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Confirmatory Method for Nine Sulfonamides in Miniature Bovine Muscle Samples Using HPLC/MS/MS without Using SPE

C. NEBOT*, P. REGAL, B. MARTÍNEZ, J. MIRANDA, A. CEPEDA AND C. FENTE

Departamento de Nutrición y Bromatología, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo 27002, Spain

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ABSTRACT

A new confirmatory method for the analysis of nine sulfonamides (sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamethoxazole, sulfapyridine, sulfachlorpyridazine, sulfamethazine, sulfamethoxine and sufaquinoxaline in small bovine muscle samples (0.1 g) is presented. The method, based on HPLC/MS/MS, identifies and quantifies the sulfonamides in bovine muscle at concentrations below the maximum residue limits (100 ng/g), established by the European Commission and by the Unites States Food and Drug Administration. The extraction protocol is rapid and easy, and does not require the use of solid phase extraction (SPE) cartridges. Validation was conducted according to the Commission Decision 2002/657/EC with fortified bovine samples and employing sulfadoxine-d_3 as internal standard. The linearity of the method was observed at the concentration range between 25 and 250 ng/g. Intra-day and inter-day recoveries obtained in the validation of the method were above 88 % for all sulfonamides.

Applicability of the method was investigated in bovine muscle samples belonging to a regional control program and results were compared with those obtained with an HPLC/DAD method accredited by ENAC (National Spanish Entity for Accreditation).

Key words: sulfonamides, HPLC/MS/MS, SPE, muscle

INTRODUCTION

Since the 1950s many classes of antimicrobials have been widely used for preventing and treating several diseases and promoting growth of food-producing animals. In some cases, recommendations for drugs withdrawal times are not respected, and risk of detecting antimicrobials in food increases. Residues of antimicrobials in food can provoke allergic reactions and antibiotic-resistance bacteria that can be transferred from food to humans[1].

Like any other antimicrobial, sulfonamides are antibiotics widely used in veterinary medicine to treat bacterial infections in livestock, poultry and in farmed fin-fish such as salmon[2]. Additionally to their therapeutic use, sulfonamides are employed for metaphylactic, prophylactic and growth promotion purposes[3-5]. The presence of these antibiotics in food, regardless of their amounts, can trigger potential adverse effects due to the possibility of developing antibiotic resistance[4].

For all the above, the EU established the Council Regulation 2377/90/EC[6] in 1990. This regulation indicates the maximum residue limits (MRL) of veterinary medicines permitted in foodstuff of animal origin. The regulation sets MRL of 100 μg/kg for sulfonamides in muscle, fat, kidney, liver and milk.

There is also the Commission Decision 2002/657/CE[7] which establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories. This regulation states that "methods based on chromatography analysis coupled with tandem mass spectrometry are suitable for use as confirmatory methods". Therefore, GC/MS and HPLC/MS are suitable techniques for confirmatory analysis of veterinary medicines in food of animal origin.

Many HPLC/DAD methods have been reported for the analysis of sufonamides from different matrices such as animal tissue[8,9], muscle[10], milk[11,12].

Nowadays, HPLC/DAD is being replaced by HPLC/MS/MS because of its sensitivity and selectivity. HPLC/MS/MS technique has already been applied for sulfonamides analysis in porcine meat, kidney and liver[13], honey[14], salmon[15], milk[16-18] and wastewater[19]. Even if these methods have the advantage of using HPLC/MS/

* Author for correspondence. Tel.: +34-982-285900; Fax: +34-982-254592; E-mail: Carolina.nebot@usc.es

MS, the problem is the extraction procedure which is laborious and time-consuming.

Available HPLC/MS/MS methods for the analysis of sulfonamides use big sample size (2 and 10 g) and consequently solid phase extraction (SPE) with cartridges[13,14] or similar extraction material, such as stir bar sorptive[18], needs to be employed.

The aim of this work is to describe a simple and rapid method for the confirmation and quantification of nine sulfonamides (Figure 1) in very small amounts of bovine muscle samples (100 mg). The method employs a single step extraction protocol and does not require the use of SPE cartridges or similar materials. Sulfonamides were detected and quantified at concentrations below MRL levels (100 ng/g) with only 100 mg of sample. The method was also validated according to Commission Decision 2002/657/EC[7].

MATERIALS AND METHODS

I. *Reagents*

Sulfonamides (sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfamethoxazole, sulfapyridine, sulfachlorpyridazine, sulfamethazine, sulfamethoxine and sufaquinoxalina and sulfadoxine-d_3 (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and all were of a purity > 96%; acetonitrile, methanol, dichloromethane and ethyl acetate of HPLC grade were from Scharlau Chemie (Sentmenat, Barcelona, Spain) and formic acid (98%) from Acros Organics (Geel, Belgium). Purified water was made in-house with a Milli-Q water system (Millipore, Bedford, MA, USA).

II. *Instrumentation*

The HPLC system consisted of a quaternary pump, degasser and auto-sampler model 1100 from Agilent Technologies (Waldbronn, Alemania). The HPLC was connected to a mass spectrometer (MS) Qtrap 2000™ from Applied Biosystems, MSD Sciex (Toronto, Canada) which integrates a TurboIonSpray® for molecules ionization. Data acquisition and control were carried out using Analyst 1.4.1 software package (MDS SCIEX). Gas nitrogen was supplied by a nitrogen generator (Peak Scientific Instruments Ltd., Chicago, IL, USA). Nitrogen was employed as curtain gas; nebulizer and collision gas on the MS. Nitrogen was also employed for extracts evaporation on a turbo-evaporator (Turbo Vap® II from Zyrmark, Hopkinton, MA, USA). The HPLC column employed was a Synergy 4 µ Polar-RP 80A (50 mm × 2.00 mm) used in conjunction with a security guard cartridge (4.0 mm × 2.0 mm) Polar-RP both from Phenomenex (Macclesfield, UK). The centrifuge was a 5415D from Eppendorf (Hamburg, Germany).

