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RESEARCH ARTICLE

Estimation of Mycophenolate Mofetil in Bulk and Tablet Dosage Form by UV-Spectroscopy

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ABSTRACT

The simple, precise and cost-effective spectrophotometric method has been developed for the determination of Mycophenolate in bulk and its pharmaceutical formulations. Mycophenolate shows λmax at 306.0nm in zero-derivative spectrum (method A), 259.0 nm in first-derivative spectrum (method B). The drug follows the Beer-Lambert's law in the concentration range of 2.0–12.0μg/ml for both the method. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Mycophenolate in bulk and pharmaceutical dosage forms.

KEYWORDS

Mycophenolate mofetil, Spectrophotometric Method, Validation

INTRODUCTION

Mycophenolate (MPH) chemically, 2-(morpholin-4-yl) ethyl (4E)-6-(4-hydroxy-6-methoxy7-methyl-3-oxo-1, 3-dihydro- 2-benzofuran-5-yl)-4-methylhex-4 enoate (Figure 1). Mycophenolate mofetil is the 2-morpholino ethyl ester of mycophenolic acid (MPA), an immunosuppressive agent, inosine monophosphate dehydrogenase (IMPDH) inhibitor. 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. Since T- and B-lymphocytes are critically dependent for their proliferation on denovo synthesis of purines, where as other cell types can utilize salvage pathways, it has potent cytostatic effects on lymphocytes. It inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Addition of guanosine or deoxyguanosine reverses the cytostatic effects of mycophenolic acid on lymphocytes. Mycophenolic acid also suppresses antibody formation by B-lymphocytes and prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection.

The different analytical methods that are reported for its determination include HPLC[8], LC/MS[9], HPTLC[10], Spectrophotometric[11], and gas chromatography[12]. The aim of the present work is to develop and validate for the estimation of Mycophenolate in bulk and pharmaceutical formulations by derivative spectrophotometry[13] and to validate as per the ICH guidelines[6]. Very

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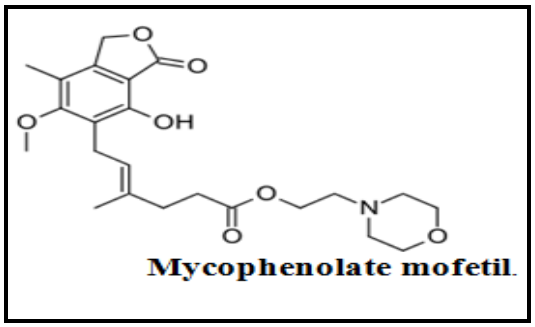
few methods are available for determination of Mycophenolate using spectrophotometry, which is very sensitive and cost- effective.



MATERIAL AND METHODS

Instrument

Shimadzu UV-1700UV/VIS Spectrophotometer was used with1cm matches quartz cell.

Materials

Standard gift sample of Mycophenolate mofetil I.P was procured from Cure Medicine PVT. LTD, Bhosari, Pune.

Derivatization UV Spectrophotometric Method

Apparatus

Shimadzu model UV-1700 UV-Visible double beam spectrophotometer was used. Shimazdu Ax200 balance was used. Calibrated glasswares were used for the study. Modern Industrial Corporation ultrasonicator.

Solvent Selection

Water and methanol was selected as the solvent for dissolving Mycophenolate Mofetil.

Preparations of Drug Stock Solution

Accurately 0.10 mg of Mycophenolate Mofetil was weighed and transferred to 100ml volumetric flask and dissolved in 50 ml of methanol. Then the volume was made up with distilled water up to the 100 ml.

Preparation of Standard Drug Dilutions

Aliquots of standard stock solution of Mycophenolate Mofetil was diluted in a series of 100ml flasks with water to get concentration in range of 2-12 µg/ml.

Method A

Zero Order Derivative Method

Selection of Analytical Wavelengths

The standard solutions were scanned separately between 400nm to 200nm. The spectrum of drug was recorded, Figure 1; from the spectrum, wavelength 306nm was selected for estimation of drug Mycophenolate Mofetil for zero order.

