Occurrence of veterinary drug residues in farmed fishery products in South Korea

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ABSTRACT

Residues of veterinary drugs were investigated in fish samples from domestic farms in South Korea to highlight the changing trends in drug usage during aquatic food production. Samples (n = 958) were collected from 32 cities and 11 provinces. In total, 41 veterinary drugs were analyzed using an LC-MS/MS. The total detection rate of veterinary drugs in fish samples was 22.7% (n = 217). Enrofloxacin and oxytetracycline were mostly detected in fishery products. Moreover, 12 samples (1.3%) exceeded the maximum residue limit set by Korea. The detection rate for veterinary drug residues varied with fish species and water type. Additionally, our findings show that of the two or more residues in 87 samples, enrofloxacin was often detected in combination with ciprofloxacin, and other compound. Our results of this study suggested the need for an appropriate withdrawal period of fishery products are required to guarantee safe residue levels.

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1. Introduction

Aquatic food production has shifted from wild-caught to farming, which is responsible for the expanded use of fishery products for human consumption. Global fish consumption per capita has increased from an average of 9.9 kg in the 1960s to 19.7 kg in 2013, indicating further growth, exceeding 20 kg. In Korea, 58.4 kg of fish per capita is consumed annually, which is higher than the global rate of fish consumption (FAO, 2016). Fishery products are a staple food for most people in Korea, resulting in increased pressure on the aquaculture industry to meet the increasing demand for aquatic food. Accordingly, many aquaculture sites have been established in Korea.

To improve the production quality of fishery products, veterinary drugs have been widely used for the treatment of parasitic and microbial diseases caused by the stressful conditions and high farming density in fisheries (Uchida et al., 2016). Misuse and improper application of antibiotics without observing the required withdrawal period can result in high residue levels in fisheries and the environment (Barani & Fallah, 2015). These antibiotic residues in fishery products can contribute to the development of antibiotic

resistance, which is a major concern for human and animal health worldwide (WHO, 2014).

Due to the health risks associated with veterinary drug residues, the European Union (EU), Codex Alimentarius Commission (CAC), and other regulatory authorities worldwide have set tolerable levels for veterinary drugs as maximum residue limits (MRLs), and harmful chemicals are banned based on risk assessment (CODEX, 2015; EU Regulation, 2010; USDA, 2017). In Korea, MRLs for veterinary drug residues in fishery products have been established for 52 compounds, including antibiotics and anthelmintics in fish, which are regulated by the Ministry of Food and Drug Safety (MFDS) in Korea. Annual surveillance program has been conducted to determine the presence of veterinary drug residues in fishery products by the MFDS and National Institution of Fisheries and Science (NIFS) in Korea.

Certain veterinary drugs have been detected in fishery products in Korea. In 2004–2005, tetracyclines were detected in a portion of 111 cultured fish samples, and fluoroquinolones were detected in a few cultured fish samples (Shim et al., 2010). Residues of 10 veterinary drugs (4 tetracyclines and 6 quinolones) were monitored in fishery products in 2009, and the results showed that 19 of 118 fish samples contained residues (Kim et al., 2010). Sulfonamide residues (14 substances) were analyzed in both domestic and imported fishery products, and no residues were detected in 99% of samples

(Won et al., 2011). The detection rate of fluoroquinolones was 7.5% in all the 268 freshwater and seawater fish samples (Park et al., 2012). Veterinary drugs, such as sulfadiazine, erythromycin, and trimethoprim, are also commonly detected in farm environments, including fish, sediment, and water (Kim, Lee, & Oh, 2017). Certain veterinary drugs, such as fluoroquinolones and tetracyclines, have been used primarily for treatment and are added directly into the water or mixed with fish feed to prevent infectious disease in fisheries in Korea.

Various analytical methods have been developed for multi-–residue analysis of veterinary drugs in fishery products using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Dasenaki & Thomaidis, 2015; Lopes, Reyes, Romero-González, Vidal, & Frenich, 2012). LC-MS/MS is the most commonly used technique for simultaneous quantification of multi-residues of chemicals in food samples owing to high sensitivity and selectivity (Dong & Xiao, 2017; Xian et al., 2016). Therefore, we used LC–MS/MS for measuring veterinary drug residues in fishery products and verified whether the detected amounts coincided with the values mentioned in the Korean Food Standard Code (MFDS, 2014).

Based on previous studies, trends in antibiotic usage during the production of aquatic products have changed continuously in the past few decades in Korea, and few comprehensive studies of veterinary drug residues in fishery products, including freshwater fish in Korean domestic markets, have been performed. Accordingly, we investigated veterinary drug residues using multiple screening and confirmation methods, including liquid chromatography tandem mass spectrometry (LC-MS/MS), in seawater and freshwater fish samples from the domestic market. Our results of study can provide occurrence trends of veterinary drug for fishery products in Korea.

