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Short communication

Development and validation of a high-performance liquid chromatographic method for the analysis of budesonide

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Abstract

A simple, rapid, and stability indicating reversed-phase high-performance liquid chromatography (HPLC) method of analysis for budesonide, a novel glucocorticoid prescribed for inflammatory bowel disease, was successfully developed. Budesonide is an epimeric mixture and both the epimers have similar anti-inflammatory activity. All the analytical methods reported in the literature are long and are based on separation of the epimers, thus our objective was to obtain a single sharp peak of the drug and to separate the drug peak from all the other degradation products. The method, was used to quantify budesonide in the developed formulation, employed a Kromasil® C_8, (150 mm × 4.6 mm) column with an isocratic mobile phase of acetonitrile–phosphate buffer (pH 3.2–0.025 M) (55:45 v/v), at a flow rate of 1.1 mL/min. Budesonide was detected by an ultraviolet detector at 244 nm. The method was validated for linearity, precision, repeatability, sensitivity, and selectivity. Selectivity was validated by subjecting stock solution of budesonide to acidic, basic, oxidative, and thermal degradation. The retention time of budesonide was about 4 min with symmetrical peaks. The method was linear over a concentration range 1–50 μg/mL ($R^2 = 0.9995$). The limit of detection of budesonide was 0.1 μg/mL and the limit of quantitation was 0.25 μg/mL. The peaks of the degradation products did not interfere with the peak of budesonide. The developed method was used to quantify budesonide in budesonide-loaded micro-particles. Excipients present in the micro-particles did not interfere with the analysis and the recovery of budesonide from micro-particles was quantitative.
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1. Introduction

Budesonide is a novel glucocorticoid with high topical anti-inflammatory activity and low systemic activity because of its high affinity to the steroid receptor and rapid conversion to metabolites with minimal or no steroid activity [1]. Budesonide is used not only for the treatment of asthma by inhalation administration (Rhinocort®) but also for treatment of inflammatory bowel disease (Entocort®).

Budesonide is an epimeric mixture of the α- and β-propyl forms of 16α,17α-butylidenedioxy-11β,21–dihydroxypregna-1,4-diene-3,20-dione. Oral administration of budesonide results in a bio-availability of approximately 10%. 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. The major metabolites, 6-β-hydroxybudesonide (5%) and 16-α-hydroxyprednisone (24%) have less than 1% of the glucocorticoid activity of unchanged budesonide. 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 feces [2].

A review of the literature revealed that all the methods published so far for the budesonide are based on separation of the two epimers [3–5] even though both the epimers are similar in their potency with respect to their anti-inflammatory activity [6]. Since our work involved the development of a colon-specific oral delivery system for budesonide our objective was to develop a simple, rapid, and stability indicating HPLC

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Table 1
Comparison of the published methods with the developed method

Parameters	Faouzi et al. [3]	Hou et al. [4]	Roth et al. [5]	Present method
Column	Hypersil C18, 5 uM 25 cm × 4.6 mm i.d.	Hypersil C18, 5 uM 12 cm × 4.6 mm i.d.	Hypersil C18, 5 uM 15 cm × mm i.d.	Kromasil® C_8, 15 cm × 4.6 mm
Mobile phase	Ethanol–water (43:57, v/v)	Acetonitrile–phosphate buffer (pH 3.2: 25.6 mM) (30:70, v/v)	Ethanol–acetonitrile–phosphate buffer (pH 3.4: 25.6 mM) (2:30:68, v/v/v)	Acetonitrile–phosphate buffer (pH 3.2–25 mM) (55:45 v/v)
Flow rate (mL/min)	1.0	1.5	1.5	1.1
Wavelength (nm)	240	240	240	244
Run time (min)	25	25	25	5

method of analysis. Thus, we developed a method, which coelutes both the epimers and also separate all the degradation products and other impurities from the main peak. A comparison of published methods with ours is mentioned in Table 1.

2. Material and methods

2.1. Chemicals and reagents

Budesonide was supplied by AstraZeneca (Lund, Sweden). Acetonitrile (HPLC grade), monobasic potassium phosphate (HPLC grade), and hydrochloric acid (HCl), 1 M, were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium hydroxide (NaOH) (USP grade) was purchased from Spectrum Quality Products (New Brunswick, NJ). All the chemicals received were used as received without any further purification.

