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SPECTROPHOTOMETRIC QUANTIFICATION OF BECLOMETHASONE DIPROPIONATE EMPLOYING MULTILINEAR REGRESSION CALIBRATION TECHNIQUE IN CREAM FORMULATIONS

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ABSTRACT

The study aims to utilize and evaluate a modest, precise, and accurate UV spectrophotometric technique for quantifying Beclomethasone Dipropionate in pharmaceutical dosage form utilizing multivariate calibration technique. The multivariate calibration methodology depends on linear regression equations and the relationships between concentration and absorbance at five distinct wavelengths. The results were treated statistically and confirmed as per the ICH guidelines. The developed method was accurate, precise, reproducible, linear within the range of 6 – 18 µg/mL. This statistical method yields the best results by minimizing variations caused by instrumental or experimental settings. Under optimum conditions, the applied statistical method provides great resolving power, sensitivity, velocity and is inexpensive for quantification, quality management, and recurrent analysis of Beclomethasone Dipropionate in pharmaceutical dosage forms.

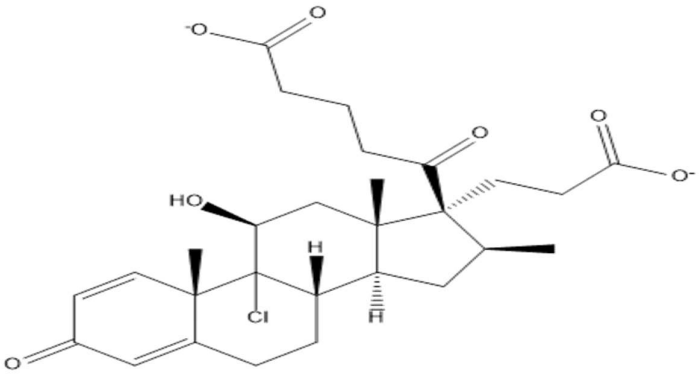
Keywords: Beclomethasone Dipropionate, Asthma, UV Spectrophotometer, Multivariate calibration

INTRODUCTION:

Current therapeutic recommendations suggest inhaled corticosteroids (ICS) as the preferred controller medicine for all patients with persistent asthma to take daily **[1]**. Beclomethasone 17, 21-dipropionate (BDP) is a synthetic chlorinated corticosteroid. It was first administered as a pressurized metered-dose inhalation (MDI),

then a nebulizer and an aqueous spray **[2]**. Beclomethasone is chemically 9α – Fluro-11β, 17α, 21- trihydroxy -16-methyl-3,20-dioxopregna- 1,4- diene- 17,21- diyl dipropionate, BDMP. The molecular formula is $C_{28}H_{37}FO_7$ **[3]**. In treating asthma, allergic or non-allergic rhinitis, it is often used as an anti-inflammatory and vasoconstrictive drug. These are also well-known medications in dermatology that are widely used to treat inflammatory illnesses such as psoriasis and eczema **[4]**.

According to the literature review, the documented techniques for determining BDP include UV **[5]**, UV Spectrophotometric **[6]**, HPLC **[7]**, RP-HPLC **[8]**, HPLC-UV, ESI-MS, NMR **[4]** UPLC-MS **[9]**, HPTLC **[10]**, LC-MS **[11-13]**, GC-MS **[14]** in pharmaceutical preparations or biologically active compounds



9-Chloro-11b-hydroxy-16b-methylpregna-1,4-diene-3,20-dione 17,21-Dipropionate
Caution: A net charge appears to be present

Figure 1: Chemical Structure of Beclomethasone Dipropionate

The presented approach is established on a high-level grade of accuracy and precision with indirect determination of BDP. It is a simple and inexpensive method, applicable to bulk medicines and formulations. The function of a UV spectral multivariate calibration technique with minimal statistical content for the estimation of BDP in the therapeutic dosage form is described in this paper.

Under optimal conditions, the statistical approach offers significant resolving power, sensitivity, rapidity, and low price for statistical analysis, quality management, and routine analysis of chemical compounds.

If the absorbance of an analyte (X) is measured at five wavelengths set (λ = 234, 236, 238, 240, 242 nm), the subsequent calculation can be written for each selected wavelength.

