C10G-E068

SHIMADZU
Excellence in Science

Liquid Chromatograph Mass Spectrometer

LCMS-8045

Application Booklet

ULTRA FAST MASS SPECTROMETRY

Speed Beyond Comparison

LCMS-8045

—*Best-in-class sensitivity*

Accomplishes Both High Sensitivity and Ultra-High-Speed Detection

Equipped with a heated ESI probe, the LCMS-8045 has the highest* sensitivity in its class. The instrument is capable of providing accurate and stable data over long periods of time. The inclusion of Shimadzu's ultra-high-speed high-voltage power supply enables the world's fastest* scan speed (30,000 u/s) and polarity switching time (5 ms). High-speed acquisition benefits the laboratory by reducing run times for increased throughput, and also shortens method development time.

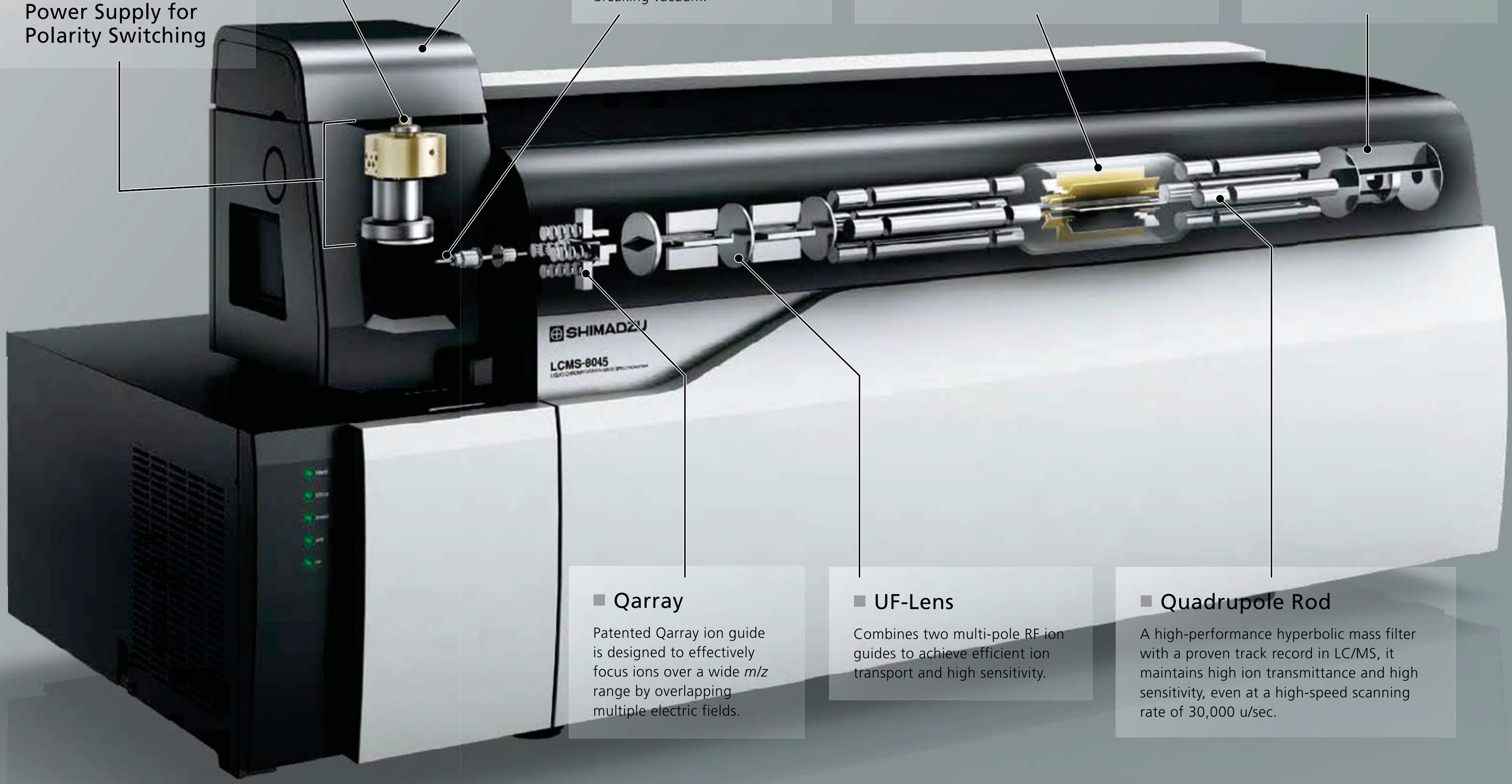
* As of August 2016, according to a Shimadzu survey

Superior Robustness Assures That High Sensitivity Is Maintained Over the Long Term

The LCMS-8045 was designed to be robust. The heated ESI probe, high-temperature heating block, heated desolvation line, drying gas, and focusing optics all act to maximize sensitivity and minimize contamination. This means long periods of continuous operation in the laboratory with reliable data collection, even with complex biological fluids or food samples.

LabSolutions LCMS Software and Wide-Ranging Method Packages

LabSolutions LCMS features an intuitive user interface, and offers the latest features designed to enhance laboratory productivity and streamline workflows. Numerous pre-configured MRM method packages are available. Predetermined LC separation conditions and MRM parameters allow analysis to be started without long hours of method development, enhancing the efficiency of your laboratory.



Heated ESI Probe

High-temperature gas supplements the nebulizer gas, improving desolvation efficiency. This facilitates the ionization of a wide range of compounds.

Ultrafast High-Voltage Power Supply for Polarity Switching

Ionization Unit

Designed without cables or tubes; utilize a one-touch lever to perform simple attachment and detachment of the unit.

DL (Desolvation Line)

Perform maintenance without breaking vacuum.

UFsweeper II Collision Cell

The UFsweeper II is a high-sensitivity, high-speed collision cell that features improved ion focusing by using high-speed ion transport technology. This yields better product ion transmission in the collision cell, maintaining signal intensity and suppressing crosstalk, even for high-speed or simultaneous multi-component analysis. The capability for high-throughput analysis is thus maintained at lower levels of detection.

Ultrafast Pulse-Counting Detector

Makes ultrafast sampling and polarity switching possible.

Qarray

Patented Qarray ion guide is designed to effectively focus ions over a wide *m/z* range by overlapping multiple electric fields.

UF-Lens

Combines two multi-pole RF ion guides to achieve efficient ion transport and high sensitivity.

Quadrupole Rod

A high-performance hyperbolic mass filter with a proven track record in LC/MS, it maintains high ion transmittance and high sensitivity, even at a high-speed scanning rate of 30,000 u/sec.

Introduction

The LCMS-8045 was designed to be robust. The heated ESI probe, high-temperature heating block, heated desolvation line, drying gas, and focusing optics all act to maximize sensitivity and minimize contamination. This means long periods of continuous operation in the laboratory with reliable data collection, even with complex biological fluids or food samples.

Equipped with a heated ESI probe, the LCMS-8045 has the highest* sensitivity in its class. The instrument is capable of providing accurate and stable data over long periods of time. The inclusion of Shimadzu's ultra-high-speed* high-voltage power supply enables the world's fastest scan speed (30,000 u/s) and polarity switching time (5 ms). High-speed acquisition benefits the laboratory by reducing run times for increased throughput, and also shortens method development time.

LabSolutions LCMS features an intuitive user interface, and offers the latest features designed to enhance laboratory productivity and streamline workflows. Numerous pre-configured MRM method packages are available. Predetermined LC separation conditions and MRM parameters allow analysis to be started without long hours of method development, enhancing the efficiency of your laboratory.

LCMS-8045 upgrade to LCMS-8060 is also available.

* As of August 2016, according to a Shimadzu survey

Method Packages and Libraries for Shimadzu LCMS

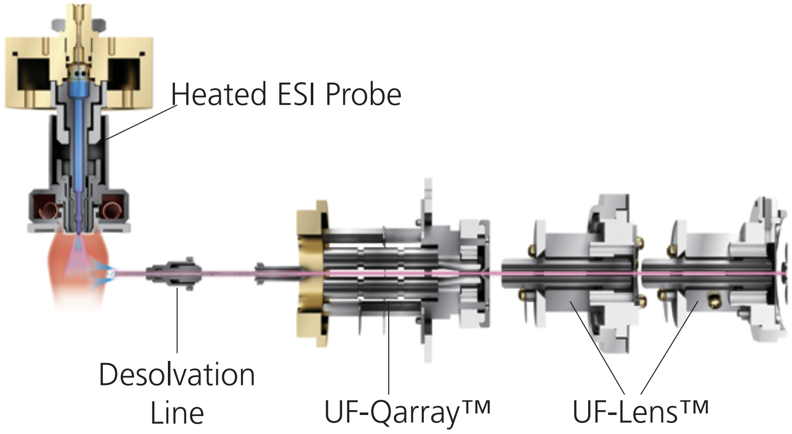
Method Packages	Residual Pesticides Veterinary Drugs Water Quality Analysis Drugs of Abuse Rapid Toxicology Screening	Primary Metabolites Lipid Mediators Cell Culture Profiling Restricted Chemicals in Textiles
MRM Library	Metabolic Enzymes in Yeast	

Click here for our full list of method packages and MRM libraries

UFMS Technology

Cutting-edge Ion Optics System

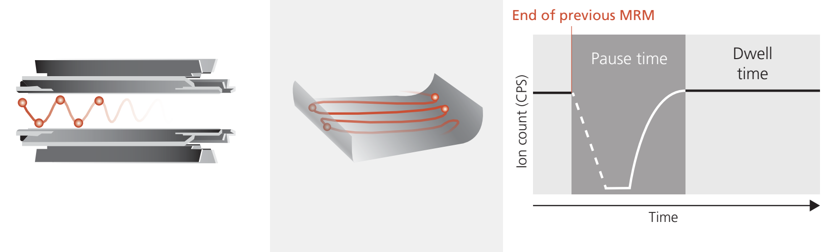
Ions are precisely converged and signal losses are minimized. In addition, the efficiency of the ionization process is improved with the use of a heated ESI probe. Together with the enhanced desolvation line and ion optics system, there is an increase in ion production and transmission, thereby generating a high-intensity and focused ion beam leading to higher sensitivity in LCMS.



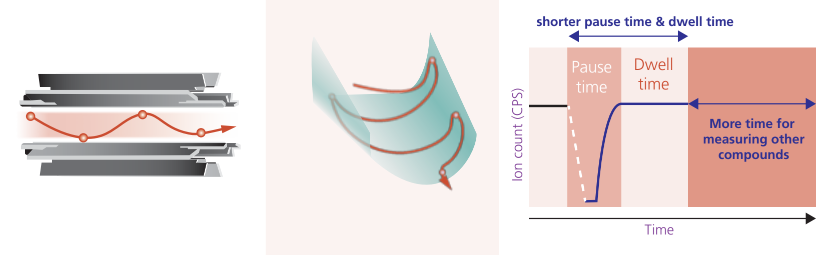
UFsweeper™ High-speed Collision Cell

With the new pseudo potential surface, ions entering the collision cell are accelerated and maintain their momentum upon collision. Under these circumstances, the efficiency of the fragmentation or CID is improved. This technology allows quicker and better ion transmission in the collision cell, maintaining signal intensity and dramatically suppressing crosstalk, even when shorter dwell and pause times are used.

Conventional design
Ions lose momentum due to collision with gas.



UFsweeper™
UFsweeper efficiently accelerates ions out of the collision cell without losing momentum.



List of Applications

Food Analysis

Determination of Nitrofuran Metabolites in Fish Matrix by LCMS-8045

Determination of Avermectin Drug Residues in Vinegar Using LCMS-8045

Determination of Sulfonamide Residues in Pork Using LCMS-8045

Determination of 5 Kinds of Estrogens in Milk using LCMS-8045

Determination of Quinolone Antibiotic Residues in Chicken by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

Multi-Residue Analysis of Antibiotics in Organic Fertilizers using the Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer

Determination of Imidocarb Residues in Milk by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

Determining Gamma-Hydroxybutyric Acid and its Precursors Gamma-Butyrolactone and 1,4-Butanediol in Suspected Drug-spiked Beverages using LCMS-8045

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Determination of 153 Pesticide Residues in Traditional Chinese Medicines by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

LC-MS/MS Compositional Analysis of Three Culture Media for Antibody Drug Production

Determination of Bile Acids in Cow-Bezoar Using LCMS-8045

Determination of 9-Propenyladenine in Tenofovir Disoproxil Fumarate Using Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer

Environmental Analysis

Determination of Pharmaceuticals and Personal Care Products (PPCPs) Residues in Tap Water with Ultra-High Performance Triple Quadrupole Liquid Chromatograph Mass Spectrometer

Determination of 20 Carbamate Pesticide Residues in Soil by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

Determination of Benzidine and 3,3-Dichlorobenzidine in Environmental Waters Using Ultra-High Performance Liquid Chromatograph and Triple Quadrupole Mass Spectrometer

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Determination of Nitrofuran Metabolites in Fish Matrix by LCMS-8045

Qisheng Zhong
Shimadzu (China), Guangzhou Analysis Center

Application News
SSL-CA14-271

Abstract

In this article, a method for the determination of nitrofuran metabolite residues in fish matrix was established using Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A in conjunction with its Triple Quadrupole Mass Spectrometer LCMS-8045. After sample preparation, they were separated by UHPLC LC-30A in 3.0 minutes and then quantitatively analyzed using the Triple Quadrupole LCMS-8045. 3-amino-2-oxazolone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD), and semicarbazide (SEM) showed good linearity over the range of 0.05-20 ng/mL and their correlation coefficients were all greater than 0.9970. The precision experiments were performed with mixed standard solutions of concentrations 0.05 and 2.0 ng/mL. The results showed that the relative standard deviations for the retention times and peak areas obtained from 6 consecutive injections were 0.09 - 0.18% and 0.85 - 1.99% respectively, indicating good precision.

Nitrofurans are a class of synthetic antimicrobials drugs which inhibits the activity of acetyl-coenzyme A and interfere with the metabolism of sugar in microbes. Nitrofurans are very unstable and can easily be converted into metabolites. In the body of animals, nitrofuran drugs are rapidly decomposed into metabolites, which are subsequently bound to the membrane proteins. As the metabolites of nitrofurans are relatively stable and are associated with carcinogenic effects, the level of nitrofuran metabolites are often monitored in food safety tests. There are four common derivatives of nitrofuran metabolites, including: 3-amino-2-oxazolone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semicarbazide (SEM).

In this article, a high-sensitivity detection method using Shimadzu UHPLC coupled with Triple Quadrupole LCMS-8045 is established for the assay of nitrofuran metabolite residues AOZ, AMOZ, AHD and SEM in fish matrix. This method was developed with reference to the standard method GB/T 21311-2007 and is aimed to serve as a reference method for regulatory testing.

EXPERIMENTAL

Instrumentation

This experiment was performed on a Shimadzu UHPLC LC-30A in conjunction with Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations includes LC-30AD×2 solvent delivery pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatography workstation.

Analytical Conditions

LC Chromatography (LC) Conditions

Column	:	Shim-pack GISS C18 Column (2.1 mm I.D.×100 mm L., 1.9 μm)
Mobile phase	:	Solvent A: 0.01% formic acid in water Solvent B: 0.01% formic acid in acetonitrile
Flow rate	:	0.50 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 μL
Elution method	:	Gradient elution with an initial ratio of 10% B

Table 1 General gradient elution program

Time (min)	Module	Command	Value (%)
1.50	Pumps	Pump B Conc.	80
1.51	Pumps	Pump B Conc.	10
3.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Analytical Instrument : LCMS-8045
Ion Source : ESI+
Nebulizar gas flowrate : 3.0 L/min
Heating gas flow rate : 15.0 L/min
Interface Temp. : 400 °C
DL Temp. : 250 °C
Heating block Temp. : 450 °C
Needle offset distance : +3.0 mm
Drying gas flow rate : 5.0 L/min
Dwell Time : 22 ms
Scan mode : Multiple Reaction Monitoring (MRM) with parameters shown in **Table 2**

Standards and Sample Preparation

Preparation of standard solutions: an appropriate amount of nitrofuran derivatives, i.e., AOZ, AMOZ, AHD and SEM, were individually weighed and dissolved in a mixture of acetone/methanol (1:1) to prepare a single-standard stock solution at a concentration of 0.5 mg/mL. Subsequently, the stock solution was diluted with methanol to 100 ng/mL and further diluted with water to prepare a series of mixed standard working solutions at concentrations of 0.050, 0.10, 0.20, 0.50, 1.0 and 2.0 ng/mL.

Sample was prepared with reference to “GB/T 21311-2007: Determination of residues of nitrofuran metabolites in foodstuffs of animal origin HPLC-MS/MS method”.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Samples

The MRM chromatogram of standard sample is shown in Figure 1.

Table 2 Compound information and MRM parameters

Number	Analyte	CAS.	Percursor Ion	Product ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	SEM	57-56-7	209.05	166.10	-14.0	-9.0	-17.0
				192.05	-15.0	-13.0	-21.0
2	AOZ	80-65-9	236.10	134.00	-11.0	-11.0	-23.0
				104.10	-17.0	-22.0	-11.0
3	AHD	2827-56-7	249.00	134.05	-26.0	-12.0	-25.0
				104.15	-17.0	-23.0	-21.0
4	AMOZ	43056-63-9	335.15	291.10	-16.0	-12.0	-30.0
				262.10	-24.0	-18.0	-30.0

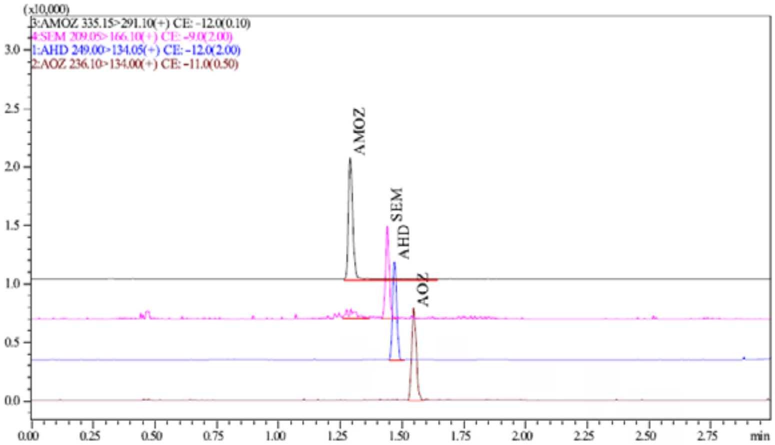

Figure 1 MRM chromatogram of 0.5 ng/mL standard sample

Calibration curve and linearity

The mixed standard working solutions at concentrations of 0.050, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10.0, and 20 ng/mL were prepared and determined according to the analytical conditions described previously. A calibration curve was created using the external standard method and shown in Figure 2. The linearity of the method was good in the range of 0.005-20 ng/mL. The linear equations, the corresponding linear range and correlation coefficient are shown in Table 3.

Limit of detection and limit of quantitation

For the injection and analysis of the mixed standard solution of concentration 0.05 ng/mL, the results for the limit of detection (S/N=3, LOD) and the limit of quantitation (S/N=10, LOQ) are shown in Table 4.

Precision experiment

The mixed standard solutions at two concentrations were consecutively injected for 6 times to test the precision. The repeatability results of retention time and peak area are shown in Table 5. The relative standard deviations of the retention time and peak area for standards at two concentrations were 0.09-0.18% and 0.85-1.99%, respectively, indicating good precision.

Table 3 Parameters of the calibration curve

Number	Analyte	Calibration curve	Linear range (ng/mL)	Accuracy (%)	Correlation coefficient (r)
1	SEM	Y = (8892.23)X + (-316.210)	0.050-20	93.2-111.6	0.9970
2	AOZ	Y = (42020.9)X + (65.5028)	0.050-20	94.0-108.4	0.9986
3	AHD	Y = (10603.3)X + (-260.028)	0.050-20	95.5-102.1	0.9996
4	AMOZ	Y = (242918)X + (-322.397)	0.050-20	94.5-103.8	0.9996

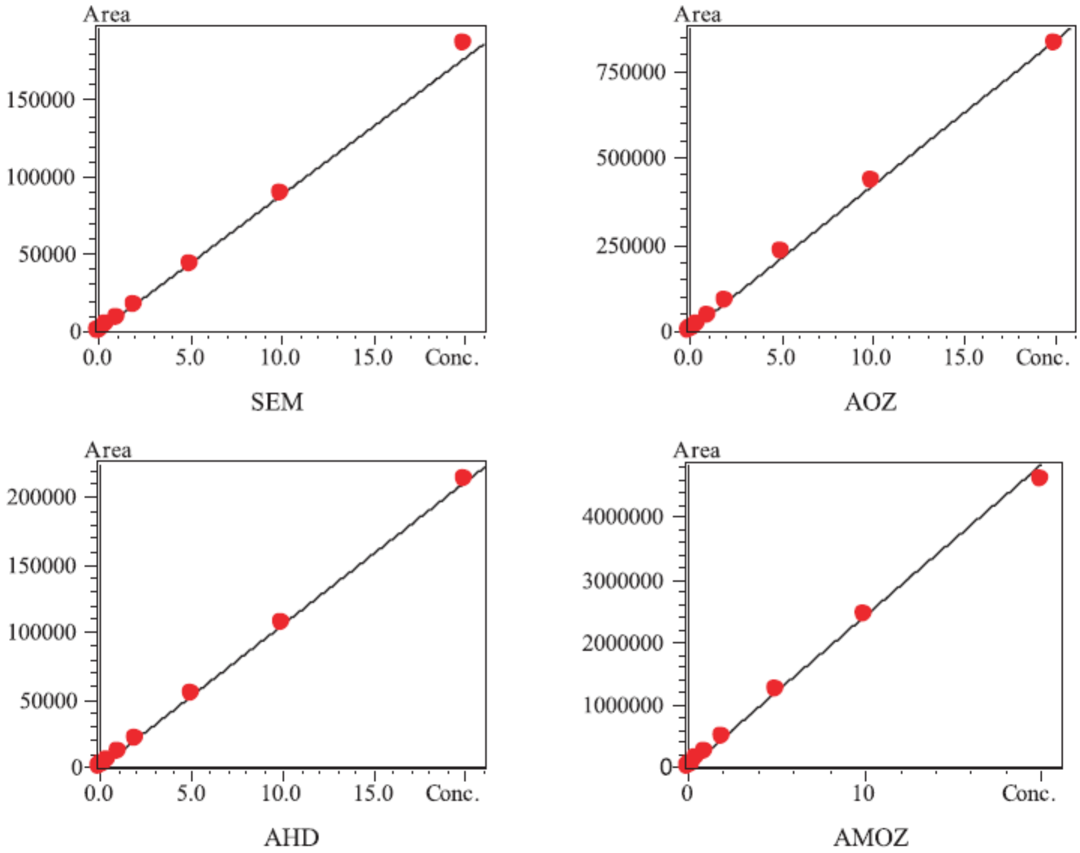

Figure 2 Standard calibration cure

Table 4 Limit of detection and limit of quantification

No.	Analyte	S/N ratio	Limit of detection (ng/mL)	Limit of quantification (ng/mL)	Correlation coefficient (r)
1	SEM	10	0.015	0.05	0.9970
2	AOZ	156	0.00096	0.0032	0.9986
3	AHD	150	0.0010	0.0033	0.9996
4	AMOZ	633	0.00024	0.00079	0.9996

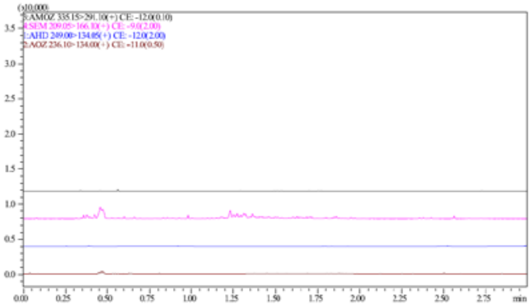

Figure 3 Chromatogram of blank matrix

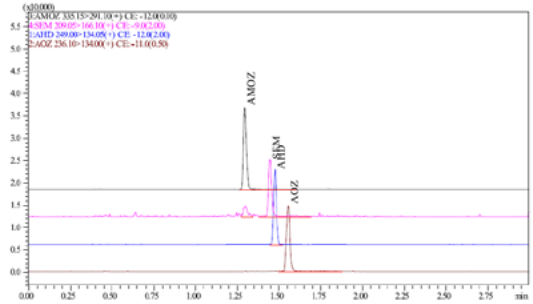

Figure 4 Chromatogram of matrix spike samples (1.0 ng/mL)

Table 5 Repeatability results of retention time and peak area (n=6)

Analyte	RSD% (0.5 ng/mL)		RSD% (2 ng/mL)	
	R.T.	Area	R.T.	Area
SEM	0.14	1.99	0.09	1.86
AOZ	0.13	1.94	0.12	1.65
AHD	0.18	1.97	0.14	1.61
AMOZ	0.18	1.97	0.09	0.85

Recovery tests

Blank fish sample was extracted according to the sample preparation method. The mixed standard solution was added with a spiked content of 1.0 μg/kg to give a final injection concentration of 1.0ng/mL. The chromatogram of blank matrix was shown in Figure 3 and the chromatogram of matrix spike samples was shown in Figure 4.

CONCLUSION

The nitrofuran metabolites in the fish matrix were determined using a Shimadzu UHPLC LC-30A in conjunction with a triple quadrupole LCMS-8045. In reference to "GB/T 21311-2007: Determination of residues of nitrofuran metabolites in foodstuffs of animal origin HPLC-MS/MS method", the measurement of AOZ, AMOZ, AHD and SEM showed good linearity in the range of 0.05-20 ng/mL, and their correlation coefficients were all greater than 0.9970. This method is fast and simple, with high sensitivity and selectivity, which meets the current requirements for the determination of nitrofuran metabolite residues in foodstuffs of animal origin.

Determination of Avermectin Drug Residues in Vinegar Using LCMS-8045

Song Lun
Shimadzu (China), Shanghai Analysis Center

Application News
SSL-CA14-351

Abstract

This application news describes a method developed for determination of avermectin drug residues in vinegar using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. The analysis was completed within 6 minutes and the external standard quantification showed good linearity with a correlation coefficient above 0.997. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.02 to 0.09% and 0.66 to 4.57%, respectively, showing good precision. The limit of detection and limit of quantitation of four avermectin drugs ranged from 0.22 to 0.25 ng/mL and 0.75 to 0.83 ng/mL respectively.

The main varieties of avermectin drugs include avermectin, eprinomectin, doramectin, and ivermectin. Due to their excellent insect repellent properties, these drugs are the widely used as anti-parasitic drugs. Although avermectin drugs are pesticides derived from microorganisms, their LD_{50} in rats is 10 mg/kg, which is similar to that of thiophos pesticides. Therefore, the World Health Organization lists avermectin drugs as highly toxic compounds. The main methods currently used for detection of avermectin drugs include liquid chromatography-ultraviolet detection, liquid chromatography-fluorescence detection, and enzyme-linked immunosorbent assay (ELISA).

In recent years, many reports have used high performance liquid chromatography-tandem mass pectrometry assays in detection of avermectin drug residues. Both ESI and APCI ion sources can be used for LC-MS/MS analysis of avermectin drugs. The limit of detection of four avermectin drugs, avermectin, eprinomectin, doramectin, and ivermectin, specified in China's national standard GB/T 21320-2008 "Determination of Avermectin Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry" is 1.5 μg/kg with an ESI source. In the People's Republic of China's entry and exit inspection and quarantine industry standard, SN/T 1973-2007 "Detection of Avermectin Residues in Import and Export Food Using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry", the limit of detection of avermectin is 5 μg/kg with an APCI source. When the positive ion scanning mode of ESI is used for analysis, precursor ions of avermectin drugs are more likely to be detected in the form of $[M+Na]^+$ ions. However, if the sodium content in extraction matrix is low or if only a trace amount of sodium ions is present in the extraction liquid due to the selected extraction method, a poor linear relationship tends to be observed when the detection is carried out in the form of $[M+Na]^+$ ions. For this reason, some literature has suggested using the negative ion mode of APCI to detect the precursor ion $[M-H]^-$ to acquire a better linear relationship. Shimadzu China has already published an application report (report No.: AP_News_LCMSMS-050) for LC-MS/MS analysis of avermectin drugs using the ESI source.

In this article, in reference to SN/T 1973-2007 and GB/T 21320-2008, Shimadzu's UHPLC LC-30A coupled with the Triple Quadrupole Mass Spectrometer LCMS-8045 and an APCI source was used to establish a highly sensitive and rapid method for detection of avermectin drug residues in vinegar. The results were superior to the requirements of the above standards and can be used as a reference method by relevant personnel.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A and triple quadrupole mass spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A$_5$ online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatography workstation.

Analytical Conditions

Liquid Chromatography (LC) Conditions

Column	:	Shim-pack XR-ODS (3.0 mm I.D.×75 mm L., 2.2 µm)
Mobile phase	:	Solvent A - 5 mM ammonium acetate in water Solvent B - methanol
Flow rate	:	1.20 mL/min
Column Temp.	:	40 °C
Injection volume	:	20 µL
Elution method	:	Gradient elution with initial concentration of mobile phase B at 75%. Refer to Table 1 for time program.

Table 1 Time program

Time (min)	Module	Command	Value (%)
3.00	Pumps	Pump B Conc.	100
4.40	Pumps	Pump B Conc.	100
4.50	Pumps	Pump B Conc.	75
6.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Analytical Instrument	:	LCMS-8045
Ionization mode	:	APCI(-)
Ionization Voltage	:	-4.5 kV
Nebulizer gas	:	Nitrogen 3.0 L/min
Drying gas	:	Nitrogen 6.0 L/min
Collision gas	:	Argon
Probe temp.	:	350 °C
DL temp.	:	100 °C
Block Heater temp.	:	200 °C
Mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	40 ms
Pause time	:	3 ms
MRM transitions	:	Refer to Table 2

Standard Solution Preparation

Mixed standard stock solution at a concentration of 1 mg/mL was prepared using acetonitrile and was subsequently diluted with acetonitrile to make a series of standard working solutions at concentrations of 1, 2, 5, 10, 20, 50, 100, 200, and 400 ng/mL.

Sample Preparation Method

Sample was prepared in accordance to the industry standard SN/T 1973-2007 "Detection of Avermectin Residues in Import and Export Food Using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry".

Table 2 MRM transition

No.	Analyte	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Avermectin	871.35	565.25*	32.0	21.0	18.0
			229.20	46.0	40.0	50.0
2	Eprinomectin	912.35	565.35*	20.0	29.0	18.0
			270.20	46.0	42.0	50.0
3	Doramectin	897.35	591.35*	16.0	28.0	18.0
			229.20	44.0	43.0	46.0
4	Ivermectin	873.35	567.30*	16.0	28.0	10.0
			229.15	44.0	40.0	50.0

RESULTS AND DISCUSSION

Q1 MS scan and Product Ion Scan of Standard Sample

The Q1 MS scan and product ion scan of the avermectin drugs are shown in Figures 1-4.

MRM Chromatogram of Standard Mixture

The MRM chromatograms of standard samples of the four avermectin drugs are shown in Figure 5.

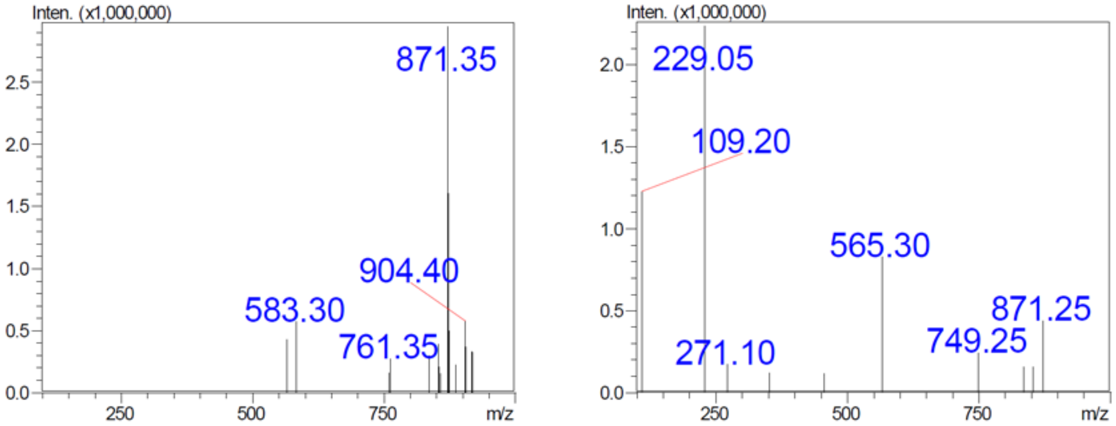

Figure 1 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of avermectin

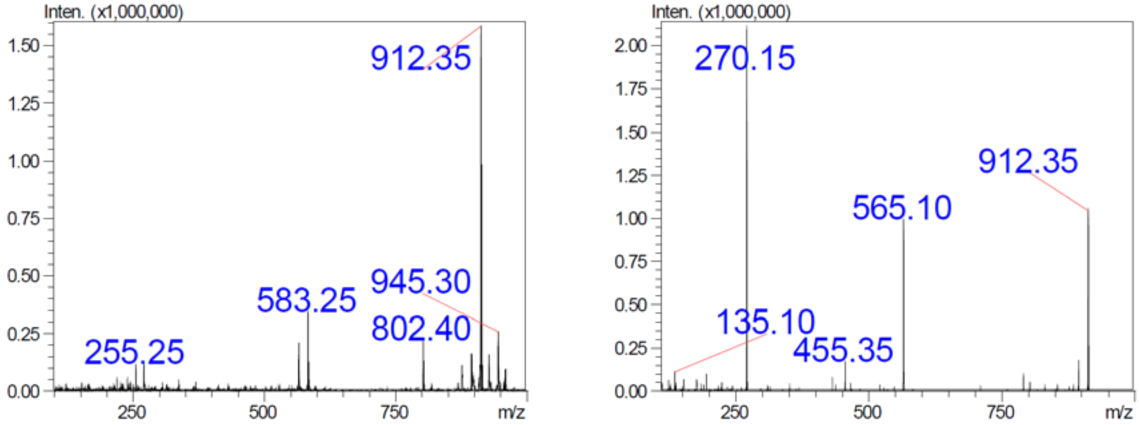

Figure 2 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of eprinomectin

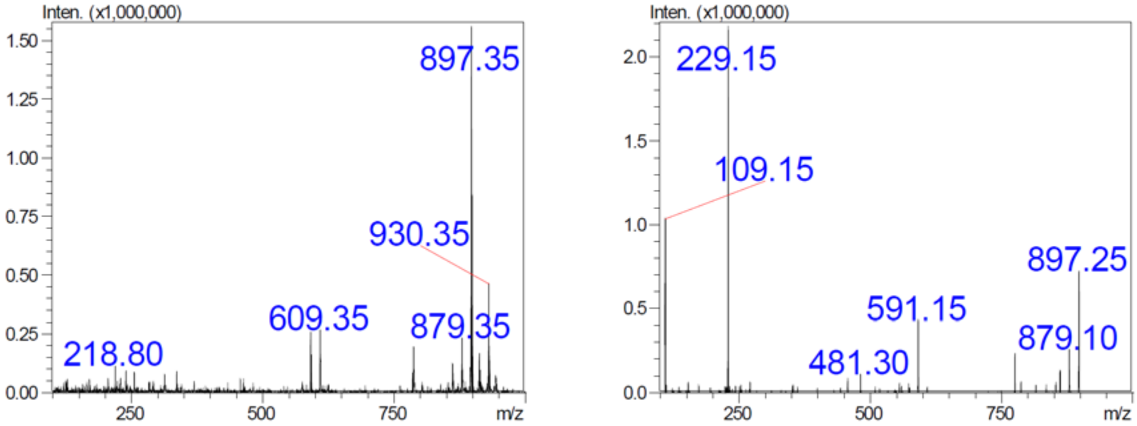

Figure 3 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of doramectin

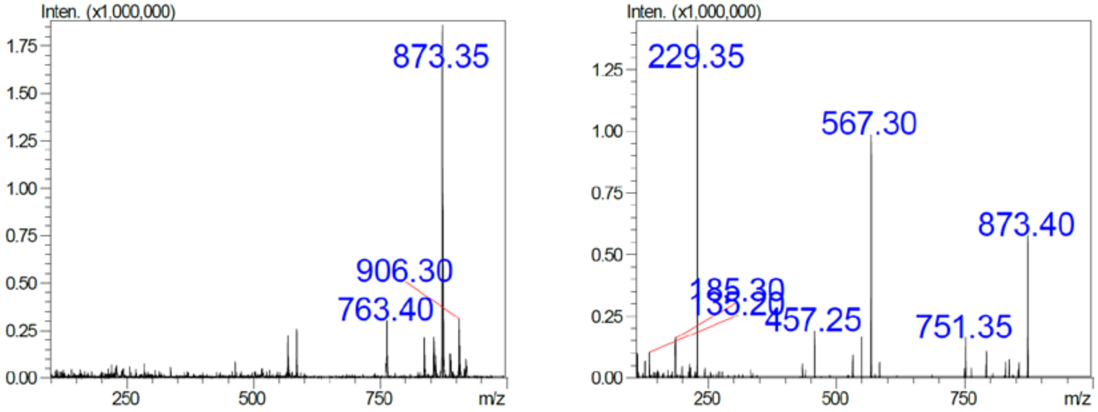

Figure 4 Q1 MS scan (left) and product ion scan (right, CE value is 40V) of ivermectin

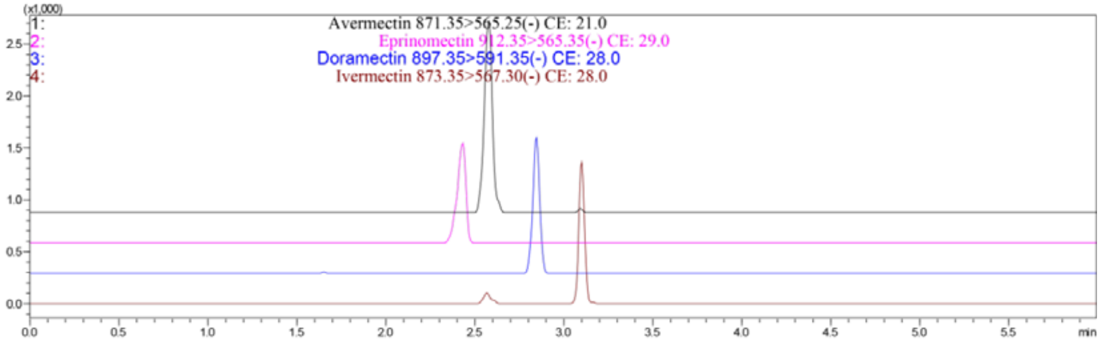

Figure 5 MRM chromatograms of 1 ng/mL standard samples of the four avermectin drugs

Calibration and Linear Range

A series of standard solutions at concentrations of 1, 2, 5, 10, 20, 50, 100, 200, and 400 ng/mL were injected and analyzed based on conditions listed in the previous section. Calibration curves were established by the external standard method and the results are shown in Figures 6-9. The four avermectin drugs showed good linearity within the range of 1 to 400 ng/mL. Linear equation, linear range, and correlation coefficients are shown in Table 3.

Limit of Detection and Limit of Quantitation

Sample solutions at a concentration of 1.0 ng/mL were injected and analyzed. A value equivalent to 3 times the noise was used as the lower limit of detection (i.e., S/N = 3, LOD), while the lower limit of quantitation was 10 times the noise (i.e., S/N=10, LOQ). Lower LOD and lower LOQ for the four avermectin drugs are shown in Table 4.

Table 4 Limit of detection and limit of quantitation

Analyte	Limit of Detection (ng/mL)	Limit of Quantification (ng/mL)
Avermectin	0.23	0.77
Eprinomectin	0.25	0.83
Doramectin	0.23	0.76
Ivermectin	0.22	0.77

Table 3 Parameters for calibration curves of 4 avermectin drugs (weight coefficient: $1/C^2$)

Compound	Calibration Curve	Linear Range (ng/mL)	Accuracy (%)	Correlation Coefficient (r)
Avermectin	Y = (6631.81) X + (-448.237)	1.00~400.00	0.9977	90.9~107.4
Eprinomectin	Y = (4314.94) X + (-2460.15)	1.00~400.00	0.9974	92.0~111.9
Doramectin	Y = (4740.90) X + (-850.905)	1.00~400.00	0.9986	91.5~105.4
Ivermectin	Y = (5160.71) X + (-1877.62)	1.00~400.00	0.9975	87.1~109.0

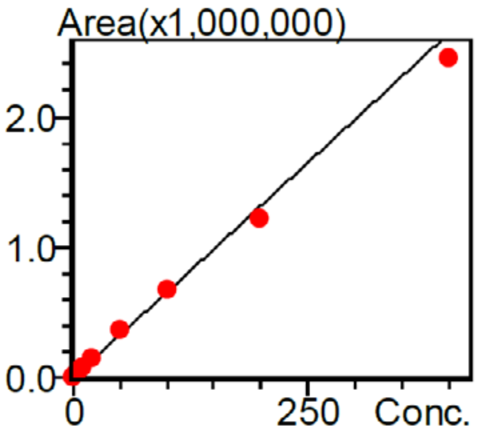

Figure 6 The standard calibration curve of avermectin

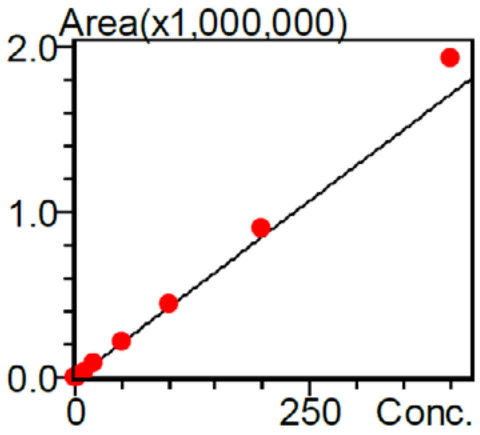

Figure 7 The standard calibration curve of eprinomectin

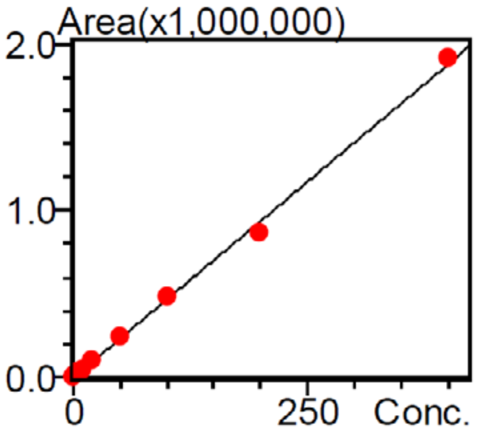

Figure 8 The standard calibration curve of doramectin

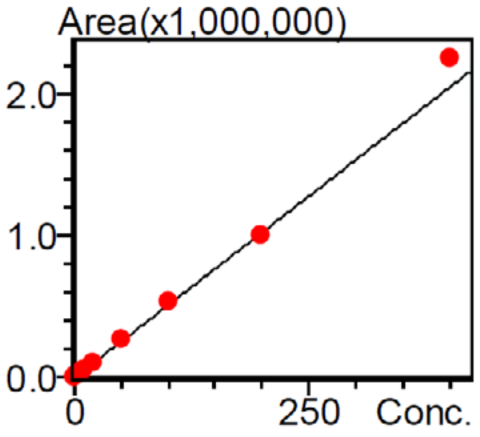

Figure 9 The standard calibration curve of ivermectin

Precision Test

After the mixed standard solutions at concentrations of 10 ng/mL, 100 ng/mL, and 200 ng/mL were analyzed by six consecutive injections, the relative standard deviations of retention time and peak area for the standards at three concentrations were 0.02 to 0.09% and 0.66 to 4.57%, respectively, indicating good precision.

Matrix Effects

Standard mixtures and spiked sample solution were prepared at low, medium and high concentrations. The matrix effects were assessed by comparing the peak area of the spiked sample solution with that of standard solution at the same concentration. If the obtained results of matrix effects are between 80% and 120%, the matrix effects showed minimal interference on detection of target substances. The experimental results are shown in Table 6. As seen from Table 6, the matrix effects showed minimal interference on detection of target substances.

Table 5 Repeatability results of retention time and peak area (n=6)

Analyte	RSD% (10 ng/mL)		RSD% (100 ng/mL)		RSD% (200 ng/mL)	
	R.T.	Area	R.T.	Area	R.T.	Area
Avermectin	0.03	2.79	0.02	3.18	0.02	0.66
Eprinomectin	0.09	4.57	0.02	1.31	0.05	1.30
Doramectin	0.05	3.76	0.02	2.68	0.02	1.30
Ivermectin	0.03	2.55	0.02	2.92	0.02	0.92

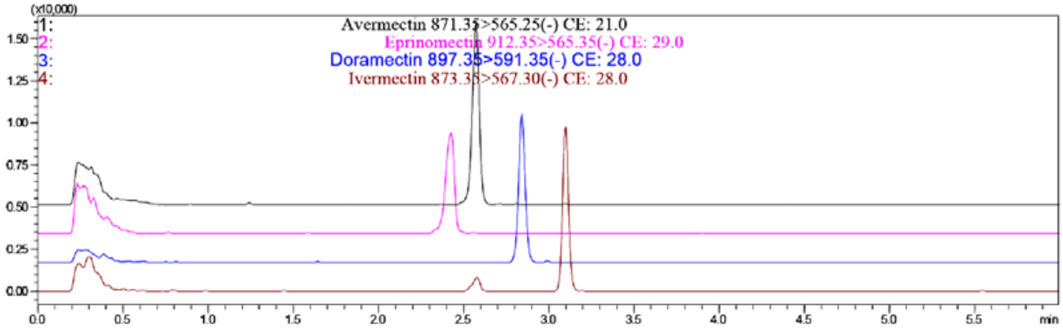

Figure 10 MRM chromatogram of blank vinegar matrix

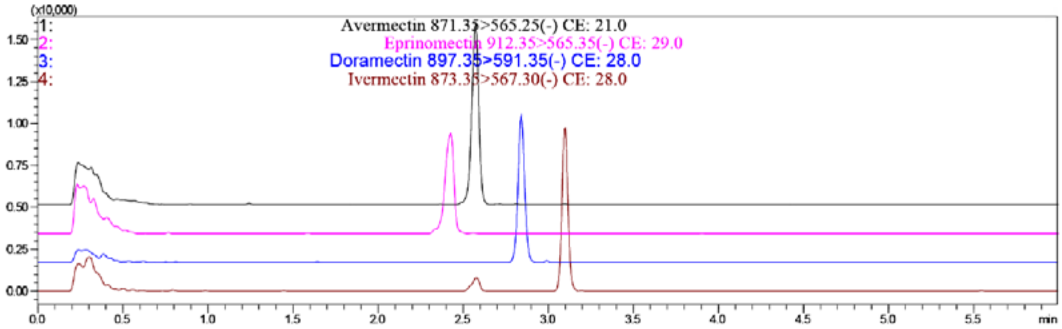

Figure 11 MRM chromatogram of vinegar matrix spike solution (5 ng/mL)

Table 6 Matrix effects

Spiking Concentration (ng/mL)	Avermectin (%)	Eprinomectin (%)	Doramectin (%)	Ivermectin (%)
10	103.8	105.7	117.1	109.7
100	84.5	91.7	101.1	90.6
200	94.7	82.0	118.4	100.4

Matrix Recovery Test

Mixed standards were first added to the blank vinegar samples. The pre-spiked samples were treated according to the method described previously, to give concentrations of pre-spiked samples of 10, 100, and 200 ng/mL.

