DETERMINATION OF SULFONAMIDES IN CHICKEN MEAT BY SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Matea, C.T., C. Bele , F. Dulf

University of Agricultural Science and Veterinary Medicine Cluj-Napoca , Department of Chemistry and Biochemistry , Manastur street 3-5 , 400372 Cluj-Napoca , Romania

Key words: Sulfonamides , solid-phase extraction, liquid chromatography, food analysis

Abstract. This paper describes a method for the simultaneous detection and quantification of six sulfonamides in chicken meat using normal phase cartridge clean-up and HPLC with UV detection. A liquid – liquid extraction and Sep- Pak silica clean-up procedure which minimizes the presence of halogenated solvents was used for sample preparation. The HPLC determination was performed using a RP C18 column and sulfonamides were detected at 266 nm. Mobile phase was 0.01 M ammonium acetate pH 4.6 ( A ) and methanol ( B ). Chromatographic separation was obtained by gradient elution ( 22 % B to 50 % within 17 min , back to 22 % in 2 min, equilibration for 5 min). Average recoveries of analytes from spiked meat were higher than 74 %.

INTRODUCTION

Sulfonamides are widely used in food-producing animals for therapeutic and prophylactic purposes [1]. Sulfonamides are also used as additives in animal feed because prolonged ingestion of sulfonamides may have a growth – promoting effect [2]. There is a risk of sulfonamide residues remaining in animal products if these antimicrobials have been improperly administered or if the proper withdrawal period has not been observed [3-5]. Drug residues may cause toxic or allergic reaction to consumers and promote antibiotic resistance. Sulfonamides are known for their negative effects on the thyroid gland in relation to the development of thyroid gland tumours. In addition, sulphadimidine applied to swine is suspected of potential carcinogen ( NCTR Technical Report Experiment No. 418, 1988). [6]. It has been estimated that approximately 5 % of human patients received unwanted effects from the drugs [7,8]. To safeguard human health, the European Union ( EU ) have established safe maximum residue limits ( MRLs ) at the total level of 100 µg / kg in food of animal origin such as a meat, milk and eggs [9]. Many methods such as LC and LC –MS , GC and GC-MS, ELISA, biosensor immunoassay ( BIA ) and high performance capillary electrophoresis ( HPCE ) have been developed for the determination of sulfonamides in tissues [10]. One of the most important clean-up procedure is liquid –liquid extraction ( LLE ) using hexane to remove fat [11-13]. The adjustment of the pH of the aqueous phase to the proper range is important for the extraction of the sulfonamides from an organic phase into an aqueous phase. Solid phase extraction ( SPE ) was used as clean-up or enrichment method for sulfonamides in tissues. The purpose of these additional clean-up steps is to extract the drugs selectively while leaving other interfering compounds behind. The extraction of sulfonamides from milk, eggs or meat is generally carried out with acetonitrile, ethyl acetate, acetone,
methylene chloride, or chloroform. Some of these solvents denature the sample protein, releasing any drug residues bound to proteins [14]. Some authors replaced the traditional liquid extraction step with matrix solid-phase dispersion or ultrafiltration [15-17]. The ultra-filter unit eliminates some problems associated with classical clean-up techniques (reduced recoveries or emulsion) and consequently improve clean-up yields. Matrix solid-phase dispersion (MSPD) reduces the solvent consumption and eliminates the possibility of emulsion formation. Supercritical fluid extraction has been used to the extraction of sulfonamides from meat, beef and chicken liver and eggs [18-20].

HPLC is the most important technique for the determination of sulfonamide residues in milk, meat and eggs and several methods have been published [21].

The use of gas chromatography to quantitate sulfonamides involves the derivatization of the drugs in order to prepare suitable volatile derivatives before analysis (N-methylation or N-methylation followed by acylation of the N4-primary amino function with pentafluoroalkane carboxylic anhydride). There is only one report on the analysis of sulfonamides in food products in which a GC method was established to analyse these drug residues in bovine milk [22].

In recent years, high-performance capillary electrophoresis (HPCE) has become an excellent alternative method for the identification and separation of sulfonamides using two modes: capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC). A method for quantification of 16 sulfonamide residues in pork meat samples using HPCE was described by Ackermans et al. [23].

Many ELISA methods have been established to detect sulfonamide residues in tissues due to the allergic reactions and the widespread use of this class of drug [24].

Thin-layer chromatography was also used to quantitate sulfonamides in tissues [25]. This analytical method can reduce solvent use and the cost of analysis, allowing the screening of a larger number of samples. Fluorescence detection for TLC method is performed at an excitation wavelength of 310 nm. The sample preparation for TLC includes membrane filtration and solid phase extraction.

This paper presents the quantitation of six sulfonamides (sulfadiazine, sulfapyridine, sulfamerazine, sulfisoxazole, sulfamethoxazole and sulfadimethoxine) in chicken meat using a silica normal phase SPE method combined with HPLC with UV detection.

