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Rapid screening of clenbuterol hydrochloride in chicken samples by molecularly imprinted matrix solid-phase dispersion coupled with liquid chromatography

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ABSTRACT

A simple and selective molecularly imprinted matrix solid-phase dispersion (MI-MSPD) method coupled with high performance liquid chromatography (HPLC) ultraviolet detection was developed for rapid screening of clenbuterol hydrochloride (CH) in chicken samples. The new molecularly imprinted microspheres (MIM) were synthesized by using butylamine and chloroaniline as dummy template with aqueous suspension polymerization and revealed good affinity to CH in aqueous solution. The application of the obtained MIM as sorbent of matrix solid-phase dispersion (MSPD) improved the selectivity of extraction procedure and avoided the effect of template leakage on quantitative analysis. Under the optimized conditions, good linearity of CH was obtained in a range of 0.059–18.30 $\mu g\,mL^{-1}$ with the correlation coefficient (R) of 0.9996. The recoveries of CH at three spiked levels were ranged from 92.0 to 99.1% with the relative standard deviation less than 4.0% ($n = 3$). The presented MI-MSPD-HPLC method combined the superiority of MIM and MSPD, and therefore could be potentially applied for the determination of CH in complicated biological samples.

1. Introduction

Clenbuterol hydrochloride (4-amino-3,5-dichloro-a-tert-butylaminomethylbenzyl alcohol hydrochloride, CH), a representative of the class of beta-adrenergic agents, had been used as a tocolytic, bronchodilator, and heart tonics in human and veterinary medicine [1,2]. It also possessed physiological effects similar to anabolic steroids, which promoted the growth of the muscular tissue and reduction of body fat [3]. As a consequence, it was extensively used in various animal species as a repartitioning agent to decrease fat deposition with enhanced protein accumulation when administered orally at high doses [4,5]. However, its long term or high dose misuse had led to serious side effects and it was prohibited to use as growth promoter for livestock in the Spain, Italy, China, and many other countries [6–8]. Therefore, a simple, accurate and reliable method for the determination of trace levels of CH in meat products was desired for the assurance of consumer healthy.

Until now, several analytical methods such as high performance liquid chromatography (HPLC) [9], gas chromatography–mass spectrometry (GC–MS) [10,11], capillary electrophoresis (CE) [12,13], liquid chromatography–mass spectrometry LC–MS [14,15], and immunoassays [16,17] had been developed for the determination of CH in different biological samples. Due to the complexity of the biological matrices and the trace levels of CH in real samples, the sample pretreatment procedures were the most tedious and time-consuming steps and the mainly possible source of imprecision and inaccuracy of the overall analysis. The common pretreatment methods were mainly including liquid–liquid extraction (LLE) [18], solid-phase extraction (SPE) [19], diphasic dialysis [20], solid-phase microextraction [4], supercritical fluid extraction [21], matrix solid-phase dispersion (MSPD) [22], and liquid–liquid microextration [23]. Among them, MSPD technique was very suitable for the simultaneous disruption, extraction and clean-up of solid, semi-solid and highly viscous samples [24–26]. It eliminated the most of the complications of performing classical LLE and SPE for solid matrixes by direct mechanical blending of sample matrix with an appropriate sorbent and a small volume of solvent for washing and elution steps. However, although each method had its advantages, further improved the selectivity, especially the selectivity for extraction the trace levels of analytes in complex samples was desired greatly.

Molecular imprinting is a synthetic approach to produce functionalized materials having specific molecular recognition properties for a given compound, its analogs, or for a single enantiomer [27–29]. The application of these synthetic polymers as sorbents allowed the analytes of interest to be pre-concentrated

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while simultaneously removed the interferences from the sample matrix, so that selective enrichment and cleanup were obtained, resulting in a higher accuracy and a lower detection limit in the subsequent analysis [30,31]. In recent years, molecularly imprinted polymers prepared using CH as template had been applied as special sorbents to extract CH from several biological samples [32–34]. However, the template leakage was always observed in its actual applications, which affected the results of quantitative analysis.

The aim of this work was to synthesize new molecularly imprinted microspheres using butylamine and chloroaniline as dummy template and apply it as special sorbent of MSPD for selective extraction and determination of CH from chicken samples. The obtained dummy imprinted microspheres showed high affinity to CH, and as special MSPD sorbent improved the selectivity of the sample pretreatment procedure and overcame the drawbacks of template leakage in real sample application. The presented MI-MSPD-HPLC method combined the superiority of MIM and MSPD, and therefore it could be potentially applied for the determination of CH in complicated biological samples.

2. Experimental

2.1. Chemicals

Tert-butylamine, chloroaniline, methacryclic acid, chloroform, 2,2-azobisisobutyronitrile, and polyvinylpyrrolidone (PVP) were obtained from Huaxin Chemical Reagent Co. (Baoding, China). Ethylene glycoldimethacrylate was purchased from Sigma–Aldrich (Missouri, USA). Clenbuterol hydrochloride was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Acetone, methanol, acetonitrile, ammonia and hydrochloric acid were purchased from Huadong Chemical Reagent Co. (Tianjin, China). All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered through a 0.45 μm fiber membrane before use.

