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Determination of sulfonamide antibiotics and metabolites in liver, muscle and kidney samples by pressurized liquid extraction or ultrasound-assisted extraction followed by liquid chromatography–quadrupole linear ion trap-tandem mass spectrometry (HPLC–QqLIT-MS/MS)

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ABSTRACT

Sulfonamides are widely used in human and veterinary medicine. The presence of sulfonamides residues in food is an issue of great concern. Throughout the present work, a method for the targeted analysis of 16 sulfonamides and metabolites residue in liver of several species has been developed and validated. Extraction and clean-up has been statistically optimized using central composite design experiments. Two extraction methods have been developed, validated and compared: i) pressurized liquid extraction, in which samples were defatted with hexane and subsequently extracted with acetonitrile and ii) ultrasound-assisted extraction with acetonitrile and further liquid–liquid extraction with hexane. Extracts have been analyzed by liquid chromatography–quadrupole linear ion trap-tandem mass spectrometry. Validation procedure has been based on the Commission Decision 2002/657/EC and included the assessment of parameters such as decision limit (CCα), detection capability (CCβ), sensitivity, selectivity, accuracy and precision. Method's performance has been satisfactory, with CCα values within the range of 111.2–161.4 μg kg^{-1}, limits of detection of 10 μg kg^{-1} and accuracy values around 100% for all compounds.

1. Introduction

Sulfonamides has been the first class of antimicrobial agents introduced in medicine [1]. These compounds are still widely used in human and veterinary medicine. In animal production, sulfonamides are used not only to treat infections but also for prophylactic purposes [2]. The potential presence of sulfonamide residues in animal tissues or products derived from animals (e.g. milk, egg, honey) is a public health concern, once these residues could provoke several side effects to humans and into the environment [3]. In order to provide food safety, maximum residue limits (MRL) have been established for numerous combinations drug/matrix. For sulfonamides, Brazil has adopted a MRL of 100 mg kg^{-1} [4]. That value comprehends the sum of sulfonamides and their metabolism products. In order to ensure the MRL compliance, analytical methods with adequate sensitivity and specificity to detect and quantify drug residues in food matrices in trace level are required.

Generally, sulfonamides residues can be determined in food matrices using several techniques, such as liquid chromatography, bioactivity-based assays, capillary electrophoresis among others [5–10]. Currently, due to their high sensitivity and selectivity, hyphenated methods based on mass spectrometry are the most applied approach to determine sulfonamides residues at low concentrations (mg kg^{-1} or μg kg^{-1}). Within hyphenated methods, the use of liquid chromatography–electrospray-quadrupole linear ion trap mass spectrometry (HPLC–(ESI)-QqLIT-MS/MS) permits analysis with high specificity and adequate limits of detection [11–16].

Extraction and clean-up techniques must be applied to food matrices, once they are generally complex samples, e.g. liver, muscle, kidney, milk and honey. Several methods are used for this purpose, from classical approaches as solid–liquid extraction to recent methods as single drop microextraction [17].

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Pressurized liquid extraction (PLE) is a relatively recent extraction technique. PLE takes advantage of the increased analyte solubility and extraction kinetics at higher temperature to speed the extraction process and reduce solvent consumption versus traditional methods [18]. Despite its advantages, PLE has not become a popular technique in analytical chemistry. In a review, Runnqvist et al. discusses some lacks of information about PLE settings optimization [19].

PLE has been mostly applied to environmental samples as plants, sediments, soil, sludge and manure [20–23]. A few reports using PLE to extract polar and moderate polar drugs from animal tissues have been published these last years [24–31]. Recently, two methods using PLE for sulfonamide residues analysis in biological and environmental samples have been reported [11,32]. García-Galán et al. developed and validated a method able to analyze 22 sulfonamide residues in soil and sewage sludge, using PLE followed by hydrophilic–lipophilic balance solid phase extraction (SPE) cartridges [11]. Yu et al. used the same approach (PLE–SPE) to determine 18 sulfonamides in muscle, kidney and liver of bovine, swine and poultry [32].

Generally, PLE produces semi-purified extracts. Thus, these extracts must be submitted to further purification procedures, generally by using SPE. Several authors report the use of PLE followed by SPE and Oasis HLB SPE cartridges were the most frequently mentioned [11,15,32–34].

Another suitable technique is ultrasound-assisted extraction (USE). The use of this technique in the analysis of food and environmental samples has been recently reviewed [35,36]. The overall advantage of this technique is the possibility of extracting several samples simultaneously. Moreover, the extraction process can be performed using an ultrasound bath, which is a simple apparatus found in most analytical laboratories. Despite that, only one report using USE for sulfonamides analysis in food has been published in recent years [37].

Throughout the present work, two extraction methods for sulfonamide residues analysis in several matrices of animal origin have been developed and validated. A fully automated PLE method and an ultrasound assisted extraction method have been reported, both without the need of further SPE purification. After extraction, samples were analyzed by HPLC–(ESI)-QqLIT-MS/MS. Methods have been validated according to the Commission Decision 2002/657/EC in terms of precision, sensitivity, decision limit (CCα) and detection capability (CCβ), among other performance parameters [38].

Table 1
Optimized mass spectrometry detection parameters. Bold SRM transitions are used for quantitative analysis.

Sulfonamide	$[M+H]^+$	SRM	Declustering potential (V)	Collsion energy (V)	Collision cell exit potential (V)	Internal standard	Rentention time (min)
SCA	215	**215 > 156**	46	21	10	SDZ-d4	9.6
		215 > 92	46	35	6		
SIM	279	**279 > 124**	76	33	8	SDZ-d4	10.3
		279 > 186	76	23	14		
STZ	256	**256 > 156**	40	25	14	SDZ-d4	14.8
		256 > 92	40	25	10		
SDZ-d4	255	**255 > 160**	46	27	10		14.9
		255 > 96	46	30	8		
S-STZ	356	**356 > 256**	71	25	16	SDZ-d4	15.0
		356 > 192	71	33	16		
N^4-SMR	307	**307 > 134**	60	35	8	SDZ-d4	15.1
		307 > 110	60	35	8		
SGD	215	**215 > 156**	56	13	10	SDZ-d4	15.1
		215 > 108	56	31	4		
SDZ	251	**251 > 156**	46	27	10	SMZ-d4	15.1
		251 > 92	46	30	8		
SPY	250	**250 > 156**	51	28	12	SMZ-d4	15.2
		250 > 92	51	31	6		
SMR	265	**265 > 92**	61	47	6	SMZ-d4	15.6
		265 > 156	61	27	8		
SMTZ	271	**271 > 156**	36	23	12	SMZ-d4	15.6
		271 > 108	36	23	8		
SMZ-d4	283	**283 > 160**	26	30	8		15.7
		283 > 96	26	35	4		
SMPZ	281	**281 > 156**	66	27	14	SMZ-d4	15.8
		281 > 126	66	27	12		
SMZ	279	**279 > 156**	26	30	8	SMZ-d4	15.9
		279 > 124	26	35	4		
SQX-OH	317	**317 > 156**	76	25	10	SMZ-d4	16.3
		317 > 108	76	47	12		
SMA-d4	258	**258 > 160**	56	25	10		16.8
		258 > 96	56	27	10		
SDX	311	**311 > 156**	46	29	12	SMA-d4	16.9
		311 > 92	46	45	4		
SMA	254	**254 > 156**	56	25	10	SMA-d4	17.0
		254 > 108	56	27	10		
SIZ	268	**268 > 156**	71	21	10	SMA-d4	17.1
		268 > 113	71	21	8		
SQX	301	**301 > 156**	76	25	10	SMA-d4	17.3
		301 > 108	76	47	12		
SDMX	311	**311 > 156**	76	31	8	SMA-d4	17.5
		311 > 92	76	31	6		
SBZ	277	**277 > 156**	56	17	10	SMA-d4	17.6
		277 > 92	56	41	6		
SNT	336	**336 > 156**	66	17	12	SMA-d4	18.2
		336 > 158	66	29	14		

2. Material and methods

2.1. Chemicals

Analytical standards with high purity (≥ 99%) were obtained from Sigma-Aldrich (St Louis, MO, USA): sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole (SMA), sulfamethoxypyridazine (SMPZ), sulfadiazine (SDZ), sulfapyridine (SPY), sulfadimethoxine (SDMX), succinyl-sulfathiazole(S-STZ), sulfaguanidine (SGA), sulfacetamide (SCA), sulfabenzamide (SBZ), sulfanitran (SNT), sulfisomidin (SIM), sulfamethizole (SMTZ), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfaisoxazole (SIX) and sulfadoxin (SDX).

