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Simultaneous analysis of four sulfonamides in chicken muscle tissue by High Performance Liquid Chromatography

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Abstract:	<p>Abstract</p> <p>The aim of this study was to develop a simple high-performance liquid chromatography (HPLC) with UV detection method, for the determination of four sulfonamides in chicken muscle tissue. The sulfonamides were extracted with acetonitrile, acetone and dichloromethane. Separation was carried out on an C18 analytical column, using as mobile phase a mixture of 6‰ di-sodium hydrogen phosphate and methanol. The analytes were detected by UV, in one run. Calibration curves were linear with very good correlation coefficients for concentration ranging from 30µg kg-1 to 150µg kg-1. The limits of detection (LOD) for sulfonamides ranged from 6.5 to 0.14 µg kg-1. The recovery for spiked chicken muscle with 50–150 µg kg-1 ranged more than 70%. The relative standard deviations (RSDs) of the sulfonamides for six measurements at 50 µg kg-1, 100 µg kg-1 and 150 µg kg-1 were less than 15%. These</p>

	<p>parameters met the EU criteria for method validation. The results were confirmed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using multiple reacting monitoring, as operating mode. Confirmation require the retention times of the analytes to be within $\pm 2.5\%$ of the retention times of the standards, presence of the parent ion and two characteristic fragment ions (product ions) per analyte, as well as the relative ion abundance ratios of the fragment ions shall correspond to ratios obtained for the standards, within permitted limits . The transition of two common product ions at m/z 155.7 and 107.5 were monitored for all sulfonamides. Each of the analytes, in all tested samples met the confirmation criteria. Thus, it was demonstrated the applicability of the HPLC–UV method for routine analysis of chicken muscle tissue.</p>

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Simultaneous analysis of four sulfonamides in chicken muscle tissue by HPLC

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Abstract

The aim of this study was to develop a simple high-performance liquid chromatography (HPLC) with UV detection method, for the determination of four sulfonamides in chicken muscle tissue. The sulfonamides were extracted with acetonitrile, acetone and dichloromethane. Separation was carried out on an C18-column, using as mobile phase a mixture of 6% di-sodium hydrogen phosphate and methanol. The analytes were detected by UV, in one run. Calibration curves were linear with very good correlation coefficients for concentration ranging from 30 $\mu\text{g kg}^{-1}$ to 150 $\mu\text{g kg}^{-1}$. The limits of detection (LOD) for sulfonamides ranged from 6.5 to 0.14 $\mu\text{g kg}^{-1}$. The recovery for spiked chicken muscle with 50–150 $\mu\text{g kg}^{-1}$ was more than 70%. The relative standard deviations (RSDs) of the sulfonamides for six measurements at 50 $\mu\text{g kg}^{-1}$, 100 $\mu\text{g kg}^{-1}$ and 150 $\mu\text{g kg}^{-1}$ were less than 15%. These parameters met the EU criteria for method validation. The results were confirmed by LC–MS/MS using multiple reacting monitoring, as operating mode. Confirmation require the retention times of the analytes to be within $\pm 2.5\%$ of the retention times of the standards, presence of the parent ion and two characteristic fragment ions (product ions) per analyte, as well as the relative ion abundance ratios of the fragment ions corresponding to ratios obtained for the standards, within permitted limits. The transition of two common product ions at m/z 155.7 and 107.5 were monitored for all sulfonamides. Each of the analytes, in all tested samples met the confirmation criteria. Thus, it was demonstrated the applicability of the HPLC-UV method for routine analysis of chicken muscle tissue.

Keywords: sulfonamides; HPLC; chicken muscle; withdrawal periods; maximum residue limits.

