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Preparation of micropipette tip-based molecularly imprinted monolith for selective micro-solid phase extraction of berberine in plasma and urine samples

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ABSTRACT

A novel berberine-imprinted polymer (MIP) monolith was prepared for extraction of berberine in aqueous medium. The MIP monolith was prepared inside a polypropylene micropipette tip by using dimethylsulfoxide as porogen, acrylamide (AA) as functional monomer and ethyleneglyol dimethacrylate (EGDMA) as cross-linker. Polymerization conditions were optimized and good permeability and selectivity was obtained when the ratio of berberine/AA/EGDMA was 1:5:30. Cross-reaction was also studied by three compounds (palmatine, coptisine, and jatrorrhizine) with similar structure. A molecularly imprinted micro solid-phase extraction (MI-μ-SPE) method was developed for selective extraction of berberine in aqueous solutions. Extraction parameters were investigated, such as sample pH value, sample flow rate, sample volume and elution solvent. By combining with HPLC/UV, MI-μ-SPE method showed a good linear range of 3–800 ng/mL with a low limit of detection limit of 1.0 ng/mL. The method was also applied for the pretreatment of berberbine in human plasma and urine samples. The result showed that proteins and other biological matrix were successfully eliminated and berberine was selectively enriched. Recoveries were tested in plasma and urine samples, and calculated to be 90.6–103.2% with relative standard deviations less than 4.7%.

1. Introduction

Berberine is a well-known alkaloid which is found in medicinal herbs such as Coptis chinensis, Phellodendron amurense, Hydrastis Canadensis, Berberis vulgaris, Arcangelisiaflava, and Berberis aquifolium. The main clinical uses of berberine preparations include the treatment of bacterial diarrhea, intestinal parasite infections and ocular trachoma infections [1]. Berberine also possesses other functions such as anti-inflammatory[2], antiarrhythmic [3], anticancer [4] and immunosuppressive [5]. In recent years, some researchers have revealed that berberine could influence drug absorption [6], glucose metabolism [7], blood contents of cyclosporine [8] et. al. The analysis of berberine in plasma, urine, and other biofluids is required in pharmacokinetic evaluation, pharmacodynamics and pharmacotoxicology study.

Generally, the matrix of biofluids is very complex, containing large amount of proteins and other endogenous components, and the determination for the analytes at trace level is often required. Therefore, extraction techniques are required before chromatographic analysis. Extraction methods with high selectivity to the analytes are most desirable to enrich the anaylates in biofluids and to clean-up interferent in the matrix [9].

One of the methods to create highly selective extraction is the incorporation of biomolecules such as enzyme and antibody or antigen in the procedures. However, although good specificity can be obtained, natural biomolecules are usually expensive and chemically unstable. Synthetic materials such as molecularly imprinted polymers (MIPs) are good alternatives to these biological substances. MIPs are tailor-made materials with high selectivity to target molecules. MIPs can be synthesized conveniently by a mixture solution containing porogenic reagent, template molecule, functional monomer, cross-linker and initiator. After polymerization, template molecules are removed and polyporous materials with selectively functional binding sites and complementary cavities for template molecules are obtained. For the high stability, ease of preparation, and high sensitivity, MIPs have been used widely in different application, such as chromatographic separation, solid phase extraction (SPE), catalysis and sensing [10–15]. MIPs-based SPE is one of the most successful and useful application. MIPs-based SPE combines both the advantages of MIPs and SPE, and exhibits good extraction efficiency, reusability and selectivity to certain kinds of analytes [16], which is promising to selectively and effectively extract drugs in biofluids.

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The most widely used method for preparing MIPs is traditional polymerization, in which polymer bulks are synthesized firstly and grounded into particles for the application in SPE. The process is time and labor consuming, and the size of particles could influence the extraction efficiency and selectivity of MIPs. Precipitation polymerization is an improved method which produces polymer particles instead of polymer bulks, the ground step could be eliminated as a result. However, tedious desorption process is also required and transfer of the particles is necessary during the extraction procedures, which is time and labor consuming and the reproducibility would also be influenced.

To use the synthesized MIP monolith directly as SPE sorbents is a promising method. Recently, Zheng et al. [17] have prepared a MIP monolith inside a fused-silica capillary and applied it in the extraction of fluoroquinolones from milk samples. Monoliths in capillaries and other microtubes can be synthesized quickly since some steps such as crushing, sieving and packing can be omitted [18]. Besides, the amount of template molecule required during monolith preparation is much less than that of other methods [19]. And the volume of sorbents and eluting solvents could be very small, so the extraction efficiency could be increased as a result [9].

