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Analytical Methods

Determination of virginiamycin M1 residue in tissues of swine and chicken by ultra-performance liquid chromatography tandem mass spectrometry

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ABSTRACT

A reliable UPLC–MS/MS method with high sensitivity was developed and validated for the determination of virginiamycin M1 in muscle, fat, liver, and kidney samples of chicken and swine. Analytes were extracted using acetonitrile and extracts were defatted by *N*-hexane. Chromatographic separation was performed on a BEH C18 liquid chromatography column. The analytes were then detected using triplequadrupole mass spectrometry in positive electrospray ionization and multiple reaction monitoring mode. Calibration plots were constructed using standard working solutions and showed good linearity. Limits of quantification ranged from 2 to 60 ng mL^{-1}.

1. Introduction

Antimicrobial feed additives are used to mitigate digestive disorders, such as lactic acid acidosis, and to improve ruminant production. Virginiamycin, a special animal-use antibiotic, is used as a feed additive to enhance growth of animals, such as cows, swines, poultry, and fish, and contains lactonic rings that are primarily composed of two anti-bacterial factors, namely M1 and S1. The structural formulae of the lactonic rings are shown in Fig. 1. An M1:S1 ratio of 7:3 induces the strongest antibacterial activity (Cocito & Kaji, 1971; Cocito, Voorma, & Bosch, 1974; Erasmus, Muya, Erasmus, Coertze, & Catton, 2008; Nott et al., 2005). In addition, virginiamycin can enhance the permeability of the intestinal wall, thus increasing nutrient influx to intestinal mucosa, as well as the absorption rate of amino acids and phosphorus (Stewart, Kim, Gramm, Nimmo, & Stein, 2010). In the Positive List System of Japan, the maximum residue limit of virginiamycin M1 is 0.05 mg kg^{-1} in chickens, 0.1 mg kg^{-1} in the meat, eggs, and milk of other animals, and 0.2–0.3 mg kg^{-1} in animal livers and kidneys. Nevertheless, antibiotics are important as feed additives for food-producing animals in China. According to the 235th Public Announcement of the Chinese Ministry of Agriculture in 2002, maximum residue limits (MRLs) of virginiamycin residue limits are 0.3, 0.5, 0.1, 0.2, and 0.2 mg kg^{-1} in the liver, kidney, muscle, fat, and skin of poultry, respectively, and 0.3, 0.4, 0.1, 0.4, 0.4 mg kg^{-1} in the liver, kidney, muscle, fat, and skin of swines, respectively (MOA, 2002). In Canada, the notice of “Proposal to amend the List of Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods” showed that M1 is now the standard virginiamycin residue marker and MRLs will be amended to 0.3, 0.4, 0.1, and 0.4 mg kg^{-1} in the liver, kidney, muscle, skin/fat of both poultry and swine (Health Products and Foods Branch, Health Canada, 2016).

Current methods for testing virginiamycin residue include microbiological methods, enzyme-linked immunosorbent assay (Situ & Elliott, 2005), thin-layer chromatography (Situ & Elliott, 2005), HPLV-UV and LC–MS/MS. Various studies of virginiamycin residue detection methods are listed in Table 1 (Boscher, Guignard, Pellet, Hoffmann, & Bohn, 2010; De Alwis & Heller, 2010; General Administration of Quality Supervision, 2007; Sin, Ho, Wong, Ho, & Ip, 2004; Tang, Lu, Lin, Shih, & Hwang, 2012; Van Poucke, Dumoulin, & Van Peteghem, 2005; Xiaoxia Chen & Ye, 2010; Zhiming Geng & Xu, 2005). However, the pre-processing method is complicated and poisonous organic solvents, such as chloroform, were used. Liquid-phase chromatography is time-consuming and laborious. Furthermore, testing methods for virginiamycin M1 in skin and fat have not been reported. Ultra-performance liquid chromatography–mass spectrometry (UPLC–MS/MS) is a drug analytical method that incorporates high-flux testing, high-performance separation, and multicomponent qualitative and quantitative analysis with less matrix interference and high sensitivity to achieve ng/kg grade testing. A simplified sample pretreatment method is combined with UPLC–MS/MS to test, optimize, and obtain a

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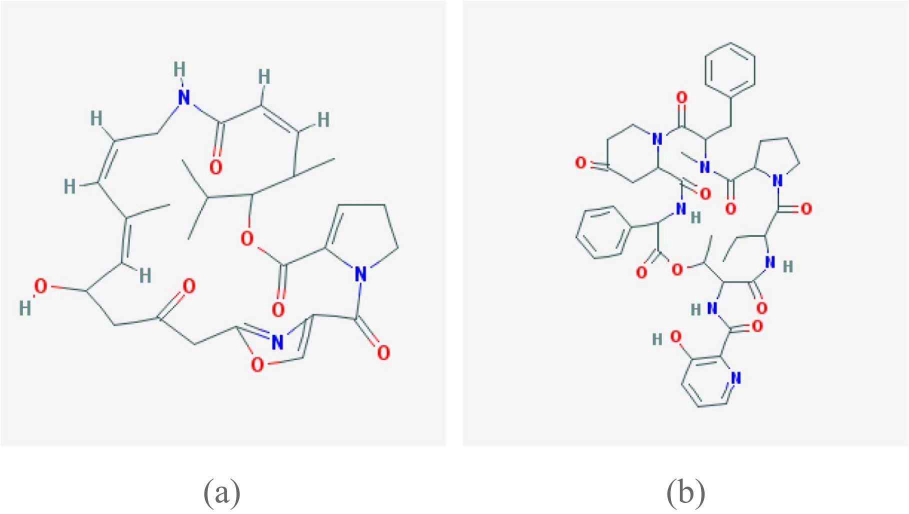

