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Simultaneous determination of sulfonamides, trimethoprim, amoxicillin and tylosin in medicated feed by high performance liquid chromatography with mass spectrometry

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Abstract

Introduction: The article presents a rapid and simple analytical procedure for determination of four sulfonamides (sulfadiazine, sulfamerazine, sulfamethazine and sulfamethoxazole), trimethoprim, tylosin and amoxicillin in animal medicated feed. **Material and Methods:** Eighteen medicated feed samples were analysed for active substances. The analytical protocol used a mixture of acetonitrile and 0.05 M phosphoric buffer, pH 4.5 for the extraction of seven antibacterial substances. After extraction, the samples were diluted in Milli-Q water and analysed by liquid chromatography with mass spectrometry. The developed procedure was subjected to validation in terms of linearity, selectivity, limits of quantification and determination, repeatability, reproducibility and uncertainty. **Results:** The validation of the method was carried out in accordance with the criteria set out in Commission Implementing Regulation (EU) 2021/808 and ICH guidelines. This method provided average recoveries of 90.8 to 104.5% with coefficients of variation for repeatability and reproducibility in the ranges of 3.2–6.9% and 5.2–8.3%, respectively for all analysed antibacterial substances. The limit of detection and limit of quantification for all seven analytes ranged from 5.4 mg/kg to 48.3 mg/kg and from 10.4 mg/kg to 119.3 mg/kg, respectively. The uncertainty of the method depending on the compound varied from 14.0% to 24.0%. The validated method was successfully applied to the 18 medicated feeds. **Conclusion:** The developed method can be successfully used to routinely control the content and homogeneity of seven antibacterial substances in medicated feed.

Keywords: sulfonamides, trimethoprim, tylosin, amoxicillin, medicated feed, liquid chromatography–mass spectrometry (LC-MS).

Introduction

Reducing the amount of antibiotics used in animal production is one of the priority actions of the European Green Deal, which aims to achieve a sustainable and ecological transformation of the economy. In the context of reducing antibiotics in the food chain, the European Green Deal sets an ambitious target for their reduction in animal husbandry by 2050. As part of this goal, the EU plans to introduce strict regulations on the use of these drugs, especially of antibiotics critical for the treatment of human infections. These activities are aimed at reducing the risk of bacterial resistance and limiting the presence of antibiotics in food products. Public health and agricultural organisations such as the World Health Organization (WHO) and the World Organisation for Animal Health (WOAH) are developing guidelines and recommendations to control the use of antibiotics in the food chain. EU Member States, including Poland, have implemented control programmes for the presence of antibiotics, sulfonamides and quinolones in drinking water for animals, medicated and non-target feed, and food and products of animal origin, which are constantly being improved and adapted to current EU and national legislation. But according to the data published in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) European Medicines Agency (EMA) report for 2021, in Poland in 2011–2021 there was a 35% increase in the total sales of veterinary antimicrobials (mg/population correction unit (PCU)). In 2021, the most frequently sold groups of antibacterial

drugs in Poland were penicillins, tetracyclines, macrolides and sulfonamides. In the same year, Poland ranked second only to Spain in terms of sales of antibacterial substances in livestock (6). According to Regulation (EU) 2019/4 of the European Parliament and of the Council of 11 December 2018 (24), one of the routes for the oral administration of veterinary medicinal products is medicated feed, which is a homogeneous mixture of feed and such products. Medicated feed should be manufactured only with veterinary medicinal products authorised for incorporation into medicated feed during manufacture, and the compatibility of all compounds used should be ensured for safety and efficacy of the product. Medicated feed should only be manufactured with approved medicinal premixes. The uniform incorporation of the veterinary medicinal product into the feed is essential for safe production and effective medicated feed to ensure food safety throughout the food chain.

