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

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A B S T R A C T

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 μg 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) < 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 C₈, C₁₈, 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...
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 β2-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). Salbutamol 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 filtering 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.
Table 1
Optimization of preparation conditions for SMIPs and SNIPs.

<table>
<thead>
<tr>
<th>The mole ratio of template:functional monomer:cross-linker</th>
<th>Adsorption capacity of MIPs (μg g⁻¹)</th>
<th>Adsorption capacity of NIPs (μg g⁻¹)</th>
<th>Polymers yields</th>
<th>Imprinting factor*</th>
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</thead>
<tbody>
<tr>
<td>1 1:1:1</td>
<td>202.4</td>
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<td>1.0</td>
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<tr>
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<td>229.3</td>
<td>Poor</td>
<td>1.0</td>
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<tr>
<td>3 1:4:8</td>
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<td>1.1</td>
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<td>288.0</td>
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<td>1.1</td>
</tr>
<tr>
<td>5 1:2:8</td>
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<td>207.4</td>
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<td>3.4</td>
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<tr>
<td>7 1:4:16</td>
<td>491.0</td>
<td>211.7</td>
<td>High</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Imprinting factor = Adsorption capacity of SMIPs/Adsorption capacity of SNIPs.

To investigate the adsorption kinetics, 80 mg of SMIPs was added to 3 mL of acetonitrile solution containing 250 μg mL⁻¹ CLB. After shaking at room temperature for different time (5–80 min), an aliquot of each sample was centrifuged for 15 min. The free CLB concentration in the supernatants was measured by HPLC.

To investigate the selectivity of SMIPs, structurally related compounds such as salbutamol, terbutaline, ractopamine, ambroxol and ephedrine were used as competing compounds. 80 mg of SMIPs or SNIPs was added to 3 mL of mixed working standard solution (100 μg mL⁻¹). After shaking at room temperature for 60 min, an aliquot of each sample was centrifuged for 15 min. The concentration of free analyte in the supernatants was measured by HPLC.

2.6. SMIPs-MEPS conditions

4 mg of SMIPs was manually packed into a 1 mL insulin syringe with a polyethylene frit was inserted at the bottom and at the top of the packed SMIPs. Prior to use, the SMIPs-MEPS syringe was conditioned with 2 mL of methanol and 2 mL water. 300 μL of CLB standard solution or sample solution was aspirated 25 times (draw−eject cycles in the syringe) at a speed of 50 μL s⁻¹ through the syringe containing the activated SMIPs. Then, the SMIPs-MEPS syringe was washed with 300 μL of acetonitrile−water solution (50:50, v/v) and eluted with 500 μL of methanol−1 mol L⁻¹ hydrochloric acid (90:10, v/v). The eluate was collected and evaporated to dryness under N₂ stream. Residues were redissolved with 50 μL of mobile phase for subsequent injection for HPLC analysis. Each extraction was performed in triplicate. Furthermore, to avoid carryover, the same SMIPs-MEPS syringe was cleaned six times with 300 μL of methanol−1 mol L⁻¹ hydrochloric acid (90:10, v/v) and then six times of 300 μL of water between extractions. The same SMIPs-MEPS syringe could be reused for 60 times without loss of extraction efficiency.

2.7. SMIPs-SPE conditions

In comparison to the SMIPs-MEPS procedure, extraction experiment onto SPE cartridges containing the same SMIPs material was carried out. The SMIPs-SPE was prepared by packing 400 mg of the SMIPs into an empty 6 mL SPE cartridge with a polyethylene frit was inserted at the bottom and at the top of the packed SMIPs. Prior to extraction, the SMIPs-SPE was conditioned with 4 mL of methanol and 4 mL water. 2 mL of CLB standard solution (0.1 μg mL⁻¹) or sample solution was loaded onto the SMIPs-SPE cartridge. The cartridge was washed with 1 mL × 1 mL of acetonitrile−water (50:50, v/v) and then eluted with 2 mL × 1 mL of methanol−1 mol L⁻¹ hydrochloric acid (90:10, v/v). The eluted fractions were collected and then evaporated to dryness under N₂ stream. Residues were redissolved with 100 μL of mobile phase for HPLC analysis.

