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Magnetic molecularly imprinted polymer beads prepared by microwave heating for selective enrichment of β-agonists in pork and pig liver samples

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ABSTRACT

Novel magnetic molecularly imprinted polymer (MIP) beads using ractopamine as template for use in extraction was developed by microwave heating initiated suspension polymerization. Microwave heating, as an alternative heating source, significantly accelerate the polymerization process. By incorporating magnetic iron oxide, superparamagnetic composite MIP beads with average diameter of 80 μm were obtained. The imprinted beads were then characterized by scanning electron microscopy, Fourier transform infrared spectroscopy, thermogravimetric analysis and vibrating sample magnetometer. Highly cross-linked porous surface and good magnetic property were observed. The adsorption isotherm modeling was performed by fitting the data to Freundlich isotherm model. The binding sites measured were 3.24 μmol g^{-1} and 1.17 μmol g^{-1} for the magnetic MIP beads and the corresponding non-imprinted magnetic beads, respectively. Cross-selectivity experiments showed the recognition ability of the magnetic MIP beads to analytes is relative to degree of molecular analogy to the template. Finally, this magnetic MIP bead was successfully used for enrichment of ractopamine, isoxsuprine and fenoterol from ultrasonically extracted solution of pork and pig liver followed by high performance chromatography with fluorescence detection. The proposed method presented good linearity and the detection limits was 0.52–1.04 ng mL^{-1}.The recoveries were from 82.0% to 90.0% and from 80.4% to 86.8% for the spiked pork and pig liver, respectively, with the RSDs of 5.8–10.0%. Combination of the specific adsorption property of the MIP material and the magnetic separation provided a powerful analytical tool of simplicity, flexibility, and selectivity.

1. Introduction

Molecular imprinting technique was originally proposed as a possible mechanism for the production of antibodies by living systems [1,2]. Molecularly imprinted polymers (MIPs) have attracted much attention due to their outstanding advantages, such as predetermined recognition ability, high stability, relative ease and low cost of preparation, and potential application to a wide range of target molecules. There have been extensive efforts directed at the synthesis of MIP materials as well as their application to analytical chemistry [3–7]. Preparation of MIPs was reported with different morphologies, such as monoliths [8], granules, membranes [9] and microspheres [10]. When MIPs were used as HPLC stationary phases or solid-phase extraction (SPE) media, it is desirable to prepare the spherical and monodispersed beads.

Molecularly imprinted solid-phase extraction (MISPE) provides a way to selectively enrich analytes which are present in low concentrations or in complex matrix, and is investigated widely in environmental and biological samples [11]. The most common mode of MISPE is in the cartridge format, which would lead to a tedious packing procedure, high back pressure, multistep pretreatment of column and slow analytical speed. Matrix solid-phase dispersion extraction (MSPD) using MIP as adsorbent is an alternative method to simplify the sample preparation process mainly for solid samples [12]. However, the mixture of sample and sorbent is still required to be packed into a column for rinsing and elution. The additional disadvantage of MSPD is that the sorbent cannot be reused, which results in higher analytical cost. Another methodology to expand the application of MIP is dispersive solid-phase extraction (DSPE) [13]. Instead of the column-like format, the sorbent is directly added into the solution without conditioning. This protocol eliminates the column packing step. But in the DSPE scheme, the MIP beads should be separated from the sample solution by centrifugation and filtration, leading to other operation difficulty.

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Magnetic separation technology has received considerable attention in recent years for its application in biological fields, including bioseparation, drug delivery, enzyme immobilization and biomolecular sensing [14,15]. Recently magnetic polymer has also been synthesized and applied as the sorbent for sample preparation. This technology used in extraction provides a relatively rapid and convenient way for withdrawal of magnetic polymers from sample matrices by applying appropriate magnetic field without additional centrifugation or filtration. If the spherical MIPs were endowed with magnetic properties, the resulting composite polymer will not only have magnetically susceptible characteristic, but also have highly selective rebinding characteristics to the target molecule. The magnetic MIP beads have been prepared in some literature. Molecular recognition investigation using magnetic MIPs as carrier was performed both for small molecule and large molecule such as protein [16,17]. Core–shell structural magnetic MIPs with aspirin as template were prepared by Zhu et al. [18]. The resulting composite had high adsorption capacity and selectivity to aspirin, and showed potential applications in drug controlled release. Drug rebinding and release experiment using magnetic MIPs were also investigated for the β-blocker (*S*)-propranolol [19]. Ding et al. [20,21] and Zhang et al. [22] applied the magnetic MIPs to environmental and food samples for trace analysis of bisphenol A, tetracycline and fluoroquinolone antibiotic. Imparting magnetism into MIPs offers a new valid approach for improvement of the operation convenience without losing selectivity. On the other hand, polymerization of the magnetic MIP beads is usually induced by conventional heating or UV light. Nevertheless the polymerization process is time-consuming by these conventional methods. In our recent work, microwave heating technique was first introduced as an efficient heating method to initiate polymerization for preparation of magnetic MIP beads using atrazine as the template. It was proven that microwave heating can significantly shorten the polymerization time of the magnetic MIPs beads by suspension polymerization. The resultant mag-MIP beads exhibited good characteristics, such as narrow size distribution, uniform morphology, superior selectivity, and showed rather higher imprinting efficiency. It is concluded that microwave heating is a powerful technique to prepare magnetic MIP beads in this simple and efficient manner [23].

Ractopamine is a β-adrenergic leanness-enhancing agent which has a repartitioning effect [24]. Although it is approved as a feed additive for swine and cattle in the United States, ractopamine is banned for use in animal feeds by regulatory agencies in China or Europe because various food poisonings have been caused by ractopamine residues as alternative of clenbuterol. Therefore, monitoring programs mandated by various government agencies have necessitated the development of assay procedures for ractopamine. The reported methods for monitoring ractopamine included immunoassays [25], gas chromatography [26], high performance liquid chromatography (HPLC) with UV, electrochemical and fluorescence detection [27–29]. Among them, HPLC along with liquid–liquid extraction (LLE), SPE and liquid-phase microextraction were the most commonly applied. In view of the complexity of the animal tissue and trace contents of the ractopamine, development of sample preparation method with high selectivity is crucial. Applying MIPs as selective adsorbent for enrichment of ractopamine in complex animal tissue would provide solutions to these problems [30–32].

In the present work, magnetic MIP beads were prepared using ractopamine as template by microwave heating. The characteristics of the magnetic MIP beads and its binding properties were investigated. The magnetic MIP beads were used as sorbents for the extraction of β-agonists from pork and pig liver samples, followed by high performance liquid chromatography and fluorescence (HPLC-FL) analysis.

