High-Pressure Liquid Chromatographic Determination of Corticosteroids in Topical Pharmaceuticals

PATRICIA A. WILLIAMS *[x] and **EDWARD R. BIEHL** [‡]

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Abstract □ Cortisone, hydrocortisone, prednisolone, and prednisone were separated on a reversed-phase microparticulate high-pressure liquid chromatographic (HPLC) column with a ternary mobile solvent containing tetrahydrofuran, methanol, and water. Cortisone acetate, hydrocortisone acetate, and prednisolone acetate were separated on the same reversed-phase column using acetonitrile–water. Various commercial topical formulations of these corticosteroids, except cortisone and prednisone, were prepared by both simple dilution and by extraction for analysis by the proposed HPLC procedure, by the blue tetrazolium procedure, and by the isoniazid procedure and/or phenylhydrazine method. Retention data are given for some common degradation products (C-17 ketones and C-17 carboxylic acid derivatives) and for methyl-, propyl-, and butylparabens with these mobile solvents.

Keyphrases □ Corticosteroids—analysis of topical pharmaceuticals, high-pressure liquid chromatography □ Steroids—analysis in topical pharmaceuticals, high-pressure liquid chromatography □ High-pressure liquid chromatography—analysis, corticosteroids in topical pharmaceuticals

High-pressure liquid chromatography (HPLC) is a powerful tool in providing a specific method of analysis for corticosteroids in pharmaceutical preparations (1–30). HPLC methods for corticosteroids in topical preparations such as creams, lotions, and ointments (5, 6, 10–14, 16, 19, 21, 22, 25–28, 30) are particularly useful because of the complex nature of the sample matrix and the potential for interference in colorimetric or UV methods.

The official blue tetrazolium (31, 32), phenylhydrazine (33), isoniazid (34), and UV methods are subject to interference from several sources (35, 36). HPLC can provide both the separation and determination steps, thereby eliminating most interference problems in other methods.

Although official methods of analysis of many corticosteroids are being changed to HPLC methods (37), all corticosteroids chosen for this study are still being determined officially by the blue tetrazolium or phenylhydrazine methods (31, 37).

The described reversed-phase HPLC procedure effectively separates the corticosteroid of interest from its most common decomposition products, closely related steroids, other common active ingredients, and product excipients. Comparisons of results of HPLC, blue tetrazolium, isoniazid, and/or phenylhydrazine methods were made for various commercial preparations. Simple dilution and extraction procedures also were compared. The corticosteroids studied were cortisone, hydrocortisone, prednisone, prednisolone, cortisone acetate, hydrocortisone acetate, and prednisolone acetate.

EXPERIMENTAL

Materials—*Standards*—Cortisone acetate[1], dexamethasone[1], hydrocortisone[1], hydrocortisone acetate[1], prednisolone[1], prednisolone acetate[1], prednisone[1], cortisone[2], androst-4-ene-3,17-dione[2] (I), 11β-hydroxyandrost-4-ene-3,17-dione[2] (II), 11β-hydroxyandrosta-1,4-diene-3,17-dione[2] (III), androsta-1,4-diene-3,11,17-trione[2] (IV), 17α-hydroxy-3,11-dioxoandrost-4-ene-17-carboxylic acid[3] (V), 11β,17α-dihydroxy-3-oxoandrost-4-ene-17-carboxylic acid[3] (VI), 11β,17α-dihydroxy-3-oxoandrosta-1,4-diene-17-carboxylic acid[3] (VII), 17α-hydroxy-3,11-dioxoandrosta-1,4-diene-17-carboxylic acid[3] (VIII), and 11β,17α-dihydroxy-3-oxo-9-fluoro-16α-methylandrosta-1,4-diene-17-carboxylic acid[3] (IX) were used.

Reagents—ACS reagent grade or equivalent chloroform, cyclohexane, acetic acid, sodium chloride, and basic aluminum oxide powder were used.

HPLC grade or distilled-in-glass grade acetonitrile, methanol, and tetrahydrofuran (no stabilizers) were used. Tetrahydrofuran was treated to remove peroxides just before use in the mobile solvent by passing it through an aluminum oxide column containing 10 g of aluminum oxide/100 ml of tetrahydrofuran (38).

