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Development and validation of an LC-UV method for the determination of sulfonamides in animal feeds

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A simple LC-UV method was developed for the determination of residues of eight sulfonamides (sulfachloropyridazine, sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxypyridazine, sulfaquinoxaline, sulfamethoxazole, and sulfadimethoxine) in six types of animal feed. C18, Oasis HLB, Plexa and Plexa PCX stationary phases were assessed for the clean-up step and the latter was chosen as it showed greater efficiency in the clean-up of interferences. Feed samples spiked with sulfonamides at 2 mg/kg were used to assess the trueness (recovery %) and precision of the method. Mean recovery values ranged from 47% to 66%, intra-day precision (RSD %) from 4% to 15% and inter-day precision (RSD %) from 7% to 18% in pig feed. Recoveries and intra-day precisions were also evaluated in rabbit, hen, cow, chicken and piglet feed matrices. Calibration curves with standards prepared in mobile phase and matrix-matched calibration curves were compared and the matrix effects were ascertained. The limits of detection and quantification in the feeds ranged from 74 to 265 μg/kg and from 265 to 868 μg/kg, respectively.

Keywords: sulphonamides; feed analysis; SPE; LC-UV

Introduction

According to a study by the European Federation of Animal Health (FEDESA), in 1999 food-producing animals consumed 4700 tons of antimicrobials in the European Union (EU).[1] Another recent report shows that in 2007, 2478 tons of antimicrobials were sold for use in food and non-food-producing animals in six European countries.[2] The same report states that sulfonamides were the second most commonly used class of antimicrobials for the production of medicated feeds. According to a US Food and Drug Administration (FDA) report, in 2009 food-producing animals consumed 13 067 tons of antimicrobials, of which 517 were sulfonamides.[3]

Aware of the threat of humans developing antimicrobial resistance as a result of the use of antimicrobials in food-producing animals, many countries have established a series of legislations and control measures.[4] In the EU, the use of antimicrobials as feed additives has been prohibited since 2006[5] and Regulation (EC) No. 470/2009 establishes Maximum Residue Limits (MRLs) for antimicrobials in food of animal origin.[6] Also, the Rapid Alert System for Food and Feed (RASFF) has been set up to exchange information about serious risks detected in food and feed. According to RASFF, 122 notifications were issued in 2009 for the presence of veterinary drug residues in a range of food products.[7] Moreover, Commission Decision 2002/657/EC was issued by the EU concerning the performance criteria and procedures for the validation of screening and confirmatory methods.[8]

In the USA, the use of antimicrobials in animals for food production has long been debated between the FDA and livestock producers, and in 2010 this agency released a draft guide on the rational use of these drugs in food-producing animals, as a step to control their application in this context.[3]

Sulfonamides, a diverse class of antimicrobials, are used in animal husbandry for prophylactic and therapeutic purposes and are administered mainly through feed. The concentration of these drugs in medicated feeds ranges between 70 and 800 mg/kg for pig and poultry feeds,[9] which are generally produced in the same production line as unmedicated feeds. The cross contamination of unmedicated feeds during production or transport and the misuse of drugs as feed additives lead to the presence of sulfonamide residues in unmedicated feeds, thus causing the accumulation of these compounds in animals. The MRL for total sulfonamides in food of animal origin is 100 μg/kg.[10] In addition to contributing to the development of antimicrobial resistance, the presence of sulfonamide residues in food-producing animals is of concern because of the potential carcinogenic activity of these drugs in humans.[11]

From an analytical perspective, animal feed is a complex matrix and its composition varies depending on the consumer and to a lesser extent on each production batch. Consequently, each kind of feed has particular characteristics and the varying interfering compounds of the feed make it difficult to develop reliable analytical methods. To overcome this problem, an efficient clean-up step or highly selective detection is required. To the best of our knowledge, there is only one method available to measure multiple sulfonamide compounds along with other compounds in feed matrices; this method is based on liquid chromatography (LC) coupled to tandem mass spectrometry.[12] Other approaches using LC with UV[13–16] or fluorescence detection can also be used to determine either one or two sulfonamide compounds.[14] A few screening methods based on enzyme linked immunosorbent

