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Original Article

Development and validation of a rapid HPLC method for simultaneous analysis of budesonide and its novel synthesized hemiesters in colon specific formulations

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Abstract

A simple and reliable reversed-phase high performance liquid chromatographic (HPLC) method was developed, validated and applied for determination of budesonide and its novel synthesized hemiesters in colon specific formulations and dissolution media. The method was employed on a μ-Bondapak C_{18} column (250 mm × 4.6 mm, 5 μm) at ambient temperature. The mobile phase consisted of acetonitrile: monobasic potassium phosphate containing orthophosphoric acid (55:45, pH 3.2) at a flow rate of 1 ml/min. The UV detection wavelength was set at 244 nm and 50 μL of sample was injected into the HPLC system. Dexamethasone was used as the internal standard. The retention times for internal standard and budesonide were 4.5 and 7.2 min, respectively. The method was linear in the concentration range of 1-20 μg/ml of budesonide (R^2 >0.999). Limit of detection and limit of quantitation were 0.05 and 0.5 μg/ml, respectively. The method presented the requisite accuracy, selectivity, sensitivity and precision and showed good resolution for separation of the drug and related derivatives in the presence of excipients. The proposed method was successfully used for analysis of the drug and its derivatives in dissolution media and oral colon specific formulations prepared in our laboratory with enough reproducibility.

Keywords: Budesonide; Budesonide conjugates; Colon drug delivery; HPLC; Validation; Dissolution

INTRODUCTION

Corticosteroids are the most effective treatment for moderate to severe active ulcerative colitis (UC). However, unacceptable side effects and lack of benefits for maintenance therapy limit their long term use. The traditional corticosteroids are gradually being replaced by a new series of anti-inflammatory glucocorticoids with enhanced topical anti-inflammatory and less systemic activity such as budesonide (1). Budesonide, a non-halogenated corticosteroid, is an epimeric mixture of α- and β- propyl forms of 16,17α-butyl dienedioxy-11β,21-dihydroxy pregna-1,4-diene-3,20-dione (2).

Budesonide is commercially available in two different pH-dependent oral formulations, for active ileal and ileocecal Crohn's disease (3). Budesonide can be considered a good candidate for development of colon specific formulations for treatment of UC.

Our work was involved with design and development of oral colon specific systems for budesonide based on several different approaches including novel synthesized prodrugs, pH-time based systems, time-enzyme based systems and pH-time-enzyme based systems with a number of different excipients. Therefore, development of a suitable rapid HPLC method was required for analysis and characterization of budesonide,

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synthesized prodrugs and related compounds.

Different HPLC methods have been developed so far for analysis of budesonide in different media including determination of budesonide in broncho-alveolar lavage of asthmatic patients (4), simultaneous quantitation of formoterole and budesonide in Symbicort Turbuhaler (5), HPLC/RIA method for determination of the drug in plasma samples (6) and HPLC method for determination of epimers and impurities of budesonide (7).

There are some other studies on the stability of budesonide and determination of its impurities (2, 8-9). Most of the above mentioned methods are based on the separation of two epimers of budesonide, even though both epimers appear to have similar potency and pharmacological effects (8).

The main novelty of our developed HPLC assay compared to similar studies is co-elution of budesonide epimers without any prior extraction or derivatization. The method was also applicable to simultaneous analysis of budesonide and its synthesized prodrugs as well as degradation product upon alkaline hydrolysis. The developed HPLC method was successfully applied for *in vitro* evaluation of the designed formulations including sequential dissolution test in HCl 0.1 N, phosphate buffer saline (PBS, pH 7.4 and 6.8) containing rat cecal and colonic contents. Determination of drug in dissolution media containing rat cecal contents in the presence of different excipients has not yet been reported elsewhere. The method was validated for linearity, specificity, selectivity, precision, accuracy and robustness.

MATERIALS AND METHODS

Materials

Budesonide was a gift from Astra Zeneca (UK). Dexamethasone base used as internal standard was a gift from Alborz Bulk (Iran). HPLC grade acetonitrile and methanol and analytical grade hydrochloric acid, potassium dihydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide and sodium lauryl sulphate (SLS) were purchased from Merck (Darmstadt, Germany). Double distilled water was used in all experiments.

Apparatus

The chromatographic system used for determination of budesonide and related compounds was composed of a Waters 515 pump; Waters 2487 dual λ absorbance detector (Waters, USA). Data were integrated using Millennium®32 software for HPLC.

