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Research Article

New Multi Wavelength Method for the Estimation of *Tazarotene* and *Hydroquinone* in Gel Formulation

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Abstract

A simple, sensitive, rapid, precise & accurate, UV spectrophotometric method has been developed for simultaneous estimation of *Tazarotene* & *Hydroquinone* from their gel formulation. This method is based on multi wavelength spectroscopic method. For the simultaneous estimation of both the drug sampling wavelength 294nm and 351nm were selected. *Tazarotene* and *Hydroquinone* showed linearity in concentration range of 1-5 µg/ml and 10-50 µg/ml respectively. Recovery for *tazarotene* and *hydroquinone* was obtained in the range of 99.38% to 100.0%. All three methods showed good reproducibility and recovery with % RSD less than 2.0%. Statistical validation of data shows that the proposed methods can be successfully applied for routine analysis of drugs in gel formulation.

Keywords: *Tazarotene*; *Hydroquinone*; Simultaneous estimation; Multicomponent

Introduction

Hydroquinone is benzene-1, 4-diol chemically or also known as quinol having the chemical formula $C_6H_4(OH)_2$ [1]. It is topical agent used for treatment of certain skin conditions. In skin creams it is also used as de-pigmenter agent and antioxidant in the photography industry. *Hydroquinone* acts by inhibiting the melanin formation. Due to toxicological effects of *hydroquinone* it can cause dermatitis. In skin toning creams *hydroquinone* and some of its derivatives are present. So the determination of *hydroquinone* and its derivative in cosmetics is very important for the protection of human health [2-4].

Tazarotene is member of the acetylenic class of retinoids. *Tazarotene* is chemically (ethyl 6-[2-(4, 4-dimethyl-3, 4-dihydro-2H-1-benzothiopyran-6-yl)-ethynyl]-pyridine-3-carboxylate). It is a third-generation topical retinoid. It is available in the form of cream, gel, or foam. *Tazarotene* is used for treatment of psoriasis, acne and photo damage skin.

Tazarotene is a prodrug which is converted to its active form by rapid de-esterification in humans and animals. Tazarotenic acid binds to all three members of the retinoic acid receptor (RAR) family: RARα, RARβ, and RARγ but shows relative selectivity for RARβ, and RARγ which may modify gene expression. The clinical significance of these findings is unknown [5] (Figure 1).

Tazarotene plus *hydroquinone* is used in treatment of photo damaged facial skin. Literature review reveals that the efficacy of *tazarotene* is improved, when applied in combination with *hydroquinone* [6]. A very few analytical methods are available for the estimation of drugs like *tazarotene* and *hydroquinone* in combination in pharmaceutical dosage formulation [7-13]. The non-availability of analytical methods as on date for the concurrent analysis of multi-component formulations made it worthwhile to pursue the present research work. The developed method is also been validated as per ICH guidelines [14]. The scope of developing and validating and analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the condition and parameters, which should be followed in the development and validation.

Materials and Methods

Instruments

A thermospectronic model is Lab India 3000+ (Double beam) spectrophotometer with 1cm matched quartz cells.

Chemicals & reagents

All chemicals are of analytical grade reagent and solutions were prepared in methanol: water (80:20). *Tazarotene* & *hydroquinone* gift samples were obtained from Lupin Pharmaceutical Ltd. Pune. Methanol is procured from Merck India Ltd. In-house formulation was prepared for gel formulation.

Procedure

Formula for preparation of carbopol gel:

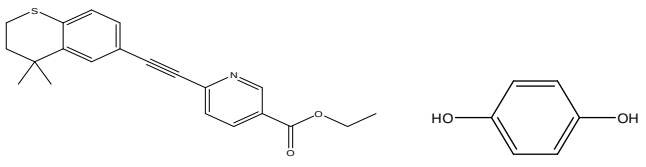
Tazarotene: 0.1 % w/w

Hydroquinone: 4% w/w

Carbopol 940: 2% w/w

Triethanolamine: q.s.

