

Development and Validation of a UV Spectrophotometric Method for Quantitative Estimation of Prednisolone Acetate in Dichloromethane**Jayesh Manikrao Wankhade¹, Dr. Sandeep Kumar Goyal¹****¹ Sabarmati University, Ahmedabad, Gujarat, India.****Received: 11-01-2024 / Revised: 10-02-2024 / Accepted: 15-03-2024****Corresponding author: Jayesh Manikrao Wankhade****Conflict of interest: Nil****Abstract**

The present study aimed to develop and validate a simple, rapid, accurate, and economical UV spectrophotometric method for the quantitative estimation of prednisolone acetate (PA) in dichloromethane. Prednisolone acetate is a synthetic corticosteroid widely used in pharmaceutical formulations for its anti-inflammatory and immunosuppressive properties. The analytical method was developed using a Shimadzu UV-1800 spectrophotometer at a wavelength of 243 nm. Calibration standards were prepared in the concentration range of 2.5–25 µg/mL, and the calibration curve exhibited excellent linearity with a correlation coefficient (R^2) of 0.9994. The developed method was validated according to ICH guidelines for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). The intra-day and inter-day precision values were found to be 1.187% and 0.659% RSD, respectively, indicating good precision. The method demonstrated satisfactory accuracy with a recovery of 99.40%. The LOD and LOQ were determined to be 0.623 µg/mL and 1.757 µg/mL, respectively. Assay of prednisolone acetate using the developed method showed a content of 99.98%, which complied with pharmacopoeial specifications. The results confirmed that the developed UV spectrophotometric method is reliable, precise, accurate, and suitable for routine quality control analysis of prednisolone acetate.

Keywords: Prednisolone acetate, UV spectrophotometry, Method validation, Analytical method development, ICH guidelines

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Introduction

Prednisolone acetate is a synthetic glucocorticoid widely employed in pharmaceutical formulations due to its potent anti-inflammatory, anti-allergic, and immunosuppressive activities. It is extensively used in ophthalmic preparations and various dosage forms for the management of inflammatory disorders. Accurate quantification of prednisolone acetate is essential to ensure the quality, safety, and efficacy of pharmaceutical products.

Analytical methods play a crucial role in pharmaceutical quality control and regulatory compliance. Various techniques, including ultraviolet (UV) spectrophotometry and high-performance liquid chromatography (HPLC), have been reported for the determination of prednisolone acetate. The drug is officially listed in the Indian Pharmacopoeia (IP), United States Pharmacopoeia (USP), and European Pharmacopoeia (Ph. Eur.). While IP and Ph. Eur. recommend UV spectrophotometric

methods, USP recommends HPLC-based analysis.

Among the available analytical techniques, UV spectrophotometry remains one of the most widely used methods because of its simplicity, rapidity, cost-effectiveness, and suitability for routine analysis. However, any analytical method intended for pharmaceutical applications must be validated to demonstrate its reliability and reproducibility. The International Council for Harmonisation (ICH) has established guidelines for validating analytical procedures with respect to linearity, precision, accuracy, sensitivity, and robustness.

Therefore, the present study was undertaken to develop and validate a UV spectrophotometric method for the estimation of prednisolone acetate in dichloromethane. The developed method was evaluated according to ICH guidelines and assessed for its suitability for routine quantitative analysis.

Materials and Methods

Materials

Prednisolone acetate (PA) working standard was obtained from a certified pharmaceutical source and used as received. Dichloromethane (DCM), of analytical reagent grade, was used as the solvent throughout the study. All chemicals and reagents employed were of analytical grade. Distilled water was used wherever required.

Instruments

UV spectrophotometric analysis was performed using a Shimadzu UV-1800 UV-Visible Spectrophotometer equipped with a 1 cm quartz cell. Analytical weighing was carried out using a calibrated electronic analytical balance. Standard laboratory glassware including volumetric flasks, pipettes, and measuring cylinders was used for solution preparation.

