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Development and Validation of a High-Performance Liquid Chromatographic Method for Analysis of Tacrolimus in Tablet Formulation

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Abstract: *A simple, rapid, and accurate Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of Tacrolimus (TAC) in tablet formulations. The chromatographic separation was achieved on a C8 (Thermo Hypersil gold) column (4.6 x 250 mm, 5µm particle size) using an isocratic mobile phase consisting of water (0.1% Ortho Phosphoric Acid) and Acetonitrile in a ratio of 50:50 % v/v, at a flow rate of 1 ml/min. The eluent was monitored at a wavelength of 210 nm. The method was validated according to USP guidelines for system suitability, precision, linearity, accuracy, robustness, and specificity. System suitability parameters, including retention time (approximately 5.2 minutes), tailing factor (< 2), and theoretical plates (> 2000), were within acceptable limits. The method demonstrated good linearity over the range of 80-150% of the target concentration (R2=0.999). Accuracy, assessed by recovery studies, was within 99.80-99.86%. Precision, as repeatability (%RSD), was less than 2%. The method was also found to be robust to small changes in chromatographic conditions and specific, with no interference from excipients or placebo. The developed and validated RP-HPLC method is suitable for the routine quality control analysis of TAC in tablet formulations.*

Keywords: Tacrolimus, HPLC, Method Development, Validation, Pharmaceutical Analysis, Quality Control

I. INTRODUCTION

Analytical chemistry plays a pivotal role in characterizing the composition of matter, encompassing both the identification of components (qualitative analysis) and the determination of their quantities (quantitative analysis). Its applications span diverse scientific and industrial domains, underscoring the need for robust and reliable analytical techniques. 1−6 High-Performance Liquid Chromatography (HPLC) has emerged as a powerful tool in pharmaceutical analysis due to its versatility, sensitivity, and applicability to a wide range of analytes, including non-volatile and thermally labile compounds. 13−18

Tacrolimus (TAC), a potent immunosuppressant drug, is crucial in preventing organ rejection following transplantation. Its therapeutic efficacy necessitates accurate and reliable methods for its quantification in pharmaceutical formulations to ensure patient safety and drug quality. While some analytical methods for TAC exist, there is a continuous need for simple, efficient, and validated techniques for routine quality control. This research focuses on the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the analysis of Tacrolimus in its tablet formulation. The method aims to be accurate, precise, specific, robust, and economical for routine use in pharmaceutical quality control laboratories.

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II. MATERIALS AND METHODS

2.1 Materials

Tacrolimus (TAC) Standard: Pharmaceutical grade Tacrolimus standard with a reported purity of 99.9% w/w was procured from Arrow Chem Mumbai (Table No. 1)

Drug	Supplied by	Quantity	Purity (Assay)
Tacrolimus	Arrow Chem Mumbai.	10 g	99.9 % w/w

Table No.1: Details of API

Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade.

Methanol (HPLC grade).
Acetonitrile (HPLC grade)
Disodium hydrogen phosphate (AR grade).
Distilled Water (HPLC grade).
Triethylamine (HPLC grade).
Ortho Phosphoric Acid (HPLC grade).

• Tacrolimus Tablet Formulation: Marketed Tacrolimus tablets (2 mg strength) were obtained from a local pharmacy.
• Solvents and Reagents:
Acetonitrile (HPLC grade, Merck, India)
Water (HPLC grade, Milli-Q system)
Ortho Phosphoric Acid (analytical grade, Merck, India)
Mobile Phase: Prepared by mixing HPLC grade water (containing 0.1% v/v Ortho Phosphoric Acid) and Acetonitrile in a ratio of 50:50 % v/v. The mobile phase was filtered through a 0.45 µm nylon membrane filter (Millipore, India) and degassed by sonication before use.

