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Determination of five sulfonamides in medicated feedingstuffs by liquid chromatography with ultraviolet detection

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Abstract

A fast and reliable method of liquid chromatography and ultraviolet detection of sulfaguanidine, sulfadiazine, sulfamethazine, sulfamethizole, and sulfamethoxazole in feedingstuffs was described. The method involves THE procedure of preparation of spiked samples, and extraction of sulphonamides from the matrix using a mixture of methanol and acetonitrile, followed by drying the extract and dissolving it in a phosphate buffer. The analysis uses octadecyl (C18) analytical column with UV detection at $\lambda = 260$ nm and a gradient programme of mobile phase composition. The analytical procedure has been successfully adopted and validated for quantitative determination of the sulfonamides in feedingstuff samples. Validation included sensitivity, specificity, linearity, repeatability, and intra-laboratory reproducibility. The mean recovery of sulfonamides was 84%, within the working range of 200–2000 mg/kg. Direct, simple sample preparation and HPLC-UV analysis allow the method to be successfully included in the scope of routine analyses. The presented results could be an answer to a need of simple and easy method for sulfonamide determination applicable in medicated feedingstuffs analysis.

Key words: medicated feedingstuffs, sulfonamides, high performance liquid chromatography, ultraviolet detection.

Introduction

Sulfonamides are amongst five most groups of chemotherapeutic agents in animal husbandry commonly used for therapeutical and prevention purposes (24). The mechanism of their action relies on the requirement of susceptible organisms to synthesise folic acid as a precursor of other essential molecules in body cells; sulfonamides act as false substrates in folic acid synthesis (22). Their use is common owing to the wide spectrum of antimicrobial activity and a relatively low price. Sulfonamides are most frequently administered in rather high doses, and as a result, elevated levels of the drugs may be found in parenchymal tissues. Their residues in edible tissues and food of animal origin may cause adverse effects, such as allergic reactions in humans, and drug resistance in microorganisms (22).

Medicated feedingstuffs are probably the easiest route of administering drugs to large animals in commercial farms. The production and use of medicated feedingstuffs are not changing in the last years, amounting to millions tons in the European Union (EU), and sulfonamides are on the second position in the rank of use after tetracyclines (21, 24). Typical levels of sulfonamides in medicated feedingstuffs range between 200 and 2000 ppm. In most cases, only one sulfonamide is added to feedingstuffs; however, it can be combined with an antibiotic or occasionally more than one sulfonamide may be present. In accordance with current legislation, it is mandatory for the manufacturer to control the parameters of medicated feedingstuffs (homogeneity, stability, and storability) to ensure that products comply with §4 of the directive 90/167/EWG (6). Concentration of drugs must be surveyed to maintain the therapeutic level and to prevent contamination of edible tissues in case of overdosing. Controlling medicated feedingstuffs, as demanded by legislation, requires a method of quantitative sulfonamide analysis

to be developed as a routine laboratory procedure. The presented results could be an answer to a need of simple and easy method for sulphonamide determination in feedingstuffs. Therefore, the study focuses on developing a fast method for determination of five sulfonamides (sulfaguanidine, sulfadiazine, sulfamethazine, sulfamethizole, and sulfamethoxazole) in medicated feedingstuffs.

Commonly used microbiological methods are not suitable for the purpose of this study due to their poor sensitivity and selectivity (21, 31). The majority of techniques for sulfonamide detection in feedingstuffs described in recent papers most commonly use chromatographic methods. These methods comprise thin-layer chromatography (TLC) (5), enzyme immunoassay (EIA) (20, 27), high performance liquid chromatography (HPLC) with ultraviolet (UV) detection (8, 10, 13, 17, 25, 26, 28), HPLC-UV in premix (28, 29), HPLC with diode array detector (DAD) (1, 11), HPLC-DAD in premix (2), HPLC with fluorescence detector (FLD) (13, 2), HPLC connected to mass spectrometry (MS) (3, 14, 15, 18, 19, 23), and micellar electrokinetic capillary chromatography (MEKC) with UV detection (12). The study of the literature allowed us to select and successfully optimise the chromatographic method with UV detection as a procedure, which can be easily implemented by any laboratory.

Material and Methods

Reagents and chemicals. Certificated analytical standards (CRM) of sulfaguanidine (CAS: 6190-55-2), sulfadiazine (68-35-9), sulfamethazine (57-68-1), sulfamethizole (144-82-1), and sulfamethoxazole (723-46-6) were obtained from Sigma-Aldrich (USA). Substance standard (RM) of sulfaguanidine (cat. no S8751), sulfadiazine (S8626), sulfamethazine (S6256), sulfamethizole (S5632), and sulfamethoxazole (S7507) were obtained from Sigma-Aldrich (USA). Acetonitrile HPLC grade, methanol HPLC grade, orthophosphoric acid pure p. a. grade were obtained from POCh Gliwice (Poland), sodium hydroxide BioXtra was delivered by Sigma-Aldrich (USA). Regenerated cellulose syringe filter (0.45 µm) was obtained from Sartorius (Germany). Ultra-pure water was obtained from Milli-Q system.

