ORIGINAL ARTICLE

New Stability Indicating RP-HPLC Method for the Estimation of Budesonide in API and Pharmaceutical Dosage Forms

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Abstract

Introduction: Budesonide is used for the treatment ofchronic obstructive pulmonary diseaseand asthma, and it belongs to glucocorticoid class. A new stability indicating RP-HPLC method has been proposed for the quantification of Budesonide in tablet dosage forms and the method was validated. **Materials and Methods:** Shimadzu HPLC system with PDA detector was used with Agilent C18 column with mobile phase mixture, formic acid: Methanol (30:70) and flow rate 1.0 ml/min (UV detection at 243 nm). **Results and Discussion:** Beer-Lambert's law was obeyed over a concentration range 0.1–100 µg/ml. The linear regression equation was found to be y = 48625x + 8107.9 (r^2 = 0.9998). The LOD and LOQ were found to be and 0.0302 and 0.0922 µg/ml. Stress degradation studies were performed and the method was validated as per ICH guidelines. The proposed method is simple, precise, accurate, and robust and can be applied for the assay Budesonide formulations.

Key words: Budesonide, ICH guidelines, RP-HPLC, Stability indicating, Stress degradation studies, Validation

INTRODUCTION

Budesonide [Figure 1] is used to prevent chest tightness, wheezing, difficult breathing and coughing which symptoms are caused by asthma. It belongs toglucocorticoid class and used for the treatment ofchronic obstructive pulmonary diseaseand asthma. Budesonide($C_{25}H_{34}O_6$ and Mo. Wt. 430.534 g/mol) is 11β,21-Dihydroxy-16α,17α-[butane-1,1-diylbis(oxy)]pregna-1,4-diene-3,20-dione.[1] Budesonide is available as pills, inhaler, nasal polyps, nasal spray and rectal forms for the treatment of Crohn's disease, microscopic colitis and ulcerative colitis.[2-6] Analytical techniques such as LC-MSMS[7] and HPLC[8-11] methods were developed for the quantification of Budesonide and in the present study a new validated stability indicating RP-HPLC method was proposed for the estimation of Budesonide.

MATERIALS AND METHODS

Preparation of solutions

Budesonide was obtained as gift sample from Natco Pharma (India). Budesonide (25 mg) was weighed and transferred carefully in to a 25 ml volumetric flask and dissolved in methanol (HPLC grade) which is known as the stock solution (1000 µg/ml). Dilutions were made as per the requirement for the linearity, precision, accuracy, robustness, and other studies using the diluent, water: methanol (45:55), and all the solutions were filtered through 0.45 µm membrane filter before injection.

Instrumentation and chromatographic conditions

Shimadzu Model HPLC system with PDA detector and Agilent C18 column was used for the chromatographic study. Mobile phase mixture consisting of formic acid: Methanol (30:70) was used for the present chromatographic study with flow rate 1.0 ml/min (UV detection at 243 nm).

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Method validation[12]

Linearity study

Budesonide solutions (0.1–100 µg/mL) were prepared from the stock using the diluent, water: methanol (45:55) and injected into the HPLC system thrice and the peak area was noted from the respective chromatograms and finally the mean peak area (n = 3) was calculated. A calibration curve was drawn by plotting the Budesonide drug concentration on the x-axis and the corresponding mean peak area on the y-axis. The LOD and LOQ were calculated from the S/N ratio.

Precision study

Intra- and inter-day precision studies were performed on the same day and on three different days (10, 20 and 40 µg/mL) and the peak area of the chromatograms were recorded during the study from which the mean peak area (n = 3) was calculated. The percentage relative standard deviation was also calculated from the mean peak area and the standard deviation.

Accuracy study

Accuracy study was performed by spiking the Budesonide (20 µg/mL) formulation solution (50, 100, 150%) with known concentration of Budesonide API (n = 3). These solutions were also injected into the HPLC system and the chromatograms were recorded followed by the peak areas from which the mean peak area, and the % RSD were calculated from the linear regression equation.

