Comparison of HPLC–DAD and LC–MS Techniques for the Determination of Tetracyclines in Medicated Feeds Using One Extraction Protocol

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Abstract

Four antibiotics, oxytetracycline, tetracycline, doxycycline, and chlortetracycline were separated and quantified in medicated feed. Tetracyclines from feed samples were extracted with 0.01 M citric buffer and acetonitrile (pH 3.0) and further purified with 0.45 µm syringe filters. The purified extract was separated on commercial RP-C18 column and analyzing using liquid chromatograph (LC) with two different detectors: diode array detector (DAD) and mass spectrometry (MS). These methods provided average recoveries from 72.2 to 101.8% for high pressure liquid chromatography (HPLC) with diode array detection and from 45.6 to 87.0% for liquid chromatography with mass spectrometry. The limit of detection in medicated feed ranged from 4.2 to 10.7 mg kg⁻¹ for HPLC–DAD and 5.6 to 10.8 mg kg⁻¹ for LC–MS. Our experiment showed that using the same extraction mixture we do not obtain the same recovery values for the analyzed compounds using two different detection techniques such as DAD and MS.

Keywords Tetracyclines · Medicated feed · Liquid chromatography · Diode array detector · Mass spectrometry

Introduction

Tetracyclines (TCs) are naturally or semi-synthetic antibacterial substances which are commonly use in both human and veterinary practices. Tetracyclines have a wide range of antibacterial activity. The mechanism of their action consists in inhibiting protein synthesis by binding to the 30S subunit of the bacterial ribosome, as a result, tRNA does not bind to the binding site on the ribosome–mRNA complex [1, 2]. In fact they have been used successfully to treat infection cause by Gram-negative and Gram-positive bacteria, *Rickettsia* and *Mycoplasma* [3, 4]. These groups of antibacterial substances are commonly used to treat bacterial infections in livestock. The beneficial therapeutic properties of tetracyclines, a broad spectrum of antibacterial activity and price considerations make them widely used in veterinary practice. In particular, they are administered for therapeutic

purposes as medicated feed to specific groups of farm animals (pigs and poultry). According to the Regulation (EU) 2019/4 of the European Parliament and of the Council of 11 December 2018, medicated feed is a homogeneous mixture of feed and veterinary medicinal products [5]. According to the ninth European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) report, which compiled sales (tonnes) of veterinary antimicrobial agents in 31 European countries during 2017, the largest quantities of antimicrobials sold were tetracyclines (30.4%), penicillins (26.9%), and sulfonamides (9.2%). Overall, these three classes accounted for 66.5% of total sales in the 31 countries [6].

Feeds are very complex and variable matrixes, and some feed components such as lipids, proteins, vegetable oils, and feed additives which can be co-extracted with the analytes may disturb the analysis. Moreover, tetracyclines are subject to chelation with the transition metal ions, such as Fe²⁺, Fe³⁺, Al³⁺, Cr³⁺, Mn²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺, Hg²⁺, and salts with alkali and alkaline earth metals and organic and inorganic acids (e.g., citric, boric, and humic acids) [7]. Therefore, for the extraction of tetracycline-class antibiotics the McIlvaine buffer containing EDTA is the most widely used solution, due to its properties of complexing metal cations [7, 8].

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Among the analytical techniques used to determination of TCs in feeds are microbiological assay [9, 10], thin layer-chromatography (TLC) [9], high performance capillary electrophoresis (HPCE) [11], HPLC [7, 12–15], LLC/MS, and LC–MS/MS [16, 17]. The advantage of chromatographic methods is good sensitivity and capability of identification of target analyte, and LC–MS/MS is effective in quantifying trace level contaminants in food and feed.

The aim of this study was to check whether the use of one extraction protocol for the determination of four tetracycline antibiotics in medicated feed will be useful for two different detection techniques, i.e., diode array detection and mass spectrometry.

Materials and Methods

Materials, Chemicals, and Reagents

Oxytetracycline hydrochloride (OXT), tetracycline hydrochloride (TC), doxycycline hyclate (DC), and chlortetracycline hydrochloride (CTC) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were from J.T. Baker (St. Louis, MO, USA). HPLC-grade 1-butanol was purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) and formic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA), citric acid and sodium citrate were from Acros Organics (New Jersey, USA), and oxalic acid dihydrate was from Chempur (Piekary Śląskie, Poland). All reagents used were of analytical grade and analytically pure. Nylon filters, 0.45 μm , were from Agilent Technology (Santa Clara, CA, USA). All solution, including electrolytes were prepared using purified Milli-Q water ($> 18 \text{ M}\Omega \text{ cm}^{-1}$) generated by a Milli-Q Plus Water Purification System (Millipore, Bedford, MA, USA).

