ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
24 (2001) 371–380

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

A stability-indicating HPLC assay method for budesonide☆

Shuguang Hou, Michael Hindle *, Peter R. Byron

Department of Pharmaceutics, Virginia Commonwealth University, Box 980533, Richmond, VA, 23298-0533, USA

Received 28 January 2000; received in revised form 15 July 2000; accepted 17 July 2000

Abstract

The official (European) pharmacopeial assay for budesonide was found to be non-specific and non-stability-indicating when used to qualify several batches of pharmaceutical grade drug substance from different sources. In contrast, the most widely cited HPLC method in the literature was found to be specific and stability-indicating with respect to drug substance stored in the dry state. However, that method failed the pharmacopeia's assay system suitability requirements because of peak tailing. Moreover, it was unable to detect or resolve two major degradation products which resulted from drug storage in non-aqueous solution. A new stability-indicating HPLC method described here overcomes these problems. This method used a Hypersil® C18 column with a mobile phase consisting of ethanol–acetonitrile–phosphate buffer (pH 3.4; 25.6 mM) (2:30:68, v/v/v), a flow rate of 1.5 ml/min and UV detection at 240 nm. The purity of budesonide EP and its impurity profile (related substances) were tested using the new assay method, and the results compared to those from the two other methods described above. Solid-state and solution stressed stability samples were used to evaluate all methods. Using the novel method, the epimers of budesonide, their related impurities and degradation products were separated successfully. Validation studies demonstrated that the novel method possessed a linear UV response, good system precision and accuracy, high sensitivity and specificity for budesonide. The novel method will be used for future studies of budesonide's degradation kinetics.

Keywords: Budesonide; Impurities; Propylene glycol; HPLC; Stability

1. Introduction

Budesonide is a potent glucocorticosteroid with a high topical anti-inflammatory activity and low systemic effects, it has been widely used for the treatment of asthma by inhalation administration [1–3]. Structurally, budesonide is a 16α, 17α-acetal prepared by reaction of the 16α, 17α-dihydroxy steroid (16α-hydroxyprednisolone) with *n*-butyraldehyde. Due to the introduction of the alkyl chain at the C_{22} atom, budesonide is a mixture of two epimers (22*R* and 22*S*) (Fig. 1) [4]. Both epimers appear to have similar pharmacological effects, however in-vitro studies suggested that the *R*-epimer was two to three times more

☆ Presented in part at the American Association of Pharmaceutical Scientists Annual Meeting, New Orleans, LA, November, 1999.

* Corresponding author. Tel.: +1-804-8286497; fax: +1-804-8288359.

E-mail address: mhindle@hsc.vcu.edu (M. Hindle).

0731-7085/01/$ - see front matter
PII: S0731-7085(00)00424-6

potent with respect to its anti-inflammatory effects [5]. Although budesonide has been widely used in the USA, the only pharmacopoeial monograph for budesonide appears in the European Pharmacopoeia (EP). The EP monograph for budesonide states that the R/S epimer ratio should be within the range of $60 \sim 49/40 \sim 51$ [6].

Presently, a novel capillary aerosol generator is being studied which is capable of producing submicrometer aerosols from solutions of budesonide in propylene glycol [7]. In order to confirm the stability of these formulations, and also of budesonide in the solid state, a stability-indicating assay is required. This method should be sensitive and specific for the quantification of budesonide, and be selective for the separation of the epimers of budesonide and their related impurities and degradation products. A review of the literature revealed that two main assay methods had been employed for the quantification of budesonide and the separation of its epimers and impurities. Wikby et al. [8] 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 [8,9]. They also evaluated the effects of different organic modifiers added to water as the mobile phase on a C18 column, and observed that ethanol produced the optimal separation of budesonide epimers. Roth et al. [10] 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 [10]. Although Roth et al.'s reversed-phase HPLC method has been employed widely for clinical pharmacokinetic studies [5,11–15], the European Pharmacopoeia describes an alternative reversed-phase HPLC method as its official assay for this drug substance. This method employs a C18 column and a mobile phase of acetonitrile/phosphate buffer pH 3.2 to determine the R/S epimer ratio, the purity of budesonide and its related substances [6]. We have been unable to find a published report of the development and validation of this official EP method.

Although both assays have been used for the determination of budesonide epimers, there are no published reports on their suitability as stability-indicating methods. In this paper, Roth et al's HPLC method and the official European Pharmacopoeia method (designated as 'EtOH method' and 'EP method', respectively) are shown to be deficient as stability-indicating techniques. We report a comparison of these two methods for the quantification of budesonide and the separation of its related substances in solid state and propylene glycol solutions, alongside the development and validation of a novel stability-indicating technique.

