SHIMADZU
Excellence in Science

C10G-E091

Honey Analysis Made Easy

Index

1.

OUR COMMITMENT TO BEES

Together, we can bee more!

Shimadzu is committed to protecting 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 honeybees, native pollinators, and insects.

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

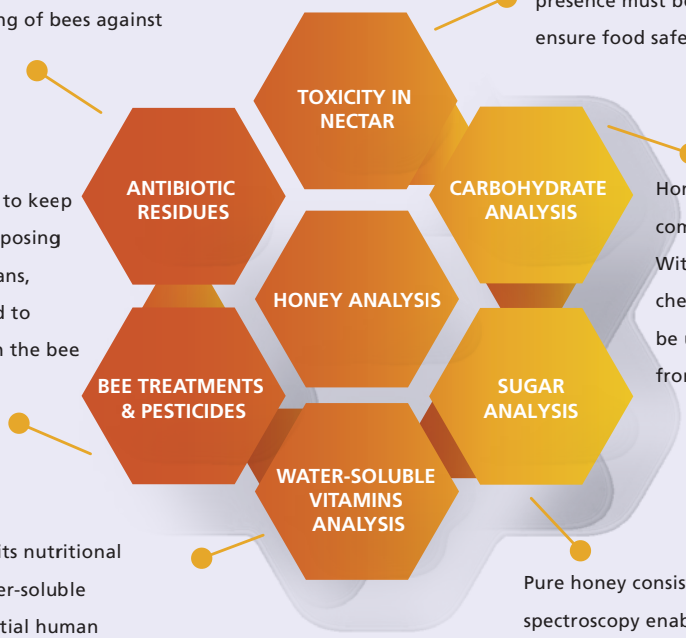
With the beeswe.love project, Shimadzu accompanies the bees, cares for them, and learns more about them to understand and protect the fragile side of nature.

2.

INTRODUCTION TO HONEY ANALYSIS

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

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



Pesticides are extensively used to keep unwanted pests away. Besides posing a potential health risk to humans, they are perceived to be linked to colony collapse disorder within the bee population.

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

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

Pure honey consists mainly of sugar. FTIR spectroscopy enables quick quantitative analysis of multiple components which can determine whether substitutes have been added to honey.

3.

APPLICATION NOTES

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 causing an undesirable increase in a microorganism's drug resistance. Various countries have defined their own maximum residue limits (MRLs) for sulfonamides accepted in honey. There are no MRLs for sulfonamides in honey in the EU. Some countries such as China and Switzerland adopt an MRL of 50 µg/kg. HPLC-MS/MS is an effective strategy to characterize and accurately measure those antibiotics because MRLs and MRPLs in food products from animals tend to get continually reduced to preserve human health and safety. A selective, fast and sensitive HPLC-MS/MS method has been developed for 15 sulfonamides and trimethoprim.

Method and Conditions

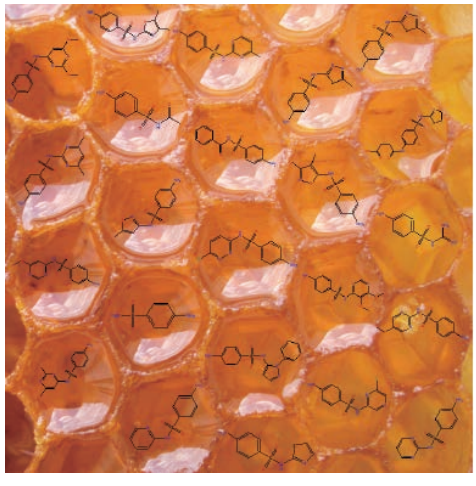
Sample Preparation

5 grams of honey, spiked with 17 SAs and trimethoprim (Table 1A), were extracted using the QuEChERS method following the 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 equipped with LabSolutions LCMS software and electrospray ionization.

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

LC Conditions

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



MS Conditions

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 was 15 L/min.

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

A. Sulfonamides used

#	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	Sulfisoxazole
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 with 1 µL injections at 400 µL/min. Three transitions from parent ions and fragments were selected using the optimization tool software.

Results

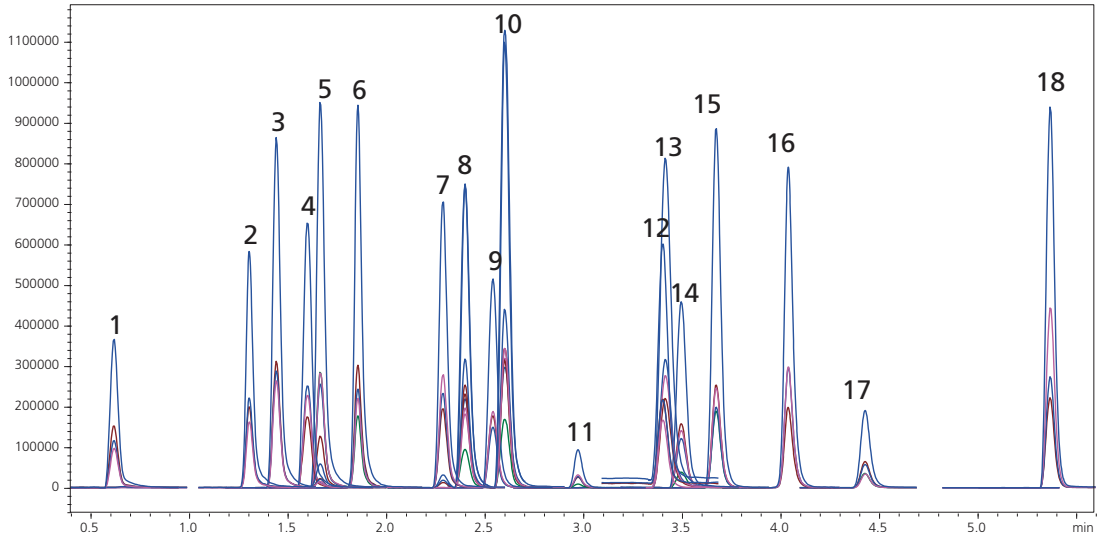


Fig. 1 Representative chromatogram of sulfonamide drugs. Standard mixture at 125 pg on-column for each standard. Peak numbers follow the order described for SA compounds in table 1A.

