Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 289–294

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Quantitative determination of penicillin V and amoxicillin in feed samples by pressurised liquid extraction and liquid chromatography with ultraviolet detection

E. Benito-Peña, J.L. Urraca, M.C. Moreno-Bondi *

Group of Optical Sensors and Laboratory of Applied Photochemistry, Department of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, E-28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form 7 November 2008
Accepted 10 November 2008
Available online 25 November 2008

Keywords:
Penicillin V
Amoxicillin
Pressurised liquid extraction
High performance liquid chromatography
Feed

ABSTRACT

A rapid and simple method is proposed for the routine determination of amoxicillin (AMOX) and penicillin V (PENV) in swine feedingstuffs. The method is based on pressurised liquid extraction (PLE) followed by high performance liquid chromatography with ultraviolet detection (PLE–HPLC–UV) for antibiotic analysis. Parameters affecting PLE procedure, such as temperature, solvent composition, number of extraction cycles and sample cell size, were evaluated in order to achieve the highest extraction efficiency. The optimised method employed 11 mL extraction cells, acetonitrile–water mixtures (25:75, v/v) for AMOX and (50:50, v/v) for PENV, as extraction solvent, 102.07 atm of extraction pressure, 50 °C of extraction temperature, 5 min of static time and 60% flush volume of the cell size. Extracts were filtered and directly analysed by HPLC–DAD/UV without further clean-up. Mean recovery rates for feed samples fortified with 200–500 mg kg^{-1} of both antibiotics were 86% for AMOX (RSD $\leq$ 6%) and 95% for PENV (RSD $\leq$ 3%). The method was successfully applied to the analysis of a commercial medicated swine feedingstuff, and the results were in good agreement with those obtained using mechanical shaking or ultrasonic extraction combined with solid phase extraction (UE-SPE), previously applied in the literature for feed analysis. The extraction efficiencies were evaluated by statistical comparison (analysis of variance, ANOVA-single factor) of the results obtained using the different extraction methods. Compared to the alternative techniques, PLE offers several practical advantages: easy to perform, fast, savings in solvent volume and in time, all steps are fully automated and further clean-up is not necessary for penicillin analysis.

1. Introduction

Antibiotics are substances produced by living organisms that are able to kill or inhibit the growth of microorganisms. Antimicrobials have been used in veterinary practice to control, prevent and treat infection, and to enhance animal growth and feed efficiency [1]. These drugs are administered to animals by injections (intravenously, intramuscularly, or subcutaneously), orally in feed or water, topically on the skin and by intramammary and intrauterine infusions [2]. Theoretically, all of these routes may lead to the appearance of residues in foods of animal origin such as milk, meat and eggs [3].

The Council Regulation 2377/90/EC and its amendments regulates the authorised drugs that can be applied for therapeutic veterinary use in animals intended for food production. β-Lactams antibiotics, specially amoxicillin and penicillin V, are among the most commonly used antimicrobials for the treatment of gastrointestinal and systemic infections. Both antibiotics are resistant to acid produced in the stomach and can be administered orally [4,5]. However, the presence of antibiotic residues in feedstuff may represent a serious problem as bacteria present in farm animals can spread to the environment and, more importantly, to the human food production chain exerting a negative impact on public health. Some cases of allergic reactions after consumption of foods containing β-lactam residues have been reported in literature [6].

Medicated feedingstuffs must undergo regular checks, including appropriate laboratory tests of homogeneity by the manufacturing establishments, to ensure that they comply with the requirements of the (90/167/EEC) Council Directive, especially with respect to its homogeneity, stability and storage conditions. Apart from these regular checks, feed contamination must also be controlled as it can be a major source of problems for feed producers. A failure to conduct adequate clean-out procedures during the manufacturing process of medicated meal may result in unsafe contamination of subsequent batches when they are processed [7]. This problem is

* Corresponding author. Tel.: +34 91394 5147 fax: +34 91394 4329.
E-mail address: mcmbondi@quim.ucm.es (M.C. Moreno-Bondi).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.11.016

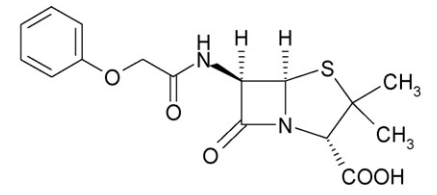
especially important in Spain where, unlike North European countries, the same factory manufactures meals for different animals [8]. So, rapid and reliable analytical methods are required to enforce the regulation and to avoid the illegal use of antibiotics.

