Journal of Chromatographic Science, 2018, Vol. 56, No. 3, 285–291
doi: 10.1093/chromsci/bmx096
Advance Access Publication Date: 13 December 2017
Article

Article

Analysis of Major Components of Bacitracin, Colistin and Virginiamycin in Feed Using Matrix Solid-phase Dispersion Extraction by Liquid Chromatography-electrospray Ionization Tandem Mass Spectrometry

Yanfei Tao*, Shuyu Xie, Yufei Zhu, Dongmei Chen, Yuanhu Pan, Xu Wang, Zhenli Liu, Lingli Huang, Dapeng Peng, and Zonghui Yuan

National Reference Laboratory of Veterinary Drug Residues (HZAU)/MOA Key Laboratory of Food Safety Evaluation, Huazhong Agricultural University, Hongshan, Shizi shan Street No. 1, Wuhan 430070, China

*Author to whom correspondence should be addressed. Email: tyf@mail.hzau.edu.cn

Received 3 June 2016; Revised 15 May 2017; Editorial Decision 5 November 2017

Abstract

A quantitative LC-MS/MS method has been developed for simultaneous determination of bacitracin A, bacitracin B, colistin A, colistin B and virginiamycin M_1 in feed. This rapid simple and effective extraction method was based on matrix solid-phase dispersion. Qualitative and quantitative analyses were performed by LC-ESI-MS/MS. CCβ of polypeptide antibiotics upon the method ranged from 9.6 to 15.8 μg kg^{-1} and 19.4 to 27.5 μg kg^{-1}, respectively. The limit of quantification of polypeptide antibiotics was 25 μg kg^{-1} in feed samples. The recoveries of polypeptide antibiotics spiked in feed samples at a concentration range of 25–100 μg kg^{-1} were found above 75.9–87.9% with relative standard deviations within days less than 15.7% and between days less than 20.6%. This rapid and reliable method can be used to efficiently separate, characterize and quantify the residues of polypeptide antibiotics in feed with advantages of simple pretreatment and environmental friendly.

Introduction

Bacitracin, colistin and virginiamycin are polypeptide antibiotics, which are produced by Paenibacillus polymyxa or Aerogenous bacillus. They are commonly used for the promotion of growth, prevention and control of diseases in farm animals (1).

Bacitracin is a polypeptide antibacterial agent produced by strains of *Bucillus lzcheniformis* and *Bacilltls subtili*. Bacitracin A (BTCA) is the major component of bacitracin which has effective activity against Gram-positive and Gram-negative bacteria, i.e., *Staphylococcus aureus*, *Streptococcus*, etc., by affecting protein synthesis, cell wall synthesis, and membrane functions (1). It has frequently been used in association with zinc, because this combination is more stable than bacitracin alone. It has been widely used as veterinary medicinal products added to feed to prevent and treat infections, promote growth and reduce feeding cost in modern animal husbandry (2–4). Colistin (also known as polymyxin E) is an important member of the polymyxin group. It is a complex mixture of at least thirty components (5), 13 of which have been isolated and identified (6, 7). Colistin is active against most Gram-negative bacteria (8). The two major components are colistin A (CLTA) and colistin B (CLTB), which differ only in the fatty acid side chain. Virginiamycin is a cyclic polypeptide produced by *Streptomyces virginae*. It is a mixture of peptolides and consists of two factors: M (M_1 and M_2) and S (S_1, S_2, S_3, S_4 and S_5), M_1 is the main component (9). Virginiamycin M_1 (VGM1) is mainly active against Gram-positive bacteria. For this reason, with Council Regulation 2821/98 virginiamycin is banned to using in animal feed (10).

Some papers about analytical methods for polypeptide antibiotics have been published. High performance liquid chromatography (HPLC) methods, liquid chromatography–mass spectrometry

© The Author(s) 2017. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024

(HPLC-MS/MS) methods and enzyme-linked immunosorbent assay (ELISA) have been considered as powerful analytical tools. As liquid chromatography–mass spectrometry (HPLC-MS/MS) methods, Wan (11) developed a method for the detection of residual bacitracin A, colistin A, and colistin B in milk and animal tissues. As methods for the assay of colistin have been developed, capillary electrophoretic (12), HPLC method (13) and HPLC-MS method (14–16). As HPLC-MS method, Sin's study (16), a selective and sensitive HPLC-MS/MS method has been developed for simultaneous determination of residual bacitracin and colistin in bovine milk samples. The practical quantification limits of bacitracin A and colistin A were 100 and 50 $\mu g\,kg^{-1}$. Kaufmann (17) developed a HPLC-MS/MS method for the detection of residual bacitracin, colistin A, colistin B, polymyxin B1 and polymyxin B2 in a variety of food matrices. Govaerts (18) investigated the major polymyxins of the B and E series by direct infusion in Q/TOF and ion trap apparatus, resulting in a complete description of the fragmentation behavior. And it had been confirmed in the commercial samples. Several chemical methods for the determination of virginiamycin in feed and edible food have been developed (19–23). Sin (20) developed a sensitive method for simultaneous determination of lincomycin and virginiamycin M_1 in swine muscle and organs using liquid chromatography with electrospray ionization tandem mass spectrometry. Van (21) analyzed the bacitracin, olaquindox, spiramycin, tylosin and virginiamycin in feeds with liquid chromatography–tandem mass spectrometry. De-Alwis (22) detected of virginiamycin M_1 and another 12 antibiotics residues in distillers grains by liquid chromatography and ion trap tandem mass spectrometry. Cronly (23) developed and validated of a rapid multi-class method for the confirmation of virginiamycin and 13 prohibited medicinal additives in pig and poultry compound feed with HPLC-MS/MS. However, there were no methods for the analysis of bacitracin A, bacitracin B, colistin A, colistin B and virginiamycin M_1 in feed with HPLC-MS/MS.