Methyl hydroxy benzoate: 0.15 % w/w



Tazarotene *Hydroquinone*

Figure 1:

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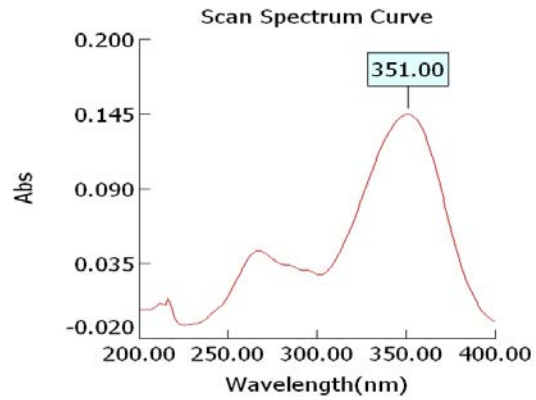

Figure 2: Selection of λ_{max} of *tazarotene*.

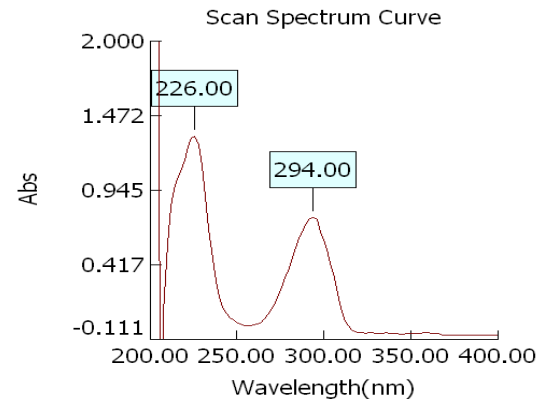

Figure 3: Selection of λ_{max} of *hydroquinone*.

Propyl hydroxy benzoate: 0.05 % w/w

Distilled water: 95.8 % w/w

Carbopol 940 was sprinkled slowly to 5ml of water as medium and the medium was continuously stirred to get a uniform dispersion of carbopol. The other ingredients that are methyl hydroxy benzoate and propyl hydroxyl benzoate were pre dissolved in separate portion of water (5ml) and added to carbopol dispersion. Final volume was adjusted with water and pH brought to neutral by using the triethanolamine.

Determination of solubility of Drug:

Preparation of standard stock solutions:

10mg of each *tazarotene* and *hydroquinone* was weighed accurately and transferred into two different 10ml volumetric flask respectively, and the volume was adjusted up to the mark with the methanol (80%), to give a stock solution of 1000ppm.

Determination of λmax of Drugs: Standard solution (10µg/ml) of pure *tazarotene* and *hydroquinone* were scanned on UV spectrophotometer, which showed maximum absorbance at 351nm and 294nm for *Tazarotene* and *Hydroquinone* respectively. The UV spectra are shown in (Figure 2 & 3).

Preparation of standard stock solution for test of linearity: From stock solutions of *tazarotene* 1 ml was taken and diluted up to 10ml. From this solution 0.1, 0.2, 0.3, 0.4, 0.5 ml solutions were

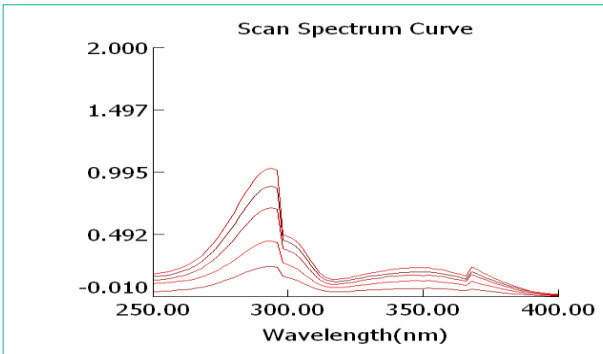

Figure 4: Overlay spectra of mixed standard of *tazarotene* and *hydroquinone*.

Table 1: Optical characteristics and linearity data.

Parameter	*Tazarotene* (TAZA)	*Hydroquinone* (HYDRO)
max (nm)	351	294
Beer's Law limit (µg/ ml)	1-5	10-50
Correlation coefficient	0.9998	0.9973
Regression Equation	1.22x-0.001	0.0053x+0.006
Intercept	0.001	0.006
Slope	1.22	0.0053
LOD (µg/ ml)	0.20	0.60
LOQ (µg/ ml)	0.35	1.20

Table 2: Analysis data of marketed formulations.

