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Analytical Methods

Molecularly imprinted solid-phase extraction for the determination of ten macrolide drugs residues in animal muscles by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A simple and sensitive method based on molecularly imprinted solid-phase extraction coupled with liquid chromatography–tandem mass spectrometry was developed for the determination of the residues of ten macrolide drugs in swine, cattle and chicken muscles samples. The molecularly imprinted polymers (MIPs) were synthesized using tylosin as a template and methacrylic acid as a functional monomer. Samples were extracted with sodium borate buffer solution and ethyl acetate, and purified by the MIP cartridge. The results showed that the cartridge exhibited good recognition performance for macrolides, and better purification effect than the traditional solid-phase extraction cartridges. Recoveries of analytes at three spiking levels 1, 5 and 20 μg kg^{-1} ranged from 60.7% to 100.3% with the relative standard deviations less than 14%. The limits of detection of the method were between 0.1 and 0.4 μg kg^{-1}. The method is useful for the routine monitoring of the residues of macrolide drugs in animal muscles.

1. Introduction

Macrolides are a group of drugs with a large lactone ring that mostly composed of 14 to 16 carbon atoms. Since the discovery of erythromycin (ERY, Fig. S1 for structure) in the 1850s, a large number of macrolide drugs have been developed as the improvement of pharmacological and microbiological effects of macrolides. Nowadays, macrolides have become one of the four most often used anti-infective drugs in the world. They have favorable antibacterial effect on gram-positive, some gram-negative bacteria and mycoplasma. Furthermore, due to the advantages of quick absorption, high oral bioavailability and long half-life, macrolides are widely applied in the clinical treatment of bacterial infections and used as feed additives to prevent diseases (Periti, Mazzei, Mini, & Novelli, 1989; Zhanel et al., 2001). However, sustained medication and illegal medical additive may lead to drug residues in food animals. The accumulation of drugs in edible animal tissues could be a potential threat to human health through the food chain, which will cause the occurrence of side effects such as gastrointestinal reaction, local stimulation, allergic reaction and hepatotoxicity (Periti, Mazzei, Mini, & Novelli, 1993). Therefore, supervising and controlling the residues of macrolide drugs in edible animal tissues have been paid a high attention in many countries. In order to ensure human health, the majority of countries have established regulations and set maximum residue limits (MRLs) for macrolide drugs. Furthermore, the macrolide antibiotics have been prohibited from using as feed additives in European Community (EC). With the rapid improvement of analytical instruments, it is available to analyze trace drugs and various analytical methods have been gradually developed to detect the drug residues in complex matrices (Kinsella et al., 2009; Reig & Toldrá, 2008).

The analytical methods of residues of macrolide drugs are mainly targeted at edible animal tissues such as muscle, liver, kidney and fat. Besides, milk, egg and honey are also the interesting animal products for analysts. There are various methods that have been reported for the determination of macrolides in animal muscle samples, including enzyme-linked immunosorbent assay (ELISA) (Galvidis, Lapa, & Burkin, 2015), capillary electrophoresis (CE) (Zhou, Chen, & Cassidy, 2000), thin layer chromatography (TLC) (Ahmed, Sree, Abdel-Fattah, Hassan, & El-Dein Saad, 2013), high performance liquid chromatography (HPLC) (Lee, Yoo, & Shin, 2013), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Dickson, 2014). Among these analytical methods, HPLC and LC–MS/MS are the two most widely used and efficient methods due to the availability and high accuracy in different complex matrices. Impurities such as protein and fat existed in animal tissues samples will not only pollute the analytical instruments,

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which leads to the decrease of the sensitivity, but also affect the separation of target analytes. With the purpose of decreasing the interference of impurities, specific pretreatment measures such as pressurized liquid extraction (PLE) (Tao et al., 2012), solid phase extraction (SPE) (Freitas, Barbosa, & Ramos, 2015), matrix solid-phase dispersion (MSPD) (García-Mayor, Gallego-Picó, Garcinuño, Fernández-Hernando, & Durand-Alegría, 2012), solid phase micro-extraction (SPME) (McClure & Wong, 2007), QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) (Kang et al., 2014), have been taken to achieve better separation, purification and preconcentration of target drugs. However, the trace amounts of drug residues, complex matrices and the interference of impurities are still the challenges in analysis. To overcome such difficulties, novel pretreatment techniques such as immunoaffinity chromatography (Zhao, Lin, Song, Pan, & Wang, 2011) and molecular imprinting (Siemann, Andersson, & Mosbach, 1997) have been introduced in the selective retention of drugs and the powerful elimination of impurities.