The official methods of the Association of American Feed Control Officials (AAFCO) [9] and the Association of Official Analytical Chemists (AOAC) [10] for the analysis of penicillins in medicated feed samples are based on a microbiological plate assays [11] which is time consuming and lacks specificity.

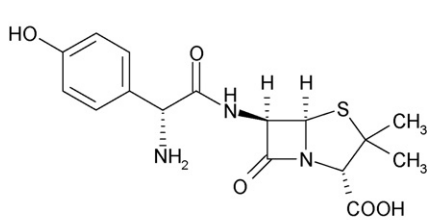
Extraction of β-lactams from feedingstuffs involve the application of lengthy mechanical shaking or ultrasonic extraction methods with different solvents, such as phosphate buffer (pH 6.0) or acetone:water mixtures [10], methanol [12], water:methanol [13], formamide [14] or acetonitrile:water mixtures [5]. Solvent selection is important for the preparation of the extracts due to limited stability of the antibiotics in some of them [15]. A further solid phase extraction step is also required, in most cases for sample clean-up before liquid chromatography with ultraviolet [12] or electrochemical [16] detection.

Pressurised liquid extraction (PLE) is a relatively new technique that has been broadly applied in recent years for the analysis of different analytes in environmental, food and feed samples [17,18]. LaCourse et al. [16] described the application of PLE to the analysis of penicillin G in chicken feed by liquid chromatography with integrated pulsed amperometric detection (HPLC–IAPD). In the optimised procedure, water was selected as the extraction solvent applying a temperature of 60 °C. The extracts were pretreated using SPE and analysed by HPLC–IPAD with recoveries of 92 ± 3%. However, quantitative analysis of the extracts by HPLC–UV, a technique broadly applied in routine analysis laboratories, was not possible due to matrix effects.

This paper describes the optimisation of an effective PLE method for the analysis of PENV and AMOX (Fig. 1) in medicated swine feed samples. The determination is carried out by reverse phase liquid chromatography with UV detection without further clean-up. Several PLE extraction parameters such as solvent mixture, cell size, flush volume, number of cycles and temperature, have been investigated using fortified feed samples in order to select the best conditions for the analysis of these two antibiotics at the usual concentration levels in commercial samples (mg kg^{-1}). The extraction efficiency of the PLE method for the analysis of a PENV in a medicated feedstuff has been compared with that obtained using mechanical shaking and ultrasonic extraction followed by SPE and HPLC–UV detection.



Penicillin V (PENV)
$pK_a = 2.7$



Amoxicillin (AMOX)
$pK_{a1} = 2.8$; $pK_{a2} = 7.2$

Fig. 1. Chemical structure, acronyms and pK_a values of the investigated penicillins.

2. Experimental

2.1. Reagents

The antibiotics penicillin V potassium salt (PENV) (94%) and amoxicillin anhydrous (AMOX) (87%) were purchased from Sigma (St. Louis, MO, USA) and used as received. Trifluoroacetic acid (TFA) (HPLC-grade, 99%) was from Fluka (Buchs, Switzerland). Cellulose acetate, glass and nylon filters were from Millipore (Madrid, Spain). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from SDS (Peypin, France) and HPLC water was purified with a Milli-Q system (Millipore, Bedford, MA). Analytical grade reagents, tetra-*n*-butylammonium hydrogen sulphate (TBA) (98%) and sodium hydroxide (NaOH) were from Merck (Darmstadt, Germany); sodium monohydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$) were from Aldrich (Steinheim, Germany). All solutions used in HPLC were passed through a 0.45 μm nylon filter before use. SPE cartridges, Oasis MAX (*n*-vinylpyrrolidone and divinylbenzene polymer, 500 mg, 6 mL) were from Waters (Barcelona, Spain).

Swine feed samples, blank and medicated were kindly supplied by Nutral S.A. (Spain).

2.2. Apparatus

Extractions of feed samples were performed using an accelerated solvent extractor ASE 200, Dionex (Sunnyvale, CA, USA), equipped with 11 or 22 mL stainless steel cells. The extracts were collected in 40 mL glass vials.

The chromatographic system consisted of a HP-1100 series high performance liquid chromatograph from Agilent Technologies (Palo Alto, CA, USA) equipped with a quaternary pump, on-line degasser, autosampler, automatic injector, column thermostat, and a diode array detector (DAD).