Matrix solid-phase dispersion (MSPD) comprises sample homogenization, cellular disruption, fractionation and purification in a single process (24). The method involves blending a viscous sample with a solid support and isolating organic compounds by adsorbing them on the suitable solid adsorbents followed by desorption with a small amount of organic solvent (25). Operational steps in MSPD and selectivity of the extraction process are conditioned by a number of factors, i.e., the physical state of the sample, the relative concentrations and properties of analytes, the interferences of the sample, the suitable combination of sorbent, etc. To start the optimization, the attention must fall upon the careful selection of sorbent materials and elution solvents to enhance the yield of the extraction (26–28).

To our knowledge, there is very few paper dedicated to the analysis of polypeptide antibiotics in feeds by MSPD. The aim of this paper is therefore the development and validation of a rugged method based on MSPD coupled to LC-MS/MS capable of detecting colistin, virginiamycin M_1 and bacitracin. The effects of several extraction parameters, such as sorbent adsorption, clean-up procedures and solvent elution were tested in order to improve recovery and sensitivity. Finally, we obtain a method allowing for the extraction of these compounds by a single extraction step, followed by a dispersive clean-up with C_{18} material and LC-MS/MS analysis.

Material and Methods

Standards

The analytical standards colistin sulfate (78%) and virginiamycin M_1 (95%) was bought from Sigma–Aldrich (Ehrenstorfer, USA). Bacitracin (77%) (Specification: 100 mg) was purchased from China Institute of Veterinary Drug Control (Beijing, China).

Individual stock solutions (1,000 $mg\,L^{-1}$) were prepared by accurately weighing 10 mg of reference substance into a 10 mL volumetric flask. The compounds colistin and bacitracin were dissolved with 0.1% formic acid in purified water separately. Virginiamycin M_1 was dissolved with methanol. Mixed stock solution (10 $mg\,L^{-1}$): the solution was prepared by transferring 1 mL of each individual stock solution (1,000 $mg\,L^{-1}$) into a 100 mL volumetric flask and diluting it to volume with mobile phase A. Mixed spiking solution (1 $mg\,L^{-1}$; 500 $\mu g\,L^{-1}$; 200 $\mu g\,L^{-1}$; 100 $\mu g\,L^{-1}$; 50 $\mu g\,L^{-1}$; 25 $\mu g\,L^{-1}$): appropriate volumes of mixed stock solution (10 $mg\,L^{-1}$) were transferred into volumetric flasks and diluted to appropriate volumes with mobile phase A.

All solutions are stored in plastic vessels. The stock solutions are kept in a freezer (−20°C). The more diluted spiking and reference solutions are stored in a refrigerator (4°C). Stability experiments indicated 1 week of stability time period.

Reagents and materials

Acetonitrile and methanolgrade for chromatography were obtained from Tedia Company Inc (Fairfield, OH, USA). Formic acidpure grade for analysis was produced by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Hypersil Gold C_{18} chromatographic column (150 mm × 2.1 mm, 5 μm) was obtained from Thermo Electron Corporation (Waltham, MA, USA). The solid-phase material used for MSPD was C_{18}-bonded silica (50 μm I.D., YMC, Japan).

0.1% formic acid methanol solution was prepared by measuring 1 mL formic acid into 1,000 mL volumetric flask contained about 800 mL Milli-Q water, then Milli-Q water was added to the volume and blending.

Mobile phase A: 1 mL of formic acid were transferred into a 1,000 mL volumetric flask and diluted to volume with purified water.

Mobile phase B: 1 mL of formic acid were transferred into a 1,000 mL volumetric flask and diluted to volume with acetonitrile.

