Journal of Chromatography B 1076 (2018) 103–109

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of eight cyclopolypeptide antibiotics in feed by high performance liquid chromatography coupled with evaporation light scattering detection

Xuqin Song[a], Jingmeng Xie[b], Meiyu Zhang[a], Yingxia Zhang[a], Jiufeng Li[b], Qiwen Huang[b], Limin He[a,b,*]

[a] National Reference Laboratory of Veterinary Drug Residues (SCAU), College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China
[b] Guangdong Provincial Key Laboratory of Veterinary Pharmaceutics Development and Safety Evaluation, South China Agricultural University, Guangzhou 510642, China

ARTICLE INFO

Keywords:
Polypeptide antibiotics
Solid-phase extraction
Biphenyl column
Liquid chromatography
Evaporative light scattering detector
Feed

ABSTRACT

A high throughput, reliable and reproducible analysis strategy based on high performance liquid chromatography combined to evaporative light scattering detector (HPLC-ELSD) was developed for simultaneous determination of eight cyclopolypeptide antibiotics including vancomycin, polymyxin B (polymyxin B1 and polymyxin B2), polymyxin E (colistin A and colistin B), teicoplanin, bacitracin A, daptomycin and virginiamycin M1 in animal Feed. Feed samples were extracted with methanol-2% formic acid aqueous solution, followed by a solid-phase extraction step using a HLB cartridge. Under the optimum chromatographic conditions and ELSD parameters, target compounds were separated well on a short column filled with biphenyl stationary phase. The method was developed in accordance with pig complete feed and then extended to detect polypeptide antibiotics in piglet premix, pig feed additive, poultry complete feed and fattening pig premix. The results showed that logarithmic calibration curves of eight analytes were linear ($r^2 > 0.99$) within the concentration range of 5–200 mg mL^{-1}. The developed method provided good accuracy and precision for quantification of eight polypeptides in five kinds of feeds with recoveries ranging from 72.0% to 105.4% with relative standard deviations < 9.5%. The limits of detection ranged from 2 to 5 mg kg^{-1}. Finally, the method was successfully applied to analyze polypeptide antibiotics in commercial feed.

1. Introduction

Polypeptide antibiotics, antibacterial agents that contain two or more moieties derived from amino acids, are often isolated from culture solutions of *Bacillus*, *Streptomyces*, and *Actinomycetes*. Since the discovery of tyrothricin [1], more and more polypeptide antibiotics have been developed, including some of the current commonly used cyclic glycopeptides (vancomycin (VCM), teicoplanin (TEC) and avoparcin), lipopeptides (daptomycin (DAP)) and other cyclopolypeptides such as polymyxin B (polymyxin B1 (PMB) and polymyxin B2), polymyxin E (also known as colistin including two main components of colistin A (CSA) and colistin B (CSB)), bacitracin A (BTCA) and virginiamycin (VGMM1). Some polypeptides such as vancomycin, teicoplanin and daptomycin have the favorable antibacterial effects against gram-positive bacteria; while polymyxin B and polymyxin E are effective against many gram-negative bacteria. These polypeptides have a long history of application in human medicine. Nevertheless, since the end of last century, some of them have been restricted in use for their serious nephrotoxicity and hepatotoxicity. For example, polymyxin E is abandoned in parenteral therapies [2]. Nowadays, because of the rapidly increasing trend of antibiotic resistance, especially the emergence of multidrug-resistant (MDR) microorganisms, people revaluate these antibiotics [3,4]. Besides, the slow development of new antibiotics makes polypeptides crucial to the treatment of gram-negative bacterial infections in human, so we call them “last-resort” antibiotics.