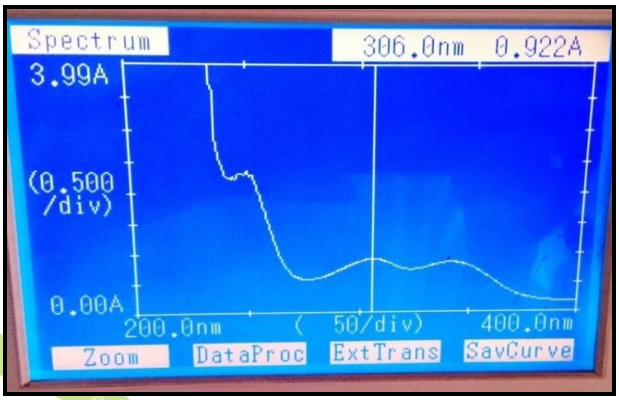


Figure 1: Zero order spectrums

Selection of Analytical Concentration Range

Aliquots of standard stock solution of Mycophenolate Mofetil was diluted in a series of 100ml flasks with water to get concentration in range of 2-12 µg/ml The solutions were scanned in the zero order derivative mode with (n = 1) and the difference in the absorbance were measured at 306 nm against reagent blank. A linear graph of absorbance Vs concentration was obtained. The standard calibration table and curve for Mycophenolate Mofetil in Zero order derivative spectrum with (n = 1) is given in table 1 and Figure 2.

Table 1: Standard calibration table for Mycophenolate Mofetil

Sr. No	Concentration (gm/ml)	Absorbance
1	2	0.038
2	4	0.059
3	6	0.096
4	8	0.120
5	10	0.159
6	12	0.174

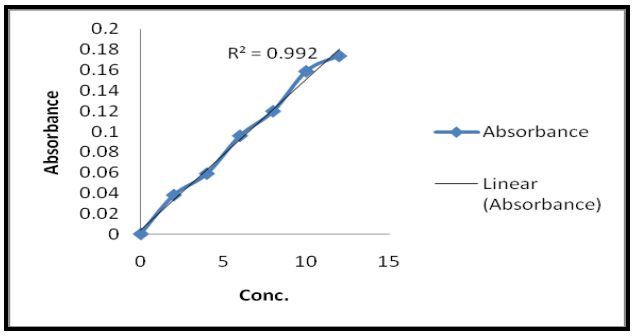


Figure 2: Calibration Curve of Mycophenolate Mofetil in zero order derivative spectrum

Estimation of Mycophenolate Mofetil in Pharmaceutical Dosage Form

Various dilution (in range of 2-12 µg/ml) of Mycophenolate Mofetil in the tablet formulation were prepared in the distilled water and analyzed at 306 nm using quantitation mode in zero order derivative spectrum with (n=1) against reagent blank.

The procedure was repeated six times and the results of the analysis of the tablet formulation.

Table 2: Assay of the Tablet

Sr. No.	Tablet	Conc.	Amount present (mg/Tab)	Amount found (mg /Tab)	% of Drug Found
1	**T_1**	2	2	1.888	98.88
2		**8**	**8**	**7.982**	**99.82**
3		10	10	10.04	100.4

Table 3: Recovery Studies

Tablet Sample	Level of Recovery (%)	Amount Present (mg/tab)	Amount Standard (mg)	Total Amount Recovered (mg)	% Recovery
T_1	80	10	8	79.90	99.87%
		10	8	79.69	99.62%
		10	8	80.01	100.1%
	100	10	10	97.01	97.01%
		10	10	98.06	98.065%
		10	10	98.21	98.21%
	120	10	12	120.11	100.09%
		10	12	119.46	99.55%
		10	12	118.99	99.15%

Method B

First Order Derivative Method

Selection of Analytical Wavelengths

The standard solutions were scanned separately between 400nm to 200nm. The spectrum of drug was recorded, Figure 3; from the spectrum, wavelength 259nm was selected for estimation of drug Mycophenolate Mofetil for first order.

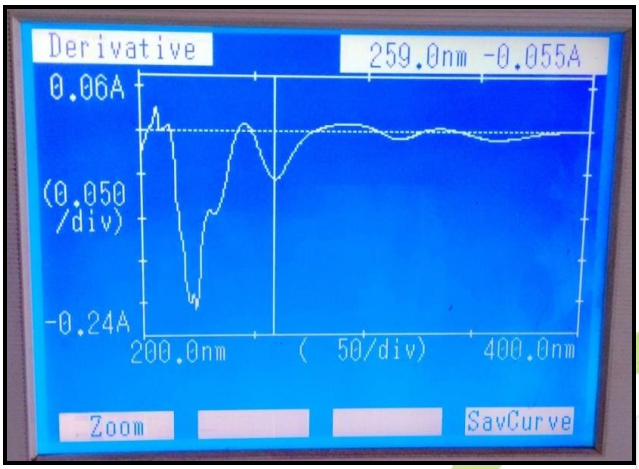


Figure 3: First order spectrum

Selection of Analytical Concentration Range

Aliquots of standard stock solution of Mycophenolate Mofetil was diluted in a series of 100ml flasks with water to get concentration in range of 2-12 µg/ml The solutions were scanned in the zero order derivative mode with (n = 1) and the difference in the absorbance were measured at 259 nm against reagent blank. A linear graph of absorbance Vs concentration was obtained. The standard calibration table and curve for Mycophenolate Mofetil in First order derivative spectrum with (n = 1) is given in Table 4 and Figure 4.

