AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

IMPURITY PROFILING OF MYCOPHENOLATE MOFETIL IN PHARMACEUTICAL FORMULATIONS BY HPLC

Verma SP.*[1], Bala Indu[3], Kumar K.[4] and Khan SA.[2]

1. Lovely Faculty of Applied Medical Sciences, Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India-144402
2. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard New Delhi, India-110062
3. Mahatma Gandhi College of Pharmacy, Jaipur, Rajasthan-302028
4. Research Scientist, Jubilant Clinsys, Noida, Uttar Pradesh, Noida.

ABSTRACT

High-performance liquid-chromatography method was developed and validated for the determination of impurities (Related Substances) of Mycophenolate mofetil in tablets and capsules using Photo diode array detector. The mobile phase was a combination of Triethylamine buffer (pH 5.3) and acetonitrile in the ratio of 65:35 and wavelength set at 250 nm. Retention time of Mycophenolate mofetil and its impurity as Mycophenolic acid was found to be approx. 21.31 and 5.79 minutes respectively. Linearity of the method for Mycophenolate mofetil and its impurity as Mycophenolic acid was found to be 0.4 to 24.163 µg mL^{-1} with the correlation coefficient of 1.000 and 0.9999 respectively. This method was validated accordingly to International Conference of Harmonization guidelines. Qualification was alone by calculating area of the peaks and peak purities. The analytical solution of Mycophenolate mofetil also has shown stability for 24 hrs at 5°C and 25°C. Present method can be applied for the impurity profiling and stability studies of Mycophenolate mofetil in tablet and capsules formulations without interference of the excipients present in the particular formulations

Keywords: - Mycophenolate mofetil, Mycophenolic acid, High-performance liquid-chromatography, Photo diode array detector, Stability studies

*Corresponding Author Email: surajpal_1982@rediffmail.com

Received 20 December 2011, Accepted 3January 2012

Please cite this article in press as: Verma SP *et al.*, Impurity Profiling of Mycophenolate Mofetil in Pharmaceutical Formulations by HPLC. American Journal of PharmTech Research 2012.

INTRODUCTION

Mycophenolate mofetil (Figure 1) (MMF) 2-morpholinoethyl (E)-6-(1, 3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate[1] is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B- lymphocytes are critically dependent for their proliferation on novo synthesis of purines, whereas other cell types can utilize salvage pathways, it has potent cytostatic effects on lymphocytes and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection [2, 3]. MMF did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 (IL-1) and interleukin-2 (IL-2), but did block the coupling of these events to DNA synthesis and proliferation. Due to clinical advantages of MMF, there has been increase in number of MMF formulations (e.g. Cellcept 500 tablets and Cellcept 250 capsules by Roche Laboratories Inc. Nutlly, New Jersey 07110) in market in recent past [4, 5] MMF is a non-official ester of mycophenolic acid (Figure 2) (MPA) so MPA present as synthetic impurity in MMF and believed so act by the inhibition of inosine monophosphate dehydrogenase (IMPDH).

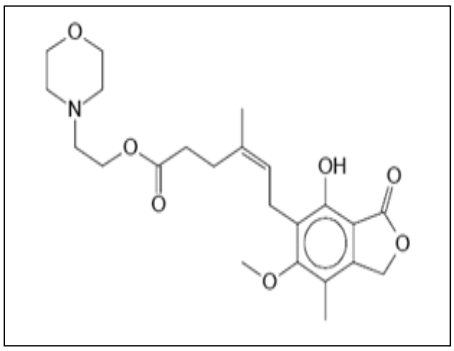

Figure 1. Mycophenolate Mofetil (MMF)

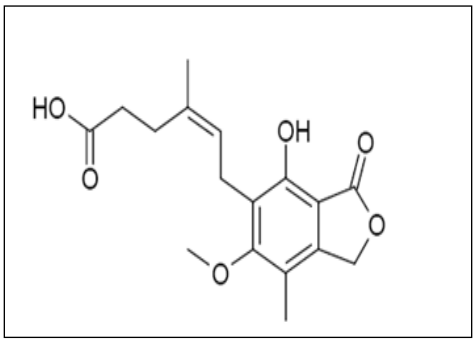

Figure 2. Mycophenolic acid (Imp acid)

It is preferably use as immunosuppressive drug in organ transplant [6] No official methods were found for the related substances (RS) determination (impurity profiling) of MMF in formulations [7]. So there is a need for method development for the RS of MMF in formulations (dosage forms) which can be used for routine analysis [8, 9].

