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Determination of total, free and saliva mycophenolic acid with a LC–MS/MS method: Application to pharmacokinetic study in healthy volunteers and renal transplant patients

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ABSTRACT

Mycophenolic acid (MPA) is the active moiety of mycophenoate mofetil (MMF), an ester prodrug widely used as an immunosuppressant. Therapeutic drug monitoring (TDM) of MPA is becoming mandatory for transplant patients received MMF therapy in the routine clinical practice because of large individual variability, dose-related toxicity and the risk of acute rejection. In this study, a rapid, sensitive and selective LC–MS/MS method was developed and validated for the quantitative analysis of total and free MPA in plasma and in saliva that uses one identical liquid chromatographic and mass spectrometric condition. Following protein precipitation for total and saliva MPA, and ultrafiltration for free MPA, chromatographic separation was performed on an Allure PFP Propyl analytical column (100 × 2.1 mm, 5 μm, RESTEK Co., Bellefonte, PA, USA) with 0.1% formic acid in acetonitrile and 0.1% formic acid in water (45:55, v/v) as the mobile phases. The compounds were quantified by positive electrospray ionization tandem mass spectrometry. Selectivity, linearity, accuracy, precision, recovery, matrix effect, and saliva stability were evaluated during method validation. The validated method was applied to a pharmacokinetic study of MPA after an oral administration of a single 1000 mg of MMF to eight healthy male volunteers and 750 mg bid of MMF to nine renal transplant patients.

1. Introduction

Mycophenolate mofetil (MMF), an ester prodrug of mycophenolic acid (MPA), is a widely used immunosuppressant that is given mainly in combination with a calcineurin inhibitor and corticosteroids, for the prevention of rejection in patients receiving solid organ transplantation [1–3]. Following oral administration, MMF is rapidly and completely hydrolyzed to MPA, the active form of the drug *in vivo*. MPA is subsequently converted to the main inactive metabolite, phenolic glucuronide (MPAG), and two minor metabolites, 7-o-glucuronide and acyl-glucuronide (AcMPAG) [4,5]. MPA is generally found to be extensively bound to serum albumin and only the free fraction (1–3%) is considered pharmacologically active [6–8]. However, some studies have demonstrated that protein binding of MPA may decrease in patients with renal dysfunction, and as a result, free MPA concentrations may dramatically increase, leading to potential severe side effects or toxicity [9–12].

MMF is known to exhibit great intra- and inter-individual pharmacokinetic variability and the MPA area under the curve (AUC) may vary more than 10-fold for the same dose [4,7,13]. Recent studies have reported a clear relationship between MPA $AUC_{0\text{-}12}$ and the risks for acute rejection and side effects, mainly from hematologic and/or gastrointestinal aspects [14–18]. Therefore, an $AUC_{0\text{-}12}$ of 30–60 mg·h/L is recommended in the initial phase of transplantation for desirable clinical outcomes [5,19,20]. For these reasons, therapeutic drug monitoring (TDM) of MPA is becoming mandatory for transplant patients received MMF therapy in routine clinical practice [19,21–23].

Considering individual variability, toxicity and the risk of acute rejection in transplant patients, TDM of MPA is definitely helpful in determining clinical therapy outcomes. So far, TDM of MPA has generally been based on total plasma concentrations or postdose AUC, through a limited sampling strategy. However, it is the free fraction of this drug that is pharmacologically active and its levels have proven to be associated with MPA-related toxicity, although the total MPA may appear to be at low concentrations [10]. Furthermore, saliva has the advantage of indicating the corresponding concentration in plasma. Salivary TDM usually offers convenient, noninvasive, cost-effective and potentially acceptable method for

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both patients and physicians [24,25]. Saliva may be used as an alternative specimen to plasma for TDM of MPA [26].

In the previous papers published on the measurement of MPA and its metabolites, most methods were based on chromatographic or immunoassay techniques. Among these published papers, a series of HPLC methods were developed for determination of MPA and its glucuronide metabolite in human plasma, which had wide clinical application [27–32]. Unlike immunoassays, which may cross-react with AcMPAG and subsequently lead to a overestimated result [33,34], the HPLC method is accurate and specific. However, its low sensitivity makes it unsuitable for free MPA analysis. LC–MS/MS has proven to be a considerably more sensitive and specific technique that is ideally suitable for MPA measurements, especially for free and saliva MPA analysis. Although LC–MS/MS methods have been described in a few studies [26,35–39], to our knowledge, none of these papers validated a method for quantification of both total and free MPA in plasma, and MPA in saliva.

In the current study, a rapid, sensitive and selective LC–MS/MS method was developed and validated for the quantitative analysis of total and free MPA in plasma and saliva, with an identical liquid chromatographic and mass spectrometric condition. Its applicability to a pharmacokinetic study and TDM of MPA in clinical practice for transplant patients receiving MMF for immunosuppressive therapy was also assessed.

