quadrupole mass spectrometer coupled with a Nexera UHPLC was used in this study. A Shim-pack XR-ODS-III UHPLC column (150x2mm, 2µm) was adopted and a gradient elution program was used for separation of the thirty-two compounds. The detailed conditions are compiled into Table 1.

Table 1: Analytical conditions of thirty-two PDE-5 inhibitors and analogues on LCMS-8040

Column	Shim-pack XR-ODSIII (150 x 2.0mm)
Flow Rate	0.3 mL/min
Mobile Phase	A: H ₂ O with 0.1% formic acid B: CAN with 0.1% formic acid
Elution Mode	Gradient elution, 8%: 10% (0.01 min) → 45% (12min) → 80% (15 to 17min)
Oven Temp.	45 °C
Injection Vol.	2.0 µL
Interface	ESI
MS mode	Positive, MRM
Block Temp.	400 °C
DL Temp.	250 °C
CID Gas	Ar (230kPa)
Nebulizing Gas Flow	N ₂ , 3 L/min
Drying Gas Flow	N ₂ , 15 L/min

Sensitive Detection and Quantification of Two Synthetic PDE-5 Inhibitors and Analogues Generated in Health Supplements Using LC/MS/MS

Results and Discussion

Establishment of MRM method for detection and quantitation of thirty-two PDE-5 inhibitors and analogues

The MRM optimization of the thirty-two PDE-5 inhibitors and analogues was carried out using an auto-optimization program of the LabSolutions. Most of the compounds produces three significant MRM transitions except two analogues which produced two MRMs. The MRM transition with highest intensity was selected as quantifier ion and the others were used as confirmation ions (Table 2). An UHPLC column (2µm particle size) was adopted with a gradient elution program to achieve high peak resolution and high sensitivity. Figure 1 shows the MRM chromatograms of thirty-two PED-5 inhibitors and analogues of mixed standard samples (50 ppb, 2 µL injection). Since this method is for targeted screening and quantitation, a wider time window of (+/-) 2.5min for each targeted MRM peak was set to be able to tolerance the peak shift that may occur in screening of actual samples. A calibrant series of ten levels from 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 ppb was prepared for establishment of calibration curves, LOD and LOQ values of the thirty-two

compounds studied. The lowest detectable levels ($S/N \geq 3$ or count ≥ 250) of every compounds in the low level mixed standards are used as the LODs. The levels with $S/N \geq 10$ or count ≥ 800 are used as LOQs of the compounds. Calibration curves were established for a range from LOQ up to 500ppb. A few selected calibration curves are shown in Figure 2, which include the five approved PDE-5 inhibitors and acetilsildenafil, an analogue of sildenafil. The complete information of the MRM quantitation method of the thirty-two PDE-5 inhibitors and analogues is compiled into Table 3. In brief, the results show that linear calibration curves with $R^2 > 0.999$ from LOQs up to 500ppb were obtained for all the compounds. The LODs and LOQs of the thirty-two compounds in neat solutions of mixed standards were determined at 0.05~2ppb and 0.2~6.7ppb, respectively. Repeatability of peak areas were evaluated with 10ppb and 100ppb mixed standard samples. The results of %RSD (n=6) are at 1.3~8.8% for 10ppb and 0.4~7.8% for 100ppb, respectively.

Table 2: Names and information of thirty-two compounds and MRM transitions on LCMS-8040

No.	Name	Formula	CAS	Quantifier ion				Conformation ion			
				MRM	Q1 (V)	CE (V)	Q3 (V)	MRM	Q1 (V)	CE (V)	Q3 (V)
1	YOHIMBINE	$C_{21}H_{26}N_2O_3$	146-48-5	354.9>144.1	-30	-32	-28	354.9>212.1	-30	-23	-16
2	ACETYLWARDENAFIL	$C_{25}H_{34}N_6O_3$	1261351-28-3	467.3>151.1	-22	-47	-30	467.3>341.2	-22	-34	-24
3	CARBODENAFIL	$C_{24}H_{32}N_6O_3$	944241-52-5	452.9>339.2	-30	-23	-25	452.9>311.2	-30	-34	-23
4	N-DESMETHYL ACETILDENAFIL	$C_{23}H_{30}N_6O_3$	N.A	439.2>99.1	-30	-31	-20	439.2>70.1	-30	-47	-28
5	N-DESETHYL VARDENAFIL	$C_{21}H_{26}N_6O_4S$	448184-46-1	461.2>151.1	-13	-46	-29	461.2>312.2	-13	-37	-23
6	HYDROXYHOMOSILDENAFIL	$C_{23}H_{32}N_6O_5S$	139755-85-4	504.9>151.1	-34	-47	-29	504.9>312.1	-34	-41	-23
7	WARDENAFIL (Levitra, Bayer)	$C_{23}H_{32}N_6O_4S$	224785-90-4	488.9>72.1	-30	-49	-29	488.9>113.1	-30	-31	-23
8	HYDROXYACETILDENAFIL	$C_{23}H_{34}N_6O_4$	147676-56-0	483.3>127.1	-23	-31	-14	483.3>143.2	-23	-35	-29
9	NORACETILDENAFIL	$C_{24}H_{32}N_6O_3$	949091-38-7	452.9>97.1	-13	-33	-20	452.9>70.1	-13	-45	-29
10	ACETILDENAFIL	$C_{25}H_{34}N_6O_3$	831217-01-7	466.9>111.1	-30	-32	-23	466.9>127.1	-30	-32	-26
11	PIPERIACETILDENAFIL	$C_{24}H_{31}N_5O_3$	147676-50-4	437.9>98.1	-30	-35	-20	437.9>297.1	-30	-37	-22
12	AVANAFIL (Stendra, Vivus)	$C_{23}H_{26}ClN_6O_3$	330784-47-9	483.9>155	-30	-43	-30	483.9>375.1	-30	-27	-28
13	HYDROXYWARDENAFIL	$C_{23}H_{32}N_6O_5S$	224785-98-2	504.9>99.1	-34	-38	-21	504.9>112.1	-34	-31	-23
14	SILDENAFIL (Viagra, Pfizer)	$C_{22}H_{30}N_6O_4S$	171599-83-0	475.2>58.1	-22	-45	-24	475.2>100.1	-22	-30	-20
15	HOMOSILDENAFIL	$C_{23}H_{32}N_6O_4S$	642928-07-2	488.9>72.2	-30	-47	-29	488.9>99.1	-30	-36	-20

High Sensitive Detection and Quantification of Thirty-Two Synthetic PDE-5 Inhibitors and Analogues Adulterated in Health Supplements Using LC/MS/MS

No.	Name	Formula	CAS	Quantifier ion				Conformation ion			
				MRM	Q1 (V)	CE (V)	Q3 (V)	MRM	Q1 (V)	CE (V)	Q3 (V)
16	DIMETHYLSILDENAFIL	$C_{23}H_{32}N_6O_4S$	1416130-63-6	488.9>99.1	-30	-38	-20	488.9>113.1	-30	-32	-23
17	UDENAFIL (Zydena, Dong-A)	$C_{25}H_{36}N_6O_4S$	268203-93-6	516.9>283.1	-40	-46	-30	516.9>112.2	-40	-35	-23
18	HYDROXYTHIOHOMOSILDENAFIL	$C_{23}H_{32}N_6O_4S_2$	479073-82-0	520.9>99.1	-36	-45	-20	520.9>299.1	-36	-42	-21
19	THIOSILDENAFIL	$C_{22}H_{30}N_6O_4S_2$	479073-79-5	490.9>58.1	-23	-48	-24	490.9>100.1	-23	-32	-20
20	THIOHOMOSILDENAFIL	$C_{23}H_{32}N_6O_4S_2$	479073-80-8	504.9>72.1	-40	-50	-29	504.9>113.1	-40	-32	-23
21	BENZYSILDENAFIL	$C_{28}H_{34}N_6O_4S$	N.A	550.9>91.1	-40	-41	-19	550.9>134.1	-40	-43	-26
22	THIODIMETHYLSILDENAFIL	$C_{23}H_{32}N_6O_4S_2$	856190-47-1	504.9>99.1	-40	-39	-20	504.9>113.1	-40	-32	-23
23	AMINOTADALAFIL	$C_{21}H_{18}N_4O_4$	385769-84-6	391.2>269.1	-27	-14	-20	391.2>135.0	-11	-20	-26
24	NORTADALAFIL	$C_{21}H_{17}N_4O_4$	171596-36-4	376.1>254.1	-17	-13	-29	376.1>169.1	-17	-38	-20
25	VARDENAFIL INTERMEDIATE*	$C_{17}H_{20}N_4O_2$	N.A	312.8>151.1	-30	-30	-30	312.8>110.1	-30	-47	-22
26	TADALAFIL (Cialis, Eli Lilly)	$C_{22}H_{19}N_3O_4$	171596-29-5	390.1>135.1	-18	-20	-15	390.1>268.1	-18	-14	-20
27	CHLOROPRETADALAFIL	$C_{22}H_{19}ClN_3O_5$	171489-59-1	426.4>135.1	-28	-20	-27	426.4>237.4	-20	-20	-28
28	PSEUDOVARDENAFIL	$C_{22}H_{29}N_5O_4S$	224788-34-5	460.2>151.1	-30	-55	-30	460.2>312.2	-30	-37	-23
29	GENDENAFIL	$C_{19}H_{22}N_4O_3$	147676-66-2	354.9>285.1	-30	-31	-21	354.9>327.1	-30	-25	-24
30	N-BUTYL TADALAFIL	$C_{25}H_{25}N_3O_4$	171596-31-9	432.2>135.1	-20	-24	-26	432.2>310.2	-20	-15	-23
31	NORNEOSILDENAFIL	$C_{22}H_{29}N_5O_4S$	371959-09-0	460.2>283.1	-17	-36	-21	460.2>299.2	-17	-37	-22
32	N-OCTYL NORTADALAFIL	$C_{28}H_{33}N_3O_4$	1173706-35-8	488.9>135.1	-14	-35	-27	488.9>366.2	-14	-16	-15

* TLC ID#: V057

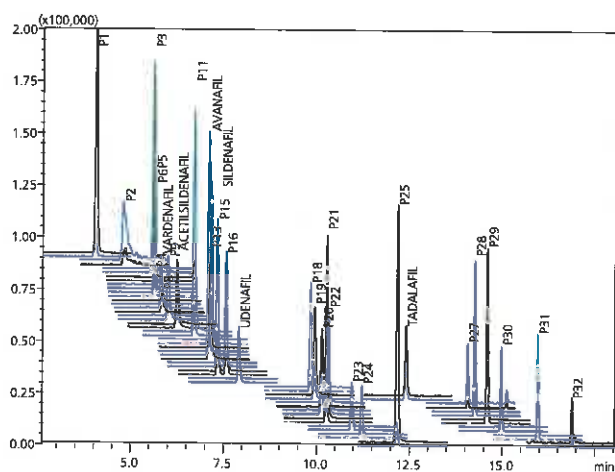


Figure 1: MRM chromatograms of 50ppb mixed standards of 32 PDE-5 inhibitors and analogues (2uL). Each compound was detected and confirmed with a pair of MRMs.

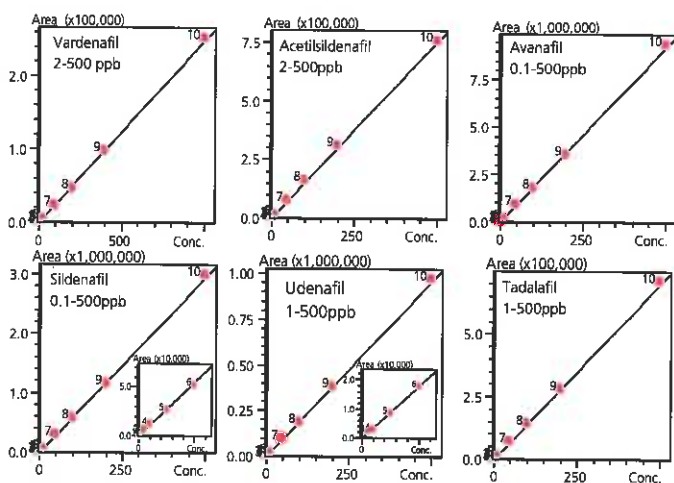


Figure 2: Calibration curves of five approved PDE-5 inhibitors and acetildenafil (sildenafil analogue).

Positive Detection and Quantification of Two Synthetic PDE-5 Inhibitors and Analogues Adulterated in Health Supplements Using LC/MS/MS

Matrix effect and reliability of targeted screening method for detection of adulteration

Aimed at detection of adulteration of the thirty-two PDE-5 inhibitors and analogues, the LC/MS/MS method established was tested with spiked samples to evaluate the detection reliability and quantitation accuracy. It is well known that health products from herbal plants and other materials are not only very complex, but also greatly different from each other in constituents and compositions. There is not a single sample that could be used as representative to study matrix effect and peak interference. In this study, we selected randomly five health supplement products from local market and carried out evaluation of matrix effect, peak interferences and their effects to screening reliability. The MeOH extracts of the five health supplement samples M, C, PP, TA and RK were analysed using the above method and the results confirmed that these samples were free of the thirty-two PDE-5 inhibitors and analogues studied. The matrix effect of every compound in a spiked sample is determined by the ratio of its peak areas in the matrix solution and in neat diluent. The results of matrix effects of the thirty-two compounds in five different matrixes are summarized in Table 4. The results show that the matrix effects of most compounds are within a range of 70% to 150%. For example, the matrix effects of sildenafil ranges from 90.9% to 149.1% in four samples except one at 162.9% (Sample RK), and acetildenafil from 99.8 to 124.7% except one at 56% (sample C). Several compounds such as thiodimethylsildenafil, thiohomosildenafil and thiosildenafil exhibit strong matrix effect in most samples. The opposite situation of ion suppression, namely ion enhancement were observed with several compounds in different samples (green color in Table 4). It is necessary to clarify whether the strong ion suppression and ion enhancement phenomena observed are due to peak interference of other species.

This could be confirmed by the peak intensity ratios of quantifier ion and confirmation ion (main ion and reference ion). As shown in Figure 3, two compounds, vardenafil which exhibits the strongest ion enhancement (ME: 131.9~221.4%, except sample C) and thiodimethylsildenafil which exhibits the strongest ion suppression (ME: 13.8~51.8%), are investigated through comparing their intensity ratios of the quantifier ion and the confirmation ion in different matrixes with that in neat diluent. The results indicate that the matrix effect values are not related to the ion suppression or ion enhancement. Therefore, peak interference due to other chemical species could be excluded. The matrix effect seems to be more related to the compound property and less to the samples under the ESI conditions. However, the same compounds and matrixes were also studied on LCMS-IT-TOF under similar conditions with a different ESI interface [4]. The matrix effect behaviour observed are different, which indicates that instrument performance and LC separation conditions may play important roles too. Based on these studies, reliable detection limits for screening of the thirtytwo PDE-5 inhibitors and analogues by the MRM method are estimated at 0.1~4ppb in extract solutions if a matrix effect factor of 50% is applied as a more secure consideration, and 1~40 ng/g in powders (dilution factor of 10). In the same way, the lowest quantitation levels are estimated at 0.4~13.4ppb in extract solutions and 4~134ng/g in powders. However, this estimation on LODs and LOQs are based assumption of 100% extraction recovery. It is worth to note that the high sensitivity of the MRM method established was achieved with 2 μ L injection. A smaller injection vol of dirty samples is preferred to reduce MS system contamination.

High Sensitive Detection and Quantification of Thirty-Two Synthetic PDE-5 Inhibitors and Analogues Adulterated in Health Supplements Using LC/MS/MS

Table 3: Calibration and performance of MRM method for 32 PDE-5 inhibitors and analogues on LCMS-8040

P. No	Compound Name	RT (min)	MRM	Range (ppb)	R ²	LOD (ppb)	LOQ (ppb)	RSD (%), n=6	
								10ppb	100b
1	YOHIMBINE	4.06	354.9>144.1	0.5-500	0.9999	0.1	0.5	1.3	0.4
2	ACETYLWARDENAFIL	4.75	467.3>151.1	5-500	0.9958	2.0	6.7	8.7	7.0
3	CARBODENAFIL	5.63	452.9>339.2	1-500	0.9999	0.3	1.0	3.3	2.2
4	N-DESMETHYL ACETILDENAFIL	5.64	439.2>99.1	2-500	0.9978	1.0	3.5	8.3	3.0
5	N-DESETHYL VARDENAFIL	5.65	461.2>151.1	1-500	0.9994	0.5	1.6	3.2	1.9
6	HYDROXYHOMOSILDENAFIL	5.67	504.9>151.1	0.5-500	0.9997	0.1	0.5	4.4	2.5
7	VARDENAFIL (Levitra, Bayer)	5.83	488.9>72.1	5-500	0.9995	2.0	6.7	7.4	6.5
8	HYDROXYACETILDENAFIL	5.85	483.3>127.1	2-500	0.9994	0.5	1.6	8.9	7.8
9	NORACETILDENAFIL	6.01	452.9>97.1	2-500	0.9997	1.0	3.5	4.3	3.4
10	ACETILDENAFIL	6.23	466.9>111.1	2-500	0.9998	1.0	3.5	7.6	6.0
11	PIPERACETILDENAFIL	6.72	437.9>98.1	1-500	0.9996	0.3	1.0	4.0	2.1
12	WARDENAFIL (Sildenafil, Vivus)	7.13	483.9>155	0.5-500	0.9994	0.1	0.5	3.1	1.4
13	HYDROXYWARDENAFIL	7.13	504.9>99.1	1-500	0.9999	0.5	1.6	5.3	2.7
14	SILDENAFIL (Viagra, Pfizer)	7.19	475.2>58.1	1-500	0.9997	0.3	1.0	3.1	1.1
15	HOMOSILDENAFIL	7.34	488.9>72.2	1-500	0.9996	0.3	1.0	3.2	1.7
16	DIMETHYLSILDENAFIL	7.58	488.9>99.1	1-500	0.9997	0.3	1.0	1.7	1.6
17	UDENAFIL (Zydene, Dong-A)	7.93	516.9>283.1	1-500	0.9999	0.5	1.6	4.5	2.3
18	HYDROXYTHIOHOMOSILDENAFIL	9.85	520.9>99.1	1-500	0.9999	0.3	1.0	4.4	2.9
19	THIOSILDENAFIL	9.95	490.9>58.1	0.5-500	0.9993	0.2	0.7	6.1	2.6
20	THIOHOMOSILDENAFIL	10.15	504.9>72.1	1-500	0.9995	0.3	1.0	4.6	4.2
21	BENZYSILDENAFIL	10.28	550.9>91.1	0.5-500	0.9999	0.2	0.7	5.1	1.7
22	THIODIMETHYLSILDENAFIL	10.34	504.9>99.1	1-500	0.9995	0.5	1.6	4.7	3.3
23	AMINOTADALAFIL	10.97	391.2>269.1	1-500	0.9998	0.5	1.6	4.0	3.3
24	NORTADALAFIL	11.24	376.1>254.1	2-500	0.9999	1.0	3.5	7.8	5.1
25	VARDENAFIL INTERMEDIATE*	12.19	312.8>151.1	0.1-500	0.9999	0.05	0.2	1.6	1.4
26	TADALAFIL (Cialis, Eli Lilly)	12.42	390.1>135.1	1-500	0.9999	0.5	1.6	4.9	1.3
27	CHLOROPRETADALAFIL	14.08	426.4>135.1	2-500	0.9994	1.0	3.5	8.8	6.9
28	PSEUDOVARDENAFIL	14.27	460.2>151.1	0.1-500	0.9996	0.05	0.2	1.7	1.6
29	GENDENAFIL	14.60	354.9>285.1	0.5-500	0.9991	0.1	0.5	2.4	1.6
30	N-BUTYL TADALAFIL	14.98	432.2>135.1	2-500	0.9998	1.0	3.5	8.8	2.2
31	NORNEOSILDENAFIL	15.97	460.2>283.1	1-500	0.9988	0.3	1.0	4.9	4.0
32	N-OCTYL NORTADALAFIL	16.89	488.9>135.1	2-500	0.998	1.0	3.5	5.9	2.4

* TLC ID#: V057

Positive Detection and Quantification of Two Synthetic PDE-5 Inhibitors and Analogues Generated in Health Supplements Using LC/MS/MS

Table 4: Matrix effects of PDE-5 inhibitors and analogues (100ppb) spiked in MeOH extracts of five health products

P. No.	Name	RT (min)	m/z	Matrix Effect (%)				
				Sample M	Sample C	Sample PP	Sample TA	Sample RK
1	YOHIMBINE	3.88	354.9>144.1	112.4	95.8	141.7	132.9	129.8
2	ACETYLVARDENAFIL	4.71	467.3>151.1	74.8	44.5	79.1	119.1	78.7
3	CARBODENAFIL	5.44	452.9>339.2	110.6	76.8	137.1	114.5	128.2
4	N-DESMETHYL ACETILDENAFIL	5.50	439.2>99.1	134.8	99.8	167.4	135.2	184.0
5	N-DESETHYL VARDENAFIL	5.55	461.2>151.1	92.2	92.8	138.8	110.6	118.4
6	HYDROXYHOMOSILDENAFIL	5.58	504.9>151.1	132.5	151.7	198.0	147.6	218.3
7	VARDENAFIL (Levitra, Bayer)	5.71	488.9>72.1	131.9	52.3	180.7	160.4	221.4
8	HYDROXYACETILDENAFIL	5.74	483.3>127.1	181.8	29.3	143.1	174.6	193.9
9	NORACETILDENAFIL	5.86	452.9>97.1	106.5	41.1	98.2	117.5	112.8
10	ACETILDENAFIL	6.09	466.9>111.1	113.8	56.0	114.6	124.7	99.8
11	PIPERACETILDENAFIL	6.53	437.9>98.1	103.1	12.7	79.9	116.8	64.7
13	AVANAFIL (Stendra, Vivus)	6.95	483.9>155	146.5	115.9	165.5	160.5	173.5
12	HYDROXYVARDENAFIL	6.96	504.9>99.1	137.9	110.9	144.6	144.7	223.4
14	SILDENAFIL (Viagra, Pfizer)	7.01	475.2>58.1	126.1	90.9	143.4	149.1	162.9
15	HOMOSILDENAFIL	7.18	488.9>72.2	129.3	80.0	129.0	128.6	141.6
16	DIMETHYLSILDENAFIL	7.43	488.9>99.1	98.0	32.1	115.8	112.3	124.7
17	UDENAFIL (Zydena, Dong-A)	7.78	516.9>283.1	133.9	109.7	146.0	148.5	200.9
18	HYDROXYTHIOHOMOSILDENAFIL	9.72	520.9>99.1	30.7	67.6	108.2	52.6	92.9
19	THIOSILDENAFIL	9.82	490.9>58.1	20.2	50.5	99.5	39.6	82.6
20	THIOHOMOSILDENAFIL	10.02	504.9>72.1	20.4	59.4	114.5	39.5	87.5
22	BENZYLSILDENAFIL	10.20	550.9>91.1	139.6	131.3	135.6	136.4	200.0
21	THIODIMETHYLSILDENAFIL	10.35	504.9>99.1	29.1	13.8	84.7	48.3	51.8
23	AMINOTADALAFIL	11.02	391.2>269.1	91.6	65.1	106.7	102.0	81.1
24	NORTADALAFIL	11.29	376.1>254.1	89.0	76.4	108.6	106.7	90.0
25	VARDENAFIL INTERMEDIATE*	12.38	312.8>151.1	99.6	108.1	114.8	113.7	102.3
26	TADALAFIL (Cialis, Eli Lilly)	12.47	390.1>135.1	99.1	88.9	113.2	117.2	101.4
27	CHLOROPRETADALAFIL	14.16	426.4>135.1	88.6	81.9	75.8	101.5	83.1
28	PSEUDOVARDENAFIL	14.34	460.2>151.1	102.6	74.4	109.2	140.6	70.4
29	GENDENAFIL	14.63	354.9>285.1	91.1	96.0	64.4	181.1	86.8
30	N-BUTYL TADALAFIL	15.01	432.2>135.1	96.4	80.1	96.1	122.1	67.3
31	NORNEOSILDENAFIL	15.99	460.2>283.1	67.3	69.3	136.1	72.6	56.4
32	N-OCTYL NORTADALAFIL	16.91	488.9>135.1	103.0	86.5	100.3	106.6	105.9

High Sensitive Detection and Quantification of Thirty-Two Synthetic PDE-5 Inhibitors and Analogues Adulterated in Health Supplements Using LC/MS/MS

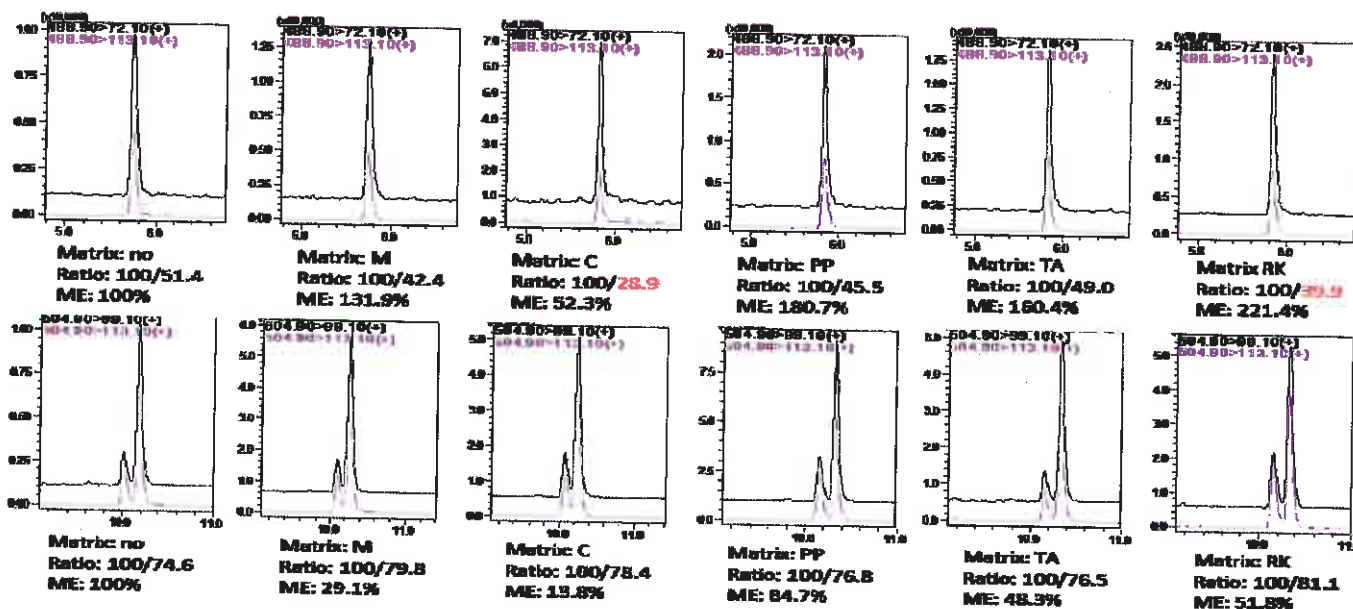


Fig. 3: Comparison of peak intensity ratios and matrix effect values for vardendafil (top) and thiodimethylsildenafilafil (bottom) in different sample matrixes.

Conclusions

A MRM targeted screening method has been developed for sensitive and reliable screening analysis of thirty-two synthetic PDE-5 inhibitors and analogues in health products. In consideration of matrix effect using spiked samples in five health products selected randomly, the

detection limits of the method are estimated at 0.1~4.0ppb for different compounds in solutions. The quantitation limits are at 0.4~13.4ppb in solutions. Further study on extract recovery of the method is needed.

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Identification of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

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Identification of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

1. Introduction

Medicinal plants have played an important role in drug discovery with many pharmaceutical products originating from plant. Isolation and characterization of antibacterial

compounds is still relevant because of continuous development of resistance of bacteria to antibiotics.

1-1. *Garcinia indica*

Garcinia indica, a plant from the mangosteen family (*Clusiaceae*), commonly known as 'Kokum' is used for culinary purposes in India. *Garcinia indica* is indigenous to the Western Ghats region of India located along the

western coast of the country. Fruits of *Garcinia indica* have been suggested in the Indian system of medicine for a number of diseases as a remedial drug^[1].



Fig. 1 *Garcinia indica*

Classification of *Garcinia indica* ^[1]

Kingdom : Plantae
Division : Mangoliophyta
Class : Magnoliopsida
Order : Malpighiales
Family : Clusiaceae
Subfamily: Clusioidae
Tribe : Garcinieae
Genus : Garcinia
Species : indica

1-2. Garcinol

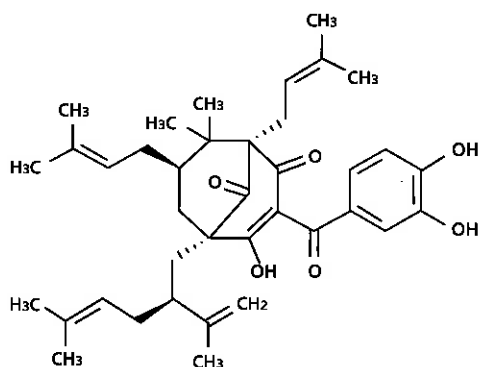


Fig. 2 Garcinol

Garcinol, a polyisoprenylated benzophenone, is extracted from the rind of the fruit of *Garcinia indica*, a plant found extensively in tropical regions (shown in Fig. 2). There is ample data to suggest potent antioxidant properties of this

compound which have been used to explain most of its observed biological activities. However, emerging evidence suggests that garcinol could be useful as an anti-cancer agent, and it is increasingly being realized that garcinol is a pleiotropic agent capable of modulating key regulatory cell signaling. Garcinol is an example of a prenylated chalcone, which has many beneficial effects in human health and disease^{[2][3]}.

In present work, we have studied antibacterial activity of extract from rinds of *Garcinia indica* by using Bioautography technique and identified this antibacterial compound by LCMS-8040 triple quadrupole mass spectrometer. The feature of 'optimisation of method' present in LCMS-8040 provides qualitative information along with Multiple Reaction Monitoring (MRM) optimisation which can be used for the probable structural assignment.

Isolation of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

2. Method of Analysis

2-1. Preparation of plant extracts

Garcinia indica fruits were collected from Ratnagiri area in the state of Maharashtra in India. The fruits rinds were separated from the fruit. Shed dried fruit rinds were

powdered using mixer grinder, sieved through the mesh size 40 and stored in the air-tight PET bottles at room temperature.

2-2. Bioautography

Target plant pathogen : *Xanthomonas axonopodis* pv. *punicae*

Information about target organism : *X. axonopodis* pv. *punicae* is a bacterial pathogen that causes serious blight disease of pomegranate. The disease was first reported in India where it is a major threat to pomegranate cultivation due to lack of effective control measures. The blight can cause 50 to 100 % production loss depending on severity of disease. In oily spot of pomegranate (also called bacterial blight, black spot) the main damage is observed on fruits, which develop black spots resulting in enormous yield losses. The disease has become an increasingly serious threat for pomegranate growers of Andhra Pradesh, Maharashtra, and Karnataka. For example in 2006-2007 in Maharashtra, oily spot was found to be the main disease with 100 percent infection in some orchards.

Plant used : Matured *Garcinia indica* fruit rinds but not fully ripe.

Extraction : Soxhlet extraction was done for 18 to 24 hrs using Ethyl acetate. Solvent was evaporated after extraction. The dried plant extract was reconstituted in Methanol.

TLC : The extract was reconstituted in methanol and used for TLC.

Mobile phase used : Toluene : Ethyl acetate : Formic acid (4 : 1 : 0.5 v/v/v) Chamber saturation : 30 mins.

Bioautography : Bioautography is a microbiological screening methods commonly used for the detection of antimicrobial activity. The screening can be defined as the first procedure, which is applied to sample, in order to establish the presence or absence of given analytes. It is a simple measurement providing a "yes/no" response. They are simple, cheap, time-saving and do not require sophisticated equipment. Bioautography screening methods are based on the biological activities, e.g. antibacterial, antifungal, antitumor, and antiprotozoae of

the tested substances. This detection method can be successfully combined with Planar Chromatography techniques, such as Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC), Overpressure Layer Chromatography (OPLC) and Planar Electrochromatography^[4]. This work was performed by Direct Bioautography technique. Nutrient broth with selected pathogen in the actively growing phase was sprayed over the TLC plates and plates were incubated for stipulated time at 28°C. To visualize the microbial growth, aqueous solution of *p*-Iodonitrotetrazolium (INT) dye (2 mg/mL) was sprayed over the plates and they were further incubated at room temperature for 6 hrs^[5].

Inference : Bacterial growth led to emergence of purple-red color on the TLC plates, formed due to reduction of INT into formazan. Clear zones (yellow) indicated the inhibition of bacteria due to the antibacterial compound present in that band with particular R_f (Retention factor) on the TLC chromatogram (shown in Fig. 3).

Isolation of active band and identification : The active band which is yellow in colour was scrapped out and taken in methanol and further subjected to LC/MS/MS for structural identification.

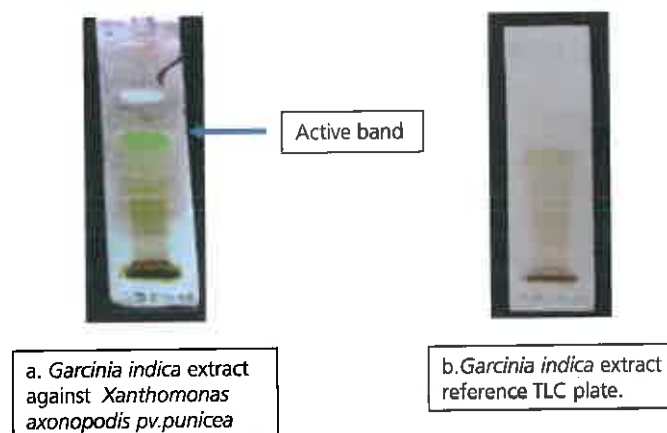


Fig. 3 Results of Bioautography

Identification of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

2-3. LC/MS/MS analysis

Column : Connector
Flow rate : 0.6 mL/min
Oven temperature : 40°C
Detector : UV detector at 238nm
Mobile phase : A: 10 mM Ammonium acetate B: methanol
Gradient program (%B) : 70
Injection volume : 20 µL
MS interface : Electro spray Ionization (ESI)
Nitrogen gas flow : Nebulizing gas 1.5 L/min; Drying gas 10 L/min
MS temperature : Desolvation line 250°C; Heat Block 400°C



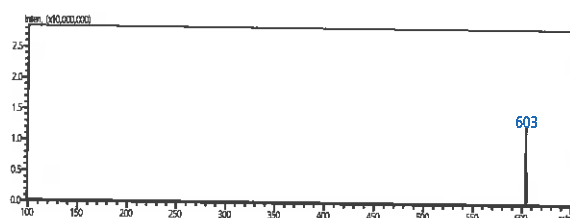
Fig. 4 LCMS-8040 triple quadrupole mass spectrometer by Shimadzu

3. Results

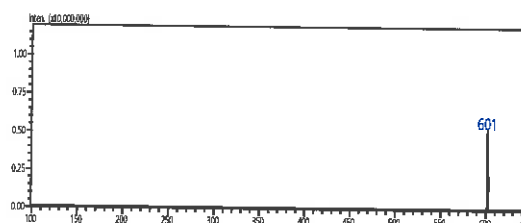
3-1. LC/MS/MS results

Q3 full scan was performed on ESI ionization mode using both polarities. The molecular ion of m/z 603 (positive ESI) and m/z 601 (negative ESI) were obtained (shown in Fig. 5).

It confirmed the molecular weight of antibacterial compound is 602.



a) ESI positive ionization mode



b) ESI negative ionization mode

Fig. 5 MS spectra of Garcinol

LCMS-8040 (shown in Fig. 4) has a feature of 'Optimisation of method' in which the mass spectrometer selects the best product ion(s) and optimises voltages and collision energies for the precursor to product transition.

For Garcinol, MRM was optimised for the precursor ion of m/z 603 using the above mentioned feature. During this

optimisation, the precursor ion was subjected to a series of collision energies ranging from -10 V to -50 V with a step width of 10 V to obtain product ions ranging from higher m/z to lower m/z (shown in Table 1). The fragmentation pathway of Garcinol at different collision energies with probable structures are given in Fig. 6.

Table1 MS/MS data of Garcinol at different Collision Energies (CE)

CE	<i>m/z</i> of Product ions										
-10			137	177	233	287	343	411	467	535	585
-20			137	177	233	287	343	411	467		
-30		81	137	177	233	287	343	411			
-40		81	137	177	233	287	343				
-50	57	81	137	177	233						

Isolation of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

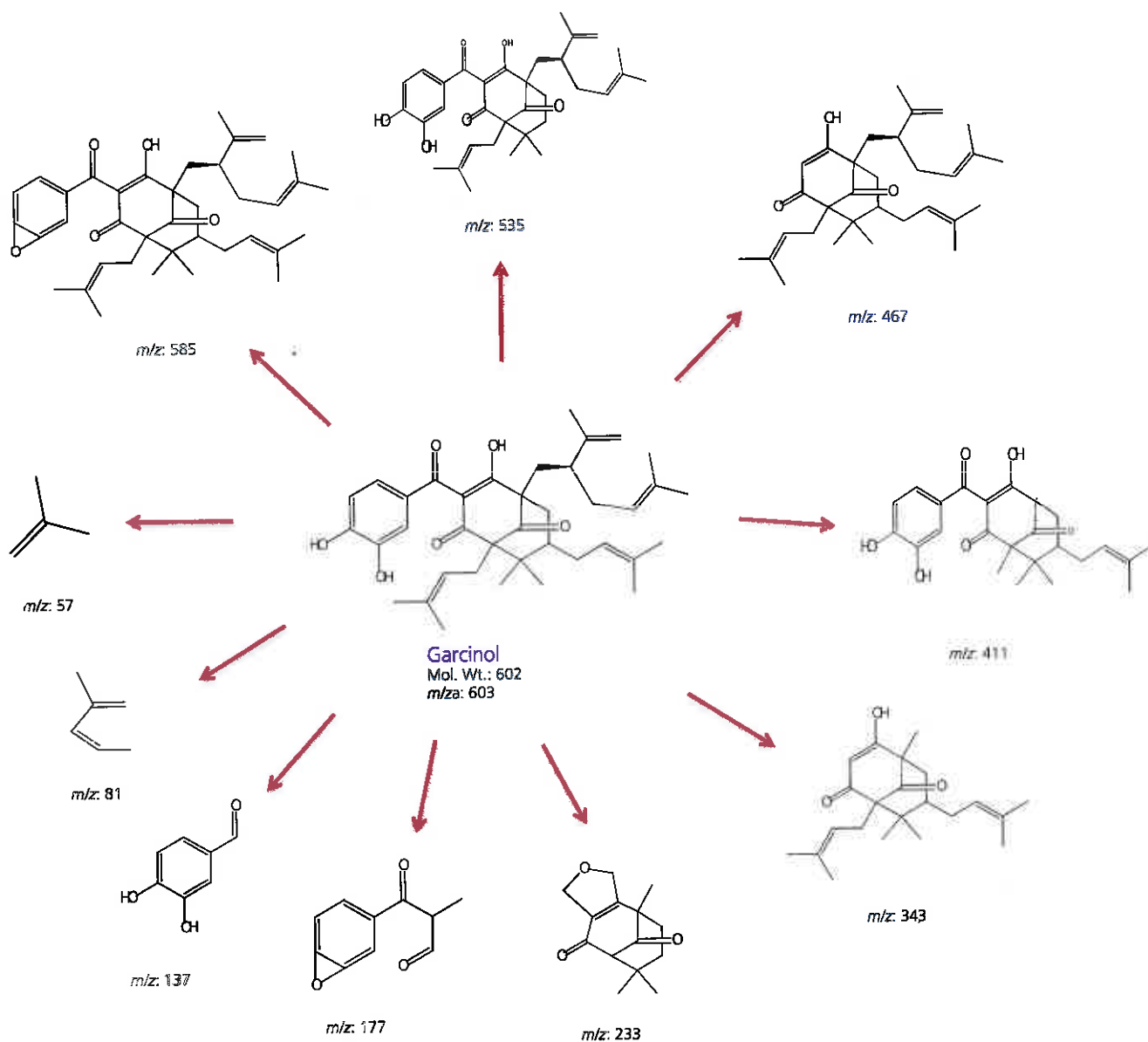


Fig. 6 Probable fragmentation pathway of Garcinol in ESI (+) tandem mass spectrometry

4. Conclusion

- The Bioautography technique was used to distinguish antibacterial component from the *Garcinia indica* fruit rinds extract.
- The antibacterial component scrapped out from TLC was subjected to LC/MS/MS analysis at different Collision Energies. Based on the product ions formed, the structure of antibacterial component was predicted and identified as 'Garcinol'

- Further structural confirmation can be established by using HRMS and NMR techniques.

Identification of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

5. References

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Multi-class pesticides analysis in challenging vegetable matrices using fast 5 msec MRM with 15 msec polarity switching

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Multi-class pesticides analysis in challenging vegetable matrices using fast 5 msec MRM with 15 msec polarity switching

Introduction

Many regulatory authorities have established multi-class residual pesticides methods for the analysis of vegetables, fruits and other food stuffs. There is, however no global agreement on the provision of a target list of pesticides and this presents a risk with products moving between different regulatory requirements. In order to eliminate this risk, food safety laboratories need to ideally screen as many compounds as possible in a single run which may reach

maximum residual limits (MRL); typically 10 ppb in food matrices.

In this study, we report the application of ultra-fast 5 msec MRM with 15 msec polarity switching for the analysis of 138 pesticides in vegetable matrices (72 and 66 compounds measured by LC-QqQ and GC-QqQ in the European Union Reference Laboratory (EURL) method).

Materials and Methods

Sample Preparation (QuEChERS EU method)



Food sample

Vegetables	Origin
Paprika (Sweet pepper)	New Zealand
Leek (Garlic chives)	Japan

Step 1 : Sample Extraction

1. Homogenize vegetables with food processor and homogenizer

2. Weigh 10 g homogenized sample

← Add 10 mL acetonitrile

← Add Salt-mixture*1

- 4 g MgSO₄
- 1 g NaCl
- 0.5 g Na₂H citrate • 1.5H₂O
- 1 g Na₂ citrate • 2H₂O

3. Shake vigorously by hand 1 min.

4. Centrifuge for 5 min. at 4000 rpm (Extract 1)

*1 : Citrate Extraction Tube (SIGMA ALDRICH)



Step 2 : Sample Clean up

5. Transfer 6 mL Extract 1 into Dispersive SPE tube*2

6. Shake vigorously by hand 2 min.

- containing
- 900 mg MgSO₄
- 150 mg PSA
- 45 mg ENVI-Carb

7. Centrifuge for 5 min. at 3000 rpm (Extract 2)

8. Transfer 1 mL Extract 2 into a vial

← Add 200 µL acetonitrile

9. Vortex sample to mix it

10. Filtrate sample with disposable filter

*2 : PSA/ENVI-Carb SPE Clean Up Tube 2 (SIGMA ALDRICH)



LC/MS/MS analysis

Analytical Conditions

HPLC : Nexera UHPLC system

Column : Shim-pack XR-ODSII (75 mm x 2 mm I.D., 2.2 µm)

Mobile phase : A ; 2 mM ammonium formate containing 0.1% formic acid – water
B ; Methanol

Gradient program : 5% B (0-2.5 min.) → 55% B (2.51-6 min.) → 80% B (6.01-12 min.) → 100% (12-15 min.) → 5% (15.01-20 min.)

Flow rate : 0.2 mL / min.

Column temperature : 40°C

MS : LCMS-8040 Triple quadrupole mass spectrometer

Ionization : ESI (Positive / Negative)

Ion spray voltage : +4.5 kV / -3.5 kV

MRM : 276 MRM transitions (2 MRMs / compound)

Dwell time 5 msec. / Pause time 1 msec.

Features of LCMS-8040

- 5 times higher sensitivity compared to LCMS-8030
- An ultra fast scan speed of 15000 u / sec.
- An ultra fast polarity switching of 15 msec.
- An ultra fast MRM transition speed of 555 ch. / sec.



Fig. 1 LCMS-8040 Triple Quadrupole Mass Spectrometer

pesticides analysis in challenging vegetable matrices fast 5 msec MRM with 15 msec polarity switching

Results

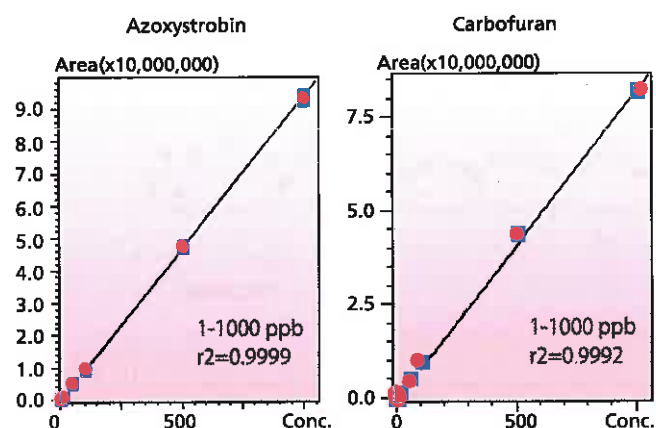
MRM of pesticide standards

Table 1 LOQs of 138 pesticides in the EURL method by LCMS-8040

Technique (on the EURL method)	LOQs < 10 ppb	LOQs > 10 ppb	Not Ionization
by LC-QqQ	72 (100%)	0 (0%)	0 (0%)
by GC-QqQ	47 (71%)	6 (9%)	13 (20%)

• 71 % of pesticides measured by GC-QqQ in the EURL method could achieved excellent LOQs (0.08-10 ppb) .

Compounds for LC-QqQ



Compounds for GC-QqQ

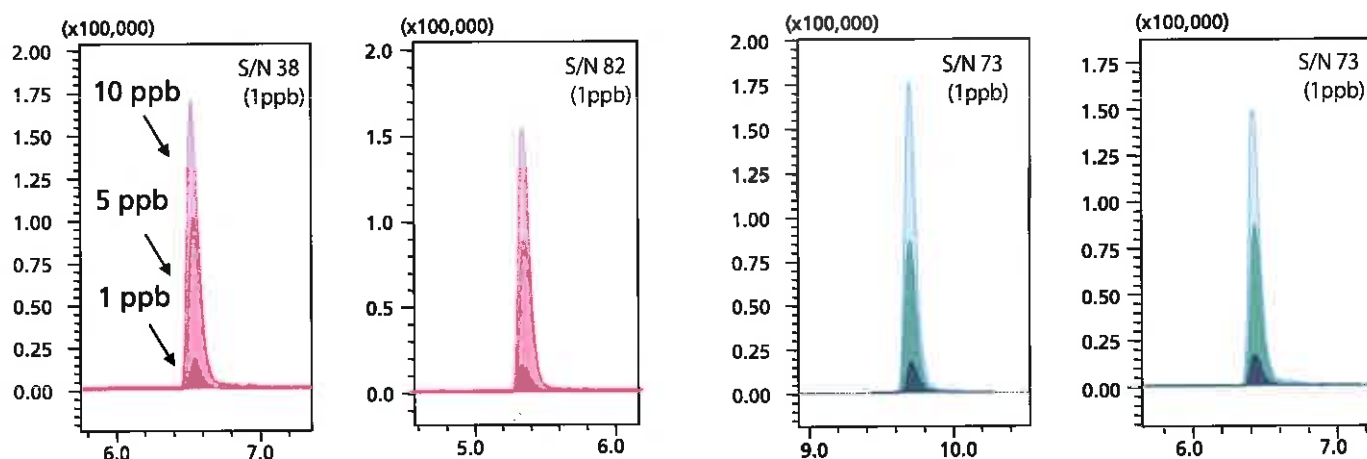
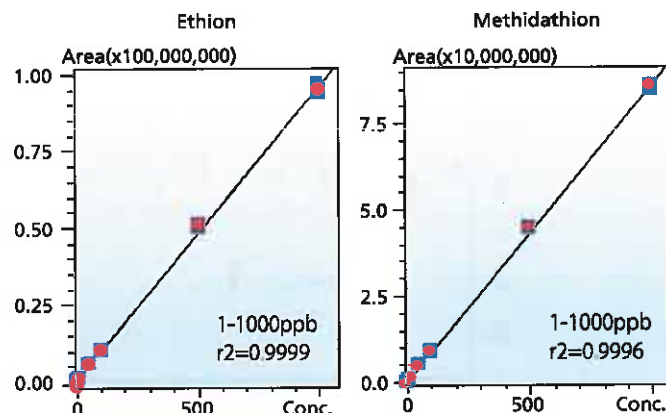


Fig. 2 Calibration curves and MRM chromatograms of typical pesticides

Multi-class pesticides analysis in challenging vegetable matrices using fast 5 msec MRM with 15 msec polarity switching

Recovery of pesticides in vegetable matrices

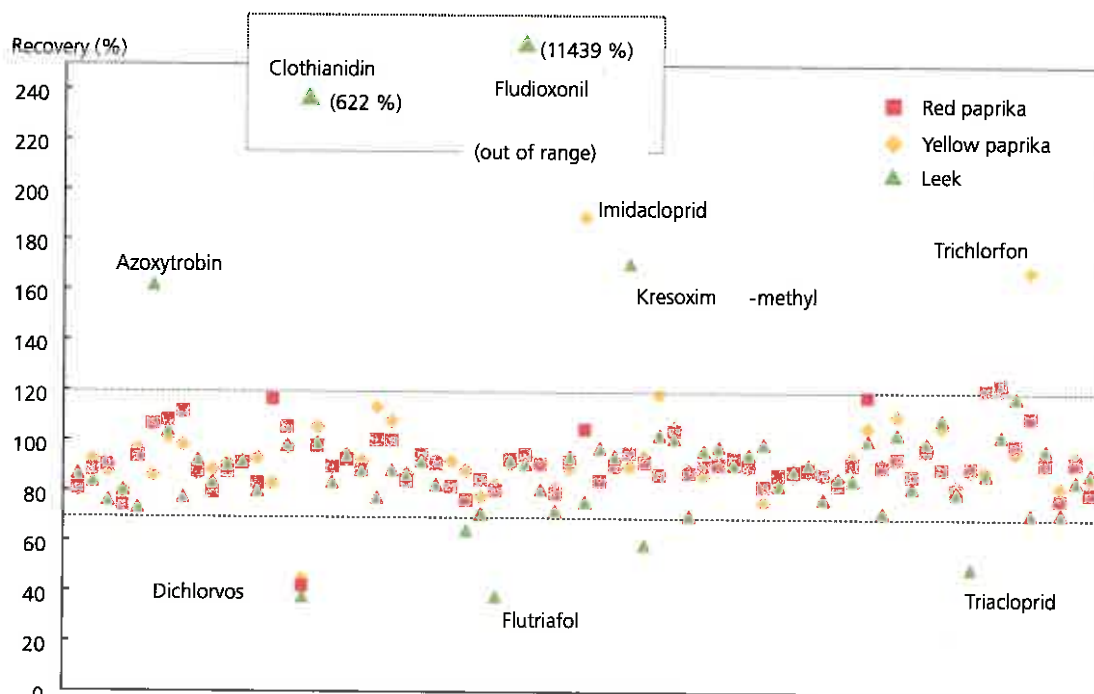


Fig. 3 Recovery of pesticides in the vegetable matrices (5 ppb spiked)

- Approximately 90% of pesticides represented good recoveries in the range of 70-120% in all studied matrices.
- Some pesticides indicating up to 120% of recovery were also detected in the matrix blank (Fig. 4).

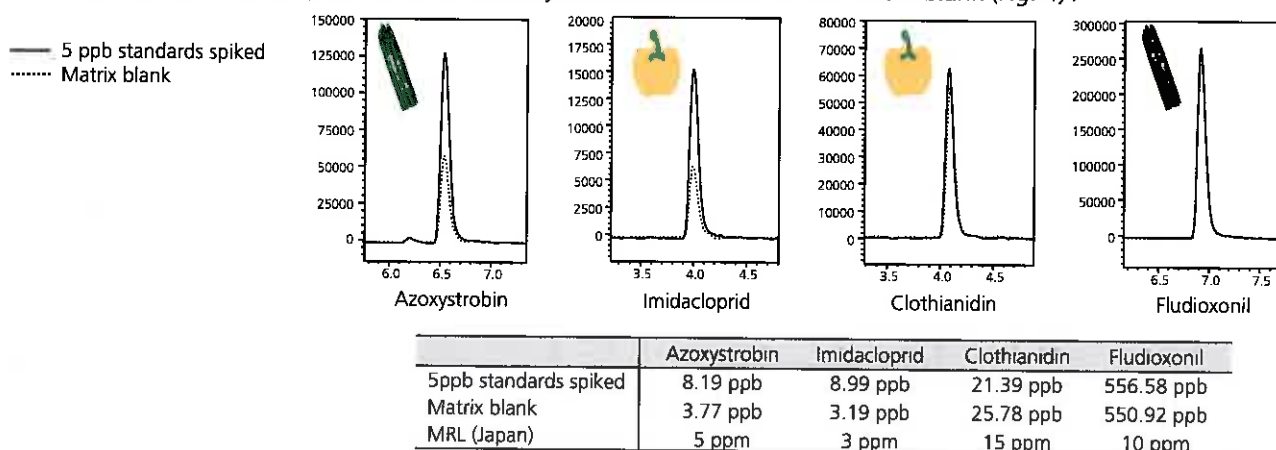


Fig. 4 MRM Chromatograms and quantitative results of 4 pesticides

Conclusion

- MRL of pesticide in vegetable matrices pre-treatment by QuEChERS method could be measured successfully using fast 5 msec MRM with 15 msec polarity switching.
- Majority of pesticides being suggested to GC-QqQ technology on the EURL method was successfully covered by LC-QqQ.



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Quantitative analysis of illegal dyes in eggs using LC/MS/MS

ASMS 2015 TP 334

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Quantitative analysis of illegal dyes in eggs using LC/MS/MS

Introduction

Generally, egg yolk color is an indication of its nutritional value and freshness. Eggs with yellow-orange hue are the most desired ones. Hence, egg producers enhance the color of their products by feeding the egg laying hens

with feed containing various synthetic dyes (shown in Figures 1 and 2). This can be proved by confirming the presence of illegal dye residues in eggs^[1].



Figure 1. Duck eggs



Figure 2. Sudan dyes in egg yolk

A method for pre-treatment and subsequent LC/MS/MS analysis has been developed for highly sensitive quantitation of these dyes from egg using LCMS-8040, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.

Ultra-fast scanning speed of LCMS-8040 enables simultaneous analysis of four Sudan dyes and para red dye. All these dyes were quantitated at low ppb levels with high specificity and good repeatability even in presence of egg matrix.

Sudan and para red dyes consist of a group of synthetic

industrial dyes like Sudan I, II, III and IV etc. The International Agency for Research on Cancer evaluated the safety of Sudan I, II, III and IV in 1987 and considered that they were unclassifiable as to their carcinogenicity to humans. Although there is some evidence that Sudan dyes may cause cancer in experimental animals and may cause damage to the genes, there is currently inadequate evidence that they cause cancer in humans.

Structure of four Sudan dyes and para red under study are as shown in Figure 3.

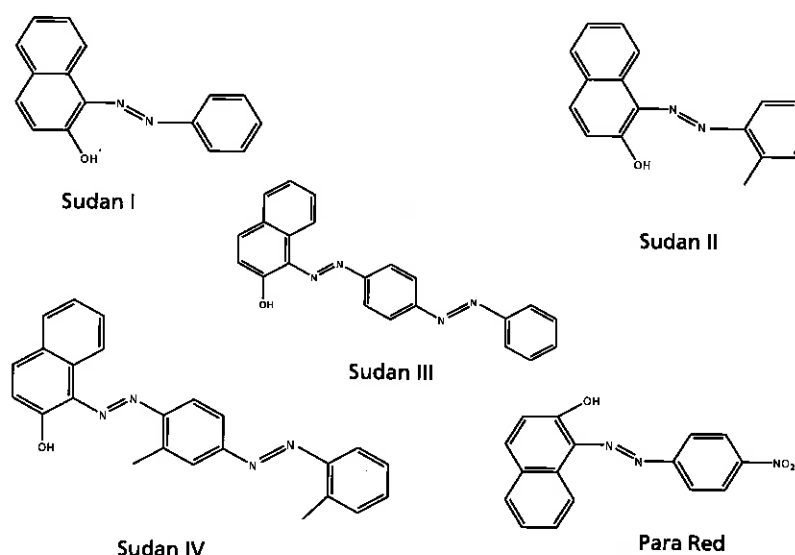


Figure 3. Structure of Sudan dyes and para red used to contaminate egg

Method of analysis

Preparation of matrix matched calibration curve

In 1 g of egg yolk, 9 mL water and 10 mL acetonitrile was added in 50 mL tarson tubes. Mixed it well for one min. For extraction, QuEChERS Kit ^[2] (1 packet EN 15662) (Phenomenex Inc.) was added into it and mixed thoroughly for one min. Centrifuged these tubes at 4000 rpm for 5 min at -10 °C and collected approximately 8 mL

of supernatant.

Passed the 3 mL of collected supernatant through a preconditioned C-18 SPE cartridge (Orochem), which finally eluted with 3 mL acetonitrile. This eluent was used to prepare dilutions for matrix matched calibration in range of 1 ppb - 100 ppb.

LC/MS/MS analysis

Compounds were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan). The details of analytical conditions are given in Table 1.

LCMS-8040 triple quadrupole mass spectrometer as

shown in Figure 4, with its ultrafast polarity switching and ultrafast MRM, along with high sensitivity is best suited instrument for food safety analysis. These features ensure analysis of multiple components with different ionizing tendency to be analyzed within same run, without any loss of sensitivity.



Figure 4. Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Quantitative analysis of illegal dyes in eggs using LC/MS/MS

Table 1. LC/MS/MS conditions for analysis of sudan dyes and para red

Column	: Shim-pack XR-C8 (75 mm L x 3 mm I.D.; 2.2 μ m)
Flow rate	: 0.4 mL/min
Oven temperature	: 40 °C
Mobile phase	: A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Gradient program (B%)	: 0.01 – 1.50 min → 50 – 80 (%); 1.50 – 2.50 min → 80 (%); 2.50 – 4.0 min → 80 – 100 (%); 4.0 – 6.0 min → 100 (%); 6.0 – 6.1 min → 100 – 50 (%); 6.1 – 10.0 min → 50 (%)
Injection volume	: 20 μ L
MS interface	: Electro Spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 3 L/min; Drying gas 10 L/min
MS temperature	: Desolvation line 230 °C; Heating block 400 °C
MRM Transitions	: Sudan I: 249.00>93.20 Sudan II: 277.00>121.15 Sudan III: 353.00>77.10 Sudan IV: 381.00>225.20 Para red: 294.20>156.10

Results

Analysis of sudan and para red dyes was carried out in both aqueous and egg yolk matrix. MRM was optimized using automatic MRM optimization feature of LabSolutions. Analysis was also checked for blank interference. The method optimized had substantially reduced background interferences.

Result of simultaneous analysis of five dyes in egg yolk is

shown in Figure 5. Overlay of blank, LOQ and highest concentrations are shown in Figure 6. Linearity and accuracy were checked for both aqueous and matrix matched calibration. These quantitative results are given in Table 2. LOQ of 1 ppb was obtained for all the five dyes in both aqueous and egg yolk matrix.