For routine analysis a simple, rapid and cost effective analytical method is preferred. A survey of literature has not revealed any simple HPLC method for RS of MMF in bulk drug and commercial formulations by PDA detector[10]. Several bioanalytical methodologies including HPLC, EMIT have been used for the quantification of MPA in plasma [11]. But there was no

method for the RS determination for MMF and MPA simultaneously in plasma as well as in formulation has been found in literature.

The main purpose of this investigation is to develop & validate reversed phase HPCL method which is simple, precise, sensitive and selective for the RS determination of MMF in dosage forms. The developed method can be easily used in stability studies at very low level of MMF concentration. Suitable statistical tests were applied on validation data [12- 14].

MATERIALS AND METHODS

Reagents and solvents

Pure MMF and MPA were obtained as gift sample from Biocon Ltd., (Bangalore, India) and certified to contain 99.7% (w/w) on dried basis. Acetonitrile was of HPLC grade & purchased from SD-Fine- Chem limited (Mumbai, India) and all other chemicals and reagents used were of analytical grade and were purchased from Merk Ltd. (Worli, Mumbai, India). Milli-Q (MA, USA) water is used for the preparation of buffer. Triethylamine buffer solution was prepared and filtered through 0.22µ filter (Millipore, USA). Formulation of MMF used for the study was MMF tablets (label claim: 500 mg/tablet) and capsules (label claim: 250 mg/capsules) procured from Jamia Hamdard, (New Delhi, India).

Instrumentation and chromatographic conditions

HPLC analysis was performed with Waters (Model 2695, Waters, USA). HPLC system equipped with PDA detector. The chromatographic separation was performed using a Zorbax SB-C8 (250 mm X 4.6 mm), 5 µm (Agilent Technologies, US). The mobile phase consisting of a mixture of triethylamine buffer, pH of which adjusted to 5.3 with diluted Orthophosphoric acid and acetonitrile in the ratio of 65:35 (%V/V) with the flow rate of 1.5 mL min-1 was employed. The detector wavelength was set at 250 nm (PDA detector model waters 2996). The injection volume was 10 µL while column was maintained at 45°C.

Preparation of standard solution and system suitability solution

A stock solution of MMF was prepared in diluent consisted mixture of water and acetonitrile in the ratio of 20:80 (%V/V) at 0.4 mg mL^{-1}. With proper dilution, standard solution was prepared of the concentration 20 µg mL^{-1}. Ten microlitres from this standard solution was injected six times as system suit in the column in the above mentioned chromatographic conditions (Section 2.2) for each parameter in method validation.

Preparation of Sample Solutions

For capsules

Determine the Average weight of not less than 10 Capsules and take out the content. Accurately weigh and transfer powdered sample equivalent to about 200mg of MMF into a 100mL volumetric flask. Add about 60mL of diluent and sonicate for about 30 min. with intermittent shaking. Make up the volume with diluent and mix. Filter through 0.45µm nylon membrane filter.

For tablets

Determine the Average weight of not less than 20 tablets and take out the content. Accurately weigh and transfer powdered sample equivalent to about 200mg of MMF into a 100mL volumetric flask. Add about 60mL of diluent and sonicate for about 30 min. with intermittent shaking. Make up the volume with diluent and mix. Filter through 0.45µm nylon membrane filter.

Method Validation

Specificity

The specificity of the method was ascertained by performing interference studies. Six injections of standard solution (for system suitability), one injection of diluents, one injection of placebo (equivalent to 200 mg mycophenolate mofetil) which has been prepared as sample solution preparation, one injection of sample solution and three injections of spiked sample solution which has been spiked with known impurity (MPA) at 0.5% level has been injected in column and analyzed. Also the peak purity of MMF was assessed.

Precision

System precision

System precision of the system was determined by injecting six replicates of the standard solution (injection volume, 10 µL) and measurement carried out of peak areas of the main peak. Data was treated to calculate % RSD.

Method precision

Method precision of method was determined by injecting six replicates (injection volume, 10 µL) of the sample solution from single batch of tablets and capsules individually and measurement carried out of peak areas of the main peak. Data was treated to calculate % RSD.

