Journal of Chromatography A, 1217 (2010) 3612–3618

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Investigation of ractopamine molecularly imprinted stir bar sorptive extraction and its application for trace analysis of β_2-agonists in complex samples

Zhigang Xu, Yufei Hu, Yuling Hu, Gongke Li*

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, China

ARTICLE INFO

Article history:
Received 5 January 2010
Received in revised form 21 March 2010
Accepted 24 March 2010
Available online 30 March 2010

Keywords:
Stir bar sorptive extraction
Molecularly imprinted polymer
Ractopamine
β_2-Agonists
Complex sample

ABSTRACT

In this paper, a novel molecularly imprinted polymer (MIP) coated stir bar with ractopamine as template by glass capillary filling with magnetic core as substrate was prepared reproducibly. The ractopamine MIP coating was homogeneous and porous with the average thickness of 20.6 μm. The extraction apparatus for the stir bar was improved to avoid coating loss. The MIP-coated stir bar showed better extraction capacity and good selectivity than that of non-imprinted polymer (NIP) coated stir bar to ractopamine and its analogues. The extraction capacities of ractopamine, isoxsuprine, clenbuterol and fenoterol for MIP-coated stir bar were 3.3, 3.1, 2.8 and 2.4 times as much as that of the NIP coated stir bar, respectively. The MIP-coated stir bars could be used at least 40 times without apparent damage and kept in dried air for 8 months without reduce of extraction ability. A method for the determination of β_2-agonists in complex samples by MIP-coated stir bar sorptive extraction coupled with high-performance liquid chromatography (HPLC) was developed. The linear ranges were 0.5–40 μg/L for ractopamine and 1.0–40 μg/L for isoxsuprine and clenbuterol. The detection limits were within the range of 0.10–0.21 μg/L. The method was successfully applied to the analysis of β_2-agonists in spiked pork, liver and feed samples with the recoveries of 83.7–92.3%, 80.5–90.2% and 73.6–86.2%, respectively. The RSDs was within 2.9–8.1%. The method is very suitable for the determination of trace β_2-agonists in pork, liver and feed samples.

1. Introduction

Stir bar sorptive extraction (SBSE) was first proposed in 1999 derived from solid-phase microextraction (SPME) [1]. It possesses a better extraction yield for its larger volume and surface area compared with SPME. At the same time, it can also avoid competitive sorption from additional stirrer. Thus, SBSE technique has been widely applied to environmental and biomedical analysis [2]. However, there is only one kind of commercial stir bar (Twister, Gerstel GmbH) with the polydimethylsiloxane (PDMS) tubing, which is a non-polar phase and shows better affinities to non-polar and weakly polar compounds. So, some polar coatings were developed. Liu et al. [3,4] prepared a thermal stable PDMS coated stir bar coupled with GC by sol–gel method. The composite coatings of PDMS/β-cyclodextrin [5] and carbowax–polydimethylsiloxane–poly(vinyl alcohol) (CW/PDMS/PVA) [6] were also prepared by the same method. Bicchi et al. [7] proposed a dual-phase stir bar coating consisted of PDMS and activated carbons. Huang et al. [8–10] reported a series of monolithic materials. Heavy metal ions were enriched by SBSE firstly [10]. Other stir bar coatings, such as restricted access material [11] and polymeric phases [12,13], were also developed. All these novel stir bars have extended the application of SBSE, but the coatings are still non-selective sorbents.

Molecularly imprinted polymer (MIP) with high selectivity has been widely used in solid-phase extraction [14], stationary phase of chromatography [15], chemical sensors [16], and membrane separation [17]. In recent years, molecularly imprinted polymer coated SPME fibers for the extraction of polar analytes in complex samples were developed [18–24]. But few literatures about SBSE with MIP coatings have been reported. Zhu et al. prepared monocrotophos [25] and L-glutamine [26] MIP-coated stir bars based on commercial PDMS stir bars by a phase inversion method.