Apparatus

Liquid chromatography coupled with tandem mass spectrometry was available from Thermo Electron Corporation (Waltham, MA, USA, TSQ Quantum Ultra). Analytical balance with a sense of weight 0.0001 g and high-speed refrigerated centrifuge (CR21G) were purchased from Shimadzu Corporation and Hitachi in Japan, respectively. High-speed homogenizer (AM-6) was obtained from Nihonseiki Kaisha Ltd. Vortex Oscillator (WX-80A micro) was bought from Shanghai Huxi Analysis Instrument Factory Co., Ltd. Nitrogen blowing instrument (N-evaptm112) was purchased from Organomation Associates, Inc. Ultra pure water system was obtained from Millipore-Q (Millipore, Bedford, MA, USA).

Samples

Different swine and poultry feed samples were collected from local farms located in Hubei (Wuhan, Yichang, Xiangfan) China. Swine feed samples were sieved at 2 mm, ground and stored at −20°C for posterior analysis.

The validation of the method was carried out using swine and poultry feed samples (each sample 1.0 gram), spiked, respectively, with 100 μL mixed standard solutions at levels ranging from 10 to 1,000 $\mu g\,L^{-1}$.

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024

Sample preparation using MSPD

One gram of the sub-samples (feed) were placed into a mortar (50 mL capacity) and were gently blended with 2 g of the C_{18} material (dispersion adsorbent) for 5 min using a pestle, to obtain a homogenous mixture. The homogeneous mixture was transferred in a glass column (300 mm × 15 mm i.d.) with degreased cotton packed at the bottom and at the top of the sample mixture. The column was tightly compressed using a galss stick.

The cartridges were simply washed with 5 mL water and 5 mL water-methanol (80/20, v/v) and the washings were discarded. Then the cartridges were dried by slight vacuum and eluted with 8 mL 0.2% formic acid methanol solution. The eluation was dried on the nitrogen dryer at 40°C under a mild stream of nitrogen. The concentrated extract was re-dissolved by 0.5 mL 0.1% formic acid water solution, filtered and subjected to LC-MS/MS analysis.

LC-MS/MS analysis

Analyses were performed with LC-MS/MS system (Thermo Electron Corp., Wyman, Waltham, MA, USA) consisting of A Finnigan Surveyor Plus system with an online degasser, a Surveyor autosampler and a TSQ Quantum triple quadrupole mass spectrometer equipped with an electrospray interface operating in the positive mode (ESI+). The LC-ESI-MS/MS system was controlled by Xcalibur Version 2.0 software. Chromatographic separation was performed on a 150 mm × 2.1 mm, 5 µm Hypersil Gold C_{18} analytical columns (Thermo Electron Corporation, Waltham, MA, USA). Mobile phases were delivered at a flow-rate of 0.2 mL min^{-1}. The gradient elution program is 0–5 min: 90% A, 10% B; 5.1–15 min: 25% A, 75% B; 15.5–20 min: 90% A, 10% B to restore the initial conditions before the next injection. The sample injection volume was 10 µL and column temperature was controlled under 40°C. Instrumental settings, data acquisition and processing were controlled via the built-in software package.

In order to optimize the tuning parameters for BTCA, BTCB, CLTA, CLTB, VGM_1, a standard solution containing 1 µg mL^{-1} BTCA, BTCB, CLTA, CLTB, VGM_1 in 0.1% formic acid methanol solution was infused into the ESI source at 10 µL min^{-1}. For the five compounds, the positive mode (ESI+) was selected and other parameters were settled with spray voltage 4,500 V, capillary temperature 350°C, sheath gas pressure 40 psi, and aux gas pressure 35arb. The parameters of the m/z and collision energy of parent ions and quantitative daughter ions are shown in Table I.

Method evaluation

The method was validated with reference to the implemented validation procedure for residues in food animal products as described in EU Commission Decision 2002/657/EC (European Commission 2002 (29)). The validation of specificity, linearity, CCα, CCβ, accuracy and precision for the method were determined by spiking feed of swine and poultry with BTCA, BTCB, CLTA, CLTB, VGM_1 standard solutions.

The specificity of the method was demonstrated by testing 20 blank feed of swine and poultry respectively. The criteria for the specificity of each analyte in feed of swine and poultry are reported in Table II. The four identification points (IPs) earned by selecting two SRM transitions were enough both to confirm BTCA, BTCB, CLTA, CLTB, VGM_1. During the validation study, the relative standard deviation of the retention time was within ±2.5% and the ion ratio between the two SRM transitions was inside the tolerances recommended by the Commission Decision 2002/657/EC (see Table II) for all analytes.

Matrix effect on the ionization of analytes was evaluated by comparing the peak area of standard solution with that of the matrix extract solution. Matrix effect was calculated by the formula: matrix effect = $[1 - (a_{matrix}/a_{standard})] \times 100$ where a_{matrix} and $a_{standard}$ are the slopes of calibration lines for solvent standards and matrix-matched calibration. Blank feed samples were used as matrices for calibration curve study. The matrix-match calibration curves were made by fortified feeds with six levels of 25, 50, 100, 250, 500 and 1,000 µg kg^{-1} for the targets before the extraction procedure ($n = 3$).

