Research Article

UV Spectroscopic Method for Estimation of Tacrolimus in Bulk and Tablet Formulation by Area Under Curve Method

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ABSTRACT

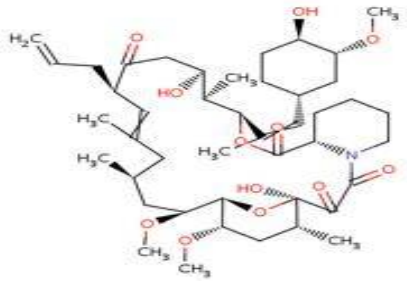
The aim behind the present study was to elaborate the novel spectroscopic method which is very simple, accurate, more precise, economic andrapid for estimation of tacrolimus by area under curve method. Tacrolimus is macrolide antibiotic acts as immunosuppressant's which is more potent than cyclosporine. The literature survey till date states that several method have been reportedfor quantification of Tacrolimus. This method involved absorbance maxima method based on absorbance measurement of tacrolimus in HCL: Water (60:40%v/v) at λ max 294 nm.This method is validated as per ICH guidelines for its accuracy, precision, reproducibity, linearity.

Keyword: Tacrolimus, Area under curve, ICH Guidelines.

INTRODUCTION

Tacrolimus is macrolide antibiotic acts as immunosuppressant's which is more potent than cyclosporine. It is used for the treatment of athopic dermatitis, designed for direct application on the surface of the skin. It binds to different imunophilliins to inhibit thecalcineurin and It inhibits the cell mediated and humeral immune response. Thedifferent methods have been reported for the determination of tacrolimus in bulk drug and in dosage form with the combination of other drug in various conditions of organ rejection conditions or organ transplantation like in kidney transplantation to avoid the rejection reactions. Among the various method available for the determination of drug, the spectroscopic method are very popular because of their specificity and their low coast on the other hand long term exposure to UV and air, it goes on degradation of active moiety in the formulation. This study presents the new method spectroscopic method for the determination of tacrolimus in bulk.

Structure



MATERIAL AND METHODS

The pre API sample of Tacrolimus obtained from Glenmark Pharmaceutical, Sinner (Nasik)as a free gift sample, while the other solvent such as methanol from the Jinendra scientific laboratory, Jalgoan.which is especially of spectroscopic grade. The water used for the whole experimental work is double distilled. The marketed preparation of tacrolimus in the dose of 1mg was purchased from Pangraf from Panacea.

Instrumentation

Shimadzu uv 1800; double beam uv visible spectrophotometer was used along with the length 1 cm. A match pair of quartz cell for spectroscopic method, digital weighing

balance and the calibrated glass wares was used for the study.

Preliminary solubility studies of drug

10 mg of (API) was weighed and solubility was checked in 10 ml water, methanol, 0.1N NaOH and 0.1 N HCl. The drug was found to be freely soluble in inorganic solvents as sulphuric acid, hydrochloric acid, acetonitrile etc. but practically poorly soluble in water, 0.1NNaOH. Therefore HCl and water (60:40 % v/v) was selected as diluent as they are freely available and drug was also found to be stable in methanol for 24 hours in stability studies. Tacrolimus.

Preparation of standard stock solution and calibration curve

Standard stock solution of pure drug containing 1000 µg/ml of tacrolimus prepared in HCl and distilled water in 60:40%v/v. The working standard solutions of the drug were obtained by dilution of the stock solution in the distilled water. Series of solutions with conc. 2, 4, 6, 8, 10 µg/ml of Tacrolimus were used to prepare calibration curve. Solutions were scanned and proposed methods were applied for the determination of area under curve. Methanol and Water 60:40 % v/v was used as blank solution.

Preparation of sample stock solution

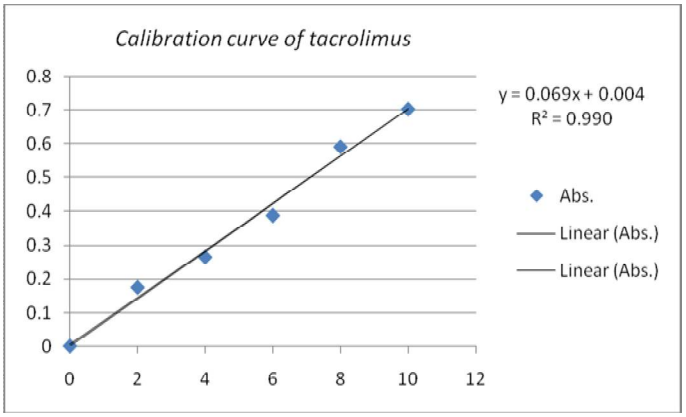
A drug equivalent to100 mg was transferred into a 100 ml volumetric flask (1000 µg/ml). From this 10 ml was withdrawn and and diluted upto 100 ml with solvent. From this further 1 ml was diluted up to 10 ml and used as stock solution**.**

Method: Area under curve (AUC)

It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths which belongs from 180 to 400 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.