2. Experimental

2.1. Material

Ractopamine, isoxsuprine and fenoterol were supplied by Sigma. Stock solutions of these β-agonists (1.0 mg mL^{-1}) were prepared in methanol, and solutions of lower concentration were prepared by serial dilution of the stock solutions. Ferric chloride ($FeCl_3{\cdot}6H_2O$) and ferrous sulfate ($FeSO_4{\cdot}7H_2O$) were purchased from Shenyang Chemical Corporation (Shenyang, Ch.). Acrylamide (AM), trimethylolpropane trimethacrylate (TRIM) were purchased from Corel Chemical Plant (Shanghai, Ch.). Styrene (St) and azo-bis (iso-butyronitrile) (AIBN) were obtained from Tianjin Reagent Plant (Tianjin, Ch.). Divinylbenzene (DVB) was from Qunli Reagent Corporation (Shanghai, Ch.). Ethylene glycol (PEG 6000) was obtained from Xilong Chemical Plant (Shantou, Ch.). Acetonitrile used for eluent was of HPLC grade and purchased from Lab-Scan (Lab-Scan Asia Co. Ltd.). Dimethyl sulfoxide (DMSO) from Damao reagent plant (Tianjin, Ch.) was of analytical grade. Water was doubly distilled. All other reagents were of analytical grade. All solutions used for HPLC were filtered through a nylon 0.45 μm filter before use.

2.2. Preparation of the magnetic MIP beads

Firstly, magnetite particles were synthesized by co-precipitation with a mixture solution (100 mL) containing $FeCl_3$ (5.4 g) and $FeSO_4$ (2.78 g) in ammonia aqueous solution (28%, weight percent), which have been reported in the literature [23]. The resultant nano-structured magnetite was aged in MAS-I microwave synthesizer from Sineo Microwave Chemistry Technology Company (Shanghai, Ch.) at 80 °C for 1 h, collected by a magnet, and washed with 10% (v/v) acetic acid and distilled water. Then the magnetite (2.00 g) was modified by PEG-6000 (10.0 g) in 30 mL distilled water with ultrasonic for 30 min to change the surface characteristics.

Magnetic MIP beads were prepared under microwave irradiation according the procedure of our previous study but with some modification [23]. Ractopamine (0.30 g) and AM (0.28 g) were dissolved in DMSO (13.4 mL). The solution was sparged with oxygen-free nitrogen and then stored in dark for 12 h, allowing self-assembly of the template and the monomer. The above pre-polymer solutions were mixed with PEG-Fe_3O_4 dispersing solution, polymer monomer (St, 8.0 mL), cross-linker (TRIM, 1.5 mL) and initiator (AIBN, 0.100 g), then dispersed in 80 mL distilled water in a 300 mL single-necked flask by vigorous agitation (600 rpm), and bubbled with a nitrogen stream throughout the procedure. Polymerization was carried out in the microwave synthesizer for 1 h, with a programmed temperature control under nitrogen protection.

The template was removed by extensive washing with mixed solution of methanol: water: acetic acid (85:5:10, v:v:v) with ultrasonic agitation, each time with fresh solution after 45 min, until no ractopamine leakage was observed from the magnetic MIP beads. Non-imprinted polymer (NIP) magnetic beads were prepared in the same way but without the addition of ractopamine to the polymerization mixture.

2.3. Physical and morphology observation

The morphology of the ractopamine imprinted magnetic beads was observed by scanning electron micrography (SEM) with a Philips XL-30 scanning electron microscope from Philips (Eindhoven, Netherlands). The particle size distribution was tested by a Malvern Master Sizer 2000 particle size analyzer from Malvern (Malvern, Britain). The infrared absorption spectrum was obtained by a Shimadzu IR-prespige-21 FTIR spectrometer from Shimadzu

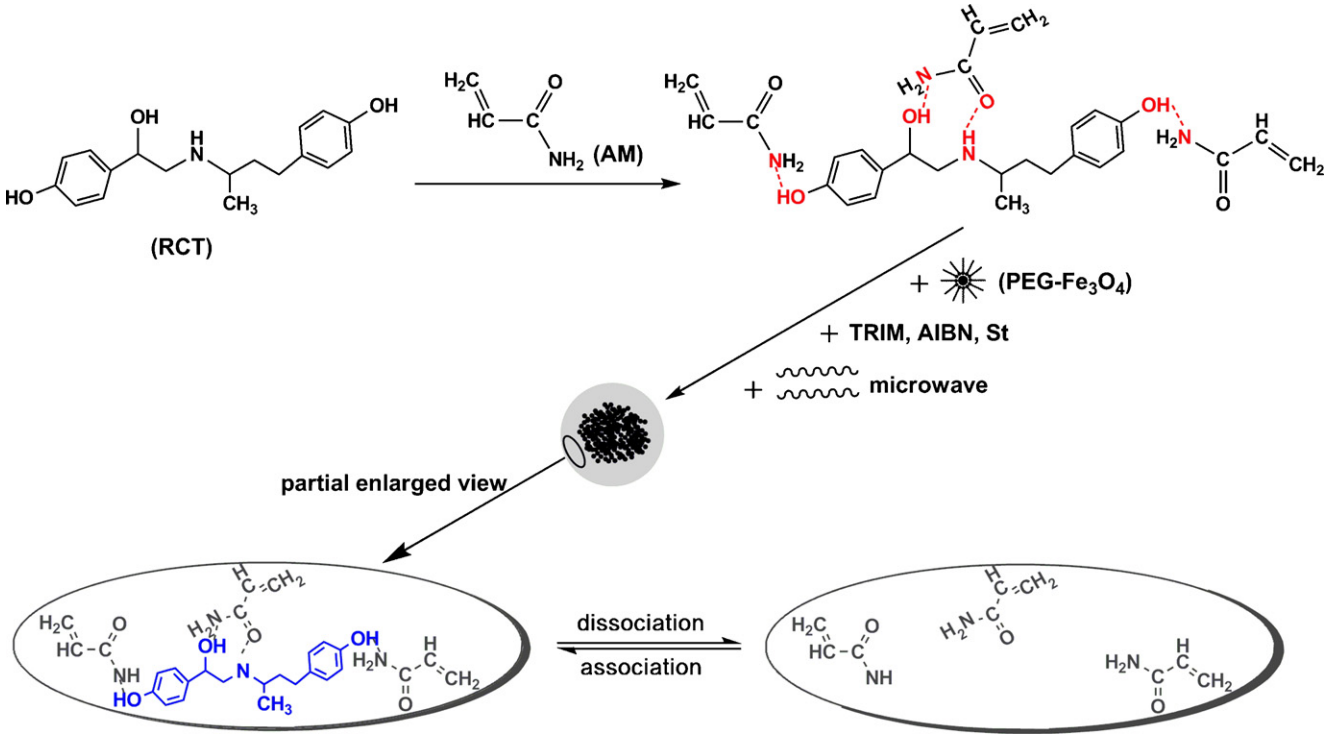

Fig. 1. Preparation scheme of the ractopamine imprinted magnetic beads.

(Tokyo, Japan). The magnetic properties of the resultant beads were measured using a SQUID-based magnetometer form Quantum Design (San Diego, USA). The thermogravimetric analysis (TGA) was performed in a Netzsch STA-409 PC thermogravimetric analyzer from Netzsch (Bavaria, Germany).

