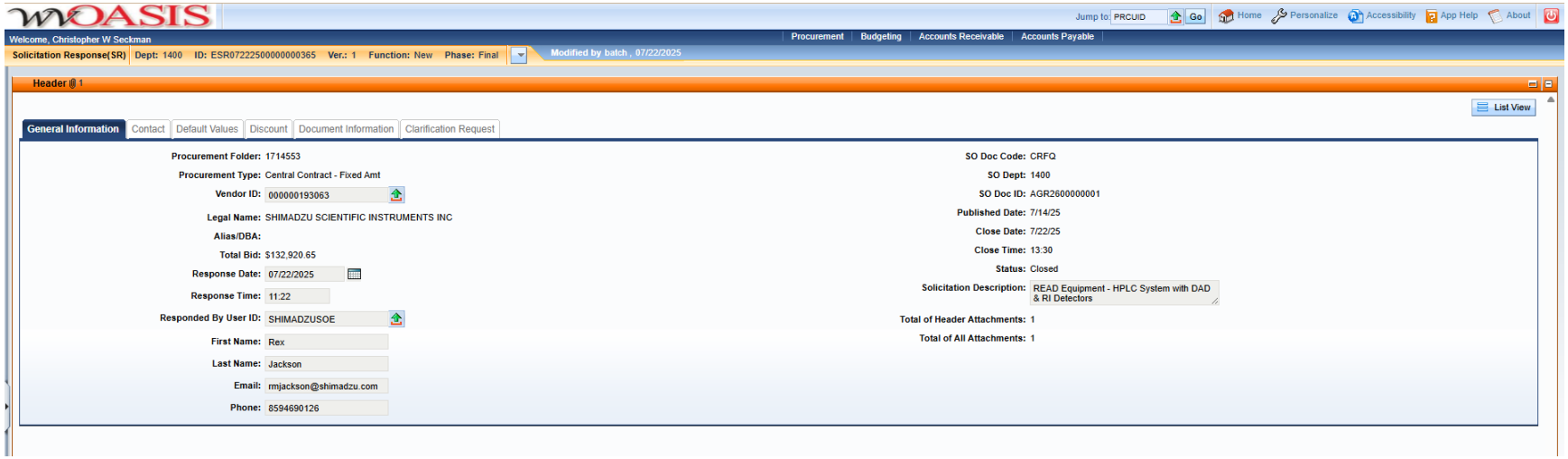
The following documentation is an electronically-submitted vendor response to an advertised solicitation from the *West Virginia Purchasing Bulletin* within the Vendor Self-Service portal at ***wvOASIS.gov***. As part of the State of West Virginia's procurement process, and to maintain the transparency of the bid-opening process, this documentation submitted online is publicly posted by the West Virginia Purchasing Division at ***WVPurchasing.gov*** with any other vendor responses to this solicitation submitted to the Purchasing Division in hard copy format.


WVOASIS
Jump to PRCUID Go
Home
Personalize
Accessibility
App Help
About
Welcome, Christopher W Seckman
Procurement
Budgeting
Accounts Receivable
Accounts Payable
Solicitation Response(SR) Dept: 1400 ID: ESR07222500000000365 Ver.: 1 Function: New Phase: Final
Modified by batch , 07/22/2025
Header @ 1
List View
General Information
Contact
Default Values
Discount
Document Information
Clarification Request
Procurement Folder: 1714553
Procurement Type: Central Contract - Fixed Amt
Vendor ID: 000000193063
Legal Name: SHIMADZU SCIENTIFIC INSTRUMENTS INC
Alias/DBA:
Total Bid: $132,920.65
Response Date: 07/22/2025
Response Time: 11:22
Responded By User ID: SHIMADZUSOE
First Name: Rex
Last Name: Jackson
Email: rmjackson@shimadzu.com
Phone: 8594690126
SO Doc Code: CRFQ
SO Dept: 1400
SO Doc ID: AGR2600000001
Published Date: 7/14/25
Close Date: 7/22/25
Close Time: 13:30
Status: Closed
Solicitation Description: READ Equipment - HPLC System with DAD & RI Detectors
Total of Header Attachments: 1
Total of All Attachments: 1

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

State of West Virginia
Solicitation Response

Proc Folder:	1714553
Solicitation Description:	READ Equipment - HPLC System with DAD & RI Detectors
Proc Type:	Central Contract - Fixed Amt

Solicitation Closes	Solicitation Response	Version
2025-07-22 13:30	SR 1400 ESR07222500000000365	1

VENDOR

000000193063
SHIMADZU SCIENTIFIC INSTRUMENTS INC

Solicitation Number: CRFQ 1400 AGR2600000001

Total Bid: 132920.6499999999941792339086 **Response Date:** 2025-07-22 **Response Time:** 11:22:00

Comments:

FOR INFORMATION CONTACT THE BUYER
Larry D McDonnell
304-558-2063
larry.d.mcdonnell@wv.gov

Vendor
Signature X **FEIN#** **DATE**

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

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Ln Total Or Contract Amount
1	HPLC System with Diode-Array and Refractive Index Detectors				132920.65

Comm Code	Manufacturer	Specification	Model #
41115707			

Commodity Line Comments:

Extended Description:

See attached documentation for further details.

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READ Equipment - HPLC System with DAD & RI Detectors
CRFQ 1400 AGR2600000001

Shimadzu Scientific Instruments, Inc Response

Prepared on: 07/15/22
Prepared for: Larry Mcdonnell

Department of Agriculture
Regulatory Protection Division
313 Gus R Douglas Ln
Bldg 11
Charleston, WV 25305

Exhibit A - Pricing Page - REVISED as of 7-14-2025

HPLC-DAD-RI

CRFQ AGR26*01

Section No.	*Description*	*Model #/Brand Name*	*Quanity*	*Unit Price*	*Extended Amount*
3.1.1	HPLC System with DAD & RI Detectors and all required parts/accessories	Shimadzu Scientific Instruments, Inc / LC-40	1	$132,920.65	$ 132,920.65
3.1.3 & 3.1.6	Warranty/Corrective Maintenance Service	Shimadzu Scientific Instruments, Inc	1	$0	$ 0 -
3.1.4	Installation	Shimadzu Scientific Instruments, Inc	1	$0	$ 0 -
3.1.4.3	Onsite Training	Shimadzu Scientific Instruments, Inc	1	$0	$ 0 -
	Failure to use this form may result in disqualification			**GRAND TOTAL**	$ 132,920.65
	Bidder / Vendor Information	Shimadzu Scientific Instruments, Inc			
Name:	Rex Jackson/Shimadzu Scientific Instruments, Inc				
Address:	7102 Riverwood Drive				
	Columbia, MD 21046				
Phone:	859-469-0126 / 800-477-1227				
Email Address:	rmjackson@shimadzu.com / Customer.service@Shimadzu.com				
Authorized Signature:	Faith Hays				

ADDENDUM ACKNOWLEDGEMENT FORM
SOLICITATION NO.: CRFQ AGR26*01

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 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

Faith Hays
Authorized Signature

07/22/25
Date

NOTE: This addendum acknowledgement should be submitted with the bid to expedite document processing.

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Included attachments in Shimadzu response:

SSI0X – Signed bid disclosure form
SSI01 – Shimadzu response
SSI02 – Shimadzu HPLC Installation Completion Report
SSI03 – Shimadzu LC-40 Brochure
SSI04 – Shimadzu LC-40 spec sheet
SSI05 – Honey Analysis made easy
SSI06 - Natural Cannabinoid and Cannflavin Profiling by HPLC-PDA
SSI07 – Shimadzu Quote for HPLC-DAD-RI system

SHIMADZU
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SSI01 Shimadzu Response

Response to technical specifications

Bid specification section 3 GENERAL REQUIREMENTS	**Shimadzu specification**
3.1.1 High-Performance Liquid Chromatography System (HPLC)	All components of Shimadzu bid are High-performance Liquid Chromatography (HPLC)
3.1.1.1 HPLC must have two completely independent flow paths capable of running different mobiles phases, column temperatures and detectors simultaneously.	• Shimadzu HPLC has 2 independent flow paths, capable of running different mobile phases, gradients, column temperatures and detectors simultaneously. • Each flow path for Shimadzu HPLC has the ability to use up to 4 solvents for high pressure gradient formation
3.1.1.2 Pump(s)	Shimadzu pumps are LC-40XS model
3.1.1.2.1 The pump(s) must have an over pressure limit setting.	Shimadzu pumps have overpressure limit settings
3.1.1.2.2 The pump(s) must have an under-pressure limit setting.	Shimadzu pumps have under-pressure limit settings
3.1.1.2.3 The pump(s) must be able to detect leaks.	Shimadzu pumps and column ovens both have integrated leak sensors
3.1.1.2.4 The pump(s) must be capable of solvent degassing.	Shimadzu pumps have solvent degassers. Up to 10 solvent lines can be degassed simultaneously
3.1.1.2.5 The pump(s) must have a piston seal wash.	Shimadzu pumps have piston seal washes
3.1.1.2.6 The pumps must be able to handle a psi of 15,000 at 5mL/min.	Shimadzu pumps have a Pmax of 15,000psi. The below snippet is taken from the LC-40 specification sheet: 0.0001 – 3.0000 mL/min (1.0 – 105 MPa) 3.0001 – 5.0000 mL/min (1.0 – 80 MPa) 5.0001 – 10.0000 mL/min (1.0 – 22 MPa)
3.1.1.2.7 The pump(s) must have a flow range of a minimum 0.001 mL/min to maximum 8.0mL/min.	Shimadzu pumps have a flow range of 0.0001-10.0000 mL/min
3.1.1.3 Autosampler	Shimadzu autosampler is Sil-40XS model
3.1.1.3.1 The autosampler must have two independent	Shimadzu autosampler has two independent injection

injection valves.	valves
3.1.1.3.2 Autosampler must have a temperature range of 4°C or lower and a high temperature of 40°C or higher.	Shimadzu autosampler has a temperature range of 4°C to 45°C
3.1.1.3.3 The injection volume must have a minimum range of 0.01 µL to 100µL.	The Shimadzu autosampler has a volume range of 0.1 µL-2000 µL
3.1.1.3.4 The autosampler tray must have a capacity of 200 vials (1.5 or 2mL in size) at a minimum.	The Shimadzu autosampler comes standard with 162 vial positions. Shimadzu has included a plate changer which holds up to 486 sample vials. The total number of 1.5mL/2mL vials Shimadzu autosampler can hold is 648 vials.
3.1.1.4 Column Oven	The Shimadzu column oven is the CTO-40C
3.1.1.4.1 The HPLC-DAD-RI system must have at a minimum two column ovens which have individual temperature controls.	The Shimadzu HPLC system has 2 column ovens which have independent temperature controls.
3.1.1.4.2 The column oven must have a temperature range of 5°C or lower to 120°C or higher.	The Shimadzu column ovens have a temperature range of 4°C to 100°C
3.1.1.5 Diode Array Detector (DAD)	The Shimadzu Diode Array Detector is referred to as a Photo Diode Array (PDA) detector. The Shimadzu PDA is SPD-M40.
3.1.1.5.1 The DAD must have a 1024 photodiode array or better.	The Shimadzu PDA has a 1024 photodiode array.
3.1.1.5.2 The DAD range must be 190nm to 800nm at a minimum with a minimum wavelength accuracy of ±1nm.	The Shimadzu PDA has a wavelength range of 190nm-800nm with wavelength accuracy of ±1nm.
3.1.1.5.3 The DAD Spectral bandwidth resolution must be 0.5nm or better.	The Shimadzu PDA has a spectral bandwidth resolution of ≤ ±1.4 nm
3.1.1.5.4 The DAD baseline noise must be ≤3uAU	The Shimadzu PDA has a baleine noise of ≤ 4.5 × 10^{-6}AU (under specified conditions)
3.1.1.5.5 The DAD drift must be ≤0.5 mAU/hr	The Shimadzu PDA drift is ≤ 0.4 × 10−3 of AU/h
3.1.1.5.6 The DAD must have 10 Data Channels at a minimum.	The Shimadzu PDA has 16 data collection channels
3.1.1.5.7 The DAD light source must be a deuterium lamp	The PDA light source has a deuterium light source
3.1.1.6 Refractive Index Detector (RI)	The Shimadzu RI detector is the RID-20A
3.1.1.6.1 The detector must have a refractive index range of 1.00 to 1.75 or broader.	The Shimadzu RID has a range of 1.00 to 1.75 RIU

3.1.1.6.2 The RI noise must be ≤1.25 nRIU	The Shimadzu RID noise is ≤ 2.5 × 10−9 RIU
3.1.1.6.3 The RI's drift must be ≤0.2 uRIU/h	The Shimadzu RID has a drift of ≤0.1 uRIU/h
3.1.1.6.4 The RI must have a temperature range of 30°C or lower and a high temperature of 55°C or higher.	The Shimadzu RID has a temperature range of 4°C to 35°C
3.1.2 Shipping	
3.1.2.1 The bidder must explain the details of its proposed packaging sizes for the deliverable(s). Vendor should provide details with their bid response but must provide upon request.	The proposed packaging size of the Shimadzu HPLC will be a standard pallet. The expected packaging size will be 1 pallet @ 48x40x60 inches with a rough weight of 150 pounds and 1 pallet @ 53x31x58 inches with a rough weight of 250 pounds.
3.1.2.2 Shipping fees must be incorporated into the unit price of the instrument.	Shipping fees are included in the unit price of the instrument.
3.1.3 Warranty	
3.1.3.1 The vendor must provide a one (1) year minimum parts and labor warranty on all items.	The Shimadzu HPLC comes standard with 1-year of bumper-to-bumper coverage on the instrument. All travel, parts, and labor costs associated with repairs are included at no charge. Shimadzu also offers technical support for **free** for the lifetime of ownership. Technical support can be answered via phone, email, or remote session.
3.1.4 Installation and Validation	
3.1.4.2 The vendor must provide a written validation of the system's performance after installation.	Shimadzu HPLC will be installed and validated according to a rigorous set of internal Shimadzu standards. More information on the pre-installation checklist is provided in Shimadzu attachment **SSI02**
3.1.4.3 The vendor must provide a minimum of three (3) days of onsite training for a minimum of three (3) Agency staff members. That means twenty-four (24) total hours of training, eight (8) hours each day.	Shimadzu will provide training for up to 10 days as needed, for up to 5 agency staff members. This means up to 80 hours of training, 8 hours each day. This training can be broken up into multiple days/weeks, if multiple shifts of employees will be required to attend. The agency is not required to have 10 days of training, but it is included in the cost of the system. The training may also be broken up into 2 sessions, which some customers prefer when a new system is

	installed. This allows for the agency to have an initial training to get started, and a deeper training 3-9 months in to owning the system.
3.1.5 Maintenance, Support, and Calibration	
3.1.5.1 Maintenance services shall be performed by the Vendor. Vendor shall be trained and certified to perform maintenance on the equipment. Vendor must have replacement parts approved by the equipment manufacture available.	Maintenance services will be performed by Shimadzu. Shimadzu has technical support scientists and service engineers that are local to West Virginia. Shimadzu's HPLC replacement parts come from Shimadzu USA manufacturing, which is based in Canby, Oregon. Lead times for replacement parts are always short, as a result.
3.1.5.2 Vendor must respond to service calls within 24 hours.	Shimadzu will respond to all service calls within 24 hours. Service calls are dispatched by emailing SOEServiceRequest@Shimadzu.com or by dialing our toll free number at 1-800-477-1227
3.1.5.3 Vendor must be capable of performing all requests for repairs and/or service within three business days of request.	Shimadzu will perform all requests for repairs and/or service within 3 business days of request.
3.1.5.4 After any maintenance or repairs have been completed the vendor shall guarantee the accuracy and precision of the instrument at the location where the instrument will be used.	Following any and all repairs, Shimadzu guarantees the accuracy and precision of the instrument at the location it will be used at.
3.1.5.5 Reports of service will be signed by State of WV authorized laboratory personnel to ensure work has been completed.	Signed work orders are a required part of any repair visit from Shimadzu. Work orders cannot be completed without signatures.

Bid terms sections 4-7	Shimadzu acceptance of terms
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 must complete the Pricing Page by entering information in each column for model/brand name, unit price and extended amount. 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. If submitting bid through the wvOASIS Vendor Self-Service Portal, vendor should enter grand total from Exhibit A Pricing Page as total in wvOASIS.	Shimadzu agrees to all terms within these sections
5. PAYMENT: **5.1 Payment:** Vendor shall accept payment in accordance with the payment procedures of the State of West Virginia.	Shimadzu agrees to all terms within
6. DELIVERY AND RETURN: **6.1 Shipment and Delivery:** Vendor shall ship the Contract Items immediately after being awarded this Contract and receiving a purchase order or notice to proceed. Vendor shall deliver the Contract Items within 90 days after 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	Shimadzu agrees to all terms within

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 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.	Shimadzu agrees to all terms within

SHIMADZU
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SSI02 – Shimadzu HPLC Installation Completion Report

10330 Old Columbia Road, Columbia, MD 21046
(410) 381-1227 ● www.ssi.shimadzu.com

Standard Installation Completion Report

Ultra High Performance Liquid Chromatograph, Nexera/Prominence series

Nexera X3, Nexera XS, Nexera XR, Nexera lite, Nexera XS inert, Nexera lite inert, Nexera CL, Nexera FV, Nexera HFIP compatible system

Installation has been completed.

:

Installation Completion Date:

Performed by:

Group/Department:

Special Notes

Nexera system Installation Checklist

No.	Item	Criteria	Result/Comment	Judge	
				Pass	Fail
1	**Confirmation before installatoin**				
1	Power supply	AC 100-240 V ±10%		○	○
2	Grounded power supply	Properly connected to the earth.		○	○
3	Appearance	No abnormal dirt, deformation, damage, etc.		○	○
4	Items	No discrepancies.		○	○
5	Standard accessories	No missing accessories.		○	○

No.	Item	Criteria	Result/Comment	Judge	
				Pass	Fail
2	**System installation**				
1	Rinse the flow lines	Flow at 1 mL/min for 30min or more with IPA. Or rinse according to the specified procedure.		○	○
2	Leakage check	No leaks in any part of the flow line.		○	○
3	Firmware check	Firmware is latest.		○	○
4	System check	Perform the system check and Pass.	☐ Attached Data	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge	
				Pass	Fail
3	**LabSolutions**				
1	Version	Same version as printing on the disk.		○	○
2	Program	The result of the program file check is “Total: Pass ".		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge	
				Pass	Fail
4	**Printer**				
1	Printing	The printer prints properly.		○	○

Special Note

System Test ☐ **Applicable** ／ ☐ **N/A**

☐ Applicable ／ ☐ N/A

<table>
<tr><th rowspan="2">No.</th><th rowspan="2">Item</th><th rowspan="2">Criteria</th><th rowspan="2">Result/Comment</th><th colspan="2">Judge.</th></tr>
<tr><th>Pass</th><th>Fail</th></tr>
<tr><td colspan="6">5 System Performance Test</td></tr>
<tr><td>1</td><td>Repeatability Test</td><td>The coefficient of variation (CV%) of the peak retention time and the peak area obtained by 6 consecutive analyses are within the range of the respective standards.
☐ System with UV-VIS Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤1.0 %</td><td>≤0.5 %</td></tr></table>☐ System with Photodiode Array Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤1.0 %</td><td>≤0.5 %</td></tr></table>☐ System with Spectrofluorometric Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤2.0 %</td><td>≤0.5 %</td></tr></table>☐ System with Differential Refractometric Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤2.0 %</td><td>≤1.0 %</td></tr></table></td><td>Peak Area:

Retention Time:

☐ Attached Data</td><td>○</td><td>○</td></tr>
</table>

Special Notes

☐Applicable(No.) ／ ☐N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
6	**Application System Performance Test**				
1	☐ Performance test of organic acid analysis system	The background conductivity value and baseline noise while the standard mobile phase and buffer are delivered. The coefficient of variation (CV%) of the water peak area and the retention time obtained by 6 consecutive analyses are within the range of the respective standards. Background conductivity value: 420±42 μS/cm Noise (ASTM method): ≤ 10 nS/cm CV% of peak area: ≤ 1 % CV% of retention time: ≤ 1 %	Background conductivity value: Noise: CV% of peak area: CV% of retention time: ☐ Attached Data	○	○
2	☐ Performance test of post-column amino acid analysis system	The coefficient of variation (CV%) of the peak area and signal noise ratio (S/N) of the peak are within the range of the respective standards. CV% of Peak area: ≤ 3.0 % S/N: ≥ 3,000	CV% of peak area: S/N of peak: ☐ Attached Data	○	○
3	☐ Performance test of reducing sugar analysis system	The coefficient of variation (CV%) of the peak area and signal noise ratio (S/N) of the peak are within the range of the respective standards. CV% of Peak area: ≤ 3.0 % S/N: ≥ 3,000	CV% of peak area: S/N of peak: ☐ Attached Data	○	○
4	☐ Performance test of Comprehensive 2D LC system (Nexera-e)	The following two values shall be within the following specified ranges, respectively. The coefficient of variation (CV%) of the peak area of 12 peaks from behind among the peaks due to modulation switching The coefficient of variation (CV%) of the elution interval time (retention time difference). CV% of Peak area: ≤ 5.0 % CV% of retention time difference: ≤ 0.5 %	CV% of Peak area: CV% of retention time difference: ☐ Attached Data	○	○

Special Notes

☐Applicable(No.) ／ ☐N/A

<table>
<tr><th rowspan="2">No.</th><th rowspan="2">Item</th><th rowspan="2">Criteria</th><th rowspan="2">Result/Comment</th><th colspan="2">Judge.</th></tr>
<tr><th>Pass</th><th>Fail</th></tr>
<tr><td>5</td><td>☐ Performance verification test of UF-Amino Station</td><td>[1] After optimizing the push volume of SIL-20ACPT, the mixing accuracy should be as specified below.
<table><tr><td>[Sample peak height] / [Control vial peak height]</td><td>1.8 -2.2</td></tr></table>
[2] All amino acid analysis results obtained by LCMS-2020 shall be as specified below.
<table><tr><td>CV % of peak retention time</td><td>≤ 1.5 %</td></tr><tr><td>CV % of peak area</td><td>≤ 10 %</td></tr></table></td><td>[Sample peak height] / [Control vial peak height]:

(reference value)
Sample Peak Height:

(reference value)
Control vial peak height:

(List the results for the amino acid with the highest coefficient of variation)
CV% of peak retention time:

(List the results for the amino acid with the highest coefficient of variation)
CV% of peak area:

☐ Attached Data</td><td>○</td><td>○</td></tr>
<tr><td>6</td><td>☐ Operation test of Nexera FV batch synthesis analysis system</td><td>[1] The rinse solution is ejected normally in the purge method.
[2] The sample is successfully aspirated in the purge method.</td><td></td><td>○</td><td>○</td></tr>
<tr><td>7</td><td>☐ Repeatability Test of Nexera FV batch synthesis analysis system</td><td>The coefficient of variation (CV%) of the peak retention time and the peak area obtained by 6 consecutive analyses are within the range of the respective standards.
☐ System with UV-VIS Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤3.0 %</td><td>≤0.5 %</td></tr></table>☐ System with Photodiode Array Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤3.0 %</td><td>≤0.5 %</td></tr></table>☐ System with Spectrofluorometric Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤6.0 %</td><td>≤0.5 %</td></tr></table>☐ System with Differential Refractometric Detector
<table><tr><th>Peak Area</th><th>Retention Time</th></tr><tr><td>≤6.0 %</td><td>≤1.0 %</td></tr></table></td><td>Peak Area:

Retention Time:

☐ Attached Data</td><td>○</td><td>○</td></tr>
</table>

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge	
				Pass	Fail
7	**Safety Inspection Notification Settings**				
1	Setting Installation Date, 10-Year Inspection Date	The component's installation date and 10-year inspection date shall be set to the date of completion of the installation and the date 10 years later.		○	○

Special Notes

Component Test ☐ Applicable ／ ☐ N/A

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
8	**Degassing Unit Performance Test**				
1	Vacuum pressure test	The green LED indicating that the component is operating at controlled pressure is lit.		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
9	**Flow Channel Selection Valve Performance Test**				
1	Flow channel selection test	The component switches the flow channel as set.		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
10	**Solvent Delivery Unit Performance Test**				
1	Flow stability test	The pressure variation at 1 mL/min is within the following criteria. Component: LC-40D X3(CL)/40B X3(CL)/ 40D XS/40D XSi/ LC-30AD(CL) — Criteria: ≤0.15 MPa Component: LC-40D XR(CL)/ 40B XR/40D/LC-40i LC-20AD(CL)/20AB/ 20AD XR — Criteria: ≤0.2 MPa Component: LC-20AT/20Ai — Criteria: ≤0.3 MPa	☐ Pump A ☐ Pump B ☐ Pump C ☐ Pump D	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
11	**Column Oven Performance Test**				
1	Temperature control test	Temperature stabilizes when the temperature is set to 40 °C.	☐ Oven A ☐ Oven B ☐ Oven C ☐ Oven D	○	○

Special Notes

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
12	**UV-VIS Detector Performance Test**				
1	Light intensity test	The output level of the reference light beam with the standard flow cell is within the following criteria. Component / Wavelength / Criteria: SPD-40(CL): [1] 220 nm / ≥200 mV SPD-40V: [1] 220 nm / ≥200 mV; [2] 540 nm / ≥250 mV SPD-20A(UFLC): [1] 220nm / ≥400 mV SPD-20AV: [1] 220nm / ≥400 mV; [2] 540nm / ≥500 mV	☐ Det A ☐ Det B	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
13	**Photodiode Array Detector Performance Test**				
1	Light intensity test	The lamp intensity with the standard flow cell is within the following criteria. Component / Wavelength / Criteria: SPD-M40: [1] 200-260 nm / ≥2.3 V; [2] 400-800 nm / ≥2.3 V SPD-M30A: [1] 200-260 nm / ≥3.5 V SPD-M20A: [1] 200-260 nm / ≥3.5 V; [2] 400-800 nm / ≥2.5 V		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
14	**Spectrofluorometric Detector Performance Test**				
1	S/N test	The signal-to-noise ratio with using raman spectrum is within the following criteria. Component / Wavelength / Criteria: RF-20A: [1] ≥600 (Standard cell); [2] ≥600 (Inert cell); [3] ≥250 (Semi-micro cell) RF-20Axs: [1] ≥1,000 (Standard cell); [2] ≥1,000 (Inert cell); [3] ≥400 (Semi-micro cell)	☐ Det A ☐ Det B	○	○

Special Notes

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
15	**Differential Refractometric Detector Performance Test**				
1	Light intensity test	The lamp light intensity is within the following criteria. Component: RID-20A; Wavelength / Criteria: ≥6,000	☐ Det A ☐ Det B	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
16	**System Controller Performance Test**				
1	Touch panel test [SCL-40 only]	There is no display defect such as missing pixels nor touch position defect on the touch panel.		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
17	**Plate Changer / Rack Changer Performance Test**				
1	Initialization test	The initial operation ends normally.		○	○
2	Temperature control test	The monitor temperature stabilizes at the setting temperature ±1 °C when the temperature is set to “room temperature -5 °C” or below.	☐ Autosampler doesn’t have a temperature control function	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
18	**Autosampler Performance Test**				
1	Needle operation test	The needle is inserted into the injection port correctly.		○	○
2	Temperature control test	The monitor temperature stabilizes at the setting temperature ±1 °C when the temperature is set to “room temperature -5 °C” or below.	☐ Autosampler doesn’t have a temperature control function	○	○

Special Notes

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
19	**Optional A/D Board Performance Test**				
1	Signal test	The monitored signal voltage corresponding to the connected detector is displayed.	☐ AD A ☐ AD B	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
20	**Chemical Reaction Box Performance Test**				
1	Temperature control test	The temperature stabilizes when the temperature is set to 60 °C.		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
21	**pH monitor Performance Test**				
1	Validation of temperature sensor	The difference between the temperature of the electrode and the actual temperature measured by the thermometer should be ±1°C.		○	○
2	Calibration of pH electrode	Calibration can be performed		○	○
3	Validation of pH electrode	Chiral potential ≤ ±30.0 mV (Potential when measuring pH6.86 standard solution) Sensitivity 90 - 105 % Repeatability pH ± 0.05		○	○
4	Confirmation of flow cell	The difference between the calibration value of pH of Test No. 2 and the measured value of pH of Test No. 4 is pH ± 0.10.		○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result/Comment	Judge. Pass	Judge. Fail
22	**Mobile Phase Monitor Performance Test**				
1	Weight Sensor Test	When 500 mL of water is weighed, the MPMChecker should display 500 mL		○	○

Special Notes

Optional unit Test ☐ Applicable ／ ☐ N/A

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result・Comment	Judge Pass	Judge Fail
23	**Low pressure gradient unit performance test**				
1	Gradient concentration accuracy	Check the gradient performance with below condition, the error of each concentration being within criteria. Concentration / Criteria / (LC-30AD) 10%B, 10%D / 8-12% / 9-11% 50%B, 50%D / 48-52% / 49-51% 90%B, 90%D / 88-92% / 89-91%	10%B: 50%B: 90%B: 10%D: 50%D: 90%D: ☐ Attached Data	○	○

☐ Applicable ／ ☐ N/A

No.	Item	Criteria	Result・Comment	Judge Pass	Judge Fail
24	**Optional cell for UV/PDA detector performance test**				
1	Detector baseline stability	Check the baseline noise and drift with below condition. (Check the cell type) SPD-40(V)/20A(V) [1] Conventional Cell Noise ≤ 0.04 mAU, Drift ≤0.50 mAU/h [2] UHPLC Cell [3] Semi micro Cell [4] UHPLC Inert Cell [5] Low Diffusion Inert Cell Noise ≤ 0.06 mAU, Drift ≤1.0 mAU/h SPD-M40/M30A/M20A [1] Conventional Cell Noise ≤ 0.05 mAU, Drift ≤5.0 mAU/h [2] UHPLC Cell [3] Semi micro Cell [4] UHPLC Inert Cell [5] Low Diffusion Inert Cell Noise ≤ 0.15 mAU, Drift ≤5.0 mAU/h [4] High sensitivity Cell Noise ≤ 0.25 mAU, Drift ≤6.0 mAU/h	☐ Det A Cell Type_____ Noise: Drift: ☐ Det B Cell Type_____ Noise: Drift: ☐ PDA Cell Type_____ Noise: Drift: ☐ Attached Data	○	○

【Condition】

Mobile phase:	Water
Restriction tubing:	4 m x 0.1 mm I.D. and 2 m x 0.5 mm I.D. SUS tube, or 3 m x 0.13 mm I.D. and 2 m x 0.5 mm I.D. PEEK tube
Flow rete:	1 mL/min
Wavelength:	250 nm (D2 lamp only) or 250 nm and 600 nm (D2 lamp and W lamp)
Measurement method:	Record for 15min.after stability and calculate the baseline noise and drift.

