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Research Article

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BUDESONIDE IN BULK AND FORMULATION

Sachin Bhusari *, Tejal Chaudhari, Gaurav Shrangare, Pravin Wakte

University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad - 431001, Maharashtra, INDIA.

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ABSTRACT

Aim: *To develop and validate a simple, precise and cost-effective UV- visible spectrophotometric method for the estimation of Budesonide according to the ICH Q2 (R1) guideline.*

Methods: *Spiked Budesonide solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of Budesonide were prepared and absorbance was recorded at wavelength of maximum absorbance. Calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness and ruggedness were calculated using QC standards.*

Results: *The maximum wavelength of Budesonide was found to be 246 nm. The correlation coefficient over the concentration range of 1-25µg/mL was found to be 0.9984. Developed UV method was found to be precise during the intra-day and inter-day study and shows percent relative standard deviation in the range of 0.8708 to 1.9535 & 0.4946 to 1.7468 respectively. The total percent recovery of Budesonide was found to be 98.12to 99.84 %. Developed method was found to be robust and rugged for the intended use. Budesonide content of marketed formulation was successfully calculated using developed UV-Visible method.*

Conclusion: *A simple, precise and cost-effective UV- visible spectrometry method for the estimation of Budesonide was developed. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV- visible method can be efficiently used for the estimation of Budesonide in bulk as well as formulation.*

KEYWORDS: *UV- visible spectrometry, Budesonide, Validation.*

INTRODUCTION

Budesonide is synthetic corticosteroids having chemical name 11-β-21-dihydroxy-16α-17α-(butylidenebis(oxy))-pregna-1,4-diene-3,20-dione, used to control and prevent symptoms caused by asthma [1, 2]. It is also used to treat the diseases like Crohn's disease, ulcerative colitis and chronic obstructive pulmonary disease (COPD) [3, 4]. Budesonide works directly to the inner lining of the inflamed airways to make breathing easier. It also reduces the irritation and swelling of the airways when used as an inhaler [5-7]. Budesonide is a steroid that is quickly metabolized by the liver and excreted through urine or faeces [8]. Till date, there are very few reports demonstrating the UV visible spectrophotometric method for estimation of Budesonide. Earlier reported UV visible spectrophotometric methods were found to contain either higher percentage of organic solvents or the use of costly solvents. Therefore, considering the therapeutic importance of the Budesonide and the need of simple yet precise and robust analytical methodology for the same, it was envisaged that development of UV-Visible spectrophotometric method for the determination of Budesonide in bulk and the formulation by using co-solvent system consisting economic percentage of organic solvent will be worth.

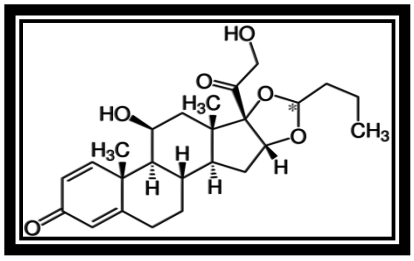

Fig. 1: Chemical structure of Budesonide

MATERIALS AND METHODS

Budesonide (> 98 % by HPLC) was purchased from TCI Chemicals (India) Pvt. Ltd. All other chemicals of analytical grade were used for study.

Instruments Used:

A double beam UV-visible spectrometer (UV-530, Jasco) with spectra manager software was used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Vibra HT, Essae) with internal calibration mode was used for the accurate weighing purpose.

***Corresponding author:**

Dr. Sachin Shivling Bhusari,

Assistant Professor,

Pharmaceutical Technology Division,

Department of Chemical Technology,

Dr. Babasaheb Ambedkar Marathwada University,

Aurangabad - 431001, Maharashtra, India.

** E-Mail: chemtech.cdmpk@gmail.com*

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Preparation of standard stock solution:

Accurately weighed 5 mg of Budesonide was transferred into the calibrated volumetric flask and dissolved in 5 ml mixture of Methanol and water (50:50v/v) to achieve a stock solution of 1000 µg/mL (Stock-I). Stock- I solution was suitably diluted with co-solvent system of Methanol and water to achieve a solution of 100µg/mL (Stock-II).