The sensitivity of BTCA, BTCB, CLTA, CLTB, VGM_1, CCα was established by the following steps: 20 blank samples were analyzed and the signal-noise ratio (S/N) is calculated at the time window in which the analytes are expected. CCα values were defined as three times of S/N. The detection capability (CCβ) is the concentration at which the method is able to detect truly contaminated samples with a statistical certainty of 1−β. CCβ was calculated by analyzing 20 blank samples spiked with the concentration at CCα and then the CCα value plus 1.64 times the corresponding standard deviation is equal to CCβ ($\beta = 5\%$).

Trueness and precisions (intra-day, inter-day, and within-laboratory) were calculated from the determination of five aliquots each feed fortified at four levels (25, 50 and 100 µg kg^{-1}). The analyses were finished by the same operator in triplicate in a 2-week period. Within-laboratory was carried out in the same laboratory, but performed by two different operators. The recovery was calculated by the following formula: (the measured level/the fortified level) ×

Table I. LC-MS/MS Parameters Needed to the Identification of Polypeptide Antibiotics, Selected in this Study, in Feed Extracts

Targets	Retention time (% R.S.D.)	Precursor ion (m/z)	SRM transition (m/z)	Collision energy (eV)	Ion ratio[a] Average (RSD)	Tolerance ion ratios 2002/657/EC (%)[b]
Bacitracin A	9.30 (0.09)	712	712/198.9	40		
			712/226.7	32	0.79 (6.9)	±20
Bacitracin B	9.36 (0.04)	705	705/198.8	40		
			705/252.8	49	0.35 (8.5)	±25
Colistin A	9.15 (0.10)	391	391/384.8	15		
			391/378.9	19	0.28 (14.2)	±25
Colistin B	9.12 (0.07)	386	386/380.0	13		
			386/374.1	17	0.90 (15.4)	±20
Virginiamycin M1	10.46 (0.08)	526	526/508.4	16		
			526/355.1	21	0.35 (13.8)	±25

[a]The relative abundance between the two SRM transitions is calculated as ratio of Qualifier Intensity/Quantifier Intensity.

[b]The EU guidelines sets criteria for the observed ratio as follows; expected ratio >0.5. Observed ratio should be within (20%). expected ratio 0.2–0.5. Observed ratio should be within (25%); expected ratio 0.1–0.2. Observed ratio should be within (30%); expected ratio <0.1. Observed ratio should be within (50%).

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024

Table II. Validation Data for Swine Feed

Analyte	Coefficient of correlation (r^2)	LOQ (μg kg^{-1})	CCβ (μg kg^{-1})	Signal suppression (-)	Precision (RSD, %)		Accuracy (%)		
					RSD % within days ($n = 6$)	RSD % between days ($n = 5$)	Level A (25 μg kg^{-1})	Level B (50 μg kg^{-1})	Level C (100 μg kg^{-1})
Bacitracin A	≥0.9984	25	20.2	0.19	7.5~13.9	10.6~15.9	75.9 ± 10.4	77.3 ± 9.7	80.9 ± 8.5
Bacitracin B	≥0.9989	25	22.8	0.23	8.4 ~15.7	9.8~17.4	79.6 ± 8.9	82.5 ± 11.4	84.7 ± 12.1
Colistin A	≥0.9991	25	27.5	0.31	6.7~12.4	11.2~19.3	80.3 ± 9.8	83.9 ± 15.2	87.9 ± 14.9
Colistin B	≥0.9993	25	25.7	0.28	9.7~11.5	14.7~14.8	78.7 ± 10.5	80.8 ± 12.7	82.9 ± 11.2
Virginiamycin M1	≥0.9996	25	19.4	0.32	8.2~9.3	12.5~20.6	76.2 ± 11.9	79.8 ± 12.8	81.3 ± 10.3

Columns: analyte (name of compounds); RSD (relative standard deviation of repeated measurements, nn = 6).The first value refers to the RSD within a day, while the value in brackets states the RSD between the days (n ($n = 5$); These values are corrected for signal suppression; The value in brackets is the current MRL; CCβ: detection capability; limit of quantification: corresponding to a S/N of 10:1; coefficient of determination: r^2 covering the dynamic range as described by the range defined by the stated levels; relative signal suppression: ratio of analyte response in matrix extract as compared to the analyte present in solvent.

100%. The precision was expressed as the RSD. The measurement of uncertainty was according SANCO/2004/2726-4th revision (30). All relevant influencing factors possibly affecting the measurement results, the within-laboratory reproducibility can be regarded as a good estimator for the combined measurement uncertainty of the individual methods.

