High-Performance Liquid Chromatographic Determination of Epimers, Impurities, and Content of the Glucocorticoid Budesonide and Preparation of Primary Standard

G. ROTH ˣ, A. WIKBY, L. NILSSON, and A. THALÉN

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Abstract □ The new glucocorticoid budesonide, a 1:1 mixture of two epimers, was evaluated analytically with respect to drug content, impurities, and epimer distribution in the drug substance. Reversed-phase high-performance liquid chromatographic (HPLC) systems using octadecylsilane columns with different ethanol–water mixtures as eluents were used. Budesonide and its epimers were determined with recoveries of 100.3% (±0.16 *SD*) and 99.7% (±0.27 *SD*), respectively. Individual foreign steroids in the ranges of 0.05–0.2 and 0.2–2% were determined with mean recoveries of 93% (±7.6 *SD*) and 97% (±2.5 *SD*), respectively. Analysis of eight small-scale production batches of budesonide gave a recovery of 99.9% (±0.39 *SD*) for the sum of budesonide and impurities. Analytical data and the procedure for preparation of a budesonide primary standard are given. The proposed HPLC procedure is faster, more accurate, more precise, and more selective than the usual pharmacopeial methods for corticosteroids.

Keyphrases □ Budesonide—high-performance liquid chromatographic analysis of drug content, impurities, and epimer distribution, preparation of primary standard □ Anti-inflammatory agents—budesonide, high-performance liquid chromatographic analysis of drug content, impurities, and epimer distribution, preparation of primary standard □ High-performance liquid chromatography—analysis, budesonide, determination of drug content, impurities, and epimer distribution

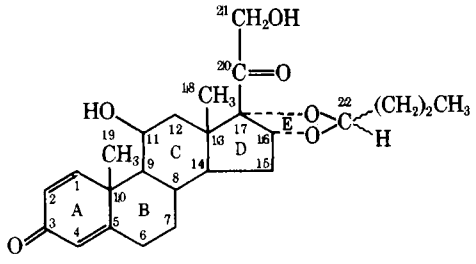
The new nonhalogenated glucocorticoid budesonide with high anti-inflammatory activity was synthesized by Thalén and coworkers (1–3). Evidence was given for the formation of two epimers in a 50:50 ratio. The epimers were isolated by chromatography on Sephadex LH-20 and studied by NMR spectroscopy and mass spectrometry (2, 3). An X-ray study of the molecular structures of the two epimers also was reported (4, 5).

The high-performance liquid chromatographic (HPLC) properties of budesonide and homologous corticosteroids were studied using both normal-phase (6) and reversed-phase (6, 7) systems. Optimal resolution of the budesonide epimers was obtained with octadecylsilane as the support and ethanol in the aqueous mobile phase.

For the assay of several steroid drugs, the USP proposes the separation of the drug substance from impurities by TLC and the final determination of the drug content by the blue tetrazolium method (8). Preliminary work indicated that this procedure was too laborious, time consuming, and not selective enough for accurate evaluation of budesonide batches with respect to drug content and impurities. Furthermore, the epimer distribution could not be determined by this method. The purpose of the present work was to develop HPLC methods for the quantitative evaluation of all of these parameters. This report also deals with the problem of preparing a reliable primary standard substance since an absolute assay for steroids is not available.

EXPERIMENTAL

Chemicals and Solvents—*Budesonide Drug Substance*—Budesonide (I) is a mixture of two epimers, 16α,17α-(22*S*)- and 16α,17α-(22*R*)-propylmethylenedioxypregna-1,4-diene-11β,21-diol-3,20-dione (A and B, respectively). The different solubilities of the epimers result in more Epimer B during purification of the crude substance, which is considered by specifying the limits for the epimer ratio of the drug substance.



I

Flucloronide[1]—Flucloronide was used as the internal standard in the assay of budesonide. The HPLC purity was >99.8%.

Impurity 1—Impurity 1 was 16α-hydroxyprednisolone, the starting material in the synthesis of budesonide. The HPLC purity (254 nm) of the substance[2] was >98%. 16α-Hydroxyprednisolone appeared only in a few budesonide batches.