Table 2
Experimental designs for PLE optimization.

Sample	First experimental design				Second experimental design			
	Categorization		Real values		Categorization		Real values	
	T (°C)	%ACN	T (°C)	% ACN	T (°C)	% acetic acid	T (°C)	% acetic acid
1	−1	−1	100	20	−1	−1	60	0.2
2	+1	−1	140	20	+1	−1	120	0.2
3	−1	+1	100	80	−1	+1	60	0.8
4	+1	+1	140	80	+1	+1	120	0.8
5 (Central point)	0	0	120	50	0	0	90	0.5
6 (Central point)	0	0	120	50	0	0	90	0.5
7 (Central point)	0	0	120	50	0	0	90	0.5
8 (Axial point)	−1.41	0	91.7	50	−1.41	0	47.58	0.5
9 (Axial point)	+1.41	0	148.3	50	+1.41	0	132.42	0.5
10 (Axial point)	0	−1.41	120	7.6	0	−1.41	90	0.08
11 (Axial point)	0	+1.41	120	92.4	0	+1.41	90	0.92

T (°C)=temperaure; %ACN=percentage of acetonitrile in water; % acetic acid: percentage of acetic acid in pure ACN.

The metabolite N_4-acetyl-sulfamerazine (AcSMR) and the isotope labeled compounds d^4-sulfamethoxazole (d^4-SMA), d^4-sulfamethazine (d^4-SMZ) and d^4-sulfadiazine (d^4-SDZ) used as surrogate and/or internal standards have been purchased from Toronto Chemical Research (North York, Ontario, Canada).

The SQX metabolites hydroxyl-sulfaquinoxaline (SQX-OH), N_4-acetyl-sulfaquinoxaline (AcSQX) and N_4-acetyl-hydroxyl-sulfaquinoxaline (AcSQX-OH) have been obtained from equine liver extract, purified using HPLC-DAD analysis, based on peak purity evaluation and also by high resolution mass spectrometry, as described elsewhere [39,40].

Water, acetonitrile (ACN), methanol (MeOH), hexane and acetone of HPLC-grade were supplied by J. T. Baker (Deventer, The Netherlands). Ethyl acetate was from Merck (Darmstadt, Germany). Formic and acetic acid and sodium chloride (NaCl) were obtained from Sigma-Aldrich. Diatomaceous earth (Hydromatrix®) was supplied by Agilent Technologies.

Individual stock standard solutions were prepared in MeOH: acetone (50:50) at 1 mg mL^{-1} and stored at −4 °C until use. Standard solutions from the mixtures of all compounds at appropriate concentrations were prepared by dilution of the individual stock standard solutions in MeOH or acetone.

2.2. Samples

Samples of ovine (muscle, liver and kidney), poultry (liver), equine (liver) and fish (muscle) were obtained from the Federal Inspection Service (SIF) of the Ministry of Agriculture, Livestock and Food Supply of Brazil (MAPA). Samples were frozen (−20 °C) until their arrival at the laboratory. After that, a representative portion of each sample has been frozen dried (−40 °C and −0.044 mbar vacuum).

2.3. Extraction and clean-up – PLE method

Samples were extracted by PLE using an ASE 350 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA). Samples (0.5 g) were grinded and homogenized in order to decrease particle size

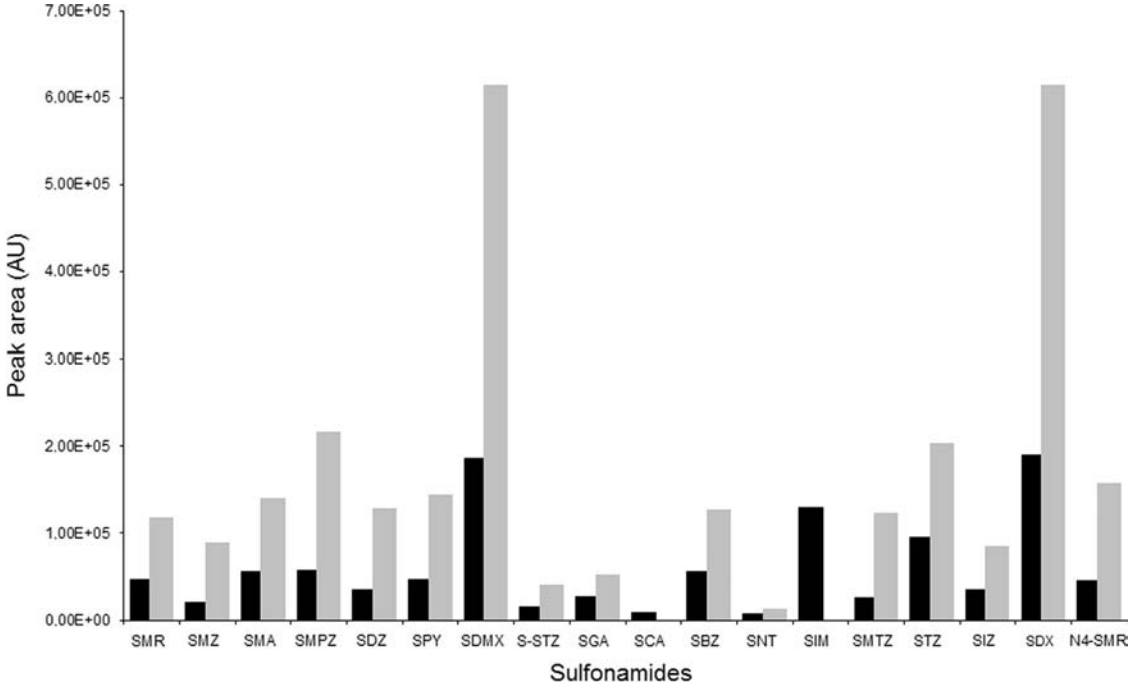

Fig. 1. Peak area comparison for extraction with pure ACN (black bars) and ACN with acetic acid (gray bars).

and promote better interaction with solvents. Prior to extraction, d^4-SMA, d^4-SMZ and d^4-SDZ were added to the sample as surrogate standards at a concentration of 100 ng g^{-1}. Samples were mixed with diatomaceous earth dispersing agent in order to avoid particle clumping and to reduce the interstitial volume of the PLE cells. Prior to extraction, samples were submitted to a clean-up method in order to remove the lipids by using hexane as solvent. PLE conditions: temperature 60 °C, 2 cycles of 5 min each one, 5 min static time, and pressure 1500 psi. Total flush volume of 80% and 300 s of purge time with nitrogen flow were applied.

After that, the same PLE cells (with the samples) were submitted to a second PLE process (extraction method). To optimize the extracting solvent composition and the extraction temperature, a central composite design experiment was performed (see Section 3). Optimized extraction solvent was ACN with 0.2% acetic acid. The optimized extraction temperature was 90 °C. There has been a preheating period of 8 min and 3 cycles of 7 min each were carried out. A total flush volume of 80% and 60 s of purge with nitrogen flow were applied. Pressure was set at a default value of 1500 psi as it has been demonstrated that this parameter is not decisive in PLE.