2. Materials and methods

2.1. Chemicals and materials

Standard chemicals (41 substances) were purchased from Dr. Ehrenstofer (Augsburg, Germany), Wako Pure Chemical Industries Inc. (Osaka, Japan), US Pharmacopeia (MD, USA), and Sigma Aldrich (MO, USA). Oxolinic acid, amoxicillin, ampicillin, cefalexin, chloramphenicol, florphenicol, thiamphenicol, praziquantel, ceftiofur, and desfuroyl ceftiofur were dissolved in 50% methanol to a concentration of 100 mg/L. Other standard stock solutions were prepared for each compound in methanol (100 mg/L). Standard solutions were stored at −20 °C when not in use. Mixture of standards for calibration curves and recovery tests were performed using these stock solutions. Analytical-grade acetonitrile, n-hexane, and methanol were purchased from Merck Inc. (Darmstadt, Germany). Centrifuge tubes were obtained from Corning (NY, USA). Polyvinylidene difluoride (PVDF) filters (0.2 μm) were purchased from Teknokroma (Barcelona, Spain).

2.2. Sample preparation

Sampling was performed from July 2014 to October 2015 in 11 provinces in Korea. In total, 958 seawater and freshwater fish were collected from retail and wholesale markets in each province (Table S1). All samples were harvested at various aquaculture farms from different provinces and were transported to markets through intermediaries. We purchased and transported these samples to our laboratory within 4 h. The edible tissues (muscle and skin) were chopped and kept frozen in plastic bags at a set storage temperature (−20 °C).

The samples were analyzed using a simultaneous multiclass detection method with LC-MS/MS as specified by the Korean Food Standard Code (MFDS, 2014). Briefly, 2.0 g of homogenized sample was transferred to a 50-mL polypropylene tube. Then, 10 mL of 2 mM ammonium formate was added to 80% acetonitrile was added. This tube was homogenized for 10 min at 10,000 × g. After centrifugation, the supernatant was poured into polypropylene tubes. A dispersive sorbent (Prep C18; 500 mg) was added, and 10 mL of hexane was then added. The mixture was mixed thoroughly for 30 s using a vortex mixer. After a 5-min centrifugation at 10,000 × g, the hexane layer was discarded, and 5 mL of the supernatant was transferred to a 15-mL polypropylene tube and concentrated to 1 mL under nitrogen gas at 40 °C. The extract was filtered through a 0.2-μm PVDF filter, and an aliquot (5 μL) was injected into LC-MS/MS.

2.3. LC-MS/MS analysis

Instrumental analysis was performed using an ACQUITY UPLC system with an XEVO TQ-S tandem quadrupole mass spectrometer (Waters, Milford, MA, USA). An X-SELECT C_{18} column (2.1 mm × 150 mm, 3.5 μm; Waters, Dublin, Ireland) was used for separation. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Chromatographic separation was performed in gradient mode: 0–1 min, 0.4 mL/min 10% B; 1–6 min, 0.4 mL/min increase to 40% B; 6–10 min, 0.4 mL/min linear increase to 95% B and hold for 15 min, 0.4 mL/min; 15–15.1 min, decrease to 10% B hold for 20 min, 0.4 mL/min. The column temperature was maintained at 40 °C, and the flow rate was set to 0.4 mL/min. The MS instrument was operated in the electrospray ionization (ESI) mode with positive and negative switching using multiple reaction monitoring (MRM). The precursor ion, three product ions, collision energy, and cone voltage for each target compound are listed in Table 1. LC-MS/MS chromatogram at MRL concentration for 41 veterinary drugs was presented in Fig. S1. The capillary voltages were ESI positive (3.5 kV) and ESI negative (−2.5 kV), and the capillary temperature was set at 350 °C. The source and desolvation temperatures were set at 150 °C and 500 °C, respectively. The cone and desolvation gas (nitrogen) flow rates were 60 and 600 L h^{-1}, respectively. The collision gas (argon) was maintained at a pressure of 4×10^{-3} mbar in the collision cell. Data collection was implemented in MRM mode using MassLynx software (Waters, UK).

2.4. Method evaluation

The validation procedure was performed taking into consideration the requirements outlined in the CODEX guidelines (CAC/GL-16 and 71) to evaluate the performance of the analytical method. The validation of the level of each veterinary drug substance was performed based on the Korean MRLs in fishery products. Matrix-match calibration curves were constructed using blank fish samples spiked with standard solutions. The representative matrices, i.e., flatfish, eels, and shrimp, were previously confirmed to be free of the target analytes. All fish samples were screened based on flatfish and eel calibration curves, and the data obtained for shrimp samples were processed using shrimp calibration curves. The precision and accuracy were expressed as recoveries and coefficient variations (CVs). Recovery experiments were performed by spiking blank fish samples at 0.5, 1, and 2 times the MRL using five replicates for each concentration level on one day. The concentration levels with the signal to noise (S/N) ≥ 3 were defined as lower limit of quantification (LLOD) and S/N ≥ 10 were defined as lower limit of quantification (LLOQ). The LLOQ and higher limit of quantification (HLOQ) were assessed on the basis of quadruple determinations of six spiked fishery product samples ranging from 0.25 to 25 ng mL^{-1} and 4–600 ng mL^{-1}.