Standard solution. Standard stock solutions of each sulfonamide (1 mg/mL) were prepared in methanol, except sulfadiazine, which was prepared in methanol:acetonitrile (50:50 v/v), and frozen for no longer than six months (-18°C). All standard stock solutions were made from a CRM. In addition, purity and water content of the standard substances were considered. Working standard solutions for the calibration curve were prepared by appropriate dilutions of stock in a phosphate buffer (0.04 M, pH = 6.5); its stability was tested for one month while being refrigerated (5 ± 3°C).

Samples. Sulfonamide-free samples of feedingstuffs used in preparation of fortified samples and as matrix samples were obtained from an animal farm, a subsidiary of the National Veterinary Research Institute in Pulawy (NVRI). The medicated feedingstuffs, designed for swine and poultry as starter and grower diets, were provided by local commercial producers. In addition, 300–500 g samples of medicated feedingstuffs from manufactures at the Polish market were obtained along surveillance programme hold by Veterinary Inspection and NVRI according to the directive 90/167/EWG (6).

The house reference material (HRM) was produced by mixing appropriate amounts of each sulfonamide standard (RM) with feedingstuff. Hence, analytical matrix constituted the feedingstuff for sows, which contained 16% of protein, 3.5% of fat, and 6% of ash and fibre. The preparation of the HRM was performed as follows: initially the RMs and blank feedingstuff were weighed to obtain 20 g portions and quantitatively transferred to an 80 mL container. The obtained material was mixed in a vortex mixer for 2 min at about 2000 rpm, then quantitatively transferred to a 1 litre glass bottle; the container was flushed three times using blank feedingstuff, and mixed with additional blank feedingstuff to obtain a total mass of 300 g. The bottle was shaken for 2 h in a horizontal shaker at 300 rpm. Afterwards, the sample was ground in a centrifugal mill (ZM 200, Retsch) with a 1.5 mm sieve at 6000 rpm. The obtained material was transferred to the same bottle and shaken for 1 h again in the horizontal shaker. The resulting HRM was checked for homogeneity by analysing six different portions of the sample. The HRM was then refrigerated (2–8°C) and used as a quality control sample.

Extraction. Prior to the analysis, all samples were ground in a centrifugal mill (ZM 200, Retsch) with a 1.5 mm sieve. A 2 g portion of the ground feedingstuff sample was precisely weighed into a 50 mL propylene centrifuge tube, and 25 mL of an extraction solvent, consisting of methanol and acetonitrile (50:50 v/v), was added. The tube was shaken for 1 min in a vortex mixer at 2000 rpm, followed by 30 min in a horizontal shaker (KS 501, IKA) at 250 rpm. The sample was then centrifuged (6K15, Sigma), 10 min at 4000 × g, at 10°C. The supernatant (1 mL) was transferred to a 6 mL conical vial and dried under a gentle stream of nitrogen at 47.5 ± 2.5°C. The residue was reconstituted in 4 mL of a phosphate buffer, pH 6.5. After mixing for 1 min, the solution was filtered through a syringe filter and 20 µL of the liquid was injected into the chromatographic system.

HPLC-UV analysis. Agilent Series 1200 HPLC system (Agilent Technologies, Germany) equipped with a degasser, binary pomp, autosampler, column oven, and ultraviolet detector (G1314D) with absorbance wavelength set at $\lambda = 260$ nm was used. The system was controlled by Chemstation software (Agilent). Gradient elution was performed on Grace

Smart C18 (250 × 4.6 mm, 5 µm) analytical column (Grace, USA) thermostated at 22°C.

The mobile phase, containing acetonitrile and phosphate buffer, pH 6.5, was split into two solutions, phases A and B of 2% and 40% of acetonitrile, respectively. Both phases were mixed in the chromatographic system pump in gradient elution; from 100% phase A held for 2 min and linearly changing to 100% phase B in 22 min. Then the initial conditions were restored. The analysis was performed at the flow rate of 1 mL/min.