Recently, two kinds of berberine imprinted polymers have been reported by Chen et al. [20] and Li et al. [21]. The polymers were prepared by conventional polymerization and the rebinding experiments were carried out by grounded particles. Although good selectivities were achieved by using the prepared MIPs, both works were focused on recognition mechanism and the extraction performance. However, the application was not studied. Moreover, the medium for berberine rebinding were chloroform and acetonitrile, which were not suitable for biological samples. Preparation of berberine MIP which exhibits good selectivity in aqueous conditions is meaningful for extraction of berberine in biological samples.

In this work, we aim to prepare a novel MIP monolith for selective extraction of berberine in biological samples. The imprinted monolith was prepared in an improved micropipette tip based device and the specific recognition of the MIP monolith for the template berberine was studied. A molecularly imprinted micro-solid phase extraction (MI-μ-SPE) method was developed for selective extraction of berberine in aqueous solutions and the parameters potential to affect MI-μ-SPE were optimized. By combining with HPLC, an analytical method was established and applied for selective pretreatment and determination of berberine in human plasma and urine samples.

2. Experimental

2.1. Instrumentation

A Shimadzu HPLC system (Tokyo, Japan) consisted of two 20AD pumps, a $20A_3$ degasser, 20A UV detector and thermostat controlled column compartment were used for chromatographic analysis. Data collection was performed in Shimadzu LC Solution software. Column for separation was a C-18 column (250 mm × 4.6 mm i.d.) with 5 μm particle size from GL Science (Tokyo, Japan). The detection wavelength was set at 345 nm and column temperature was 25 °C. The micropipette tips made of polypropylene were purchased from Shenshi Chemical Company (Wuhan, China).

2.2. Chemicals

Berberine, jatrorrhizine, coptisine and palmatine of chloride forms were obtained from Aladdin-reagent (Shanghai, China). Ethyleneglyol dimethacrylate (EGDMA), which was used as cross linker, was obtained from Alfa-Aesar (Lancashire, UK). Acrylamide (AA), methacrylic acid (MAA), 4-vinylpyridine (4-VP) and 2,2′-azobis (2-methylpropionitrile) (AIBN) were obtained from Shanghai Reagent Factory (Shanghai, China) and were analytical reagent grade. Methanol and acetonitrile were HPLC grade and purchase from Tedia (OH, USA). And water was purified by a Milli-Q system (MA, USA).

2.3. Preparation of berberine imprinted polymer monolith

A polypropylene micropipette tip was firstly washed with methanol and dried in an oven. 2 mm of the bigger end was cut to facilitate the connection of micropipette tip and the syringe. Another end was burned slightly to seal the micropipette tip.

The template molecule (berberine chloride, 0.0048 g) was mixed with AA (5.6 mg) in a 1 mL screwcapped glass vial, followed by addition of 0.30 mL DMSO as solvent. The solution was put in dark for 2 h. After self-assembly, the cross-linker EGDMA (75 μL) and the initiator AIBN (3.0 mg) were added to the above solution. To remove dissolved oxygen, ultrasonication was applied for 20 min. Finally, 60 μL of the pre-polymerization solution was translated into the micropipette tip carefully, which was ultrasonicated for another 10 min. The micropipette tip was then sealed and put into a water bath (60 °C, 3 h) for polymerization. After removing the seal in the two ends of the micropipette tip, it was connected with a syringe and the resultant polymer monolith was washed thoroughly with hot methanol/acetic acid (9:1, v/v) to remove the templates. The non-imprinted polymer monolith was prepared by the same procedures but in the absence of berberine.

2.4. MI-μ-SPE procedures

The prepared berberine imprinted monolith was applied for extraction of berberine in aqueous solutions. Solutions were put in the syringe and loaded by a KDs 100 syringe pump (MA, USA). For precondition, 0.3 mL methanol/acetic acid (9:1, v/v) acetonitrile and 0.2 mL phosphate buffer (pH 7.0) were injected respectively (0.2 mL/min). For sampling, 2 mL sample solution was loaded at the rate of 0.2 mL/min. 0.2 mL chloroform was then introduced for washing. Finally, 0.1 mL methanol/acetic acid (9:1, v/v) was injected at 0.1 mL/min and the eluate was collected for HPLC analysis.

2.5. Analysis of berberine in human plasma and urine samples

Human plasma sample was supplied by Dongfeng Hospital. 0.5 mL spiked plasma was added with 0.5 mL 10% trichloroacetic acid solution for protein deposition. After centrifugalization (10,000 rpm, 10 min), pH value of the supernatant was adjusted to about pH 7 with 1 M NaOH, then the solution was loaded onto the MIP monolith, extracted and analyzed as described in 2.4.

Urine sample was obtained from a healthy man. 0.5 mL spiked urine sample was firstly diluted with 0.5 mL water, the pH value of the mixture was then adjusted pH value to 7. After centrifugalization (10,000 rpm, 10 min), the solution was further filtered with a membrane filter (0.22 μm) and the filtrate was loaded onto the MIP monolith, extracted and analyzed as described in 2.4.

