An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

METHOD DEVELOPMENT AND VALIDATION FOR ASSAY STUDIES OF TACROLIMUS OINTMENT IN MARKETED FORMULATION BY RP-HPLC

Mr. Kishor Yuvraj Khairnar[1*], Prof. Vikas B. Kandekar[1*]

[1]Department of Quality Assurance Techniques, Chaudhary Attarsingh Yadav Memorial Education Trust's Siddhant College of Pharmacy, Pune (412109)

*Corresponding author E-mail: kishorkhairnar106@gmail.com

ARTICLE INFO

Key Words
RP-HPLC, Tacrolimus, Assay, Chromatography, ICH Guidelines.

Access this article online Website: *https://www.jgtps.com/*
Quick Response Code:

ABSTRACT

A simple, economical, specific, accurate, precise and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the assay study of Tacrolimus in the pharmaceutical dosage form. The chromatographic separation was achieved on Inertsil ODS-3V, C-18 column (150 mm x 4.6 mm, 5 µ particle size, packing L1) at 25^0C temperature using mobile phase buffer (6.8 gm potassium dihydrogen phosphate in 1000 ml water, sonicated to dissolve, then added 0.6ml Ortho Phosporic Acid and mixed well): Acetonitrile(ACN) : Methanol (30:60:10% v/v/v) (ph 3.0 ± 0.05) at flow rate 1.5 ml/min. Quantification was achieved with a UV detector at 209 nm. The retention time of Tacrolimus was found to be 6.699 ± 0.05 min. The proposed method was validated according to ICH guidelines concerning assay studies for Tacrolimus ointment. The developed method with good separation successfully applied for the determination of Tacrolimus in its pharmaceutical dosage form.

INTRODUCTION:

One of the most common skin disorders in children is Atopic dermatitis (AD). It is a chronic, highly pruritic (itchy) inflammatory skin disease [1]. The said disorder adversely affects quality of life and also results in significant morbidity [2]. Not only the patients are affected by the stigma of a visible skin condition, but the whole itching characteristic of the disease often leads to significant sleep disturbances. For such disease condition management, it necessitates the frequent application of emollients (agents that soothe, moisturize and soften the skin) and topical medications, as well as physician visits [3]. Tacrolimus is a highly potent immunosuppressive agent and has proven activity in both in-vivo and in-vitro experiments, also calcineurin inhibitor. It is isolated from fungus Streptomycin tsukubaensis [4]. It is the basis of immunosuppressive regimens after liver and kidney transplantation and it has also been used for heart, pancreas, bone marrow, small bowel, lung transplantation and for the treatment of T-cell mediated autoimmune disease such as allergic encephalomyelitis [5].

Assay techniques that provide specific Tacrolimus concentration measurement with greater sensitivity, such as liquid chromatography–tandem mass spectrometry (LC– MS/MS) are now widely employed [7]. Further objective in the present investigation is to develop and validate RP-HPLC method for Assay study of Tacrolimus bulk drug. The proposed RP-HPLC method utilizes economical solvent system having advantages like better retention time, very sharp and symmetric peak shapes. The proposed method is validated according to ICH guidelines [8]. So we can conclude that above said method is simple, rapid, sensitive, specific, robust and novel that makes it a validated procedure in high throughput in the sense of analytical work.

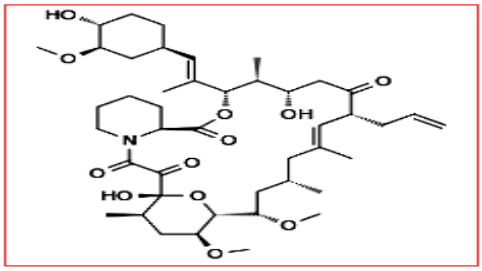


Fig 1: Chemical Structure of Tacrolimus

MATERIALS & METHOD

Instrument and apparatus: A HPLC Instrument (LC-2010 CHT, Shimadzu, Japan) equipped with UV detector, auto injector and LC-Solution Software was used. The chromatographic analysis was performed on Inertsil ODS-3V, C-18 column (150 mm x 4.6 mm, 5 µ particle size, packing L1). Analytical balance (Mettler Toledo), digital pH meter (Hanna pH meter) was used during the analysis.