Introduction

Sulfadimethoxine, sulfamethoxazole, sulfaquinoxaline and sulfadiazine are the most common sulfonamides (SA) used in poultry farms. They can be easily absorbed and distributed through the body of the chicken, accumulated in various tissues and transferred into their

1 37 products (Kan and Petz, 2000; Kishda and Furusawa 2002). The recommended withdrawal
2 38 periods, if not observed before slaughtering of the medicated animals, may impact on the
3 39 safety of chicken meat and by-products.
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7 41 In order to ensure the reduction to an acceptable level of sulfonamide residues in edible
8 42 tissues, these substances must be administered only in recommended concentrations and their
9 43 withdrawal times must be observed. The maximum residue level (MRL) of sulfonamides in
10 44 poultry tissues and eggs is 100 µg kg⁻¹ (Council Regulation 2377/90-EEC, 1990; Codex
11 45 Alimentarius Commission, *CAC/MRL 02-2006*, Maximum residue limits for veterinary drugs
12 46 in foods; and Code of Federal Regulation, USA, 1996).
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20 48 Despite the efforts of national and international bodies involved in food residue control, still
21 49 there are people affected by the presence of drug residues in food. Unfortunately, about 10-
22 50 15% of the human population is considered to be hypersensitive to antimicrobials especially
23 51 penicillin and sulfonamides (Slatore, 2004; Cochrane *et al.*, 1995) and suffer from allergic
24 52 reactions like skin rashes, hives, asthma and anaphylactic shock. It has been estimated that
25 53 approximately five percent of human patients medicated with sulfonamides received
26 54 unwanted effects from the drugs (Montanaro, 1998; Korpimäki, 2004).
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34 56 The interest in having reliable methods able to detect low amounts of sulfonamides in food is
35 57 very real. Chicken meat is the second most popular meat in Romania, with a weight of about
36 58 33% of total meat consumption (average consumption in 2009 was 20.5kg/person/year),
37 59 contributing to increased population exposure to residues of sulfonamides, if this level is not
38 60 well controlled (Peligrad, 2010).
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43 61
44 62 Sulphonamide residue analysis involves extraction with an appropriate solvent followed by
45 63 one or more clean-up processes and then quantitative determination. Traditionally, the
46 64 extraction of sulphonamides from meat, has been performed with organic solvents.
47 65 Sulphonamides are not very soluble in non-polar solvents, but have good solubility in more
48 66 polar solvents. Extraction is generally carried out with chloroform, methylene chloride
49 67 (Thomas, 1998), acetone, acetonitrile, or ethyl acetate. Sample clean-up procedures include
50 68 column chromatography, solid phase extraction (SPE) (Bele *et al.*, 2007; Hela *et al.*, 2003;
51 69 Kao, *et al.*, 2001), matrix solid phase dispersion (MSPD) (Kishda and Furusawa , 2001),
52 70 supercritical fluid extraction (SPE) (Maxwell, Lightfield,1998).
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A variety of methods have been used to measure sulfonamide residue in biological materials, including thin-layer chromatography (TLC) (Babić, *et al.* 2005), high-performance liquid chromatography (HPLC) with UV, UV-DAD (Gratacós-Cubarsí, *et al.*, 2006; Furusawa and Kishida, 2001) or fluorescence detection (Gehring, *et al.*, 2006), liquid chromatography - mass spectrometry (LC/MS) (Jung, *et al.*, 2004), micellar liquid chromatography (Szyman, 2008), high-performance capillary electrophoresis (HPCE), gas chromatography (GC), along with enzyme-linked immunosorbent assay (ELISA) (Zhang, *et al.*, 2007), biosensor immunoassay (BIA) (Ploum, *et al.* 2005) and microbiological methods (Braham, *et al.*, 2001).

Instrumental methods such as LC/MS and GC are both sensitive and specific, but are laborious and expensive. These methods are suitable for confirmation but not for screening of large numbers of samples. Microbiological methods do not require highly specialized and expensive equipment, but they have not enough sensitivity and assay precision. Currently, TLC has been almost replaced by other instrumental analysis. Although HPCE has powerful separation ability, the precision is poor and the instrument still needs to be improved. Immunochemical methods such as ELISA can be simple, rapid and cost-effective, with enough sensitivity and specificity to detect small molecules (Wang *et al.*, 2005). The official method AOAC uses pre-column derivatization and liquid chromatography with fluorescence detection (Salisbury, 2004).