2.2. Apparatus

A Hewlett Packard series 1100 (HP 1100) system equipped with a multiple-wavelength ultraviolet (UV) detector and a HP A.6.2 Chemstation integrator. The stationary phase was a Kromasil® C_8 column, (150 mm × 4.6 mm i.d., Column engineering, Ontario, CA).

2.3. Chromatographic conditions

The mobile phase consisted of acetonitrile–phosphate buffer (pH 3.2–0.025 M) (55:45 v/v). The flow rate was 1.1 mL/min. The wavelength of detection was 244 nm (λ_{max} for budesonide). The column temperature was ambient and the injection volume was 10 μl.

2.4. Procedures

2.4.1. Preparation of stock and standard solutions

Mobile phase 55% v/v of acetonitrile and 0.025 M phosphate buffer (pH adjusted to 3.2 using phosphoric acid) was used as a solvent for the preparation of both stock as well as standard solutions. The stock solution of budesonide was prepared by dissolving 10.0 mg of budesonide in 100.0 mL solvent, creating a 100 μg/mL solution of budesonide. This solution was diluted with the solvent as needed to prepare different standard solutions (50, 33.3, 20, 10, 5, and 1 μg/mL).

2.4.2. Linearity

Standard solutions (50, 33.3, 20, 10, 5, and 1 μg/mL), each in three replicates, were injected into the system. The method of linear regression was used for data evaluation. Peak area ratios of standard compounds were plotted against theoretical concentrations of standards. Linearity was expressed as a correlation coefficient; the value must be >0.9990.

2.4.3. Precision

The precision of the method was tested by injecting a standard solution of budesonide (50 μg/mL) five times. Peak areas were determined and compared. Precision was expressed as percentage relative standard deviation (R.S.D.); results must be less than 2% R.S.D.

2.4.4. Repeatability

Inter and intra-day variation was performed by injecting the standard solutions (50, 33.3, 20, 10, 5, and 1 μg/mL), each in three replicates, twice on the same day, and once on the next day. Peak areas were determined and compared by using one-way ANOVA.

2.4.5. Determination of limit of quantitation and limit of detection

The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte which can be quantitatively determined with suitable precision. The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution under the chromatographic conditions mentioned in Section 2.3. The lowest concentrations assayed where the signal/noise ratio was at least 10:1, this concentration was regarded as LOQ. The LOD was defined as a signal/noise ratio of 3:1.

2.4.6. Oxidation of budesonide

For oxidation of budesonide a 10.0 mL of stock solution of budesonide (100 μg/mL) was transferred to 20 mL amber-

colored volumetric flask and the volume was made up to 20 mL with 5% hydrogen peroxide (H_2O_2 solution). The flask was sealed and placed at 85 °C for 2 h and then cooled to room temperature, the volume was readjusted with 5% hydrogen peroxide, the solution was filtered through a 0.45 μm syringe filter, and injected into the liquid chromatographic system to detect peaks of the oxidation.

2.4.7. Thermal degradation of budesonide

For thermal degradation of budesonide a 10.0 mL of stock solution of budesonide (100 μg/mL) was transferred to 20 mL amber-colored volumetric flask and the volume was made up to 20 mL with the mobile phase. The flask was closed and placed at 85 °C for 2 h; then cooled to room temperature and the volume was readjusted with the mobile phase. The solution was filtered through a 0.45 μm syringe filter, and injected into liquid the chromatographic system to detect peaks of the thermal degradation.

2.4.8. Degradation of budesonide by acid

For acidic degradation of budesonide a 10.0 mL of stock solution of budesonide (100 μg/mL) was transferred to 20 mL amber-colored volumetric flask and the volume was made up to 20 mL with 1 M hydrochloric acid (HCl). The flask was sealed and placed at 85 °C for 2 h; then cooled to room temperature, and the volume was readjusted with 1 M HCl. The pH of the solution was adjusted to neutrality by adding 1 M sodium hydroxide (NaOH), the solution was filtered through a 0.45 μm syringe filter, and injected into the liquid chromatographic system in to detect peaks of the degradation products.

2.4.9. Degradation of budesonide by alkali

For basic degradation of budesonide a 10.0 mL of stock solution of budesonide (100 μg/mL) was transferred to 20 mL amber-colored volumetric flask and the volume was made up to 20 mL with 1 M NaOH. The flask was sealed and placed at 85 °C for 2 h; then cooled to room temperature; the volume was readjusted with 1 M NaOH. The pH of the solution was adjusted to neutrality by adding 1 M HCl, the solution was filtered through a 0.45 μm syringe filter, and injected into the liquid chromatographic system to detect peaks of the degradation products.

