APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Determination of Sulfonamides in Honey by UHPLC-MS/MS Method

Introduction

Antibiotics are often used in animal husbandry to help prevent or treat diseases in livestock, and improve productivity. The wide usage of antibiotics in animals not only results in contamination of food but also leads to substantial residues in the environment, leading to adverse health effects in humans and the development of antibiotic-resistant bacterial strains. In apiculture, sulfonamides are the most commonly used antibiotics to treat American and European Foulbrood, a type of disease that infects bees. This can result in bee honey being contaminated with sulfonamides. Regarding all the above, developing a fast, reliable and robust method for the quantitation of sulfonamides in honey is very important.

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Sample Preparation

1. Extraction

Weigh 5 g of honey sample into a 150 mL beaker; then add 25 mL of phosphoric acid solution (pH=2.0). Vortex/mix until honey is completely dissolved.

2. Clean-up

The sample clean-up consists of the following two solid phase extraction (SPE) steps:

Step 1. Load the dissolved sample onto a preconditioned aromatic sulfonic cation-exchange cartridge at a flow rate of ~2 mL/min, then wash the cartridge with 5 mL of phosphoric acid solution (pH=2) and 5 mL of water. Use 40 mL of potassium phosphate buffer (0.2 mol/L, pH=8) to elute the analytes from cartridge. Add 1.5 mL of sodium heptanesulfonate solution (0.5 mol/L) to the eluent, and adjust its pH to six by adding phosphoric acid.

Step 2. Load the above-mentioned solution onto a preconditioned HLB cartridge at a flow rate of ~2 mL/min. Wash the cartridge with 3 mL of water, and then dry under vacuum. Elute the analytes from the cartridge using 10 mL of methanol. Evaporate the eluent to dryness, and then dissolve the residue into1 mL of mobile phase A.

Experimental

Hardware/Software

Separation and detection of the analytes were performed on a PerkinElmer QSight® 210 UHPLC-MS/MS system. All instrument control, analysis and data processing was performed using the Simplicity 3Q™ software platform.

Method parameters

The LC and MS/MS method parameters are shown in Tables 1 and 2, respectively.

Table 1. LC method parameters.

Column: PerkinElmer Brownlee™ SPP C18,100 mm*2.1 mm,2.7 µm

Mobile Phase:

Solvent A: Water containing 0.1% of formic acid (FA)

Solvent B: Acetonitrile (ACN)

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.4	90	10	
2	1.00	0.4	90	10	6
3	4.00	0.4	70	30	6
4	5.00	0.4	70	30	6
5	6.00	0.4	10	90	6
6	6.50	0.4	10	90	6
7	7.00	0.4	90	10	6
8	9.00	0.4	90	10	6

Oven Temp.: 40 °C

Injection Volume: 3 µL

Table 2. MS/MS parameters and retention times of the analytes.

Ion source	ESI Positive
ElectroSpray/V	4500
Heating Gas Temp/°C	500
HSID Temp/°C	320
Dry Gas	200
Nebulizer Gas	180

Analytes	RT	Precursor ion/product ion 1	EV (entrance voltage)	CC (collision energy)	Precursor ion/product ion 2	EV	CC
Sulfadiazine	1.34	251.2/156.1	28	-21	251.2/92.2	22	-40
Sulfathiazole	1.63	256.1/156.1	26	-20	256.1/108.1	25	-40
Sulfapyridine	1.77	250.2/156.1	24	-24	250.2/184.1	28	-24
Sulfisoxazole	2.65	268.1/156.1	27	-21	268.1/113.2	26	-29
Sulfamethizole	2.86	271.1/156.1	19	-20	271.1/108.0	25	-38
Sulfamethazine	3.02	279.2/186.1	18	-24	279.2/156.1	18	-25
Sulfachloropyidazine	3.66	285.1/156.1	23	-23	285.1/108.1	21	-37
Sulfamethoxazole	3.79	254.2/156.1	29	-23	254.2/108.1	28	-36
Sulfamonomethoxine	4.15	281.0/155.8	19	-24	281.0/108.0	30	-38
Sulfadimethoxine	5.11	311.3/156.1	35	-27	311.3/218.1	24	-26
Sulfaquinoxaline	5.18	301.1/156.1	22	-23	301.1/108.0	18	-40