Special Notes

No.	Item	Description	Check
25	**Safety Instruction**		
1	Safety	Introduce the Safety Guideline and inform to read it before use. Explain the action in an emergency.	☐
2	Warranty	Explain the warranty.	☐

No.	Item	Description	Check
26	**Instruction**		
1	Outline of the equipment	Explain items below; · Role of each module. · Structure of the flow path. · Operation of the main power switch. · Operation of the power button. · Operation of the direct key. · LED indicator.	☐
2	Basic operation [System installation Only]	Introduce System Guide and instruction manuals. Explain basic operations as follows; · Power on and set up the system. · Shut down the system. · Points to be followed when not used for a long term.	☐
3	Single Analysis [System Installation Only]	Explain single analysis as follows; · Create method and perform single run · Perform post run analysis · Prepare data report.	☐
4	Batch Analysis [System Installation Only]	Explain batch analysis as follows; · Create batch table and perform batch analysis · Perform multi-data analysis · Prepare summary report.	☐
5	Maintenance	Introduce periodic inspections and maintenances.	☐

Special Notes

SHIMADZU
Excellence in Science

SSI03 – Shimadzu LC-40 Brochure

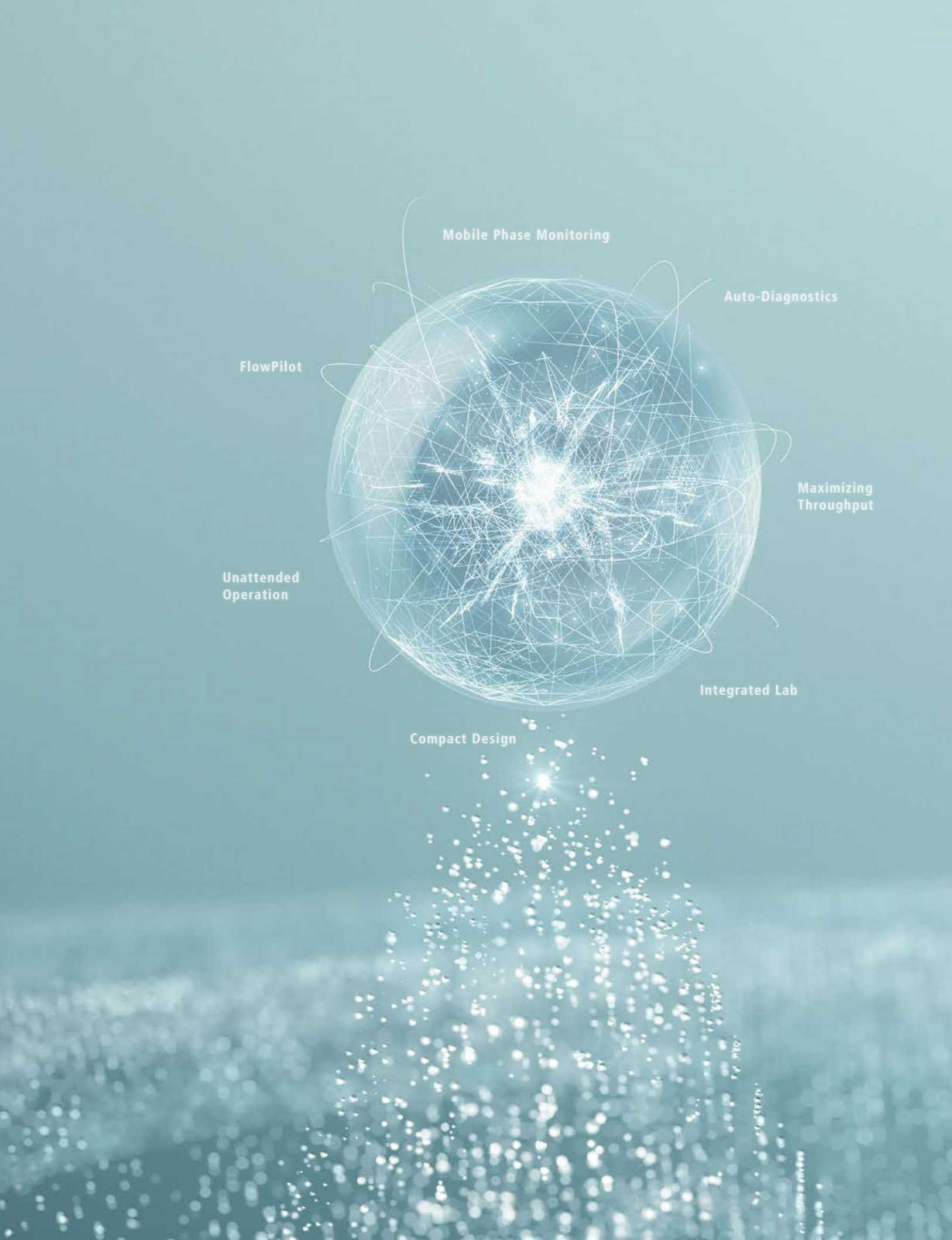
10330 Old Columbia Road, Columbia, MD 21046
(410) 381-1227 ● www.ssi.shimadzu.com

C196-E096

SHIMADZU
Excellence in Science

Ultra High Performance Liquid Chromatograph

Nexera series


Mobile Phase Monitoring
Auto-Diagnostics
FlowPilot
Maximizing Throughput
Unattended Operation
Integrated Lab
Compact Design

EXPERIENCE NEW BENCHMARKS

- A New Benchmark of Intelligence
- A New Benchmark of Efficiency
- A New Benchmark of Design

Shimadzu has long been advancing the analytical performance of HPLC systems. At the same time, we recognize that overall efficiency depends not only on the performance of one instrument, but on the management of all devices within a lab. This realization leads us to now, a time in which AI capabilities have been incorporated to allow devices to detect and resolve issues automatically. In addition, lab management has been integrated using the Internet of Things (IoT) and device networking, making it simple to review the status of instruments and optimize resource allocation.

Building upon 40 years of experience in LC technology, the Nexera series is a family of UHPLC systems that marries these AI and IoT enhancements to set new industry standards in terms of intelligence, efficiency, and design.

Nexera™ series

Key Features

UV-VIS Absorbance Detector SPD-40 / SPD-40V
Photodiode Array Detector SPD-M40

- Temperature control improves performance stability. P. 13
- Analytical data is linked to information about consumables to ensure traceability.

Solvent Delivery Unit LC-40 Series

- Auto-diagnostics to detect problems during analysis, and an auto-recovery function. P. 6
- Reduces space requirements with a dual solvent delivery system. P. 12

Autosampler SIL-40 Series
PLATE CHANGER

- The injection speed is twice as fast as previous models, shortening multi-analyte processing times. P. 10
- Can perform continuous analysis on up to 44 microtiter plates. P. 11
- High reproducibility and ultra-low carryover for micro-volume injections. P. 13
- Automated sample preparation functions such as diluting samples, adding internal standards, and performing derivatization reactions, reduce labor.

Mobile Phase Monitor MPM-40

- Reservoir tray weight sensors monitor the remaining mobile phase in real time. P. 7

System Controller SCL-40, CBM-40

- Supports remote monitoring via a smart device. P. 8
- Mobile phases can be purged and baselines checked easily via the touch panel.

Column Oven CTO-40 Series

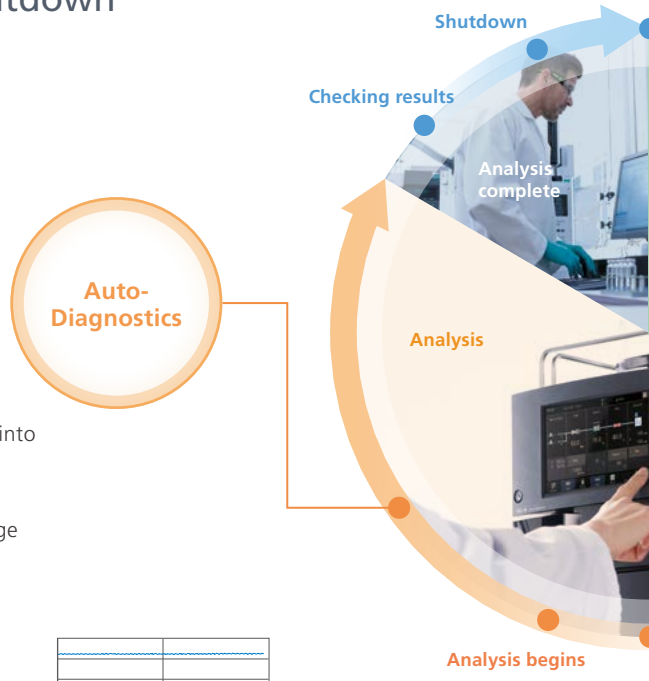
- Slim-type column oven with half the width of the previous model. P. 12
- Easy column attach/detach mechanism prevents peak broadening.

A New Benchmark of Intelligence
Maximizing Reliability, Minimizing Down Time

Fully Unattended Operation from Startup to Shutdown

The Nexera can be set to start up at a specified time, so that it can complete auto-purge, equilibration and baseline checks in advance, and be ready for analysis as soon as you arrive at the lab. Moreover, the system can be set up in advance to run without user intervention all the way from startup through analysis to shutdown.

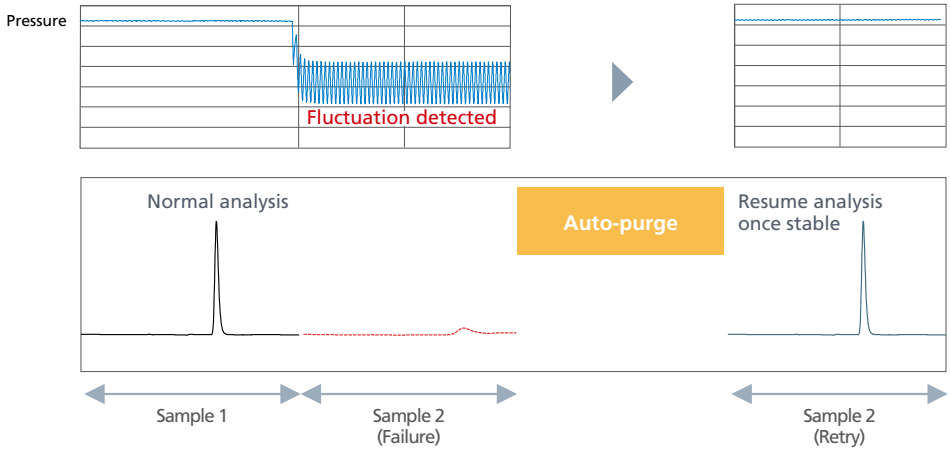
You can view the status and predicted analysis completion time for multiple systems from any location via a smart device. None of these features requires any special software.



Auto-Diagnostics and Recovery

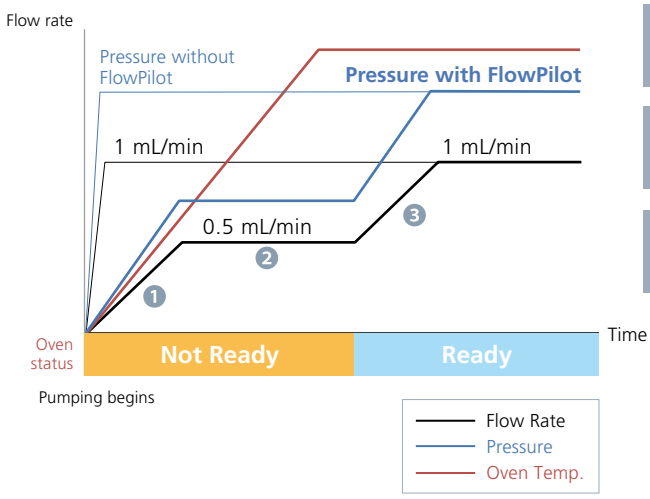
In rare cases, air bubbles can form in the mobile phase and cause problems if inhaled into the pump. The Nexera has the ability to monitor baseline changes and pressure fluctuations to check for abnormalities.

When it detects an unusual fluctuation, it can automatically pause the analysis, purge the flow path, and restart analysis once it has confirmed recovery to normal pressure. (Pat. Pending)

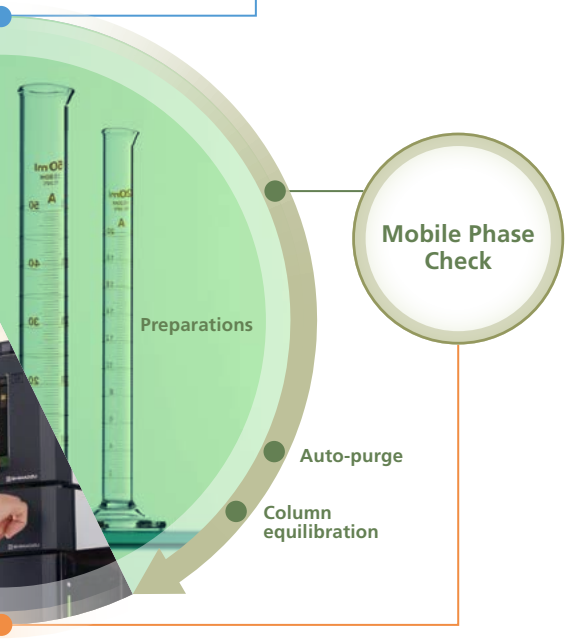


FlowPilot Protects Columns

HPLC columns can be damaged by sudden pump starts and stops or extreme gradient changes. The Nexera automatically uses FlowPilot (Smart Flow Control) to increase the flow rate gradually to the set point. There is no need to create startup protocols for each analysis.
(Pat. Pending)



1. The flow rate is gradually increased up to half of the set value.
2. The flow rate is kept constant until the oven is ready.
3. The flow rate is gradually increased up to the set value.



Mobile Phase Levels Measured in Real Time

Reservoir tray weight sensors (optional) can be used to monitor the volume of mobile phase or autosampler rinse solution in up to twelve containers. The containers can also be checked remotely from a smart device.

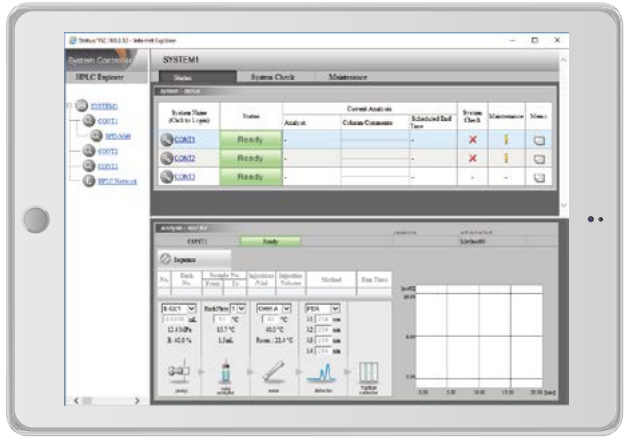
You will no longer need to worry about running out of mobile phase mid-analysis, because the device will notify you before starting the run if the volume remaining is too low.
(Pat. Pending)

A New Benchmark of Intelligence
Remote Monitoring and Integrated Lab Management

Check Chromatograms Online

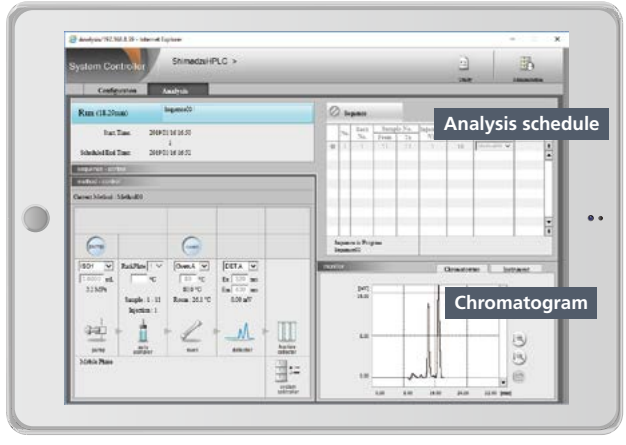
View the operating status of instruments from a web browser using a smart device. This allows you to confirm parameters such as oven temperature and pump status directly from the web, and to monitor chromatograms in real time without returning to the lab.

In addition, SHIMADZU LabTotal™ Smart Service Net (optional) saves operating data from your instruments in a cloud server. Error information, including the date and time the errors occurred, can be sent via email.



Check the operating status of your LC systems

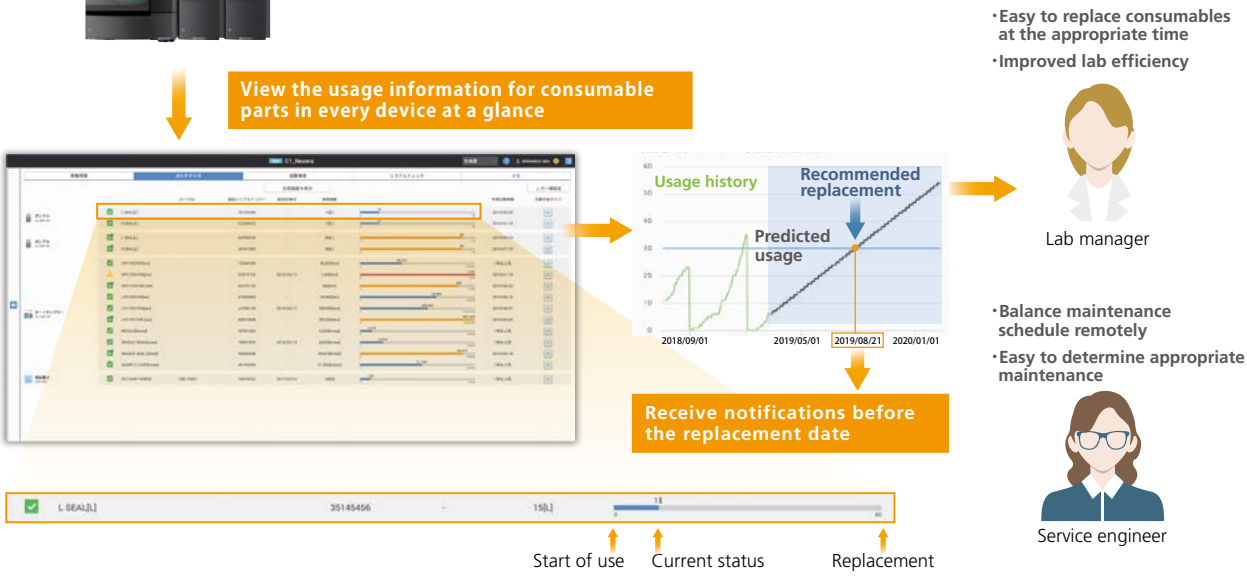
Select one system



Monitor chromatograms currently being taken

Remote Instrument Maintenance Ensures Stable Operation

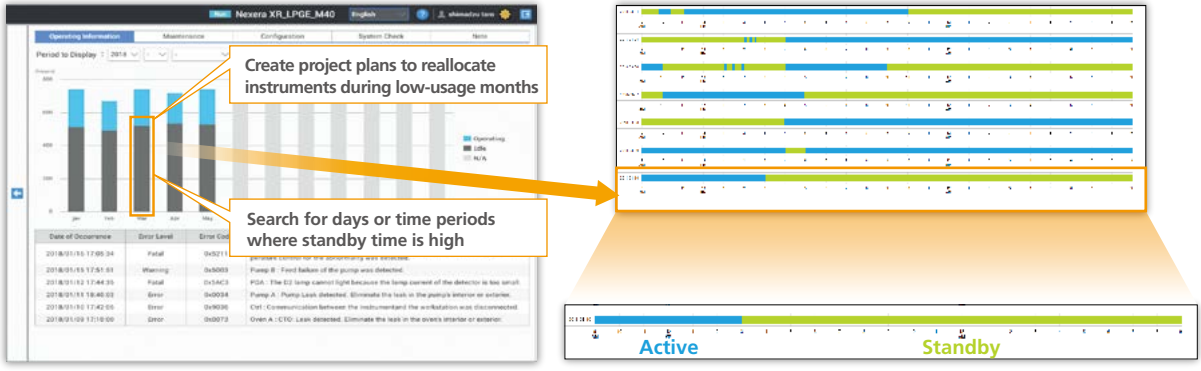
Each Nexera component automatically transfers the status of consumable parts, the traceability information of consumable parts after shipment from the factory, and various logs and error information to the SHIMADZU LabTotal Smart Service Net cloud system. Using this information, the system recommends the timing for consumable part replacement. Problems can be diagnosed remotely by Shimadzu service engineers. This means the system can be maintained in optimal condition and equipment management costs can also be reduced.



Usage display for consumable pump parts

Allocate Resources Efficiently

Manage the overall operation of your lab with SHIMADZU LabTotal Smart Service Net (optional). Review and compare instrument usage to maximize available analysis time.



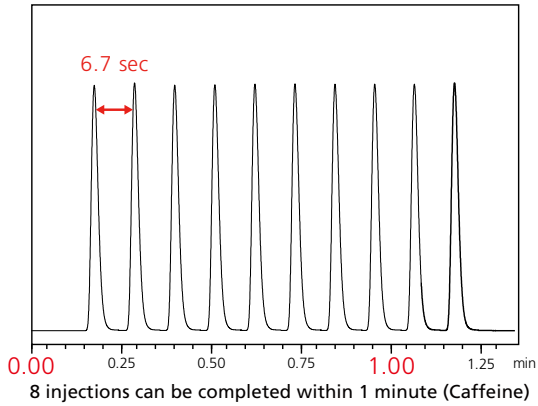
View graphs of monthly operation status

Zoom in further to view the daily operation status of each instrument

A New Benchmark of **Efficiency**
Automating Workflow, Maximizing Throughput

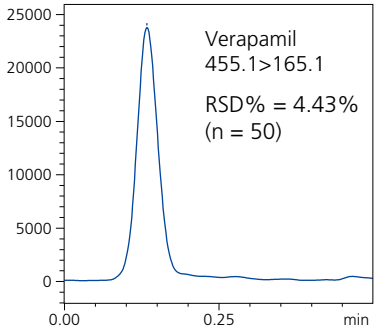
Analysis Cycle Time Less Than 10 Seconds

The SIL-40 autosampler can process the entire injection cycle time in as little as seven seconds, twice as fast as the previous model. In addition, continuous analysis can be carried out on up to 44 MTPs (using 3 PLATE CHANGERS). Together these features dramatically increase analysis throughput.

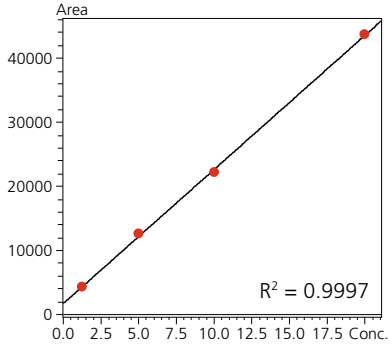


8 injections can be completed within 1 minute (Caffeine)

Pharmacokinetic analysis requires not only speed but also high reliability at low concentrations. With its ultra-fast injection and ultra-low carryover, the SIL-40 autosampler delivers high reproducibility and reliability, even during an ultra-fast 30-second analysis.



Verapamil in plasma (spiked 2.5 ppb)



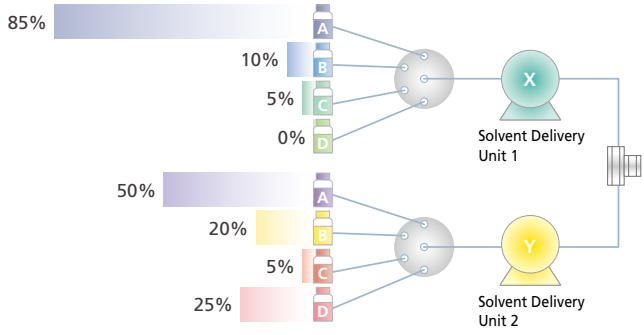
Linearity

Automated Analysis of Thousands of Samples

Non-stop temperature-controlled analysis of thousands of samples is now possible with the SIL-40's optional plate changers. Set all your samples in advance with up to 14 MTPs or vial racks in each PLATE CHANGER. The autosampler's excellent temperature control also allows the insertion of additional vials and MTPs during analysis.

Quick and Reliable Mobile Phase Blending

The Nexera can automatically blend mobile phases at any set ratio. This speeds up the preparation of buffer solutions and the dilution of solvents, and can be used to easily prepare the exact amounts required for analysis, reducing waste as well as labor.

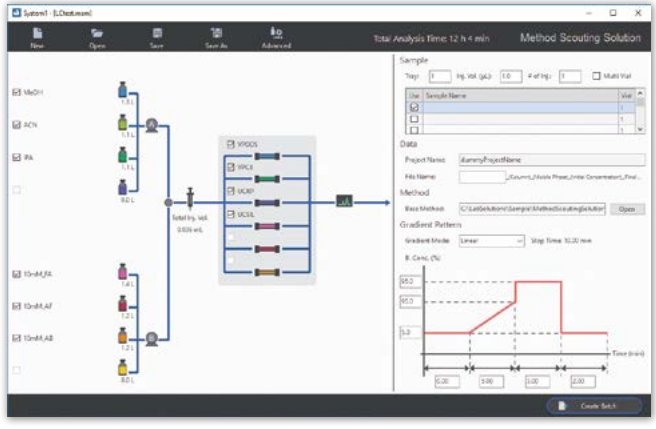


Mobile phases are automatically prepared using the pumps.

Binary Gradient

Simplified Method Development

The Nexera Method Scouting System is capable of automatically switching between combinations of up to 6 columns and 8 mobile phases. It can run unattended, maximizing available uptime. In addition, Method Scouting Solution Software can automatically create methods with different columns, mobile phases, and gradient conditions from a single base method.



A New Benchmark of Design
Compact and Inventive

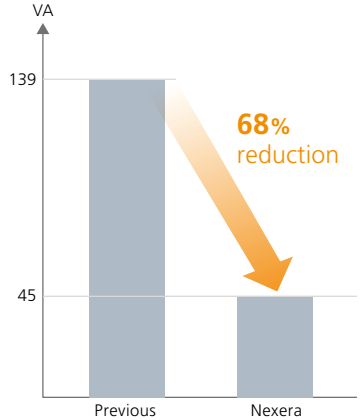
Space-Saving Design

The Nexera frees up bench space with a compact design two thirds the size of Shimadzu's previous model.

Nexera Series
Standard Model

Energy-Saving Standby Mode

The Nexera uses over 80% less electricity when in standby mode, significantly reducing running costs and supporting an environmentally-friendly lab.



Power Consumption on Standby

Dual Injection Enables Simultaneous Analysis

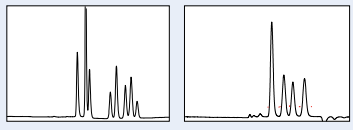
Injection ports for two separate flow paths can be installed, allowing two different types of analysis (such as analysis of amino acids, organic acids or vitamins) to be performed using one system.
(Pat. Pending)

Begin analysis

Inject the samples simultaneously using the 2 injection ports

Analysis

Analyze 2 classes of compounds simultaneously

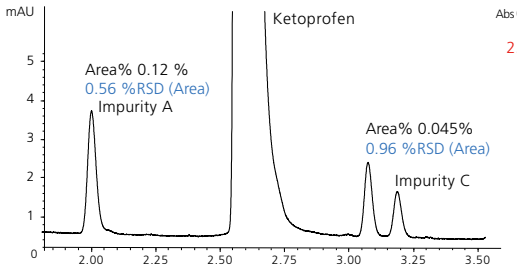


Results

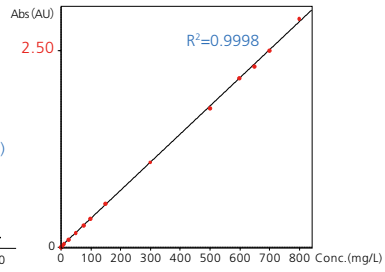
Generate a report summarizing the results of both analyses

High-Sensitivity Impurity Analysis

The SPD-M40 detector achieves an extremely high level of sensitivity and linearity (up to 2.5 AU). This allows quantitation of very lowconcentration impurities even in high-concentration samples. The UV cut-off filter installed in the detector prevents sample degradation due to UV light, helping to maintain good linearity at low concentrations.
(Pat. Pending)



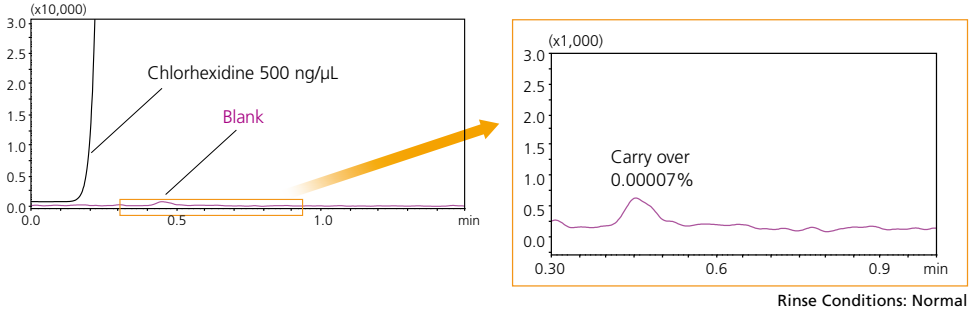
Example: Ketoprofen analysis reveals impurities of area% < 0.2%



Detector linearity for ketoprofen

Ultra-low Carryover

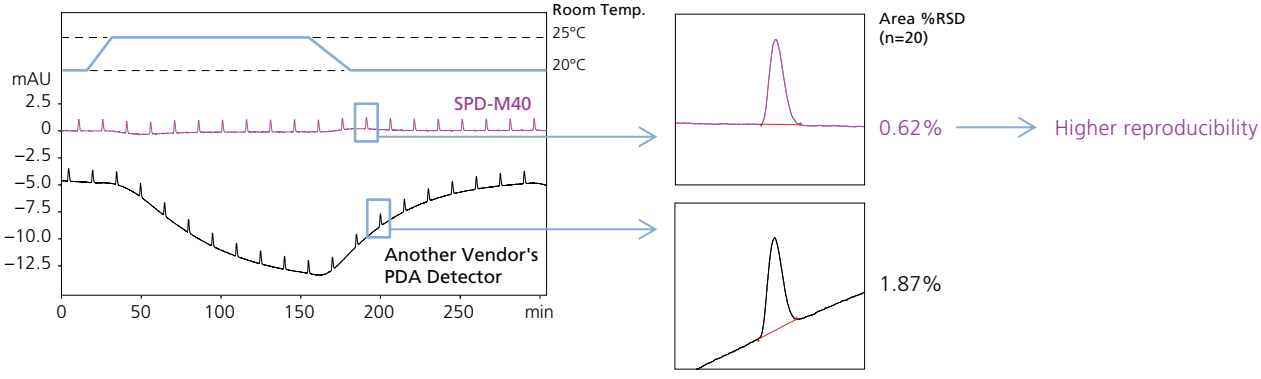
The Nexera boasts ultra-low carryover, even on a high-sensitivity LC/MS/MS. This reduces time spent on rinsing, resulting in a shorter overall analysis time.
(Pat. Pending)



After analysis of Chlorhexidine, the carryover to a blank solution is negligible.

