ISSN: 0976-822X

Available online on www.ijcpr.com

International Journal of Current Pharmaceutical Review and Research 2022; 14(3); 79-85

Review Article

A REVIEW ON VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF MYCOPHENOLATE MOFETIL IN PURE AND PHARMACEUTICAL TABLET DOSAGE FORM

Mo. Firoj Tanwar[1], Ritika Bhatia[1], Sulekha Mandal[2], Dilip Agarwal[3]

[1]Research Scholar, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur
[2]Professor, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur
[3]Principal, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur

Received: 25-08-2022 / Revised: 02-09-2022 / Accepted: 25-09-2022
Corresponding author: Mo. Firoj Tanwar
Conflict of interest: Nil

Abstract

Immunosuppressant medications, or those that weaken the body's immunological response, such mycophenolate mofetil (CellCept) is used to treat a variety of autoimmune illnesses. The prevalence of normal phase chromatography in HPLC has dwindled as a result of its inefficiencies, such as the short solvent retention times for its mobile phase solvents. Consequently, liquid chromatography systems frequently use reverse phase chromatography. The goal of this study is to describe and create a straightforward, precise, and focused technique for the RP-HPLC method of mycophenolate mofetil determination in pure and pharmaceutical dosage form. The accuracy, system appropriateness, precision, linearity, robustness, limit detection and limit of quantitation are just a few of the factors that may be used to verify the RP-HPLC method.

Keywords: RP-HPLC, Mycophenolate Mofetil, Immunosuppressant, CellCept. autoimmune illnesses

INTRODUCTION

Mycophenolate mofetil, often known as MMF or CellCept, is a reversible inhibitor of inosine monophosphate dehydrogenase and a prodrug of mycophenolic acid (IMPDH)This immunosuppressant is used in conjunction with Cyclosporine and corticosteroids to avoid organ rejection after hepatic, renal, and cardiac transplants. Roche Pharmaceuticals markets it, and the FDA approved it in 1995 for the prevention of transplant rejection.

Mycophenolate mofetil has also been examined for the treatment of nephritis and other autoimmune disease problems. Unlike another type of immunosuppressant known as a calcineurin inhibitor, MMF seldom causes nephrotoxicity or fibrosis. Previously, mycophenolic acid (MPA) was supplied to people with autoimmune illnesses beginning in the 1970s, but it was discontinued due to gastrointestinal side effects and concerns about carcinogenicity. To eliminate the gastrointestinal symptoms associated with MPA treatment, a novel semi-synthetic 2-morpholinoethyl ester of MPA was created. When compared to MPA, it has greater bioavailability, higher effectiveness, and less gastrointestinal side effects.

Mechanism of action of Mycophenolate Mofetil: Mycophenolic acid, mycophenolate's active metabolite, inhibits T-cell and B-cell proliferation as well as

the formation of cytotoxic T-cells and antibodies. MPA prevents lymphocyte and monocyte adherence to endothelial cells of blood arteries, which is ordinarily part of inflammation.

MPA inhibits de novo purine production (which enhances immune cell proliferation) by inhibiting the enzyme inosine 5'-monophosphate dehydrogenase (IMPDH), with preference for IMPDH II.6 Inosine monophosphate (IMP) is generally converted by IMPDH to xanthine monophosphate (XMP), a metabolite that contributes to the synthesis of guanosine triphosphate (GTP).Ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein are all made from GTP, which is a crucial component. Due to the aforementioned chain of events, mycophenolate mofetil inhibits the synthesis of the DNA, RNA, and protein needed to produce immune cells by reducing the amount of guanosine nucleotides that are produced from scratch. Additionally reducing the action of the enzyme inducible nitric oxide synthase, which in turn reduces the synthesis of peroxynitrite, a chemical that causes inflammation, MMF depletes tetrahydrobiopterin, which furthers the aforementioned anti-inflammatory properties.[3, 4]

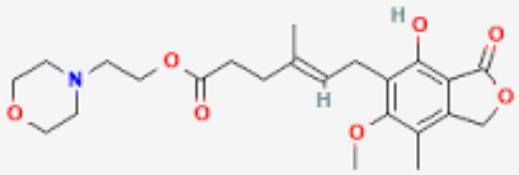

Figure 1:Mycophenolate Mofetil Structure

Analytical Method Development:

In order to determine the identification, purity, physical properties, and potency of pharmaceuticals, as well as the medications' bioavailability and stability, analytical technique development is done. It is possible to think of the process of developing and validating analytical methods as demonstrating that analytical processes are sufficient for evaluating pharmaceuticals, and notably the active pharmacological component (API).[5]

To evaluate certain substance properties against the established acceptability criteria for such qualities, analytical processes are devised. The examination and selection of the most accurate assay techniques to ascertain the composition of a medicine are therefore part of the analytical method development process. Every drug development programmed should place a high priority on the development and validation of analytical methods. For any biotechnology business creating novel drug candidates, there are at least three key reasons why establishing analytical methods is essential.

