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RESEARCH ARTICLE

SPECTROPHOTOMETRIC AND VALIDATED RP – HPLC METHOD FOR THE ESTIMATION OF RETINOD DRUG TAZAROTENE IN GEL FORMULATION

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ABSTRACT

The retinoid are classes of chemical compounds that are related chemically to vitamin a Retinoid are used in medicine, primarily due to the way they regulate epithelial cell growth. Tazarotene is chemically Ethyl6-[2-(4,4-dimethylthiochroman-6-yl) ethynyl] Nicotinate. Tazarotene is a synthetic acetylenic retinoid that is applied topically. It is de-esterified in the skin to its active form, tazarotene acid, which affects cell proliferation and differentiation by modulating gene expression in acne and psoriasis

Key Words: Retinoid, Nicotinate, , tazarotene acid, RP-Hplc

INTRODUCTION:

Tazarotene is chemically Ethyl6-[2-(4,4-dimethylthiochroman-6-yl) ethynyl] Nicotinate. Tazarotene is a synthetic acetylenic retinoid that is applied topically. It is de-esterified in the skin to its active form, tazarotene acid, which affects cell proliferation and differentiation by modulating gene expression in acne and psoriasis. Tazarotene is a retinoid prodrug which is converted to its active form, the cognate carboxylic acid of tazarotene by rapid desertification in animals and man.

The mechanism of tazarotene action in psoriasis is not defined. Topical tazarotene blocks induction of mouse epidermal ornithine decarboxylase (od) activity, which is associated with cell proliferation and hyperplasia. In cell culture and in vitro models of skin, a marker of inflammation present in the epidermis of psoriasis patients at high levels. In human keratinocyte cultures, it inhibits cornified envelope formation, whose build-up is an element of the psoriatic scale. Tazarotene also induces the expression of a gene which may be a growth suppressor in human keratinocytes and which may inhibit epidermal hyper proliferation in treated plaques. However, the clinical significance of these findings is unknown. And mechanism of action in acne vulgaris is not defined. However, the basis of tazarotene therapeutic effect in acne may be due to its anti-hyper proliferative, normalizing-of-differentiation and anti-inflammatory effects. Tazarotene inhibited corneocyte accumulation in rhino mouse skin and cross-linked envelope formation in cultured human keratinocytes. The clinical significance of these findings is unknown.

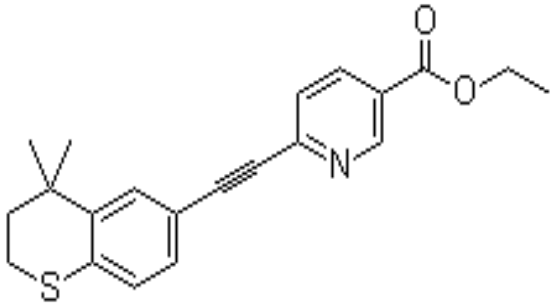

Fig 1. Molecular structure of Tazarotene

Experimental method:

1) By UV spectrophotometer :

Instrumentation

The instrument used was Sytronic R 2203 double beam spectrophotometer. Weighing was done on electrical balance (wensar).

Materials

Tazarotene drug sample was gifted from Glenmark pharmaceuticals, Solan (India). Methanol was purchase form RFCL laboratories, New Delhi, India. Gel formulation containing Tazorac (Tazarotene 20 mg) Glenmark pharmaceuticals. All other chemical were used A.R. grade.

Solubility

Tazarotene is soluble in methanol and insoluble in water, 0.1 N HCL, 0.1 N NaOH. For the method validation methanol solvent system is used.

Preparation of Stock solution:

An accurately weighted quantity of about 2 gm of tazarotene was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol in volumetric flask, to give a stock solution of 1000 ppm.

Determination of Absorption Maxima

The standard solution of tazarotene was scanned at different concentration in range of 200 – 400 nm and the λmax was found to be 346.4 nm. The spectrum of Tazarotene is shown in fig 2.