A rapid, sensitive and specific assay is required to detect sulphonamides positive samples in routine analysis, by liquid chromatography with ultraviolet detection (LC-UV). The goal of this paper is to promote a HPLC-UV method, which does not need to be performed on the latest generation equipment, but is able to be used for the simultaneous detection of sulfonamides residues in meat. This method could be welcomed both by the laboratories of the veterinary medicine manufacturers, where withdrawal times have to be established, and Official Control Laboratories that regularly evaluate the implementation of good veterinary practices, detect and regulate deviations in veterinary drugs usage. The authors are expecting that this method to be of real help especially for the laboratories from the EU new member states, in which expensive analytical techniques are not widely available. This multi-residue analysis was performed to simultaneously determine four sulfonamides in chicken muscle tissue: sulfadimethoxine sulfamethoxazole, sulfaquinoxaline and sulfadiazine, the most common used sulfonamides in poultry farms.

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Materials and methods

Apparatus: Cutter/mixer, electronic balance (Precision Balance, KERN Abj - Germany), centrifuge (Centra MP 4R- USA), sonicator (Sororex RK 100H - Germany), vortex (Ultraturax IKA T25 - Germany), filter unit 0.45 µm and 0.2µm (Whatman – Germany), piston – operated pipette 100 - 1000µl (Transferpette – Brand - USA).

The HPLC system consisted of a UV/VIS detector (Waters LC Module I - USA), a HPLC auto sampler (Waters model 717 Plus - USA), and two pumps (Waters model 510 and 590 - USA). The HPLC column used was Zorbax SB- C18, 5 µm 4.6 × 250 mm, 5µ (Agilent Technologies - USA).

Chemicals and reagents:

Marker residue for sulfonamides is represented by parent drugs. Standards used are: sulfadimethoxine (Sigma Chemical Co – USA), sulfamethoxazole (Sigma Chemical Co – USA), sulfaquinoxaline (EDQM), and sulfadiazine (EDQM). Reagents: Acetonitrile (Lab-Scan - Poland), methanol (Merck, Germany), n-hexane (Fluka-Switzerland), di-natriumhydrogenphosphat (Merck, Germany), dichloromethane (Chimactiv – Romania), acetone (Chimactiv - Romania) and N – dymethylformamide (Fisher Scientific-UK). All reagents were HPLC grade.

Preparation of standard solutions:

Stock standard solutions were prepared by dissolving 100 mg of each SA standard with 100 mL of N-dimethylformamide separately (1mg ml⁻¹). Mix standard solution was prepared by combining 1 mL of each stock standard solution and was made up to 50mL with 50% methanol in di-natrium hydrogen phosphate solution 6g/1000ml. Working mix standard solution at a concentration of 0.1 – 2µg ml⁻¹ was prepared by diluting the mix standard solution with mobile phase.

Tissue samples:

Blank matrix comes from previously unmedicated chicken, which were provided by the Biobase S.N. Pasteur Institute, Bucharest, Romania – Gnotobiology Laboratory and Experimental Testing for chickens and turkeys. Incurred samples come from medicated chicken, under normal farm condition, which were provided by the Biobase S.N. Pasteur Institute, Bucharest, Romania – Gnotobiology Laboratory and Experimental Testing for chickens and turkeys.

137 Six chicken of approximately 1.5kg, were treated with sulfadiazine in their water ration at
138 rate of 50mg kg⁻¹ d⁻¹, for five days. After 12 and 24 h withdrawal from medication, three
139 chickens were removed for slaughter.

140 Another group of six chicken of approximately 1.5kg, was treated with sulfadimethoxine in
141 their water ration at rate of 50mg kg⁻¹ d⁻¹, for five days. After 24 and 48h withdrawal from
142 medication, three chickens were removed for slaughter.

143 Another group six chicken of approximately 1.5kg, were treated with sulfamethoxazole in
144 their water ration at rate of 8mg kg⁻¹ d⁻¹, for four days. After 48 and 72h withdrawal from
145 medication, three chickens were removed for slaughter.

146 Another group six chicken of approximately 1.5kg, were treated with sulfaquinoxaline in
147 their water ration at rate of 50mg kg⁻¹ d⁻¹, for five days. After 48 and 72 h withdrawal from
148 medication, three chickens were removed for slaughter.