Standard Solution

The stock standard solutions were prepared by weighting $50 \text{ mg} \pm 0.1 \text{ mg}$ of standard substances of OTC, TC, CTC, and DC and dissolving in 5 mL methanol. The solution were stable for 6 months, stored at -18°C .

Calibration Curves

A series of working standard solutions were prepared at the concentrations of 0.01, 0.05, 0.1, 0.2, and 0.3 $\text{mg}\cdot\text{mL}^{-1}$ of OTC, TC, CTC, and DC in 1:1; v/v acetonitrile: 0.01 M citric buffer (pH 3) mixture. These solutions were analyzed by HPLC–DAD and calibration curve was plotted. To plot the LC–MS calibration curve, working standard solutions was diluted 100-fold in a mixture of acetonitrile and 0.01 M

citric buffer (pH 3) and analyzed by LC–MS. The three linearity studies for both HPLC–DAD and LC–MS were compared and the accuracy was calculated.

Extraction and Clean-Up

The presented extraction procedure was previously described by Patyra et al. in 2013 [15]. 2 g feed sample was weighed into a 50 mL Erlenmeyer flask. For the extraction of TCs from feed samples 10 mL of 1:1; v/v acetonitrile and 0.01 M citric buffer mixture (pH 3) were added. The samples were shaken for 30 min on a horizontal shaker, vortexed for 1 min, and centrifuged for 20 min, at $4000\times g$. The supernatants were filtered through 0.45 μm syringe filters and injected into the HPLC–DAD system. For LC–MS analysis feed extract after filtration was diluted a 100-fold in extractant mixture (acetonitrile: 0.01 M citrate buffer (pH 3) and analyzed by LC–MS.

Apparatus and Chromatographic Conditions

Micellar Liquid Chromatography—DAD Analysis

A micellar liquid chromatography with diode array detector (MLC-DAD) was carried out in accordance with the methodology developed by Patyra et al. in 2013 [15]. MLC was used for the separation and quantitative analysis of four tetracyclines. This was performed on an HP 1100 Series Agilent Technologies (Santa Clara, CA, USA). Chromatographic analysis was performed on a Thermo BDS column C18, $150\times 4.6 \text{ mm}$, 5 μm (Thermo Scientific, San Jose, CA, USA), using a micellar mobile phase consisting of 0.03 M sodium dodecyl sulfate/0.02 M oxalic acid/7% 1-butanol (pH 2.5) mixture prepared in one glass bottle. The chromatographic separation was accomplished with isocratic elution, flow rate was 0.8 mL min^{-1} , injected volume was 20 μL , and the column thermostat was set at 20°C . The UV detection was monitored at 360 nm for OTC and TC, 350 nm for DC and 370 nm for CTC and all compounds were eluted within 33 min.

LC–MS Analysis

LC–MS was carried out using an LC–MS System form Agilent Technologies (Santa Clara, CA, USA). The HPLC system was coupled to a single MS equipped with an ESI interface operating in positive ion mode using a capillary voltage of 2000 V. The other optimum values of the ESI–MS in positive parameters were drying gas temperature 350°C , drying gas flow 13 L/min, nebulising gas pressure 40 psi. Molecular masses of the precursor ions of OTC, TC, CTC, and DC were 461, 445, 479, and 445 m/z, respectively.

LC–MS separation of tetracyclines was achieved with Zorbax Eclipse XDB C18 column (150×4.6 mm, 5 μ m), (Agilent Technologies, Santa Clara, CA, USA). Eluent A was formic acid 0.1% in acetonitrile and eluent B was ultrapure water containing 0.1% formic acid (v/v). The gradient was as follows. The initial 22% A was increased linearly to 32% in 11 min and held for 1 min. Finally the gradient was returned to the initial of 22% A for 3 min. The flow rate was 0.6 mL min^{−1}, and the column thermostat was set at 30 °C. The injection volume was 3 μ l and all compounds were eluted within 15 min. The LC–MS parameters were published by Patyra and Kwiatek in 2016 [8].