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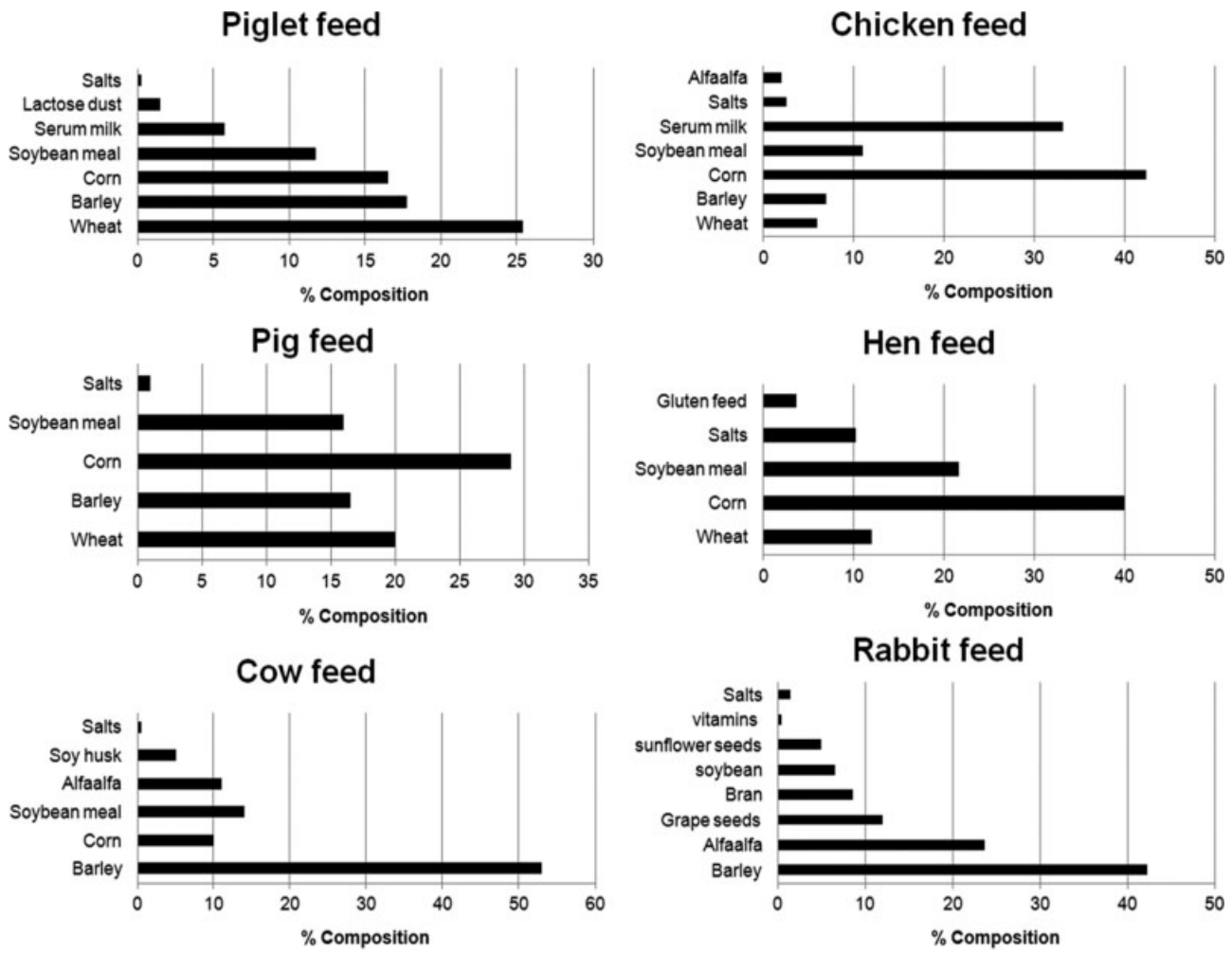


Figure 1. Composition of animal feed samples.

assays (ELISA) have also been proposed.[17–18] Sample treatment usually involves solid-liquid extraction or pressurized liquid extraction (PLE),[12] followed by a clean-up step based on solid phase extraction (SPE).[13,19] In the case of foods of animal origin, many chromatographic methods are available to analyze sulfonamide residues.[11]

Here we studied four distinct SPE phases to achieve efficient clean-up of feed extracts. In addition, we developed and validated an analytical method that applies LC-UV for the determination of sulfachloropyridazine (SCP), sulfadiazine (SDZ), sulfadimidine (SDD), sulfadoxine (SDX), sulfamethoxypyridazine (SMP), sulfaquinoxaline (SQX), sulfamethoxazole (SMX), and sulfadimethoxine (SDM) in various animal feed matrices.

Experimental

Chemicals and solutions

Sulfadiazine, sulfadimidine, sulfamethoxazole, sulfachloropyridazine, sulfadoxin, sulfadimethoxin, sulfaquinoxaline, and sulfamethoxypyridazine, all Vetranal grade, were purchased from Riedel-de Haen (Buchs, Switzerland). Methanol (Merck, Darmstadt, Germany) and acetonitrile (Panreac Quimica SAU, Barcelona, Spain) of LC gradient grade were used. Ultrapure water (Milli Q, Millipore, Molsheim, France) of 18.2 MΩcm^{-1} resistivity was used. All other reagents used were of analytical grade.

A single stock standard solution of all sulfonamides with a concentration of 50 mg/l of each sulfonamide was prepared from solid standards in methanol. The stock solution was stored in amber glass vials at 4 °C for up to 6 months.

Calibration standards (50, 70, 100, 150, and 200 µg/l) were prepared from the stock solution by diluting with initial mobile phase solution (83% buffer: 17% acetonitrile) in a 5-ml volumetric flask.

Matrix-matched calibration standards (50, 70, 100, 150, and 200 µg/l) were prepared by diluting 0.85 ml of blank SPE extracts of the respective feeds and a range of volumes of the stock standard solution (50 mg/l) with the aqueous mobile phase in a 5-ml volumetric flask. Calibration standards were prepared daily.

Preparation of spiked feeds

Pig, rabbit, hen, cow, chicken, and piglet feed samples were kindly provided by the *Associacio Catalana de Fabricants de Pinsos* (ASFAC). They were stored at 4 °C in polyvinylchloride (PVC) flasks. Samples were tested to be free of any analytes, with an LC/MS/MS method. Figure 1 shows the composition of the feeds.

