Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 844 (2006) 142–147
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Molecularly imprinted solid-phase extraction for rapid screening of mycophenolic acid in human plasma

Junfa Yin [a], Sumin Wang [b], Guanqun Yang [b], Gengliang Yang [a,*], Yi Chen [a]

[a] *Beijing National Laboratory for Molecular Sciences, Laboratory of Analytical Chemistry for Life Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China*
[b] *College of Pharmacy, Hebei University, Baoding 071002, PR China*

Received 10 April 2006; accepted 7 July 2006
Available online 7 August 2006

Abstract

A molecularly imprinted solid-phase extraction coupled with high performance liquid chromatography (MISPE–HPLC) method was developed for rapid screening of mycophenolic acid (MPA) in human plasma. MPA imprinted polymers (MPA-MIP) were synthesized and then tested for their performance both in organic and in aqueous solution. MPA was selectively trapped and preconcentrated on the MPA-MIP sorbent using different loading and washing conditions. The good selectivity of MPA-MIP enabled further clean-up of the interfering components in human plasma. For the proposed MISPE–HPLC method, the linearity between responses (peak area) and concentration was found over the range of 1–100 μg/ml with a linear regression coefficient (R^2) of 0.9989. The limit of detection (LOD) and theoretical limit of quantification (LOQ) for MPA in plasma were 0.10 and 0.32 μg/ml, respectively. The precisions were 7.3, 3.5 and 4.7% RSD for intra-day assay and 9.2, 4.1 and 5.5% RSD for inter-day reproducibility, respectively, at three concentration levels of MPA in spiked plasma (1, 10 and 100 μg/ml). Both recoveries for the extraction (more than 74%) and for the analytical method (more than 87%) were acceptable for screening MPA in plasma samples. Twelve-hour pharmacokinetic profile of MPA for a renal transplant recipient receiving chronic oral dosing of 500 mg mycophenolate mofetil (MMF) was investigated. Results indicated that this method could be applied for therapeutic drug monitoring of mycophenolic acid in patient plasma.

Keywords: Mycophenolic acid; Molecularly imprinted polymer; Molecularly imprinted solid-phase extraction

1. Introduction

Mycophenolate mofetil (MMF) is the current primary immunosuppressant for the prevention of renal allograft rejection. Mycophenolic acid (MPA), the active metabolite of the prodrug MMF, is an immunosuppressive agent which inhibits lymphocyte proliferation by blocking the *de novo* synthesis pathway of guanosine nucleotides. MPA is primarily metabolized by glucuronidation at the phenolic hydroxyl group to mycophenolic acid glucuronide (MPAG), which is the major urinary excretion product of the drug and pharmacologically inactive but may be hydrolyzed *in vivo* to form free MPA [1–3]. Various analytical methods, such as reverse-phase HPLC [4–9], HPLC–mass spectrometry (HPLC/MS) [10,11], ion-pair liquid chromatography [12,13] and capillary electrophoresis (CE) [14] have been developed and used in quantitating MPA in patient samples from clinical studies.

One common problem in developing HPLC methods for screening MPA in human plasma is that extensive sample clean-up procedures are often required. Solid-phase extraction (SPE) has been used to simplify and improve the efficiency of sample preparation [15–19]. C.E. Jones et al. [16,17] developed a typically SPE–HPLC method for simultaneous determination of MPA and MPAG in plasma samples from renal transplant recipients and then compared it with a proposed enzyme-multiplied immunoassay technique (EMIT). They found that both results of the two different methods were acceptable but EMIT overestimated the concentration of MPA. In those literatures, most SPE media for MPA extraction were traditional ODS C18 bonded silica particles. Another kind of commercial Strata-X polymeric sorbent was employed by D.G. Watson et al. [18] for simultaneous determination of MPA and MPAG in plasma samples. Those commercial SPE sorbents offered significant advantages such as ease of operation, a wide spectrum of materials avail-

* Corresponding author. Tel.: +86 10 82627290; fax: +86 10 62559373.
E-mail address: glyang@iccas.ac.cn (G. Yang).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.07.009

able, high load ability, high recovery and high reproducibility. Despite their attractive features, they also suffered from a major problem, namely generic selectivity, in conventional analytical methods. The lack of specific selectivity could be attributed to the custom SPE sorbents differentiated only by generic properties such as hydrophobic and Van der Waal forces.