Chromatographic separation for PENV and AMOX was performed on a Luna^{TM} $C_{18}(2)$ reverse phase column (150 mm × 4.6 mm, 5 μm) protected by a RP18 guard column (4.0 mm × 3.0 mm, 5 μm), both from Phenomenex (Torrance, CA, USA).

2.3. Sample preparation and extraction

Following the guidelines of AAFCO [19], samples were stored in a plastic bag at 4 °C until use. For sample analysis, swine feed was grounded in a mill Ika M20 from Ika-Werke (Staufen, Germany) and passed through a US Std. No. 20 sieve from Filtra (Barcelona, Spain). Sub-samples (5 g) were weighed and transferred directly into the 22 or 11 mL stainless steel extraction cells from Dionex.

For method optimisation blank feed sub-samples were directly spiked into the extraction cells with AMOX and PENV at two concentration levels 200 and 500 mg kg^{-1} (three replicates for each concentration level, $n = 3$), for each antibiotic. This was done by adding the appropriate volume of a standard solution of the antibiotics in water (0.8 g L^{-1}) followed by 3 min vortex mixing. The samples were allowed to equilibrate about 15 h (overnight) before extraction.

For the recovery studies, matrix matched calibration plots were prepared for each antibiotic by spiking blank feedstuff extracts, with 25, 50, 100, 150 and 250 mg L^{-1} of PENV and AMOX. The blank extracts were obtained with the same extraction conditions than the samples and were tested not to contain the antibiotics at the method detection limits. All the measurements were carried out in triplicate.

Table 1
Selected PLE operating conditions for antibiotic extraction from feed samples.

Extraction solvent	ACN–H_2O 25–75 (%) for AMOX and 50:50 (%) for PENV
Pressure (atm)	102.07
Temperature (°C)	50
Heat-up time (min)	5
Static time (min)	5
Flush volume (%)	60
Purge time (min)	1
Number of cycles	1
Cell volume (mL)	11
Total extraction time (min)	(13)[a]
Total solvent used (mL)	(16)[a]

[a] Per sample.

The PLE parameters optimised in this work were: the composition of the extraction solvent (acetonitrile, water and acetonitrile/water mixtures 27:75, 50:50 and 75:25, v/v, were tested), extraction temperature (25 °C and 50 °C), number of PLE extraction cycles (1–2), flush volume (60–120% of the extraction cell volume, i.e. the volume of fresh extraction solvent mixture used for flushing of the extraction cell after static extraction) and the cell size (11 and 22 mL). The optimised conditions are summarised in Table 1.

The extracts were made up to a final volume of 20 or 25 mL, depending on the volume of the extraction cell (11 or 22 mL, respectively), with the solvent extraction mixture and passed through a 0.22 μm nylon syringe filter from Tecknokroma (Barcelona, Spain). Finally they were analysed by HPLC–DAD/UV without further clean-up.

To calculate the overall method accuracy, recovery studies were carried out spiking 5 g samples of a medicated swine feedstuff (Sample 1, nominal value 200 mg kg^{-1} PENV, RSD 10%) with 50 and 100 mg kg^{-1} of PENV. Samples were analysed using the optimised PLE–HPLC–DAD/UV method and recoveries were calculated as described previously. All the analysis were carried out in triplicate.

2.4. Comparison with other extraction procedures

For validating purposes, a medicated swine feed sample (Sample 2, nominal value 100 mg kg^{-1} PENV, RSD 10%,) was extracted using the PLE optimised procedure and two other extraction methods based on mechanical shaking (SHA) or ultrasonic extraction (UE). These methods, applied in routine feed analysis in Spanish quality control laboratories, were kindly provided by Labocor S.L. (Madrid, Spain) and are described below.

In the first method, a 2 g of sample was suspended in 25 mL of milli-Q water and shaken for 45 min using a P-Selecta Vibromatic-340 mechanical arm shaker from Selecta (Barcelona, Spain). The supernatant was filtered through a 0.20 μm filter from Whatman (Maidstone, UK) and injected in the HPLC–DAD.

