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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR QUANTIFICATION OF TACROLIMUS IN BULK AND A CAPSULE DOSAGE FORM BY UV SPECTROSCOPY METHOD

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Abstract:

Quantitative and qualitative analysis of one or more analytes with stepwise instructions involves specific analytical technique. UV Spectroscopy is one of the analytical methods which involve the study of amount of light absorbed at each wavelength of UV and visible region. The present study is aimed to develop and validate a rapid, robust, effective, specific and accurate UV-Vis method for the determination of Tacrolimus in pharmaceutical dosage forms according to International Conference on Harmonization (ICH Q2 R1) guidelines. The method development was done using phosphate buffer pH 7.4.The method validation parameters like linearity, precision, accuracy, robustness and ruggedness were assessed for the developed method. Pure drug solutions were prepared using Phosphate buffer pH 7.4 in the concentration range of 10-100 µg/ml and the linear regression coefficient (R^2) was found to be 0.999.The λ_{max} was found to be at 294 nm and the developed method was found to be precise within the interday and intraday studies and showed percentage Relative standard deviation of 1.759 and 1.566 % respectively. Thus a precise, simple and cost-effective UV-Vis method for the determination of tacrolimus was developed. The method developed was found to be precise, robust, simple and cost-effective method for the determination of tacrolimus in the bulk and in the pharmaceutical dosage form with regression coefficient (R^2) of 0.999.

Key words: *Tacrolimus, regression coefficient, UV spectrophotometry, Method validation*

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INTRODUCTION:

Validation of developed method is a demonstration of the method suitability by determining an accuracy of the test results as well as an uncertainty and a traceability of measurements. [1,2]Method validation is needed to check performance characteristic of an analytical procedure meeting the requirements for intended analytical applications in laboratory studies. Method development is carried out with the objectives of obtaining accurate data which are reliable and consistent.[3]

Isolated from streptomyces tsukubanesis tacrolimus is a 23-membered macrolide lactone with molecular weight of 803.5 Da, widely used immunosuppressant in transplant patients. Half life of tacrolimus in human is found to be 8.7-11.3 h with mean bioavailability of approximately 21%.It is employed clinically for the prevention of organ transplant rejection, such as liver, kidney, heart, pancreas and bone marrow. The drug is additionally indicated for the treatment of atopic dermatitis, eczema, psoriasis and vitiligo. Tacrolimus is highly soluble in lipids and few organic solvents, slightly soluble in saturated hydrocarbons and insoluble in water. Pharmaceutical dosage forms like capsules, injection and ointment are available for clinical use. Tacrolimus has the below mentioned structure [4].

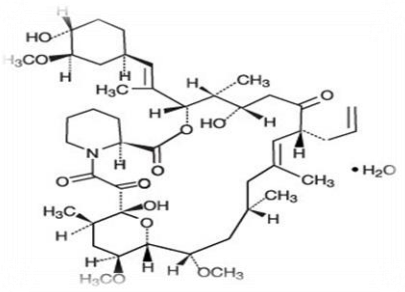

Figure-1: Chemical Structure of Tacrolimus

According to ICH Q2(R2) guidelines method development provides information and specifications for establishing, submitting, and maintaining evidence that an analytical method developed is fit for its intended purpose.[5]

Method validation is that the process of establishing documented evidence which gives high degree of assurance that product or equipment would meet the requirements for the intended applications. The major existing method for the determination of tacrolimus is by high-performance liquid chromatography (HPLC) as they require longer and are not economical. Hence an attempt was made to develop UV method using aqueous medium i.e. Phosphate buffer pH 7.4 as diluent and UV method does not require the elaborate procedures that are usually related to the chromatographic method.

MATERIALS AND METHODS:

Instrumentation: UV-1601 UV-VISIBLE spectrophotometer make: Shimadzu

Chemicals and Reagents:

Tacrolimus was supplied from Dr. Reddy's Laboratories, Acetonitrile was purchased from SDFCL. All other chemicals used were of analytical grade.