For the preparation of the post-spiked samples, the blank vinegar sample matrix was first prepared according to the method described in the previous section. Mixed standards were then subsequently added into the blank vinegar matrix, so that the concentrations of post-spiked samples were 10, 100, and 200 ng/mL.

Both pre-spiked and post-spiked samples were injected and analyzed. The recovery was then calculated as the ratio between the peak area of pre-spiked samples and the peak area of post-spiked samples. The recovery rates obtained at various concentrations are shown in Table 7.

Table 7 Recovery rate

Theoretical Concentration of Sample (ng/mL)	Avermectin (%)	Eprinomectin (%)	Doramectin (%)	Ivermectin (%)
10	77.5	74.3	78.0	71.9
100	81.2	72.8	80.4	75.3
200	88.0	76.4	78.0	77.4

CONCLUSION

This application news demonstrates a method for determination of avermectin drug residues in vinegar using Shimadzu's UHPLC LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. When quantified by the external standard method, the calibration curves of the method showed adequate linearity with correlation coefficients all above 0.997. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.02 to 0.09% and 0.66 to 4.57% respectively, showing good precision. The limit of detection and limit of quantitation ranged from 0.22 to 0.25 ng/mL and 0.75 to 0.83 ng/mL respectively, thus complying with the current requirements for detection of avermectin drug residues in food. This method can provide a reference for relevant personnel in carrying out the detection of avermectin drug residues in food.

SHIMADZU
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Determination of Sulfonamide Residues in Pork Using LCMS-8045

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Application News
SSL-CA14-359

Abstract

This application news demonstrates a method for determination of sulfonamide residues in pork using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A together with the triple quadrupole mass spectrometer LCMS-8045. The linearity of the 11 sulfonamides was excellent and their correlation coefficients were all greater than 0.999. The limit of detection was 0.002 to 0.026 μg/L, and its limit of quantitation was 0.006 to 0.080 μg/L. The matrix spike recovery rate was between 86.6 and 119.8%. As this method meets the requirements in terms of lower limit of detection of 0.5 μg/kg as specified in the Department of Agriculture's announcement No. 1025-23-2008, it can be used to quickly and accurately determine sulfonamide residues in pork.

Sulfonamides (SAs) refer to synthetic antibiotics with sulfanilamide structures and it can be used to suppress most gram-positive bacteria and some gram-negative bacteria. When used in combination with antibacterial synergists, such as trimethoprim, SAs can enhance the antibacterial effect and expand the scope of treatment. Due to their advantages of having wide antibacterial spectra, strong curative effect and low cost, SAs are widely used in the prevention and treatment of diseases. However, one of its main drawbacks is that it can easily bring about side effects such as allergies and hematopoietic disorders, thereby causing a gradual reduction in clinical applications and usage. Instead, SAs are widely used in livestock breeding and aquaculture. Most of these drugs cannot be fully metabolized in animals and these SA residues can enter human body through the food chain, thus causing harm to human health. At present, the European Union, the United States, and Japan all list SAs as drugs with a restricted use in animal husbandry, with the maximum amount of SA residues generally limited to 50–100 μg/kg. China has also established relevant standards for detection of SA residues in animal-derived food, such as GB 29694-2013 "Determination of 13 Types of SA Residues in Animal-Derived Food Using High Performance Liquid Chromatography", GB 21316-2007 "Determination of SA Residues in Animal-Derived Food Using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry", and SN/T 4057-2014 "Determination of SA Residues in Animal-Derived Food for Export Using Immunoaffinity Column Chromatography-HPLC and LC-MS/MS Method".

In reference to the sample preparation method No. 1025-23-2008 listed by the Department of Agriculture "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry", this application news demonstrates the use of Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A together with the triple quadrupole mass spectrometer LCMS-8045 to determine SA residues in pork.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

Analytical Conditions

Liquid Chromatography (LC) Conditions

Column : Shim-pack XR-ODS III (2.0 mm I.D.×50 mm L., 1.6 µm)
Mobile phase : Mobile phase A-0.1% formic acid in water, Mobile phase B-methanol
Flow rate : 0.3 mL/min
Column Temp. : 40 °C
Injection volume : 5 µL
Elution method : Gradient elution program with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for detailed gradient elution program.

Table 1 Time program

Time (min)	Module	Command	Value (%)
0.20	Pumps	Pump B Conc.	10
1.00	Pumps	Pump B Conc.	30
2.00	Pumps	Pump B Conc.	30
4.00	Pumps	Pump B Conc.	90
5.00	Pumps	Pump B Conc.	90
5.01	Pumps	Pump B Conc.	10
8.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Ion sources : ESI
Interface temperature : 300 °C
DL temperature : 250 °C
Mode : Multiple reaction monitoring (MRM)
Nebulizing gas flow rate : 3.0 L/min
Drying gas flow rate : 10.0 L/min
Heated gas flow rate : 10.0 L/min
MRM transition : Refer to Table 2

Sample Preparation

There were 11 SA substances in total, including sulfathiazole, sulfapyridine, sulfamethiazole, sulfamethazine/ sulfadimidine, sulfameter/ sulfamethoxydiazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfamethoxazole, sulfisoxazole, sulfamethoxine, and sulfaquinoxaline were prepared.

Preparation of standard solutions: mixed standard stock solutions at a concentration of 10 mg/L were prepared using acetonitrile. The mixed standard stock solutions were subsequently diluted with a methanol/water solution (V/V, 10:90) to obtain mixed standard working solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 µg/L.

Preparation of sample: samples were prepared and injected for analysis according to the sample extraction and clean-up method listed in the Department of Agriculture's No. 1025-23-2008 "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry".

RESULTS AND DISCUSSION

MRM Chromatograms of Standard Samples

MRM chromatograms of mixed standard samples are shown in Figure 1.

Calibration and Linearity

The mixed standard calibration solutions at concentrations of 0.1, 0.5, 1, 5, 10, and 50 µg/L were prepared and determined according to analytical conditions described previously. Linearity was good over the concentration range of 0.1-10 µg/L and 0.1-50 µg/L where the external standard method was used to generate the calibration curve. Linear equation, linear range, and coefficients of the determination are shown in Table 3.

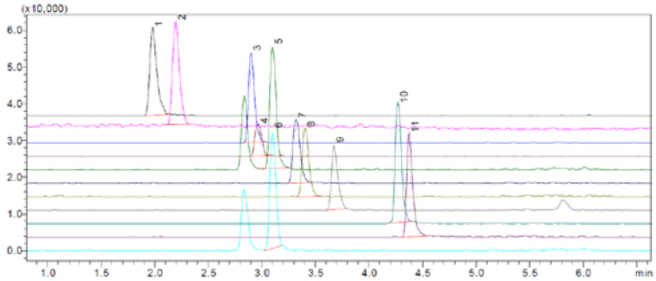

Figure 1 MRM chromatogram of mixed standard sample (0.5 μg/L)

(1. Sulfathiazole; 2. Sulfapyridine; 3. Sulfamethiazole; 4. Sulfamethazine/sulfadimidine; 5. Sulfameter/sulfamethoxydiazine; 6. Sulfamethoxypyridazine; 7. Sulfachloropyridazine; 8. Sulfamethoxazole; 9. Sulfisoxazole; 10. Sulfamethoxine; 11. Sulfaquinoxaline)

Table 2 MRM transition

No.	Analyte	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Sulfathiazole	256.10	156.05*	-28.0	-16.0	-29.0
			108.10	-18.0	-25.0	-21.0
2	Sulfapyridine	250.15	156.05*	-28.0	-17.0	-29.0
			184.00	-12.0	-17.0	-19.0
3	Sulfamethiazole	271.05	156.05*	-13.0	-14.0	-29.0
			108.10	-13.0	-27.0	-21.0
4	Sulfamethazine/ sulfadimidine	279.20	186.10*	-14.0	-18.0	-19.0
			156.00	-30.0	-20.0	-28.0
5	Sulfameter/ sulfamethoxydiazine	281.15	156.05*	-30.0	-18.0	-30.0
			108.15	-20.0	-28.0	-20.0
6	Sulfamethoxypyridazine	281.15	156.10*	-30.0	-18.0	-30.0
			108.10	-20.0	-27.0	-20.0
7	Sulfachloropyridazine	285.05	156.10*	-14.0	-14.0	-29.0
			108.00	-14.0	-27.0	-21.0
8	Sulfamethoxazole	254.10	156.05*	-29.0	-16.0	-30.0
			108.10	-12.0	-24.0	-21.0
9	Sulfisoxazole	268.10	156.10*	-29.0	-14.0	-28.0
			113.15	-13.0	-15.0	-22.0
10	Sulfamethoxine	311.15	156.10*	-15.0	-23.0	-30.0
			108.05	-15.0	-30.0	-22.0
11	Sulfaquinoxaline	301.15	156.10*	-15.0	-17.0	-29.0
				-15.0	-27.0	-19.0

Note: * indicates quantification ion

Table 3 Parameters for calibration curve (linear regression, the weight coefficient was 1/C)

No.	Compound	Calibration Curve	Linear Range (ng/mL)	Accuracy (%)	Correlation Coefficient (r)
1	Sulfathiazole	Y = (208213) X + (3090.87)	0.1~50	81.9~106.9	0.9994
2	Sulfapyridine	Y = (244596) X + (-547.080)	0.1~10	91.9~104.1	0.9998
3	Sulfamethiazole	Y = (183474) X + (-83.6554)	0.1~10	95.6~104.9	0.9999
4	Sulfamethazine/sulfadimidine	Y = (163835) X + (1324.90)	0.1~10	89.2~108.3	0.9997
5	Sulfameter/sulfamethoxydiazine	Y = (250149) X + (5313.05)	0.1~10	88.5~108.1	0.9997
6	Sulfamethoxypyridazine	Y = (242793) X + (4103.03)	0.1~10	95.2~103.3	0.9996
7	Sulfachloropyridazine	Y = (157837) X + (175.633)	0.1~10	95.0~104.5	0.9998
8	Sulfamethoxazole	Y = (154848) X + (4259.61)	0.1~50	82.0~109.0	0.9991
9	Sulfisoxazole	Y = (145057) X + (1448.37)	0.1~50	94.9~106.6	0.9999
10	Sulfamethoxine	Y = (245919) X + (11612.2)	0.1~50	97.3~103.3	0.9999
11	Sulfaquinoxaline	Y = (200233) X + (6393.09)	0.1~50	88.1~104.2	0.9997

Limit of Detection and Limit of Quantitation

Pork samples were treated according to the method specified in the previous section to obtain spiked samples at a concentration of 0.5 μg/L. After injection and analysis, the lower limit of detection (LOD, S/N=3) and the lower limit of quantitation (LOQ, S/N=10) for 11 SAs were calculated as shown in Table 4.

Precision Test

Mixed standard solutions at various concentrations were injected consecutively 6 times to determine precision. Repeatability results of retention time and peak area are shown in Table 5. The relative standard deviations of retention time and peak area were within ranges of 0.02 to 0.13% and 0.66 to 5.15%, respectively, indicating good precision.

Matrix Spike Samples Test

Pork samples were treated according to the method specified in the previous section to obtain a blank matrix, which was then used to prepare spiked samples at a concentration of 0.5 μg/L for injection and analysis. The spike recovery rate of samples ranged from 86.6 to 119.8%. The chromatograms of the blank matrix are shown in Figure 2, while the chromatograms of the spiked samples are shown in Figure 3.

Table 4 Limit of detection and limit of quantification for 11 SAs

No.	Compound Name	Limit of Detection (μg/L)	Limit of Quantitation (μg/L)
1	Sulfathiazole	0.002	0.006
2	Sulfapyridine	0.010	0.032
3	Sulfamethiazole	0.002	0.006
4	Sulfamethazine/sulfadimidine	0.006	0.017
5	Sulfameter/ sulfamethoxydiazine	0.025	0.076
6	Sulfamethoxypyridazine	0.026	0.080
7	Sulfachloropyridazine	0.003	0.011
8	Sulfamethoxazole	0.021	0.062
9	Sulfisoxazole	0.004	0.014
10	Sulfamethoxine	0.002	0.008
11	Sulfaquinoxaline	0.009	0.028

Table 5 Repeatability results of retention time and peak area (n=6)

No.	Compound Name	RSD% (1 μg/L)		RSD% (10 μg/L)	
		R.T.	Area	R.T.	Area
1	Sulfacetamide	0.13	2.62	0.07	0.92
2	Sulfathiazole	0.08	2.47	0.05	1.28
3	Sulfapyridine	0.13	2.39	0.05	1.00
4	Sulfamethiazole	0.03	1.86	0.04	0.83
5	Sulfamethazine/sulfadimidine	0.09	4.95	0.05	0.66
6	Sulfameter/sulfamethoxydiazine	0.05	1.33	0.03	1.56
7	Sulfamethoxypyridazine	0.04	5.15	0.03	1.72
8	Sulfachloropyridazine	0.07	2.11	0.03	1.04
9	Sulfamethoxazole	0.03	3.44	0.04	0.91
10	Sulfisoxazole	0.03	2.46	0.03	1.31
11	Sulfamethoxine	0.01	1.29	0.03	1.41
12	Sulfaquinoxaline	0.06	1.91	0.02	1.26

Figure 2 Chromatogram of blank matrix

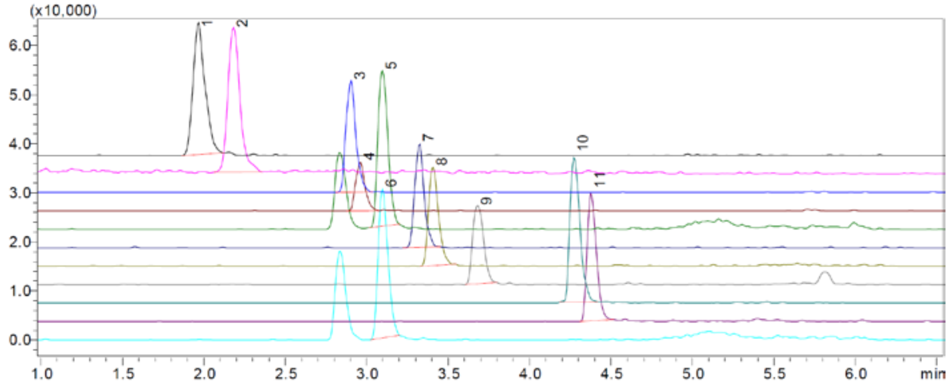

Figure 3 Chromatogram of spike recovery test

(1. Sulfathiazole; 2. Sulfapyridine; 3. Sulfamethiazole; 4. Sulfamethazine/sulfadimidine; 5. Sulfameter/sulfamethoxydiazine; 6. Sulfamethoxypyridazine; 7. Sulfachloropyridazine; 8. Sulfamethoxazole; 9. Sulfisoxazole; 10. Sulfamethoxine; 11. Sulfaquinoxaline)

Table 6 Results of spiked sample recovery

No.	Compound Name	Measured Concentration (μg/L)	Recovery (%)
1	Sulfathiazole	0.599	119.8
2	Sulfapyridine	0.553	110.6
3	Sulfamethiazole	0.509	101.8
4	Sulfamethazine/ sulfadimidine	0.468	93.6
5	Sulfameter/ sulfamethoxydiazine	0.494	98.8
6	Sulfamethoxypyridazine	0.508	101.6
7	Sulfachloropyridazine	0.479	95.8
8	Sulfamethoxazole	0.513	102.6
9	Sulfisoxazole	0.515	103.0
10	Sulfamethoxine	0.433	86.6
11	Sulfaquinoxaline	0.478	95.6

CONCLUSION

In the determination of SA residues in pork using Shimadzu's UHPLC LC-30A coupled with the triple quadrupole mass spectrometer LCMS-8045, the linearity of each of these 11 SAs was good, and their correlation coefficients were all greater than 0.999. The limit of detection was 0.002 to 0.026 μg/L, and its limit of quantification was 0.006 to 0.080 μg/L. The matrix spike recovery rate was between 86.6 and 119.8%. As this method meets the requirements of lower limit of detection of 0.5 μg/kg as specified in the Department of Agriculture's No. 1025-23-2008 "Detection of SA Residues in Animal-Derived Food Using Liquid Chromatography-Tandem Mass Spectrometry", it can be used to detect SA residues in pork.

Determination of 5 Kinds of Estrogens in Milk using LCMS-8045

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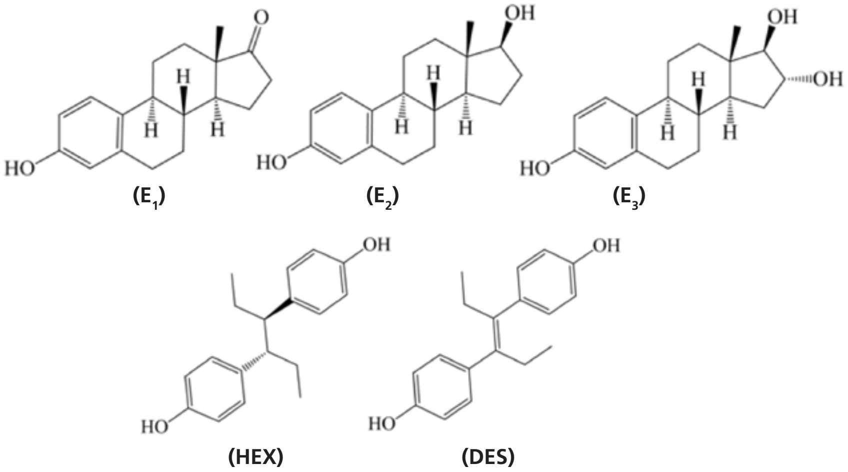
Application News
SSL-CA14-360

Abstract

A method for determination of three kinds of natural estrogen (estrone, 17β-estradiol, and estriol) and two kinds of synthetic estrogen (hexestrol and diethylstilbestrol) in milk was developed using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A coupled with the triple quadrupole mass spectrometer LCMS-8045. Proteins in milk samples were precipitated with acetonitrile and extracted by sonication. Without any further pretreatment and derivatization, the samples were analyzed by the ESI -MS/MS in negative ion mode. The samples were quantified by an external standard method. The linearity of the calibration curve was good and all linear correlation coefficients were at or above 0.9993. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.05 to 0.12% and 0.42 to 8.26%, respectively. The method's limit of quantitation was 0.006 to 0.033 ng/mL. This method, characterized by simple sample preparation, high sensitivity and good repeatability, can be used for determination of estrogen content in milk products.

Estrogen is a type of steroid hormones and is widely used in dairy farming to increase milk yield in dairy cows. While the use of estrogen can increase economic efficiency, it may also give rise to estrogen residues in milk. In recent years, a large number of studies have demonstrated that estrogen can enter the human body through the food chain and may induce cancers of the breast, uterus, testis, bone, kidney, and other tissues. Therefore, the analysis and detection of estrogen in milk are of practical significance.

In this application news, esterone (E1), 17β-estradiol (E2), estriol (E3), hexestrol (HEX) and diethylstilbestrol (DES) were used as target substances to establish a method for detection of estrogen content in milk using Shimadzu LCMS-8045. First, proteins in milk samples were precipitated with acetonitrile and extracted by sonication. Subsequently, without further pretreatment and derivatization, the samples were ionized by ESI negative ion mode and data was analyzed and collected under MRM mode. The samples were then quantitated by the external standard method. This method, characterized by simple sample preparation, high sensitivity and good repeatability, can be used for determination of estrogen content in milk products.



(E_1) (E_2) (E_3)

(HEX) **(DES)**

Figure 1 Structural formulas of five estrogen molecules

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A5 online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatography workstation.

Analytical Conditions

LC Chromatography (LC) Conditions

Column	:	Shim-pack GISS Column (2.1 mm I.D.×100 mm L., 1.9 µm C18)
Mobile phase	:	Mobile phase A - 0.03% ammonia in water Mobile phase B-acetonitrile
Flow rate	:	0.4 mL/min
Column Temp.	:	40 °C
Injection volume	:	5 µL
Elution method	:	Gradient elution with initial concentration of mobile phase B at 20%. Refer to Table 1 for detailed elution program.

Table 1 Time program

Time (min)	Module	Command	Value (%)
0.30	Pumps	Pump B Conc.	20
0.50	Pumps	Pump B Conc.	50
3.50	Pumps	Pump B Conc.	50
3.70	Pumps	Pump B Conc.	95
4.50	Pumps	Pump B Conc.	95
4.60	Pumps	Pump B Conc.	20
7.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Ion source	:	ESI (-)
Heated air	:	Air 12.0 L/min
Nebulizing gas	:	Nitrogen 12.0 L/min
Drying gas	:	Nitrogen 8.0 L/min
Collision gas	:	Argon
Interface temperature	:	350 °C
DL temperature	:	150 °C
Block heater temperature	:	350 °C
Mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	100 ms
Pause time	:	3 ms
MRM transitions	:	Refer to Table 2

Standard Solution Preparation

Preparation of standard solution: mixed standard stock solution at a concentration of 100 mg/L was serially diluted with an aqueous solution of 50% methanol to obtain standard solutions at concentrations of 0.1, 0.2, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 ng/mL.

Sample Preparation Method

200 µL of milk sample were added to 800 µL of acetonitrile and vortexed for 1 min. Subsequently, the sample was subjected to 20 min of sonication extraction and centrifuged at 10,000 rpm for 3 min. Finally, the supernatant was collected for testing.

Table 2 MRM transition

No.	Compound	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Estriol (E3)	50-27-1	287.20	171.10*	20.0	40.0	27.0
				145.10	20.0	47.0	22.0
2	17 β-Estradiol	50-28-2	271.20	145.10*	13.0	44.0	21.0
				183.20	28.0	45.0	30.0
3	Estrone (E1)	53-16-7	269.20	145.10*	30.0	41.0	25.0
				143.00	13.0	66.0	12.0
4	Hexestrol (HEX)	84-16-2	269.20	119.05*	19.0	43.0	18.0
				134.10	19.0	16.0	11.0
5	Diethylstilbestrol (DES)	6898-97-1	267.20	251.10*	18.0	27.0	23.0
				237.10	18.0	31.0	22.0

Note: * indicates quantification ion

RESULTS AND DISCUSSION

Chromatogram of Standard Mixture

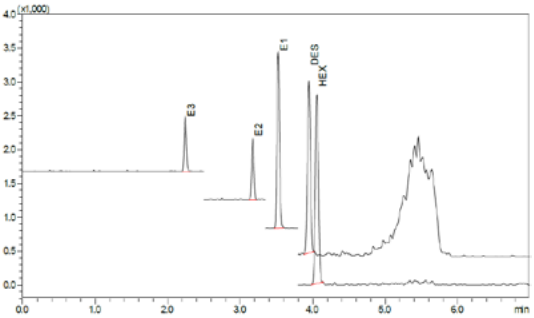


Figure 2 MRM chromatograms of standard mixture (0.2 ng/mL)

Calibration and Linearity

Standard calibration solutions of the four target compounds at concentrations of 0.1, 0.2, 0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 ng/mL were determined based on conditions listed in the experimental section. An example of the MRM chromatograms of a standard sample (0.2ng/mL) is shown in Figure 2. Calibration curves were established using the external standard method and shown in Figure 3. The linear equation, linear range, and correlation coefficients are tabulated in Table 3.

Precision Test

Mixed standard samples containing 0.2, 5.0, and 100 ng/mL of target substances were prepared and tested in 6 replicates to determine the repeatability. The retention time and peak area are shown in Table 4 and it demonstrates good repeatability.

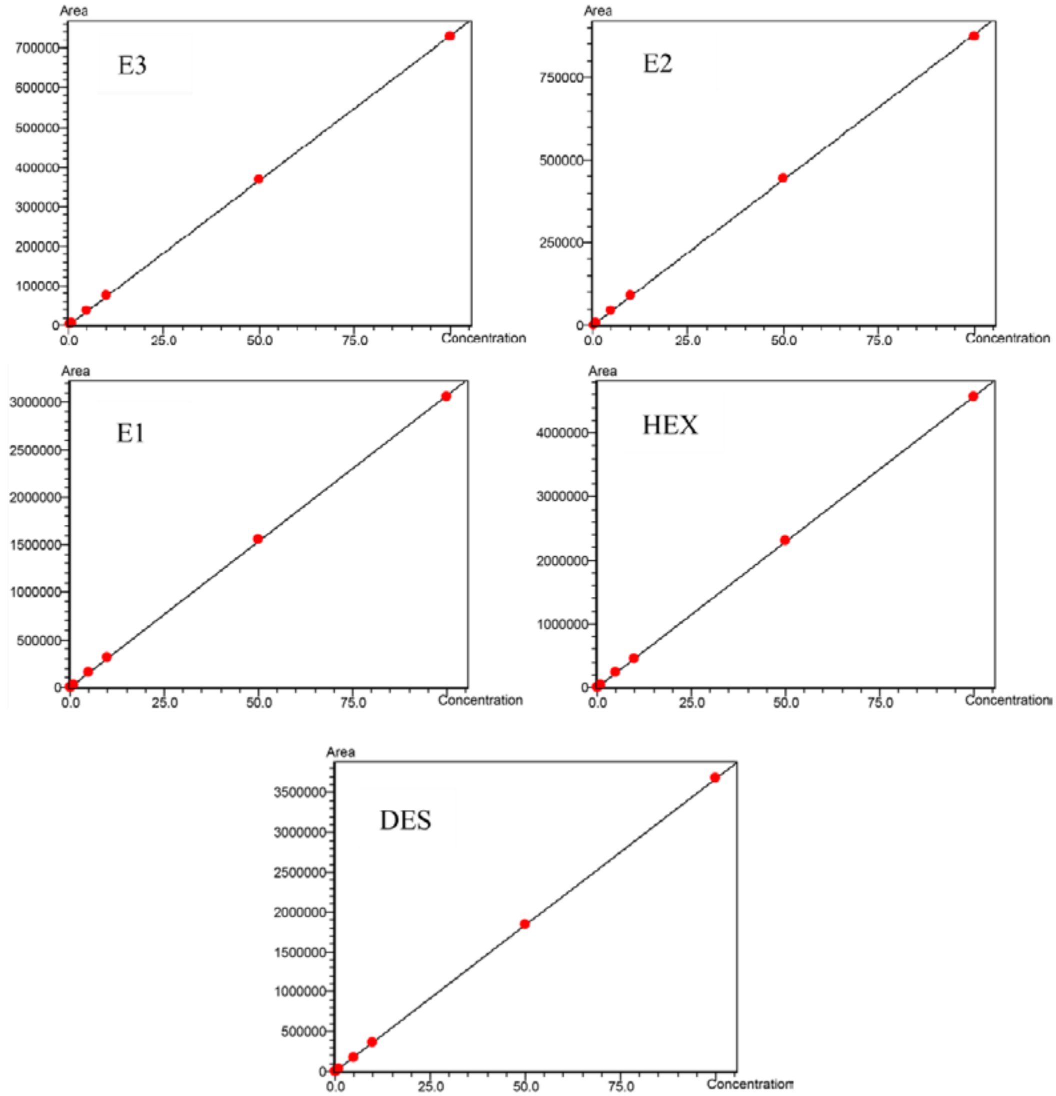


Figure 3 Calibration curve

Table 3 Parameters of calibration curve

Compound	Calibration Curve	Linear Range (ng/mL)	Correlation Coefficient (r)	Accuracy (%)
E3	Y = (7335.5) X +150.426	0.2-100	0.9999	96.01-103.4%
E2	Y = (8932.6) X -2.7160	0.2-100	0.9999	95.5-104.7%
E1	Y = (30778.2) X -18.0396	0.1-100	0.9998	94.7-104.9%
HEX	Y = (45714.2) X-376.316	0.1-100	0.9999	96.5-106.1%
DES	Y = (36057.1) X -196.210	0.1-100	0.9997	93.4-105.1%

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

Compound	RSD% (0.2 ng/mL)		RSD% (5.0 ng/mL)		RSD% (100 ng/mL)	
	R.T.	Area	R.T.	Area	R.T.	Area
E3	0.12	3.99	0.11	1.33	0.08	0.42
E2	0.12	5.34	0.09	1.48	0.06	0.54
E1	0.10	2.20	0.09	0.62	0.06	0.51
HEX	0.11	3.35	0.09	0.84	0.05	0.49
DES	0.08	8.26	0.09	1.62	0.05	0.39

Sensitivity Test

Standard solutions containing 0.20 ng/mL of target compounds were injected and analyzed. The limit of detection (ILOD, S/N=3) and limit of quantitation (ILOQ, S/N=10) for each target component were calculated based on the signal to noise ratios (S/N). The results are shown in Table 5.

Table 5 Limit of detection and limit of quantitation

Compound	Limit of Detection (ng/L)	Limit of Quantitation (ng/L)
E3	0.008	0.025
E2	0.007	0.021
E1	0.002	0.006
HEX	0.007	0.020
DES	0.010	0.033

Recovery Test

A certain brand of milk purchased from a supermarket was processed, injected and analyzed according to the method listed in the experimental section. The results showed that none of the five target compounds were detected, as shown in Figure 4. The five target compounds were added into the milk to determine the spike recovery. The results are shown in Table 6.

CONCLUSION

This application news describes a method for determination of three kinds of natural estrogen and two kinds of synthetic estrogen in milk using Shimadzu's ultra-high performance liquid chromatograph (UHPLC) LC-30A and triple quadrupole mass spectrometer LCMS-8045. Proteins in milk samples were precipitated with acetonitrile and extracted by sonication.

Table 6 Spike Recovery Test

Compound	Spiked Concentration (ng/mL)	Recovery (%)	Spiking Concentration (ng/mL)	Recovery (%)	Spiking Concentration (ng/mL)	Recovery (%)
E3	0.2	103.3	4.0	86.5	80	92.0
E2	0.2	87.8	4.0	103.3	80	106.0
E1	0.2	86.8	4.0	102.4	80	106.3
HEX	0.2	81.0	4.0	90.8	80	95.9
DES	0.2	83.3	4.0	102.9	80	109.4

Without any further pretreatment or derivatization, the samples were ionized by the ESI negative ion mode and the data was obtained under MRM mode. The samples were then quantified by an external standard method. The linearity of the calibration curve was good and all linear correlation coefficients were at or above 0.9993. Samples of low, medium and high concentrations were tested in 6 replicates. The relative standard deviations of retention time and peak area were 0.05 to 0.12% and 0.42 to 8.26%, respectively. The limit of quantitation was 0.006 to 0.033 ng/mL. This method, characterized by simple sample preparation, high sensitivity and good repeatability, can be used for determination of estrogen in milk products.

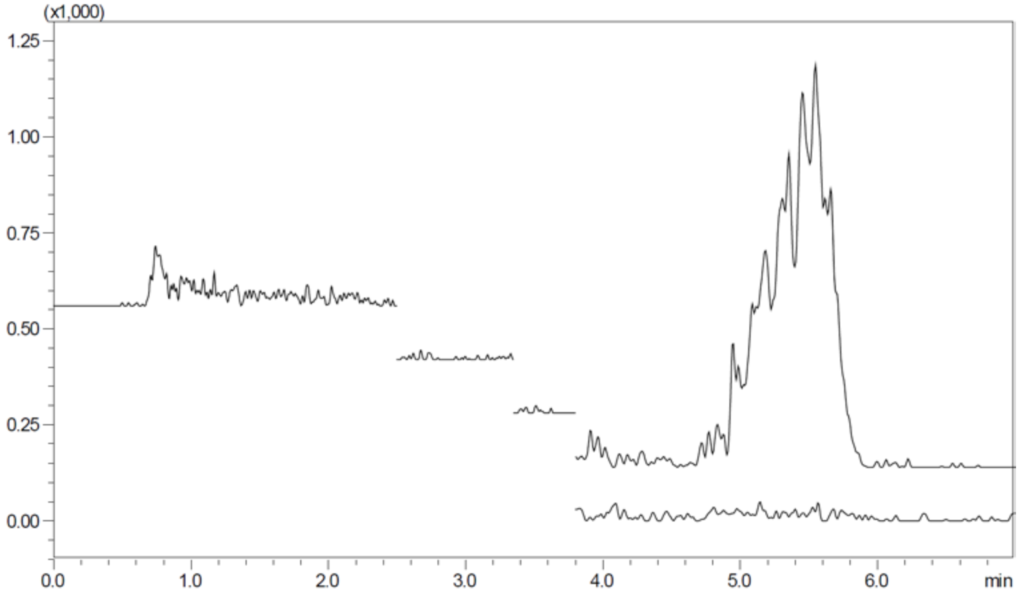


Figure 4 Chromatogram of milk sample found to not contain any targeted estrogen

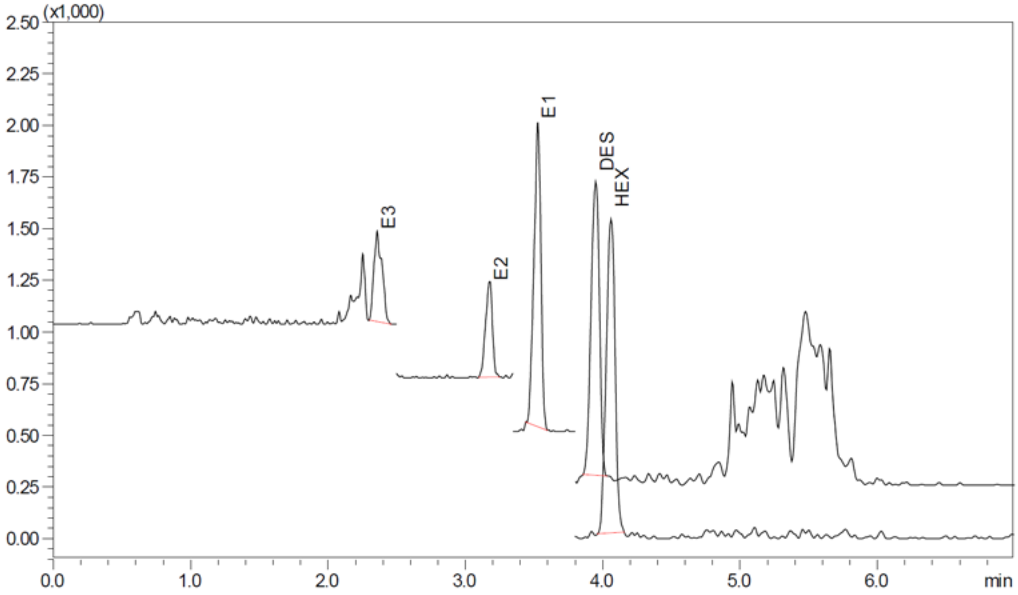


Figure 5 Chromatogram of blank sample matrix spiked with 0.2 ng/mL estrogens

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Determination of Quinolone Antibiotic Residues in Chicken by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

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Application News
SSL-CA14-397

Abstract

A method was developed for the determination of 12 quinolone antibiotics in chicken using Shimadzu's ultra-high performance liquid chromatograph LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. The analysis of 12 antibiotics was completed within 9 min and the correlation coefficients of the calibration curves were all above 0.997. The mixed standard solutions with various concentrations of antibiotics were tested in 6 replicates. The relative standard deviations of retention time and peak area of the 12 target compounds were 0.03 - 0.27% and 1.13 - 4.93%, respectively, and the precision was good. The range of matrix spike recovery was 91.90 - 108.60% at different concentrations. The method can be applied to the simultaneous detection of 12 quinolone antibiotic residues in chicken.

Quinolones (QNs) are synthetic drugs with broad-spectrum bactericidal effect. Due to their strong antibacterial activity and wide spectrum range, they are widely used in the prevention and treatment of various infectious diseases in human beings, poultry and livestock However, drug overdose or improper use will lead to a high level of QNs residues in animals, especially for food-producing animals. In addition to the immediate and direct toxic effects of QNs on human body, the long-term consumption of animal-derived food containing QNs can readily induce drug resistance, thus affecting the clinical efficacy of QNs on human body. Therefore, the issue of QN residues has raised more and more concerns. The U.S., Japan, E.U. and China have regulated the maximum residue limit of QNs in food and it varies according to the different classification, properties and characteristics of QNs and is in the range of 10-6000 μg/kg.

Chicken is a meat widely consumed in China, so the determination of QNs residues in chicken is of great significance. High performance liquid chromatography-tandem mass spectrometry is a rapid developing analytical technology in recent years. With its ability to perform highly sensitive and selective quantitative and qualitative analyses as well as provide high accuracy for antibiotic compounds in complex matrices, it is the preferred technique for ultra-trace residue analysis. A method was established in this application news for determination of 12 QNs antibiotics in chicken using a Shimadzu UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and triple quadrupole mass spectrometer LCMS-8045. The specific configurations are two LC-30AD pumps, DGU-20A5 online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

Analytical Conditions

Liquid Chromatography (LC) Conditions

Column	:	Shim-pack GISS (2.1 mm I.D.× 100 mm L, 1.9 μm)
Mobile phase	:	Phase A - 0.2% formic acid in water Phase B - acetonitrile/methanol (6:4)
Flow rate	:	0.40 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 μL
Elution method	:	Gradient elution with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for elution program.

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Environmental Analysis

Table 1 Time program

Time (min)	Module	Command	Value (%)
4.50	Pumps	Pump B Conc.	40
4.60	Pumps	Pump B Conc.	95
5.50	Pumps	Pump B Conc.	95
5.60	Pumps	Pump B Conc.	10
9.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Mass Spectrometer : LCMS-8045
Ion sources : ESI (+)
Heating gas : Air 10.0 L/min
Nebulizing gas : Nitrogen 3.0 L/min
Drying gas : Nitrogen 10.0 L/min
Collision gas : Argon
Interface temp. : 300 °C
DL temp. : 250 °C
Heater temp. : 400 °C
Mode : Multiple reaction monitoring (MRM)
Dwell time : 15 ms
Delay time : 3 ms
MRM parameters : Refer to Table 2

Table 2 MRM optimized parameters

No.	Analyte	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Pipemidic Acid	51940-44-4	304.1	286.1*	-16	-21	-20
				215.1	-30	-38	-24
2	Enoxacin	84294-96-2	321.1	303.1*	-13	-22	-11
				232.1	-13	-35	-16
3	Ofloxacin	82419-36-1	362.1	318.2*	-12	-21	-22
				261.1	-12	-29	-18
4	Norfloxacin	70458-96-7	320.0	302.1*	-11	-22	-21
				231.1	-11	-39	-24
5	Pefloxacin	149676-40-4	334.1	316.1*	-28	-23	-22
				290.1	-13	-18	-14
6	Ciprofloxacin	93107-08-5	332.0	314.1*	-11	-16	-24
				231.0	-11	-37	-25
7	Lomefloxacin	98079-52-8	352.1	265.1*	-16	-25	28
				308.1	-16	-17	-21
8	Danofloxacin	119478-55-6	358.1	340.1*	-14	-24	-12
				255.1	-14	-40	-17
9	Enrofloxacin	93106-60-6	360.1	316.2*	-12	-20	-11
				342.1	-12	-20	-11
10	Cinoxacin	28657-80-9	263.0	245.0*	-28	-15	-26
				217.0	-28	-23	-22
11	Oxolinic Acid	14698-29-4	262.1	244.0*	-17	-18	-26
				216.1	-30	-28	-23
12	Flumequine	42835-25-6	262.1	244.1*	-16	-16	-17
				202.0	-16	-36	-22

Note: * indicates quantification ion

Standard Solution Preparation

Quinolone standards were weighed and dissolved in methanol to prepare mixed standard stock solutions of 1.0mg/ml. The mixed standard solutions were stored at -18 °C. Accurate volumes of mixed standard stock solution were added to blank chicken extract solutions to prepare mixed standard working solutions with concentrations of 0.2, 0.5, 1, 5, 10, 20 and 50 ng/mL.

Sample Preparation Method

Chicken samples were prepared with reference to the national standard GB/T 21312-2007 "Analysis of 14 Quinolone in Food of Animal Origin by High Performance Liquid Chromatography Tandem Mass Spectrometry".

5.0g (accurate to 0.1g) of homogeneous chicken sample was weighed in a 50mL polypropylene centrifuge tube. 20mL of 0.1m/L EDTA-Mcllvaine buffer solution was added. The mixture was vortexed, ultrasonically extracted for 10 mins and centrifuged at 10,000 rpm for 5 mins. The extraction was repeated 3 times in total and the supernatant was combined.

SPE clean-up was performed using HLB SPE cartridges (200mg, 6mL). The SPE was first activated with 6mL of methanol and 6mL of water before use. 6mL of extracted supernatant was added to the SPE column and rinse with 2mL of 5% methanol solution. The filtrate was discarded and the SPE column drained. Elution of SPE was carried out with 6mL of methanol. The eluate was collected and completely dried with nitrogen. The dried extract was reconstituted with 1ml of mobile phase and vortexed. The reconstituted extracts were filtered through a 0.22µm filter membrane and injected into LC-MS/MS for analysis.

RESULTS AND DISCUSSION

MS Scan and Product Ion Scan of Standard Samples

The Q1 MS scan and product ion scan of the quinolone drugs are shown in Figures 1-12.