Spiked feed was prepared by mixing blank pig feed with solid sulfonamide standards at a concentration of 2 mg/kg. Initially, a

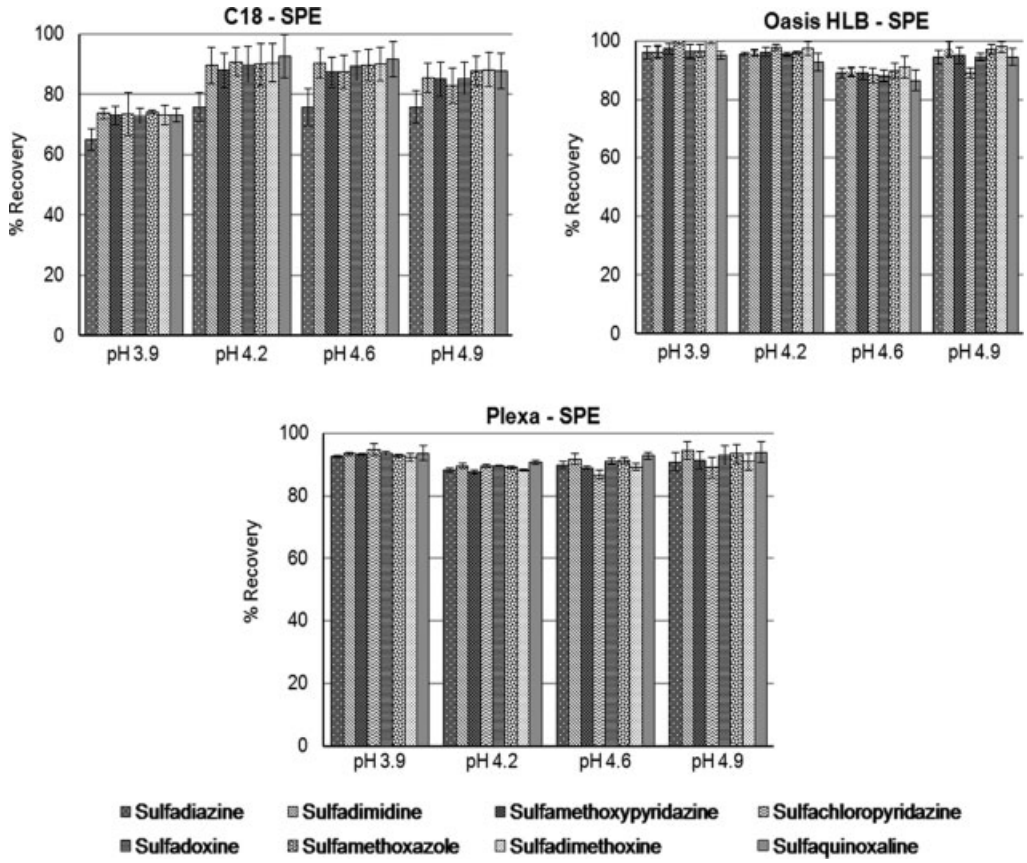


Figure 2. Effect of pH on the retention of sulfonamides in various SPE stationary phases during sample preparation. Standard deviations (n = 3 replicates) are represented as error bars.

sulfonamide concentration of 500 mg/kg material was prepared by adding 62.5 mg of each sulfonamide to 125 g of the pig feed. The mixture was then placed in a PVC flask containing teflon balls and rolled on a rolling table for at least 90 h. Homogeneity of the material was checked by taking samples from several parts of the material and determining the concentration of the sulfonamides by LC-UV. After checking the homogeneity, 10 g of this material was diluted with 90 g of blank pig feed to obtain a new material with a sulfonamide concentration of 50 mg/kg. Homogeneity was checked as described before. The feeds spiked at a concentration of 2 mg/kg were prepared by diluting 4g of the 50 mg/kg pig feed with the respective blank feeds. After homogenization and checking homogeneity, the spiked feed samples were stored in a refrigerator at 4 °C. In these conditions, these materials were stable for up to 18 months.[17]

Extraction and clean-up

To optimize the SPE step of the method, we studied the following four sorbents: Oasis HLB (3 ml × 60 mg cartridges), an N-vinylpyrrolidinedivinylbenzene copolymer sorbent with a hydrophilic-lipophilic balance was purchased from Waters (Milford, MA, USA); Bond Elut C18 (1 ml × 100 mg cartridges), a hydrophobic silica-based sorbent; Bond Elut Plexa (3 ml × 60 mg cartridges), a restricted access material based on a polymeric sorbent with hydroxyl groups on the surface and a hydrophobic polystyrene – divinylbenzene copolymer core; and Bond Elut Plexa PCX (3 ml × 60 mg cartridges), a polymeric sorbent with a strong cation exchange functionality.[20] All Bond Elut cartridges were purchased from Varian (Lakeforest, CA, USA). Rapid Trace® SPE Workstation by Caliper Lifesciences (Hopkinton, MA, USA), an automated SPE system, was used to load sample and solvents onto the cartridge by positive pressure.

Pig feed was used in all the optimization experiments. The effect of pH on sulfonamide retention in C18, Oasis HLB, and Plexa SPE was assessed by loading 500 µg/l standard solutions (in triplicate) prepared at pH 3.9, 4.2, 4.6 and 4.9 and elution with 2 ml of acetonitrile without any washing step. The pH was measured with a Crison GLP21 (Alella, Spain) pH meter equipped with a Crison 52-02 Ag/AgCl combined glass electrode. In Oasis HLB and Plexa, the effect of washing solvent strength on sulfonamide recoveries and clean-up of matrix components was studied by loading blank extracts and standard solutions prepared at pH 3.9. Several solutions of methanol in water (10%, 25%, and 50% *v/v*) were tested as washing solvents.

