SHIMADZU
Excellence in Science

S-FEM-B18

Honey Analysis **Made Easy**

Index

1.

OUR COMMITMENT TO BEES

Together, we can bee more!

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

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

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

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

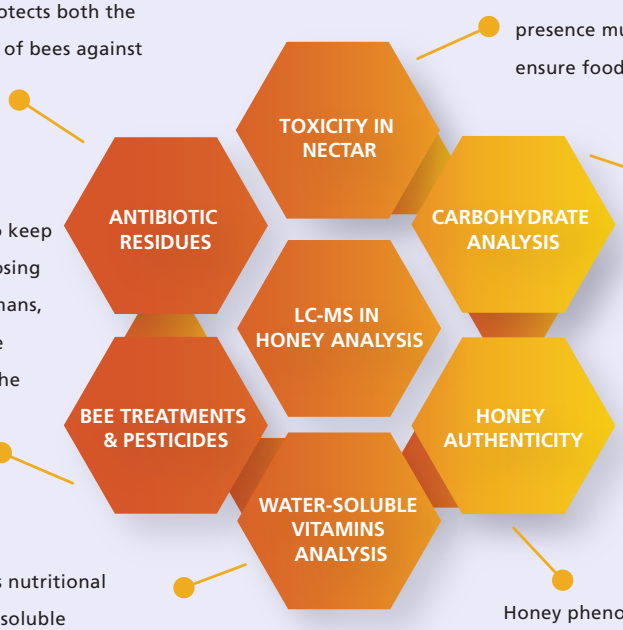
2.

INTRODUCTION TO HONEY ANALYSIS

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

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

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



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

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

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

3.

APPLICATION NOTES

3.1 Detection of Antibiotics

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

INTRODUCTION

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

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

EXPERIMENTAL

Apparatus

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

Condition of Analysis

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

See Table 1 for the time program.

Table 1 Time Program

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

MS Conditions

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

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

Sample Preparation

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

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

Table 2 Optimized MRM Parameters

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

*Note: * refers to quantitative ion*

RESULTS AND DISCUSSION

Mass Spectrum and MS/MS Spectrum

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

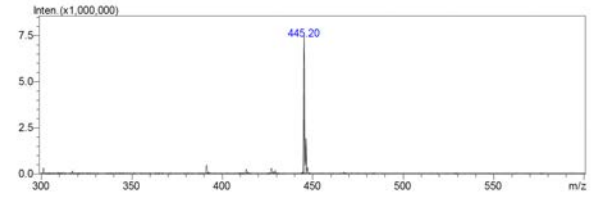


Fig. 1 Mass spectrum of tetracycline

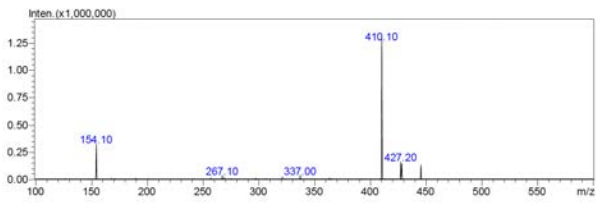


Fig. 2 MS/MS spectrum of tetracycline (CE -20V)

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

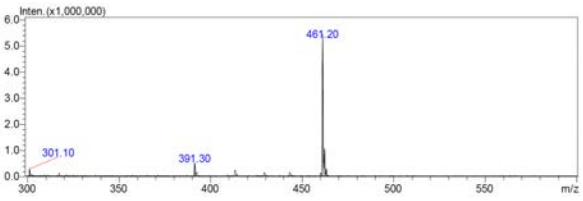


Fig.3 Mass spectrum of terramycin

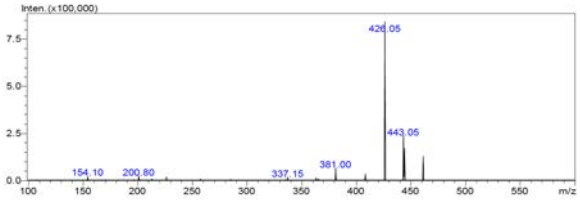


Fig.4 MS/MS spectrum of terramycin (CE -19V)

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

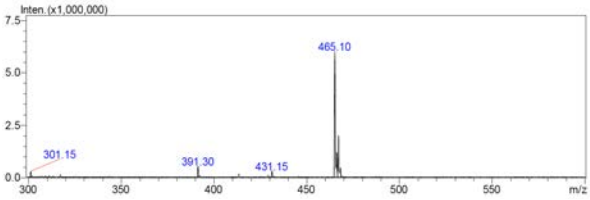


Fig. 5 Mass spectrum of ledermycin

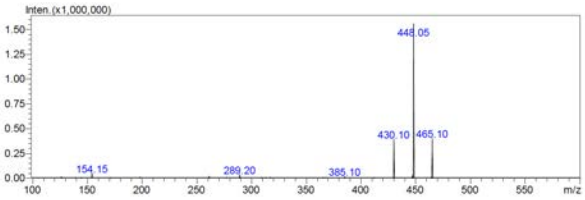


Fig. 6 MS/MS spectrum of ledermycin (CE -19V)

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

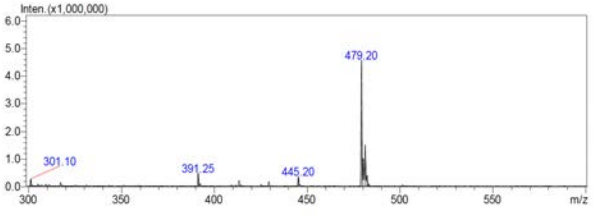


Fig. 7 Mass spectrum of aureomycin

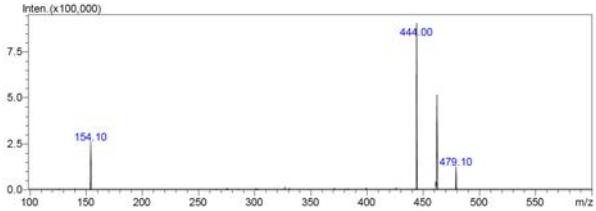


Fig. 8 MS/MS spectrum of aureomycin (CE -22V)

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

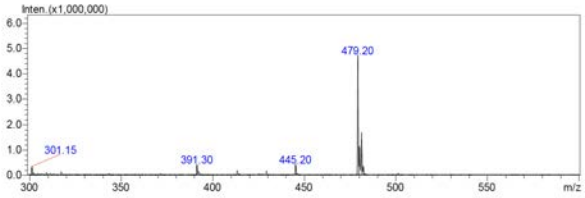


Fig. 9 Mass spectrum of doxycycline

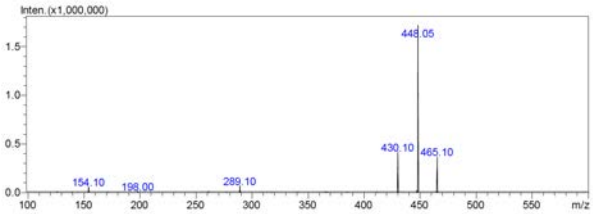


Fig. 10 MS/MS spectrum of doxycycline (CE -19V)

MRM Chromatogram of Standard Mixture

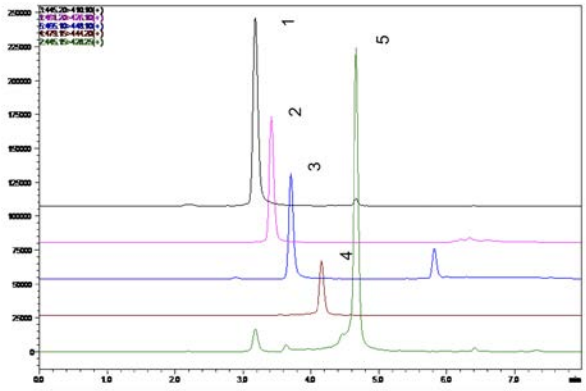


Fig. 11. MRM chromatograms of standard mixture (100 μg/L) (1. Tetracycline; 2. Terramycin; 3. Ledermycin; 4. Aureomycin; 5. Doxycycline)

Linear Range

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

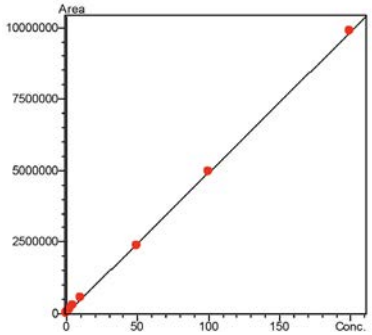


Fig. 12 Calibration curve of tetracycline

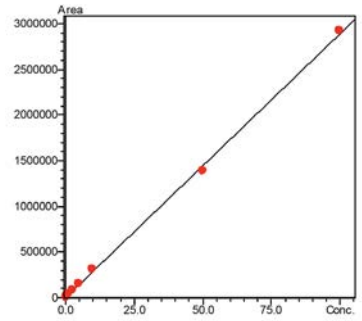


Fig.13 Calibration curve of terramycin

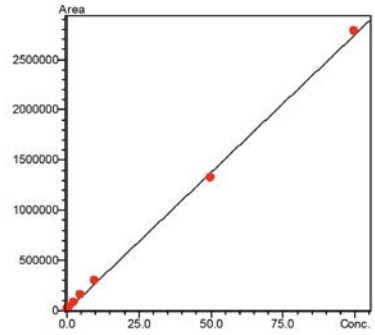


Fig. 14 Calibration curve of ledermycin

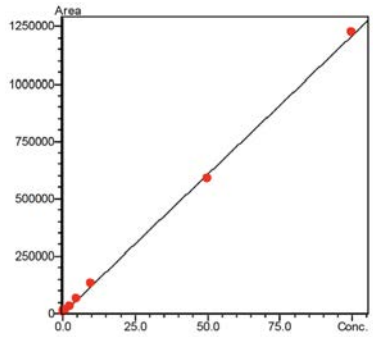


Fig. 15 Calibration curve of aureomycin

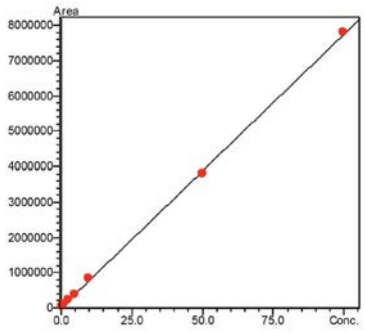


Fig. 16 Calibration curve of doxycycline

Table 3 Parameters of Calibration Curves

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

Precision Test

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

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

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

LOD

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

Table 5 LODs and LLOQs of Tetracyclines

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

Recovery Test

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

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

MRM Chromatogram of Actual Samples

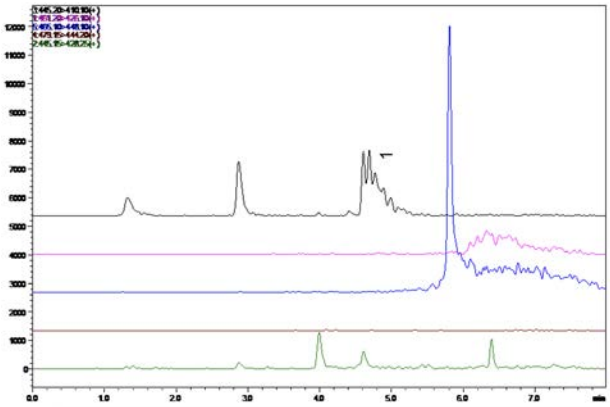


Fig.17 MRM chromatograms of honey sample (1 tetracycline detected)

