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Comparative Study of UV Spectroscopy, RP-HPLC, and HPLC Methods for Quantification of Clobetasol Propionate.

Bharath. B*, Jayakumar. A , Karthi. J

Department of Pharmaceutical Chemistry, Pallavan Pharmacy College, India.

ABSTRACT

This study presents a comparative analysis of UV spectroscopy, High-Performance Liquid Chromatography (HPLC), and Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) methods for the quantification of Clobetasol Propionate (CP), a potent topical glucocorticosteroid widely used in the treatment of inflammatory skin disorders. The methods were developed and validated as per ICH Q2(R1) guidelines with respect to linearity, accuracy, precision, robustness, and system suitability. UV spectroscopy was performed at 239 nm in ethanol, with a linear concentration range of 2–40 µg/mL. HPLC and RP-HPLC methods employed C18 columns with optimized mobile phase compositions and flow rates to achieve efficient separation. The calibration curves for all methods demonstrated excellent linearity ($r^2 \geq 0.999$), with recoveries ranging between 97–102%, indicating high accuracy. Precision studies showed %RSD values below 2%, confirming reproducibility. Robustness was evaluated by deliberate variations in flow rate, column temperature, and mobile phase composition, with minimal impact on the analytical performance. Among the three techniques, RP-HPLC provided superior sensitivity and resolution with shorter run times, while UV spectroscopy was found to be a simple, rapid, and cost-effective alternative for routine analysis. This comparative study provides a reliable basis for the selection of appropriate analytical techniques for CP quantification in pharmaceutical formulations.

Keywords Clobetasol Propionate; UV Spectroscopy; HPLC; RP-HPLC; Method Validation; Linearity; Accuracy; Precision; Robustness

INTRODUCTION

A powerful topical glucocorticosteroid, clobetasol propionate (CP), is frequently used to treat inflammatory skin conditions such as vitiligo, eczema, psoriasis, and atopic dermatitis in the short term. With the chemical formula $C_{25}H_{32}ClFO_5$, CP is a dihalogenated corticosteroid that resembles a white to cream-coloured crystalline powder. It has demonstrated outstanding clinical results with quick symptom alleviation and fewer relapses in a variety of topical dose forms, including creams, ointments, gels, foams, and solutions[1].

Various analytical methods, such as spectrophotometry, HPLC, RP-HPLC, and HPTLC, have been used to estimate CP either alone or in combination with other drugs in pharmaceutical formulations. However, there are few validated UV-visible spectrophotometric techniques for its determination in bulk and ointment dosage forms. This highlights the need for straightforward, quick, and cost-efficient analytical methods that do not involve complex procedures like derivatization or extraction.[1]

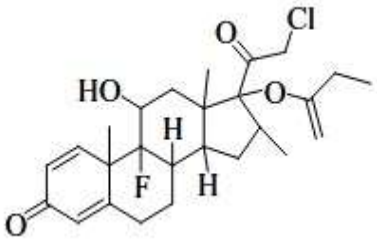

Fig.1 Clobetasol propionate Chemical structure

METHODS

Materials

Clobetasol 17-Propionate (CP) was obtained from Sigma Aldrich, USA. Ethanol was procured from S D FineChem Ltd, Mumbai, India. Formulation (ointment) collected from the market with a drug equivalent to 0.05% w/w of CP. All the other reagents and chemicals used were of analytical grade [1].

Instrumentation.

Double beam UV Visible Spectrophotometer (Variance Carry 5000, India [1]. The equipment used for the method was an Analytical balance Sartorius (model: TE214S). HPLC was Dionex Ultimate 3000(equipped with autosampler and UV-visibledetector. HPLC Shimadzu Prominence (equipped with autosampler and PDA detector). The Column selected for the method was 250 mm x 4.6 mm 5 µm (Zorbax Eclipse XDB-C18) [2].HPLC-grade methanol, acetonitrile and trifluoroacetic acid were procured from Merck, Mumbai, India. Samples of Clobetasol propionate (CLBPR) and all its impurities were received as gift sample from SynZeal Research Pvt Ltd., Ahmedabad, Gujarat, India .HPLC-grade water was obtained using Milli-Q water purification[3]

Sample preparation for UV, HPLC, and RP-HPLC development

Standard solution preparation

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 concentrations by suitable dilutions varying between 2 and 40 µg/ml using ethanol[1].

