Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 803 (2004) 191–200
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Direct separation, identification and quantification of epimers 22*R* and 22*S* of budesonide by capillary gas chromatography on a short analytical column with Rtx®-5 stationary phase

Jan Krzek [a,*], Janusz S. Czekaj [a], Włodzimierz Rzeszutko [a], Anna Jończyk [b]

[a] *Department of Inorganic and Analytical Chemistry, Collegium Medicum of Jagiellonian University, Kraków, Poland*
[b] *Narodowy Instytut Ochrony Zdrowia, Warszawa, Poland*

Received 28 July 2003; received in revised form 15 December 2003; accepted 15 December 2003

Abstract

The conditions for separation, identification and quantitative determination of epimers 22*R* and 22*S* of budesonide by capillary gas chromatography (GC) with FID detection and two various sample injection methods, namely split–splitless and cool on-column, were established. In analysis helium as carrier gas and Rtx®-5 capillary column of 7 m in length along with stationary phase Crossbond® 5% diphenyl–95% dimethyl polysiloxane were used. The individual epimers were identified under specified conditions by using standard samples of different declared concentration of each epimer under investigation: (1) 51.2% of epimer 22*R* and 47.3% of epimer 22*S*, and (2) 95.1% of 22*R* and 4.4% of 22*S*, as well as Pulmicort®, a preparation containing micronized budesonide as an active substance.

It seems that good parameters of preliminary validation achieved by the proposed methods can confirm its suitability for quantitative analysis purpose. The retention times obtained for epimers 22*R* and 22*S*, depending on injection technique are about 7.7 and. 8.3 min for split and, approx. 10.3 and 10.9 min for cool on-column. The limits of detection and quantitation are 5.7 and 6.2 ng, for 22*R* respectively, and 4.3 and 4.8 ng for 22*S*. The linearity is maintained for concentrations ranging from 0.01 to 0.20 mg/ml. The quantitative analysis features of repeatability, high precision and accuracy confirmed by the obtained results and its statistical evaluation.

Keyword: Budesonide

1. Introduction

Stereochemistry is an important determinant of the efficacy of drugs [1–4]. While naturally occurring compounds and many semisynthetic compounds are available as pure stereoisomers, synthetic drugs are typically manufactured as racemates [3] and they are used as such in therapy. There is now considerable effort underway to produce drugs that are stereoisomers [5,6]. This is justified if considering differences in action of particular isomers [6,7] as well as its pharmacodynamical and pharmacological properties in the absorption, distribution, biotransformation, transport and isomer elimination processes discussed by numerous papers [8,9].

Due to differences in stereoisomer therapeutic effects it is necessary to develop new methods for determining them in pharmacological preparations. In reviewed literature for determination of enantiomers and diastereoisomers the HPLC and gas chromatography (GC) as well as capillary electrophoresis are mainly used along with the spectroscopic and thermal methods [10–12].

In separation methods the determination of enantiomers is reduced to direct analysis based on chiral stationary phases or indirect analysis in which individual epimers reacting with chiral reagents are converted into diastereoisomers [11,12].

Budesonide of chemical formula $C_{25}H_{34}O_6$, molecular weight of 430.54 and spatial configuration as in Fig. 1 is an example of mixture of two diastereoisomers, epimers 22*R* and 22*S* [13] that differ from each other both in activity and pharmacological properties. Depending on the means of synthesis budesonide may contain isomers in the

* Corresponding author. +48-12-657-0480; fax: +48-12-657-0262.
E-mail address: jankrzek@cm-uj.krakow.pl (J. Krzek).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.12.038

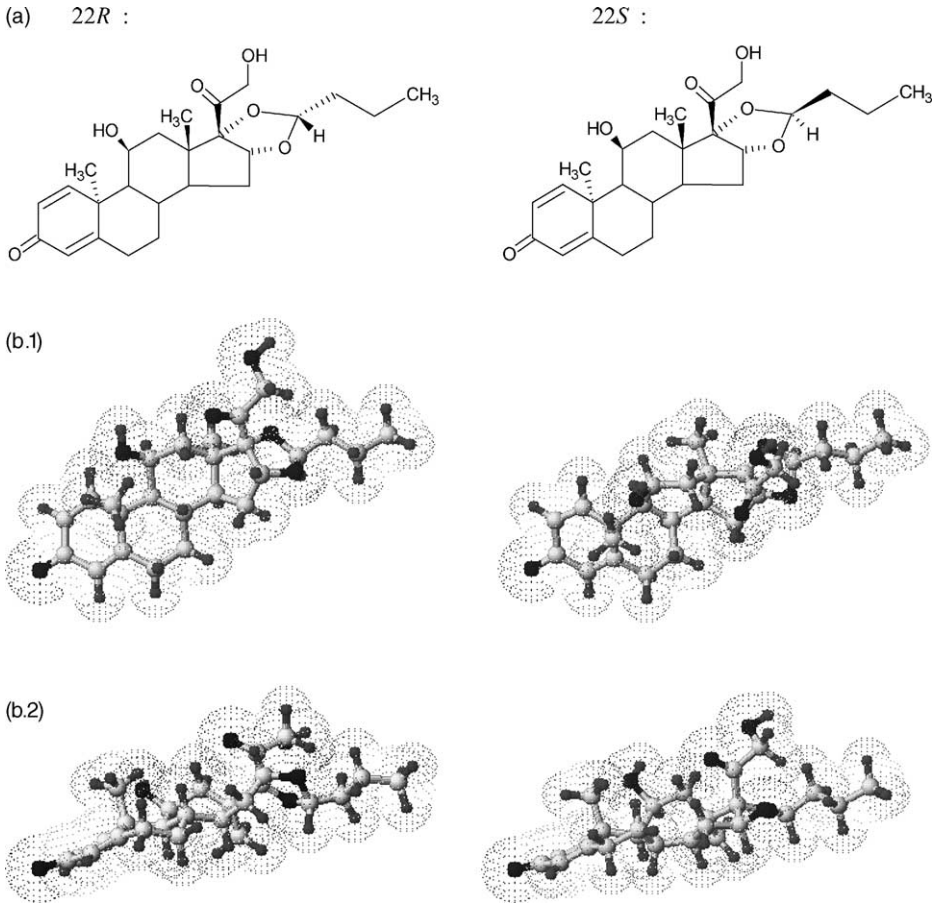

Fig. 1. (a) Structural formulae for budesonide epimers. (b) 3D formulae obtained from spatial optimization based on the ACD/ChemSketch version 4.55 (6.05.2000) and ACD/3D version 4.52 (10.04.2000) software (Advanced Chemistry Development Inc., Toronto, Kanada): (b.1) view in the direction perpendicular to the second six-membered ring, (b.2) view in the direction parallel to the second six-membered ring.

following ratios 22*R*:22*S* = 90:10 or 55:45 [14]. In contrast to enantiomers, diastereoisomers differ in physical and chemical properties, what creates the possibility of their direct determination.

