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# A NEW VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF MYCOPHENOLATE MOFETIL IN PURE AND TABLET DOSAGE FORM

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**ABSTRACT**

A simple, rapid, sensitive and precise high performance liquid chromatographic (HPLC) method has been developed for the estimation of mycophenolate mofetil in bulk and tablet dosage form. In this method RP-$C_{18}$ column (250mmx4.6mm I.D., 5µm particle size) with mobile phase consisting of acetonitrile and 0.03M phosphate buffer in the ratio of 60:40 v/v in isocratic mode was used. The detection wavelength is 254 nm and the flow rate is 0.8 ml/min. In the range of 20-120 µg/ml, the linearity of mycophenolate mofetil shows a correlation coefficient of 0.9995. The proposed method was validated by determining sensitivity, accuracy, precision and system suitability parameters. The mobile phase selected for the method is simple, fast, accurate and precise and hence can be applied for routine quality control analysis of mycophenolate mofetil in bulk and its tablet dosage form.

**KEYWORDS:** HPLC, Mycophenolate mofetil, Validation.

## INTRODUCTION

Mycophenolate mofetil[1] (MMF), an ester prodrug of immunosuppressant, is a morpholinoethyl ester of mycophenolic acid (MPA), which is its active metabolite. MPA, a fermentation product of several *Penicillium* species, is a potent, non-competitive, reversible inhibitor of eukaryotic inosine monophosphate dehydrogenase. MPA thus inhibits the synthesis of guanosine monophosphate. This enzyme plays an important role in purine metabolism of lymphocytes. Chemically[2], mycophenolate mofetil is 6-[(7-Hydroxy-5-methoxy-4-methyl-1-oxo-3H-isobenzofuran-6-yl)]-4-methyl-hex-4-enoic acid 2-morpholinoethyl ester. Few HPLC[3-10] and LC-MS[11-16] methods for its determination have been reported. A simultaneous determination of mycophenolic acid and valproic acid in human plasma by HPLC[17] was reported. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of mycophenolate mofetil in bulk sample and tablet dosage forms.

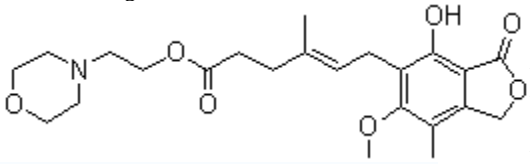


**Figure 1. Chemical structure of mycophenolate mofetil**

## EXPERIMENTAL PROCEDURE:

### Instrumentation

The separation was carried out on isocratic HPLC system (Shimadzu) with Shimadzu Binary HPLC pump, Shimadzu LC- 10ATP UV-Visible Detector, Spinchrom SPD-10AVP software and RP-$C_{18}$ column (250mmx4.6mm I.D; particle size 5µm).

### Drugs and chemicals

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and triethylamine of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Mycophenolate mofetil was a gift sample by Intas Pharmaceuticals Ltd., Ahmedabad. The commercially available mycophenolate mofetil tablets were procured from the local market.

### Chromatographic conditions

The mobile phase consisting of acetonitrile (HPLC grade) and 0.03M phosphate buffer pH 2.9 (1ml of triethylamine was added to buffer solution before adjusting the pH) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40 v/v was pumped into the column at a flow rate of 0.8 ml/min. The detection was monitored at 254 nm and the run time was 6 min. The volume of injection loop was 20 µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

### Procedure

Stock solution of mycophenolate mofetil was prepared by dissolving 100 mg of mycophenolate mofetil in 100 ml standard volumetric flask containing 25 ml of acetonitrile and the solution was sonicated for 20 min. and then made upto the mark with acetonitrile to get a concentration of 1 mg/ml. Subsequent dilutions of this solution were made with mobile phase to get concentration of 20-120 µg/ml.

The standard solutions prepared as above were injected into the 20 µl loop and the chromatogram was recorded in Fig. 2.

The retention time of mycophenolate mofetil was found to be 3.24 min. The calibration curve was constructed by plotting concentration vs peak area ratio. The amount of mycophenolate mofetil present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method.

