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Short communication

Determination of valnemulin in swine and bovine tissues by ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive and reliable method has been developed and validated for the determination of valnemulin in swine and bovine muscle, liver, and kidney using solid-phase extraction (SPE) and ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS). The tissue samples were extracted with mixture solution of acetonitrile and 0.01 mol/L hydrochloric acid, defatted by *n*-hexane, and further cleaned up using SPE cartridges with polymeric sorbent. Gradient UHPLC separation was performed using an Acquity BEH C_{18} column with water and acetonitrile as the mobile phase. Multiple reaction monitoring mode of two precursor-product ion transitions for valnemulin was used. Mean recoveries from fortified samples ranged from 93.4 to 104.3% with 3.3–10.7% relative standard deviation. The limit of detection and quantification was 0.2 and 1 μg/kg for the analyte, respectively.

1. Introduction

Valnemulin (VML) is a semi-synthetic pleuromutilin antibiotic derivative, which acts by inhibition of bacterial protein synthesis [1–4]; therefore, it has very modest antimicrobial effect on mycoplasma and spirochaetes [3,5–7]. VML is widely used in livestock for therapeutic, prophylactic and somatotrophic purposes. However, more and more people have realized another potential human health risk due to the toxicity of the veterinary antibiotic residues used in food-producing animals. The European Medicines Agency eastblished a maximum residue limit (MRL) of 50 μg/kg for muscle, 100 μg/kg for kidney and 500 μg/kg for liver [8], and Japan recommended the MRL at 50 μg/kg for swine muscle, fat, kidney and liver in the Positive List System for Agriculture Chemical Residues in Food [9]. Up to now, few methods have been published for the determination of VML in various matrices [10]. Concerning about food safety and protection of human health, it is necessary to establish an ideal analytical method for the determination of residues of VML in animal tissues.

In recent decades, there has been interest in VML residues due to a number of reports of food contamination. Huang et al. determined the residue of VML in swine tissues using high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [11]. And a multi-residue method was developed to determine several antibiotic groups including VML in honey by LC–MS/MS. The CCα and CCβ calculated for VML were 8.0 and 10.7 μg/kg, respectively [12]. In addition, a comprehensive screening and quantitation of veterinary drugs including VML was performed in milk using ultra-high performance liquid chromatography combined with time-of-flight mass spectrometry (UHPLC-TOF/MS). The VML was extracted with acetonitrile and cleaned-up by StrataX-SPE column. The validation level of VML was 6.1 μg/L and CCβ was 68.2 μg/L [13]. Guo et al. developed a method for determination of VML residues in porcine tissues by molecularly imprinted solid-phase extraction coupling with HPLC-UV [14]. However, existing methods suffered from some drawbacks, such as low sensitivity, time-consuming sample preparation, and limited sample matrices.

In this work, we present an UHPLC–MS/MS method for the determination of VML in different animal muscle, liver, and kidney samples. Sample preparation and detection procedures were optimized to achieve high throughput and sensitivity. The proposed method was validated and incurred samples were analyzed to test the performance of the method.

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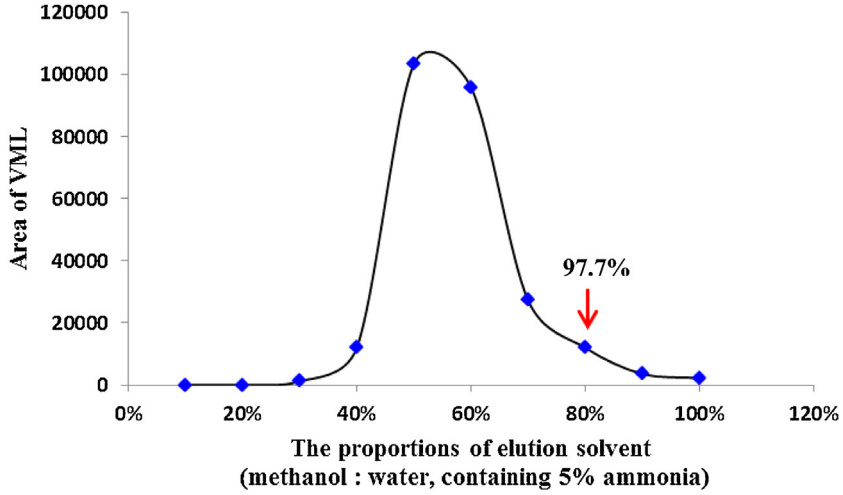

Fig. 1. The elution curve of valnemulin from SPE cartridge (the horizontal axis indicates the ratio of methanol in the elution solution; the vertical axis indicates the response of different eluent strength).

