Analytical Method of Development and Validation of Hydrocortisone by UV Spectroscopic Method

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Article Info

ISSN (online): 3049-0421
Volume: 03
Issue: 04
Received: 06-05-2026
Accepted: 04-06-2026
Published: 02-07-2026
Page No: 01-05

Abstract
The present investigation aimed at establishing and validating a UV spectrophotometric method for the quantitative determination of hydrocortisone (HC) in its pure form and pharmaceutical dosage formulations, employing multivariate linear regression analysis. Analytical approach exploited the quantitative relationship between drug concentration and measured absorbance values at five selected wavelengths (238, 240, 242, 244, and 246 nm). The method was validated according to the International Conference on Harmonization (ICH) Q2(R1) guidelines for linearity, range, precision, accuracy, and assay. Linearity was demonstrated over a concentration range of 6–14 µg/mL with a correlation coefficient exceeding 0.999. The accuracy, assessed by recovery studies, was found within 98.2–102%, and the precision (% RSD) was below 2% for both interday and intraday studies, with the exception of the HISONE 5 mg formulation. This method provides a viable alternative for routine quality control of hydrocortisone in pharmaceutical formulations.

DOI: https://doi.org/10.54660/IJPGRR.2026.3.4.01-05

Keywords: Analytical, Hydrocortisone, ICH Q2(R1), Multivariate, Regression, UV, Validation

Introduction

Hydrocortisone (HC), also known as Cortisol, is chemically designated as (11β)-11,17,21-trihydroxypregn-4-ene-3,20-dione with the molecular formula $C_{21}H_{30}O_5$. Its chemical structure is shown in Figure 1.

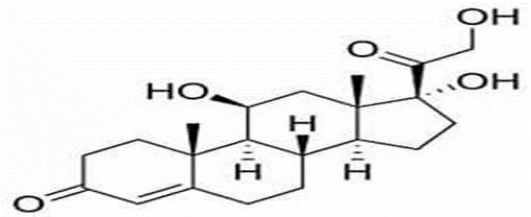

Fig 1: Chemical Structure of Hydrocortisone ($C_{21}H_{30}O_5$).

Hydrocortisone is a short-acting synthetic corticosteroid with glucocorticoid and mineralocorticoid properties. It is widely used in the management of autoimmune disorders, inflammatory conditions, dermatological diseases, and as replacement therapy for adrenocortical insufficiency, particularly in classic congenital adrenal hyperplasia (CAH).

Precise quantification of hydrocortisone is critical for maintaining pharmaceutical quality. The literature shows various analytical methods, including high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and simultaneous UV spectrophotometric determination. While reliable, some methods are resource-intensive. UV spectrophotometry is commonly preferred for its simplicity and affordability. Integrating chemo metric tools like multivariate linear regression (MLR)

can potentially enhance its performance. However, there is a recognized analytical gap in demonstrating a meaningful advantage of MLR over conventional single-wavelength measurement for relatively simple dosage forms like HISONE tablets, particularly regarding specificity and robustness in the presence of common excipients. This study aims to develop and rigorously validate an MLR-based UV method for hydrocortisone, specifically evaluating its potential for routine use according to ICH guidelines.

Material and Method

1. Experimental Chemicals and Solvents Employed

- Analytical grade methanol was used as the solvent.
- The hydrocortisone reference standard (purity stated by source, batch number not provided) was a gift sample from Ideal Testing Laboratory, Pondicherry, and was used without further purification or moisture correction. It was stored according to laboratory protocol.
- HISONE Tablets (Samarth Life Sciences Pvt. Ltd.), labeled to contain 5 mg, 10 mg, and 20 mg of HC, were purchased from the local market. Information regarding the batch number, manufacturing date, and expiry date of the specific samples analyzed is not available.

Instruments

- UV-Vis Double Beam Spectrophotometer (Lab India UV-3092) with a fixed spectral bandwidth and scan speed (details not specified). Measurements were performed using 1 cm quartz cuvettes with auto-baseline correction.
- Electronic Analytical Balance (SHIMADZU AY-220H).

2. Method Development Selection of Solvent

Due to its free solubility in methanol, it was selected as the dissolution medium and diluent. Preparation of Standard Stock Solution: A primary stock solution of 1 mg/mL was prepared by dissolving an accurately weighed 100 mg of HC reference standard in methanol and making the volume up to 100 mL in a volumetric flask. A working standard solution of 100 µg/mL was prepared by diluting 1 mL of the primary stock to 10 mL with methanol. This solution was further diluted with methanol to prepare working concentrations.

Determination of Absorption Maximum (λmax) and Wavelength Selection: A 10 µg/mL solution of HC was scanned in the range of 200–400 nm against methanol. The absorption maximum (λmax) was observed at 242 nm (Figure 2). For the multivariate calibration model, five wavelengths surrounding the λmax (238, 240, 242, 244, and 246 nm) were selected.