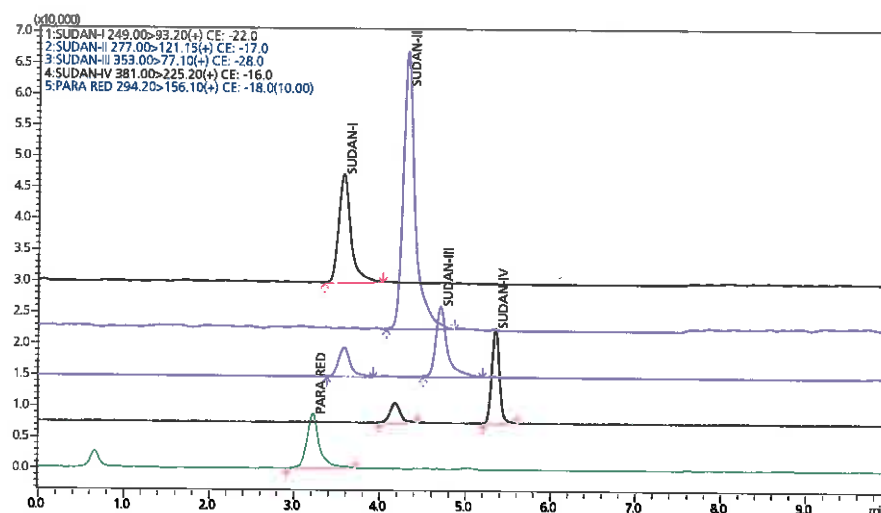


Figure 5. Overlay of sudan dyes and para red for 5 ppb standard in egg yolk

Qualitative analysis of illegal dyes using LC/MS/MS

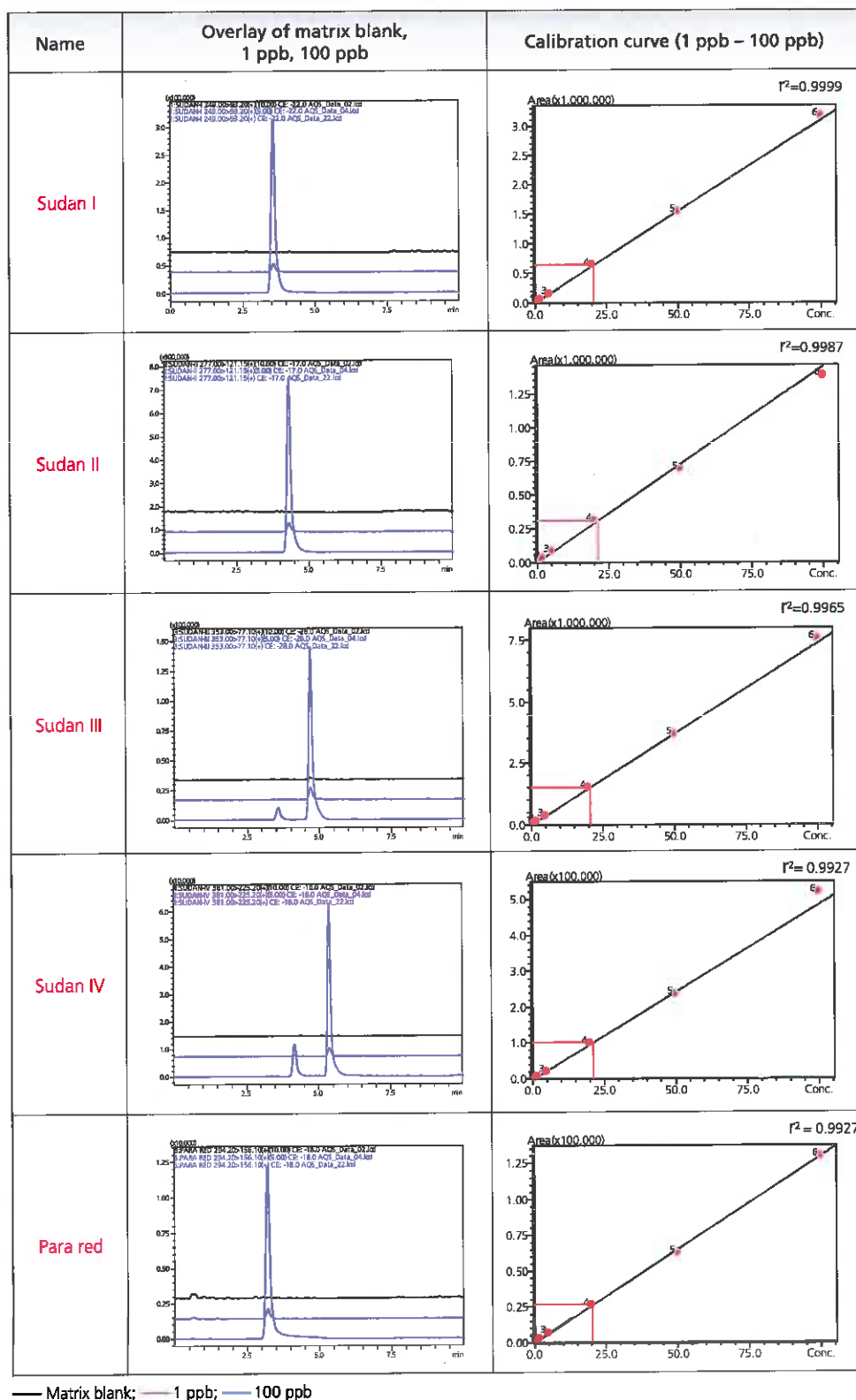


Figure 6. Overlay mass chromatograms and calibration curve for sudan dyes and para red in egg yolk matrix

Quantitative analysis of illegal dyes in eggs using LC/MS/MS

Table 2. Results of sudan dyes and para red calibration curve

Name	Nominal concentration (ppb)	Measured concentration* (ppb)	Avg. area*	%RSD cal.point area*	% accuracy*
Sudan I	1	1.0	35597	7.45	100.4
	2	1.9	69150	0.86	99.2
	5	4.9	172070	3.59	99.8
	20	19.9	684163	1.62	99.7
	50	50.3	1722867	0.70	100.6
	100	100.1	3426385	0.65	100.1
Sudan II	1	0.9	79040	2.74	97.6
	2	2.1	176546	1.75	105.7
	5	5.0	428791	2.47	101.2
	20	19.6	1677856	2.20	98.2
	50	49.1	4199978	0.83	98.2
	100	99.8	8537248	1.07	99.7
Sudan III	1	0.9	21571	2.09	98.2
	2	2.0	41915	9.33	100.3
	5	5.3	109039	2.54	107.7
	20	20.7	413989	2.84	103.7
	50	49.6	987767	1.21	99.3
	100	93.0	1848722	1.48	93.0
Sudan IV	1	1.0	20996	4.96	106.9
	2	2.2	38519	1.36	111.3
	5	5.6	91127	1.21	113.3
	20	19.9	307351	0.98	99.8
	50	50.8	774778	1.20	101.6
	100	91.2	1387058	2.02	91.2
Para red	1	1.0	1605	7.70	100.1
	2	1.9	3256	9.29	99.2
	5	5.1	8498	5.48	102.1
	20	19.6	32836	3.55	98.1
	50	49.8	83635	1.88	99.8
	100	100.9	169136	1.75	100.8

* Average result for n=3

Simultaneous analysis of illegal dyes in egg yolk like four sudan dyes and para red using LC/MS/MS

Conclusion

- Highly sensitive method for simultaneous analysis of illegal dyes in egg yolk like four sudan dyes and para red was developed.
- LOQ of 1 ppb was obtained for all five dyes by matrix matched calibration standards, with linearity > 0.99 and accuracy between 90-110%.
- An analytical method was developed to minimize the background interference, hence giving high level of sensitivity.

References

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Rapid simultaneous assay of 23 mycotoxines in a variety of food samples by UHPLC-MS/MS using fast polarity switching



ASMS 2013 TP-746

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Rapid simultaneous assay of 23 mycotoxins in a variety of food samples by UHPLC-MS/MS using fast polarity switching

1. Introduction

Mycotoxins are contaminants in grains. For consumer food safety, quality control of food and beverages has to assay such contaminants. Rapid determination of the presence and then quantification of hazardous mycotoxins is essential. UHPLC-MS/MS offers the best combination of

selectivity, sensitivity, and speed for detection of these compounds in complex matrices. In this study, a high throughput method for the quantification of 25 mycotoxins in various matrices was established.

2. Methods and Materials

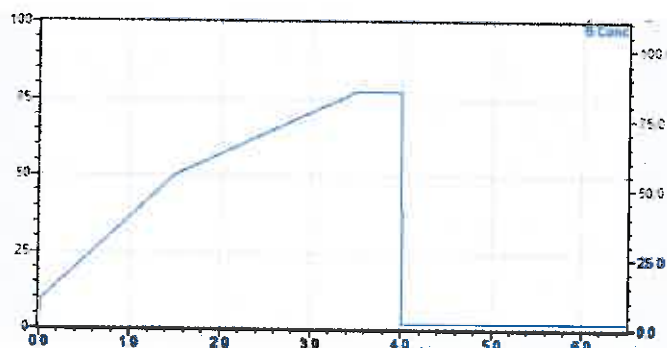
Sample preparation (modified QuEChERS)

Samples (5 g for grains or animal food + 10 mL of water or 10 g for fruits), were mixed with 10 mL of acetonitrile. After maceration, salts were added to allow phase separation. Then the supernatant was 5-fold diluted with mobile A and the internal standard mix was added.

UHPLC conditions



System	: UFLCXR
Column	: Phenomenex Kinetex XB-C18 50*2 mm 2.6μm
Column temperature	: 50°C
Mobile phase A	: Water + 0.5% acetic acid
B	: Isopropanol + 0.5% acetic acid
Flow rate	: 0.4 mL/min
Time program	: Time (min) Pump B Conc
	Initial 2
	0.01 10
	1.50 55
	3.50 85
	4.00 85
	4.01 2
	6.50 Stop
Injection vol.	: 20 μL



Simultaneous assay of 23 mycotoxins in a variety of samples by UHPLC-MS/MS using fast polarity switching

MS conditions (LCMS-8040)

Ionization : ESI, Positive and Negative MRM mode

Ion source temperatures: Desolvation line: 250°C

Heater Block : 500°C

Gases: Nebulization: 3 L/min

Drying : 15 L/min

MRM Transitions: Two transitions were selected for each mycotoxin

Dwell time: 9 msec

Pause time : 1 msec

Loop time : 0.27 sec (maximum)

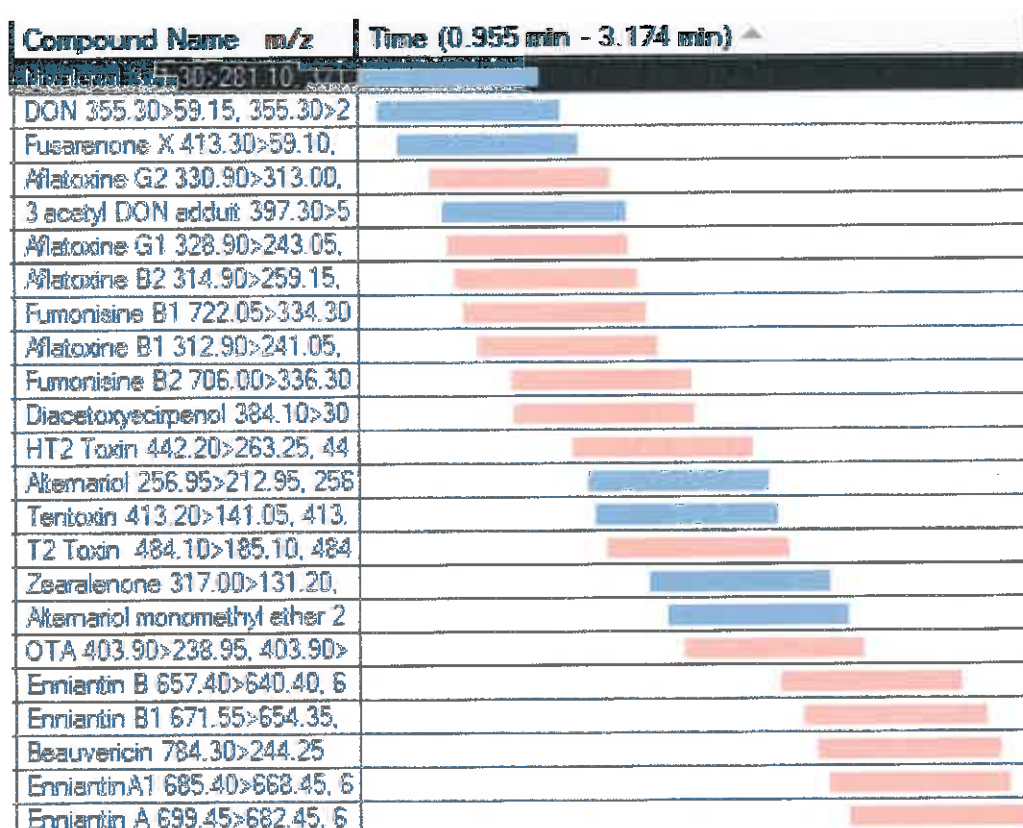


Fig. 1 MRM scheduling for positive (red) and negative (blue) ionization

Rapid simultaneous assay of 23 mycotoxins in a variety of food samples by UHPLC-MS/MS using fast polarity switching

3. Results

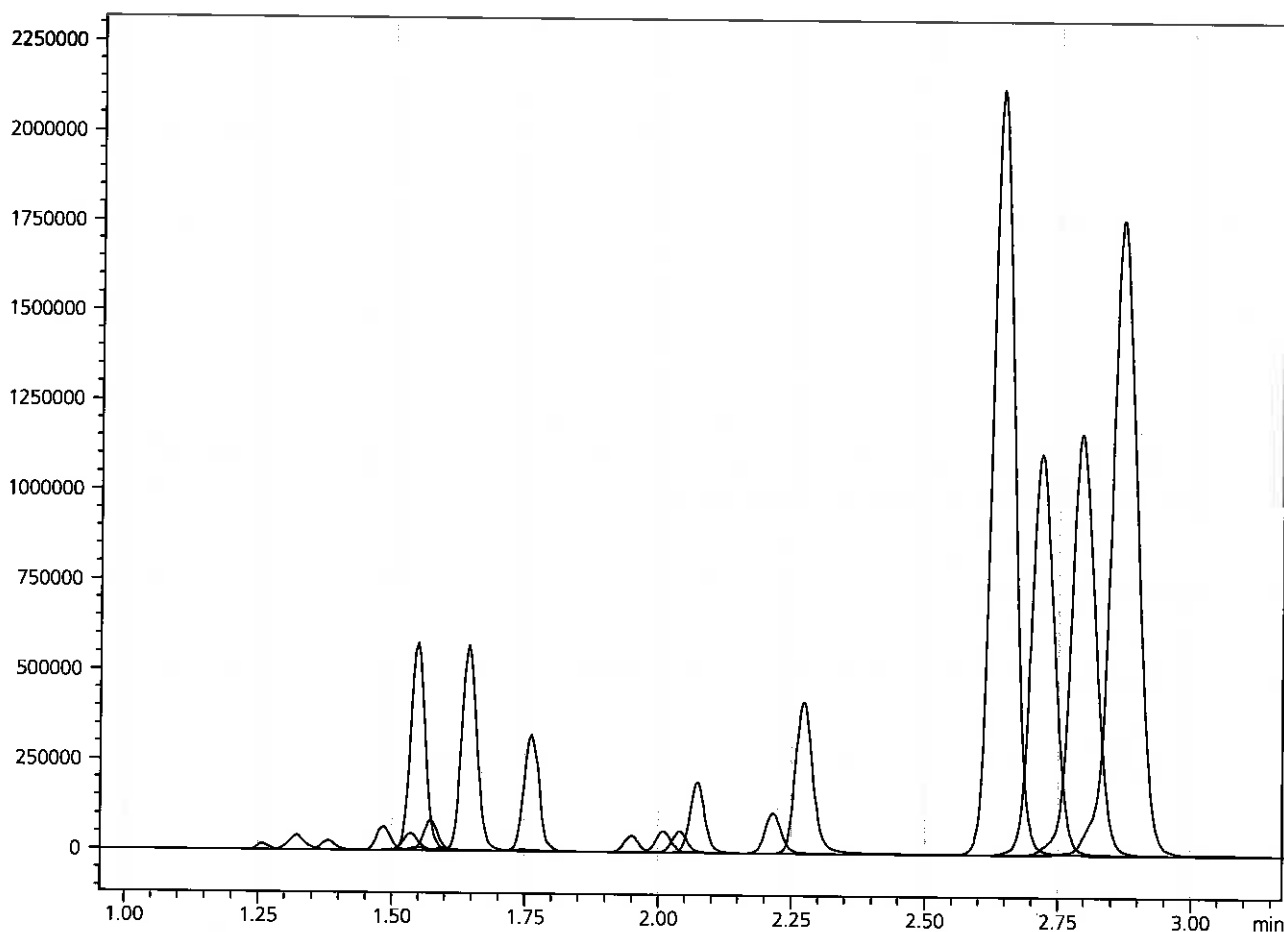


Fig. 2 typical chromatogram at 50 ppb (see Fig. 1 for elution order)

Specificity and matrix effect

The assay specificity was studied in various matrices to show the correlation of results in neat standards with matrix standard.

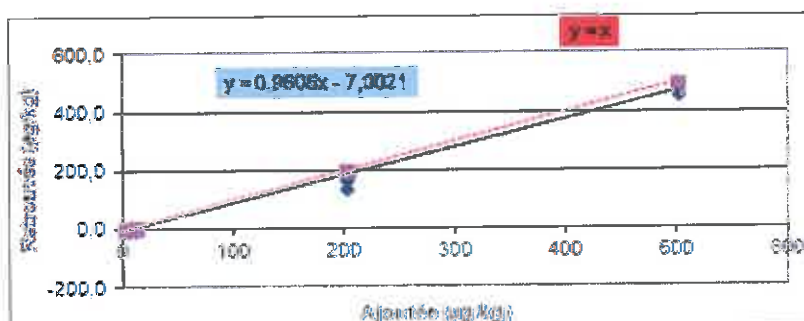
The concentration obtained was compared to the theoretical one. The specificity was validated for example in the following matrices : rice, maize, dry pastas, banana, muesli, wheat, carrot, apple compote, flour, etc....

The figure 3 shows the correlation data for exemplary mycotoxins in the cited matrices at different levels.

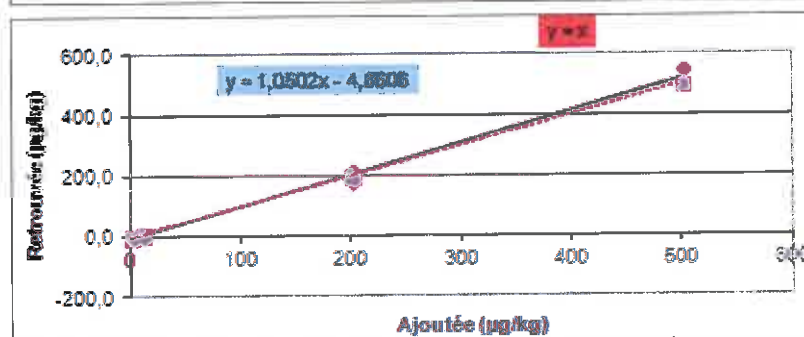
These data shows that the method gives accurate concentration results of the mycotoxins whatever the matrix assayed against neat solution standards. This suggests that the method is specific and free of significant matrix effect.

Simultaneous assay of 23 mycotoxines in a variety of samples by UHPLC-MS/MS using fast polarity switching

Aflatoxin B2



Ochratoxin A



Toxin T2

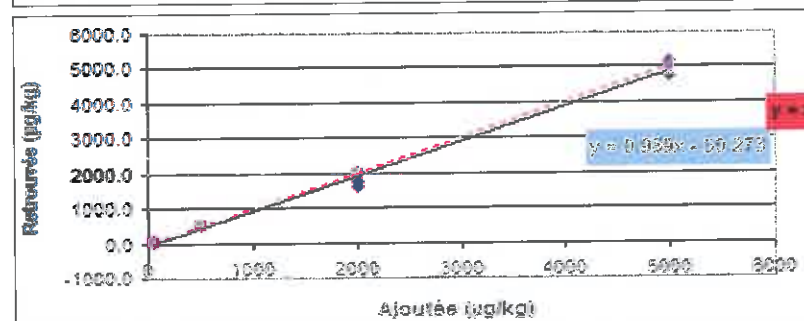


Fig. 3 Correlation data

4. Conclusions

- The fast polarity switching and the low electronic pause time allow simultaneous analysis of co-eluted compounds,
- The method is fast and accurate,
- The sample preparation and the selectivity of the method lowered the matrix affect, thus neat standards can be used for a variety of different samples. This increases system productivity.

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Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

ASMS 2013 WP-098

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Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

1. Introduction

Thyroid hormones are essential hormones involved in growth regulation and development. Free circulating thyroxine concentrations are constant and are correlated with the tissue hormone level. Highly sensitive and specific

methods are needed because most circulating thyroid hormone is bound to plasma proteins and only a few pg/mL are in the unbound free form.

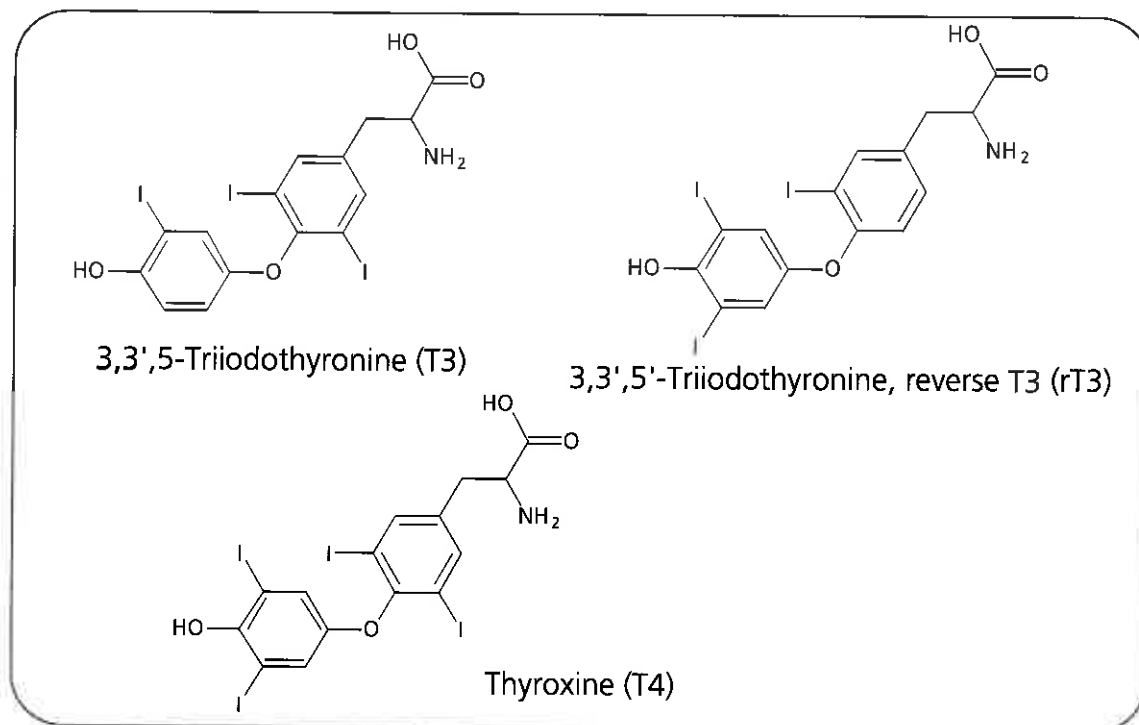


Fig. 1 Structure of studied thyroid hormones

2. Methods and Materials

Thyroxine (T4) and 3,3',5-Triiodothyronine (T3) were assayed by online SPE-UHPLC-MS/MS.

3,3',5'-Triiodothyronine, reverse T3 (rT3) is isobaric with T3, it was not assayed but was monitored in order to control that only T3 was effectively quantified. Free thyroid hormones were first isolated from plasma proteins by ultrafiltration. Then filtrate was injected on online SPE column. Free thyroid hormones were then separated and assayed by UHPLC-MS/MS.

Ultrafiltration

500 µL of plasma were ultra filtrated using an centrifree device (Millipore) at 2000 g for 20 minutes. About 150-200 µL of ultrafiltrate were obtained. 120 µL of ultrafiltrate were transferred to a vial with glass insert. Internal standards (¹³C₆ analogues) were added at a final concentration of 10 pg/mL.

Assay of free thyroid hormones online SPE-UHPLC-MS/MS in human plasma

Online Solid Phase Extraction

Column : Phenomenex STRATA-XC 20 × 2 mm
 Mobile phases : Sample loading (A): Water + 0.1% formic acid
 Washing (B): Methanol/acetonitrile/formic acid (50/50/0.1 v/v)
 Column cleaning (C): Methanol/acetonitrile/isopropanol/water/TFA (25/25/25/25/0.1 v/v)
 Flow rate : 2 mL/min
 Time program : 0-0.5 min : 100% A sample loading
 0.5-1 min : 100%B interferences elution
 1-1.5 min : column backflush with UHPLC mobile phase B'
 1.5-3.5 min: 100%C column cleaning
 3.5 – 8.5 min 100% A column conditioning
 Injection vol. : 100 µL

UHPLC conditions (Nexera MP system)

Column : Phenomenex Synergi Fusion-RP C18 50 × 2 mm 2.5 µm
 Mobile phase A : Water + 0.05% Ammonia
 B : Acetonitrile + 0.05% Ammonia
 Flow rate : 0.4 mL/min
 Time program : B conc. 10%(0-1.5 min) -10 to 100%(1.5 – 5.5 min) - 10%(5.51 – 8.5 min)
 Column temperature : 30°C

MS conditions (LCMS-8040)

Ionization : ESI, Negative MRM mode
 Ion source temperatures: Desolvation line : 250°C
 Heater Block : 400°C
 Gases : Nebulization : 3 L/min
 Drying : 15 L/min

MRM Transitions:

Compound	MRM	Dwell time (msec)
T3	649.70>126.90 (Quan)	20
	649.70>632.75 (Qual)	20
T4	775.50>126.90 (Quan)	20
	775.50>604.80 (Qual)	20
rT3	649.50>126.90 (Quan)	20
	649.50>604.80 (Qual)	20

Pause time : 3 msec
 Loop time : 0.164 sec (minimum 15 points per peak for each MRM)

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

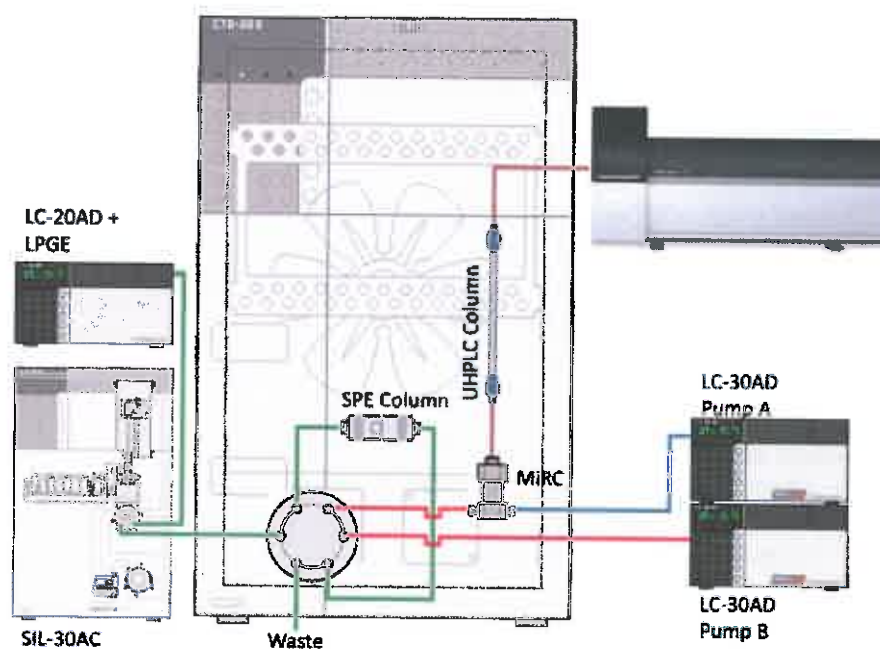


Fig. 2 Scheme of the analytical system

3. Results

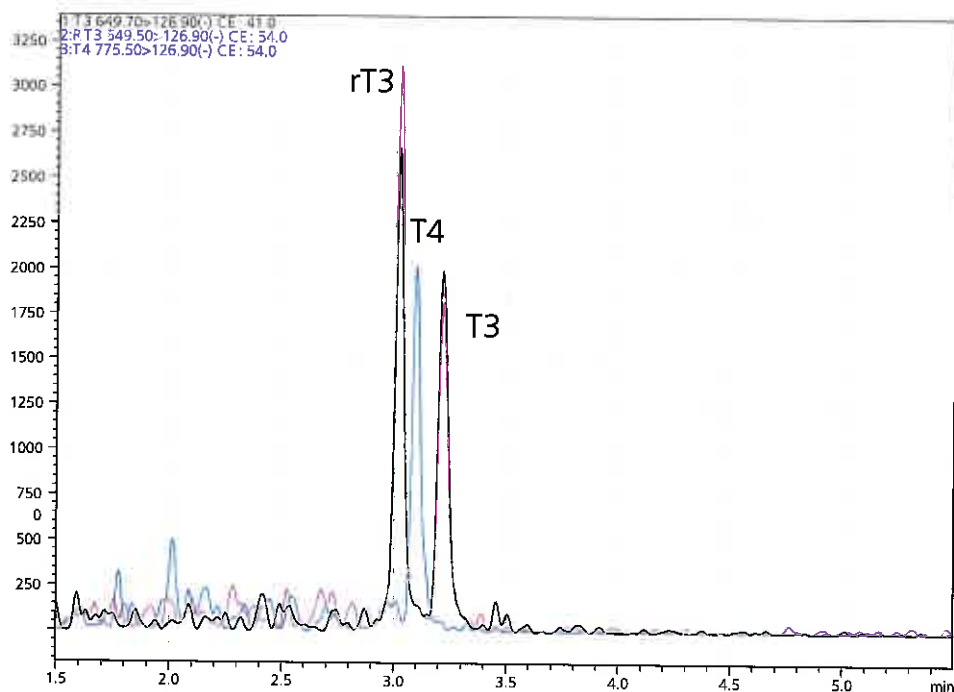


Fig. 3 Typical patient sample with hormone levels of about 10 pg/mL

Assay of free thyroid hormones by online SPE-UHPLC-MS/MS in human plasma

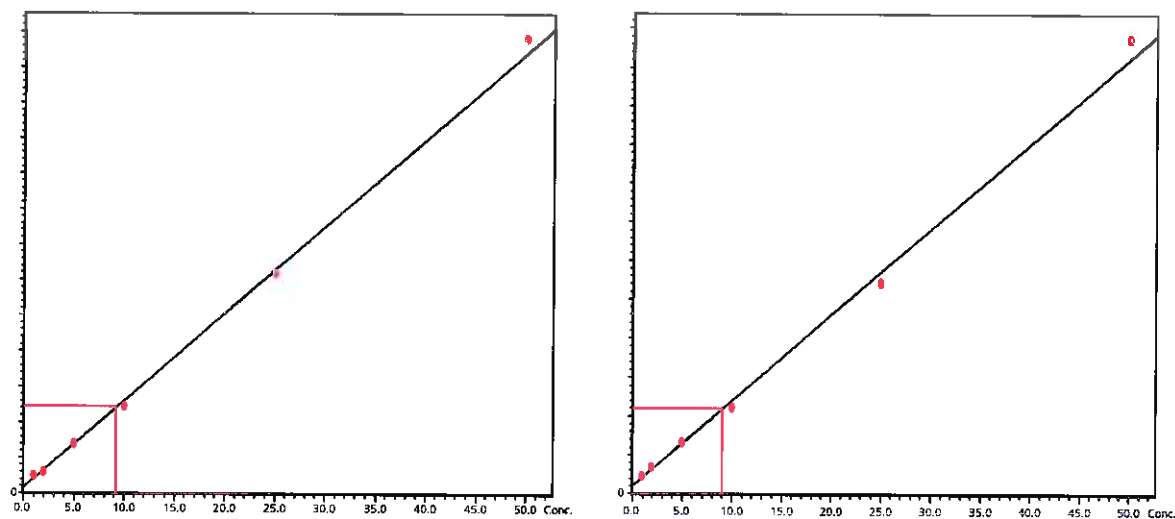


Fig. 4 Calibration curves of T3 (left) and T4 (right) from 1 to 50 pg/mL

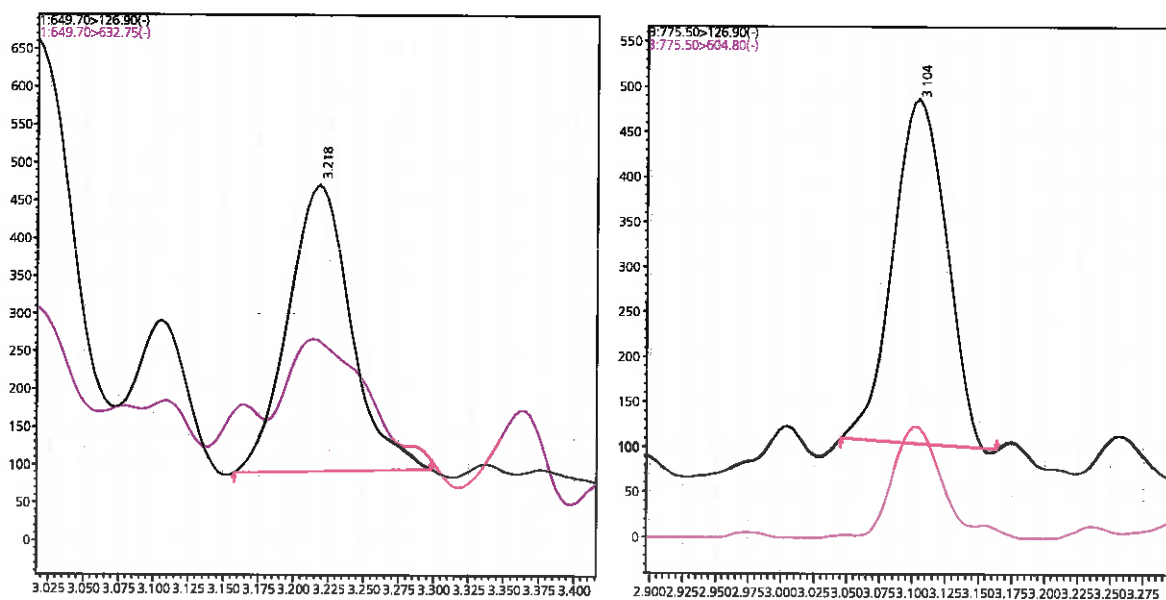


Fig. 5 Lowest calibration point for T3 (left) and T4 (right) at 1 pg/mL

Sensitive assay of free thyroid hormones by on line SPE-UHPLC-MS/MS in human plasma

4. Conclusions

- The method enable fast and sensitive assay of free thyroid hormone levels in plasma sample,
- The sensitivity required to measure low levels is obtained and the matrix effect are limited by the use of a mixed-mode (cation exchange and hydrophobic) on line SPE column,
- The purification of small volumes of ultra filtrate is facilitated by on line SPE,
- The monitoring of the rT3 is required to be sure that there is no bias in T3 quantification.

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Targeted Screening and Quantification of Pesticide Residuals in Tobaccos by Ultra Fast LC/MS/MS

ASMS 2013 ThP-530

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Targeted Screening and Quantification of Pesticide Residuals in Tobaccos by Ultra Fast LC/MS/MS

1. Introduction

Pesticides are used widely during cultivation of plants including tobaccos.

Although they are not foodstuffs, there has been a global concern in the tobacco industry and public about pesticide residues being taken into the body. LC/MS/MS methods have been employed increasingly in detection and quantification of pesticide residuals in foods and agriculture products. One of the challenges in food safety analysis is the large numbers of pesticides on the watching lists imposed by authorities, which leads to screening analysis using conventional MRM method on triple quadrupole

LC/MS/MS to be difficult.

The new generation ultra-fast LC/MS/MS technique introduced recently features with ultra fast MRM speed (>500 MRM transitions per second), which enhances greatly the capacity of MRM method when it is used for screening analysis [1].

Both screening analysis and quantification could be carried out using a single method on the same system. Here we report an example of screening analysis and quantification of pesticides in tobaccos using a ready-to-use Method Package on LCMS-8040 system.

2. Experimental

Three samples of dried tobacco leaves labelled as A, B and C were obtained from a manufacture for this study.

The QuEChERS (Restek) method was employed for extraction of pesticides from the samples for screening analysis using a ready-to-use MRM based Method Package developed by Shimadzu [2].

The details of the pre-treatment method are described in Fig. 1.

The Method Package includes the complete analytical conditions from retentions and MRM transitions of 167 pesticides which are on the Positive List (Japanese Regulation).

By applying this method directly, screening analysis for the pesticides listed could be easily carried out without any method development efforts.

The results of this direct screening analysis were treated as the preliminary results, which were required to be further confirmed.

The LC/MS/MS system used was LCMS-8040 (Shimadzu Corporation, Japan) coupled with Nexera UHPLC.

The details of the LC and MS conditions are shown in Table 1.

The purposes of this study are in two aspects. Since the pesticides included in the Method Package (167 in current version) may be not sufficient for particular projects running in a testing laboratory, expanding of the compound list of the Method Package may become a task to be carried out for users. Therefore, the first aspect of the study was to practise the procedure of adding new registration of some concerned pesticides which are not in the pesticide list.

The second aspect was to validate and quantify the screening results obtained from the direct screening analysis. Nine concerned pesticides standards were obtained from Sigma-Aldrich.

A mixed stock solution of the nine pesticides standards (Tables 2 & 3) was prepared and spiked into the extraction solution of Samples A.

The pesticide standard in the mixed stock solution was 2500 ng/mL and was further diluted into calibration series.

Screening and Quantification of Pesticide Residuals Accos by Ultra Fast LC/MS/MS

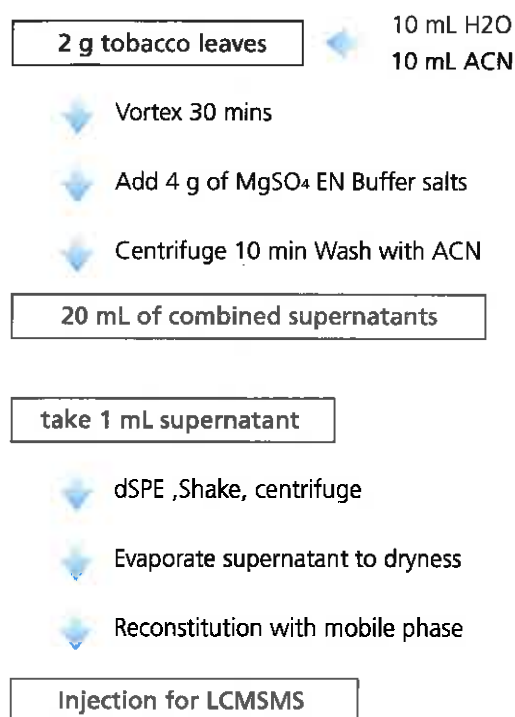


Fig. 1 QuEChERS Extraction

Table 1 MRM Based Screening Method

LC Conditions	
Instrument	Shimadzu LCMS-8040
Column	Shim-pack FC-ODS 150 × 2 mm, 3 μm
Mobile Phase	A: 5 mmol/L Ammonium Acetate – Water B: 5 mmol/L Ammonium Acetate – Methanol
Gradient (B Conc.)	15%, 0 min → 40% 1~3.5 min → 50%, 6 min → 55%, 8 min → 95%, 17.5 min ~ 30 min → 15%, 30.01 → 40 min (stop)
Flow rate	0.2 mL/min
Oven temperature	40°C
Injection volume	5 μL
MS Conditions	
MS interface	ESI
MS mode	Two MRM for each compound
Heat Block temp.	400°C
DL temp.	250°C
Nebulizing Gas	1.5 L/min
Drying Gas	10 L/min

Table 2 New registration of Pesticides onto the Shimadzu Method Package

Additional Pesticide	RT (min)	MRM	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Clomazone	14.050	240.1 > 125.1	-28	-19	-26
		240.1 > 89.1	-28	-45	-18
Profenofos	18.442	373.0 > 303.0	-27	-17	-23
		373.0 > 345.1	-27	-12	-19
Chlorpyrifos	19.299	350.0 > 198.1	-26	-18	-23
		350.0 > 125.1	-26	-20	-26
Flumetralin	19.416	422.1 > 143.1	-30	-25	-30
		422.1 > 107.0	-30	-60	-22
Pendimethalin	19.439	282.2 > 212.1	-20	-10	-25
		282.2 > 194.1	-20	-18	-22
Butralin	19.656	296.2 > 240.2	-22	-13	-28
		296.2 > 222.2	-22	-21	-27

Targeted Screening and Quantification of Pesticide Residuals in Tobaccos by Ultra Fast LC/MS/MS

3. Results and Discussion

3-1 Targeted Screening Analysis using ready-to-use method package

The ready-to-use Method Package for pesticides consists of 167 pesticides for screening analysis.

In addition to these pesticides, six other concerned pesticides as shown in Table 2 were registered onto the Method Package using their standards and tested under the same conditions.

As a result, the modified method package was expanded to include 173 compounds with completed parameters of retentions and optimized MRMs.

The expanded method package was applied to the samples A, B and C for screening analysis of the 173 pesticides. The results were summarized in Table 3.

It can be seen that a total of six pesticides were found in the three samples at significant levels as shown in Fig. 2. The screening results are considerably reliable because of matching of two MRM transitions as well as retention of each pesticide found.

However, further confirmation by spiked samples of the found pesticides is required to reach solid conclusions.

3-2 Validation and Quantification of Screening Results

It is necessary to use spiked samples to validate the screening results above.

At the same time, quantification of the found pesticides could be obtained if calibration curves were established using spiked samples.

A series of spiked samples were prepared by adding mixed standards (six pesticides) into the extract solutions of samples A, B and C. The chromatograms of sample A and its 100 ppb spiked samples are shown in Fig. 3.

It can be seen that the MRM peak pairs of every found pesticide (except Methomyl) in sample A were in accordance with the spiked samples in terms of MRM peak ratio and peak intensity which increased proportionally with the spiked amount. This results firmly confirm the screening results above.

MRM calibration curves of the six found pesticides were established using the spiked samples into Sample A as matrix.

The calibration curves with excellent linearity for ranges from 1 ppb to 500 (or 2500) ppb are shown in Fig. 4.

The performance of the MRM quantification method was evaluated and the results are summarized in Tables 4-6. The repeatability of the method was evaluated and the RSD (% , n = 6) of peak area obtained for 50 ng/mL and 10 ng/mL concentration were found to be below 5% except chlorpyrifos (11.5%).

Table 6 shows the results of matrix effect (ME), recovery (RE) and process efficiency (PE) of the six pesticides in Samples A, B and C by the QuEChERS pre-treatment and MRM quantification method.

Both post-spiked and pre-spiked samples were prepared and analyzed using the MRM quantification method established. The results indicate that the current method from sample pre-treatment by the modified QuEChERS method and MRM quantification method generated considerably excellent results in low matrix effect, high recovery and high process efficiency.

Screening and Quantification of Pesticide Residuals in Tobacco by Ultra Fast LC/MS/MS

Table 3 Screening results using the expanded method package of 173 pesticides

No	Found Pesticides	Result of Screening Analysis		
		Sample A	Sample B	Sample C
1	Methomyl	Detected	-	-
2	Thiodicarb	Detected	-	Detected
3	Azoxystrobin	Detected	-	-
4	Profenofos	Detected	Detected	Detected
5	Chlorpyrifos	-	-	Detected
6	Butralin	-	Detected	-

Note: pesticide No. 4-6 were new registrations into the Method Package in this study

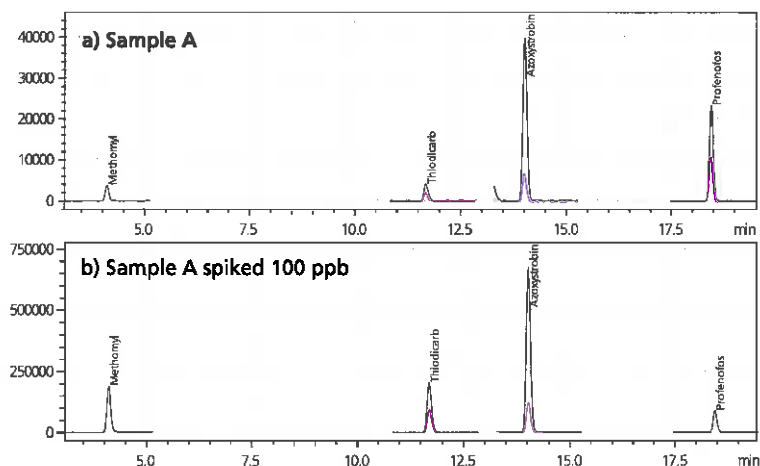


Fig. 3 MRM Chromatograms of Sample A (a) and Sample A spiked with 100 ppb pesticide standards (b)

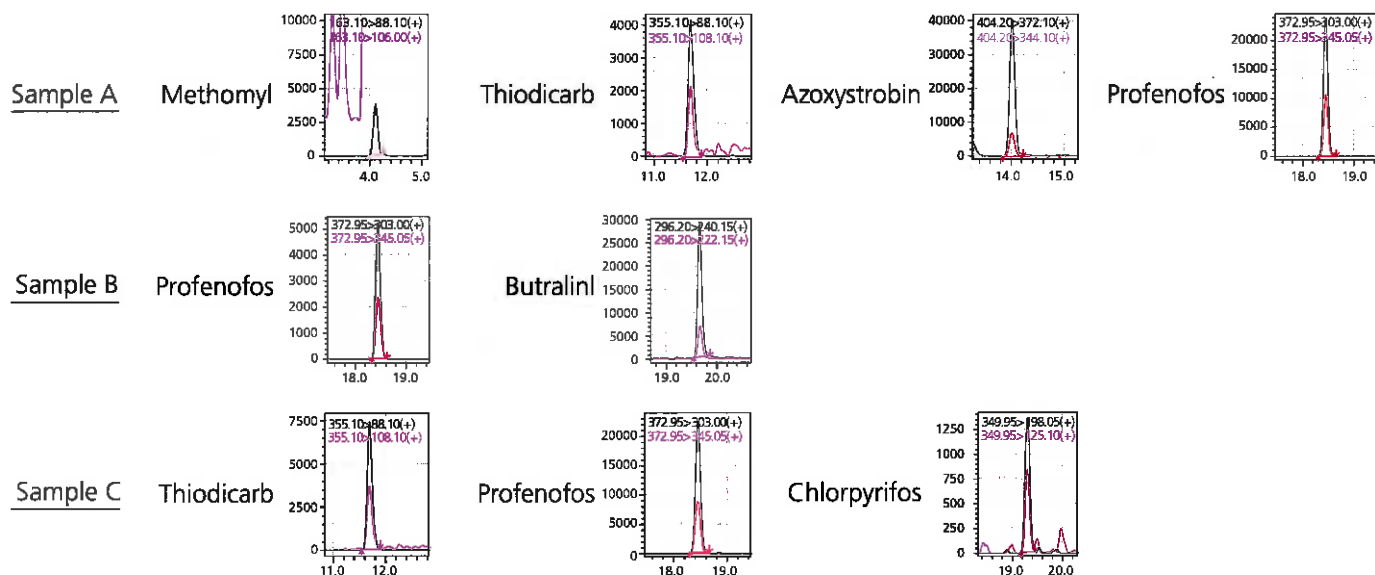


Fig. 2 MRM peak pairs of found pesticides in Samples A, B and C using the expanded method package of 173 pesticides on LCMS-8040

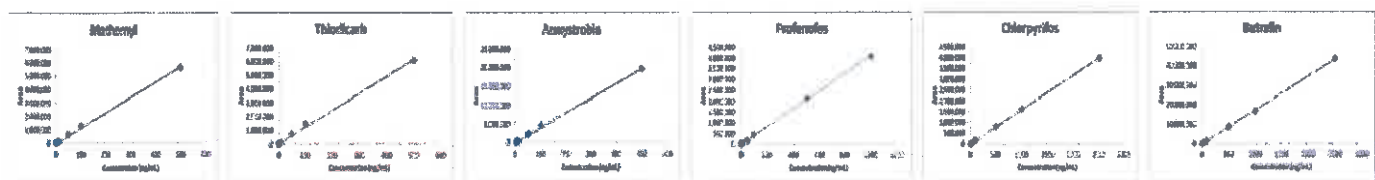


Fig. 4 Calibration Curves of six pesticides in tobacco leaf extract by MRM method

Targeted Screening and Quantification of Pesticide Residuals in Tobaccos by Ultra Fast LC/MS/MS

Table 4 Range and linearity of calibration curves

No.	Compounds	Ret. Time	Calibration	
			Range (ppb)	R ²
1	Methomyl	4.110	1 – 500	0.9984
2	Thiodicarb	11.693	1 – 500	0.9965
3	Azoxystrobin	14.019	1 – 500	0.9987
4	Profenofos	18.442	1 – 1000	0.9996
5	Chlorpyrifos	19.299	1 – 2500	0.9998
6	Butralin	19.656	1 – 2500	0.9998

Table 5 Repeatability of MRM method

No.	Compounds	% RSD (10 ppb, n = 6)		% RSD (50 ppb, n = 6)	
		Ret Time	Area	Ret Time	Area
1	Methomyl	0.127	3.22	0.025	2.39
2	Thiodicarb	0.055	1.18	0.030	1.74
3	Azoxystrobin	0.031	0.97	0.009	2.13
4	Profenofos	0.032	2.92	0.024	2.47
5	Chlorpyrifos	0.042	11.46	0.019	2.08
6	Butralin	0.017	3.22	0.014	4.33

Table 6 Matrix effect (ME), recovery (RE) and process efficiency (PE)

No.	Compounds	Average (%), n = 3		
		ME	RE	PE
1	Methomyl	98.0	84.7	82.9
2	Thiodicarb	113.4	89.2	101.0
3	Azoxystrobin	115.4	81.0	91.2
4	Profenofos	104.5	85.7	89.4
5	Chlorpyrifos	95.1	83.2	79.1
6	Butralin	88.3	80.0	70.7

The calculation of ME, RE and PE

$$\text{Matrix Effect (\%): ME} = \frac{\text{Conc. of Post Spiked} - \text{Conc. Sample blank}}{\text{Conc. of Standard}} \times 100\%$$

$$\text{Recovery (\%): RE} = \frac{\text{Conc. of Pre Spiked} - \text{Conc. Sample blank}}{\text{Conc. of Pre Spiked} - \text{Conc. Sample blank}} \times 100\%$$

$$\text{Process Efficiency (\%): PE} = \frac{\text{Conc. of Pre Spiked} - \text{Conc. Sample blank}}{\text{Conc. of Standard}} \times 100\%$$

The quantitative MRM method established was then applied to the samples by processing the raw data in the Postrun program of the LabSolutions. The quantification

results in extract solutions and converted to tobacco leaves in ng per gram are shown in Table 7.

Table 7 Quantification results of pesticides in tobacco leaves samples by MRM method

No	Found pesticide	Quantification result in extract solution (ppb)			Quantification result in tobacco leaves (ng/g)		
		Sample A	Sample B	Sample C	Sample A	Sample B	Sample C
1	Methomyl	2.20	-	-	22.00	-	-
2	Thiodicarb	2.48	-	4.06	24.80	-	40.60
3	Azoxystrobin	6.92	-	-	69.20	-	-
4	Profenofos	36.41	8.18	36.10	364.10	81.80	361.00
5	Chlorpyrifos	-	-	6.20	-	-	62.00
6	Butralin	-	10.10	-	-	101.00	-

4. Conclusions

This study shows that the ready-to-use MRM based Method Package provides an easy and reliable workflow for both screening analysis and quantification of residual pesticides in tobacco leaves on the LCMS-8040. Because of the expandability of the Method Package, users can add easily any desired new registration of pesticides or other concerned compounds.

This flexibility makes it even more valuable in research, manufacture and testing laboratories targeting for different groups of pesticides in tobacco leaves etc. The procedure as well as the strategy of using the Method Package for both targeted screening analysis followed by quantification of found pesticides can be applied to other food safety analysis.

Screening and Quantification of Pesticide Residuals Accos by Ultra Fast LC/MS/MS

5. References

1. Shimadzu Corporation, Catalogue, LC/MS/MS Method package for Residual Pesticides, C146-E160
2. Michelle Misselwitz, Jack Cochran, Julie Kowalski.
The QuEChERS Sample Preparation Approach with Dispersive and Cartridge SPE Cleanup, GCxGC-TOFMS, and LC-MS/MS for the Analysis of Pesticides in Tobacco. Retrieved from:
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Rapid development of quantitative method for determination of plant growth regulators and streptomycin in fruits using LC/MS/MS

ASMS 2016 WP 234

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Rapid development of quantitative method for determination of plant growth regulators and streptomycin in fruits using LC/MS/MS

Introduction

Plant growth regulators (PGRs) and antibiotics are commonly used by farmers for enhancing growth of crops and to protect against infections respectively. However, extensive usages of these compounds is known to have adverse effect on human health^{[1],[2]}. Each standard has different linearity range as shown in Table 2. It is challenging to quickly develop multi-analyte quantitation method for determination of varied compounds simultaneously^[3]. One interesting approach can be Method Scouting, which allows user to conduct all

method development exercises automatically. This results in swift development of an optimum method which will suit all analytes.

Highly sensitive quantitative method was developed quickly using Nexera Method Scouting system for determination of eight PGRs and streptomycin (shown in Figure 1) on LCMS-8040 (shown in Figure 2), a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.

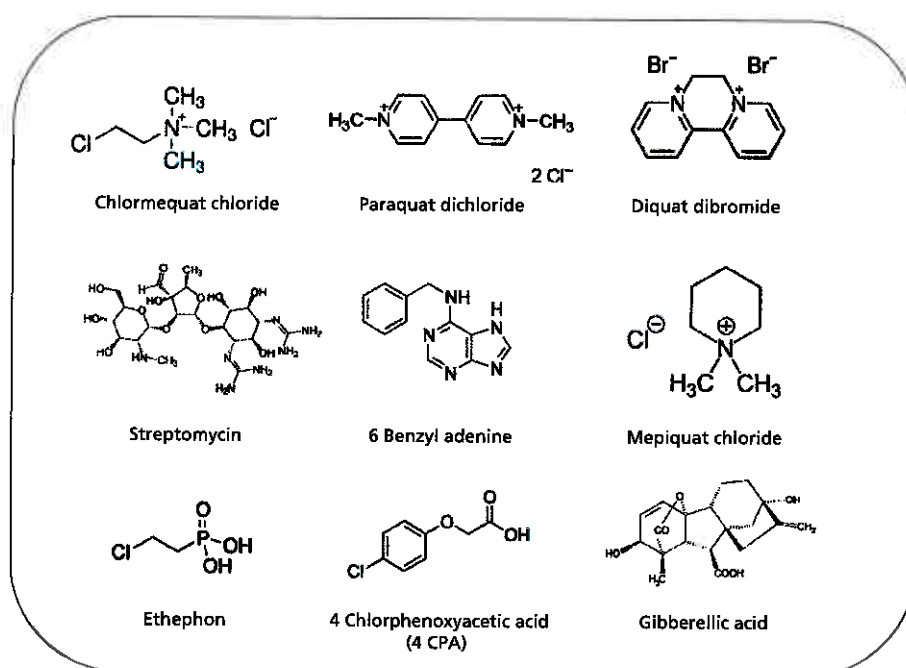


Figure 1. Structures of PGRs and antibiotics

Method of analysis

Sample preparation

Preparation of aqueous calibration levels

The standards of PGRs and streptomycin procured from Sigma Aldrich were used for analysis. Mixed standard stock of PGRs and streptomycin was prepared in the water : methanol (1:1 v/v). Further this stock was serially diluted to prepare calibration levels ranging from 0.1 ng/mL to 1000 ng/mL in water : methanol (1:1 v/v).

Development of quantitative method for determination of plant growth regulators and streptomycin in plants using LC/MS/MS

Preparation of matrix matched calibration levels

Matrix matched standards were prepared in fresh watermelon juice purchased from local market. In 50 mL centrifuge tube, 10 mL of watermelon juice sample was taken. To this sample, 10 mL of acidified methanol was added and vortexed for few minutes. Solution was then centrifuged at 5000 rpm for 5 min. Supernatant was

collected and filtered through 0.2 micron filter. This filtered extract was used as a diluent for matrix matched calibration levels.

The matrix matched calibration levels were prepared in a same way as aqueous standards.



Figure 2. LCMS-8040 triple quadrupole mass spectrometer

LC/MS/MS analysis

In a typical method scouting procedure, data was collected using a number of mobile phase and column combinations. Analyzers switching manually between these combinations are limited by the number of work hours in a single day, making it impossible to perform method scouting efficiently. The Nexera Method Scouting System is capable of automatically investigating up to 96 combinations of mobile phases and columns, without such time restrictions, thereby significantly improving method development productivity.

For primary method scouting, different mobile phases were checked e.g. 0.1 % formic acid in water, 5 mM ammonium acetate, 10 mM ammonium acetate etc. as aqueous phases and methanol, acetonitrile, 0.1 % formic acid in acetonitrile etc. as organic phases. Columns like C18 and HILIC of different makes and dimensions were

tested (shown in Figure 3). Various gradient programs were also tried. Automatic batch file was created using Method Scouting software and data were acquired by LabSolutions software (Shimadzu Corporation, Japan). Solvent blending is a powerful tool in order to save the labor and time required for preparing mobile phases. For example, a wide analysis range can be performed with varied concentrations of the buffer and organic solvent by just assigning aqueous solutions to pump A and organic solutions to pump B. When chromatographic elution patterns are investigated with various compositions of the solvents, using an automated solvent blending eliminates wastage of solvent and reduce labors.

By using solvent blending function, formic acid concentration in 20 mM ammonium acetate was optimized.

Rapid development of quantitative method for determination of plant growth regulators and streptomycin in fruits using LC/MS/MS

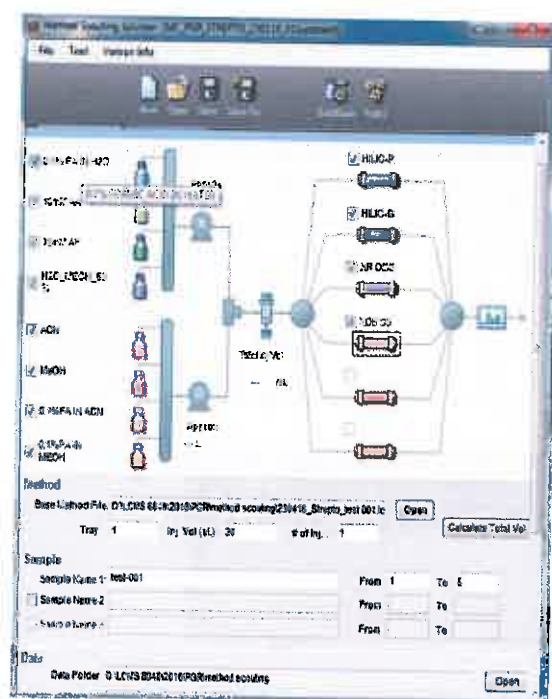


Figure 3. Method scouting software

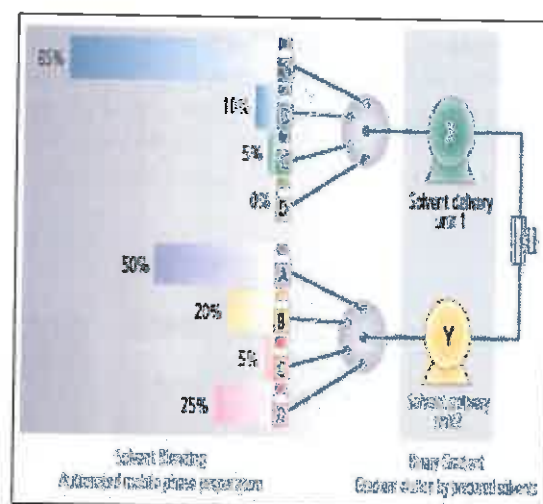


Figure 4. Solvent blending

Table 1. Optimized LC/MS/MS conditions for PGRs and antibiotic analysis

Column	: Grace Alltima HP HILIC (50 mm L x 2.1 mm I.D.; 3 μ m)
Mobile phase	: A: 20 mM ammonium acetate containing 0.05 % formic acid in water B: 0.1 % formic acid in acetonitrile
Gradient program (B%)	: 0.0-1.0 min $\rightarrow$ 80 (%); 1.0-2.5 min $\rightarrow$ 80-57 (%); 2.5-4.0 min $\rightarrow$ 5 (%); 4.0-4.1 min $\rightarrow$ 5 (%); 4.1-5.0 min $\rightarrow$ 80 (%); 5.0-12.0 min $\rightarrow$ 80 (%)
Flow rate	: 0.8 mL/min
Oven temperature	: 35 $^{\circ}$ C
Injection volume	: 20 μ L
MS interface	: Electro Spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 3 L/min; Drying gas 15 L/min
MS temperature	: Desolvation line 300 $^{\circ}$ C; Heating block 400 $^{\circ}$ C

Development of quantitative method for determination of plant growth regulators and streptomycin in plants using LC/MS/MS

Results

LC/MS/MS analysis results of PGRs and streptomycin

The preliminary data (Figures 5A to 5D) showed that the combination of 20 mM ammonium acetate containing 0.15 % formic acid and 0.1% formic acid in acetonitrile with HILIC column gives good peak shapes and separation. This mobile phase composition was further optimized with solvent blending software (as shown in Figure 4).

It was observed that the formic acid concentration in aqueous phase regulates the peak shapes. Therefore, automated batch file was created for optimization of formic acid concentration by using solvent blending

software and 20 mM ammonium acetate containing 0.05 % formic acid showed maximum response compared to all other combinations especially for streptomycin (shown in Figure 6).

The LC/MS/MS method was developed using method scouting for simultaneous quantitation of PGRs and streptomycin. MRM transitions used for these compounds are given in Table 2. Linearity studies were carried out using external standard calibration method and results of linearity studies for both aqueous and matrix matched standards are tabulated in Table 2.

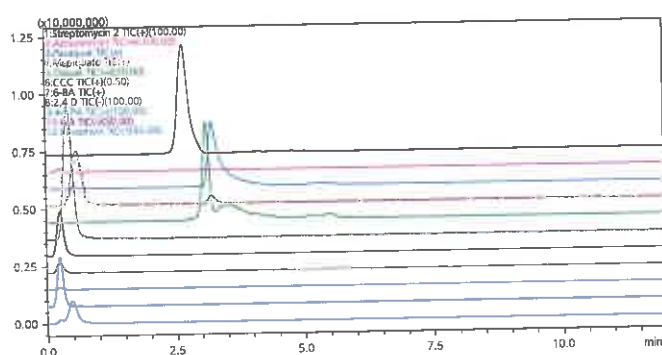


Figure 5A. A=20 mM ammonium acetate 0.1% formic acid
B= 0.15% formic acid in acetonitrile

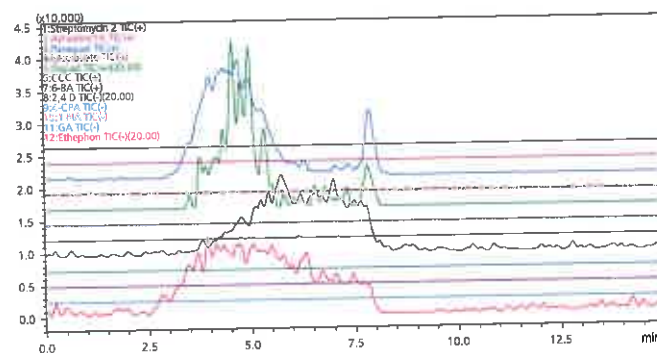


Figure 5B. A= 0.1 % formic acid in water
and B= methanol

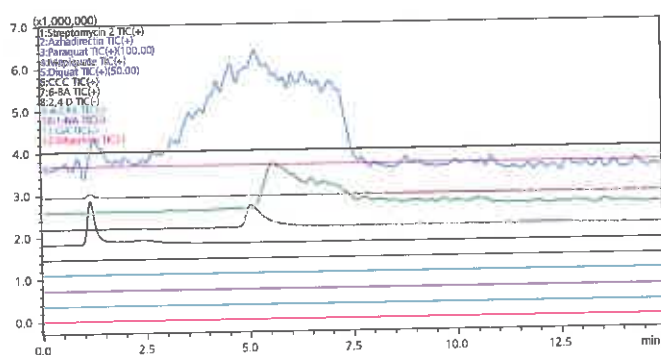


Figure 5C. A= 10 mM ammonium formate and
B= 0.1 % formic acid in methanol

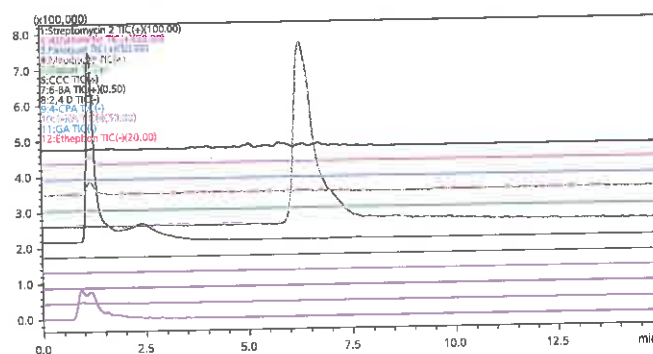


Figure 5D. A=10 mM ammonium acetate and
B= 0.1 % formic acid in methanol

Rapid development of quantitative method for determination of plant growth regulators and streptomycin in fruits using LC/MS/MS

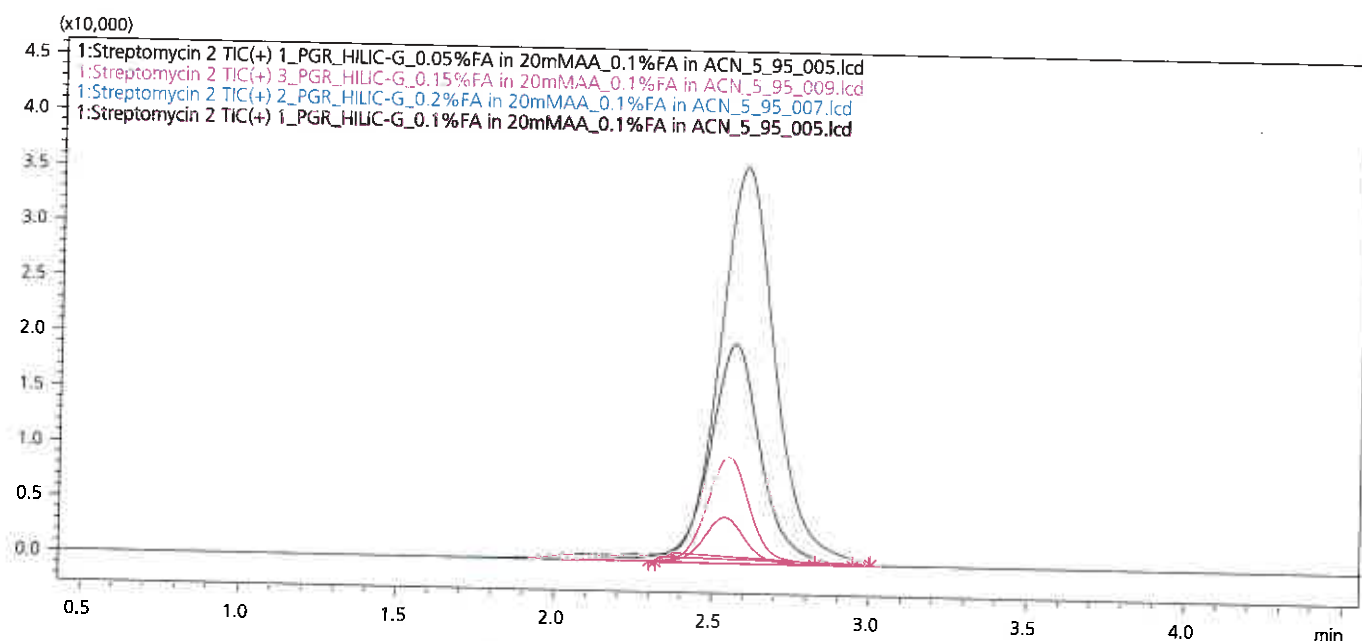


Figure 6. Optimization of formic acid concentration by solvent blending

The matrix matched calibration levels were prepared and injected in triplicate. The calibration curve of PGRs and streptomycin are shown in Figure 7 to Figure 15 and the correlation coefficient of > 0.99 was observed for all the compounds.

