



Molecularly imprinted polymer-high performance liquid chromatography for the determination of tetracycline drugs in animal derived foods



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ARTICLE INFO

Article history:

Received 3 January 2016
Received in revised form
26 April 2016
Accepted 29 April 2016
Available online 30 April 2016

Keywords:

Molecularly imprinted polymer
Solid phased extraction
High performance liquid chromatography
Tetracycline drugs

ABSTRACT

In this study, a molecularly imprinted polymer solid phase extraction method combining high performance liquid chromatography was developed to determine the residues of tetracycline drugs in animal derived foods. The polymer was synthesized with chlortetracycline as template molecule, methacrylic acid as functional monomer at template/monomer ratio of 1:4. The prepared solid phase extraction column was able to capture four tetracyclines simultaneously with high adsorption capacities (3560–4700 ng) and high recoveries (>87%), and was able to be reused for at least thirty times. The limits of detection were in a range of 20–40 ng/g, and the recoveries from fortified blank samples (milk, egg and pork) were in a range of 74%–93%. Furthermore, the polymer-based column achieved better purification effect than three commercial solid phase extraction columns. This is the first study reporting the use of chlortetracycline based molecularly imprinted polymer for determination of tetracyclines in animal derived foods.

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1. Introduction

Tetracycline drugs (TCs), such as tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and doxycycline (DC), are a class of broad-spectrum antibacterial drugs that have been widely used to treat various bacterial-inducing diseases in animals. However, TCs residues in foods of animal origin become a series problem nowadays, because their residues may pose many health threats to the consumers, e.g. allergic reactions, antimicrobial resistance and gastrointestinal disturbance and tetracycline pigmentation teeth (Jeon, Kim, Paeng, Park, & Paeng, 2007; Michalova, Novotna, & Schlegelova, 2004; Roesch et al., 2006). For protection of consumer health, China, the European Union and the United States have established maximum residue limits for single or total amount of TCs in foods of animal origin, e.g. 100 ng/g (ng/mL) in milk and meat. Therefore, it is urgent to monitor TCs residues in foods of animal origin.

By now, many methods have been reported to determine TCs residues in various samples. Among the reported methods, high performance liquid chromatography (HPLC) (Cinquina, Longo,

Anastasi, Giannetti, & Cozzani, 2003; Furusawa, 2003; Nikolaidou, Samanidou, & Papadoyannis, 2008; Samanidou, Nikolaidou, & Papadoyannis, 2007; Spisso, de Oliveira e Jesus, Gonçalves de Araújo Júnior, & Monteiro, 2007; Zhao, Zhang, & Gan, 2004) and liquid chromatography mass spectrometry (Al-Mazeedi et al., 2010; Capriotti, Cavaliere, Piovesana, Samperi, & Laganà, 2012; Cherlet, Schelkens, Croubels, & De Backer, 2003; Gaugain-Juhel et al., 2009) were usually used as confirmatory methods. In these methods, the first step was to extract and purify low level of TCs from different samples, and the most commonly used extraction and purification methods were liquid–liquid extraction (LLE) and solid phase extraction (SPE). However, these two methods all have their respective disadvantages. LLE requires large volume of organic solvents that are unfriendly to the environment, and its procedure is tedious and time consuming. SPE is the mainstream purification technique due to its simplicity, speed, less consumption of organic solvent, and availability of a wide range of sorbents types. However, SPE sorbents are prone to interferences by impurities in the biological samples, and cartridges are for one-time use only. Therefore, it is desirable to develop simple, rapid, environment-friendly methods using reusable cartridges.

In recent years, a novel purification sorbent named molecularly imprinted polymer (MIP) has drawn the attentions of many

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researchers, because MIP overcomes the above-mentioned drawbacks of conventional SPE sorbents. MIP is synthesized by cross-linking the functional monomers with a specific molecule as template. After the template molecules are removed, the countless cavities remaining in the polymer can specifically capture the template molecule and its structurally related molecules. Furthermore, MIP is stable under different harsh conditions (extreme pH, high pressure and high temperature), and can be reused for dozens of times. By now, MIP based purification methods have been widely used to determine the residues of fluoroquinolones (Urraca, Castellari, Barrios, & Moreno-Bondi, 2014), sulfonamides (Zhao et al., 2014), erythromycin (Zhang et al., 2011), β -lactam antibiotics (Zhang et al., 2010), aminoglycosides (Ji et al., 2013), pharmaceuticals (Duan, Dai, Zhang, & Chen, 2013), estrogens (Lin et al., 2013), rhodamine B (Su et al., 2015) and DDT (Graham, Carlson, & Edmiston, 2002) in various samples. Therefore, MIP is a promising sorbent in the field of residual analysis.