Determination of wavelength of maximum absorbance (λmax):

Stock-II solution was scanned using full scan mode with medium scanning speed for the entire range of UV and visible i.e. 800 to 200 nm with co-solvent system as a blank. After obtaining the spectrum, λmax was identified with the help of software. In order to achieve reproducible results, above method was repeated five times.

Preparation of calibration curve:

Calibration curve was prepared by diluting the stock-II solution to achieve the six different calibration standards representing 1, 5, 10, 15, 20, 25µg/mL strength. Absorbance of each calibration standard was measured at pre-identified λmax; 246 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted using Excel program of Microsoft Office 2010. Above mentioned procedure was repeated five times so that reproducible results can be obtained.

Method Validation:

Developed UV method for the estimation of Budesonide was validated as per the ICH guidelines. Different parameters like linearity and range, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) were calculated using predefined calibration standards or quality control standards as described below [9, 10].

Linearity and Range:

Linearity of the proposed UV method was established using six different calibration standards. After analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy:

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of analyte of interest. Three different solutions of Budesonide were prepared in pentaplicate at level of 80%, 100% and 120% of its predefined concentrations (2, 12 and 24µg/mL). To the predefined concentrations, different amounts of Budesonide were added (standard addition method) and the accuracy was calculated on the basis of percent recovery. For calculating the percent recovery, following formula was used

$$\%RC = (SPS-S/SP) \times 100$$

Where,
SPS = Amount found in the spiked sample
S = Amount found in the sample
SP = Amount added to the sample
% RC = Percent recovery

Precision:

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of quality control samples (2, 12 and 24µg/mL). Budesonide solutions of 2, 12 and 24µg/mL strength (n=5 for each concentration) were analyzed at morning, afternoon and evening time of three consecutive days. Deviation in the results was calculated in terms of % relative standard deviation (% RSD).

Robustness:

Robustness of the developed UV method was established using different percentage of methanol in co-solvent system. Methanol percentage in co-solvent system was intentionally adjusted to 47 and 53 % and middle level quality control sample (12µg/mL) of Budesonide was prepared using above mentioned co-solvent system separately. Samples (n=5) were analyzed at 246 nm for Budesonide content. The results were calculated in terms of % RSD.

Ruggedness:

Ruggedness study of the method was carried out by analyzing triplicate samples of Budesonide solution (12µg/mL) using two different instruments (V-530, Jasco and BA-UV-2600, Bioage). Results were expressed in terms of % RSD.

Limit of Detection (LOD):

The LOD of the developed UV method was calculated by using following formula

$$LOD = 3.3 \times SD/S$$

Where, SD= Standard deviation of Y-intercepts
S= Slope

Limit of Quantitation (LOQ):

The LOQ of the developed UV method was calculated by using following formula

$$LOQ = 10 \times SD/S$$

Where, SD= Standard deviation of Y-intercepts
S= Slope

Estimation of Budesonide content in marketed formulation:

The Budesonide content in its marketed formulation (Bodecort rotacaps) was estimated using pre-validated UV-Visible spectrophotometric method. Capsule formulation contents (labeled strength: 100µg/capsule) were dissolved in 1 ml of co-solvent system to achieve a stock solution of 100 µg/mL (n=5). Said solution was suitably diluted with co-solvent system and analyzed for the Budesonide content using proposed UV method.

RESULTS AND DISCUSSION

Determination of wavelength of maximum absorbance:

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for Budesonide solution (20µg/mL) was carried out using full scan mode of UV-Visible spectrophotometer (figure 2). Full scan was processed using Jasco UV software and the λ_{max} was identified with the help of software. The λ_{max} was found to be 246 nm for Budesonide.

Preparation of calibration curve:

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and a mathematical equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Budesonide, calibration curve for Budesonide was developed using six different calibration standards. The absorbance of different calibration standards at 246 nm was recorded using fixed wavelength mode. Calibration curve was repeated five times and the mean values ± standard deviation was reported as shown in Table 1.