Recently, a novel SPE sorbent based on molecularly imprinted polymer (MIP) has attracted increasing attention in chromatography because of its excellent selectivity. MIPs are stable synthetic polymers possessing selective molecular recognition sites, which are obtained by using certain amounts of cross-linker and functional monomers in the presence of the template molecule [20–24]. Once the template is removed from the polymer, the resulting MIP can recognize the template molecule depending on geometrical fit and chemical interactions. As a result of the chemical and physical robustness of MIPs, they have proven to be good sorbents for molecularly imprinted solid-phase extraction (MISPE). B. Sellergren [25] first reported the use of MIPs as sorbents for SPE, since then a wide range of analytes of biological, pharmaceutical, food and environmental samples have been involved [26–30].

Herein, we report a simple and robust method for screening MPA in human plasma using a combination of MISPE and HPLC method. The potential of the developed method for carrying out pharmacokinetic studies was also demonstrated.

2. Experimental

2.1. Materials

MMF, MPA, MPAG and cyclosporine A (CsA) were kindly donated by North China Pharmaceutical Co. (Shijiazhuang, China). Suprofen was purchased from J&K Chemical (Beijing, China). Ethylene dimethacrylate (EDMA) and 4-vinylpyridine (4-VP) from Acros (NJ, USA) were refined by distillation. The initiator 2,2′-azobisisobutyronitrile (AIBN) was purchased from Shanghai Chemical Plant (Shanghai, China) and recrystallized prior to use. HPLC grade acetonitrile and methanol was purchased from Fisher (NJ, USA). All chemicals of analytical grade were purchased from Beijing Chemical Reagent (Beijing, China). Water was prepared by a Millipore system (resistivity 18.2 MΩ cm) and, unless otherwise specified, all solutions were filtered through a 0.45 μm membrane filter from Millipore before use.

2.2. MPA imprinted and nonimprinted polymers preparation

The template MPA (0.16 g, 0.5 mmol), functional monomer 4-VP (0.24 ml, 2 mmol) and cross-linker EDMA (2 ml, 10 mmol) were dissolved in 10 ml acetonitrile–toluene (7:3, v/v) in a round-bottom flask. To this solution, 15 mg AIBN was added in steps as an initiator of the radical polymerization reaction. The mixture was saturated with nitrogen for 10 min, followed by degasification under vacuum for 5 min and then sealed. The polymerization was carried out by heating the mixture in a 55 °C water bath for 24 h. The obtained bulk polymer was ground to fine powders and subsequently sieved to obtain 25–40 μm particles. The MPA-MIP particles were packed into a 4.6 mm i.d. × 50 mm steel column, washed on-line with methanol–acetic acid (4:1) and then with methanol until no residue of MPA was found in the rinses.

A reference, nonimprinted polymer (NIP) for comparison experiments was prepared similarly to MIP described above except that the polymerization mixture did not contain the imprinted molecule MPA.

2.3. Chromatographic evaluation

Chromatographic investigations were performed on Shimadzu HPLC system, which consists of a LC-10AT pump, a SPD-10A variable-wavelength UV detector and a Rheodyne 7725i injector equipped with a 20-μl loop. Data processing was carried out with a HW2000 chromatography workstation (Nanjing Qianpu Software, China). An Apollo C18 column (4.6 mm i.d. × 150 mm, 5 μm, Alltech) was employed for determination of MPA in plasma samples and methanol–20 mM phosphate buffer (pH 3.3) (55:45) was used as the mobile phase at a flow rate of 1.0 ml/min. The detection was selected at 254 nm, which was the maximum absorption wavelength of MPA.

To evaluate the chromatographic characteristics of the imprinted polymer, the column packed with MPA-MIP (4.6 mm i.d. × 50 mm) was connected into HPLC system. Methanol, methanol aqueous solutions and acetic acid–methanol solutions were tested as mobile phase at a typical flow rate of 1.0 ml/min.

2.4. MISPE protocol for plasma samples

Two hundred milligrams of the cleaned-up MPA-MIP (or NIP) was put into a 10 ml screw-cap centrifuge vial and incubated with 4 ml methanol, standing at ambient temperature with occasional shaking for 24 h. Then the slurry was transferred into a 1 ml polypropylene SPE cartridge equipped with a perforated frit and then stood for 15 min so that the polymer could settle down to the bottom of the cartridge. After that, another polyethylene frit was carefully put onto the polymer to stabilize the sorbent bed. MPA-MIPs of the size 25–40 μm proved to be an acceptable compromise between bed homogeneity and permeability, allowing appropriate flow rates in the range of 0.1–0.2 ml/min with the solvents employed in this paper.