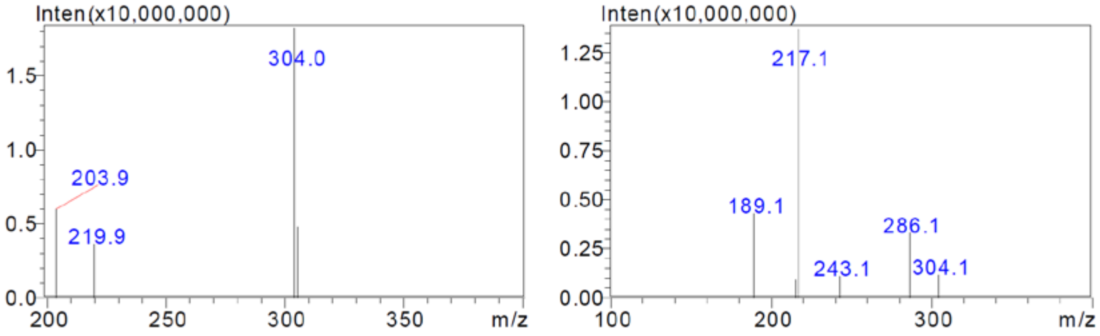

Figure 1 Q1 MS Scan (left) and product ion scan (CE value-20 V, right) of pipemidic acid

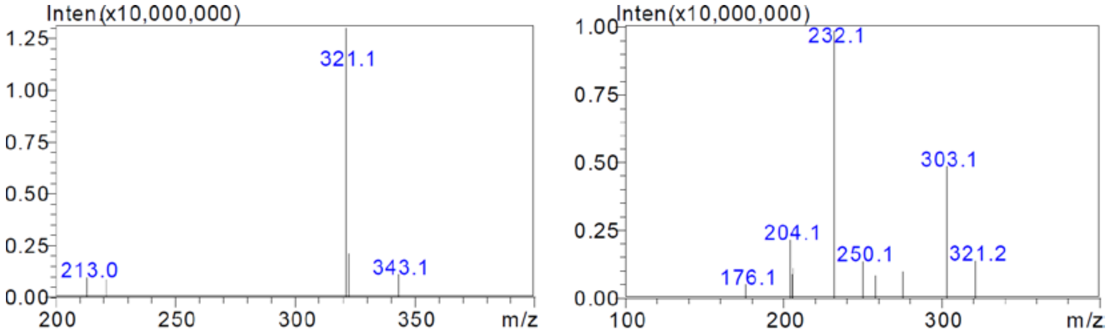

Figure 2 Q1 MS Scan (left) and product ion scan (CE value-30 V, right) of enoxacin

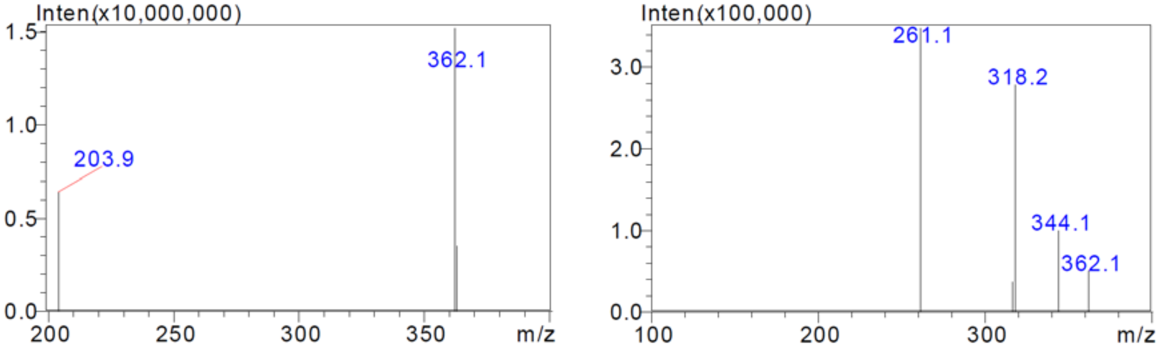

Figure 3 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of ofloxacin

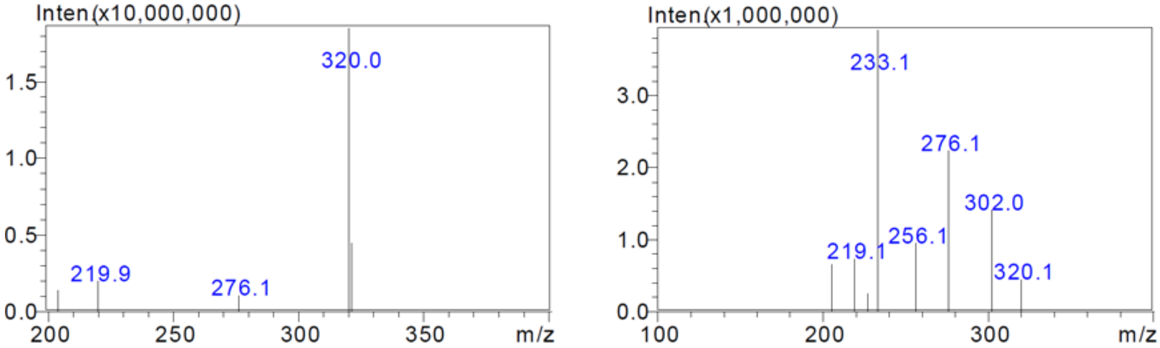

Figure 4 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of norfloxacin

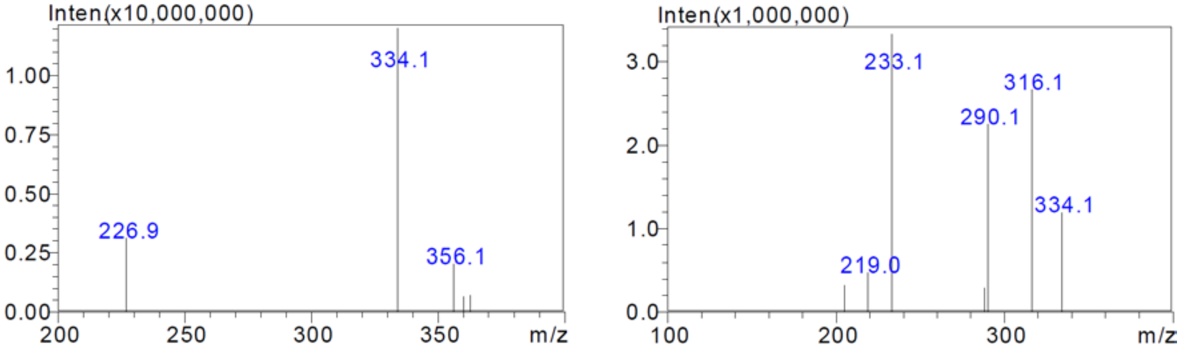

Figure 5 Q1 MS scan (left) and product ion scan(CE value-25 V, right) of pefloxacin

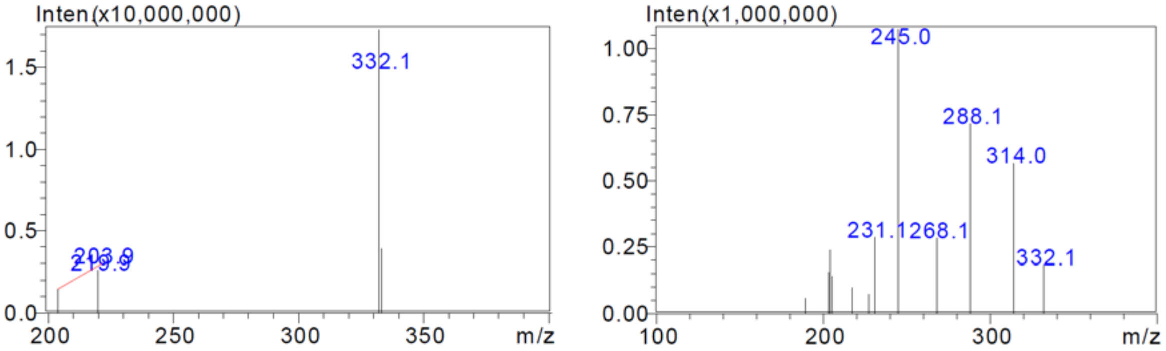

Figure 6 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of ciprofloxacin

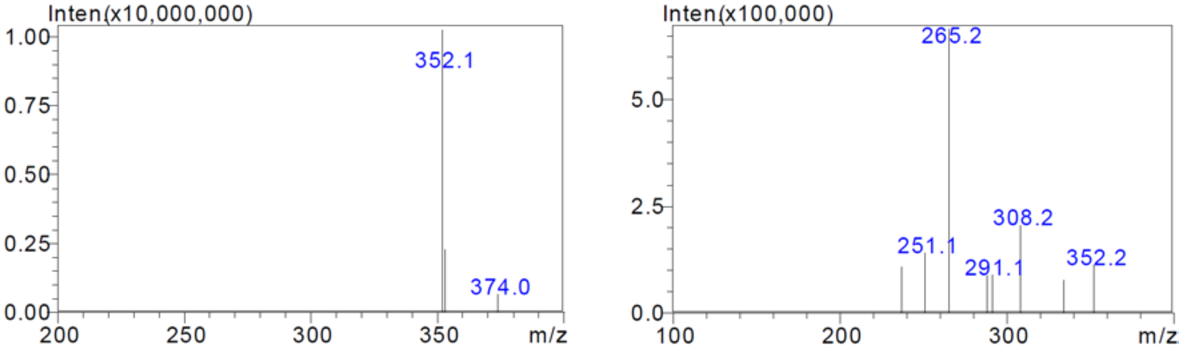

Figure 7 Q1 MS scan (left) and product ion scan CE value-25 V, right) of lomefloxacin

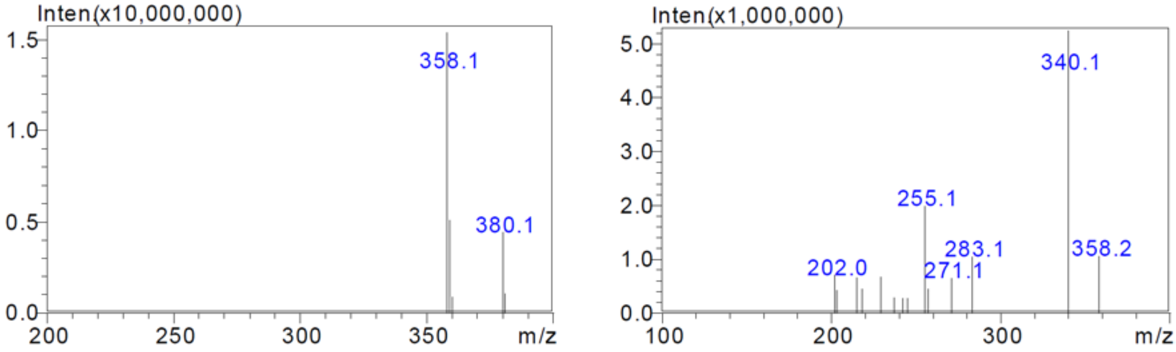

Figure 8 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of danofloxacin

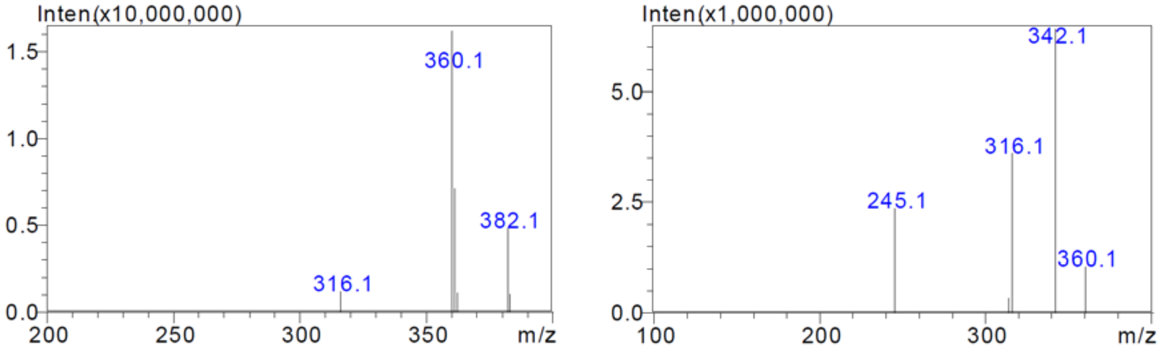

Figure 9 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of enrofloxacin

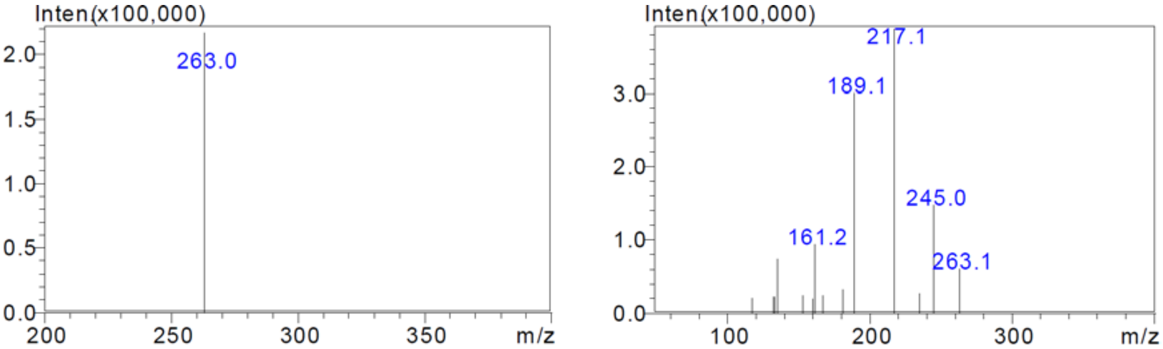

Figure 10 Q1 MS scan (left) and product ion scan (CE value-30 V, right) of cinoxacin

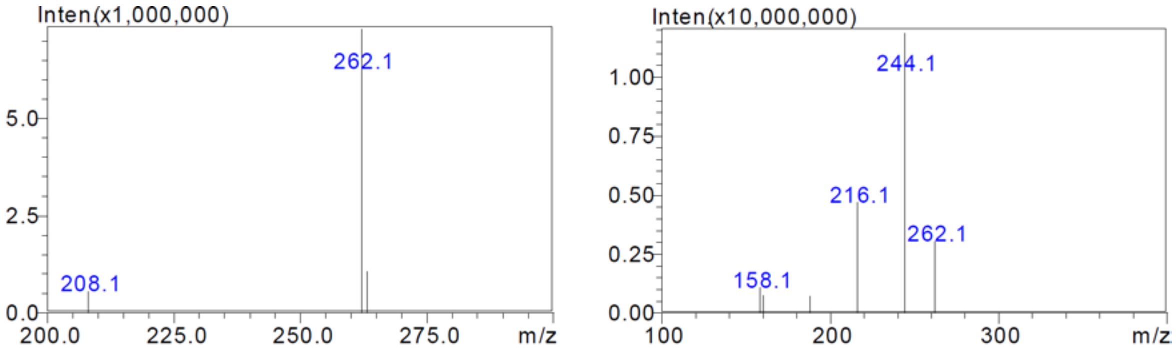

Figure 11 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of oxolinic acid

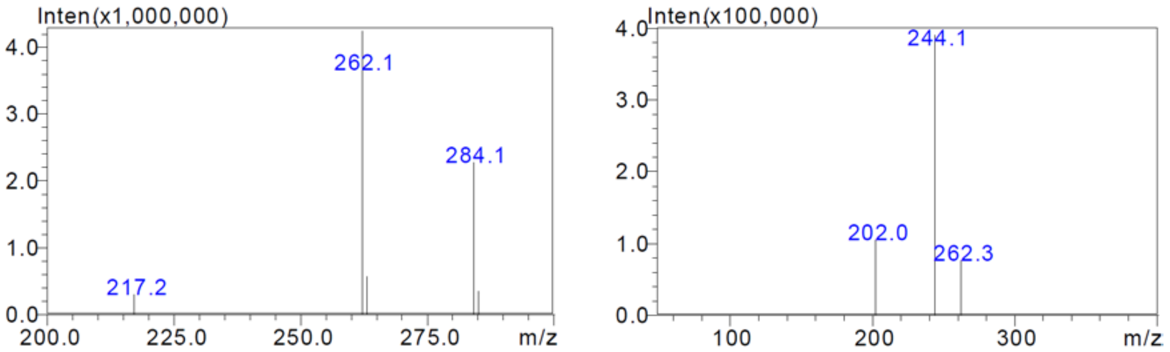

Figure 12 Q1 MS scan (left) and product ion scan (CE value-35 V, right) of flumequine

MRM chromatograms of 12 quinolone standard solutions (1 ng/mL)

The MRM chromatograms of the quinolone drugs are shown in Figure 13.

Calibration and linearity

About 60mL of combined liquid extract was collected after the extraction of 5.0g chicken sample with EDTA-McIlvaine buffer solution. The combined liquid extract went through similar SPE clean-up as described previously and was reconstituted to give 10mL of blank chicken extraction solution. The mixed standard calibration solutions of concentrations 0.2, 0.5, 1, 5, 10, 20 and 50 ng/mL were prepared by diluting appropriate amounts of the standard stock solution (1mg/mL) with the blank chicken extract solution. A calibration curve was plotted showing concentration of working solution against peak area (see Figure 13). The linearity was good and the linear equation and correlation coefficient are shown in Table 3.

Precision test

The retention times and peak areas of the mixed standard working solutions at different concentrations (6 replicates for each concentration) were determined to evaluate the precision. The repeatability results of retention time and peak area are shown in Table 4. The results indicate that the relative standard deviations of retention time and peak area of standard samples at different concentrations are 0.03 - 0.27 % and 1.13 - 4.93% respectively, showing good precision.

Sensitivity test

To determine the sensitivity, a low concentration (0.2ng/mL) mixed standard antibiotics solution was prepared and analyzed. The signal-to-noise (S/N) ratio, limit of detection (LOD) and limit of quantitation (LOQ) was determined with the use of RMS calculation method and LabSolutions Ver. 5.86. The S/N ratio, LOD and LOQ of the 12 compounds are shown in Table 5.

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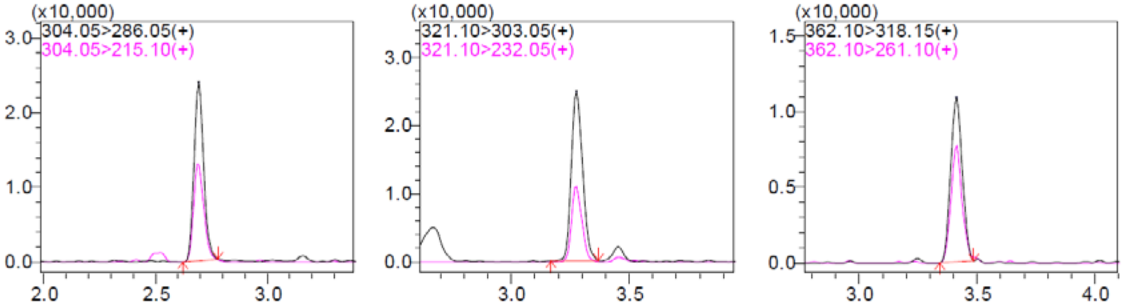
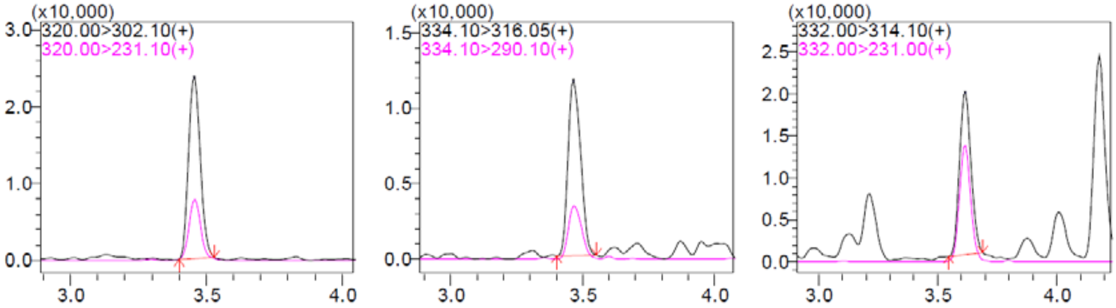
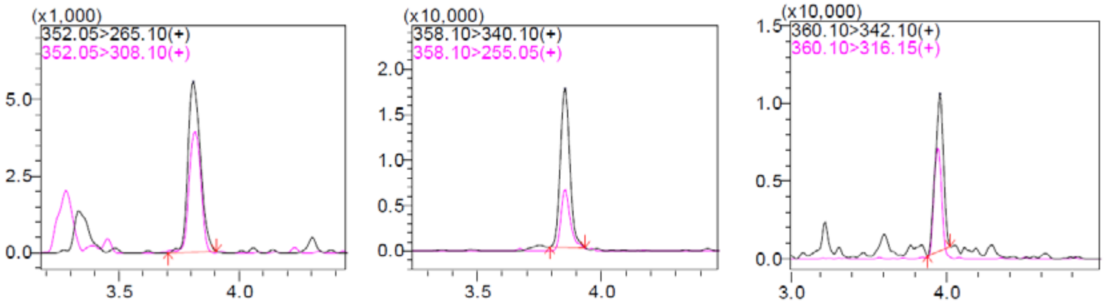


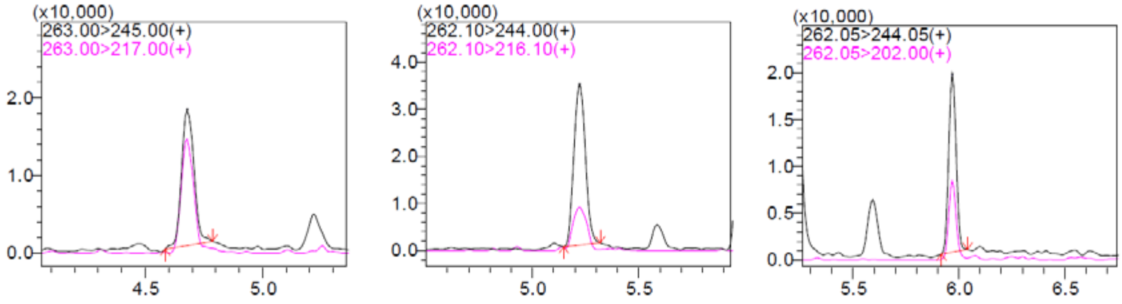
Pipemidic Acid Enoxacin Ofloxacin



Norfloxacin Pefloxacin Ciprofloxacin



Lomefloxacin Danofloxacin Enrofloxacin



Cinoxacin Oxolinic Acid Flumequine

Figure 13 Standard curves of 12 quinolones

Table 3 Calibration curve

	Analyte	Calibration Curve	Linear Range (ng/mL)	Correlation Coefficient (r)	Accuracy (%)
1	Pipemidic Acid	Y = (76616.3) X + (-2517.68)	0.2-50	0.9998	96.1-104.1
2	Enoxacin	Y = (75235.4) X + (832.686)	0.2-50	0.9996	87.9-109.4
3	Ofloxacin	Y = (30271.5) X + (-849.705)	0.2-50	0.9998	90.9-106.6
4	Norfloxacin	Y = (64434.1) X + (1680.13)	0.2-50	0.9988	85.7-112.4
5	Pefloxacin	Y = (30801.7) X + (579.667)	0.2-50	0.9990	89.5-109.8
6	Ciprofloxacin	Y = (58040.6) X + (4785.98)	0.2-50	0.9990	90.7-114.2
7	Lomefloxacin	Y = (19629.0) X + (-749.499)	0.2-50	0.9997	89.8-107.5
8	Danofloxacin	Y = (44434.3) X + (911.073)	0.2-50	0.9995	91.1-110.6
9	Enrofloxacin	Y = (23178.6) X + (76.5354)	0.2-50	0.9975	87.2-111.3
10	Cinoxacin	Y = (69528.6) X + (284.320)	0.2-50	0.9995	92.1-111.6
11	Oxolinic acid	Y = (124807) X + (4369.16)	0.2-50	0.9999	87.5-113.0
12	Flumequine	Y = (18436.0) X + (814.851)	0.2-50	0.9987	91.2-108.2

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

Compound Name	RSD% (0.2 ng/mL)		RSD% (10 ng/mL)		RSD% (50 ng/mL)	
	R.T.	Area	R.T.	Area	R.T.	Area
Pipemidic Acid	0.23	4.93	0.21	2.54	0.24	1.85
Enoxacin	0.16	3.73	0.18	1.21	0.19	1.79
Ofloxacin	0.27	4.93	0.17	1.59	0.18	1.66
Norfloxacin	0.17	4.91	0.16	1.92	0.19	1.13
Pefloxacin	0.16	4.77	0.17	2.06	0.19	2.01
Ciprofloxacin	0.07	3.88	0.15	1.34	0.18	2.29
Lomefloxacin	0.15	3.37	0.15	1.70	0.18	1.45
Danofloxacin	0.17	3.23	0.15	2.61	0.16	3.74
Enrofloxacin	0.23	4.88	0.13	3.16	0.15	1.81
Cinoxacin	0.10	3.32	0.11	1.40	0.10	1.34
Oxolinic acid	0.09	2.15	0.09	1.69	0.07	1.44
Flumequine	0.04	3.43	0.03	3.37	0.03	2.00

Table 5 Signal-to-noise ratio (S/N), LOD and LOQ

Compound	Concentration Level (ng/mL)	S/N	Limit of Detection (ng/L)	Limit of Quantitation (ng/L)
Pipemidic Acid	0.20	15.43	0.04	0.13
Enoxacin	0.20	31.11	0.02	0.07
Ofloxacin	0.20	21.89	0.03	0.10
Norfloxacin	0.20	39.04	0.02	0.06
Pefloxacin	0.20	19.21	0.04	0.12
Ciprofloxacin	0.20	56.02	0.01	0.04
Lomefloxacin	0.20	39.68	0.02	0.06
Danofloxacin	0.20	29.48	0.02	0.07
Enrofloxacin	0.20	13.34	0.05	0.16
Cinoxacin	0.20	29.57	0.07	0.21
Oxolinic acid	0.20	44.84	0.02	0.05
Flumequine	0.20	18.66	0.03	0.09

Matrix spike test

A matrix spike using the blank chicken sample was prepared accordingly to give spiked sample at concentrations of 0.5 ng/mL, 10 ng/mL and 40 ng/mL. 3 replicates were tested and the average results are shown in Table 6. The test results indicate that the spike recovery of the 12 samples of antibiotics were 91.90–108.60%.

CONCLUSION

A method was established for the determination of quinolone antibiotics in chicken using Shimadzu UHPLC LC-30A coupled with triple quadrupole mass spectrometer LCMS-8045. This method analyzed 12 antibiotics within 9 min, and the correlation coefficients of the calibration curve are all above 0.997. The mixed standard antibiotics solutions at concentrations of 0.2 ng/mL, 10 ng/mL and 50 ng/mL were tested in 6 replicates. The relative standard deviations of retention time and peak area of the 12 target compounds are 0.03-0.27 % and 1.13-4.93% respectively, showing good precision. The chicken sample matrix at spiked concentrations of 0.5 ng/mL, 10 ng/mL and 40 ng/mL were tested in 3 replicates, and the spike recovery was 91.90-108.60%. The described method is fast and ensures high sensitivity and excellent reproducibility. It can be used for the determination of various antibiotic residues in chicken.

Table 6 Results of spike-recovery test

No.	Compound Name	Spiked Sample Concentration (0.5 ng/mL)		Spiked Sample Concentration (10 ng/mL)		Spiked Sample Concentration (40 ng/mL)	
		Average (ng/mL)	Recovery (%)	Average (ng/mL)	Recovery (%)	Average (ng/mL)	Recovery (%)
1	Pipemidic Acid	0.54	108.60	10.22	102.20	39.02	97.55
2	Enoxacin	0.50	100.23	9.72	97.23	37.34	93.38
3	Ofloxacin	0.51	101.93	9.59	95.90	38.84	97.08
4	Norfloxacin	0.48	96.35	9.82	98.20	37.16	92.90
5	Pefloxacin	0.47	94.08	10.01	100.08	37.37	93.43
6	Ciprofloxacin	0.47	94.23	9.90	99.03	37.12	92.83
7	Lomefloxacin	0.46	93.70	9.33	93.40	38.51	96.30
8	Danofloxacin	0.47	93.05	9.72	97.20	36.77	91.90
9	Enrofloxacin	0.48	95.85	9.86	98.63	39.70	99.25
10	Cinoxacin	0.48	95.78	9.82	98.25	37.71	94.28
11	Oxolinic acid	0.50	100.65	10.24	102.45	39.36	98.43
12	Flumequine	0.50	100.50	10.05	100.60	39.40	98.50

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Multi-Residue Analysis of Antibiotics in Organic Fertilizers using the Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer

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Application News
SSL-CA14-453

Abstract

This report describes a method for the determination of 15 antibiotics, such as tetracyclines, penicillin, quinolones and sulfonamides, in organic fertilizer using the Shimadzu Ultra-High Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. In this method, 15 antibiotics are analyzed within 12 minutes, and the correlation coefficients of the calibration curve are all above 0.995 with good linearity. Six parallel tests of the mixed standard solutions with low, medium and high concentrations were performed, and the relative standard deviations of retention time and peak area of the 15 antibiotics were 0.01–0.46% and 0.29–7.09% respectively. These results show good precision. The sensitivity test result indicates that the quantitation limit of the 15 antibiotics is at 0.004–0.135 ng/mL; the samples were also tested with the standard addition concentrations of 0.5 ng/mL and 5 ng/mL, and the recovery rates were measured to be in the range 82.2–104.8%. Characterized by fast analysis speed, good repeatability and high sensitivity, the described method can determine antibiotic residues in organic fertilizers and at the same time meet requirements specified in the new national standard (GB/T32951-2016).

With the rapid growth and developments in the livestock and poultry industry, antibiotics have been widely used as a feed additive to prevent and treat diseases and promote growth. Research shows that the amount of antibiotics discharged in an animal's urine or feces in the form of matrix or metabolites accounts for 40%–90% of the antibiotic dose. Organic fertilizer, consisting of mainly livestock and poultry feces, is widely used in agricultural production because of its rich organic compounds and nitrogen and phosphorus contents. However, due to limitations in the treatment process, antibiotics cannot be effectively degraded and removed during the production of organic fertilizer. The use of organic fertilizer containing antibiotics directly cause soil pollution, especially when livestock manure containing adsorbable tetracycline antibiotics is applied to farmland. These antibiotics combines with soil particles forming stable compounds with long lifetime, polluting the soil, as well as inducing and spreading various antibiotic-resistant bacteria. This poses a major threat to human health and our environment.

The current organic fertilizer standards in China are referenced mainly from the "Organic Fertilizer" (NY525-2012) and "Bio-organic Fertilizer" (NY884-2012) standards. These standards stipulate the contents of organic material, total nutrients and heavy metal, but the amount of residual antibiotics is not included. In view of the lack of detection methods and standards for antibiotic content in organic fertilizers in China, the State General Administration of Quality Supervision, Inspection and Quarantine and the State Standardization Administration Committee approved and issued the standard GB/T32951-2016 "High Performance Liquid Chromatography for Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline Content in Organic Fertilizer" in August 2016, and implemented it on March 1st, 2017. This standard has not only provided an important method for determining tetracycline antibiotic residues in organic fertilizer products, but has also provided technical support for the analysis and optimization of treatment processes in livestock and poultry manure processing enterprises.

With reference to the sample pretreatment method specified in GB/T32951-2016 "High Performance Liquid Chromatography for Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline Content in Organic Fertilizer", this paper demonstrates a method that uses the Shimadzu Ultra-High Performance Liquid Chromatograph LC-30A and the Triple Quadrupole Mass Spectrometer LCMS-8045 to determine the amount of 15 antibiotic residues in organic fertilizer.

EXPERIMENTAL

Instrumentation

A system with a Shimadzu Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045 is used in this experiment. The specific configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC automatic sampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 Chromatographic Workstation.

Analytical Conditions

LC Chromatography (LC) Conditions

Column	:	Shim-pack XR-ODS III, 2.0 mm I.D.× 75 mm L., 1.6 µm
Mobile Phase	:	Phase A - 0.1% formic acid solution Phase B - acetonitrile
Flow rate	:	0.30 mL/min
Column temp.	:	40 °C
Injection volume	:	10 µL
Type of elution	:	Gradient elution with the initial concentration of Mobile Phase B at 10%. Refer to Table 1 for elution program.

Table 1 Gradient elution time program

Time (min)	Module	Command	Value (%)
0.50	Pumps	Pump B Conc.	10
3.00	Pumps	Pump B Conc.	35
5.50	Pumps	Pump B Conc.	90
8.00	Pumps	Pump B Conc.	90
8.10	Pumps	Pump B Conc.	10
12.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Analytical Instrument	:	LCMS-8045
Ion sources	:	ESI
Nebulizer gas flow rate	:	3.0 L/min
Drying gas flow rate	:	10.0 L/min
Interface temp.	:	300 °C
DL temp.	:	250 °C
Heating module temp.	:	400 °C
Heating gas flow rate	:	10.0 L/min
Scanning mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	8 ms
MRM Parameters	:	Refer to Table 2

Preparation of Standard Solutions

An appropriate amount of each of the 15 antibiotic standards was weighed, combined and dissolved in methanol to prepare a mixed standard stock solution containing 15 antibiotics, each of concentration 10µg/mL. It is further diluted with blank matrix to obtain mixed standard solutions of concentrations 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 ng/mL (taking oxytetracycline calibration curve concentration as an example).

The sample pretreatment method was conducted in reference to the sample extraction and clean-up method specified in GB/T 32951-2016 "Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline in Organic Fertilizers".

Table 2 MRM optimized parameters

Compound Name	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Amoxicillin	26787-78-0	366.2	114.00*	-26.0	-22.0	-20.0
			349.20	-26.0	-10.0	-17.0
Lincomycin	154-21-2	407.3	126.10*	-20.0	-40.0	-17.0
			359.30	-20.0	-20.0	-27.0
Sulfadiazine	68-35-9	251.0	156.00*	-30.0	-15.0	-29.0
			92.10	-30.0	-25.0	-17.0
Ampicillin	69-53-4	350.2	106.10*	-17.0	-23.0	-11.0
			114.10	-17.0	-30.0	-19.0
Sulfathiazole	72-14-0	256.0	156.00*	-30.0	-14.0	-30.0
			92.10	-30.0	-28.0	-17.0
Ciprofloxacin	85721-33-1	332.2	314.10*	-13.0	-16.0	-24.0
			231.00	-13.0	-44.0	-18.0
Levofloxacin	100986-85-4	362.1	318.10*	-11.0	-20.0	-21.0
			261.10	-11.0	-28.0	-17.0
Sulfapyridine	144-83-2	250.0	156.00*	-30.0	-16.0	-29.0
			92.10	-30.0	-27.0	-17.0
Oxytetracyclin	79-57-2	461.2	426.00*	-14.0	-18.0	-23.0
			443.10	-14.0	-14.0	-24.0
Tetracycline	60-54-8	445.2	410.20*	-11.0	-22.0	-30.0
			427.00	-11.0	-16.0	-23.0
Aureomycin	57-62-5	479.2	444.10*	-12.0	-24.0	-23.0
			154.10	-12.0	-28.0	-12.0
Doxycycline	564-25-0	445.1	428.05*	-16.0	-18.0	-30.0
			154.05	-11.0	-32.0	-30.0
Sulfamethazine	57-68-1	279.0	186.00*	-30.0	-17.0	-20.0
			92.10	-30.0	-31.0	-17.0
Chloramphenicol	56-75-7	321.00	152.10*	22.0	17.0	27.0
			257.10	22.0	11.0	26.0
Oxacillin	66-79-5	400.1	259.10*	29.0	13.0	19.0
			356.25	30.0	8.0	14.0

Note: * indicates quantification ion

RESULTS AND DISCUSSION

MRM Chromatograms of Standard Samples

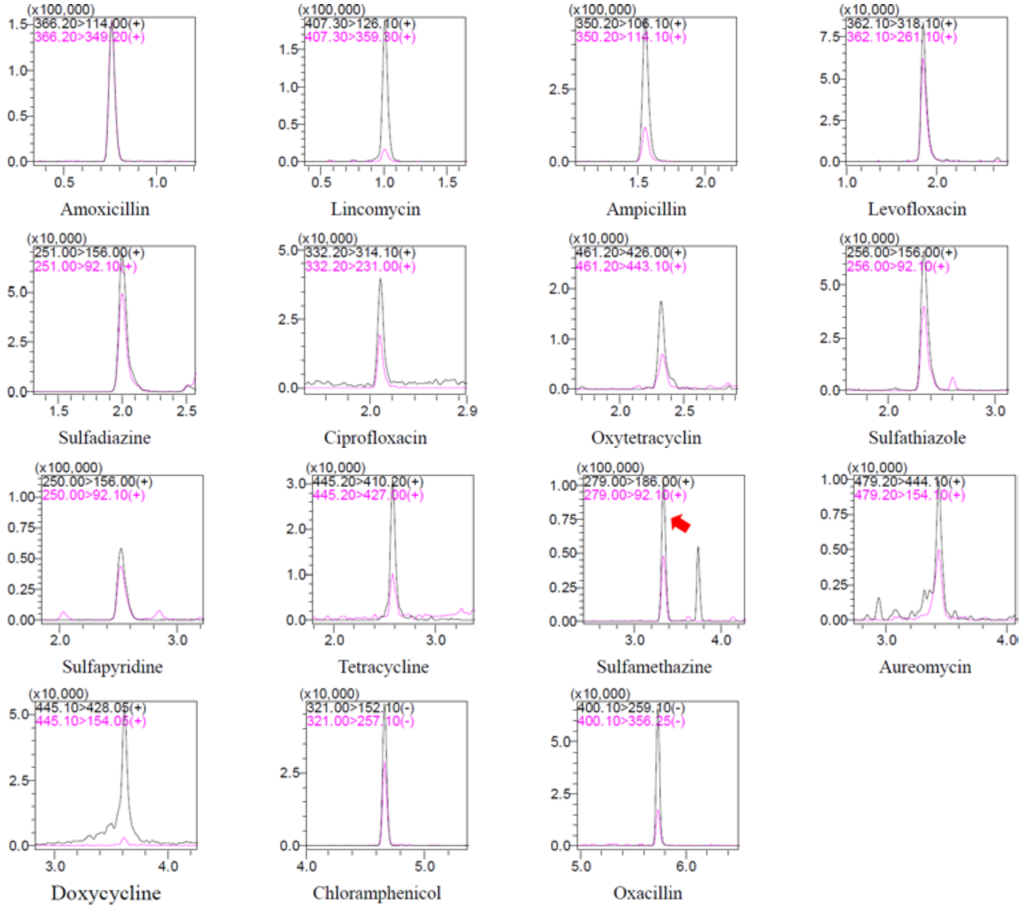
The MRM chromatograms for the mixed standard samples are displayed in Figure 1.

Calibration curve and linearity

The samples were first extracted and purified to obtain blank matrix extract solutions. The stock solution was added to the blank matrix solution to prepare standard working solutions at different concentrations. LC-MS/MS analysis was conducted as specified in previously. A calibration curve was obtained using the external standard method. Refer to Table 3 for linear equations, linear range and the corresponding correlation coefficient.

Precision experiment

Mixed standard solutions were prepared at different concentrations with blank matrix and injected for 6 consecutive times (taking oxytetracycline calibration curve concentration as an example) to check the precision. As shown in Table 4, the relative standard deviation (RSD%) of retention time and peak area are at 0.01–0.46% and 0.29–7.09% respectively, showing good precision.



Amoxicillin Lincomycin Ampicillin Levofloxacin
Sulfadiazine Ciprofloxacin Oxytetracyclin Sulfathiazole
Sulfapyridine Tetracycline Sulfamethazine Aureomycin
Doxycycline Chloramphenicol Oxacillin

Figure 1 MRM chromatograms of standard samples with concentration of 1 ng/mL (taking the calibration curve concentration of Oxytetracyclin as an example)

Table 3 Parameters for the calibration curve (linear regression, the weight coefficient was $1/C^2$)

No.	Compound	Calibration curve	Linear Range (ng/mL)	Accuracy (%)	Correlation Coefficient (r)
1	Amoxicillin	Y = (31256.8)X + (5907.03)	1~200	93.8~113.7	0.9967
2	Lincomycin	Y = (373554)X + (186159)	0.1~50	94.0~110.4	0.9976
3	Ampicilin	Y = (151576)X + (-9683.21)	1~500	91.2~107.0	0.9984
4	Levofloxacin	Y = (107361)X + (215994)	0.2~50	90.4~111.7	0.9963
5	Sulfadiazine	Y = (263530)X + (26112.5)	0.1~50	88.6~110.1	0.9965
6	Ciprofloxacin	Y = (45160.1)X + (95808.1)	0.2~50	91.3~109.4	0.9972
7	Oxytetracyclin	Y = (41615.1)X + (16467.2)	0.1~50	92.1~105.4	0.9989
8	Sulfathiazole	Y = (276746)X + (27313.8)	0.1~20	93.1~104.6	0.9988
9	Sulfapyridine	Y = (237308)X + (30237.9)	0.1~20	90.5~107.7	0.9979
10	Tetracycline	Y = (97960.6)X + (4577.06)	0.1~50	95.6~107.4	0.9987
11	Sulfamethazine	Y = (314461)X + (10821.7)	0.1~50	94.3~109.6	0.9984
12	Aureomycin	Y = (24763.0)X + (9419.09)	0.1~50	86.7~111.0	0.9955
13	Doxycycline	Y = (114771)X + (30987.1)	0.2~50	91.5~109.1	0.9976
14	Chloramphenicol	Y = (11132.6)X + (1469.11)	1~100	92.2~108.0	0.9971
15	Oxacillin	Y = (15531.9)X + (-1257.09)	1~500	95.5~106.2	0.9993

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

No.	Compound Name	RSD% (0.5 ng/mL)		RSD% (2 ng/mL)		RSD% (10 ng/mL)	
		R.T.	Area	R.T.	Area	R.T.	Area
1	Amoxicillin	0.09	3.62	0.08	2.65	0.03	1.61
2	Lincomycin	0.26	3.56	0.25	1.73	0.10	1.41
3	Ampicilin	0.37	2.02	0.39	0.89	0.13	0.29
4	Levofloxacin	0.45	3.51	0.46	1.34	0.16	0.79
5	Sulfadiazine	0.17	4.13	0.26	2.82	0.08	0.88
6	Ciprofloxacin	0.39	6.47	0.40	3.08	0.18	3.02
7	Oxytetracyclin	0.33	4.53	0.34	5.43	0.16	2.29
8	Sulfathiazole	0.22	5.11	0.21	2.73	0.09	1.41
9	Sulfapyridine	0.13	3.35	0.21	1.98	0.09	1.96
10	Tetracycline	0.29	6.96	0.23	3.68	0.09	1.08
11	Sulfamethazine	0.06	4.92	0.12	2.14	0.05	0.53
12	Aureomycin	0.13	5.45	0.14	5.70	0.06	5.40
13	Doxycycline	0.07	5.93	0.10	4.01	0.06	4.39
14	Chloramphenicol	0.04	3.75	0.05	2.77	0.01	1.12
15	Oxacillin	0.01	7.09	0.02	2.53	0.01	2.27

Limit of detection and limit of quantitation

The organic fertilizer samples are prepared according to the treatment method described in the experimental section. A matrix spike sample of concentration 0.2 ng/mL was prepared (taking the standard curve concentration of oxytetracycline as an example), and injected for analysis. The lowest detection limit (S/N=3) and the lowest quantitative limit (S/N=10) of the 15 antibiotics were calculated by software and the results are shown in Table 5.

Matrix Spike and Recovery Experiment

The organic fertilizer sample was prepared according to the treatment method described in experimental section to obtain a blank matrix., Matrix spike samples at concentrations of 0.5 ng/mL and 5 ng/mL are prepared and injected to determine the recovery rate. The results in Table 6 show that the spike recovery rates are in the range of 82.2–104.8%.

Table 5 Detection Limit and Quantitation Limit of 15 Antibiotics

No.	Compound Name	Spiked Sample Concentration (0.5 ng/mL)		Spiked Sample Concentration (5 ng/mL)	
		Average (ng/mL)	Recovery (%)	Average (ng/mL)	Recovery (%)
1	Amoxicillin	4.72	94.4	46.66	93.3
2	Lincomycin	0.51	101.8	4.13	82.6
3	Ampicilin	4.67	93.5	47.25	94.5
4	Levofloxacin	0.42	83.9	4.34	86.9
5	Sulfadiazine	0.48	96.6	4.11	82.2
6	Ciprofloxacin	0.41	82.8	4.31	86.2
7	Oxytetracyclin	0.49	98.4	4.98	99.5
8	Sulfathiazole	0.49	97.8	4.52	90.3
9	Sulfapyridine	0.48	96.5	4.34	86.7
10	Tetracycline	0.47	93.0	4.39	87.8
11	Sulfamethazine	0..48	95.4	4.58	91.6
12	Aureomycin	0.45	89.7	5.24	104.8
13	Doxycycline	0.43	86.3	4.52	90.4
14	Chloramphenicol	4.85	96.9	46.43	92.9
15	Oxacillin	4.49	89.9	53.89	107.8

CONCLUSION

This paper establishes a method for the rapid determination of 15 antibiotics in organic fertilizer using the Shimadzu UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. With this method, 15 antibiotics are analyzed within 12 minutes, and the correlation coefficients of the calibration curve are all above 0.995 with good linearity. Six parallel tests of the mixed standard solutions with low, medium and high concentrations were performed, and the relative standard deviations of retention time and peak area of the 15 antibiotics are at 0.01–0.46% and 0.29–7.09% respectively, showing good precision. Prepared samples with concentrations of 0.5 ng/mL and 5 ng/mL had a recovery rate of 82.2–104.8%, indicating characteristics of fast analysis speed, good repeatability and high sensitivity. This method has broadened the scope of antibiotic analysis and demonstrated the multi-residue analysis of antibiotics at the same time meet requirements specified in the new national standard (GB/T32951-2016).

Determination of Imidocarb Residues in Milk by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

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Application News
SSL-CA14-551

Abstract

In this application news, Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A was coupled with Triple Quadrupole Mass Spectrometer LCMS-8045 for the determination of imidocarb residues in milk. This method is made in reference to the "Inspection and Quarantine Industry Standard, SN/T 4252-2015: Method for Detection of Imidocarb Residues in Animal-Derived Food for Export". The linearity was excellent over the range of 0.02 – 50 ng/mL and the correlation coefficient of the method was 0.9999. In the precision experiment, the RSD% of the retention time and peak area of imidocarb samples were 0.1 – 0.2% and 1.5 – 5.4% respectively, indicating good precision. In the spike recovery test, the spike recovery of the matrix at different concentrations ranged from 86.0 to 98.6%.

Imidocarb is a derivative of 1,3-Diphenylurea and is commonly used as a novel antiprotozoal chemical drug (veterinary medicine) used in animals. In clinical applications, imidocarb preparations are usually used in forms of dipropionate salt or bis-hydrochloride salt. Through intramuscular or subcutaneous injection, imidocarb is used in the treatment and prevention of various parasitic infections such as babesiosis, piroplasmosis, trypanosomiasis, eperythrozoonosis, anaplasmosis and theileriosis. Imidocarb is concentrated in kidneys and resorbed in its original forms. The detoxification (metabolism) of imidocarb occurs in the liver. Therefore, the amount of imidocarb use is small and its efficacy is long-lasting, thus inducing minimal stimulation. As a veterinary medicine, imidocarb is characterized by small dosage, convenient administration, short course of treatment and low drug resistance.

Studies have shown that imidocarb has a long metabolic period and high residue levels in animals. High concentrations of imidocarb residues may pose a threat to human health. CODEX, EU, Japan, and Australia have all established standards regarding the Maximum Residue Level (MRL) of imidocarb in foods. In particular, the MRL of imidocarb in both milk and beef fat is 50 μg/Kg.

Milk contains a high nutritional content and is the most ideal natural food for humans. Therefore, the detection of imidocarb residues in milk is of great significance. In this experiment, in reference to the "Inspection and Quarantine Industry Standard, SN/T 4252-2015: Method for Detection of Imidocarb Residues in Animal-Derived Food for Export", Shimadzu's UHPLC LC-30A was used together with the Triple Quadrupole Mass Spectrometer LCMS-8045 to establish a method for determination of imidocarb residues in milk. UHPLC-tandem mass spectrometry is characterized by high selectivity, high sensitivity, strong qualitative and quantitative capabilities, and high accuracy. Therefore, in the inspection and quarantine industry, UHPLC-tandem mass spectrometry is ideal for the analysis of veterinary drug residues in animal-derived foods.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler,

Food Analysis
Pharmaceutical Analysis
Environmental Analysis

CTO-20AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions LCMS DB Ver. 6.80 Chromatographic Workstation.

Analytical Conditions

Liquid Chromatography (LC) Conditions

Column	:	Shim-pack GISS, 2.1 mm I.D. × 100 mm L., 1.9 µm
Mobile Phase	:	Mobile Phase A-0.1% formic acid in water Mobile Phase B-methanol
Flow rate	:	0.40 mL/min
Column temp.	:	40 °C
Injection volume	:	1 µL
Type of elution	:	Gradient elution with the initial concentration of Mobile Phase B at 25%. Refer to Table 1 for elution program.

Table 1 Gradient elution time program

Time (min)	Module	Command	Value (%)
0.50	pumps	B Conc.	25
1.00	pumps	B Conc.	50
2.00	pumps	B Conc.	50
2.10	pumps	B Conc.	90
3.00	pumps	B Conc.	90
3.10	pumps	B Conc.	25
5.00	controller	Stop	

Mass Spectrometry (MS) Conditions

Analytical Instrument	:	LCMS-8045
Ion source	:	ESI (+)
Heating gas	:	Air 10.0 L/min
Nebulizing gas	:	Nitrogen 3.0 L/min
Drying gas	:	Nitrogen 10.0 L/min
Collision gas	:	Argon
Interface temp.	:	300 °C
DL temp.	:	250 °C
Heating block temp.	:	400 °C
Scanning mode	:	Multiple reaction monitoring (MRM)
Interface voltage	:	0.2 kV
MRM parameters	:	See Table 2

Standard Solution Preparation

An appropriate amount of imidocarb standard was weighed and dissolved in methanol to prepare a standard stock solution at a concentration of 1.0 µg/mL. The standard stock solution was stored at 4 °C. The standard stock solution was diluted with the mobile phase at its initial ratio (0.1% formic acid in water: methanol = 75:25, v/v) to obtain standard working solutions at concentrations of 0.02 ng/mL, 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 50 ng/mL.