149 Muscle tissue samples were collected and stored frozen (-17°C) until they were analyzed.

150 *Sample preparation:*

151 The sulfonamide extraction was carried out using a method based on the one described by
152 Furusawa and Hanabusa (2002), Stoev and Michailova (2000) in which acetonitrile, acetone
153 and dichloromethane were utilized as organic solvents.

154 A volume of 30 ml acetonitrile was added to 10 g of minced and homogenized muscle tissue
155 weighed in a glass centrifuge tube. The sample was homogenized for 1 min in a Vortex and
156 centrifuged at 3500 rpm for 10 min. The supernatant was transferred into a pear-shaped flask.

157 Twenty milliliter of acetone was added to the sediment before the mixture was sonicated for
158 10 min. The solution was centrifuged once again and the supernatant was added into the
159 same pear-shaped flask. The mixed solution was evaporated at 40°C until near to dryness.

160 Afterwards, 5 mL of dichloromethane was added, homogenized by vortexing and transferred
161 into a test tube. The step after the addition of dichloromethane was repeated three times and
162 the combined dichloromethane was dried at 40°C. The residue was reconstituted with 1mL of
163 50% methanol in di-sodium hydrogen phosphate solution (6g /1000ml) and mixed properly
164 by vortexing. n-Hexane (2 mL) were added into the test tube for defeating, and vortexed
165 before being removed. The remaining solution was filtered through the filter of 0.2 µm or no
166 more than 0.45 µm, and was ready for injection into HPLC system.

167 *HPLC Analysis:*

168 The HPLC analyses were performed by using the following mobile phase:

169 75:25 = di-sodium hydrogen phosphate solution 6g/1000 ml: methanol (v/v), according USP
170 29 – Sulfadimethoxine Monographs, without pH adjustment.

The flow rate: 1 mL/min.
Injection volume: 20µl
Detection was performed at 245 nm, in order to achieve a greater sensitivity than in the method described by Ismail and co-workers (2008), which used 266 nm.
Run time: 10min

Calculation formula:

$$\mu\text{g kg}^{-1} \text{ sulfonamide} = C \times 100 \times 100 \times R^{-1}$$

C = measured concentration ($\mu\text{g kg}^{-1}$)
R = recovery (%)

LC-MS/MS

Identification and quantification of analytes were carried out on Waters 2695 (USA) liquid chromatography, equipped with a MS-MS Quatro Micro (Micromass, USA) tandem mass spectrophotometer. The X Bridge Shield RP18 column, 150mm x 2.1, 3.5µm, (Waters, USA) was used for separation. The flow rate was 20µl/min, and the temperature of the column was 40 °C. Mobile phase was methanol (B) and a 0.1% formic acid solution (A) following gradient

Time	A	B
0	95	5
1.5	95	5
10	60	40
15	10	90
15.5	95	5
20	95	5

MS/MS conditions: the mass spectrophotometer was operated in electrospray positive ion mode. The capillary voltage was held at 4.5KV, cone voltage, at 30V and extractor voltage, at 2V. The source temperature was 100° C, desolvation gas (nitrogen) temperature, 350 °C and flow: 350l/h. Collision gas (argon) pressure: 3x10⁻³mbar. Data acquisition was made in multiple reaction monitoring mode. The transition of two common product ions were monitored.

200 *Ions used for LC/MS-MS confirmation:*

Sulfonamide types	Parent ion m/z	Product ions m/z	Collision energy(eV)	Retention time (min)	Relative ion intensities %
Sulfadiazine	251,2	155.7* 107.5	16	7.64	18.6
Sulfamethoxazole	245.2	155.7* 107.5	18	9.94	19.5
Sulfadimethoxine	311.2	155.7* 107.5	18	13.05	7.9
Sulfaquinoxaline	301.2	155.7* 107.5	16	14.5	7.1

201 * Quantifying ions

203 Results and discussion

204 Under the condition of HPLC-UV analyses, sulfonamides eluted in the following order with
 205 approximate retention times in minutes: sulfadiazine 2,7; sulfamethoxazole 3,2;
 206 sulfadimethoxine 4.0; sulfaquinoxaline 6,8. The validation data of HPLC-UV method are
 207 presented in the Table 1 and are discussed below:

209 Validation of analytical procedure

210 The proposed analytical method was validated according to the following criteria: specificity,
 211 accuracy, precision, limit of detection, limit of quantification, and linearity, according to
 212 EMEA - "Notice to applicant and Guideline – Veterinary Medicinal Products (vol. 8) ",and
 213 Commission Decision (EEC) No. 657/2002.