Discussion

Optimization of LC-MS/MS

The optimization of MS/MS parameters were carried out by infusing individual standard solutions of polypeptide antibiotics. Formation of positively charged polypeptides in acidic media through protonation of free amine groups in the amino acid moieties was expected. Doubly charged at m/z 712 were found to be the dominant species in the respective mass spectra for BTC, m/z 391 for CLTA, m/z 386 for CLTB, m/z 525 for VGM_1. The results are summarized in Table I. According to Commission Decision 2002/657/EC, two specific transitions must be acquired for each compound reaching the minimum number of identification points (IPs) required for unambiguous confirmation. In the present work, two transitions for reliable confirmation were possible for these targets. The MRM ratio utilized for confirmation has to be derived from a blank matrix extract which was fortified with analyte.

Regarding the chromatographic analysis, it was necessary to find a compromise to guarantee a good peak shape and reproducible retention times according to the physical–chemical characteristics of BTCA, CLTA, CLTB, VGM_1. Terminal amine groups in BTCA, CLTA, CLTB, VGM_1 were found to exert considerable adsorption affinity with the stationary phase of the LC column, thus causing significant peak tailing. According the articles (Artemis 2006 [38]) and previous experience, such undesirable interaction could be minimized if mild acidic mobile phases were used. We selected 0.1% formic acid in water as the aqueous phase, and the organic phase was compared to that of methanol, acetonitrile and acetonitrile in the presence of 0.1% formic acid.The results showed that the retention times is stable and the signal suppression effects is the less than the other two conditions when using the 0.1% formic acid in water and 0.1% formic acid in acetonitrile as the mobile phase.

Optimization of MSPD condition

Polypeptide antibiotics are weakly polar compounds, and bacitracin can be transformed into 1-epibacitracin through molecular rearrangement in acidic medium, unnecessary delay of SPE clean-up or overnight storage of acidic extracts was therefore avoided. The effect of purification can be improved by MSPD step. With the aim to find the most effective conditions, the most suitable elution solvents and the polarity of solid-phase were assessed. For the optimization and development of the extraction, all the tests were performed in triplicate.

Classic applications of the MSPD technique employ octadecylsilica (C_{18}) and octylsilica (C_8). As it was explained in the previous work, C_{18}-bonded (HLB) was proved to be a good solid support for the extraction of polypeptide antibiotics. In this work, C_{18}-bonded was chosen because it presented good affinity and good mechanical strength for polypeptide antibiotics, resulting in acceptable recoveries of polypeptide antibiotics from samples of feeds.

Another critical parameter in the MSPD is the ratio between matrix and sorbent material. This ratio depends on the sample nature, although the ratios often applied are 1–4. For further optimization different amounts of C_{18} (1, 2, 3 and 4 g) were added to a glass mortar and blended with 1 g of sample, and then elution was performed with 8 mL methanol. Figure 1 shows that there were no remarkable differences between the recoveries obtained with the different ratios except the ratio of 1:1. In order to minimize the use of inorganic material sorbent, the ratio of 1:2 (1 g of sample and 2 g of C_{18}) was selected for this study.

Optimization of the elution sequence was performed using A: 8 mL methanol; B: 8 mL methanol-acetonitrile (1:1, v/v); C: 8 mL acetonitrile; D: 8 mL methanol (with 0.1% formic acid); E: 8 mL acetonitrile (with 0.1% formic acid). The comparison study (Figure 2) demonstrated that methanol (with 0.1% formic acid) provided satisfactory recoveries and acceptable matrix influence.

Results

Validation results

Selectivity was evaluated by analyzing a blank sample and a blank sample spiked polypeptide antibiotics. No interfering peaks could be detected near the retention time of the analytes. The retention time was shown in Table I. The relative retention times of polypeptide antibiotics in feed samples are corresponded to that of the standard solution at a tolerance of ±2.5% according to EU Commission Decision 2002/657/EC.

The matrix-matched calibration curves were established. Table II summarizes the result of calibration curve and detection limits in samples. The squared correlation coefficient (r^2) of standard

