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Development and Validation of UV Spectrophotometric Method for Quantitative Estimation of Clobetasol 17-Propionate

Neelam Devi[1], Sunil Kumar[1], Sunny Rajain[1], Jagbir Gagoria[2], Sheefali Mahant[2] and Rekha Rao[3*]

[1]Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar - 125001, Haryana, India

[2]Department of Pharmaceutical Sciences, Maharishi Dayanand University, Rohtak - 124001, Haryana, India

[3]Assistant Professor, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar - 125001, Haryana, India; rekhaline@gmail.com

Abstract

Clobetasol 17-propionate is used most potent topical glucocorticoid clinical effective in treatment of topical dermatitis, vitiligo and psoriasis. A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of Clobetasol 17-Propionate (CP) in bulk forms and dosage formulations. The spectrophotometric detection was carried out at an absorption maximum of 239 nm using ethanol as solvent. The method was validated for specificity, linearity, accuracy, precision, and robustness. The detector response for the CP was linear over the selected concentration range 2 to 40 µg/ml with a correlation coefficient of 0.9999. The accuracy was between 99.1 and 101.4 %. The precision of 4 µg/ml sample preparation three times in a day (intraday) was 0.1325%. The Limit of Detection (LOD) and Limit of Quantification (LOQ) are 0.84 and 2.55 µg/ml, respectively. The recovery of CP was about 101.84%. The results demonstrated that the excipients in the commercial formulation did not interfere with the method and can be conveniently employed for daily routine quality control analysis of CP in bulk drug, marketed formulations.

Keywords: Clobetasol 17-Propionate, ICH Guidelines, UV-Visible Spectroscopy, Validation

1. Introduction

High potency dihalogenated corticosteroid, Clobetasol 17-Propionate (CP) is used for skin diseases such as vitiligo, psoriasis and atopic dermatitis due to its anti-inflammatory, vasoconstrictive, antiproliferative and immunosuppressive activities. It has been approved for the topical use in dosage forms like such as gel, cream, ointment, solution and foam[1–2]. Clobetasol 17-Propionate is used to relieve redness, itching, sweeling, or other discomfort caused by skin conditions. The treatment of more severe skin disorders using CP with or without the inclusion of other drug substances were compared in several clinical studies. CP has demonstrated excellent recovery, rapid relief and reduced relapses of different skin conditions and symptoms[3–5]. It was also proven to be the first topical corticosteroid that demonstrated satisfactory results in the treatment for psoriasis[2,4]. Literature search reveals HPLC, RP-HPLC and liquid chromatography methods were reported for determination of various salts of Clobetasol in formulations like ointment, creams and suspensions[6–11]. Beside these, some simultaneous analytical estimations of Clobetasol 17-Propionate with other drugs have been reported in literature[12–16]. Till date, no studies have been reported for estimation of CP in bulk and ointment formulation using a validated UV-visible spectrophotometric assay method. Therefore, the aim of the present work is to develop and validate analytical method by UV-Visible spectrophotometer which is simple, rapid and advantageous and in which no complexation agent, extraction, derivatization, or evaporation steps are involved.

* *Author for correspondence*

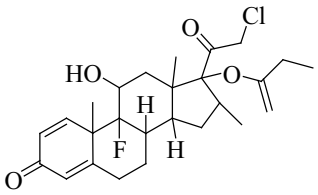

Figure 1. Structure of Clobetasol 17-Propionate.

2. Materials and Method

2.1 Materials

Clobetasol 17-Propionate (CP) was obtained from Sigma Aldrich, USA. Ethanol was procured from S D Fine-Chem Ltd, Mumbai, India. Formulation (ointment) collected from market with drug equivalent to 0.05% w/w of CP. All the other reagents and chemicals used were of analytical grade.

2.2 Method Development

2.2.1 Instrument

Double beam UV Visible Spectrophotometer (Variance Carry 5000, India)

2.2.2 Preparation of Standard Stock Solution

Accurately weighed 10 mg of standard CP was dissolved in 100 ml of ethanol (standard stock solution). From this standard stock solution, prepare the aliquots of different concentration by suitable dilutions varying in between 2 and 40 µg/ml using ethanol. These diluted solutions were checked for Linearity, Precision, Accuracy, Robustness, Limit of Quantification (LOQ) and Limit of Detection (LOD).

