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DEVELOPMENT OF ASSAY METHOD FOR MYCOPHENOLATE MOFETIL IN PHARMACEUTICAL DOSAGE FORM

Mehta Hiralben Satishchandra*[1], Dr. Indrajeet Singhvi[2] and Dr. Hasumati Raj[3]

[1]Research Scholar, Pacific University.

[2]Professor and Head, Pacific Academy of Higher Education and Research University.

[3]Principal and Professor, Laxminarayandev College of Pharmacy, Bharuch, Gujarat.

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***Corresponding Author**
Mehta Hiralben Satishchandra
Research Scholar, Pacific University.

ABSTRACT

A simple, rapid and reproducible high performance reverse phase liquid chromatographic method has been developed for quantitative estimation of Mycophenolate mofetil in tablets form using a, Phenomenex 250 mm x 4.9 mm C18 , 5 µm, inertsil and UV detection at 250nm. The isocratic elution was used to quantify the analyte and the mobile phase was Acetonitrile: acetate buffer (40:60) was pumped at 1.0 mL/min. The method was linear between 50-300 µg/mL, statistically validated for its linearity, precision and accuracy. It was found that the excipients in the commercial tablet did not interfere with the method.

KEYWORDS: HPLC, Mycophenolate mofetil Validation ICH guidelines.

1. INTRODUCTION

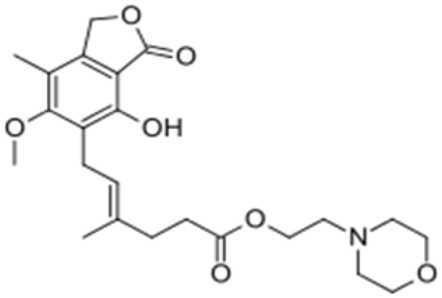
Mycophenolic acid is important because of its selective effects on the immune system. It prevents the proliferation of T-cells, lymphocytes, and the formation of antibodies from B-cells. It also may inhibit recruitment of leukocytes to inflammatory sites.[2]

Mycophenolic Acid is an antineoplastic antibiotic derived from various Penicillium fungal species. Mycophenolic acid is an active metabolite of the prodrug Mycophenolate mofetil. Mycophenolic acid inhibits inosine monophosphate dehydrogenase (IMPDH), preventing the formation of guanosine monophosphate and synthesis of lymphocyte DNA that results in inhibition of lymphocyte proliferation, antibody production, cellular adhesion, and migration

of T and B lymphocytes. Mycophenolic acid also has antibacterial, antifungal, and antiviral activities. (NCI04).[2]

Several methods have been described for determination of Mycophenolate mofetil in pharmaceutical preparations including HPTLC, HPLC and NMR had been used for the determination of Mycophenolate mofetil.[1],[3],[4],[12],[13] the proposed method was validated as per ICH guidelines.

1.1 CHEMICAL STRUCTURE



IUPAC NAME: 2-morpholin-4-ylethyl (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1H-2-benzofuran-5-yl)-4-methylhex-4-enoate

2. MATERIALS AND METHOD

2.1 MATERIALS

All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Acetonitrile HPLC grade, Water HPLC grade, Sodium Acetate buffer A.R, Orthophosphoric acid HPLC grade, and Mycophenolate mofetil standard.

2.2 METHOD

2.2.1 PREPARATION OF SOLUTIONS

A) 0.05M ACETATE BUFFER: 2.7 gm of acetic acid and 0.41 gm of sodium acetate in 1000 ml beaker dissolve in HPLC grade water and dilute to the mark with the same HPLC grade water. PH adjusted to 5.4 with ortho phosphoric acid Filtered through 0.45 micron membrane filter.

B) PREPARATION OF STANDARD STOCK SOLUTION

150ppm of Mycophenolate mofetil (Prepared standard in duplicate for similarity) accurately weigh and transfer 50 mg of Mycophenolate mofetil standard into a 50 ml volumetric flask. Add 3ml acetonitrile and sonicate to dissolve and dilute up to mark with same medium and mix it well.

Dilute 1.5 ml of this solution to 10 ml with ACN solution.

