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Research Article

Development and Validation of UV-Spectrophotometric Method for Determination of Pimecrolimus in Bulk and in Cream Formulation

Jain Pritam[1]*, Dhulgunde Snehal[2] and Nandre Mayur[2]

[1]Department of Pharmaceutical Analysis, R. C. Patel Institute of Pharmaceutical Education and Research, Maharashtra, India

[2]M. Pharm. Student, R. C. Patel Institute of Pharmaceutical Education and Research, (M.S.), India

***Corresponding Author:** Jain Pritam, Associate Professor, Department of Pharmaceutical Analysis, R. C. Patel Institute of Pharmaceutical Education and Research, Maharashtra, India.

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Abstract

A simple, rapid, accurate and sensitive UV-spectrophotometric method has been developed for estimation of Pimecrolimus from bulk and pharmaceutical cream formulation. The method based on the sulfuric acid reaction and the λ_{max} of Pimecrolimus in acetonirile was found to be 360 nm. The drug follows linearity in the concentration range 10-60 µg/ml with correlation coefficient value 0.9978. The proposed method was applied to pharmaceutical cream formulation and % amount of drug estimated 98.16% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120%. The % recovery was found to be in the range 98.54%– 99.69%. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. For routine analyses of pimecrolimus in bulk and in pharmaceutical dosage form, the mentioned procedure was rapid and employed as a quality-control tool.

Keywords: Pimecrolimus; UV; Validation; Quantitative Determination

Introduction

Pimecrolimus is a crystalline powder that ranges in colour from white to off-white. It is insoluble in water but soluble in methanol and ethanol. Pimecrolimus belongs to the ascomycin class of macrolactam immunosuppressives, acting by the inhibition of T-cell activation by the calcineurin pathway and inhibition of the release of numerous inflammatory cytokines, thereby preventing the cascade of immune and inflammatory signals [2]. Pimecrolimus is chemically (1R,9S,12S,13R,14S,17R,18E,21S,23S,24R,25S,27R)-12-[(1E)-2-{(1R,3R,4S)-4-chloro-3-methoxycyclohexyl}-1-methylvinyl]-17-ethyl-1,14-dihydroxy-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-aza-tricyclo [22.3.1.04,9] octacos-18-ene-2,3,10,16-tetraone, (Figure 1) having a molecular formula C43H68CINO11, with molecular mass 810.4 g/ml [1].

The literature survey revealed that various methods of analysis for pimecrolimus alone or in combination with other drugs have been reported, which included, HPLC-MS/MS [3], RP-HPLC [2] and LC-TMS [4].

To our knowledge, there are currently no UV Spectrometric method in bulk and pharmaceutical formulation described for

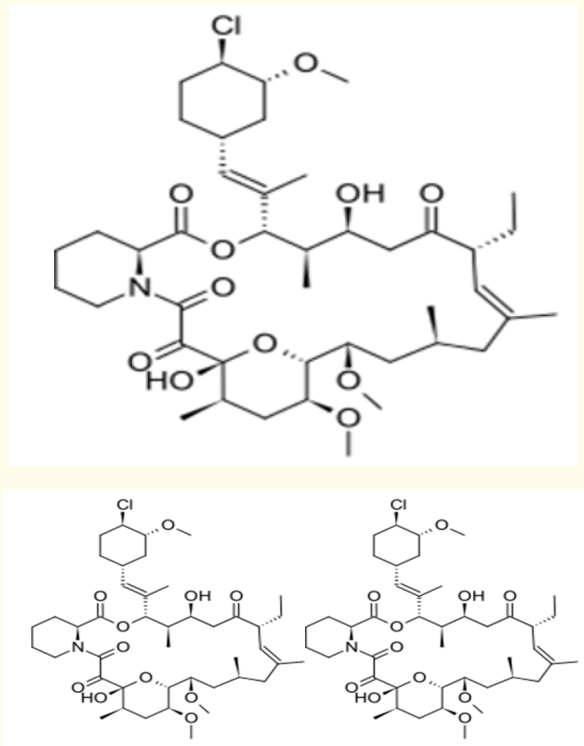

Figure 1: Chemical structure of Pimecrolimus.

pimecrolimus. When there is no interference from excipients, the spectrophotometric methods are reliable and simple to use. As a result, they are frequently utilised for the quantification of medicines in formulations. There is no Pharmacopoeia that currently describes pimecrolimus.