Sample solution preparation

An aliquot of 100 µg/ml solution was made by weighing 10 mg of the drug in 100 ml of ethanol in order to find the wavelength of absorption maxima (λ max) of CP [1].

UV method development

Determination of clobetasol propionate maximum absorbance (λ max)

The prepared solution is measured using a UV-Visible spectrophotometer against ethanol as a blank in the 400–200 nm wavelength range. Figure 2 displayed the obtained spectra, and the absorption curve revealed that Clobetasol 17-propionate had a distinctive maximum absorption at 239 nm. The wavelength chosen for additional examination is 239 nm, where the highest absorption was detected [1]

HPLC method development

Optimization of HPLC method

To achieve accurate separation, and sensitivity requires optimisation. During trial runs, a number of chromatographic parameters are Accurate analyte separation, repeatability, and stability within a tolerable analysis time are the goals of HPLC method development. Choosing the right stationary phase, mobile phase composition, detection wavelength, and sample preparation methods is the first step in the development process for corticosteroids like clobetasol propionate. Choosing a stationary phase: Because C18 reverse-phase columns can hold moderately polar substances like clobetasol propionate, they are typically chosen . For Mobile phase composition, to get crisp peaks, little tailing, and good resolution, various ratios of organic solvents (methanol, acetonitrile) and aqueous buffers (phosphate, acetate) are tested. For wavelength of detection, A compromise wavelength between 240 and 254 nm can be selected for simultaneous detection based on the UV absorption maxima of clobetasol, which are approximately 240 to 245 nm. For preparing the sample, before injection, standard and sample solutions are made in methanol or methanol–water mixtures, sonicated, and filtered. When developing a process, the main objective is to reduce run time while maintaining a distinct separation between excipients and active pharmaceutical ingredients (APIs)[2].

Optimization of RP-HPLC Conditions

Establishing the established RP-HPLC method's robustness, repeatability,methodically changed until the ideal peak shape, retention duration, and resolution are attained. The Optimised parameters should be considered as follows, For mobile phase ratio, acetonitrile:buffer or methanol:water (50:50 to 80:20, v/v) combinations are tested. A composition with symmetrical peaks, retention durations of 3–7 minutes, and a resolution factor greater than 2 is chosen. For adjusted flow rate, 0.8–1.2 mL/min. In order to maintain high peaks with little tailing, a standard rate of 1.0 mL/min is frequently selected. The Column temperature is necessary to increase reproducibility, it is typically maintained between 25 and 30 °C. Depending on the sensitivity of the detector, the injection volume is usually between 10 and 20 µL. Based on the UV absorbance of both medicines, the detection wavelength is optimised. Wavelength scanning is then carried out, and the final λmax is selected to ensure sufficient sensitivity for both analytes.[4]

RESULTS

Method validation

System suitability parameters

For HPLC, system suitability was confirmed if %RSD of Clobetasol Propionate and Salicylic Acid peak areas from six standard injections was ≤2.0%, tailing factor ≤2.0, and resolution <15[2].For RP-HPLC, %RSD of CLBPR peak area from replicate standard injections must be ≤5.0%, and resolution between Clobetasol Propionate and its related compound A must be ≥1.5 in system suitability solution [3].

Linearity

For HPLC, Calibration curves of salicylic acid and clobetasol propionate at 80–120% concentrations were used to evaluate linearity; the results showed correlation values of 0.9999 and 1.0000, respectively[2]. For RP_HPLC,Clobetasol Propionate standard solutions (4–12 µg/mL) were prepared in the mobile phase, showing linear peak area-concentration plots with $r^2 = 0.999$[4]. For UV, the linearity of this method was checked at concentrations ranging between 2-40 µg/ml[1] . The linear equations of UV and RP-HPLC are y=0.0353x + 0.0162 and y=137519x.

Accuracy

HPLC accuracy, defined as the closeness of test results to the true value, showed an average recovery of 100.33%. RP-HPLC accuracy was assessed using nine determinations at 80%, 100%, and 120% of the expected concentration, with all recoveries exceeding 97%, confirming method accuracy. For UV analysis, accuracy was evaluated by spiking pre-analyzed samples with 50%, 100%, and 150% of standard CP (10 µg/mL), and recovery was determined using a UV–visible spectrophotometer. The fact that the percentage RSD was less than two suggests that the excipients had no influence on the Clobetasol measurement. The procedure is accurate as a result.