2.4.10. Assay of budesonide in micro-particles of pectin

Budesonide-loaded pectin micro-particles were prepared by incorporating a solution of budesonide into an aqueous pectin solution and then cross-linking it with calcium chloride (5%). The matrix was dried and the product was crushed and passed through sieve #80.

For extraction of budesonide weighed amount of micro-particles were suspended in 10 mL methanol. This suspension was stirred on a horizontal shaker for 8–12 h. Samples were collected, and filtered through a 0.45 μm syringe filter, and injected into the liquid chromatographic system for the analysis of budesonide. The drug content of each batch was determined as follows:

Table 2
Samples analyzed for drug recovery, containing varying amounts of pectin and budesonide

Sample	Pectin (% w/w)	Budesonide (mg)
1	LM 12 CG® (3%)	100
2	LM 20 AS® (3%)	150
3	LM 105 AS® (3%)	100
4	LM 12 CG® (5%)	200
5	LM 20 AS® (5%)	150
6	LM 105 AS® (5%)	200

$$\text{concentration of the sample} = \frac{(A \times B)}{C}$$

A: concentration of standard solution;
B: mean peak area of the sample;
C: mean peak area of the standard solution.

Table 2 summarizes the contents of each batch and gives the percentage weight of each ingredient. Percent recovery of budesonide from micro-particles was calculated by dividing the determined amount of budesonide by the theoretical amount.

3. Results and discussion

The molecular structure of the budesonide is presented in Fig. 1. The proportions of the organic and aqueous phases were adjusted to obtain a rapid and simple assay method for budesonide with a reasonable run time, suitable retention time and the sharpness of the peak. Under the experimental conditions, the chromatogram of budesonide (Fig. 2) showed a single peak of the drug around 4 min.

3.1. Linearity and range

The standard curves for budesonide were linear over the investigated range (1–50 μg/mL) with a percent relative standard deviation (% R.S.D.) of less than 2% based on three successive readings Table 3. A correlation coef-

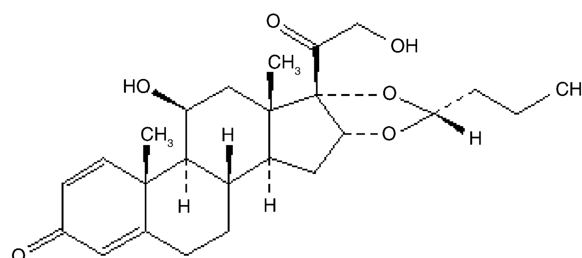

Fig. 1. Structure of budesonide.

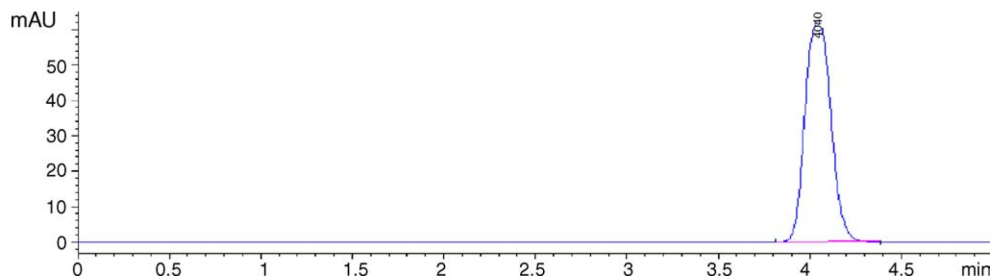

Fig. 2. A typical chromatogram of budesonide.

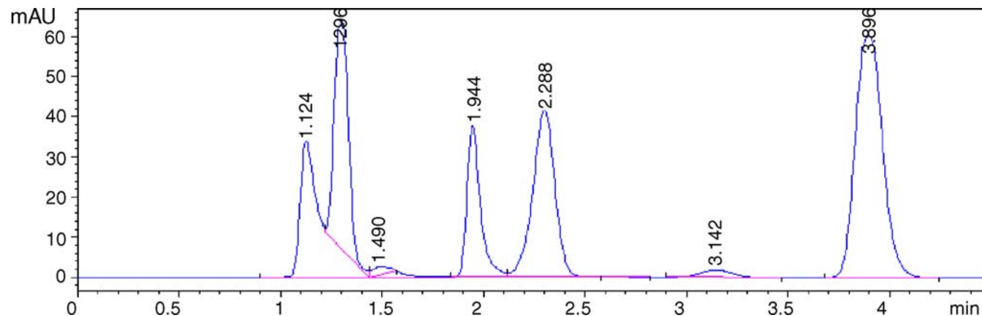

Fig. 3. A chromatogram of budesonide exposed to 1 N NaOH and then spiked with budesonide solution.

