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Performance characterization of a quantitative liquid chromatography–tandem mass spectrometric method for 12 macrolide and lincosamide antibiotics in salmon, shrimp and tilapia

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ABSTRACT

This paper describes an extension and performance characterization of a quantitative confirmatory multi-residue liquid chromatography–tandem mass spectrometric method for residues of macrolide and lincosamide antibiotics, originally validated for application to bovine kidney tissues, to tissues of salmon, shrimp and tilapia. The 12 analytes include clindamycin, erythromycin A, gamithromycin, josamycin, lincomycin, neospiramycin 1, oleandomycin, pirlimycin, spiramycin 1, tildipirosin, tilmicosin and tylosin A. The limit of detection was 0.5 μg/kg. Within-laboratory precision evaluated over the analytical range of 5.0–50.0 μg/kg ranged from 4 to 17%. The accuracy of the method ranged from 80 to 112%. Recoveries ranged from 47 to 99% with all but one recovery above 60%. This is the first report of a quantitative confirmatory method for gamithromycin, pirlimycin and tildipirosin in fish and shrimp.

1. Introduction

Intensive fish farming practices have been adopted to meet the increasing global demand for fish and other seafood products. The use of high densities and volumes which foster the spread of diseases have led to the increased use of compounded feed formulated with antibiotics, other veterinary medicinal products, pesticides and disinfectants [1].

Some of the antibiotics approved for use in aquaculture include macrolides and lincosamides. The macrolides are a class of semi-synthetic medium spectrum antibiotics characterized by a macrocyclic lactone nucleus of 14–16 carbon atoms to which various sugars are attached. Lincosamides are structurally unrelated antibiotics often grouped together with macrolides due to their similar modes of action. They act by binding to the 50S ribosomal subunit, thus inhibiting protein synthesis and are used to treat diseases caused by Gram-positive bacilli and some Gram-negative coccii [2].

Fish and seafood can be exposed to macrolide and lincosamide antibiotics both by deliberate application and by inadvertent exposure in the aquatic environment. A limited number of macrolide and lincosamide antibiotics have been approved for therapeutic use in aquaculture. Canada has approved the use of erythromycin in aquaculture under "Emergency Drug Release" regulations and has established an MRL of 30 μg/kg [3,4]. In the European Union (EU), erythromycin can only be administered off-label to treat conditions such as lactococcosis and bacterial kidney disease in fish as it is not registered for use in most EU countries [5]. However, the EU has established MRLs in fish for erythromycin (200 μg/kg), lincomycin (200 μg/kg), tilmicosin (75 μg/kg) and tylosin (100 μg/kg) [6,7]. Given the paucity of macrolide and lincosamide antibiotics approved for use in aquaculture as compared to those approved for use in terrestrial livestock production, some producers might resort to off-label use of these antibiotics registered for use in other food animals, or use non-registered or banned products [8,9]. Antibiotics administered to livestock can also reach the aquatic environment by surface run-off from agricultural land receiving manure or slurry or from pastures with animals excreting directly to the land [10]. Some macrolide and lincosamide antibiotics such as erythromycin, lincomycin, roxithromycin and tylosin have been detected in river water and wild fish [11].

To enforce regulatory limits and monitor the use of both approved and unapproved antibiotics in aquaculture, and to conduct surveys of the prevalence of residues in wild aquatic organisms, it is desirable to have sensitive multi-residue methods with as wide a scope of analytes as possible regardless of specific human or animal health concerns. There are few multi-residue quantitative confirmatory analytical methods for macrolide and lincosamide antibiotics which have been demonstrated to be applicable to fish and shrimp [6,11–16]. Most of these methods have a limited

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number of macrolide and lincosamide analytes, three to seven, in their scope. The most commonly included macrolide antibiotics are erythromycin [6,11–16], tylosin [6,11,13–15], roxithromycin [6,11–13,16], josamycin [6,11–14], spiramycin [6,11–14] and tilmicosin [6,12,14]. Only three methods include any lincosamide antibiotics, lincomycin and clindamycin, in their scope [12,14,16]. The method reported by Tang et al. [14] has the broadest range of analytes, 12, in its scope and includes analytes not commonly included: clarithromycin, kitasamycin and natamycin. None of these methods includes two macrolides recently approved for use to prevent and treat respiratory diseases in cattle and swine: gamithromycin and tildipirosin [17]. The lincosamide pirlimycin, used to treat mastitis in ruminants [18], is also not included in the scope of these methods. As the availability of these antibiotics increases and their cost decreases, the likelihood that they might be used off-label in aquaculture will increase, prompting regulatory agencies to include these analytes in their monitoring programs.

Recently, a quantitative confirmatory multi-residue liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based method was developed and validated for macrolide and lincosamide antibiotics, including clindamycin, erythromycin A, josamycin, lincomycin, neospiramycin 1, oleandomycin, pirlimycin, spiramycin 1, tilmicosin and tylosin A, in bovine kidney with a working range of 5.0–50.0 μg/kg [19]. This method is routinely used by the Canadian Food Inspection Agency to support the National Chemical Residue Monitoring Program.

In the proof-of-concept study reported here, the objective was to demonstrate the feasibility of extending the applicability of the method validated in bovine kidney to salmon, shrimp and tilapia tissues. The advantage of extending the scope of the bovine kidney method to seafood is that a single method can be employed for a broader range of matrices rather than having several methods specific to different matrices. To broaden the scope of the method, two additional macrolide antibiotics not included in the original validation study, gamithromycin and tildipirosin, were added for evaluation. This is the first report of a quantitative confirmatory method for gamithromycin, pirlimycin and tildipirosin in fish and shrimp.