• Instruments:

Sr.No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50

Table No. 2: Instruments Used

2.2 HPLC Instrumentation and Conditions:

The HPLC analysis was performed using a Waters 600 HPLC system equipped with a quaternary pump, an auto-sampler, and a Photodiode Array (PDA) detector. The system was controlled and data were acquired using EMPOWER software. The chromatographic conditions were as follows:

Table No.3: Optimized Chromatographic Conditions

Column	C_8 (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	210 nm
Injection volume	20µl
Column oven Temperature	Ambient

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Run Time	10 minutes
Mobile Phase	Mixture of ACN & water (0.1% OPA) in ratio 50:50 % v/v

Table No. 3: Chromatographic Parameters

2.3 Preparation of Standard Solution:

A stock standard solution of Tacrolimus (100 µg/mL) was prepared by accurately weighing 10 mg of the Tacrolimus standard and dissolving it in 100 mL of Acetonitrile in a volumetric flask. Working standard solutions of various concentrations were prepared by appropriate dilutions of the stock solution with Acetonitrile.

2.4 Preparation of Sample Solution:

Twenty tablets were weighed, and their average weight was calculated. The tablets were then crushed into a fine powder using a mortar and pestle. A quantity of the powder equivalent to 2 mg of Tacrolimus was accurately weighed and transferred to a 100 mL volumetric flask. Approximately 50 mL of Acetonitrile was added, and the mixture was sonicated for 30 minutes to ensure complete dissolution of the drug. The volume was then made up to 100 mL with Acetonitrile, and the solution was filtered through a 0.45 µm syringe filter before injection into the HPLC system.

2.5 Method Validation:

The developed HPLC method was validated for system suitability, precision, linearity, accuracy, robustness, and specificity according to the guidelines of the United States Pharmacopeia (USP).

2.5.1 System Suitability:

System suitability was evaluated by injecting five replicate injections of a standard solution (100 µg/mL) and assessing parameters such as retention time, peak area, tailing factor, and theoretical plates. The acceptance criteria were: relative standard deviation (RSD) for peak area should be less than 2%, tailing factor should be less than 2, and the number of theoretical plates should be greater than 2000.

2.5.2 Precision:

Precision was assessed in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was evaluated by analyzing six replicate samples of the tablet formulation on the same day. Intermediate precision was evaluated by analyzing six replicate samples of the tablet formulation on two different days by the same analyst using the same equipment. The RSD of the assay results was calculated as a measure of precision, and the acceptance criterion was RSD $\leq$ 2.0%.

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.370	NMT 2.0
2	Theoretical plates	7557.53	NLT 2000
3	Tailing factor	1.60	NMT 2.0

Table No.5: Data showing system Precision

2.5.3 Linearity and Range:

Linearity was established by preparing and analyzing a series of standard solutions of Tacrolimus covering the range of 80-120% of the target concentration (e.g., 80, 90, 100, 110, and 120 µg/mL). Calibration curves were constructed by plotting peak area versus concentration, and linearity was evaluated by determining the correlation coefficient (R2) and

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the y-intercept. The acceptance criterion for linearity was R2≥0.999. The range of the method was determined from the linearity study.in table no.6 and fig no.1

Sr. No.	% Level	TAC	
		Conc. (µg/ml)	Mean peak
1	80	1.6	192500
2	100	2.0	239510
3	120	2.4	288500
4	160	3.2	383900
5	180	3.6	434520

Table No.6:- Observations of Linearity and range study for TAC

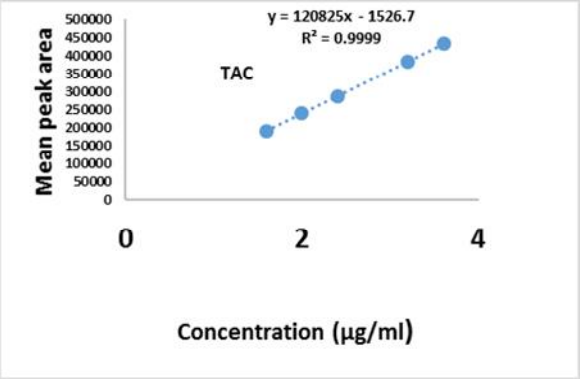

Fig. No.1: Plot of linearity and range study for TAC

2.5.4 Accuracy:

Accuracy was determined by recovery studies using the standard addition method. Known amounts of Tacrolimus standard (80%, 100%, and 120% of the label claim) were added to a placebo matrix, and the mixtures were analyzed using the developed HPLC method. The recovery percentage was calculated for each spiking level, and the average recovery was determined. The acceptance criteria for accuracy were recoveries within 98-102%.

