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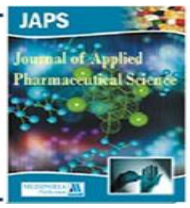

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Development and validation of selective UV spectrophotometric analytical method for budesonide pure sample

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ABSTRACT

Budesonide is a very potent corticosteroid, used for bronchial asthma and inflammatory bowel disease. Objective of the present investigation is to develop the simple and selective UV spectrophotometric method for quantification of budesonide in bulk sample. Absorption maximum of budesonide was found to be 246.0 nm and obeyed the beers law in the concentration range of 1.4 to 25 µ g/ml. Calibration curve shows a linear relationship between the absorbance and concentration in the range of 2 to 10 µ g/ml and the limit of detection is 0.01 µ g/ml. The limit of quantification was found to be 1.4 µg/ml. The method was validated for repeatability, accuracy and precision. The percent amount of recovery was 99 - 100% with minimum standard deviation less than 1%. Obtained results showed there is minimum intra day and inter day variation. The excipients present in the preparation did not interfered during the analysis. Developed analytical UV spectrophotometric method is simple, rapid and reproducible and further it can be used for estimation of drug in bulk and colon matrix tablet dosage form.

***Key words*:** Budesonide, UV method development, Validation studies

INTRODUCTION

Crohn's disease is a chronic inflammatory bowel disease of unknown origin primarily affecting the terminal ileum and proximal colon. Complications associated with Crohn's disease include deficiency of nutrients, arthritis, skin problems, and inflammation in the eyes or mouth (Krishnamachari. et al., 2007). Budesonide, a second generation glucocorticoid, exhibits high affinity to the corticosteroid receptors with a high ratio of topical to systemic anti-inflammatory activity. Oral administration of budesonide results in a bio-availability of approximately10 %. Once absorbed distribution of budesonide is extensive and protein binding is approximately 88 %. It has poor systemic availability due to extensive first pass metabolism in the liver. Budesonide has a terminal half-life of about 2–4 h. Excretion occurs primarily through urine (2/3 of a dose) as metabolites. The remainder is excreted in faeces (www.rxlist.com. 2011). It is designated chemically as (RS)-11-beta, 16-alpha,17,21-tetrahydroxypregna-l,4-diene-3,20-dionecyclic 16, 17-acetal with butyraldehyde. Due to the introduction of the alkyl chain at the C22 atom, budesonide is a mixture of two epimers (22R and 22S) as shown in figure 1. Both epimers appear to have similar pharmacological effects; however *in vitro* studies suggested that the R-epimer was two to three times more potent with respect to its anti-inflammatory effects. A review of the literature revealed that analytical methods had been employed for the quantification of budesonide and the

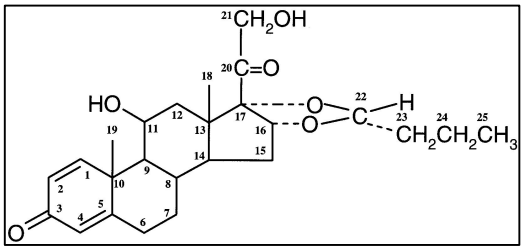

Fig 1 Chemical structure of budesonie.

separation of its epimers and impurities. Wikby et al. reviewed normal and reversed phase HPLC systems, and concluded that the separation of budesonide and its homologous corticosteroids was based mainly on their relative lipophilicity and solubility. Roth et al. developed and validated this ethanol based HPLC method for separation and quantification of budesonide epimers and their related impurities. The authors proposed their method as a suitable compendial method for budesonide. Although Roth et al. reversed-phase HPLC method has been employed widely for clinical pharmacokinetic studies, the European Pharmacopoeia describes an alternative reversed phase HPLC method as its official assay for this drug substance. This method employs a C18 column, mobile phase of acetonitrile: phosphate buffer pH 3.2 to determine the R:S epimer ratio, the purity of budesonide and its related substances (Shuguang Hou. et al., 2001). Above all methods are developed by HPLC and are expensive, complex. There is no report for UV spectrophotometric method for budesonide. Hence in the present study aimed to develop and validate the UV spectrophotometric method for quantification of budesonide in bulk and colon targeted matrix tablet.

MATERIAL AND METHODS

Pure gift sample of budesonide was received from Ajantha pharma, Mumbai. The potassium die hydrogen orthphosphate and sodium hydroxide was purchased from Sdfine chemicals, Limited, Bangalore. UV-Visible double beam spectrophotometer (UV-1700, Pharmaspec, SHIMADZU Limited, Japan) with 1cm matched quartz cells and Digital balance (Citizen Co.) and wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells.

