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Method Development and Validation of Tacrolimus in Pharmaceutical dosage form by RP-HPLC Method

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ABSTRACT

A simple, economical, specific, accurate, precise and validated Reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for Tacrolimus in pharmaceutical dosage form. The chromatographic separation was achieved on Princeton SPHER C8 column (150 mm x 4.6 mm id, 5 µ particle size) at 50^{0}c temperature using mobile phase buffer (0.05 M Potassium dihydrogen phosphate) : Acetonitrile (ACN) : tert-butyl methyl ether (35:60:5% v/v/v) (pH 3.0 ± 0.05) at flow rate 1.0 ml/min. Quantification was achieved with UV detector at 210 nm. Retention time of Tacrolimus was found to be 6.02 ± 0.05 min. Linearity was studied in the concentration range 20-120 µg/ml for Tacrolimus with a correlation coefficient of 0.9986. The proposed method was validated according to ICH guidelines with respect to linearity, accuracy, precision, robustness, LOD, and LOQ. The developed method with good separation, successfully applied for determination of Tacrolimus in its pharmaceutical dosage form.

Keywords: RP-HPLC, Tacrolimus, Validation.

INTRODUCTION

Tacrolimus is a highly potent immunosuppressive agent and has proven activity in both invivo and invitro experiments, also calcineurin inhibitor. It is isolated from fungus S*treptomycin tsukubaensis*. It is the basis of immunosuppressive regimens after liver and kidney transplantation and it has also been used for heart, pancrease, bone marrow, small bowel, lung transplantation and for the treatment of T-cell mediated autoimmune disease such as allergic encephalomyelitis [1-4]. Human data show that Tacrolimus cross the placenta. Limited data from organ transplant recipients show no evidence of an increased risk of adverse effect on the course and outcome of pregnancy under Tacrolimus treatment compared with other immunosuppressive medicinal products [3]. It is officially in USP [5]. Assay techniques that provide specific Tacrolimus concentration measurement with greater sensitivity, such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) are now widely employed [10]. Literature survey reveals that very few methods have been reported for the determination of Tacrolimus in pharmaceutical dosage form such as RP-HPLC, UPLC and mass Spectrometry methods [6-10]. Our objective in the present investigation is to develop and validate RP-HPLC method for Tacrolimus. The proposed RP-HPLC method utilises economical solvent system having advantages like better retention time, very sharp and symmetric peak shapes. The proposed method is validated according to ICH guidelines [12]. So our method is simple, rapid, sensitive, specific, robust and novel that makes it an attractive procedure in high throughput.

Chemical structure of Tacrolimus [6]

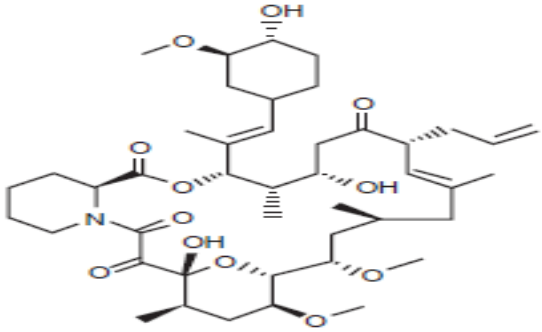

Figure 1: Chemical structure of Tacrolimus

MATERIALS AND METHOD

Instrument and apparatus: A HPLC Instrument (LC-2010 CHT, Shimadzu, Japan) equipped with UV detector, auto injector and LC-Solution Software was used. The chromatographic analysis was performed on Princeton SPHER C8 Column (150mm x 4.6mm id, 5 µ particle size). Analytical balance (Mettler Toledo), digital pH meter (Eutech instruments pH tutor) was used during the analysis.

Reagents and Materials: Working standards of Tacrolimus (Potency = 97.40%) was obtained as a gift sample from Biocon Limited Bangalore. HPLC grade Acetonitrile, tet-butyl methyl ether, AR grades Orthophosphoric acid and Potassium dihydrogen phosphate were procured from Merck Ltd. Mumbai India. Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a 0.45µ membrane filter paper. The commercial fixed dose product containing 1 mg of Tacrolimus was procured from the local market.

Chromatographic Condition

HPLC system	LC-2010 CHT , Shimadzu
Software	LC Solution
Detector	UV Detector
Wavelength	210 nm
Pump	Isocratic Pump
Stationary phase	Princeton SPHER, C8(150 mm x 4.6 mm id, 5µ particle size)
Mobile phase	0.05 M Potassium dihydrogen phosphate buffer : ACN : tert-butyl ether (35:60:5 % v/v/v) (pH 3.0 ± 0.05)
Flow rate	1.0 mL/min
Injection volume	50 µL
Diluent	Water : ACN (40:60 v/v)

Preparations of Solutions:

Preparation of stock and standard solutions: A Stock solution of Tacrolimus (0.25 mg/mL) was prepared by dissolving 25 mg Tacrolimus in 100 ml volumetric flask and volume make up by diluent. Appropriate volumes of the stock solution were transferred to appropriate volumetric flask and solution was diluted with diluent to furnish final concentration of Tacrolimus in the range 20-120 µg/mL respectively.