Chromatographic separation of budesonide and synthesized conjugates was conducted using the method previously published with some modifications (10). Waters μ-Bondapak C_{18} (250 mm × 4.6 mm, 5 μm particle size) analytical column was used for separation. The mobile phase constituted of acetonitrile–monobasic sodium phosphate buffer 0.025 M (55:45, pH 3.2). Before each run, the mobile phase was filtered under vacuum through a 0.45 μm filter (Millipore®, USA) and degassed in an ultrasonic bath for 10 min.

The mobile phase was pumped to the column at a flow rate of 1.0 ml/min and the HPLC system was operated at 25 ± 2°C. The injection volume was 50 μl and detection wavelength was set at 244 nm. Dexamethasone was used as internal standard (IS). Quantification was performed by measuring the ratios of peak area of the drug to that of the internal standard.

Calibration procedure

Primary standard stock solutions of budesonide and dexamethasone were prepared in acetonitrile at the concentrations of 0.2 mg/ml. Calibration solutions of budesonide in different dissolution media were prepared at concentrations of 1, 2, 4, 6, 8, 12, 16, 18 and 20 μg/ ml by serial dilution of the stock solution with the various dissolution media.

Standard solutions of synthesized hemiesters, budesonide hemisuccinate and budesonide hemiglutarate, were prepared in acetonitrile at concentrations of 10, 20, 50 and 100 μg/ml in the corresponding media. 10 ml of these standard solutions along with 6.0 ml of 0.2 mg/ml of dexamethasone solution in acetonitrile (internal standard) were added to a 100 ml volumetric flask and made up to volume with corresponding dissolution media. Solutions were prepared in triplicates and filtered through a 25 mm nylon membrane syringe filter (pore size 0.45 μm) before

analysis. Calibration solutions were prepared daily and analyzed immediately after preparation.

Method validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. To meet current pharmaceutical regulatory guidelines i.e. USP (11), ICH (12-13) and EP (14), a number of criteria such as specificity, linearity, precision, accuracy, sensitivity and robustness must be investigated in order to validate analytical methods.

For budesonide hemisuccinate and budesonide hemiglutarate, the validation parameters were only investigated in PBS (pH 6.8) contained rat cecal contents and 0.5% SLS as the most interfering medium.

System suitability

System suitability test as an integral part of method development was used to ensure adequate performance of the chromatographic system. Retention time (R_T), number of theoretical plates (N) and tailing factor (T) were evaluated for three replicate injections of the drug at a concentration of 8 μg/ml.

Specificity

To evaluate the specificity of the method, the absence of interference from the excipients used in the designed formulations (placebo samples) was investigated. The placebo solutions containing mannitol, microcrystalline cellulose, lactose, lactulose, pectin, dextran and hydroxypropyl methyl cellulose were prepared in acetonitrile. The placebo samples were analyzed in three replicates.

Selectivity

The selectivity of the analytical method was confirmed by the analysis of a solution containing 20 μg/ml of budesonide and a known added quantity of hemiesters. The ability to separate hemiesters from budesonide in the sample was demonstrated by assessing the resolution between the peaks corresponding to various substances. The tailing factor for budesonide was also determined.

Linearity

To accomplish this experiment, standard calibration curve in dissolution medium was prepared with different concentrations of budesonide in the range of 1-20 μg/ml or 1-10 μg/ml of hemiesters. Solutions were prepared in triplicate and injected into the HPLC column. The ratios of the peak areas of the drug or hemiestes to those of the internal standard were plotted against the corresponding concentrations to obtain the calibration curve. The linearity was evaluated by linear regression analysis calculated by the least square method (15).

Accuracy

Accuracy was measured as the percent of deviation from the nominal concentration. Five accuracy solutions of budesonide (9.6, 8.8, 8, 6.4 and 5.6 μg/ml) were accurately prepared and injected to the HPLC in triplicate. For budesonide hemiesters, four concentrations of 1, 2, 5 and 10 μg/ml were accurately prepared and injected into the HPLC column in triplicate. The response was used for back calculation of concentration according to the calibration curve equation. The back-calculated concentration was compared to the nominal concentration and the percent deviation was calculated (16).

Precision and repeatability

The precision of the HPLC method was determined by repeatability (intra-day) and intermediate precision (inter-day). Each level of precision was investigated by three determinations of three solutions of budesonide at concentrations of 1, 4 and 12 μg/ml and three determinations of four solutions of budesonide hemiesters at concentrations of 1, 2, 5 and 10 μg/ml.