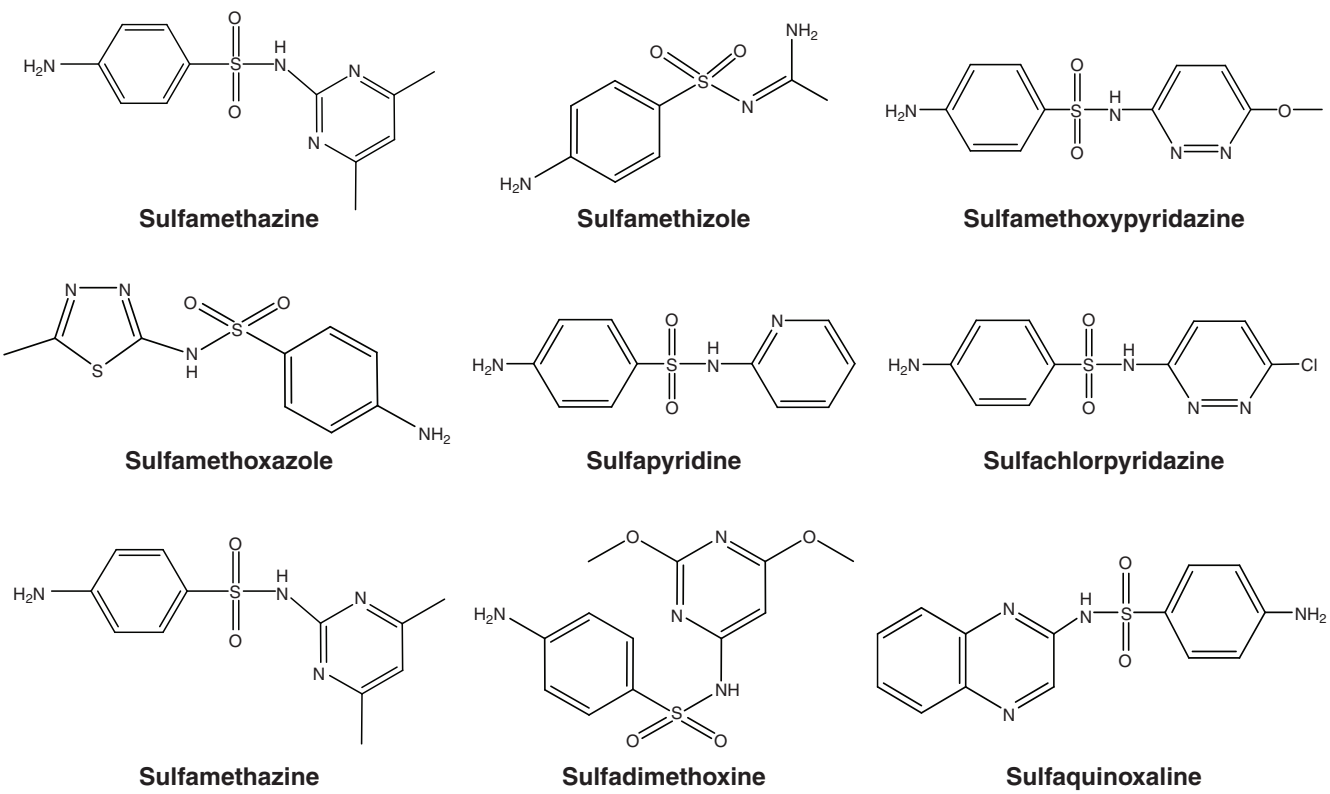

Figure 1. Chemical structures of sulfonamides

III. *Standard Solutions*

Stock solutions of individual sulfonamides were prepared by dissolving 10 mg of the compound in 4 mL of methanol (2.5 mg/mL). One hundred miroliters of each stock solution was then transferred into a 25-mL volumetric flask (10 μg/mL) and the volume made up with methanol. This stock solution was further diluted with 0.1% formic acid in methanol to obtain working standard solutions of 1 μg/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 75 ng/mL, 50 ng/mL, 25 ng/mL, and 10 ng/mL. All standards were stored in the dark at -18°C. Ten milligrams of sulfadoxine-d_3 was diluted into 50 mL of methanol (200 μg/mL). This solution was then diluted to a final concentration of 1 μg/mL and was used to spiked samples with the internal standard (IS).

Table 1. Mobile phase gradient

Time*	Acetonitrile with 0.1% formic acid (%)	Water with 0.1% formic acid (%)
0	2	98
2	2	98
6	30	70
9	35	60
14	45	55
17	60	40
18	75	15
19	100	0
21	30	70
22	2	98
30	2	98

*Flow rate was 0.2 mL/min.

IV. *Sample Preparation and Extraction*

Homogenized bovine muscle sample (100 mg) was mixed with 1.5 mL of dichloromethane and 10 μL of IS solution. The mixture was vortex, sonicated (10 min) and centrifuged at 3500 rpm (10 min), the organic phase was then transferred into a 10-mL Pyrex® glass conical tube. The extraction procedure was repeated with additional 1.5 mL of dichloromethane added to the muscle sample. The two extracts were mixed and evaporated to dryness at about 40°C. The volume was then made up to 0.1 mL with 0.1% formic acid in methanol and vortex. Final extracts were transferred directly into amber autosampler vials (2 mL, containing 0.2-mL insert vials) and stored at -18°C prior to analysis by HPLC-MS/MS.

The analysis of real samples was conducted simultaneously with four types of control samples: blank sample (bovine muscle known to be negative), fortified samples (bovine muscle spiked to a known concentration of sulfonamides), blank of reagent (only reagents, no muscle), fortified reagents (reagents spiked to a known concentration of sulfonamides).