2.8. Sample pretreatment

The pork samples, which were kindly supplied by a local farmer, were verified to be free of CLB by HPLC-MS. For the spiking analysis, 2 g of pork sample was spiked with different CLB amounts to achieve CLB concentration of 0.02, 0.04, 0.1, 1, 5, 25 and 50 μg kg⁻¹. After the sample was homogenized in a disintegrator, 4 mL of acetonitrile was added to precipitate the protein. The mixture was ultrasonicated for 5 min and then separated by centrifugation at 8000 rpm for 10 min. The supernatant was collected and dried under N₂ stream. Finally, the residual was dissolved in 1 mL ultrapure water for SMIPs-MEPS process.

3. Results and discussion

3.1. Preparation of SMIPs and SNIPs

In this study, APTES (functional monomer) was used to provide amines groups to interact with the hydroxyl and chlorine substituent groups of CLB. TEOS (cross-linker) was used to establish three-dimensional polymer network around the silica gel. Fig. S1 in Supplementary material shows the recommended scheme for the preparation process. Important parameters affecting the adsorption properties were optimized, including the mole ratio of the template (CLB):functional monomer (APTES):cross-linker (TEOS) and stirring rate. The optimization results are listed in Table 1. When the mole ratio of template: APTES:TEOS was 1:2:8, the resulting SMIPs were optimal because they afforded the highest imprinting factor. It can be explained that insufficient functional monomer led to insufficient functional groups, whereas excess functional monomer resulted in non-specific adsorption. In addition, insufficient cross-linker could not result in sufficient number of effective imprinting sites, whereas excess cross-linker deeply embedded template molecules into the network and reduced the number of effective imprinting sites. The influences of different stirring rates ranging from 200 to 600 rpm were investigated. It was found that the agglomeration of the SMIPs particles deceased with the increasing stirring rate. Therefore, 600 rpm was selected as the stirring rate in the polymerization process.

3.2. Characterization of SMIPs and SNIPs

Fig. 1 shows the SEM images of activated silica gel and SMIPs. The surface of activated silica gel was relatively smooth, whereas the SMIPs particles exhibited a rough surface with porous morphological structures, indicating that the SMIPs were synthesized on the surface of activated silica gel. Porous structures enhanced the adsorption rate of analyte and provided specific recognition cavities for the template molecules.
Fig. S2 in Supplementary material shows the FTIR spectra of activated silica gel, SMIPs and SNIPs. Fig. S2A shows strong bands at 3436.10 and 1652.40 cm\(^{-1}\) which were attributed to the –OH group vibration of activated silica gel. The band at 1120.46 and 973.69 cm\(^{-1}\) were attributed to the stretching vibration of Si–O–Si and Si–O–H, respectively. The bands at 794.64 cm\(^{-1}\) and 464.25 cm\(^{-1}\) were due to the Si–O vibrations. Compared with those of the activated silica gel, the characteristic spectral features of SMIPs (Fig. S2B) and SNIPs (Fig. S2C) were the peak of N–H at around 1522.33 cm\(^{-1}\) and C–H vibrations at 2799.63 cm\(^{-1}\), respectively [13]. These results indicated that the –NH\(_2\) groups were successfully grafted onto the surface of activated silica gel. The FTIR spectra of the SMIPs and SNIPs shows major bands in similar locations, indicating that the template added during the polymerization process did not affect the chemical composition of the polymers [25].

The surface areas of SMIPs, SNIPs and activated silica gel were 80.1, 110.3, and 277.8 m\(^{2}\)g\(^{-1}\), respectively. These results indicated that the SMIPs and SNIPs were synthesized on the surface of activated silica gel. It is notable that the surface area of SMIPs is smaller than that of SNIPs, suggesting that specific adsorption by SMIPs could be attributed to the imprinting effect but not to the high surface area. This inference agreed well with that reported in the literature [14].

Fig. S3 in supplementary material shows the thermograms of SMIPs and SNIPs. The small amount loss of SMIPs or SNIPs at 104 °C could be attributed to the vaporization of volatiles. Significant loss of mass occurred at 270 °C. The results suggested that the SMIPs and SNIPs exhibited relatively good thermal stability.