Limit of detection and limit of quantitation (LOD and LOQ)

The LOQ and LOD of the method were determined based on signal to noise ratios. LOD of the method was determined as lowest budesonide concentration which can be detected by the HPLC system producing a signal-to-noise ratio of about 3. LOQ was the concentration produced a signal-to-noise ratio of 10 (2).

Robustness

The robustness of the HPLC method was evaluated according to the variety of conditions such as small changes in the percentage of mobile phase and buffer concentration, pH and flow rate of the mobile phase (17). Robustness was also studied by quantitation in another liquid chromatograph. Shimadzu liquid chromatograph equipped with a model LC-20AD pump with manual injector, Shimadzu SPD-20A Dual λ absorbance detector and data were integrated using ChromGate chromatography Data system LC solution version 1.2. The effect on retention time and peak parameters were studied.

Practical application of the method

Determination of drug content in conjugates

Budesonide-dextran conjugates were synthesized using two spacers (succinate and gluatarate) and different molecular weights of dextran by the method we described previously (18). Since there is no carboxylic acid group in budesonide structure (Fig. 1), succinic and glutaric anhydride were used to insert a carboxylic group at C_{21} of budesonide. To determine the budesonide content in dextran conjugates, basic hydrolysis of the conjugates was performed by the method previously reported for methylprednisolone succinate-dextran conjugates (19). 5.0 ml NaOH 0.1 N and 3.0 ml methanol were added to 5.0 mg of each conjugate and vortexed. There is an acetal group between C_{16} and C_{17} of budesonide which renders the drug susceptible to alkaline hydrolysis and degrades under basic condition. To control this degradation, solutions of budesonide at concentration range of 10-200 µg/ml were prepared in methanol and subjected to basic hydrolysis by adding 5.0 ml NaOH 0.1 N. All mixtures were then left at room temperature overnight. A 100 µl sample of each solution was mixed with 100 µl HCl 0.1 N and 50 µl of the resulting solution was injected into the HPLC. The concentration of the degraded product was determined and proportioned to the initial concentration of the drug. Finally, a standard curve was constructed to determine the drug content of each conjugate.

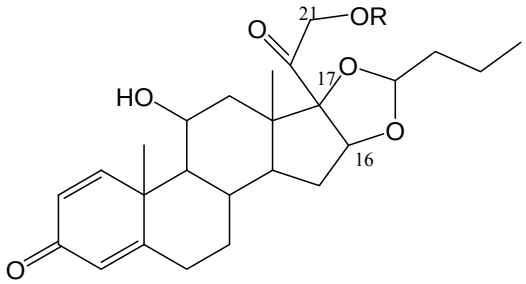
Budesonide assay

The assay test was performed to determine the budesonide content in the prepared formulations (pellets, tablets). Dosage forms were finely powdered and an accurately weighed amount of powder equivalent to 3 mg budesonide was used for the test. The powder was transferred into a 100 ml volumetric flask, 50 ml HCl 0.1 N was added and stirred for 1 h. Then 10 ml acetonitrile with 6.0 ml of 0.2 mg /ml of dexamethasone solution in acetonitrile as internal standard were added to the flask and made up to volume with HCl 0.1 N.

Release studies and dissolution condition

A modified USP dissolution apparatus II in accordance with the United States Pharmacopoeia (USP) (11), general methods was used. Sequential dissolution studies in three media were performed to evaluate the *in vitro* release profiles of the final formulations. The dissolution studies were started with 250 ml of 0.1 N HCl for 2 h. Afterward, 0.49 g $NaH_2PO_4.2H_2O$ was added and pH was adjusted to 7.4 with 5 N NaOH and maintained for 4 h. Finally, the dosage forms were transferred to 100 ml of PBS (pH 6.8) containing 4% rat caecal contentsfor 18 h under continuous supply of CO_2.

Release behavior of conjugates were measured in acetate buffer pH 4.4 containing gastric contents of rat, PBS (pH 7.4) containing contents of rat small intestine and PBS (pH 6.8) containing rat caecal contents.



Budesonide: R=H
Budesonide hemisuccinate: R=$CO(CH_2)_2COOH$
Budesonide hemiglutarate: R=$CO(CH_2)_3COOH$

Fig. 1. Chemical structure of budesonide and hemiesters.

All above mentioned buffer solutions contained 0.5% SLS. Both the esteric bond between the drug and the spacer and the bond between the spacer and the polymer are sensitive to hydrolysis and the developed method was used to determine the percentage of release of both drug and its hemiesters in dissolution media.

