RESEARCH ARTICLE

An Ultra-sensitive Photo-luminescent Method for the Quantification of Mycophenolate Mofetil: Validation and Appliance to Marketed Formulations

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ABSTRACT

An ultra-sensitive spectrofluorimetric work is developed for Mycophenolate mofetil, an immunosuppressant drug using Shimadzu RFPC-5301 Spectrofluorimeter, Shimadzu, Japan, equipped with 150W Xenon arc lamp, assisted by RFPC software. The excitation and emission wavelengths for Mycophenolate mofetil were found to be 350 nm and 438 nm respectively. A linear relationship was in the concentration range of 0.1–1.0 µg/mL for Mycophenolate mofetil. The assay results obtained for the marketed formulation were 102.5% for MMF. The method was validated for different parameters as per the International Conference for Harmonization Guidelines.

Keywords: Fluorimetry, Mycophenolate mofetil, Photo-luminescent.

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INTRODUCTION

Fluorescence, a photo-luminescent process is observed with a limited number of molecules and hence considered as a selective method. This is due to the molecular requirement for the phenomena of fluorescence. Apart from the presence of chromophore in the molecule, it should have structural rigidity, and the latter will be lacking in many of the compounds; hence, all the molecules are not fluorescent, despite possessing chromophore. Another reason for the selective nature of the fluorescent technique is the requirement of two wavelengths, namely excitation and emission wavelengths, which can hardly be the same for different molecules. Another benefit is its inherent sensitivity, which compounds can be analyzed even in nanogram quantities. The greater selectivity and sensitivity, the desirable features of analytical methods, confer superiority for photo-luminescent methods over absorption techniques.

Mycophenolate Mofetil (MMF), the ester derivative of mycophenolic acid is a nucleic acid synthesis inhibitor, and it belongs to the category of immunosuppressant drugs and is very useful for autoimmune diseases. It is also helpful in treating patients with organ transplants as the drug can reduce the strength of ones own immune system. Chemically it 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]

Recently, it has been the drug of choice for rheumatoid arthritis, lupus, vasculitis, inflammatory eye disease, inflammatory bowel disease etc. Mycophenolate mofetil, an immunosuppressant drug is used along with other drugs to treat AIDS.

An exhaustive literature review has shown that various techniques analyze the drug and the reported works include UV spectroscopy,[2-6] fourier transform infrared spectroscopy (FTIR),[7] high-performance thin-layer chromatography (HPTLC),[8] high performance liquid chromatography (HPLC)[9-15] ultra-performance liquid chromatography (UPLC)[16]

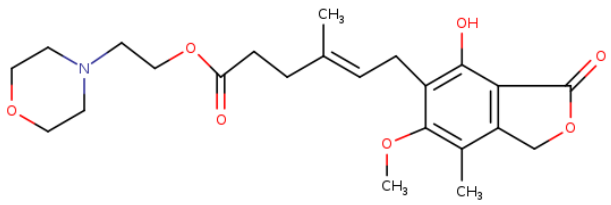

Figure 1: The chemical structure of MMF

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and LC-MS/MS[17]. Since many drugs are coming to the market, it is convenient to have some simple and sensitive methods for quantifying the drug. But up to now, no spectrofluorimetric method is reported for MMF. As spectrofluorimetric methods are a preferred choice because of the inherent sensitivity and the literature shows the deficit of this method, we have developed, optimized and validated a very sensitive photo-luminescent method for mycophenolate mofetil that can be used for the drug estimation of Mycophenolate mofetil, in bulk and its tablet dosage forms.

MATERIALS AND METHODS

Instrumentation

Shimadzu RF-5301 PC Spectrofluorophotometer, Shimadzu, Japan, equipped with 150W Xenon arc lamp, 1-cm non-florescence quartz cell, connected to RFPC software was used for recording the photo-luminescent process.

Materials

Reference Standards and Marketed Formulation

Gratis sample of MMF was obtained from Airis Pharma Pvt Ltd, Hyderabad. Commercially available tablets (CellCept) containing MMF (500 mg) manufactured by Roche Products (India) Pvt. Ltd.

Chemicals

Methanol was purchased from Qualigens, Mumbai and was used without further purification as solvent.

