Note

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Validation of a method for the determination of apramycin in livestock products by LC-MS/MS

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Abstract

As reported in previously, we established a sample preparation method for determining residual apramycin in livestock products by LC-MS/MS, where we utilized an external calibration method. In this study, the *n*-hexane–trichloroacetic acid (hexane–TCA) method by us was applied to the sample preparation method for LC-MS/MS analysis, and evaluated the linearity of the calibration curve and the validity of the quantitative value. Bovine tissue samples (e.g., muscle, fat, and liver) were extracted using the hexane–TCA method, and cleanup was performed using two different hydrophilic–lipophilic balance (HLB) cartridge columns and a MCX cartridge column for these samples. The apramycin in the resulting samples was quantified by LC-MS/MS by utilizing an external calibration curve. The validation study was performed on bovine tissues spiked with apramycin at maximum residue limits (MRLs; muscle and fat: 0.5 ppm; liver: 5 ppm) and a value equivalent to 1/10 of MRLs (1/10 MRLs; muscle and fat: 0.05 ppm, liver: 0.5 ppm). The trueness (n = 5) values of apramycin based on the used three kinds of bovine tissue were 84.3%–92.7% at MRLs and 79.2%–97.8% at 1/10 MRLs, and the relative standard deviations (RSD) were 2.1%–5.9% at MRLs and 2.8%–5.7% at 1/10 MRLs. The limit of quantification (LOQ) of the developed method were 0.05 mg/kg (0.05 ppm) for bovine muscle and fat and 0.5 mg/kg (0.5 ppm) for the bovine liver according to the results of the validation study.

Keywords : apramycin, aminoglycoside antibiotic, validation study, livestock products

I Introduction

Apramycin (Figure 1) is an aminoglycoside antibiotic with a broad antibacterial spectrum, and it is used to treat livestock infections[1, 2)]. The Positive List System has been introduced in Japan, and maximum residue limits (MRL) for residues regarding livestock products are set accordingly[3)]. A notification method for measuring apramycin using *Bacillus subtilis* (ATCC 6633) has been reported[4)], but the preparation of test samples for this method is highly complicated and entails problems related to specificity and detection sensitivity.

Recently, extensive application of liquid chromatography-tandem mass spectrometry (LC-MS/MS), several measurement methods for aminoglycoside antibiotics that remain in biological samples have been reported; these methods include the reversed-phase ion-pair chromatography[5-11)], matrix-

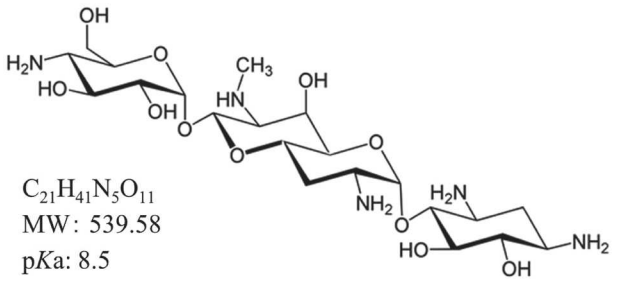

Fig. 1. Apramycin chemical structure
citation: https://chem.nlm.nih.gov/ChemIDplus/name/apramycin

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