Asian Journal of Chemistry Vol. 15, No. 2 (2003), 961–966

Development and Validation of a Reversed-Phase HPLC Method for the Analysis of Budesonide in Pharmaceutical Dosage Forms

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A rapid and sensitive high-performance liquid chromatographic method was developed for the estimation of budesonide in pharmaceutical dosage forms. Budesonide was chromatographed on a reverse phase C-18 column using nimesulide as internal standard in a mobile phase consisting of methanol and water in the ratio of 80 : 20 v/v. The mobile phase was pumped at a flow rate of 0.8 mL/min, and the eluents were monitored at 241 nm. The calibration curve was linear in the range of 0.1 to 40 g/mL. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solutions containing 5, 10 or 20 μg/mL was 98.47 ± 0.37% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining budesonide in bulk drug samples or in capsules.

Key Words: Budesonide, Nimesulide, Dosage forms, Reversed-phase HPLC

INTRODUCTION

Budesonide is a synthetic glucocorticosteroid having a potent glucocorticoid and weak mineralocorticoid activity. It has an approximately 200-fold higher affinity for the glucocorticoid receptor and a 1000-fold higher topical anti-inflammatory potency than cortisol[1]. It has been recently approved by the FDA for the use in the treatment of inflammatory bowel diseases.

A few analytical methods have been reported for the estimation of budesonide in pharmaceutical dosage forms using HPLC; a stability indicating method and a selective HPLC/radioimmuno assay[2, 3]. The HPLC methods using the most commonly available columns and detectors like UV are preferred. The present study describes the determination of budesonide in bulk drug samples and pharmaceutical dosage forms by using RP C-18 column with UV detection. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating budesonide. The aim of this study is to develop a simple, precise, rapid and accurate reversed phase HPLC method for the determination of budesonide either in bulk drug samples or in pharmaceutical dosage forms.

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EXPERIMENTAL

Budesonide (98–101% purity) and nimesulide were gift samples obtained from M/s Astra-Zeneca Ltd, Bangalore, India and M/s Dr. Reddy's Laboratories Pvt., Ltd., Hyderabad respectively. Methanol (Qualigens) and water used were of HPLC grade. All other reagents used in the study were of AR quality (Qualigens).

Instrumentation: A gradient HPLC (Shimadzu HPLC class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10A VP, CTO-10AS VP Column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard™, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (150 mm × 4.6 mm I.D., particle size 5 μm; YMC Inc., USA) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)"

HPLC conditions: The contents of the mobile phase, methanol and water in the ratio of 80 : 20 v/v, were filtered before use through 0.45 μm membrane filter and degassed with a helium spurge for 15 min. The components of the mobile phase were pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 mL/min which yielded a column back pressure of 120–130 kg/cm^2. The run time was set at 7 min and the column temperature was maintained at 40°C. The volume of the injection loop was 20 L. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The eluents were monitored at 241 nm and the data were acquired, stored and analyzed with the software "Class-VP series version 5.03 (Shimadzu)".

Procedure: The solutions were prepared on a weight basis and volumetric flasks were used to minimize solvent evaporation. The HPLC estimation was accomplished by an internal standard method. Stock solution of drug and internal standard were prepared by dissolving 100 mg of budesonide and nimusulide separately in 100 mL volumetric flasks containing 70 mL of methanol, sonicated for about 15 min and then made up to volume with methanol. Daily working standard solutions of budesonide and nimesulide (internal standard) were prepared by suitable dilution of the stock solution with water.

Six sets of the budesonide solution were prepared in methanol at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 20 and 40 μg/mL along with a fixed quantity (10 μg) of nimesulide as internal standard. Each of this sample (20 μL) was injected six times into the column and the peak area of the drug and internal standard were recorded.

Assay of budesonide in capsules: Twenty capsules were weighed, the contents of the capsules were emptied, an accurately weighed sample of powder equivalent to 3 mg of budesonide was placed in a 100 mL volumetric flask. 70 mL of methanol were added, shaken well and the flasks allowed to stand for 6 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then made up to volume with methanol, thoroughly mixed and filtered through a 0.45 μm membrane filter. An aliquot of the filtrate (1 mL) was transferred to a volumetric flask along with appropriate volume of nimesulide solution and made up to volume with water to give an expected concentration of 20 μg/mL of

budesonide and 1 μg/mL of nimesulide (internal standard). All determinations were conducted in triplicate. The same procedure was used to estimate the amount of budesonide in one more commercial brand of budesonide capsule.

Validation: The proposed HPLC method was validated for its linearity, precision and accuracy. The precision (% coefficient of variation) was expressed with respect to the inter- and intra-day variation in the expected drug concentration. The accuracy was expressed in terms of per cent recovery of known amount of drug added to the pre-analyzed samples of drug solution.

Linearity: The linearity of the proposed HPLC method was determined in terms of the correlation coefficient between the concentration of the drug and its respective peak area ratio to that of internal standard. The data were subjected to regression analysis using least squares method of analysis.

Precision: The precision of the assay was determined in terms of intra- and inter-day variation in the peak area ratio for a set of drug solutions on three different days (n = 5). The intra- and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of coefficient of variation (C.V.), and obtained by multiplying the ratio of standard deviation to the mean with 100 [C.V = (SD/mean) × 100].

Accuracy: The accuracy of the HPLC assay method was assessed by adding known amount (5, 10 or 20 μg /mL) of the drug to a drug solution of known concentration along with 1 μg /mL internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (5, 10 or 20 μg/mL) was added to the volumetric flask containing the powder sample of the capsule formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of budesonide in capsule formulations. In both the cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the per cent recovery.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in the recent years because of their importance in quality control of drugs and drug products. The goal of this study was to develop a rapid HPLC method for the analysis of budesonide in bulk drug samples and its tablet formulations using the most commonly employed RP C-18 column with UV detection.