The conditions for identification and quantitative analysis of budesonide epimers by the HPTLC with β-cyclodextrin in mobile phase method used for evaluating of pharmaceutical preparations were established in another paper prepared at our department [15].

In parallel with the above, the research studies on separation and quantitative analysis of budesonide epimers by GC without derivatization were taken on. Two sample injection modes: split–splitless and cool on-column were applied.

Initially, the separation of epimers 22*R* and 22*S* budesonide being a common drug constituent was examined by using the Rtx®-5 column of 7 m in length, 0.32 mm in inner diameter with Crossbond® 5% diphenyl–95% dimethyl polysiloxane stationary phase of 0.25 μm in film thickness, of relatively high thermal resistance (minimal bleed at 330 °C), low polarity and polymer chains structure schematic presented in Fig. 2.

The lack of information about adopted assumptions and goals justified our attempt to deal with them.

2. Experimental

2.1. Apparatus

2.1.1. GC system

TRACE GC gas chromatograph (Thermo Finnigan, Rodano, Italy), equipped with:

- FID (3 pg C/s, linearity of 10^6), base temperature of 320 °C, air flow at 350 ml/min, hydrogen flow at 35 ml/min and nitrogen (make-up gas) 33 ml/min and
- split–splitless and cool on-column injectors:

  (1)split: temperature of 220 °C, split flow 17 ml/min, split ratio 1:10; syringe: capacity of 10 μl, needle length of 50 mm (MICROLITER® #701, Hamilton-Bonaduz, Switzerland); injected volume of 1.0 μl; oven temperature program: from 220 to 310 °C at rate of 10°/min;

  (2)cool on-column: secondary cooling time 0.05 min; syringe: capacity of 1.0 μl, needle length of 70 mm (MICROLITER® #7001, Hamilton-Bonaduz, Switzerland); injected volume of 0.10 μl; oven temperature programs: (A) from 70 to 220 °C at rate of

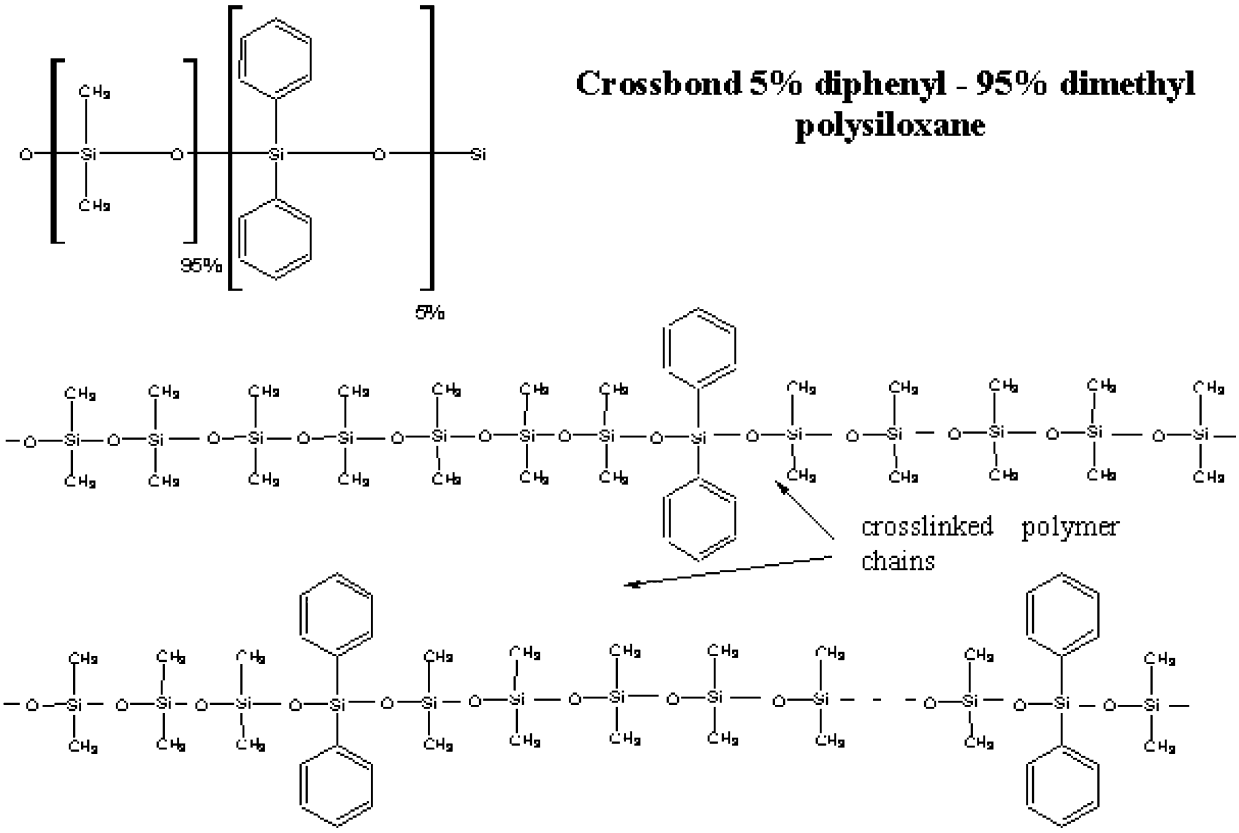

Fig. 2. Stationary phase used in the analysis (in ACD/ChemSketch version 4.55).

