ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF HALOBETASOL AND TAZAROTENE IN BULK AND PHARMACEUTICAL FORMULATIONS

Ramanjaneyulu K.V*[1], Venkata Ramana K [1,2], M. Prasada Rao[3]

[1]*Department of Pharmaceutical Analysis, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India*

[2]*A.S.N College of Pharmacy, Tenali, Guntur, Andhra Pradesh, India*

[3]*M.A.M College of Pharmacy, Kesanupalli, Narasaraopet, Guntur, Andhra Pradesh, India*

**Corresponding author: ram.ramanji@gmail.com*

ABSTRACT

The objective of the present study is to develop simple RP-HPLC method for the simultaneous determination of Halobetasol and Tazarotene without prior separation. In this method, Luna C_{18} (250 mm×4.6 mm, 5µm) column was used. The mobile phase used was methanol and 0.1 M sodium perchlorate in the ratio of 87:13 (v/v) at pH 5.8, at flow rate of 1 ml/min. UV detection was monitored at 231 nm. Calibration graphs were established in the range of 4.5 to 27µg/mL for Tazarotene and 1 to 6µg/mL for Halobetasol. The retention time for Halobetasol and Tazarotene was found to be 3.4 min and 2.6 min, respectively. The intraday and interday precision expressed as percent relative standard deviation, were below 2%. The mean recovery of paracetamol and lornoxicam was found to be in the acceptable range. Hence it can be concluded that the validated HPLC method was found to be rapid, precise and accurate and can be readily utilized for analysis of Halobetasol and Tazarotene in bulk and in pharmaceutical formulations.

Keywords: Halobetasol, Tazarotene, HPLC method, Forced Degradation, lotion formulation.

1. INTRODUCTION

Halobetasol is an ultra potent corticosteroid having anti-inflammatory and antiproliferative effects. It is prescribed for the treatment of eczema, dermatitis, rash and psoriasis [1]. It reduces the itching, redness and swelling that occur in these types of conditions. Burning, stinging, itching, dryness or redness are the possible side effects associated with the use of Halobetasol. Halobetasol diffuses across cell membranes and interact with cytoplasmic corticosteroid receptors located in both the dermal and intradermal cells, thereby activating gene expression of anti-inflammatory proteins mediated via the corticosteroid receptor response element [2-3]. Stretch marks, skin discoloration, excessive hair growth and hair bumps are the possible side effects associated with the use of Halobetasol.

Tazarotene is a third-generation retinoid drug belongs to acetylenic class and is prescribed for the treatment of psoriasis, acne, and photodamaged skin (photodamage) [4]. In animals and humans, Tazarotene rapidly de-esterified in to its active carboxylic acid derivate which binds to retinoic acid receptors and modify gene expression [5]. Sensitivity to sunlight, dry skin, itchiness and redness are the major side effects associated with the use for Tazarotene.

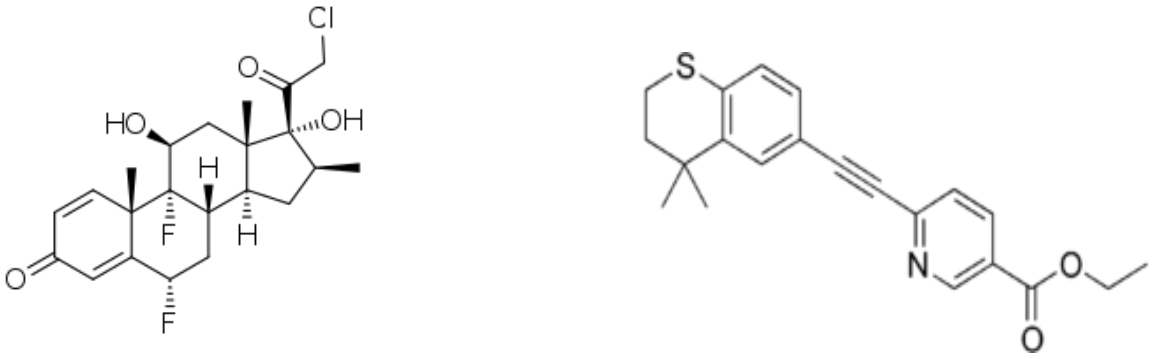
Halobetasol and Tazarotene are the combined medication available in lotion formulation and is used for the treatment of plaque psoriasis in adults [6]. The literature survey for the analysis of Halobetasol and Tazarotene confirms that there is only one HPLC method reported for the simultaneous estimation of Halobetasol and Tazarotene [7]. One HPLC [8] and One UV spectro-photometric [9] method reported for the estimation of Halobetasol in combination with other drugs. One HPLC method [10] is reported for the estimation of Tazarotene and its related impurities. Hence, the present work aimed to develop a simple, precise accurate HPLC method for the simultaneous estimation of Halobetasol and Tazarotene in pharmaceutical formulations. The molecular structure of Halobetasol and Tazarotene was given in fig. 1.

2. MATERIAL AND METHODS

2.1. Instrumentation

The separation and quantification of Tazarotene and Halobetasol was carried in waters spherisorb ODS1 C18

column (250 mm x 4.6 mm, 5µ) equipped in isocratic LC-100 S-HPLC™ (Cyberlab- USA) LC 20AT pump for solvent delivery and variable wavelength programmable LC-100 UV-visible detector for detection. The samples were injected using Rheodyne manual inject port and data was analyzed by using WS-100 Workstation software (Cyberlab- USA). DENVER (SI-234) electronic balance and ultrasonic batch sonicator (1.5 L) were used in the study.