The run time of the method was set at 7 min and budesonide and nimesulide appeared on the chromatogram at 4.96 min and 3.23 min respectively (Fig. 1). When the same drug solution was injected 6 times, the retention time of the drug and internal standard were same. The ratio of peak areas of budesonide to peak area of the internal standard was calculated and the average values for 6 such determinations were shown in Table-1. When the concentration of budesonide and its respective peak area ratios were subjected to regression analysis by least squares method, a high correlation coefficient was observed ($r = 0.9999 \pm 0.058$)

in the range of 0.1 to 40 μg/mL only. The regression of budesonide concentration over its peak area ratio was found to be Y = –0.01108 + 1.03363X where 'Y' is the peak area ratio and 'X' is the concentration of budesonide. This regression equation was used to estimate the amount of budesonide either in tablet formulations or in validation study.

TABLE- 1
CALIBRATION AND PRECISION OF THE HPLC ASSAY

Concentration of budesonide (μg/mL)	Peak area ratio*	C.V. (%)
0.0	0	0.00
0.1	0.10201	2.12
0.2	0.20398	1.42
0.4	0.40248	2.42
0.8	0.79903	0.47
1.0	0.98233	1.21
2.0	1.97926	0.27
5.0	5.17520	0.88
10.0	10.42096	1.52
20.0	20.68524	0.92
40.0	41.30102	0.85

*Mean of six determinations
Regression equation: Y=– 0.01108 + 1.03363X (r = 0.9999)

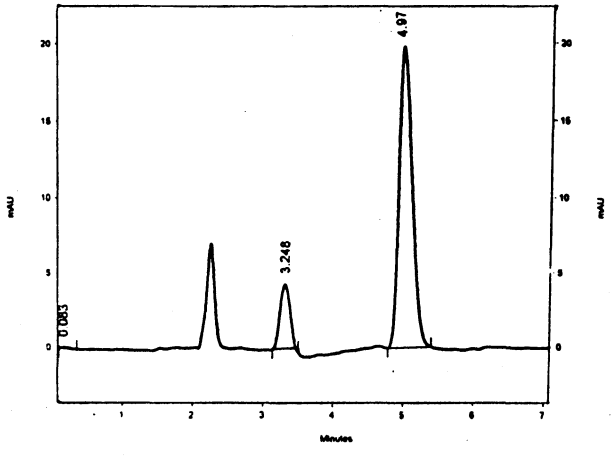

Fig. 1. Typical HPLC chromatogram of budesonide

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 10, 20 or 50 μg/mL of budesonide along with 1 μg/mL of nimesulide were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area ratio of the drug for five replicate injections was found to be less than 1%. Also, the inter-day variation (3 days and five injections) was found to be less than 1% (Table 2). Thus, the results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (10, 20 or 50 μg/mL) was added to a known amount of drug solution (20 μg/mL), there was a high recovery (98.47 ± 0.37%) of budesonide (Table-3) indicating that the proposed method is highly accurate.

TABLE- 2
INTER- AND INTRA-DAY PRECISION FOR BUDESONIDE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Actual concentration of budesonide (μg/mL)	Observed concentration of budesonide (μg/mL)			
	(Intra-day)		(Inter-day)	
	Mean*	CV (%)	Mean*	CV (%)
10	10.03	0.94	10.01	0.81
20	20.01	0.65	19.99	0.47
50	49.99	0.53	50.01	0.33

* Mean of five determinations

TABLE 3
RECOVERY OF BUDESONIDE USING THE PROPOSED HPLC METHOD

Amount of drug added (μg) to pre-analysed drug solution	Recovery of budesonide	
	Mean (±s.d.) amount (μg) found (n = 5)	Mean (±s.d.) % recovery (n = 5)
10	9.95 ± 0.18	99.50 ± 1.20
20	19.98 ± 0.32	99.90 ± 0.86
50	49.96 ± 0.80	99.92 ± 1.21

The HPLC method, developed in the present study, has also been used to quantify budesonide in capsule dosage forms. Budesonide capsules (containing 3 mg of the drug) were analyzed as per the procedure described above. The average drug content was found to be 98% of the labeled amount (Table 4). No interfering peaks were found in the chromatogram indicating that excipients used in the capsule formulation did not interfere with the estimation of the drug by the proposed HPLC method. A known amount of the drug solution was added to the powder sample of the capsule dosage form and subjected to the estimation of the drug by the proposed method. There was a high recovery of budesonide (98.47 ± 0.31%) indicating that the proposed procedure for the determination of budesonide in the capsule dosage forms is highly accurate.

TABLE 4
MEAN (±s.d.) AMOUNT OF BUDESONIDE IN CAPSULE DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the capsule	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AA	3	2.94 ± 0. 07	98.00 ± 2.33
BB	3	2.92 ± 0.03	97.33 ± 1.00

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from Government of India, Department of Science and Technology (DST) and All India Council for Technical Education (under MODROBS) in establishing the infrastructure for HPLC. The authors acknowledge M/s Astra-Zeneca, Bangalore, India and Dr. Reddy's Laboratories Pvt. Ltd., Hyderabad, India for providing the gift sample of budesonide and nimesulide respectively. One of the authors Mr. S.S. Shyale, is thankful to Dr. S.M. Shantakumar, Principal, V.L. College of Pharmacy, Raichur, and also to the Management of V.L. College of Pharmacy, Raichur, in encouraging to pursue Ph.D. program.

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(*Received*: 28 October 2002; *Accepted:* 8 January 2003) AJC-2961