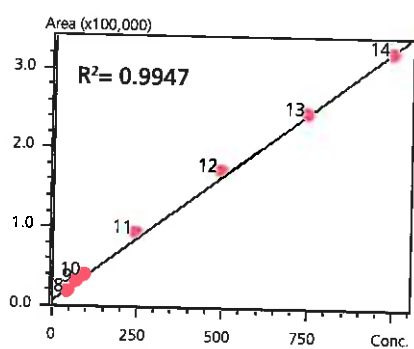


Figure 7. Streptomycin

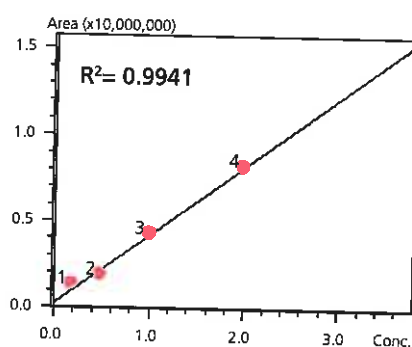


Figure 8. Paraquat

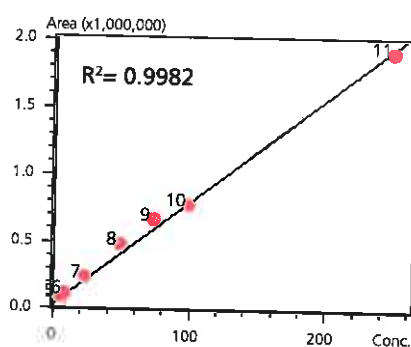


Figure 9. Mepiquat

Development of quantitative method for determination of plant growth regulators and streptomycin standards using LC/MS/MS

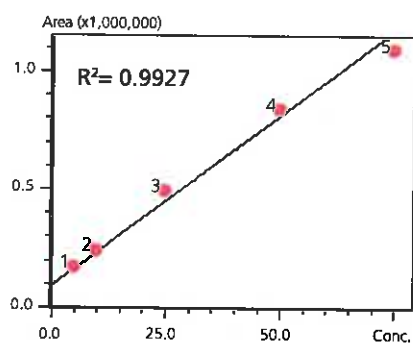


Figure 10. Diquat

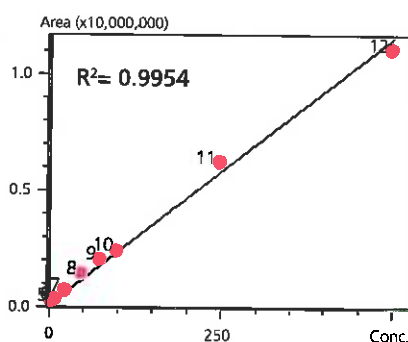


Figure 11. Chlormequat

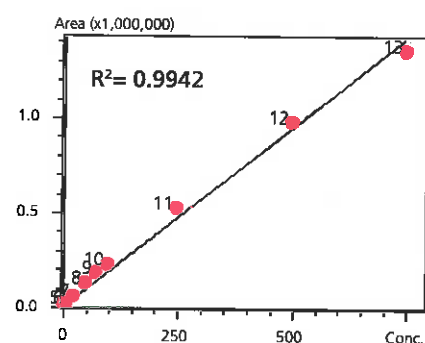


Figure 12. 6-Benzyl adenine

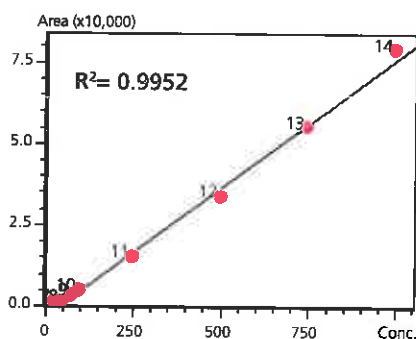


Figure 13. Ethephon

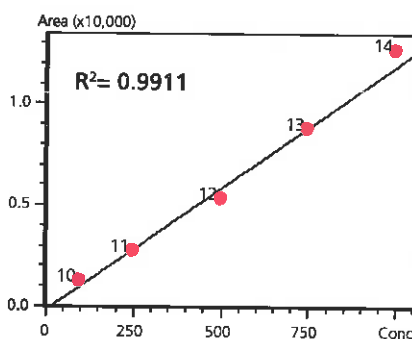


Figure 14. Gibberellic acid

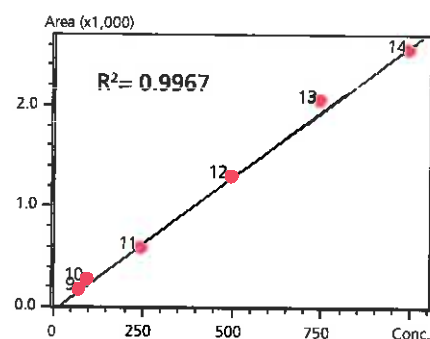


Figure 15. 4-CPA

Table 2. Result table of aqueous and matrix matched calibration standards

Sr.No.	Name of molecule	MRM transition	Polarity	Linearity range in ppb	Correlation coefficient (r^2)	
					Aqueous standard	Matrix matched standards
1	Streptomycin	582.00>176.05	Positive	50-1000	0.9947	0.9983
2	Paraquat	186.00>171.05	Positive	5-75	0.9941	0.9941
3	Diquat	183.90>128.05	Positive	5-100	0.9927	0.9927
4	6-Benzyl adenine	225.80>91.00	Positive	5-750	0.9972	0.9942
5	Mepiquat	114.00>98.15	Positive	5-250	0.9982	0.9954
6	Chlormequat	122.10>58.15	Positive	5-500	0.9958	0.9954
7	Gibberellic acid	345.05>239.10	Negative	100-1000	0.9992	0.9911
8	Ethephon	143.00>106.90	Negative	50-1000	0.9948	0.9952
9	4-CPA	184.70>127.05	Negative	100-1000	0.9977	0.9967

Rapid development of quantitative method for determination of plant growth regulators and streptomycin in fruits using LC/MS/MS

Conclusion

- The Nexera Method Scouting System enhances method development efficiency.
- An automated solvent blending system can provide the same level of accuracy as manual premixing.
- With the help of method scouting and solvent blending techniques, it was very easy to develop a quantitative multi-residue analytical method for different class of compounds.

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[2] Ana Coste, Laurian Vlase. Plant Cell Tiss Organ Cult 106, (2011), 279-288.
[3] X. Esperza, E. Moyano,. Journal of Chromatography A, Volume 1216, (2009), 4402-4406.

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A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

ASMS 2015 WP074

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A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

Introduction

Aflatoxins are metabolites produced by fungi (*Aspergillus flavus* and *Aspergillus parasiticus*) in high humidity environment in crops such as maize nuts and processed food. Aflatoxin contamination in food is monitored with strict regulations worldwide due to high toxicity of the compounds [1]. Recently, several media reports revealed the exceed levels of aflatoxins found in peanut butters in the USA and Taiwan. Aflatoxins in food have been

analyzed by LC/MS/MS using various sample pre-treatment methods. We describe a high sensitivity LC/MS/MS method for quantitative analysis of aflatoxins in peanut butters using QuEChERS sample pre-treatment procedure [2], as opposed to the use of immunoaffinity column or other methods for sample pre-treatment which are more expensive and tedious. High sensitivity and good recoveries were achieved using this LC/MS/MS method.

Experimental

A mixed standard of aflatoxin B1, B2, G2 and G2 was obtained from Supelco. A stock solution was prepared using methanol as the diluent, from which calibrant series and spiked samples were prepared. The QuEChERS kits were purchased from RESTEK. Two grams of peanut butter was first extracted with the extraction kits followed by cleaning up using dSPE tubes. The procedure was adjusted and optimized to obtain highest recovery. A

LCMS-8050 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A C18 column (Kinetex, 2.1 x 100mm, 1.7 μ) was used for fast separation of aflatoxins using a gradient elution program. The method development and performance evaluation were carried out using spiked aflatoxins in peanut butter samples. Table 1 shows the analytical conditions on LCMS-8050.

Table 1: LC/MS/MS analytical conditions of aflatoxins on LCMS-8050

Column	Kinetex C18 (2.1mmI.D x 100mmI.D, 1.7 μ m)
Flow rate	0.5 mL/min
Mobile phase	A: 5mM ammonium acetate in water with 0.1% FA B: 5mM ammonium acetate in MeOH
Oven temp.	40 °C
Injection vol.	5 μ L
Elution mode	Gradient elution, B%: 5% (0 to 0.5 min) $\rightarrow$ 50% (4 to 5.5 min) $\rightarrow$ 85% (6 to 7.5 min) $\rightarrow$ 5% (8.1 to 10 min)
Interface	ESI
MS mode	Positive, MRM, 2 transitions for each compound
Interface temp.	350 °C
Block temp.	400 °C
DL temp.	250 °C
CID gas	Ar (350 kPa)
Nebulizing gas flow	3.0 mL/min
Drying gas flow	10.0 L/min
Heating gas flow	10.0 L/min

ensitivity LC/MS/MS Method with QuEChERS Pre-treatment for Analysis of Aflatoxins in ut Butter Samples

Results and Discussion

QuEChERS sample pre-treatment

Hexane was used in the procedure to remove fats, oils and non-polar components from the peanut butter samples. The extraction step was completed using Q-sep QuEChERS extraction salt packet (4 g MgSO₄, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate). Dispersive SPE tube containing MgSO₄, PSA and C18 was used in the clean-up process to remove remaining water, organic acid and non-polar components respectively. The process of the sample preparation is illustrated in Figure 1.

Method Development

Automated MRM optimisation of aflatoxins was carried out using the LabSolutions workstation. The precursors of aflatoxins B1, B2, G1 and G2 were their protonated ions (m/z 313.1, m/z 315.1, m/z 329.1 and m/z 331.1, respectively). Two MRM transitions of every aflatoxin were chosen as quantifier and confirmation ion (Table 2). A peanut butter matrix free from aflatoxins was used as a "blank" and matrix for the preparation of post-spiked calibrants to build calibration curves. The blank and every post-spiked calibrant was injected thrice and the average area was calculated to obtain reliable results. A chromatogram of spiked sample is shown in Figure 2. Linear calibration curves were obtained for all four aflatoxin compounds. Good linearity with correlation coefficient (r^2) greater than 0.999 across the range of 10 pg/mL - 10 ng/mL was obtained. The calibration curves of aflatoxins spiked in peanut butter matrix are shown in Figure 3.

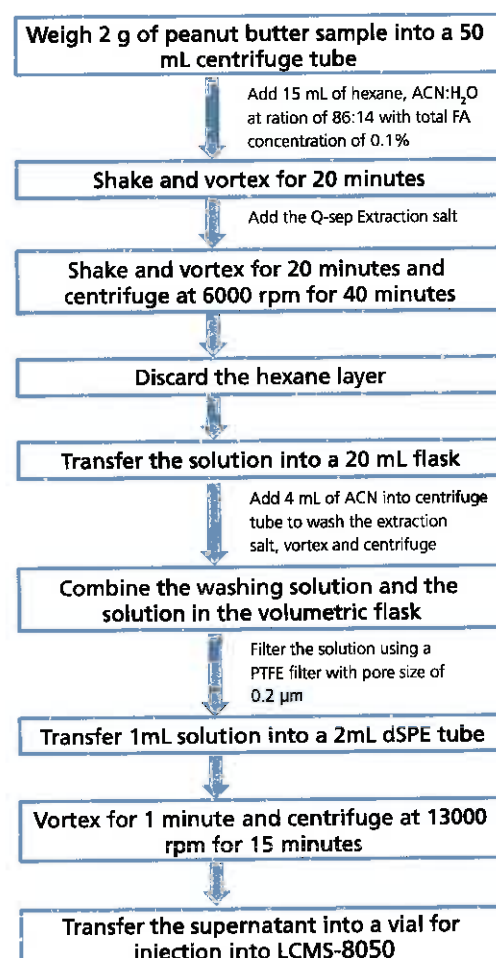


Figure 1: Flow chart of sample pretreatment for aflatoxins in peanut butter by modified QuEChERS method.

Table 2: LC/MS/MS analytical conditions of LCMS-8050 for aflatoxins

Compound	MRM (m/z)	CID Voltage (V)		
		Q1	CE	Q3
Aflatoxin B1	313.1>241.0*	-15	-37	-26
	313.1>213.0	-15	-46	-22
Aflatoxin B2	315.1>259.1*	-15	-30	-28
	315.1>287.0	-15	-27	-20
Aflatoxin G1	329.1>243.0*	-30	-27	-27
	329.1>200.0	-30	-42	-21
Aflatoxin G2	331.1>245.0*	-16	-33	-25
	331.1>189.0	-16	-42	-19

*MRM transitions used as quantifiers.

A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

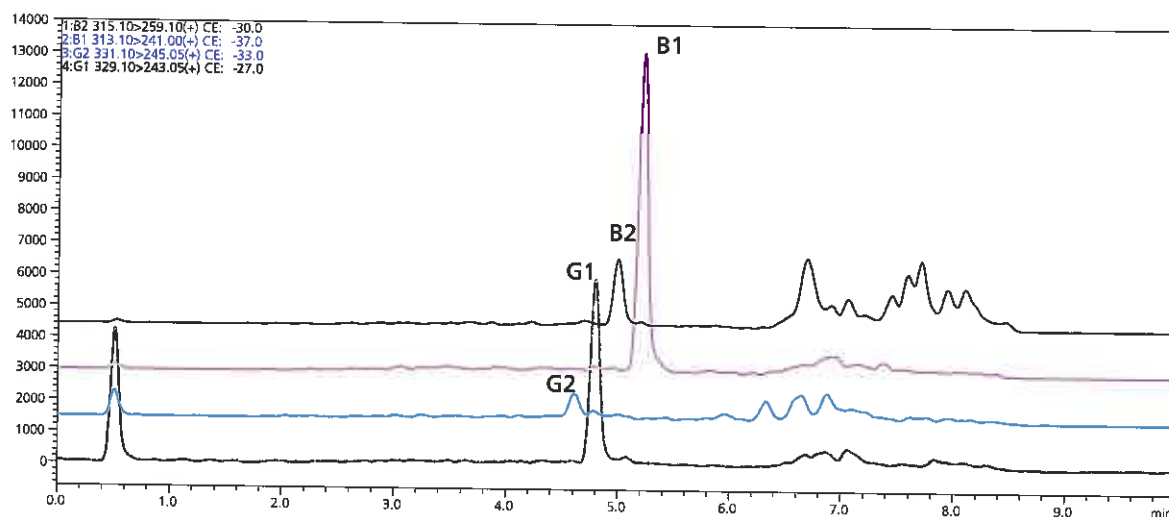


Figure 2: MRM chromatograms of aflatoxins spiked in peanut butter sample
(B1 and G1 at concentration of 100 pg/mL; B2 and G2 at concentration of 30 pg/mL).

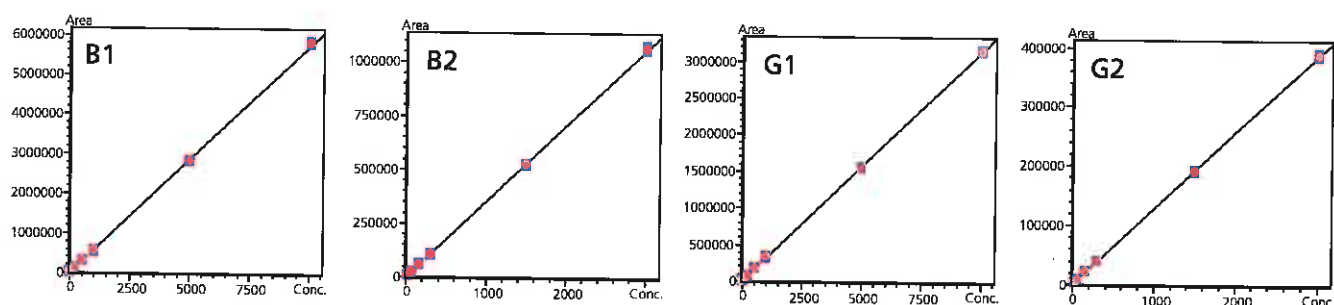


Figure 3: Calibration curves of aflatoxins B1, B2, G1 and G2 in peanut butter matrix.

Table 3: LOD, LOQ and repeatability of aflatoxin spiked samples at different concentrations

Compound	Concentration range (pg/mL)	%RSD (n=6)						LOQ	LOD	r2
		15 ppt	30 ppt	50 ppt	60 ppt	100 ppt	200 ppt			
B1	10 - 10000			2.71		2.22	1.20	4.6	1.5	0.9995
B2	11 - 3000	7.73	7.51		2.96			8.7	2.9	0.9997
G1	10 - 10000			2.21		1.82	1.21	4.2	1.4	0.9995
G2	30 - 3000		12.12		9.10			22.4	7.4	0.9995

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Method Performance Evaluation

As shown in Table 4, the LOD and LOQ of aflatoxins in peanut butter matrix are lower than 7.4 pg/mL and 22.4 pg/mL respectively. The repeatability of the method was evaluated using using spiked samples at lower concentrations. The peak area %RSD of aflatoxins were found to be lower than 7.5% except for G2 with %RSD of 12.1%.

The matrix effect was evaluated by using the average of spiked samples injected thrice at different concentrations. The recoveries of aflatoxins were determined by using a duplicate set of samples at different concentrations. Each duplicate was obtained from the average of three injections. The results are shown in Table 4.

Table 4: Matrix effects of the MRM method for aflatoxins in spiked peanut butter samples

Concentration (pg/mL)	Matrix effect (%)		Concentration (pg/mL)	Matrix effect (%)	
	B1	G1		B2	G2
50	78.35	80.63	60	72.31	70.14
100	71.88	71.24	150	73.81	74.72

Table 5: Recoveries of aflatoxins in spiked peanut butter samples

Compound	Recovery (%)							
	30 pg/mL		50 pg/mL		60 pg/mL		200 pg/mL	
	Dup 1	Dup 2	Dup 1	Dup 2	Dup 1	Dup 2	Dup 1	Dup 2
B1			70.24	74.00			81.52	80.49
B2	65.22	71.20			87.40	85.12		
G1			90.92	95.11			77.47	79.38
G2	79.97	79.58			87.76	86.52		

Analysis of aflatoxins in actual samples

Three peanut butter samples from local supermarket were analysed using the established MRM method. The results showed that aflatoxins were found in two of the samples (Table 6). While the aflatoxins in sample M is within the

EU regulatory limits (sum of aflatoxins below 4 µg/kg), the aflatoxin B1 amount in sample UL exceeds the allowed concentration (aflatoxin B1 below 2 µg/kg).

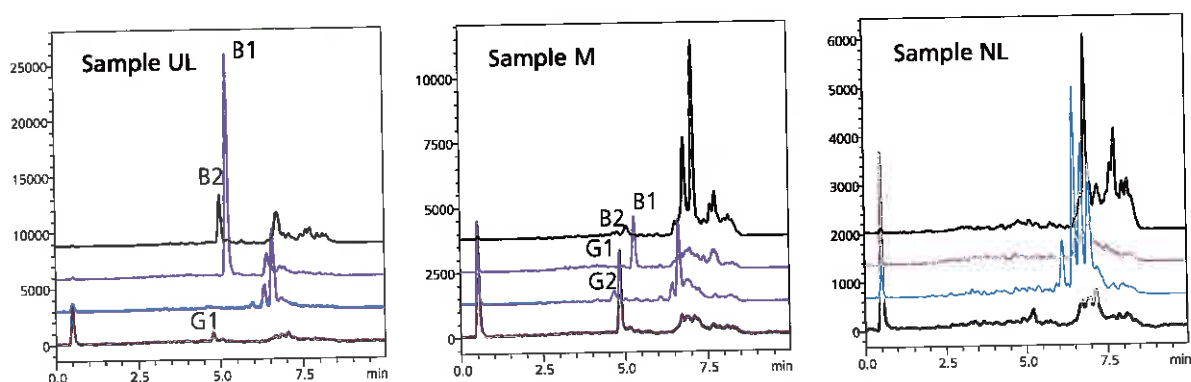


Figure 4: Chromatograms of peanut butter samples.

A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Peanut Butter Samples

Table 6: The amount of aflatoxins found in peanut butter samples from supermarket

Sample	Concentration (ng/g)			
	B1	B1	G1	G2
UL	2.09	0.79	0.03	Not detected
M	0.16	0.06	0.50	0.18
NL	Not detected	Not detected	Not detected	Not detected

Conclusions

A high sensitivity LC/MS/MS method with QuEChERS for sample pre-treatment was established using Shimadzu LCMS-8050 system. The QuEChERS sample preparation method was proven effective and easy to operate. The method performance including sensitivity, linearity,

repeatability, matrix effect and recovery were carried out and the results confirm that the method is feasible and reliable for determination of aflatoxins in peanut butter samples.

References

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A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

ASMS 2015 WP 057

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A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

Introduction

The antibacterial sulfonamides (SA) and trimethoprim are widely used in veterinary and human medicine. Diverse foods from animals potentially contain residues of these drugs posing possible threats to people by triggering allergic reactions and undesirable increasing of microorganism's drug resistance. Various countries have defined their own maximum residue limits (MRLs) for sulfonamides accepted in honey, There are no MRL's for sulfonamides in honey in the UE but in 2002 a minimum

required performance level (MRPL) was set for analytical methods at a level of 10 µg/kg. HPLC-MS/MS is an effective strategy to characterize and accurately measure those antibiotics considering MRLs and MRPLs in food products from animal origin tend to be continually reduced to preserve human health safety. A selective, fast and sensitive HPLC-MS-MS method has been developed for 15 sulfonamides and trimethoprim.

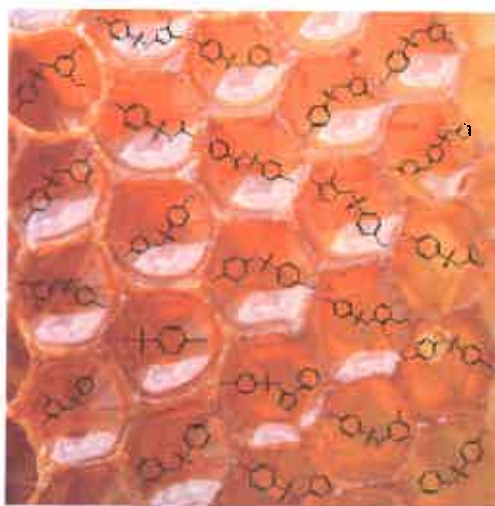
Materials and Method

Sample preparation

5 grams of honey, spiked with 17 SAs and trimethoprim (Table 1A), were extracted using QuEChERS method following manufacturer's procedure with a final 1:5 extract dilution using methanol. A multiple reaction monitoring MRM method was optimized for quantitation for each sulfonamide compound using a Shimadzu Nexera UHPLC with an LCMS-8050 fast-scanning triple quadrupole mass spectrometer model equipped with

software Labsolution LCMS version 5.65 and electrospray ionization ESI.

Stock standard solutions of each sulfonamide were prepared dissolving appropriate amounts in DMSO and methanol, diluting to 100 ppm and 1 ppm at the end with mobile phase A:B 50:50. Table 1B shows the concentrations at each level used to build calibration curves for external calibration method.



LC conditions

A Kinetex 2.6µ PFP 100 Å column (100 × 2.1 mm) was used at 40 °C, flow rate of 0.5 mL/min, and 10 µL injection volume using QuEChERS extraction method. A binary gradient of 10% methanol (mobile phase A) and methanol, 0.3% formic Acid (mobile phase B) was used with the gradient program described in Table 1C.

and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with HPLC-Chromatography-Tandem Mass Spectrometry

Mass Spectrometry:

Electrospray ionization was used in positive mode, spray voltage was 4.5 kV, desolvation line temperature was 250 °C, nebulization gas was 2.0 L/min, heater block was 400 °C, and drying gas 15 L/min.

Table 1. A. Sulfonamide compounds used in this study; B. Concentration levels to define calibration curves, and C. HPLC gradient used.

A. Sulfonamides used				B. Calibration Curve		C. LC Gradient	
#	SULFONAMIDE	#	SULFONAMIDE	Level	Conc. (ng/ml)	Time (min)	%B
1	Sulfaguanidine	10	Sulfamethoxypyridizine	1	1000	0	5
2	Sulfacetamide	11	Succinylsulfathiazole	2	500	1	15
3	Sulfadiazine	12	Sulfamethoxazole	3	250	4.5	35
4	Sulfathiazole	13	Trimethoprim	4	125	5	60
5	Sulfapyridine	14	Sulfamonomethoxine	5	62.5	5.01	95
6	Sulfamerazine	15	Sulfisoxazole	6	31.3	5.5	95
7	Sulfamethazine	16	Sulfabenzamide	7	15.6	5.51	5
8	Sulfameter	17	Sulfaclozine	8	7.8	7	5
9	Sulfamethizole	18	Sulfadimethoxine	9	3.9		
				10	2		
				11	1		

To implement sulfonamide quantitation, MRM transitions were optimized using a 0.5 µg mixture of SAs, 1 µL injections at 400 µL/min. Three transitions from parent ions and fragments were selected using the optimization tool software.

Results

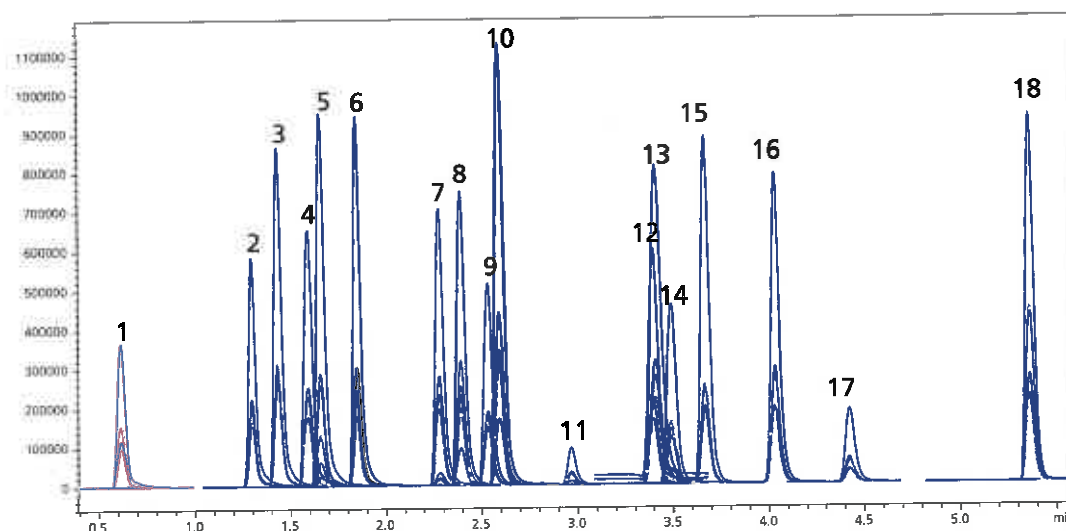


Figure 1. Representative chromatogram of sulfonamide drugs. Standard mixture at 125 pg on-column for each standard. Peak numbers follow the order described for SA compounds in table 1A.

A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

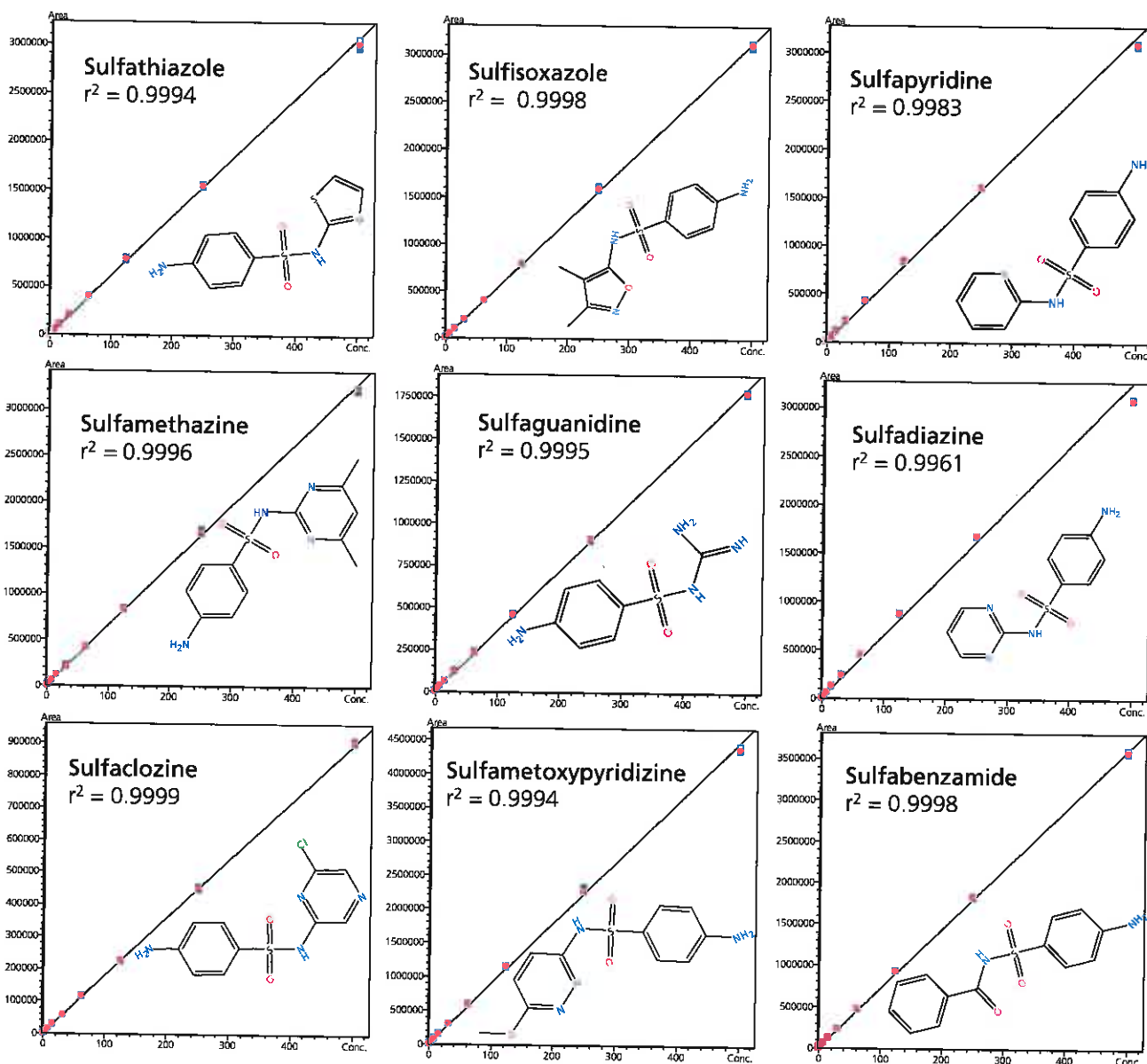
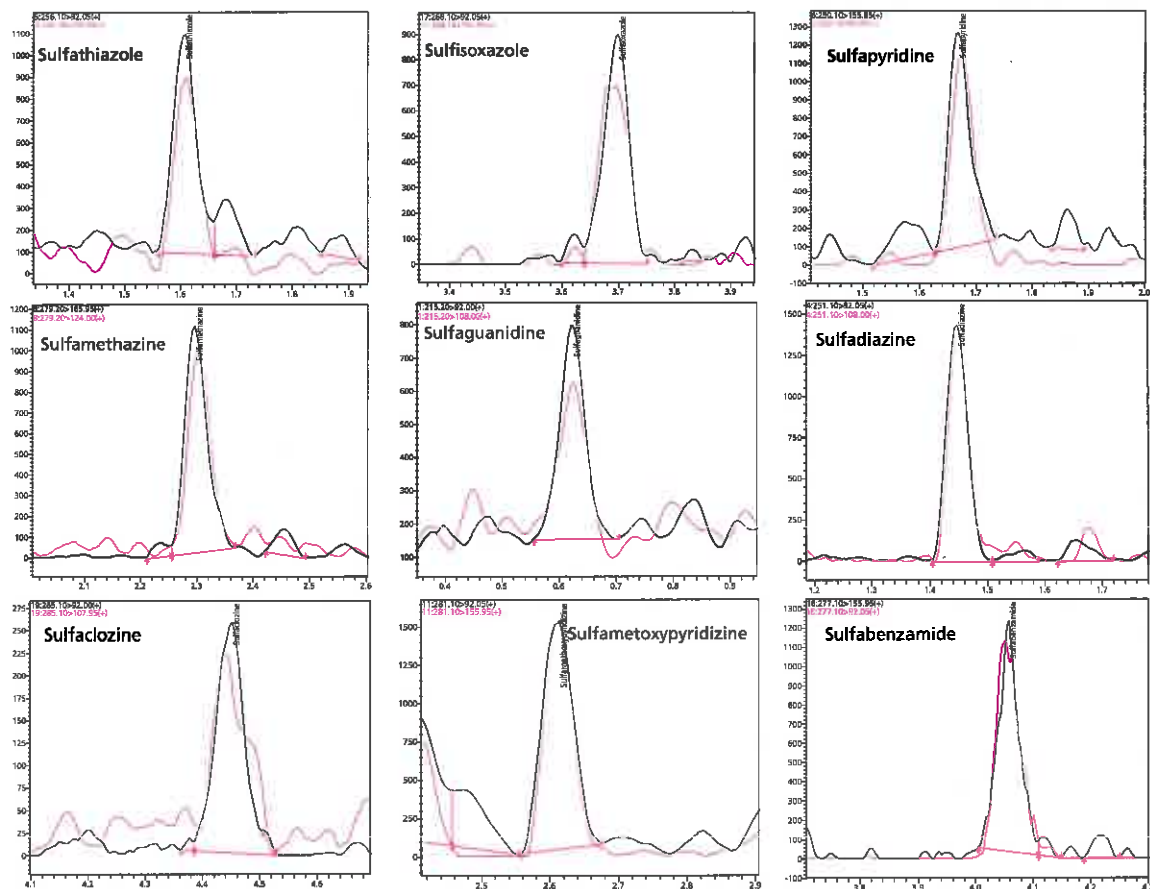


Figure 2. High degree of linearity was observed over the concentration range 0.5–500 pg on column, with values of $r^2 \geq 0.990$ for all analytes.

Authentic SAs standards were fully characterized by HPLC and MS/MS with an MRM optimized assay. The calibration curves of standards in 50% methanol matrix were linear with $r^2 > 0.990$ (Figure 2) in the tested range of 1 to 1000 µg/Kg (0.5 to 500pg on column). The limits of quantification were 1 µg/Kg (0.5pg on column) for all

compounds except succinylsulfathiazole and sulfacetamide, which were 2 µg/Kg (1pg on column). The recovery ranged from 53.9 to 91.4% for all but two compounds measured using drug residue-free organic honey. Succinylsulfathiazole and sulfaguanidine exhibited recovery below 20% using the QuEChERS method for extraction.

and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with HPLC-Chromatography-Tandem Mass Spectrometry



	Sulfathiazole		Sulfisoxazole		Sulfapyridine		Sulfamethazine		Sulfaguanidine		Sulfadiazine		Sulfaflozine		Sulfamethoxypyridazine		Sulfabenzamide	
Level	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD	Cal point	%RSD
1	1.7	97.8	1.0	98.9	0.5	96.5	0.6	98.1	0.5	98.3	0.4	94.3	0.5	100.4	1.0	97.5	0.8	98.8
2	0.9	100.4	1.4	101.2	0.3	100.6	1.5	101.8	0.7	99.8	0.2	102.3	1.1	99.9	1.9	101.5	0.3	100.3
3	2.1	102.6	0.4	100.3	0.9	105.0	1.1	101.0	0.9	101.2	1.0	106.0	0.8	99.3	1.1	102.8	0.7	101.6
4	1.7	103.6	0.3	102.0	1.3	107.7	0.8	102.5	0.6	103.6	1.5	109.7	1.7	100.0	0.9	102.7	1.2	102.8
5	0.4	106.3	2.0	101.0	1.4	107.8	1.0	102.1	1.7	105.5	1.3	114.0	1.0	98.4	1.5	104.3	1.4	101.7
6	1.6	106.1	3.3	102.6	0.5	110.8	0.9	106.3	1.8	108.3	0.6	116.8	1.5	99.3	3.2	104.0	0.4	104.2
7	3.8	109.4	1.5	101.1	6.3	103.1	0.7	105.8	3.0	113.9	2.8	115.4	3.4	100.9	1.2	108.1	0.6	103.6
8	4.4	108.0	1.2	104.1	8.2	102.2	3.3	103.7	2.3	114.6	1.6	111.9	5.0	98.5	6.3	105.9	3.3	102.6
9	4.4	115.7	1.5	100.2	4.0	104.4	2.0	106.4	6.4	114.3	6.5	110.8	8.1	87.9	2.7	112.3	2.6	104.7
10	3.9	107.4	5.0	88.0	9.2	103.7	15.2	90.6	8.0	114.1	9.1	118.5	8.8	90.9	5.7	121.4	2.7	97.6
11	12.7	114.7	10.5	100.3	6.2	109.9	5.9	100.9	9.9	126.9	16.5	116.5	6.9	95.5	7.3	105.9	6.4	91.3

Figure 3. Representative chromatograms of sulfonamide drugs at lowest concentration showing limit of quantitation and statistics for diverse concentration levels.

A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

Conclusions

LC-MS/MS with QuEChERS as extraction method provides a fast, simple, sensitive and accurately measuring for sulfonamide drugs and trimethoprim in honey with an acceptable recovery range. Matrix matched calibration and use of internal standards can be tested to improve performance.

First Edition: June, 2015

Low level quantitation of steroids in milk using LC/MS/MS

ASMS 2015 TP 346

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Introduction

Increase in incidences of some hormone-related cancers throughout the world is of great concern^[1]. Dairy cows continue to lactate during the later half of pregnancy, when the concentration of estrogens in blood is high, and hence increases in milk. Main concern is about cows' milk, which contains considerable quantity of estrogens. Increased consumption of such animal-derived food may have adverse effects^{[2][3][4]}.

Hormones are chemicals that are naturally produced in the body of animals and human beings and have a

number of important functions in life, such as reproduction or growth. However, due to the important role of these chemicals in several body functions, they also have been exogenously applied to animals and humans in order to obtain some kind of benefit in health or even to improve physical growth and performance. As a matter of fact, anabolic steroid hormones have played a key role among veterinary products in farming history and they have been one the most used and controversial components among veterinary drugs.

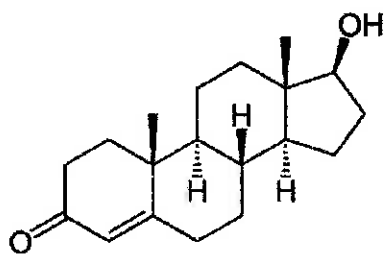


Figure 1A. Testosterone

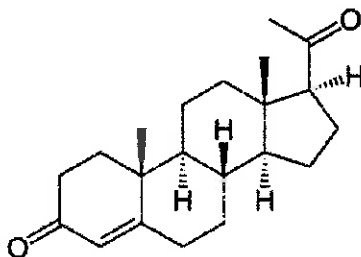


Figure 1B. Progesterone

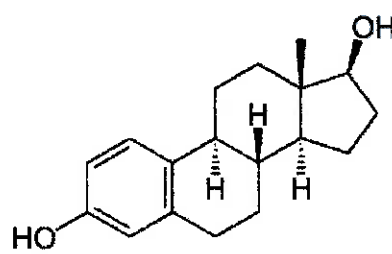


Figure 1C. Estradiol

The February 2010 article in the journal *Pediatric International* (February 2010, Vol. 52 #1) showed a potentially disturbing issue related to milk and its impact on the hormonal system.

The modern dairy cow has been converted to a full time milk factory. It is not often talked about, but modern industrialized dairy cattle continue to produce milk throughout their pregnancy. This milk goes directly into the food supply and contains varying amounts of bovine estrogen and progesterone. These hormones are then

directly absorbed by consumers.

A highly specific LC/MS/MS method has been developed for trace level quantitation of steroids in milk using LCMS-8050, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan. Ultra high sensitivity of LCMS-8050 due to heated ESI source, enabled development of ppt level quantitation for testosterone, progesterone and estradiol.

(shown in Figures 1A, 1B and 1C) with good repeatability and specificity even in presence of complex matrix.

Method of analysis

Simple and straightforward sample preparation technique of QuEChERS was used which involves liquid-liquid extraction followed by dispersive Solid Phase Extraction (d-SPE). Sample preparation using QuEChERS allows for

fast throughput and high sensitivity in food analysis. Various trials were taken for optimization of pre-treatment method. Such simple methodology can be used by small dairy farms and corporate milk producers.

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Sample preparation

Extraction (QuEChERS):

Place 10 mL whole milk into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of Q-Sep pouch salts for European Committee for Standardization (CEN)

QuEChERS (cat # 26236) and shake vigorously for 1 minute. Centrifuge for 5 minutes at 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup:

Transfer 1 mL aliquot of supernatant to a 2 mL d-SPE cleanup tube that contains 150 mg of magnesium sulfate, 50 mg PSA sorbent and 50 mg C18 sorbent (cat # 26243) and shake vigorously for 1 minute. Centrifuge for 3 minutes at 4000 rpm and take a 0.5 mL aliquot as a sample for LCMS/MS analysis.

LC/MS/MS analysis

LCMS-8050 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 2A), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UF sensitivity) with scanning speed of 30,000 Da/Sec (UF scanning) and polarity switching speed of 5 msec (UF switching). This system ensures highest quality of data, with very high degree of reliability. In order to improve ionization efficiency, the newly developed heated ESI probe combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This

development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background. Presence of heated electro spray interface in LCMS-8050 (shown in Figure 2B) ensured good quantitative sensitivity for steroids even in presence of a complex matrix like milk. MS voltages and collision energies were optimized to achieve maximum transmission of precursor and product ion. Gas flow rates and source temperature conditions were optimized, and linearity graph was plotted for three steroids.

Table 1. MRM conditions

Compound name	MRM transition (m/z)	Collision Energy (CE)	Dwell time (msec)
Testosterone	289.40>109.20	27	10
Progesterone	315.10>97.15	23	10
Estradiol	255.10>159.20	22	40



Figure 2A. LCMS-8050 triple quadrupole mass spectrometer by Shimadzu

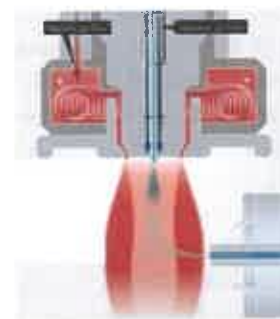


Figure 2B. Heated ionization probe

Low level quantitation of steroids in milk using LC/MS/MS

Table 2. LC conditions

Column	: Shim-pack XR-ODS (75 mm L x 2.0 mm ID; 2.2 μm)																		
Mobile Phase	: A: 0.1 % formic acid in water B: 0.1 % formic acid in acetonitrile																		
Gradient Program	: <table><tr><th>Time (min)</th><th>A conc. (%)</th><th>B conc. (%)</th></tr><tr><td>0.01</td><td>70</td><td>30</td></tr><tr><td>3.5</td><td>10</td><td>90</td></tr><tr><td>4.0</td><td>10</td><td>90</td></tr><tr><td>4.3</td><td>70</td><td>30</td></tr><tr><td>6.0</td><td colspan="2">Stop</td></tr></table>	Time (min)	A conc. (%)	B conc. (%)	0.01	70	30	3.5	10	90	4.0	10	90	4.3	70	30	6.0	Stop	
Time (min)	A conc. (%)	B conc. (%)																	
0.01	70	30																	
3.5	10	90																	
4.0	10	90																	
4.3	70	30																	
6.0	Stop																		
Flow Rate	: 0.4 mL/min																		
Oven Temperature	: 40 °C																		
Injection Volume	: 20 μL																		

Table 3. LCMS conditions

Interface	: Electro Spray Ionization (ESI)
Polarity	: Positive
Nebulizing Gas Flow	: 2 L / min
Drying Gas Flow	: 10 L / min
Heating Gas Flow	: 10 L / min (zero air)
Interface Temp.	: 350 °C
Desolvation Line (DL) Temp.	: 250 °C
Heating Block Temp.	: 500 °C

Results

LC/MS/MS analysis

Nexera UHPLC coupled with LCMS-8050 system was used for quantitation of progesterone, testosterone and estradiol in cow milk. The analysis was carried out using Shim-pack XR-ODS column with mobile phase containing 0.1 % formic acid in water & acetonitrile. The optimized gradient method was preferred to ensure complete elution of the matrix components (shown in Table 2). MRM was optimised for all three steroids and following transitions were used for quantitation; testosterone, progesterone and estradiol in ESI positive mode (shown in Table 1). MS voltages and collision energy were optimised to achieve maximum transmission of mentioned precursor and product ions using the automatic MRM optimization feature of LCMS-8050. In addition, gas flow rates (Heating gas, Nebulizing gas, Drying gas), source temperature conditions (Interface temperature, DL

temperature, Heat block temperature) and collision gas were also optimised (shown in Table 3).

Ultralow carryover is required with very high sensitive LC/MS/MS system. Nexera system coupled with fast autosampler demonstrates exceptional carry over performance for this analysis.

Under the optimized MRM conditions no interference was found in blank at the retention time of the steroids (shown in Figures 3A, 3B and 3C). Linearity solutions were prepared in methanol from 0.05 to 10 ppb for testosterone and estradiol and 0.25 to 50 ppb for progesterone. The linearity standards show correlation coefficient >0.999 for all three steroids, demonstrating the low level quantitation sensitivity of the instrument (shown in Figures 4A, 4B and 4C). Accuracy was found within acceptance criteria as shown in Table 4.

Quantitation of steroids using LC/MS/MS

Specificity and interference

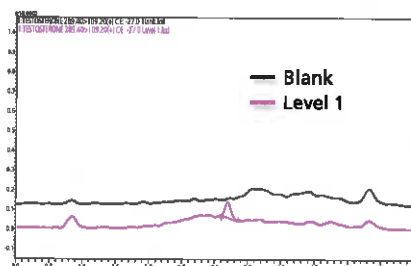


Figure 3A. Testosterone

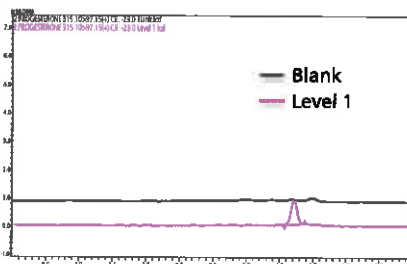


Figure 3B. Progesterone

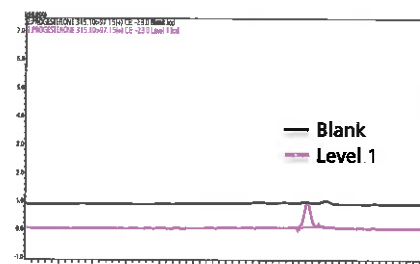


Figure 3C. Estradiol

Linearity

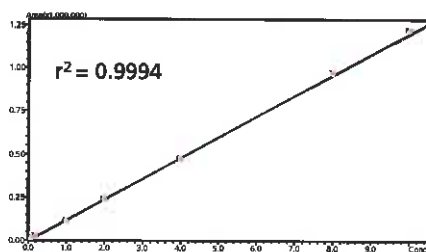


Figure 4A. Testosterone

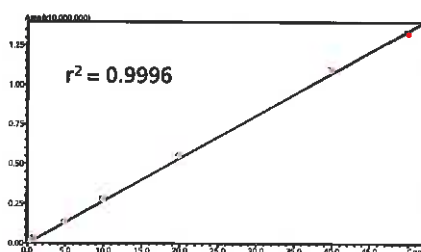


Figure 4B. Progesterone

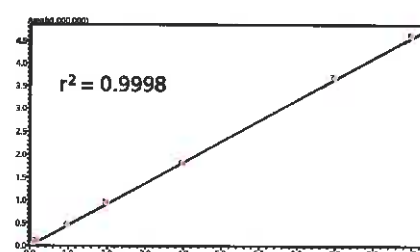


Figure 4C. Estradiol

Table 4. Result table

Level	Testosterone			Progesterone			Estradiol		
	Nominal conc. (ppb)	Measured conc. (ppb)	Accuracy (%)	Nominal conc. (ppb)	Measured conc. (ppb)	Accuracy (%)	Nominal conc. (ppb)	Measured conc. (ppb)	Accuracy (%)
Std 1	0.05	0.059	118.0	0.25	0.273	109.1	0.05	0.052	103.9
Std 2	0.10	0.094	93.7	0.5	0.474	94.8	0.10	0.099	99.2
Std 3	0.20	0.197	98.3	1.0	0.979	97.9	0.20	0.199	99.5
Std 4	1.0	0.920	91.9	5.0	4.767	95.3	1.0	0.973	97.3
Std 5	2.0	1.960	98.1	10.0	10.131	101.3	2.0	2.028	101.4
Std 6	4.0	3.910	97.8	20.0	20.351	101.8	4.0	3.913	97.8
Std 7	8.0	8.070	100.9	40.0	40.500	101.2	8.0	8.021	100.3
Std 8	10.00	10.140	101.4	50.0	49.274	98.5	10.0	10.065	100.7

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Recovery

Recovery was checked for each steroid hormone at low, mid and high concentration levels. Recovery was calculated by comparing the MRM peak area of samples spiked prior to QuEChERS extraction with calibrations standards linearity prepared in methanol. Progesterone was found around 5 ppb in sample and the same was taken for recovery study. Testosterone and estradiol of

0.1 ppb each and 0.5 ppb of progesterone was spiked in milk sample as a low spiked sample. For high spike sample 10 ppb of testosterone and estradiol and 50 ppb of progesterone was spiked in milk. The QuEChERS extraction procedure was followed to prepare sample and recovery was calculated which was found within the acceptance criteria (shown in Figures 5A, 5B and 5C).

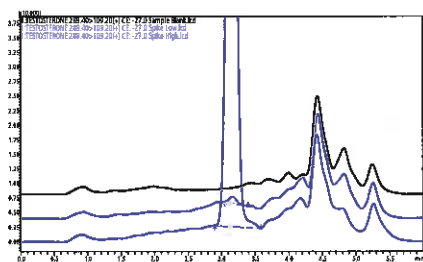


Figure 5A. Spiked sample Testosterone

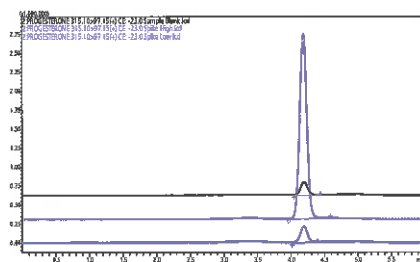


Figure 5B. Spiked sample Progesterone

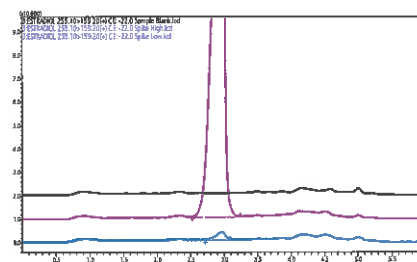


Figure 5C. Spiked sample Estradiol

Conclusion

- The QuEChERS is effective method for extraction of steroid hormones from milk.
- D-Sep dispersive sample preparation is an easy and straightforward technique that requires minimal user training, resulting in high throughput sample preparation of complex milk matrix.
- Nexera - LCMS-8050 enables sensitive quantitation of steroids in milk at low ppt concentrations.

References

- [1] Kazumi Maruyama, Tomoe Oshima and Kenji Ohya, Pediatric International, 52, (2010), 32-38.
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Multi-residue analysis of pyrethroids in soil and sediment using QuEChERS by LC/MS/MS

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Multi-residue analysis of pyrethroids in soil and sediment using QuEChERS by LC/MS/MS

Introduction

Pyrethroids are one of the most widely used commercial household insecticides in agricultural or non-agricultural application sites. Synthetic pyrethroids are poorly water-soluble, but are strongly adsorbed to soil, therefore these compounds are increasingly being found in soil or sediments. Recently, soil and sediment contamination by pyrethroids has been detected in both urban and agricultural area, and it's becoming a global concern due

to the influence on the insects and aquatic invertebrates. Therefore, quick, high-sensitive and universal analysis methods are required. The analysis of pyrethroids is typically performed by GC or GC/MS because of their hydrophobicity. In this study, we report the development of a simultaneous analysis technique for trace amounts of pyrethroids by LC/MS/MS.

Materials and Methods

Materials

Sample	Sampling point
Soil	Residential garden (Kyoto, Japan)
Sediment	Lake Biwa (Shiga, Japan)

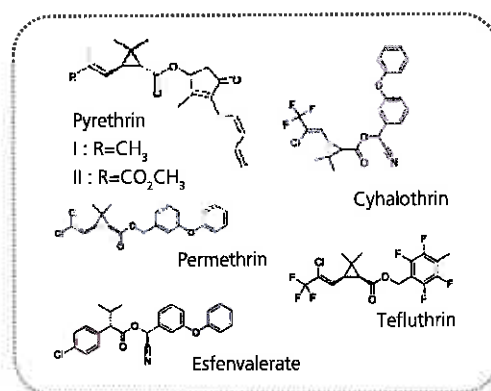


Figure 1 Chemical structure of pyrethroids

Sample preparation

Sample preparation was carried out by the use of the QuEChERS method. In case of the soil samples, hydration of sample with water before acetonitrile extraction is required to improve the recovery and operability. Result of several different extraction methods that changed the

amount of the soil and water added, we finally adopted a combination of 5 g soil (or 10 g sediment) and 5 mL water, and the following procedures were based on the original QuEChERS method.

Residue analysis of pyrethroids in soil and sediment by QuEChERS by LC/MS/MS

Step 1 : Acetonitrile extraction



*1 : Q-sep QuEChERS Extraction Salts (RESTEK)

Step 2 : Clean-up



*2 : Q-sep QuEChERS dSPE Tubes (RESTEK)

LC/MS/MS analysis

HPLC conditions (Nexera UHPLC system, Shimadzu)

Column	: Phenomenex Kinetex 2.6 µm PFP 100Å (100 mm x 2.1 mm I.D.)
Mobile phase	: A 5mM ammonium acetate - water : B Methanol
Gradient program	: 40 % B (0 min.) → 100 % B (10 -12 min.) → 40 % B (12.01-15 min.)
Flow rate	: 0.2 mL / min.
Column temperature	: 40 °C
Injection volume	: 1 µL

MS conditions (LCMS-8050, Shimadzu)

Ionization	: ESI (positive / negative)
Interface temperature	: 100 °C
DL temperature	: 100 °C
Heat block temperature	: 400 °C
Nebulizing gas	: 3.0 L / min.
Drying gas	: 15.0 L / min.
Heating gas	: 3.0 L / min.

Multi-residue analysis of pyrethroids in soil and sediment using QuEChERS by LC/MS/MS

Table 1 MRM transitions of pyrethroids

Compounds	Polarity	Quantitative ion (<i>m/z</i>)	Confirmation ion (<i>m/z</i>)
pyrethrin-I	+	329.20>161.20	329.20>105.20
pyrethrin-II	+	373.20>161.20	373.20>105.20
fenpropathrin	+	367.20>125.20	367.20>181.20
cycloprothrin	+	498.90>181.10	498.90>229.20
deltamethrin	+	522.80>280.90	522.80>181.10
esfenvalerate	+	437.10>167.30	437.10>125.30
cypermethrin	+	433.10>191.10	433.10>181.20
cyfluthrin	+	450.90>191.00	450.90>206.10
ethofenprox	+	394.20>177.30	394.20>107.20
permethrin	+	408.10>183.30	408.10>355.20
cyhalothrin	+	467.10>225.10	467.10>141.10
bifenthrin	+	440.00>181.20	440.00>166.10
acrinathrin	+	559.00>208.20	559.00>181.10
acrinathrin	-	540.10>372.20	540.10>345.30
silafuofen	+	426.20>287.10	426.20>168.20



High Speed Mass Spectrometer

Ultra Fast Scanning

- 30,000 u / sec.

Ultra Fast Polarity Switching

- 5 msec.

Ultra Fast MRM

- Max. 555 transitions / sec

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

Result

MRM of pyrethroid standards

In this study, we selected and evaluated 15 pyrethroids (pyrethrin, fenpropathrin, cycloprothrin, deltamethrin, esfenvalerate, cypermethrin, cyfluthrin, ethofenprox, permethrin, cyhalothrin, bifenthrin, acrinathrin, tefluthrin, silafuofen) which are the most widely used for household or

agrochemical insecticides worldwide.

Except for tefluthrin, which was not ionized by LC/MS under conditions tested, all other 14 compounds were successfully detected in ESI positive mode or in both positive and negative mode.

Residue analysis of pyrethroids in soil and sediment QuEChERS by LC/MS/MS

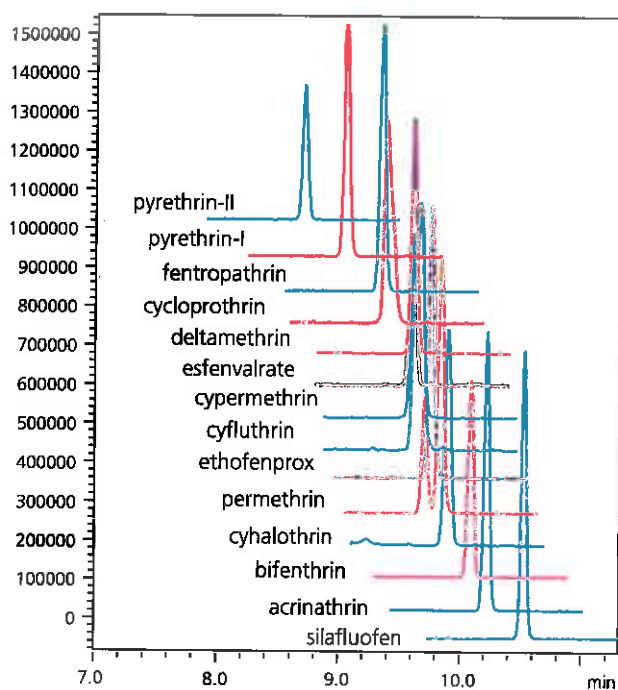


Figure 3 MRM chromatograms

Table 2 Calibration curves

compounds	min. conc.	max. conc.	r ²
pyrethrin I	0.5	500	0.9996
pyrethrin II	0.5	500	0.9997
fenpropathrin	0.02	100	0.9993
cycloprothrin	0.5	100	0.9991
deltamethrin	0.05	100	0.9992
esfenvalerate	0.5	100	0.9990
cypermethrin	0.05	100	0.9986
cyfluthrin	0.5	100	0.9976
ethofenprox	0.01	100	0.9993
trans-permethrin	0.02	100	0.9996
cis-permethrin	0.02	100	0.9994
cyhalothrin	0.1	100	0.9993
bifenthrin	0.02	100	0.9995
acrinathrin (+)	0.1	100	0.9987
acrinathrin (-)	0.5	500	0.9993
silafluofen	0.01	100	0.9999

(ppb)

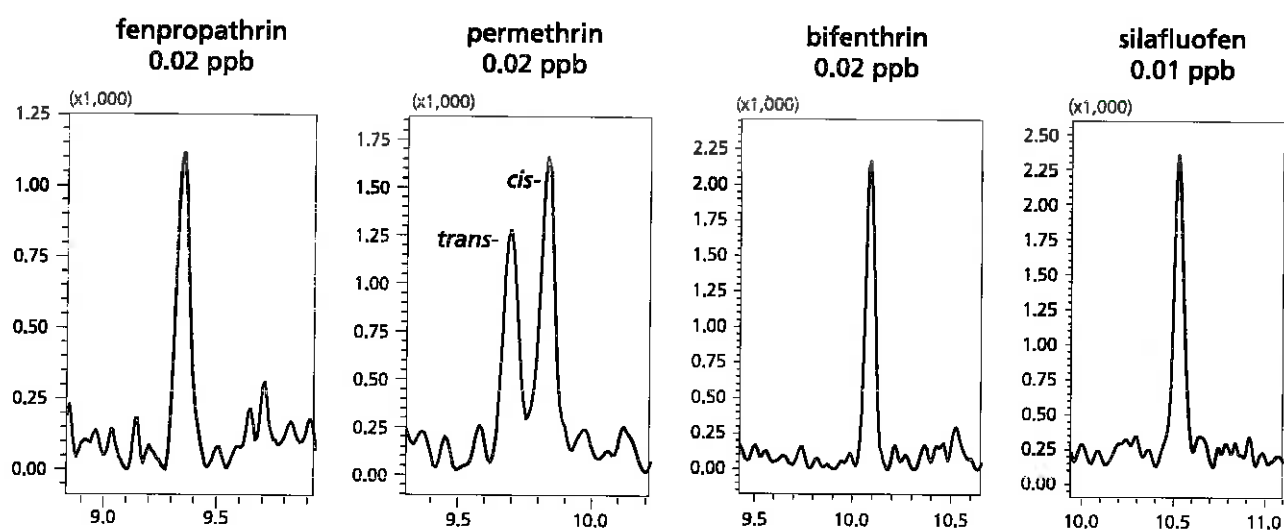


Figure 4 MRM chromatograms of the LOQs of typical pyrethroids

Multi-residue analysis of pyrethroids in soil and sediment using QuEChERS by LC/MS/MS

Recovery from soil and sediment matrices

All target compounds showed good recoveries from soil and sediment matrices in the range 70-120% by the QuEChERS

method. Neither matrix effect (ion suppression or enhancement) nor sample preparation losses were observed.

