Research Article

Development and Validation of a Simple HPTLC Method for Estimation of Mycophenolate Mofetil in Bulk Drug and in Tablet Dosage Form

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ABSTRACT

A simple, specific and precise high performance thin layer chromatographic method of analysis of Mycophenolate mofetil, both as a bulk drug and in formulation was developed and validated. This method can be used for routine quality testing. The method employed TLC (Thin Layer Chromatography) aluminum plates pre-coated with silica gel 60 F254 as the stationary phase manufactured by Merck Ltd. The solvent system consisted of acetonitrile: triethylamine: glacia acetic acid (9:1:0.1 v/v). This system was found to give compact bands for Rf value 0.58±0.02. Densitometric analysis of Mycophenolate mofetil was carried out in the absorbance mode at 340nm. Linear regression analysis data for the calibration spots showed good relationship with regression coefficient r^2 = 0.9964 in the range of 200-800 ng/ band. The limits of detection and quantitation were 20 ng/ band and 60 ng/ band respectively. The proposed method was found to be simple, precise, accurate, and reproducible for the estimation of Mycophenolate mofetil in pure drugs and its formulations.

Keywords: Mycophenolate mofetil, HPTLC method validation, Densitometric.

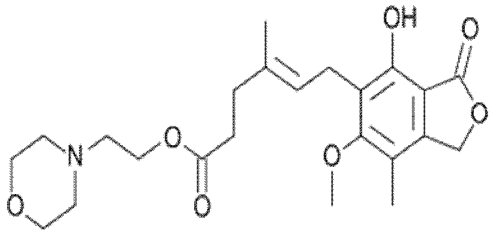
1. INTRODUCTION

The chemical name for Mycophenolate mofetil is 2-morpholinoethyl (E)-6-(1, 3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. (Franklin et.al.1969)

Its empirical formula is $C_{23}H_{31}NO_7$ and molecular weight 433.50. Mycophenolate mofetil is morpholinoethyl ester of mycophenolic acid, which is used to mask the carboxyl group. Mycophenolate mofetil (MMF) (brand names CellCept, Myfortic) is an Immunosuppressant and prodrug of mycophenolic acid, used extensively in transplant medicine. It is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH) in purine biosynthesis (specifically guanine synthesis) which is necessary for the growth of T cells and B cells. Other cells are able to recover purines via a separate, scavenger, pathway and are, thus, able to escape the effect.MMF is a less toxic alternative to Azathioprine.MMF is also used in the treatment of autoimmune diseases, such as Behçet's disease, pemphigus vulgaris, and systemic lupus erythematosus. Suppressing T cells and B cells stops them from attacking healthy cells, but also weakens their ability to defend against infections.

Few HPLC (Barzoki et.al.2005, Srivatsan et.al.2004, Teshima et.al.2002, Renner et.al.2001, Hosotsubo et.al.2001.Na-Bangchang et.al.2000, Tisna et.al.1996, Sugioka et.al.1994) and LC-MS (Kuhn et.al.2009, Kuhn et.al.2010, Shen et.al.2009. Marie-Odile et.al.2007, Benech et.al.2007, Platzer et.al.2001) methods for its determination have been reported. HPTLC is one of the best techniques in chromatography. Hence this HPTLC method was developed which is simple, accurate, and precise for the determination of bulk drug and its formulation and can be used for routine quality testing.

Molecular structure



2. MATERIALS AND METHODS

Mycophenolate mofetil (MMF) (purity 99.78%) was provided as a gift sample by RPG Life science Ltd. Mumbai, India and was used without further purification. All the other reagents used were of analytical grade. Toluene (AR grade), Methanol (AR grade), Acetone (AR grade) were purchased from Merck (chemicals) Pvt Limited, Germany.

2.1 INSTRUMENTATION

Chromatographic separation of drug was performed on Merck TLC plate pre-coated with silica gel 60 F254 (10cm ×10cm with 250 mm layer thickness) from E.Merck, Germany. The samples were applied onto the plates as a band with 8 mm width using *CAMAG* 100 µl sample syringe (Hamilton, Switzerland) with Linomat V applicator (*CAMAG*,Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using *CAMAG* TLC scanner 3 in the range of 200-800 ng per band and operated by winCATS software (V 1.4.6, *CAMAG*).

2.2 SELECTION OF DETECTING WAVELENGTHS

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that the drug showed considerable absorbance at 340 (Figure I). So, 340nm were selected as the wavelength for detection

2.3. METHOD VALIDATION

2.3.1 Linearity

A stock solution of Mycophenolate mofetil (100 µg /µl) was prepared in methanol and diluted suitably to obtain concentration of 0.08µg /µl. Different volumes of the dilution, 2, 3,4,5,6,7,8 µl were spotted on TLC plate to obtain concentration of 200 to 800 ng/ band of Mycophenolate mofetil , respectively. The data of peak area v/s drug amount were treated by linear least-square regression analysis.

2.3.2. Precision

The intra and inter-day variation for the determination of Mycophenolate mofetil was carried out at three different concentration levels of 40, 80 and 120 ng per spot. The % RSD values were determined for intra-day and inter-day variation.

2.3.3. Accuracy

The analysed samples were spiked with 80, 100 and 120 % of the standard Mycophenolate mofetil and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate.This was done to check for the recovery of the drug by standard addition method.

2.3.4. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard formula as per the ICH guidelines.

2.3.5. Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peaks was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on win CATS software.

