A novel surface molecularly imprinted polymer as the solid-phase extraction adsorbent for the selective determination of ampicillin sodium in milk and blood samples

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Abstract
Surface molecularly imprinted polymers (SMIPs) for selective adsorption of ampicillin sodium were synthesized using surface molecular imprinting technique with silica gel as a support. The physical and morphological characteristics of the polymers were investigated by scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), elemental analysis and nitrogen adsorption–desorption test. The obtained results showed that the SMIPs displayed great adsorption capacity (13.5 μg/mg), high recognition ability (the imprinted factor is 3.2) and good binding kinetics for ampicillin sodium. Finally, as solid phase extraction adsorbents, the SMIPs coupled with HPLC method were validated and applied for the enrichment, purification and determination of ampicillin sodium in real milk and blood samples. The averages of spiked accuracy ranged from 92.1% to 107.6%. The relative standard deviations of intra- and inter-day precisions were less than 4.6%. This study provides a new and promising method for enriching, extracting and determining ampicillin sodium in complex biological samples.

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1. Introduction
Ampicillin sodium, a member of β-lactam antibiotics (BLAs), has been extensively used in treating a wide range of diseases and infections which were caused by both gram-positive and gram-negative bacteria in humans and animals, such as infections by sensitive bacteria, gastrointestinal and urinary tract infections, meningitis, and septicemia [1]. As a broad-spectrum antimicrobial of the amino penicillin family, ampicillin sodium is active against microorganisms by inhibiting the cell wall synthesis during active multiplication [2].

Currently, the misuse and abuse of antibiotics are becoming a great concern along with drug resistance arising in the target microorganisms or the organisms being treated [1,2]. Antibiotic residues in human, medical wastes, farms, and pharmaceutical and hospital sewage can contaminate natural environments. Long-term exposure to antibiotics in aquatic environment may be associated with an increased risk of development and spread of antibiotic resistance, posing a serious threat to public health [3]. As a veterinary drug, ampicillin sodium is widely used to increase the rate of weight gain or to improve the feed efficiency in cattle breeding. In recent years, the excessive or improper usage of ampicillin sodium in some cases may lead to the presence of residues in edible animal tissues and body fluids, which is potentially toxic and dangerous for human health [4]. At present, the reasonable and correct use of BLAs for animals feeding has been emphasized in China, Europe and some other countries. Nevertheless, the illegal use of BLAs still exists because of their low price and consistent antibiotic effectiveness. Therefore, it is very necessary to establish a sensitive and selective analytical method to detect the residual of BLAs in environmental water and biological samples.

Several analytical methods have been reported to determine the levels of BLAs in environmental water and foods, such as capillary electrophoresis (CE) [5–7], chemiluminescence [8,9] and liquid chromatography (LC) [10,11]. Because of the interference of...
complex matrix in samples, these analytical methods require sample preparation to obtain the desired sensitivity before chromatographic separation and determination, including extraction, purification and enrichment. Generally, the samples are pretreated using liquid–liquid extraction (LLE) or solid-phase extraction (SPE). SPE is the currently dominating technique for the extraction of a target compound from different samples including food, environment, and pharmaceutical and biological samples [12–14]. Compared with LLE, SPE is simple and fast, and less solvent consumption. However, the traditional media (including C8, silica gel, Al2O3, etc.) of SPE have the disadvantage of poor selectivity and low recovery for target molecules. Therefore, it is necessary to develop high selectivity and specific recognition adsorbents for SPE.

