Development Of RP-HPLC Procedure And Forced Stability Studies For Lotion Formulations Of Tazarotene And Halobetasol

T. Sravanthi, N. Madhavi

Abstract : In the current research, an isocratic, novel RP-HPLC method was developed for quantifying tazarotene and halobetasol simultaneously. The HPLC method was optimized using the Waters C18 column (150 mm × 4.5 mm; 5 µm), employing simple isocratic method. Experimental parameters were standardized, including mobile phase, pH, wavelength detection and flow rate. The method has been validated in conformance with the guideline frame of ICH. This method was apt for determination of tazarotene and halobetasol in bulk and lotion formulation samples with suitable precision (relative standard deviation: 0.135-0.189% for tazarotene and 0.229-0.472% for halobetasol), accuracy (recovery: 98.66-100.70% for tazarotene and 98.21-100.09% for halobetasol) and linearity (2.25-11.25 µg/ml for tazarotene and 0.5-2.5 µg/ml for halobestol). This method has a better ability in the routine analysis of tazarotene and halobetasol in bulk and lotion formulations. The present method was successfully applied to lotion formulations for assessment of stability of tazarotene and halobetasol under basic, acidic, oxidative, photolytic and thermal conditions.

Index Terms : Tazarotene, Halobetasol, Topical lotion, Analysis, Stability study

1 INTRODUCTION

Tazarotene is a representative of the retinoid acetylene group. Tazarotenic acid, an active metabolite of tazarotene is produced by deesterification in the cells [1,2]. Tazarotenic acid binds to the forms of retinoic acid receptor alpha, beta and gamma. Tazarotenic acid shows selectivity for retinoic acid receptor forms of beta and gamma, and may alter the expression of genes [3]. Tazarotene is used for treating skin damaged by sun, psoriasis and acne [4-7]. Halobetasol is a corticosteroid of synthetic nature with antipruritic, vasoconstrictor and anti-inflammatory activities [8]. Halobetasol binds to corticosteroid receptors of intradermal and dermal cells, and activates anti-inflammatory protein gene expression. Prostaglandin and leukotrienes are strong inflammatory mediators. Arachidonic acid induces the synthesis of prostaglandin and leukotrienes. Halobetasol inhibits arachidonic acid release by inducing phospholipase A2 inhibitory proteins [9]. Halobetasol reduces skin conditions like eczema, dermatitis, psoriasis and rash [10,11]. Duobrii™ topical lotion was authorized by the US FDA in April 2019 for the therapy of plaque psoriasis in adults [12-14]. Each gram of Duobrii™ topical lotion contains (0.01%) 0.1 mg of halobetasol and (0.045%) 0.45 mg of tazarotene. The literature survey revealed that no analytical method is stated to simultaneously quantify halobetasol and tazarotene simultaneously in lotion formulation. The aim of this study was to develop a new stability indicating liquid chromatographic method for the simultaneous estimation of halobetasol and tazarotene in lotion formulation.

- *T. Sravanthi, Department of Chemistry, Acharya Nagarjuna University, Nagarjuna nagar, Guntur, Andhra Pradesh, India - 522510.*
- *N. Madhavi , PG Department of Chemistry, Jagarlamudi Kuppuswamy Chowdary College, Guntur, Andhra Pradesh, India - 522006. Email: madhavijkcchempg@gmail.com*

2 EXPERIMENTAL SECTION

2.1 Solvents and chemicals

Reference drugs of halobetasol and tazarotene were obtained from Glenmark pharmaceuticals limited, Mumbai. Duobrii™ topical lotion formulation (Bausch Health Americas, Inc. USA) was purchased. Solvents (acetonitrile and methanol) of HPLC grade were obtained from Qualigens Fine Chemicals Ltd, Mumbai, India. Milli-Q water processed by water purification system, Milli-Q (Millipore, Merck, Germany), was used. Analytical grade acids (HCl and orthophosphoric acid) and analytical grade chemicals (potassium dihydrogen phosphate, sodium hydroxide and peroxide) were obtained from M/s. Rankem Chemicals Ltd, India.

