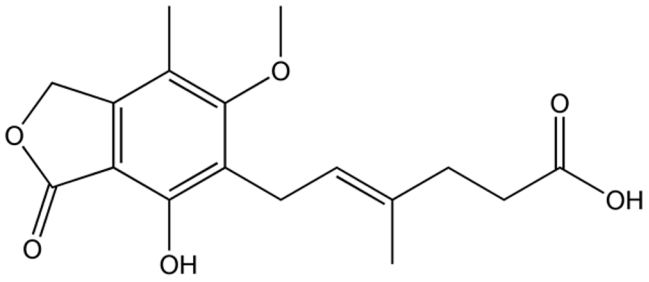
Tom Fleischmann[1], James Merdink[1], Sue Faulconbridge[2] and Tony Clarke[2]
BASi (Bioanalytical Systems, Inc.) [1]McMinnville, Oregon and [2]Warwickshire, United Kingdom

LC/MSMS Methods for Quantitative Determination of Mycophenolic Acid in Human Plasma

Mycophenolic acid (**F1**) is an immunosuppressive agent used in therapy following solid organ transplantation. The active drug component is marketed as the sodium salt (Myfortic, Novartis) or as an ester (Cellcept, Roche) that is metabolized *in vivo* to mycophenolic acid.

F1. Chemical Structure of Mycophenolic Acid



Liquid chromatography-based methods using either UV or mass spectroscopic detection is the standard for determination of mycophenolatic acid (MPA) in biological samples. Patel et. al.[1] reported using 100 mL sample extracted by SPE method analyzed by reverse phase chromatography with either UV detection (18 minute cycle time) or MS/MS detection (7 minute cycle time) with a quantification range of 1-1000 mg/mL. Analysis of plasma and plasma ultrafiltrate used LC-MS/MS with detection range of 0.5-1000 mg/mL range[2]. LC-UV method used 50 mL sample size with protein precipitation, and chromatographic separation on a monolithic column with isocratic elution gave linear response for MPA at 1-40 mg/mL range[3]. Fluorescence detection was used to analyze 100 mL plasma sample extracted by protein precipation with methanol or direct injection of plasma ultrafiltrate with quantification range of 5-1000 mg/mL[4]. A method that included analysis of urine used reverse phase chromatography with ion-pairing by UV detection with quantification range of 5-400 mg/mL[5].

Stability of mycophenolate acyl glucronides was reported in biological samples prepared for analysis by protein precipitation procedure followed by reverse phase HPLC separation with UV detection. (6)

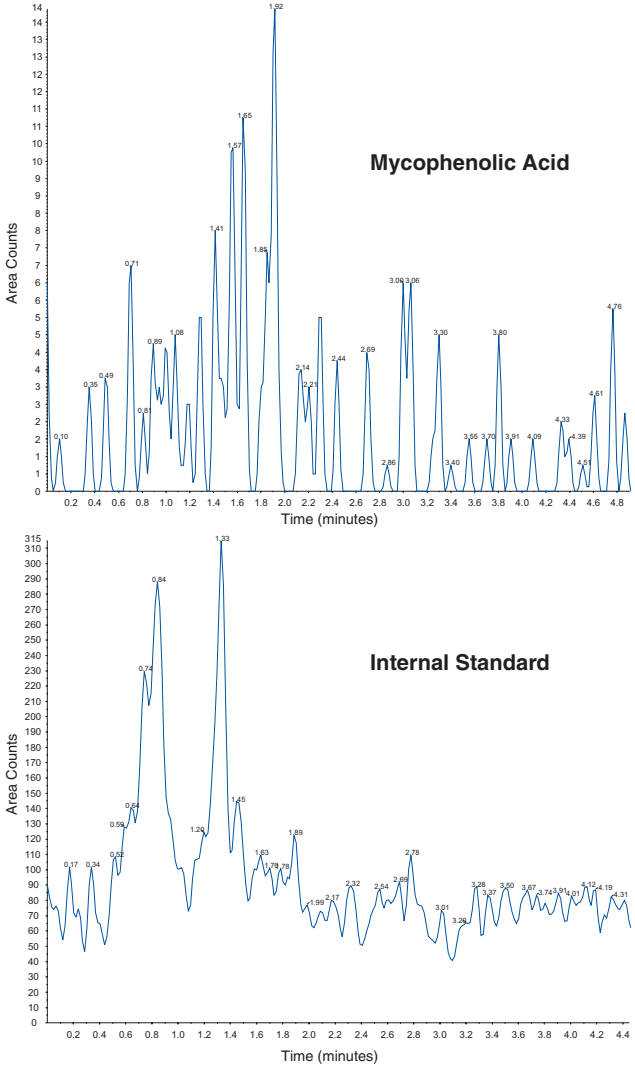
T1. Inter-run (n=14) Accuracy and Precision for Standard Calibrators

