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Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Tacrolimus in Bulk and Pharmaceutical Nanoparticles

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

A UV-visible spectrophotometric method for quantifying tacrolimus in nanoparticles has been developed and validated, and it is accurate, simple, reproducible, and affordable. For drug analysis, optimal conditions have been identified. 291 nm was discovered to be the maximum wavelength (max). In the range of 0.2-1.8 mg/ml, the response was linear (r2 = 0.9989). The intra- and inter-day relative standard deviations for precision studies were found to be less than 2%, indicating that the procedure is precise.

Keywords: Tacrolimus; UV visible spectrometry; validation; ICH.

1. INTRODUCTION

Tacrolimus (TAC), a macrolide agent, derived from Streptomyces Tsukubaensis, inhibits T-lymphocyte activation by binding to an intracellular protein called FKBP- 12 [1,2]. Tacrolimus is mainly used in post organ transplant patients to prevent organ rejection [3].

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It is also used as a topical formulation to treat severe atopic dermatitis, severe refractory inflammation after bone marrow transplantation, and white spots of skin disease [4]. It's insoluble in water, soluble in saturated hydrocarbons just slightly, and highly soluble in lipids and other organic solvents [5]. For therapeutic use, pharmaceutical dose forms such as capsules, injections, and ointments are available. We developed and validated a new UV visible spectrophotometric method for measuring tacrolimus in nanoparticles in this study. Following the ICH criteria for analytical performance characteristics, the method was verified. The goal of our study was to create and test a UV visible spectrophotometric method for determining tacrolimus in pharmaceutical dose forms.

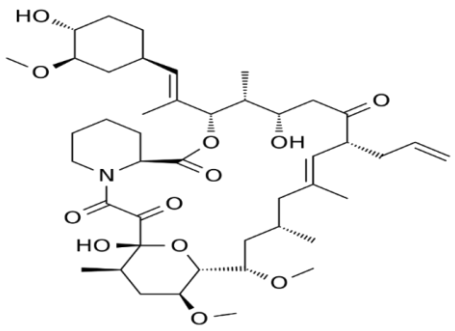

Fig. 1. Structure of tacrolimus

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 produced solutions was then measured against the reagent blank at the absorbance maxima, 291 nm. The readings were taken three times. The standard deviation (SD) and mean value (n=3) are recorded. The average absorption values were graphically plotted against the concentrations, and the regression coefficient was calculated.

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 Fig. 2.

Table 1. Calibration curve of tacrolimus drug in methanol

Concentration (mg/ml)	Absorbance ± 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)

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

Specificity, accuracy, linearity, and range, as well as LOD and LOQ, have all been validated for this approach. For all parameters, percentage relative standard deviation values were obtained.

According to ICH requirements, the suggested UV-visible spectrophotometry has been validated.

2.6.1 Accuracy

The accuracy of the tacrolimus was determined by adding three different quantities[6,7] [Low, Medium, and High] to a sample solution with a concentration of 1 mg/ml. Table 2 displays the results.

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 [6,7]. The results are shown in Table 2.

2.6.3 Repeatability

Six replicates of each concentration of the standard solution were used to determine repeatability [6]. The results are shown in Table 2.

2.6.4 Limit of detection and limit of quantification

Low quantities of standard solutions were used to determine the developed method's limit of detection (LOD) and limit of quantification (LOQ)