2.2.3 Method Optimization

2.2.3.1 Selection and Optimization of Solvent

As reported in literature, the solvent have a profound influence on the shape and quality of the peak[17]. The choices of solvents for ultra violet method development are: ethanol, methanol, acetone, etc. Various solvents were checked and ethanol was found to fulfill all the conditions relating to quality and non-interference of peak at the specified wavelength.

2.2.3.2 Selection of Wavelength

In order to determine the wavelength of absorption maxima (λ_{max}) of CP, aliquot of 100 µg/ml solution was prepared by taking weighed amount of drug (10 mg) in 100 ml of ethanol and scanned by UV-Visible spectrophotometer in the wavelength range of 400-200 nm against ethanol as a blank. The resulting spectrum was shown in Figure 2 and absorption curve showed characteristic maximum absorption at 239 nm for Clobetasol 17-propionate. The wavelength at which maximum absorption observed is 239 nm, which is selected for further analysis.

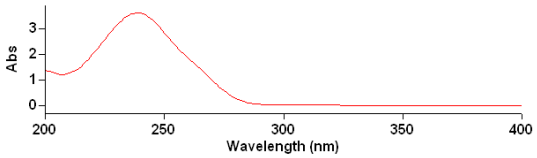

Figure 2. UV spectrum of clobetasol 17-propionate in ethanol (λ_{max} at 239nm).

2.3 Method Validation

Method validation was performed as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005)[18,19] and all the parameters were evaluated.

2.3.1 Linearity

The linearity of this method was checked at concentrations ranging between 2-40 µg/ml. The curve of absorbance v/s concentration (Figure 3) of CP was found to be linear as given in Table 1. The investigated concentrations followed Beer's Lambert law[20].

Table 1. Absorbance for standard curve of clobetasol propionate (CP) at 239 nm

Concentration (µg/ml)	Absorbance at 239 nm
2	0.0765
4	0.1558
6	0.2327
8	0.3004
10	0.3749
20	0.7273
40	1.4255

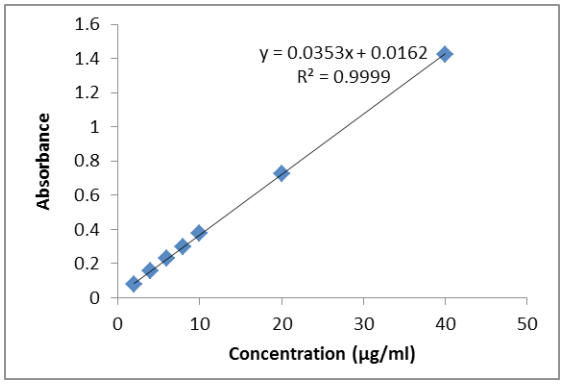

Figure 3. Standard curve of clobetsaol 17-propionate in ethanol.

2.3.2 Precision

The precision of the UV method was performed by intermediate precision (inter-day) and repeatability (intra-day).

2.3.2.1 Repeatability

Repeatability (intra-day) was carried out by analyzing CP having concentration 4 µg/ml, three times a day. To assess the intra-day variation, the % RSD was calculated from absorbance as obtained.

2.3.2.2 Intermediate Precision

Intermediate precision (inter-day) was assessed by analyzing 4 µg/ml concentration of CP for three different days. The % RSD was calculated for absorbance thus obtained, to measure the interday variation[21].

Table 2. Repeatability and intermediate precision

Concentration (µg/ml)	Repeatability (n=3) Absorbance at 239 nm	Intermediate precision (n = 3) Absorbance at 239 nm
4µg/ml	0.1154	0.1153
4µg/ml	0.1152	0.1153
4µg/ml	0.1151	0.1150
Mean	0.1152	0.1152

2.3.3 Accuracy

Accuracy is defined as closeness between the actual (true) value and value obtained by repeating test method for a number of times. Accuracy may be expressed as % Recovery by the assay of known analyte which is added. It gives exact measure of the analytical method. The pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard CP (10 µg/ml) and the mixtures were analyzed using UV visible spectrophotometer. The experiment was performed in triplicate[22].

