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Manual and automated (robotic) high-performance liquid chromatography methods for the determination of mycophenolic acid and its glucuronide conjugate in human plasma

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Abstract

A manual and an automated (Zymark PyTechnology robot) HPLC method for simultaneous determination of plasma mycophenolic acid (MPA) and its glucuronide conjugate (MPAG) are described here. Both methods are reproducible and accurate, and both are equivalent in all respects, including quantification limits (MPA, 0.100 μg/ml; MPAG, 4.00 μg/ml), range (using 0.05–0.5 ml of plasma: MPA, 0.0500–20.0 μg/aliquot; MPAG, 2.00–200 μg/aliquot), precision, and accuracy. MPA and MPAG were stable under the conditions used with both methods. Results from aliquots of paired control samples, analyzed by the manual method over three years at six analytical laboratories, showed excellent agreement in precision and accuracy.

Keywords: Mycophenolic acid; Mycophenolic acid glucuronide

1. Introduction

Mycophenolate mofetil (RS-61443-000, I; Fig. 1), the 2-(4-morpholino)ethyl ester of mycophenolic acid (MPA, II; Fig. 1), is being evaluated as an immunosuppressive agent following solid organ transplantation. It has been shown in double-blind, randomized, controlled clinical trials to be effective in adjunctive therapy with cyclosporine and corticosteroids for the prevention of acute rejection in patients receiving kidney transplants [1–3].

Following oral administration, mycophenolate mofetil is rapidly absorbed and hydrolyzed to form free MPA, which is the active metabolite [4]. MPA is conjugated to form a phenolic glucuronide conjugate (MPAG, III; Fig. 1) [4], which is pharmacologically inactive but may be hydrolyzed in vivo to form free MPA. MPA potently, selectively, and reversibly inhibits inosine monophosphate dehydrogenase (IMPDH) and therefore inhibits the de novo pathway of purine synthesis in T and B cells (T

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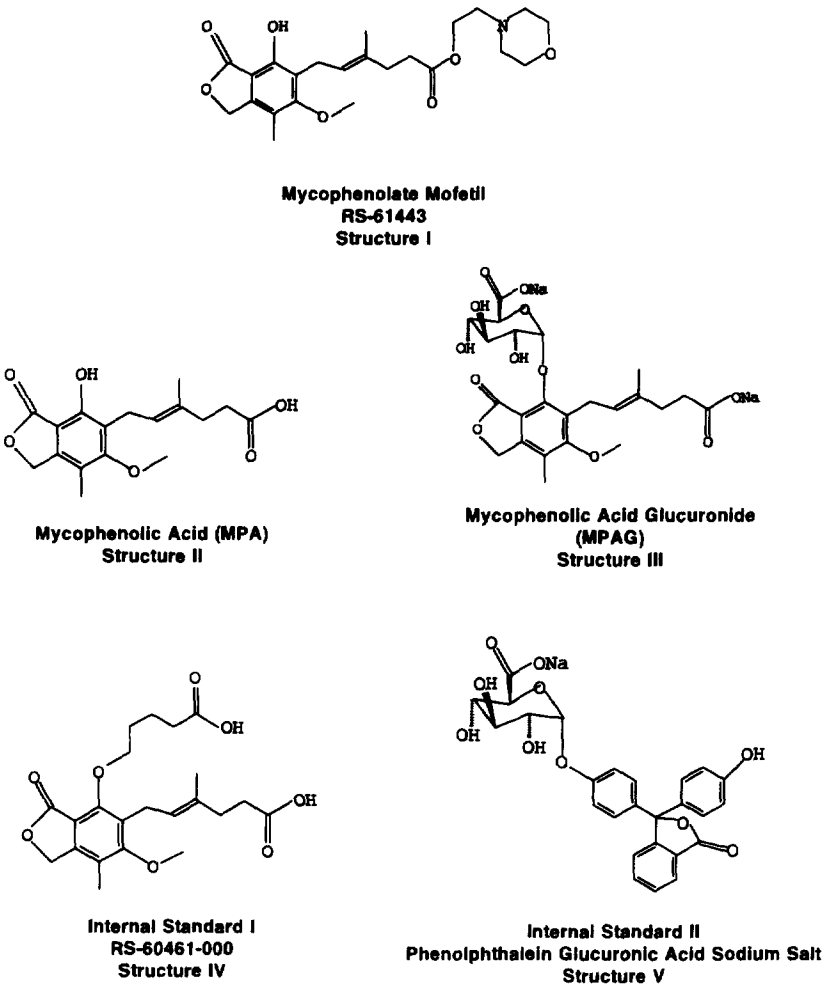

Fig. 1. Structures of mycophenolate mofetil, its metabolites, and internal standards.

and B lymphocytes) [5,6]. Unlike most other cells, lymphocytes rely on the de novo pathway more than the salvage pathway (hypoxanthine-guanine phosphoribosyl transferase; HGPRT) for purine biosynthesis. Addition of guanosine or deoxyguanosine reverses the antiproliferative effects of MPA on lymphocytes [7]. MPA also inhibits antibody formation by B cells [5,6].