V. *HPLC/MS/MS Analysis*

Two mobile phases (water and acetonitrile, both with 0.1% formic acid) mixed on a gradient mode (Table 1) were used for the separation of sulfonamides.

Selected sulfonamides were identified by their retention times (Rt) and four selected reaction monitoring (SRM) summarized in Table 2. Quantification was conducted with the SRM transition which gave the most intense signal to noise ratio. The MS parameters employed which were constant during the whole run were: Vacuum Gauge: 2.2

Table 2. Rt, precursor ion and product ions employed to identify nine sulfonmaides

Sulfonmaides	Rt (min)	Precursor ion *(m/z)*	Product ion 1* *(m/z)*	Product ion 2 *(m/z)*	Product ion 3 *(m/z)*	Product ion 4 *(m/z)*
Sulfadiazine	10.2	251.1	92.2	156.0	108.2	96.1
Sulfamethizole	11.7	271.1	156.2	92.2	108.1	116.0
Sulfamethoxypyridazine	11.7	281.0	156.2	108.1	92.1	112.1
Sulfamethoxazole	12.9	254.1	92.1	156.0	108.1	93.0
Sulfapyridine	10.3	250.1	92.1	156.1	108.2	184.2
Sulfachlorpyridazine	12.4	285.0	156.1	92.2	108.0	
Sulfamethazine	11.2	279.0	186.1	92.1	124.1	108.1
Sulfadimethoxine	13.8	311.1	156.1	92.2	108.2	245.1
Sulfaquinoxaline	14.0	301.1	156.1	92.1	108.1	146.1

*Quantification was performed with SRM between precursor ion and product ion 1.

atm; source temperature: 400°C; Ion spray 5500 V; curtain gas ; 25 psi; Ion source 1: 55 psi; Ion source 2: 50 psi. A dwell time of 20 ms was set between transitions of the ions.

VI. *Extraction Procedure Optimization*

Three replicates of bovine muscle samples fortified

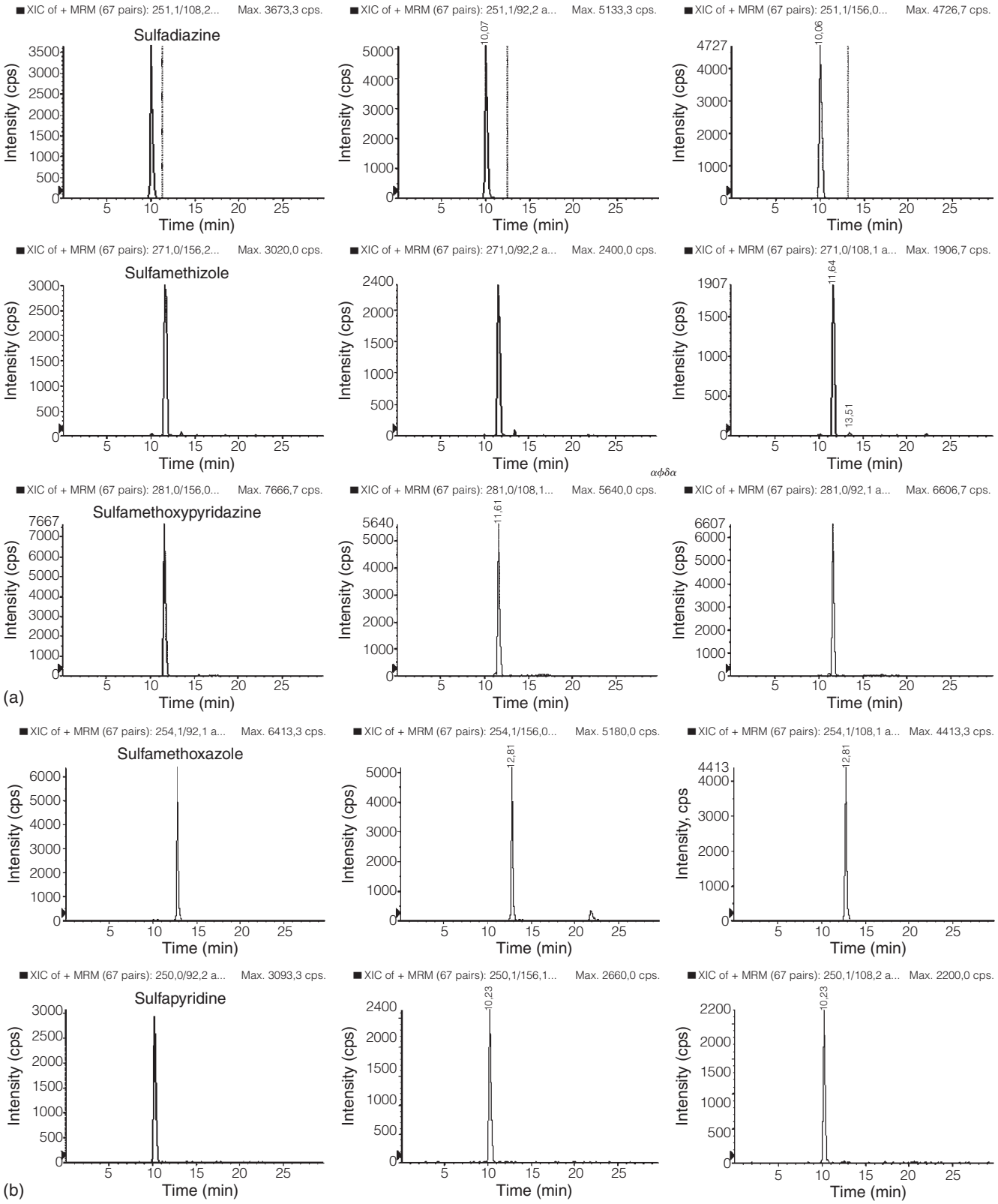

Figure 2. SRM chromatograms selected for sulfonamides identifiaction: a) sulfadiazine, sulfamethizole and sulfamethoxypyridazine; b) sulfamethoxazole, sulfapyridine and sulfamethazine; c) sulfachlorpyridazine, sulfadimethoxine and sulfaquinoxaline.