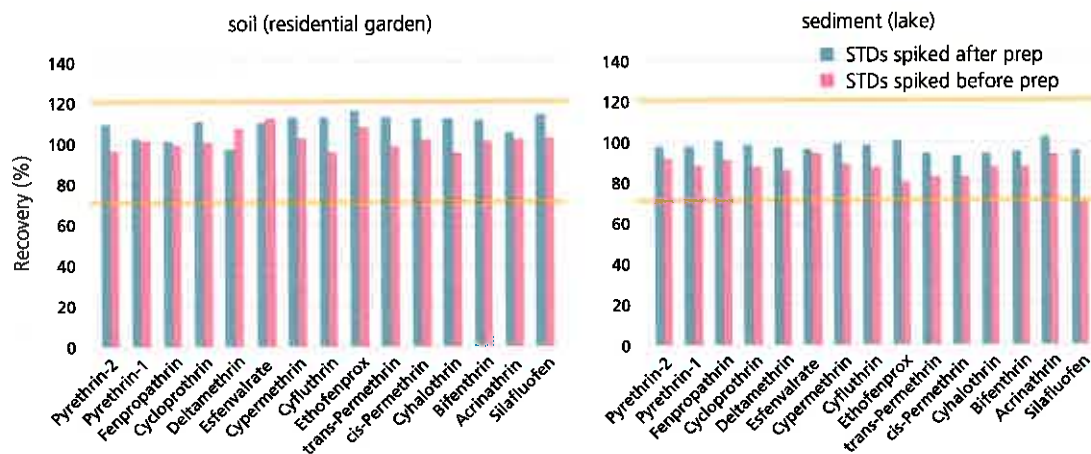


Figure 5 Recovery of 14 pyrethroids from soil and sediment matrices (10 ppb spiked)

Quantitative analysis of soil and sediment

The quantitative analysis of the soil and sediment sample was performed. Ethofenprox and permethrin was detected

from the soil sample at approximately 0.02 and 0.06 $\mu\text{g}/\text{kg}$, respectively.

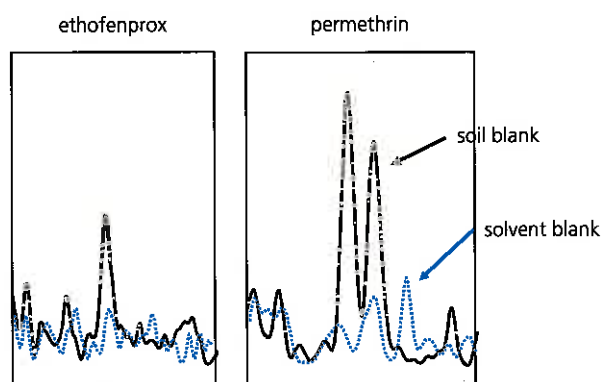


Figure 6 Chromatograms of pyrethroids in the soil

Table 3 Result of quantitative analysis in the soil and sediment

	soil	sediment
pyrethrin-I	n.d.	n.d.
pyrethrin-II	n.d.	n.d.
fenpropathrin	n.d.	n.d.
cycloprothrin	n.d.	n.d.
deltamethrin	n.d.	n.d.
esfenvalrate	n.d.	n.d.
cypermethrin	n.d.	n.d.
cyfluthrin	n.d.	n.d.
ethofenprox	0.01 ppb*	n.d.
permethrin	0.03 ppb	n.d.
cyhalothrin	n.d.	n.d.
bifenthrin	n.d.	n.d.
acrinathrin	n.d.	n.d.
silafluofen	n.d.	n.d.

n.d. : not detected

* : <LOQ

Residue analysis of pyrethroids in soil and sediment QuEChERS by LC/MS/MS

Conclusions

- A method for quantification of 14 pyrethroids in soil and sediment at ppt-level concentrations was developed by LC/MS/MS.
- In this study, neither matrix effect nor sample preparation losses were observed in the recovery test, demonstrating the applicability of QuEChERS method to sample preparation of soil and sediment.

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Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

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Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Table 3 – MRM transitions

Name	Ret. Time (min)	MRM Quan	MRM Qual
15-acetyldeoxynivalenol (15ADON) [M+H] ⁺	3.37	339 > 297.1	339 > 261
3-acetyldeoxynivalenol (3ADON) [M+H] ⁺	3.37	339 > 231.1	339 > 231.1
Aflatoxine B1 (AFB1) [M+H] ⁺	3.78	312.6 > 284.9	312.6 > 240.9
Aflatoxine B2 (AFB2) [M+H] ⁺	3.57	315.1 > 259	315.1 > 286.9
Aflatoxine G1 (AFG1) [M+H] ⁺	3.46	329.1 > 242.9	329.1 > 199.9
Aflatoxine G2 (AFG2) [M+H] ⁺	3.26	330.9 > 244.9	330.9 > 313.1
Aflatoxine M1 (AFM1) [M+H] ⁺	3.30	329.1 > 273	329.1 > 229
Alternariol [M-H] ⁻	4.78	257 > 214.9	257 > 213.1
Alternariol monomethyl ether [M-H] ⁻	5.81	271.1 > 255.9	271.1 > 228
Beauvericin (BEA) [M+H] ⁺	8.03	784 > 244.1	784 > 262
Citrinin (CIT) [M+H] ⁺	4.16	251.3 > 233.1	251.3 > 205.1
D5-OTA (ISTD)	5.22	409.2 > 239.1	N/A
Deepoxy-Deoxynivalenol (DOM-1) [M-H] ⁻	3.02	279.2 > 249.3	279.2 > 178.4
Deoxynivalenol (DON) [M-CH3COO] ⁻	2.61	355.3 > 295.2	355.3 > 265.1
Deoxynivalenol 3-Glucoside (D3G) [M+CH3COO] ⁻	2.45	517.5 > 457.1	517.5 > 427.1
Deoxynivalenol 3-Glucoside (D3G) [M+CH3COO] ⁻	2.45	517.5 > 457.1	517.5 > 427.1
Diacetoxyscirpenol (DAS) [M+NH4] ⁺	1.20	384 > 283.3	384 > 343
Enniatin A (ENN A) [M+H] ⁺	8.51	699.2 > 682.2	699.3 > 210
Enniatin A1 (ENN A1) [M+H] ⁺	8.22	685.3 > 668.3	685.3 > 210.1
Enniatin B (ENN B) [M+H] ⁺	7.57	657 > 640.4	657 > 195.9
Enniatin B1 (ENN B1) [M+H] ⁺	7.92	671.2 > 654.2	671.2 > 196
Fumagillin (FUM) [M+H] ⁺	6.16	459.2 > 131.1	459.2 > 338.7
Fumonisin B1 (FB1) [M+H] ⁺	4.10	722.1 > 334.2	722.1 > 352.2
Fumonisin B2 (FB2) [M+H] ⁺	4.71	706.2 > 336.3	706.2 > 318.1
Fumonisin B3	4.38	706.2 > 336.2	706.2 > 688.1
Fusarenone-X (FUS-X) [M+H] ⁺	2.84	355.1 > 247	355.1 > 175
HT2 Toxin [M+Na] ⁺	4.58	446.9 > 344.9	446.9 > 285
Moniliformin (MON) [M-H] ⁻	1.16	97.2 > 40.9	N/A
Neosolaniol (NEO) [M+NH4] ⁺	2.90	400.2 > 215	400.2 > 185
Nivalenol (NIV) [M+CH3COO] ⁻	2.41	371.2 > 280.9	371.2 > 311.1
Ochratoxin A (OTA) [M+H] ⁺	5.53	404.2 > 239	404.2 > 358.1
Ochratoxin B (OTB) [M+H] ⁺	4.83	370.2 > 205.1	370.2 > 187
Patulin (PAT) [M-H] ⁻	2.35	153 > 81.2	153 > 53
Sterigmatocystin [M+H] ⁺	5.60	325.3 > 310	325.3 > 281.1
T2 Tetraol [M+CH3COO] ⁻	1.64	356.8 > 297.1	356.8 > 59.1
T2 Toxin [M+NH4] ⁺	4.94	484.2 > 215	484.2 > 305
Tentoxin [M-H] ⁻	4.77	413.1 > 140.9	413.1 > 271.1
Tenuazonic acid (TEN) [M-H] ⁻	4.51	196.1 > 138.8	196.1 > 112
Wortmannin (M-H)	3.95	426.9 > 384	426.9 > 282.1
Zearalanol (alpha) (ZANOL) [M-H] ⁻	5.17	321.3 > 277.2	321.3 > 303.2
Zearalanol (beta) (ZANOL) [M-H] ⁻	4.85	321.3 > 277.2	321.3 > 303.1
Zearalanone (ZOAN) [M-H] ⁻	5.43	319 > 275.1	319 > 301.1
Zearalenol (alpha) (ZENOL) [M-H] ⁻	5.25	319.2 > 275.2	319.2 > 160.1
Zearalenol (beta) (ZENOL) [M-H] ⁻	4.94	319.2 > 275.2	319.2 > 160.1
Zearalenone (ZON) [M-H] ⁻	5.52	316.8 > 174.9	316.8 > 131.1

Sensitive and rapid simultaneous method for 45 mycotoxins by food samples by HPLC-MS/MS using fast polarity switching

Results and discussion

Method development

LC conditions were transferred from a previously described method (Tamura et al., Poster TP-739, 61st ASMS). In particular, the column was chosen to provide very good peak shape for chelating compounds like fumonisins thanks to its inner PEEK lining.

Small adjustments in the mobile phase and in the gradient program were made to handle more mycotoxins, especially the isobaric ones. These modifications are reported in the Table 1.

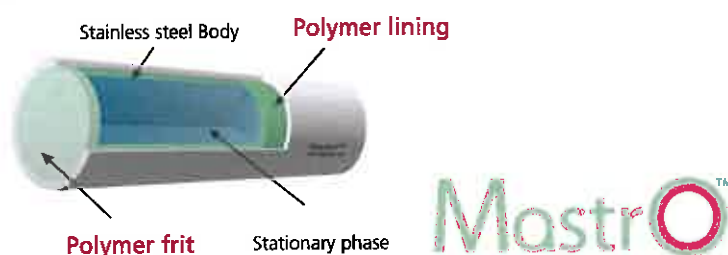


Figure 1 – Structure of the Mastro™ column

Also, autosampler rinsing conditions were kept to ensure carry-over minimisation of some difficult compounds. Electrospray parameters (gas flows and temperatures) were cautiously optimized to find the optimal combination for the most critical mycotoxins (aflatoxins). Since these parameters act in a synergistic way, a factorial design experiment is needed to find it. Manually testing all combinations in the chromatographic conditions is very

time consuming. Therefore, new assistant software (Interface Setting Support) was used to generate all possible combinations and generate a rational batch analysis. Optimal combination was found in chromatographic conditions. The difference observed between optimum and default or worst parameters was of 200 and 350%, respectively.

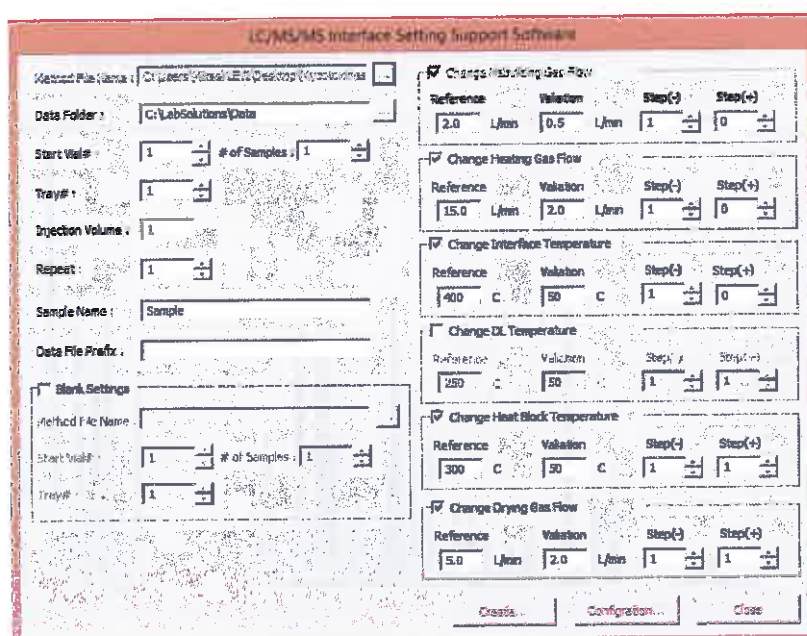


Figure 2 – Parameters selection view in the Interface Setting Support Software

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Results

Extraction and ionisation recovery for aflatoxins was measured in the three matrices by comparing peak areas of the raw sample extract to extract spiked at 50 ppb after or before extraction and to standard solution. Results in table

4 showed that the total recovery was quite acceptable to ensure accurate quantification. Results from other matrices were not significantly different.

Table 4 – Extraction and ionisation recoveries in puree

	AFB1	AFB2	AFG1	AFG2	AFM1
Extraction recovery	101%	109%	104%	114%	118%
Ionisation recovery	49%	90%	96%	106%	91%
Total recovery	49%	98%	100%	121%	108%

Repeatability was evaluated at low level for aflatoxins. Figure 3 shows an overlaid chromatogram (n=4) for aflatoxins.

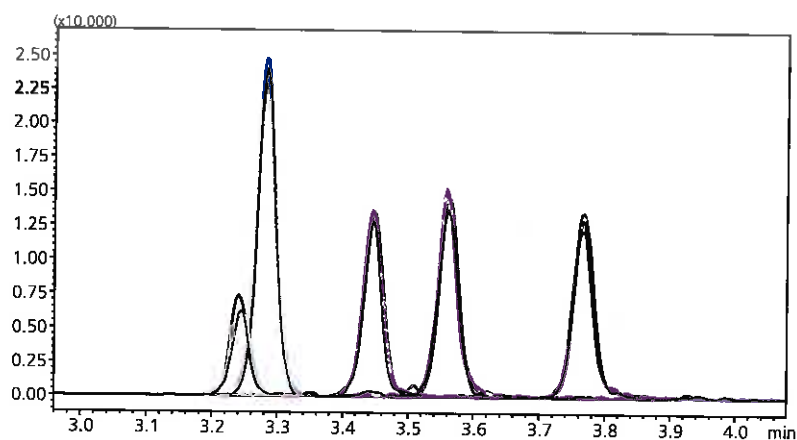


Figure 3 – Chromatogram of aflatoxins at 0.1 ppb in milk thickening cereals

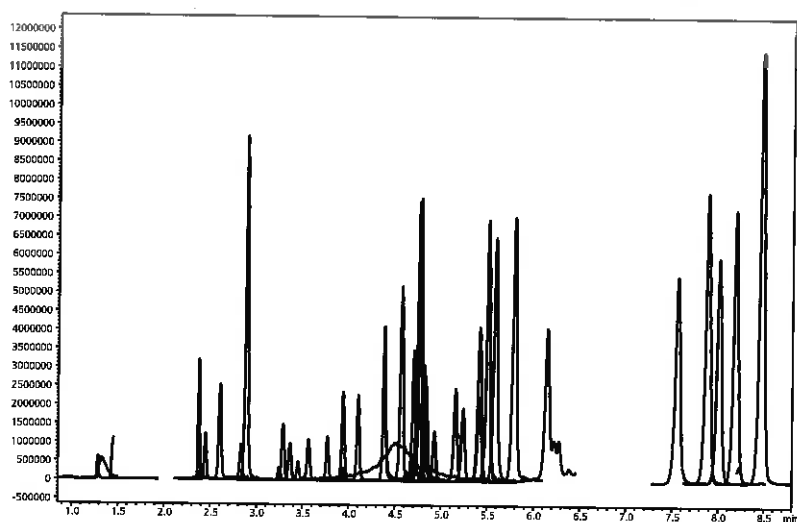


Figure 4 – Chromatogram of the 45 mycotoxins in standard at 50 ppb (2 ppb for aflatoxins and ochratoxines)

Sensitive and rapid simultaneous method for 45 mycotoxins by food samples by HPLC-MS/MS using fast polarity switching

Conclusion

- A very sensitive method for multiple mycotoxines was set up to ensure low LOQ in baby food sample,
- Thanks to high speed polarity switching, a high number of mycotoxines can be assayed using the same method in a short time,
- The extraction method demonstrate good recoveries to ensure accurate quantification.

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Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

ASMS 2015 TP 320

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Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

Introduction

The multi-residue determination of pesticides is extremely challenging due to the uncertainty concerning adverse effects that those residues may have on human health after a lengthy exposure at low levels. Consequently, stricter food safety regulations are being enforced around the world, placing pesticide analysis laboratories under increasing pressure to expand the list of targeted pesticides, to detect analytes at lower levels and with greater precision, to reduce analysis turnaround times, and all the while maintaining or reducing costs. Most pesticides are generally analyzed by gas chromatography but this

technique however, is not suitable for polar pesticides or thermolabile. A well established tool for the determination of residues of such pesticides is liquid chromatography combined with tandem mass spectrometry. The aim of this study was to develop and compare two fast, sensitive and simple methodologies for the multi-residue of pesticides, that require the online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry, thus the limits of quantification and matrix effects can be compared.

Materials and Methods

Sample preparation

Samples of 94 pesticides spiked at different concentrations (5 to 300ng/L) in spring water and different environmental waters were prepared adding ascorbic acid (800 mg/L) and phosphoric acid (0.2% v/v) and three internal standards (d5-Atrazine, d6-Bentazone, d6-Diuron).

LC-MS/MS analysis

Sample were analyzed using Nexera X2 UHPLC system (Shimadzu Corp.) with or without online SPE. Detection was performed using a highly sensitive triple quadrupole mass spectrometer (LCMS-8050, Shimadzu Corp.) in MRM mode. Thanks to the high speed polarity switching

capability of the MS, measurements were made in positive and negative electrospray ionization in one injection. Electrospray parameters (gas flows and temperatures) were optimized to find the optimal combination. The whole acquisition method lasted 15 min.

Table 1 – Direct injection conditions

Analytical column	: Zorbax Eclipse Plus C18 2.1*150 mm, 3.5 µm.
Mobile phases	A = Water + 0.05% acetic acid B = Acetonitrile + 0.05% acetic acid
Gradient	10% B (0-1.5min), 50% B (3.5 min), 100% B (9.5 min), 100% B (13.4 min), 10%B (13.5 min), Stop (15.0min)
Column temperature	: 40 °C
Injection volume	: 100 µL
Flow rate	: 0.4 mL/min

Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

Table 2 – online SPE conditions

Analytical column	: Restek Raptor ARC-18 100*2.1 mm 2.7 µm
SPE cartridge	: Spark holland PLRP-S 10x2mm 15µm
Mobile phases	: A = Water + 0.05 % acetic acid B = Methanol
SPE Mobile phases	: Loading (A'): Water + 1% acetic acid Elution/Wash (B'): Methanol
Gradient	: 0% B (0-1.55), 100% B (1.6), 100% B (9.95 min), 10% B (10 min), Stop (15.0min)
Column temperature	: 30 °C
Injection volume	: 1000 µL
Flow rate	: 0.6 mL/min
Flow rate SPE	: 0.2 to 2 mL/min

Results and discussion

Comparison between direct injection and online SPE

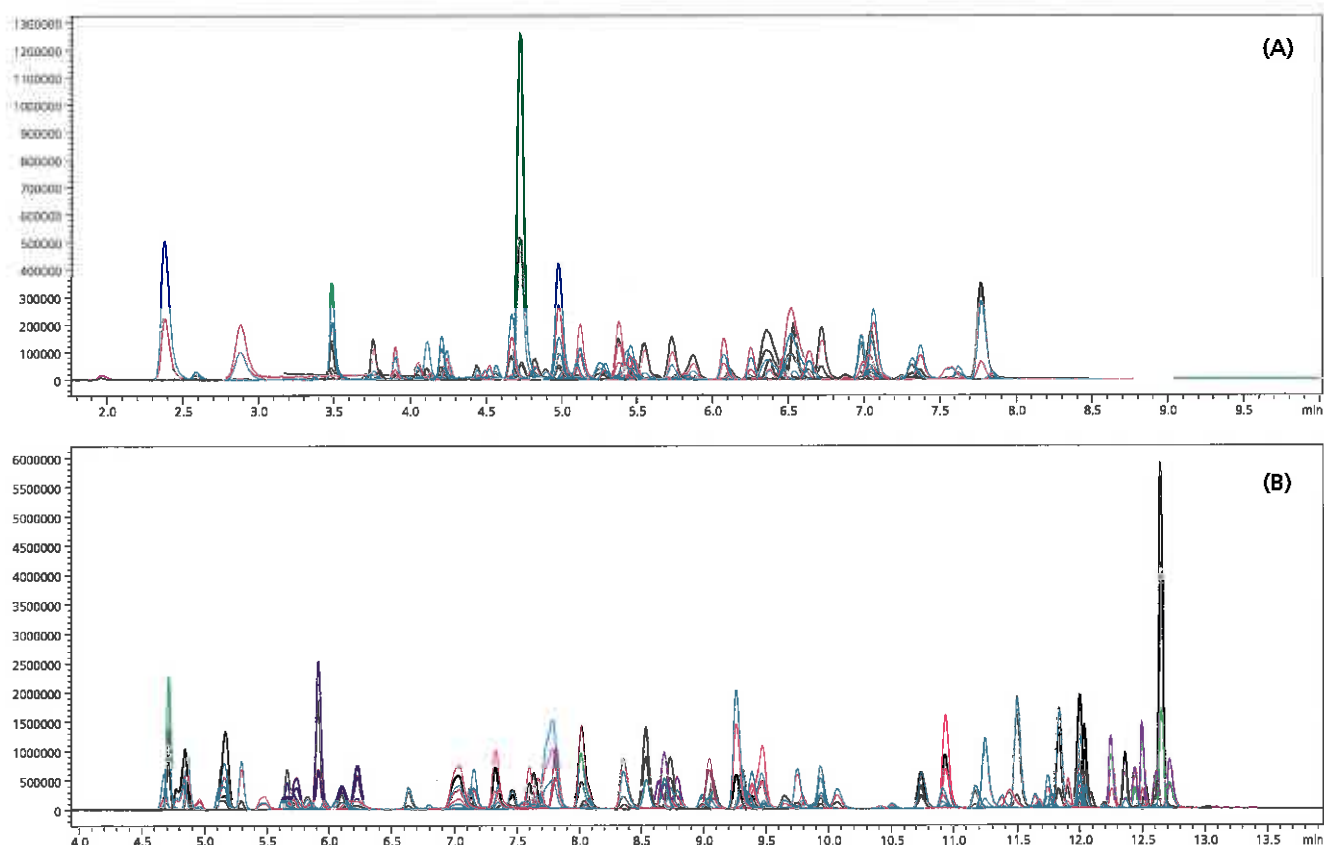


Figure 1 – Chromatograms of the 94 pesticides in spring water at 200 ng/L using (A) Direct injection or (B) online SPE.

Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

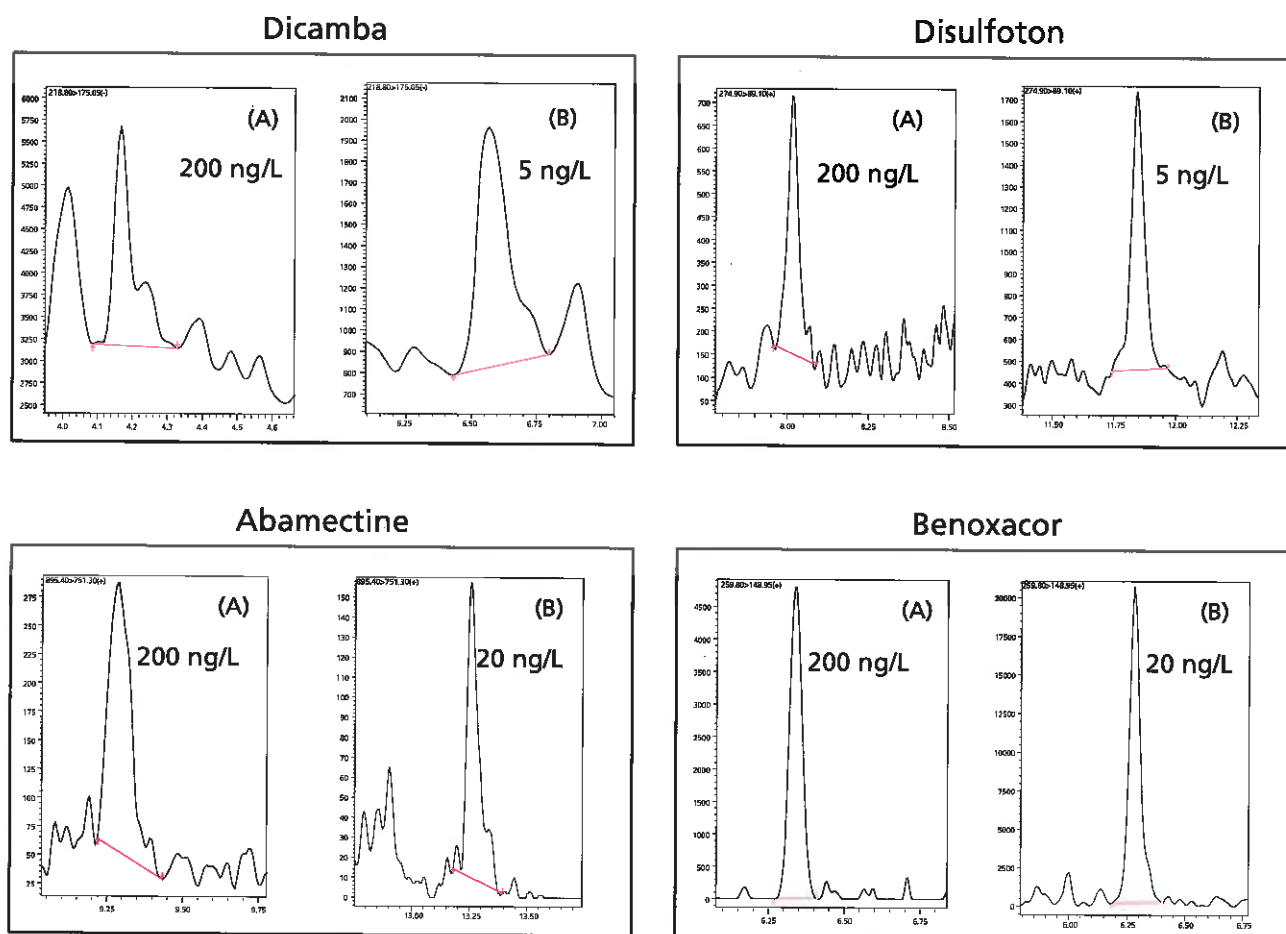


Figure 2 – Chromatogram of selected compounds at their LOQ using (A) Direct injection or (B) online SPE.

Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

Evaluation of matrix effect

Table 3 – Limits of Quantification in spring water

Target compound	Direct Injection		SPE	
	LOQ (ng/L)	S/N	LOQ (ng/L)	S/N
2,4-D	20	5	5	95
2,4-5T	50	9	5	29
Abamectine	100	14	20	7
Acetamiprid	10	58	5	127
Acetochlor	10	29	5	18
Aldicarb sulfone	20	10	5	7
Amidosulfuron	10	45	5	190
Atrazine	10	37	5	92
Atrazine hydroxy	20	26	5	104
Azamectophos	20	11	5	57
Azoxystrobin	10	15	5	109
Benoxacor	100	8	5	8
Bromconazole 1	100	5	5	30
Bromconazole 2	10	5	5	48
Buturon	100	13	5	43
Carbenthiolam	20	140	5	98
Carbofuran	20	20	5	107
Chlorobromuron	50	32	5	28
Chloridazon	50	5	5	19
Chloroxuron	200	7	5	57
Chlorothalonil	50	15	5	11
Cyprothiazole	>200	5	10	10
Cymoxanil	200	5	5	55
Cyprodinil	10	11	5	152
DC PMU	50	15	5	28
Desethylatrazine	10	16	5	65
Desisopropylatrazine	20	6	5	8
Dicamba	100	4	5	5
Difenoconazole	20	20	5	900
Dimethuron	100	13	5	25
Dimethachlor	20	500	5	14
Dimethoate	10	480	5	56
Dimethomorph	50	5	5	900
Disulfoton	200	3	5	8
Diuron	10	3	5	99
Epoxiconazole	10	6	5	285
Ethidimuron	20	5	5	154
Fenbuconazole	20	10	5	10
Fenpropidin	20	30	5	92
Fenpropimorph	10	20	5	133
Fenuron	10	25	5	100
Flazasulfuron	100	5	5	35
Flupyrifluor methyl	50	10	5	171
Fluquinconazole	50	6	5	75
Furitanone	10	11	5	272
Foransulfuron	10	9	5	4500
Hexaconazole	10	8	5	500
Imazalil	10	3	5	18
Imazamethabenz	10	40	5	344
Imazapyr	10	5	5	189
Imidacloprid	100	12	5	47
Iodosulfuron methyl	20	14	5	44
Isoproturon	10	14	5	121
Isosab	20	20	5	92
Kresoxim-methyl	50	15	5	107
Mesosulfuron methyl	10	100	5	209
Metaxyl	10	5	5	20
Metamifon	10	5	5	5
Metazachlor	10	14	5	114
Methabenzthiazuron	10	6	5	205
Methiocarb	10	80	5	111
Methomyl	20	15	5	171
Metolachlor	10	100	5	37
Metosulam	10	160	5	118
Metosulfuron	20	25	5	7
Metribuzin	10	10	5	23
Metsulfuron methyl	10	36	5	141
Nicosulfuron	20	10	5	2900
Omethoate	20	34	5	84
Oxadetyl	20	15	5	9
Oxydemeton methyl	20	20	5	20
Phosphate de butyl	10	80	5	78
Picloram	50	10	10	4
Primicarb	10	26	5	132
Prochloraz	10	40	5	444
Propoxur	50	25	5	66
Pyridostrobin	10	15	5	1400
Pyriproxyfen	20	5	5	10
Pyriproxyfen 2	20	5	5	10
Pymethanil	50	200	5	29
Quinmerac	10	15	5	42
Simazine	10	182	5	70
Simazine hydroxy	50	60	5	340
Sulcotrione	100	16	5	6
Sulfosulfuron	50	20	5	43
Tebuconazole	10	32	5	329
Tebuthiazuron	10	11	5	168
Terbutylazine desethyl 2 hydroxy	10	62	5	300
Tetraconazole	20	10	5	900
Thiadenozol	10	10	5	33
Thifensulfuron methyl	10	40	5	129
Thiodicarb	20	8	5	109
Triazamate	10	20	5	6200
Trichlorphon	100	8	5	13
Trinexapac-ethyl	100	10	5	10
Vamidothion	10	20	5	88

Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

Table 4 – Comparison of accuracy between direct injection and online SPE for several water samples

Target compound	Direct Injection				Online SPE			
	Surface water spiked at 200 ng/L		Deep water spiked at 150 ng/L		Tap water spiked at 300 ng/L			
	Accuracy (%)	%RSD	Accuracy (%)	%RSD	Accuracy (%)	%RSD	Accuracy (%)	%RSD
2,4-D	99	8	201	8	92	5	105	6
2,4-5T	87	2	163	1	92	11	86	7
Abamectine	122	27	199	27	148	8	118	6
Acetamiprid	112	5	100	7	96	5	98	1
Acetochlor	86	10	109	11	95	2	96	3
Aldicarb sulfone	71	27	75	9	100	7	90	7
Amidosulfuron	91	7	104	8	95	6	94	4
Atrazine	112	21	101	5	106	4	104	2
Atrazine hydroxy	151	20	130	7	103	5	119	6
Azamethphos	112	3	107	6	100	6	108	3
Azoxystrobin	118	7	115	4	112	4	115	4
Benoxacor	115	23	92	6	98	6	99	4
Bromuconazole 1	103	6	97	5	109	5	110	3
Bromuconazole 2	108	12	97	6	117	6	129	4
Buturon	99	11	99	5	100	4	107	2
Carbetamide	117	4	100	7	96	3	96	4
Carbofuran	112	4	95	7	100	6	102	3
Chlorbromuron	99	13	101	8	96	6	101	6
Chloridazon	90	16	107	7	101	5	105	3
Chloroxuron	100	29	101	7	99	5	103	4
Chlorothalonil	105	5	107	9	96	6	103	5
Clopyralid			93	12	104	4	100	2
Cymoxanil	61	17	144	5	89	2	85	2
Cyprodinil	95	2	80	9	85	8	97	3
DCPMU	121	10	94	6	100	2	106	3
Desethylatrazine	108	19	134	10	98	8	104	3
Desisopropylatrazine	100	19	88	7	90	4	84	6
Dicamba	74	16	72	5	100	2	96	3
Difenoconazole	98	5	180	12	103	9	124	15
Dimethuron	126	12	105	14	103	4	109	1
Dimethachlor	110	7	100	6	101	4	106	1
Dimethoate	104	6	93	9	101	5	101	3
Dimethomorph	113	6	96	4	102	3	103	3
Disulfoton	118	20	124	4	112	4	84	2
Diuron	97	3	102	10	106	6	108	3
Epoxiconazole	102	3	88	10	98	3	102	5
Ethidimuron	95	3	66	7	82	3	78	3
Fenbuconazole	112	5	71	13	85	12	88	3
Fenpropiadin	106	5	106	13	99	4	108	3
Fenpropimorph	N/A	N/A	107	2	97	6	104	4
Fenuron	102	2	109	10	94	6	98	2
Flazasulfuron	89	17	109	2	99	10	92	8
Fluopyrusulfuron methyl	99	4	133	3	100	3	106	7
Fluquinconazole	122	8	91	3	99	6	94	6
Furilamone	108	2	109	4	101	3	104	2
Foramsulfuron	104	4	119	6	121	11	167	9
Hexaconazole	109	3	96	5	102	2	103	5
Imazalil	83	6	114	6	105	5	113	2
Imazamethabenz	91	3	110	8	102	6	113	3
Imazequin	91	6	120	7	100	8	110	5
Imidacloprid	104	6	92	10	99	2	107	3
Iodosulfuron methyl	113	6	100	5	100	9	95	6
Isoproturon	115	7	116	8	116	8	109	3
Isoxaben	113	3	106	4	102	4	104	4
Kresoxim-ethyl	111	2	109	5	113	8	116	2
Mesosulfuron methyl	104	7	141	3	92	4	99	9
Metabenz	112	7	123	12	122	11	140	3
Metamitron	97	6	102	8	94	5	100	3
Metazachlor	112	2	103	5	97	2	102	4
Methabenzthiazuron	97	3	103	6	88	4	92	4
Methiocarb	108	5	96	6	101	4	104	4
Methomyl	96	7	107	2	97	2	89	2
Metolachlor	116	5	106	8	96	3	103	4
Metosulam	126	11	165	4	97	3	97	6
Metoxuron	121	35	136	13	133	9	148	7
Metribuzin	108	3	101	6	98	0	98	8
Metsulfuron methyl	117	1	124	9	105	9	115	3
Nicosulfuron	101	2	128	7	93	7	95	8
Omethoate	94	2	87	10	83	4	58	4
Oxadiazyl	112	2	103	6	105	7	107	6
Oxydemeton methyl	111	6	97	5	93	3	94	2
Phosphite de tributyl	141	5	105	18	93	6	98	4
Picloram	100	2	88	7	97	7	113	2
Prinmipar	100	2	100	7	92	4	100	4
Prochloraz	97	2	96	3	104	4	101	4
Propoxur	115	0	98	11	97	7	104	1
Pyraclostrobin	117	1	94	5	101	6	108	4
Pyrioxon 1	105	5	115	12	111	10	130	6
Pyrioxon 2	99	2	103	8	99	5	104	7
Pyrimethanil	100	1	111	2	99	5	107	3
Quinmerac	83	3	102	10	94	4	96	2
Simazine	103	23	105	6	96	4	96	5
Simazine hydroxy	124	22	89	3	101	4	107	2
Sulfotione	136	9	74	4	91	8	92	6
Sulfosulfuron	125	7	94	1	97	1	80	5
Tebuconazole	111	2	81	28	105	2	109	2
Tebuconazole	121	1	94	6	90	7	104	6
Terbutylazine desethyl 2 hydroxy	88	21	108	7	104	5	93	2
Tetraconazole	113	2	73	9	97	11	85	14
Thiacloprid	105	4	79	9	110	4	102	4
Thifensulfuron methyl	111	3	102	7	97	9	104	7
Thiodicarb	128	4	92	7	98	6	103	5
Triazamate	124	4	105	12	107	9	101	9
Trichlorophen	129	7	92	9	106	4	101	2
Trinexapac-ethyl	118	19	97	3	96	6	107	4
Vamidothion	97	1	122	9	92	3	90	4

Comparison of matrix effects in multi-residue pesticide analysis when using online SPE or direct injection in Liquid Chromatography-tandem Mass Spectrometry

Conclusion

Linearity of response in LCMS quality water of the multi-residue pesticides was demonstrated ($r > 0.99$). Limits of quantification (LOQ) for 97 % of pesticides were less than 5 ng/L for SPE and 70% less than 20 ng/L for the direct injection. During the validation process the methods were tested for matrix effect with several environmental

waters (tap, surface and deep waters). A difference of matrix effect were highlighted between the two injection modes (SPE and direct injection), which helped to set up an analytical strategy based on these effects and the sensitivity of pesticides.

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Cooked meat product analysis by LCMS/MS to determine between animal species using proteotypic peptide quantitative proteomic analysis

ASMS 2015 WP 099

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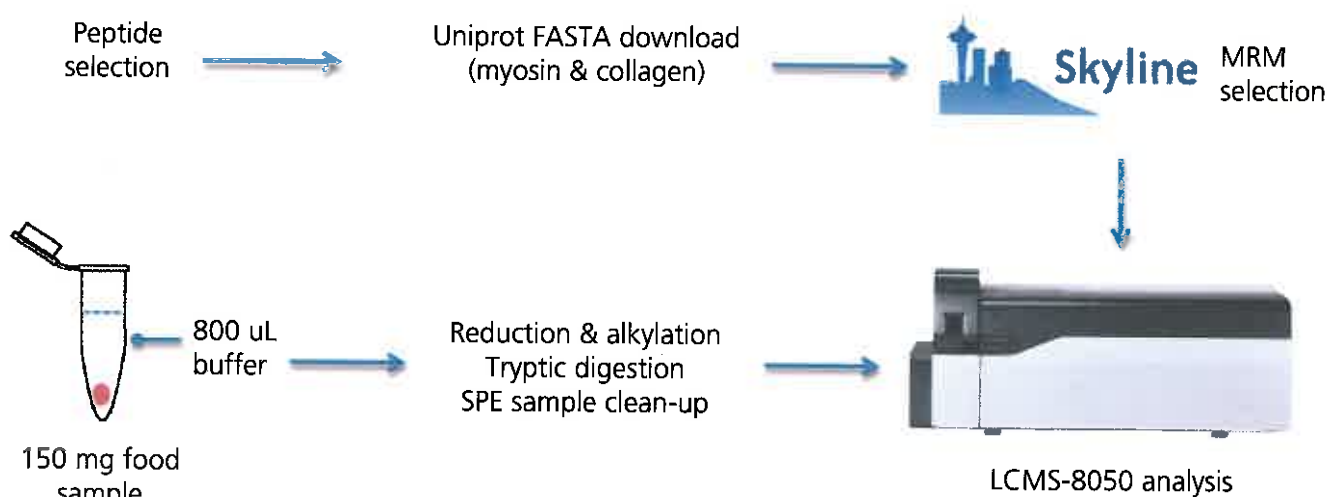
Cooked meat product analysis by LCMS/MS to determine between animal species using proteotypic peptide quantitative proteomic analysis

Introduction

With the emergence of new food safety challenges innovative analytical technologies are being developed to enable industry stakeholders and regulators to make sound scientific decisions. These advances have been made in response to changing regulations and needs and also to help maintain the safety of the food chain. In the case of unwanted or undeclared ingredients food authenticity testing is important component in many quality and safety programs. In 2013, horse meat issues received intense scrutiny and Polymerase Chain Reaction (PCR) techniques were used to screen for horse DNA in processed food samples. However, despite its widespread use in Europe for food fraud, PCR is problematic. DNA extraction kits are

expensive and often require up to 8 h for the extraction, followed by cleanup and the PCR process. DNA is also prone to degradation especially in processed food. In contrast, mass spectrometry enables a promising approach to the detection of marker peptides in tryptic digests of horse, pig, beef and chicken with high sensitivity. In this work previously published proteotypic peptides were selected to detect horse, pork, beef and chicken using Skyline software (MacCoss Lab Software - University of Washington). These were used to interrogate Uniprot database FASTA download for myosin (43263 proteins) and collagen (44531 proteins) to determine no other species cross reactivity to pig myosin and collagen.

Method Development



meat product analysis by LCMS/MS to determine animal species using proteotypic peptide quantitative isotopic analysis

Materials and Methods

Sample preparation

Food samples were prepared by weighing 150mg into a microfuge tube, crushed using a micro-pestle then addition of 800uL extraction buffer (water containing 6M urea, 1M thiourea, 50mM Tris-HCl). Vigorous vortexing (2500rpm 15 sec), followed by centrifugation (13,000g, 10min), 100uL supernatant removed. Samples were reduced with addition of 30uL DTT (45mM) in 25mM ammonium bicarbonate 15min at 50C, then alkylated with addition of 30uL iodoacetamide (100mM) in 25mM ammonium bicarbonate 15min in darkness at room temperature, then diluted with 800uL 25mM ammonium bicarbonate. 30uL trypsin at 6.6uM in 25mM ammonium bicarbonate was added then incubated overnight at 37C. Sample clean-up following tryptic digestion was performed using off-line solid phase extraction (SPE) using Phenomenex Strata-X 33u polymeric reversed-phase

(RP) cartridges filled with 30 mg/mL RP material. These were conditioned with 1mL methanol followed by 1mL acetonitrile, then equilibrated with 2mL water containing 5% acetonitrile + 0.1% formic acid. The sample was loaded, followed by cartridge washing (2mL water containing 5% acetonitrile + 0.1% formic acid) and elution (1mL 90% acetonitrile + 0.1% formic acid). Eluate was reduced by a centrifugal vacuum evaporation for 1h to approximately 50uL then increased to exactly 100uL with 3% acetonitrile + 0.1% formic acid. Samples included: pork, chicken, beef and horse meat. Processed and mixed foodstuffs included: horse sausage, horse burger, beef haché, beef-chicken paste, beef-ham-chicken paste. Lamb mince was also analysed to represent a control sample. Cooked food was subjected to 200C for 1h.

Liquid chromatography

UPLC	: Nexera LC system	
Analytical column	: Phenomenex Aeris C18 (150 x 2.1mm, 1.7um particle size).	
Column temp.	: 50 °C	
Injection cycle	: 2 µL injections	
Flow rate	: 0.3mL/minute	
Solvent A	: Water + 0.1% formic acid	
Solvent B	: Acetonitrile + 0.1% formic acid	
Binary Gradient	Time (mins)	%B
	0	5
	3	5
	35	40
	37.5	100
	45	100
	45.1	5
	50	Stop

Mass spectrometry

LC/MS/MS	: LCMS-8050
Ionisation mode	: Heated ESI
Polarity switching time	: 5 ms
Dwell times	: 47-117ms
Interface temperature	: 400 °C
Heating block	: 250 °C
Desolvation line	: 150 °C
Heating gas	: 15 L/min
Drying gas	: 5 L/min
Nebulising gas	: 2 L/min
CID gas pressure	: 350kPa

Cooked meat product analysis by LCMS/MS to determine between animal species using proteotypic peptide quantitative proteomic analysis

Targeted peptides

Species	Protein	Accession	Peptide	RT (min)	Q1 prec.	MRM 1 (q)	MRM 2	MRM 3	MRM 4	CE (q)
Pig	MYH4	Q9TV62	TLAFLFAER	21.24	534.3 >	853.46	782.42	635.35	522.27	-18.9
	COL1A2	Q1T7B0	GVGAGPGPMGLMGPR	16.77	677.34 >	915.45	858.43	630.34	460.23	-34.6
Chick	MLE1	P02604	DQGTFFDFVEGLR	22.27	756.85 >	964.47	835.43	720.4	474.27	-27.8
	MLR5	P02609	GADPEDVIMGAFK	21.55	675.32 >	422.24	880.46	666.36	553.28	-28.5
			SFLEELLTTQCDR	24.20	777.88 >	723.31	949.48	836.39	622.26	-26.6
	MYSS	P13538	NLTEEMAVLDETIK	22.90	838.92 >	789.44	959.54	888.5	676.35	-25.0
			VAEQELLDATER	13.87	687.35 >	591.27	817.44	704.36	476.25	-23.0
Bovin	MLR5	Q0P571	EASGPINFVFLNMFGEK	30.60	667.66 >	985.48	838.41	725.33	480.25	-21.9
	CO1A1	P02453	GAPGADGPAGAPGTPGPGIAGQR	30.79	686.34 >	883.47	729.4	601.34	-	-24.6
Horse	MYH1	Q8MJV0	TLALLFSGPASADAEAGGK	20.77	888.46 >	1030.48	973.46	805.37	532.27	-31.0
			VVETMQTMLDAEIR	19.90	818.41 >	488.28	948.48	847.43	603.31	-38.2
	MYH2	Q8MJV1	EFEIGNLQSK	14.72	582.8 >	646.35	888.48	759.44	475.29	-20.8

Acquisition parameters for food tryptic digest peptides: pig, chicken, bovine and horse. Q1 and Q3 bias used tuning file default settings, MS2 setting - Unit resolution, positive

ionisation mode. Collision energy (CE) was set using predicted setting from Skyline software. CE optimisation was later performed once peptide RT was defined.

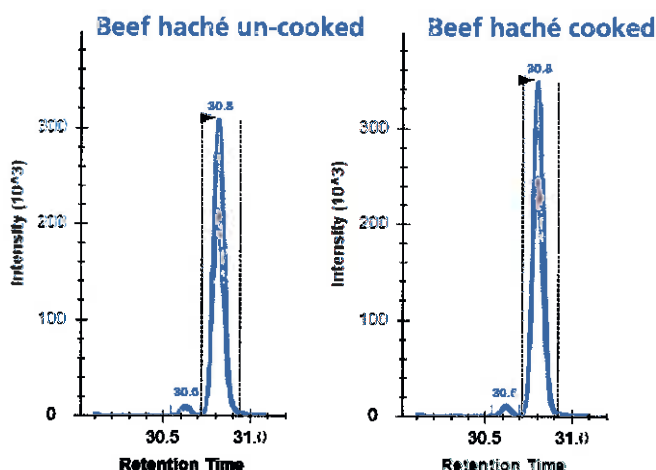
Results

Heat degradation effect

Heat degradation through cooking is a concern for food testing as this may impair measurement of target peptides and possibly reduce the apparent levels of animal material present. As a consequence of cooking however, the water and lipid content of the meat was significantly reduced

ranging from 19% reduction in weight (chicken sausage) to 52% weight reduction (lamb mince). As a consequence of net weight loss in cooked food products, the anticipated signal loss through heat degradation was offset by net increase in concentration of target analyte.

Bovine myosin before and after cooking



Bovine myosin regulatory light chain 2, skeletal muscle isoform tryptic peptide: EASGPINFVFLNMFGEK
Signal intensity from 150mg of beef haché increased from un-cooked to cooked however the process of cooking decreased the net weight by 29%.

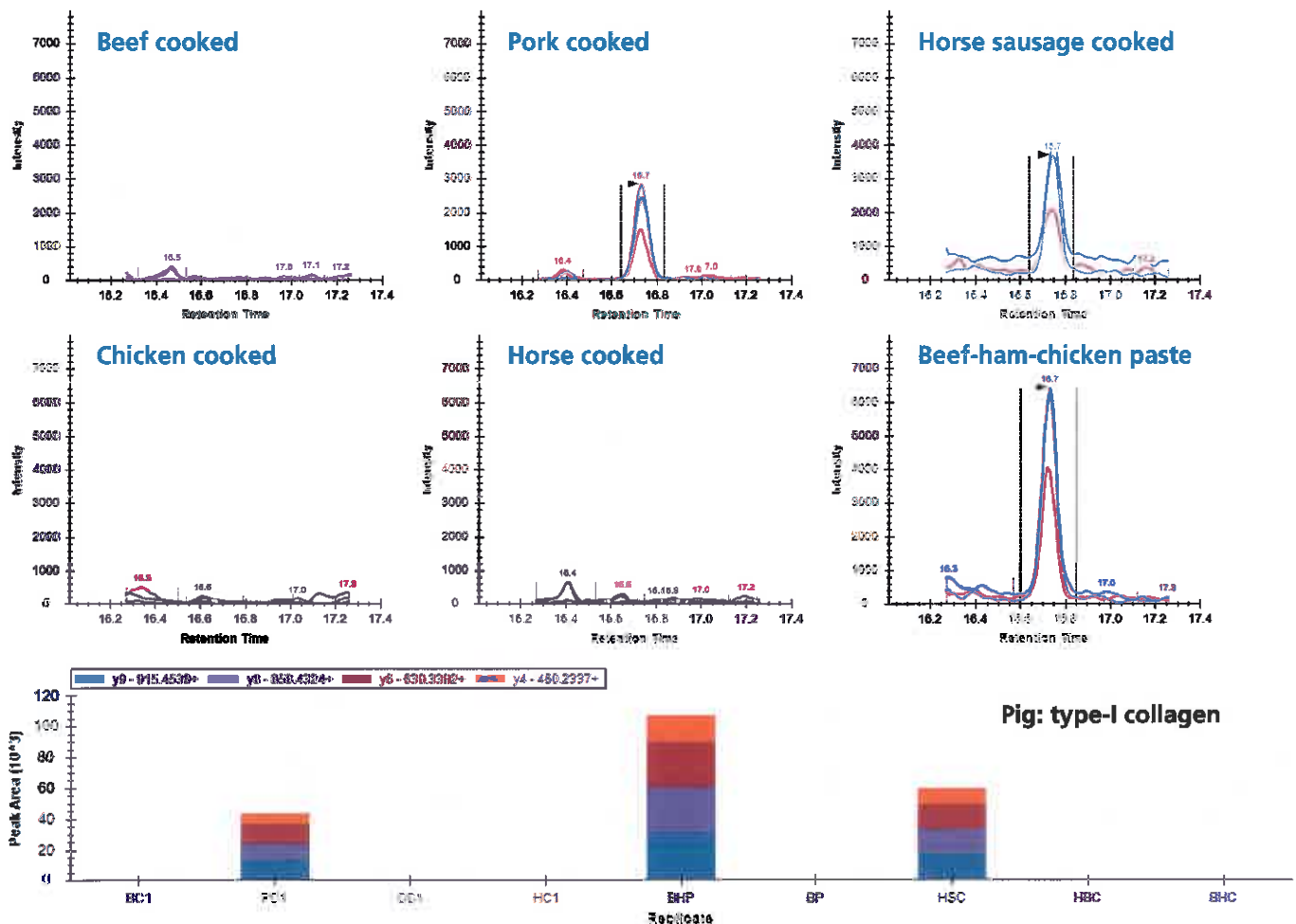
meat product analysis by LCMS/MS to determine een animal species using proteotypic peptide quantitative teomic analysis

Sample drying / desiccation is not specified in food testing guidelines (LGC DNAS-13000036) however when testing precooked food products borderline quantities of contaminating material may be detected even if original raw ingredients were below a reporting threshold.

Likewise if heat degradation plays a significant part in reducing the limit of detection testing of uncooked food would be recommended in addition to testing of cooked food in order to prevent reportable levels of contamination being missed.

Pig: type I collagen

Measurement of pig collagen type I successfully detected pig collagen in mixed food types horse sausage and beef-ham-chicken sandwich paste.

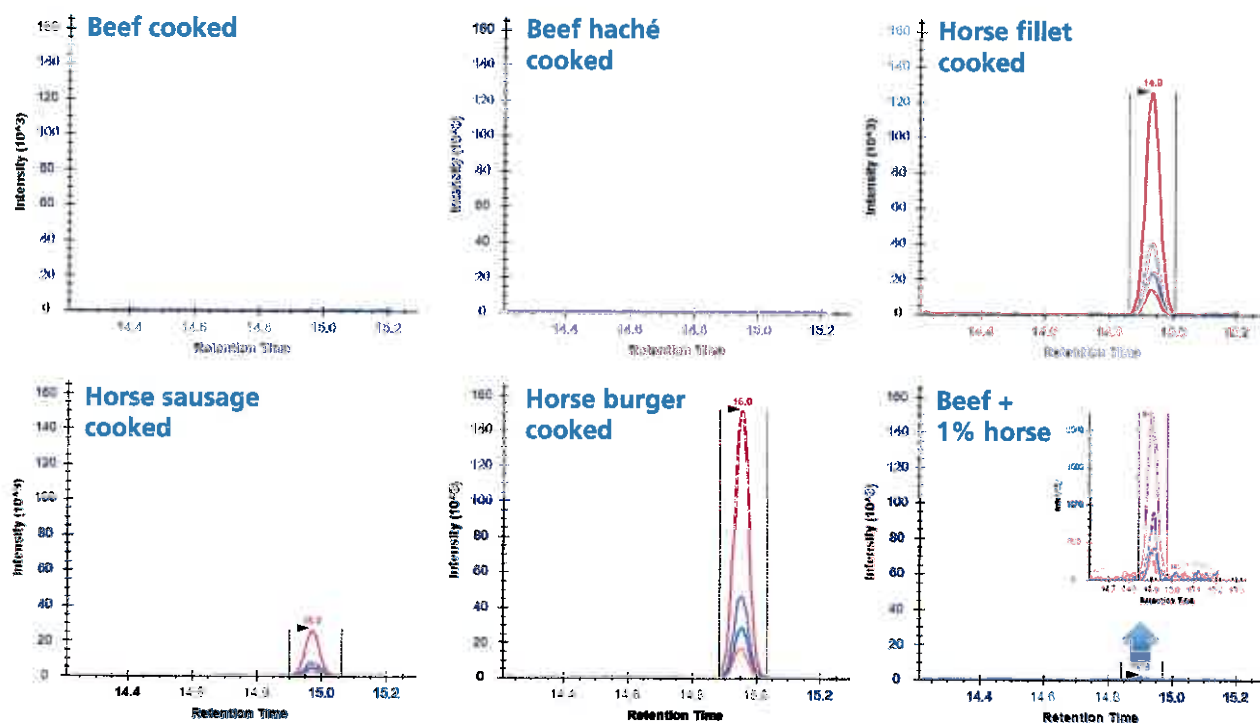


Peak area comparison between different food types by type-I collagen alpha-2 chain tryptic peptide. (BC – beef cooked, PC – pork cooked, CC – chicken cooked, HC –

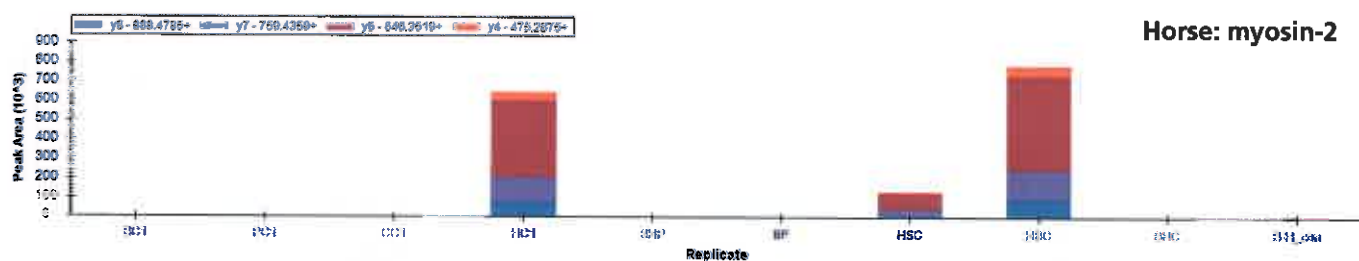
horse cooked, BHP – beef-ham-chicken paste, BP – beef-chicken paste, HSC – horse sausage cooked, HBC – horse burger cooked, BHC – beef haché cooked).

Cooked meat product analysis by LCMS/MS to determine between animal species using proteotypic peptide quantitative proteomic analysis

Horse myosin analysis



Proteotypic peptides from two different horse myosin proteins, myosin-1 and myosin-2, were measured from food samples. These successfully differentiated horse meat from other food types and was detected at 1% spiked level in beef sample.



Horse: myosin-2

meat product analysis by LCMS/MS to determine animal species using proteotypic peptide quantitative proteomic analysis

Conclusions

Current food authenticity testing focuses on animal speciation testing by PCR measurement of the mitochondrial gene cytochrome b. The strength of this approach offers any tissue type from an animal can be determined, however, it does not provide information on the type of animal tissue measured. Guidelines on food labelling and composition of meat products recommend limits on the percentage fat and connective tissue at 30%

and 25% in pork products. In products containing meat from two or more species, measuring the level of connective tissue from more than one animal species adds further complexity to routine analysis. In these targeted proteomic experiments animal species can be determined in addition to measuring different proteins specific to a tissue type, therefore offering an added level of information not routinely performed in food testing.

References

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Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

ASMS 2014 TP 583

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Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

Introduction

Many pharmaceuticals from medical treatments are metabolized or partially degraded in the body. An even larger amount of these compounds is excreted intact and pollutes the aquatic environment. Relevant classes of drugs are human or veterinary antibiotics, antiepileptics, analgetics and lipid lowering drugs or radio-opaque substances. The extent of damage caused to the environment and the resulting health risk for humans or animals should not be underestimated, even though it is

not understood in detail so far. The requirement for universal, reliable and fast methods for drug determination in water is steadily increasing. Highly sensitive triple-quad-MS systems are suitable tools for the analysis of residues in ground-, surface- and wastewater, but development of a simple, rapid and robust method for simultaneous measurement of trace levels of various different classes of analytes in complex matrices is a challenge.



Figure 1. LCMS-8050 triple quadrupole mass spectrometer

Method

This study describes a fast LC-MS/MS method for the determination of trace levels of different classes of drugs in water. With online SPE no further sample pretreatment is necessary. The quaternary system with low pressure gradient eluent (LPGE) and solvent blending functionality renders addition of a third LC-Pump unnecessary. The

solvent blending function was further used for method development. High speed values for MRM recording and the fastest polarity switching time of 5 ms are essential physical parameters for a maximum of data points on various classes of analytes in different polarities during one single analysis.