2. Experimental

2.1. Chemicals

Mycophenolic acid (purity 99.5%) was purchased from Sigma–Aldrich, Inc (St. Louis, MO, USA). Sulfadimethoxypyrimidine (Internal standard, purity 99.9%) was provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile, formic acid were supplied by Tedia Company Inc. (Fairfield, OH, USA). Mycophenolate mofetil (MMF) capsules were supplied by Shanghai Roche Pharmaceuticals (Shanghai, China). All other reagents were of analytical grade. Double distilled water was used throughout the study.

2.2. Instrument and chromatographic conditions

A Shimadzu (Shimadzu Corporation, Kyoto, Japan) HPLC system, equipped with two LC-20AD pumps, a SIL-HTC autosampler and an online DGU-20A3 vacuum degasser, was used to perform the chromatographic separation on an Allure PFP Propyl analytical column (100 × 2.1 mm, 5 μm, RESTEK Co., Bellefonte, PA, USA) coupled with a Phenomenex C18 guard column (4.0 mm × 3.0 mm, 5 μm), which was kept at room temperature. The mobile phase consisted of 0.1% formic acid in acetonitrile and 0.1% formic acid in water (45:55, v/v), was eluted at an isocratic flow rate of 0.3 mL/min.

A triple quadrupole tandem mass spectrometer API 3000 (Applied Biosystems/MDS Sciex, Toronto, Canada) equipped with Turbo Ionspray source was operated in positive ionization mode. Multiple reaction monitoring (MRM) analysis was applied to detect ion transitions at *m/z* 321.2/207.1 and 311.1/156.1 for MPA and internal standard, respectively. The ion spray voltage was set at 1800 V and the source temperature at 400 °C. The collision activated dissociation (CAD) was set at 0.5 MPa using nitrogen as collision gas. Q1 and Q3 quadrupoles were set on unit resolution. Analyst 1.4 software was used for instrument control and data acquisition.

2.3. Preparation of stock solutions, calibration samples and quality control (QC) samples

Standard stock solutions of MPA and internal standard were separately prepared at 1 mg/mL in methanol and kept at −20 °C. The calibrators and QC samples were prepared by spiking appropriate MPA stock solution into a blank plasma or saliva sample collected from normal subjects.

2.4. Sample preparation

For the determination of total mycophenolic acid (tMPA) in human plasma, a simple protein precipitation procedure was employed. In a polystyrene tube, a 100 μL aliquot of plasma and 200 μL of acetonitrile (containing 200 ng/mL of internal standard) were vortex-mixed for 10 s, followed by centrifugation for 3 min at 12,000 rpm on Hettich Mikro 22R Microcentrifuge (Global Medical Instrumentation, Inc, Germany). The supernatant (20 μL) was further diluted with 60 μL of mobile phase and 2 μL was injected onto the analytical column.

For the determination of free mycophenolic acid (fMPA) in human plasma, an ultrafiltration procedure was employed. Two hundred microliters of plasma was added to a Millipore Microcon YM-3 Centrifugal Filter (Millipore Co., Billerica, MA, USA) and centrifuged at 12,000 rpm for 30 min. The filtrate (50 μL) was mixed with 5 μL of internal standard (200 ng/mL in acetonitrile) and 10 μL was injected onto the analytical column.

For the determination of mycophenolic acid (sMPA) in human saliva, 100 μL of saliva and an equal volume of acetonitrile (containing 20 ng/mL of internal standard) were added and vortex-mixed in a polystyrene centrifuge tube, followed by centrifugation at 12,000 rpm for 3 min. 10 μL of supernatant was injected onto the analytical column.

2.5. Method validation

To assay MPA in human plasma and saliva, all method validation steps were carried out according to the FDA guidance for industry bioanalytical method validation.

Linearity was evaluated by constructing linear regression equation fitted with peak area ratio of MPA to IS vs. nominal concentration using a 1/× weighting. The sensitivity of the analytical procedure was expressed as the lower limit of quantification (LLOQ) or the lowest concentration on the calibration curve that can be quantitatively determined with acceptable accuracy and precision and should be atleast 10 times of signal to noise (S/N). The specificity of assay was determined by analysis of six blank plasmas and saliva from different subjects. There should be no interference from endogenous or exogenous materials observed at the retention time in each analyte channel.

The accuracy and precision were assessed by determining QC samples at three concentration levels on three different validation batches. The QC samples were prepared for six duplicates together with calibration samples.

Matrix effect and recovery were assessed by comparing the peak areas of the neat analyte standards, standards spiked before and after extraction in six different lots of plasma and saliva at three concentration levels.The stabilities of MPA in plasma, including freeze-thaw, short-term, long-term, and autosampler or postpreparative stability, have been investigated in previous studies. The results showed good stability in these conditions. In the present study, the stabilities of MPA in saliva were evaluated by three freeze-thaw cycles from −70 °C to 37 °C and by placing processed QC samples in the autosampler at 4 °C for 15 h.

