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UV SPECTROPHOTOMETRY AIDED MULTIVARIATE CALIBRATION TECHNIQUE FOR THE QUANTIFICATION OF HYDROCORTISONE AND ITS APPLICABILITY IN ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The purpose of the study is to develop and validate a simple, sensitive, and accurate UV spectrophotometric method for the quantification of Hydrocortisone in bulk drug and its pharmaceutical dosage form employing multivariate linear regression analysis. Multivariate linear regression analysis was based on the relationship between concentration and absorbance, where the absorbance was measured from five distinct wavelengths, and the results were obtained statistically. The developed approach was linear throughout a concentration range of 5-15 µg/mL, with a correlation coefficient value greater than 0.998. The Absorption maximum (λmax) of Hydrocortisone was observed at 242 nm. The developed method was found to be simple, rapid, accurate, and precise in accordance with the ICH guidelines Q2(R1). This approach using statistics gives reliable results without any deviations coming out of the instrument or by experimental conditions.

Keywords: Hydrocortisone, Multivariate Linear Regression Analysis, Validation, UV Spectrophotometry

INTRODUCTION

Hydrocortisone (HC) is chemically 11, 17-Dihydroxy-3, 20-dioxopregn-4-en-21-yl acetate with a molecular formula of $C_{21}H_{30}O_5$ **(Figure 1)**. HC is a short-acting, mild, and synthetic corticosteroid that has both glucocorticoid and mineralocorticoid

action, however to a lower level. Several studies have reported that HC is used to treat a variety of skin conditions and as a replacement drug for adrenocortical insufficiency in tandem with a stronger mineralocorticoid **[1]**, effective in treating cutaneous conditions (such as inflammatory dermatitis) and suppresses major inflammatory and immunological reactions **[2]** and also often used to treat classic Congenital adrenal hyperplasia (CAH) **[3]**. On literature survey, several analytical methods have been identified to be reported for the quantification of HC employing techniques such as HPLC **[4-8]**, TLC **[9]**, FTIR Multivariate calibration of chloramphenicol and HC **[10]**, and Simultaneous estimation of HC with other drugs using UV spectrophotometer **[11 & 12]** and HPLC **[13 -19]**.

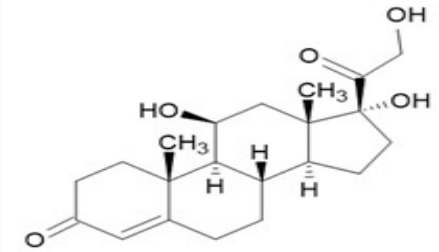


Figure 1: Chemical Structure of Hydrocortisone

Spectrophotometric methods are used as the preferred approach in most labs because of their accuracy, precision, repeatability, and cheap cost. The favored method is based on a high degree of accuracy and precision on the direct estimate of HC. The technique is simple and cost-effective, and it can be used to analyze HC. The suggested approach describes how to evaluate HC in pharmaceutical formulations using a UV spectral multilinear regression methodology with basic mathematical elements.

Multilinear regression is the conversion of a sole common class determination from single dependent variable to "m" dependent variables that may be incorporated into the calibration model at any time **[20]**. This statistical approach provides substantial resolving ability and sensitivity at a cheap cost for routine quality control analysis under optimum experimental circumstances **[21]**. Validation of the established method's applicability according to the International Conference on Harmonization (ICH) **[22]**. To ensure the validity of the developed method, it was recommended that the method to be validated using ICH Q2(R1) procedure for analytical method validation.

Amongst some of the recently reported complex analytical techniques, the current study was conceived with the goal of developing a simple, accurate, precise, and sensitive, rapid analytical approach for the measurement of HC. A simple analytical approach based on UV spectrophotometry facilitated multivariate calibration procedure was suggested to be created based on the preceding assertion **[23 & 24]**.

EXPERIMENTAL:

Chemicals and solvents employed:

- Methanol
- The reference standard of HC (purity – 99.8 %w/w) was obtained as a gift sample from Ideal testing laboratory, Pondicherry.
- HISONE TABLETS – (Label claim – 5 mg, 10 mg and 20 mg of HC), manufactured by Samarth Life Sciences Pvt. Ltd., The marketed tablet formulations were procured from the local market.

Solubility:

- Impractically soluble in water.
- Sparingly soluble in ethanol.
- Freely soluble in methanol.

Instrumentation:

- UV-Vis double beam Spectrophotometer (Lab India UV-3092).
- Electronic balance (SHIMADZU AY-220H).

METHOD DEVELOPMENT:

Selection of solvent:

HC was reported to be freely soluble in the methanol, which was employed as the solvent to solubilize the drug throughout the analysis.