Last decades, it is because of their restricted use in human medicine, polypeptide antibiotics have been widely utilized as veterinary drugs and feed additives in animal husbandry [5]. Despite the advantages of polypeptides in livestock production, their abuse in feed and the ignorance of withdrawal time may result in drug residues in animal-

Abbreviations: ACN, acetonitrile; BTCA, bacitracin A; CSA, colistin A; CSB, colistin B; DAP, daptomycin; ELSD, evaporative light scattering detection; FA, formic acid; MeOH, Methanol; MDR, multidrug-resistant; MRLs, maximum residue limits; PMB, polymyxin B1; TEC, teicoplanin; VCM, Vancomycin; VGMM1, virginiamycin M1

* Corresponding author at: National Reference Laboratory of Veterinary Drug Residues (SCAU), College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong, PC 510642, China.
E-mail address: liminokhe@scau.edu.cn (L. He).

https://doi.org/10.1016/j.jchromb.2018.01.020
Received 7 November 2017; Received in revised form 11 January 2018; Accepted 15 January 2018

derived food. Thus, they may threat to human health for a long-term consumption of food with drug residues. However, more worrying is the mass propagation of MDR bacteria caused by the extensive use and the abuse of these compounds in animal husbandry [6]. As MDR pathogens move along the food supply chain from farms to consumers' plates, humans may be infected through the consumption of contaminated animal products [7,8]. These MDR pathogens can diminish the therapeutic effects of antibiotics for bacterial infection diseases, which results in more deaths [9,10]. Astonishingly, recent study has confirmed a new mobilized colistin resistance gene (*mcr-1*), which means our 'last-resort' is in jeopardy [11]. Polypeptide antibiotics are so important in human medicine that many governments have taken measures to supervise these compounds. According to commission regulation 2788/98, bacitracin and virginiamycin have been banned in European Union (EU) to use as growth promoters since 1999 [12]. In China, Bulletin 168 of the Ministry of Agriculture (MOA) sets the concentrations of bacitracin, colistin, virginiamycin, enramycin and nosiheptide in feed additives ranging from 2.5 to 500 g kg^{-1}. The recommended amounts of bacitracin, colistin (colistin has been banned as a growth promoter from 2017) and virginiamycin in livestock feeds as antimicrobial growth promoters are 2–100 mg kg^{-1} [13]. Furthermore, to safeguard public health, some countries and community such as China [14], European Union (EU) [15], and United States [16] have established maximum residue limits (MRLs) for bacitracin, colistin and virginiamycin in animal-derived food at ranges of 50–500 μg kg^{-1}. Thus, there is a need for developing simple, effective and reproducible methods to simultaneously monitor and detect polypeptide antibiotics in animal feed.

Due to large molecular masses, poor chromophores and fluorophores and multi-component of polypeptide antibiotics, few reports described analytical approaches for quantifying them. Recently, there are several analytical methods based on high-performance liquid chromatography-ultraviolet detector (HPLC-UV) to detect bacitracin, virginiamycin, teicoplanin and daptomycin in animal feeds or pharmaceutical formulations [17–20]. Besides, HPLC-diode array detector (DAD) and fluorescence detector (FLD) combined with pre-column derivatization were applied to detect colistin, which is the absence of UV absorption [21,22]. The evaporative light scattering detection (ELSD) is a general-purpose detector with no need for the derivatization process of non-absorbing analytes. Its signal response only depends on the concentration of compounds in the effluent rather than their optical properties. ELSD has been widely applied in the determination of contents of bioactive constituents in traditional Chinese medicines [23,24]. We also successfully established analytical methods based on HPLC-ELSD for determining macrolides and aminoglycoside antibiotics (lack of chromophores and fluorophores) in feed [25,26]. So far, only few HPLC-ELSD methods for the determination of colistin or bacitracin have been published [27,28]. These methods just focused on the chromatography analysis of main components of colistin or bacitracin in pharmaceutical formulations, and additionally, trifluoroacetic acid had to be used as ion pair reagent in mobile phase.