2.6. Pharmacokinetic study in healthy subjects and renal transplant patients

Eight healthy Chinese male volunteers participated in this study. The age was 23.4 ± 2.58 y (20–25 y), height 170 ± 2.86 cm (162–179 cm), and body weight 67.4 ± 4.10 kg (62–75 kg). They were

free of significant cardiac, hepatic, renal, pulmonary, neurologic, gastrointestinal and hematologic diseases, as assessed by physical examination. Electrocardiography and clinical laboratory tests including hematology, biochemistry, electrolytes and urinalysis were conducted. Volunteers were fasted overnight and a single dose of 1000 mg of MMF was administrated with 200 mL of water at 8:00 AM. Blood was drawn from the ulnar vein into tubes containing the anticoagulant EDTA at predose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48 h postdose. Saliva was simultaneously collected at the above time points.

Nine renal transplant patients were also recruited for the study. There were nine males with age 32.5 ± 8.18 y (18–48 y), height 167 ± 5.65 cm (156–182 cm), and body weight 65.4 ± 9.60 kg (50–76 kg). The patients were 2–3 weeks post-renal allograft transplant and continuously received triple immunosuppressive therapy included MMF, a calcineurin inhibitor, and prednisone. The patients were fasted overnight and administrated with 17.5–20 mg of prednisone at 6:00 AM, then with 750 mg bid MMF and 125–200 mg cyclosporine A or 2–3 mg tacrolimus at 8:00 AM. Blood was drawn from the ulnar vein into tubes containing the anticoagulant EDTA at predose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 9, 10, 11, 12 h postdose. Saliva was simultaneously collected at the same time points.

The study protocols and informed consent forms were approved by the relevant Ethical Committee of Shanghai First People's Hospital, affiliated by Shanghai Jiao Tong University.

The blood samples were centrifuged for 10 min at 1500 rpm at room temperature and the separated plasma was stored at −70 °C until analysis. Collected saliva was placed at 4 °C for 30 min, followed by centrifugation for 10 min at 1500 rpm at room temperature. The clear upper layer was stored at −70 °C until analysis.

2.7. Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed using a noncompartmental method. The maximal concentration (C_{max}) and the time to maximal concentration (T_{max}) were obtained directly for the concentration–time curves of MPA. The area under the concentration–time curve from the time of drug administration to the last plasma sampling time (AUC_{0-t}) was calculated according to the linear trapezoidal rule. The area under the concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t is the last measured concentration and λ_z is the slope of the linear regression of the log-transformed concentration–time. The half-life of MPA ($T_{1/2}$) was calculated as $0.693/\lambda_z$.

Pearson correlation between total, free and saliva MPA was analyzed by using SPSS 11.5 software for windows. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Optimization of LC–MS/MS conditions

The MS ionization was achieved at electrospray ionization mode (ESI) by infusing a single standard solution of MPA. Positive ESI showed obvious advantages for MPA analysis, including favorable ionization efficiency, high signal response and low noise background, over negative ESI. In addition to the protonated molecule $[M+H]^+$ at *m/z* 321.2, we also found other adducts such as $[M+NH_4]^+$ at *m/z* 338.2, and $[M+Na]^+$ at *m/z* 343.2 in Q1 full scan (Fig. 1). In fact, the intensity of $[M+NH_4]^+$ at *m/z* 338.2 is a little higher than that of $[M+H]^+$ at *m/z* 321.2. Kuhn et al. described a LC–MS/MS method by using an ammonium adduct ion transition at *m/z* 338 → 207 for MPA quantification [39]. Product ions were obtained in product ion scan (MS2) by collisionally activated

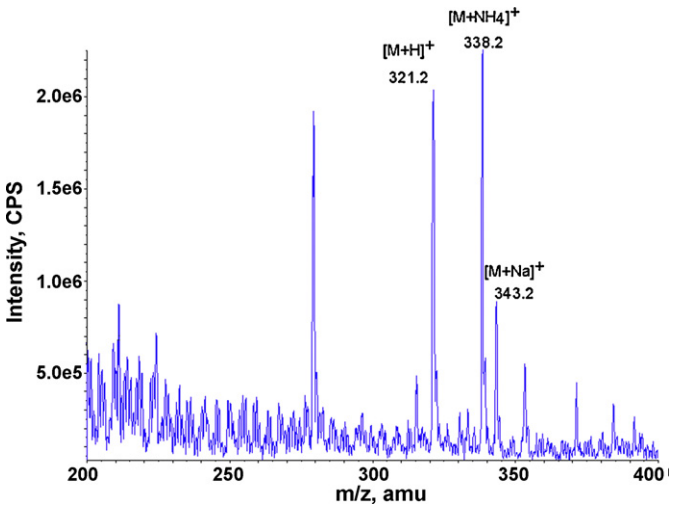

Fig. 1. Q1 scan spectra of mycophenolic acid, the ion adducts $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$ are shown.