1 A **1 B**

Fig. 1: Molecular structure of Halobetasol (1 A) and Tazarotene (1 B)

2.2. Chemicals and Reagents

Tazarotene and Halobetasol active pharmaceutical ingredients (APIs) were obtained from Glenmark pharmaceuticals limited, Mumbai. The marketed formulation DUORBII® (Tazarotene-0.045% and Halobetasol-0.01%) was purchased from local pharmacy. HPLC grade acetonitrile, methanol and water were purchased from Merck chemicals, Mumbai. Laboratory reagent grade sodium perchlorate and percloric acid were purchased from Fisher scientific, Mumbai. The membrane filter papers (0.2µ nylon) were purchased from millipore (India).

2.3. Preparation of standard solutions:

Fifty (50) mg of Tazarotene active pharmaceutical ingredient was accurately weighed and was dissolved in 50 ml methanol. Standard stock solution of Tazarotene at a concentration of 1000µg/ml was obtained and resulting solution was filtered through 0.45 µ nylon membrane filter paper. Tazarotene standard solution at a concentration 100 µg/ml was prepared by diluting 10 ml from 1000 µg/ml to 100 ml. The same procedure was used for the preparation of Halobetasol standard solution separately. For preparing calibration curve dilutions, equal volume of known and fixed concentration of Tazarotene and Halobetasol were mixed separately. The combined solution of Tazarotene and Halobetasol having known concentrations were used for method development and validation study.

2.4. Preparation of formulation solution

An accurately weighed 1gram of Tazarotene and Halobetasol with brand DUORBII® (Tazarotene-0.045% and Halobetasol-0.01%) was dissolved in 100 mL methanol. Then it was filtered and was further diluted to get a concentration of 18 µg/mL of Tazarotene. As per the label claim of the drugs in the formulation, sample solution having 4 µg/mL of Halobetasol. This solution is used for the determination of the applicability of the developed method for the analysis of Tazarotene and Halobetasol in pharmaceutical formulations.

2.5. Method development

In the method development, various mobile phase compositions with different pH ranges, wavelength of the detector and stationary phase was studied. One condition is changes in each developmental studies and remaining parameters were kept intact. In each studied condition, system suitability conditions like number of theoretical plates, tail factor for each drug and the resolution factor between both the drugs was studied in consideration with ICH guidelines [10]. All the results observed were summarized and the conditions that produce best results were considered as the suitable conditions and the conditions were compared with the existing literature. The best suitable and advance method conditions for the simultaneous quantization of Tazarotene and Halobetasol obtained and the method was further validated.

2.6. Method validation

The best suitable conditions that produced in the method development studies was further validated as per ICH guidelines and the validation parameters like linearity range, accuracy, ruggedness, robustness, precision.

The range of analysis of the developed method was determined by serial diluting the standard drug solution having different concentration ranges of both the drugs Tazarotene and Halobetasol peak area response for both the drugs in each level of analysis was noted and the calibration curve was constructed by plotting peak area against the concentration of the analyte prepared. From the obtained calibration curve, the range of analysis for both the drugs in the developed method was obtained.

In the calibration curve range obtained, one concentration is selected degerming the precision, ruggedness and robustness of the method. The selected concentration was repeatedly analyzed six times in the same day for intraday precision, six times in three different days for interday precision and six times by change of analysts for ruggedness. The % relative standard deviation (RSD) of the peak area response of both the analytes was calculated and the % RSD of less than 2 was considered as acceptable.

The accuracy and recovery of the developed method confirmed by spiked recovery study. Three spiked levels *i.e.* 50%, 100% and 150 % were studied with a target concentration of 9µg/mL of Tazarotene and 2µg/mL Halobetasol. The % recovery in each studied concentration and the % recovery in each spiked level was studied. A % recovery of 98-102 % and the % RSD of less than 2 was considered as accurate.

The change in the peak area response when small and deliberate changes were made in the developed method confirms the robustness of the developed method. The mobile phase composition (±5 %), detector wavelength (±5 nm) and pH of the mobile phase (±0.1) were studied. In each changed condition, the % change in the peak area response was calculated and the % change of less than 2 was considered as acceptable.

The limit of detection (LOD) and limit of quantification (LOQ) was considered as sensitive expression parameter for the developed method. The signal and noise ratio (s/n) of both the analytes in the developed method was used for evaluating the sensitivity of the method developed for the analysis of Tazarotene and Halobetasol. S/N ratio of 3 was considered as LOD and 10 were considered as LOQ for both the drugs in the developed method.

The stability of both the drugs in different stress conditions confirms the stability indicating nature of the developed method. Both the standard drugs Tazarotene and Halobetasol was expressed in different stress conditions like acidic, base, peroxide, thermal and ultra violet (UV) light. Fifty (50) mg of standard drug was mixed with 50mL of 0.1N HCl for acid hydrolysis study, 50mL of 0.1N NaOH in base hydrolysis study and 50mL of 3% hydrogen peroxide solution for oxidative degradation study. These conditions were carried separately for both the drugs and the solutions were incubated 24 H and then neutralized separately. The equal volume of selected concentration of both the drugs were mixed and then neutralized. The neutralized solutions were analyzed in the developed method condition. In photolytic and thermal degradation conditions, standard drug was kept under UV light at 254nm and oven at 60°C for 24 hours respectively. Then the standard drug was diluted to and was analyzed in the developed method condition. The % degradation, number of degradation products formed in the degradation study and the % effectiveness of the method for the separation of degradation products was evaluated.

The sample solution prepared from the marketed formulation of Tazarotene and Halobetasol was analyzed in the developed method and the peak area observed was used for the determination of formulation assay which confirms the applicability of the method for the analysis of Tazarotene and Halobetasol in the pharmaceutical formulations.