2.2.2 SELECTION OF WAVELENGTH FOR ANALYSIS OF MYCOPHENOLATE MOFETIL

Accurately weigh and transfer 50 mg of Mycophenolate mofetil standard into a 50 ml volumetric flask. Add 3ml acetonitrile and sonicate to dissolve and dilute up to mark with same medium and mix it well.

Dilute 1.5 ml of this solution to 10 ml with ACN solution.

The resulting solution was scanned in UV range (200nm–400nm). In spectrum Mycophenolate mofetil showed absorbance maximum at 250 nm.

2.2.3 VALIDATION OF THE METHOD

The method was validated in terms of linearity, accuracy, precision, sensitivity, repeatability and ruggedness.

A) LINEARITY STUDY

Different aliquots of Mycophenolate Mofetil in range 50 - 300 µg/ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with acetonitrile to get concentrations, respectively.

B) ACCURACY

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120%. The solutions were reanalyzed by proposed method.

C) PRECISION

Precision of the method was studied as intra-day and inter-day variations. Method Intra-day precision was determined by analyzing the 150ppm solution of Mycophenolate Mofetil for method precision and system precision have been. Inter-day precision was determined by

analyzing the 150 ppm solution of Mycophenolate Mofetil daily for three days over the period of week.

D) SENSITIVITY

The sensitivity of measurements of Mycophenolate Mofetil by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

E) REPEATABILITY

Repeatability was determined by analyzing 150 ppm concentration of Mycophenolate Mofetil solution for six times.

F) RUGGEDNESS

Ruggedness of the proposed method is determined for 150 ppm concentration of Mycophenolate Mofetil by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions.

2.2.4 DETERMINATION OF MYCOPHENOLATE MOFETIL IN BULK

TEST SOLUTION: 150 ppm of Mycophenolate mofetil

Take 20 tablets, find out its average weight and take powder equivalent about 50mg of Mycophenolate mofetil corresponding into a 50ml volumetric flask. Add acetonitrile and shaken well. The solution is sonicated for approx. 15 minutes.

Following cooling to room temperature, the solution is filled up to volume with the same solvent acetonitrile, mixed well and then filtered through a 0.45µm nylon membrane. The first 5 ml of filtrate are discarded. 1.5ml of this solution are diluted to 10 ml with Acetonitrile and mixed well.

3. RESULTS AND DISCUSSION

3.1 METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

3.1.1 LINEARITY STUDIES

The linear regression data for the calibration curves showed good linear relationship over the concentration range 50-300 µg/ml for Mycophenolate mofetil. Linear regression equation was found to be y = 7942.x - 4.729

$R^2 = 1.00$

Table I.

Mycophenolate mofetil linearity result			
Conc. µg/ml	**Avg. Area**	**SD**	**%RSD**
50	397127	1.48	0.0003%
100	794254	1.48	0.0001%
150	1191381	1.41	0.0001%
200	1588408	1.48	0.00009%
300	2382767	1.48	0.00006%
---	**1270787**	--	---
---	**1.00**	--	---