Preparation of calibration curve

From above working std. stock solution of Tacrolimus (100 µg/ml), pippete out from stock solution 0.2 to 1 ml and transferred to series of 10 ml volumetric flasks and final volume made upto mark with diluent to form solutions of 2 to 10µg/ml of Tacrolimus. These solutions were then scanned in the range of 1800-400 nm against diluent as blank. The absorbance maxima (λmax) was found to be 294 nm for Tacrolimus and then calibration curve was plotted as absorbance vs. concentration.



Sample preparation for analysis of Tablet formulation

Twenty tablets containing 1 mg of Tacrolimus weighed, average weight calculated and triturated to fine powder and then weight equivalent 100mgof Tacrolimus transferred to 100 ml of volumetric flask containing proposed diluent, then sonicated for 15 to20 minutes and filtered through Whatman filter paper no. 42 to form 500µg/ml of Tacrolimus std. stock solution and final volume made upto mark with diluent. From this, 0.2 ml of aliquot transferred in 10 ml of volumetric flask containing diluent to form 2 µg/ml of Tramadol hydrochloride stock solution and scanned in the range of 180-400 nm against methanol as blank at 294

nm and then drug content of solution was calculated by using standard calibration curve.

RESULTS AND DISCUSSION

Method validation

The Selected method was validated as per ICH guidelines using different parameter.

Linearity

The linearity was evaluated by analyzing different concentration of standard solution of Tacrolimus. The Beer Lambert's law was obeyed in the concentration range of 2-10 µg/ mL with regression coefficient of 0.9904.

Ruggedness

Ruggedness of the selected method is determined by analysis of stock from homogenous slot by two analyst using same operational and environmental conditions in laboratory.

Accuracy (% recovery)

The accuracy of the methods was performed by calculating recovery of Tacrolimus by the standard addition method. Known amounts of standard solutions of Tacrolimus were added at60%, 100% and 140% levels to pre quantified Tacrolimus sample solutions of 20 µg/ml. The amount of Tacrolimus was estimated by applying obtained values to the respective regression equations.

Precision

To determine the precision of the method, Tacrolimus solutions at linear concentration were analyzed each three times. Solutions for the standard curves were prepared fresh every day.

CONCLUSION

Till date there is no Area Under Curve spectrophotometric methods have been described for the determination of Tramadol. The present study was undertaken with an objective of developing simple, sensitive and reliable analytical method like UV-Visible spectrophotometry for estimation of Tacrolimus. The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate.

Table 1: Spectrophotometric characteristics and statistical data of the regression equations

Parameters	Results
λmax (nm)	260-310
Beer`s range (µg/ mL)	2-10
Regression equation	Y=0.0697X +0.0049
Correlation coefficient	0.9904
Intercep	0.0039
Slope	0.0077

Table 2: Results of Analysis of Tablet Formulation

S. No	Label claim	Amount in test solution	Amount found
Sample-1	1 mg	20 µg/mL	19.50 µg/mL

Table 3: Intra and interday Precision

Label Claim	Amount in solution	Intraday Precision Interday			Precision
		Set 1	Set 2	Set 3	
1 mg per Tab	20µg/mL	99.10%	99.14%	99.24%	99.16%

Table 4: Recovery data of Tcrolimus

Level of % Recovery	Concentration Taken (µg/ mL)	Concentration estimated(µg/ mL)	% Analytical Recovery
60%	20	19.56	97.55%
100%	22	21.86	99.30%
140%	24	23.79	98.77%

Preparation of calibration curve

From the stock solution working standard solution (0.2 – 1 ml) were transferred into a series of 10 ml volumetric flask, diluted up to mark with double distilled water and scanned in the uv spectrophotometer on spectrum mode from the wavelength range 180-400 nm. A calibration curve was prepared by plotting the area versus concentration. The calibration curve was linear in concentration range of 2-10 µg/ml.

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