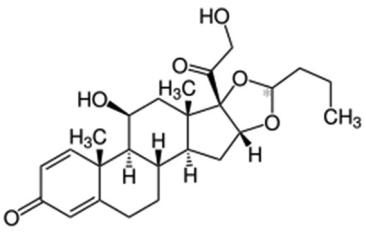

Figure 1: Structure of Budesonide

Robustness study

The robustness of the method was proved by incorporating a very small changes in the optimized chromatographic conditions such as flow rate (± 0.1 mL; 1.1 and 0.9 mL/min), mobile phase composition (Formic acid: Methanol) (± 2%v/v; 28:72 and 32:68), and detection wavelength (± 2 nm; 241 and 245 nm).

Stress degradation studies[13]

Forced degradation or stress degradation studies were performed to determine the stability of Budesonide (20 µg/mL) toward acidic hydrolysis, basic hydrolysis, oxidation, and thermal degradation. The specificity of the method was determined from the stability studies and therefore Budesonide was exposed to different stress conditions as explained below.

Acidic degradation was performed by heating Budesonide (20 µg/mL) solution with 1 mL of 0.1 N HCl solution at 50° for 30 min on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL 0.1N sodium hydroxide solution, diluted with diluent, and then 20 µl of the solution was injected in to the HPLC system.

Alkaline degradation was performed by heating Budesonide (20 µg/mL) solution with 1.0 mL 0.1N sodium hydroxide solution at 50° for 30 min on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL of 0.1 N

Table 1: Literature survey of Budesonide

Mobile phase (v/v)	Column	λ (nm)	Linearity (µg/mL)	Comment	Ref
Tertbutyl methyl ether: n-hexane (70:30)	Agilent Zorbax Eclipse XDB-C8 (100 mm×4.6 mm)	-	-	LC-MS/MS	7
Acetonitrile: Phosphate buffer (pH 3.2–0.025M) (55:45)	Kromasil C8 (150 mm×4.6 mm)	244		HPLC (PDA)	8
Ethanol: Acetonitrile: Phosphate buffer (pH 3.4; 25.6 mM) (2:30:68)	Hypersil C18	240		HPLC (UV)	9
Methanol: water (80:20)	Phenomenex C18 (250×4.6 mm)	244		HPLC (PDA)	10
Formic acid: Acetonitrile (25:75)	Agilent C18	247	10-100	UFLC (PDA) Stability indicating	11
Formic acid: Methanol (30:70)	Agilent C18	243	0.1-100	HPLC (PDA) Stability indicating	Present method