3. RESULTS AND DISCUSSION

The present work is aimed to develop a simple, precise and accurate stability indicating HPLC method for the separation and simultaneous quantification of Tazarotene and Halobetasol in pharmaceutical formulations. The optimized separation was achieved using isocratic elution at a flow rate of 1.0 mL/min using mobile phase of methanol and 0.1 M sodium perchlorate in the ratio of 87:13 (v/v) at pH 5.8. Luna C_{18} (250 mm×4.6 mm, 5 µm) column was used as stationary phase and UV detection was monitored at 231 nm. In the optimized conditions, Tazarotene and Halobetasol were well resolved and retained with clear base line were observed within run time of 10 min. The method obeys system suitability conditions (Table 1) for both Tazarotene and Halobetasol. Figure 2 shows the optimized chromatogram of Tazarotene and Halobetasol in the developed method.

Table 1: System suitability results

Parameter	Tazarotene	Halobetasol
Api Concentration	18 µg/ml	4 µg/ml
RT (min)	2.6	3.4
Area	242195	63712
Resolution	8.54	---
Theoretical Plates	5540	6827
Tailing Factor	1.59	0.79

An accurately fit calibration curve was observed in the concentration range of 4.5 to 27 µg/mL for Tazarotene and 1 to 6 µg/mL for Halobetasol with regression equation of y = 12118x +19407 (R^2 = 0.9993) and y = 14566x + 5369 (R^2 = 0.9987) for Tazarotene and Halobetasol, respectively. The correlation coefficient was found to be very high for both the drugs confirms that the calibration range was found to be accurate in the concentrations studied. The results of linearity study were given in table 2 and calibration curve was shown in Fig.3 and 4 for Halobetasol and Tazarotene respectively. In robustness study, small change in the analytical conditions doesn't influence the separation, detection of both the drugs. There is no change in the baseline, retention times and system suitable parameters of both Halobetasol and Tazarotene. The % change in each changed condition was found to be within the acceptable of less than 2 which is in the acceptable limits. This confirms that the method was found to be robust. In the recovery study the % recovery within the range of 98-102 and the % RSD of less than 2 (table 3 and 4) was observed for both the drugs in the developed method. This confirms that the method was found to be accurate.

Table 2: Linearity results

S. No	Tazarotene		Halobetasol	
	Concentration in µg/ml	Peak Area	Concentration in µg/ml	Peak Area
1	4.5	72345	1	19081
2	9	129511	2	34154
3	13.5	180913	3	50839
4	18	242195	4	63712
5	22.5	292774	5	78398
6	27	343845	6	91925