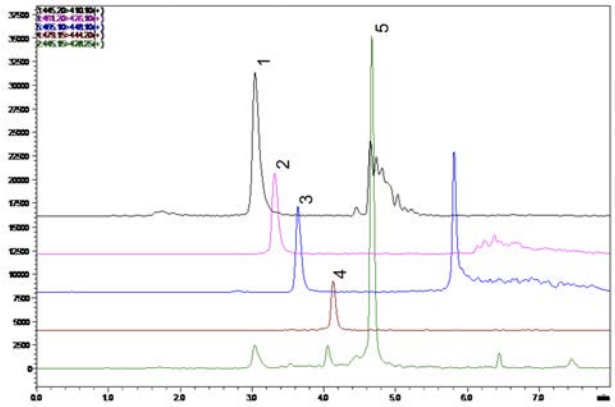


Fig. 18 MRM Chromatograms of a spiked honey sample (2 μg/kg) (1.Tetracycline; 2.Terramycin; 3.Ledermycin; 4.Aureomycin; 5. Doxycycline)

Table 6. Spike Recoveries of Tetracyclines

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

CONCLUSION

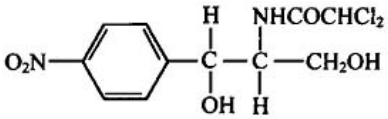
A method was proposed for detection of tetracyclines residues in honey using Shimadzu LC-30A UHPLC and LCMS-8040 triple quadrupole mass spectrometer. The method is of high sensitivity, good precision and wide linear range with the correlation coefficient greater than 0.9996. Detection of trace tetracycline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

3.1 Detection of Antibiotics

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

INTRODUCTION

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



Chlorampenicol(CAP)

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

EXPERIMENTAL

Instrument

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

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

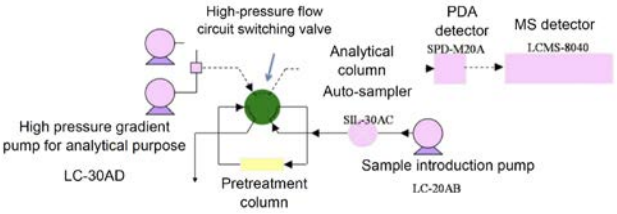


Fig.1 Flow circuit diagram of sample introduction

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

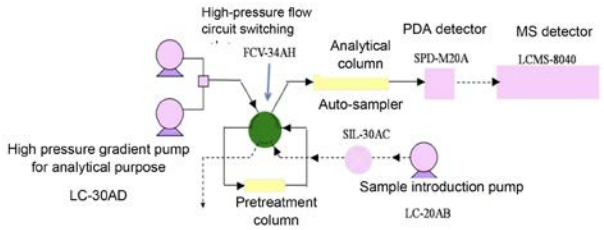


Fig.2 Flow circuit diagram of sample analysis

Conditions of Analysis

LC conditions

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

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

Flow Rate : 2 mL/min

Injection Volume : 5 μL

Conditions of Analysis

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

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

Flow Rate : 0.35 mL/min

Column Temperature : 40°C

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

See Table 1 for the elution program.

Table 1 Time Program

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

MS Conditions

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

Table 2 Optimized MRM Parameters

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

*Note: * refers to quantitative ion*

Preparation of Standard Solutions

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

Sample Pretreatment Method

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

RESULTS AND DISCUSSION

Optimization of Loading Time

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

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

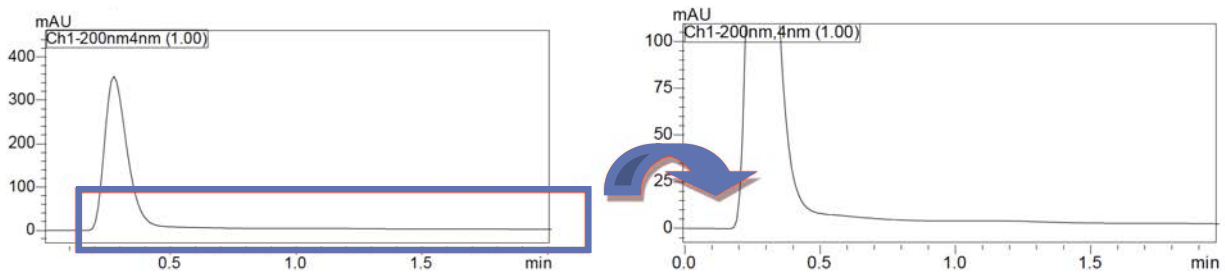


Fig.3 UV chromatogram of sample retention in the pretreatment column

Mass Spectrum and MRM Chromatogram

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

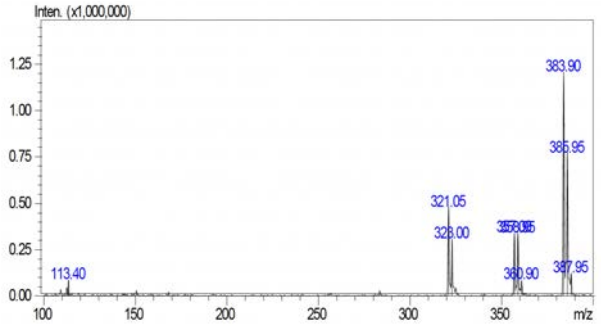


Fig.4 Scan chromatogram of a 100 ng/mL standard solution in Q3 Scan mode

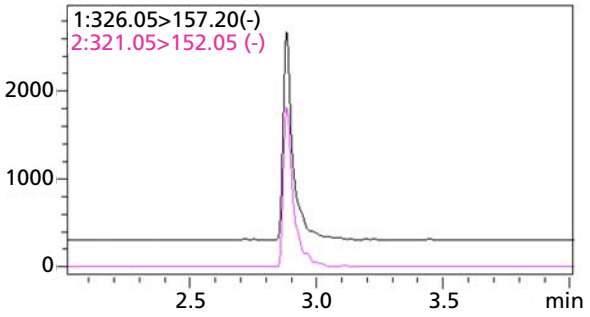


Fig.5 MRM chromatogram of a 10 ng/mL standard solution

Linear Range

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

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

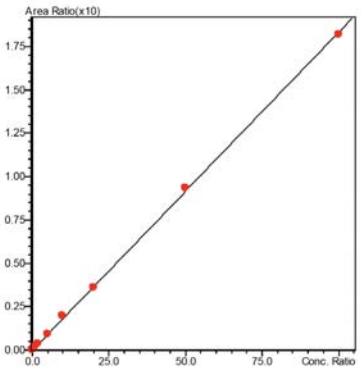


Fig.6 Calibration curve of chloramphenicol

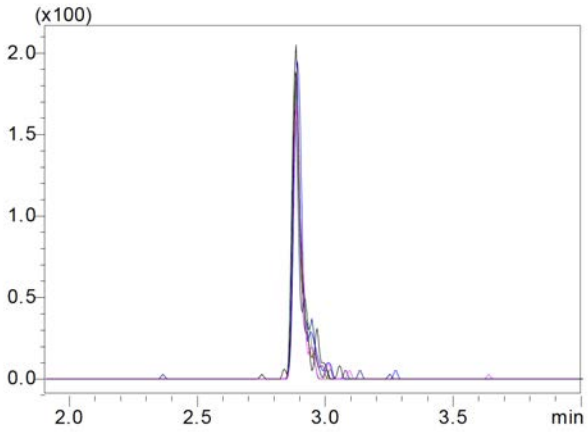


Fig.7 Overlapping chromatogram of 6 replicate injections of a 0.5 ng/mL standard solution

Precision Test

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

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

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

Sensitivity Test

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

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

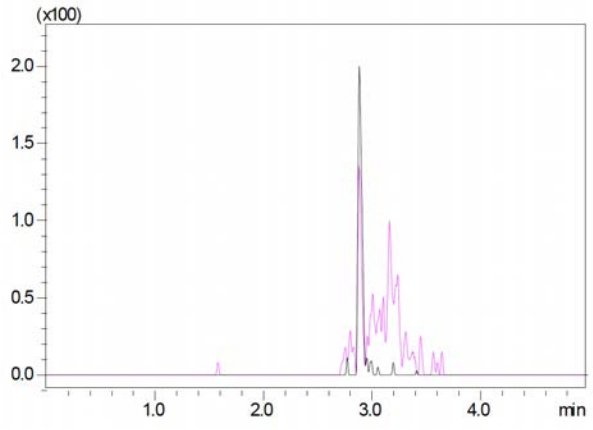


Fig.8 Chromatogram of a loading of 5 uL honey matrix spiked with 5 µg/kg standard

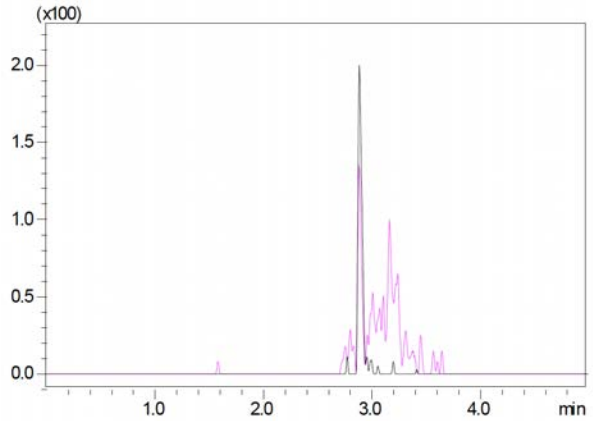


Fig.9 Chromatogram of a loading of 50 uL honey matrix spiked with 0.5 µg/kg standard