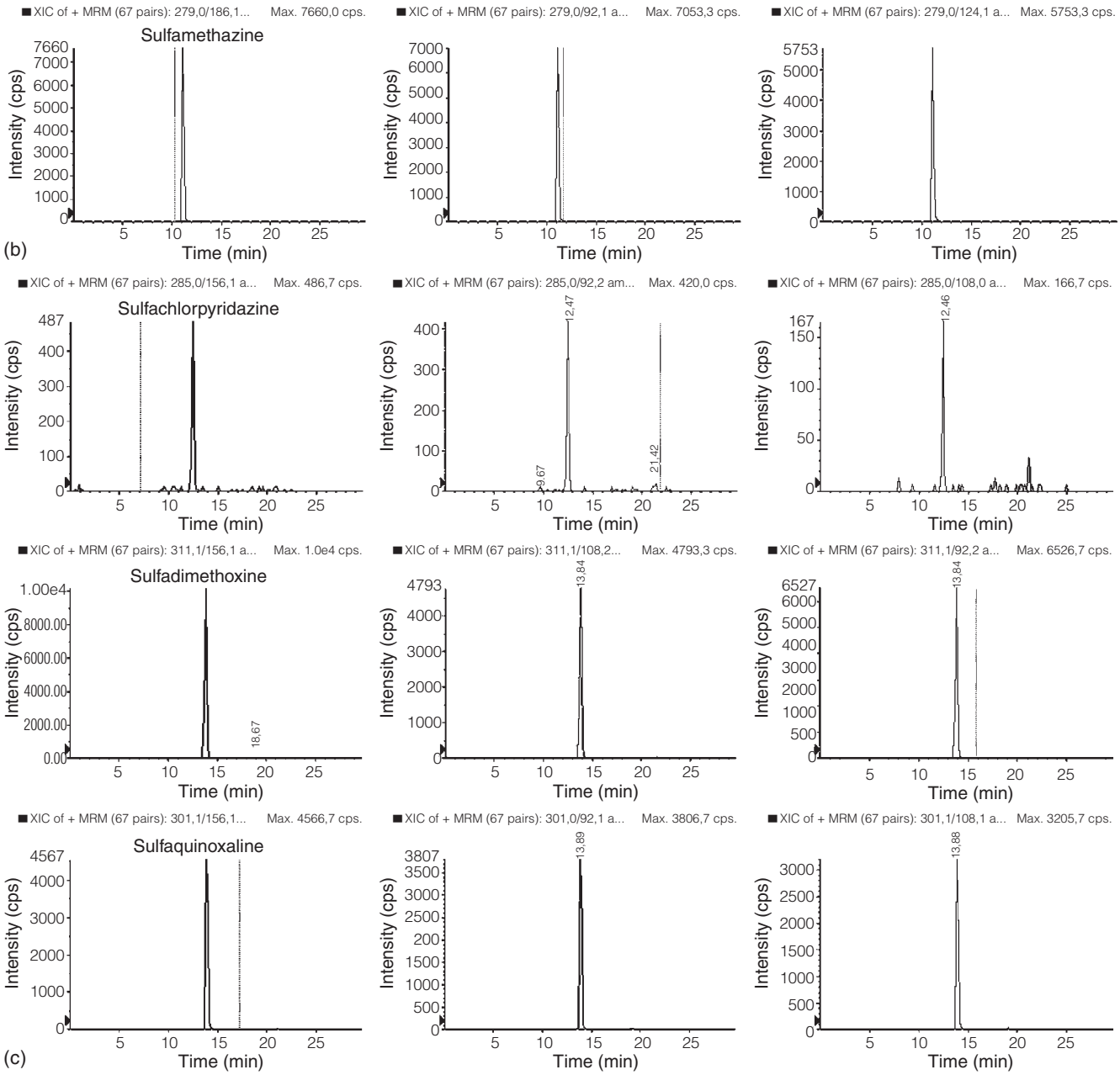

Figure 2. Continued

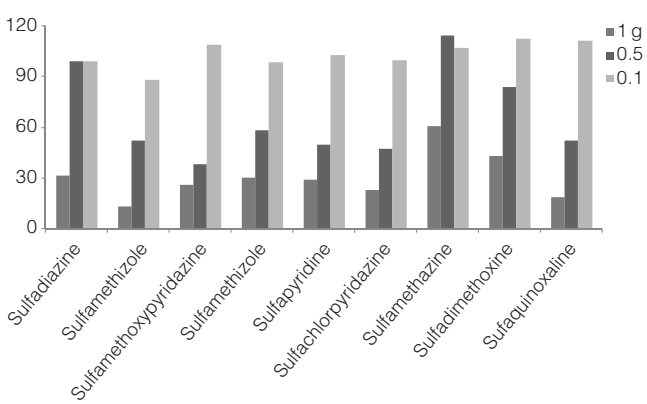

Figure 3. Recoveries of sulfonamides from 1, 0.5 and 0.1 g of bovine muscle samples.

with sulfonamides at different concentration (50, 100 and 150 ng/g) were extracted with 1 mL of acetonitrile, methanol, dichloromethane, ethyl acetate and these organic solvents with 0.1, 0.2, 0.5 and 1% formic acid. The best recoveries were achieved with dichloromethane acidified with 0.1% formic acid (results have not been included).