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024

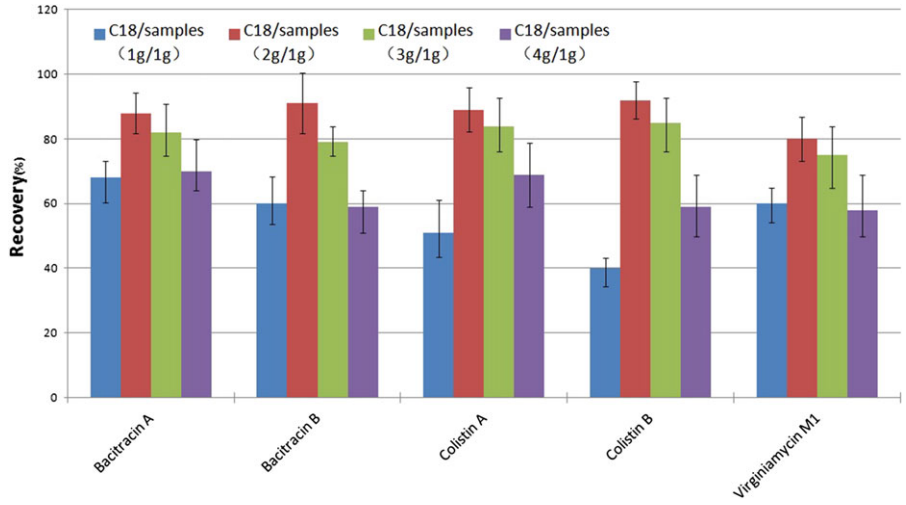

Figure 1. The influence of the ratio and the amounts between matrix and sorbent material on the recoveries of the analytes.

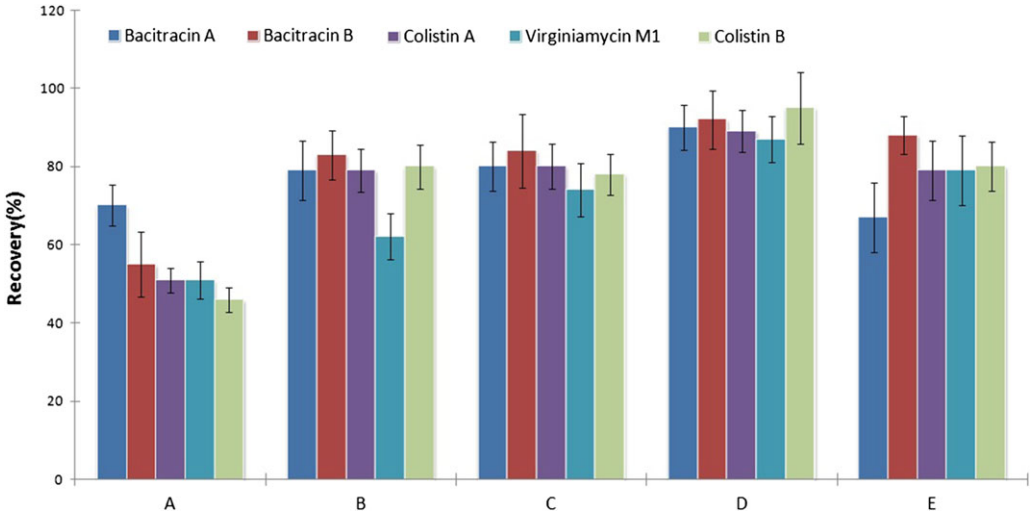

Figure 2. The influence of elution solvents on the recoveries of the analytes. (A) 8 mL methanol; (B) 8 mL methanol-acetonitrile (1:1, v/v); (C) 8 mL acetonitrile; (D) 8 mL acetonitrile-0.1% formic acid (1:4, v/v); (E) 8 mL acetonitrile-0.1% formic acid (1:1, v/v).

calibration curves were above 0.99 for BTCA, BTCB, CLTA, CLTB, VGM_1. Results of matrix effects on the ionization of analytes (the signal suppression is less than 32%) showed that the MSPD process could efficiently remove non-polar interfering compounds that may cause ion suppression and affect the analytical performance of LC-MS/MS. As Table II showed that LOQ of BTCA, BTCB, CLTA, CLTB, VGM_1 were 25 $\mu g\,kg^{-1}$. CCβ of BTCA, BTCB, CLTA, CLTB, VGM_1 was 19.4 to 27.5 $\mu g\,kg^{-1}$, respectively. A chromatogram of each of the matrix blanks and spiked feed samples at LOQ levels is shown in Figure 3.

The recoveries of BTCA, BTCB, CLTA, CLTB, VGM_1 were calculated by interpolation of matrix-matched calibration curve. The data about average recoveries and precision (RSD of within days and between days) in feed samples at four levels (25, 50, 100 $\mu g\,kg^{-1}$) are shown in Table II. The values of recoveries were 75.9% ~87.9% at spiking levels. The RSD of within days were less than 15.7% and between days less than 20.6%.

The stability of BTCA, BTCB, CLTA, CLTB, VGM_1 standard stock solutions were storage in a freezer at −20°C for three months. The results showed that BTCA, BTCB, CLTA, CLTB, VGM_1 are good stable.

Applications of the method

The application of the MSPD developed method was evaluated carrying out 100 swine feed samples and 100 chicken feeds purchased from different markets, which were sampled and analyzed by this validated method in the present study. In light of these results, the trueness of the method was in good agreement with this European Commission performance criterion for qualitative analysis. In the 200 tested samples, BAC was detected at the level range from 52.5 $\mu g\,kg^{-1}$ to 119.3 $\mu g\,kg^{-1}$ in these swine feeds, from 92.6 $\mu g\,kg^{-1}$ to 236.1 $\mu g\,kg^{-1}$ in chicken feed samples. And other compounds were undetectable.