2.4. Adsorption and selectivity experiments

For adsorption experiments, 150 mg of magnetic MIP beads or magnetic NIP beads was incubated with ractopamine in toluene with various concentrations from 10 to 150 ng mL^{-1}. For cross-selectivity investigation, the same amounts of polymer beads were incubated with standard solution of ractopamine, isoxsuprine, fenoterol, sulfamerazinum and bisphenol A. After incubation on a rocking table for 2 h at room temperature to facilitate the adsorption of analytes onto the sorbent, the polymer beads were separated from the suspension by external magnetic field. The concentration of free substrate in the supernatant was measured by HPLC and the adsorption amounts were then calculated. The amount of binding amount (B) was calculated by subtracting the amounts of free analytes (F) at equilibrium from the initial solution. All tests were conducted in triplicate.

The binding isotherms were plotted in concentrations of log bound (B) versus log free (F) analyte and were fitted to the Freundlich model (Eq. (1)), where m and a are binding parameters. The parameter m is the heterogeneity index, which varies from zero to one. In addition, the number of binding sites (N) and association constant (K) can be estimated for the subset of binding sites that are accessed within the concentration limits of the binding isotherm. This range is set by the experimental binding isotherm ($F1$ to $F2$) as defined by Eq. (2). The weighted average affinity constant (K_{K1-K2}) and number of binding sites (N_{K1-K2}) for the polymers was calculated using Eqs. (3) and (4) [33].

$$\log B = m \log F + \log a \tag{1}$$

$$K_{\max} = \frac{1}{F_{\min}}, \quad K_{\min} = \frac{1}{F_{\max}} \tag{2}$$

$$N_{K_1-K_2} = a(1-m^2)({K_1}^{-m} - {K_2}^{-m}) \tag{3}$$

$$\bar{K}_{K_1-K_2} = \frac{m}{m-1} \frac{{K_1}^{1-m} - {K_2}^{1-m}}{{K_1}^{-m} - {K_2}^{-m}} \tag{4}$$

2.5. Extraction procedure

30 mg magnetic MIP beads were suspended in 5 mL ractopamine standard solution in toluene in a glass sample vial. Samples were agitated by rocking with a reciprocating shaking-table at room temperature. After appropriate extraction time, the polymer beads were separated from the solution by a magnetic separation step with an adscititious magnet. Subsequently the target analytes were desorbed by 1.0 mL methanol, dealt with a nitrogen drying step and re-dissolved in a 100 μL methanol. Then 20 μL of the portion were injected in HPLC system and analyzed.

2.6. Sample analysis

Pork and pig liver were selected for the spiked sample analysis. 10.0 g of pork and pig liver were minced and mixed with 50 μL of ractopamine, isoxsuprine and fenoterol standard solution, homogenized for 1 h. The spiking concentrations for each β-agonist were 5.0 ng g^{-1}. The spiked sample was then extracted with 20 mL of acetonitrile by ultrasonic agitation for 30 min. After being centrifuged at 3800 r min^{-1} for 10 min, the supernatant was collected, dried with a rotary evaporator, and re-dissolved with 5 mL of toluene, then subjected to the extraction procedure by magnetic MIP beads.

All chromatographic measurements were performed using Shimadzu LC-20A system, which consists of a pump with gradient elution function, an auto-sampler, a RF-10AxL detector. The analytical column was a C_{18} column (250 mm × 4.6 mm I.D., 5 μm, Dikma) with an attached 7.5 mm C_{18} security guard column (Phenomenex, USA). A linear gradient of the mobile phase from 10 to 48% (v/v) of

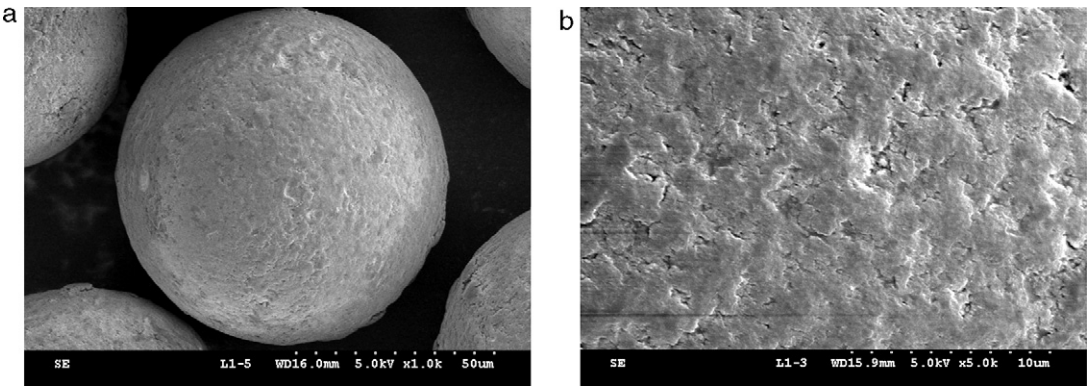

Fig. 2. SEM image of the magnetic MIP beads with magnifications of 200 (a) and 5000 (b).

methanol/buffer (0.1% phosphoric acid in water) over 8 min at the flow rate of 1.0 mL min^{-1} was used. The excitation wavelength is 226 nm, emission wavelength is 305 nm.

3. Results and discussion

3.1. Synthesis of the magnetic ractopamine imprinted polymer beads

The preparation of the magnetic ractopamine imprinted polymer beads involved synthesis of the superparamagnetic Fe_3O_4 nanoparticles, encapsulation of Fe_3O_4 nanoparticles in the polymer network, formation of the homogenous spherical beads, and removal of the template ractopamine thus generation of the recognition sites. Fig. 1 is a "theoretical" interaction mode to show the self-assembly of the template and the monomer, as well as the polymerization process.

The superparamagnetic Fe_3O_4 nanoparticles were prepared by the coprecipitation method. Because of the anisotropic dipolar attraction, the obtained Fe_3O_4 nanoparticles tend to aggregate into large clusters [17]. In order to address this problem, the Fe_3O_4 nanoparticles were modified with PEG 6000 by surface adsorption. The PEG shell helps to avoid electrostatic agglomeration, and ensures uniform dispersion of magnetic nanoparticles in the prepolymerization solution and homogeneous embedding. MAA, AA and AM were chosen as the monomer for investigating their function concerning the beads morphology and imprinting efficiency. Results show that AM which could provide multiple hydrogen-binding sites exhibited the best imprinting effect. The St plays important role to form spherical shape structure during the suspension polymerization procedure. Although the imprinted polymer beads may be produced using either thermal or UV-initiated polymerization, microwave heating was employed as the energy to initiate polymerization. The distinguished advantage by microwave heating-initiated polymerization mainly lies in two aspects. One is significant promotion of reaction rate. This could be proved that the polymerization had been accomplished within 1 h in this experiment, which is superior to the conventional heating method, usually taken for 14–24 h reported in other literature [17,20–22]. The other advantage is that uniform morphology and narrow diameter can be obtained for the magnetic polymer beads by the microwave heating protocol. The well shaped beads with diameter distribution from 50 to 120 μm (up to 90% polymer beads) were achieved by the microwave heating method (see Supplementary material, Fig. S1). However, a significant amount of polymer beads exhibited irregular shape by conventional heating, and wider diameter distribution was also observed.