Prior to use, the MIP (or NIP) SPE-cartridges were conditioned by washing with 2 ml methanol–acetic acid (8:2, v/v) and 2 ml methanol, followed by 2 ml water at a flow rate of 0.4 ml/min. For the MISPE process, several aliquots of 400 μl spiked plasma standards and the real plasma samples from a renal transplant patient were then loaded onto the MISPE cartridges at a migration flow rate of 0.2 ml/min. A series of solvents [water (2 ml), methanol–water (25:75, 2 ml) and methanol (1 ml)] were employed for selected washing, and then the SPE cartridge was eluted with 4 ml methanol–acetic acid (8:2) at a flow rate of 0.4 ml/min. The last eluate was immediately dried under a stream of nitrogen at 40 °C. The residue was reconstituted in 200 μl mobile phase for HPLC analysis, spiked with internal standard to a final concentration 10 μg/ml of supro-

fen. Unlike the traditional SPE protocols, the internal standard should be added into the samples only after the extraction; otherwise it would be eluted into the rinses. Aliquots of 20 μl of each solution were analyzed by HPLC.

As a comparison experiment, solid-phase extraction based on commercial reverse-phase ODS C18 sorbent was also performed for pretreating MPA-containing samples. The SPE cartridges were preconditioned with methanol (2 ml), followed by water (2 ml) and then the sample supernatants (400 μl) were applied onto the respective cartridges. The loaded cartridges were washed with 2 ml methanol–20 mM phosphate buffer (pH 3.3) (40:60) and then 1 ml water. The analytes were eluted with 2 ml methanol–20 mM phosphate buffer (pH 5.0) (80:20), followed by 1 ml methanol. The eluate was dried under a nitrogen stream at 40 °C then the residue was reconstituted in 200 μl mobile phase and spiked with internal standard to a final concentration 10 μg/ml of suprofen.

2.5. Plasma samples preparation and calibration curves

Aliquots of 1.9 ml of blank human plasma were spiked with 0.1 ml each of MPA (20, 40, 100, 200, 400, 1000 and 2000 μg/ml) working solutions (in acetonitrile) to yield MPA spiked plasma concentrations corresponding to 1, 2, 5, 10, 20, 50 and 100 μg/ml. These calibration standard samples were vortex-mixed for 2 min and allowed to equilibrate at room temperature for 10 min. Subsequently, protein precipitation was carried out by adding 2 ml of acetonitrile to each of the above samples, followed by vigorous centrifugation at 8000 rpm for 5 min at 4 °C. Human plasma samples were from anonymous renal transplant patients who were receiving MMF and co-medications (cyclosporine A) for immune suppression. Aliquots of 800 μl of the samples were diluted with 800 μl of acetonitrile and then centrifuged at 8000 rpm for 5 min. If not used immediately, these calibration standards and plasma samples were stored at −20 °C in a refrigerator.

Aliquots of 400 μl clear supernatant of the calibration standards and plasma samples were loaded onto the MIP sorbent, and the calibration curves were obtained by the manipulations described in Section 2.4.

3. Results and discussion

3.1. Molecular recognition properties of MPA-MIPs

One merit of MISPE is that the imprinted polymer sorbents have good selectivity for the template molecule. It was expected that a great imprinting effect would be achieved if MPA was dissolved in solvent before being loaded onto the SPE cartridges. To confirm the selectivity of this kind of SPE materials, the molecular recognition properties of the MPA-MIP were investigated on HPLC.

Fig. 1 shows the chromatogram of MPA on MIP, indicating that the related compounds have no interferences on the recognition of MPA. MMF, CsA and MPAG were rapidly eluted while MPA was strongly bonded on the MIP column when (A) methanol (B) acetic acid–methanol (2:98) and (C) water–methanol (75:25) were used as mobile phase. However, the similar separation was not found on the NIP column. The strong retention of MPA on the imprinted polymer showed the high selectivity and affinity of MPA-MIP, which originated from the imprinted molecules matching in steric structure and functionalities of the sites. In the rebinding process, only MPA could be accommodated into the *print*, whereas MMF, CsA and MPAG could not interact with those sites strongly for their incoordinations of chemical groups and/or dimensional structures.