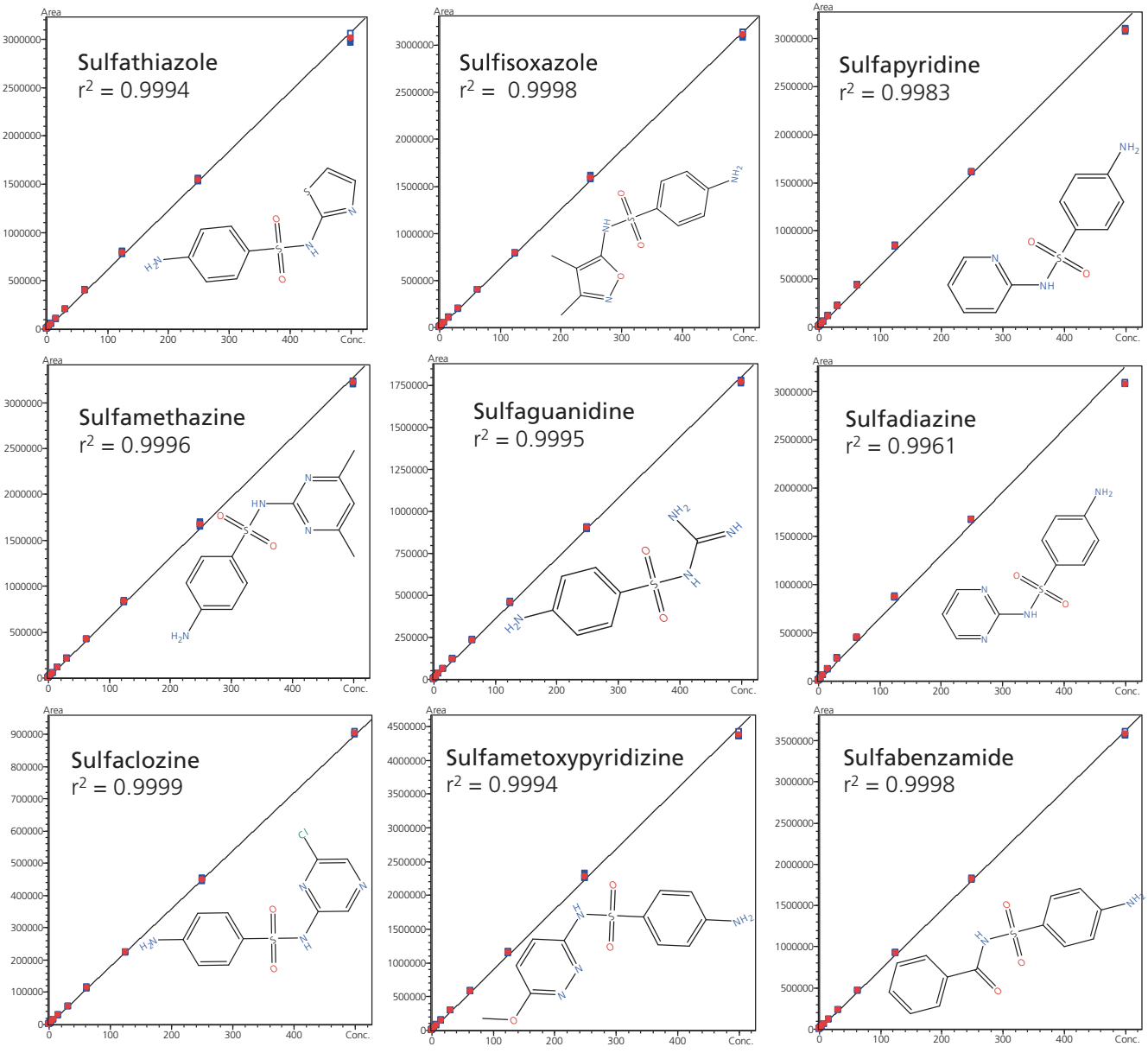
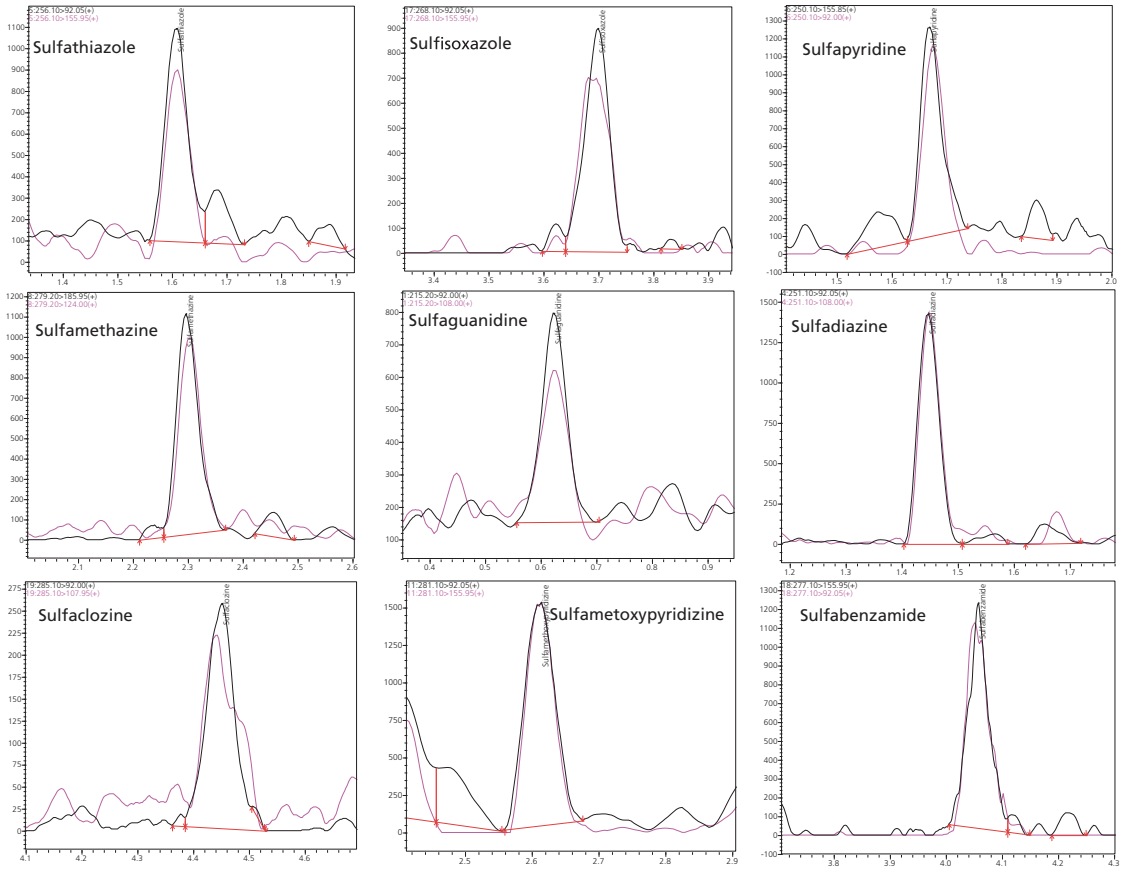


Fig. 2 High degree of linearity was observed over the concentration range of 0.5–500 pg on column, with values of $r^2 \geq 0.990$ for all analytes.

Authentic SA standards were fully characterized by HPLC and MS/MS with an MRM optimized assay. The calibration curves of standards in the 50% methanol matrix were linear with $r^2 > 0.990$ (Fig. 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

Fig. 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 the extraction method provides a fast, simple, sensitive and accurate method for measuring sulfonamide drugs and trimethoprim in honey with an acceptable recovery range. Matrix-matched calibration and the 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 has become controversial as they are suspected of being a cause of colony collapse disorder affecting honeybees. Since pollination is essential for agriculture, extensive studies have been conducted to evaluate the impact of neonicotinoids on bee health. As a result, the European Food Security Authority (EFSA) has limited the use of thiamethoxam, clothianidin and imidacloprid. Fipronil, a pesticide from a different chemical class, has also been banned by EFSA for maize seed treatment due to its high risk to 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 a Shimadzu UHPLC system with the triple quad 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 ultrapure water were added and the samples were homogenized by vortex mixing for one minute. 10 mL of acetonitrile were then added followed by vortex mixing for one 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 five 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 five 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 a local supermarket was extracted with and 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 of 70-120%, as specified in 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%