To support the development of mycophenolate mofetil, an HPLC method was developed for the simultaneous determination of MPA and MPAG in plasma. Subsequently, an automated method using the Zymark PyTechnology XP robotic system (Zymark, Hopkinton, MA, USA), equipped with two high-performance solid-phase extraction Pysections (HPSPE) and on-line HPLCs, was developed. Both the manual and automated methods are described here and are shown to be equivalent.

2. Experimental

2.1. Chemicals and supplies

Mycophenolic acid, (*E*)-6-(1,3-dihydro-4-hydr-

oxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (MPA, II; Fig. 1); mycophenolic acid glucuronide disodium salt (MPAG, III; Fig. 1); and internal standard I, RS-60461-000, (*E*)-6-[1,3-dihydro-4-(4-carboxybutoxy)-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoic acid (I.S. I, IV; Fig. 1) were obtained from Syntex Research (Palo Alto, CA, USA), and internal standard II, phenolphthalein mono-β-glucuronic acid, sodium salt (I.S. II, V; Fig. 1) was purchased from Sigma (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were purchased from Burdick and Jackson Laboratories (Muskegon, MI, USA), and water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Reagent-grade phosphoric acid, 85%, analytical-grade sodium acetate, glacial acetic acid, potassium phosphate monobasic, 0.10 *M* hydrochloric acid, and citric acid were purchased from Mallinckrodt (St. Louis, MO, USA). Heparinized human control plasma was obtained from normal, healthy volunteers from the Clinical Studies Unit, Syntex Research (Palo Alto, CA, USA). Solid-phase extraction columns (Bond Elut and Bond Elut LRC) C_{18}, 3 cm^3, containing 200 mg of sorbent, were purchased from Varian Sample Preparation Products (Harbor City, CA, USA). Aqueous solutions of 0.125 *M* and 0.025 *M* citric acid, 0.1 *M* sodium acetate buffer, pH 4, and 0.025 *M* monobasic potassium phosphate were prepared in house.

2.2. Instrumentation

For the manual method, the HPLC system consisted of a Hewlett-Packard Model 1090 L ternary solvent delivery system and autosampler (Santa Clara, CA, USA), a Shimadzu SPD-10A UV–Vis detector (Shimadzu Scientific Instruments, Columbia, MD, USA), and a Nelson 6000 Laboratory Data System (P.E. Nelson, Cupertino, CA, USA). For the automated method, two Hewlett-Packard Model 1050 systems equipped with two Shimadzu SPD-10A UV–Vis detectors were configured in the robotic system (see below) for chromatographic analysis.

2.3. Chromatographic conditions

For the determination of MPA and MPAG using the manual method, aliquots of sample extract (25–35 μl for MPA and 10–25 μl for MPAG) were injected onto two separate 5-μm columns (BDS Hypersil C_{18}, 250 × 4.6 mm for MPA and 150 × 4.6 mm for MPAG [Keystone Scientific, Bellefonte, PA, USA]) using a mobile phase of acetonitrile to 0.05% aqueous phosphoric acid in the ratio of 45:55 and a flow-rate of 0.8 ml/min for MPA and in the ratio of 21:79 and a flow-rate of 1 ml/min for MPAG. The UV detector was set at 254 nm for both MPA and MPAG. A C_{18} guard column (Keystone Scientific, Bellefonte, PA, USA) and a 0.5-μm precolumn filter (Upchurch Scientific, Oak Harbor, WA, USA) were connected to each analytical column and were replaced after every 200 to 500 injections.

For the determination of MPA and MPAG using the automated method, two aliquots of the sample extract were loaded into two separate injection loops (50 μl for MPA and 10 μl for MPAG) for injection onto two separate columns. For MPA, HPLC was performed on a 150 × 4.6 mm, 5 μm, Adsorbosphere HS C_{18} column (Alltech, Deerfield, IL, USA) using a mobile phase of acetonitrile–0.05% aqueous phosphoric acid (39:61, v/v) and a flow-rate of 0.8 ml/min, and for MPAG, HPLC was performed on a 150 × 4.6 mm, 5 μm, Keystone Scientific BDS Hypersil C_{18} column using a mobile phase of acetonitrile–0.05% aqueous phosphoric acid (21:79, v/v) and a flow-rate of 0.8 ml/min. The UV detector was set at 254 nm for both MPA and MPAG. The precolumn frit described in the manual method was also used here; however, the guard column was an Applied Biosystems Newguard RP-8 column, 15 × 3.2 mm (Rainin Instruments, Woburn MA, USA).

2.4. Robotic system

A Zymark PyTechnology robotic system, equipped with two high-performance solid-phase extraction Zymark Pysections and on-line HPLCs was used to automate the manual meth-

od. The robot was controlled by a Zymate System V controller linked to a PC. The program utilized Easilab software. The components and configuration of the robotic Pysections are shown in Fig. 2.

