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

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Overview

A LC-MS/MS method for the simultaneous quantification of nine sulfonamides (sulfamerazine, Sulfadiazine, Sulfamethazine, Sulfadimethoxine, Sulfamethoxypyridazine, Sulfamethoxazole, Sulfadoxine, Sulfathiazole, and Sulfapyridine) on SCIEX Triple Quad™ 3500 was developed with a simplified sample preparation to detect veterinary residues, The method presented here demonstrated adequate linearity with correlation coefficients above r≥0.99 for all the nine sulfonamides analyzed.

Introduction

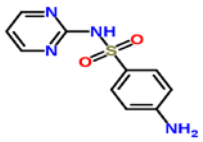
Sulfonamides (SAs) are used to treat a wide variety of bacterial and protozoal infections in animals. The presence of these antimicrobials can be a potential risk for consumers health if present above the allowed limits. Sulfonamides are illegally used as additives in animal feed as a growth promoters and thus they can generate serious threats in human health such as allergic or toxic reactions, carcinogenic.

For the purpose of monitoring the presence of these residues, an LC-MS/MS method was established to identify and quantify the nine sulfonamide residues in milk with a very simple sample preparation and shorter runtime. The Committee for Veterinary Medicinal Products considers that the sum of all substances belonging to the sulfonamide group in bovine milk should not exceed 100 µg/kg (EMEA, 1995a)

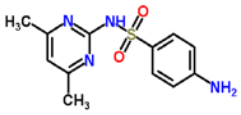
Figure 1. SCIEX Triple Quad™ 3500

Figure 2. Re-engineered quadrupole to maximize efficiency

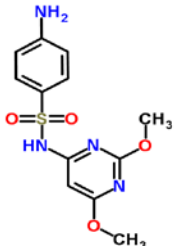
1. Sulfamerazine



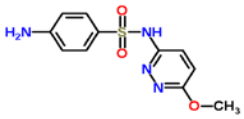
2.Sulfadiazine



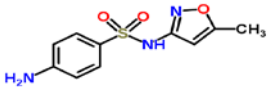
3. Sulfamethazine



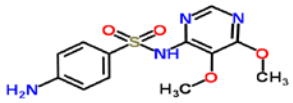
4.Sulfadimethoxine



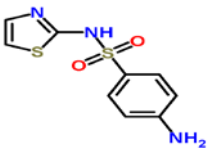
5. Sulfadimethoxypridazine



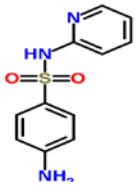
6. Sulfamethoxazole



7. Sulfadoxine



8.Sulfathiozole



9. Sulfapyridine

Figure 3: Structures of Sulfonamide Analytes.

Materials and Methods

Chemicals

Sulfonamides Standards were purchased from Sigma Aldrich ≥99% Purity. All other chemicals used were of LC-MS grade, commercially available.

Milk samples

Milk samples were procured from local market of Delhi and Gurgaon, India and was stored at 2−8 °C until end of analysis.

Sample Preparation

1. 1 ml Milk sample is mixed with 5ml of acidified acetonitrile
2. Add 1 gm of Sodium Chloride and Vortex followed by centrifugation at 4000 rpm
3. Transfer the supernatant and evaporate with N2 steam to dryness
4. Reconstitute with 1ml of Methanol: water: Formic Acid (80:20:0.1%) and use it for LC-MS/MS 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 with (0.1% formic acid) as mobile phase A and Acetonitrile with (0.1% formic acid) as 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 1. Gradient Time Program

MS/MS Conditions

The SCIEX Triple Quad™ 3500 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 Analyst® 1.6.2 Software and MultiQuant™ Software version 3.0.2.

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 2. MS Transition for the nine sulfonamides.