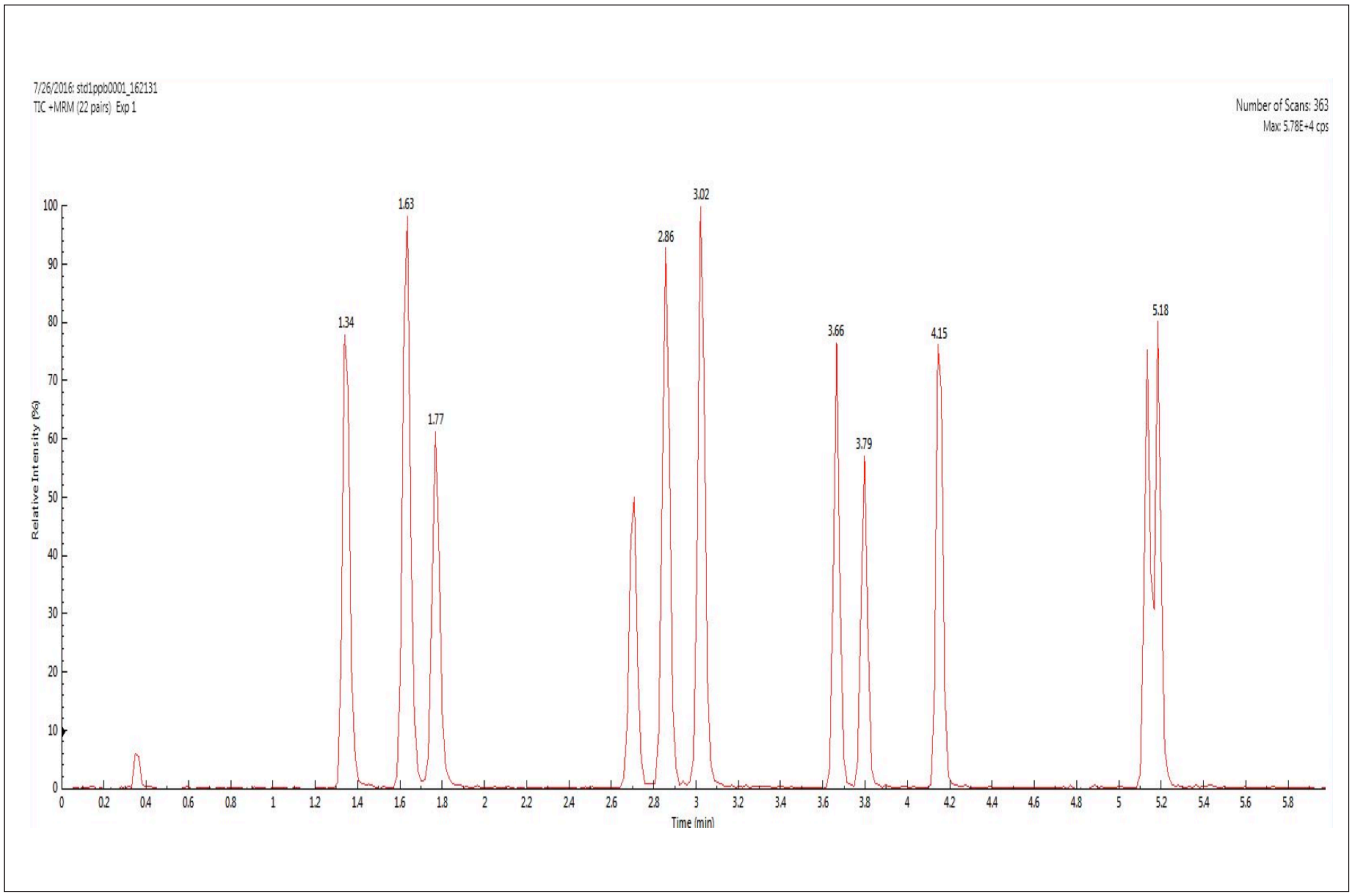

Figure 1. TIC of 11 sulfonamides analyzed in MRM transition mode.

Results

Figure 1 shows the total ion chromatogram (TIC) of 11 sulfonamides analyzed in MRM transition mode. Signal-to-noise ratios obtained at 0.02 ng/mL, and recoveries of individual sulfonamides spiked in honey taken through the entire extraction process are summarized in Table 3.

Quantitation of the sulfonimides in honey was performed using external standard calibration curves generated by dissolving standards at 0.1-10 ng/mL in 90% water/ACN. Results show very good linearity within the concentraton range, with regressional coefficients (R^2) ≥ 0.99. The limit of quantitation (LOQ) was 0.01 ng/mL for all analytes, with good reproduicibility (CV<8%).

Table 3. Signal-to-noise ratio of the analytes at 0.02 ng/mL, and recovery of the analytes from sample at 0.02 µg/kg.

Analytes	S/N at 0.02 ng/mL	Recovery at 0.02 µg/kg (%)
Sulfadiazine	18	81.6
Sulfathiazole	40	85.9
Sulfapyridine	30	83.2
Sulfisoxazole	50	86.2
Sulfamethizole	100	90.1
Sulfamethazine	400	91.5
Sulfachloropyidazine	30	79.8
Sulfamethoxazole	30	81.1
Sulfamonomethoxine	20	77.5
Sulfadimethoxine	25	78.0
Sulfaquinoxaline	40	80.7

Table 4. Linear dynamic range, regressional coefficients, LOQ and reproducibility at LOQ.

Analytes	Linear dynamic range	Regressional coefficients (R^2)	LOQ (ng/mL)	CV% at LOQ n=6
Sulfadiazine	0.01~10.0	0.9943	0.01	5.41
Sulfathiazole	0.01~10.0	0.9950	0.01	6.25
Sulfapyridine	0.01~10.0	0.9936	0.01	4.77
Sulfisoxazole	0.01~10.0	0.9958	0.01	4.90
Sulfamethizole	0.01~10.0	0.9943	0.01	3.22
Sulfamethazine	0.01~10.0	0.9950	0.01	2.15
Sulfachloropyidazine	0.01~10.0	0.9948	0.01	6.54
Sulfamethoxazole	0.01~10.0	0.9934	0.01	7.03
Sulfamonomethoxine	0.01~10.0	0.9972	0.01	6.05
Sulfadimethoxine	0.01~10.0	0.9958	0.01	7.12
Sulfadiazine	0.01~10.0	0.9959	0.01	5.79

Conclusion

A quick and reliable UHPLC-MS/MS method was developed for the determination of 11 sulfonamides in honey. The extraction and two-step clean-up sample preparation showed good recoveries (>80%) for all analytes in the sample. The LOQs for all analytes were 0.01 ng/mL (0.01 µg/kg). An EU Community Reference Laboratories' (CRLs) Guidance Paper recommended a maximum concentration level of 50 µg/kg for sulfonamides in honey. The LOQs achieved using this method are well below that level, suggesting that the QSight 200 System provides a very sensitive and robust platform for the analysis of sulfonamides in honey.

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