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# A novel Reverse Phase-HPLC method development and validation of Mycophenolate Sodium-An Immunosuppressant drug

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**ABSTRACT**

A simple and accurate RP-HPLC method has been developed for the estimation of Mycophenolate Sodium in pharmaceutical dosage forms, using USP L7 octyl silane chemically bonded to porous silica C8 (4.6X 250 mm) partical size in isocratic mode, with mobile phase comprising of acetonitrile and buffer in the ratio of 50:50 v/v. The flow rate was 1.5 ml/min and the detection was monitored out by Photodiode array detector at 254 nm. The retention time for Mycophenolate Sodium was found to be 4.872 min and the method produced linear response in the concentration range of 288-468 µg/ml (r~0.9993). The recovery studies were also carried out and % RSD from reproducibility was found to be 0.2423. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Mycophenolate Sodium.

**Keywords:** RP-HPLC, Mycophenolate Sodium, Validation, Immunosuppressant, Isocretic

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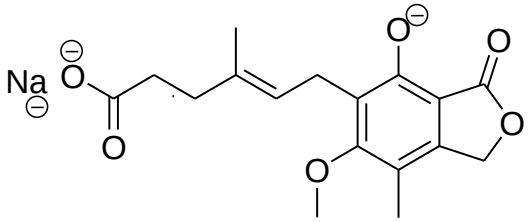
## INTRODUCTION

Mycophenolic acid is an immunosuppressant drug used to prevent rejection in organ transplantation. It was initially marketed as the prodrug Mycophenolate Mofetil to improve oral bioavailability [1-4]. More recently, the salt Mycophenolate Sodium has also been introduced. It acts as a non-competitive, selective and reversible inhibitor of Inosine-5'-monophosphate dehydrogenase (IMPDH) [5-7], Mycophenolate is the prodrug of Mycophenolic acid which inhibits purine synthesis by inhibiting Inosine Monophosphate Dehydrogenase [8-11]. Currently two products are available, they are Mycophenolate Mofetil and Mycophenolate Sodium (1). Both are converted to mycophenoloic acid.




(1)

## EXPERIMENTAL

### Chemicals

Acetonitrile and Orthophosphoric acid were supplied by E.Merck Ltd, Germany. Methanol and HPLC Water were obtainted from Sun Pharma Advanced Research Centre, Mycophenolic acid sodium salt was purchased from Sigma-aldrich, Mycophenolic acid sodium salt Tablet was purchased from Moral. All reagents used were of HPLC grade.

### Preparation of Stock solution

180 mg of Mycophenolate sodium was taken into a 100 ml volumetric flask, and dissolved using Sonicater and made upto the volume with methanol. Working standards in the range of 0.1 to 10 µg/ml were prepared by using mobile phase as diluent.

### Buffer preparation

6.8 gm of potassium dihydrogen orthophosphate was dissolved in 1000 ml of distilled water and 5 ml of triethylamine was added and the pH was adjusted to 6.0 with ortho phosphoric acid.

### Preparation of mobile phase

65 volumes of 0.01 M Potassium dihydrogen orthophosphate was added and the pH was adjusted to 3.0 with 25 volumes of acetonitrile and 10 volumes of methanol.

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**Instruments and Chromatographic conditions**

The HPLC system (Shimadzu Co, Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVp, A Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 240nm),

| | | |
|---|---|---|
| Column | : | USP L7 Octyl silane chemically bonded to porous silica C8, (5µm), (4.6 x 250mm) |
| Flow rate | : | 1.5ml/min |
| Mobile phase | : | Acetonitrile : Buffer (50: 50) |
| Buffer | : | 0.1% v/v solution of orthophosphoric acid |
| Diluent | : | Methanol |
| Injection volume | : | 10 µg/ml |
| Detector | : | 254 nm |
| Temperature | : | 28ºC |
| Retention time | : | 4.872 min |

**Calculation of Mycophenolate Sodium**

The amount of drug present was calculated by comparing the peak area values of standard with that of samples as follows:

$$\frac{\text{Spl.Area}}{\text{Std.Area}} \times \frac{\text{Std.wt}}{100} \times \frac{10}{50} \times \frac{100}{\text{Spl.wt}} \times \frac{50}{10} \times \frac{\text{Std. purity (as is)}}{100} \times \text{Avg.wt} \times \text{conv.factor}$$

$$\text{Conversion factor} = \frac{\text{Mol.wt of Mycophenolic acid (320.34)}}{\text{Mol.wt of Mycophenolic acid sodium (342.32)}}$$

conv.factor = 0.9358

**Validation parameters**

**System Suitability**

System suitability parameters were evaluated by injecting five replicates of 360 µg/ml concentration of standard Mycophenolate Sodium solution. Resolution factor, theoretical plate and tailing factor were evaluated by following ICH guidelines.

**Specificity**

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by comparing the blank chromatogram with the chromatogram obtained for the drug spiked with internal standard (placebo) to trace out the interfering peaks.