Recovery Test

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

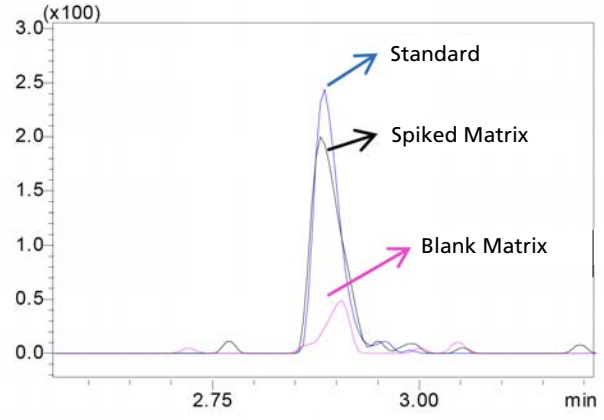


Fig.10 Overlapping chromatogram of blank matrix, spiked matrix and standard

CONCLUSION

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

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

3.1 Detection of Antibiotics

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

INTRODUCTION

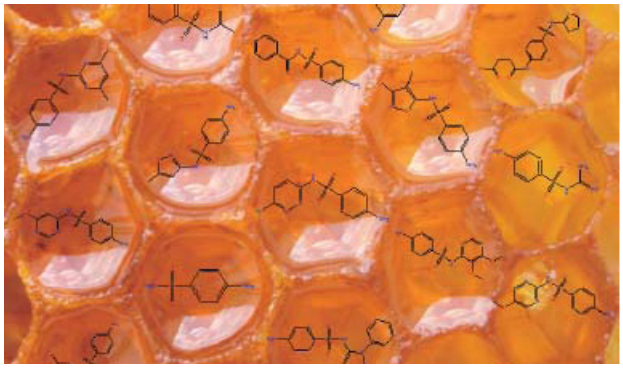
The antibacterial sulfonamides (SA) and trimethoprim are widely used in veterinary and human medicine. Diverse foods from animals potentially contain residues of these drugs posing possible threats to people by triggering allergic reactions and undesirable increasing of microorganism's drug resistance. Various countries have defined their own maximum residue limits (MRLs) for sulfonamides accepted in honey.

There are no MRL's for sulfonamides in honey in the UE but in 2002 a minimum required performance level (MRPL) was set for analytical methods at a level of 10 µg/kg. HPLC-MS/MS is an effective strategy to characterize and accurately measure those antibiotics considering MRLs and MRPLs in food products from animal origin tend to be continually reduced to protect human health safety. A selective, fast and sensitive HPLC-MS/MS method has been developed for 15 sulfonamides and trimethoprim.

MATERIALS AND METHOD

Sample Preparation

5 grams of honey, spiked with 17 SAs and trimethoprim (Table 1A), were extracted using QuEChERS method following manufacturer's procedure with a final 1:5 extract dilution using methanol. A multiple reaction monitoring MRM method was optimized for quantitation for each sulfonamide compound using a Shimadzu Nexera UHPLC with an LCMS-8050 fast-scanning triple quadrupole mass spectrometer model equipped with software Labsolution LCMS version 5.65 and electrospray ionization ESI.



Stock standard solutions of each sulfonamide were prepared dissolving appropriate amounts in DMSO and methanol, diluting to 100 ppm and 1 ppm at the end with mobile phase A:B 50:50. Table 1B shows the concentrations at each level used to build calibration curves for external calibration method.

LC Conditions

A Kinetex 2.6µ PFP 100 Å column (100 × 2.1 mm) was used at 40 °C, flow rate of 0.5 mL/min, and 10 µL injection volume using QuEChERS extraction method. A binary gradient of 10% methanol (mobile phase A) and methanol, 0.3% formic Acid (mobile phase B) was used with the gradient program described in Table 1C.

Mass Spectrometry:

Electrospray ionization was used in positive mode, spray voltage was 4.5 kV, desolvation line temperature was 250°C, nebulization gas was 2.0 L/min, heater block was 400°C, and drying gas 15 L/min.

Table 1. A. Sulfonamide compounds used in this study; B. Concentration levels to define calibration curves, and C. HPLC gradient used.

A. Sulfonamide Used

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

B. Calibration Curve

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

C. LC Gradient

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

To implement sulfonamide quantitation, MRM transitions were optimized using a 0.5 µg mixture of SAs, 1 µL injections at 400 µL/min. Three transitions from parent ions and fragments were selected using the optimization tool software.

RESULTS

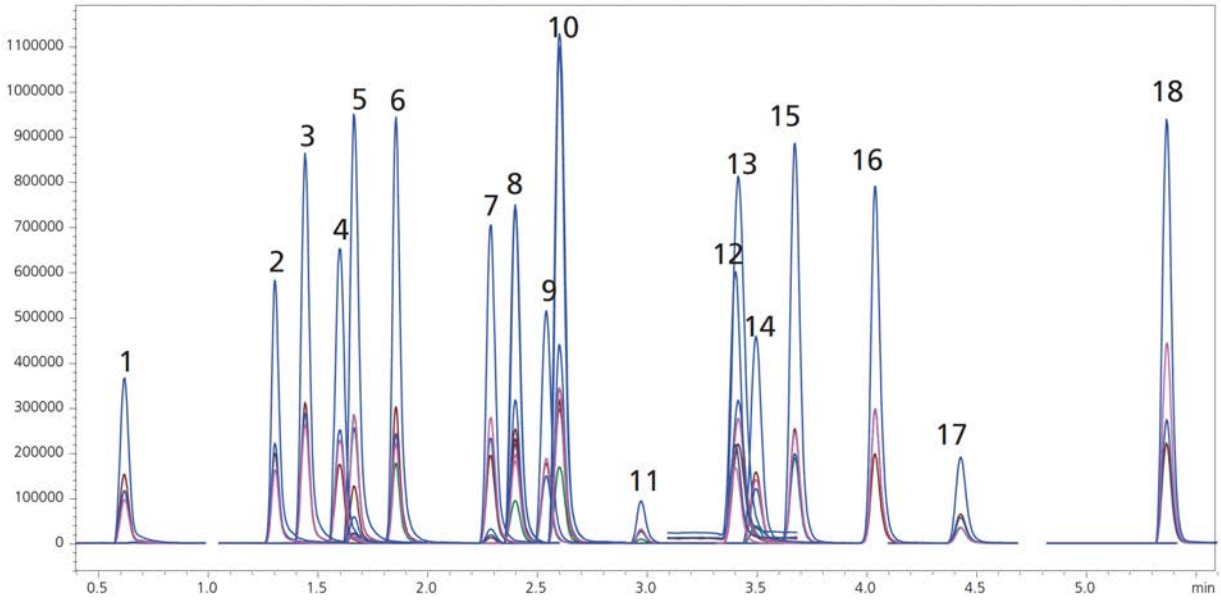
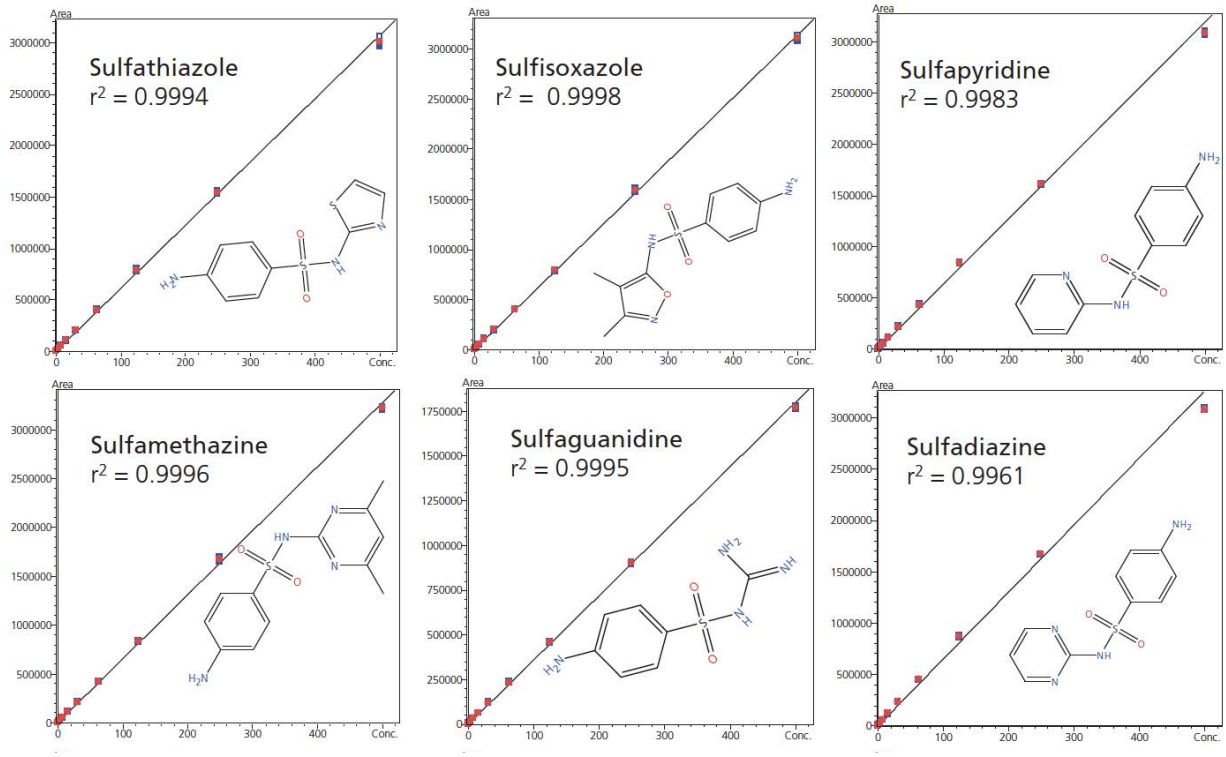


Figure 1. Representative chromatogram of sulfonamide drugs. Standard mixture at 125 pg on-column for each standard. Peak numbers follow the order described for SA compounds in table 1A.