Preparation of standard stock solution:

10 mg of HC was precisely weighed and transferred to a 10 mL volumetric flask. To dissolve the contents of the volumetric flask, 5 mL of the solvent was added. The final volume was made up to 10 mL and well mixed (1 mg/mL) with the solvent. 1 mL of the aforementioned solution was pipetted into a 10 mL volumetric flask, and the total volume was built up to the mark using the solvent and well mixed. Using the solvent, the resulting solution was further diluted to obtain concentrations ranging from 5 to 15 µg/mL.

Determination of λ_{max}:

The solvent was employed to dilute the standard stock solution of HC to a concentration of 10 µg/mL. The UV range of 400-200 nm was used to scan this solution. The UV spectra of HC is shown in **Figure 2** and the absorbance maxima of HC was found to be 242 nm. Five wavelengths were selected in and around the absorbance maxima of HC, such as 238, 240, 242, 244 and 246 nm for the study.

Preparation of standard solution for linearity:

The standard stock solution of HC was further diluted with the solvent to prepare concentrations of 5, 7.5, 10, 12.5, and 15 µg/ mL for linearity studies.

Preparation of sample solution:

20 tablets of HC (HISONE TABLETS – Label claim – 5 mg, 10 mg and 20 mg of HC) were weighed and their average weights were determined. The tablets were crushed and made into a fine powder and the contents were mixed well. A weight

equivalent to 10 mg of HC was weighed from the mixed content and dissolved in 5 mL of solvent with the aid of sonication for 15 min and the volume was finally made up to 10 mL with the solvent. The above solution was mixed well and filtered. The filtrate was suitably diluted for further analysis.

METHOD VALIDATION:

The developed approach was validated in accordance with the ICH Q2(R1) protocol, which examined validation parameters such as linearity, precision, and accuracy.

Linearity:

To establish linear correlation and eliminate instrumental fluctuations, the absorbance of the prepared linearity concentrations was measured at five different wavelengths around the drug's maximum (242 nm), namely 238, 240, 242, 244, and 246 nm **(Table 1)**, and the overlay UV spectra demonstrating linearity is shown in Figure 3. At each of the five wavelengths, the correlation coefficient values for the developed linear regression equations were calculated individually **(Table 2)**. **Figure 4 (a & b)** represents the multivariate calibration linearity constructed at five distinct wavelengths and the sum of all absorbance.

Precision:

Intraday and interday precision studies were carried out by quantifying the absorbance of the linearity solution at 100 % concentration (10 µg/mL) at all five wavelengths. The specified concentration was scanned six times a day (intraday precision) and on three distinct days (interday precision). **Tables 3 & 5** provides the absorbance values obtained at the specified wavelengths for intraday and interday precision experiments, whereas **Figures 5 & 6** show the overlay UV spectra for intraday and interday precision studies. The percentage relative standard deviation (% RSD) and standard deviation (SD) values were determined and represented in **Table 4 & 6** for intra and inter day precision respectively.

Accuracy:

Recovery studies were carried out using the standard addition method to assess the accuracy of the developed technique at concentration levels of 50 %, 100 %, and 150 %. From the prepared stock solutions of standard and sample, 0.1 mL of sample solution was pipetted into three separate 10 mL volumetric flasks, and 0.1, 0.5, and 0.7 mL of standard stock solution were pipetted into the aforementioned volumetric flasks, respectively. Methanol was used to get the final volume up to the required level. The recovery percentages were determined. **Table 7** summarizes the findings of the recovery studies, and **Figure 7** depicts the overlay UV spectra showing accuracy.

Assay:

The absorbance of the extracted sample solutions were recorded at 242 nm. The amount of drug present in the formulations was calculated and the assay results are tabulated in **Table 8**.

RESULTS AND DISCUSSION:

The absorption maxima of HC were observed at 242 nm using methanol as solvent.

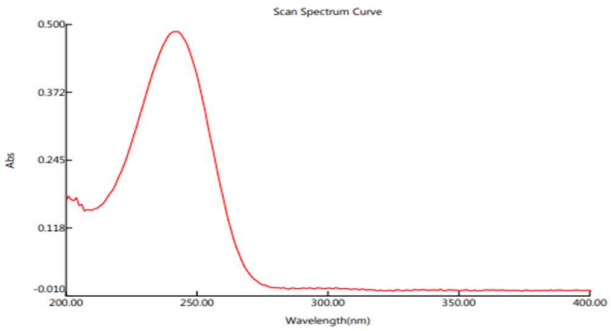


Figure 2: UV spectra of Hydrocortisone

Linearity:

Within the defined concentration range of 5 – 15 µg/mL, the devised technique was found to be linear. All five wavelengths of 238, 240, 242, 244, and 246 nm were used to construct a linear regression equation. The resulting correlation coefficient values were determined to be more than 0.998.

