DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TACROLIMUS IN BULK AND PHARMACEUTICAL NANOPARTICLES

ABSTRACT

An accurate, simple, reproducible and cost-effective UV visible spectrophotometric method was developed and validated for the determination of Tacrolimus in nanoparticles. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 291 nm. The response was linear in the range of 0.2-1.8 mg/ml (r^2 = 0.9989). The relative standard deviations for intra- and inter-day precision studies were found to be less than 2% which indicates method is precise.

***Keywords**: Tacrolimus, UV visible spectrometry, Validation, ICH.*

1. INTRODUCTION

Tacrolimus (TAC), a macrolide agent, derived from Streptomyces Tsukubaensis, inhibits T-lymphocyte activation through a process that is thought to involve it binding to an intracellular protein, FKBP- 12.[1] Tacrolimus is primarily used in post organ transplant patients to prevent organ rejection.[2] 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.[3] It is insoluble in water, slightly soluble in saturated hydrocarbons, and highly soluble in lipids and other organic solvents.[4] Pharmaceutical dosage forms such as capsules, injection and ointment are available for clinical use. In this study, we developed and validated a new UV visible spectrophotometric method for quantitation of tacrolimus in nanoparticles. The method was validated by following the analytical performance parameters suggested by the ICH guidelines. The aim of our investigation was to develop and validate an UV visible spectrophotometric method for the determination of tacrolimus in pharmaceutical dosage forms.

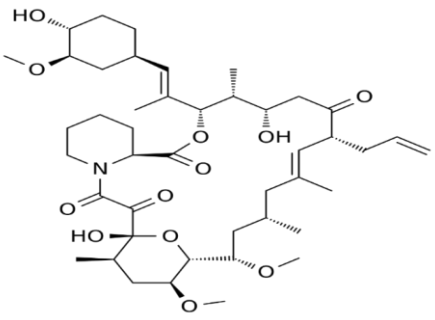

Figure 1: Chemical structure of tacrolimus

2. MATERIALS AND METHODS

2.1 CHEMICALS AND REAGENTS

Tacrolimus was procured from Centurion laboratories private limited (Vadodara, India). Methanol was purchased from the Merck (India).

2.2 DETERMINATION OF λ_{max}

First step in development of UV spectroscopic method is screening of each formulation component expected to be present in sample prepared for estimation of drug content in formulation and diffusion media over the entire UV range. Excipients should not interfere with drug peak at absorption maxima (λ_{max}) of drug and if any excipients interfere with drug peak, then method should be modified accordingly.

The λ_{max} (maximum absorbance) of Tacrolimus was determined by screening of 1.8 mg/ml drug solution respectively in methanol over entire UV range 200 – 400 nm.

2.3 CALIBRATION CURVES OF DRUGS IN METHANOL

200 mg of TAC was dissolved in 10 ml of distilled water and then the volume was made upto 100 ml with distilled water. Appropriate aliquots from the stock solution of TAC were transferred to 10 ml volumetric flasks and were diluted up to the mark with distilled water to prepare final drug concentration of 0.2, 0.6, 1, 1.4 and 1.8 mg/ml. The absorption of all the prepared solutions was then measured at the absorbance maxima, 291 nm against the reagent blank. The readings were recorded in triplicate. Mean value (n=3) along with the standard deviation (SD) are recorded. The average values of absorption were plotted graphically against the concentrations and regression coefficient was obtained.

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2.4 CALIBRATION CURVE OF TACROLIMUS DRUG IN METHANOL

Calibration curve was plotted for Tacrolimus drug in methanol (as a solvent) using UV-Visible spectrophotometer at 291 nm (λ_{max}). It shows the linearity in the range 0.2 mg/ml – 1.8 mg/ml with regression coefficient of 0.9989. Various values are shown in Table 1 and represented graphically in Figure 2.

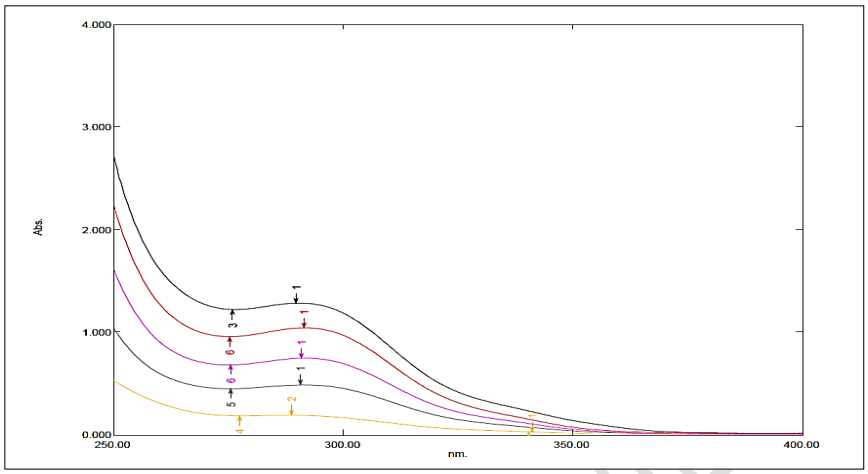
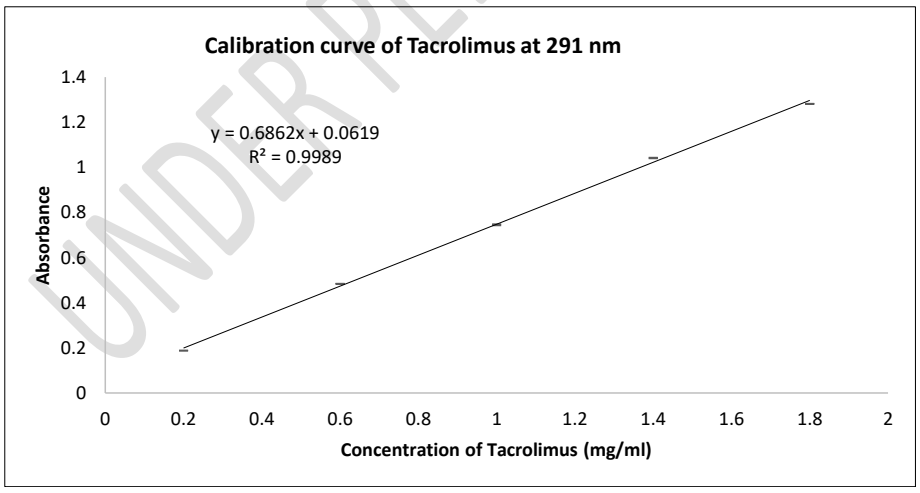