Intermediate precision

Intermediate precision of method was determined by injecting six replicates (injection volume, 10 µL) of the sample solution from single batch of tablets and capsules individually as per the method by a different analyst on different instrument using different column and on a different

day. Measurements carried out of peak areas of the main peak. Data was treated to calculate % RSD.

Linearity

Linearity of the method was studied in two replicate of each level in the range of 2% - 125% of the standard concentration. A stock solution of 500 ppm of MMF standard was prepared and diluted the stock standard solution for the preparation of solution of different concentrations of MMF i.e. 0.403 µg mL^{-1}, 2.0 µg mL^{-1}, 8.0 µg mL^{-1}, 12.0 µg mL^{-1}, 16.0 µg mL^{-1}, 20.0 µg $mL^{-1}$and 24.163 µg $mL^{-1}$and same was done for MPA also. In order to assess the linearity of the method data were plotted in the form of linearity curve and slope, intercept and correlation coefficient of the curve has been calculated.

Accuracy

Accuracy studies were carried out by applying the method to samples solution to which known amount of MPA(Imp acid) corresponding to LOQ level, 50% level of specification, 100% level of specification and 120% level of specification. At each level of the amount, samples prepared in triplicate and determination was performed. Data was treated to calculate % R.S.D. at each level and overall.

Robustness

Robustness was studied in six replicate at a concentration level of 20 ppm. In this study, five parameters (wavelength of detection, column oven temperature, and flow rate of mobile phase, pH of buffer in the mobile phase and composition of the mobile phase) were investigated and the effects on the results were expressed as standard deviation and % R.S.D. The wavelength of detection was varied by +5 nm (at 245 nm and 255 nm), column oven temperature was varied by +5ºC (at 40°C and 50°C).The flow rate of mobile phase and pH of the buffer in mobile phase were varied by +10% (at 0.9 mL min^{-1} and 1.1 mL min^{-1}) and +0.2 (at pH 5.1 and pH 5.5), respectively. In the mobile phase, the minor component was varied by + 2% absolute of minor component i.e. 38% of buffer and 42% of buffer. Data were treated to calculate % R.S.D. in each case.

Limit of Detection and Limit of Quatitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), single injection of blank and six injections of standard solution at concentration level of 20 µg mL^{-1} were injected in column and system suitability was determined by determining % R.S.D. of six injections. Several lower concentrations of MMF and MPA (Imp acid) were prepared and

analyzed six times for determination of LOD and LOQ based on % R. S. D. The LOD was expressed as 10% R. S. D. whereas LOQ was expressed as 33% R. S. D. of six injections.

Stability of analytical solution

In order to determine the stability of the analytical solution, standard solution and sample solution to be analyzed initially and at different time intervals at 25ºC for around 24 hours and / or standard solution and sample solution to be analyzed initially and at different time intervals at 5ºC for around 24 hours. For that six injections of standard solution were injected in column for the determination of system suitability and one injection of each standard solution as well as sample solution were injected at different time intervals for around 24 hours.

Forced degradation studies

To perform the forced degradation studies first injected five injections of standard solution (for system suit). Then the sample solution and placebo solution were treated separately in each condition as followed:

(a) Two milliliters of 2N HCl was added and mixture was heated at 70°C for 30 minutes and neutralized by addition of 1N NaOH solution and 10 mL of diluent. (Acid induced degradation)

(b) Two milliliters of 1N NaOH was added and mixture placed at room temperature for 5 minutes. (Base induced degradation)

(c) Two milliliters of 30% w/v H_2O_2 was added and mixture was heated at 70°C for 15 minutes. (Hydrogen peroxide H_2O_2 induced degradation)

(d) Tablets and capsules of MMF were placed in UV chamber at 6250 LUX for 15 days. (UV induced degradation)

(e) Tablets and capsules of MMF were placed in oven at 105°C for 15 days. (Thermal induced degradation)

In all degradation studies, 10 µL of the resultant solutions were injected in column and chromatograms were run as described in section 2.2. The peak area of each peak has been determined and peak purity determined in each case. Data was treated to calculate the degradation in each case.

System suitability

System suitability in each parameter of validation has been determined and the acceptance criteria for the system suit were as followed:

- Tailing factor of MMF peak from the standard solution should not be more than 2.0.
- Theoretical plates of MMF peak from the standard solution should not be less than 7000.

