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Forced degradation studies: A Stability indicating liquid chromatographic method for the quantification of Mycophenolate mofetil in tablets

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ABSTRACT

A simple and sensitive stability-indicating reverse phase high-performance liquid chromatographic (HPLC) method was developed and validated for determination of Mycophenolate mofetil in tablet dosage forms. Mycophenolate mofetil is an immunosuppressant drug used extensively in transplant medicine. The chromatographic separation (isocratic mode) was performed on Shimadzu Model LC-Class-Vp with Lichrocart / Lichrosphere 100 (250 × 4.6 mm, 5 µm particle size) using C-18 column and mobile phase (52: 48, v/v) containing tetra butyl ammonium hydrogen sulphate and methanol with flow rate 1.2 mL/min. Ultraviolet detection was carried out at 216 nm. Lamotrigine has been used as internal standard. Mycophenolate mofetil has shown linearity over the concentration range 0.5–160 µg/mL (r^2 = 0.999) and the method was validated. The limit of quantitation and limit of detection 0.321 and 0.102 µg/mL respectively. Forced degradation studies were performed and Mycophenolate mofetil is found to be more sensitive towards alkaline conditions.

KEY WORDS: Mycophenolate mofetil, RP-HPLC, stability-indicating, Lamotrigine, validation.

1. INTRODUCTION

Mycophenolate mofetil (MPT) (Fig.1), is an immunosuppressant and prodrug of mycophenolic acid, used extensively in transplant medicine (Fulton, 1996). MPL is chemically 2-morpholinoethyl(E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5isobenzofuranyl)-4-methyl-4-hexenoate with molecular formula $C_{23}H_{31}NO_7$ and molecular weight 433.50 g/mol (Merck index, 2006). It is a potent, selective, uncompetitive, and reversible inhibitor of inosine mono phosphate dehydrogenase and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Analytical techniques such as liquid chromatography (Khoschsorur, 2004; Watson, 2004; Tsina, 1996; Jones, 1998; Bopp, 1972; Hosotsubo, 2001; Gummert, 1999; Shipkova, 1998; Teshima, 2002; Zambonin, 2004; Huang, 1997;, 2002; Shipkova, 2001; Yau, 2004; Hosotsubo, 2001, Sugioka, 1994; Na-Bangchang, 2001; Srivatsan, 2004; Mehdi, 2005; Saunders, 1997; Wiwattanawongsa, 2001; Teshima, 2003), LC/MS (Atcheson, 2004; Marie, 2007; Benech, 2007; Kuhn, 2009) and UPLC/MS (Kuhn, 2010) were reported for the determination of Mycophenolate mofetil in biological fluids. The pharmacokinetics (Kuriata, 2003; Shaw, 2000; David, 2003; Brusa, 2000; Brunet, 2000; Cattaneo, 2001; Shaw, 1998) as well as the enzymatic immuno assay methods (Yeung, 1999; Schütz, 1998) were developed and compared with HPLC methods during the transplantation in biological fluids. Mycophenolate mofetil was also studied by turbidometric bio assays (Noto, 1970) and HPTLC (Suraj, 2011) methods. Gainer and Wesselman developed GLC (Gainer, 1970) for mycophenolic acid and its related compounds. Lee et al., improved the bioavailability of mycophenolic acid through amino ester derivatization (Lee, 1990). Liquid chromatographic (Gopalakrishnan, 2010; Lakshmana Rao, 2010) and spectrophotometric (Vinay, 2012; Narendra, 2013) methods were also established for the determination of Mycophenolate mofetil in pharmaceutical dosage forms. In the present study the authors have proposed a stability indicating liquid chromatographic method for the assay of Mycophenolate mofetil in presence of an internal standard (IS), Lamotrigine.

2. MATERIALS AND METHODS

Instrumentation: RP-HPLC analysis was performed with a Shimadzu Model LC-Class-Vp version 6.12 SPI, equipped with UV-VIS detector Model SPD-10A maintained at 25 ºC. A Lichrocart / Lichrosphere 100 C-18 (25 0mm × 4.6 mm i.d., 5 µm particle size) column employed for chromatographic separation. The specificity of the method was conducted by using heating oven, photo stability chamber and heating mantle (Thermo Lab, India).

Chemicals and reagents: Mycophenolate mofetil was obtained from Cipla Ltd. Mumbai (India). Mycophenolate mofetil is available in pharmacy with brand name CELLCEPT (Roche Products Limited, India); label claim 500 mg and 250 mg as tablets and also as suspension with label claim 200 mg and CELLMUNE (Cipla Ltd, India). HPLC grade methanol, sodium hydroxide, hydrochloric acid and hydrogen peroxide, tetra butyl ammonium hydrogen sulphate (TBHS) were purchased from Merck (India).

