Research Article

Novel RP-HPLC Method for Precise Quantification of Trifarotene in Topical Cream: Development and Validation

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ABSTRACT

Trifarotene is a topical retinoid used for treating acne vulgaris, requiring a reliable analytical method for its quantification in pharmaceutical formulations. This study aims to develop and validate a simple, rapid, and accurate RP-HPLC method for the quantification of Trifarotene in a cream formulation. Chromatographic separation was performed on a Waters Spherisorb 5 µm ODS C18 column (250 × 4.6 mm, 5 µm) using an isocratic mobile phase composed of 0.1% trifluoroacetic acid, methanol, and acetonitrile (44:20:36, v/v/v) maintaining flow rate of 1.0 ml/min. Detection was conducted at 262 nm, with Trifarotene eluting at 9.3 minutes. The validation of developed method was carried out according to ICH guidelines and exhibited excellent linearity (R^2 = 0.9995) over a concentration range of 0.5–3.5 µg/ml, with an average recovery of 99.27%. System suitability, specificity, precision, accuracy, and robustness met the required validation criteria. The developed RP-HPLC method provides a precise, accurate, and reliable approach for the routine quantification of Trifarotene in pharmaceutical formulations, ensuring compliance with regulatory standards.

Keywords: RP-HPLC, Method Validation, Trifarotene, Acne Vulgaris, Chromatographic Analysis, topical formulation.

INTRODUCTION

Trifarotene (Aklief) is a fourth-generation retinoid approved by the FDA in October 2019 for treating Acne vulgaris[1,2]. Trifarotene, available in a 0.005% w/w cream formulation, is an effective treatment for acne, targeting both facial and truncal breakouts. Acne vulgaris is a widespread skin condition, impacting almost 80% of young adults aged 11 to 30[3]. It leads to disfigurement, and permanent scarring, and can significantly harm psychological development, resulting in social phobias, withdrawal from society, and clinical depression[4,5]. Trifarotene is a type of topicalretinoid designed to target the retinoic acid receptor (RAR) specifically γ. The most predominant isoform of RARs in the skin is RAR-γ and Trifarotene's strong selectivity for RAR-γ allows it to be effective evenin low concentrations. Similar to other topical retinoids, Trifarotene promotes keratinocyte differentiation and reduces prolieration, which helps decrease hyperkeratinization[6–8]. Trifarotene's selectivity for RAR-γ sets it apart from existing first generation topical retinoids like tretinoin, isotretinoin, and third-generation topical retinoid like adapalene, all of which are nonspecific and target both RARβ and RARγ[9]. Trifarotene's chemical structure consists of a naphthoic acid core with additional functional groups that enhance its receptor specificity and pharmacological activity (Figure 1). The presence of a carboxyl functional group contributes to its interaction with RAR-γ, while the overall molecular framework ensures stability and bioavailability. This selective structure differentiates Trifarotene from other retinoids, leading to targeted efficacy in acne treatment with potentially fewer side effects. A thorough review of analytical literature found only one LC–MS method reported for trifarotene estimation in plasma samples[10]. However, the method reported only discusses the analysis in the plasma while performing the clinical trial. No official Pharmacopeia guidelines exist for the analytical evaluation of trifarotene.

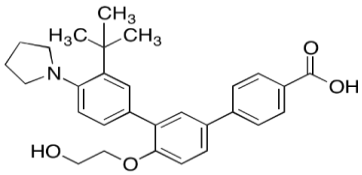

Figure 1: Structure of Trifarotene[8]

As of March 2025, the United States Pharmacopeia (USP) has not yet published an official monograph or update specifically for trifarotene. Due to its low-dose application, a sensitive and selective method is needed to measure it accurately among potential excipients. The current study aimed to develop a liquid chromatography method to estimate trifarotene in topical dosage forms, following ICH guidelines[11]. Given this gap, an in-depth exploration of structurally related drugs was conducted to guide the development of a robust and reliable analytical method[12–15]. Thus, current study was designed to conduct a validated simple RP-HPLC method todetermine Trifarotene in a cream formulation and potential impurities including nitrosamine impurities[16].

MATERIALS AND METHODS

Materials:

The chromatographic analysis was performed using a Shimadzu HPLC system (Separation Module 2695) equipped with a UV detector and controlled by LC Solution software. The system included a pump, autosampler, and auto-injector

for precise sample handling. A Waters Spherisorb ODS C18 column (4.6 mm × 250 mm, 5 µm) was used for separation. A UV-visible spectrophotometer with UV WinLab software was employed for spectral analysis. Additional equipment included an Electrolab centrifuge for sample processing, a Sartorius analytical balance for accurate weighing, and nylon syringe filters (0.45 µm pore size) for sample filtration.

