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Research Article

REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (RP-HPLC) METHOD FOR DETERMINATION OF TACROLIMUS IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

An accurate, simple, and reproducible liquid chromatographic method was developed and validated for the determination of Tacrolimus in capsules. The analysis was performed at ambient temperature on a reversed-phase C18 column with UV detection at 213 nm. The mobile phase was acetonirile (100 %) used at a constant flow rate of 0.9 ml/min. The method was validated in terms of linearity, precision, accuracy, and specificity .The response was linear in the range of 5-250 µg/ml (r^2 = 0.9978). The relative standard deviations for intra- and inter-day precision studies were found to be less than 2% with 98.90 to 101.12% accuracy, respectively.

Keyworlds: Tacrolimus, RP-HPLC

INTRODUCTION

Tacrolimus was discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria Streptomyces tsukubaensis Tacrolimus, 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. Tacrolimus is primarily used in post-organ transplant patients to prevent 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 is insoluble in water, slightly soluble in saturated hydrocarbons, and highly soluble in lipids and other organic solvents. Pharmaceutical dosage forms such as capsules, injection and ointment are available for clinical use.

In this study, we developed and validated a new chromatographic method for quantitation of TCR in capsules 1 mg. The method was validated by following the analytical performance parameters suggested by the ICH guidelines.

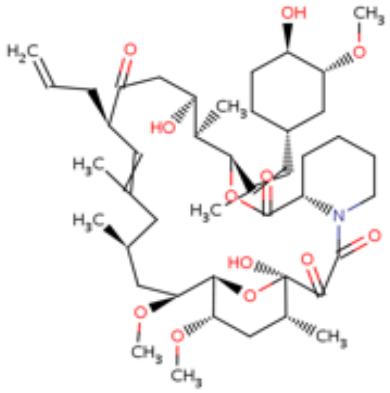

Fig. 1: Structure of Tacrolimus

Till now most of the published methods for Tacrolimus determination in blood are based on LC-MS, HPLC tandem mass spectroscopy and ELISA essay[5, 6, 7]. Author Maria and co workers published method based on HPLC-UV detector for determination of Tacrolimus in pharmaceutical dosage form in the presence of its degradation products[8]. The aim of our investigation was to develop and validate an LC method for the determination of TCR in pharmaceutical dosage forms. Finally we developed method to detect Tacrolimus at ambient temperature using single mobile phase, acetonitrile at detection wavelength 213 nm.

MATERIALS AND METHODS

Tacrolimus was kindly gifted from Alembic Pharmaceutical, Baroda. Marketed formulation of Tacrolimus (Pangraf from Panacea) was purchased from Indian market contains 1 mg of Tacrolimus. Acetonitrile (HPLC grade) was purchased from the Merck (INDIA).

Apparatus and Chromatographic Conditions

The HPLC system consisted of a Young Lin 9101 vaccum degasser, a Young Lin 9110 quaternary pump and a Young Lin 9160 photodiode array detector (Seoul, South Korea). An YL-clarity chromatography data system was used to record and evaluate the data collected during and following chromatographic analysis. The chromatographic separation was achieved on a Purospher® 5µm, 250mm X 4.6mm. The mobile phase was acetonitrile (100%) pumped at a constant flow rate of 0.9 ml/min. The eluent was monitored using Photodiode array detector at a wavelength of 213 nm. The column was maintained at room temperature and injection volume of 20µl was injected. The mobile phase was filtered through 0.45µm Chrom Tech Nylon-66 filter to use. Under these conditions, the retention time (t_R) of Tacrolimus was approximately 5.3 min. The peak purity was checked with the photodiode array detector.

Preparation of Standard Solution and Calibration Graphs

For the preparation of standard stock solution, 10mg of Tacrolimus was taken and dissolved in the 10ml acetonirile to get the concentration of 1000 µg/ml. Then appropriate dilutions were done to adjust the final concentration 5, 50 100, 150, 200, 250 µg/ml. The results of calibration curve are shown in table 1.

