RESEARCH PAPER

Simple Cost Effective Stability Indicating Method Development of Tazarotene (TAZA) and Halobetasol (HALO) Using RP-HPLC

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Abstract

A simple, rapid, cost-effective, and stability-indicating reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Tazarotene (TAZA) and Halobetasol propionate (HALO) in combined cream formulation. Chromatographic separation was achieved using a C18 column with an optimized mobile phase at a flow rate of 1.0 mL/min and detection at 254 nm. The method exhibited excellent linearity over the concentration range of 2–10 µg/mL for HALO and 1–5 µg/mL for TAZA, with correlation coefficients (r^2) of 0.9994 and 0.999, respectively. The method was validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for parameters including linearity, accuracy, precision, robustness, specificity, limit of detection (LOD), and limit of quantitation (LOQ). Recovery studies showed results within 98–102%, and %RSD values for precision were less than 2%, indicating high reproducibility. Forced degradation studies under acidic, alkaline, oxidative, and thermal conditions demonstrated significant degradation without interference from degradation products, confirming the stability-indicating nature of the method. Assay results of marketed cream formulation were within acceptable limits. Hence, the developed RP-HPLC method is suitable for routine quality control and stability studies of combined TAZA and HALO pharmaceutical formulations.

Keywords: Tazarotene; Halobetasol propionate; RP-HPLC; Stability-indicating method; Method validation; Forced degradation; Cream formulation; ICH guidelines; Linearity; Precision.

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Introduction

Topical combination therapy is widely used in the management of inflammatory and hyperproliferative skin disorders such as psoriasis and dermatitis [1-3]. Tazarotene (TAZA) is a third-generation acetylenic retinoid that modulates keratinocyte differentiation and proliferation through selective binding to retinoic acid receptors (RARs). It is primarily indicated for psoriasis, acne, and photodamaged skin [4-5].

Halobetasol propionate (HALO) is a super-potent topical corticosteroid that exhibits strong anti-inflammatory, antipruritic, and vasoconstrictive properties. It acts by suppressing inflammatory mediators and reducing immune responses in affected skin tissues [6-7].

The fixed-dose combination of Tazarotene and Halobetasol propionate has shown enhanced therapeutic efficacy due to the complementary mechanisms of action retinoid-mediated normalization of keratinocyte differentiation and corticosteroid-mediated inflammation control. However, both drugs are chemically sensitive and prone to degradation under stress conditions such as light, heat, oxidation, acidic, and alkaline environments. Therefore, the development of a reliable stability-indicating analytical method is essential to ensure product quality, safety, and efficacy [8-10].

Among various analytical techniques, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is widely preferred for pharmaceutical analysis due to its high sensitivity, accuracy, reproducibility, and suitability for non-volatile and moderately polar compounds. A simple and cost-effective RP-HPLC method reduces solvent consumption, analysis time, and operational expenses while maintaining compliance with regulatory requirements [11-12].

A stability-indicating method is defined as a validated analytical procedure that accurately and specifically measures active pharmaceutical ingredients (APIs) in the presence of degradation products, impurities, and excipients. According to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines (ICH Q1A and Q2(R1)), forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions

are necessary to demonstrate the specificity and stability-indicating capability of the method [13].

Despite the availability of individual analytical methods for Tazarotene and Halobetasol, there is limited literature describing a simple, rapid, and economical stability-indicating RP-HPLC method for their simultaneous estimation in combined dosage forms. Therefore, the present study aims to develop and validate a precise, accurate, robust, and cost-effective RP-HPLC method for the simultaneous determination of TAZA and HALO in pharmaceutical formulations, in accordance with ICH guidelines.

The developed method is expected to provide effective separation of both drugs and their degradation products within a short run time, making it suitable for routine quality control and stability studies in pharmaceutical industries.

Material and Methods

Material

Pure drug samples of Tazarotene (TAZA) and Halobetasol propionate (HALO) were obtained as gift samples from pharmaceutical company. HPLC grade methanol and acetonitrile were procured from Merck (India), and analytical grade orthophosphoric acid and other reagents were used throughout the study. Ultra-pure water was prepared using a Milli-Q water purification system and filtered through a 0.45 µm membrane filter prior to use. The commercial cream formulation containing TAZA and HALO was purchased from the local market for assay analysis. Chromatographic analysis was performed using a standard Reverse Phase High Performance Liquid Chromatography (RP-HPLC) system equipped with a UV detector and a C18 analytical column. All chemicals and reagents used were of HPLC or analytical grade to ensure accuracy and reproducibility of results.

Methods

Selection of Mobile Phase

Initially to estimate Tazarotene and Halobetasol in fix dosage form number of mobile phase in different ratio were tried.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20mM KH_2PO_4: Acetonitrile (pH Adjust with OPA 3.0): Methanol in the ratio of 20:80v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution:

Accurately weighed 10mg API of HALO and TAZA was transferred into 10 ml volumetric flask separately and added 5ml of mobile phase as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50ml with diluent (mobile phase) to give concentration of 100µg/ml of HALO and TAZA respectively (Stock-B).