Validation of the method. The procedure was assessed by evaluation of sensitivity, linearity, specificity, repeatability, and intra-laboratory reproducibility according the ISO/IEC 17025:2005/AC. Validation was conducted according to the procedures described below. The specificity of the method was evaluated by determining the limits of detection (*LoD*) and quantification (*LoQ*). For this purpose, gradients of HRMs calibration curves and standard deviations were studied using samples containing analytes in the range of the limits of detection. *LoD* and *LoQ* were calculated according to equation $LoD = 3.3*SD_{low}/a$ and $LoQ = 10*SD_{low}/a$, where SD_{low} was defined as a standard deviation for samples containing the analyte at 50 mg/kg and "a" as a slope of the calibration curve. Moreover, 24 blank samples of feedingstuffs for swine, poultry, and cattle were used as blank matrix samples and absence of interfering peaks was checked in the 5% range of the retention time window for each peak. Linearity was evaluated by determining the regression line using the least squares method and calculating the coefficient of determination (R^2). The linearity of each sulfonamide was established on five concentration levels (50, 100, 500, 1000, and 2000 mg/kg) excluding the blank matrix. Precision of the method was evaluated over the linear range at the same concentration levels by analysis of earlier pre-prepared HRMs. Repeatability was assessed by analysing the same HRMs sample in six repetitions during one day; whereas, intra-laboratory precision was checked by HRMs analysis at monthly intervals by two different operators. Recovery parameters for each sulfonamide were calculated from the results obtained for intra-laboratory precision. The stability of the stock standard solution was tested monthly for over a period of six months. Working standard solution was tested in the same manner as working standards by injection of freshly prepared dilutions.

Results

The typical chromatogram of the standard solution and a blank sample were compared on Fig. 1. Fig. 2 presents a chromatogram obtained from the extracts of HRMs with sulfonamides at different concentrations (50–2000 mg/kg). The percentage of calculated R.S.D. was lower than 0.3% for each of the compounds, which indicates stability of the chromatographic method (Table 1).