The second method consists of ultrasonic extraction followed by SPE using Oasis MAX cartridges. To that aim, 1 g of the homogenised sample was thoroughly mixed with 25 mL of phosphate buffer (0.1 mol L^{-1}, pH 8.0). Sonication was carried out using a Selecta sonicator bath (mod. 514) from Selecta (Barcelona, Spain) for 10 min. After that the sample was centrifuged at 3400 rpm for 15 min. The supernatant was filtered through a 0.45 μm filter, Whatman (Maidstone, UK), to remove all suspended matter and pH was adjusted to 7.5. The samples were cleaned up and preconcentrated using the Oasis MAX SPE cartridges as described previously [20].

For quantification purposes matrix matched calibration plots were prepared by extracting blank feedstuff samples, spiked with increasing concentrations of PENV (25, 50, 100, 150 and 200 mg kg^{-1}), using the three extraction procedures. The extracts were analysed by HPLC–DAD/UV and sample concentration was evaluated from the corresponding calibration plot. All the analysis were carried out in triplicate.

2.5. Chromatographic analysis

A gradient programme was used with the mobile phase, combining solvent A (Milli-Q water with 0.01% TFA, v/v) and solvent B (acetonitrile with 0.01% TFA, v/v) as follows: 100% A (3 min), 100–63% A (5 min), 63% A (11 min), 63–33% A (5 min), 33% A (5 min), as described previously [20]. Analyses were performed at a flow rate of 1.5 mL min^{-1} and the column temperature was kept at 35 °C. The injection volume was 20 μL and all the compounds were eluted within 15 min. The DAD detector wavelength was set at 220 nm.

3. Results and discussion

3.1. Optimisation of the PLE method for AMOX and PENV extraction in swine feed

3.1.1. Extraction solvent and temperature

The choice of the extraction solvent is probably one of the most critical parameters in a PLE procedure. The presence of an unstable four-member ring in the β-lactam structure makes these compounds prone to degradation by heat or in the presence of alcohols. These antibiotics are also readily isomerised in an acidic environment. Thus, solvent and temperature applied in the PLE procedure must be carefully selected for penicillins determination [15,21].

Usually mixtures of organic solvents as hexane, chloroform, MeOH and ACN among them or with water have been applied to the extraction of analytes such as mycotoxins, pesticides, fatty acids or antibiotics in cereals, feed samples or eggs [18], [22–24]. However, the low solubility of penicillins in hexane or in chlorinated solvents prevents their use for this application [25].

To evaluate the thermal stability of the antibiotics for further PLE extraction, solutions of acetonitrile, water and methanol containing 50 (200 mg kg^{-1}) and 125 mg L^{-1} (500 mg kg^{-1}) of PENV and AMOX were heated at 50 °C for 10 and 20 min. The analysis of the solutions by HPLC showed no degradation in acetonitrile and water. However, recoveries obtained for the methanolic solution were lower than 80% and 42%, for both antibiotics after 10 and 20 min heating, respectively. Consequently, acetonitrile, water and combinations of both solvents at different ratios (75:25, 50:50, 25:75, v/v) were tested for the optimisation of the PLE method at a temperature of 50 °C.

First of all, the non-specific retention of analytes in the filters used in the PLE cells was evaluated. Cellulose acetate, glass fibre and nylon membrane filters were tested by percolating 10 mL of a solution containing 50 mg L^{-1} of AMOX and PENV through the different materials and evaluating the recoveries in each case. The experiment confirmed that none of the antibiotics were retained in any of the materials. Finally, cellulose acetate filters were selected for the assay.

Table 2 collects the recovery rates obtained in the extraction of AMOX and PENV from spiked ground feed (200 and 500 mg kg^{-1}, for each antibiotic) as a function of the solvent composition used in the PLE method.

The best recoveries for AMOX, 86% (RSD = 6%, $n = 3$)) and 80% (RSD = 2%, $n = 3$) for spiking levels of 200 and 500 mg kg^{-1}, respectively, were obtained using an acetonitrile–water ratio of 25:75 (v/v). In the case of PENV, the optimum extraction mixture was acetonitrile–water 50:50 (v/v) which yielded recoveries of 95% (RSD = 2%, $n = 3$) for 200 mg kg^{-1} and 94% (RSD = 3%, $n = 3$) at 500 mg kg^{-1}. The recoveries obtained for both antibiotics were independent of the assayed concentration level.

Table 2
Influence of the solvent composition on the extraction efficiency (*R*%) of PENV and AMOX from swine feed samples using PLE.