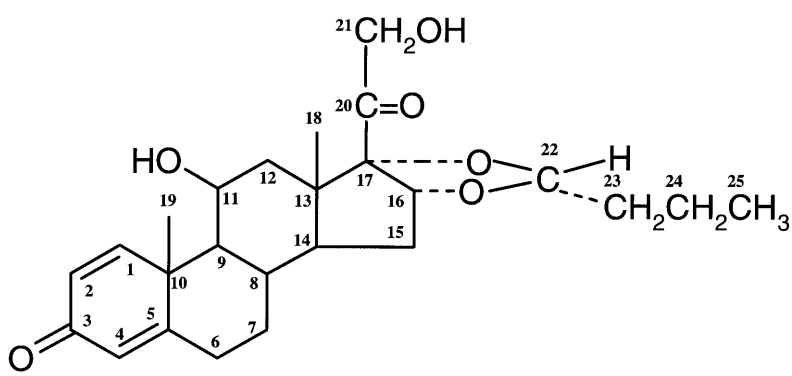

Fig. 1. The chemical structure of epimer 22R of budesonide. The S epimer inverts the position of the proton and the propyl grouping on C-22.

Table 1
The chromatographic conditions and parameters of the three HPLC assay methods

Parameters		EtOH method[a]	EP method[b]	Novel method
Column		Hypersil C18 column 5 μm 25 cm × 4.6 mm i.d.	Hypersil C18 column, 5μm 12 cm × 4.6 mm i.d.	Hypersil C18 column, 5 μm 15 cm × 4.6 mm i.d.
Mobile phase		Ethanol–water (43:57, v/v)	Acetonitrile–phosphate buffer (pH 3.2; 25.6 mM) (30:70, v/v/v)[c]	Ethanol–acetonitrile –phosphate buffer (pH 3.4; 25.6 mM) (2:30:68, v/v/v)
Flow rate (ml/min)		1.0	1.5	1.5
Wavelength (nm)		240	240	240
Retention time (min)	*R*-epimer	16	16	18
	S-epimer	18	18	20
Mean number of theoretical plates (SD; $n = 6$)	*R*-epimer	7500 (300)	8400 (300)	10000 (200)
	S-epimer	8200 (700)	8300 (200)	9950 (100)
Mean height of plate (SD; $n = 6$) (μm)	*R*-epimer	33 (1)	15 (1)	14 (1)
	S-epimer	30 (1)	15 (1)	15 (1)
Mean symmetry factor (SD; $n = 6$)	*R*-epimer	1.6 (0.1)	1.0 (0.0)	1.0 (0.0)
	S-epimer	1.6 (0.1)	1.0 (0.0)	1.0 (0.0)
Mean resolution between two epimers (SD; $n = 6$)		2.5 (0.0)	2.3 (0.0)	2.5 (0.0)

[a] Reference 10.
[b] Reference 6.
[c] Ratio adjusted to provide 16 min retention time for *R* epimer as required in reference.

2. Experimental

2.1. Materials

Two batches of budesonide EP (Batch numbers: NM0172 and NT0038) were purchased from Spectrum Quality Products Inc. (New Brunswick, NJ). A sample of budesonide EP was obtained from a Pulmicort Turbuhaler® (Lot number: YD291, Astra Draco, Lund, Sweden; 9 months prior to its expiry date). A primary reference standard of budesonide CRS (Lot number: 1, 99.9% purity) was purchased from the European Pharmacopoeia. Propylene Glycol USP, ethanol (HPLC grade), acetonitrile (HPLC grade), *o*-phosphoric acid 85% (HPLC grade), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and sodium hydroxide 1 N solution were purchased from Fisher Scientific Co. (Swannee, GA).

2.2. Instrumentation

The HPLC system consisted of a Shimadzu LC-10AD VP liquid chromatography pump, a Shimadzu SIL-10AD VP auto-injector, an Applied Biosystems 783A UV detector, and a Shimadzu C-R5A Chromatopac integrator.

2.3. Chromatographic conditions

Table 1 summarizes the chromatographic conditions for the EtOH, EP and novel assay methods, respectively. All chromatographic analyses were performed at ambient temperature.