Figure 2: UV visible spectra of Tacrolimus drug

Table 1: Calibration curve of Tacrolimus drug in methanol

Conc (mg/ml)	ABS ± SD
0.2	0.188 ± 0.001
0.6	0.484 ± 0.001
1	0.745 ± 0.003
1.4	1.041 ± 0.002
1.8	1.281 ± 0.001

(n=3)



(n=3)

Figure 3: Calibration curve of Tacrolimus drug in methanol

2.5 PREPARATION OF SAMPLE SOLUTION

4 mg of formulated nanoparticles was added to 10 ml of methanol to precipitate protein. Followed by centrifugation at 3000 rpm for 5 min. After suitable dilution, the supernatant was scanned in the uv region of 200-400 nm. The conc. of tacrolimus was determined at 291nm by using regression equation of calibration curve.

2.6 METHOD VALIDATION

The method was validated in terms of parameters like specificity, precision, accuracy, linearity and range, LOD and LOQ. For all the parameters percentage relative standard deviation values were calculated. The proposed UV visible spectrophotometric method was validated as per ICH guideline.

2.6.1 ACCURACY

Accuracy was determined by adding the three different quantities[5, 6] [Low, Medium, and High] of the tacrolimus to the sample solution containing the concentration of 1 mg/ml. The results are shown in table 2.

2.6.2 PRECISION

The precision of an analytical procedure defines the degree of closeness of agreement between a series of measurements obtained from multiple samplings of homogenous sample under prescribed conditions. Precision of the method was reported as RSD% at different levels- repeatability, Intra-day precision and Inter-day precision.[5, 6] The results are shown in table 2.

2.6.3 REPEATABILITY

Repeatability was determined on 6 replicates of each concentration of the standard solution.[5] The results are shown in table 2.

2.6.4 LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by low concentrations of the standard solutions using the developed UV visible spectrophotometric method.[5] The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for tacrolimus found to be 0.09 mg/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 0.2 mg /ml for tacrolimus.

Table 2: Validation Parameters

Parameters	TAC
Detection wavelengths (nm)	291
Linearity range (mg/mL)	0.2-1.8
Correlation coefficient (r^2)	0.9989
Regression Equation	y= 0.6862x + 0.0619
Precision, %RSD Intra-day (n=3) Inter-day (n=3) Repeatability of measurement (n=6)	 100.65, 0.47% RSD 100.71, 0.69% RSD 101.54, 1.62% RSD
Accuracy (% Recovery, n=3) 50 % 100% 150%	 99.80±0.34 101.02±1.87 100.48±1.22
LOD (mg/mL)	0.09
LOQ (mg/mL)	0.2

3. RESULT AND DISCUSSION

The UV visible spectrophotometric method described in this research article was developed to provide a rapid quality control determination of tacrolimus in any pharmaceutical nanoparticles. The proposed method is less time consuming and simple. Method was evaluated for their accuracy and precision. The method was validated according to ICH guidelines. The linearity of the detector responses was determined by preparing calibration graphs. The linearity of the peak response versus concentration was studied from 0.2 to 1.8 mg/ml. The representative linear equation was y= 0.6862x + 0.0619 and a correlation coefficient (r^2) is 0.9989. Recovery study was performed in triplicate and average recovery was found in range of 99.80% - 101.02% indicating that the proposed method for the determination of tacrolimus in pharmaceutical nanoparticles was highly accurate. The precision was found to be 100.65, 0.47% RSD for intra-day and 100.71, 0.69% RSD for inter-day.

4. CONCLUSION

A convenient and rapid UV visible spectrophotometric method has been developed for analysis of tacrolimus in nanoparticles. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day %RSD coupled with excellent recoveries. The proposed method is simple, economic fast, accurate and precise for the quantification of tacrolimus in dosage form as well as bulk drugs for quality control purpose.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and

producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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