Chemicals and Reagents:

A pharmaceutical-grade working standard of Trifarotene was obtained from the Central Drug Testing Laboratory, Mumbai. Trifarotene cream (Aklief, 50 µg/g) was sourced from Galderma Laboratories. High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were procured from Merck Life Science Pvt. Ltd. Ultra-purified HPLC-grade distilled water was obtained from a Milli-Q® system to ensure analytical precision. Trifluoroacetic acid (98%, AR grade) was supplied by Finer Chemicals, while sodium chloride (99.9%, AR grade) was acquired from Sisco Research Laboratories Pvt. Ltd.

RP-HPLC Method Development

Determination of wavelength of maximum absorbance:

The prepared solution was scanned in the wavelength range of 200–400 nm using a UV-visible spectrophotometer. Trifarotene exhibited maximum absorbance at 262 nm, which was subsequently selected as the ideal wavelength for its analysis.

Chromatographic conditions:

Chromatographic separation was achieved by injecting 50 µL of the sample into an HPLC system equipped with a Waters Spherisorb ODS C18 analytical column (5 µm, 250 mm × 4.6 mm), maintained at 30°C. Methanol was used as the common solvent. The analysis was performed under isocratic conditions with 1 mL/min flow rate. Detection wavelength was 262 nm, with a total run time of 20 minutes.

Preparation of the mobile phase:

A mixture of 0.1% Trifluoroacetic acid, Methanol and Acetonitrile in the proportion of 44:20:36 v/v/v was used as the mobile phase. Trifluoroacetic acid (0.1% v/v) was prepared by dissolving one millilitre of Trifluoroacetic acid (98%) in 1000mL of milli-Q water. The solution was sonicated for 10 minutes to degas it.

Preparation of standard solution:

Accurately weighed about 10 mg of Trifarotene standard was transferred in a 100 ml volumetric flask and dissolved by sonication in sufficient diluent then volume made with diluent (100µg/ ml). Then 2 ml from the above stock solution was diluted up to 100 ml with methanol (2µg/ ml), which was used as the standard solution for all the analysis.

Preparation of sample solution:

2gm cream (Equivalent 1mg Trifarotene) was accurately weighed and transferred to six 50 mL volumetric flasks.2 ml of saturated Sodium Chloride (NaCl) and a diluent was added. The solution was subjected to sonication for 15 minutes. The prepared solution was adjusted to the required volume using the diluent (2 µg/ml). The resulting solution was then centrifuged for 5 minutes at 1500 rpm. Additionally, the clarified solution was passed through a 0.45µ Nylon membrane syringe filter.

Method Validation

System suitability: This test evaluates the effectiveness of the RP-HPLC system. A single injection of blank preparation and a standard preparation (six replicates) of Trifarotene were analyzed, followed by the recording of chromatograms to assess parameters like retention time (RT), number of theoretical plates (N), and tailing factor.

Specificity: Specificity of the developed RP-HPLC method was evaluated to establish its ability to accurately identify and quantify Trifarotene in the presence of formulation excipients and other potential interfering components. Blank solution was injected under optimized chromatographic conditions to examine the presence of any interfering peaks at the retention time of the analyte. The absence of overlapping or co-eluting peaks confirmed the specificity and selectivity of the proposed analytical method, thereby ensuring reliable identification and quantification of Trifarotene in the topical cream formulation.

Accuracy: Recovery estimation was conducted at three distinct levels viz., 110%, 120%, and 130%. The recovery studies were carried out by adding a known amount of standard solution to the pre-analyzed sample concentration at three different levels. At each level, three determinations were performed and me and recovery was calculated and reported.

Linearity and concentration range: The linearity Trifarotene was performed by suitably diluting the standard stock solution within the concentration range of 0.5-3.5µg/were prepared with a concentration of 50% to 150% and all were filled in a vial.

Robustness: The robustness was considered unaffected when minor variations were made in the chromatographic method. To analyze the robustness of the proposed method, deliberate changes were, made in flow rate (± 2%ml/min), wavelength (± 2nm), and column temperature (± 2°C).

RESULTS AND DISCUSSION

Trifarotene is a newer generation topical retinoid widely employed in the treatment of acne vulgaris due to its high selectivity toward retinoic acid receptor-γ. With the increasing therapeutic application of Trifarotene-containing dermatological products, the establishment of reliable analytical procedures for its estimation in semisolid formulations has gained significant importance. The RP-HPLC technique plays a critical role in the qualitative and quantitative assessment of pharmaceutical dosage forms by enabling accurate estimation of active pharmaceutical ingredients in the presence of excipients, degradation

products, and related impurities, thereby ensuring product quality, safety, and regulatory compliance[17]. A review of the available scientific literature indicates that only limited analytical methods have been reported for the determination of Trifarotene in topical dosage forms. In addition, the intricate composition of cream formulations, containing lipophilic bases, surfactants, and stabilizing excipients, may interfere with analyte separation and quantification, thereby demanding the development of a sensitive and selective chromatographic method.