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024

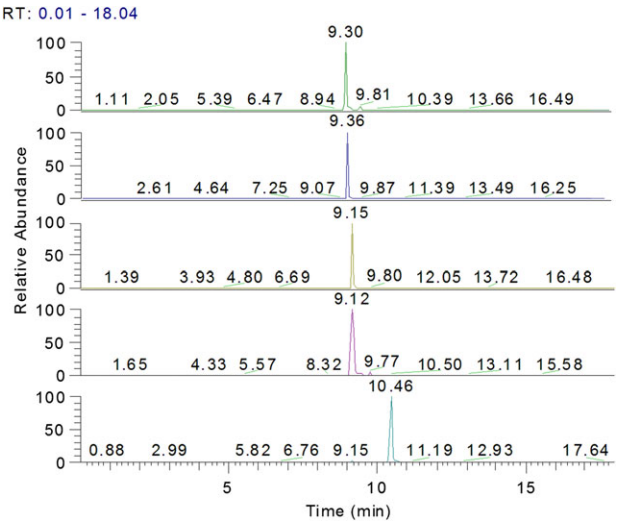

Figure 3. The Chromatogram of polypeptide antibiotics at the level of 50 μg kg^{-1} in swine feed sample.

Conclusions

This study was the preparation of BTCA, BTCB, restriction, total removal rate, vgm1 residue in feed by MSPD method. The MSPD method presented is a good starting point for further BTCA, BTCB, CLTA, CLTB, VGM_1 analysis and it can be regarded as a valuable future alternative in this field. It has good recovery and high sensitivity. The method was validated with reference to the implemented validation procedure for residues in products as described in EU Commission Decision 2002/657/EC. The good performances of this method satisfy the demand of the detection of polypeptide antibiotics in feed.

Funding

The authors thank the National Natural Science Foundation of China (grant number 31602115), the Natural Science Foundation of Hubei Province (grant number 2016CFB553).

