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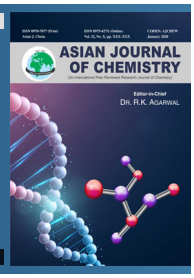

Stability Indicating RP-HPLC Assessment and Stability Testing of Tazarotene and Halobetasol in Lotion Formulation

V.L.N. Balaji Gupta Tiruveedhi[1,*], Venkateswara Rao Battula[2] and Kishore Babu Bonige[2]

[1]Department of Basic Science, Vishnu Institute of Technology (A), Bhimavaram-534202, India
[2]Department of Engineering Chemistry, Andhra University, Visakhapatnam-530003, India

*Corresponding author: E-mail: tvlbalaji79@gmail.com

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A novel liquid chromatographic method was established and validated for the quantification of tazarotene and halobetasol in the presence of degradation products obtained during stress conditions. The liquid chromatographic method was based on isocratic elution on Knauer Eurospher II C18 (5 µm particle size, 250 mm × 4.5 mm) column using a mobile phase consisting of methanol, acetonitrile and 0.5 mM perchloric acid (40:45:15 v/v/v) mixture at flow rate of 0.9 mL/min. Quantization of tazarotene and halobetasol was done with UV detection at 234 nm. The method validity was assessed in agreement with the recommendations of the International Conference on Harmonization. Linearity, accuracy and precision were satisfactory over the concentration ranges of 2.25-13.5 and 0.5-3.0 µg/mL for tazarotene and halobetasol, respectively. Detection limit values of 0.006 µg/mL and 0.01 µg/mL were found for halobetsol and tazarotene, respectively, while the quantization limit values of 0.02 µg/mL and 0.033 µg/mL were found for halobetsol and tazarotene, respectively. The developed method is appropriate for routine analysis and quality control of tazarotene and halobetasol combination in lotion dosage form.

Keywords: RP-HPLC, Tazarotene, Halobetasol, Lotion formulation.

INTRODUCTION

Tazarotene (Fig. 1a) is an associate group of retinoid acetylene drugs. De-esterification of tazarotene in cells generates tazarotenic acid, an active tazarotene's metabolite [1,2]. Tazarotenic acid attaches to α-, β- and γ-types of retinoic acid receptors. Tazarotenic acid displays selectivity for types of β- and γ-receptors of retinoic acid and even can modify gene expression [3]. Tazarotene is applicable to medicate the disease psoriasis, detriment of skin due to sun and acne [4-7].

Halobetasol (Fig. 1b) is a vasoconstrictive, antipruritic and anti-inflammatory drug belonging to synthetic corticosteroids category of drugs [8]. After binding to intradermal and dermal cell corticosteroid receptors, halobetasol propels the expression of anti-inflammatory protein genes. Prostaglandin and leukotrienes are powerful mediators of inflammation which are produced from arachidonic acid. The curbing of deliverance of arachidonic acid by halobetasol is executed *via* the induction of phospholipase A2 inhibitory proteins [9]. The reduction in severity of skin conditions like eczema, dermatitis, psoriasis and rash are supported by halobetasol [10,11].

The authorization of the topical lotion by name Duobrii™ to treat plaque psoriasis in adults was given in April, 2019 by the US FDA [12-14], of which every gram has a composition consist of (0.01%) 0.1 mg of halobetasol and (0.045%) 0.45 mg of tazarotene. The literature study disclosed that so far there isn't any analytical method in quantification of lotion formulation of halobetasol and tazarotene. The intention of the current study was to develop a novel stability indicating liquid chromatographic method to estimate lotion formulation of halobetasol and tazarotene simultaneously.