In the present study, a reverse-phase high-performance liquid chromatographic method was successfully designed and optimized for the quantitative analysis of Trifarotene in topical cream. Critical chromatographic variables such as mobile phase ratio, stationary phase selection, flow rate, and detection wavelength were systematically investigated to obtain optimum chromatographic performance characterized by satisfactory peak shape, suitable retention time, and effective analyte resolution[18]. The optimized analytical procedure was subsequently validated in compliance with ICH Q2 (R1) recommendations to confirm its suitability for routine assay and quality control evaluation of Trifarotene topical formulations.

Method development and optimization of chromatographic conditions:

The chemical structure of Trifarotene suggests that it is a weakly acidic molecule with a pKa value of 5.69. Hence, non-end capping ODS columns such as Spherisorb (Waters) were selected for the study. The aqueous portion of mobile phase was modified with 0.1%TFAA to control the ionization of themolecule with a pH of about 2.

The molecule will be in neutral form and will retain in the Hydrophobic phase. To attain effective separation of Trifarotene, Variations were made in mobile phase proportion, injection volume and concentration of Standard solution. However, good peak shape with acceptable system suitability parameters was found with the mobile phase composition of 0.1% TFAA:Methanol: Acetonitrile in the ratio of 44:20:36 (v/v/v) using Waters Spherisob C18 ODS column (4.6mm × 250mm × 5µm) to obtain optimal results at a flow rate of 1 mL/min[19], measured at a detection wavelength of 262 nm.

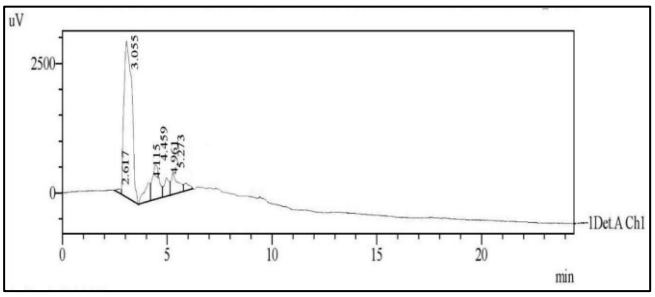

Figure 2: Chromatogram of Blank

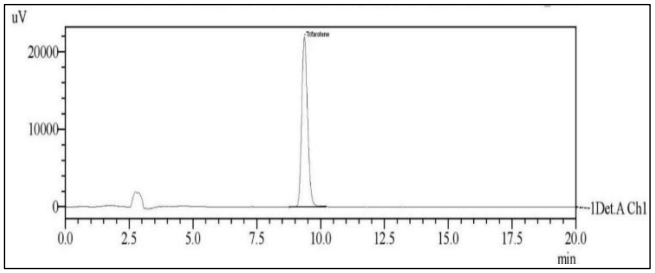

Figure 3: Chromatogram of Standard

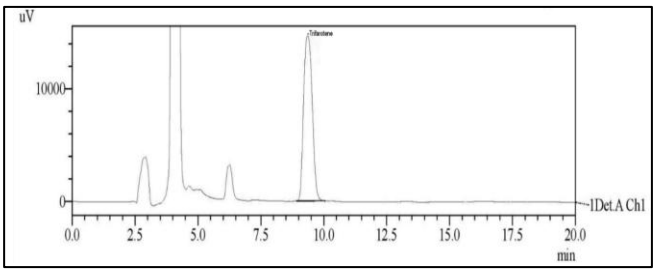

Figure 4: Chromatogram of Sample

Analytical method validation

Specificity: The specificity of an analytical method is its ability to accurately measure the analyte of interest amidst other sample components. No co-eluting peaks were observed at the retention time of Trifarotene. This indicates that the peak of the analyte was pure and confirms the specificity of the method (Figure 4).

System suitability: The system suitability parameters, including retention time, tailing factor, and theoretical plates for the optimized standard mixture chromatogram, are presented in Table 1.

Table 1: System suitability results

Sr No.	Area (mAu*min)	Retention Time (min)	Tailing Factor	Theoretical Plates (N)
1	369748	9.32	1.15	9628.682
2	369975	9.34	1.15	9613.183
3	370061	9.36	1.14	9669.299
4	371703	9.34	1.15	9652.867
5	370376	9.36	1.15	9646.406
6	368060	9.37	1.14	9690.23
Average	369987	9.35	1.15	9650
SD	1172.352	0.018	0.005	27.647
% RSD	0.317	0.196	0.450	0.286
Limit	NMT 2%	NMT 2%	NMT 2%	NLT 2000

Linearity:

The standard calibration curve was plotted between 0.5 and 3.5 µg/ml concentrations. Three injections from each concentration were analyzed under the same conditions. The average peak area ratio of Trifarotene was plotted against the corresponding concentrations to establish the calibration graph (Figure 5).

