Development of andrographolide molecularly imprinted polymer for solid-phase extraction

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ABSTRACT

A method employing molecularly imprinted polymer (MIP) as selective sorbent for solid-phase extraction (SPE) to pretreat samples was developed. The polymers were prepared by precipitation polymerization with andrographolide as template molecule. The structure of MIP was characterized and its static adsorption capacity was measured by the Scatchard equation. In comparison with C18-SPE and non-imprinted polymer (NIP) SPE column, MIP-SPE column displays high selectivity and good affinity for andrographolide and dehydroandrographolide for extract of herb Andrographis paniculata (Burm.f.) Nees (APN). MIP-SPE column capacity was 11.9 ± 0.6 μmol/g and 12.1 ± 0.5 μmol/g for andrographolide and dehydroandrographolide, respectively and was 2–3 times higher than that of other two columns. The precision and accuracy of the method developed were satisfactory with recoveries between 96.4% and 103.8% (RSD 3.1–4.3%, n = 5) and 96.0% and 104.2% (RSD 2.9–3.7%, n = 5) for andrographolide and dehydroandrographolide, respectively. Various real samples were employed to confirm the feasibility of method. This developed method demonstrates the potential of molecularly imprinted solid phase extraction for rapid, selective, and effective sample pretreatment.

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1. Introduction

Molecularly imprinted polymers are polymeric materials specially designed to offer high recognition to specific templates just like the recognition between enzymes and antibodies. Due to its efficient, stable and distinct selectivity characteristics, MIP has been applied to many fields, such as sensor [1], biomedical application [2], catalysis [3] and separate technology. In particular, they are often packed into the solid phase extraction (SPE) column as sorbents to concentrate and determine environmental samples [4,5]. Also it is used to extract and separate the active ingredients from traditional Chinese herb [6,7]. That mainly involved the qualitative separation for some compounds with simple structure, such as alkaloids [8–10], flavones [11–13] and polyhydric phenols [14]. However, little research focused on the quantitative analysis employing MIP materials to pretreat samples [15,16]. Different preparation conditions such as the difference of the template molecule, functional monomer, cross-linker and other factors may cause large differences of adsorption performance of the polymer.

The terpenoid is the largest class of compounds in herbal medicine, but they were rarely used as template molecule to prepare MIP. Andrographolide is called as a natural antibiotic and widely used in anti-inflammation and adjusting the immune function [17]. So andrographolide was selected as the template molecule. In this paper, andrographolide molecularly imprinted polymers were prepared with precipitation polymerization. The synthesized MIP was identified by FTIR and the adsorption performance of the andrographolide MIP was investigated by the static adsorption method. Furthermore the MIP was packed into the SPE column to enrich and preconcentrate quantitatively the active components from extract of real herb sample APN and its preparations, such as tablets. The selective adsorption performance of MIP-SPE was compared with that of NIP-SPE and C18-SPE. Our approach is to expand the application of molecular imprinting technology in traditional Chinese medicine field, especially for those compounds with unstable, low content and complicated structures. Moreover, the feasibility of the method of quantitative analysis to use MIP as adsorbent to determine samples is further explored.

2. Experimental

2.1. Materials and apparatus

Ethylene glycol dimethacrylate (EDMA) was purchased from TCI Development Co., Ltd. (Shanghai, China). Acrylamide (AM) was obtained from Sinopharm Chemical Reagent (Shanghai, China).
2.2-Azo-bis-isobutyronitrile (AIBN) was Shanghai No. 4 Reagent & H.V. Chemical Co., Ltd. (Shanghai, China) product. APN and its different preparations were purchased from Dunshoutang Drugstore (Jiangxi, China). Andrographolide (more than 99% purity) was obtained from Yixin Corporation (Shanghai, China). Dehydroandrographolide was purchased from Chinese National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Lot numbers: 110854–200306. Organic solvents such as methanol, acetic acid and acetonitrile were obtained from Tianjin Chemical Reagent Company (Tianjin, China). All chemicals and solvents were analytical or HPLC grade.