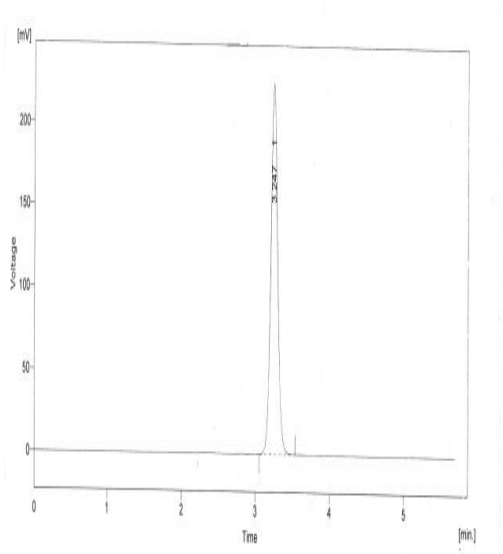


**Figure 2. Typical chromatogram of mycophenolate mofetil.**

**Assay**

Twenty tablets were weighed and powdered. A quantity equivalent to 500 mg of mycophenolate mofetil was weighed accurately and transferred to 500 ml volumetric flask, dissolved in acetonitrile and made upto 500 ml with acetonitrile. From this solution, further dilutions were made in mobile phase to get 80 µg/ml. This solution was injected and the chromatogram was recorded. The amount of mycophenolate mofetil was determined from the regression equation. The results are furnished in Table 1.

| Formulation | Label claim (mg) | % Amount found* | % C.V. | Amount added (µg/ml) | Amount found | % Recovery |
|---|---|---|---|---|---|---|
| Brand-1 | 500 | 99.97 | 0.51 | 20 | 20.05 | 100.27 |
| Brand 2 | 500 | 100.02 | 0.90 | 20 | 19.95 | 99.75 |

*Mean of five determinations.

TABLE-1 **ASSAY AND RECOVERY STUDIES**

**Validation of proposed method**

Selectivity of the method was assessed on the basis of elution of mycophenolate mofetil using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of mycophenolate mofetil. The results are furnished in Table 2.

**Specificity**

The specificity was established by preparing a mycophenolate mofetil standard at 0.5% level of test concentration and injected 5 times into HPLC system as per the test procedure.

**Linearity**

The standard curve was obtained in the concentration range of 20-120 µg/ml. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

**Precision**

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100.

**Limit of detection (LOD) and limit of quantitation (LOD)**

The LOD and LOQ for mycophenolate mofetil were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of mycophenolate mofetil.

**Robustness**

The robustness was checked by changing the temperature to $30^0$ and $35^0$C and the method suits best.

**Accuracy**

The accuracy of the HPLC method was assessed by adding known amount of drug solution to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug

concentration to the expected drug concentration with 50 so as to give the percentage recovery.

| System suitability parameters | Results |
|---|---|
| Linearity (µg/ml) | 20-120 |
| Correlation coefficient | 0.9995 |
| Retention time (min.) | 3.24 |
| Theoretical plates (N) | 6000 |
| Tailing factor | 1.032 |
| Asymmetric factor | 1.148 |
| LOD (µg/ml) | 0.08 |
| LOQ (µg/ml) | 0.28 |

TABLE-2 VALIDATION SUMMAR

**RESULTS AND DISCUSSION**

By applying the proposed method, the retention time of mycophenolate mofetil is found to be 3.24 min. which indicates a good base line. When the same drug solution was injected 5 times, the retention time of the drug was same. Linearity was obeyed in the concentration range of 20-120 µg/ml. The regression equation of mycophenolate mofetil concentration over its peak area ratio was found to be Y=571.4902+18066.19X (r =0.9995) where Y is the peak area ratio and X is the concentration of mycophenolate mofetil (µg/ml). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 5 replicate injections was found to be less than 1%. The asymmetry factor was found to be 1.148, which indicates asymmetric nature of peak. The number of theoretical plates was found to be 6000, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.08 µg/ml and 0.28 µg/ml, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of acetonitrile and phosphate buffer were tested. The use of acetonitrile and phosphate buffer in the ratio of 60:40 v/v resulted in peak with good shape and resolution. The high percentage of recovery of mycophenolate mofetil ranging from 99.75 to 100.27 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

**CONCLUSION**

The proposed HPLC method was found to be highly accurate, sensitive and precise. Therefore this method can be applied for the routine quality control analysis of mycophenolate mofetil in pure and its tablet dosage form.