To our knowledge, there is still no HPLC method for the simultaneous determination of multiple polypeptides in feed. For this purpose, we first established a high throughput, reliable and reproducible HPLC-ELSD method for the determination of eight cyclopolypeptide antibiotics, including vancomycin, colistin A, colistin B, polymyxin B1, teicoplanin, bacitracin A, daptomycin and virginiamycin M1 (Fig. S1). Appropriate extraction and clean up approaches for 8 cyclopolypeptides in feed was optimized. Good retention and separation for target compounds were realized on the biphenyl column. The proposed method could be used for routinely supervising of cyclopolypeptide antibiotics in animal feed and quality control of their preparations.

2. Materials and methods

2.1. Reagents and chemicals

HPLC-grade solvents, including acetonitrile (ACN) and formic acid (FA), were from Fisher Scientific (Fairlawn, NJ, USA). Ammonium acetate was also HPLC grade from TEDIA (Fairfield, OH, USA). Methanol (MeOH) and ammonium hydroxide were of analytical grade and bought from Guangzhou Chemical Reagent Factory (Guangzhou, China). Ultrapure water was made by a Millipore MilliQ system (Molsheim, France). Oasis HLB SPE cartridge (60 mg, 3 mL) was supplied from Waters Co. (Milford, MA, USA).

2.2. Standards and stock solution

Reference standards of DAP, CSA, CSB and VGMM1 were purchased from TRC (Toronto, Canada). VCM, TEC and BTCA were obtained from National Institutes for Food and Drug Control (Beijing, China). PMB was available from Dr. Ehrenstrofer GmbH (Augsburg, Germany). The purity of each standard was > 88.6%. For preparing individual stock solution (5 mg mL^{-1}), DAP and VGMM1 were prepared by dissolving each compound in methanol; VCM, TEC, PMB, CSA, CSB and BTCA were respectively dissolved by 0.1% formic acid in water. These stock solutions could be stored in dark for 3 months at −20 °C. Mixed standard working solutions (250 μg mL^{-1} for VCM, DAP and VGMM1; 500 μg mL^{-1} for TEC, PMB, CSA, CSB and BTCA), were prepared by mixing each stock solution into a brown volumetric flask and then diluting with a mixture of methanol-0.1% formic acid aqueous solution (50:50, v/v). Mixed standard solutions were stored at 4 °C and were kept stable for a maximum of a month.

2.3. Sample preparation

Five kinds of animal feeds including piglet premix (A), pig feed additive (B), poultry complete feed (C), pig complete feed (D) and fattening pig premix (E) were obtained from local feed markets (Guangzhou, China). Complete feed samples were ground and passed through a 1 mm size sieve (16 mesh). A previous LC-MS/MS analysis was conducted to ensure that these samples were free from polypeptide antibiotics.

One gram of sample was accurately weighed into a 15 mL polypropylene centrifuge tube. For recovery experiments, feed samples were spiked with mixed standard working solutions to prepare the desired concentration levels (5, 10 and 25 mg kg^{-1} for VCM, DAP and VGMM1; 10, 20 and 50 mg kg^{-1} for TEC, PMB, CSA, CSB, and BTCA), and incubated for 30 min at room temperature to make sure the permeation of target drugs into the matrix before proceeding.

5 mL of methanol-2% formic acid aqueous solution (2:5, v/v) was dispensed into the centrifuge tube. Samples were performed with an ultrasonic bath for 5 min, and then mechanically shaken for 20 min. After centrifugation at 9000 rpm for 10 min, the supernatant was transferred into a 50 mL pear-shaped flask and the residue was re-extracted with 5 mL of methanol-2% formic acid aqueous solution (5:2, v/v). The supernatant was collected into the previous pear-shaped flask and evaporated to near 5 mL in a water bath at 40 °C.

Purification and enrichment were carried out on an Oasis HLB cartridge, which was previously conditioned with 1 mL methanol and 1 mL water. The extracts were diluted with 10 mL of 2% formic acid aqueous solution prior to loading to the cartridge. Then, the cartridge was rinsed with 3 mL of 5% methanol in water. After drying the cartridge under a low pressure, analytes were eluted with 5 mL of 2% formic acid in methanol. The eluates were evaporated to dryness under