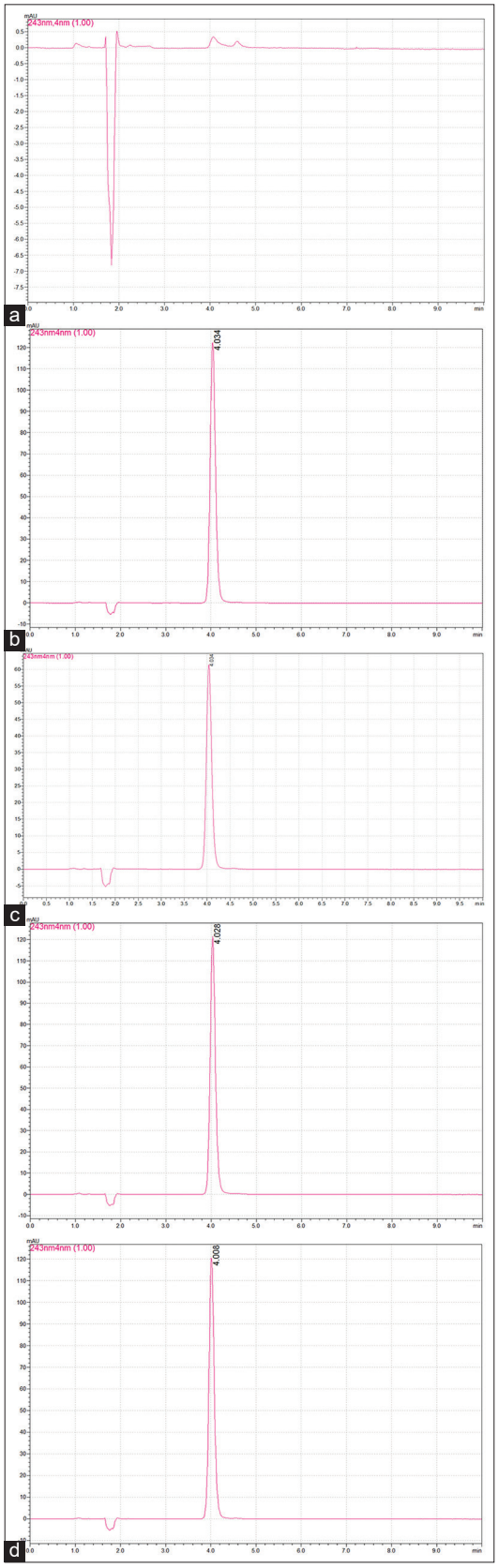

Figure 2: Typical chromatograms of (a) Placebo (b) Budesonide (10 µg/mL) (API) (Rt: 4.034 min) (c) Budesonide formulation (20 µg/mL) (Brand I) (Rt: 4.028 min) (d) Budesonide formulation (20 µg/mL) (Brand II) (Rt: 4.008 min)

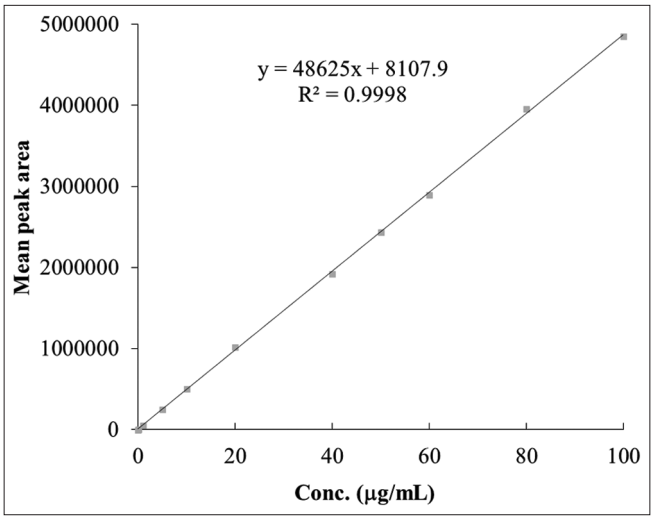

Figure 3: Calibration curve of Budesonide

Table 2: Linearity of Budesonide

Conc. (µg/mL)	*Mean peak area	% RSD
0	0	-
0.1	6019	0.51
1	51652	0.33
5	252431	0.39
10	504658	0.41
20	1015557	0.26
40	1924349	0.45
50	2439276	0.61
60	2895476	0.37
80	3953687	0.43
100	4847853	0.59

*Mean of three replicates

HCl solution, diluted with mobile phase and then 20 µl of the resulting solution was injected in to the HPLC system.

Thermal degradation was performed by heating the Budesonide (20 µg/mL) solution at 50° for 30 min on a water bath and then cooled, diluted with mobile phase and 20 µl of the resulting solution was injected in to the HPLC system.

Oxidative degradation was performed by heating Budesonide (20 µg/mL) solution with 1.0 mL 30% hydrogen peroxide solution at 50° for 30 min on a water bath. The stressed sample was then cooled, diluted with mobile phase and then 20 µl of the resulting solution was injected in to the HPLC system.

Assay of budesonide formulations

Budesonide is available as capsules with brand name Budecort (Label claim 3.0 mg) (Natco Pharma), Budenase

AQ nasal spray (Cipla Ltd), Rotacaps (Inhaler suspension) (Label claim: 0.5 mg/2 ml and 1 mg/2 ml), Budamate Transcaps (Lupin Ltd), Breemax (Intra Labs), Budecort inhaler (200 mcg/dose), Budez enema and Budamate inhaler, etc. Twenty capsules of Budesonide of two different brands were collected, and the contents accurately transferred to two different volumetric flasks and extracted with HPLC grade methanol. The mixture of the contents was sonicated, filtered and then diluted with the methanol. This extracted solutions of these two brands were diluted with the diluent and the resulting solutions were injected (n = 3) in to the system and the mean peak area was calculated from the peak areas obtained from the resultant chromatograms. The mean peak area so calculated was substitute in the linear regression equation and the percentage of purity was calculated.