Short term stability of budesonide in dissolution media

Since the prepared formulations of budesonide were designed to prevent drug release in the stomach and small intestine, release studies were carried out in simulated stomach, small intestine and colon pH, consecutively. Therefore, the short term stability of budesonide in each dissolution medium was examined by keeping the stock solutions of budesonide at two studied concentrations, low (2 µg/ml) and high (20 µg/ml), at 37 ± 0.5°C for 24 h. The concentration of budesonide after the storage period was determined and compared to the concentration of the freshly prepared solutions.

RESULTS

A typical chromatogram of budesonide and budesonide hemiester and internal standard using the proposed method is shown in Fig. 2.a. The sharp and symmetric peak with an almost flat baseline for each compound facilitated the accurate measurement of peak areas. Under the described HPLC conditions, the tested compounds including internal standard, budesonide and its hemiesters were clearly separated and appeared at reasonable retention times.

System suitability

The results of system suitability which is summarized in Table 1 were within acceptablelimits (11). Acceptable limits for each parameter are also presented in Table 1.

Specificity and selectivity

The specificity test demonstrated that the used excipients, did not interfere with the peak of the main compound. Thus, the HPLC method is useful to quantify budesonide in the developed formulations and dissolution was observed after injection of the placebo samples (chromatogram not shown). There was no interference between the peaks of budesonide and hemiesters (Fig. 2a and 2b). The chromatograms of dissolution samples in the presence of rat caecal contents did not show any interfering peak (Fig. 3a and 3b).

Table 1. System suitability data. Mean± SD (n=5)

Parameter	Budesonide	Budesonide hemisuccinate	Budesonide hemiglutarate	Acceptable limits
Retention time (R_T)	7.25 ± 0.06 (RSD = 0.79)	9.16 ± 0.05 (RSD = 0.55)	10.25 ± 0.08 (RSD = 0.78)	RSD ≤ 1%
Theoretical plates (N)	3235 ± 114	2876 ± 203	2341 ± 289	*N* > 2000
Tailing factor (T)	1.85 ± 0.14	0.88 ± 0.08	1.09 ± 0.27	T ≤ 2

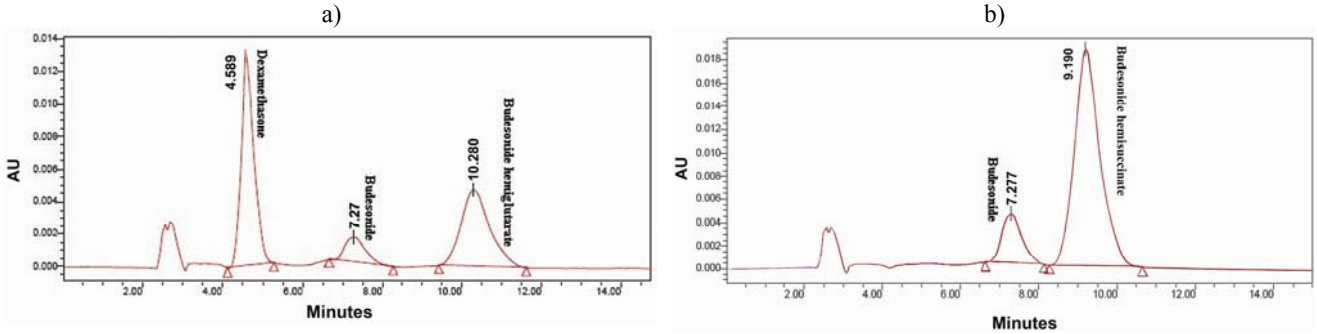


Fig. 2. Representative chromatograms for mixtures of a) budesonide, dexamethasone and budesonide hemieglutarate and b) budesonide and budesonide hemisuccinate.