In Poland, medicated feeds are subject to official control by the Veterinary Inspection and quality control by manufacturers, which ascertains that the actual content of the active substance added to the feed is the declared content of that substance and tests the homogeneity of the feed. The first methods used for this purpose were microbiological methods. However, with the development and availability of other analytical methods, such as chromatographic methods with various types of detectors, and the impossibility of using microbiological methods for some groups of antibacterial substances, such as sulfonamides or phenicols, other methods began to be developed and implemented in laboratory practice.

In scientific publications, methods can be found of quantitative analysis of single substances in medicated feed (*e.g.* sulfaguanidine, tylosin, tiamulin, amoxicillin, florfenicol and sulfamethazine) (5, 10, 11, 18, 20, 23, 25, 26), and of analysis of several compounds belonging to the same chemical group (sulfonamides, phenicols, β-lactams and tetracyclines) (1, 2, 4, 8, 12–17, 21, 22). However, few developed analytical methods exist for several antibacterial substances belonging to different chemical groups (19). For the analysis of antibacterial substances in medicated feeds, the technique of liquid chromatography with various types of detectors is used: diode array detector, fluorescence detector, single mass spectrometer or tandem mass spectrometer (1, 2, 4, 5, 7, 8, 10–23, 25, 26).

In this publication, an analytical method for the analysis of seven antibacterial substances such as: sulfadiazine (SDZ), sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole (SMX), trimethoprim (TRIM), amoxicillin (AMO) and tylosin (TYL) in one analytical procedure in medicated feeds using only liquid extraction and liquid chromatography with mass spectrometry is presented. The method was „in-house" validated, verified by analysis of real samples, and successfully applied in laboratory practice. The development of multi-analyte protocols is practical for laboratories monitoring the content and homogeneity of antimicrobial substances in medicated feeds, as it allows for much greater analysis of feed samples without changing the extraction or analytical method.

Material and Methods

Reagents and chemicals. The requisite solvents, namely acetonitrile and methanol HPLC grade, were purchased from J.T. Baker (Deventer, the Netherlands). 85% orthophosphoric acid and disodium hydrogen phosphate anhydrous p.a. were obtained from POCH (Gliwice, Poland) and formic acid ≥98% was from Sigma Aldrich. Purified water was prepared in-house with a Milli-Q water system from Millipore (Bedford, MA, USA). The reference standards were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany): sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, trimethoprim, tylosin and amoxicillin.

Standards. Standard solutions of antibacterial substances were prepared in separate 5 mL volumetric flasks by weighing the appropriate amount of mg of standard substances to obtain the concentrations indicated in Table 1 and using the solvents listed in the table. Standard solutions were stored at a temperature below −18ºC for no longer than six months.

Table 1. Preparation of standard antimicrobial solutions

Analyte	Concentration (mg/mL)	Solvent
Sulfadiazine	2	Acetonitrile
Sulfamerazine	5	Methanol
Sulfamethazine	5	Methanol
Sulfamethoxazole	5	Methanol
Trimethoprim	2	Methanol
Tylosin	2	Methanol
Amoxicillin	2	Milli-Q water

Feed samples. Samples of medicated feeds were collected and sent to the laboratory by the Veterinary Inspection as part of official control or directly by feed mills that are authorised to produce medicated feed. Samples were to be analysed for their content and homogeneity. The collected samples were transported to the laboratory and stored at room temperature until analysis. Fourteen medicated feed samples were tested to determine the content of sulfadiazine and trimethoprim or amoxicillin, and four feed samples were tested in five portions of each feed to evaluate the homogeneity of the produced medicated feed.