Mycophenolate mofetil stock solution (1000 µg/mL) was prepared by accurately weighing 25 mg of Mycophenolate mofetil in a 25 mL volumetric flask and making up to volume with mobile phase. Working solutions for HPLC injections were prepared on a daily basis from the stock solution in a solvent mixture of TBAHS and methanol (52:48, v/v). Solutions were filtered through a 0.45 µm membrane filter prior to injection.

Lamotrigine, the internal standard stock solution was prepared in methanol and 2 µg/mL solution was added during the validation study.

Validation: The method was validated for the following parameters: system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness (ICH guidelines, 2005).

Linearity: A series of Mycophenolate mofetil solutions were prepared from the stock solution by diluting with mobile phase along with 2 µg/mL internal standard (IS) solution. 20 µL of each of these solutions were injected in to the HPLC system and the peak area of both MPT and IS were noted from the resulting chromatogram. The peak area ratio MPT/IS was plotted on the y axis against the concentration of MPT on the x axis.

Precision and Accuracy: The intra-day and inter-day precision method were evaluated by carrying out 9 independent assays of Mycophenolate mofetil on the same day and on three different days and the %RSD was calculated. The accuracy of the assay method was evaluated by spiking the formulation with the pure drug solution (80, 100 and 120%) and the percentage recovery was calculated.

Assay of marketed formulations: Twenty tablets were purchased from the local market, weighed and crushed to a fine powder and powder equivalent to 25 mg Mycophenolate mofetil was accurately weighed into a 25 mL volumetric flask and made up to volume with methanol. The volumetric flask was sonicated for 30 min to enable complete dissolution of Mycophenolate mofetil. The solution was filtered and the filtrate was diluted with mobile phase to yield required concentrations. Internal standard was incorporated before making up the solution. All the solutions were filtered through a 0.45 µm nylon filter before injecting in to HPLC system.

Forced degradation studies: Forced degradation studies (ICH guidelines, 2003) were performed to evaluate the stability indicating properties and specificity of the method. All solutions for use in stress studies were prepared with an initial concentration 1 mg/mL of Mycophenolate mofetil and refluxed for 30 mins at 80 ºC in thermostat. All samples were then diluted in mobile phase to give a final concentration of 20 µg/mL and filtered before injection. Acid decomposition was carried out in 0.1 M HCl at a concentration of 1.0 mg/mL Mycophenolate mofetil and after refluxation for 30 mins at 80 ºC the stressed sample was cooled, neutralized and diluted with mobile phase. Similarly, stress studies in alkaline conditions were conducted using a concentration of 1.0 mg/mL in 0.1 M and 0.01 M NaOH and refluxed for 30 mins at 80 ºC. After cooling the solution was neutralized and diluted with mobile phase. Solutions for oxidative stress studies were prepared using 3% H_2O_2 at a concentration of 1 mg/mL of Mycophenolate mofetil and after refluxation for 30 mins at 80 ºC on the thermostat the sample solution was cooled and diluted accordingly with the mobile phase. For thermal stress testing, the drug solution (1 mg/mL) was heated in thermostat at 80 ºC for 30 mins, cooled and used. The drug solution (1 mg/mL) for photo stability testing was exposed to UV light for 1 hour (365 nm) UV light chamber and analyzed.

3. RESULTS AND DISCUSSION

The authors have developed a validated stability indicating RP-HPLC method for the determination of Mycophenolate mofetil using Lamotrigine as internal standard. UV detector was employed in the present study. The MPT solution was scanned in the UV region prior to the chromatographic study to identify the λ_{max} (Figure 2). Both MPT and IS were eluted with UV detection at 216 nm. The study was performed using a mixture of TBHS and methanol (52:48, v/v) as mobile phase with flow rate 1.2 mL/min and two sharp peaks were eluted at 7.033 min for Mycophenolate mofetil and 3.442 mins for Lamotrigine (Figure.3).

Linearity: Mycophenolate mofetil has shown linearity over the concentration range 0.1–160 µg/mL (Table 1). The calibration curve was obtained by plotting the peak area ratio (MPT/IS) against the concentration of MPT. The linear regression equation to be y = 1.1295 x + 0.6887 (Figure 4) with correlation coefficient 0.9998. The LOQ was found to be 0.043 µg/mL and the LOD was found to be 0.012 µg/mL. The proposed method was applied for the determination of Mycophenolate mofetil tablets and percentage recovery was found to be 98.89 -99.83

Precision and Accuracy: During the precision study the % relative standard deviation (% RSD) was found to be 0.18-0.31 and 0.34-0.83 for intra-day and inter-day indicating that the method is precise. The method has shown recovery 98.93-99.24% with %RSD (0.28-0.74) less than 2.0 indicating that the method is accurate.