Different conditions were also tested for the re-dilution of the final extract after evaporation; tested conditions included acetonitrile: water (50: 50, v/v) with 0.1% formic acid, methanol: water (50: 50, v/v) with 0.1% formic acid, acetonitrile: methanol (50: 50, v/v), acetonitrile on its own and methanol on its own (results not included). The best peak shapes and chromatographic separation were achieved with methanol with 0.1%

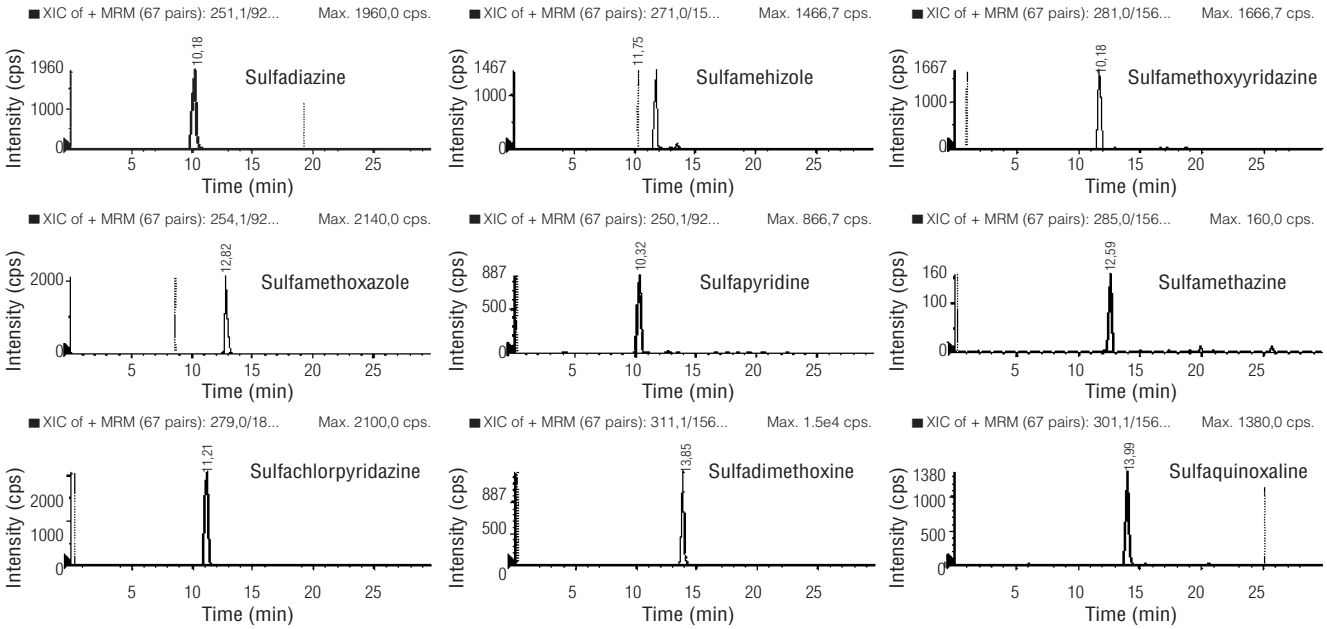

Figure 4. SRM transition of sulfonamides in a fortified sample with 50 ng/g of sulfonamides.

formic acid and not with acetonitrile with 0.1% formic acid, as it was expected. Figure 4 shows selected MRM chromatograms for each of the sulfonamides in a muscle sample spiked to a concentration of 100 ng/mL in each sulfonamide.

Three different sample sizes were tested (1, 0.5 and 0.1 g) to compare the matrix effects on the recoveries and signal response. Six replicates samples were used for each size and the protocol described above was used for the different sample sizes tested.

VII. *Validation*

The method was validated according to the criteria of the European Commission Decision 2002/657/EC[7] using the program ResVal version 2.2 obtained from the Community Reference Laboratory CRL (Bilthoven, Netherlands). The following solutions were used for instruments calibration curves: blank (methanol with 0.1% formic acid), 25, 50, 75, 100, 150, 200, 250, 500 ng/mL. Fortified samples employed to build the samples calibration curves were prepared as follows: twelve pork muscle samples (10 g) were homogenized and spiked with the analytes to the final concentration of 25, 50, 75, 100, 250 ng/g. From each sample, six subsamples of 100 mg were obtained and transferred into a 2-mL eppendorf to perform the extraction protocol. Samples of bovine's muscle (100 mg) negative in sulfonamides were processed with the fortified samples and the procedure was repeated during three consecutive days. On the fourth day, 10 blank samples (100 mg of negative bovine's muscle from different animals) and 10 fortified samples (100 mg of bovine's muscle spiked with sulfonamides to a final concentration of 50 ng/g) were processed. Sulfonamides were extracted and analysed as described above.

This procedure was carried out to validate the method in terms of selectivity, specificity, linearity, accuracy, repeatability (interday and intraday), decision limit (CCα) and detection capability (CCβ). CCα and CCβ are intended to replace the following method characteristics: limit of detection (LOD) and limit of quantification (LOQ)[20]. Even if for sulfonamides there are MRL levels it was decided to treat them as drugs without MRL. Therefore, CCα is defined as "the concentration at and above it can be concluded with an error probability of 1% that a sample is non-compliant (positive)"[7]. CCβ is defined as "the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of 95%"[10].

VIII. *Real Samples*

The laboratory takes part in a quality control program of a regional brand where bovine samples (muscle, kidney, liver, etc.) are analyzed for the presence of different veterinary medicines such as sulfonamides, clenbuterol and tetraciclines. Some of the non-compliant and compliant samples belonging to this control program were used to investigate the applicability of the method presented.