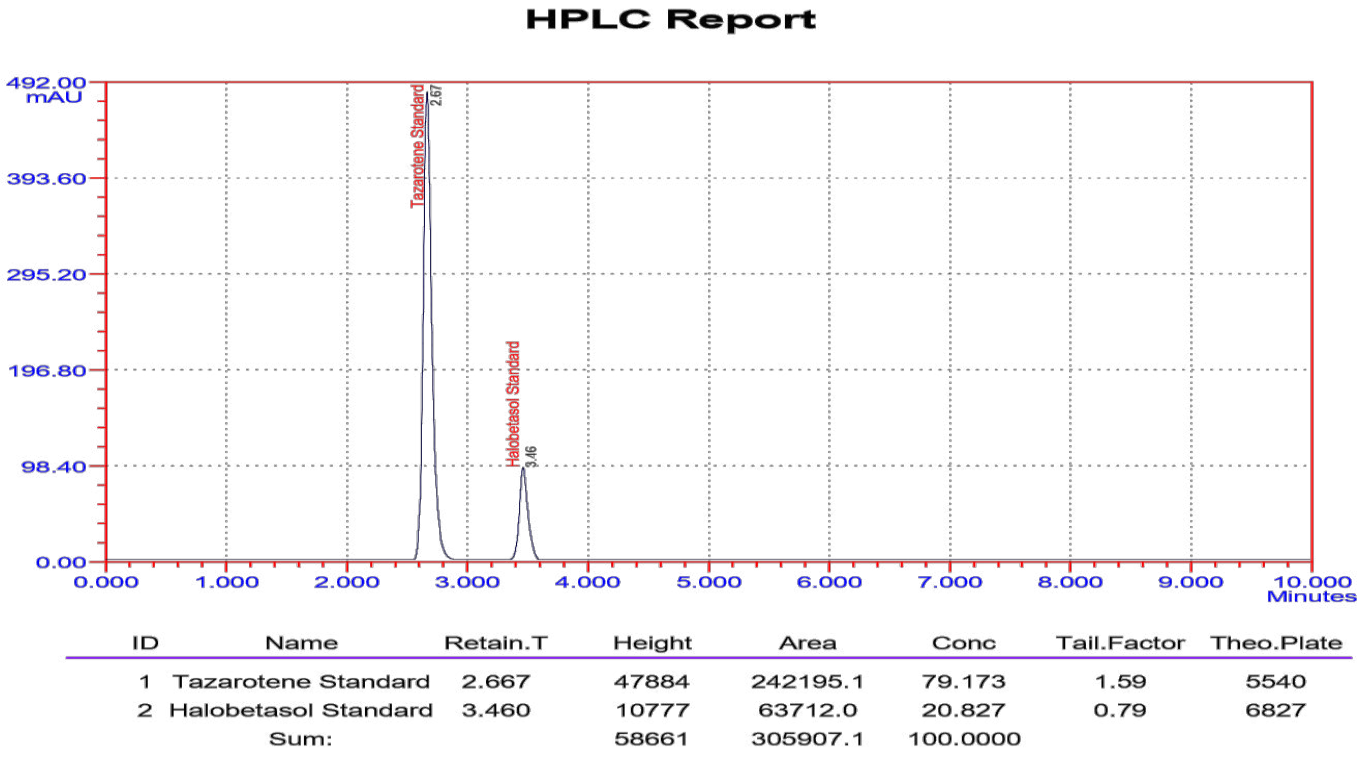


Fig. 2: Standard chromatogram in the optimized conditions

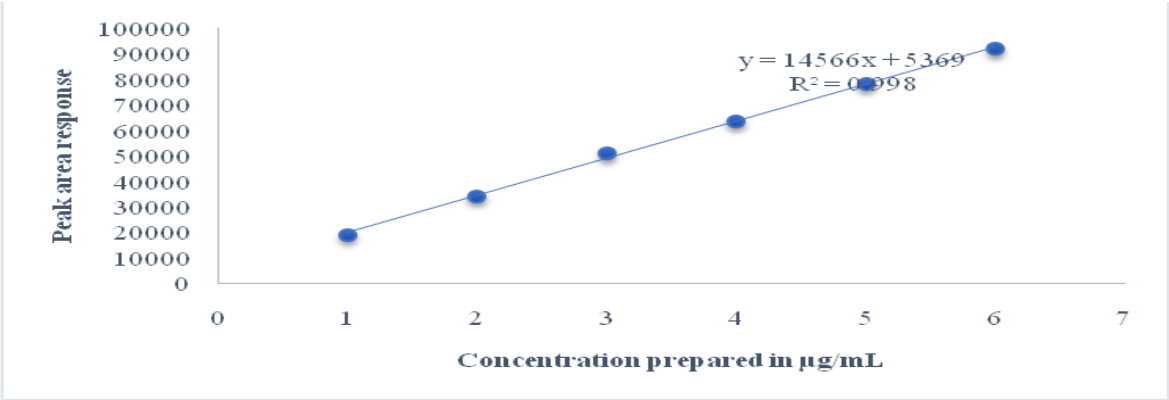


Fig. 3: Linear calibration curve for Halobetasol

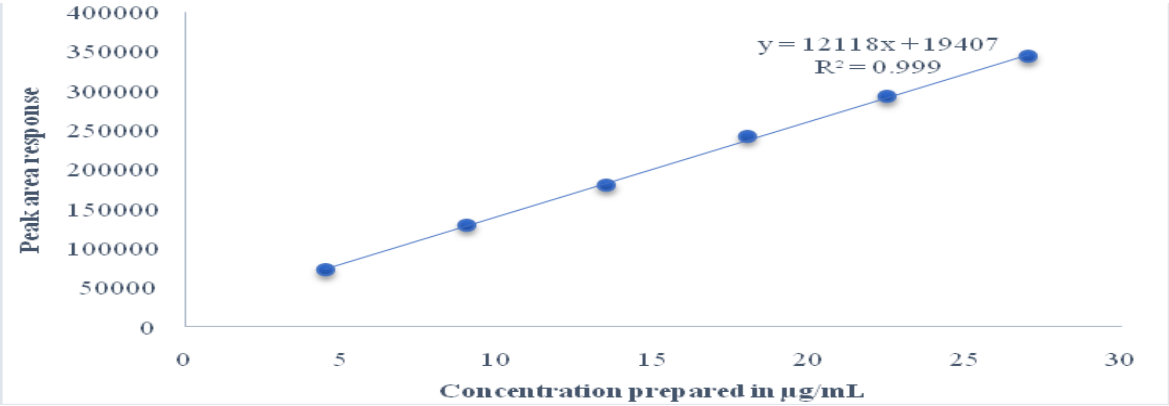


Fig. 4: Linear calibration curve for Tazarotene

Table 3: Linearity results for Halobetasol

S No	Recovery level	Concentration in µg/mL			Peak Area	Concentration Obtained (µg/mL)	% Recovery	% RSD of recovery
		Target	Spiked	Total				
1	50%	2	1	3	50641	2.98832	99.6105	0.001
2		2	1	3	50493	2.97958	99.3194	
3		2	1	3	50571	2.98419	99.4728	
4	100%	2	2	4	63104	3.96183	99.0457	0.003
5		2	2	4	63487	3.98587	99.6468	
6		2	2	4	63052	3.95856	98.9641	
7	150%	2	3	5	77787	4.96103	99.2206	0.007
8		2	3	5	77185	4.92264	98.4528	
9		2	3	5	78386	4.99923	99.9847	

Table 4: Linearity results for Tazarotene

S. No	Recovery level	Concentration in µg/mL			Peak Area	Concentration Obtained (µg/mL)	% Recovery	% RSD of recovery
		Target	Spiked	Total				
1	50%	9	4.5	13.5	180181	13.4454	99.5954	0.641
2		9	4.5	13.5	178290	13.3043	98.5501	
3		9	4.5	13.5	180376	13.4599	99.7032	
4	100%	9	9	18	241898	17.9779	99.8774	0.204
5		9	9	18	241737	17.966	99.8109	
6		9	9	18	240975	17.9093	99.4963	
7	150%	9	13.5	22.5	288018	22.1345	98.3755	0.795
8		9	13.5	22.5	292571	22.4844	99.9307	
9		9	13.5	22.5	290984	22.3624	99.3886	

The %RSD intraday, interaday precision and ruggedness study was found to be within the acceptable limits of less than 2 confirms that there is change in the results observed for both the drugs when analysis was carried in different days or different analysts. This confirms that the method was found to be precise and rugged. The results of precision and ruggedness study are given in table 5.

The method cam applied for the separation of unknown impurities formed during the stress study and the stress effect on the standard drugs in the developed method was assessed by forced degradation study. In all the stress conditions studied, both the drugs were detected and the degradation products were successfully separated in the developed method. The chromatogram of acid degradation study (fig. 5) shows three impurity products with a % degradation of 94.82 and 93.52 for Tazarotene and Halobetasol respectively. The % degradation of 7.40, 2.70, 3.22 and 4.52 was observed for Tazarotene and 6.21, 4.69, 14.40 and 2.56 for Halobetasol in Base, Peroxide, Thermal and UV light conditions. During the stress study, there is no change in the retention time, system suitability conditions of both the analytes in the developed method and the degradation compounds formed were successfully separated. Hence the method can be used for the identification of known or unknown impurities formed during the stress study. Hence the method was considered as stability indicating method. The stress degradation chromatograms were given in fig. 5 to 9.