Well first of all, the success of a pharmaceutical development programme clearly depends on the quality of the drugs being developed, thus biotech businesses creating novel compounds must take the development of analytical methods extremely seriously

Secondly, regulatory agencies throughout the world want analytical technique validation for requests for marketing authorizations as well as clinical trial applications.

In early phase clinical trials (first in human / Phase 1 studies), people will ultimately get the investigational medicinal product (IMP), therefore the quality of a drug's research and manufacture is crucial to assure patient safety and, ideally, observe promising efficacy in the new therapies.[6]

Any new or improved method is typically developed by adapting existing approaches and instrumentation to the current analyte as well as to the method's final needs or requirements. The selection of the method requirements and the determination of the type of instrumentation to be used and why are typically necessary before method development can begin.

There are a number of good reasons to create novel analytical techniques-

•Including the possibility that analytes in a given sample matrix cannot be analysed using an existing technique.

•Existing techniques could be overly costly, time- or energy-consuming, or they might be difficult to automate.
•There may be a need for an alternative method to confirm, for legal or scientific reasons, analytical data initially obtained by existing methods. These reasons could be related to science or law. Newer instruments and techniques may have developed that would present opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision, or better return on investment.
The characteristics of the analyte(s) of interest must be taken into account while creating a technique in order to determine the ideal analyte parameter value ranges.
After the apparatus has been put together and the analyte parameters have been taken into account, standards should be utilized for the method's ongoing development, optimization, and first assessment.
It should only be carried out using analytical standards whose purity is already known, have been thoroughly recognized and defined. By taking such steps, future issues may be avoided, and variables can be eliminated when trying to optimize or enhance the starting circumstances during method development.
Analytical methods for the drugs, as per instrumentation criteria include;
A. Non-instrumental analytical techniques such as
•Titrimetric
•Gravimetry
•Voltammetry
B. Analytical techniques using instruments
•Electrical technique
•Optical technique
•Emission technique
•Chromatography

Classification of Analytical Methods:

Analytical methods can be broadly classified into following types:

•Class A: Identification tests for either bulk drug compounds or specific ingredients in completed dosage forms.
•Class B: Techniques intended to identify and quantify contaminants in a completed dosage form or a bulk pharmacological material.
•Methods used to quantify the concentration of a principal constituent or a bulk drug substance in a completed dosage form are classified as Class C methods.
•Methods used to evaluate the properties of completed dosage forms, such as dissolution profiles and content homogeneity, fall within the classification of Class D.
Analytical methods as per USP are:
•Category I: Analytical techniques for measuring the main elements of bulk medicinal compounds or active chemicals, such as preservatives, in pharmaceutical finished goods.
•Category II: Analytical techniques for identifying contaminants in bulk pharmaceuticals or for identifying degradation chemicals in pharmaceutical finished goods.
•Analytical techniques for identifying performance characteristics fall under category III (e.g. dissolution, drug release).[7-9]

Analytical Method Devlopment by RP-HPLC:

Introduction to Chromatography
Excipients, disintegrants, colours, and flavours are only a few examples of the inert substances that are present in modern pharmaceutical formulations, which are complex combinations comprising one or more therapeutically active compounds. Prior to quantitative analysis, the pharmaceutical analyst must be able to separate the mixtures into distinct components in order to assure the quality and stability of the final product.
Chromatography is ascribed to the Russian botanist "Tswett." Using a solid polar stationary phase, he was successful in separating leaf pigments in 1903. [10, 11]

High Performance Liquid Chromatography In HPLC, many separation methods are used. These include

•Size exclusion chromatography
•Affinity chromatography
•Reverse phase ion pair chromatography
•Normal phase mode (gel permeation and gel filtration chromatography)[12]

Reversed phase:

In the fields of chemical, biological, pharmaceutical, food science, and biomedical research, the most common mode for analytical and preparative separations of target compounds is reversed phase mode. In this manner, a silica gel-based nonpolar hydrophobic packing containing an octyl or octa-decyl functional group serves as the stationary phase, while a polar solvent serves as the mobile phase. With an aqueous mobile phase, retention and selectivity may be controlled by secondary solute chemical equilibrium processes such ionization control, ion suppression, ion pairing, and complexation. In this mode, nonpolar molecules are held for a longer period of time while the polar chemical is eluted first. Being polar in nature, most medications and pharmaceuticals are not kept for lengthy periods of time and elute quickly. The various columns include octa decyl silane (ODS), often known as C18, C8, C4, etc (in the order of increasing polarity of the stationary phase).[2]

Validation of Method:

The act of validating a technique, process, piece of machinery, activity, or system involves demonstrating that it operates as predicted under a certain set of circumstances and provides the necessary accuracy, precision, sensitivity, ruggedness, etc. Depending on the application, when applied to an analytical technique, it indicates that a method is repeatable whether it is used by the same or other people, in the same or various laboratories, with different chemicals, equipment, etc.

Advantages of Analytical Method Validation:

The primary benefit of method validation is that it fosters a sense of confidence in both the user and the developer. The validation exercise may seem expensive and time-consuming, but it really ends up being affordable, gets rid of tedious repeats, and improves time management in the long run. Minor adjustments to circumstances like reagent supplier or grade or analytical setup are inevitable for obvious reasons, but method validation smooths out the shock of these changes and generates a return on the process investment that exceeds the initial investment.

Key Parameters of the Analytical Method Validation:

Performance parameters that are covered in a validation exercise.

- accuracy,
- precision (repeatability and reproducibility),
- linearity and range,
- limit of detection (LOD)/ limit of quantitation (LOQ),
- selectivity/ specificity
- robustness/ ruggedness[13, 14]

RP-HPLC Method for Estimation of Mycophenolate Mofetil:

Through the process of reversed-phase high-performance liquid chromatography, molecules are distinguished according to their hydrophobicity. The solute molecule from the mobile phase must bind hydrophobically to the stationary phase's immobilized hydrophobic ligands in order for the separation to take place. In the presence of aqueous buffers, the solute mixture is first applied to the sorbent; the solutes are then eluted by adding an organic solvent to the mobile phase. Either gradient elution, in which the quantity of organic solvent is raised gradually over time, or isocratic circumstances, in which the concentration of organic solvent is constant, can be used to carry out the elution process. As a result, the solutes are ejected in ascending order of molecular hydrophobicity.

Table 1: A brief description of research work on HPLC / RP-HPLC methods responsible for the estimation of Mycophenolate Mofetil

S. No.	Drug	Method	Instrument, mobile phase,	Results of validation process	Ref.
1	Mycophenolate Mofetil	HPLC	Hplc Phenomenex C18 column, wavelength 250 nm. methanol and water (75:25 v/v)	LOD and LOQ values 3.660 ng/mL and 11.091 ng/ml, linear in the range of 0.1 to 10 µg/ml.	[15]
2	Mycophenolate Mofetil	RP-HPLC	RP-HPLC (LC-2010, Shimadzu, Japan), RP C18, 5 µm, 4.6 × column, wavelength 250nm. mobile phase: acetonitrile, phosphate buffer, methanol: (25:60:15) v/v	LOD and LOQ values 11.4163 (µg/ml) 32.56454 (µg/ml), linearity range for MMF 2-7 µg/ml.	[16]
3	Mycophenolate Mofetil	HPLC	Hplc Merck (Mumbai, India), C18 (250 mm × 4.6 mm, 5.0 μ) column, wavelength 251nm. mobile phase: methanol acetate buffer (75:25 v/v).	LOD and LOQ values 0.121 μg/mL, 0.366 μg/ml.	[17]
4	Mycophenolate Mofetil	RP-HPLC	HPLC Shimadzu, RP C18 (250 mm × 4.6 mm, 5.0 μ), (250 mm × 4.6 mm, 5.0 μ), mobile phase: acetonitrile, 0.02M phosphate buffer 50:50 v/v. wavelength 230nm	linearity range for MMF10-60 µg/ml. correlation coefficient of 0.999.	[18]
5	Mycophenolate Mofetil	RP-HPLC	RP-HPLC, Shimadzu, Hypersil BDS C18(250mm x 4.6mm x 5.0 µm) column, phosphate buffer (7.0 pH) and acetonitrile (65:35 v/v), as mobile phase. Wavelength 250nm.	retention time 6.520. good recovery range of 97.0% to 103.0%	[19]
6	Mycophenolate Mofetil	RP-HPLC	RP-Hplc, Shimadzu, RP-C18 column (250mmx4.6mm I.D., 5µm particle size), mobile phase consisting of acetonitrile and 0.03M phosphate buffer in the ratio of 60:40 v/v in isocratic mode, wavelength 254nm,	flow rate is 0.8 ml/min, range of 20-120 µg/ml. correlation coefficient of 0.9995.	[20]