$A_{\lambda234}$= a xC_x + k1 ……..... [1]

$A_{\lambda236}$ = b xC_x + k2 …….. [2]

$A_{\lambda238}$= c x C_x + k3 …….. [3]

$A_{\lambda240}$= d xC_x + k4 …….. [4]

$A_{\lambda242}$= e xC_x + k5 …….. [5]

where A is the absorbance of the analyte; a, b, c, d, e are the slopes of the analyte's linear regression functions; k1, k2, k3, k4, k5 are the intercepts of the linear regression functions at the five specified wavelengths; and Cx is the analyte's concentration. The five equation systems (1–5) listed above can be summarised as follows:

A_T = a x C_x+ b x C_x+ c x C_x+ d x C_x+ e x C_x+ K_T…….. [6]

this may be simplified further to

$A_T = C_x (a+b+c+d+e) + K_T$ …….. [7]

A_T and K_T denotes the total of the determined absorbance and the total of the intercepts of linear regression at five wavelengths, respectively. The equation can be used to calculate the concentration of analyte X in a solution.

$C_x = A_T - K_T (a + b+c + d+e)$ …….. [8]

EXPERIMENTAL:

Chemicals and solvent:

- Methanol
- Analytical grade BDP was obtained as a gift sample from Ideal test laboratories, Puducherry. The retail cream formulation used was BETAFOAM cream, containing 0.1 %w/w of BDP, Cipla Pharma, India, obtained from the market place.

Solubility:

- Very easily soluble in methanol
- Sparingly soluble in Acetone.

Instrumentation:

- Perkin-Elmer UV- Spectrophotometer
- Sonicator
- Electronic balance

METHOD DEVELOPMENT:

Solvent Selection:

BDP was extensively soluble in methanol and sparingly soluble in acetone. As a result, methanol was utilized as a solvent to dissolve the standard medication and the sample.

Developing a standard stock solution:

The BDP reference standard stock solution was obtained by dissolving 10 mg of the drug in 10 mL of solvent to achieve 1 mg/mL concentration. The solution was further diluted to achieve concentrations ranging from 6 to 18 µg/mL for linearity analysis.

Determination of λmax:

The BDP stock solution was diluted with the selected solvent to yield a concentration of 10 µg/mL. This solution was scanned in the UV range between 200 and 400 nm. The maximum wavelength was observed to be at 238 nm **(Figure 2)**.

The absorbance of the linearity solutions were measured at the five selected wavelengths around the absorbance maxima (238 nm) of the drug, i.e., 234,

236, 238, 240, 242 nm, to enhance the correlation and decrease the instrumental variations.

Sample solution preparation:

An amount equivalent to 100 mg of BDP from commercially available BETAFOAM cream, containing 0.1 %w/w of BDP (CIPLA PHARMA) was weighed accurately and transferred into a 100 mL volumetric flask, half the volume of methanol was added and sonicated for 20 min and the volume was made upto the mark with methanol. The contents were mixed well and filtered. The filtrate was diluted suitably and used for further analysis.

METHOD VALIDATION [15]:

The methodology was evaluated for linearity, sensitivity, precision, and accuracy using ICH Q2(R1) parameters.

Linearity:

BDP stock solution was diluted with methnol to obtain concentrations of 6, 9, 12, 15 and 18 µg/mL. To enhance the correlation and decrease instrumental variations, the absorbance of the solution was determined throughout a range encompassing 238 nm, viz 234, 236, 238, 240, and 242 nm. **Figure 3 and Table 1** represents the overlay spectra demonstrating linearity at the max wavelength and the absorbance of various concentrations measured at five different wavelengths respectively.

Precision:

The 100 % target concentration (12 µg/mL) solution was scanned at all five selected wavelengths to determine intraday and interday precision. For intraday and inter-day precision, the selected target concentration was scanned six times each day (intra day) and on three different days (inter day). The Mean, Standard deviation (SD) and % Relative standard deviation (% RSD) values were computed and the results were represented in **Table 3, 4, 5 & 6** and the overlay spectra's showing precision in **Figure 5 & 6**.

Accuracy:

The proposed method's accuracy was determined by standard addition method at 50%, 100%, and 150% levels. 3 µg/mL of sample solution is contained in three different 10 mL volumetric flask and 3, 9 and 15 µg/mL of standard solutions were pipetted into the above volumetric flasks respectively and the final volume was made upto the mark employing Methanol and the percent recovery was computed. The overlay UV spectra representing accuracy is presented in **Figure 7** and the results are tabulated in **Table 7**.

RESULTS AND DISCUSSION:

Using methanol as the solvent, the maximum wavelength of BDP was determined to be 238 nm.

