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Research Article

Validated Spectrophotometric Methods for the Determination of Mycophenolate: An Anti-Neoplastic Agent in Bulk and Pharmaceutical Dosage Forms

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Three simple, precise and cost-effective spectrophotometric methods have been developed for the determination of Mycophenolate in bulk and its pharmaceutical formulations. Mycophenolate shows λ_{max} at 250.0 nm in zero-derivative spectrum (method A), 258.0 nm in first-derivative spectrum (method B) and method C is based on the calculation of area under curve (AUC) for analysis of Mycophenolate in the wavelength range of 240.0–260.0 nm. The drug follows the Beer-Lambert's law in the concentration range of 1.0–150.0 μg/mL for all the methods. 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 methods were successfully applied to estimate the amount of Mycophenolate in bulk and pharmaceutical dosage forms.

1. 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-methyl hex-4enoate (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 de novo synthesis of purines, whereas 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 [1–14], LC/MS [15], HPTLC [16], spectrophotometry [17], and gas chromatography [18]. The aim of the present work is to develop and validate for the estimation of Mycophenolate in bulk and pharmaceutical formulations by derivative spectrophotometry [19] and to validate as per the ICH guidelines [20]. Very few methods are available for the determination of Mycophenolate using spectrophotometry, which is very sensitive and o cost-effective. The present proposed method was developed using 0.02 M Octane 1-sulfonic acid buffer solution and validated.

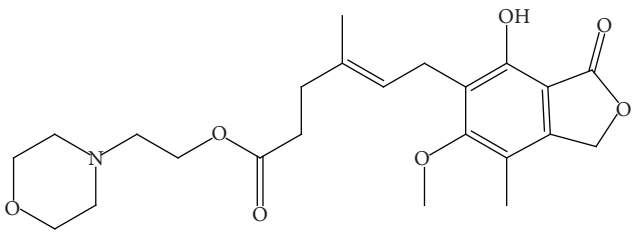

Figure 1: Chemical structure of Mycophenolate.

2. Experimental

2.1. Chemicals and Reagents. Mycophenolate working standard was kindly provided by Alembic Ltd., (Vadodara, India) and was used as received. A commercial formulation was purchased from the local market. Octane 1-sulphonic acid sodium salt of analytical grade solution was prepared in Milli-Q water.

2.2. Instrumentation. A double-beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Denver, Germany). For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

2.3. Preparation of (0.02 M) Octane 1-Sulfonic Acid Buffer Solution. 4.35 grams of octane 1-sulfonic acid was dissolved in 800 mL of distilled water and the pH was adjusted to 3.5 with *o*-phosphoric acid in a 1000 mL volumetric flask.

2.4. Preparation of Standard Stock Solution. The standard solution of MPH was prepared by dissolving accurately weighed 10 mg of the drug in methanol and diluted to 10 mL with methanol to obtain a final concentration of 1000 μg/mL. From this solution, 2.5 mL was taken and diluted with 0.02 M octane 1-sulfonic acid buffer in a 25 mL volumetric flask to prepare a working standard solution (100 μg/mL).

3. Method A

3.1. Zero-Derivative Spectrometry. Series dilutions of standard solutions were prepared in 10 mL volumetric flasks and diluting to volume with 0.02 M Octane 1-sulfonic acid buffer (pH 3.5 ± 0.05) to produce the concentrations ranging from 1.0–150.0 μg/mL. The above solutions were scanned over the range of 400 nm to 200 nm against blank. The λ_{max} was found to be at 250.0 and 305.0 nm (Figure 2). But the present study was carried out at 250.0 nm where the Beer-Lambert's law was following properly and statistical data was shown in Table 1. A calibration curve was constructed by plotting the concentration on the *x*-axis and the corresponding absorbance (at 250 nm) on *y*-axis.

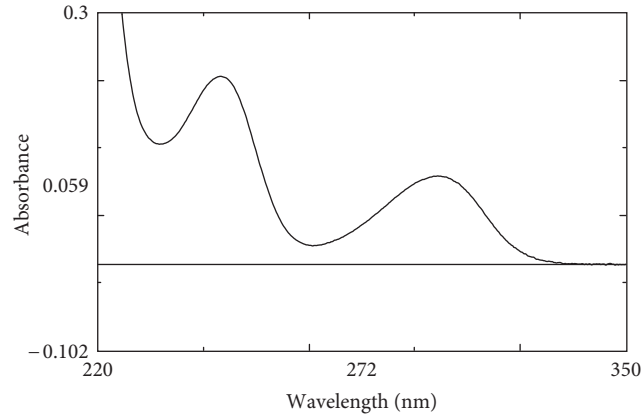

Figure 2: UV absorption spectrum of Mycophenolate (D_0) (10 μg/mL).

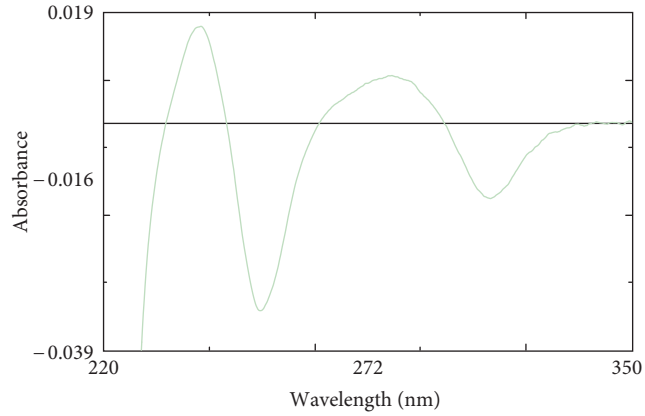

Figure 3: First-derivative absorption spectrum of Mycophenolate (D_1) (20 μg/mL).

