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Research Article

NEW STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF MYCOPHENOLATE MOFETIL CAPSULE IN PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for separation of Mycophenolate mofetil with impurity-c and validated for the determination Mycophenolate mofetil activity in Mycophenolate mofetil capsule formulation. Separation of Mycophenolate mofetil was successfully achieved on Hypersil BDS C18(250mm x 4.6mm x 5.0 µm) column utilizing phosphate buffer (7.0 pH) and acetonitrile (65:35 v/v), as mobile phase at a flow rate of 2mL/min and the eluate was monitored using PDA Detector at 250 nm. The retention time was found to be 6.520. The developed method was validated as per ICH guidelines for specificity, linearity and range, precision, accuracy, robustness and ruggedness. The results of all the validation parameters were well within their acceptance values. The method gave good recovery in the range of 97.0% to 103.0% for the given Capsule form when it was applied for its determination in pharmaceutical dosage form.

Key words: : Mycophenolate mofetil, Stability indicating RP-HPLC Method

INTRODUCTION

Mycophenolate mofetil (MMF) is a white to off-white crystalline powder which is slightly soluble in water, sparingly soluble in ethanol and freely soluble in acetone and methanol. It is official in E.p. Chemically Mycophenolate mofetil, (Fig 1.) is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate
Mycophenolate mofetil is morpholinoethyl ester of mycophenolic acid.[1,2] It is used as immunosuppressant agent .[3,4] MMF acts by irreversible inhibitor of inosine monophosphate dehydrogenase and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA.[5,6,7]According to the literature survey it was found that few analytical methods on Colorimetry, UV Spectrophotometry, HPLC were reported for Mycophenolate mofetil.[8,9,10,11] The scope of the study is to Development and validation of Mycophenolate mofetil capsules (250mg) using parameters like system precision and system suitability, specificity, precision, accuracy, linearity, robustness.The objective of the proposed method is to develop simple and accurate method for the estimation of Mycophenolate mofetil in Mycophenolate mofetil capsule in pharmaceutical dosage forms by HPLC.

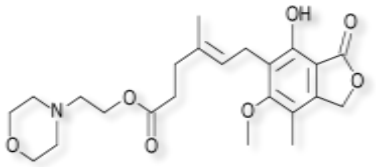

Fig.1: Structure of Mycophenolate mofetil

MATERIALS AND METHODS

Drugs and Chemicals

Mycophenolate mofetil gift sample from Local pharmaceutical industry, water (grade Mill Q), acetonitrile (HPLC Grade), and all other chemicals used were of analytical grade.

Preparation of Mobile Phase

The preparation of the mobile phase and the diluents was done by using mixture of phosphate Buffer (pH7.0)and Acetonitrile in the ratio of 65:35(v/v) respectively.

Preparation of Standard Solution

Standard Stock Preparation

Weigh accurately 40mg of Mycophenolate mofetil working standard was transferred into a 100mL volumetric flask. 30mL of diluents was added, sonicated to dissolve and upto volume with diluents.

Standard Preparation

10ml of standard stock solution was pipette into a 20 mL volumetric flask and the volume was made up to mark with diluents. Filter about 2ml through 0.45µm pall pharma lab nylon 66 membrane filter or 0.45µm Durapore PVDF hydrophilic membrane filter.

Sample Preparation

20Capsules were weighed accurately and the average weight was calculated. Capsules were crushed to fine powder and equivalent to 500mg of Mycophenolate mofetil was weighed and transferred into 100mL volumetric flask. 75mL of diluent was added and sonicated for 30min with intermediate shaking. Volume was made up with diluent. The above solution was centrifuged for 10min at 4000rpm. 2mL of the above solution was pipette into 50mL volumetric flask and made up with diluent.

Optimization of the chromatographic conditions[12, 13, 14]

Around six trials were carried out to separate out the main peak from the impurity –c peak and to obtain the desired retention time .The following desired chromatographic parameters obtained as in table 1.

Table 1: Chromatographic parameters

CHROMATOGRAPHIC PARAMETERS	
Column	Hypersil BDS C18 (150mm x 4.6 mm, 5µm)
Mobile phase	mix buffer and Acetonitrile in the ratio of (650:350) V/V Respectively
Diluent	Mobile phase
.Flow Rate	2.00 mL/min
Column Temperature	50^0C
Sample temperature	20^0C
Injection Volume	10µL
Wave Length	250nm
Run time	15 min
USP Plate Count	10348.12
Resolution	1.658551

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RESULTS AND DISCUSSION

Several trials has made until getting good peak resolution of Mycophenolate mofetil with impurity-c, acceptable plate count and tailing factor. Method was optimized and the retention time was reported as 6.520 min for Mycophenolate mofetil.