LC-MS/MS Method Optimization

One of the first steps during this automated process is the precursor ion selection, followed by m/z adjustment of the precursor. The collision energy is optimized for the most abundant fragments and finally the fragment m/z is

adjusted. Six optimization steps were performed via flow injection analysis, each taking 30 seconds (Figure 2). The result of these automated steps was the automatic generation of a final MRM method (Table 1).

and highly sensitive analysis multiple drugs in ground-, surface- and wastewater

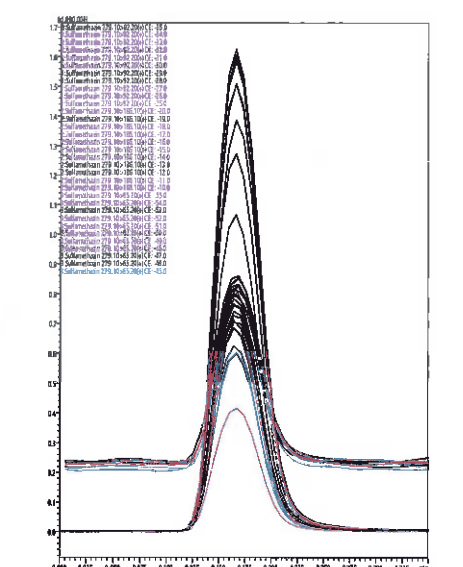
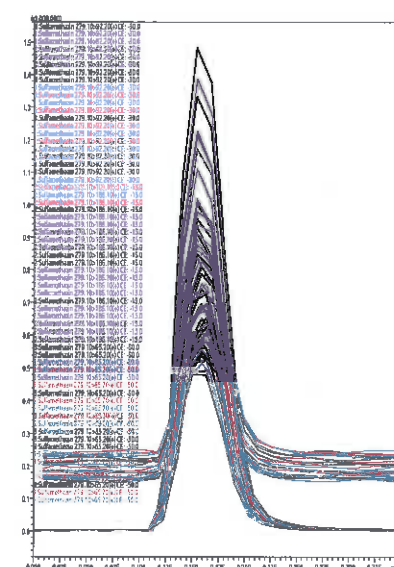
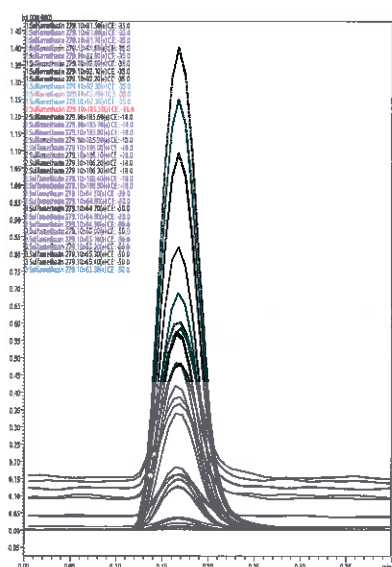
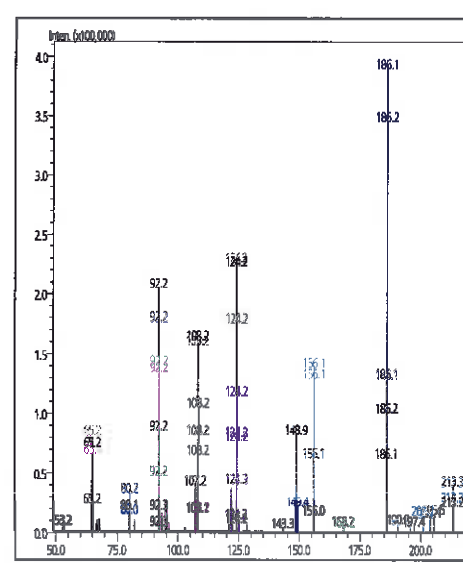
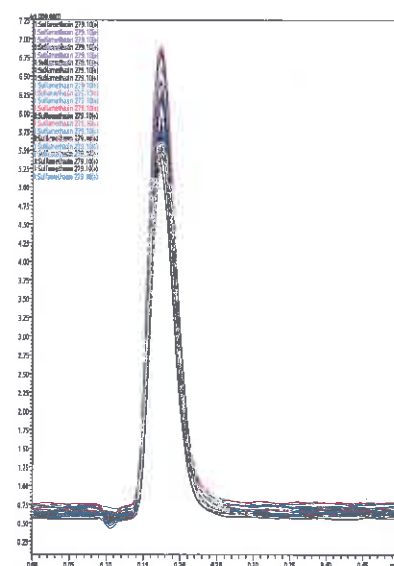
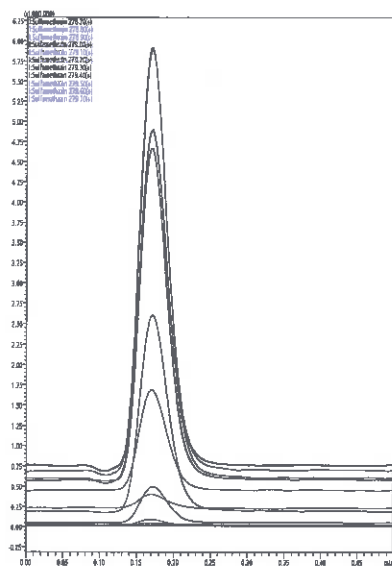


Figure 2. Automated MRM Optimization of the drug Sulfamethazin

Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

Table 1. Optimized MRM transitions of 9 drugs

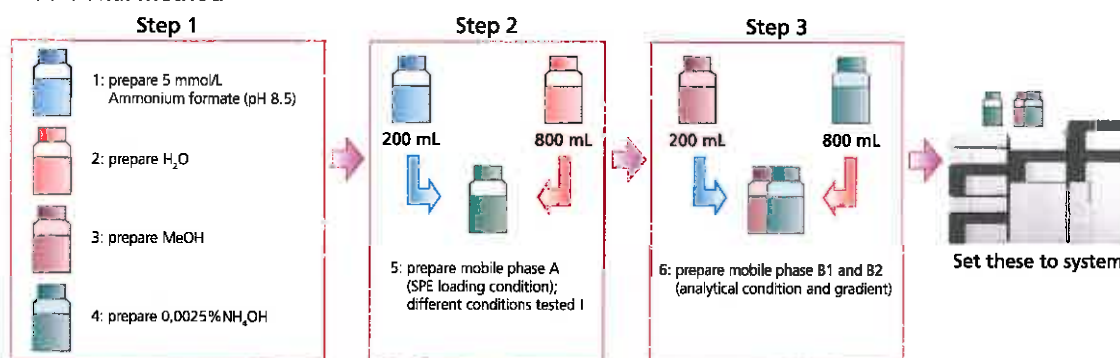
Compound	Mode	MRM transitions	Collision energy (kV)
Sulfamethazin	ESI positive	279,10>186,10 / 279,10>92,20	-17 / -31
Sulfamethoxazol	ESI positive	253,90>92,20 / 253,90>156,15	-26 / -15
Bezafibrat	ESI positive	362,10>139,15 / 362,10>316,25	-25 / -15
Carbamazepine	ESI positive	237,10>194,20 / 237,10>179,20	-19 / -34
Diclofenac	ESI positive	296,00>214,15 / 296,00>215,15	-34 / -19
Clofibric acid	ESI negative	213,00>127,00 / 213,00>85,00	15 / 15
Ibuprofen	ESI negative	205,10>161,30	7
Iopamidol	ESI negative	775,80>126,95	22
Iopromid	ESI negative	790,00>127,00	26

Solvent Blending

The solvent blending functionality entails automated mobile phase preparation on a LPGE (low pressure gradient) unit which is integrated in the binary LC pumps. The blending function eliminates the need of mobile phase pre-mixing, as necessary with ordinary binary pumps. Mobile phase composition can simply be changed in the

method without physically changing the solvents. Therefore solvent blending is a powerful tool for easy and efficient elucidation of the SPE, the gradient and the starting conditions. During this study the solvent blending function was used for optimization of the SPE conditions. A second LPGE unit was used for the analytical gradient.

Traditional method



Mobile phase blending function

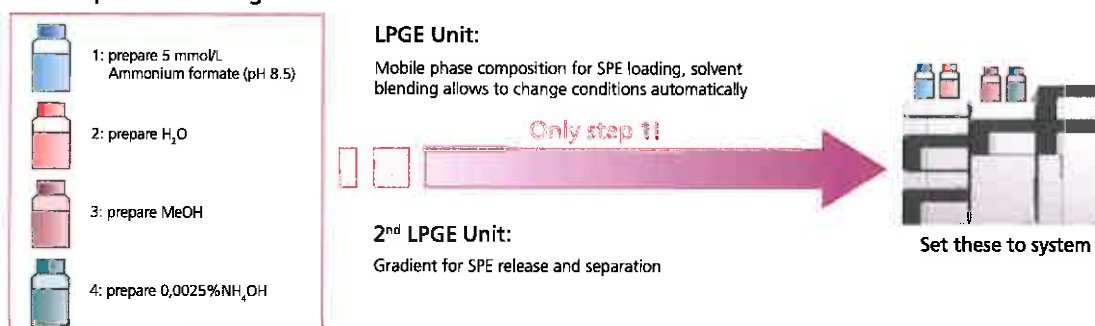


Figure 3. Solvent blending functionality

Highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

HPLC/MS Workflow

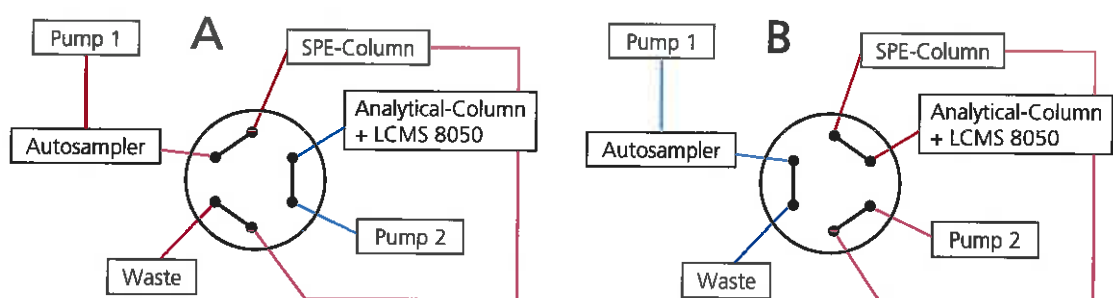


Figure 4. Scheme of online-SPE extraction (A) and analytical separation (B)

Final method

SPE Conditions

Injection volume	: 250 µl
SPE-column	: Strata-X, 25 µm, 20*2 mm
SPE-flow rate	: 1 mL/min
SPE-loading buffer	: 1 mmol/L ammonium formate (LPGE Pump B)

Analytical Conditions (LPGE Pump A)

Column	: Kinetex C8, 2.6 µm, 100*2.1 mm
Flow rate	: 0.5 mL/min
Solvent A	: 0.0025% NH ₄ OH
Solvent B	: MeOH 1 min – 2.5 min analytical separation
Gradient	: 0 min : 30% B
	: 1 min : 30% B
	: 1.5 min : 95% B
	: 4.5 min : 95% B
	: 4.51 min : 30% B
	: 6 min : 30% B (Stop)

LCMS Conditions

Interface	: ESI
Nebulizing Gas Flow	: 2.2 L/min
Heating Gas Flow	: 12 L/min
Interface Temperature	: 400 °C
Desolvation Line Temperature	: 150 °C
Heat Block Temperature	: 400 °C
Drying Gas Flow	: 6 L/min
Polarity Switching Time	: 5 ms

Fast and highly sensitive analysis of multiple drugs in ground-, surface- and wastewater

Results

In this study we developed a fast method for direct online SPE LC-MS/MS analysis of 9 different drugs in water with a minimal LC configuration of two binary pumps equipped with LPGE units. The solvent blending function was used for method development of the SPE extraction. The second LPGE unit was used for SPE release and analytical gradient

separation. Each compound was quantified in a concentration range from 0.05 ng/ml up to 2 ng/ml. Measurements were performed on Shimadzu's LCMS-8050 Triple Quad MS System. The calibration curves and lowest calibration point is shown in figure 5.

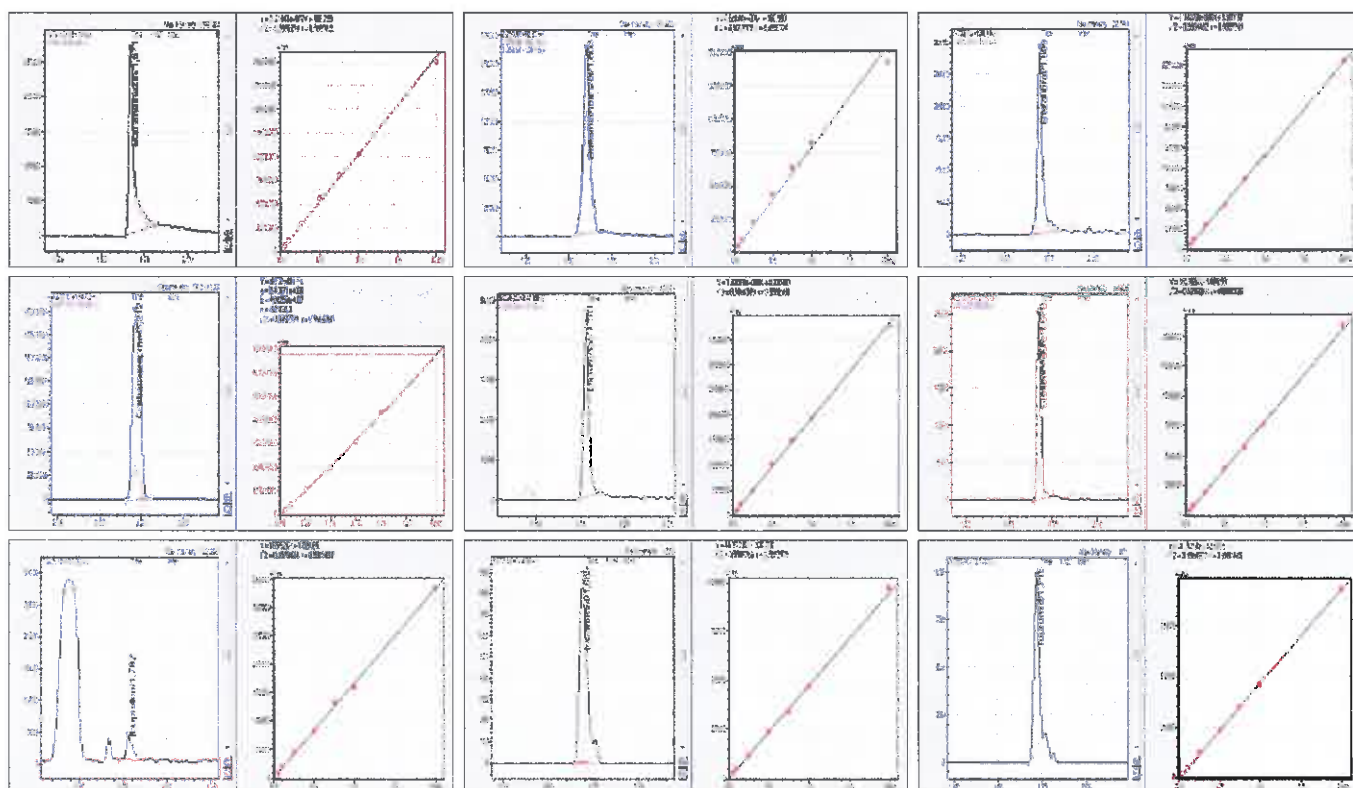


Figure 5. Calibration curve and lowest calibration point at 0.05 ng/ml of each compound

First Edition: June, 2014

High Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Toxins Using Liquid Chromatography Tandem Mass Spectrometry

ASMS 2015 TP 349

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High Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Toxins Using Liquid Chromatography Tandem Mass Spectrometry

Introduction

For the past decades marine toxins in shellfish have been monitored by the mouse bioassay (MBA) in many countries including Japan. Recently several alternative testing methods have been developed and a few of them have been validated. The most widely accepted method for many kinds of the marine toxins is liquid chromatography (LC) combined with mass spectrometry (MS), deemed to be the powerful tool than the MBA in sensitivity and accuracy. Diarrhetic shellfish poisoning (DSP) toxins, okadaic acid (OA) and dinophysistoxins (DTXs), are very important target for marine bio-toxin

monitoring in Japan. The MBA for DSP toxin monitoring was replaced with a LC/MS/MS method on the new regulation issued in March 2015 in Japan (Notification No. 1 issued by the Dept. of Food Safety, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare on March 6, 2015). In this presentation, we demonstrate the developed LC/MS/MS methods for the screening of OA, dinophysistoxin1 (DTX1), pectenotoxin1, 2, 6 (PTX) and yessotoxin (YTX) as well as for the routinely quantification of OA and DTX1.

Materials & Methods

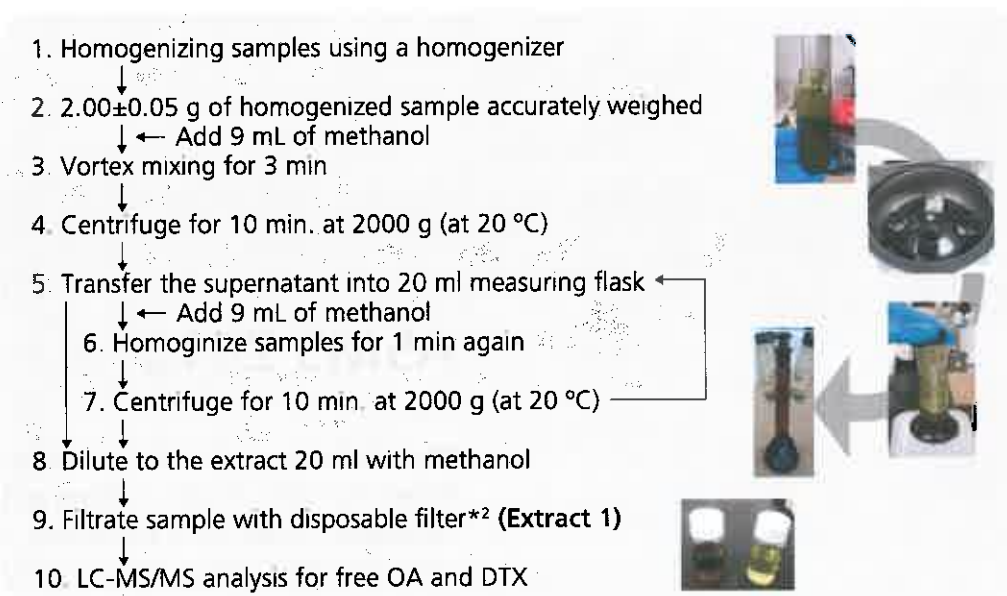
Sample Preparation (EU-RL-MB*1)

Table 1 Sample List

Sample	Food	Origin
1	The naturally contaminated midgut gland of scallops with toxins	Japanese National Research Institute of Fisheries Science
2	Midgut gland of scallops	Market
3	Oyster	Market



Step 1 : Sample Extraction



Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Using Liquid Chromatography Tandem Mass Spectrometry

Step 2 : Hydrolysis (In order to detect the total content of OA and DTX toxins)

1. Transfer 1 mL **Extract 1** into a 1.5 mL HPLC vial
 ↓ ← Add 125 µL of 2.5 mol/L sodium hydroxide aqueous solution
2. Vortex mixing for 0.5 min
 ↓
3. Heat the mixture at 76 °C for 40 min
 ↓
4. Cooling down to room temperature
 ↓ ← Add 125 µL HCl 2.5 M for neutralise
5. Vortex mixing for 0.5 min
 ↓
6. Filtrate sample with disposable filter*2 (**Extract 2**)
 ↓
7. LC-MS/MS analysis

*1 EU-Harmonised Standard Operating procedure for determination of lipophilic marine biotoxins in molluscs by LC-MS/MS Ver.4

*2 TORAST DISC 0.22 µm P/N GLCTD-PTFE1322

Standard Solutions

- The mixture of six standards solution (OA, DTX1, PTX1, PTX2, PTX6, YTX) was provided by courtesy of Dr. Toshiyuki Suzuki in the Japanese National Research Institute of Fisheries Science for the purpose of this research.
- The certified solutions for calibration of OA and DTX1 standards were purchased from a National Research Council Canada.
 [CRM-OA-c (Lot #20070328) CRM-DTX1 (Lot #20071024)]

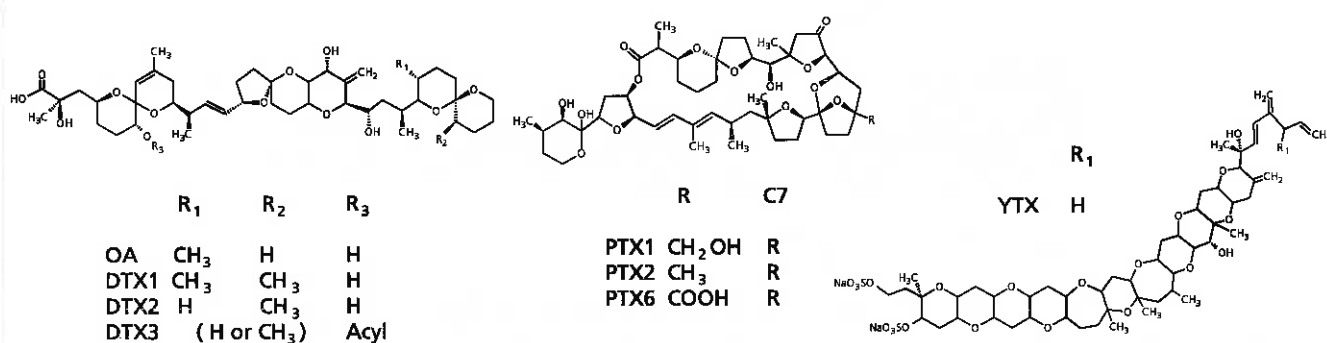


Figure 1. Structure of DSP toxins

High Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Toxins Using Liquid Chromatography Tandem Mass Spectrometry

LC/MS/MS analysis

Table 2 Analytical Conditions

HPLC : Nexera UHPLC system	
Column	: L-column2 ODS (50 mmL x 2.1 mm, 2 μm, CERI, Japan)
Mobile phase	: A - Aqueous solution of 2 mM ammonium formate with 50 mM formic acid B - Acetonitrile / Water : 95 / 5 (v/v) including 2 mM ammonium formate with 50 mM formic acid
Gradient program	: 30% B concentration (0 min) to 100% B concentration (5 to 10 min)
Flow rate	: 0.2 mL / min
Column temperature	: 20 °C
Injection volume	: 5 μL
MS : LCMS-8050 Triple quadrupole mass spectrometer	
Ionization	: ESI (Negative)
Ion spray voltage	: -3.0 kV
Heating Gas Flow	: 10 L/min
Nebulizing Gas Flow	: 2 L/min
Drying Gas Flow	: 10 L/min
IF Temp.	: 350 °C
DL Temp.	: 150 °C
HB Temp.	: 450 °C
MRM	: OA : m/z 803.5>255.2 , m/z 803.5>113.0 DTX1 : m/z 817.5>255.2 , m/z 817.5>113.0 Dwell time 200 msec / Pause time 3 msec



Triple Quadrupole LC/MS/MS [LCMS-8050]

Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) is Using Liquid Chromatography Tandem Mass Spectrometry

Result

Screening Analysis

- Figure 2(B) shows the MRM chromatograms of the sample1 acquired from the naturally contaminated scallops (Extract 1). Principle analytical condition is shown in Table 2 MRM transitions of PTX1,2,6 and YTX were in Figure 2. Six ingredients of sensitive screening analysis was successfully performed shown as Figure 2.

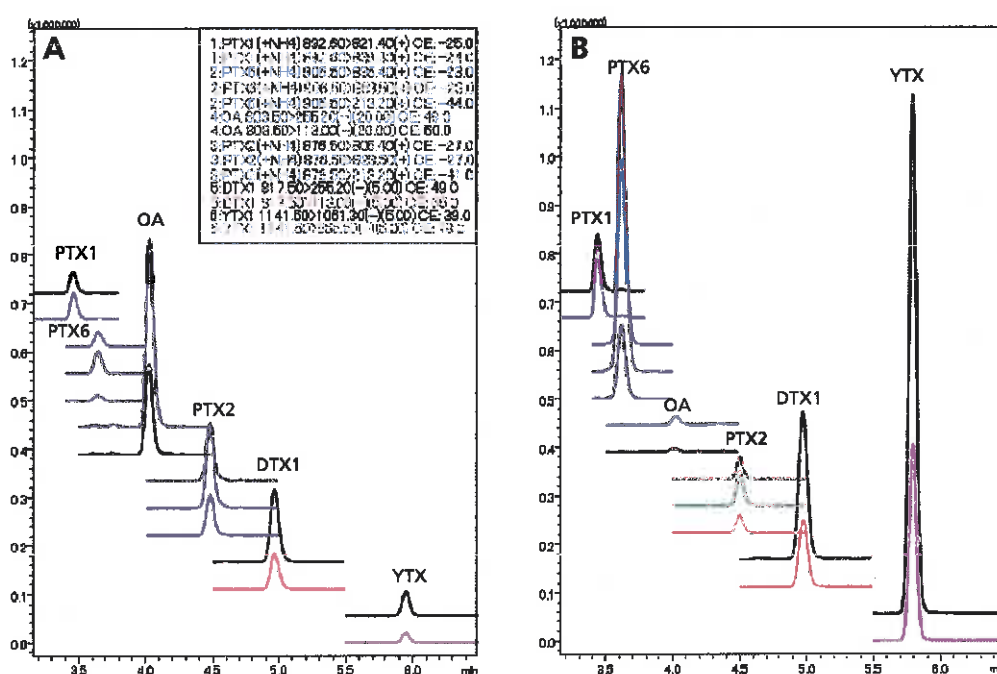


Figure 2. MRM chromatograms of DSP toxins
A) 10 ppb standard solution, B) The naturally contaminated midgut gland of scallops sample

MRM of OA and DTX1 standards

- Highly precise and sensitive DSP toxin analysis can be performed by LC/MS/MS.
- Linear calibration curves of both OA and DTX1 were established with a correlation coefficient (r^2) of 0.999 in the range 0.04 - 20 ppb.
- Repeatability of peak area of standard solutions were %RSD 4.2 (OA m/z 803.5>255.2) and 8.1 (DTX1 m/z 817.5>255.2) at 0.1 ppb (n=5).

High Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Toxins Using Liquid Chromatography Tandem Mass Spectrometry

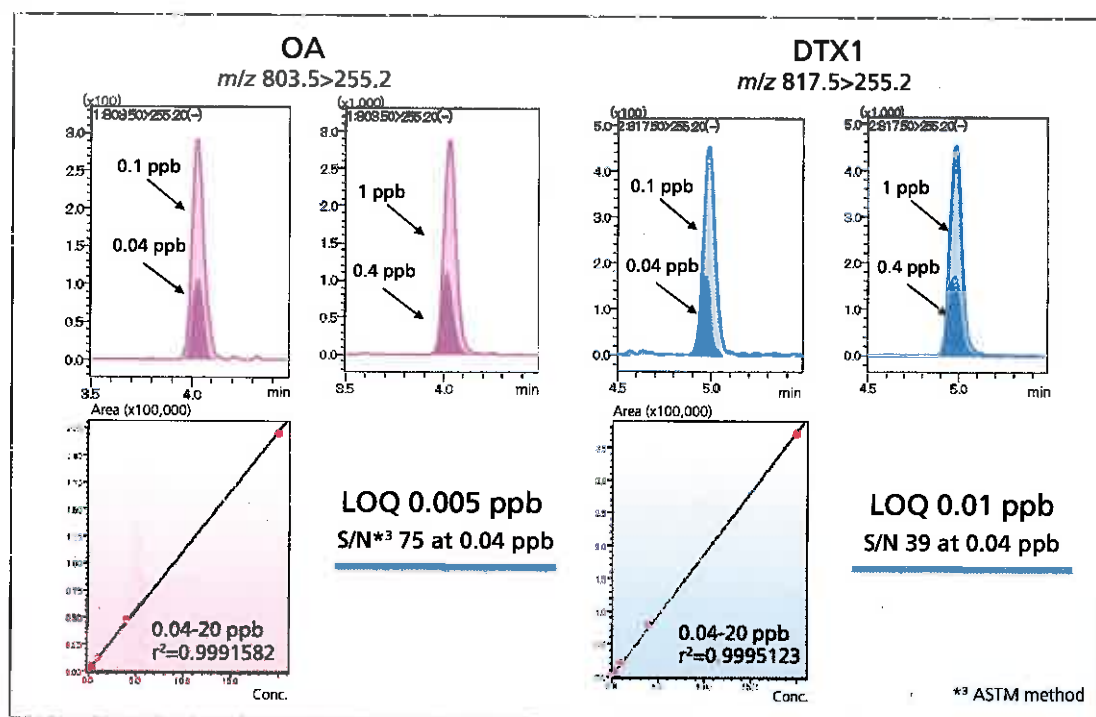


Figure 3. Calibration curves and MRM chromatograms of OA and DTX1

Recovery and Matrix Correction

- Convenient sample preparation is one of the advantage in this method.
- There is some consideration for a matrix effect.
- Correction due to the matrix effect should be estimated.
- QC parameter requires the response drift of 25% slope variation between the two sets of the calibration curve.
- In this study, the results of spiked 1 ppb of OA and DTX1 standard in the sample (2 and 3) are demonstrated with QC parameter of response drift within the 2 - 6%.
- According to the investigation, samples of 10- and 100-fold dilution can be recommended. Result of the recovery is shows in Figure 4.

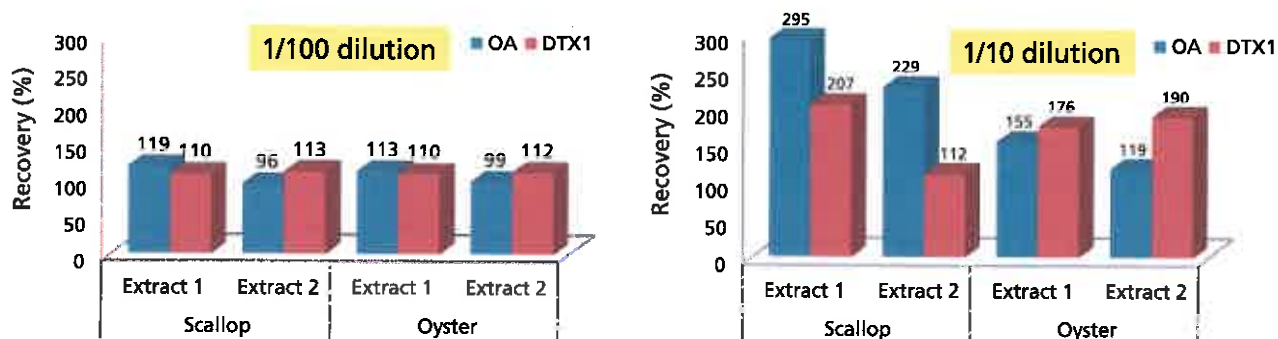


Figure 4. Recovery (1 ppb spiked)

Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Using Liquid Chromatography Tandem Mass Spectrometry

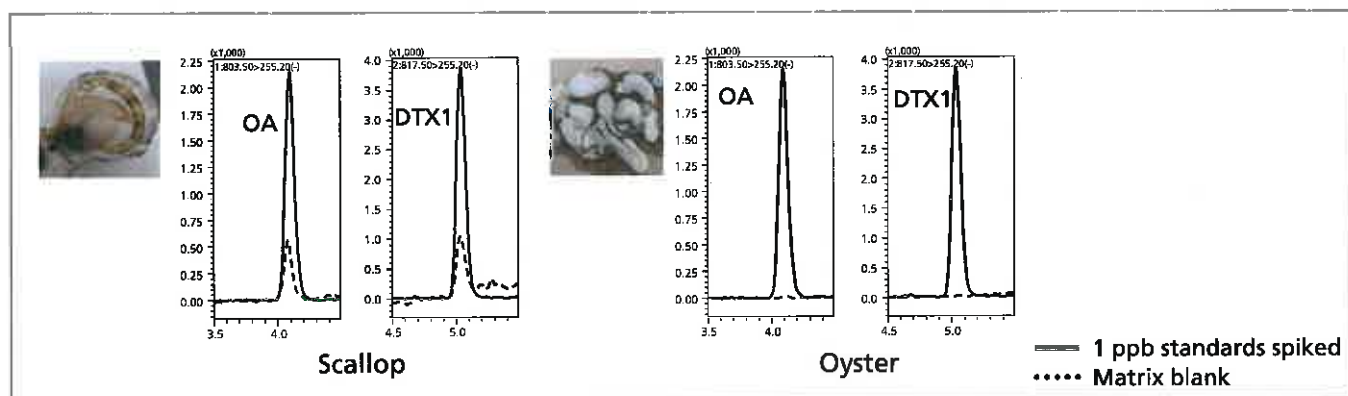


Figure 5. MRM chromatograms of 10 times dilution matrix sample (Extract 2)

Table 3 Quantity Results of Sample2 and Sample3 (μg/Kg)

Toxin	Sample 2 Scallop	Sample 3 Oyster
OA (ester)	17.4	N.D.
DTX1 (ester)	32.8	N.D.
DTX1 (free)	6.0	N.D.
	56.2 μg OA equivalent/Kg	

N.D.:Not Detected

Good scallop dish!!



Conclusion

- LC/MS/MS is a powerful methodology for an analysis of DSP toxins.
- LCMS-8050 has highly precise and sensitivity features.
- LCMS-8050 can be applicable for the SOP of EU-RL-MB.
- OA and DTX1 was detected in the midgut gland of scallop bought at the market, but it was lower enough than the regulated value in Japan (160 μg OA equivalent/Kg).
- Further study, we would like to investigate the matrix removal by solid phase extraction.

Reference

- EU-Harmonized Standard Operating procedure for determination of lipophilic marine biotoxins in molluscs by LC-MS/MS Ver.4
- Toshiyuki SUZUKI and Michael A. QUILLIAM, *Anal. Sci.* 27 (2011) 571-584

High Sensitivity Analysis of Diarrhetic Shellfish Poisoning (DSP) Toxins Using Liquid Chromatography Tandem Mass Spectrometry

Acknowledgements

- We are grateful to Dr. Toshiyuki Suzuki for providing the naturally contaminated scallops with toxins and toxins standard for this research.

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Determination of Oleanolic acid and Ursolic acid in loquat leaf extract By Liquid Chromatography-Tandem Mass Spectrometry

ASMS 2016 ThP 335

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Determination of Oleanolic acid and Ursolic acid in loquat leaf extract By Liquid Chromatography-Tandem Mass Spectrometry

Introduction

Ursolic acid (UA) and oleanolic acid (OA) belong to a class of triterpenic acid, which play significant bioactive roles in anti-inflammatory, antitumor as well as enhancing the cellular immune system. However, due to the lack of suitable chromophoric or ionization groups in molecular structures, both UV and MS responses are far from satisfaction. In this study, a highly sensitive method based on derivatization and LC-MS/MS has been developed.

After being extracted, a pair of stable isotope probes, 2-dimethylaminoethylamine (DMED) and d4-2-dimethylaminoethylamine (d4-DMED) were added for derivatization. The generated heavy labeled triterpenic acids were used as internal standards for quantification, the detection sensitivities of analytes improved sharply, the detection limits of OA and UA were 0.92 and 1.06 ng/L, respectively.

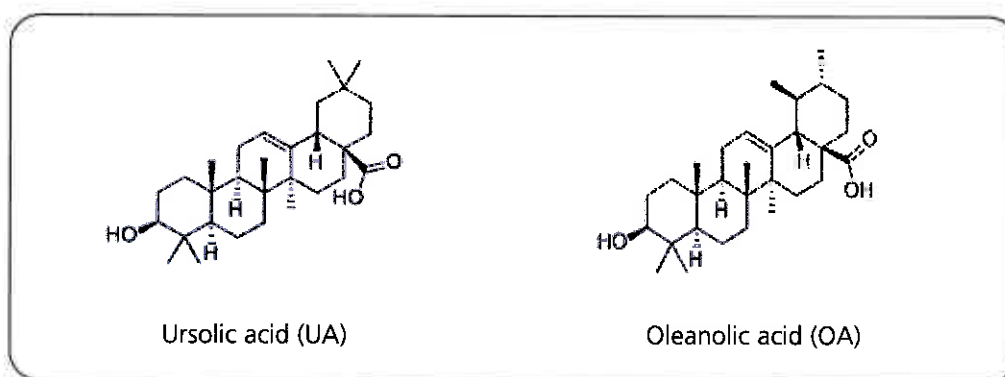


Figure 1 Structure of Ursolic acid (UA) and oleanolic acid (OA)

Methods

The derivatization of standard solution

Take 200 μ L of working standard solution, after adding 10 μ L 20 μ mol/mL of triethylamine (TEA) and 10 μ L 20 μ mol/mL of 2-chloro-1-methylpyridinium iodide (CMPI), the solution was vortexed for 5 min. Then 10 μ L 40 μ mol/mL of DMED was added, and the mixture was vibrated at

1500 rpm for 1 h at 40 $^{\circ}$ C. The sample was evaporated to dryness under a mild nitrogen steam at room temperature. The residue was dissolved in 100 μ L of mobile phase, and then analyzed by LC-MS/MS.

Preparation of Samples

0.5 g of sample were added into 50 mL volumetric flask, and then diluted with saturated salt water to the required volume. 1 mL above solution was withdrawn and added 200 μ L of ethyl acetate. After being vortexed and

centrifuged, and the resulting supernatant was used for derivatization. The procedure of derivatization was the same as that of standard solution.

Preparation of Internal Standard

The derivatization process of internal standard was almost the same as that of standard solution except that the DMED was replaced with d4-DMED. The evaporated residue was dissolved in 1 mL of mobile phase, took 10 μ L of that to each standard solution and real samples, and then used for analysis.

Quantification of Oleanolic acid and Ursolic acid in loquat leaf by Liquid Chromatography-Tandem Mass Spectrometry



High Speed Mass Spectrometer

Ultra Fast Polarity Switching

- 5 msec

Ultra Fast MRM

- Max. 555 transition /sec

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

The LC-MS/MS system were Prominence LC-20A and triple quadrupole mass spectrometry (Shimadzu Corporation, Kyoto, Japan). Shimadzu LC-20A system consists of a CBM-20A system controller, two LC-20AD pumps, a SIL-20AC autosampler, a CTO-20AC column

oven, and a DGU-20A3 online degasser. MS/MS detection was performed by LCMS-8050. Data acquisition and processing were performed with Labsolution software Version 5.72. Electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

Result

Method development for UA and OA

HPLC conditions	
Column	: InertSustain C18 Column (2.1 mm I.D.x250 mm L., 3 µm)
Mobile phase A	: 0.1% formic acid aqueous solution B: Acetonitrile A:B=47:53 (v/v)
Flow rate	: 0.25 mL/min
Injection vol.	: 10 µL
Column temperature	: 35 °C
MS conditions (LCMS-8050)	
Ionization	: ESI, Positive MRM mode
Nebulizer Flow	: 2.0 L/min
Heating Gas Flow	: 10.0 L/min
Interface Temperature	: 300 °C
DL Temperature	: 250 °C
Heat block Temperature	: 400 °C
Dry Gas	: 10.0 L/min

Determination of Oleanolic acid and Ursolic acid in loquat leaf extract By Liquid Chromatography-Tandem Mass Spectrometry

Table 1. MRM transition

Compound	MRM transition	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
Oleanolic acid	527.30>482.30*	-20	-34	-23
	527.30>203.20	-20	-41	-20
Ursolic acid	527.30>482.30*	-20	-34	-23
	527.30>203.20	-20	-41	-20
d4-Oleanolic acid	531.30>482.30	-20	-34	-23
d4-Ursolic acid	531.30>482.30	-20	-34	-23

OA and UA are pentacyclic triterpenoids, the structural difference is merely a methyl group at C-20 on the OA shift to C-19 position. It is very difficult to separate these two compounds that belong to the geometric isomers. In this study, 3 μm of InertSustain C18 column was used, the resolution is 1.110, which is better than the results

reported in the literature. Figure 3 shows MRM chromatograms of 0.1 $\mu\text{g/mL}$ standard solution of UA and OA(a), as well as 0.1 $\mu\text{g/mL}$ standard solution of d4-UA and d4-OA(b). It took 12 minutes per one LC-MS/MS analysis, and excellent separation and high sensitive detection were obtained.

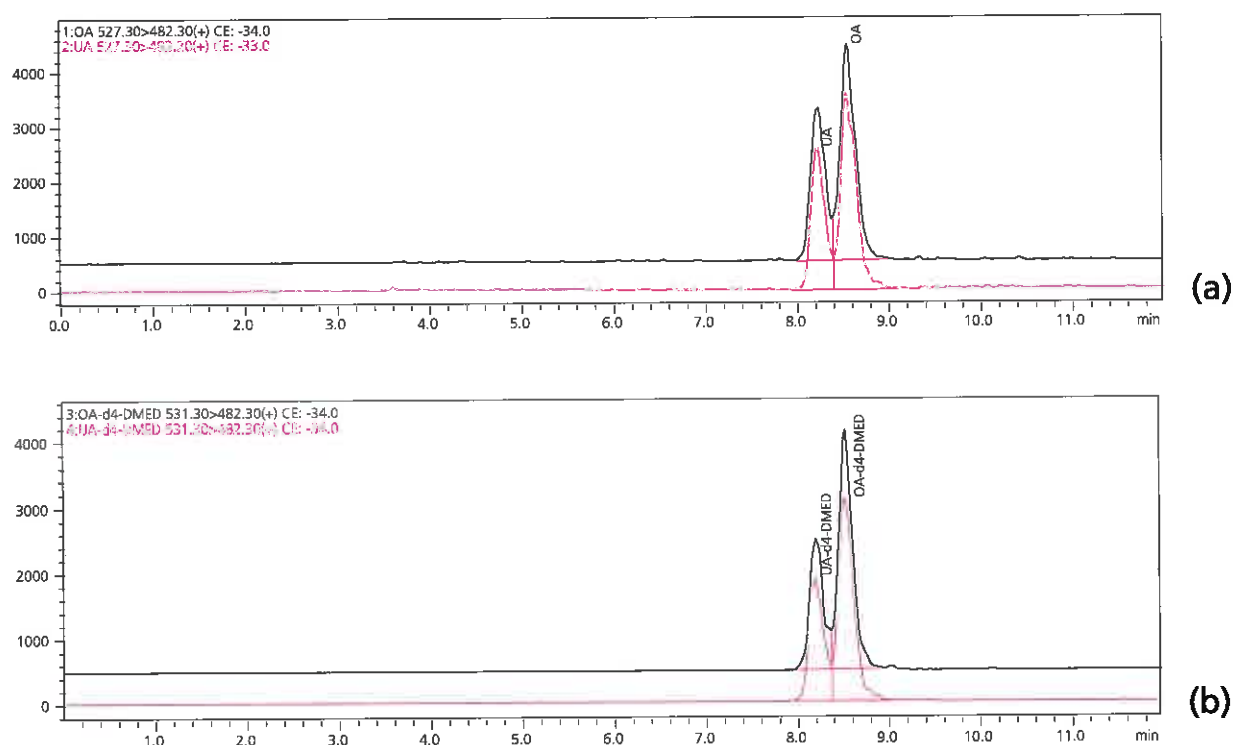


Figure 3 MRM chromatograms of standard solution of UA and OA(a), as well as internal standard solution (Concentration of each compound were 0.1 $\mu\text{g/mL}$)

Determination of Oleanolic acid and Ursolic acid in loquat leaf extract By Liquid Chromatography-Tandem Mass Spectrometry

Analytical Performance

Linearity

The determination of UA and OA was verified using an internal standard for quantification. The internal calibration was performed by plotting peak area ratios versus concentration ratios of DMED derivatives and d4-DMED derivatives (As seen in Figure 4). The sample

solutions were spiked with stock solution to get final concentrations of UA and OA at 0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 ng/mL.

The detailed calibration curves, ranges, correlation coefficients and precisions were shown in Table 2.

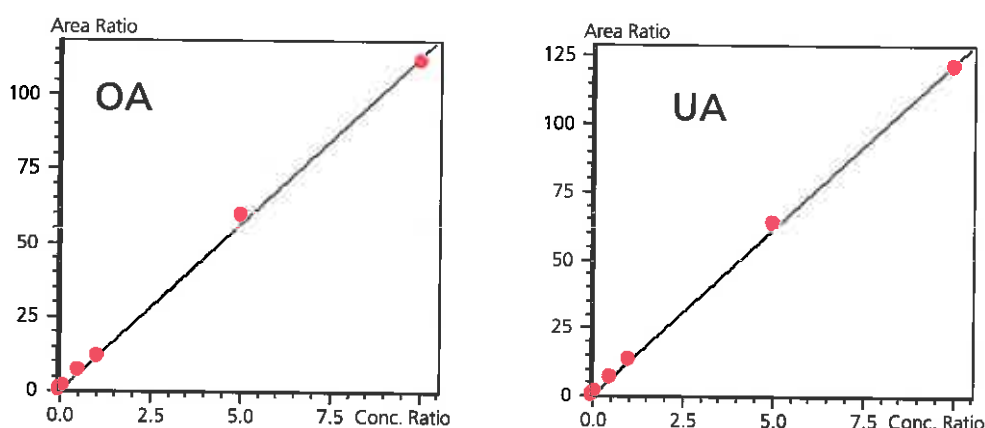


Figure 4 Calibration curve of OA and UA

Table 2. Parameters of Calibration Curves

Compound	Calibration Curves	Range (ng/mL)	Coefficient (r ²)	Precision (%)
OA	$Y=(11.230) X + (0.480)$	0.01~10.0	0.9995	81.7~117.8
UA	$Y=(12.197) X + (0.467)$	0.01~10.0	0.9998	94.6~119.7

Sensitivity

Detection and quantification limits were calculated as the concentration corresponding to a signal 3 and 10 times of the baseline noise, and the limit of detection oleanolic acid and ursolic acid were 0.92 and 1.06 ng/mL, the quantification limits were 3.07 and 3.53 ng/L, respectively.

Recovery

Preparation of blank loquat leaf extract samples as well as blank loquat leaf extract samples spiked at 100 mg/kg. According to the mentioned method before, each sample was measured three times in parallel. The recovery is

calculated by subtracting the content of OA and UA in blank samples. The results showed that the recoveries of OA and UA were in the range of 98.7~102.7 % and 97.2~105.0 %, respectively.

Determination of Oleanolic acid and Ursolic acid in loquat leaf extract By Liquid Chromatography-Tandem Mass Spectrometry

Quantitative Analysis of real sample

According to the sample preparation method, loquat leaf extracts from market were analyzed. The sample was determined parallel twice. The content of oleanolic acid and ursolic acid in this sample were 7.40 and 23.6 mg/Kg, respectively. The results were shown in Figure 5.

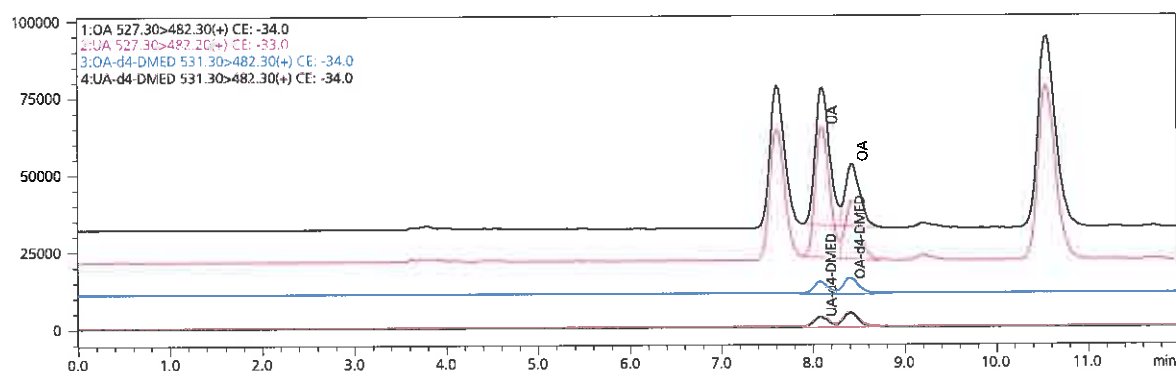


Figure 5 MRM chromatograms of real samples

Conclusions

In this paper, a highly sensitive method based on derivatization of oleanolic acid and ursolic acid coupled with LC-MS/MS detection was established. This method has good linearity, with correlation coefficient greater than 0.999, the limit of detection were

0.92 and 1.06 ng/mL, the quantification limits were 3.07 and 3.53 ng/L, respectively. The recoveries were between 97.2~105.0 %. This method is simple, rapid, accurate and can be applied to detect OA and UA in loquat leaf extract.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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Determination of Wilfordine and Wilforine in honey using Liquid Chromatography with Tandem Mass Spectrometry

ASMS 2016 ThP 110

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Determination of Wilfordine and Wilforine in honey using Liquid Chromatography with Tandem Mass Spectrometry

Introduction

Tripterygium wilfordii, which contains a lot of biological toxic compounds such as Wilfordine and Wilforine, is one of the toxic nectar plants. The Wilfordine and Wilforine may be transferred to honey by honey bees. Due to the low content and complex matrix, determination of Wilfordine and Wilforine in honey is not easy. In this

study, a highly sensitive method based on liquid-liquid extraction (LLE) and LC-MS/MS has been developed. The results showed that the detection limits of Wilfordine and Wilforine in honey sample were 5.16 and 10.80 ng/kg, respectively.

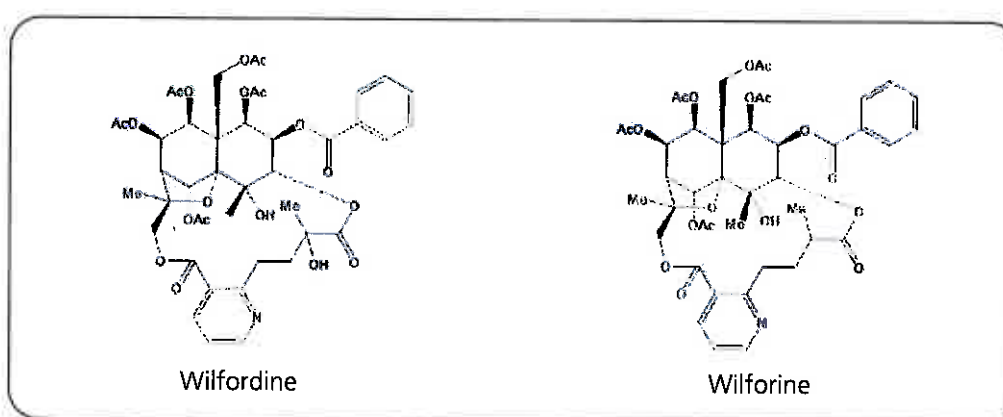


Figure 1 Structure of Wilfordine and Wilforine

Methods

Preparation of Samples

1.0 g of honey sample was added into 10 mL centrifuge tube, and then diluted with 2 mL of pure water. After adding 2 mL of acetonitrile, 0.3 g of NaCl, and 1.2 g of MgSO₄ in order, the mixture was vortexed for 2 min and

centrifuged at 8000 rpm for 5 minutes. The above solution was withdrawn and filtered (Organic membrane, 0.22 µm) for detection.

Instruments

The LC-MS/MS system were Prominence LC-20A and triple quadrupole mass spectrometry (Shimadzu Corporation, Kyoto, Japan). Shimadzu LC-20A system consist of a CBM-20A system controller, two LC-20AD pumps, a SIL-20AC autosampler, a CTO-20AC column

oven, and a DGU-20A3 online degasser. MS/MS detection was performed by LCMS-8050. Data acquisition and processing were performed with Labsolution software Version 5.72. Electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

Simultaneous determination of Wilfordine and Wilforine in honey using HPLC and Chromatography with Tandem Mass Spectrometry



High Speed Mass Spectrometer

Ultra Fast Polarity Switching

- 5 msec

Ultra Fast MRM

- Max. 555 transition /sec

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

Result

Method development for Wilforine and Wilfordine

HPLC conditions

Column	: InertSustain C8-3 Column (2.1 mm I.D.×150 mm L., 5 µm)
Mobile phase A	: 0.1% formic acid aqueous solution
B	: Acetonitrile
Elution Mode	: Gradient Elute, the initial concentration of MP B was 30%,

Table 1. LC Time Programme

Time	Module	Command	Value
1.00	Pumps	Pump B Conc.	30
4.00	Pumps	Pump B Conc.	90
5.00	Pumps	Pump B Conc.	90
5.10	Pumps	Pump B Conc.	30
10.00	Controller	Stop	

Injection vol.	: 10 µL
Column temperature	: 35 °C

MS conditions (LCMS-8050)

Ionization	: ESI, Positive MRM mode
Nebulizer Flow	: 3.0 L/min
Heating Gas Flow	: 8.0 L/min
Interface Temperature	: 400 °C
DL Temperature	: 150 °C
Heat block Temperature	: 300 °C
Dry Gas	: 12.0 L/min

Determination of Wilfordine and Wilforine in honey using Liquid Chromatography with Tandem Mass Spectrometry

Table 2. MRM transition

Compound	MRM transition	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
Wilfordine	884.30>856.20*	-12	-25	-30
	884.30>176.10	-12	-50	-18
Wilforine	868.30>178.10*	-12	-60	-18
	868.30>206.10	-12	-43	-20

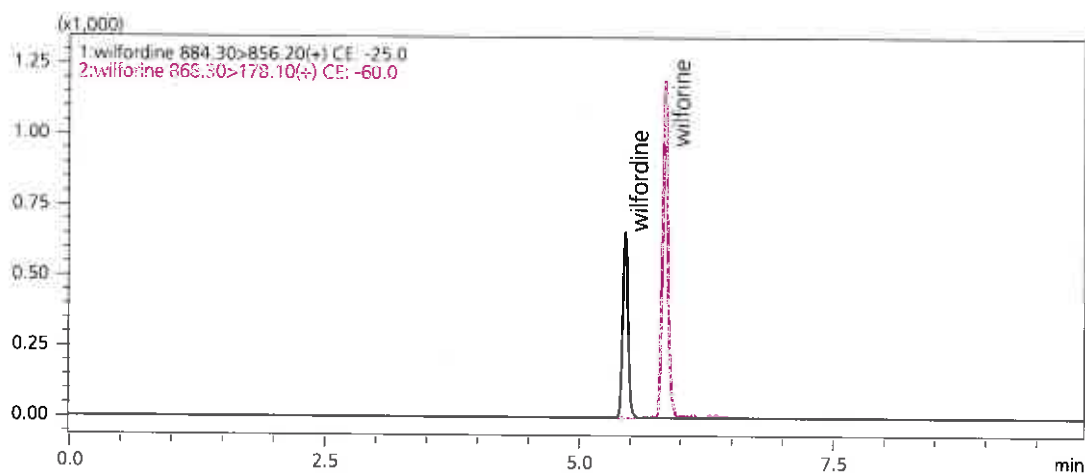


Figure 3 MRM chromatograms of standard solution of Wilforine and Wilfordine (Concentration of each compound were 0.05 ng/mL)

Analytical Performance

Linearity

The determination of Wilfordine and Wilforine were verified using an external standard method. The external calibration was performed by plotting peak area versus concentration of Wilfordine and Wilforine (As seen in Figure 4). The sample solutions were spiked with stock

solution to get final concentrations of Wilfordine and Wilforine at 0.01, 0.02, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 ng/mL.

The detailed calibration curves, ranges, correlation coefficients and precisions were shown in Table 2.

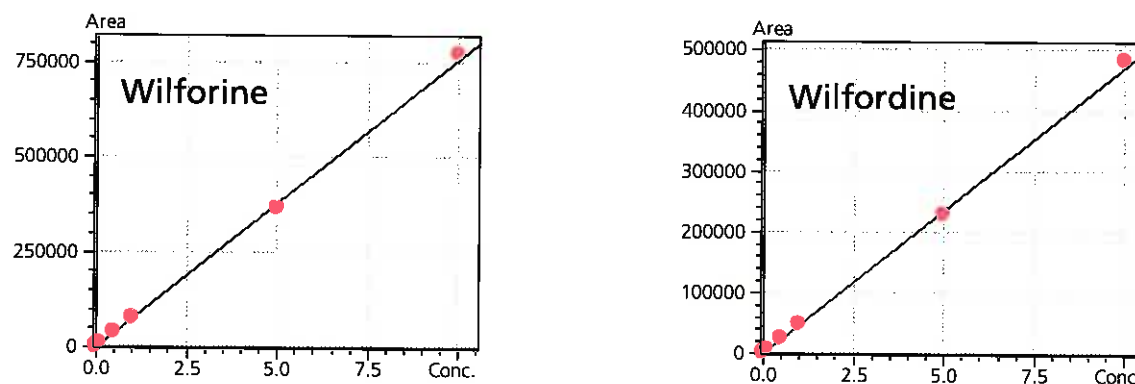


Figure 4 Calibration curve of Wilfordine and Wilforine

Determination of Wilfordine and Wilforine in honey using Liquid Chromatography with Tandem Mass Spectrometry

Table 3. Parameters of Calibration Curves

Compound	Calibration Curves	Range (ng/mL)	Coefficient (r^2)	Precision (%)
Wilforine	$Y=(75959.6) X -45.2$	0.01~10.0	0.9996	92.1~113.8
Wilfordine	$Y=(47426.1) X+ 206.6$	0.01~10.0	0.9997	87.7~108.3

Sensitivity

Detection and quantification limits were calculated as the concentration corresponding to a signal 3 and 10 times of the baseline noise, and the detection limits of Wilforine and Wilfordine were 1.3 and 4.3 ng/L, the quantification limits were 2.7 and 9.0 ng/L, respectively.

Recovery

Preparation of blank honey samples as well as blank honey samples spiked at 0.05 ng/g and 5.0 ng/g. According to the mentioned method before, each sample was measured three times in parallel. The recovery is calculated by subtracting the content of Wilfordine and Wilforine in blank honey samples. The recovery results were shown in table 4.

Table 4. Recovery results

No.	Compound	Spiked at 0.05 ng/g (%)	Spiked at 5.0 ng/g (%)
1	Wilfordine	104.0	99.6
2	Wilforine	116.0	98.8

Conclusions

In this paper, a fast and effective method for the sensitive and reliable analysis of Wilfordine and Wilforine using LC-MS/MS was established. The method has good linearity, with correlation coefficient greater than 0.999,

the limit of detection were 1.3 and 4.3 ng/mL, the quantification limits were 2.7 and 9.0 ng/L, respectively. The recoveries were between 98.8~116.0%.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

Multi-residue analysis of pesticides in crude food extracts using a simple extraction technique and LC/MS/MS

ASMS 2015 WP 077

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Multi-residue analysis of pesticides in crude food extracts using a simple extraction technique and LC/MS/MS

Introduction

Analysis of pesticide residues in food is typically tedious and time-consuming due to the necessary extraction and clean up procedures. Furthermore, to deal with the ever-growing number of pesticides, the food safety laboratories need to ideally screen as many compounds as possible in a single run which may reach maximum residual limits (MRL); typically 10 ppb in food matrices.

In this study, we illustrate the results utilizing an UF MRM capability (just 5 msec. MRM measurement includes dwell and pause time) with 5 msec. polarity switching (UF switching) for the analysis of 146 pesticides in crude food extracts by using easy and simple sample preparation technique.

Methods & Materials

Sample preparation

Food samples were purchased from a local grocery store in Japan. Each sample, with dry ice, was finely ground by milling until it became a powder and then extracted with acetonitrile. After filtration, the sample extracts were

directly injected 1 μ L to LC/MS/MS. This sample preparation technique is much easier and simpler than QuEChERS.

Sample	Origin
Soybeans	Japan
Brown rice	Japan
Spinach	Japan
Cucumber	Japan

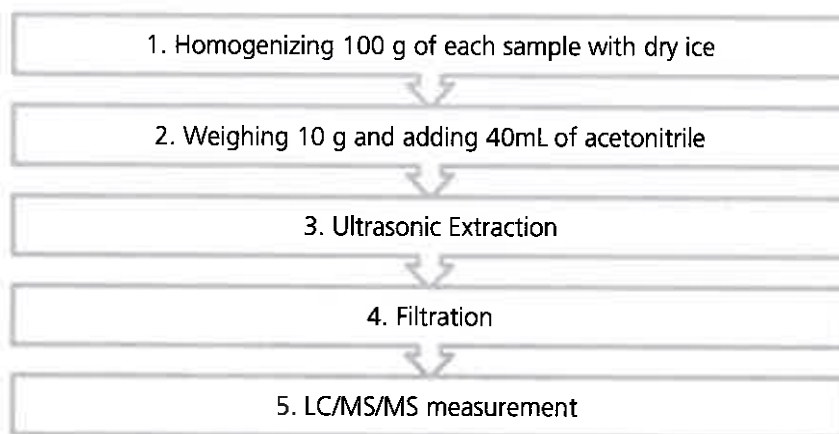


Figure 1 Protocol of sample preparation

Residue analysis of pesticides in crude food extracts using a simple extraction technique and LC/MS/MS



High Speed Mass Spectrometer
Ultra Fast Scanning
- 30,000 u / sec.
Ultra Fast Polarity Switching
- 5 msec.
Ultra Fast MRM
- Max. 555 transitions /sec

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

LC/MS/MS analysis

HPLC conditions (Nexera UHPLC system)	
Column	Shim-pack XR-ODSII (75 mm x 2 mm I.D., 2.2 μ m)
Mobile phase	A – 5 mM ammonium acetate - water B – 5 mM ammonium acetate - methanol
Gradient program	10% B (0min) → 40% (1-2min.) → 95% (10-15min.) → 10% (15.01-20min.)
Flow rate	0.2 mL / min.
Column temperature	40 °C
MS conditions (LCMS-8050)	
Ionization	ESI (Positive / Negative)
MRM	Max MRMs simultaneously monitored: 72ch. (36 event) Max loop time: 0.442 sec Dwell time 5 msec. / Pause time 1 msec.

Result

Pesticide standards

146 compounds were analyzed in a single run by just 5 msec. MRM event with 5 msec. polarity switching. All studied compounds have shown excellent LOQs and linearity of calibration curves ranging from 0.1-100 μ g/L.

Multi-residue analysis of pesticides in crude food extracts using a simple extraction technique and LC/MS/MS

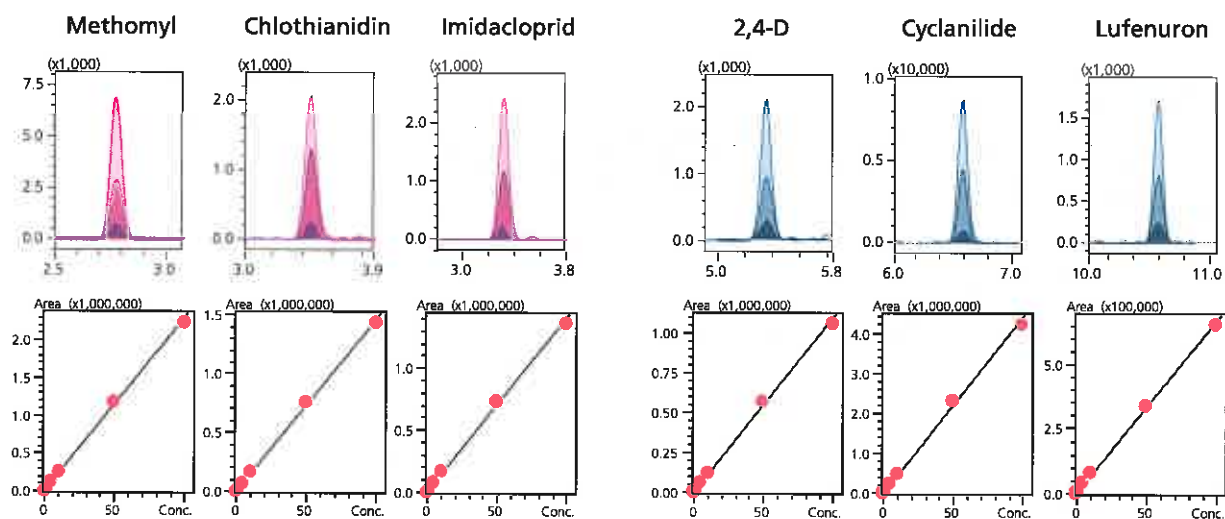


Figure 3 MRM chromatograms and calibration curves of typical pesticides (left: positive / right : negative)

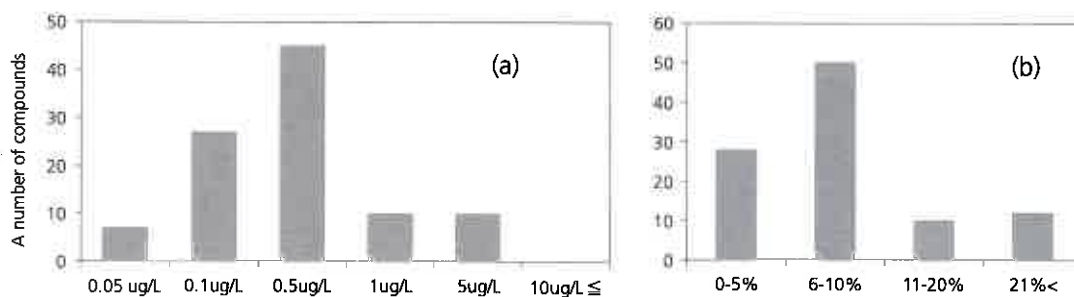


Figure 4 (a) : LOQs of all tested compounds
(b) : CV(%) of all tested compounds at 1 µg/L (n=5)

Matrix effect

The matrix samples spiked with 10 µg/L standards were prepared for the recovery test in all matrices. More than 80% of target compounds have shown good recoveries ranging from 70-120% in all matrices, neither ion suppression nor enhancement was observed.