Preparation of Stock Solution

The stock solution of budesonide is prepared by dissolving 100 mg of drug in 100 ml methanol in volumetric flask with continuous shaking. 1 ml of sample was withdrawn and diluted to 100 ml of phosphate buffer of ph 6.8 to get 10µg / ml of solution. The solution was than scanned in UV range between 200–400 nm UV-VIS Spectrophotometer, (double beam) Shimadzu, Japan to determine the absorption maxima of the drug against blank as pH 6.8 buffer.

Wavelength scanning and determination of absorption maximum

From the stock solution of budesonide, known concentration of 10µg/ml is prepared by suitable dilution with ph 6.8 buffer. Wavelength scanned for the maximum absorption of drug solution using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against blank phosphate buffer. Obtained spectra shows the peak with a highest absorbance is considered as absorbance maximum of the drug.

Calibration curve of Budesonide

The prepared stock solution was subsequently diluted to get 2 g/ml, 4µg/ml, 6µg/ml 8µg/ml, 10µg/ml. The resulting solutions absorbance was measured at wavelength of 246.0 nm using (double beam) UV spectrophotometric against blank of pH 6.8 buffer. The results obtained were tabulated and plotted a calibration curve of absorbance versus concentration. The slope of the calibration curve is determined by regression equation.

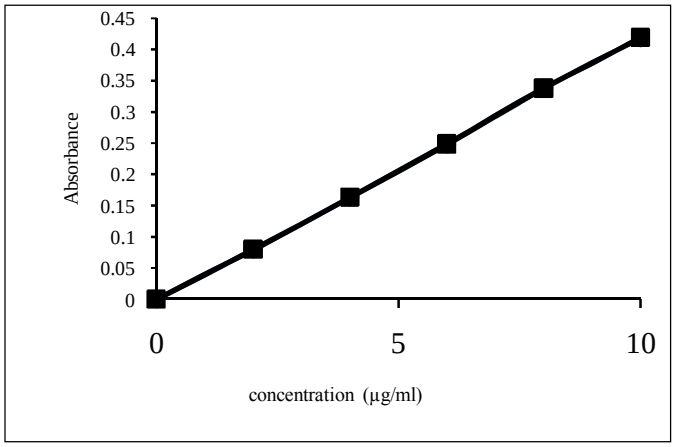

Fig 2 Standard graph of budesonie.

Linearity studies for budesonide analytical method

Stock solution was subsequently diluted with ph 6.8 buffer to get 0.1µg/ml, 0.5µg/ml, 1µg/ml,1.2 µg/ml,1.4 µg/ml,1.6 µg/ml,1.8 µg/ml 2 µg/ml, 4 µg/ml, 6 µg/ml 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml, 20 µg/ml, 25 µg/ml, 30 µg/ml. The results are tabulated and the linearity curve was constructed by plotting absorbance versus concentration.

Inter day and intraday studies for budesonide analytical method

The prepared stock solution was subsequently diluted to get 2µg/ml, 4µg/ml, 6µg/ml 8µg/ml, and 10µg/ml. The resulting solutions absorbance was measured at wavelength of 246.0 nm using double beam UV spectrophotometer against blank of pH 6.8 buffer. The findings was made three times in a day, morning, afternoon, evening and performed continuously for three days. The results obtained were tabulated and studied for inter day and intra-day variation.

Accuracy and Precision study (Parambi et al., 2010)

Previously analytical method was developed and has been reported. A part of validation was also reported. Further validation of the method was conducted to determine accuracy and precision. The accuracy and recovery studies were carried out by

adding a known amount of drug from the pre analyzed tablet powder and percentage recoveries were calculated. The reproducibility of estimation was determined by performing the tablet drug content of different samples. The results of precisions were expressed in % SD.

RESULTS AND DISCUSSION

Budesonide is a glucocorticosteriod highly potent, water insoluble and soluble in organic solvent drug. The physico chemical characteristic study of budesonide like melting point is 238°C nearer to the literature value 221–236°C (www.drugbank.com. 2011). In previous literature G. Hochhaus et.al developed the selective HPLC method for determination of budesonide in bulk sample and tablet dosage form. Sonali R. Naikwade et.al developed and validated specific HPLC method for budesonide and characterization of its alkali degradation product. The literature survey ascertains that HPLC analytical method is developed for budesonide, which is cost effective. In our laboratory we developed UV spectrophotometric method for the analysis of budesonide. The known concentration of budesonide is prepared and scanned for absorption maximum. Spectrum with highest absorbance maximum is 246.0 nm corroborate with literature value 244.3 nm (Prabhakara et al., 2010). Different

Table 1. Spectrophotometric data for Budesonide at λ_{max} 246.0 nm.

