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Colorimetric estimation of mycophenolatemefotel

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ABSTRACT

A simple, reliable, accurate, and sensitive method for the determination of Mycophenolate Mofetil (MMF) was developed and validated in tablets and capsules. The maximum absorbance of Mycophenolate Mofetil was found to be 700nm.The linearity was observed in the range of 1µg/ml to 45 µg/ml in hydrochloric acid. Mycophenolate mofetil in 0.1N hydrochloric acid was estimated at 700 nm with the help of 0 .008M potassium ferri cyanide and 0.1M ferric chloride in 0.1M hydrochloric acid. The method was found to have high degree of accuracy and precision in both inter and intra day.

Keywords - Mycophenolate Mofetil, Colorimetric method, Validation.

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INTRODUCTION

Mycophenolate Mofetil (MMF) is 2-(Morpholin-4-yl) ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate [1]. Mycophenolate Mofetil is a semi synthetic derivative of a fungal antibiotic. In body it is converted to mycophenolic acid, which restains proliferation of both T and B lymphocytes and reduces the production of cytotoxic T cells by inhibiting inosine monophosphate dehydrogenase, an enzyme crucial for denova purine biosynthesis in both T and B cells. So drug has fairly selective action. It is mainly used to curtail transplant rejection [2]. Mycophenalte Mofetil is indicated for proophylaxis of transplant rejection and typically in combination with glucocorticoids and a calcineurin inhibitor [3].

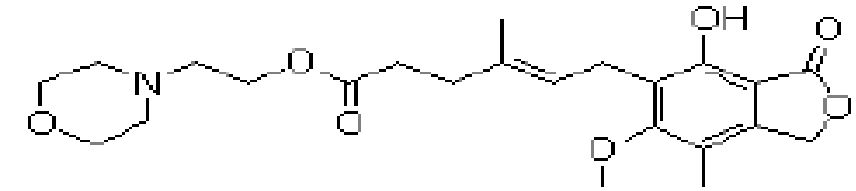

Figure 1: Chemical structure of Mycophenolate Mofetil.

The literature survey reveals that, Mycophenolate Mofetil reported in British Pharmacopoeia. But till now there is no colorimetry method and is only one publication describing determination of Mycophenolate Mofetil by spectrophotometry [4]. There are three methods published for the assay of Mycophenolate Mofetil by HPLC [5-7] and several methods for the assay of Mycophenolic Acid (MPA) in plasma by HPLC and LCMS [8-20]. But HPLC techniques are tedious, time consuming, high priced, requires skilled expert and are not suitable for the routine analysis. So the aim of this work was to develop a simple, easy, economical, and preferred for routine analysis.

MATERIALS AND METHODS

Experimental Procedures

Material and Reagents

MMF was obtained as gift sample from Strides Arco lab Limited, Bangalore, India. Marketed formulations of MMF tablets and capsules were procured from the market which contains 500 mg of MMF per tablet and capsule respectively. The reagents and chemicals used in this procedure are Hydrochloric acid, Potassium ferri cyanide, and Ferric Chloride. All chemicals and reagents used were of analytical grade.

Instruments

Double-beam UV-visible spectrophotometer (Systronics) connected to a computer equipped with software is used. The instrument has an automatically checked wavelength

accuracy of 0.1 nm and is equipped with matched quartz cells of 10 mm (1.0 cm) cell path length.

Analytical method development

1ml of 0.005M Potassium ferri cyanide and 1ml of 0.05M Ferric Chloride were added to the MMF standard solution and MMF in formulations. The absorbance of MMF in the standard and formulation after development of bluish green colour was measured at respective wavelength and determined the quantity of the MMF presence in the formulation.