- % R.S.D. of the area of MMF peak from the six injection of the standard solution should not be more than 5.0

RESULTS AND DISCUSSION

Development of the optimal mobile phase

The RS procedure was optimized with a view to develop a stability indicating RS method to quantify the MMF from marketed formulation and manufactured tablets and capsules. In order to obtain good separation of MMF, various compositions of triethylamine buffer pH 5.3 and acetonitrile were tried but the best results were obtained with the mobile phase consisting triethylamine buffer pH 5.3 and acetonitrile (65:35 v/v) at the maximum temperature of 45°C and flow rate 1.5 mL/min. The main peak of MMF was quite sharp, narrow and also not presented any fronting and tailing problem in this mobile phase. The injection volume used in method was 10 µL for each injection, because higher injection volume produced broad main peak of mycophenolate mofetil. The scanning of the standard solution represented wavelength maxima at 250 nm, and therefore 250 nm was chosen as the wavelength for UV detector**.** (Figure 3, 4, 5, 6)

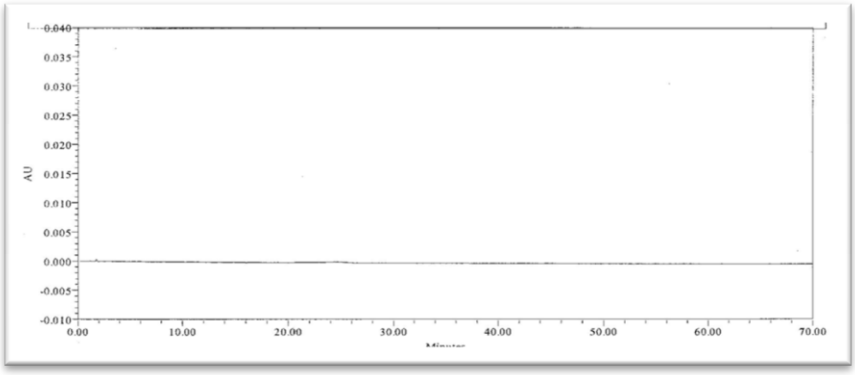

Figure 3. HPLC chromatogram of placebo of MMF capsules in Related Substances

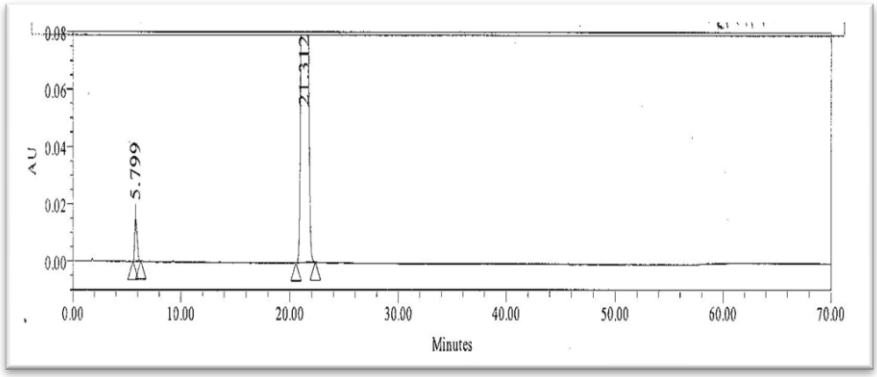

Figure 4. HPLC chromatogram of spiked sample at 1% level of MMF capsules in Related Substances

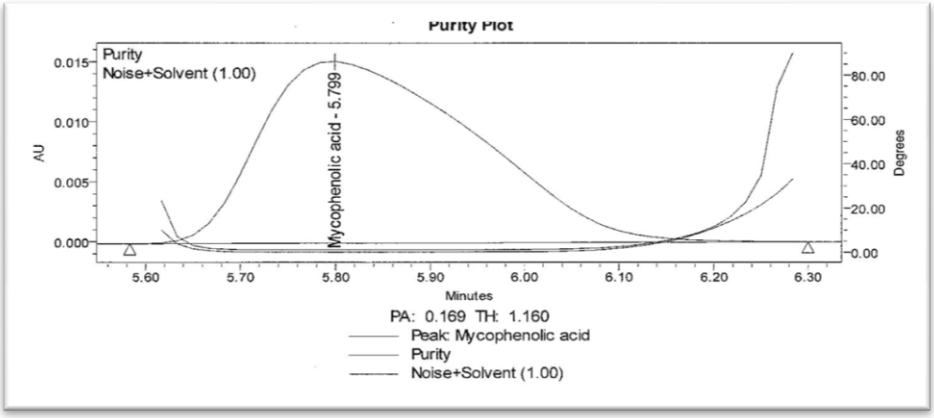

Figure 5. Peak Purity of Imp Acid in spiked sample at 1% level of MMF capsules in Related Substances