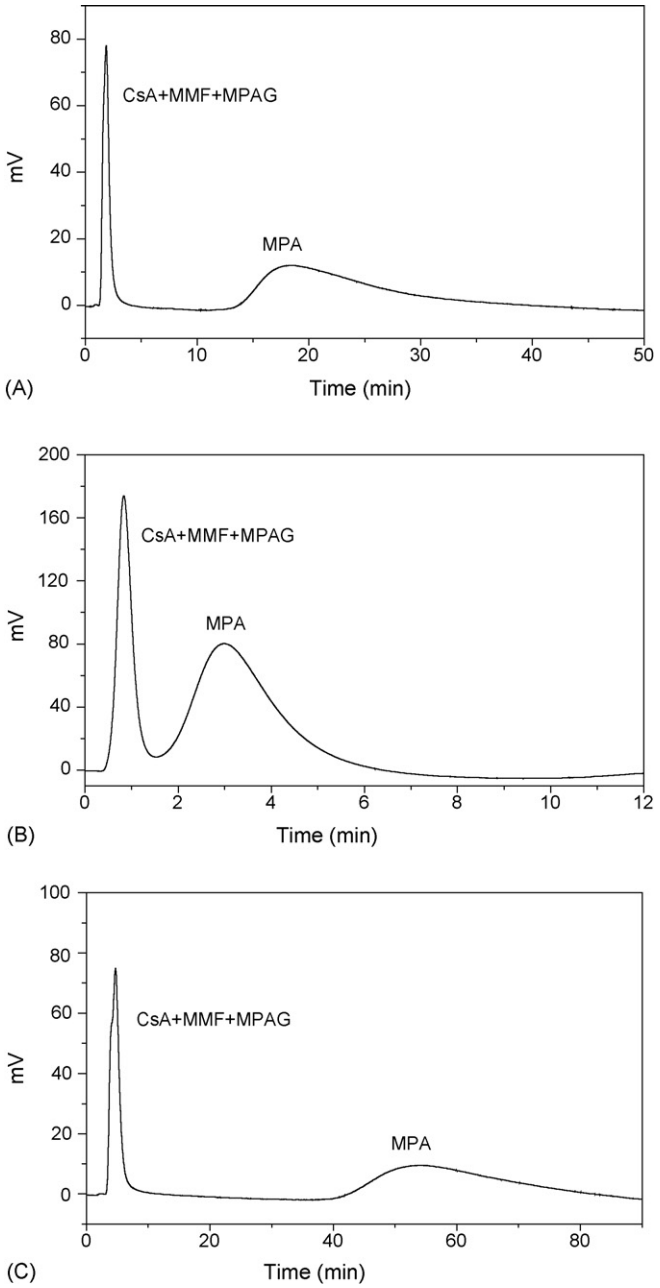

Fig. 1. Chromatograms of MPA and related compounds (CsA, MMF and MPAG) using methanol (A), 2% (v/v) acetic acid in methanol (B) and 75% water in methanol (C). Other HPLC conditions: column size, 4.6 mm i.d. × 50 mm; flow rate, 1.0 ml/min; UV detection, 254 nm, ambient temperature.

Although the selectivity and affinity of MIPs are usually high, they are generally associated with a poor chromatographic effi-

ciency and the elution of broad and asymmetric peaks [22]. One of the causes of the poor performance may be the nonspecific binding which comes from incomplete monomer–template association and nonequivalence of the different binding sites. Another reason is slow mass transfer. The irregular size and shape, and the low surface area together with low mesoporosity lead to low template recognition owing to slow analyte diffusion to sites located in micropores. A key to improve the performance of imprinted polymers would thus be either to achieve a narrower site distribution or to increase the accessibility of the binding sites. The former requires chemical modifications whereas the latter can be affected by changing the polymer morphology. We have prepared monolithic MIP which showed good properties: ease of preparation, all binding sites beyond destruction during grinding, fast mass transport and hence rapid separation, homogeneous and continuous construction and hence lower pressure drops, and high efficiencies even at high flow rate [24]. In this study, the size and shape of MIP particles for MISPE were very important, as well as the inner polymer morphology. MPA imprinted polymer was prepared by using 10 ml acetonitrile–toluene (7:3) as porogenic solvent, generating lots of homogeneous mesopores in the body of polymer. MPA-MIP particles in the size of 25–40 μm proved to be an acceptable compromise between homogeneity and permeability, allowing appropriate flow rates of the washing and eluting solvents.

As solvents could influence the selectivity and affinity of MIPs, the effects of mobile phase compositions on retention of MPA were investigated. When methanol was employed as mobile phase, the retention time (t_R) of MPA was 18.2 min whereas it was about 2.0 min for MMF, MPAG and CsA. Addition of 0.2–5% (volume proportion) of acetic acid into methanol would lead to sharp decline of t_R and the total MPA loaded on an MIP column (4.6 mm i.d. × 50 mm) could be eluted within 3 ml mobile phase when 20% acetic acid in methanol was employed. Similarly, t_R significantly decreased with the addition of water in methanol from 0.2 to 10%, whereas it rapidly increased in the ranges of 10–80%. To be emphasized, MPA was not eluted within 180 min when 90% water in methanol or pure water was employed as mobile phase. It was suggested that the imprinted polymer bound through hydrogen bonds was suppressed by the increased concentration of a substance with higher hydrogen bonding capacity. However, another noncovalent interaction contributing to the molecular recognition, hydrophobic interaction, would play an important role to let the analyte remain on the MIP when the proportion of water in methanol was more than 10% [31,32]. On the basis of the above observations, we decided to select water, methanol–water (25:75) and methanol for selective clean-up of the proteins and the related compounds, and methanol–acetic acid (4:1) for elution of MPA for the MISPE protocols.