EXPERIMENTAL

Agilent 1100 series HPLC system with Quaternary pump (model no. G1311 A), column temperature control (model no. G1316A), autosampler (model no. G 1329A) and UV detector (model no. G1314 A) was used to develop and validate the assay

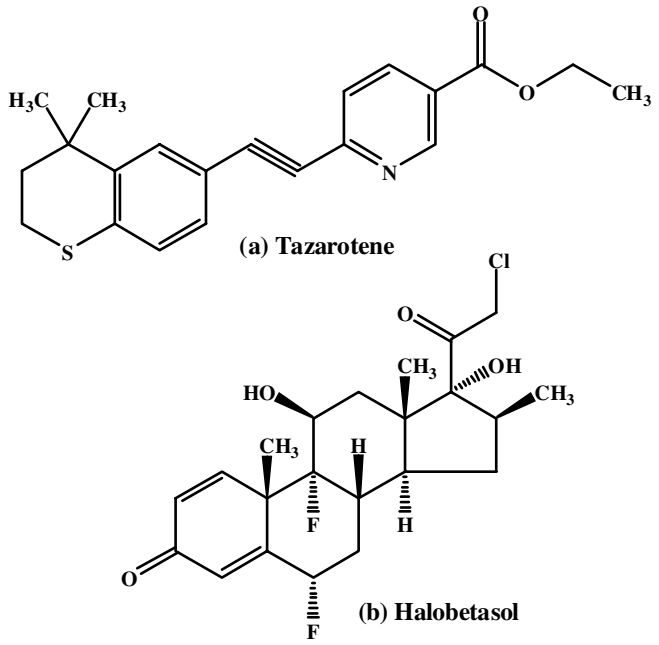

Fig. 1. Chemical structure of (a) tazarotene and (b) halobetasol

method. Agilent Chem Station LC software was employed to control and integrate the HPLC system. Knauer Eurospher II C18 column (250 mm × 4.5 mm; 5 µm) column was utilized to separate and assay halobetasol and tazarotene simultaneously.

Glenmark Pharmaceuticals Limited gifted the reference drugs of halobetasol and tazarotene. Duobrii™ topical lotion formulation (Bausch Health Americas, Inc. USA) was purchased locally. HPLC class acetonitrile and methanol were acquired from Qualigens Fine Chemicals Ltd. Milli-Q water was obtained through processing with Milli-Q (Millipore, Merck, Germany) water purification system. Analytical class hydrochloric acid, perchloric acid, sodium hydroxide and peroxide were obtained from M/s. Rankem Chemicals Ltd.

HPLC assay conditions: Methanol, acetonitrile and 0.5 mM perchloric acid (40:45:15 v/v/v) mixture at an isocratic flow rate of 0.9 mL/min was employed as mobile phase. The pH was set to 5.6 with 0.5 mM perchloric acid. The injection volume, column temperature and detector were fixed at 20 µL, 25 ± 2 ºC and 234 nm, respectively.

Standard solutions: In the preparation of standard solutions, the mobile phase was employed as diluent. With mobile phase, stock solution of halobetasol (100 µg/mL) and tazarotene (450 µg/mL) were prepared. The sequential dilution of halobetasol and tazarotene stock solution was done to get five-level calibration solutions of concentrations as underneath:

Tazarotene: 2.25, 4.5, 6.75, 9.0, 11.25 and 13.50 µg/mL

Halobetasol: 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL

The halobetasol and tazarotene stock solution was diluted to get solution of 1 µg/mL halobetasol and 4.5 µg/mL tazarotene concentration for the purpose of study of validation parameters.

Calibration curve: Preparation of concentrations of 6 units, from 0.5-3.0 µg/mL of halobetasol and 2.25-13.50 µg/mL of tazarotene was done for calibration curves. Every solution of concentration was injected into the system and analyzed using the method recommended. The peak areas of selected analytes (halobetasol and tazarotene) are mapped against the corresponding concentrations of selected analytes (halobetasol and tazarotene) in attempt to prepare the calibration curves. Regression equation was computed using concentration data and peak area data.