Accordingly, the objective of this study was to develop and validate the spectroscopic method for the estimation of Pimecrolimus in bulk and pharmaceutical formulation as per ICH guidelines [8]. The aim of the present study was to develop and validate simple, fast, selective and economical spectrophotometric methods for the routine analysis of pimecrplimus in both industrial-scale and small-scale manipulated pharmaceutical formulations, so as to improve and accelerate quality control, and to ensure the therapeutic effectiveness of the medicinal products.

Experimental

Chemical derivatization

Due to the presence of weak chromophore group in pimecrolimus, detection and quantification of pimecrolimus by UV spectroscopic method is not possible. The spectroscopic method based on chemical derivatization by sulfuric acid addition reaction was developed and used to identify and quantify pimecrolimus. Dehydration caused by the addition of sulfuric acid improves the potency of chromophore in the molecule by the induction of α, β unsaturated enone system.

Accurately weighing 10 mg of pimecrolimus and dissolving it in 20 ml of acetonitrile produced a 500 µg/ml concentration. Aliquots of the stock solution, ranging from 0.2 to 1.2 ml, were transferred to a 10 ml volumetric flask, to which 0.4 ml of concentrated sulfuric acid was added. The mixture was then diluted to volume using acetonitrile. For three hours, the solution is stable. Dehydration of the molecule occurred after treatment with sulfuric acid. Unsaturated enone system was introduced into the molecule as a result of dehydration, and λ max was found to be 360 nm.

Materials

Chemicals and reagents

Pimecrolimus was a gift sample from Sumar Biotech LLP, Gujarat. All chemicals and reagents used were of analytical grade and purchased from Rankem Chemicals, mumbai, India. Pimecrolimus Cream 1% w/w strength were purchased from the local pharmacy under commercial available brand name Pacroma (Ajanta pharma Ltd.).

Instrumentation

Spectrophotometer: UV-2450 and UV-1601 Shimadzu, Japan

Software: UV Probe 2.21

Sample cell: 1 cm matched quartz cell

Lamp: Deuterium Lamp

Wavelength range: 200-800 nm

Scan speed: Medium

Spectral slit width: 1.0 nm

Weighing Balance: Shimadzu AUX-120

Preparation of standard stock solution:

The stock solution was prepared by accurately weighing 10 mg of pimecrolimus, which was transferred to a 20 ml volumetric flask, dissolved, and diluted to volume with acetonitrile to obtain a concentration of 500 µg/ml.

Selection of wavelength for analysis of Pimecrolimus

Appropriate volume 1 ml of the standard stock solution of pimecrolimus was transferred into a 10 ml volumetric flask, and 0.4 ml of concentrated sulfuric acid was added. The mixture was diluted to volume with acetonitrile to give a concentration of 50 µg/ml. The solutions were stable for 3 hours. The resulting solution was scanned in the UV range (200 nm–800 nm). In the spectra, Pimecrolimus showed an absorbance maximum at 360 nm (Figure 2).

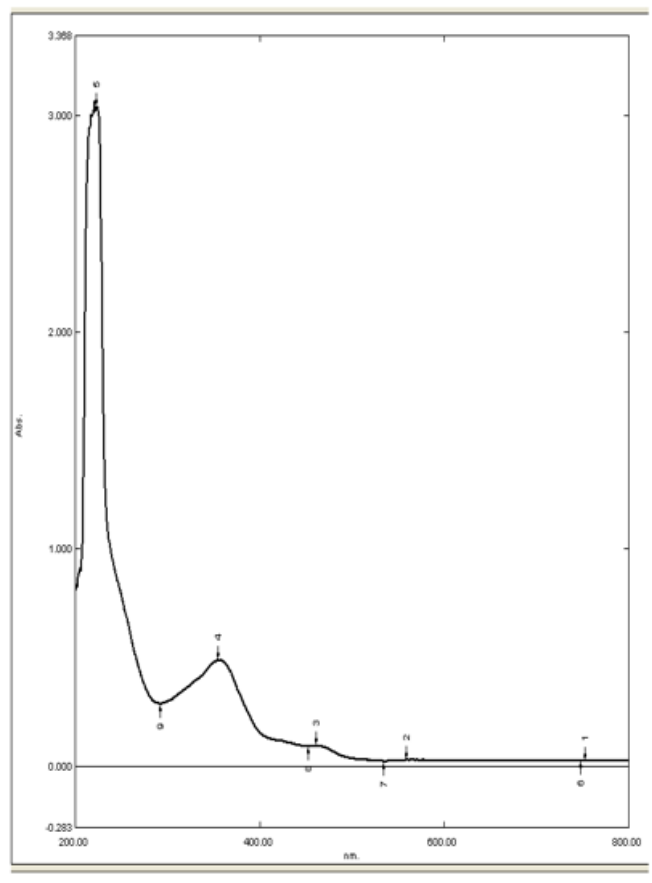

Figure 2: UV Spectrum of Pimecrolimus 360 nm.