40°/min, from 220 to 310 °C at the rate of 10°/min, (b) from 40 °C (3 min) to 280 °C at rate of 20°/min, from 280 to 310 °C at the rate of 5°/min.

2.1.2. Capillary column

Rtx®-5, 7 m × 0.32 mm i.d., with stationary phase: Crossbond® 5% diphenyl–95% dimethyl polysiloxane, film thickness of 0.25 μm (category #54042, serial #231227, Restek; under license of Hewlett-Packard Company, US Patent 4,293,415). Retention gap: 2 m, 0.50 mm i.d. of uncoated fused silica tubing, PRECOL. F.S. 0.5MM—MT2.MONT, Thermo Finnigan (Rodano, Italy).

2.1.3. Computer

PC, Pentium 266 MHz MMX, 160MB RAM (Adax Land-JTT Computers, Kraków, Poland); software: Chrom Card for TRACE, version 1.07, Microsoft® Office 2000; ACD/ChemSketch version 4.55 (6.05.2000) and ACD/3D version 4.52 (10.04.2000; Advanced Chemistry Development Inc., Toronto, Canada).

2.2. Chemicals and reagents

2.2.1. Carrier gas

Helium of purity class 5.0 (Linde Gaz Polska, Kraków, Poland), additionally cleaned with OT3-2 oxygen/moisture trap (R&D Separations, Inc., Rancho Cordova, CA, USA). Chromatograms were recorded at constant gas flow of 1.7 ml/min (38 cm/s).

2.2.2. Detector gases

Synthetic air: Synthetische Luft KW-Frei 20.0000% Sauerstoff rest Stickstoff (350 ml/min); hydrogen (35 ml/min) and nitrogen (make-up gas, 33 ml/min) of purity class 5.0 (Linde Gaz Polska, Kraków, Poland).

2.2.3. Sample solvent

Methanol gradient grade for liquid chromatography, LiChrosolv® (Merck KgaA, Darmstadt, Germany).

2.2.4. Standard samples

(1) Budesonide-R micronized, 99.5%, concentration of diastereoisomer 22*S* 4.4% (serial number 210598; Instytut Farmaceutyczny, Warsaw; "Budesonide R"), (2) Budesonid, 98.0%; isomer concentrations (HPLC): 22*R* 51.2%, 22*S* 47.3%; (serial number 4422/M1, manufacturer SICOR, Italy; "Budesonide RS").

2.2.5. Preparation

Pulmicort® 1 mg budesonide/2 ml ("suspension pour inhalation buccale en recipients unidoses", BH 1160, Laboratoires Astra, France).

2.2.6. Standard solutions

(1) For identification. Amounts of 10.0 ± 0.3 mg of standard samples were weighed up to ±0.1 mg, dissolved in methanol and filled up to 10.0 ml.
(2) For linearity checking. Standard samples were weighed up to ±0.1 mg and dissolved in methanol to get solutions of budesonide concentrations ranging from 0.01 to 0.20 mg/ml.

2.2.7. Preparation solutions

A volume of 8.0 ml of methanol were added to 2.0 ml of water solution of suspension, mixed to dissolve suspension solids and to get a solution of 0.1 mg/ml in budesonide concentration.

3. Results

3.1. Preliminary analyses

Preliminary analyses were carried out by two independent analysts to check chromatographic separation conditions for budesonide epimers and determine preliminary validation parameters for establishing optimal analysis conditions [16].

An effect of parameter changes on the results. Solutions of standard samples of Budesonide RS and Budesonide R were injected into the chromatographic column at constant flow rate (1.0–2.0 ml/min, 26–43 cm/s) or constant pressure (10–30 kPa) of the mobile phase, isothermally or at thermostat temperature programmed within 40–325 °C, while changing the temperature rise rate from 5 to 40 °C/min as well as temperature and duration of isotherms used. Injection methods:

(a) Split: sample volumes of 1.0–3.0 μl were injected by employing the "air plug" technique [17] with injected volume control before and after injection. The injector temperature was altered within 150–300 °C, and sample distribution ratio from 1:10 to 1:50 (split–vent flow rate, 10–100 ml/min).
(b) Cool on-column: volumes of 0.1–0.3 μl were injected, while changing the column initial temperature within 40–100 °C and secondary cooling time—from 0.05 to 0.2 min.

Since measurements were made in series of cycles, the system tightness and baseline stability were checked and the capillary column was evaluated under specified separation conditions prior to each cycle. Three chromatograms with baseline compensation were recorded for each sample.

The results of preliminary chromatographic analyses related to separation of epimers of the substance under investigation caused that further identification and quantitative analyses were performed by using the same capillary column. In addition, the use of this capillary column with this stationary phase was justified by its high thermal resistance mentioned above and reaching temperature levels above 300 °C, as chromatogram peaks of the analyzed substance appeared at chromatograph thermostat temperature ranging from 280 to 300 °C.

The chromatograms of standard samples were used to check separation, identification (by determining the absolute and relative retention times), linearity and to determine epimer concentrations. The chromatograms of pharmaceutical preparation were recorded to confirm the obtained results pertaining separation, identification and quantitative analysis of budesonide epimers under established conditions, and to validate that the developed method can be used for analysis of available preparations.