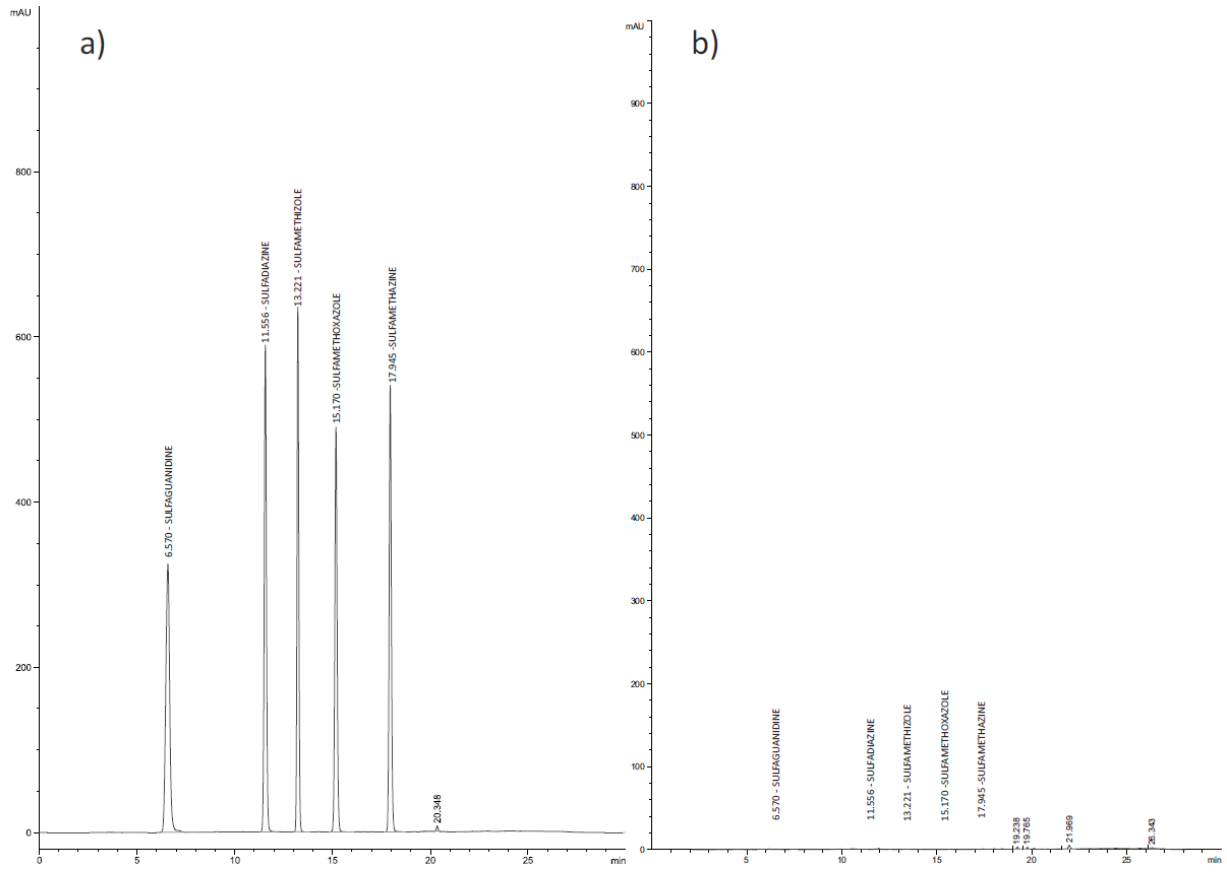


Fig. 1. Typical chromatogram of a) standard solution 50 µg/mL, b) blank feedingstuffs extract

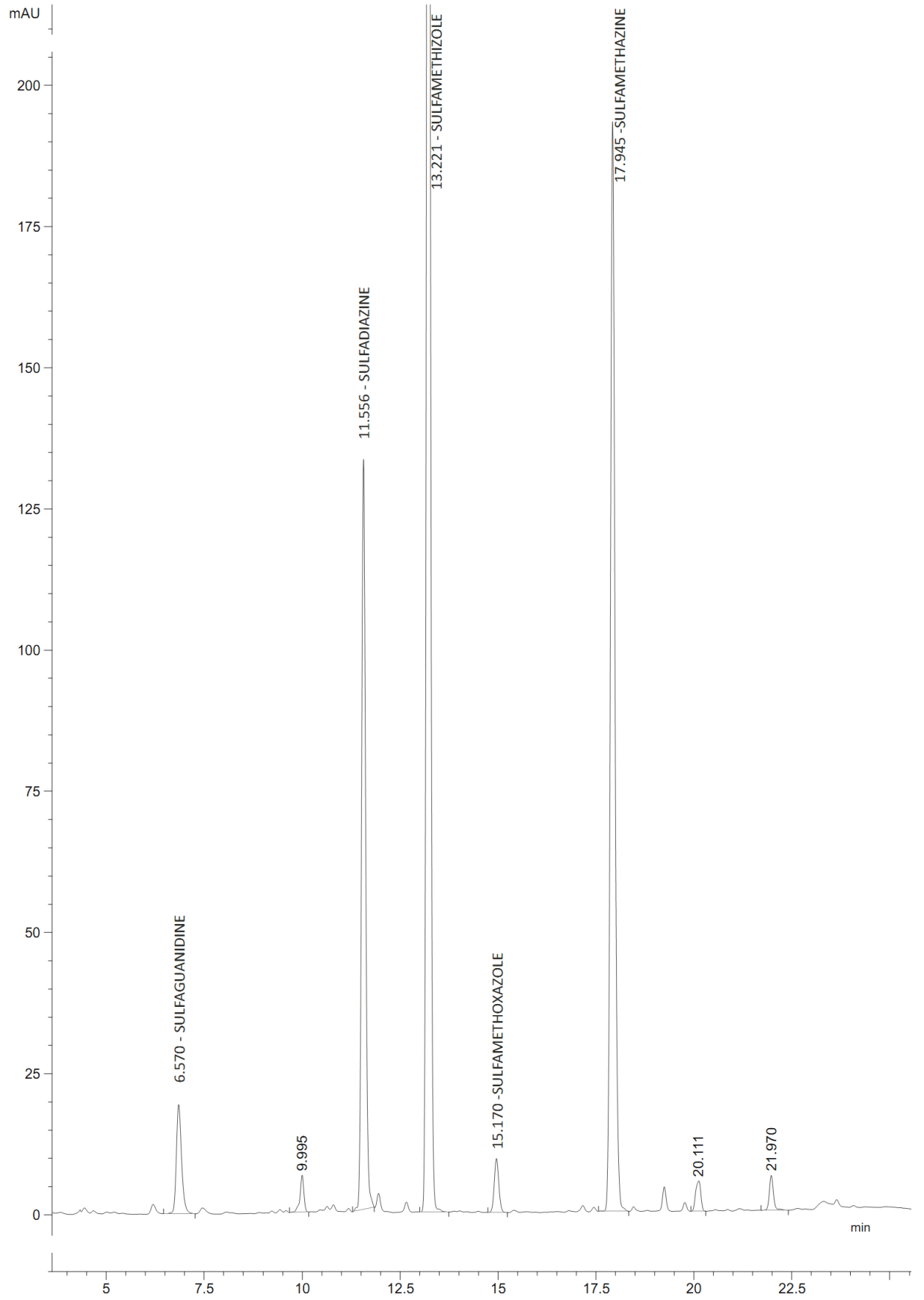


Fig. 2. Chromatogram of feedingstuff extract containing sulfaguanidine (100 mg/kg), sulfadiazine (500 mg/kg), sulfamethizole (2000 mg/kg), sulfamethoxazole (50 mg/kg), and sulfamethazine (1000 mg/kg)