References

1. Smith, J.L., Weinber, E.D.; Mechanisms of antibacterial action of bacitracin; *Journal of General Microbiology*, (1962); 28: 559–569.
2. Kyriakis, S.C., Tsinas, A., Lekkas, S., Sarris, K., Bourtzi-Hatzopoulou, E.; Clinical evaluation of in-feed zinc bacitracin for the control of porcine intestinal adenomatosis in growing/fattening pigs; *Veterinary Record*, (1996); 138: 489–492.
3. Engberg, R.M., Hedemann, M.S., Leser, T.D., Jensen, B.B.; Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers; *Poultry Science*, (2000); 79(9): 1311–1319.
4. Li, D., Zang, S., Li, T., Qiao, Q., Thacker, P.A., Kim, J.H.; Effect of feed antibiotics on the performance and intestinal microflora of weanling pigs in China; *Asian-Australasian Journal of Animal Sciences*, (2000); 13(11): 1554–1560.
5. Ikai, Y., Oka, H., Hayakawa, J., Kawamura, N., Mayumi, T., Suzuki, M., *et al.*; Total structures of colistin minor components; *The Journal of Antibiotics*, (1998); 51(5): 492–498.
6. Elverdam, I., Larsen, P., Lund, E.; Isolation and characterization of three new polymyxins in polymyxins B and E by high-performance liquid chromatography; *Journal of Chromatography B*, (1981); 218: 653–661.
7. Orwa, J.A., Van Gerven, A., Roets, E., Hoogmartens, J.; Development and validation of a liquid chromatography method for analysis of colistin sulphate; *Chromatographia*, (2000); 51(7): 433–436.
8. Orwa, J.A., Govaerts, C., Gevers, K., Roets, E., Van Schepdael, A., Hoogmartens, J.; Study of the stability of polymyxins B_1, E_1 and E_2 in aqueous solution using liquid chromatography and mass spectrometry; *Journal of Pharmaceutical and Biomedical Analysis*, (2002); 29: 203–212.
9. Sin, D.W.M., Ho, C., Wong, Y.C., Ho, S.K., Ip, A.C.B.; Simultaneous determination of lincomycin and virginiamycin M1 in swine muscle, liver and kidney by liquid chromatography–electrospray ionization tandem mass spectrometry; *Analytica Chimica Acta*, (2004); 517: 39–45.
10. Commission of the European Communities., Council Regulation 2821/98, Off. J. Eur. Communities: Legis, L351 (1998) 4.
11. Wan, E.C.H., Ho, C., Sin, D.W.M., Wong, Y.C.; Detection of residual bacitracin A, colistin A, and colistin B in milk and animal tissues by liquid chromatography tandem mass spectrometry; *Analytical and Bioanalytical Chemistry*, (2006); 385: 181–188.
12. Kristensen, H.K., Hansen, S.H.; Separation of polymxins by micellar electrokinetic capillary chromatography; *Journal of Chromatography B*, (1993); 628: 309–315.
13. Potts, A.R., Psurek, T., Jones, C., Parris, L., Wise, A.; Validation of a quantitative HPLC method for bacitracin and bacitracin zinc using EDTA as a mobile-phase modifier; *Journal of Pharmaceutical and Biomedical Analysis*, (2012); 70: 619–623.
14. Van Poucke, C., De Keyser, K., Baltusnikiene, A., McEvoy, J.D.G., Van Peteghem, C.; Liquid chromatographic–tandem mass spectrometric detection of banned antibacterial growth promoters in animal feed; *Analytica Chimica Acta*, (2003); 483: 99–109.
15. Lee, S.C., Matus, J.L., Gedir, R.G., Boison, J.O.; A validated LC-MS method for the determination of bacitracin drug residues in edible pork tissues with confirmation by LC-tandem mass spectrometry; *Journal of Liquid Chromatography and Related Technologies*, (2011); 34: 2699–2722.
16. Sin, D.W., Ho, C., Wong, Y.C., Ho, S., Ip, C.; Analysis of major components of residual bacitracin and colistin in food samples by liquid chromatography tandem mass spectrometry; *Analytica Chimica Acta*, (2005); 535: 23–30.
17. Kaufmann, A., Widmer, M.; Quantitative analysis of polypeptide antibiotic residues in a variety of food matrices by liquid chromatography coupled to tandem mass spectrometry; *Analytica Chimica Acta*, (2013); 797: 81–88.
18. Govaerts, C., Orwa, J., Van Schepdael, A., Roets, E., Hoogmartens, J.; Liquid chromatography–ion trap tandem mass spectrometry for the characterization of polypeptide antibiotics of the colistin series in commercial samples; *Journal of chromatography. A*, (2002); 976: 65–78.
19. Gossele, F., Blain, P., Marneffe, T., Biot, A.; High-performance liquid chromatographic determination of virginiamycin in Stafac, premixes and animal feeds; *Analyst*, (1991); 116: 1373–1380.
20. Sin, D.W.M., Ho, C., Wong, Y.C., Ho, S.K., Ip, A.C.B.; Simultaneous determination of lincomycin and virginiamycin M1 in swine muscle, liver and kidney by liquid chromatography–electrospray ionization tandem mass spectrometry; *Analytica Chimica Acta*, (2004); 517: 39–45.
21. Van Poucke, C., Dumoulin, F., Van Peteghem, C.; Detection of banned antibacterial growth promoters in animal feed by liquid chromatography–tandem mass spectrometry: optimisation of the extraction solvent by experimental design; *Analytica Chimica Acta*, (2005); 529: 211–220.
22. De-Alwis, H., Heller, D.N.; Multiclass, multiresidue method for the detection of antibiotic residues in distillers grains by liquid chromatography and ion trap tandem mass spectrometry; *Journal of chromatography. A*, (2010); 1217: 3076–3084.
23. Cronly, M., Behan, P., Foley, B., Malone, E., Earley, S., Gallagher, M., *et al.*; Development and validation of a rapid multi-class method for the confirmation of fourteen prohibited medicinal additives in pig and poultry compound feed by liquid chromatography–tandem mass spectrometry; *Journal of Pharmaceutical and Biomedical Analysis*, (2010); 53: 929–938.
24. Barker, S.A.; Applications of matrix solid-phase dispersion in food analysis; *Journal of chromatography. A*, (2000); 880: 63–68.
25. Barker, S.A.; Matrix solid phase dispersion (MSPD); *Journal of Biochemical and Biophysical Methods*, (2007); 70: 151–162.

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024

26. Govaerts, C., Li, C., Orwa, J., Schepdael, A.V., Adams, E., Hoogmartens, J.; Sequencing of bacitracin A and related minor components by liquid chromatography/electrospray ionization ion trap tandem mass spectrometry; *Rapid Communications in Mass Spectrometry*, (2003); 17: 1366–1379.
27. Kristenson, E.M., Brinkman, U.A.T., Ramos, L.; Recent advances in matrix solid-phase dispersion; *Trends in Analytical Chemistry*, (2006); 25 (2): 96–111.
28. Capriotti, A.L., Cavaliere, C., Foglia, P., Samperi, R., Stampachiacchiere, S., Ventura, S., *et al*.; Recent developments in matrix solid-phase dispersion extraction; *Journal of Chromatography. A*, (2010); 1217: 2521–2532.
29. Official Journal of the European Union, 125., 10. European Union. (2002). Commission Decision 2002/657/EC.
30. Document SANCO/2004/2726-revision 4. Guidelines for the Implementation of Decision 2002/657/EC. 13 European Union (2010).

Downloaded from https://academic.oup.com/chromsci/article/56/3/285/4734952 by guest on 24 April 2024