Mycophenolate mofetil linearity Peak

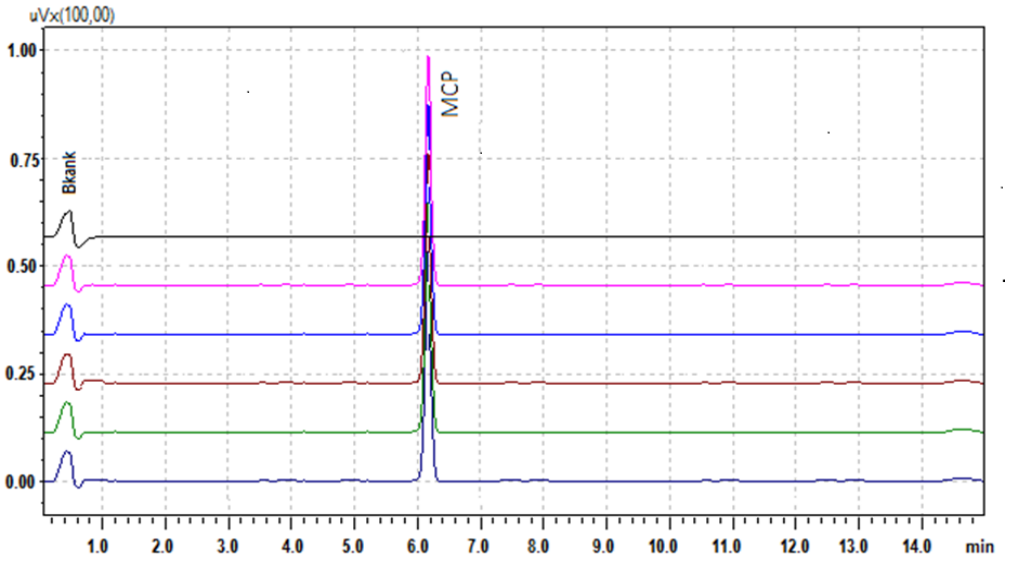


Fig. 1.

Mycophenolate mofetil linearity plot

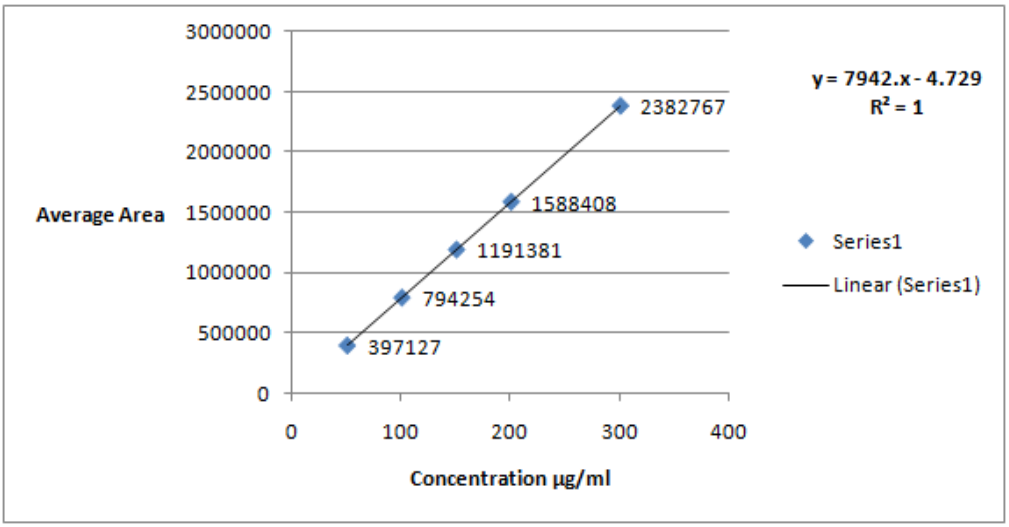


Fig. 2.

3.1.2 ACCURACY

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 99.32- 101.54 with % R.S.D. <2.

3.1.3 PRECISION

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation.

3.1.4 SENSITIVITY

The linearity equation was found to be Y = 7942.x - 4.729. The LOQ and LOD for mycophenolate mofetil were found to be 0.038 µg and 0.32 µg, respectively.

3.1.5 REPEATABILITY

Repeatability was determined by analyzing 150 ppm concentration of mycophenolate mofetil solution for six times and the % amount found was between 99.32- 101.54 with % R.S.D. less than 2.

3.1.6 RUGGEDNESS

Peak area was measured for same concentration solutions, six times. The result showed that the % R.S.D. was less than 2%.

Table II.

SUMMARY REPORT		
Sr. No	**Parameters**	**TMP**
1	Linearity range	50- 300µg/ml
2	Correlation coefficient (r^2)	1.00
3	Intercept	0
4	Slope	7942
5	Precision	0.35
	Intraday Average % RSD (n = 5)	1.54
	Inter day Average % RSD (n = 5)	0.31
	Reproducibility of measurements	99.32- 101.54
	%RSD	0.000167
	% Recovery	99.32- 101.54
6	Limit of detection (µg/ml)	0.013
7	Limit of quantification (µg/ml)	0.0562

3.2 Determination of Mycophenolate mofetil in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 99.32- 101.54.

3.3 Application of proposed method for pharmaceutical formulation

The spectrum was recorded at 250 nm. The concentrations of the drug were calculated from linear regression equation. The % amount was found between 99.32- 101.54.

