DETERMINATION OF SULFAGUANIDINE IN MEDICATED FEEDINGSTUFFS BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract

A liquid chromatography-mass spectrometric method (LC-MS) for the determination of sulfaguanidine in medicated feedingstuffs was developed and validated. The samples were extracted using acidified methanol, centrifuged, and a portion of the extract was diluted with water. An appropriately diluted aliquot of the extract was analysed on a column 150 mm x 4.6 mm, 5 µm using a mixture of 0.1% formic acid in water and in acetonitrile as the mobile phase. The LC-MS was used in the positive atmospheric pressure chemical ionisation MS mode. The LC-MS method was validated for its linearity, precision, and specificity. Recovery from spiked samples was from 90.4% to 96.0% depending on sulfaguanidine concentration. The method was used for the quantitative determination of sulfaguanidine and its homogeneity in commercial medicated feedingstuffs.

Key words: sulfaguanidine, medicated feedingstuffs, liquid chromatography-mass spectrometry.

Since January 1, 2006, when a ban on using antibiotic growth promoters was introduced, an increasing demand for the production of medicated feedingstuffs has been observed. Medicated feedingstuffs are defined as “any mixture of veterinary medicinal product or products and feed or feeds, which is ready prepared for marketing and intends to be the fed to animals without further processing, because of its curative or preventive properties or other properties as a medicinal product” (1). Medicated feedingstuffs may be prepared only from premixes, which have been authorised under the suitable acts. Currently in Poland, there are a few dozen medicated premixes authorised. There are a lot of premixes, which contain sulfonamides.

Sulfonamides are one of the groups of antibacterial substances, which are widely used in veterinary medicine for the treatment of bacterial infections caused by Gram-negative and Gram-positive organisms and for the production of medicated feeds. Sulfonamides are veterinary drugs with wide spectrum of activity, which inhibit growth of bacteria, chlamydia, toxoplasma, and other protozoan agents, especially coccidia in the poultry, rabbits, and other economically important animal species (5). Furthermore, sulfonamide treatment of piglets against neonatal coccidiosis and infectious enteritis has frequently been recommended. Many parenteral, intramammary, and oral preparations are authorised for the treatment of a variety of conditions in food and domestic animal species in the EU. An evaluation of production orders for medicated feedingstuffs showed sulfonamides and combinations of sulfonamides and trimethoprim as frequently used antibiotical ingredients, especially for pigs (4). Sulfonamides are bacteriostatic agents. They are structural analogues of p-amino benzoic acid (PABA). They compete with PABA for enzyme dihydropteroate synthetase (DHPS), which is essential for synthesis of folic acid in bacteria.

Sulfaguanidine is a sulfonamide and as Suibicol® premix is added to swine feeds because of its antimicrobial activity and is frequently used as preventive and therapeutice agents for treatment of polyaetiological post-weaning diarrhoeas. The dosage of sulfaguanidine is 1–3 g/10 kg of bodyweight and usually the content of premix is 1 kg/100 kg of the final feed and corresponds with the dose of 2 g/kg of feed.

The content of drug in medicated feedingstuffs is strictly determined in a prescription and the evidence of homogeneity must be presented to demonstrate that adequate mixing of the active ingredient in the feed has been achieved. The purpose of the test of the quality requirements for medicated feedingstuffs is to ensure that a homogenous feedingstuffs was produced, and to provide information on the content of active substances. The criterion for the assessment of homogeneity is the coefficient of variation (CV), whose value is ≤15% (9). This supposes the availability of suitable methods for the quantitative determination of sulfaguanidine in medicated feed.
Taking these facts into account, there is a necessity to control the content and homogeneity of active substances in medicated feedingstuffs. The aim of the presented study was to develop and validate a reliable and rapid method capable of the quantitative analysis of sulfaguanidine in medicated feedingstuffs.

Material and Methods

Reagents. Sulfaguanidine standard was purchased from Sigma–Aldrich Chemical Company (USA). Methanol, acetone, sodium hydroxide, and water were obtained from POCH (Poland); acetonitrile and formic acid were from Merck (Germany). Acidified methanol was prepared by adding 100 µl of formic acid to 100 ml methanol.

Standard solutions. A stock standard of sulfaguanidine (1.5 mg mL\(^{-1}\) = 10 g kg\(^{-1}\)) was prepared by dissolving sulfaguanidine standard in acetone with 2 ml of 0.2 N NaOH in order to completely dissolve the compound. Working standard solutions for calibration curve at five concentration levels (0.2, 1, 2, 5, 10 g kg\(^{-1}\)) were prepared on the day of analysis by dilution in water. All working solutions were diluted 1,000 times before LC-MS analysis.

Sample preparation. The sample was grinded and sieved through 1 mm sieve. Three-gram portion of the samples was weighed into 250 ml Erlenmeyer flask. The sample was soaked with 10 ml of water and then 10 ml of acidified methanol was added. This mixture was prepared procedure we used the isocratic elution on C8 column (150 mm x 4.6 mm, 5 µm) with the mobile phase consisting of a 0.1% solution water of formic acid (A) and 0.1% formic acid solution in acetonitrile (B) in 40:60 v/v, respectively. The monitored ion for sulfaguanidine compound (six replicates for each concentration level). The samples were processed through the whole procedure. The within-laboratory reproducibility was determined by fortifying other set of blank samples at the same concentration levels of analysed compound, as for the repeatability and analysing on different days with the same instrument and the different operator. Limit of detection - LOD and limit of quantitation – LOQ of the method were calculated using data from the determination of specificity.

