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Research Article

Development and Validation of HPTLC Method for Estimation of Pimecrolimus in Formulations

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

[1]Associate Professor, R. C. Patel Institute of Pharmaceutical Education and Research, India

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

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

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Abstract

To identify the quantitative determination of pimecrolimus, a novel, straightforward, and rapid high-performance thin-layer chromatographic method with a derivatization process was created and validated. On a silica gel 60 F254 TLC plate, pimecrolimus was chromatographed using toluene-acetonitrile-glacial acetic acid (6:4:0.5, by volume) as the mobile phase. Pimecrolimus was measured using densitometric analysis in the reflectance mode at 690 nm after being visualised with a derivatization reagent that contained anisaldehyde-sulfuric acid in absolute alcohol. It was discovered that the technique produced compact spots for the drug (Rf= 0.55gb ± 0.03). In the concentration range of 800-4800 ng/spot, the results from the calibration plots' linear regression analysis demonstrated a strong linear relationship with r^2=0.9989. According to the International Conference on Harmonization's guidelines, the method's precision, recovery, repeatability, and robustness were all verified. The limit of quantitation was found to be 97.04 ng, while the lowest detectable amount was found to be 28.90 ng. The technique is precise, accurate, reproducible, and selective for the analysis of pimecrolimus, according to statistical analysis of the data. The technique worked well for quantifying and estimating the equilibrium solubility of pimecrolimus both as a bulk drug and in commercially available cream formulations.

Keywords: Pimecrolimus; HPTLC; 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 is member of the ascomycin class of macrolactam immunosuppressives. It work by preventing the release of multiple inflammatory cytokines and inhibiting T-cell activation via the calcineurin pathway, which prevents the cascade of immunological and inflammatory signals [2]. As a result, Pimecrolimus prevents mast cells and T-cells from producing or secreting inflammatory cytokines and other proinflammatory mediators [8]. Pimecrolimus cream 1% has been demonstrated to be beneficial in treating other inflammatory skin conditions such allergic contact dermatitis and seborrheic dermatitis in addition to treating patients with mild to moderate atopic eczema (atopic dermatitis) [5-6]. 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[1] (figure 1), having a molecular formula C43H68CINO11, with molecular mass 810.4 g/ml [1].

Analysis part is an important from formulation development of any drug molecule. A suitable and validated method should be vacant for the drug delivery system for the analysis of bulk drug and in pharmaceutical cream formulation. Various methods are reported for the analysis of pimecrolimus alone or in combination with other drugs have been reported, which included, HPLC-MS/MS [3], RP-HPLC [2] and LC-TMS [4] but pimecrolimus has not yet been subject to the HPTLC method of analysis, as far as we are aware. Methods based on high-performance thin layer chromatography (HPTLC), which are being investigated as a key tool in routine drug analysis, could be thought of as an appropriate alternative. The capacity of HPTLC to analyse multiple samples at once while only using a small amount of mobile phase is a significant benefit. Thus, research takes less time and costs less money. Additionally, it greatly reduces the difficulties associated with disposing of toxic organic effluents, minimising the danger of environmental pollution. Additionally, HPTLC makes it easier to repeatedly identify chromatograms with the same or different parameters. Additionally, there are no limitations on the selection of solvents and mobile phases when using HPTLC; drugs and lipophilic excipients can be dissolved in an appropriate solvent that would evaporate during spotting on a TLC plate, leaving the analyte as a narrow band behind. As a result, such methods could be developed for drug analysis without excipient interference and do not always require an extraction process. In light of this, the current research details the creation of a routine estimation of pimecrolimus from bulk and pharmaceutical dosage forms, such as marketed cream, that is simple, quick, economical, and validated.

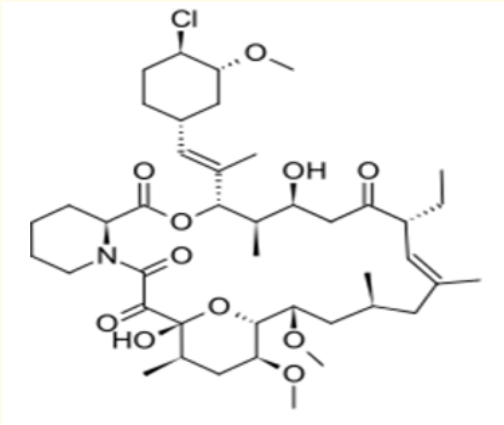

Figure 1: Chemical structure of Pimecrolimus.