2.5. Sample preparation (manual method)

Spiking procedure

Stock solutions were prepared by dissolving MPA and MPAG in methanol. The stock solutions were further diluted with methanol–water (9:1, v/v) to prepare spiking solutions at concentrations of 0.5, 1, 2, 3, 5, 10, 100, and 200 μg/ml for MPA and 20, 30, 50, 100, 200, 500, 1000, and 2000 μg/ml for MPAG. Stock internal standard solutions were prepared by dissolving the internal standards in methanol and were further diluted in methanol–water (9:1, v/v) to prepare a single internal standard (I.S.) spiking solution containing 10 μg/ml of I.S. I and 200 μg/ml of I.S. II. For preparation of the calibration standards used for construction of the calibration curve and for validation of the method, 0.1 ml of each of the MPA and MPAG spiking solutions were added to 0.5-ml aliquots of blank human plasma so that the samples were spiked in the concentration ranges of 0.1–40 μg/ml for MPA and 4–400 μg/ml for MPAG.

Extraction of calibration standards

To each calibration standard were added 1.5 ml of water, 0.1 ml of the internal standard spiking solution, and 0.75 ml of 0.1 N HCl solution. Each combination was mixed in a vortex mixer for 10 s and applied to a C_{18} solid-

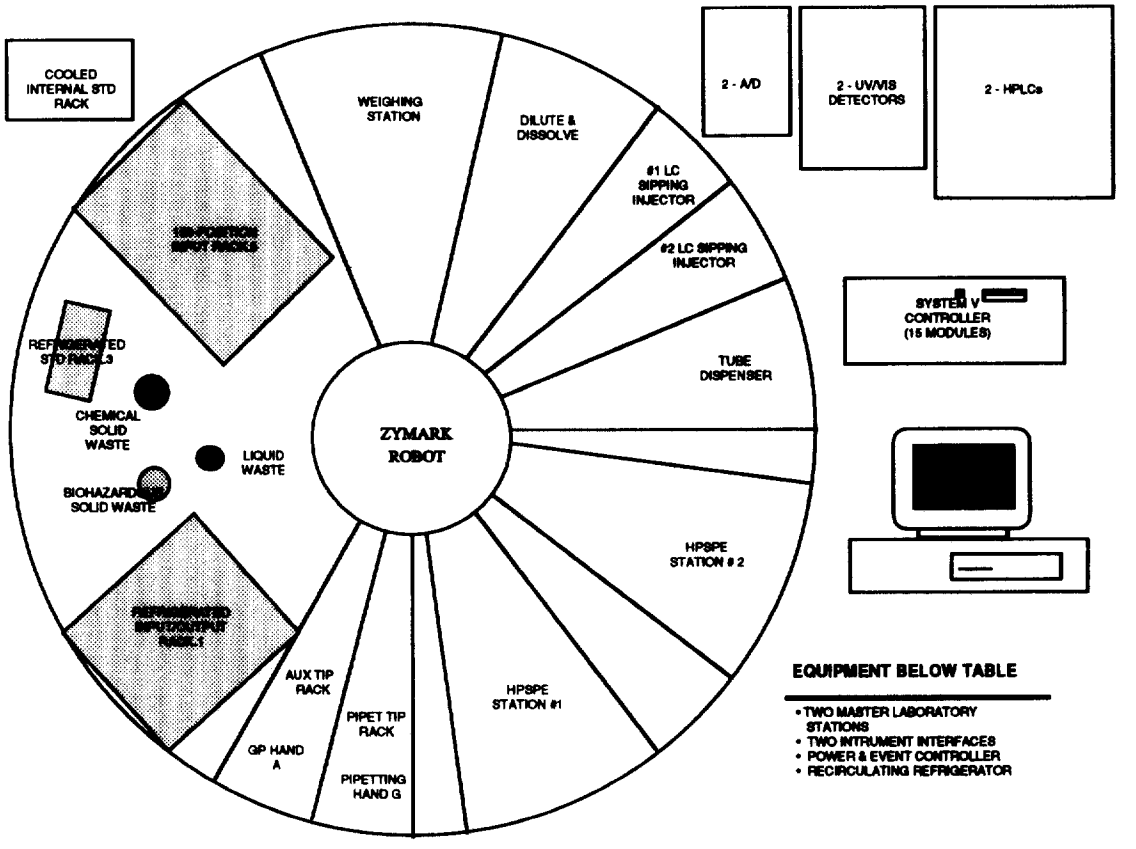

Fig. 2. Zymark PyTechnology robotic system configuration for MPA/MPAG assay (HPSPE = high-performance solid-phase extraction PySection).

phase extraction column that had been preconditioned with 2 ml of methanol followed by 2 ml of water using gravity flow and then allowing the column to drip dry. The plasma mixture was allowed to pass through the column under the force of gravity. The test tube that had contained the plasma was washed with 1 ml of water, and the wash was applied to the same solid-phase extraction column. The column was allowed to drip dry and the eluate was discarded. The column was then eluted with 1 ml of methanol–0.1 *M* acetate buffer (80:20, v/v), pH 4, and the eluate was collected in an HPLC autosampler vial. The eluate was mixed briefly, and an aliquot was injected onto HPLC for analysis. The analysis of MPA and MPAG was performed either sequentially using one HPLC system or simultaneously by splitting the extract into two portions and injecting them into two separate HPLC systems.