The specificity of the method was investigated by the analysis of the two blank preparation spiked with two different concentration of standard Mycophenolate Sodium, sample Mycophenolate sodium and internal standard (placebo) is also added.

**Limit of Quantitation:(LOQ)**

LOQ is the peak area response was determined by analyzing five times of 14.4 µg/ml concentration of standard Mycophenolate sodium. The % RSD was calculated.

**Limit of Detection: (LOD)**

LOD is the peak area response was determined making twelve measurements at four different concentration points in the range of 3.6 µg/ml-6.48 µg/ml. The % RSD was calculated

**Linearity**

Linearity of the peak area response was determined by making six measurements at five different concentration points in the range of 288-468 µg/ml for Mycophenolate Sodium respectively.

**Accuracy**

Accuracy was assessed by using a minimum of three different concentration (standard Mycophenolate sodium 80%, 100%, and 120%) and 130 mg/ml of placebo spiked into the standard solution of Mycophenolate Sodium. The mean, standard deviation and %RSD were calculated.

**Precision**

The precision of an analytical method is the closeness of a series of individual measurements of an analyte when the analytical preocedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. The precision is calculated as coefficient of variation (C. V.), i.e., relative standard deviation (RSD). The measure RSD can be subdivided into three categories: repeatability (intra-day precision) and reproducibility (between laboratories precision).

**Reproducibility**

Reproducibility of the method assessed by analyzing five times of 360 µg/ml of standard solution of Mycophenolate Sodium. The % RSD was calculated.

**Repeatability**

Repeatability of the peak area response was determined by making six measurements at six concentration points in the range of (305-310) mg/ml of sample

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Mycophenolate Sodium respectively and it is compared with the standard Mycophenolate Sodium of 180 mg/ml.

**Robustness**

Robustness was determined by injecting triplicate injection of standard and three sample solutions in single and at different concentrations with respect to control condition.

Robustness of the method was checked by varying the instrumental condition wavelength ±2 nm, temperature ±2°C. The sample and standard solution were injected in each condition and the %RSD was calculated.

## RESULTS AND DISCUSSION

**Method development**

The present RP-HPLC method for the quantification of Mycophenolate Sodium in bulk and Pharmaceutical dosage forms, revealed as simple, accurate, presise, robust, specific and stability indicating. The method is significant retention time of 4.872 min.

**System suitability**

System suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification. System suitability results are presented in Table-1. The typical chromatogram, of Mycophenolate Sodium is shown in Fig.1

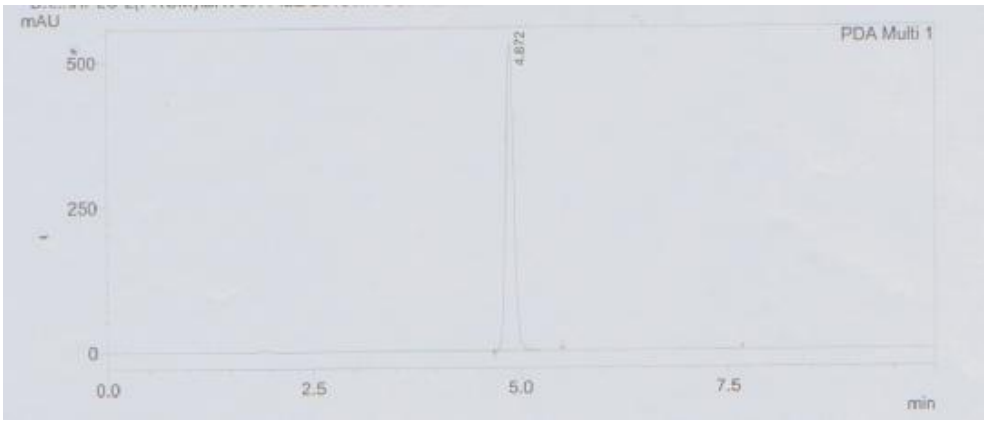


**Fig-1. Typical chromatogram of Mycophenolate sodium**

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**Table-1. System suitability parameters of Mycophenolate sodium**

| | |
|---|---|
| Retention Time | 4.872 |
| Theoretical Plates | 10587.206 |
| Tailing Factor | 1.191 |

**Table-2. Linearity of Mycophenolate sodium**

| S. No | Concentration (µg / ml) | Volume of stock solution (ml) | Volume of made up to (ml) | Mean Peak Area |
|---|---|---|---|---|
| 1 | 288 | 8 | 50 | 2733418 |
| 2 | 324 | 9 | 50 | 3076968.5 |
| 3 | 360 | 10 | 50 | 3411253.5 |
| 4 | 396 | 11 | 50 | 3785974.5 |
| 5 | 432 | 12 | 50 | 4072193 |
| 6 | 468 | 13 | 50 | 4389634 |
| Linear Regression Coefficient | | | | 0.9993 |
| Correlation coefficient | | | | 0.994 |