Solvent mixture ACN–H_2O		Spiking level (mg kg^{-1})	AMOX			PENV		
			Level found (mg kg^{-1})	*R* (%)	RSD (%)	Level found (mg kg^{-1})	*R* (%)	RSD (%)
100	0	200	5	3	9	84	42	5
		500	58	12	6	226	45	2
75	25	200	49	24	4	150	76	9
		500	111	22	5	419	84	3
50	50	200	113	57	3	190	95	2
		500	296	59	3	470	94	3
25	75	200	171	86	6	122	61	5
		500	400	80	2	301	60	7
0	100	200	98	50	15	83	41	13
		500	163	33	12	245	49	14

Conditions: pressure 102.07 atm, static time 5 min, flush volume 60%, 1 cycle, cell size 11 mL ($n = 3$)

3.1.2. Flush volume, number of extraction cycles and cell size

The extraction efficiency of the PLE technique depends on the number of cycles applied. To evaluate the effect of the total volume and the number of extraction cycles on the recovery of the antibiotics from a feed matrix, experiments were performed using a flush volume of 60% or 120% of the cell size (11 or 22 mL) and one or two extraction cycles, respectively, with 5 min static time. As it is shown in Fig. 2, the variation of these parameters had no significant effect on the recoveries of both penicillins at the concentration levels tested (200 and 500 mg kg^{-1}).

PLE usually requires the dispersion of the sample with an inert material to prevent extraction cell clogging, avoid sample aggregation and/or improve the sample/solvent contact favouring analyte extraction. A diatomaceous earth sorbent, usually applied in PLE, was tested to evaluate its effect on the extraction of the antibiotics from the swine feed samples using 11 mL extraction cells. Recoveries were not significantly different (95% confidence level) in the absence and in the presence of the dispersion agent and its use was discarded.

Fig. 2 shows the effect of the cell size (11 and 22 mL) on the recoveries of AMOX and PENV from feedingstuffs fortified with 200 and 500 mg kg^{-1} of each antibiotic. No significant differences (95% confidence level) were obtained in both cases. However, the use of the 11 mL cells allowed a decrease in the volume of extracting solvent and lower detection limits, so it was selected for further experiments.

The PLE optimised parameters are collected in Table 1. The total PLE extraction time was 13 min per sample and the instrument can extract up to 24 samples, unattended.

3.2. Analytical characteristics

The specificity of the method was assessed by analysing blank feed extracts. The absence of background peaks at the retention time of PENV and AMOX above a signal to noise ratio of 3 demonstrated that the optimised procedure is free of endogenous interferences. The chromatogram obtained for the PLE extract is shown in Fig. 3. The calibration curve for each penicillin was built by spiking blank swine feed PLE extracts with five different concentrations of AMOX and PENV in the range 25–250 mg L^{-1} [25]. The linearity of the plots in the investigated range was good ($R^2 > 0.998$ for both penicillins).

In order to evaluate the occurrence of matrix effects, observed in previously reported methods [16], calibration curves obtained with standard solutions of the antibiotics were compared to those obtained using spiked blank feed extracts. The slope and the origin ordinates of the two regression curves, compared using a *t*-test, were not significantly different (95% confidence level) demonstrating the lack of matrix effect in the analysis of penicillins using the PLE optimised method.

The optimised extraction procedure was first applied to spiked feedingstuffs. The absolute recovery rates and precision are pre-