3.2. Characterization of the synthesized polymer beads

To determine the morphological and physical properties of the synthesized magnetic MIP beads, SEM, FT-IR spectrum, TGA analysis, and magnetic analysis were performed.

Representative SEM images of the magnetic MIP beads are provided in Fig. 2. From these images, it is obvious that spherical shape and rough surface was obtained for the synthesized magnetic MIP beads. The polymer beads are robust, which is suitable for regeneration for repeated usage. In fact, it was verified in the stability test that the polymer beads can be used at least 100 times for extraction and desorption without losses of the adsorption/desorption capacities. The average diameter of the magnetic MIP beads observed from the SEM is 80 μm.

The FT-IR spectrum of the magnetic MIP beads, the magnetic NIP beads, the MIP beads without magnetism and the Fe_3O_4 nanoparticles were illustrated in Fig. 3. A distinct absorption band at 571 cm^{-1} in magnetic MIP and NIP beads attributed to Fe–O bond in Fe_3O_4 particles, which suggests the Fe_3O_4 was successfully embedded into the magnetic polymer beads, but was not observed in the MIP beads without the partition of Fe_3O_4 particles. The other spectrum matched for magnetic MIP beads, magnetic NIP beads and the MIP beads. For example, peaks at about 1727 cm^{-1}(C=O stretch) and 1450 cm^{-1} (C–N stretch) are observed, which indicates the existence of acylamino groups in the polymer. The absorptions corresponded to O–H stretch-

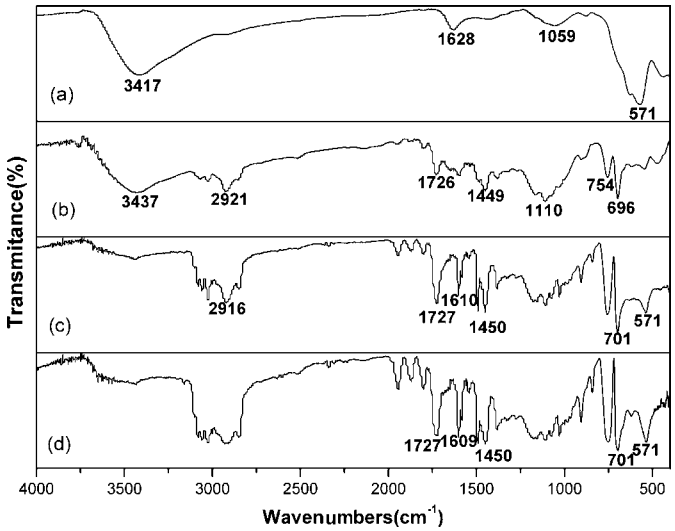

Fig. 3. FT-IR spectra of the Fe_3O_4 (a), MIP beads without magnetism (b), magnetic MIP beads (c) and magnetic NIP beads (d).

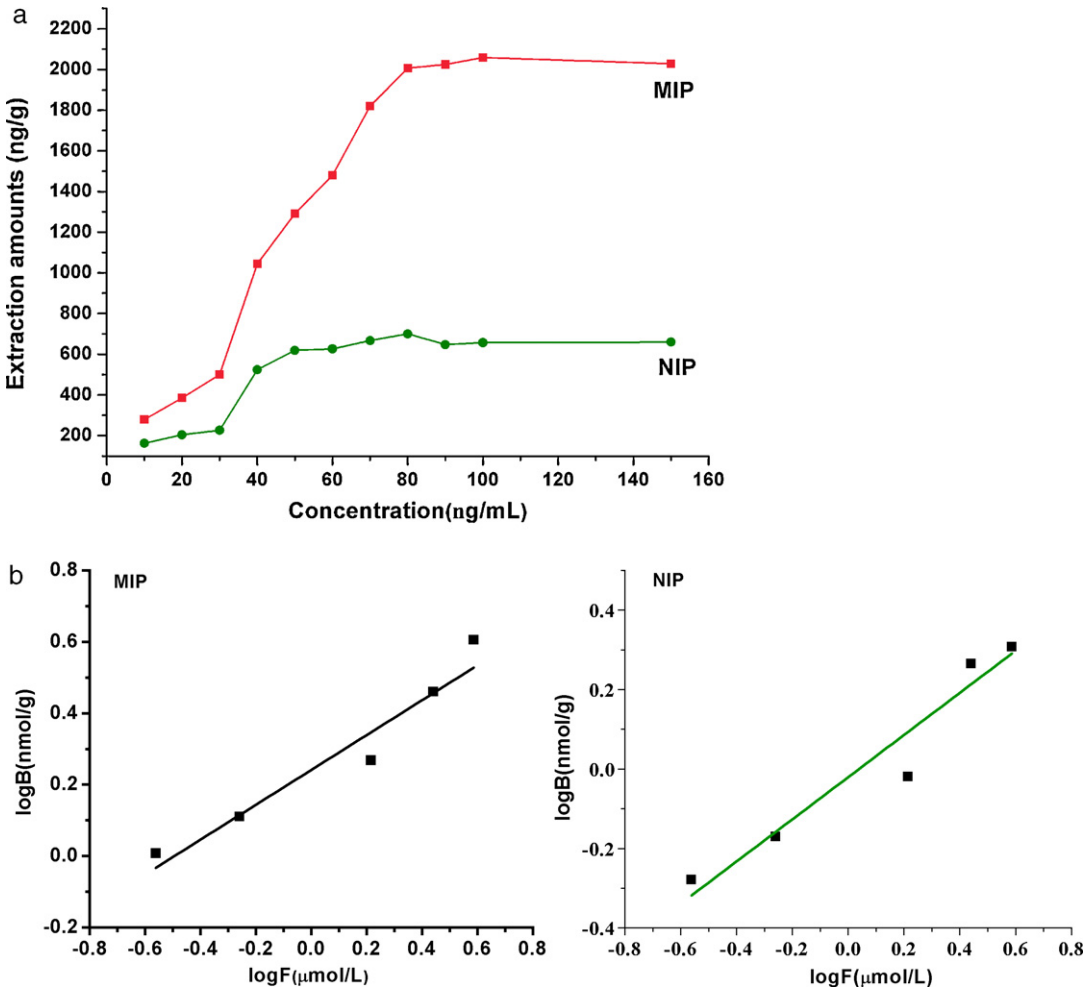

Fig. 4. Adsorption isotherm of ractopamine on the magnetic MIP and NIP beads in the range of 10–150 ng mL^{-1} (a) and the fitting plots with Freundlich isotherm model (b).

ing vibration at about 3437 cm^{-1}, C–H stretching vibration at 2916 cm^{-1}.