Residue analysis of pesticides in crude food extracts by a simple extraction technique and LC/MS/MS

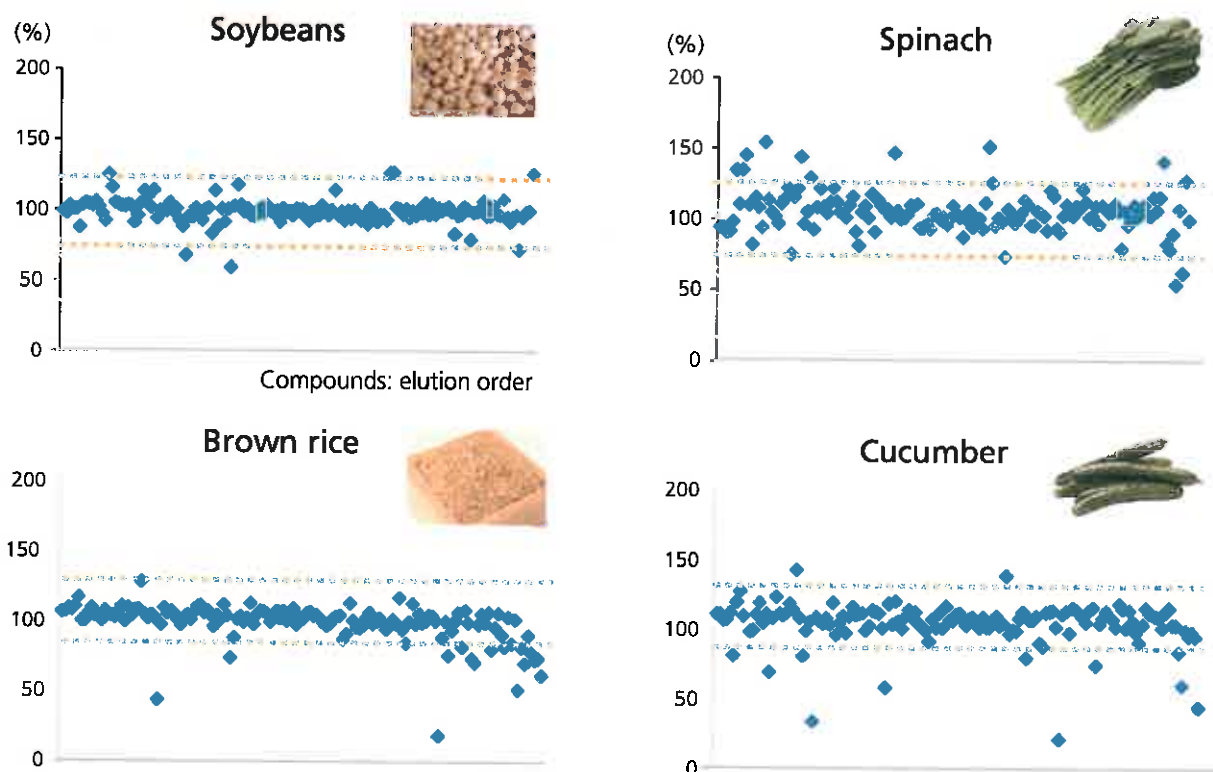


Figure 5 Recoveries of all target compounds in each matrix

Extraction efficiency

Extraction efficiency was determined from ratios of peak area of pre-extraction / post-extraction spiked sample (10 µg/kg). Almost all compounds have shown the good recoveries in high water content foods such as spinach and cucumber.

However, in low water content foods like brown rice and

soybeans, especially, high polar compounds showed poor recovery. To improve this, 9 mL of water is added to the homogenized sample before acetonitrile extraction. As a result, recoveries of these compounds were dramatically improved.

Foods	Water content (%)
Soybeans	13
Brown rice	15
Spinach	92
Cucumber	95

Multi-residue analysis of pesticides in crude food extracts using a simple extraction technique and LC/MS/MS

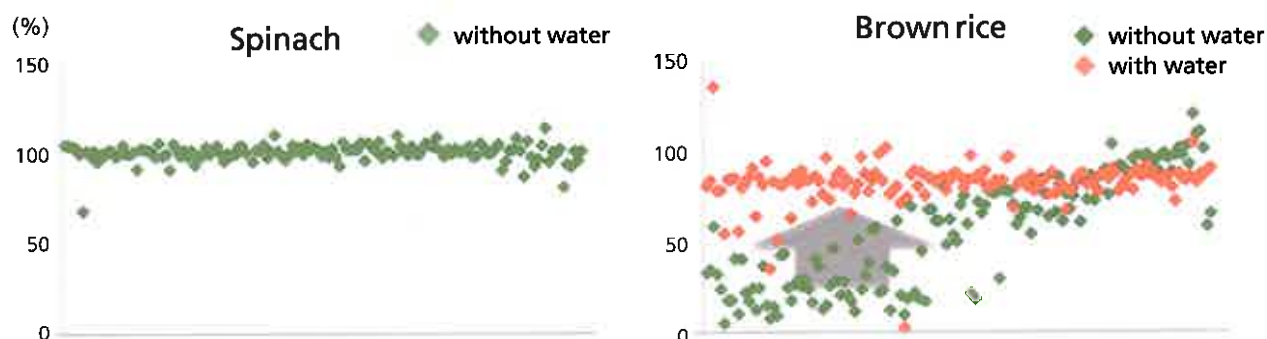


Figure 6 Recoveries in each matrix

Comparison between LCMS-8050 & LCMS-8060

■ Sensitivity

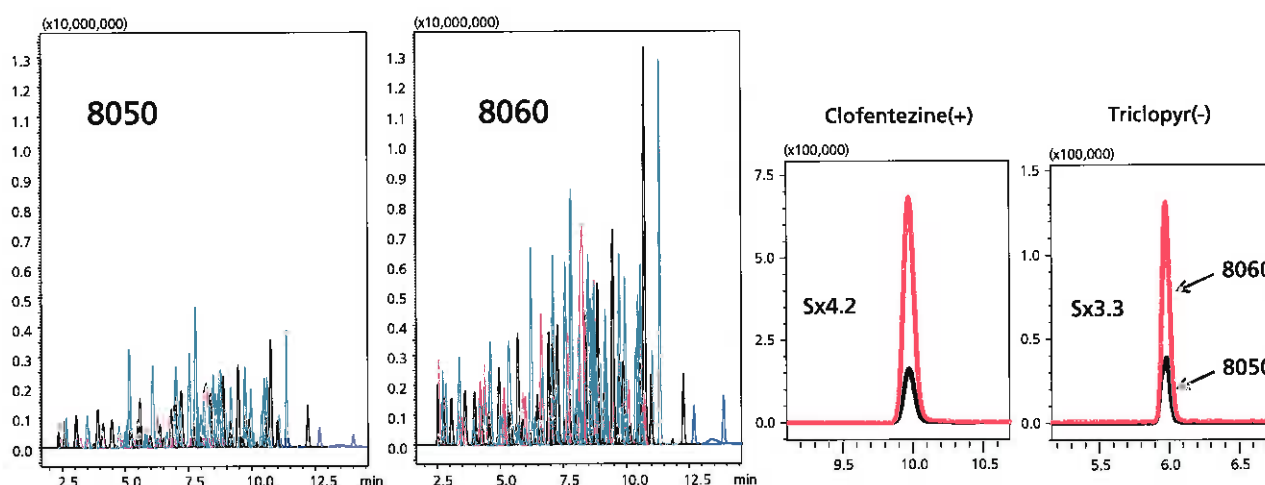


Figure 7 MRM chromatograms in LCMS-8050 and LCMS-8060

The sensitivity of pesticides was compared between LCMS-8050 and 8060 under the same analytical conditions. In LCMS-8060, signal response was improved average about 3 times higher than LCMS-8050, and

lower LOQs were achieved. The increased sensitivity of the LCMS-8060 enables the accurate quantitation below MRLs even in high degree of dilution in the matrix.

Residue analysis of pesticides in crude food extracts using a simple extraction technique and LC/MS/MS

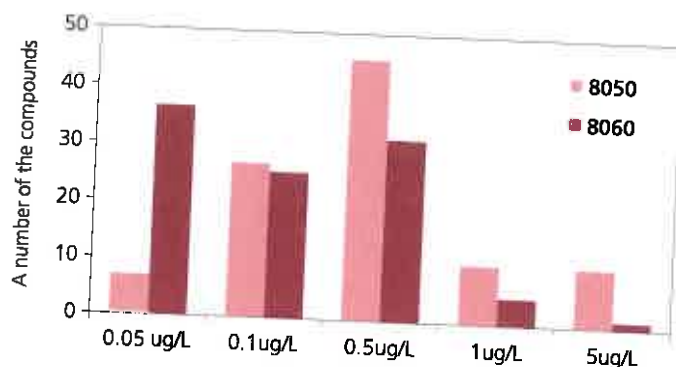


Figure 8 LOQs of the compounds

Matrix effect

The matrix effect was also compared between LCMS-8050 and 8060. As a result, there is no difference of the recoveries between two instruments.

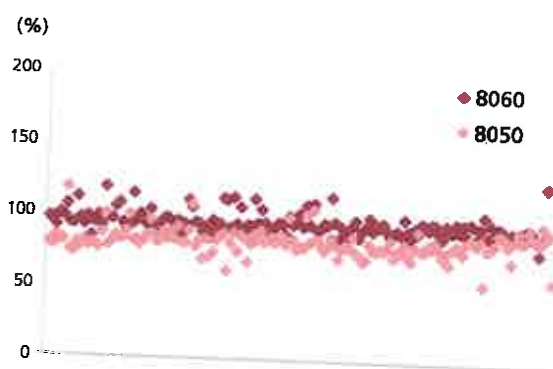


Figure 9 Recoveries of pesticides in soybeans



Figure 10 LCMS-8060

Conclusions

- This method is able to be applied to the quantification of pesticides in complex food matrices.
- The increased sensitivity of the LCMS-8060 enables the accurate quantitation below MRLs even in high degree of dilution.

Comprehensive analysis of primary & secondary metabolites in citrus using an automated method changeover UHPLC system coupled to LC/MS/MS

ASMS 2015 · MP 397

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Comprehensive analysis of primary & secondary metabolites in citrus using an automated method changeover UHPLC system coupled to LC/MS/MS

Introduction

There is a demand for better tasting, healthier and safer food-stuffs which better meet the needs of both industry and the consumer. New technology has been required to monitor and improve the quality of food, metabolomics has become an important tool for food processing, plant breeding and so on. In-food science, comprehensive analysis of primary metabolites through to secondary

metabolites is very important. However, there is no application which can analyze them at the same time. In this study, we developed the analytical methods using LC/MS/MS for monitoring the primary and secondary metabolites in foods, focus on the major compound's categories such as organic acids, amino acids, sugars, carotenoids and flavonoids.

Methods & Materials

Sample preparation

7 varieties of citrus fruits (Mikan, Ponkan, Shiranui, Amakusa, Hassaku, Buntan, Hyuganatsu) in citrus genus were selected and purchased from local grocery store in Kyoto, Japan.

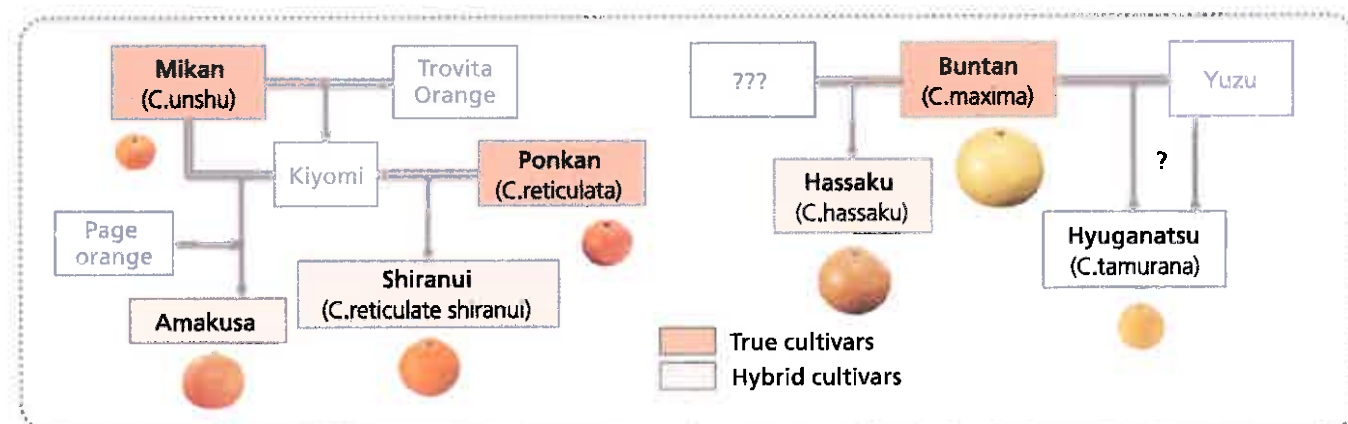


Figure 1 The breed trees of tested citrus fruits (the cultivars with grey were not surveyed in this study)

Comprehensive analysis of primary & secondary metabolites in citrus using an automated method changeover UHPLC system coupled to LC/MS/MS

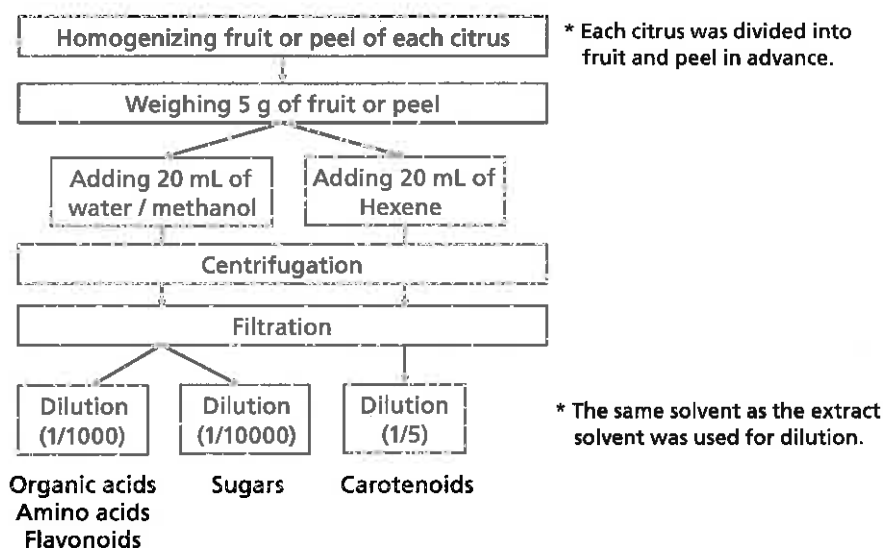


Figure 2 Protocol of sample preparation

LC/MS/MS analysis

We developed 3 analytical conditions for separation of target compounds. These 3 analytical conditions, comprising combinations of 4 mobile phases and 3 columns, were automatically performed using UHPLC

multi-method system in 40 minutes. This system enables multiple separations based on the combination of up to 8 different mobile phases and 6 different columns.

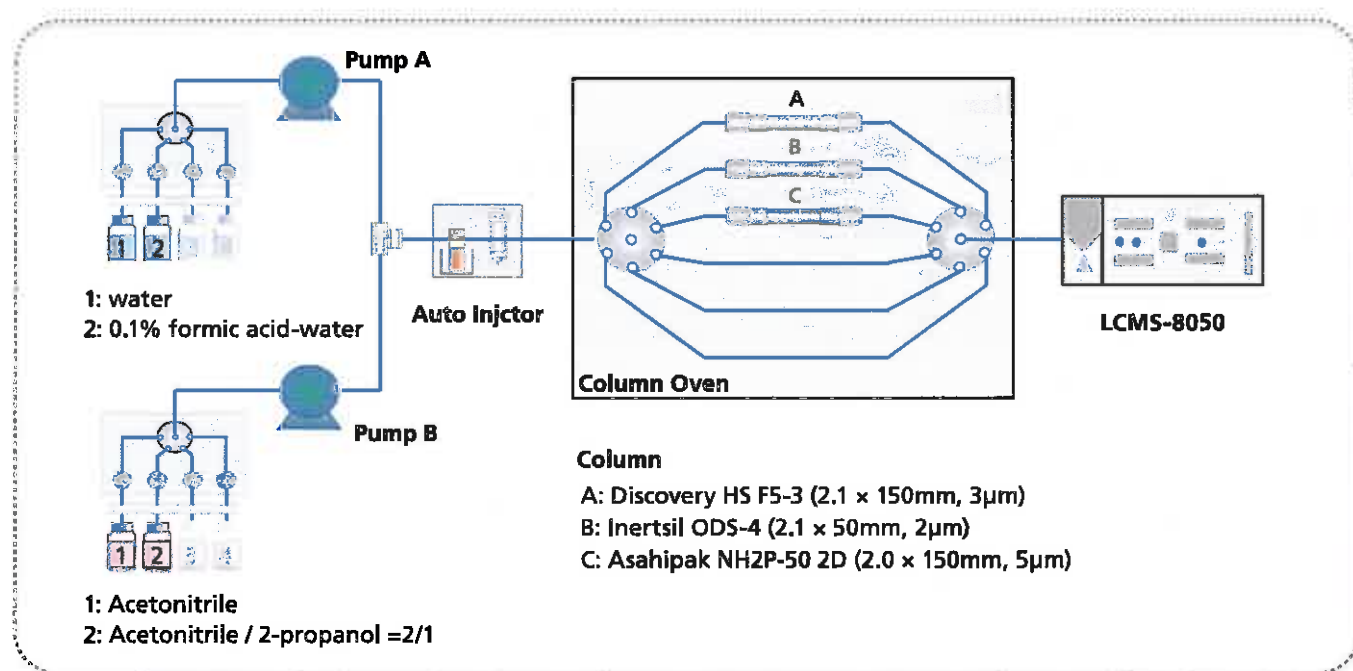
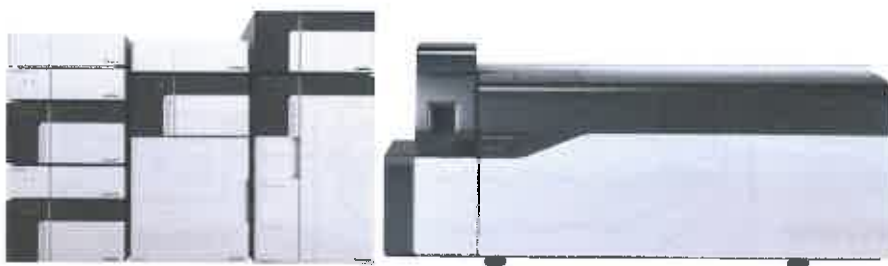


Figure 3 System configuration

Comprehensive analysis of primary & secondary metabolites in citrus using an automated method changeover UHPLC system coupled to LC/MS/MS

Table 1 Analytical conditions

Conditions		Condition 1	Condition 2	Condition 3
HPLC	Instrument	UHPLC Nexera system (Shimadzu)		
	Target compounds	Organic acids Amino acids Flavonoids	Carotenoids	Sugars
	Column	A: Discovery HS F5-3 (2.1×150mm, 3µm) Sigma-aldrich	B: Inertsil ODS-4 (2.1×50mm, 2µm) GL-science	C: Asahipak NH2P-50 2D (2.0×150mm, 5µm) Shodex
	Column oven temp.	40°C		
	Mobile phase A	2: 0.1% formic acid-water	1: water	1: water
	Mobile phase B	1: Acetonitrile	2: Acetonitrile / 2-propanol =2/1	1: Acetonitrile
	Flow rate	0.25 mL/min.	0.4 mL/min.	0.4 mL/min.
	Time program	0%B (0-2min.) → 95% (10-13min.) → 0% (13.01-16min.)	60%B (0min.) → 100% (5-8min.) → 60% (8.01-10min.)	65%B (0-8min.) → 30% (8-11min.) → 65% (11.01-15min.)
	Measurement time	16 min.	10 min.	15 min.
	Total run time	41 min.		
	Injection volume	2 µL		
MS	Instrument	LCMS-8050		
	Ionization	ESI (+ / -)		
	Mode	MRM		



High Speed Mass Spectrometer
Ultra Fast Scanning
- 30,000 u / sec.
Ultra Fast Polarity Switching
- 5 msec.
Ultra Fast MRM
- Max. 555 transitions /sec

Figure 4 LCMS-8050 triple quadrupole mass spectrometer

Comprehensive analysis of primary & secondary metabolites in *Trichoderma reesei* using an automated method changeover UHPLC system coupled to LC/MS/MS

Result

Standards of primary & secondary metabolites

All tested compounds (10 organic acids, 24 amino acids, 5 sugars, 5 flavonoids and 9 carotenoids) were successfully separated using 3 analytical conditions (Fig. 5). Calibration range and LOQs of all compounds were showed in Table 2.

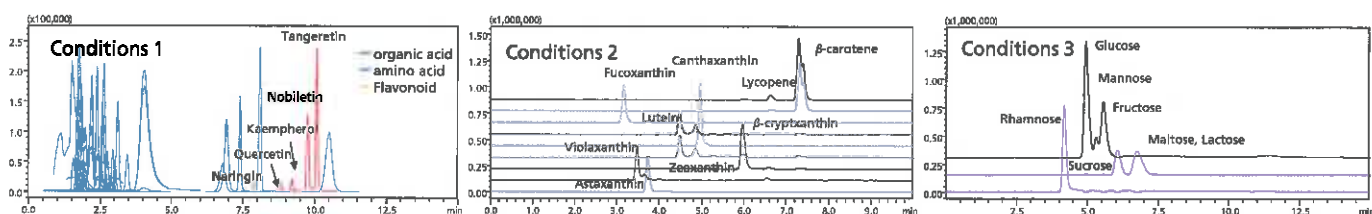


Figure 5 MRM chromatograms in each analytical conditions

Table 2 Calibration range of tested compounds

Amino acids	CAL of range
Cystine	1-100
Aspartic acid	5-100
Asparagine	5-100
Serine	5-100
4-Hydroxyproline	1-100
Glycine	5-100
Lysine	1-100
Cysteine	50-100
Threonine	5-100
Glutamic acid	1-100
Alanine	5-100
Proline	1-100
Ornithine	5-1000
Glutamine	5-100
Histidine	5-100
Arginine	5-100
GABA	5-100
Valine	1-100
Methionine	5-100
Tyrosine	5-100
Isoleucine	5-100
Leucine	10-100
Phenylalanine	1-100
Tryptophan	5-500

($\mu\text{g/L}$)

Organic acids	CAL of range
Tartaric acid	50-10000
2-Ketoglutaric acid	10-1000
Isocitric acid	50-10000
Malic acid	10-5000
Lactic acid	50-10000
Citric acid	50-10000
Pyroglutamic acid	10-10000
Succinic acid	10-1000
Fumaric acid	500-1000
Maleic acid	50-10000

($\mu\text{g/L}$)

Carotenoids	CAL of range
Fucoxanthin	0.1-100
Violaxanthin	1-100
Astaxanthin	0.5-100
Lutein	0.1-100
Zeaxanthin	0.5-100
Canthaxanthin	0.05-100
β -Cryptoxanthin	0.05-100
Lycopene	50-100
β -carotene	0.1-100

($\mu\text{g/L}$)

Sugars	CAL of range
Rhamnose	50-1000
Fluctose	50-5000
Glucose	50-5000
Sucrose	100-5000
Maltose, Lactose	200-2000

($\mu\text{g/L}$)

Flavonoids	CAL of range
Naringin	10-1000
Quercetin	5-1000
Kaempferol	5-1000
Nobiletin	0.1-1000
Tangeretin	0.1-1000

($\mu\text{g/L}$)

Comprehensive analysis of primary & secondary metabolites in citrus using an automated method changeover UHPLC system coupled to LC/MS/MS

Sample analysis

■ Comparison between fruit and peel

Principal component analysis (PCA) was performed to compare fruit with peel in all tested citrus cultivars. Fruit and peel samples were tend to be separated into 2 groups in the score plot. Analysis of the loading plot

reveals some compounds responsible of the separation between fruit and peel samples (sugars, flavonoids and carotenoids).

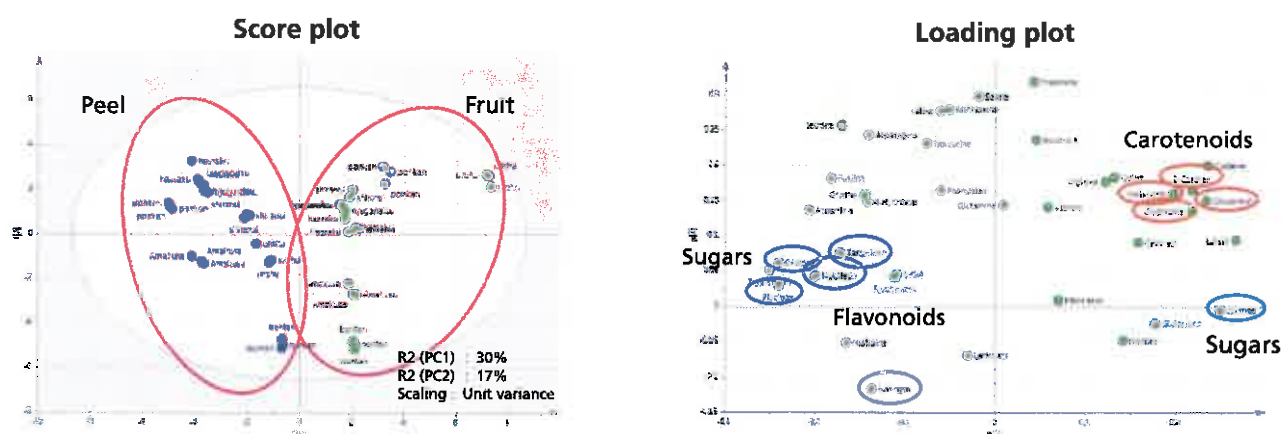


Figure 6 Result of PCA in both fruit and peel samples (SIMCA-P)

■ Primary metabolites (sugars)

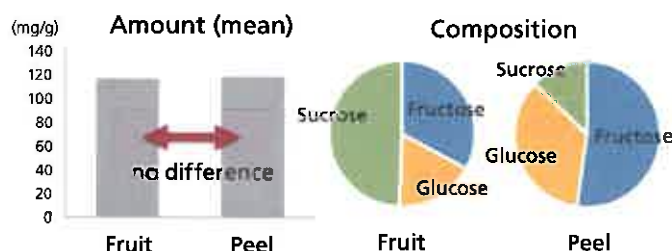


Figure 7 Comparison of amount (mean) and composition between fruit and peel

Result of the quantification, there is no difference in total amount of sugars between fruit and peel samples. However, composition of them was obviously different.

■ Secondary metabolites

In secondary metabolites, total amount was significantly different between fruit and peel. We confirmed that fruit is more rich in carotenoids than peel but poor in flavonoids.

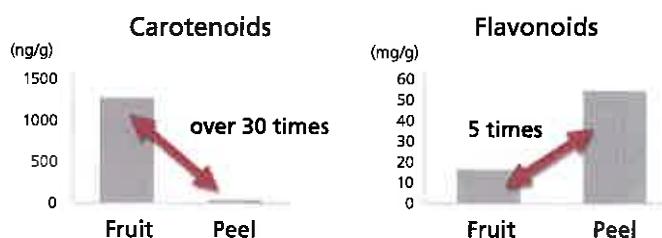


Figure 8 Comparison of amount (mean) in secondary metabolites

Comprehensive analysis of primary & secondary metabolites of citrus using an automated method changeover UHPLC system coupled to LC/MS/MS

■ Comparison between cultivars (fruit)

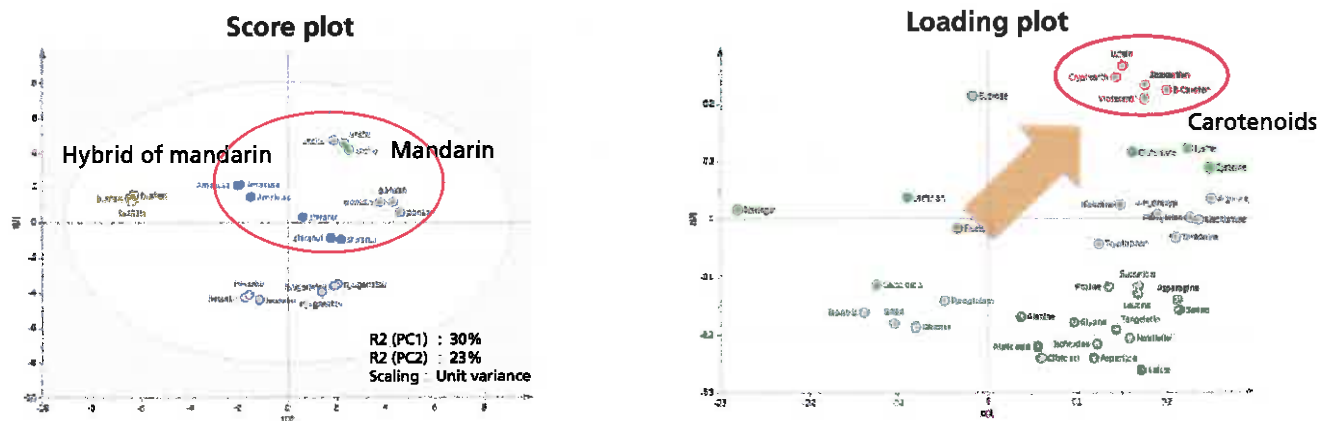


Figure 9 Result of PCA in fruit samples (SIMCA-P)

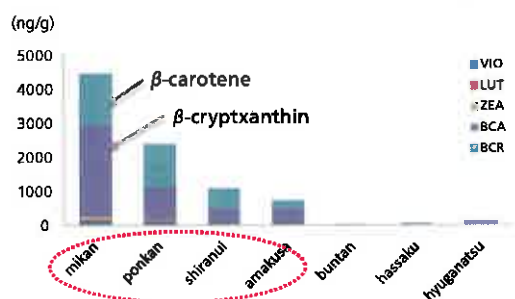


Figure 10 Comparison of amount of carotenoids between 7 citrus

The cultivars in mandarin and hybrid of mandarin, their plots tend to be located in the upper right in the score plot. The loading plot showed that carotenoids are characteristic of these cultivars. Result of the quantification, the cultivars in mandarin and hybrid of mandarin showed a high level of carotenoids, especially β -cryptoxanthin and β -carotene.

Conclusions

- Primary & secondary metabolites were successfully separated using 3 analytical conditions, and UHPLC multi-method system enables contentious analysis without replacement of the columns or mobile phases.
- We confirmed that the cultivars in mandarin group showed high level of carotenoids.

Fast and sensitive analysis of drug residues in water using on line SPE-UHPLC-MS/MS with ultra-fast polarity switching

ASMS 2015 TP 315

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Fast and sensitive analysis of drug residues in water using on line SPE-UHPLC-MS/MS with ultra-fast polarity switching

Introduction

As monitoring of drug residue in drinking and surface water is becoming of major concern in western countries, the environmental laboratories must face the increase in analysis request as well as the increase in the number of compounds to be screened. In parallel, as long as no consensus as been found on regulatory reporting levels, the assay sensitivity must be very high to not miss any compound. Thus, laboratories need a simple, rapid, reliable, sensitive and cost effective method to screen and to accurately quantify drug residues in a variety of water samples.

A method was then set up to fulfill these requirements. On line SPE is a useful tool to enable large sample volume injections. But due to the chemical diversity of target compounds, sample clean up cannot be pushed too far to not lose polar compounds. Then matrix effect or ion suppression can be problematic for accurate quantification. Then, a very sensitive Mass Spectrometer as well as very efficient chromatography were necessary to compensate the relatively low sample volume injection. An innovative SPE set up was developed to combine low pressure on line SPE and high pressure UHPLC analysis.

Materials and Methods

Online SPE-UHPLC-MS/MS

System	: Shimadzu Nexera X2 with online SPE features (fig. 1)
Columns	: SPE : Spark Holland cartridge OASIS HLB 15µm 10x2mm (@RT) UHPLC : Restek Raptor C18 2.7µm 100x2mm (@30°C)
Mobile Phases	: SPE : A : Water B : Methanol C : Methanol/Isopropanol 1/1 UHPLC : A : Water + NH ₄ OH 0.05% B : Methanol + NH ₄ OH 0.05%
Flow rate:	: SPE : Loading : 2 mL/min Elution : 0.4 mL/min Cleaning : 3 mL/min UHPLC : 0.5 mL/min
Injection volume	: 1000 µL
Sample Temperature	: +5°C

High sensitive analysis of drug residues in water using Online SPE-UHPLC-MS/MS with ultra-fast polarity switching

Time Program

Online SPE				UHPLC		
Time (min)	Step	Action	Value	Time (min)	Step	Value
	Loading			0.01	Initial conditions	0%B
0.90		Loading	2 mL/min			
0.91		Changing flow rate	0.4 mL/min			
1.00	Elution	Switching Elution loop	Elution position			
1.00		Switching Storage loop	Storage position			
1.50		Switching Storage loop	Elution position			
1.60	Cleaning	Changing Solvent	C			
1.70		Changing flow rate	3 mL/min			
				2.50	Starting Gradient	0%B
6.00	Filling elution loop	Changing Solvent	B			
8.90		Switching Elution loop	Loading position			
9.00		Changing Solvent	A			
				10.50	Holding gradient	90%B
				12.50	Ending gradient	90%B
				12.60	Reconditioning	0%B
14.00	Stop			14.00	Stop	

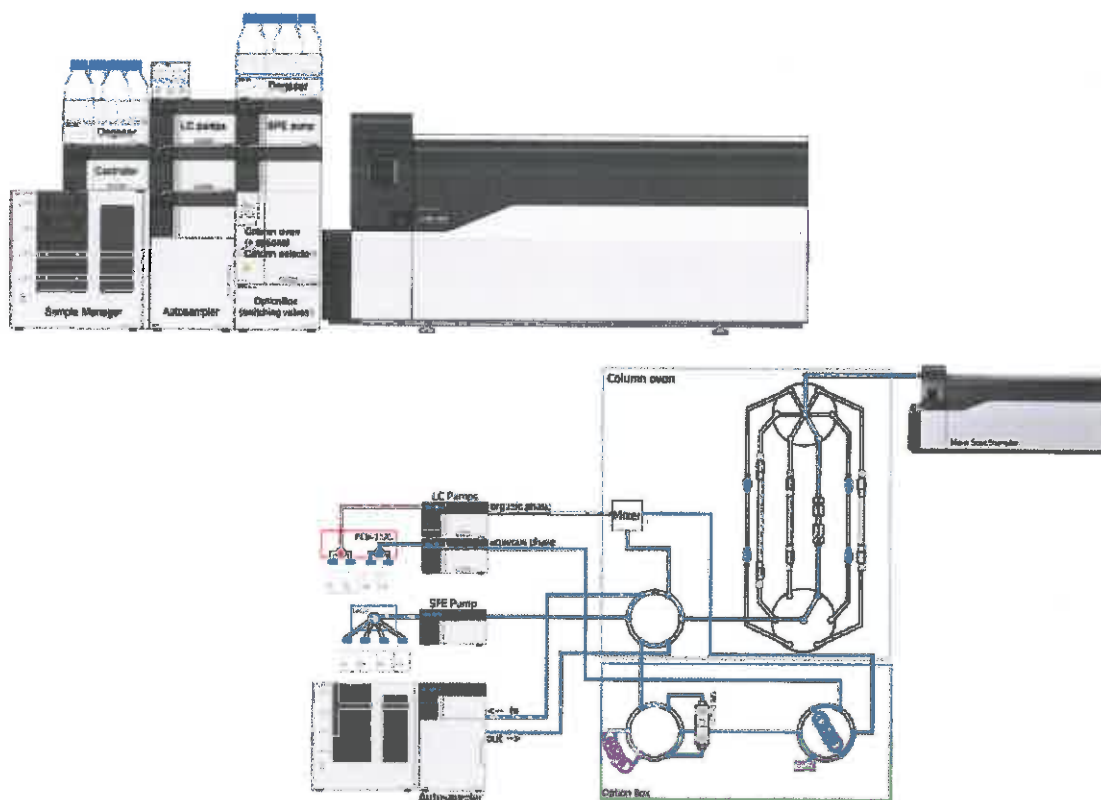


Figure 1 – scheme of the analytical system (patent pending)

Fast and sensitive analysis of drug residues in water using on line SPE-UHPLC-MS/MS with ultra-fast polarity switching

Mass Spectrometry

System	: Shimadzu LCMS-8050
Ionization mode	: Heated ESI
Temperatures	: Interface : 400°C
	DL : 250°C
	Heater Block : 400°C
Gas flow	: Nebulizer : 3L/min
	HESI : 10 L/min
	Drying : 10 L/min
Polarity switching time	: 5 ms
Pause time	: 1 ms
Dwell time	: 5 to 30 ms to obtain at least 25 points per peak,
Compound table	: see table 1

Results

Out of the 130 compounds assayed with this method, 40 compounds were selected for evaluation of sensitivity and matrix effect.

A calibration curve ranging from 0.1 to 100 ng/L was prepared by spiking Evian water (spring water).

Independent QCs in Evian, tap water and superficial water

were prepared for accuracy and matrix effect evaluation. ISTD compounds added in the calibration standards and QCs were only use for process and injection monitoring. Therefore external calibration was performed and ion suppression/enhancement was not compensated by the internal standards.

and sensitive analysis of drug residues in water using the SPE-UHPLC-MS/MS with ultra-fast polarity switching

Table 1: List of the compounds

Name	Polarity	MRM Quantifier	MRM Qualifier
14-Hydroxy-clarithromycin	Pos	764.3 > 158.25	764.3 > 606.1
19-Norethisterone	Pos	299.2 > 109.2	299.2 > 91.1
1-OH-Ibuprofen	Pos	221 > 159.1	221 > 177.2
2-OH-Ibuprofen	Pos	221.4 > 177.35	N/A
4-epitetracycline	Pos	444.9 > 410	444.9 > 347
4-Methylbenzylidene camphor	Pos	255.2 > 237	255.2 > 171.15
Acebutolol	Pos	337.2 > 116.2	337.2 > 319.1
Alprazolam	Pos	309.1 > 281.1	309.1 > 205.1
Aminopyrine (aminophenazone)	Pos	232.1 > 113.25	232.1 > 111.2
Amitriptyline	Pos	278.2 > 91.05	278.2 > 105.05
Amlodipine	Pos	410.1 > 239	410.1 > 238.1
Androstenedione	Pos	286.7 > 97.2	286.7 > 109.15
Antipyrindine	Pos	189.2 > 57	189.2 > 77.1
Atenolol	Pos	266.8 > 145.05	266.9 > 190.1
Betaxolol	Pos	308.2 > 116.2	308.2 > 72.2
Bezafibrate	Pos	361.9 > 316	361.9 > 138.95
Bezafibrate	Pos	360.1 > 274.1	360.1 > 154.05
Bezafibrate d6 (ISTD)	Pos	367.9 > 138.95	N/A
Bisoprolol	Pos	326.2 > 116.2	326.2 > 74.15
Bromazepam	Pos	316 > 182	317.7 > 182.2
Bupivacaine	Pos	289.2 > 140.25	289.2 > 84.05
Caffeine	Pos	194.9 > 138.2	194.9 > 42.2
Carbamazepine	Pos	237.1 > 194.2	237.1 > 192.15
Carbamazepine 10,11 epoxide	Pos	253.1 > 180.2	253.1 > 236
Carbamazepine c13 (ISTD)	Pos	239.1 > 194.2	239.1 > 192.15
Cetiofur	Pos	524 > 240.85	524 > 165.95
Chloramphenicol	Pos	320.8 > 152.1	323 > 152.1
Chlormadinone	Pos	405.1 > 309	405.1 > 345
Chlorophene	Neg	217 > 181.2	217 > 153.2
Chlortetracycline	Neg	479.1 > 444.05	479.1 > 260
Ciprofloxacin	Neg	332 > 231.15	332.1 > 288.15
Clarithromycin	Neg	748.5 > 158.2	748.5 > 83
Clenbuterol	Neg	279.2 > 204.9	279.2 > 181.2
Cliindamycin	Neg	425.2 > 126.2	425.2 > 377.15
Clofibric acid	Neg	213 > 127	213 > 85.1
Cortisol	Neg	362.7 > 121.2	362.7 > 327.1
Cotinine	Neg	177.2 > 80.1	177.2 > 98.15
Cyclophosphamide	Neg	260.7 > 140	263 > 142.05
Danofloxacin	Neg	358.1 > 314	358.1 > 283.05
Dexamethasone	Neg	393.2 > 373.05	393.2 > 355.05
Diazepam	Pos	285.1 > 154.1	285.1 > 193.05
Diclofenac	Neg	296 > 214	298 > 217.1
Dihydrocodeine	Neg	302.3 > 199.1	302.3 > 128.1
Dilantin	Neg	251 > 101.9	251 > 208.25
Diltiazem	Neg	415.1 > 178.05	415.1 > 150.05
Doxepine	Neg	280.2 > 107.1	280.2 > 235
Doxycycline	Neg	444.9 > 428.15	444.9 > 147.3
Enoxacin	Neg	321.1 > 303	321.1 > 232.05
Enrofloxacin	Neg	360 > 316.05	360 > 245.15
Erythromycin A	Neg	734.3 > 158.2	734.3 > 576.25
Fenobric acid	Neg	317 > 231	317 > 249.1
Fenofibrate	Neg	362.1 > 234.15	362.1 > 139.1
Fenoprofen	Neg	241.1 > 197.2	241.1 > 93
Florfenicol	Neg	356 > 335.85	356 > 185.1
Flumequine	Neg	261.9 > 202.15	261.9 > 126.2
Fluoxetine	Neg	310.1 > 251	310.1 > 44.1
FOSA	Neg	497.9 > 77.95	497.9 > 477.9
Furosemide	Neg	329 > 285.05	329 > 205
Gemfibrozil	Neg	249.2 > 121	249.2 > 106.1
Ibuprofen	Neg	204.9 > 161.1	N/A
Imipramine	Neg	281.2 > 86.1	281.2 > 58.15
Indomethacin	Neg	355.8 > 297.1	355.8 > 312.1
Irbesartan	Neg	429.2 > 206.95	429.2 > 195.25
Isosfamide	Neg	261 > 154	261 > 92.1
Isoquinoline	Pos	130.1 > 77.15	130.1 > 103.15
Ketoprofen	Pos	254.8 > 105.1	254.8 > 209
Ketorolac	Pos	256.1 > 105.1	256.1 > 77.15
Levonorgestrel	Pos	313.2 > 245	313.2 > 91.15
Uncomycin	Pos	407.2 > 126.3	407.2 > 359
Lorazepam	Pos	320.8 > 275.05	322.6 > 276.9

Name	Polarity	MRM Quantifier	MRM Qualifier
Marbofloxacin	Pos	363.1 > 72.25	363.1 > 320
Meclocycline	Pos	476.9 > 460	476.9 > 235
Mefenamic acid	Pos	242.1 > 223.95	242.1 > 209
Mepivacaine	Pos	247.2 > 98.15	247.2 > 70.15
Methotrexate	Pos	454.9 > 308	454.9 > 175.2
Metoprolol	Pos	268 > 116.35	268 > 55.95
Monensin sodium	Pos	693.2 > 675.2	693.2 > 461.05
Morphine	Pos	286.2 > 165.2	286.2 > 152.1
Nadolol	Pos	310.2 > 254	310.2 > 201.15
Naproxen	Pos	229 > 169.3	229 > 170.2
n-ETFOA	Pos	526 > 169.15	526 > 219
Nicotine	Pos	163.2 > 130.1	163.2 > 117.2
Norfloraxacin	Pos	320.1 > 276.05	320.1 > 233.2
Norfluraxetine	Pos	296 > 213.95	296 > 214.95
Odesmethyl-tramadol	Pos	250.2 > 58.15	250.2 > 171.05
Ofloxacin	Pos	361.9 > 318.05	361.8 > 260.95
Ofloxacin d3 (ISTD)	Pos	364.9 > 321.05	364.8 > 260.95
Oxazepam	Pos	286.7 > 241.1	286.7 > 268.95
Oxycodone	Pos	316.1 > 298	316.1 > 241
Oxytetracycline	Pos	460.9 > 426.1	460.9 > 201
Pentoxifylline	Pos	279.2 > 205.1	279.2 > 181.15
PFBA	Pos	213 > 169.1	213 > 93
PFBS (L-PFBS)	Pos	298.9 > 79.9	298.9 > 98.95
PFDA	Pos	512.9 > 468.9	512.9 > 269
PFDaA	Pos	612.9 > 568.75	612.9 > 169.05
PFDS (L-PFDS)	Pos	598.9 > 79.9	598.9 > 98.95
PFHDA (PFHxDA)	Pos	768.9 > 269	768.9 > 168.9
PFHpA	Pos	363 > 318.95	363 > 169.15
PFHpS (L-PFHpS)	Pos	448.9 > 79.9	448.9 > 98.9
PFHxA (PFHA)	Pos	313 > 269.1	313 > 91.05
PFHxS (PFHS)	Pos	398.9 > 79.9	398.9 > 99
PFNA	Pos	463.1 > 418.9	463.1 > 219
PFOA	Pos	412.9 > 368.9	412.9 > 169.15
PFODA	Pos	912.9 > 868.8	912.9 > 168.95
PFOS	Pos	498.9 > 98.95	498.9 > 79.95
PFPeA	Pos	263 > 219	263 > 18.8
PFTeA	Pos	712.9 > 219	712.9 > 219
PFTFA (PFTFDA)	Pos	663 > 618.85	663 > 169
PFUnDA	Pos	562.9 > 518.8	562.9 > 219.05
Piroxicam	Pos	330.1 > 146.15	330.1 > 266.1
Prednisolone	Pos	361.2 > 343.05	361.2 > 307
Prilocaine	Pos	221.2 > 86	221.2 > 44.2
Pristinamycin IIA	Pos	526.1 > 355.15	526.1 > 508.05
Progesterone	Pos	314.8 > 109.2	314.8 > 97.15
Propranolol	Pos	260.15 > 183.15	260.15 > 116.15
Propyphenazone	Pos	231.1 > 189.2	231.1 > 56.1
Ramiprilat	Pos	389.2 > 206	389.2 > 156.2
Ranitidine	Pos	314.8 > 176.1	315.1 > 130.1
Roxithromycin	Pos	837.3 > 158.05	837.3 > 679.3
Salbutamol	Pos	240.2 > 148.15	240.2 > 222
Sertraline	Pos	306.1 > 159.05	306.1 > 274.95
Simvastatin	Pos	419.1 > 199.2	419.1 > 225.05
Sotalol	Pos	272.9 > 213.1	273.1 > 133.1
Spiramycin	Pos	422.3 > 174.05	422.3 > 101.15
Sulfamethazine	Pos	278.9 > 185.95	278.9 > 124.2
Sulfamethoxazole	Pos	253.9 > 156.1	253.9 > 92.1
Sulfaminoxaline	Pos	302 > 156.1	302 > 157.15
Sulfaminoxaline	Pos	300.8 > 156.05	300.8 > 92.1
Terbutaline	Pos	226.2 > 152	226.2 > 107.15
Testosterone	Pos	289.1 > 96.9	289 > 109
Tetracycline	Pos	444.9 > 410	444.9 > 241
Ticlopidine	Pos	264.1 > 125.15	264.1 > 154.15
Timolol	Pos	317.1 > 260.95	317.1 > 74.15
Tramadol	Pos	264.2 > 58.15	N/A
Trimethoprim	Pos	290.8 > 230	290.8 > 123
Trimethoprim d3 (ISTD)	Pos	293.8 > 230	293.8 > 123
Zolpidem	Pos	308.1 > 237.05	308.1 > 235.05

Fast and sensitive analysis of drug residues in water using on line SPE-UHPLC-MS/MS with ultra-fast polarity switching

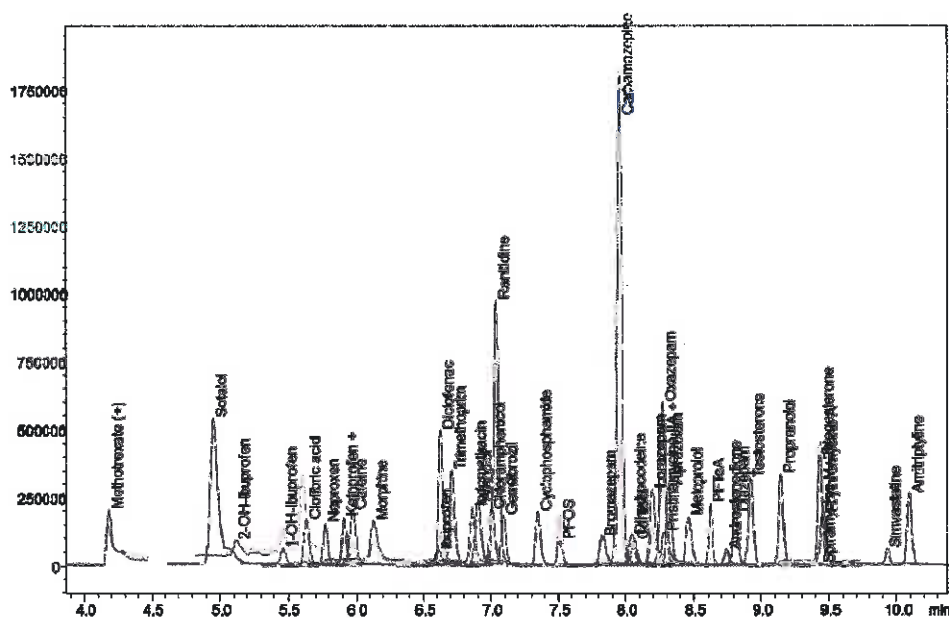


Figure 2 – Superficial water spiked at 10 ng/L

Table 2: Quantitative results

Compound	LOQ (ng/L)	Accuracy in QC (50 ng/L)		
		Evian	Tap water	Superficial water
1-OH-Ibuprofen	5	75%	90%	17%
2-OH-Ibuprofen	10	80%	95%	43%
Alprazolam	0.5	95%	100%	99%
Amitriptyline	1	84%	84%	51%
Androstenedione	0.5	98%	113%	20%
Atenolol	0.5	99%	116%	89%
Bromazepam	0.5	100%	104%	97%
Cafeine	5	104%	162%	98%
Carbamazepine	0.1	95%	103%	110%
Chloramphenicol	1	106%	183%	123%
Clofibric acid	0.5	90%	102%	72%
Cortisol	2.5	110%	161%	70%
Cyclophosphamide	0.5	104%	126%	104%
Diazepam	1	97%	99%	104%
Diclofenac	0.5	107%	160%	104%
Dihydrocodeine	0.5	96%	106%	116%
Erythromycin A	0.1	92%	96%	74%
Gemfibrozil	0.5	89%	93%	74%
Ibuprofen	10	67%	61%	67%
Indomethacin	2.5	90%	124%	69%

Compound	LOQ (ng/L)	Accuracy in QC (50 ng/L)		
		Evian	Tap water	Superficial water
Ketoprofen	0.5	110%	272%	107%
Lorazepam	0.5	103%	128%	103%
Methotrexate	0.5	64%	57%	5%
Metoprolol	0.5	96%	96%	93%
Morphine	2.5	88%	122%	69%
Naproxen	1	94%	96%	49%
n-ETFOA	5	18%	17%	5%
Oxazepam	0.5	104%	111%	100%
PFOS	0.5	52%	73%	58%
PFTeA	0.1	72%	117%	52%
Pristinamycin IIA	1	93%	97%	80%
Progesterone	2.5	91%	98%	14%
Propranolol	0.5	93%	92%	72%
Ranitidine	0.5	97%	105%	92%
Simvastatin	2.5	41%	48%	7%
Sotalol	0.5	93%	131%	122%
Spiramycin	0.5	85%	128%	37%
Testosterone	0.5	97%	109%	18%
Trimethoprim	0.5	99%	110%	86%

Highly sensitive analysis of drug residues in water using online SPE-UHPLC-MS/MS with ultra-fast polarity switching

Conclusion

A fast and sensitive method was set up to assay a large number of drug residue in water. Limits of quantification are ranging from 0.1 to 5 ng/L with only 1 mL of sample and an automated sample preparation. Most of the compounds do not suffer from matrix effect without using internal standards. Some investigations are running to improve accuracy using selected internal standards.

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Toxicological General Unknown Screening (GUS) by triple quadrupole LCMS/MS with high sensitivity product ion scan library identification

ASMS 2016 ThP 731

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Toxicological General Unknown Screening (GUS) by triple quadrupole LCMS/MS with high sensitivity product ion scan library identification

Introduction

Forensic toxicological sample measurement can take two paths commonly referred to as targeted and untargeted analysis. Targeted approaches on triple quadrupole platforms are well established with MRM/SRM detection and quantitation for pre-determined lists of compounds. For untargeted analysis, general unknown screening (GUS) acquires full scan MS data to trigger MS/MS spectra at a broad range of collision energies above a predefined threshold. This work describes the application of a

nominal mass library acquired with certified reference materials to screen and identify forensic toxicology samples in a routine clinical laboratory. The LC-MS/MS method was designed to acquire MRM and scanning data with polarity switching in a single data file. The MRM data was used to provide quantitative results on commonly encountered target compounds and full scan data to help confirm identification.

Methods and Materials

Whole blood samples were spiked with a panel of the most commonly detected compounds reported in toxicological screening. Among them included: psychoactive drugs (antidepressants, anxiolytic drugs, antipsychotics), analgesic, antipyretics and anti-inflammatory agents, antiepileptics. These samples

were prepared by QuEChERS method with the inclusion of ten internal standard compounds for quality control purposes. Data acquisition was set to pos/neg Q3 scan with data dependant MS/MS in addition to MRM for a panel of commonly encountered compounds.

Table 1. LC-MS/MS data acquisition conditions. The method included full scan and MRM data acquisition in both positive and negative ion mode. Full scan data was acquired with three collision energies to generate a broad range of fragment ions (events 1-8; polarity switching time 5msecs). MRM data was acquired for targeted analysis (events 10-35) and each MRM event contained two reference ions. 10 internal standard compounds were also included in the method.

Liquid chromatography																	
UHPLC	: Nexera LC system																
Analytical column	: Restek Raptor Biphenyl 2.7 μm 100 x 2.1 mm																
Column temp.	: 50°C																
Injection cycle	: 5 μL injection volume																
Flow rate	: 0.3 mL/min																
Solvent A	: Water + 2mM ammonium formate + 0.002% formic acid																
Solvent B	: Methanol + 2mM ammonium formate + 0.002% formic acid																
Binary Gradient	<table><tr><th>Time (mins)</th><th>%B</th></tr><tr><td>1.00</td><td>5</td></tr><tr><td>2.00</td><td>40</td></tr><tr><td>10.50</td><td>100</td></tr><tr><td>13.00</td><td>100</td></tr><tr><td>13.01</td><td>5</td></tr><tr><td>17.00</td><td>Stop</td></tr><tr><td>11-14.2</td><td>0.5 mL/min</td></tr></table>	Time (mins)	%B	1.00	5	2.00	40	10.50	100	13.00	100	13.01	5	17.00	Stop	11-14.2	0.5 mL/min
Time (mins)	%B																
1.00	5																
2.00	40																
10.50	100																
13.00	100																
13.01	5																
17.00	Stop																
11-14.2	0.5 mL/min																

Mass spectrometry	
LC-MS/MS	: LCMS-8060
Ionisation mode	: Heated ESI
Scan speed	: 30,000 u/sec
Polarity switching time	: 5 msec
MRM Dwell time	: 5 msec
Pause time	: 3 msec
Interface temp.	: 300°C
Heating block	: 400°C
Desolvation line	: 250°C
Heating gas	: 10 L/min
Drying gas	: 10 L/min
Nebulising gas	: 3 L/min
CID gas pressure	: 250kPa
Interface voltage	: 4 kV

Biological General Unknown Screening (GUS) Triple quadrupole LCMS/MS with high sensitivity Product ion scan library identification

LC-MS/MS method set up for simultaneous full scan and MRM data with polarity switching

Type	Event	Polarity	Name m/z	Time (0-13mins)
Q3 Scan	1	+	Mass range 100-1000	
Product Ion Scan	2	+	> CE: -10, 30.00-1000.00	
Product Ion Scan	3	+	> CE: -35, 30.00-1000.00	
Product Ion Scan	4	+	> CE: -50, 30.00-1000.00	
Q3 Scan	5	-	Mass range 100-1000	
Product Ion Scan	6	-	> CE: 10, 30.00-1000.00	
Product Ion Scan	7	-	> CE: 35, 30.00-1000.00	
Product Ion Scan	8	-	> CE: 50, 30.00-1000.00	
MRM	9	-	Metformin 130.10>71.10, 130.10>60.05, 130.10>85.05	
MRM	10	+	Paracetamol 152.05>110.10, 152.05>65.05, 152.05>93.10	
MRM	11	+	Levetiracetam 171.10>126.10, 171.10>154.10, 171.10>69.10	
MRM	12	+	Codeine 300.15>152.10, 300.15>215.15, 300.15>165.15	

Spectral Library >1200 compounds

Each library spectrum was acquired by authentic standard flow injection at collision energies 10-55V. Compounds that ionised efficiently in more than one adduct state were saved resulting in 1476 Library entries from 1207 compounds (1278 positive mode, 229 negative mode). Spectral Library information was registered for three collision energies corresponding to CE 10, 35 and 55V.

Optimised MRM transitions were also determined for all compounds together with retention time and peak area data ratio of response between quantifier and qualifier transitions. RT analysis included internal standard compounds for relative RT calculation. Compound information was populated including: CAS number, formula, synonyms,.

Table 2. The spectral library data base included full scan spectrum at three defined collision energies and optimized MRM transitions for multi-targeted screening and quantitation. Expected retention times correspond to the retention on a Biphenyl phase.

Library data base									
Compound	CAS	Formula	Ion	Precursor ion	Product ion	Pre-bias	CE	Pre-Bias	RT (min) Note 1
				Q1 m/z	Q3 m/z	Q1		Q3	
Ibuprofen	51146-56-6	C13H18O2	[M-H]-	205.10	161.20	22.00	10.00	16.00	7.70
Ibuprofen	51146-56-6	C13H18O2	[M-H]-	205.10	146.30	10.00	19.00	18.00	7.70
Ibuprofen	51146-56-6	C13H18O2	[M-H]-	205.10	159.10	10.00	13.00	15.00	7.70
Codeine	76-57-3	C18H21NO3	[M+H]+	300.16	152.10	-15.00	-63.00	-28.00	3.82
Codeine	76-57-3	C18H21NO3	[M+H]+	300.16	215.15	-15.00	-24.00	-22.00	3.82
Codeine	76-57-3	C18H21NO3	[M+H]+	300.16	165.15	-15.00	-42.00	-16.00	3.82
Levomepromazine	60-99-1	C19H24N2OS	[M-H]-	329.17	100.15	-12.00	-21.00	-22.00	7.83
Levomepromazine	60-99-1	C19H24N2OS	[M-H]-	329.17	58.05	-12.00	-36.00	-10.00	7.83
Levomepromazine	60-99-1	C19H24N2OS	[M-H]-	329.17	242.05	-12.00	-22.00	-16.00	7.83

Toxicological General Unknown Screening (GUS) by triple quadrupole LCMS/MS with high sensitivity product ion scan library identification

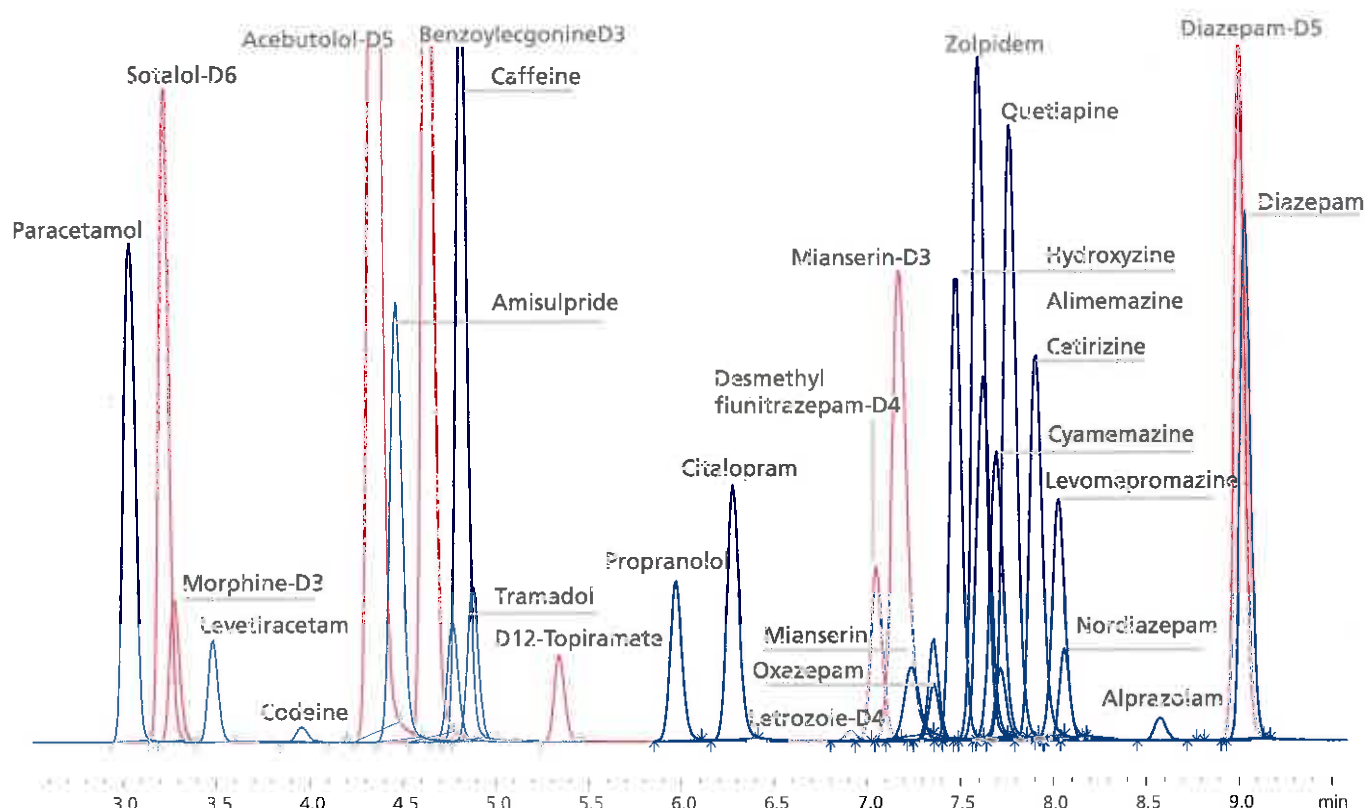


Figure 1. A whole blood sample was spiked with a panel of drugs which are commonly encountered in routine forensic toxicology and extracted using a QuEChERS method. The triple quadrupole mass spectrometer acquired full scan and MRM data throughout the analysis switching polarity with a speed of 5msecs. MRM chromatograms for the panel of drugs (blue MRM chromatograms) and associated stable isotope labelled internal standards (highlighted in red) are shown above.