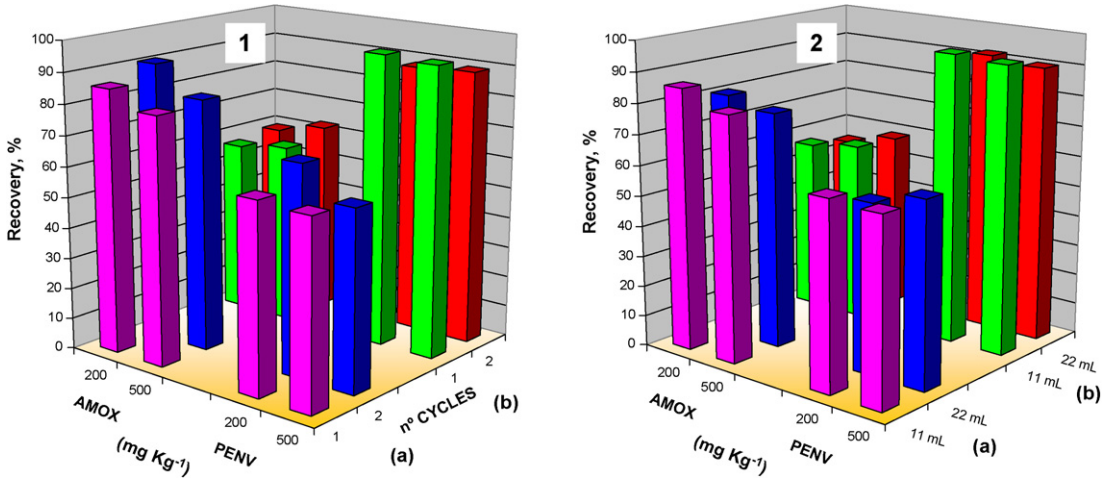


Fig. 2. (1) Influence of the number of extraction cycles on the recoveries of 200 and 500 mg kg^{-1} of AMOX and PENV from feed samples. (a) Acetonitrile–water (25:75 v/v) and (b) acetonitrile–water (50:50, v/v). PLE conditions: 102.07 atm, temperature 50 °C, static time 5 min, flush volume 60%, cell size 11 mL. (2) Influence of the cell size on the extraction recoveries of 200 and 500 mg kg^{-1} AMOX and PENV from feed samples. (a) Acetonitrile–water (25:75 v/v) and (b) acetonitrile–water (50:50, v/v). PLE conditions: 102.07 atm, temperature 50 °C, static time 5 min, flush volume 60%, 1 cycle.

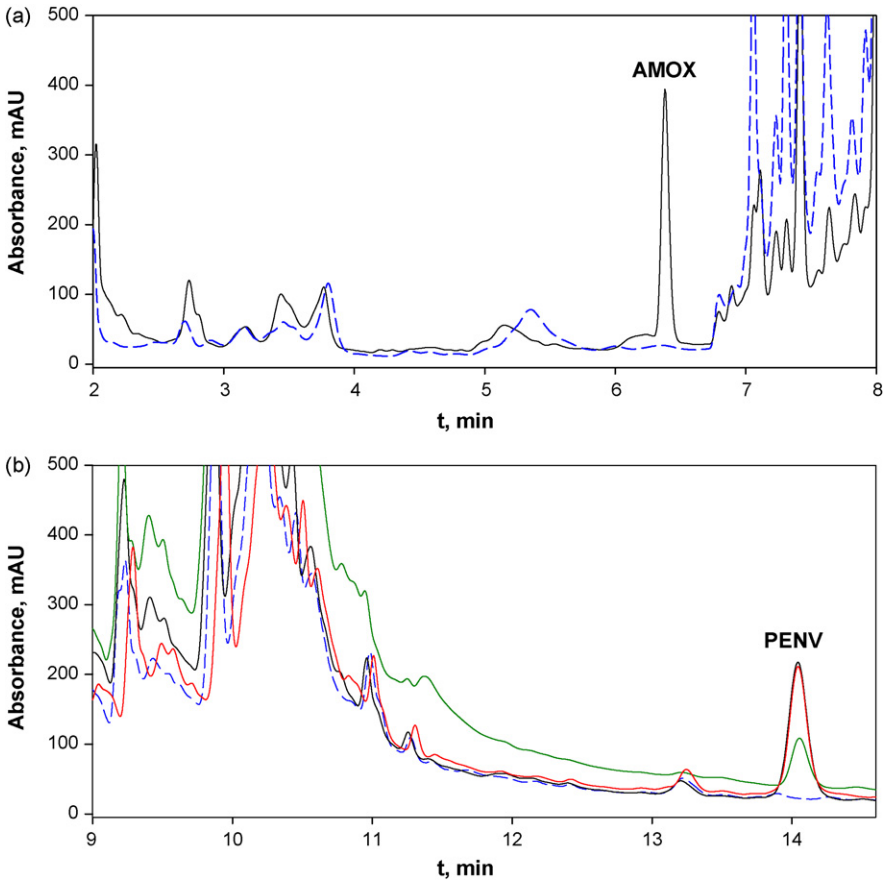


Fig. 3. (a) HPLC–UV chromatogram obtained for a swine feed sample spiked with 500 mg kg^{-1} of AMOX extracted using the optimised PLE method (acetonitrile–water 25:75, v/v; 102.07 atm; temperature 50 °C; static time 5 min; flush volume 60%; cell size 11 mL). (b) HPLC–UV chromatogram obtained for a medicated swine feed sample (nominal value 200 mg kg^{-1}, RSD 10%) extracted: (▬) using the optimised PLE method; (—) UE-SPE and (▬) SHA. (▬ ▬ ▬) HPLC–UV chromatogram corresponding to a blank feed extract obtained by PLE. Chromatographic conditions are summarised in Section 2.5.