Experimental

Materials

Chemicals and reagents

Pimecrolimus was a gift sample from Sumar Biotech LLP, Gujarat. All chemicals and reagents used were of HPLC 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

- Balance SHIMADZU AUX -120
- Ultrasonicator Enertech Electronics Pvt. Ltd
- HPTLC System CAMAG (Muttenz, Switzerland)
- Applicator Linomat 5 with 100-µL syringe connected to a nitrogen cylinder
- Scanner Camag TLC Scanner 3
- Data Processor winCATS (version 1.3.0).

Chromatographic conditions

Chromatography was performed on 10 cm × 10 cm aluminum-backed TLC plates coated with 200 µm layers of silica gel 60F_{254} S (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). The plates were prewashed by methanol and activated at 100 – 110° C for 10 min prior to chromatography. The samples were applied on the plates as 6 mm wide bands, by means of a CAMAG (Muttenz, Switzerland) Linomat-5 sample applicator fitted with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland). Plate was developed to a distance of 8 cm using Toluene: Acetonitrile: Glacial acetic acid (6:4:0.5 v/v) as mobile phase in a Camag twin-trough glass chamber previously saturated with mobile phase vapors for 10 min at ambient temperature. After spraying with derivatizting reagent densitometric scanning was performed at 690 nm using Camag TLC Scanner 3 equipped with winCATS software version 1.3.0. A typical chromatogram of Pimecrolimus in bulk is shown in Figure 2.

Selection of derivatization reagent

For the identification of pimecrolimus, a number of reagents including iodine vapours, ceric sulphate, sulfuric acid, and

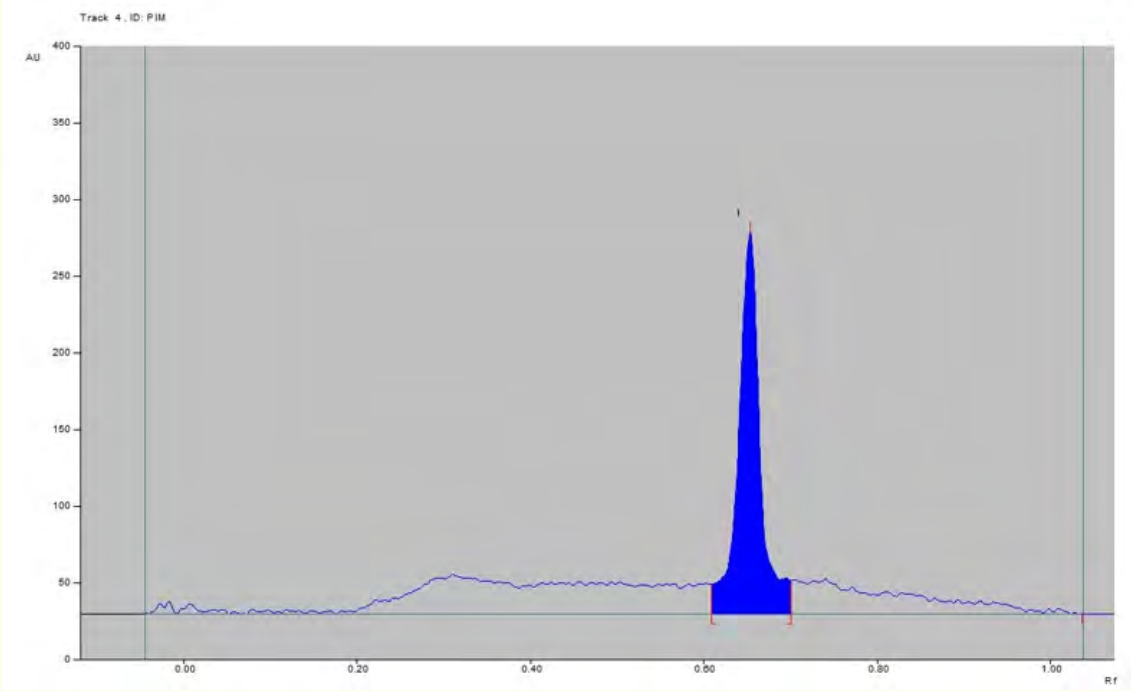

Figure 2: Chromatogram of standard Pimecrolimus (Rf: 0.55), UV detection at 690 nm, Mobile phase- Toluene: Acetonitrile: Glacial acetic acid (6:4:0.5 v/v).

Dragendorff reagent were tested; however, they were not acceptable for the drug's quantitative measurement. As a macrolide lactone with structural similarities to macrolide antibiotics, pimecrolimus was studied using derivatization reagents that are used to visualise these drugs, including those based on p-anisaldehyde (4-methoxybenzaldehyde), molybdophosphoric acid, and Folin and Ciocalteu's reagent. Other than the p-anisaldehyde-based reagent, no other reactions could produce colour with pimecrolimus. Pimecrolimus showed a bright colouring and created well-defined spots that could be used for quantitative analysis when anisaldehyde and sulfuric acid in 100% alcohol (1:1:9, by volume) were combined with plate activation at 110°C for 60 s.