Stable Baseline

Baseline fluctuations can affect peak area calculation, reducing the accuracy of quantitative results. The SPD-M40 photodiode array detector's "Advanced TC-Optics" function adjusts the temperature of the flow cell, lamp, and optical system to lessen the impact of external temperature changes. Noise and drift have also been reduced by 40% compared to the previous model.
(Pat. Pending)

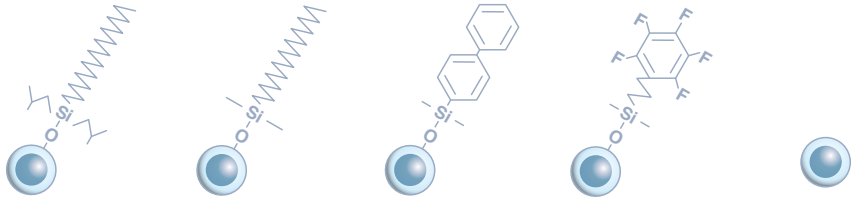


Faster Analysis Without Sacrificing Precision

It is important to use the most appropriate column in your LC system to achieve the highest efficiency and most accurate chromatograms. The Shim-pack series consists of a range of columns designed with the increased capabilities of the Nexera in mind. The superior ruggedness of Shim-pack columns ensures a long lifetime even with demanding sample matrices.

Shim-pack™ Velox

The superficially porous particle (SPP) technology allows for optimum separation and analysis times. The range of column types available enables you to choose the most appropriate column for each application.



	SP-C18	C18	Biphenyl	PFPP	HILIC
USP classification	L1	L1	L11	L43	L3
Stationary phase	Sterically protected C18	C18	Biphenyl	Pentafluorophenyl propyl	None
Particle size (µm)	1.8, 2.7, 5	1.8, 2.7, 5	1.8, 2.7, 5	1.8, 2.7, 5	2.7

*To maximize column lifetime, columns with 1.8 µm particle size are recommended for use with a pressure under 80 MPa.

Shim-pack™ XR-ODS II & III

Although the Shim-pack XR-ODS II has a particle size of 2.2 µm, it can be used up to a pressure of 60 MPa, making it appropriate for a wide range of analyses. The particle size of the XR-ODS III column has been reduced to 1.6 µm. With a maximum pressure of 100 MPa, it achieves high separation even with a short column length.

These columns are suitable for shortening analysis times while taking full advantage of the high separation power of UHPLC.

	XR-ODS II	XR-ODS III
USP classification	L1	L1
Stationary phase	C18	C18
Particle size (µm)	2.2	1.6

Shim-pack™ GIS/GIST/GISS

The Shim-pack GIS/GIST/GISS series lineup includes columns with particle sizes ranging from 5 µm, appropriate for HPLCs, to 2 µm, appropriate for UHPLCs. With a variety of substrates available, they are ideal for method development.

GIS Series: HPLC columns packed with a high-purity silica gel material as a base. With high particle uniformity to secure the mobile phase path, these columns are ideal for low-pressure analysis.

GIST Series: An increase in the inertness of the silica results in improved peak shapes and ruggedness. Can be used in pH 1–10 environments. Easy to use for a wide range of chemicals and environments.

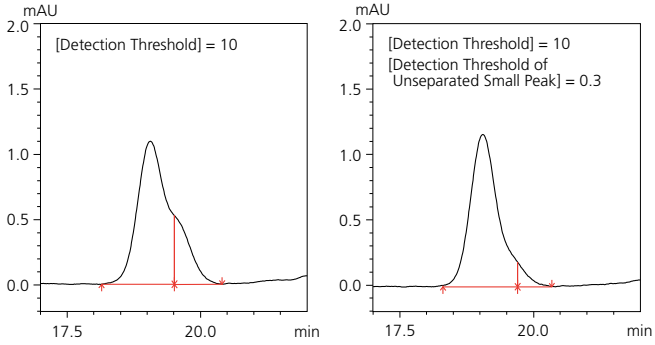
GISS Series: Adding to the advantages of the GIST series, a faster elution time has been achieved to provide even sharper peaks.

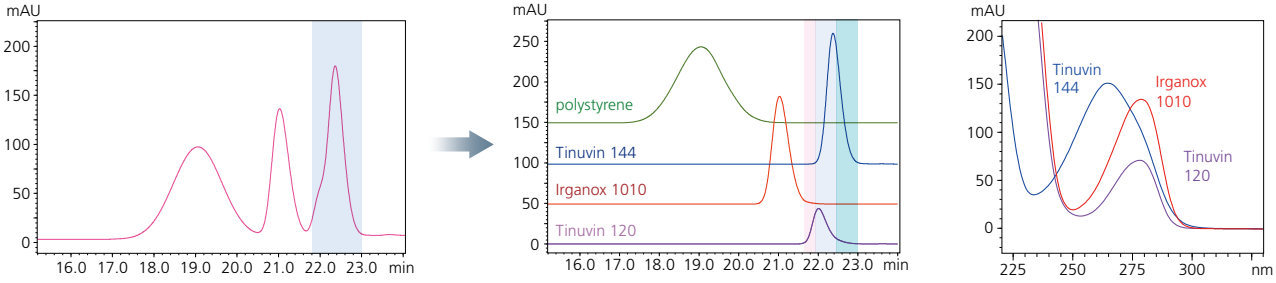
LabSolutions™ Chromatography Software

Extract the Smallest of Shoulder Peaks

The i-PeakFinder tool can automatically recognize peaks even in complex chromatograms with high noise, and accounts for baseline drift for higher integration accuracy. Adjustable parameters in i-PeakFinder, such as peak detection threshold, allow the user to detect smaller peaks.

In addition, the i-PDeA II function can quantify peaks that cannot be completely separated by a column. Separate peaks by simply specifying the time and wavelength in LabSolutions.

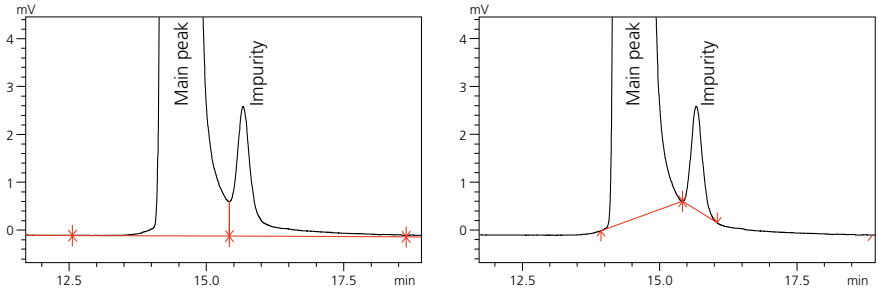




Application example: i-PDeA II

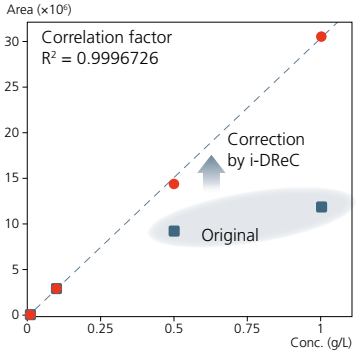
Consistent Peak Splitting

Manually setting the baseline for fused peaks is time-consuming, and the peaks may be split differently depending on the user. The i-PeakFinder has adjustable parameters for different analyses, allowing the consistent application of the best baseline in each situation.



Dynamic Range Extension Function for Accurate Calibration

i-DReC is a new analytical method that significantly extends the dynamic range. It enables the analysis of high-concentration compounds without diluting them, and ensures a correct calibration curve.



Application example: i-DReC

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SSI04 – Shimadzu LC-40 spec sheet

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C196-E095

SHIMADZU
Excellence in Science

Ultra High Performance Liquid Chromatograph

Nexera series

Specifications

System Configuration

UV-VIS Detector
SPD-40/40V
Photodiode Array (PDA) Detector
SPD-M40

Baseline stability and linearity have been improved, and stability remains even under fluctuating temperatures. The PDA detector is equipped with a UV cut-off filter to improve the quantitation accuracy of photodegradable compounds. The cell and lamp are traceable via individual IDs.

Solvent Delivery Pump
LC-40 series

In addition to the four parallel double plunger models based on the maximum pressure limit, the XR and X3 models have a dual pump that reduces gradient delay volume and enables an ultra-fast high-pressure gradient. Other pumping environments (low-pressure gradient, mobile phase blending) can also be provided.

Autosampler
SIL-40 series

The autosampler boosts ultra-low carryover, less than 0.0003% (under specified conditions). Its ultra-fast injection cycle and auto pretreatment functions also contribute to more efficient analysis. The optional dual-injection system consists of two separate injection ports and flow lines, enabling different analyses to be carried out simultaneously.

Plate Changer

The installation area has been greatly reduced to 170 mm. It is possible to load up to 7 racks of 1.5 mL vials or 14 microtiter plates. Up to 3 plate changers can be connected, allowing up to 44 MTPs with up to 16896 samples to be loaded at once (using 384-well MTPs).

Mobile Phase Monitor
MPM-40 (Optional)

The monitoring device can be placed in the reservoir tray. The volume of liquid remaining in each mobile phase bottle is measured in real time and can be checked from a PC or mobile device. Before a batch analysis is started, the amount of mobile phase required is calculated and a warning is displayed if the amount remaining is insufficient.

System Controller
SCL-40, CBM-40/40lite

The SCL-40 system controller features a touch panel and allows the user to control the instrument and carry out analysis preparation directly without the need for a PC. A graphical UI makes the controller easy to use.

Degassing Unit
DGU-403/405

3-channel and 5-channel types available. Since the degassing unit is built into the LC-40B X3 pump, a separate unit is not required.

Column Oven
CTO-40 series

The circulation oven has a slim 130 mm model (maximum temperature: 85°C) and a standard 260 mm model (maximum temperature: 100°C). Both are able to accommodate a 300 mm column and have connection ports for CMD or mixer ID recognition. Active preheater tubing is available as an option.

Specifications

System Controller


SCL- 40

	SCL-40	CBM-40	CBM-40lite
Monitor	Touch panel LabSolutions™ Web monitor	LabSolutions Web monitor	LabSolutions Web monitor
Connectable unit	Solvent delivery unit: max. 4, Autosampler: 1, Column oven: max. 4, Detector: max 2, etc.		
Number of connectable units	8 (Using option: 12)		4 (Excluding built-in solvent delivery unit)
Event input/output	Input: 1, output: 2		
Analog board	Up to two channels (option)	Up to one channel (option)	—
Communication	Ethernet		
Reservoir tray	Built-in	—	
Dimensions [mm], weight	W 260 × D 500 × H 140, 6 kg	W 260 × D 500 × H 72, 5 kg	—
Operating temperature range	4 to 35°C		
Power supply	AC 100–240 V, 50 VA, 50/60 Hz		Supplied from solvent delivery unit

Solvent Delivery Pump


LC-40B XR

	LC-40D	LC-40D XR LC-40B XR	LC-40D XS	LC-40D X3 LC-40B X3
Pumping method	Parallel-type double plunger (approx. 10 µL/1 stroke)			
Allowable maximum pressure	44 MPa	70 MPa	105 MPa	130 MPa
Flow rate settings range	0.0001 – 5.0000 mL/min (1.0 – 44 MPa) 5.0001 – 10.0000 mL/min (1.0 – 22 MPa)	0.0001 – 3.0000 mL/min (1.0 – 70 MPa) 3.0001 – 5.0000 mL/min (1.0 – 44 MPa) 5.0001 – 10.0000 mL/min (1.0 – 22 MPa)	0.0001 – 3.0000 mL/min (1.0 – 105 MPa) 3.0001 – 5.0000 mL/min (1.0 – 80 MPa) 5.0001 – 10.0000 mL/min (1.0 – 22 MPa)	0.0001 – 3.0000 mL/min (1.0 – 130 MPa) 3.0001 – 5.0000 mL/min (1.0 – 80 MPa) 5.0001 – 10.0000 mL/min (1.0 – 22 MPa)
Flow rate accuracy	± 1% or ± 2 µL/min, whichever greater (under specified conditions)		± 1% (under specified conditions)	
Flow rate precision	≤ 0.06% RSD or 0.02 minSD, whichever greater			
Gradient mode	High-pressure gradient (2 or 3 solvents) Quaternary low-pressure gradient	High-pressure gradient (2 solvents (LC-40B XR standard) or 3 solvents) Quaternary low-pressure gradient (Only available for LC-40D XR)	High-pressure gradient (2 or 3 solvents) Quaternary low-pressure gradient	High-pressure gradient (2 solvents (LC-40B X3 standard) or 3 solvents) Quaternary low-pressure gradient (Only available for LC-40D X3)
Gradient range of set concentrations	0 to 100% (0.1% step)			
Gradient concentration accuracy	± 0.5% (under specified conditions)			
Wetted materials	SUS316L, Hastelloy® C, PEEK, PTFE, Sapphire, Ruby	SUS316L, Hastelloy C, PEEK, PE, Sapphire, Ruby		
Available pH range	1 to 14			
Automatic rinsing kit	Option	Standard equipment		
Degassing unit	1 unit connectable	LC-40D XR: 1 unit connectable LC-40B XR: 2 units connectable	1 unit connectable	LC-40D X3: 1 unit connectable LC-40B X3: pre-installed (5 port built-in), 1 unit connectable
Dimensions [mm]	W 260 × D 500 × H 140			LC-40D X3: W 260 × D 500 × H 140 LC-40B X3: W 260 × D 500 × H 210
Weight	10 kg	LC-40D XR: 10 kg LC-40B XR: 13 kg	12 kg	LC-40D X3: 12 kg LC-40B X3: 21 kg
Operating temperature range	4 to 35°C			
Power supply	AC 100–240 V, 50/60 Hz			
	150 VA	LC-40D XR: 150 VA LC-40B XR: 180 VA	150 VA	LC-40D X3: 150 VA LC-40B X3: 180 VA

Degassing Unit


DGU-403

	DGU-403	DGU-405
Number of degassed solvents	3	5
Degassed flow line capacity	400 µL/1 line	
Dimensions [mm], weight	W 260 × D 500 × H 72, 4 kg	
Operating temperature range	4 to 35°C	
Power supply	Supplied from solvent delivery unit	

Autosampler

SIL-40C XR

		SIL-40 SIL-40C	SIL-40 XR SIL-40C XR	SIL-40C XS	SIL-40C X3
Injection method		Total-volume Injection (standard), loop injection (optional)			
Allowable maximum pressure		44 MPa	80 MPa	105 MPa	130 MPa
Injection volume		0.1 to 100 µL	0.1 to 50 µL		
		0.1 to 2000 µL (optional)			
Injection volume accuracy		≤ ± 1% (5 µL injection, n = 20)			
Linearity		≥ 0.9999			
Injection cycle time		≤ 6.7 seconds (under specified conditions)			
Samples for processing		288 (microtiter plate, 96 well × 3 plates), 1152 (microtiter plate, 384 well × 3 plates), 252 (1 mL sample vial, 84 × 3 plates), 162 (1.5 mL sample vial, 54 × 3 plates), 84 (4 mL sample vial, 28 × 3 plates), 36 (10 mL sample vial, 12 × 3 plates), 72 (1.5 mL micro tube, 24 × 3 plates)			
Injection volume reproducibility		RSD ≤ 1.0% (0.5 to 0.9 µL), RSD ≤ 0.5% (1.0 to 1.9 µL), RSD ≤ 0.25% (2.0 to 4.9 µL), RSD ≤ 0.15% (More than 5.0 µL), RSD < 0.5% (typically, 0.5 µL), RSD < 0.25% (typically, 1.0 µL)			
Carryover		≤ 0.0025% (without rinse) ≤ 0.0005% (with rinse, typically) (under specified conditions)	≤ 0.0015% (without rinse) ≤ 0.0003% (with rinse, typically) (under specified conditions)		
Dip rinsing outside the needle and injection port rinsing		Standard equipment			
Pumping rinse outside the needle		Option	Standard equipment		
Internal rinsing (3 dil)		Option			Standard equipment
Sample cooler		SIL-40: None SIL-40C: Standard equipment (Air-circulation temperature control type)	SIL-40 XR: None SIL-40C XR: Standard equipment (Air-circulation temperature control type)	Standard equipment (Air-circulation temperature control type)	
Sample cooler temperature setting range		4 to 45°C (Room temperature needs to be less than 30°C and humidity needs to be less than 70% to set 4°C)			
Sample cooler temperature accuracy		± 2°C (sensor position ± 0.5°C)			
Wetted material		SUS316L, DLC, PEEK, GFP, PTFE, FEP, ETFE, sapphire, ceramics, PPS, FFKM			
Available pH range		1 to 14			
Dimensions [mm], weight		W 260 × D 500 × H 280 (SIL-40C/40C XR/40C XS/40C X3: Protrusion adds 140 mm to the depth)			
		SIL-40: 17 kg SIL-40C: 24 kg	SIL-40 XR: 17 kg SIL-40C XR: 24 kg	24 kg	
Operating temperature range		4 to 35°C			
Power supply	Cooler model	AC 100–240 V, 400 VA, 50/60 Hz			
	Non cooler model	AC 100–240 V,150 VA, 50/60 Hz		—	

Plate Changer

		PLATE CHANGER
Samples for processing (includes two plates of autosampler)	1 PLATE CHANGER	1536 (microtiter plate, 96 well × 16 plates), 864 (deep-well plate, 96 well × 9 plates) 6144 (microtiter plate, 384 well × 16 plates), 3456 (deep-well plate, 384 well × 9 plates) 756 (1 mL sample vial, 84 × 9 plates), 486 (1.5 mL sample vial, 54 × 9 plates) 252 (4 mL sample vial, 28 × 9 plates), 108 (10 mL sample vial, 12 × 9 plates)
	3 PLATE CHANGERS	4224 (microtiter plate, 96 well × 44 plates), 2208 (deep-well plate, 96 well × 23 plates) 16896 (microtiter plate, 384 well × 44 plates), 8832 (deep-well plate, 384 well × 23 plates) 1932 (1 mL sample vial, 84 × 23 plates), 1242 (1.5 mL sample vial, 54 × 23 plates) 644 (4 mL sample vial, 28 × 23 plates), 276 (10 mL sample vial, 12 × 23 plates)
Sample cooler temperature setting range		Air-circulation temperature control type, 4 to 45°C (Room temperature needs to be less than 30°C and humidity needs to be less than 70% to set 4°C)
Dimensions [mm], weight		W 170 × D 500 × H 560 (Protrusion adds 140 mm to the depth), 26 kg
Operating temperature range		4 to 35°C
Power supply		AC 100–240 V, 400 VA, 50/60 Hz

Column Oven

CTO-40S

	CTO-40C	CTO-40S
Temperature control type	Forced air circulation	
Temperature control range	Room temperature –10°C to 100°C	Room temperature –10°C to 85°C
Temperature accuracy	± 0.5°C	± 0.8°C
Temperature precision	± 0.05°C	± 0.1°C
Containable column size and number	Up to 250 mm L. column × 6 or 300 mm L. column × 3	Up to 100 mm L. column × 6 or 300 mm L. column × 3
Dimensions [mm], weight	W 260 × D 500 × H 415, 21 kg	W 130 × D 500 × H 553, 15 kg
Operating temperature range	4 to 35°C	
Power supply	AC 100–120 V / 220–240 V (Automatic switching), 400 VA, 50/60 Hz	AC 100–240 V, 300 VA, 50/60 Hz

UV-VIS Detector

SPD-40V

	SPD-40	SPD-40V
Light source	Deuterium (D_2) lamp	Deuterium (D_2) lamp, tungsten lamp
Wavelength range	190 to 700 nm	190 to 1000 nm
Bandwidth	8 nm	
Wavelength accuracy	± 1 nm	
Wavelength reproducibility	± 0.1 nm	
Drift	≤ 0.1×10^{-3} of AU/h (under specified conditions)	
Noise	1 Wavelength mode: ≤ 4.0×10^{-6} AU, 2 Wavelength mode: ≤ 10.0×10^{-6} AU (under specified conditions)	
Linearity	2.5 AU (under specified conditions)	
Standard flow cell	Optical path length: 10 mm, Cell volume: 12µL, Pressure: 12 MPa Material of wetted parts: SUS316L, PFA, quartz	
Cell temperature control range	19 to 50°C, 1°C Step	
Optional flow cell	UHPLC cell (optical path length: 10 mm, cell volume: 8 µL, equipped with temperature control function) Semi-micro cell (optical path length: 5 mm, cell volume: 2.5 µL, equipped with temperature control function) Conventional cell (optical path length: 10 mm, cell volume:12 µL, equipped with temperature control function) Inert cell (optical path length: 10 mm, cell volume: 12 µL, equipped with temperature control function) Preparative cell (optical path length: 0.1/0.2/0.5 mm, cell volume: 0.8/1.6/4.0 µL) Micro flow cell (optical path length: 3 mm, cell volume: 0.21µL) Maximum pressure cell (optical path length: 10 mm, cell volume: 12 µL)	
Available pH range	1 to 13 (Cell quartz might be damaged by a mobile phase of pH >10.)	
Dimensions [mm], weight	W 260 × D 500 × H 140, 11 kg	
Operating temperature range	4 to 35°C	
Power supply	AC 100–240 V, 150 VA, 50/60 Hz	

Photodiode Array Detector

SPD-M40

	SPD-M40
Light source	Deuterium (D_2) lamp, Tungsten lamp
Number of diode elements	1024
Wavelength range	190 to 800 nm
Wavelength accuracy	± 1 nm
Wavelength reproducibility	± 0.1 nm
Slit width	1.2 nm, 8 nm
Spectral resolution	≤ ± 1.4 nm
Drift	≤ 0.4×10^{-3} of AU/h (under specified conditions)
Noise	≤ 4.5×10^{-6} AU (under specified conditions)
Linearity	2.5 AU (under specified conditions)
Standard flow cell	Optical path length: 10 mm, Cell volume: 12 µL, Pressure: 12 MPa Material of wetted parts: SUS316L, PFA, quartz
Cell temperature control range	19 to 50°C, 1°C Step
Optional flow cell	UHPLC cell (optical path length: 10 mm, cell volume: 8 µL, equipped with temperature control function) Semi-micro cell (optical path length: 5 mm, cell volume: 2.5 µL, equipped with temperature control function) Conventional cell (optical path length: 10 mm, cell volume:12 µL, equipped with temperature control function) Inert cell (optical path length: 10 mm, cell volume: 12 µL, equipped with temperature control function) Preparative cell (optical path length: 0.1/0.2/0.5 mm, cell volume: 0.8/1.6/4.0 µL, equipped) Micro flow cell (optical path length: 3 mm, cell volume: 0.21µL) Maximum pressure cell (optical path length: 10 mm, cell volume: 12 µL)
Available pH range	1 to 13 (Cell quartz might be damaged by a mobile phase pH >10.)
Dimensions [mm], weight	W 260 × D 500 × H 140, 10 kg
Operating temperature range	4 to 35°C
Power supply	AC 100–240 V, 180 VA, 50/60 Hz

Capillary cell type Photodiode Array Detector

	SPD-M30A
Light source	Deuterium (D_2) lamp
Number of diode elements	1024
Wavelength range	190 to 700 nm
Wavelength accuracy	± 1 nm
Wavelength reproducibility	± 0.1 nm
Slit width	1 nm, 8 nm
Spectral resolution	≤ 1.4 nm
Drift	≤ 0.5×10^{-3} AU/h (under specified conditions)
Noise	≤ 0.4×10^{-6} AU (under specified conditions)
Linearity	2.0 AU (under specified conditions)
Cell	Standard cell: Optical path length: 10 mm, Capacity: 1 µL, Pressure: 8 MPa Optional high-sensitivity cell: Optical path length: 85 mm, Capacity: 9 µL, Pressure: 8 MPa
Dimensions [mm], weight	W 260 × D 500 × H 140, 12 kg
Operating temperature range	4 to 35°C
Power supply	AC 100–240 V, 150 VA, 50/60 Hz

Spectrofluorometric Detector

	RF-20A	RF-20Axs
Light source	Xenon lamp	Xenon lamp Low-pressure mercury lamp (to check wavelength accuracy)
Wavelength range	200 to 650 nm	200 to 750 nm
Spectral bandwidth	20 nm	
Wavelength accuracy	± 2 nm	
Wavelength precision	± 0.2 nm	
S/N	Water Raman peak S/N ≥1200 Low background S/N ≥ 9000	Water Raman peak S/N ≥ 2000 Low background S/N ≥ 12000
Range of cell temperature control	—	Room temperature –10°C to 40°C, 1°C step
Cell	Standard conventional cell: volume 12 µL, maximum pressure 2 MPa Optional semi-micro cell: volume 3 µL, maximum pressure 2 MPa	
Function	Simultaneous measurement of four wavelengths, Wavelength scanning	
Dimensions [mm], weight	W 260 × D 500 × H 210, 16 kg	W 260 × D 500 × H 210, 18 kg
Operating temperature range	4 to 35°C	
Power supply	AC 100–240 V, 400 VA, 50/60 Hz	

Differential Refractive Index Detector

	RID-20A
Measurement range	1 to 1.75 RIU
Noise	≤ 2.5×10^{-9} RIU
Drift	≤ 1×10^{-7} RIU/h
Range	A mode: 0.01×10^{-6} to 500×10^{-6} RIU P, L-mode: 1×10^{-6} to 5000×10^{-6} RIU
Response	0.05 to 10 sec, 10 steps
Polarity – Change	Available
Zero adjustment	Auto zero, Optical zero, Fine zero
Maximum flow rate	20 mL/min (150 mL/min in option)
Range of cell temperature control	30 to 60°C
Cell	Volume 9 µL, Maximum pressure 2 MPa
Dimensions [mm], weight	W 260 × D 420 × H 140, 12 kg
Operating temperature range	4 to 35°C
Power supply	AC 100–240 V, 150 VA, 50/60 Hz

Conductivity Detector

	CDD-10Avp
Cell volume	0.25 µL
Cell constant	25 µS·cm^{-1}
Material of wetted parts	PEEK, SUS316
Maximum use pressure	2.9 MPa (30 kgf/cm^2)
Response	0.05 to 10 s, 10 steps
Zero adjustment	Auto-zero function, Baseline-shifting function
Dimensions [mm], weight	W 260 × D 420 × H 140, 6 kg
Operating temperature range	4 to 35°C
Power supply	AC 100–240 V, 250 VA, 50/60 Hz

Evaporative Light-Scattering Detector

	ELSD-LT II
Nebulizing method	Siphon Splitting
Light source	LED
Detection	Photomultiplier
Scope of set temperature	Room temperature to 80°C
Gas nebulizer	Nitrogen or air*
Gas flow rate, gas pressure	Up to 3.0 mL/min, up to 450 kPa
Standard mobile phase flow rate	0.2 to 2.5 mL/min
Analog output	0 to 1 V
Dimensions [mm], weight	W 260 × D 550 × H 450, 20 kg
Operating temperature range	5 to 40°C
Operation humidity range	≤ 80% (Room temperature 5 to 31°C), ≤ 50% (Room remperature 31 to 40 °C)
Power supply	AC 100 V, 210 VA, 50/60 Hz

*Requires a gas supply source, such as an air compressor, nitrogen generator and gas piping.

[Note] • Please use a regulator with filter (option) in order to remove small foreign matters in the gas.
• Please make sure that nitrogen or air doesn't contain oil, dust, or moisture when you use nitrogen generator and/or air compressor.
• Please use the instrument in a room with exhaust facilities.