Sample preparation and extraction. A feed sample of 2.00 ± 0.01 g, previously ground, was weighed into a 50 mL polypropylene centrifuge tube. Samples were fortified with all the selected antibacterial substances, shaken on a vortex mixer for 30 s, and then allowed to stand at room temperature for 12 h to enable

sufficient equilibration with the feed matrix. For the extraction, 5 mL of acetonitrile was added and mixed on a vortex mixer for 30 s. Then 5 mL of 0.05 M phosphate buffer at pH 4.5 ± 0.1 was added and the samples were shaken for 60 min on a horizontal shaker and centrifuged at 4,000 rpm for 15 min at 20°C. Transfer of 990 µL of Milli-Q water to the chromatography vial and dispensing of 10 µL of the obtained extract took place next. Samples prepared for analysis should be mixed on a vortex.

Liquid chromatography–mass spectrometry analysis. A liquid chromatography system consisting of an HP 1200 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, degasser system, automatic injector and column thermostat, and an Agilent 6140 single quadrupole mass spectrometer (Agilent Technologies) was used for the analysis. Separations were performed on a reverse-phase Kinetex C18 column (100 mm × 4,6 mm; 2,6 µm) and an RP18 guard column (4.0 mm × 3.0 mm, 5 µm), both from Phenomenex (Torrance, CA, USA). The column thermostat temperature was set to 20°C. The flow rate was 0.5 mL/min and injection volume 10 µL. The composition of mobile phases A and B was set as 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution started from 10% of solvent B at 0 min, increased to 20% from 4.01 to 8.0 min, to 40% from 8.01 to 12.0 min, to 50% from 12.01 to 15.0 min, reached 100% from 15.01 to 17.0 min, and was held for 1 min; next reduced to 10% from 18.01 to 19.0 min and was held from 19.01 min to 23 min which was the end of the analysis run time. Electrospray ionisation (ESI) was set in a positive mode, the capillary voltage was set at 2,000 V, drying gas temperature was 350°C, drying gas flow was 12 L/min, and nebulising gas pressure was 40 psi. Selected ion monitoring and retention time for all antibiotic substances intended for detection are listed in Table 2.

Validation studies. The method was in-house validation with the criteria specified by Commission Implementing Regulation (EU) 2021/808 and the guidelines of the International Council for the Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (3, 9). Evaluation of the method's linearity was based on the analysis of the matrix calibration curves and the assignment of an R^2 determination coefficient. Blank feed samples were fortified to five different concentrations of all analytes (Table 3) before the extraction procedure. Correlation coefficient values in this concentration range were >0.99 for all analysed antibacterial substances. To verify the absence of interfering endogenous compounds around the retention time of analytes, 10 different blank feed samples for poultry and pigs were analysed. The limit of detection and limit of quantification for all analytes SDZ, SMR, SMZ, SMX, TRIM, TYL and AMO were determined with the use of signal-to-noise (S/N) rations of 3 and 10, respectively. Recovery values were calculated by comparing the concentration obtained from the feed samples with the added amounts. Repeatability was assessed by comparing the results of six replicates prepared the same day at three different concentrations (Table 4). Within-laboratory reproducibility was assessed by spiking two other sets of blank feed samples at the same concentrations as for repeatability and having them analysed on different days with the same instrument but by another technician. Standard deviations (SD) and coefficients of variation (CV, %) were calculated for each level. The uncertainty (U) was calculated as the ratio of coverage factor (k = 2) and SD of within-laboratory reproducibility, and expressed as a percentage.

Table 2. Monitored ions (m/z) and retention time of analysed antibacterial substances

Analyte	Monitored ion (m/z)	Retention time (min)
Sulfadiazine	251.0	7.2
Sulfamerazine	265.0	9.9
Sulfamethazine	279.0	11.5
Sulfamethoxazole	254.0	15.1
Trimethoprim	291.0	10.4
Tylosin	916.0	15.7
Amoxicillin	366.0	3.5

m/z – mass-to-charge ratio

Table 3. Spiking levels in matrix-matched calibration curves for all analysed antibacterial substances

Analyte	Concentration level (mg/kg)				
	I	II	III	IV	V
Sulfadiazine	150	300	450	600	750
Sulfamerazine	150	300	450	600	750
Sulfamethazine	150	300	450	600	750
Sulfamethoxazole	150	300	450	600	750
Trimethoprim	45	90	120	160	200
Tylosin	20	40	80	100	150
Amoxicillin	150	250	400	500	600