Precision:

Intraday and interday precision studies were carried out. The % RSD values for intraday and interday precision were determined to be in the ranges of 0.516 – 0.550 and 0.524 - 0.570, respectively, which were considered within the ICH guidelines' acceptability requirement of 2%. The low estimated % RSD value illustrates the precision of the established approach. **Tables 5 & 6** provide the mean, Standard deviation, and % RSD Relative standard deviation at five selected wavelengths.

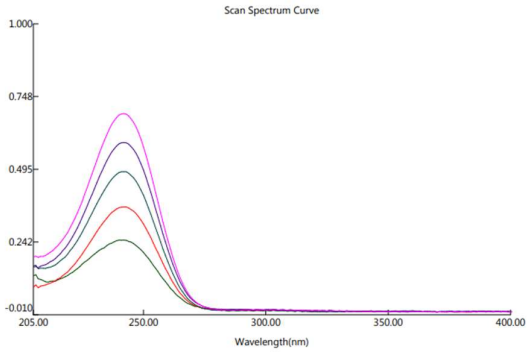
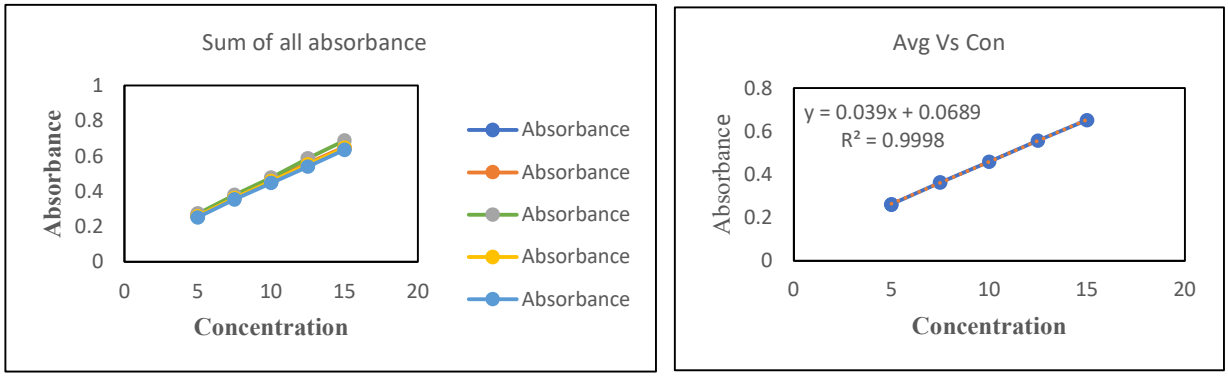


Figure 3: Overlay spectra of HC showing Linearity



(a) **(b)**

Figure 4: Multivariate Calibration linearity graph (a) and Sum of all Absorbance (b) of HC

Table 1: Absorbance values at five selected wavelengths

Concentration (µg/mL)	Absorbance				
	238 nm	240 nm	242 nm	244 nm	246 nm
5	0.257	0.266	0.273	0.259	0.252
7.5	0.358	0.368	0.379	0.362	0.353
10	0.453	0.464	0.479	0.458	0.447
12.5	0.548	0.558	0.588	0.552	0.539
15	0.641	0.651	0.688	0.647	0.636

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

Wavelength	Slope	Intercept	Regression equation	r^2
238	0.03832	0.0026331	y= 0.0383x + 0.0682	0.9998
240	0.0384	0.0032249	y= 0.0384x + 0.0774	0.9997
242	0.04156	0.0023875	y= 0.0416 + 0.0658	0.9998
244	0.03864	0.0031411	y= 0.0386x + 0.0692	0.9997
246	0.03816	0.0026833	y= 0.0382x + 0.0638	0.9998

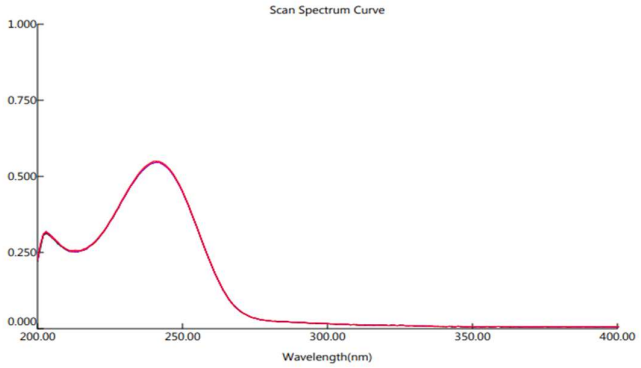


Figure 5: Overlay UV Spectra of HC showing intraday precision studies

Table 3: Intraday precision at five selected wavelengths

Concentration (µg/ mL)	No. of Repetitions	Absorbance (nm)				
		238	240	242	244	246
10	1	0.534	0.544	0.546	0.535	0.516
	2	0.535	0.545	0.546	0.535	0.516
	3	0.534	0.544	0.545	0.536	0.516
	4	0.535	0.545	0.546	0.535	0.516
	5	0.535	0.546	0.546	0.535	0.516
	6	0.538	0.550	0.550	0.539	0.520