3. Results and discussion

3.1. Fabrication of micropipette tip-based extraction device

In this work, we fabricated a micropipette tip-based device for evaluating the properties extraction performance of the molecularly imprinted monolith (Fig. 1). Micropipette tip-based device is a simple and effective device that has been used for microextraction [18]. Fig. 1a showed the structure of the device, where the monolith was synthesized in the end of the narrow end. However, the tip of the monolith was found to drop easily, for the tip of the monolith was apt to get out of the micropipette tip under pressure from the solution. In this work, an improved method was developed, in which the monolith was synthesized about 2 mm away from the narrow end of the micropipette tip (Fig. 1b). The pre-polymerization solution was put into the sealed micropipette tip carefully and gently. A small chamber was formed and the solution was supported by air pressure (Fig. 1c). The sealed micropipette tip was then put in the water bath for polymerization under heat. The monolith was restricted inside the micropipette tip and the drop of the monolith was significantly improved by this device.

3.2. Preparation and evaluation of berberine-imprinted monolith

3.2.1. Optimization of preparation conditions

The berberine-imprinted monolith was prepared through copolymerization of functional monomer and cross-linker in the presence of berberine. The evaluation and extraction were carried out by pushing solutions through the monolith, therefore, the permeability of the monolith one of the most important parameters.

The ratio of functional monomer to cross-linker was investigated firstly, for it was essential for the permeability of the polymer. The ratio between monomer and cross-linker can affect the pore size and capacity of the reticular structure of the polymer [22]. The polymer cannot be generated in a lower percentage of cross-linker, and too much cross-linker would result in large density and bad permeability of the polymer, which would decrease the extraction efficiency, therefore, proper ratio is required. The ratios of berberine/AA/EGDMA were studied from 1:40:400 to 1:5:30, smaller ratios were not studied for the consideration of adequate berberine-AA interaction. The ratio of 1:40:400 was applied in literature [20], however, the permeability was found to be so bad that the sample solution was difficult to flow through the monolith. Good permeability was obtained when the ratio was 1:5:30, and it was selected for further optimization.

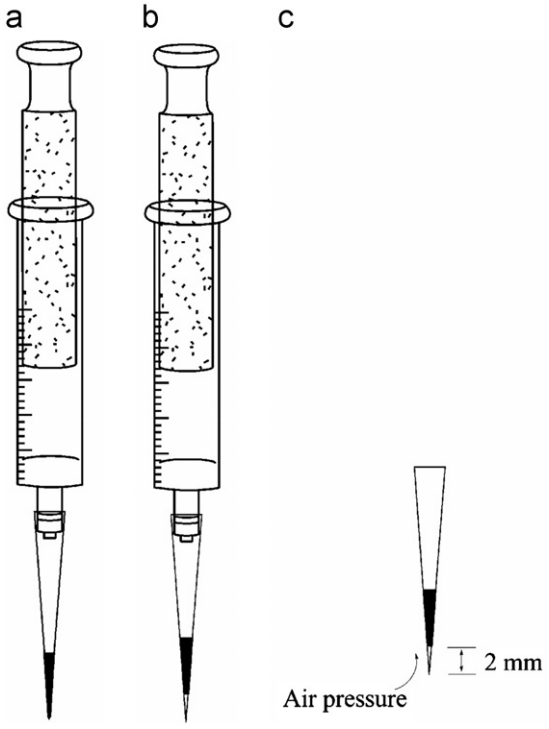

Fig. 1. Schematic diagram of micropipette tip based device.

In order to evaluate the selectivity of the MIP monolith, imprinted factor (IF) was introduced:

$$IF = \frac{Q_{MIP}}{Q_{NIP}}$$

Where the Q_{MIP} is the binding amount of berberine (μg) in MIP monolith (per gram) and E_{NIP} is binding amount of berberine in NIP monolith under the same conditions.

The binding of berberine onto the monolith was mainly based on the interaction between BER and functional monomer, which is essential for the selectivity of MIP. Three kinds of functional monomers, AA, MAA and 4-VP were used respectively and the selectivity of MIPs were investigated.

The IF value of MIPs using AA, MAA and 4-VP as functional monomer was listed in Table 1. The sample was 1 mL berberine aqueous solution (0.5 μg/mL) and 0.1 mL methanol/acetic acid (9:1, v/v) was used as eluting solvents. The results showed that MIP using AA as functional monomer exhibited bigger IF value (2.41) than that using MAA and 4-VP. To realize best selectivity, AA was chosen as functional monomer.