Procedure for calibration curve

First stock solution of 1000 µg/ml of MMF was prepared by dissolving 100 mg in 100ml of 0.1N hydrochloric acid (HCl) and second stock of 100 µg/ml MMF is prepared by pipetting out 10ml of first stock into a 100ml volumetric flask and diluting it upto the volume with distilled water. To prepare samples of different concentrations, aliquots of stock solutions were transferred into a series of 10 ml standard volumetric flasks and the volumes made up with distilled water. Five different concentrations were prepared in the range of 5–25 µg/ml of MMF in distilled water and the quantity of MMF was estimated at 700 nm.

Sample preparation

MMF tablets were powdered and extracted with 0.1N HCl and sonicated to dissolve the particles. The solutions were then filtered by using whatmann filter paper and suitably diluted with distilled water to get final concentrations.

Analytical method validation

Specificity and selectivity

Standard MMF solutions (1,5,10,15,20,25,30,35,40,45,µg/ml) are prepared along with tablets and capsules separately. Standard solutions were scanned from 400nm to 900 nm at a speed of 400 nm/min and analyzed for any change and shift in absorbance at the respective wavelengths. In a separate study, the drug concentration of 20µg/ml was prepared independently from the pure drug stock solution, tablets and capsules for the analysis of the content. The standard deviations were determined.

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RESULTS AND DISCUSSION

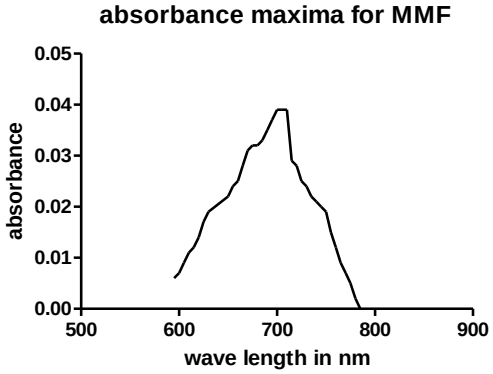

Figure 2:

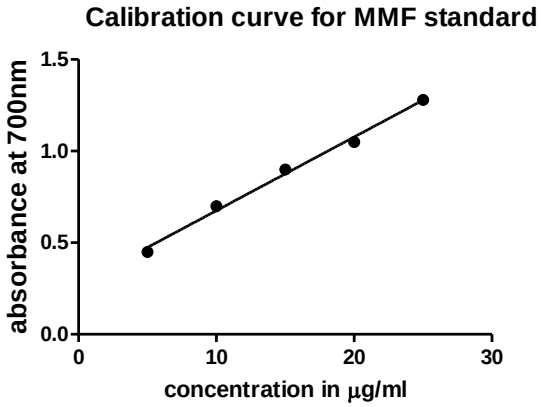

Figure 3: Calibration curve of MycophenolateMofetil

Table 1: Absorbance values of standard MMF at different concentrations

Concentration in µgm/ml	Absorbance
05.0	0.450
10.0	0.700
15.0	0.900
20.0	1.050
25.0	1.280

Figure 3 shows the calibration curve of MMF. It can be seen that MMF obeys Beer's law in the concentration range of 5µg/ml to 25 µg/ml and there is a linear relationship between absorbance and the concentration with a high correlation coefficient value of 0.999. The solutions prepared for study of the linearity were stored for 24 hours at room temperature and the readings were again taken. There was no significant change in the readings obtained after 24 hours indicating the stability of the solutions over the period of 24 hours.

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Table: 2 Linear Regression Data for the Linearity Curve

Parameter	Values
λ max	700nm
Beers law limit	1 - 45µgm/ml
Sandell's sensitivity (µg/cm^{2-}/0.001 A.U)	0.00588235
Molar absorbtivity 1 mol^{-1} cm^{-1}	7.3913 X 10^4
Correlation Coefficent r	0.9969
r squared	0.9938
Best fit slope	"0.0402 ± 0.001829"
95% confidence interval slope	"0.03438 to 0.04602"
Standard deviation of results	0.02893
P value	<0.0002

The P value is 0.0002, considered extremely significant. The 95% confidence limit for the slope is "0.03438 to 0.04602" and Y-intercept when X=0.0 is "0.1765 to 0.3695".

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