Forced Degradation Studies: Mycophenolate mofetil was subjected to stress conditions. It was found that the drug was more sensitive to alkaline conditions because when the drug solution was treated with 0.1 N sodium hydroxide the entire drug has undergone degradation and therefore the study was performed by modifying the condition as 0.01 N sodium hydroxide where a very slight degradation was observed. During the oxidation, thermal and photolysis also MPT shows about 20.32%, 25.91% and 36.86% degradation. The drug peak was well separated even in the presence of degradation products and therefore this method is specific and selective and can be used for the determination of Mycophenolate mofetil in pharmaceutical formulations. The theoretical plates are found to be more than 2000 and the tailing factor was found to be less than 1.5.

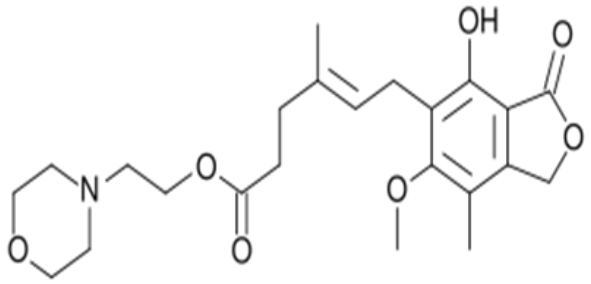

Figure.1. Chemical structure of Mycophenolate mofetil

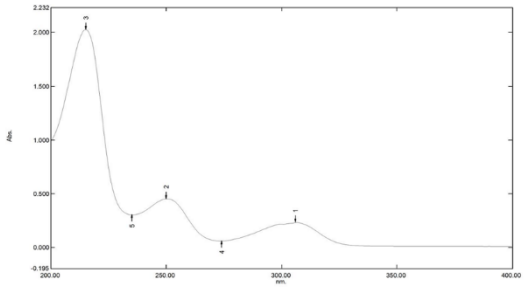

Figure.2. Absorption spectrum of Mycophenolate mofetil (20 µg/ml) (λ_{max} 216 nm)

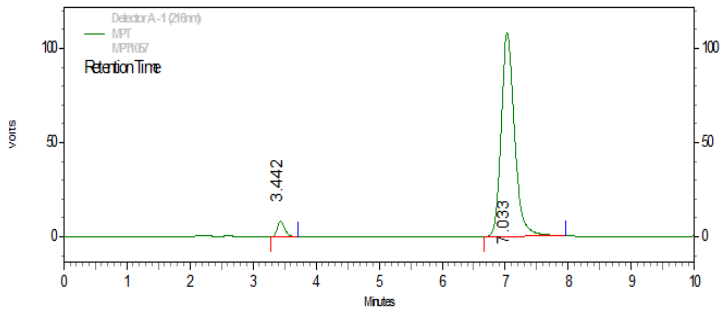

Figure.3. Typical chromatogram of Mycophenolate mofetil (20 µg/ml) (Rt 7.033 mins) with Lamotrigine (IS) (2 µg/ml) (Rt 3.442 mins)