HPLC—The high-pressure liquid chromatograph was equipped with a 6000-psi pump, a high-pressure injector or automatic injector, a 254-nm detector[4], a 10-mv recorder[5], and an electronic digital integrator[6]. The instrument was operated at ambient temperature, and the detector sensitivity used was 0.05 aufs.

Column—The column[7] (25 cm × 4.6 mm) was purchased prepacked with octylsilane chemically bonded to porous microsilica particles, 5 μm in diameter. The theoretical plates (N) for hydrocortisone were determined to be 3467 with the formula $N = 16(V/W)^2$, where V is the retention volume of the component and W is the width of the base of the peak.

Mobile Solvents—Each mobile solvent was filtered through a micropore filter[8] just before use. Solvent A (for hydrocortisone, prednisolone, and prednisone determinations) was 25% (v/v) tetrahydrofuran and 12.5% (v/v) methanol in water. A flow rate of 1 ml/min was used at a column back-pressure of 1800 psi. The retention volume for hydrocortisone ranged from 9 to 10.5 ml. The retention volume for dexamethasone ranged from 14 to 16.5 ml. The resolution was 1.3 for prednisone–cortisone, 1.6 for cortisone–prednisolone, and 1.0 for prednisolone–hydrocortisone. Resolution (R_s) was determined by the formula $R_s = 2(V_2 - V_1)/(W_2 + W_1)$, where V_2 and V_1 are the retention volumes of the two components and W_2 and W_1 are the corresponding widths of the bases of the peaks.

Solvent B (for cortisone acetate, hydrocortisone acetate, and prednisolone acetate determinations) was 45% (v/v) acetonitrile in water. A flow rate of 1 ml/min was used at a column back-pressure of 800 psi. The retention volume for cortisone acetate ranged from 10 to 11 min. The resolution (R_s) was 0.80 for prednisolone acetate–hydrocortisone acetate and 3.0 for hydrocortisone acetate–cortisone acetate.

Internal Standard Preparations—*For Hydrocortisone, Prednisolone, and Prednisone Determinations*—The dexamethasone stock internal standard solution was ~150 μg of dexamethasone/ml of methanol. To prepare the dexamethasone diluted internal standard solution, the stock internal standard solution was diluted quantitatively one to five with methanol to obtain a solution with ~30 μg of dexamethasone/ml.

For Corticosteroid Acetate Determinations—Cortisone acetate was used for hydrocortisone acetate and prednisolone acetate determinations, and hydrocortisone acetate was used for cortisone acetate determinations.

[1] USP reference standards.

[2] Research Plus Steroids Laboratories, Denville, N.J.
[3] Prepared by Millard Maienthal, Division of Drug Chemistry, Food and Drug Administration, Washington, D.C.
[4] Model 204 liquid chromatograph with model 6000 A pump, model U6K loop injector or WISP 710A sample processor, and model 440 absorbance detector, Waters Associates, Milford, Mass.
[5] Model A-25 strip-chart recorder, Varian Instrument Division, Aerograph Products, Palo Alto, Calif.
[6] Supergrator 3, Columbia Scientific Industries, Austin, Tex.
[7] Zorbax C_8, DuPont Chemical Co., Wilmington, Del.
[8] Millipore type LS, 5.0-μm pore size, Millipore Corp., Bedford, Mass.

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Table I—Comparison of HPLC Columns

Corticosteroid	Octadecylsilane[a] Column RRV[c]	R_s	Octylsilane[b] Column RRV[c]	R_s
Prednisone	0.77	1.2	0.80	1.3
Cortisone	0.85	1.2	0.86	1.6
Prednisolone	0.92	1.1	0.94	1.0
Hydrocortisone	1.00		1.00	
Cortisone acetate	2.30		2.15	
Prednisolone acetate	2.33		2.12	
Hydrocortisone acetate	2.60		2.32	
Methylparaben	1.26		1.24	
Propylparaben	4.01		3.30	
Butylparaben	4.56		3.52	

[a] Zorbax ODS (25-cm × 4.6-mm) column, DuPont Chemical Co., N (hydrocortisone) = 3364 plates. [b] Zorbax C_8 (25-cm × 4.6-mm) column, DuPont Chemical Co., N (hydrocortisone) = 3467 plates. [c] Retention volume relative to hydrocortisone, V = 9–10 ml.