Table 4. Validation spiking levels

Analyte	Spiking level (mg/kg)		
	I	II	III
Sulfadiazine	150	450	750
Sulfamerazine	150	450	750
Sulfamethazine	150	450	750
Sulfamethoxazole	150	450	750
Trimethoprim	45	90	200
Tylosin	20	100	150
Amoxicillin	150	400	600

Results

Method validation results. The procedure presented in the current article was selective and able to detect seven different antibacterial substances from medicated feed in one analytical protocol. The developed procedure obtained a qualitative and quantitative method of determination of sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, trimethoprim, amoxicillin and tylosin in medicated feed. The chosen reversed-phase Phenomenex Kinetex C18 column and gradient of the formic acid in water and formic acid in acetonitrile successfully separated seven antibacterial substances chromatographically within 23 min. Typical chromatogram obtained for a blank feed sample, spiked feed sample and real medicated feed sample with sulfadiazine and trimethoprim are presented in Figs 1, 2 and 3.

After extraction process optimisation of analytes, it is necessary to validate the method, which shows the performance of analytical method. The validation of the method was carried out in accordance with the criteria set out in the Commission Implementing Regulation (EU) 2021/808 and ICH guidelines (3, 9).

The range of linearity was between 20 and 750 mg/kg depending on the antibacterial substance, and the correlation coefficient was higher than 0.99 for all analytes. The recovery for the presented analytes ranged from 90.8% to 104.5%. The repeatability expressed as a coefficient of variation ranged from 3.2% to 6.9%, and within-laboratory reproducibility ranged from 4.8% to 8.3% depending on the concentration level investigated. The limit of detection and limit of quantification for all seven analytes ranged from 5.4 mg/kg to 48.3 mg/kg and from 10.4 mg/kg to 119.3 mg/kg, respectively. The uncertainty of the method varied from 14.0% to 24.0%, depending on the compound. All validation data are presented in Tables 5a and 5b.

Medicated feed sample analysis. The developed method was used for routine analyses of medicated feed samples delivered for testing by the Veterinary Inspection as a part of the Official Feed Control in Poland and directly from the feed factory. Fourteen medicated feed samples were evaluated to determine the content of sulfadiazine and trimethoprim or amoxicillin, and 4 samples (5 samples each) to test the homogeneity of the produced medicated feed. The tested feeds were consistent with the manufacturer's declaration as to the content of active substances in the feeds, *i.e.* they contained the stated 450 mg/kg of sulfadiazine and 400 mg/kg of amoxicillin. One sample of medicated feed containing sulfadiazine and trimethoprim had an excessive trimethoprim content of over 122 mg/kg, whereas the manufacturer's declaration was 90 mg/kg. Considering the method uncertainty of 23% for trimethoprim, the sample taken was considered inconsistent with the manufacturer's declaration. The samples tested for the homogeneity of the active substances added to the feed were homogeneous, as evidenced by the coefficient of variation >15% calculated for them. The list of medicated feed samples that were analysed with the developed method is presented in Table 6.