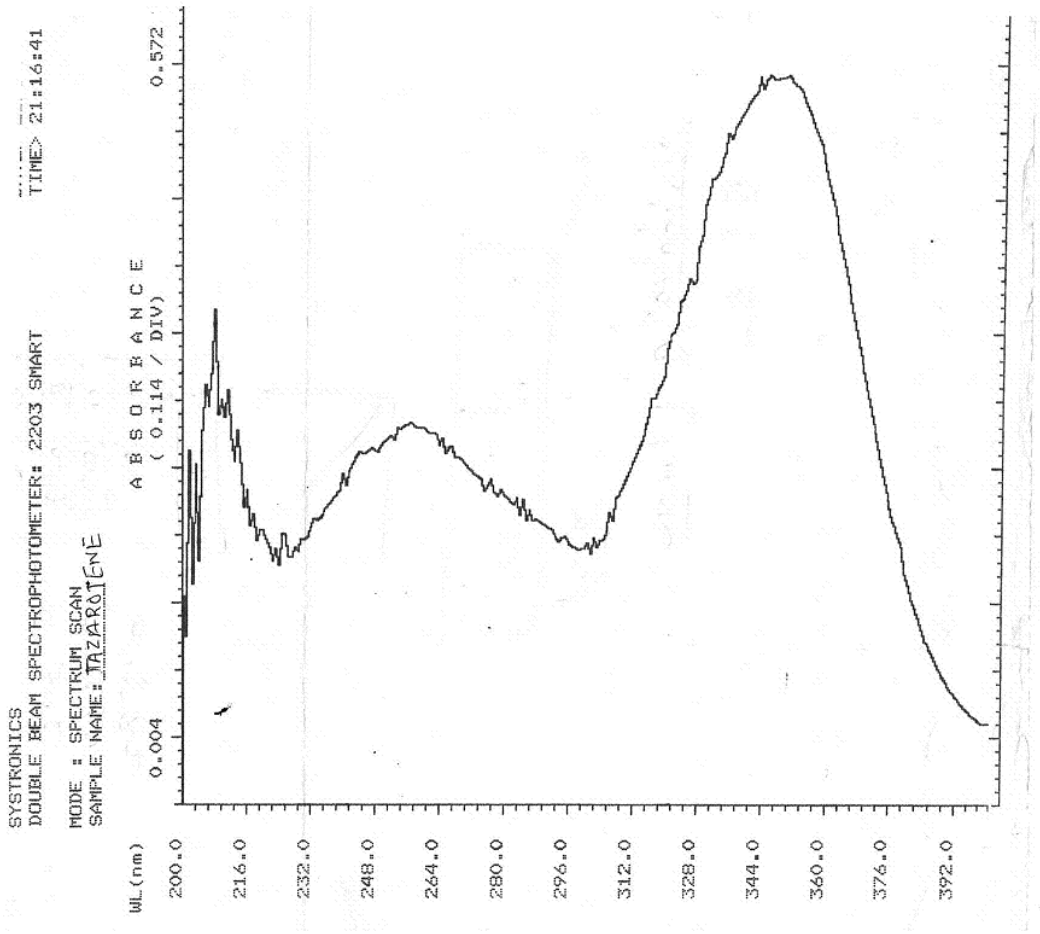

Fig 2. Determination of λmax of tazarotene

Determination of Linearity:

Calibration curve of absorbance vs concentration were studied by taking concentration ranging from 1-10 µg/ml and data revealed that Beer's Lambert law was obeyed. The linearity curve for Tazarotene was shown in Fig 3 and statically parameter reported in table 1.

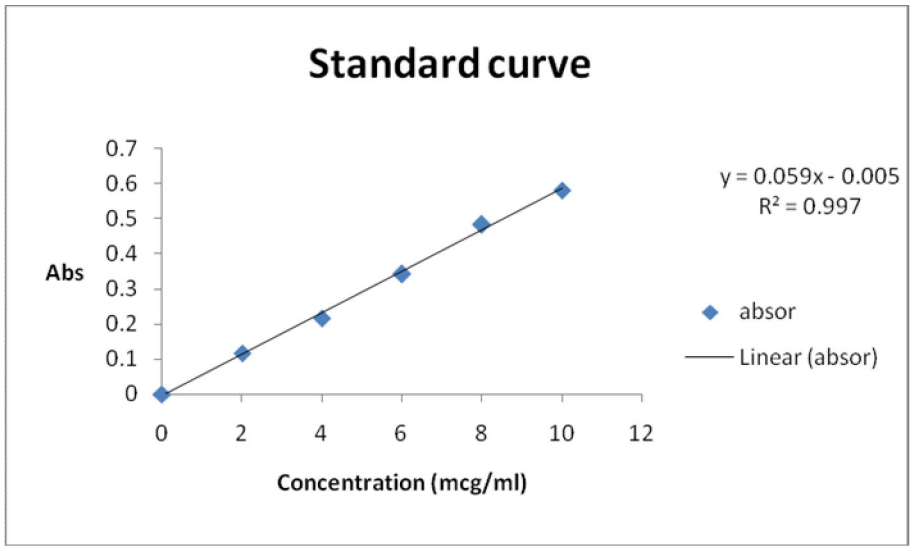

Fig 3. Linearity for Tazarotene

Table 1. Statically Parameter form the Calibration Plot

S. No.	Parameters	Observation
1	λ_{max}	346.4nm
2	Beer's law limit (µg/ml)	2-20 µg/ml
3	Regression equation	Y = 0.059 conc.
4	Correlation coefficient (r^2)	0.997
5	Molar absorbtivity (l mol^{-1} cm^{-1})	2 $x10^4$
6	Sandell's sensitivity µg/ml 0.001 absorbance unit	0.0335 $x10^3$

Assay of Tazarotene in Gel dosage form

2 gm of Tazorac (Tazarotene) were accurately weighted accurately and transferred to the conical falsk and made up to volume with methanol and shake for 20 min. This solution was filtered through Whatman filter paper. The resultant 1mg/ml of the solution was further diluted to get a concentration of 100 µg/ml. transferred 1 ml of the above solution into a 10 ml standard flask and volume was makeup using water. This gave sample solution having concentration 10 µg/ml the absorbance was measured and the result of analysis of gel formulation are shown in Table 2.

