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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF TAZAROTENE AND HALOBETASOL PROPIONATE IN FIXED-DOSE COMBINATION

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ABSTRACT
Simple, precise and accurate UV-Spectrophotometric simultaneous equation method for estimation of Halobetasol propionate (HALO) and Tazarotene (TAZA) were developed and validated as per ICH guidelines. This Method involves solving of simultaneous equations based on measurement of absorbance at two wavelengths 238 nm and 351 nm (λ_{max} of HALO and TAZA) in methanol. HALO obey the Beer's law in the concentration ranges 5-25µg/ml and TAZA (1-5µg/ml). % Recovery for both the drugs were in the range of 98.46±0.854% to 99.21±0.688 for halo and 97.10±0.781 to 99.04±0.770 for TAZA respectively indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus, method can be used for routine monitoring of drugs in industry for the assay of bulk drugs and commercial formulation.

Keywords: Halobetasol propionate, Tazarotene, Spectrophotometric analysis, Simultaneous equation method.

1. INTRODUCTION
Halobetasol propionate is a topical corticosteroid drug with molecular weight of 484.965g/mol and pKa value of 12.46. Chemically it is (6S, 8S, 9R, 10S, 11S, 13S, 14S, 16S, 17R)-17-(2-chloroacetyl)- 6,9-difluoro-11,17-dihydroxy-10, 13, 16-trimethyl6, 7, 8, 11, 12, 14, 15, 16-octahydrocyclopenta[a] phenanthren-3-one [1] (Fig. 1). Halobetasol propionate belongs to a Class I topical synthetic corticosteroids used to treat a variety of skin conditions, for example, eczema, dermatitis, allergies and rash, in which it reduces swelling, itching and redness. It also acts as an anti-inflammatory and antipruritic agent [2] and available in the form of ointment and cream, 0.05% (w/w). Tazarotene (TA), ethyl 6-[(4,4-dimethyl-3,4-dihydro-2*H*-thiochromen-6-yl) ethynyl] nicotinate (Fig. 2), is a member of a new generation of receptor-selective, synthetic retinoids for the topical treatment of mild to moderate plaque psoriasis, acne vulgaris, and photoaging [3-5]. Psoriasis is one of the most common human skin diseases and is characterized by excessive growth and aberrant differentiation of corneocytes, but is fully reversible with appropriate therapy [6-8].