Chuanxinlian tablets were manufactured by Huacheng Pharmaceutical Factory (Guangzhou, China), Lot number: 201000104, and Yikang Pharmaceutical Co., Ltd. (Guangdong, China), Lot number: 100401. Compound Chuanxinlian tablets were produced by Zaitian Pharmaceutical Co., Ltd. (Guangdong, China), Lot number: 102100021. Chuanwang Xiaoyan tablets were made in Luoyu Pharmaceutical Co., Ltd. (Guangdong, China), Lot number: 091103.

Infrared spectra were measured with a VERTEX70 Fourier transform infrared spectrometer (Bruker, Germany). Absorption spectra were measured on a Unico UV spectrometer (St. Louis, USA). The HPLC system was an Agilent-1200 (Agilent, USA). The incubation temperature was controlled from 500 to 4000 cm⁻¹ with sample scanning for 32 times.

2.2. Polymers preparation

Uniform the imprinted and non-imprinted polymers were prepared by precipitation polymerization. Andrographolide (0.3 mmol), AM (1.5 mmol), EDMA (2.4 mmol), and AIBN (10 mg) were dissolved by 60 mL mixture of acetonitrile and toluene (3:1, v/v) in a 100 mL round-bottomed flask. The mixture was sparged with oxygen-free nitrogen for 15 min and sealed under vacuum. The polymerization was carried out in a water bath at 60 °C for 24 h. After reaction, the obtained polymers were collected by centrifugation at 10,000 rpm for 10 min. Methanol–acetic acid (9:1, v/v) was used to remove the andrographolide until there was no template molecule to be detected by UV (at 225 nm) in the eluate. The polymers were then eluted by methanol to remove the remaining acetic acid and dried under vacuum.

The NIP was also prepared by the same protocols as like the MIP, except the addition of andrographolide.

2.3. FTIR measurements

FTIR spectroscopic measurements were performed by KBr pellet method. The wave numbers of FTIR measurement range were controlled from 500 to 4000 cm⁻¹, and collected at one data point per 2 cm⁻¹ with sample scanning for 32 times.

2.4. Static adsorption tests

MIP or NIP particles (30 mg) were placed in a 10 mL flask, then added 3 mL of andrographolide–acetonitrile solution with the initial concentration ranging from 10 μmol/L to 30 μmol/L. The conical flasks were shaken by the oscillator for 12 h and then filtrated. The free concentration of andrographolide in the filtrate was determined by UV–visible spectrophotometer and the HPLC instrument. And the acquired experimental data were analyzed by the Scatchard equation.

2.5. Sample preparation

Chinese traditional medicine APN and Chuanxinlian tablets were prepared according to the method of Chinese Pharmacopoeia 2010 Edition [18]. Compound Chuanxinlian tablets and Chuanwang Xiaoyan tablets were treated according to the method of Drug Standards for Ministry of Health [19,20]. At the same time, the above herbs and tablets were also treated in accordance with the following method. The method includes two steps: (1) the contents were ground into powders and weighted 0.5 g into a flask with a plug and added 25 mL 60% (v/v) methanol–water solution, then extracted by ultrasonic wave for 40 min. The extract was cooled down to room temperature. Methanol–water solution (60% v/v) was added to compensate solvent loss during the procedure of Ultrasonic extraction. After filtration through a paper filter, the extract was again filtered by a 0.45 μm filter. (2) 200 mg MIP were packed into a SPE cartridge. At first, the polymers were pre-equilibrated with 10 mL 60% (v/v) methanol–water solution. Secondly, 2 mL extract above was added gradually into the column and the filtrate was collected. After that the column was washed with 5 mL methanol. The eluate was collected. NIP was performed the same process. All of the liquid samples were filtered by a 0.45 μm filter for HPLC analysis. C₁₈-SPE was dealt with the above method according to the same ratio.

2.6. HPLC analytical conditions

Elite C₁₈ column (150 mm × 4.6 mm i.d., 5 μm particle size, Dalian, China) was used. An isocratic elution was conducted with the mobile phase of 0.2% glacial acetic acid aqueous solution and methanol (45:55). The flow rate was 1.0 mL/min, and the injection volume was 20 μL. The column temperature was maintained at 30 °C. The detection wavelength was set at 225 nm.