Impurity 2—Impurity 2 was a 1:1 mixture of two epimers, 16α,17α-(22*S*)- and 16α,17α-(22*R*)-methylmethylenedioxypregna-1,4-diene-11β,21-diol-3,20-dione (the propyl group of budesonide is replaced by a methyl group). The epimers were isolated by column chromatography and identified by NMR spectroscopy and mass spectrometry. Their HPLC purity was >99%.

Impurities 3 and 4—Impurities 3 and 4 were isolated by column chromatography. Work is now in progress[3] to identify them. Their HPLC purity was >99%.

Epimers A and B of Budesonide—Epimers A and B were isolated by column chromatography (2, 3), and the HPLC purity was >99.8%.

Solvents—Analytical or spectroscopic (for ethanol) grade solvents were used.

Apparatus—The liquid chromatograph was equipped with a pump[4], a high-pressure injector[5], and a 254-nm detector[6]. Peak areas were determined with an electronic digital integrator[7].

The columns (3.9 mm i.d. and 300 mm long) were prepacked with porous siliceous microbeads to which stationary phases of octadecylsilane or aminopropylsilane were chemically bonded[8].

Preparation of Budesonide Primary Standard—Budesonide was synthesized according to the method described previously (1, 3). Its purification was divided into seven steps.

The crude product was recrystallized three times from methanol–water (Steps 1–3). Since the repeated recrystallizations led to a significant change in the epimeric distribution, the product was partially resolved by chromatography on Sephadex LH-20, using chloroform as the eluent. Two fractions with different epimeric ratios were collected and evaporated to dryness, dissolved in methylene chloride, and precipitated with petroleum ether. Calculated amounts of the two precipitates were mixed to give budesonide with an epimeric ratio near 50:50 (Step 4). This

[1] Astra Syntex.
[2] Lark, Italy.
[3] A. Thalén, unpublished data.
[4] Model M 6000 solvent delivery system, Waters Associates.
[5] Model CV-6-UHPa-N60, Valco.
[6] Model M 440 UV detector, Waters Associates.
[7] Autolab System IV computing integrator, Spectra-Physics.
[8] μBondapak C_{18} and μBondapak NH_2, Waters Associates.

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Table I—Analytical Data of the Budesonide Primary Standard

Test for	Analytical Method	Content, %
Epimer A of budesonide	HPLC (reversed phase)	46.8
Epimer B of budesonide	HPLC (reversed phase)	53.0
Impurities	HPLC (reversed phase)	0.1
Impurities[a]	HPLC (normal phase)	0.1
Methylene chloride	GLC	<0.05
Petroleum ether	GLC	<0.02
Methanol	GLC	0.01
Water	Coulometric Karl Fischer titration	0.05

[a] The impurities obtained by normal-phase HPLC were identical to those detected by reversed-phase HPLC.

product was purified further by repeated recrystallizations from methanol–water (Step 5), ethanol–water (Step 6), and methanol–water (Step 7). A portion of the substance was dried *in vacuo* to constant weight and then was used for an assay of budesonide. The product was analyzed for foreign steroids and epimeric distribution by HPLC (System 2).

Analytical Procedures—*HPLC Conditions*—The mobile phase for reversed-phase chromatography (on μBondapak C_{18}) was ethanol–water in a 48:52 ratio (v/v) in the assay of budesonide (System 1) or in a 43:57 ratio (v/v) for determination of the epimer ratio and foreign steroids (System 2). Five microliters of the sample or of the standards was injected.

The mobile phase for normal-phase chromatography (on μBondapak NH_2) was heptane–ethanol (91:9 v/v). Five microliters of the samples dissolved in ethanol was injected.

HPLC Assay of Budesonide—For the assay of budesonide drug substance, samples of 14.00–16.00 mg were weighed into 25-ml volumetric flasks. The samples were dissolved and diluted to volume with a flucloronide internal standard solution. The latter was prepared by dissolving 35 mg of flucloronide in 100 ml of ethanol.

The samples were compared with a budesonide primary standard, from which solutions were prepared as for the samples.

Standard Addition Experiments—About 0.2 mg of each of the four impurities was weighed with a precision of 0.2% and dissolved together in 25.00 ml of ethanol. A second stock solution was prepared in the same way with 2 mg of the impurities. Aliquots of these solutions were transferred into flasks containing 14.00–16.00 mg of the budesonide primary standard substance. In this way, seven samples with known contents were prepared. They contained impurities in the range of 0.2–7.5%; *i.e.*, the budesonide content was 92.5–99.8%.