Reagents and Materials:

Working standards of Tacrolimus (Potency = 97.40%) was obtained as a gift sample from Biocon Limited Bangalore. HPLC grade Acetonitrile, AR grades Ortho-phosphoric acid and Potassium dihydrogen phosphate were procured from Merck Ltd. Mumbai India. Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a 0.45µ membrane filter paper. The commercial fixed dose product (Ointment) containing 0.03% of Tacrolimus was procured from the local market.

Experimental Work:

Preparation of Buffer: Weigh accurately about 6.8 gm potassium dihydrogen phosphate in 1000 ml water, sonicate to dissolve, add 0.6ml Ortho Phosporic Acid and mix well.

Preparation of Mobile Phase: Prepare mixture of Buffer, Acetonitrile and Methanol in ratio of 30:60:10 % v/v/v respectively, degas and filter the mixture.

Preparation of Solution A (For Diluent): Prepare 6 M Phosphoric acid by dissolving 0.4 ml Ortho-Phosporic acid in 1000 ml Milli-Q water.

Preparation of Diluent: Prepare the diluent by mixing solution A and Acetonitrile in the ratio of 20:80% v/v

Preparations of Solutions:

Preparation of standard solution: Weigh accurately and transfer about 30.00 mg of Tacrolimus working standard to a 50 ml volumetric flask. Add about 30 ml diluent, sonicate to dissolve and dilute the above solution to the final volume with diluent and mix well. Further dilute 5.0 ml of this solution to 50 ml with diluent.

Preparation of sample solution: Weigh accurately and transfer about 3.00 gm of ointment sample to a 200 ml volumetric flask, add 50ml of n-heptane, sonicate to disperse the ointment and add 50ml of diluent with volumetric pipettes ad sonicate for 15 min, followed by vertex for 5 min with intermediate shaking. Allow to stand for 1 hour at room temperature to form transparent lower layer. Use lower transparent layer for analysis.

HPLC system	LC-2010 CHT, Shimadzu Corporation
Software	LC Solution
Detector	UV Detector
Wavelength	209 nm
Pump	Isocratic Pump
Stationary phase	Inertsil ODS-3V, C-18 column (150 mm x 4.6 mm, 5 µ particle size, packing L1)
Mobile phase	Buffer : Acetonitrile (ACN) : Methanol (30:60:10% v/v/v) (ph 3.0 ± 0.05)
Flow rate	1.5 ml/min
Injection volume	50 µL
Diluent	Solution A : ACN (20:80 v/v)