2.2 Instrumentation

Agilent 1100 series HPLC system with Quaternary pump (G1311 A), column temperature control (COLCOM G1316A), autosampler (G 1329A) and UV detector (G1314 A) was used in this analysis. The HPLC system was controlled and integrated with Agilent Chem Station LC software. Waters C18 (150 mm × 4.5 mm; 5µm) column was utilized to separate and analyze halobetasol and tazarotene.

2.3 System condition for the analysis

Methanol and 0.1 M potassium phosphate buffer (45:55 v/v) mixture at a flow rate of 0.9 ml/min in an isocratic elution mode was employed as mobile phase. The pH was set to 5.8 with 1% orthophosphoric acid. The detector wavelength, injection volume and column temperature were set at 231 nm, 20 µl and ambient temperature, respectively.

2.4 Standard solutions

The mobile phase was utilized as diluent for preparing standard solutions. Stock solution of halobetasol (100 µg/ml) and tazarotene (450 µg/ml) were prepared in mobile phase. Appropriate dilution of halobetasol and tazarotene stock solution was made to provide five-level calibration solutions with the following concentrations:

Halobetasol – 0.5, 1.0, 1.5, 2.0 and 2.5 µg/ml.

Tazarotene – 2.25, 4.5, 6.75, 9.0 and 11.25 µg/ml.

For validation study, halobetasol and tazarotene stock solution was diluted to get solution with concentration 1 µg/ml of halobetasol and 4.5 µg/ml of tazarotene.

2.5 Calibration curve

Five concentrations ranging from 0.5 µg/ml to 2.5 µg/ml of halobetasol and 2.25 µg/ml to 11.25 µg/ml of tazarotene was prepared for calibration curves. Each concentration solution was infused into the system and assessed using the suggested method. To prepare the calibration curves, the peak areas of chosen analytes have been plotted against the respective concentrations of chosen analytes. The equation of regression was determined using data of concentration and peak area.

2.6 Assay of tazarotene and halobetasol in lotion formulation

An accurately weighed 10 gm of Duobrii™ topical lotion (equal to 1 mg of halobetasol and 4.5 mg of tazarotene) was transferred into 100 ml volumetric flask. 30 ml of solvent mixture (acetonitrile and methanol - 40:60 v/v) was added and heated on water bath set with 50 °C for 10 min. Cool to room temperature and volume was made up to 100 ml using the same solvent system. The solution was filtered via 0.45 µm nylon membrane filter. Approximately 1 ml of the above solution was diluted to 10 ml with diluent to obtain a solution containing halobestol and tazarotene at concentrations of 1.0 µg/ml and 4.5 µg/ml, respectively. This solution was infused into the system and assessed using the suggested method. Halobestol and tazarotene content in lotion formulation was calculated using the corresponding calibration curve or regression equation.

2.7 Degradation studies

In order to get an indication of the stability indicating ability and specificity of the current method, forced degradation studies have been conducted for halobetasol and tazarotene lotion sample. The forced degradation study was conducted under the following criteria [15]:

2.7.1 Acid hydrolysis

1 ml of halobetasol (10 µg/ml) and tazarotene (45 µg/ml) lotion sample and 5 ml of 0.1N HCl were added to a 10 ml flask. The contents were kept for 24 hr at room temperature. The solution was subsequently neutralized with 0.1 N NaOH and diluted in the mobile phase to obtain a concentration of 1 µg/ml halobetasol and 4.5 µg/ml tazarotene. This solution was infused into the system and assessed using the suggested method.