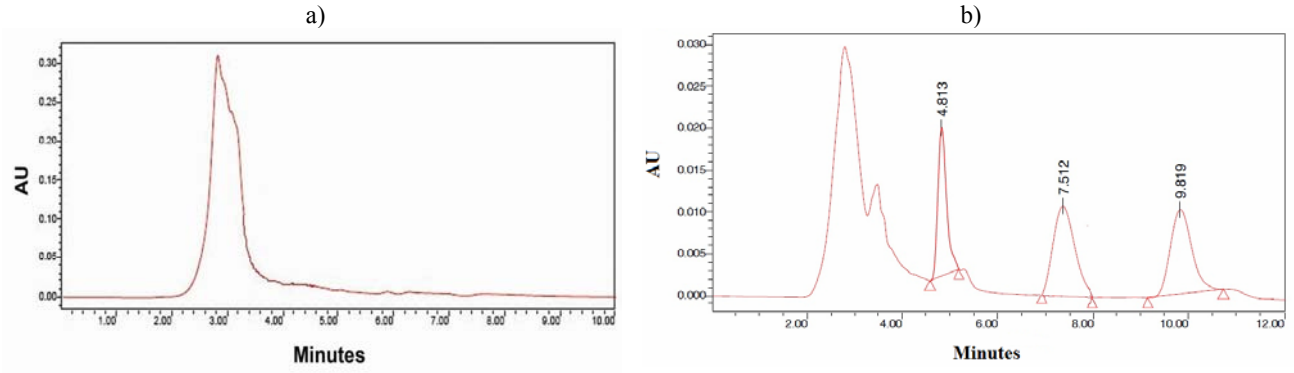


Fig. 3. HPLC chromatogram of a) blank dissolution sample of PBS (pH 6.8) and b) dissolution sample of conjugate containing budesonide hemiglutarate in 0.5% SLS and 4% rat cecal contents.

Table 2. Linear regression analysis data of budesonide and its hemiesters in standard solutions in different dissolution media (each point in the regression line is the mean of three experiments).

Compound	Calibration range (µg/ml)	R^2	Slope	95% CI of slope*	Intercept	95% CI of intercept
Budesonide						
HCl 0.1 N	1-20	0.9998	0.195	0.005	-0.044	0.033
PBS (pH 6.8)	1-20	0.9993	0.124	0.006	0.051	0.042
PBS (pH 7.4)	1-20	0.9997	0.186	0.006	0.043	0.041
Budesonide hemisuccinatePBS (pH 6.8)	1-10	0.9995	0.174	0.012	-0.034	0.068
Budesonide hemiglutaratePBS (pH 6.8)	1-10	0.9995	0.186	0.015	-0.011	0.082

*CI: Confidence interval

The results showed that the developed method was selective for determination of budesonide and its derivatives in formulations and dissolution samples.

Linearity

To assess the linearity, three calibration plots of budesonide and budesonide hemiesters in each dissolution medium were constructed in the concentration range 1-20 µg/ml and 1-10 µg/ml, respectively. The representative regression equations and correlation coefficients are presented in Table 2. These results show that the developed method was linear in the mentioned ranges with correlation coefficients of over 0.999 in most cases.

Accuracy

The accuracy of the developed method was evaluated by back calculation method. The results are expressed as percent recoveries of the components in the samples. As shown in Table 3, the overall recovery of budesonide and hemiesters in the samples was more than 95% (RSD <5%) which is sufficient for determining the drug in the dissolution media and the formulations.

Precision and repeatability

The results obtained for inter- and intra-day variability are presented in Table 4. In all cases for budesonide and hemiesters, the relative standard deviation (RSD) of inter- and intra-day variations was less than 5%.

LOD and LOQ

The LOD and LOQ and corresponding SDs for tested compounds are reported in Table 4. LOD and LOQ for budesonide were 0.05 and 0.5 µg/ml, respectively. The LOQ was calculated to show that the method could be applied for lower concentrations of analytes. LOQ were good enough for determination of the drug in the colon specific

Table 3. Evaluation of accuracy of the proposed method for determination of budesonide and hemiesters (n=3).

	Nominal concentration (µg/ml)	Real concentration (µg/ml)			
		Mean	SD	RSD	% Recovery
Budesonide					
	5.6	5.38	0.23	4.27	96.07
	6.4	6.17	0.10	1.62	96.41
	8.0	8.4	0.18	2.14	105.00
	8.8	8.70	0.26	3.02	98.86
	9.6	9.67	0.44	4.53	100.73
Budesonide hemisuccinate					
	1	1.01	0.05	4.57	95.90
	2	2.09	0.09	4.14	100.53
	5	5.23	0.15	2.87	101.18
	10	9.80	0.17	1.76	97.40
Budesonide hemiglutarate					
	1	0.97	0.02	2.13	96.80
	2	2.09	0.03	1.40	104.38
	5	5.00	0.14	2.87	99.76
	10	9.93	0.19	1.94	99.24

Table 4. Results from the determination of the inter- and intra-day variability of the method for determination of budesonide and its hemiesters (n=3).