### 3.3. Adsorption study

#### 3.3.1. Adsorption isotherm

The amount of CLB adsorbed to SMIPs or SNIPs (\(Q_e, \mu g g^{-1}\)) was calculated through the following formula (Eq. (1)):

\[
Q_e = \frac{(C_0 - C_e)V}{m}
\]  

(1)

where \(C_0\) (\(\mu g mL^{-1}\)) is the initial CLB concentration, \(C_e\) (\(\mu g mL^{-1}\)) the equilibrium CLB concentration, \(V\) (mL) the sample volume, and \(m\) (mg) is the mass of the polymers.

Fig. 2A shows the adsorption isotherms of CLB to the SMIPs and SNIPs. The adsorbed CLB amount increased gradually with increased initial CLB concentration. In the meantime, the adsorbed CLB amounts to the SMIPs were higher than those to the SNIPs. Furthermore, when \(C_e\) was above 250 \(\mu g mL^{-1}\), \(Q_e\) approached saturation (1000 \(\mu g g^{-1}\)), which was higher than the reported value [26], indicated that the porous structures on the surface of activated silica gel could produce more specific recognition sites.

Subsequently, the Langmuir (Eq. (2)) and Freundlich (Eq. (3)) equations were used to analyze the equilibrium experimental data [27]. Linearized forms of the two isotherms are:

\[
\frac{C_e}{Q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}
\]  

(2)

\[
\ln Q_e = \ln K_F + \frac{1}{n} \ln C_e
\]  

(3)
where $q_m$ (µg g$^{-1}$) and $K_f$ (L g$^{-1}$) are the theoretical maximum adsorption capacity and Langmuir equilibrium constant, respectively. $K_f$ and $n$ are the Freundlich constants which are the indicators of adsorption capacity and adsorption intensity.

Calculated data corresponding to the Langmuir and Freundlich isotherms are shown in Supplementary material Table S1. The Freundlich isotherm was found to be more suitable than the Langmuir isotherm for describing the adsorption because of its larger correlation coefficient, illustrating that the adsorption of CLB to SMIPs took place at multilayers.

3.3.2. Adsorption kinetics

**Fig. 2** shows the adsorption kinetics of CLB to the SMIPs. The adsorption capacity increased rapidly with increased adsorption time and reached the equilibrium at about 25 min. This result indicated that the adsorption of CLB to the SMIPs exhibited a fast mass transfer rate, which might be attributed to the surface imprinting [25].

The adsorption kinetics was described by the Lagergren’s pseudo-first-order (Eq. (4)) and pseudo-second-order (Eq. (5)) equations [27]. Both the pseudo-first-order and pseudo-second-order rate equations are commonly employed in parallel, and one is often claimed to be better than the other according to a marginal difference in correlation coefficient.

$$\log(Q_e - Q_t) = \log Q_e - \frac{k_1 t}{2.303}$$  \hspace{1cm} (4)

$$\frac{t}{Q_t} = \frac{1}{k_2Q_e^2} + \frac{t}{Q_e}$$  \hspace{1cm} (5)

where $Q_t$ (µg g$^{-1}$) is the amount of CLB adsorbed to SMIPs at time $t$ (min), $k_1$ (min$^{-1}$) and $k_2$ (g min µg$^{-1}$) are the pseudo-first-order and pseudo-second-order adsorption rate constants, respectively.

The pseudo-first-order and pseudo-second-order kinetic models were used to evaluate the kinetics of CLB adsorbed to SMIPs. As illustrated in Supplementary material Table S2, the pseudo-second-order model with a higher value of the correlation coefficient ($R^2 = 0.9454$), provided a better fit with kinetics data than did the pseudo-first-order model. This result indicated that the adsorption of CLB to SMIPs was a chemical adsorption process, and the adsorption rate was controlled by chemical reaction mechanism.

3.3.3. Selectivity test

The selectivity of the SMIPs was investigated by using salbutamol, terbutaline, ractopamine, ambroxol and ephedrine as structure analogues of CLB. **Fig. 3** shows the amount of analytes adsorbed to SMIPs or SNIPs. The amount of CLB adsorbed to SMIPs was higher than that of salbutamol, terbutaline, ractopamine, ambroxol and ephedrine. In contrast, SNIPs showed similar selectivity for CLB as for salbutamol, terbutaline, ractopamine, ambroxol and ephedrine. These results indicated that the SMIPs selective recognized CLB and did not recognize the structure analogues.

