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UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF TACROLIMUS IN BULK AND FORMULATION

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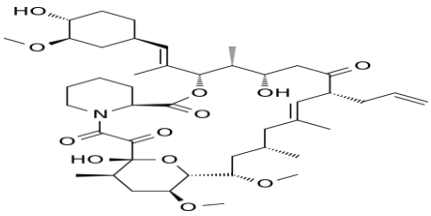
ABSTRACT

Objective: The objective of the present work is to develop a new, simple, sensitive and precise UV spectrophotometric method for Tacrolimus in bulk and pharmaceutical formulation as per ICH guidelines. **Method**: The UV spectrophotometric method has been developed using by methanol as solvent to determine the Tacrolimus in bulk and pharmaceutical formulation. The λ_{max} of Tacrolimus in methanol was found to be 292 nm. **Results**: The drug was proved linear in the concentration range of 10-50 µg/ml and regression coefficient was found to be 0.999. The LOD and LOQ of Tacrolimus was found to be 0.453 and 0.256 respectively. This method was successfully applied to Tacrolimus in marketed formulation and results were in good agreement with label claims. **Conclusion**: Depending on the results, the given method can be successfully applied for assay of Tacrolimus in Capsule formulation.

INTRODUCTION

Tacrolimus (also FK-506 or Fujimycin) is an immunosuppressive drug whose main use is after organ transplant to reduce the activity of the patient's immune system and so the risk of organ rejection. It is also used in a topical preparation in the treatment of severe atopic dermatitis, severe refractory uveitis after bone marrow transplants, and the skin condition vitiligo. It was discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria Streptomyces tsukubaensis. Tacrolimus is chemically known as a macrolide. It reduces peptidyl-prolyl isomerase activity by binding to the immunophilin FKBP-12 (FK506 binding protein) creating a new complex. This FKBP12-FK506 complex inhibits calcineurin which inhibits T-lymphocyte signal transduction and IL- 2 transcription [1].

Figure 1: Chemical structure of Tacrolimus



Molecular formula and weight of Tacrolimus is $C_{44}H_{71}N_{013}$ and 822.03 respectively. Tacrolimus is practically insoluble in water but soluble in methanol, ethanol, acetone, ether and ethyl acetate. Analytical methods are reported for determination of Tacrolimus by UV Visible spectroscopy[2-4]. The aim of this study is to give a new, simple, sensitive, precise and reproducible UV spectroscopic method was developed for Tacrolimus in capsule formulation.

MATERIALS AND METHODS:

Materials: Tacrolimus was obtained as gift sample from Aadhar life Sciences, Solapur. Methanol was taken from Aadhar life Sciences Pvt. Ltd

Instruments: Analytical balance (AczetCY224C), Sonicator (Labman), UV-Visible Spectrophotometer (LMSP UV-1900)

Experimental:

Preparation of standard stock solution:

Accurately weighed 10 mg of Tacrolimus was transferred to a 10 ml volumetric flask; dissolved in methanol and volume was made upto the mark with methanol. (Conc: 1000µg/ml)

Working Standard:

Add 0.1 ml of standard stock solution in 10 ml volumetric flask and add 5 ml of methanol, mix for 2 min and make up the volume upto 10 ml with methanol. (Conc: 10 µg/ml)

Selection of analytical wavelength was done by scanning above solution in the range 200-400 nm

Procedure for plotting calibration curve:

For calibration curve, in a series of 10 ml volumetric flasks, 0.1 to 0.5 ml of standard stock solution of 1000µg/ml was pipetted out separately and the volume was made upto the mark using Methanol. The absorbance was measured at wavelength 292 nm against the blank solution.

Sample stock solution:

20 capsule content were weighed and mixthem in mortar and pestle. Powder weight equivalent to 10 mg Tacrolimus was weighed and transferred into the 10 ml volumetric flask and add 5 ml of methanol, sonicate for 10 minutes and make the volume to 10 ml with methanol. (Conc: 1000µg/ml)

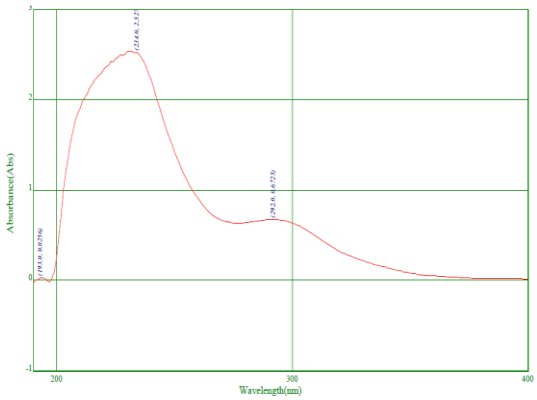
Sample solution:

ml of above solution was then transferred into a 10 ml volumetric flask and 5 ml of methanol was added, sonicate for 10 minutes and make the volume upto 10 ml with the methanol and analysed at 292 nm. Then % purity of Tacrolimus was calculated. (Conc: 50 µg/ml)

RESULTS AND DISCUSSION:

The absorption spectrum shows λmax of Tacrolimus at 292 nm

Fig 2: UV Scan of Tacrolimus



The proposed method was validated according to ICH Q2 R1 guidelines for validation of analytical procedure.