Optional accessories

Solvent Delivery Unit

Part Name		P/N	Description
Low-pressure gradient unit		228-65016-58	Low-pressure gradient unit for LC-40D/40D XR/40D XS/40D X3
Reservoir selection valve		228-65017-58	Two-solvent switching unit to be incorporated in solvent delivery unit
FCV-11AL		228-65611-58	The mobile phase switching valve of 3 flow lines that connects to solvent delivery unit (external)
FCV-11ALS		228-65610-58	The mobile phase switching valve of 1 flow line that connects to solvent delivery unit (external)
Automatic rinsing kit		228-56201-41	Automatic rinsing kit for plunger seal cleaning
Mixer	MR 20 µL	228-72652-41	High-efficiency mixer for high-pressure gradient system (volume 20 µL)
	MR 40 µL	228-72652-42	High-efficiency mixer for high-pressure gradient system (volume 40 µL)
	MR 100 µL	228-72652-43	High-efficiency mixer for high-pressure gradient system (volume 100 µL)
	MR 180 µL	228-72652-44	High-efficiency mixer for high-pressure gradient system (volume 180 µL)
	MR 40 µL LPGE	228-65020-41	High-efficiency mixer for low-pressure gradient system (volume 40 µL)
	MR 300 µL LPGE	228-72653-42	High-efficiency mixer for low-pressure gradient system (volume 300 µL)

Autosampler

Part Name		P/N	Description
Sample loop	50 µL	228-63132-44	Sample loop for 50 µL injection (standard configuration of SIL-40 XR/40C XR/40C XS/40C X3)
	100 µL	228-63132-45	Sample loop for 100 µL injection (standard configuration of SIL-40/40C)
	500 µL	228-45405-45	Sample loop to increase the injection volume up to 500 µL (Connect sample loop 100 µL (228-63132-45))
	2000 µL	228-45405-46	Sample loop to increase the injection volume up to 2 mL (Connect sample loop 100 µL (228-63132-45))
Dual-injection kit		228-72568-41, -42	Tubing kits for dual injection (228-72568-41 is for CTO-40S and 228-72568-42 is for CTO-40C)
Sample loop for loop injection	5 µL	228-71759-42	Sample loop for loop injection mode (volume 5 µL)
	20 µL	228-71759-43	Sample loop for loop injection mode (volume 20 µL)
	50 µL	228-71759-44	Sample loop for loop injection mode (volume 50 µL)
Sample plate	1.5 mL	228-71762-46	Plate for 1.5 mL sample vial (54)
	1 mL	228-71762-42	Plate for 1 mL sample vial (84)
	4 mL	228-71762-43	Plate for 4 mL sample vial (28)
	10 mL	228-71762-44	Plate for 10 mL sample vial (12)
Identification labels	For 96-well microplates	228-71840-41	Identification label affixed to the 96-well microtiter plate (100 set)
	For 96-well deep-well plates	228-71840-42	Identification label affixed to the 96-well deep-well plate (100 set)
	For 384-well microplates	228-71840-43	Identification label affixed to the 384-well microtiter plate (100 set)
	For 384-well deep-well plates	228-71840-44	Identification label affixed to the 384-well deep-well plate (100 set)

Column Oven

Part Name		P/N	Description
Active pre-heater		228-72084-41	Pre-heater device for thermostatting mobile phase before the column inlet
FCV kits	For CTO-40S	228-72438-41	This is a kit for attaching a flow line switching valve to CTO-40S
	For CTO-40C	228-72589-41	This is a kit for attaching a flow line switching valve to CTO-40C
Two FCV tubing kits	ID 0.3	228-72437-41	Tubing kit to connect the flow line switching valve and columns
	ID 0.1	228-72437-42	
Six FCV tubing kits	ID 0.3	228-72437-43	
	ID 0.1	228-72437-44	
Nexlock™ SS (with fitting)	ID 0.1 mm × 600 mm	228-62544-11	Finger-tight high-pressure fitting
	ID 0.3 mm × 600 mm	228-62544-22	

UV Detector / PDA Detector

Part Name	P/N	Description
UHPLC cell	228-64724-41 (PDA), -42 (UV)	Flow cell for high-speed analysis (volume 8 µL)
Semi-micro cell	228-64725-41 (PDA), -42 (UV)	Flow cell for semi-micro analysis (volume 2.5 µL)
Conventional cell	228-68250-41 (PDA), -42 (UV)	Flow cell with the same cell volume (12 µL) as standard cell of SPD-20A and SPD-M20A
Inert cell	228-64728-41 (PDA), -42 (UV)	Inert-type flow cell with metal-less wetted parts
Preparative cell	228-64727-41 (PDA), -42 (UV)	Preparative flow cell with variable optical path length
Micro flow cell	228-64737-41 (PDA), -42 (UV)	Flow cell for micro analysis (volume 0.21 µL)
Maximum pressure cell	228-64726-41 (PDA), -42 (UV)	High-pressure resisting flow cell for Nexera™ UC
Solvent recycle valve	228-56808-42 (UV)	Valve to recycle mobile phase by attaching to SPD-40/40V

Others

Part Name	P/N	Description
Mobile phase monitor (controller)	228-65525-58	MPM-40 controller to monitor remaining mobile phase in real-time Up to six bottle holders can be connected (228-65526-58, set of two)
Power outlet unit 6P	228-65523-42 (socket type B) 228-65523-43 (socket type D) 228-65523-46 (socket type I) 228-65523-58 (socket type F)	Power tap to turn off the main power of the instrument completely at one time. Switches can be installed in front of the reservoir tray. It provides six outlets.
Power outlet unit 2PS	228-65524-46 (for China) 228-65524-58 (for other than China)	Outlet to supply power to main units that need to be connected to service outlets, such as SIL-10A and FRC-10A. It provides two outlets.
Tubing kit A, ID 0.3 for high-pressure GE	228-70254-41	Tubing kits for high-pressure gradient system. Column inlet tubing ID 0.3 mm
Tubing kit B, ID 0.1 for high-pressure GE	228-70254-42	Tubing kits for high-pressure gradient system. Column inlet tubing ID 0.1 mm
Tubing kit C, ID 0.3 for low-pressure GE	228-70254-43	Tubing kits for low-pressure gradient system. Column inlet tubing ID 0.3 mm
Tubing kit D, ID 0.1 for low-pressure GE	228-70254-44	Tubing kits for low-pressure gradient system. Column inlet tubing ID 0.1 mm
Cable kit A	228-70247-41	Optical link cable kit, 600 mm × 1 pc, 800 mm × 1 pc
Cable kit B	228-70247-42	Optical link cable kit, 600 mm × 2 pcs, 800 mm × 1 pc
Cable kit C	228-70247-43	Optical link cable kit, 600 mm × 3 pcs, 800 mm × 1 pc
Cable kit D	228-70247-44	Optical link cable kit, 600 mm × 4 pcs, 800 mm × 1 pc
Reservoir tray	228-65508-58	Reservoir tray for up to 8 bottles (1L)
AD board	228-55519-41	Board for analog–digital conversion. It takes in detector signals as analog signals.
Optical cable connector expansion board	228-70481-41	The board to expand the number of optical cable connector channels to 12ch from 8ch (standard) by attaching to SCL-40/CBM-40

Valve

Part Name	P/N	Description
FCV-DR	228-65602-58	Drive unit and control board for incorporating valve into CTOs (1 FCV valve is required separately)
FCV-0206	228-65603-58	2-position 6-port valve (Maximum pressure: 44 MPa)
FCV-0607	228-65604-58	6-position 7-port valve (Maximum pressure: 44 MPa)
FCV-0206H	228-65607-58	2-position 6-port valve (Maximum pressure: 80 MPa)
FCV-0607H	228-65608-58	6-position 7-port valve (Maximum pressure: 80 MPa)
FCV-0206H3	228-65624-58	2-position 6-port valve (Maximum pressure: 130 MPa)
FCV-0607H3	228-65625-58	6-position 7-port valve (Maximum pressure: 130 MPa)

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Honey Analysis **Made Easy**

Index

1.

OUR COMMITMENT TO BEES

Together, we can bee more!

Shimadzu is committed to protecting the bees for the conservation of nature and its biodiversity.

With the beeswe.love project in Europe, Shimadzu took over a partnership for a bee colony and enabled the creation of 100 m^2 of bee pasture, a natural meadow to provide forage for the honeybees, native pollinators, and insects.

This commitment may appear small, but the idea is big to allow everyone to take part in sustainability efforts. We want to show with our commitment that everyone can make a difference.

With the beeswe.love project, Shimadzu takes the effort to accompany the bees, take care of them, and learn more about them to understand and protect the fragile side of nature.

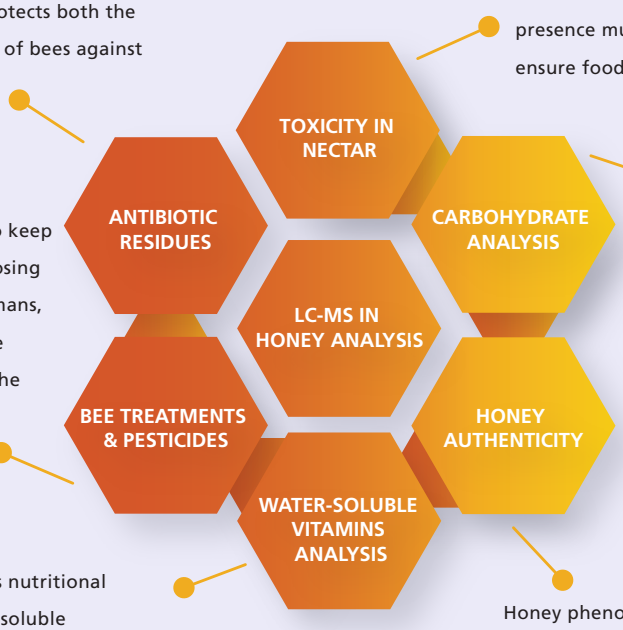
2.

INTRODUCTION TO HONEY ANALYSIS

Antibiotic drugs are used in apiculture to prevent bacterial infections among the bees. The analysis of antibiotic residues helps to protects both the public health and the well-being of bees against improper usage of medicines.

Pesticides are extensively used to keep unwanted pests away. Besides posing as a potential health risk for humans, it is perceived to be linked to the colony collapse disorder within the bees as well.

Honey is highly valued due to it's nutritional benefits. It contains many water-soluble vitamins that are vital to essential human body functions – these can be analyzed to understand its composition in detail.



Honey poisoning has been widely reported due to the contamination from plant-derived toxins such as Tripterygium wilfordii. Their presence must be detected and quantified to ensure food safety.

Honey is mostly made up of a complex mixture of carbohydrates. With suitable LC-MS method, the chemical composition of honey can be understood to differentiate honey from various varieties and sources.

Honey phenolic compounds are commonly used as potential authenticity markers to ensure quality and prevent food fraud for the benefit of the consumers.

3.

APPLICATION NOTES

3.1 Detection of Antibiotics

Determination of Residues of Five Tetracyclines in Honey with UHPLC Triple Quadrupole Mass Spectrometry

INTRODUCTION

In this paper, a method is proposed for the determination of 5 tetracyclines residues in honey using Shimadzu Ultra-High-Performance Liquid Chromatography (UHPLC) and triple quadrupole mass spectrometer. Tetracyclines in honey sample were first enriched by solid-phase extraction, then fast separated with LC-30A UHPLC, and finally quantitatively assayed with LCMS-8040 triple quadrupole mass spectrometer. The calibration curves of 5 tetracyclines were plotted by an external standard method and all demonstrated a wide linear range and correlation coefficients greater than 0.9996. Precision tests were performed on 5 μg/L, 10 μg/L and 50 μg/L multi-standard solutions and the %RSDs of retention time and peak area of 6 successive injections fell in the ranges of 0.20%~1.14% and 0.62%~3.79%, respectively, suggesting that the method's precision was good. LODs fell in the range of 31.9~63.4 ng/L and LOQs were 127~254 ng/L. The recovery of spiked samples fell in the range of 86.9~ 98.1%. Tetracyclines (TCs) are a category of broad-spectrum antibiotics that is widely used clinically. However, irrational use of such drugs, such as excessive use of agents, prolonged drug use, drug abuse and non-compliance with withdrawal period to slaughter ahead, causes such drugs and their metabolites residual in animal muscle, eggs, milk, organ tissues and secretions. Tetracyclines cannot be completely absorbed by animals and a considerable part enters the food chain and the environment in the primary form or metabolite form, indirectly affecting human health.

The honey industry has developed rapidly in recent years. Our bees are mostly imported from abroad with relatively high incidence rate. Though China advocates biological control, some people still use chemical drugs and antibiotics to treat the bees, resulting in higher level of antibiotics in honey. Therefore, there is an urgent need to establish an effective and sensitive method to detect tetracylines in honey. High performance liquid chromatography (HPLC)-tandem mass spectrometry has been developed rapidly in recent years. It has merits such as high selectivity and sensitivity and accurate quantitation of drug residues in complex matrices. A method was proposed for determination of five tetracyclines in honey with Shimadzu LC-30A UHPLC and LCMS-8040 triple quadrupole mass spectrometer.

EXPERIMENTAL

Apparatus

A combined system of Shimadzu UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8040 was used in the experiment. The configuration included two LC-30AD pumps, a DGU-20A$_5$ online degasser, a SIL-30AC autosampler, a CTO-30A column oven, a DGU-20A$_5$ communication bus module, a LCMS-8040 triple quadrupole mass spectrometer, and a LabSolutions ver. 5.53 chromatography workstation.

Condition of Analysis

LC Conditions	: Shim-pack XR-ODS II 2.0 mm I.D.× 100 mm L., 2.2 μm
Mobile Phase	: A:0.1% formic acid aqueous solution
Mobile Phase	: B:methanol
Flow Rate	: 0.25 mL/min
Column Temperature	: Room Temperature
Injection Volume	: 20 μL
Elution Mode	: Gradient elution with initial concentration of mobile phase B of 20%

See Table 1 for the time program.

Table 1 Time Program

Time (min)	Module	Command	Value
0.00	Pumps	B Conc.	20
5.00	Pumps	B Conc.	95
6.00	Pumps	B Conc.	95
6.01	Pumps	B Conc.	20
8.00	Pumps	B Conc.	20
8.00	Controller	Stop	

MS Conditions

Ionization Mode	: ESI(+)
Ionization Voltage	: 4.5 kV
Nebulizing Gas	: Nitrogen, 3.0 L/min
Drying Gas	: Nitrogen, 15 L/min
Collision Gas	: Argon
DL Temperature	: 250°C
Heater Block Temperature	: 400°C
Scan Mode	: Multiple Reaction Monitoring (MRM)

Dwell Time : 10 ms
Pause Time : 3 ms
MRM Parameters : See Table 2

Sample Preparation

Preparation of standard solution: take appropriate standard substances of tetracycline, terramycin, ledermycin, aureomycin and doxycycline, and prepare 1000 mg/L multi-standard stock solutions with them and methanol, and then dilute the stock solutions with methanol and 0.1% formic acid aqueous solution (1:4, v/v) to get multi-standard working solutions of various concentrations.

Sample pretreatment method: refer to "GB/T 23409-2009 Determination of residues of oxytetracycline, tetracycline, chlortetracycline, and doxycycline in royal jelly - LC-MS/MS method" for the preparation of honey samples and purification and extraction of analytes.

Table 2 Optimized MRM Parameters

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Tetracycline	445.20	410.10*	-22	-20	-29
		427.15	-22	-14	-30
Terramycin	461.20	426.10*	-23	-19	-30
		443.20	-23	-14	-21
Ledermycin	465.10	448.10*	-23	-19	-30
		430.10	-23	-22	-30
Aureomycin	479.15	444.20*	-24	-22	-30
		462.15	-24	-18	-22
Doxycycline	445.15	428.25*	-22	-19	-30
		154.20	-22	-34	-28

*Note: * refers to quantitative ion*

RESULTS AND DISCUSSION

Mass Spectrum and MS/MS Spectrum

The mass spectrum of tetracycline is shown in Fig. 1 and the MS/MS spectrum is shown in Fig. 2.

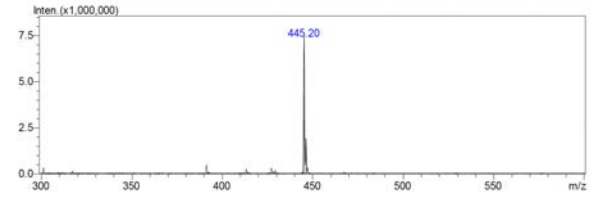


Fig. 1 Mass spectrum of tetracycline

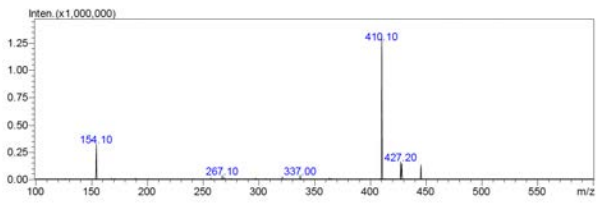


Fig. 2 MS/MS spectrum of tetracycline (CE -20V)

The mass spectrum of terramycin is shown in Fig. 3 and the MS/MS spectrum is shown in Fig. 4.

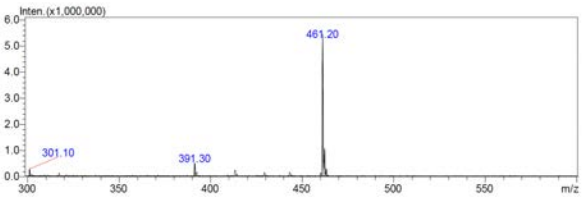


Fig.3 Mass spectrum of terramycin

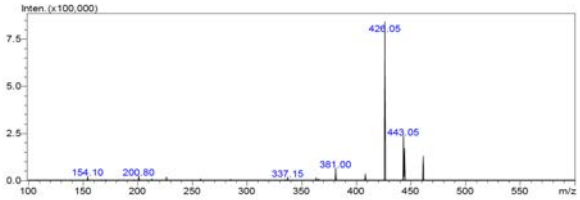


Fig.4 MS/MS spectrum of terramycin (CE -19V)

The mass spectrum of ledermycin is shown in Fig. 5 and the MS/MS spectrum is shown in Fig. 6.

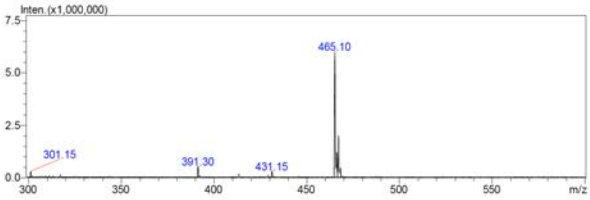


Fig. 5 Mass spectrum of ledermycin

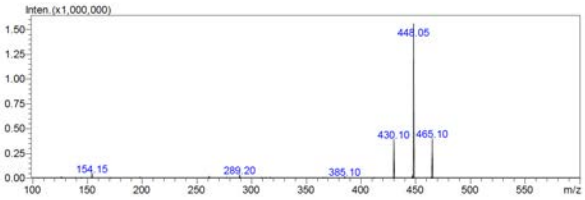


Fig. 6 MS/MS spectrum of ledermycin (CE -19V)

The mass spectrum of aureomycin is shown in Fig. 7 and the MS/MS spectrum is shown in Fig. 8.

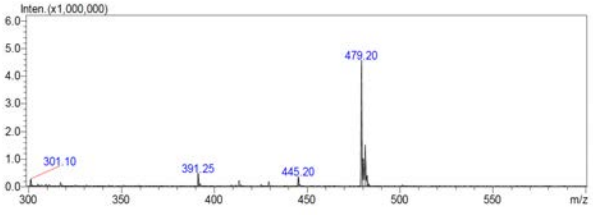


Fig. 7 Mass spectrum of aureomycin

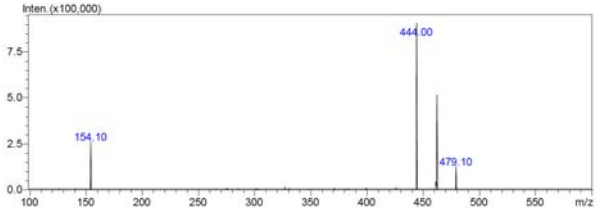


Fig. 8 MS/MS spectrum of aureomycin (CE -22V)

The mass spectrum of doxycycline is shown in Fig. 9 and the MS/MS spectrum is shown in Fig. 10.

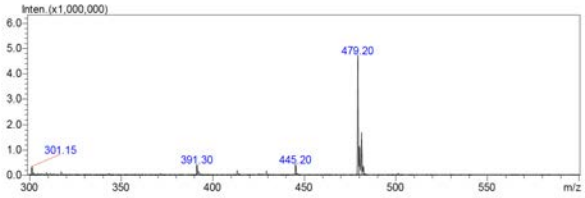


Fig. 9 Mass spectrum of doxycycline

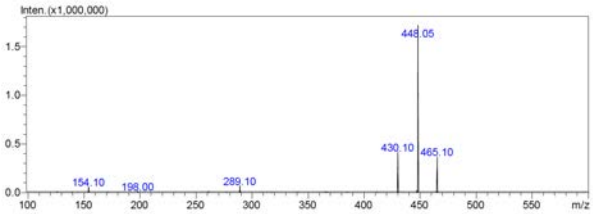


Fig. 10 MS/MS spectrum of doxycycline (CE -19V)

MRM Chromatogram of Standard Mixture

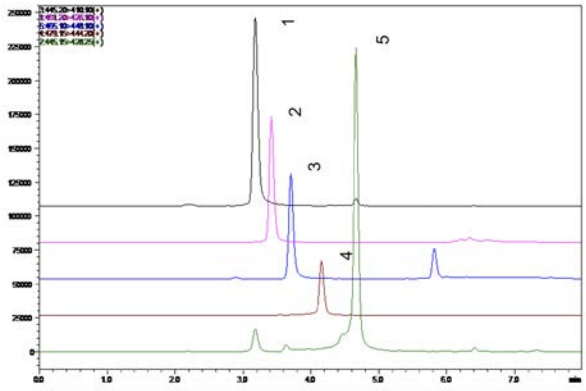


Fig. 11. MRM chromatograms of standard mixture (100 μg/L) (1. Tetracycline; 2. Terramycin; 3. Ledermycin; 4. Aureomycin; 5. Doxycycline)

Linear Range

Multi-standard solutions at concentrations of 0.2 μg/L, 0.5 μg/L, 1 μg/L, 2.5 μg/L, 5 μg/L, 10 μg/L, 50 μg/L, 100 μg/L and 200 μg/L were subjected to quantitative assay by external calibration method under the analysis conditions as specified. Calibration curves were plotted as shown in Fig. 12 to Fig. 16 with concentration as abscissa and peak area as ordinate; the calibration curves were of satisfactory linearity and their linear equations and correlation coefficients are shown in Table 3.

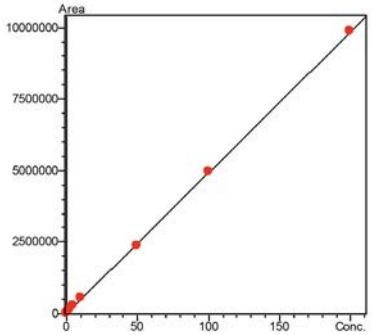


Fig. 12 Calibration curve of tetracycline

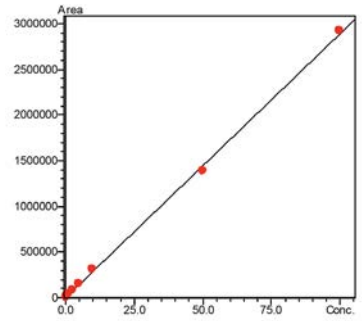


Fig.13 Calibration curve of terramycin

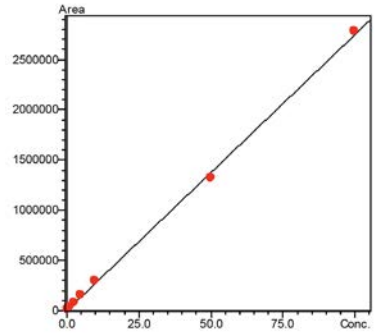


Fig. 14 Calibration curve of ledermycin

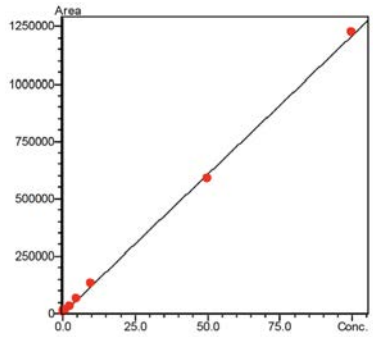


Fig. 15 Calibration curve of aureomycin

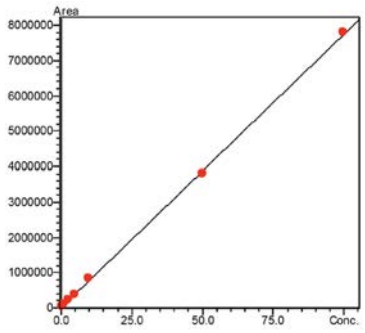


Fig. 16 Calibration curve of doxycycline

Table 3 Parameters of Calibration Curves

No.	Compound	Calibration Curve	Linear Range (μg/L)	Correlation Coefficient (r^2)
1	Tetracycline	Y = (49259.9)X + (-1866.27)	0.2~200	0.9999
2	Terramycin	Y = (28905.9)X + (-348.296)	0.5~100	0.9997
3	Ledermycin	Y = (27468.4)X + (1698.49)	0.5~100	0.9996
4	Aureomycin	Y = (12102.7)X + (571.906)	0.5~100	0.9997
5	Doxycycline	Y = (77333.8)X + (5973.94)	0.2~100	0.9998

Precision Test

Multi-standard working solutions of various concentrations were determined for 6 times in succession to assess the method's precision. Repeatability of retention time and peak area was shown in Table 4. The results showed that the %RSDs of retention time and peak area data of standard solutions of various concentrations fell in the ranges of 0.20%~1.14% and 0.62%~3.79% respectively, suggesting the method had satisfactory precision.

Table 4 Repeatability - retention time and peak area (n=6)

Compound	%RSD (5 μg/L)		%RSD (10 μg/L)		%RSD (50 μg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Tetracycline	1.14	3.21	0.93	2.65	1.10	1.42
Terramycin	0.82	2.76	0.91	2.87	0.79	0.62
Ledermycin	0.88	3.27	0.78	3.04	0.70	2.90
Aureomycin	0.48	3.79	0.46	2.98	0.38	1.80
Doxycycline	0.20	2.71	0.22	1.72	0.20	1.20

LOD

Seven standard samples at 200 ng/L were prepared and directly injected for analysis. After discounting the outliers from the results, the standard deviation S of these 7 measurements was calculated. The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were calculated using these formulae LOD=3.14×S, LOQ=4×MDL. The assay results are shown in Table 5.

Table 5 LODs and LLOQs of Tetracyclines

No.	Compound	Standard deviation (S)	MDL (ng/L)	LLOQ (ng/L)
1	Tetracycline	14.9	46.8	187
2	Terramycin	20.2	63.4	254
3	Ledermycin	17.8	55.9	224
4	Aureomycin	18.4	57.8	231
5	Doxycycline	10.2	31.9	127

Recovery Test

Honey samples were analyzed for the 5 tetracyclines in honey. Tetracycline was detected in 2 g of honey samples at concentration of 0.249 μg/kg. The resulted chromatograms are shown in Fig. 17.

In order to assess the method's actual detection effect of tetracyclines in honey samples, honey samples were spiked with five tetracyclines standard substances at concentration of 2 μg/kg. The chromatograms of a spiked sample are shown in Fig. 18 and recoveries of a spiked sample are shown in Table 6.

MRM Chromatogram of Actual Samples

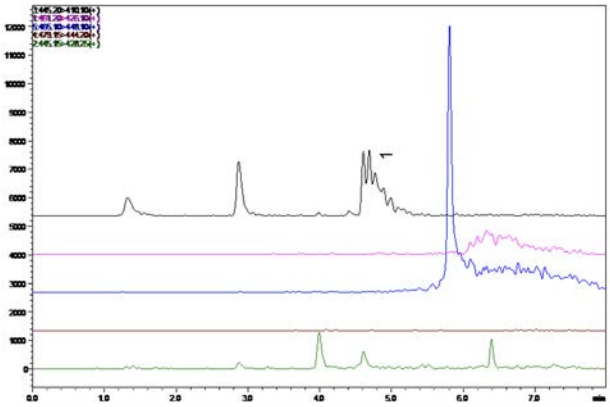


Fig.17 MRM chromatograms of honey sample (1 tetracycline detected)

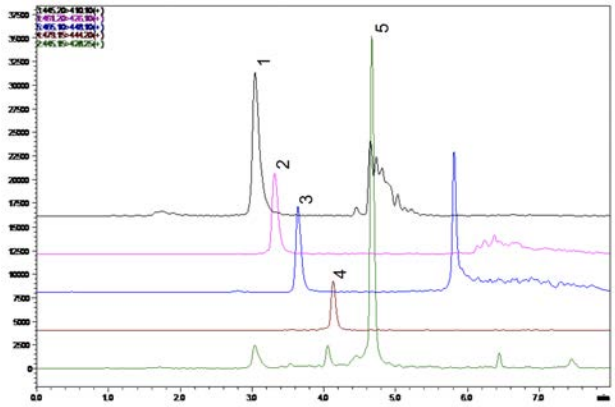


Fig. 18 MRM Chromatograms of a spiked honey sample (2 μg/kg) (1.Tetracycline; 2.Terramycin; 3.Ledermycin; 4.Aureomycin; 5. Doxycycline)

Table 6. Spike Recoveries of Tetracyclines

No.	Compound	Tested concentration of Sample 1 (μg/kg)	Tested concentration of Sample 2 (μg/kg)	Average Recovery (%)
1	Tetracycline	2.18	2.25	98.1
2	Terramycin	1.93	1.87	95.2
3	Ledermycin	1.67	1.81	86.9
4	Aureomycin	1.78	1.76	88.3
5	Doxycycline	1.89	1.92	98.1

CONCLUSION

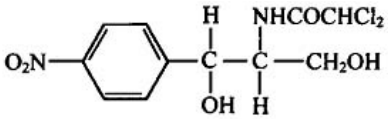
A method was proposed for detection of tetracyclines residues in honey using Shimadzu LC-30A UHPLC and LCMS-8040 triple quadrupole mass spectrometer. The method is of high sensitivity, good precision and wide linear range with the correlation coefficient greater than 0.9996. Detection of trace tetracycline was realized by determining commercial honey samples. The recoveries of spiked samples were in the range of 86.9~98.1% by spiked analysis at high, medium and low levels for the reagent samples, proving that the method is suitable for analysis and detection of tetracycline in honey samples.

3.1 Detection of Antibiotics

Determination of Chloramphenicol in Honey with Online Pretreatment LC System-Mass Spectrometery

INTRODUCTION

A method is proposed in this paper for determination of chloramphenicol in honey with online pretreatment LC system-mass spectrometer. The proposed method utilizes online pretreatment and concentration of samples by a specifically established valve switching system which significantly cut down users' time needed for sample pretreatment. The method was simple, convenient, highly sensitive, and capable of determining of chloramphenicol in honey with high reproducibility and an LOQ as low as 0.5 μg/kg. Chloramphenicol (CAP, CAS:56-75-7), also called chloromycetin, is a broad-spectrum antibiotic which is commonly used for the treatment of bacterial infectious diseases in fishery and poultry husbandry production. Its chemical structural formula is as follows.



