Journal of Chromatography B 1065–1066 (2017) 104–111

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Application of molecularly imprinted polymer based matrix solid phase dispersion for determination of fluoroquinolones, tetracyclines and sulfonamides in meat

Geng Nan Wang, Lei Zhang, Yi Ping Song, Ju Xiang Liu, Jian Ping Wang*

College of Veterinary Medicine, Agricultural University of Hebei, Baoding, Hebei, 071000, China

ARTICLE INFO

Keywords:
Molecularly imprinted polymer
Matrix solid phase dispersion
Ultra performance liquid chromatography
Fluoroquinolones
Sulfonamides
Tetracyclines

ABSTRACT

In this study, a type of novel mixed-template molecularly imprinted polymer was synthesized that was able to recognize 8 fluoroquinolones, 8 sulfonamides and 4 tetracyclines simultaneously with recoveries higher than 92%. Then the polymer was used to develop a matrix solid phase dispersion method for simultaneous extraction of the 20 drugs in pork followed by determination with ultra performance liquid chromatography. During the experiments, the MMIP amount, washing solvent and elution solvent were optimized respectively. The limits of detection of this method for the 20 drugs in pork were in the range of 0.5–3.0 ng g^{-1}, and the intra-day and inter-day recoveries from the fortified blank samples were in the range of 74.5%–102.7%. Therefore, this method could be used as a rapid, simple, specific and sensitive method for multi-determination of the residues of the three classes of drugs in meat.

1. Introduction

The residues of veterinary drugs in foods of animal origin can pose many potential risks to the consumers. For example, the residues of fluoroquinolones (FQs) can induce the drug resistant pathogens [1], the residues of tetracyclines (TCs) can cause gastrointestinal disturbances and allergic reactions to the consumers [2], and the residues of sulfonamides (SAs) have potential carcinogenic effects on humans [3]. To ensure food safety and human health, China and the European Union have established different maximum residue limits (MRLs) for these drugs in meat, i.e. the sum of SAs, 100 ng g^{-1}; FQs, 20–200 ng g^{-1}; single or total amount of TCs, 100 ng g^{-1} [4,5]. Therefore, it is urgent to monitor their residues in foods of animal origin.

The first thing for determination of residual veterinary drugs in foods of animal origin is to extract and purify the low level of analytes in the samples. By now, many sample preparation methods, such as solid phase extraction [6], solid-phase microextraction [7], stir bar sorptive extraction [8], QuEChERS [9], immunoaffinity chromatography [10], and dispersive solid phase extraction [11], have been reported for extraction and purification of FQs, SAs and TCs in various samples. However, the first step of these extraction methods is to transfer the analytes from the samples into various solvent phases, and then different purification procedures are performed. This meant that the solubilization procedure is a rate-limiting step.

Matrix solid phase dispersion (MSPD) is a novel sample preparation method that was first developed in 1989 [12]. For a MSPD method, the solid sample, viscous sample or liquid sample is blended with a type of suitable dispersing sorbent, and then the sample-sorbent mixture is transferred into an empty syringe barrel. After the sample impurities are washed out with proper solvent, the analytes are eluted for analysis. This sample preparation method combines extraction and purification into one step that can be finished within several minutes, so this technique is simpler and more flexible than the commonly used sample preparation methods. Therefore, there have been many articles reporting the use of MSPD technique for determination of various analytes in different samples [13], including SAs [14–16], TCs [17] and FQs [18,19]. However, the usually used dispersing sorbents in the previous reports (C_{18}, SiO_2, HLB material, diatomite, *N*-propylethylenediamine, alumina, Florisil) are easily interfered by the sample impurities, and these sorbents may lead to competitive adsorption when one sample simultaneously contains other compounds besides the target analytes. Therefore, it is desirable to find a specific dispersing sorbent for MSPD technique.

Over the past 10 years, molecularly imprinted polymer (MIP) has drawn the interests of many researchers due to its specific recognition ability. Therefore, MIP based materials have been widely used for the determination of different analytes in various samples [20], including TCs [21,22], FQs [23] and SAs [24–26]. In 2007, a novel sample

* Corresponding author.
E-mail address: chinawangjp@hotmail.com (J.P. Wang).

http://dx.doi.org/10.1016/j.jchromb.2017.09.034
Received 21 June 2017; Received in revised form 15 August 2017; Accepted 20 September 2017
Available online 21 September 2017
1570-0232/

pretreatment method combining MIP and MSPD was first developed for extraction of FQs residues in egg and swine tissues [27]. Results showed that this technique avoided competitive adsorption and achieved highly selective purification effect, and the sorbent MIP showed better purification effect and higher recoveries than four tested sorbents (silica, C18, sand and Florisil). Thereafter, MIP-MSPD has been used for the determination of BPA [28], β-estradiol [29], steroids [30], clenbuterol [31], FQs [32–34] and Sudan dyes [35] in various samples. However, there has been no article reporting the use of MIP-MSPD method for the determination of SAs and TCs so far.

The commonly synthesized MIP is only able to recognize a group of structurally similar analytes, so there has been no article reporting the use of MIP-MSPD method for multi-determination of different classes of veterinary drugs in foods of animal origin so far. In the present study, three molecules (pipemidic acid for FQs, sulfabenzamide for SAs, and chlortetracycline for TCs) were used to synthesize a novel mixed-template MIP (MMIP), and its recognition performance for FQs, SAs and TCs were studied. Then, a MMIP-MSPD method was developed for extraction and purification of the three classes of drugs in pork followed by determination with ultra performance liquid chromatography (UPLC).