CONCLUSION

The intent of this review article is to provide a succinct elaboration of the RP-HPLC approach for the determination of mycophenolate mofetil. Literature data indicated that certain analytical techniques have been reported for this purpose. The analysis of mycophenolate mofetil in pure and pharmaceutical tablet dosage forms presently necessitate the implementation of effective, sensitive, accurate, and consistent methodologies.

REFERENCES

1. Fulton B. and A. Markham Mycophenolate mofetil. Drugs, 1996; 51(2):278-298.
2. Prathap B., et al., A review-importance of RP-HPLC in analytical method development. International Journal of Novel Trends in Pharmaceutical Sciences, 2013; 3(1): 15-23.
3. Allison A.C. and E.M. Eugui. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology, 2000; 47(2-3): 85-118.
4. Sievers T.M., et al., Mycophenolate mofetil. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 1997; 17(6):1178-1197.
5. Breaux J., K. Jones and P. Boulas. Analytical methods development and validation. Pharm. Technol, 2003; 1: 6-13.
6. Chan C.C., et al., Analytical method validation and instrument performance verification. Vol. 18. 2004: Wiley Online Library.
7. Swartz M.E. and I.S. Krull. Analytical method development and validation. 2018: CRC press.
8. Kalra K., Method development and validation of analytical procedures. Quality Control of Herbal Medicines and Related Areas, 2011; 4:3-16.
9. Sharma N., T. Barstis and B. Giri. Advances in paper-analytical methods for pharmaceutical analysis. European Journal of Pharmaceutical Sciences, 2018; 111: 46-56.
10. Smith I., Chromatography. 2013: Elsevier.
11. Lederer E. and M. Lederer, Chromatography. 1953: Elsevier Publishing.
12. Snyder L.R., J.J. Kirkland and J.W. Dolan. Introduction to modern liquid chromatography. 2011: John Wiley & Sons.
13. Trullols E., I. Ruisanchez and F.X. Rius. Validation of qualitative analytical methods. TrAC Trends in Analytical Chemistry, 2004; 23(2): 137-145.
14. Gumustas M., et al., UPLC versus HPLC on drug analysis: advantageous, applications and their validation parameters. Chromatographia, 2013; 76(21): 1365-1427.
15. Kaur P., M. Kumar and U.K. Mandal. Development and validation of a simple HPLC method for estimation of mycophenolate mofetil in microemulsion formulation. Int J Pharm Pharm Sci, 2020; 12(4): 16-20.
16. Kumar P., et al., Development of RP-HPLC method for simultaneous estimation of mycophenolate mofetil and tacrolimus. J Mater Environ Sci, 2018. 9: p. 1357.
17. Choudhari V.P. and A.P.G. Nikalje, Development and validation of stability indicating LC-PDA method for mycophenolate mofetil in presence of mycophenolic acid and its application for degradation kinetics and pH profile study. Advances in Chemistry, 2014: 1-9.
18. Prasad K.R. and S. Kathirvel, Development and validation of RP-HPLC method for estimation of mycophenolate mofetil in bulk and pharmaceutical dosage form. Research Journal of Pharmaceutical Dosage Forms and Technology, 2013; 5(1): 42-45.

19. Nareshbabu A., P. Afroz and V. Anjaneyulu, New Stability Indicating Rp-Hplc Method for Estimation of Mycophenolate Mofetil Capsule in Pharmaceutical Dosage Form. International Research Journal of Pharmaceutical and Applied Sciences. 2012; 2(5): 149-154.

20. Rao A.L. and P.V.S.J. RAO, A new validated RP-HPLC method for the estimation of mycophenolate mofetil in pure and tablet dosage form. Asian Journal of Pharmaceutical Research and Health Care. 2010; 2(3).