precursor ion fragmentations using nitrogen as the collision gas (Fig. 2). The most intense transition, with *m/z* 321.2 → 207.1 for MPA and *m/z* 311.1 → 156.1 for the internal standard, was selected for quantitative analysis in multiple reaction monitoring (MRM) mode. The compound-dependant parameters and source/gas conditions were optimized to produce the most intense MS signal. When assaying the total MPA in real plasma samples collected from patients after administration of MMF, an intense peak with chromatographic retention time 1.1 min was found. The prepared sample was scanned in Q1 MS and then fragmented in MS2, the chromatographic peak was showed as $[M+Glu+18]^+$ *m/z* 514.3 with same fragmentation ions including *m/z* 303, 275, 207 as produced by MPA, which was subsequently identified as phenolic MPA glucuronide (MPAG). Although MPAG is a pharmacologically inactive metabolite from MPA, it is essential to achieve its chromatographic separation in real sample analysis.

Liquid chromatographic condition optimization is also very important during method development. In order to avoid the interference from its glucuronide metabolite and to enhance the selectivity and sensitivity, several types of analytical columns including Gemini C18, Capcell C18, and Allure PFP propyl column were tested. By comparison, the Allure PFP propyl column was found more suitable for MPA analysis because of its high retention,

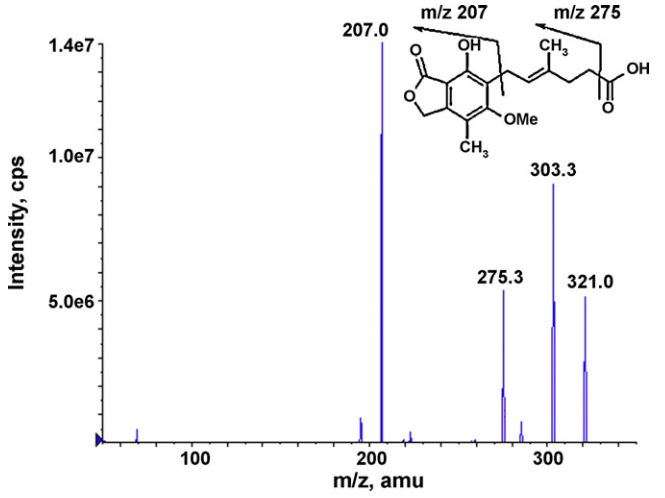

Fig. 2. Product ion spectra from protonated molecule and the fragmentation pattern of mycophenolic acid.

excellent selectivity, and better compatibility with high-organic mobile phases for better ESI-MS sensitivity.

Considering that the sensitivity was sufficiently high for MS detection, we adopted a simple preparation procedure for plasma and saliva samples using acetonitrile protein precipitation. For determination of the free fraction MPA in plasma, an ultrafiltration procedure was employed to separate the unbound portion, the pharmacologically active form, from the protein-bound portion. Using a 3k Millipore Centrifugal Filter and centrifuging at 12,000 rpm for 30 min, about 80 μL filtrate solution could be obtained from 200 μL plasma.

In this study, sulfadimethoxypyrimidine was used as internal standard for the quantification of MPA in plasma, ultrafiltrate, and saliva. This selected internal standard, though with different retention time to MPA, showed no really much difference in recovery and matrix effect between its analyte. Moreover, a simple protein precipitation procedure was employed to prepare sample, therefore sulfadimethoxypyrimidine was used to correct the loss during sample preparation and MS ionization.

3.2. Method validation

3.2.1. Selectivity

Six different batches of blank human plasma/saliva, spiked plasma/saliva, and real plasma/saliva sample from volunteers and patients were collected. The samples were prepared by following the previously established sample preparation procedures for that type of sample. Selectivity was assessed by comparing the chromatograms of blank samples with corresponding spiked samples and real samples. Fig. 3 shows the typical chromatograms of tMPA acquired from blank plasma, LLOQ, plasma sample from a healthy volunteer and a patient 0.5 h after oral administration with MMF. Figs. 4 and 5 present chromatograms of fMPA and sMPA in corresponding ultrafiltrates and saliva. There was no significant interference from endogenous substances in plasma, ultrafiltrate or saliva observed at the retention times for the analyte or the IS. The typical retention times for MPA and IS were about at 3.7 min and 2.5 min, in tMPA, fMPA and sMPA analysis.

