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).
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.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A%</th>
<th>Mobile phase B%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>5.50</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>6.00</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>8.00</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>11.00</td>
<td>Controller</td>
<td>Stop</td>
</tr>
</tbody>
</table>

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.
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.

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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Recovery 10 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamerazine</td>
<td>93.93</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>101.34</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>95.13</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>99.43</td>
</tr>
<tr>
<td>Sulfamethoxypyridazine</td>
<td>100.95</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>94.78</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td>100.17</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>102.26</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>98.84</td>
</tr>
</tbody>
</table>

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 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

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