Determination of λ_{max}:

For the determination of the optimum $\lambda_{max,}$ 2 µg/ml of tacrolimus solution was scanned in the UV wavelength range of about 200-400nm using Phosphate buffer pH 7.4+Conc.sulphuric acid and acetonitrile solutions as blank. The drug showed the maximum absorbance at 294nm, which was considered as λ_{max} for the further method development. The spectrum of the drug solution was recorded and as shown in the figure-2.

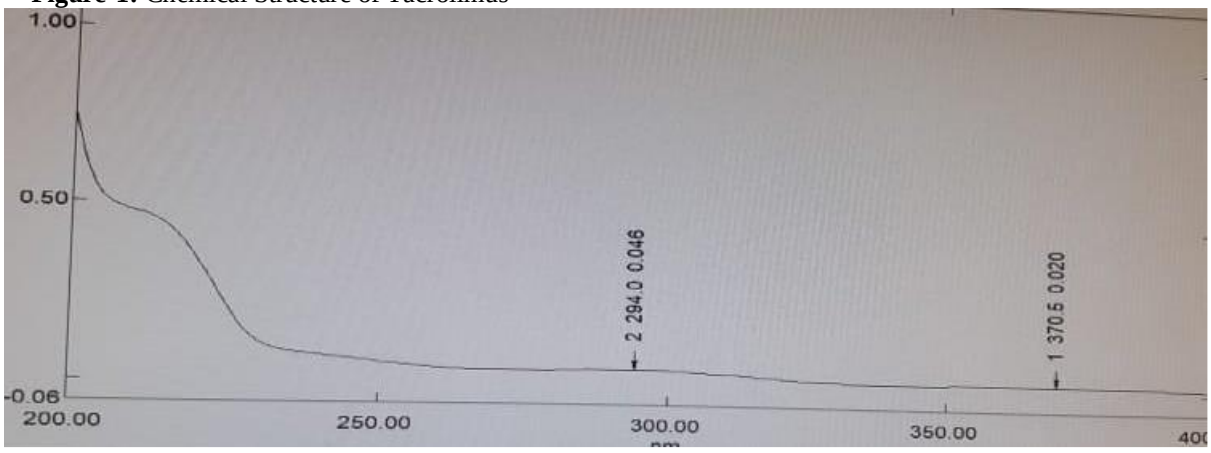

Figure-2: Tacrolimus drug showing **λmax at 294 m**

Preparation of Primary standard stock Solution:
Accurately weighed, 20 mg of Tacrolimus was dissolved in 10 ml of acetonitrile to result in a concentration of 2 mg/ml.

Preparation of secondary standard stock solution:
0.5 ml of primary standard stock solution was mixed with 0.5 ml of Sulphuric acid in a 10 ml volumetric flask and diluted with Phosphate buffer pH 7.4 up to the mark. The concentration of the obtained solution is 100µg/ml.

METHOD VALIDATION:
The developed method was validated for the parameters as below,

a) Linearity:
As per the method validation ICH Q2 (R1) guideline, the linearity of an analytical method can be explained as its capability to show "results that are directly proportional to the concentration of the analyte in the sample". .Aliquots of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 1.0 ml were withdrawn from secondary stock solution and diluted with 0.5ml of conc. sulphuric acid and made up with Phosphate buffer pH 7.4 solution to get 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml respectively. Absorbance of each aliquot was measured by UV spectrophotometer at 294nm using Phosphate buffer pH 7.4+Coc.sulphuric acid and acetonitrile solutions as a blank. The regression correlation coefficient [R^2] was found to be 0.998 (Fig-3) and the linearity data is shown in the Table-1.

Precision:
Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings.[6] It is determined by measuring the absorbance of different solutions of the same concentration on the same day (intraday) and on the next day (interday) and %RSD is calculated as shown in the Table-2.