Preparation of Different Solution

0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (mobile phase). This gives the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml, for HALO. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of TAZA also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 2-10µg/ml for HALO and 1-5µg/ml for TAZA were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 245.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of HALO 10µg/ml for HALO and 5µg/ml TAZA was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 2 to 10µg/ ml for HALO) and (1 to 5µg/ ml for (TAZA) and areas for each

concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 2, 4, 6, 8 and 10µg/ml for HALO and 1, 2, 3, 4 and 5µg/ml for TAZA indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for HALO and TAZA reported in table.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM KH_2PO_4: Acetonitrile (80:20% v/v) to (85:15 % v/v). Results of robustness are reported in table.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in lotion formulation

Determined the content of HALO and TAZA in lotion, weight equivalent to 1mg TAZA was calculated and dissolved in 10ml mobile phase and the extraction was sonicated for 15 min and centrifuge at 300rpm. Then 1ml solution from it was diluted with 10 ml mobile phase. The resulting solution was injected in HPLC and drug peak area was noted. The peak area regression equation and amount of both the drug in sample was calculated. Analysis procedure was repeated six times with formulation. Results of lotion analysis are reported in table.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

10mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

10mg of the drug sample was taken into a 50ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs

Oxidative degradation:

10mg of the drug sample was taken into a 50ml separate round bottom flask, 50ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

10mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Results and Discussion

The present study describes the development and validation of a simple, rapid, cost-effective, and

stability-indicating RP-HPLC method for the simultaneous estimation of Tazarotene (TAZA) and Halobetasol propionate (HALO) in combined cream formulation using Reverse Phase High Performance Liquid Chromatography (RP-HPLC) as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.

Linearity and Calibration

The method showed excellent linearity over the concentration range of 2–10 µg/mL for HALO and 1–5 µg/mL for TAZA (Table 1). The correlation coefficients (r^2) of 0.9994 for HALO and 0.999 for TAZA confirm a strong linear relationship between concentration and peak area.

The regression parameters (slope and intercept) indicate consistent detector response and minimal systematic error. Additionally, low %RSD values in response ratio studies (Table 3) further support the reliability and proportional analytical response of the method.

he chromatograms (Figures 1–3) demonstrate well-resolved, sharp, and symmetrical peaks for both drugs without interference from excipients or blank. HALO and TAZA were eluted at retention times of 4.115 min and 6.314 min respectively (Table 2), indicating adequate separation within a short run time.

System suitability parameters such as theoretical plates (>2500) and tailing factor (<1.5) were within acceptable limits, confirming good column efficiency and peak symmetry.

Accuracy

Recovery studies performed at 80%, 100%, and 120% levels (Table 4) yielded results within 98–102% for both drugs. The overall %RSD values were less than 1.2%, demonstrating high accuracy and absence of matrix interference from formulation excipients.

Precision

Precision studies including repeatability, day-to-day, and analyst-to-analyst variation (Table 5) showed %RSD values well below 2% for both drugs. This confirms that the developed method is highly reproducible and reliable under normal laboratory conditions.

Robustness

Robustness testing (Table 6) showed minimal variation in assay values when small deliberate changes were introduced in chromatographic conditions. %RSD values remained below 1.1% for HALO and 0.15% for TAZA, indicating the method's stability and operational reliability.

Sensitivity (LOD and LOQ)

The low LOD and LOQ values (Table 7) indicate high sensitivity of the method. HALO exhibited LOD and LOQ values of 0.15 µg/mL and 0.45 µg/mL respectively, while TAZA showed 0.10 µg/mL and 0.30 µg/mL, confirming the method's suitability for detecting trace levels of analytes.

Assay of Cream Formulation

Assay results (Table 8) showed 100% for HALO and 97.78% for TAZA, both within pharmacopeial acceptance limits (95–105%). The low %RSD values confirm uniformity and suitability of the method for routine quality control analysis of marketed cream formulations.

Forced Degradation Studies

Forced degradation studies (Table 9) demonstrated significant degradation of both drugs under acidic, alkaline, oxidative, and thermal stress conditions. HALO showed maximum degradation under alkaline conditions (13.30%), whereas TAZA showed notable degradation under thermal and alkaline stress.

Importantly, degradation products did not interfere with the main drug peaks, confirming that the method is stability-indicating. The separation of degradation peaks from the parent drug peaks validates the specificity and selectivity of the developed RP-HPLC method.