Concentration (ng/mL)	SC 50 50.0 ng/mL	SC 100 100 ng/mL	SC 250 250 ng/mL	SC 1000 1000 ng/mL	SC 2500 2500 ng/mL	SC 5000 5000 ng/mL	SC 7500 7500 ng/mL	SC10000 10000 ng/mL
Mean	50.2	99.2	248	1000	2570	5120	7220	9900
%CV	3.8	4.8	2.7	3.6	3.5	3.2	3	2.9
%Bias	0.4	-0.8	-0.8	0	2.8	2.4	-3.7	-1
n	14	14	14	14	14	14	14	13

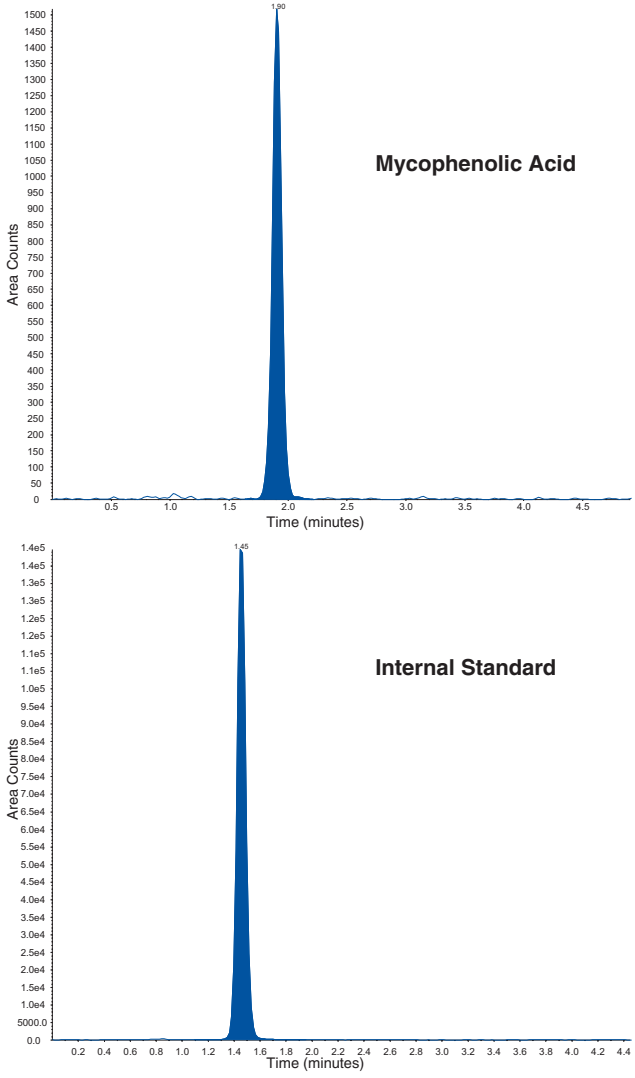
T2. Inter-run (n=18) Accuracy and Precision for Quality Control Samples

	Curve Number	QC 50 50.0 ng/mL	QC 150 150 ng/mL	QC 500 500 ng/mL	QC 8000 8000 ng/mL
Intrarun Mean	1	44.2	142	508	7360
Intrarun %CV		6.3	4.7	3.0	0.9
Intrarun %Accuracy		88.4	94.7	101.6	92.0
n		6	6	6	6
Intrarun Mean	3	54.4	153	506	7660
Intrarun %CV		4.1	1.5	1.5	2.7
Intrarun %Accuracy		108.8	102.0	101.2	95.7
n		6	6	6	6
Intrarun Mean	4	53.3	154	487	7750
Intrarun %CV		5.8	2.6	2.0	8.2
Intrarun %Accuracy		106.6	102.7	97.4	96.9
n		6	6	6	6
Inter-run Mean		50.6	150	500	7590
Inter-run %CV		10.6	4.8	2.9	5.3
Inter-run %Accuracy		101.2	100.0	100.0	94.9
n		18	18	18	18