Linearity:

A graph was plotted to standard peak area obtained versus "Actual concentration of standard" in linearity of the detector response section. The linearity of this method evaluated by linear regression analysis..From the Linearity data it was observed that the method was showing linearity in the concentration range of 15-90 µg / ml for Mycophenolate mofetil and Correlation coefficient was found to be 0.999. The slope and intercept was for Mycophenolate mofetil as shown in fig2 and table-1.

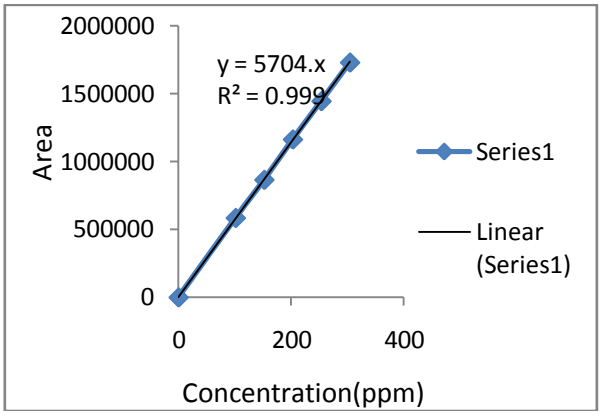

Fig 2: Calibration curve for the Mycophenolate mofetil of the proposed assay method

Table 2: Analytical validation parameters: Linearity and system suitability

Linearity range (ppm)	15-90ug/ml
r^2 ± S.D.	0.9999
Slope	5704
Intercept	10098
No of thretical plates	8535
Tailing factor	0.8
%Relative standard deviation	0.4

Accuracy

A study of accuracy was conducted .drug assay was performed in six times for lower and higher levels and triplicate for remaining levels by adding mycophenolate mofetil drug substance with the equivalent amount of placebo at 50%.75%,100% ,125% ,and 150% of the targeted assay concentration into each volumetric flask .The average % recovery was found to be wit in the limits . The recoveries of pure drug from the analyzed solution of formulation were 98.0 % to 101.4 % for Mycophenolate mofetil, which shows that the method was accurate. The summary of Accuracy results were expressed in table 3.

Table 3: Accuracy studies of Mycophenolate mofetil

Sample No.	Spike level atabout (in %)	Sample area	Amount of mycophenolate mofetil added(ppm)	Amount of mycophenolate mofetil found(ppm)	%Recovery	Mean recovery%
1	50	569399	100.29	100.79	100.50	99.86
2	75	853027	150.33	152.99	101.44	
3	100	1129844	200.06	199.99	99.97	
4	125	1624527	290.20	288.53	99.42	
5	150	1663567	300.46	294.46	98.00	

System Suitability

From the system suitability and system precision studies it was observed that % RSD for Mycophenolate mofetil was found to be 0.4%, peak area of Mycophenolate mofetil was found to be 1168291. As per USP Theoretical plates were found to be 8535 for Mycophenolate mofetil. Tailing factor was found to be 0.8 for Mycophenolate mofetil. All the parameters were within the limit. Peak obtained for Mycophenolate mofetil was sharp and have clear base line.

Specificity

Specificity is confirmed by there is no interference of Mycophenolate mofetil Retention time with other known impurities Retention time.

Precision

The method precision studies were performed for six sample preparations of marketed formulations. A study was carried out for the intermediate precision with same analyst on different day for five sample preparations of marketed formulations.Robustness of method was determined by small deliberate changes in flow rate, mobile phase $P^{H,}$ column temperature and mobile phase ratio. The contents of the drug was not affected by these changes as evident from the low value of Relative standard deviation indicates that the method was rugged and robust.The %RSD of for six Sample Chromatograms of repeatability precision was found to be 0.69 for Mycophenolate mofetil and for Intermediate precision found to be 0.80 for day-1 and 1.4 for day-2.It passes repeatability and Intermediate precision. The results of precision were summarized in table 4.

Table 4: Intraday and interday precision of Mycophenolate mofetil

Drug	Interday precision(%RSD)	Intraday precision(%RSD)
Mycophenolate mofetil	0.69	0.80

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analysed over a period of 24hr at room temperature. The results show that for both solutions, the retention time and peak area of mycophenolate mofetil %RSDnot more than 2 and no significant degradation indicated that both solutions stable for at least 24hr which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed.

Control sample

Weigh 20 capsules and transfer weight equivalent to 500mg of Mycophenolate mofetil transfer to 100ml volumetric flask and add 70ml of diluents and sonicate for 30min , further add 5ml water then make up the solution upto 100 ml with diluent.From the stock solution take 2ml and make up to 50ml with diluent.