Sample Preparation

Sample preparation was performed according to the milk extraction and purification method in the "Inspection and Quarantine Industry Standard, SN/T 4252-2015: Method for Detection of Imidocarb Residues in Animal-Derived Food for Export". 5.00 g of the sample (to the nearest 0.01 g) was weighed and placed

Table 2 MRM optimized parameters

Compound Name	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Imidocarb	27885-92-3	349.1	188.2*	-17	-30	-18
			162.2	-17	-25	-15

Note: * indicates quantification ion

into a 50 mL centrifuge tube. After adding the extraction solution (acetonitrile: water = 8:2, v/v) to a final volume of 25 mL, the solution was vortexed and mixed for 2 min. Subsequently, the solution was centrifuged at 4500 r/min for 8 min, and 5 mL of the supernatant was transferred into another centrifuge tube. After adding sodium hydroxide solution into the supernatant (sample solution) to adjust the pH to approximately 8.0, the solution was centrifuged at 4000 r/min for 5 min. The sample solution was added to a WCX solid-phase extraction (SPE) column, followed by washing with 2 mL of water and 3 mL of methanol. The entire wash solution was discarded and the column was eluted with 5 mL of methanol. The eluate was collected and completely dried with nitrogen. The residues were dissolved in 1 mL of solution (0.1% formic acid in water : methanol = 75:25, v/v) and filtered through a 0.22 µm membrane filter to obtain the final sample for analysis

RESULTS AND DISCUSSION

Q1 MS Scan and Product Ion Scan of Imidocarb Standard

The Q1 MS scan and product ion scan of the imidocarb standard are shown in Figures 1 and 2.

MRM Chromatogram of 0.02 ng/mL Imidocarb Standard Solution

The MRM chromatogram is shown in Figure 3.

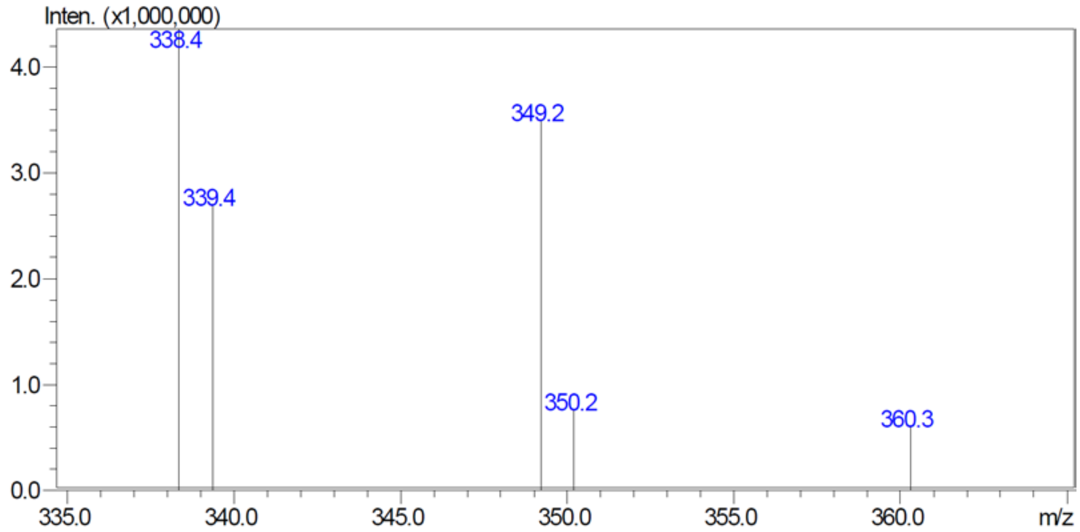

Figure 1 Q1 MS scan of imidocarb

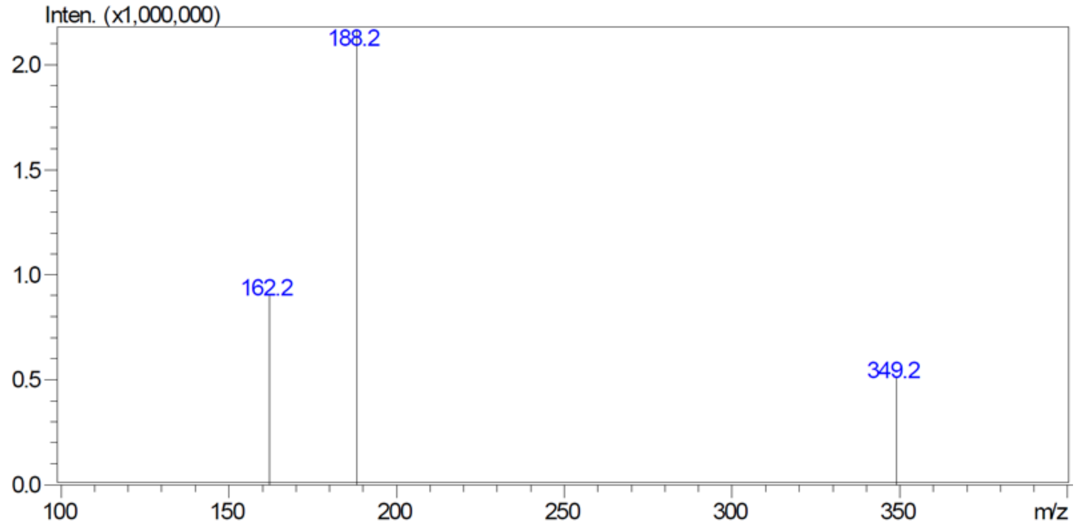

Figure 2 Product ion scan of imidocarb (the CE value was -25 V)

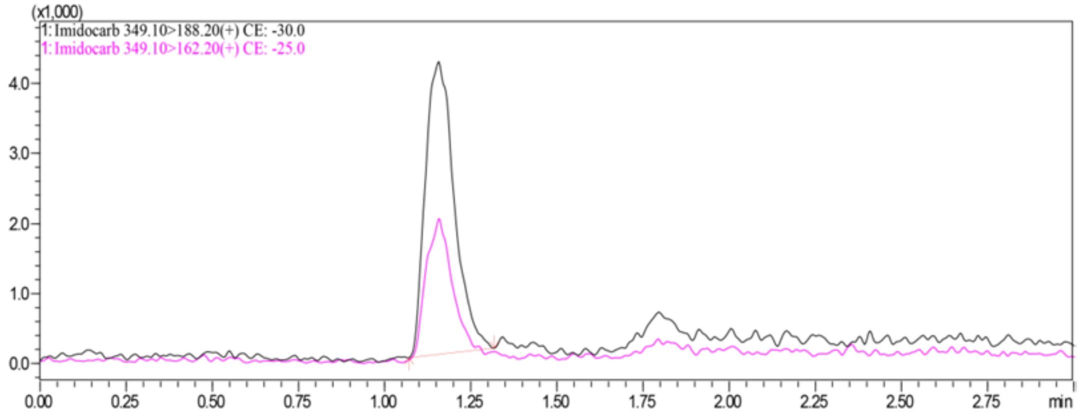

Figure 3 MRM chromatogram of 0.02 ng/mL imidocarb standard solution

Calibration Curve and Linear Range

After the measurement was performed according to the analytical conditions specified in the experimental section, a standard curve was established by the external standard method. The results are shown in Figure 4. In the linear concentration range of 0.02-50 ng/mL, the correlation coefficients of imidocarb were all greater than 0.9999 and the linear correlation of the results was excellent. The detailed results are shown in Table 3.

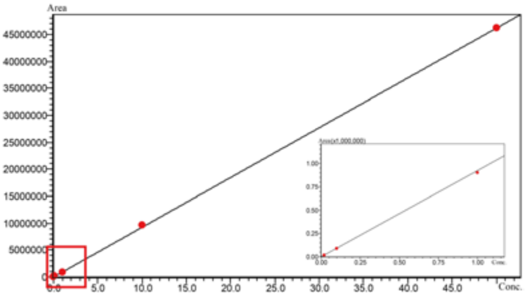

Figure 4 The standard curve of imidocarb

Precision Test

According to the analytical conditions specified in previously, the standard solutions of imidocarb at concentrations of 0.02 ng/mL, 1 ng/mL, and 50 ng/mL were injected consecutively and tested in 6 replicates. The relative standard deviation (RSD) for the retention time of imidocarb was in the range of 0.1 – 0.2%. The RSD for the peak area was in the range of 1.5 – 5.4% (see Table 4 for detailed results). The results showed that the precision was adequate.

Sensitivity Test

In order to examine the sensitivity, the imidocarb standard solution at a low concentration of 0.02 ng/mL was measured under the analytical conditions specified in the experimental section. The signal-to-noise ratio, limit of detection, and limit of quantitation were calculated using the LabSolutions LCMS DB Ver. 6.80 software. The results are shown in Table 5.

Table 3 Parameters of the calibration curve

Analyte	Linear Equation	Correlation Coefficient	Concentration Point of the Curve (ng/mL)	Accuracy (%)
Imidocarb	Y = 923549X	0.9999	0.02	92.8
			0.1	94.6
			1	99.4
			10	103.3
			50	99.9

Table 4 Precision results (n=6)

Analyte	0.02 ng/mL		1 ng/mL		50 ng/mL	
	Retention Time (Min)	Peak Area	Retention Time (Min)	Peak Area	Retention Time (Min)	Peak Area
Imidocarb	1.158	22343	1.147	688961	1.143	40201400
	1.162	21251	1.148	697204	1.141	40360042
	1.155	24061	1.152	690845	1.141	40507473
	1.156	24457	1.152	718771	1.143	40860853
	1.156	22054	1.147	711057	1.142	41454168
	1.160	22913	1.147	718038	1.145	41716697
Average	1.157	22846	1.149	704146	1.143	40850106
RSD%	0.2	5.4	0.2	1.9	0.1	1.5

Table 5 Signal-to-noise ratio(S/N), limit of detection and limit of quantitation

Analyte	Conc. (ng/mL)	S/N	LOD (ng/mL)	LOQ (ng/mL)
Imidocarb	0.02	19.2	0.003	0.01

Spike Recovery

The milk sample solutions, prepared as mentioned in experimental section, were injected and analyzed to obtain the chromatogram shown in Figure 5. The imidocarb standard solution was added into 5.00 g of milk sample and the matrix spike sample was prepared according to the pretreatment method specified in experimental section. The final volume of the sample was 1.0 mL.

As specified in SN/T 4252-2015, the lower limit of detection for imidocarb is 25 μg/kg. No imidocarb was detected in the sample. The samples were spiked to obtain the final spiked concentrations of 0.02 ng/mL (0.02 μg/kg), 1 ng/mL (1 μg/kg) and 50 ng/mL (50 μg/kg). Each spiked sample was continuously injected 6 times. The chromatograms of the spiked samples and the spike recovery rates are shown in Figure 6 and Table 6 respectively. From

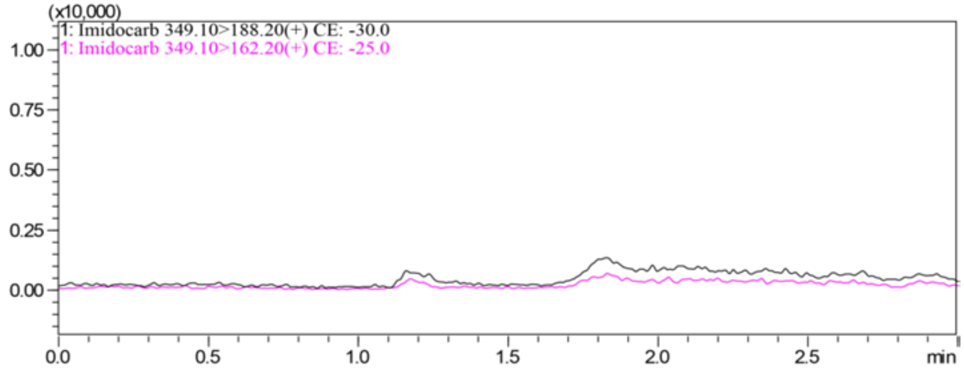

Figure 5 Chromatogram of milk samples

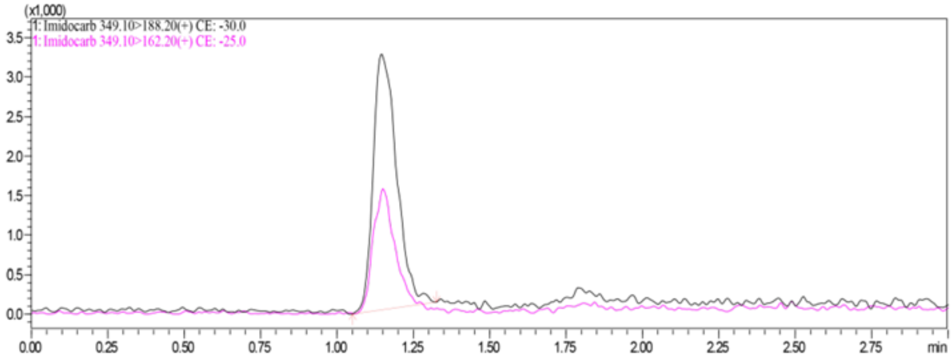

Figure 6 Chromatogram of milk samples using 0.02 μg/kg of imidocarb standard addition

Table 6 Spike recovery (n=6)

Analyte/Spiked Amount	Detected Concentration µg/kg	Spike Recovery (%) 0.02 µg/kg	1 µg/kg	50 µg/kg
Imidocarb	N.D.	92.0	89.0	99.0
		83.5	90.9	97.7
		82.0	87.5	99.8
		85.5	86.8	97.9
		89.5	87.9	99.5
		85.5	88.7	97.7
Average Recovery (%)		86.0	88.5	98.6
RSD%		5.0	1.6	0.9

(N.D.: Not Detected)

Table 6, it is observed that when the level of imidocarb spiked in the matrix was 0.02 µg/kg, the recovery was in the range of 82.0-92.0%; at the level of 1 µg/kg, the recovery was in the range of 86.8–90.9%; at the level of 50 µg/kg, the recovery was in the range of 97.7–99.5%. The spiked samples showed an adequate response above the limit of quantitation. Therefore, the method can meet the requirements for the quantitative analysis of the samples.

CONCLUSION

This application news established a method for determination of imidocarb residues in milk using Shimadzu's UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The linearity was excellent over the concentration range of 0.02–50 ng/mL and the correlation coefficient was 0.9999. The precision was evaluated and the RSD% of the retention time and peak area of imidocarb samples were 0.1 – 0.2% and 1.5 – 5.4% respectively, indicating the adequate precision. In the spike recovery test, the spike recovery of the matrix at different concentrations ranged from 86.0 to 98.6%. This method, characterized by fast analysis, high sensitivity and excellent reproducibility, is suitable for the detection and analysis of imidocarb residues by the inspection/quarantine department and related industries.

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Determining Gamma-Hydroxybutyric Acid and its Precursors Gamma-Butyrolactone and 1,4-Butanediol in Suspected Drug-spiked Beverages using LCMS-8045

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Application News
SSL-CA14-679

Abstract

This paper established a method to determine the quantity of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in suspected drug-spiked beverages using Shimadzu's Ultra-High Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. Analysis was completed within 2 min with good linearity. The standard samples at various concentrations were tested in 6 replicates, and the relative standard deviations of retention time and peak area were 0.26 – 0.48% and 0.70 – 2.31% respectively, showing good precision. The spike recovery of the sample was 92.6 – 104.3%. Given the fast analysis and accurate results acquired, this method can be used for the determination of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in beverages.

Media recently reported that energy drinks are being used as alcohol-substitute beverages in karaoke establishments (KTVs) and other entertainment venues. These beverages have been detected to contain high concentrations of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL). GHB is listed as a psychotropic drug strictly controlled in China. Substance abuse or drug misuse can cause temporary memory loss, nausea, vomiting, headache, loss of reflex, immediate loss of consciousness, coma and even death. In addition, the consumption of GHB with alcoholic beverage further increase these risks. Both GBL and 1,4-butanediol (1,4-BD) are precursors of GHB and they are rapidly metabolized to GHB inside the human body.

For all these reasons, it is necessary to accurately detect GHB and its precursors in beverages. Traditional detection methods mainly involve the use of GCMS, which requires derivatization of target substances before sample analysis. In this paper, a rapid and accurate method using Shimadzu's LCMS-8045 is established to determine the quantity of GHB and its precursors in beverages. The method preparation is simple, which doesn't call for derivatization. Sample preparation consists of dilution and filtration of samples. The analysis is fast and ensures good precision, which is ideal for quantitative analysis of such drug substances in beverages.

EXPERIMENTAL

Instrumentation

The experiment used Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are LC-30AD×2 pumps, DGU-20A5 online degassing unit, SIL-20AC autosampler, CTO-20AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.89 chromatographic workstation.

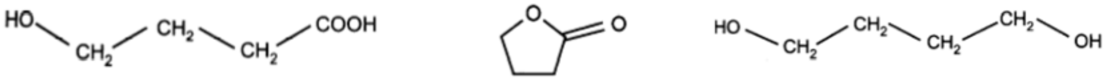

Figure 1 Structures of the target drug substances (left: GHB, middle: GBL, right: 1,4-BD)

Analytical Conditions

LC Chromatograpy (LC) Conditions

Column : Shim-pack XR-ODS III (150 mm. L×2.0 mm I.D., 2.2 µm)
Mobile phase : Phase A - 0.1% acetic acid in water
Phase B - acetonitrile
Flow rate : 0.30 mL/min
Column Temp. : 25 °C
Injection volume : 10 µL
Elution method : Isocratic elution, where the concentration of Phase B is 5%

Mass Spectrometry (MS) Conditions

Analytical Instrument : LCMS-8045
Ion Source : ESI, simultaneous scanning of positive and negative ions
Heating gas : Air 10.0 L/min
Nebulizer gas : Nitrogen 3.0 L/min
Drying gas : Nitrogen 10.0 L/min
Scan mode : Multiple reaction monitoring (MRM)
Dwell time : 50 ms
DL temp. : 200 °C
Heating block temp. : 400 °C
Scan mode : Multiple reaction monitoring (MRM)
Dwell time : 50 ms
Collisions gas : Argon
Interface temp. : 300 °C
DL temp. : 200 °C
Heat block temp. : 400 °C
Delay time : 3 ms
MRM Parameters : Refer to Table 1

Standard solution preparation

Preparation of Standard Working Solutions: The drug standards were weighed and dissolved in methanol or ultra-pure water to give a standard stock solution at concentration of 1000 µg/mL. The stock solution were further diluted with ultra-pure water to obtain standard working solutions of the following concentrations: 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0 ng/mL.

Sample Preparation Method

1mL of the beverage sample were collected and diluted with ultra-pure water. The solution was filtered with a 0.22 µm filter membrane prior to analysis.

RESULTS AND DISCUSSION

MRM Chromatogram of Standard Drug Samples

The MRM chromatogram is shown in Figure 2.

Calibration Curve and Linear Range

A series of standard solutions with concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0 and 500.0 ng/ml were prepared and injected for analysis according to the conditions in the experimental section and quantified with external standard calibration method. A calibration curve, shown in Figure 3, was plotted showing peak area against concentration,. The linear equation, linear range, and correlation coefficients are shown in Table 2.

Table 1 MRM optimized parameters

Compound	Ionization Mode	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Gamma-Hydroxybutyric Acid (GHB)	ESI (-)	103.1	85.1*	10.0	15.0	11.0
			57.1	10.0	12.0	16.0
Gamma-Butyrolactone (GBL)	ESI (+)	87.2	45.1*	-13.0	-25.0	-18.0
			43.1	-15.0	-25.0	-20.0
1,4-Butanediol (1,4-DB)	ESI (+)	91.2	55.1*	-15.0	-12.0	-22.0
			73.1	-15.0	-10.0	-13.0

Note: * indicates quantification ion

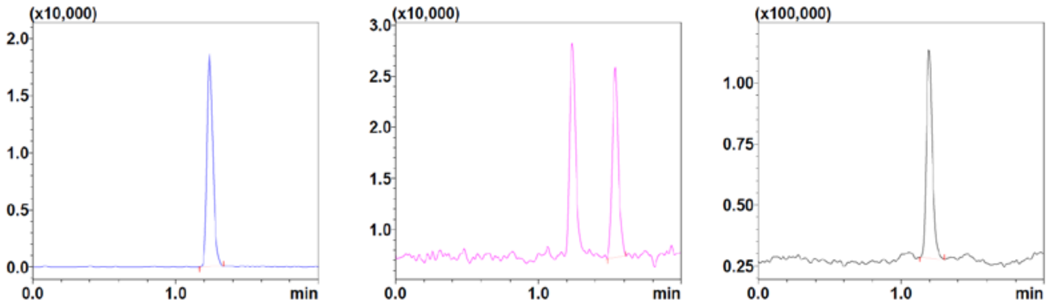

Figure 2 Chromatogram of 20 ng/ml standard samples (left: GHB, middle: GBL, right: 1,4-BD)

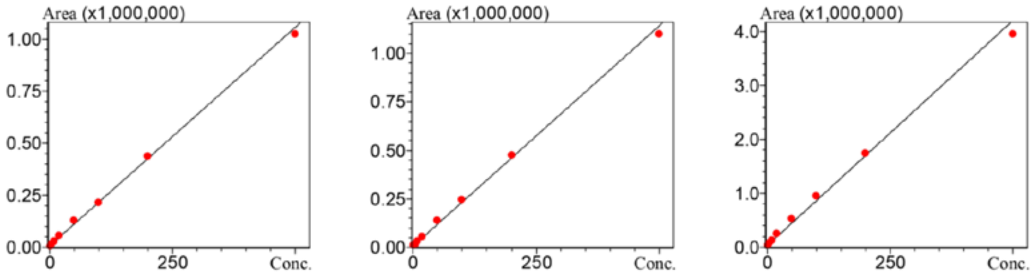

Figure 3 Calibration curve (left: GHB, middle: GBL, right: 1,4-BD)

Table 2 Parameters of the calibration curve

Compound Name	Calibration curve	Linear range (ng/mL)	Correlation coefficient (R)	Accuracy (%)
GHB	Y = (2413.4) X + (945.6)	1-500	0.9979	85.4-111.1
GBL	Y = (2379.9) X + (3704.9)	2-500	0.9982	88.3-114.6
1,4-BD	Y = (10000.5) X + (9547.5)	2-500	0.9986	86.7-113.6

Precision Test

The standard samples with concentrations of 10.0 and 200 ng/ml were consecutively injected for 6 times to investigate the repeatability; and the result of retention time and peak area were shown as Table 3. It shows good repeatability.

Table 3 Repeatability results of retention time and peak area (n=6)

Compound Name	RSD% (10 ng/ml)		RSD% (200 ng/ml)	
	R.T.	Area	R.T.	Area
GHB	0.47	2.31	0.41	0.81
GBL	0.30	2.08	0.26	0.70
1,4-BD	0.44	1.87	0.48	0.97

Actual Sample Test and Spike Recovery Test

The samples were prepared and injected for analysis. The results are shown as Table 5. The spike recovery at a spiking concentration of 0.5 mg/mL was calculated.

CONCLUSION

A method was established in this paper to determine the quantity of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in suspected drug-spiked beverages using Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. Analysis was completed within 2 min with good linearity. The standard samples at various concentrations were tested in

Table 5 Sample detection and spike recovery test

Compound Name	Detected concentration (μg/ml)	Dilution Factor	Actual Sample concentration (mg/ml)	Spiking concentration (mg/ml)	Detected concentration of spiked samples (mg/ml)	Recovery (%)
GHB	0.11	4000	0.44	0.50	0.91	93.1
GBL	0.18		0.70	0.50	1.23	104.3
1,4-BD	N.D.		N.D.	0.50	0.46	92.6

Note: The samples were provided by the Drug Control Branch of the Wuhan Municipal Public Security Bureau

N.D. = Not detected

6 replicates, and the relative standard deviations of retention time and peak area were 0.26 – 0.48% and 0.70 – 2.31% respectively, showing good precision. The spike recovery of the sample was in the range of 92.6 – 104.3%. Given the fast analysis and accurate results acquired, this method can be used for the determination of gamma-hydroxybutyric acid and its precursors gamma-butyrolactone and 1,4-butanediol in beverages.

Determination of 153 Pesticide Residues in Traditional Chinese Medicines by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

Jiaqi Liu
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Application News
SSL-CA14-416

Abstract

This application news established a method for the determination of 153 pesticide residues in traditional Chinese medicines using Shimadzu's Ultra-High Performance Liquid Chromatograph LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. This method separated and quantified 153 pesticides within 22 min. The linearity was good in the range of 0.5–200 μg/L, and the correlation coefficients were all above 0.995. The analysis is fast and provides excellent repeatability and high sensitivity. Hence, the described method meet the requirements for the determination of pesticide residues in Chinese medicines in Chinese Pharmacopoeia (2015 Edition).

There is a significant amount of traditional Chinese medicines that are conventionally-grown (i.e. not naturally grown in the wild or forest) out in the market today. Due to the excessive use and consumption of pesticides and the harm inflicted to the human body, the level of pesticide residues must be determined. Using the 2010 Edition of the Chinese Pharmacopoeia as a basic reference, the 2015 Edition not only included more pesticide categories, it introduced mass spectrometry (MS) technique to detect 153 pesticide residues in traditional Chinese medicines. The use of LC-MS/MS greatly increase the number of detectable pesticide types.

This application news established a LC-MS/MS method of for the detection of 153 pesticides in traditional chinese medicines using Shimadzu's Ultra-high Performance Liquid Chromatograph LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The described method can serve as a reference for relevant personnel.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are two LC-30A pumps, DGU-20A$_5$ on-line degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

Analytical Conditions

LC Chromatography (LC) Conditions

Analytical Instrument	:	LC-30A system
Column	:	Shim-pack XR-ODS III (2.0 mm I.D.× 150 mm L., 2.2 μm)
Mobile phase	:	Phase A - 10 mM ammonium formate + 0.1% formic acid in water Phase B - methanol
Flow rate	:	0.40 mL/min
Column Temp.	:	40 °C
Injection volume	:	5 μL
Elution method	:	Gradient elution with the initial concentration of mobile Phase B at 10%. Refer to Table 1 for time program.

Table 1 Gradient elution program

Time (min)	Module	Command	Value
1.00	Pumps	Pump B Conc.	30
4.00	Pumps	Pump B Conc.	70
14.00	Pumps	Pump B Conc.	98

Mass Spectrometry (MS) Conditions

Analytical Instrument	:	LCMS-8045
Ion Source	:	ESI, simultaneous scanning of positive and negative ions
Nebulizing gas	:	Nitrogen 2.0 L/min
Drying gas	:	Nitrogen 10 L/min
Heating gas	:	Air 10.0 L/min
Collision gas	:	Argon
Interface temp.	:	300 °C
DL temp.	:	200 °C
Heating block temp.	:	400 °C
Scan mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	1.0 - 8.0 ms
Delay time	:	1 ms
MRM parameters	:	Refer to Table 2

Sample Preparation

With reference to Method 2341 in Chinese Pharmacopeia (2015 Edition), sample preparation was conducted with Shimadzu's QuEChERS SPE product. The specific steps are as follows: weigh 3 g of homogeneous sample in a 50 ml polystyrene centrifuge tube, then add 15 mL of 1% glacial acetic acid solution, vortex to fully mix and leave it to react for 30 minutes; add 15 mL of acetonitrile, vortex and mix well, then the tube was placed on shaker for 5 min (500 times/min); add 7.5 g of mixed powder of anhydrous magnesium sulfate and anhydrous sodium acetate (4:1) (Item No.: 5010-050020) and immediately shake to disperse, then place on shaker for another 3 min (500 times/min), after cool in ice-bath for 10 min, centrifuge (4,000 r/ min) for 5 min; take 9 mL of the supernatant and put in a dispersive solid phase extraction purification tube pre-filled with purified materials (900 mg of anhydrous magnesium sulfate, 300 mg of N-primary secondary amine (PSA), 300 mg of octadecylsilane bonded silica gel, 90 mg of graphitized carbon black, 300 mg of silica gel, item No. 5010-015050), thoroughly mix by vortex, and place on shaker (500 times/min) for another 5 min for complete purification; centrifuge at 4,000 r/min for 5 min, exactly pipette 5 mL of supernatant, and evaporate to near dryness under a stream of nitrogen; then add 1mL of acetonitrile, filter with microporous membrane (0.22 µm), and the supernatant of filtrate was used for analysis.

Preparation of standard solution: prepare standard samples at different concentrations of 0.5 µg/L, 1.0 µg/L, 2.0 µg/L, 5.0 µg/L, 10.0 µg/L, 20.0 µg/L, 50.0 µg/L, 100.0 µg/L and 200.0 µg/L.

RESULTS AND DISCUSSION

Chromatogram of Standard Samples

The chromatogram of 153 pesticide (10.0 µg/L) standard samples is shown in Figure 1.

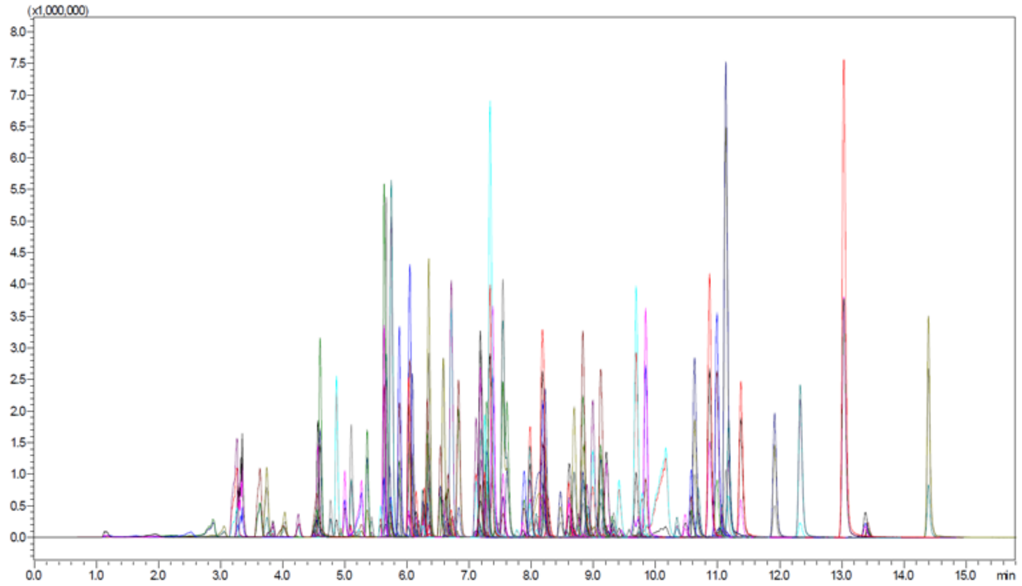

Figure 1 Standard sample chromatogram of 153 pesticides (10.0 µg/L)

Table 2 MRM parameters

No.	Compound Name	Retention Time	CAS No.	Precursor Ion m/z	Product Ion 1 m/z	Product Ion 2 m/z	Product Ion 1			Product Ion 2		
							Q1 Pre Bias	CE	Q3 Pre Bias	Q1 Pre Bias	CE	Q3 Pre Bias
1	Methamidophos	2.12	10265-92-6	142.05	94.00	125.05	-16	-15	-17	-16	-16	-23
2	Acephate	2.407	30560-19-1	184.2	143.00	95.00	-20	-8	-15	-20	-23	-16
3	Omethoate	2.613	1113-02-6	214.10	183.00	155.00	-23	-10	-19	-23	-14	-28
4	Aldicarb-sulfoxide	2.731	1646-87-3	207.10	89.00	132.00	-14	-14	-15	-14	-8	-23
5	Aldicarb-sulfone	2.869	1646-88-4	240.10	86.00	223.00	-26	-21	-15	-26	-8	-23
6	Oxamyl	2.926	23135-22-0	237.05	72.10	90.10	-17	-16	-29	-17	-8	-17
7	Methomyl	2.407	16752-77-5	163.05	88.00	106.05	-18	-8	-16	-18	-10	-19
8	Thiamethoxam	3.269	153719-23-4	292.00	211.10	181.10	-30	-11	-22	-30	-23	-19
9	Methacrifos	3.409	62610-77-9	241.00	126.85	108.9	-17	-14	-22	-17	-28	-19
10	Monocrotophos	3.493	6923-22-4	224.10	127.05	193.00	-25	-15	-13	-25	-8	-20
11	Carbendazim	3.489	10605-21-7	192.05	160.05	132.05	-30	-17	-30	-30	-30	-24
12	Dicrotophos	3.645	141-66-2	238.10	112.15	193.05	-18	-12	-21	-18	-10	-20
13	Imidacloprid	3.666	105827-78-9	256.05	175.10	209.05	-29	-17	-18	-29	-14	-22
14	Thiabendazole	3.919	148-79-8	202.00	175.05	131.10	-30	-24	-30	-30	-33	-24
15	Mevinphos	4.176	26718-65-0	225.00	127.05	193.05	-25	-17	-23	-25	-8	-20
16	Carbofuran-3-hydroxy	4.185	16655-82-6	238.10	163.10	181.20	-27	-14	-17	-27	-10	-19
17	Acetamiprid	4.237	135410-20-7	222.95	126.00	56.00	-15	-20	-23	-26	-16	-22
18	Fenthion-oxon-sulfoxide	4.157	6552-13-2	278.95	263.95	104.00	-19	-18	-28	-14	-29	-19
19	Dimethoate	4.279	60-51-5	230.00	198.95	125.00	-26	-9	-21	-26	-22	-22
20	Trichlorfon	4.278	52-68-6	256.90	108.95	220.85	-29	-17	-19	-29	-10	-23
21	Phorate-oxon-sulfone	4.307	2588/4/7	277.00	111.00	127.00	-21	-22	-11	-21	-15	-22
22	Thiacloprid	4.666	111538-49-9	253.00	126.05	99.00	-28	-20	-22	-28	-43	-17
23	Phosphoric acid	4.606	14086-35-2	295.05	217.10	233.10	-22	-20	-22	-22	-17	-24
24	Fensulfothion-oxon	4.787	6552-21-2	292.95	236.95	264.95	-20	-19	-25	-20	-14	-29
25	Tricyclazole	4.887	41814-78-2	190.00	163.00	136.00	-21	-21	-30	-21	-26	-24
26	Aldicarb	4.946	116-06-3	208.00	116.10	89.10	-22	-10	-24	-10	-21	-21
27	Oxadixyl	4.278	77732-09-3	279.10	219.05	133.10	-30	-11	-23	-30	-21	-24
28	Phosphamidon	5.016	13171-21-6	300.00	174.05	127.00	-15	-12	-17	-15	-29	-22
29	Metolcarb	5.109	1129-41-5	166.05	109.05	94.00	-11	-11	-20	-11	-30	-16
30	Fenamiphos-sulfoxide	5.256	31972-43-7	319.80	233.00	292.05	-30	-23	-26	-30	-16	-21

31	Desmethylnitrophos	5.25	950-35-6	274.95	201.95	108.95	-15	-19	-21	-15	-31	-19
32	Propoxur	5.3	114-26-1	210.10	168.10	111.10	-23	-7	-18	-23	-13	-20
33	Malaoxon	5.304	1634-78-2	314.90	98.95	127.00	-15	-24	-19	-15	-13	-23
34	Carbofuran	5.341	1563-66-2	222.10	165.10	123.10	-25	-11	-17	-25	-21	-22
35	Fenamiphos-sulfone	5.348	31972-44-8	335.90	266.00	188.10	-16	-21	-29	-16	-28	-21
36	Metribuzin	5.432	21087-64-9	215.10	187.10	84.10	-25	-18	-18	-25	-21	-30
37	Hexazinone	5.44	51235-04-2	253.15	171.10	85.10	-30	-15	-18	-30	-31	-15
38	Fenthion-sulfoxide	5.417	3761-41-9	295.00	279.95	109.00	-11	-18	-10	-11	-31	-20
39	Imazalil	5.403	35554-44-0	297.00	159.00	201.00	-15	-24	-15	-15	-18	-21
40	Pirimicarb	5.503	23103-98-2	239.15	72.05	182.15	-30	-25	-30	-30	-19	-30
41	Carbaryl	5.571	63-25-2	202.05	145.05	127.10	-14	-10	-29	-14	-25	-21
42	Ethiofencarb	5.749	29973-13-5	226.10	107.05	164.05	-26	-15	-19	-26	-8	-30
43	RH5849	5.733	112225-87-3	295.10	121.10	77.25	13	18	22	13	32	30
44	Fosthiazate	5.757	98886-44-3	284.10	228.00	104.05	-30	-10	-24	-30	-21	-19
45	Fenthion-sulfone	5.813	3761-42-0	311.00	124.90	278.90	-15	-21	-24	-15	-17	-13
46	Disulfoton-sulfoxide	5.818	2497/7/6	291.00	185.00	212.95	-30	-15	-19	-30	-9	-23
47	Disulfoton-sulfone	5.901	2497/6/5	306.80	96.90	124.95	-15	-30	-18	-15	-17	-23
48	Paraoxon	5.891	311-45-5	276.00	219.80	94.05	-11	-14	-23	-11	-36	-17
49	Phorate Sulfone	5.946	2588/4/7	292.90	171.05	142.90	-11	-12	-29	-11	-16	-28
50	Isoprocarb	6.013	2631-40-5	194.10	95.00	137.10	-21	-14	-17	-22	-10	-14
51	Fensulfothion	6.048	115-90-2	309.00	281.00	253.00	-11	-15	-30	-11	-18	-26
52	Fensulfothion-oxon-sulfone	6.053	6132-17-8	309.05	253.00	281.05	-23	-15	-12	-23	-10	-30
53	Atrazine D5* (ethylamino D5)	6.087	163165-75-1	221.15	179.10	101.2	-18	-18	-18	-18	-24	-18
54	Atrazine	6.116	1912-24-9	216.10	174.05	96.05	-30	-17	-18	-30	-25	-17
55	Metalaxyl	6.116	70630-17-0	280.10	220.20	192.20	-30	-13	-24	-30	-18	-20
56	Fensulfothion-sulfone	6.171	14255-72-2	324.90	268.90	296.90	-23	-16	-29	-23	-12	-20
57	Pirimiphos-methyl-N-desethyl	6.331	67018-59-1	278.00	246.00	125.00	-21	-18	-26	-21	-24	-24
58	Phorate-oxon	6.526	2600-69-3	244.95	75.05	81.20	-17	-12	-30	-17	-55	-14
59	Chlorantraniliprole	6.283	500008-45-7	484.00	452.90	285.85	-24	-19	-30	-24	-16	-30
60	Methidathion	6.472	950-37-8	303.00	145.00	85.10	-21	-8	-15	-21	-22	-30

61	Fenthion-oxon	6.526	6522/12/1	263.05	231.00	215.95	-20	-16	-16	-20	-24	-22
62	Azinphos-methyl	6.567	86-50-0	318.10	132.10	261.00	-15	-14	-23	-15	-7	-28
63	Azoxystrobin	6.589	131860-33-8	404.10	372.05	329.00	-30	-14	-26	-30	-31	-23
64	Phosemet	6.624	732-11-6	317.95	160.05	77.00	-12	-18	-30	-12	-54	-29
65	Ametryn	6.637	834-12-8	228.10	186.10	68.05	-30	-18	-19	-30	-39	-27
66	Demeton	6.664	8065-48-3	258.90	88.95	60.95	-13	-13	-16	-13	-35	-25
67	Methiocarb	7.06	2032-65-7	226.00	121.10	169.05	-16	-20	-23	-16	-9	-17
68	Propanil	7.114	709-98-8	218.00	162.00	127.00	-24	-15	-17	-24	-26	-23
69	Dimethenamid	7.085	87674-68-8	276.10	244.10	168.10	-14	-14	-25	-14	-24	-17
70	Boscalid	7.114	188425-85-6	343.00	307.10	271.10	-12	-18	-30	-12	-30	-26
71	Flutolanil	7.158	66332-96-5	324.10	262.10	242.00	-16	-18	-27	-16	-26	-25
72	Promecarb	7.203	2631-37-0	208.20	109.10	151.10	-22	-15	-19	-22	-8	-16
73	Paclobutrazol	7.229	76738-62-0	294.10	70.05	125.05	-15	-21	-28	-15	-40	-22
74	Methoxyfenozide	7.251	161050-58-4	369.20	149.10	313.10	-18	-16	-16	-18	-8	-22
75	Malathion	7.258	121-75-5	331.00	127.05	99.00	-17	-12	-13	-17	-23	-18
76	Isoprothiolane	7.294	50512-35-1	291.10	231.10	189.10	-14	-11	-25	-14	-21	-20
77	Mepronil	7.361	55814-41-0	270.15	119.05	228.05	-30	-25	-30	-30	-18	-30
78	Myclobutanil	7.445	88671-89-0	289.10	70.05	125.05	-30	-21	-28	-30	-30	-22
79	Propetamphos	7.437	31218-83-4	282.10	138.00	156.05	-22	-19	-28	-22	-12	-29
80	Bifenazate	7.548	149877-41-8	301.15	198.05	170.10	-30	-10	-21	-30	-19	-18
81	Dichlofenthion	7.55	97-17-6	314.90	258.85	286.95	-12	-16	-26	-12	-11	-30
82	Triazophos	7.562	24017-47-8	314.05	162.15	119.15	-23	-19	-17	-23	-35	-21
83	Isazofos	7.593	42509-80-8	313.95	120.00	162.00	-12	-25	-22	-12	-16	-17
84	Molinate	7.735	2212-67-1	188.10	126.10	98.10	-21	-13	-13	-20	-20	-18
85	Azinphos-ethyl	7.907	2642-71-9	346.05	136.95	97.00	-13	-23	-25	-13	-33	-17
86	Mecarbam	7.928	2595-54-2	329.90	96.95	199.00	-16	-40	-18	-16	-16	-22
87	Fenarimol	7.935	60168-88-9	331.00	268.10	259.10	-16	-22	-28	-17	-26	-26
88	Ethoprophos	7.989	13194-48-4	243.10	131.00	97.00	-26	-20	-23	-27	-32	-17
89	Fenchlorphos-oxon	8.039	3983-45-7	304.80	109.00	272.85	-22	-22	-19	-22	-21	-29
90	Napropamide	8.002	15299-99-7	272.20	129.15	171.10	-30	-16	-23	-30	-17	-18

91	Alachlor	8.189	15972-60-8	270.10	238.05	162.15	-30	-10	-26	-30	-19	-30
92	Fenbuconazole	8.185	114369-43-6	336.90	125.05	70.00	-26	-27	-25	-26	-20	-28
93	Metolachlor	8.204	51218-45-2	284.10	252.10	176.20	-30	-14	-27	-30	-25	-19
94	Iprodione	8.236	36734-19-7	330.00	245.00	108.15	-23	-12	-29	-23	-11	-21
95	Fenamiphos	8.275	22224-92-6	304.10	217.10	202.00	-15	-22	-23	-15	-36	-21
96	Flusilazole	8.311	85509-19-9	316.10	247.10	165.10	-30	-18	-27	-30	-29	-30
97	Tebufenozide	8.305	112410-23-8	353.20	133.10	297.10	-18	-20	-24	-18	-8	-15
98	Diflubenzuron	8.404	35367-38-5	309.10	289.05	156.15	21	8	18	21	10	27
99	Phenthoate	8.574	2957/3/7	321.00	246.95	107.10	-12	-11	-25	-12	-25	-17
100	Isofenphos-methyl	8.644	99675-03-3	354.00	252.85	312.00	-14	-18	-26	-14	-16	-22
101	Sulfotep	8.707	3689-24-5	323.00	115.00	171.10	-16	-31	-20	-16	-15	-18
102	Quinalphos	8.711	13593-03-8	299.00	163.05	147.05	-15	-20	-30	-15	-21	-27
103	Tolylfluanid	8.753	731-27-1	364.00	237.90	137.00	-28	-14	-25	-28	-30	-23
104	Edifenphos	8.786	17109-49-8	311.00	282.95	111.05	-24	-13	-30	-24	-21	-21
105	Cyprodinil	8.824	121552-61-2	226.10	93.10	108.10	-30	-34	-16	-30	-27	-19
106	Tebuconazole	8.888	107534-96-3	308.10	70.10	125.00	-22	-22	-27	-22	-38	-23
107	Benalaxyl	8.934	71626-11-4	326.20	148.20	294.10	-16	-21	-15	-16	-11	-20
108	Etrimfos	8.953	38260-54-7	293.00	265.00	125.00	-15	-16	-28	-15	-24	-23
109	Fenthion	9.085	55-38-9	279.20	169.05	247.05	-30	-17	-18	-30	-12	-18
110	Chlorfenvinphos	9.012	470-90-6	358.90	155.10	99.00	-18	-12	-30	-18	-33	-18
111	Coumaphos	9.068	56-72-4	363.00	227.00	307.10	-18	-26	-23	-18	-18	-21
112	Diazinon	9.083	333-41-5	305.00	169.10	153.10	-30	-19	-18	-30	-20	-16
113	Propiconazole	9.1	60207-90-1	342.05	159.10	205.10	-17	-30	-29	-17	-18	-21
114	Fonofos	9.174	994-22-9	247.10	109.00	137.10	-27	-19	-19	-26	-10	-14
115	Pyraclostrobin	9.266	175013-18-0	388.10	194.05	163.05	-19	-13	-21	-19	-24	-30
116	Phoxim	9.266	14816-18-3	299.00	77.00	129.05	-11	-33	-29	-11	-15	-24
117	Hexaconazole	9.307	79983-71-4	314.10	70.20	159.15	-15	-21	-28	-15	-29	-30
118	Prochloraz	9.329	67747-09-5	376.00	308.00	266.00	-19	-11	-21	-19	-17	-29
119	Bitertanol	9.406	55179-31-2	338.20	269.15	99.10	-17	-9	-29	-17	-15	-18
120	Isofenphos	9.413	25311-71-1	368.00	266.95	326.15	-14	-19	-27	-14	-18	-22

121	Phosalone	9.466	2310-17-0	367.85	181.95	110.95	-28	-12	-19	-28	-38	-20
122	Pirimiphos methyl	9.526	29232-93-7	306.05	108.10	95.00	-30	-31	-19	-30	-29	-17
123	Phorate	9.595	298-02-2	260.95	75.10	142.90	-18	-14	-30	-18	-20	-25
124	Disulfoton	9.642	298-04-4	275.00	89.15	61.05	-19	-7	-16	-19	-34	-24
125	Tolclofos methyl	9.675	57018-04-9	300.95	268.85	124.90	-22	-16	-29	-22	-18	-23
126	Cyhalofop-butyl	9.825	122008-85-9	375.15	256.05	120.00	-14	-18	-29	-14	-32	-30
127	Diniconazole	9.747	76714-88-0	326.10	70.00	159.00	-16	-25	-28	-16	-30	-30
128	Cadusafos	9.806	95465-99-9	271.10	159.00	97.00	-30	-14	-29	-30	-37	-18
129	EPN	9.832	2104-64-5	324.00	156.85	295.95	-12	-23	-29	-12	-13	-20
130	Difenoconazole	9.829	119446-68-3	406.10	251.00	337.05	-30	-25	-27	-30	-17	-24
131	Indoxacarb	9.878	144171-61-9	528.10	249.10	292.95	-26	-17	-27	-26	-15	-21
132	Haloxyfop-methyl	9.904	69806-40-2	376.05	316.00	288.05	-15	-17	-22	-15	-26	-30
133	Trifloxystrobin	9.954	141517-21-7	409.10	186.05	145.00	-20	-18	-20	-20	-44	-26
134	Pretilachlor	10.248	51218-49-6	312.20	252.15	176.15	-15	-16	-28	-15	-28	-18
135	Profenofos	10.563	41153-08-7	372.90	302.80	345.00	-18	-19	-30	-18	-12	-24
136	Fluazifop-p-butyl	10.625	79241-46-6	384.20	282.20	328.10	-19	-21	-30	-19	-17	-23
137	Furathiocarb	10.687	65907-30-4	383.20	195.05	252.10	-27	-19	-21	-27	-13	-27
138	Tetramethrin	10.756	7696-12-0	332.05	164.10	135.10	-23	-24	-30	-23	-19	-24
139	Buprofezin	10.887	69327-76-0	306.10	201.10	116.10	-30	-11	-22	-30	-16	-12
140	Pirimiphos-ethyl	11.007	23505-41-1	334.10	198.05	182.10	-25	-22	-21	-25	-22	-19
141	Allethrin	11.065	584-79-2	303.10	135.10	93.15	-15	-10	-26	-15	-15	-19
142	Butachlor	11.089	23184-66-9	312.15	238.05	162.10	-12	-13	-25	-12	-22	-17
143	Clethodim	11.1	99129-21-2	362.00	345.00	302.95	-14	-11	-24	-14	-18	-21
144	Oxadiazon	11.114	19666-30-9	345.00	302.95	219.85	-13	-13	-21	-13	-20	-23
145	Chinomethionate	11.091	2439/1/2	235.00	207.15	163.05	-19	-16	-21	-19	-28	-16
146	Piperonyl butoxide	11.132	1951/3/6	356.30	177.10	119.00	-24	-13	-19	-24	-37	-22
147	Ethion	11.199	563-12-2	385.00	199.00	142.95	-19	-10	-22	-19	-26	-25
148	Pyriproxyfen	11.382	95737-68-1	322.10	96.05	185.05	-30	-14	-10	-30	-22	-20
149	Propargite	11.848	2312-35-8	368.10	231.10	175.05	-14	-11	-11	-14	-15	-17
150	Fenpyroximate-E	12.226	111812-58-9	422.20	366.10	138.10	-30	-15	-26	-30	-33	-26
151	Pyridaben	12.83	96489-71-3	365.10	309.05	147.10	-18	-12	-22	-18	-25	-27
152	Tau-Fluvalinate	13.07	102851-06-9	503.05	181.05	208.00	-40	-30	-18	-40	-13	-22
153	Prothiophos	13.229	34643-46-4	344.90	240.85	268.90	-13	-18	-24	-13	-13	-18
154	Etofenprox	14.659	80844-07-1	394.00	177.10	106.95	-19	-17	-20	-19	-40	-19

*Note: Atrazine D5 is internal standard. All interface voltages have been optimized and are different in optimal interface voltages of tune methods of some analytes.