Recently, molecularly imprinted polymers (MIPs) have been recognized as useful sorbent materials for SPE [15,16]. Molecular imprinting, as an outstanding technique for tailor-made preparation of synthetic polymer-receptors for given molecules, is a rapidly developing field in recent years [17,18]. Because of the specific recognition and high selectivity, MIPs have been applied as a potential clean-up system in many different sample matrices. MIPs also exhibit other favorable properties such as low cost, high stability, reusability, and long-term storage [19,20]. Molecularly imprinted solid-phase extraction (MISPE), as a relatively new concept in the clean-up of samples, has attracted much attention in different areas. However, most of conventional MIPs have been prepared by traditional bulk polymerization, which show conspicuous imprinting properties but have some limitations including irregular materials shape, poor site accessibility, slow mass transfer, incomplete templates removal, low binding capacity and serious templates leakage [21–23]. Compared with traditional polymerization, surface molecular imprinting is another approach receiving significant attention in the past few years. The surface molecularly imprinted polymers (SMIPs) are a new type of selective sorbent which is prepared by anchoring MIPS shells on the spherical surface (silica, chitosan or Fe3O4 particles) via a surface molecular imprinting process [24,25]. The surface-imprinted technique can get SMIPs with uniform morphology size, high specific surface, improved recognition, high binding capacity and fast mass transfer rate [26,27].

In this work, we reported the preparation of silica-SMIPs particles via surface molecularly imprinted polymerization using silica gel as the carrier, ampicillin sodium as the template molecule, methacrylic acid (MAA) as the functional monomer, and ethylene glycol dimethacrylate (EGDMA) as the cross-linker. The morphology and structure of SMIPs were characterized with scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), elemental analysis, and nitrogen adsorption–desorption analysis (TGA). The adsorption characteristics of SMIPs were also investigated. Finally, coupled with high performance liquid chromatography (HPLC), the SMIPs were employed for SPE and applied to determine the trace ampicillin sodium in milk and blood samples.

2. Experimental

2.1. Ampicillin sodium and reagents

Ampicillin sodium was purchased from Xi’an Woersen Biotechnology Co. (Shaanxi, China). Penicillin G was provided by Xi’an Renda Trading Co. (Shaanxi, China). Mezlocillin sodium was supplied by Jiangsu Hansi Medicine Co. (Jiangsu, China). 6-Aminopenicillanic acid (6-APA) was obtained from Zhengzhou Gehua Trading Co. (Henan, China). Penicilloic acid was synthesized in Pharmaceutical Analysis Laboratory in Xi’an Jiaotong University [28]. Clenbuterol hydrochloride was supplied by Huainan Jiameng Medicine Co. (Anhui, China). Silica gel (diameter: 10 μm; surface area: 407.34 m2/g) was purchased from Tokyo Chemical Co. (Tokyo, Japan). 3-Aminopropyltriethoxysilane (APTES) was purchased from J&K Chemical Co. (China). MAA was purchased from Tianjin Chemical Reagent Plant (Tianjin, China) and distilled under vacuum to remove inhibitors prior to use. EGDMA was obtained from Sigma-Aldrich (New Jersey, USA) and refined by distillation. 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai No. 4 Reagent Factory (Shanghai, China) and recrystallized from methanol before use. Methanol and acetonitrile of HPLC grade were supplied by Kemite Co. (Tianjin, China). Water was purified with Molement 1805b (Shanghai, China). All other chemicals were of analytical grade and obtained from local supplies. Empty SPE cartridges (2.5 mL) were purchased from Shanghai Zhiyu Medical Co. (Shanghai, China). Milk samples were purchased from a local supermarket in Xi’an, China. Blood samples were collected from the rats after gavage. The rats were identified by the Ethics Committee of Xi’an Jiaotong University (certificate no. 22-9601018).

2.2. Instruments and HPLC analysis

The adsorption selectivity of the SMIPs and surface molecularly non-imprinted polymers (SNIPs) was analyzed by HPLC. HPLC analysis was performed with a Shimadzu HPLC system (LC-2010A HT, Kyoto, Japan), equipped with an LC-2010AHT pump, an SPD-20A UV–vis detector and a CBM-102 workstation. The chromatographic separation was carried out with a Kromasil C8 column (250 mm × 4.6 mm, 5 μm) using acetonitrile–0.02 mol/L ammonium acetate solution (15:85, v/v) as mobile phase. The detection wavelength for ampicillin sodium was 254 nm. The flow rate was kept at 1.0 mL/min. The column temperature was maintained at 30 °C. The injection volume was 20 μL.