Validation results. Linearity of the detector was evaluated using seven standard concentrations at the range of 0.2–100 µg/mL. Determination coefficients were higher than 0.999 for each of the analysed compounds. Good linearity parameters were obtained for HRMs analysis in the range of 50-2000 mg/kg with R^2 higher than 0.989. For each concentration level, the percentage of R.S.D. values of linearity coefficients were calculated over 18 repetitions of HRMs. All parameters are listed in Table 1.

Precision and absolute recovery of the method were evaluated at five concentrations over the linear dynamic range. Repeatability and intermediate precision of all compounds were less than 8% and 11%, respectively, except sulfadiazine, which variation was 15.8% for the lowest and 13.1% for the highest concentration tested. Recovery of all analytes varied from 68.8% to 116.6%. Recovery variation was less than 7% for all the compounds, except sulfadiazine (R.S.D. = 15%), which recovery was inversely related to concentration. The obtained relative standard deviation was compared with the results calculated from the Horwitz curve (8). Validation results were lower than values estimated from the Horwitz function except sulfadiazine at the lowest level in reproducibility studies. Precision parameters are listed in Table 2 and an overview of the results obtained for recovery can be seen in Table 3.

High coefficients for sulfadiazine in intra-laboratory RSD may be caused by poor homogeneity of the HRMs samples especially on a low concentration level. The sulfadiazine substance standard has fluffy texture making it hard to obtain homogeneity within the feedingstuffs. *LoD* and *LoQ* values for all compounds were listed in Table 4. The analytes were stable in matrix for at least 10 months when refrigerated (2–8°C). The uncertainty was calculated as the single result separately according to the rules for chromatographic methods (16), and was expressed as a sum of A and B uncertainty. Values of extended uncertainty are shown in Table 5.

Table 1. Linearity of detector and method

Compound	t_R (min)		a	b	r^2
Sulfaguanidine	6.80	solution	1.00064 (0.04)	-0.02 (8.7)	0.99989 (10^{-04})
	(0.28)	matrix	0.8614 (1.6)	14.92 (57.6)	0.9968 (0.1)
Sulfadiazine	11.52	solution	1.00030 (0.05)	-0.02 (0.91)	0.99999 (10^{-04})
	(0.13)	matrix	0.7823 (13.1)	60.64 (35.5)	0.9896 (0.9)
Sulfamethizole	13.02	solution	1.00018 (0.04)	-0.012 (3.4)	0.99999 ($8*10^{-06}$)
	(0.19)	matrix	0.8160 (4.9)	-20.82 (43.0)	0.9993 (0.01)
Sulfamethoxazole	15.21	solution	0.99992 (0.02)	0.005 (20.4)	0.99999 ($5*10^{-05}$)
	(0.10)	matrix	0.9690 (4.1)	-24.95 (26.6)	0.9981 (0.09)
Sulfametazine	17.78	solution	0.99582 (0.01)	0.013 (54.7)	0.99991 ($2*10^{-03}$)
	(0.06)	matrix	0.9277 (0.45)	3.45 (103.0)	0.9998 (0.02)

t_R – retention time; ( ) – relative standard deviation %

Table 2. Precision of the method

Concentration (mg/kg)	50	100	500	1000	2000
Compaund			Repeatability precision (R.S.D.%; n = 6)		
Sulfaguanidine	5.20	5.28	3.22	1.84	2.11
Sulfadiazine	0.82	2.13	3.69	4.46	7.38
Sulfamethizole	1.01	2.50	1.85	2.38	2.38
Sulfamethoxazole	3.90	1.57	1.91	2.82	2.37
Sulfametazine	1.65	1.76	2.29	2.25	1.16
			Reproducibility precision (R.S.D.%; n = 18)		
Sulfaguanidine	8.65	5.93	3.47	2.79	2.67
Sulfadiazine	15.79	6.92	3.95	6.58	13.11
Sulfamethizole	10.86	5.33	4.13	4.01	4.24
Sulfamethoxazole	5.05	2.97	3.60	4.44	3.93
Sulfametazine	10.56	4.40	2.24	1.94	1.53
Horwitz*	8.83	7.96	6.25	5.64	5.08

* - values estimated from Horwitz function

Table 3. Recovery parameters (%) (n = 18)