Assay of tazarotene and halobetasol concentration in lotion formulation: An exact weight 10 g Duobrii™ topical lotion (equivalent to 1 mg halobetasol and 4.5 mg tazarotene) was transferred to flask with capacity 100 mL. A mixture of 30 mL solvent (acetonitrile and methanol 40:60 v/v) was appended then heated using a water bath till 50 ºC for a period of 10 min and later brought to room temperature making its volume to 100 mL with the help of solvent system already used. The filtration of this solution was performed with a membrane made of nylon with thickness 45 µm. Apx 1 mL of the solution made was diluted for 10 mL with a diluent so as to acquire a solution comprising halobestol and tazarotene at 1.0 µg/mL and 4.5 µg/mL concentrations, respectively. The solution thus prepared was incorporated into the system and then analyzed as per the method suggested. Halobetasol and tazarotene concentration in the lotion formulation was determined through its calibration curve or regression equation.

Degradation studies: In attempt to achieve an indication about the stability indicating ability and specificity of the present method, forced degradation tests have been performed on lotion sample of halobetasol and tazarotene. The stress degradation study was undertaken [15] in accordance with the criteria which are follows:

Acid based degradation: In a 10 mL flask, 1 mL of lotion sample (halobetasol 10 µg/mL and tazarotene 45 µg/mL) was mixed with 5 mL of 0.1N HCl and maintained at room temperature exactly for 24 h. Thereupon neutralization was done using 0.1 N NaOH and diluted properly by the diluent to get 1 µg/mL of halobetasol and 4.5 µg/mL of tazarotene concentration solution. The solution thus obtained was injected into the system and assessed as per the method suggested.

Alkali based degradation: In a 10 mL flask, 1 mL of lotion sample (halobetasol 10 µg/mL and tazarotene 45 µg/mL) was mixed with 5 mL of 0.1N HCl and maintained at room temperature for 24 h. Thereupon neutralization was done using 0.1 N HCl and diluted using diluent so as to get 1 µg/mL of halobetasol and 4.5 µg/mL of tazarotene concentration solution. The solution thus obtained was injected into the system and assessed as per the method suggested.

Peroxide based degradation: In a 10 mL flask, 1 mL of lotion sample (halobetasol 10 µg/mL and tazarotene 45 µg/mL) was mixed with 5 mL of 3% peroxide. The mixture was maintained at room temperature for 24 h and diluted using diluent so as to get 1 µg/mL of halobetasol and 4.5 µg/mL of tazarotene concentration solution. This solution was injected into the system and assessed as per the method suggested.

UV based degradation: A 1 mL lotion sample (halobetasol 10 µg/mL and tazarotene 45 µg/mL) was placed for a period of 24 h in a UV chamber (254 nm). Later, the volume was diluted by using diluent in order to get 1 µg/mL of halobetasol and 4.5 µg/mL of tazarotene concentration solution, which was then injected into the HPLC for assessment as per the method suggested.

Dry heat based degradation: A 1 mL lotion sample (halobetasol 10 µg/mL and tazarotene 45 µg/mL) was placed for a

period of 24 h in a oven (60 ºC). Later, the volume was diluted by using diluent in order to get 1 µg/mL of halobetasol and 4.5 µg/mL of tazarotene concentration solution which was then injected into the HPLC for assessment.

RESULTS AND DISCUSSION

Optimization of assay method: In order to obtain the good resolution between peaks, better peak shape and best peak parameters (tailing factor, plate count), chromatographic parameters were optimized. For selecting best column, initially three different columns namely Zodiac C18, Waters C18 and Knauer Eurospher II C18 were tried. The best peak resolution and peak shape with adequate system suitability values were obtained with Knauer Eurospher II C18 column. Therefore, the same column was chosen for the assay of halobetasol and tazarotene. For selecting better mobile phase, the following combination of solvents with different ratios, flow rate and pH were tried. (a) Methanol:water; (b) methanol:acetonitrile; (c) methanol:acetonitrile:1 mM phosphate buffer; (d) methanol: 1mM acetate buffer; and (e) methanol: acetonitrile:0.5 mM perchloric acid.

All combinations except the last one provided poor peak shape and reduced system suitability parameters. Finally, methanol:acetonitrile: 0.5 mM perchloric acid (40:45:15 v/v/v) mixture tried at pH 5.6 at a flow rate of 0.9 mL/min was found to be satisfactory with good system suitability parameters (Table-1). Halobetasol and tazarotene peaks were well resolved with good peak shape using these conditions optimized (Fig. 2). Therefore, the best chromatographic response given by these conditions are used for further studies. Quantification of halobetasol and tazarotene was accomplished on the basis of peak area determined with UV detection at 234 nm (isobestic point of halobetasol and tazarotene UV spectra, Fig. 3).