Accuracy (%Recovery):

The recovery studies confirm the accuracy of the proposed method, with known concentrations of the pure drug spiked at three different levels, i.e., 110%, 120%, and 130%, and the study was carried out in triplicates for each level. The average percentage recovery was calculated. The results are presentedin Table 2.

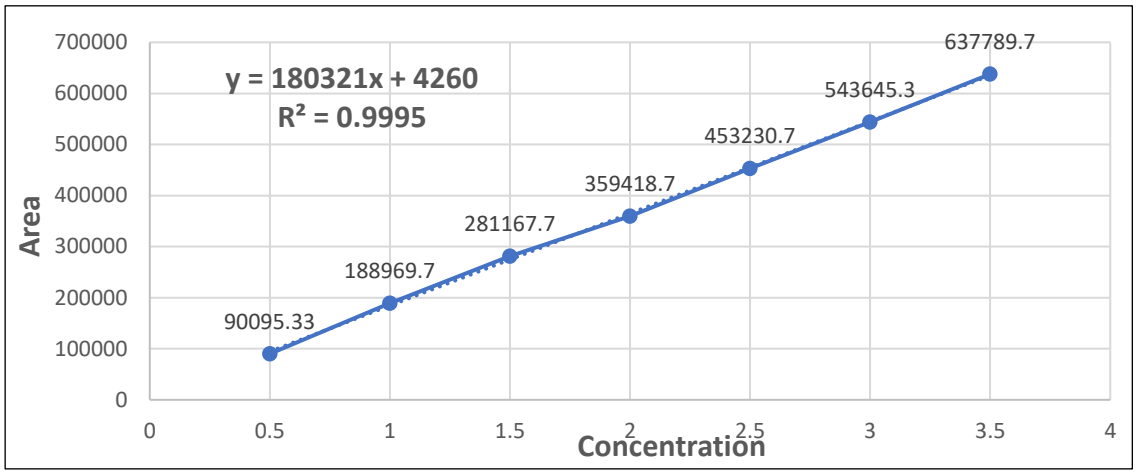

Figure 5: Calibration Curve for Linearity Data for Trifarotene

Table 2: Result for Accuracy

Level (in %)	Amount Spiked (ml)	Amount recovered (µg/g)	% Amount recovered	Mean recovery
100	0	49.77	99.54%	99.27%
110	1	54.69	109.2%	
120	2	59.60	119.2%	
130	3	64.61	129.22%	

Table 3: Result of Robustness

Parameters	Conditions	% Assay	Mean
Flow (± 2%ml/min) (± 0.02ml/min)	0.98ml	99.32%	99.34%
	1ml	99.54%	
	1.02ml	99.16%	
Column Temperature (± 2°C)	28°C	99.38%	99.48%
	30°C	99.65%	
	32°C	99.43%	
Wavelength (± 2nm)	260nm	99.65%	99.63%
	262nm	99.81%	
	264nm	99.45%	

Precision:

The reliability of the system and methodology was assessed by evaluating the consistency of data across multiple measurements. Six injections at 100% concentration were analyzed to determine precision. The results demonstrated excellent reproducibility, with a %RSD of 0.31%, confirming that the proposed approach ensures a high level of precision for analytical applications.

Limit of Quantification and Detection:

The limits of quantification and detection were determined by dividing the standard deviation of the intercept by the slope and then multiplying the obtained value by a factor of 10 for LOQ and 3.3 for LOD. The quantitation concentration limit was recorded 0.03µg/ml, and the detection concentration limit was 0.01µg/ml.

Robustness:

The robustness was considered to remain unaffected when minor variations were introduced in the chromatographic method. To analyze the robustness of the proposed method, deliberate changes were, made in flow rate, wavelength, and column temperature performed at 100% test (2ppm) concentration**.** The results are presented in Table 3.

CONCLUSION

A highly accurate, precise, specific, and stability-indicating isocratic RP-HPLC method was successfully developed and validated for the estimation of Trifarotene. The method met all ICH guideline criteria, demonstrating its reliability for quantitative analysis. Its robustness and compliance with regulatory standards make it a valuable tool for routine quality control and assay determination of Trifarotene, particularly in pharmaceutical formulations such as creams. This validated method ensures consistency and accuracy in drug analysis, contributing to the advancement of pharmaceutical research and development.

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