The formulation assay was found to be 99.84 % for Tazarotene and 98.21% for Halobetasol in the developed method. In the formulation chromatogram, both the drugs Halobetasol and Tazarotene were well retained and the retention time was found to be similar to the standard. There is no detection of lotion formulation excipients and clear base line was observed (fig. 10).

Table 5: Precision and ruggedness results

S No	Intraday Precision		Interday Precision		Ruggedness	
	Tazarotene	Halobetasol	Tazarotene	Halobetasol	Tazarotene	Halobetasol
1	242188	63789	242444	63140	242541	63125
2	244727	63495	242335	63511	238490	62619
3	241856	63076	242149	63518	243970	63031
4	242627	63511	241523	63600	241711	63400
5	243010	63201	241974	63669	241576	63042
6	242796	63005	243617	63939	241360	62408
% RSD	**0.41**	**0.48**	**0.29**	**0.41**	**0.75**	**0.57**

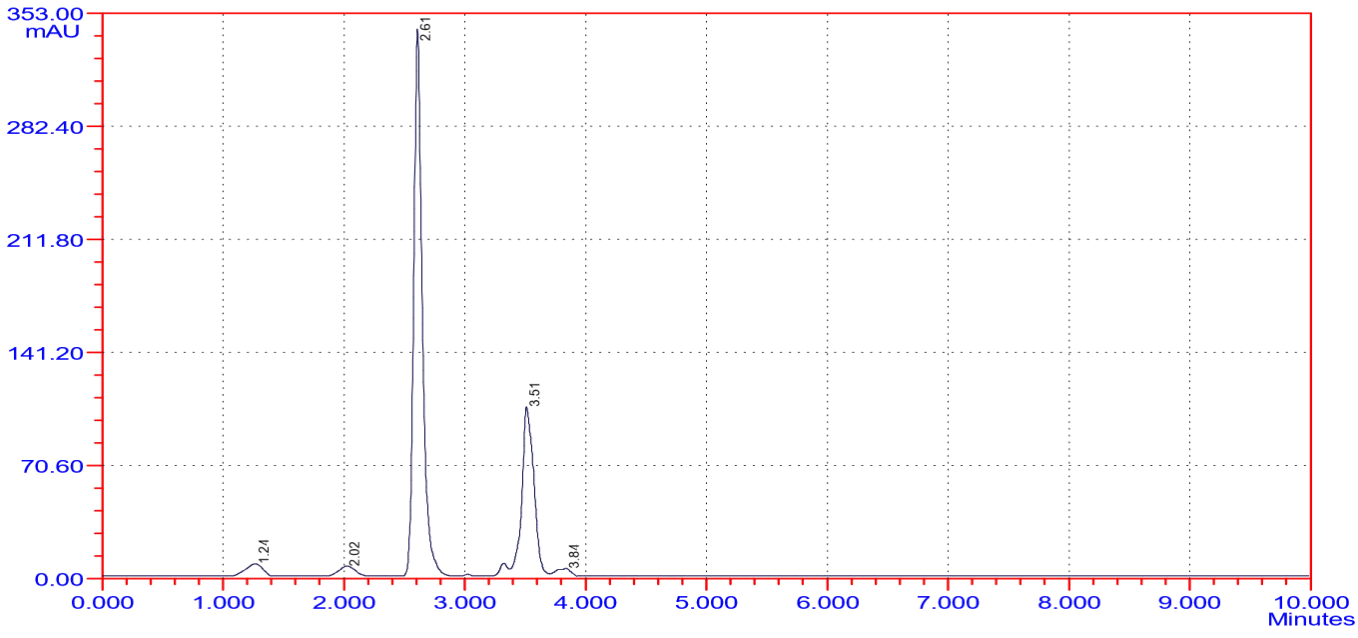


Fig. 5: Acid degradation chromatogram

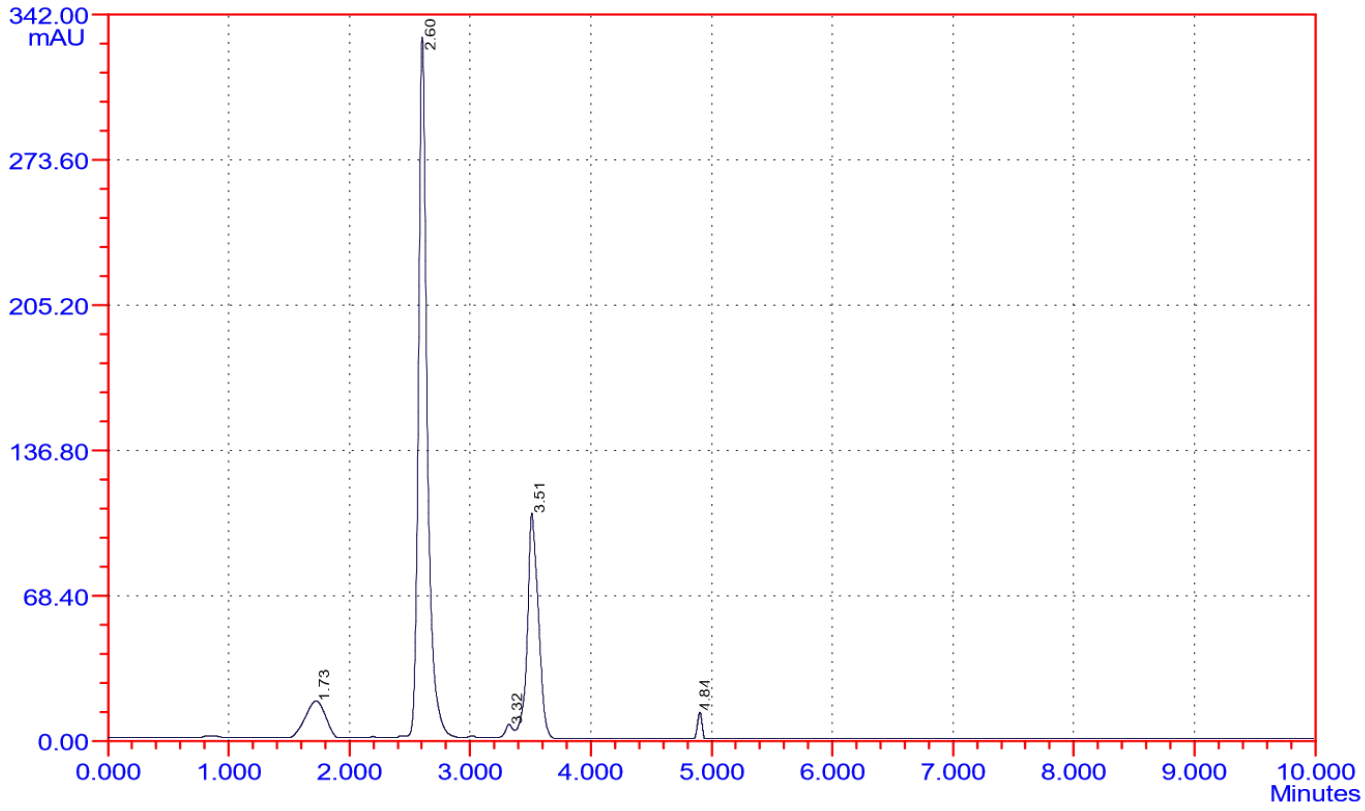


Fig. 6: Base degradation chromatogram

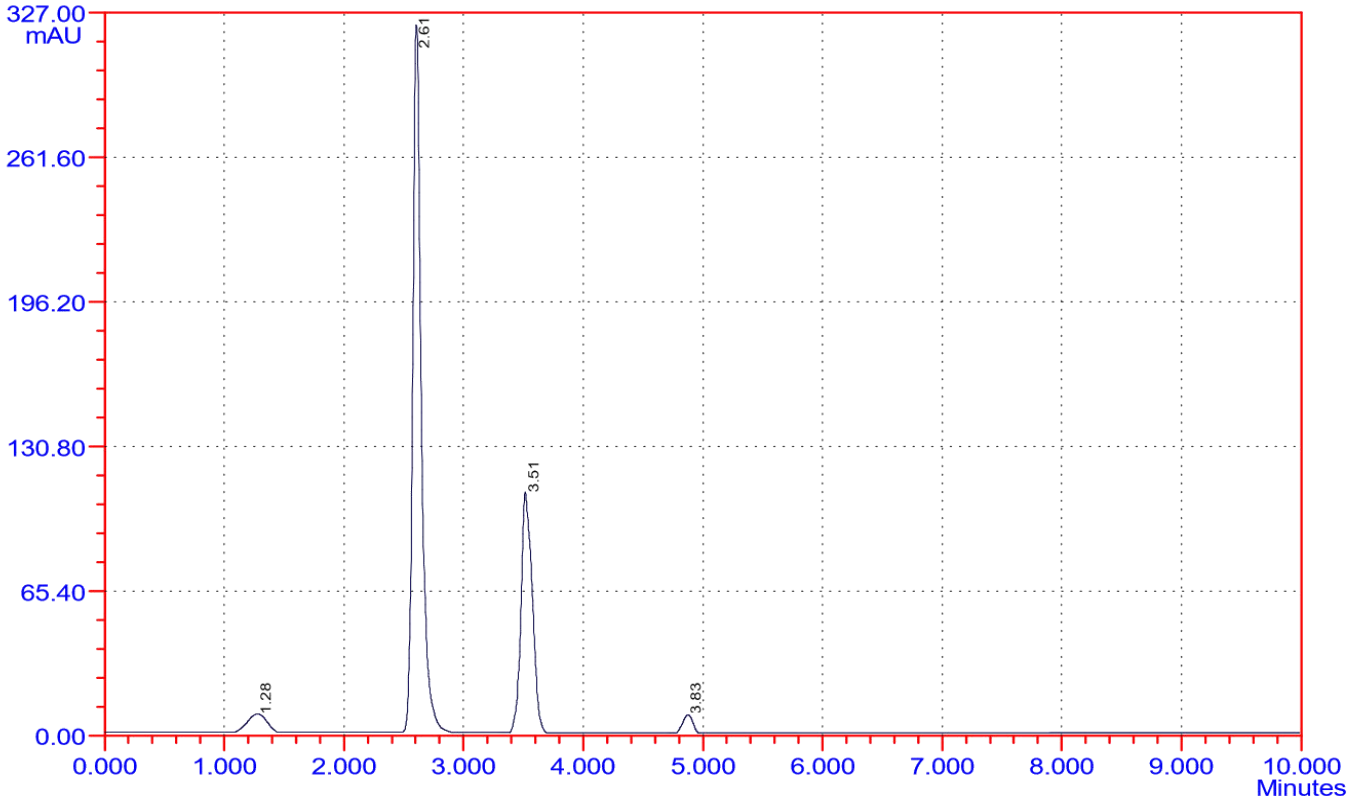


Fig. 7: Peroxide degradation chromatogram

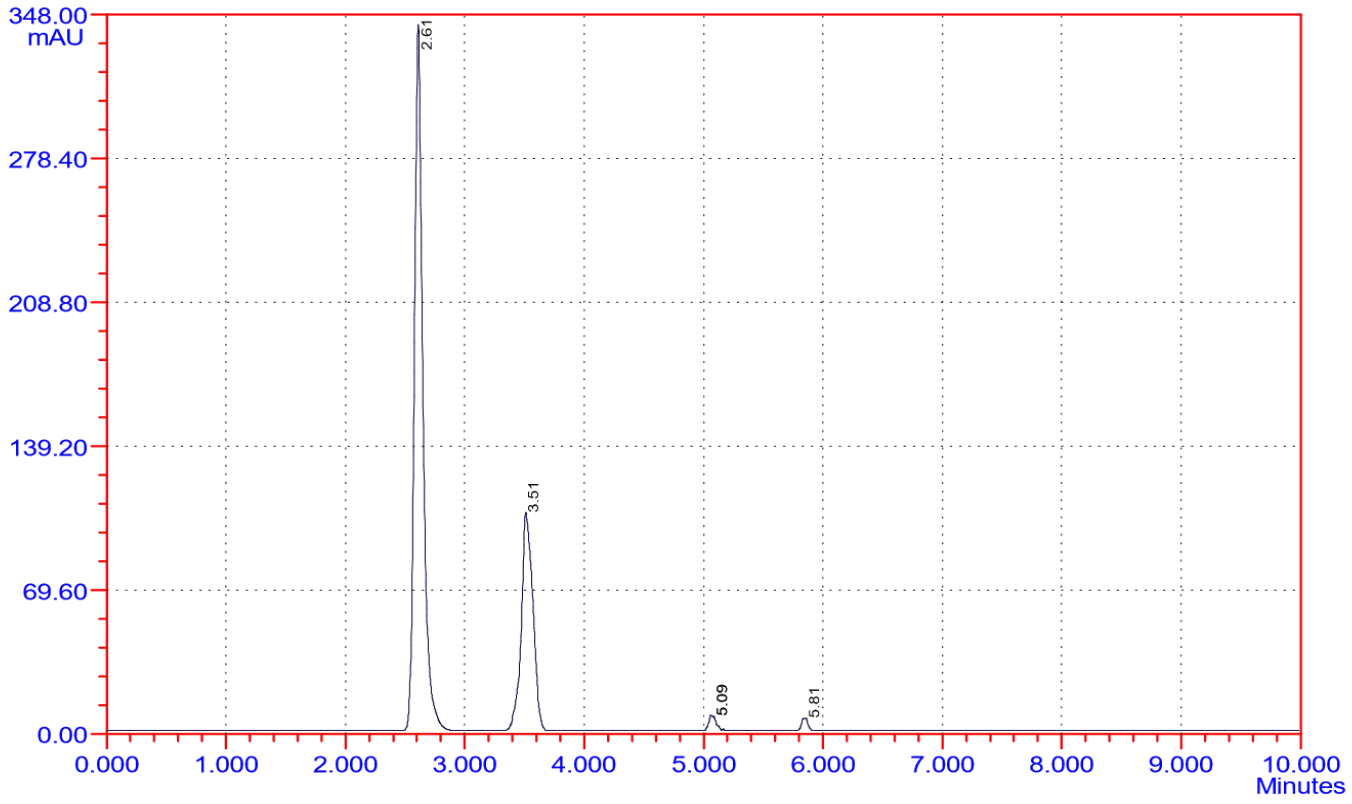


Fig. 8: Thermal degradation chromatogram

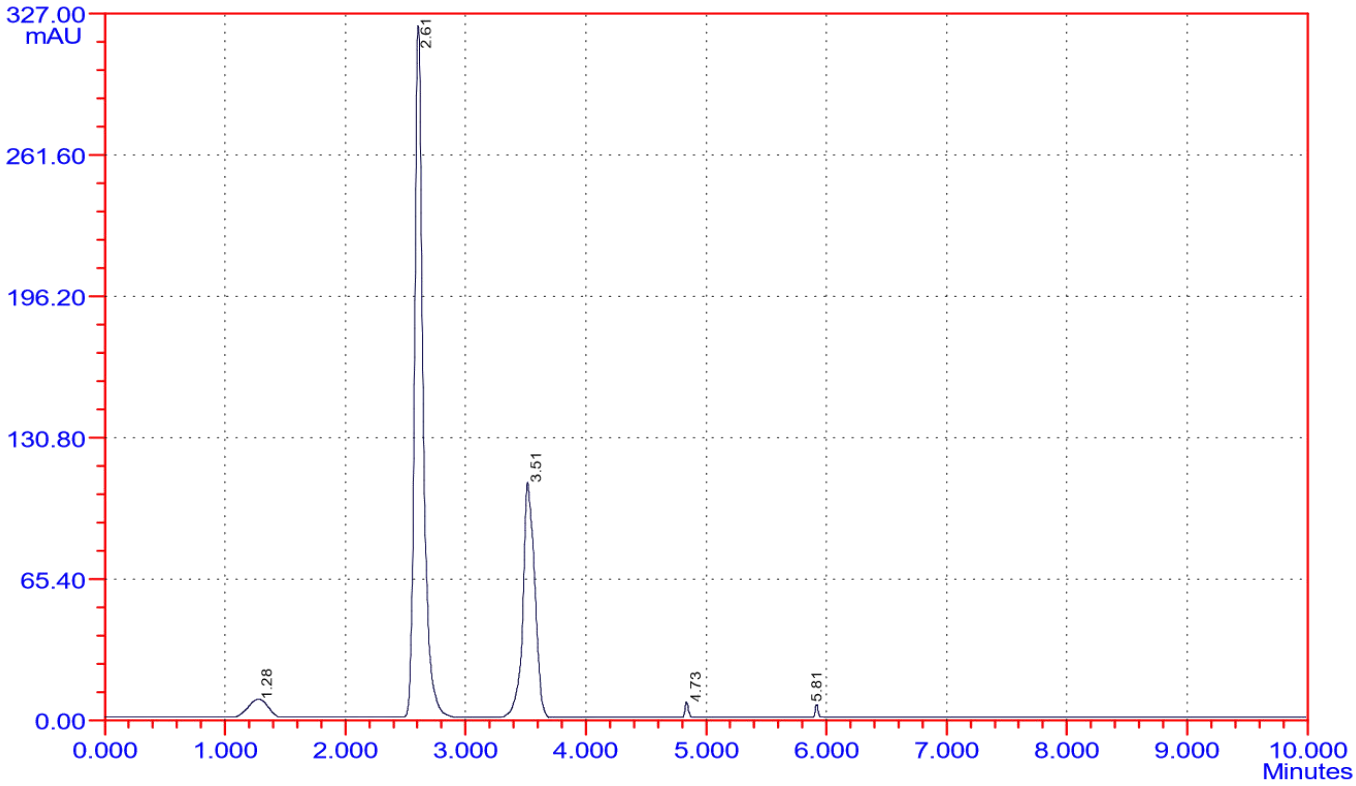
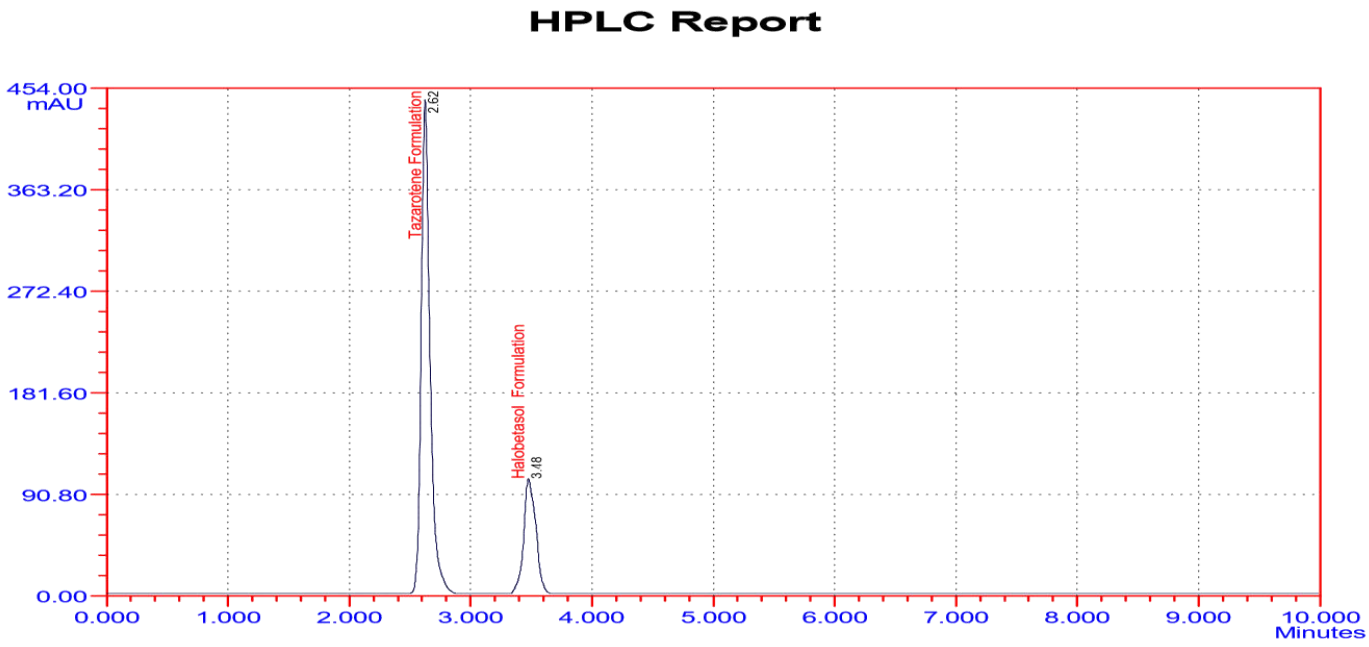


Fig. 9: UV light degradation chromatogram



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Tazarotene Formulation	2.623	45044	241816.8	81.305	1.14	4759
2	Halobetasol Formulation	3.477	10251	55603.2	18.695	1.90	8188
	Sum:		55295	297419.9	100.0000		

Fig. 10: Formulation chromatogram

The novelty of the developed method was proved by comparing the developed method conditions with the literature available for the simultaneous analysis