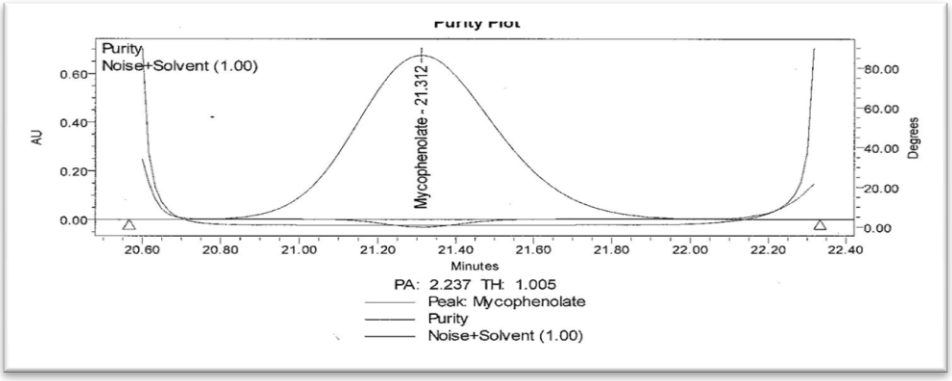

Figure 6. Peak Purity of MMF capsules in spiked sample at 1% level of MMF capsules in Related Substances

Validation of the method

Specificity

In the interference studies, diluents run as well as placebo run were not produced any peak or fluctuation at the run time of main peak of Mycophenolate mofetil and MPA (Imp acid). The % R.S.D. of six injection of standard solution was found to be 1.2 and other data given in (Table 1).

Table 1.Specificity table for related substances

Parameters	Value
Purity threshold of sample solution	1.054
Purity angle of sample solution	0.223
Purity threshold of spiked sample	1.042
Purity angle of spiked sample	0.223
Purity threshold of Imp acid	1.741
Purity angle of Imp acid	0.415

Precision

System Precision

In the system precision; the USP tailing, USP plates and % R.S.D. of the six injections of standard solution were found to be 1.02, 14778 and 1.7 respectively.

Method Precision

For the method precision; the USP tailing, USP plates and % R.S.D. of the five injections of standard solution were found to be 1.02, 14778 and 1.70 respectively. The % R.S.D. of the six sample solutions of Imp acid was found to be 2.25.

Intermediate Precision

In the intermediate precision the USP tailing, USP plates and % R.S.D. of the five injections of standard solution were found to be 1.04, 16074 and 1.20 respectively. The % R.S.D. of the six sample solutions of Imp acid was found to be 4.55.

Linearity

The USP tailing, USP plates and % R.S.D. of the eight injections of MMF standard solution and MPA (Imp acid) presented in (Table 2).

Table2. Linearity data for MMF and MPA (Imp acid) (n=8)

Parameters	Values (n=8)	
	MMF	MPA
Linearity range (μg mL^{-1})	0.403- 24.163	0.400- 24.163
Correlation coefficient (r)	1.000	0.9999
Slope	8179	11029
Response factor	1.00	0.74
Intercept(y)	-101	528

Accuracy

The standard deviation, % R.S.D. and % Recovery of three replicates of each level individually and the overall were given in (Table 3).

Table3. Accuracy of HPLC method (n=3)

Parameter	Accuracy		
	S.D.	R.S.D.	%Recovery
LOQ level	0.00	0.00	100
50 Accuracy level	1.617	1.66	97.47
100% Accuracy level	0.922	0.94	98.20
120% Accuracy level	0.574	0.59	97.00
Overall %RSD	1.452	1.48	98.17

Robustness of the method

The S.D. of standard solution, % R.S.D. of standard solution, theoretical plate of sample solution, tailing factor of sample solution, % R.S.D. of sample solution and RRT of MPA (Imp acid) are summarized in (Table 4). The low values of % R.S.D. after introducing small deliberate changes in the developed HPLC method indicated the robustness of the method.