Table 1: Chromatographic Conditions

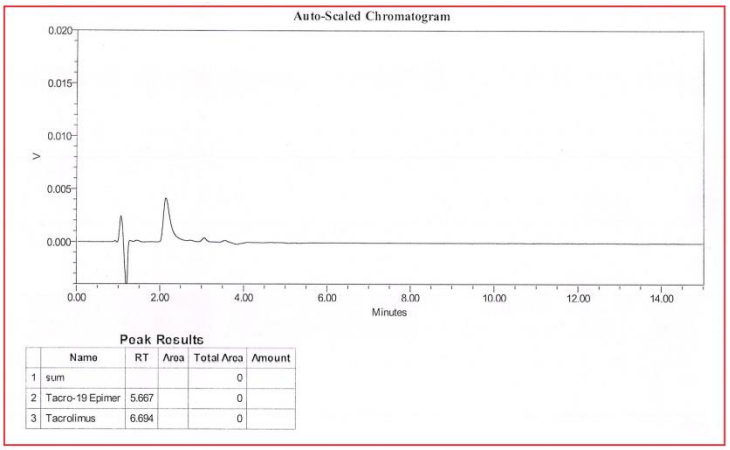


Fig 2: Chromatogram of Blank injection of Tacrolimus

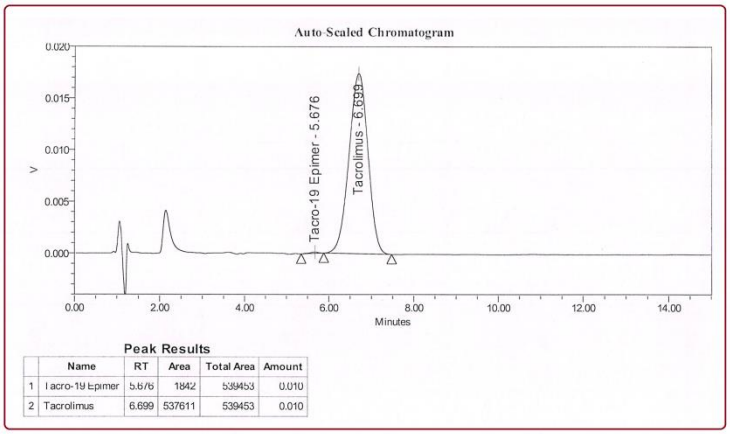


Fig 3: Chromatogram of Standard injection no. 1 of Tacrolimus

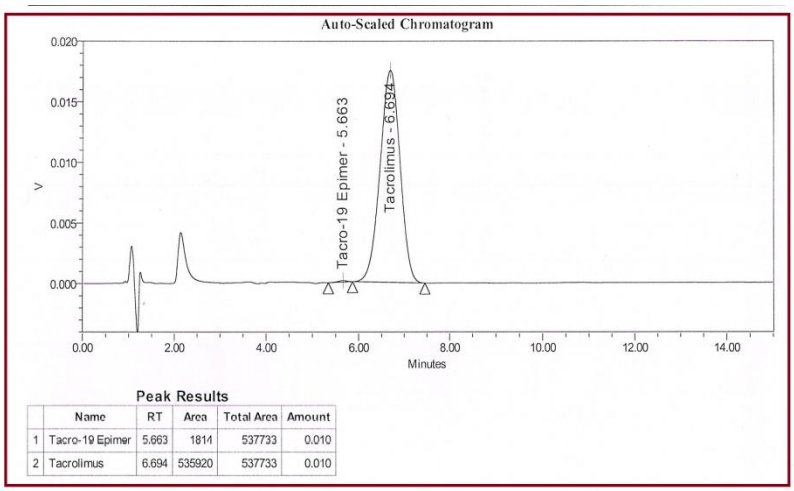


Fig 4: Chromatogram of Standard injection no. 2 of Tacrolimus

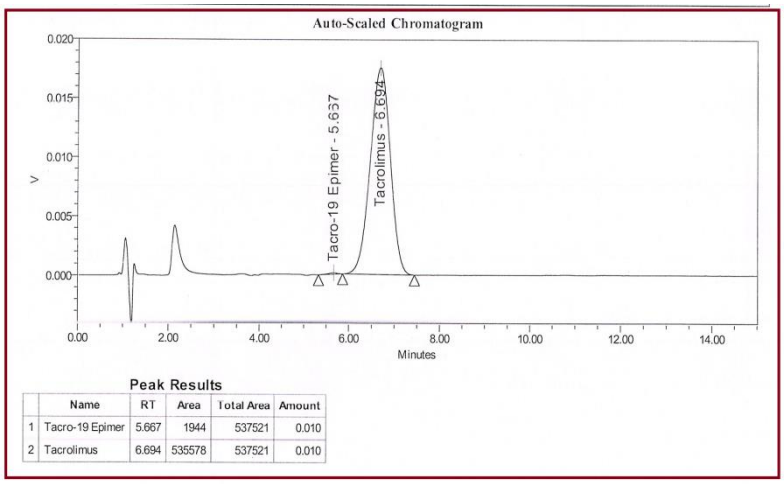