The stock internal standard solution was ~100 μg of internal standard/ml in methanol. To prepare the diluted internal standard solution, the stock internal standard solution was diluted quantitatively one to five with 0.5% acetic acid in methanol to obtain a solution with ~20 μg of corticosteroid acetate/ml.

Standard Preparations—*For Hydrocortisone, Prednisolone, and Prednisone Determinations*—Corticosteroid reference standard, about 10 mg accurately weighed, was transferred to a 100-ml volumetric flask and dissolved and diluted to volume with methanol. A 10.0-ml aliquot was transferred to a 50-ml volumetric flask, 10.0 ml of the dexamethasone stock internal standard solution was added, and the solution was diluted to volume with methanol. The final dilution contained 20 μg of corticosteroid reference standard/ml and 30 μg of dexamethasone internal standard/ml.

For Cortisone Acetate, Hydrocortisone Acetate, and Prednisolone Acetate Determinations—Corticosteroid acetate reference standard, about 10 mg accurately weighed, was transferred to a 100-ml volumetric flask and dissolved and diluted to volume with 0.5% acetic acid in methanol. A 10.0-ml aliquot was transferred to a 50-ml volumetric flask, 10.0 ml of the appropriate corticosteroid acetate stock internal standard solution was added, and the solution was diluted to volume with 0.5% acetic acid in methanol. The final dilution contained 20 μg of corticosteroid acetate reference standard/ml and 20 μg of corticosteroid acetate internal standard/ml.

Sample Preparations for Hydrocortisone, Prednisolone, and Prednisone Determinations—*Direct Dilution*—An accurately weighed quantity of product, equivalent to ~10 mg of corticosteroid, was transferred to a 150-ml beaker, and 25 ml of methanol was added. The solution was heated on a steam bath with periodic agitation until the sample material was thoroughly dispersed. The solution was cooled in an ice bath

Table II—Retention Data of Octylsilane Microsilica Particle (5-μm) Column

Compound	Mobile Solvent A RRV[a]	K'[b]	Mobile Solvent B RRV[c]	K'[b]
Cortisone	0.86	2.3	1.07	0.9
Cortisone acetate	2.15	7.1	2.44	3.4
I	0.96	—	2.01	—
V	0.44	—	0.74	—
Hydrocortisone	1.00	2.8	1.00	0.8
Hydrocortisone acetate	2.32	7.7	2.08	2.7
II	1.13	—	1.82	—
VI	0.63	—	0.78	—
Prednisolone	0.94	2.6	0.97	0.7
Prednisolone acetate	2.12	7.0	2.03	2.6
III	1.02	—	1.72	—
VII	0.54	—	0.76	—
Prednisone	0.80	2.0	1.04	0.9
IV	0.90	—	1.90	—
VIII	0.37	—	0.64	—
Dexamethasone	1.64	5.2	1.30	1.3
IX	1.59	—	0.87	—
Methylparaben	1.24	—	1.22	—
Propylparaben	3.30	—	2.33	—
Butylparaben	3.52	—	3.77	—

[a] Retention volume relative to hydrocortisone, V = 9–10 ml. [b] $K' = (V - V_0)/V_0$, where V_0 = 2.4 ml. [c] Retention volume relative to hydrocortisone, V = 4–5 ml.

Table III—Replication Study with Standards

Mobile Solvent	Amount Injected, μg	Retention Volume, ml	Coefficient of Variation[a] Peak Height	Peak Area
		Hydrocortisone		
A	0.25	9.35–9.49	1.38	3.19
B	0.15	4.34	1.62	0.89
		Dexamethasone		
A	0.375	15.30–15.62	1.34	2.33
B	0.225	5.62–5.65	1.61	3.61
		Cortisone Acetate		
A	0.53	20.10–20.47	1.30	1.39
B	0.30	10.60–10.66	1.14	1.83

[a] For 10 replicate injections.

until the residue solidified, and then the liquid was decanted into a 100-ml volumetric flask. The extraction was repeated with three 20-ml portions of methanol, decanting each into a volumetric flask after cooling. After the methanol solution reached room temperature, it was diluted to volume with methanol and filtered if necessary.