Method Development

Selection of Analytical Method

A literature survey and pharmacopoeial review indicated that prednisolone acetate is officially listed in the Indian Pharmacopoeia (IP), United States Pharmacopeia (USP), and European Pharmacopoeia (Ph. Eur.). The IP and Ph. Eur. recommend UV spectrophotometric analysis, whereas the USP recommends HPLC analysis. Considering the simplicity, rapidity, and cost-effectiveness of UV spectroscopy, a UV spectrophotometric method was selected for the quantitative estimation of prednisolone acetate.

UV Spectrophotometric Method for Prednisolone Acetate (PA)

Stock Solution of PA

The stock solution of prednisolone acetate (PA) was prepared by accurately weighing 25 mg of PA working standard and dissolving it in dichloromethane. The volume was then made up to 100 mL with the same solvent to obtain a stock solution having a concentration of 0.25 mg/mL (250 µg/mL).

Calibration Curve

UV spectrophotometric analysis was performed using a Shimadzu UV-1800 spectrophotometer equipped with a 1 cm quartz cell. Appropriate aliquots were withdrawn from the stock solution and diluted with dichloromethane to obtain concentrations of 2.5, 5, 10, 15, 20, and 25 µg/mL of PA.

The prepared solutions were analyzed at 243 nm (λ_{max}) against dichloromethane as the blank. All measurements were performed in triplicate, and for each replicate determination, the quartz cell was filled with a fresh sample solution. The mean absorbance values were recorded and used for the construction of the calibration curve.

Analytical Method Validation

The developed UV spectrophotometric method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ) in accordance with ICH guidelines.

Linearity

Linearity is defined as the ability of an analytical method to obtain test results that are directly proportional to the concentration of analyte present in the sample within a given range.

For the evaluation of linearity, the mean absorbance values obtained at different concentrations were plotted against the corresponding concentrations to construct a calibration curve. The least squares regression method was employed to determine the regression equation and correlation coefficient (R^2). The method was considered linear when the value of R^2 was close to 1.0.

Precision

Precision is the degree of agreement among individual test results when the analytical procedure is applied repeatedly to multiple samplings of a homogeneous sample.

The precision of the method was evaluated in terms of intra-day and inter-day precision, expressed as the percentage relative standard deviation (%RSD). Three different concentrations were selected, and the analysis was performed three times within the same day for intra-day precision and on three different days for inter-day precision.

Acceptance Criteria: %RSD $\leq$ 2.0%, as recommended by ICH guidelines.

Accuracy

Accuracy refers to the closeness of agreement between the experimental value and the true value.

The accuracy of the method was determined by the recovery study using the standard addition method at three concentration levels, namely 80%, 100%, and 120% of the working concentration. The percentage recovery of prednisolone acetate from the placebo solution was calculated, and the obtained values were used to assess the accuracy of the developed analytical method.

Limit of detection and limit of quantitation: LOD is the lowest concentration of the analyte that can be reliably detected and LOQ is the lowest concentration at which the analyte cannot only be reliably detected. The LOD and LOQ were measured as follows;

$$\text{LOD} = 3.3 \cdot (\sigma/S) \text{ and } \text{LOQ} = 10 \cdot (\sigma/S)$$