Table 4. Robustness of the HPLC method (n=3)

Parameters	Control	Minus	Plus
Wavelength (±5nm) (245 nm and255 nm)			
S.D. (peak Area) of standard	2926.2	4775.1	3379.1
% R.S.D. (peak Area) of standard	1.7	3.1	3.22
Theoretical plate of sample	14776	14757	14946
Tailing factor of sample	1.02	1.03	1.04
% R.S.D. of sample	1.70	3.10	2.22
RRT of Imp Acid	0.31	0.31	0.31
Colum oven temperature (45±5 ºC) (40 and 50 ºC)			
S.D. (peak Area) of standard	838.1	657.6	1142.7
% R.S.D. (peak Area) of standard	0.51	0.40	0.69
Theoretical plate of sample	14504	14375	14636
Tailing factor of sample	0.97	1.0	0.97
% R.S.D. of sample	0.51	0.40	0.69
RRT of Imp Acid	0.34	0.30	0.32
Change in mobile phase (± 2% absolute of minor component) (38% of buffer and 42% of buffer)			
S.D. (peak Area) of standard	1161.2	2024.3	917.1
% R.S.D. (peak Area) of standard	0.67	1.18	0.53
Theoretical plate of sample	8844	9631	8787
Tailing factor of sample	1.34	1.26	1.37
% R.S.D. of sample	0.67	1.18	0.53
RRT of Imp Acid	0.32	0.28	0.30
Flow rate (±10%) (0.9ml min^{-1} and 1.1ml min^{-1})			
S.D. (peak Area) of standard	1171.7	850.3	552.5
% R.S.D. (peak Area) of standard	0.67	0.43	0.34
Theoretical plate of sample	15172	16126	14588
Tailing factor of sample	1.02	1.02	1.01
% R.S.D. of sample	0.67	0.43	0.34
RRT of Imp Acid	0.30	0.30	0.30
pH of buffer of mobile phase (±0.2) (5.1 and 5.5)			
S.D. (peak Area) of standard	503.8	1100.5	552.3
% R.S.D. (peak Area) of standard	0.29	0.62	0.31
Theoretical plate of sample	15102	15031	15026
Tailing factor of sample	1.08	10.6	1.06
% R.S.D. of sample	0.29	0.62	0.31
RRT of Imp Acid	0.35	0.25	0.30

Table 5. LOD and LOQ for related substances

Name	LOD(µg mL^{-1})	LOQ (µg mL^{-1})
Imp Acid (MPA)	0.399	0.412
MMF	0.399	0.412

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and quantitation limit with % R. S. D. of 10 and 33 were considered as LOD and LOQ and were represented in (Table 5)

Stability of analytical solution

At 5ºC, in the study of the stability of the analytical solution the % R.S.D. of the five injection of the standard solution at the concentration of 400 µg mL^{-1} of MMF was found to be 1.24. The USP tailing and USP plates of the sample solution spiked with Imp acid in the starting of the method were found to be 0.93 and 13463 respectively. After the time interval of 24 hours the USP tailing and USP plates were found to be 0.95 and 12695 respectively. The cumulative % R.S.D. for the each time intervals was less than 1.00. Hence the analytical solution of MMF was stable at 5ºC for 24 hours. The % R.S.D. of the five injections of standard solution of the drug at 25 ºC and the concentration level of 400 µg mL^{-1} was found to be 0.1. The USP tailing and USP plates of the sample solution spiked with Imp acid in the starting of the method were found to be 0.99 and 14536 respectively. After the time interval of 24 hours the USP tailing and USP plates were found to be 0.99 and 14557 respectively. The cumulative % R.S.D. for the each time interval was less than 4.00. Hence the analytical solution of MMF was stable at 25ºC for 24 hours.