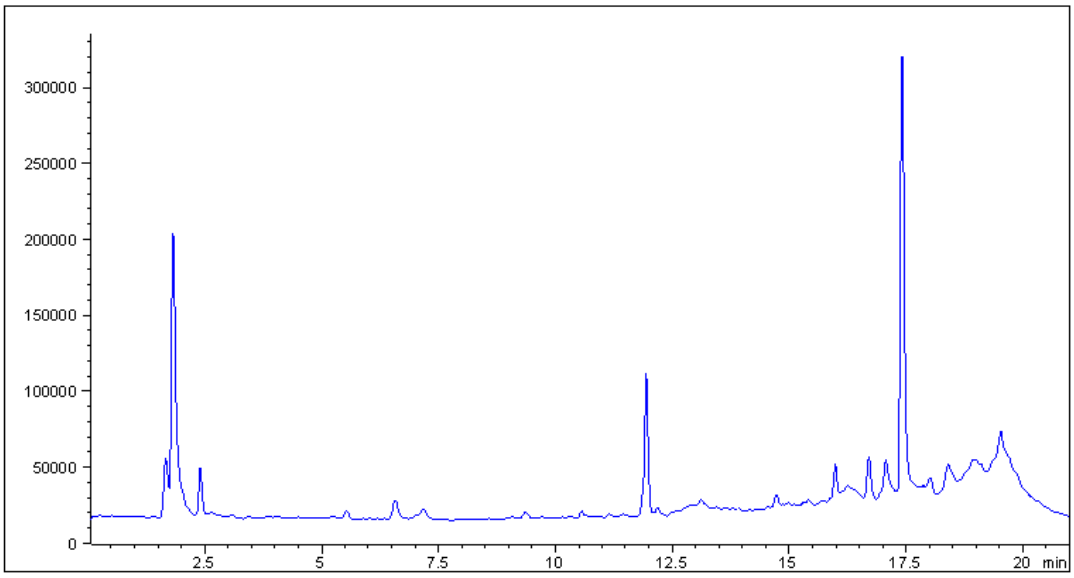

Fig. 1. Selected-ion monitoring chromatogram of blank feed sample

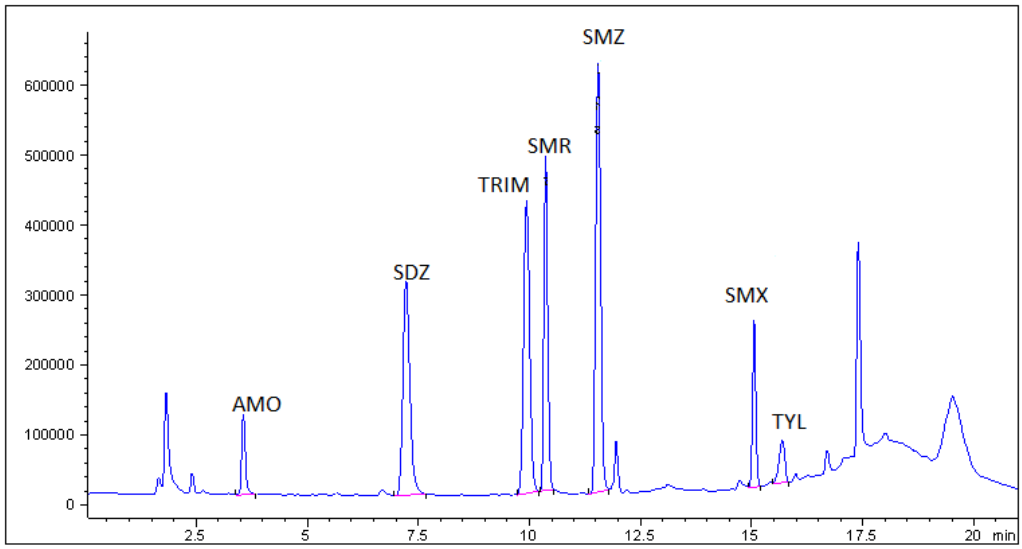

Fig. 2. Selected-ion monitoring chromatogram of feed matrix spiked with all analysed antibacterial substances at first validation level