Validation Procedure

The HPLC–DAD and LC–MS methods were validated for linearity, intra-day and inter-day precision, and accuracy. The validation of the methods included the following parameters according to the International Conference on Harmonization (ICH) guidelines: linearity, limit of detection (LOD), limit of quantification (LOQ), and precision and recovery [18].

The linearity was determined by a standard solution calibration curve which was prepared at five concentration levels (0.01, 0.05, 0.1, 0.2, and 0.3 mg mL^{−1} for HPLC–DAD and 100-fold dilution for LC–MS). The repeatability was calculated as the coefficient of variation (CV, %) of results obtained after fortifying six blank feed samples at three concentration levels (50, 500, and 1500 mg kg^{−1} for each level). The within-laboratory reproducibility was calculated as the CV (%) of the results obtained after fortifying another two sets of blank samples at the concentration levels of analyzed compound as for the repeatability and analyzing them on two days with the same instrument and different operators.

Sensitivity was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). LOD was defined as the amount of analyte that gives a peak with a signal-to-noise ratio of 3, whereas LOQ was the lowest amount of analyte with a signal-to-noise ratio of 10. Specificity was checked by analysis of blank feed samples to verify the appearance of possible interfering substances at the retention times of the tetracyclines.

Statistical Study

With two detection techniques at their disposal: HPLC–DAD and LC–MS, was examined to what extent they influence the result of testing medicated feed samples for which an identical method of preparation was used. For this purpose were used Student's *t* test and Fisher–Snedecor's test. The Student's *t* test was applied to compare the average values of the two groups. Fisher–Snedecor's test was used to evaluate

the differences between the average values in a two groups, at the assumed significance level $\alpha=0.05$.

Results and Discussion

Chromatographic Conditions

The available literature describes few analytical methods enabling the determination of tetracyclines in medicated feed. Some of them are intended for the analysis of a single analyte [19] or several analytes from the tetracyclines group [2, 14, 15, 20–24].

Liquid chromatography with UV detection or diode array detector is a classic technique for detection and determination of tetracyclines. Another way to detection of tetracyclines is to use the fluorescent ability of analyte derivatives with divalent metal ions [24]. However, the synthesis of derivatives extends the time of analysis, which has not found widespread use in laboratory practice. Mass spectrometry seems to be most useful in the analysis of antibacterials in feed [2, 20, 21, 25]. Currently, the use of liquid chromatography with mass spectrometry is recommended for the identification and quantification of antibacterial substances in medicated feeds and non-target feeds.

In our work, we compared two analytical techniques, i.e., micellar liquid chromatography with diode array detection and liquid chromatography by mass spectrometry using the same extraction protocol for tetracyclines from feed samples. First time micellar mobile phase and UV detector was used by Callabero and co-workers in 2002 for analysis tetracyclines from feed samples. They used a conventional unprotected C18 chromatographic column and eluted five tetracycline antibiotics (oxytetracycline, tetracycline, chlortetracycline, doxycycline, and minocycline) with a mobile phase of 0.05 M sodium dodecyl sulfate/5% 1-butanol/0.01 M oxalic acid at pH 3. Good resolution was achieved for the five compounds, whereas OTC and TC co-eluted with an optimized aqueous-organic mobile phase of methanol/acetonitrile/0.01 M oxalic acid at pH 3 [7]. In this work, was used method described by Patyra et al. (2013) where authors using micellar mobile phase with a diode array detector for determination of tetracyclines in medicated feeds. Tetracyclines were analyzed on a Thermo BDS C18 column and a micellar mobile phase consisting of 7% butanol, 0.02 M oxalic buffer, and 0.03 M sodium dodecyl sulfate (pH 2.5). Diode array detector was used and three UV wavelengths were collected for OTC and TC 360 nm, for CTC 370 nm, and for DC 350 nm [15].

In the next step of the work, a sensitive and selective mass spectrometry technique was used to detection and determination this group of compounds. The use of mass spectrometry in multi-analysis, including tetracyclines, to a large extent

imposes the need for such mobile phase components that will not crystallize in the ion source. The most commonly used solution is formic acid [21, 25, 26], as well as acetic acid [27]. For the analysis of tetracyclines with mass spectrometry detection some researchers also used 0.01 M aqueous solution of oxalic acid [20]. However, oxalic acid can be crystallization in the ion source.