3.4. Investigation of SMIPs-MEPS conditions

The performance of SMIPs-MEPS was measured by comparison with the commercial C$18$-MEPS and mixed-mode MEPS for the preconcentration of CLB in pork sample. The recovery using SMIPs-MEPS (92.5%) was much higher than that of the commercial C$18$-MEPS (75.1%) and mixed-mode MEPS (83.9%), indicating that the SMIPs-MEPS exhibited higher affinity to CLB.

To evaluate the applicability of SMIPs-MEPS, parameters affecting the extraction efficiency of SMIPs-MEPS were optimized, including the pH of sample solution, number of draw-eject cycles, volume of sample, type and volume of washing solution, and type and volume of elution solution.

3.4.1. pH of sample solution

**Fig. 4A** shows the influence of sample pH (pH was adjusted 3.0–10.0 with hydrochloric acid or sodium hydroxide) on the recovery of CLB. The recovery increased with the pH from 3.0 to 7.4, and then remained at a specific value at pH 7.4. So, the optimal sample pH was 7.4. This behavior was attributed to the protonation of CLB in acid conditions, in which clenbutrol formed hydrogen bonds with amino groups on the imprinted sites.

3.4.2. Number of draw-eject cycles and volume of sample

In MEPS, the sample can be drawn into and expelled from the syringe for a number of cycles without discarding it. **Fig. 4B** shows the effect of the number of draw–eject cycles (from 2 to 30) and the impact of sample volume (from 100 to 300 µL) on the recovery during the optimization process. The recovery increased from 22.1% to 89.9% with increased sample volume and the number of extraction cycles. No significant differences in recovery were observed when the number of draw-eject cycles was increased from 25 to 30. A sample volume of 300 µL provided good recovery and did not require increasing the number of extraction cycles. Therefore, 25 of extraction cycles and 300 µL of sample were selected conditions for further experiments to save extraction time and to extend the lifetime of SMIPs-MEPS syringe. The total extraction time was about 2 min.

3.4.3. Type and volume of washing solvent

**Fig. 4C** shows the effect of different washing solvents on the recovery of CLB, including acetone, hexane, water, methanol, acetonitrile, methanol–water (50:50, v/v) and acetonitrile–water (50:50, v/v) of different volumes. The highest recovery of CLB was obtained when acetonitrile–water (50:50, v/v) was employed as the washing solvent. Furthermore, the effect of different volumes (from 100 to 600 µL) of acetonitrile–water (50:50, v/v) was investigated. No significant differences were observed when the volume...
was varied from 100 to 300 μL, whereas a slight decrease in recovery was observed when the volume was varied from 400 to 600 μL. Therefore, 300 μL of acetonitrile-water (50:50, v/v) was used as the washing solvent for further work.

3.4.4. Type and volume of eluent

Fig. 4D shows the effect of different eluents on the recovery of CLB, including dichloromethane, acetonitrile, methanol, acetonitrile–1 mol L⁻¹ hydrochloric acid (90:10, v/v), methanol–1 mol L⁻¹ hydrochloric acid (90:10, v/v), methanol–0.2% ammonia (90:10, v/v), and acetonitrile–0.2% ammonia (90:10, v/v). Methanol–1 mol L⁻¹ hydrochloric acid (90:10, v/v) offered the best recovery of CLB. To optimization of the volume of eluent, various volumes of methanol–1 mol L⁻¹ hydrochloric acid (90:10, v/v) (ranging from 100 to 600 μL) were investigated. The results showed that the recovery of CLB increased as the volume of eluent increased from 100 to 500 μL, and then retain constant at 500–600 μL. Thus, to reduce the consumption of solvent, 500 μL of methanol–1 mol L⁻¹ hydrochloric acid (90:10, v/v) was used as the eluent.