RESULTS AND DISCUSSION

The authors have proposed a new stability indicating RP-HPLC method for the estimation of Budesonide in pharmaceutical formulations. The literature survey collected regarding the analytical methods so far published for the estimation of budesonide were explained in Table 1.

Method optimization

During the optimization process different columns such as Phenomenex column, Zorbox were used initially but finally Agilent C18 column was chosen and mobile phases such as Acetonitrile: water, Formic acid: acetonitrile were used for trials but the system suitability parameters were not within the acceptable criteria and therefore finally mobile phase mixture containing formic acid: methanol (30:70) was selected for the present study with a diluent containing water : methanol (45:55) and the optimized chromatogram of Budesonide (Rt 4.034 min) along with the placebo were shown in Figure 2.

Method validation

Budesonide shows linearity over the concentration range 0.1–100 µg/mL [Table 2], and the linear regression equation was found to be y = 48625x + 8107.9 with correlation coefficient 0.9998 [Figure 3]. The LOD and LOQ were found to be 0.0302 and 0.0922, respectively. The % RSD was found to be 0.0056–0.0102 (Intraday) [Table 3] and 0.0149–0.5929 (Inter-day) [Table 3] in precision studies which is <2.0 indicating that the method is precise. The % recovery in accuracy studies was found to be 99.30–99.84% [Table 4] and % RSD was (0.84–0.92) <2% indicating that the method is accurate. The % RSD in robustness study was found to be 0.129–0.913 which was <2% indicating that the method is robust [Table 5].