3.2.2. Characterization of berberine imprinted monolith

FT-IR was performed to testify the successful preparation of berberine imprinted monolith. As shown in Fig. 2, the characteristic peak of AA was around at 3400, 1725, and 1635 cm^{-1}, which were corresponding to the N–H, C=O and C=C stretching of AA. The peak of C=O at 1725 cm^{-1} in the MIP shows that AA has been polymerized with EGDMA, and the weak absorbance peaks of at 1635 cm^{-1} demonstrated that only a few remained unlinked and most AA were cross-linked with EGDMA. Moreover, the peak intensity at 1625 cm^{-1} (the stretching vibration of C=O) in the NIP was lower than that of MIP, which probably results from the form of hydrogen bonds between berberine and the AA.

3.2.3. The effect of washing solvent on MIP monolith

To decrease non-specific interaction between berberine and monolith, and to achieve selective extraction, a washing step was carried out after sample loading. Low-polarity organic solvents are most widely used, however, good results could also be obtained with high-polarity solvents, and some authors have also stated that recognition is better when the porogen is used as the solvent for the environment established during the synthesis is reproduced. Therefore, in this work, three common used solvents with different polarity (isopropanol, acetone, and chloroform) and the porogen (DMSO) were used as washing solvents respectively

Table 1
IF values of MIPs using different kinds of functional monomers.

Monomer	IF (Q_{MIP}/Q_{NIP})
AA	2.41
MAA	1.37
4-VP	1.41

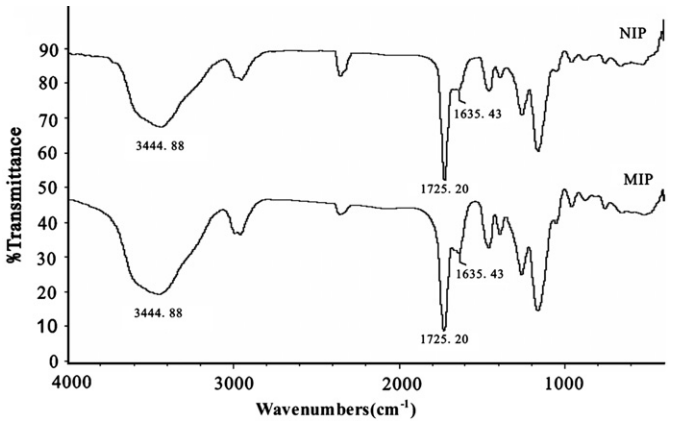

Fig. 2. IR spectra of MIP and NIP monolith.

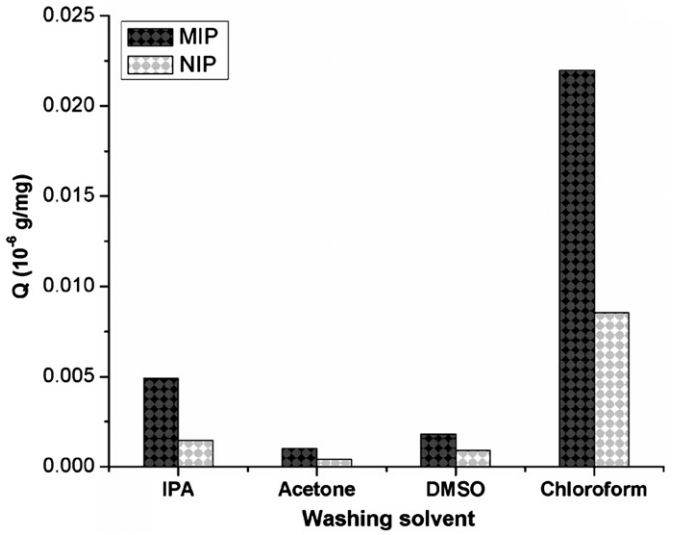

Fig. 3. Effect of washing solvent on the MIP monolith.

and the recognition ability of MIP and NIP were studied. As shown in Fig. 3, large IF value could be obtained with isopropanol, acetone, and chloroform, however, the extraction efficiencies were decreased significantly with isopropanol and acetone, which could desorb most of berberine molecules. Chloroform was selected as washing solvent when taking the selectivity and extraction efficiency into consideration.

3.2.4. Selectivity of the MIP monolith to structure analogs

The selectivity of berberine MIP monolith to some structure analogs was studied. Jatrorrhizine, coptisine and palmatine aqueous solution (100 ng/mL) was loaded onto the MIP and NIP monolith respectively, the conditions and procedures was same as described in Section 2.4. The eluents was collected and analyzed by HPLC. As shown in Fig. 4, cross-reaction was observed, as the berberine MIP could also recognize jatrorrhizine, coptisine and palmatine well, enrichment folds from 6.71 to 9.03 were obtained. The reason for cross-reaction was probably that the sizes and functional groups of these molecules are similar to that of berberine. The result indicated that the MIP monolith was also applicable for extraction of jatrorrhizine, coptisine and palmatine.