Extraction of clinical samples

Samples of heparinized plasma obtained from healthy volunteers or patients treated orally with mycophenolate mofetil were stored at −20°C prior to analysis. Samples were thawed at room temperature, mixed in a vortex mixer for 30 s, and centrifuged for 5 min, and an aliquot of 0.05 to 0.5 ml was removed for analysis. When less than 0.5 ml was used for analysis, water was added to bring the total volume to 0.5 ml. The samples were extracted using the same procedure described for the calibration standards. Concentrations of MPA and MPAG in the samples were calculated by reference to calibration curves generated from calibration standards analyzed along with each batch of clinical samples.

Data handling and calculations

Linear least-squares regression was performed on the peak-height ratio (analyte peak height/internal standard peak height) versus concentration data generated by the calibration standards to construct a linear standard curve of the form peak-height ratio = m(concentration) + b. For laboratories such as ours that have chromatography systems incapable of performing *weighted* linear regression analysis to construct the standard curve, calibration standards from 0.1 to 1.0 μg/ml for MPA and from 4.0 to 40 μg/ml for MPAG were used in the *unweighted* linear regression to construct calibration curves, and the calibration standards of higher concentrations were used to verify extrapolations of the curve up to 40 μg/ml for MPA and 400 μg/ml for MPAG. A verification standard must be within 10% of its nominal value, as calculated by the standards used in the linear regression, to verify the curve between the highest standard in the regression and the verification standard concentration. This procedure of verifying the upper range of the calibration curve in every run with verification calibration standards avoided the dominating effect of the upper points on the low end of the unweighted standard curve and minimized the need for sample dilution to achieve concentrations in a narrow curve range. Other groups with capability for weighted linear regression used all calibration standards in Tables 1 and 2 for curve construction but weighted the peak-height ratios in the linear regression by $1/x$, where x is the either the nominal concentration of analyte or the peak-height ratio. Concentrations in unknowns were then determined from their peak-height ratios by the standard curve equation, with appropriate corrections for sample aliquot volumes that differed from 0.500 ml.

Preparation of quality control samples

Quality control samples (QCs) prepared by spiking MPA and MPAG into control human plasma were stored at −20°C in a manner similar to that used for the clinical samples. QCs were prepared at the following four concentrations: QC 1 (0.216 μg/ml of MPA, 8.02 μg/ml of MPAG), QC 2 (2.14 μg/ml of MPA, 78.1 μg/ml of MPAG), QC 3 (19.9 μg/ml of MPA, 153 μg/ml of MPAG), and QC 4 (36.2 μg/ml of MPA, 359 μg/ml of MPAG). To prepare the bulk QCs, MPA and MPAG were dissolved in methanol–water (1:1, v/v, to 9:1, v/v) and added to the appropriate amount of blank human plasma, which was then swirled briefly and

Table 1
Intra- and inter-assay precision and accuracy data for MPA (manual method)

	Concentration[a] (μg/ml)	n		Mean concentration found (μg/ml)		C.V. (%)		Recovery (%)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay	Intra-assay	Inter-assay	Inter-assay	Inter-assay
Calibration standards									
	0.100	4	4	0.105	0.105	10.2	2.85	105	105
	0.200	4	4	0.198	0.204	1.87	0.566	99.0	102
	0.400	4	4	0.390	0.390	2.87	1.69	97.5	97.5
	0.600	4	4	0.610	0.586	3.51	1.91	102	97.7
	1.00	4	4	1.01	1.01	2.06	1.40	101	101
	2.00	4	4	2.02	1.99	1.82	1.86	101	99.5
	20.0	4	4	19.9	19.0	0.891	1.85	99.5	95.0
	40.0	4	4	38.0	37.0	4.23	0.681	95.0	92.5
QC samples									
QC 1	0.216	4	34	0.213	0.223	3.96	4.56	98.6	103
QC 2	2.14	4	34	2.03	2.11	3.20	3.98	94.9	98.8
QC 3	19.9	4	34	18.4	19.2	3.91	3.79	92.5	96.3
QC 4	36.2	4	34	33.9	34.5	1.97	3.16	93.6	95.3

[a] Concentration added to calibration standards or nominal concentration for control samples.