2.7.2 Alkali hydrolysis

1 ml of halobetasol (10 µg/ml) and tazarotene (45 µg/ml) lotion sample and 5 ml of 0.1 N NaOH were added to a 10 ml flask. The contents were kept for 24 hr at room temperature. The solution was subsequently neutralized with 0.1 N HCl and diluted in the mobile phase to obtain a concentration of 1 µg/ml halobetasol and 4.5 µg/ml tazarotene. This solution was infused into the system and assessed using the suggested method.

2.7.3 Peroxide oxidation

1 ml of halobetasol (10 µg/ml) and tazarotene (45 µg/ml) lotion sample and 5 ml of 3% peroxide were added to a 10 ml flask. The contents were kept for 24 hr at room temperature. The solution was subsequently diluted in the mobile phase to obtain a concentration of 1 µg/ml halobetasol and 4.5 µg/ml tazarotene. This solution was infused into the system and assessed using the suggested method.

2.7.4 Photolysis

1 ml of halobetasol (10 µg/ml) and tazarotene (45 µg/ml) lotion sample was kept for 24 hr in UV chamber (254 nm). The solution was subsequently diluted in the mobile phase to obtain a concentration of 1 µg/ml halobetasol and 4.5 µg/ml tazarotene. This solution was infused into the system and assessed using the suggested method.

2.7.5 Thermal lysis

1 ml of halobetasol (10 µg/ml) and tazarotene (45 µg/ml) lotion sample was kept for 24 hr in oven (60 °C). The solution was subsequently diluted in the mobile phase to obtain a concentration of 1 µg/ml halobetasol and 4.5 µg/ml tazarotene. This solution was infused into the system and assessed using the suggested method.

3 RESULTS AND DISCUSSION

3.1 Optimizing the method

The technique was configured to develop a method for separating and analyzing halobetasol and tazarotene with good system suitability findings. The chromatography conditions were established after testing two stationary phases (Zodiac C18 column and Waters C18 column) and mobile phases having different proportions of organic solvent (acetonitrile/methanol) and water, with and devoid of buffer solutions (0.1 M phosphate buffer/0.1 M acetate buffer) at distinct pH values. Finally, methanol and 0.1 M potassium phosphate buffer (45:55 v/v) mixture with pH 5.8 value at a flow rate of 0.9 ml/min in isocratic elution mode was adopted. The halobetasol and tazarotene were determined with UV detection at 231 nm (isobestic point). Retention times of halobetasol and tazarotene were 5.95 and 4.65 min (Fig. 1), respectively, allowing quick determination of halobetasol and tazarotene.

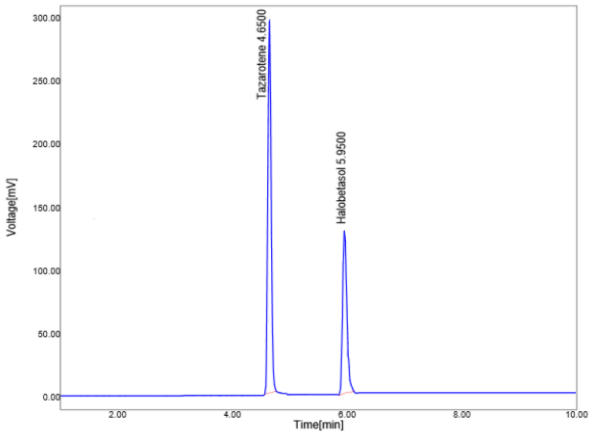

***Fig. 1**. Obtained chromatogram after optimization of the method*

Table 1 describes the system suitability variables obtained under the conditions developed for the current method. According to these findings (Table 1), the system and method demonstrated that they are capable of providing acceptance

quality data [16-18].