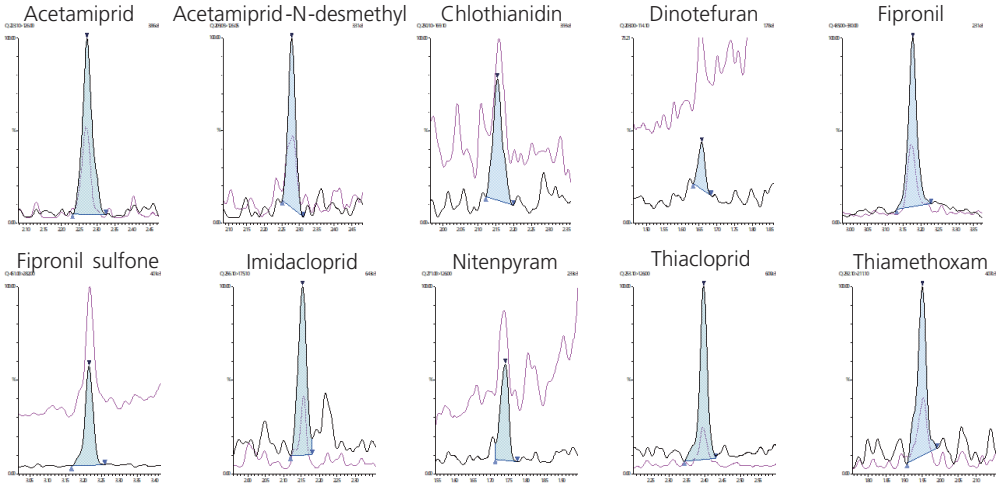


Fig. 1 Chromatogram of the Target Compounds at Their Lower Limit of Quantification

Table 2 Analytical Conditions

System	: Shimadzu UHPLC
Column	: Fully porous C18 brand A (100 mm × 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 having 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 correspond 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% (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

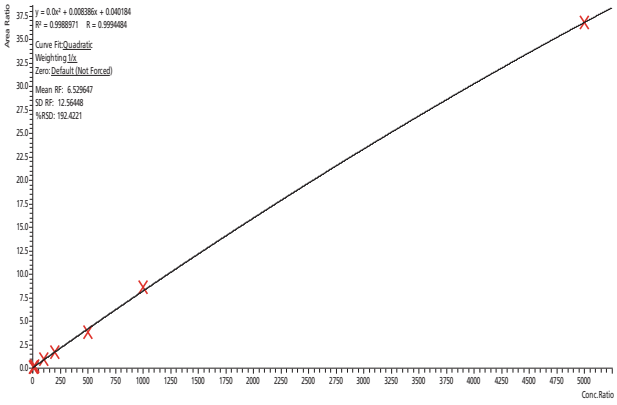


Fig. 2 Calibration Curve of Acetamiprid

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

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	---	---

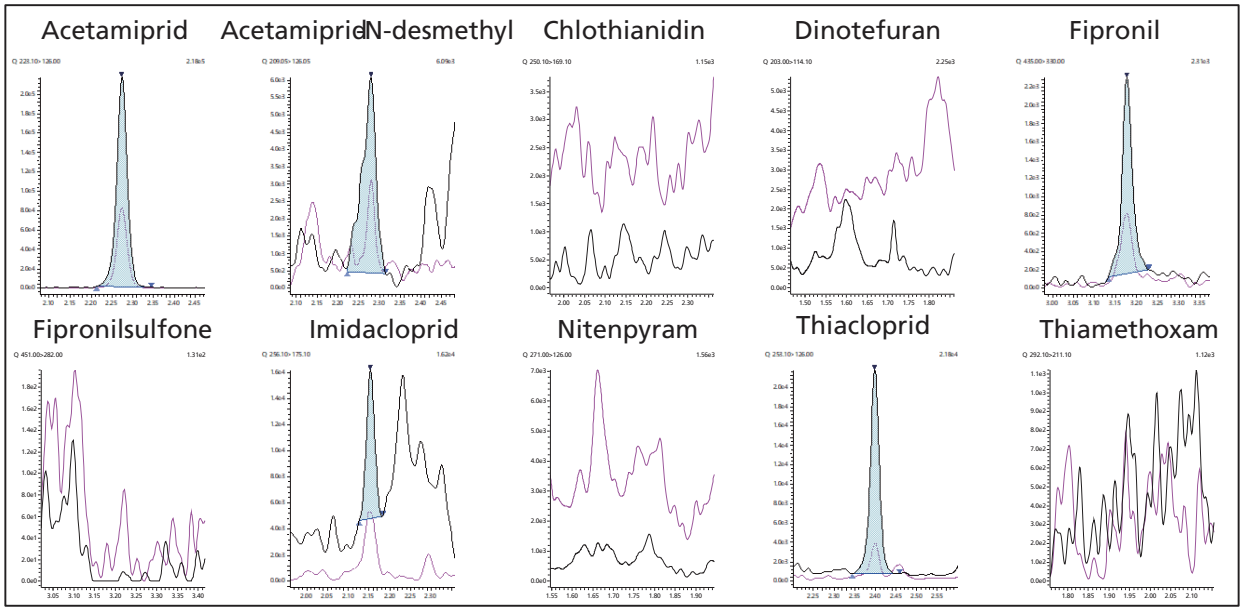


Fig. 3 Chromatogram of a Sample Honey (Pyrenees)

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 excellent sensitivity can be maintained over a long series of real sample analysis thanks to the ruggedness of the ion source.

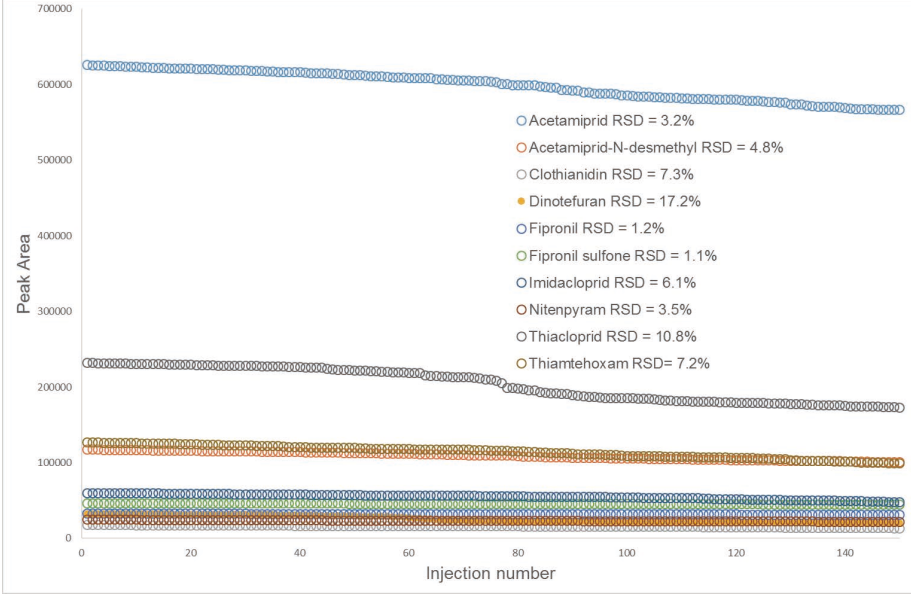


Fig. 4 Stability of Peak Areas in Real Honey Samples

Conclusion

A method for an 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.

The high sensitivity obtained enabled an 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 a long analytical series of real samples. This method can help 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

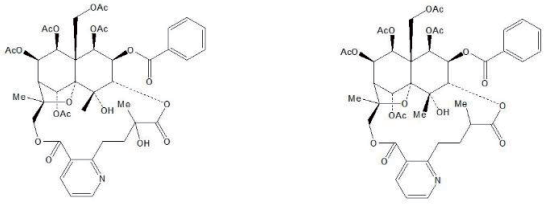
Introduction

Tripterygium wilfordii, which contains a lot of biological toxic compounds such as Wilfordine and Wilforine, is a toxic nectar plant. 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 a honey sample were 5.16 and 10.80 ng/kg, respectively.

Method and Conditions

Sample Preparation

1.0 g of a honey sample was added into a 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 two minutes and centrifugated at 8000 rpm for five minutes. The above solution was withdrawn and filtered (Organic membrane, 0.22 µm) for detection.