In the presented work, optimization of the separation conditions was carried out using a mobile phase consisting of 0.1% aqueous formic acid solution and 0.1% formic acid in ACN, in a gradient elution, which allowed satisfactory separation and obtaining symmetrical peaks of all analyzed TCs. For the LC–MS technique ZORBAX Eclipse XDB C18 column was selected. Chromatograms of blank feed samples and samples fortified all analyzed tetracyclines for both methods (HPLC–DAD and LC–MS) are shown in Figs. 1 and 2.

Sample Preparation

The analytical procedure was optimized in such a way that enable the effective determination of tetracycline antibiotics as simply as possible in a short time. In addition, during the development of procedures, attempts were made to consider economic and ecological aspects, mainly by limiting the use of organic solvents in the preparation of samples for chromatographic analysis.

The main problem in the extraction of tetracyclines from the biological matrices is their ability to form complexes with metal ions and strong binding to matrix proteins. In addition, these antibiotics are sensitive to light and acidic environment, because under the influence of these factors undergo epimerization processes, creating a less active epimeric form [28].

For the extraction of tetracyclines from biological matrices the most common using solvent is McIlvaine buffer with EDTA pH 4 [2, 8, 12–14]. Many researchers also used a solution of succinic [1, 4], hydrochloric, perchloric, phosphoric, oxalic acid or imidazolic buffer for extraction tetracyclines [7, 26, 28–32], as well as mixtures such as: ethyl acetate and citric buffer [28] but also citric buffer and acetonitrile [7, 15].

Feed consists on cereals seeds, fats (vegetable and/or animal), and many different additives such as enzymes, acidifiers, mineral, vitamins or mineral-vitamin premixes all of these component make this matrix very complex and variable making its analysis complex due to the variability of feed composition. Therefore, the analysis of antibacterial substances from feeds is much more labor-intensive and complicated compared to matrices such as animal tissues, milk, eggs or honey.

Several methods have been reported for the extraction of tetracyclines from medicated feed. Considering that

tetracyclines form chelation complexes with different cations, the use of EDTA is a common practice. Therefore, the use of the McIlvaine–Na₂EDTA buffer for the extraction of tetracyclines from the medicated feed described by Patyra and Kwiatek was tested, but the use of this buffer did not give good results for the recovery of tetracyclines using the micellar liquid chromatography technique with a diode detector. On the other hand, satisfactory results were obtained for the LC–MS technique. Gavilán and co-workers (2015) for extraction tetracyclines from feed samples used mixture consisting on methanol and concentrated hydrochloric acid (980 mL + 20 mL), next they diluting of this sample and analysis with the use LC–MS/MS [33]. Methanol is not good solution for extraction antibiotics from feed samples because clean-up step is difficult and a lot of substances from feed matrix is co-eluted with organic extractant. Fernandez-Gonzalez et al. (2002) for the extraction of OTC from medicated and standard fortified blank feed samples used methanol:water mix by up-and-down shaking. The extract was separated and two additional extractions for each sample were repeated. The aqueous extracts for each sample were combined and diluted to 100 mL with the methanol:water mix. This solution was diluted with the methanol mix that was taken to pH 9–10 with triethylamine to keep the OTC level within the calibration range. OTC was analyzed by synchronous spectrofluorimetry [34].

In our work, the analytical procedure was optimized in such a way that enable the effective determination of tetracycline antibiotics as simply as possible in a short time. In addition, during the development of procedures, attempts were made to consider economic and ecological aspects, mainly by limiting the use of organic solvents in the preparation of samples for chromatographic analysis.

In the presented work we tested extraction method described by Caballero et al. [7] and Patyra et al. [15]. Antibacterial substances were extracted with the use of acetonitrile and 0.01 M citric buffer at pH 3. Next feed extract was analyzing with the use HPLC–DAD technique. This arrangement enabled the isolation of tetracyclines to the greatest extent and gave good results for HPLC–DAD technique but did not give satisfactory results using the LC–MS technique because lower recovery values for the analyzed compounds were obtained, especially for chlortetracycline.