Susceptibility of budesonide molecule to thermal decomposition observed with split injections in higher then 220 °C temperature and probable existence of matrix constituents (not identified) of unknown thermal stability in the analyzed preparation decided that in preparation analyses the direct injection into the capillary column was used with a short duration cooling with compressed air of its initial section (cool on-column). In addition, both initial column temperature and temperature rise rate in the revised temperature program were lowered. In that case such parameters as analyte thermal stability, duration of analysis, peak

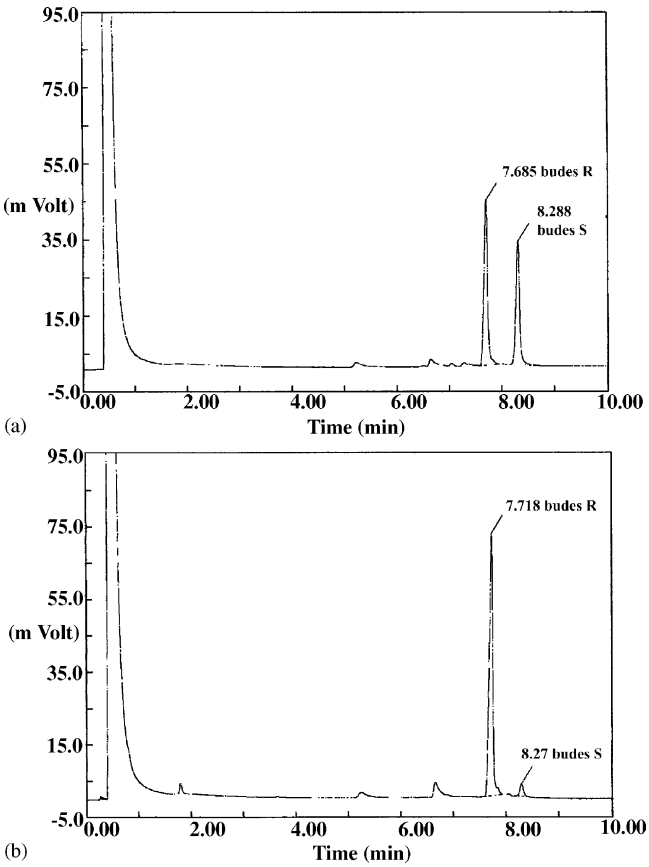

Fig. 3. Chromatograms recorded for standard solutions of Budesonide RS (a) and Budesonide R (b) by using the split injection method.

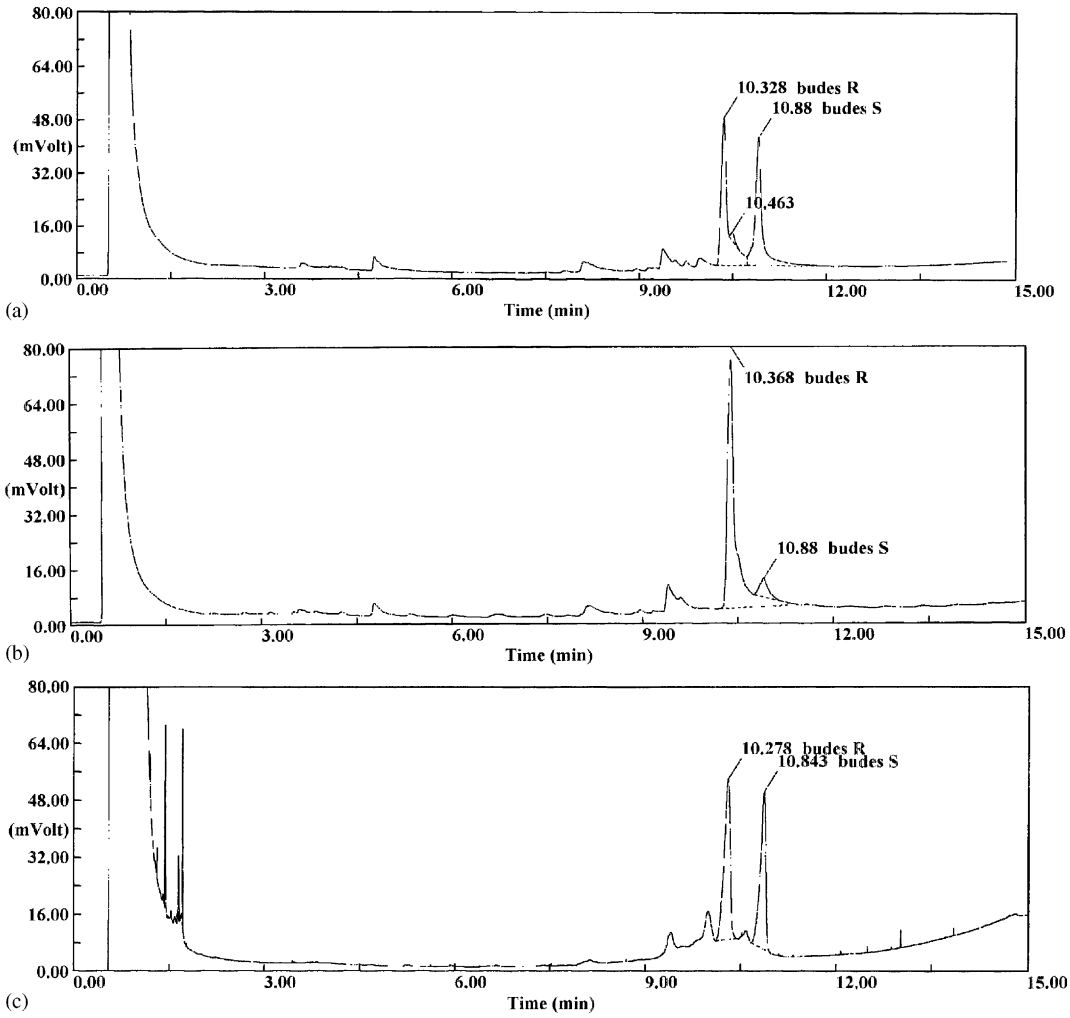

Fig. 4. Comparison of chromatograms obtained for standard solutions of Budesonide RS (a), Budesonide R (b) and Pulmicort (c), by using the cool on-column method (A) and shorter version of the oven temperature program.

resolution, results accuracy and precision were taken into account.

3.2. Selectivity and specificity

The selectivity of the method was checked by establishing optimal separation conditions for epimers. The separation conditions were controlled by computing resolution R [18] for peaks of epimers 22*R* and 22*S* marked on chromatograms as "budes R" and "budes S". The criterion $R \geq 1.0$ [19] was adopted.

The chromatograms of standard solutions were recorded by using both injection methods and comparing retention times, peak heights and areas (Figs. 3–5).