3.2.2. Linearity and LLOQ

Calibration curves were constructed with a linear regression with $1/x$ weighting using three different matrixes. The plasma-based calibration curve was linear over the quantification range of 0.1–51.2 μg/mL for total mycophenolic acid (tMPA), the ultrafiltrate-based calibration curve over 2–256 ng/mL for free mycophenolic acid (fMPA), and the saliva-based calibration curve over 2–256 ng/mL for saliva mycophenolic acid (sMPA). The typical equations for the calibration curves were $y = 1.04x - 0.00487$ ($r = 0.9982$), $y = 0.0106x + 0.00216$ ($r = 0.9992$), and $y = 0.016x - 0.00531$ ($r = 0.9990$) for tMPA, fMPA, and sMPA, respectively. The LLOQ for the three forms of MPA was the lowest concentrations of calibration curve with S/N > 10.

3.2.3. Precision and accuracy

The intra- and inter-batch precision and accuracy results are listed in Table 1, as determined by assaying the QC samples in plasma, ultrafiltrate and saliva at three concentration levels. In the assay, the intra-batch accuracy was 92.3–101.6%, and the inter-batch accuracy was 94.7–99.1%. The precision, presented by relative standard deviation (RSD), was less than 15%. These results are acceptable by US FDA's requirements for bioanalytical method validation.

3.2.4. Matrix effect and recovery

The matrix effect and recovery of mycophenolic acid were evaluated by assay the quality controls in plasma and saliva from six different subjects. The results are summarized in Table 2. We also investigated the matrix effect by using a 10 μL/min continuous post column infusion (FIA) a solution containing 100 ng/mL of MPA and IS in mobile phase to MS, and simultaneously injection of mobile phase (A), blank plasma (B), blank ultrafiltrate (C) and blank saliva (D) with autosampler (Fig. 6). As shown in the figure, no significant ion suppression was observed at the retention time of MPA and IS. The mean extraction recoveries of tMPA and sMPA ranged from 90.6% to 93.4% and from 84.1% to 86.7%, respectively. The corresponding data for the matrix effect were 90.7–94.3% and 96.4–100.1%. The RSD of these values were less than 10%. These results demonstrated that recovery and matrix effects

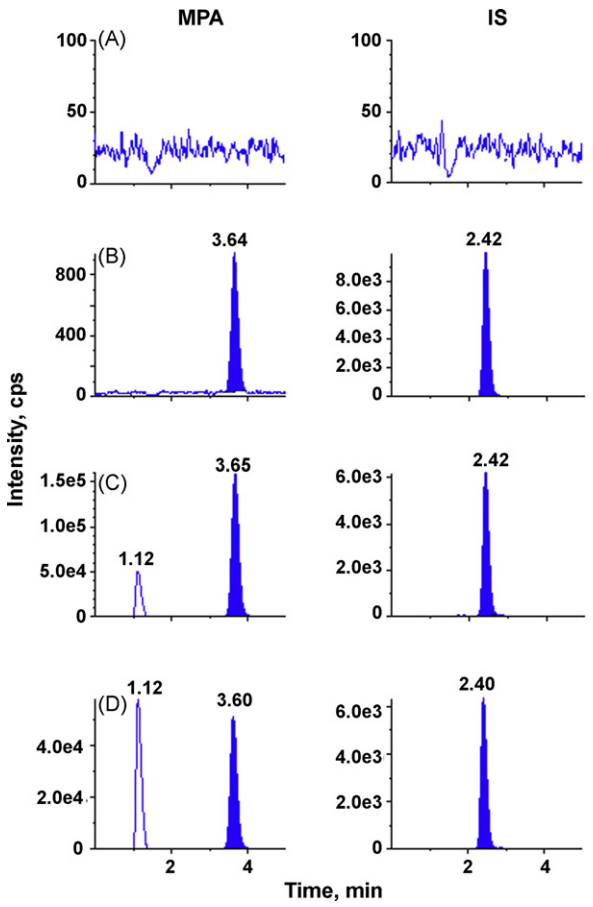

Fig. 3. Typical chromatograms of total MPA of blank plasma (A) LLOQ (B) plasma sample collected from a healthy volunteer (C) and a renal transplant patient (D) 0.5 h after oral administration of MMF.

Table 1
Precision and accuracy of mycophenolic acid assay in plasma, ultrafiltrate and saliva.