2.3. Preparation of SMIPs

The preparing procedure of SMIPs consists of three steps, i.e., surface activation of silica particles, surface amino-functionalization of activated silica particles, and surface molecularly imprinting of amino-functionalized silica particles. Firstly, 12 g of silica particles were mixed with 100 mL of 10% HCl and refluxed with continuous stirring for 24 h at 110 °C. Then the silica gel particles were collected by filtration, washed with ultrapure water until the pH was neutral, and dried at 60 °C for 24 h. Secondly, the activated silica particles (10 g), APTES (4 mL) and triethylamine (2 mL) were dispersed into 100 mL of toluene with continuous stirring in a 250 mL flask, and refluxed for 24 h at 110 °C. The particles were filtered, washed several times with methanol, and dried at 60 °C for 24 h. Then the amino-functionalized silica gels were obtained, and defined as modified silica gels. Finally, 0.25 mmol ampicillin sodium (0.0928 g) and 1.0 mmol MAA (84 μL) were dissolved in the mixed solvent (15 mL) of methanol–acetonitrile (2:1, v/v) in a conical flask. Then the obtained amino-functionalized silica particles (1.0 g) were dispersed in the solution. The mixed solution was put in a water bath for prepolymerization with continuous stirring at 25 °C for 12 h. After that, the cross-linker EGDMA (476 μL, 2.4 mmol) and the initiator AIBN (0.028 g) were added into the above solution. The mixture was thoroughly mixed and degassed under ultrasonication and stirring condition for 20 min. After purging with nitrogen gas for 20 min, the mixture was set in a water bath for polymerization at 50 °C for 24 h under stirring condition. The products were separated by filtration and washed with methanol to remove any residual solvents. Afterwards, the polymers were eluted by Soxhlet extraction with a mixture of methanol and acetic acid (8:2, v/v) for 48 h to remove the
template molecule. Then the polymers were sequentially washed with the mixture of acetonitrile–water (2:8, v/v) and methanol to neutral, and dried at 60 °C for 24 h. The SMIPs were obtained.

For comparison, the SNIPs were also prepared following the similar procedure in the absence of template molecules.

2.4. Morphological characterization

The surface morphology of silica gels, SMIPs and SNIPs were observed with a TM–1000 Scanning Electron Microscope (Hitachi, Japan). All samples were sputter-coated with gold before scanning. The infrared spectra of silica gels, SMIPs and SNIPs were recorded on a Thermo Nicolet Nexus 330 FT–IR spectrometer (Madison, USA) by pressed KBr tablets in the scanning range of 400–4000 cm⁻¹.

The adsorption isotherms were described by the Langmuir equation (Eq.(3)) and Freundlich equation (Eq.(4)) [29,30]:

\[ Q = \frac{Q_{c} Q_{m}}{C_{e} + Q_{m}} \]

where \( Q \) (μg/mg) is the adsorption amount; \( C_{c} \) (μg/mL) and \( C_{e} \) (μg/mL) are the initial and final solution concentration of ampicillin sodium, respectively; \( v \) (mL) is the solution volume; and \( m \) (mg) is the weight of the polymer particles.

The imprinting factor (IF) was defined as Eq. (2):

\[ IF = \frac{Q_{SMIPS}}{Q_{SNIPS}} \]

\[ Q_{SMIPS} \] or \( Q_{SNIPS} \) is the adsorption amount of SMIPs or SNIPs.

The adsorption isotherms were described by the Langmuir equation (Eq. (3)) and Freundlich equation (Eq. (4)) [29,30]:

\[ \log Q_{c} = \log K_{c} + \log \frac{1}{(K_{b} Q_{m})} \]

\[ \log Q_{c} = \log K_{c} + \log \frac{Q_{m}}{n} \]

where \( K_{c} \) (μg/mL) and \( Q_{c} \) (μg/mg) are the equilibrium concentration and the amount of ampicillin sodium adsorbed at equilibrium, respectively; \( Q_{max} \) (μg/mL) and \( K_{b} \) are the so-called maximum adsorption capacity and Langmuir equilibrium constant, respectively; \( K_{c} \) and \( n \) are the Freundlich constants, which are indicators of adsorption capacity and adsorption intensity.