TABLE 1
VALUES OF SYSTEM SUITABILITY FOR SELECTED ANALYTES

Parameter	Results	Recommended value [16-18]
Retention time	Tazarotene – 4.65 min Halobetasol – 5.95 min	-
Resolution	Tazarotene– ……. Halobetasol – 6.41	> 2.0
Theoretical Plates	Tazarotene – 4183 Halobetasol - 6756	> 2000
Tailing Factor	Tazarotene – 0.84 Halobetasol-1.11	≤ 2.0

3.2 Method validation
This was achieved in alignment with ICH guidance [16].

3.2.1 Selectivity
Selectivity was assessed by injecting blank mobile phase standard solution (halobetasol-1 µg/ml and tazarotene-4.5 µg/ml) and lotion sample (halobetasol-1 µg/ml and tazarotene-4.5 µg/ml) to ensure no obstruction peaks at halobetasol and tazarotene retention times. The chromatograms are given in Fig. 2a, 2b and 2c. Halobetasol and tazarotene peaks were not co-eluted with any extra peak. Therefore, It was determined that the method was selective to analyze halobetasol and tazarotene.

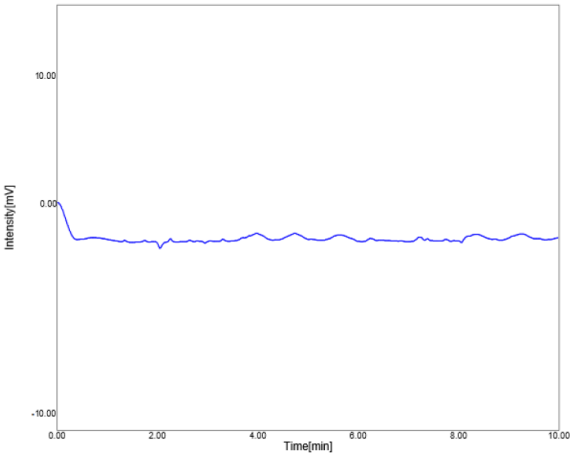

Fig. 2a*. Blank mobile phase chromatogram*

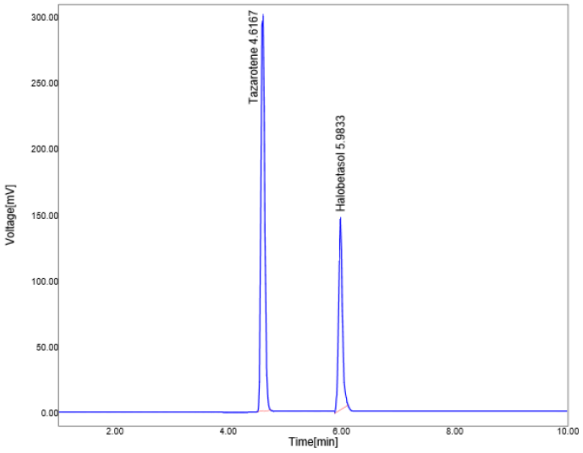

Fig. 2b. *Standard solution chromatogram*

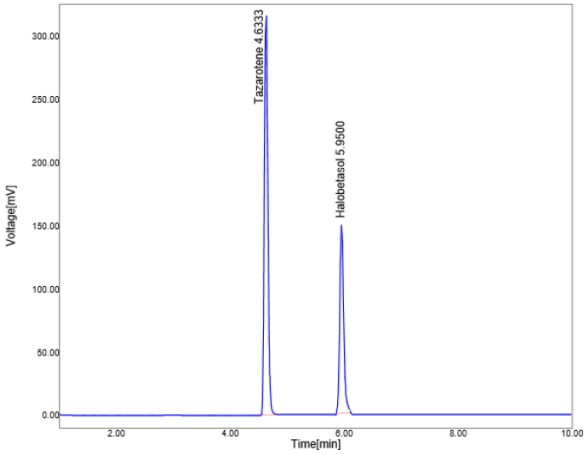

Fig. 2c. *Lotion sample chromatogram*

3.2.2 Linearity
Linearity of the procedure was determined over a quantitative range of 0.5 µg/ml-2.5 µg/ml for halobetasol and 2.25 µg/ml-11.25 µg/ml for tazarotene (Fig. 3a and 3b). Excellent association among analyte peak area and drug concentration was accomplished with R^2 >0.999 for halobetasol and tazarotene (Fig. 3a and 3b).