The samples were analyzed for their content of budesonide and impurities by reversed-phase HPLC.

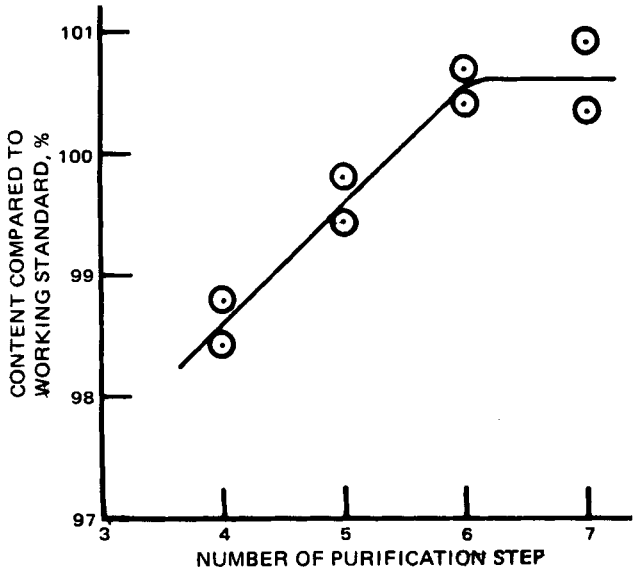

Figure 1—*Content* versus *number of purification step for preparation of budesonide primary standard. Purification step numbers correspond to the steps described in the text.*

Table II—Molar Absorptivities (254 nm), Absorbance (*A*) (1%, 1 cm) Values (254 nm), and Detector Response Factors for Epimers and Impurities of Budesonide

Compound	Molar Absorptivity	*A*	*A* (Relative)[a]	Response Factor (*R*)[b] Determined by HPLC
Budesonide primary standard	1.26×10^4	292	1.00	1.00
Epimer A of budesonide	1.26×10^4	292	1.00	0.99
Epimer B of budesonide	1.26×10^4	292	1.00	0.99
Impurity 1	1.26×10^4	334	1.14	1.14
Impurity 2	1.22×10^4	303	1.04	1.07
Impurity 3	1.21×10^4	282	0.97	—
Impurity 4	1.18×10^4	275	0.94	0.92

[a] Ratio of *A* (compound) to *A* (budesonide). [b] See text for definition.

Ethanolic solutions of Epimer A (0.103 mg/ml) and Epimer B (0.104 mg/ml) also were prepared. Aliquots of the two solutions were mixed to obtain samples with known epimeric distributions. These samples were used to evaluate the accuracy of the HPLC determination of the epimer distribution.

Detector Response Experiments—Four ethanolic solutions of budesonide were prepared. Each solution contained a known content of Impurity 1, 2, 3, or 4. Five microliters was injected in reversed-phase HPLC System 2. The peak areas of the impurity and of budesonide were determined for each sample. The relative detector response, *R*, was calculated according to:

$$R = \frac{\text{peak area of impurity}}{\text{concentration of impurity}} \times \frac{\text{concentration of budesonide}}{\text{peak area of budesonide}} \quad \text{(Eq. 1)}$$

From spectrophotometric measurements, absorbance (*A*) (1%, 1 cm) values and molar absorptivities were calculated for budesonide, Epimers A and B, and the four impurities.

Analysis of Solvent Residues—The water content of budesonide was analyzed by the coulometric Karl Fischer titration method (9). Organic solvent residues in budesonide originating from the synthesis were analyzed by GLC. The gas–liquid chromatograph[9] was equipped with a flame-ionization detector and a 1.5-m × 1.3-mm Porapak Q glass column. Nitrogen was the carrier gas at a flow rate of 20 ml/min. The injector temperature was 220°, and the oven temperature was 150°.

Two samples were prepared by dissolving 20.0–30.0 mg of budesonide bulk substance in 1.0 ml of absolute methanol and 1.0 ml of ethanol, respectively; 0.02% methanolic solutions of methylene chloride and petroleum ether and 0.02% ethanolic solutions of methanol were prepared and used as standards. One microliter of the samples and standards was injected. Solvent residues of >0.01% could be detected. Corrections for trace impurities in methanol and ethanol were made.