The obtained PLE extracts were maintained in a freezer for one hour (at approximately −18 °C) to promote protein precipitation. Following that, samples were centrifuged at 3500 rpm for 10 min in a 5810R centrifuge (Eppendorf). The supernatant was evaporated at 40 °C under nitrogen flow using a Turbo-Vap system (Zymark) until dryness. Extracts were redissolved in 1.0 mL of the HPLC mobile phase (water-ACN, 85:15) and transferred to an HPLC vial.

2.4. Extraction and clean-up – USE method

Samples (0.5 g) were weighted in polypropylene centrifuge tubes of 15 mL and spiked as previously described for the PLE extraction method. After that, 10 mL of ACN was added and tubes were mixed in a mechanical vortex by approximately 10 s. After that, all samples were placed into an ultrasonic bath by 60 min. After the extraction time, samples were stored in the freezer (−18 °C) for 1 h to promote protein precipitation. Then, samples were centrifuged at 3500 rpm for 10 min, the supernatant was dried at 40 °C under nitrogen flow. The extracts were redissolved in 2.0 mL of the HPLC mobile phase (water-ACN, 85:15). An aliquot of 2 mL of hexane was added to remove the fat content. Tubes were mixed in a vortex by approximately 5 s followed by centrifugation (3500 rpm for 10 min). The lower layer was carefully transferred to an HPLC vial.

2.5. Instrumental analysis

Sulfonamide separation was performed in a Symbiosis™ Pico System (Spark Holland, Emmen, The Netherlands), equipped with a HPLC system consisting of an Alias™ autosampler, a loop injector and two binary pumps with a four-channel solvent selector for

Table 3
Validation data for sulfonamides in liver by PLE and USE: decision limits (CCα) and detection capability (CCβ). Bold numbers represent the lower values obtained for each sulfonamide.

Compound	USE		PLE	
	CCα (μg kg^{-1})	CCβ (μg kg^{-1})	CCα (μg kg^{-1})	CCβ (μg kg^{-1})
SMR	**119.3**	**138.6**	119.9	139.6
SMZ	122.5	144.9	**111.2**	**122.4**
SMA	125.1	150.2	**122.5**	**145.0**
SMPZ	124.9	149.7	**118.0**	**136.0**
SDZ	125.4	150.9	**120.5**	**141.0**
SPY	121.4	142.8	**114.2**	**128.3**
SDMX	133.6	167.1	**127.2**	**154.4**
SCA	**139.5**	**179.0**	161.4	222.8
SBZ	140.3	180.7	**134.7**	**169.4**
STZ	**132.1**	**164.3**	ND	ND
SMTZ	**142.7**	**185.5**	154.1	208.2
SQX	130.5	161.1	**129.6**	**159.3**
SIZ	128.5	157.1	**121.7**	**143.3**
SDX	**124.1**	**148.3**	124.4	148.9
N4-SMR	**138.4**	**176.7**	160.6	221.2
SIM	ND	ND	**152.9**	**205.8**

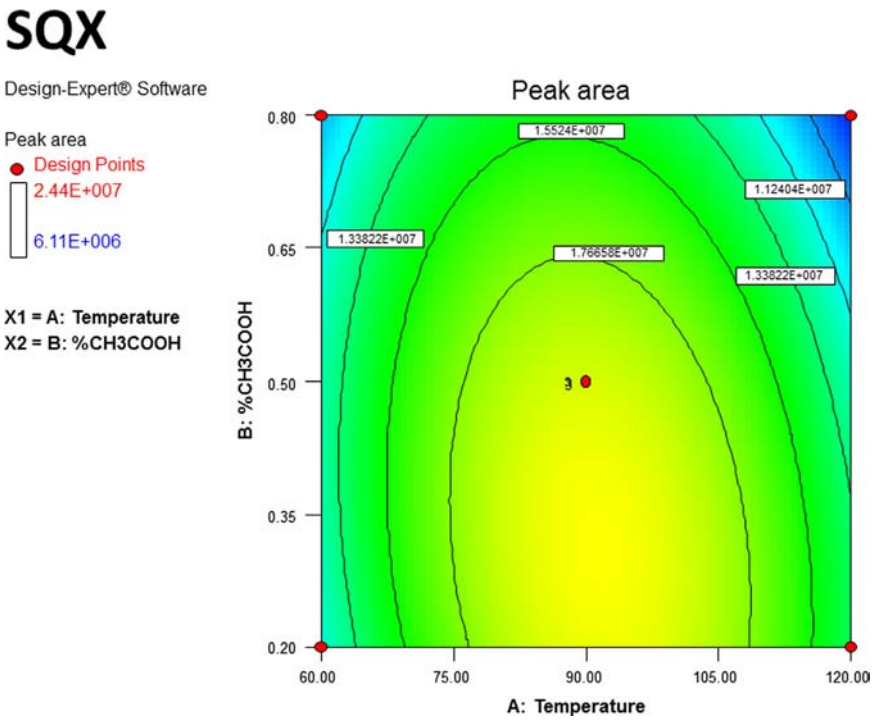

Fig. 2. Surface plot examples for second CCD corresponding to sulfaquinoxaline.

Table 4
Linearity data for sulfonamides in liver by PLE and USE.

Compound	PLE method			USE method		
	Equation	r	RSD_r (%)	Equation	r	RSD (%)
SMR	$y=1.2478x-0.105$	0.99329	4.9	$y=1.0298x+0.0336$	0.99512	1.2
SMZ	$y=0.8381x-0.059$	0.97533	5.8	$y=0.8356x+0.0044$	0.99582	1.1
SMA	$y=1.0683x-0.082$	0.98920	11.3	$y=1.1480x+0.0447$	0.99421	2.0
SMPZ	$y=1.7707x-0.229$	0.98371	2.4	$y=1.7707x-0.229$	0.98371	2.4
SDZ	$y=1.4485x-0.0253$	0.98560	3.6	$y=1.6161x+0.0802$	0.99505	1.9
SPY	$y=1.0822x-0.0662$	0.99206	11.6	$y=1.0830x+0.0345$	0.99325	1.1
SDMX	$y=3.6084x-0.2738$	0.98432	11.0	$y=3.9274x+0.2113$	0.99165	2.0
SCA	$y=0.4932x-0.0095$	0.98287	17.3	$y=0.4868x-0.0314$	0.97769	1.0
SBZ	$y=0.5028x-0.0731$	0.98036	18.2	$y=0.5554x-0.0233$	0.93560	1.8
STZ	$y=2.3348x-0.1152$	0.98174	38.5	$y=1.2153x+0.6426$	0.94848	2.3
SMTZ	$y=0.66398x-0.0953$	0.96309	6.4	$y=0.4931x-0.0737$	0.91017	1.6
SQX	$y=1.3288x-0.1037$	0.98455	5.9	$y=1.5243x+0.0265$	0.98953	2.5
SIZ	$y=0.8747x-0.1281$	0.97131	0.7	$y=0.7934x+0.0179$	0.99430	0.8
SDX	$y=3.2228x-0.2638$	0.95470	5.7	$y=3.6125x+0.1117$	0.99343	1.2
N4-SMR	$y=1.3565x+0.3449$	0.97670	16.4	$y=0.9441x+0.2720$	0.97553	2.3

RSD: relative standard deviation for slope ($n=3$).