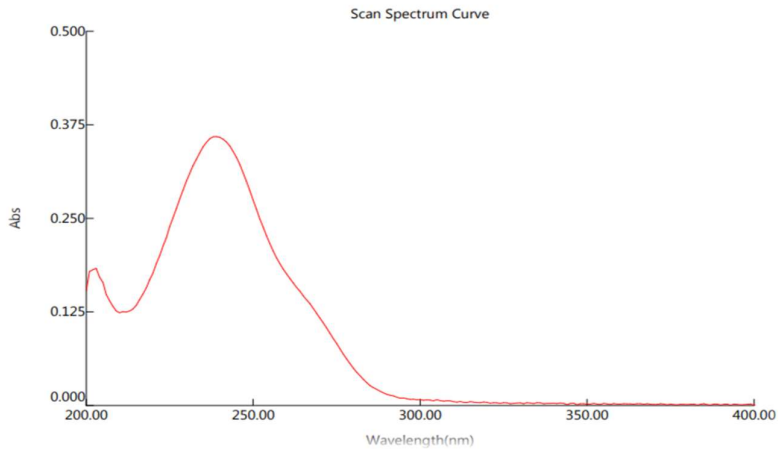


Figure 2: Maximum wavelength of Beclomethasone Dipropionate

Linearity:

All of the calibration curves were linear over the concentration range of 6-18 µg/mL. The calibration plots linear regression analysis results revealed a good linear relationship with r^2 value more than 0.998. The linearity used for various concentrations 6-18 µg/mL was recorded at 234, 236, 238, 240, and 242 nm. The results of statistical parameters of linearity data is depicted in **Table 2**, and their calibration graphs in **Figures 4 (a) and (b)**, respectively.

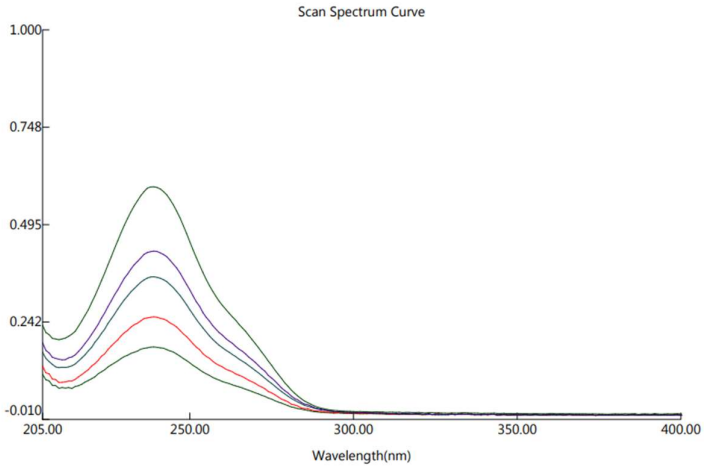
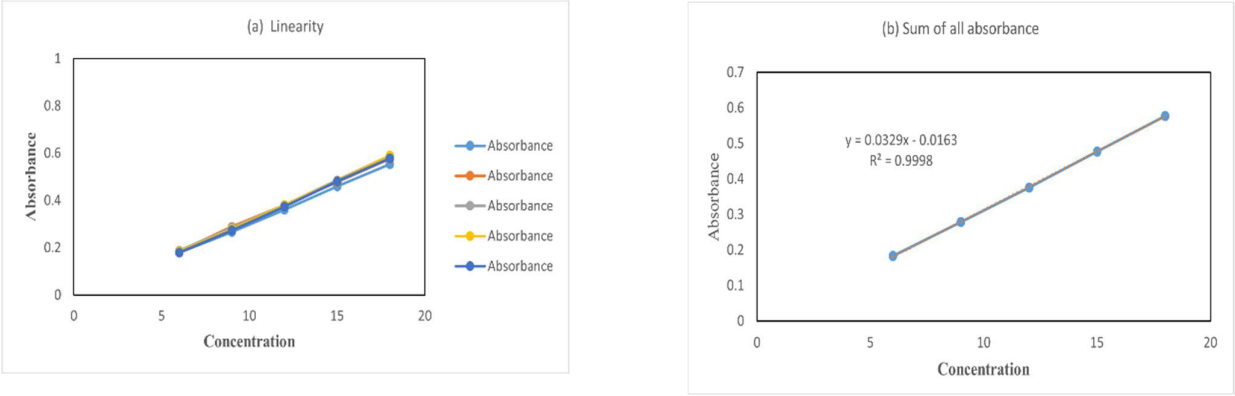