Fig 5: Chromatogram of Standard injection no. 3 of Tacrolimus

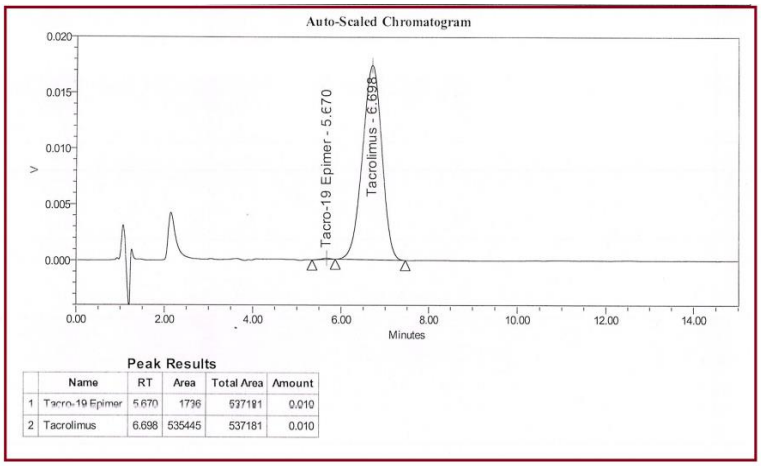
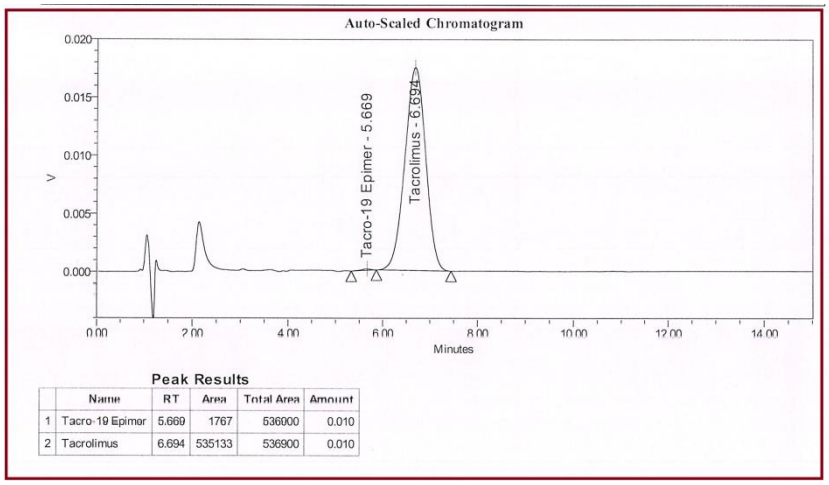
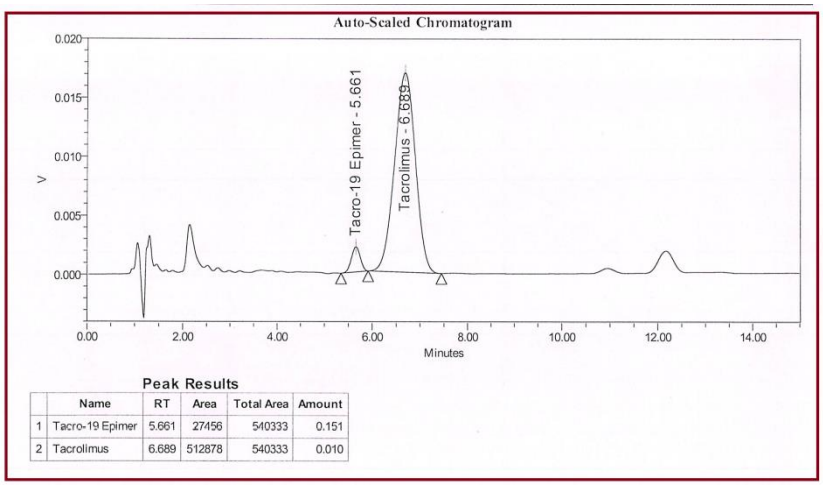


Fig 6: Chromatogram of Standard injection no. 4 of Tacrolimus



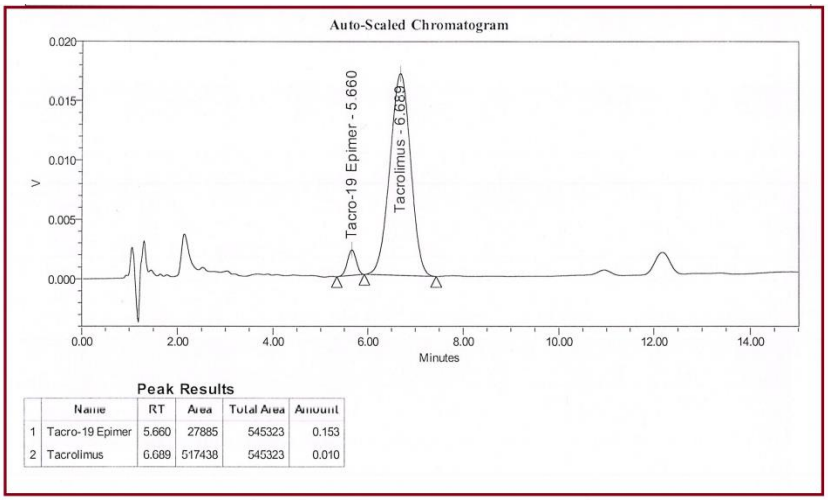
	Name	RT	Area	Total Area	Amount
1	Tacro-19 Epimer	5.669	1767	536900	0.010
2	Tacrolimus	6.694	535133	536900	0.010

