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Analytical Methods

Development of a liquid chromatography–tandem mass spectrometry method with modified QuEChERS extraction for the quantification of mebendazole and its metabolites, albendazole and its metabolites, and levamisole in edible tissues of aquatic animals

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ABSTRACT

A liquid chromatography–tandem mass spectrometry quantitative method was developed for determining mebendazole and its metabolites, albendazole and its metabolites, and levamisole in muscles of bluntnose black bream, shrimp, eel and turtle based on modified QuEChERS methodology. The method included 2 g of the muscle matrix with 10 mL acetonitrile, and 0.8 g of magnesium sulphate and 0.2 g of sodium chloride for liquid-liquid partitioning. After vortex and centrifugation, the resulting liquid (5.5 mL) was purified by C_{18} (50 mg) and Al-N (50 mg). The limits of detection were lower than 0.3 μg kg^{-1} and the limits of quantitation were no more than 1 μg kg^{-1} for all analytes. The recoveries of the analytes ranged from 80.0% to 113.7% with the relative standard derivation less than 10.0%. The preparation procedure provided efficient extraction and purification that enabled a sensitive and rugged determination of target compounds.

1. Introduction

Mebendazole (MBZ) and albendazole (ABZ) belong to broad-spectrum anthelmintic agents of benzimidazoles drugs, against gastrointestinal worms, tapeworms and flukes. However, the imidazolthiazole drug–levamisole (LVS) plays an important role in killing nematodes and improving immunity (Cuesta, Meseguer, & Esteban, 2004). These drugs are widely used in food producing animals for treatment of parasitic infections or maintaining high weight gains and reproductive performance.

With the development of intensive aquaculture, frequent outbreaks of parasitic diseases have resulted in large of the economic losses. The pathogenic parasites involved Dactylogyrus (Nitta & Nagasawa, 2016), Pseudodactyl (Buchmann & Bjerregaard, 1990), Gyrodactylus (Zhou et al., 2017), Leucochloridium paradoxum (Ataev, Zhukova, Tokmakova, & Prokhorova, 2016), Benedenia (Hirazawa, Tsubone, & Takano, 2016), Neobenedenia (Hirazawa, Ishizuka, & Hagiwara, 2016), and so on. Consequently, MBZ, ABZ and LVS were employed to therapy those infections in many countries. MBZ and ABZ have been approved in aquaculture in China, but LVS was not permitted to use in aquatic animals. Generally, if the users followed the properly dosing and complied with the withdrawal period, it is concluded that the drug residues can be controlled within safe range and will not produce harm to humans. Counterproductively, many phenomena have occurred concerning to drug off-label and non-compliment with the withdrawal period. The residue levels may exceed the maximum residue limit in food. What is worry is that some of these drugs and its metabolites possess embryotoxicity, teratogenicity, neurotoxicity and mutagenicity (Danaher, De Ruyck, Crooks, Dowling, & O'Keeffe, 2007; EMEA, 1996, 1999a, 1999b, 2001, 2004). Therefore, the drug residues were the major concern of humans and regulatory bodies. In order to avoid the health risk, the development of a rapid and easy analytical method for monitoring these drugs and its metabolites is an urgent need.

Many quantitative methods have been developed for the analysis of MBZ, ABZ, LVS and their metabolites in food producing animals such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC–MS/MS). Although GC (Lafuente, Tadeo, & Tuset, 1987) or GC–MS

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(Jacob, Carlin, Walker, Wolf, & VandenHeuvel, 1975) methods were used to determine benzimidazoles in the early stages of the study, it is very difficult due to the basic nature and low volatility of these substances. Afterwards, HPLCs with ultraviolet detection are the most widely applied. Simple liquid-liquid extraction and solid-phase extraction were mainly employed to complete the analysis tasks (Fletouris, Botsoglou, Psomas, & Mantis, 1997; Fletouris, Papapanagiotou, Nakos, & Psomas, 2005; Hajee & Haagsma, 1996; Iosifidou, Haagsma, Olling, Boon, & Tanck, 1997). The most common problems faced in the extraction of MBZ in biological matrices were producing a large amount of waste liquid and exhaust gas and reducing extraction efficiencies. The sensitivity of HPLC with ultraviolet was also not high enough. Currently, many methods of LC–MS/MS were performed to determine those drugs. Although, sensitivity and precision are improved, the extraction method was not developed (Ruyck, Daeseleire, Ridder, & Renterghem, 2003; Xia et al., 2010). Recently, more advanced methods were applied to measure the target compounds. Chen et al. determined 11 benzimidazoles and 10 their metabolites in the muscles and livers of swine, cattle, sheep and chicken by LC–MS/MS using liquid pressurized liquid extraction (Chen et al., 2011). Kinsella et al. developed a LC–MS/MS method with QuEChERS extraction for analysis of 38 residues of anthelmintic veterinary including benzimidazoles in milk and liver (Kinsella et al., 2009). However, little information was available on measuring MBZ, ABZ, LVS and their metabolites in meat origin of aquatic animals using a more concise and quicker method.

In the preset study, it was developed to a rapid, easy and reliable LC–MS/MS with modified QuEChERS extraction for analysis of MBZ, ABZ, LVS and their metabolites in muscle of bluntnose black bream, shrimp, eel and turtle. The sample preparation by modified QuEChERS in this study adopted fewer samples and fewer reagents compared to traditional QuEChERS method. Moreover, the limit of detection, the limit of quantitation, accuracy and precision achieved a desired effect.

