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Method Development and Validation of Mycophenolate Mofetil by RP-HPLC

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A B S T R A C T

Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. A schematic diagram showing the binding of a peptide or a protein to a reversed-phase surface. The present objective is to develop a new simple and rapid analytical method to estimate Mycophenolate mofetil in bulk form by hplc method. Hence, on the basis of literature survey it was thought to develop a precise, accurate, simple and reliable method for the estimation. Validation of the method will be done in accordance with ICH guidelines for the assay of active ingredients. The methods will be validated for parameters like accuracy, linearity, precision, robustness, and system suitability. These proposed methods are suitable for the pharmaceutical analysis in analytical laboratories.

Keywords: Mycophenolate mofetil, RP-HPLC, ICH guidelines

A R T I C L E   I N F O

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1. Introduction

Analytical chemistry is the science to analyze morphologies, compositions, and quantities of analytical targets. These analytical results have played critical roles from the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, and forensic science, to name a few. Modern analytical chemistry is dominated by instrumental

analysis. There are so many different types of instruments today that it can seem like a confusing array of acronyms rather than a unified field of study. Many analytical chemists focus on a single type of instrument. Academics tend to either focus on new applications and discoveries or on new methods of analysis. An effort to develop a new method might involve the use of a tunable laser to increase the specificity and sensitivity of a spectrometric method. Many methods, once developed, are kept purposely static so that data can be compared over long periods of time. This is particularly true in industrial quality assurance (QA), forensic and environmental applications. Analytical chemistry plays an increasingly important role in the pharmaceutical industry where, aside from QA, it is used in discovery of new drug candidates and in clinical applications where understanding the interactions between the drug and the patient are critical. (S, M.Khopkar2008)

Chromatography:

Introduction:

Chromatography (from Greek: chroma, colour and: "grafein" to write) is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated.(L.R .Syder 1997)

History:

It was the Russian botanist Mikhail Semyonovich Tswet who invented the first chromatography technique in 1900 during his research on chlorophyll. He used a liquid-adsorption column containing calcium carbonate to separate plant pigments. In 1952 Archer John Porter Martin and Richard Laurence Millington Synge were awarded the Chemistry Nobel Prize for their invention of partition chromatography.

Chromatography theory:

Chromatography is method of separating mixtures and identifying their components i.e. it's a separation method that exploits the differences in partitioning behavior of analyte between a mobile phase and a stationary phase to separate components in a mixture. Components of a mixture may be interacting with the stationary phase based on charge (ion-ion-interactions, ion- dipole-interactions), Vander Waal's forces, relative solubility or adsorption (hydrophobic interactions, specific affinity). There are two theories of chromatography, the plate and rate theories.

High-performance liquid chromatography (HPLC)

Introduction: In the field of analytical chemistry high performance liquid chromatography (HPLC) is considered by many to be most exciting and dynamic technique of past decade. Since its advent in 1969, tremendous improvements have been realized in pumping system, sample introduction modes, column design and detector to make it a rapid, accurate, and precise technique for analytical determination of compounds. The numbers of mobile phases in HPLC are infinite and thus separation possibilities are limited only to the analyst's imagination. Nonvolatile, polar, and thermally degradable compounds that are difficult to analyze by gas chromatography are particularly suited for modern liquid chromatography. High performance liquid chromatography is a convenient separation technique used for wide types of samples, with exceptional resolving power, speed and nano molecular detection levels. It is presently used in pharmaceutical research and developments in the following ways:

- To purify synthetic or natural products
- To characterize metabolites
- To assay active ingredients, impurities, degradation products and in dissolution assays
- In pharmacodynamics and pharmacokinetic studies

Early, liquid chromatography was carried out in glass columns with diameters of 1 to 0.5 cm and lengths of 50 to500cm. The average diameter of the solid stationary phase particles was usually in the 100 to 200 micron range. Recent technology has allowed for the development of packing material with relatively small particles size diameter (3-10 micron). This technology resulted in the development of columns with very high efficiencies, and consequently has involved the use of more sophisticated instrumentation to perform at increased pressures and flows; hence the term High Performance Liquid Chromatography (HPLC). (E .Katz,et.al, 1998)

2. Materials and Methods

Materials:

HPLC Grade Methanol, HPLC Grade Acetonitrile, Double Distilled Water, Mycophenolate mofetil.

Methodology: Spectral and absorbance measurements were made on a TECHCOMP UV-Visible double beam spectrophotometer (model: UV- 2310) with 1cm matched quartz cells. AR grade Diluent was used.