2. Experimental

2.1. Chemicals and reagents

Valnemulin standard was obtained from J & K Chemical Ltd. (Beijing, China). HPLC grade methanol, acetonitrile, formic acid and *n*-hexane were purchased from Dikma Technology Inc. (Muskegon, MI, USA). Ammonia was obtained from Alfa-Aesar (Ward Hill, MA, USA). Hydrochloric acid was purchased from Beijing Chemical Reagent Co., (Beijing, China). HPLC water was purified through a Milli-Q Synthesis system (Millipore, Bedford, MA).

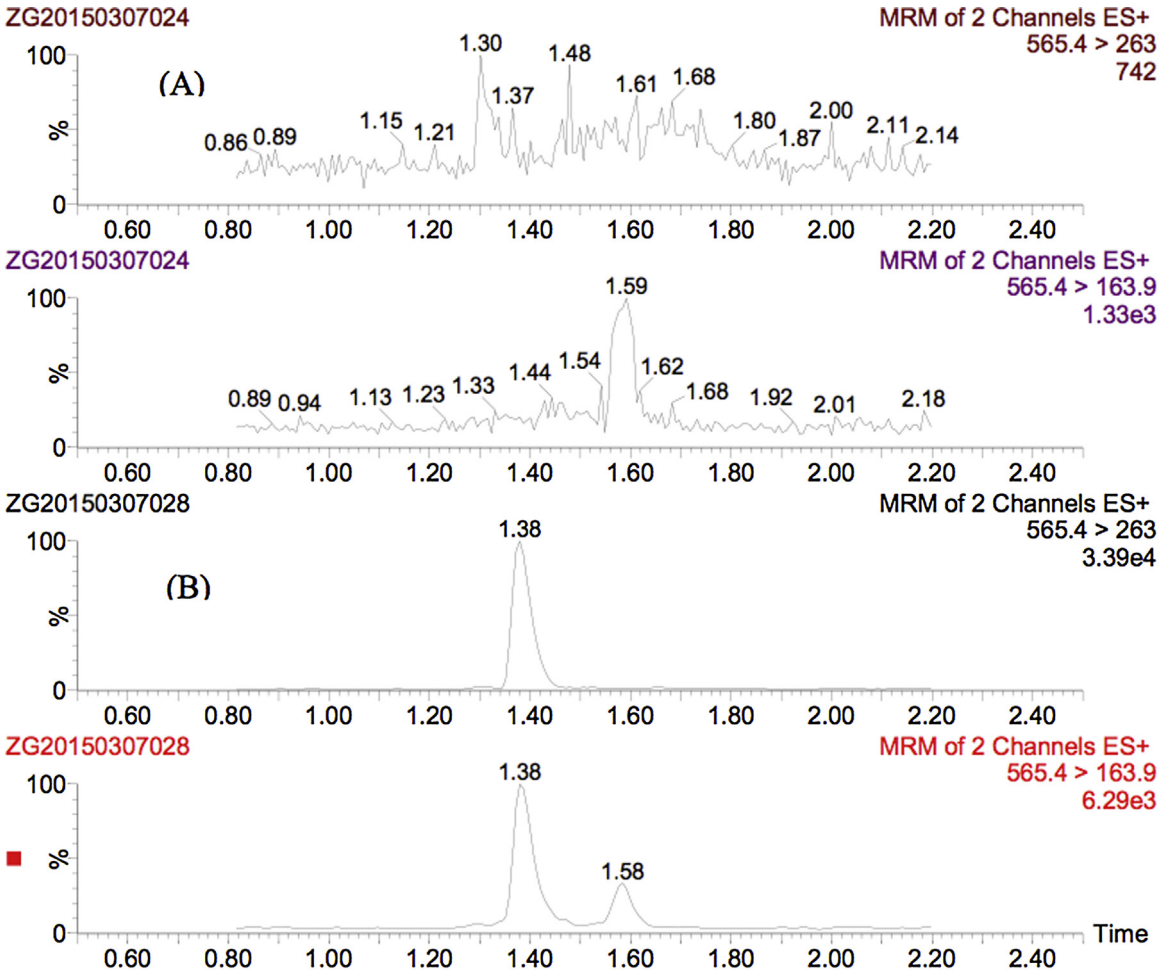

Fig. 2. UHPLC–MS/MS chromatograms of blank swine liver sample (A) and fortified swine liver sample at LOQ level (1 μg/kg).