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Determination of clenbuterol from pork samples using surface molecularly imprinted polymers as the selective sorbents for microextraction in packed syringe

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ABSTRACT

In this study, a selective sample pretreatment procedure combing surface molecularly imprinted polymers and microextraction in packed syringe (SMIPs-MEPS) was developed for the analysis of clenbuterol (CLB) from pork samples. SMIPs for CLB were synthesized on silica gel particles through a sol–gel process. A series of characterization and adsorption experiments revealed that the SMIPs exhibited porous structures, good thermal stability, high adsorption capacity and a fast mass transfer rate. The obtained SMIPs were employed as selective sorbents of SMIPs-MEPS for extraction of CLB from pork samples. Several parameters affecting the extraction efficiency were investigated, including the pH of sample solution, number of draw-eject cycles, volume of sample, type and volume of washing solution, and the type and volume of elution solution. Under the optimized conditions, a simple and rapid method for the determination of CLB from pork samples was established by coupling with high performance liquid chromatography (HPLC). The whole pretreatment process was rapid and it can be accomplished with 2 min. The limit of quantitation and the limit of detection for CLB were 0.02 and 0.009 $\mu g\,kg^{-1}$, respectively. The average recoveries of CLB at three spiked levels ranged from 86.5% to 91.2% with the relative standard deviations (RSD) $\leq$6.3%.

1. Introduction

In recent years, simplification and miniaturization in sample pretreatment techniques by reducing the amount of solvents used has been the dominant trend in green analytical chemistry. Microextraction in packed syringe (MEPS), which was introduced by Abdel-Rehim, is a new, fast, environmentally friendly, and efficient method for sample pretreatment [1]. In MEPS, a small amount of packed sorbent is packed inside the barrel of a syringe, and sample extraction is achieved in the packed bed. The MEPS sorbent bed is integrated into a liquid handling syringe that allows for low void volume sample manipulations either manually or in combination with laboratory robotics [2]. MEPS can be connected on-line to the analytical instrument for automated methods or it can be used for on-site sampling. This new extraction method has been widely used in environmental water analysis [3,4] and biological sample analysis [5,6]. Nevertheless, the commercially available MEPS sorbents (such as C_8, C_{18}, strong cation exchanger (SCX), etc.) are lack of selectivity, which commonly lead to coextraction of impurities from sample matrix. Thus, new sorbents such as molecularly imprinted polymers (MIPs) were increasingly developed to meet the need of selective extraction of analytes from complex matrix.

MIPs are synthetic polymers which exhibited specific recognition sites for a target analyte, which have the advantages of high selectivity, easy preparation, and high chemical stability [7–11]. Techniques that combine MIPs and MEPS have been applied for the extraction of drugs from biological samples [10,11]. However, the MIPs in the previous studies were prepared by traditional bulk polymerization. The resultant polymers had to be crushed, ground and sieved to obtain microparticles, which have irregular size, low binding efficiency, poor site accessibility and poor kinetics of binding toward the template molecules [12].

A promising solution to the aforementioned problems with MIPs is the development of surface molecularly imprinted polymers (SMIPs). SMIPs are usually fabricated on the surface of the

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support through sol–gel process and are used to incorporate template molecules into rigid inorganic or inorganic–organic networks [13]. The imprinted binding sites are often situated on the polymer layer. Preparation of SMIPs offers the inherent advantage of avoiding crushing and sieving steps, and the resultant SMIPs also lead to a more homogeneous distribution of binding sites, higher selectivity, faster mass transfer, and improved binding kinetics [14–16]. These polymers have attracted great attention for their advantages over the traditional MIPs [15,17]. Consequently, the study of SMIPs for developing an economic and efficient sorbent seems worthwhile.

To evaluate the potential of SMIPs as a selective sorbent for MEPS, clenbuterol (CLB) was studied as the model compound. CLB is a β_2-adrenergic agonist that can be used for the treatment of asthma. However, it is illegally used as a nutrient-repartitioning agent for commercially grown livestock [18]. Accumulation of CLB in human foodstuffs causes severe threats to human health, such as muscle tremors, headache and palpitations, etc. [19,20]. Although it is banned for growth promotion in animal production in China and European Union, the use of CLB remains attractive to swine producers because it can improve feed efficiency. Therefore, a rapid, simple, and sensitive analytical method is required to monitor residual CLB in food samples. Current techniques of sample preparation for extracting CLB from complex samples involve liquid–liquid extraction (LLE) [21], SPE [22], liquid–liquid microextraction (LLME) [23] and solid-phase microextraction (SPME) [24]. However, the conventional LLE and SPE procedures are time consuming, and require large quantities of toxic organic solvents. The LLME technique needs a dedicated syringe pump for every extraction. SPME fibers are expensive, and the fiber coating is fragile and easily broken while handling. Therefore, there is a need to develop green analytical methodologies or to modify older methods to incorporate procedures that are simple, rapid and use smaller amounts of hazardous chemicals. To our knowledge, the application of SMIPs combined with MEPS (SMIPs-MEPS) for extracting CLB from samples has not been reported.