Biological General Unknown Screening (GUS) Triple quadrupole LCMS/MS with high sensitivity Product ion scan library identification

Results

Library searching

Library searches were performed on spiked blood at low and high concentrations. Each collision energy acquired was searched against a library with search parameters set to search with same precursor ion and collision energy.

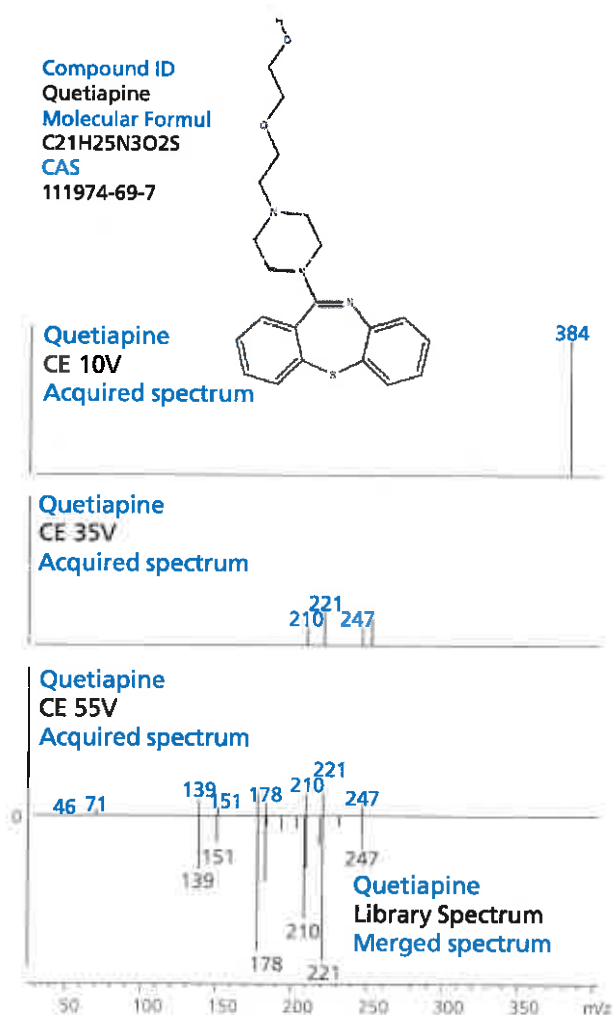


Figure 2. Intensity triggered product ion spectrum for quetiapine spiked into a whole blood sample at a high level toxicological sample (corresponding to a concentration of 0.5mg/L). Product ion spectra are shown for each of the three collision energies (10, 35 and 55V) together with a merged spectrum and library spectrum (CE10-55V).

There are 4 spectral library databases;

Positive ion | Merged Spectra Positive ion | 3 collision energies

Negative ion | Merged Spectra Negative ion | 3 collision energies

Libraries can be searched individually or collectively in automated search routines.

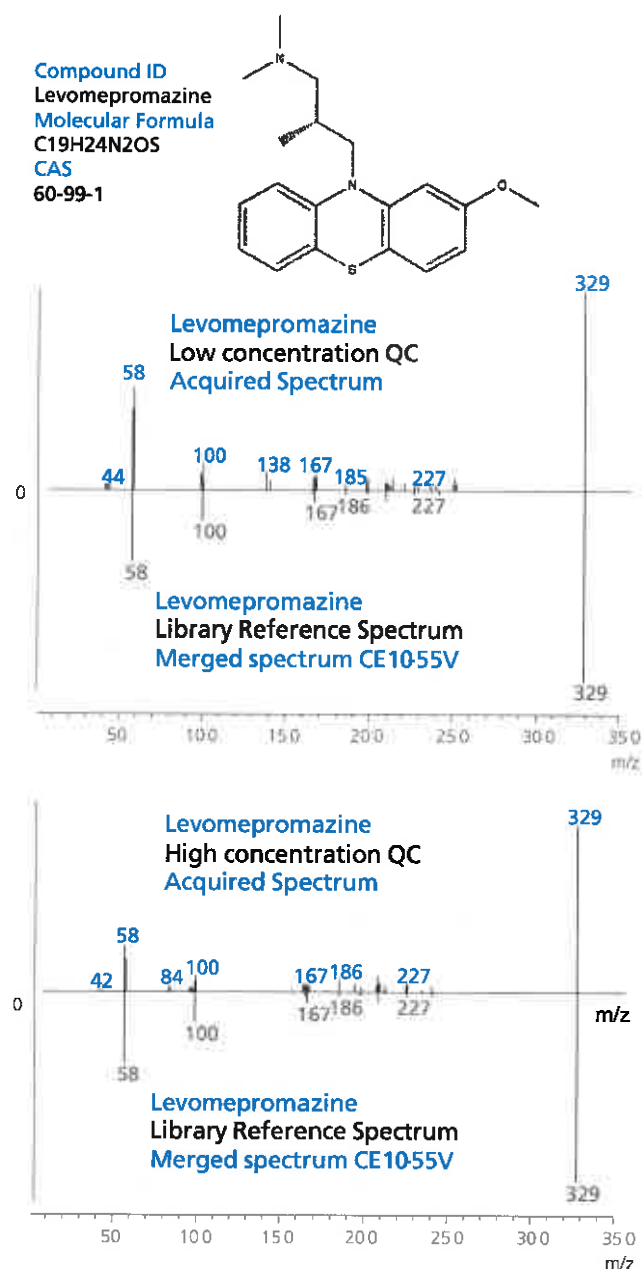


Figure 3 Merged product ion spectra (CE 10-55V) for levomepromazine spiked into whole blood at a low concentration QC (100 µg/L) and a high concentration QC (500 µg/L). Both spectra result in a high library match score despite the difference in concentration levels.

Toxicological General Unknown Screening (GUS) by triple quadrupole LCMS/MS with high sensitivity product ion scan library identification

Quantitation

The method was designed to deliver robust quantitation data together with higher confidence in compound identification using threshold triggered product ion spectra.

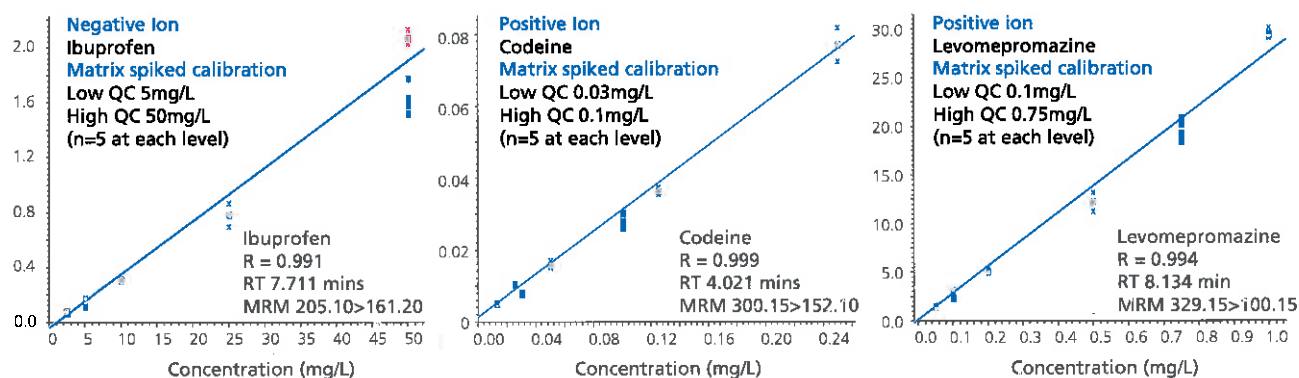


Figure 4. Calibration curve data for ibuprofen (negative ion mode, calibration range 2-50mg/L), codeine (positive ion mode, calibration range 0.01-0.25mg/L) and levomepromazine (positive ion mode, calibration range 0.05-1.0mg/L) spiked into whole blood and extracted using QuEChERS.

Table 3. Reproducibility for 2 quality control levels spiked into blood (low and high level at differing concentrations for each level; n=5) for a panel of drug compounds.

Compound	RT (min)	Low (mg/L)	CV (area)	Library Hit	High (mg/L)	CV (area)	Library Hit	Cal range (mg/L)	R2
		Therapeutic			Toxic				
Metformin	1.03	1	1.0	-	5	5.4	1	1 - 10	0.985
Paracetamol	3.05	10	2.8	1	100	2.7	SatR	5 - 50	0.973
Levetiracetam	3.49	10	6.6	1	50	3.6	SatR	5 - 100	0.997
Codeine	4.02	0.025	1.6	1	0.1	5.6	1	0.0125 - 0.375	0.999
Amisulpride	4.53	0.1	1.2	1	0.5	4.1	1	0.05 - 1	0.992
Tramadol	4.95	0.1	2.4	1	1	5.9	1	0.05 - 1	0.994
Metoprolol	4.84	0.1	5.2	1	0.5	4.5	1	0.05 - 1	0.998
Caffeine	4.82	5	5.0	1	15	2.8	1	2.5 - 50	0.998
Propranolol	6.06	0.1	5.0	1	0.5	5.4	1	0.05 - 1	0.999
Citalopram	6.37	0.1	2.2	1	0.5	8.0	1	0.05 - 1	0.997
Mianserin	7.34	0.05	1.6	1	0.5	4.2	1	0.025 - 0.5	0.994
Hydroxyzine	7.56	0.05	1.4	1	0.2	4.9	1	0.025 - 0.5	0.994
Oxazepam	7.36	0.2	5.4	-	0.5	4.5	-	0.1 - 2	0.994
Alimemazine	7.73	0.1	2.9	1	0.5	4.8	1	0.05 - 1	0.999
Zolpidem	7.64	0.1	2.2	1	0.5	4.6	1	0.05 - 1	0.998
Quetiapine	7.84	0.1	1.8	1	0.5	13.7	1	0.05 - 1	0.997
Cyamemazine	7.79	0.1	2.8	1	0.5	3.8	1	0.05 - 1	0.996
Ibuprofen	7.71	5	6.4	-	50	5.8	1	2.5 - 50	0.98
Levomepromazine	8.13	0.1	3.2	1	0.5	4.7	1	0.05 - 1	0.988
Cetirizine	7.92	0.05	1.8	1	0.2	5.8	1	0.025 - 0.5	0.998
Nordiazepam	8.06	0.2	2.1	1	0.5	3.5	1	0.1 - 2	0.995
Alprazolam	8.57	0.01	2.2	-	0.1	5.0	1	0.005 - 0.1	0.993
Diazepam	9.04	0.2	1.2	1	0.5	4.4	1	0.1 - 2	0.997

Biological General Unknown Screening (GUS) Triple quadrupole LCMS/MS with high sensitivity Product ion scan library identification

Conclusions

Screening by GUS successfully identified spiked compounds at therapeutic and toxic concentrations using generic LC and MS conditions with few exceptions. This combined screening and MRM approach has been used, not only to screen but also to quantify samples for toxicological screening and reduce false reporting by

confirming compound identification using spectral based library searching.

Future work will aim to validate a larger panel of compounds and determine which compounds are suitable for routine GUS forensic and toxicological screening.

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First Edition: June, 2016



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Pesticide analysis in complex food extracts by multi-transition MRM and library searching for enhanced residue confirmation

ASMS 2016 TP 231

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Pesticide analysis in complex food extracts by multi-transition MRM and library searching for enhanced residue confirmation

Introduction

Multiple Reaction Monitoring (MRM) based LC-MS/MS techniques are widely used on triple quadrupole platforms for targeted quantitation as a result of high selectivity and sensitivity and for compound confirmation using specific MRM transitions. In a regulated environment such as food safety there is a growing need to enhance the capability in routine monitoring programs by increasing the number of pesticides measured in a single analysis and at the same time deliver the highest confidence in compound identification to reduce false reporting. In this work, a conventional MRM method developed for routine pesticide analysis (typically monitoring 2-3 MRM

transitions for each pesticide) has been modified and extended to include a higher number of MRM transitions specific to each pesticide compound (dependent on the pesticide in question, typically 6-12 fragment ions per-compound are monitored). This approach has not been possible on previous MS instruments due to the limited duty cycle resulting in shorter dwell times or an increased cycle time for each MRM and as a consequence lower peak sampling rates and lower sensitivity. Using a fast scanning triple quadrupole mass spectrometer this approach was applied to the routine analysis of a panel of 202 pesticides spiked into a matrix.

Materials and Methods

Pesticide spiked samples, extracted using QuEChERS based methods, were provided by Scientific Analysis Laboratories. Samples were then directly injected into the LC-MS/MS. The MRM method was setup for 202 pesticides. To increase the confidence in compound identification, each target pesticide was fragmented to

generate at least 6 fragment ions per-compound (the highest number of fragment ions was 12). This approach enabled sufficient MRM data to create a MRM product ion spectrum which could be confirmed using library based searching. In total 1,236 transitions were acquired (1,179 in positive ion mode and 57 in negative ion mode).

Table 1. LC and MS/MS acquisition parameters used to create the LC-MS/MS method.

Liquid chromatography																							
UHPLC	: Nexera LC system																						
Analytical column	: HSS T3 (100 x 2.1, 1.7µm)																						
Column temperature / Flow rate	: 40°C ; 0.4mL/minute																						
Solvent A	: 5 mmol/L ammonium formate and 0.004% formic acid																						
Solvent B	: 5 mmol/L ammonium formate and 0.004% formic acid in methanol																						
Binary Gradient	<table border="1"> <thead> <tr> <th>Time (mins)</th><th>%B</th><th>Time (mins)</th><th>%B</th></tr> </thead> <tbody> <tr> <td>1.00</td><td>10</td><td>20.50</td><td>100</td></tr> <tr> <td>2.00</td><td>35</td><td>20.51</td><td>5</td></tr> <tr> <td>19.00</td><td>95</td><td>22.50</td><td>Stop</td></tr> <tr> <td>19.50</td><td>100</td><td></td><td></td></tr> </tbody> </table>			Time (mins)	%B	Time (mins)	%B	1.00	10	20.50	100	2.00	35	20.51	5	19.00	95	22.50	Stop	19.50	100		
Time (mins)	%B	Time (mins)	%B																				
1.00	10	20.50	100																				
2.00	35	20.51	5																				
19.00	95	22.50	Stop																				
19.50	100																						
Mass spectrometry																							
Mass spectrometer	: Shimadzu LCMS-8060																						
Pause time/dwell time	: 1 msec./5 msec.																						
Target number of compounds	: 202 (including 3 internal standards)																						
Total Number of MRM transitions	: Multi-transition MRM method = 1,236 transitions (1,179 is ESI+ and 57 in ESI-) 2 MRM method = 403 transitions																						
Source temperatures (interface; heat block; DL)	: 350°C; 300°C; 150°C																						
Gas flows (nebulising; heating; drying)	: 3L/min; 10 L/min; 10L/min																						

Wide analysis in complex food extracts by multi-transition MRM and library searching for enhanced residue confirmation

Results

To help increase the confidence in compound identification the collision energy was optimized for each product ion in the pesticide compound list. This resulted in a MRM method of 1,236 transitions for 202 pesticides. However,

despite the high number of MRM transitions acquired a pause time of 1msec and dwell time of 5 msec was used (Figure 1).

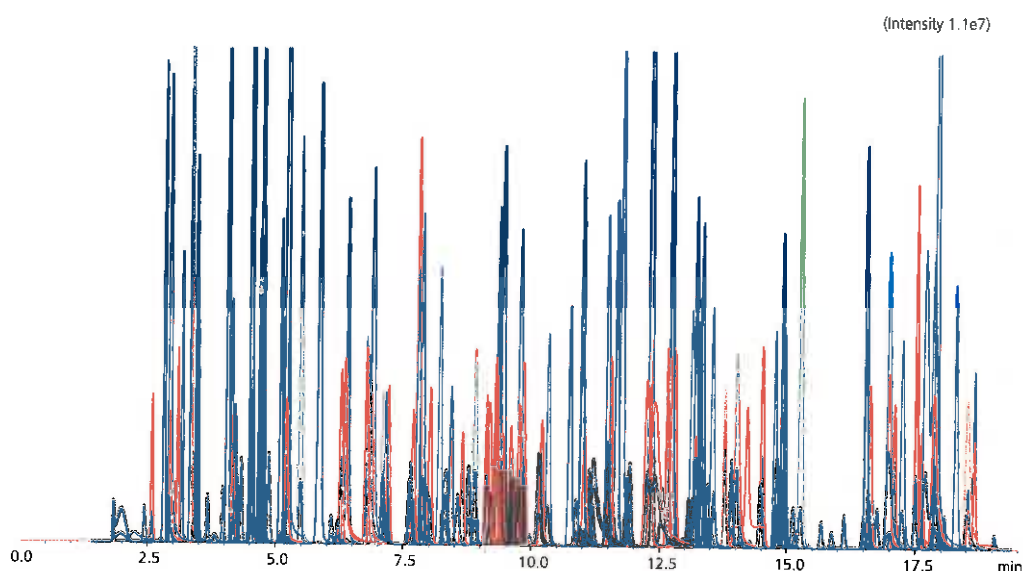


Figure 1. MRM chromatogram for 202 pesticides spiked into a tomato extract corresponding to a concentration of 0.1mg/kg. [For clarity only 2 transitions are displayed for each compound; data acquired with a polarity switching speed of 5 msec].

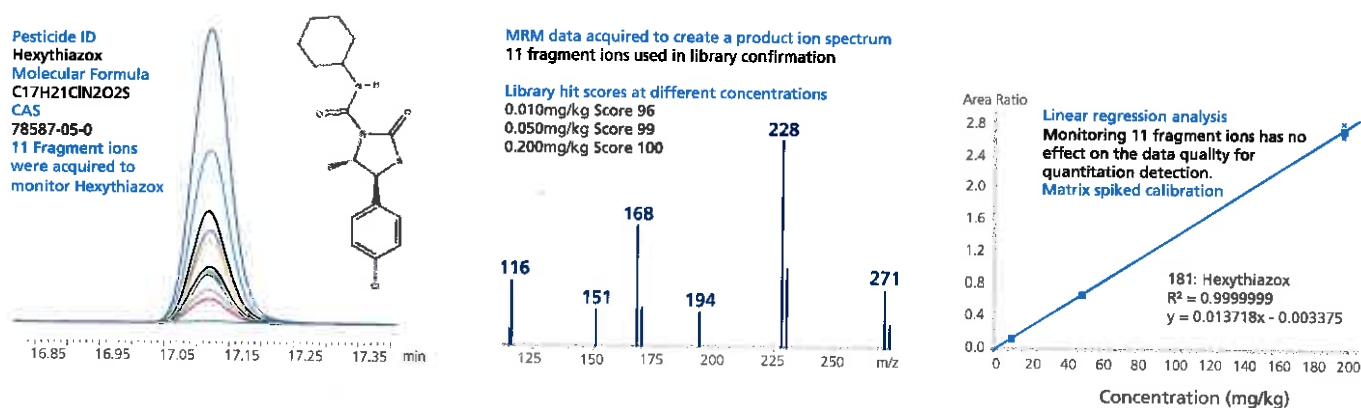


Figure 2. MRM chromatogram, calibration curve, and MRM product ion spectrum results at each calibration level (0.010mg/kg, 0.050 mg/kg and 0.2 mg/kg) for hexythiazox. Library search results were consistently high at all calibration levels.

Pesticide analysis in complex food extracts by multi-transition MRM and library searching for enhanced residue confirmation

Fragment ion	Precursor ion m/z	Product ion m/z	Pause time msec	Dwell time msec	Q1 Bias (V)	CE	Q3 Bias (V)
1	355.1	273.1	1	5	-18	-13	-19
2	353.1	270.9	1	5	-18	-13	-29
3	355.1	229.9	1	5	-18	-16	-25
4	353.1	228.0	1	5	-18	-16	-24
5	353.1	193.9	1	5	-18	-24	-20
6	355.1	170.0	1	5	-18	-24	-11
7	353.1	168.0	1	5	-18	-24	-30
8	353.1	151.0	1	5	-18	-32	-15
9	355.1	118.0	1	5	-18	-47	-22
10	353.1	116.0	1	5	-18	-47	-22
11	355.1	115.2	1	5	-18	-44	-21

Multi-transition MRM and data quality

To assess the data quality generated using a multi-transition MRM method against a conventional MRM approach with 2 MRM transitions the same samples were acquired with both methods. The conventional MRM method included 403 MRM transitions compared to 1,236 MRM transitions used in the multi-MRM method. As one typical example, the repeatability and linearity for

Linuron is in good agreement between the conventional 2 MRM method and a 9 MRM method. Using 10 replicates for each method the %RSD is below 5% for all MRM transitions monitored and despite the multi-MRM method monitoring 1,236 transitions the linear regression analysis is in close agreement.

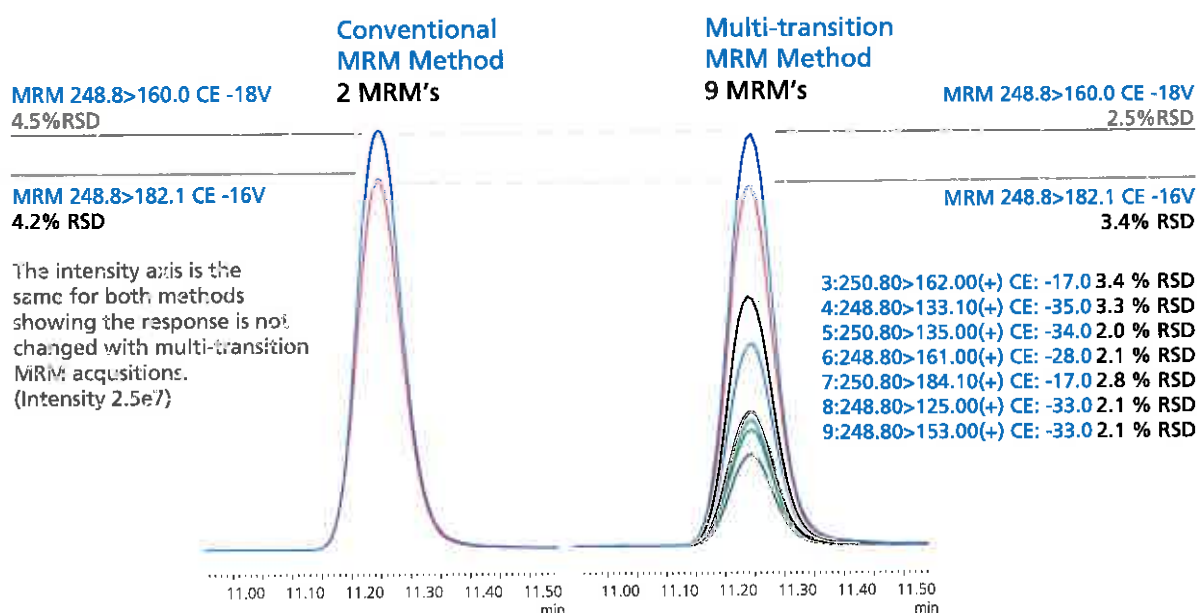


Figure 3. The reproducibility for each MRM method was evaluated by repeatedly injecting a tomato extract corresponding to a concentration of 0.1 mg/kg. The response for linuron was assessed by using 10 replicate injections for each method. In each MRM method the %RSD was less than 5%.

Multi-residue analysis in complex food extracts by multi-transition MRM and library searching for enhanced residue confirmation

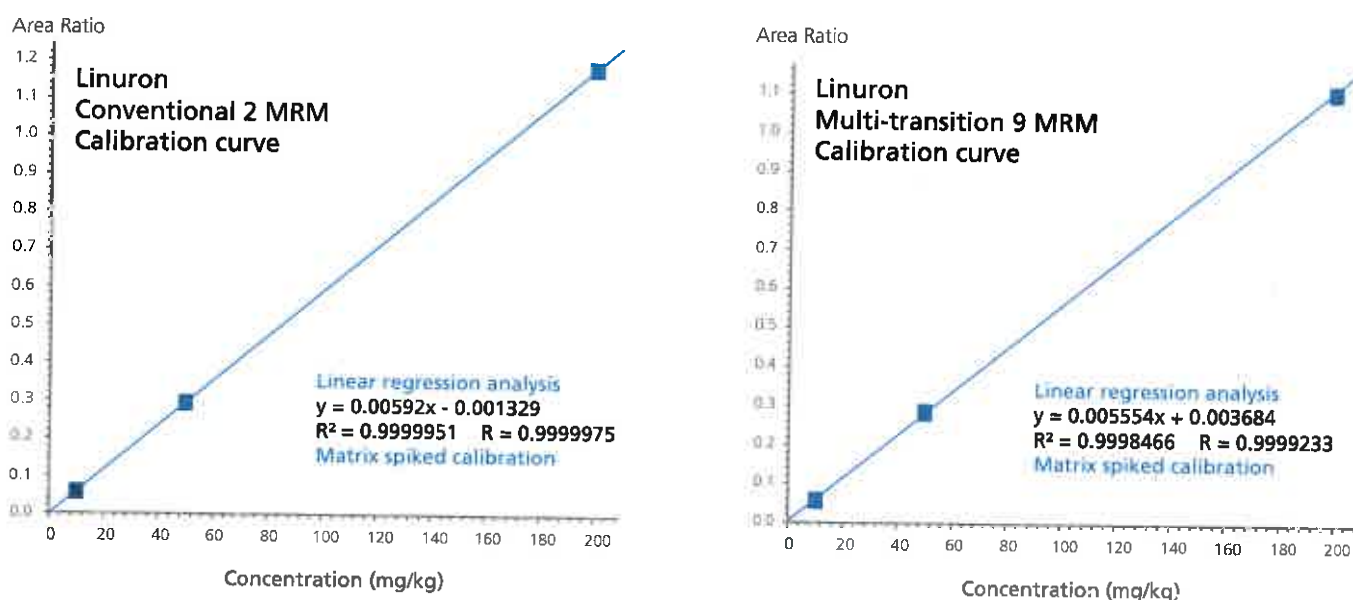


Figure 4. Calibration curve data for linuron acquired using 2 MRM methods (corresponding to a concentration range from 0.010 mg/kg to 0.2 mg/kg). The conventional MRM method acquired 2 MRM transitions (403 MRM's were used in total) whilst the multi-transition MRM method acquired 9 MRM transitions (1,236 MRM's in total).

Multi-transition MRM to help reduce false reporting

In multi-residue pesticide analysis reporting results with a high degree of confidence in compound identification relies on the ratio of response between quantifier and qualifier transitions. To increase the confidence in reporting results the number of qualifier transitions was increased for each pesticide (a minimum of 6 transitions) and the combined transitions were used to create a product ion spectrum. The product ion spectrum can then

be automatically compared against a reference spectrum to generate a product ion spectrum match score using conventional library matching. One well known example is desmedipham which has a similar retention time and several transitions that are common with phenmedipham. Using the multi-transition MRM approach and comparing to a library, desmedipham is positively identified.

Pesticide analysis in complex food extracts by multi-transition MRM and library searching for enhanced residue confirmation

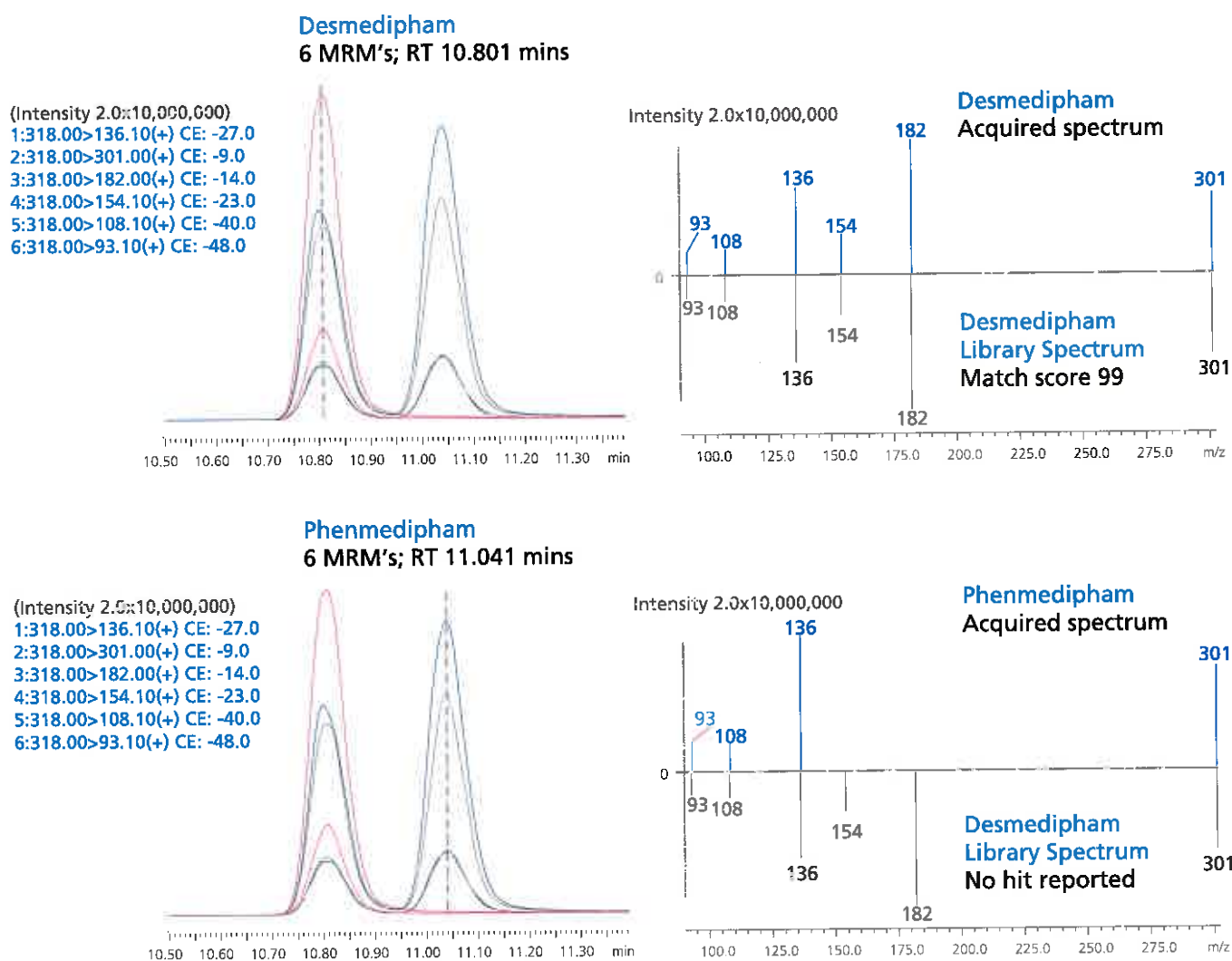


Figure 5. MRM chromatogram for desmedipham spiked into a cumin extract at 0.1 mg/kg. As phenmedipham shares common transitions and elutes at a similar retention time as desmedipham the product ion spectrum is used to distinguish between both pesticides to avoid false reporting.

ide analysis in complex food extracts by multi-transition M and library searching for enhanced residue confirmation

Conclusions

- In this presentation we have shown that a multi-transition MRM approach with library searching against a reference product ion spectrum can be used to reduce false positive reporting.
- Comparing this multi-MRM approach (1,236 MRMs in total) to a conventional 2 MRM method (403 MRMs) demonstrated that the additional MRM data can be acquired without effecting signal response, repeatability and linearity.
- As the data for all the MRMs is acquired during the entire MRM window, quantifier/qualifier transitions can easily be changed post acquisition if matrix interference is observed on a particular MRM channel.

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Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

ASMS 2016 TP-233

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Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

Introduction

More than a thousand different types of pesticides are being used worldwide. In recent years, due to increased awareness of food safety, the amount of pesticide residues in food are controlled by local regulatory agencies that define the maximum residue levels (MRLs) and enforce compliance by regular inspection. LC-MS/MS is widely employed as an analytical method for pesticide residues in the food safety because it features excellent

selectivity and sensitivity even in the matrix-matched samples. As for the separation with LC, ODS has long been the first choice column chemistry but biphenyl columns are emerging as an alternative. Here we performed simultaneous LC-MS/MS analysis of 167 pesticides using both ODS and biphenyl columns for comparison.

Methods

A mixture of 167 pesticides was diluted to working concentrations (80 ng/mL) in water/acetonitrile (60/40, v/v). Separation was achieved within 10 min using Kinetex XB-C18 (100 mmL x 2.1 mmID., 2.6 µm) and Kinetex Biphenyl (100 mmL x 2.1 mmID., 2.6 µm) maintained at 35 °C on a UHPLC system (NexeraX2, Shimadzu corporation, Kyoto, Japan). Data acquisition was performed on triple quadrupole mass spectrometer

LCMS-8060 (Shimadzu Corporation, Kyoto, Japan). All samples were analyzed by multiple reaction monitoring (MRM) with fast polarity switching (5 msec) used for simultaneous detection of positively and negatively charged analytes. The dwell time was set to 4 msec for target ion and 1 msec for reference ion, and the pause time was 1 msec.

Table 1 Analytical condition.

UHPLC (Nexera X2)	
Mobile Phase A	: 2 mmol/L Ammonium formate + 0.002% Formic acid – Water
Mobile Phase B	: 2 mmol/L Ammonium formate + 0.002% Formic acid – Methanol
Gradient Program	: 3%B (0 min) – 10%B (1 min) – 55%B (3 min) – 100%B (10.5 – 12 min) – 3%B (12.01 -15 min)
Flow Rate	: 0.4 mL/min
Column Temperature	: 35 °C
Injection Volume	: 2 µL
MS (LCMS-8060)	
Ionization	: ESI positive and negative
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
Interface Temperature	: 350 °C
DL Temperature	: 150 °C
HB Temperature	: 300 °C
Pause Time	: 1 msec
Dwell Time	: 5 msec

Residue pesticides analysis by LC-MS/MS using ODS column and the biphenyl column



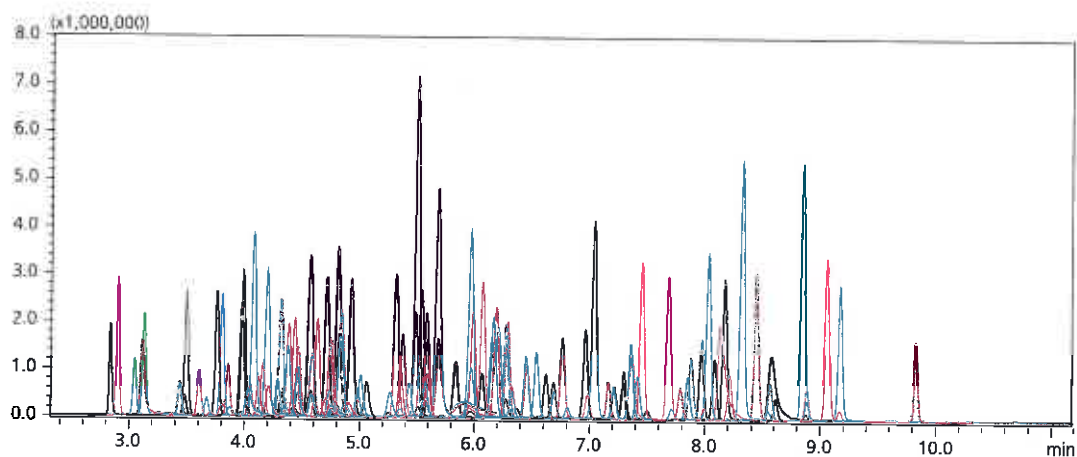
Figure 1 LCMS-8060 Triple Quadrupole Mass Spectrometer

Result

Pesticides encompass a wide range compound classes and chemical properties and consequently it is necessary to employ both positive and negative electrospray ionization for complete analysis in a single run. All of the

compounds could be detected in 10 minutes by LC-MS/MS with fast polarity switching (5 msec) using both the ODS column and the biphenyl column.

a) ODS column



b) Biphenyl column

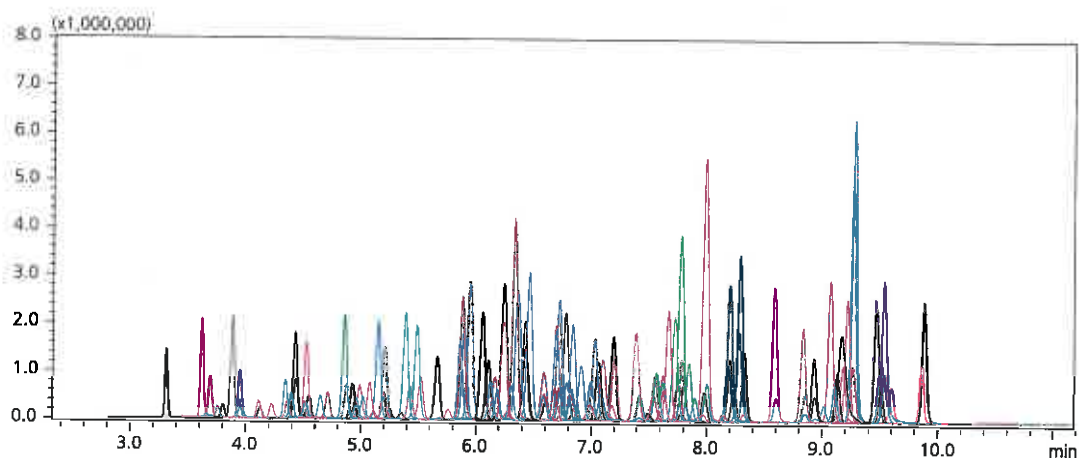


Figure 2 MRM chromatograms for 167 pesticides.

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

While both columns efficiently separated 167 compounds by the same gradient program, the overall distribution of retention times was slightly shorter for the biphenyl column than for the ODS column. The resulting overlap of MRM acquisition was 42 events at maximum that included both positive and negative mode.

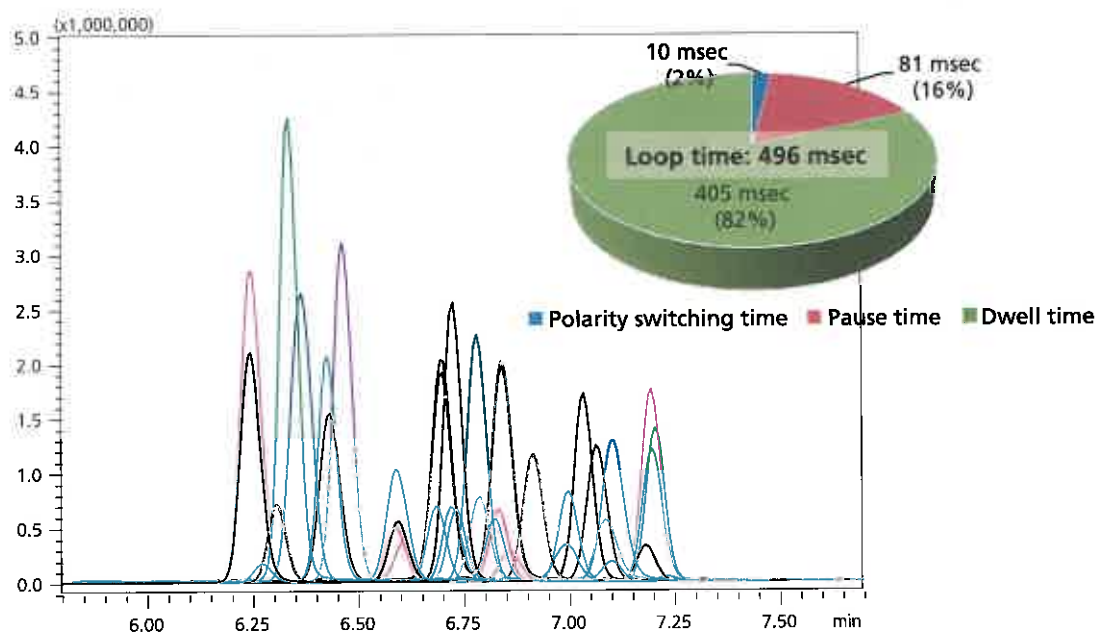


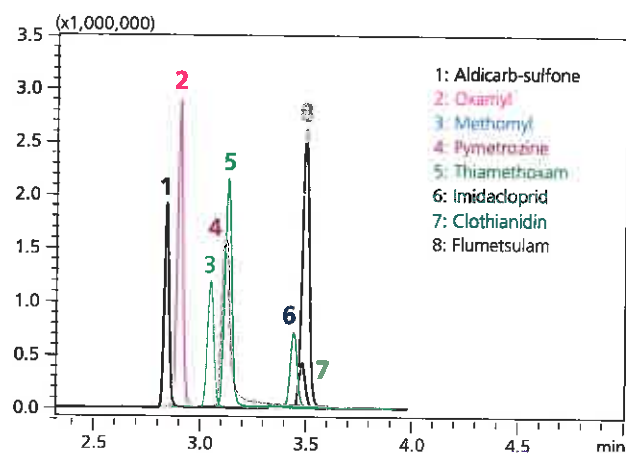
Figure 3 MRM chromatograms for 42 pesticides detected with retention times of 6.720 to 6.723 min (One/Two MRM events per compound, one polarity switching) using the biphenyl column and ratio of dwell time, pause time and polarity switching time in 496 msec of loop time.

Figure 3 shows the MRM chromatograms of 42 pesticides detected in retention time 6.20 – 7.30 min using the biphenyl column, and the pie chart that shows the breakdown of acquisition loop time during the period. Less than 20% of the total loop time of 496 msec was needed for polarity switching and pause time that may be considered as instrumental inefficiency associated with fast detection. The LCMS-8060 ensures the collection of sufficient data points (20 points per peak for peak width of 10 sec) without compromising ion loss.

The biphenyl column is characterized by its high retention capability and separation of aromatic compounds. In this experiment, the retention times of nearly half of all compounds increased by 1 min on average with the biphenyl column than with the ODS. Figure 4 shows MRM chromatograms of early-eluting 8 pesticides in the case of using ODS column. The retention times of all 8 compounds increased and the result indicates the high retention capability of a biphenyl column.

pesticide residue analysis by LC-MS/MS using ODS column and the biphenyl column

a) ODS column



b) Biphenyl column

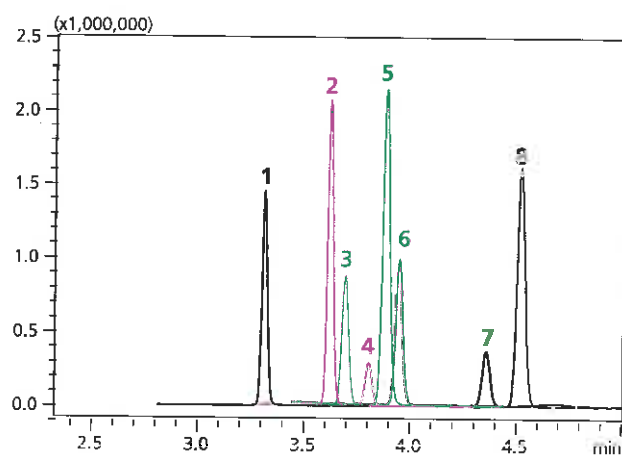


Figure 4 MRM chromatograms for early eluting 8 pesticides.

Table 2 Retention time (RT) of 8 compounds.

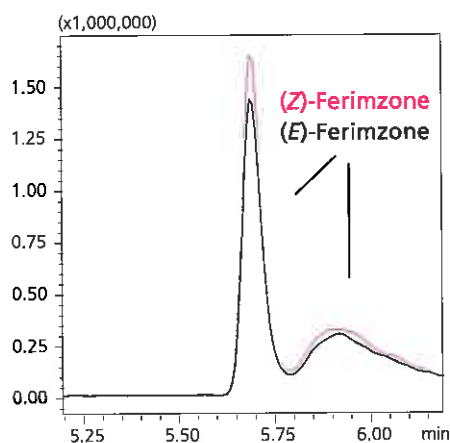
#	Compound	Transition	Retention Time (RT) / min		ΔRT^* / min
			ODS column	Biphenyl column	
1	Aldicarb-sulfone	240.20>86.10	2.844	3.318	0.474
2	Oxamyl	237.10>72.10	2.905	3.624	0.719
3	Methomyl	163.10>88.10	3.055	3.696	0.641
4	Pymetrozine	218.10>105.10	3.121	3.886	0.765
5	Thiamethoxam	292.00>211.10	3.136	3.951	0.815
6	Imidacloprid	256.10>175.10	3.446	4.364	0.918
7	Clothianidin	250.10>132.00	3.488	3.808	0.32
8	Flumetsulam	326.10>129.10	3.501	4.527	1.026

* $\Delta RT = RT \text{ (Biphenyl column)} - RT \text{ (ODS column)}$

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

The separation was also enhanced with the biphenyl column, even when the retention was not improved, such as the *cis-trans* isomers of (*E*)-Ferimzone and (*Z*)-Ferimzone.

a) ODS column



b) Biphenyl column

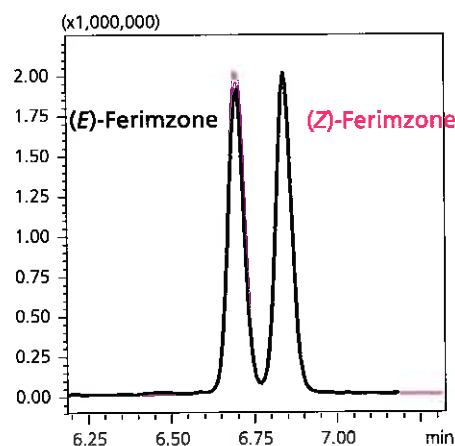
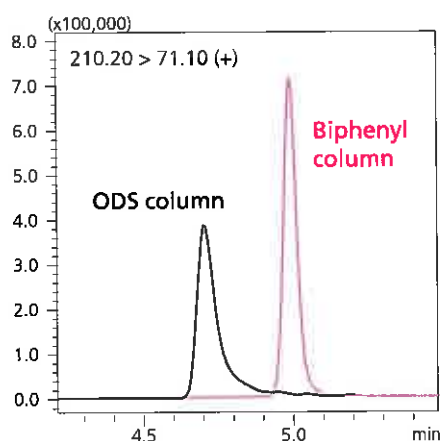


Figure 5. MRM chromatograms for (*E*)-Ferimzone and (*Z*)-Ferimzone.

Using the biphenyl column, the peak shapes improved for Dimethirimol and Iprovalicarb, which have aromatic groups, and hence sensitivity was higher than when using the ODS column. This tendency was also observed for other aromatic compounds.

a) Dimethirimol



b) Iprovalicarb

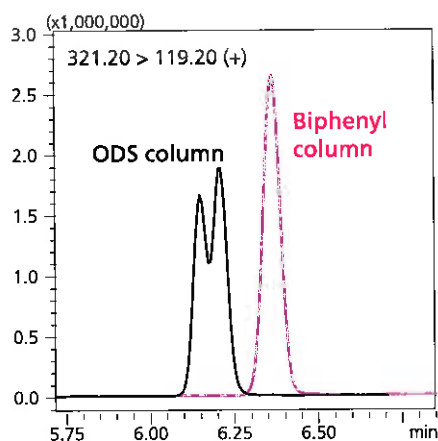


Figure 6. MRM chromatograms for Dimethirimol (a) and Iprovalicarb (b), black: ODS column and pink: biphenyl column

Multi-residue pesticides analysis by LC-MS/MS using an ODS column and the biphenyl column

Conclusions

- A biphenyl column as well as an ODS column is useful for the multi-residue pesticides analysis by LC-MS/MS.
- By developing unique technologies for fast data acquisition, the LCMS-8060 system creates new opportunities for high density analysis and expanded pesticides screening programs.

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Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

ASMS 2016 TP 223

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Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Introduction

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food. National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRL's) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL

enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This paper describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (typically 3 MRM transitions for each targeted pesticide resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Materials and Methods

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of

LCMS quality purchased from Sigma-Aldrich. A six point calibration curve from 0.002 – 0.1 mg/kg (2 – 100 pg/ μ L) were generated using an internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

Quantitative analysis of 646 pesticides (1,919 MRMs) LC-MS/MS with a fast 10.5 minute cycle time

Table 1. LC and MS/MS acquisition parameters used to create the generic LC-MS/MS method.

Liquid chromatography																	
UHPLC	: Nexera LC system																
Analytical column	: Restek Raptor biphenyl (100 x 2.1 mm, 2.7 µm)																
Column temperature	: 35°C																
Flow rate	: 0.4mL/minute																
Solvent A	: 2 mmol/L ammonium formate in 0.002% formic acid solution																
Solvent B	: 2 mmol/L ammonium formate in 0.002% formic acid solution																
Binary Gradient	: <table border="1"> <thead> <tr> <th>Time (mins)</th><th>%B</th></tr> </thead> <tbody> <tr> <td>0</td><td>3</td></tr> <tr> <td>1</td><td>10</td></tr> <tr> <td>3</td><td>55</td></tr> <tr> <td>10.5</td><td>100</td></tr> <tr> <td>12</td><td>100</td></tr> <tr> <td>12.01</td><td>3</td></tr> <tr> <td>15</td><td>Stop</td></tr> </tbody> </table>	Time (mins)	%B	0	3	1	10	3	55	10.5	100	12	100	12.01	3	15	Stop
Time (mins)	%B																
0	3																
1	10																
3	55																
10.5	100																
12	100																
12.01	3																
15	Stop																
Injection volume	: 2µL sample (plus 40µL water)																
Mass spectrometry																	
LC/MS/MS	: LCMS-8060																
Ionisation mode	: Heated electrospray																
Polarity switching time	: 5 msec																
Pause time	: 1 msec																
Total MRM transitions	: 1919 (1819 positive; 100 negative)																
MRM Dwell	: 4msec (target ion); 1msec (reference ion)																
Interface temperature	: 350°C																
Heating block	: 300°C																
Desolvation line	: 150°C																
Heating gas	: 10 L/min																
Drying gas	: 10 L/min																
Nebulising gas	: 3 L/min																

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Results

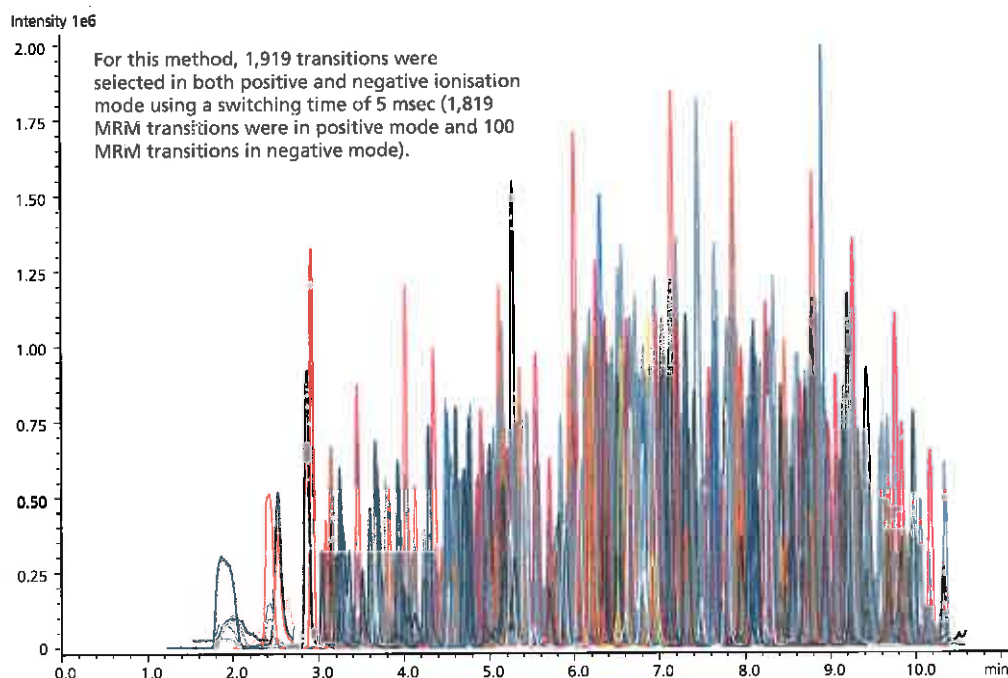


Figure 1. Chromatogram of 646 pesticides spiked into a mint extract at 0.010 mg/kg.
(Up to 3 MRMs per compound and 5 msec polarity switching time).

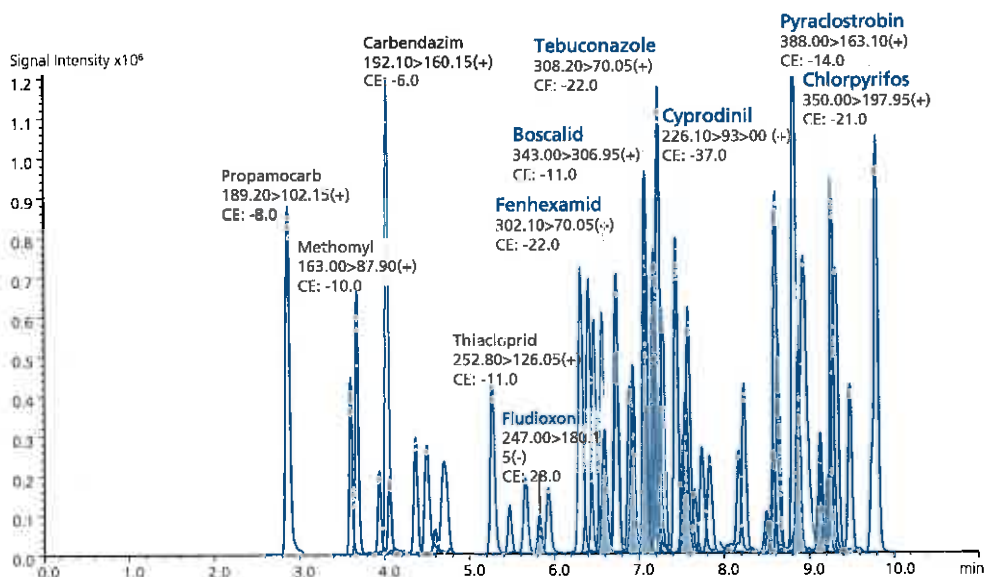


Figure 2. MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyrifos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4% of the samples analyzed. The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

Quantitative analysis of 646 pesticides (1,919 MRMs) LC-MS/MS with a fast 10.5 minute cycle time

High data density acquisitions

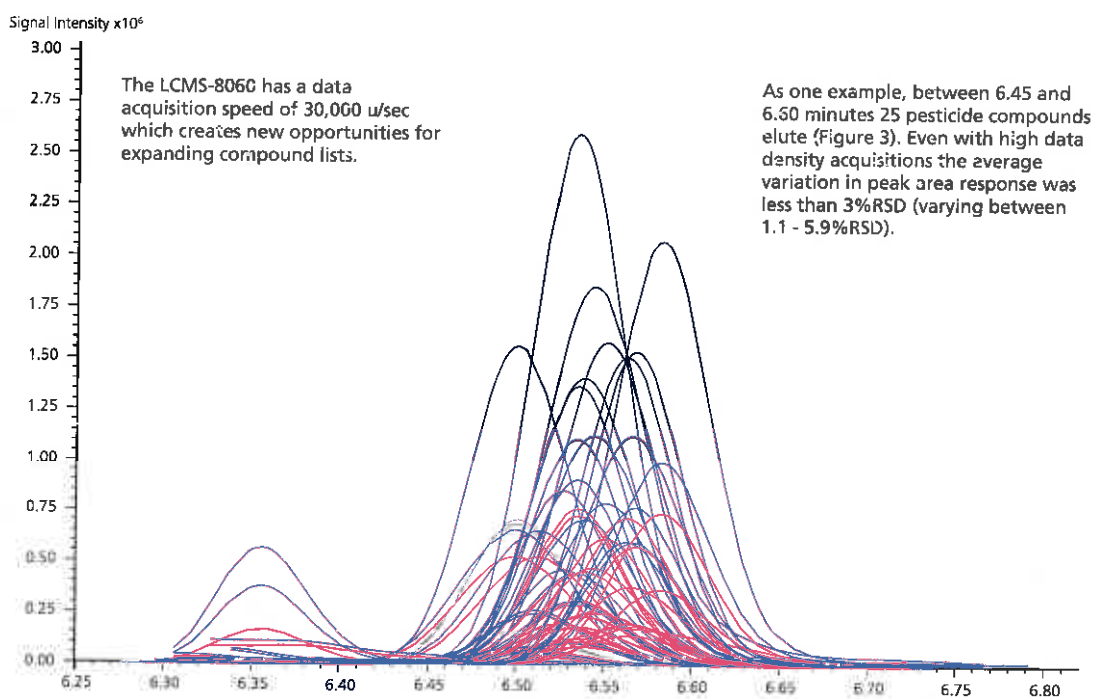


Figure 3. The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Table 2. The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density.
Between 6.45 and 6.60 minutes 25 compounds were monitored with a peak area variation less than 6% (%RSD; n=6).
(The sample is a mint matrix extract at the reporting limit of 0.01 mg/kg).

Compound Name	CAS number	Formula	M	Polarity	MRM	RT (mins)	%RSD (n=6)
Identifiers					Quantitation Ion		
Trinexapac-ethyl	95266-40-3	C ₁₃ H ₁₆ O ₅	252.0998	+	252.90>69.05	6.45	3.1
iprovalicarb	140923-17-7	C ₁₈ H ₂₈ N ₂ O ₃	320.2100	+	321.20>119.15	6.46	2.8
Dodemorph	1593-77-7	C ₁₈ H ₃₅ NO	281.2719	+	282.30>116.15	6.47	4.2
Fluopyram	658066-35-4	C ₁₆ H ₁₁ ClF ₆ N ₂ O	396.0464	+	397.00>145.00	6.47	1.9
Flutolanil	66332-96-5	C ₁₇ H ₁₆ F ₃ N ₂ O ₂	323.1133	+	324.10>242.00	6.48	2.7
Trifloxysulfuron	145099-21-4	C ₁₄ H ₁₄ F ₃ N ₅ O ₆ S	437.0617	+	438.00>182.15	6.48	2.5
Azaconazole	60207-31-0	C ₁₂ H ₁₁ Cl ₂ N ₃ O ₂	299.0228	+	300.00>159.00	6.50	2.0
Terbutryn	886-50-0	C ₁₀ H ₁₉ N ₅ S	241.1361	+	242.10>157.95	6.50	3.4
Prometryn	7287-19-6	C ₁₀ H ₁₉ N ₅ S	241.1361	+	242.10>158.00	6.50	2.6
Azimsulfuron	120162-55-2	C ₁₃ H ₁₆ N ₁₀ O ₅ S	424.1026	+	425.10>182.10	6.50	1.8
Metominostrobin	133408-50-1	C ₁₆ H ₁₆ N ₂ O ₃	284.1161	+	285.10>193.95	6.51	1.7
Thiifluzamide	130000-40-7	C ₁₃ H ₆ Br ₂ F ₆ N ₂ O ₂ S	525.8421	+	528.60>148.05	6.51	5.9
Nicarbazin	330-95-0	C ₁₃ H ₁₀ N ₄ O ₅	302.0651	-	301.10>137.15	6.52	2.6
Bromobutide	74712-19-9	C ₁₅ H ₂₂ BrNO	311.0885	+	312.10>194.10	6.53	2.1
Saflufenacil	372137-35-4	C ₁₇ H ₁₇ ClF ₄ N ₄ O ₅ S	500.0544	+	501.00>198.00	6.53	2.3
Cyproconazole	94361-06-5	C ₁₅ H ₁₈ ClN ₃ O	291.1138	+	292.10>70.05	6.54	1.7
Clomazone	81777-89-1	C ₁₂ H ₁₄ ClNO ₂	239.0713	+	239.90>125.00	6.54	1.7
Fensulfothion	115-90-2	C ₁₁ H ₁₇ O ₄ PS ₂	308.0306	+	309.00>281.00	6.54	1.4
Oxasulfuron	144651-06-9	C ₁₇ H ₁₈ N ₄ O ₆ S	406.0947	+	407.10>150.15	6.54	1.1
Rimsulfuron	122931-48-0	C ₁₄ H ₁₇ N ₅ O ₇ S ₂	431.0569	+	432.00>182.00	6.55	1.8
Fenthion-oxon	6552-12-1	C ₁₀ H ₁₅ O ₄ PS	262.0429	+	263.10>231.00	6.55	1.4
Nitrothal-isopropyl	10552-74-6	C ₁₄ H ₁₆ N ₂ O ₆ Na	317.0875	+	295.10>230.95	6.56	3.0
Chlorantraniliprole	500008-45-7	C ₁₈ H ₁₄ BrCl ₂ N ₅ O ₂	480.9708	+	483.90>452.90	6.57	2.7
Fipronil-sulfone	120068-36-2	C ₁₂ H ₄ Cl ₂ F ₆ N ₄ O ₂ S	451.9336	-	451.00>414.90	6.57	2.0
Valifenalate	283159-90-0	C ₁₉ H ₂₇ ClN ₂ O ₅	398.1608	+	399.20>155.00	6.59	1.9

Quantitative analysis of 646 pesticides (1,919 MRMs) LC-MS/MS with a fast 10.5 minute cycle time

Robustness

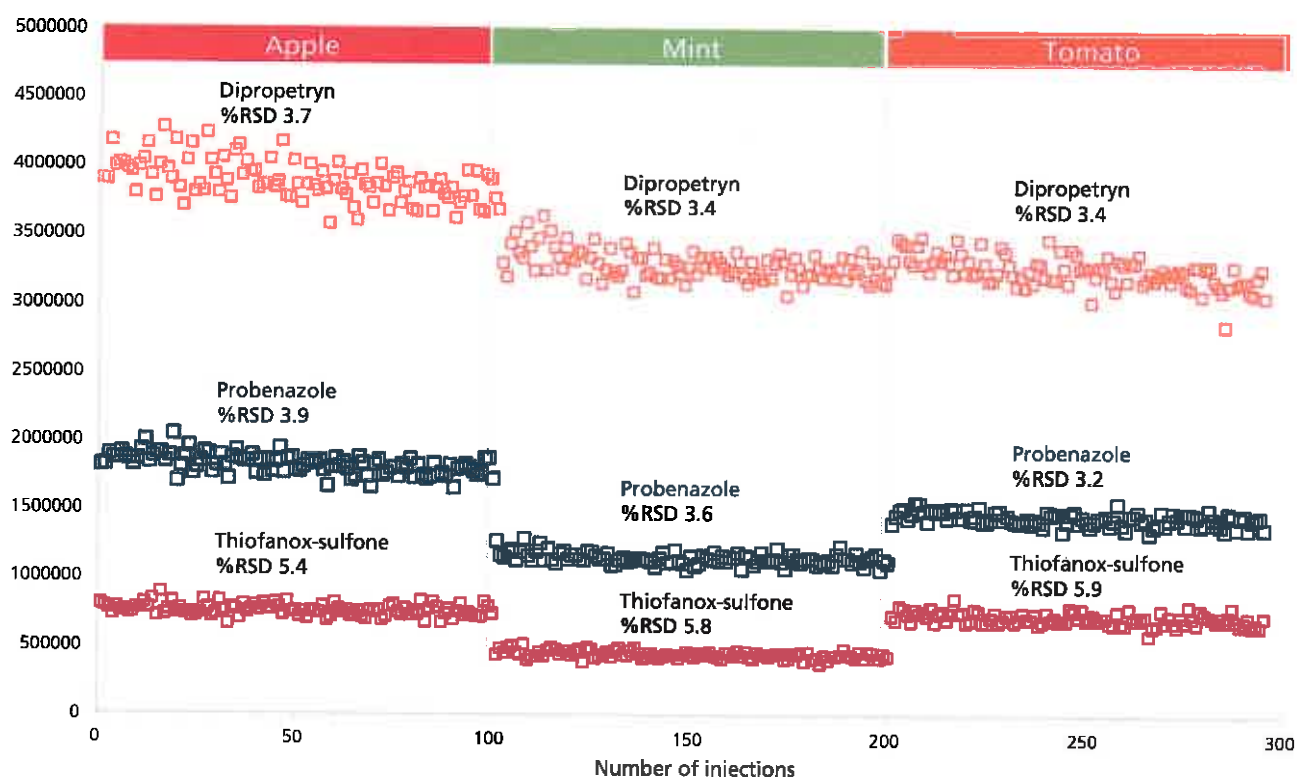


Figure 4. Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Figure 3, compounds were selected to reflect peak area response throughout the chromatographic run (Table 2).