To investigate the adsorption kinetic, 30 mg of SMIPs and 10 mL of ampicillin sodium water solution (300 μg/mL) were added into a conical flask. The mixture was shaken at 25 °C. At different time intervals (5, 10, 20, 30, 40, 50, 60, 90, and 120 min), the concentration of ampicillin sodium in the supernatant was analyzed by HPLC. The adsorption kinetic curve of SNIPs was carried out by the same procedure as SMIPs.

The adsorption selectivities were investigated by testing the binding capacities of SMIPs and SNIPs towards four structural analogues, i.e., 6-APA, penicillin G, penicillioic acid and mezlocillin sodium, and a non-analogue, i.e., clenbuterol. Test conditions were set as follows: the mass of polymer was 30 mg; the concentration of each solution was 300 μg/mL, \( v = 10 \) mL; the adsorption time was 90 min at 25 °C. The supernatants were diluted and the residue concentrations of the substances in the solution were determined by HPLC analysis.

2.6. Surface molecularly imprinted solid phase extraction (SMISPE) procedure

200 mg of SMIPs was packed in an empty SPE cartridge and used for SMISPE, in which two sieve plates were placed on both the top and bottom, respectively. The SMISPE column was washed with 10 mL of methanol–acetic acid (4:1, v/v) and activated by 5 mL of water and 5 mL of methanol, respectively. After loading 1 mL of sample, the SMISPE column was washed with 1 mL of dichloromethane, and then eluted with 3 mL of methanol–acetic acid (4:1, v/v). The eluents were evaporated to dryness at room temperature under nitrogen stream. The residues were redissolved in 0.5 mL of water and analyzed by HPLC.

2.7. Samples preparation

0.5 mL of milk sample was mixed with 1.5 mL of acetonitrile, and centrifuged at 12,000 rpm for 10 min. The supernatant was obtained and evaporated under gentle nitrogen to dryness. The residues were redissolved in 2 mL of water and loaded onto the SMISPE cartridge. The loading volume of each sample was 1 mL.

Blood samples were collected from the rats which were given 2.5 mL of ampicillin sodium (40 mg/mL) by gavage, and then collected blood after half an hour. Blood samples were treated as similar steps as milk samples described above.

2.8. Method validation and application to different samples

The SMISPE column coupled to liquid chromatography–ultraviolet (HPLC–UV) was developed to determine the trace ampicillin sodium in milk and blood samples. The calibration curve of the analytical method was constructed by measuring six different concentrations of ampicillin sodium in spiked biological samples in the range of 5, 10, 25, 50, 100, and 200 μg/mL after the SMISPE procedure. The limit of detection (LOD) and limit of quantification (LOQ) were defined as three or ten times of the ratio of signal to noise, respectively, and calculated by measuring the injection of the spiked samples after SMISPE process. To evaluate the accuracy and precision, samples spiked with ampicillin sodium were tested. The accuracy was evaluated by recovery. The precision was estimated by detecting relative standard deviation (RSD%) of intra- and inter-day tests.

3. Results and discussion

3.1. Characterization

3.1.1. SEM analysis

SEM was used to characterize the modified silica gels, SMIPs and SNIPs with different magnification. Fig. 1 shows that the modified silica gels display a smooth surface. On the contrary, SMIPs and SNIPs are different from the modified silica gels, and show visual differences in morphology. Due to the existence of the
porogen, a porous and loose structure was formed on the surface of silica gels. The SNIPs had rough surface, which was merely caused by the radical polymerization between the functional monomer and the cross-linker. Due to the prearranged polymer of template and functional monomer, the image of SMIPs structure appeared rougher and more porous than that of SNIPs, indicating that the presence of recognition sites in SMIPs attributed to the removal of template molecules.