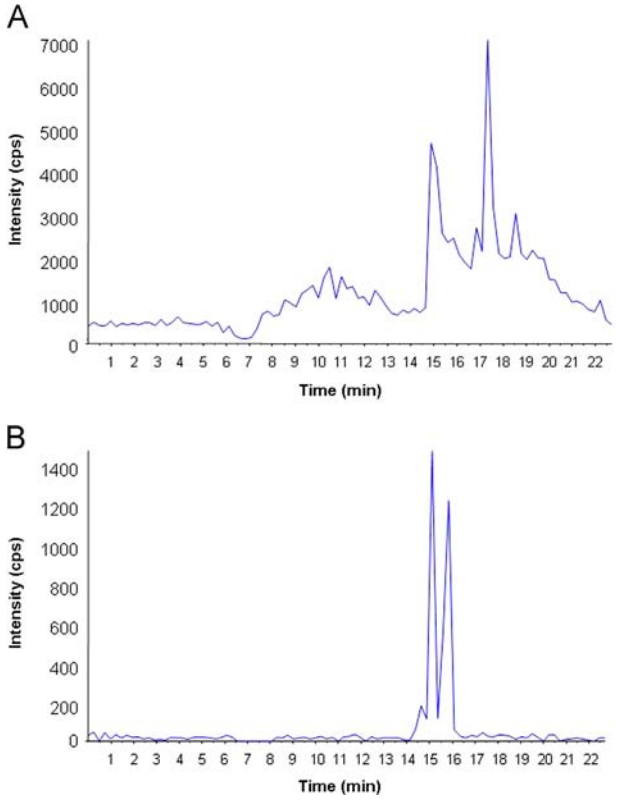

Fig. 3. Total ion chromatogram (TIC) for blank extracts of the PLE (A) and USE (B) extraction methods.

each one. Chromatographic separation was performed using a HPLC column Purospher® STAR (C18, ec, 150 × 4.6 mm, 5 μm) preceded by a guard column with the same packing material. The flow rate was set to 0.2 mL min^{-1}. Mobile phase was used in a gradient mode and was composed by a binary system: (A) HPLC grade water acidified with 10 mM of formic acid, and (B) ACN with 10 mM of formic acid. The elution gradient started with 25% of eluent (B), increasing to 80% in 10 min and to 100% in 11 min. During the next 2 min, the column was kept at 100% (B), it has been readjusted to the initial conditions in 3 min and equilibrated for 7 min. MS/MS analysis was carried out in a 4000 QTRAP hybrid triple quadrupole-linear ion trap-mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a turbospray ionization source working in the positive mode (ESI+). The optimization of the MS/MS experimental conditions was carried out in previous studies [11,41,42]. For increased sensitivity and selectivity, MS/MS data acquisition was performed in the selected reaction monitoring (SRM) mode, selecting the two most abundant transitions precursor ion/product ions. The optimal MS/MS parameters are listed in Table 1.

Table 5
Matrix effect estimation and relative recovery values for PLE and USE methods.

Analyte	PLE		USE	
	R_R (%)	ME (%)	R_R (%)	ME (%)
SMR	38	−80	42	−80
SMZ	33	−79	40	−78
SMA	32	−90	45	−89
SMPZ	24	−77	35	−77
SDZ	40	−87	45	−81
SPY	42	−83	41	−74
SMDX	28	−79	42	−79
S-STZ	39	−78	13	−73
SGA	18	−88	53	−86
SCA	28	−27	16	−6
SBZ	21	−96	45	−97
SNT	78	−90	49	−97
SIM	41	−76	22	−75
SMTZ	9	−85	8	−80
SQX	29	−86	35	−84
STZ	41	−63	29	−75
SIZ	23	−93	35	−93
SDX	29	−75	45	−74
N4-SMR	61	−65	57	−73

R_R=relative recovery; ME=matrix effects, as signal supression in percentage.

3. Results and discussion

3.1. PLE optimization

Despite the fact that PLE is considered a useful extraction technique in analytical chemistry, some drawbacks have reduced its applicability. Main limitations are the high cost of the equipment and the frequent need of additional clean up and/or concentration steps [19]. Moreover, the optimization of a PLE method is time consuming. In order to improve the development and enhance the yield of extraction, a

central composite design experiment to statistically evaluate the major parameters in PLE has been performed.

As all samples included in this study showed high lipidic content, a protocol for fat removal from PLE extracts, including refrigeration, centrifugation, evaporation and liquid–liquid extraction (LLE) with hexane was firstly evaluated.

In order to avoid additional steps and to provide a higher degree of automation to the method, two PLE methods were tested successively in which the same cell was submitted to clean-up, extraction and elution, according to the solvent used in PLE process. Hexane was selected as extracting solvent for lipids considering that sulfonamides are virtually insoluble in hexane. The PLE method for fat removal was evaluated using 2, 3 and 4 cycles. The results showed that the number of cycles delivered practically equivalent results, although 4 cycles removed approximately 16% of sample dry weight in fat content. To estimate fat content, the resulting hexane extracts have been concentrated and residual fat has been exactly weighted. However, extracts obtained with only 2 cycles did not present apparent fat and were clear enough to be directly injected after the evaporation step. The hexane phase was evaporated until dryness and redissolved in the HPLC mobile phase to evaluate potential losses of analytes. Analyte signal was not observed in the corresponding chromatograms.

After lipids removal, the same PLE cells were submitted to an extraction method. Two solvent mixtures were initially evaluated: MeOH and ACN. Both solvents were evaluated separately and in distinct mixture degrees with water. The obtained PLE extracts were further purified using a salting-out assisted liquid–liquid extraction

Table 6
Validation data for sulfonamides in liver by PLE: precision and accuracy results ($n=21$ for each level).

Compound	Accuracy (%)	RSD_r (%)	RSD_R (%)	Accuracy (%)	RSD_r (%)	RSD_R (%)	Accuracy (%)	RSD_r (%)	RSD_R (%)
	50 μg kg^{-1}			100 μg kg^{-1}			150 μg kg^{-1}		
SMR	112	9.2	10.7	105	8.6	8.8	104	6.9	10.7
	110	13.5		106	9.0		101	10.3	
	101	5.4		115	7.0		110	13.2	
SMZ	112	6.4	9.7	98	7.5	6.5	98	4.3	4.7
	102	3.8		100	7.4		101	3.8	
	93	6.2		95	3.5		102	5.3	
SMA	110	12.9	15.1	109	7.8	11.6	97	4.4	9.6
	103	14.1		107	8.1		97	12.4	
	88	8.6		89	6.0		87	6.9	
SMPZ	97	3.8	4.5	104	6.5	7.6	97	8.4	9.9
	97	4.9		104	9.5		96	10.5	
	92	2.8		104	7.8		100	11.5	
SDZ	108	13.4	11.2	99	9.1	7.9	95	7.0	10.5
	113	8.2		100	8.4		102	13.6	
	109	12.7		99	7.5		90	4.0	
SPY	119	9.2	11.0	107	7.2	8.1	108	5.9	6.6
	113	8.0		100	5.7		105	7.9	
	99	6.1		116	3.8		111	5.6	
SDMX	119	11.3	12.0	119	14.7	18.0	104	5.4	11.1
	111	11.3		108	9.8		107	14.1	
	101	6.6		86	11.2		94	7.1	
SCA	120	13.6	22.6	92	18.1	28.6	86	11.5	33.5
	136	16.5		143	27.8		141	34.9	
	93	21.8		111	14.0		107	15.7	
SBZ	109	20.3	16.7	109	25.2	20.7	100	18.3	16.2
	95	9.5		88	9.4		88	13.5	
	96	14.9		89	13.3		95	15.4	
SIM	96	16.2	17.9	84	9.9	27.4	87	7.1	27.3
	115	16.0		96	14.6		96	30.0	
	126	12.2		143	13.3		128	21.6	
SMTZ	55	29.5	22.8	84	10.9	15.1	57	13.8	17.4
	76	10.6		88	14.5		88	14.4	
	61	16.0		89	19.9		63	22.7	
SQX	113	9.3	12.3	108	16.9	17.3	92	7.9	12.2
	111	11.4		100	10.3		100	15.7	
	95	8.1		82	9.6		87	11.5	
SIZ	103	8.4	8.4	98	17.9	12.3	85	6.7	9.0
	99	8.1		92	6.5		92	10.5	
	100	9.5		92	9.2		93	7.2	
SDX	113	7.1	12.1	109	15.0	13.9	91	6.4	10.3
	98	16.3		108	6.7		108	14.0	
	99	7.1		88	5.9		92	5.0	
N4-SMR	141	20.7	19.6	74	13.0	13.2	77	16.2	20.4
	139	16.1		79	7.2		79	27.4	
	126	22.6		82	17.3		82	14.6	

RSD_r: relative standard deviation for intra-day precision; RSD_R: relative standard deviation for inter-day precision.