4. Method B

4.1. First-Derivative Spectrometry. The zero-order spectrum was derivatised to get first-order derivative spectra (Figure 3) by the inbuilt spectra manager software "UV Probe." The derivative spectrum shows maxima at 258 nm, and the derivative absorbance ($dA/d\lambda$) was noted at 258 nm for all the analytical calculations [21].

A graph was drawn by taking the concentration of the drug solution on the *x*-axis and the corresponding $dA/d\lambda$ values (maxima, at 258.0 nm) on the *y*-axis.

5. Method C

5.1. Area-Under-Curve Method. The AUC (area-under-curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 (240 nm) and λ_2 (260 nm). In method A the λ_{max} was observed at 250 nm, and therefore for method C the AUC was selected between the wavelength 240–260 nm (i.e., $\lambda_{max} \pm 5$ or 10 nm;

Table 1: Optical characteristics and validation parameters of Mycophenolate.

Parameters	Method A	Method B	Method C
Beer-Lambert's range (μg/mL)	1–150	1–150	1–150
λ_{max}/wavelength range (nm)	250	258	240–260
Molar absorptivity (L/mol·cm)	9.71×10^3	7.37×10^2	4.68×10^4
Sandell's sensitivity (μg cm^2/0.001 absorbance unit)	0.05	—	—
Slope	0.022	0.001	0.107
Standard deviation of slope	0.0252	0.0002	0.0036
Intercept	0.001	0.000	0.025
Standard deviation of intercept	0.0003	0.000	0.0025
Correlation coefficient	0.999	0.999	0.999
Precision (RSD, %)			
Intraday ($n = 3$)	0.61–1.25	0.78–0.90	0.92–1.11
Interday ($n = 3$)	0.96–1.09	0.66–1.12	0.87–1.04
Accuracy (% recovery) (% RSD)	99.87–101.80 (0.23)	98.56–100.54 (0.41)	99.75–100.90 (0.38)

Table 2: Assay results of Mycophenolate in pharmaceutical dosage form.

Formulation (tablets)	Labelled amount (mg)	% Recovery		
		Method A	Method B	Method C
Renofix	500	100.05	99.82	100.04
Mycept	500	99.87	100.02	99.89
Cellcept	500	99.96	99.97	99.93

λ_1 and λ_2 = 240–260 nm). A graph can be plotted by taking the concentration of the drug solution on the x-axis and the corresponding AUC value on the y-axis.

6. Method Validation

6.1. Linearity. For all the methods, (1.0–150.0 μg/mL) calibration curves were prepared on three different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression method.

6.2. Assay of Mycophenolate Tablets. Mycophenolate is available in the local market with different brand names in India such as Renofix (500 mg, UNICHEM Laboratories Ltd.), MYCEPT (500 mg, PANACEA Biotech Ltd.), and CELLCEPT (500 mg, ROCHE Pharmaceuticals).

Twenty tablets of Mycophenolate from three different brands were collected from pharmacy store, weighed and finely powdered. Powder equivalent to 10 mg of the drug was transferred to a 100 mL volumetric flask and dissolved in about 40 mL 0.02 M octane-sulphonic acid buffer, ultra sonicated for 30 minutes, filtered through Whatman filter paper (number 41), and suitably diluted as per the requirement. The results obtained with three brands were summarised in Table 2.

6.3. Precision. The intraday and interday precision of the proposed methods was performed on the same day with three different concentration levels ($n = 3$) and on three different days at three different concentration levels ($n = 3$) of MPH (10.0, 20.0, and 50.0 μg/mL), and the results (% RSD) were reported (Table 1).

6.4. Accuracy. This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding known amounts of MPH pure drug solution to a preanalysed formulation solution. An extracted formulation solution containing Mycophenolate (10.0 μg/mL) was spiked with pure drug solution (80, 100, and 120%) in different 10 mL volumetric flasks, and the absorbance was measured (18, 20, and 22 μg/mL). The % recovery as well as the % RSD was calculated (Table 1).

7. Results and Discussion

Beer-Lambert's law was obeyed in the concentration range of 1–150 μg/mL for all the three methods A, B, and C. The regression equations were given in Table 1.

The % recovery from the formulation (Table 2) was found to be 99.87–100.05 for method A, 99.82–100.02 for method B, and 99.89–100.04 for method C. The proposed methods were validated as per the ICH guidelines. The % RSD in precision and accuracy was found to be less than 2.0 indicating that the proposed methods are precise and accurate. The % recovery was found to be within the acceptable range in all the three methods. Therefore, the present methods can be employed for the determination of Mycophenolate in pharmaceutical formulations successfully.

8. Conclusion

The three validated methods can be successfully applied for the determination of Mycophenolate in tablet dosage forms. The three methods are very simple and cost-effective for the routine analysis of pharmaceutical formulations.

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