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SPECTOPHOTOMETRIC METHOD DEVELOPMENT FOR THE ESTIMATION OF COMBINED ANTIPSORIATICS DRUGS IN BULK AND FORMULATION

Deepak Nagar*, Pushpendra Soni, Lavakesh K Omray

Radharaman Institute of Pharmaceutical Sciences, Bhopal (M.P.)

***Correspondence Info:**

Deepak Nagar
Radharaman Institute of Pharmaceutical Sciences, Bhopal (M.P.)

Email:
nagardeepak48@gmail.com

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ABSTRACT

Simple Accurate, precise, rapid and economical method was developed for the simultaneous estimation of Tazarotene and Halobetasol in marketed formulations. Method is based on the simultaneous equations and Halobetasol showed an absorbance peak at 238.0 nm, whereas Tazarotene at 351.0 nm. The overlain spectra also showed isoabsorptive points at 305.0 nm. The linearity was obtained in the concentration range of 5-25µg/ml for Halobetasol and Tazarotene respectively. The correlation coefficient of Halobetasol and Tazarotene were found to be 0.999 respectively. The proposed procedure was successfully applied for the simultaneous determination of both drugs in commercial preparation. The results of the analysis have been validated statistically and by recovery studies have confirmed the accuracy of proposed method.

Key words: Method development, Validation, Simultaneous estimation, Halobetasol, Tazarotene

INTRODUCTION:

Spectrometry deals with instruments based on the absorption or emission of electromagnetic radiations as a result of its interaction with matter (Sethi, 1996; Davidson *et al.*, 1987). Absorption spectrometry is the measurement of the selective absorption by atoms, molecules or ions of electromagnetic radiation having a definite and a narrow wavelength range (approximating monochromatic energy) (Jeffery *et al.*, 1989; Swarbrick *et al.*, 1998; Sahu *et al.*, 2006; Jain *et al.*, 2009; Khan and Jain, 2006). Tazarotene and Halobetasol combination recently launched in the market for the treatment of skin infection in the strength of 0.045:0.01%. Till date there is no cost effective method for the spectrophotometric method for the estimation of Tazarotene and Halobetasol in combination. Following are the marketed formulation to be estimated by using UV. The objective of

present work is, to develop UV method for combined antipsoriatics drugs in pharmaceutical dosage form (Roy *et al.*, 2012; Patel *et al.*, 2010). To validate the developed methods according to ICHQ2R1 & ICHQ2R2 guidelines to ensure their precision, accuracy, repeatability, reproducibility and other analytical method validation parameters. Finally developed methods were compared for more suitability and usefulness.

MATERIAL AND METHODS

Material

Both standard drugs were obtained from pharmaceutical Industries. Methanol, Acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Other chemicals used were of analytical grade.

Methods

Simultaneous equation method

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared as in concentration 10 µg/ml of Halobetasol and 10 µg/ml of Tazarotene 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. Halobetasol showed an absorbance peak at 238.0 nm, whereas Tazarotene 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 Halobetasol and Tazarotene 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.

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

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

Where, A_1 and A_2 are absorbances of mixture at 238.0 nm and 351.0 nm respectively, ax_1 and ax_2 are absorptivities of Halobetasol at λ_1 (238.0 i.e. λ_{max} of Halobetasol) and λ_2 (351.0 i.e. λ_{max} of Tazarotene) respectively and ay_1 and ay_2 are absorptivities of Tazarotene at λ_1 and λ_2 respectively. C_{HALO} and C_{TAZA} are concentrations of Halobetasol and Tazarotene respectively. 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 Halobetasol and Tazarotene.

Validation of Simultaneous Equation Method

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of Halobetasol and Tazarotene to preanalysed lotion. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in table 4.16.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Methanol (100 % v/v) to Methanol: Water (90:10 % v/v). Results of robustness are reported in table 4.24-4.25.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of marketed formulation (Lotion)

Amount equal to 1mg of Halobetasol 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.

Results and Discussion

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

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 then 2 indicate the precision of method.

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs.

Table 1: Response Ratio for lineraty of of Halobetasol and Tazarotene

S. No.	HALO		TAZA	
	Conc. (µg/ml)	Response Ratio	Conc. (µg/ml)	Response Ratio
1.	0	0	0	0
2.	5	0.0294	5	0.0194
3.	10	0.0285	10	0.0212
4.	15	0.0289	15	0.0209
5.	20	0.0287	20	0.0211
6.	25	0.0282	25	0.0205

Table 2: Recovery study of Tazarotene

Tazarotene	80%	98.9±0.798
	100%	98.7±0.513
	120%	98.3±1.075

* Mean of 3 replicate and 5 concentrations

Table 3: Recovery study of Tazarotene

Halobetasol	80%	98.3±1.125
	100%	98.3±0.921
	120%	97.3±1.338

* Mean of 3 replicate and 5 concentrations

Table 4: Results of other validation parameters

Repeatability	
Tazarotene	98.8±0.143
Halobetasol	98.7±0.166
Day-to-Day Variation	
Tazarotene	99.1±0.098
Halobetasol	99.0±0.146
Analyst-to-Analyst	
Tazarotene	99.5±0.093
Halobetasol	98.8± 0.143
Reproducibility	
Tazarotene	98.3±0.145
Halobetasol	99.6±0.039
Robustness	
Tazarotene	99.0±0.117
Halobetasol	98.9±0.126

Table 5: LOD and LOQ of Tazarotene and Halobetasol

Name	LOD (μg/ml)	LOQ (μg/ml)
Tazarotene	0.45	0.145
Halobetasol	0.35	0.110

Table 6: Results of assay of marketed formulation

	Tazarotene	Halobetasol
MEAN*	98.471	97.757
SD*	1.305	1.481
% RSD*	1.325	1.515

CONCLUSION

The proposed method was found to be linear with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent.

The result obtained shows the developed methods to be Cost effective, Rapid (Short

retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

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