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# A sample preparation method from livestock products for quantitative apramycin measurement by LC-MS/MS

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## Abstract

This study aimed to develop a preparation method for apramycin residue in livestock products for liquid chromatography–tandem mass spectrometry (LC-MS/MS). Apramycin was extracted from samples via alkaline hydrolysis or with trichloroacetic acid (TCA) in the presence of *n*-hexane (hexane). Next, matrix components were removed from the extracted apramycin using two types of hydrophilic-lipophilic balanced (HLB) solid-phase extraction (SPE) cartridges, and apramycin was retained with strong cation-exchange SPE cartridges. Apramycin recovery from the extract was evaluated using bovine muscle, liver, and fat samples with apramycin at the maximum residue limit-value (muscle and fat: 0.5 ppm, liver: 5 ppm). The recovery rate of alkaline hydrolysis extraction was 75.5-90.1% and hexane-TCA extraction was 77.3-92.6%, demonstrating that this method could sufficiently remove matrix components that would affect the measurement, as indicated by guidelines of the Ministry of Health, Labor and Welfare. The method examined in this study is effective for apramycin residue sample preparation in livestock products for quantitative LC-MS/MS measurements.

Keywords : apramycin, aminoglycoside antibiotic, extraction method, livestock productions, LC-MS/MS

## I Introduction

Apramycin ($C_{21}H_{41}N_5O_{11}$, MW: 539.58, p*K*a: 8.5), also known as nebramycin factor 2, belongs to a class of aminoglycoside antibiotics produced by the actinomycete *Streptomyces tenebrarius*[1], inhibiting protein synthesis at the level of peptidyl rearrangements[2-4]. It also exhibits a broad antibacterial spectrum, strong antibacterial activity against bacteria resistant to conventional aminoglycoside antibiotics (e.g., kanamycin and fradiomycin), and stability against aminoglycoside antibiotic–inactivating enzymes. Due to these traits, it was developed as a veterinary drug and is used to treat livestock infections, including cattle, pigs, poultry, and rabbits, affected by gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa* (causing bronchial septicemia), as well as *Salmonella* and certain gram-positive bacteria, including *Staphylococcus aureus* and *Mycoplasma*[5].

To treat pigs with bacteriogenic diarrhea as an indication, apramycin sulfate was approved by Japan as a feed and drinking water additive, and the maximum residue limit (MRL)-value in foods was set accordingly following the introduction of the positive list system[6]. Therefore, to develop a quantitative method for determination of the amount of apramycin residue in livestock products is necessary. Regarding apramycin, an aminoglycoside antibiotic, a measurement method has not been established yet owing to its characteristics including 1) high affinity for livestock products and 2) high polarity with poor solubility in organic solvents[7, 8].

A microbiological quantification method for samples prepared through ion-exchange chromatography of alkaline hydrolysis extraction by bioautography using *Bacillus subtilis* (ATCC 6633) has been reported as a quantitative measurement

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