A 10.0-ml aliquot was transferred to a 50-ml volumetric flask, 10.0 ml of the dexamethasone stock internal standard solution was added, and the solution was diluted to volume with methanol.

Extraction—A 10.0-ml aliquot of the first sample dilution from the direct dilution procedure, equivalent to ~1 mg of corticosteroid, was transferred to a 125-ml separator. Then 2 ml of aqueous saturated sodium chloride solution and 25 ml of cyclohexane were added, and the separator was shaken vigorously for 1 min. After the layers separated, the lower methanol layer was drained into a 250-ml separator containing 50 ml of distilled water. The cyclohexane layer was extracted with two 2-ml portions of 80% methanol–water. These extracts were added to the solution in the 250-ml separator, and the cyclohexane layer was discarded.

The solution in the 250-ml separator was extracted with three 50-ml and one 45-ml portions of chloroform, with shaking of the separator for 2 min for each extraction. Each chloroform extract was filtered through a cotton plug into a 200-ml volumetric flask. The solution was diluted to volume with chloroform and mixed.

A 20.0-ml aliquot of the chloroform solution was transferred to a 25-ml conical flask, and the chloroform was evaporated just to dryness on a steam bath under a stream of air. Then 5.0 ml of the dexamethasone diluted internal standard solution was added to the residue in the flask.

Sample Preparation for Cortisone Acetate, Hydrocortisone Acetate, and Prednisolone Acetate Determinations—The method for corticosteroid alcohols was followed, except that 0.5% acetic acid in methanol was substituted for methanol in both the direct dilution and extraction procedures. Also, the appropriate corticosteroid acetate internal standard solution was used in place of the dexamethasone internal standard solution.

HPLC Determination—Ten microliters of each standard and sample preparation was injected onto an HPLC column that had been previously

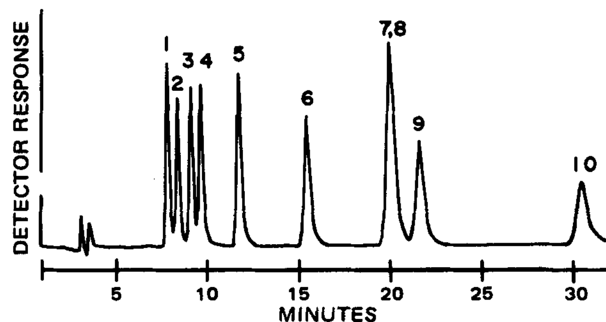

Figure 1—*Chromatogram of a standard mixture. The column was octylsilane bonded to microsilica (5 μm). Solvent A was 25% tetrahydrofuran–12.5% methanol in water. The flow rate was 1 ml/min, the pressure was 1800 psi, the temperature was ambient, and the detector was at 254 nm (0.05 aufs). Key: 1, prednisone; 2, cortisone; 3, prednisolone; 4, hydrocortisone; 5, methylparaben; 6, dexamethasone; 7, prednisolone acetate; 8, cortisone acetate; 9, hydrocortisone acetate; and 10, propylparaben.*

Table IV—Linearity Study with Standards

Mobile Solvent	Amount Injected, μg	Coefficient of Determination (r^2)	
		Peak Height	Peak Area
		Hydrocortisone	
A	0.05–0.4	0.9993	0.9977
B	0.05–0.4	0.9995	0.9999
		Dexamethasone	
A	0.075–0.6	0.9994	0.9977
B	0.075–0.6	0.9993	0.9986
		Cortisone Acetate	
A	0.1–0.8	0.9996	0.9934
B	0.1–0.8	0.9995	0.9993