3.2. MISPE for plasma samples

For a recommendable MISPE protocol, high selectivity, high recoveries, low-cost and low-toxicity solvent for washing and operational simplicity should be involved and comprehensively considered [27]. Results showed that the developed method accorded with these requirements mentioned above.

Firstly, the proposed MISPE protocol showed highly efficient and selective clean-up of plasma samples, resulting in chromatographic traces with few peaks (Fig. 2). Most of the

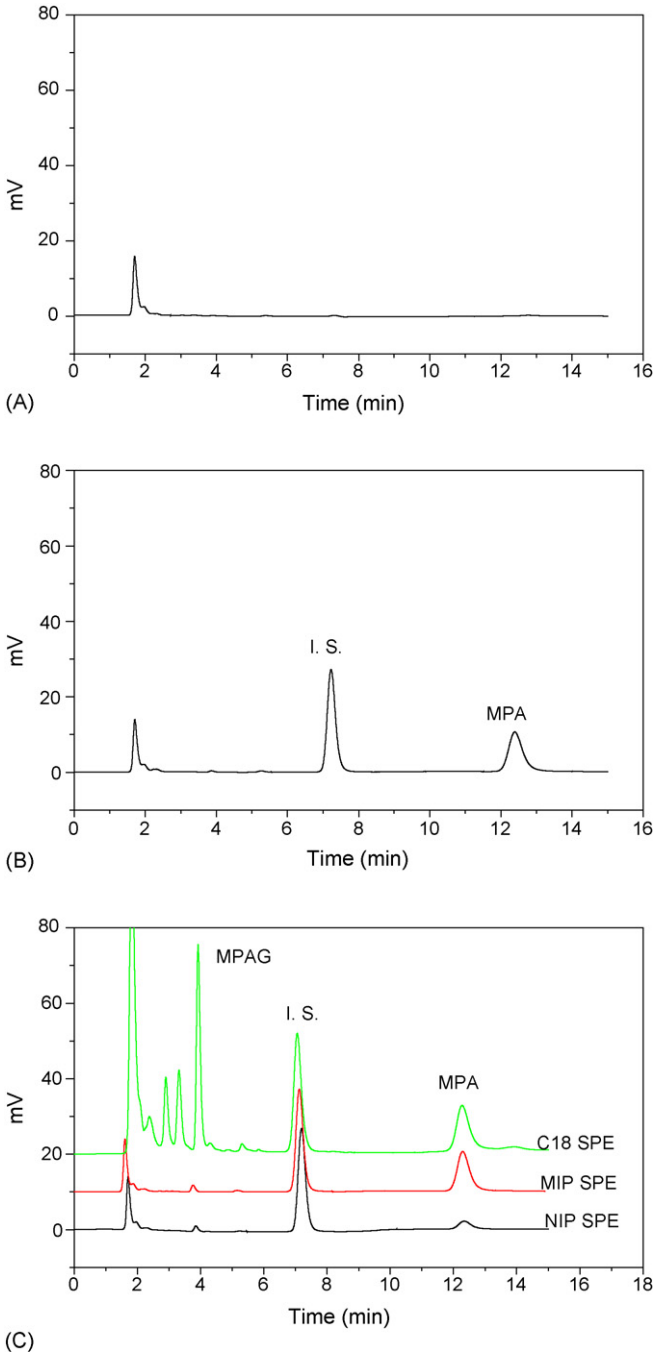

Fig. 2. HPLC chromatograms of MPA in human plasma: (A) blank plasma; (B) blank plasma spiked with MPA (10 μg/ml) and I.S. (suprofen, 10 μg/ml); (C) plasma sample at 1 h from a subject administered a single 500 mg oral dose of MMF (by ODS C18 SPE, MISPE and NIP SPE, respectively). HPLC conditions: column, Apollo C18 column (4.6 mm i.d. × 150 mm, 5 μm, Alltech); mobile phase, methanol–20 mM phosphate buffer (pH 3.3) (55:45, v/v); flow rate, 1.0 ml/min; UV detection, 254 nm, ambient temperature.

