

DEVELOPMENT AND VALIDATION OF VISIBLE SPECTROPHOTOMETRIC METHOD OF MYCOPHENOLATE MOFETIL IN BULK AND PHARMACEUTICAL DOSAGE FORM

Rajendra Prasad K and Kathirvel S*

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India.

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ABSTRACT

A simple, sensitive and accurate spectrophotometric method has been developed for the estimation of mycophenolate mofetil in bulk and pharmaceutical formulation. The method is based on the formation of green colored chromogen with 3-methyl-2-benzothiazolinone- hydrazonehydrochloride reagent in the presence of 0.03M ferric chloride and obeys Beer's law in the range of 2–10 µg/mL exhibiting the maximum absorbance at 659 nm. The limit of detection and quantification were found to be 0.87 µg/mL and 2.66 µg/mL respectively. The calibration curve demonstrated a linear relationship between the absorbance and concentration, with the correlation coefficient higher than 0.999. The regression equation of the curve is $Y=0.082x-0.019$. The method is fully validated according to ICH guidelines.

Key words: *Mycophenolate mofetil, 0.03M Ferric chloride, 3-methyl-2-benzothiazolinone hydrazonehydrochloride (MBTH) reagent, Validation, ICH guidelines.*

INTRODUCTION

Mycophenolate mofetil (MMF) is chemically 2-(Morpholin-4-yl)ethyl(4E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate¹. Mycophenolate mofetil is an immunosuppressant and prodrug of mycophenolic acid, extensively used to prevent rejection in organ transplantation. It acts as a non-competitive, selective and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH)^{2,3} in purine biosynthesis, to be specific guanine synthesis, which is necessary for the growth of T cells and B cells.

Analysis plays an important role in the formulation development of any drug molecule. A suitable and validated method has to be available for the analysis of drugs in bulk, in drug delivery systems, in dissolution studies (in vitro), and in biological samples (in vivo). Few HPLC⁴⁻⁷ and LC-MS⁸⁻¹¹ methods for its determination have been reported. A simultaneous determination of mycophenolic acid and valproic acid in human plasma by HPLC¹² reported. After an extensive literature survey there is no reported visible spectrophotometric method for the determination of MMF using MBTH reagent and 0.1N HCl as solvent. Thus the present study was undertaken to develop a colorimetric method of mycophenolate mofetil by using MBTH reagent and validate the method as per ICH guidelines¹³.

EXPERIMENTAL

Preparation of the standard stock solution

A standard drug solution of mycophenolate mofetil was

prepared by dissolving 100 mg of drug in 10 mL of 0.1 N HCl and this was transferred into a 100 mL volumetric flask to obtain a stock solution of 1000 µg/mL. From the above stock solution, 10 mL of the sample was transferred into a 100 mL volumetric flask and the volume was made up to the mark with 0.1N HCl to get a concentration of 100 µg/mL. From the above solution pipetted out 1 mL, added 2 mL of 0.03M ferric chloride and 2 mL of MBTH reagent and heated for 15 min in water bath, then cooled to obtain green color finally made up to 10 mL with 0.1 N HCl and simultaneously blank was prepared without using the sample and scanned by a UV-VIS spectrophotometer in the range of 400-800 nm using the blank as a reference solution. The wavelength corresponding to the maximum absorbance (λ_{max}) was found to be 659 nm. This was further utilized to obtain a calibration curve.

Preparation of sample solution

The proposed method was applied to analyze the commercially available mycophenolate mofetil tablets (Brand name: MYCOFIT, 250 mg Intas pharmaceuticals Ltd.). Twenty tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of mycophenolate mofetil was weighed accurately and transferred to a 100 mL volumetric flask containing 10 mL of 0.1N HCl, the volume was brought up to 100 mL by using 0.1N HCl.

ASSAY PROCEDURE

Aliquots of 0.2 to 1 mL stock solutions were transferred to a series of 10 mL volumetric flasks, and 2 mL of 0.03M ferric chloride was added, shaken vigorously for

*Correspondence : kathirvel2007@gmail.com

few minutes and 2 mL of MBTH reagent was added, heated for 15 min in water bath, then cooled. Green color was formed with subsequent volume adjustment by 0.1N HCl up to 10 mL. Simultaneously blank was prepared without using the sample. The absorbance of the colored solution was measured at 659 nm. The calibration curve was plotted (Fig. 1) and the optical characteristics summarized (Table 1)

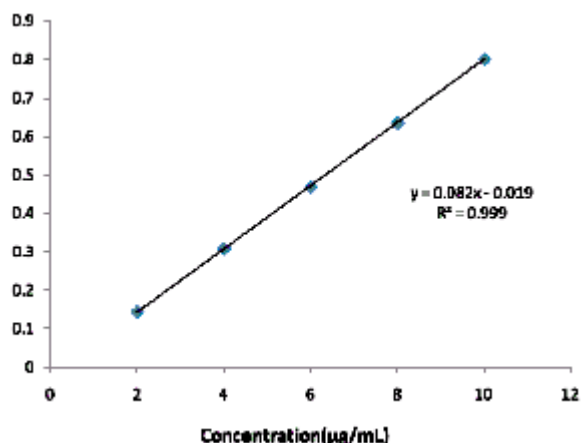


Fig. 1: Calibration curve of Mycophenolate mofetil with MBTH reagent

Table 1: Optical Characteristics of Mycophenolate Mofetil

Parameter	Result
Absorption maxima λ_{max} (nm)	659nm
Beer's law limits ($\mu\text{g/mL}$)	2-10 $\mu\text{g/mL}$
Standard regression equation $Y=bC+a$	$Y=0.082x-0.019$
Correlation coefficient	0.999
Sandell's sensitivity (mg/cm ² /0.001 absorbance unit)	0.012800
Molar absorptivity (lit.mol ⁻¹ cm ⁻¹)	0.338804×10^4
LOD	0.87 $\mu\text{g/mL}$
LOQ	2.86 $\mu\text{g/mL}$

SAMPLE ANALYSIS

Appropriate aliquots of the sample solution were prepared, the absorbance was measured and the amount of mycophenolate mofetil was determined from the calibration curve.