For the temperature programs mentioned above the following retention times were obtained:

- split: approx. 7.7 min and approx. 8.3 min,
- cool on-column: (A) about 10.3 min and about 10.9 min, (B) about 16.6 min and about 17.1 min,

for "Budesonide R" and "Budesonide S" peaks, respectively.

The peak location on chromatograms determined by retention times and its relative values (RRT; Table 1) is repeatable, thus enabling easy identification. For the "Budesonide S" peak, RRT computed with the "Budesonide R" retention time as a reference, the ±0.5% criterion was adopted. The results of analyses mentioned above are presented in Table 1.

The average ($n = 6$) resolutions R of epimer peaks computed from chromatograms recorded by split injection of the Budesonide RS and Budesonide R solutions of total budesonide concentration of 0.2 mg/ml, were 4.5 and 3.4, respectively.

3.3. Accuracy and precision

The method accuracy was defined by relative error (E, in %) of percentage epimer concentrations in the Budesonide RS and Budesonide R solutions under examination based on

Table 1
Validation of retention parameters

Parameter	t_R (min)		RRT[a]	Epimer peak resolution, R ($c = 0.2$ mg/ml), $n = 6$	
	Budesonide RS, split, n[b] = 25			Split	
	22*R*	22*S*	22*S*/22*R*	Budesonide RS	Budesonide R
Mean	7.663	8.267	1.079	4.54	3.44
Minimum	7.642	8.243	1.078	3.93	2.94
Maximum	7.688	8.293	1.080	5.02	4.08
S.D.	0.014	0.015	0.0005	0.35	0.45
μ, 95%[c]	0.005	0.005	0.0002	0.28	0.36
R.S.D. (%)	0.181	0.181	0.048	7.7	13.0
	Budesonide RS, cool on-column (A), $n = 15$			Cool on-column (A)	
	22*R*	22*S*	22*S*/22*R*	Budesonide RS	Budesonide R
Mean	10.339	10.874	1.033	2.51	1.27
Minimum	10.285	10.855	1.032	2.51	1.12
Maximum	10.368	10.912	1.033	2.52	1.43
S.D.	0.030	0.022	0.0003	0.005	0.155
μ, 95%	0.024	0.018	0.0002	0.006	0.176
R.S.D. (%)	0.294	0.202	0.029	0.20	12.2
	Budesonide RS, cool on-column (B), $n = 6$			Cool on-column (B)	
	22*R*	22*S*	22*S*/22*R*	Budesonide RS	Budesonide R
Mean	16.562	17.096	1.055	2.37	1.34
Minimum	16.537	17.065	1.055	2.35	1.31
Maximum	16.585	17.123	1.055	2.39	1.40
S.D.	0.017	0.021	0.0002	0.020	0.049
μ, 95%	0.010	0.012	0.0002	0.023	0.056
R.S.D. (%)	0.102	0.124	0.018	0.84	3.67

[a] RRT: relative retention time.
[b] n: number of analyses taken into account.
[c] μ, 95%: confidence level 95%.

integrated peak areas and peak heights compared to epimer concentrations declared by the manufacturers.

The precision was defined as the degree of compliance between the results of measurements repeated many times. The precision was expressed by absolute standard deviation (S.D.) and relative standard deviation (R.S.D.), while assuming that the result of individual determination $x = \bar{x} \pm 2$ S.D.

3.4. Linearity

The linearity of a relationship between peak area (A, expressed in 0.1 μV s) and peak height (H, in μV) versus concentrations was checked by using the Budesonide RS standard solution of total budesonide concentration (c, expressed in mg/ml) ranging from 0.01 to 0.20 mg/ml. The linear regression method was used for data handling. The following relationships were established: for epimer 22*R*: $A = 1312000c - 1060$ ($r = 0.9972$), $H = 29920c - 20$ ($r = 0.9949$), and for epimer 22*S*: $A = 957100c - 734$ ($r = 0.9957$), $H = 19800c - 12$ ($r = 0.9949$).

3.5. Quantitation and detection limits

The determinations were carried out with split–splitless injections by recording chromatograms of the Budesonide RS standard solutions and analyzing the ratio of detector signal for a sample containing a specified amount of budesonide to the baseline noise level. For detection limit, the amount of the substance for which the detector signal-to-baseline noise ratio ≥3, while for limit of quantitation the ratio ≥6 were adopted. The detection and quantitation limits recomputed to determined mean content of individual epimers were 5.7 and 6.2 ng for epimer 22*R* and 4.3 and 4.8 ng for 22*S* (see Fig. 6).

3.6. Quantitative analysis

The chromatograms were recorded at constant helium flow rate of 1.7 ml/min (38 cm/s). Detector—FID: base temperature of 320 °C, air flow of 350 ml/min, hydrogen flow of 35 ml/min and nitrogen (make-up gas) 33 ml/min.

Injection methods:

1. Split: temperature 220 °C, split flow 17 ml/min, split ratio 1:10, injected sample volume of 1.0 μl. Oven temperature program: from 220 to 310 °C at rate of 10°/min.
2. Cool on-column: secondary cooling time 0.05 min, injected volume of 0.10 μl GC oven temperature programs: (A) from 70 to 220 °C at rate of 40°/min, from 220 to 310 °C at the rate of 10°/min, (B) from 40 °C (isotherm