Chlorampenicol(CAP)

Because of the hematopoietic function inhibiting action of chloramphenicol, its application in animal-derived food is banned in many countries and a maximum residue limit (MRL) of zero is set for chloramphenicol in edible tissues of food animals. It is stipulated by the Ministry of Agriculture of China (in No. 227 announcement of the year 2002) that the afore-mentioned ban also applied in China and chloramphenicol was included in the *List of Food Additives That May Be Illegally Added into Food and Abused (the fifth batch)*. China is a major honey exporting country and chloramphenicol is a mandatory test item for imported/exported honey products. Therefore, it is absolutely necessary to develop simple, convenient, and sensitive detection methods for chloramphenicol. In this paper, an online pretreatment LC system-mass spectrometer was used in conjunction with Shimadzu LCMS-8040 triple quadrupole mass spectrometric detector for fast and highly sensitive assay of trace amount of chloramphenicol at the same time significantly simplifying the pretreatment procedures for honey samples.

EXPERIMENTAL

Instrument

An LC-30A based online pretreatment system was used in the experiment in conjunction with LCMS-8040 triple quadrupole mass spectrometer. The flow circuit diagram of the system was shown below:

1) Sample introduction flow circuit: A sample introduction pump was used for introduction of samples into the pretreatment column, where the target analyte was retained while the matrix was carried away by the mobile phase into waste liquid bottle, thereby achieving the purpose of sample pretreatment.

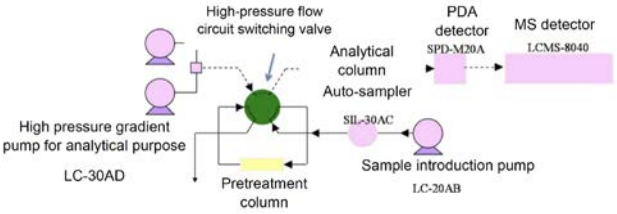


Fig.1 Flow circuit diagram of sample introduction

2) Sample analysis flow circuit: When sample flow path was switched to this circuit, analytical mobile phase would be transported by a high pressure gradient pump to the pretreatment column, where the mobile phase would elute the target analyte enriched in the pretreatment column out into the analytical column for separation and analysis with PDA and MS detectors.

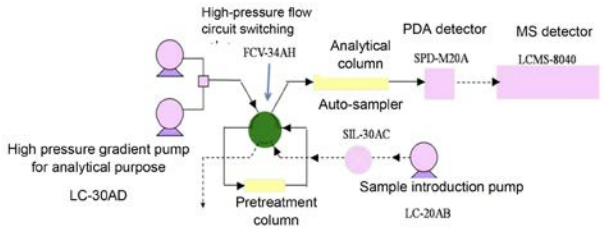


Fig.2 Flow circuit diagram of sample analysis

Conditions of Analysis

LC conditions

Loading Conditions Column: MAYI-ODS (2.0 mm I.D.×10 mm L., 5 μm)

Introduction Mobile Phase : A: aqueous solution; mobile phase B:acetonitrile

Flow Rate : 2 mL/min

Injection Volume : 5 μL

Conditions of Analysis

Column :Shim-pack XR-ODS (2.0 mm I.D.×75 mm L., 2.2 μm)

Mobile Phase : A: aqueous solution; Mobile phase B:acetonitrile

Flow Rate : 0.35 mL/min

Column Temperature : 40°C

Elution Mode :Gradient elution with initial concentration of mobile phase B of 5%

See Table 1 for the elution program.

Table 1 Time Program

Time(min)	Module	Command	Value
1.00	Column Oven	CTO.RVL	1
1.00	Pumps	Pump B Conc.	5
1.00	Pumps	Pump C B.Conc	5
1.01	Pumps	Pump C B.Conc	90
2.50	Pumps	Pump B Conc.	95
3.00	Column Oven	CTO.RVL	0
3.00	Pumps	Pump B Conc.	95
3.00	Pumps	Pump C B.Conc	90
3.01	Pumps	Pump B Conc.	5
3.01	Pumps	Pump C B.Conc	5
5.00	Controller	Stop	

MS Conditions

Ionization Mode : ESI(-)
Ionization Voltage : -3.5 kV
Nebulizing Gas : Nitrogen 2.5 L/min
Drying Gas : Nitrogen 15 L/min
Collision Gas : Argon
DL Temperature : 250°C
Heater Block Temperature : 300°C
Acquisition Mode : Multiple Reaction Monitoring (MRM)
Dwell Time : 100 ms
Pause Time : 3 ms
MRM Parameters : See Table 2

Table 2 Optimized MRM Parameters

Compound	Pre-cursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q1 Pre Bias (V)
Chloramphenicol	321.05	152.05*	12.0	18.0	29.0
		257.05	12.0	10.0	16.0
D5-chloramphenicol(IS)	326.00	262.15	23.0	11.0	16.0

*Note: * refers to quantitative ion*

Preparation of Standard Solutions

Preparation of standard working solutions: A 1.0 mg/mL standard stock solution was prepared using acetonitrile as solvent, then progressively diluted with water to get a series of working solutions of concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 50, and 100 ng/mL, respectively.

Sample Pretreatment Method

The proposed method made use of online pretreatment, therefore honey samples were simply diluted and filtered for direct analysis. The specific procedures were as follows: 5 g honey was accurately weighed (with a precision of 0.01 g) and added 50 mL water, subjected to a shaker for mixing evenly followed by filtration with 0.22 μm micropore film before injection for assay.

RESULTS AND DISCUSSION

Optimization of Loading Time

The determination of sample loading time can have significant impact on the results when a sample pretreatment system is used. If the loading time is too short, the matrix may not be completely eluted; if the loading time is too long, the target analyte may suffer from wider peak span and lower recovery. In the light of this, sample loading time need to be determined early in the development of the method.

In consideration of that the analyte chloramphenicol in honey, which contained a lot of carbohydrates, the mass spectrometer was not connected to the system during determination of loading time. A UV detector working at 200 nm was used instead for monitoring the elution of matrix. The sample introduction flow circuit was used at this time and the exit of the circuit was connected to the UV detector. The results showed that all carbonhydrates in samples were almost completely eluted within 1 min. Therefore, the sample loading time of the method was set to 1 min.

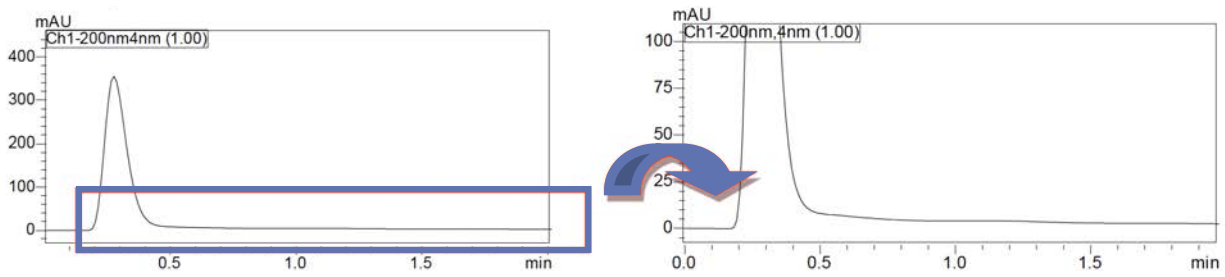


Fig.3 UV chromatogram of sample retention in the pretreatment column

Mass Spectrum and MRM Chromatogram

Mass spectrum of chloramphenicol was obtained by analyzing a 100 ng/mL standard solution in Q3 Scan mode. Chloramphenicol responded well to the method in negative ion mode, [M-H]-=321.05. MRM chromatogram of a 10 ng/mL standard solution was shown in Fig.5.

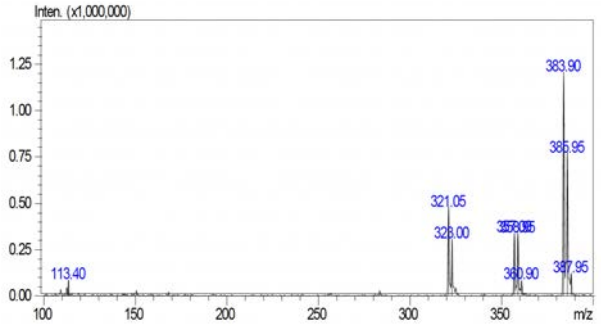


Fig.4 Scan chromatogram of a 100 ng/mL standard solution in Q3 Scan mode

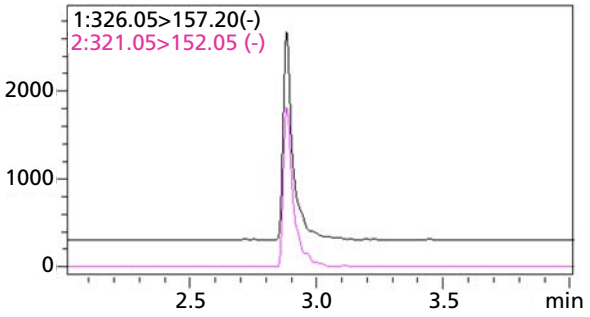


Fig.5 MRM chromatogram of a 10 ng/mL standard solution

Linear Range

A series of standard working solutions of concentrations of 0.1, 0.5, 1, 2, 5, 10, 20, 50, and 100 ng/mL was subjected to quantitative analysis under the analytical conditions specified using internal standard. A calibration curve was plotted as shown in Fig 6 with concentration ratio as abscissa and peak area ratio as ordinate.

The resulted calibration curve was of satisfactory linear relation and had a linear equation of Y=(0.183317)X + (-0.00508229) and a correlation coefficient of r=0.9997.

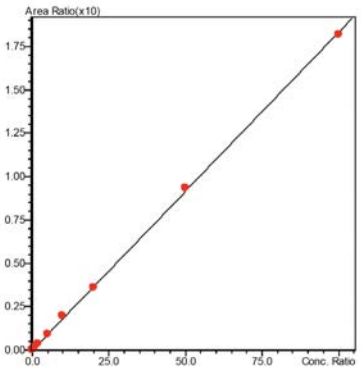


Fig.6 Calibration curve of chloramphenicol

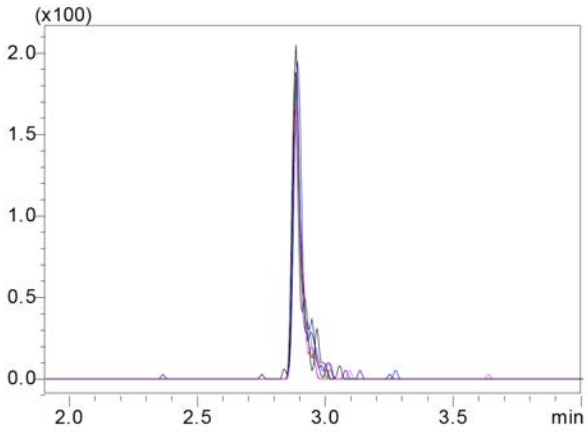


Fig.7 Overlapping chromatogram of 6 replicate injections of a 0.5 ng/mL standard solution

Precision Test

The system's precision was assessed on 6 replicate injections of 0.5 ng/mL standard working solution. The resulted overlapping chromatogram is shown in Fig. 7. The %RSDs of retention time and peak area data were 0.13% and 3.12%, respectively, suggesting that the system had good precision.

Table 5 Repeatability - Retention Time and Peak Area (n=6)

No.	R.T.	Area
1	2.885	457
2	2.886	432
3	2.892	462
4	2.886	433
5	2.882	434
6	2.881	435
Average	2.885	442
RSD%	0.13	3.12

Sensitivity Test

In order to assess the system's sensitivity, honey matrix samples spiked with standard at the spiked level of 5 μg/kg were analyzed and demonstrated good response to the method. The resulted chromatograms are shown in Fig.8.

Since the pretreatment column in the system was provided with sample concentrating function, large volume samples could be loaded to the system. When 50 μL honey matrix sample, spiked with 0.5 μg/kg standard, was loaded, the system's S/N ratio was 36.65. The resulted chromatograms are shown in Fig. 9.

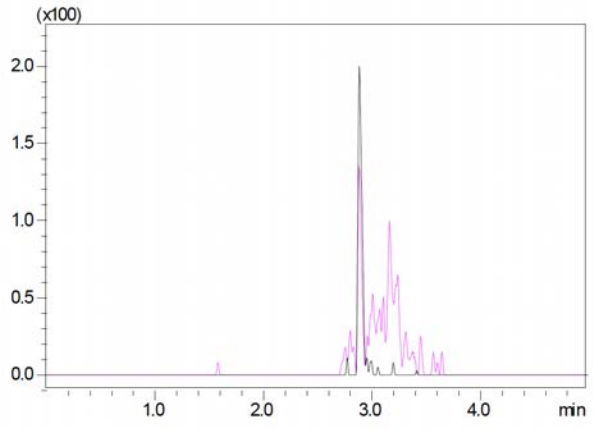


Fig.8 Chromatogram of a loading of 5 uL honey matrix spiked with 5 µg/kg standard

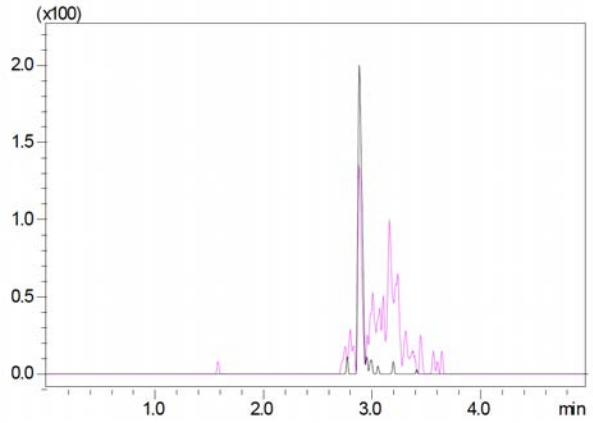


Fig.9 Chromatogram of a loading of 50 uL honey matrix spiked with 0.5 µg/kg standard

Recovery Test

The method's recovery of 5 µg/kg chloramphenicol from spiked samples was carried out. The results show recovery of 83.0%.

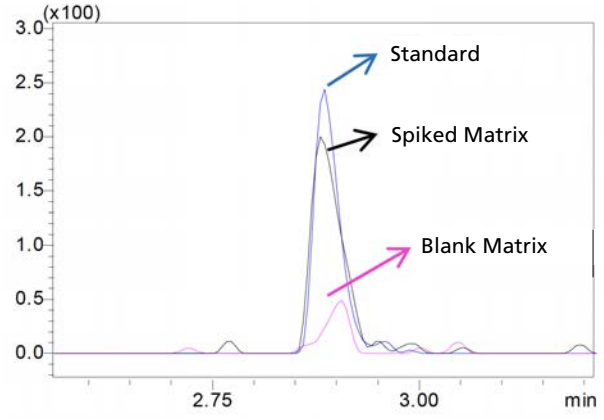


Fig.10 Overlapping chromatogram of blank matrix, spiked matrix and standard

CONCLUSION

A method was proposed in this paper for detection of chloramphenicol in honey with online pretreatment LC system-mass spectrometer. The method was capable of online pretreatment and concentrating honey samples and demonstrated good linearity for chloramphenicol in the concentration range of 0.1 ~100 ng/mL with a correlation coefficient of 0.9997. The method is suitable at level of 0.5 µg/kg chloramphenicol by loading large volume sample and making use of its online concentrating function.

The 6 replicate injections of 0.5 ng/mL standard working solution shows %RSDs of retention time and peak area as 0.13% and 3.12%, respectively, showing that the system had good precision. The method achieved a recovery of 83.0% of 5 µg/kg samples.

3.1 Detection of Antibiotics

A Sensitive and Repeatable Method for Characterization of Sulfonamides and Trimethoprim in Honey using QuEChERS Extracts with Liquid-Chromatography-Tandem Mass Spectrometry

INTRODUCTION

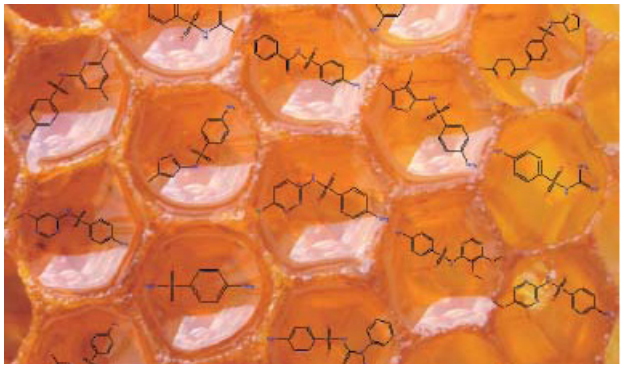
The antibacterial sulfonamides (SA) and trimethoprim are widely used in veterinary and human medicine. Diverse foods from animals potentially contain residues of these drugs posing possible threats to people by triggering allergic reactions and undesirable increasing of microorganism's drug resistance. 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 protect 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.

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. Sulfonamide Used

#	SULFONAMIDE	#	SULFONAMIDE
1	Sulfaguanidine	10	Sulfamethoxypyridizine
2	Sulfacetamide	11	Succinylsulfathiazole
3	Sulfadiazine	12	Sulfamethoxazole
4	Sulfathiazole	13	Trimethoprim
5	Sulfapyridine	14	Sulfamonomethoxine
6	Sulfamerazine	15	Sulsoxazole
7	Sulfamethazine	16	Sulfabenzamide
8	Sulfameter	17	Sulfaclozine
9	Sulfamethizole	18	Sulfadimethoxine

B. Calibration Curve

Level	Conc. (ng/ml)
1	1000
2	500
3	250
4	125
5	62.5
6	31.3
7	15.6
8	7.8
9	3.9
10	2
11	1

C. LC Gradient

Time (min)	%B
0	5
1	15
4.5	35
5	60
5.01	95
5.5	95
5.51	5
7	5

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

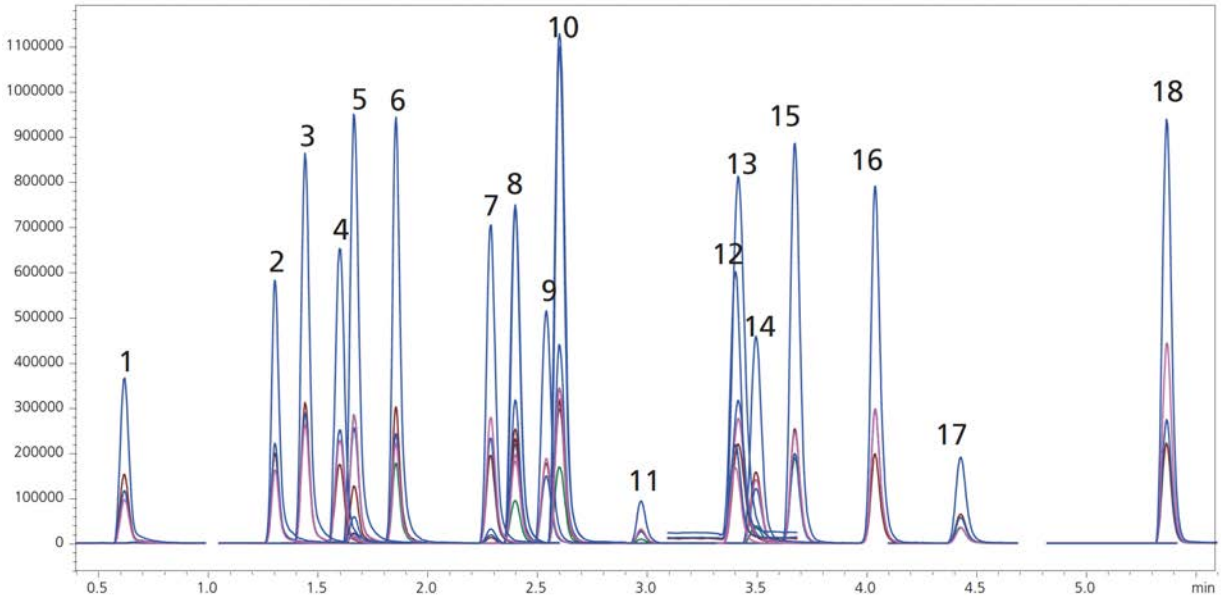
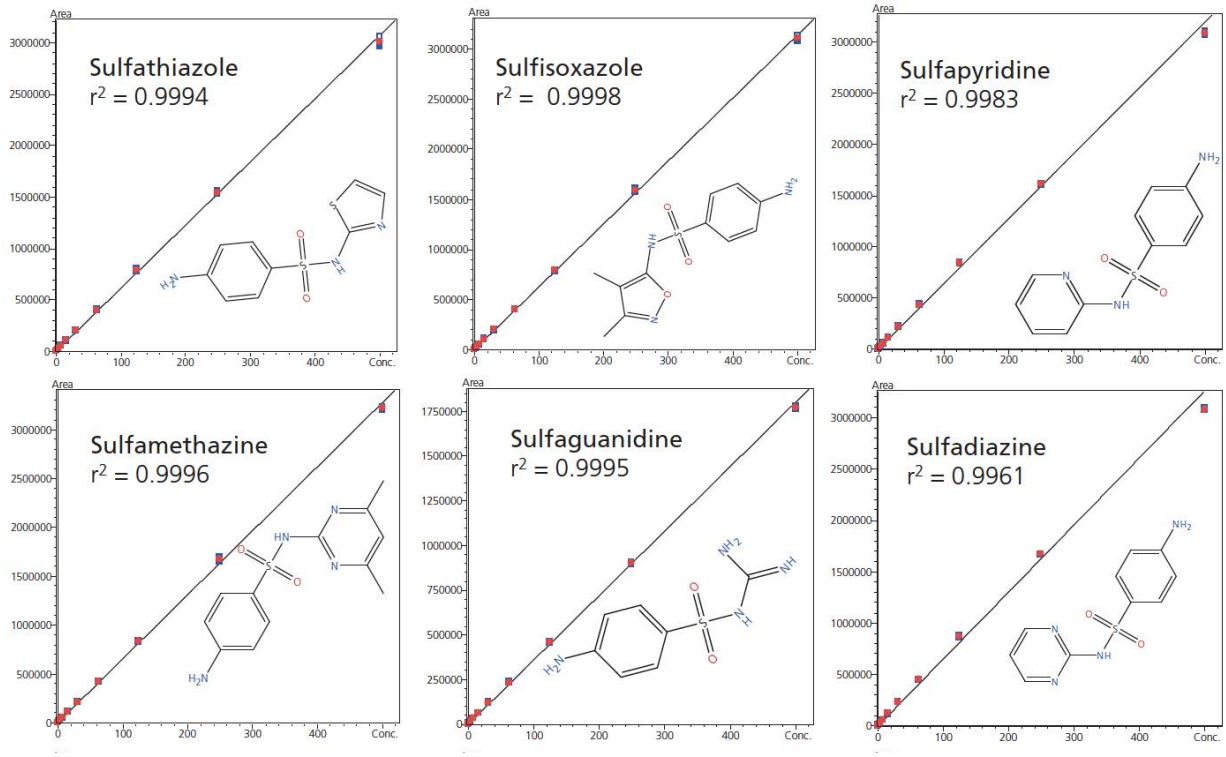


Figure 1. Representative chromatogram of sulfonamide drugs. Standard mixture at 125 pg on-column for each standard. Peak numbers follow the order described for SA compounds in table 1A.



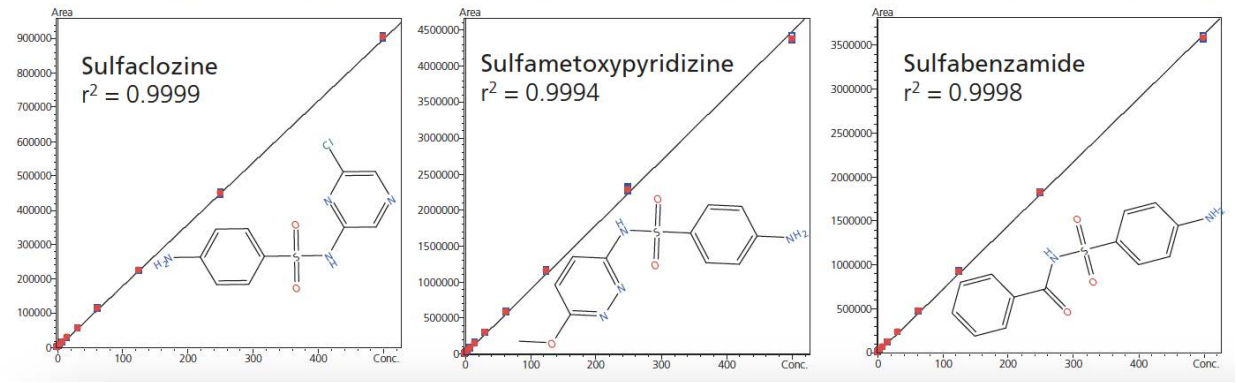
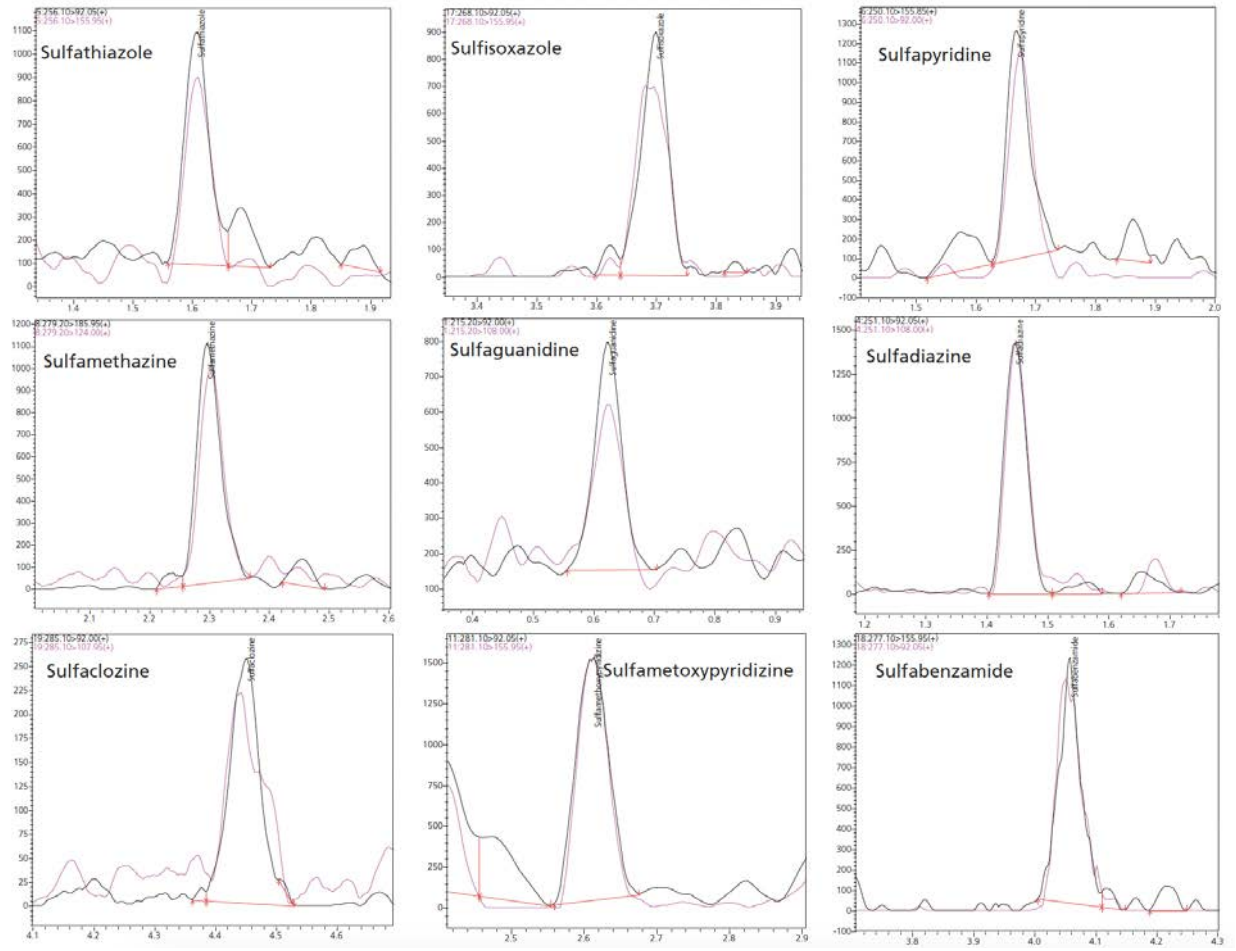


Figure 2. High degree of linearity was observed over the concentration range 0.5–500 pg on column, with values of $r^2 \geq 0.990$ for all analytes.

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 with $r^2 > 0.990$ (Figure 2) in the tested range of 1 to 1000 µg/Kg (0.5 to 500 pg on column). The limits of quantification were 1 µg/Kg (0.5 pg on column) for all compounds except succinylsulfathiazole and sulfacetamide, which were 2 µg/Kg (1 pg 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.



	Sulfathiazole		Sulfisoxazole		Sulfapyridine		Sulfamethazine		Sulfaguanidine		Sulfadiazine		Sulfaclozine		Sulfamethoxypyridizine		Sulfabenzamide	
Level	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %
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.

CONCLUSION

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.

3.2 Detection of Pesticide Residues

Ultra-Sensitive and Rapid Assay of Neonicotinoids, Fipronil and Some Metabolites in Honey by UHPLC-MS/MS [LCMS-8060]

Neonicotinoids are a class of insecticides widely used to protect fields as well as fruits and vegetables. Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybees 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 European Food Security Authoritiy (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. A method was set up using Nexera X2 with LCMS-8060.

Sample Preparation

Thiamethoxam-d3, imidacloprid-d4 and chlothianidin-d3 were used as internal standards. 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.

After incubation at room temperature for one hour with gentle shaking, a commercially available salt mix from Biotage (4 g $MgSO_4$, 1 g Sodium Citrate, 0.5 g Sodium Citrate sesquihydrate, 1 g NaCl) was added. After manual shaking, samples were centrifuged at 3000 g for 5 minutes at 10°C. Supernatant (6 mL) was transferred into a 15 mL tube containing 1200 mg of $MgSO_4$, 400 mg PSA and 400 mg C18 from Biotage. After centrifugation at 3000 g and 10°C for 5 minutes the supernatant was transferred into a LCMS certified inert glass vial for analysis (Shimadzu LabTotal 227-34001-01).

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 1. Calculated recoveries are within acceptance values 70-120% from EU SANTE/11945/2015.