2. Experimental

2.1. Drugs and reagents

Analytical standards of mebendazole (MBZ), albendazole (ABZ), amino mebendazole (MBZ-NH_2), hydroxyl mebendazole (MBZ-OH), Albendazole sulphoxides (ABZ-SO), albendazole sulphone (ABZ-SO_2), albendazole-2-amino-sulphone (ABZ-NH_2-SO_2), MBZ-d_3 and ABZ-d_3 were obtained from Dr. Ehrenstorfer (Gmbh, Augsburg, Germany). HPLC-grade Acetonitrile, methanol, water and formic acid were purchased from Fisher (Bar–Bel, France) and J–T Baker (Philipsburg, USA). Ammonium acetate for LC–MS was supplied by Shanghai Anpel Laboratory Technologies (Shanghai, China). Anhydrous magnesium sulphate and sodium chloride were supplied by Shanghai Guoyao Company (Shanghai, China). Cleanert C_{18} sorbent (40–60 μm, analytical grade), Cleanert PSA sorbent (40–60 μm, analytical grade), Cleanert NH_2 sorbent (40–60 μm, analytical grade), Cleanert Alumina-N sorbent (40–60 μm, analytical grade) was obtained from CNW Technologies (Shanghai, China).

2.2. Standard solutions

Individual primary stock standard solutions (400 $\mu g\,mL^{-1}$) of all analytes were prepared by dissolving each pure standard in dimethyl sulfoxide. A 10 $\mu g\,mL^{-1}$ working standard fortification solution was made by combining 0.5 mL of each stock standard and dilute to corresponding volume with methanol. A 1 in 10 dilution of the standard was conducted to get a 1 $\mu g\,mL^{-1}$ working standard solution. A 1.0 $\mu g\,mL^{-1}$ labeled internal standard solution (MBZ-d_3 and ABZ-d_3) was prepared with methanol, respectively. Stock and mixed standard solution were prepared every 3 and 1 months and were stored in amber vials at or below −20 °C.

2.3. Blank samples and sample preparation

The muscles of bluntnose black bream, shrimp, eel and turtle were purchased from local markets. After being homogenized in a high-speed food blender, the samples were stored below −20 °C in a freezer.

2 g of muscle sample was thawed and weighed into 50 mL centrifuge tube. Samples were spiked with the internal standards (10 μL MBZ-d_3 and 5 μL ABZ-d_3) or the analytes. After standing 15 min, 10 mL of acetonitrile was added into spiked sample following by 1 g of MgSO4 and NaCl (4:1, w/w) for dewatering and liquid-liquid partitioning. Samples were immediately shaken for 30 s and centrifuged 5 min at 3500*g*. Consequently, 5.5 mL of the supernatant was transferred into a 10 mL centrifuge tube containing C_{18} (50 mg) and Alumina-N (50 mg). The sample mixture was shaken for 30 s and centrifuged 5 min at 7000*g*. The resulting supernatant (5 mL) was transferred into another 10 mL centrifuge tube and evaporated to dryness under gentle nitrogen stream in water bath at 45 °C. The remaining residue was re-dissolved in 1 mL of methanol and 0.01% formic acid water (50:50). The mixture was also shaken for 1 min and centrifuged 5 min at 7000*g*, and then filtered through a 0.22 μm membrane filter into an auto-sampler vial for LC–MS/MS analysis.

2.4. LC–MS/MS analysis

An LC–MS/MS system (TSQ Quantum Access MAX, Thermo Fisher, USA) was employed to analyze all samples, equipped with a triple quadrupole mass spectrometer, an LC binary pump and an auto-sampler. The sample separation was carried out on a Hypersil Golden (150 mm×2.1 mm, 3 μm) with appropriate column temperature at 30 °C. Data were obtained and processed using the Thermo Xcalibur software (Copyright 2.1.0). The mobile phase of component A was a buffer solution consisting of 10 $mmol\,L^{-1}$ ammonium formate at pH 4 and that of component B was acetonitrile. The mobile phase gradient profile was as follows (t in min): $t_{0'}$, A = 90%, B = 10%; $t_{5'}$, A = 10%, B = 90%; $t_{10'}$, A = 90%, B = 10%. The flow rate was 0.2 mL/min and injection volume was 10 μL.

The heated electrospray ionization in positive mode was operated to optimize the ion source parameters by monitoring the MS/MS spectra of the analytes. Selective reaction monitoring (SRM) was conducted to simultaneously monitor corresponding protonated molecular ions for all analytes using a spray voltage of 3500 V, vaporizer temperature of 350 °C, ion transport tube temperature of 350 °C, sheath gas (high purity nitrogen) of 40 psi, auxiliary gas (high purity nitrogen) of 10 arb, collision gas (ultra-high purity argon) pressure of 1.50 mTorr, Q1 peak width of 0.70 amu, Q3 peak width 0.50 amu, and a scan time of 0.2–0.4 s. Collision energies were set at 18–30 eV.

2.5. Validation procedure

The validation procedure of LC–MS/MS method was achieved by referring to the guideline of the EU Commission Decision 2002/657/EC. The specific indicators of the method for validation were determined, such as linearity, specificity, decision limits (CCα) and the detection capability (CCβ), recovery and precision. The matrix-matched curves of MBZ and its metabolites, ABZ and its metabolites, and LVS were prepared by analyzing spiked blank samples with standard mixtures at different concentrations of 1, 2, 5, 10, 20 and 50 $\mu g\,L^{-1}$. All curves were constructed by linear regression of the ratios of chromatographic peak area of the standards and suitable IS versus nominal concentrations. The correlation coefficients and the slope were calculated. Such calibration curves were obtained with each series of samples.

2.5.1. Specificity

In order to evaluate possible exogenous and endogenous interferences in samples, 10 different blank samples from different sources