Nominal concentration	Intra-batch ($n = 6$)		Inter-batch ($n = 18$)	
	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
tMPA (μg/mL)				
0.15	2.69	93.8	4.54	94.7
22.5	2.88	93.1	6.84	98.4
45	6.95	92.3	7.09	96.7
fMPA (ng/mL)				
3	3.23	92.8	10.6	99.1
102	4.31	95.0	9.29	97.4
205	5.34	99.4	7.09	98.5
sMPA (ng/mL)				
3	2.47	95.9	2.50	95.9
102	2.00	98.4	5.75	99.1
205	3.22	101.6	4.79	98.7

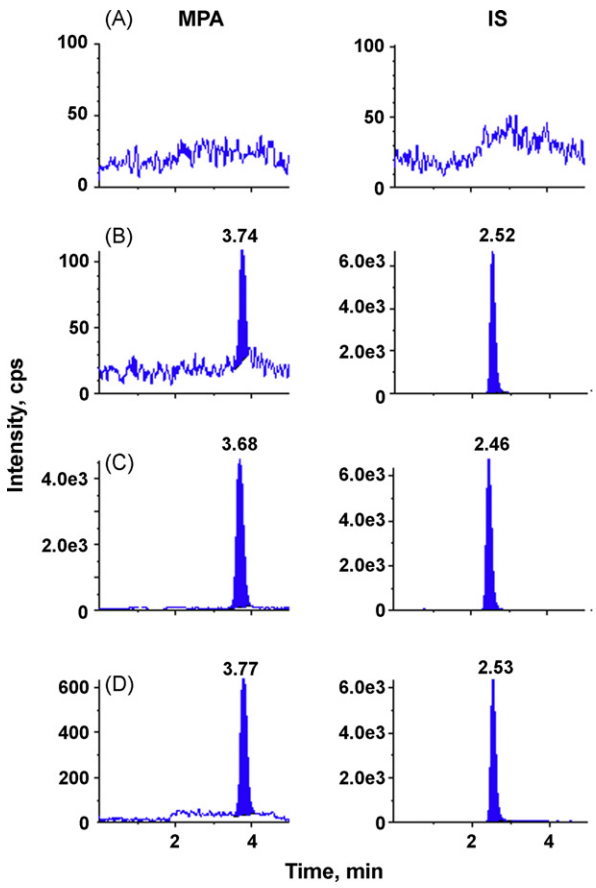

Fig. 4. Typical chromatograms of free MPA of blank ultrafiltrate (A), LLOQ (B), ultrafiltrate from plasma sample collected from a healthy volunteer (C) and a renal transplant patient (D) 0.5 h after oral administration of MMF.

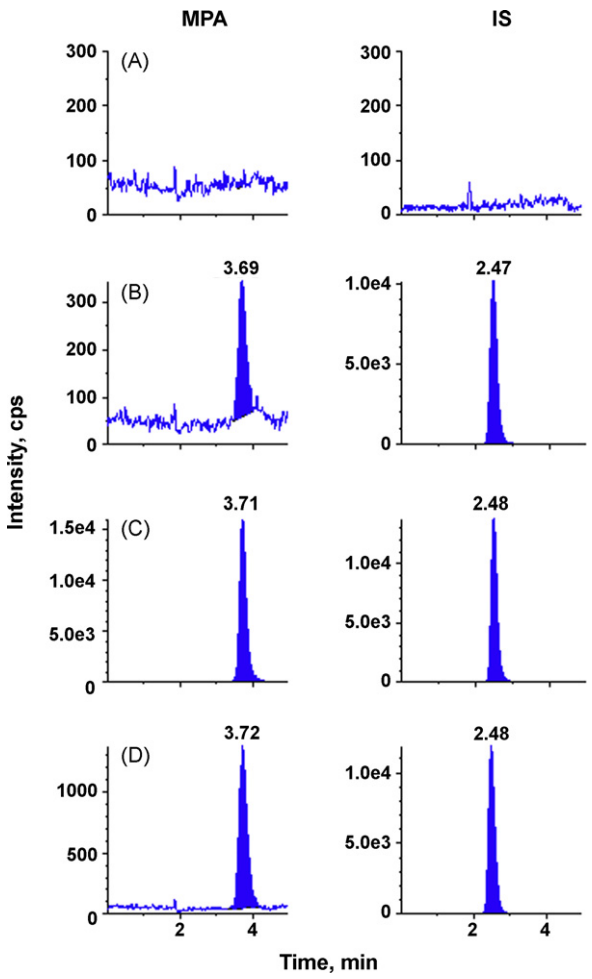

Fig. 5. Typical chromatograms of saliva MPA of blank saliva (D), LLOQ (B), saliva sample collected from a healthy volunteer (C) and a renal transplant patient (D) 0.5 h after oral administration of MMF.

in different resources of plasma and saliva were consistent and repeatable.

3.2.5. Stability

Previous reports demonstrated that MPA stock solution in methanol was stable at 4 °C for atleast 6 months and at room temperature for atleast 24 h [19]. Total and free mycophenolic acid in plasma was stable after three freeze-thaw cycles, as determined by placing samples on the bench top for 24 h at room temperature, by freezing samples for 6 months and by storage in an autosampler post-preparation for 1 day, 1 week and 1 month at room temperature [32,37–40]. Consequently, we did not repeat investigation of the stability in plasma.