For Plexa PCX, blank feed extracts and extracts spiked at 500 µg/l, evaporated and reconstituted in 4% phosphoric acid, were loaded (in triplicate) onto the cartridges to assess sulfonamide recovery and clean-up of matrix components. The washing was done in two steps. In the first, 1% formic acid in water was used to keep the sulfonamides in a protonated form while in the second step formic acid in methanol and methanol-water mixtures were studied for their clean-up efficiency. The analytes were eluted with 2 ml of 0.5% ammonia in MeOH: ACN (1 : 1).

The method adopted was as follows: 1 g of spiked or blank feed sample and 10 ml of acetonitrile : water (95 : 5 *v/v*) were added to a 25-ml centrifuge tube, which was then capped, shaken manually for 1 min and then centrifuged at 3500 rpm for 10 min.[17]

A Heraeus Christ Labofuge 400 centrifuge (Osterode am Harz, Germany) with a range of 600 to 6000 rpm was used. An aliquot of 8.5 ml of the supernatant was decanted into a glass test tube, evaporated to dryness at 50 °C for about 30 min in a TurboVap® LV Evaporator Workstation by Caliper Lifesciences (Hopkinton) and reconstituted with 4.25 ml of 4% Phosphoric acid. The sample was then vortexed and placed in the sample rack of the Rapid Trace SPE. The Plexa PCX SPE cartridge was conditioned with 1 ml of methanol, followed by 1 ml of water. Four ml of sample was loaded onto the cartridge at a flow rate of 2 ml/min. The cartridge was washed in two steps with 1 ml of 1% formic acid in water and 1 ml of 2% formic acid in methanol. It was then dried with a nitrogen flow for 0.5 min and eluted with 2 ml of 0.5% ammonia in MeOH: ACN (1 : 1). A portion of 0.85 ml of the eluate was taken and made up to 5 ml in a volumetric flask with pH 4.7 buffer to match initial mobile phase conditions. It was then filtered into injection vials using syringes and 0.45-µm nylon filters from Micron Analitica, S. A. (Madrid, Spain).

Liquid chromatography with UV detection

Chromatographic separation was carried out with an Agilent 1100 (Santa Clara, CA, USA) series liquid chromatography instrument consisting of a degasser, a quaternary pump, an automatic liquid sampler and a diode array detector with tungsten and deuterium lamps. A C8 Inertsil chromatographic column from GL Sciences Inc. (Tokyo, Japan) with a length of 250 mm and diameter of 4.6 mm (5 µm) was used. A pre-column, matching the characteristics of the column and with dimensions of 7.5 × 4.6 mm, was used. Mobile Phase A consisted of 0.01 M aqueous acetic acid – sodium acetate buffer (pH 4.7) and mobile phase B comprised acetonitrile. The LC gradient, at room temperature, began with mobile phase B at 17%, reaching 25% in 15 min, 40% in 20 min and then returning to initial conditions at 25 min with total run time of up to 40 min. The flow rate was maintained at 0.6 ml/min, injection volume was 50 µl and the diode array detector was set at 268 nm.

Method validation

For pig feed, linearity was assessed with matrix-matched standards of 50, 70, 100, 150, and 200 µg/l of sulfonamides, which were prepared and measured on three days. For rabbit, piglet, hen, chicken, and cow feeds, matrix-matched standards at the same levels were prepared and measured on one day. Limits of detection (LODs) and limits of quantification (LOQs) were calculated from ordinary least squares regression data[21] from calibration curves obtained with matrix-matched standards of lower concentrations of 20 to 60 µg/l in pig feed extracts. LOD and LOQ correspond to the analyte concentrations for which the peak area is equal to 3 and 10 times the standard deviation of the intercept, respectively. Inter-day and intra-day precisions of the methodology were assessed by analyzing the pig feed spiked at 2 mg/kg in six replicates on three consecutive days. For rabbit, piglet, hen, chicken, and cow feed samples, intra-day precision was assessed by analyzing six replicates of 2 mg/kg-spiked feeds on the same day. Matrix effect was assessed by comparing the slopes of the calibration curves in solvent (external standards) and matrix-matched calibration curves obtained in the feeds. For this purpose, we used the following *t*-test:[22]

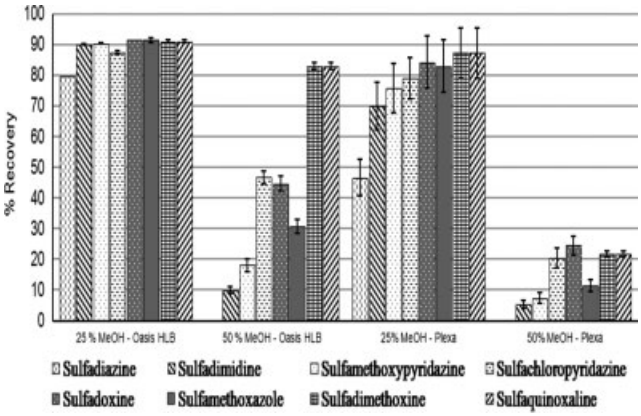


Figure 3. Effect of washing solvent strength on retention of sulfonamides in Oasis HLB – SPE and Plexa – SPE during sample preparation. Standard deviations (n = 3 replicates) are represented as error bars.

$$t_{calc} = \frac{b_1 - b_2}{\sqrt{s_{b_1}^2 + s_{b_2}^2}} \quad (1)$$

where b_1 and b_2 are the slopes and S_{b1} and S_{b2} are the standard errors of slopes of the external calibration curve and matrix-matched calibration curve, respectively. The *t* values calculated were compared with the critical ones at 95% confidence level.

