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A STABLE HPLC BIOANALYTICAL METHOD DEVELOPMENT FOR THE ESTIMATION OF BUDESONIDE IN PLASMA

Lonikar N. B[*1], Mallikarjuna Gouda M[2], Baby Sudha Lakshmi[3], Ramakrishna Shabaraya A[4]

[1]Department of Chemistry, Shivalingeshwara College of Pharmacy, Latur-Maharastra.
[2]Department of Pharmaceutics, V. L. College of Pharmacy, Raichur.
[3]Department of Chemistry, Ramachandra College of Engineering, Eluru - Andhra Pradesh.

Corresponding Author: Dr. M. Mallikarjuna Gouda

Department of Pharmaceutics, V. L. College of Pharmacy, Raichur.

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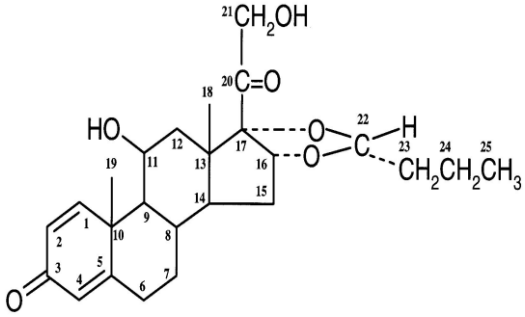
ABSTRACT

In pharma industry the development of analytical method is very important in drug analysis, development and in pharmacokinetics study of the drug. Hence in the present investigation the HPLC bioanalytical method of liquid-liquid extraction of drug from plasma is established and the linearity study of Budesonide was found in range of 50% to 150 % concentration, the regression equation was found to be Y = 22326X + 15677. The method checked for Pression and % RSD was found to be 1.72 % and the accuracy was in the range 100.4 % to 101.1%. The limit of detection and the limit of quantification were calculated by the non instrumental that is by equation mentioned in the methodology. The LOD was found to be 191.3µg/ml and LOQ was found to 579.9 µg/ml. Thus the developed bioanalytical HPLC is selective, stable and quantity the budesonide in plasma.

KEYWORDS: Budesonide, nimusolide, Linearity, HPLC, Relative standard deviation.

INTRODUCTION

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 approximately 10 %. 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 C_{22} 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 [1-2]. A review of the literature revealed that analytical methods had been employed for the quantification of budesonide and the 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.[3] 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 above all different analytical methods gave an idea to develop the stable bioanalytical HPLC method and it was further validated for linearity and some other parameter to estimate the budesonide in plasma accurate and precise.

MATERIALS AND METHODS

Gift sample of Budesonide and nimusolide was obtained from Ajanta Pharma Ltd and Aurobindo Pharma Ltd, Hyderabad. Ammonium acetate phosphate, Acetonitrile, potassium die hydrogen orthophosphate and sodium hydroxide was received from S.d. Fine chemicals, Bangalore.

INSTRUMENTATION[4]

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software , reversed phase C18 column (Phenomenex C18

column (4.6 , 3.5µm)) as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

HPLC Conditions[4]

The contents of the mobile phase of ammonium acetate phosphate buffer pH 3.5 and Acetonitrile at ratio of (3:5) is used for Budesonide Before using, the mobile phases were filtered through 0.45- µm membrane filter and degassed with a helium spurge for 15 minutes. The components of the mobile phase were pumped from the respective solvent reservoirs in to the column at a flow rate of 1.5 ml/min which yielded a column back pressure of 120 – 130 kg/cm2. The run time was set at 20 min and the column temperature was maintained at 40 ^{o} C. The volume of the injection loop was 10 µL. Prior to the injection of the sample solutions the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The eluents were monitored at the wavelength of 246 nm for Budesonide. The data were acquired, stored and analyzed with the software Class – VP series version 5.03 (Shimadzu).

PREPARATION OF STOCK SOLUTION

Budesonide stock solution and Nimusolide internal standard stock solution

Accurately weighed and transferred 10 mg of Budesonide sample in a 100 ml volumetric flask then dissolved by sonication and made the volume up to the mark with mobile phase, similarly the internal standard nimusolide stock solution was prepared by transferring the 10 mg of accurately weighed drug into 100 ml volumetric flask and then dissolved by sonication and made the volume up to the mark with mobile phase.

From above stock solution of 10 µg/mL of budesonide and 10 µg/mL of Internal standard is prepared by diluting 1 ml of standard stock solution and 1mL of internal standard stock solution to 10 ml with mobile phase. The resulting solutions are scanned for wavelength at their respective peaks.

Extraction and sample preparation[5]

Transferred 0.2mL of plasma which was previously taken from the rabbit animal in a conical shape test tube, added 1ml of sample to the plasma and samples were vortexed briefly for proper mixing. Added 3mL of Chilled methanol and then also added 1mL of internal standard solution, swirl it. Made the volume up to 10mL with methanol. Centrifuge the mixture and collected the supernatant.

The above established HPLC method was further studied for the following parameter like linearity, precision, accuracy, LOD, LOQ.

Linearity[6]

The linearity of the proposed HPLC method was determined in terms of correlation coefficient between concentration of the drug and its respective peak area. The data were subjected to regression analysis using least square method.