3.2.5. Recognition mechanism of MIP monolith to berberine

The schematic procedure of berberine MIP monolith was shown in Fig. 5. Hydrogen bonds and electrostatic force were

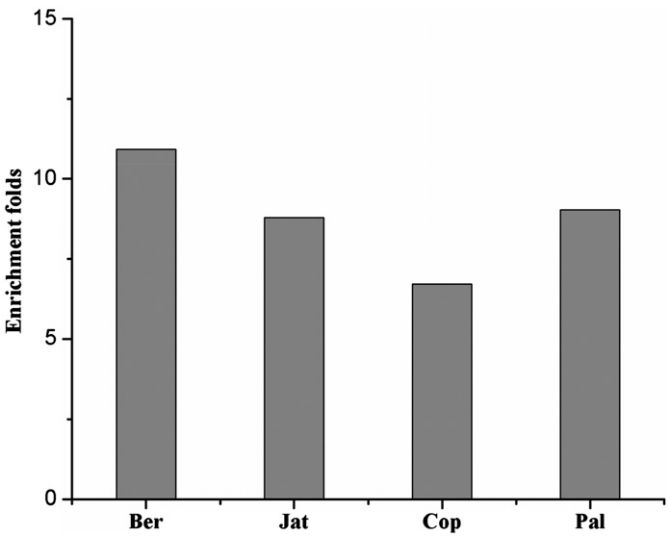

Fig. 4. Synthesis process for berberine MIP monolith.

formed between beberine and AA, which led to the formation of a self-assemblied complex of berberine and AA [20]. According to the structure information of AA and berberine, three AA molecules would form four hydrogen bonds with four oxygen atoms of berberine, another AA would form electrostatic interaction with quaternary amine in berberine structure. The ratio of berberine/AA was 1:4, which was in agreement with our result, in which a ratio of 1:5 was used. A bit more functional monomers were always required to ensure complete interaction between functional monomers and templates, because some functional monomers remained dissociative with the templates.

3.3. Development of MI-μ-SPE method

3.3.1. The extraction performance of the MIP monolith

The imprinted monolith was applied to extract berberine in aqueous solutions. The extraction performance was studied firstly. A series of berberine standard solutions of 10–2×10^4 ng/mL were loaded onto the monoliths, the sample volumes were 1 mL. After washing with chloroform, the analytes were eluted by methanol/acetic acid (9:1, v/v) and injected into HPLC system for quantitative analysis. Fig. 6 showed the extraction yield curves of imprinted and non-imprinted monoliths. The extraction yields of MIP were obviously higher than that of NIP. The extraction capacity was 0.21 μg/mg for MIP, which was about 2 times over the NIP. This difference was due to the different extraction mechanism between the two kinds of monolith, which was resulted from the synthesis process. In the synthesis process of MIP, hydrogen bond was formed between the template (berberine) and monomers (AA) and thus produced an ordered structure, which was then fixed by the polymerization process. After polymerization, the template was eluted, and the binding sites could recognize berberine selectively, while the interaction between the NIP monolith and berberine was just non-specific sorption. Therefore, the MIP monolith exhibited higher affinity to the template molecule and exhibited better extraction capacity.

3.3.2. Optimization of parameters for MI-μ-SPE

A MI-μ-SPE method was established for the extraction of berberine in aqueous solutions. Some parameters possible to effect extraction were systematically studied, such as sample pH value, sample flow rate, sample volume and eluting solvents.

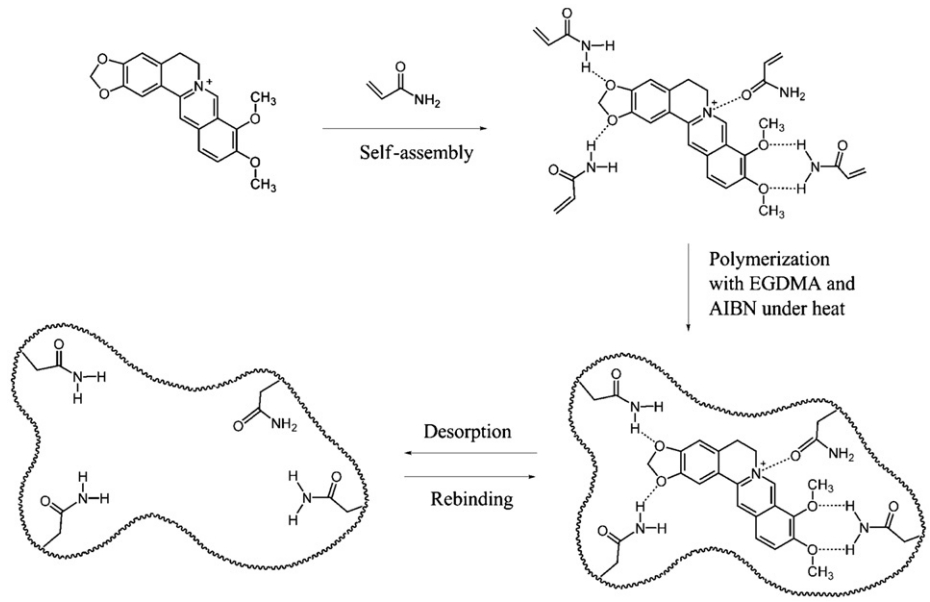

Fig. 5. Extraction efficiency of berberine MIP monolith to jatrorrhizine, coptisine and palmatine.