MATERIAL AND METHOD

Materials and reagents

Chicken muscle tissues were purchased from local food markets and deep frozen until analyses. Organic solvents such as methanol, acetonitrile and 1-propanol were all pesticide residue grade, commercially available from Merck (Darmstadt, Germany). Anhydrous sodium sulfate was analytical grade (Bucarest, Romania). Deionized and redistilled water was prepared on Milli – Q Plus (Millipore, France).Sulfonamides were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All standards were stored at –20°C. Stock solutions of each sulfonamide were prepared in acetonitrile. The working solutions for liquid chromatography and sample spiking were prepared by dilution of 1 ml of each stock solution in mobile phases to concentration of 10, 5, 2.5, 1.25 and 0.5 µg ml⁻¹. All solutions were stored in the dark at 4°C.

Chromatographic conditions

All experiments were carried out with an Shimadzu VP Series liquid chromatograph equipped with a UV-VIS detector. The chromatographic analyses were performed on a Altima RP- C18 column (250x4.6 μm) with a mobile phase 0.01 M ammonium acetate pH
4.6 ( A ) and methanol ( B). Flow 1 ml/ min was used for the separation of analytes in
gradient mode at the following program: 22 % B to 50 % within 17 min, back to 22 % in 2
min, equilibration for 5 min. The injection volume was 20µl and the detection of
sulfonamides was conducted at 266 nm.

Sample preparation and sample clean-up

Ten grams of minced chicken tissue was placed into a 50 ml polypropylene tube. 15
ml acetonitrile and 5 grams baked anhydrous sodium sulfate was then added. The sample was
homogenized with a refiner for about 1 min, and then centrifuged at 5000 rpm for 10 min.
The supernatant was decanted into a separatory funnel. The residue was extracted two times
with 15 ml acetonitrile. Then 20 ml n-hexane was added into acetonitrile extracts, and each
samples was mixed and separated. The upper layer was discarded and 4 ml 1-propanol was
added. The solvent was evaporated to near dryness by a rotary evaporator at 40°C. The
residue was dissolved by ultrasonication for 30 s with 4 ml chloroform and 4 ml n-
hexane. The solution was then passed through the Sep-Pak Silica solid phase extraction
cartridge preconditioned by 5 ml n-hexane without any pressure. The analytes were eluted
with 5 ml 0.05 M phosphate buffer pH 10 at a rate of about 10 ml/ min. The pH of the eluent
was adjusted to 4.5-5 with 0.5 M ortho-phosphoric acid and the solution was filtered through
a 0.45 µm filter unit. The filtrate was analyzed by HPLC with UV detection.

RESULTS AND DISCUSSIONS

Traditionally, sulfonamides are extracted by treating the sample with an organic
solvents and clean-up in solid phase by passage through disposable cartridge [26]. The present
procedure uses for extraction no halogenated solvents, which are toxic and expensive to
dispose of. The liquid–liquid extraction using n-hexane was followed by normal phase SPE in
order to improve the purification of the sample and enhance the method’s sensitivity.

A typical chromatogram of a mixture of sulfonamide standards is shown in Figure 1a.
Typical chromatograms for blank and spiked chicken meat sample are shown in Figure 1b
and c.
The calibration curves for sulfonamides were obtained by plotting peak area versus concentrations of working standard solutions for each compound respectively. Quantitation was carried out by using the external calibration technique. The linearity of each compound measured by HPLC method was good from 0.5 to 10 µg/ml and acceptable correlation ($r^2 = 0.99$) over the range examined was determined (Table 1).

<table>
<thead>
<tr>
<th>Sulfonamides</th>
<th>Retention time (min)</th>
<th>Curve equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td>7.599</td>
<td>$Y=124093x -3603$</td>
<td>0.9999</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>8.915</td>
<td>$Y= 109393x -24470$</td>
<td>0.9997</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>9.748</td>
<td>$Y=122642x -9996$</td>
<td>0.9999</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>12.955</td>
<td>$Y= 98068x +3225$</td>
<td>0.9999</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>13.440</td>
<td>$Y= 111981x +17623$</td>
<td>0.9996</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>20.852</td>
<td>$Y= 84497x -13984$</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

The recoveries of analytes were evaluated by spiking 1µg of each standard analyte to 10.0 g tissues. The results are listed in Table 2.
Recoveries of spiked chicken meat tissue (100 µg/kg). Table 2

<table>
<thead>
<tr>
<th>Sulfonamides</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadiazine</td>
<td>78</td>
</tr>
<tr>
<td>Sulfapyridine</td>
<td>74</td>
</tr>
<tr>
<td>Sulfamerazine</td>
<td>82</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>75</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>83</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>77</td>
</tr>
</tbody>
</table>

Good recoveries ranging from 74 to 83% were determined, which indicated that this sample preparation method was suitable for the analysis of sulfonamides in chicken meat samples. The recoveries obtained in this sample treatment procedure are similar to the values found in the literature for the analysis of these compounds in chicken meat matrices.

As an application, 25 samples of commercial chicken meat purchased from local food markets were analysed using the present method. No samples contained detectable concentrations of sulfonamides. All HPLC chromatograms were free from interferences.

CONCLUSIONS

In this study, a clean-up procedure which minimizes the use of halogenated solvents and HPLC-UV method has been developed to measure the residues of six sulfonamides in chicken meat tissues. A liquid–liquid extraction and Sep-Pak silica clean-up was used for sample preparation. Average recoveries from spiked meat were higher than 74%.

BIBLIOGRAPHY