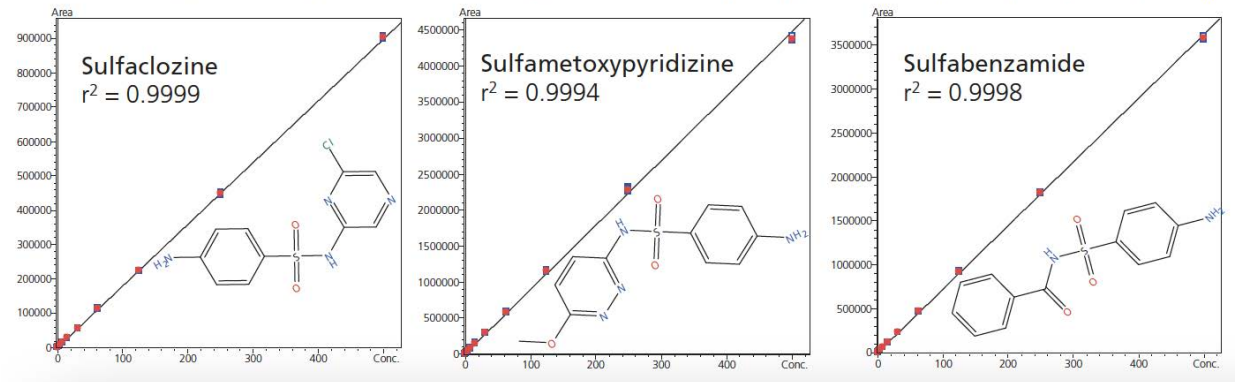
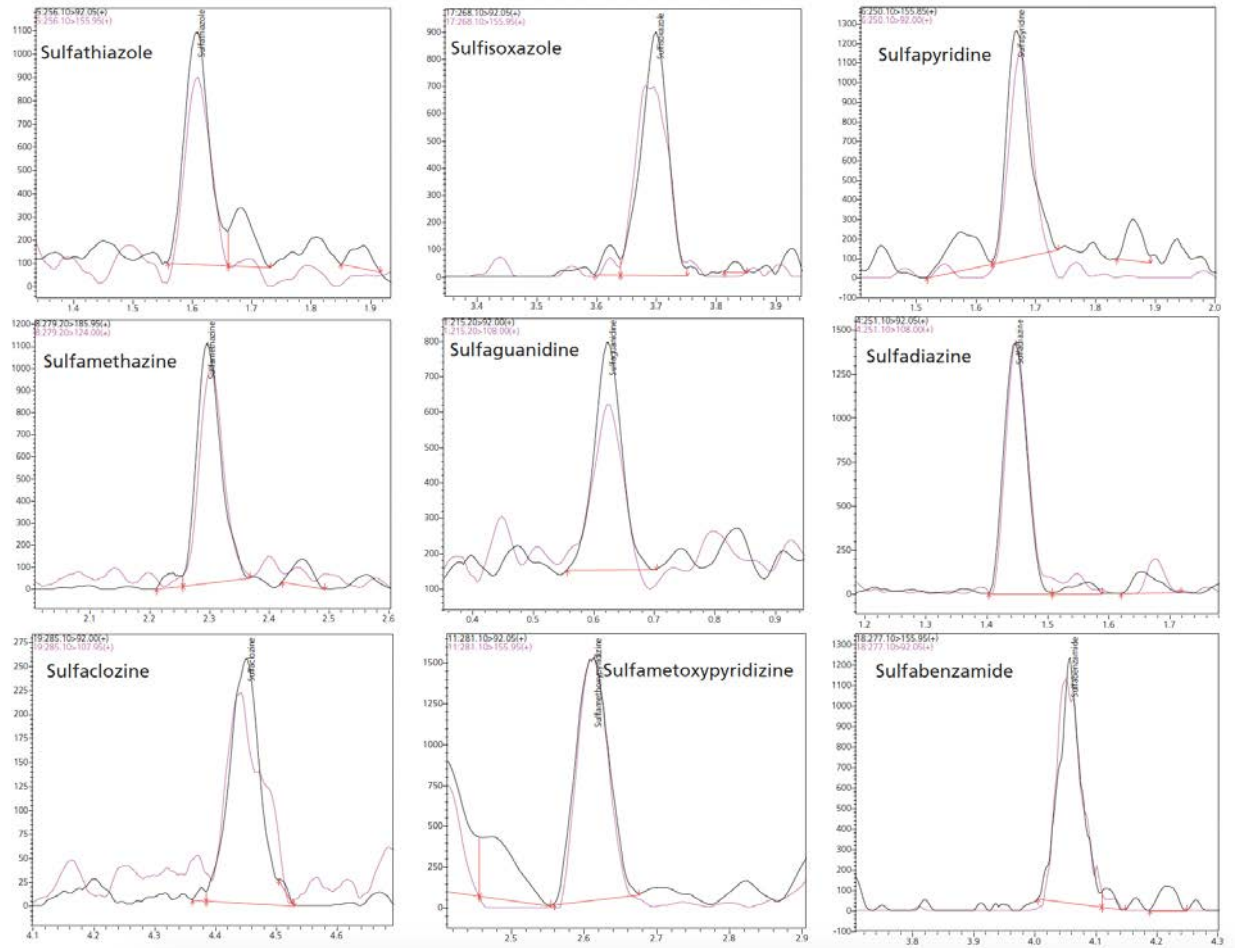


Figure 2. High degree of linearity was observed over the concentration range 0.5–500 pg on column, with values of $r^2 \geq 0.990$ for all analytes.

Authentic SAs standards were fully characterized by HPLC and MS/MS with an MRM optimized assay. The calibration curves of standards in 50% methanol matrix were linear with with $r^2 > 0.990$ (Figure 2) in the tested range of 1 to 1000 µg/Kg (0.5 to 500 pg on column). The limits of quantification were 1 µg/Kg (0.5 pg on column) for all compounds except succinylsulfathiazole and sulfacetamide, which were 2 µg/Kg (1 pg on column). The recovery ranged from 53.9 to 91.4% for all but two compounds measured using drug residue-free organic honey. Succinylsulfathiazole and sulfaguanidine exhibited recovery below 20% using the QuEChERS method for extraction.



	Sulfathiazole		Sulfisoxazole		Sulfapyridine		Sulfamethazine		Sulfaguanidine		Sulfadiazine		Sulfaclozine		Sulfamethoxypyridizine		Sulfabenzamide	
Level	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %
1	1.7	97.8	1.0	98.9	0.5	96.5	0.6	98.1	0.5	98.3	0.4	94.3	0.5	100.4	1.0	97.5	0.8	98.8
2	0.9	100.4	1.4	101.2	0.3	100.6	1.5	101.8	0.7	99.8	0.2	102.3	1.1	99.9	1.9	101.5	0.3	100.3
3	2.1	102.6	0.4	100.3	0.9	105.0	1.1	101.0	0.9	101.2	1.0	106.0	0.8	99.3	1.1	102.8	0.7	101.6
4	1.7	103.6	0.3	102.0	1.3	107.7	0.8	102.5	0.6	103.6	1.5	109.7	1.7	100.0	0.9	102.7	1.2	102.8
5	0.4	106.3	2.0	101.0	1.4	107.8	1.0	102.1	1.7	105.5	1.3	114.0	1.0	98.4	1.5	104.3	1.4	101.7
6	1.6	106.1	3.3	102.6	0.5	110.8	0.9	106.3	1.8	108.3	0.6	116.8	1.5	99.3	3.2	104.0	0.4	104.2
7	3.8	109.4	1.5	101.1	6.3	103.1	0.7	105.8	3.0	113.9	2.8	115.4	3.4	100.9	1.2	108.1	0.6	103.6
8	4.4	108.0	1.2	104.1	8.2	102.2	3.3	103.7	2.3	114.6	1.6	111.9	5.0	98.5	6.3	105.9	3.3	102.6
9	4.4	115.7	1.5	100.2	4.0	104.4	2.0	106.4	6.4	114.3	6.5	110.8	8.1	87.9	2.7	112.3	2.6	104.7
10	3.9	107.4	5.0	88.0	9.2	103.7	15.2	90.6	8.0	114.1	9.1	118.5	8.8	90.9	5.7	121.4	2.7	97.6
11	12.7	114.7	10.5	100.3	6.2	109.9	5.9	100.9	9.9	126.9	16.5	116.5	6.9	95.5	7.3	105.9	6.4	91.3

Figure 3. Representative chromatograms of sulfonamide drugs at lowest concentration showing limit of quantitation and statistics for diverse concentration levels.

CONCLUSION

LC-MS/MS with QuEChERS as extraction method provides a fast, simple, sensitive and accurately measuring for sulfonamide drugs and trimethoprim in honey with an acceptable recovery range. Matrix matched calibration and use of internal standards can be tested to improve performance.

3.2 Detection of Pesticide Residues

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

Neonicotinoids are a class of insecticides widely used to protect fields as well as fruits and vegetables. Recently the use of these compounds became very controversial as they were pointed as one cause of the honeybees colony collapse disorder. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. Following this the European Food Security Authoritiy (EFSA) limited the use of thiamethoxam, clothianidin and imidacloprid. Fipronil, a pesticide from a different chemical class, has been also banned by EFSA for maize seed treatment due to its high risk for honeybee health. In order to better understand the effect of these compounds on bees and their contamination in pollen and honey, a highly sensitive assay method was necessary. A method was set up using Nexera X2 with LCMS-8060.

Sample Preparation

Thiamethoxam-d3, imidacloprid-d4 and chlothianidin-d3 were used as internal standards. Compound extraction was performed using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with an additional dispersive Solid Phase Extraction (dSPE) step. 5 g of honey (±1%) were weighted in a 50 mL polypropylene tube. 5 µL of internal standard solution at 5 µg/mL of each compound in acetonitrile was added on honey and let dry for 10 minutes. 10 mL of ultra pure water were added and the samples were homogenized by vortex mixing for 1 minute. 10 mL of acetonitrile were then added followed by vortex mixing for 1 minute.

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

Recovery

An "all-flowers" honey from the local supermarket was extracted with or without spike at 50 ppt. A blank extract (no honey) was prepared to evaluate losses or non specific interactions. Results are presented in Table 1. Calculated recoveries are within acceptance values 70-120% from EU SANTE/11945/2015.