Results

The optimal conditions for the detection and quantitative determination of sulfaguanidine were obtained with the use of flow injection analysis. The solution used for the optimisation was at the concentration level of 1 µg mL\(^{-1}\). Different extraction techniques and solvents were tested to obtain the optimal conditions for sample preparation. In the prepared procedure we used the isocratic elution on C8 column (150 mm x 4.6 mm, 5 µm) with the mobile phase consisting of a 0.1% solution water of formic acid (A) and 0.1% formic acid solution in acetonitrile (B) in 40:60 v/v, respectively. The above chromatographic conditions allowed obtaining stable retention time and symmetric peaks of the analysed substance. No interferences were observed. Typical obtained chromatograms are shown in Fig. 1.

The equation for the calibration curve was \( y = 29.028x + 1.528 \) and the correlation coefficient \( R^2 \) equalled 0.9998. The high value of the coefficient indicated good linearity of the method. The precision and accuracy of the method was determined by repeatability. Six samples at the same concentration were examined. The standard deviation (SD) was 0.024 to 0.068. The repeatability represented by the coefficient of variation for three levels ranged from 1.5% to 4.9% depending on the concentration. The average recovery was 90.4%–96.0%. The variation coefficient and the mean recovery were satisfactory. The method was linear and showed good precision. The results obtained for the validation are shown in Table 1.

Discussion

Sulfonamides are very popular and frequently used in veterinary medicine to cure the diseases of animals and improve their antibacterial ability. However, such treatment can lead to the sulfonamide residues in food. The occurrence of residues of veterinary drugs in food of animal origin is systematically monitored. There are many analytical procedures, which describe the determination of sulfonamides in food (meat, honey), as well in biological matrices and wastewater (2, 8, 10, 11).
Fig. 1. Chromatograms: a) blank sample b) blank sample and sulfaguanidine solution at the concentration level of 0.2 g kg\(^{-1}\); c) feedingstuff sample spiked at the level of 2 g kg\(^{-1}\); d) medicated feedingstuff sample with declared content of sulfaguanidine 2 g kg\(^{-1}\).

Table 1
Results of validation of analytical procedure for the determination of sulfaguanidine in medicated feeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear regression equation, (y = ax + b)</td>
<td>(y = 29,028 + 1,528)</td>
</tr>
<tr>
<td>LOD, g kg(^{-1})</td>
<td>0.04</td>
</tr>
<tr>
<td>LOQ, g kg(^{-1})</td>
<td>0.07</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
</tr>
<tr>
<td>Linearity (working range), g kg(^{-1})</td>
<td>0.2 – 10.0</td>
</tr>
<tr>
<td>Level of spiked samples, g kg(^{-1})</td>
<td>0.5  2.0  5.0</td>
</tr>
<tr>
<td>Recovery, %</td>
<td>96.0  91.0  90.4</td>
</tr>
<tr>
<td>Repeatability, CV %</td>
<td>4.9   2.8  1.5</td>
</tr>
<tr>
<td>Reproducibility, CV %</td>
<td>6.2   5.9  4.3</td>
</tr>
<tr>
<td>Uncertainty combined, (uc)</td>
<td>0.029 0.080 0.158</td>
</tr>
<tr>
<td>Coverage factor, (k)</td>
<td>2  2  2</td>
</tr>
<tr>
<td>Expanded, (U)</td>
<td>0.058 0.160 0.316</td>
</tr>
<tr>
<td>(U), %</td>
<td>11.6  8.0  6.3</td>
</tr>
</tbody>
</table>
Simultaneously there are considerably fewer procedures for the determination of sulfonamides in animal feedingstuffs (6, 7). At the same time, there has been no available analytical procedure for the determination of sulfaguanidine in feeds (6, 7).

In an official control of medicated feedingstuffs, microbiological methods are used to determine different active substances in medicated feedingstuffs. Microbiological methods are not suitable for determination of sulfonamides in feeds. Therefore, it was necessary to develop chemical method for the quantitative determination of sulfaguanidine in feeds.

In our preliminary study, we tried to find the most efficient way for sample extraction. In spite of high concentration of sulfaguanidine in medicated feedingstuffs, the complexity of matrix can create some difficulties with obtaining sufficient recovery of the active substances. Few extraction solvents were tested. An acidified methanol was used as the first solvent. The recovery obtained was in the range of 80%, but the additional drawback of the use of methanol was quite high level of signal noise. Some attempts of extraction with acetonitrile and water were also undertaken. The recovery with acetonitrile gave pure results; however, water proved to give good recovery. The optimal way of extraction was obtained by combining water and acidified methanol. The sample of spiked feedingstuff was firstly damped with water and subsequently the methanol was added. The solvent combination gave the most satisfactory results of the recovery.

After some tests it was decided to adopt dilution instead of solid phase extraction as the step of sample clean up. Appropriate dilution is a less time and money consuming way for sample preparation in comparison to solid phase extraction. Moreover, the repeatability and recovery results confirmed the suitability of this way of sample preparation.

The acidic conditions were chosen since most of the veterinary sulfonamides have at least two nitrogen-containing functional groups and most of these compounds are positively charged under acidic conditions (3). During the optimisation of sulfaguanidine in full scan MS, the most intensive ion was 215.1 – the molecular mass of sulfaguanidine and one hydrogen atom.

In summary, an analytical procedure based on a liquid chromatography coupled to mass spectrometry was presented. The procedure allowed the separation and quantification of sulfaguanidine in medicated feedingstuffs. The presented LC-MS method is fast, the sample preparation is simple, and the method has good repeatability and within-laboratory reproducibility. These parameters were less than 10%. The satisfactory results of validation proved that the method is efficient and precise. Furthermore, the method has been applied for the analysis of sulfaguanidine in real medicated feeds samples, demonstrating its usefulness for routine analyses. Therefore, this method can be applied for the official control to verify the producers’ declarations with regard to the amount of the active substances in medicated feedingstuffs and their homogeneity.

References

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