proteins were precipitated and cleaned-up in the pretreating steps, for instance, precipitating with acetonitrile and washing loaded cartridges with water. The signals of MPA and internal standard were well separated from interfering matrix component and could be reliably quantified. As a control experiment, extraction on NIP was investigated in similar conditions. Results showed that NIP extraction led to a low recovery, generally less than 40%. This was because most of the analyte was cleaned-up with the matrices selective washing solutions due to its low affinity to MPA. On the contrary, reverse-phase ODS C18 extraction provided a high recovery and strong retention protocol for pretreating human plasma samples. The extraction recoveries were more than 90% for C18 SPE, which led to a precious and acute determination of MPA. However, the chromatogram of ODS C18 extracted samples showed more complex matrices peaks due to some trace interfering components that were also preconcentrated in the samples (Fig. 2C). Although ODS C18 extraction had high recoveries of the analyte, it was generally associated with a poor specific selectivity for MPA. MISPE protocols afforded both good selectivities and acceptable extraction recoveries; therefore, it was predicted to be a promising high-selective SPE technique in clinical and pharmaceutical analysis [25].

Furthermore, all washing and elution solvents (water, methanol, acetic acid and mixture thereof) were inexpensive and low- or nontoxic. Dichloromethane (1 ml) had been employed as a washing solvent followed by methanol–water (25:75, 2 ml) washing step in our original experiments. The obtained sample extracts showed a better clean-up effect and their chromatograms were fewer interfering peaks but suffered from the poor recoveries (less than 60% at a concentration level of 10 μg/ml). This result was unacceptable, hence this step was subsequently omitted.

In addition, the washing and elution steps for MISPE and HPLC analysis for screening of MPA were rapid and simple. A single run of the sample could be accomplished within 1.5 h from MISPE to HPLC analysis.

3.3. Validation assay

The developed MISPE method for MPA analysis was validated using plasma standards. The linearity of the standard calibration curve for MPA was evaluated over the range of 1–100 μg/ml in plasma and produced a linear regression coefficient (R^2) of 0.9987. The limit of detection (LOD) and the theoretical limit of quantification (LOQ) for MPA in plasma at the concentrations, where the signal to noise ratio was equal to 3 for LOD and 10 for theoretical LOQ, were calculated to be 0.10 and 0.32 μg/ml, respectively. The operational LOQ is the lower limit of linearity (1.0 μg/ml). The intra-assay precision was evaluated by five repeated injections of each spiked samples (1, 10 and 100 μg/ml) and calculated to be 7.3, 3.5 and 4.7% RSD. Similar results of 9.2, 4.1 and 5.5% RSD were obtained for inter-day reproducibility of these samples. The values were well within the 10% limits required for the assay to be validated.

The recoveries of SPE and the MISPE–HPLC analysis method were investigated and the results were listed in Table 1.

Table 1
Recoveries and precisions of the proposed method.

Concentration (μg/ml)	Recovery (%)		Precision (RSD, %)	
	SPE	Method	Intra-day	Inter-day
1	73.8	87.4	7.3	9.2
10	86.7	94.2	3.5	4.1
100	82.2	91.3	4.7	5.5

The recoveries of MPA for MISPE were calculated from the formula shown below:

$$\text{Recovery (SPE)} = \frac{A_{\text{Plasma}}}{A_{\text{Acetonitrile}}} \times 100\%$$

where A_{Plasma} is the peak area of MPA in plasma obtained by HPLC analysis with MISPE pretreating, $A_{\text{Acetonitrile}}$ is the peak area of MPA standard in acetonitrile (1, 10, or 100 μg/ml) obtained by HPLC analysis without SPE pretreating. The recoveries of MISPE–HPLC method were investigated using three injections of each plasma sample pretreated by MISPE and the values could be calculated from the formula:

$$\text{Recovery (method)} = \frac{C_{\text{Found}}}{C_{\text{Spiked}}} \times 100\%$$

where C_{Found} is the concentration of MPA obtained by HPLC analysis with MISPE, which can be calculated from the calibration curve, C_{Spiked} is the concentration of initial MPA of spiked plasma standard. As shown in Table 1, the recoveries of MPA for SPE are more than 74% and the recoveries for method are no less than 87% at the top, middle and bottom concentration levels. The acceptable recoveries were sufficient for quantitative determination of MPA in human plasma.

MMF was usually given as part of a combination therapy with CsA or sirolimus (rapamycin) and corticosteroids for the prevention of rejection in the above-mentioned transplant populations. So, drug monitoring of MPA was required regarding the potential interferences caused by others immunosuppressant drugs. CsA, rapamycin, ibuprofen, amlodipine, nicardipine and flunarizine have been investigated and results showed they had no interferences in the determination of MPA.