Wilfordine　　Wilforine

Fig. 1　Structure of Wilfordine and Wilforine

Instruments

The LC-MS/MS system consisted of a Prominence LC-20A and a triple quadrupole mass spectrometer (Fig. 2). Shimadzu LC-20A system included the following components: 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 a LCMS-8050. Data acquisition and processing were performed with LabSolutions software. Electrospray ionization was operated in multiple reaction monitoring (MRM) mode.

Fig. 2　LC-MS/MS System (LC-20A + LCMS-8050)

HPLC Conditions

Column　　: Fully porous C8 brand B (150 mm × 2.1 mm I.D., 5 µm)

Mobile phase A　: 0.1% formic acid aqueous solution

B　: Acetonitrile

Elution Mode　: Gradient Elute, the initial concentration of MP B was 30%

Table 1　LC Time Program

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 Transitions

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

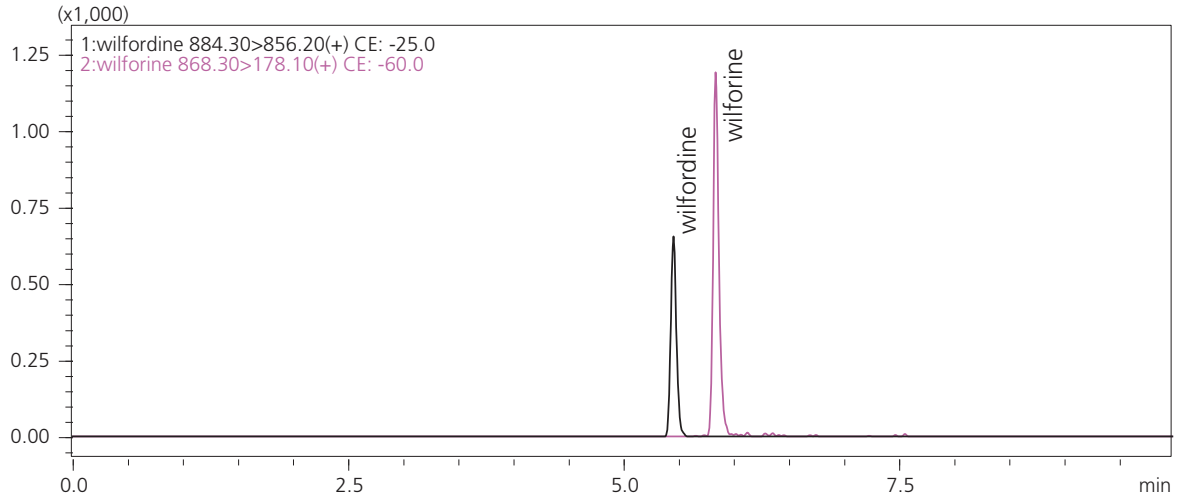


Fig. 3 MRM chromatograms of a standard solution of Wilforine and Wilfordine
(Concentration of each compound was 0.05 ng/mL)

Analytical Performance

Linearity

The determination of Wilfordine and Wilforine was verified using an external standard method. The external calibration was performed by plotting peak area versus concentration of Wilfordine and Wilforine (as seen in Fig. 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 precision values are shown in Table 3.

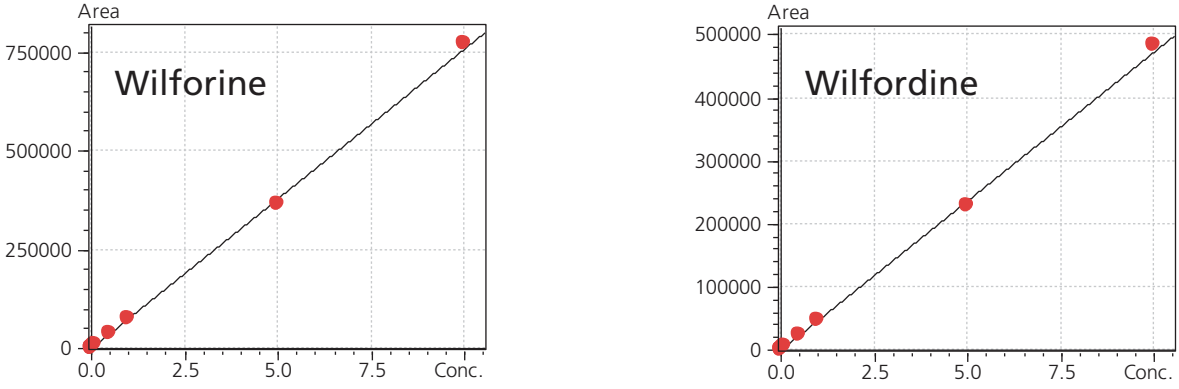


Fig. 4 Calibration curves of Wilfordine and Wilforine

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 to 10.0	0.9996	92.1 to 113.8
Wilfordine	Y=(47426.1) X+ 206.6	0.01 to 10.0	0.9997	87.7 to 108.3

Sensitivity

Detection and quantification limits were calculated at concentrations corresponding to a signal 3 and 10 times of the baseline noise. The detection limits of Wilforine and Wilfordine were 1.3 and 4.3 ng/L and 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. 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 are 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 article, 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 a correlation coefficient greater than 0.999. The limits of detection were 1.3 and 4.3 ng/mL and the quantification limits were 2.7 and 9.0 ng/L, respectively. The recoveries were between 98.8 and 116.0%.

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

3.4 Analysis of Carbohydrates

Optimization of a Sugar Analysis using an HPLC Method Scouting System Coupled to a Single Quadrupole Mass Spectrometer

Introduction

Optimization of peak separation and sensitivity is important for determining LC/MS analytical conditions. However, this can be a tedious and time-consuming operation. The HPLC method scouting system, coupled to a single quadrupole mass spectrometer, used in this study can dramatically shorten total run times compared to a conventional system because it can make enormous searching conditions and run batch programs automatically. In this study, we developed an optimized method for the simultaneous analysis of seventeen kinds of sugars based on results from evaluating 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

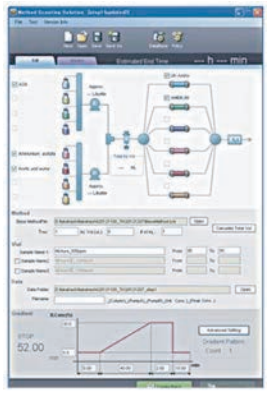


Fig. 1 Main screen of the Method Scouting Solution

Easy Operation

Mobile phases and columns can be selected in the same window. An 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.

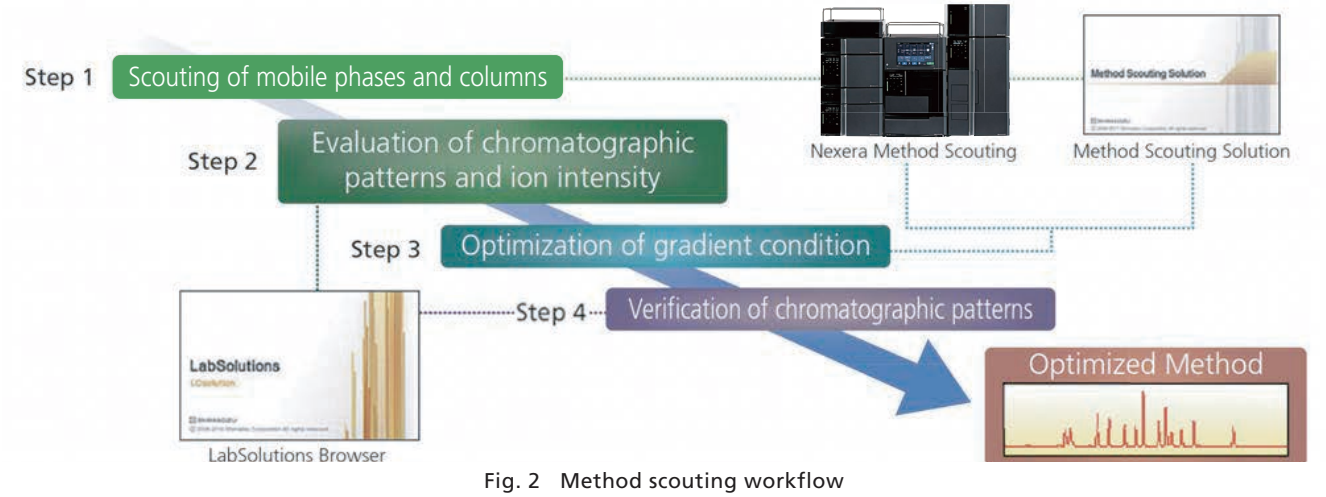


Fig. 2 Method scouting workflow

Scouting of mobile phases and columns (Step 1)