IX. *HPLC/DAD Method Accredited by ENAC*

To 10 g of bovine muscle, 2 mL of 50 mM sodium phosphate was added and vortex. The pH of the mixture

was adjusted to 3.5 with phosphoric acid and 25 mL of dichloromethane was added. After sonication for 10 min at temperature < 40ºC, the sample was centrifuged at 2500 rpm for 15 min. The organic phase was collected and filtered and a second extraction was conducted with additional 25 mL of dichloromethane. To the mixture, 25 mL of benzene was added.

After loading the whole extract into Spe-Pak® cartridges (Waters, USA), sulfonamides were eluted form the cartridges with 4 mL of methanol, evaporated to dryness and re-dissolved into 0.3 mL of 10 mM NH_4Ac.

The final extract (50 µL) was injected into a X-Terra® C18 hybrid silica column (250 mm × 4.6 mm, 5 µm) from Waters (USA) with a guard column (4.0 × 4.6 mm, 5 µm) of the same stationary phase. Two mobile phases were employed: acetonitrile (phase A) and 10 mM NH_4Ac (phase B) combined on a gradient mode. The flow was held at 1 mL/min throughout the 20 min run. The gradient elution was as follows: 0-4 min, 90% A; 4-6 min, 75% A; 6-15 min, 50% A; 15-20 min, 90% A. The spectrums monitored were from 200 to 400 nm and the signal selected 266 nm.

RESULTS AND DISCUSSION

The aim of this work was to develop and validate a simple and rapid method for the detection of sulfonamides below MRL levels, in miniature bovine's muscle samples (100 mg) without using SPE.

I. *HPLC/MS/MS Determination and Quantification*

For the detection of each sulfonamide by the MS, standard solution of individual compounds (1000 ng/mL in 0.1% formic acid in methanol) was infused directly into the MS. The objective was to select representative ions (precursor and product ions) and to tune the MS to optimise the detection of the sulfonamides. The main problem found was the ions formed by the sulfonamides when they fragment as three ions are normally generated by sulfonamides: $[M\text{-}RNH_2]^+$ 196 (*m/z* 156), $[M\text{-}RNH_2\text{-}SO]^+$ (*m/z* 108) and $[M\text{-}RNH_2\text{-}SO_2]^+$ 197 (*m/z* 92). Therefore, for a reliable identification of each sulfonamide, it was decided to use four SRM transitions.

Based on previous publication on sulfonamides analysis, several gradient profiles were tested in order to elute all the sulfonamides in the same run and with Gaussian peak shape. Separation was achieved starting with 98% water with 0.1% of formic acid and 2% of acetonitrile both with 0.1% of formic acid (Table 1). Even if sulfonamides eluted at about 65% of acetonitrile, the percent of acetonitrile was increased to 100% to clean the HPLC column of other organic molecules.

Even if Rt and precursor ion of each sulfonamide were different (Table 2), for a reliable identification, it was decided to use four MRM transitions (three for sulfachlorpyridazine). Consequently, even if the Rt of the peaks slightly moved (due to matrix effect) confirmation of each sulfonamide could still be conducted. Figure 2 shows three of the four SRM transitions selected, SRM which gave the higher signal to noise ratio and employed for the correct sulfonamide identification.

II. *Sample Extraction Procedure*

Available analytical techniques for the detection of sulfonamides in muscle samples consisted in laborious extraction procedure as bovine muscle is a complex matrix. However, if small amounts of samples are employed the amount of interferences should be reduced and extraction procedure could be simplified.

The development of new analytical techniques such as GC/MS/MS and HPLC/MS/MS make possible the analysis of complex matrix. However, if large amounts of samples are employed again, laborious extraction processes are required. To date, the protocols for the analysis of sulfonamides in muscle samples have to be performed on large amounts of samples, weight range between 1 and 10 g[8,13,21,22]. In this study, the use of small sample size was investigated to reduce interference and its applicability in sulfonamides analysis by HPLC/M/MS.

Available methods for analysis of sulfonamides from muscle samples generally employ two steps for the extraction of the analytes. The first step consists of simple extraction with water[23] or an organic solvent such as acetonitrile[8,13,22], ethyl acetate[10] and dichloromethane[15], and the second step involve extraction with SPE cartridges[8,10,24] or solid-phase micro extraction[23].

The extraction of sulfonamides with 0.1% formic acid in dichloromethane and from different sample sizes (1, 0.5 and 0.1 g) were investigated. Recoveries were calculated by comparing the amount of sulfonamides measured in the fortified samples, calculated with the use of the instruments calibration curves, and the amount of sulfonamides spiked in the samples.

The best recoveries were achieved for 100 mg of sample (Figure 3). Purification of the extracts were not conducted to reduce the sample procedure time, therefore it was expected to observe less matrix effect in 100 mg of sample.

III. *Method Validation*

The software Resval version 2.2 was employed to calculate automatically all the parameters related to the validation.

Instruments calibration curves were built by representing the ratio (analyte peak area / IS peak area) against the correspondent concentration of the pharmaceutical in pork muscle samples expressed in ng/g. Instruments calibration curves were used to calculate recovered concentrations of pharmaceuticals.