Table 1: Optimized Method Parameters

Parameters	Conditions
Mobile Phase	Acetonitrile: water (30:70)
Column (Stationary Phase)	Symmetry C18 (4.6 x 100 mm, 3.5µlm,
Flow rate (ml/min)	Make: Phenomenax)
Column temperature (°C)	0.9 ml/min
Volume of injection loop (µl)	Ambient
Detection wavelength (nm)	20µl
Retention Time (min)	254nm
Plate count	2.248 min
Tailing factor	2,615

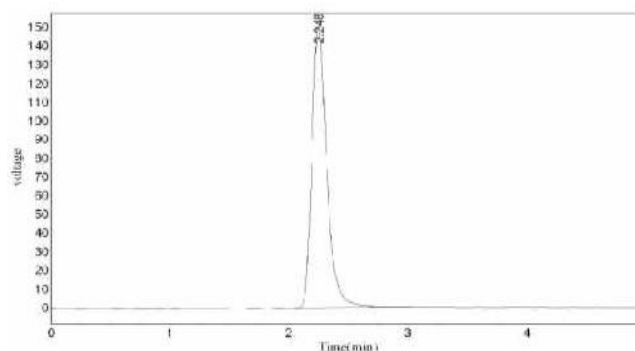

Figure 1: optimized chromatogram

3. Results and Discussions

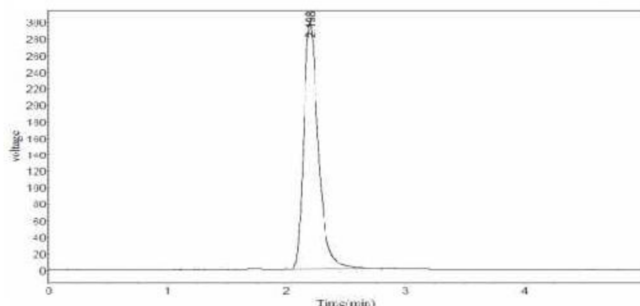

Figure 2: Accuracy 150% chromatogram -3

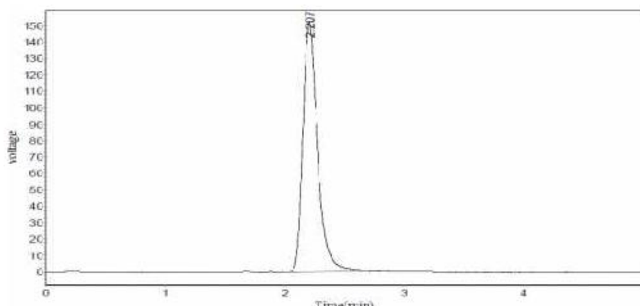

Figure 3: Precision chromatogram -1

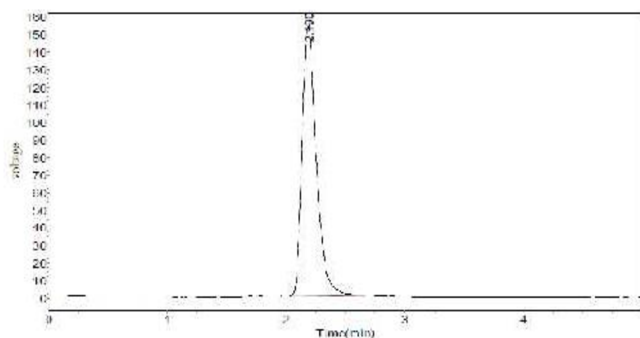

Figure 4: FTIR spectrum of pure Glipizide pure drug

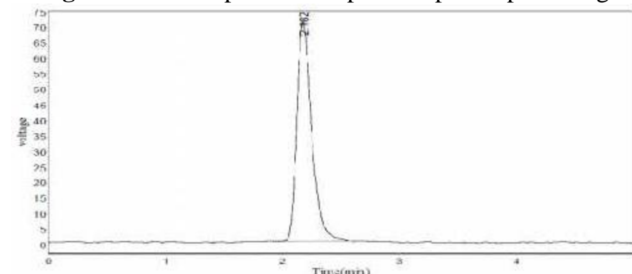

Figure 5: Linearity chromatogram -1

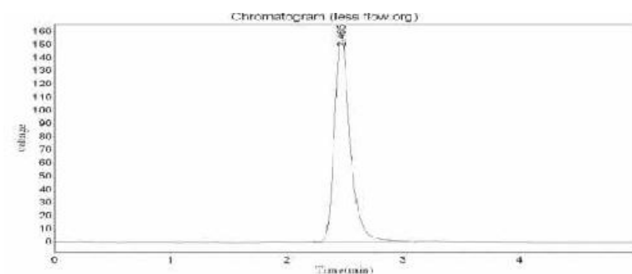

Figure 6: Less Flow Rate: (0.8ml/min)

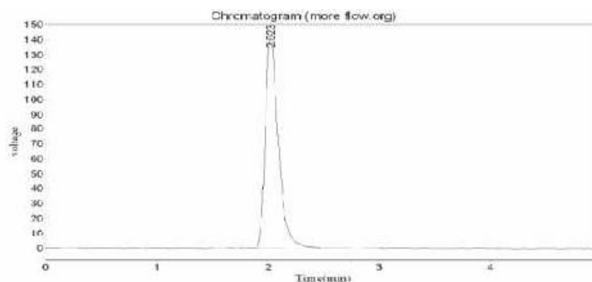

Figure 7: More Flow Rate (1.0ml/min)