One of the major advantages of MIP for SPE is their high chemical robustness, providing the opportunity to clean and reactivate them under relatively harsh conditions for multiple uses in SPE applications [27]. Those ODS C18 bonded silica sorbents and immunoaffinity sorbents are generally not encouraged to multi-use because the exposure to protein-denaturating environments may lead to irreversible changes in the binding domains. However, MIPs sorbent can avoid these problems. For the assessment of the reusability of the MPA-MIPs, five consecutive clean-up cycles for a plasma sample (10 μg/ml) were performed with a single MISPE cartridge. After each cycle, the MISPE cartridge was reactivated by washing with 2 ml methanol–acetic acid (8:2, v/v) and 2 ml methanol–water (6:4, v/v), followed by 2 ml methanol at a flow rate of 0.2 ml/min. The results showed an average recovery of 91.7% and precision of 5.2% RSD ($n = 5$). In agreement with the requirement of recovery >90% and precision <10% RSD, this MISPE sorbent could

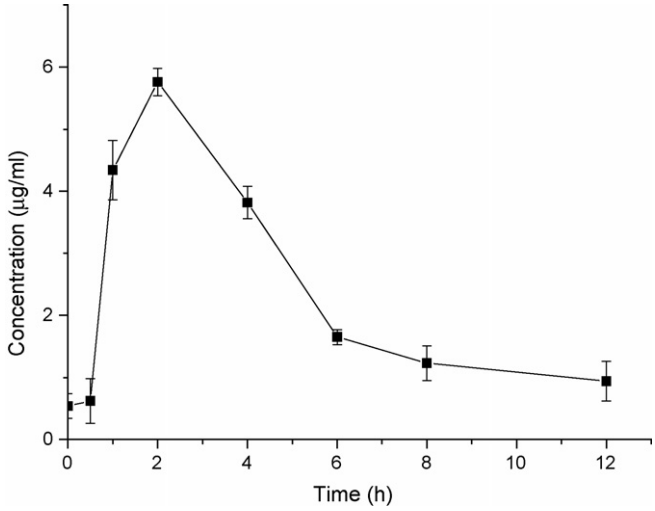

Fig. 3. Pharmacokinetic profiles of MPA of a renal transplant recipient receiving chronic oral dosing of MMF (500 mg) co-administered with CsA.

be reused seven to nine times at the same concentration level of samples. To be mentioned, this MISPE protocol combined with a protein precipitation procedure (adding acetonitrile to plasma samples), which prolonged the reuse times of MIP sorbent. Without protein precipitation, it could be used three times according to the recovery and precision requirement.

3.4. Clinical application

The proposed method was subsequently employed to the pharmacokinetic study of MPA in patient plasma. Blood samples were colleted from a subject administered a single 500 mg oral dose of MMF. Fig. 3 shows the curve of concentration of MPA in plasma versus time. Following oral administration of 500 mg MMF, the highest concentration of MPA was observed at to be 5.76 ± 0.22 μg/ml at 2 h. The area under the curve ($AUC_{0-12\,h}$) was calculated to be 30.64 ± 2.12 h μg/ml.

4. Conclusion

This study highlighted the potential of MPA-MIP as solid-phase extraction sorbent for pretreating MPA plasma samples. The imprinted polymers showed good selectivity for MPA, avoiding the interferences of the related compounds such as MMF, MPAG and CsA. The developed method was validated with linearity, precision, LOD, LOQ and recoveries. Furthermore, this MISPE protocol was more direct and selective than conventional C18 SPE, avoiding time-consuming dilution, pH adjustment and sometimes tedious liquid–liquid extraction steps. Simultaneously, the high physical and chemical robustness, high selectivity and perfect clean-up effects of MISPE sorbents enabled their good reusability. Those good properties enabled the applications of MPA–MISPE for selective extraction and rapid screening MPA in human plasma of renal transplant patients.

The limitation of this method is that simultaneous screening of MPA and MPAG cannot be accomplished using a single MPA-MIP sorbent. Work is in progress on preparation of MPAG–MIP for simultaneous determination of MPA and MPAG.

Acknowledgements

This work was supported by *Bairen* Project of Chinese Academy of Sciences, National Natural Science Foundation of China (Grant No.20375010, No. 20435030) and Excellent Youth Program of Chinese Education Ministry.