Ractopamine, one kind of β_2-agonists, is prohibited to use as growth promoter in the European Union (EU) and China. The selective sample preparation for β_2-agonists analysis in complex samples is necessary. Wang et al. [27] reported ractopamine molecularly imprinted polymer used as solid-phase extraction sorbent for the determination of trace ractopamine in pork. In this study, ractopamine molecularly imprinted stir bar sorptive extraction was firstly prepared. The extraction apparatus of stir bar was improved to avoid coating loss. The extraction capability and selectivity of the MIP-coated stir bar were also investigated under the opti-

* Corresponding author at: Sun Yat-Sen University, Institute of Analytical Sciences, School of Chemistry and Chemical Engineering, Xingang West Road 135, Guangzhou, Guangdong 510275, China. Tel.: +86 20 84110922; fax: +86 20 84115107.
E-mail address: cesgkl@mail.sysu.edu.cn (G. Li).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.03.046

mized conditions. A method for the determination of β_2-agonists by MIP-coated stir bar sorptive extraction coupled with HPLC was developed and applied to the analysis of ractopamine, isoxsuprine and clenbuterol in pork, liver and feed samples, respectively.

2. Experimental

2.1. Chemicals and materials

Ractopamine (RCT), isoxsuprine (ISOX) and clenbuterol (CLEN) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fenoterol (FENO) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile (HPLC grade) was obtained from LAB-SCAN (Bangkok, Thailand). Methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were purchased from Damao Regent Plant (Tianjin, China). Acrylamine (AM) was purchased from Sigma–Aldrich. Ethylene glycol dimethacrylate (EGDMA) was purchased from Corel Chemical Plant (Shanghai, China). 3-(Methacryloxy) propyltrimethoxysilane was purchased from Shengda Fine Chemical Industry Corporation (Beijing, China). Glass capillary was obtained from West China University of Medical Sciences Instrument Plant. Water solutions used for HPLC was doubly distilled and filtered through a 0.45 μm nylon filter. Other chemicals were of analytical grade.

2.2. Instrumentation

HGC-12 nitrogen Evaporator (Quandao, Shanghai, China) was used for the polyreaction. An S-4300 scanning electron microscope (HITACHI, Japan) was used to investigate the coating surface of the MIP-coated stir bar. A NICOLET AVATAR 330 Fourier transform infrared (FT-IR) spectrometer was applied to investigate the composition of the coating. A thermal gravity (TG) analyzer (Netzsch-209, Bavaria, Germany) was applied to evaluate the thermal stability of the coating. An LC-2010AT$_{VP}$ HPLC system (Shimadzu, Japan) performed with RF-10A$_{XL}$ fluorescence detector (FL) was used to analyze ractopamine, isoxsuprine and fenoterol. An LC-10AT$_{VP}$ HPLC system (Shimadzu, Japan) performed with SPD-10A$_{VP}$ ultraviolet detector (UV) was utilized to analyze clenbuterol, benzyl alcohol and para-methylphenol.

2.3. Preparation of molecularly imprinted polymer coated stir bar

The glass capillary substrate should be silanized before the polymerization. It was immerged in 1 mol/L sodium hydroxide for 8 h at room temperature. Subsequently, the glass capillary was washed with water and soaked in 1 mol/L hydrochloric acid for 1 h. It was washed with water again and dried in an oven at 150 °C for 1 h. Then the glass capillary was silylated for 3 h in a 25% (v/v) 3-(methacryloxy) propyltrimethoxysilane solution in acetone at room temperature. The glass capillary was finally washed with methanol and dried with a stream of nitrogen.