Sl.no	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.079 ± 0.002
3	4	0.401 ± 0.003
4	6	0.578 ±0.002
5	8	0.787 ± 0.013
6	10	0.993 ±0.010
Y =0 .042181*X ± 0.00324		

Table 2. Linearity studies of Budesonide:

Sl.no	Concentration (µg/ml)	Absorbance (nm)	Regression Data
1	0.00	0.00	
2	1.4	0.063	
3	1.6	0.064	*m = 0.040509
4	1.8	0.070	
5	2	0.08	
6	4	0.162	
7	6	0.244	
8	8	0.324	C = 0.0002
10	10	0.410	
11	12	0.490	
12	14	0.569	
13	16	0.640	
14	18	0.727	
15	20	0.815	r = 0. 9999
16	25	1.153	

measured for absorbance at wavelength 246.0 nm and plotted the curve absorbance versus concentration. Budesonide obeys the beers law in the concentration range 1.4 to 25 µg/ml. Linearity study indicates the curve is linear in the range of 2 to 10 µg/ml. The linear regression equation is Y =0 .042181*X ± 0.00324 with correlation coefficient (r^2) = 0. 9999. The developed method is validated for repeatability, reproducible and the accuracy and pression. In the interday and intraday study of standard graph the %SD is less than 2% indicating the developed method is reproducible. The different levels of standard concentration solutions are measured for absorbance and actual concentration is calculated by regression equation (n= 3). The results showed that the amount recovered is 99% to 101% with very less 0.1 to 1 % SD indicating the UV spectrophotometric method is accurate and pressise.

Table 3. Inter day and Intraday Validation study data of budesonide (n = 3).

Morning Conc. (µg /ml)	Day 1 Absorbance	Day 2	Day3	Avg± SD
0	0.000	0.000	0.000	0.000
2	0. 068	0.1	0.11	0.092 ± 0.021
4	0.156	0.221	0.246	0.207 ± 0.046
6	0.242	0.371	0.385	0.333 ± 0.078
8	0.329	0.427	0.486	0.414 ± 0.079
10	0.403	0.549	0.570	0.507 ± 0.091
Afternoon	Day 1	Day 2	Day3	Avg± SD
0	0.00	0.00	0.000	0.00
2	0.07	0.081	0.109	0.086 ± 0.020
4	0.177	0.204	0.259	0.213 ± 0.041
6	0.260	0.346	0.36	0.322 ± 0.053
8	0.346	0.437	0.463	0.415 ± 0.061
10	0.412	0.522	0.559	0.497 ± 0.076
Evening	Day 1	Day 2	Day3	Avg± SD
0	0.000	0.000	0.00	0.000
2	0.056	0.089	0.092	0.079 ± 0.019
4	0.143	0.185	0.193	0.173 ± 0.026
6	0.235	0.356	0.350	0.314 ± 0.068
8	0.317	0.397	0.435	0.383 ± 0.060
10	0.400	0.516	0.555	0.490 ± 0.080

Conc. (µg /ml)	Day1 M Avg Absorbance	Day 2 A Avg	Day 3 E Avg	Avg±SD
0	0.000	0.00	0.000	0.000
2	0.092 ± 0.021	0.086 ± 0.020	0.079 ± 0.019	0.086 ± 0.006
4	0.207 ± 0.046	0.213 ± 0.041	0.173 ± 0.026	0.198 ± 0.021
6	0.333 ± 0.078	0.322 ± 0.053	0.314 ± 0.068	0.323 ± 0.009
8	0.414 ± 0.079	0.415 ± 0.061	0.383 ± 0.060	0.404 ± 0.018
10	0.507 ± 0.091	0.497 ± 0.076	0.490 ± 0.080	0.498 ± 0.008

Table 4. Accuracy and Precision study of Budesonide.

	Amount drug added (µg/ml)	Amount drug recovered (µg/ml	Accuracy	Precision
Budesonide	6	6.07	101	0.02
	8	7.89	99.07	0.01
	10	10	100	0.03

CONCLUSION

The developed analytical method for budesonide by using UV spectrophotometer is found to simple, rapid and selective and the amount of drug recovered will be same as the label claimed and precise. It can be conveniently employed for the routine analysis and quantification of budesonide in bulk and colon matrix tablet.

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