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree assurance that a specific activity will consistently produce a desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of detection (LOD), and Limit of quantification (LOQ).

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations, that is, 80, 100, 120% in which the amount of marketed formulation was kept constant (25 mg) and the amount of pure drug was varied, that is 20 mg, 25 mg, 30 mg for 80, 100, 120%, respectively. The solutions were prepared in triplicate and the accuracy was indicated by % Recovery.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, the solutions of same concentration (6 $\mu\text{g/mL}$) were prepared and analyzed thrice, for three consecutive days, and the absorbances were recorded. In the intra-day variation study, six different solutions of the same concentration (6 $\mu\text{g/mL}$) were prepared and analyzed thrice a day (morning, afternoon, and evening). The result indicated % RSD.

Ruggedness

The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbances were noted.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

Limit of quantification

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

RESULTS AND DISCUSSIONS

Mycophenolate mofetil reacts with MBTH in the presence of FeCl_3 to form a green colored complex and the absorbance was measured at 659 nm. It is an iron catalyzed oxidative coupling reaction of MBTH with mycophenolate mofetil. Under the reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the green colored product¹⁴. The mechanism of reaction is shown in Figure 2. The proposed method obeyed Beer's law in the concentration range of 2-10 $\mu\text{g/mL}$ with MBTH reagent respectively. The optical characteristics and the data concerning the proposed method are presented in Table 1. Linearity data for the proposed method is incorporated in Table 2. The recovery studies were carried out for the developed method by the addition of standard drug solution of mycophenolate

mofetil to pre-analyzed solution. The recovery studies were satisfactory and the percentage of drug recovered is shown in Table 2, which shows that there was no interference from excipients. The results of analysis of marketed formulation are also shown in Table 2. The precision of the method expressed as % RSD of intraday and interday validation is found to be 0.57 and 0.61%, respectively. The sensitivity was estimated in terms of limit of quantification (LOQ), the smallest amounts detected were estimated in terms of limit of detection (LOD). The results are shown in Table 3. Ruggedness data for the developed method is incorporated in Table 3. The method was validated and found to be simple, sensitive, accurate, and precise. The study has acceptable correlation coefficient and % RSD. It was successfully applied for determination of drugs in newly formulated pharmaceutical formulations.

and 0.1N HCl as the solvent. All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and can be applied successfully for the estimation of mycophenolate mofetil in bulk and pharmaceutical formulations.

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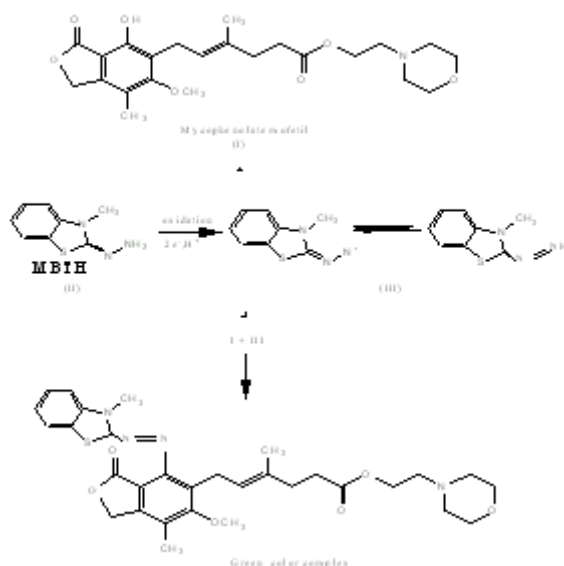


Fig. 2: Reaction scheme of Mycophenolate mofetil with MBTH reagent

Table 2: Result of Analysis of Marketed Formulation

FORMULATION	Concentration Level (µg)	Level of Addition(%)	Amount found (µg) (Average)	%Recovery
Mycophenolate mofetil	4+10	80	14.897	105.4
	5+10	100	14.824	98.8
	6+10	120	15.638	97.7

Table 3: Ruggedness study of Mycophenolate mofetil

Concentration (µg/mL)	Analyte 1		Analyte 2	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis
5	0.4934	%RSD=0.4951	0.4703	%RSD=0.4977
5	0.4955	S.D=0.0043	0.4991	S.D=0.0028
5	0.4493	%RSD=0.91	0.4992	%RSD=0.385
5	0.4994	Standard error=0.001753	0.4994	Standard error=0.001621
5	0.4983		0.4703	
5	0.4718		0.4993	

CONCLUSION

The manuscript presents Visible- Spectrophotometric method for the determination of mycophenolate mofetil in bulk and its dosage form by using MBTH reagent