RESULTS AND DISCUSSION

Like other diastereoisomers, Epimers A and B of budesonide differ in solubility. This fact had to be considered in the preparation of the primary standard. During the recrystallizations (Steps 1–3), the ratio of Epimer A to B changed from about 50:50 to about 40:60. In purification Step 4, the epimeric distribution was adjusted back to 49.9:50.1. The final recrystallizations (Steps 5–7) resulted in 46.9% Epimer A and 53.1% Epimer B. The changes in epimeric distribution with the number of recrystallizations limit the purification procedure if the requirements for the epimer ratio in the drug substance specifications are to be fulfilled.

Figure 1 shows a plot of the budesonide content relative to a budesonide working standard *versus* the number of purification step. The content increased with purification to a constant value. The substance obtained in the ethanol–water recrystallization (Step 6) could not be purified further and, therefore, was defined as the primary standard. Reversed-phase HPLC with System 2 revealed a few traces of foreign steroids, and no further impurities were detected by normal-phase HPLC. Traces of methanol and water were detected by GLC and coulometric Karl Fischer titration, but no other chemicals used in purification could be traced. These results are summarized in Table I.

[9] Model 1400, Varian.

Table III—Recovery for Budesonide and Impurities in HPLC Analysis

Sample	Budesonide, %		Impurity 1, %		Impurity 2, %		Impurity 3, %		Impurity 4, %		Sum of Detected Impurities, %		Total Sum, %	
	Added	Found	Added	Found	Added	Found	Added	Found	Added	Found	Added	Found	Added	Found
1	99.78	99.89	0.06	0.05	0.05	0.05	0.05	0.04	0.05	—	0.16	0.14	99.99	100.05
2	99.60	100.06	0.11	0.10	0.11	0.10	0.10	0.07	0.10	—	0.32	0.27	100.02	100.33
3	99.18	99.45	0.21	0.20	0.21	0.19	0.20	0.17	0.20	—	0.62	0.56	100.00	100.01
Recovery, %				101.7		100.2		78.3		—		92.8		
SD				7.55		9.75		7.64		—		5.39		
4	99.01	99.07	0.25	0.25	0.23	0.21	0.26	0.26	0.25	—	0.74	0.72	100.00	99.79
5	97.93	98.26	0.53	0.51	0.48	0.45	0.54	0.55	0.52	0.52	2.07	2.03	100.00	100.29
6	96.03	96.50	1.02	0.96	0.92	0.85	1.04	1.08	0.98	0.89	3.96	3.78	99.99	100.28
7	92.42	92.70	1.95	1.86	1.77	1.62	1.98	2.04	1.88	1.78	7.58	7.30	100.00	100.00
Recovery, %				96.4		92.3		102.2		95.2		96.8		
SD				2.54		1.14		1.65		4.62		1.14		
Recovery, %		100.29												100.11
SD		0.162												0.197

Known detector response factors are necessary for a correct evaluation of foreign steroids and epimer distribution. Such factors are listed in Table II. They were used for all of the reported content determinations of the four impurities and the two epimers. These factors are close to one since the molar absorptivities of the impurities and of Epimers A and B are similar to that of budesonide. This result is due to the fact that the UV absorption originates from ring A in the steroid nucleus. This structure probably is common to each of the four impurities.

The HPLC assay for budesonide using System 1 was tested by a standard addition procedure (Table III). Known samples containing 92–100% of the budesonide primary standard were assayed with a mean recovery of 100.3% and a standard deviation of 0.16. In the same experiment, the four impurities were evaluated analytically (Table III). In the range of 0.05–0.2% of individual foreign steroids, recoveries of 78–102% were obtained. The corresponding standard deviations were 7.6–9.8. For samples with individual impurities between 0.2 and 2%, the recoveries were 92–102% with standard deviations of 1.1–4.6.