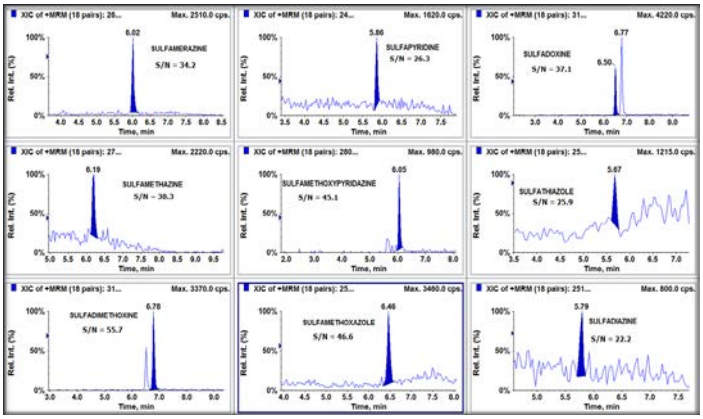


Figure 4: Signal to Noise (S/N) of all sulfonamides at 5 ng/ml concentration.

Results and Discussions

The matrix matched calibration curve shows excellent linearity (5 to 300ng/ml), with a correlation coefficient r≥0.98 for all sulfonamides in milk using linear regression and weighing factor 1/X2. The lowest calibration point for quantitation of sulfonamides was 5 ng/ml. The SCIEX Triple Quad™ 3500 was found to be capable of analyzing concentrations well below the MRPL required by EU. The signal to noise ratio for all sulfonamides compound at 5 ng/ml is ≥ 60.

The calibration curves and chromatographs are shown in Figure 4 and Figure 5.

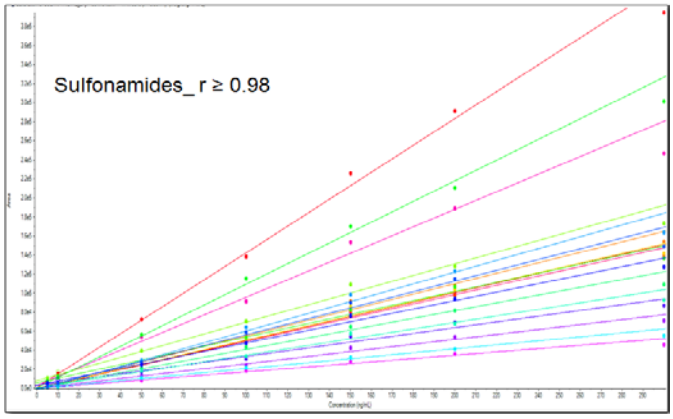


Figure 5. Linear range of the detection of sulfonamides from 5 to 300 ng/mL (r ≥ 0.98)

The Results of accuracy data obtained for Sulfonamides in the milk matrix is given in Table 3.

Recovery was assessed by performing tests where fortified milk

All Sample Types

Sample Name	Sample Type	Component Name	Mass Info	Actual Concentration	Area	Retention Time	Used	Calculated Concentration	Accuracy
EXT_SULFO_BLK	Blank	SULFADOXINE_1	311.0 / 155.9	N/A	754.34	6.51	☑	0.08	N/A
EXT_SULFO_5PPB	Standard	SULFADOXINE_1	311.0 / 155.9	5.00	6148.68	6.50	☑	5.13	102.59
EXT_SULFO_10PPB	Standard	SULFADOXINE_1	311.0 / 155.9	10.00	10710.14	6.50	☑	9.40	93.96
EXT_SULFO_50PPB	Standard	SULFADOXINE_1	311.0 / 155.9	50.00	56054.20	6.50	☑	51.81	103.61
EXT_SULFO_100PPB	Standard	SULFADOXINE_1	311.0 / 155.9	100.00	105314.54	6.51	☑	97.88	97.88
EXT_SULFO_150PPB	Standard	SULFADOXINE_1	311.0 / 155.9	150.00	171744.02	6.50	☑	160.01	106.68
EXT_SULFO_200PPB	Standard	SULFADOXINE_1	311.0 / 155.9	200.00	222819.90	6.50	☑	207.79	103.89
EXT_SULFO_300PPB	Standard	SULFADOXINE_1	311.0 / 155.9	300.00	293796.37	6.51	☑	274.17	91.39

Table 3. Accuracy data obtained for sulfonamides (Sulfadoxine)

samples at the MRL level were analyzed (n=6) respectively. The recovery of all sulfonamides was ≥ 93%. The recovery data for sulfonamides are shown in Table 4.