sented in Table 3. Data obtained from samples spiked at two levels (200 and 500 mg kg^{-1}) for each antibiotic demonstrate that the recoveries do not depend on the analyte concentration in the evaluated range. Intra day repeatability and within laboratory inter day reproducibility were estimated from three replicate determinations of each penicillin at two concentration levels (200 and 500 mg kg^{-1}) on the same day and on three different days, respectively. The results, collected in Table 3, compare favourably those obtained using alternative techniques such as SHA-SPE [12] or UE [13] for the determination of penicillins in such samples. Recoveries were always higher for PENV than for AMOX, probably due to the amphoteric character of this antibiotic, and ranged between 80 and 86% (RSDs < 6%) for AMOX and 94 and 95% (RSDs < 3%) for PENV, demonstrating the good accuracy of the PLE method. The limits of detection (LOD), calculated as three times signal to noise ratio, were 7.3 and 3.4 mg kg^{-1} for AMOX and PENV, respectively, and are of the same order [5,12] or better [10] than those reported in the literature using other extraction techniques.

The optimised PLE–HPLC–DAD/UV method was applied to the determination of PENV in medicated swine feedingstuffs S1 and S2 (nominal value 200 and 100 mg kg^{-1}, RSD 10%, respectively).

Fig. 1S of the supplementary material shows the results of the analysis of sample S1 using the optimised procedure in three different days (n = 9) [26]. A mean PENV concentration of 192 mg kg^{-1} was obtained with a RSD value of 5%. This value is not significantly different (95% confidence level) from the nominal value reported by the supplier demonstrating both, the homogeneity of the medicated feedstuff and the reproducibility of the PLE method.

Moreover, for validating purposes, a recovery study was performed by spiking 5 g of S1 with increasing amounts (0.25 and

Table 3
Evaluation of the repeatability (n = 3) and within laboratory reproducibility (n = 9) for the determination of PENV and AMOX in swine feedingstuffs.

Antibiotic	Added (mg kg^{-1})	Repeatability			Reproducibility		
		Found (mg kg^{-1})	R (%)	RSD (%)	Found (mg kg^{-1})	R (%)	RSD (%)
AMOX	200	168	84	2	171	86	6
	500	401	80	2	401	80	2
PENV	200	190	95	1	190	95	2
	500	465	93	2	470	94	3

Table 4
PENV analysis in a medicated swine feed using PLE–HPLC–DAD/UV ($n = 3$).

Medicated feed sample		Added (mg kg^{-1})	Found (mg kg^{-1})	RSD (%)	R (%)
Nominal value[a] (mg kg^{-1})	RSD (%)				
200	10	0	196	4	98
		50	240	3	96
		100	286	3	95

[a] RSD 10%.

Table 5
Recovery study of PENV ($n = 3$) in a medicated swine feed using different extraction techniques.

Extraction technique	R[a] (%)	RSD (%)	Analysis time/ sample (min)
PLE	102	2	13
UE-SPE	105	9	60
SHA	91	14	50

[a] Nominal value: 1.0 kg ton^{-1} of Pen V potassium (RSD 10%).

0.5 mg) of PENV potassium salt. As shown in Table 4, recoveries ranging from 95 to 98% were obtained in the assayed interval and the results do not differ significantly from the nominal value reported by the supplier.

3.3. Comparison of the optimised PLE method with other extraction procedures

Table 5 collects the results of the analysis of sample S2 (PENV, 100 mg kg^{-1}, RSD 10%) by PLE, mechanical shaking (SHA) and ultrasonic extraction-SPE (UE-SPE), as described in Section 2.4.