Table 1: Linearity and Regression Parameters of HALO and TAZA

Parameter	HALO	TAZA
Linearity Range	2–10 µg/mL	1–5 µg/mL
Correlation Coefficient (r^2)	0.9994	0.999
Slope (m)	1243.6	1422
Intercept (c)	71.344	8.348

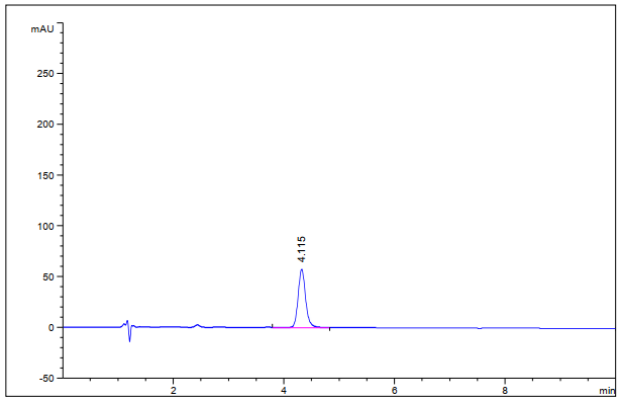

Figure 1: Chromatogram of HALO

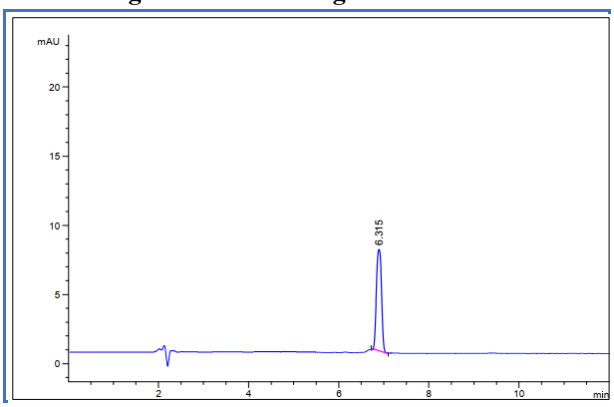

Figure 2: Chromatogram of TAZA

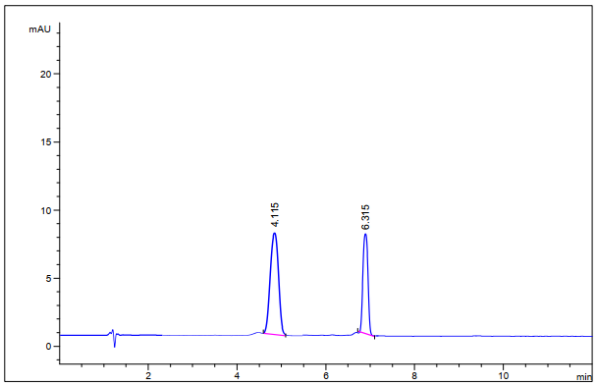

Figure 3: Chromatogram of Both the drug

Table 2: System Suitability Parameters

Drug	RT (min)	Mean AUC	Theoretical Plates (N)	Tailing Factor
HALO	4.115	12375.814	~2880	1.05
TAZA	6.314	10192.887	~2584	1.15

Table 3: Response Ratio data for Linearity

Drug	Mean Response Ratio	%RSD
HALO	1264.568	1.791
TAZA	2027.482	1.025

Table 4: Results of Recovery Study

Drug	80% Level (%)	100% Level (%)	120% Level (%)	Overall %RSD
HALO	99.26	96.92	99.07	< 1.2
TAZA	99.03	98.48	98.89	< 1.0

Table 5: Results of precision

Parameter	HALO (%RSD)	TAZA (%RSD)
Repeatability	< 0.20	< 0.15
Day-to-Day	< 0.20	< 0.16
Analyst-to-Analyst	< 0.15	< 0.12

Table 6: Results of Robustness

Drug	Mean % Assay	%RSD
HALO	95.14–99.70	< 1.1
TAZA	93.20–98.36	< 0.15

Table 7: Results of LOD and LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
HALO	0.15	0.45
TAZA	0.10	0.30

Table 8: Assay of Cream Formulation

Drug	Label Claim	% Assay	%RSD
HALO	0.01%	100%	0.125
TAZA	0.045%	97.78%	0.224

Table 9: Results of Forced Degradation Studies

Drug	Stress Condition	Drug Recovered (%)	Drug Decomposed (%)
HALO	Acidic	88.85	11.10
HALO	Alkaline	86.65	13.30
HALO	Oxidative	93.32	6.63
HALO	Thermal	88.87	11.08
TAZA	Acidic	92.25	7.20
TAZA	Alkaline	89.96	9.49
TAZA	Oxidative	94.45	5.00
TAZA	Thermal	89.45	10.00

Conclusion

A simple, rapid, economical, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Tazarotene (TAZA) and Halobetasol propionate (HALO) in combined cream dosage form. The method demonstrated excellent linearity, accuracy, precision, robustness, and sensitivity within the selected concentration ranges. All validation parameters complied with the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. The forced degradation studies confirmed that the method is

stability-indicating, as degradation products did not interfere with the drug peaks. The assay results of the marketed formulation were within acceptable limits, proving the applicability of the method for routine quality control analysis.

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