Concentration (mg/kg)	Sulfaguanidine	Sulfadiazine	Sulfamethizole	Sulfamethoxazole	Sulfamethazine
50	94.0	116.6	73.4	101.4	93.5
100	90.0	109.7	68.8	94.7	90.8
500	84.9	98.9	72.0	84.1	96.1
1000	91.4	91.2	79.1	91.8	93.7
2000	85.7	79.1	81.0	96.8	92.7
Average	89.2	99.1	74.9	93.7	93.3
R.S.D.%	4.3	15.0	6.8	6.9	2.0

Table 4. Method specificity

	LoD (mg/kg)	*LoQ* (mg/kg)
Sulfaguanidine	10.0	30.2
Sulfadiazine	1.7	5.2
Sulfamethizole	2.0	6.2
Sulfamethoxazole	6.6	20.1
Sulfamethazine	2.9	8.9

SD_{low} - standard deviation for samples containing analytes at 50 mg/kg; a – slop of HRMs calibration curve

Table 5. Extended uncertainty (k = 2) %

Concentration (mg/kg)	Sulfaguanidine	Sulfadiazine	Sulfamethizole	Sulfamethoxazole	Sulfamethazine
50	22	18	17	11	36
100	13	13	11	7	21
500	9	11	7	6	12
1000	9	11	7	6	12
2000	9	11	7	6	12

Discussion

The key point of developing chromatographic methods are selectivity and detection limit. Although mass spectrometry detection leads primacy due to selectivity and sensitivity, nonetheless, UV detection also has good characteristics. When adequate sample preparation and efficiency of chromatographic analysis are provided, a satisfactory level of selectivity may be achieved. Sensitivity is not a crucial parameter in terms of this study, since a low limit of detection is not demanded due to 200 to 2000 ppm therapeutic levels of sulphonamides in feedingstuffs. Some of the available methods require a post-column derivatisation, which are problematic and require an additional equipment (25, 26). Derivatisation before separation and FLD are well suited; however, it is dedicated more for low concentrations of analytes. Highly sensitive HPLC tandem mass spectrometry detection is associated with a high sensitivity but also with expensive equipment. Thus, HPLC-UV analysis is a simple and low-cost technique; however, it involves appropriate sample preparation step (8, 10, 13, 17, 30). Furthermore, no papers were found concerning five sulfonamides analysis in feedingstuffs in the range of concentrations used in this study. Particularly, it concerns determination of sulfaguanidine in feedingstuffs by HPLC-UV technique.

Sample fortification. The task of samples fortification implies difficulties such as placing analytes into sample, type of matrix interaction of analytes, and solvent with the matrix. Moreover, in the case of medicated feedingstuffs, preparation of HRMs should be as reproducible as possible, and particularly it refers to the preparation step of grinding in feed mill. Moreover, adding the analytes in the methanolic or acetonitilic solutions, and leaving for 24 h, causes some interactions of solvent and analytes with the feedingstuff. As a result, recovery lowering (13) and exclusion of contamination are difficult to achieve (11). Final overcoming, based on addition of the solid sulfunamide standards into the feedingstuff (11, 13, 17), followed by accurate mixing, allowed to obtain homogenous matrix. Both satisfactory precision and recovery were reported when the above preparation of HRMs grinding step was conducted by Smallidge *et al.* (25). These samples were used to establish an analytical impact on the ingredient differences of feedingstuffs obtained from veterinary inspections when compared to HRMs.

Sample preparation. Feedingstuff is a complex matrix, composed of many different compound classes, which are co-extracted and co-eluted with the analyte of interest. The first step in the development of a feedingstuff extraction method was the selection of extraction solution and purifying procedure. Aqueous solution showed poor recovery and numerous impurities were reported in other papers describing the

methods of sulfadiazine and sulfadimidine analysis (13, 28). Following buffer extraction, a special sample cleaning process, *e.g.* by solid phase extraction (SPE), is needed to avoid interference in UV detection. The octadecyl (J.T. Baker) and strong cation exchange (Phenomenex) cartridges were used in the study, but none provided satisfactory results for all compounds analysed. Octadecyl cartridges were suitable for all sulfonamides except sulfaguanidine, which is fully ionised at pH lower than 12, and no retention in C18 cartridges took place. Despite the fact that SCX cartridges were adequate to sulfaguanidine determination, variability of results and low recovery factors for other sulfonamides excluded them. Considering the high concentration of sulfonamides in samples, SPE phase was eliminated, which allowed to shorten the time of sample preparation and simplified the method (13, 17).