2.4. Budesonide EP purity assay

Using budesonide CRS as a primary reference standard, the purity of two batches of budesonide EP (Batch numbers: NM0172 and NT0038, Spectrum) were assayed using the three HPLC meth-

ods following the general procedures described in the EP monograph for budesonide. Thus, budesonide samples were dried to constant weight at 100°C in a vacuum oven before use. Test solutions of budesonide EP (500 μg/ml) were prepared by transferring 25.0 mg of budesonide EP into a 50-ml volumetric flask, dissolving and diluting to volume in the respective mobile phase. The reference solution of budesonide CRS (500 μg/ml) was produced using the same procedures. Solutions were allowed to stand for at least 15 min before use. The following procedure was employed for each HPLC assay method: 20 μl of budesonide CRS reference solution was first injected six times to ensure that the relative standard deviation of the total peak areas (the sum of the areas under the two epimer peaks) for budesonide was $< 1.0\%$ [6]. The budesonide EP test solution and the budesonide CRS reference solution were then injected alternately six times. The mean (SD) purity of budesonide EP was calculated from the total peak area due to the budesonide epimers compared with that due to the budesonide CRS epimers. To satisfy the EP purity requirement, budesonide should contain not less than 98% and not more than 102% of a mixture of the *R* and *S* epimers of budesonide.

2.5. Budesonide EP related substance test

The EP related substance test was performed using each of the three HPLC assay methods. The EP specification for budesonide requires that (1) the area of any individual impurity peak following a 500 μg/ml injection, should be less than the total peak area of the budesonide epimers in a chromatogram obtained from a 2.5 μg/ml injection (e.g. no single impurity $\geq 0.5\%$ with respect to budesonide) and (2) the sum of the peak areas due to impurities, following a 500 μg/ml injection, should be less than the total peak area of the budesonide epimers in a chromatogram obtained from a 7.5 μg/ml injection (e.g. not more than 1.5% total impurities). Accordingly, two budesonide solutions with concentrations of 7.5 and 2.5 μg/ml were prepared by appropriate dilution with mobile phase of the 500 μg/ml test solution of budesonide EP. Twenty microlitres of the 500, 7.5 and 2.5 μg/ml solutions were injected into each of the separate HPLC assay systems.

2.6. Assessment of the individual HPLC methods as stability indicating assays

A stressed solid state stability sample of budesonide was used to evaluate the three HPLC methods. Budesonide powder (Lot number: YD291, Astra Draco) was exposed to environmental conditions of 40°C and 85% RH for 9 months. Test solutions containing 500 μg/ml of this powder were prepared in mobile phase and assayed using each of the three methods.

Stressed solution stability samples of budesonide were produced using 0.5% w/w budesonide (Batch number: NT0038, Spectrum) in propylene glycol solutions, stored at 40°C for 3 months with continuous oxygenation. Test solutions were prepared in mobile phase as if they contained 500 μg/ml of non-degraded budesonide. These were assayed using each of the three methods.

2.7. Validation of the novel HPLC method

A stock solution of budesonide EP was prepared with a concentration of approximately 1000 μg/ml. Six standard solutions ranging from 2.5 to 25 μg/ml were prepared by volumetric dilution with mobile phase. Each standard solution was assayed three times, budesonide peak area was calculated as the sum of the peak areas of the *R* and *S* epimers of budesonide. A calibration curve for the mean (SD) budesonide peak areas against original budesonide concentration was constructed. Linear regression analysis was performed to calculate the regression equation and the limit of detection (LOD) from

$$\mathrm{LOD} = 3.3\sigma/S \quad (1)$$

where σ was the standard deviation of the y-intercept and S was the slope of the calibration curve.

A 10.0 μg/ml budesonide EP standard solution was used to test system precision. This solution was injected six times and 'within day' precision calculated as the relative standard deviation (RSD, $n = 6$) of the total peak areas due to budesonide *R* and *S* epimers. The same solution was

assayed again for six injections on the next day, and 'between day' precision calculated as the relative standard deviation (RSD, $n = 12$) of the peak areas due to drug substance. In order to evaluate the accuracy of the assay method for the determination of budesonide in propylene glycol formulations, a solution of known concentration (0.5% w.w) was prepared accurately in propylene glycol. About 100 mg of this solution was accurately weighed, transferred into a 50 ml volumetric flask and diluted to volume with the mobile phase to produce a test solution of about 10 μg/ml. Six such test solutions were prepared and assayed using the novel HPLC method. The measured concentration of budesonide in propylene glycol solution was calculated from the calibration curve. Accuracy, expressed as the percentage difference from the nominal value (%DFN, $n = 6$), was obtained by comparing the mean measured concentration of budesonide with its nominal value.