The purpose of this step is to determine the best combination of mobile phase and column using a typical gradient condition (Table 1). In these experiments we used two combinations of mobile phases and two 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)

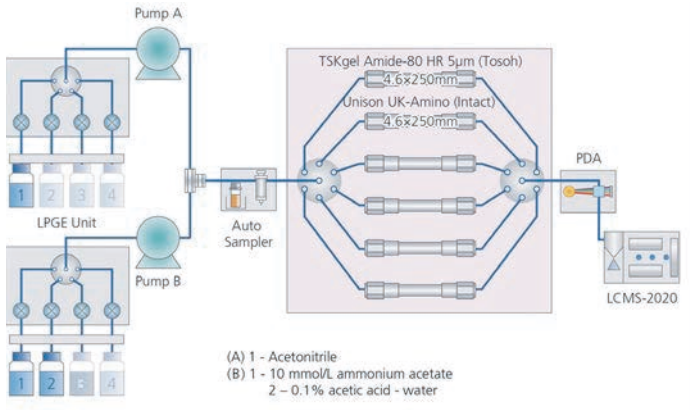


Fig. 3 Schematic representation and features of the Nexera Method Scouting System

Analysis by the Nexera Method Scouting System

We targeted seventeen sugars and analyzed them simultaneously.

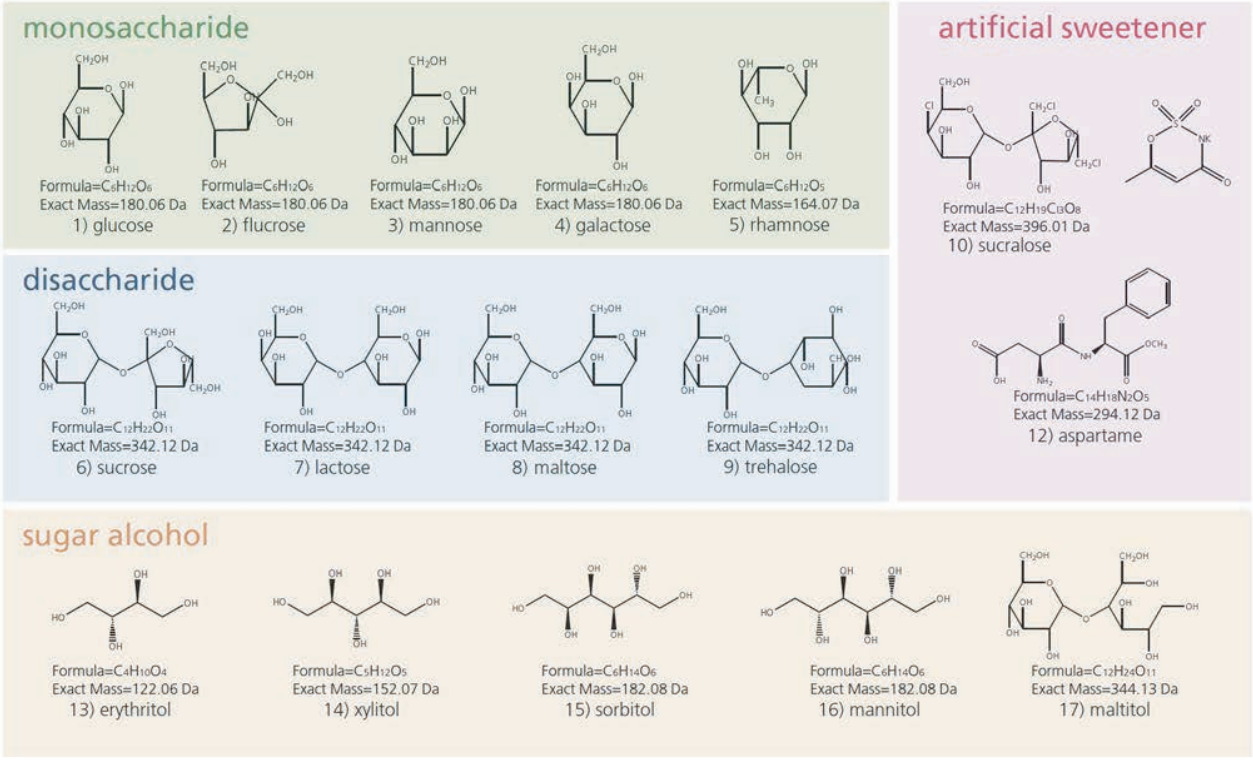


Fig. 4 Structures of analyzed compounds

Evaluation of chromatographic patterns and ion intensity (Step 2)

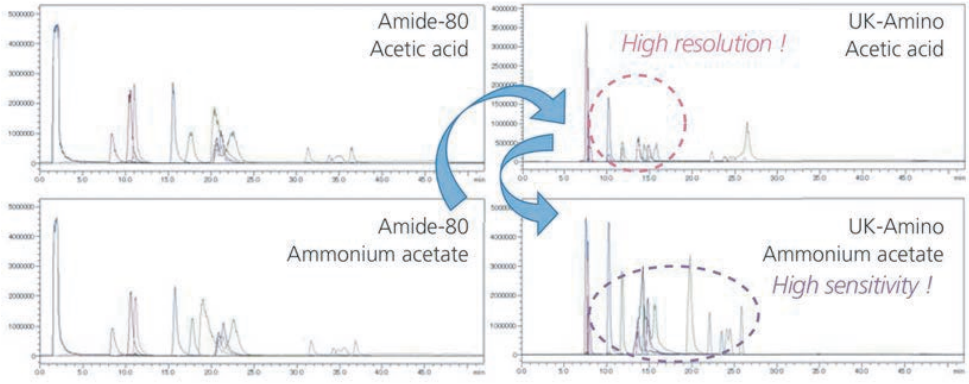


Fig. 5 Typical chromatograms in selected mobile phases and column conditions

Optimization of gradient conditions (Step 3 and 4)