Derivatization methodology

A solution of 1 mL anisaldehyde in 9 mL absolute alcohol was combined with 1 mL concentrated sulfuric acid to create the derivatization reagent. Before usage, the reagent was newly produced. A hair dryer was used to dry the TLC plate after chromatographic development. Derivatization was carried out by newly prepared derivatization reagent being sprayed onto the created plate. TLC plate was heated for one minute at 110°C after drying.

Preparation of standard stock solution

An accurately weighed Pimecrolimus (10 mg) was transferred to 10 mL volumetric flask; dissolved in methanol and the volume was made up to mark with the same solvent to give 1000 ng/µL solution.

Linearity study

Linearity was performed using working standard of Pimecrolimus. Calibration was done by applying standard stock solution ranging from 0.8-4.8 µL on TLC Plate; which gives concentration of 800 – 4800 ng/band. The plate was developed and scanned as described under above chromatographic conditions. Calibration curve was constructed by plotting the peak area *vs.*

corresponding drug concentration. The results are reported in Table 1 and the calibration curve in figure 4. The 3-D linearity chromatogram is shown in Figure 3.

Calibration curve

The linear regression data for the calibration curves showed good linear relationship over the concentration range 800 – 4800 ng/spot. Linear regression equation was found to be Y = 1.3353 X + 1173.9 (r^2 = 0.997) with Peak display of Pimecrolimus in Figure 2.

Sr. no.	Concentration µg/ml	AUC* Mean ± S.D. (n = 6)	% R.S.D.
1	800	2380.60 ± 45.17	1.89
2	1600	3244.76 ± 30.11	0.92
3	2400	4328.66 ± 34.50	0.79
4	3200	5319.36 ± 50.91	0.95
5	4000	6490.26 ± 67.07	1.03
6	4800	7712.66 ± 66.17	0.85

Table 1: Linearity study Pimecrolimus.

* average of Six estimations.

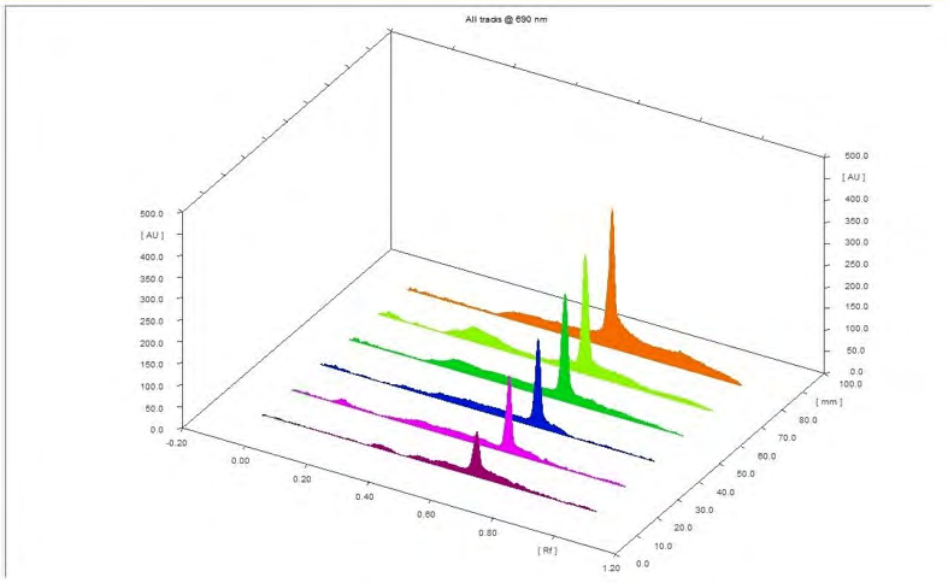

Figure 3: 3-D linearity chromatogram of pimecrolimus.

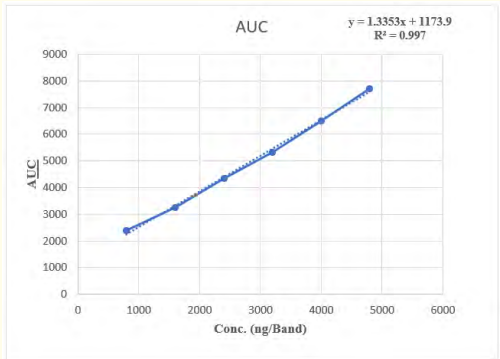

Figure 4: Calibration curve.