TGA was performed to further estimate the relative composition of the polymer and Fe_3O_4 particles, which was shown in Supplementary material, Fig. S2. When the temperature was changed from 25 to 800 °C, there is dramatic decrease at about 425 °C. The decreased weight of magnetic MIP beads was approximately 96.0%, which demonstrated the content of polymer. The remaining mass was attributed to the thermal resistance of Fe_3O_4 particles, and the quantity of Fe_3O_4 particles in the beads was about 4.0%.

The magnetic hysteresis loops analysis was employed to study the magnetic property of magnetic MIP beads. The magnetization curves (Supplementary material, Fig. S3) showed their superparamagnetic property. This feature illustrated that the materials respond magnetically to external magnetic field and that this response vanishes upon the removal of the field. The magnetic MIP and NIP beads achieved a saturation magnetization value of 0.43 emu/g and 0.26 emu/g, respectively. The saturation magnetization value measured is lower than the magnetic nanoparticles reported [16,18]. This was expected because the polymeric coating had effectively shielded the magnetite. However, the magnetic MIP beads with less magnetite encapsulation also possess enough magnetic response to meet the need of magnetic separation within a short time. Higher contents of MIP polymer were benefit for creating more recognition cavity.

3.3. Recognition properties of the magnetic MIP beads

3.3.1. Adsorption isotherm

The adsorption isotherm for the magnetic MIP and NIP beads was plotted by the batch rebinding experiments which were conducted in toluene using a range of ractopamine concentrations from 10.0 to 150.0 ng mL^{-1} (Fig. 4). It is observed that all the magnetic polymer beads showed increased binding amounts as the initial concentration increased. However, the extraction capacities with the magnetic MIP beads were 2007 ng g^{-1} for ractopamine and only 619 ng g^{-1} obtained by magnetic NIP beads. This data is indicative of a true molecular imprinting effect being observed with the magnetic ractopamine imprinted polymer beads.

To further thoroughly investigate the imprinting effect, the binding properties of isotherms could be estimated by application of specified binding models. As for non-covalent MIPs, which possess a heterogeneous binding site distribution, it is common to apply the Freundlich isotherm ($B = a \cdot Fm$) from where a (a measure of the affinity and average capacity) and m (the heterogeneity index) of the polymer can be determined. m varies between 0 and 1, where 1 refers to a totally homogeneous binding site distribution, with higher values corresponding to more homogeneous systems. In addition, the number of binding sites ($N_{K1\text{-}K2}$) and the average binding affinity ($K_{K1\text{-}K2}$) can be calculated in a given concentration range. The values of $N_{K1\text{-}K2}$, $K_{K1\text{-}K2}$, and m are shown in Table 1. The binding sites measured were 3.24 μmol g^{-1} and 1.17 μmol g^{-1} for the magnetic MIP beads and the corresponding magnetic NIP beads,

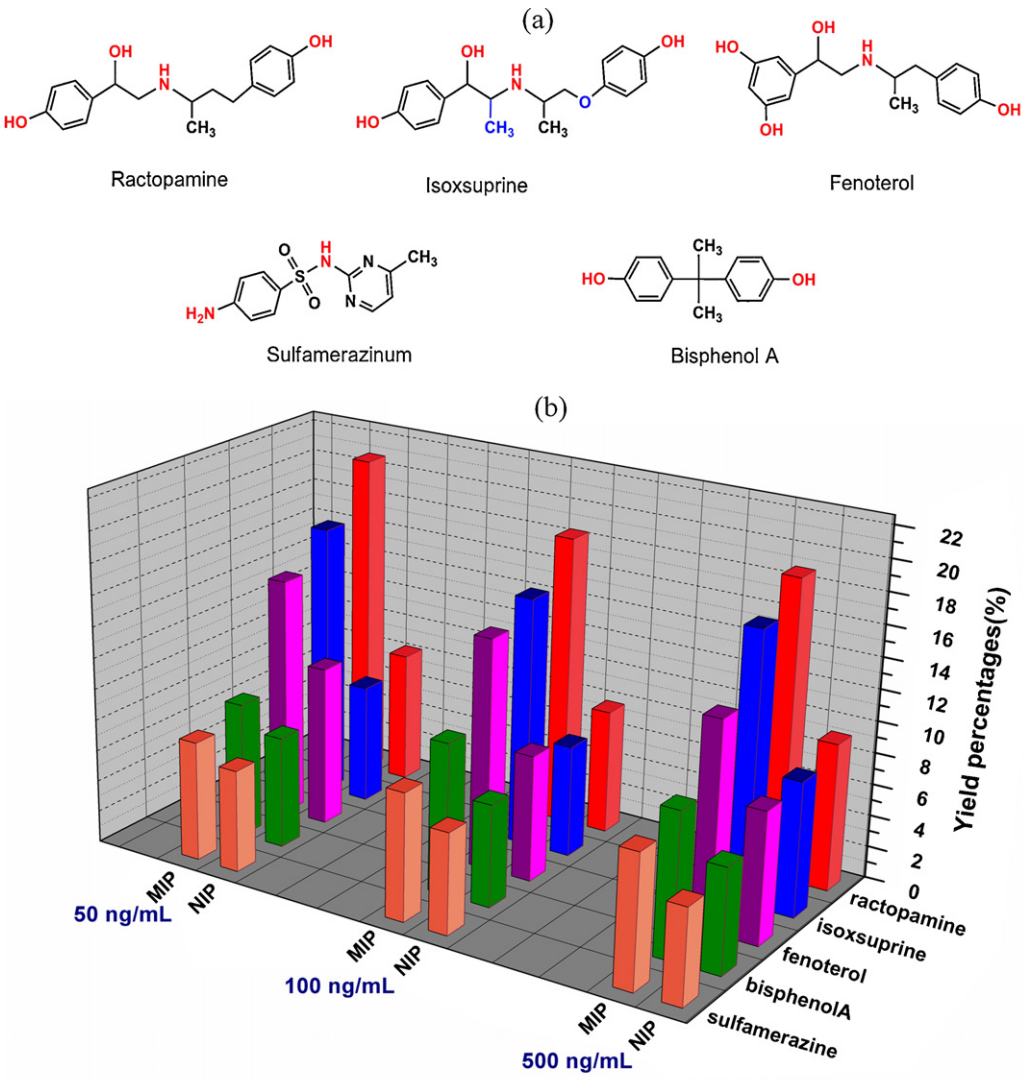

Fig. 5. Chemical structure of ractopamine, its structural analogues, and the reference compounds (a), and their yield percentages on the magnetic MIP, NIP beads at three different initial concentrations in solution (b).

respectively. These results indicate the successful imprinting of the magnetic MIP beads. The average association constants (K_{K1-K2}) for the magnetic MIP and NIP beads were very similar using this analysis. Finally, both of the magnetic beads contained a heterogeneous distribution of binding sites with heterogeneity indexes (m) of 0.488 for MIP and 0.535 for NIP. This is consistent with previous studies, which found that non-covalent MIPs generally tend to be more heterogeneous than NIPs [34].