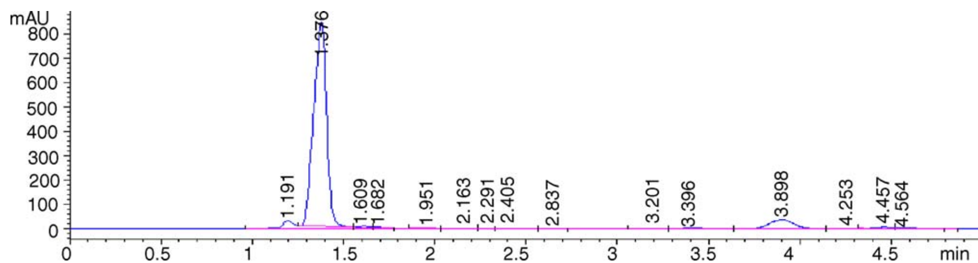

Fig. 4. A chromatogram of budesonide exposed to 5% H_2O_2.

ficient of 1 suggests that the developed HPLC method had an excellent linearity over the concentration range of 1–50 μg/mL.

3.2. Precision

Results for precision tests performed on each of the standard solution of budesonide of 50 and 1 μg/mL showed that the % R.S.D. was less than 0.29 for the former and 0.44 for the latter. Therefore, the system precision is considered to be satisfactory.

3.3. Repeatability

The mean peak area, the standard deviation and the relative standard deviation were calculated and are reported in Table 4. After injecting budesonide standard concentrations (50, 33.3, 20, 10, 5, 1 μg/mL) in triplicate, on same day,

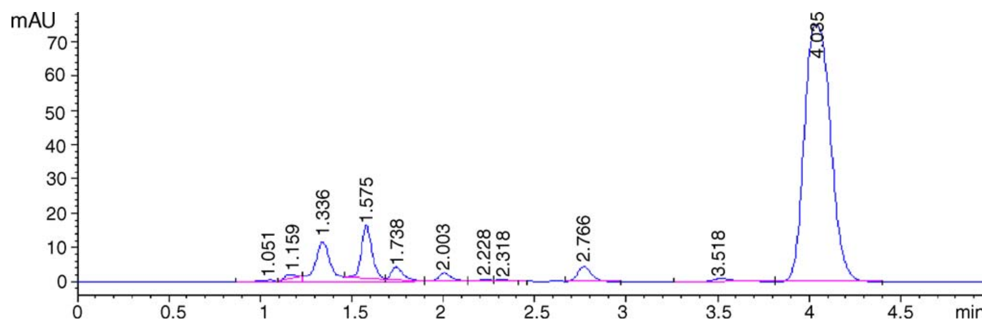

Fig. 5. A chromatogram of budesonide exposed to 1 M HCl.

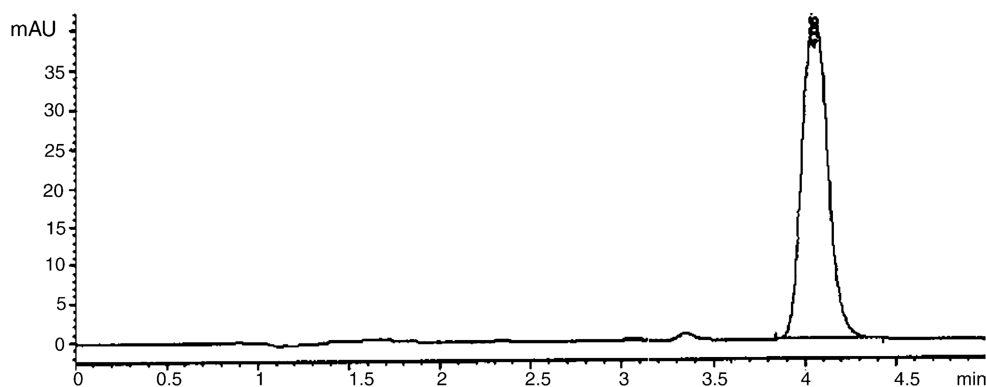

Fig. 6. A Chromatogram of budesonide exposed to heat.