Figure 3: UV spectra showing linearity of Beclomethasone Dipropionate at 238 nm

Table 1: UV Multivariate calibration absorbance obtained at five selected wavelengths

Concentration (µg/mL)	Absorbance				
	234 nm	236 nm	238 nm	240 nm	242 nm
6	0.18	0.184	0.187	0.185	0.18
9	0.267	0.289	0.285	0.279	0.274
12	0.361	0.381	0.381	0.381	0.374
15	0.459	0.478	0.485	0.483	0.481
18	0.53	0.577	0.589	0.589	0.579



(a) **(b)**

Figure 4: Multivariant Calibration linearity graph (a) and Sum of all Absorbance (b) of Beclomethasone Propionate

Table 2: Statistical parameters of linearity data at five selected wavelengths

Wavelengths (nm)	Regression equation	Slope	Intercept	R^2
234	Y =0.0313x - 0.0112	0.0313	-0.0112	0.9996
236	Y = 0.0325x - 0.0082	0.0325	-0.0082	0.9998
238	Y = 0.0335x - 0.0162	0.0335	-0.0162	0.9997
240	Y = 0.0939x - 0.0214	0.0337	-0.0214	0.9996
242	Y = 0.0810x – 0.0244	0.0335	-0.0244	0.9996

Precision:

The low reported %RSD values for precision below 2, shows that the developed method was found to be precise.

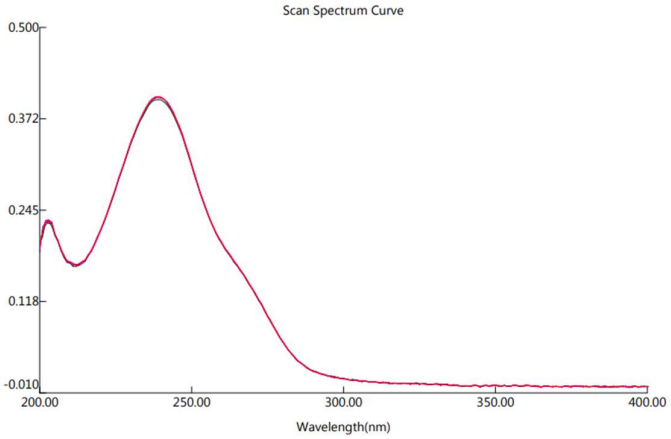


Figure 5: Overlay UV Spectrum showing Intraday precision

Table 3: Absorbance values for Intraday Precision

Con (µg/mL)	No. of Repetitions	Absorbance				
		234nm	236nm	238nm	240nm	242nm
12	1	0.359	0.377	0.374	0.372	0.365
	2	0.360	0.378	0.376	0.375	0.368
	3	0.361	0.381	0.381	0.381	0.374
	4	0.360	0.375	0.377	0.376	0.370
	5	0.358	0.370	0.365	0.374	0.366
	6	0.362	0.379	0.380	0.378	0.372

Table 4: Statistical data for Intraday Precision

Concentration (µg/mL)	Description	234nm	236nm	238nm	240nm	242nm
12	Mean	0.360	0.3766	0.375	0.376	0.369
	Standard Deviation	0.001414	0.00383	0.005753	0.003162	0.003488
	% RSD	0.003928	0.0101	0.015322	0.00841	0.009449