Method validation:

Linearity and Range:

Linearity and range are the key parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, six point calibration curve of Budesonide covering a range of 1-25 µg/mL was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.0376x+0.0031 with correlation coefficient 0.9984 as shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear over the concentration range of 1 to 25µg/mL.

Accuracy:

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Budesonide, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of Budesonide was found to be 99.84% whereas at 100 and 120 % standard addition, it was found to be 98.12 and 99.06% respectively. % RSD was found to be less than 2 for the Budesonide recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 100% and the % RSD was well below 2%.

Precision:

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 2, 12 and 24 µg/mL levels of Budesonide. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 0.87 and 1.95 whereas those of inter-day precision study were in between 0.49 and 1.74. Overall, % RSD values of less than 2 demonstrated the precision of developed UV method.

Robustness:

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition, buffer strength, pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the analytical method. Therefore, robust analytical method is preferred. Robustness of proposed UV method was established by modifying the composition of co-solvent system. Change in methanol percentage (47 to 53 %) in co-solvent system did not affect the method performance. % RSD values were found to be in between 0.15 and 0.68 as shown in Table 5. % RSD values below 2 depicted that proposed UV method is robust in nature.

Ruggedness:

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from impact of environmental/external factors. In order to establish the ruggedness of proposed UV method, Budesonide solution was analyzed using two different UV-Visible spectrophotometers of two different labs. Sample analysis and data processing resulted into % RSD values between 0.46 and 0.84. Results revealed that proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Limit of Quantitation (LOQ) and Limit of Detection (LOD):

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.66and 2.019µg/mL respectively as shown in Table 7. Lower LOQ value indicated that proposed method would be sensitive enough to quantify the Budesonide content of samples at its lower level.

Estimation of Budesonide content in marketed formulation:

The developed UV method was successfully applied for estimation of Budesonide content in Budecort rotacaps. By proposed UV method, Budesonide content in the capsule was found to be 101.55 ±0.0071 %.

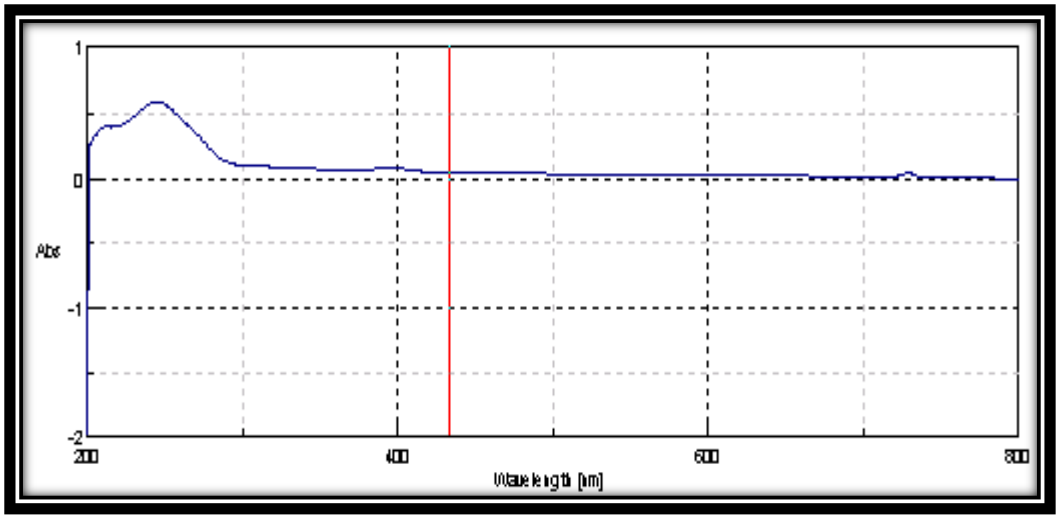

Fig. 2: UV-visible spectra of Budesonide

Table No. 1: Calibration standard data for Budesonide

S. NO.	Concentration (µg/mL)	Absorbance
1	1	0.0490±0.0027
2	5	0.1985±0.0041
3	10	0.3690±0.0052
4	15	0.5436±0.0059
5	20	0.7599±0.0066
6	25	0.9540±0.0065

