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A sensitive and repeatable method for characterization of sulfonamides and trimethoprim in honey using QuEChERS extracts with Liquid-Chromatography-Tandem Mass Spectrometry

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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 preserve 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.

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

Results

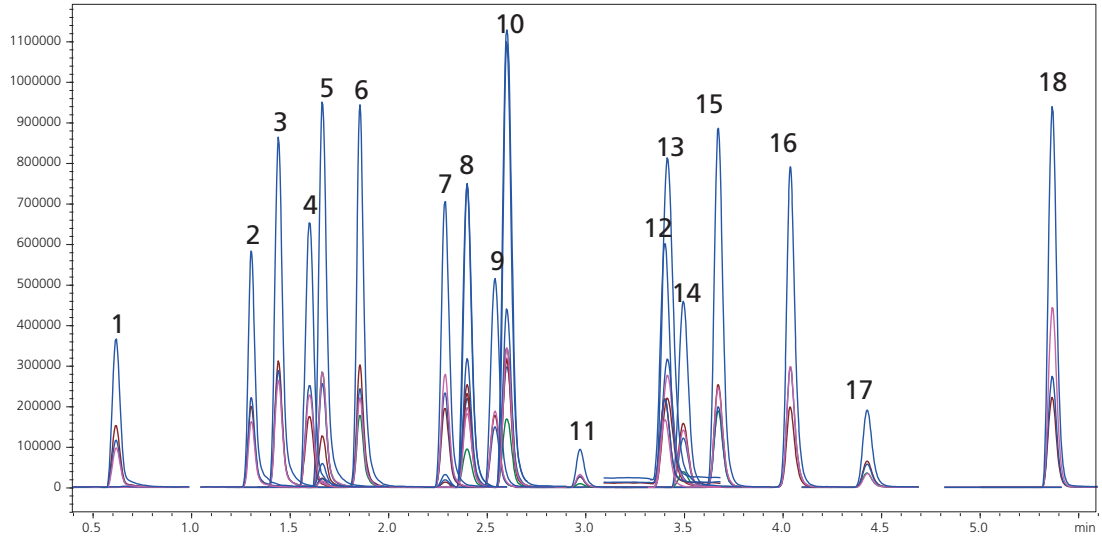

Figure 1. Representative chromatogram of sulfonamide drugs. Standard mixture at 125 pg on-column for each standard. Peak numbers follow the order described for SA compounds in table 1A.

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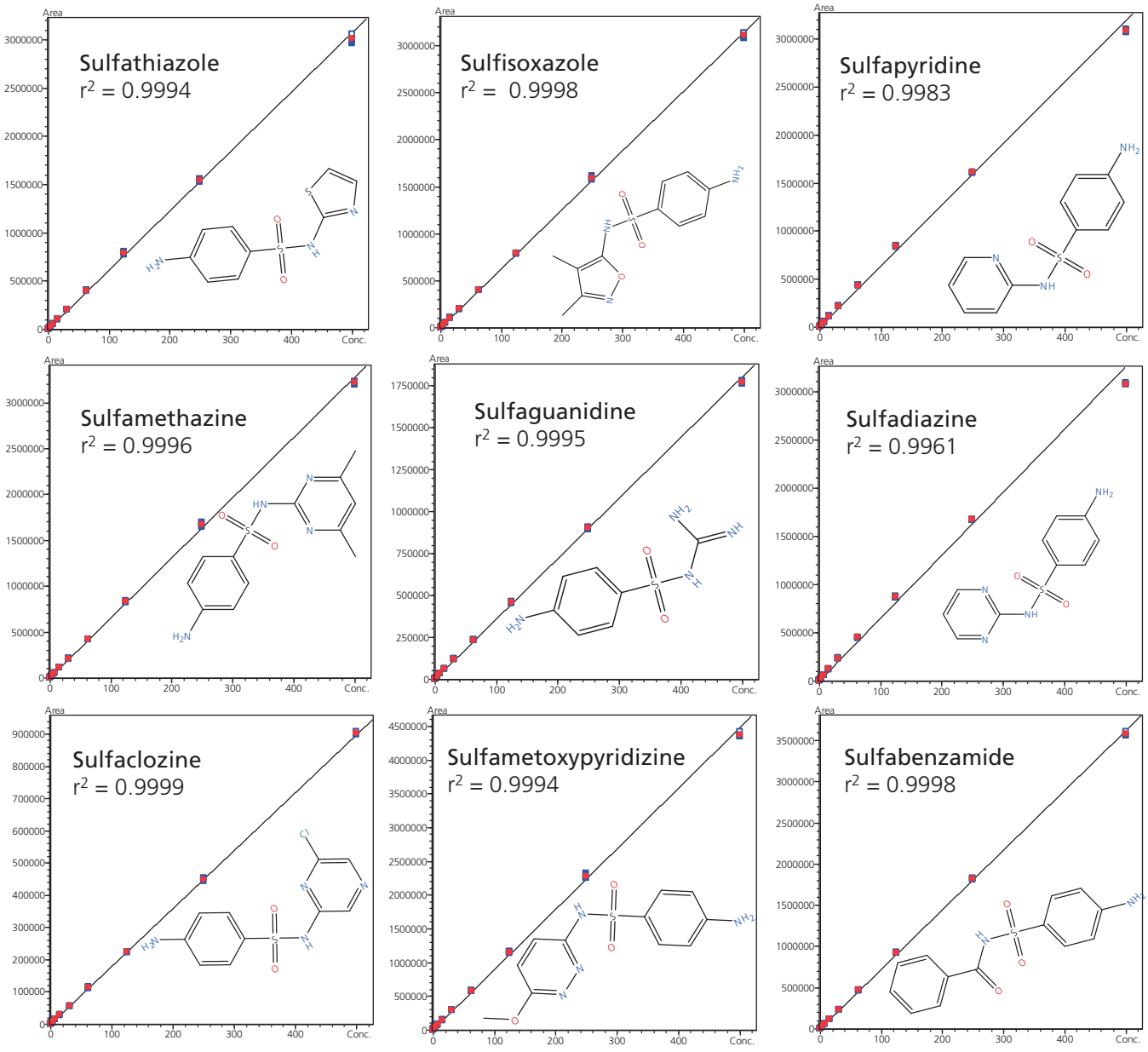