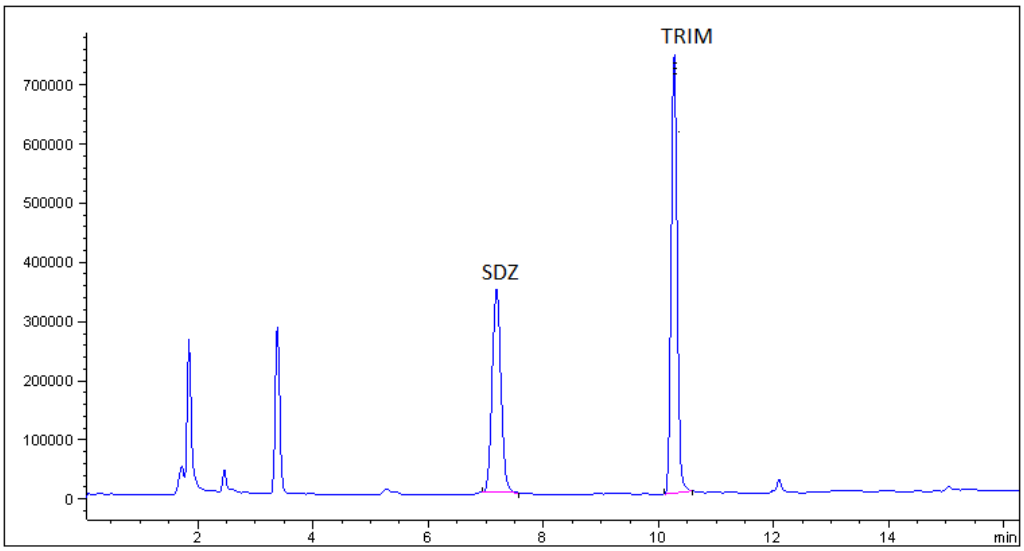

Fig. 3. Selected-ion monitoring chromatogram of real medicated feed sample for pigs with sulfadiazine and trimethoprim at the concentrations of 450 mg/kg and 90 mg/kg

Table 5a. Validation report for antibacterial substances

Analyte	Linearity R^2	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)		
				I	II	III
Sulfadiazine	1.00	48.3	69.6	96.6	104.5	99.6
Sulfamerazine	0.998	32.0	55.6	90.8	101.3	97.7
Sulfamethazine	1.00	35.3	64.0	97.2	99.3	96.6
Sulfamethoxazole	0.993	37.6	60.3	102.9	102.8	97.8
Trimethoprim	1.00	17.4	28.2	94.8	98.0	95.5
Tylosin	0.998	5.4	10.4	103.2	101.7	97.4
Amoxicillin	0.990	91.0	119.3	101.9	100.7	96.2

LOD – limit of detection; LOQ – limit of quantification

Table 5b. Validation report for antibacterial substances

Analyte	Repeatability (CV %)			Reproducibility (CV %)			Uncertainty (%)		
	I	II	III	I	II	III	I	II	III
Sulfadiazine	3.2	3.6	4.8	6.5	5.7	5.6	19.0	19.0	17.0
Sulfamerazine	3.4	3.9	6.6	5.2	4.8	6.0	24.0	14.0	18.0
Sulfamethazine	6.1	4.0	5.7	6.4	7.4	5.3	18.5	21.0	17.0
Sulfamethoxazole	5.9	4.1	5.9	7.3	5.1	6.3	21.0	16.0	18.0
Trimethoprim	6.9	5.6	4.1	7.1	5.4	6.4	22.0	16.0	20.0
Tylosin	3.5	5.6	6.7	6.6	5.4	7.1	20.0	16.0	20.0
Amoxicillin	5.6	3.3	6.0	8.3	6.1	5.8	24.0	18.0	18.0

Table 6. The result for the tested samples of medicated feeds provided by the Veterinary Inspection and feed factories from Poland