215 Sample preparation

216 As for many antibiotics, selective extraction of sulfonamides from biological tissues is
 217 complicated due to the polar character of the analytes and matrix components (Stolker, et.,al,
 218 2005). Sulfonamides have a good solubility in more polar solvents. Acetonitrile was chosen
 219 to extract these compounds because of its polar character and its good proprieties to
 220 denaturing the sample proteins, which results in a cleaner extraction and a better release of
 221 any sulfonamides residues bound to proteins. Acetone is also a polar solvent that leads to
 222 depletion of sample. Dichloromethane is less polar than acetonitrile and acetone, separating
 223 the compound of interest from the initial extract. n-Hexane was used to obtain defatted
 224 extract. During the extraction it was important to respect the proportion between solvents.

1 225 Specificity and recovery results were in acceptable limits, which show the extraction
2 226 efficiency and a good removal of matrix interference. The detection limit was also
3 227 comparable with the other reported method.
4 228
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6
7 229 *Calibration curves* of each component were established by plotting the peak area of each
8
9 230 active component against its associated concentrations. The concentration used for each drug
10 231 ranged from 30 $\mu\text{g kg}^{-1}$ to 150 $\mu\text{g kg}^{-1}$ (0.5MRL – 1.5MRL), at five levels, according to the
11 232 recommendation made by the Commission Decision No. 657/2002.
12
13
14 233 A statistic linear regression was performed for each component. For the tested domain,
15 234 calibration curves were linear with very good correlation coefficients ($R^2 > 0.99$).
16
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18 235
19
20 236 *Specificity* was study by analyzing blank samples and spiked samples at relevant
21 237 concentration (0.5, 1, 1.5 x MRL) and checking any interferences in the region of interest.
22
23 238 The peak of each analite width at half-maximum height was within the 90-110% range of the
24 239 standard width, and the retention times were identical within a margin of 5 %. No interfering
25 240 peaks from endogenous compounds were found in the retention time of the target
26 241 sulfonamides.
27
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29 242
30
31
32 243 *Repeatability:* 3 test samples were spiked at analyte levels, which encompass 0.5x and 2x the
33 244 MRL. Then, 6 test portions of each level were taken, analyzed and the residue concentration
34 245 of each test portion was determined. At each spiked concentration, the CV ($S_{\text{rel}} = S \times 100 /$
35 246 Mean) was calculated. The relative standard deviations (RSDs) of the sulfonamides for six
36 247 measurements at 50 $\mu\text{g kg}^{-1}$, 100 $\mu\text{g kg}^{-1}$ and 150 $\mu\text{g kg}^{-1}$ were from 7.8 to 13.5%. These
37 248 values are within the criteria stipulated for residue analysis by the Commission Decision No.
38 249 657/2002: RSD <20%.
39
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41 250
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43
44 251 *The accuracy (recovery)* was determined by recovery experiments using blank matrices.
45
46 252 - 18 aliquots of a blank material was spiked (six aliquots) at each of 0.5, 1 and 1.5 x MRL
47 253 - the samples were analyzed and the sulfonamides concentration present in each sample was
48 254 calculated;
49
50 255 - using the equation below, the recovery for each sample was calculated.
51
52 256 % Recovery (R) = $100 \times \text{measured content} / \text{spiked level}$
53
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55 257 - it was calculated the mean recovery and CV from the six results at each level.
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A recovery between 70 – 110 % in the spike range of 0.5MRL- 1.5MRL, is acceptable by EU regulations. The recovery for spiked chicken muscle with 50–150 $\mu\text{g kg}^{-1}$ ranged from 70 to 84 %.