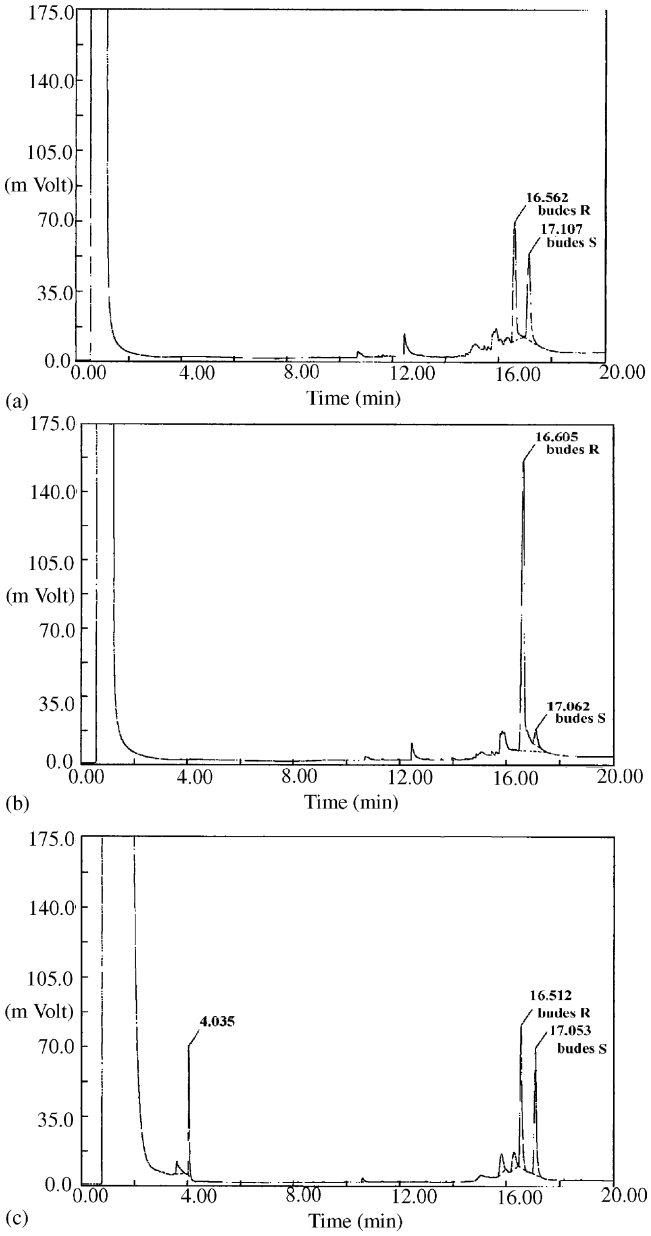

Fig. 5. Comparison of chromatograms obtained for standard solutions of Budesonide RS (a), Budesonide R (b) and Pulmicort (c), by using the cool on-column method (B) and longer version of the oven temperature program.

duration of 3 min) to 280 °C at rate of 20°/min, from 280 to 310 °C at the rate of 5°/min.

In computations the absolute calibration method was employed based on epimer integrated peak areas and peak heights. The epimer concentration levels (%) in the analyzed solutions were illustrated by examining relationships between the peak areas and heights. The internal normalization method was used. The results are presented in Tables 2–4.

Table 5 presents comparison of the results obtained in established methods which differ in injection technique (split,

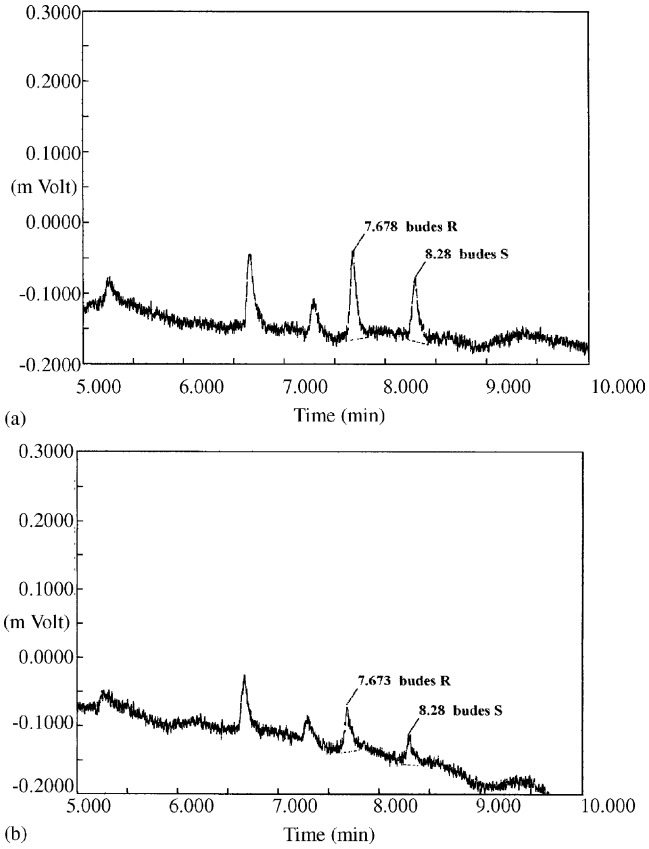

Fig. 6. Chromatograms recorded for establishing the limits of quantitation (a) and detection (b) for budesonide epimers (split injection).

cool on-column) or in GC oven temperature program (cool on-column (A) and (B)).

4. Discussion

The sample chromatograms presented in Figs. 3–5 are easy to interpret. In spite of the fact that two different sample injection methods were employed, i.e. split–splitless and cool on-column, on chromatograms recorded for various standard samples (Figs. 3a,b, 4a,b and 5a,b) there are peak pairs of corresponding retention times. For these peaks, the relative relationship between integrated areas is consistent with the declared epimer contents in the analyzed substances (Tables 2–4). The peak pairs of identical retention times are also present on chromatograms obtained for the preparation solution under examination (Figs. 4c and 5c) in which budesonide is the only active component, thus confirming that they originate from budesonide epimers. Some additional peaks are also present on the preparation chromatograms. These peaks probably come from the preparation matrix. These peaks have no effect on budesonide determination results under established conditions. For both injection methods, despite the small length of the capillary column and relatively low volume of the stationary phase, good separation

Table 2
Quantitative analysis of budesonide epimer concentrations in the analyzed standard solutions—split; $n = 10$