(SALLE). To each extract (around 20–25 mL), a certain amount of NaCl was added in order to obtain approximately 1.0 mol L^{-1}. An aliquot of 5 mL of ethyl acetate was added and the tubes were vortexed and centrifuged at 3500 rpm for 10 min. The upper phase (organic layer) was collected using a Pasteur pipette. This organic extract was evaporated at 40 °C under nitrogen until dryness. Extracts were redisolved in 1.0 mL of the HPLC mobile phase and transferred to an HPLC vial.

For samples extracted with pure MeOH or ACN, the final LLE was unnecessary. In this case, samples were evaporated to dryness and then reconstituted in 1.0 mL of the HPLC mobile phase and transferred to an HPLC vial.

As expected, MeOH extracts were not able to be submitted to the SALLE procedure, as reported by other authors [42,43]. Moreover, pure MeOH extracts were much more turbid than pure ACN extracts. Thus, ACN was the chosen solvent to perform the extraction step.

Table 8
Calculated SQX amount in naturally incurred samples using PLE, USE and a reference method[a].

Sample	PLE (ng g^{-1})	USE (ng g^{-1})	Reference method (ng g^{-1})
Fish (*Astyanax* sp.).	25	15	19
Ovine kidney	325	284	295
Ovine muscle	17	7.5	12

[a] Ref. [7].

Table 7
Validation data for sulfonamides in liver by US: precision and accuracy results (n=21 for each level).

Compound	Accuracy (%)	RSD_r (%)	RSD_R (%)	Accuracy (%)	RSD_r (%)	RSD_R (%)	Accuracy (%)	RSD_r (%)	RSD_R (%)
	50 μg kg^{-1}			100 μg kg^{-1}			150 μg kg^{-1}		
SMR	120	13.6	22.6	92	18.1	28.6	86	11.5	33.5
	96	14.1		103	6.1		103	12.6	
	104	16.0		103	8.0		111	10.8	
SMZ	94	8.2	11.5	101	7.8	6.5	99	12.2	14.5
	96	11.6		100	6.6		109	16.1	
	98	15.0		100	5.9		107	15.0	
SMA	94	13.3	14.0	107	7.2	9.4	108	6.5	12.2
	96	14.0		106	9.2		113	13.8	
	90	16.1		104	12.5		109	16.2	
SMPZ	100	6.6	7.5	102	7.8	7.7	100	13.8	13.4
	105	7.3		104	7.4		108	13.8	
	105	8.7		105	9.0		103	13.7	
SDZ	93	9.1	12.9	103	6.5	6.5	105	10.9	13.3
	93	13.3		101	7.9		106	13.8	
	97	16.5		105	5.2		105	17.4	
SPY	91	8.6	11.7	105	9.3	8.1	101	10.8	11.1
	95	13.5		109	7.6		111	11.4	
	100	12.0		104	7.7		107	11.3	
SDMX	95	9.2	13.9	107	10.8	10.9	108	10.2	14.5
	97	15.3		109	7.9		115	15.5	
	90	17.1		105	14.7		111	18.9	
SCA	85	15.1	18.8	83	10.5	13.5	90	16.0	13.6
	91	20.7		89	12.7		91	14.0	
	93	21.2		85	17.9		96	12.5	
SBZ	107	18.4	22.8	102	25.3	18.9	108	15.2	19.7
	89	22.0		100	15.4		100	21.8	
	78	18.3		94	15.3		111	23.6	
STZ	54	40.0	41.7	91	11.0	12.7	102	14.2	24.0
	62	39.9		91	12.4		91	22.2	
	58	49.9		94	15.7		113	32.8	
SMTZ	96	22.7	17.7	87	23.8	21.1	81	19.3	19.3
	108	11.2		90	21.3		90	18.0	
	106	18.9		91	21.2		83	20.6	
SQX	97	11.2	12.3	103	7.5	9.6	106	11.6	20.4
	103	11.2		106	7.9		106	19.1	
	93	14.2		103	13.5		120	26.5	
SIZ	99	12.3	12.6	107	8.8	10.2	101	9.2	18.2
	94	11.8		106	8.5		106	16.4	
	94	14.8		104	14.0		110	25.9	
SDX	94	12.2	12.7	102	7.8	9.6	106	5.4	15.7
	94	13.7		103	10.0		103	13.1	
	90	13.7		103	11.8		115	23.2	
N4-SMR	97	16.5	24.6	98	10.9	13.8	107	13.0	15.9
	84	40.7		89	11.7		89	14.8	
	83	9.7		109	11.0		119	19.4	

RSD_r: relative standard deviation for intra-day precision; RSD_R: relative standard deviation for inter-day precision.