Discussion

A new isocratic reverse-phase high performance liquid chromatography with UV- detection at 254nm was developed for quantitative determination of Mycophenolate mofetil in bulk form. The mobile phase used was of Acetonitrile and water. The chromatographic method was performed Symmetry C18 (4.6 x 100 mm, 3.5 m, Make: Phenomenax) at a flow rate of 0.9 ml/min. Column Temperature was set a ambient temperature and injection volume was 20μ l.

Estimation of Mycophenolate mofetil bulk form by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The peak area ratio of standard and sample solutions was calculated. The sample shows percentage purity value as 99.64%.

The method was validated according to the ICH guidelines. The validated parameters were system suitability, Precision, Accuracy, Specificity, linearity, LOD, LOQ and Robustness. The resulting chromatograms exhibited retention time at 2.248 min. The number of theoretical plates were more than 2000,as per limits i.e 2,615. The tailing factor was less than 2 that is 1.423. Hence all the system suitability parameters were with the specified limits. Results are presented in the tables. The specificity of the method was established by injecting sample and standard. The linearity for the drug from concentration range of 5ppm was established by constructing the calibration curve with concentration on x-axis and peak area on y-axis with the correlation coefficient of 0.999. Results are within specified limits.

Precision was determined by preparing the standard solution at working concentration and analysis was carried for five replicate injections. The percentage relative standard deviation (% RSD) was calculated for the peak area of Mycophenolate mofetil was found to be 1.656 which is within the acceptance criteria of not more than 2.0%.Intermediate precision was determined by preparing different samples solutions from working concentration and analysis was carried out for five replicate injections. The percentage relative standard deviation (% RSD) was calculated for the peak areas of Mycophenolate mofetil was found to be 1.738 which is within the acceptance criteria of not more than 2.0%. The accuracy studies were shown as % recovery for Mycophenolate mofetil at 50%,

100% and 150% the limits of % recovered should be in range of 98-102% the results obtained for Mycophenolate mofetil were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of Mycophenolate mofetil was 99.4. The limits of % recovery of drugs were 98-102% and the above results which indicates that the method was accurate.

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) was determined by injecting progressively low concentrations of the standard solutions using the developed RP- HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The detection limit (LOD) was found to be 0.429µ g/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10).The quantitative limit (LOQ) was found to be 1.432µ g/ml. In case of LOD and LOQ the S/N ratio were found to be with in the limits. The Robustness of the method was established by changing the parameters like Flow rate and mobile phase composition. The small changes in system suitability parameters were with in the limits which ensures that the method developed can withstand slight changes in the experimental conditions and produce results with good reproducibility and repeatability.

Table 1: Validation parameters

S.NO	Parameter	Specifications	No interference
1	Specificity	No interference	1.423
2	Tailing factor	NMT 2	2,615
4	Number of Theoritical Plates	NLT 2000	1.656
5	Precision	%RSD NMT 2.0	1.738
6	Intermediate precision	%RSD NMT 2.0	0.999
7	Linearity range	Correlation coefficient NLT 0.999	99.4
8	Accuracy	% Mean Recovery 98–102%	0.429µ g/ml
9	Limit of Detection	Signal noise ratio should be more than 3:1 for the conc	No interference
10	Limit of Quantitation	Signal noise ratio should be more than 10:1 for the conc	1.432µ g/ml
11	Robustness by change in flow rate and mobile phase		No effect on SSP

4. Conclusion

A simple, rapid, cost effective and accurate RP-HPLC method was developed for the determination of Mycophenolate mofetil in bulk form by isocratic mode elution. The analytical conditions and the solvent system developed provided good separation within a short run time. The RP-HPLC method was validated and demonstrated good linearity, precision, accuracy and specificity. Thus, the developed RP-HPLC method can be utilized for routine analysis during the analysis of Mycophenolate mofetil. All the parameters for the drug Mycophenolate mofetil had met the criteria of ICH guidelines for method validation. However, RP-HPLC method is considered more specific and sensitive. For routine analytical purpose it is desirable to establish methods capable of analysing huge number of samples in a short time period with good robust, accuracy and precision without any prior separation step. Good agreement was seen in the assay results of bulk form as well as in laboratory prepared mixtures by developed methods. We concluded that all the proposed methods are a good approach for obtaining reliable results and were found to be suitable for the routine estimation of Mycophenolate mofetil. The % RSD of proposed method was found to be less than 2% shows its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Result of validation parameter demonstrates that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2A/B. Hence it is recommended to be used in routine testing release and stability samples testing.

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