3.2.3 Detection limit & Quantitation limit
For sensitivity assessment, the limit of quantitation and limit of detection were determined at a signal to noise ratio of around 10 and 3, respectively. The results pointed out that the limit of detection were 0.02 µg/ml for tazarotene and 0.01 µg/ml for halobetasol. The limit of quantitation for tazarotene was 0.066 µg/ml and for halobetasol, limit of quantitation was 0.033 µg/ml.

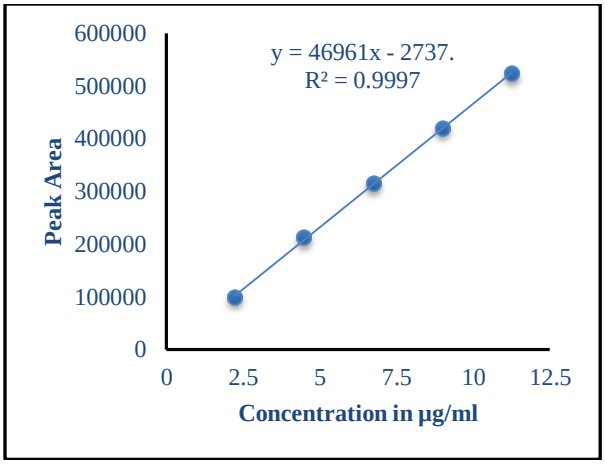

Fig. 3a*. Linearity graph for tazarotene*

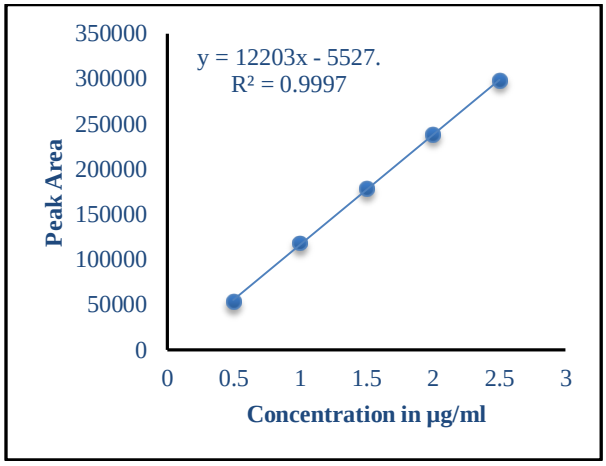

***Fig. 3b**. Linearity graph for halobetasol*

3.2.4 Precision

Repeatability (Intra-day precision) was established following analysis of samples (n=6) at the concentration of 1 µg/ml (halobetasol) and 4.5 µg/ml (tazarotene). The study was carried out over a short period in a single day. Reproducibility (Inter-day precision) was assessed at the same concentration on three distinct days. The relative standard deviation percentage was used to assess intra- and inter-day levels of precision. Data (Table 2) for intra- and inter-day precision studies suggest that the current method is precise.

TABLE 2
REPEATABILITY AND REPRODUCIBILITY DATA FOR HALOBETASOL AND TAZAROTENE

Sample No.	Tazarotene peak area	Halobetasol peak area
Repeatability (Intra-day precision)		
1	212039	118476
2	211985	118546
3	211758	118021
4	212236	118622
5	212575	118847
6	212326	118474
Average	212153	118498
RSD percentage	0.135	0.229
Reproducibility (Inter-day precision)		
Day1	212153	118498
Day 2	211598	117456
Day 3	212374	117642
Average	212042	117865
RSD percentage	0.189	0.472

RSD – relative standard deviation; No. - number

3.2.5 Accuracy

The accuracy of the current method was checked by analyzing lotion sample (halobetasol-1 µg/ml and tazarotene-4.5 µg/ml) spiked with halobetasol and tazarotene at 50, 100 and 150% levels of concentrations. The percent recovery values (Table 3) ranging from 98.66-100.70% for tazarotene and 98.21-100.09% for halobestol indicating that the current method is accurate.