Calibration curve and linearity

According to the analytical conditions in previously, the prepared standard solutions at different concentrations of 0.5 μg/L, 1.0 μg/L, 2.0 μg/L, 5.0 μg/L, 10.0 μg/L, 20.0 μg/L, 50.0 μg/L, 100.0 μg/L and 200.0 μg/L were determined. The Atrazine D5 was used as internal standard and the calibration curve was plotted with internal standard method, showing good linearity. Refer to Table 3 for linear equation, correlation coefficient and linear range.

Precision Test

Mixed standard solutions of 10 μg/L were consecutively injected for 6 times to evaluate its precision, and the repeatability results of retention time and peak area is shown in Table 4. The relative standard deviations of retention time and peak area were 0.06–1.19% and 0.93–8.46% respectively, showing good precision.

Table 3 Parameters of calibration curve, limit of detection and limit of quantification for 153 pesticides

No.	Compound Name	Calibration Curve	Correlation Coefficient (R)	Linear Range (μg/L)	Limit of Detection (μg/L)	Limit of Quantitation (μg/L)
1	Methamidophos	Y = (0.0173353) X + (-0.00180141)	0.9991	0.5-200	0.047	0.143
2	Acephate	Y = (0.0218573) X + (-0.00214913)	0.9997	0.5-200	0.137	0.414
3	Omethoate	Y = (0.0532375) X + (-0.00193726)	0.9992	0.5-200	0.005	0.014
4	Aldicarb-sulfoxide	Y = (0.00969561) X + (-0.000378735)	0.9989	0.5-200	0.047	0.144
5	Aldicarb-sulfone	Y = (0.0900362) X + (-0.00172303)	0.9975	0.5-200	0.359	1.086
6	Oxamyl	Y = (0.0556160) X + (-0.00190643)	0.9992	0.5-200	0.004	0.012
7	Phorate-oxon	Y = (0.0957374) X + (-0.00290804)	0.9982	0.5-200	0.060	0.181
8	Fenchlorphos-oxon	Y = (0.107259) X + (-0.00773397)	0.9988	5-200	1.068	3.237
9	Alachlor	Y = (0.00322822) X + (8.05752e-006)	0.9965	0.5-200	0.184	0.557
10	Fenthion	Y = (0.00142541) X + (0.00128385)	0.9983	0.5-200	0.138	0.419
11	Tolclofos methyl	Y = (0.00141366) X + (0.00128898)	0.9955	2-200	0.237	0.717
12	Clethodim	Y = (0.00519241) X + (0.00287075)	0.9967	2-200	0.312	0.945
13	Oxadiazon	Y = (0.00539541) X + (0.000460442)	0.9959	0.5-200	0.199	0.603
14	Prothiophos	Y = (0.00292960) X + (-0.000150432)	0.9978	0.5-200	0.042	0.128
15	Methomyl	Y = (0.0227831) X + (-0.000448149)	0.9961	0.5-50	0.062	0.188
16	Thiamethoxam	Y = (0.0470597) X + (0.00247671)	0.998	0.5-200	0.026	0.079
17	Methacrifos	Y = (0.0194474) X + (-0.000454077)	0.9989	0.5-200	0.035	0.107
18	Monocrotophos	Y = (0.00552705) X + (-0.00109676)	0.9956	0.5-200	0.061	0.186
19	Carbendazim	Y = (0.0181203) X + (-0.00573927)	0.9996	1-200	0.325	0.986
20	Dicrotophos	Y = (0.00841206) X + (0.000945306)	0.9974	0.5-200	0.039	0.118
21	Imidacloprid	Y = (0.0159962) X + (-0.000918301)	0.9973	0.5-200	0.037	0.112
22	Thiabendazole	Y = (0.0556340) X + (-0.00331513)	0.9993	0.5-200	0.056	0.169
23	Phosphoric acid	Y = (0.0176711) X + (0.000424968)	0.9994	5-200	0.335	1.014
24	Mevinphos	Y = (0.00574826) X + (-0.000599411)	0.9966	0.5-200	0.057	0.172
25	Fenthion-oxon-sulfoxide	Y = (0.00185623) X + (-0.000842991)	0.9991	1-200	0.044	0.134
26	Carbofuran-3-hydroxy	Y = (0.00566732) X + (-0.000319504)	0.9952	0.5-200	0.036	0.109
27	Acetamiprid	Y = (0.0718501) X + (0.000675243)	0.9983	0.5-200	0.043	0.129
28	Dimethoate	Y = (0.0726079) X + (0.00225958)	0.9984	0.5-200	0.036	0.108
29	Trichlorfon	Y = (0.00336367) X + (-0.00238820)	0.9961	1-200	0.128	0.386

30	Phorate-oxon-sulfone	Y = (0.0103364) X + (-0.00102264)	0.9973	0.5-200	0.011	0.033
31	Desmethylnitrophos	Y = (0.00289893) X + (-0.000112794)	0.9974	5-200	0.183	0.555
32	Thiacloprid	Y = (0.103314) X + (0.00214825)	0.9991	0.5-100	0.040	0.122
33	Fensulfothion-oxon	Y = (0.0238216) X + (-0.00411365)	0.999	0.5-200	0.040	0.122
34	Tricyclazole	Y = (0.0444021) X + (-0.00149661)	0.9996	0.5-200	0.043	0.130
35	Aldicarb	Y = (0.000610067) X + (-0.000137804)	0.9951	1-200	0.052	0.157
36	Oxadixyl	Y = (0.0551181) X + (-0.00214564)	0.998	0.5-200	0.036	0.109
37	Phosphamidon	Y = (0.0541566) X + (0.00131704)	0.999	0.5-200	0.046	0.139
38	Metolcarb	Y = (0.00636940) X + (0.000327561)	0.996	0.5-200	0.029	0.087
39	Fenamiphos-sulfoxide	Y = (0.0101179) X + (-0.00258725)	0.9988	0.5-200	0.040	0.121
40	Propoxur	Y = (0.0646245) X + (0.00482929)	0.9993	0.5-200	0.031	0.093
41	Malaoxon	Y = (0.122202) X + (0.00438514)	0.9986	0.5-100	0.034	0.102
42	Carbofuran	Y = (0.0996865) X + (0.00603730)	0.9993	0.5-100	0.056	0.169
43	Fenamiphos-sulfone	Y = (0.00833445) X + (-0.000746579)	0.9981	0.5-200	0.016	0.047
44	Hexazinone	Y = (0.349242) X + (0.0139541)	0.9951	0.5-50	0.027	0.083
45	Fenthion-sulfone	Y = (0.0442730) X + (0.00136725)	0.9986	2-200	0.104	0.315
46	Metribuzine	Y = (0.0199684) X + (-0.000446527)	0.996	0.5-100	0.030	0.092
47	Imazalil	Y = (0.0332150) X + (-0.000936020)	0.9988	0.5-200	0.063	0.192
48	Fenthion-sulfoxide	Y = (0.0300062) X + (-0.00159435)	0.9956	0.5-100	0.038	0.116
49	Pirimicarb	Y = (0.114895) X + (-0.00145338)	0.9992	0.5-100	0.041	0.124
50	Propanil	Y = (0.00138421) X + (-0.000598214)	0.9972	2-200	0.214	0.648
51	Carbaryl	Y = (0.0118318) X + (0.000443472)	0.9989	0.5-200	0.024	0.071
52	Ethiofencarb	Y = (0.0569713) X + (-0.00327528)	0.9994	0.5-200	0.041	0.125
53	Fosthiazate	Y = (0.103612) X + (-0.00283316)	0.9981	0.5-200	0.016	0.049
54	Disulfoton-sulfoxide	Y = (0.0621498) X + (-0.00239611)	0.9993	0.5-200	0.025	0.076
55	Disulfoton-sulfone	Y = (0.0149672) X + (-0.000427309)	0.9991	0.5-200	0.016	0.048
56	Phorate Sulfone	Y = (0.00532230) X + (-0.00248869)	0.9987	1-200	0.011	0.034
57	Isoprocarb	Y = (0.0138536) X + (0.000705791)	0.9962	0.5-200	0.013	0.039
58	Fensulfothion-oxon-sulfone	Y = (0.0105975) X + (-0.000313356)	0.995	0.5-200	0.222	0.674
59	Fensulfothion	Y = (0.0250156) X + (-0.00232943)	0.9978	0.5-200	0.081	0.246
60	Atrazine	Y = (0.0510443) X + (0.00102152)	0.9995	0.5-200	0.021	0.063
61	Metalaxyl	Y = (0.124794) X + (0.00161438)	0.9998	0.5-100	0.031	0.093
62	Fensulfothion-sulfone	Y = (0.00689199) X + (-0.00130067)	0.9969	0.5-200	0.021	0.065
63	Pirimiphos-methyl-N-desethyl	Y = (0.0379220) X + (-0.00172146)	0.9989	0.5-200	0.030	0.091
64	Chlorantraniliprole	Y = (0.0458920) X + (0.00163813)	0.9977	0.5-200	0.016	0.047
65	Methidathion	Y = (0.0123281) X + (-0.00298717)	0.9979	0.5-200	0.062	0.188
66	Fenthion-oxon	Y = (0.0259217) X + (-0.000930436)	0.9987	0.5-200	0.075	0.228
67	Azinphos-methyl	Y = (0.00652514) X + (0.000774298)	0.9967	0.5-200	0.059	0.177
68	Azoxystrobin	Y = (0.268560) X + (0.000189122)	0.9994	0.5-100	0.020	0.062
69	Phosemet	Y = (0.0304063) X + (0.00234112)	0.9981	0.5-200	0.038	0.115
70	Ametryn	Y = (0.142928) X + (-0.00809511)	0.9994	0.5-100	0.055	0.167

71	Demeton	Y = (0.00285777) X + (-0.000808611)	0.9983	0.5-200	0.040	0.122
72	Methiocarb	Y = (0.0315795) X + (-0.00304621)	0.9989	0.5-200	0.031	0.095
73	Dimethenamid	Y = (0.130715) X + (-0.00309240)	0.9987	0.5-100	0.043	0.132
74	Boscalid	Y = (0.0179079) X + (0.000850326)	0.9983	0.5-200	0.094	0.284
75	Flutolanil	Y = (0.0852652) X + (-0.00341973)	0.9995	0.5-200	0.013	0.039
76	Promecarb	Y = (0.0265943) X + (-0.00191192)	0.9971	0.5-200	0.012	0.036
77	Paclobutrazol	Y = (0.103195) X + (0.00837101)	0.9974	0.5-100	0.013	0.039
78	Methoxyfenozide	Y = (0.0626557) X + (-0.00250302)	0.9965	0.5-100	0.013	0.038
79	Propetamphos	Y = (0.0224913) X + (-0.000776337)	0.9995	2-200	0.156	0.472
80	Malathion	Y = (0.0334706) X + (0.000253787)	0.996	0.5-200	0.014	0.042
81	Isoprothiolane	Y = (0.208073) X + (0.00147689)	0.997	0.5-100	0.007	0.022
82	Mepronil	Y = (0.147294) X + (-0.0133369)	0.9996	0.5-100	0.009	0.026
83	Myclobutanil	Y = (0.0340615) X + (0.000242268)	0.9966	0.5-200	0.008	0.025
84	Triazophos	Y = (0.286234) X + (-0.00522228)	0.9996	0.5-50	0.002	0.006
85	Bifenazate	Y = (0.00121915) X + (-0.00183178)	0.998	5-200	0.948	2.872
86	Dichlofenthion	Y = (0.000442886) X + (-0.000290401)	0.9968	2-200	0.536	1.624
87	Isazofos	Y = (0.0467123) X + (0.000751323)	0.998	0.5-200	0.033	0.100
88	Molinate	Y = (0.00189652) X + (-0.000502486)	0.9951	1-200	0.041	0.125
89	Azinphos-ethyl	Y = (0.00570105) X + (-0.000550611)	0.9963	0.5-200	0.084	0.254
90	Mecarbam	Y = (0.0255949) X + (-0.000567316)	0.9997	1-100	0.026	0.079
91	Fenarimol	Y = (0.0209948) X + (0.000926640)	0.996	0.5-100	0.076	0.231
92	Ethoprophos	Y = (0.0295620) X + (-9.52104e-005)	0.9987	0.5-200	0.034	0.102
93	Napropamide	Y = (0.0527870) X + (-0.00348479)	0.9984	0.5-200	0.047	0.141
94	Iprodione	Y = (0.00637109) X + (-0.00144636)	0.9995	5-200	1.055	3.198
95	Paraoxon	Y = (0.00326359) X + (-0.000339883)	0.9991	1-200	0.100	0.304
96	Fenbuconazole	Y = (0.0483557) X + (0.00235492)	0.9955	0.5-100	0.048	0.146
97	Metolachlor	Y = (0.182702) X + (-0.00384197)	0.9998	0.5-100	0.040	0.121
98	Fenomiphos	Y = (0.0992224) X + (0.00328418)	0.9976	0.5-200	0.050	0.150
99	Flusilazole	Y = (0.101733) X + (0.00595385)	0.9958	0.5-100	0.035	0.105
100	Tebufenozid	Y = (0.0615690) X + (0.00568526)	0.9989	0.5-200	0.027	0.081
101	Phenthoate	Y = (0.0194230) X + (-0.00130668)	0.9995	0.5-200	0.052	0.159
102	Isofenphos-methyl	Y = (0.00449961) X + (-0.000780418)	0.9977	0.5-200	0.088	0.268
103	Quinalphos	Y = (0.0504904) X + (-0.000486238)	0.9992	0.5-200	0.034	0.103
104	Sulfotep	Y = (0.0302779) X + (-0.00355803)	0.9993	0.5-200	0.020	0.061
105	Tolylfluanid	Y = (0.00626669) X + (-0.000634101)	0.9973	0.5-200	0.012	0.036
106	Edifenphos	Y = (0.0580984) X + (0.00183308)	0.9994	0.5-200	0.016	0.049
107	Cyprodinil	Y = (0.0151117) X + (-0.00149677)	0.9981	0.5-200	0.116	0.351
108	Tebuconazole	Y = (0.0681602) X + (-0.000840069)	0.9979	0.5-200	0.023	0.068
109	Benalaxyl	Y = (0.130753) X + (-0.00215157)	0.9967	0.5-200	0.006	0.017
110	Etrimfos	Y = (0.0563576) X + (0.00187836)	0.9991	0.5-200	0.012	0.035
111	Chlorfenvinphos	Y = (0.0158212) X + (-0.00117776)	0.9967	0.5-200	0.044	0.133
112	Coumaphos	Y = (0.0170368) X + (-0.00225346)	0.9996	0.5-200	0.091	0.276
113	Diazinon	Y = (0.0895419) X + (-0.00135594)	0.9988	0.5-200	0.015	0.046
114	Propiconazole	Y = (0.0717623) X + (0.00116199)	0.9994	0.5-100	0.021	0.064

115	Fonofos	Y = (0.00893680) X + (-0.000476958)	0.9983	0.5-200	0.118	0.356
116	Pyraclostrobin	Y = (0.118964) X + (0.00544848)	0.9978	0.5-200	0.027	0.080
117	Phoxim	Y = (0.0320844) X + (-0.00101591)	0.9991	0.5-200	0.052	0.158
118	Prochloraz	Y = (0.148279) X + (-0.00697379)	0.9994	0.5-100	0.023	0.070
119	Hexaconazole	Y = (0.0690465) X + (0.00217636)	0.9974	0.5-50	0.018	0.054
120	Bitertanol	Y = (0.0713641) X + (-0.00116535)	0.9957	0.5-100	0.039	0.119
121	Isofenphos	Y = (0.00688474) X + (-0.000950220)	0.9999	0.5-200	0.022	0.065
122	Phosalone	Y = (0.0136274) X + (0.000618539)	0.9989	0.5-200	0.025	0.077
123	Pirimiphos methyl	Y = (0.0516377) X + (-0.00136225)	0.9985	0.5-200	0.015	0.047
124	Disulfoton	Y = (0.0250855) X + (-0.000588861)	0.9993	2-200	0.269	0.815
125	Phorate	Y = (0.00623685) X + (-0.000838612)	0.9961	0.5-200	0.022	0.067
126	Cyhalofop-butyl	Y = (0.00395028) X + (-0.000363730)	0.997	5-200	0.252	0.763
127	Diniconazole	Y = (0.0438988) X + (0.00285677)	0.9956	0.5-200	0.062	0.187
128	Cadusafos	Y = (0.116215) X + (-0.00436567)	0.9989	0.5-200	0.014	0.041
129	EPN	Y = (0.0208078) X + (-0.000862546)	0.9991	2-200	0.085	0.259
130	Difenoconazole	Y = (0.158463) X + (0.00968258)	0.9988	0.5-200	0.079	0.240
131	Indoxacarb	Y = (0.00625472) X + (0.000853360)	0.9978	0.5-200	0.071	0.215
132	Haloxyfop-methyl	Y = (0.0297634) X + (-0.00129085)	0.9986	0.5-200	0.012	0.037
133	Trifloxystrobin	Y = (0.197587) X + (0.00438362)	0.9969	0.5-200	0.012	0.037
134	Pretilachlor	Y = (0.130854) X + (-0.00478905)	0.9988	0.5-200	0.036	0.109
135	Profenofos	Y = (0.00950039) X + (-0.000836209)	0.9958	0.5-200	0.032	0.097
136	Fluazifop-p-butyl	Y = (0.0322093) X + (0.00192199)	0.9983	0.5-200	0.005	0.015
137	Furathiocarb	Y = (0.0830947) X + (-0.000832066)	0.9992	0.5-200	0.005	0.015
138	Tetramethrin	Y = (0.0136805) X + (-0.000213357)	0.9977	0.5-200	0.086	0.261
139	Buprofezin	Y = (0.178774) X + (-0.00337155)	0.9988	0.5-100	0.007	0.022
140	Pirimiphos-ethyl	Y = (0.230854) X + (-0.00144221)	0.9959	0.5-100	0.008	0.025
141	Allethrin	Y = (0.00224286) X + (-0.000607295)	0.9984	1-200	0.037	0.113
142	Butachlor	Y = (0.0163092) X + (-0.00268724)	0.9973	0.5-200	0.058	0.174
143	Piperonyl butoxide	Y = (0.310692) X + (-0.00373548)	0.9999	0.5-50	0.013	0.039
144	Ethion	Y = (0.0459216) X + (-0.00112365)	0.9997	0.5-200	0.006	0.019
145	Chinomethionate	Y = (0.00946778) X + (-0.000884029)	0.9991	5-200	0.155	0.469
146	Pyriproxyfen	Y = (0.0436088) X + (-0.00272298)	0.9994	0.5-100	0.016	0.047
147	Propargite	Y = (0.0436088) X + (-0.00272298)	0.9985	0.5-200	0.007	0.022
148	Fenpyroximate-E	Y = (0.342880) X + (-0.00421878)	0.9996	0.5-50	0.008	0.024
149	Pyridaben	Y = (0.205135) X + (0.000458842)	0.9975	0.5-100	0.009	0.026
150	Tau-fluvalinate	Y = (0.00217148) X + (-7.99458e-005)	0.998	0.5-200	0.012	0.036
151	Etofenprox	Y = (0.0815944) X + (0.00239759)	0.9981	0.5-200	0.010	0.031
152	RH5849	Y = (0.00217439) X + (-0.00212373)	0.9951	2-200	0.071	0.214
153	Diflubenzuron	Y = (0.0342957) X + (-0.00300925)	0.9988	0.5-200	0.003	0.008

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

No.	Compound Name	RSD% R.T.	RSD% Area	No.	Compound Name	RSD% R.T.	RSD% Area
1	Methamidophos	1.19	2.36	**40**	Propoxur	0.07	2.47
2	Acephate	0.59	1.90	**41**	Malaoxon	0.05	1.95
3	Omethoate	0.50	1.43	**42**	Carbofuran	0.07	3.56
4	Aldicarb-sulfoxide	0.45	1.23	**43**	Fenamiphos-sulfone	0.05	4.12
5	Aldicarb-sulfone	0.35	2.06	**44**	Hexazinone	0.06	1.83
6	Oxamyl	0.35	1.13	**45**	Fenthion-sulfone	0.05	5.01
7	Phorate-oxon	0.06	1.88	**46**	Metribuzine	0.08	3.22
8	Fenchlorphos-oxon	0.15	6.66	**47**	Imazalil	0.08	2.32
9	Alachlor	0.21	3.65	**48**	Fenthion-sulfoxide	0.08	3.01
10	Fenthion	0.21	3.65	**49**	Pirimicarb	0.08	1.59
11	Tolclofos methyl	0.14	2.16	**50**	Propanil	0.10	4.55
12	Clethodim	0.36	4.48	**51**	Carbaryl	0.06	4.40
13	Oxadiazon	0.30	4.74	**52**	Ethiofencarb	0.06	2.03
14	Prothiophos	0.09	4.95	**53**	Fosthiazate	0.07	3.73
15	Methomyl	0.21	4.80	**54**	Disulfoton-sulfoxide	0.06	1.62
16	Thiamethoxam	0.21	2.92	**55**	Disulfoton-sulfone	0.06	4.47
17	Methacrifos	0.19	2.38	**56**	Phorate Sulfone	0.05	5.07
18	Monocrotophos	0.26	4.97	**57**	Isoprocarb	0.08	4.56
19	Carbendazim	0.25	3.70	**58**	Fensulfothion-oxon-sulfone	0.08	4.76
20	Dicrotophos	0.19	3.37	**59**	Fensulfothion	0.06	1.46
21	Imidacloprid	0.15	1.50	**60**	Atrazine	0.06	1.76
22	Thiabendazole	0.15	1.96	**61**	Metalaxyl	0.06	1.68
23	Phosphoric acid	0.07	6.80	**62**	Fensulfothion-sulfone	0.11	2.43
24	Mevinphos	0.16	4.79	**63**	Pirimiphos-methyl-N-desethyl	0.08	1.58
25	Fenthion-oxon-sulfoxide	0.13	5.22	**64**	Chlorantraniliprole	0.08	4.30
26	Carbofuran-3-hydroxy	0.13	5.22	**65**	Methidathion	0.05	4.00
27	Acetamiprid	0.13	2.02	**66**	Fenthion-oxon	0.07	3.95
28	Dimethoate	0.13	1.84	**67**	Azinphos-methyl	0.06	4.25
29	Trichlorfon	0.14	4.54	**68**	Azoxystrobin	0.06	2.17
30	Phorate-oxon-sulfone	0.12	4.13	**69**	Phosemet	0.07	3.83
31	Desmethylnitrophos	0.13	4.44	**70**	Ametryn	0.06	1.73
32	Thiacloprid	0.10	0.95	**71**	Demeton	0.07	3.70
33	Fensulfothion-oxon	0.09	2.02	**72**	Methiocarb	0.04	4.42
34	Tricyclazole	0.09	2.01	**73**	Dimethenamid	0.06	1.77
35	Aldicarb	0.06	9.94	**74**	Boscalid	0.08	4.04
36	Oxadixyl	0.08	3.32	**75**	Flutolanil	0.07	3.12
37	Phosphamidon	0.07	2.28	**76**	Promecarb	0.07	4.42
38	Metolcarb	0.07	2.28	**77**	Paclobutrazol	0.07	1.37
39	Fenamiphos-sulfoxide	0.05	3.03	78	Methoxyfenozide	0.08	3.84
79	Malathion	0.08	3.27	117	Phoxim	0.08	4.04
80	Propetamphos	0.06	6.64	118	Prochloraz	0.08	1.27
81	Isoprothiolane	0.07	0.93	119	Hexaconazole	0.08	1.45

82	Mepronil	0.08	1.72
83	Myclobutanil	0.09	3.30
84	Triazophos	0.10	1.94
85	Bifenazate	0.15	2.82
86	Dichlofenthion	0.10	4.46
87	Isazofos	0.09	2.39
88	Molinate	0.12	4.63
89	Azinphos-ethyl	0.14	2.18
90	Mecarbam	0.10	3.81
91	Fenarimol	0.12	4.93
92	Ethoprophos	0.10	3.00
93	Napropamide	0.13	2.13
94	Paraoxon	0.08	4.12
95	Iprodione	0.11	6.74
96	Fenbuconazole	0.13	2.98
97	Metolachlor	0.12	1.75
98	Fenomiphos	0.13	1.08
99	Flusilazole	0.13	1.71
100	Tebufenozid	0.12	3.55
101	Phenthoate	0.13	2.37
102	Isofenphos-methyl	0.17	4.66
103	Quinalphos	0.11	2.76
104	Sulfotep	0.13	4.29
105	Tolylfluanid	0.15	4.13
106	Edifenphos	0.11	2.59
107	Cyprodinil	0.14	3.56
108	Tebuconazole	0.11	1.84
109	Benalaxyl	0.10	1.79
110	Etrimfos	0.10	1.03
111	Chlorfenvinphos	0.12	4.07
112	Coumaphos	0.07	2.21
113	Diazinon	0.09	1.29
114	Propiconazole	0.10	2.43
115	Fonofos	0.10	3.36
116	Pyraclostrobin	0.09	0.92
120	Bitertanol	0.09	1.74
121	Isofenphos	0.06	4.81
122	Phosalone	0.08	3.53
123	Pirimiphos methyl	0.07	2.24
124	phorate	0.12	2.47
125	Disulfoton	0.06	6.71
126	Diniconazole	0.10	3.36
127	Cyhalofop-butyl	0.12	8.46
128	Cadusafos	0.12	0.74
129	Difenoconazole	0.13	3.04
130	EPN	0.05	4.68
131	Indoxacarb	0.15	3.70
132	Haloxyfop-methyl	0.16	4.03
133	Trifloxystrobin	0.18	1.26
134	Pretilachlor	0.20	2.11
135	profenofos	0.29	4.91
136	Fluazifop-p-butyl	0.32	2.71
137	Furathiocarb	0.30	1.91
138	Tetramethrin	0.31	2.73
139	Buprofezin	0.31	1.62
140	Pirimiphos-ethyl	0.31	0.62
141	Allethrin	0.29	4.85
142	Butachlor	0.32	3.20
143	Piperonyl butoxide	0.31	1.08
144	Ethion	0.31	1.88
145	Pyriproxyfen	0.30	1.13
146	Chinomethionate	0.05	4.82
147	Propargite	0.23	1.03
148	Fenpyroximate-E	0.17	1.15
149	Pyridaben	0.13	1.05
150	Tau-fluvalinate	0.08	2.57
151	Etofenprox	0.05	0.7
152	RH5849	0.09	4.91
153	Diflubenzuron	0.14	2.46

Spike Experiment of Samples

According to the sample preparation method, the mixed standard was added into the purified blank samples with spiked concentration of 10 μg/kg and injection concentration of 10 μg/L. The chromatogram of the matrix spike sample was shown in Figure 2.

CONCLUSION

The 153 pesticides listed in Method 2341 of Chinese Pharmacopoeia (2015 Edition) are readily analyzed with a Shimadzu LCMS-8045 Triple Quadrupole Liquid Chromatograph Mass Spectrometer. The described method can separate 153 pesticides within 22 min. The linearity was good and the correlation coefficients of

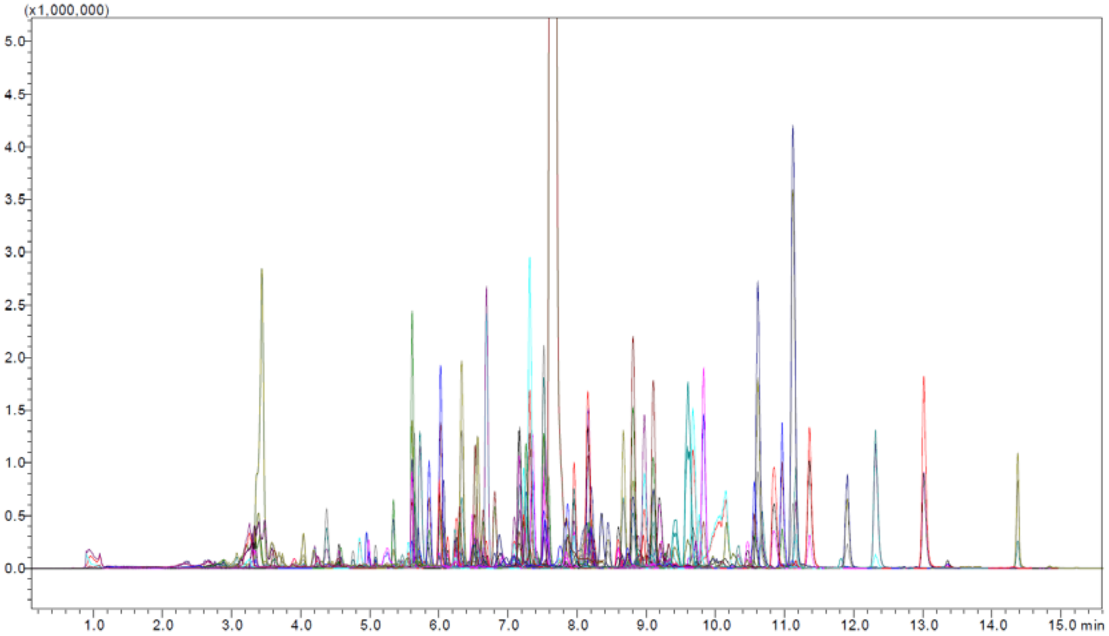

Figure 2 Chromatogram of matrix spike samples (injection concentration of 10 µg/L)

calibration curve were all greater than 0.995 in the range of 0.5–200 µg/L. This method is fast and ensures excellent repeatability and high sensitivity, which is applicable to the highly sensitive and rapid detection of pesticides in traditional Chinese medicines.

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LC-MS/MS Compositional Analysis of Three Culture Media for Antibody Drug Production

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Application News
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Abstract

This paper establishes a method for the determination of 95 compounds in culture media for antibody drug production using a Shimadzu Ultra-High Performance Liquid Chromatograph LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. This method is able to analyze 95 different compounds in 17 minutes. With the high speed, good repeatability and high sensitivity of LCMS-8045, the described method is suitable for the detection of amino acids, vitamins and other compounds in culture media intended for antibody drug production. The method can quickly identify the compositional differences among the various types and batches of culture media. In doing so, it increases production efficiency by enabling the selection of the appropriate media according to the antibody production requirements.

Large-scale culturing of animal cells and antibody quality analysis have become the main limiting factors for the industrialization and development of antibody drugs. As a key link in the large-scale culturing of animal cells, the optimization of culture media has a particularly important effect on antibody quality. Furthermore, the development of culture media is widely known to be closely related to the characteristics of the production cell line. Therefore, the design and optimization of a customized culture media shall be based on the characteristics of the production cell line during culture media development. This results in understanding each cell in the culture process, especially the consumption of various nutrients by each cell as well as the influence of its own secretions on cell production and reproduction. In order to expedite the rapid and comprehensive analysis of cell culture media components, the basic carbon sources, nitrogen sources, nucleotides, vitamins and other major metabolites were detected and analyzed to obtain more detailed information about the biological processes involved. From this data, we have developed a “Cell Culture Analysis Method Package”. The described method utilizes an Ultra-High Performance Liquid Chromatograph (UHPLC) Triple Quadrupole Mass Spectrometer, which takes only 17 minutes to simultaneously monitor and analyze the relatively abundance of 95 nutrients and metabolites in cell culture media.

With the use of Shimadzu “Cell Culture Analysis Method Package”, UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045, this paper established a method for the simultaneous LC-MS/MS compositional analysis of nutrient substances and cell metabolites in culture media for antibody drug production.

EXPERIMENTAL

Instrumentation

Shimadzu Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045 are used in this experiment. The specific configurations are two LC-30AD pumps, DGU-20A$_5$ online degassing unit, SIL-30AC autosampler, CTO-30AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 Chromatography Workstation.

Analytical Conditions

<u>LC Chromatography (LC) Conditions</u>

Analytical Instrument	:	LC-30A system
Column & Mobile Phase	:	Refer to “Cell Culture Analysis Method Package”)
Flow rate	:	0.35 mL/min
Column Temp.	:	40 °C
Injection volume	:	1 µL
Elution method	:	Gradient elution, refer to “Cell Culture Analysis Method Package” for detailed gradient elution program

Food Analysis
Pharmaceutical Analysis
Environmental Analysis

Mass Spectrometry (MS) Conditions

Analytical Instrument	:	LCMS-8045
Ion Source	:	ESI, simultaneous scanning of positive and negative ions
Interface voltage of ion source	:	+4.0 kV; -3.0 kV
Nebulizing gas	:	Nitrogen 3.0 L/min
Drying gas	:	Nitrogen 15.0 L/min
Heating gas	:	Air 10.0 L/min
Collision gas	:	Argon
DL temp.	:	250 °C
Heater temp.	:	400 °C
Interface temp.	:	300 °C
Scanning mode	:	Multiple reaction monitoring
Delay time	:	4.0 ms - 97.0 ms
MRM parameters	:	Refer to "Cell Culture Analysis Method Package"
Compounds	:	Indicated in Table 1

Culture Media

The three culture media for antibody drug production are simply labeled as 1, 2, and 3.

Data Processing

Software	:	Labsolutions Traverse MS

Sample Preparation

Sample pretreatment method: 500 μL of cell culture solution was centrifuged at room temperature and 3000 rpm for 1 minute. 100 μL of the supernatant was transferred into a new centrifuge tube. 20 μL of 0.5mmol/L 2-isopropyl malic acid solution (internal standard) and 200 μL of acetonitrile were added and vortexed. The mixture was centrifuged at room temperature and 15 000 rpm for 15 minutes. 100 μL of the supernatant was added to 900 μL of water. The solution was vortexed and diluted 10 times with pure water for LC-MS/MS analysis.

RESULTS AND DISCUSSION

Compositional Analysis of Culture Media Used for Antibody Drug Production

The culture media components for antibody drug production were analyzed according to the method described in the "Cell Culture Analysis Method Package". The target compounds are detected to different degrees and the chromatogram chart is shown in Figure 1.

Figure 1 Analysis chromatogram of 1# culture medium supernatant

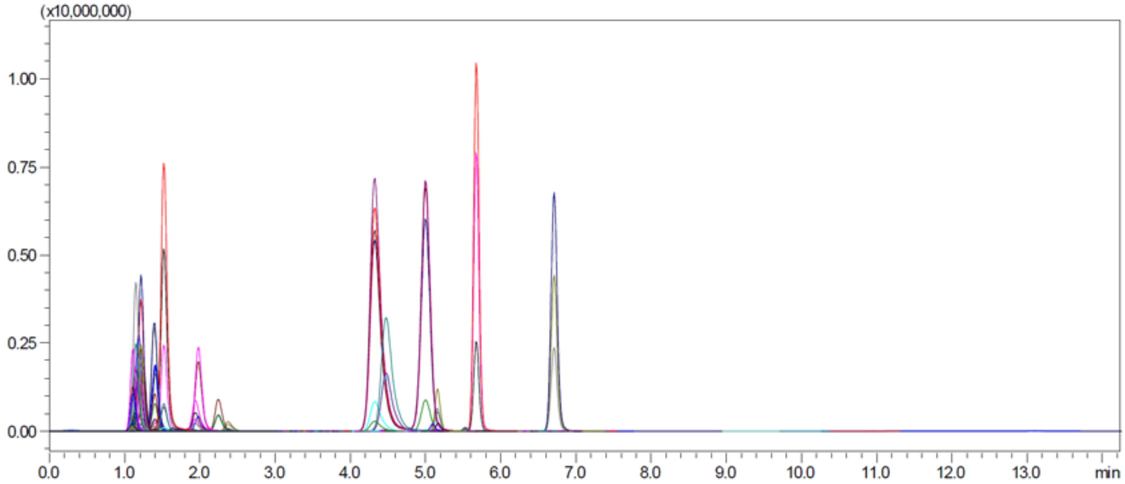

Table 1 96 Compounds in Cell Culture Method Package

No.	Compound Name	Category	No.	Compound Name	Category	No.	Compound Name	Category
1	2-Isopropylmalic acid	Internal standard	**33**	N-Acetylaspartic acid	Amino Acid	**65**	Cytidine	Nucleotide
2	Gluconic acid	Carbohydrate	**34**	N-Acetylcysteine	Amino Acid	**66**	Cytidine monophosphate	Nucleotide
3	Glucosamine Carbohydrate	Carbohydrate	**35**	Ornithine	Amino Acid	**67**	Deoxycytidine	Nucleotide
4	Hexose (Glucose) Carbohydrate	Carbohydrate	**36**	Oxidized glutathione	Amino Acid	**68**	Guanine	Nucleotide
5	Sucrose Carbohydrate	Carbohydrate	**37**	Phenylalanine	Amino Acid	**69**	Guanosine	Nucleotide
6	Threonic acid Carbohydrate	Carbohydrate	**38**	Pipecolic acid	Amino Acid	**70**	Guanosine monophosphate	Nucleotide
7	2-Aminoadipic acid	Amino Acid	**39**	Proline	Amino Acid	**71**	Hypoxanthine	Nucleotide
8	4-Aminobutyric acid	Amino Acid	**40**	Serine	Amino Acid	**72**	Inosine	Nucleotide
9	4-Hydroxyproline	Amino Acid	**41**	Threonine	Amino Acid	**73**	Thymidine	Nucleotide
10	5-Glutamylcysteine	Amino Acid	**42**	Tryptophan	Amino Acid	**74**	Thymine	Nucleotide
11	5-Oxoproline	Amino Acid	**43**	Tyrosine	Amino Acid	**75**	Uracil	Nucleotide
12	Alanine	Amino Acid	**44**	Valine	Amino Acid	**76**	Uric acid	Nucleotide
13	Alanyl-glutamine	Amino Acid	**45**	4-Aminobenzoic acid	Vitamin	**77**	Uridine	Nucleotide
14	Arginine	Amino Acid	**46**	Ascorbic acid	Vitamin	**78**	Xanthine	Nucleotide
15	Asparagine	Amino Acid	**47**	Ascorbic acid 2-phosphate	Vitamin	**79**	Xanthosine	Nucleotide
16	Aspartic acid	Amino Acid	**48**	Biotin	Vitamin	**80**	Penicillin G	Antibiotic
17	Citrulline	Amino Acid	**49**	Choline	Vitamin	**81**	2-Aminoethanol	Others
18	Cystathionine	Amino Acid	**50**	Cyanocobalamin	Vitamin	**82**	2-Ketoisovaleric acid	Others
19	Cysteine	Amino Acid	**51**	Ergocalciferol	Vitamin	**83**	3-Methyl-2-oxovaleric acid	Others
20	Cystine	Amino Acid	**52**	Folic acid	Vitamin	**84**	4-Hydroxyphenyllactic acid	Others
21	Glutamic acid	Amino Acid	**53**	Folinic acid	Vitamin	**85**	Citric acid	Others
22	Glutamine	Amino Acid	**54**	Lipoic acid	Vitamin	**86**	Ethylenediamine	Others
23	Glutathione	Amino Acid	**55**	Niacinamide	Vitamin	**87**	Fumaric acid	Others
24	Glycine	Amino Acid	**56**	Nicotinic acid	Vitamin	**88**	Glyceric acid	Others
25	Glycyl-glutamine	Amino Acid	**57**	Pantothenic acid	Vitamin	**89**	Histamine	Others
26	Histidine	Amino Acid	**58**	Pyridoxal	Vitamin	**90**	Isocitric acid	Others
27	Isoleucine	Amino Acid	**59**	Pyridoxine	Vitamin	**91**	Lactic acid	Others
28	Kynurenine	Amino Acid	**60**	Riboflavin	Vitamin	**92**	Malic acid	Others
29	Leucine	Amino Acid	**61**	Tocopherol acetate	Vitamin	**93**	O-Phosphoethanolamine	Others
30	Lysine	Amino Acid	**62**	Adenine	Nucleotide	**94**	Putrescine	Others
31	Methionine	Amino Acid	**63**	Adenosine	Nucleotide	**95**	Pyruvic acid	Others
32	Methionine sulfoxide	Amino Acid	**64**	Adenosine monophosphate	Nucleotide	**96**	Succinic acid	Others

Compositional Differences of Some Amino Acids, Vitamins and Other Compounds in Three Types of Culture Media for Antibody Drug Production

In Figures 2–4, the y-axis indicates the peak area ratio and the x-axis indicates the three types of culture medium samples. The results show negligible differences in the peak area ratios of corresponding amino acids, vitamins and other compounds for culture media 1 and 2. This indicates similar compositions and ratios between the two media, while there is a substantial difference between the culture medium 3 and culture media 1 and 2.

CONCLUSION

The compositional analysis of 95 compounds in three types of culture media used for antibody drug production was performed with the Shimadzu LCMS-8045 Triple Quadrupole Mass Spectrometer. The relative quantities of 95 compounds in the culture media were rapidly analyzed with the Shimadzu "Cell Culture Analysis Method Package". In this application news, the distribution of amino acids, vitamins and other compounds in the culture media are investigated and illustrated in detail in the form of graphs.