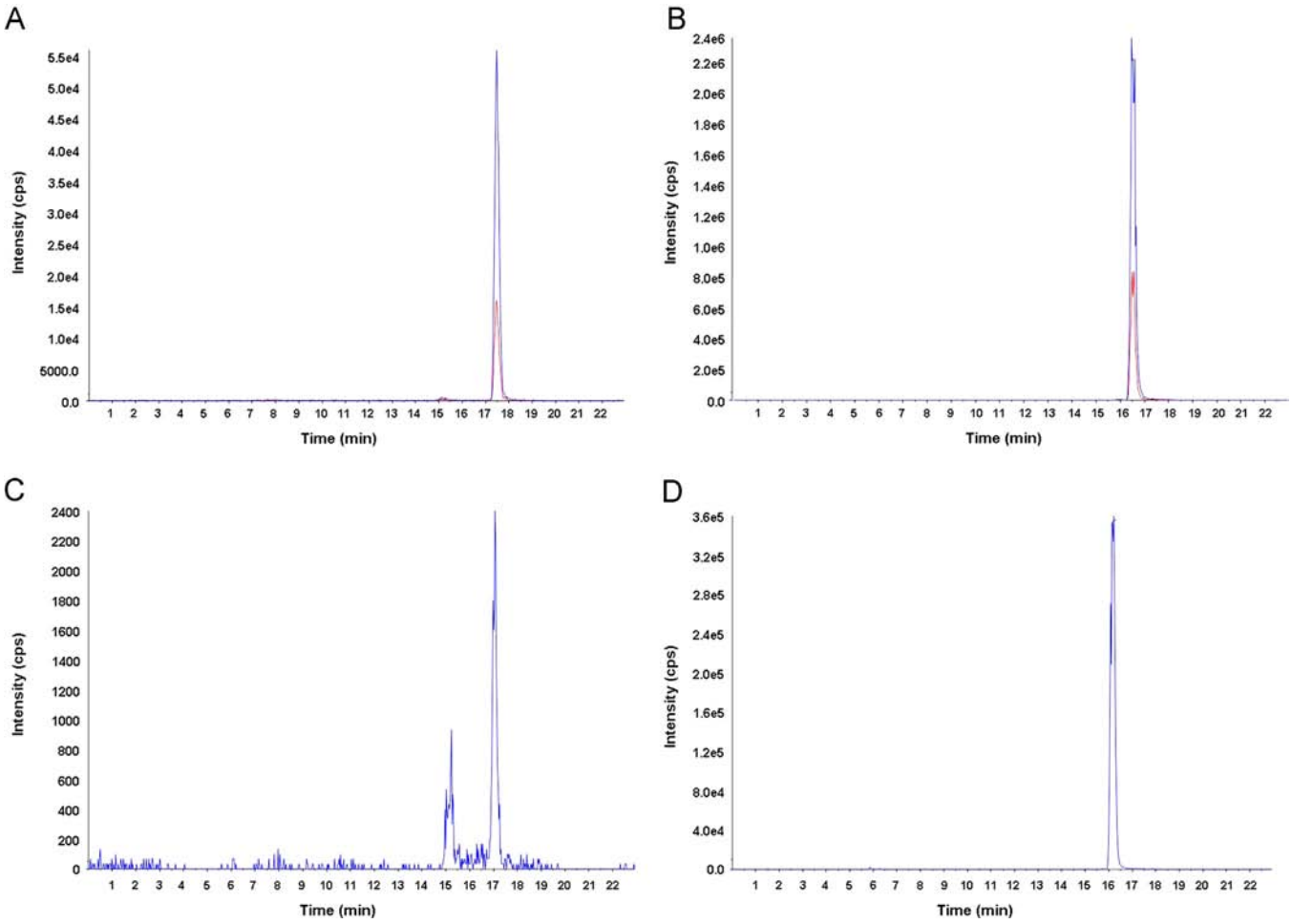

Fig. 4. Extracted ion chromatogram for the presence of SQX (A), SQX-OH (B), AcSQX (C) and AcSQX-OH (D) in ovine kidney using USE extraction method.

3.2. Optimization using a central composite design

Univariate optimization procedure is based on varying "one variable at-a-time". This approach does not guarantee a real approximation from optimal conditions. For PLE extraction optimization, a central composite design (CCD) was applied. Control variables were ACN percentage in water (%) and temperature of extraction (°C). The response variable was sulfonamide peak area. Table 2 shows the experimental design, including 4 axial points and 3 replicates for the center point. Center point conditions were established as the initial extraction levels obtained in the solvent selection stage. Data analysis and mathematical modeling were processed using Minitab 16 statistical software (Minitab, State College, PA, USA) and Design-Expert 7.0.0 (Stat-Ease, Minneapolis, MN, USA). Raw data was tabled and regression analysis was performed. Mathematical models were validated using ANOVA. Thus, contour plots for surface responses data were plotted. The results were very similar for all analytes. Data showed that the most intense peak areas were obtained using high percentages of ACN and lower temperatures. As the optimal conditions were not achieved, a second CCD was performed, now using a lower range of temperatures (48–132 °C) and pure ACN with acid additive as extraction solvent (acetic acid from 0.08% to 0.92%). Table 2 also shows this second experimental design. The use of acid as additive was based on our previous report published elsewhere [44]. A simple experiment was included to compare the extraction efficiency with and without the acid additive in the ACN, using 3 samples spiked at MRL level. Fig. 1 shows the differences observed in the extraction yield. The results obtained in the second CCD were much more varied, showing that SFAs with a group attached to the aniline moiety, such as succinyl-STZ and N_4-acetyl-SMR, has an increasing signal at lower temperatures and higher amounts of acid additive. Some analytes were more affected by changes in control variables (e.g. SQX and STZ), whereas the most part of analytes have shown low signal variability. In order to obtain a compromise between higher extraction yield and analytes response, we have chosen to use 90 °C and 0.2% of acetic acid in the PLE extraction method. Fig. 2 presents some examples of the analytes which showed heterogeneous response in the second CCD experiment.

3.3. Method validation

Method validation is absolutely necessary in residue analysis because of the important role in statutory programs involved in international trade of commodities. European Union (EU) has issued specific regulation (Commission Decision 2002/657/EC) concerning the performance of analytical methods and the interpretation of results in the official control of residues in products of animal origin. According to it, several parameters must be calculated such as limit of decision (CCα) and detection capability (CCβ).

In the present study, the HPLC–MS/MS methods were validated according to Commission Decision 2002/657/EC: method performance parameters were determined and evaluated using samples of liver spiked with the appropriate volume of the standard solution of sulfonamides at various concentration levels. The parameters studied included linearity, accuracy, precision, specificity, matrix effects, together with the parameters CCα and CCβ. The linear response was assessed using standard solutions injected thrice, covering the range of 25–400 ng mL^{-1}. The calibration curves were constructed using the ratio [peak area of analyte/area

of internal standard peak] versus the concentration of analyte. Precision and accuracy were determined by the analysis of samples spiked at three concentration levels (50, 100 and 150 ng mL^{-1}) corresponding to 0.5, 1.0 and 1.5 times the MRL. The intra-day precision test was carried out using seven measurements in replicate for the three concentration levels, whereas the inter-day precision test was performed during the execution of three batches into three consecutive days. Although the method was applied to several tissues (muscle, kidney, fish), liver was chosen as the matrix for validation studies because of its more complex matrix among all the analyzed samples. With the exception of matrix effects estimation experiments, all parameters were determined using standard addition calibration curves.

3.4. Decision limit (CCα) and detection capability (CCβ)

The decision limit (CCα) and the detection capability (CCβ) were calculated plotting all data obtained from the precision determination and applying the calibration curves approach as described in Commission Directive 2002/657/EC and also in conformity with the ISO 11843. Briefly, the signal was plotted against the added concentration and the corresponding concentration at the *y*-intercept plus 1.64 times the standard deviation of the within-laboratory reproducibility gave the CCα values. CCβ was calculated by summing of the concentration at the CCα and 1.64 times the standard deviation of the within-reproducibility of the mean measured content at the MRL concentration level. Table 3 reports the CCα and CCβ values for both PLE and USE methods. Although these parameters do not present criteria for upper limits, some sulfonamides present values considered unacceptably high and were removed from the method. That was the case for STZ in the PLE method and SIM in the USE method.

3.5. Determination of limit of detection (LOD), limit of quantification (LOQ) and linearity

Considering that the mathematical approach for LOD and LOQ determination using the deviation of blank samples resulted in improbable low values, these parameters were established using data from spiked samples. To carry out the experimental determination of the lowest concentration detectable as required by guidelines for implementation of the Commission Decision (LOD and LOQ), calibration curves with lower concentrations than those used in previous tests (0.10 and 0.25 × MRL) were analyzed. The lowest spiked points were correctly identified and quantified. Based on these experimental data, LOD and LOQ were defined as 10 and 25 μg kg^{-1}, respectively, for each compound for both PLE and USE extraction methods. Table 4 shows correlation coefficients and linearity data that match the internal criteria of our laboratory ($r > 0.95$ for standard addition calibration curves) for PLE and USE methods, respectively. To define the relationship between concentration and analytical response, a calibration curve with five levels of concentration, taking off the zeros, was prepared for quantification of each matrix studied. For linearity, a standard addition calibration curve with nine levels of concentration was analyzed, which was linear into the studied range (10–400 ng g^{-1}).

3.6. Specificity

Blank samples ($n=20$) were tested for verification of interference, using both PLE and USE extraction procedures. No significant difference in retention times of analytes and internal standard were observed. Typical results for blank samples (for both extraction methods) are shown in Fig. 3.

3.7. Recovery and matrix effect estimation

Relative recoveries (RE_R) were determined using the approach proposed by Matuszewski to quantitative estimation of matrix effects, as described elsewhere [45,46]. In this method, losses caused by matrix effects are not taken into account to calculate recovery: just the losses caused by the sample preparation method are considered. RE_R is calculated using the raw signal of each analyte peak, by the comparison between matrix-matched samples (TS from "tissue standard"), considered as 100% and standard addition samples (R from "recovery samples"). Calculations were performed according to the following equation:

$$RE_R(\%) = R/TS \times 100 \quad (1)$$

Results shown in Table 5 demonstrate that PLE method and USE method provide similar recoveries and matrix effect values. Matrix effects are highly intense in both PLE and USE method. Moreover, both methods have considerable losses in extraction process, which results in low recovery values. This fact lead us to the use of isotope labeled internal standards associated with standard addition calibration curves, in which standard solutions were added in the beginning of the analysis and suffer all the extraction and concentration process. This approach takes into account the various variables present in these matrices and it is adequate for both extraction procedures. Since this method is based on standard addition calibration, recovery values are not considered for calculations. A detailed matrix effects report comparing several approaches using data from PLE and USE methods has recently been accepted for publication [47].