Methanol extraction revealed high levels of extracted fats causing problems at the evaporation phase. Moreover, numerous impurity peaks on chromatogram appeared, which decreased the method precision. Application of acetonitrile extraction resulted in a low recovery for sulfadiazine. This problem may be solved by the use a microwave-assisted extraction; however additional equipment is needed (13). Another approach based on a mixture of acetonitryle and methanol (50:50 v/v), selected as extraction phase, enabled to achieve optimal solubility for sulfadiazine, and resulted in the best recovery and lowest amount of impurities for all studied sulfonamides.

Since the temperature is a critical parameter in the evaporation process, samples were dried at different temperatures ranging from 30 to 50°C in the gentle stream of nitrogen. In the studied temperature range, no impact on the analysis results was observed, which was also confirmed in other papers (13, 11). The residue was reconstituted in 200 µL of methanol, and then diluted in a phosphate buffer up to 4000 µL mixed by means of vortex, which significantly increased recovery compared to direct buffer reconstituted. The remaining insoluble solid particles were eliminated by filtration through a cellulose syringe filter.

Separation of analytes was performed in a gradient programme, starting with a mobile phase containing 2% of organic components. Evaporation of supernatant before injection was necessary as introducing analytes in an organic solution impacted the shape of peaks and diminished selectivity of the separation. Moreover, additional filtration after the residue dissolving reduced the amount of interfering contaminants.

Chromatographic conditions. Gradient elution with a phosphate buffer (pH 6.5) and acetonitile on the 250 mm octadecyl column allowed for a good separation of all tested compounds, and a relatively short time of analysis. Low concentration of acetonitryle (2%) in the mobile phase at the beginning of the chromatographic separation was necessary due to relatively short retention time of sulfaguanidine, and its poor separation from impurities. The best separation of analysed sulphonamides was achieved using a mobile phase containing an ortophosphoric buffer adjusted to pH 6.5. Separation, especially at lower pH (>5.0), was not sufficient for sulphametoxazole and sulphametizole. Stability of the chromatographic method was evaluated by calculation of the variation of retention times obtained for 18 injections over six months.

To conclude, an efficient, precise and relatively fast method of determination of five sulphonamides in medicated feedingstuffs was developed. Direct sample preparation and HPLC-UV analysis allow the method to be successfully included in the scope of routine analyses. The results obtained from validation confirmed that the method can be recommended for the analysis of medicated feedingstuffs quality, homogeneity, and stability. These parameters are essential to prudent use of antimicrobial medications for animals, and are important in terms of public health and safety, as high demands on the quality of food of animal origin are currently being set.

Reference

1. Biancotto G., Angeletti R., Piro R.D.: Detection of veterinary drugs in foodstuffs using gel permeation. Analyst 1996, 121, 229–232.
2. Borras S., Companyo R., Guiteras J.: Analysis of sulfonamides in animal feeds by liquid chromatography with fluorescence detection. J. Agric. Food Chem 2011, 59, 5240–5247.
3. Boscher A., Guignard C., Pellet T., Hoffmann L., Bohn T.: Development of a multi–class method for the quantification of veterinary drug residues in feedingstuffs by liquid chromatography–tandem mass spectrometry. J. Chromatogr A 2010, 1217, 6394–6404.
4. Cancho–Grande B., Garcia–Falcon M.S., Rodriguez–Comesana M., Simal–Gandara J.: Determination of sulfamethazine and trimethoprim in liquid feed premixes by HPLC and diode array detection, with an analysis of the uncertainty of the results. J Agric Food Chem 2001, 49, 3145–3150.
5. Cieri U.R., Detection of sulfonamides in animal feeds J Assoc Off Anal Chem 1976, 59, 56–59.
6. Council Directive (EEC) 90/167/EWG of 26 March 1990 laying down the conditions governing the preparation, placing on the market and use of medicated feedingstuffs in the Community.
7. European Medicines Agency: Trends in the sales of veterinary antimicrobial agents in nine European countries (2005–2009). 2011, EMA/238630/2011.
8. Hormazabal V., Steffenak I., Yndestad M.: Simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by high–performance liquid–chromatography. J Chromatogr 1993, 648, 183–186.
9. Horwitz W., Kamps L.R., Boyer K.W.: Quality assurance in the analysis of foods for trace constituents. J Assoc Off Anal Chem 1980, 63, 1344–1354.
10. Houglum J.E., Larson R.D., Neal R.M.: Liquid–chromatographic determination of sulfamethazine in feeds. J Assoc Off Anal Chem 1988, 71, 1054–1056.
11. Iammarino M., Palermo C., Nardiello D., Muscarella M.: Optimization and validation of a confirmatory method for determination of ten sulfonamides in feeds by LC and UV–diode array detection. Chromatographia 2011, 73, 75–82.
12. Injac R., MLinaric A., Djorjevic–Milic V., Karljikovic–Rajic K., Strukelj B.: Optimal conditions for determination of zinc