3.4.5. Carryover

In contrast to the conventional SPE process, which uses disposable cartridges or membranes, MEPS requires a detailed evaluation of potential carryover, as it involves reuse of the sorbent. For this reason, it is necessary to choose a suitable cleanup solution after each extraction process. Thus, the SMIPs-MEPS syringe was cleaned six times with 300 μL of methanol–hydrochloric acid (90:10, v/v) and six times of 300 μL of water between extractions, which gave a carryover value below 0.6%.

3.5. Reproducibility and stability

To investigate the syringe-to-syringe and batch-to-batch reproducibility, three SMIPs-MEPS syringes prepared in the same batch and three SMIPs-MEPS syringes from different batches were used to extract CLB in the spiked samples, and the relative standard deviations (RSD%) were calculated. The SMIPs-MEPS syringes had satisfactory reproducibility of extraction efficiency for syringe-to-syringe (RSD% < 6.5%) and batch-to-batch (RSD% < 10.3%). Moreover, the SMIPs-MEPS syringes showed high stability and could be reused for least 60 times without significant loss of extraction efficiency.

3.6. Comparative study of SMIPs-MEPS and SMIPs-SPE

The SMIPs-MEPS protocol was compared against the SMIPs-SPE protocol for CLB extraction. The principal parameters of SMIPs-MEPS such as the type of washing and elute solution, were transferred to the SMIPs-SPE protocol. The results in Table 2 demonstrated that the SMIPs-MEPS protocol required a shorter extraction time, lower sample volume, and less consumption of organic solvents.
3.7. Method validation

The SMIPs-MEPS-HPLC method was validated in terms of specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), repeatability, intermediate precision, and accuracy. The specificity of this method was evaluated by analyzing of blank samples. Typical chromatograms shown in Fig. 5 indicates that there were no interfering peaks from endogenous compounds at the retention time of CLB, and the SMIPs-MEPS could remove potential matrix interferences in the matrix and effectively enrich CLB to sufficient purity. The calibration curve was constructed by using the areas of the chromatographic peaks measured at seven spiked levels ranging from 0.02 to 50 μg kg⁻¹. For each point, three replicate extractions were performed. The results are summarized in Table 3. Good linearity of the analytical response was obtained in the range of 0.02–50 μg kg⁻¹ ($R^2 = 0.9989$). The LOD and LOQ based on signal-to-noise ratio of 3:1 and 10:1 were 0.009 and 0.02 μg kg⁻¹, respectively. This level of sensitivity effectively enabled trace residue determination in the target matrix.

The repeatability (intra-day) and intermediate (inter-day) precision of this method were assessed using spiked samples at three concentrations (0.2, 1 and 50 μg kg⁻¹). Analyses were done five replicates for each concentration on the same day and on three consecutive days, respectively. The results showed that the RSD of repeatability ranged from 4.2% to 6.4%, and that of intermediate precision ranged from 6.8% to 10.1%. Averages of intra- and inter-day accuracy ranged from 93.2% to 101.6% and from 90.7% to 95.5%, respectively. Recoveries of CLB at three spiked concentrations (0.2, 1 and 50 μg kg⁻¹) were in range of 86.5–91.2% with RSD of ≤6.3%.

Table 4 compares the proposed method with previously reported methods. The LOD of this method was either similar or better than those previously published methods for CLB determination. The SMIPs-MEPS-HPLC method developed in this work exhibited better characteristics such as sensitivity and facility. This study also offered a new method to determine other β2-adrenergic agonists in different samples.

Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SMIPs-MEPS</th>
<th>SMIPs-SPE</th>
</tr>
</thead>
<tbody>
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<td>Sorbent amount (mg)</td>
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<tr>
<td>Sample volume (mL)</td>
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<td>Extraction time (min)</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Consumption of organic solvents (mL)</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Reuse times</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. (A) Effect of pH on the recovery of CLB, (B) effect of draw-eject cycles and sample volume on the recovery of CLB, (C) effect of washing solvent on the recovery of CLB and (D) effect of eluent on the recovery of CLB.

Fig. 5. Typical chromatograms (A) standard CLB solution, (B) blank pork sample, (C) spiked pork sample before SMIPs-MEPS, and (D) spiked pork sample after SMIPs-MEPS.
Table 3
Linearity, LOQ, LOD, recovery, precision and accuracy of CLB in spiked pork samples (n = 5).