Concentration (µg/ml)	Intra-day precision		Inter-day precision		Limit of Quantitation (µg/ml)	
	SD	RSD	SD	RSD	SD	RSD
Budesonide						
1	0.01	4.55	0.04	3.73	0.5	
4	0.01	2.19	0.01	2.22	0.04	8
12	0.06	4.44	0.06	4.40		
Budesonide hemisuccinate						
1	0.01	1.55	0.01	3.57	1	
2	0.01	1.55	0.02	3.65		
5	0.04	3.51	0.03	2.72	0.02	2
10	0.01	0.61	0.03	1.72		
Budesonide hemiglutarate						
1	0.01	4.63	0.01	2.22	1	
2	0.01	2.60	0.01	1.43		
5	0.04	4.26	0.03	2.90	0.05	5
10	0.03	1.32	0.04	2.00		

currently marketed dosage forms (20). LOD of budesonide in our study was lower than the value reported in a study on multiparticulate system for colon specific delivery which was reported to be 0.4 µg/ml (21).

Robustness

The results of the robustness of the method are presented in Table 5. Data showed that the minor changes in operating conditions did not result in huge difference in resolution and suitability of the separation parameters. Based on the robustness studies, in all studied conditions, the tailing factor of budesonide was less than 2. The recovery of budesonide and hemiesters was within the acceptable range and no significant change was observed when the critical parameters were modified. Quantitation in another liquid chromatograph demonstrated that although the retention time formulations containing 3 mg of budesonide which is the usual dose of budesonide in

Table 5. Robustness studies after small changes in chromatographic conditions

Parameter	Modification	Recovery of budesonide (%)
Mobile phase ratio (v/v) acetonitrile:buffer	55:45	99.42
	60:40	98.81
pH	3.2	99.42
	3.5	100.45
Flow rate (ml/min)	1	99.42
	1.5	101.80

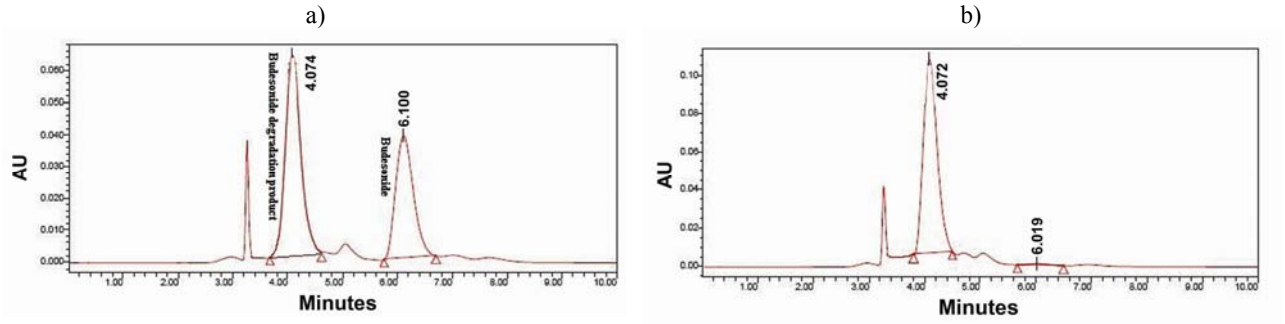


Fig. 4. HPLC chromatograms of progressive degradation of budesonide a) 0 min and d) 60 min after performing alkaline hydrolysis of BSD-70.

was slightly different (about 5.4 min versus 7 min), quantification of the drug was performed satisfactorily which again confirmed that the method was robust.

Practical application

The described HPLC method was used to evaluate the stability of the drug in the dissolution media while studying release profiles of prepared formulations. The stability test of the solutions showed that budesonide was stable in the dissolution media at least for 24 h at room temperature and can therefore be precisely analyzed in dissolution experiments.

Results of basic hydrolysis on the conjugates showed that budesonide is highly sensitive to strong alkaline pH and the acetal group is hydrolyzed under these circumstances. Fig. 4 shows the progressive degradation of budesonide after alkaline hydrolysis of conjugates. HPLC chromatograms show that degradation of the drug is completed after 60 min. Therefore, all solutions were stored at room temperature for 120 min after alkaline hydrolysis to ensure that the reaction is completed. A calibration curve was constructed based on the alkaline hydrolysis of the standard solutions of budesonide and the drug content in conjugates was determined accordingly. Linear regression analysis showed that in the concentration range of 10-200 µg/ml, the regression was significant (P<0.05) with R^2 of 0.9992. Coefficient of variation (CV%), which indicates precision of the method was less than 8% for all the studied concentrations. Error percentage indicating the accuracy of the method was less than 9%. Drug content in conjugates was determined by this constructed calibration curve (results are not shown).