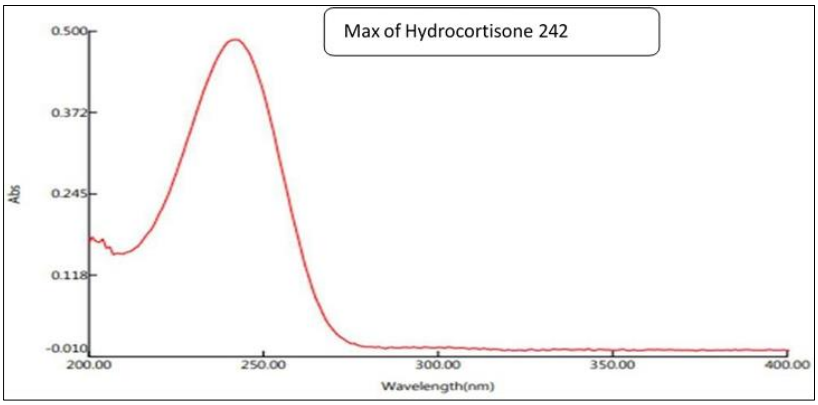

Fig 2: UV spectrum of Hydrocortisone (10 µg/mL).

Preparation of Standard Solutions for Linearity and Validation: Aliquots of the working standard solution (100 µg/mL) were appropriately diluted with methanol in a series of 10 mL volumetric flasks to yield standard solutions at five concentrations of 6, 8, 10, 12, and 14 µg/mL.

Preparation of Sample Solution: Assay preparation was conducted on the HISONE formulations (5 mg, 10 mg, and 20 mg). For each strength, twenty tablets were accurately weighed, and their average weight was calculated. The tablets were ground to a fine powder and thoroughly mixed. An amount of the powder equivalent to 10 mg of hydrocortisone was accurately weighed, transferred to a 10 mL volumetric flask, and dissolved in 5 mL of methanol by sonicating for 15 minutes (specific conditions not detailed). The volume was then made up to the mark with methanol and mixed. The resulting solution was filtered through Whatman filter paper No. 41. The filtrate was appropriately diluted for analysis. The absorbance of these solutions was measured, and the content was calculated using the validation data.

3. Method Validation

The analytical method was validated according to ICH Q2(R1) guidelines, evaluating linearity, precision (repeatability and intermediate precision), accuracy (recovery), and assay. The procedures did not explicitly include testing for specificity (especially against a placebo), robustness (to variations in bandwidth, wavelength accuracy, filtration, or sonication time), LOD, LOQ, or filter compatibility and solution stability.

Linearity

Linearity was assessed by analyzing standard HC solutions at concentrations of 6, 8, 10, 12, and 14 µg/mL (corresponding to the prepared linearity range) at all five selected wavelengths. The responses were used to construct a multivariate calibration model based on linear regression. Details regarding the software used, calibration design, validation set, model coefficients, residuals, or prediction errors of the MLR approach were not part of the standard calculations provided.

Results and Discussion

The UV absorption maximum (λmax) of hydrocortisone in methanol was confirmed at 242 nm, and five wavelengths (238, 240, 242, 244, and 246 nm) were utilized for the calibration model.

Linearity: The absorbance values for hydrocortisone across the concentration range of 6–14 µg/mL at the five wavelengths are presented in Table 1. This experimental range is slightly narrower than the stated linearity range of 5–15 µg/mL.

Table 1: Absorbance values of Hydrocortisone at five selected wavelengths.

Concentration(µg/mL)	Absorbance				
	238nm	240nm	242nm	244nm	246nm
6	0.283	0.292	0.300	0.285	0.278
8	0.359	0.369	0.383	0. 364	0.354
10	0.453	0.464	0.479	0.458	0.447
12	0.529	0.540	0.562	0.537	0.523
14	0.606	0.618	0.645	0.616	0.600

The linearity data, including slope, intercept, and correlation coefficient (r^2 and r), for each wavelength are summarized in Table 2. It is important to note that certain equations in Table 2, particularly for 238 nm (e.g., Y= 0.083x + 0.0682), are mathematically incompatible with their reported slope (e.g., 0.03832), indicating a need for careful recalculation.

Table 2: Linearity data showing statistical parameters at all five wavelengths.

Wavelength (nm)	Slope	Intercept	Regression Equation	r2	r (Corr. Coeff.)
238	0.03832	0.0026	Y= 0.083x + 0.0682	0.9998	0.9999
240	0.03840	0.0032	Y= 0.0384x + 0.0774	0.9997	0.9998
242	0.04256	0.0024	Y =0.0416x + 0.0658	0.9998	0.9999
244	0.03864	0.0031	Y = 0.0386x+0.0692	0.9997	0.9998
246	0.03816	0.0027	Y = 0.0382x+ 0.0638	0.9998	0.9999

Precision: Precision was evaluated for repeatability (intraday) and intermediate precision (interday). The study design regarding the number of separate preparations, replicate measurements per preparation, the number of days, and potential variations in analysts or instruments was not fully detailed. The intraday precision results (Table 3), intermediate precision results (Table 5), and the overall interday results (Table 6) show that the values calculated as % RSD generally remained below the acceptable 2% limit. However, the calculation verification is warranted, as several reported figures in Table 6 appear to represent decimal ratios rather than true percentages.