TABLE 3
RECOVERY OF TAZAROTENE AND HALOBETASOL

Level	Target (µg/ml)	Spiked (µg/ml)	Total (µg/ml)	Recovered (µg/ml)	Recovery (%)
Tazarotene					
50%	4.5	2.25	6.75	6.65	98.66
	4.5	2.25	6.75	6.71	99.53
	4.5	2.25	6.75	6.70	99.27
100%	4.5	4.5	9	8.90	98.98
	4.5	4.5	9	8.99	99.90
	4.5	4.5	9	8.97	99.67
150%	4.5	6.75	11.25	11.22	99.98
	4.5	6.75	11.25	11.30	100.66
	4.5	6.75	11.25	11.30	100.70
Halobetasol					
50%	1.0	0.5	1.5	1.49	99.55
	1.0	0.5	1.5	1.47	98.53
	1.0	0.5	1.5	1.47	98.58
100%	1.0	1.0	2	1.96	98.21
	1.0	1.0	2	1.97	98.51
	1.0	1.0	2	1.97	98.63
150%	1.0	1.5	2.5	2.50	100.09
	1.0	1.5	2.5	2.47	99.13
	1.0	1.5	2.5	2.47	99.01

3.2.6 Robustness

The composition of the mobile phase, pH and wavelength have been slightly changed to lower and upper sides of the optimized values to determine if there was any effect on halobetasol and tazarotene peak areas. The mobile phase composition was changed to 50:50 ratio from 40:60, pH was changed to 5.9 from 5.7 and wavelength was varied to 236 nm from 226 nm. Change in the peak areas of halobetasol and tazarotene were observed and percentage change values were calculated (Table 4). The percentage change values were less than 2.0% indicating that the current method is robust.

3.2.7 Ruggedness

Ruggedness was established following analysis of samples (n=3) at the concentration of 1 µg/ml (halobetasol) and 4.5 µg/ml (tazarotene) by two different analysts. The relative standard deviation percentage was used to assess ruggedness. Data (Table 5) for ruggedness studies suggest that the current method is rugged.

3.2.8 Specificity

Forced degradation tests for halobetasol and tazarotene lotion sample were performed in order to obtain an indication of the stability indicating potential and specificity of the current method. The amount of halobetasol and tazarotene remained following acid degradation was 90.82% and 91.82%, respectively and the resultant chromatogram (Fig. 4a) revealed the presence of four degradation peaks with retention times 1.121 min, 3.766 min, 5.516 min and 8.383 min that were well resolved from the peaks for halobetasol and tazarotene.

TABLE 4
ROBUSTNESS DATA FOR TAZAROTENE AND HALOBETASOL

Parameter	Condition changed	Tazarotene		Halobetasol	
		Peak area	% Change	Peak area	% Change
Standard	No Change	212543	--	118573	--
MP 1	Methanol:0.1 M potassium phosphate buffer (50:50 v/v)	212025	0.24	117636	0.79
MP 2	Methanol:0.1 M potassium phosphate buffer (40:50 v/v)	212234	0.16	118020	0.47
pH 1	5.9	211858	0.32	118878	0.26
pH 2	5.7	211693	0.40	118949	0.32
WL 1	226 nm	211017	0.72	117413	0.98
WL 2	236 nm	211357	0.57	117858	0.60

MP – mobile phase; WL - wavelength

TABLE 5
RUGGEDNESS DATA FOR HALOBETASOL AND TAZAROTENE

Sample No.	Tazarotene peak area	Halobetasol peak area
	Analyst 1	
1	212968	118974
2	212893	118843
3	213043	117636
Average	212968	118484
RSD percentage	0.035	0.622
	Analyst 2	
Day1	213414	117143
Day 2	212246	117952
Day 3	212515	118503
Average	212725	117866
RSD percentage	0.288	0.580

RSD – relative standard deviation; No. – number

The amount of halobetasol and tazarotene remained following degradation with alkali were 92.66% and 94.04%, respectively and the resultant chromatogram (Fig. 4b) revealed the occurrence of three extra peaks with retention times 2.400 min, 5.383 min and 6.683 min that were well resolved from the peaks for halobetasol and tazarotene.