Response linearity:

- 6 test sample were spiked at level of: 0.5, 0.75, 1, 1.25, 1.5, 1.75 $\mu\text{g ml}^{-1}$
- the samples were analyzed and the sulfonamides concentration present in each sample was calculated;
- linearity curves of each component were established by plotting the measured concentration of each active component against its fortification level of concentrations.

Correlation coefficients for the analytes show an acceptable linearity of the analytical response, across the range of tested concentrations.

The limits of detection and quantification

In the Commission Decision 2002/657/EC, the decision limit ($\text{CC}\alpha$) and detection capability ($\text{CC}\beta$) are intended to replace the LOD and LOQ, as method characteristics. On the other hand, in EMEA- *Notice to applicants and Guideline - Veterinary medicinal products, Volume 8: Establishment residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin*, LOD and LOQ are still required, and furthermore, the limit of quantification must be validated at least at 0.5 MRL. This Guidance recommended as one possible way to estimate the detection limit, the following: arithmetic mean of the analytes concentration, in at list 20 blank samples, plus three time standard deviation. In *Guidance for the validation of analytical method in depletion studies* - VICH 49 (2009), LOD is estimated in the same mode like in *EMEA Guidance*, and LOQ is estimated as three time LOD. Another possibility to calculate LOD and LOQ is using the linearity curves.

In respect with all these regulation, the detection limit of HPLC-UV method was calculated as arithmetic mean of the analytes concentration in 20 blank samples plus three time standard deviation and LOQ was estimated as three times LOD.

An LOD for sulfonamides in LC-UV detection method is acceptable in general on values $\leq 10\mu\text{g kg}^{-1}$ (Stolker, et.,al, 2005), and the values obtained in this validation were lower.

290 The limits of quantification (LOQ) for the three sulfonamides were in the range of 0.4-1.7 μg
291 kg^{-1} . Sulfaquinoxaline LOQ is 19.6 $\mu\text{g kg}^{-1}$, still below 0.5 MRLs, and for all analytes LOQ
292 was significant lower then $\text{CC}\beta$, reducing the false negative result rate.
293
294 *Decision limit ($\text{CC}\alpha$) and detection capability ($\text{CC}\beta$)*
295 In this validation $\text{CC}\alpha$ and $\text{CC}\beta$ was established considering the permitted limit, in that case
296 MRL, in respect with Commission decision 2002/657/EC, by analyzing 20 blank samples
297 fortified with the analytes at the permitted limit, and calculating the decision limit ($\text{CC}\alpha$) as
298 arithmetic mean of analyte concentration at MRL level plus 1.64 times the standard deviation
299 of repeatability ($\alpha = 5\%$), and detection capability ($\text{CC}\beta$), as arithmetic mean of analyte
300 concentration at $\text{CC}\alpha$ level plus 1.64 times the standard deviation of repeatability ($\beta = 5\%$)
301 Decision limits ($\text{CC}\alpha$) and detection capabilities ($\text{CC}\beta$) were in the range of 104.64 – 112.95
302 $\mu\text{g kg}^{-1}$ and 108.5 - 125 $\mu\text{g kg}^{-1}$, respectively.
303
304 *LC-MS/MS confirmation*
305 A method for measuring residue level by liquid chromatography–tandem mass spectrometry
306 (LC-MS/MS) was applied, using the same extraction procedure, as confirmatory method.
307 The confirmatory analysis is based on MS-MS monitoring of two product ion and a
308 precursor ion, which provide four identification points (IPs). For sulfonamides, listed in
309 Group B (Council Directive 96/23/EC), a minimum three IPs are required. Relative retention
310 time of analytes was within the tolerance of $\pm 2.5\%$. Relative ion intensities for fortified
311 samples correspond to those of the calibration standards, in the permitted tolerance.
312
313 The residue was reconstituted with 1mL of 50% methanol in 0.1% formic acid solution. For
314 determination, analytes were separated by a different gradient LC procedure, ionized by
315 electrospray ionization (ESI), and detected by MS-MS with a triple quadrupole mass
316 spectrometer.
317
318 MS parameters were optimized by direct flow infusion of each standard. The calibration
319 curves show a good linearity in the concentration range of 0.5 – 10 $\mu\text{g/kg}$, for each analyte,
320 with correlation coefficients between 0.9988 – 0.9996. The chromatograms of a spiked
321 sample contain 10 $\mu\text{g/kg}$ of each compound is shown in Figure 1.
322
323 The LC-MS/MS method was used for verify the identity of marker residue and to ensure the

absence of false positives, in fortified tissues, incurred samples and real sample.