3.1.2. FT-IR analysis

To confirm the successful modification on the surface of silica particles and preparation of SMIPs and SNIPs, we employed FT-IR to characterize them. FT-IR spectra of activated silica gels, modified silica gels, SMIPs and SNIPs are shown in Fig. 2. In Fig. 2a, the modified silica gels showed the vibration absorptions of C–N (1020–1300 cm\(^{-1}\)) and C–H (2943 cm\(^{-1}\)), indicating that APTES was successfully grafted onto the activated silica gels. Compared with the modified silica gels, the FT-IR spectra of SMIPs and SNIPs displayed the similar characteristic bands (Fig. 2b and c). The bands at about 3390 cm\(^{-1}\), 3275 cm\(^{-1}\), 1730 cm\(^{-1}\), and 1550 cm\(^{-1}\) were attributed to the stretching vibrations of –OH, –CH\(_3\), C=O and C=C of MAA or EGDMA, respectively, indicating that functional monomer MAA and cross-linking agent EGDMA were grafted on the surface of silica-gels and the SMIPs were successfully prepared.

3.1.3. TGA

Fig. 3 describes the TGA curves of the activated silica gels, modified silica gels, SMIPs and SNIPs. From room temperature to 100 °C, activated silica-gel particles had only about 5% weight loss (Fig. 3a), and this stage was around 100 °C corresponding to the

![Fig. 1. SEM images of modified silica gels (A and D), SMIPs (B and E) and SNIPs (C and F).](image)

![Fig. 2. The FT-IR spectra of modified silica gels (a), SMIPs (b) and SNIPs (c).](image)

![Fig. 3. The TGA curves of activated silica gels (a), modified silica gels (b), SMIPs (c) and SNIPs (d).](image)
release of physically adsorbed water on the surface of all the particles. All polymer particles were thermally stable up to 300 °C, and the complex processes of decomposition started at this temperature. With the temperature increasing to 800 °C, activated silica-gel particles had less weight loss. The weight loss of modified silica gels was about 10% and it had another remarkable decrease about 5% around 300–600 °C, indicating that APTES had been grafted onto the surface of the activated silica particles. During the whole process, the weight loss of SMIPs and SNIPs had similar trend. They both had a sharp loss around 300–600 °C corresponding to the degradation of polymer particles. The weight loss of SMIPs and SNIPs was 27% and 34%, respectively, which was more than that of modified silica gels (10%), further indicating that monomers and cross-linking agents were successfully grafted on the surface of silica gels. The difference of weight loss between SMIPs and SNIPs may be attributed to the template molecules, which made the grafting density of polymer different in polymerization. All these results demonstrated that the MIPs were grafted on the surface of silica gels successfully.

3.1.4. Elemental analysis

The element variation of activated silica gels, modified silica gels, SMIPs and SNIPs was characterized by elemental analysis (Table 1). Compared with activated silica gels, nitrogen element appeared in modified silica gels, demonstrating that APTES were grown on the surface of silica particles. The percentages of carbon and hydrogen elements were all increased in SMIPs and SNIPs. The carbon and hydrogen contents increased to 16.46% and 3.36% for SMIPs, and 17.11% and 3.98% for SNIPs, respectively. The results indicated that the imprinted polymers were prepared successfully.

3.1.5. Nitrogen adsorption–desorption of SMIPs and SNIPs

Nitrogen adsorption and desorption isotherms were obtained by nitrogen adsorption and desorption test. Since specific surface area and pore size influenced the efficiency of MISPE adsorption, the parameters were obtained using a Brunauer–Emmet–Teller (BET) analysis routine (Table 2). The parameters of SMIPs were all greater than those of SNIPs, indicating that SMIPs had more mesopore and were much looser than SNIPs. Therefore, SMIPs could provide more accessible cavities and binding sites for target analytes than SNIPs.

3.2. Evaluation of the adsorption characteristics

The binding capacity of SMIPs for ampicillin sodium was an important parameter to estimate how many SMIPs were required to bind a specific amount of ampicillin sodium from solution. Therefore, the adsorption isotherms and adsorption kinetics were determined.