Table 2. Assay of tazarotene gel formulation

Brand name	Tazarotene	
	Label claim (w/w)	% purity
Tazarotene	0.005%	98%

Validation:

The methods were validated with respect to linerality, accuracy, precision, LOD and LOQ.

Accuracy:

The accuracy of the method was carried out by recovery studies at three different levels to cover both minimum and maximum (80%, 100%, 120%), the normal levels expected in the sample, the accuracy studied were carried out at each level of recovery. The result of studied along with its evaluation is given in table 3.

Table 3. Results of accuracy

Level of recovery (%)	80	100	120
Tazarotene			
Amount present(µg/ml)	5	5	5
Amount of std.added(µg/ml)	4	5	6
Mount recovered(µg/ml)	0.550 0.549 0.546	0.575 0.576 0.579	0.606 0.608 0.605
Mean	0.548	0.576	0.606
% recovery	107	95.24	87.83

Repeatability

Standard dilution was prepared and three replicated of each dilution were analyzed in same day repeatability and results were subjected to statistical analysis. (Table 4) Standard dilution was prepared and three replicates of each dilution were analyzed in different days and different analysts. Statistical analysis was carried out.

Table 4. Results of analysis data of gel formulation

Drug	Label claim Gm	Amount found* Gm	Label claim (%)	S.D.	% RSD
Tazarotene	10	0.98	99.98	0.005	0.001

Precision

The reproducibility of the proposed method was determined by performing the assay for the same day (Intraday precision) and on three different days (Inter day precision). The result of intraday and interday precision were expressed in %RSD was tabulated in Table 5.

Table 5. Intra-day and inter-day precision

Intra-day precision		**Inter day**	
	%label claim		**% label claim**
	Tazarotene		**Tazarotene**
After1hr	99.70	First day	98.60
After2hr	99.55	Second day	96.30
After3hr	98.60	Third day	95.50
After4hr	98.56		
After5hr	97.90		
After6hr	97.80		
Mean	98.68	**Mean**	96.80
SD	0.800	**SD**	1.609
%RSD	0.810	**%RSD**	1.662

By RP-HPLC:

- **Selection of flow rate**

Different flow rate such as 0.8, 1.0 and 1.2ml/min were used and chromatograms were recorded. At these flow rates symmetrical peaks with acceptable tailing factor and high plates count were observed. For the present study 2ml/min was selected.

1) **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 and areas for each concentration was recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given in (Table 6) and the standard calibration curve of the drugs is shown in (Fig 4). From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration (Table 7).

Table 6. Linearity of tazarotene

S.No	Concentration	AUC
1	0	0
2	4	272.12
3	6	365.18
4	8	485.25
5	10	665.44
6	12	790.12
7	15	910.25
8	20	1170.21

Regression Equation

Y = 59.18+27.52; **Y** = mx +c

Whereas Y= AUC, M = slope = 9.18, X = Conc. in µg/ml

C = Intercept = 27.52, r^2=0.992

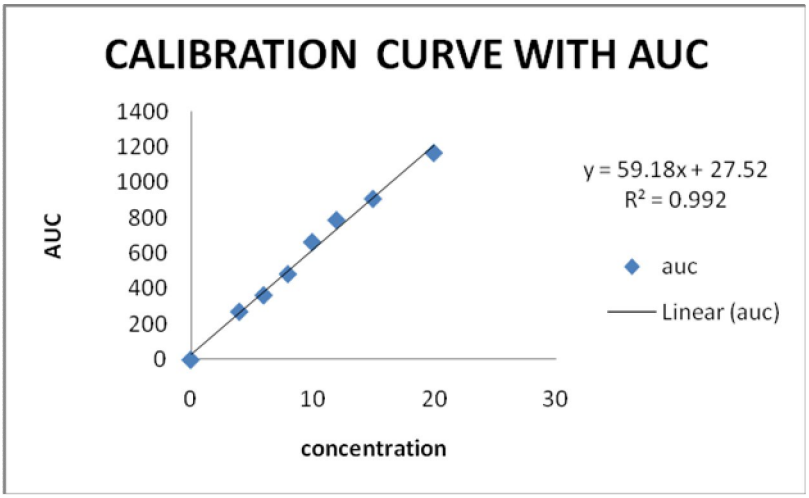

Fig 4: Calibration curve of tazarotene

Table 7. Result of Linearity of Tazarotene

Std. Conc. µg/ml	0	4	6	8	10	12	15	20
1	0	272.13	364.12	486.21	666.46	790.9	911.20	1171.20
2	0	271.14	366.09	484.20	664.41	790.12	910.21	1170.19
3	0	274.11	365.11	485.23	665.45	791.11	911.19	1171.20
Mean	0	272.13	365.18	485.25	665.44	790.12	910.25	1170.21
SD	0.00	1.5122	0.98500	1.0051	1.0250	0.5216	0.5687	0.5831
%RSD	0.000	0.5550	0.2697	0.2071	0.1540	0.06597	0.06243	0.04980