**Table-3. Accuracy of Mycophenolate sodium**

| S.No | %Recovery/ Concentration | Placebo Weight in mg | Standard Weight in mg | Syn.mix Weight in mg | Standard Area | Syn.mix. Area | %Recovery |
|---|---|---|---|---|---|---|---|
| 1 | Standard | ---- | 180.2 | ---- | 3458956.5 | ---- | ---- |
| 2 | 80 | 132.5 | ---- | 144.5 | ---- | 2818745 | 100.62 |
| 3 | 80 | 133.4 | ---- | 144.3 | ---- | 2815804 | 100.66 |
| 4 | 80 | 132.8 | ---- | 144.8 | ---- | 2825478 | 100.66 |
| 5 | 100 | 133.2 | ---- | 180.1 | ---- | 3523378 | 100.92 |
| 6 | 100 | 133.7 | ---- | 179.9 | ---- | 3521387 | 100.97 |
| 7 | 100 | 133.2 | ---- | 180.2 | ---- | 3520848 | 100.79 |
| 8 | 120 | 132 | ---- | 215.1 | ---- | 4182086 | 100.29 |
| 9 | 120 | 132.7 | ---- | 215.5 | ---- | 4195902 | 100.43 |
| 10 | 120 | 132.4 | ---- | 215.8 | ---- | 4196615 | 100.31 |
| | | | | | | Mean | 100.63 |
| | | | | | | Standard Deviation | 0.2463 |
| | | | | | | RSD in % | 0.2423 |

**Table-4. Summary of Validation parameters**

| S.no | Parameters | Results |
|---|---|---|
| **1** | Limit of detection(LOD) (µg/ml) | 3.60 |
| **2** | Limit of quantitation (LOQ) (µg/ml) | 14.4 |
| **3** | Reproducibility (% RSD) | 0.0612 |
| **4** | Repeatability (% RSD) | 0.3629 |
| **5** | Robustness | |
| | (1) Change in wavelength (Mean % Assay) | 98.41% |
| | (2) Change in Temperature (Mean % Assay) | 98.54% |
| **6** | specificity | No interference of other peak, so the system is specific. |

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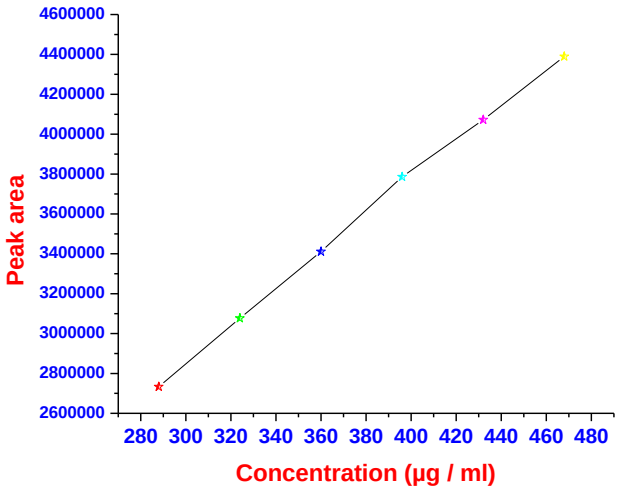


**Fig.2. Linearity of Mycophenolate sodium**

## Linearity

Linearity was evaluated by plotting peak area as a function of analyte concentration for Mycophenolate Sodium. From the linear studies the specified range determined was 288-468 µg / ml. The linear regression coefficient was found to be 0.9993. It obeys the linear equation Y=9239.2 X + 85798.6 (n=6). Results are shown in Table-2 and Fig-2.

## Precision

Reproducibility of the method was studied by injecting Standard Mycophenolate Sodium for five times (n=5). The % RSD was found to be 0.0612.

Repeatability of the method was studied by obtained data from the precision experiments for six multiple injections at six different dilutions (305.8, 309.2, 306.3, 310, 304.8, 305.5). The % RSD was found to be 0.3629.

## Accuracy

Accuracy was evaluated by injecting three times of three different concentrations equivalent to 80, 100 and 120% of the active ingredient, by adding a known amount of Mycophenolate Sodium standard to a sample of known concentration and calculating the recovery of Mycophenolate sodium with % RSD and % recovery for each concentration are presented in Table-3.

## LOD and LOQ

The LOD of Mycophenolate sodium was found to be 3.60 µg/ml and the LOQ was 14.4. Overall summary of validation parameters are presented in Table-4

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## CONCLUSION

This developed RP-HPLC method for estimation of Mycophenolate sodium is accurate, precise, robust, specific, and stability-indicating. The method has been found to be better because of its less retention time, use of an economical and readily available mobile phase, and good resolution of peaks. The run time is relatively short, which will enable rapid quantification of many samples in routine and quality-control analysis of various formulations containing Mycophenolate Sodium. All these factors make this method suitable for quantification of Mycophenolate Sodium in bulk drugs and in pharmaceutical dosage forms without any interference. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating.

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