Even in the low impurity range (0.05–0.2%), quantification gives fairly good accuracy and precision. This result is possible since the detection limit, 0.005%, is below this range and since the chromatographic system works linearly and is stable down to the highest sensitivity, *i.e.*, 0.005 absorbance unit full scale. For Impurity 4, the analytical evaluation depends on its concentration; in the range of 0.05–0.25%, the impurity could not be separated chromatographically from the budesonide Epimer B. Therefore, the impurity peak is added to the budesonide peaks. This addition results in a slightly higher recovery for budesonide. However, if Impurity 4 has to be determined in the range of 0.05–0.25%, System 2 can be used since it results in larger retention times and, thus, in higher separation efficiency.

Mixtures with a known content of Epimers A and B were investigated with ethanol–water in ratios of 43:57 (v/v) (System 2) and 48:52 (v/v) (System 1) as the mobile phases. For the control of the epimer distribution of budesonide batches, System 2 was used since it was optimal with respect to the epimer separation. The effect of increasing the water content in the mobile phase to improve the epimer separation was due to increased retention and to an increase in the separation factor (7).

Figures 2 and 3 show chromatographic separations of the impurities and the epimers of budesonide using Systems 1 and 2. The resolution was improved significantly by increasing the mobile phase water content from 52 to 57%. This change in resolution had little effect on the quantification of the epimers. Although no baseline separation was obtained, System

Table IV—Recovery for Individual Epimers of Budesonide in HPLC Analysis

Sample	Percent of Epimer A in Standard Mixtures of Epimers A and B[a]		
	Added	Found[b]	Found[c]
1	62.41	62.16	62.41
2	55.46	55.30	55.72
3	49.90	49.62	49.95
4	44.34	44.20	44.58
5	37.50	37.56	37.75
Recovery, %		99.7	100.3
SD		0.27	0.29

[a] Since the standard mixtures contained only Epimers A and B, the percentage of Epimer B was obtained from 100% − % of Epimer A. [b] Mobile Phase System 1 was ethanol–water in a ratio of 48:52 (v/v). [c] Mobile Phase System 2 was ethanol–water in a ratio of 43:57 (v/v).