Compound	% Recovery 10 ng/ml
Sulfamerazine	93.93
Sulfadiazine	101.34
Sulfamethazine	95.13
Sulfadimethoxine	99.43
Sulfamethoxypyridazine	100.95
Sulfamethoxazole	94.78
Sulfadoxine	100.17
Sulfathiazole	102.26
Sulfapyridine	98.84

Table 4. Recovery of sulfonamides in the milk matrix at (10ng/ml).

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.

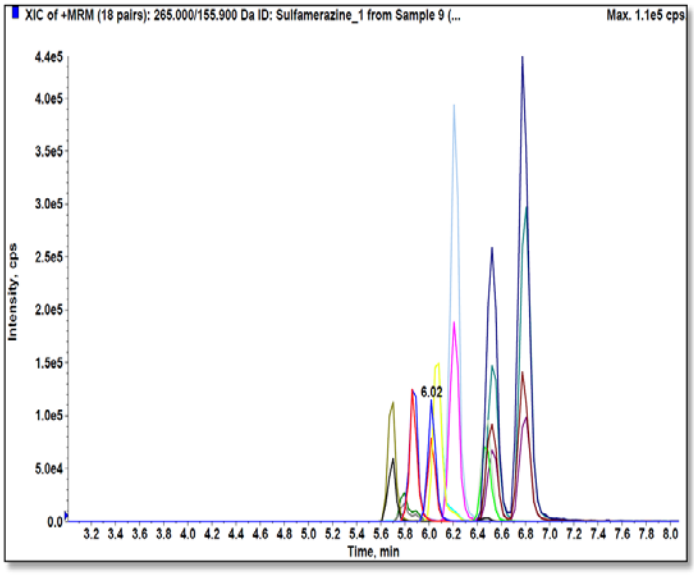


Figure 6. Representative chromatogram of Sulfonamides at 100ng/ml

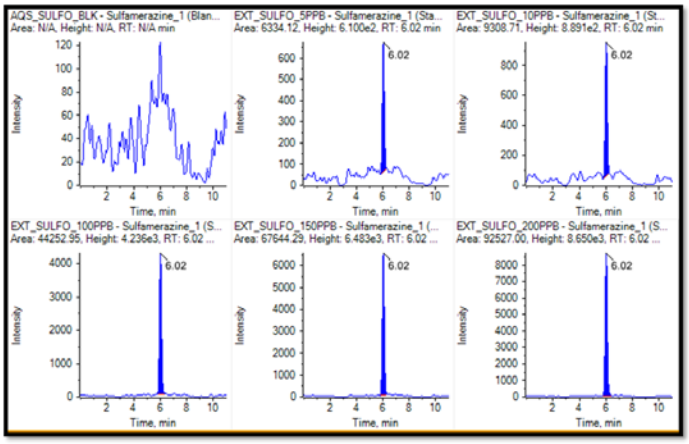


Figure 6. Representative chromatogram of Sulfonamide (Sulfamerazine; 5ng/ml to 200ng/ml)

Repeatability experiment was evaluated by 06 repeated injections at the lowest calibration point (5 ng/ml). Repeatable injections (n= 06) at 5ng/ml level gives the % relative standard deviation of ≤ 5.0%.

Conclusions

- The developed method on SCIEX Triple Quad™ 3500 was simple, sensitive and reproducible.
- This method found to be simple, linear, reproducible and rugged.
- Trueness (Average recovery %) for this method found to be ≥ 93%.

Summary

A SCIEX Triple Quad™ 3500 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 showcase the fast and accurate solution for the quantitation and identification of Sulfonamides in milk samples by LC-MS/MS.

References

- U.S. Food and Drug Administration Center for Food Safety Applied Nutrition Food Compliance Program Chapter 03 – Foodborne Biological Hazards (10-01-97)

- Veterinary Drug MRL Database http://www.mrldatabase.com/?selectvetdrug=1

- Elizabeta Dimitrieska Stojkovic, Hajrulai Musliu Zehra, Biljana Stojanovska Dimzoska and Risto Uzunov; Screening of veterinary drug residues in milk from individual farms in Macedonia. Macedonian Veterinary Review 34(1):5-13, May 2011

- N.Furusava and K.Kishida; High performance liquid chromatographic procedure for routine residue monitoring of seven sulfonamides in milk. Fresenius J Anal Chem (2001) 371: 1031

- EC (2010): Council Regulation 37/2010/EU of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of European Union, L15, 1-72.

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