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Rapid analysis of ractopamine in pig tissues by dummy-template imprinted solid-phase extraction coupling with surface-enhanced Raman spectroscopy

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ABSTRACT

Ritodrine has similar skeleton structure to ractopamine and it was selected as the dummy-template molecule to synthesize the molecular imprinted polymers (MIPs). The MIPs exhibited better selectivity to ractopamine than to the dummy-template molecule: the imprint factor for ractopamine was 8.9, while 7.6 for ritodrine. The MIPs were used as sorbents in solid-phase extraction for selective enrichment of ractopamine, and some key parameters were optimized. After that, a rapid surface-enhanced Raman spectroscopy method was developed for analysis of ractopamine and isoxuprine in pig tissue samples. Under the optimal conditions, good linearity was achieved in the range of 20.0–200.0 μg/L for ractopamine and isoxsuprine at 842 cm^{-1} and 993 cm^{-1}, respectively. The limits of detection were 3.1–4.3 μg/L, which were lower than the maximum allowed by U. S. Food and Drug Administration. The recoveries of ractopamine and isoxsuprine were 72.4–79.7% and 71.0–78.2% for the spiked pork and pig liver, respectively, while the relative standard deviations ranging from 7.4% to 13.0%. The results suggest that the proposed method is sensitive and selective, and it has good potential on the quantitative analysis of trace amounts of β-agonists in complex samples.

1. Introduction

The demand for faster, more sensitive and cost-effective analytical methods is rapidly increasing during the last decades, especially on the determination of trace analytes in complex matrix such as foods, environmental or biological samples [1]. Normally, an analytical process has several steps including sample preparation, sampling, separation, determination, data handling and treatment, etc. Of these steps, selective sample preparation and sensitive analytical methods might be the most important. Chromatographic methods such as liquid chromatography–mass spectroscopy, gas chromatography–mass spectroscopy and immunoassay such as enzyme-linked immunosorbent assay (ELISA) were typically used in the most cases [2]. However, the chromatographic methods are relatively expensive and complex operation while ELISA is likely to be influenced by matrix interferences and so lead false-positive results. Surface-enhanced Raman spectroscopy (SERS) is a special surface-enhanced optical phenomenon on the nano-scale metal surfaces, which has overcome the low sensitivity of normal Raman spectroscopy via adsorption of analytes on rough nano-scale surfaces typically made from gold and silver [3]. The magnitude of Raman signals in SERS can be enhanced to 10^4–10^7 due to the effects of electromagnetic field and chemical enhancement [4]. Hence, the ultrasensitive SERS has been used to characterize and detect organic chemicals and microorganisms such as melamine [5–7], malachite green [8–10], pesticides [11–13], antibiotics [14–16], β-agonists [17,18], etc. Although the limits of detection (LODs) of these methods were acceptable, their further application to detect specific analytes in complex matrices, especially for the quantitative analysis of trace analytes, were greatly limited by the serious matrix interferences and poor selectivity. Therefore, sample pretreatment to reduce or remove undesirable interferences is required before SERS detection.

Conventional liquid–liquid extraction method is considered the most time-consuming and error-prone part of the analytical scheme. Some new extraction techniques such as solid-phase extraction (SPE), solid-phase microextraction (SPME) [19] or liquid-phase microextraction (LPME) [20], were developed to reduce the initial sample sizes, and to minimize the amount of hazardous organic solvents. Among them, both SPE and SPME were well established in analytical laboratories. However, the main drawback associated to them is the lack of selectivity of the sorbents. Molecularly imprinted polymers (MIPs), the synthetic materials with artificially generated recognition sites which are able to specifically rebind target molecule, have attracted more and more attention for their high affinity and selectivity for target analyte and its analogues. Molecular imprinted solid-phase extraction (MISPE)

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was also developed as a relatively new concept in the pretreatment of biological sample, it had been successfully used for enrichment of ractopamine from complex matrices [21]. MISPE had also been combined with SERS for the detection of chemical compounds from complicated samples. Feng [18] reported a MISPE-SERS biosensor system for the detection of α-tocopherol from four different types of vegetable oils based on using a dendritic silver nanostructure as SERS substrate. This biosensing system demonstrated good sensitivity and selectivity for the quantitative detection of different spiking levels of α-tocopherol in vegetable oils. Meanwhile, the further applications of traditional MIPs were greatly limited owing to the unavoidable template leaking, which may influence the accuracy of identification and quantization of the analytes. The structural analogues of the analyte itself, namely dummy-template, can avoid the leakage of the template. To guarantee the selectivity and capacity of the sorbents, the selection of the dummy template is a key factor, while the available dummy template molecules are limited especially for simultaneous determination of homologue compounds. In most case, the imprinting factor or selectivity for dummy template was significantly higher than that for the cross-recognition targets in these molecular imprinted polymers [22].