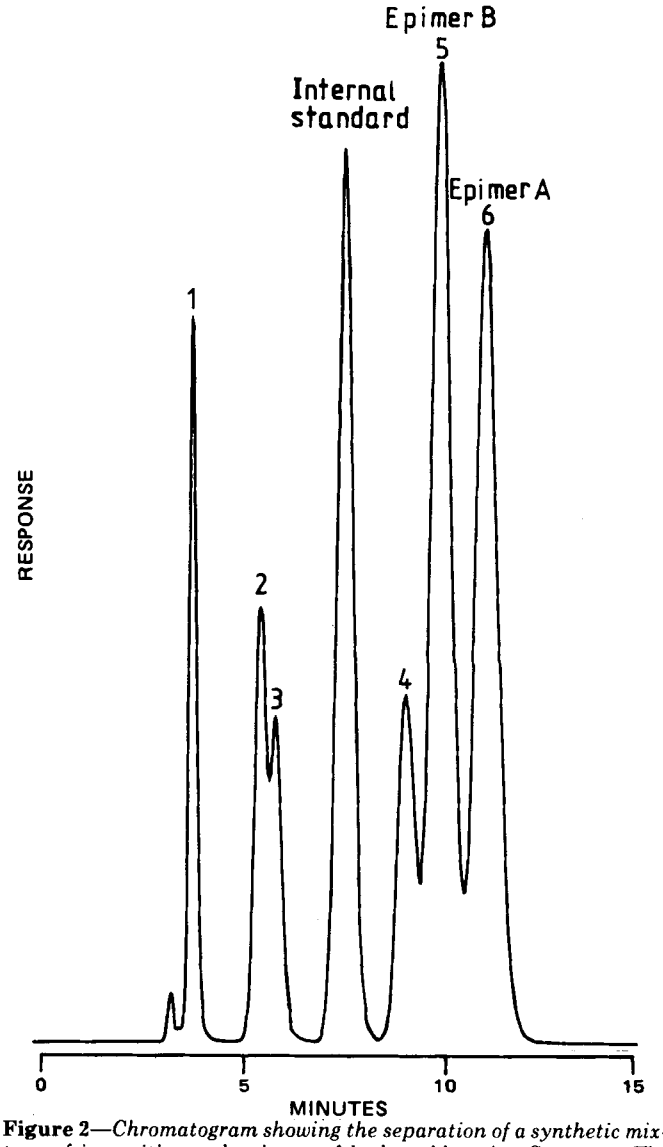

Figure 2—*Chromatogram showing the separation of a synthetic mixture of impurities and epimers of budesonide using System 1. The support was μBondapak C_{18}, the mobile phase was ethanol–water (48:52 v/v), and the flow rate was 1.0 ml/min. Key: 1, Impurity 1 (16α-hydroxyprednisolone); 2, Impurity 2 (homolog of budesonide; see text); 3, Impurity 3 (unknown); 4, Impurity 4 (unknown); 5, Epimer B of budesonide; and 6, Epimer A of budesonide.*

Table V—Analytical Data of Budesonide Batches

	Batch							
	372-40	2/76	22/77	23/77	24/77	33/77	34/77	35/77
Budesonide, %	97.6	98.6	99.2	98.7	98.6	98.5	98.4	98.7
Epimer A of budesonide, %	49.3	48.4	46.4	46.5	46.7	47.2	47.0	47.4
Impurities by HPLC (reversed phase), %	0.9	0.9	0.8	0.7	0.7	0.9	0.9	0.7
Impurities by HPLC (normal phase), %	0.0	0.1	0.4	0.4	0.3	0.4	0.3	0.3
Methylene chloride by GLC, %	0.6	0.0	0.0	—	—	—	—	—
Petroleum ether by GLC, %	0.1	0.0	0.0	—	—	—	—	—
Loss on drying, %	0.1	0.2	0.3	0.2	0.4	0.2	0.3	0.1
Total sum[a], %	99.3	99.8	100.7	100.0	100.0	100.0	99.9	99.8

[a] Mean of total sum is 99.9%. Standard deviation of total sum is 0.39.

1 gave similar accuracy in the epimer distribution determination to that of System 2 (Table IV). This result must be a consequence of the ideal behavior of the chromatographic system; *i.e.*, symmetrical peaks were obtained. As for the epimeric resolution, the separation of impurities was improved by changing from System 1 to System 2. Several tests showed that even the quantification of individual foreign steroids was not significantly affected by the differences in resolution.

The ratio between the peak area of budesonide and the peak area of the flucloronide internal standard was plotted *versus* the concentration of budesonide between 0 and 0.6 mg/ml. The result was a straight line with an intercept of 0.096, a slope of 6.01 ml/mg, and a correlation coefficient of 0.999989. The data indicate that the chromatographic system behaves properly and that the detector responds with high linearity. The use of an internal standard is necessary to minimize the injection error. Repeated injections gave a relative standard deviation of ~0.1% for the peak area ratio. Furthermore, the internal standard must not interfere with budesonide or impurity peaks. This condition is satisfied by flucloronide (Fig. 2).

In all budesonide batches analyzed, Impurities 1–4 represented ~75% of the total foreign steroids found by reversed-phase liquid chromatography. The remaining content (*i.e.*, traces of other impurities in budesonide) was quantified by calculation of the area under the chromatographic peaks since the detector response factors are unknown. The same procedure was used for traces of several nonpolar impurities, which could not be eluted by reversed-phase chromatography but were eluted and detected by normal-phase chromatography on μBondapak NH_2. In general, impurities that eluted before budesonide in the reversed-phase system eluted after the drug substance in the normal-phase system, as illustrated for the four impurities in Fig. 4. Figure 4 also shows the separation of the two epimers of Impurity 2, in contrast to the reversed-phase systems where they were not separated (Figs. 2 and 3).

Analytical results from eight small-scale production batches of budesonide (Table V) gave a mean for the sum of budesonide and impurities of 99.9% with a standard deviation of 0.39. This result suggests that the detector response factors are similar for budesonide and for all unknown impurities.

The results show that the necessary prerequisites for an accurate evaluation of budesonide substance by HPLC are fullfilled. The proposed analysis method is highly selective, linear, precise, and sensitive. Another advantage of the method is its high analytical capacity. The budesonide content, the impurity content, and the epimer distribution can be evaluated accurately by one chromatographic system. Comparison of retention times in sample and standard chromatograms may serve as an identity test for budesonide. The proposed HPLC method meets the main points in monographs for steroid substances and seems analytically superior to the usual procedures suggested in pharmacopeias.