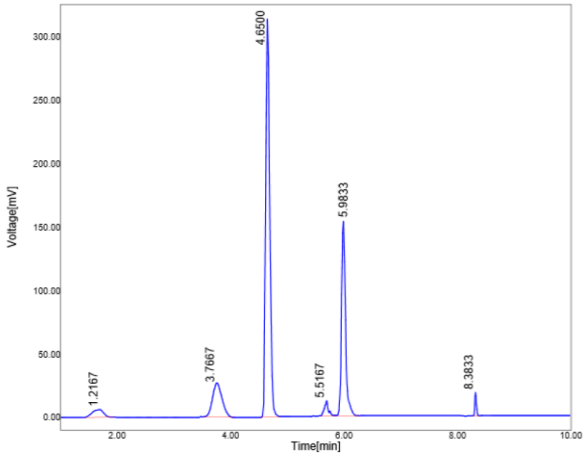

Fig. 4a. *Chromatogram of halobetasol and tazarotene lotion sample degraded with acid*

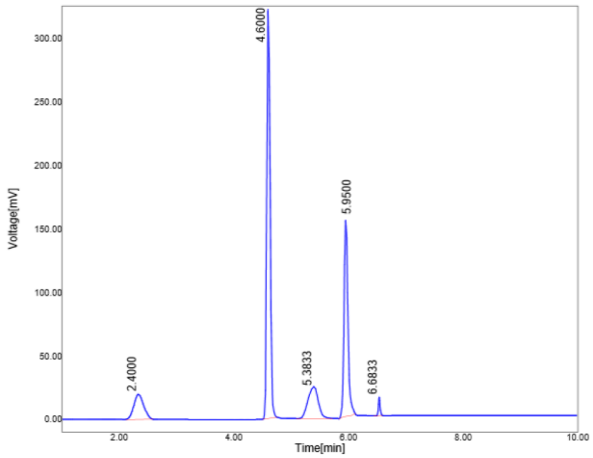

Fig. 4b. *Chromatogram of halobetasol and tazarotene lotion sample degraded with alkali*

Halobetasol and tazarotene were oxidized with 3% peroxide. Approximately 94.89% of halobestol and 9.3.43% of tazarotene was recovered after oxidation with three degradants observed at retention times of 1.500 min, 6.333 min and 8.533 min in the chromatogram (Fig. 4c).

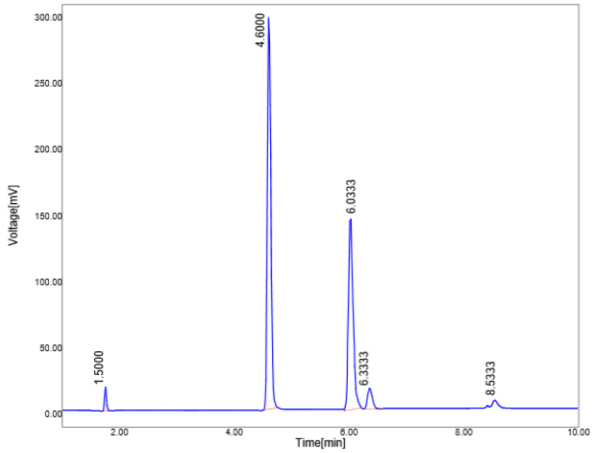

Fig. 4c*. Chromatogram of halobetasol and tazarotene lotion sample degraded with peroxide*

The thermal degradation of halobetasol and tazarotene resulted in the formation of three degradation products eluted

at 2.200 min, 3.716 min and 7.150 min (Fig. 4d). The degradants are separated well from approximately 93.07% of halobetasol recovered and 91.73% of tazarotene recovered.