The adsorption isotherms of ampicillin sodium onto SMIPs and SNIPs are demonstrated in Fig. 4A. The adsorption amount of SMIPs toward ampicillin sodium rapidly increased with the increase of the concentrations of ampicillin sodium in the initial stage, and followed by a slow increase till the adsorption equilibrium, resulting from the tailor-made recognition cavities during the imprinting process. When the concentration of ampicillin sodium was 300 μg/mL, the IF reached its maximum, i.e., 3.2. When the concentration of ampicillin sodium was up to 600 μg/mL, the adsorption was saturated. The saturated adsorption capacity of SMIPs was 18.2 μg/mg. The adsorption amount of SMIPs was dramatically higher than that of SNIPs at the same initial concentration, indicating that the resultant SMIPs showed a higher affinity to ampicillin sodium than SNIPs. The equilibrium data were modeled with the Langmuir equation and Freundlich equation, respectively. The plot log Qe versus log Ce was used to validate the linearized Langmuir isotherm. The equation for SMIPs can be described as y = 0.6307x + 13.8214, with the correlation coefficient of 0.8305. The plot log Qe versus log Ce was used to validate the linearised Freundlich isotherm, and the equation for SMIPs can be described as y = 0.7546x – 0.7918, with the correlation coefficient of 0.9888, indicating that the Freundlich isotherm model was more suitable for the experimental data than the Langmuir isotherm model because of the higher correlation coefficient. It suggests that the adsorption of SMIPs for ampicillin sodium was multilayer adsorption.

### Table 1

| Elements | Activated silica gels (%) | Modified silica gels (%) | SMIPs (%) | SNIPs (%) |
|----------|---------------------------|--------------------------|-----------|-----------|
| N        | 0                         | 3.28                     | 2.18      | 1.91      |
| C        | 0.12                      | 8.96                     | 16.46     | 17.11     |
| H        | 1.71                      | 2.75                     | 3.36      | 3.98      |

### Table 2

Nitrogen adsorption/desorption analysis.

| Samples | Surface area (m²/g) | Pore volume (m³/g) | Pore size (nm) |
|---------|---------------------|--------------------|---------------|
| SMIPs   | 183.41              | 0.36               | 2.75          |
| SNIPs   | 173.18              | 0.26               | 2.26          |

Fig. 4. (A) Adsorption isotherm curves and (B) adsorption kinetic curves of SMIPs and SNIPs.
The adsorption kinetic curves are illustrated in Fig. 4B. Before the adsorption equilibrium was reached, the absorption capacity of SMIPs for ampicillin sodium increased with adsorption time, and SMIPs could bind ampicillin sodium from the solution with a much faster rate than SNIPs. At 60 min, the adsorption gradually reached equilibrium. Thus, the adsorption process could be divided into two phases: rapid adsorption in the first 10 min and slow adsorption thereafter, suggesting that the binding sites of the SMIPs were on the surface or in the proximity of the surface for easy diffusion of target analytes into imprinting cavities. SMIPs showed high binding capacity and rapid mass transfer rate which are especially favorable for use in the pretreatment of complex biological samples by SPE.

3.3. Selectivity experiments

The selectivity of SMIPs were performed using 300 µg/mL of six different solutions, including ampicillin sodium, four structural analogues and a reference compound. The structures of ampicillin sodium and its analogues are shown in Fig. 5. SNIPs were used as comparison. The adsorption capacity and selectivity of SMIPs for the analogues were relatively higher than those of SNIPs, indicating that the binding results were generated by the specific interactions. The IFs of SMIPs for 6-APA, penicillin G, penicilloic acid, mezlocillin sodium, clenbuterol and ampicillin sodium were 1.9, 1.5, 1.7, 1.4, 1.1 and 3.2, respectively. The 6-APA molecule possesses a smaller spatial diameter than other analogues, which could make it easier to access the imprinted cavities. In contrast, clenbuterol has little structural similarity with ampicillin sodium, so its binding capacity was the lowest among the five compounds. The results indicated that SMIPs showed the highest selectivity for ampicillin sodium. It is attributed to the mutual matched three-dimensional cavity structure between the template molecule and molecularly imprinted polymers.

