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Dual-dummy-template molecularly imprinted polymer combining ultra performance liquid chromatography for determination of fluoroquinolones and sulfonamides in pork and chicken muscle

Yi Ping Song [1], Lei Zhang [1], Geng Nan Wang, Ju Xiang Liu, Jing Liu, Jian Ping Wang*

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

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ABSTRACT

In this study, a dual-dummy-template molecularly imprinted polymer capable of simultaneous recognizing 8 fluoroquinolones and 8 sulfonamides was synthesized. Its recognition performance was investigated by comparing the 3D conformations of four dummy templates and the two classes of drugs based on computational simulation. Then a solid phase extraction column was prepared and optimized that was combined with ultra performance liquid chromatography for determination of the 16 drugs in pork and chicken. The column could be reused for at least eighty times, and it showed high absorbency capacities (34.9–74.2 μg) and high recoveries (92%–99%) to these drugs. The limits of detection of this method for the two classes of drugs in meat were in the range of 1.0–3.4 ng/g, and the recoveries from the standards fortified blank samples were in the range of 86.1%–109.4%. Therefore, this method could be used as a specific, sensitive and accurate method for determination of fluoroquinolones and sulfonamides in meat.

1. Introduction

Now days, the residues of veterinary drugs in foods of animal origin have drawn the attentions of all over the world, because the residual veterinary drugs may cause allergic reactions to the consumers and induce the generation of drug resistant pathogens (Horii, Monji, Uemura, & N agura, 2006; Blasco, Torres, & Picó, 2007). For protection of consumer health, China and the European Union have established the maximum residue limits (MRLs) for different veterinary drugs in various sample species, i.e. fluoroquinolones (FQs), 10–200 ng/g in meat; sulfonamides (SAs), 100 ng/g in milk (Franek, Diblikova, Cernoch, Vass, & Hruska, 2006; Ministry of Agriculture of China, 2002). Therefore, it is urgent to monitor the residues of these veterinary drugs in foods of animal origin.

Up to now, there have been many analytical methods reported to determine the residues of FQs and SAs in food samples (Tang, Yang, Tan, & Luo, 2009; Zhao et al., 2009; Hou et al., 2014; Meng et al., 2015; Zhang, Li et al., 2016; Yin et al., 2016; Zhang, Liu et al., 2016). In these methods, the first step is to extract and purify the low level of analytes in the samples, and the most commonly method is solid phase extraction technique (SPE). Therefore, many commercial SPE products containing different absorbents are available (e.g. HLB, MCX, C_8, C_{18}, Al_2O_3 and silica). However, the commercial SPE columns are disposable products, and their absorbents are easily interfered by the sample impurities. Furthermore, these conventional absorbents may lead to competitive adsorption when one sample simultaneously contains different classes of analytes. Therefore, it is desirable to find a durable, recyclable and specific absorbent.

In recent years, molecularly imprinted polymer (MIP) as a novel absorbent has drawn the attentions of many researchers, because this material can circumvent the drawbacks of traditional SPE absorbents mentioned above. MIP is prepared by cross-linking different functional monomers to form a polymer with a specific molecule as the template. After the template molecules are removed, the left cavums in the polymer can specifically capture the template molecule and its structurally related molecules. Furthermore, MIP can be reused for dozens of times. Therefore, MIP based extraction and purification methods have been widely used for determination of FQs (Mirzajani & Kardani, 2016; Urraca,

* Corresponding author.
E-mail address: chinawangjp@hotmail.com (J.P. Wang).
[1] The two authors contributed equally to this study.

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Castellari, Barrios, & Moreno-Bondi, 2014; Wang, Yang, Liu, Feng, & Wang, 2016; Yang, Wang, Liu, Liu, & Wang, 2017; Yang et al., 2014; Zheng, Gong, Zhao, & Feng, 2010), SAs (Kong, Gao, He, Chen, & Zhang, 2012; Shi et al., 2011; Xu, Song, Hu, & Li, 2011; Zhao et al., 2014), tetracyclines (Feng, Wang, Yang, Liu, & Wang, 2016), penicillins (Zhang et al., 2010), and aminoglycosides (Ji et al., 2013) in various samples. However, these MIP based methods have different advantages. Firstly, these MIPs are only able to purify one analyte or a group of structurally similar analytes. Secondly, the template molecules in these MIPs may be leaked during SPE procedure (template bleeding) that can interfere with the detection result for a real sample. Thirdly, the MIP-analyte interactions and the MIP recognition differences for the respective analytes are not discussed in these reports.