Table 1 Measured Recoveries in Honey

Compound	Recovery	Compound	Recovery
Acetamiprid	78.8%	Fipronil sulfone	74.2%
Acetamiprid-N-desmethyl	93.4%	Imidaclorpid	83.2%
Chlothianidin	70.6%	Nitenpyram	87.0%
Dinotefuran	76.5%	Thiacloprid	82.2%
Fipronil	78.1%	Thiamethoxam	75.6%

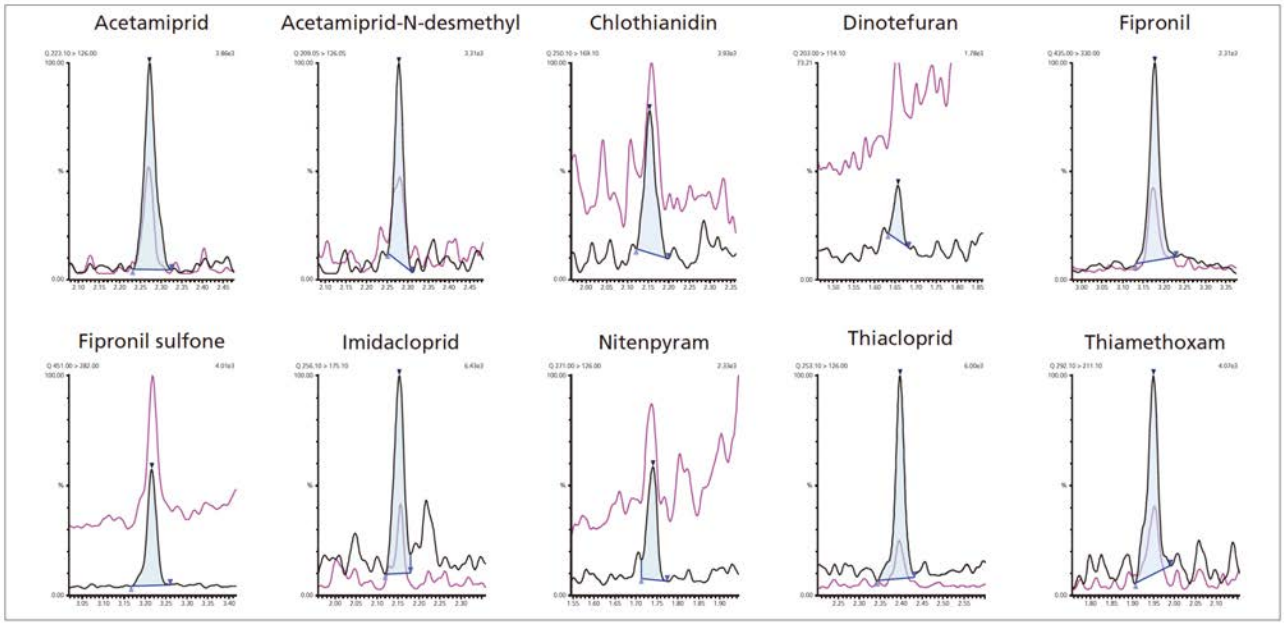


Fig. 1 Chromatogram of the Target Compounds at Their Lower Limit of Quantification

Table 2 Analytical Conditions

System	: Nexera X2
Column	: ACE SuperC18 (100 mm L. × 2.1 mm I.D., 2 µm)
Column Temperature	: 30 °C
Mobile Phases	: A: Water = 0.05% ammonia B: Methanol + 0.05% ammonia
Flowrate	: 600 µL/min
Gradient	: 5%B to 100%B in 3 min 100%B to 5%B in 0.1 min
Total Run Time	: 4 min
Injection Volume	: 2 µL (POISe mode with 10 µL of water)

System	: LCMS-8060
Ionization	: Heated ESI
Probe Voltage	: +1 kV (positive ionization) / -1.5 kV (negative ionization)
Temperature	: Interface: 400°C
Desolvation Line	: 200°C
Heater Block	: 400°C
Gas Flow	: Nebulizing Gas: 3 L/min Heating Gas: 10 L/min Drying Gas: 5 L/min

Table 3 MS/MS Acquisition Parameters

Name	Polarity	MRM Quan	MRM Qual	ISTD
Acetamiprid	+	223.1 > 126.0	223.1 > 56.1	2
Acetamiprid-N-desmethyl	+	209.1 > 126.0	211.1 > 128.0	2
Clothianidin	+	250.1 > 169.1	250.1 > 132.0	3
Dinotefuran	+	203.0 > 114.0	203.0 > 87.0	1
Fipronil	-	435.0 > 330.0	435.0 > 250.0	3
Fipronil sulfone	-	451.0 > 415.0	451.0 > 282.0	3
Imidacloprid	+	256.1 > 175.1	258.1 > 211.1	2
Nitenpyram	+	271.0 > 126.0	271.0 > 225.0	3
Thiacloprid	+	253.1 > 126	253.1 > 90.1	1
Thiamethoxam	+	292.1 > 211.1	292.1 > 181.1	1
Thiamethoxam-D3	+	295.1 > 214.05	---	1
Imidacloprid-D4	+	260.1 > 179.1	---	2
Clothianidin-D3	+	253.1 > 132.05	---	3

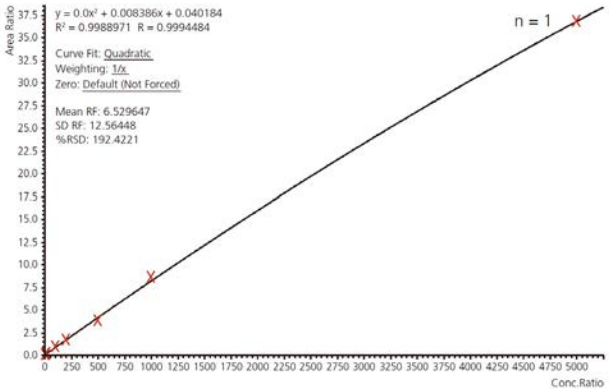
Dwell Time	3 to 34 msec depending upon the number of concomitant transitions to ensure to have at least 30 points per peak (max total loop time 140 msec).
Pause Time	1 msec
Quadrupole Resolution	Q1: Unit Q3: Unit

Calibration

Calibration curves were prepared in acetonitrile to obtain final concentrations ranging from 0.5 pg/mL (1 fg on column) to 5 ng/mL. These concentrations corresponds to 1 ng/kg and 10 µg/kg in honey, respectively. For each compound, the lower limit of quantification was selected to give an accuracy between 80-120% (see table 4). A typical calibration curve is shown in Fig. 2.

Table 4 Limits of Quantification in Honey

Compound	LOQ (µg/kg)	Compound	LOQ (µg/kg)
Acetamiprid	0.005	Fipronil sulfone	0.001
Acetamiprid-N-desmethyl	0.005	Imidacloprid	0.020
Chlothianidin	0.020	Nitenpyram	0.020
Dinotefuran	0.010	Thiacloprid	0.005
Fipronil	0.001	Thiamethoxam	0.005



Standard (µg/kg)	Accuracy (%)
0.005	106
0.010	97.2
0.020	95.6
0.100	107
0.200	98.4
0.500	91.5
1.000	104
5.000	99.9
10.000	100

Fig. 2 Calibration Curve of Acetamiprid

Real Samples Analysis

Nine honey samples purchased at the local supermarket or used as raw materials in cosmetics (orange tree honey) were assayed as unknowns. All tested honeys showed concentrations far below the authorized maximum residue limit. But thanks to the very high sensitivity reached, even low concentrations of neonicotinoids were quantified. Results are presented in table 5. A representative chromatogram of a sample honey is shown in Fig. 3.

Table 5 Honey Samples Results (concentrations in µg/kg)

Honey	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
1. Provence creamy	---	---	0.20	---	0.010
2. Italy creamy	0.15	---	0.17	---	---
3. Pyrenees liquid	0.38	---	0.043	0.020	---
4. French-Spanish creamy	0.27	---	0.047	0.020	---
5. Thyme liquid	---	---	---	---	---
6. Lemon tree creamy	1.7	---	0.15	0.033	---
7. Orange tree liquid	1.2	---	0.62	---	---
8. Flowers creamy	0.14	---	0.055	0.39	---
9. Flowers liquid	0.34	---	0.11	0.010	---

Honey	Dinotefuran	Nitenpyram	Acetamiprid-Ndesmethyl	Fipronil	Fipronil sulfone
1. Provence creamy	---	0.052	0.005	---	---
2. Italy creamy	---	0.040	---	---	---
3. Pyrenees liquid	---	---	0.015	0.004	---
4. French-Spanish creamy	---	0.032	---	---	---
5. Thyme liquid	---	---	---	---	---
6. Lemon tree creamy	---	---	0.020	---	---
7. Orange tree liquid	---	0.024	0.018	---	---
8. Flowers creamy	---	---	0.016	---	---
9. Flowers liquid	---	---	0.006	---	---

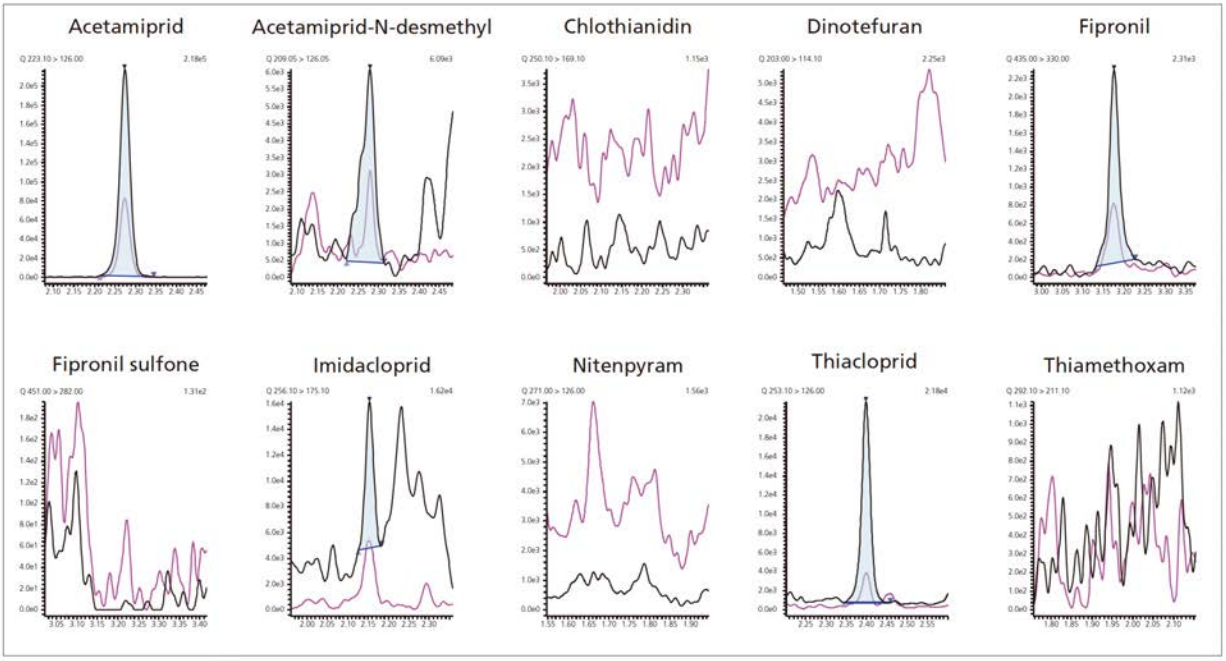


Fig. 3 Chromatogram of a Sample Honey (Pyrenees)

Stability

The thyme honey sample with no detectable target compound was spiked at 50 ng/kg with all compounds prior to extraction. The extract obtained was then consecutively injected 150 times in the system. The results presented in Fig. 4 show excellent stability of the signal even at these low concentrations. This demonstrates that the excellent sensitivity can be maintained over long series of real sample analysis thanks to the ion source ruggedness.

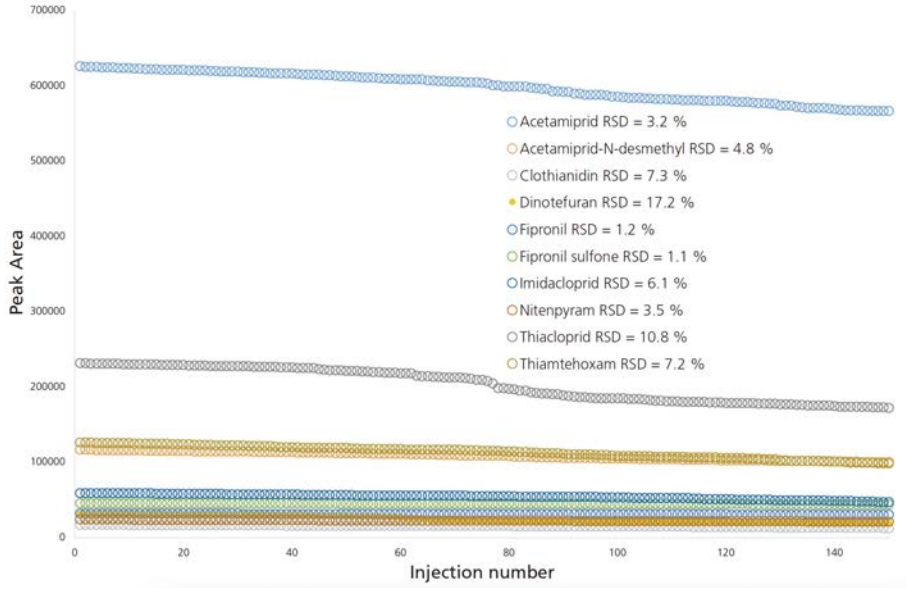


Fig. 4 Stability of Peak Areas in Real Honey Samples

Conclusion

A method for ultra sensitive assay of neonicotinoids in honey was set up. The sample preparation was simple but provided excellent recoveries. The injection mode used prevented the use of tedious evaporation/reconstitution or dilution steps. Thanks to the high sensitivity obtained enabled assay in real samples at very low levels far under the regulated residue levels. Furthermore, even at low measured concentrations, the system demonstrated its stability after long analytical series of real samples. This method can be a very efficient support tool to better understand the impact of neonicotinoids on honey bee colonies and could be easily transposed to pollen or bee samples.

3.3 Evaluation of Toxicity in Nectar

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.

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 $MgSO_4$ in order, the mixture was vortexed for 2 min and centrifugated at 8000 rpm for 5 minutes. The above solution was withdrawn and filtered (Organic membrane, 0.22 μm) for detection.

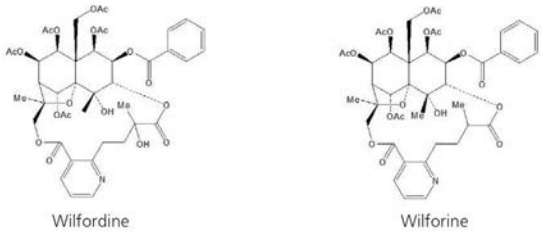


Figure 1 Structure of Wilfordine and Wilforine

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.

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

High Speed Mass Spectrometer

Ultra Fast Polarity Switching

- 5 msec

Ultra Fast MRM

- Max. 555 transition /sec

RESULT

Method Development for Wilforine and Wilfordine

HPLC Conditions

Column : InertSustain C8-3 Column (2.1 mm I.D.×150 mm L., 5 m)
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
5.10	Controller	Stop	

Injection Vol. : 10 μL
Column Temp. : 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

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

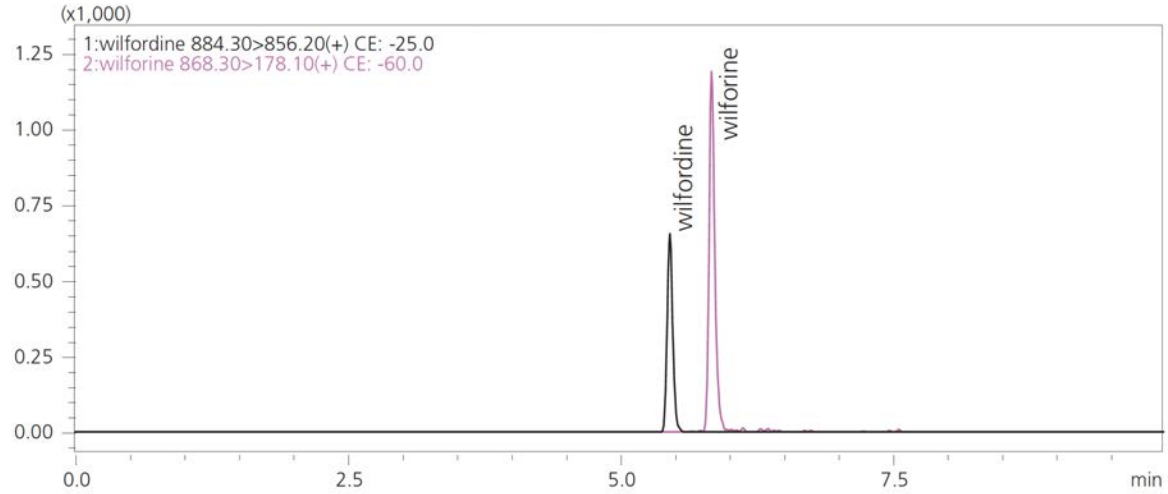


Figure 3 MRM chromatograms of standard solution of Wilforine and Wilfordine (Concentration of each compound were 0.05 ng/mL)

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.

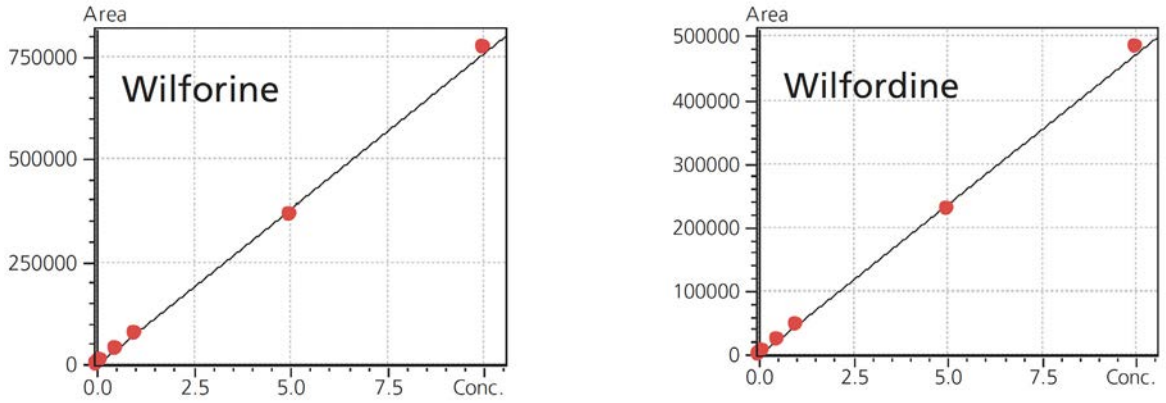


Figure 4 Calibration curve of Wilfordine and Wilforine

Table 3. Parameters of Calibration Curves

Compound	Calibration Curves	Range (ng/mL)	Coefcient (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

CONCLUSION

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.

3.4 Analysis of Carbohydrates

Examination of the Sugar Analysis using HPLC Method Scouting System Coupled to Single Quadrupole Mass Spectrometer

INTRODUCTION

Optimization of peak separation and sensitivity is important for decision of LC/MS analytical conditions. However, the evaluation of them has been tedious and time-consuming operation. The HPLC method scouting system coupled to single quadrupole mass spectrometer used in this study can dramatically shorten total run time compared with the conventional system, because this system can make enormous combinatorial analysis methods and run batch program automatically. In this study, we developed the optimized method for the simultaneous analysis of seventeen kinds of sugars based on the result of evaluation for columns, mobile phases and gradient programs using this system.

OVERVIEW OF THE NEXERA METHOD SCOUTING SYSTEM

- Capable of searching conditions based on a maximum of six columns and sixteen mobile phases
- Can be used with basically all current UHPLC columns (100 MPa valve pressure resistance)
- Easily configured scouting conditions enabled through proprietary software (Fig. 1)
- Automated control of entire analysis from system checks to scouting, and then shut down

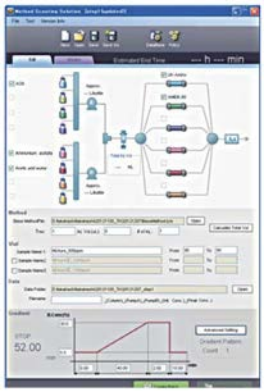


Fig. 1 Main screen of the Method Scouting Solution

Easy Operation

Mobile phases and columns can be selected in the same window. Integrated user interface allows simple operation.

- Seamless Connection

Software links with LabSolutions Ver. 5.53 SP3 or later versions.

- Improved Workflow

Batch analysis files are automatically created.

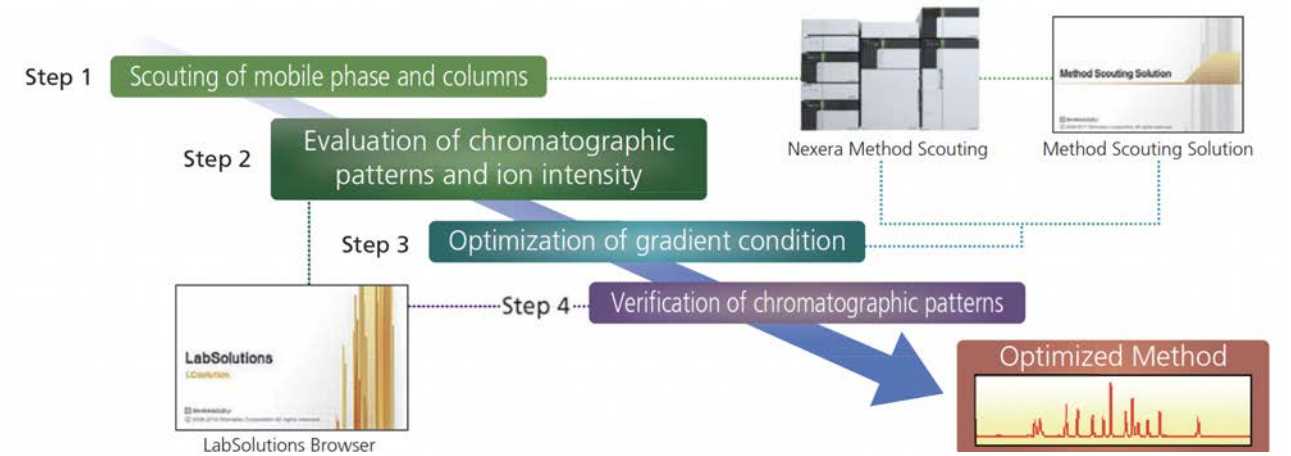


Fig. 2 Work-flow of the method scouting

Scouting of mobile phases and columns (Step 1)

The purpose of this step is to find out for the best combination of mobile phase and column using a typical gradient condition (Table 1). In these experiments we used 2 combinations of mobile phases and 2 different columns (Fig. 3).

Table 1 Analytical conditions of Step 1

Binary gradient	: B conc. 5% (0 min)
	→30% (40-42 min)
	→5% (42.01-52 min)
Flow Rate	:1.0 mL/min
Injection Vol.	: 5 µL
Column Temp.	: 55 deg. C
Ionization	: ESI (Negative)
Detection	: SCAN (range: m/z 100-500)

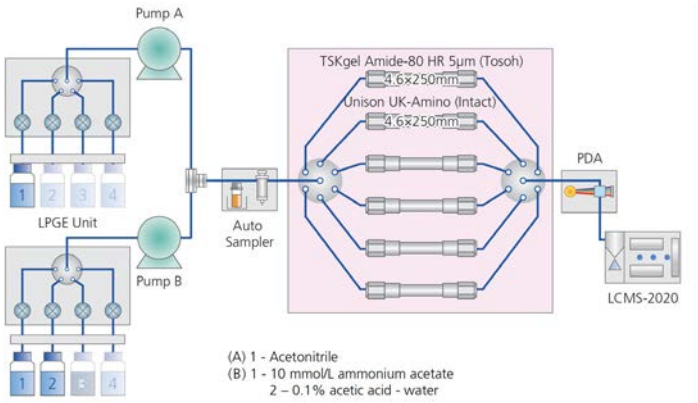


Fig. 3 Schematic representation and features of the Nexera Method Scouting System

Analysis by the Nexera Method Scouting System

We targeted seventeen sugars and analyzed them simultaneously. (Fig. 4)

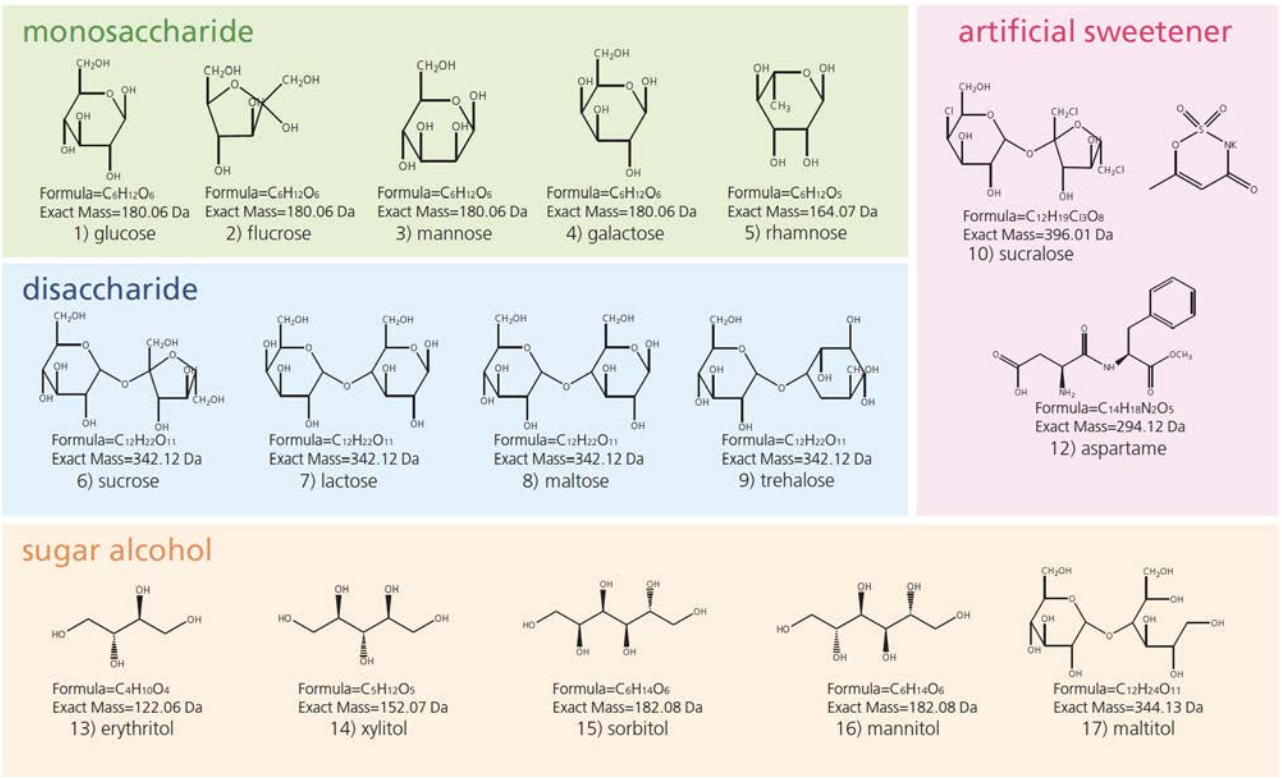


Fig. 4 Structures of analyzed compounds

Evaluation of chromatographic patterns and ion intensity (Step 2)

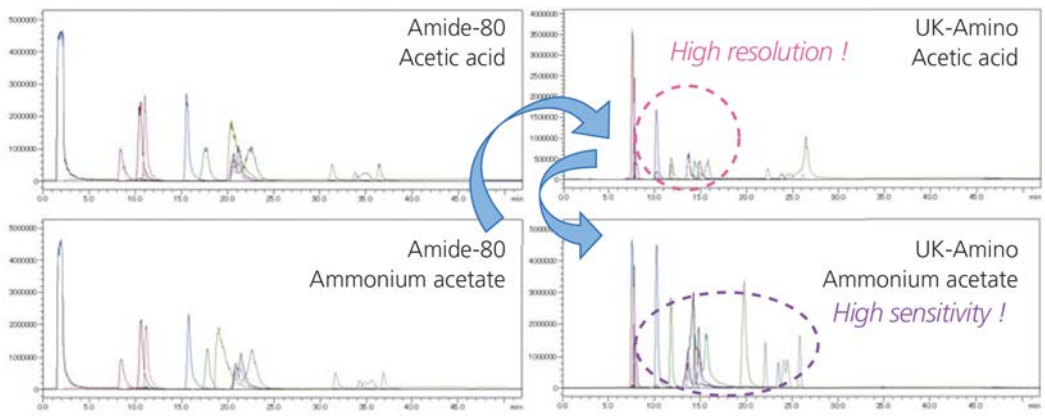


Fig. 5 Typical chromatograms in selected mobile phases and column conditions

Optimization of gradient condition (Step 3 and 4)

For improved separation and sensitivity for sugars, we optimized the gradient condition using method scouting system.