Results and discussion

Extraction and clean-up

Sulfonamides can be extracted from feed matrices by manual shaking for 1 min with acetonitrile containing 5% water.[17] Therefore we adopted this extraction procedure in this study.

The suitability of an SPE phase relies on its capacity to retain the analytes and to remove matrix components to the fullest extent possible. The performance of the three non-functionalized SPE sorbents to retain analytes was studied at several pH values. Under the conditions tested, C18 had less retentive capacity for sulfonamides than Oasis HLB and Plexa, and provided less repeatable results (Figure 2). Recoveries at pH 3.9 in Oasis HLB and Plexa were slightly higher than at the other pH values. Indeed, the average recoveries for all the analytes at pH 3.9 were 98% (Oasis HLB) and 94% (Plexa) compared with 95–98% (Oasis HLB) and 89–92% (Plexa) at the other pH values. Thus pH 3.9 was selected for further studies.

In order to ascertain the clean-up capacity of the sorbents, several washing solutions were assessed. A preliminary study using a 10% solution of methanol in water showed that SDZ was washed off in C18 cartridges. Therefore C18 was not studied further. When a 25% aqueous solution of methanol was applied as washing solvent, Oasis HLB gave similar recoveries for all analytes and more repeatable results than Plexa (Figure 3). When 50% methanol was used, significantly lower recoveries were obtained for most analytes in both cartridges. Moreover, chromatograms of blank feed extracts showed that the removal of interferences (matrix peaks) improved in Oasis HLB (Figure 4A), with an increase in the methanol percentage; however, no improvement was detected in Plexa (Figure 4B). On the basis of these observations, we concluded that Oasis HLB shows the highest clean-up capacity of the three non-functionalized phases.

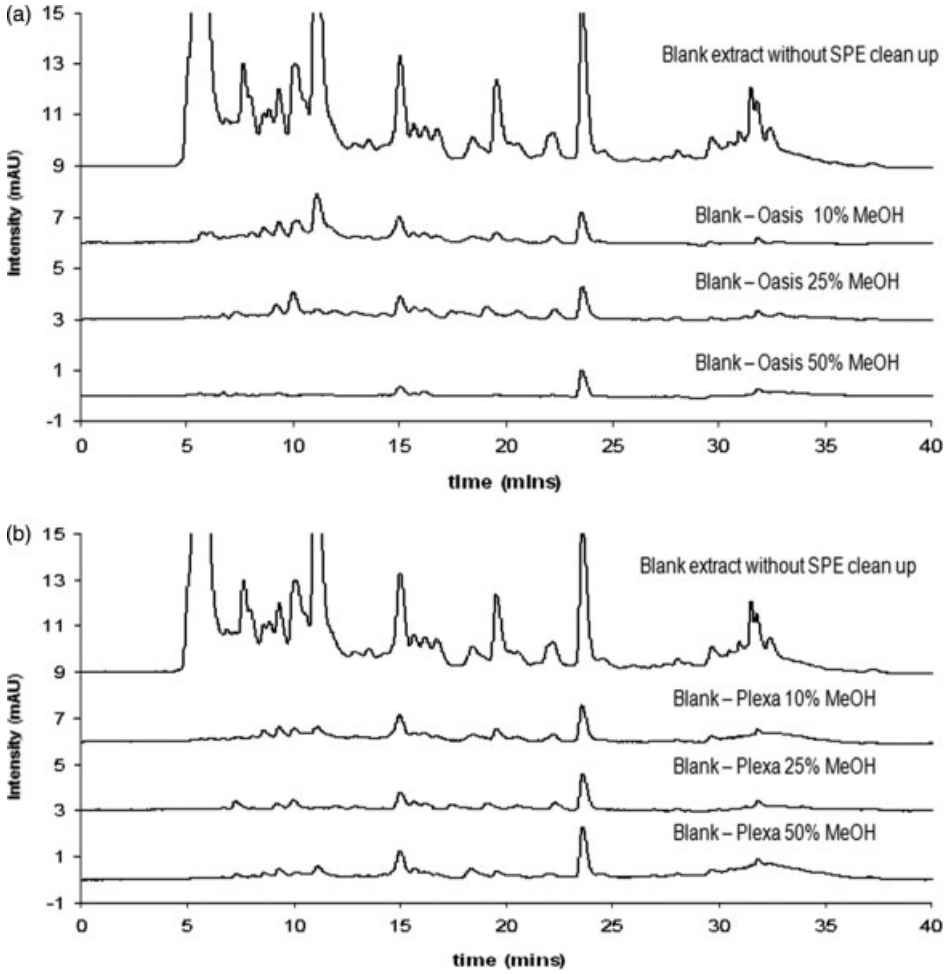


Figure 4. (A) Chromatograms of blank pig feed samples after Oasis HLB – SPE and (B) Plexa – SPE during sample preparation, measured at 268 nm wavelength.