3. Results and discussion

3.1. Preparation and characterization of andrographolide MIP

Fig. 1 depicts the structure of andrographolide. It was dissolved in the mixed solution of acetonitrile and toluene, which has a good solubility and a weak polarity as the solvent. AM was selected as the functional monomer because a strong hydrogen-bonding interaction can generate between it and andrographolide in the solvent [21], the structure of AM lists in Fig. 2. Such strong interaction will produce excellent stability in the resultant polymers, which results in the high recognition specificity of the MIP. And in the followed thermal polymerization, EDMA was used as a cross-linker to form a “frozen” cavity structure. After the template is removed, the specific binding sites will be maintained. These sites will selectively adsorb andrographolide molecule.

![Structure of andrographolide.](image1)

![Structure of acrylamide (AM).](image2)
The structure of MIP was identified by infrared spectrometer. Fig. 3 shows FTIR spectrum of andrographolide. Two strong O–H stretching vibration absorbance peaks in the molecule of andrographolide are at 3628.0 cm⁻¹, 3537.3 cm⁻¹. A stretching vibration peak of C=O is found at 1757 cm⁻¹.

FTIR spectrum of AM is shown in Fig. 4. The absorbance peaks of two N–H stretching, C=O stretching and C=C stretching are at 3477.60 cm⁻¹, 3363.70 cm⁻¹, 1691 cm⁻¹, 1618 cm⁻¹, respectively.

Fig. 5a and b presents the FTIR spectra of MIP after polymerization. At 1638 cm⁻¹, the weak absorbance peak of C=C suggests that the repeated cross-linking units of EDMA have been polymerized. The broad peak at 3565 cm⁻¹ is assigned to the stretching vibration of O–H in MIP, and the peak at 1729 cm⁻¹ is the C=O stretching vibration, the two absorbance peaks indicate the existence of specific binding sites. Another strong split peaks at 2987 cm⁻¹, 2954 cm⁻¹ is the stretching vibration of CH₃– and CH₂–. As to NIP (see Fig. 5c), the characteristic signals of FTIR are almost the same. Fig. 5a presents the FTIR spectrum of MIP before removing the template molecule, and two weak O–H stretching vibration absorbance peaks at 3628.0 cm⁻¹, 3537.3 cm⁻¹ for the molecule of andrographolide can still be seen. After the polymers were eluted, the FTIR spectrum of MIP is very close to that of NIP. The results display that the template molecule have been removed from the MIP.

3.2. Adsorption analysis of andrographolide MIP

Fig. 6a and b illustrates the adsorption isotherms of andrographolide MIP and its corresponding NIP. The curves were obtained by plotting the saturated adsorption capacity with equilibrium concentrations of the andrographolide in acetonitrile solution. The adsorption capacity (Q) of andrographolide can be calculated according to the formula as following:

\[
Q = \frac{(C_0 - C_s) \times V}{m}
\]

where \(Q\) is the amount of andrographolide bound to the MIP; \(C_0\) is the initial concentration of substrate; \(C_s\) represents adsorption equilibrium concentration of substrate; \(m\) is the application amount of MIP.

As Fig. 6 displays, \(Q\) of MIP is significantly greater than that of NIP. It is obvious that the absorption performance of MIP is distinctly superior to the NIP.

Scatchard analysis was employed to further study the binding isotherms, which is an approximate model commonly used to estimate the binding parameters of MIP. The Scatchard equation is defined as below [22, 23]:

\[
\frac{Q}{[C]} = \frac{Q_{\text{max}} - Q}{K_d}
\]

where \(Q_{\text{max}}\) is the maximum binding capacity; \(K_d\) is the equilibrium dissociation constant; \([C]\) represents the equilibrium concentration.
of andrographolide. Based on the data the Scatchard equations of the two polymers can be expressed as following:

$$\frac{Q}{[C]} = -0.0827Q + 0.4095 \quad \text{(MIP)}$$  \hspace{2cm} (3)

$$\frac{Q}{[C]} = -0.1584Q + 0.2213 \quad \text{(NIP)}$$  \hspace{2cm} (4)

Also, Fig. 6 (the upper part of this figure) describes the plot based on the Scatchard equations. The correlation coefficients \((R)\) of regression analysis for the two curves are 0.9872 and 0.8086 for MIP and NIP, respectively. It is noted that the MIP has only one distinct section within the plot and inclined to a straight line, which presents that only the homogeneous affinity binding sites are formed in the polymers. As to the NIP, the bad linearity reveals that there are no selective adsorption sites for andrographolide in the polymers.