Validation of the Methods

Gavilán et al. conducted study that compared the results for four tetracyclines in medicated feed using HPLC–MS/MS and HPLC–FLD techniques. They used two different detection techniques but one extraction protocol to quantified of tetracycline antibiotics from feed samples. They obtained similar results compared to our work. They found that the use of the same extraction technique gives different values

Fig. 1 HPLC–DAD chromatogram of blank feed sample (a) and feed sample spiked four tetracyclines at a concentration 50 mg/kg (b)

Fig. 2 LC–MS chromatogram blank feed sample (a) and feed sample spiked four tetracyclines at a concentration 50 mg/kg (b)

for the recovery of tetracyclines from feed samples. They obtained better recoveries for the tetracyclines using the HPLC–MS/MS technique compared to HPLC–FLD. The results presented by Gavilán show that recoveries for four

tetracyclines obtained with the HPLC–FLD technique were ranged from 50 to 62%, while much higher results were obtained with the HPLC–MS/MS technique, which ranged from 92 to 98% for the four antibiotics [33]. The results

obtained show that the use of one extraction protocol for the two detection techniques differs significantly.

Our results for two different techniques: micellar liquid chromatography with diode detection and liquid chromatography with mass spectrometry also showed this.

In our work, animal feeds were fortified with concentrations of 50 to 1500 mg kg⁻¹ of DC, OTC, TC, and CTC. The linearity was studied in the range of concentration levels from 0.01 to 0.3 mg mL⁻¹ for HPLC–DAD and for LC–MS method. Correlation coefficient (*R*) values in this concentration range were 0.99 for all analyzed TCs. The accuracy of the method and repeatability were evaluated by analyzing OTC, TC, CTC, and DC spiked feeds at levels of 50, 500, and 1500 mg kg⁻¹ feed (six replicates for each level).

The selectivity/specificity study, 20 blank feed samples from different animal species (swine, poultry, and cattle) were analyzed. The results show that the assay recovery for HPLC–DAD method was satisfactory and was in the range of 72.2% to 101.8%. Caballero et al. analyzed feed samples in a similar way. They used an acetonitrile/water mixture buffered at pH 3 mixture for the extraction and a micellar mobile phase consisting of 0.05 M sodium dodecyl sulfate/5% 1-butanol/0.01 M oxalic acid at pH 3. They obtained mean recoveries from spiked feed samples were 79, 83, 86, 87, and 95% for OTC, TC, CTC, DC, and MINO, respectively, in the 15–100 µg g⁻¹ concentration range [7].

In our research recovery for LC–MS was lower than HPLC–DAD, the recoveries were between 45.6 and 87%. The repeatability (CVs, %) for all analyzing tetracyclines were between 2.0 and 5.3% for HPLC–DAD and 3.4%–10.0% for LC–MS. CVs for within-laboratory reproducibility were between 3.4–10.0 and 3.9–17.7%, respectively for HPLC–DAD and LC–MS. LOD and LOQ values for OTC, TC, CTC, and DC in medicated feed ranged from 4.2 to 10.7 mg kg⁻¹ and from 5.2 to 12.6 mg kg⁻¹ for HPLC–DAD and from 5.6 to 10.8 mg kg⁻¹ and from 7.9 to 24.3 mg kg⁻¹ for LC–MS, respectively. All validation parameters for both method are shown in Table 1.

Statistical Results

In this work, the impact of two detection techniques (DAD and MS) on the recovery of tetracyclines from medicated feed which was prepared the same extraction protocol was assessed. Antibiotics were extracted with a mixture of 0.01 M pH 3 citric buffer and acetonitrile (1:1; v/v) and analyzed using liquid chromatography. Samples of medicated feeds for pigs and poultry fortified with antibiotics were tested at three concentration levels. For each sample series, the mean concentration of the analyte in the tested samples, the recovery and the standard deviation were calculated using two detection techniques: diode array detection and mass spectrometry. Based on the obtained results,