Because sulfonamides become protonated at pH values below 4, we also studied a clean-up approach based on an SPE cation exchange sorbent, Plexa PCX. In this case, after solvent evaporation, the extracts were reconstituted with a 4% phosphoric acid solution and loaded onto the cartridges. A first washing step with 1% formic acid did not cause any loss of analytes. In order to improve extract clean-up, a second washing step with acidic solutions containing methanol was applied. In contrast to the other phases, an increase in the methanol percentage did not lead to greater loss of analytes (Figure 5). Moreover, 2% formic acid in MeOH produced cleaner extracts than 2% formic acid in MeOH: water mixtures (Figure 6).

Finally, pig feed spiked at 2 mg/kg was analyzed in six replicates using the optimized Oasis HLB and Plexa PCX SPE approaches. Plexa PCX cartridges gave recoveries ranging between 47% and 66% with RSD% between 4% and 15% while those for Oasis HLB cartridges ranged between 47% and 77% with RSD% between 5% and 21% (Figure 7). Although Plexa PCX produced lower recovery values than Oasis HLB, it gave more repeatable results for all the analytes and thus, Plexa PCX was finally selected.

Method validation

Calibration curves with matrix-matched standards of the six feeds were prepared in the range 50–200 µg/l. Table 1 shows the linearity parameters of the calibration curves obtained in pig feed. Curves corresponding to the other feeds showed similar linearity parameters. Figure 8A shows the chromatogram of a 200 µg/l matrix-matched standard in pig feed.

LOD and to a lesser extent LOQ are not robust parameters. Depending on the method applied to estimate LOD, mathematical artifacts without analytical significance are sometimes obtained. These artifacts are attributed to the fact that LOD and LOQ must be calculated from calibration curves at the lowest concentration levels of the method linear range, where the precision of signal values is low. Here we applied the approach based on signal-to-noise ratio and the one based on standard deviation of the intercept of the calibration curve. The first approach gave LOD values between two and five times lower than the second. On the basis of our practical experience using this method, we concluded that the values obtained from the regression data were more realistic. They were therefore adopted and consigned in Table 1. Figure 8B shows a chromatogram of a 20 µg/l matrix-matched standard overlaid on a chromatogram of a blank feed extract. This concentration was close to the LOD for most of the analytes, except SDZ and SMX, which showed twice the LOD values. The analyte peaks were well distinguished from the matrix peaks and their areas were measured for quantification purposes.

Precision and trueness expressed as recovery values are summarized in Table 2. Matrix matched calibration standards

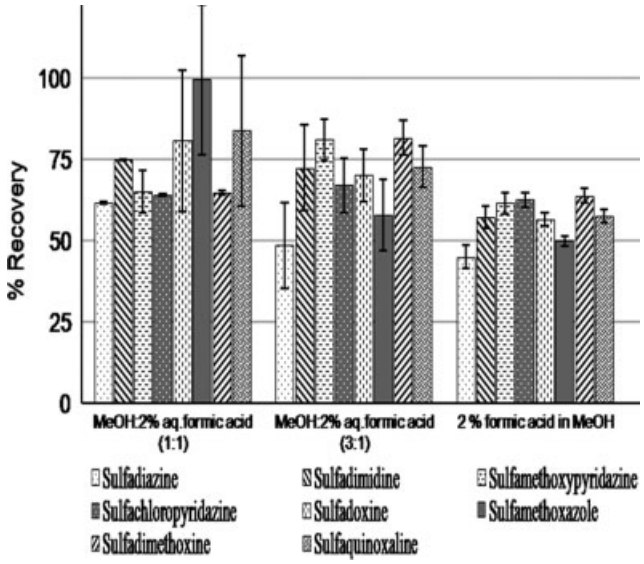


Figure 5. Effect of washing solvent in second washing step on recovery % using Plexa PCX – SPE. Standard deviations (n = 3 replicates) are represented as error bars.

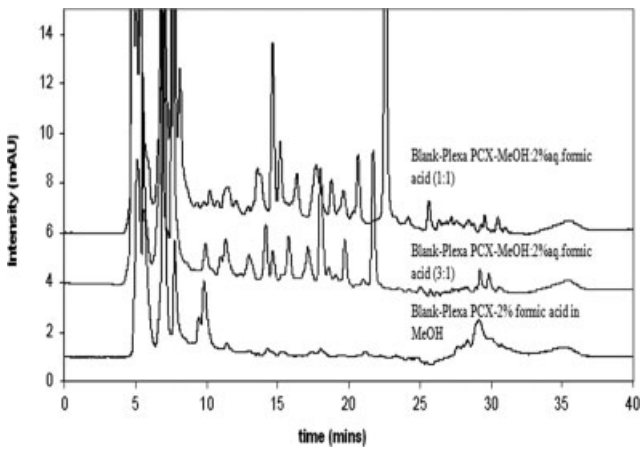
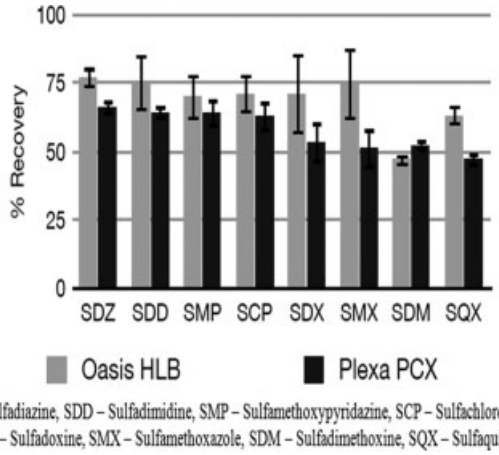


Figure 6. Chromatograms of blank pig feed samples after Plexa PCX-SPE with a range of washing solvents in a second washing step, measured at 268 nm wavelength.