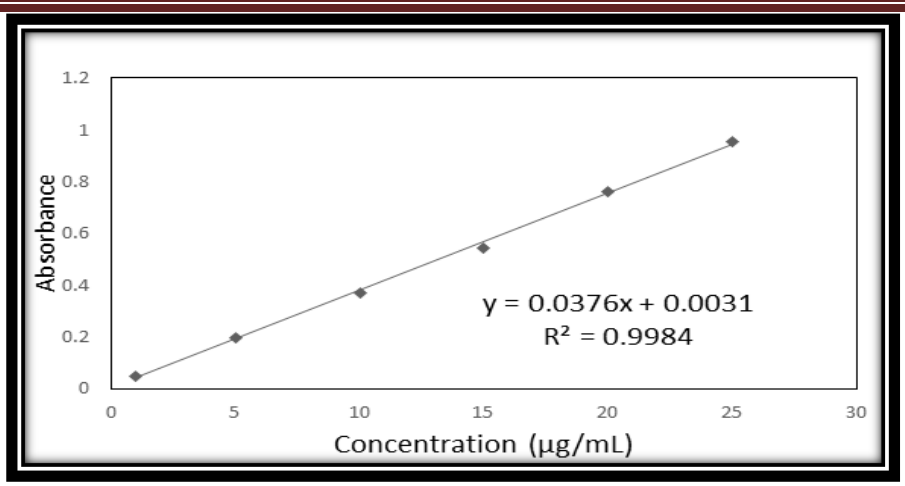

Fig. 3: Calibration curve for Budesonide

Table No. 2: Accuracy data of UV method for Budesonide

S No.	Concentration (%)	Origin level (µg/mL)	Amount added (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	80	2	1.6	100.29	99.84	0.8308
2	80	2	1.6	99.27		
3	80	2	1.6	100.10		
4	100	12	12	101.34	98.12	1.90
5	100	12	12	99.28		
6	100	12	12	101.84		
7	120	24	28.8	98.56	99.06	1.85
8	120	24	28.8	100.17		
9	120	24	28.8	100.84		

Table No. 3: Intra-day precision data of UV method for Budesonide

S No.	Conc. (µg/mL)	Morning			Afternoon			Evening		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	2	0.0758	101.86	1.7505	0.0732	99.37	1.3240	0.0746	100.78	1.7947
2	12	0.4447	100.43	1.5743	0.4419	99.81	1.8941	0.4436	100.18	1.9535
3	24	0.8773	99.06	0.8708	0.8760	98.92	1.4896	0.8885	100.33	1.1571

Table No. 4: Inter-day precision data of UV method for Budesonide

S No.	Conc. (µg/mL)	Day 1			Day 2			Day 3		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	2	0.0743	100.67	0.5686	0.0734	99.47	0.4946	0.0742	100.65	1.2624
2	12	0.4436	101.41	0.0652	0.4384	99.61	1.5639	0.4424	99.44	1.7468
3	24	0.8806	99.44	0.5343	0.8698	98.22	0.6415	0.8641	97.67	1.062

Table No. 5: Robustness data of UV method for Budesonide

S. NO	Concentration (µg/mL)	% Methanol	Absorbance	% RSD
1	12	47	0.4324	0.1516
2	12	53	0.4363	0.4169

Table No. 6: Ruggedness data of UV method for Budesonide

S. No.	Concentration (µg/mL)	Instrument	Absorbance	% RSD
1	12	Jasco	0.4341	0.4624
2	12	Bio-age	0.4336	0.8473

Table No. 7: LOD & LOQ data for UV method for Budesonide

1	**LOD**	**0.666 µg/mL**
2	LOQ	2.06 µg/mL

CONCLUSION

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of Budesonide was developed and validated. The Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of Budesonide.

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