Table 1 Measured Recoveries in Honey

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

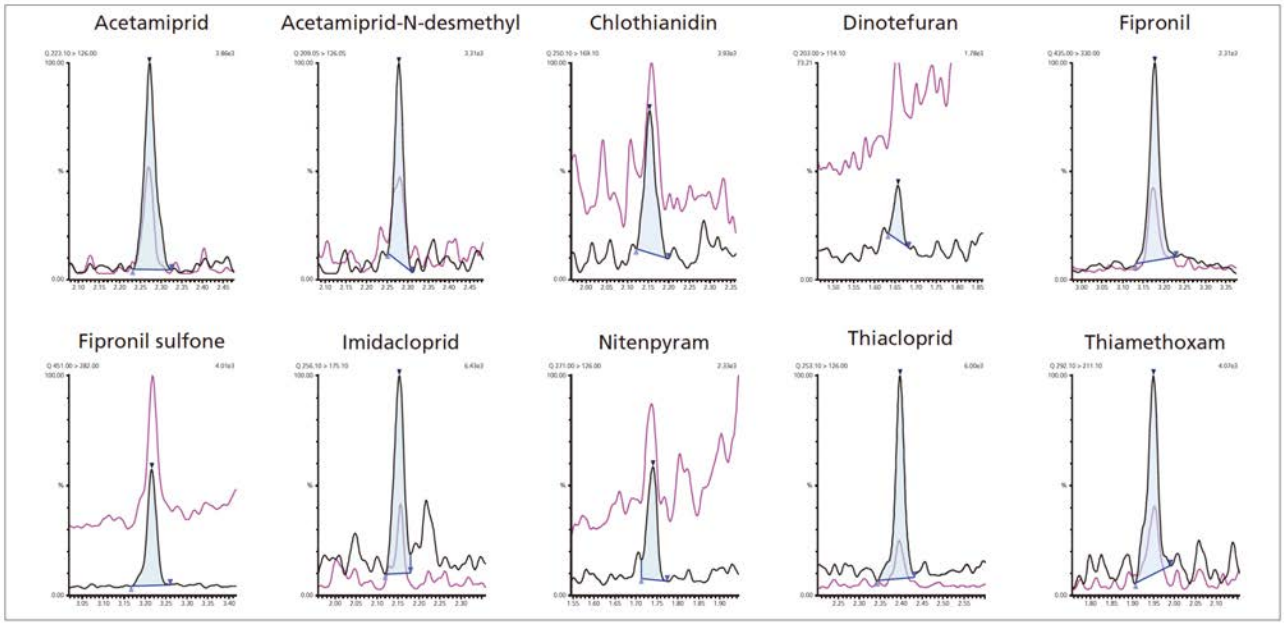


Fig. 1 Chromatogram of the Target Compounds at Their Lower Limit of Quantification

Table 2 Analytical Conditions

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

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

Table 3 MS/MS Acquisition Parameters

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

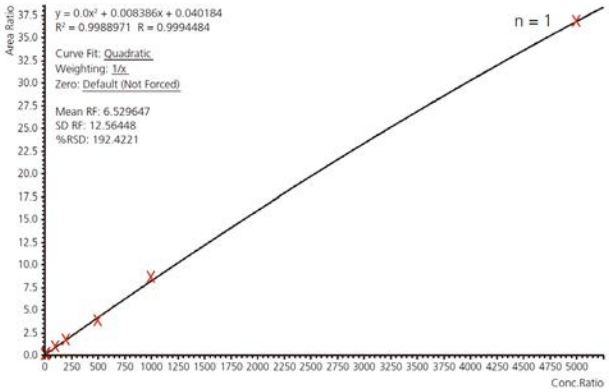
Dwell Time	3 to 34 msec depending upon the number of concomitant transitions to ensure to have at least 30 points per peak (max total loop time 140 msec).
Pause Time	1 msec
Quadrupole Resolution	Q1: Unit Q3: Unit

Calibration

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

Table 4 Limits of Quantification in Honey

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



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

Fig. 2 Calibration Curve of Acetamiprid

Real Samples Analysis

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

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

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

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

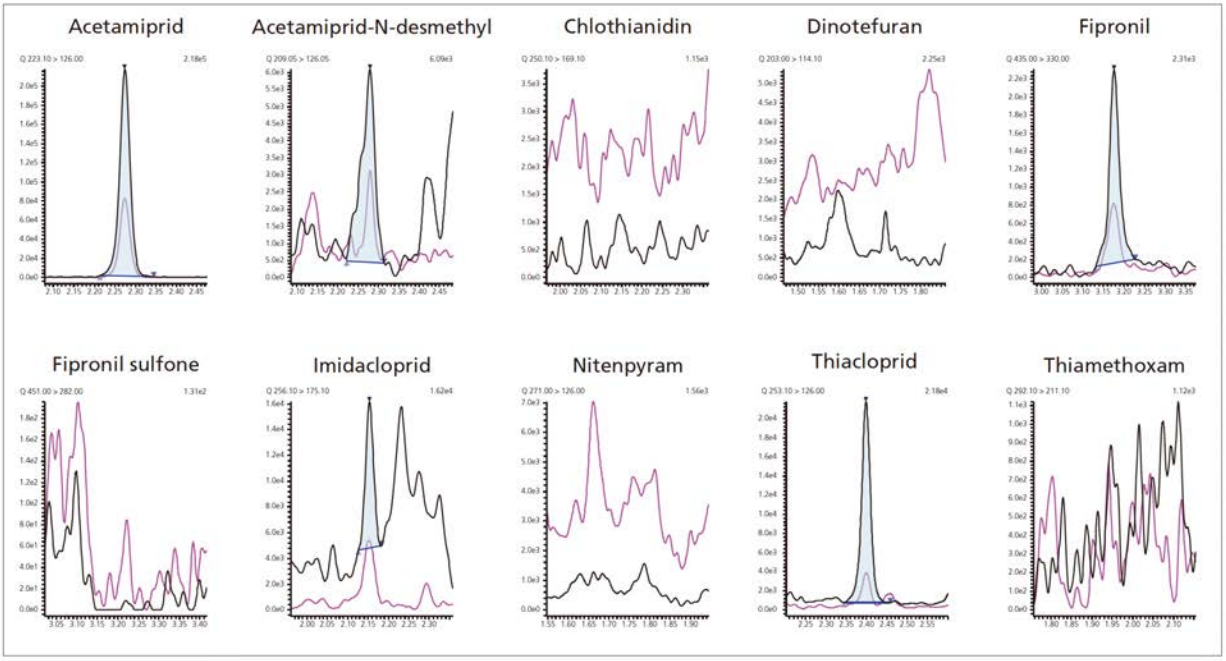


Fig. 3 Chromatogram of a Sample Honey (Pyrenees)

Stability

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

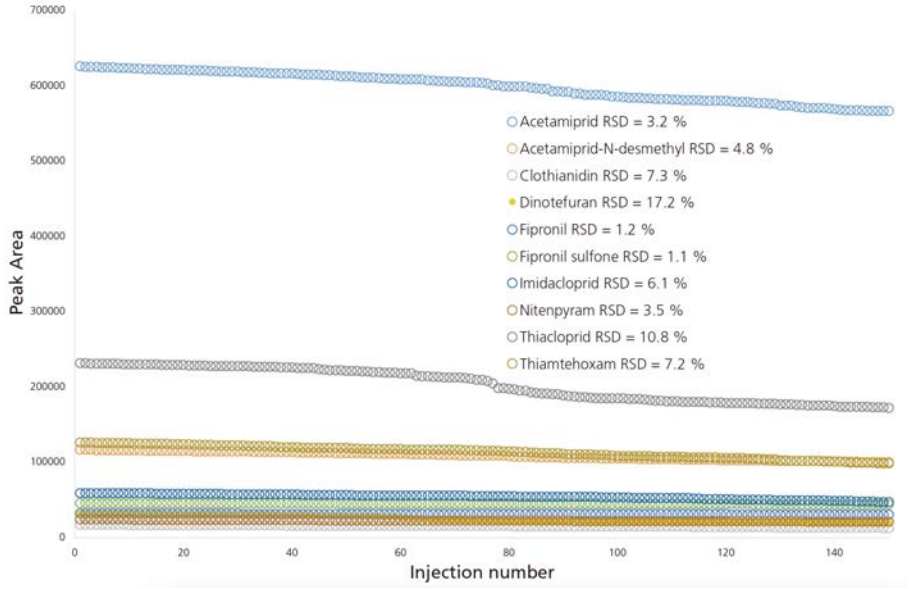


Fig. 4 Stability of Peak Areas in Real Honey Samples

Conclusion

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

3.3 Evaluation of Toxicity in Nectar

Determination of Wilfordine and Wilforine in Honey using Liquid Chromatography with Tandem Mass Spectrometry

INTRODUCTION

Tripterygium wilfordii, which contains a lot of biological toxic compounds such as Wilfordine and Wilforine, is one of the toxic nectar plants. The Wilfordine and Wilforine may be transferred to honey by honey bees. Due to the low content and complex matrix, determination of Wilfordine and Wilforine in honey is not easy. In this study, a highly sensitive method based on liquid-liquid extraction (LLE) and LC-MS/MS has been developed. The results showed that the detection limits of Wilfordine and Wilforine in honey sample were 5.16 and 10.80 ng/kg, respectively.

METHODS

Preparation of Samples

1.0 g of honey sample was added into 10 mL centrifuge tube, and then diluted with 2 mL of pure water. After adding 2 mL of acetonitrile, 0.3 g of NaCl, and 1.2 g of $MgSO_4$ in order, the mixture was vortexed for 2 min and centrifugated at 8000 rpm for 5 minutes. The above solution was withdrawn and filtered (Organic membrane, 0.22 μm) for detection.

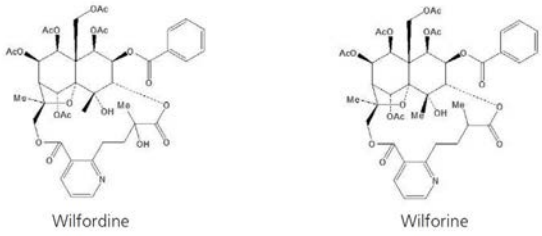


Figure 1 Structure of Wilfordine and Wilforine

Instruments

The LC-MS/MS system were Prominence LC-20A and triple quadrupole mass spectrometry (Shimadzu Corporation, Kyoto, Japan). Shimadzu LC-20A system consist of a CBM-20A system controller, two LC-20AD pumps, a SIL-20AC autosampler, a CTO-20AC column oven, and a DGU-20A3 online degasser. MS/MS detection was performed by LCMS-8050. Data acquisition and processing were performed with Labsolution software Version 5.72. Electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

High Speed Mass Spectrometer

Ultra Fast Polarity Switching

- 5 msec

Ultra Fast MRM

- Max. 555 transition /sec

RESULT

Method Development for Wilforine and Wilfordine

HPLC Conditions

Column : InertSustain C8-3 Column (2.1 mm I.D.×150 mm L., 5 m)
Mobile phase A : 0.1% formic acid aqueous solution
B : Acetonitrile
Elution Mode : Gradient Elute, the initial concentration of MP B was 30%

Table 1. LC Time Programme

Time	Module	Command	Value
1.00	Pumps	Pump B Conc.	30
4.00	Pumps	Pump B Conc.	90
5.00	Pumps	Pump B Conc.	90
5.10	Pumps	Pump B Conc.	30
5.10	Controller	Stop	

Injection Vol. : 10 μL
Column Temp. : 35°C

MS conditions (LCMS-8050)

Ionization : ESI, Positive MRM mode
Nebulizer Flow : 3.0 L/min
Heating Gas Flow : 8.0 L/min
Interface Temperature : 400°C
DL Temperature : 150°C
Heat block Temperature : 300°C
Dry Gas : 12.0 L/min

Table 2. MRM Transition

Compound	MRM transition	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
Wilfordine	884.30>856.20*	-12	-25	-30
	884.30>176.10	-12	-50	-18
Wilforine	868.30>178.10*	-12	-60	-18
	868.30>206.10	-12	-43	-20