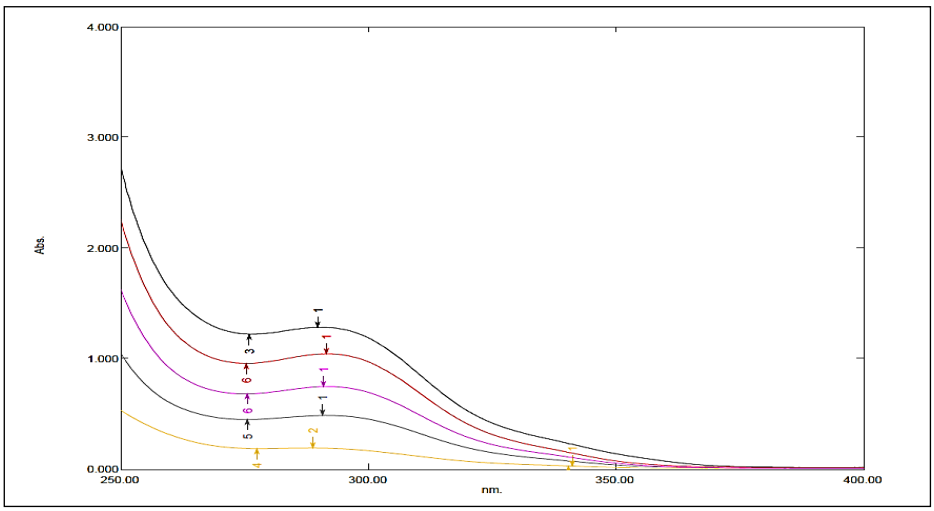

Fig. 2. UV visible spectra of tacrolimus drug

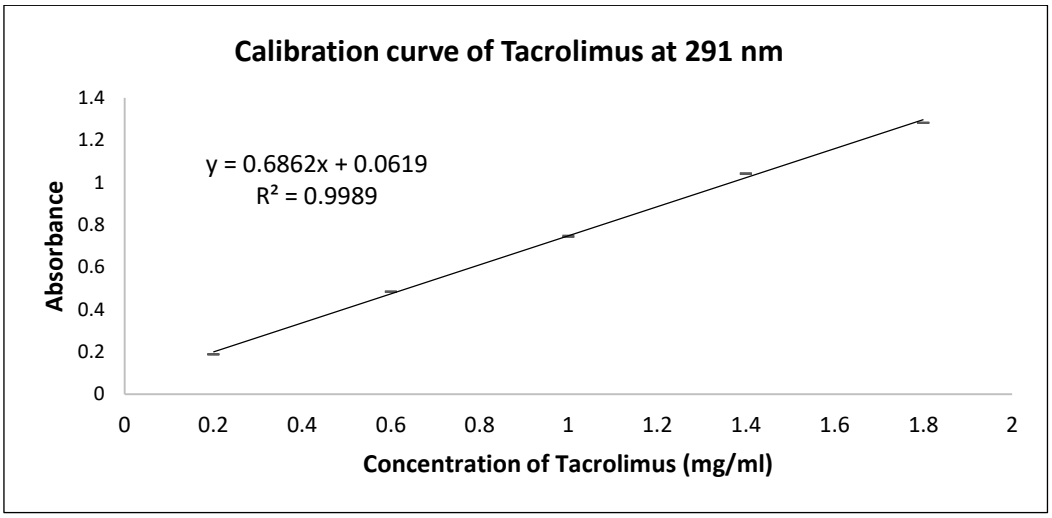

Fig. 3. Calibration curve of tacrolimus drug in methanol
(n=3)

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)	100.65, 0.47% RSD
Inter-day (n=3)	100.71, 0.69% RSD
Repeatability of measurement (n=6)	101.54, 1.62% RSD
Accuracy (% Recovery, n=3)	
50 %	99.80±0.34
100%	101.02±1.87
150%	100.48±1.22
LOD (mg/mL)	0.09
LOQ (mg/mL)	0.2

using the UV visible spectrophotometric approach[6]. The LOD is the analyte concentration at which a detectable response can be obtained (signal to noise ratio of 3). For tacrolimus, the LOD was found to be 0.09 mg/ml. The LOQ is the minimum concentration of analyte that produces an accurately quantifiable data (signal to noise ratio of 10). The LOQ for tacrolimus was 0.2 mg/ml.

3. RESULTS AND DISCUSSION

The UV visible spectrophotometric approach presented in this study was created to give a quick qualitative determination of tacrolimus in pharmaceutical nanoparticles. The proposed method is more concise and straightforward. The accuracy and precision of the methods were assessed. According to ICH criteria, the approach was validated. Calibration graphs were used to determine the linearity of the detector responses. From 0.2 to 1.8 mg/ml, the linearity of the peak response vs concentration was investigated. y= 0.6862x + 0.0619 was the sample linear equation, and the correlation coefficient (r2) was 0.9989. The recovery research was done in triplicate, and the average recovery was found to be between 99.80% and 101.020%, showing that the suggested method for determining tacrolimus in pharmaceutical nanoparticles was extremely 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

For the analysis of tacrolimus in nanoparticles, a simple and quick UV visible spectrophotometric approach has been established. The assay has a linear response over a large concentration range. Low percent RSD intra-day and inter-day, with excellent recoveries. For the quantification of tacrolimus in dosage form as well as bulk medicines for quality control purposes, the proposed approach is simple, economical, rapid, accurate, and exact.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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