Fig 7: Chromatogram of Standard injection no. 5 of Tacrolimus



	Name	RT	Area	Total Area	Amount
1	Tacro-19 Epimer	5.661	27456	540333	0.151
2	Tacrolimus	6.689	512878	540333	0.010

Fig 8: Chromatogram of Sample injection no. 1 of Tacrolimus Ointment



	Name	RT	Area	Total Area	Amount
1	Tacro-19 Epimer	5.660	27885	545323	0.153
2	Tacrolimus	6.689	517438	545323	0.010

Fig 9: Chromatogram of Sample injection no. 2 of Tacrolimus Ointment

Evaluation of System Suitability:

- Injected 50µL Blank solution (single injection vial no: 1)
- Injected 50µL standard solution in 5 replicates (vial no: 2) and record the chromatograph
- Measure the area counts of Tacrolimus & Tacrolimus 19-epimer from standard solution injection peaks. The relative standard deviation for five replicate injections of standard solution for Tacrolimus and Tacrolimus 19-epimer should not be more than 2.0%
- Inject 50µL Sample in duplicate (vial no: 3 & vial no: 4 respectively) and record the chromatograph. Measure the response for the major peaks.
- RRT (Relative Retention time) for Tacrolimus 19-epimer is about 0.85.

RESULT & DISCUSSION:

Calculations for Assay:

$$\%\,Tacrolimus = \frac{AT}{AS} \times \frac{Std.wt(mg)}{50} \times \frac{5}{50} \times \frac{50}{Sample\ wt(mg)} \times \frac{P}{100} \times \frac{0.1}{L.C} \times 100$$

Where,

AT= Sum of average peak area count of Tacrolimus in chromatogram of Sample solution.
AS= Sum of average peak area count of Tacrolimus in chromatogram of Standard solution.
LC= Label Claim
P= % Potency of Tacrolimus Working Standard on as is Basis.

$$\%\,Tacrolimus = \frac{535937.4}{515158} \times \frac{30.01}{50} \times \frac{5}{50} \times \frac{50}{3.03} \times \frac{7.5}{25} \times \frac{97.40}{100} \times \frac{0.1}{0.03} \times 100$$

% Tacrolimus = 100.3 %

CONCLUSION:

The simple and economic chromatographic method can be used to carry out Assay studies of Tacrolimus in Marketed Formulation by RP-HPLC. Although of cost-effective and minimal maintenance, the present chromatographic method can be preferred at a small scale industry and successfully applied and suggested for the quantitative analysis of Tacrolimus in pharmaceutical dosage formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

REFERENCES

1. Ohtsuki, Morimoto, Nakagawa H. Tacrolimus ointment for the treatment of adult and pediatric atopic dermatitis: Review on safety and benefits. *J Dermatol*. 2018; 45(8):936–942. doi:10.1111/1346-8138.14501
2. Watson, Kapur. Atopic dermatitis. *Allergy Asthma Clin Immunol*. 2011;7 Suppl 1(Suppl 1):S4. Published 2011 Nov 10. doi:10.1186/1710-1492-7-S1-S4
3. Barbeau, Burden of atopic dermatitis in Canada. Int J Dermatol. 2006; 45:31–36. doi: 10.1111/j.1365-4632.2004.02345.x.
4. Wong et al. Therapeutic drug monitoring for immunosuppressive. Clin Chim Acta. 2001, 313; 241-253.
5. Deshmukh, Inam Method Development and Validation of Tacrolimus in Pharmaceutical dosage form by RP-HPLC Method. International Journal of Research in Biosciences, Agriculture & Technology Feb. 2015, Special Issue (2), Page 355-361.
6. The USP, the United state Pharmacopoeia Commission, America. 2014, vol. 36(6); 1570.
7. Arunadevi, J. Pharm. Sci. & Res. 2014, vol. 6(12); 425-435.
8. ICH Harmonized Tripartite Guideline, Validation of analytical procedure text and methodology Q2 [R1]. In ICH of technical requirements for registration of pharmaceuticals for human use, 2005.