References

[1] R.E. Bullingham, A.J. Nicholls, B.R. Kamm, Clin. Pharmacokinet. 34 (1998) 429.
[2] E. Schutz, M. Shipkova, V.W. Armstrong, E. Wieland, M. Oellerich, Clin. Chem. 45 (1999) 419.
[3] M. Shipkova, C.P. Strassburg, F. Braun, F. Streit, H.J. Grone, V.W. Armstrong, R.H. Tukey, M. Oellerich, E. Wieland, Br. J. Pharmacol. 132 (2001) 1027.
[4] K. Wiwattanawongsa, E.L. Heinzen, D.C. Kemp, R.E. Dupuis, P.C. Smith, J. Chromatogr. B 763 (2001) 35.
[5] D. Indjova, L. Kassabova, D. Svinarov, J. Chromatogr. B 817 (2005) 327.
[6] G.A. Khoschsorur, W. Erwa, J. Chromatogr. B 799 (2004) 355.
[7] D.U. Renner, C. Thiede, M. Bornhauser, G. Ehninger, H.M. Thiede, Anal. Chem. 73 (2001) 41.
[8] J. Shen, Z. Jiao, Y.Q. Yu, M. Zhang, M.K. Zhong, J. Chromatogr. B 817 (2005) 207.
[9] Z. Jiao, Y. Zhong, J. Shen, Y.Q. Yu, Chromatographia 62 (2005) 363.
[10] B. Atcheson, P.J. Taylor, D.W. Mudge, D.W. Johnson, P.I. Pillans, S.E. Tett, J. Chromatogr. B 799 (2004) 157.
[11] C. Willis, P.J. Taylor, P. Salm, S.E. Tett, P.I. Pillans, J. Chromatogr. B 748 (2000) 151.
[12] H. Hosotsubo, S. Takahara, Y. Kokado, S. Permpongkosol, J.D. Wang, T. Tanaka, K. Matsumiya, M. Kitamura, A. Okuyama, H. Sugimoto, J. Pharm. Biomed. Anal. 24 (2001) 555.
[13] W.P. Yau, A. Vathsala, H.X. Lou, E. Chan, J. Chromatogr. B 805 (2004) 101.
[14] S. Noe, J. Bohler, A.W. Frahm, J. Pharm. Biomed. Anal. 22 (2000) 197.
[15] I. Tsina, F. Chu, K. Hama, M. Kaoostian, Y.L. Tam, T. Tarnowski, B. Wong, J. Chromatogr. B 675 (1996) 119.
[16] J.L. Beal, C.E. Jones, P.J. Taylor, S.E. Tett, Ther. Drug Monit. 20 (1998) 685.
[17] C.E. Jones, P.J. Taylor, A.G. Johnson, J. Chromatogr. B 708 (1998) 229.
[18] D.G. Watson, F.G. Araya, P.J. Galloway, T.J. Beattie, J. Pharm. Biomed. Anal. 35 (2004) 87.
[19] D. Teshima, K. Otsubo, N. Kitagawa, S. Yoshimura, Y. Itoh, R. Oishi, J. Clin. Pharm. Ther. 28 (2003) 17.
[20] G. Wulff, Angew. Chem. Int. Ed. Eng. 34 (1995) 1812.
[21] M. Kempe, K. Mosbach, J. Chromatogr. A 694 (1995) 3.
[22] B. Sellergren, K.J. Shea, J. Chromatogr. A 690 (1995) 29.
[23] G. Yang, J. Yin, Z. Li, H. Liu, L. Cai, D. Wang, Y. Chen, Chromatographia 59 (2004) 705.
[24] J. Yin, G. Yang, Y. Chen, J. Chromatogr. A 1090 (2005) 68.
[25] B. Sellergren, Anal. Chem. 66 (1994) 1578.
[26] L.I. Andersson, E. Hardenborg, M. Sandberg-stall, K. Moller, J. Henriksson, I. Bramsby-Sjostrom, L.I. Olsson, M. Abdel-Rehim, Anal. Chim. Acta 526 (2004) 147.
[27] N.M. Maier, G. Buttinger, S. Welhartizki, E. Gavioli, W. Lindner, J. Chromatogr. B 804 (2004) 103.
[28] E.P.C. Lai, S.G. Wu, Anal. Chim. Acta 481 (2003) 165.
[29] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatography 46 (1997) 57.
[30] C. Chassaing, J. Stockes, R.F. Venn, F. Lanza, B. Sellergren, A. Holmberg, C. Berggren, J. Chromatogr. B 804 (2004) 71.
[31] C. Yu, O. Ramstrom, K. Mosbach, Anal. Lett. 30 (1997) 2123.
[32] C. Yu, K. Mosbach, J. Chromatogr. A 888 (2000) 63.