Table 2
Intra- and inter-assay precision and accuracy data for MPAG (manual method)

	Concentration[a] (μg/ml)	n		Mean concentration found (μg/ml)		C.V. (%)		Recovery (%)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay	Intra-assay	Inter-assay	Inter-assay	Inter-assay
Calibration standards									
	4.00	4	4	4.02	3.98	1.88	2.53	101	99.5
	6.00	4	4	6.06	6.00	2.49	0.980	101	100
	10.0	4	4	9.72	10.0	2.05	1.07	97.2	100
	20.0	4	4	20.2	19.7	1.04	1.50	101	98.5
	40.0	4	4	40.2	40.2	1.95	0.627	101	101
	100	4	4	95.4	98.6	1.85	2.00	95.4	98.6
	200	4	4	202	202	0.948	1.13	101	101
	400	4	4	398	394	0.503	1.74	99.5	98.5
QC samples									
QC 1	8.02	4	30	8.10	8.30	4.80	3.15	101	104
QC 2	78.1	4	30	71.9	74.1	3.92	1.87	92.1	94.9
QC 3	153	4	30	150	157	4.01	1.72	98.0	103
QC 4	359	4	30	356	365	2.49	3.23	99.2	102

[a] Concentration added to calibration standards or nominal concentration for control samples.

stirred on a magnetic stirrer for 10 min before being apportioned into polypropylene tubes for storage at −20°C. Two QCs at each of the four different concentrations were analyzed with each batch of clinical samples to monitor the performance of the method during routine use.

2.6. Sample preparation (automated method)

Spiking solutions of MPA and MPAG were prepared in the same manner as described for the manual method, except that the MPAG solution was diluted into methanol–water (1:9, v/v) instead of methanol–water (9:1, v/v). Concentrations of internal standards I.S. I and I.S. II were 2.5 μg/ml and 50 μg/ml, respectively, and 400 μl of this solution was added to each sample. Also, the automated method used calibration standards with MPA concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 μg/ml (samples of 10 and 40 μg/ml were used for extrapolation) and with MPAG concentrations of 4, 6, 10, 20, 30, 40, 60, and 100 μg/ml (with 200 and 400 μg/ml samples used for extrapolation). QC samples for the automated method were prepared in the same manner as for the manual method. The sample preparation procedure of the manual method was modified to adapt the samples to the robotic system. Instead of the HCl solution and water used in the manual method, 2 ml of 0.125 *M* aqueous citric acid was used to acidify the plasma. Solid-phase extraction columns were conditioned using 3 ml of methanol and washed with 2 ml of water and 4 ml of 0.025 *M* citric acid instead of 2 ml of methanol and 2 ml of water. Finally, MPA and MPAG were eluted from the solid-phase extraction column using 3 ml of methanol–0.1 *M* acetate buffer (80:20, v/v), pH 4, instead of the 1 ml used in the manual method. Unlike the manual method, in which samples were processed in a batch mode, in the robotic method samples were processed serially. A flow-rate of 1.3 ml/min was used for loading, washing, and eluting the samples from the solid-phase extraction columns.

3. Results and discussion

3.1. Quantification limits

The quantification limits for both the manual and automated methods are 0.100 μg/ml for MPA and 4.00 μg/ml for MPAG using 0.5 ml of plasma for analysis. Concentrations below 0.100 μg/ml for MPA and 4.00 μg/ml for MPAG are reported as below the quantification limit of the method (BQL). At the quantification limit, the signal-to-noise ratios of the HPLC peaks were approximately 8:1 and 35:1 for MPA and MPAG, respectively.

3.2. Precision and accuracy

Manual assay

The precision of the manual assay was assessed by the intra- and inter-assay coefficients of variation (C.V., %) of the method. The accuracy of the method was evaluated by the recovery, defined as the ratio of the concentration of MPA or MPAG found to that added (found/added). Data for the intra- and inter-assay % C.V. and for the recoveries obtained using calibration standards are presented in Tables 1 and 2. Data for the intra- and inter-assay % C.V. and recoveries for the QC samples are also presented in Tables 1 and 2. All % C.V. were less than 5%, except for the lowest spiked calibration standard for MPA, which had an intra-assay % C.V. of 10.2%. All recoveries were between 92% and 105%.

For the manual method, using calibration standards, the intra-assay (within day) and inter-assay (between days) coefficients of variation (% C.V.) for MPA at the quantification limit are 10.2% and 2.85%, respectively, and the intra- and inter-assay % C.V. for MPAG at the quantification limit are 1.88% and 2.53%, respectively (Tables 1 and 2).

Automated assay

For the automated assay, % C.V. for concentrations of both MPA and MPAG in the calibration standards and QCs were all less than 8%. The recovery for MPA from the calibration

standards and QCs ranged from 94.3% to 107%, except for a 118% recovery for the lowest calibration standard. The recovery for MPAG calibration standards and QCs ranged from 93.0% to 114%.