Linearity:

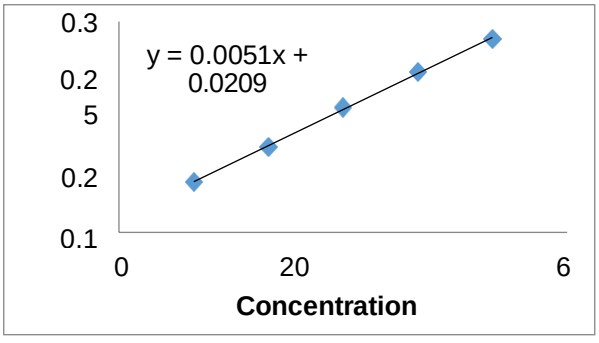
Five different concentrations of Tacrolimus were prepared and analysed at wavelength 292 nm. The regression coefficient was found to be 0.999. The absorbance was found in limit i.e. 0-1. (table no 1)

Table No2: Optimisation parameters of Tacrolimus

Table no 1: Linearity data of Tacrolimus

Sr.No	Conc (µg/ml)	Abs
1	10	0.0717
2	20	0.1215
3	30	0.1775
4	40	0.2283
5	50	0.2748

Figure 3: Linearity



Parameters	Method values
Maximum Wavelength	292
Beers law	10-50µg/ml
Correlation Coefficient	0.9991
Regression Equation	Y=0.0051x + 0.0209
Slope	0.0051
Intercept	0.0209

Accuracy:

The Samples were prepared of 80%, 100%, 120% concentrations by spiking 0.24 ml ,0.3ml,and 0.32 ml of Tacrolimus stock solution of 1000 µg/ml Samples were injected in triplicate to calculate % RSD % recovery was found to be in range 99%-101%. Hence the parameter was found to be validated [5].

Range is an interval between highest and lowest concentration limit of the analyte i.e. 10-50 µg/ml.

Repeatability:

A single sample of 30 µg/ml was prepared as described and 5 times absorbance was measured. The RSD of 5 readings was calculated. The obtained results were found within limit i.e. less than 2% RSD [5].

Limit of Detection: It was calculated by ANOVA technique. The limit of detection was found to be 1.87 µg/ml.

Limit of Quantification: The limit of quantification was found to be 5.68µg/ml.

Assay:

Sample solution of concentration 50 µg/ml was analysed at wavelength 292 nm and the % purity was calculated.

Range:

Sample ID	Conc (µg/ml)	Abs	Amount recovered	% Recovery	AVG	STDEV	RSD
80% Rep 1	23.93	0.1423	23.97	100.19	100.21	0.18	0.18
80% Rep 2	23.93	0.1426	24.02	100.40			
80% Rep 3	23.93	0.1421	23.94	100.05			
100% Rep 1	29.91	0.1775	29.90	99.98	99.90	0.09	0.09
100% Rep 2	29.91	0.1774	29.89	99.92			
100% Rep 3	29.91	0.1772	29.85	99.81			
120% Rep 1	35.89	0.2132	35.95	100.17	100.12	0.05	0.05
120% Rep 2	35.89	0.2134	35.92	100.07			
120% Rep 3	35.89	0.2133	35.93	100.12			

Table No 3: Accuracy

Table No 4: Repeatability

Sample ID	Absorbance
Rep 1	0.1775
Rep 2	0.1773
Rep 3	0.1776
Rep 4	0.1779
Rep 5	0.1774
Avg	0.17754
STDEV	0.00023
RSD	0.13

Table no 5: Result of Assay

Formulation	LabeledAmount	Amount obtained	% purity
Prograf Capsules	1 mg	99.64	99.64%

CONCLUSION:

An analytical UV spectrophotometric method was developed & validated thoroughly for quantitative determination of Tacrolimus in Capsule Formulation. The presented method was found to be simple, precise, accurate, and reproducible gives an acceptable recovery of the analyte.

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