2.2. Instrumentation and conditions

HPLC analysis was performed using a LC-20A system equipped with two LC-20AT Solvent Delivery Units, a SUS-20A gradient controller, and a SPD-20A UV-VIS Detector (Shimadzu, Kyoto, Japan). An N-2000 Chromatographic workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as the data acquisition system. The analytical column (Venusil XBP C_{18}, 5 μm, 250 mm × 4.6 mm I.D.) was obtained from Bonna-Agela Tech. (Tianjin, China). The mobile phase was water–methanol (65:35, *v/v*, containing 0.2‰ trifluoroacetic acid, pH 2.8) with a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL and the detection wavelength of the detector was set at 210 nm.

2.3. Synthesis of the MIM

1.0 mmol of tert-butylamine, 1.0 mmol of 2-chloroaniline, 4.0 mmol of methacryclic acid, 25.0 mmol of ethylene glycoldimethacrylate, and 120 mg of 2,2-azobisisobutyronitrile were dissolved in 15 mL chloroform and ultrasonically vibrated for 3.0 min. Then, this chloroform solution was added dropwise to 60 mL of water solution (1.5 g of PVP was dissolved) at 600 rpm under a nitrogen stream. After polymerization at 60 °C for 24 h, the obtained MIM in the polymerization solution was filtered with 0.45 μm membrane, and washed with methanol-acetic acid (9:1, v/v) and methanol to remove the template and residual monomer, and then dried at 40 °C under vacuum. Non-imprinted microsphere (NIM,) was prepared in a fashion analogous to that of the MIM but without the inclusion of templates.

2.4. MI-MSPD procedure

0.1 g of chicken sample and 0.1 g of MIM sorbent were placed in a small glass mortar and blended together using a glass grinder until complete disruption and dispersion of the sample on the solid support. The homogenized mixture was transferred into an empty cartridge (50 mg of MIM was pre-packed in the bottom) and rinsed with 2.0 mL of water, and then eluted by 3.0 mL of acetonitrile-acetic acid (95:5, v/v). The eluate was evaporated to dryness under vacuum and then re-dissolved in 150 μL of mobile phase for further HPLC analysis. The extraction efficiency was calculated as the percentage of the analyte (n_a) extracted in the final solvent for HPLC analysis with the total analyte (n_0) in samples.

3. Results and discussion

3.1. Synthesis of the MIM

Several imprinted polymers had been synthesized using CH as template and applied them as SPE sorbents for extraction of CH from various samples, however, there always suffered from template leakage in real application which affected the results of quantitative analysis. Therefore, in order to obtain the MIM with special recognition to CH and eliminate the effect of template leaking on quantitative analysis, tert-butylamine and 2-chloroaniline were chosen as dummy template (similar tridimensional structures or recognition site to CH) to prepare MIM. The MIM obtained at the molar ratio of 2:4:25 (template/monomer/crossing reagent) showed good mechanical strength and affinity to CH. To further improve the molecular recognition of the MIM in biological samples, aqueous suspension polymerization using PVP as dispersion agent was adopted. The morphology of the MIM and NIM (Fig. 1) evaluated by scanning electron microscope (SEM) revealed that the MIM were monodisperse and spherical with average diameters distribution from 2 to 5 μm. Moreover, the surface of them was porous and rough, which was suitable for rebinding or releasing the target molecules from its surface. The pore diameter distribution of the MIM and NIM determined by a JW-BK112 specific surface area and pore size analyzer revealed that both of them are multi-porous polymers (less than 20 nm). The pore volumes and specific surface areas from nitrogen adsorption experiments were 0.312 $cm^3\ g^{-1}$ and 205.6 $m^2\ g^{-1}$ for MIM, 0.315 $cm^3\ g^{-1}$ and 209.3 $m^2\ g^{-1}$ for NIM, respectively. The similar surface areas and pore volumes of MIM and NIM indicated the selectivity of the MIM was due to special imprinted recognition.

Dynamic adsorption experiment was employed to evaluation the adsorption ability of the sorbent to CH. 0.25 g of MIM, NIM, OASIS HLB and C_{18} were employed as sorbents for SPE column and 0.2 mL of 10 μg mL^{-1} CH solution was loaded on each column per time, the post-column solution was used for determination the level of the existing CH. The results showed that the maximum bear volume of MIM, NIM, OASIS HLB and C_{18} was 9.8 mL, 5.2 mL, 8.8 mL and 7.6 mL respectively. Furthermore, a CH structural analog of salbutamol (10 μg mL^{-1}) was also employed for dynamic adsorption to further evaluation the recognition properties of the MIM. The results indicated that the maximum bear volume of salbutamol on MIM was 8.0 mL, which was below CH on the MIM. All the above indicated that MIM had higher affinity and adsorption capacity to CH, it demonstrated the special imprinted recognition of the MIM.

The molecular recognition ability of MIM was much dependent on shape and functional group complementarities. The MIM should