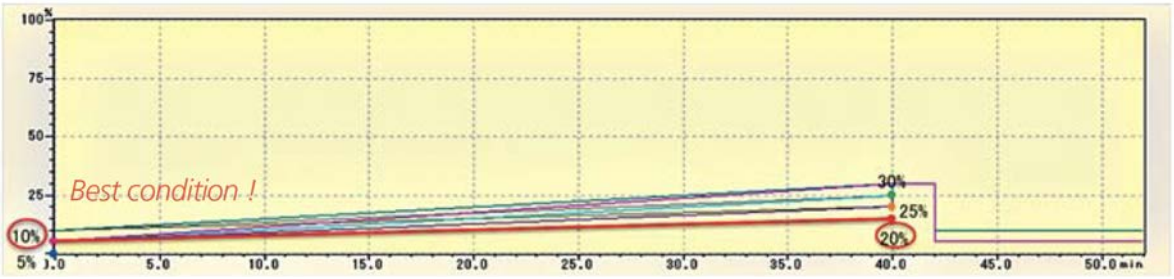


Fig. 6 Optimization of gradient conditions for separation of sugars

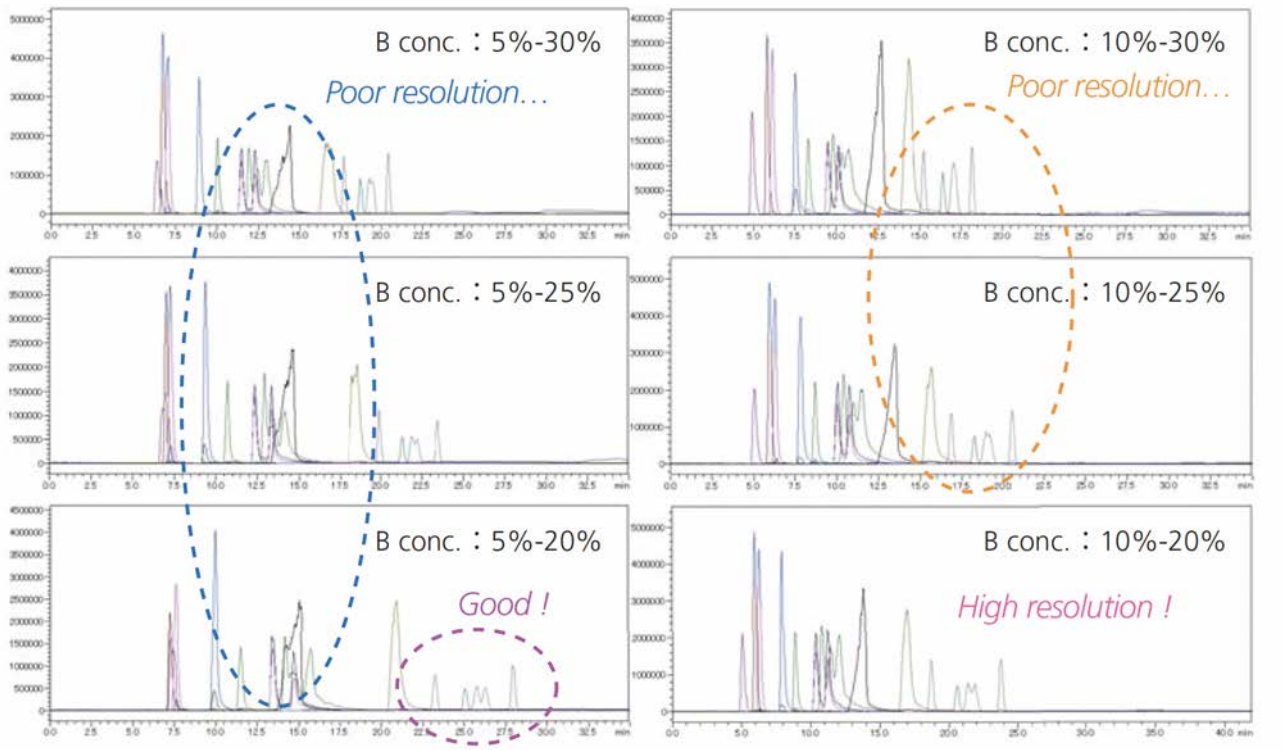


Fig. 7 Typical chromatograms in selected gradient conditions using ammonium acetate and Amide-80 column

Optimized Method

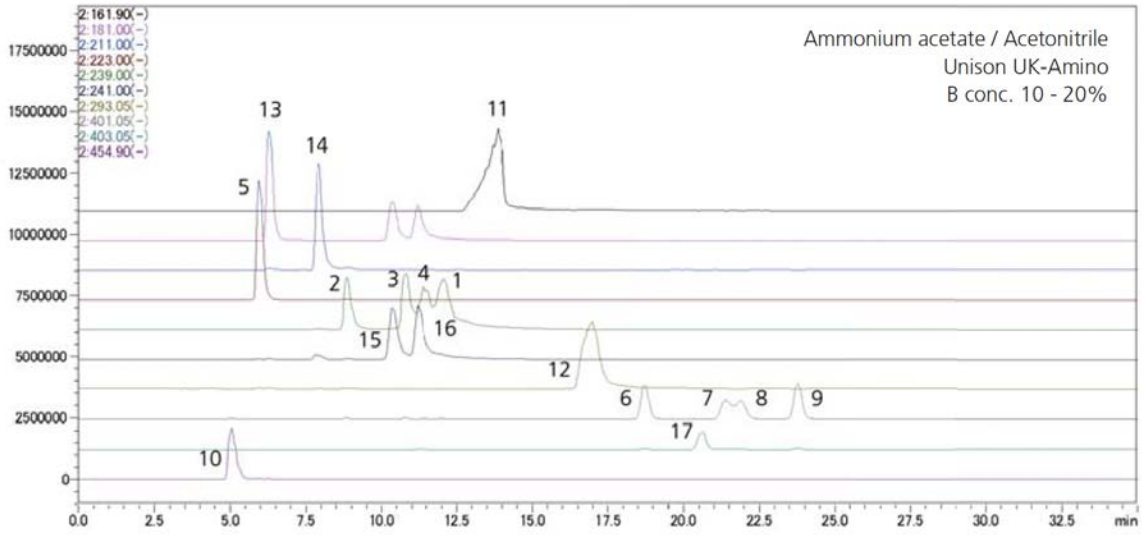


Fig. 8 Optimized method for seventeen sugars

Calibration Curves

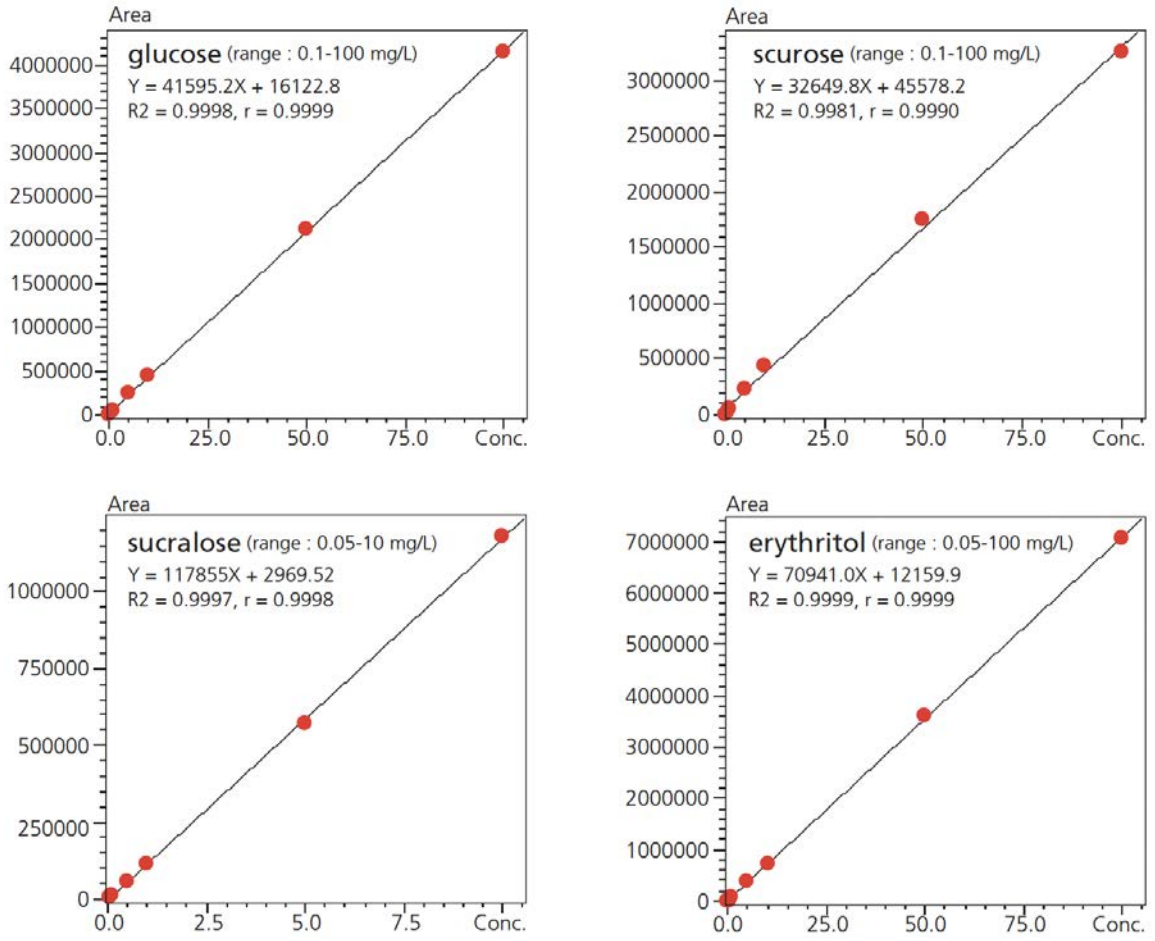


Fig. 9 Calibration curves of each sugar using optimized method

CONCLUSION

- Method Scouting Solution, dedicated software for controlling method scouting system, enabled optimization of the analytical method separating compounds of differing properties in a single batch.
- The most suitable method for a single compound class could be chosen, alternatively a generic method could also be selected allowing separation of all compounds.
- Through method optimization LC/MS sensitivity was enhanced significantly.
- Seamless integration of software provided improved speed and efficiency in method development processes.
- Using an optimized method file, high quantifiability was provided.

3.5 Analysis of Water-Soluble Vitamins

Ultra High-Sensitivity Analysis of Water-Soluble Vitamins

Explanation

The Nexera SR is a high-end model within the Nexera X2 series of ultra high performance liquid chromatographs. It features the SPD-M30A high-sensitivity photodiode array detector which incorporates the newly designed capillary SR-Cell (Sensitivity and Resolution Cell). Optimization of the optical path length and diameter results in both high sensitivity and low noise. Introduced here is an example of high-speed, high-sensitivity simultaneous analysis of water-soluble vitamins using the Nexera SR ultra high performance liquid chromatograph with high-sensitivity cell (option).

Simultaneous Analysis of 6 Water-Soluble Vitamins

High-sensitivity cell (option) of the Nexera SR ultra high performance liquid chromatograph incorporates 85 mm optical path length. Low noise levels and long optical path length have achieved excellent S/N, not only high signal response. In this simultaneous analysis of water-soluble vitamins, S/N has increased by 7.0 times compared to the previous instrument. High sensitivity detection is achieved even for compounds with low molar absorptivity.

Analytical Conditions

Column	: Kinetex 2.6 µm C18 100 Å (100 mm L. x 4.6 mm I.D., 2.6 µm)
Mobile Phase	: A: 20 mmol/L (Sodium) Phosphate Buffer (pH 2.5) 2 mmol/L Sodium 1-Hexanesulfonate B: Mobile Phase A/ Acetonitrile = 2/3 Gradient Elution Method
Time Program	: B 5% (0.0 min.) → 23% (1.0 min.) → 100% (2.0-2.5 min.)
Flow Rate	: 2.5 mL/min
Column Temp.	: 40°C
Injection Volume	: 5 µL

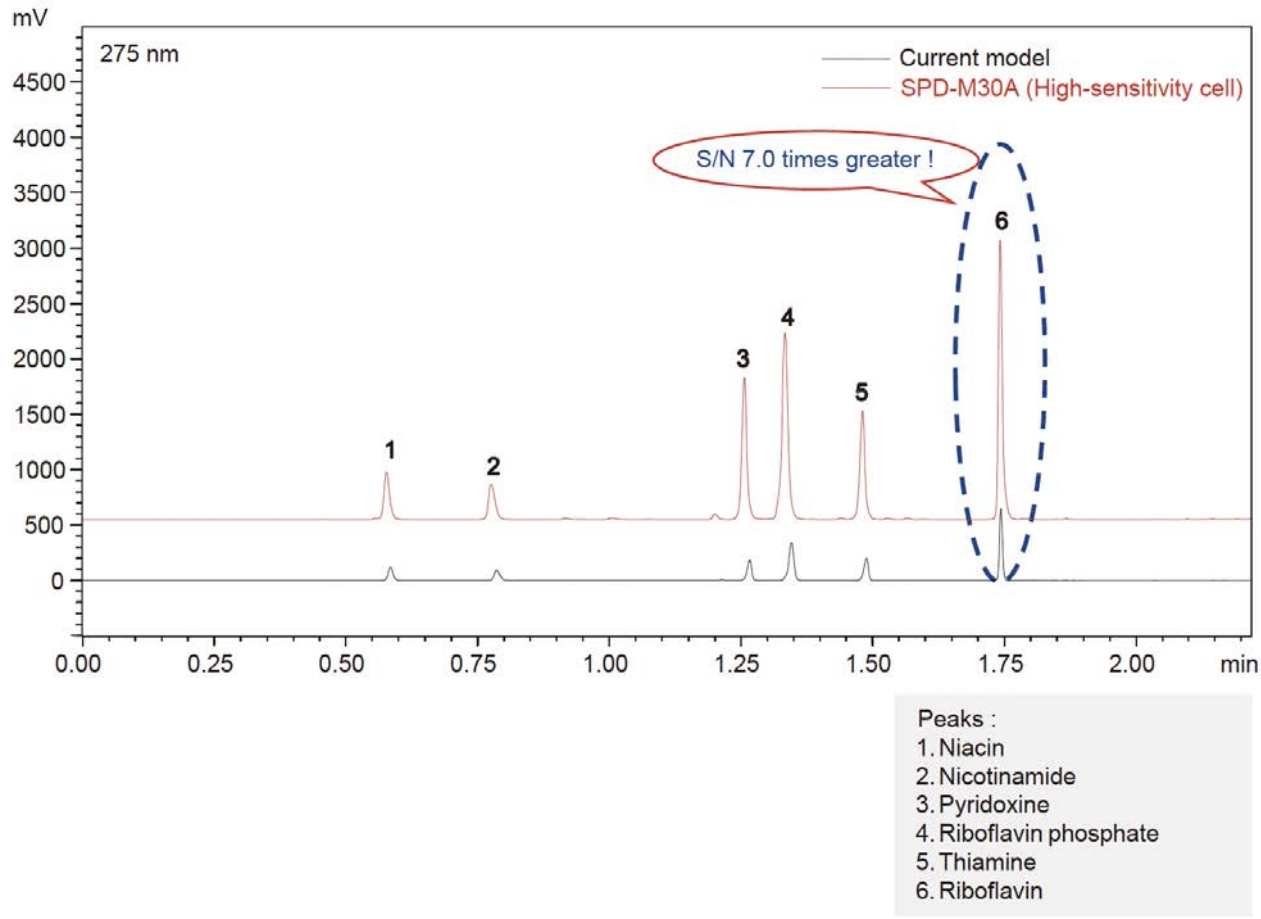


Fig. 1.11.1 Chromatogram of a Standard Mixture Solution of 6 Water-Soluble Vitamin

3.6 Determination of Honey Authenticity

High Speed Analysis of Phenolic Acids

Explanation

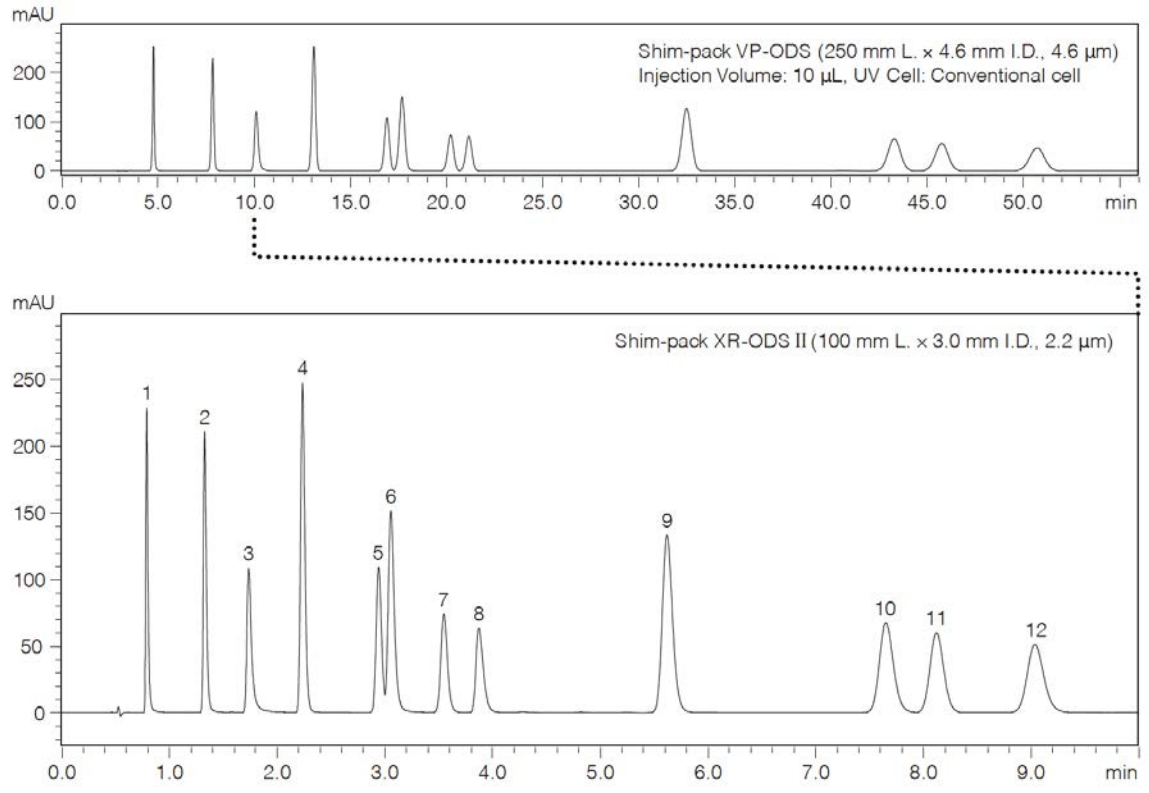
Phenolic acid exists in higher plants as esters, ethers or in its free state, and is a compound that has been receiving increased attention in recent years due to its antioxidant effects. HPLC is often used for quantitative analysis of phenolic acid in processed foods containing fruits and fruit materials, but the analysis is often quite time-consuming because a relatively long column and gradient elution are required to achieve separation of the contaminants in actual samples. Here we present an example of high speed, high resolution analysis of phenolic acids using the Shimadzu Prominence UHPLC$_{XR}$ ultra high-speed LC system with the SPD-M20A photodiode array detector.

Simultaneous Analysis of 11 Phenolic Acids and Benzoic Acid

Fig. 1.22.1 shows a chromatogram of a standard mixture of 11 phenolic acids and benzoic acid (50 mg/L each), analyzed using a Shim-pack XR-ODS II high speed, high resolution column. For comparison, data acquired using a conventional Shim-pack VP-ODS column are also shown.

Analytical Conditions

Column	: Shim-pack XR-ODS II (100 mm L. x 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 50 mmol/L Ammonium Formate Buffer (pH 3.6) B: Methanol Gradient Elution Method
Time Program	B 20% (0-10 min) ⟶ 80% (10.01-11 min) ⟶ 20% (11.01-15 min) 0.5 mL mixer
Flowrate	: 0.9 mL/min
Column Temp.	: 40°C
Injection Volume	: 4 µL
Detection	: SPD-M20A (Max plot 230-350 nm)
UV Cell	: Semi-micro cell



■ Peaks
1. Gallic acid, 2. Protocatechuic acid, 3. Chlorogenic acid, 4. *p*-Hydroxybenzoic acid, 5. Vanillic acid, 6. Caffeic acid, 7. Syringic acid
8. Salicylic acid, 9. *p*-Coumaric acid, 10. Ferulic acid, 11. Benzoic acid, 12. Sinapic acid

Fig. 1.22.1 Chromatograms of a Standard Mixture of 11 Phenolic Acids and Benzoic Acid (50 mg/L each)

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Application News

No. HPLC-045

High Performance Liquid Chromatography

Natural Cannabinoid and Cannflavin Profiling by HPLC-PDA

This application was developed by our partner Front Range Biosciences with generous support from MilliporeSigma.

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■ Introduction

Cannabinoids are a diverse group diterpenoid compounds primarily observed in Cannabis and Rhododendron species. To date, over 120 phytocannabinoids have been identified and quantified in Cannabis extracts using analytical techniques such as High Performance Liquid Chromatography (HPLC). With the federal legalization of hemp, a type of Cannabis, and state-supported legalization measures for high-THC Cannabis, HPLC testing of dried plant material for psychotropic potency and therapeutic dosing has become part of nearly every piece of legislation. While numerous chromatographic methods have been developed for the detection and quantification of THCA, CBDA, CBGA, CBNA, and their decarboxylated forms, many do not account for the possibility of coelutions with other secondary metabolites in plant samples such as cannabinoids, flavonoids, and terpenes. To complicate analyses further, the metabolomes of different Cannabis varieties can vary greatly, resulting in chromatographic coelutions that are present in some extracts but not in others.

The method presented in this application note attempts to resolve most of the significant coelutions common to different types of Cannabis and was designed for laboratories interested in the quantification of minor cannabinoid and cannflavin constituents. Using this method, a total of 34 unique Cannabis analytes were quantified in less than 32 minutes. The method described has been successfully applied to not only leaf and flower Cannabis tissue, but cannabis/hemp products such as concentrates, oils, and cosmetic products.

■ Sample Preparation

Air-dried samples were milled to a powder using stainless steel ball-bearings with stems and seeds mechanically removed after pulverization. Between 0.2 and 0.5 grams of powder aliquots were solvent extracted in 10mL of HPLC-grade acetone using ultrasonication for a total of 30 minutes at a water temperature no greater than 35°C. Sample extracts were syringe-filtered with 0.22 µm PTFE filters, followed by either a 2-fold dilution for leaf extracts or a 5-fold dilution for floral extracts.

■ Experimental

A Shimadzu Prominence-i LC-2030 C 3D Plus system, equipped with an MilliporeSigma Ascentis-C18 Express column and a photodiode array detector (PDA) was utilized to quantitate cannabinoid and cannflavin analytes in dried hemp tissues.

Table 1: Instrument and Mobile Phase Conditions

Column	Ascentis® Express C18, 2.7 µm x 150mm x 3mm
Mobile Phase A	HPLC Water, 8% (v/v) Methanol, 0.035% (v/v) Formic Acid, 1.8mM Ammonium Formate
Mobile Phase B	HPLC Acetonitrile
Column Temperature	24°C
Autosampler Temperature	15°C
Injection Volume	2 µL
Flow Rate	0.45 mL/min

Table 2: Gradient Conditions

Time (min)	% Mobile Phase A	% Mobile Phase B
0	59	41
1	58	42
10	37	63
16	32	68
26	19	81
28	13	87
29.5	0	100
30.5	0	100
31	59	41

Recommended Equilibration Time: 4 Minutes

Table 3: Photodiode Array Detector Conditions

Analyte	Quantitative Wavelength	Analyte	Quantitative Wavelength	Analyte	Quantitative Wavelength
Cannflavin B	340nm	CBG	230nm	CBL	230nm
CBDO	230nm	CBD	230nm	Δ9-THCA	270nm
CBDVA	270nm	THCV	230nm	CBC	280nm
CBDV	230nm	Δ9-THCVA	270nm	CBCA	258nm
CBGV	230nm	CBCV	280nm	CBLA	270nm
CBGVA	270nm	CBDPA	270nm	CBDM	230nm
CBE	230nm	CBCVA	258nm	CBGM	230nm
CBDB	230nm	CBN	280nm	Δ9-THCP	230nm
CBCO	280nm	CBNA	258nm	CBT	230nm
CBDA	270nm	CBDP	230nm	Δ9-THCPA	270nm
Cannflavin A	340nm	Δ9-THC	230nm	**PDA Conditions** Lamp: D2 Cell Temperature: 40°C Polarity: + Slit Width: 8nm	
CBGA	270nm	Δ8-THC	230nm		

Calibrations

Calibration standards were prepared gravimetrically for 34 unique cannabinoids and cannflavins at concentrations ranging from 0.1 ug/mL to 800 ug/mL. Certified Reference Materials (CRM) standards or research grade isolates were obtained from MilliporeSigma, Restek, Caymen Chemical, Purisys, and Toronto Research Chemicals.

The linearity for all compounds was ≥ 0.99 R^2 using linear correlations and a best-fit weighting of 1/Concentration. The UV spectra of each analyte was recorded in a spectral library to assist in positive identification of cannabinoids and cannflavins in plant tissue extracts.

Figure 1: Calibration Curves and UV Spectra with Lambda Max Values

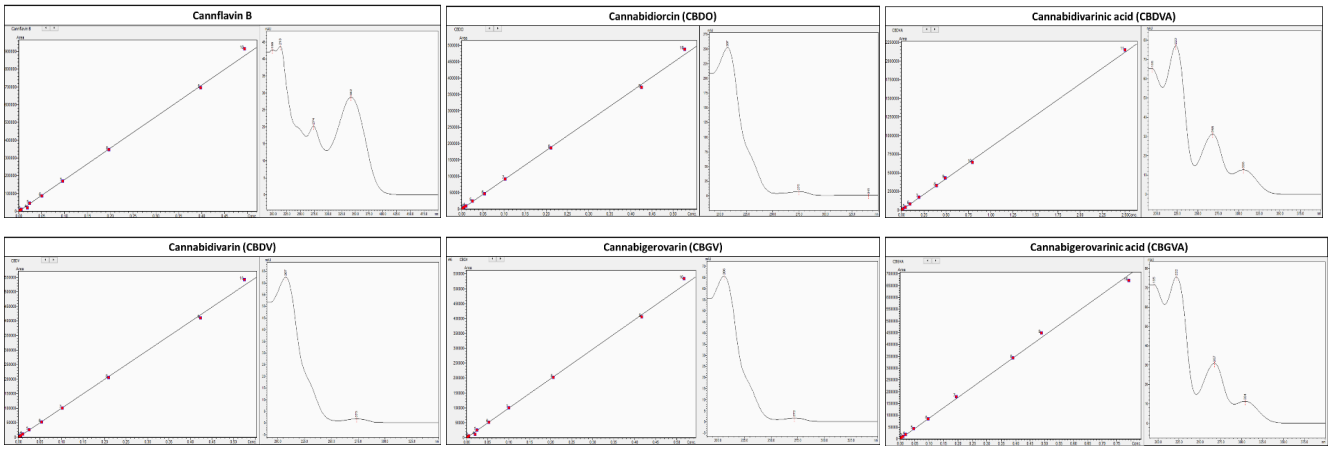


Figure 1 *(Continued)*: Calibration Curves and UV Spectra with Lambda Max Values

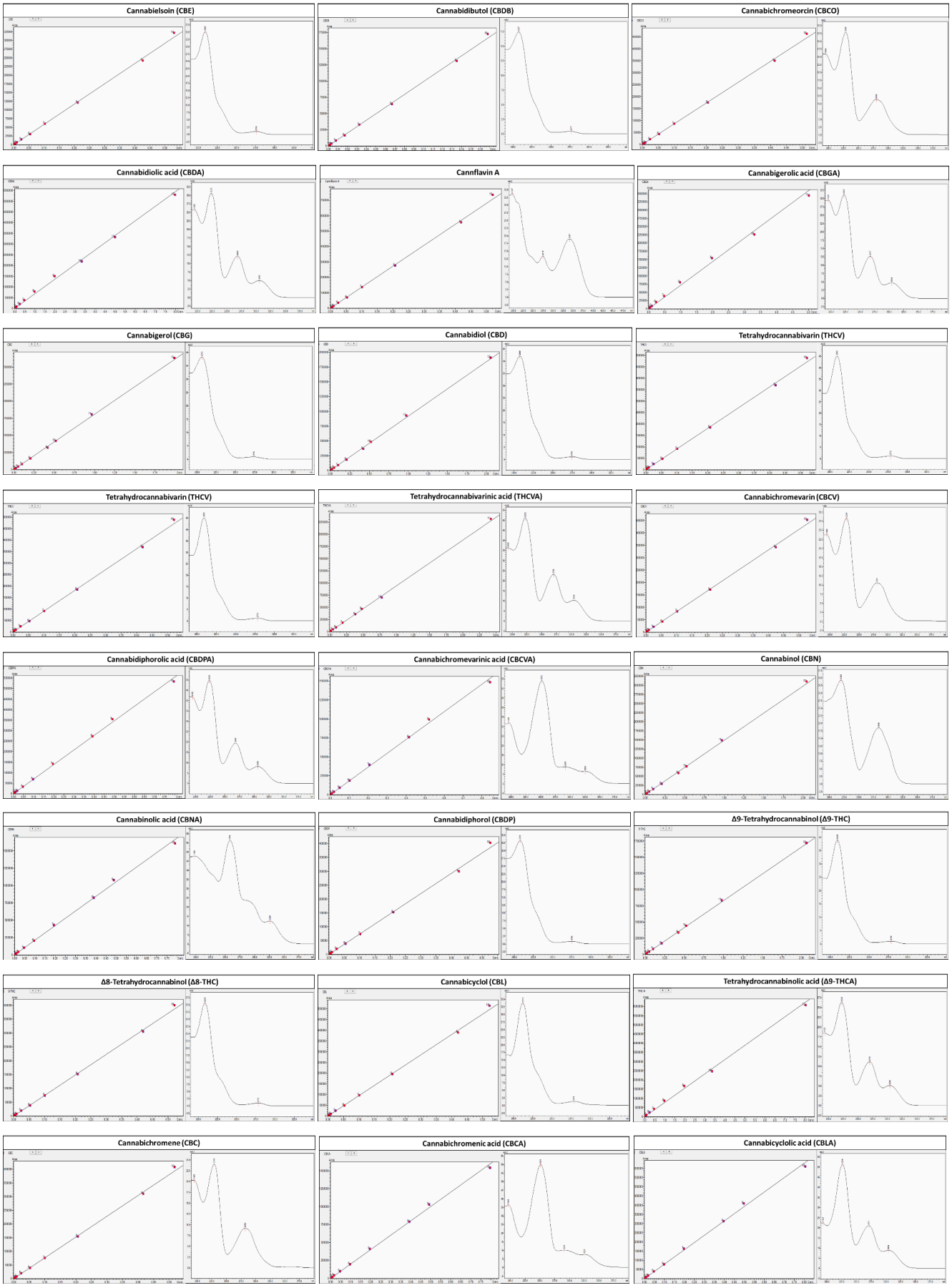
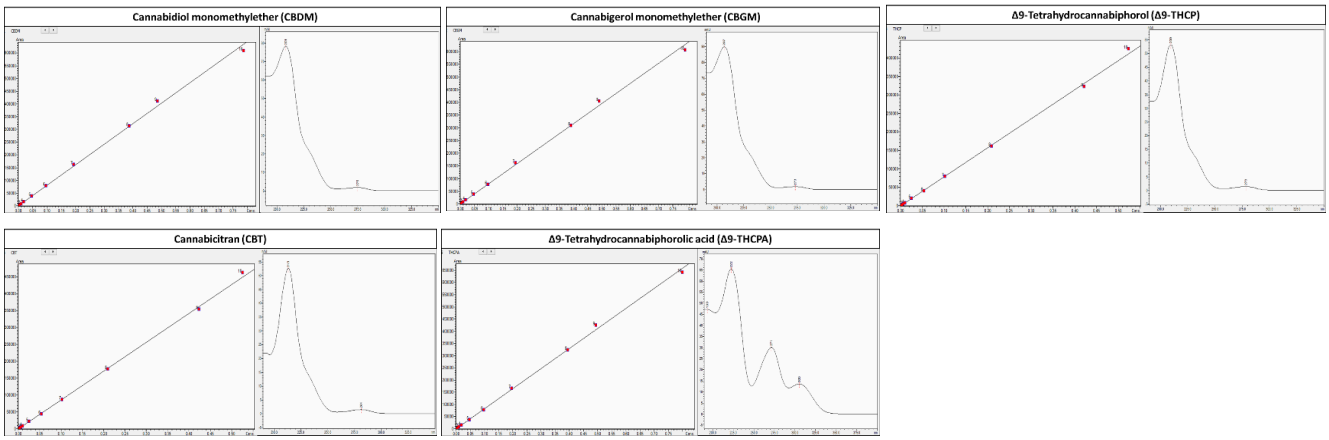