TABLE-1
VALUES OF SYSTEM SUITABILITY
FOR SELECTED ANALYTES

Parameter	Tazarotene	Halobetasol	Criteria limit [16-18]
Retention time	7.116	8.933	–
Resolution	–	7.13	> 2.0
Plate count	4512	9470	> 2000
Tailing factor	0.91	1.09	0.9-1.4

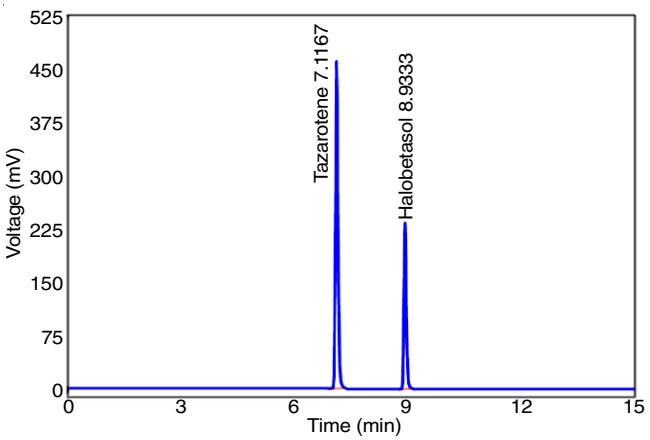

Fig. 2. Obtained chromatogram after optimization of the method

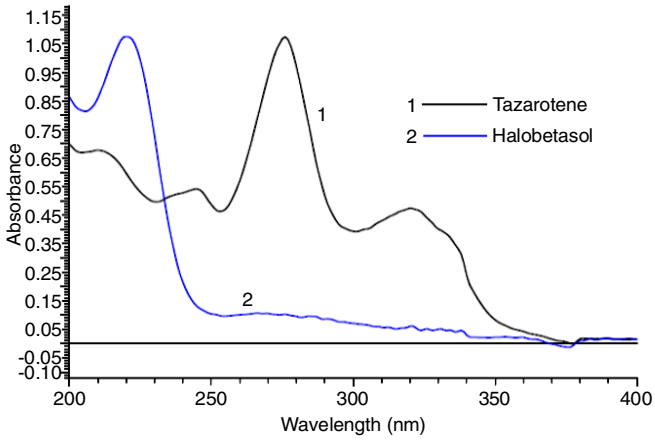

Fig. 3. UV spectra of halobetasol and tazarotene

Method validation: This was accomplished in compliance with the ICH criteria [16].

Selectivity: Selectivity was assessed through comparing chromatograms of diluent (Fig. 4a), standard solution of halobetasol and tazarotene with concentration of 1 µg/mL and 4.5 µg/mL, respectively (Fig. 4b) and lotion sample of halobetasol and tazarotene with concentration of 1 µg/mL and 4.5 µg/mL, respectively (Fig. 4c). No obstruction peaks were seen at halobetasol and tazarotene retention times in chromatograms of diluent (Fig. 4a) and lotion sample of halobetasol and tazarotene (Fig. 4c). Halobetasol and tazarotene peaks were not interfered with