3.4. Method validation

The reliability of HPLC–UV coupled to SMISPE was evaluated with ampicillin sodium standard solution, different blank and spiked samples (Fig. 6). Ampicillin sodium was well separated from endogenous compounds, indicating that this method had good specificity to detect ampicillin sodium in water, milk and blood samples.

The optimized condition described above was validated before application. Under the optimized HPLC conditions, the linear calibration curves of ampicillin sodium in milk and blood samples were obtained from the concentration range of 5–200 µg/mL.

The linear regression equation of milk sample was \( y = 283.4x / C0 + 418.5 \) with a correlation coefficient of 0.9991, where \( y \) is the peak area and \( x \) is the analyte concentration. The LOD and LOQ were 0.05 µg/mL and 0.2 µg/mL, respectively.

The linear regression equation of blood sample was \( y = 192.2x + 536.6 \) with a correlation coefficient of 0.9994. The LOD and LOQ were 0.15 µg/mL and 0.5 µg/mL, respectively.

To estimate the accuracy and precision of the developed method, the milk and blood samples were determined by spiking with ampicillin sodium at three different concentration levels (5, 25 and 200 µg/mL). As shown in Table 3, the accuracy ranged from 92.1% to 107.6%, and the RSDs of intra- and inter-day precisions were less than 4.6%. The recovery of ampicillin sodium for the...
SMISPE-HPLC–UV analysis was also assessed (Table 3). All the extraction recoveries for the proposed method were in the range of 63.8%–82.5% at the three typical concentration levels. These results demonstrated that the method had good accuracy, precision, selective, and extraction recovery, which was sufficient for enrichment and quantitative determination of trace ampicillin sodium in different samples.

### 3.5. Application to real samples

The applicability of the developed method was further validated by analyzing two milk samples and blood samples. No ampicillin sodium residues were detected at the detectable level in milk samples (Fig. 7A), indicating that the use of ampicillin sodium was mainly controlled in animal-derived food. Ampicillin sodium may be below the detectable level and was not detected in rat blood sample without SMISPE pretreatment (Fig. 7B). However, ampicillin sodium was detected at the detectable level in rat blood samples after extraction. The results demonstrated that SMISPE could enrich and purify ampicillin sodium in blood samples. Therefore, the SMISPE can be used for selective enrichment, extraction and purification of trace ampicillin sodium.

**Table 3**

| Sample | Spiked level (µg/mL) | Accuracy (%) | Precision (RSD, %) | Extraction recovery (RSD, %) |
|--------|----------------------|--------------|--------------------|----------------------------|
| Milk   | 200                  | 94.9         | 0.6 – 3.3          | 82.5                       |
|        | 25                   | 92.1         | 1.1 – 2.2          | 77.9                       |
|        | 5                    | 107.6        | 3.1 – 3.4          | 63.8                       |
| Blood  | 200                  | 100.9        | 0.4 – 1.4          | 78.5                       |
|        | 25                   | 95.7         | 3.4 – 4.6          | 79.1                       |
|        | 5                    | 102.1        | 3.5 – 3.4          | 67.3                       |

Fig. 6. (A) The chromatograms of spiked milk samples. (a) standard solution of ampicillin sodium; (b) blank milk sample; (c) before eluting step of spiked milk samples; and (d) after eluting step of spiked milk samples. (B) The chromatograms of spiked blood samples. (a) standard solution of ampicillin sodium; (b) blank blood sample; (c) before eluting step of spiked blood samples; and (d) after eluting step of spiked blood samples. 1-ampicillin sodium.
the SMISPE procedure was easy to operate.

4. Conclusions

In this study, a novel surface molecular imprinted polymer has been prepared to extract and determine ampicillin sodium in milk and blood samples combining with HPLC. The SMIPs displayed great adsorption capacity, high selectivity and good stability for ampicillin sodium. This developed SMISPE coupled with HPLC method was applicable for determination of ampicillin sodium in various samples and exhibited good specificity, accuracy, precision and recovery. Consequently, this study could provide a promising method for the selective separation and analysis of ampicillin sodium in milk and blood samples.

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