Forced degradation studies

The system suitability data like theoretical plates, tailing factor and % R.S.D. from the five injections of standard solution were found to be 7468, 1.08 and 0.53 respectively. The % degradation in acid degradation, base degradation, peroxide degradation, photolytic degradation and thermal degradation were found to be 13, 20, 18, 3 and 26 respectively. The purity angle and purity threshold in acid degradation; base degradation; peroxide degradation; photolytic degradation and thermal degradation were found to be 0.157 and 0.441; 0.171 and 0.464; 0.150 and 0.462; 0.370 and 1.563; 0.234 and 0.645 respectively. (Table 6)

Table 6. Degradation data of MMF (n=3)

S. No.	Exposure conditions	Purity angle	Purity Threshold	Recovery (%)
1.	None (control samples)	0.189	0.807	99.5
2.	2ml, 2N HCl, 70ºC. 30 min, neutralized with 1N NaOH and 10 ml diluent	0.127	0.381	89.27
3.	2 ml, 1N NaOH, RT, 5min	0.169	0.455	89.67
4	2 ml, 30% w/v H_2O_2, 70ºC, 15 min	0.162	0.362	74.74
5.	UV light (6250 LUX) (15 days)	0.355	1.489	72.20
6.	Dry heat 105ºC, (15days)	0.238	0.588	75.23

CONCLUSION:

In summary, the described method is rapid, sensitive, specific, accurate and reproducible. It was successfully used to MMF stability under various stress degradation conditions. It is of potential value for the analysis of MMF as bulk drug and commercial formulations.

ACKNOWLEDGEMENT

The authors are grateful to Jamia Hamdard, New Delhi for assistance. The authors grateful acknowledge Biocon Ltd., Bangalore, India for providing MMF as gift sample.

REFERENCES:

1. Tripodi VP, Lucangioli SE, Barbara CL, Rodriguez VG, Carducci CN. Analysis of immunosuppressive Drugs and Their Pharmaceuticals by micellar Elctrokinetic chromatography. Chromatographia 2001; 54: 93-98.
2. Birnbaum F, Mayweg S, Reis A, Bahringer D, Seitz B, Engelmann K, Messmer EM, Reinhard T. Mycophenolate mofetil (MMF) following penetrating high-risk keratoplasty: long-term results of a prospective, randomised, multicentre study. Eye 2009: 402.
3. Haentzchel I, Freiberg-Richter J, Platzbecker U, Kiani A, Schetelig J, Illmer T, Ehninger G, Schleyer E, Bornhauser M. Targeting mycophenolate mofetil for graft-versushost disease prophylaxis after allogenic blood stem cell transplantation. Bone Marrow Transplantation 2008; 42: 113-120.
4. Sepe V, Libetta C, Gluliano MG, Adamo G, Dal Canton A. Mycophenolate mofetil in primary glomerulopathies. Kidney International 2007; 73: 154-162.
5. Tjeertes IFA, Bastiaans DET, van Ganzewinkel CJLM, Zegers SHJ. Neonatal anemia and hydrops fetalis after maternal mycophenolate mofetil use. J Perinatology 2007; 27: 62-64.
6. Tang S, Leung JCK, Chan LYY, Lui YH, Tang CSO, Kan CH, Ho YW, Lai KN. Mycophenolate mofetil alleviates persistent proteinuria in IgA nephropathy. Kidney International 2005; 68: 802-812.
7. Benech H, Hascoet S, Furlan Valerie, Pruvost A, Durrbach A. Development and validation of an LC/MS/MS assay for mycophenolic acid in human peripheral blood mononuclear cells. J Chromatography B 2007; 853: 168-174.
8. Snyder LR, Glajch JL, Kirkland JJ. Practical HPLC Method Development, 2nd edn. Wiley-Chichester, New York, 1997:110-210.

9. Martindale, The Extra Pharmacopoeia, Pharmaceutical Press, Wallingford, 31st edn. 1996
10. Protic A, Zivanovic Lj, Zecevic M, Jocic B. Development of liquid chromatographic method for simultaneous determination of mycophenolate mofetil and its degradation product mycophenolic acid in dosage form. J chromatographic sci 2009; 47(2):149-55.
11. Toseland PA in Clarke's Isolation and Identification of Drugs, ed. Moffat, AC, pharmaceutical Press, London, 2nd edn.,1986:111–117.
12. International Conference on Harmonisation (ICH) Validation of Analytical Procedures, Note for Guidance, Commission of the European Communities, Brussels1995.
13. Reviewer Guidance- Validation of Chromatographic Methods. Center for Drug evaluation and Research, Food and Drug Administration 1994.
14. The United States Pharmacopoeia USP Convention Inc., Mack Publishing Company, 2000; 24:168 & 187.