Figure 2. High degree of linearity was observed over the concentration range 0.5–500 pg on column, with values of $r^2 \geq 0.990$ for all analytes.

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 500pg on column). The limits of quantification were 1 µg/Kg (0.5pg on column) for all compounds except succinylsulfathiazole and sulfacetamide, which were 2 µg/Kg (1pg 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.

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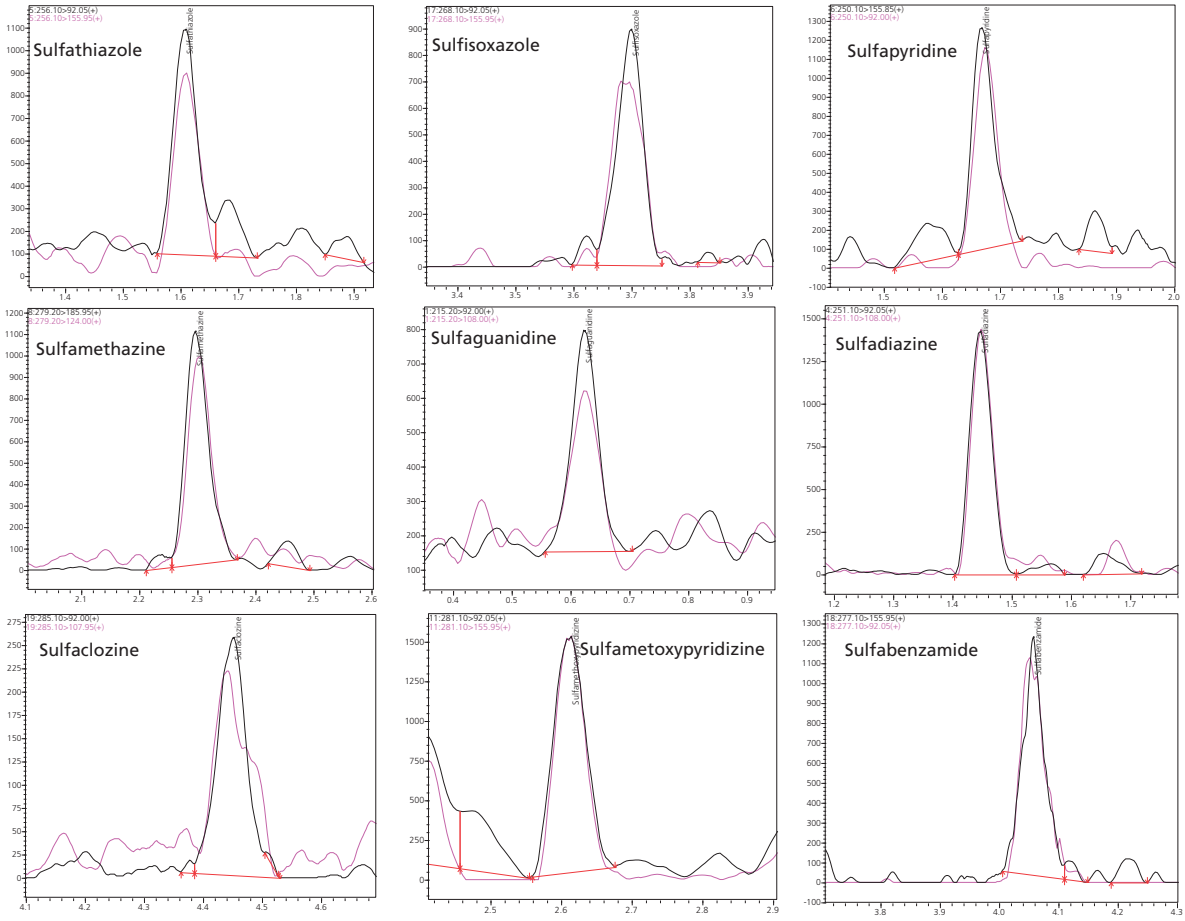

	Sulfathiazole		Sulfisoxazole		Sulfapyridine		Sulfamethazine		Sulfaguanidine		Sulfadiazine		Sulfaclozine		Sulfamethoxypyridizine		Sulfabenzamide	
Level	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy %	Cal point %RSD	Accuracy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

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Conclusions

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.

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