The stability of MPA in saliva, however, has not previously been reported. In the present method, quality control samples were prepared in saliva at three levels of 3, 102, and 205 ng/mL. The freeze-thaw stability was performed for three cycles by freezing samples at −70 °C and then thawing at 37 °C. The accuracy was 90.8–99.7% and RSD was 5.83–8.84%. Autosampler stability was assessed by keeping the prepared quality control samples at 4 °C at intervals of 0, 3, 9, and 15 h. The results showed that MPA was stable in an autosampler for at least 15 h, with accuracies ranging from 90.9% to 96.8% and RSD from 2.47% to 5.50%.

Table 2
Matrix effect and recovery of mycophenolic acid in plasma and saliva.

Compound	Recovery ($n=6$)		Matrix effect ($n=6$)	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)
tMPA (μg/mL)				
0.15	93.4	6.85	94.3	8.23
22.5	91.0	7.68	90.7	9.25
45	90.6	5.32	92.5	4.56
IS (200 ng/mL)	89.7	8.33	91.7	9.92
sMPA (ng/mL)				
3	86.3	8.67	96.4	5.83
102	84.1	9.02	100.1	4.54
205	86.7	5.68	98.8	3.63
IS (20 ng/mL)	95.6	4.70	82.9	1.84

3.3. Application to pharmacokinetic study

Mycophenolate mofetil (MMF) is rapidly absorbed following oral administration and hydrolyzed to mycophenolic acid (MPA), the active metabolite form. In the present study, the tMPA and fMPA concentration in plasma, and sMPA concentration in saliva were determined using the validated LC–MS/MS method following an oral administration of a single 1000 mg dose of MMF to eight healthy male volunteers and 750 mg bid MMF to nine male renal transplant patients. Good correlation between tMPA, fMPA, and sMPA in healthy volunteers and renal transplant patients were observed by Person Correlation analysis with SPSS 11.5. The correlation coefficients were 0.980 (tMPA vs. fMPA), 0.914 (tMPA vs. sMPA), 0.849 (fMPA vs. sMPA) in healthy volunteers, and 0.992 (tMPA vs. fMPA), 0.838 (tMPA vs. sMPA), 0.816 (fMPA vs. sMPA) in renal transplant patients. Therefore saliva may present an alternative to plasma total and free MPA monitoring for renal transplant patients. The mean drug concentration–time profiles for tMPA,

