Analysis of Sulfonamides in Honey Using the SCIEX Triple Quad™ 3500 System

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Introduction

Honey is widely consumed as food and medicine. Many different antibiotics are used in Apiculture to keep bees away from various bacterial infections. Accumulation of antibiotic residues in the raw material from bees, lead to adverse health effects during human consumption.

According to European Union regulations, honey is considered as a natural product and must be free of chemicals. Antibiotics used in honey and other bee products production are usually veterinary medicines. Beekeepers use high doses of antibiotics to prevent and treat bacterial infections in honey. Antibiotic residues have a relatively long half-life and may have direct toxic effects on consumers. Extensive use of antibiotics and its accumulation makes the trade of honey difficult globally. To reduce the chances of health risks to consumers, regulatory legislations were laid down for various antibiotic classes for honey. Minimum required performance limit (MRPLs) of antibiotics have been set to levels as small as parts-per-billion (ppb).

The LC-MS/MS method was developed using the Multiple Reaction Monitoring (MRM) that detects antibiotics as per European Union regulatory guidelines with the consideration of two Transitions to one analyte (ratio of quantifier and qualifier ion) for all the nine sulfonamides (Sulfamerazine, Sulfadiazine, Sulfamethazine, Sulfadimethoxine, Sulfamethoxypyridazine, Sulfamethoxazole, Sulfadoxine, Sulfathiazole, and Sulfapyridine) on SCIEX Triple Quad™ 3500 LC/MS/MS System with minimum required performance limit (10.0 ng/ml).

Figure 1. SCIEX Triple Quad™ 3500

Compound	Precursor ion	Product ion Quantifier	Product ion Qualifier
Sulfamerazine	265.0	155.9	107.9
Sulfadiazine	251.0	156.0	91.9
Sulfamethazine	279.0	186.0	124.0
Sulfadimethoxine	311.0	156.1	92.0
Sulfamethoxypyridazine	280.9	91.9	107.9
Sulfamethoxazole	254.0	155.8	92.0
Sulfadoxine	310.9	155.9	92.0
Sulfathiazole	256.3	156.1	92.0
Sulfapyridine	249.9	155.7	108.1

Table 1. MS Transition for Sulfonamides

Materials and Methods

Chemicals

Sulfonamides Standards were purchased from Sigma Aldrich. All other chemicals used were of LC-MS grade, commercially available.

Honey samples

Honey samples were purchased from local market of Delhi and Gurgaon, India and were stored at room temperature for analysis.

Sample Preparation

Accurately weighed 1 g of honey, mixed with 2ml of 0.1 M HCl, sonicated for 30 min. Followed by the addition of 3ml of 0.3M Citric acid (final vol. around 5 ml) and Vortex well. Then 10 ml Acetonitrile with 0.1% Formic Acid (FA) was added and mixed well for 10 min. followed by the addition of 2 g Sodium chloride (NaCl), vortexed again, centrifuged, collected supernatant and evaporated at 500C. The residue was reconstituted in 1 ml Methanol: Water (80:20) with 0.1% Formic Acid (FA) and transfer into vial for analysis.

LC Conditions

LC separation was achieved using the Shimadzu prominence system with a Zorbax SB C-18 (4.6×150 mm) 5 µm column with a gradient of water containing 0.1% formic acid (mobile phase A) and Acetonitrile containing 0.1% formic acid(mobile phase B) at flow rate of 0.5 mL/min. The injection volume was set to 10 µL.

Time (min)	Mobile phase A%	Mobile phase B%
0.01	98	2
5.50	2	98
6.00	2	98
8.00	98	2
11.00	Controller	Stop

Table 2. Gradient method

MS/MS Conditions

The SCIEX Triple Quad™ 3500 LC/MS/MS system was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in positive polarity. Two selective MRM transitions were monitored for all sulfonamides using the ratio of quantifier and qualifier ion for compound identification. Analyst 1.6.2 software was used for method development and data acquisition. LC-MS/MS data was processed using the MultiQuant™ software version 3.0.2

Results and Discussions

The calibration curve shows excellent linearity, with a correlation coefficient greater than 0.98 for nine sulfonamides using linear regression and weighing factor 1/X2. Matrix based calibration curves were made with standard levels ranging from 1.0 ng/ml to 100 ng/ml spiked concentration; linear graph was obtained with regression co-efficient (r) ≥0.99 for all the nine sulfonamides. The calibration curve was shown in Figure 3 and a representative chromatogram was shown in Figure 4 & 5

The retention times of the analytes were ranging from 5.50 min to 7.00 min. A representative chromatogram obtained from a standard mixture of the sulfonamides with minimum background noise in 11.0 minutes chromatographic run.