Computational basis	Peak area		Peak height	
	%22*R* (determined)	%22*S* (determined)	%22*R* (determined)	%22*S* (determined)
Analyzed solution: Budesonide RS; declared composition: %22*R*:%22*S* = 52.24:47.76				
Mean, $\bar{x}$	56.53	43.47	59.69	40.31
Minimum	55.79	42.91	58.72	38.51
Maximum	57.09	44.21	61.49	41.28
S.D.	0.41	0.41	0.80	0.80
$\bar{x} \pm$ 2S.D.	56.53 ± 0.82	43.47 ± 0.82	59.69 ± 1.60	40.31 ± 1.60
μ, 95%[a]	0.25	0.25	0.50	0.50
R.S.D. (%)	0.73	0.94	0.83	1.23
E (%)[b]	8.21	8.98	14.27	15.64
Analyzed solution: Budesonide R; declared composition: %22*R*:%22*S* = 95.58:4.42				
Mean, $\bar{x}$	96.63	3.37	96.77	3.23
Minimum	96.24	2.96	96.56	3.08
Maximum	97.04	3.76	96.92	3.45
S.D.	0.28	0.28	0.11	0.11
$\bar{x} \pm$ 2S.D.	96.63 ± 0.56	3.37 ± 0.56	96.67 ± 0.22	3.23 ± 0.22
μ, 95%[a]	0.17	0.17	0.07	0.07
R.S.D. (%)	0.28	8.16	0.11	3.25
E (%)	1.10	23.83	1.25	26.94

[a] μ, 95%: confidence level 95%.
[b] *E*: relative error (%).

Table 3
Quantitative analysis of budesonide epimer concentrations in the analyzed standard solutions and preparation solution, cool on column (A); $n = 6$

Computational basis	Peak area		Peak height	
	%22*R* (determined)	%22*S* (determined)	%22*R* (determined)	%22*S* (determined)
Analyzed solution: Budesonide RS; declared composition: %22*R*:%22*S* = 52.24:47.76				
Mean, $\bar{x}$	53.47	46.53	53.32	46.68
Minimum	53.02	46.08	53.04	46.39
Maximum	53.92	46.98	53.61	46.96
S.D.	0.40	0.40	0.40	0.40
$\bar{x} \pm$ 2S.D.	53.47 ± 0.80	46.53 ± 0.80	53.32 ± 0.80	46.68 ± 0.80
μ, 95%[a]	0.51	0.51	0.32	0.32
R.S.D. (%)	1.20	1.37	0.76	0.87
E (%)[b]	2.35	2.57	2.07	2.27
Analyzed solution: Budesonide R; declared composition: %22*R*:%22*S* = 95.58:4.42				
Mean,$\bar{x}$	92.66	7.34	93.26	6.74
Minimum	92.42	7.14	92.54	6.18
Maximum	92.86	7.58	93.82	7.46
S.D.	0.22	0.22	0.65	0.65
$\bar{x} \pm$ 2S.D.	92.66 ± 0.44	7.34 ± 0.44	93.26 ± 1.30	6.74 ± 1.30
μ, 95%[a]	0.18	0.18	0.52	0.52
R.S.D. (%)	0.24	3.03	0.68	9.70
E (%)	3.05	65.98	2.43	52.57
Analyzed solution: Pulmicort®				
Mean, $\bar{x}$	54.30	45.70	51.13	48.87
Minimum	52.94	44.74	50.70	48.19
Maximum	55.26	47.06	51.81	49.30
S.D.	1.14	1.14	0.60	0.60
$\bar{x} \pm$ 2S.D.	54.30 ± 2.28	45.70 ± 2.28	51.13 ± 1.20	48.87 ± 1.20
μ, 95%[a]	0.91	0.91	0.48	0.48
R.S.D. (%)	2.11	2.50	1.18	1.23

[a] μ, 95%: confidence level 95%.
[b] *E*: relative error (%).

Table 4
Quantitative analysis of budesonide epimer concentrations in the analyzed standard solutions and preparation solution, cool on column (B); $n = 6$

Computational basis	Peak area		Peak height	
	%22*R* (determined)	%22*S* (determined)	%22*R* (determined)	%22*S* (determined)
Analyzed solution: Budesonide RS; declared composition: %22*R*:%22*S* = 52.24:47.76				
Mean, $\bar{x}$	52.25	47.75	55.08	44.60
Minimum	51.09	46.12	54.18	43.18
Maximum	53.88	48.91	56.39	45.82
S.D.	1.01	1.01	0.84	1.09
$\bar{x} \pm$ 2S.D.	52.25 ± 2.02	47.75 ± 2.02	55.08 ± 1.68	44.60 ± 2.18
μ, 95%[a]	0.81	0.81	0.68	0.87
R.S.D. (%)	1.93	2.12	1.53	2.44
E (%)[b]	0.02	0.02	5.43	6.61
Analyzed solution: Budesonide R; declared composition: %22*R*:%22*S* = 95.58:4.42				
Mean, $\bar{x}$	95.52	4.48	95.68	4.32
Minimum	95.38	4.62	95.49	4.13
Maximum	95.67	4.33	95.87	4.51
S.D.	0.21	0.21	0.26	0.26
$\bar{x} \pm$ 2S.D.	95.52 ± 0.42	4.48 ± 0.42	95.68 ± 0.52	4.32 ± 0.52
μ, 95% (1)	0.29	0.29	0.36	0.36
R.S.D. (%)	0.22	6.41	0.27	6.08
E (%)	0.06	1.25	0.10	2.26
Analyzed solution: Pulmicort®				
Mean, $\bar{x}$	50.70	49.30	52.16	47.84
Minimum	50.39	48.82	51.03	46.96
Maximum	51.18	49.61	53.04	48.97
S.D.	0.36	0.36	0.71	0.71
$\bar{x} \pm$ 2S.D.	50.70 ± 0.72	49.30 ± 0.72	52.16 ± 1.42	47.84 ± 1.42
μ, 95%[a]	0.29	0.29	0.57	0.57
R.S.D. (%)	0.71	0.73	1.36	1.48

[a] μ, 95%: confidence level 95%.
[b] *E*: relative error (%).

of epimers was achieved that is of great importance for the identification and quantitative determination. The mean resolutions for epimer peaks, calculated from chromatograms recorded with split the Budesonide RS and Budesonide R solutions of total budesonide concentration of 0.2 mg/ml, were 4.5 and 3.4, respectively (see Table 1).