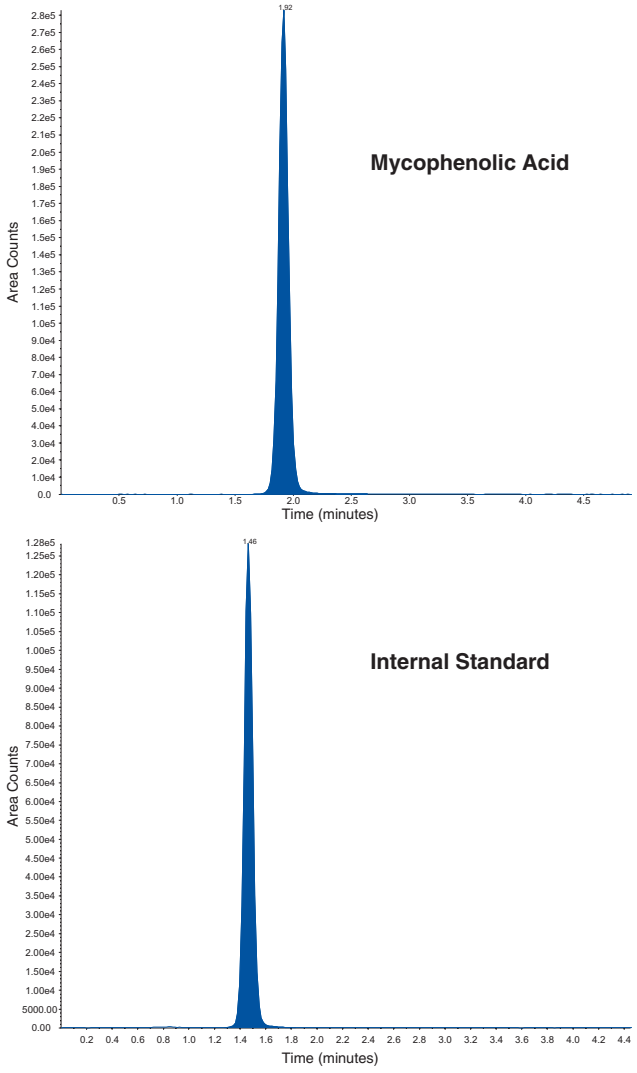
F2. Extracted Blank



F3. LLOQ Sample (50 ng/mL)



F4. ULOQ Sample (10000 ng/mL)



BASi has developed and validated an LC/MSMS method for analysis of mycophenolic acid (immunosuppressant drug) in human plasma. The method uses protein precipitation for quick sample preparation. Processing a sample volume of only 0.1 mL plasma yields a detection limit of 50 ng/mL. The method for analysis of mycophenolate plasma was shown to be robust with standard calibrator accuracy (CV) well within acceptance guidelines of ±15% over the course of seven independent sequences (T1). The between-run accuracy and precision of quality control samples prepared at four different levels [LLOQ (QC-50), low-QC (QC-150), mid-QC (QC-500) and high-QC (QC-8000)] run in three sequences with n=6 replicates in each sequence also showed excellent method performance (T2). Representative chromatograms are shown in **F2** thru **F4**. Ruggedness tests of freeze/thaw stability, heat treatment stability (for deactivation of HIV virus), short-term room temperature stability and autosampler stability were performed with acceptable accuracy and precision. No matrix interferences were detected testing six different lots of plasma.

This analytical method for determination of MPA is a reliable and convenient procedure that meets the criteria for application in routine clinical drug monitoring and pharmacokinetic studies.

Reference

1. *C. G. Patel, A. E. Mendonza, F. Akhlaghi, O. Majib, A. K. Trull, T. Lee, D. W. Holt J Chrom B, 813 (2004) 287-294.*
2. *F. Streit, M. Shipkova, V. W. Armstrong, M. Oellerich, Clin Chem 50:1 (2004) 152-159.*
3. *D. Indjova, L. Kassabova, D. Svinarov, J Chrom B, 817 (2005) 327-330.*
4. *J. Shen, Z. Jiao, Y. Yu, M. Zhang, M. Zhong, J Chrom B, 817 (2005) 207-213.*
5. *W. Yau, A. Vathsala, H. Lou, E. Chan, J Chrom B, 805 (2004) 101-112.*
6. *M. Shipkova, E. Schutz, V. W. Armstrong, P. D. Niedmann, M. Oellerich, E. Wieland. Clin Chem 46:3 (2000) 365-372.*