No.	Medicated feed	Active substance content (manufacturer's declaration) (mg/kg)		Content of active substances determined by the developed method (mg/kg) ± U mg/kg	
		Sulfadiazine	Trimethoprim	Sulfadiazine	Trimethoprim
1	for piglets	450	90	421.6 ± 84.3	87.4 ± 20.1
2	Porcus Silver			476.3 ± 95.3	100.8 ± 23.2
3	for pigs			425.2 ± 85.0	86.7 ± 19.9
4	Porcus Silver			455.8 ± 91.2	96.9 ± 22.3
5	Pig Lead			518.6 ± 103.7	109.6 ± 25.2
6	Pig Lead			453.7 ± 90.7	110.5 ± 25.4
7	for piglets			411.7 ± 82.3	96.2 ± 22.1
8	Pig Lead			488.9 ± 97.8	100.9 ± 23.2
9	Porcus Silver			521.0 ± 104.2	101.4 ± 23.3
10	for pigs			484.5 ± 96.9	91.0 ± 20.9
11	for pigs			480.2 ± 96.0	122.3 ± 28.1*
12	for piglets			492.0 ± 98.4	92.1 ± 21.2
		Amoxicillin			
13	for pigs	400 mg/kg		381.3 ± 91.5	
14	for pigs			432.8 ± 103.9	
		Medicated feed samples with sulfadiazine (450 mg/kg) and trimethoprim (90 mg/kg) tested for homogeneity			
No.	Medicated feed	Sample No.		Sulfadiazine	Trimethoprim
15	Porcus Silver	1		505.5	101.1
		2		483.2	96.6
		3		554.5	110.9
		4		447.7	89.5
		5		537.4	107.5
		Mean value (mg/kg)		505.7 ± 101.1	116.7 ± 26.8
		CV (%)		8.4	10.5
16	Starter for piglets	1		502.8	100.6
		2		413.7	82.7
		3		480.5	96.1
		4		448.2	89.6
		5		399.6	79.9
		Mean value (mg/kg)		448.9 ± 96.0	96.0 ± 22.1
		CV (%)		9.7	9.3
17	Pig Lead	1		400.3	80.1
		2		458.4	91.7
		3		407.6	81.5
		4		405.2	81.0
		5		463.2	92.6
		Mean value (mg/kg)		426.9 ± 85.4	90.7 ± 20.9
		CV (%)		7.3	6.4
18	Porcus Silver	1		513.9	104.4
		2		583.9	116.8
		3		494.1	98.8
		4		549.4	109.9
		5		512.8	102.6
		Mean value (mg/kg)		530.8 ± 106.2	107.2 ± 24.7
		CV (%)		6.7	7.2

***** – result inconsistent with the declaration

Discussion

There are few chromatographic methods described in the scientific literature that allow the analysis of several antibacterial substances in medicated feeds in one analytical procedure (19). This may be due to the long-standing application of cheap and simple microbiological methods that have been used for years to control the content of active substances declared by the manufacturer or to test the homogeneity of manufactured medicated feeds. Unfortunately, these methods are not useful for ascertaining the content of all antimicrobial substances approved for use in medicated feeds, including sulfonamides and phenicols. Therefore, work has been undertaken on the optimisation and validation of a new, multicomponent analytical method involving seven antibacterial substances: sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, trimethoprim, tylosin and amoxicillin. Trimethoprim is the drug that is most commonly administered in medicated feed in combination with sulfadiazine or sulfamethoxazole. So far, no chemical or microbiological analytical