3.8. Precision, accuracy and reproducibility

Precision and reproducibility data are summarized in Table 6 (PLE) and Table 7 (USE). The accuracy for each concentration is also included. Accuracy was determined using the comparison between the calculated concentration (C_C) and the analyte amount added to the sample in the spiking procedure (C_S). At first, all concentration values were determined using the equations provided by the calibration curves. After that, the accuracy calculation was performed according to the following equation:

$$\text{Accuracy}(\%) = C_C / C_S \times 100 \quad (2)$$

3.9. Application to real samples

Both validated methods were used to analyze real incurred samples, which contain SQX and some metabolites. Results are showed in Table 8. Sulfaquinoxaline was correctly detected using both techniques, although a significant difference between calculated concentrations was observed. In the case of ovine liver samples, which were previously analyzed in a sulfonamide residues method with ISO 17025 accreditation and used for routine analysis in our laboratory since 2009 [42], USE method provided closest results than PLE method. However, the use of both methods in a proficiency test to sulfonamides residues analysis in liver is still necessary to perform a more precise comparison. Fig. 4 shows the extracted ion chromatogram for the presence of SQX and some metabolites in ovine kidney using USE extraction. Even though SQX metabolites could not be determined because standards were not available, the SQX metabolites can be qualitatively detected using the current method. The optimization of the MS/MS determination parameters for SQX metabolites was performed using a semi-purified equine liver extract.

3.10. Methods comparison

In general terms, both methods were able to correctly extract and determine more than 15 sulfonamides residues in tissues. PLE and USE extraction procedures show similar performance regarding several parameters.

There are just a few reports comparing PLE and USE for drugs residues extraction. However, the similarity found in the results from PLE and USE is in agreement with other authors [48,49]. Both reports deal with similar matrices (soil and sewage sludge). However, for vegetal matrices, some reports suggest better responses for PLE, especially for extraction efficiencies [50,51].

Some more hydrophilic sulfonamides as SGD, SNT and S-STZ were recovered in low yields and without acceptable precision. Thus, those compounds were removed from the method scope. The majority of the analytes showed similar responses in terms of linearity, precision and accuracy for both extraction methods. Interestingly, STZ could not be satisfactorily determined using PLE method and was removed from this method scope. A similar behavior was demonstrated for SIM responses when USE method was being performed. Regarding the detection and quantification limits, these parameters were firstly estimated using the methods of noise standard deviation of blank samples and the mathematical approach based on calibration curves. As experienced previously in other methods, the first method produced values unrealistically low and the calibration curve also resulted in levels unrealistically high. Thus, LOD and LOQ were determined using real spiked samples as described before. Although the established LOD and LOQ (10 and 25 ng g^{-1}, respectively) can be seen as relatively high, these values were considered as satisfactory, taking into account that the methods do not include an SPE procedure as additional purification step. In other words, a compromise between sensitivity and feasibility of the methods was chosen.

Furthermore, USE method was able to extract analytes not just in spiked samples but also in incurred samples. It was expected that PLE method would have promoted a more efficient extraction in incurred samples, due to the advantages of PLE as high pressure and high temperature. Interestingly, both methods showed similar results.

Naturally incurred samples were analyzed using both the PLE and the USE developed methods. Previously, those samples were analyzed using a reference method. USE method showed results closest to those achieved by the reference method, which use conventional extraction with ACN followed by clean-up with sodium sulfate and concentration of organic extract [42]. Considering that PLE showed similar extraction efficiencies than USE, this last technique must be considered a low-cost alternative for routine analysis. Although PLE can be totally automated, initial investment costs can be inhibitory for many laboratories. Thereby, but also considering the rapidity, simplicity and low cost of USE, this approach was considered the best method for sulfonamide residue analysis in animal tissues.

4. Conclusions

Two new extraction methods for sulfonamide residues determination in biological samples were developed and validated. All figures of merit were established, such as decision limits, detection capability, accuracy, precision and linearity. PLE method was statistically optimized. Both PLE and USE methods are suitable as routine methods, although USE method seems to be more efficient and easier to perform. Results lead us to point out PLE and USE as useful extraction techniques for the trace analysis of sulfonamides and metabolites, with a slight advantage of USE method, in terms of time and solvent consumption. Considering the satisfactory results obtained using both techniques, the same strategies could be evaluated for other veterinary drugs groups or other target matrices. Both techniques are potentially versatile to be applied in further studies with physico-chemical characteristics related to sulfonamides, such as fluoroquinolones. Moreover, complementary studies must be performed to include other matrices for sulfonamides analysis, such as honey or feed. Concisely, the reported methods may be applied for routine analysis as is or be validated for various scope extensions.

