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ORIGINAL ARTICLE

Determination of mycophenolic acid in human plasma by ultra performance liquid chromatography tandem mass spectrometry

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Abstract A simple, sensitive and high throughput ultra performance liquid chromatography tandem mass spectrometry method has been developed for the determination of mycophenolic acid in human plasma. The method involved simple protein precipitation of MPA along with its deuterated analog as an internal standard (IS) from 50 μL of human plasma. The chromatographic analysis was done on Acquity UPLC C18 (100 mm × 2.1 mm, 1.7 μm) column under isocratic conditions using acetonitrile and 10 mM ammonium formate, pH 3.00 (75:25, v/v) as the mobile phase. A triple quadrupole mass spectrometer operating in the positive ionization mode was used for quantitation. In-source conversion of mycophenolic glucuronide metabolite to the parent drug was selectively controlled by suitable optimization of cone voltage, cone gas flow and desolvation temperature. The method was validated over a wide concentration range of 15–15000 ng/mL. The mean extraction recovery for the analyte and IS was >95%. Matrix effect expressed as matrix factors ranged from 0.97 to 1.02. The method was successfully applied to support a bioequivalence study of 500 mg mycophenolate mofetil tablet in 72 healthy subjects.

1. Introduction

Mycophenolic acid (MPA) is the active metabolite of ester prodrug mycophenolate mofetil (MPM) and is widely used as an immunosuppressant drug to prevent the rejection of organ transplantation and in the treatment of autoimmune disease [1,2]. To improve its oral bioavailability MPA is administered as MPM, which is completely absorbed and rapidly hydrolyzed by esterases to MPA. It is a selective, reversible and non-competitive inhibitor of inosine

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monophosphate dehydrogenase (IMPDH), the rate limiting enzyme in the *de novo* synthesis of guanosine nucleotides [3]. MPA is primarily metabolized by uridine diphosphate glucuronosyl enzyme in the liver, kidney and intestine to phenolic mycophenolic acid glucuronide (MPAG) metabolite which is recovered in urine. MPAG is pharmacologically inactive and can hydrolyze back to MPA by β-glucuronidase during entrohepatic recirculation [4,5]. This leads to a second concomitant peak in the plasma concentration-time profile between 6–12 h after administration and might contribute to the gastrointestinal toxicity [6]. Additionally, MPA is further metabolized to two minor metabolites namely acyl glucuronide (AcMPAG) and phenolic glucoside of MPA. MPA is highly bound to plasma proteins, mainly to human serum albumin (97–99%) [7]. Clinical pharmacokinetics of MPA reveal significant inter and intra subject variation in plasma concentration [8], which can be associated with different factors such as renal allograft function, hepatic function, MPA unbound fraction, entrohepatic recirculation, and concomitant immunosuppressant therapy [9]. Hence therapeutic drug monitoring for MPA is necessary to optimize outcomes, especially in patients with high rejection risk [10]. Moreover, due to low therapeutic levels it is essential to develop sensitive, rugged and rapid bioanalytical methods for its determination in biological fluids to minimize the risk of drug accumulation, and for optimization of therapy to reduce the frequency of adverse effects.

Several methods have been reported for the analysis of MPA and its metabolites in different biological fluids by electrophoresis [11–14], immunoassay technique [15–17], high performance liquid chromatography using diode array [18], fluorescence [19–21], UV [22–29], and mass spectrometry detection [30–41]. Relatively few UPLC–MS/MS based methods are available in literature for rapid analysis of MPA in biological samples [42,43]. Kuhn et al. [42] have developed a rapid method to determine MPA and MPAG with a limit of quantitation (LOQ) of 50 ng/mL and 2300 ng/mL respectively in serum and plasma. A comparable method with LOQ of 100 ng/mL for MPA was used for a pharmacokinetic study in kidney transplant recipients [43]. A summary of salient features of liquid chromatographic methods with mass spectrometry detection for determination of total MPA concentration in human plasma is presented in Table 1.

Ultra performance liquid chromatography (UPLC) is an ideal tool for rapid separation of complex mixtures in both isocratic and gradient modes. Improved separation efficiency and a decrease in the analysis time can be realized by reducing the particle size of the column packing material. The advantage of UPLC over conventional HPLC is the ability to increase the speed without sacrificing efficiency [44]. In the present work, a simple, sensitive, selective and rapid UPLC–MS/MS method has been developed

Table 1 Comparative summary of liquid chromatographic methods with mass detection developed for determination of total MPA in plasma.

Sr. no.	Detection technique	Human plasma volume (μL)	Extraction procedure; internal standard	Recovery (%)	Linear range (ng/mL)	Run time (min)	Application	Ref.
1[a]	LC–MS/MS	100	PP with perchloric acid and sodium tungstate; carboxy butoxy ether of MPA	91–110	100–50000	4.0	–	[31]
2[a]	LC–MS/MS	100	SPE on Phenomenex Strata-X cartridges; indomethacin	82–92	50–50000	7.0	Pharmacokinetic study with 1.5 g MPM in 52 healthy volunteers	[33]
3[a]	LC–MS/MS	100	PP with acetonitrile and formic acid; carboxy butoxy ether of MPA	98.5–101.7	50–30000	4.0	Pharmacokinetic study with MPM in healthy volunteers and patients	[35]
4[a]	LC–MS/MS	100	LLE with diethyl ether at pH 4.0; PPA	43.3–60.0	50–100000	3.0	Method comparison with commercially available EMIT	[36]
5[a]	LC–MS/MS	10	Ultrafiltration followed by on-line SPE; mycophenolic acid-d3	89.3–99.1	100–40000	2.0	Analysis in dried blood/plasma spots	[37]
6	LC–MS/MS	50	SPE on Waters MAX Oasis plates; MPA cyclopropane analog	81.3–84.7	19.95–19955	–	Bioequivalence study with 500 mg MPM in 103 healthy subjects	[38]
7	LC–MS/MS	250	LLE; pioglitazone	–	75.43–24965	4.0	Bioequivalence study with 500 mg MPM in 117 healthy subjects	[39]
8[a]	LC–MS/MS	20	PP with acetonitrile; [^{13}C, ^{2}H$_3$]–MPA	82.0	80–20000	2.0	Pharmacokinetic study with 500 mg MPM twice daily in a renal pediatric patient	[40]
9[a]	UPLC–MS/MS	10	PP with acetonitrile; PPA	78.1–129.7	50–1000000	2.0	Analysis of 121 heart transplantation patients who used MPM as part of multiple-drug regime	[42]
10[a]	UPLC–MS/MS	50	PP with acetonitrile; mycophenolic acid-d3	–	100–20000	2.0	Analysis of clinical samples collected from 15 *de novo* kidney transplant recipients	[43]
11	UPLC–MS/MS	50	PP with acetonitrile; mycophenolic acid-d3	96.8–101.1	15–15000	2.0	Bioequivalence study with 500 mg MPM in 72 healthy subjects	PM

[a]Along with metabolites; MPA: mycophenolic acid; MPM: mycophenolate mofetil; PP: protein precipitation; SPE: solid phase extraction; LLE: liquid-liquid extraction; PPA: N-phthaloyl-L-phenylalanine; EMIT: enzyme multiplied immunoassay technique; PM: present method.