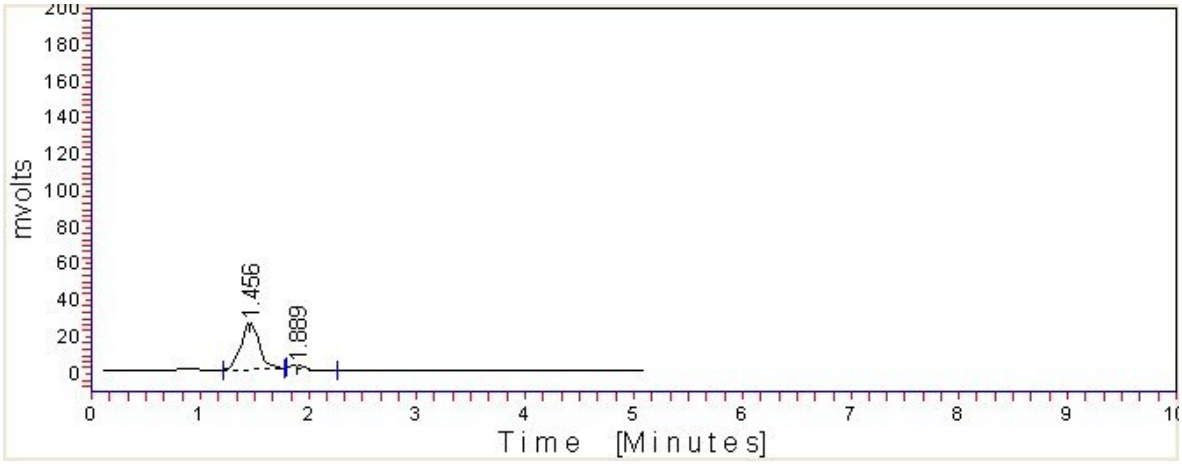

Fig. 5 Linearity graph of tazarotene at 4 µg/ml by HPLC

Result-A Table							
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Width@50%**	**Name**
1	1.456	272.133	24.805	94.913	92.668	0.05	-
2	1.888	14.586	1.963	5.087	7.332	0.05	-
Total		**286.719**	**26.768**	**100**	**100**		

PerformanceB Table											
Pk.No	**RT**	**Width@50%**	**Asymmetry**	**Tailing**	**Capacity**	**Efficiency**	**Efficiency/L**	**Resolution**	**Selectivity**	**HETP**	**RRT**
1	1.456	0.05	1.286	1.134	0.456	4696.703	4696.703	Nil	1.95	2.129	0.456
2	1.888	0.05	3.831	2.416	0.889	7903.214	7903.214	5.105	Nil	1.265	0.889

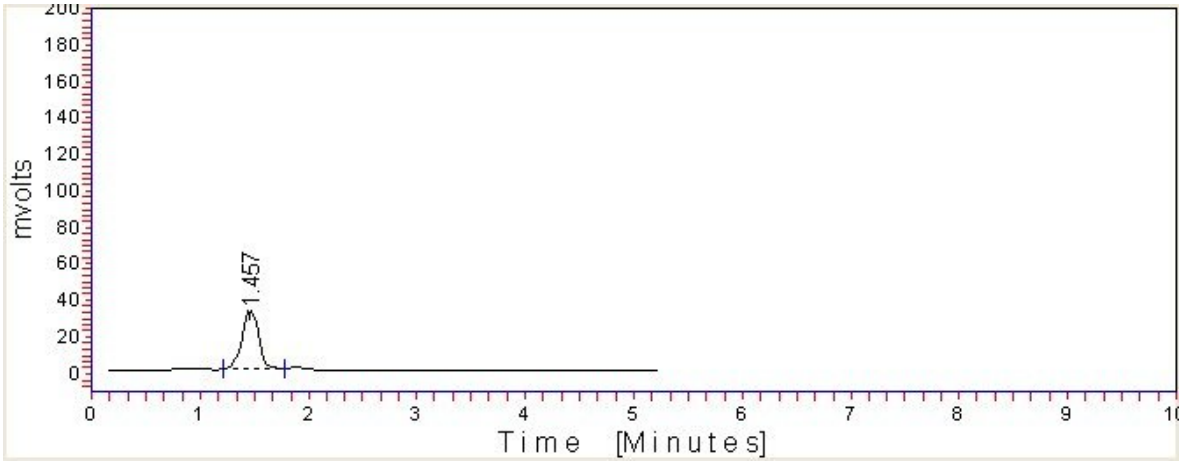

Fig. 6 Linearity graph of tazarotene at 6 µg/ml by HPLC

Result-A Table							
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Width@50%**	**Name**
1	1.457	365.18	31.515	100	100	0.05	-
Total		**365.18**	**31.515**	**100**	**100**		