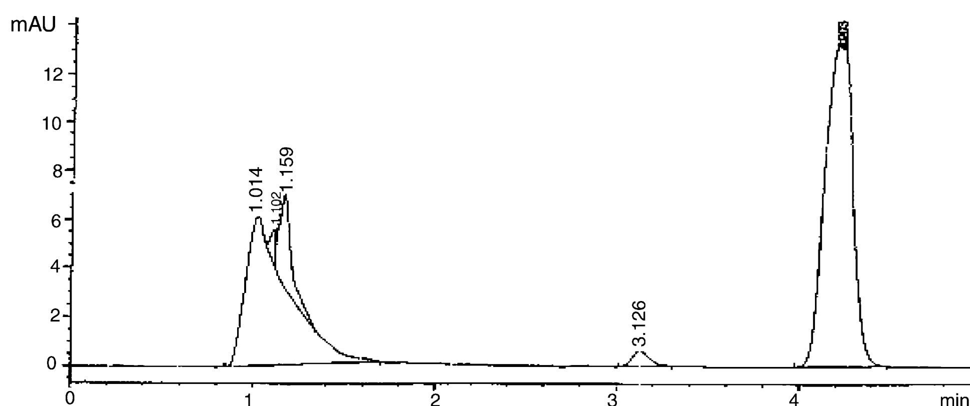

Fig. 7. A chromatogram of budesonide after extraction from pectin micro-particles.

Table 3
Range of linearity

Concentration (μg/mL)	Peak area (mean and S.D.)[a]
50	919.32 ± 2.63
33.3	612.02 ± 1.35
20	367.05 ± 1.23
10	178.97 ± 0.85
5	91.92 ± 0.23
1	19.12 ± 0.35

[a] Mean ± S.D. ($n = 3$).

and on two consecutive days the peak areas were compared. The relative standard deviation for the peak areas on same day, and on two consecutive days, were found to be with in the acceptable limits less of than 2%. Moreover one-way ANOVA was used to compare the data. p-Value obtained was 0.936 ($p > 0.05$).

Table 4
System repeatability

Concentration (μg/mL)	Day 1 peak area[a]	Day 1 peak area[a]	Day 2 peak area[a]
1	20.52 ± 0.46	20.53 ± 1.43	20.73 ± 0.97
5	91.70 ± 1.53	91.79 ± 2.34	92.59 ± 1.97
10	178.93 ± 2.91	178.53 ± 3.43	179.59 ± 2.45
20	367.06 ± 1.49	367.57 ± 2.78	369.43 ± 3.59
33.3	612.96 ± 2.54	613.26 ± 4.53	624.51 ± 2.68
50	943.03 ± 5.95	944.32 ± 2.34	959.60 ± 3.43

[a] Mean ± S.D. ($n = 3$).

3.4. Limit of quantitation and limit of detection

Under the developed HPLC conditions, the limit of quantitation was determined to be 0.25 μg/mL with a % R.S.D. of less than 1.33% for three successive injections of the sample. Also, the limit of detection was found to be 0.1 μg/mL.

3.5. Selectivity

Fig. 2 shows the typical chromatogram of budesonide. Figs. 3–6 are the chromatograms of budesonide samples subjected to various stress conditions then analyzed using chromatographic conditions mentioned in Section 2.3. The method proved to be both selective as well as stability indicating. There was very little degradation of budesonide either by heat, oxidation or acid hydrolysis. Budesonide did degrade by alkaline hydrolysis. The peaks of the degradation products were separate from the drug peak.

3.6. Recovery

The results indicate that the developed method can be used to quantify budesonide from budesonide-loaded pectin

Table 5
Assay of budesonide in micro-particles of pectin

Sample	Budesonide (theoretical value) (mg)	Budesonide (determined value) (mg)	Recovery (%)
1	100	102.4	97.7
2	150	148.7	100.9
3	100	99.2	100.8
4	200	198.4	100.8
5	150	148.9	100.7
6	200	197.6	101.2

micro-particles. The recovery of budesonide from the micro-particles was quantitative (Table 5), and there was no interference from the excipients present in the dosage form (Fig. 7) when compared to the control (figure not shown).

4. Conclusions

A simple, rapid and stability indicating HPLC method was developed and validated. Total run time was 5 min. The method is rapid, easy, and reliable compared to other methods published for the analysis of budesonide (Table 1). The method has been proven to be stability indicating. The peaks of the degradation products, as well as those of the excipients in the budesonide-loaded pectin micro-particles did not interfere with the analysis. The recovery of budesonide from the micro-particles was essentially quantitative.

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