Precision

The precision of the UV technique was assessed using 4 µg/mL CP for repeatability (intra-day) and intermediate precision (inter-day). Three analyses in a single day were used to evaluate intra-day accuracy, and three analyses across three days were used to evaluate inter-day precision[1]. The percentage RSD was computed for each analysis. Six replicates at 100% test concentration were examined for HPLC, and the percentage RSD was calculated[2]. RP-HPLC precision was expressed as %RSD (<2%) and was evaluated as repeatability, intermediate precision, and reproducibility [4].

Robustness

Robustness was confirmed in accordance with ICH Q2(R1) using the UV technique (λ max = 239 nm in ethanol)[1]. Variations in column temperature (±5 °C), mobile phase composition, and flow rates (0.80–1.70 mL/min) were used to evaluate robustness for HPLC [2]. Deliberate adjustments to flow rate, pH, and methanol percentage at three different levels were used to assess the resilience of the RP-HPLC while keeping an eye on the retention duration of Clobetasol Propionate [4].

Discussion

The comparative evaluation of UV spectroscopy, HPLC, and RP-HPLC methods for the quantification of Clobetasol Propionate (CP) highlights the advantages and limitations of each technique in terms of sensitivity, accuracy, reproducibility, cost, and applicability for routine quality control. UV spectrophotometry, though simple and economical, demonstrated good linearity ($r^2 \geq 0.999$) within the concentration range of 2–40 µg/mL, making it suitable for preliminary or routine analysis where sophisticated instrumentation may not be available. Its main strength lies in rapid analysis and minimal sample preparation, which reduces operational time and cost. However, the technique lacks specificity, as excipients or degradation products can interfere with absorbance at 239 nm. This limitation restricts its use in stability studies and complex formulations where selectivity is critical. Conventional HPLC using a C18 column provided excellent separation, with recoveries around 100% and %RSD values <2%, meeting ICH Q2(R1) criteria. The robustness evaluation confirmed the methods reliability under minor variations in chromatographic conditions. Despite its reliability and wide adoption, the relatively longer run time and the requirement for more solvent compared to RP-HPLC increase cost and environmental burden.

RP-HPLC proved to be the most sensitive and selective method among the three. Optimized chromatographic conditions, including acetonitrile–buffer mobile phases and a 1.0 mL/min flow rate, provided sharp peaks with minimal tailing and shorter retention times. The method exhibited superior resolution (resolution factor >2) and high reproducibility, with recoveries consistently above 97%. Moreover, the RP-HPLC method showed robustness against deliberate changes in pH, flow rate, and solvent composition, reinforcing its suitability for routine quality control, stability studies, and regulatory submissions.When comparing the three techniques, RP-HPLC emerges as the method of choice for pharmaceutical industries due to its balance of specificity, speed, and sensitivity. HPLC remains a reliable standard, particularly where RP-HPLC optimization may not be feasible. UV spectrophotometry, despite its limitations, continues to be a valuable tool for rapid, cost-effective assays in academic research laboratories and routine in-process checks.Overall, the study demonstrates that while UV spectroscopy offers simplicity and cost-effectiveness, chromatographic methods particularly RP-HPLC are indispensable for regulatory compliance, quality assurance, and advanced pharmaceutical analysis of Clobetasol Propionate.

Conclusion

The comparative study of UV spectroscopy, HPLC, and RP-HPLC methods for the quantification of Clobetasol Propionate (CP) demonstrated that all three techniques are reliable and conform to ICH Q2(R1) validation parameters. UV spectroscopy proved to be a simple, rapid, and economical approach suitable for routine quality control where high sensitivity is not critical. HPLC offered precise and reproducible results with acceptable accuracy and system suitability for standard analytical needs. RP-HPLC emerged as the most robust and sensitive method, providing superior resolution, shorter analysis time, and higher reproducibility, making it ideal for advanced pharmaceutical quality assurance and regulatory submissions. The findings of this study provide a scientific basis for selecting the appropriate analytical method depending on the required sensitivity, resource availability, and application in pharmaceutical formulations.

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