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Original Article

Rapid confirmatory analysis of avermectin residues in milk by liquid chromatography tandem mass spectrometry

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ABSTRACT

Our study developed a quick method for confirmatory analysis of avermectins (abamectin B_{1a}, doramectin, ivermectin B_{1a}, eprinomectin B_{1a}, and moxidectin) in bovine milk according to the European Commission Decision 2002/657/EC requirements. Avermectins were liquid–liquid extracted with acetonitrile, followed by an evaporation step, and then analyzed by liquid chromatography/electrospray ionization tandem mass spectrometry in the negative ion mode. An in-house method validation was performed and the data reported on specificity, linearity, recovery, limit of detection, limit of quantitation, decision limit, and detection capability. The advantage of this method is that low levels of avermectins are detectable and quantitatively confirmed at a rapid rate in milk.

1. Introduction

Avermectins (AVMs) are widely used as an active substance for endo- and ectoparasites in veterinary medicine. AVMs are isolated from a fermentation broth of the soil actinomyete *Streptomyces avermitilis*. AVMs in commercial use are abamectin (ABA), doramectin (DORA), ivermectin (IVER), emamectin, eprinomectin (EPRI), moxidectin (MOXI), and selamectin. These compounds are registered for use in cattle and other food animals, fish farming, and pet animals [1].

The European Medicines Agency has proposed maximum residue limits (MRLs) of 20 ppb and 40 ppb of EPRI and MOXI for milk, respectively. They are not used in animals from which milk is produced for human consumption for ABA,

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DORA, and IVER in the Turkish Food Codex based on European Commission Regulations [2,3]. *Codex Alimentarius* (International Food Standards) has proposed MRLs of 10 ppb, 15 ppb, and 20 ppb for IVER, DORA and EPRI, respectively, but not for ABA and MOXI [4].

AVM residues are usually determined using solid phase extraction techniques and liquid chromatography with fluorescence detection [5]. Immunoaffinity chromatography clean-up of AVMs is an alternate method of sample preparation [6]. Mass spectrometry (MS) is the preferred technique for confirmation of suspect residues due to its inherent specificity and sensitivity [1,7,8].

Our study developed a quick method of confirmatory analysis for AVMs (ABA B_{1a}, DORA, IVER B_{1a}, EPRI B_{1a}, and MOXI) in bovine milk, which are liquid–liquid extracted with acetonitrile (ACN), followed by an evaporation step and, finally, analysis by liquid chromatography/electrospray ionization tandem MS (LC/ESI-MS/MS) in the negative ion mode, according to European Commission Decision 2002/657/EC [9].

2. Materials and methods

2.1. Reagents and standards

HPLC-grade methanol and ACN, triethylamine (TEA), and anhydrous sodium sulfate ($NaSO_4$) were purchased from Bilgen Kimya in Istanbul (Turkey). Purified water was obtained from a Milli-Q purifying system (Elga PureLab Prima).

ABA (purity: 92.3%), IVER (99.2%), EPRI (99.3%), DORA (81.1%), MOXI (92.3%), and selamectin (94%; internal standard) were obtained from Sigma–Aldrich (St Louis, MO, USA). An individual standard stock solution (1000 ppm) of AVMs was prepared by dissolving each pure reference compound in methanol. The mixed standard solution was prepared as 0.05 ppm for ABA, DORA, and IVER based on detection limit and 0.5 ppm for EPRI and 1 ppm for MOXI based on MRL limits. The solution of internal standard selamectin was prepared at 3 ppm in methanol. All stock solutions were stored at −20°C stable for 1 year in a freezer. Working standard and internal standard solutions were prepared in a dilution of methanol and stable for 2 months in a refrigerator.

2.2. Sample preparation

Milk obtained for use as a negative control was separated into 50 mL aliquots and stored at −20°C. The milk was dissolved in a water bath at 45°C and then mixed by gentle shaking. A 5 mL milk sample was pipetted into 50 mL polypropylene centrifuge tubes. Mixed standard solutions were added at 100 μL, 200 μL, 300 μL, and 400 μL and the internal standard at 100 μL to all tubes and then this sample was added to 10 mL of ACN. The tubes were capped, vortexed briefly and left for 15 minutes in a Multi Reax (multiple vortex). Next, 5 g of $NaSO_4$ was added, vortexed, and centrifuged at 4000 g at −4°C for 15 minutes. The 5-mL supernatant was removed using a pipette and transferred into a 15 mL graduated tube. The organic fraction was evaporated to full dryness under a stream of nitrogen in a water bath at 45°C. The dry residue was redissolved in 0.5 mL of ACN (0.1% TEA). The sample was filtered using a 0.45 μm syringe filter to place it into an autosampler vial.

2.3. Instrumentation

Chromatographic analysis was performed on an LC-MS/MS; equipment consisted of a Thermo Electron TSQ Quantum Discovery Max and mass spectrometer controlled by Xcalibur 1.4 software.

Chromatographic separations were achieved on an Agilent Extend C18 column (100 mm × 2.0 mm, 5 μm) and protected with a C18 guard column from Agilent. The isocratic mobile phases used 0.1% TEA in water (20%) and acetonitrile (80%) at a flow rate of 0.3 mL/min and with an injection volume of 50 μL. Detection of the analytes was carried out in the negative ESI-MS-MS ion mode.

2.4. MS

MS/MS parameters and precursor–product ions of each compound were tuned by direct infusion in the selective reaction-monitoring mode at a 0.3 mL/min flow rate mobile phase.

Desolvation temperature was 350°C; capillary voltage 5500 V; sheath gas pressure (air) 40 (arbitrary units); auxiliary gas pressure (air) 10 (arbitrary unit); collision gas pressure 10 (arbitrary units) and collision-induced dissociation source voltage 5 eV. The collision gas was argon and the desolvation gas was nitrogen. MS/MS parameters and precursor–product ions of each compound are given in Table 1.

3. Results and discussion

Method validation was performed with consideration of the criteria and recommendations of European Commission Decision 2002/657/EC and implementation of Council Directive 96/23/EC.

3.1. Specificity/selectivity

Specificity/selectivity were evaluated via analysis of blank matrix samples fortified separately with mixed benzimidazole

Table 1 – Liquid chromatography–tandem mass spectrometry parameters for the analytes.

Compounds	Precursor ion *m/z*	Product ion *m/z*	Collision energy	Retention time (min)
Abamectin	871.4	565.1*	26	3.12
		228.9	36	
Doramectin	897.6	591.1*	26	3.84
		228.9	30	
İvermectin	873.4	567.1*	20	5.43
		228.9	30	
Eprinomectin	912.5	565.1*	26	2.42
		269.9	30	
Moxidectin	638.5	528.2*	26	4.12
		236.0	35	

* Confirmative ion.