The highest extraction recoveries (102% and 105%) were obtained using PLE or UE-SPE. However, the RSD values for the PLE method, 2%, were significantly better than those obtained by UE-SPE (9%). Moreover, the PLE method allowed shorter analysis times than UE-SPE and it did not require a further clean-up step. The worst extraction yields were obtained with the SHA method (91%, RSD 14%) and the extraction times were also longer than with PLE. These results demonstrate that a more efficient contact solvent/matrix is achieved at the temperature and pressure conditions applied in PLE, or using the ultrasonic energy (US-SPE), than with mechanical shaking. Statistical analysis by ANOVA-single factor, of the results obtained by the three extraction methods showed that they did not differ significantly.

3.4. Conclusions

A simple, fast, and cost effective method has been optimised for the analysis of AMOX and PENV in swine feed samples. The procedure is based on a PLE method and HPLC–UV analysis, without derivatisation, and avoids the use of chlorinated organic solvents with the associated environmental safeguard. In comparison to the AOAC official method and other alternative extraction procedures for the analysis of penicillin antibiotics in medicated feeds, this procedure is faster (13 min) and can be easily applied to routine analysis of AMOX and PENV in swine feeds with very little sample manipulation.

Acknowledgements

This work has been funded by the Spanish Ministry of Science and Technology (Project no. CTQ2006-15610-C02-02), by the Madrid Community (ref. S-0505/AMB/0374), the European Social Fund and the European Regional Development Funds.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2008.11.016.

References

[1] L. Tollefson, M.A. Miller, J. AOAC Int. 83 (2000) 245–254.
[2] J.M. Mitchell, M.W. Griffiths, S.A. McEven, W.B. McNab, J. Yee, J. Food Prot. 61 (1998) 742–756.
[3] A. Johnston, Br. Med. J. 317 (1998) 663–665.
[4] www.octagon-services.co.uk/articles/potencil.htm, May 2007.
[5] V. Gamba, G. Dusi, Anal. Chim. Acta 483 (2003) 69–72.
[6] A.D. Dayan, Vet. Microbiol. 35 (1993) 213–216.
[7] D.G. Kennedy, A. Cannavan, R.J. McCracken, J. Chromatogr. A 882 (2000) 37–52.
[8] www.e-campo.com/media/news/nl/ganporcinosnutricion13.htm, Mayo 2007.
[9] www.aafco.org, May 2007.
[10] Reference method AOAC 967.41 (Code AAFCO 074.00) in AOAC Feed Check Samples, 2006.
[11] A. Kabay, Appl. Microbiol. 22 (1971) 752–755.
[12] M.J. Gramse, P.E. Jacobson, J AOAC Int. 88 (2005) 679–683.
[13] M. Dousa, R. Hosamnova, J. Pharm. Biomed. Anal. 37 (2005) 273–279.
[14] R.C. Kersey, F.V. Leghorn, Appl. Environ. Microb. 1 (1953) 150–152.
[15] Chemistry and biology of beta-lactam antibiotics, in: R.B. Morin, M. Gorman (Eds.), in: Penicillins and Cephalosporins, vol. 1, Academic Press, New York, 1982.
[16] W.R. LaCourse, C.O. Dasenbrock, J. Pharm. Biomed. Anal. 19 (1999) 239–252.
[17] E. Björklund, S. Sporring, K. Wiberg, P. Haglund, C. Holst, Trends Anal. Chem. 25 (2006) 207–218.
[18] J.L. Urraca, M.D. Marazuela, M.C. Moreno-Bondi, Anal. Chim. Acta 524 (2004) 175–183.
[19] Association of American Feed Control Officials, Inc., Oxford IN, Method, SP-11, 2.11.
[20] E. Benito-Peña, A.I. Partal-Rodera, M.E. León-González, M.C. Moreno-Bondi, Anal. Chim. Acta 556 (2006) 415–422.
[21] A.A.M. Stolker, U.A.T. Brinkman, J. Chromatogr. A 1067 (2005) 15–53.
[22] M.C. Herrera, R.C. Prados-Rosales, J.L. Luque-García, M.D. Luque de Castro, Anal. Chim. Acta 463 (2002) 189–197.
[23] K. Schäfer, Anal. Chim. Acta 358 (1998) 69.
[24] S. Herranz, M.C. Moreno-Bondi, M.D. Marazuela, J. Chromatogr. A 1140 (2007) 63–67.
[25] L. Pallaroni, C. Von Holst, C.S. Eskilsson, E. Björklund, Anal. Bioanal. Chem. 374 (2002) 161–166.
[26] S. De Baere, P. De Backer, Anal. Chim. Acta 586 (2007) 319–325.