Preparation of capsule dosage form: 20 capsules each contained 1 mg Tacrolimus was accurately weighed. Its average weight determined and finally powdered. Quantity of the powder containing weight equivalent to 10 mg of Tacrolimus was transferred to 100 ml volumetric flask and 50 ml diluent was added followed by ultrasonication for 10 minute and make up the volume up to 100 ml with diluent. The resulting solution stirred for 1 hour. After that centrifuged at 2000 RPM for 5 min further dilution was performed with diluent to reach the calibration range for each compound.

Method Validation: The proposed method was validated according to ICH (Q2) B Guidelines for validation of analytical procedures. As per the ICH guidelines the method validation parameters checked were linearity, accuracy, precision, assay, robustness, LOD, LOQ.

Linearity (Calibration Curve): For constructing calibration curve, series of six dilutions in the concentration range 20-120 (20, 40, 60, 80, 100, and 120) µg/mL of Tacrolimus was taken. Calibration curve was constructed by plotting peak area vs. concentration of Tacrolimus and regression equation calculated from calibration curve. Linearity curves have shown in figure 4.

Accuracy (% Recovery): The accuracy of the method was determined by calculating recovery of Tacrolimus by the standard addition method. Known amounts of standard solutions of Tacrolimus added at 80,100 and 120% level to prequantified sample solution of Tacrolimus. Three samples were prepared for each recovery level then solutions were analysed and the percentage recovery was calculated by using formula.

Precision: The precision of analytical procedure express degree of the agreement among individual tests when the procedure is applied repeatedly to multiple sampling of homogenous samples. Precision are considered at two levels: Repeatability and Reproducibility.

Method Precision (Repeatability): The precision of the instrument was checked by repeatedly injecting (n =6) standard solutions of Tacrolimus. Under the same Chromatographic condition and measurements of % RSD of peak area, retention time, theoretical plate and tailing factor should not be more than 2%.

Intermediate Precision (Reproducibility): The intraday and interday precision of the proposed method was determined by analysing the corresponding responses 3 times of the same day and on three different days over a period of 1 week for three different concentration of standard solutions of Tacrolimus (80, 100 and 120 µg/mL) the results was reported in the terms of % RSD.

Robustness: The robustness of the method was established by introducing small changes in various parameters like, pH of mobile phase, flow rate, wavelength and column temperature. The method was evaluated by calculating % RSD.

Limit of Detection & Limit of Quantification: Limit of detection (LOD) was lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of quantification (LOQ) the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (m) was used for determining the detection and quantification limits. LOD can be calculated according to formula LOD = 3.3* (SD/m) and LOQ = 10*(SD/m). The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 & LOQ as the amount for which the signal to noise ratio was 10:1.

Results and Discussion

To optimize the HPLC parameters, several mobile phase composition were tried. A good peak symmetry, a satisfactory resolution for Tacrolimus was obtained with mobile phase 0.05 M Potassium dihydrogen phosphate buffer : ACN : tert-butyl ether (35:60:5 % v/v/v) (pH 3.0 ± 0.05) at a flow rate 1 ml/min to get better reproducibility and repeatedly optimization of method was done by changing mobile phase composition, pH of mobile phase, column packing, flow rate, temperature, detection wavelength and the effect on retention time and peak shape were monitored of Tacrolimus.

Typical chromatogram of Tacrolimus

The characteristics absorption frequencies of various functional groups

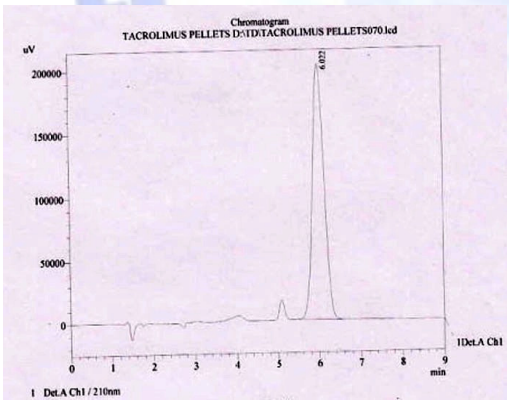

Figure 2: Chromatogram of Tacrolimus in sample solution.