Conc. (µg/ml)		Conc. found (µg/ml)		% Found	
TAZA	HYDRO	TAZA	HYDRO	TAZA	HYDRO
2	20	2.05	19.90	102.50	99.50
2	20	2.00	19.95	100.00	99.75
2	20	2.01	19.98	100.50	99.90
2	20	1.98	20.00	99.00	100.00

transferred to five different 10ml volumetric flasks respectively and make volume up to 10ml with methanol (80%), gives standard drug solution of 1, 2, 3, 4, 5 µg/ml concentration and From stock solutions of *hydroquinone* 1ml was taken and diluted up to 10 ml. From this solution 1.0, 2.0, 3.0, 4.0, 5.0 ml solutions were transferred to five different 10ml volumetric flasks respectively and make the volume up to 10ml with methanol (80%), gives standard drug solution of 10, 20, 30, 40, 50 µg/ml concentration. The absorbance of resulting solutions for these drugs was measured at 294.0nm, and 351.0nm respectively.

Multi-component method: In this method six mixed standards of *tazarotene* and *hydroquinone* in the ratio of 0.1:4 having concentrations in µg/ml 1:10, 2:20, 3:30, 4:40, and 5:50 were prepared in methanol: water (80:20) solution by diluting appropriate volumes of the standard stock solutions and scanned in the region of 400nm to 200nm. Sampling wavelengths (294.0nm and 351.0nm) were selected on the trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards and concentration of each component were obtained by spectral data of sample solution with reference to that of six mixed standards (Figure 4).

Table 3: Recovery studies for accuracy of formulation.

Level of Recovery (%)	80		100		120	
	TAZA	HYDRO	TAZA	HYDRO	TAZA	HYDRO
Amount Present (mg)	2	10	2	10	2	10
	2	10	2	10	2	10
	2	10	2	10	2	10
Amount of Std. Added (mg)	1.6	8	2	10	2.4	12
	1.6	8	2	10	2.4	12
	1.6	8	2	10	2.4	12
Amount Recovered (mg)	1.58	8.01	2	10.01	2.39	11.98
	1.59	7.99	1.99	10	2.4	12
	1.6	8	2.01	9.99	2.39	11.99
% Recovery	98.75	100.13	100	100.1	99.58	99.83
	99.38	99.88	99.5	100	100	100
	100	100	100.5	99.9	99.58	99.92

Table 4: Statistical validation of recovery studies.

Level of Recovery (%)	Drug	% Recovery	Standard Deviation*	% RSD
80	*Tazarotene*	99.38	0.625	0.628931
	Hydroquinone	100	0.125	0.125
100	*Tazarotene*	100	0.5	0.5
	Hydroquinone	100	0.1	0.1
120	*Tazarotene*	99.72	0.240563	0.241233
	Hydroquinone	99.92	0.083333	0.083403

Table 5: Results of analysis data of gel formulation.

Drug	% Label claim	Amount found*	Label claim (%)	S.D. *	% RSD
Tazarotene	0.1	0.099	99.66	0.052	0.058
Hydroquinone	4	3.99	98.6	0.252	0.125

*Denotes average of three determinations.

Table 6: Intra-day and Inter-day precision.

Intra-day Precision			Inter-day Precision		
	% Label claim			% Label claim	
	Tazarotene	*Hydroquinone*		*Tazarotene*	*Hydroquinone*
After 1hr	99.52	99.15	First day	98.00	98.00
After 2hr	99.10	99.10	Second day	97.10	98.10
After 3hr	98.78	99.05	Third day	97.05	97.50
After 4hr	98.70	98.56			
After 5hr	98.60	98.00			
After 6hr	98.50	98.00			
Mean	98.87	98.64	Mean	97.38	97.87
S.D.*	0.38	0.54	S.D.*	0.53	0.32
% RSD	0.38	0.55	% RSD	0.55	0.33

*Denotes average of three determinations.

Analysis of the gel formulations: A quantity of Gel equivalent to 0.1mg of *tazarotene* was transferred to separate 10ml volumetric flask and dissolved in 5ml of diluent with frequent shaking for 15 minutes and final volume was made up with diluent. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 1.0ml of solution was taken and diluted to 10ml with diluent to get a solution containing 1µg/ml of *tazarotene*, and 40µg/ml of *hydroquinone* respectively. Absorbance of the sample was recorded at 294.0 and 351.0nm respectively and analysis procedure was repeated six times with gel formulation. Concentration of drugs in the sample was determined using absorbance of sample.