Validation of the method

The method was validated in terms of linearity, accuracy, precision, Sensitivity and ruggedness.

Linearity study

Different aliquots of Pimecrolimus in the range of 0.2–1.2 ml were transferred into a series of 10 ml volumetric flasks, and 0.4 ml of concentrated sulfuric acid was added to each flask, and the volume was made up to the mark with acetonitrile to get concentrations of 10, 20, 30, 40, 50, and 60 µg/ml, respectively. The solutions were stable for 3 hours. The solutions were scanned on a spectrophotometer in the UV range of 200–800 nm. The spectrum was recorded at 360 nm. The calibration plot was constructed as absorbance vs. concentration (Figure 3).

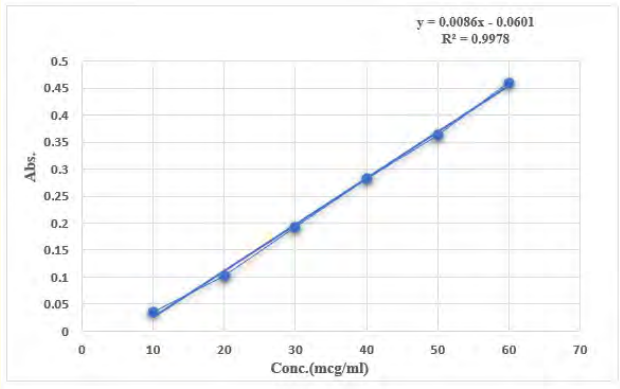

Figure 3: Calibration curve of Pimecrolimus 360 nm.

Accuracy

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were reanalyzed by proposed method.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 20, 30 and 50 µg/ml of Pimecrolimus solutions for three times in the same day. Inter-day precision was determined by analyzing the 20, 30 and 50 µg/ml of Pimecrolimus solutions daily for three days over the period of week.

Sensitivity

The Limit of Quantification (LOQ) and Limit of Detection (LOD) were used to determine the sensitivity of readings of pimecrolimus using the suggested approach (LOD). Equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is the standard deviation of the drug peak areas (n = 3), considered as a measure of noise, and 'B' is the slope of the associated calibration curve, were used to determine the LOQ and LOD.

Repeatability

Six analyses of a pimecrolimus solution at a concentration of 30 µg/ml were used to determine repeatability.

Ruggedness

For a Pimecrolimus concentration of 30 µg/ml, the suggested method's robustness is assessed through the analysis of aliquots from homogenous slots by two analysts under identical operational and environmental conditions.

Determination of Pimecrolimus in bulk

Accurately weighed 10 mg of Pimecrolimus was transferred into 20 ml volumetric flask and volume was made up to the mark using acetonitrile. Appropriate volume 0.6 ml of this solution was transferred to 10 ml volumetric flask, add 0.4 ml of sulfuric acid and volume was adjusted to mark using acetonitrile to give a concentration of 30 µg/ml The solutions were stable for 3 hours. The resulting solution was scanned on spectrophotometer in the UV range 200 - 800 nm. The concentrations of the drug were calculated from linear regression equations.

Application of proposed method for pharmaceutical cream formulation

For the analysis of commercial formulation, weigh accurately 1 gm of the cream and dissolve in 10 ml acetonitrile, sonicate for 15 minute and add sufficient acetonitrile to produce 20 ml. filter the resulting solution with whatmann filter paper. From this 0.6 ml was taken and transferred to 10 ml volumetric flask and add 0.4 ml of sulfuric acid and volume was made up to the mark with acetonitrile to give 30 µg/ml concentration. The solutions were stable for 3 hours. It was scanned on spectrophotometer in the UV range 200 - 800 nm. The spectrum was recorded at 360 nm. The concentrations of the drug were calculated from linear regression equation.

Results and Discussion

Method validation

The suggested procedure was approved in accordance with ICH rules. The drug solutions were made using the previously used method described in the experiment.