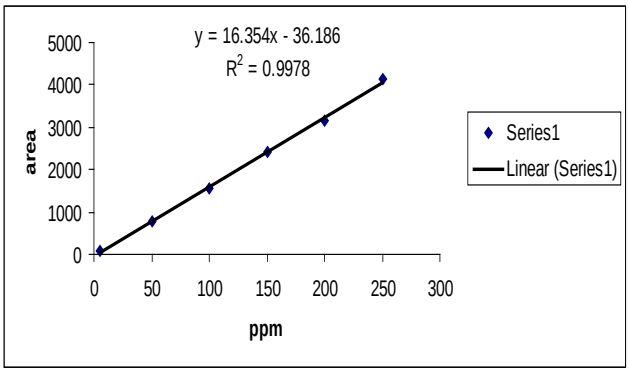

Fig. 2: Standard curve of Tacrolimus

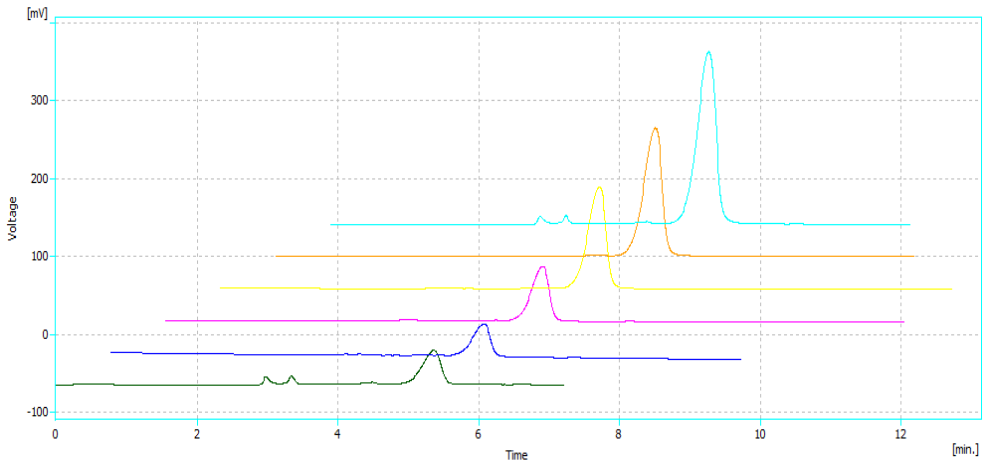

Fig. 3: Chromatogram of Tacrolimus

Preparation of Sample Solution

The contents of 20 capsules were placed in a mortar, and an amount of powder equivalent to 10mg of Tacrolimus was taken and dissolved in the 100 ml of acetonitrile.Stock solution was diluted to obtain the final concentration of 100 µg/ml of Tacrolimus. The results are shown in table 2.

Table 1: Data derived from standard curve.

Parameters	Tacrolimus
Linear Range (µg/ml)	5-250
Retention time (min)	5.32
Slope	16.35
Intercept	36.18
Standard deviation of slope	0.269
Standard deviation of intercept	2.92
Limit of Detection (µg/ml)	0.20
Limit of Quantification (µg/ml)	0.60
Linear equation	y = 16.354x - 36.186
R^2 value	0.9978
Tailing factor (As)	0.815

Table 2: Assay result of market formulation

Formulations	Actual concentration (µg/ml)	%Tacrolimus
Pangraf (Panacea)	100	98.91

Method Validation

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

Accuracy

Accuracy was determined by adding the three different quantities [Low, Medium, and High] of the Tacrolimus to the sample solution containing the concentration of 150 µg/ml. The results are shown in table 3.

Precision

Precision was determined by performing Intra day and Inter day determination concentration on three different concentrations as shown in table 3.

Repeatability

Repeatability was determined on 6 replicate of each concentration of the standard solution. The results are shown in table 3.

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. 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 0.20µg/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 0.60 µg /ml for Tacrolimus.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments. The average %RSD values with two different instruments were 1.32 and 1.46 respectively.

Table 3: Summary of validation parameter

Parameters	Tacrolimus
Recovery (%)	98.93 - 101.16
Repeatability (RSD, n=6)	0.817-1.920
Specificity	Specific
Precision Range (CV)	
Intra-day (n=3)	0.454-1.217
Inter-day (n=3)	0.817-1.920

RESULT AND DISCUSSION

The reversed-phase LC method described in this paper was developed to provide a rapid quality control determination of TCR in capsules. 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. All samples were analyzed by using the chromatographic conditions described. The linearity of the detector responses was determined by preparing calibration graphs. The linearity of the peak response versus concentration was studied from 5 to 250 µg/ml. The representative linear equation was Y = 16.354X - 36.186 and a correlation coefficient (*r*) is 0.9978. Recovery study was performed in triplicate and average recovery was found in range of 98.93% - 101.16% indicating that the proposed method for the determination of Tacrolimus in capsule was highly accurate (table 2).The precision is usually expressed as the %RSD and it was found to be. 0.817-1.920.The Inter-day and intra-day precision were 0.817-1.920 and 0.454-1.217 respectively.

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of Tacrolimus in capsule dosage form. 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 simultaneous quantification of Tacrolimus in dosage form as well as bulk drugs for quality control purpose.

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