From the slope \((-1/K_d)\) and intercept \(Q_{\text{max}}/K_d\) of the Scatchard plot, the dissociation constants \(K_d\) and the maximum binding capacity \(Q_{\text{max}}\) of the MIP and NIP are \(K_d = 12.09 \mu\text{mol/L}\), \(Q_{\text{max}} = 4.951 \mu\text{mol/g}\) and \(K_d = 6.313 \mu\text{mol/L}\), \(Q_{\text{max}} = 1.388 \mu\text{mol/g}\), respectively. As \(Q_{\text{max}}\) is an important parameter to evaluate the adsorption capacity of MIP, it can be concluded that the MIP adsorption performance is better than NIP by comparing their \(Q_{\text{max}}\).

### 3.3. Comparison of the performance with C18-SPE and NIP-SPE column

The particles of MIP and NIP were packed into SPE column as the sorbent to pretreat the extracts of real sample. The extract of APN was as the sample, the adsorption performance of MIP-SPE, C18-SPE and NIP-SPE columns was made a comparison. Fig. 7a depicts HPLC chromatogram of the extract of APN. In this chromatogram, two main peaks are identified as andrographolide and dehydroandrographolide. After 2 mL extract flowing through MIP-SPE column, no compound can be detected in the filtrate, as Fig. 7b shown. However, the two peaks of andrographolide and dehydroandrographolide are all found in the filtrate through NIP-SPE and C18-SPE columns, as shown in Fig. 7c and d. Moreover, from Fig. 7e–g can be seen that the concentration of the two compounds in the eluate of 5 mL methanol from MIP-SPE column was obviously higher than that of from other two columns. These results meant that andrographolide and dehydroandrographolide are almost completely adsorbed by the MIP-SPE column and the absorption to target compounds depends on the hydrogen bonding interaction between AM molecule and andrographolide molecule. While the absorption of the NIP-SPE and C18-SPE columns to target molecules depends mainly the non-specific adsorption. It is also indicated that MIP-SPE column has high selectivity to the target component and its similar ingredients of structure.

The others performance parameters of SPE column were also investigated. The extract of APN was as a sample, the column capacity of andrographolide and dehydroandrographolide was measured in the above three SPE columns. The enrichment factor \((EF)\) was introduced and it was defined as the ratio of the concentration of analyte in the concentrated phase \((C_{\text{con}}, \mu\text{mol/mL})\) and the initial concentration of analyte within the sample \((C_0, \mu\text{mol/mL})\):

$$EF = \frac{C_{\text{con}}}{C_0}$$  \hspace{2cm} (5)

![Fig. 7. HPLC chromatograms of the extraction of APN. Initial solutions before SPE column (a), filtrate of 2 mL extracts through MIP-SPE column (b), filtrate of 2 mL extracts through NIP-SPE column (c), filtrate of 2 mL extracts through C18-SPE column (d), eluates of 5 mL methanol for MIP-SPE column (e), eluates of 5 mL methanol for NIP-SPE column (f) and eluates of 5 mL methanol for C18-SPE column (g).]
andrographolide and dehydroandrographolide, respectively, and the above three SPE columns are detected as shown in Table 1. These data results display that the column capacity of MIP-SPE, respectively. The limit of detection (LOD) and the limit of quantity (LOQ) were evaluated on the basis of a signal-to-noise ratio of 3 and 10. The relative standard derivations (RSDs) of peak areas were 1.40% and 0.59% for andrographolide and dehydroandrographolide, respectively. The relative standard derivations (RSDs) of retention time for andrographolide and dehydroandrographolide were below 0.30% and 0.31%, respectively. The relative standard derivations (RSDs) of retention time are 0.65% and 0.29% for andrographolide and dehydroandrographolide, respectively.