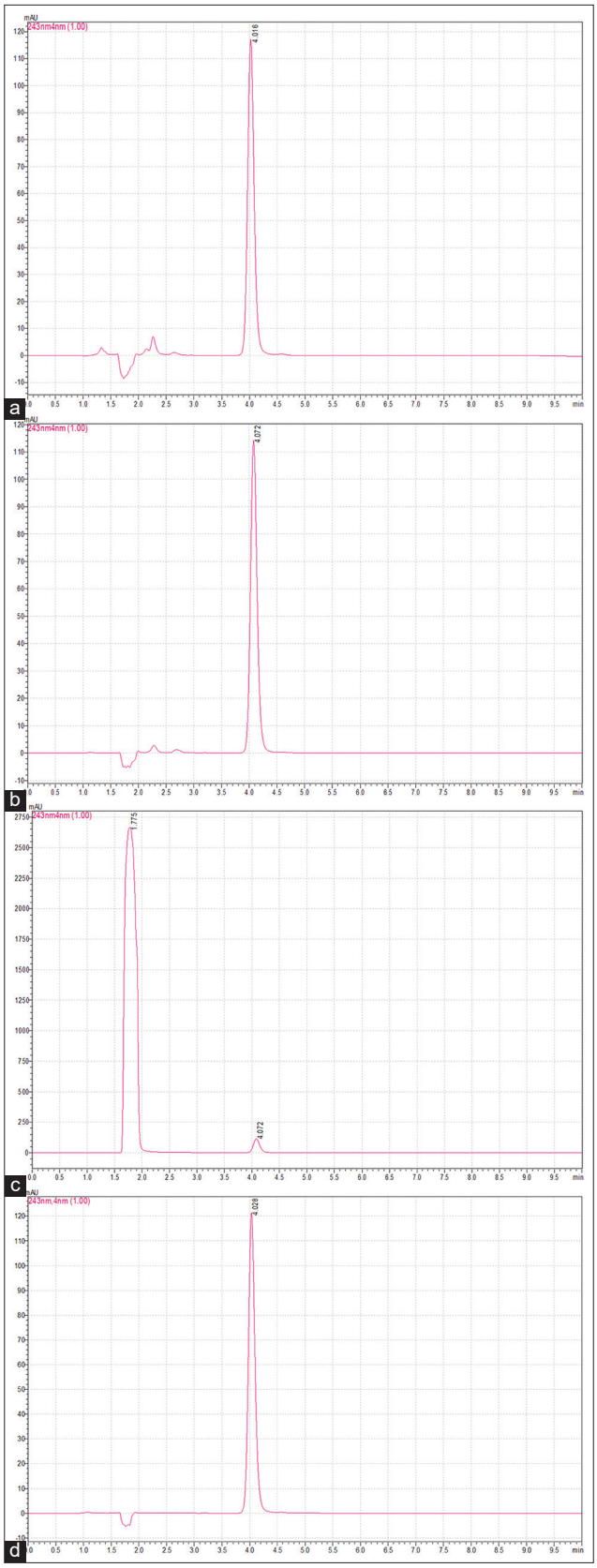

Figure 4: Representative chromatograms of Budesonide (20 µg/mL) (a) Acidic degradation (b) Alkaline degradation (c) Oxidative degradation (d) Thermal degradation

Table 3: Precision studies of Budesonide

Intraday study of precision		
Conc. (µg/ml)	**Mean peak area**	**Statistical analysis *Mean peak area±SD (% RSD)**
10	504654	504623±28.4781 (0.0056)
10	504598	
10	504617	
20	1015557	1015584±103.6066 (0.0102)
20	1015698	
20	1015496	
40	1924349	1924550±185.2917 (0.0096)
40	1924587	
40	1924714	

Inter-day study of precision				
Conc. (µg/ml)	**Day 1**	**Day 2**	**Day 3**	**Statistical analysis *Mean peak area±SD (% RSD)**
10	504654	505843	510352	506949.7±3005.883 (0.5929)
20	1015557	1015698	1015394	1015550±152.1326 (0.01498)
40	1924349	1925691	1926314	1925451±1004.184 (0.0522)

*Mean of three replicates

Table 4: Accuracy study of Budesonide

Conc. (µg/mL)	Formulation (µg/mL)	Total Conc. (µg/mL)	*Conc. obtained(µg/mL)±SD (%RSD)	% Recovery
10 (50%)	20	30	29.79±0.2741 (0.92)	99.30
	20	30		
	20	30		
20 (100%)	20	40	39.82±0.3345 (0.84)	99.55
	20	40		
	20	40		
30 (150%)	20	50	49.92±0.4343 (0.87)	99.84
	20	50		
	20	50		

*Mean of three replicates

Table 5: Robustness study of Budesonide (20 µg/ml)

Parameter	Condition	*Mean peak area	Statistical analysis *Mean±SD (% RSD)
Flow rate (±0.1 ml/min)	0.9	1016548	1016373±9279.49 (0.913)
	1.0	1015557	
	1.1	1017015	
Detection wavelength (±2 nm)	241	1015633	1015596±1310.12 (0.129)
	243	1015557	
	245	1015598	
Mobile phase composition Formic acid: Methanol (30:70, v/v)(±5%)	25:75	1015496	1015616±6367.91 (0.627)
	30:70	1015557	
	35:65	1015795	

*Mean of three replicates

Table 6: Assay of Budesonide formulations

Brand name	Label claim (mg)	*Observed amount (%w/w)	% Recovery*
Brand I	3.0	2.98	99.33
Brand II	3.0	2.92	97.33

*Mean of three replicates

Assay of Budesonide formulations

The optimized RP-HPLC method was applied for the existing pharmaceutical formulations and the percentage of purity of budesonide was calculated with the help of the linear regression equation. The percentage of purity was found to be 97.33-99.33 [Table 6].