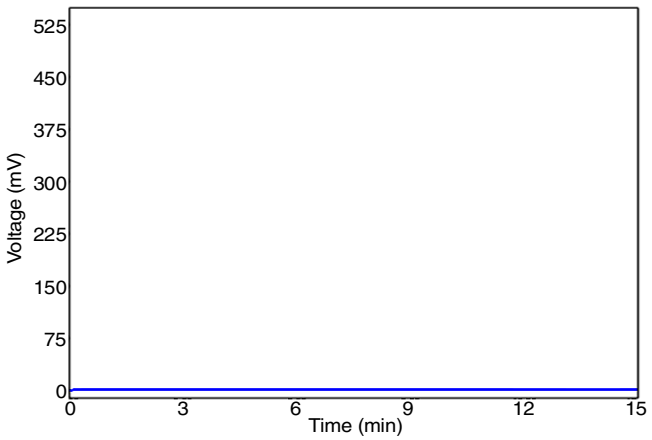

Fig. 4a. Chromatogram of diluent

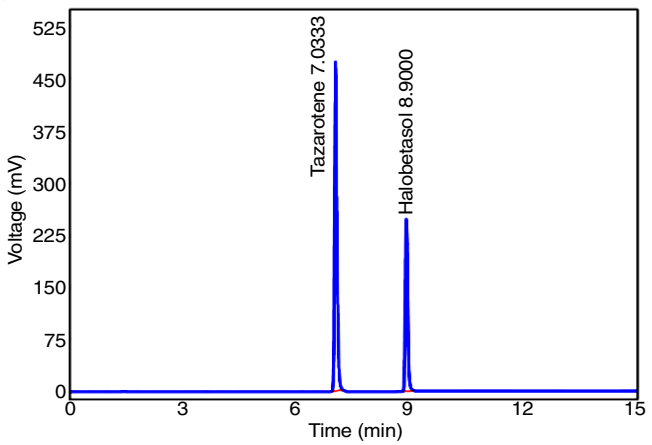

Fig. 4b. Chromatogram of standard solution of halobetasol and tazarotene

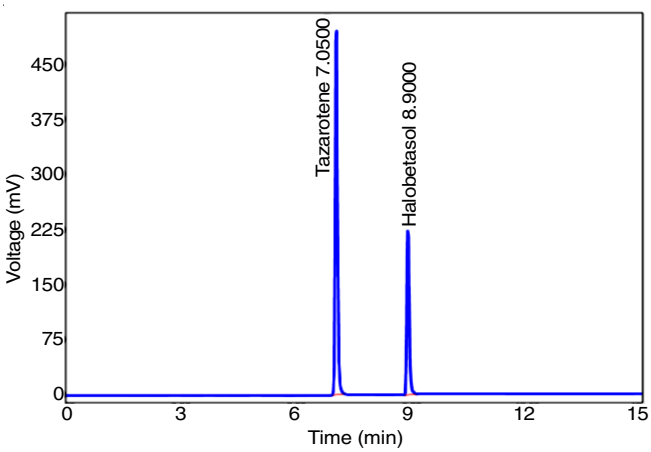

Fig. 4c. Chromatogram of lotion sample of halobetasol and tazarotene

any other additional peaks and hence the method was concluded to satisfy the selectivity in the analysis of halobetasol and tazarotene.

Linearity: Linearity was identified over a spatial range between 0.5-3.0 µg/mL for halobetasol and 2.25-13.50 µg/mL for tazarotene. Good relation between the peak area of analyte and concentration of analyte was achieved with R^2 (regression coefficient) more than 0.9999 for halobetasol and tazarotene.

Regression equation for tazarotene: y = 70515x + 2866 (R^2 = 0.9994)

Regression equation for halobetasol: y = 17225x - 5325 (R^2 = 0.9991)

In the above equation, 'y' is peak area of tazarotene/halobetasol and 'x' is concentration of tazarotene/halobetasol in µg/mL.

Limit of detection and limit of quantization: Signal to noise ratio approach was applied to assess sensitivity parameters like quantization limit and the detection limit. The concentration at signal-to-noise ratio of 10 was taken as quantization limit. The concentration at signal-to-noise ratio of 3 was taken as detection limit. The results pointed out that the detection limit was 0.01 µg/mL and 0.006 µg/mL for tazarotene and halobetasol, respectively. The quantization limit was 0.033 µg/mL and 0.02 µg/mL for tazarotene and halobetasol, respectively.

Accuracy: The analysis of lotion sample which was spiked at 50, 100 and 150% levels with reference halobetasol and tazarotene revealed the accuracy of the present method. The values of percent recovery of halobetasol and tazarotene are shown in Table-2. The values were in the range 98.67-99.18% for tazarotene and 98.36-100.80% for halobetasol proved the accuracy of the suggested method.