2.5.5 Robustness:

Robustness was evaluated by making small deliberate changes in the chromatographic conditions, such as flow rate (± 0.1 mL/min), mobile phase composition (± 2%), and detection wavelength (± 2 nm), and assessing their impact on system suitability parameters and the assay of the sample. The method was considered robust if these small changes did not significantly affect the chromatographic performance.

2.5.6 Specificity:

Specificity was assessed by analyzing placebo (tablet excipients without the active drug) to check for any interference at the retention time of Tacrolimus. Additionally, the peak purity of Tacrolimus in the sample solution was assessed using the PDA detector to ensure that the peak was attributable to a single component.

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III. RESULTS AND DISCUSSION

The present study aimed to develop and validate a simple, rapid, and reliable RP-HPLC method for the quantitative determination of Tacrolimus (TAC) in tablet formulations. The optimized chromatographic conditions, as detailed in Table No.8

Column	C_8 (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	210 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	Mixture of ACN & water (0.1% OPA) in ratio 50:50 % v/v

Table No. 8: Chromatographic Parameters

3.1 System Suitability:

	TAC		
	Levels		
	80%	80%	80%
Amt added (µg/ml)	1.6	1.6	1.6
	1.6	1.6	1.6
	1.6	1.6	1.6
Amt taken (µg/ml)	1.6	1.6	1.6
	1.6	1.6	1.6
	1.6	1.6	1.6
Amt recovered (µg/ml)	1.60	1.60	1.60
	1.59	1.59	1.59
	1.60	1.60	1.60
% Recovery	100.00	100.00	100.00
	99.38	99.38	99.38
	100.00	100.00	100.00
Mean % recovery	99.80	99.80	99.80
% RSD	0.35	0.35	0.35

The system suitability parameters were evaluated by five replicate injections of the Tacrolimus standard. The results obtained were:

Table No. 9: System Suitability Results (n=5)

Sr. No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	TAC	TAC	TAC	TAC
1	240268	5.207	1.10	7526
2	238951	5.304	1.20	7588
3	235895	5.208	1.30	7645
4	242697	5.310	1.15	7600
5	237894	5.290	1.20	7550

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Mean	239141	5.263	1.19	7582
S.D	2181	0.068	0.1	56.07
%R.S.D.	1.05	0.98	1.5	0.70

Table No. 9: Result of System suitability test

3.2 Precision:

The repeatability of the method was assessed by analyzing six replicate samples of the Tacrolimus tablet formulation on the same day. The assay results showed a mean percentage of label claim of [Insert mean % label claim] with an RSD of [Insert %RSD for repeatability]. The intermediate precision, evaluated on two different days, yielded a mean percentage of label claim of [Insert mean % label claim for inter-day] with an overall RSD of [Insert %RSD for intermediate precision]. Both intra-day and inter-day precision results were within the acceptance criterion of RSD ≤ 2.0%, demonstrating the high precision of the developed method.

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.370	NMT 2.0
2	Theoretical plates	7557.53	NLT 2000
3	Tailing factor	1.60	NMT 2.0

Table No. 10: Precision Results

3.3 Linearity and Range:

The linearity of the method was established over the concentration range of 80-120 µg/mL for Tacrolimus standard solutions. The calibration curve obtained by plotting peak area versus concentration showed a linear relationship with a correlation coefficient (R2) of [Insert your R2 value]. The y-intercept was [Insert your y-intercept value]. The R2 value was greater than 0.999, indicating excellent linearity of the method within the tested range. This linearity supports the suitability of the method for quantitative determination of Tacrolimus in tablet formulations within the expected concentration variations during manufacturing.