3.3.2. Selectivity investigation

Recognition properties cannot solely be evaluated on the ability of the MIP to rebind the template but also on its discrimination between analogue molecules. Therefore, the cross-selectivity of the magnetic MIP beads was investigated with ractopamine, isoxsuprine and fenoterol, the three β-agonists with similar molecular structure, as well as two reference compounds, sulfamerazinum and bisphenol A. The chemical structure of these compounds is illustrated in Fig. 5(a). The rebinding experiment was performed at initial concentration of 50, 100 and 500 ng mL^{-1}, respectively. The yield percentages, defined as the percentage of extracted amounts of analytes on the polymer over the total amounts in the initial solution, was calculated and illustrated in Fig. 5(b). It can be seen that the magnetic NIP beads cannot discriminate among different compounds because these analytes were extracted on the magnetic NIP beads with similar yield percentages from 6.5% to 9.1%. However, the yield percentages of these compounds differed from 7.6% to 21.0% on the magnetic MIP beads. In addition, the recognition ability of the magnetic MIP beads is relative to the degree of molecular analogy to the template. The structure of isoxsuprine is very similar to ractopamine except for an additional

Table 1
Isotherm parameters for ractopamine on the magnetic MIP and NIP beads estimated by fitting data to the Freundlich isotherm models.

Beads	Equation	Relative coefficient	m	$N_{K_1-K_2}$ (μmol g^{-1})	$\bar{K}_{K_1-K_2}$ (M^{-1})
Magnetic MIPs	Log B = 0.4879 log F + 0.2411	0.950	0.4879	3.24	1.2×10^3
Magnetic NIPs	log B = 0.5351 log F +0.1111	0.932	0.5351	1.17	1.3×10^3

Table 2
Selectivity coefficients of the MIP and NIP beads.

	K_d (mL g^{-1})					k				k'			
	K_{d1} ractopamine	K_{d2} isoxsuprine	K_{d3} fenoterol	K_{d4} sulfamerazine	K_{d5} bisphenol A	k_1	k_2	k_3	k_4	k'_1	k'_2	k'_3	k'_4
Magnetic MIPs	8.8	7.1	5.0	3.1	3.4	1.2	1.8	2.9	2.6	1.1	1.7	2.0	2.0
Magnetic NIPs	3.1	2.7	3.0	2.1	2.4	1.1	1.0	1.4	1.3				

K_d, the distribution coefficient. $K_d = (c_0 - c_{final})/c_{final} \times$ (solution volume [mL]/absorbent mass [g]); k, the selectivity coefficient. $k_1 = K_{d1}/K_{d2}$; $k_2 = K_{d1}/K_{d2}$, $k_3 = K_{d1}/K_{d3}$; $k_4 = K_{d1}/K_{d4}$; k', the relative selectivity coefficient. $k'_1 = k_{1MIP}/k_{1NIP}$, $k'_2 = k_{2MIP}/k_{2NIP}$, $k'_3 = k_{3MIP}/k_{3NIP}$, $k'_4 = k_{4MIP}/k_{4NIP}$.

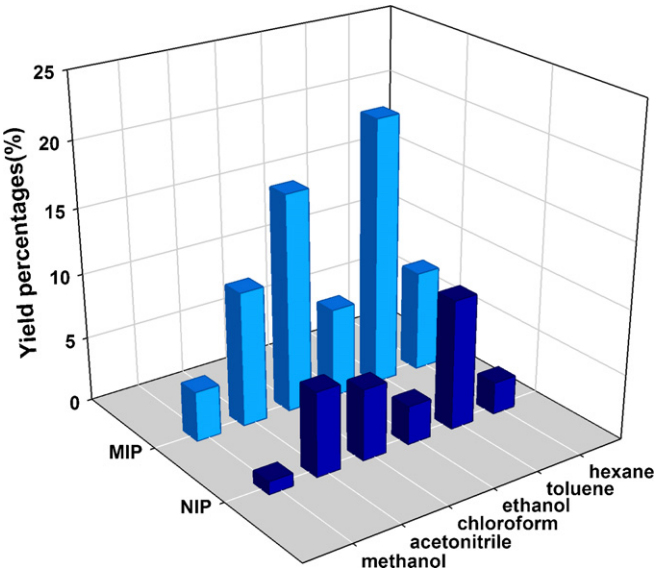

Fig. 6. Influence of the extraction solvents on the extraction yield percentages of ractopamine.

methyl-group in the molecule, resulting in its relatively higher recovery. Whereas the molecular size of fenoterol is smaller for less methylene group between two benzene rings. Bisphenol A and sulfamerazinum were extracted with lower yield percentages, owing to their larger difference to ractopamine in molecular structure and size. The discrimination ability of the beads was also demonstrated by the distribution coefficient (k_d), the selectivity coefficient (k), and the relative selectivity coefficient (k') (Table 2). These selectivity parameters were measured at the initial concentration of 50.0 ng mL^{-1} of each compound. From the demonstrated parameters, it can be implied that the created binding sites of the magnetic MIP beads can distinguish between ractopamine and other compounds based on their molecular size and chemical structure.

3.4. Application to the selective enrichment of β-agonists in pork and pig liver samples

As described above, the magnetic MIP beads have specific affinity to ractopamine, as well as its structural analogues such as isoxsuprine and fenoterol. It was used as a highly selective extraction material coupled with HPLC for simultaneous determination of these compounds in pork and pig liver samples.

3.4.1. Optimization of the extraction conditions

Several factors govern the enrichment effect of the magnetic MIP beads. These include the extraction and desorption solvents, extraction and desorption time. In order to obtain the optimized conditions we used 20 ng mL^{-1} ractopamine standard solutions to evaluate the extraction efficiency under different conditions.

The choice of the extraction solvent can be critical for the quantitative extraction of the target β-agonists. Therefore, the first step of the quantitative approach consisted of testing different solvents. Results are shown in Fig. 6, from which we can see that toluene enables higher extraction efficiency than *n*-hexane, ethanol, chloroform, acetonitrile and methanol. The desorption process is the reversed procedure of extraction. The satisfactory desorption solvent is usually the poor solvent for extraction. In addition, acidified solvent were commonly supposed to accelerate desorption. Based on these considerations, several solvents were thus screened as possible desorption solvents, including methanol, methanol–acetic acid (9:1, v/v), acetonitrile and acetonitrile–acetic acid (9:1, v/v). The desorbed amounts of ractopamine by these desorption solu-