Table 7: Stress degradation studies of Budesonide

Stress condition	Rt (min)	Mean peak area	% Recovery	% Drug degradation	Theoretical plates (>2000)	Tailing factor (<1.5)	Resolution (>2)
Standard drug	4.034	1015557	100	-	4953.212	1.195	-
Acidic degradation 0.1N HCl/50ºC/30 min	4.016	959598	94.49	5.51	4920.422	1.185	-
Alkaline degradation 0.1NNaOH/50ºC/30 min	4.072	957679	94.31	5.69	4919.672	1.191	-
Oxidation degradation H_2O_2/50ºC/30 min	4.072 1.775	946734	93.22	6.78	5006.090	1.204	8.203
Thermal degradation Water/50ºC/30 min	4.028	1010619	99.51	0.49	4919.693	1.193	-

*Mean of three replicates

Stress degradation studies

Budesonide elutes at 4.034 min with theoretical plates 4953.212 (>2000) and tailing factor 1.195 (<1.5). During the acidic degradation study Budesonide was eluted at 4.016 min with 5.51% degradation with theoretical plates 4920.422 and tailing factor 1.185, respectively. During the alkaline degradation study Budesonide was eluted at 4.072 min with 5.69% degradation with theoretical plates 4919.672 and tailing factor 1.191 respectively. During the oxidative degradation study, Budesonide was eluted at 4.072 min with an extra degradation peak 1.775 min and during this study about 6.78% degradation was observed with theoretical plates 5006.090, tailing factor 1.204 and resolution 8.203 (>2.0), respectively. During the thermal degradation study, Budesonide was eluted at 4.028 min with less than 1.0% degradation was reported with theoretical plates 4919.693 and tailing factor 1.193, respectively. The method is specific and selective as Budesonide drug peak was not interfering with any other degradant peak. The system suitability parameters observed during the stress degradation studies were shown in Table 7 and the respective chromatograms were shown in Figure 4.

CONCLUSION

A new stability indicating RP-HPLC method has been developed for the estimation of Budesonide and validated as per ICH guidelines.The proposed method is simple, precise, accurate, and robust.

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REFERENCES

1. Christophi GP, Rengarajan A, Ciorba MA. Rectal budesonide and mesalamine formulations in active ulcerative proctosigmoiditis: Efficacy, tolerance, and treatment approach. Clin Exp Gastroenterol 2016;9:125-30.
2. Stanaland BE. Once-daily budesonide aqueous nasal spray for allergic rhinitis: A review. Clin Ther 2004;26:473-92.
3. Ellul-Micallef R, Hansson E, Johansson SA. Budesonide: A new corticosteroid in bronchial asthma. Eur J Respir Dis 1980;61:167-73.
4. Silverman J, Otley A. Budesonide in the treatment of inflammatory bowel disease. Expert Rev Clin Immunol 2011;7:419-28.
5. O'Donnell S, O'Morain CA: Therapeutic benefits of budesonide in gastroenterology. Ther Adv Chronic Dis 2010;1:177-86.
6. Todd GR, Acerini CL, Buck JJ, Murphy NP, Ross-Russell R, Warner JT, *et al*. Acute adrenal crisis in asthmatics treated with high-dose fluticasone propionate. Eur Respir J 2002;19:1207-9.
7. Gudimitla RB, Atmakuri LR, Jangala VR. A novel method for the estimation of Budesonide in human plasma by using LC-MSMS. Pharm Chem 2018;10:181-5.
8. Gupta M, Bhargava HN. Development and validation of a high-performance liquid chromatographic method for the analysis of budesonide. J Pharm Biomed Anal 2006;40:423-8.
9. Hou S, Hindle M, Byron PR. A stability-indicating HPLC assay method for budesonide. J Pharm Biomed Anal 2001;24:371-80.
10. Sonali RN, Amrita NB. Development of a validated specific HPLC method for Budesonide and characterization of its alkali degradation product. Can J Anal Sci Spectrosc 2008;53:113-22.
11. Sheela AS, Annapurna MM, Yasaswini RS. New validated stability indicating RP-UFLC method for the determination of budesonide. Res J Pharm Technol 2020;13:2819-24.
12. ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1), International Conference on Harmonization; 2005.
13. ICH Stability Testing of New Drug Substances and Products Q1A (R2), International Conference on Harmonization; 2003.

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