<table>
<thead>
<tr>
<th>Spiked level (μg kg⁻¹)</th>
<th>Linearity</th>
<th>LOQ (μg kg⁻¹)</th>
<th>LOD (μg kg⁻¹)</th>
<th>Recovery (%)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Precision (RSD %)</td>
<td>Accuracy (%)</td>
<td>Precision (RSD %)</td>
</tr>
<tr>
<td>0.02</td>
<td>Y = 937990X - 8941.0</td>
<td>0.02</td>
<td>0.009</td>
<td>85.5</td>
<td>6.4</td>
<td>93.2</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>91.2</td>
<td>5.5</td>
<td>95.0</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td>90.4</td>
<td>4.2</td>
<td>101.6</td>
</tr>
</tbody>
</table>

Table 4
Comparison of different methods for CLB determination.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>LOD</th>
<th>Recovery (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLE-HPLC</td>
<td>Porcine liver</td>
<td>1.2 ng g⁻¹</td>
<td>90.6–91.5%</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>0.006 μg kg⁻¹</td>
<td>97.5–106.2%</td>
<td></td>
</tr>
<tr>
<td>SPE-HPLC-MS/MS</td>
<td>Milk</td>
<td>0.07 μg kg⁻¹</td>
<td>87.9–103.6%</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>0.07 μg kg⁻¹</td>
<td>95.2%</td>
<td>[29]</td>
</tr>
<tr>
<td>SPE-HPLC-MS/MS</td>
<td>Milk</td>
<td>0.10 μg L⁻¹</td>
<td>99.6–55.2%</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Porcine meat</td>
<td>0.07 μg kg⁻¹</td>
<td>93.79–109.04%</td>
<td>[23]</td>
</tr>
<tr>
<td>SPE-LLME-HPLC</td>
<td>Porcine liver</td>
<td>0.07 μg kg⁻¹</td>
<td>85.6–91.2%</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Bovine muscle</td>
<td>0.15 ng g⁻¹</td>
<td>95.2%</td>
<td>[29]</td>
</tr>
<tr>
<td>MISPE-HPLC-MS</td>
<td>Water</td>
<td>0.07 μg kg⁻¹</td>
<td>95.2%</td>
<td>[28]</td>
</tr>
<tr>
<td>MISPE-LC-MS/MS</td>
<td>Calves urine</td>
<td>0.03 ng mL⁻¹</td>
<td>49.6–55.2%</td>
<td>[30]</td>
</tr>
<tr>
<td>LLME-GC-MS</td>
<td>Urine</td>
<td>0.10 ng mL⁻¹</td>
<td>93.79–109.04%</td>
<td>[23]</td>
</tr>
<tr>
<td>SMIPs-MEPS-HPLC</td>
<td>Pork</td>
<td>0.009 μg kg⁻¹</td>
<td>85.6–91.2%</td>
<td>[28]</td>
</tr>
</tbody>
</table>

3.8. Real samples analysis

To verify the practical applicability of the newly developed method, 15 pork samples obtained from different markets were analyzed through the SMIPS-MEPS-HPLC method. The results indicated that CLB was not detected in the pork samples. The accuracy of this method was restudied by a recovery test carried out with three different spiked levels of CLB in real pork samples. The results showed that the accuracy ranged between 92.8% and 104.2% for CLB, indicating that this method was reliable and could be used for the determination of CLB in pork samples.

4. Conclusions

In this work, SMIPS for CLB were prepared by using a sol–gel process and then applied for the first time as the selective sorbents of MEPS. Characteristics and adsorption experiments revealed that the SMIPS exhibited porous structures, good thermal stability, high adsorption capacity and a fast mass transfer rate. The SMIPS-MEPS procedure followed by HPLC was applied for rapid and selective determination of CLB in pork samples. Compared with SMIPS-SPE, the SMIPS-MEPS procedure required lower sample volume, shorter extraction time, and less consumption of organic solvents. Results of practical application revealed that the SMIPS-MEPS-HPLC method could be used to develop an economic, environmentally friendly, and efficient method for CLB analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2013.12.022

References