Molecular imprinting technology (MIT) is an emerging and increasingly applied technique that originally derived from the immunological mechanism of the receptor and molecular recognition. Molecularly imprinted polymers (MIPs) are synthesized using the template, functional monomer, cross-linker and initiator, which form the corresponding spatial structure and binding site. Once the template is removed, the MIPs will leave behind a cavity, whose spatial structure and binding site are complementary in size and shape with the target template. Due to the "lock-key" relationship, the MIPs possess the characteristic of selectivity toward the target molecules, and in a consequence can specifically recognize the template or analogues. As a novel molecular recognition material, molecularly imprinted polymers are widely used in the fields of environmental monitoring, bioanalysis and food detection, owing to the selective enrichment characteristic of the MIPs which can specifically concentrate trace amounts of target compounds such as pesticides, traditional Chinese medicine, veterinary drugs or environmental toxins from complicated matrix samples (Cirillo et al., 2011; Hantash et al., 2006; Zhang et al., 2006). In terms of drugs residues analysis in food, molecularly imprinted polymers are generally used as the materials of solid phase extraction in the sample cleanup step. Considerable attention has focused on MIP solid phase extraction (MISPE) method for purification and preconcentration of macrolides drugs residues in samples. The molecularly imprinted polymers of erythromycin were prepared by non-covalent bulk polymerization and successfully applied to the cleanup and enrichment of the ERY in pig muscle (Song et al., 2008). The MIPs exhibited powerful affinity toward template molecule with the maximum adsorption amount of 72.09 g kg^{-1} and the recoveries of ERY were more than 80%. It is suggested that the MIPs as solid phase extraction sorbents had high selective performance and could easily absorb the ERY in tissue extracts and efficiently remove the impurities co-extracted with ERY. Piletsky et al. (Piletsky et al., 2004) synthesized the MIPs of tylosin (TYL, Fig. S1 for structure) that could rebind with the template or related metabolites. Furthermore the MIP could adsorb tylosin from complex matrices with easy removal of lipids and other impurities. Zhang et al. (Zhang et al., 2011) reported that the multi-walled carbon nanotube-molecularly imprinted polymer of ERY prepared was used as solid-phase extraction sorbent, and exhibited good selectivity toward template molecule. The recoveries in chicken muscle sample were between 85.3% and 95.8%. To the best of our knowledge, researches on molecularly imprinted solid-phase extraction mainly focused on the detection of the template or an analogue, while it's feasible for finding a MIP that can purify and enrich a class of structural analogues in accordance with the class-specific recognition sites in the polymers.

The purpose of the present study is to synthesize a class-selective MIP using tylosin as a virtual template and then used as the selective absorbent for packing SPE cartridge. Finally, a simple and sensitive method based on MISPE combining with LC–MS/MS was established for the simultaneous determination of the residues of ten macrolides (Azithromycin (AZI), tulathromycin (TUL), tilmicosin (TIL), erythromycin (ERY), kitasamycin (KIT), spiramycin (SPM), roxithromycin (ROX), josamycin (JOS), clarithromycin (CLA), and medecamycin (MED)) in swine, cattle and chicken muscles samples. The proposed method is appropriate to clean up, enrich and determine macrolide drugs residues in complex biological matrix samples.

2. Experimental

2.1. Reagents and materials

The required template tylosin was obtained from Hengtong Guanghua (Xian, China). The synthetic materials methacryclic acid (MAA) and 2,2′-Azobisisobutyronitrile (AIBN) were from Kermel Chemical Reagents Development Center (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). MAA and EGDMA were removed inhibitor by active carbon before they were used.

The purity of each reference standard (Fig. S1 for structure) was more than 90%. Azithromycin, tulathromycin, tilmicosin, erythromycin, kitasamycin and tiamulin (TIA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Spiramycin, roxithromycin, josamycin and clarithromycin were obtained from European Pharmacopoeia (EDQM, Strasbourg, France) and medecamycin was purchased from China Institute of Veterinary Drug Control (Beijing, China). Each of the appropriate standards was dissolved in methanol to prepare the stock solutions at the concentration of 1000 mg L^{-1}. The stock solutions were stored in volumetric flask and could keep stable for 6 months at 4 °C. The working solutions of ten macrolides (100 mg L^{-1}) were prepared monthly by diluting the stock solutions with acetonitrile.

Acetonitrile, methanol and formic acid were chromatographic grade and purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Other reagents including ethyl acetate, acetone and ammonia, etc. were analytical grade or better and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Oasis HLB (60 mg, 3 mL) SPE cartridge was obtained from Waters Co. (Milford, MA, USA), C_{18} (200 mg, 3 mL) and SCX (60 mg, 3 mL) SPE cartridges were purchased from Agilent Technologies Co. (Santa Clara, CA, USA). De-ionized water was obtained by Milli-Q water system (Molsheim, France).

2.2. Preparation of the imprinted polymers

The MIPs were synthesized according to our method previously reported (Zheng et al., 2011). Briefly, 1.0 mmol tylosin was dissolved in 6 mL chloroform and then sonicated for 5 min. 8 mmol MAA was added into the dispersion and incubated at 4 °C for 4 h. 20 mmol cross-linker EGDMA and 20 mg initiator AIBN were added. After sonication for 5 min, the mixture was purged with nitrogen for 5 min to remove oxygen. The polymerization reaction would accomplish by incubating the mixture at 60 °C for 24 h in a water bath. After grinding and sieving, the polymers were sedimented with acetone to discard the tiny particles. The template was removed by methanol-acetic acid (90:10, v/v) and finally the polymers were dried under vacuum at 60 °C.

2.3. Filling amount of MISPE cartridge

The polymers particles were packed into 1 mL empty SPE cartridge at the amount of 10, 20, 30 and 40 mg, respectively.