3.3. Specificity

The analysis of blank human plasma from six different sources showed no interfering peaks at the retention times of MPA, MPAG, and the internal standards. A number of drugs that might potentially be coadministered with mycophenolate mofetil were tested for assay interference by their retention time when injected into the HPLC under the assay conditions for MPA and for MPAG. The following drugs showed no interference in either assay: mycophenolate mofetil, cyclosporine, prednisone, methylprednisolone, cimetidine, acyclovir, ganciclovir, methotrexate, and salicylic acid. Also, there were no cross-interferences between MPA, MPAG, and their respective internal standards.

Chromatograms from blank plasma, from spiked calibration standards, and from a patient sample are shown in Fig. 3 for MPA and in Fig. 4 for MPAG.

A small percentage of all samples from dosed post-transplant patients contained unidentified endogenous substances that produced interfering peaks in the chromatogram; presumably this was because of some combination of multiple-drug administration and altered metabolism. Generally, a small change (±2%) in the acetonitrile content of the mobile phase was sufficient to resolve these interferences. When this modification was unsuccessful, an alternative mobile phase consisting of acetonitrile–methanol–0.025

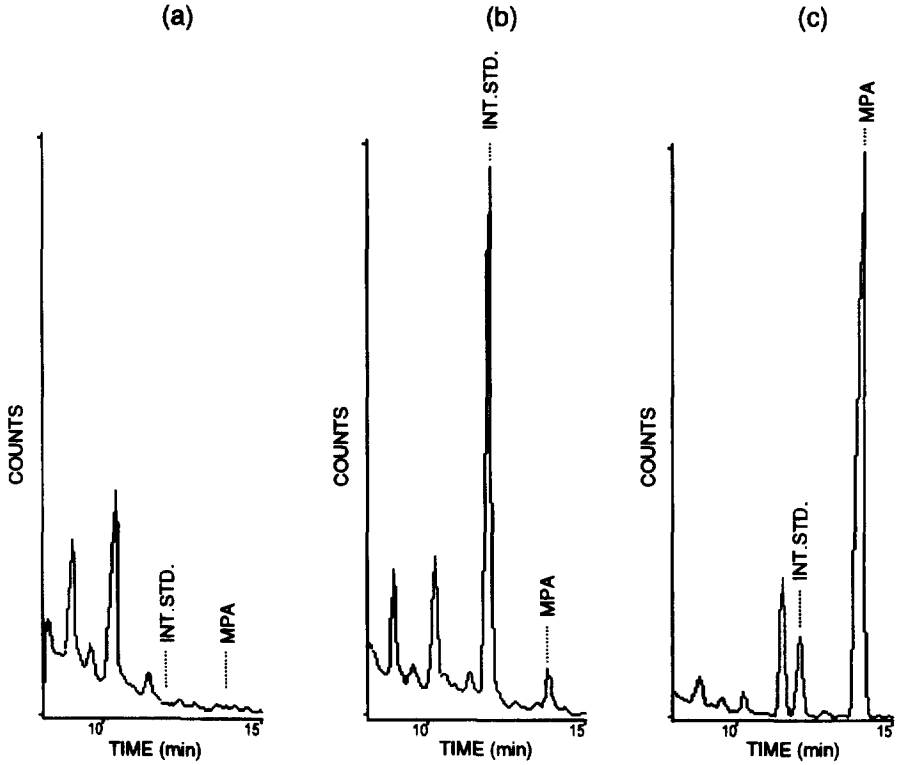

Fig. 3. Typical chromatograms obtained from the analysis of MPA in (a) blank human control plasma, (b) blank human control plasma spiked with 0.100 μg/ml of MPA, or (c) plasma sample from a patient 1 h following oral administration of 1500 mg of mycophenolate mofetil twice daily.