PerformanceB Table											
Pk.No	**RT**	**Width@50%**	**Asymmetry**	**Tailing**	**Capacity**	**Efficiency**	**Efficiency/L**	**Resolution**	**Selectivity**	**HETP**	**RRT**
1	1.457	0.05	1.285	1.134	0.457	4703.157	4703.157	Nil	Nil	2.126	0.457

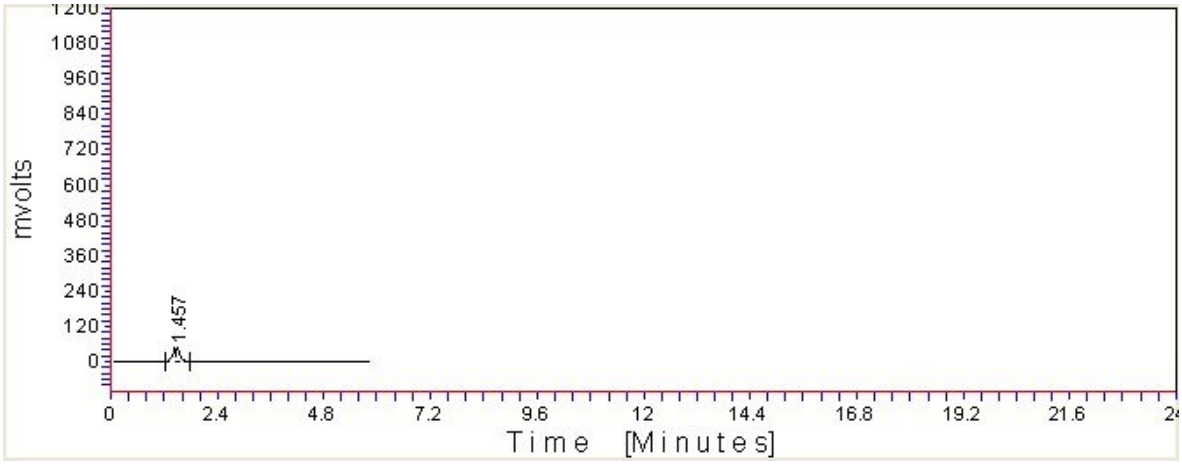

Fig. 7 Linearity graph of tazarotene at 8 µg/ml by HPLC

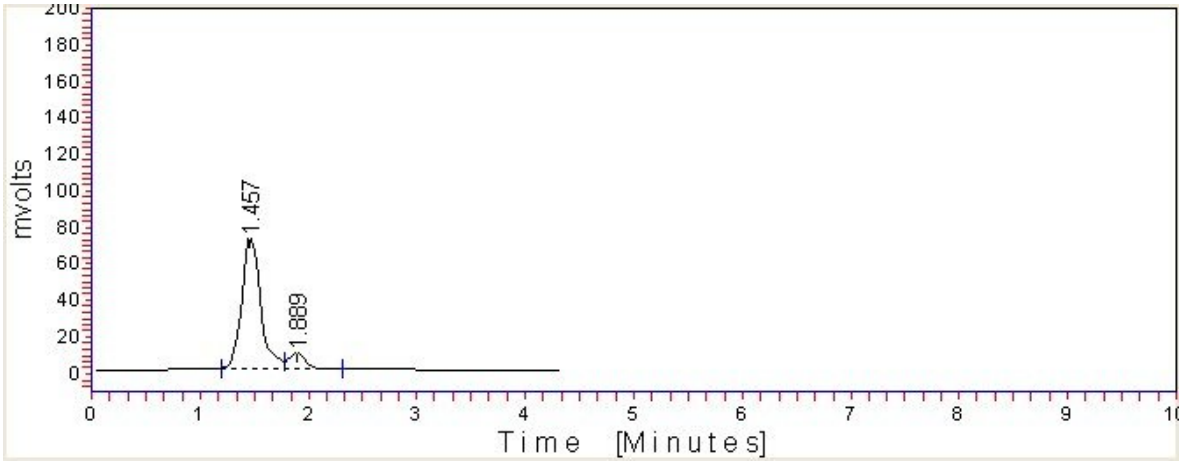

Fig. 8 Linearity graph of tazarotene at 10 µg/ml by HPLC

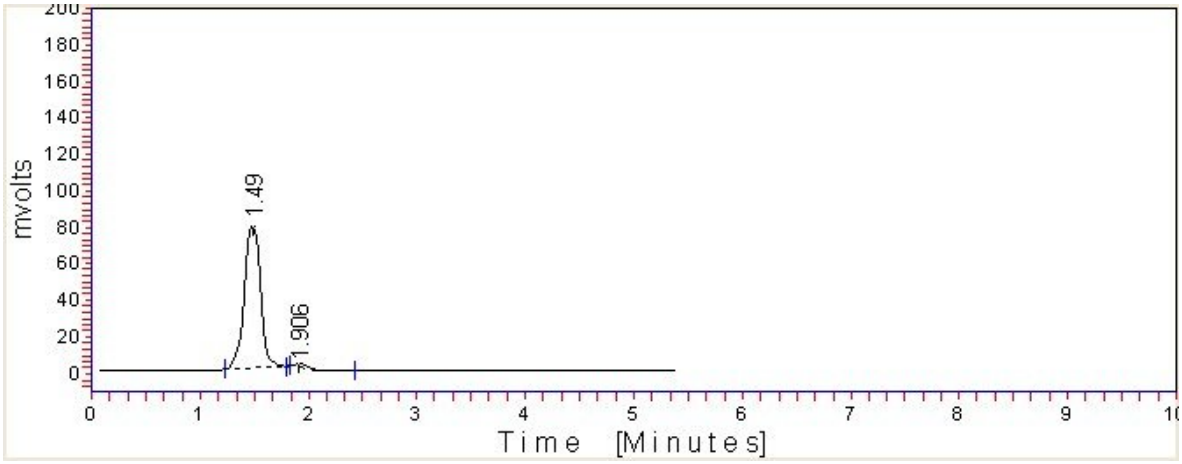