2.4. ANALYSIS OF MARKETED FORMULATION

To determine the content of Mycophenolate mofetil in tablet (label claim: 500mg per tablet). Methanol was added gradually upto 50 ml. then from this stock solution 1ml diluted to 10 ml, again from this diluted stock solution we took 0.8 ml in volumetric flask. This volumetric flask was kept covered with aluminum foil. Finally volume was made upto 10ml with methanol to get stock solution of (0.08 µg/µl). The solution was suitably diluted. Appropriate volume of solution was applied on TLC plate followed by development and scanning.

3. RESULTS AND DISCUSSION

3.1. Development of the optimum mobile phase

TLC procedure was optimized with a view to develop an accurate assay method. The drug reference standard was spotted on the TLC plate and developed in different solvent

systems. The mobile phase methanol: toluene: 25%ammonia (7:3:0.1v/v) gave sharp and symmetrical peak with Rf 0.65. Well-defined bands were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature. The representative densitogram is given in (Figure III).

3.2 Validation of the method

3.2.1 Linearity

The response for the drugs was found to be linear in the concentration range 200-800 ng / band with correlation co-efficient of 0.9964. The representative linearity graph is given in (Figure II).

3.2.2 Precision

The % RSD value for intra-day and inter-day variation study was found to be not more than 0.725 % and 1.21 % respectively, thus confirming precision of the method.

3.2.3 Recovery

Acceptable recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80 %, 100 %, 120 % level) indicated the mean recovery 98.28%.

3.2.4 Limit of Detection and Limit of Quantitation

The limit of detection and limit of quantitation as calculated by standard formula as given in ICH guidelines was found to be 20 ng / band and 60 ng/ band respectively.

3.2.5 Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s, m) 0.9978 and r (m, e) 0.9959 for Mycophenolate mofetil , 0 indicating the non interference of any other peak of degradation product, impurity or matrix. The validation results are listed in Table I.

3.3 Analysis of marketed formulation

There was no interference from the excipients present in the suspension. The drug content was found to be 102.3%.

4. CONCLUSION

The developed method was found to be simple, precise, and sensitive. High throughput ability of HPTLC makes it a very useful method for routine analysis of bulk drug as well as formulation.

Table 1: Validation Parameters

S. No.	Validation Parameter	Mycophenolate mofetil
1	Linearity Equation (r^2) Range	**Y = -209.5 + 46.08 * X** (0.9964) 20 0– 800 ng per band
2	Precision (% RSD) Intraday Interday	 NMT 0.725 % NMT 1.21 %
3	Accuracy (% mean recovery)	**98.28%**
4	LOD	20ng
5	LOQ	60ng
6	Specificity Peak Purity	Specific r(s,m) = 0.9978 r(m,e) = 0.9959

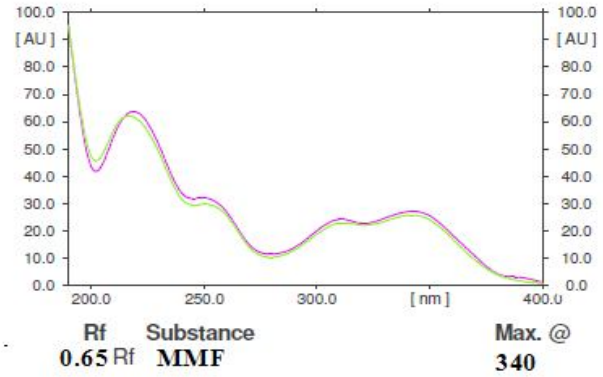


Fig. 1: Spectrum of Mycophenolate mofetil

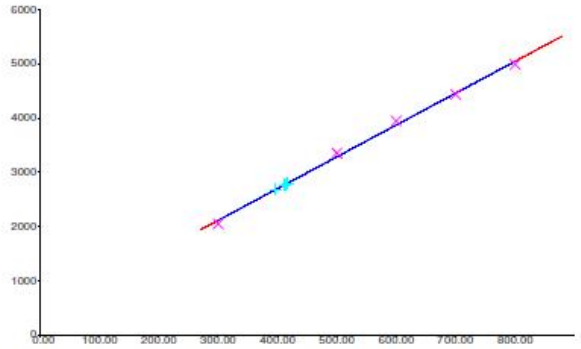


Fig. 2: Linearity of Mycophenolate mofetil

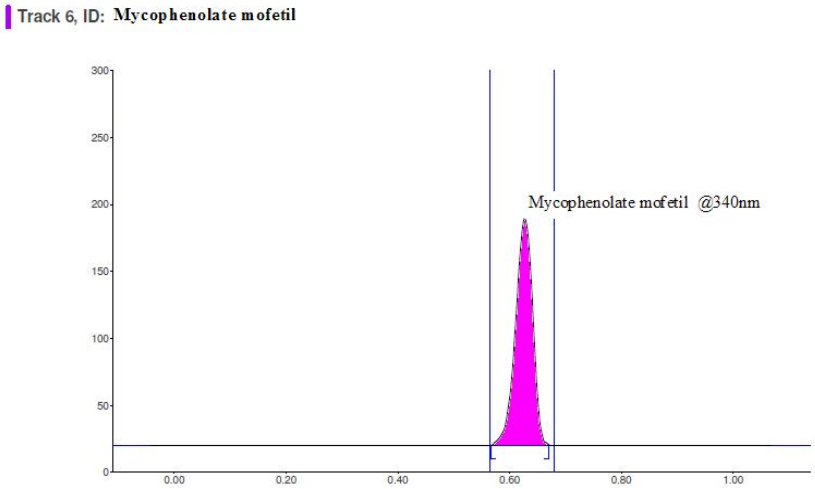


Fig. 3: Representative Densitogram of Mycophenolate mofetil

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