To determine the linearity relationships between concentrations and the corresponding peak areas of the two compounds, a series of concentrations of the mixed standard solution range from 50 \mu g/mL to 1600 \mu g/mL was tested at the same situations. The influence of detection (LOD) and the limit of quantity (LOQ) were evaluated on the basis of a signal-to-noise ratio of 3 and 10. The results of regression analysis on calibration curves, linear ranges and detection limits are summarized in Table 2.

The repeatability was assessed by analyzing six independently prepared samples of the extract of herb APN. The relative standard deviations of retention time for andrographolide and dehydroandrographolide were below 0.30% and 0.31%, respectively. The relative standard deviations of peak area for the same two compounds were 2.40% and 1.18%, respectively.

### 3.4.2 Reproducibility

The reproducibility was assessed by analyzing six independently prepared samples of the extract of herb APN. The relative standard deviations of retention time for andrographolide and dehydroandrographolide were below 0.30% and 0.31%, respectively. The relative standard deviations of peak area for the same two compounds were 2.40% and 1.18%, respectively.

### 3.4.3 Recovery and practical sample analysis

The recovery experiments used the MIP-SPE column to quantitative analysis under the same conditions (see Section 2.5) were also conducted to evaluate the precision and accuracy of the method.

### 3.4. Methodology validation for MIP-SPE column used for quantitative analysis

#### 3.4.1 Precision, linearity and detection limits of analytes

The method was validated for precision of the peak area of the analytes. The precision is estimated by making six replicate injections of a standard mixture solution (50 \mu g/mL andrographolide methanol solution and 80 \mu g/mL dehydroandrographolide methanol solution) under the above optimum conditions. The relative standard derivations (RSDs) of peak areas are 1.40% and 0.59% for andrographolide and dehydroandrographolide, respectively. The relative standard derivations (RSDs) of retention time are 0.65% and 0.29% for andrographolide and dehydroandrographolide, respectively.

### Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>MIP-SPE</th>
<th>C18-SPE</th>
<th>NIP-SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC (\mu mol/g)</td>
<td>EF (%)</td>
<td>CC (\mu mol/g)</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>11.9 ± 0.6</td>
<td>90.6</td>
<td>5.91 ± 0.42</td>
</tr>
<tr>
<td>Dehydroandrographolide</td>
<td>12.1 ± 0.5</td>
<td>93.2</td>
<td>5.71 ± 0.52</td>
</tr>
</tbody>
</table>

CC stands for the column capacity of SPE column.

### Table 2

The regression equations, detection limits and quantity limits for andrographolide and dehydroandrographolide.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression equation</th>
<th>Correlation coefficient (R²)</th>
<th>Linear range (\mu g/mL)</th>
<th>Detection limits (\mu g/mL)</th>
<th>Quantity limits (\mu g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrographolide</td>
<td>y = 29.46x + 18.80</td>
<td>0.9991</td>
<td>50–1000</td>
<td>0.27</td>
<td>1.44</td>
</tr>
<tr>
<td>Dehydroandrographolide</td>
<td>y = 24.35x – 6.557</td>
<td>0.9992</td>
<td>80–1600</td>
<td>0.48</td>
<td>1.60</td>
</tr>
</tbody>
</table>

### Table 3

The recoveries using MIP-SPE column to enrich andrographolide and dehydroandrographolide in the extracts of APN (n = 5).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Original amount (\mu g)</th>
<th>Added amount (\mu g)</th>
<th>Found (\mu g)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrographolide</td>
<td>418</td>
<td>320</td>
<td>730</td>
<td>97.5</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>418</td>
<td>416</td>
<td>820</td>
<td>96.6</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>418</td>
<td>512</td>
<td>950</td>
<td>104</td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td>646</td>
<td>480</td>
<td>1106</td>
<td>95.8</td>
<td>2.90</td>
</tr>
<tr>
<td></td>
<td>646</td>
<td>624</td>
<td>1284</td>
<td>102</td>
<td>3.60</td>
</tr>
<tr>
<td>Dehydroandrographolide</td>
<td>646</td>
<td>720</td>
<td>1394</td>
<td>104</td>
<td>3.70</td>
</tr>
</tbody>
</table>