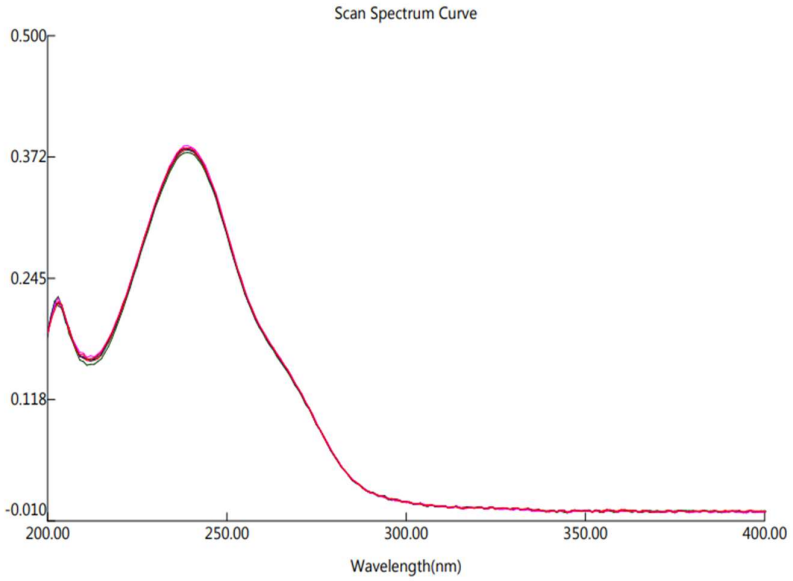


Figure 6: Overlay UV Spectrum showing Interday Precision

Table 5: Absorbance values for Interday Precision

Con (µg/mL)	Day	Absorbance				
		234	236	238	240	242
12	1	0.361	0.381	0.381	0.381	0.374
	2	0.362	0.379	0.380	0.378	0.372
	3	0.359	0.377	0.374	0.372	0.365
	4	0.360	0.378	0.376	0.375	0.368
	5	0.360	0.375	0.377	0.376	0.370
	6	0.358	0.370	0.365	0.374	0.366

Table 6: Statistical data for Interday Precision

Concentration (µg/mL)	Description	234nm	236nm	238nm	240nm	242nm
12	Mean	0.3766	0.375	0.369	0.376	0.360
	Standard Deviation	0.00383	0.005753	0.003488	0.3162	0.001414
	%RSD	0.01017	0.015341	0.009453	0.00841	0.003928

The percentage recovery was found to be within the specified limit. As a result, all parameters were determined to be within the ICH standard acceptance requirements.

Recovery: The percentage recovery of the drug from the synthetic mixture was determined to be in the range of 98.50 - 101.10 % w/w, which was well within the ICH criteria of 97 - 103 % w/w.

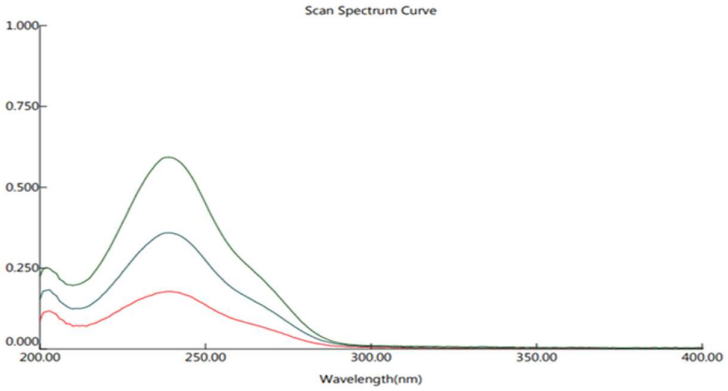


Figure 7: Overlay UV spectra showing accuracy

Table 7: Recovery studies

Wavelengths (nm)	Amount present (µg/mL)	Added amount (µsg/mL)	Recovered amount (µg/mL)	% Recovery
234	3	3	5.91	98.50
		9	11.99	99.91
		15	17.92	99.55
236	3	3	6.01	100.10
		9	11.92	99.33
		15	17.98	99.88
238	3	3	6.07	101.10
		9	11.95	99.58
		15	17.97	99.83
240	3	3	5.97	99.50
		9	12.01	100.08
		15	17.92	99.44
242	3	3	5.95	99.16
		9	12.01	100.08
		15	17.99	99.94

The low SD value at all wavelengths shows that the technique was accurate, and the % RSD for intraday and interday precision was determined, which was well within the acceptance criteria of less than 2% at all wavelengths. The lower percentage RSD value shows that the suggested approach was precise and accurate.

Assay:

The absorbance of the sample solution was recorded at 238 nm and the quantity of BDP present in the cream formulation was calculated. The assay percentage of the drug was found to be 99.46 % w/w and the calculated % RSD was found to be less than 2%.

CONCLUSION:

A new advanced spectrophotometric multivariate calibration method was developed and verified by assessing several validation parameters under ICH standards and was confirmed to be within limits. The technique utilized in this study was proven to be specific, accurate, precise, and reliable for determining BDP in its preparations. In conclusion, this

methodology is more accurate than alternative spectrophotometric approaches with relatively simple mathematical components. The suggested method is strongly recommended for frequent monitoring of BDP in pharmaceutical dosage forms.

CONFLICT OF INTEREST:

Authors declare no conflict of interest

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