Where, σ – Standard deviation of intercepts and S = Slope of calibration plot

UV spectrophotometric analysis of PA

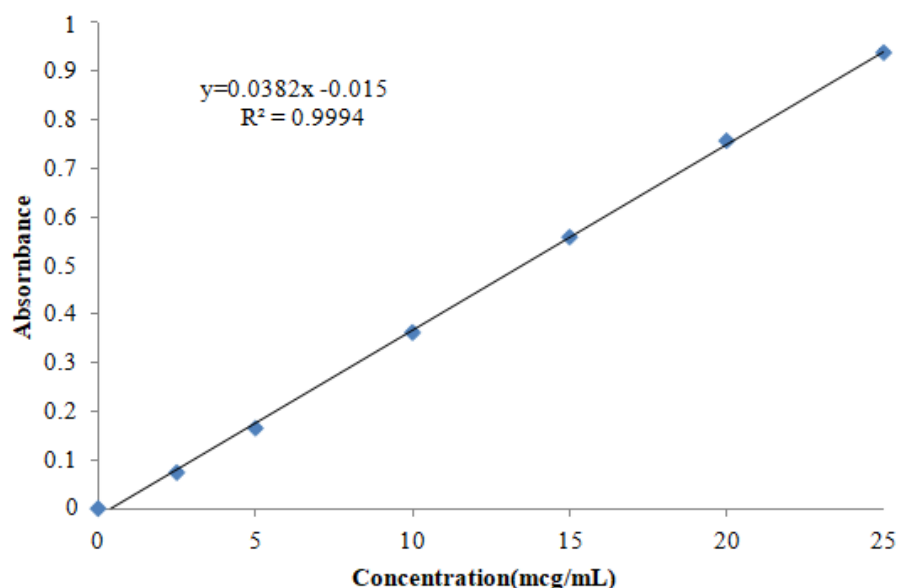
The assay of PA was performed using developed UV method. 100 mg of PA was dissolved in DCM and diluted up to 100 mL. 2 mL of this solution was diluted upto 100 mL with DCM and absorbance was measured at 243 nm λ_{max} .

Results and discussion:

Calibration curve for UV method is represented in Table 1 and Figure 1 Regression analysis was performed on the experimental data. Regression equation for standard plot was $y = 0.0382x - 0.015$. Correlation coefficient was found to be 0.9994 signifying that a linear relationship existed between absorbance and concentration of the drug.

Table 1: Calibration curve for estimation of PA

Sr. No.	Concentration (µg/mL)	Absorbance			Average absorbance ± SD
		I	II	III	
1.	0	0.000	0.000	0.000	0.000±0.000
2.	2.5	0.071	0.074	0.078	0.074±0.0035
3.	5	0.162	0.166	0.169	0.166±0.0035
4.	10	0.358	0.364	0.365	0.362±0.0038
5.	15	0.558	0.556	0.562	0.559±0.0031
6.	20	0.752	0.761	0.758	0.757±0.0046
7.	25	0.931	0.94	0.944	0.938±0.0067

**Figure 1: Calibration curve of PA in dichloromethane at 243nm**

UV spectrophotometric analysis method was validated by measuring different validation parameters like linearity, precision, accuracy, limit of detection and limit of quantification as per ICH guidelines. Validation parameters for the UV spectrophotometric method in DCM are listed in Table 2.

Table 8: Validation of UV method of PA in dichloromethane

Parameters	Results
Linearity (µg/mL)	2.5to25
Co-relation coefficient (R2)	0.9994
Intra day precision (%RSD)	1.187
Inter day precision (%RSD)	0.659
Accuracy (%recovery)	99.40
LOD(µg/mL)	0.623
LOQ(µg/mL)	1.757

The result of assay of PA using UV method is presented in the Table 2.

Table 2: Result of assay of PA using UV method

Test	Result	Acceptance criteria
Assay of PA	99.98%	97.0%–102.0% on the dried basis

The UV method was found to be accurate and precise under the linearity range mentioned in the validation table. It was validated as per ICH guidelines and can be used for estimation of the PA. Samples shall be diluted to get the absorbance within this range to determine the concentration.

The developed UV spectrophotometric method successfully quantified prednisolone acetate at 243 nm using dichloromethane as the solvent. The calibration curve exhibited excellent linearity over the concentration range of 2.5–25 µg/mL with a correlation coefficient (R^2) of 0.9994, indicating a strong linear relationship between concentration and absorbance.