Acid degradation sample:

Weigh 20 capsules and transfer weight equivalent to 500mg of Mycophenolate mofetil transfer to 100ml volumetric flask and add 75ml of

diluents and sonicate for 30min further add 5ml of 1N HCL heat for 1hr at 65^0C then cool to room temperature and neutralized the above solution with 5ml 1NNaOH and make up to 100ml with diluent. Take 2ml of above solution and make upto 50 ml with diluents. The typical chromatogram of acid degradation was given by fig 3.

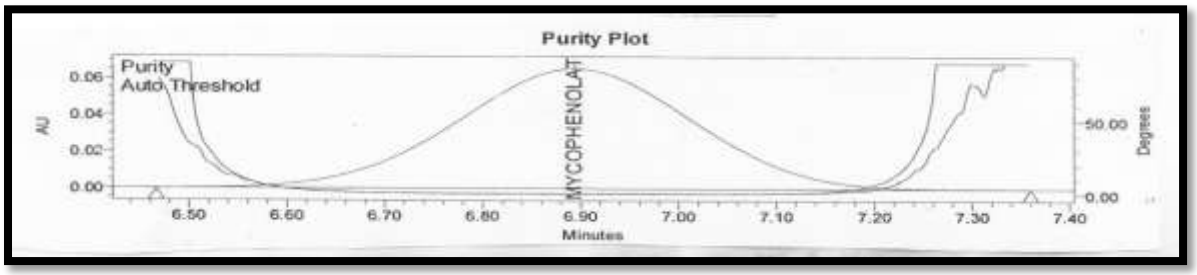

Fig 3: Acid degradation chromatogram of Mycophenolate mofetil

Base degradation sample:

Weigh 20 capsules and transfer weight equivalent to 500mg of Mycophenolate mofetil transfer to 100ml volumetric flask and add 50ml of diluents and sonicate for 30min further add 5ml of 1N NaOH heat for 1hr at 65^0C then cool to room temperature and neutralized the above solution with 5ml1N HCL and make up to 100ml with diluent. Take 2ml of above solution and make upto 50 ml with diluents. The typical chromatogram of acid degradation was given by fig 4.

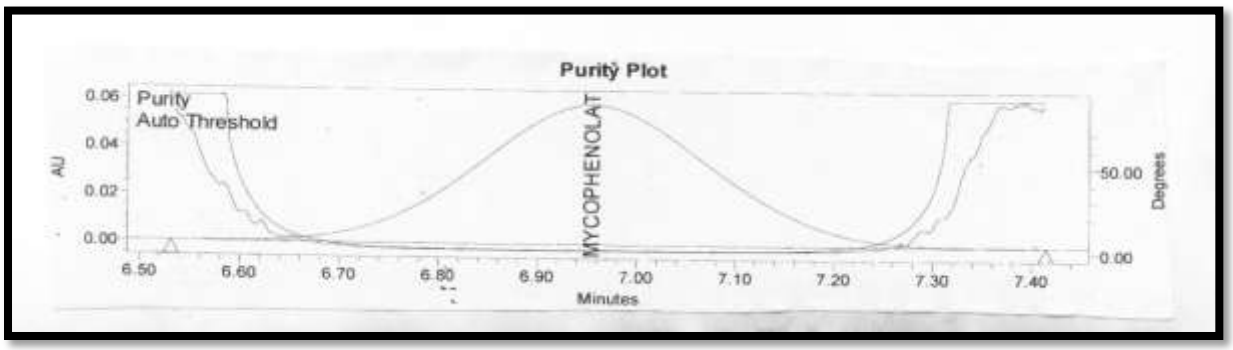

Fig 4: Base degradation chromatogram of Mycophenolate mofetil

Peroxide degradation sample:

Weigh 20 capsules and transfer weight equivalent to 500mg of Mycophenolate mofetil transfer to 100ml volumetric flask and add 75ml of diluents and sonicate for 30min further add 5ml of 3%H_2O_2heat for 10min at 65^0C then cool to room temperature and neutralized the above solution with 5ml1N HCL and make up to 100ml with diluent. Take 2ml of above solution and make upto 50 ml with diluents. The typical chromatogram of acid degradation was given by fig 5.