The method demonstrated good precision and accuracy batch. No interferences with the peaks of interest were observed throughout the chromatographic run.

The recovery study was carried out by spiking the honey samples with 10 ng/ml concentration of Sulfonamides and found the recoveries ≥ 86% at MRPL level. The recovery was performed with six replicates (n=6) respectively. The recovery data for sulfonamides are shown in Table 4.

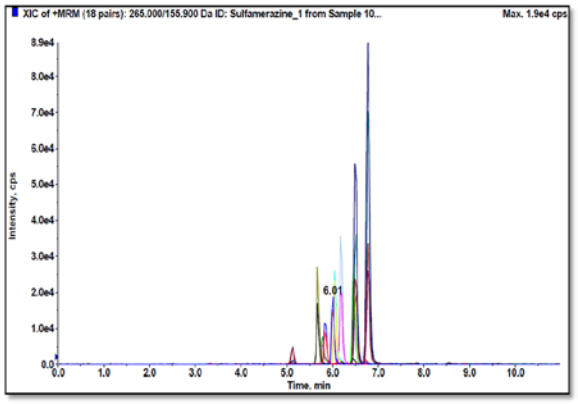

Figure 3. Representative chromatogram of Sulfonamide (Sulfamerazine) at MRPL Level (10ng/ml) Concentration.

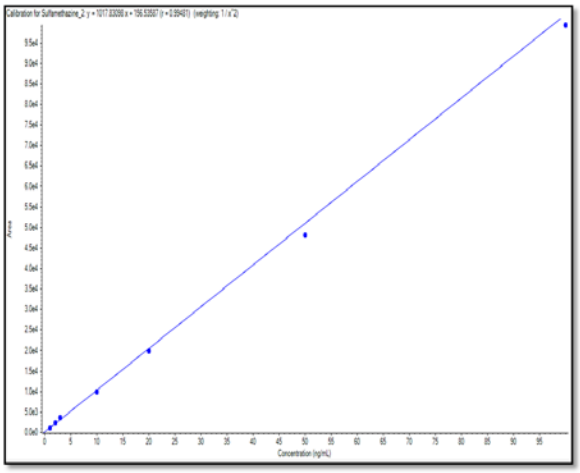

Figure 2. Linear range of the detection of Sulfamethazine from 1.0 to 100ng/mL (r ≥ 0.98)

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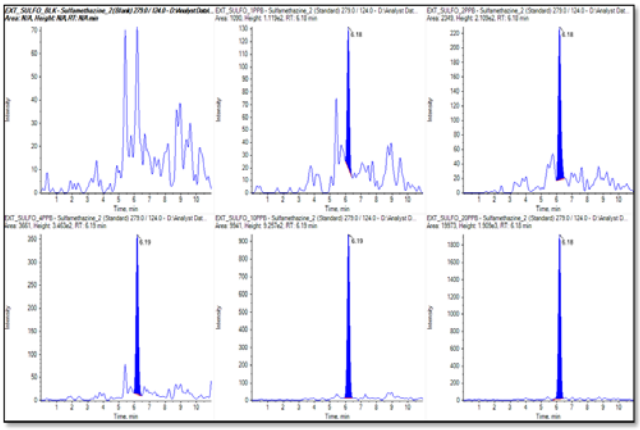

Figure 4. Representative Calibration Linearity chromatogram of Sulfonamide (Sulfamerazine) from 1.0 – 20.0ng/ml.

