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Determination of six polyether antibiotic residues in foods of animal origin by solid phase extraction combined with liquid chromatography–tandem mass spectrometry

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ABSTRACT

A new method using solid phase extraction (SPE) combined with liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed for the determination of six polyether antibiotics, including lasalocid, salinomycin, monensin, narasin, madubamycin and nigericin residues, in foods of animal origin. The samples were extracted with acetonitrile and purified by ENVI-Carb SPE columns after comparing the impurity effect and maneuverability of several SPE cartridges. Subsequently, the analytes were separated on a Hypersil Gold column (2.1 × 150 mm, 5 µm) and analyzed by MS/MS detection. The limit of quantization (LOQ) for milk and chicken was 0.4 µg/kg, and for chicken livers and eggs, it was 1 µg/kg. The linearity was satisfactory with a correlation coefficient of >0.9995 at concentrations ranging from 2 to 100 µg/L. The average recoveries of the analytes fortified at three levels ranged from 68.2 to 114.3%, and the relative standard deviations ranged from 4.5 to 12.1%. The method was suitable for quantitative analysis and confirmation of polyether antibiotic residues in foods of animal origin.

1. Introduction

Polyether antibiotics are ionophore antibiotics produced by various strains of Streptomyces; these antibiotics possess potent microbiological activities against Gram-positive bacteria, fungi and protozoa and are relatively large molecules with molecular masses of 613–940. These compounds readily form cyclic complexes with cations, particularly sodium ions and have the ability to cross biological membranes. They are widely used as anticoccidial agents because they can prevent coccidiosis in different animal species, causing little or insignificant drug resistance problem. They have growth promoting properties and increase the rate of weight gain [1].

These antibiotics should be carefully handled to ensure that they are used at the appropriate concentration. The carry-over of the antibiotics in non-target feed may result in the presence of residues of coccidiosis in animal products and pose a health risk for sensitive individuals. Therefore, the use of polyether antibiotics as feed additives was licensed by the European Union in 2003 (1831/2003/EC, 2003) to allow for the prevention of coccidiosis and must meet a number of legislative criteria of Regulation 1831/2003/EC. MRLs have been laid down for coccidiostats in tissues of target species in the Council Regulation 1831/2003 (Anon, 2003) [2], and Maximum Limits (MLs) have been established by the Commission Regulation No. 124/2009 (Anon, 2009b) [3].

Due to the widespread use of these drugs on farms, there is a risk that residues will be present in animal products intended for human consumption. Suitable methods must therefore be available for detecting the presence of such residues in original animal food, arising from improper use of anticoccidials or from cross-contamination. Amongst some of the earliest methods designed to detect the presence of ionophore drugs were cell culture procedures. Immunoassay techniques are the most popular and highly sensitive screening methods, but they can normally detect only one, or occasionally two coccidiostats [4–6]. At present, the residues of coccidiostats in food of animal origin are determined mainly by HPLC. Lasalocid, alone among the carboxylic acid ionophores, has a fluorescent chromophore. The HPLC-based assays that have

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been developed to determine other carboxylic acid ionophores do not have suitable UV or fluorescent chromophores and require derivatization to introduce a suitable chromophore [7–9]. Using mass spectrometry for the detection allows for sensitivity and for processing a wide range of analytes. The coupling of high-performance liquid chromatography with a mass spectrometer detector (LC–MS/MS) has been employed in the development of satisfactory multiresidue methods in eggs and (or) in chicken [10–15], in milk [16] [17] and in chicken livesr [18–20].

Sample preparation is a very important step in the analysis of polyether antibiotics in food matrices. Because the structures and properties of coccidiostats are significantly different, they are difficult to selectively isolate from sample matrices using extraction. Consequently, more intensive sample clean-up procedures are employed for HPLC based applications. Solvent extraction followed by purification using liquid–liquid partitioning or solid phase extraction is probably the most widely reported approach for the preparation of samples for coccidiostat analysis. Pang et al. developed a method for isolating clopidol residues from chicken tissue based on acetonitrile extraction followed by alumina SPE clean-up [21]. Recovery ranged from 92 to 97%. Rosén developed a method for the isolation of ionophore residues from egg and liver samples using automated SPE clean-up with a Gilson AspecXL system [20]. Ionophore residues were extracted using 87% methanol, applied onto 100 mg C_{18} columns and eluted with 800 μL of methanol prior to direct LC–MS/MS analysis. Matabudul et al. developed a simple sample preparation protocol for isolating the residues of four ionophores and dinitrocarbanilide from eggs and liver [22]. Samples were mixed with Na_2SO_4, extracted with acetonitrile and purified on silica SPE cartridges. The recovery was 86% and 92% for eggs and liver, respectively. Malgorzata Olejinik et al. developed a method for the determination of residues for 12 coccidiostats in chicken liver. Samples preparation was based on extraction with acetonitrile and clean-up on Oasis HLB SPE columns [18]. Szilárd Nász et al. developed an HPLC–MS method with simple solvent extraction and purification by solid phase extraction (SPE) for the determination of coccidiostats in milk. The compounds determined include lasalocid, narasin, salinomycin, monensin, semduramicin, maduramicin, robenidine, decoquinate, halofuginone, nicarbazin and diclazuril. The calculated recoveries ranged between 77.1% and 118.2% [17]. There are several other articles reporting the determination of ionophores in different matrices using SPE-HPLC–MS methods [23–25]. To our knowledge, most of the studies aim at the analysis of polyether antibiotics in a specific matrix, and the established SPE method is suitable for the one or two matrixes. The purpose of this paper is to compare five commonly used SPE methods and establish a widely used SPE-HPLC–MS/MS method for the determination of six polyether antibiotics in four different matrices.