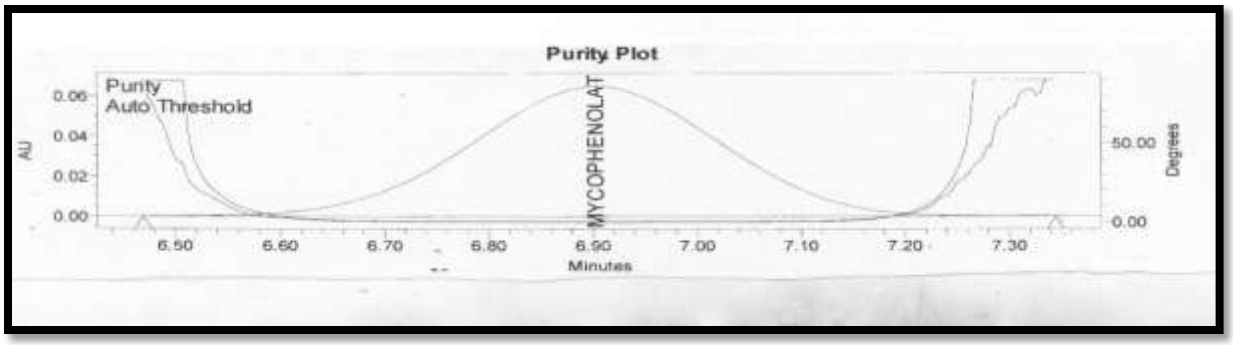

Fig 5: Peroxide degradation chromatogram of Mycophenolate mofetil

Thermal degradation sample:

Weigh 20 capsules and the powder exposed to heat at 105^0 C for 48 hrs transfer weight equivalent to 500mg of Mycophenolate mofetil transfer to 100ml volumetric flask and add 70ml of diluents and sonicate for 30min , further add 5ml water then make up the solution upto 100 ml with diluent. From the stock solution take 2ml and make up to 50ml with diluent. The typical chromatogram of acid degradation was given by fig-6 and Table-5.

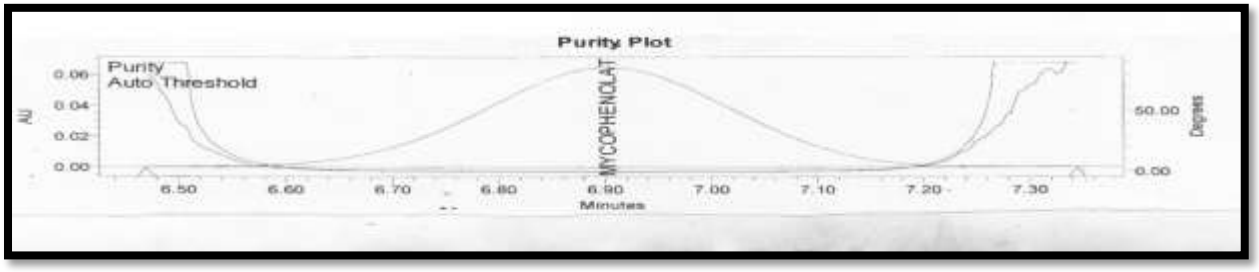

Fig 6: Thermal degradation chromatogram of Mycophenolate mofetil

Table 5: Forced degradation studies of Mycophenolate mofetil

S.NO	Stress condition	% Assay	Degraded sample area	Peak purity Angle	Peak purity Threshold
1	SAMPLE AS SUCH	99.10	1136560	0.183	0.459
2	1N HCL for 2hrs at 60°C	95.72	1099156	0.136	0.377
3	1N NaOH for 2min on BT	53.67	615738	0.223	0.480
4	30% H_2O_2 for 1hr at 60^0C	91.23	1047033	0.229	0.156
5	Thermal SPL	92.47	1058971	0.337	0.596

CONCLUSION

The study presents a simple and validated stability indicating high performance liquid chromatographic method was developed for separation of Mycophenolate mofetil with impurity-c and estimation of Mycophenolate mofetil capsules in pharmaceutical dosage in the presence of degradation products. The method was validated As per ICH Guidelines by using various validation parameters like linearity, accuracy, precision, range, specificity, ruggedness and robustness. All the degradation products formed during the forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability indicating.It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Mycophenolate mofetil Capsule formulation.

Acceptance Criteria:

The net degradation should be in between 1% to 50%. Purity angle should be less than purity Threshold.

An efficient high performance liquid chromatographic method was developed for separation of Mycophenolate mofetil with impurity-c and validated for estimation of Mycophenolate mofetil capsules in pharmaceutical dosage forms using Hypersil BDS C_{18}, 150mm×4.6mm, 5µ column with mobile phase phosphate buffer (pH 7.0) and acetonitrile (65:35).The method was validated As per ICH Guidelines by using various validation parameters like linearity, accuracy, precision, range, specificity, solution stability and robustness. Thus a simple, sensitive, rapid and economic RP-HPLC method was developed. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Mycophenolate mofetil Capsule formulation.

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