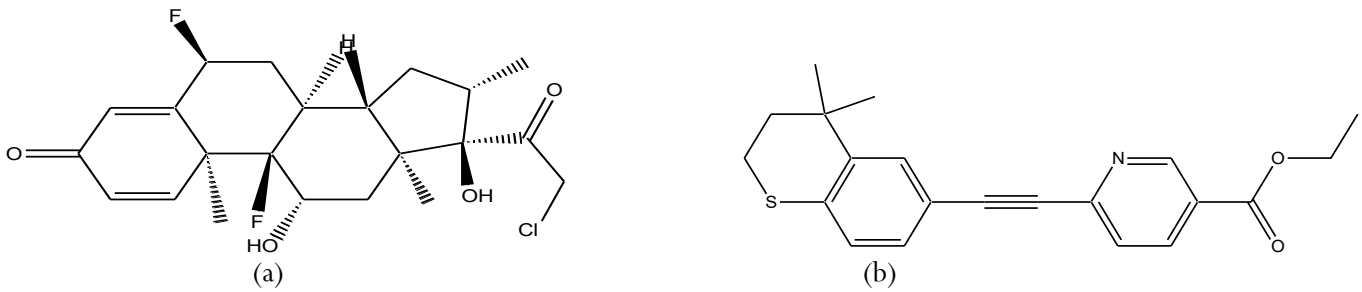

Fig. 1: Chemical structure of (A) Halobetasol and (B) Tazarotene

No analytical methods are reported for this combination (Halobetasol propionate and Tazarotene) in gel formulation however; analytical methods of single drug as well as with other combination are reported like, a spectrophotometric determination of clobetasol propionate, Halobetasol propionate, quinagolide hydrochloride, through charge transfer complexation [9], simultaneous determination of halobetasol propionate and fusidic acid-related substances by reversed phase high performance liquid chromatographic method [10], Development and validation of RP-HPLC Method for simultaneous estimation Prednicarbate, Mupirocin and Ketoconazole in topical dosage form [11], spectroscopic tools and implementation strategies for the chemical and pharmaceutical industries [12], Formulation and evaluation of novel combined halobetasol propionate and fusidic acid ointment [13], Practical High Performance Liquid Chromatographic method development [14], Development and validation of a liquid chromatographic method for *in-vitro* mupirocin quantification in both skin layers [15]. However, no UV-Spectrophotometric simultaneous equation method is available for simultaneous determination of the TAZA and HALO in combined pharmaceutical dosage form. In the present study, an attempt was made to develop a simple, precise and accurate method for the simultaneous estimation of these drugs in combined pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines.

2. MATERIAL AND METHOD

2.1. Reagents and chemicals

TAZA and HALO standard were obtained from pharmaceutical company as gift sample. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Other chemicals used were of analytical or analytical grade.

2.2. Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

2.3. Method development

2.3.1. Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80 mL methanol and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with methanol to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

2.3.2. Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of HALO and TAZA and transferred into 25 ml volumetric flask separately and diluted up to 25ml with methanol that gave concentration of 100µg/ml (Stock-B).

2.3.3. Preparation of Working Standard Solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2 ml and 2.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with methanol. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for HALO. 0.5 ml, 1.0 ml, 1.5 ml, 2 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with methanol. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20 µg/ml and 25µg/ml respectively for TAZA.

2.3.4. Selection of wavelength for linearity

Solutions of 10µg/ml of HALO and 10µg/ml TAZA were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of HALO and TAZA was observed at 238.0 nm and 351.0 nm, respectively. HALO and TAZA showed linearity in the concentration range of 5-25µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