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References

[1] GerhardDomagk, Nobel Lectures in Physiology or Medicine 1922–1941, Elsevier Publishing Company, Amsterdam, 1965.
[2] W.C. Campbell, J. Parasitol. 94 (2008) 934–945. http://dx.doi.org/10.1645/GE-1413.1.
[3] J.F. Acar, G. Moulin, Rev. Sci. Tech. Off. Int. Epiz. 25 (2006) 775–792.
[4] A. de Queiroz Mauricio, E.S. Lins, Food Addit. Contam. A 29 (2012) 482–489. http://dx.doi.org/10.1080/19440049.2011.620987.
[5] R. Hoff, T.B.L. Kist, J. Sep. Sci. 32 (2009) 854–866. http://dx.doi.org/10.1002/jssc.200800738.
[6] R. Hoff, F. Ribarcki, I. Zancanaro, L. Castellano, C. Spier, F. Barreto, et al., Food Addit. Contam. A 29 (2012) 577–586. http://dx.doi.org/10.1080/19440049.2011.641508.
[7] R.B. Hoff, F. Barreto, T.B.L. Kist, J. Chromatogr. A 1216 (2009) 8254–8261. http://dx.doi.org/10.1016/j.chroma.2009.07.074.
[8] I. Maia Toaldo, G. Zandonadi Gamba, L. Almeida Picinin, G. Rubensam, R. Hoff, M. Bordignon-Luiz, Talanta 99 (2012) 616–624. http://dx.doi.org/10.1016/j.talanta.2012.06.047.
[9] M. Lillenberg, S. Yurchenko, K. Kipper, K. Herodes, V. Pihl, K. Sepp, et al., J. Chromatogr. A 1216 (2009) 5949–5954. http://dx.doi.org/10.1016/j.chroma.2009.06.029.
[10] A.M. Bueno, A.M. Contento, Á. Ríos, Anal. Methods 5 (2013) 6821–6829. http://dx.doi.org/10.1039/c3ay41437j.
[11] M.J. García-Galán, S. Díaz-Cruz, D. Barceló, J. Chromatogr. A 1275 (2013) 32–40. http://dx.doi.org/10.1016/j.chroma.2012.12.004.
[12] M.J. García-Galán, M. Silvia Díaz-Cruz, D. Barceló, TrAC – Trends Anal. Chem. 27 (2008) 1008–1022. http://dx.doi.org/10.1016/j.trac.2008.10.001.
[13] M.S. Díaz-Cruz, M.J. García-Galán, D. Barceló, J. Chromatogr. A 1193 (2008) 50–59. http://dx.doi.org/10.1016/j.chroma.2008.03.029.
[14] M. Gros, S. Rodríguez-Mozaz, D. Barceló, J. Chromatogr. A 1292 (2013) 173–188. http://dx.doi.org/10.1016/j.chroma.2012.12.072.
[15] A. Jelić, M. Petrović, D. Barceló, Talanta 80 (2009) 363–371. http://dx.doi.org/10.1016/j.talanta.2009.06.077.
[16] Y. Chen, Q. Cao, S. Deng, J. Huang, B. Wang, G. Yu, Int. J. Environ. Anal. Chem. 93 (2013) 1159–1173. http://dx.doi.org/10.1080/03067319.2012.717271.
[17] M. Kaykhaii, A. Abdi, Anal. Methods 5 (2013) 1289–1293. http://dx.doi.org/10.1039/c2ay26560e.
[18] S.J. Lehotay, C.-H. Lee, J. Chromatogr. A 785 (1997) 313–327. http://dx.doi.org/10.1016/S0021-9673(97)00551-7.
[19] H. Runnqvist, S.A. Bak, M. Hansen, B. Styrishave, B. Halling-Sørensen, E. Björklund, J. Chromatogr. A 1217 (2010) 2447–2470. http://dx.doi.org/10.1016/j.chroma.2010.02.046.
[20] P.Viñas, M. Bravo-Bravo, I. López-García, M. Pastor-Belda, M. Hernández-Córdoba, Talanta 119 (2014) 98–104. http://dx.doi.org/10.1016/j.talanta.2013.10.053.
[21] A.L. Oliveira, E. Destandau, L. Fougère, M. Lafosse, Food Chem. 145 (2014) 522–529. http://dx.doi.org/10.1016/j.foodchem.2013.08.065.
[22] E.Y. Ordoñez, J.B. Quintana, R. Rodil, R. Cela, J. Chromatogr. A 1320 (2013) 10–16. http://dx.doi.org/10.1016/j.chroma.2013.10.049.
[23] C. Fernández-Ramos, O. Ballesteros, A. Zafra-Gómez, F.J. Camino-Sánchez, R. Blanc, A. Navalón, et al., Environ. Sci. Pollut. Res. 21 (2014) 4286–4296.
[24] B. Huerta, A. Jakimska, M. Gros, S. Rodríguez-Mozaz, D. Barceló, J. Chromatogr. A 1288 (2013) 63–72. http://dx.doi.org/10.1016/j.chroma.2013.03.001.
[25] Y. Liu, H. Yang, S. Yang, Q. Hu, H. Cheng, H. Liu, et al., J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 917–918 (2013) 11–17. http://dx.doi.org/10.1016/j.jchromb.2012.12.036.
[26] D. Chen, X. Cao, Y. Tao, Q. Wu, Y. Pan, L. Huang, et al., J. Chromatogr. A 1253 (2012) 110–119. http://dx.doi.org/10.1016/j.chroma.2012.06.095.
[27] D. Chen, Y. Tao, H. Zhang, Y. Pan, Z. Liu, L. Huang, et al., J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 1659–1667. http://dx.doi.org/10.1016/j.jchromb.2011.04.004.
[28] V. Jiménez, R. Companyó, J. Guiteras, Talanta 85 (2011) 596–606. http://dx.doi.org/10.1016/j.talanta.2011.04.021.
[29] V. Carretero, C. Blasco, Y. Picó, J. Chromatogr. A 1209 (2008) 162–173. http://dx.doi.org/10.1016/j.chroma.2008.09.011.

[30] G. Font, A. Juan-García, Y. Picó, J. Chromatogr. A 1159 (2007) 233–241. http://dx.doi.org/10.1016/j.chroma.2007.03.062.
[31] L. Kantiani, M. Farré, J.M. Grases, I Freixiedas, D. Barceló, Anal. Bioanal. Chem. 398 (2010) 1195–1205. http://dx.doi.org/10.1007/s00216-010-4005-0.
[32] H. Yu, Y. Tao, D. Chen, Y. Wang, L. Huang, D. Peng, et al., J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 2653–2662. http://dx.doi.org/10.1016/j.jchromb.2011.07.032.
[33] L. Kantiani, M. Farré, J.M.G.I. Freixiedas, D. Barceló, J. Chromatogr. A 1217 (2010) 4247–4254. http://dx.doi.org/10.1016/j.chroma.2010.04.029.
[34] A. Pamreddy, M. Hidalgo, J. Havel, V. Salvadó, J. Chromatogr. A 1298 (2013) 68–75. http://dx.doi.org/10.1016/j.chroma.2013.05.014.
[35] S. Seidi, Y. Yamini, Cent. Eur. J. Chem. 10 (2012) 938–976. http://dx.doi.org/10.2478/s11532-011-0160-1.
[36] Y. Picó, TrAC – Trends Anal. Chem. 43 (2013) 84–99. http://dx.doi.org/10.1016/j.trac.2012.12.005.
[37] S. Gao, X. Yang, W. Yu, Z. Liu, H. Zhang, Talanta 99 (2012) 875–882. http://dx.doi.org/10.1016/j.talanta.2012.07.050.
[38] Commission of the European Communities, Commission Decision 2002/657/EC, (2002).
[39] R.B. Hoff, F. Barreto, J. Melo, L. Jank, M.D.C.R. Peralba, T.M. Pizzolato, Anal. Methods 4 (2012) 2822–2830. http://dx.doi.org/10.1039/c2ay25197c.
[40] R.B. Hoff, L. Meneghini, T.M. Pizzolato, M.D.C.R. Peralba, M.S. Díaz-Cruz, D. Barceló, Anal. Chem. 86 (2014) 5579–5586. http://dx.doi.org/10.1021/ac501132r.
[41] R.B. Hoff, F. Barreto, J. Melo, M.T. Martins, T.M. Pizzolato, M.do Carmo Ruaro Peralba, Food Addit. Contam. A 31 (2014) 39–47. http://dx.doi.org/10.1080/19440049.2013.861082 (131106185253008).
[42] I.M. Valente, L.M. Gonçalves, J.A. Rodrigues, J. Chromatogr. A 1308 (2013) 58–62. http://dx.doi.org/10.1016/j.chroma.2013.08.014.
[43] J. Liu, M. Jiang, G. Li, L. Xu, M. Xie, Anal. Chim. Acta 679 (2010) 74–80. http://dx.doi.org/10.1016/j.aca.2010.09.013.
[44] M.S. Bittencourt, M.T. Martins, F.G.S. de Albuquerque, F. Barreto, R. Hoff, Food Addit. Contam. A 29 (2012) 508–516. http://dx.doi.org/10.1080/19440049.2011.606228.
[45] R.B. Hoff, F. Barreto, J. Melo, M.T. Martins, T.M. Pizzolato, M.C.R. Peralba, Food Addit. Contam. – Part Chem. Anal. Control Expo. Risk Assess. 31 (2014) 39–47. http://dx.doi.org/10.1080/19440049.2013.861082.
[46] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019–3030. http://dx.doi.org/10.1021/ac020361s.
[47] R.B. Hoff, G. Rübensam, L. Jank, F. Barreto, M.do C.Ruaro Peralba, T.M. Pizzolato, et al., Talanta 132 (2014) 443–450. http://dx.doi.org/10.1016/j.talanta.2014.08.046.
[48] N. Dorival-García, A. Zafra-Gómez, F.J. Camino-Sánchez, A. Navalón, J.L. Vílchez, Talanta 106 (2013) 104–118. http://dx.doi.org/10.1016/j.talanta.2012.11.080.
[49] N. Barco-Bonilla, J.L.M. Vidal, A. Garrido Frenich, R. Romero-González, Talanta 78 (2009) 156–164. http://dx.doi.org/10.1016/j.talanta.2008.10.048.
[50] V.F. Péres, J. Saffi, M.I.S. Melecchi, F.C. Abad, R. De Assis Jacques, M.M. Martinez, et al., J. Chromatogr. A 1105 (2006) 115–118. http://dx.doi.org/10.1016/j.chroma.2005.07.113.
[51] D.L. Luthria, R. Biswas, S. Natarajan, Food Chem. 105 (2007) 325–333. http://dx.doi.org/10.1016/j.foodchem.2006.11.047.