of Halobetasol and Tazarotene. In the literature, there is only one HPLC method reported by Sravanthi et al. [7] for the simultaneous analysis of Halobetasol and Tazarotene. The less buffer composition that facilitates column long life, wide calibration range give more applicability and early retention time which completes the analysis in less time are the advantages for the developed method than the existing literature. The other methods reported were found to be the one analyte in the present study with other combination drugs available in market. Hence the method is successively applied to pharmaceutical formulation; No chromatographic inter-ferences from the tablet excipients were found. The suitability of this HPLC method for quantitative determination of the compounds is proved by validation in accordance with the requirements of ICH guidelines. Statistical data showed that RP-HPLC methods are robust, rugged, sensitive and accurate as compared to existing analytical methods. RP-HPLC methods are costly and time consuming but produce more accurate and precise results.

4. CONCLUSION

The optimization of RP-HPLC method showed that the mobile phase composition, pH and flow rate were more crucial parameters to be controlled for reproducible and quantitative estimation of Halobetasol and Tazarotene. Simple and reproducible sample extraction for lotion formulation provided higher sensitivity for determination of combination of drugs by RP-HPLC. The developed RP-HPLC method could further be applicable for the stability analysis of Halobetasol and Tazarotene in their combined dosage forms.

Conflicts of interest

None declared

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