4. CONCLUSION

This HPLC technique is quite simple, accurate, precise, reproducible and sensitive. The HPLC method has been developed for quantification of Mycophenolate mofetil tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

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REFERENCE

1. Magdalena Korecka, Leslie M. Shaw, Ann Transplant, "Review of the newest HPLC methods with mass spectrometry detection for, determination of immunosuppressive drugs in clinical practic" Ann Transplant, 2009; 14: 61-72.
2. http://www.drugbank.com.
3. Sudha T., Krishana Kanth V., Nukala Poorana Chandra Sainath, Mishal, Saloman, Raja T, Ganesan V," Method Development and Validation" Journal of Advanced Pharmacy Education & Research, 2012; 3: 146-176.
4. Vijaya Bhaskara Reddy T, Sowjanya Reddy N, Ramu G, Ravi Prakash Reddy S, Rambabu C, "Determination of mycophenolate mofetil in bulk and pharmaceutical formulations by UV derivative spectrophotometric method MycophenolateMofetil (MMF), an immunosuppressant is extensivelyused in transplantmedicine" Asian journal of chemistry, 2014; 8: 296-301.
5. Gopalakrishnana S, Vadivela E, Krishnavenia P and Jeyashreeb B," A novel Reverse Phase-HPLC method development and validation of Mycophenolate Sodium-An Immunosuppressant drug" Research Journal of Pharmaceutical Biological and Chemical Sciences, 2010; 4: 200.
6. Vijaya bhaskara reddy T, Ramu G, Sravan kumar M and Rambabu C" Validated RP-HPLC Method for the Determination of Mycophenolate Mofetil in Tablet Dosage Forms"Asian Journal of Chemistry, 2013; 9: 4788-4790.
7. Lakshmana rao A, Vijay srinivas P and J.v.l.n.s. rao." A new validated rp-hplc method for the estimation of mycophenolate mofetil in pure and tablet dosage form", Asian journal of pharmaceautical research and health care, 2010; 3: 266-269.
8. Kailas Chitalkar, Shardul Kulkarni, Prafullachandra Tekale, Ratnakar Lanjewar and Ranjeet Kaur Bajwa "Development and Validation of a Simple HPTLC Method for Estimation of Mycophenolate Mofetil in Bulk Drug and in Tablet Dosage Form", International journal of pharmaceutical and chemical sciences, 2277-5005.
9. Vijaya Bhaskara Reddy Tummala, Sowjanya Reddy Nallagari, Ramu Golkonda, Veera Venkatrao Sure, Rambabu Chintala "Development of stability indicating liquid chromatography-mass tandem spectrometric method for the estimation of mycophenolate mofetil in bulk and pharmaceutical formulations" Journal of pharmacy research, 2013; 7:

640-646.

10. Vasanth kumar kunithala, kiran kumar chinthakindi, sateesh kumar vemula, prasad garepally, vijay kumar bontha.” A new rapid and simple analytical method development and validation of estimation the mycophenolate in dosage form by uplc technique” Asian Journal of Pharmaceutical and Clinical Research, 2012; 3: 0974-2441.
11. Shraddha Digole, Swarali Hingse, Ashwini Tilay, Uday Annapure” Development and validation of HPTLC method for quantification of mycophenolic acid” Journal of Biochem Tech, 2014; 3: 756-759.
12. P. Raja Abhilash, K. Vasanth Kumar and V. Sateesh Kumar” Development and Validation of New RP-UPLC Method for the Quantitative Analysis of Mycophenolate in Tablet Dosage Form” British Journal of Pharmaceutical Research, 2013; 3: 363-373.
13. Suman Pattanayak, A. Alekhya Prasanna, Ch. Kiranmayi, K. Padmalatha “Analytical UV Spectroscopic Method Development and Validation for the Estimation of Mycophenolate Mofetil” Asian Journal of Pharmaceautical Analysis, 2015; 4: 209-213.