Table 4: Intraday Precision of HC showing Mean, SD, and % RSD

Concentration (µg/mL)	Description	238 nm	240 nm	242 nm	244 nm	246 nm
10	Mean	0.535	0.545	0.546	0.535	0.516
	SD	0.001472	0.002251	0.001761	0.001602	0.001633
	% RSD	0.00275	0.004125	0.003222	0.00299	0.003161

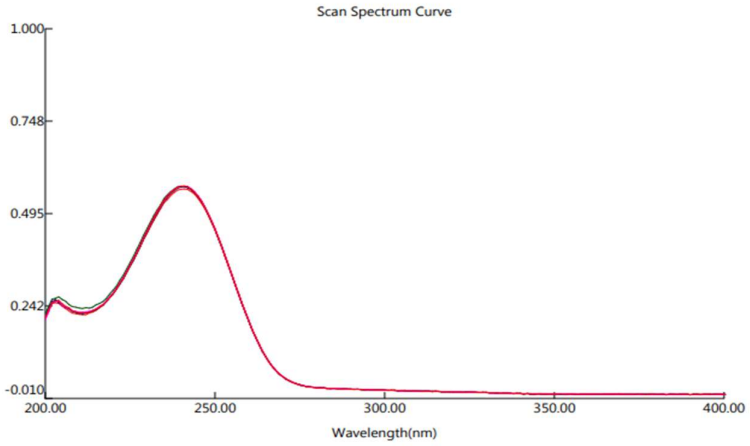


Figure 6: Overlay UV Spectra of HC showing interday precision studies

Table 5: Interday precision at five selected wavelengths

Concentration (µg/ mL)	No. of Repetitions	Absorbance (nm)				
		238	240	242	244	246
10	1	0.562	0.570	0.568	0.552	0.529
	2	0.553	0.561	0.561	0.548	0.524
	3	0.558	0.567	0.565	0.552	0.528
	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

Concentration (µg/mL)	Description	238 nm	240 nm	242 nm	244 nm	246 nm
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

Table 6: Interday Precision of HC showing Mean, SD, and % RSD

Recovery:

The percentage recovery of the drug was calculated and determined to be between 98.2-102 % w/w, which was found to be within the ICH protocol's limit of 97 – 103 percent w/w. As a result, the approach can be considered accurate.

Assay:

The absorbance of the sample solution was recorded at 242 nm and the quantity of HC present in the tablet formulations was calculated. The assay percentage of the drug was found to be 99.58 % w/w and the calculated % RSD was found to be less than 2%.

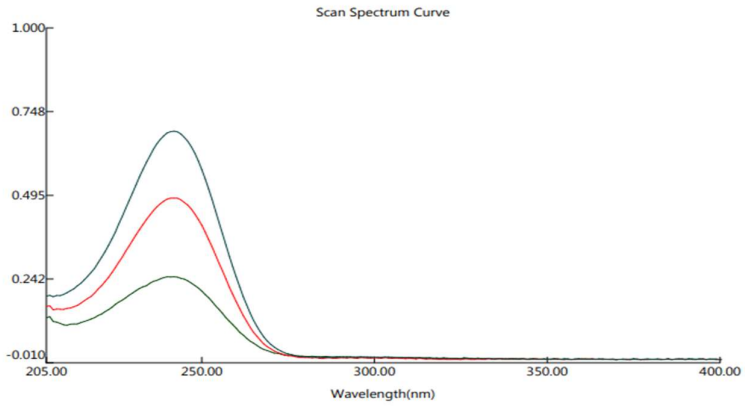


Figure 7: Overlay UV Spectra of HC showing Accuracy

Table 7: Recovery studies of HC at five selected wavelengths

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery
238	3	2	4.91	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

Table 8: Assay of HC in marketed pharmaceutical formulations

Label claim (mg)	Amount estimated (mg)	%Assay	Average (n = 3)	SD	% RSD
HISONE – 20 (20 mg)	19.21	99.10	99.43	0.31	0.31
	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
	5.04	100.80			
	4.71	94.20			

CONCLUSION:

The developed simple and rapid UV spectrophotometric-assisted Multivariate calibration approach was found to be linear, sensitive, accurate, and precise for assessing HC in pharmaceutical formulation. The multivariate calibration technique has been stated to be superior to the other reported procedures since the drug's absorbance is measured at five distinct selected wavelengths, making the method more reliable. As an outcome, a

simple and rapid method based on mathematical components was developed, which is more predictable than earlier spectrophotometric techniques and is highly advised for routine quality control testing of HC in pharmaceutical formulations.

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CONFLICT OF INTEREST:

The authors report no conflicts of interest in the study**.**

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