Fig. 1. Virginiamycin M1 (a) and S1 (b).

pretreatment method for virginiamycin M1, thereby reducing matrix interference to the maximum extent and establishing liquid-phase mass spectrometry conditions for UPLC–MS/MS testing. Moreover, the method is validated for sensitivity, linearity, recovery rate, and precision to obtain a quantitative analytical method for virginiamycin M1, thereby performing drug residue detection test and field sampling validation. However, no studies regarding the use of UPLC–MS/MS to determine M1 in all kinds of tissues have been reported to date. Therefore, establishing a series of sensitive and comprehensive detection methods of M1 as a virginiamycin residue marker is critical.

This study established a simple, effective and sensitive quantitative method to detect residues of virginiamycin M1 for all residue tissues of swine and chicken using UPLC–MS/MS.

2. Experimental materials and methods

2.1. Reagents and apparatus

Virginiamycin M1 (> 93% purity) was purchased from Sigma CO (USA). Stafac 500@ was a gift from the Phibro Animal Health Corporation Ltd. Shanghai Rep. Office. LC-grade acetonitrile and formic acid were purchased from Fisher (Fair Lawn, NJ, USA). *N*-hexane was of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

UPLC–MS/MS was performed on a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to an Acquity tandem triple quadrupole mass spectrometer (TQ-S, Waters, Milford, MA, USA) equipped with an electrospray interface source. The UPLC–MS/MS instrument was controlled by MassLynx 4.1 (Waters, Milford, MA, USA). A high-speed grinder (Waring Products, Torrington, CT, USA), a ultrasonic instrument (KQ-500DE, Kunshan Ultrasonic Instrument Ltd. CO., China), a vortex mixer (Genie-2, Scientific Industries, Inc., Bohemia, NY, USA), and a centrifuge (Shanghai Anting Scientific Instruments, Shanghai, China) were used for sample preparation.

2.2. Preparation of standard solutions

Standard stock solution (50 μg mL^{-1}) was prepared in acetonitrile which was further diluted stepwise with acetonitrile-water (60:40) to obtain a series of working standard solutions in the range from 0.5 to 10 μg mL^{-1}. The stock and working standard solutions were stored at 4 °C in the dark and were stable for 30 days.

2.3. UPLC–MS/MS conditions

An Acquity UPLC BEH C18 (1.7 μm, 2.1 × 100 mm) (Waters, Milford, MA, USA; Ireland) equipped with an Acquity UPLC BEH C18 VanGuard Pre-column (1.7 μm, 2.1 mm × 5 mm) was used to separate the analytes at a temperature of 30 °C. The mobile phase was composed of ultrapure water containing 0.1% formic acid (eluent A) and acetonitrile (eluent B) at a flow rate of 0.2 mL min^{-1}. The gradient elution conditions were optimized as follows: 0 min, 70%A; 4 min, 50%A; 6 min, 50%A; 6.5 min, 5%A; 7.5 min, 5%A; 8 min, 70%A; 10 min, 70%A. The sample injection volume was 5 μl.

Tandem MS detections were performed using positive electrospray ionization. The detection parameters were as follows: capillary voltage, 3.0 kV; source temperature, 150 °C; and nitrogen desolvation temperature, 200 °C with a flow rate of 500 L h^{-1}. Argon was used in the collision cell as collision gas. Analyte detection by tandem MS was performed in multiple reaction monitoring (MRM) mode of $[M+H]^+$ ion for virginiamycin M1. The MRM transitions parameters selected for determination were as follows: m/z 526.3 > 355.1 (collision energy, 18 eV; as the quantitative ion), m/z 526.3 > 355.1 (collision energy, 22 eV); m/z 526.3 > 109.1 (collision energy, 38 eV). The cone voltages were set at 20 V for all analyses.

2.4. Sample preparation

Edible tissue samples (muscle, fat, liver, and kidney of chicken and swine) were purchased from local markets in Shanghai, China, homogenized in a high-speed grinder, and then stored at −40 °Cuntil use.

After thawing, samples (2.00 ± 0.02 g) were accurately weighed into 50 mL centrifuge tubes. Exactly 4 mL of acetonitrile was added to each tube. Then the tubes were thoroughly vortexed for 2 min, and the analysts were extracted by ultrasonication for 30 min and then centrifugation at 3500 × *g* for 10 min. The supernatant was transferred to another centrifuge tube. Then, 2 mL of acetonitrile was added to the residue. The acetonitrile–residue mixture was vortexed for 2 min, extracted by ultrasonication for 30 min, and centrifuged for 10 min (3500 × *g*) again. The supernatant was transferred to the same centrifuge tube and the extract from these two steps were combined. The mixed extract was added to a volume of 9.5 mL with ultrapure water. Then, 3 mL *N*-hexane was added to the extract. The mixture was vortexed for 30 s and centrifuged for 10 min (3500 × *g*). The *N*-hexane at the upper layer was discarded. Defatting was repeated once. After completely removing *N*-hexane from the upper layer, the remaining layer was transferred to a 10 mL test tube and mixed with ultrapure water to a volume of 10 mL. The mixture was vortexed for 30 s. After