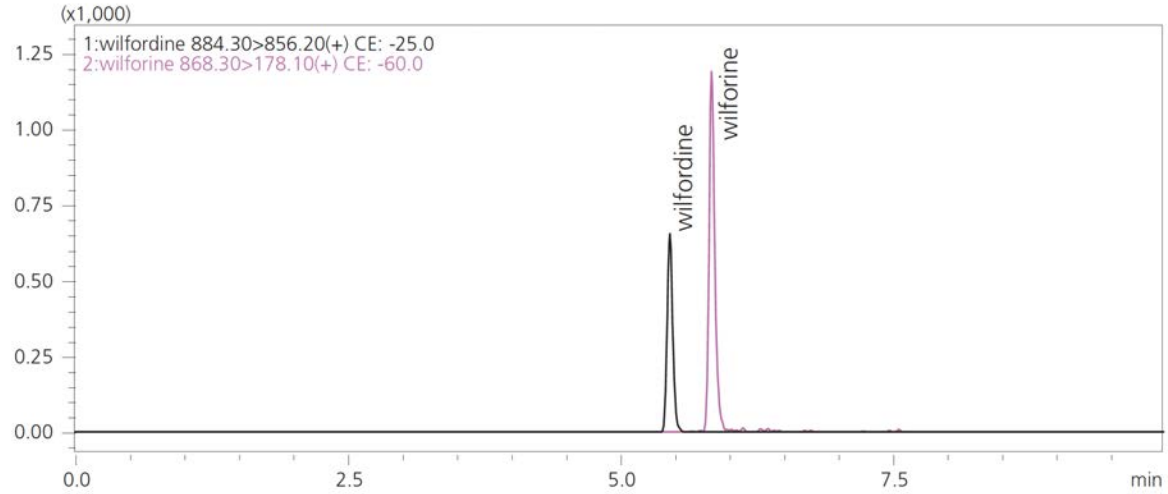


Figure 3 MRM chromatograms of standard solution of Wilforine and Wilfordine (Concentration of each compound were 0.05 ng/mL)

Analytical Performance

Linearity

The determination of Wilfordine and Wilforine were verified using an external standard method. The external calibration was performed by plotting peak area versus concentration of Wilfordine and Wilforine (As seen in Figure 4). The sample solutions were spiked with stock solution to get final concentrations of Wilfordine and Wilforine at 0.01, 0.02, 0.05, 0.1, 0.5, 1.0, 5.0 and 10 ng/mL. The detailed calibration curves, ranges, correlation coefficients and precisions were shown in Table 2.

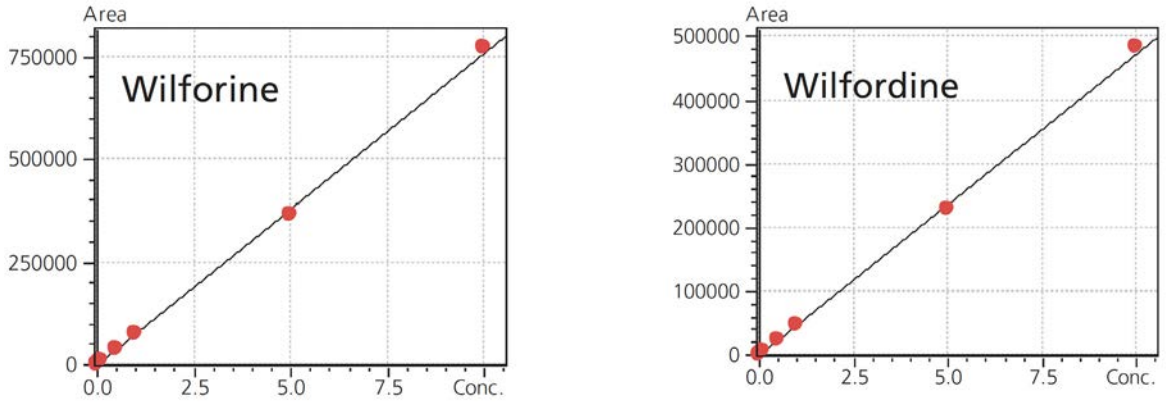


Figure 4 Calibration curve of Wilfordine and Wilforine

Table 3. Parameters of Calibration Curves

Compound	Calibration Curves	Range (ng/mL)	Coefcient (r^2)	Precision (%)
Wilforine	Y=(75959.6) X -45.2	0.01~10.0	0.9996	92.1~113.8
Wilfordine	Y=(47426.1) X+ 206.6	0.01~10.0	0.9997	87.7~108.3

Sensitivity

Detection and quantification limits were calculated as the concentration corresponding to a signal 3 and 10 times of the baseline noise, and the detection limits of Wilforine and Wilfordine were 1.3 and 4.3 ng/L, the quantification limits were 2.7 and 9.0 ng/L, respectively.

Recovery

Preparation of blank honey samples as well as blank honey samples spiked at 0.05 ng/g and 5.0 ng/g. According to the mentioned method before, each sample was measured three times in parallel. The recovery is calculated by subtracting the content of Wilfordine and Wilforine in blank honey samples. The recovery results were shown in table 4.

Table 4. Recovery Results

No.	Compound	Spiked at 0.05 ng/g (%)	Spiked at 5.0 ng/g (%)
1	Wilfordine	104.0	99.6
2	Wilforine	116.0	98.8

CONCLUSION

In this paper, a fast and effective method for the sensitive and reliable analysis of Wilfordine and Wilforine using LC-MS/MS was established.The method has good linearity, with correlation coefficient greater than 0.999, the limit of detection were 1.3 and 4.3 ng/mL, the quantification limits were 2.7 and 9.0 ng/L, respectively. The recoveries were between 98.8~116.0%.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

3.4 Analysis of Carbohydrates

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

INTRODUCTION

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

OVERVIEW OF THE NEXERA METHOD SCOUTING SYSTEM

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

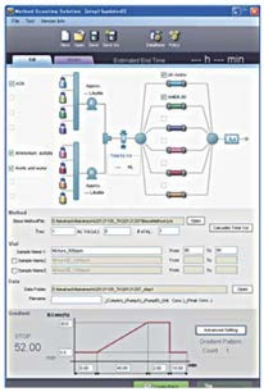


Fig. 1 Main screen of the Method Scouting Solution

Easy Operation

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

- Seamless Connection

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

- Improved Workflow

Batch analysis files are automatically created.

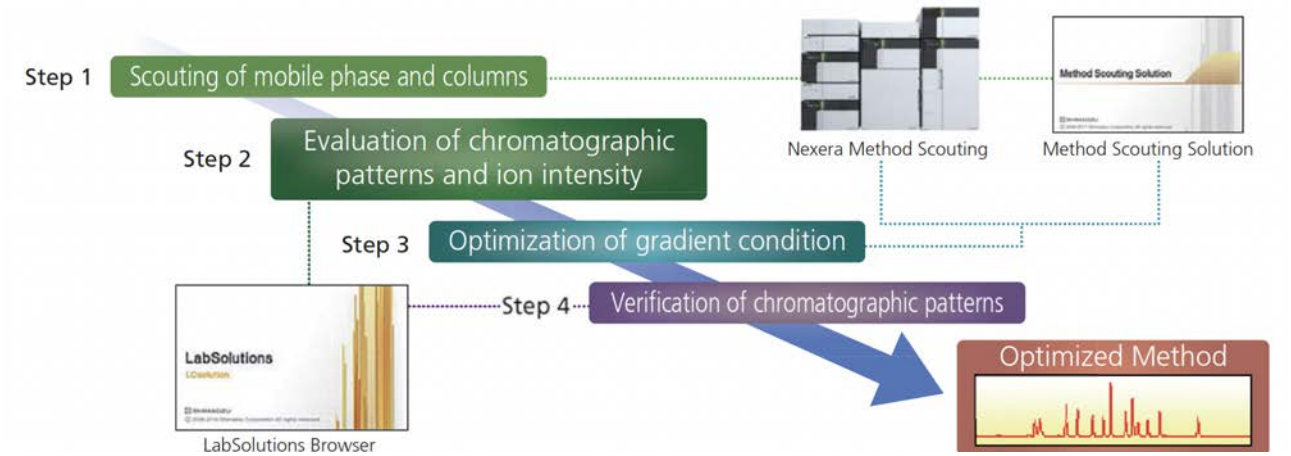


Fig. 2 Work-flow of the method scouting

Scouting of mobile phases and columns (Step 1)

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

Table 1 Analytical conditions of Step 1

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

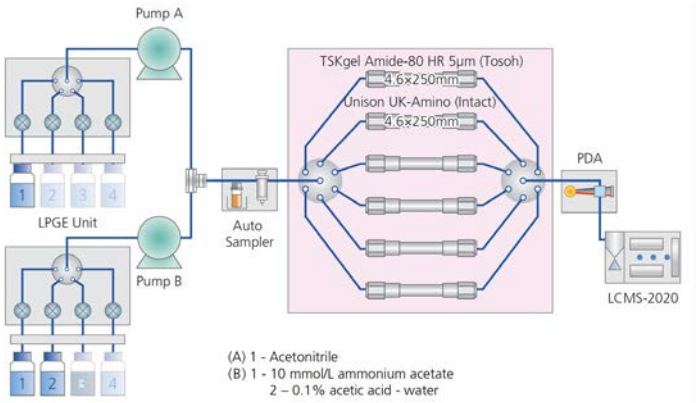


Fig. 3 Schematic representation and features of the Nexera Method Scouting System

