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Original Article

Development of stability indicating liquid chromatography-mass tandem spectrometric method for the estimation of mycophenolate mofetil in bulk and pharmaceutical formulations

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ABSTRACT

Aim: To develop a simple LC/MS/MS method for the determination of mycophenolate mofetil in bulk and pharmaceutical formulations and study of stability of the drug in different stressed conditions.

Methods and materials: The LC/MS/MS analysis was carried out on Applied Biosystems API 3200 triple quadrupole mass spectrometer attached to Shimadzu LC 10 AT VP series HPLC system using chromosil ODS-3, C18, 4.6 × 50 mm, 2.5 μm column. The mass spectral analysis was carried out by direct infusion of 10 μg/mL solution into the ESI source at a flow rate of 10 μL/min along with the mobile phase flow rate of 600 μL/min. All chemicals and reagents were of either analytical grade or chromatographic grade.

Results: The obtained mass spectrum showed *m/z* 434 as a major ion which can be attributed to the MH^+ ion of the analyte. This ion was subjected to collision induced dissociation (CID) using nitrogen as a collision gas. The collision energy was tuned in such a way that the intensity of MH^+ ion was reduced to a minimum of 20%. The obtained mass spectrum after CID showed *m/z* 114 as a major fragment. Hence the transition *m/z* 434 → 114 was used to monitor the analyte peak in LC/MS/MS analysis. The developed method was validated in terms of precision, accuracy, linearity, robustness and ruggedness.

Conclusions: The developed method was found to be simple, rapid, repeatable, reproducible, robust, rugged and economic hence it can be used as a new analytical method for the analysis of pharmaceutical formulations in any pharmaceutical industries.

1. Introduction

Mycophenolate mofetil (MMF) is an immunosuppressant and prodrug of mycophenolic acid, used extensively in transplant medicine. It is a reversible inhibitor of inosine monophosphate dehydrogenase[1] in purine biosynthesis, more specifically guanine synthesis. MMF is also used in the treatment of autoimmune diseases, such as Behcet's disease, pemphigus vulgaris

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and systemic lupus erythematosus. The chemical name for MMF is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. The empirical formula and molecular weight of the drug are $C_{23}H_{31}NO_7$ and 433.50 g respectively. The chemical structure of MMF is presented in Fig. 1. An extensive literature surrey is carried out and found a few HPLC[2–7] methods have been reported for the determination of MMF present in biological fluids or biological matrixes. Very few reverse phase-HPLC methods[8,9] are reported for the determination of the drug in dosage forms. But no LC/MS method is reported to determine the quantity of MMF in pharmaceutical formulations; therefore the authors are interested in developing a new LC/MS method for the assay of MMF in pharmaceutical formulations. The scope of the present investigation is to apply this method to determine the amount of MMF and to study the stability of MMF under forced degradation. This manuscript gives the first report for the application of proposed LC/MS method in stability testing and assay of pharmaceutical dosage forms with less-time consuming analysis.

2. Materials and methods

2.1. Materials

HPLC grade methanol (sd Fine-Chem Limited, Mumbai, India), acetonitrile (Qualigens Fine Chemicals, Mumbai, India) and ammonium acetate (Qualigens Fine Chemicals, Mumbai, India); AR grade glacial acetic acid (Loba Chemie Pvt. Ltd., Mumbai, India), hydrochloric acid, sodium hydroxide, methanol and hydrogen peroxide (Qualigens Fine Chemicals, Mumbai, India) and Milli-Q water (RANKEM Laboratories, Mumbai, India) were used for the present investigation. MMF, gifted sample by Dr Reddy's Laboratory, Hyderabad, India was used as such without any further clean up. The commercially available tablets were purchased from the local market.

2.2. Preparation of stock and sample solutions

Stock solution of 1000 μg/mL was prepared by accurately weighing 5.00 mg of MMF, transferred into a 5.0 mL clean and dry volumetric flask, and dissolved in methanol. The primary standard solution of concentration of 10 μg/mL was prepared by taking 10 μL stock solutions and diluted to 1.0 mL with methanol. Further a series of working standard solutions of different concentrations were sequentially diluted to the required volume.

2.3. LC/MS/MS analysis

The LC/MS/MS analysis was carried out on Applied Biosystems API 3200 triple quadrupole mass spectrometer attached to LC 20 Series Shimadzu Corporation (Kyoto, Japan), equipped with pump (Shimadzu LC-10AT VP), auto sampler (Shimadzu SIL-HTC), degasser (Shimadzu FCV-10AL VP) and system controller (Shimadzu SIL-HTC ver 6.03) in NISHKA Scientific and Research Laboratories, Hyderabad. The chromatographic analysis was performed under isocratic conditions using 75% acetonitrile containing 2 mM ammonium acetate at pH 5.0 at a flow rate of 600 μL/min and Chromosil ODS-3, C18, 4.6 × 50 mm, 2.5 μm column. The ionization was carried out by ESI. The source heater temperature was maintained at 300 °C. The analysis was carried out in multiple reaction monitoring (MRM) mode for the transition *m*/z 434 → 114 at collision energy 30 V.

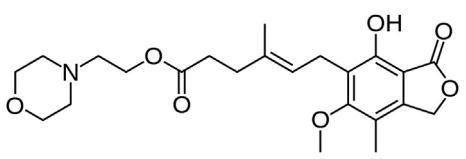

Fig. 1 – Chemical structure of mycophenolate mofetil.

2.4. Mass spectral studies of MMF

The mass spectral analysis was carried out by direct infusion of 10 μg/mL solution of MMF in to the ESI source at a flow rate of 10 μL/min along with the mobile phase flow rate of 600 μL/min. The obtained mass spectrum showed *m*/z 434 as a major ion which can be attributed to the MH^+ ion of the analyte. This ion was subjected to collision induced dissociation (CID) using nitrogen as a collision gas. The collision energy was tuned in such a way that the intensity of MH^+ ion was reduced to a minimum of 20%. The obtained mass spectrum after CID showed *m*/z 114 as a major fragment. Hence the transition *m*/z 434 → 114 was used to monitor the analyte peak in LC/MS/MS analysis. The ESI mass spectra of MMF obtained before and after fragmentation were presented in Figs. 2 and 3 respectively.

3. Results and discussion

3.1. Precision

Intra/inter day precision was calculated at three different concentrations of working standard solution of reference MMF by taking measurements of six replicates at each concentration on different occasions. Mean, standard deviation (SD) and percent of relative standard deviation (%RSD) were calculated at each concentration and found to be within the acceptable limits. The results of intra day and inter day precision were presented in Table 1.

3.2. Accuracy

In proposed method, accuracy was determined at three different concentrations of working standard sample solution of MMF (Tablet) by taking measurements of three replicates at each concentration. The proposed method was found to be highly accurate. The calculated %RSD of peak area, weight found and percent of weight found were found to be 2.382, 0.133 and 0.153; 1.178, 1.880 and 1.857 and 2.151, 1.543 and 1.542 at HQC, MQC and LQC levels respectively. The experimentally determined accuracy of the proposed method was presented in Table 2. Typical LC/MS/MS chromatograms for standard and test were presented in Figs. 4 and 5.