### Table 4

Comparison of the content determination of real samples by different methods (n = 3).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Medicinal origin</th>
<th>Standard method</th>
<th>MIP-SPE</th>
<th>C18-SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C1⁺</td>
<td>C2⁺</td>
</tr>
<tr>
<td>APN</td>
<td>Guangxi</td>
<td>0.48 ± 0.05%</td>
<td>0.69 ± 0.04%</td>
<td>1.3 ± 0.09%</td>
</tr>
<tr>
<td></td>
<td>Anshui</td>
<td>0.22 ± 0.04%</td>
<td>0.59 ± 0.04%</td>
<td>0.70 ± 0.05%</td>
</tr>
<tr>
<td>Chuanxinlian</td>
<td>Huacheng</td>
<td>0.33 ± 0.03%</td>
<td>0.14 ± 0.09 mg</td>
<td>0.54 ± 0.04 mg</td>
</tr>
<tr>
<td>tablets</td>
<td>Yikang</td>
<td>0.46 ± 0.04 mg</td>
<td>1.8 ± 0.11 mg</td>
<td>0.74 ± 0.06 mg</td>
</tr>
<tr>
<td>Chuanwang Xiaoyan tablets</td>
<td>Luoyu</td>
<td>0.25 ± 0.03 mg</td>
<td>2.3 ± 0.15 mg</td>
<td>0.49 ± 0.03 mg</td>
</tr>
<tr>
<td>Compound Chuanxinlian tablets</td>
<td>Zaitian</td>
<td>-</td>
<td>-</td>
<td>0.34 ± 0.04 mg</td>
</tr>
</tbody>
</table>

⁺ C1 and C2 represent the contents of andrographolide and dehydroandrographolide, respectively.

The recovery experiments used the MIP-SPE column to quantitative analysis under the same conditions (see Section 2.5) were also conducted to evaluate the precision and accuracy of the method.
Recovery was determined with standard addition method in sample with three different fortification levels total 15 samples. The precision of determination of andrographolide is in the range of 3.1–4.3% and dehydroandrographolide in the range of 2.9–3.7% with the recovery of 96.6–104.0% and 95.8–104.0% in the three levels, respectively. As Table 3 displays, it is noticeable that the method has good precision and accuracy.

Various real samples were applied to test the feasibility of the MIP-SPE column used to quantitative analysis. These real samples involved 2 APN, 2 Chuanxinlian tablets, 1 Compound Chuanxinlian tablet and 1 Chuanwang Xiaoyan tablet, total 6 samples. At first, their determination of the content was in accordance with Chinese Pharmacopoeia methods or ministerial standards. Moreover, three SPE columns with different sorbent, such as MIP, NIP, and C18, were used to determine the content of these real samples. The results are shown in Table 4. It can be seen that the content measured by MIP-SPE column is higher than the content measured by other methods. Because of high selectivity and high capacity to target compound for MIP-SPE column, more the target compounds can be adsorbed in the unit weight. While the target compounds are just partially be adsorbed in the C18-SPE or Al2O3 columns (used in the official method), it will lead to the value of determination is lower. Results suggest that the higher contents can be obtained by using MIP-SPE column to enrich the target compound and the values are close to the actual contents.

4. Conclusion

In this work, a selective method of quantitative analysis using MIP-SPE column to enrich target components has been developed. The MIP with high affinity was prepared by precipitation polymerization. The results indicated that MIP-SPE column shows a advantage compared to C18 and NIP column, namely higher selectivity, greater column capacity and enrichment factor to target compositions. The method employing MIP-SPE column to quantitative analysis has a low detection limit, high recovery and good reproducibility. Moreover, the feasibility of quantitative analysis to use MIP-SPE column to enrich target compounds has been confirmed by real samples. Therefore, MIP-SPE column is an ideal material to enrich the active target component from the complex samples. It suggests that molecularly imprinting technique (MIT) should have a very good application prospect in enriching and separating the active component, especially for those compounds with unstable, low content and complicated structures from traditional Chinese medicines.

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