3. Results and discussion

3.1. Budesonide EP purity assay and related substances tests

The pharmacopoeial specifications for assay validation require that (1) the resolution between the two epimer peaks of budesonide should not be less than 1.5, (2) the number of theoretical plates for epimer *R* be at least 4000 and (3) the symmetry factor for epimer *R* be less than 1.5. Table 1 summarizes these parameters for the three assay methods used in this study. Both the EP method and the novel HPLC method satisfied these requirements. The EtOH method failed to meet the *R*-epimer symmetry factor requirement and showed significant tailing for both epimer peaks (symmetry factor = 1.6 in each case). In addition, the plate height for each budesonide epimer with the EtOH method was double that following either the EP or the novel method (indicating a lower separation efficiency). Wikby et al. (1978) concluded that this was a consequence of the higher viscosity of the ethanol–water mobile phase [8]; lower solute diffusion coefficients causing greater plate heights.

The results from each of the three HPLC methods are shown in Table 2. Both batches of drug met EP purity specifications (98–102%; [6]) and there was no significant difference observed between the purity determinations using the three HPLC methods for Batch NM0172 (ANOVA, $\alpha = 0.1$). This batch of budesonide also passed the EP related substances test when analyzed using each of the three HPLC methods. This was not the case for Spectrum batch NT0038 which clearly failed the EP related substances specification when tested using the novel method (Table 2), despite the supplier's valid claim that the batch was of EP quality. The reasons for this observation relate to the EP method's failure to resolve a significant impurity from the R budesonide peak, as discussed further below.

Figs. 2 and 3 compare the chromatographic separations for each batch of budesonide EP using the three HPLC methods (baseline separation of the *R* and *S* epimers was not observed in these chromatograms due to the high concentration of the test solution). Although the resolution for the two epimers of budesonide was consistently > 2.3 (Table 1; EP states that > 1.5 is acceptable), the

Table 2
Mean percentage purity (SD; $n = 6$) (Spectrum batches NM0172 and NT0038) and results of related substances test

Method	Budesonide EP (NM0172)		Budesonide EP (NT0038)	
	Mean purity (SD)	Related substances	Mean purity (SD)	Related substances
EtOH	99.4 (0.1)	Pass	99.1 (0.1)	Pass
EP	99.6 (0.3)	Pass	99.8 (0.3)	Pass
Novel	99.5 (0.1)	Pass	99.1 (0.3)	Fail

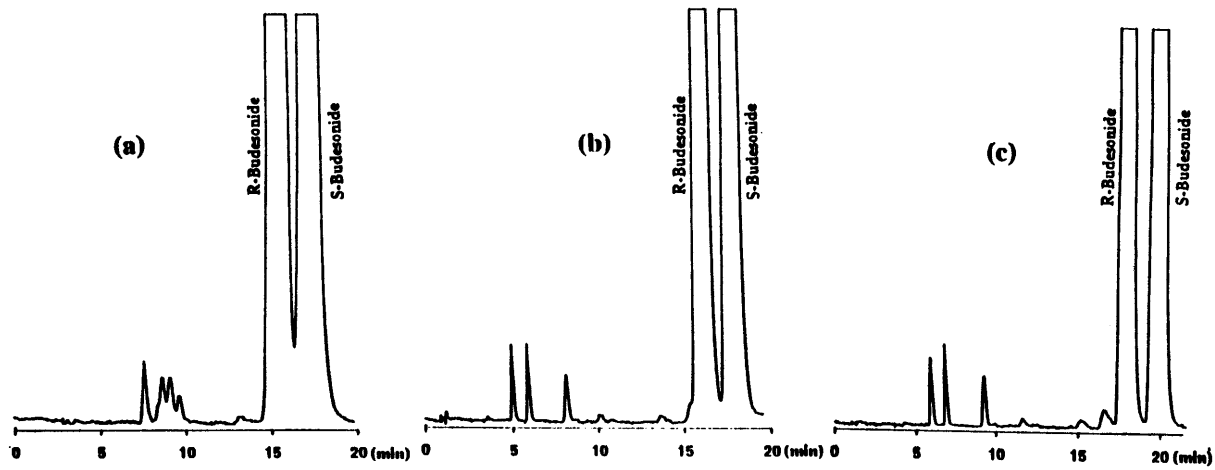

Fig. 2. The chromatograms of budesonide EP (Batch number: NM0172, Spectrum) ($C = 500$ μg/ml) assayed using (a) EtOH, (b) EP and (c) novel methods.