Table 3. Validation results of the method developed

Sulfonmaides	Instrumental calibration	Fortified samples	Intra-day[a]		Inter-day[b]		Critical levels (ng/g)		Measurment of uncertainty (%)	Recoveries percentage reported (Reference)
	Linearity (r^2)	Linearity (r^2)	Mean recovery (%)	CV (%)	Mean recovery (%)	CV (%)	CCα	CCβ		
Sulfadiazine	0.992	0.992	99.2	5.8	105.8	9.5	4.3	7.2	20.4	84 (2); 92 (1); 98 (3)
Sulfamethizole	0.996	0.988	88.0	9.7	106.6	8.6	5.9	10.2	26.1	86 (1)
Sulfamethazine	0.984	0.988	108.8	6.5	114.7	5.5	4.8	8.2	19.1	88 (1); 86 (2)
Sulfamethoxypyridazine	0.984	0.977	98.7	10.2	106.6	8.6	5.9	10.1	26.0	86 (1); 89 (3)
Sulfamethoxazole	0.983	0.986	102.7	6.4	106.9	6.3	5.3	9.1	34.4	92 (1); 80 (2); 111(3)
Sulfapyridine	0.985	0.972	99.7	10.7	101.3	7.0	7.5	12.7	22.9	89 (1); 81 (2); 105(3)
Sulfachlorpyridazine	0.989	0.994	107.2	6.3	104.2	6.7	2.8	4.7	17.6	79 (1); 82 (2); 90 (3)
Sulfadimethoxine	0.998	0.9853	112.4	7.4	127.0	7.6	4.6	7.7	17.2	85(1); 75 (2); 78(3)
Sulfaquinoxaline	0.985	0.9853	111.2	6.1	127.0	7.6	4.5	7.7	19.8	81 (1); 77 (2); 60 (3)

[a]Average of three days (n = 6) for synthetic samples with 50 ng/g of each sulfonamides.
[b]Average of ten fortified samples with 50 ng/g of each sulfonamides.
(1)Gentili *et al.* (2004); (2) Pecorelli, Bibi, Fioroni, & Galarini (2004); (3) Shao *et al.* (2005).

The samples' calibration curves were built like the instruments calibration curves but in this case, concentrations spiked in sample and expressed in ng/g were represented against the ratio analyte peak area / IS peak area. Samples calibration curves were employed to calculate CCα and CCβ. In all cases, calibrations curves were described by the equation y = mx + b. Figure 4 shows SRM transitions of a fortified sample spiked with sulfonamides to a final concentration of 50 ng/g. The figure shows Rt of each sulfonamides, intensity of the SRM transitions in a fortified sample and the quality of the peaks' shape. Instruments calibration curves, samples calibration curves, mean recoveries, variation coefficients CCα, CCβ and uncertainty resulted from inter-day and intra-day experiments conducted over a four-day period.

Correlation coefficients (r^2) were above 0.980 for both instrument and fortified samples; this indicated a good linearity of the method for sulfonamides between 0 and 250 ng/g in bovine muscle samples. Values of CCα were between 2.8 and 5.9 ng/g (sulfachlorpyridazine and sulfathiazole), and those of CCβ between 4.7 and 12.7 ng/g (sulfachlorpyridazine and sulfapyridine).

Intra- and inter-day assay precision and accuracy were calculated by replicate analysis of quality control samples (fortified samples) containing known amounts of the analyzed substances at each level of 25, 50, 75, 100, and 250 ng/g that were tested within one day (n = 6) and on three different occasions, respectively. Because all validation results at all sulfonamides concentration could not be included; Table 3 shows intra- and inter-day mean recoveries and CV at the validation levels (50 ng/g).

The measurement of uncertainty (U) of the method was measured with the use of all variance which are the variance of the reproducibility and of the matrix effects. The matrix effect variance was determined by subtracting the repeatability of experiment conducted on day 4 from the reproducibility variance of experiment conducted on day 1, 2 and 3. The equation 1 shows how uncertainty was calculated.

$$U = 2\sqrt{Sr^2 + Smartrix^2} \quad \text{..... Equation 1}$$

Where Sr is the variance of the reproducibility and Smatrix is the variance of the matrix.

U values were between 19.1 and 34.4%, which are higher than expected. This is probably due to the effect of no using SPE cartridges. It is know that SPE cartridge perform a selective extraction of analytes and eliminate most of the unwanted interferences. However, in this research the use of SPE cartridges was avoided to reduce cost and time of analysis which would have clearly led to higher uncertainty values. Interferences present in the final extract may have had an effect on the electrospray formation and gave higher standard deviation than expected.

It should be noted that recoveries achieved for each sulfonamides depend on the U values and they should