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**REFERENCES**

1. Franklin T J and Cook J M, The inhibition of nucleic acid synthesis by mycophenolic acid, Biochem. J., 1969, 113: 515.
2. www.chemblink.com
3. Barzoki M A, Rouini M, Gholami K, Lessan-Pezeshki M and Rezaee S, Determination of mycophenolic acid in human plasma by high-performance liquid chromatography, DARU, 2005, 13: 120.
4. Srivatsan V, Dasgupta A K, Kale P, Verma R, Joshi P, Soni D, Patel M, Soni G, Patel J and Modi H, Determination of mycophenolic acid in human plasma by high-performance liquid chromatography, J. Chromatogr. A, 2004, 1031: 259.
5. Teshima D, Kitagawa N, Otsubo K, Makino K, Itoh Y and Oishi R, Simple determination of mycophenolic acid in human serum by column-switching high-performance liquid chromatography, J. Chromatogr. B, 2002, 780: 21.
6. Renner U D, Thiede C, Bornhauser M, Ehninger G and Hans-Michael Thiede, Determination of mycophenolic acid and mycophenolate mofetil by high-performance liquid chromatography using postcolumn derivatization, Anal. Chem., 2001, 73: 41.
7. Hosotsubo H, Takahara S, Kokado Y, Permpongkosol S, Wang J D, Tanaka T, Matsumiya K, Kitamura M, Okuyama A and Sugimoto H, Rapid and simple determination of mycophenolic acid in human plasma by ion-pair RP-LC with fluorescence detection, J. Pharm. Biomed. Anal., 2001, 24: 555.
8. Na-Bangchang K, Supasyndh O, Supaporn T, Banmairuroi V and Karbwang J, Simple and sensitive high-performance liquid chromatographic method for the determination of mycophenolic acid in plasma, J. Chromatogr. B, 2000, 738: 169.

9. Tisna I, Kaloostian M, Lee R, Tarnowski T and Wong B, High-performance liquid chromatographic method for the determination of mycophenolate mofetil in human plasma, J. Chromatogr. B, 1996, 681: 347.
10. Sugioka N, Odani H, Ohta T, Kishimoto H , Yasumura T and Takada K, Determination of a new immunosuppressant, mycophenolate mofetil, and its active metabolite, mycophenolic acid, in rat and human body fluids by high-performance liquid chromatography, J. Chromatogr. B, 1994, 654: 249.
11. Kuhn J, Gotting C and Kleesiek K, Sample cleanup-free determination of mycophenolic acid and its glucuronide in serum and plasma using the novel technology of ultra-performance liquid chromatography–electrospray ionization tandem mass spectrometry, Talanta, 2010, 80, 1894.
12. Kuhn J, Prante C, Kleesiek K and Gotting C, Measurement of mycophenolic acid and its glucuronide using a novel rapid liquid chromatography–electrospray ionization tandem mass spectrometry assay, Clin. Biochem., 2009, 42: 83.
13. Shen B, Li S, Zhang Y, Yuan X, Fan Y, Liu Z, Hu Q and Yu C, Determination of total, free and saliva mycophenolic acid with a LC–MS/MS method: Application to pharmacokinetic study in healthy volunteers and renal transplant patients, J. Pharm. Biomed. Anal., 2009, 50: 515.
14. Marie-Odile Benoit-Biancamano, Caron P, Levesque E, Delage R, Couture F and Guillemette C, Sensitive high-performance liquid chromatography–tandem mass spectrometry method for quantitative analysis of mycophenolic acid and its glucuronide metabolites in human plasma and urine, J. Chromatogr. B, 2007, 858: 159.
15. Benech H, Hascoet S, Furlan V, Pruvost A and Durrbach A, Development and validation of an LC/MS/MS assay for mycophenolic acid in human peripheral blood mononuclear cells, J. Chromatogr. B, 2007, 853: 168.
16. Platzer M, Jahn K, Wohlrab J and Neubert R H H, Quantification of mycophenolate mofetil in human skin extracts using high-performance liquid chromatography–electrospray mass spectrometry, J. Chromatogr. B, 2001, 755: 355.
17. Zhong Y, Jiao Z and Yunqiu Y, Simultaneous determination of mucophenolic acid and valproic acid based on derivatization by high-performance liquid chromatography with fluorescence detection, Biomed. Chromatogr., 2006, 20: 319.



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