Accuracy:
Accuracy of an analytical method is the degree of closeness between the 'true' value of analytes in the sample and the value determined by the method. The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (80%, 100% and 120% concentration) by addition of known amount of standard to the placebo. For each concentration three sets were prepared. The results are mentioned as in the Table-3.

Robustness:
Robustness is the evaluation of an analytical method wherein the results obtained are found to be reliable even when performed in a slightly varied condition [7]. It is the ability of a method to remain unaffected when slight variations are applied. Robustness of the developed method was determined by analyzing appropriate concentrations (60 µg/ ml) at different wavelengths and values of % RSD calculated using regression coefficient and the calculations are as represented in the Table 4.

Ruggedness:
Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst". As per USP the robustness of an analytical procedure is defined as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability in normal usage".Appropriate concentrations of tacrolimus were analyzed using different UV spectrophotometry equipment, by a different analyst to absorbance values. % RSD was calculated using regression coefficients obtained and the results are as shown in the Table-5.

Assay of Marketed Formulation:
Ten marketed formulations i.e., capsules of Tacrolimus containing 2mg drug per each capsule were taken, emptied into a mortar and pestle. Weighed amount of the capsule powder, which is equivalent to 20 mg of Tacrolimus was transferred into a 10 ml volumetric flask, into which 10 ml acetonitrile was added and the solution is sonicated for 3 minutes on bath sonicator. It is extracted with acetonitrile and filtered. The prepared solution was found to be a clear and having the strength 2000 µg/ml. From this solution 0.5 ml of solution was transferred into 100 ml volumetric flask and diluted with pH 7.4 Phosphate buffer to get required concentration.
From this solution, aliquots were prepared and were analyzed at 294 nm and the results are summarized in Table-6.

RESULTS:

Table 1: Linearity

Sl No	Concentration (µg/ ml)	Absorbance
1	10	0.078
2	20	0.198
3	30	0.291
4	40	0.385
5	50	0.486
6	60	0.596
7	70	0.697
8	80	0.798
9	90	0.897
10	100	0.987

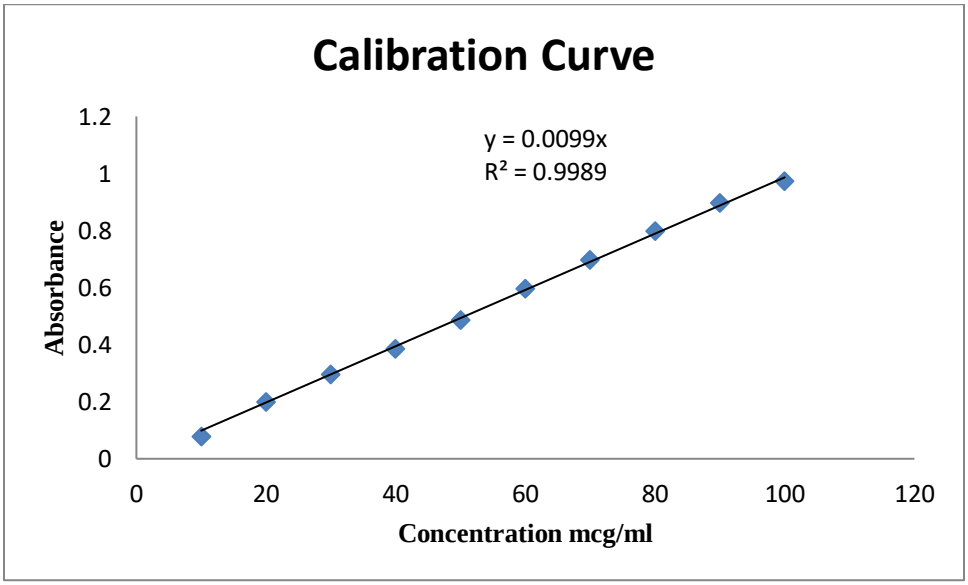

Figure 3: Calibration Curve of Tacrolimus drug in Phosphate buffer pH 7.4

Table 2: Intraday and interday precision studies

Sl No	Concentration (µg/ ml)	Absorbance (Intraday)	Absorbance (Interday)
1	60	0.597	0.478
2	60	0.581	0.476
3	60	0.592	0.477
4	60	0.586	0.472
5	60	0.594	0.471
6	60	0.596	0.473
7	60	0.589	0.475
8	60	0.591	0.478
9	60	0.583	0.493
% RSD		1.1759 ± 0.02	1.5667±0.06