Table V—Recovery following Extraction

Sample[a]	Percent of Recovered Standard		
	HPLC	Blue Tetrazolium	Isoniazid
1	103.2	102.6	103.6
3	99.1	103.0	100.3
4	98.9	99.5	102.3
5	99.1	99.3	101.4
7	101.4	102.8	103.1
9	99.4	97.3	100.2
10	100.4	101.7	98.2
12*a*	91.6	91.6	98.2
12*b*	100.1	115.8	110.6
13*a*	95.1	92.8	100.9
13*b*	102.1	109.5	107.0
14	100.2	100.6	—
16	99.8	101.2	102.3
17	99.3	102.9	102.0
18	98.4	108.4	103.7
19	100.4	98.0	100.8
20	99.3	106.8	105.1
21	98.4	103.0	—
22	99.3	103.6	—
Average	99.24	102.13	102.48
SD	2.48	5.60	3.16
CV	2.5	5.48	3.08

[a] Sample numbers correspond to samples in Table VII.

equilibrated for 0.5–1 hr with the appropriate mobile solvent (Solvent A for hydrocortisone, prednisolone, and prednisone; Solvent B for cortisone acetate, hydrocortisone acetate, and prednisolone acetate). The corticosteroid peak response should be ~50% full scale at 0.05 aufs. If not, the injection volume should be adjusted.

From the chromatogram, the peak height ratios of the corticosteroid to the internal standard in the sample and standard were determined. The amount of corticosteroid in the sample is found using:

$$\frac{\text{mg of corticosteroid}}{\text{g of sample}} = \frac{\text{sample ratio}}{\text{standard ratio}} \frac{\mu\text{g of standard}}{\text{ml}} \times \frac{\text{sample dilution}}{\text{g of sample}} \frac{1\text{ mg}}{1000\ \mu\text{g}} \quad \text{(Eq. 1)}$$

For lotion samples, the value, in milligrams per gram, is multiplied by sample density to determine the value in milligrams per milliliter.

Blue Tetrazolium Determination—The USP (31) "Assay for Steroids" procedure was used.

Phenylhydrazine Method—The Silber–Porter (33) procedure was followed.

Isoniazid Method—The Umberger (34) procedure was followed using twice the recommended concentration of hydrochloric acid to increase sensitivity. Prednisolone, prednisolone acetate, and prednisone reactions were run in a 50° water bath[9].

RESULTS AND DISCUSSION

HPLC Parameters—Two reversed-phase columns, octadecylsilane and octylsilane chemically bonded to microparticle silica (5 μm), were used. Column efficiency results (theoretical plates) were comparable, but

[9] Suggested by Sharon R. Reed and Stephen M. Walters, Food and Drug Administration, Detroit, Mich., personal communication.

Table VI—Precision of HPLC Analysis of Samples

Run	Percent of Declared			
	0.25% Hydrocortisone Lotion[a]		0.25% Hydrocortisone Acetate Lotion	
	Methanol Dilution	Chloroform Extraction	Methanol Dilution	Chloroform Extraction
1	50.8	49.7	96.1	94.7
2	51.0	50.7	97.7	95.6
3	51.4	49.7	96.7	95.3
4	51.7	51.8	96.4	96.0
5	51.5	49.5	96.3	95.7
6	51.0	51.3	96.3	94.7
Mean	51.23	50.45	96.58	95.33
SD	0.35	0.96	0.58	0.54
CV	0.68	1.90	0.68	0.57

[a] Hydrocortisone decomposed. See Sample 8 in Table VIII.