Table 4: Standard calibration table for Mycophenolate Mofetil

Sr. No.	Conc. (µg/ml)	Absorbance
1	2	-0.034
2	4	-0.055
3	6	-0.092
4	8	-0.116
5	10	-0.155
6	12	-0.170

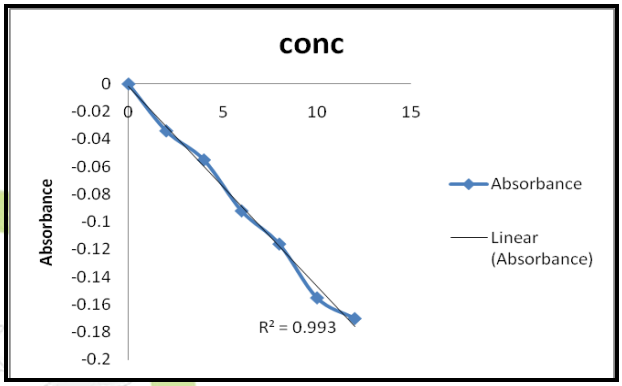


Figure 4: Calibration Curve of Mycophenolate Mofetil in First order derivative spectrum

Estimation of Mycophenolate Mofetil in Pharmaceutical Dosage Form

Various dilution (in range of 2-12 µg/ml) of Mycophenolate Mofetil in the tablet formulation were prepared in the distilled water and analyzed at 259 nm using quantitation mode in zero order derivative spectrum with (n=1) against reagent blank. The procedure was repeated six times and the results of the analysis of the tablet formulation.

Table 5: Assay of the Tablet

Sr. No.	Tablet	Conc.	Amount present (mg/Tab)	Amount found (mg /Tab)	% of Drug Found
1	**T_1**	2	2	1.928	96.40
2		**8**	**8**	**7.994**	**99.92**
3		10	10	10.03	100.3

Table 6: Recovery Studies

Tablet Sample	Level of Recovery (%)	Amount Present (mg/tab)	Amount Standard (mg)	Total Amount Recovered (mg)	% Recovery
T_1	80	10	8	80.90	101.2%
		10	8	81.69	102.1%
		10	8	82.01	102.51%
	100	10	10	98.01	98.01%
		10	10	99.06	99.06%
		10	10	98.08	98.08%
	120	10	12	118.25	98.54%
		10	12	117.30	97.75%
		10	12	116.46	97.05%

RESULTS AND DISCUSSION

Table 7: Optical characteristics and other parameters

Parameters	Method A	Method B
λ Max (nm)/wavelength range (nm)	306	259
Beer's – Lambert's range (µg/ml)	2-12	2-12
Coefficient of correlation (r^2)	0.992	0.993
Regression equation : Y = mx + c	0.013x+0.0012	-0.002x +0.000
a – Slope (m)	0.013	-0.002
b – Intercept (c)	0.0012	0.000
LOD	0.048	2.694
LOQ	0.161	6.448

Mycophenolate Mofetilis an immunosuppressant drug used to prevent rejection in organ transplantation. It inhibits an enzyme needed for the growth of T cells and B cells. Literature scan revealed no Derivatization UV spectrophotometric method using methanol and water was developed for the determination of Mycophenolate Mofetil Fig1shows Zero order spectrum of Mycophenolate Mofetil and Fig. 3 shows First order spectrum of Mycophenolate Mofetil The linearity of method was statistically confirmed. The correlation coefficients (r^2) for calibration curves were not less than 0.99. The relative standard deviation values of the slope were not more than 2%. The analytical recovery at three different concentrations of Mycophenolate Mofetil was determined. In Derivatization method 306 nm which was λmax of Mycophenolate Mofetil for zero order and 259nm for first order were selected for analysis. Derivatization UV spectrophotometric method was validated. Therefore proposed validated method was successfully applied to determine Mycophenolate Mofetilin tablet dosage form.

CONCLUSION

Derivatization method was developed and validated as per ICH guidelines for estimation Mycophenolate Mofetil. This method was applied for estimation of the compounds in the marketed formulation. The method has been evaluated for the linearity, accuracy, precision and Robustness in order to ascertain the suitability of the method. It has been proved that the developed method was linear in the concentration range of 2.0-12.0µg/ml. High percentage recovery showed that method was free from interference of excipients used in the formulations. The results of the study indicates that the proposed absorbance ratio UV spectrophotometric method of analysis can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Mycophenolate Mofetil.

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