AMINO ACIDS

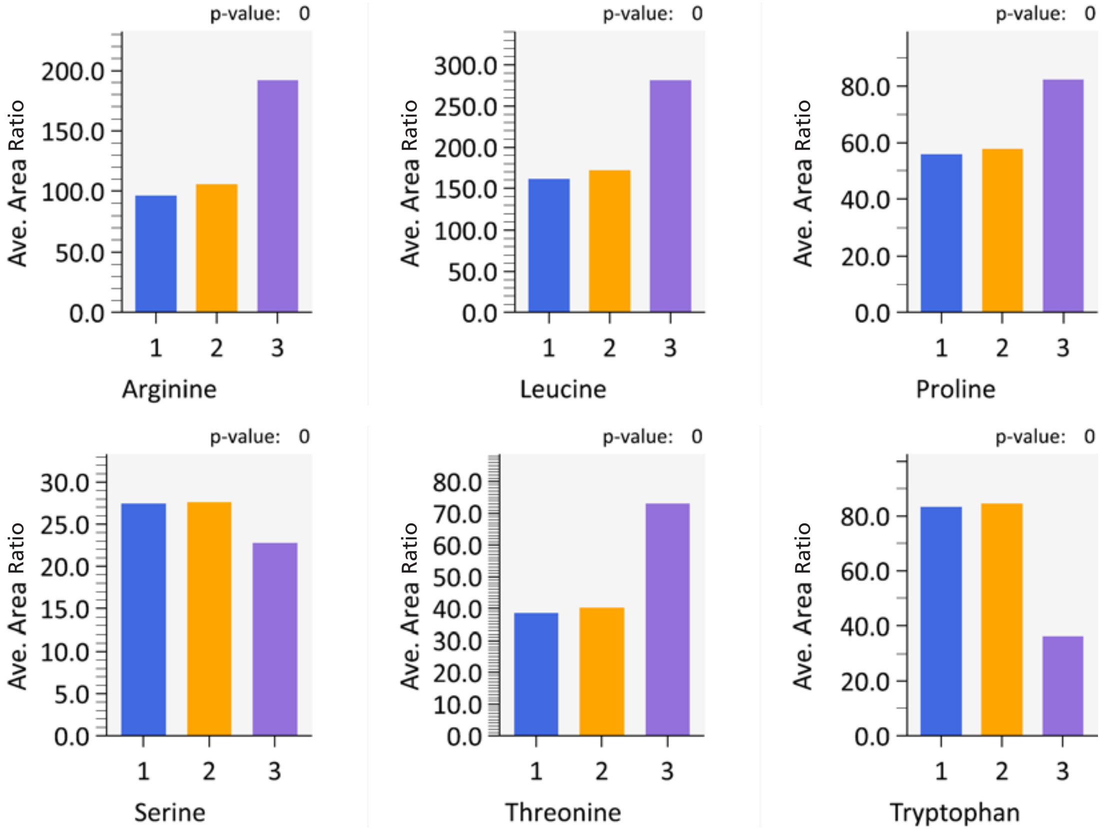

Figure 2 Relative Compositional Differences of Some Amino Acids in Three Types of Culture Media for Antibody Drug Production

VITAMIN COMPOUNDS

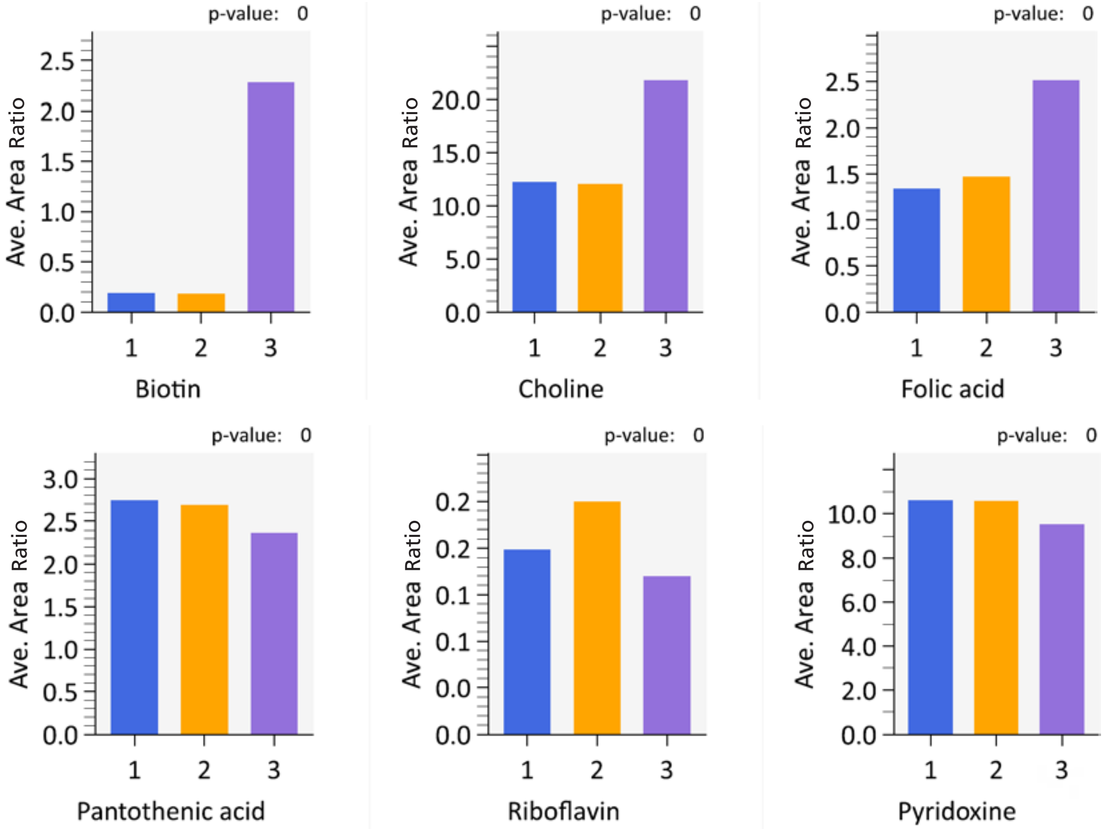

Figure 3 Relative Compositional Differences of Some Vitamins in Three Types of Culture Media for Antibody Drug Production

OTHER COMPOUNDS

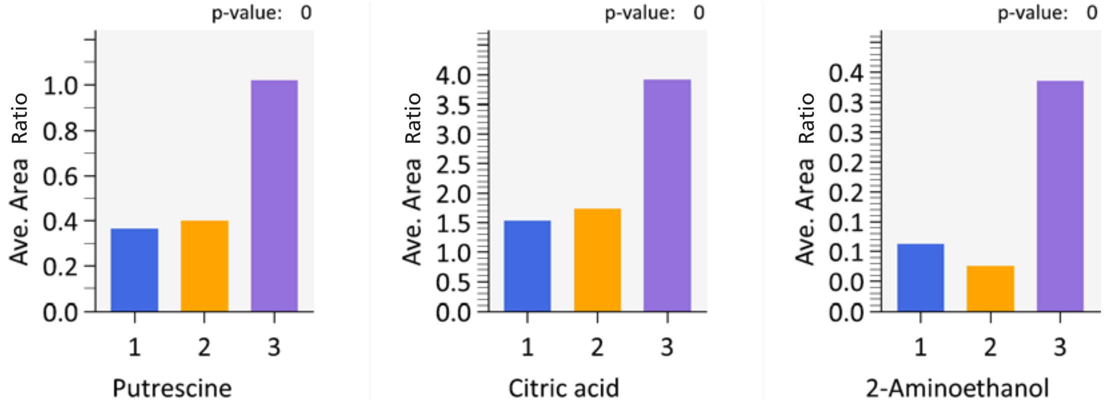

Figure 4 Relative Compositional Differences of Other Compounds in Three Kinds of Culture Media for Antibody Drug Production

Food Analysis
Pharmaceutical Analysis
Environmental Analysis

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Determination of Bile Acids in Cow-Bezoar Using LCMS-8045

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Application News
SSL-CA14-520

Abstract

This paper established a method for determination of bile acids (hyodeoxycholic acid, cholic acid, deoxycholic acid, chenodeoxycholic acid and ursodeoxycholic acid) in cow-bezoar using Shimadzu's UHPLC Nexera XR and Triple Quadrupole Mass Spectrometer LCMS-8045. The cow-bezoar samples were fully dissolved in methanol and separated using a Shim-pack XR-ODS III (2.0 mm I.D. × 75 mm L., 1.6 µm) column. The sample analysis was carried out in MRM mode. The results showed good linearity in the range of 20–2000 ng/mL for cholic acid and the four deoxycholic acids and the linear correlation coefficients were more than 0.997. The detection accuracy was in the range of 87.0 – 109.8%. After 6 consecutive injections of 40 ng/mL, the RSDs of retention time and peak area were 0.227 – 0.400% and 1.145 – 5.042% respectively. This method can be used for determination of bile acids in natural and artificial cow-bezoar.

Cow-bezoar, the dried gallstone of bovine, is a valuable traditional Chinese medicine that can eliminate toxins from the body, treat liver diseases, remove phlegm and calm the mind and body. The main components in cow-bezoar consists of cholic acid and bile acids.

Natural cow-bezoar is difficult to obtain. Therefore, China has developed artificially produced and artificially-cultivated bezoar. Artificial cow-bezoar is processed from bovine gall powder, cholic acid, hyodeoxycholic acid, taurine, bilirubin, cholesterol, trace elements and more.

The Chinese Pharmacopoeia (2015 Edition) only specified the content of cholic acids in cow-bezoar, and the use of thin-layer chromatography has difficulty distinguishing between artificial and natural cow-bezoar. In addition, there have been no reports on the separation and analysis of cholic and bile acids in cow-bezoar.

In this paper, a method for the quantitative analysis of hyodeoxycholic acid, cholic acid, deoxycholic acid, chenodeoxycholic acid and ursodeoxycholic acid in cow-bezoar was established with the use of Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC), Nexera XR, and Triple Quadrupole Mass Spectrometer, LCMS-8045. This method serves to provide a reference for quality control and method development for analysis of bile acids in cow-bezoar.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC Nexera XR and Triple Quadrupole Mass Spectrometer LCMS-8045. The configurations are two LC-20AD$_{XR}$ pumps, DGU-20A$_5$ on-line degassing unit, SIL-30ACMP autosampler, CTO-20AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86.002 Chromatography Workstation.

Analytical Conditions

LC Chromatography (LC) Conditions

Column	:	Shim-pack XR-ODS III (2.0 mm I.D. × 75 mm L., 1.6 µm)
Mobile phase	:	Mobile Phase A - 2 mM ammonium acetate in water Mobile Phase B - acetonitrile
Flow rate	:	0.30 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 µL
Elution method	:	Gradient elution; with initial concentration of Mobile Phase B at 60%. Refer to Table 1 for elution program

Food Analysis
Pharmaceutical Analysis
Environmental Analysis

Mass Spectrometry (MS) Conditions

Ion Source : ESI-
Nebulizing gas : 3.0 L/min
DL temp. : 250 °C
Interface temp. : 350 °C
Heater temp. : 500 °C
Heated gas flow rate : 12.0 L/min
Scan Mode : Multi reaction monitoring, parameters are shown in Table 2
Drying gas flow rate : 8.0 L/min
Dwell time : 50 ms
Delay time : 3ms

Sample Preparation Method

1 g of cow-bezoar powder was placed in a centrifuge tube and dissolved in an appropriate amount of methanol. After 5 min of sonication, the solution was diluted to a final volume of 10 ml using a 6:4 (v/v) methanol in water. It was further diluted 1000 times with the same diluent and injected for analysis.

Table 1 Gradient elution program

Time (min)	Module	Command	Value
5.00	Pumps	Pump B Conc.	90
6.00	Pumps	Pump B Conc.	90
6.10	Pumps	Pump B Conc.	60
12.00	Controller	Stop	

Table 2 MRM parameters

Compound Name	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Hyodeoxycholic acid	83-49-8	391.15	355.20*	13	39	14
		391.15	373.40	29	35	23
Cholic acid	81-25-4	407.10	289.10*	15	41	27
		407.10	389.10	12	36	21
Deoxycholic acid	83-44-3	391.15	373.40*	29	35	23
		391.15	355.20	13	39	14
Ursodeoxycholic acid	128-13-2	391.15	355.20*	13	39	14
		391.15	373.40	29	35	23
Chenodeoxycholic acid	474-25-9	391.15	355.20*	13	39	14
		391.15	373.40	29	35	23

Note: * quantification ion pairs

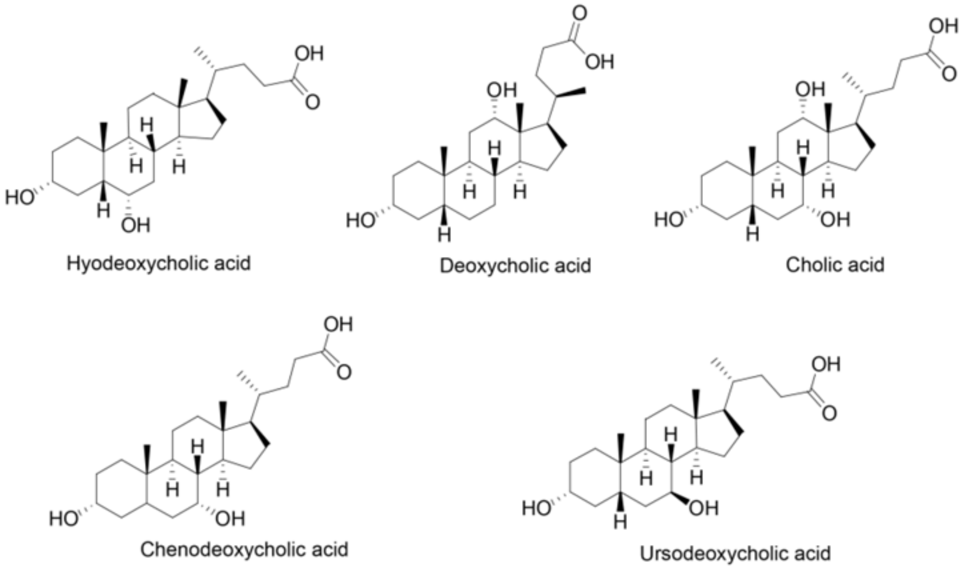

Hyodeoxycholic acid
Deoxycholic acid
Cholic acid
Chenodeoxycholic acid
Ursodeoxycholic acid

Figure 1 Structures of hyodeoxycholic acid, deoxycholic acid, cholic acid, chenodeoxycholic acid and ursodeoxycholic acid.

RESULTS AND DISCUSSION

Chromatogram of Standards

Figure 2 shows the MRM chromatograms of cholic acids and 4 bile acids.

Calibration Curve and Linear Range

Mixed standard working solutions at concentrations of 20 ng/mL, 40 ng/mL, 80 ng/mL, 400 ng/mL, 1000 ng/mL, and 2000 ng/mL were determined according to the analytical conditions specified in the experimental section and quantified using the external standard method. Calibration curves were established and shown in Figure 3. The linear equation, linear range, and correlation coefficients are shown in Table 3.

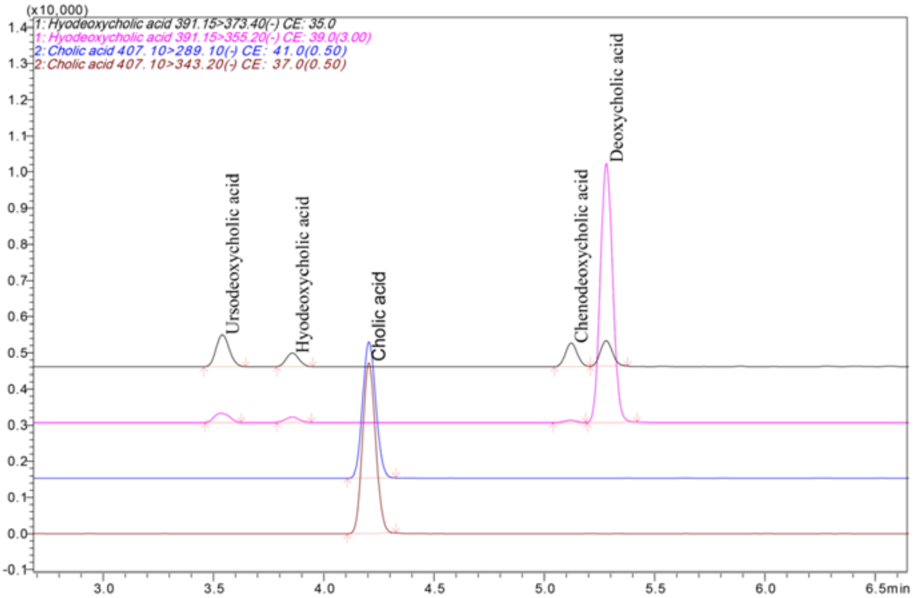
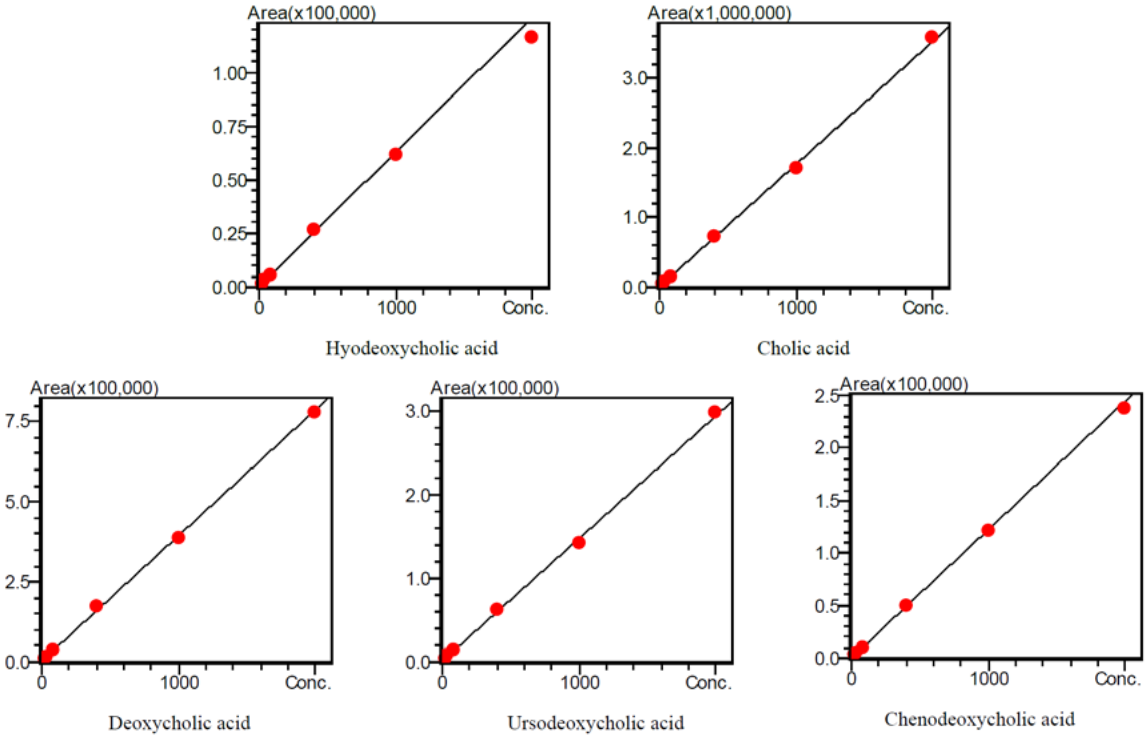


Figure 2 MRM chromatograms of hyodeoxycholic acid, deoxycholic acid, cholic acid, chenodeoxycholic acid and ursodeoxycholic acid (20 ng/ml).



Hyodeoxycholic acid

Cholic acid

Deoxycholic acid

Ursodeoxycholic acid

Chenodeoxycholic acid

Figure 3 Calibration curves for 5 cholic and bile acids

Table 3 Parameters of calibration curve

No.	Compound Name	Calibration Curve	Linear Range (ng/mL)	Correlation Coefficient (R)	Accuracy (%)
1	Hyodeoxycholic acid	Y = (62.6764) X + (333.395)	20-2000	0.9979	92.4-107.9
2	Cholic acid	Y = (1763.19) X + (4359.56)	20-2000	0.9997	96.0-103.9
3	Deoxycholic acid	Y = (392.484) X + (2570.14)	20-2000	0.9992	87.0-109.8
4	Ursodeoxycholic acid	Y = (146.490) X + (1080.33)	20-2000	0.9996	91.3-107.6
5	Chenodeoxycholic acid	Y = (122.303) X + (162.162)	20-2000	0.9990	96.5-108.6

Sensitivity Test

The mixed standard solution at a concentration of 20 ng/mL was injected and analyzed to calculate the lower limit of detection (S/N=3, LOD) and the lower limit of quantitation (S/N=10, LOQ) of the 5 cholic and bile acids. The results are shown in Table 4.

Table 4 Limit of detection and limit of quantitation

No.	Compound Name	LOD (ng/mL)	LOQ (ng/mL)
1	Hyodeoxycholic acid	1.25	3.80
2	Cholic acid	0.03	0.10
3	Deoxycholic acid	0.01	0.02
4	Ursodeoxycholic acid	0.55	1.67
5	Chenodeoxycholic acid	0.68	2.07

Repeatability

The mixed standard solution at a concentration of 40 ng/mL was analyzed by six consecutive injections. The relative standard deviations (RSDs) for the retention time and peak area of each compound are shown in Table 5.

Table 5 Repeatability of retention time and peak area (n=6)

Compound Name	Repeatability (RSD%)	
	Retention Time	Peak Area
Hyodeoxycholic acid	0.400	5.042
Cholic acid	0.294	1.145
Deoxycholic acid	0.227	2.733
Ursodeoxycholic acid	0.297	1.674
Chenodeoxycholic acid	0.237	2.972

Real Sample Analysis

A total of 5 cow-bezoar samples were tested: three suspect samples, one natural cow-bezoar sample, and one artificial cow-bezoar, all were ground into fine powders. 1.00 g of each homogenous powdered sample was weighed and placed into a 1.5 mL centrifuge tube and dissolved in an appropriate amount of methanol. After

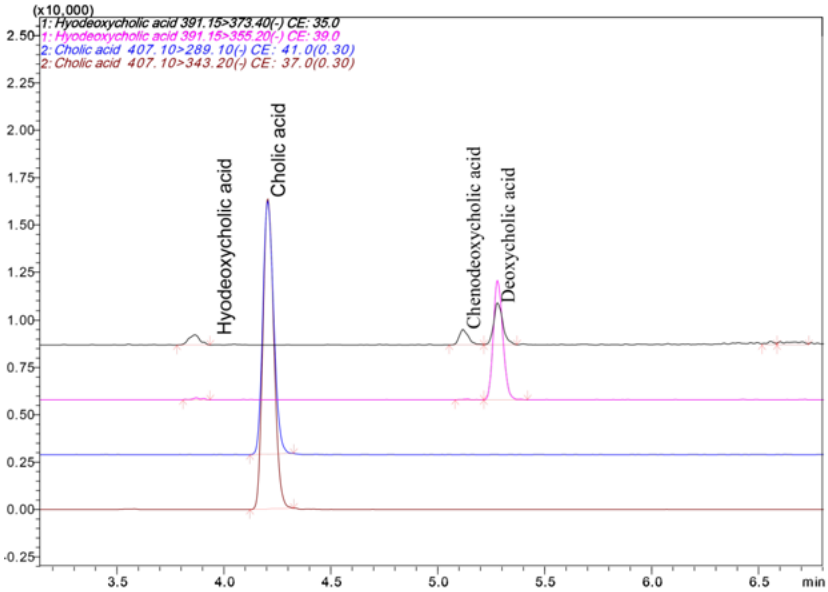


Figure 4 MRM chromatogram of a suspected cow-bezoar sample

5 min of sonication, the solution was diluted to a final volume of 10 mL and then diluted 1000 times using a 6:4 methanol in water. The samples were analyzed according to the conditions specified in the experimental section. The results are shown in Figure 4 and Table 6.

CONCLUSION

In this paper, hyodeoxycholic acid, cholic acid, deoxycholic acid, chenodeoxycholic acid and ursodeoxycholic acid in cow-bezoar were determined using Shimadzu's UHPLC Nexera XR coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The results showed good linearity in the range of 20 – 2000 ng/mL for the cholic acid and four deoxycholic acids and the linear correlation coefficients were more than 0.997. The detection accuracy was in the range of 87.0 – 109.8%. After 6 consecutive injections of 40 ng/mL, the RSDs of retention time and peak area were 0.227 – 0.400% and 1.145 – 5.042% respectively. This method can be used for the determination of cholic acid and bile acids in natural and artificial cow-bezoar.

Table 6 Test results of bile and cholic acids in a cow-bezoar sample

No.	Name	Concentration (μg/L)				
		Sample 1	Sample 2	Sample 3	Natural bezoar	Artificial bezoar
1	Hyodeoxycholic acid	26.00	60.95	51.43	/	1065.22
2	Cholic acid	107.48	99.12	98.53	168.63	457.35
3	Deoxycholic acid	43.71	32.10	34.76	122.65	114.36
4	Ursodeoxycholic acid	/	/	/	/	/
5	Chenodeoxycholic acid*	18.65	19.09	17.40	27.61	45.26

*The quantitative results of chenodeoxycholic acid are lower than the lower limit of the calibration curve.

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Determination of 9-Propenyladenine in Tenofovir Disoproxil Fumarate Using Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer

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Application News
SSL-CA14-537

Abstract

This paper established a method for determination of 9-propenyladenine in Tenofovir Disoproxil Fumarate using Shimadzu's Ultra-High Performance Liquid Chromatograph (UHPLC) LC-30A in conjunction with Triple Quadrupole Mass Spectrometer LCMS-8045. In this method, gradient elution is used and samples are quantified by the external standard method. The linear range is 0.5 to 20 ng/mL, the correlation coefficient is 0.9999, the limit of quantitation is 0.5 ng/mL, and the repeatability (RSD) is 4.62%. The method, characterized by fast analysis, high sensitivity, and good repeatability, can meet the requirements for accurate quantification of 9-propenyladenine in Tenofovir Disoproxil Fumarate, and therefore proves to be a suitable method for the monitoring of genotoxic impurities in drugs.

Tenofovir disoproxil fumarate (TDF) is a new type of nucleoside reverse transcriptase inhibitor, and a prodrug of tenofovir with potential anti-HIV-1 activity. TDF is effective against a variety of viruses and therefore is widely used in clinical treatments of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections. Various impurities can be introduced during the synthesis and storage of TDF. Among these impurities, 9-propenyladenine is a genotoxic impurity with mutagenic effects. The World Health Organization (WHO) has strictly limited the content of TDF to ≤ 5 mg/L. Therefore, a sensitive and highly efficient detection method for 9-propenyladenine is crucial for controlling its content. This method used LCMS-8045 to establish a highly sensitive method for the detection of 9-propenyladenine in TDF, thus providing a basis for quality control of TDF. Results obtained by this method can be used by relevant personnel as a reference.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A5R on-line degassing unit, SIL-30AC autosampler, CTO-20AC column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.89 Chromatography Workstation.

Analytical Conditions

<u>LC Chromatography (LC) Conditions</u>

Column : Shim-pack GIST C18 Column (2.1 mm I.D.×100 mm L., 2.0 μm)
Mobile phase : Mobile Phase A-10 mM ammonium acetate in water
Mobile Phase B-acetonitrile
Flow rate : 0.40 mL/min
Column Temp. : 30 °C
Injection volume : 5 μL
Elution method : Gradient elution with initial concentration of Mobile Phase B at 20%. Refer to Table 2 for elution program.

Table 1 Compound information

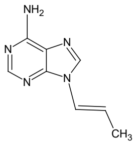
Compound Name	Synonym	CAS No.	Formula	Structure
9-Propenyladenine	(E)-9-(Prop-1-enyl-9H-purin-6-amine)	4121-40-8	$C_8H_9N_5$	

Table 2 Gradient elution program

Time (min)	Module	Command	Value (%)
4.00	Pumps	B.Conc	70
4.10	Pumps	B.Conc	20
6.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Analytical instrument	:	LCMS-8045
Ion source and mode	:	ESI (+)
Ion source interface voltage	:	4.0 kV
Nebulizing gas	:	Nitrogen 3.0 L/min
Heating gas	:	Air 15.0 L/min
Drying gas	:	Nitrogen 5.0 L/min
Collision gas	:	Argon
Interface temp.	:	300 °C
DL temp.	:	250 °C
Heater temp.	:	400 °C
Scan Mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	97 ms
Delay time	:	3 ms
MRM parameters	:	Refer to Table 3

Pretreatment of Standards and Samples

Preparation of standard: An appropriate amount of 9-propenyladenine standard was precisely weighed and dissolved in methanol. The standard solution was diluted with the mobile phase to establish a standard curve at concentrations of 0.5 ng/mL, 1 ng/mL, 2 ng/mL, 4 ng/mL, 6 ng/mL, 8 ng/mL, 10 ng/mL, 12 ng/mL, 14 ng/mL, and 20 ng/mL.

Preparation of sample: An appropriate amount of TDF was precisely weighed and dissolved in methanol to prepare a test sample at a concentration of 2 mg/mL.

RESULTS AND DISCUSSION

Q1 MS Scan and Product Ion Scan of Standard

From the Q1 MS scan, 9-propenyladenine mainly generated a quasi-molecular ion peak for $[M+H]^+$ with a m/z value of 176.15. The product ion scan of the quasi-molecular ion peak mainly generated fragment ions with m/z values of 136.10 and 119.10. The Q1 MS scan and product ion scan of 9-propenyladenine are shown in Figures 1 and 2, respectively.

Table 3 MRM optimized parameters

Compound Name	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
9-propenyladenine	176.15	136.10*	-19.0	-21.0	-23.0
		119.10	-10.0	-31.0	-10.0

Note: * indicates quantification ion

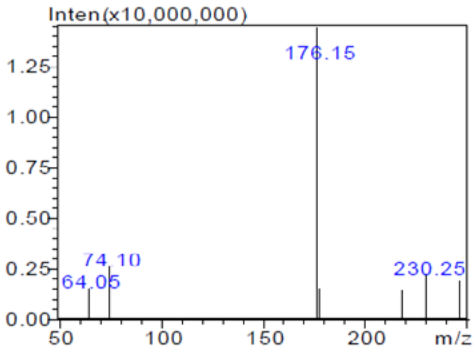


Figure 1 Q1 MS scan of 9-propenyladenine

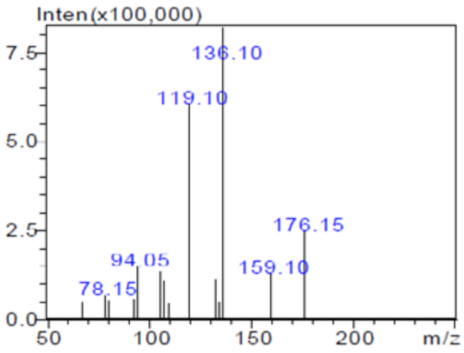


Figure 2 Product ion scan of 9-propenyladenine (CE value: -28 V)

Optimization of Liquid Chromatography Conditions

In the TDF inspection standard (YBH01472017) issued by the State Food and Drug Administration, 9-propenyladenine undergoes isocratic elution using an acetonitrile - 10 mM ammonium acetate in water (40/60, v/v). Under isocratic elution analysis of standard samples, interference peaks were present in the front of the target peak, as shown in Figure 3.

Liquid chromatography conditions, such as the use of gradient elution, were investigated experimentally for more accurate quantification of 9-propenyladenine. After the optimization of the gradient elution method, the results showed that the interference peak was clearly separated from the target peak. Therefore, in this experiment, gradient elution was used for the detection and the chromatogram obtained is shown in Figure 4.

Calibration Curve, Linear Range and the Lower Limit of Quantitation

Standard samples were prepared according to the method specified in the experimental section. Samples were injected, analyzed and quantified by the external standard method. The standard curves were established and a linear regression analysis was carried out using a weight coefficient of 1/C. The obtained standard curve is shown in Figure 5, while the linear regression equation and correlation coefficient are shown in Table 4. The results showed that the linear relationship between the concentration of 9-propenyladenine and its peak area was good over the concentration range of 0.5-20 ng/mL.

According to the method established in this paper, the lower limit of quantitation of 9-propenyladenine was determined to be 0.5 ng/mL. Six standard replicates at the concentration equivalent to the lower limit of quantitation were prepared and determined. The obtained RSD was 4.62% (n=6), the accuracy was 97.1 to 111.4%, and average value of S/N was 16.5. The results showed that the precision and accuracy of the method were all in line with the standard specifications at the above concentration level. Therefore, the method can meet the requirements for detection.

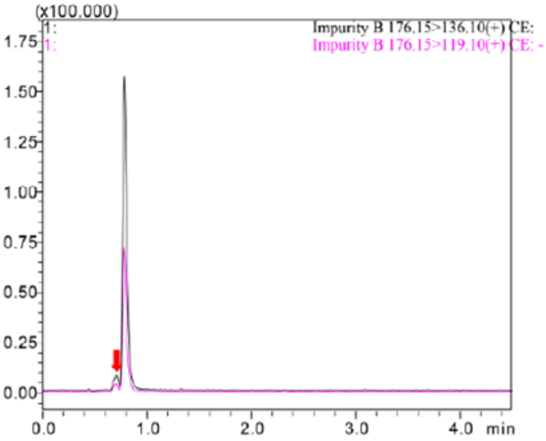


Figure 3 The chromatogram of 10 ng/mL standard at isocratic elution conditions

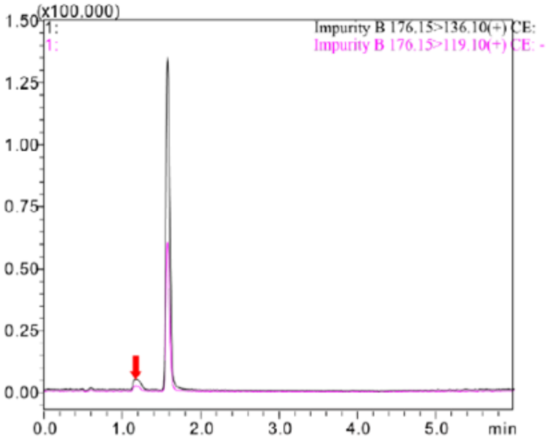


Figure 4 The chromatogram of 10 ng/mL standard at gradient elution program

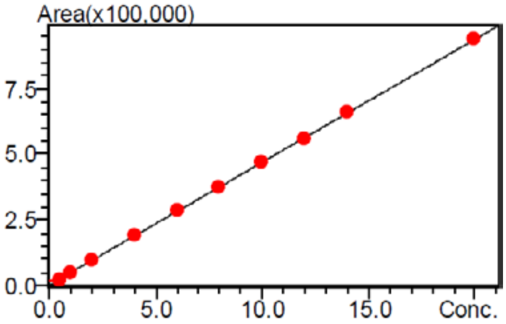


Figure 5 The standard curve of 9-propenyladenine

Table 4 Parameters for calibration curve (linear regression, the weight coefficient was 1/C)

Compound Name	Calibration Curve	Linear Range (ng/mL)	Accuracy (%)	Correlation Coefficient (R)
9-propenyladenine	Y = (46814.1) X + (1997.28)	0.5~20	93.1~104.6	0.9999

Table 5 Accuracy of the standard curve at each concentration point

No.	Standard Concentration. (ng/mL)	Measured Concentration. (ng/mL)	Accuracy (%)
1	0.5	0.466	93.1
2	1	1.046	104.6
3	2	2.032	101.6
4	4	4.036	100.9
5	6	6.083	101.4
6	8	7.958	99.5
7	10	9.962	99.6
8	12	11.868	98.9
9	14	14.084	100.6
10	20	19.965	99.8

Detection of 9-Propenyladenine in Drug Samples

The samples of TDF were prepared and analyzed in 6 parallel injections according to the sample preparation method and instrument conditions described in the experimental section. The content of 9-propenyladenine in the samples was determined by the external standard method. The results showed that the quantity of 9-propenyladenine in TDF was determined to be 0.216 µg/g (n=6).

CONCLUSION

This paper established a method for determination of 9-propenyladenine in Tenofovir Disoproxil Fumarate using Shimadzu's UHPLC LC-30A together with Triple Quadrupole Mass Spectrometer LCMS-8045. The analysis was completed within 6 mins and samples were quantified by the external standard method. The limit of quantitation, linear range and correlation coefficient were 0.5 ng/mL, 0.5 to 20 ng/mL, and 0.9999, respectively. This rapid and simple method, with high sensitivity and excellent reproducibility, meets the requirements for the quantitative analysis of 9-propenyladenine and is suitable for the monitoring of genotoxic impurities in drugs.

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Determination of Pharmaceuticals and Personal Care Products (PPCPs) Residues in Tap Water with Ultra-High Performance Triple Quadrupole Liquid Chromatograph Mass Spectrometer

Dianbao Yu
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Application News
SSL-CA14-385

Abstract

A method has been established in this application news for determination of 21 pharmaceuticals and personal care products (PPCPs) residues in tap water with a Shimadzu Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. Pretreatment of samples were conducted according to EPA Method 1694. 21 PPCP compounds showed good linearity over the concentration range of 0.05 – 10 μg/L; correlation coefficient of calibration curve was 0.9954 – 0.9999; limit of detection was 0.0025 – 0.015 μg/L; and limit of quantitation was 0.0075 – 0.045 μg/L. Standard working solutions at concentrations of 1.0 μg/L and 10.0 μg/L were evaluated six consecutive times, relative standard deviations (RSD) of retention time were 0.13 – 0.53% and 0.12 – 0.55% respectively, and relative standard deviations of peak area were 1.91 – 8.64% and 0.83 – 4.31% respectively, showing good precision. Spike recovery rates of 21 PPCP compounds were in the range of 75.1 – 99.7% in blank water samples. This method meets detection requirements of PPCPs in water and demonstrates a fast and high sensitivity analysis of PPCPs with good repeatability.

With the large-scale development of pharmaceutical and household chemical industries, pharmaceuticals and personal care products (PPCPs) have witnessed rapid growth both in production and consumption These products are continuously released into the environment leaving residues in water, soil and atmosphere, and these concentrations continue to increase. Since the 1990s, there has been a great concern for these chemicals as environmental pollutants.

There are as many as several thousand different PPCPs, but only hundreds of which are of higher concern and interest. Due to absence of laws or standards for determination of PPCP pollutants in water in China, relevant researches are usually carried out according to EPA Method 1694 of the U.S. Environmental Protection Agency. This method adopts a solid-phase extraction (SPE) cleanup and a pre-concentration step prior to analysis and this causes the sample preparation to be complicated and inefficient.

With reference to EPA Method 1694, and the local water characteristics, 21 PPCPs were selected and analyzed using Shimadzu UHPLC Triple Quadrupole Mass Spectrometer LCMS-8045.

EXPERIMENTAL

Instrumentation

The experiment employed Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler, CTO-20A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 Chromatography Workstation.

Analytical Conditions

LC Chromatography (LC) Conditions

Column	:	Shim-pack XR-ODS III (2.0 mm I.D.× 75 mm L., 1.6 μm)
Mobile phase	:	Mobile phase A - 0.1% formic acid in water Mobile phase B - methanol
Flow rate	:	0.30 mL/min
Column Temp.	:	40 °C
Injection volume	:	10 μL
Elution method	:	Gradient elution with the initial concentration of mobile phase B at 5%. Refer to Table 1 for detailed gradient elution program.

Table 1 Gradient elution program

Time (min)	Module	Command	Value (%)
0.50	Pumps	Pump B Conc.	25
5.00	Pumps	Pump B Conc.	35
6.00	Pumps	Pump B Conc.	95
9.00	Pumps	Pump B Conc.	95
9.10	Pumps	Pump B Conc.	5
13.00	Controller	Stop	

Mass Spectrometry (MS) Conditions

Analytical instrument	:	LCMS-8045
Ionization mode	:	ESI, simultaneous acquisition of positive and negative ions
Ion source interface voltage	:	+4.5 kV; -3.5 kV
Nebulizing gas	:	Nitrogen 3.0 L/min
Heating gas	:	Air 10.0 L/min
Drying gas	:	Nitrogen 10.0 L/min
Collision gas	:	Argon
Interface temp.	:	300 °C
DL temp.	:	250 °C
Heater temp.	:	400 °C
Scanning mode	:	Multiple reaction monitoring (MRM)
Dwell time	:	3.0 - 6.0 ms
Delay time	:	2.0 ms
MRM parameters	:	Refer to Table 2

Sample Preparation

With reference to the sample preparation and cleanup method for water specified in EPA Method 1694, water samples were first subjected to physical filtration and then SPE cleanup and elution. The sample was then evaporated and concentrated to constant volume by nitrogen blowing and injected for analysis.

RESULTS & DISCUSSION

MRM Chromatogram of Standard Samples

Figure 1 shows the MRM chromatogram of 21 PPCP standard samples.

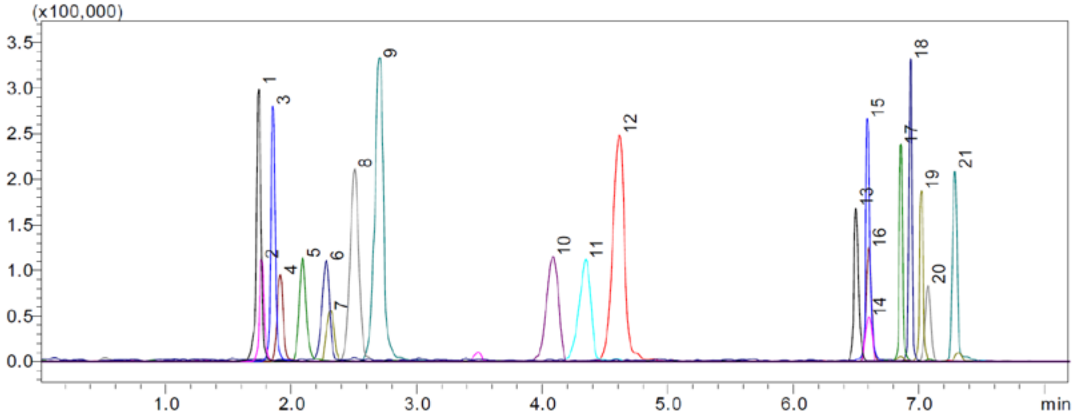


Figure 1 MRM chromatogram of 21 PPCP standard samples (1.0 μg/L)
(Note: peak numbers correspond to compound names in Table 2)

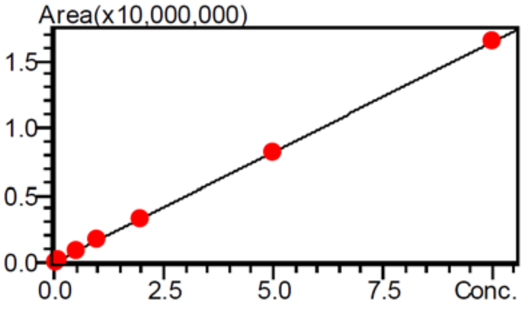
Table 2 MRM optimized parameters

No.	Compound Name	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Lincomycin	407.20	126.15*	-14.0	-27.0	-23.0
			359.10	-14.0	-12.0	-13.0
2	Trimethoprim	291.10	230.15*	-10.0	-22.0	-25.0
			123.15	-10.0	-24.0	-24.0
3	Pirenzepine	352.15	113.10*	-12.0	-21.0	-21.0
			70.15	-12.0	-40.0	-29.0
4	Ofloxacin	362.10	318.20*	-24.0	-19.0	-22.0
			261.05	-24.0	-26.0	-29.0
5	Ciprofloxacin	332.05	314.10*	-15.0	-20.0	-22.0
			231.00	-15.0	-38.0	-24.0
6	Sulfapyridine	250.05	156.05*	-11.0	-15.0	-29.0
			92.15	-11.0	-26.0	-17.0
7	Carbazochrome	237.05	220.10*	-11.0	-7.0	-23.0
			194.05	-11.0	-16.0	-17.0
8	Metoprolol	268.10	74.15*	-12.0	-22.0	-28.0
			116.25	-12.0	-19.0	-23.0
9	Disopyramide	340.20	239.10*	-12.0	-18.0	-26.0
			195.05	-12.0	-30.0	-20.0
10	Sulfamethoxazole	254.00	156.05*	-17.0	-15.0	-16.0
			92.15	-17.0	-28.0	-16.0
11	Antipyrine	189.05	56.20*	-12.0	-31.0	-22.0
			77.15	-12.0	-40.0	-29.0
12	Ifenprodil	326.20	308.20*	-11.0	-19.0	-22.0
			176.10	-11.0	-22.0	-19.0
13	Erythromycin	734.50	576.30*	-26.0	-19.0	-28.0
			158.10	-26.0	-33.0	-29.0
14	Azithromycin	749.55	116.10*	-26.0	-49.0	-22.0
			158.10	-26.0	-40.0	-17.0
15	Clarithromycin	748.50	590.35*	-26.0	-20.0	-27.0
			158.10	-26.0	-31.0	-30.0
16	Roxithromycin	837.40	679.35*	-28	-22	-34
			158.05	-28	-36	-16
17	Carbamazepine	237.00	194.05*	-15.0	-19.0	-20.0
			119.00	-15.0	-30.0	-25.0
18	DEET	192.10	119.10*	-13.0	-17.0	-21.0
			91.15	-13.0	-30.0	-16.0
19	Crotamiton	204.10	69.15*	-13.0	-22.0	-28.0
			136.15	-13.0	-15.0	-36.0
20	Bezafibrate	360.10	274.05*	16	17	27
			154.00	16	30	24
21	Triclocarban	314.90	162.05*	14.0	14.0	29.0
			126.05	14.0	15.0	26.0

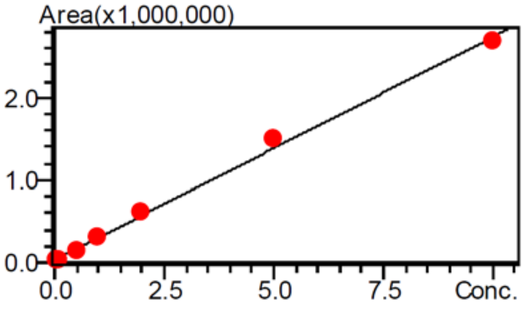
Note: * indicates quantification ion

Calibration curve and method validation

The mixed standard working solutions at concentrations of 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0 and 10.0μg/L were prepared and analyzed according to analytical conditions in experimental section. External standard calibration method was used and linearity was good within the concentration range of 0.05–10.0 μg/L. The limit of detection (LOD) and limit of quantitation (LOQ) of each substance were calculated based on signal-to-noise ratio where LOD and LOQ is determined at S/N=3and S/N=10 respectively. Refer to Figure 2 for calibration curves and Table 3 for method validation results (e.g. linearity range, correlation coefficient, LOD and LOQ).