Table VII—Composition of Commercial Corticosteroid Samples Studied

Sample	Sample Type and Declared Amount of Corticosteroid	Other Active Ingredients
Cortisone acetate		
1	Ophthalmic ointment, 1.5%	Neomycin sulfate, 0.5%
Hydrocortisone		
2	Cream, 0.25%[a,b]	—
3	Cream, 0.25%[a,b]	Neomycin sulfate, 0.5%
4	Cream, 0.5%[a]	Diiodohydroxyquin, 1%
5	Cream, 0.5%	Diiodohydroxyquin, 1%
6	Gel, 1%	—
7	Lotion, 0.125%[a,b]	—
8	Lotion, 0.25%	—
9	Lotion, 0.25%[a,b]	—
10	Lotion, 0.25%[a]	Coal tar solution, 2%; diiodohydroxyquin, 1%
11	Lotion, 0.5%[a,b]	—
12	Lotion, 0.5%[a,b]	Benzoyl peroxide, 5.5%; chlorhydroxyquinoline, 0.25%
13	Lotion, 0.5%[a,b]	Benzoyl peroxide, 5.0%; chlorhydroxyquinoline, 0.25%
14	Ointment, 0.5%	—
15	Ointment, 0.5%	Polymyxin B sulfate, 5000 U/g; bacitracin zinc, 400 U/g; neomycin sulfate, 0.5%
Hydrocortisone acetate		
16	Cream, 0.5%[a]	Polymyxin B sulfate, 10,000 U/g; neomycin sulfate, 0.5%; gramicidin, 0.25%
17	Cream, 0.5%[a,b]	Lidocaine, 3%; iodochlorhydroxyquin, 3%
18	Cream, 0.5%[a,b]	Lidocaine, 3%
19	Cream, 0.5%[a]	Chlorcyclizine hydrochloride, 2%
20	Cream, 0.5%	Salicylic acid, 2%
Prednisolone		
21	Cream, 0.5%[a,c]	Aluminum chlorhydroxy allantoinate, 0.25%
22	Ophthalmic ointment, 0.5%	Chloramphenicol, 1%
Prednisolone acetate		
23	Ophthalmic ointment, 0.2%	Sulfacetamide sodium, 10.0%
24	Ophthalmic ointment, 0.5%[a,b]	Sulfacetamide sodium, 10.0%

[a] Methylparaben is present in the sample. [b] Propylparaben is present in the sample. [c] Butylparaben is present in the sample.

the octylsilane column gave slightly better resolution for prednisone, cortisone, prednisolone, and hydrocortisone with Solvent A (Table I).

Attempts were made to find a mobile solvent that would separate the seven steroids with resolutions greater than 1.0. Binary mixtures of methanol–water, acetonitrile–water, and tetrahydrofuran–water and ternary mixtures of methanol-acetonitrile-water, tetrahydrofuran-acetonitrile-water, and tetrahydrofuran–methanol–water were tried. In

Table VIII—Assay Results (Percent of Declared Value) of the Commercial Corticosteroid Samples

Sample	Proposed HPLC		Blue Tetrazolium		Other Methods	
	Methanol Dilution	Chloroform Extraction	Methanol Dilution	Chloroform Extraction	Methanol Dilution	Chloroform Extraction
1a[a]	76.2	75.2	74.6	76.1	78.3[b]	—
1b	55.3	55.5	55.3	58.5	56.5[b]	57.2[b]
2	74.6[c]	72.4[c]	92.8	85.0[c]	—	78.4[d]
3	95.2[c]	92.0	101.4	96.3	105.3[b]	105.3[b]
4	77.9	76.9	80.6	80.7	101.4[b]	89.7[b]
5	93.2	93.9	—	96.0	—	107.2[b]
6	90.2[c]	87.3[c]	98.7	96.2[c]	—	95.9[d]
7	83.9	83.2	91.0	88.8	99.0[b]	99.4[b]
8	53.0[c]	51.8[c]	74.2	59.4[c]	—	57.1[d]
9	55.2[c]	54.7[c]	86.3	68.2[c]	—	55.8[d]
10	96.6	95.9[c]	—	102.3	—	126.7[b]
11	94.5[c]	92.4[c]	100.2	98.2[c]	—	93.1[d]
12a[e]	99.6	92.3	139.4	91.9	148.3[b]	124.5[b]
12b	92.0	95.8	177.3	97.4	164.6[b]	112.4[b]
13a[e]	104.7	88.9	88.1	86.4	133.3[b]	110.0[b]
13b	103.3	105.7	108.4	116.7	130.4[b]	130.7[b]
14	88.3	92.2	92.6	94.5	—	92.7[d]
15	101.0	98.3	109.8	100.4	99.5[b]	100.4[b]
16	100.0[c]	100.1[c]	107.4	102.2[c]	97.7[b]	103.8[b]
17	102.6	100.9	110.1	108.3	104.7[b]	102.7[b]
18	100.7	99.7	279.1	213.1	103.1[b]	103.8[b]
19	99.7[c]	99.5[c]	104.2	104.8[c]	103.5[b]	105.6[b,c]
20	108.4	105.3	182.1	113.3	112.6[b]	109.3[b]
21	82.7	81.4	85.9	88.0	—	—
22	106.8	106.4	110.8	110.4	—	—
23	104.8	102.0	109.9	108.4	—	—
24	96.7	100.6	96.9	101.2	—	—