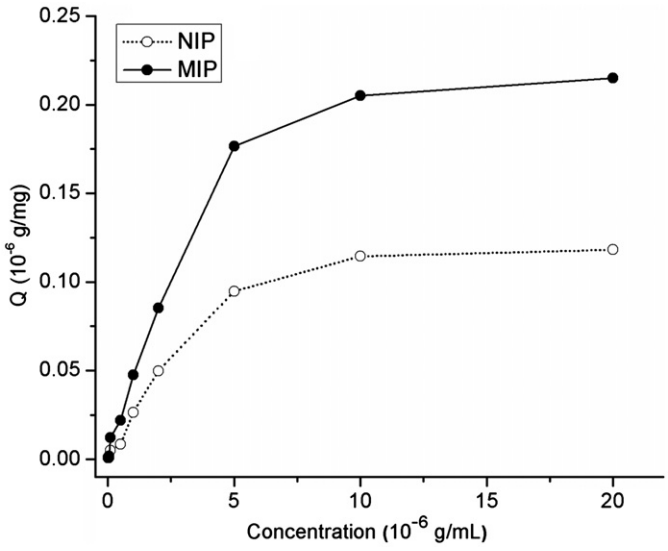

Fig. 6. Extraction yield curves of MIP and NIP monoliths.

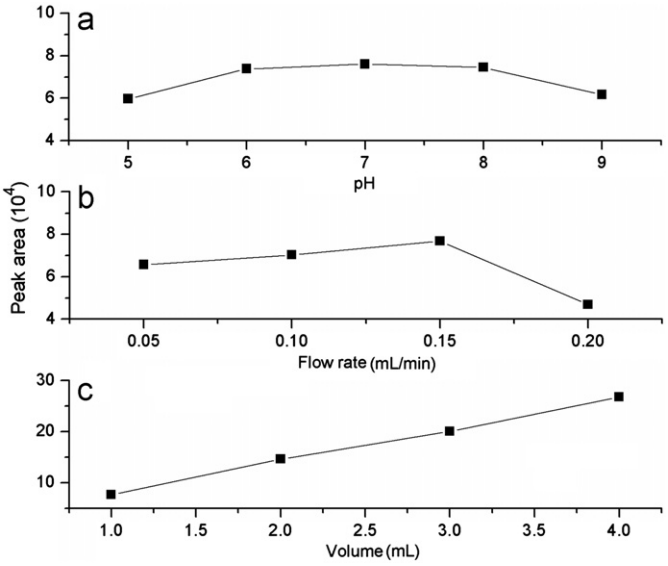

Fig. 7. Effect of sample pH (a), sample flow rate (b) and sample volume (c) on the extraction efficiency of MI-μ-SPE.

The binding of berberine molecules onto the MIP monolith was mainly based on the hydrogen bonds and electrostatic force, which were related to pH of the medium, therefore, pH value of sample solution was one of most important parameters for MI-SPE of berberine. By adjusting with phosphate buffer, sample (100 ng/mL) with pH values from 5 to 9 were extracted and investigated. The result was shown in Fig. 7a. From pH 5 to 7, the extraction efficiency of berberine increased along with the increase of pH, and decreased when pH was higher. The possible reasons were that under acidic condition, the amino group of AA was apt to binding with H^+, which was disadvantageous for the formation of hydrogen bonds between AA and berberine. Under basic condition, the amino group of AA kept uncharged and it was beneficial for the formation of hydrogen bonds, however, anions such as OH^- was easy to bind with quaternary ammonium of the berberine molecule, thus weakening the electrostatic force between AA and berberine and the extraction efficiency decreased as a result. pH 7 was selected for the best extraction efficiency.

Sample flow rate was another important parameter for MI-μ-SPE, which was possible to affect extraction efficiency of berberine and the time of analysis. Sample flow rate from 0.05 mL/min to 0.2 mL/min was investigated. As shown in Fig. 7b, no significant difference of peak areas between different flow rate was observed, indicating that sample flow rate have little influence on extraction efficiency in this system. Considering analysis time and monolith pressure which increased along with the flow rate, 0.15 mL/min was selected for further studies.