1 Lincomycin



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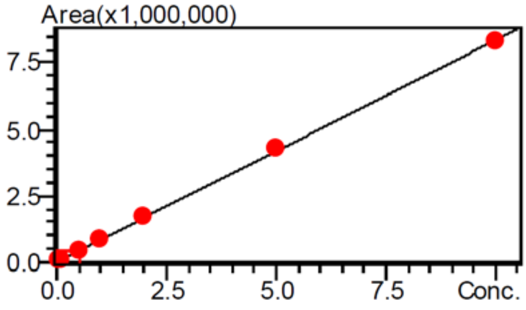
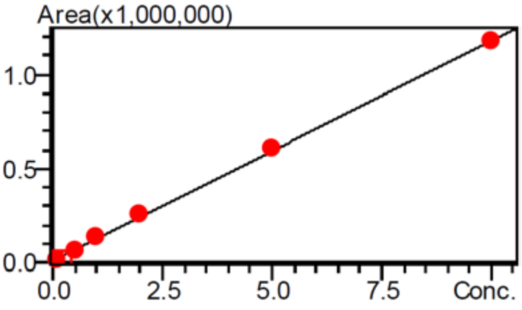
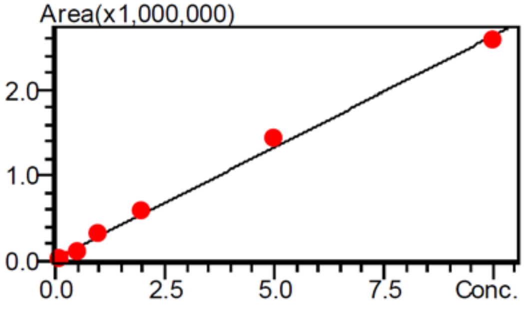


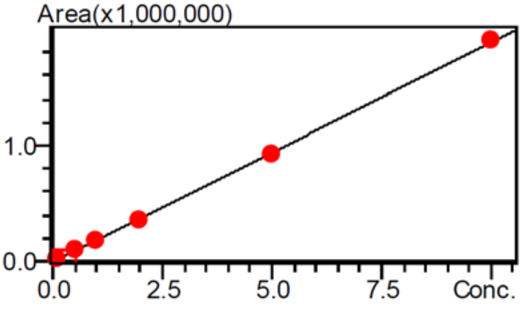
3 Pirenzepine



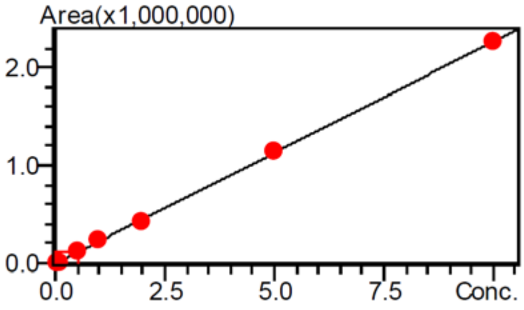
4 Ofloxacin



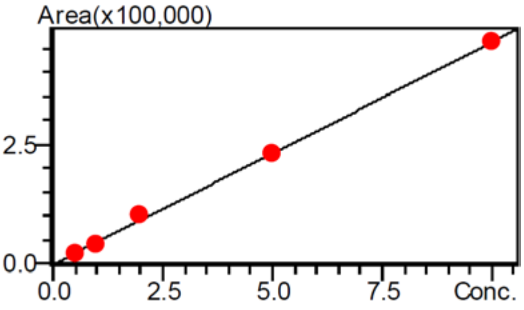
5 Ciprofloxacin



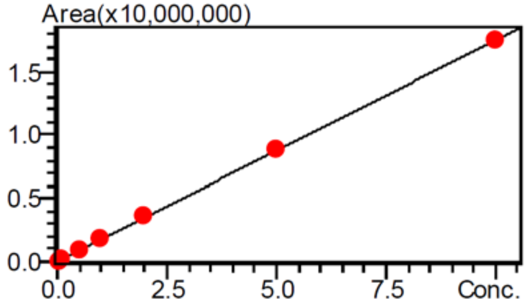
6 Sulfapyridine



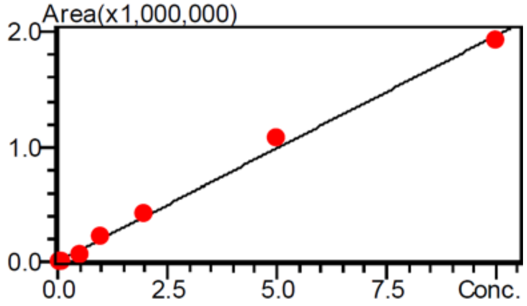
7 Carbazochrome



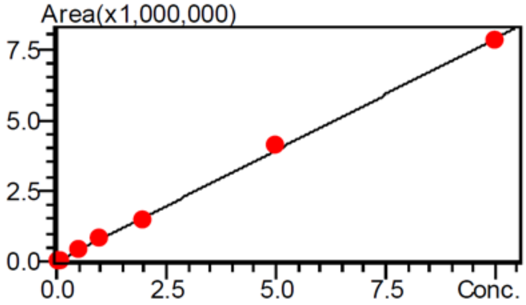
8 Metoprolol



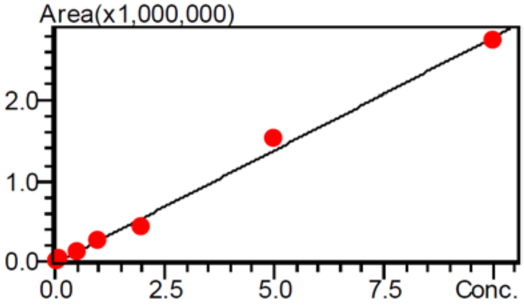
9 Disopyramide



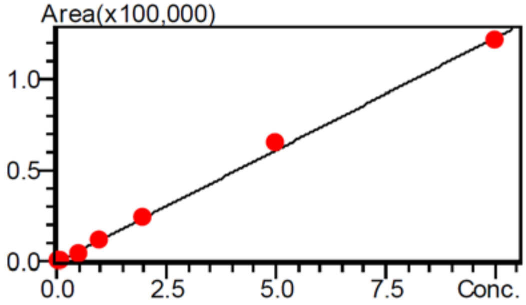
10 Sulfamethoxazole



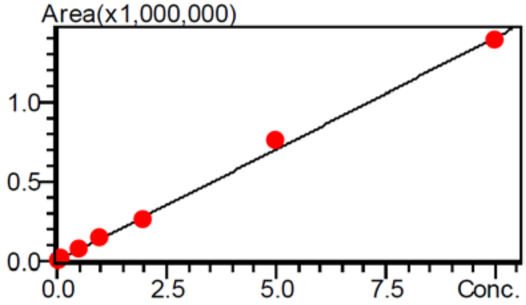
11 Antipyrine



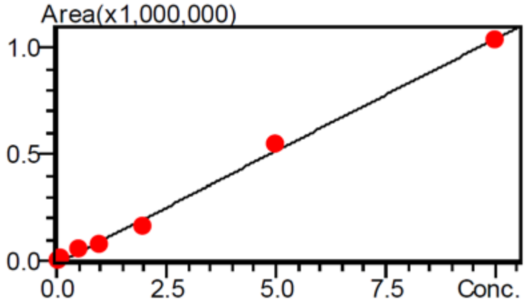
12 Ifenprodil



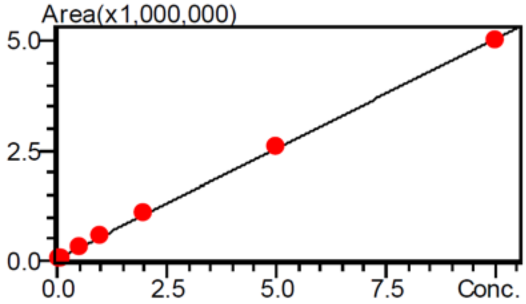
13 Erythromycin



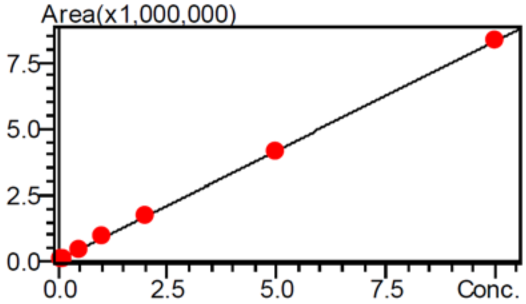
14 Azithromycin



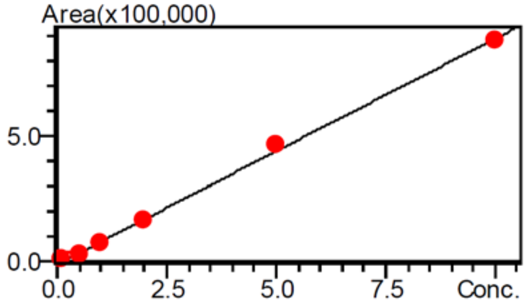
15 Clarithromycin



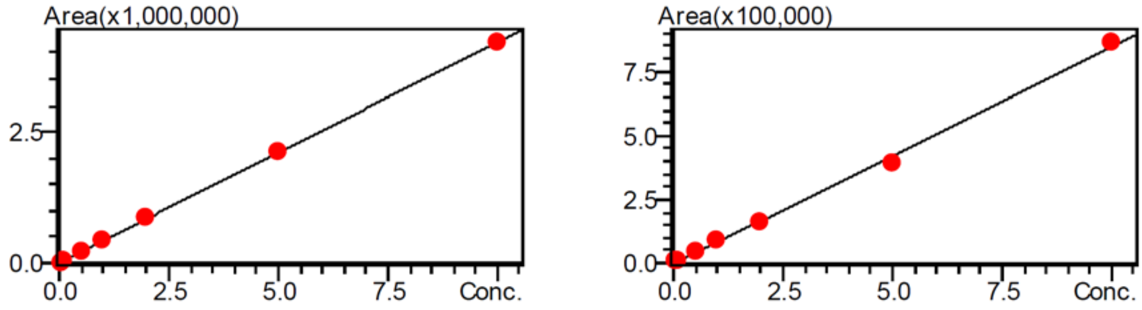
16 Roxithromycin



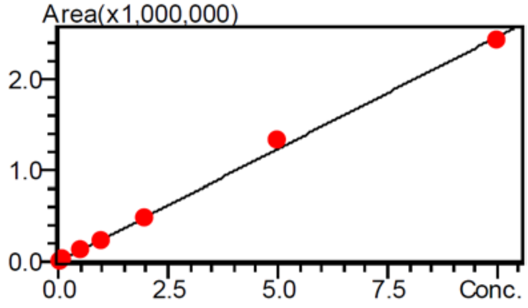
17 Carbamazepine



18 DEET



19 Crotamiton 20 Bezafibrdined



21 Triclocarban

Figure 2 Standard calibration curve

Table 3 Method validation results

No.	Compound name	Calibration Curve	Accuracy (%)	Correlation Coefficient (r^2)	Limit of Detection (μg/L)	Limit of Quantitation (μg/L)
1	Lincomycin	Y = (1.64138e+006) X + (-10050.9)	83.8~101.5	0.9999	0.010	0.040
2	Trimethoprim	Y = (213614) X + (23648.7)	92.3~110.3	0.9991	0.010	0.040
3	Pirenzepine	Y = (833594) X + (19297.3)	80.7~102.7	0.9998	0.010	0.030
4	Ofloxacin	Y = (117101) X + (4863.62)	90.3~105.2	0.9998	0.010	0.030
5	Ciprofloxacin	Y = (542632) X + (-1996.77)	89.2~111.3	0.9994	0.002	0.006
6	Sulfapyridine	Y = (273639) X + (2209.94)	89.9~111.2	0.9993	0.002	0.006
7	Carbazochrome	Y = (53874.6) X + (-1841.75)	90.2~112.4	0.9954	0.015	0.045
8	Metoprolol	Y = (228179) X + (-4012.05)	83.6~111.4	0.9996	0.013	0.040
9	Disopyramide	Y = (1.75782e+006) X + (-7623.46)	96.6~104.9	0.9999	0.005	0.015
10	Sulfamethoxazole	Y = (205361) X + (1940.27)	91.2~113.9	0.9997	0.003	0.010
11	Antipyrine	Y = (161552) X + (-1798.87)	90.3~107.9	0.9996	0.010	0.030
12	Ifenprodil	Y = (789714) X + (-8527.81)	94.5~105.6	0.9993	0.0075	0.022
13	Erythromycin	Y = (123693) X + (-2316.57)	92.8~110.3	0.9958	0.015	0.045
14	Azithromycin	Y = (12303.9) X + (-428.492)	85.6~105.7	0.9990	0.010	0.030
15	Clarithromycin	Y = (437746) X + (-14408.8)	96.4~109.8	0.9978	0.0034	0.010
16	Roxithromycin	Y = (252138) X + (-4464.83)	77.9~102.3	0.9957	0.0071	0.022
17	Carbamazepine	Y = (518201) X + (4883.88)	87.6~105.9	0.9990	0.010	0.030
18	DEET	Y = (833190) X + (22136.7)	98.0~106.9	0.9995	0.0062	0.0183
19	Crotamiton	Y = (426081) X + (-472.990)	92.8~108.6	0.9998	0.005	0.0015
20	Bezafibrate	Y = (83466.0) X + (1201.69)	94.7~107.0	0.9989	0.0025	0.0075
21	Triclocarban	Y = (17631.2) X + (131.172)	86.0~115.1	0.9984	0.010	0.030

Precision Test

Mixed standard solutions of PPCPs at concentration 1.0 μg/L and 10.0 μg/L were injected 6 consecutive times to check the precision. Repeatability results of retention time and peak area are shown in Table 4. Relative standard deviations of retention time of the 1.0 μg/L and 10.0 μg/L standard working solutions were 0.13–0.53% and 0.12–0.55% respectively, and relative standard deviations of peak area were 1.91–8.64% and 0.83–4.31% respectively, showing good precision.

Spike Recovery Test

After collecting three blank water samples, mixed standard solutions of PPCPs were added to two of them (the theoretical concentration of the spiked concentration solution was 1.0 μg/L), and the remaining one was left as a blank matrix sample. Preparation was carried out according to the sample preparation method in the experimental section to check the spike recovery rate of samples. The chromatogram of the blank water matrix is shown in Figure 3, and the chromatogram of the matrix spike solution is shown in Figure 4. Figure 5 shows the spike recovery rates. Statistically, the 21 PPCP compounds have higher recovery rates in the blank water sample.

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

No.	Compound Name	RSD% (1.0 μg/L)		RSD% (10.0 μg/L)	
		R.T.	Area	R.T.	Area
1	Lincomycin	0.26	2.79	0.32	1.35
2	Trimethoprim	0.26	3.13	0.33	2.39
3	Pirenzepine	0.29	2.71	0.29	1.22
4	Ofloxacin	0.41	8.64	0.28	2.76
5	Ciprofloxacin	0.52	8.44	0.28	2.30
6	Sulfapyridine	0.27	6.71	0.12	1.40
7	Carbazochrome	0.26	7.52	0.29	2.16
8	Metoprolol	0.41	5.31	0.19	2.33
9	Disopyramide	0.53	3.21	0.21	0.83
10	Sulfamethoxazole	0.49	6.23	0.55	1.66
11	Antipyrine	0.51	3.62	0.54	3.01
12	Ifenprodil	0.39	3.62	0.72	1.48
13	Erythromycin	0.14	6.21	0.13	2.45
14	Azithromycin	0.17	3.65	0.12	2.24
15	Clarithromycin	0.18	3.43	0.12	2.30
16	Roxithromycin	0.18	6.29	0.11	2.23
17	Carbamazepine	0.13	3.72	0.09	2.48
18	DEET	0.14	2.25	0.09	0.83
19	Crotamiton	0.15	1.91	0.09	1.95
20	Bezafibrate	0.15	5.92	0.10	2.59
21	Triclocarban	0.18	5.45	0.16	4.31

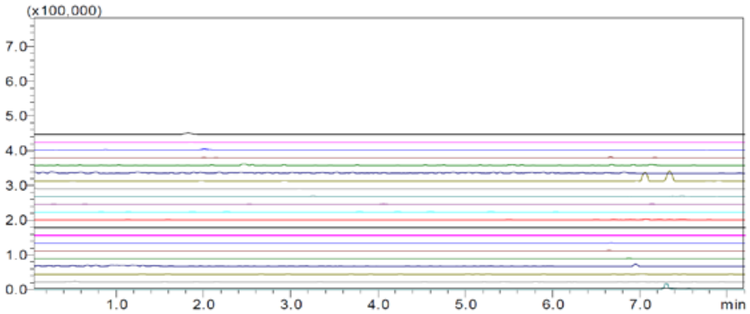


Figure 3 Chromatogram of blank matrix

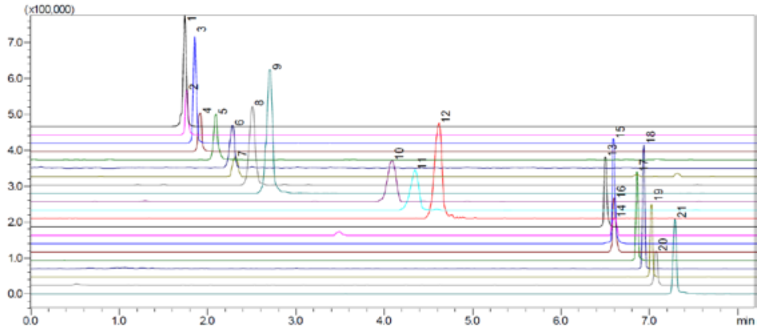


Figure 4 Chromatogram of spike recovery test (1.00 ng/mL)

Table 5 Spike recovery rate of blank water sample (1.0 μg/L)

No.	Compound Name	Blank Matrix Concentration (μg/L)	Actual Concentration of Sample 1 (μg/L)	Actual Concentration of Sample 2 (μg/L)	Ave. Rec. (%)
1	Lincomycin	ND	0.968	0.918	94.3
2	Trimethoprim	ND	0.920	0.914	91.7
3	Pirenzepine	ND	0.951	0.987	96.9
4	Ofloxacin	ND	0.903	0.843	87.3
5	Ciprofloxacin	ND	0.990	1.005	99.7
6	Sulfapyridine	ND	0.922	0.946	93.4
7	Carbazochrome	ND	0.932	1.003	96.8
8	Metoprolol	ND	0.945	0.890	91.8
9	Disopyramide	ND	1.002	0.960	98.1
10	Sulfamethoxazole	ND	1.002	0.958	98.0
11	Antipyrine	ND	0.920	0.972	94.6
12	Ifenprodil	ND	0.774	0.728	75.1
13	Erythromycin	ND	0.841	0.805	82.3
14	Azithromycin	ND	0.822	0.752	78.7
15	Clarithromycin	ND	0.894	0.823	85.9
16	Roxithromycin	ND	0.937	0.862	90.0
17	Carbamazepine	ND	0.762	0.813	78.8
18	DEET	ND	0.980	0.921	95.0
19	Crotamiton	ND	0.847	0.810	82.9
20	Bezafibrate	ND	0.826	0.742	80.7
21	Triclocarban	ND	0.854	0.890	87.2

Note: ND = not detected

CONCLUSION

A method has been established in this application news for determination of 21 PPCPs residues in tap water with a Shimadzu Ultra-High Performance Liquid Chromatograph coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. Pretreatment of samples were conducted according to EPA Method 1694. 21 PPCPs showed good linearity over the concentration range of 0.05–10 μg/L; correlation coefficient of calibration curve was 0.9954 – 0.9999; limit of detection was 0.0025–0.015 μg/L; and limit of quantitation was 0.0075– 0.045 μg/L. Standard working solutions at concentrations of 1.0 μg/L and 10.0 μg/L were evaluated six consecutive times, and the relative standard deviations (RSD) of retention time were 0.13 – 0.53% and 0.12 – 0.55% respectively, and relative standard deviations of peak area were 1.91 – 8.64% and 0.83 – 4.31% respectively. Spike recovery rates of 21 PPCP compounds were 75.1 – 99.7% in blank water samples. This method, characterized by fast analysis, good repeatability and high sensitivity, meets detection requirements of PPCPs in water.

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Determination of 20 Carbamate Pesticide Residues in Soil by Ultra-High Performance Liquid Chromatograph Coupled with Triple Quadrupole Mass Spectrometer

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Application News
SSL-CA14-461

Abstract

This paper established a method for the determination of 20 carbamate pesticides in soil using Shimadzu's Ultra-High Performance Liquid Chromatograph LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The analysis of 20 carbamates was completed within 12 min, and the correlation coefficients of calibration curve were all above 0.997. The mixed standard solutions with different concentrations of carbamates were tested in 6 replicates. The relative standard deviations of the retention time and peak area of the 20 target compounds were 0.015 – 0.263% and 0.554 – 4.917% respectively, showing good precision. The matrix spike recovery at difference concentrations were in the range of 88.1 – 108.4%. The described method can be applied to the simultaneous determination of 20 carbamate pesticide residues in soil.

Carbamates are a group of highly effective and broad-spectrum pesticides. It plays a vital role in crop protection and accounts for about 15% of the global pesticide market, with increasing usage each year. The accumulation and toxic effects of carbamates in the environment have raised concerns and become a pressing environmental issue. Carbamates inhibit acetylcholinesterase enzymes and the effects and symptoms of acute poisoning includes lachrymation, fasciculation and pupil constriction. Currently, research is mainly focused on the analysis and method development of carbamate pesticide residues in water and food. Nevertheless, soils directly affect the quality of agricultural products. Furthermore, soils play an important role in the migration of water pollutants and can be the source and sink of environmental pollutants. Research and analysis on carbamate residues in soil and sediments are rare at present, and most of these existing research uses liquid chromatography with post-column derivatization and fluorescence detection or gas chromatography. The former is complicated to perform and limited in qualitative analyses. The application and scope of gas chromatography is limited since most carbamate pesticides are thermally unstable.

High performance liquid chromatography-tandem mass spectrometry system is a rapidly developing analytical instrument in recent years. With high selectivity and sensitivity for both qualitative and quantitative analyses as well as high accuracy for carbamate pesticide residues in complex matrices, it is the preferred method for trace residue analysis. This paper established a method for the determination of 20 carbamates residues in soil using Shimadzu's UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045.

EXPERIMENTAL

Instrumentation

This experiment employed Shimadzu's UHPLC LC-30A and Triple Quadrupole Mass Spectrometer LCMS-8045. The specific configurations are two LC-30AD pumps, DGU-20A$_{5R}$ online degassing unit, SIL-30AC autosampler, CTO-30A column oven, CBM-20A system controller, LCMS-8045 triple quadrupole mass spectrometer and LabSolutions Ver. 5.86 chromatographic workstation.

Analytical Conditions

Column : Shim-pack XR-ODS III (2.0 mm I.D.× 75 mm L., 1.6 μm)
Mobile phase : Mobile Phase A-5 mM ammonium acetate in water; Mobile Phase B-methanol
Flow rate : 0.30 mL/min
Column Temp. : 45 °C
Injection volume : 2 μL
Elution method : Gradient elution; with the initial concentration of Mobile Phase B at 20%. Refer to Table 1 for elution program.

Table 1 Gradient elution program

Time (min)	Module	Command	Value (%)
1.0	Pumps	Pump B Conc.	20
2.0	Pumps	Pump B Conc.	60
6.0	Pumps	Pump B Conc.	95
7.5	Pumps	Pump B Conc.	95
7.6	Pumps	Pump B Conc.	20
12	Controller	Stop	

LC Chromatography (LC) Condition

Analytical instrument : LCMS-8045
Ionization mode : ESI (+)
Nebulizing gas : Nitrogen 3.0 L/min
Heating gas : Air 10.0 L/min
Drying gas : Nitrogen 10.0 L/min
Collision gas : Argon
Source temp. : 200 °C
DL temp. : 150 °C
Heater temp. : 400 °C
Scanning mode : Multiple reaction monitoring (MRM)
Dwell time : 9.0 ms
Delay time : 3.0 ms
MRM parameters : Refer to Table 2

Mass Spectrometer (MS) Condition

Standard Solution Preparation

Appropriate amounts of carbamate pesticide standards were weighed, combined and dissolved in methanol to prepare a mixed standard stock solution of 1.0mg/mL and stored at -18oC. A series of mixed standard working solutions at various concentrations were prepared by diluting the 1.0mg/mL mixed standard stock solution with 20% methanol solution.

The following standard stock solutions and working solutions were prepared:

(1) Standard solutions of propoxur, methomyl, dioxacarb, bendiocarb, carbaryl, ethiofencarb, isoprocarb, fenobucarb, carbaryl, promecarb, BDMC, indoxacarb, benfuracarb with the concentrations of 1, 2, 10, 20, 50, 100 and 200 ng/mL.

(2) Standard solutions of carbofuran, fenothiocarb, aldicarb and alanycarb with the concentrations of 0.5, 1, 5, 10, 25, 50 and 100 ng/mL.

(3) Standard solutions of oxamyl, furathiocarb and pirimicarb with the concentrations of 0.25, 0.5, 2.5, 5, 12.5, 25 and 50 ng/mL.

The internal standards, carbaryl-D7 and methomyl-D3, were both added into the solutions to get the final concentrations of 50 ng/mL. Methomyl-D3 was selected as internal standard for analytes eluting till 4.5 min, and carbaryl-D7 is used as internal standard for analytes eluting after 4.5 min.

Sample Preparation Method

The extraction of carbamate in soil samples is referenced from the standard HJ 783-2016 “Soil and Sediment - Extraction of Organic Compounds - Pressurized Fluid Extraction”. 10 g (accurate to 0.1 g) of soil sample was weighed and extracted using pressurized fluid extraction. The extracting solvent dichloromethane-methanol (1:1) was added to the soil samples. The mixture was heated at 80 °C, with 3 static cycles and flush volume of 80% of the cell volume. The extract was concentrated to 1.0 mL using a rotary evaporator. The concentrated extract was clean-up using GCB/PSA solid phase extraction (SPE), cartridge was pre-activated with 6.0 mL of dichloromethane-methanol mixed solvent (1:1) and elution was performed with 6 mL dichloromethane-methanol mixture (1:1). The eluate was concentrated to near dryness with nitrogen blower, and the internal standards were added to give a final concentration of 50 ng/mL. The sample solutions were topped up to 1.0 mL with the initial mobile phase and injected for LC-MS/MS analysis.

Table 2 MRM optimized parameters

No.	Compound Name	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Oxamyl	23135-22-0	237.1	72.1*	-11	-13	-27
				90.2	-11	-8	-17
2	Methomyl	16752-77-5	163.1	88.1*	-16	-8	-17
				106.1	-16	-10	-11
3	Dioxacarb	6988-21-2	224.0	123.0*	-10	-14	-24
				167.1	-10	-8	-17
4	Aldicarb	116-06-3	208.1	116.1*	-19	-10	-20
				89.1	-21	-20	-20
5	Bendiocarb	22781-23-3	224.1	167.1*	-16	-9	-17
				109.1	-16	-18	-21
6	Propoxur	114-26-1	210.1	111.1*	-10	-14	-21
				168.1	-10	-9	-18
7	Carbofuran	1563-66-2	222.1	165.1*	-10	-11	-17
				123.1	-10	-22	-22
8	Carbaryl	63-25-2	202.1	145.1*	-21	-9	-15
				127.1	-20	-26	-13
9	Ethiofencarb	29973-13-5	226.1	107.1*	-16	-15	-20
				164.1	-16	-9	-17
10	Pirimicarb	23103-98-2	239.2	72.1*	-11	-21	-28
				182.2	-12	-15	-19
11	Isoprocarb	2631-40-5	194.2	95.1*	-14	-15	-19
				137.2	-14	-11	-14
12	Fenobucarb	3766-81-2	208.1	95.1*	-10	-14	-19
				152.1	-10	-10	-16
13	Methiocarb	2032-65-7	226.1	169.1*	-16	-10	-18
				121.1	-16	-18	-23
14	Promecarb	2631-37-0	208.1	109.1*	-10	-16	-20
				151.2	-21	-10	-17
15	4-bromo-3, 5-dimethylphenyl N-methylcarbamate (BDMC)	672-99-1	258.0	201.0*	-12	-10	-21
				122.1	-10	-22	-23
16	Fenothiocarb	62850-32-2	254.1	72.1*	-12	-16	-27
				160.0	-18	-9	-17
17	Alanycarb	83130-01-2	400.2	238.1*	-15	-9	-18
				91.0	-19	-35	-19
18	Indoxacarb	144171-61-9	528.1	249.1*	-20	-16	-12
				218.0	-20	-24	-22
19	Benfuracarb	82560-54-1	411.3	190.2*	-15	-11	-13
				252.0	-15	-13	-18
20	Furathiocarb	65907-30-4	383.2	195.1*	-14	-17	-20
				252.1	-14	-12	-18
21	Methomyl-D3	398109-07-3	165.9	88.1*	-12	-10	-17
				106.1	-12	-10	-11
22	Carbaryl-D7	362049-56-7	208.9	152.1*	-15	-10	-28
				133.1	-15	-30	-26

Note: * indicates quantification ion

RESULTS AND DISCUSSION

Q1 MS Scan and Product Ion Scan of Pesticides Standards

The MS scan and product ion scan of 20 pesticide standards are shown in Figure 1 - 20.

MRM Chromatograms of 20 Carbamate Standard Solutions

Figure 21 shows the MRM chromatograms of the 20 carbamate standard solutions. The concentrations of the

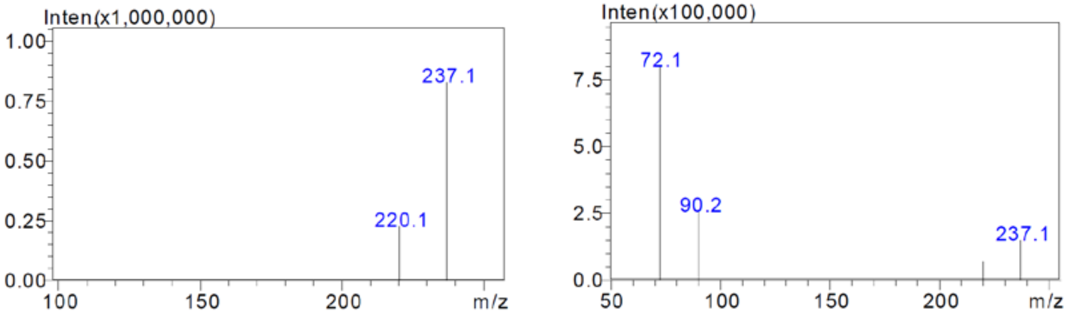


Figure 1 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of oxamyl

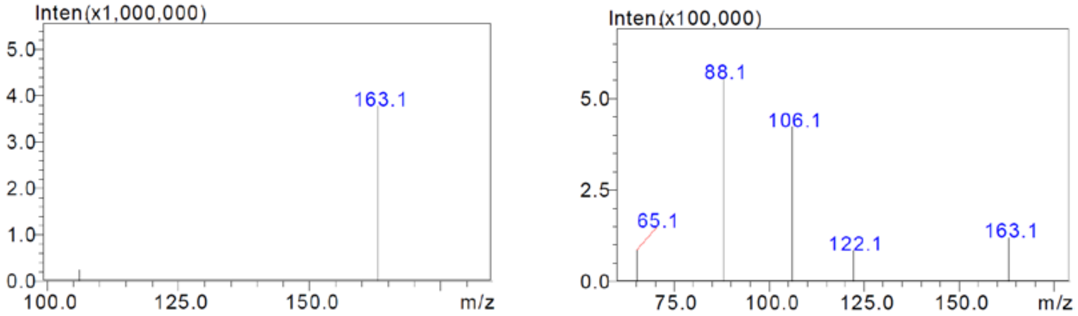


Figure 2 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of methomyl

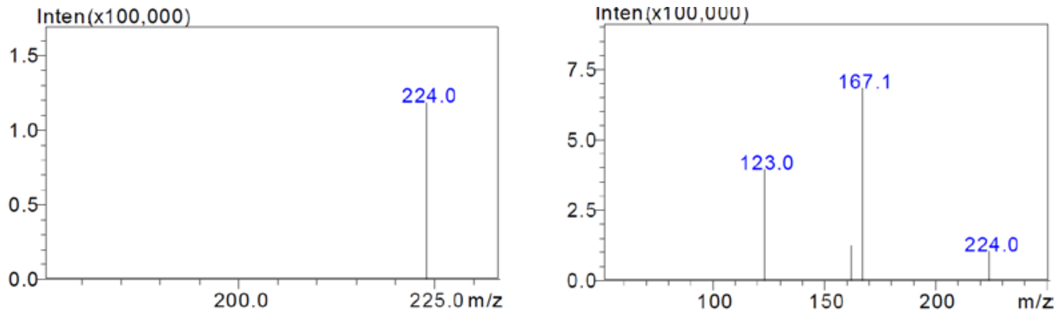


Figure 3 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of dioxacarb

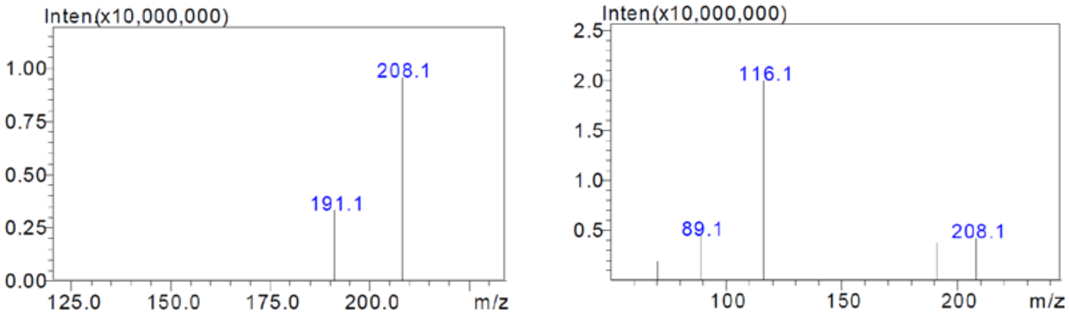


Figure 4 Q1 MS scan (left) and product ion scan (CE value-8 V, right) of aldicarb

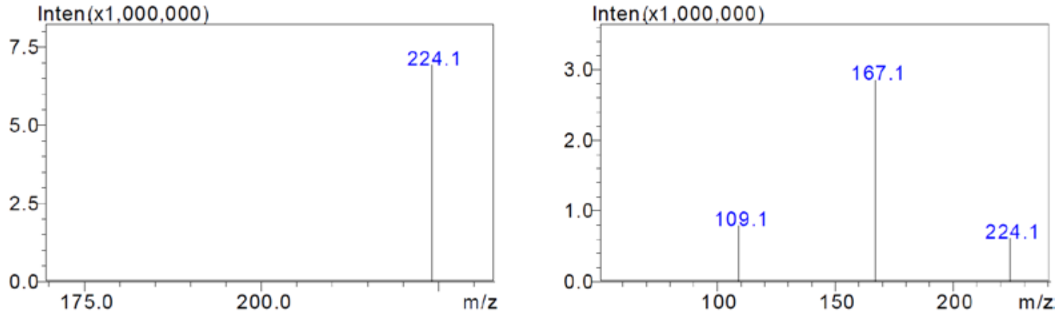


Figure 5 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of bendiocarb

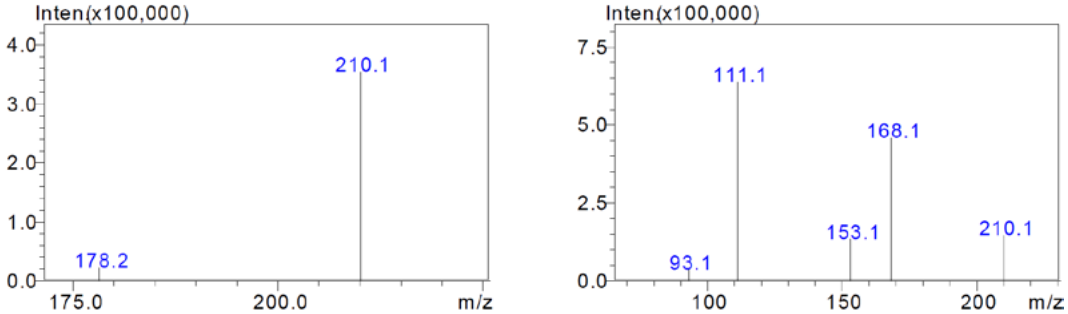


Figure 6 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of propoxur

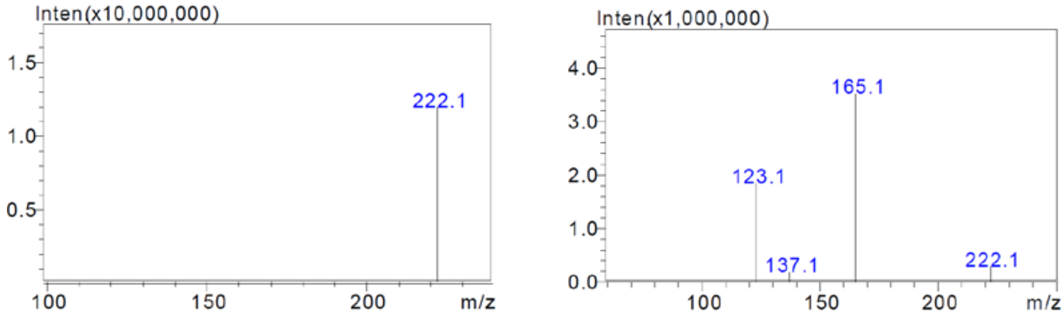


Figure 7 Q1 MS scan (left) and product ion scan (CE value-15 V, right) of carbofuran

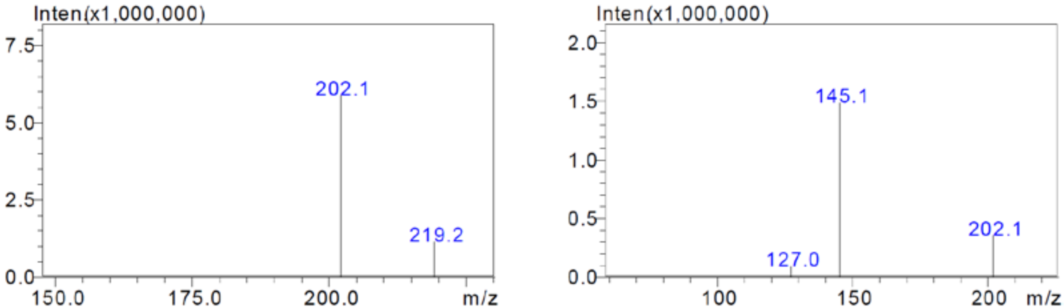


Figure 8 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of carbary

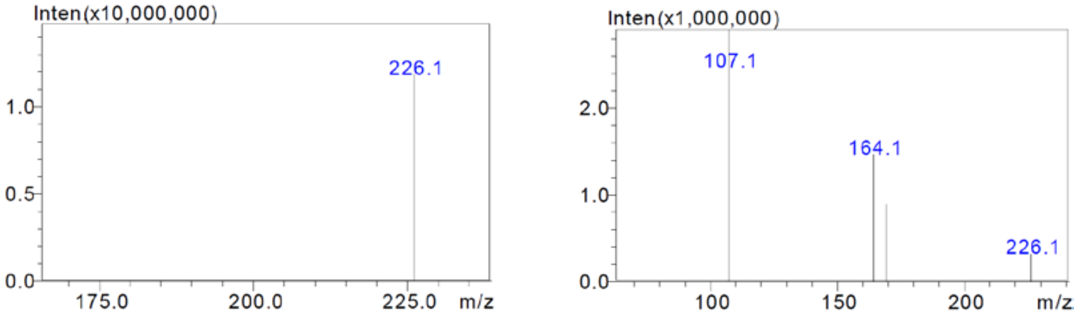


Figure 9 Q1 MS scan (left) and product ion scan (CE value-8 V, right) of ethiofencarb

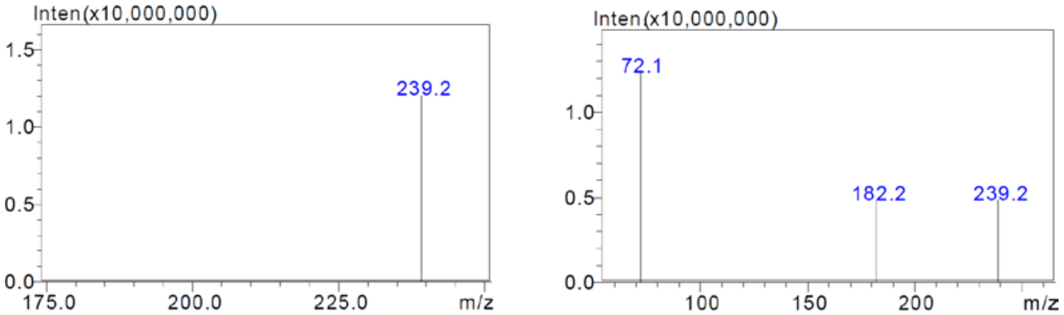


Figure 10 Q1 MS scan (left) and product ion scan (CE value-15 V, right) of pirimicarb

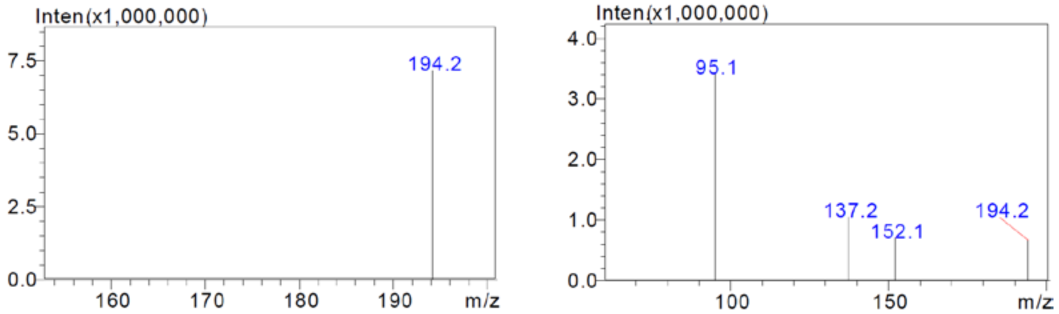


Figure 11 Q1 MS scan (left) and product ion scan (CE value-25 V, right) of isoprocarb

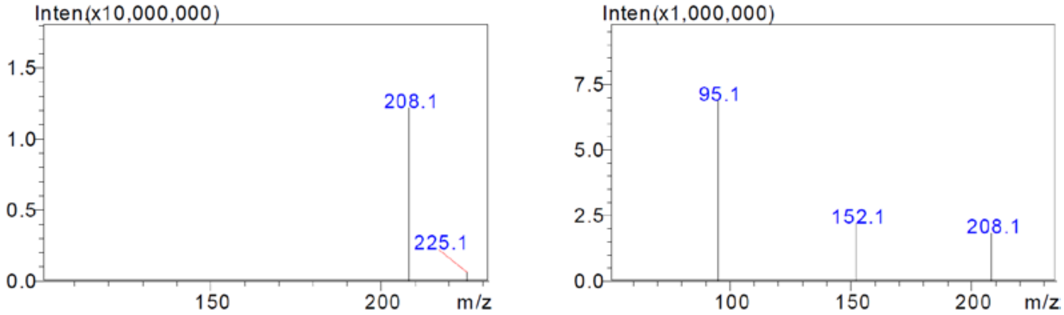


Figure 12 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of fenobucarb

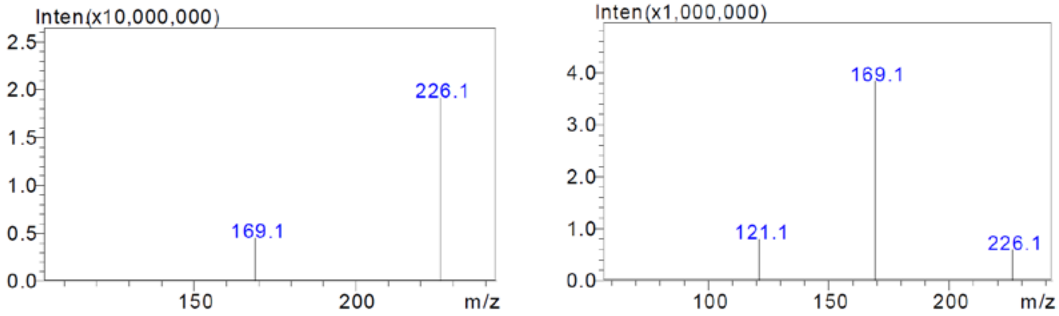


Figure 13 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of methiocarb

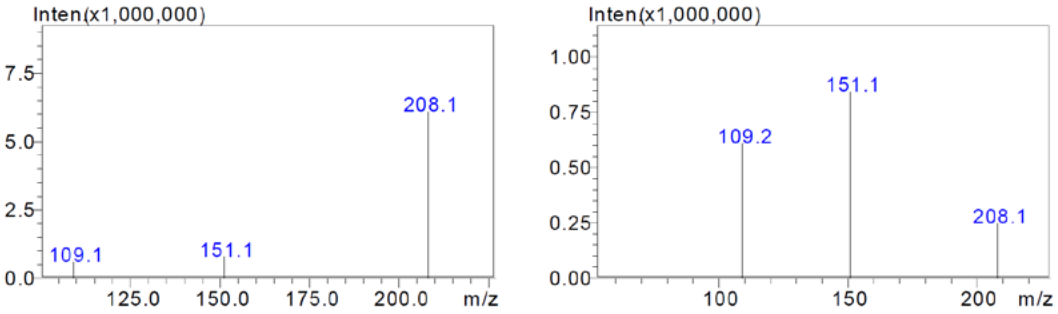


Figure 14 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of promecarb

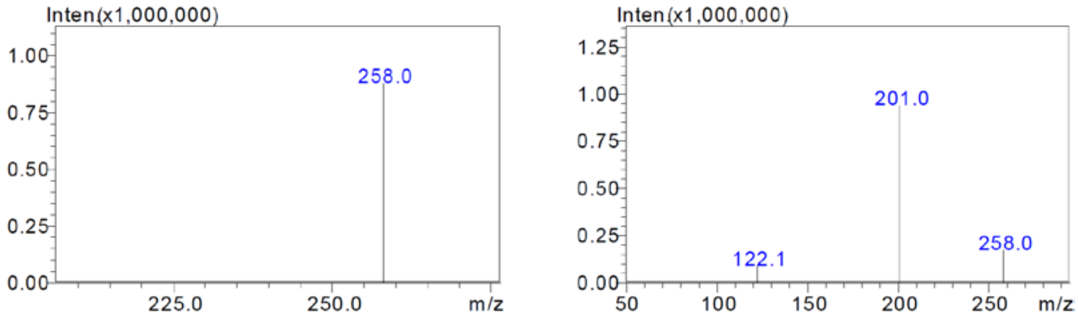


Figure 15 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of BDMC

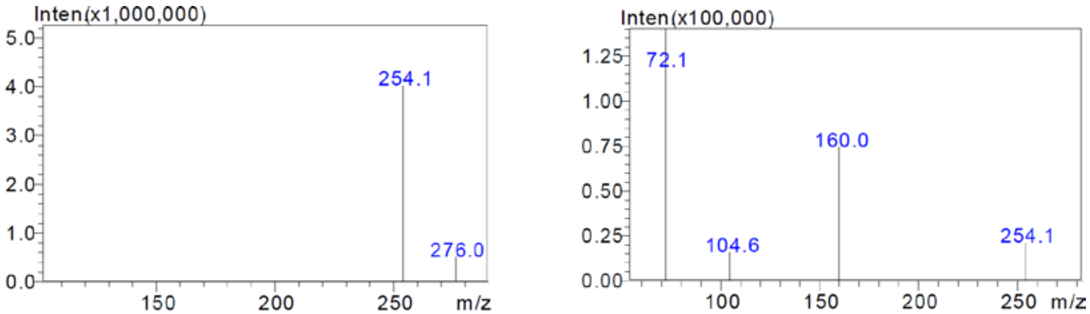


Figure 16 Q1 MS scan (left) and product ion scan (CE value-10 V, right) of fenothiocarb

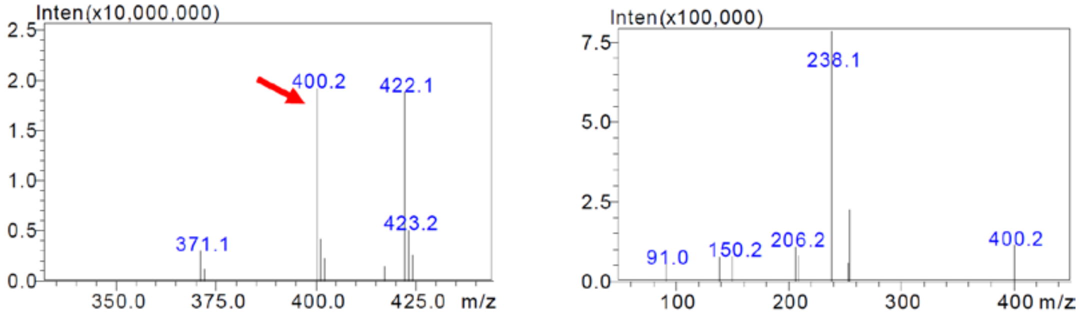


Figure 17 Q1 MS scan (left) and product ion scan (CE value-8 V, right) of alanycarb

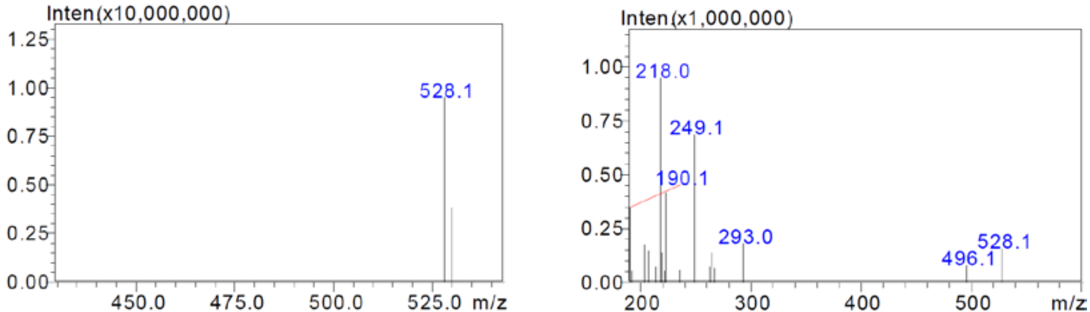


Figure 18 Q1 MS scan (left) and product ion scan (CE value-20 V, right) of indoxacarb

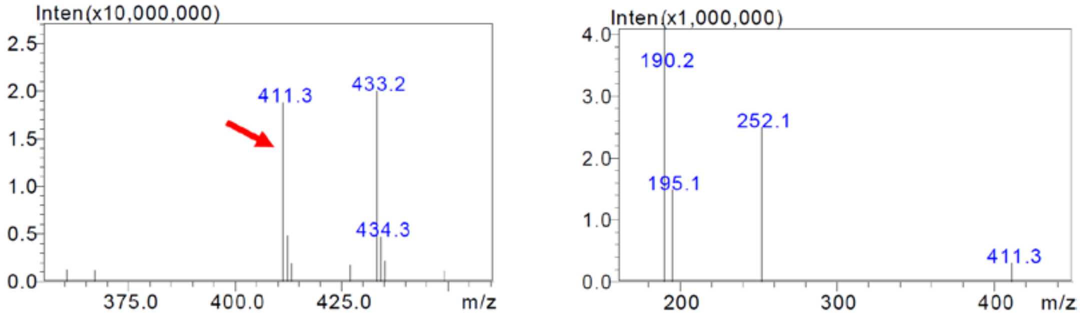


Figure 19 Q1 MS scan (left) and product ion scan (CE value-15 V, right) of benfuracarb

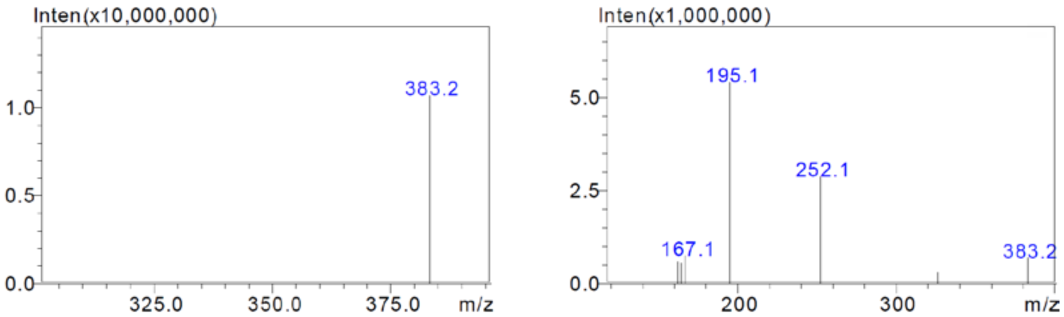


Figure 20 Q1 MS scan (left) and product ion scan (CE value-15 V, right) of furathiocarb

carbamate standard solutions used are as follows: 10 ng/mL standard solutions for propoxur, methomyl, dioxacarb, bendiocarb, carbaryl, ethiofencarb, isoprocarb, fenobucarb, carbaryl, promecarb, BDMC, indoxacarb and benfuracarb, 5 ng/mL standard solutions for carbofuran, fenothiocarb, aldicarb and alanycarb, 2.5 ng/mL standard solutions for oxamyl, furathiocarb and pirimicarb. The final concentrations for internal standards carbaryl-D7 and methomyl-D3 were 50 ng/mL.