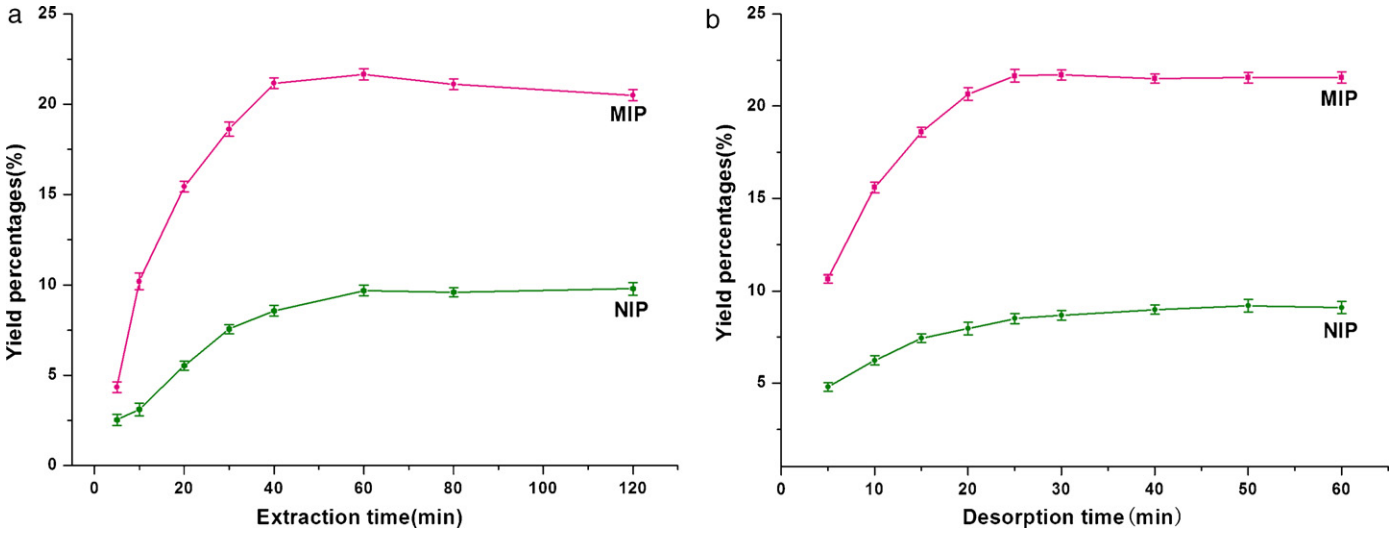

Fig. 7. The adsorption and desorption time curve of ractopamine on the magnetic MIP beads.

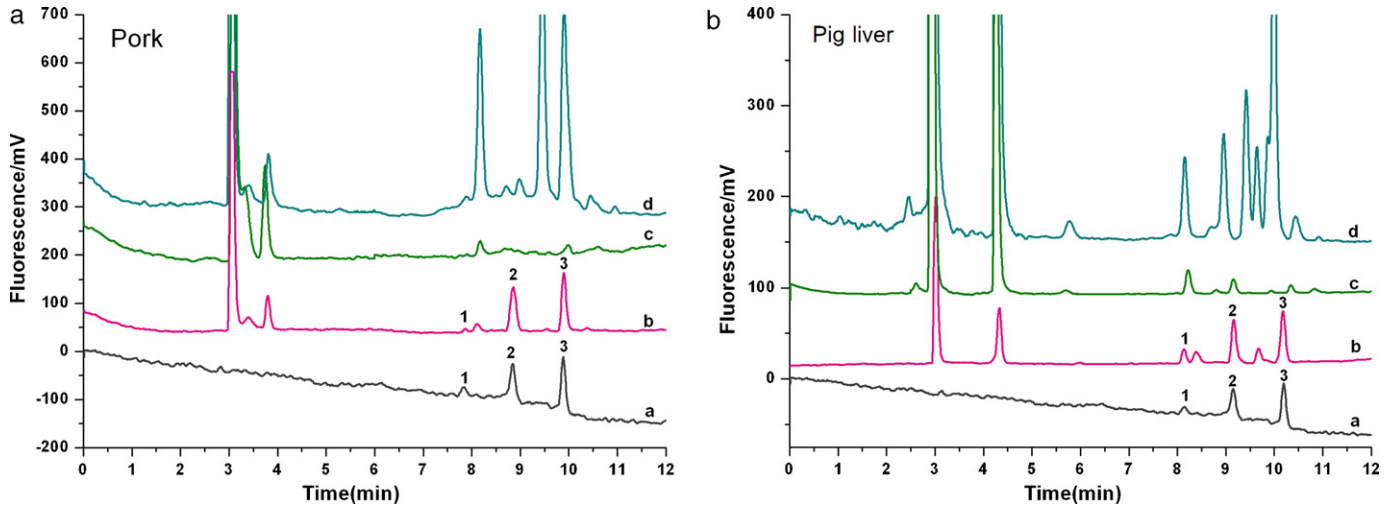

Fig. 8. Chromatograms of β-agonists in pork and pig liver samples with fluorescence detection. (a) Aliquots of 50.0 ng mL^{-1} β-agonists mixed standard solution, (b) 5.0 ng kg^{-1} β-agonists spiked sample solution extracted with MIP, (c) the above sample extracted with NIP, (d) direct injection of ultrasonic assisted extraction solutions of the spiked sample. Peaks: (1) fenoterol, (2) ractopamine, (3) isoxsuprine.

Table 3
Analytical performance data for β-agonists.

Analytes	Linearity range (ng mL^{-1})	Correlation coefficient	Limits of detection (ng mL^{-1}) (n = 5)	RSD (%) (n = 5)	
				Intra-day	Inter-day
Ractopamine	1.0–50.0	0.9952	0.52	2.1	4.5
Isoxsuprine	1.0–50.0	0.9953	0.71	2.7	4.7
Fenoterol	1.5–50.0	0.9902	1.04	3.0	8.0

tions were 4.2, 10.1, 5.1 and 3.7 ng, respectively. So the solution of methanol–acetic acid (9:1, v/v) was selected for the optimal desorption solvent.

The adsorption and desorption kinetic were investigated to optimize the extraction and desorption time. It was demonstrated the equilibrium was established after 40 min for adsorption and 20 min for desorption, and finally these conditions were applied to sample analysis (Fig. 7).

3.4.2. Analytical performance and the application to real samples

Based on the aforementioned conditions under optimization, the selectivity of the magnetic MIP beads was demonstrated by extracting spiked β-agonists in pig tissues, and analyzing the final extracts by HPLC-FL method.

Firstly, the analytical performance was tested regarding linearity, precision and sensitivity, and was illustrated in Table 3. To test the linearity of the calibration curves, various concentrations of ractopamine, isoxsuprine and fenoterol mixed solution in the range of 0.05–50.0 ng mL^{-1} were analyzed. The linearity was acceptable for all β-agonists with correlation coefficients higher than 0.9902. The sensitivity of this analytical procedure was evaluated in terms of the limit of detection (LOD) calculated using S/N = 3 and the limit of quantification (LOQ) defined as the lowest concentration within linear range. The LOD and LOQ of each β-agonist by this method were in the range of 0.52–1.04 ng mL^{-1} and 1.0–1.5 ng mL^{-1}, respectively. The intra-day and inter-day precision was calculated by analysing samples spiked with 20 ng mL^{-1}, and ranged from 2.1% to 3.0% and 4.5% to 8.0%, respectively.