Analysis by the Nexera Method Scouting System

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

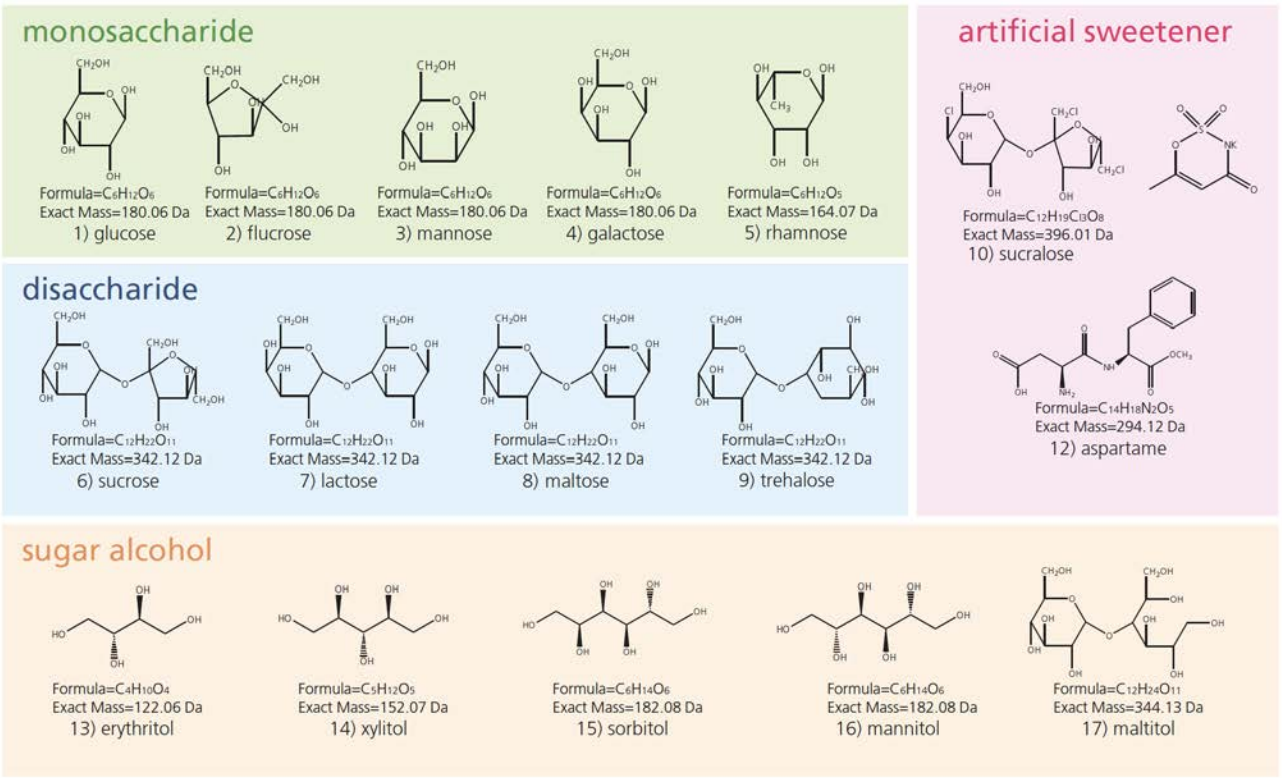


Fig. 4 Structures of analyzed compounds

Evaluation of chromatographic patterns and ion intensity (Step 2)

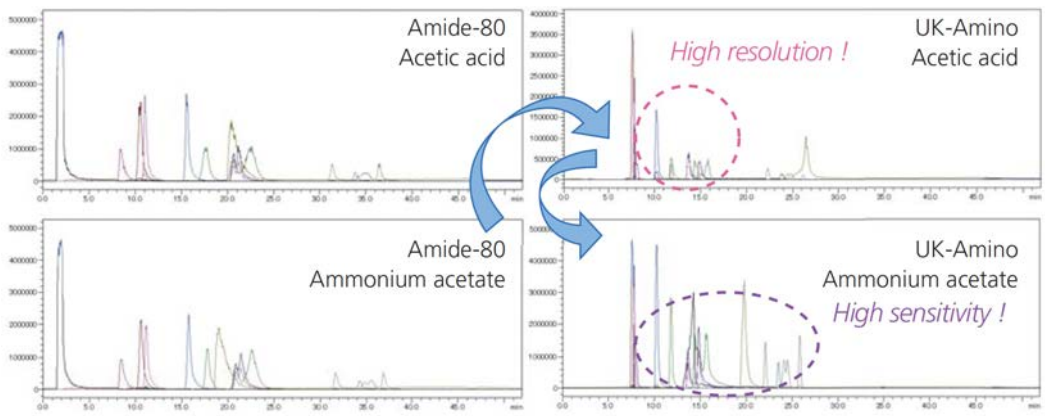


Fig. 5 Typical chromatograms in selected mobile phases and column conditions

Optimization of gradient condition (Step 3 and 4)

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

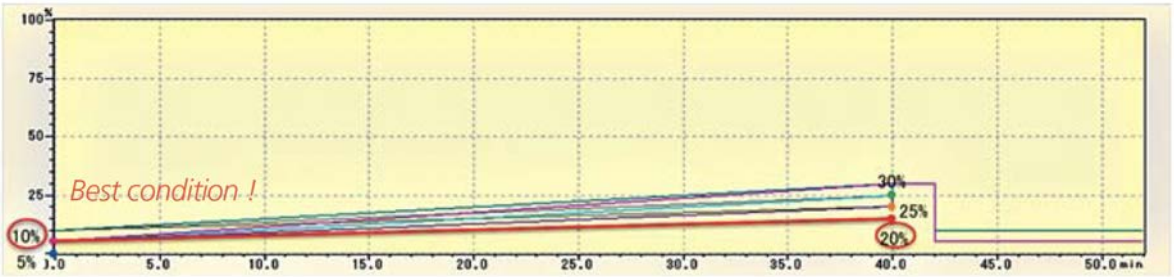


Fig. 6 Optimization of gradient conditions for separation of sugars

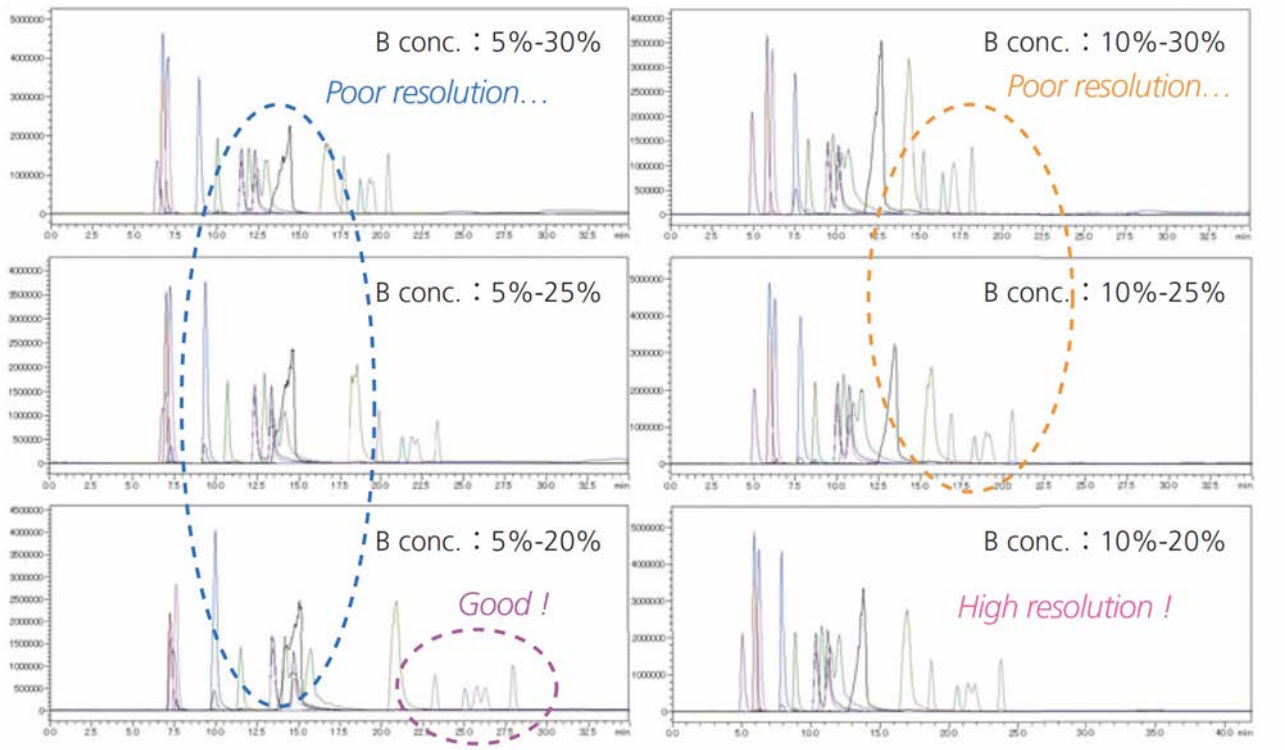


Fig. 7 Typical chromatograms in selected gradient conditions using ammonium acetate and Amide-80 column