Calibration Curve and Linear Range

Mixed standard working solutions of different concentrations were prepared as described. The calibration curves (Figure 22) were plotted using the internal standard method, with the peak area ratio of carbamate to internal standard against the concentration ratio. The linearity was good, and the linear equation and correlation coefficient were shown in Table 3.

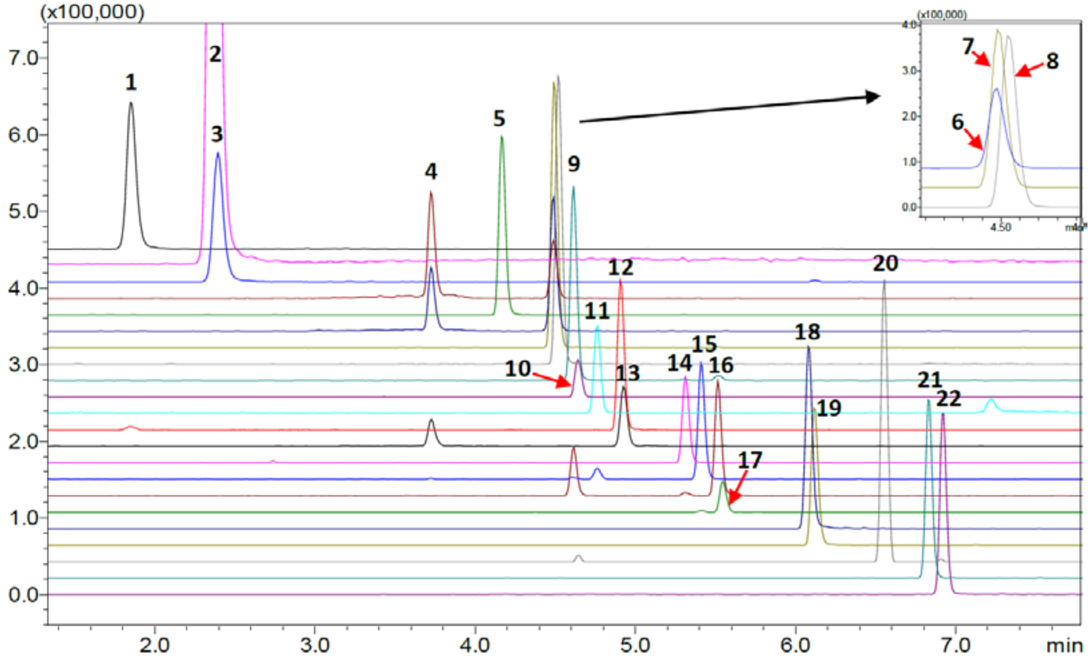
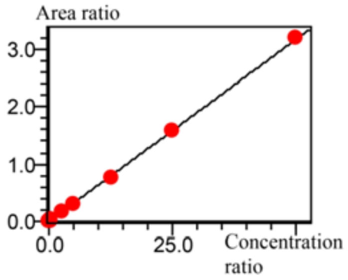
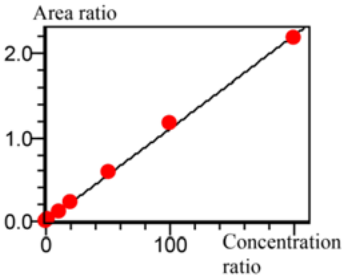


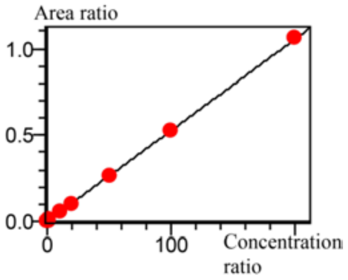
Figure 21 MRM chromatogram of 20 carbamate pesticide residues
(1 oxamyl, 2 methomyl-D3, 3 methomyl, 4 dioxacarb, 5 aldicarb, 6 bendiocarb, 7 propoxur, 8 carbofuran, 9 carbaryl-D7, 10 carbaryl, 11 ethiofencarb, 12 pirimicarb, 13 isoprocarb, 14 fenobucarb, 15 methiocarb, 16 promecarb, 17 BDMC, 18 fenothiocarb, 19 alanycarb, 20 indoxacarb, 21 benfuracarb, 22 furathiocarb)



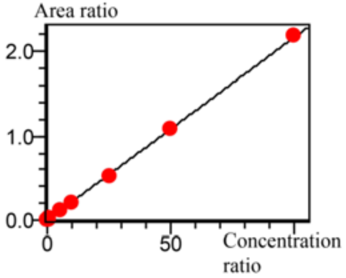
Oxamyl



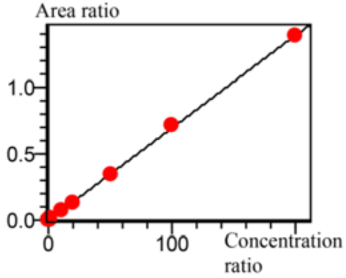
Methomyl



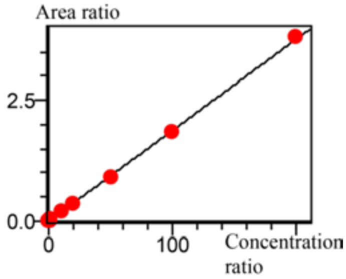
Dioxacarb



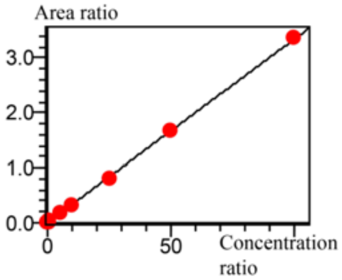
Aldicarb



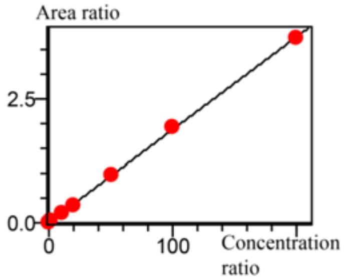
Bendiocarb



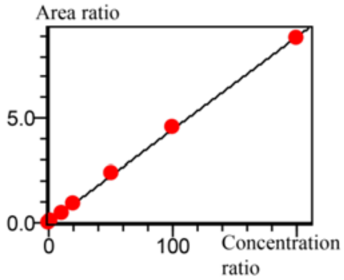
Propoxur



Carbofuran



Carbaryl



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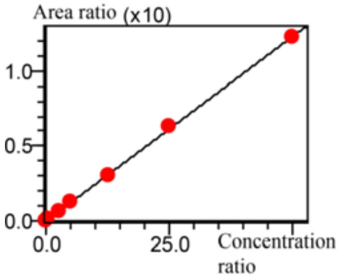

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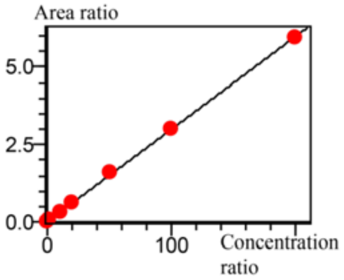

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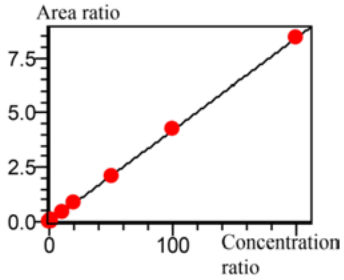
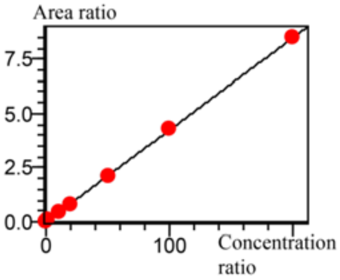
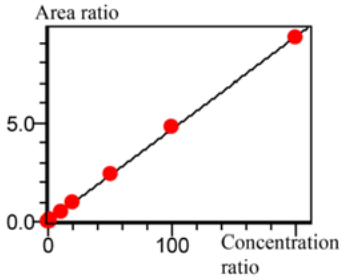


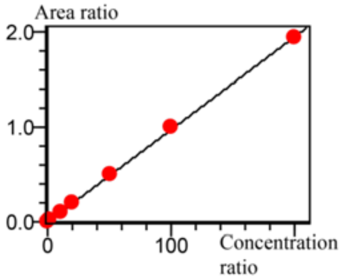
Fenobucarb



Methiocarb



Promecarb



BDMC

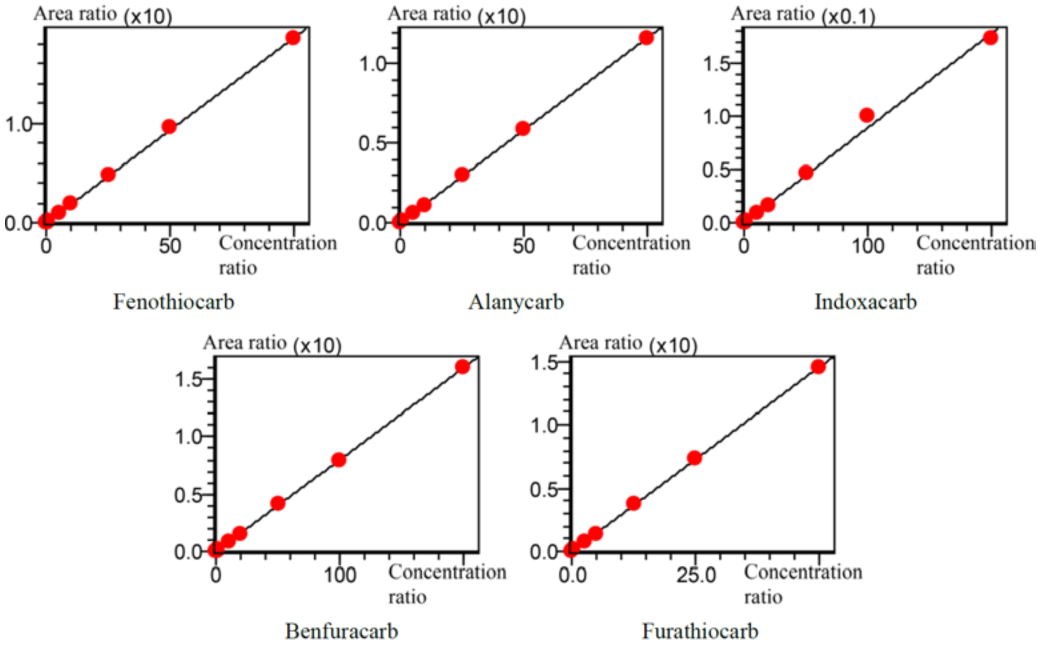


Figure 22 Standard calibration curves of 20 carbamates

Table 3 Method validation results

No.	Compound Name	Calibration Curve	Linear Range (μg/L)	Correlation Coefficient (r)	Accuracy %
1	Oxamyl	Y = 0.0634370X	0.25~50	0.9999	87.4~100.5
2	Methomyl	Y = 0.0110982X	1~200	0.9994	98.4~109.7
3	Dioxacarb	Y = 0.00528174X	1~200	0.9999	86.1~100.6
4	Aldicarb	Y = 0.0217154X	0.5~100	0.9999	87.6~100.6
5	Bendiocarb	Y = 0.00693232X	1~200	0.9999	95.8~110.3
6	Propoxur	Y = 0.0188432X	1~200	0.9998	87.6~100.8
7	Carbofuran	Y = 0.0334726X	0.5~100	0.9999	92.9~100.4
8	Carbaryl	Y = 0.0187430X	1~200	0.9997	93.7~112.6
9	Ethiofencarb	Y = 0.0446606X	1~200	0.9999	93.2~113.3
10	Pirimicarb	Y = 0.245836X	0.25~50	0.9999	92.5~108.7
11	Isoprocarb	Y = 0.0299024X	1~200	0.9998	87.8~112.2
12	Fenobucarb	Y = 0.0421050X	1~200	0.9999	86.8~102.5
13	Methiocarb	Y = 0.0424351X	1-200	0.9999	86.3~110.9
14	Promecarb	Y = 0.0467029X	1-200	0.9998	90.0~106.3
15	BDMC	Y = 0.00973255X	1-200	0.9999	99.3-114.4
16	Fenothiocarb	Y = 0.186315X	0.5-100	0.9998	87.9-102.6
17	Alanycarb	Y = 0.116527X	0.5-100	0.9999	87.0-111.6
18	Indoxacarb	Y = 0.000892791X	1-200	0.9968	85.8-111.7
19	Benfuracarb	Y = 0.0800311X	1-200	0.9998	87.9-104.2
20	Furathiocarb	Y = 0.291325X	0.25-50	0.9999	99.5-106.8

Precision Test

The mixed standard working solutions were prepared at 3 different concentrations and injected 6 consecutive times for each concentration to evaluate the precision. The repeatability results of retention time and peak area are shown in Table 4. The results indicated that the relative standard deviations of retention time and peak area of standard samples at the three concentrations were 0.015–0.263 % and 0.554–4.917% respectively, showing good precision.

Sensitivity Test

The sensitivity was determined using the mixed standard working solution at low-concentration. With reference to Method HJ 168-2010 “Environmental monitoring – Technical Guideline on Drawing and Revising Analytical Method Standards”, the method detection limit is calculated by MDL = t(6,0.99) x S where S is defined as the average standard deviation of 7 blank tests and the limit of quantitation (LOQ) is calculated to be 4 times of the limit of detection (LOD). The signal-to-noise ratio (S/N) and method detection limit of the 20 compounds are shown in Table 5.

Table 4 Repeatability and sensitivity results of retention time and peak area.

Compound Name	RSD% (1 µg/L)		RSD% (20 µg/L)		RSD% (160 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Propoxur	0.034	4.152	0.058	1.735	0.042	1.353
Methomyl	0.180	3.434	0.215	1.017	0.198	0.843
Dioxacarb	0.056	4.833	0.092	3.487	0.069	1.119
Bendiocarb	0.056	3.010	0.056	2.125	0.042	0.911
Carbaryl	0.075	3.335	0.049	2.276	0.042	1.520
Ethiofencarb	0.066	4.261	0.051	2.675	0.037	1.126
Isoprocarb	0.077	2.536	0.051	3.565	0.037	1.186
Fenobucarb	0.077	4.775	0.040	1.483	0.031	1.604
Methiocarb	0.077	4.145	0.045	1.611	0.026	0.835
Promecarb	0.076	2.368	0.042	1.802	0.028	1.539
BDMC	0.038	4.917	0.051	4.815	0.027	2.000
Indoxacarb	0.104	4.318	0.015	4.806	0.026	4.255
Benfuracarb	0.022	3.739	0.036	1.369	0.023	1.394
	RSD% (0.5 µg/L)		RSD% (10 µg/L)		RSD% (80 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Carbofuran	0.068	4.98	0.058	1.735	0.040	0.592
Fenothiocarb	0.035	3.904	0.049	2.455	0.018	1.346
Aldicarb	0.056	2.035	0.069	2.639	0.055	1.434
Alanycarb	0.062	4.033	0.049	2.455	0.016	1.501
	RSD% (0.25 µg/L)		RSD% (5 µg/L)		RSD% (40 µg/L)	
	R.T.	Area	R.T.	Area	R.T.	Area
Oxamyl	0.263	4.031	0.256	1.624	0.201	0.554
Furathiocarb	0.022	3.739	0.036	1.369	0.024	1.171
Pirimicarb	0.072	2.723	0.058	1.735	0.035	0.809

Table 5 Signal-to-noise ratio (S/N), limit of detection and limit of quantitation

Compound Name	Concentration Level (μg/kg)	S/N	Limit of Quantitation (μg/kg)	Limit of Detection (μg/kg)
Oxamyl	0.025	27.59	0.020	0.005
Methomyl	0.100	24.98	0.048	0.012
Dioxacarb	0.100	10.22	0.092	0.023
Aldicarb	0.050	27.59	0.036	0.009
Bendiocarb	0.100	19.37	0.068	0.017
Propoxur	0.100	55.33	0.036	0.009
Carbofuran	0.050	28.28	0.040	0.010
Carbaryl	0.100	54.07	0.052	0.013
Ethiofencarb	0.100	12.22	0.024	0.006
Pirimicarb	0.025	31.67	0.016	0.004
Isoprocarb	0.100	15.49	0.076	0.019
Fenobucarb	0.100	30.92	0.060	0.015
Methiocarb	0.100	72.88	0.032	0.008
Promecarb	0.100	32.27	0.088	0.022
BDMC	0.100	58.56	0.040	0.010
Fenothiocarb	0.050	13.64	0.044	0.011
Alanycarb	0.050	64.20	0.036	0.009
Indoxacarb	0.100	10.73	0.052	0.013
Benfuracarb	0.100	39.73	0.084	0.021
Furathiocarb	0.025	19.13	0.012	0.003

Matrix Spike Test

3 replicates of each matrix spike samples were prepared and tested. The spiked concentrations and spike recovery are shown in Table 6. It showed that the spike recovery of 20 carbamate samples were in the range of 88.1 – 108.4%.

Actual Analysis of Soil Samples

The soil samples were prepared and tested in 3 replicates. The MRM chromatogram results are shown in Figure 23. The soil sample was analyzed to contain 0.296 ± 0.026 μg/kg of Methomyl, 0.491 ± 0.047 μg/kg of Propoxur, 0.235 ± 0.012 μg/kg of Carbofuran, 0.237 ± 0.013 μg/kg of Bendiocarb, 0.379 ± 0.025 μg/kg of Isoprocarb, 0.462 ± 0.053 μg/kg of Fenobucarb, 0.517 ± 0.048 μg/kg of Promecarb and 0.095 ± 0.015 μg/kg of Fenothiocarb.

CONCLUSION

This paper established a method for the determination of carbamate pesticide residues in soil using Shimadzu's UHPLC LC-30A coupled with Triple Quadrupole Mass Spectrometer LCMS-8045. The analysis of 20 carbamates was completed within 12 min, and the correlation coefficients of calibration curve were all above 0.997. The mixed standard solutions with different concentrations were tested in 6 replicates. The relative standard deviations of the retention time and peak area of the 12 target compounds were 0.015% – 0.263% and 0.554% – 4.917% respectively, showing good precision. The blank matrix was injected into the instrument for analysis after sample preparation, and the recovery was 88.1 – 108.4%. This demonstrates a rapid, high sensitivity and excellent reproducibility method for the analysis of multi carbamate pesticide residues in soil.

Table 6 Test results of matrix spike

Compound Name	Spiked Concentration (2 μg/kg)		Spiked Concentration (16 μg/kg)	
	Detection Value	Recovery	Detection Value	Recovery
Propoxur	1.812±0.023	90.6	16.016±0.285	94.3
Methomyl	2.123±0.033	106.2	16.584±0.193	103.7
Dioxacarb	1.949±0.051	94.1	15.911±0.181	91.4
Bendiocarb	1.832±0.028	91.6	16.278±0.167	101.7
Carbaryl	2.041±0.106	97.7	16.353±0.278	102.2
Ethiofencarb	2.049±0.082	92.2	16.260±0.397	96.6
Isoprocarb	2.067±0.056	103.4	16.652±0.348	98.1
Fenobucarb	2.042±0.087	102.1	16.597±0.491	103.7
Methiocarb	1.980±0.055	95.0	16.393±0.318	99.5
Promecarb	2.038±0.080	101.9	16.731±0.419	94.6
BDMC	2.071±0.086	103.6	16.971±0.269	106.1
Indoxacarb	1.761±0.096	88.1	17.385±0.428	108.4
Benfuracarb	1.923±0.086	96.3	17.145±0.270	107.2
	Spiked Concentration (1 μg/kg)		Spiked Concentration (8 μg/kg)	
	Detection Value	Recovery	Detection Value	Recovery
Carbofuran	0.922±0.011	92.2	8.158±0.863	102.0
Fenothiocarb	0.925±0.039	92.5	7.628±0.161	95.3
Aldicarb	0.904±0.018	90.4	7.610±0.106	95.1
Alanycarb	0.995±0.046	99.5	8.323±0.190	104.0
	Spiked Concentration (0.5 μg/L)		Spiked Concentration (4 μg/L)	
	Detection Value	Recovery	Detection Value	Recovery
Oxamyl	0.473±0.010	94.7	3.935±0.036	98.9
Furathiocarb	0.489±0.020	97.9	4.022±0.685	100.6
Pirimicarb	0.503±0.018	100.8	3.864±0.0927	96.6

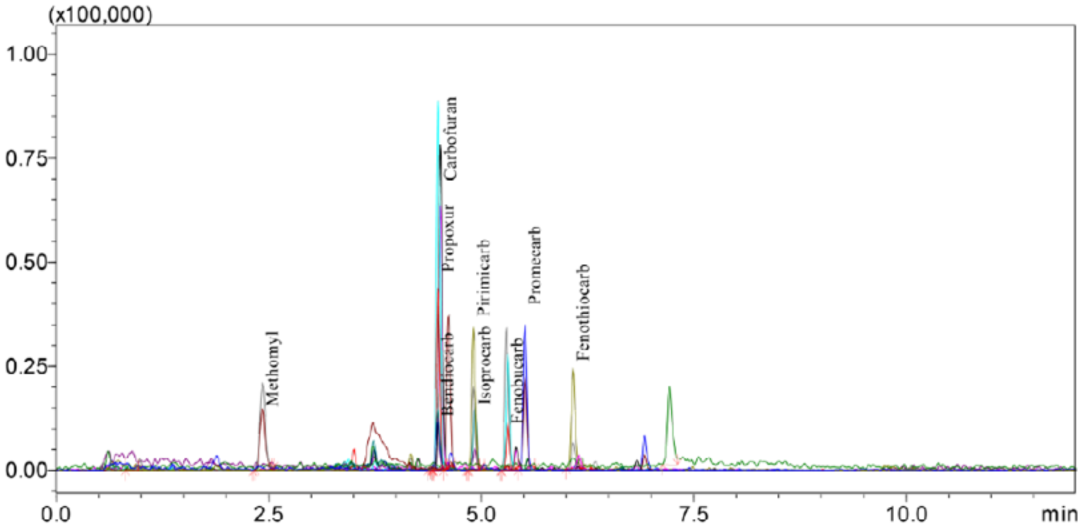


Figure 23 Chromatogram of 20 carbamates in soil samples

SHIMADZU
Excellence in Science

Determination of Benzidine and 3,3-Dichlorobenzidine in Environmental Waters Using Ultra-High Performance Liquid Chromatograph and Triple Quadrupole Mass Spectrometer

Long Zhen
Shimadzu (China), Beijing Analysis Center

Application News
SSL-CA14-576

Abstract

A method was established in this experiment to detect benzidine and 3,3-dichlorobenzidine (DCB) in environmental water using Shimadzu's Nexera XR and LCMS-8045 system. Using the MRM mode, this paper is the first to establish a rapid method to detect benzidine and DCB in environmental waters. The method has a wide linear range (0.39 - 6.25 µg/L), good repeatability (2.80% - 5.94%), good recovery (91% - 98%) and high detection sensitivity. Compared with the electrochemical detection (ECD) method documented in EPA605, the described method has better selectivity in detection of river water and sewage and can eliminate the false positive problem encountered in ECD.

Benzidine and 3,3'-dichlorobenzidine (DCB) are important pigment intermediates (Figure 1). They are used for the synthesis of medium and high-grade diaromatic amine azo pigments such as pigment yellow 12, 13, 14, 17, 35, 55, pigment orange 13 and pigment red 38. The diaromatic amine azo pigments are widely applied in ink, plastic, rubber, paint and dye industries due to the good solubility and migration resistance, as well as good crystallization resistance and thermal stability.

In addition, the diaromatic amine azo pigments have the double coloring power compared to monoazo pigments, bright color and low price. For example, pigment yellow 17 is an irreplaceable dye for high transparency printing inks because of its strong coloring power and good transparency. The current total yield is 240,000 tons/year worldwide, among which pigments yellow share 25%. The demand of DCB is 25,000 tons/year. Due to the high environmental protection requirements in Europe, US and Japan, the production of such dyes has been stopped in the above-mentioned regions and they are mostly produced by China.

Both benzidine and DCB have strong carcinogenic effects. The Environmental Protection Agency of US issued a standard method, "EPA Method 605", which uses electrochemistry as a detector to achieve high sensitivity detection of the substance. However, the complex environmental water samples, especially sewage samples, are prone to give false positive test results. In addition, benzidine and DCB residues are tend to retain in the sampler, which will interfere the accuracy and repeatability of test results. Mass Spectrometry (MS) can provide compound quality information (molecular, ion information and fragment information) and increase the accuracy of compound quantitation. The unique MRM mode of the Triple Quadrupole Mass Spectrometer identifies and quantifies specific fragment ions of compounds to increase the quantitative and qualitative accuracy and exclude false positives of test results to the greatest extent possible. In this paper, a rapid method was established for quantitation of benzidine and DCB using MRM mode and Shimadzu's Nexera XR coupled with a Triple Quadrupole Mass Spectrometer LCMS-8045. The method is applied to the detection and quantitation of benzidine and DCB in surface water and waste water. Meanwhile, an SIL-30AC was used as the autosampler, which can simultaneously wash the inside and outside of the needle, and uses SL injection (split loop, where the injection needle is located in the flow path after sample injection for continuous flushing) to achieve low residue analysis and detection of benzidine and DCB.

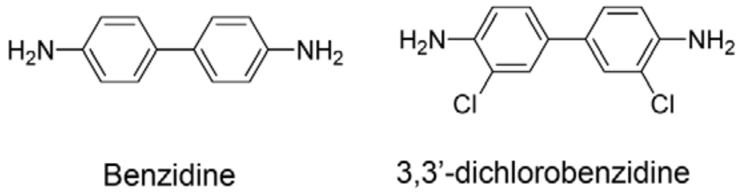


Figure 1 Structures of benzidine and DCB

EXPERIMENTAL

Instrumentation

LC-20AD$_{XR}$ pump, SIL-30ACMP autosampler, CTO-20AC column oven, CBM-20A system controller; LCMS-8045 triple quadrupole mass spectrometer equipped with ESI Ionization Source; LabSolutions Ver5.86 (Chromatographic Workstation).

Sample Information and Preparation

Standard Solutions: Reference standards of benzidine (purchased from Sigma, CAS #: 92-87-5) and DCB (3,3-dichlorobenzidine, purchased from Sigma, CAS #: 91-94-1) were weighed and dissolved in acetonitrile to prepare 1 mg/L of standard stock solution. The standard stock solution was used for serial dilution with 0.1% of formic acid in water (formic acid : water, 1:1,000, v/v) to obtain standard solutions of concentrations 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.185, 0.093 and 0.046 µg/L.

Repeatability: Seven injections were performed for samples with concentrations of 6.25, 3.13 and 1.56 µg/L.

Actual Samples: Water samples were collected and filtered using 0.22 µm polytetrafluoroethylene membrane and are ready for analysis. Actual samples include: lake water (1#), river water (2# and 3#), sewage (4#, 5# and 6#).

Spike recovery: 100 µL of 50 µg/L standard solution was diluted to 1 mL with 0.1% FA water (reference); 100 µL of 0.1% FA aqueous solution was added to 900 µL of sample (spiked sample); 100 µL of 50 µg/L standard solution was added to 900 µL of sample (spiked sample). Recovery = ((spiked sample A - sample A) / reference A) x 100%, where A: peak area of target compound.

Analytical Conditions

LC Chromatography (LC) Condition

Column : Phenomenex Kinetex C8 (2.1 x 150 mm, 2.6 µm)
Mobile Phase : A- 0.05% formic acid solution; B- acetonitrile containing 0.05% formic acid
Column temperature : 30 °C
Gradients : 0 - 1.5 min 5% - 95% B; 1.5 - 3.9 min 95% B; 3.9 - 4.0 min 95% B; 4.0-6.5 min 5% B
Flow rate : 0.3 mL/min
Injection volume : 10 µL
Washing : External washing (washing before and after injection)

Mass Spectrometry (MS) Conditions:

Analytical instrument : LCMS-8045
Ionization mode : ESI (+)
Nebulizing gas flow rate : 3.0 L/min
Drying gas flow rate : 10 L/min
Dwell time : 100 ms
Interface temperature : 400 °C
MRM Parameters : Refer to Table 1
DL temperature : 200 °C
Dwell time : 9 ms
Interface voltage : 4.0 kV

Table 2 MRM optimized parameters

No.	Compound Name	CAS No.	Precursor Ion	Product Ion	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
1	Benzidine	92-87-5	185.3	168.1*	-10.0	-20.0	-28.0
				151.0	-14.0	-30.0	-25.0
2	3,3-dichlorobenzidine (DCB)	91-94-1	253.2	182.1*	-27.0	-30.0	-19.0
				154.0	-20.0	-50.0	-29.0

Note: * indicates quantification ion

RESULTS & DISCUSSION

Optimization of Analytical Conditions

Sensitivity of benzidine and DCB is greatly influenced by column, mobile phase gradient and flow rate. Kinetex C8 (2.1 x 150 mm, 2.6 μm) column was used as stationary phase, and the sensitivity of the target substance is 10 times higher than that of C18 column of the same brand. Compared with the flow rate of 0.3 mL/min, the response to benzidine increases by about 68% with flow rate of 0.4 mL/min, while the response to DCB decreases by about 70%. The increase in sensitivity of benzidine comes from sharp peaks produced by high flow, while the decrease in sensitivity of DCB comes from the influence of flow rate on nebulization efficiency. This experiment used a steep gradient (0 - 1.5 min, 5% - 9 5% gradient compression) and flow rate of 0.3 mL/min to simultaneously obtain a high response to the two analytes.

Among DCB fragment ions, m/z 217.0 has a higher response, followed by m/z 182.1 and 154.0, but m/z 217.0 channel has higher baseline noise with a steep gradient. Therefore, m/z 182.1 and 154.0 are selected as quantitative ion and reference ion, respectively.

Calibration Curve, LOD and LOQ

Standard solutions at different concentrations were prepared as specified and determined according to the analytical conditions described previously (as shown in Figure 2). By using the ASTM method, and taking the S/N of target compound with LOD concentration not lower than 3 and S/N with LOQ concentration not lower than 10 as the standard, LOD of benzidine was determined to be 0.046 μg/L (S/N: 4.3) and LOQ was 0.093 μg/L (S/N: 10.5) in this method; LOD of DCB was 0.185 μg/L (S/N: 5.17) and LOQ was 0.39 μg/L (S/N: 10.5) , which met the requirements of EPA 605 on determining the sensitivity to benzidine (LOD not higher than 0.08 μg/L) and DCB (LOD not higher than 0.20 μg/L).

The range of the calibration curve was chosen by taking the approximate

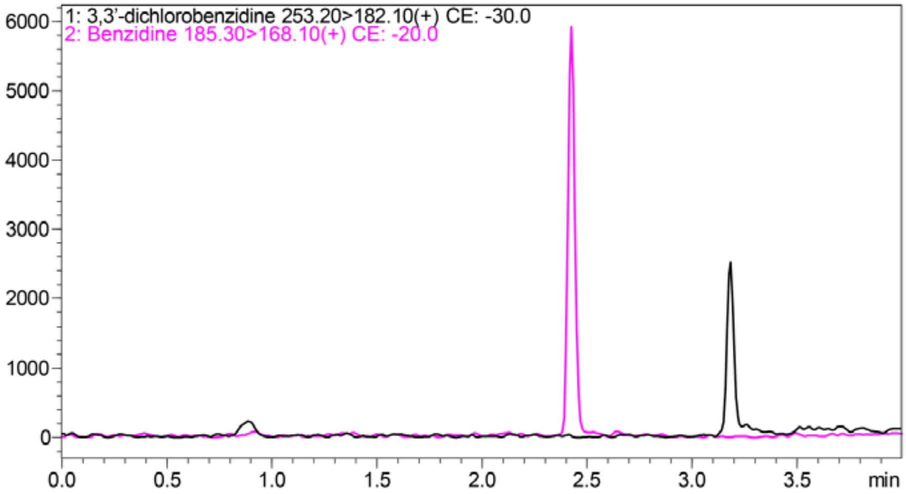


Figure 2 Chromatogram of benzidine and DCB

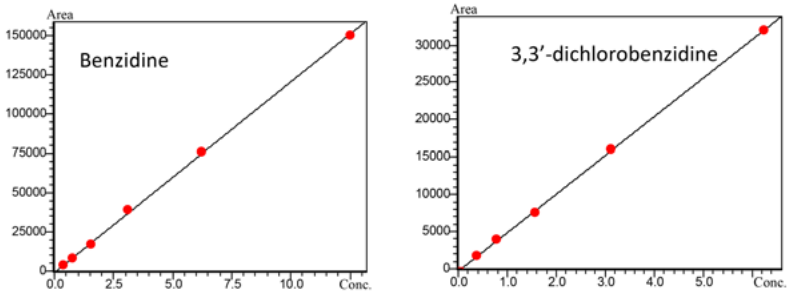


Figure 3 Calibration curves of benzidine and DCB

Table 2 Calibration curves and correlation coefficients of each analyte

No.	Analyte	Linear Range (μg/L)	Linear Equation	Correlation Coefficients	Accuracy (%)
1	Benzidine	0.39-12.5	A = 11787.1 C-204.9	0.9998	95.7-103.5
2	DCB	0.39-6.25	A = 3486.2 C-220.6	0.9994	96.1-104.5

concentration of the sample to be tested as the center and five concentration points (0.39-12.5 μg/L) were selected. A calibration curve was plotted with peak area against concentration and using external standard calibration method. The obtained standard calibration curve was shown in Figure 3. The linear regression equations and correlation coefficients of each analytes were shown in Table 2. The results indicated good linearity of both benzidine and DCB within the range of investigated concentration, while R was greater than 0.999 and the accuracy of each standard point was 95%-105%.

Repeatability

The standard solutions with concentrations of 1.56, 3.13 and 6.25 μg/L were injected for 7 replicates to calculate the relative standard deviations (RSD %) of peak area and retention time of the target compound. The results were shown in Table 3. It indicated that the RSD of the retention time and peak area of the two target analytes were 0.02 - 0.41% and 2.80 - 5.94% for the three concentrations, which met the requirements of quantitative analysis.

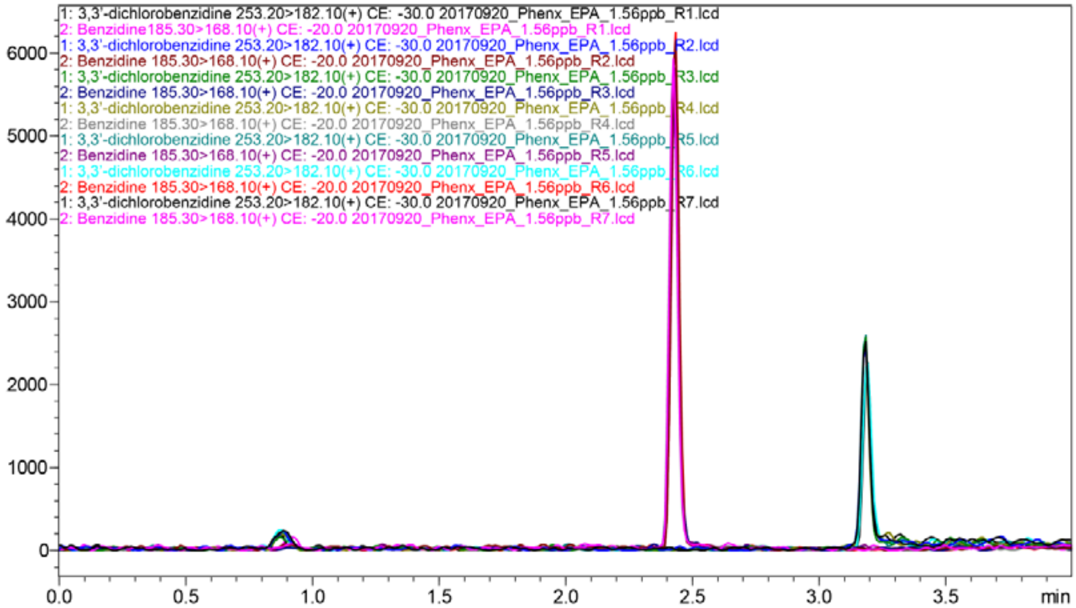


Figure 4 Repeatability analysis of benzidine and DCB with concentration of 1.56 μg/L (n=7)

Table 3 Instrument repeatability investigation results of each component (n=7)

	Benzidine			DCB		
	1.56 μg/L	**3.13 μg/L**	**6.25 μg/L**	**1.56 μg/L**	**3.13 μg/L**	**6.25 μg/L**
Retention Time %RSD	0.15	0.41	0.19	0.15	0.13	0.019
Peak Area Ratio %RSD	3.69	5.94	3.22	3.69	2.84	2.80

Sample Test and Recovery

With regards to the actual sample preparation and analysis, this experiment has also investigated the adsorption of benzidine and DCB to the filter membrane. The results showed that the water film and nylon membrane had significant adsorption of the two analytes, while the membrane made of polytetrafluoroethylene had less adsorption.

After optimization of chromatographic conditions and sample treatment conditions, this method is applied to the determination of benzidine and DCB in lake water (1#), river water (2# and 3#) and sewage (4#, 5# and 6#). The chromatogram of some samples were shown in Figure 5, and the test results were shown in Table 4. From Table 4, it can be seen that no DCB is detected in any samples, but benzidine is clearly detected, and the content of benzidine in sewage is higher than that of surface water (lake water and river water), indicating that the collected water samples are all polluted by benzidine to various degrees. According to the requirements

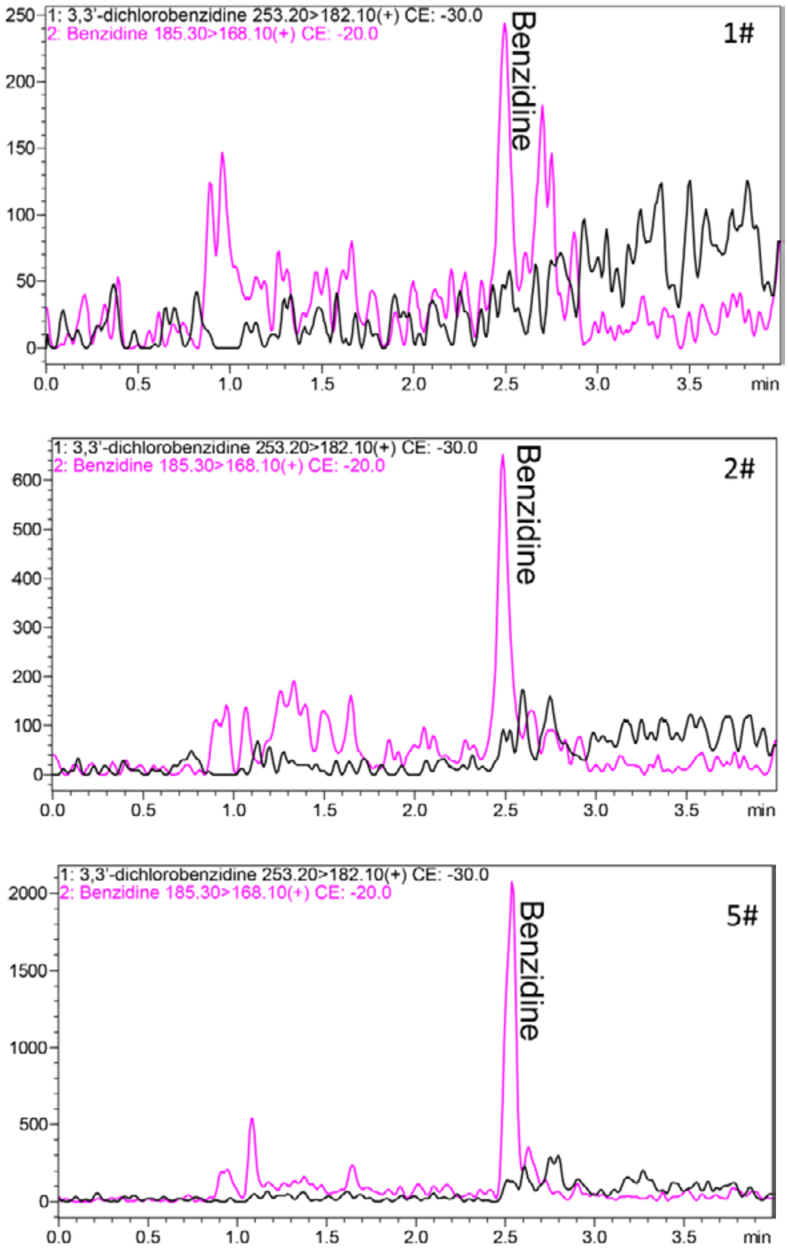


Figure 5 Sewage (5#), lake water (1#) and river water (2#)

Table 4 Determination results of actual samples

Sample Name	Benzidine Concentration (μg/L)	DCB (μg/L)
1# (Lake water)	detected	-
2# (River water)	0.45	-
3# (River water)	0.73	-
4# (Sewage)	1.18	-
5# (Sewage)	1.20	-
6# (Sewage)	1.11	-

Detected: Response is around LOD; -: Not detected

of EPA 605, the actual sample recovery of 5 μg/L spike was investigated. The recovery was determined to be 98.3% with 1# sample as the matrix, and 91.5 % with 5# sample as the matrix.

CONCLUSION

A method was established in this experiment to detect benzidine and DCB in environmental water using Shimadzu's Nexera XR and LCMS-8045 system. The method has good sensitivity, repeatability, recovery and wide linear range. The sensitivity and repeatability meet the requirements of EPA 605 and can be applied to the detection of benzidine and DCB in surface water and sewage.

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