The validity of the method were then checked with real samples of pork and pig liver purchased in market. The samples spiked with 5.0 ng kg^{-1} β-agonist were extracted by the magnetic MIP beads, and the chromatogram is illustrated in Fig. 8. For comparison, the chromatogram of the spiked samples that were extracted by the magnetic NIP beads and the direct injection of sample solutions after ultrasonic extraction were also illustrated. Comparison of results from these chromatograms showed high selectivity of the magnetic MIP beads, in that direct injection of sample solutions appeared as some undefined interfering peaks in the chromatograms that were mostly disappeared after extraction by the magnetic MIP beads. By contrast, the magnetic NIP beads apparently lack specific enrichment properties to the target β-agonists. From these results it can be concluded that the proposed magnetic MIP beads has good applicability to selective extraction of the mentioned compounds from complex samples. The recoveries were from 82.0% to 92.0% for the spiked samples with RSDs in the range of 5.8–10.0%.

4. Conclusions

Magnetic MIP beads imprinted by ractopamine were developed as extraction adsorbent for analysis of β-agonists in pork and pig liver samples. The achievement of well formed magnetic MIP beads depended upon several parameters of the polymerization conditions, such as surface modification of the Fe_3O_4 nanoparticles, the selection of monomer and crosslinker. It should be mentioned polymerization initiated by microwave heating provide an efficient approach to obtain uniform beads with good spherical shape in significantly shortened time. The obtained magnetic MIP beads were characterized by SEM, FT-IR, TGA and magnetization curve. Investigation of recognition properties showed high adsorption capacity and selectivity of the magnetic MIP beads to the template ractopamine. Pleasingly, the imprints also showed cross-selectivity for isoxsuprine and fenoterol. This enables the simultaneous extraction and analysis of these β-agonists followed by HPLC-FL. The complete elimination of matrix components and some sample interferents from the target compounds was accomplished, provid-

ing improved isolation and identification of β-agonists in complex biological fluids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.01.045.

References

[1] G. Wulff, Angew. Chem. Int. Ed. 34 (1995) 1812.
[2] B. Sellergren, Molecularly Imprinted Polymers Man-made Mimics of Antibodies and their Application in Analytical Chemistry, Elsevier, New York, 2001.
[3] E. Turiel, A. Martín-Esteban, Anal. Chim. Acta 668 (2010) 87–99.
[4] E. Turiel, A. Martín-Esteban, J. Sep. Sci. 32 (2009) 3278–3284.
[5] S. Tokonami, H. Shiigi, T. Nagaoka, Anal. Chim. Acta 641 (2009) 7–13.
[6] Y.P. Huang, Z.S. Liu, C. Zheng, R.Y. Gao, Electrophoresis 30 (2009) 155–162.
[7] C.J. Tan, Y.W. Tong, Anal. Bioanal. Chem. 389 (2007) 369–376.
[8] M.C. Prieto-Blanco, P. López-Mahía, P. Campíns-Falcó, Anal. Chem. 81 (2009) 5827–5832.
[9] M. Ulbricht, J. Chromatogr. B 804 (2004) 113–125.
[10] S. Wei, A. Molinelli, B. Mizaikoff, Biosens. Bioelectron. 21 (2006) 1943–1951.
[11] C.Y. He, Y.Y. Long, J.L. Pan, K. Li, F. Liu, J. Biochem. Biophys. Methods 70 (2007) 133–150.
[12] H.Y. Yan, F.X. Qiao, K.H. Row, Anal. Chem. 79 (2007) 8242–8248.
[13] Q. Lu, X.M. Chen, L. Nie, J. Luo, H.J. Jiang, L.N. Chen, Q. Hu, S.H. Du, Z.P. Zhang, Talanta 81 (2010) 959–966.
[14] Y.B. Sun, X.B. Ding, Z.H. Zheng, X. Cheng, X.H. Hu, Y.X. Peng, Talanta 78 (2009) 327–332.
[15] H.M. Chen, C.H. Deng, Y. Li, Y. Dai, P.Y. Yang, X.M. Zhang, Adv. Mater. 21 (2009) 2200.
[16] X. Wang, L.Y. Wang, X.W. He, Y.K. Zhang, L.X. Chen, Talanta 78 (2009) 327–332.
[17] L. Li, X.W. He, L.X. Chen, Y.K. Zhang, Chem. Asian J. 4 (2009) 286–293.
[18] X.W. Kan, Z.R. Geng, Y. Zhao, Z.L. Wang, J.J. Zhu, Nanotechnology 20 (2009) 165601.
[19] J.A. Richard, M. Klaus, Analyst 121 (1998) 611–1616.
[20] L.G. Chen, X.P. Zhang, Y. Xu, X.B. Du, X. Sun, L. Sun, H. Wang, Q. Zha, A.M. Yu, H.Q. Zhang, L. Ding, Anal. Chim. Acta 662 (2010) 31–38.
[21] L.G. Chen, J.L.Q.L. Zeng, H. Wang, A.M. Yu, H.Q. Zhang, L. Ding, J. Chromatogr. A 1216 (2009) 3710–3719.
[22] Y.S. Ji, J.J. Yin, Z.G. Xu, C.D. Zhao, H.Y. Huang, H.X. Zhang, Anal. Bioanal. Chem. 395 (2009) 1125–1133.
[23] Y. Zhang, R.J. Liu, Y.L. Hu, G.K. Li, Anal. Chem. 81 (2009) 967–976.
[24] A.D. Mitchell, Animal 3 (2009) 173–180.
[25] J.Z. Shen, Z. Zhang, Y. Yao, W.M. Shi, Y.B. Liu, S.X. Zhang, Anal. Bioanal. Chem. 387 (2007) 1561–1564.
[26] L.M. He, Y.J. Su, Z.L. Zeng, Y.H. Liu, X.H. Huang, Anim. Feed Sci. Tech. 132 (2007) 316–323.
[27] E. Shishani, S.C. Chai, S. Jamokha, G. Aznar, M.K. Hoffman, Anal. Chim. Acta 483 (2003) 137–145.
[28] Z.Y. Qiang, F.Q. Shentu, B. Wang, J.P. Wang, J.Y. Chang, J.Z. Shen, J. Agric. Food Chem. 55 (2007) 4319–4326.
[29] M.P. Turberg, T.D. Macy, J.J. Lewis, M.R. Coleman, J. AOAC Int. 78 (1995) 1394–1402.
[30] S. Wang, L. Liu, G.Z. Fang, C. Zhang, J.X. He, J. Sep. Sci. 32 (2009) 1333.
[31] Z.G. Xu, Y.F. Hu, Y.L. Hu, G.K. Li, J. Chromatogr. A 1217 (2010) 3612–3618.
[32] Y.L. Hu, R.J. Liu, Y.W. Li, G.K. Li, J. Sep. Sci. 33 (2010) 2017–2025.
[33] R.J. Umpleby, S.C. Baxter, A.M. Rampey, G.T. Rushton, Y. Chen, K.D. Shimizu, J. Chromatogr. B 804 (2004) 141–149.
[34] R.J. Umpleby, S.C. Baxter, M. Bode, J.K. Berch, R.N. Shah, K.D. Shimizu, Anal. Chim. Acta 435 (2001) 35–42.