[a] Results *a* and *b* are for two separate units of product. [b] Isoniazid method. [c] Result is the average of two or three replicate determinations. [d] Phenylhydrazine method. [e] Result *a* from product with benzoyl peroxide added and *b* from product without benzoyl peroxide.

the preliminary search for a suitable mobile solvent, a solvent programmer was used with the HPLC instrument[10].

The best separation of the four steroids, prednisone, cortisone, prednisolone, and hydrocortisone, was obtained using the ternary solvent of 25% tetrahydrofuran–12.5% methanol in water (Solvent A). The resolution with this ternary system was optimized (39) by combining the two binary mixtures, 30% tetrahydrofuran–water and 50% methanol–water. Although baseline separation was not achieved, each steroid could be analyzed successfully in the presence of up to 10% of the other three steroids. An experiment was performed utilizing hydrocortisone standard (20 μg/ml) with 2 μg/ml each of the other three steroids added and dexamethasone (internal standard, 30 μg/ml). The hydrocortisone in this mixture was determined to be 99.9% (area ratio) and 98.6% (peak height ratio).

The best separation of the steroid acetates, cortisone acetate, prednisolone acetate, and hydrocortisone acetate, was obtained using 30% tetrahydrofuran in water. However, the retention times were too long (~30 min) for routine analytical work. The best separation within an acceptable time was obtained with 45% acetonitrile in water (Solvent B).

Table II gives the retention data, and Figs. 1 and 2 provide typical chromatograms obtained using the octylsilane microparticle (5 μm) silica column with Solvents A and B.

Replication Study—A mixture of hydrocortisone, dexamethasone, and cortisone acetate reference standards in 0.5% acetic acid in methanol was used. Ten 10-μl injections were made onto the octylsilane-bonded column with the automatic injector (Table III).

In general, the coefficient of variation was more acceptable for peak height values. Therefore, peak heights were used for all sample determinations.

Linearity Study—Standard mixtures containing hydrocortisone, dexamethasone, and cortisone acetate in 0.5% acetic acid in methanol were injected onto the octylsilane-bonded column. A straight line was fit to the peak height and area data by linear least squares, and the coefficient of determination (r^2) was calculated (Table IV).

These corticosteroids give linear responses in the given concentration ranges. Peak height data appeared to be more acceptable for quantitation of these corticosteroids.

Extraction Recovery Studies—A known amount of reference standard (~0.5 mg) was added to a 5.0-ml aliquot of the first dilution of the sample preparation and 5 ml of methanol in a 125-ml separator. The extraction was followed beginning with "Then 2 ml of aqueous saturated sodium chloride solution. . . ."

The recovery of standard added to the sample was determined by the proposed HPLC, blue tetrazolium (31), and isoniazid (34) methods (Table V). The results show that extraction is suitable.

Precision of HPLC Analysis of Samples—Six replicate determinations of two commercial samples were performed by the proposed direct dilution and extraction procedures (Table VI).

Analysis—The results of the analysis of commercial samples (Table VII) are given in Table VIII. The HPLC results for most samples by both procedures were comparable, indicating that either is suitable. However, since continuous injections of methanolic extracts of cream, lotion, or ointment samples could plug or otherwise shorten the life of the HPLC column, the proposed extraction procedure is the sample preparation method of choice.