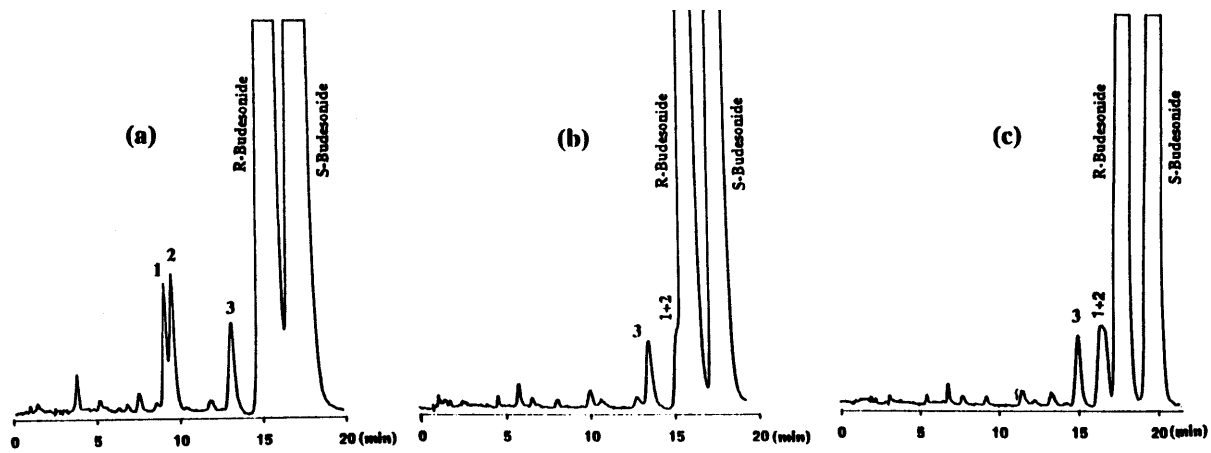

Fig. 3. The chromatograms of budesonide EP (Batch number: NT0038, Spectrum) ($C = 500$ μg/ml) assayed using (a) EtOH, (b) EP and (c) novel methods.

EtOH method showed poor impurity separation. This was improved by using the EP method, however a small shoulder was evident on the *R*-epimer peak for budesonide. Using the novel method, this small shoulder was fully resolved from the *R*-epimer and, in addition, there was an excellent separation of all the impurities.

Spectrum batch NT0038 appeared to be significantly more pure (99.8%) when assessed using the EP method as compared to either the EtOH (99.1%) or the novel (99.1%) technique ($P < 0.05$; unpaired *t*-test). The impurity profile of this batch of drug substance was substantially different to that of NM0172 (Figs. 2 and 3). Using the EtOH method, three main impurity peaks were observed in NT0038 which separated from the epimers of budesonide (impurity peaks labeled as 1, 2, and 3). Using the EP method, however, only one of these was resolved from budesonide (impurity peak 3) while impurities 1 and 2 co-eluted as a shoulder on the *R*-epimer peak. The EP method was clearly not specific with respect to budesonide and, due to the different separation mechanisms employed by the EP and EtOH techniques, the

elution order of the major impurities in this batch was altered (peak fractionation and re-injection techniques were employed in order to correlate the impurity peaks observed in these chromatograms; Fig. 3). Use of the EP method resulted in over-estimation of budesonide purity due to inclusion of these significant co-eluting peaks along with the *R*-epimer of budesonide when Spectrum Batch NT0038 was assayed.

The novel assay method combined the separation mechanisms of the EtOH method and the EP methods. The addition of a small percentage of ethanol (2%) to the EP mobile phase enabled successful resolution of impurity peaks 1 and 2 as a single peak from the *R*-epimer of both batches of budesonide EP (Figs. 2 and 3). Furthermore, the use of this novel method caused Batch NT0038 to fail EP specifications when impurities 1 and 2 were resolved and quantified at 0.7% with respect to the drug substance. However, this 'failure' of Batch NT0038 on the basis of its related substances was illusory and due only to the novel technique's inability to separate, and thus separately quantify, impurities 1 and 2 (Fig. 3a) at levels $< 0.5\%$ with respect to budesonide.