The developed HPLC method was successfully applied to study the drug release profiles after incubating BSD-70 (conjugate synthesized using succinate spacer and dextran 70000) as the sample conjugates with contents of different segments of the rat gastrointestinal tract. The method was capable of simultaneous determination of the drug and its hemiesters. Fig. 5a and 5b present the release profiles of budesonide and budesonide hemisuccinate in three consecutive dissolution media. Our developed method could also be applied as a stability indicating method for quality control of formulations. Table 6 presents the results of stability studies of budesonide in different dissolution media.

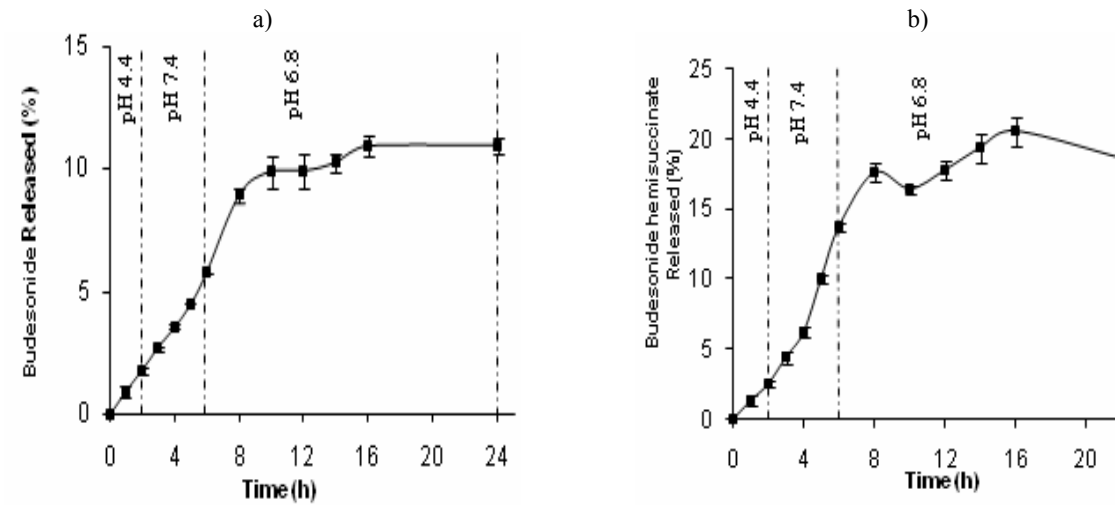


Fig. 5. Release profiles of a) budesonide and b) budesonide hemisuccinate, after incubation of BSD-70 with contents of different segments of the rat GI tract (n=3).

Table 6. Stability studies of budesonide in different dissolution media.

Buffer type	Budesonide concentration (µg/ml)	Peak ratio of budesonide /IS		
		At time 0	After 24 h	Shift (%)
HCl 0.1 N	2	0.152	0.159	4.600
	20	1.510	1.506	-0.260
Phosphate buffer pH 6.8	2	0.147	0.154	4.430
	20	1.452	1.494	2.890
Phosphate buffer pH 7.4	2	0.152	0.155	1.970
	20	0.912	0.925	-3.280

DISCUSSION

We developed and validated a simple and efficient reversed phase HPLC method for analysis of budesonide and synthesized hemiesters in the formulations and dissolution samples. Although the developed HPLC method presented in this study is based on the HPLC method described by Gupta et al. (10), but the drug and synthesized hemiesters could be determined simultaneously in biorelevant dissolution media and colon specific formulations. In addition, in the present study, an internal standard was used to provide higher accuracy and precision. Of several substances tested, dexamethasone was chosen as the most appropriate internal standard. This substance is stable and does not interfere with the excipients present in matrix of formulations and composition of the dissolution media. Indeed, in the developed method, dexamethasone was adequately separated from budesonide and hemiesters. Moreover, its elution time was shorter than that of budesonide which resulted in a short run time of less than 8 min.

The described HPLC method was successfully applied to the simultaneous determination of budesonide and hemiesters in different media. To the best of our knowledge, there is no published method for the simultaneous measurement of these compounds in the literature. Previously published methods for analysis of budesonide are limited to the determination of the drug in biological fluids such as broncho-alveolar lavage (4), determination of drug in a dosage form for example in Symbicort Turbohaler (5) or determination of impurities in tablet formulations (8). The most similar study to the current study is a method reported for determination of the drug in colon specific formulations in order to evaluate its stability under different conditions which still lacked quantification in different dissolution media without the risk of interference (10).