In the present study, a novel strategy was developed to prepare SMIPs as sorbent for MEPS for selective adsorption of CLB. The SMIPs were directly synthesized on silica gel particles through a sol–gel process. Characteristics of the SMIPs, such as surface morphology, thermal stability, and adsorption performances, were investigated in detail. SMIPs-MEPS procedure followed by high performance liquid chromatography (HPLC) was applied for selective determination of CLB in pork samples. Surface molecularly imprinted solid-phase extraction (SMIPs-SPE) procedure was also used for comparison with SMIPs-MEPS procedure. The scientific novelty of the present work is the use of this new MEPS technique with SMIPs as the packing sorbents.

2. Experimental

2.1. Chemicals and reagents

CLB was purchased from Jinhe Pharmaceutical Co., Ltd. (Wuhan, China). Ractopamine and ambroxol hydrochloride were purchased from Sigma-Aldrich (New Jersey, USA). Salbutomol was purchased from Cunyi Chemical Co., Ltd. (Jiangsu, China). Terbutaline sulfate was purchased from Gangzheng Pharmaceutical Co., Ltd. (Wuhan, China). Ephedrine hydrochloride was purchased from Aike Pharmaceutical Co., Ltd. (Chifeng, China). 3-Aminopropyltriethoxysilane (APTES) and tetraethoxysilane (TEOS) were purchased from J&K Chemical Co., Ltd. (Beijing, China). Ultrapure water from a Milli-Q purification system (Millipore, USA) was used in preparing mobile phase and sample solutions. HPLC-grade methanol and acetonitrile were purchased from Kemite Co. (Tianjin, China). All other chemicals were of analytical grade and obtained from local suppliers. Silica gel particles (400 μm, i.d.,) were provided by Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China). Empty SPE cartridges (6 mL) were purchased from Shenzhen Doudian Co. (Shenzhen, China).

Individual stock solutions were prepared at a concentration of 5 mg mL^{-1} in acetonitrile. Mixed working standard solution (100 μg mL^{-1}) was prepared daily by diluting the stock solutions with water.

2.2. Instrumentation

The LC system was composed of a LC-10ATvp pump, a SPD-10Avp spectrophotometer, a CBM-10Avp communications bus module, a LC Solution work station (all from Shimadzu, Kyoto, Japan), a AT-330 column oven(Tianjin, China). The LC conditions were: Himadzu VP-ODS column (250 × 4.6 mm i.d., 5 μm), a 25 °C column temperature, methanol–0.1% ammonium acetate (30:70, v/v) mobile phase, a 1.0 mL min^{-1} flow rate and detection at 225 nm. The shaker was performed by a SHZ-82 Vapour-bathing Constant Temperature Vibrator (Jintan, China).

2.3. Preparation of SMIPs

Silica gel particles were activated by a reported method [13]. Silica gel particles (20 g) and 6 mol L^{-1} hydrochloric acid (250 mL) were mixed in a round-bottom flask equipped with a mechanical stirring. The mixture was refluxed at 110 °C with continuous stirring for 10 h. Through filtrating and washing with water, activated silica gel was collected and then dried at 70 °C for 10 h.

CLB (template, 5 mmol) were dissolved in methanol (10 mL), and then APTES (functional monomer, 10 mmol) and TEOS (cross-linker, 40 mmol) were added to the solution. The resulting mixture was stirred for 30 min before activated silica gel (3 g in 8 mL methanol) was added to it. After the mixture was stirred for 10 min at 600 rpm, 1 mol L^{-1} hydrochloric acid (catalyst, 2 mL) was added to it. Polymerization was performed at room temperature under magnetic stirring at 600 rpm for 20 h. After polymerization, the polymers were isolated by centrifugation and then washed with methanol–1 mol L^{-1} hydrochloric acid solution (90:10, v/v) until no CLB in the washings was detected by HPLC. Finally, the resulting SMIPs were washed with water until the washings became neutral, and dried at 70 °C for 10 h. As a control, surface non-imprinted polymers (SNIPs) were also prepared without CLB in the same procedure.

2.4. Characterization of SMIPs and SNIPs

Fourier transform infrared (FTIR) spectra (4000–400 cm^{-1}) were recorded on a Thermo Nicolet Nexus 330 FTIR spectrometer (Madison, USA). Scanning electron microscopy (SEM) images were obtained using a TM-1000 scanning microscope (Hitachi, Japan). The surface areas of SMIPs and SNIPs were measured by nitrogen adsorption experiments using a physical chemistry analyzer ASAP-2020 C (Mckesson, USA). The specific surface areas were calculated by Brunauer–Emmett–Teller (BET) method. Thermogravimetric analysis (TGA) (80–800 °C) was performed with a SDT Q600 thermogravimetric analyzer (TA, New Castle, USA).

2.5. Adsorption experiments

To investigate the adsorption capacities, 80 mg of SMIPs or SNIPs was added to 3 mL of acetonitrile solution containing 5–750 μg mL^{-1} CLB. After shaking for 60 min at room temperature, an aliquot of each sample was centrifuged for 15 min. The free CLB concentration in the supernatants was measured by HPLC.