SDZ – Sulfadiazine, SDD – Sulfadimidine, SMP – Sulfamethoxypyridazine, SCP – Sulfachloropyridazine, SDX – Sulfadoxine, SMX – Sulfamethoxazole, SDM – Sulfadimethoxine, SQX – Sulfaquinoxaline.

Figure 7. Sulfonamide recoveries and standard deviation of the Oasis HLB and Plexa PCX SPE approaches assessed with spiked pig feed (2 mg/kg). Standard deviations (n = 3) are represented as error bars.

Table 1. Linearity parameters (n = 3 days, 5 levels), LODs and LOQs of the sulfonamides in LC-UV calculated from ordinary least square regression data using matrix-matched standards in pig feed

	Slope	Intercept	R^2	LOD in extract µg/l	LOQ in extract µg/l	LOD in sample µg/kg	LOQ in sample µg/kg
SDZ	0.3956	−2.4985	0.9998	13	45	191	662
SDD	0.3544	−3.2839	0.9985	5	18	74	265
SMP	0.3272	1.0656	0.9964	10	33	147	485
SCP	0.2563	−1.7394	0.9999	9	29	132	426
SDX	0.3687	−4.1828	0.9966	5	18	74	265
SMX	0.465	−6.661	0.9996	18	59	265	868
SDM	0.3284	−2.3985	0.9983	5	18	74	265
SQX	0.4047	−2.3437	0.9997	5	18	74	265

SDZ – Sulfadiazine, SDD – Sulfadimidine, SMP – Sulfamethoxypyridazine, SCP – Sulfachloropyridazine, SDX – Sulfadoxine, SMX – Sulfamethoxazole, SDM – Sulfadimethoxine, SQX – Sulfaquinoxaline.

were used for quantification. Table 2 shows that the average of the recovery values of all sulfonamides in each feed varied considerably between matrices. This finding could be attributed to the difference in the composition of the feed.

Analysis of complex samples such as animal feeds calls for the consideration of possible effects of sample matrix on the analytical signal. Matrix-matched calibration standards are generally used for quantification purposes in order to compensate for the effects of matrix mismatch between samples and standards. The use of calibration standards for solvents simplifies the analytical methodology and they can be used for quantification provided there are no significant matrix effects.[23]

In order to ascertain the presence of matrix effects, we compared the slopes of the calibration curves in solvent with those obtained with matrix-matched standards. No significant differences at 95% confidence level between the slopes of any analyte were observed in pig and rabbit feed (Table 3). In the case of chicken feed, only SQX showed a significant difference. Matrix effects were more severe in the other three feeds studied. When matrix effects cause non-acceptable errors in analyte quantification, the method of standard additions should be used for quantification when blank feeds are not available to prepare matrix-matched standards. To assess the effect of matrix mismatch in quantification, feeds spiked at a concentration of 2 mg/kg (six replicates) were analyzed and quantified with both matrix-matched calibration curve and external calibration curve. Maximum relative errors in the concentrations of analytes quantified with external standards were 32% in hen and piglet feed and 46% in cow feed.

Conclusions

Of the few methods reported for the determination of sulfonamides in animal feeds, all of them address the analysis of only one[12–13,17–18] or two[15–16] kinds of feeds, mostly those used for pigs. Moreover, except for a recent study[12] devoted to LC-MS/MS analysis of 22 veterinary drugs belonging to the β-lactam and sulfonamide families, the remaining ones deal with the analysis of one or two compounds. With regard to sample treatment,