In the proposed micropipette tip based MI-μ-SPE device, sample solution was pushed through the imprinted monolith, berberine was extracted onto the monolith based on the hydrogen bonds and electrostatic force, which was a nonequilibrium absorption process, therefore, extraction efficiency was related to sample volume. Volume of berberine standard solution (100 ng/mL, pH 7.0) in the range of 1.0–4.0 mL was studied. Good linearity was observed in Fig. 7c, indicating that the breakthrough volume for MI-μ-SPE was above 4.0 mL and the monolith would be in

good performance for extraction when the sample volume was within 4.0 mL. Better pre-concentration efficiency could be obtained by loading sample with large volume, however, for the application in biological analysis, the sample volumes were always small, therefore, 1.0 mL was chosen in this work.

Another advantage of the micropipette based extraction device is that only small volume of eluting solvent was required, as the amount of sorbent is small (about 60 μL) and the eluting solvent could reach all of the surface of imprinted monolith easily and rapidly. Dozens of microlitre was enough for desorption of berberine, considering the reproducibility of the eluting procedure, 100 μL methanol/acetic acid (9:1, v/v) was used as eluting solvent.

Under optimized conditions, berberine was effectively extracted by MI-μ-SPE and a representative chromatogram of standards (200 ng/mL) was shown in Fig. 8. About one magnitude of enhancement in sensitivity was observed by MI-μ-SPE.

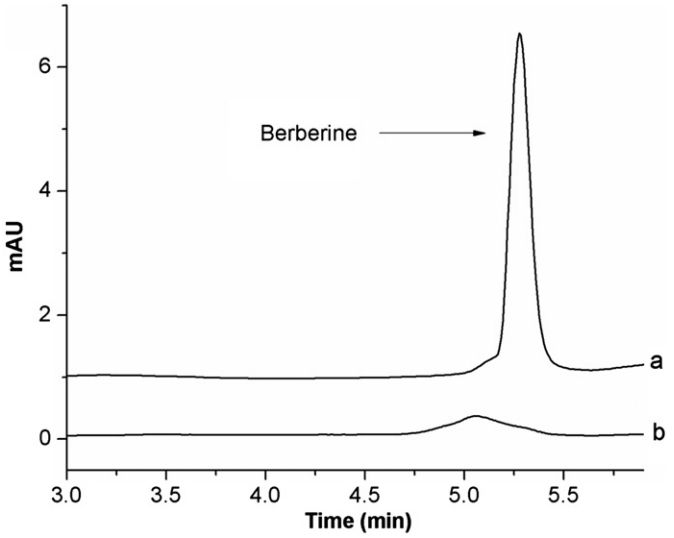

Fig. 8. Chromatographic comparison of 200 ng/mL berberine standard solusion with MI-μ-SPE (a) and without extraction (b).

Table 2
Analytical performance of MI-μ-SPE-HPLC method.

Analyte	Linear range (ng/ml)	Slope	Intercept	R	LOD (ng/ml)	LOQ (ng/ml)	RSD%
Berberine	3–800	267.2	399.9	0.9991	1.0	2.5	2.4

3.4. Analytical performances of MI-μ-SPE-HPLC method

By coupling with HPLC, a MI-μ-SPE-HPLC method was established for the pretreatment and determination of berberine in aqueous samples. Analytical performance of the method was validated, including linear range, coefficient (R^2), low limits of detection (LOD) and reproducibility, and was listed in Table 2. Good linearity (R=0.9991) was obtained in the range of 3–800 ng/mL. LOD, which indicated the sensitivity of the analytical method, was evaluated and found to be 1.0 ng/mL (S/N=3). By loading five replicates of berberine standards (200 ng/mL), RSDs for peak areas were calculated and found to be 2.4%, indicating good reproducibility of the method.

3.5. Application in plasma and urine samples

The developed method was applied to the determination of berberine in human plasma and urine samples. 0.5 mL samples were used for each analysis. The chromatograms of spiked plasma samples (100 ng/mL) before and after treated by MI-μ-SPE were shown in Fig. 9a, and the chromatograms for urine samples were shown in Fig. 9b. Most of the peaks for matrix were removed while berberine was efficiently enriched. The main components of plasma are proteins, inorganic salts and non-protein nitrogen molecules. And the main components of urine are urea, inorganic salts, amino acids and other small organic acids. When passing through the poly(AA-EGDMA) monolith, the size of proteins are