2.3.4 LOD and LOQ

The Detection Limit (DL) is the lowest concentration of analyte present in a sample, which can be analyzed but not necessarily quantitated. The Quantitation Limit (QL) is the lowest concentration of analyte present in a sample, which can be quantitatively analyzed with acceptable precision and accuracy. The limit of detection and limit of quantification were assessed based on the technique of signal-to-noise ratio10 using the Equations (1) and (2).

$$QL = 10\ \sigma / S \quad (1)$$

$$DL = 3.3\ \sigma / S \quad (2)$$

Where, σ is the standard deviation of the intercept of the calibration plot and S is the slope of the calibration curve[23].

3. Result and Discussion

The CP was found to be soluble in ethanol. The λ_{max} of drug was found to be 239 nm as shown in (Figure 1). From the result obtained from Table 1, it was observed that CP obeys linearity within the concentration range of 2 µg/ml-40 µg/ml and coefficient correlation was 0.9999. The regression value from the curve was y = 0.0353x + 0.0162 as shown in Figure 2. The detection and quantitation limits were calculated as LOD (k = 3.3) and LOQ (k = 10) and these were found to be 0.84 µg/ml and

Table 3. Recovery data for the accuracy analysis of the UV method

Excess of CP added (%)	Concentration of sample (µg/ml)	Theoretical concentration of spiked sample (µg/ml)	Concentration of spiked sample ± SD (µg/ml) (n=3)	Recovery± SD (%)	%RSD
50	10	15	14.99±0.010	99.93±0.065	0.067
100	10	20	19.95±0.010	99.75±0.050	0.050
150	10	25	25.46±0.025	101.84±0.59	0.098

Table 4. Validation Parameters

Validation parameters	Data (Mean±SD)		
λ_{max} (nm)	239 nm		
Range (µg ml^{-1})	2-40 µg/ml		
Correlation coefficient	0.9997±0.00015		
Intercept	0.0162±0.00882		
Slope	0.03533±0.0016		
Accuracy	99.93-101.84%		
Precision (%RSD)	0.1325		
LOD (µg ml^{-1})	0.84		
LOQ (µg ml^{-1})	2.55		
Precision (%RSD)	Concentration (µg/ml) 4µg/ml	Intra-day (% RSD) 0.1325	Inter-day (%RSD) 0.1503

Table 5. Recovery studies of marketed ointment formulation

Conc. level	Sample no.	Drug	Formulation	Amount added (µg/ml)	Abs.	Amount recovered	% recovery	Mean % recovered ± SD (N=3)	% RSD
50%	1		10 ml of 20 µg/ml	15 µg/ml	0.5698	14.80	98.67	98.56±0.25	0.25
	2				0.5676	14.74	98.27		
	3				0.5703	14.81	98.75		
100%	1	10 ml of 10g/ml	10 ml of 30 µg/ml	20 µg/ml	0.7204	19.81	99.05	99.04±0.01	0.01
	2				0.7302	19.80	99.02		
	3				0.7204	19.81	99.05		
150%	1		10 ml of 40 µg/ml	25 µg/ml	0.9323	24.53	98.10	98.18±0.07	0.07
	2				0.9335	24.55	98.23		
	3				0.9333	24.55	98.21		

2.55 µg/ml respectively. The precision (measurements of intra-day and inter-day) results demonstrated (Table 2.) significant reproducibility with % RSD below 2.0 observed. This showed that method is highly precise. The percent recovery value (Table 3.), was observed higher than 100%, indicating the accuracy of the method. The estimation of CP in marketed ointment formulation was found to be 98-99%.

5. Conclusion

The proposed method was observed as a simple, accurate, precise, sensitive, economical, reproducible and rapid for the routinely estimation of CP. The developed method is specific for estimation commercial formulations like ointments without interference of excipients.

6. Conflicts of Interest

All authors have none to declare.

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