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

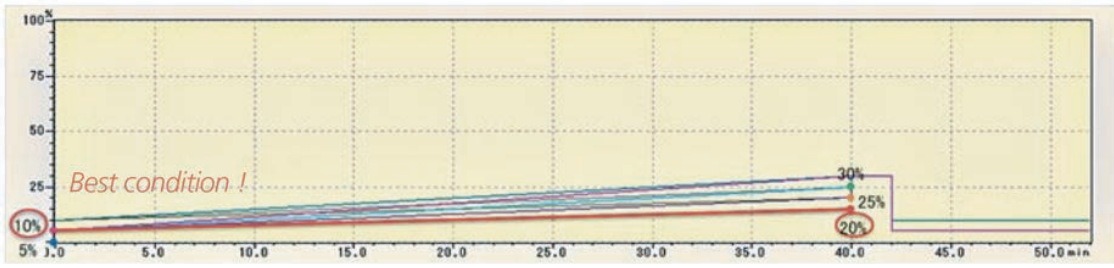


Fig. 6 Optimization of gradient conditions for separation of sugars

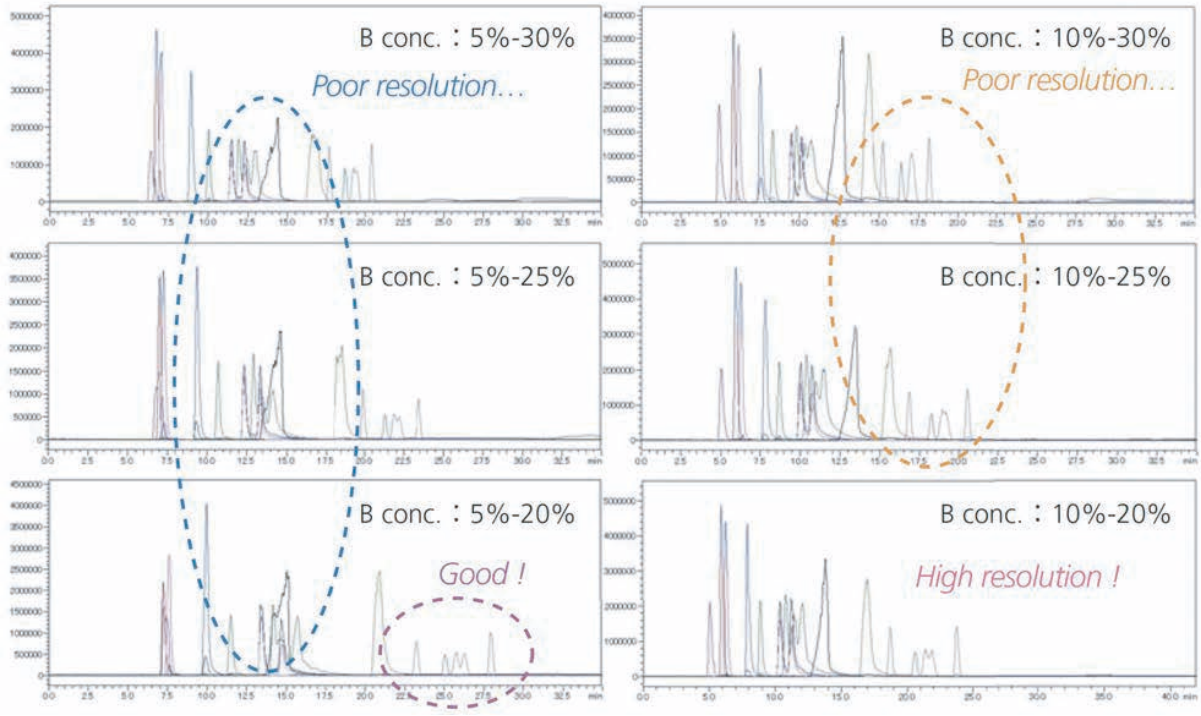


Fig. 7 Typical chromatograms in selected gradient conditions using ammonium acetate and an Amide-80 column

Optimized Method

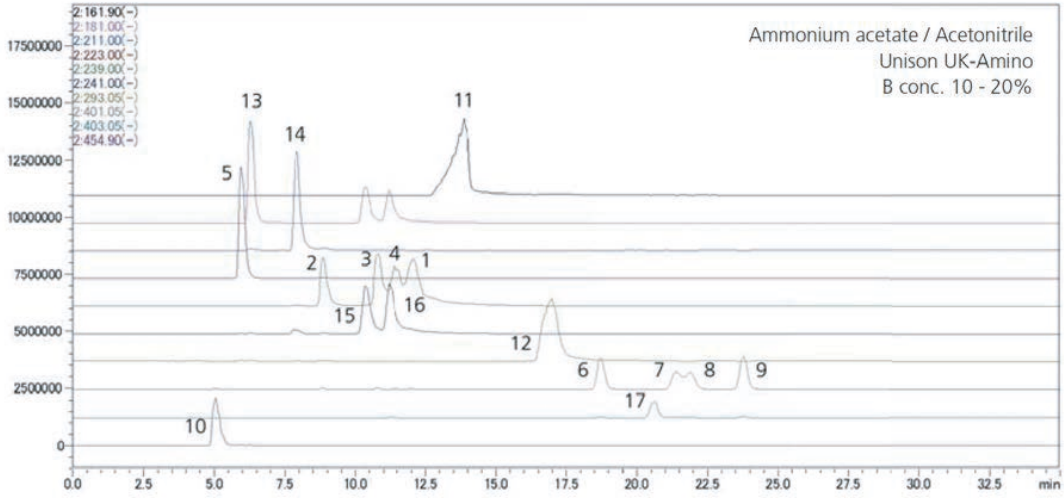


Fig. 8 Optimized method for seventeen sugars

Calibration Curves

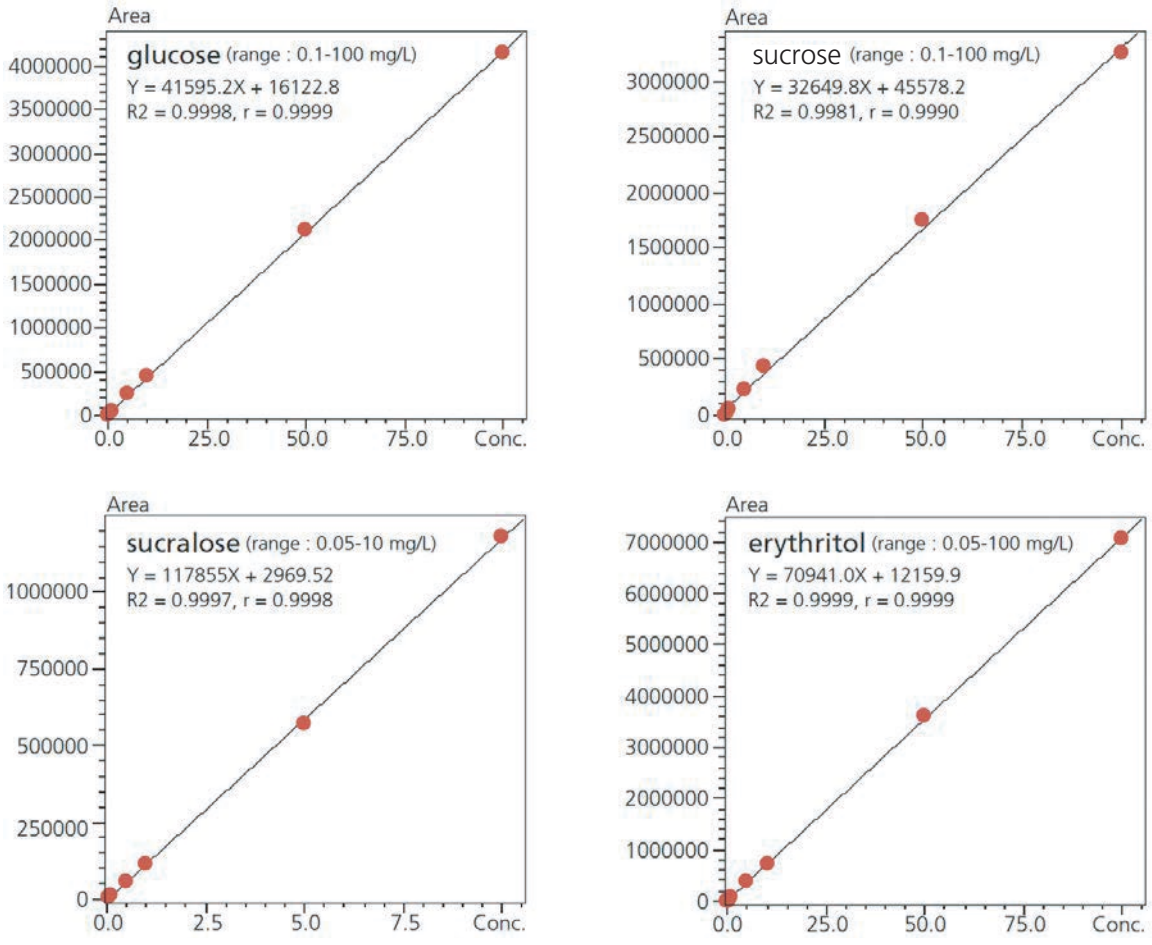


Fig. 9 Calibration curves of four sugars using the optimized method

Conclusion

- Method Scouting Solution, dedicated software for controlling the method scouting system, enabled optimization of an analytical method for 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 to separate all compounds.
- Method optimization significantly enhanced LC/MS sensitivity.
- Seamless integration of software provided improved speed and efficiency in method development processes.
- An optimized method file provided high quantifiability.