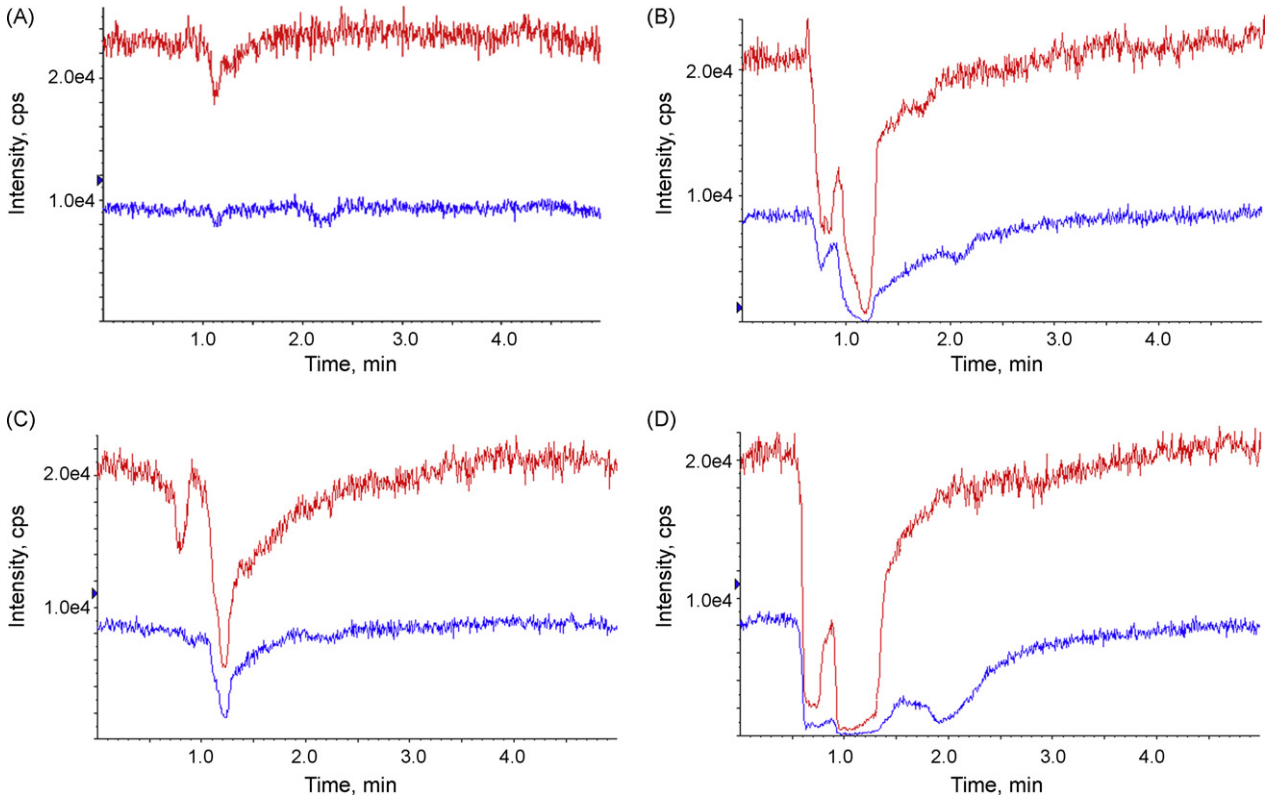

Fig. 6. Chromatograms of ion suppression for MPA and IS, acquired from injection of mobile phase (A) blank plasma (B) blank ultrafiltrate (C) and blank saliva (D) with autosampler and simultaneously continuous infusion 100 ng/mL of MPA (blue) and IS (red) with flow injection analysis at 10 μL/min (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

fMPA and sMPA obtained from healthy volunteers and renal transplant patients are presented in Fig. 7. The main pharmacokinetic parameters for tMPA are summarized in Table 3. As shown in the mean MPA pharmacokinetic curve, the main *c*–*t* profile trends of total, free and saliva MPA, both in healthy volunteers and renal transplant patients, remained the same overall. The tendency can also be seen from the significant correlation among the three forms of MPA. A secondary peak in the plasma MPA *c*–*t* profile was observed at 6–12 h postdose in the two subject groups due to enterohepatic recirculation. Although significant individual differences were observed, the main pharmacokinetic parameters obtained in present study are comparable with those previously reported in other populations [5,7,41]. An obviously higher bioavailability of MPA in healthy volunteer was noted over that seen in renal transplant patients, which can be deduced from the difference in the area under *c*–*t* curve (AUC_{0-t}) between the two groups.

Table 3
The main pharmacokinetics parameters of tMPA after an oral administration of 1000 mg of MMF to eight healthy male volunteers and 750 mg MMF bid to nine renal transplant patients ($\bar{x} \pm s$).

Parameter	Healthy volunteers	Renal transplant patients
AUC_{0-t} (mg·h/L)	99.1 ± 26.6	37.1 ± 11.5
$AUC_{0-\infty}$ (mg·h/L)	104.3 ± 29.0	40.1 ± 13.6
$T_{1/2}$ (h)	11.6 ± 2.6	3.0 ± 1.5
T_{max} (h)	0.81 ± 0.51	1.06 ± 0.53
C_{max} (mg/L)	41.0 ± 13.4	17.7 ± 5.5

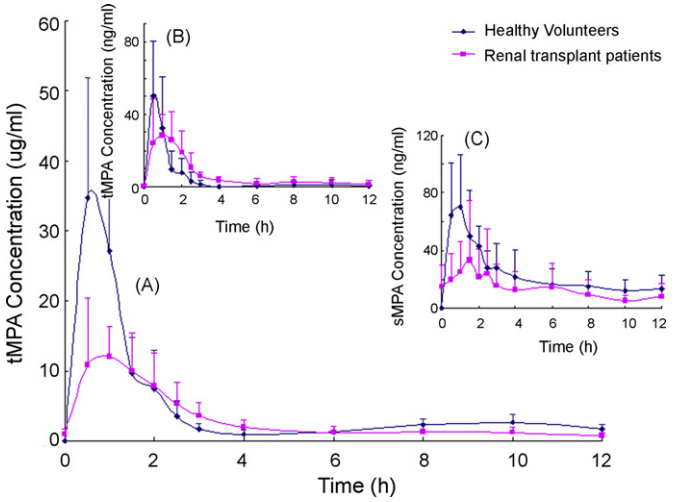

Fig. 7. Mean drug concentration–time profiles of tMPA (A), fMPA (B) and sMPA (C) following an oral administration of a single dose of 1000 mg MMF to eight healthy volunteers and 750 mg bid MMF to nine renal transplant patients.

4. Conclusions

A liquid chromatography-positive electrospray tandem mass spectrometry method was developed for the determination of mycophenolic acid concentration in plasma and saliva that uses a simple protein precipitation or ultrafiltration procedure. The three forms of mycophenolic acid (tMPA, fMPA and sMPA) were analyzed with an identical LC–MS/MS condition and a 5 min turnaround was achieved. After validation for selectivity, linearity, precision, accuracy, recovery, matrix effect and stability, the proposed method was shown to be ideally suitable for MPA analysis in human plasma and saliva. This method was successfully applied to a pharmacokinetic study of MPA after an oral administration of a single 1000 mg dose

of MMF to eight healthy male volunteers and 750 mg bid of MMF to nine renal transplant patients.

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