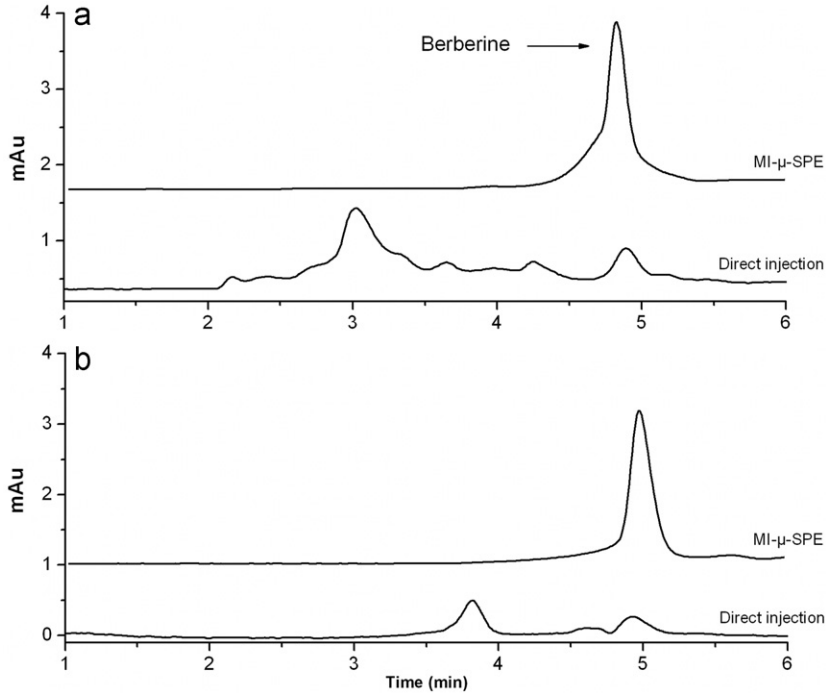

Fig. 9. Chromatograms of spiked plasma (a) and urine (b) samples with MI-μ-SPE and without extraction.

Table 3
Evaluation of selectivity and extraction efficiency of MI-μ-SPE in plasma and Urine samples.

Samples		Total area	Berberine area	%Area	Total height	Berberine height	%Height
Plasma	MI-μ-SPE	28227	27727	96.7	2073	2078	97.3
	Direct inj	41761	3068	7.5	2362	339	14.3
	Ratio	0.67	9.04	12.89	0.88	6.13	6.80
Urine	MI-μ-SPE	27793	26270	94.5	2021	1945	96.7
	Direct inj	15807	3103	19.6	1175	292	24.9
	Ratio	1.76	8.46	4.82	1.72	6.66	3.89

Table 4
Recoveries of the MI-μ-SPE-HPLC method for berberine in plasma and urine samples.

Samples	Added (ng/ml)	Found (ng/ml)	Recovery (%)	RSD (%)
Plasma	10	10.3	103.0	2.9
	100	95.9	95.9	1.9
	500	462.5	92.5	2.3
Urine	10	9.4	93.4	3.3
	100	90.6	90.6	3.6
	500	455.4	91.1	4.7

too large to absorb into the imprinted cavities and the proteins are high polar that is easy to be taken by aqueous media. For molecules such as urea and creatinine, the repel interaction of amino group in the MIP monolith with the nitrogen in these molecules possibly make it difficult to be extracted. Amino acids and other organic acid could bond with the amino group in the MIP monolith. However, the interactions are also not strong and they are incapable to form rigid structure as berberine, and the size of these molecules also mismatch with the imprinted cavities in the surface of monolith. Therefore, the interferences in plasma and urine could be removed through the MI-μ-SPE process. The static analysis comparison for plasma sample was listed in Table 3. The results showed that berberine was extracted effectively, the enrichment folds was 9.04 for peak area and 6.80 for peak height for plasma sample; in urine sample, the values were 8.46 and 6.66, respectively. Meanwhile, most of interference from the samples matrix was removed after MI-μ-SPE process, as shown in Table 3, the ratio of berberine peak area increased from 7.5% to 96.7% for plasma sample and increased from 19.6% to 94.5% for urine sample. In the aspect of peak height, the increases were 14.3% to 97.3% and 24.9% to 96.7%, respectively. The results demonstrated the high selectivity of the MIP monolith to berberine and high clean-up efficiency of the proposed method.

To evaluate the accuracy of the established method, the extraction recoveries were carried out in plasma and urine samples spiked with standard berberine of three different concentrations. The results were listed in Table 4. The recoveries were in the range of 92.5–103.2% for plasma sample and 90.6–93.4% for urine sample, showing the effectiveness of the method for the pretreatment and determination of berberine in plasma and urine samples.

4. Conclusions

In this work, a novel berberine MIP monolith has been synthesized for selective extraction of berberine in aqueous conditions. A micropipette tip based micro device was fabricated for MI-μ-SPE and it was improved to avoid the drop of extraction materials. The MIP monolith showed good selectivity by comparing with NIP monolith and the capacity was calculated to be 0.21 μg/mg. MI-μ-SPE followed HPLC was developed as analytical method for determination of berberine in aqueous conditions. The method has been applied for determination of berberine in human plasma and urine samples, by using MIP monolith as selective sorbent, most of the matrix in the plasma sample was eliminated and berberine was selectively extracted and well determined. The results showed that micropipette tip based MI-μ-SPE method exhibited high clean-up efficiency, low organic solvent consumption, and good extraction efficiency. The method could be used for pretreatment and determination of berberine in plasma and urine samples, and is potential to be applied in other biological samples.

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