Fig. 9 Linearity graph of tazarotene at 12 µg/ml by HPLC

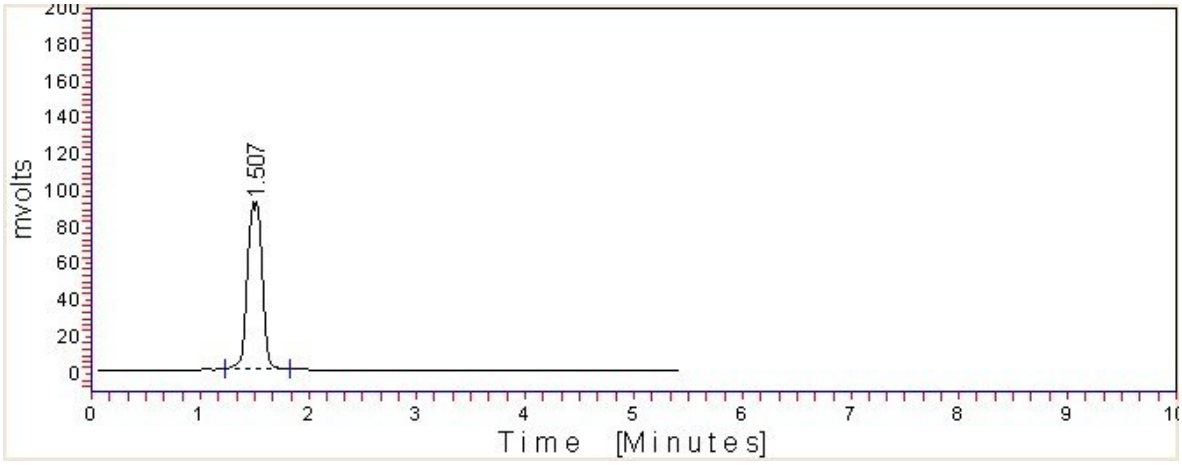

Fig. 10 Linearity graph of tazarotene at 15 µg/ml by HPLC

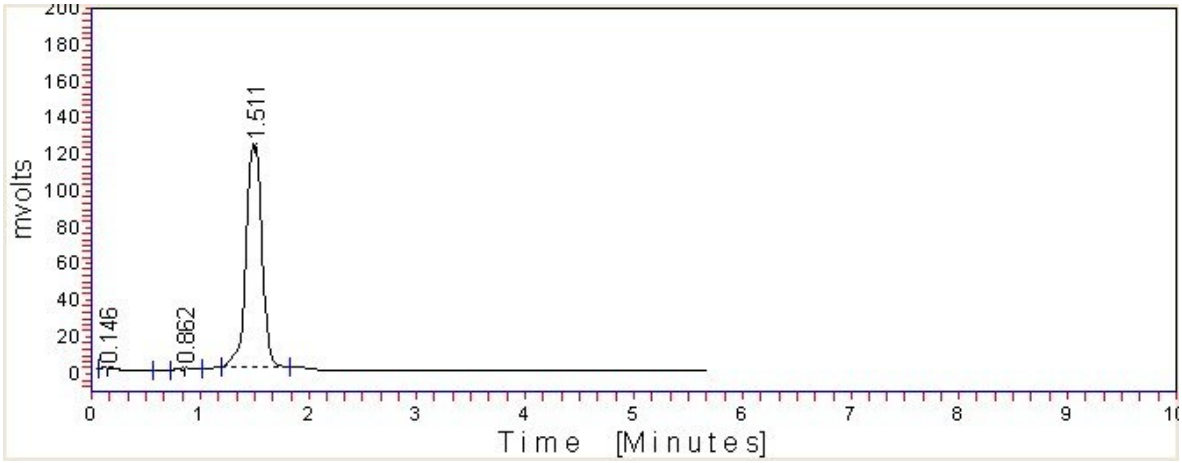

Fig. 11 Linearity graph of tazarotene at 20 µg/ml by HPLC

2) Accuracy

Table 8 Results for Accuracy

Sr. No	Level of Recovery (%)	Level (about)	RT(min)	Area Response	% Recovery	Mean % Recovery
1	80(5+4)		1.44	799.137	101.15	
2	100(5+5)	100%	1.49	723.09	98.96%	98.07%
3	120(5+6)		1.458	720.556	96.11%	