3.5 Analysis of Water-Soluble Vitamins

Ultra-High-Sensitivity Analysis of Water-Soluble Vitamins

Introduction

The Nexera SR is a high-end UHPLC model. 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 Six Water-Soluble Vitamins

The high-sensitivity cell (option) for the Nexera SR UHPLC incorporates an 85 mm optical path length. Low noise levels and a long optical path length enable an excellent S/N ratio, not only high signal response. In this simultaneous analysis of water-soluble vitamins, the S/N ratio 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	: Coreshell C18 brand C (100 mm × 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.)
Flowrate	: 2.5 mL/min
Column Temp.	: 40 °C
Injection Volume	: 5 µL

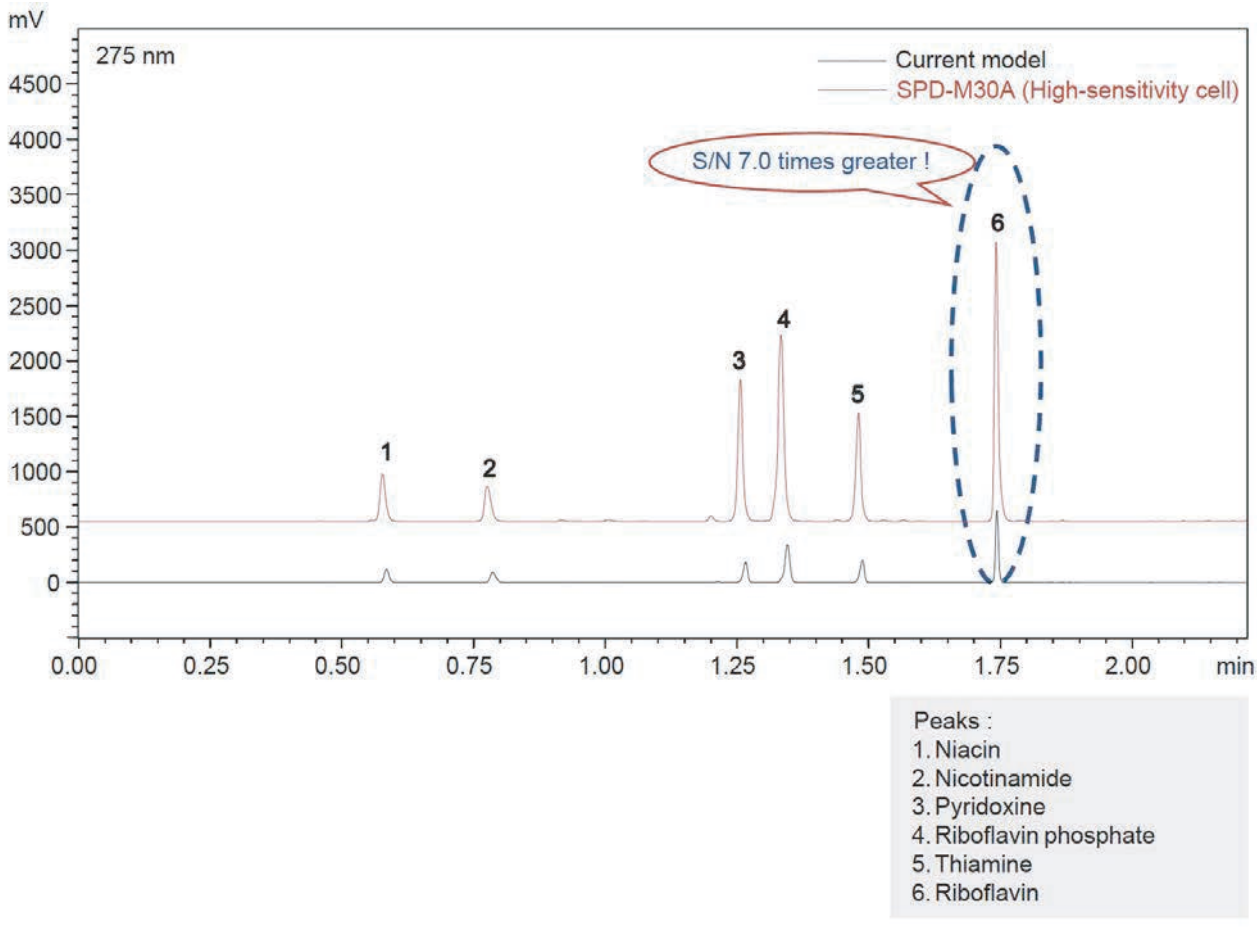


Fig. 1.11.1 Chromatogram of a Standard Mixture Solution of Six Water-Soluble Vitamins

3.6 Analysis of Sugars in Honey

Quantitative Analysis of Sugars (Fructose, Glucose, and Sucrose) in Honey by FTIR

Introduction

Honey has attracted attention because it offers a number of health benefits in the form of vitamins, minerals, and other nutrients. However, it is susceptible to adulteration, for example, by intentionally adding cheap corn syrup, in order to reduce manufacturing costs. Although adulteration of honey with corn syrup does not cause any serious health problems, the resulting loss of consumer confidence can have an adverse effect on market growth. Therefore, in quality control, the development of a simple analytical technique which makes it possible to determine whether substitutes have been added to honey has been demanded.

Infrared (IR) spectrophotometry is an effective technique for identifying the components contained in honey because organic compounds each display a different spectrum. Chemometrics (PLS: partial-least squares method) and multiple regression analysis of the IR spectra obtained by IR spectrophotometry enable quick quantitative analysis of multiple components.

In this article, a quantitative analysis of the sugars contained in several honey samples was conducted using a Shimadzu Fourier transform IR (FTIR) spectrophotometer.

Method and Conditions

Pure honey consists mainly of sugar. Virtually the entire sugar content is fructose and glucose, with a small amount of sucrose. As percentage values, pure honey consists of 33-43 % fructose, 25-35 % glucose, and 0-2 % sucrose, and its fructose : glucose ratio is 1.2 : 1. On the other hand, adulterated products containing corn syrup consist mainly of glucose. Therefore, if a honey product displays an elevated level of glucose, it can be inferred that corn syrup has been added in order to reduce the cost of production.

In this experiment, nine types of commercial honey were prepared and diluted to 10 % w/w with pure water. The samples were then measured with a Shimadzu IRTracer-100 FTIR spectrophotometer and a Quest single-bounce attenuated total reflectance (ATR) accessory (ZnSe prism), and their fructose, glucose, and sucrose contents were quantified by a chemometric analysis (PLS method).