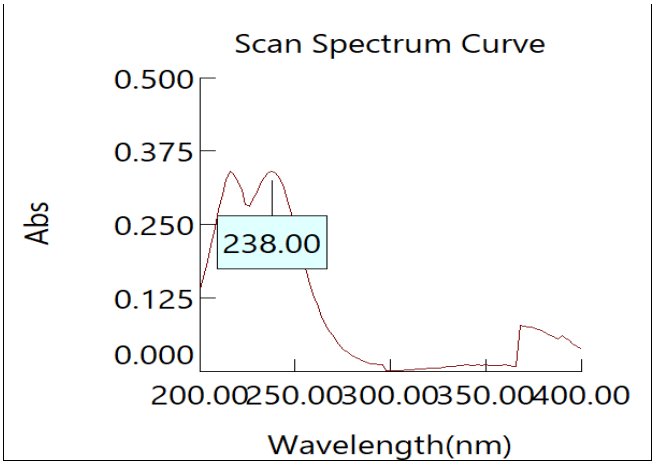

Fig. 2: Determination of λ_{max} of HALO

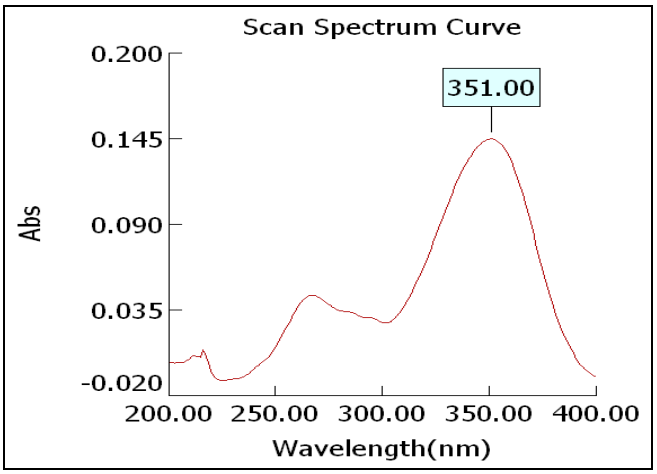

Fig. 3: Determination of λ_{max} of TAZA

2.4. Simultaneous equation method (Vierordt's)

Working standard solution from the standard stock solution prepared as in concentration 10µg/ml of HALO and 5µg/ml of TAZA were scanned in the spectrum mode over the range of 200-400 nm against methanol as blank and the overlain spectra of the two were recorded. HALO showed an absorbance peak at 238.0 nm, whereas TAZA at 351.0 nm. The overlain spectra also showed isoabsorptive points at 305.0 nm.

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 238.0 nm and 351.0 nm that are λ_{max} of HALO and TAZA respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_{HALO} = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \text{..... Eq. (1)}$$

$$C_{TAZA} = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \text{..... Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 222.0 nm and 276.0 nm respectively, ax_1 and ax_2 are absorptivities of SAXA at λ_1 (222.0 i.e. λ_{max} of SAXA) and λ_2 (276.0 i.e. λ_{max} of DAPA) respectively and ay_1 and ay_2 are absorptivities of DAPA at λ_1 and λ_2 respectively. C_{DAPA} and C_{SAXA} are concentrations of SAXA and DAPA respectively. Fig. 4 represent the overlain spectra of both the drugs in 2:40 ratio and the criteria for obtaining maximum precision [i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the SAXA and DAPA [28].

2.5. Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 [16].

2.5.1. Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in table 1.

Table 1: Results of Linearity of Halobetasol and Tazarotene

PARAMETER	Halobetasol	Tazarotene
Concentration (µg/ml)	5-25	1-5
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.028	0.020
Intercept (c)*	0.003	0.000

**value of three replicates*

2.5.2. Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicates and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study is shown in table 2.

Table 2: Results of Recovery Study

% LEVEL	% MEAN±SD*	
	Halobetasol	**Tazarotene**
80%	98.46±0.854	98.33±0.519
100%	99.21±0.688	97.10±0.781
120%	99.19±0.513	99.04±0.770

** Value of three replicate and five concentrations*

2.5.3. Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision is shown in table 3.

2.6. Analysis of marketed formulation (Lotion)

Amount equal to 1mg of HALO was taken in 10 ml volumetric flask. Then 5 ml of methanol was added and the flask was sonicated for about 10 min to solubilize the drug present in formulation and the volume was made up to the mark with methanol. After sonication, filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with methanol to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

Table 3: Results of Precision

PARAMETER	% MEAN±SD*	
	Halobetasol	Tazarotene
Repeatability	99.00±0.10	97.69±0.04
Intermediate precision		
Day to day precision	98.67±0.09	97.67±0.05
Analyst-to-Analyst	99.02±0.08	96.98±0.06
Reproducibility	98.83± 0.09	95.97±0.08

** Value of five replicate and five concentrations*

Table 4: Assay of Tablet Formulation

% Conc. Found		
Drug	**HALO**	**TAZA**
Mean*	98.82	97.20
SD*	1.157	1.351
% RSD*	1.171	1.390

**Average of three replicate and five concentrations*

3. RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials. To develop suitable methods of analysis, various solvents were studied. Based on solubility of both the drug in methanol, methanol was selected as a solvent for the methods. UV spectra shows that at λ_{max} of HALO (238 nm) and at λ_{max} of TAZA (351nm) no interference of wavelength occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with near to 100. The standard deviation, relative standard deviation was obtained for HALO and TAZA were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine HALO and TAZA simultaneously.

4. CONCLUSION

A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of HALO and TAZA in their commercial lotion formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied for determination and dissolution testing of HALO and TAZA in commercial formulation.

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