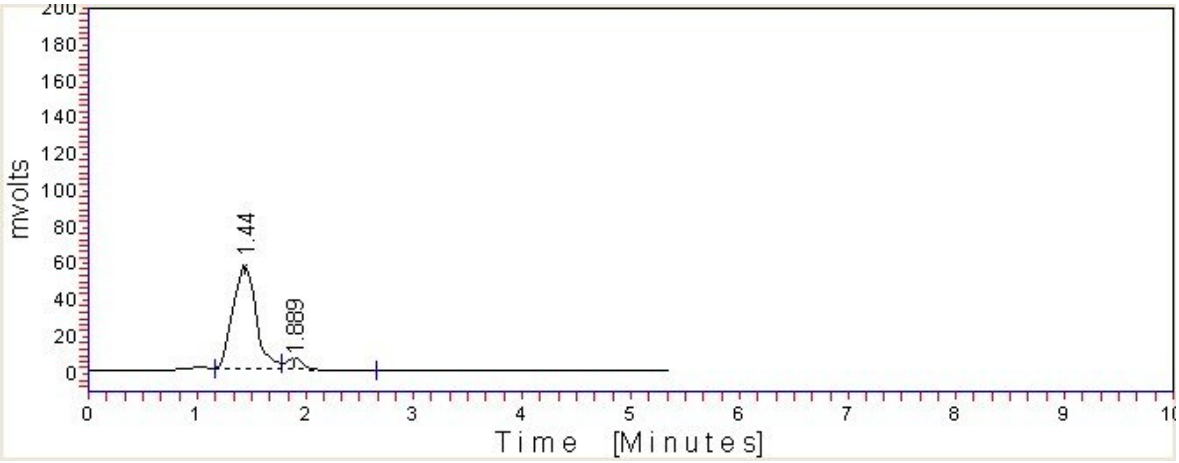

Fig. 12 level of recovery 80% (5 + 4)

Result-A Table							
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%	Name
1	1.44	799.137	56.281	92.848	90.495	0.05	-
2	1.889	61.559	5.911	7.152	9.505	0.05	-
Total		860.696	62.192	100	100		

PerformanceB Table											
Pk.No	RT	Width@50%	Asymmetry	Tailing	Capacity	Efficiency	Efficiency/L	Resolution	Selectivity	HETP	RRT
1	1.44	0.05	1.262	1.164	0.44	4595.098	4595.098	Nil	2.02	2.176	0.44
2	1.889	0.05	6.569	3.785	0.889	7908.795	7908.795	5.3	Nil	1.264	0.889

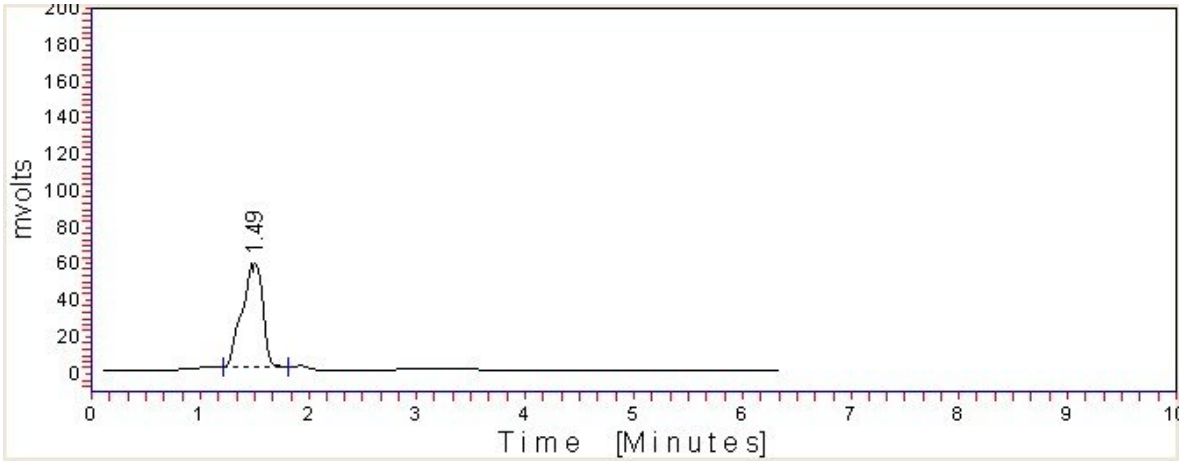

Fig. 13 level of recovery at 100% (5 + 5)

Result-A Table							
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%	Name
1	1.49	723.09	56.76	100	100	0.05	-
Total		723.09	56.76	100	100		