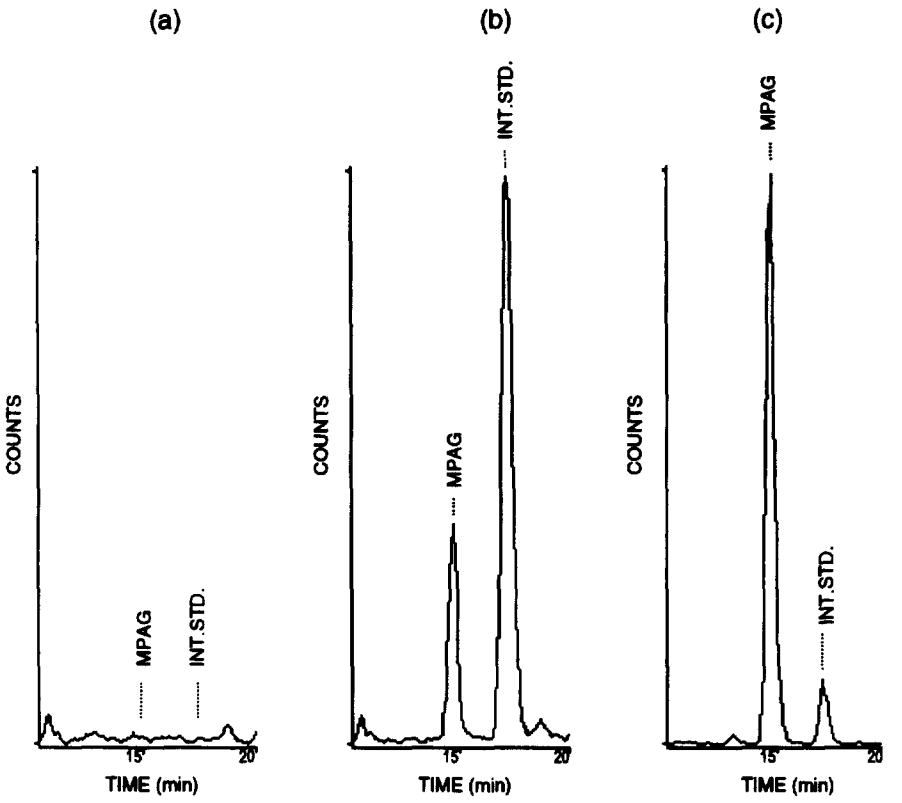

Fig. 4. Typical chromatograms obtained from the analysis of MPAG in (a) blank human control plasma, (b) blank human control plasma spiked with 4 μg/ml of MPAG, or (c) plasma sample from a patient 1 h following oral administration of 1500 mg of mycophenolate mofetil twice daily.

M monobasic potassium phosphate (32:6:62, v/v/v) was used for MPA. Use of this alternative mobile phase gave an assay with precision and accuracy equivalent to the precision and accuracy observed with the standard mobile phase.

3.4. Linearity

The linear range of both the manual and automated methods, using 0.05 to 0.5 ml of plasma for analysis, was 0.050–20 μg per aliquot of plasma for MPA and 2.00–200 μg per aliquot of plasma for MPAG.

3.5. Absolute recovery

The absolute recoveries of MPA and MPAG from plasma, determined by the analysis of plasma spiked with [^{14}C]MPA and [^{14}C]MPAG, were 92% and 91%, respectively, for the manual method. For the automated method, the recoveries of MPA and MPAG were 76.9% and 73.2%, respectively.

3.6. Effect of volume

For the manual method, the effect of varying the volume of plasma used in the range of 0.050 to 0.500 ml was examined using QC samples. For both MPA and MPAG, the results with aliquots of 0.05, 0.1, and 0.2 ml were equivalent to results with 0.500-ml aliquots. Similar results were obtained for the automated method. These data

indicate that volumes of plasma from 0.05 to 0.5 ml may be used for the analysis.

3.7. Stability

Spiking [^{14}C]MPA and [^{14}C]MPAG separately into freshly collected human blood and plasma and monitoring them at room temperature (20–23°C) and at 1–4°C showed that MPA and MPAG were stable at room temperature for at least 4 h and were stable at 1–4°C for at least 8 h. Use of radiolabeled materials for these stability studies offered a convenient and sensitive method for differentiating between loss of analytes in the plasma fraction that was due to degradation and loss due to slow partitioning of analytes into the cells. Thus, in addition to HPLC analysis of the plasma fraction for MPA and MPAG, scintillation counting was used to monitor both analytes in whole blood and in the plasma and cell fractions. At the time and temperature conditions under which MPA and MPAG in whole blood were found to be stable (room temperature for 4 h or at 1–4°C for 8 h), partitioning of MPA and MPAG into the cells, as indicated by scintillation counting, was not significant.

QC samples containing MPA and MPAG at therapeutic levels were found to be stable when stored in a −20°C freezer for at least 11 months. Using refreezing in a −20°C freezer after thawing samples, MPA and MPAG were found to be stable in the plasma after three freeze–thaw cycles. After extraction from plasma, MPA and MPAG were stable in the extract and could be stored for at least six days at room temperature (20–23°C) under normal laboratory conditions or for at least two weeks when refrigerated (1–4°C) prior to analysis by HPLC.

With the automated method, MPA and MPAG concentrations determined for QC samples stored refrigerated (1–4°C) on instrument for up to nine days were equivalent to concentrations determined for freshly thawed QCs analyzed in the same run; the % C.V. were also equivalent. MPA and MPAG concentrations for QCs stored on the benchtop at room temperature for one day were equivalent to the concentrations in freshly thawed QCs analyzed in the same run.

3.8. Comparison of manual and automated methods

The automated method had quantification limits for MPA and MPAG identical to those reported for the manual method. Forty-three samples of plasma collected from subjects during a clinical study were analyzed for MPA and MPAG using both methods. Ninety-two percent of MPA values and 98% of MPAG values determined by the manual method were within 15% of the values determined by the automated method. The data were compared by performing a linear regression analysis of the two sets of data (see Figs. 5 and 6). The regression line had a slope near 1.0 and an intercept near zero for both MPA and MPAG, indicating excellent agreement between the results obtained by the two methods.