Precision: Repeatability (intra-day) analysis of standard solution of halobetasol (1 µg/mL) and tazarotene (4.5 µg/mL) for six times was done using the suggested method. Computation of relative standard deviations (RSD) for peak areas of halobetasol and tazarotene was done. The above procedure was repeated (inter-day analysis) for three continuous days for the analysis of standard solution of halobetasol (1 µg/mL) and tazarotene (4.5 µg/mL). Computation of relative standard deviations (RSD) for peak areas of halobetasol and tazarotene was done. The data (%RSD value less than 2.0%, Table-3) of intra-day and inter-day analysis established the preciseness of the method.

TABLE-2
RECOVERY FINDINGS

Level of spiking	Concentration of drug (µg/mL)			Recovery (%)
	Target	Spiked	Total recovered	
Tazarotene accuracy findings				
50%	4.5	2.25	6.70	99.32
	4.5	2.25	6.69	99.18
	4.5	2.25	6.71	99.46
100%	4.5	4.5	8.94	99.43
	4.5	4.5	8.96	99.63
	4.5	4.5	8.96	99.59
150%	4.5	6.75	11.08	98.67
	4.5	6.75	11.18	99.63
	4.5	6.75	11.16	99.45
Halobetasol accuracy findings				
50%	1.0	0.5	1.49	99.37
	1.0	0.5	1.48	98.84
	1.0	0.5	1.48	99.10
100%	1.0	1.0	1.96	98.36
	1.0	1.0	2.01	100.80
	1.0	1.0	2.01	100.27
150%	1.0	1.5	2.48	99.20
	1.0	1.5	2.48	99.42
	1.0	1.5	2.49	99.62

TABLE-3
INTRA-DAY AND INTER-DAY FINDINGS

Test No.	Tazarotene peak area	Halobetasol peak area
Intra-day analysis		
1	324419	164682
2	329213	164661
3	326743	164286
4	321113	165003
5	325240	165078
6	324858	165745
Average	325264	164909
RSD	0.824	0.302
Inter-day analysis		
Day1	325264	164909
Day 2	326273	163303
Day 3	323730	163875
Average	325089	164029
RSD	0.394	0.496

Ruggedness: Ruggedness was established by analysis of same standard solution of halobetasol (1 µg/mL) and tazarotene (4.5 µg/mL) by two analysts using the same operating conditions. Computation of relative standard deviations (RSD) for peak areas of halobetasol and tazarotene was done. The current method is proved rugged based on the data shown in Table-4 (%RSD value less than 2.0%) of ruggedness analysis.

Robustness: Robustness was established by analysis of same standard solution of halobetasol (1 µg/mL) and tazarotene (4.5 µg/mL) with the following deliberate changes in the operating conditions: acetonitrile ratio (± 5%); mobile phase with pH (± 0.1 unit); and wavelength (± 2 nm).

The robustness evaluation was carried out by comparing the peak areas of halobetasol and tazarotene in each altered parameter with the peak areas of halobetasol and tazarotene

TABLE-4
RUGGEDNESS FINDINGS

Sample No.	Analyst 1		Analyst 2	
	Tazarotene peak area	Halobetasol peak area	Tazarotene peak area	Halobetasol peak area
1	325842	165374	322895	162946
2	330623	165072	324737	163835
3	328726	163749	325148	165785
Average	328397	164732	324260	164189
RSD	0.733	0.525	0.370	0.884

in optimized operating conditions. The percent change was computed. Data (percent change was less than 2.0%, Table-5) obtained from these studies suggest the robustness of the current method.

Stability testing: To confirm the stability of halobetasol and tazarotene, forced degradation test were done on the lotion sample. The lotion sample was subjected to 0.1 N HCl, 0.1 N NaOH, 3% peroxide, UV light of 254 nm and dry heat of 60 ºC. The peak area of halobetasol and tazarotene obtained in each condition was compared with peak area of undegraded sample. The percent of drug remained and percent of drug degraded were determined. Table-6 revealed that halobetasol and tazarotene were degraded under the conditions applied. The order of stability was given as follows:

Tazarotene: dry heat (60 ºC) > 0.1 N NaOH > 3% peroxide > 0.1N HCl > UV light (254 nm).