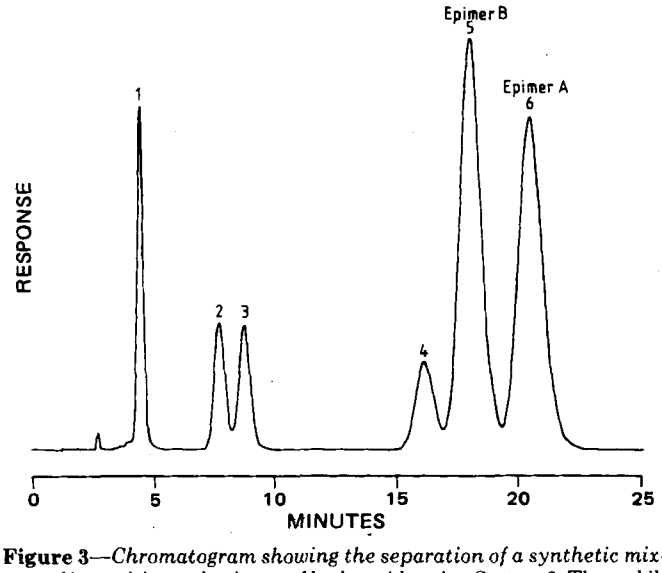

Figure 3—*Chromatogram showing the separation of a synthetic mixture of impurities and epimers of budesonide using System 2. The mobile phase was ethanol–water (43:57 v/v). The other conditions were the same as those described for Fig. 2.*

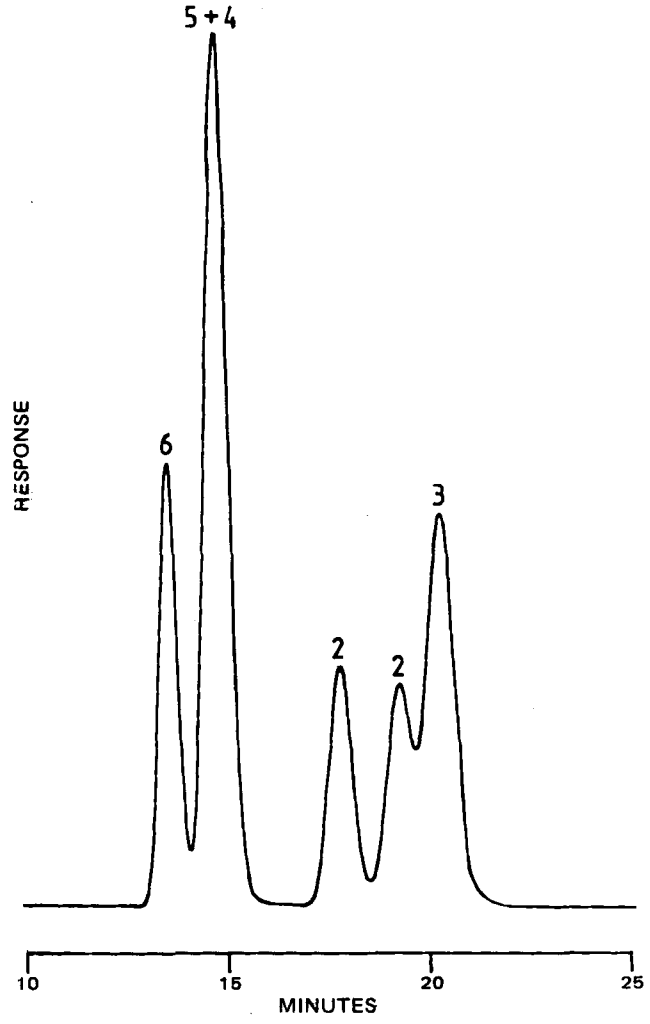

Figure 4—*Chromatogram showing the separation of a synthetic mixture of impurities and epimers of budesonide using a normal-phase system. The support was μBondapak NH_2, the mobile phase was heptane–ethanol (91:9 v/v), and the flow rate was 1.0 ml/min. The peak designations are the same as in Fig. 2. Impurity 2 (a mixture of two epimers; see text) appeared in this system as two peaks. Impurity 1 was not eluted.*

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Physical Model Evaluation of Topical Prodrug Delivery—Simultaneous Transport and Bioconversion of Vidarabine-5′-valerate III: Permeability Differences of Vidarabine and *n*-Pentanol in Components of Hairless Mouse Skin

CHENG DER YU *, WILLIAM I. HIGUCHI ˣ, NORMAN F. H. HO, JEFFREY L. FOX, and GORDON L. FLYNN

Received August 2, 1979, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication December 13, 1979. *Present address: Syntex Research, Stanford Industrial Park, Palo Alto, CA 94304.