Analytical Method Development and Validation

According to ICH guidelines, we extended the method validation study for accuracy, precision, linearity, limit of detection (LoD), and limit of quantitation (LoQ).[18] One important step in any instrumental method is the proper solvent selection and for spectrofluorimetric works, it has a key role as the solvents can enhance or diminish the fluorescence. Different solvents like methanol, sodium lauryl sulfate and ethanol were attempted and in methanol, the emission intensity was found to be maximum compared to other solvents. Hence, methanol was used as the solvent for the stock solution and dilutions. The excitation wavelength of Mycophenolate mofetil was found to be at 350 nm. Using the excitation wavelength, the emission spectrum of Mycophenolate mofetil was recorded after scanning in a wavelength range of 450–650 nm, and the emission wavelength was found to be at 438 nm.

Linearity and Range

Standard stock solution of Mycophenolate mofetil was prepared with 10 mg of the drug in 10 mL of methanol which on further dilution with the same solvent yields calibration solutions of 0.1, 0.2, 0.4, 0.6, 0.8, and 1-µg/mL. The fluorescence data versus concentration were used to determine the regression equation and correlation coefficient value (R^2) to measure the degree of linearity.

Accuracy

The standard addition process did the accuracy, assessing the degree of closeness between the actual and the value found. Standards were spiked at three levels (80, 100 and 120%) to commercially available tablets in triplicate. The method's accuracy and reproducibility are proved by calculating the amount of drug recovered and the values of % relative standard deviation (RSD) that should be <2.0.

Precision

The precision assessment measures the closeness of test results upon multiple sampling of the homogeneous sample. The method repeatability (intra-day precision) was determined by the triplicate analysis of three standard solutions of MMF at 0.2, 0.6 and 1.0 µg/mL of concentrations. Inter-day (n=3) analysis of three standard solutions of MMF at the concentration of 0.2, 0.6 and 1.0-µg/mL for MMF was used for Intermediate precision. The statistical analysis on precision data proved good precision of the methods as the % RSD was <2.0 for both drugs in interday and intraday precision studies.

LoD and LoQ

We used samples containing low concentrations of the analyte to determine the LoD and LoQ. Formulae 1 and 2 are used for calculating LoD and LoQ.

$$LoD = 3.3\ \sigma/S \qquad \text{Formula 1}$$

$$LoQ = 10\ \sigma/S \qquad \text{Formula 2}$$

S = slope of the calibration curve and σ = standard deviation of the response

Analysis of the Pharmaceutical Dosage Form

The marketed formulation of MMF, CellCept is available in 500 mg tablets. Twenty tablets were powdered and a quantity corresponding to 10 mg of MMF was transferred to a 10 mL volumetric flask, added 10 mL of methanol and dissolved and further diluted with the same solvent with the help of sonication for 15 minutes. The solutions were filtered. An aliquot was diluted to get a sample solution of 0.5 µg/mL of MMF. The substitution of the emission intensity into the straight-line equation yields the concentration of MMF.

RESULTS AND DISCUSSION

Method Optimization

Various solvents like SLS, methanol and ethanol were used to study the fluorescent properties of MMF. A comparison of the fluorescence intensity, as shown in Figure 2, was highest

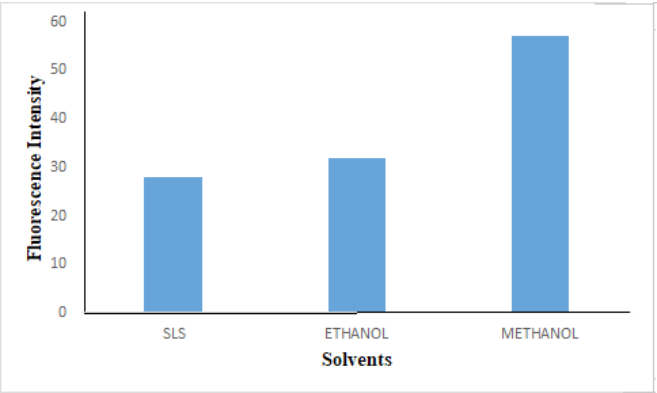

Figure 2: The effect of solvents on the fluorescence intensities of MMF of 1-µg/mL

in methanol and hence selected as the solvent. The Excitation wavelength of Mycophenolate mofetil (MMF) in methanol as solvent was found at 350 nm. Using the excitation wavelength, the emission spectrum of Mycophenolate mofetil was recorded after scanning in a wavelength range of 320–770 nm and the emission wavelength was found to be at 438 nm.

Method Validation

Calibration Graph of Mycophenolate Mofetil

Linearity data show a linear relationship between the concentration of MMF (µg/mL) and the corresponding emission intensities in the 0.1–1.0 µg/mL range. By linear regression analysis, the coefficient of determination, R^2 was 0.999. From Figure 3, it was observed that with the increase in Mycophenolate mofetil concentration, the fluorescence intensity was increased at the emission wavelength of 438 nm. Figure 3 represents the fluorescence spectra which indicate the linearity between the emitted light and the drug concentration. System suitability parameters are given in Table 1.