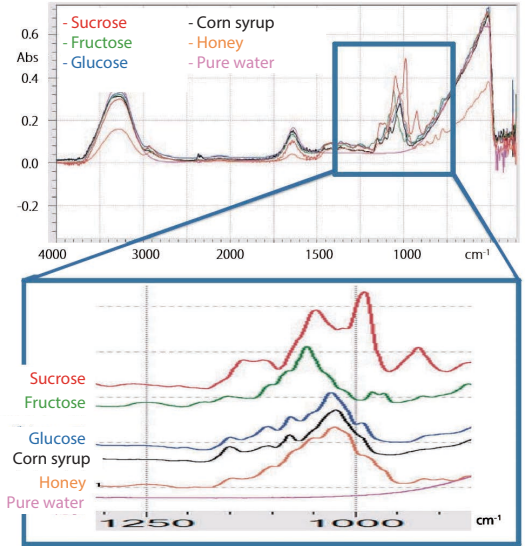


Fig. 1 IR Spectra of Sucrose, Fructose, Glucose, Corn Syrup, Honey (All 50 % Aqueous Solutions), and Pure Water

Table 1 shows the measurement conditions, Fig. 1 shows the IR spectra of sucrose, fructose, glucose, corn syrup, honey (in all cases, 50 % aqueous solutions), and pure water, and Fig. 2 shows the IR spectra of the nine commercial honey samples (10 % w/w).

Table 1 Measurement Conditions

Instruments	: IRTracer-100 Quest ATR accessory
Resolution	: 4 cm^{-1}
Accumulation	: 32 times
Wavenumber range	: 4000 - 600 cm^{-1}
Apodization function	: Happ-Genzel
Detector	: DLATGS

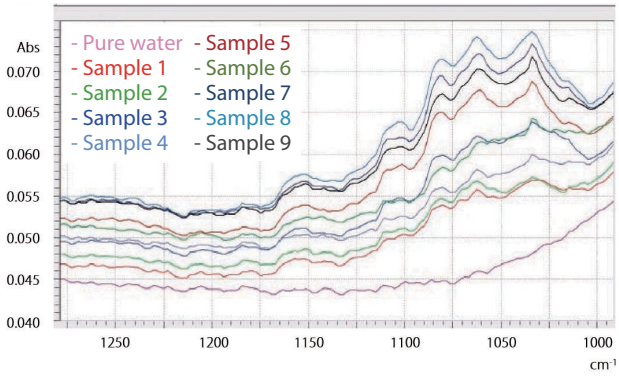


Fig. 2 IR Spectra of Commercial Honey Samples (10 % w/w)

Quantitative Analysis of Sugars in Honey

In order to prepare a calibration curve from a mixed aqueous solution of fructose, glucose, and sucrose, a 3-dimensional sample training matrix (model showing the mixing ratios of the three components in the standard sample) was prepared, as shown in Fig. 3, and the concentration of the standard sample necessary for quantitation of the sugars contained in the honey samples was studied. The percentages (0-15 %) of fructose, sucrose, and glucose are shown on the X, Y, and Z axes of the model in Fig. 3, and the concentration of the standard sample was decided so as to include the entire 3-dimensional space of the model.

Chemometrics (PLS method) was used in the quantitative analysis. Table 2 shows a list of the standard samples. The PLS calibration curve was prepared using 25 of the 34 samples, and the calibration curve was verified using the remaining nine samples.

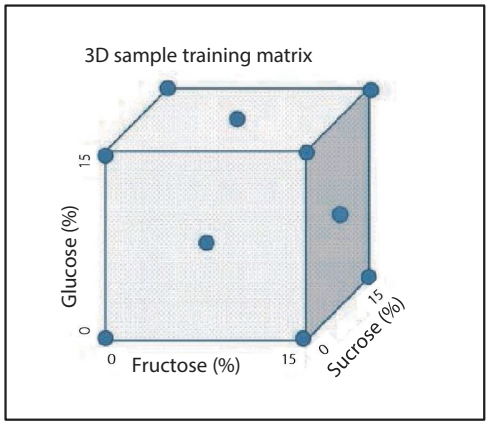


Fig. 3 Three-Dimensional Sample Training Matrix (Model Showing Mixing Ratios of Three Components in the Standard Sample)

Table 2 List of Standard Samples

Concentration of sugars (% w/w)			
Sample	Fructose	Glucose	Sucrose
1	0.00	0.00	0.00
2	5.08	0.00	0.00
3	10.06	0.00	0.00
4	0.00	5.00	0.00
5	0.00	10.22	0.00
6	0.00	0.00	4.97
7	0.00	0.00	9.94
8	4.92	4.91	0.00
9	0.00	5.10	4.95
10	4.95	0.00	4.91
11	10.10	10.55	0.00
12	0.00	9.83	9.88
13	0.43	0.00	10.03
14	5.10	5.06	5.04
15	10.11	9.84	9.94
16	3.84	7.83	2.68
17	7.94	5.03	1.75
18	1.83	4.67	0.73
19	0.48	2.94	3.07
20	4.95	6.39	1.47
21	3.99	2.66	7.21
22	3.56	3.53	9.63
23	4.97	4.96	9.95
24	10.13	5.05	5.05
25	4.92	9.84	4.94
26	14.95	0.00	0.00
27	0.00	14.99	0.00
28	0.00	0.00	14.72
29	15.14	15.26	0.00
30	15.31	0.00	15.24
31	0.00	15.15	15.16
32	0.65	14.98	7.59
33	14.89	7.45	14.97
34	7.53	15.14	15.21

Table 3 PLS Calibration Report

Algorithm	PLS I		
Number of components	3		
Number of standard samples	25		
Wavenumber range (cm^{-1})	963 - 1486		
Component	Fructose	Glucose	Sucrose
Number of factors	5	5	5
Correlation coefficient	0.9990	0.9987	0.9986
Square of correlation coefficient	0.9980	0.9973	0.9973
MSEP	0.0019	0.0026	0.0026
SEP	0.0441	0.0506	0.0513

Table 3 shows the PLS calibration report. The correlation coefficients of all sugars were satisfactory, at 0.99 or more, and the values of MSEP (mean square error of prediction) and SEP (standard error of prediction) were also small.

Table 4 shows the results of the quantitative analysis of the sugars contained in the nine commercial honey samples. Samples 1 to 5, which were labelled "100 % pure honey," had high ratios of glucose to fructose, suggesting addition of corn syrup. Sample 6 was labelled "7 % pure honey," but because it contained a high percentage of sucrose, it is considered possible that refined sugar was used in the preparation of that product.

Conclusion

A simple quantitative analysis of the sugars contained in honey was possible by FTIR measurement and analysis by chemometrics. The results of a quantitative analysis of commercial honeys showed component compositions different from the label information, suggesting the addition of low-cost substitutes. The FTIR analysis method can be used as an efficient technique for analysis of sugars in quality control of food products.

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The content of this article was presented by Shimadzu Scientific Instruments at Pittcon 2015 in March 2015.

Table 4 Results of Quantitative Analysis of Sugar in Commercial Honey Samples

Sample	Label information	Concentration of sugars (% w/w)			Ratio
		Fructose	Glucose	Sucrose	Fructose / Glucose
1	100 % pure honey	0.081	0.242	0.00	0.33
2	100 % pure honey	0.119	0.180	0.00	0.66
3	100 % pure honey	0.186	0.367	0.00	0.51
4	100 % pure clover honey	0.031	0.236	0.00	0.13
5	100 % pure honey	0.279	0.404	0.00	0.69
6	Made with 7 % pure honey	0.00	0.299	0.089	0.00
7	None	0.462	0.428	0.00	1.08
8	None	0.536	0.464	0.00	1.16
9	Grade A	0.379	0.363	0.00	1.04

First Edition: January, 2022

Shimadzu Corporation
www.shimadzu.com/an/

Printed in Japan 3655-10108-PDFIK, C10G-E091