Analysis of real samples

The result obtains from the HPLC analyses of incurred samples are given in Table nr. 2.

The HPLC assay result showed all these animals tissues to contain sulfonamides at level above MRL. Thirty samples of chicken muscles collected from a local market in Romania, were investigated for sulfonamides residues used HPLC method. Twelve muscle samples was found to contain sulfadiazine with concentration level ranging from 300 µg/kg to 180 µg/kg, the level of which exceeded the regulated tolerance.

Conclusion

A multi-residue method has been developed for the simultaneous determination of sulfadiazine, sulfamethoxazole, sulfadimethoxine and sulfaquinoxaline in chicken muscle tissue by HPLC-UV, after extraction with organic solvents (acetonitrile, acetone, dichloromethane). The method is simple, rapid, sensitive, and capable of detecting sulfonamides residues below the maximum residue limits (MRL).

Criteria of validation: specificity, accuracy, precision, decision limit, detection capability, and linearity, according to the European Commission Decision 2002/657/EC, show that the method can detect different kinds of sulfonamides within one run, without fluorimetric derivatization of the analytes.

Compared to other methods, this one is easy to use for on routine samples, in laboratories that are equipped with HPLC-UV. The method takes just 10 min to be performed, without extraction time, which is 60 min. The HPLC results were confirmed by LC-MS/MS, demonstrating the usefulness of HPLC technique as rapid and specific method.

The proposed HPLC method is very suitable for determination of withdrawal periods in chicken muscle tissue for any medicinal product which contains any of the sulfonamides studied in this group, or for monitoring a large number of samples in order to observe that the recommended withdrawal period is followed. It can be really useful for checking whether Good Veterinary Practices are in place in poultry farms. The results of this investigation could be a reference for authorities to further monitor the residue of veterinary drugs in chicken products and reinforce the administration of veterinary drug users.

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Table 1: Validation parameter of HPLC-UV method for target sulfonamides residue

Targhet residues	RT min	LOD $\mu\text{g kg}^{-1}$	LOQ $\mu\text{g kg}^{-1}$	Mean RSD%	Mean Recovery %	CC α $\mu\text{g kg}^{-1}$	CC β $\mu\text{g kg}^{-1}$	Linearity R^2
Sulfadiazine	2.7	0.14	0.42	11	84	104	108	0.9958
Sulfadimethoxine	3.2	0.36	1.08	13	77	109	121	0.9834
Sulfamethoxazole	4.0	0.58	1.68	9	70	111	119	0.9913
Sulfaquinoxaline	6.8	6.53	19.6	8	71	113	125	0.9800

Table 2: Result for incurred samples

Sulfonamide types	Withdrawal time (h)	HPLC assay ($\mu\text{g kg}^{-1}$)
Sulfadiazine	12	305
		257
		312
	24	124
		97
		184
Sulfaquinoxaline	48	547
		438
		470
	72	210
		185
		162
Sulfadimethoxine	24	328
		456
		412
	48	115
		205
		156
Sulfamethoxazole	48	465
		305
		375
	72	157
		186
		255

Figure caption

Figure 1. The chromatograms of spiked samples contain 0.1 µg/ml (10 µg/kg) of each compound. The extracted ions are from top to bottom: sulfadiazine m/z 251.2 →155.7, 251.2 →107.5, Rt =7.64; sulfamethoxazole m/z 254.2 → 155.7, 254.2 → 107.5 , Rt =9.94; sulfadimethoxine m/z 311.2 → 155.7; 311.2 → 107.5 Rt =13.05 and sulfaquinoxaline m/z 301.2 → 155.7, 301.2 → 107.5, Rt = 14.50.

Figure1

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