Table 3: Accuracy

Conc(mcg/ml)	Spike level	Absorbance	% recovery	Mean % recovery	SD	% RSD
60	80 %	0.532	134.3434343			
		0.531	134.0909091		0.385739	
		0.539	136.1111111	134.8484848		0.816± 0.014
60	100 %	0.641	129.4949495			
		0.634	128.0808081		1.833085	
		0.631	127.4747475	128.3501684		0.807± 0.036
60	120 %	0.678	114.1414141			
		0.674	113.4680135		1.833085	
		0.671	112.962963	113.5241302		0.520± 0.045

Table 4: Robustness

Sl No	Concentration (µg/ ml)	292 nm	296 nm
1	60 µg/ml	0.898	0.789
2	60 µg/ml	0.891	0.786
3	60 µg/ml	0.896	0.784
4	60 µg/ml	0.893	0.785
5	60 µg/ml	0.895	0.782
6	60 µg/ml	0.892	0.78
% RSD		0.2951 ± 0.034	0.4004 ± 0.049

Table 5: Ruggedness

Sl No	Concentration (µg/ ml)	Analyst 1	Analyst 2
1	30 µg/ ml	0.276	0.278
2	30 µg/ ml	0.274	0.276
3	30 µg/ ml	0.271	0.272
4	30 µg/ ml	0.27	0.273
5	30 µg/ ml	0.279	0.277
6	30 µg/ ml	0.272	0.274
% RSD		1.237 ± 0.011	0.865 ± 0.019

TABLE 6: ASSAY OF CAPSULE FORMULATION

Formulation	Label Claimed	% Drug recovered
Tacrolimus capsule	2mg	99.1 %

DISCUSSION:

As per the ICH guidelines each method used for the analysis of parameter has been validated. Linearity studies carried out showed good linear relationship over the concentration range of 10-100 µg/ml.

The drug solutions were analyzed for accuracy by proposed method and the % recovery was found to be 134.84, 128.35, 113.52% with the %RSD<2 which indicated that there is no interference of the excipients used in the formulation indicating the developed method accuracy which can be used for the determination of tacrolimus both in bulk and in formulation than the more tedious and time consuming HPLC method.

Robustness was analysed by making small changes in analytical wavelength by ± 2nm i.e., 292nm and 296nm. The %RSD was found to be 0.2951 and 0.4004 respectively signifying that the developed method is robust.

The ruggedness was obtained by different analyst's i.e, analyst 1 and analyst 2. The % RSD was found to be 1.2 and 0.86 respectively which is not more than 2 indicating that the method is rugged enough to variation in the analysts.

Marketed formulations were analysed by developed method at 294nm. The drug concentration was calculated from the calibration curve of the drug and the % amount was found to be 99.1 %.

CONCLUSION:

The developed analytical method was validated as per ICH Q2 (R1) guidelines and found to be robust meeting the acceptance criteria of each parameter. The developed analytical method showed linearity in the concentration range of 10 to 100µg/ml. The λ_{max} was found to be at 294nm with percentage Relative standard deviation of 1.759 and 1.566 % respectively. It is concluded that the developed method is specific, linear, precise accurate, robust and sensitive to analyze tacrolimus in bulk and commercial dosage form. The main advantage of developed UV method over HPLC method is that it is less time consuming and also economical. Thus, the developed method can be used for routine analysis of Tacrolimus in pure form as well as in the pharmaceuticals.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interests regarding the publication.

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