All Sample Types

Sample Name	Sample Type	Component Name	Mass Info	Actual Concentration	Calculated Concentration	Accuracy	Retention Time	Used	MRM Ratio
EXT_SULFO_BLK	Blank	Sulfamethazine_2	279.0 / 124.0	N/A	N/A	N/A	N/A	☑	0.000
EXT_SULFO_1PPB	Standard	Sulfamethazine_2	279.0 / 124.0	1.00	0.92	91.69	6.18	☑	0.970
EXT_SULFO_2PPB	Standard	Sulfamethazine_2	279.0 / 124.0	2.00	2.15	107.72	6.18	☑	0.742
EXT_SULFO_3PPB	Standard	Sulfamethazine_2	279.0 / 124.0	3.00	3.46	115.32	6.19	☑	0.778
EXT_SULFO_10PPB	Standard	Sulfamethazine_2	279.0 / 124.0	10.00	9.61	96.13	6.19	☑	0.812
EXT_SULFO_20PPB	Standard	Sulfamethazine_2	279.0 / 124.0	20.00	19.47	97.35	6.18	☑	0.825
EXT_SULFO_50PPB	Standard	Sulfamethazine_2	279.0 / 124.0	50.00	47.17	94.34	6.19	☑	0.791
EXT_SULFO_100PPB	Standard	Sulfamethazine_2	279.0 / 124.0	100.00	97.46	97.46	6.19	☑	0.826

Table 3. Accuracy data obtained for sulfonamides (Sulfamethazine) with MRM Ratio

Compound	% Recovery 10 ppb
Sulfamerazine	91.33
Sulfadiazine	96.93
Sulfamethazine	89.33
Sulfadimethoxine	93.02
Sulfamethoxypyridazine	86.52
Sulfamethoxazole	91.05
Sulfadoxine	91.58
Sulfathiazole	97.35
Sulfapyridine	90.43

Table 4. Recovery of sulfonamides at MRPL (10ng/ml) in honey matrix

Repeatable injections (n= 06) at MRPL gives the % relative standard deviation of ≤5.0%.

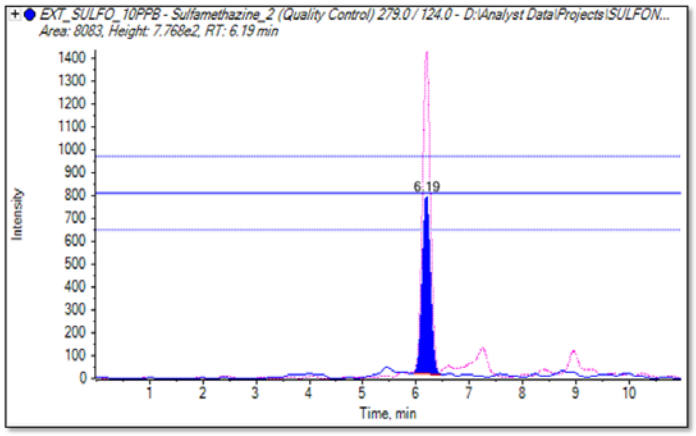

Figure 5. MRM Ratio of sulfonamides (Sulfamethazine) at RT-6.19 was ≤ 1.0

Summary

A SCIEX Triple Quad™ 3500 LC/MS/MS system reduces analysis time and improves sensitivity and resolution, detecting and quantifying several classes of sulfonamides drugs. Nine sulfonamide analytes were determined with a single extraction and the proposed method could be applied in routine analysis.

The method and data presented here showcased the fast and accurate solution for the quantitation and identification of Sulfonamides in honey samples by LC-MS/MS.

Matrix interferences study was conducted to understand the matrix effects. Automatic MRM ratio calculation in MultiQuant™ Software can be used for confirmation in compound identification.

References

[1]Method validation and quality control procedures for pesticide residue analysis in food and feed Document NO. SANCO/12495/2011, Implemented by 01/01/2012

[2]Guidelines for the design and implementation of National regulatory food safety assurance programme associated with use of veterinary drugs in food producing animals CAC/GL 71-2009

[3]Rongyuan Liu, Pingli He, Zhen Li, and Ruiguo Li; Simultaneous Determination of 16 Sulfonamides in Animal Feeds by UHPLC–MS–MS. Journal of Chromatographic Science, (2011) Vol. 49 (8):640-646

[4]Neha Bhasin, Prasanth Joseph, Praveen Sharma, Manoj Pillai, Jens Dahlmann, André Schreiber and Christopher Borton; Targeted Multi-Residue LC-MS/MS Method for Sulfonamide and Nitroimidazole Antibiotics in Honey. (2015), AOAC poster

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