To confirm our observations with respect to impurity peaks 1, 2, and 3 in Batch NT0038, a LC-MS method (ZMD 4000, Waters Corp.) was employed for preliminary identification of these impurities following the EtOH and novel HPLC separations. The molecular weights of impurity peaks 1 and 2 were shown to be identical (428.5 daltons) for both methods, indicating that these impurities were probably epimers. A preliminary identification of impurities 1 and 2 is the two epimers of 21-dehydro-budesonide.

3.2. Assessment of the HPLC methods as stability-indicating assays for budesonide

Accelerating the degradation of budesonide in the solid state or in non-aqueous solution caused substantial alteration in its chromatograms (Figs. 4 and 5). Budesonide powder (Lot number: YD291, Astra Draco) stored at 40°C and 85% RH for 9 months was relatively stable; the percentage budesonide remaining was 97.2, 97.4 and 97.6%, respectively, following EtOH, EP and novel assay methods. Fig. 4 shows the chromatograms obtained using the three HPLC methods. Only one major degradation product ($> 0.5\%$) was observed in the solid state and all three methods were capable of resolving this from budesonide. Once again however, with this batch of budesonide from a separate supplier, the EP method failed to resolve impurities 1 and 2 from the *R*-epimer. The separation of budesonide epimers, impurities and degradation products appeared to be optimal using the novel method.

For budesonide stored in propylene glycol solution at 40°C for 3 months, the percentage budesonide remaining was 87.4, 92.3 and 88.7%,

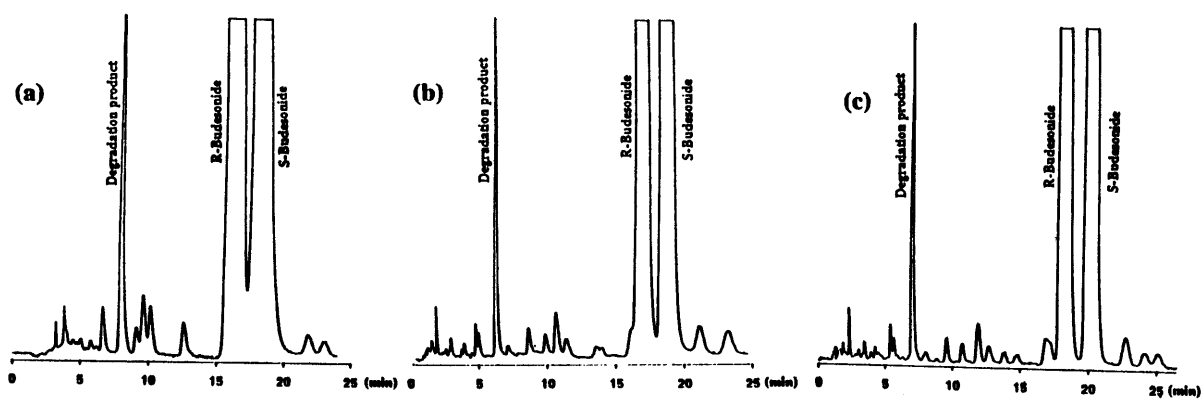

Fig. 4. The chromatograms of budesonide EP (lot number: YD291, Astra) solid-state stability sample ($C = 500$ μg/ml) assayed using (a) EtOH, (b) EP and (c) novel methods.

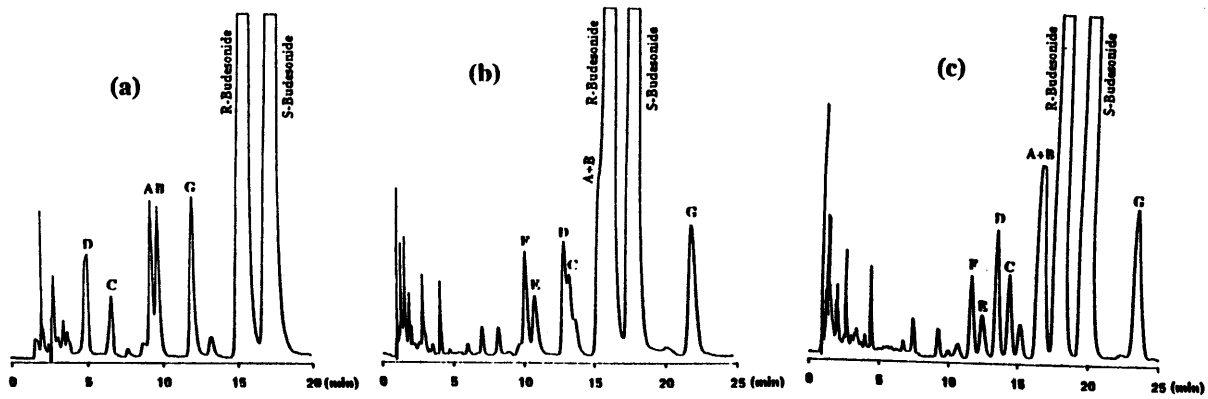

Fig. 5. The chromatograms of budesonide EP following storage in solution in propylene glycol USP and assayed using (a) EtOH, (b) EP and (c) novel methods.