2. Materials and methods

2.1. Chemicals and materials

Lasalocid (purity ≥98.0%), Monensin (purity ≥98.0%), were purchased from Dr. Ehrenstorfer (Wesel, Germany). Narasin (purity ≥98.0%) was purchased from TRC (Toronto, Canada). Nigericin (purity ≥98.0%), Salinomycin (purity ≥98.0%), and Maduramicin (purity ≥99.0%) was purchased from Apollo Scientific Limited (Bredbury, England). Formic acid (98–100%) and ammonium acetate (NH_4Ac) were provided by Dikma (Lake Forest, USA). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were provided by Fisher Scientific (Geel, Belgium). Purified water was obtained from a Waters Milli-Q system (Milford, USA) and was used throughout all experiments. Stock solutions, containing 1 mg/mL of polyether antibiotics were prepared individually. Maduramicin, Narasin, Nigericin, Monensin, Salinomycin and Lasalocid were dissolved in methanol. Working solutions were prepared by diluting the individual stock solutions with formic acid-methanol-water (0.1:20:80, V/V/V).

For solid phase extraction (SPE), biological materials were subjected to solid-phase extraction (SPE) on an SPE column. The purification effectivity of Waters Oasis HLB cartridges (60 mg, 3 mL, Waters, USA), preconditioned with 3 mL of methanol followed by 5 mL of water; ENVI-Carb cartridges (250 mg, 3 mL, Supelclean, USA), preconditioned with 5 mL of acetonitrile; Agilent Florisil cartridges (500 mg, 6 mL, Agilent, USA), Cleanert Alumina N cartridges (200 mg, 3 mL, Agela, USA) and Silica cartridges (250 mg, 3 mL, Waters, USA), preconditioned with 5 mL of isooctane, were investigated for different matrices.

The samples of milk, chicken, eggs, and chicken livers were used as blanks and, after spiking with different polyether antibiotics, as specimens.

2.2. Equipment

In the sample preparation, a vortex mixer (Scientific, USA), nitrogen evaporator (Zymark, USA), SPE system (Supelco, USA), homogenizer (Kinematica, Switzerland), rotatory evaporator (Buchi, Switzerland) and laboratory centrifuge (Hitachi, Japan) were used. The analysis was performed using a Finnigan Surveyor liquid chromatograph (Thermo Electron Corporation, USA) connected to a Finnigan TSQ Quantum ultra AM mass spectrometer (Thermo Electron Corporation, USA) controlled by Analyst software.

2.3. Sample extraction and SPE clean-up

A 5 g portion of blank or spiked sample (milk or chicken) or a 2 g portion of blank or spiked sample (egg or chicken liver) was weighed into a 50 mL Falcon tube, then 20 mL of acetonitrile and 5 g of anhydrous sodium sulfate were added to the tube. The samples of chicken, chicken livers and eggs were vigorously mixed and centrifuged (10,000 rpm, 1 min). The milk sample was vortex-mixed for 1 min and then vigorously mixed and centrifuged (10,000 rpm, 10 min). The supernatant was collected in a 100 mL heart-shaped bottle. Extraction with 20 mL of acetonitrile was repeated, and the combined extracts were evaporated to approximately 10 mL (N_2, 50 °C) and transferred to the preconditioned ENVI-Carb column, 10 mL acetonitrile was passed through the cartridge, and the eluate was combined with the loading solutions and then evaporated to dryness (N_2, 50 °C). Finally, the dry residue was redissolved in 1 mL of methanol, filtered and transferred into an LC vial.

2.4. LC–MS/MS conditions

All chromatographic and mass spectrometric measurements were performed using a Finnigan Surveyor liquid chromatograph (Thermo Electron Corporation, USA) connected to a Finnigan TSQ Quantum ultra AM mass spectrometer (Thermo Electron Corporation, USA), controlled by Analyst software.

2.4.1. Chromatography

The chromatographic separation conditions of the polyether antibiotics were as follows: the column used was a reverse phase Thermo Hypersil Gold C_{18} (150 mm × 2.1 mm, 5 μm) column and the separation was performed under gradient elution conditions. Eluent A was water containing 0.1% formicacid-1 mmol ammonium acetate; eluent B was acetonitrile, which was acidified with 0.1% formic acid. The flow rate was 0.3 mL/min with the following gradient program: 40% eluent B for 1 min, then linear increase of eluent B to 95% over 3 min, finally holding for 5 min, decrease of eluent B