Table 1 Validation parameters for HPLC–DAD and LC–MS optimized methods

Validation parameters		HPLC–DAD				LC–MS			
		OTC	TC	CTC	DC	OTC	TC	CTC	DC
Calibration range [µg mL ⁻¹]		0.01–0.3				0.01–0.3			
Calibration curve parameters	<i>R</i> ²	0.9999	0.9997	0.9978	0.9963	0.9995	0.9979	0.9991	0.9976
	a	30.832	43.295	20.312	26.775	2145.4	2832.1	596.87	1095.0
	b	– 24.813	– 37.118	– 19.936	– 40.125	9895.8	3222.4	1681.1	2955.4
Repeatability [%] [<i>n</i> = 6]	50 mg/kg	5.3	3.4	4.5	5.2	6.1	10.0	9.4	9.4
	500 mg/kg	3.3	2.0	2.9	1.6	3.4	6.2	9.8	6.3
	1500 mg/kg	3.9	2.0	2.4	2.8	4.6	4.9	8.0	5.7
Within-laboratory reproducibility [%] [<i>n</i> = 18]	50 mg/kg	6.0	5.2	5.8	4.7	9.5	12.2	17.7	12.6
	500 mg/kg	2.9	2.8	7.5	2.8	9.2	9.8	14.4	9.4
	1500 mg/kg	5.3	3.4	3.2	4.9	3.9	5.2	13.5	3.9
Recovery [%]	50 mg/kg	76.4	96.3	90.7	101.8	74.3	77.0	45.6	77.0
	500 mg/kg	74.8	88.4	83.8	90.4	87.0	81.9	47.2	81.9
	1500 mg/kg	72.2	78.8	76.2	80.4	74.2	70.3	73.7	70.3
Mean recovery [%]		74.5	87.8	83.6	90.9	78.5	76.4	55.5	76.4
LOD [mg/kg]		4.2	7.1	4.5	10.7	8.2	6.3	10.8	5.6
LOQ [mg/kg]		5.4	10.8	5.2	12.6	15.1	14.9	24.3	7.9
Uncertainty (U %)	50 mg/kg	7.21	7.83	13.82	8.41	9.51	12.20	17.68	12.61
	500 mg/kg	4.36	4.95	12.32	4.73	9.24	9.83	14.43	9.40
	1500 mg/kg	2.31	4.38	10.83	3.63	3.87	5.16	13.52	3.91

Table 2 Influence of detection type on the results of tetracycline analysis in medicated feed samples

Comparision of detec- tion techniques	Analyt	F_{calc}	F_{crit}	Result	t_{calc}	t_{crit}	Result
LC–MS	OTC	4.87	2.27	$F_{\text{crit}} > F_{\text{calc}}$	2.01	2.03	$t_{\text{calc}} < t_{\text{crit}}$
LC–MS	TC	0.67	0.44	$F_{\text{crit}} > F_{\text{calc}}$	4.88	2.03	$t_{\text{calc}} > t_{\text{crit}}$
LC–MS	CTC	4.90	2.27	$F_{\text{crit}} > F_{\text{calc}}$	7.32	2.03	$t_{\text{calc}} > t_{\text{crit}}$
LC–MS	DC	0.42	0.44	$F_{\text{calc}} < F_{\text{crit}}$	5.27	2.03	$t_{\text{calc}} > t_{\text{crit}}$

a statistical analysis was carried out to check the hypothesis whether the results of testing samples made with the same extraction method depend on the type of detection used. Student's t test was used to compare the accuracy of the two analytical methods and to compare the significance of the differences between the two mean values, while the Fisher–Snedecor's test was used to compare the values of standard deviations. The hypothesis was verified by the significance test of the difference in precision Fisher–Snedecor at the significance level $\alpha=0.05$. The results of the experiment are shown in the Table 2.

The obtained recoveries of the analyzed tetracyclines from medicated feeds by two different detection methods differ from each other, especially for chlortetracycline where mean recovery is 55% for LC–MS technique. The conducted statistical analysis also indicates that the dispersion of results as well as their differences between the average values of recoveries of the tested substances differ in a statistically significant manner. Therefore, the use of one protocol for the extraction of tetracyclines from medicated feed using citrate buffer and acetonitrile at pH 3, presented in this work is not useful for the analysis of these antibiotics with two types of detection (DAD and MS). The extraction method presented is not suitable for analyzing these drugs using the LC–MS technique.

Conclusion

The results obtained show that one protocol for the extraction of tetracyclines from medicated feed is not suitable for the two analytical techniques MLC–DAD and LC–MS. Statistical analysis using the student t test and Fisher–Snedecor's test showed that the obtained results for tetracycline recoveries from medicated feed are statistically significantly different. Developed extraction method using a mixture of citrate buffer and acetonitrile is useful only for analysis of tetracyclines by HPLC–DAD.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the author.

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