Abstract □ The permeation behavior of ^{3}H-vidarabine (^{3}H-9-β-D-arabinofuranosyladenine) and ^{14}C-*n*-pentanol through different strata of hairless mouse skin was studied using a diffusion cell at 37° under steady-state conditions. Partition coefficients for the skin components *versus* 0.9% aqueous NaCl solution also were obtained. Various skin preparations including full-thickness skin, cellophane-stripped skin, and dermis membranes of different thicknesses were employed. The dermis membranes were considered to be diffusionally homogeneous, and the product of the permeability coefficient and the thickness was taken as the apparent diffusivity. The apparent diffusivities for both compounds investigated were independent of thickness. Therefore, it was concluded that the molecular diffusivity is constant throughout the dermis. Comparisons of permeability coefficients in various strata of the skin revealed that, while the stratum corneum is the major diffusional barrier, the epidermis appears to be significantly less permeable than the dermis.

Keyphrases □ Vidarabine valerate prodrug—topical dosage forms, permeability differences through various mouse skin strata □ Prodrugs, topical—based on simultaneous transport and bioconversion of vidarabine, permeability differences in various components of mouse skin □ Diffusivity—vidarabine and *n*-pentanol, effect of skin strata and thickness on diffusivity □ Models, physical—based on simultaneous transport and bioconversion, effect of homogeneous distribution on prodrug evaluation

The basic experimental methods for determining key parameters and evaluating the transport and metabolism of a prodrug of vidarabine (9-β-D-arabinofuranosyladenine, I) in hairless mouse skin were discussed previously (1). Among the parameters needed for the mechanistic quantification of the problem are the transport parameters, the permeability coefficients, and the diffusivities in the various components of the skin. These parameters and the metabolizing enzyme constants, k_1 and k_2, permit a quantitative analysis of the prodrug delivery behavior in the skin.

The barrier nature of the various skin components is not fully understood. Several studies concerned the possible differences between the diffusional resistances of the epidermis and the dermis (2, 3). The purposes of this investigation were to study mechanistically the barrier nature of the various components of the hairless mouse skin using membrane preparations of different strata of the skin and to investigate the possible variations in the diffusivity with respect to position in the dermis component.

Studies were conducted on the permeabilities of I in various membrane preparations of the hairless mouse skin

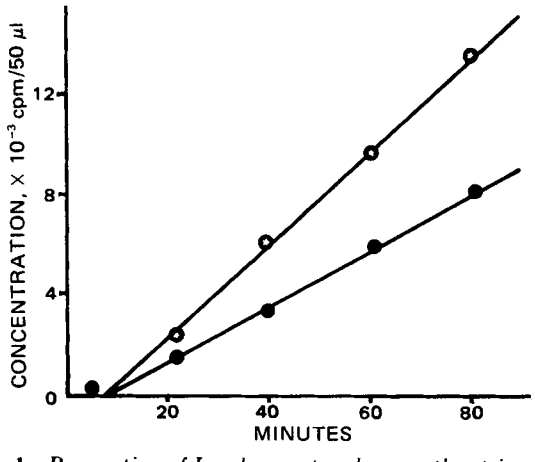

Figure 1—*Permeation of I and* n*-pentanol across the stripped skin. Key:* ●, ^{3}*H-2-I; and* ✪, ^{14}*C-*n*-pentanol. Both permeants were run concurrently, and the* Δ*C values were* 6.68×10^{5} *and* 2.55×10^{5} *cpm/50 µl for I and* n*-pentanol, respectively. The fluxes may be calculated according to flux = (slope) (volume/area), where the volume is 3.0 ml and the area is 1.767 cm^{2}.*

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