Halobetasol: 0.1 N NaOH > dry heat (60 ºC) > 0.1 N HCl > 3% peroxide > UV light (254 nm).

Specificity: The results of forced degradation tests done on the lotion sample were also utilized to confirm the specificity and stability indicating capability of method. The number of additional peaks produced during forced degradation tests and their relative retention times are given in Table-7. Table-7 revealed that halobetasol and tazarotene were well separate from each other and also from the degradants produced during the applied conditions with distinct retention times. The chromatograms which prove specificity and stability indicating capability of method are shown in Fig. 5a-e.

Assay of halobetasol and tazarotene in lotion formulation: The method suggested was applied to analyze the content of halobetasol and tazarotene simultaneously in lotion formulation (Duobrii™, label claim 0.045% tazarotene and 0.01% halobetasol) to verify the validity of method. The amount measured was 0.988 µg/mL (halobetasol) and 4.452 µg/mL (tazarotene) corresponding to 98.76% (halobetasol) and 98.94% (tazarotene) of the label claim. Data obtained in this study have shown that the method is accurate and selective enough to evaluate halobetasol and tazarotene content simultaneously in lotion formulation.

Conclusion

A novel liquid chromatographic method was established and validated for the quantification of halobetasol and tazarotene in the vicinity of degradation products during stress conditions. Validation parameters for halobetasol and tazarotene are adequate for the concentration ranges of 0.5-3.0 µg/mL and

TABLE-5
ROBUSTNESS FINDINGS

Parameter	Condition changed	Tazarotene		Halobetasol	
		Peak area	Change (%)	Peak area	Change (%)
Standard	Optimized operating conditions	324979	–	169679	–
Mobile phase 1	Methanol: acetonitrile: 0.5 mM perchloric acid (45:40:15 v/v)	324399	0.178	170573	0.527
Mobile phase 2	Methanol: acetonitrile: 0.5 mM perchloric acid (35:50:15 v/v)	324719	0.080	171247	0.924
pH 1	5.5	324143	0.257	172611	1.728
pH 2	5.7	323891	0.335	171406	1.018
Wavelength 1	232 nm	322856	0.653	170954	0.751
Wavelength 2	236 nm	323376	0.493	168419	0.743

TABLE-6
STABILITY TEST FINDINGS

Condition	Tazarotene			Halobetasol		
	Area	Recovered (%)	Degraded (%)	Area	Recovered (%)	Degraded (%)
0.1 N HCl	299583	92.18	7.82	161157	94.97	5.03
0.1 N NaOH	302546	93.09	6.91	162035	95.49	4.51
3% Peroxide	301042	92.63	7.37	160484	94.58	5.42
UV Light	295874	91.04	8.96	158496	93.41	6.59
Dry heat	306425	94.29	5.71	161472	95.16	4.84

TABLE-7
STABILITY TEST FINDINGS

Condition	Number of additional peaks formed	Retention time (min) of additional peaks	Retention time (min) of halobetasol	Retention time (min) of tazarotene
0.1 N HCl	4	3.316, 5.919, 11.950 and 13.550	8.933	7.116
0.1 N NaOH	3	1.666, 3.883 and 11.716	8.883	7.016
3% Peroxide	2	1.956 and 5.950	8.883	7.016
UV Light	3	1.766, 3.050 and 9.993	8.933	7.050
Dry heat	2	3.033 and 6.050	8.933	7.050