3.9. Applications

These methods have been applied to the analysis of plasma from healthy subjects and from patients from various post-transplant populations treated with oral mycophenolate mofetil. A representative profile for a stable transplant

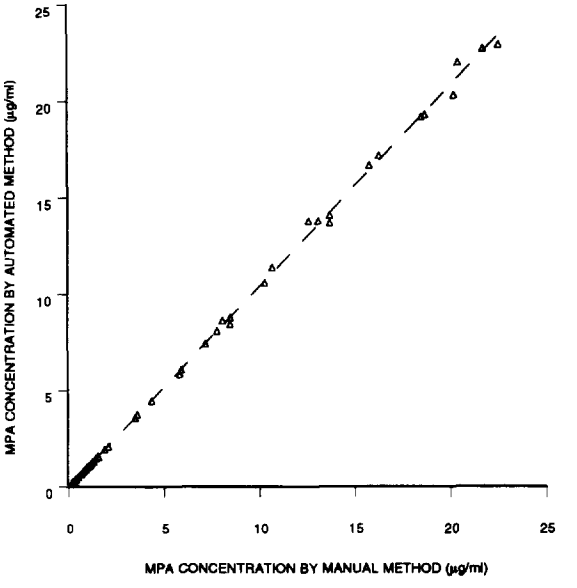

Fig. 5. Comparison of the manual method and the automated method: plasma MPA. $y = -0.0348 + 1.0416x$; $r = 0.9995$.

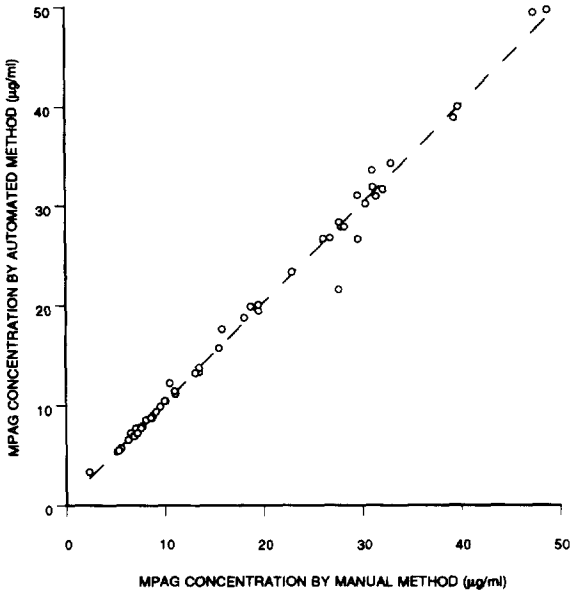

Fig. 6. Comparison of the manual method and the automated method: plasma MPAG. $y = 0.3909 + 0.9959x$; $r = 0.9950$.

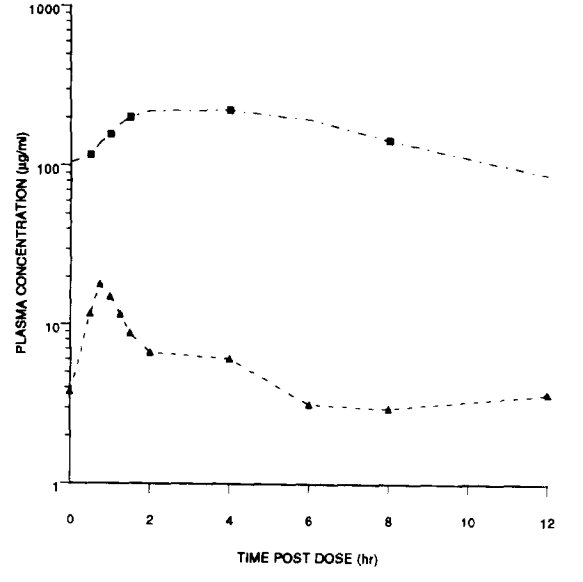

Fig. 7. Plasma concentration versus time profile from a stable renal transplant patient following oral administration of 1500 mg of mycophenolate mofetil twice daily. ▲ = MPA; ■ = MPAG.

patient receiving oral mycophenolate mofetil is shown in Fig. 7.

Over a period of three years the MPA/MPAG method has been set up in at least five contract laboratories (two in the United States, two in Europe, and one in Japan) and one U.S. research laboratory. Results from aliquots of paired control samples analyzed with these or slightly modified HPLC conditions at our laboratory and at each outside laboratory have shown excellent agreement in precision (C.V. < 5% except for 11% for the low control at a European laboratory) and accuracy (found/added of 89–106%).

4. Conclusions

Both the manual and automated methods described here for the determination of MPA and MPAG in plasma are precise and accurate and can be used in samples obtained during clinical studies from subjects receiving mycophenolate mofetil. The two methods are equivalent.

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