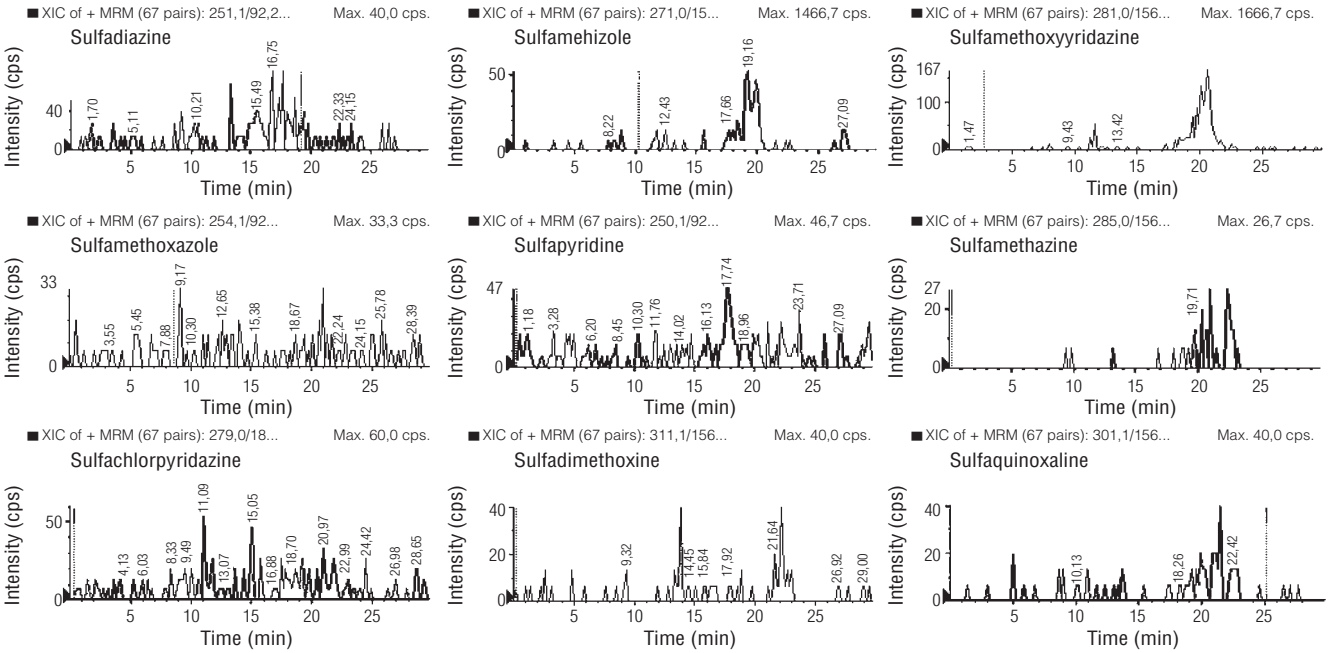

Figure 5. SRM transition of sulfonamides in a compliant bovine muscle sample.

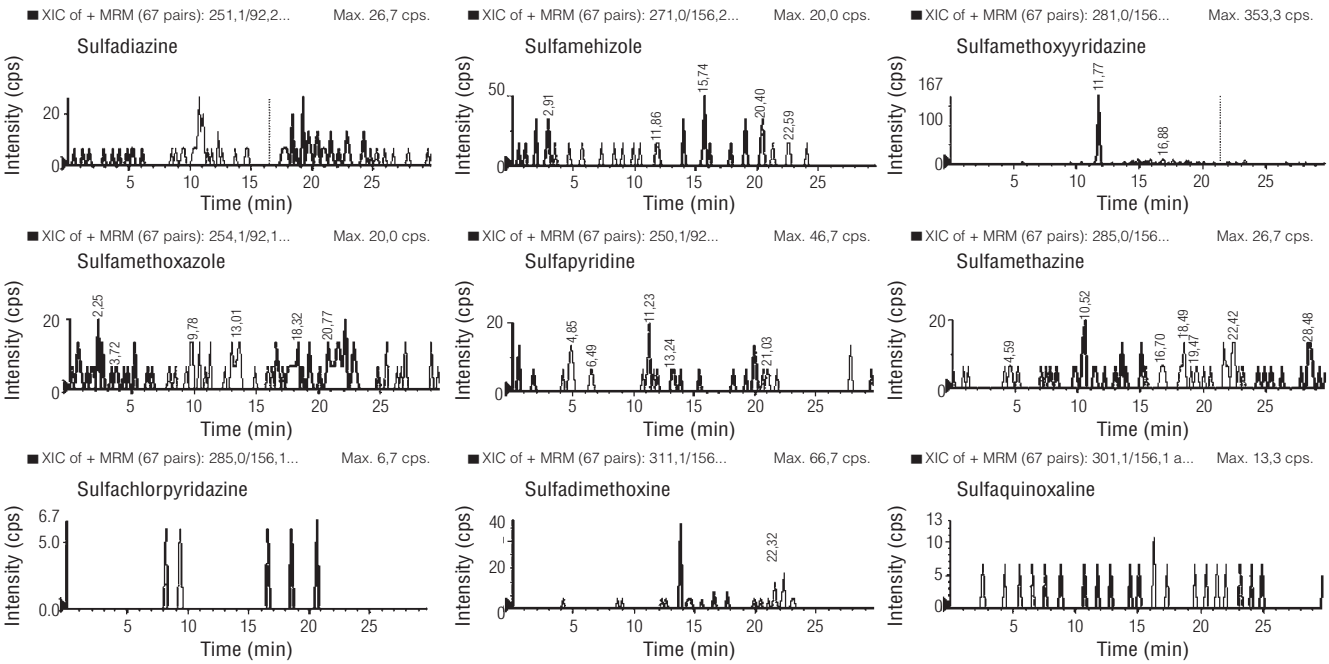

Figure 6. SRM transition of complaint bovine muscle sample but positive for sulfamethoxypyridazine (10 ng/g).

be expressed as X ± U%. Recoveries of sulfonamides achieved during this work were between 88 and 112 %, similar to those reported by other researchers[8,12,17]. For example, recoveries for sulfamethazine are 108.8 ± 19.1%, which are in the same than 88% and 86% reported by Gentili *et al.* and Pecorelli *et al.* (Table 3).

IV. *Application*

The applicability of the method presented has been demonstrated in real bovine muscle samples. Our laboratory takes part in a quality control program for bovine samples. Non-compliant and compliant samples,

belonging to this control program, were analysed for the presence of sulfonamides with the method presented in this article. One of the compliant samples resulted to be positive and these results were in accordance with those obtained with an SPE and HPLC/DAD method accredited by ENAC (National Spanish Entity for Accreditation). The SRM transitions of the sulfonamides investigated are shown in Figure 5. Figure 6 shows SRM transition of sample which was positive in sulfamethoxypyridine (10 ng/g) as its concentration was below the MRL level is was catalogue as compliant sample. It may be also possible to be positive on sulfadimetoxine however its concentration was much lower than CCα.

The method presented has a number of advantages compared to other available methods for the analysis of sulfonamides in muscle samples which include the short time of analysis (approximately 3 hours), the reduction in the amount of solvents required, no use of SPE cartridges and consequently, the reduction in the cost of the analysis.

CONCLUSIONS

This work presents a suitable method for the extraction, detection and quantification of nine sulfonamides by HPLC-MS/MS in bovine muscle samples of small size, rapidly and reliably. The method could then be applied in routine analysis and surveillance programs for the control of the presence of sulfonamides residues in bovine muscle.

ACKNOWLEDGMENTS

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