Ractopamine was a typical β-agonists, which can promote bronchodilation, vasodilatation and increase heart rate. It is also used in animal feeding, effects seen in the skeletal muscle include speed up feeding efficiency and carcass leanness. However, the residual of ractopamine in pork products may pose health risks, particularly to those with asthma or cardiovascular disease. The use of ractopamine in swine has been banned by the majority of countries and areas in the world, such as China, Japan, and European Union, the maximum residue limit of ractopamine in swine liver is strictly limited to 0.15 μg/g or lower in the United States. In recent years, monitoring the residues of β-agonists in swine liver or pork products had been attracted great interest to the government regulatory agencies and the food industry [18]. Since the residue of ractopamine in complex samples is lower than μg/g, efficient sample pretreatments as well as sensitive analytical methods are significantly important. Solid-phase extraction (SPE) [23] or solid-phase microextraction (SPME) [24] with chromatographic methods were commonly used to analysis β-agonists in different matrices. Recently, Du et al. [25] reported a dummy-template MISPE method for selective analysis of ractopamine in pork, in which salbutamol was used as the dummy-template, the selectivity factors of dummy-template MIPs for salbutamol was significantly higher than that for ractopamine.

In the present work, ritodrine was selected as the dummy-template molecule to synthesize the molecular imprinted polymers due to its highly similar structure skeleton to ractopamine. The MIPs were used as sorbents of SPE for the selective extraction of β-agonists, then a rapid SERS method for analysis of ractopamine in pig tissue samples was developed.

2. Experimental

2.1. Chemicals and materials

Ritodrine hydrochloride (RTD) was purchased from Ruihe Chemical Plant (Taiyuan, China); Ractopamine hydrochloride (RAT), isoxsuprine hydrochloride (ISP) and clenbuterol hydrochloride (CLB) were purchased from Sigma (Shanghai, China); 4-nitrophenol (4-NP) and trisodium citrate was purchased from Guangzhou Reagent Plant (Guangzhou, China). Methacrylic acid (MAA) and azo-bis(isobutyronitrile) (AIBN) were purchased from Damao Reagent Plant (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Rohm-Haas AG (USA). Chloroauric acid was purchased from Guangfu Chemical Research Institute (Tianjin, China). Methanol (MeOH) and acetonitrile (ACN) of HPLC grade were purchased from Merck (Darmstadt, Germany). Deionized water was used thoroughly. All other reagents were of analytical grade.

The individual stock solutions of standards were prepared at the concentration of 100.0 mg/L in acetonitrile, and further solutions of lower concentration were prepared by serial dilution of the stock solutions. The mixed standard solution was prepared with iso-proportional mass of each solution.

Au nanoparticles (Au NPs) was prepared according to the method reported previously [3]. Briefly, 200 mL deionized water consist of 5.0 mL 10 mmol/L $HAuCl_4$ was heated to boiling under vigorous stirring, then 1.2 mL 1% (w/v) trisodium citrate was injected rapidly and kept boiling for 40 min. The red solution was detected by Cary-100 UV–vis spectrophotometer (Varian, American) and its absorption wavelength was 530 nm, the average diameter of Au NPs was about 55 nm.

2.2. Instruments

An S-4300 scanning electron microscope (Hitachi, Japan) was used to investigate the surface of the MIP polymer. A Nicolet Avatar 330 Fourier transform infrared (FT-IR) spectrometer with a scanning range from 400 to 4000 cm^{-1} was applied to investigate the composition of the polymer. An LC-20A HPLC system (Shimadzu, Japan) consists of a RF-10AXL fluorescence detector and a diode-array detector was utilized to analyze ractopamine and its analogues. Aspirator A-3S circulating pump was applied to promote the solid-phase extraction. The solid-phase extraction tube and frits were purchased from Anpel company (Shanghai, China).

A battery-powered Raman spectrometer with 785 nm laser excitation wavelength (Delta Nu Inspector Raman, Laramie, WY) was used to perform SERS detection. This system consists of a CCD detector (ModelSpec-10:400B, Roper Scientific, Trenton, NJ) with a spectral resolution of 8 cm^{-1}, and a data acquisition system (Photometrics, Tucson, AZ).

2.3. Synthesis of the dummy-template MIPs

The dummy-template MIPs were prepared according to our reported work [26] with some modifications. Briefly, 0.33 g RTD, 347 μL MAA and 3.1 mL EGDMA were dissolved in 6.8 mL methanol to prepare the pre-polymer solution. This solution was stirred for 12 h at room temperature, and then 30 mg AIBN was added and dissolved adequately. After purging with nitrogen stream for 5 min, the bottle was sealed immediately and allowed to perform polymerization at 60 °C for 24 h. Non-imprinted polymers (NIPs) were prepared by the same way with the imprinted polymers except without the addition of RTD.

The polymers were ground and sieved with a mesh gauge. The template molecule was removed by soxhlet extraction with methanol-acetic acid (9:1, v/v) until no template molecule was detected by HPLC. The polymers were heated at 120 °C for 12 h before use.

2.4. Optimization of molecular imprinted solid-phase extraction procedures

200 mg of MIP particles were packed into a 3.0 mL empty polypropylene cartridge with glasswool frit at each end. The cartridge was activated with 3.0 mL of methanol and equilibrated with 3.0 mL of acetonitrile. Then, the loading solutions in different solvent including deionized water, methanol, acetonitrile and their corresponding aqueous solutions were investigated. 5.0 mL of the pre-treated sample was added into the cartridge at 0.5 mL/min. After that, the cartridge was washed with methanol and eluted with methanol containing 20% (v/v) acetic acid. The eluent was