Table 3: Interday precision at five selected wavelengths.

Concentration(µg/mL)	No. of Repetition	Absorbance				
		238nm	240nm	242nm	244nm	246nm
10	1	0.534	0.544	0.546	0.535	0.516
	2	0.535	0.545	0.546	0.535	0.516
	4	0.560	0.568	0.567	0.553	0.530
	5	0.560	0.570	0.568	0.554	0.532
	6	0.560	0.569	0.568	0.554	0.529

Table 4: Interday Precision of Hydrocortisone showing Mean, SD, and % RSD.

Concentration(µg/mL)	Description	238nm	240nm	242nm	244nm	246nm
10	Mean	0.558	0.567	0.566	0.552	0.528
	SD	0.003125	0.003391	0.002787	0.002229	0.002658
	% RSD	0.005592	0.005976	0.004922	0.004036	0.005028

Recovery: The accuracy of the method was studied using the standard addition technique (Table 7). Spiking was reportedly performed at levels of 50%, 100%, and 150%, achieved by adding 2, 7, and 12 µg/mL to a base concentration of 3 µg/mL, which requires verification to align mathematically with the claimed percentages. The recovery calculations for several of these spiked levels are inconsistent. For instance, at 238 nm with 2 µg/mL added, the "Amount recovered" is listed as 4.19 µg/mL, while the recovery is stated as 98.20%. These and several other entries require mathematical verification, as the reported amount present, amount added, amount recovered, and recovery percentages do not align.

Table 5: Recovery studies of Hydrocortisone at five selected wavelengths.

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	Recovery
238	3	2	4.19	98.20
		7	9.92	99.20
		12	14.91	99.40
240	3	2	5.02	100.4
		7	9.94	99.4
		12	14.96	99.70
242	3	2	4.95	99.00
		7	9.93	99.30
		12	14.95	99.60
244	3	2	4.99	99.80
		7	10.2	102
		12	15.03	100.2
246	3	2	4.96	99.20
		7	9.89	98.90
		12	15.12	100.8

Assay: The validated method was applied to the analysis of hydrocortisone in marketed HISONE tablet formulations (Table 8). While the mean assay values were reported around 99% for the 10 mg and 20 mg formulations, mathematical inconsistencies exist within the table, where certain estimated amounts do not mathematically align with their reported percentage assays, nor do the overall averages. Furthermore, the assay for the HISONE 5 mg formulation yielded a % RSD of 3.58%, which exceeds the standard 2% acceptance limit for pharmaceutical assays, indicating high variability at this dosage level.

Table 6: Assay of Hydrocortisone in marketed pharmaceutical formulations.

Label claim (mg)	Amount estimation (mg)	% Assay	Average (n=3)	SD	% RSD
HISONE– 20	19.21	99.10	99.43	0.31	0.31
(20 mg)	19.44	99.72			
	19.98	99.49			
HISONE– 10 (10 mg)	9.87	98.70	99.63	0.80	0.81
	9.94	99.40			
	9.78	97.80			
HISONE– 5 (5 mg)	4.98	99.60	98.20	3.51	3.58

Conclusion

A UV spectrophotometric method for the quantitative determination of hydrocortisone in bulk drug and pharmaceutical tablet formulations was established using a multivariate linear regression approach involving five wavelengths (238, 240, 242, 244, and 246 nm). The experimental data demonstrated linearity across the concentration range of 6–14 µg/mL with a correlation coefficient greater than 0.999. Precision studies (intraday and interday) yielded % RSD values within acceptable limits for the 10 mg and 20 mg formulations, although the assay of the HISONE 5 mg formulation showed a % RSD of 3.58%, exceeding the acceptance criteria. Recovery studies generally indicated good accuracy, despite inconsistencies found in some calculations. Key limitations of this study included the interchangeable use of chemical names (hydrocortisone vs. hydrocortisone acetate), non-standard presentation of some precision and recovery data, mathematical inconsistencies in linear regression equations, and incomplete reporting of specific validation criteria such as specificity against placebo and robustness. Furthermore, a comparative validation against conventional single-wavelength measurement at 242 nm would be required to experimentally establish a definitive advantage for the multivariate approach for this particular drug formulation. The method, however, demonstrates potential for use in routine analysis with necessary procedural corrections.

Author Contributions

A.Sai Susmitha (Method Development, Investigation, Writing - Original Draft) and M. Sumithra (Conceptualization, Methodology, Supervision, Project Administration, Writing - Review & Editing). All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Funding Source

The authors did not receive any specific funding for this work.

Data Availability

The data presented in this study are available upon request from the corresponding author.

Acknowledgements

The authors wish to thank Ideal Testing Laboratory, Pondicherry, for providing the gift sample of hydrocortisone and the supporting institution for laboratory facilities.

Disclosure regarding the use of AI: The authors did not use any AI tool during the research process or manuscript preparation.

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How to Cite This Article

Mane SR, Ingale MB, Dilip VK. Analytical method of development and validation of hydrocortisone by UV spectroscopic method. Int J Pharma Growth Res Rev. 2026;3(4):1-5. doi:10.54660/IJPGRR.2026.3.4.01-05.

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