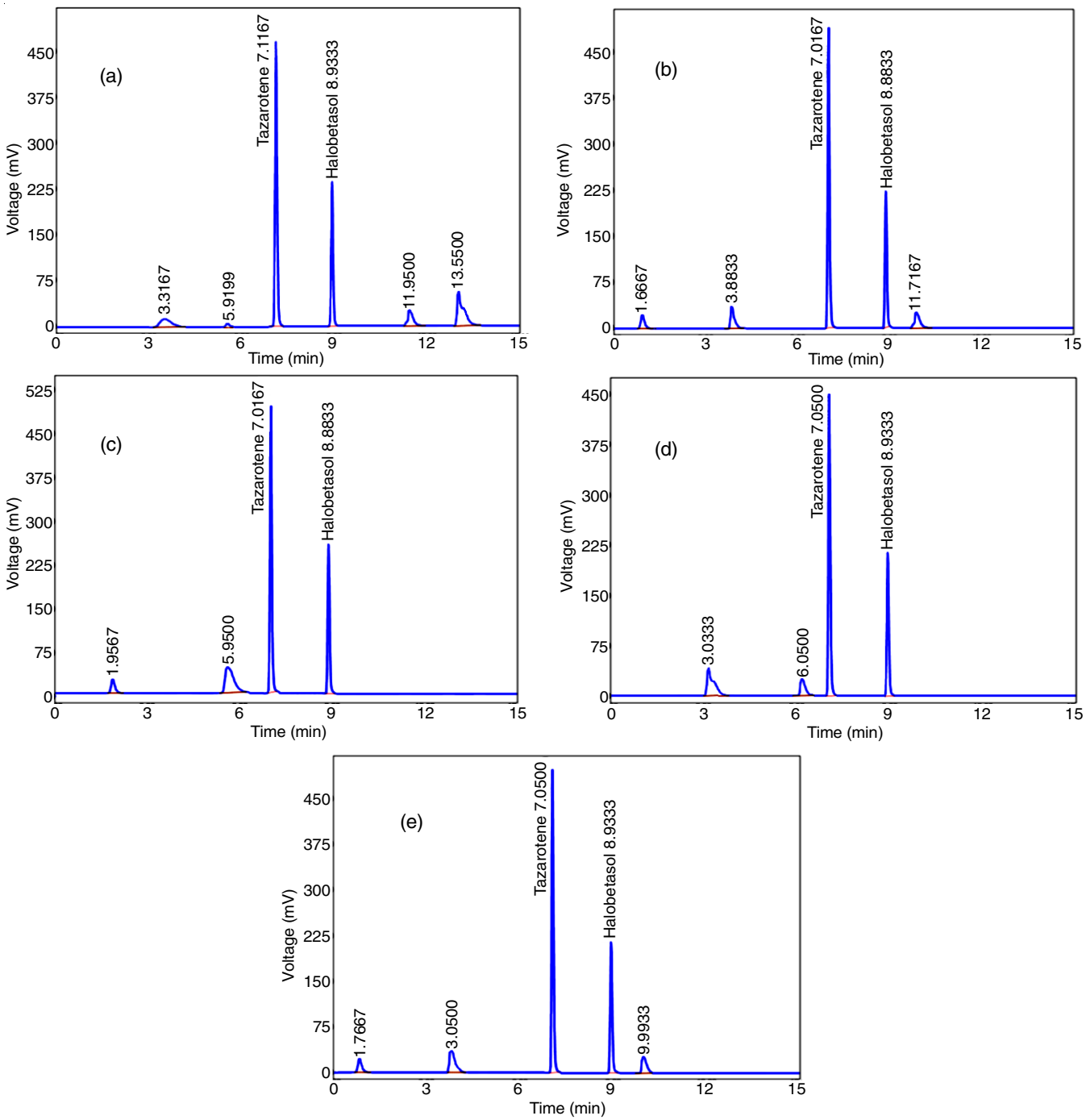

Fig. 5. Chromatogram obtained after degrading lotion sample with (a) 0.1 N HCl, (b) 0.1 N NaOH, (c) 3% peroxide, (d) dry heat (60 °C), (e) UV light (254 nm)

2.25-13.5 µg/mL, respectively as per the International Conference on Harmonization standards. The method has the necessary selectivity and accuracy to be used in laboratories for halobetasol and tazarotene routine analysis and quality control.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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