2. Materials and methods

2.1. Reagents and chemicals

Pipemidic acid (PA) and sulfabenzamide (SB) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The standards of enrofloxacin (ENR), sarafloxacin (SAR), ciprofloxacin(CIP), lomefloxacin (LOM), ofloxacin (OFL), pefloxacin (PEF), danofloxacin (DAN), marbofloxacin (MAR), sulfadiazine (SD), sulfadimidine (SM_2), sulfamethoxypyridazine (SMP), sulfadimethoxine (SDM), sulfamonomethoxine (SMM), sulfamethoxazole (SMZ), sulfaquinoxaline (SQ), sulfachloropyridazine sodium (SCP) tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DC) were purchased from Sigma (St. Louis, MO, USA). Ethylene glycol dimethacrylate (EGDMA) was purchased from Aladdin Industrial Corporation (Shanghai, China). Methacrylic acid (MA) and 2,2-azobis (isobutyronitrile) (AIBN) were purchased from Kermer Chemical Company (Tianjin, China). Other chemical reagents were of analytical grade or better from Beijing Chemical Company (Beijing, China). Liquid chromatographic grade acetonitrile and methanol were purchased from Dikma (Richmond Hill, USA). Standard stock solutions of the 20 drugs were prepared with methanol respectively (100 μg mL^{-1}), and these solutions were stable for four months at −20 °C. The working solutions of these drugs were diluted from the stock solutions with water (1–1000 ng mL^{-1}), and these solutions were stable for one week at 4 °C.

2.2. UPLC conditions

UPLC system consisted of a ACQUITY H-CLASS liquid chromatography, a PDA detector and a BEH C_{18} column (2.1 × 50 mm, 1.7 μm) (Waters, USA). The mobile phase consisted of (A) 0.2% formic acid and (B) acetonitrile/methanol (1:1, *v/v*) with gradient elution at a flow rate of 0.4 mL min^{-1}. The gradient elution program was: started with 10% (A), linearly increased to 12% (A) in 7.9 min, decreased to 1% (A) in 0.6 min, then increased to 12% (A) in 0.1 min, linearly increased to 65% (A) in 2.4 min, decreased to 10% (A) in 0.1 min, linearly increased to 50% (A) in 0.9 min, finally brought back to 10% (A) in 1.0 min and maintained for 1.0 min with a total running time of 14 min. The injection volume was 10 μL, and the detection wavelengths were 270, 279 and 289 nm.

2.3. Synthesis of MMIP particles

The MMIP was synthesized according to our recent reports [22,23]. Briefly, the mixture of three templates (PA, SB and TC, 1 mmol of each template), the functional monomer MA (18 mmol) and the porogen chloroform (18 mL) were added into a glass bottle to be sonicated for 20 min, and kept at 4 °C overnight. Then, the cross-linker DGDMA (40 mmol) and the initiator AIBN (40 mg) were added. The bottle was filled with nitrogen for 10 min and sealed to be shaken in a 60 °C water bath for 24 h. The obtained MMIP particles were extracted on a Soxhlet apparatus for 72 h successively by using methanol/acetic acid (9:1, *v/v*). Finally, the MMIP particles were dried at 110 °C for 2 h for the subsequent use. For comparison, the controlled non-imprinted polymer (NIP) was also synthesized as described above but without the three templates. The two types of polymers were all scanned by using scanning electron microscopy (SEM) (JSM-7500F, JEOL, Japan).

Furthermore, the MMIP specificity for the 20 drugs was determined according to a previous report [24]. Briefly, 20 mg MMIP or NIP was added into 5 mL mixed standard solution (100 ng of each drug) to be stirred for 5 min. Then the supernatant was analyzed by UPLC. The partition coefficient (K) was calculated as: $K = C_A/C_S$ (C_A is the drug amount absorbed by MMIP or NIP, and C_S is the drug amount in supernatant). The specificity was evaluated based on imprinting factor (IF): $IF = K_{MMIP}/K_{NIP}$ (K_{MMIP} and K_{NIP} represent the K value of each drug from MMIP and NIP respectively).

2.4. Sample preparation with MMIP-MSPD

An amount of 0.2 g homogenous pork sample was added into a mortar. At this stage, the mixed standards were fortified at different concentrations, and the mixture was ground with a pestle to obtain a homogenous sample. Then, 0.15 g MMIP was added, and the mixture was gently ground until a homogenous mixture was obtained (about 3 min). At the same time, an empty cartridge (100 mm × 9.9 mm, i.d.) with a lower frit was packed with 50 mg MMIP that was conditioned by washing with 1 mL methanol and 1 mL water in turn. Then the homogenous mixture was transferred into the cartridge, and compressed with a syringe plunger to create a compact column bed. The cartridge was washed with 3 mL methanol/water (2:8, v/v), and the solutions passed through the cartridge at a flow rate of 1 mL min^{-1} under the assistance of vacuum pump (about 3 min). Then, the analytes were eluted with 4 mL methanol/acetic acid (9:1, v/v) under the assistance of vacuum pump (about 4 min), and the eluate was evaporated to dry under gentle nitrogen. Finally, the dry residue was reconstituted in 200 μL water and filtered with a 0.22 μm membrane for UPLC analysis. During the experiments, the MMIP amount, washing solvent and eluting solvent were optimized respectively.

2.5. Method validation

Some blank pork samples obtained from the controlled slaughterhouses were used to evaluate the method. The limits of detection (LODs) and the limits of quantification (LOQs) for the 20 analytes were calculated as the concentrations corresponding to peak/noise ratio (S/N) of 3:1 and 10:1 respectively. Then the 20 drugs were fortified into the blank pork samples respectively at levels of 20–1000 ng g^{-1} for analysis. The intra-day recoveries (six repetitions for each fortification level in a single day) and the inter-day recoveries (duplicate injections for each fortification level on six successive days) were determined respectively. Finally, 70 pork samples purchased from several supermarkets in China were analyzed by the developed method.