Optimized Method

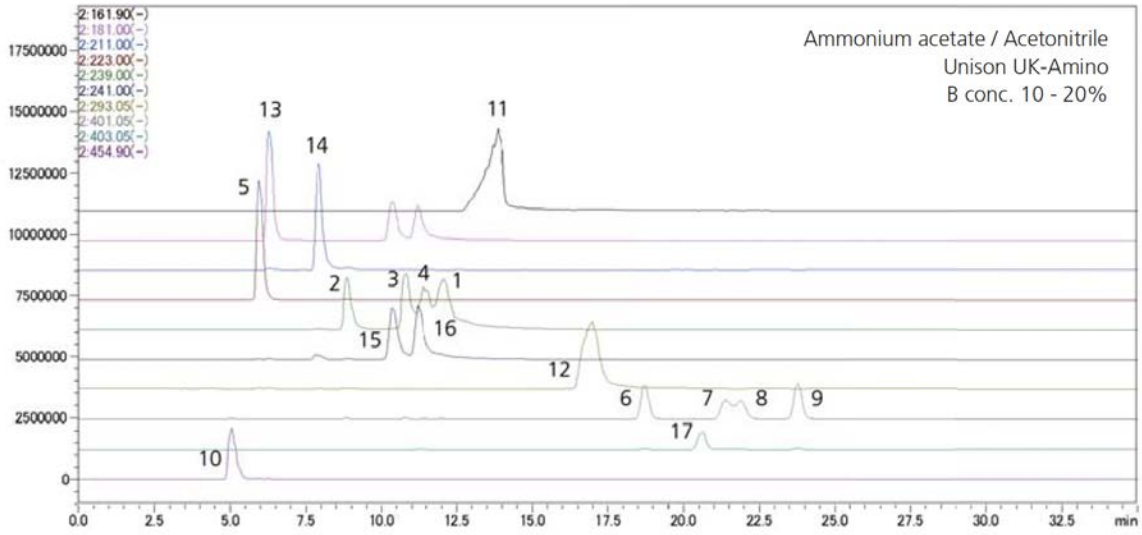


Fig. 8 Optimized method for seventeen sugars

Calibration Curves

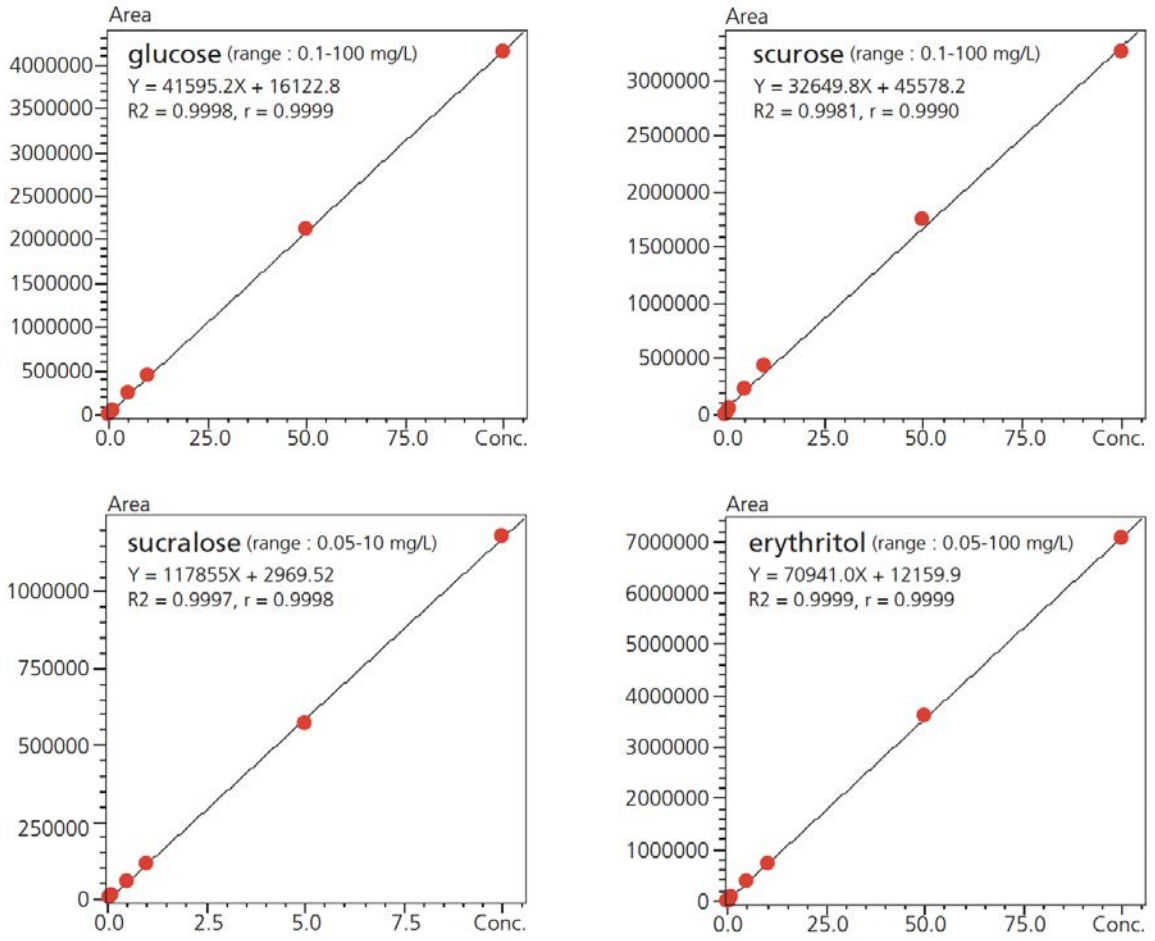


Fig. 9 Calibration curves of each sugar using optimized method

CONCLUSION

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

3.5 Analysis of Water-Soluble Vitamins

Ultra High-Sensitivity Analysis of Water-Soluble Vitamins

Explanation

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

Simultaneous Analysis of 6 Water-Soluble Vitamins

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

Analytical Conditions

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

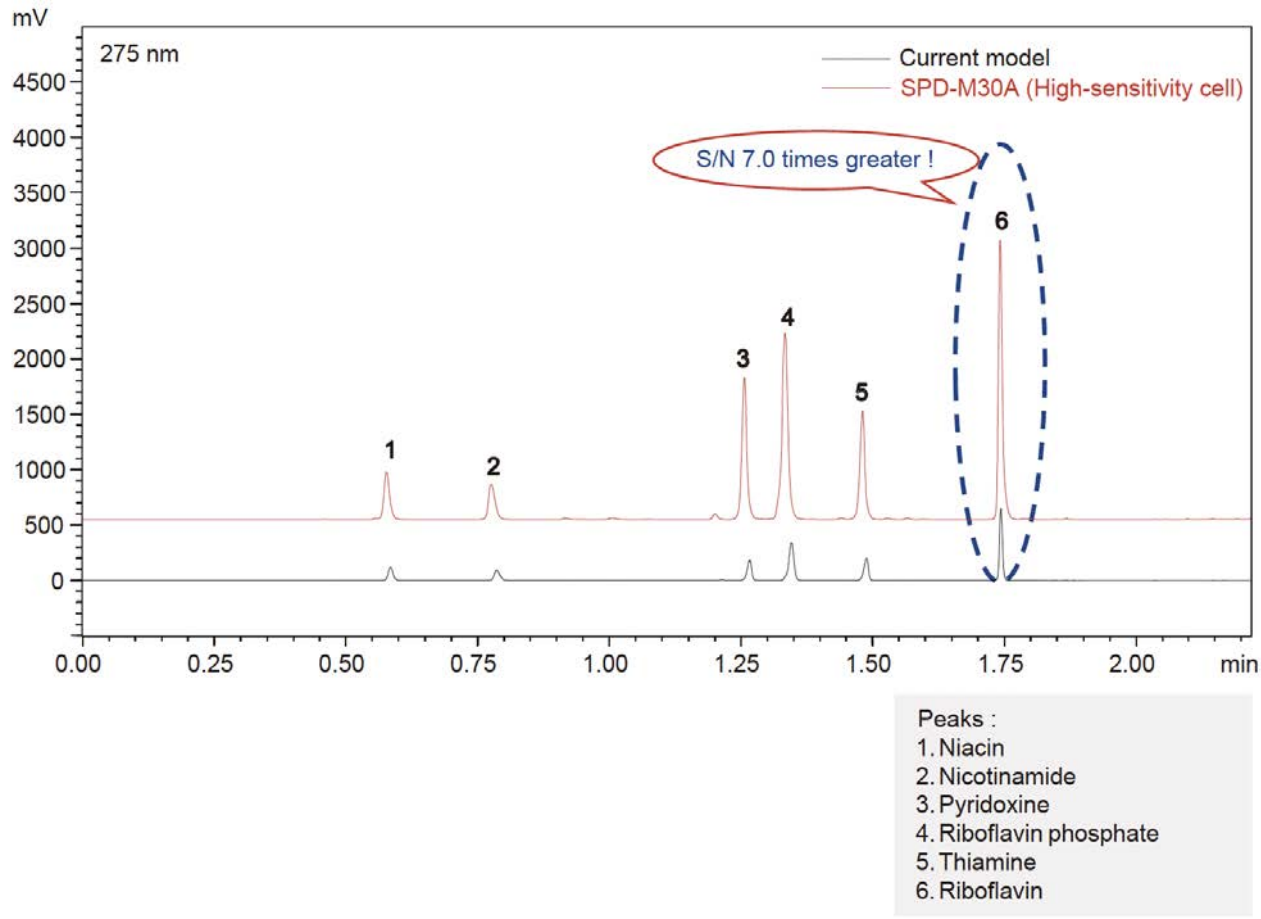


Fig. 1.11.1 Chromatogram of a Standard Mixture Solution of 6 Water-Soluble Vitamin

3.6 Determination of Honey Authenticity

High Speed Analysis of Phenolic Acids

Explanation

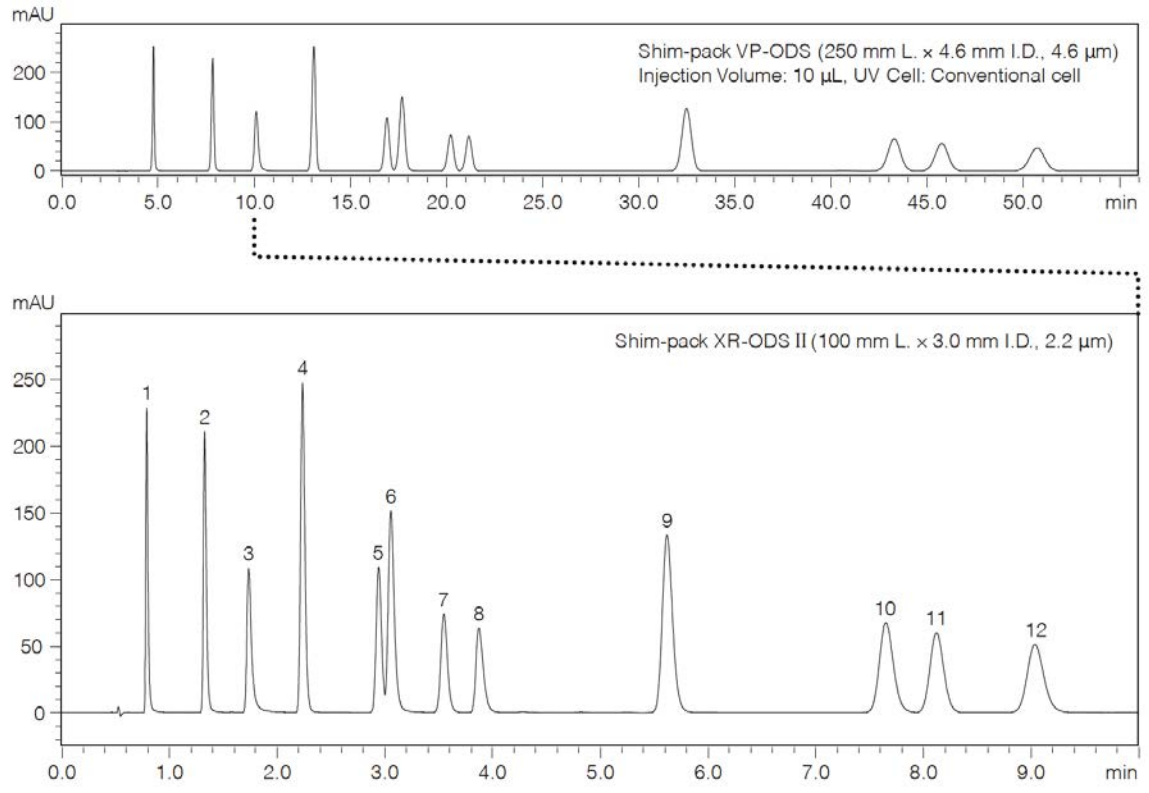
Phenolic acid exists in higher plants as esters, ethers or in its free state, and is a compound that has been receiving increased attention in recent years due to its antioxidant effects. HPLC is often used for quantitative analysis of phenolic acid in processed foods containing fruits and fruit materials,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

Simultaneous Analysis of 11 Phenolic Acids and Benzoic Acid

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

Analytical Conditions

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



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

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

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