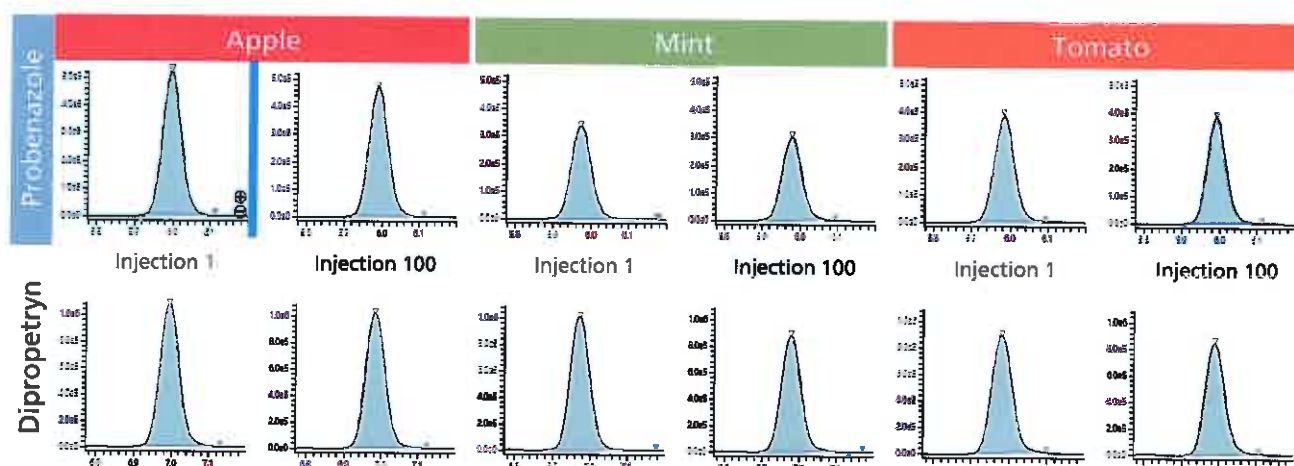


Figure 5. Peak area response for probenazole (RT 5.995 min) and dipropetryn (RT 6.999 min) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

Conclusions

- A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.
- As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.
- The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120%.

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A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

ASMS 2016 WP230

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*Student

A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

Introduction

Aflatoxins (B1, B2, G1 and G2) are metabolites produced by fungi (*Aspergillus favus* and *Aspergillus parasiticus*) in crops, animal feed and dairy products. Aflatoxins are highly toxic contaminants in food and feed and their amounts increase under bad storage conditions favourable for fungal growth. Aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1 found in milk of cow fed with a diet contaminated with aflatoxin B1^[1]. Aflatoxin B1 is known the most carcinogenic among all the aflatoxins, and hence its metabolite aflatoxin M1 is given critical attention. Strict regulations for aflatoxin M1 in milk and

dairy products have been set. For example, European Union (EU) limits the level of aflatoxin M1 to no more than 0.05 µg/kg in milk and dairy products and 0.025 µg/kg in infant food. We present a high sensitivity LC/MS/MS method for quantitative analysis of the five aflatoxins (B1, B2, G2, G2 and M1) in milk powder incorporating QuEChERS sample pre-treatment procedure, which is more cost effective as compared to the traditional procedure using immunoaffinity column (IAC)^[2]. High sensitivity and good recoveries were achieved using this LC/MS/MS method.

Experimental

A mixed standard of aflatoxin B1, B2, G1 and G2 was obtained from Supelco. Aflatoxin M1 was obtained from Romer Labs. A stock solution of the mixture of 5 aflatoxins was prepared using methanol as the diluent, from which calibrant series and spiked samples were prepared. The QuEChERS kits were purchased from RESTEK. Two grams of milk powder was first extracted with the extraction kits followed by cleaning up using

dSPE tubes. A LCMS-8060 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. A C18 column (Kinetex, 2.1 x 100mm, 1.7µm) was used for fast separation of aflatoxins using a gradient elution program. Method performance evaluation were carried out using spiked aflatoxins in milk powder samples. Table 1 shows the analytical conditions on LCMS-8060.



Shimadzu LCMS-8060, an UFMS triple quadrupole system with a heated ESI interface

High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

Table 1: LC/MS/MS analytical conditions of aflatoxins on LCMS-8060

Column	: Kinetex C18 (2.1mmI.D x 100mmL, 1.7 µm)
Flow rate	: 0.5 mL/min
Mobile phase	: A: 5 mM ammonium acetate in water with 0.1% FA B: 5 mM ammonium acetate in MeOH
Oven temp.	: 40°C
Injection vol.	: 5 µL
Elution mode	: Gradient elution, B%: 5% (0-5 min) 50% (4- 5.5 min) 85% (6-7.5 min) 5% (8.1-10 min)
Interface	: ESI (Heated)
MS mode	: Positive, MRM, 2 transitions each compound
Interface temp.	: 350°C
Block temp.	: 400°C
DL temp.	: 250°C
CID gas	: Ar (350 kPa)
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min

Results and Discussion

QuEChERS sample pre-treatment

Hexane was used in the procedure to remove fats, oils and non-polar components from the milk powder samples. The extraction step was completed using Q-sep QuEChERS extraction salt packet (4 g MgSO₄, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen

citrate). Dispersive SPE tube containing MgSO₄, PSA and C18 was used in the clean-up process to remove remaining water, organic acid and non-polar components respectively. The process of the sample preparation is illustrated in Figure 1.

Method Development

Automated MRM optimisation of the five aflatoxins was carried out using the LabSolutions workstation. Two MRM transitions for every aflatoxin were chosen as quantifier and confirmation ion (Table 2).

A milk powder matrix free from aflatoxins was used as a “blank” and matrix for the preparation of post-spiked calibrants to build calibration curves. The blank and every post-spiked calibrant was injected thrice and the average area was calculated to obtain reliable results.

A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

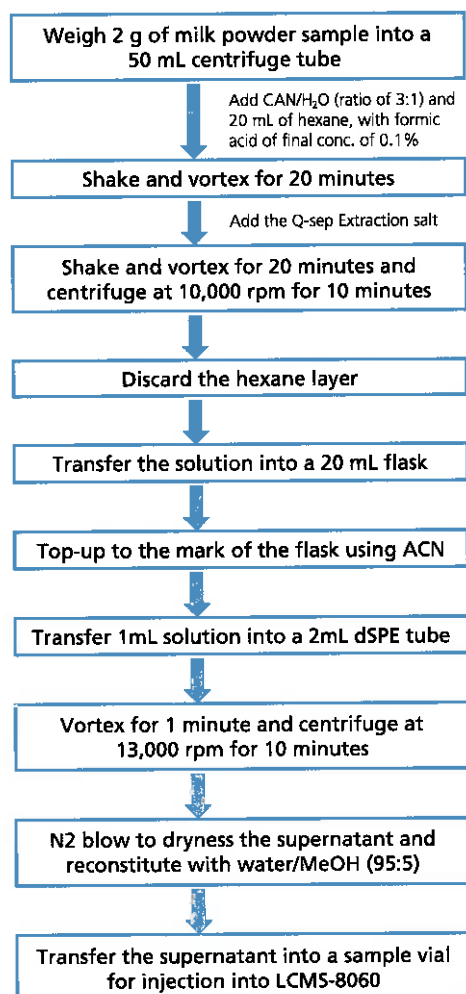


Figure 1: Flowchart of sample pre-treatment for aflatoxins in milk powders by modified QuEChERS method.

Table 2: LC/MS/MS analytical conditions of LCMS-8050 for aflatoxins

Compound	MRM (m/z)	CID Voltage (V)		
		Q1	CE	Q3
Aflatoxin B1	313.1>241.0*	-12	-40	-17
	313.1>213.0	-21	-44	-15
Aflatoxin B2	315.1>287.0*	-22	-27	-20
	315.1>259.1	-11	-30	-18
Aflatoxin G1	329.1>243.0*	-12	-28	-17
	329.1>200.0	-12	-40	-22
Aflatoxin G2	331.1>189.0*	-24	-43	-19
	331.1>245.0	-12	-31	-18
Aflatoxin M1	329.0>273.0*	-12	-23	-18
	329.0>259.0	-23	-24	-29

* MRM transitions used as quantifiers.

High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

A chromatogram of spiked sample is shown in Figure 2. Linear calibration curves were obtained for all five aflatoxin compounds with good linearity ($r^2 > 0.999$). The calibration curves of aflatoxins spiked in milk powder matrix are shown in Figure 4.

Method Performance Evaluation

The LOD and LOQ of aflatoxins in milk powder matrix are lower than 0.83 pg/mL and 2.50 pg/mL respectively (Table 3). The repeatability of the method was evaluated using spiked samples at two concentrations. The peak area %RSD of aflatoxins were found to be lower than 7.46%.

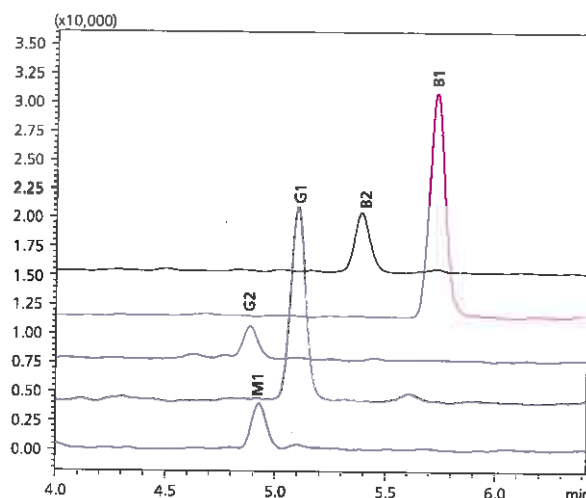


Figure 2: Total ion chromatogram of 5 aflatoxins (Concentrations of B1, G1 and M1 at 10 pg/mL; B2 and G2 at 3 pg/mL)

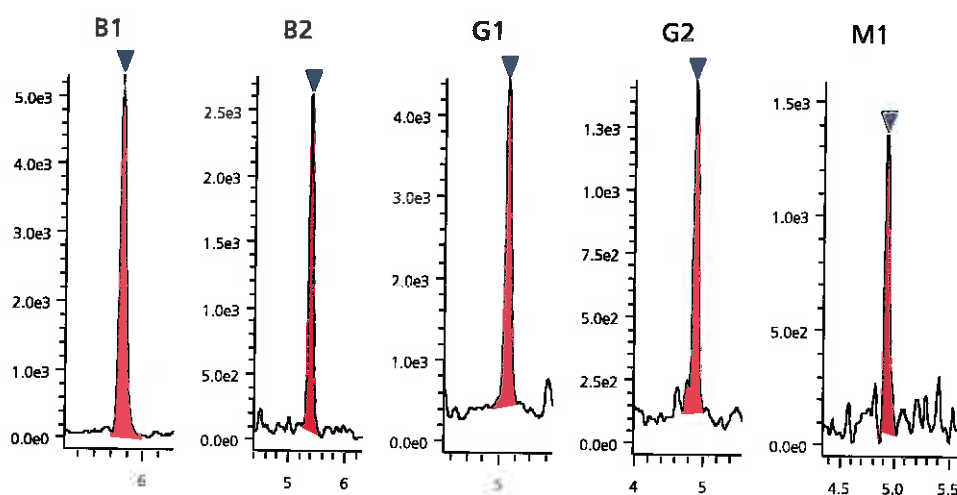


Figure 3: Single LOQ MRM chromatograms of 5 aflatoxins (Concentrations of B1, G1 and M1 (5 pg/mL); B2 and G2 (3 pg/mL))

A High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

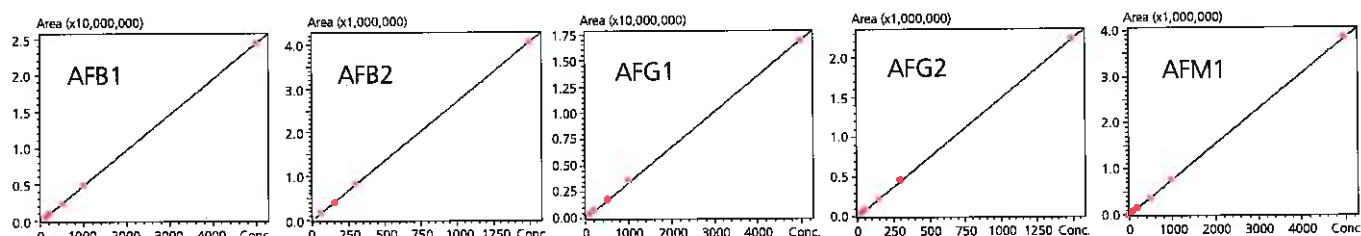


Figure 4: Calibration curves of aflatoxins B1, B2, G1, G2 and M1 in milk powder matrix.

Table 3: LOD, LOQ and repeatability of aflatoxin spiked samples at different concentrations

Aflatoxin	Range (pg/mL)	Linearity	LOD (pg/mL)	LOQ (pg/mL)	%RSD (n=6)			
					5 pg/mL	6 pg/mL	30 pg/mL	50 pg/mL
B1	1-5000	0.9999	0.14	0.44	3.1			2.3
B2	3-1500	0.9999	0.36	1.09		6.4	2.4	
G1	3-5000	0.9998	0.71	2.16	4.0			2.44
G2	3-1500	0.9999	0.41	1.22		5.8	3.5	
M1	3-5000	0.9999	0.83	2.50	7.5			2.7

Both the matrix effect and recoveries of aflatoxins were evaluated by using a duplicate set of samples at different concentrations. Each duplicate was obtained from the average of three injections. The results are shown in Table 4 and Table 5.

Table 4: Matrix effects of the MRM method for aflatoxins in spiked milk powder samples

Concentration (pg/mL)	Matrix effect (%)			Concentration (pg/mL)	Matrix effect (%)	
	B1	G1	M1		B2	G2
5.0	105.1	116.0	99.4	6.0	105.8	116.3
50.0	105.3	107.9	105.4	30.0	110.2	109.3

Table 5: Recoveries of aflatoxins in spiked milk powder samples

Concentration (pg/mL)	Recovery (%)			Concentration (pg/mL)	Recovery (%)	
	B1	G1	M1		B2	G2
5.0	76.6	87.3	83.8	6.0	71.6	70.8
50.0	73.8	76.5	75.6	30.0	73.9	75.6

Analysis of aflatoxins in actual milk powder samples

Three milk powder samples from local supermarket were analysed using the established MRM method. The results showed that no aflatoxin was detected in all three samples.

High Sensitivity LC/MS/MS Method with QuEChERS Sample Pre-treatment for Analysis of Aflatoxins in Milk Powder Samples

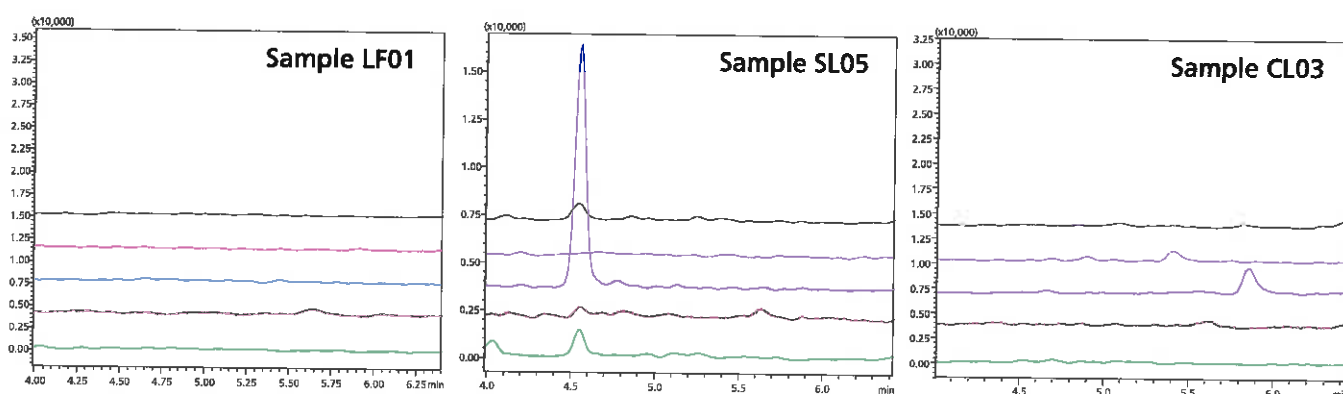


Figure 5: MRM Chromatograms for Aflatoxin B2, B1, G2, G1 and M1 (top to bottom) of three milk powder samples from local supermarket. Targets were not detected in all samples.

Conclusions

A high sensitivity LC/MS/MS method with QuEChERS for sample pre-treatment was established using Shimadzu LCMS-8060 system. The QuEChERS sample preparation method was proven effective and easy to operate. The method performance including sensitivity, linearity,

repeatability, matrix effect and recovery were carried out and the results confirm that the method is feasible and reliable for determination of aflatoxins in milk powder samples.

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1. Iqbal, S. Z.; Jinap, S.; Pirouz, A. A.; Ahmad Faizal, A.R. *Trends in Food Science & Technology* 2015, **46**, 110-119.
2. Cherkani-Hassani, A.; Mojemmi, B.; Mouane, N. *Trends in Food Science & Technology* 2016, **50**, 56-69.

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Technical Report

Comprehensive Two-dimensional Liquid Chromatography for Determination of Polyphenols in Red Wines

LCxLC-PDA-MS/MS for polyphenol analysis in red wine

Paola Dugo^{1,2}, Francesca Rigano¹, Francesco Cacciola¹, Paola Donato¹, Luigi Mondello^{1,2}

Abstract:

A comprehensive two-dimensional liquid chromatography method was developed and applied to the determination of polyphenols in red wines. To fulfil such a task, a micro cyano and a partially porous octadecylsilane columns were employed in the first and the second dimension, respectively in combination with photodiode array and mass spectrometry (LCxLC-PDA-MSMS) detection. To increase the peak capacity values by using RP modes in both dimensions, a comparison of a conventional full-in-fraction and shifted second dimension gradient was carried out. The separation capabilities of the comprehensive LC approaches tested allowed the analysis of such a complex natural sample without any pre-treatment to effectively reduce the interferences coming from the matrix.

Keywords: comprehensive LC, polyphenols, red wines, mass spectrometry

1. Introduction

Phenolic compounds are secondary metabolites synthesized by plants during normal development and in response to stress conditions. They embrace a considerable range of substances possessing an aromatic ring bearing one or more hydroxyl moieties. Produced and consumed world-wide, wine is an excellent natural source of various polyphenol families that go from phenolic acids (benzoic- or cinnamic-like derivatives) to different classes of flavonoids (flavones, flavan-3-ols, flavonols and anthocyanins).

Sometimes, the polyphenol content in real world-samples can be so complex that they cannot be resolved in a one-dimensional HPLC analysis. In order to overcome this problem, comprehensive two-dimensional liquid chromatography (LCxLC) employing two columns with different selectivity in the two dimensions could be a viable tool. In addition, in order to improve the peak distribution, different gradient elution strategies could be investigated to enhance the orthogonality degree, by means of specific elution gradient approaches to be used in the second dimension.

This technical report describes a novel LCxLC-PDA-MSMS (Fig. 1) instrument, capable of extremely high-resolution power, as well as targeted and untargeted analysis, that was successfully applied to the characterization of the polyphenol content a red wine sample (Fig. 2).



Fig. 1 LCxLC-PDA-MSMS instrumentation

2. Experimental

2-1. Reagents and Materials

LC-MS grade solvents for LCxLC analyses: water (H₂O), acetonitrile (ACN); Acetic acid 99–100%, (glacial). All the solvents and chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Chromatographic separations were carried out using columns provided by Supelco (Bellefonte, PA, USA): Ascentis Cyano (250 mmL. × 1 mmI.D., 5 µm d.p.), and Ascentis Express C₁₈ (30 mmL. × 4.6 mmI.D., 2.7 µm d.p.).

The red wine was purchased in a local market. The sample was filtered through a 0.45 µm Acrodisc nylon membrane (Pall Life Sciences, Ann Arbor, MI, USA) before injection.

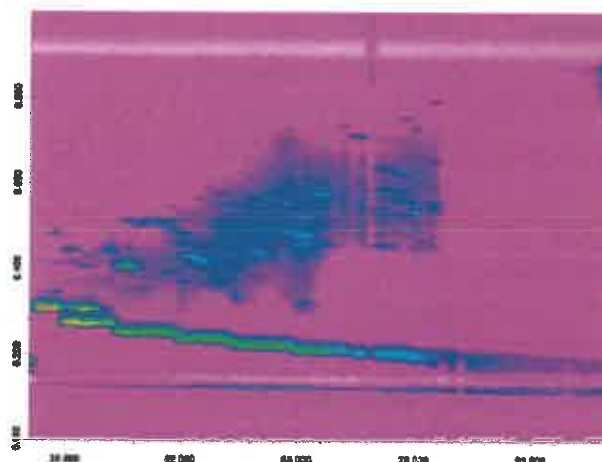


Fig. 2 RP-LCxRP-LC Plot of a red wine sample

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² Chromaleont S.r.l.

2-2. Instrument

- Shimadzu CBM-20A controller
- Shimadzu LC-30AD dual-plunger parallel-flow pumps (D1-LC)
- Shimadzu DGU-20A_{SR} degassing unit (D1-LC)
- Shimadzu LC-30AD dual-plunger parallel-flow pumps (D2-LC)
- Shimadzu DGU-20A_{SR} degassing unit (D2-LC)
- Shimadzu CTO-20AC column oven
- Shimadzu SIL-30AC autosampler
- Shimadzu SPD-M30A photo diode array detector (1 μ L flow cell)
- Shimadzu LCMS-8030 (ESI source)

For connecting the two dimensions: two electronically-controlled 2-position, 6-port high pressure switching valves FCV-32AH (with two 20 μ L empty loops).

2-3. Software

- Shimadzu LabSolutions (Version 5.60 SP2)

2-4. 2D Software

- LCxLC-Assist
- ChromSquare (Version 2.0) from Chromaleont, Messina, Italy

3. LCxLC-MS Conditions

First dimension (D1) separations	
Column	: Ascentis Cyano
Flow rate	: 20 μ L/min
Mobile phases	: (A) 0.1% acetic acid in water (pH around 3); (B) acetonitrile 0.1% acetic acid.
Gradient elution	: 0.01 min, 2% B; 10 min, 2% B; 60 min, 50% B; 75 min, 100% B; 100 min, 100% B.
Backpressure (at analysis start)	= 40 bar
Injection volume	: 5 μ L
Second dimension (D2) separations	
Column	: Ascentis Express C18
Flow rate	: 2.5 mL/min
Mobile phases	: (A) 0.1% acetic acid in water (pH around 3); (B) acetonitrile 0.1% acetic acid.
Gradient elution	: FIF, full in fraction: 0.01 min, 0% B; 0.10 min, 0% B; 0.75 min, 50% B; 1.00 min, 0% B. SG, Shifted gradient: illustrated in Fig. 3
Backpressure (at analysis start)	= 170 bar
Modulation time of the switching valves	: 1 min.

MS conditions

MS acquisition performed using the ESI interface operating in negative ionization mode:

mass spectral range: 100–800 m/z ; event time: 0.1 sec; scan speed: 7500 u/s ; nebulizing gas (N_2) flow: 2 L/min; drying gas (N_2) flow: 15 L/min; Heat block temperature: 250 $^{\circ}$ C; desolvation line (DL) temperature: 250 $^{\circ}$ C; Interface voltage: 3.5 kV; detector voltage: 1.80 kV; The flow eluting from the second column was splitted before the MS instrument (approximately 0.4 mL/min to the MS).

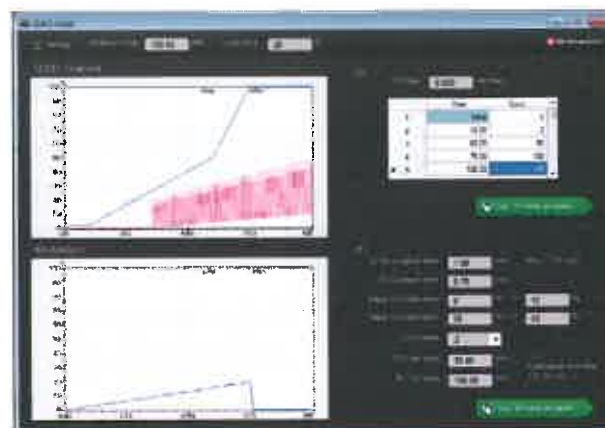


Fig. 3 Scheme of the LCxLC-Assist software

4. Results and Discussion

In an LCxLC system, with two not orthogonal dimensions, an LCxLC separation most likely results in peaks concentrated around the main diagonal line of the separation area. A typical example is the LCxLC analysis of a red wine sample, illustrated in Fig. 4A, by employing a Cyano column in the D1 and a C18 column in the D2 using the conventional full-in-fraction approach.

As a matter of fact, a clear correlation of the D1 and D2 and a small peak-distribution area were observed because the separation mechanisms in the two dimensions were similar. The analytes eluted early in the D1 were only weakly retained in the D2; the analytes eluted in the middle of the D1 were eluted in the middle of the D2 and the analytes eluted late in the D1 were strongly retained in the D2.

To overcome such a limitation, we used a narrower organic solvent span changing the gradient program according to the elution properties. The shifted gradient program, led to a greater coverage of the separation space (Fig. 4B). The blue line is the program of the D1 run and the red line is that of the D2 run. The D2 gradient covered a narrow organic solvent range, which varied continuously during the LCxLC run. The gradient program started with 0% acetonitrile and rose to 20% ACN over 0.75 min; at the end of the analysis, the gradient program in the D2 starts at 10% acetonitrile and rose to 50% acetonitrile. At the end of the analysis, the higher percentage of organic solvent made possible the efficient elution of the strongly retained compounds.

As can be seen from Fig. 4B regarding the red wine sample analyzed with LCxLC with a shifted gradient in the D2, a significant improvement in the retention space was attained (Fig. 5). In fact, the use of a shifted gradient with a gradual increase of the proportion of organic solvent gave better separation in the D2 with a less typical diagonal-line distribution. In addition, because of the narrower solvent range, the backpressure was much smoother and steadier.

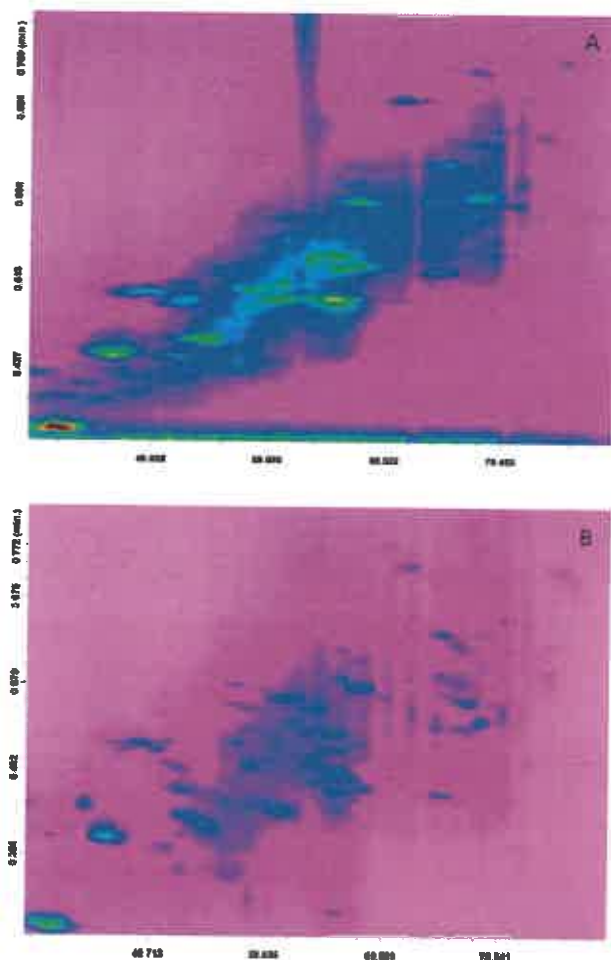


Fig. 4 Separation of a red wine sample by using the FIF (A) and the shifted gradient (B) approaches

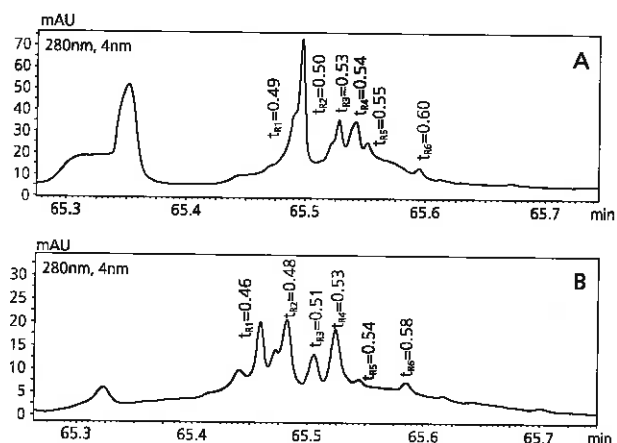


Fig. 5 Analysis of a red wine sample. A comparison of the second-dimension separation of the 65th fraction for both LCxLC plots shown in Fig. 4A–B.

An evaluation of the performance, in terms of peak capacity (n_c), of the two different set-ups tested was carried out, considering both theoretical and practical peak capacity. The theoretical peak capacity values, multiplicative of the individual values obtained for the two dimensions ($^1n_c \times ^2n_c$), yielded values as high as 690 and 570 for the full in fraction and for the shifted gradients, respectively.

As expected, due to the partial correlation of the two dimensions, “practical” peak capacity values, corrected for both undersampling (number of fractions effectively transferred from the D1 to the D2) and orthogonality (separation space effectively covered by sample compounds), were significantly lower at 75 and 216. The set-up with the use of the shifted second dimension gradients was the most efficient one since it less suffered from the correlation of the two dimensions tested (Table 1).

Selected ion extracted chromatograms for some target compounds occurring in the red wine sample along the relative mass spectra are illustrated in Fig. 6.

Table 1 Relative performances, in terms of peak capacity, n_c , of the two set-up investigated

	Full in fraction gradient	Shifted gradient
^1n_c	15	15
^2n_c	46	38
Theoretical D2 n_c	690	570
Practical D2 n_c	75	216

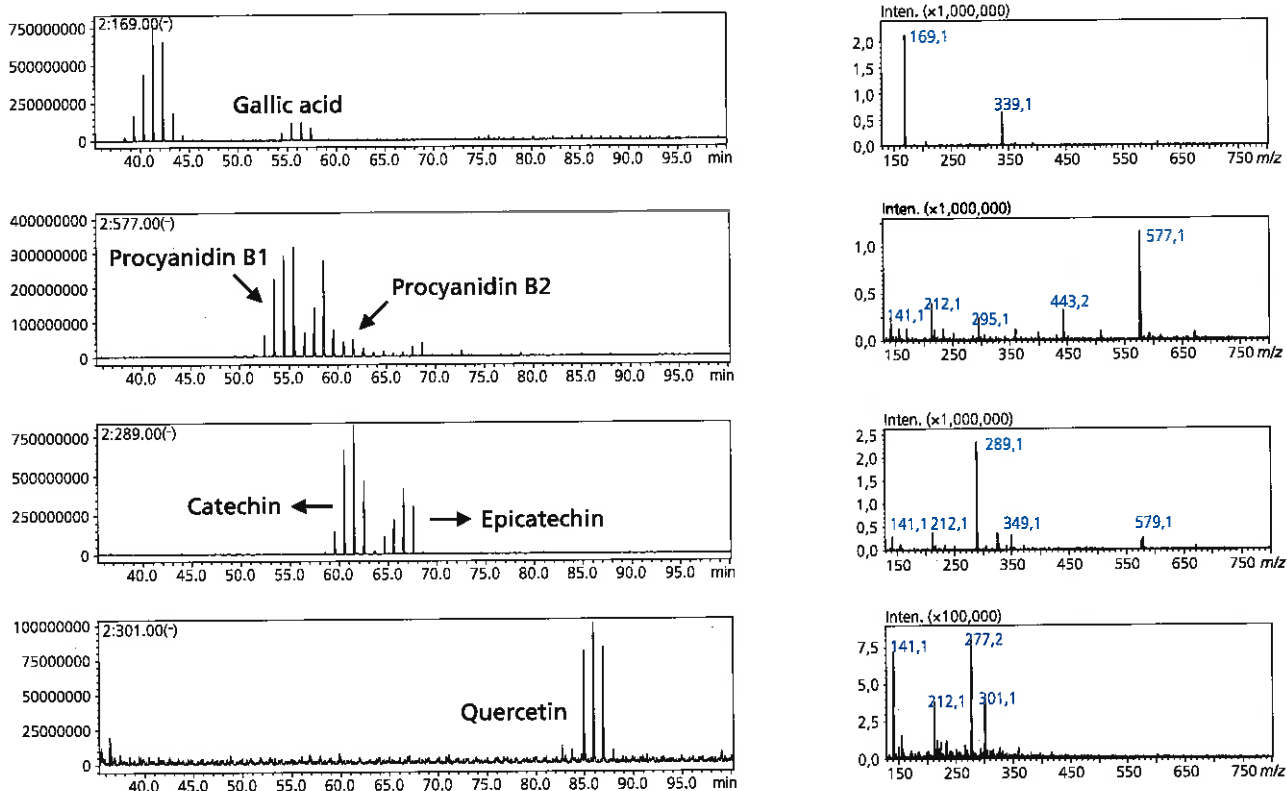


Fig. 6 Ion extracted chromatograms (on the left) along with relative mass spectra (on the right) of the main polyphenolic compounds identified in the red wine sample investigated

5. Conclusions

A comprehensive two-dimensional liquid chromatography system, based on the use of a micro cyano column and a partially porous (C_{18}) column in the first and second dimension, respectively, in combination with photodiode array and mass spectrometry detection, is presented.

Two second dimension gradient approaches, namely full in fraction and shifted were investigated and compared in terms of peak capacity.

The shifted gradient method used increased the effective peak-distribution area in the LC $\times$ LC analysis of a red wine sample.

Therefore, the use of a shifted gradient in an LC $\times$ LC system brings about a significant improvement in separation power and is a great advantage in the analysis of such complex samples.

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Technical Report

Screening and Individual Quantitative Works of Regulated Substances in Food Using Triple Quadrupole Mass Spectrometer LCMS-8030

1. Introduction

Our environment is overflowing with a variety of chemical substances, and the effect of these substances on our health is becoming a matter of worldwide concern. Looking at agrochemicals alone, more than a thousand different types of compounds are widely used, therefore it is significantly important to develop a screening method capable of analyzing large number of compounds at low concentration levels. The LC/MS/MS is widely employed for the food safety because it features excellent selectivity and sensitivity by using Multiple Reaction Monitoring (MRM). However, it is well-known that the number of compounds which can be monitored is limited by the length of dwell and pause time because the length of both parameters affects the MRM sensitivity.

The dwell time is the time during which an ion is monitored by the detector in MRM, and the pause time is the time required for MRM switching. Since the dwell time and pause time are parameters which affect the sensitivity of the method as well as the instrument's data sampling rate, the dwell and pause time is a critical parameter in achieving appropriate sensitivity to quantify the regulated substances in a complex food matrix. The LCMS-8030 features extremely fast pause and dwell times of 1 msec.

In this Technical Report, we used the LCMS-8030 triple quadrupole mass spectrometer (Fig. 1) to evaluate whether or not 10 pesticide compounds used to spike a matrix at 10 ppb each could be detected automatically by using generic peak detection method in the screening for 176 pesticides using 1 msec pause and 5 msec dwell times.



Fig. 1 LCMS-8030 Triple Quadrupole Mass Spectrometer

2. Method

A matrix consisting of green tea was used and sample preparation was conducted according to the methods of simultaneous measurement of pesticides using LCMS, specified by Japan's Ministry of Health, Labour and Welfare. The test sample used to evaluate the screening performance consisted of green tea extract solution spiked with 10 pesticides (Carbofuran, Chlorfluazuron, Fosthiazate, Hexathiazox, Indanofan, Lufenuron, Mevinphos, Propoxur, Pyrimidifen, and Tricyclazole), each at a 10 ppb concentration.

A 100 ppb spiked sample was also prepared to develop the generic peak integration parameters used for compound identification. For each MRM mass chromatogram, the largest peak was automatically selected as the target compound to permit automatic identification of target analytes without retention time data. Fig. 2 shows the actual LabSolutions LCMS software (hereafter, LabSolutions) parameters (left: Peak Integration, right: Peak Identification). Of course, the LabSolutions software also supports peak identification using a compound's retention time but the retention time information was not used for peak identification in this experiment.

For the screening, 352 MRM transitions were monitored. Since 2 MRM transitions were set for each compound, 176 compounds could be screened. Of the 176 compounds, the MRM conditions for 167 of these compounds that are included in the Residual Agrochemicals Method Package (Fig. 3) were used.

Since the MRM conditions for each compound can be saved using LabSolutions, multiple MRM transitions can be imported into any LabSolutions HPLC method. Fig. 4 shows the window that is displayed when adding MRM events to the HPLC method (window at left), and the MS event window after the MRM conditions have been imported (window at right). The light blue-colored items are the MRM events in negative mode, and the pink-colored items are the MRM events in positive mode. The width of each band indicates the time window each MRM will be monitored. In this case, all MRM transitions were monitored over the entire chromatographic run, from 0 minutes to 20 minutes.

The dwell and pause time for each MRM was set to 5 msec and 1 msec, respectively. Including polarity switching, the time required for all 352 MRM transitions, including polarity switching, is 2.058 sec. This is called as the Loop Time. Since the chromatographic peak widths are expected to be about 20 sec, approximately 10 data points for each peak will be acquired.

The liquid chromatograph conditions that were used were as follow:
Column: Shim-pack XR-ODS II (75 mmL. x 2.0 mmI.D., 2.2 µm)
Mobile phase A: 5 mmol/L ammonium acetate - water
Mobile phase B: 5 mmol/L ammonium acetate - methanol

Gradient conditions: 30% B (0 min.) → 80% B (4 min.) → 95% B (10 min.) → 30% B (15.01–20 min.)
Flow rate: 0.2 mL/min.
Column oven temperature: 40°C
Injection volume: 2 µL

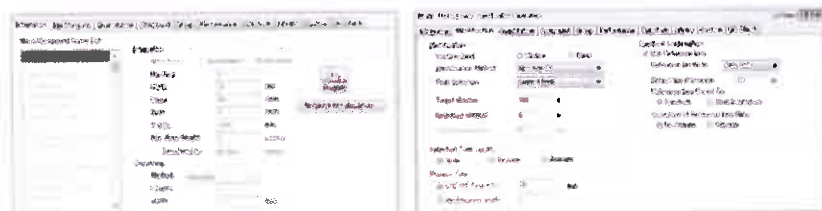
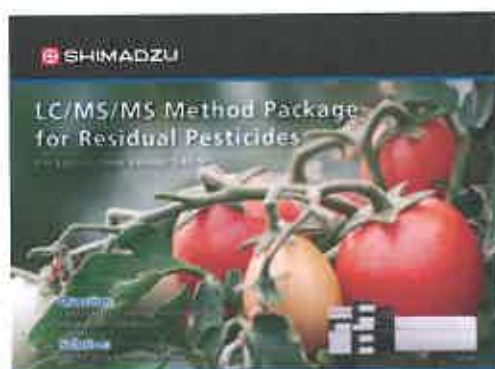


Fig. 2 Peak Integration Parameters (left) and Peak Identification Parameters (right) Screens Used for Screening



Analytical column : Shim-pack FC-ODS (2.0 mmI.D. x 150 mmL., 3 µm)
Mobile phase A : 5 mmol/L ammonium acetate - Water
Mobile phase B : 5 mmol/L ammonium acetate - Methanol
Flow rate : 0.2 mL / min.
Injection volume : 1 µL

- MRM for 167 compounds
- Retention time information
- HPLC conditions

[illegible]

00%	10%	20%	30%	40%	50%	60%	70%	80%	90%
0	0	85	15		5	17.5	5	95	
1	1	60	40		6	30	5	95	
2	3.5	60	40		7	30.1	85	15	
3	6	50	50		8	40	85	15	
4	8	45	55						

Fig. 3 Residual Agrochemicals LC/MS/MS Method Package

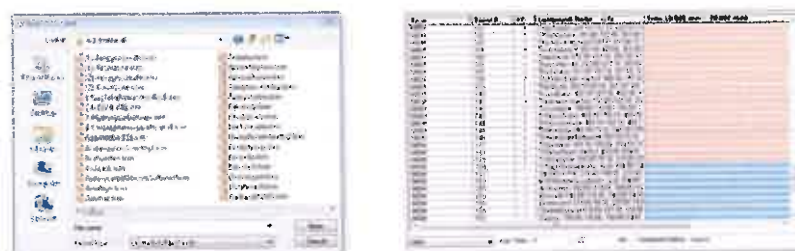


Fig. 4 Adding MRM Events (left), Analysis Screen After Loading MRM Conditions (right)
Light blue-colored items are negative MRM, and pink-colored items are positive MRM.
The width of band indicates the monitoring time span.

3. Screening Results for Green Tea Matrix

Fig. 5 shows the detection results for a sample consisting of green tea spiked with 10 pesticides, each at a concentration of 100 ppb. In the data processing window of LabSolutions, the respective tables and chromatograms are linked, and show the pesticide chromatogram associated with the corresponding green-colored row in the table. The ▼ at the peak center is the peak detection mark, whose retention time is displayed in the table. The red up arrow and down

arrow indicate the peak start point and end point, respectively. The "Auto" in the Mode column of the table indicates that the peak was automatically detected. Compounds which were not detected display the phrase "No peak is detected."

In the Mevinphos chromatogram (second from top chromatogram at right in Fig. 5), 3 peaks are detected including Mevinphos, but the

correct peak was identified because the largest peak was specified in the peak identification conditions. The remaining 9 pesticides were also correctly identified.

The detection results for green tea extraction spiked with the 10 pesticides at a concentration of 10 ppb each are also displayed in Fig. 6. Seven of the ten pesticides were detected and identified, while Chlorfluazuron, Indanofan, and Lufenuron were detected but not automatically identified. It was clear that the dwell time of 5 msec was too short for detection and automatic identification of 10

substances present at 10 ppb without retention time information, but all 10 substances were identified when retention time information was used.

Fig. 7 shows the chromatograms of the 10 pesticides measured using a dwell time of 20 msec. The 3 substances (Chlorfluazuron, Indanofan, Lufenuron) that were not detected in the screening with generic peak detection parameters were detected without using retention time information.



Fig. 5 Detection Results for 100 ppb-Spiked Sample (Green Tea)
"Auto" in the Mode column indicates peaks that were detected automatically.

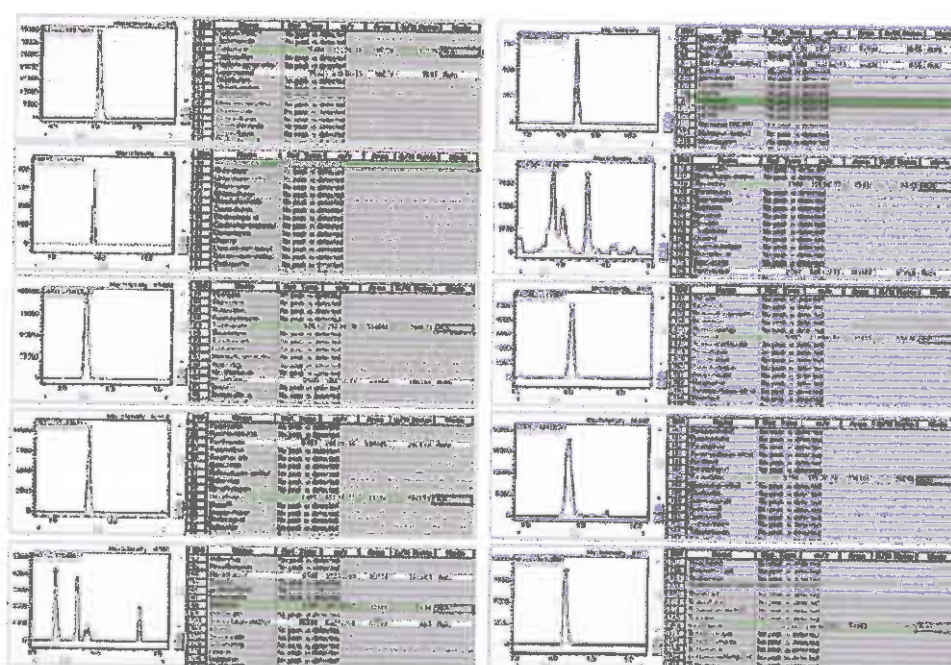


Fig. 6 Detection Results for 10 ppb-Spiked Sample (Green Tea)

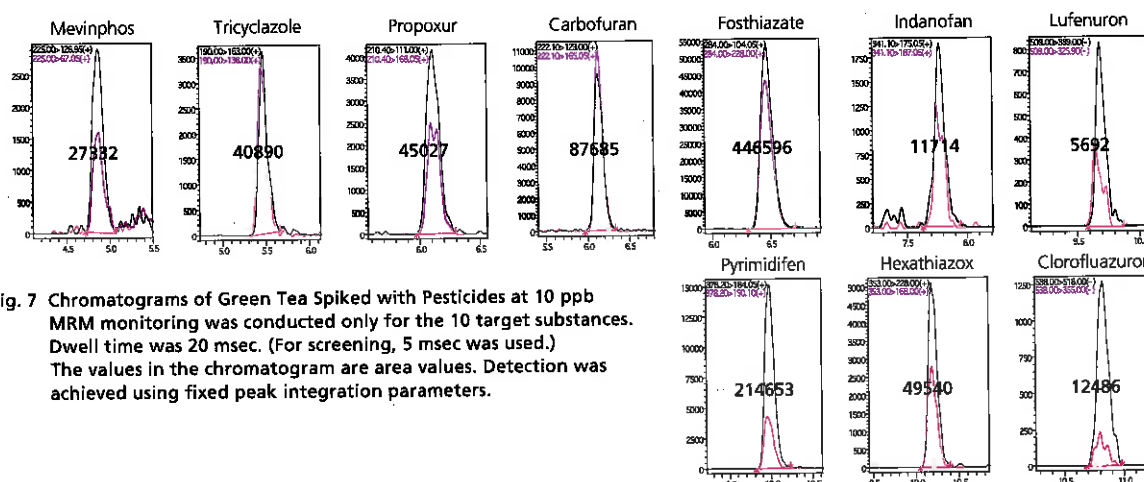


Fig. 7 Chromatograms of Green Tea Spiked with Pesticides at 10 ppb
MRM monitoring was conducted only for the 10 target substances.
Dwell time was 20 msec. (For screening, 5 msec was used.)
The values in the chromatogram are area values. Detection was
achieved using fixed peak integration parameters.

4. Individual Quantitation of 10 Pesticides in Green Tea

Due to the excessive presence of caffeine in the green tea matrix, it was necessary to assess the influence of caffeine on the quantitation values of the various pesticides. When analysis was conducted on the matrix-spiked sample, the area values of the respective pesticides showed great variation. For example, after a 100 ppb spiked sample was injected in the midst of injecting a 100 ppb standard, the %RSD ranged from 5.4% (Tricyclazole) to 37.3% (Chlorfluazuron). To suppress the influence of caffeine, a divert valve was inserted between the column outlet and the MS to eliminate the caffeine. As a result, the area value %RSD of each pesticide in the samples spiked at 10 ppb was less than 10%.

There was no attempt during screening to increase the sensitivity by optimizing the ionization parameters, but here, the ionization parameters were optimized in order to obtain better sensitivity. Table 1 shows the details of the optimized parameters. Fig. 8 shows the chromatograms of Propoxur, Carbofuran, Indanofan and Hexathiazox obtained before and after optimization of the ionization parameters. With the pesticide standards, the area values of the pesticides increased about 2-fold as a result of optimizing the ionization parameters.

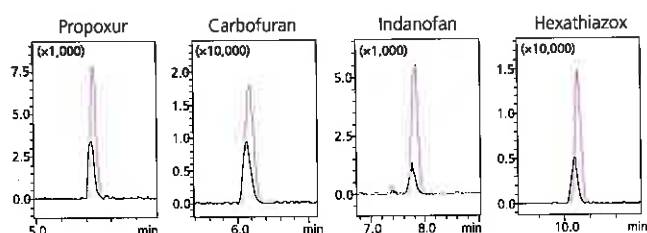


Fig. 8 Comparison of Chromatograms
Before and After Optimization
of Ionization Parameters

Table 1 Optimization of Ionization Parameters

Nebulizing Gas Flow	1.5 L / min. → 3.0 L / min.
Drying Gas Flow	10 L / min.
DL Temperature	250°C → 300°C
BH Temperature	400°C → 500°C
Probe Position	+2 → +3

5. Conclusion

As shown in Fig. 7, a 20 msec dwell time was best for detection and automatic identification of the pesticides added to green tea at 10 ppb. If the minimum dwell time that satisfies the target quantitation limit can be determined, the number of compounds that can be screened in a single measurement will depend on the HPLC method that will be used and the instrument's polarity switching speed. Since polarity switching takes only 15 msec with the LCMS-8030, and assuming that 10 sampling points can be acquired for each substance due to the approximately 20 sec required for elution of each peak based on the employed HPLC conditions, 100 compounds can be screened and quantitated using a dwell time setting of 20 msec and limiting the number of monitored MRMs to one per compound.

By using the retention time information, each of the 10 analyte pesticides could be detected at a concentration of 10 ppb in green tea, even with the extremely short dwell time of 5 msec. Simultaneous analysis methods, which also include HPLC conditions, have been published by each country's regulating authority. The sensitivity and speed of the LCMS-8030 more than satisfy these requirements for multi-component screening and quantitation.



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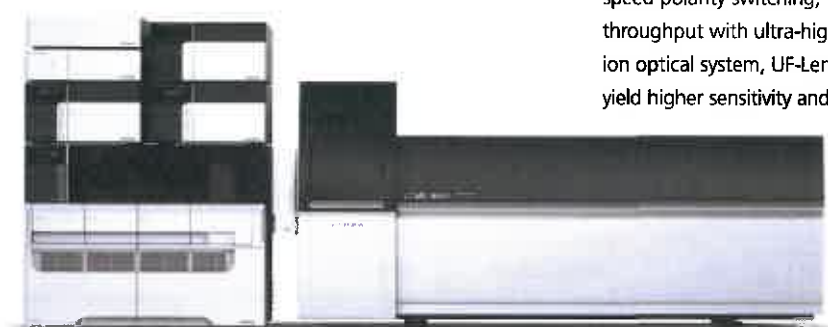
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Specification Sheet

Liquid Chromatograph Mass Spectrometer

LCMS-8040



Triple Quadrupole Mass Spectrometry is the method of choice for definitive identification and reproducible quantification of trace level analytes in complex samples for a variety of applications such as pharmacokinetics in pharma/CRO, food, environmental, forensic/toxicology and newborn screening. Combined with chromatographic resolving power of our world leading UHPLC systems and maintaining Shimadzu's proprietary ultrafast technologies (UF Technologies) which includes high-speed MRM transitions, MS/MS acquisitions, and ultra-high speed polarity switching, the LCMS-8040 can dramatically improve analytical throughput with ultra-high speed performance. In addition, the newly improved ion optical system, UF-Lens™ and unique UFsweeper™ II collision cell technology yield higher sensitivity and expand the potential range of LC/MS/MS applications.

Instrument

Model	LCMS-8040
Mass range	<i>m/z</i> 2 to 2,000
Sensitivity	ESI positive: 1 pg reserpine, <i>S/N</i> >10,000:1 (RMS) ESI negative: 1 pg chloramphenicol, <i>S/N</i> >10,000:1 (RMS)
Resolution	<i>R</i> < 0.7 <i>u</i> FWHM
Mass stability	0.05 <i>u</i> / 12 hr
Cross talk	< 0.003%
Minimum pause time	1 msec
Minimum dwell time	0.8 msec
Scan speed	Max 15,000 <i>u</i> /sec (0.1 <i>u</i> step: 150,000 data points/sec)
Polarity switching time	15 msec
Interface	ESI (Standard), APCI (Optional), DUIS (Optional)
Applicable LC flow rate	ESI 1 μ L/min to 2 mL/min
MRM transition speed	Max 555 channels/sec
DL maximum temperature	300 °C
Block heater maximum temperature	ESI/DUIS: 500 °C APCI: 300 °C
Interface maximum temperature	APCI: 500°C

Analysis mode	Q1 Scan/SIM Q3 Scan/SIM MRM Precursor ion scan Product ion scan Neutral loss scan

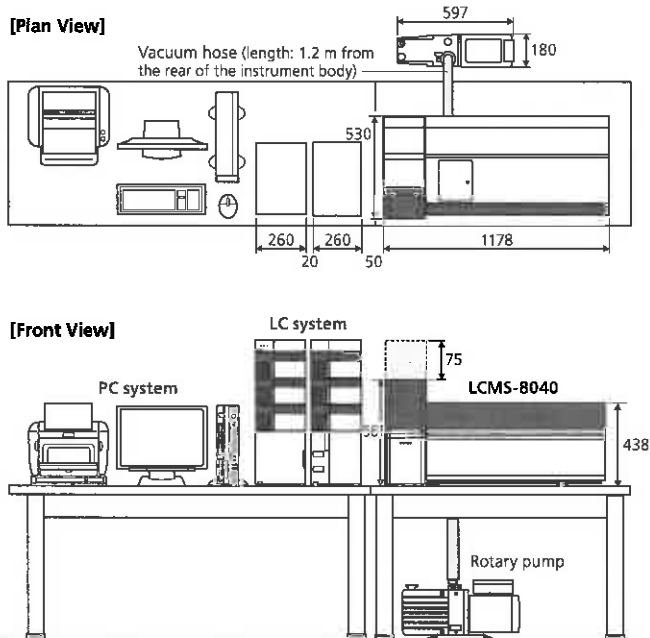
Mass Analyzers and Detector

Mass analyzers	Q1 & Q3 are molybdenum hyperbolic mass filters with pre-rods; Q1 includes post-rods
Collision cell	Tapered multipole type ultra-high-speed collision cell (UFsweeper™ II collision cell)
Detector	Secondary electron multiplier with off-axis conversion dynode
Ion optics	Q-array focus optics operating in Field-Flow mode, multipole transfer optics
Digital detection system	Operates in pulse counting mode for fastest operation
Detection mode	Ultra-fast positive/negative ion switching
Vacuum system	Rotary pump: 1 unit Vacuum pumping speed: 28 m ³ /hr Triple-inlet turbo molecular pump: 1 unit 40 L/sec, 260 L/sec, 210 L/sec

Software

Workstation	LabSolutions LCMS
Operation system	Windows 7 32 Bit, 64 Bit
Instrument control	Prominence and Nexera series
MS acquisition mode	Scan (Max. 1,000 events), SIM (Max. 1,000 events × 32 channels)
MS/MS acquisition mode	MRM (Max. 1,000 events × 32 channels) Product ion scan Precursor ion scan Neutral loss scan
Auto-tuning	Possible to optimize sensitivity and resolution in both positive and negative ionization mode as well as mass calibration

Installation Example



Units: mm

Installation Conditions

Temperature	18 to 28 °C
Humidity	20 to 70 % (Non-condensing)
Size	1180 mm (W) × 530 mm (D) × 560 mm (H)
Weight	130 kg
Power supply	MS unit: AC 230 V 15 A (50/60 Hz) Single-phase
Gas requirements	Nitrogen gas: Maximum consumption 25 L/min, Purity greater than 97 % Argon: Purity greater than 99.99 % as CID gas

The above are not standard installation specification. All LCMS-8040 instruments will be installed and tested in accordance with standard performance tests as detailed in the Shimadzu document ZEAH-0450, Shimadzu High-Performance Liquid Chromatograph Mass Spectrometer LCMS-8040 Installation Standard.
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Specification Sheet

Liquid Chromatograph Mass Spectrometer

LCMS-8045



Instrument

The LCMS-8045 offers the proven high sensitivity, high speed, and robustness of Shimadzu's UFMS series to provide highly reliable data for applications that demand the sensitivity and speed of a mass spectrometer, such as for simultaneous analysis used in the food safety and environmental measurement fields. Due to the heated-ESI probe and UFsweeper™ II collision cell, it offers the **highest sensitivity in the middle-range class (UFsensitivity)**. LCMS-8045 also includes other various patented proprietary ultrafast technologies (UF technologies), such as ultra-high-speed polarity switching (UFswitching) and ultra-high-speed scanning (UFscanning). In combination with Shimadzu's UHPLC system, which is among the fastest in the world, the LCMS-8045 can shorten analysis times even further. It also features excellent durability and ease-of-maintenance refined over many years.

Model	LCMS-8045
Mass range	<i>m/z</i> 2 to 2,000
Sensitivity	ESI positive: 1 pg reserpine, S/N > 100,000:1 (RMS) ESI negative: 1 pg chloramphenicol, S/N > 100,000:1 (RMS)
Resolution	R < 0.7 μ FWHM
Mass stability	0.05 μ /12 hr
Cross-talk	< 0.003 %
Minimum pause time	1 msec
Minimum dwell time	0.8 msec
Scan speed	Max 30,000 u/sec (in all modes of scanning) (0.1 u step: 300,000 data points/sec)
Polarity switching time	5 msec
Interface	ESI (Standard), APCI (Optional), DUIS (Optional)
Applicable LC flow rate	ESI 1 μ L/min to 2 mL/min
MRM transition speed	Max 555 channels/sec
DL maximum temperature	300 °C
Block heater maximum temperature	ESI/DUIS: 500 °C APCI: 300 °C
Interface maximum temperature	ESI/DUIS: 400 °C APCI: 500 °C

Analysis mode	Q1 Scan/SIM Q3 Scan/SIM MRM Precursor ion scan Product ion scan Neutral loss scan

Mass Analyzers and Detector

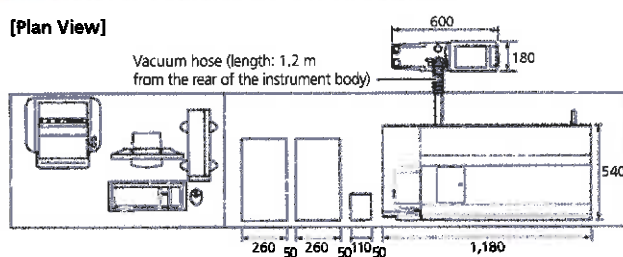
Mass analyzers	Q1 & Q3 are molybdenum hyperbolic mass filters with pre-rods; Q1 includes post-rods
Collision cell	Tapered multipole type ultra-high-speed collision cell (UFsweeper™ II collision cell)
Detector	Secondary electron multiplier with off-axis conversion dynode
Ion optics	Q-array focus optics operating in Field-Flow mode, multipole transfer optics
Digital detection system	Operates in pulse counting mode for fastest operation
Detection mode	Ultra-fast positive/negative ion switching
Dynamic range (pulse counting)	2×10^7 cps
Vacuum system	Rotary pump: 1 unit Vacuum pumping speed: 28 m ³ /hr Triple-inlet turbo molecular pump: 1 unit 40 L/sec, 260 L/sec, 210 L/sec

Software

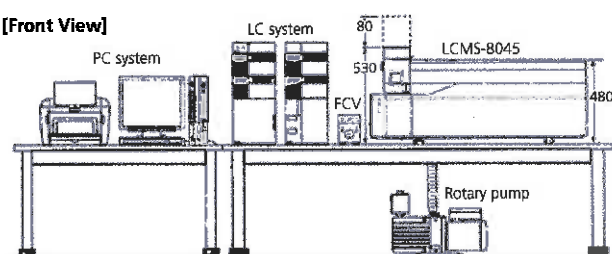
Workstation	LabSolutions LCMS
Operating system	Windows 7 32 Bit, 64 Bit
Instrument control	Prominence and Nexera series
MS acquisition mode	Scan (Max. 1,000 events), SIM (Max. 1,000 events × 32 channels)
MS/MS acquisition mode	MRM (Max. 1,000 events × 32 channels) Product ion scan Precursor ion scan Neutral loss scan
Auto-tuning	Possible to optimize sensitivity, resolution, and mass calibration in both positive and negative ionization mode

Installation Example

[Plan View]



[Front View]



Units: mm

Installation Conditions

Temperature	18 to 28 °C
Humidity	20 to 70 % (Non-condensing)
Size	1,180 mm (W) × 540 mm (D) × 610 mm (H)
Weight	140 kg
Power supply	MS unit: AC 230 V 15 A (50/60 Hz) Single-phase
Gas requirements	Nitrogen gas: Maximum 24.4 L/min, Purity greater than 97 % Argon: Purity greater than 99.99% as CID gas Dry air: Maximum 20 L/min, oil/water-free Total nitrogen plus air: 25L/min maximum

The above are not standard installation specification. All LCMS-8045 instruments will be installed and tested in accordance with standard performance tests as detailed in the Shimadzu document ZEAH-0500, Shimadzu High-Performance Liquid Chromatograph Mass Spectrometer LCMS-8045 Installation Standard.



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Ultra High Performance Liquid Chromatograph

Nexera X2



Liquid Chromatograph Mass Spectrometer

LCMS-8040

UFMS
ULTRA FAST MASS SPECTROMETRY



Shimadzu Scientific Instruments

Company Overview



Ultra High Performance Liquid Chromatograph

Nexera X2 Specifications



Shimadzu's Mass Spectrometry Solutions

Mass Spectrometry General Product Catalog

UPLC
ULTRA FAST MASS SPECTROMETRY



Liquid Chromatograph Mass Spectrometer

LCMS-8040

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