Figure 1 *(Continued)*: Calibration Curves and UV Spectra with Lambda Max Values

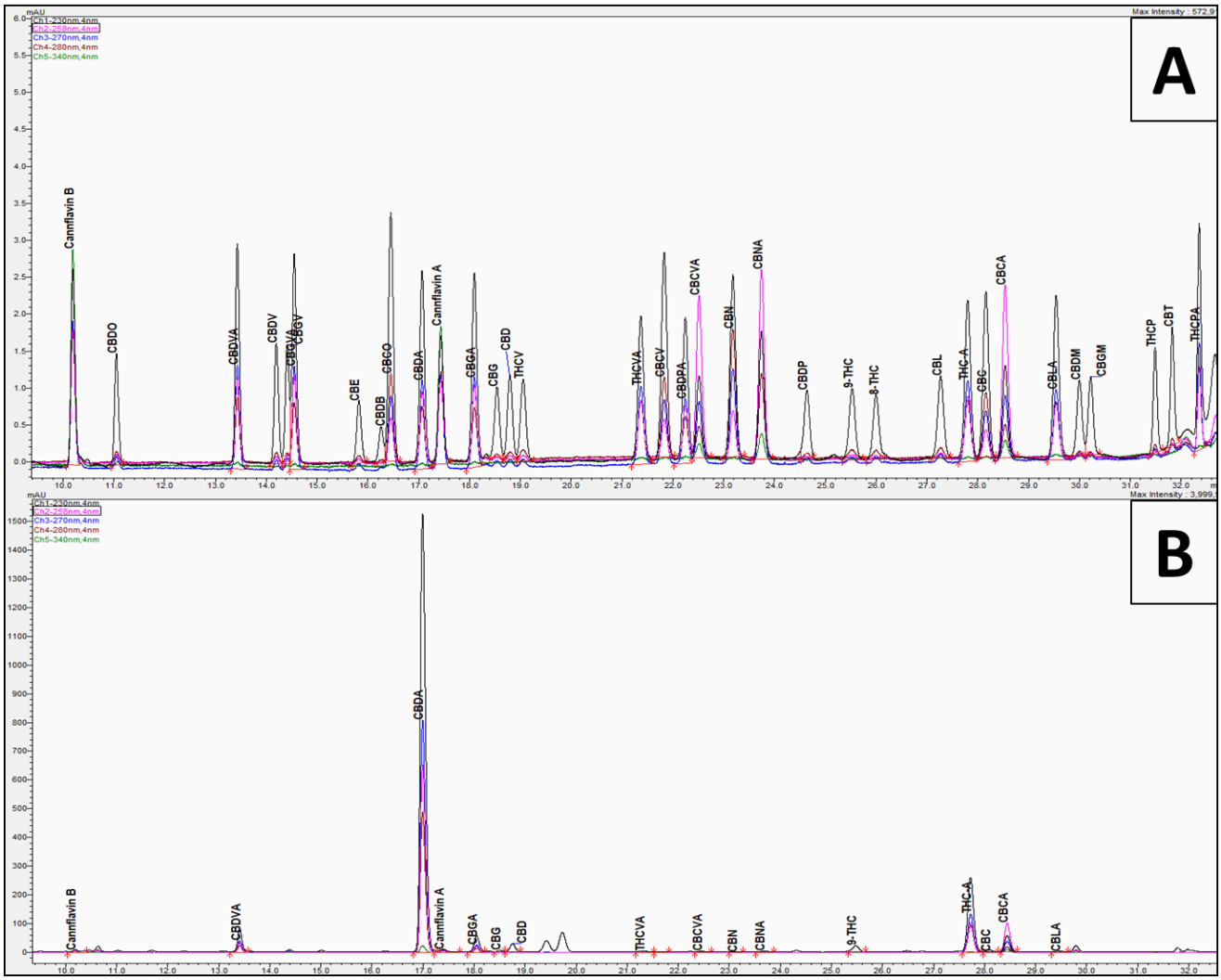


■ Results

Calibrations

USP resolution greater than 1.0 was observed for all analytes. A solvent containing no analytes was applied to all standards and samples for consistent baseline identification.

Figure 2A: Chromatogram of 34 cannabinoids/cannflavins at approximately 1 ug/mL. Single injection with quantitation wavelengths overlaid.
Figure 2B: Chromatogram of mix of hemp acetone extracts at a total dilution of 50X. Single injection with quantitation wavelengths overlaid.



Accuracy and Precision

Accuracy and precision were evaluated by spiking all 34 analytes on to homogenized low-cannabinoid producing Cannabis plant material (Table 4). The concentration of cannabinoids and cannaflavins present in non-spiked Cannabis plant material was subtracted from the observed concentrations in the spiked samples.

To further evaluate the method's accuracy and precision, performance tests were provided by MilliporeSigma and diluted by 5X (Table 5).

Table 4: Average Percent Recoveries and Percent Relative Standard Deviations at Approximately 5 ug/mL On-Column or Approximately 0.05 Weight %. N=3 Replicates.

Analyte	Average Recovery (%)	Relative Standard Deviation (%)	Analyte	Average Recovery (%)	Relative Standard Deviation (%)
Cannflavin B	101	3.5	CBDPA	102	2.4
CBDO	120	7.5	CBCVA	97.4	2.3
CBDVA	97.6	2.5	CBN	95.3	2.8
CBDV	112	2.8	CBNA	97	2.4
CBGV	108	2.0	CBDP	114	3.3
CBGVA	108	3.2	Δ9-THC	99.8	1.9
CBE	111	2.9	Δ8-THC	103	1.3
CBDB	99.5	1.8	CBL	105	5.0
CBCO	101	2.8	Δ9-THCA	99.7	2.1
CBDA	103	2.3	CBC	85.6	4.9
Cannflavin A	111	7.5	CBCA	98.4	2.4
CBGA	100	1.5	CBLA	98.7	2.6
CBG	89.7	3.7	CBDM	101	1.4
CBD	103	3.7	CBGM	98.9	2.1
Δ9-THCV	94.2	3.3	Δ9-THCP	103	2.6
Δ9-THCVA	91.5	0.63	CBT	101	2.1
CBCV	102	2.0	Δ9-THCPA	96.0	0.70

Table 5: Average Percent Recoveries and Percent Relative Standard Deviations of MilliporeSigma PEC6001 (8 Part Neutral Potency) and PEC6002 (6 Part Acid Potency). N=3 Replicates Per Performance Test

Analyte	Average Recovery (%)	Relative Standard Deviation (%)	Analyte	Average Recovery (%)	Relative Standard Deviation (%)
CBDVA	106	2.0	Δ9-THCVA	98.8	1.9
CBDV	103	1.3	CBN	101	1.3
CBDA	123	1.8	Δ9-THC	111	1.2
CBGA	113	2.0	Δ8-THC	107	1.4
CBG	113	1.1	Δ9-THCA	108	1.9
CBD	102	1.5	CBC	106	1.3
Δ9-THCV	104	1.4	CBCA	105	2.1

Limits of Detection

Table 6: Calculated Method Limits of Detection at 50X Total Dilution Factor and 0.2 Grams of Sample.

Analyte	LOD (S/N > 3:1) Wt %	Analyte	LOD (S/N > 3:1) Wt %
Cannflavin B	0.002	CBDPA	0.006
CBDO	0.003	CBCVA	0.006
CBDVA	0.006	CBN	0.003
CBDV	0.003	CBNA	0.006
CBGV	0.003	CBDP	0.003
CBGVA	0.006	Δ9-THC	0.003
CBE	0.003	Δ8-THC	0.003
CBDB	0.001	CBL	0.003
CBCO	0.003	Δ9-THCA	0.005
CBDA	0.005	CBC	0.003
Cannflavin A	0.003	CBCA	0.006
CBGA	0.005	CBLA	0.006
CBG	0.003	CBDM	0.006
CBD	0.003	CBGM	0.006
Δ9-THCV	0.003	Δ9-THCP	0.003
Δ9-THCVA	0.006	CBT	0.003
CBCV	0.003	Δ9-THCPA	0.006

■ Conclusions

A gradient HPLC method was developed for the quantification of 34 unique compounds in Cannabis within a single injection. Solvent consumption per injection was less than 16mL with an injection-to-injection runtime of 35 minutes. The method described allows for the quantitation of major and minor phytocannabinoids in Cannabis with minimal coelutions from flavonoids or terpenes; thus, reducing limits of detection while maintaining accuracy at ≤ ±20% and precision at ≤ ±10%.

First Edition: January 2023

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
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Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com

SHIMADZU
Excellence in Science

SSI07 – Shimadzu Quote for HPLC-DAD-RI system

10330 Old Columbia Road, Columbia, MD 21046
(410) 381-1227 ● www.ssi.shimadzu.com

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Shimadzu Scientific Instruments
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Durham, NC 27703
Regional Office: (800) 951-9167 Ext.
Direct Dial:
E-mail: rmjackson@shimadzu.com

Sales Proposal

Quotation: SSI-270843-D6Y1

Expiration: 10/22/2025

Quote Description:
Modular HPLC system HPLC-DAD-RI response to AGR26*01

Proposed Ship Date: 7-10 Days/ARO **FOB:** DESTINATION **Ship Method:** BEST WAY
Shipping Terms: PREPAID & ADD **Incoterms:** FOB
Additional Information:

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

For Order Placement:

Reference Quotation Number on Purchase Order

Please note that state taxes, local taxes and freight, if applicable, will be added at invoicing.
Forward official tax exempt documentation if applicable.

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

Thank you for your interest in Shimadzu Scientific Instruments.

Authorization Signature*

ESTIMATE ONLY

*Sales Proposal is not valid without Authorized Signature.

Quote Created On: 7/22/2025

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Company Name: State of West Virginia -Department of Agriculture
Quote Number: SSI-270843-D6Y1
Activation Date: 7/22/2025
Expiration Date: 10/22/2025

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

Bid request AGR26*01

	Product #	Qty	Description	Price Per Unit	Ext'd Price
1	220-91657-01	1	SCL-40 with Installation Kit	$5,073.00	$5,073.00
2	228-65077-58	4	LC-40D XS UHPLC Pump	$13,663.00	$54,652.00
3	220-91656-05	1	SIL-40CXS Autosampler with Installation Kit	$34,311.00	$34,311.00
4	228-65202-58	2	CTO-40C Column Oven	$6,452.00	$12,904.00
5	228-65019-58	2	DGU-405 5-channel Degasser	$4,715.00	$9,430.00
6	228-72568-44	1	Dual Injection Kit for CTO-40C	$4,329.00	$4,329.00
7	228-65624-58	1	FCV-0206H3 2-position 6-port UHPLC Valve	$4,441.00	$4,441.00
8	228-70254-42	2	Piping Kit B, for High Pressure GE, ID0.1	$1,666.00	$3,332.00
9	228-71759-44	2	Sample Loop for Loop Injection, 50 uL	$602.00	$1,204.00
10	228-72652-44	2	180 uL MiRC Mixer with Recognition Device	$1,990.00	$3,980.00
11	228-65302-58	1	SPD-M40 Photodiode Array Detector	$19,187.00	$19,187.00
12	228-65306-58	1	RID-20A Refractive Index Detector	$16,004.00	$16,004.00
13	228-65110-58	1	Plate Changer	$19,637.00	$19,637.00
14	228-71762-46	1	Vial Plate, 12 x 32mm 1.5 mL Vials, 54-Position, SIL-40	$89.00	$89.00
15	220-91494-06	1	HPLC Startup Kit w/o Res Tray #2 5-GAL Poly	$1,866.00	$1,866.00
16	228-57647-43	1	Tool Kit, Common LC-40	$1,298.00	$1,298.00
17	TIER 3 I&F	1	Tier 3 Installation and Customer Familiarization	$5,408.00	$0.00
18	220-91545-01	1	Solvents, LC, Honeywell B&J LCMS Grade Solvent Kit for Installations	$626.00	$626.00
19	1YW	1	1 YEAR WARRANTY	$0.00	$0.00

LabSolutions Workstation (WS)

	Product #	Qty	Description	Price Per Unit	Ext'd Price
20	223-62726-51	1	(LC/PDA) LabSolutions LCGC WS, 1 LC, and 1 PDA licenses; software separate - WS	$5,439.00	$5,439.00
21	223-19335-51	1	(LC/GC) LabSolutions LCGC Workstation software disk	$641.00	$641.00
22	220-97321-10	1	(PC Hardware) Dell widescreen 24" monitor	$517.00	$517.00
23	220-97317-85	1	(PC Hardware) Standard Dell Computer; includes Microsoft Office/mouse/keyboard	$3,841.00	$3,841.00

Company Name:	State of West Virginia -Department of Agriculture
Quote Number:	SSI-270843-D6Y1
Activation Date:	7/22/2025
Expiration Date:	10/22/2025

Total List Price:	**$208,209.00**
Total Line Item Discount:	**$5,408.00**
State of WV Discount:	**$70,980.35**
Quote Sub-Total:	**$131,820.65**
Estimated Freight:	**$1,100.00**
Total Amount:	**$132,920.65**

By placing an Order for any Products related to this Sales Proposal and Quotation, Customer agrees to the terms and conditions at https://www.ssi.shimadzu.com/terms/General-Terms-Conditions-of-Sale.html, which are incorporated herein and applicable hereto. SSI reserves the right to update its Terms and Conditions at any time, however the terms and conditions in effect at the date of delivery of Products related to this Sales Proposal and Quotation shall apply to that purchase.

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Company Name:	State of West Virginia -Department of Agriculture
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Activation Date:	7/22/2025
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Sales Quotation - please reference the quotation when submitting purchase order.

Optional Items

Optional Items:

Bid request AGR26*01

	Product #	Qty	Description	List Price	Ext'd Price
1	228-64724-41	1	High-Sensitivity UHPLC Flow Cell, SPD-M40	$1,964.00	$1,964.00

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Company Name:	State of West Virginia -Department of Agriculture
Quote Number:	SSI-270843-D6Y1
Activation Date:	7/22/2025
Expiration Date:	10/22/2025

Service Agreement Plans Available

As Quoted

Quantity	Plan Name	Plan Price	Disc't %	Ext'd Price	Products Covered
1	Premium Preventative Maintenance	$19,375.00	20.00%	$15,500.00	DGU-405 5-channel Degasser, LC-40D XS UHPLC Pump, Plate Changer, CTO-40C Column Oven, SPD-M40 Photodiode Array Detector, RID-20A Refractive Index Detector, FCV-0206H3 2-position 6-port UHPLC Valve, SCL-40 with Installation Kit, SIL-40CXS Autosampler with Installation Kit
1	Extended Warranty	$15,505.00	20.00%	$12,404.00	DGU-405 5-channel Degasser, LC-40D XS UHPLC Pump, Plate Changer, CTO-40C Column Oven, SPD-M40 Photodiode Array Detector, RID-20A Refractive Index Detector, FCV-0206H3 2-position 6-port UHPLC Valve, SCL-40 with Installation Kit, SIL-40CXS Autosampler with Installation Kit
1	Extended Warranty Plus	$29,235.00	20.00%	$23,388.00	DGU-405 5-channel Degasser, LC-40D XS UHPLC Pump, Plate Changer, CTO-40C Column Oven, SPD-M40 Photodiode Array Detector, RID-20A Refractive Index Detector, FCV-0206H3 2-position 6-port UHPLC Valve, SCL-40 with Installation Kit, SIL-40CXS Autosampler with Installation Kit
1	Three Year(36 month) Value Plan Service Agreement	$48,405.00	20.00%	$38,724.00	DGU-405 5-channel Degasser, LC-40D XS UHPLC Pump, Plate Changer, CTO-40C Column Oven, SPD-M40 Photodiode Array Detector, RID-20A Refractive Index Detector, FCV-0206H3 2-position 6-port UHPLC Valve, SCL-40 with Installation Kit, SIL-40CXS Autosampler with Installation Kit

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.

Three Year Value Plan

Three Year Value Plan Service Agreement:
Following system installation, provides 3 consecutive years of FULL warranty coverage. Customer Care follow-up including PM level service Year1. Scheduled Premium Preventative Maintenance (PPM) on-site service during Year2 and Year3.

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Company Name:	State of West Virginia -Department of Agriculture
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Activation Date:	7/22/2025
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Sales Quotation - please reference the quotation when submitting purchase order.

Line Item Descriptions

Product # **Description**

Bid request AGR26*01

220-91657-01 **SCL-40 with Installation Kit**

The SCL-40 system controller is the central communication and control module for the Nexera series. An advanced, color touchscreen allows system control and monitoring. A built-in reservoir tray for HPLC mobile phase bottles is included.

The SCL-40 system controller is the central communication and control module for the Nexera series. Advanced pressure-sensitive color touchscreen allows system control and monitoring. System components connect via fiber optic cables for easy configuration. Internal web server offers convenient control and monitoring of the HPLC system through a web browser. A network switch and built-in reservoir tray are included.

228-65077-58 **LC-40D XS UHPLC Pump**

The LC-40DXS is a next-generation UHPLC pump that provides reliable and robust UHPLC performance up to 105 MPa (~15,000 psi). This pumping system incorporates a dual plunger, reciprocating pump for stable solvent delivery across a wide analytical flow rate range. The -40DXS pump can be configured for HPGE, LPGE, and blending functions for solvent delivery. The -XS pump is ideal for semi-micro LCMS applications and for analysis using <2 um particle columns.

The LC-40D XS uses dual 10 uL sapphire plungers for pulse-free flow. An automatic rinse kit is included as standard. Flow rates from 0.0001 to 3.000 mL (105MPa) and 0.0001 to 10.000 mL (22MPa) are accomodated. PMax is 105 MPa (15,000 psi). The XS is ideal for semi-micro LCMS applications, and for analyses using sub-2 micron particle columns.

220-91656-05 **SIL-40CXS Autosampler with Installation Kit**

The SIL-40C XS provides high speed injections with ultra-low carryover achieved through advanced material technology and rinse chemistries with up to 4 solvents, when equipped with the optional Multi Rinse Kit. 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, 10, or 20 µL) is user-changeable to reduce delay volume. The sample compartment provides temperature control from 4-40 C. An external rinse pump is standard, and provides additional rinsing to the outer surface of the needle. Sample capacity is three racks, for 1.5 (2mL) vials, 1 mL vials, 4 mL vials, 10 mL vials, 1.5 mL microcentrifuge tubes, or 96/384 well plates. Sample capacity is 252 x 1 mL vials, 162 x 1.5 mL vials, 84 x 4 mL vials, or 3 x MTP (MTP 96, DWP 96, or MTP 384). Maximum pressure is 105 MPa (15200 psi).

The SIL-40C XS provides high speed injections with ultra-low carryover achieved through advanced material technology and rinse chemistries with up to 4 solvents, when equipped with the optional Multi Rinse Kit. 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, 10, or 20 µL) is user-changeable to reduce delay volume. The sample compartment provides temperature control from 4-40 C. An external rinse pump is standard, and provides additional rinsing to the outer surface of the needle. Sample capacity is three racks, for 1.5 (2mL) vials, 1 mL vials, 4 mL vials, 10 mL vials, 1.5 mL microcentrifuge tubes, or 96/384 well plates. Sample capacity is 252 x 1 mL vials, 162 x 1.5 mL vials, 84 x 4 mL vials, or 3 x MTP (MTP 96, DWP 96, or MTP 384). Maximum pressure is 105 MPa (15200 psi).

228-65202-58 **CTO-40C Column Oven**

Full-sized forced air column oven that provides temperature control from (ambient - 10 C) to 100 C. Can accommodate a manual injector, mixer, and 2 switching valves. For columns up to 300 mm length.

Linear temperature programming is possible; Optional Column Management Device and Mixer Recognition Device can be used with CTO-40C dual ports.

228-65019-58 **DGU-405 5-channel Degasser**

A 5-channel degassing unit for the Nexera Series (U)HPLC. Degassing reduces the occurrence of bubbles in the HPLC which can lead to poor chromatographic outcomes. This degasser provides efficient vacuum degassing for four pump lines plus the rinse line for the autosampler.

Inline membrane degasser utilizing PTFE AF® for rapid degassing of HPLC mobile phases. Convenient 5th channel for degassing the autosampler rinse phase for optimum injection reproducibility performance.

228-72568-44 **Dual Injection Kit for CTO-40C**

Optional kit for SIL-40 and CTO-40C that allows parallel dual injection capability. Requires dual binary gradient pump configuration, second injection valve, sample loop kits, dual detectors, and LabSolutions software.

228-65624-58 **FCV-0206H3 2-position 6-port UHPLC Valve**

Quick-exchange UHPLC valve for use with Nexera series. Max pressure = 19000 psi.

For use in CTO-40, FCV-S, FCV-Box, and SIL-40. Requires FCV-DR for CTO and FCV applications, mounting kit required for CTO.

228-70254-42 Piping Kit B, for High Pressure GE, ID0.1

Kit with stainless steel tubing for connecting two UHPLC pumps to mixer (0.3x600 mm), mixer to injector (0.3x300 mm), and injector to column (0.1x600 mm).

Required for new Nexera series installations.

228-71759-44 Sample Loop for Loop Injection, 50 uL

50 ul sample loop for loop injection method, comes with two UHPLC fittings and needle loop expansion pipe

50 ul sample loop for loop injection method, comes with two UHPLC fittings and needle loop expansion pipe

228-72652-44 180 uL MiRC Mixer with Recognition Device

High efficiency 180 uL mixer using micro reactor technology to thoroughly blend solvents in a small volume. This mixer is recommended for (U)HPLC systems with an PDA detector and with additives like trifluoroacetic acid (TFA). Effective at mixing solvents at analytical flow rates (1-5 mL/min) and LCMS flow rates with solvents from pH 1-14. This mixer comes with a Mixer Recognition Device which will log the volume of the mixer in the system configuration when used with the CTO-40S/C oven and LabSolutions.

pH 1-14, P. Max = 19000 psi

228-65302-58 SPD-M40 Photodiode Array Detector

The SPD-M40 is a variable slit width photodiode array detector with D2 and W lamps for a wavelength range of 190-800 nm. The standard temperature controlled flow cell coupled with dual temperature control of the optical bench and new digital signal processing results in extremely low noise and a greatly extended linear range. UV cutoff filter can be used to prevent degradation of UV-labile compounds being analyzed in the visible range.

Includes standard temperature controlled flow cell (ambient +5 C) to 50 C; 10 mm path length; linear to 2.0 AU; D2 and W lamps can be lit simultaneously; 1.2 & 8 nm slit widths; slit switching possible. Optional flow cells available.

228-65306-58 RID-20A Refractive Index Detector

The RID-20A differential refractive index detector features a dual temperature-controlled optical compartment for less than 30 min warm-up time and exceptional baseline stability. The temperature controlled flow cell minimizes drift if the ambient temperature changes. Optional flow selection blocks are available for high sensitivity, prep, and large scale prep applications.

Safety features include a leak sensor and pressure relief valve to protect the flow cell. Maximum cell pressure is 2MPa (290psi). An optional recycle valve is available for solvent conservation.

228-65110-58 Plate Changer

Temperature Controlled robotic unit for loading microtiter plates into the SIL-40C, -40CXR, -40XS, -40CX3. 14 MTP capacity (96 position, shallow or deep well). Up to 3 plate changers may be multiplexed.

Temperature Controlled robotic unit for loading microtiter plates into the SIL-40C, -40CXR, -40XS, -40CX3. 14 MTP capacity (96 position, shallow or deep well). Up to 3 plate changers may be multiplexed.

228-71762-46 Vial Plate, 12 x 32mm 1.5 mL Vials, 54-Position, SIL-40

1.5 mL rack for the SIL-40 autosampler. 54 vial capacity. Skeletal construction provides increased airflow around vials for more efficient sample cooling. Built in bar code allows the autosampler or Plate Changer to automatically detect the type of plate for injection.

1.5 mL rack for the SIL-40 autosampler. 54 vial capacity. Skeletal construction provides increased airflow around vials for more efficient sample cooling. Built in bar code allows the autosampler or Plate Changer to automatically detect the type of plate for injection.

220-91494-06 HPLC Startup Kit w/o Res Tray #2 5-GAL Poly

Complete installation kit for HPLC that includes a set of 5 1-L bottles with 3-hole caps, PEEK tubing and fittings kit, and a 5-gallon (20 L) PE waste can with polypropylene quick-disconnect manifold fittings.

Bottle caps have a solid plug and filter plug with a stainless steel frit for use with He sparging. The tubing kit contains 2 each of 5' rolls of Blue (0.01" i.d.) and Red (0.005" i.d.) PEEK tubing, plus a cutter, fingertight fittings, PEEK unions, column plugs, and spare nuts and ferrules for the DGU-403 or -405 degassers. The waste can comes complete with manifold and fittings for 1 HPLC system (autosampler and detector); additional ports are present so 3 systems can feed 1 can; additional fittings sold separately. Also included is an activated carbon vapor filter for the waste can to minimize solvent vapors in the lab environment.

228-57647-43 Tool Kit, Common LC-40

Shared tool kit for the i-Series and LC-40. Includes wrenches, seal installer tool, syringes and adapters, rotor replacement tool, and other items for routine maintenance on i-Series and LC-40 Series units.

Shared tool kit for the i-Series and LC-40.

TIER 3 I&F **Tier 3 Installation and Customer Familiarization**

220-91545-01 **Solvents, LC, Honeywell B&J LCMS Grade Solvent Kit for Installations**

Honeywell B&J brand LCMS grade solvents. Packaged as a case of four 1L bottles. Case contains the following solvents:

1L of Acetonitrile
1L of Methanol
1L of Water
1L of 2-propanol

Please note that overnight shipping is not possible for this item due to hazmat restrictions

Honeywell B&J brand LCMS grade solvents. Packaged as a case of four 1L bottles. Case contains the following solvents:

1L of Acetonitrile
1L of Methanol
1L of Water
1L of 2-propanol

Size: 1L bottles

Please note that overnight shipping is not possible for this item due to hazmat restrictions

1YW **1 YEAR WARRANTY**

1 YEAR WARRANTY

1 YEAR WARRANTY

LabSolutions Workstation (WS)

223-62726-51 **(LC/PDA) LabSolutions LCGC WS, 1 LC, and 1 PDA licenses; software separate - WS**

This is an electronic license for control of 1 LC and 1 PDA instrument concurrently in LabSolutions Workstation (WS).

Quote licenses for the number of instruments running concurrently in LabSolutions.

Quote licenses for the number of instruments running concurrently in LabSolutions.

223-19335-51 **(LC/GC) LabSolutions LCGC Workstation software disk**

Shimadzu LabSolutions® Workstation Software provides system control, data acquisition and reporting for Shimadzu HPLC and GC systems.

LabSolutions Workstation provides user-friendly workstation software for HPLC and GC instruments. LabSolutions has built in system security and system management functions. Data can be saved, in a proprietary format, to a computers local hard drive or to network location.

LabSolutions Workstation supports digital data acquisition of data through RS-232, USB, or Ethernet connection to Shimadzu and third party instruments. Analog signal acquisition can also be performed when an optional A/D board is used.

This item includes an installation disc for the current version of LabSolutions Workstation software.

It does not include any LabSolutions licenses. Any licenses must be purchased seperatly.

220-97321-10 **(PC Hardware) Dell widescreen 24" monitor**

24” Widescreen LCD display, resolution to 1920 x 1080, contrast ratio (typical): 1000:1. Brightness (typical): 250 cd/m2, 0.277 mm pixel pitch.

Limited warranty: Three years Advanced Portable Exchange through Dell.

- Resolution: 1920 x 1080
- Panel Type: In-Plane Switching Technology
- DisplayPort
- Limited warranty: Three years Advanced Portable Exchange through Dell
- Dell PN: P2411H

220-97317-85 (PC Hardware) Standard Dell Computer; includes Microsoft Office/mouse/keyboard

Dell computer suitable for most Shimadzu software packages. It is a small form factor computer (approximately 11.5"x11.5"x3.5") and includes an 13th gen Intel i7 processor, 16Gb of RAM, a 1 TB SSD, a DVD+ R/W drive, 2 ethernet ports, Windows 11 Pro, and Microsoft Office Home & Business. Wired keyboard and mouse included.

Model: Dell Precision 3460
Design: Small form factor tower
Dimensions: 11.53 x 11.42 x 3.65"
Operating System (OS): Windows 11 Pro
RAM: 16 GB
Hard Drive: Single 1 TB SSD
Processor: 13th gen Intel i7
Ports:
- Ethernet (2)
- DisplayPort (3)
- USB A (9)
- USB C (1)

Additional devices: DVD+ R/W, Dell Wired Keyboard, Dell Optical Mouse
Additional software: Microsoft Office Home & Business
Warranty: 3-year on-site repair

Optional Item Descriptions

Product # **Description**

Bid request AGR26*01

228-64724-41 High-Sensitivity UHPLC Flow Cell, SPD-M40

Recommended for the detection of low concentration analytes; mm path length, 9 uL

Recommended for the detection of low concentration analytes; 10 mm path length, 9 uL