Validation of developed method

Linearity: Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to absorbance of analyte in the sample. The calibration plot was constructed after analysis of five different (from 1 to 5 µg/ml for *tazarotene* and 10 to 50 µg/ml for *hydroquinone*) concentrations and absorbance for each concentration were recorded three times, and mean absorbance was calculated (Table 1 & 2).

Accuracy: Recovery studies were performed to validate the accuracy of developed method. To pre-analysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed and result was shown in Table 3 and statistical validation of recovery studies shown in Table 4.

Precision:

(A) Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out (Table 5).

(B) Intermediate Precision

(a) Day to Day

(b) Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out and the data is presented in Table 6.

LOD (Limit of Detection): The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table 1.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

LOQ (Limit of Quantification): The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 1.

$$LOQ = 10 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of

the response.

Results and Discussion

Result of Precision

(A) Repeatability:-

(B) Intermediate Precision (Inter-day and Intra-day precision)

Conclusion

The validated spectrophotometric method employed here proved to be simple, economical, rapid, precise and accurate. The method can be used for routine simultaneous determination of *tazarotene* and *hydroquinone* in gel dosage form instead of processing and analyzing each drug separately.

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References

1. Multum C. Drugs.com., 2009, version. 3.04.
2. Sheliya K, Shah K, Kapupara P. Development and validation of analytical method for simultaneous estimation of mometasone furoate, *hydroquinone* and tretinoin in topical formulation by RP-HPLC. J Chem Pharm Res. 2014; 6: 934-940.
3. Odumosu P, Ekwe T. Identification and spectrophometric determination of *hydroquinone* levels in some cosmetic creams. Afr J Pharm Pharmacol. 2010; 4: 231-234.
4. Claudia D, Luigia O, Salvatore, F. Analysis of *hydroquinone* and some of its ethers by using capillary electrochromatography. J Chromatogr A. 2000; 887: 489-496.
5. Brenna E, Frigoli S, Fronza G, Serra S. Impurities of *tazarotene*: isolation and structural characterization. J Pharm Bio A. 2008; 46: 574-576.
6. Lowe N, Horwitz S, Tanghetti E, Draelos Z, Menter A. *Tazarotene* versus *tazarotene* plus *hydroquinone* in the treatment of photo damaged facial skin: a multicenter. J Cosmet Laser Ther. 2006; 8: 121-127.
7. Elzanfaly E, Saad A, Elaleem A. Simultaneous determination of retinoic acid and *hydroquinone* in skin ointment using spectrophotometric technique (ratio difference method). Saudi Pharm J. 2012; 20: 249-253.
8. Jogarami R, Jain P, Sharma, S. Validated UV spectrophotometric method development for simultaneous estimation of *tazarotene* and *hydroquinone* in gel preparation. J Pharm Res. 2012; 5: 2273-2275.
9. Patel M, Patel R, Parikh J, Patel B. HPTLC method for estimation of *tazarotene* in topical gel formulations and *in vitro* study. Anal Methods. 2010; 2: 275-281.
10. Pathar D, Jadhav S, Shingare M. A validated stability indicating RPLC method for *tazarotene*. Chromatographia. 2007; 66: 247-250.
11. Badawy A, Abd E, Saad A. Stability-indicating spectrophotometric methods for determination of *tazarotene* in the presence of its alkaline degradation product by derivative spectrophotometric techniques. Drug Test Anal. 2010; 2: 130-136.
12. Roy C, Chakrabarty J. Development and validation of a stability-indicating RP-HPLC method for the simultaneous determination of phenoxyethanol, methylparaben, propylparaben, mometasone furoate, and *tazarotene* in topical pharmaceutical dosage formulation. J Sci Pharm. 2013; 81: 951-967.
13. Roy C, Patel H, Chakrabarty. Stability indicating RP-HPLC method development and validation for determination of process related impurities and degradation products of *tazarotene* in *tazarotene* topical formulation. Indo Am J Pharm Res. 2012; 3: 1400-1413.
14. International Conference on Harmonization. Validation of Analytical Procedures Text and Methodology, 2005, Accessed October 10, 2016.

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