bacitracin, polymyxin B, oxytetracycline and sulfacetamide in animal feed by micellar electrokinetic capillary chromatography. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008, 25, 424–431.
13. Jimenez V., Companyo R., Guiteras J.: Preparation of quality control materials for the determination of sulfonamides in animal feed. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2009, 26, 969–977.
14. Kantiani L., Farre M., Freixiedas J., Barcelo D.: Development and validation of a pressurised liquid extraction liquid chromatography–electrospray–tandem mass spectrometry method for β–lactams and sulfonamides in animal feed. J Chromatogr A 2010, 1217, 4247–4254.
15. Kantiani L., Farre M., Grases M., Freixiedas J., Barcelo D.: Determination of antibacterials in animal feed by pressurized liquid extraction followed by online purification and liquid chromatography–electrospray tandem mass spectrometry. Anal Bioanal Chem 2010, 398, 1195–1205.
16. Konieczka P., Namiesnik J.: Estimating uncertainty in analytical procedures based on chromatographic techniques. J Chromatogr A 2010, 1217, 882–891.
17. Kumar P., Companyo R.: Development and validation of an LC–UV method for the determination of sulfonamides in animal feeds. Drug Test Anal 2012, 4, 368–375.
18. Liu R., He P., Li Z., Li R.: Simultaneous determination of 16 sulfonamides in animal feeds by UHPLC–MS–MS. J Chromatogr Sci 2011, 49, 640–646.
19. Lopes R.P., Passos E.E.D., de Alkimim J.F., Vargas E.A., Augusti D.V., Augusti R.: Development and validation of a method for the determination of sulfonamides in animal feed by modified QuEChERS and LC–MS/MS analysis. Food Control 2012, 28, 192–198.
20. Lynas L., Currie D., McCaughey W.J., McEvoy J.D.G., Kennedy D.G.: Contamination of animal feeding stuffs with undeclared antimicrobial additives. Food Addit Contam 1998, 15, 162–170.
21. Patyra E., Kowalczyk E., Kwiatek K.: Determination of chlorotetracycline and doxycycline in medicated feedingstuffs by liquid chromatography. Bull Vet Inst Pulawy 2012, 56, 329–333.
22. Riviere J.E., Papich M.G.: Veterinary Pharmacology and Therapeutics, in: H. Richard Adams, Sulfonamides and Potentiated, Wiley–BlackWell, Hong Kong, 2009, pp. 835–861.
23. Qin Y., Zhang M., Lin H.: Qualification and quantification of 10 sulfonamides in animal feedingstuff by high performance liquid chromatography–electrospray tandem mass spectrometry. Chinese J Chromatogr 2005, 23, 397–400.
24. SANCO, E.C.D. Evaluation of the EU Legislative Framework in the Field of Medicated Feed–Final Raport, Food Chain Evaluation Consortium: Berlin, Germany, 2010.
25. Smallidge R.L., Albert K.: Determination of sulfamethazine in swine and cattle feed by reversed–phase liquid chromatography with post–column derivatization: Collaborative study. J Assoc Off Anal Chem 2000, 83, 260–268.
26. Smallidge R.L., Kentzer E.J., Stringham K.R., Kim E.H., Lehe C., Stringham R.W., Mundell E.C.: Sulfamethazine and sulfathiazole determination at residue levels in swine feeds by reverse–phase liquid–chromatography with post–column derivatization. J Assoc Off Anal Chem 1988, 71, 710–717.
27. Spinks C.A., Schut C.G., Wyatt G.M., Morgan M.R.: Development of an ELISA for sulfachlorpyridazine and investigation of matrix effects from different sample extraction procedures. Food Addit Contam 2001, 18, 11–18.
28. Stringham R.W., Mundell E.C., Smallidge R.L.: Use of post–column derivatization in liquid–chromatographic determination of sulfamethazine and sulfathiazole in feeds and feed premixes. J Assoc Off Anal Chem 1982, 65, 823–827.
29. Torel J., Cillard J., Cillard P., Vie M.: Determination of oxytetracycline, sulfamethazine and sulfame–thoxypyridazine in feed premixes. J Chromatogr 1985, 330, 425–428.
30. Torel J., Cillard J., Cillard P., Vie M.: Simultaneous analysis of 3 antimicrobial agents in feed premixes by reversed–phase high–performance liquid–chromatography. J Chromatogr 1985, 323, 447–450.
31. Wang S., Zhang H.Y., Wang L., Duan Z.J., Kennedy I. Analysis of sulphonamide residues in edible animal products: a review. Food Addit Contam 2006, 23, 362–384.