respectively, determined using the EtOH, EP and novel methods. The EP method significantly overestimated the drug substance remaining due to its failure to resolve products A and B which were chromatographically identical to impurities 1 and 2. This showed conclusively that the pharmacopeial technique was not stability-indicating when degradation occurred in solution. A complex degradation pathway with multiple degradation products was observed (Fig. 5). Using the EtOH method, five main degradation products were observed. These were successfully separated from the epimers of budesonide, each of the degradation products eluting before budesonide. Two further solution degradation products, E and F, were detected by the EP method which were not detectable with the EtOH technique (both products were practically insoluble in the EtOH mobile phase). Using the novel HPLC method, all seven major degradation products were successfully detected and separated from the epimers of budesonide (Fig. 5). The percentage of the ethanol in the mobile phase and the pH value of the phosphate buffer were optimized during development of this novel method. By adding 2% ethanol to the EP mobile phase, the resolution of the degradation products A and B from the *R*-epimer peak of budesonide was achieved. Nevertheless, they were eluted as a single peak (the EtOH method showing that they were formed in similarly concentrations). The retention of the degradation products C and D was mainly affected by the pH value of phosphate buffer in the mobile phase; optimal separation was achieved at pH 3.4. The novel method's specificity for budesonide was also studied by comparing the assay result with that from the EtOH method. The lack of significant difference between the results implied that either of these methods could be used to quantify budesonide integrity, but the EP method should not be used when liquid formulations were being studied. The novel method was most able to resolve individual degradation products but the EtOH technique appeared to be essential to resolve products A and B (impurities 1 and 2).

3.3. Validation of the novel method

The novel method was studied for its capacity to quantify budesonide. A linear calibration curve of the mean budesonide peak areas versus original budesonide concentrations was obtained in the concentration range from 2.5 to 25.0 μg/ml ($r = 1.00$, slope $= 263600 \pm 200$, y-intercept $= 2400 \pm 2000$, $n = 3$). The limit of detection (LOD) for budesonide was 0.30 μg/ml. The 'within day' precision (RSD, $n = 6$) and 'between day' precision (RSD, $n = 12$) were 1.1 and 1.6%, respectively at a budesonide concentration of 10 μg/ml. The accuracy (%DFN, $n = 6$) for the determination of budesonide in propylene glycol solutions was − 0.59% at the same budesonide concentration.

An appropriate system suitability test for this novel method should meet all the following specifications: In a chromatograph obtained with a 500 μg/ml budesonide standard solution, (1) the retention time for *R*-epimer is 18 ± 1.5 min; (2) the resolution between the peaks corresponding to *R*-epimer and *S*-epimer is not less than 1.5 (typical values obtained in our studies were $>$ 2.0); (3) the number of theoretical plates determined from the *R*-epimer peak is at least 4000; (4) the symmetry factor for the *R*-epimer peak is less than 1.5. In order to confirm assay specificity an addition test is required. The budesonide standard solution is spiked with known amounts of impurities 1 and 2 (preliminarily identified as the two epimers of 21-dehydrobudesonide), resolution between the peaks corresponding to *R*-epimer and impurities 1 and 2 is not less than 1.0.