PerformanceB Table											
Pk.No	RT	Width@50%	Asymmetry	Tailing	Capacity	Efficiency	Efficiency/L	Resolution	Selectivity	HETP	RRT
1	1.49	0.05	1.125	1.063	0.49	4920.842	4920.842	Nil	Nil	2.032	0.49

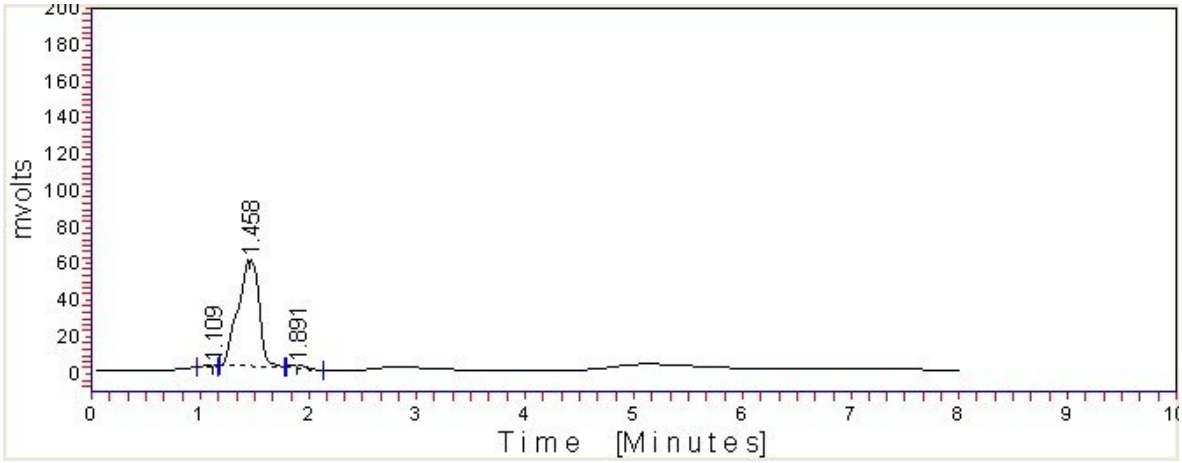

Fig. 13 Level of recovery at 120% (5+6)

Result-A Table							
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%	Name
1	1.109	3.631	0.43	0.477	0.714	0.05	-
2	1.458	720.556	58.125	97.75	96.407	0.05	-
3	1.891	13.505	1.736	1.773	2.879	0.05	-
Total		737.692	60.291	100	100		

PerformanceB Table											
Pk.No	RT	Width@50%	Asymmetry	Tailing	Capacity	Efficiency	Efficiency/L	Resolution	Selectivity	HETP	RRT
1	1.109	0.05	0.376	0.688	0.109	2723.778	2723.778	Nil	4.202	3.671	0.109
2	1.458	0.05	1.124	1.029	0.458	4711.77	4711.77	4.124	1.945	2.122	0.458
3	1.891	0.05	2.501	1.75	0.891	7921.359	7921.359	5.104	Nil	1.262	0.891

3) Method Precision

Table 9. Method Precision

Inj.	Area counts	Retention Time (min)	Acceptance Criteria
1	1465.004	2.954	**% RSD should not be more than 2%.**
2	1470.042	2.957	
3	1472.11	2.958	
4	1478.45	2.959	
5	1480.58	2.960	
6	1485.45	2.965	
Mean Peak area	8851.636		
SD	1421.91		
RSD (%)	1.21%		

Summary & Conclusion

Summary:

From the developed method satisfactory result is obtained. A RP-HPLC method has been optimized with a view to develop an accurate and reproducible method for tazarotene. Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results.

The final chromatographic conditions are set for stationary phase giving satisfactory resolved peak and run time with reversed phase Hypersile BDS C_{18}, (250 mm X 4.6 mm, 5µm particle size) column. A series of mobile phases varying the pH and volume fractions of methanol and water are also tested and the best results were obtained by the use of mobile phase consisting only methanol well resolved, sharp peak for tazarotene with a retention time (RT) of 1.456 as shown in graph The flow rate of 2.0 mL/min at 346.5 nm and ambient temperature (25^0 C) for column oven was found to be the best for analysis. The newly developed method has been validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. % RSD was less than 2 in intraday, interday precision and all parameters of robustness are in the limit. So the proposed method is more precise, accurate and robust.

CONCLUSSION:

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Tazarotene from bulk and formulation. A mobile phase composed of only methanol with a short run time (05 min) and isocratic elution used are advantageous and made the routine analysis easy. Among the significant advantages of this method are simplicity, selectivity, accuracy and precision ensuring that it is suitable for determining the content of Tazarotene in gel formulation. Thus, the proposed method can be used for routine analysis of Tazarotene alone and also in combination; likewise the same can be applied to other formulations. We have also got the similar results from the method that was developed by UV Visible spectroscopy. This assures us to our work of analysis. Future plan includes further evaluation of degradants & stability indicating method.

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