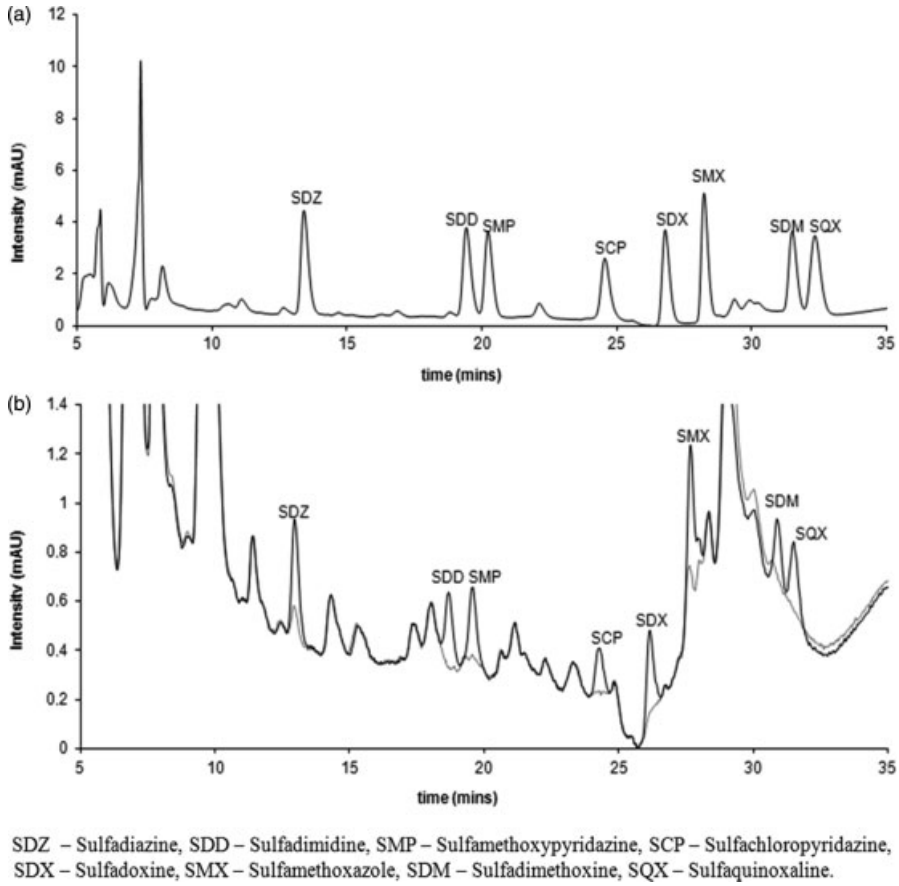


Figure 8. (A) Chromatogram of a 200 µg/l matrix-matched standard in pig feed and (B) chromatogram of a 20 µg/l matrix-matched standard overlaid on blank pig feed sample, measured at 268 nm wavelength.

Table 2. Intra-day precision (n = 6), inter-day precision (n = 18) and accuracy of the whole LC-UV method assessed with the spiked feed (2 mg/kg) in several feed matrices prepared by homogenising blank feeds with solid sulfonamide standards

	Pig feed		Rabbit feed	Piglet feed	Hen Feed	Chicken feed	Cow feed
	Recovery % (Intra-day precision - RSD%)	Inter-day Precision (RSD%)	Recovery % (Intra-day precision - RSD%)	Recovery % (Intra-day precision - RSD%)	Recovery % (Intra-day precision - RSD%)	Recovery % (Intra-day precision - RSD%)	Recovery % (Intra-day precision - RSD%)
SDZ	66(4)	7	60(5)	52(3)	61(4)	56(5)	52(6)
SDD	64(4)	11	51(14)	37(4)	67(5)	56(12)	48(11)
SMP	64(8)	18	58(6)	47(12)	64(9)	66(10)	51(9)
SCP	63(9)	17	59(11)	68(8)	55(7)	67(4)	55(14)
SDX	53(14)	16	75(20)	42(11)	63(17)	52(19)	46(20)
SMX	51(15)	14	43(19)	40(17)	45(12)	45(15)	40(10)
SDM	52(4)	14	48(5)	38(3)	52(5)	46(3)	43(5)
SQX	47(6)	11	38(8)	33(3)	42(4)	38(7)	43(11)
Average recovery %	58		54	45	56	53	47

SDZ – Sulfadiazine, SDD – Sulfadimidine, SMP – Sulfamethoxypyridazine, SCP – Sulfachloropyridazine, SDX – Sulfadoxine, SMX – Sulfamethoxazole, SDM – Sulfadimethoxine, SQX – Sulfaquinoxaline.

Croubels *et al.*[13] applied cation exchange SPE to purify sulfadiazine extracts, achieving a recovery of 31%. After performing a pressurised liquid extraction, Kantiani *et al.*[12] used Oasis HLB cartridges but only relative recoveries (corrected by the surrogates) were reported. Here we propose a method that comprises a rapid solid-liquid extraction and an SPE clean-up with Plexa PCX cartridges. This approach is suitable for the determination of eight sulfonamide residues in six feed matrices. It can also be used for quality control and inspection purposes for feed containing higher concentrations of these drugs, such as in medicated feeds. Concerning the calibration, external standards can be used in the analysis of pig and rabbit feed, as studied in the present work.

Table 3. Study of matrix effect by comparing slopes of external calibration curve (prepared in solvent) and matrix- matched calibration curve (prepared in blank SPE eluates). (+) Presence of matrix effect (slopes of two regression lines are significantly different at 95% confidence level). (−) No matrix effect (slopes of two regression lines are not significantly different at 95% confidence level). Measurement was done with LC-UV

	Pig feed	Rabbit feed	Piglet feed	Hen feed	Chicken feed	Cow feed
SDZ	−	−	+	+	−	+
SDD	−	−	−	−	−	+
SMP	−	−	+	−	−	+
SCP	−	−	+	+	−	+
SDX	−	−	+	−	−	−
SMX	−	−	+	−	−	+
SDM	−	−	−	−	−	+
SQX	−	−	+	+	+	+

SDZ – Sulfadiazine, SDD – Sulfadimidine, SMP – Sulfamethoxypyridazine, SCP – Sulfachloropyridazine,
SDX – Sulfadoxine, SMX – Sulfamethoxazole, SDM – Sulfadimethoxine, SQX – Sulfaquinoxaline.

In contrast, matrix-matched standards or the standard additions approach should be used with piglet and cow feeds. For hen and chicken feeds, only a few analytes would require these approaches. Given its simplicity and the instrumentation involved, the proposed method can be implemented in most routine laboratories as a quantitative method, provided that confirmation of analyte identity is not required. According to EU legislation,[8] a confirmatory analysis calls for the use of LC-MS/MS, which is a more powerful but also more expensive technique.

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