The separation has been achieved despite the capillary column was short and analyte was not derivatized. It seems

Table 5
The comparison of results obtained in three method versions used for budesonide epimers analysis

Method version	Split		Cool on-column (A)		Cool on-column (B)	
Epimer	22*R*	22*S*	22*R*	22*S*	22*R*	22*S*
t_R (min)	7.663	8.267	10.339	10.874	16.562	17.096
RRT[a]	1	1.079	1	1.033	1	1.055
Resolution, *R* (for Budesonide RS)	4.54		2.51		2.37	
Determined: %22*R*:%22*S*						
Budesonide RS (declared 52.24:47.76)						
Mean	56.53	43.47	53.47	46.53	52.25	47.75
R.S.D. (%)	0.73	0.94	1.20	1.37	1.93	2.12
E[b] (%)	8.21	8.98	2.35	2.57	0.02	0.02
Budesonide R (declared 95.58:4.42)						
Mean	96.63	3.37	92.66	7.34	95.52	4.48
R.S.D. (%)	0.28	8.16	0.24	3.03	0.22	6.41
E (%)	1.10	23.83	3.05	65.98	0.06	1.25
Pulmicort®						
Mean	Not analyzed		54.30	45.70	50.70	49.30
R.S.D. (%)	Not analyzed		2.11	2.50	0.71	0.73

[a] RRT: relative retention time.
[b] *E*: relative error (%).

that the separation of analyzed isomers could be induced by differences in its spatial structure presented in Fig. 1, sufficiently large to differentiate its physical and chemical properties and in consequence its affinity to the stationary phase used under the established determination conditions (Fig. 2). It is confirmed by the results of quantitative measurements presented in Tables 2–4 with statistical analysis. Obtained results are repeatable and of high accuracy and precision. The shortest retention times (approx. 7.7 and 8.3 min), maximum resolution and precision of the quantitative analysis results (R.S.D. = approx. 1%) were obtained for split injection, while the maximum accuracy of the results (relative error $E < 0.1\%$) at good precision (R.S.D. = approx. 2%), were achieved for cool on-column (B) method, increasing analysis duration: epimer retention times were 16.6 and 17.1 min. Cool on-column (A) instead, just like split method, is not time-consuming but many times less accurate than cool on-column (B) version (Table 5).

The method features of high detectability and determinability. The limits of detection and quantitation were: 5.7 and 6.2 ng for 22*R*, and 4.3 and 4.8 ng for 22*S*, respectively (see Fig. 6). The relationship between peak area and peak height versus concentration shows linearity within the concentration range under consideration.

5. Conclusions

A sensitive and accurate method has been developed to enable simultaneous determination of epimers 22*R* and 22*S* of budesonide by capillary gas chromatography on a short (7 m) analytical column of inner surface coated with achiral stationary phase consisting of Crossbond® 5% diphenyl–95% dimethyl polysiloxane. The satisfactory results were obtained during the method validation phase, thus indicating its suitability for identification and quantitative determination of individual budesonide isomers in pharmaceutical preparations. The newly developed method allows direct determination of budesonide epimers, without their prior derivatization and without necessity to use chiral reagents during analysis. The analyses were carried out on a short analytical column with one of the most commonly used stationary phases to demonstrate the possibility of a simple mode separation of these diastereoisomers. Alternative sample injection methods (split and cool on-column) and chromatograph oven temperature programs were proposed with pointing to cool on-column technique in (B) version because of very high accuracy results (relative error $E < 0.1\%$) and good precision (R.S.D. = approx. 2%). Considering the results of the preliminary analyses which may suggest susceptibility of budesonide molecule to thermal decomposition the choice of cool on-column injection technique seems to be reasonable.

References

[1] K. Wiliams, K. Lee, Drugs 30 (1985) 333.
[2] E.J. Ariens, Pharmacol. Toxicol. 64 (1989) 319.
[3] S. Gõrõg, M. Gazdag, J. Chromatogr. B 659 (1994) 51.
[4] J.M. Daniels, E.R. Nestmann, A. Kerbb, Drug Inform. J. 31 (1977) 639.
[5] FDA's policy statement for the development of new stereoisomeric drugs, US Food and Drug Administration, May 1992.
[6] J. Pawlaczyk, Farmacja Polska (Pol. Pharm.) 55 (1999) 183 (in Polish).
[7] A. Czarnecki, A. Maciejczyk, M. Kosek, A.P. Mazurek, Acta Polon. Pharm. 50 (1993) 5.
[8] D.E. Drayer, Cl. Pharmacol. Therapeut. 40 (1986) 125.
[9] E.J. Aries, Trends Pharmacol. Sci. 7 (1986) 200.
[10] F. Lai, A. Mayer, T. Sheenhan, J. Pharm. Biomed. Anal. 11 (1999) 117.
[11] N. Sadlej-Sanowska, Biuletyn Instytutu Leków (Bull. Inst. Drugs) 42 (1998) 94 (in Polish).
[12] A. Wren, R. Rowe, J. Chromatogr. 603 (1992) 235.
[13] European Pharmacopoeia, fourth ed., Council of Europe, Strasbourg, 2002.
[14] M. Zając, E. Pawełczyk, Chemia Leków (Chemistry of Drugs), Akademia Medyczna, Poznań, 2000 (in Polish).
[15] J. Krzek, U. Hubicka, M. Dąbrowska-Tylka, E. Leciejewicz-Ziemecka, Chromatographia 56 (2002) 759.
[16] The Validation of Analytical Procedure Used in the Examination of Pharmaceutical Materials, WHO/Pharm. 85.541/Rev.2 Genf., 1989.
[17] D. Rood, A Practical Guide to the Care, Maintaince and Troubleshooting of Capillary Gas Chromatographic Systems, second enlarged and revised ed., Hütig Verlag, 1995.
[18] The United States Pharmacopoeia/The National Formulary (VSP/NF 19), United States Pharmacopoeial Convention, Inc., Rockville, MD, USA, 2000.
[19] British Pharmacopoeia, Her Majesty's Stationary Office, London, UK, 1999.