Pre-polymer solution for molecularly imprinted polymer was prepared with 405.0 mg ractopamine and 0.41 mL MAA dissolved in 20 mL methanol. The solution was mixed thoroughly and kept for 12 h at room temperature. Then 4.59 mL EGDMA and 45.9 mg AIBN were added, degassed in an ultrasonic bath for 5 min. 1.5 mL of the degassed solution was transferred into a test-tube, deoxygenized with a nitrogen stream for 5 min. Subsequently, the silylated glass capillary was inserted in and the polymerization was performed at 60 °C. When the polymer solutions became solid polymers after 2.5 h, the glass capillary was pulling out. The polymer coatings were still heated for 24 h under nitrogen atmosphere. A suitable thickness was obtained by repeating the coating procedure. A NIP-coated stir bar was prepared by the same way but without template in the synthesis. New coated stir bars were eluted by 10% (v/v) acetic acid solution to remove ractopamine template until it could not be detected by HPLC.

2.4. HPLC conditions

All separations were performed on a DIKMA-C$_{18}$ column (250 mm × 4.6 mm i.d., 5 μm packing, Diamonsil). The mobile phase was composed by acetonitrile (A) and 0.1% (v/v) phosphoric acid buffer (B), and the flow rate was 1.0 mL/min. Ractopamine, isoxsuprine and fenoterol were detected by fluorescence detector with an excitation wavelength at 226 nm and an emission wavelength at 305 nm. Elution was carried out with a gradient of A and B. The content of A was increased linearly from 10% to 48% (v/v) during 8 min, and held for 9 min, then adjusted back to 10% (v/v). Isocratic elution was carried out for ultraviolet detection. The mobile phase consisted of a mixture 16.5% (v/v) A for clenbuterol and atenolol detection at 243 and 220 nm, respectively. And the mobile phase consisted of a mixture 45% (v/v) A for benzyl alcohol and para-methylphenol detection at 257 and 278 nm, respectively.

2.5. Sample preparation

Pork, liver and feed were selected for the spiked samples. Pork and liver were shredded and the feed sample was sieved with a 20-mesh gauge. Two levels of 5.0 and 10 μg/kg for each β_2-agonists were prepared in the spiked samples. 5 g sample was set into a flask and mixed with β_2-agonists mixed standard solutions. It was mixed thoroughly and kept for 0.5 h. It was applied to investigate the specific selectivity of MIP coatings in complex matrix. The spiked sample was not made by slurry and subsequent aging process so it might be different from the real samples. The extraction was performed in 20-mL acetonitrile with a MAS-I microwave oven (Sineo, Beijing, China) at 60 °C for 10 min. The extraction was repeated and the extraction solutions were dried with the reduced pressure distillation, and then it was dissolved with 10 mL 0.1 mol/L hydrochloric acid. 10 mL petroleum ether was added and the water solution was collected after 10 min. The pH value of the solution was adjusted to 10 by 1 mol/L sodium hydroxide. Then 20 mL chloroform was added to extract β_2-agonists for 10 min. The organic phase was collected and dried with the reduced pressure distillation, then dissolved with 5 mL toluene for stir bar sorptive extraction. The concentrations of each β_2-agonists for three kinds of spiked sample solutions were 5.0 and 10 μg/L, respectively.

2.6. SBSE procedure

The stirring speed of stir bar sorptive extraction was 500 rpm at room temperature. The volume of sample was 5 mL. After extraction and the solvent evaporation, the stir bar was inserted in a 200 μL glass vial with a conical insert. Then 150 μL 10% (v/v) acetic acid/water solution was added and it was treated with ultrasonic bath for 10 min. Subsequently, the stir bar was taken out by a magnet and 20 μL desorption liquid was injected into HPLC for analysis.

3. Results and discussion

3.1. Preparation of the MIP-coated stir bar

3.1.1. Stir bar sorptive extraction apparatus

The stir bar sorptive extraction was performed in a flat bottle [28]. The rubbing between the stir bar coating and the bottom was inevitable. Furthermore, the homemade molecularly imprinted polymer coating was very thin with the thickness about 20 μm. The frequent stirring with high speed could lead to the desquamation of the stir bar coating. In order to solve this problem, Richter et al. [29] developed a kind of rotating-disk sorptive extraction