2. Experimental

2.1. Chemicals and reagents

Clindamycin hydrochloride, gamithromycin and josamycin were obtained from Sigma-Aldrich Canada (Oakville, Ont., Canada). Erythromycin A was obtained from USP (Rockville, MD, USA). Lincomycin hydrochloride, tilmicosin and tylosin A tartrate were obtained from Fluka (Millwaukee, MI). Neospiramycin 1 and spiramycin 1 were obtained from Toronto Research Chemicals (Toronto, Ont., Canada). Oleandomycin phosphate dihydrate was obtained from EQ Laboratories (Atlanta, GA, USA). Pirlimycin hydrochloride was a gift from Pfizer Animal Health (Kalamazoo, MI, USA). Tildipirosin was obtained from Intervet Canada (Whitby, Ont., Canada). The structures of these analytes are presented in Fig. 1.

Ammonium acetate, anhydrous disodium hydrogen phosphate, *o*-phosphoric acid, sodium dihydrogen phosphate monohydrate and sodium hydroxide pellets were of reagent grade and obtained from Fisher Scientific (Ottawa, Ont., Canada). Concentrated formic acid, puriss p. a. for mass spectrometry, was obtained from Sigma-Aldrich. Water was purified by reverse osmosis followed by deionization, absorption and filtration.

Phosphate buffer (0.3 M, pH 8) was prepared by dissolving 9.8 g of disodium hydrogen phosphate and 0.56 g of sodium dihydrogen phosphate monohydrate in 250 mL of water. The pH was adjusted as required by the addition of concentrated sodium hydroxide or *o*-phosphoric acid. Methanolic ammonium acetate solution (0.1 M) was prepared by dissolving 0.77 g of ammonium acetate in 100 mL of methanol. The solid-phase extraction (SPE) conditioning solution, 12% volume fraction acetonitrile in water, was prepared by mixing 120 mL of acetonitrile with 880 mL of water. The 0.1% volume fraction formic acid aqueous LC mobile phase was prepared by adding 1.0 mL of formic acid to freshly purified water in a 1000 mL volumetric flask then brought up to volume with water.

2.2. LC–MS/MS analysis

The separation was carried out on a Waters Alliance 2695 LC (Waters, Milford, MA) using a Kinetex 2.6 μm XB-C18 2.1 mm ID × 100 mm LC analytical column and SecurityGuard Ultra C18 2.1 mm guard (Phenomenex, Torrance, CA). The mobile phases were 0.1% (v/v) formic acid in water and acetonitrile. The LC column was maintained at 40 °C. The flow rate was 0.2 mL/min. The elution started with 2% volume fraction eluent B for 0.5 min then was linearly increased to 98% volume fraction eluent B over 8.5 min. These conditions were maintained for 4 min before returning to initial conditions over 0.5 min. Initial conditions were maintained for 7.5 min to flush any remaining components out of the column and return to equilibrium conditions. Total run time was 21 min. The injection volume was 5 μL.

Mass spectrometric detection was carried out on a Waters Micromass Quattro Micro triple quadrupole MS/MS equipped with an electrospray ionization source (ESI) through a Z-spray interface (Waters, Milford, MA). General MS source parameters were set as follows: The instrument was operated in the ESI positive mode. Source temperature and desolvation temperature were set at 120 and 500 °C, respectively. The capillary and extractor potentials were set at 0.6 and 2.0 V, respectively. The cone gas and desolvation gas, both nitrogen, were set at flow rates of 50 and 1000 L/h, respectively. The collision gas was argon at a pressure of 0.42 Pa. The specific MS/MS parameters for each analyte are given in Table 1.

2.3. Preparation of standards and quality control samples

Individual stock standard solutions of each analyte were prepared by transferring 25 mg of each standard, calculated as free analyte and corrected for purity, into separate 25 mL volumetric flasks. The standards were dissolved in methanol, brought to volume and mixed. The solutions were stored in polypropylene screw-cap vials at −20 °C and prepared fresh every 6 months. Working mixed standard solutions at concentrations of (0.50, 1.25, 2.5, 3.75 and 5.0) μg/mL were prepared by transferring the appropriate volumes of the stock solutions to a 10 mL volumetric flask and diluting to volume with methanol. The solutions were stored in polypropylene screw-cap vials at −20 °C and prepared fresh every 3 months.

Matrix-fortified calibration standard samples were prepared by fortifying five (5.00 ± 0.05) g blank tissue portions with 50.0 μL of one of the working mixed standard solutions to give nominal concentrations of 5.0, 12.5, 25.0, 37.5 or 50.0 μg/kg tissue. Matrix-matched recovery check QC samples were prepared by fortifying processed blank tissue samples with 20.0 μL of the appropriate working mixed standard solution just before reconstitution in a 1:1 mixture of methanol and water. This smaller volume of working mixed standard solution accounts for the 40% subsample volume.

2.4. Preparation of samples

A 5 g homogenized sample of tissue was transferred to a 50 mL polypropylene centrifuge tube. To the tube were added three 9.5 mm diameter steel bearing balls, 1 mL of water and 10 mL of acetonitrile. The capped tube was shaken vertically for 5 min at