A 0.5% solution of acetic acid in methanol was recommended as the diluting solvent for corticosteroid acetate esters so that ester hydrolysis

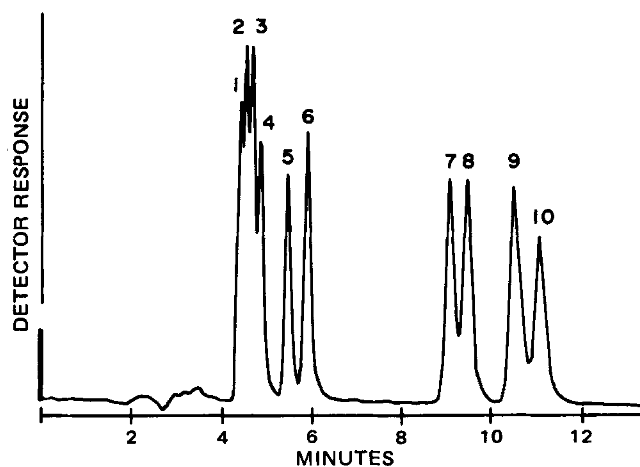

Figure 2—*Chromatogram of standard mixture. The column was octylsilane bonded to microsilica (5μm). Solvent B was 45% acetonitrile in water. The flow rate was 1 ml/min, the pressure was 800 psi, the temperature was ambient, and the detector was at 254 nm (0.05 aufs). Key: 1, prednisolone; 2, hydrocortisone; 3, prednisone; 4, cortisone; 5, methylparaben; 6, dexamethasone; 7, prednisolone acetate; 8, hydrocortisone acetate; 9, propylparaben; and 10, cortisone acetate.*

[10] Model 201 liquid chromatograph with two model 6000 pumps and a model 660 solvent programmer, Waters Associates, Milford, Mass.

will not occur in the sample preparation. Without acetic acid, Samples 23 and 24 had almost completely hydrolyzed within 1–2 hr. The 10% sulfacetamide sodium in these samples probably caused the rapid hydrolysis, but other corticosteroid acetate ester samples and standards also had small amounts of free corticosteroid alcohol present (<2%) after being in methanol solution overnight.

Samples 2, 7–9, 11, and 14, which were official USP hydrocortisone preparations, illustrate the need for a more specific assay such as HPLC. The official blue tetrazolium results for these samples were 5–30% higher than those from the proposed HPLC method. Chromatograms for Samples 2, 7–9 (hydrocortisone), and 21 (prednisolone) had one or more small peaks (<5% of the main peak) in addition to the main steroid peak. The retention volume for one of these small peaks in each chromatogram corresponded to that of the C-17 ketone degradation standard (Table II, Compounds I and III). Samples 2, 4, 8, and 9 also had an early eluting peak with a retention volume close to that of the C-17 carboxylic acid degradation product of hydrocortisone, which was reduced or completely removed by extraction (Table II, Compound VI).

Other active ingredients in the samples did not interfere with the proposed HPLC determination. Most of these compounds either were not detected by the 254-nm absorbance detector or were probably retained completely by the column (iodohydroxyquin and related compounds). Sulfacetamide and salicylic acid eluted mainly in the column dead volume, 2–3 ml. Chloramphenicol (V = 22 ml) was detected but was well separated from prednisolone (V = 9.5 ml). Samples 12 and 13 were analyzed with and without benzoyl peroxide (which was packaged in a separate vial to be added to the lotion before dispensing), because decomposition appeared to begin soon after addition of benzoyl peroxide.

The parabens were well separated from the steroids studied, except cortisone acetate. With Solvent B, the recommended solvent for acetate esters, propylparaben and cortisone acetate eluted too close for accurate quantitation of cortisone acetate. Therefore, cortisone acetate cannot be used as an internal standard for other acetate esters; another mobile solvent must be used for cortisone acetate analysis in the presence of propylparaben.

The proposed HPLC procedure is recommended to replace or at least to supplement the blue tetrazolium, phenylhydrazine, isoniazid, and UV spectrophotometric methods in the analysis of corticosteroids in topical pharmaceuticals based on the data presented in this and other (1–30) papers.

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