Method robustness was assessed as a function of changing the ethanol and acetonitrile concentrations. Ethanol concentrations between the range of 1.5–2.5% v/v (this represented $\pm 25\%$ relative change from target (2% v/v)) were investigated. Changes over this range did not significantly alter budesonide retention times (maximum observed retention time change compared to the novel mobile phase $= \pm 1\%$) or the resolution between *R* and *S* epimers (maximum observed resolution change compared to the novel mobile phase $= -4\%$). However, decreased ethanol concentrations did effect the resolution between the *R*-epimer and impurities 1 and 2. Acceptable resolution was achieved when the ethanol concentration was within the range from 1.8 to 2.5% v/v. Acetonitrile concentrations between the range 29–31% v/v (this represented $\pm 3.3\%$ relative change from target (30% v/v)) were investigated. The system suitability test criteria were met using acetonitrile concentrations between the range 29.6–30.4% v/v. As expected decreasing the acetonitrile concentration improved peak resolution, however this was at the expense of peak tailing and longer retention times (>20 min). Changes in the pH of phosphate buffer over the range 3.0–4.6 did not significantly affect the retention times of the budesonide epimers (maximum observed retention time changes compared to the novel mobile phase $= -5.5\%$). Optimal resolution of budesonide degradation products was observed within the pH range 3.3–3.4. These studies were performed using five separate columns (Hypersil C18, 5 μm, 15 cm $\times$ 4.6 mm, lot # 5/100/5040 (3 columns) and # 5/100/4828 (2 columns)). The system suitability test was acceptable for each of the columns.

This novel technique appeared to be sensitive, precise, robust and accurate for studying the stability of budesonide in this non-aqueous solution matrix.

4. Conclusions

The current official EP assay method for budesonide was not specific with respect to budesonide. This method over-estimated the purity of several budesonide samples, due to its co-elution of a major impurity along with the *R*-epimer of budesonide itself. The EP method also over-estimated budesonide concentrations in non-aqueous solution stability samples for a similar reason. Although the EtOH method was specific for budesonide, it showed severe tailing for both epimer peaks, and failed to meet pharmacopeial HPLC assay suitability specifications. This method also showed lower separation efficiencies for both budesonide epimers and related impurities. For solution stability studies, the EtOH method was unable to elute and resolve all of the major degradation products.

A novel HPLC method that combined the separation capacity of the EtOH and EP methods was developed. This technique enabled successful resolution of budesonide epimers and their impurities and was suitable for simultaneous determination of budesonide and limitation of its related substances. It was also capable of eluting and resolving the degradation products of budesonide formed following storage of non-aqueous solutions. The new technique was shown to be sensitive, accurate, robust and precise for drug substance, and thus acceptable as a stability-indicating assay for budesonide in solution and solid states.

Acknowledgements

Shuguang Hou acknowledges the receipt of a pre-doctoral fellowship from the School of Pharmacy, VCU. This work was supported by Philip Morris USA. The authors thank Clark March for his helpful discussions of this work.

References

[1] R. Brattsand, A. Thalen, K. Roempke, L. Kallstrom, E. Gruvstad, Eur. J. Respir. Dis. 63 (suppl. 122) (1982) 62–73.
[2] A. Thalen, R. Brattsand, E. Gruvstad, Acta Pharm. Suec. 21 (1984) 109–124.
[3] S.P. Clissold, R.C. Heel, Drugs 28 (1984) 485–518.
[4] J. Albertsson, A. Oskarsson, C. Svensson, Acta Cryst. B34 (1978) 3027–3036.
[5] R. Brattsand, A. Thalen, K. Roempke, L. Kallstrom, E. Gruvstad, J. Steroid Biochem. 16 (1982) 779–786.
[6] European Pharmacopoeia, 1997, pp. 496–498.
[7] M. Hindle, P.R. Byron, R.N. Jashnani, T.M. Howell, K.A. Cox, Respiratory Drug Delivery VI, Interpharm Press, Buffalo Grove, IL, 1998, pp. 97–102.
[8] A. Wikby, A. Thalen, J. Chromatogr. 157 (1978) 65–74.
[9] A. Wikby, L. Nilsson, G. Hallsas, J. Chromatogr. 157 (1978) 51–64.
[10] G. Roth, A. Wikby, L. Nilsson, A. Thalen, J. Pharm. Sci. 69 (1980) 766–770.
[11] A. Ryrfeldt, M. Tonnesson, E. Nilsson, A. Wikby, J. Steroid Biochem. 10 (1979) 317–324.
[12] P. Andersson, S. Edsbacker, A. Ryrfeldt, C. von Bahr, J. Steroid Biochem. 16 (1982) 787–795.
[13] A. Ryrfeldt, P. Andersson, S. Edsbacker, M. Tonnesson, D. Davies, R. Pauwels, Eur. J. Respir. Dis. 63 (suppl. 122) (1982) 86–95.
[14] A. Ryrfeldt, S. Edsbacker, R. Pauwels, Clin. Pharmacol. Ther. 35 (1984) 525–530.
[15] Y. Li, B. Tattam, K.F. Brown, J.P. Seale, J. Chromatogr. B 683 (1996) 259–268.