methods have been implemented in Poland that would allow for quantitative determination of the combination of these two antibacterial substances in the feed mixture. In the case of methods available for the quantitative determination of amoxicillin, microbiological and chromatographic methods are available, but the chemical methods developed for this compound most often allow only the analysis of amoxicillin or amoxicillin and penicillin V or G or ampicillin in one analytical procedure (1, 7). For this purpose, liquid chromatography with ultraviolet-visible spectroscopy (UV/Vis) and diode array detection (DAD) is also most often used, which may hinder the quantitative analysis of these compounds because the wavelength of UV light that amoxicillin absorbs is about 200–210 nm. In the UV wavelength range similar to that of amoxicillin, other endogenous compounds present in the analysed extract also show significant absorption, which may significantly hinder the quantitative analysis of this antibiotic. The presence of interfering substances can be partly eliminated by purifying the sample, but in this case the development of the method requires more work and increases the cost of the analysis. Patyra and Kwiatek (18) described a method of analysing amoxicillin in medicated feed using the HPLC-DAD technique. They used 0.01 M of phosphate buffer at pH 5.0 and acetonitrile as the mobile phase and gradient elution. Tylosin is an antibiotic from the group of macrolides, which is quite often used in the production of medicated feed. The content of this compound in medicated feed is 50 or 100 mg/kg. The content of this drug is most often determined using the microbiological method, but chromatographic analysis methods for this compound in medicated feed are available in scientific publications. The author of such a method is Pietroń *et al.* (20), who used liquid chromatography with UV/Vis detection to quantify tylosin in medicated feed. They used a mobile phase consisting of methanol and acetonitrile (50:50, v/v), and solution triethylamine 70 mM adjusted to pH 2.5 with orthophosphoric acid. The wavelength of UV light for tylosin was set at 282 nm.

However, there are few described chromatographic methods that concern the analysis of several antibacterial substances from different chemical groups in one course of analytical procedure. One method which can analyse several antibacterial substances in one performance of a procedure is the method described by a Polish–Spanish team of scientists (19). The method allows for the analysis of five antibacterial substances in medicated feeds (sulfadiazine, sulfamethazine, trimethoprim, tylosin and tiamulin) using liquid chromatography with tandem mass spectrometry (LC-MS/MS). In the developed method, a combination of 0.1% formic acid in water and acetonitrile with 0.1% formic acid was used as the mobile phase, and chromatography was in a biphenyl column. In our work, we developed an analytical method using liquid chromatography with single mass spectrometry for the analysis of seven antibacterial substances belonging to 3 chemical groups. The most important and labour-intensive stage of work was the optimisation of the stage of extraction and purification of analytes from the feed matrix. The physical and chemical properties of the antibiotics in the optimised method were different because the drugs were from three antibiotic classes; for this reason, solvents that would allow the extraction of all analytes were challenging to select. Sulfonamides and trimethoprim are most often extracted with organic solvents such as methanol, acetonitrile and ethyl acetate (2, 17, 21). For tylosin, methanol in citrate buffer (20), sodium bicarbonate solution with acetonitrile (10) or a mixture of water and acetonitrile with the addition of formic acid are used (20). However, antibiotics from the penicillin (amoxicillin, ampicillin and penicillin) class are most often extracted from biological matrices using acetonitrile, an acetonitrile and water mixture or phosphate buffer at different pH ranges (1, 5, 12, 18). In order to optimise the extraction process, we had to find the all-round best, which meant selecting the solvents which could obtain the highest possible recoveries for all the analysed antibacterial substances. Various extraction mixtures were tested for this purpose, such as a combination of methanol and acetonitrile, a mixture of acetonitrile and water with the addition of formic acid, and a combination of acetonitrile with a phosphate buffer at a range of pH (3, 3.5, 4, 4.5 and 5). The best extraction mixture was a combination of acetonitrile and phosphate buffer at pH 4.5 (1:1; v/v). The high concentrations of antibacterial substances in medicated feed and their wide range from several dozen to several hundred milligrams per kilogram of feed necessitate the obtained extracts' dilution for analyses using the LC-MS technique. When optimising the method, we used 0.1% formic acid in water and pure water to dilute the extract. Different dilutions of the extract were also tested: 100-fold and 50-fold. It was found that the best results were obtained by diluting the extract 100-fold in water. The developed method was validated, giving satisfactory results for all seven analysed compounds.

Conclusion

The conducted research on market samples of medicated feed showed that in Poland there is no problem with the concentration of active substances in the feed declared by the manufacturer, because the antibiotic contents are consistent with the manufacturers' declarations. Moreover, medicinal feeds produced in Poland are homogeneous, which was confirmed by our research.

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