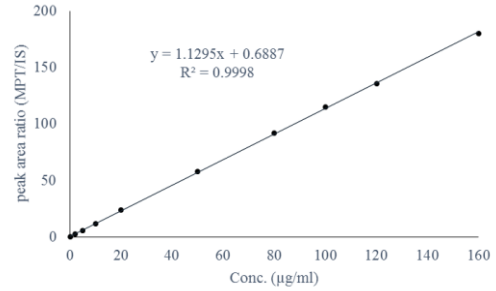

Figure.4. Calibration curve of Mycophenolate

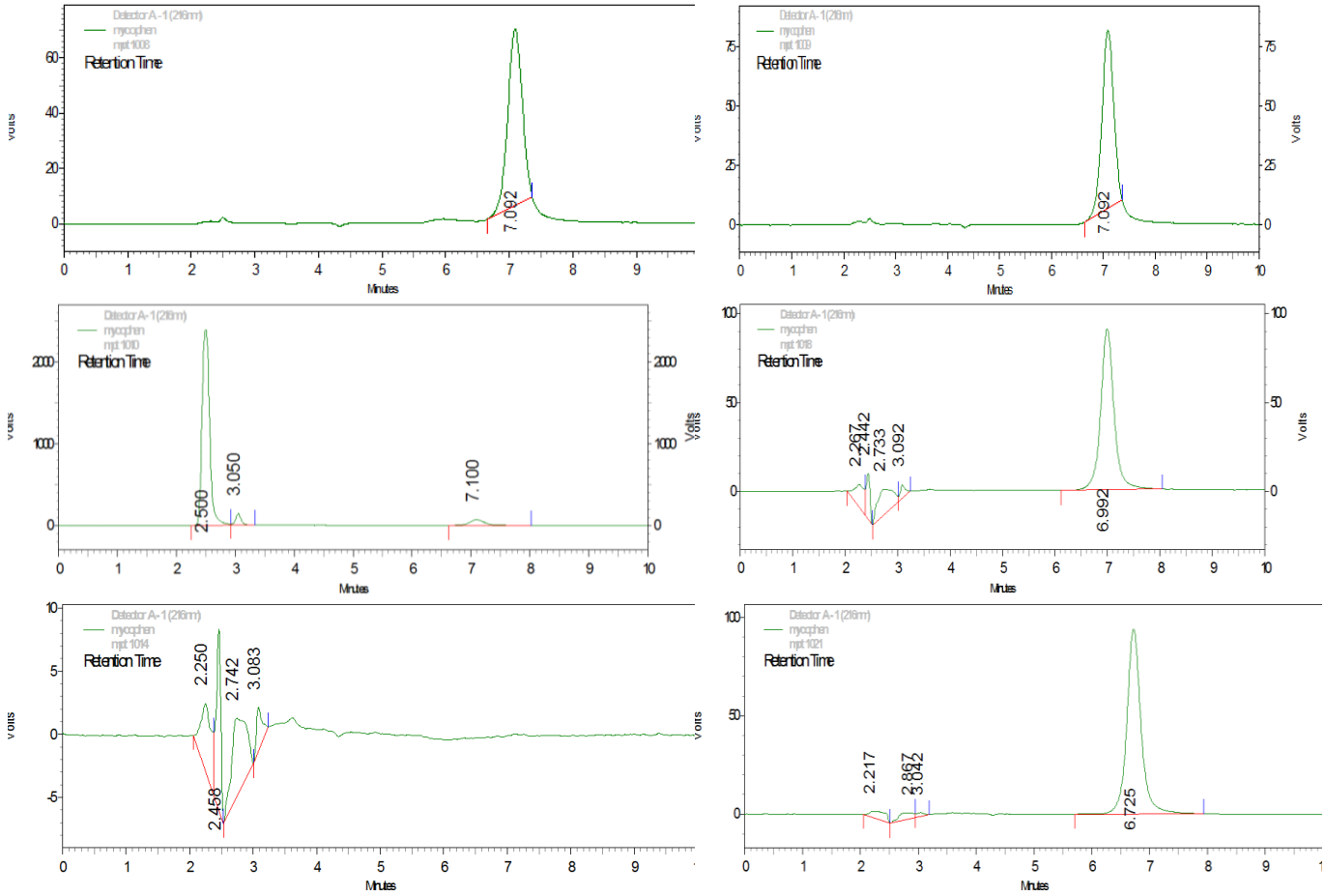

Figure.5. Typical chromatograms of Mycophenolate mofetil (a) photolysis (b) thermal (c) oxidative (d) acidic (e) alkaline (0.1N NaOH) (f) alkaline (0.01N NaOH) degradations

Table.1. Linearity of Mycophenolate mofetil

MPT Conc. (µg/mL)	IS Conc. (µg/mL)	Mean peak area MPT	Mean peak area IS	peak area ratio (MPT / IS)
0.1	2	39208	6054	0.154407

2	2	75455	179824	2.383195
5	2	68033	383404	5.635559
10	2	67791	795342	11.73227
20	2	66476	1595001	23.99364
50	2	65348	3800049	58.15096
80	2	65019	5962531	91.70444
100	2	43638	5025502	115.1634
120	2	63505	8630712	135.906
160	2	66143	11907476	180.0262

*Mean of three replicates

Table.2. Forced degradation studies of Mycophenolate mofetil

Stress Conditions	***Mean peak area**	***Drug recovered (%)**	***Drug decomposed (%)**
Standard drug (Untreated)	1595001	100	-
Acidic degradation	1593328	99.89511	0.10489
Alkaline degradation	1594440	99.96483	0.035172
Oxidative degradation	1270881	79.67901	20.32099
Thermal degradation	1181668	74.08572	25.91428
Photolytic degradation	1007116	63.14203	36.85797

*Mean of three replicates

4. CONCLUSION

A stability-indicating HPLC method was developed, validated and applied for the determination of Mycophenolate mofetil in pharmaceutical dosage forms. The developed method was validated and as per ICH guidelines and was found to be accurate, precise and specific.

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