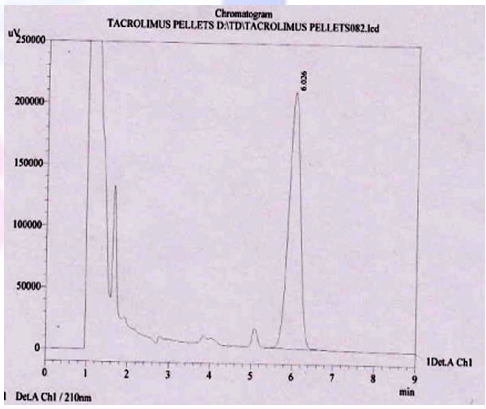

Figure 3: Chromatogram of Tacrolimus in Standard solution.

1] System suitability test parameters of Tacrolimus for the developed method are reported in table number 1.

Table 1: System suitability test parameters of Tacrolimus

Parameters	Tacrolimus ± RSD (n=6)
Retention Time (min)	6.023 ± 0.068
Tailing Factor	0.985 ± 0.085
Theoretical plates	2127.223 ± 0.509
Symmetry factor (k')	0.00 ± 0.00

RSD: - Relative standard deviation

2] The method showed good linear response in concentration range 20-120 µg/ml of Tacrolimus (r^2 = 0.9986).

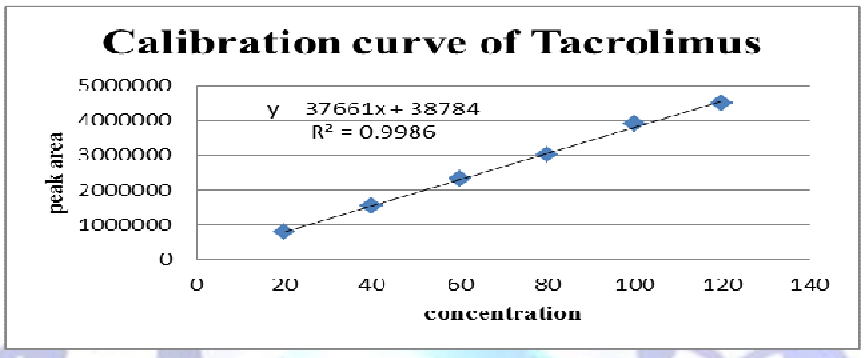

Figure 4: linearity curve of Tacrolimus

Table 2: Regression analysis of the calibration curves of Tacrolimus (n=6).

Parameters	Tacrolimus
Linear range (µg/mL)	20-120
Slope	37661
Intercept	38784
Correlation Coefficient (r^2)	0.9986

3] The method was found to be precise and RSD was found to be less than 2% are given in table number 3.

Table 3: Precision of RP-HPLC method

Drug	Level (n=3)	Intraday amount found (%) ±RSD	Interday amount found (%) ±RSD
Tacrolimus	1	100.62±0.541	99.28±0.682
	2	101.55±1.201	101.25±0.861
	3	100.13±0.925	100.75±1.152

4] The results of recovery of Tacrolimus with the RSD less than 2% are given in table number 4.

Table 4: Recovery studies of Tacrolimus

Drug	Level (n=3)	% Recovery±% RSD	Mean recovery
	80 %	100.96±1.18	

Tacrolimus	100 %	100.95±1.88	100.47%
	120 %	99.50±0.95	

5] Assay of Tacrolimus shown in table number 5.

Table 5: Results of assay of Tacrolimus

Drug	Label claim (mg/caps)	Amount of drug estimated (mg/caps)	% Amount found
Tacrolimus	1.0	1.006	100.62

6] LOD and LOQ value of Tacrolimus was determined by residual standard deviation method. The results are given in table number 6.

Table 6: LOD and LOQ of Tacrolimus

Drugs	LOD (µg/mL)	LOQ (µg/mL)
Tacrolimus	0.088	0.267

7] Robustness was evaluated by varying different parameters. The results of these variations are given in table number 7. **Table 7: Results of robustness of Tacrolimus**

Parameters	Variation	Tacrolimus	
		Retention time(min)	Assay (%)
Flow rate(ml/min)	0.8	7.52	98.52
	1.0	6.02	100.21
	1.2	5.03	98.59
pH	2.8	6.02	99.56
	3.0	6.02	100.23
	3.2	6.02	101.98
Column temperature (^{0}C)	45	6.03	100.95
	50	6.02	99.56
	55	5.99	101.60
Wavelength(nm)	205	6.04	101.85
	210	6.02	100.98
	215	6.04	101.37

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Tacrolimus in capsule dosage form. The developed method is simple, rapid, linear, accurate, precise and specific. Results from the validation experiments shows that the method is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of Tacrolimus in capsule dosage form.

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