In the past few years, several novel methods have been used to resolve these problems. In several previous articles, the dual-template and the multi-template MIPs were synthesized, and the recognition spectra of the prepared MIPs were broadened (Dai et al., 2012; Duan, Dai, Zhang, & Chen, 2013; Jing et al., 2010). For avoiding the template bleeding problem, some researchers employed the dummy templates to synthesize MIPs for FQs (Sun, Wang, Li, Yang et al, 2014), bisphenol A (Sun, Wang, Li, Jin et al, 2014), salicylic acid (You, Peng, Zhang, Guo, & Shi, 2014), phthalate esters (Hu et al., 2014), phenothiazines (Song et al., 2017) and capsaicin and dihydrocapsaicin (Ma et al., 2015). Results showed that the dummy-MIPs showed excellent and specific recognition abilities for the target analytes. For the MIP-analyte interaction, computational simulation was proved to be a useful method. In several previous reports, the authors used different computational software to construct the 3D structures of the templates and analytes, calculate the MIP-analyte binding energies, and compare the volumes of the templates and analytes (Feás et al., 2009; Han et al., 2012; Li et al., 2009; Liu, Wang, Tan, & Lei, 2007; Song et al., 2017; Sun, Wang, Li, Jin et al, 2014). Results showed that the MIP-analyte interaction energy and/or the shape + size of the cavities in MIPs were responsible for the MIPs' selectivity and affinity.

To the best of knowledge of the authors, there has been no article reporting the use of MIP based extraction method for simultaneous determination of FQs and SAs so far. In the present study, two dummy templates for FQs (pipemidic acid and nalidixic acid) and two dummy templates for SAs (sulfanilamide and sulfabenzamide) were used to synthesize four MIPs, and their recognition differences for the two classes of drugs were studied respectively by using computational simulation. Then, a dual-dummy-template MIP (DDMIP) was synthesized, and a solid phase extraction column was prepared for extraction of the two classes of drugs in meat 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). Nalidixic acid (NA) was purchased from Beijing Solarbio Company (Beijing, China). Sulfanilamide (SA) was purchased from Tianjin Fuchen Chemical Company (Tianjin, China). 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) and sulfachloropyridazine sodium (SCP) were obtained 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 these drugs were prepared with methanol respectively (100 μg/mL), and their working solutions were diluted from the stock solutions with water (1–1000 ng/mL).

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) water containing 0.2% formic acid and (B) acetonitrile/methanol (1:1, *v/v*) with binary gradient elution at a flow rate of 0.4 mL/min. The gradient elution program was: started with 10% (A), linearly decreased to 8.5% (A) in 2.0 min, increased to 14% (A) in 0.1 min and maintained for 1.5 min, then decreased to 0% (A) in 0.1 min and maintained for 1.5 min, then linearly increased to 74% (A) in 1.3 min, further increased to 87% (A) in 0.5 min, finally brought back to 10% (A) in 1.0 min with a total running time of 8 min. The injection volume was 10 μL, and the detection wavelength was 289 nm.

2.3. Synthesis of MIPs

During the experiments, the four dummy templates (PA and NA for FQs, SA and SB for SAs) were used to synthesize four single template MIPs respectively according to our recent reports (Feng et al., 2016; Wang et al., 2016). Briefly, 1.0 mmol the template, 6.0 mmol functional monomer MA and 6 mL of porogen chloroform were added into a capped glass bottle. The bottle was shaken for 10 min, sonicated for 10 min, and kept at 4 °C for 4 h. Then, 20 mmol cross-linker DGDMA and 40 mg initiator AIBN were added into the above bottle. The bottle was deoxygenated with nitrogen for 10 min and sealed to be shaken in a 60 °C water bath for 24 h. The bulk polymer was ground and sieved to obtain the MIP particles. The MIP particles were extracted with methanol/acetic acid (9/1, *v/v*) on a Soxhlet apparatus for 48 h to remove the templates. Finally, the MIP particles were dried at 110 °C for 2 h for the subsequent use. For comparison, the non-imprinted polymer (NIP) was synthesized as the procedures described above but without the template. The recognition performances of the four single-template MIPs for the respective class of drugs were evaluated. Then, the two optimal dummy templates were used to synthesize the DDMIP (1 mmol of each template) as the procedures described above. The DDMIP and NIP were characterized respectively with scanning electron microscopy (SEM) (JSM-7500F, JEOL, Japan) and infrared spectrum (IR) (FTIR-8400S Spectrometer, Shimadzu, Japan).

During the experiments, the specificities of the five MIPs for the 8 FQs and the 8 SAs were determined according to a previous report (Kong et al., 2012). Briefly, 10 mg MIP particle or NIP particle was put into 5 mL of mixed standard solution (containing 1.0 μg of each drug) to be stirred for 10 min. Then, the supernatant and the analytes eluted from the particles were analyzed by UPLC. The partition coefficient (K) was calculated as: $K = C_B/C_S$ (C_B is the drug amount absorbed by MIP or NIP, and C_S is the drug amount in the supernatant). The specificity for each drug was evaluated based on imprinting factor (IF): $IF = K_{MIP}/K_{NIP}$ (K_{MIP} and K_{NIP} represent the partition coefficient of each drug from MIP and NIP respectively).