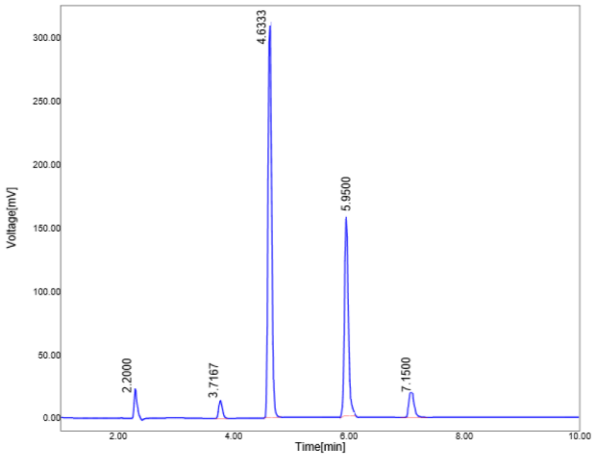

Fig. 4d. *Chromatogram of halobetasol and tazarotene lotion sample degraded with dry heat*

After exposure to UV light, 90.60% of tazarotene and 91.57% of halobestol was recovered and the chromatogram represented in Fig. 4e revealed the occurrence of four extra peaks with retention times 1.950 min, 3.250 min, 5.200 min and 8.516 min that were well resolved from the peaks for halobetasol and tazarotene.

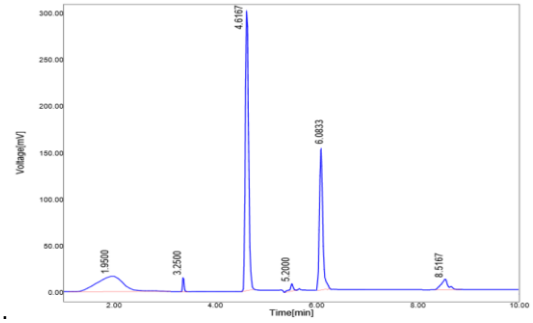

Fig. 4e. *Chromatogram of halobetasol and tazarotene lotion sample degraded with UV light*

The current method was regarded as specific and stability indicating, because the additional peaks formed during forced degradation research didn't interfere with halobetasol and tazarotene peaks.

3.3 Method application to assay halobetasol and tazarotene in lotion formulation

The data from analysis of halobetasol and tazarotene in lotion formulation are summarized in Table 6. The quantity of halobetasol and tazarotene analyzed in the product was 4.44 µg/ml (tazarotene) and 0.98 µg/ml (halobetasol) equivalent to 98.65% (tazarotene) and 98.63% (halobetasol) of the label claim. The percent recovery determined lies within the assay specifications.

4 CONCLUSION

Stability indicating HPLC method developed in this study was used in the analysis of tazarotene and halobetasol simultaneously. Good separation of tazarotene and halobetasol with acceptable recovery and relative standard deviation has indicated high accuracy and precision of the method. The low values of limit of detection and limit of quantification demonstrates the high sensitivity in detection and quantification of tazarotene and halobetasol in bulk and lotion formulation samples. Satisfactory results were obtained when the method was adopted to analyze tazarotene and halobetasol in lotion formulation.

TABLE 6
ASSAY HALOBETASOL AND TAZAROTENE IN LOTION FORMULATION

Drug	Brand Name	Label Claim (%)	Amount Prepared (µg/ml)	Amount Found (µg/ml)	Assay (%)
Tazarotene Halobetasol	Duobrii	0.045% 0.01%	4.5µg/ml 1.0µg/ml	4.44 0.98	98.65 98.63

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CONFLICTS OF INTEREST

None exists in the current study.

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