Linearity studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 10-60 µg/ml for Pimecrolimus. Linear regression equation was found to be Y = 0.0086 X - 0.0601 (r^2 = 0.9978). The result is expressed in Table 1.

Sr. no.	Concentration µg/ml	Absorbance* Mean ± S.D. (n = 6)	% R.S.D.
1	10	0.036 ± 0.0005	1.61
2	20	0.102 ± 0.001	1.68
3	30	0.192 ± 0.001	0.89
4	40	0.283 ± 0.002	0.81
5	50	0.363 ± 0.001	1.63
6	60	0.460 ± 0.001	1.83

Table 1: Linearity study Pimecrolimus.

* average of Six estimations.

Accuracy

The solutions were reanalyzed using the suggested approach, and the recovery study findings are shown in Table 2. They revealed that the amount found ranged from 98.54% to 99.69% with an %R.S.D. of less than 2.

Pre-analyzed sample solution (µg/ml)	Amount of drug added (µg/ml) (n = 3)	Amount recovered* (µg/ml) (n = 3)	% Recovery	% R.S.D.
30	24	23.92	99.69	1.01
	30	29.81	99.39	1.57
	36	35.47	98.54	1.50

Table 2: Recovery studies.

*average of three estimates.

Precision

Relative standard deviation (% RSD) was used to measure the accuracy of the developed approach. These outcomes demonstrate the assay's repeatability. The percentage R.S.D. values were less than 2, which shows that this method is accurate for determining both the drugs in bulk and in formulation (Table 3).

Component	Concentration (µg/ml)	Intra-day precision* (n = 3)		Inter-day Precision* (n = 3)	
		Conc. found	% R.S.D.	Conc. found	% R.S.D.
Pimecrolimus	20	0.099	1.01	0.113	1.53
	30	0.154	1.62	0.177	1.41
	50	0.413	0.73	0.405	1.11

Table 3: Precision studies.

*average of three estimates.

Sensitivity

The linearity equation was found to be Y = 0.006X + 0.0215. The LOD and LOQ for Pimecrolimus were found to be 0.68 µg and 2.08 µg, respectively.

Repeatability

Repeatability was determined by analyzing 30 µg/ml concentration of Pimecrolimus solution for six times and the % amount found was between 98% to 102% with % R.S.D. less than 2 (Table 4).

Component	Amount taken (µg/ml) (n = 6)	Amount found* (%)	%R.S.D.
Pimecrolimus	30	98.10 ± 1.01	1.03

Table 4: Repeatability studies.

Average of six estimations.

Ruggedness

Six times the identical concentration of solutions' peak areas were measured. The outcomes are within the drug's approved range. Table 5 presents the findings. The outcome revealed that the %R.S.D. was also under 2%.

Component	Amount taken (µg/ml) (n = 3)	Amount Found (%) *	
		Analyst I ± S.D.	Analyst II ± S.D.
Pimecrolimus	30	98.87 ± 1.40	98.74 ± 1.74

Table 5: Ruggedness studies.

* average of six estimations.

Determination of pimecrolimus in bulk

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 98.03% to 100.42% (Table 6).

Concentration (µg/ml)	Amount found (µg)	Amount found (%)
30	30.12790	100.42
	29.43023	98.10
	28.96511	96.55
	29.54651	98.48
	28.96511	96.55
	29.430233	98.100
Mean ± S.D.	29.410 ± 0.431	98.03 ± 1.43
% R.S.D	1.46	1.46

Table 6: Analysis of Pimecrolimus in bulk.

Application of proposed method for pharmaceutical formulation

The spectrum was recorded at 360 nm. The concentrations of the drug were calculated from linear regression equation. The % amount found was between 98.16% to 101.58% (Table 7).

Conc. (µg/ml)	Amount found (µg)	Amount found (%)
30	29.43023	98.10
	29.19767	97.32
	29.54651	98.48
	29.08139	96.93
	28.96511	96.55
	30.47674	101.58
Mean ± S.D.	29.44961 ± 0.54	98.16 ± 1.82
% R.S.D.	1.85	1.85

Table 7: Analysis of formulation.

Conclusion

This UV spectrophotometric method is sensitive, reliable, accurate, and precise. For the purpose of quantifying pimecrolimus in bulk and in cream formulations, the UV technique has been developed. The validation process validates that this is a suitable method for their bulk and in formulation quantification. It is also employed in routine quality monitoring of the raw ingredients and formulations that incorporate the entire chemical.

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