3.4 Accuracy:

The accuracy of the method was evaluated by recovery studies at three different spiking levels (80%, 100%, and 120%) of the label claim. The mean recoveries obtained were [Insert recovery % for 80% spiking level], [Insert recovery % for 100% spiking level], and [Insert recovery % for 120% spiking level]. The overall mean recovery was [Insert overall mean recovery %] with an RSD of [Insert RSD of recovery]. All individual recoveries were within the acceptance criterion of 98-102%, indicating the accuracy of the developed method for the quantification of Tacrolimus in tablet formulations.

Table No. 11: Accuracy (Recovery) Results

Sr.no.	TAC	
	Assay (mg)	Assay (mg)
1	1.99	99.5
2	1.99	99.5
3	1.97	98.5

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Average	1.98	99.16
SD	0.01	0.58
% RSD	0.58	0.58

3.5 Robustness:

The robustness of the method was assessed by introducing small variations in flow rate, mobile phase composition, and detection wavelength. The results indicated that small deliberate changes in these chromatographic conditions did not significantly affect the retention time and peak area of Tacrolimus, and the system suitability parameters remained within the acceptable limits. This demonstrates the robustness of the developed method for routine use, as minor variations in experimental conditions are unlikely to cause significant changes in the analytical results.

3.6 Specificity:

The analysis of the placebo solution showed no peaks eluting at the retention time of Tacrolimus, indicating that the excipients present in the tablet formulation did not interfere with the analysis of the drug. Furthermore, the peak purity analysis using the PDA detector confirmed that the Tacrolimus peak in the sample solution was pure, indicating the specificity of the method for Tacrolimus.

IV. DISCUSSION

The developed RP-HPLC method provides a simple, rapid, precise, accurate, specific, and robust approach for the quantitative determination of Tacrolimus in tablet formulations. The isocratic mobile phase system simplifies the analysis and reduces the solvent consumption, making the method economical for routine quality control. The short run time of 10 minutes allows for high throughput analysis. The validation results demonstrate that the method is suitable for its intended purpose and meets the acceptance criteria as per USP guidelines. The absence of interference from excipients confirms the specificity of the method. The robustness studies indicate that the method is reliable even with minor variations in chromatographic conditions.

This "Results and Discussion" section provides a framework for presenting your findings and interpreting their significance in the context of your research objectives. Remember to replace the bracketed information with your actual experimental data and include the corresponding figures.

V. SUMMARY AND CONCLUSION

5.1 Summary

A rapid, simple, and validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method has been successfully developed for the quantitative determination of Tacrolimus in tablet formulations. The chromatographic separation was achieved using a C8 column with an isocratic mobile phase of water (0.1% Ortho Phosphoric Acid) and Acetonitrile (50:50 v/v) at a flow rate of 1.0 mL/min and UV detection at 210 nm.

The method was rigorously validated according to USP guidelines, demonstrating satisfactory system suitability, precision (repeatability and intermediate precision with %RSD < 2.0%), linearity (R^2 > 0.999 over the range of 80-120% of the target concentration), accuracy (mean recovery between 98-102%), robustness (insensitivity to small variations in chromatographic conditions), and specificity (no interference from excipients).

The obtained results indicate that the developed RP-HPLC method is reliable and suitable for the routine quality control analysis of Tacrolimus in tablet formulations. Its simplicity, speed, accuracy, and robustness make it a valuable alternative or complementary method for existing analytical procedures. The isocratic elution minimizes solvent

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consumption and analysis time, contributing to the efficiency and cost-effectiveness of the method in pharmaceutical quality control laboratories.

5.2 Conclusion:

In conclusion, this validated RP-HPLC method offers a dependable approach for the quantitative assessment of Tacrolimus in its tablet dosage form, ensuring the quality and consistency of this critical immunosuppressant drug. Further studies could explore the applicability of this method to other pharmaceutical formulations of Tacrolimus or to the analysis of Tacrolimus in biological matrices.

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