In the past few years, several articles have been reported to prepare MIPs for TCs (Dai et al., 2012; Hou et al., 2016; Jing et al., 2009, 2010; Lian et al., 2012; Lv, Wang, Yang, Zhao, & Sun, 2012; Sánchez-Polo, Velo-Gala, López-Penlver, & Rivera-Utrilla, 2015; Wang et al., 2011), and the typical template molecule was TC. Among the TC-based MIPs, only one MIP was able to recognize four TCs in animal derived foods (Jing et al., 2009), whereas other TC-based MIPs were only able to recognize TC (Dai et al., 2012; Hou et al., 2016; Sánchez-Polo et al., 2015; Wang et al., 2011). Lv et al. synthesized MIP with OTC as template, but it was only able to recognize OTC (Lv et al., 2012). Lian et al. synthesized CTC specific MIP that was used to prepare an electrochemical sensor for determination of CTC in foods (Lian et al., 2012). Besides these single template MIPs, Jing et al. used a mixture of OTC and CTC as templates, and the resulting MIP was able to recognize TC, OTC, CTC and DC simultaneously (Jing et al., 2010). The details of these MIPs for TCs are shown in Table 1.

As mentioned above, there has been only one article so far on a single-template MIP that can enrich four TCs residues (Jing et al., 2009). In the present study, a template candidate was selected among DC, OTC and CTC, and then a MIP-SPE procedure was developed to extract and purify TCs residues in foods of animal origin followed by determination with HPLC method.

2. Materials and methods

2.1. Reagents and chemicals

The standards of TC, OTC, CTC and DC were purchased from

Sigma (St. Louis, MO, USA). Ethylene glycol dimethacrylate (EGDMA), 2-vinyl pyridine (2-VP) and 4-vinyl pyridine (4-VP) were purchased from Aladdin Industrial Corporation (Shanghai, China). Methacrylic acid (MA) and 2,2-azobis (isobutyronitrile) (AIBN) were purchased from Kermer Chemical Company (Tianjin, China). All other chemical reagents were of analytical or better grade from Beijing Chemical Company (Beijing, China). Liquid chromatographic grade acetonitrile was purchased from Dikma (Richmond Hill, USA). Standard stock solutions of the four TCs were prepared in methanol (100 $\mu\text{g}/\text{mL}$), and their working solutions with series concentrations (50–1000 ng/mL) were diluted from the stock solutions with 0.01 mol/L trifluoroacetic acid (pH 3.0). All these standard solutions were stored at 4 °C to be stable for 4 weeks.

2.2. HPLC conditions

The HPLC system consisted of a Waters 1525 liquid chromatography (Waters, Milford, MA, USA), a Waters 2998 DAD detector and a C_{18} column (150 \times 4.6 mm, 5 μm). The mobile phase consisted of (A) acetonitrile and (B) 0.01 mol/L trifluoroacetic acid (pH 3.0) with binary gradient elution at a flow rate of 1.0 mL/min. The gradient elution started with 10% (A), linearly increased to 20% (A) in 3.0 min and further linearly increased to 30% (A) in 11.0 min, then linearly decreased to 20% (A) in 2.0 min, brought back to 10% (A) in 2.0 min and maintained for 2.0 min with a total running time of 20 min. The injection volume was 20 μL and the detection wavelength was 350 nm. Identification was based on retention time; and quantification was based on peak area.

2.3. Synthesis of MIPs

In the present study, three templates (DC, OTC and CTC), three functional monomers (MA, 2-VP and 4-VP), and different molar ratios of template/monomer (1:4–1:10, mmol ratio) were used to optimize the synthesis conditions. The MIP was synthesized by the following precipitation polymerization method. Briefly, a template molecule, a functional monomer and 7 mL chloroform were added into a screw-capped glass bottle. The mixture was shaken for 1 min, sonicated for 15 min, and kept at 4 °C overnight to achieve pre-polymerization. Then, a cross-linker (EGDMA, 0.3 mmol) and an initiator (AIBN, 40 mg) were added. The bottle was deoxygenated with nitrogen for 10 min and sealed under the protection of nitrogen to be shaken in a 60 °C water bath for 24 h to accomplish polymerization. The obtained bulk polymer was crushed, ground and sieved to obtain MIP particles in 32–60 μm size range. The template molecules were removed with methanol/acetic acid (8/2,

Table 1
Details of the MIPs for TCs in previous reports and this study.

Reference	Template	Monomer	Method	Recognition ability	Application
Jing et al., 2009	TC	MA	Precipitation polymerization	TC, CTC, OTC, DC	MIP-SPE of four TCs in egg, duck, honey and lobster
Wang et al., 2011	TC	MA	Precipitation polymerization	TC	Employed in electrochemical sensor for detection of TC in egg and fish
Dai et al., 2012	TC	MA	Atom transfer radical emulsion polymerization	TC	Nano magnetic MIP-SPE of TC in pork
Sánchez-Polo et al., 2015	TC	MA	Precipitation polymerization	TC	As MIP particles to remove TC in water
Hou et al., 2016	TC	Carbon quantum dots	Microwave assisted polymerization	TC	Employed in fluorescence spectroscopy for detection of TC in milk
Lv et al., 2012	OTC	MA and KH570	Precipitation polymerization	OTC, DC, metacycline	MIP-SPE of three TCs in milk
Lian et al., 2012	CTC	Chitosan derivative	Sonication assisted	CTC	Employed in electrochemical sensor for detection of CTC in milk and honey
Jing et al., 2010	OTC/CTC	MA	Precipitation polymerization	TC, CTC, OTC, DC	MIP-SPE of four TCs in milk, honey and lobster
This study	CTC	MA	Precipitation polymerization	TC, CTC, OTC, DC	MIP-SPE of four TCs in milk, egg and pork

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