Analysis of bulk material

Accurately weighed 10 mg pimecrolimus was transferred to 10 mL volumetric flask, dissolved in methanol and volume was made up to mark. The solution (0.8 µL, containing 800 ng of pimecrolimus) was applied; the plate was developed and scanned. The concentration was determined by regression equation and the results are shown in table 2.

Formulation	Component	Amount Taken (ng/band)	Amount Found (ng) ± SD	% RSD
Bulk	PIM	2400	2368.1 ± 18.9	0.801
Cream formulation			2391.5 ± 42.3	1.77

Table 2: Analysis of bulk and in cream formulation.

* mean of three estimation.

Analysis of pharmaceutical dosage form

To determine the content of pimecrolimus in cream, weigh accurately 1 gm of the cream and dissolve in 4 ml methanol, sonicate for 20 minute and add sufficient methanol to produce 10 ml. filter the resulting solution with whatmann filter paper. from it, the sample solution (2.4 µL, containing 2400 ng of pimecrolimus) was applied on TLC plate, developed, scanned and sprayed. Results of the assay are shown in Table 2.

Validation of the proposed method

The method was validated in compliance with ICH guidelines.

Accuracy

Recovery study was carried out by over spotting at 80, 100 and 120 % level where known amount of standard pimecrolimus was added to pre analyzed sample (2400 ng of pimecrolimus) and subjected them to the proposed TLC method. Results are shown in Table 3.

Drug	Label claim (mg/tablet)	(%) Amount of standard drug added	Drug Recovered	% RSD
PIM	2400	80	99.35	1.86
		100	98.68	1.97
		120	101.31	1.56

Table 3: Recovery study.

* mean of three estimations at each level.

Precision

Intra–day and inter–day precision

Intraday precision was determined by analyzing, the three different concentrations 800 ng, 1600 ng and 2400 ng of Pimecrolimus, for three times within the day. Day to day variability was assessed using above mentioned three concentrations and analyzing it for three consecutive days, which shows reproducibility of the method. Results are shown in Table 4.

Drugs	Conc. ng/spot	Intra-day		Inter-day	
		% Amount found	% RSD	% Amount found	% RSD
PIM	1600	98.43	1.38	98.06	1.09
	2400	98.35	1.08	98.45	1.41
	3200	98.04	0.59	98.36	0.62

Table 4: Intra-day and Inter-day precision of HPTLC method.

* mean of three estimation.

Repeatability

Repeatability of sample application was assessed by applying 2.4 µL (2400 ng) of drug solution six times on a TLC plate followed by development of plate and recording the peak height and area for 6 bands Table 6.

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD

and LOQ were calculated by the method which was based on the SD of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3(SD/S) and LOQ = 10(SD/S). Stock solution of Pimecrolimus was prepared and different volume of stock solution in the range 800 to 1600 ng were applied in triplicate Table 6.

Ruggedness

Ruggedness of the method was checked by analyzing 2400 ng (n = 6) of pimecrolimus, with the help of two analysts and the variations in the results were checked. The results of the studies are shown in Table 5.

Component	Amount taken (µg/ml) (n = 3)	Amount Found (%) *	
		Analyst I ± S.D.	Analyst II ± S.D.
Pimecrolimus	2400	98.41 ± 1.10	99.30 ± 1.25

Table 5: Ruggedness studies.

* average of six estimations.

Parameter Data	PIM
Linearity range (ng per spot)	800 - 4800
Correlation coefficient	0.997
Limit of detection (ng per spot)	72.76
Limit of quantification (ng per spot)	194.94
Recovery (n = 6)	99.44
Ruggedness a(% RSD)	
Analyst-I (n=6)	1.10
Analyst-II (n=6)	1.25
Precision (% RSD)	
Repeatability of application (n = 6)	1.00
Inter-day (n = 6)	0.62 - 1.41
Intra-day (n = 6)	0.59 - 1.38
Specificity	Specific

Table 6: Summery of validation parameter.

Robustness

Robustness was studied at the concentration level of 2400 ng/band. In this study, few parameters (mobile phase composition, development distance and duration of saturation) were studied and the effects on the results were examined. The results of the studies are shown.

Conclusion

Pimecrolimus can now be identified and measured using a brand-new HPTLC technique. The main advantages of this technique include low ingredient costs, quick processing, and satisfactory precision and accuracy. The method was effectively validated in accordance with ICH guidelines, and statistical analysis shows that it is precise, repeatable, and sensitive. Without excipient interference, it is easily applied for regular quality control analysis of pimecrolimus as the active ingredient in marketed cream formulations.

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