Validation studies demonstrated that the method possesses adequate precision, as evidenced by the low intra-day (1.187%) and inter-day (0.659%) %RSD values, which were within the acceptable ICH limit of less than 2%. The recovery value of 99.40% confirmed the accuracy of the method and indicated minimal interference from excipients or solvents. Furthermore, the low LOD (0.623 µg/mL) and LOQ (1.757 µg/mL) values demonstrated the sensitivity of the method for detecting and quantifying small amounts of prednisolone acetate.

The assay result of 99.98% complied with the pharmacopoeial acceptance criteria (97.0–102.0%), confirming the suitability of the method for routine pharmaceutical analysis. Overall, the developed method proved to be simple, reliable, and efficient for the estimation of prednisolone acetate.

Conclusion

A simple, rapid, accurate, and precise UV spectrophotometric method was successfully

developed and validated for the estimation of prednisolone acetate in dichloromethane. The method exhibited excellent linearity within the concentration range of 2.5–25 µg/mL and fulfilled all validation requirements specified by ICH guidelines. The low values of %RSD, high percentage recovery, and acceptable sensitivity parameters confirmed the reliability of the method. The assay results further demonstrated compliance with pharmacopoeial standards. Therefore, the developed UV spectrophotometric method can be effectively employed for routine quality control and quantitative analysis of prednisolone acetate in pharmaceutical formulations.

References

1. International Council for Harmonisation (ICH). Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva; 2005.
2. Indian Pharmacopoeia Commission. *Indian Pharmacopoeia*. Ghaziabad, India; 2022.
3. United States Pharmacopeial Convention. *United States Pharmacopeia and National Formulary (USP–NF)*. Rockville, MD; 2023.
4. European Directorate for the Quality of Medicines. *European Pharmacopoeia*. 11th ed.; 2023.
5. Beckett AH, Stenlake JB. *Practical Pharmaceutical Chemistry*. 4th ed. CBS Publishers; 2004.
6. Chatwal GR, Anand SK. *Instrumental Methods of Chemical Analysis*. Himalaya Publishing House; 2019.

7. Skoog DA, Holler FJ, Crouch SR. *Principles of Instrumental Analysis*. 7th ed. Cengage Learning; 2018.
8. Sharma BK. *Instrumental Methods of Chemical Analysis*. Goel Publishing House; 2014.
9. Willard HH, Merritt LL, Dean JA. *Instrumental Methods of Analysis*. CBS Publishers; 2012.
10. Mendham J, Denney RC, Barnes JD, Thomas MJK. *Vogel's Textbook of Quantitative Chemical Analysis*. 6th ed. Pearson Education; 2016.
11. Ermer J, Miller JHM. *Method Validation in Pharmaceutical Analysis*. Wiley-VCH; 2005.
12. Snyder LR, Kirkland JJ, Dolan JW. *Introduction to Modern Liquid Chromatography*. 3rd ed. Wiley; 2011.
13. Ahuja S, Dong MW. *Handbook of Pharmaceutical Analysis by HPLC*. Elsevier; 2005.
14. Swartz ME, Krull IS. *Analytical Method Development and Validation*. CRC Press; 2018.
15. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability-indicating studies. *J Pharm Anal*. 2014;4(3):159–165.
16. Kazakevich Y, Lobrutto R. *HPLC for Pharmaceutical Scientists*. Wiley; 2007.
17. Gurupadayya BM, Kumar CA, Gupta NV. UV spectrophotometric method development and validation in pharmaceutical analysis. *Int J Pharm Sci Rev Res*. 2011;8(2):1–8.
18. Christian GD. *Analytical Chemistry*. 7th ed. Wiley; 2014.
19. Douglas A, Skoog FJ, Holler SR. *Fundamentals of Analytical Chemistry*. 9th ed. Cengage Learning; 2014.
20. United States Food and Drug Administration. *Analytical Procedures and Methods Validation for Drugs and Biologics*. FDA Guidance; 2015.