CONCLUSION

A simple reversed phase HPLC method for the analysis of budesonide and its synthesized hemiesters in the colon specific formulations and dissolution media was developed and validated. The proposed method is simple, accurate, precise, specific and linear over the analysis ranges and was able to resolve the drug from internal standard and other related compounds in a short analytical run time. Other advantages are determination of the drug in various formulations and matrices without any interfering peaks from formulation excipients. Simultaneous determination of budesonide and its hemiesters in synthesized conjugates was also assured by this method. The presented novel method merges the two peaks of the epimers in a single one and can be used for assay of drug in different dosage forms.

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REFERENCES

1. Hanauer SB. New steroids for IBD: Progress report. Gut. 2002; 51: 182-183.
2. Naikwade SR and Bajaj AN. Development of a validated specific HPLC method for budesonide and characterization of its alkali degradation product. Canad J Anal Sci Spect. 2008;53:113-122.
3. Fedorak RN and Bistritz L. Targeted delivery, safety, and efficacy of oral enteric-coated formulations of budesonide. Adv Drug Deli Rev. 2005;57:303-316.
4 Faouzi MA, Dine T, Luyckx M, Brunet C, Gressier B, Cazin M, et al. High-performance liquid chroma-tographic method for the determination of budesonide in bronchoalveolar lavage of asthmatic patients. J Chromatogr B: Biomed Sci and Appl. 1995;664:463-467.
5. Assi KH, Tarsin W, Chrystyn H. High performance liquid chromatography assay method for simultaneous quantitation of formoterol and budesonide in symbicort turbuhaler. J Pharm Biomed Anal. 2006;41:325-328.
6. Hochhaus G, Frehlich P, Hochhaus R, Mllmann A, Derendorf H, Mllmann HW. A selective HPLC/RIA for the determination of budesonide. J Pharm Biomed Anal. 1998;17:1235-1242.
7. Roth G, Wikby A, Nilsson L and Thalen A. High-performance liquid chromatographic determination of epimers, impurities, and content of the glucocorticoid budesonide and preparation of primary standard. J Pharm Sci. 1980;69:766-770.
8. Ferraboschi P, Bertacche V, Maccone I, Pini E, Ragonesi L, Venturini A, et al. Estimation and characterisation of budesonide tablets impurities. J Pharm Biomed Anal. 2008;47:636-640.
9. Hou S, Hindle M and Byron PR. A stability-indicating HPLC assay method for budesonide. J Pharm Biomed Anal. 2001;24:371-380.
10. Gupta M, Bhargava HN. Development and validation of a high-performance liquid chroma-tographic method for the analysis of budesonide. J Pharm Biomed Anal. 2006;40:423-428.
11. The united states pharmacopeia. United States Pharmacopeial Convention Inc., Rockville: 2007
12. International conference of harmonization Q2A: Text on validation of analytical procedures. US FDA federal register. 1995. p. 11260-11266.
13. International conference of harmonization Q2B: Validation of analytical procedures- methodology. US FDA federal register. 1997. p. 27463-27467.
14. The European Pharmacopoeia. Directorate for the Quality of Medicines of the Council of Europe, 5th ed. France: Strassbourg; 2005.
15. Daraghmeh N, Al Omari M, Badwan AA and Jaber AMY. Determinaton of sildenafil citrate and related substances in the commercial products and tablet dosage form using HPLC. J Pharm Biomed Anal. 2001;25:483-492.
16. den Brok MWJ, Nuijen B, Hillebrand MJX, Grieshaber CK, Harvey MD, Beijnen JH. Development and validation of an LC-UV method for the quantification and purity determination of the novel anticancer agent C1311 and its pharmaceutical dosage form. J Pharm Biomed Anal. 2005;39:46-53.
17. Emami J, Ghassami N, Ahmadi F. Development and validation of a new HPLC method for determination of lamotrigine and related compounds in tablet formulations. J Pharm Biomed Anal. 2006;40:999-1005.
18. Varshosaz J, Emami J, Tavakoli N, Fassihi A, Minaiyan M, Ahmadi F, et al. Synthesis and evaluation of dextran-budesonide conjugates as colon specific prodrugs for treatment of ulcerative colitis. Int J Pharm. 2009;365:69-76.
19. Mehvar R. Simultaneous analysis of dextran-methylprednisolone succinate, methylprednisolone succinate, and methylprednisolone by size-exclusion chromatography. J Pharm Biomed Anal. 1999;19: 785-792.
20. PDR. Physician desk reference. Montvale: Thomson Healthcare Inc.; 2007
21. Rodriguez M, Vila-Jato JL and Torres D. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. J Control Rel. 1998;55:67-77.