Recovery Study

The accuracy was determined by the standard addition method. Three different levels 80, 100 and 120% of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and %RSD values calculated are presented in Table 2. The %recovery of Mycophenolate mofetil was in the range of 99.70–100.27% and the %RSD was less than 2.

Precision Study

The method repeatability (intra-day precision) was determined by inter-day (n = 3) analysis of three of the selected standard solutions of MMF at concentrations of 0.2, 0.6 and 1.0 µg/mL. The inter-day precision analysis was also performed at the same concentration levels but on different days, and the precision data is presented in Table 3. The statistical analysis on precision data proved good precision of the method as the % RSD was <2.0 for MMF in inter-day and intra-day precision studies.

LoD and LoQ

LoD and LoQ of Mycophenolate mofetil were found to be 3.3 and 10.02 ng/mL, respectively.

Analysis of Commercial Tablets (Assay)

The utility of the method was done by extending the method to analyze the drug content in marketed formulations. The assay of CellCept tablets containing 500 mg of MMF was used for assay. The assay results obtained for MMF were compared with the labeled amounts and reported in Table 4. The %RSD for

Table 1: System suitability parameters

Parameters	*Values*
Excitation wavelength (nm)	350
Emission wavelength (nm)	438
Slope	56.85
Intercept	1.135
Correlation coefficient	0.999
Regression equation	y = 56.85x + 1.135
LOD (ng/mL)	3.3
LOQ (ng/mL)	10.02

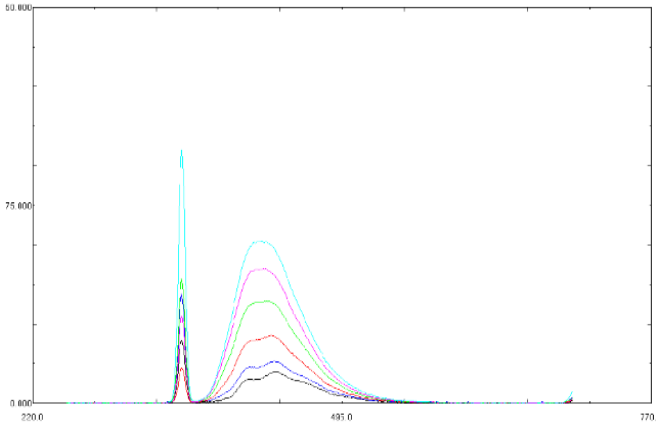

Figure 3: Overlay emission spectra of Mycophenolate mofetil (0.1–1.0 µg/mL)

Table 2: Accuracy data of MMF (recovery studies)

%Spiking level	*Conc. of sample (µg/mL)*	*Conc. of std spiked (µg/mL)*	*Total conc. (µg/mL)*	*Conc. recovered (µg/mL) (AM ± SD) (n=3)*	*Recovery (%)*	*%RSD*
80%	0.4	0.32	0.72	0.718 ± 0.00624	99.70	0.86
100%	0.4	0.4	0.8	0.8103 ± 0.0047	101.2	0.58
120%	0.4	0.48	0.88	0.882 ± 0.00112	100.27	0.12

Table 3: Precision data of MMF

	Intra-day		*Inter-day*	
Conc. (µg/mL)	*Conc. found (µg/mL) AM ± SD (n=3)*	*% RSD*	*Conc. found (µg/mL) AM ± SD (n=3)*	*%RSD*
0.2	0.198 ± 0.0018	0.90	0.210 ± 0.0019	0.904
0.6	0.612 ± 0.0062	1.01	0.573 ± 0.0059	1.02
1.0	0.989 ± 0.0116	1.17	0.993 ± 0.0119	1.19

Table 4: Analysis of commercial tablet formulation (assay of MMF)

Drug name/ Brand name	*Label claim (mg)*	*Amount found AM ± SD (n = 3) (mg)*	*Assay (%)*	*%RSD*
MMF/ CellCept	500	512.5 ± 4.23	102.5	0.83

the assay results of the formulation was <2, which indicated the developed methods' accuracy.

CONCLUSIONS

A very sensitive and simple photo-luminescent method is developed for the routine estimation of MMF in bulk and the tablet dosage form. The %RSD for all parameters was found to be < 2, which indicates the method's validity and the assay results obtained by this method are in good agreement with the label claim.

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