Precision[7]

The precision of the analytical procedure express the closeness of agreement between series of measurement obtained from the multiple sampling of same homogeneous solution. Budesonide solution of 100% concentration at six determinations is injected into the column, the peak area and the retention time is recorded and tabulated. The precision is expressed in terms of coefficient of variation (CV) which is calculated by multiplying the ratio of standard deviation to the mean with 100.

$$CV = \frac{SD}{Mean} X\ 100$$

Accuracy[6]

The accuracy of an analytical method is the closeness of test results to the true value. The accuracy is expressed in terms % recovery, which is calculated by multiplying the ratio of measured % drug concentration to the expected % drug concentration with 100 so as to give the percent recovery.

Limit of detection[7]

The limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessicerly quantiated under the stated experimental conditions and it is calculated by.

$$LOD = \frac{SD}{Slope} X\ 3.3$$

Limit of quantification[7]

The limit of quantification is the lowest amount of analyte in a sample that can be quantified with the acceptable accuracy and precision under the stated experimental conditions and it is calculated by.

$$LOQ = \frac{SD}{Slope} X\ 10$$

RESULTS

Table: 1. Spectrophotometric data of Budesonide at λ_{max} 241 nm.

Concentration (%)	AVG. Peak Area	SD	% RSD
50	1154997	392	0.30
80	1817156	1412	0.08
100	2208566	23800	1.08
130	2965893	538	0.18
150	3333696	2062	0.06

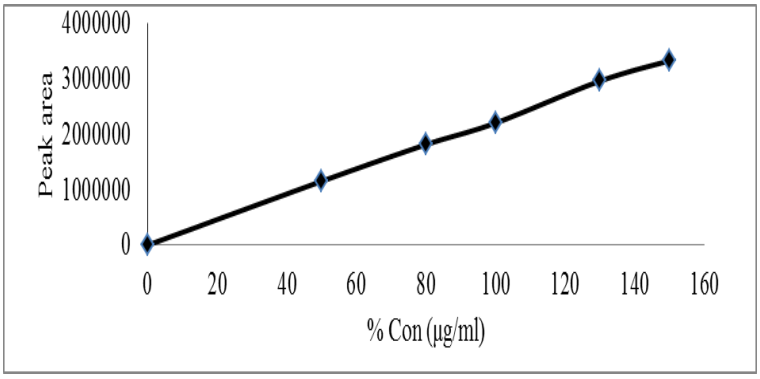


Figure: 2. Calibration curve of Budesonide

Table No 2.0. Linearity study of Budesonide

Concentration (%)	AVG. Area	SD	% RSD	Regression equation Data
50	1154997	392	0.30	M = 11480817 C= 15677 r = 0.99916
80	1817156	1412	0.08	
100	22085665	23800	1.08	
130	2965893	538	0.18	
150	3333696	2062	0.06	

Table No 3.0. Precision study of Budesonide

Inj.No	Timings (hrs)	Peak area
1	7.30 AM	2244854
2	10.30 AM	2247417
3	1.30 PM	2251033
4	4.30 PM	2243731
5	7.30 PM	2242130
6	9.30 PM	2243874
Average		2245507
SD		3220
%RSD		0.14

Table No 4.0. Accuracy study of Budesonide

Standard solution		Test sample solution				% con founded	% Con Recovery
Trial	Peak area	% Con Added	Peak area	AVG. Peak area	SD		
1 2 3 4 5 6	2216623 2208010 2218043 2219562 2225893 2222273	50	1121628 1120412 1122041	1121360	847	50.55	101.1
		100	2233862 2235114 2232874	2233950	1123	100.7	100.7
AVG	2218401	150	3342370 3348217 3340726	3343771	3937	150.73	100.49
SD	6062						

DISCUSSION

To estimate the plasma drug concentration, the simple, stable bioanalytical HPLC method has been established and studied the following parameters like, linearity, accuracy, precision and LOD and LOQ. The linearity of Budesonide was found in the range of 50 % to 150 % concentration and it showed the linear relationship with concentration and peak area. The correlation of coefficient was found to be 0.99916 indicating the greater dependence between concentration and peak area and the slope was found to 22325.97 The regression equation obtained as Y= 22325.97 + 15677. The developed methods were checked for precision by spiking the same concentration at six determinations. The results observed from table no 03 indicates that peak area is consistent and % RSD was found to be 1.72, which is within the acceptable limit. Accuracy measures the closeness of the test sample value to true value. It is performed at three different concentration levels and the result of % recovery was found to be 100.5% to 101.1%, for Budesonide, which is within the acceptable limit. The limit of detection and the limit of quantification were calculated by the non instrumental that is by equation mentioned in the methodology. The LOD was found to be 191.3µg/ml and LOQ was found to 579.9µg/ml.

CONCLUSION

The established bioanalytical HPLC methods were found to be simple, stable and specific in separating the peak area of internal standard and sample peak area. Accurate and precise in estimating the drug in plasma, hence the above HPLC methods can be used for quantifying the plasma drug concentration of Budesonide colon targeted microbial degradable matrix tablet.

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