CONCLUSIONS

O-Methylhydroxylamine rapidly reacts with tautomers II and III at neutral pH to quantitatively produce an ~60:40 mixture of the *E* and *Z* isomers of aldophosphamide *O*-methyl oxime, XI and XII. Assuming that oxime formation occurs by condensation of the amine with aldehyde III, it follows that the rate of ring-opening of *cis*- and *trans*-II to give III must be relatively fast, since ^{31}P-NMR analysis of the reaction mixture showed that these hemiaminals are no longer detectable after ~10 min at 37°. The *O*-methyl oximes of III are resistant toward hydrolysis of the oxime functionality, fragmentation into IV and V, and transoximination with either acetone, acetaldehyde, or formaldehyde. In concert, these features lend themselves to the use of *O*-methylhydroxylamine as an effective trapping agent for studies of cyclophosphamide metabolites II and III. Investigations of enzymatic and chemical regeneration of III from XI/XII in the design of new anticancer prodrugs will be reported in another study.

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High-Performance Liquid Chromatographic Analysis of Hydrocortisone Drug Substance, Tablets, and Enema

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Abstract □ Methods for the analysis of hydrocortisone drug substance, tablets, and enema were developed using adsorption high-performance liquid chromatography (HPLC). This HPLC system was shown to be capable of isolating hydrocortisone from its degradation products, synthesis precursor, and related corticosteroids. The accuracy, precision, and linearity of the HPLC assay methods and their applicability to commercial products has been demonstrated.

Keyphrases □ High-performance liquid chromatography—analysis of hydrocortisone drug substance, tablets, and enema □ Hydrocortisone—analysis of drug substance, tablets, and enema using high-performance liquid chromatography □ Degradation products—separation from hydrocortisone, high-performance liquid chromatography

The majority of reported analytical methods for corticosteroids utilize blue tetrazolium (1–10), isoniazid (11), or phenylhydrazine (12, 13) reactions, UV spectrophotometry (14), or high-performance liquid chromatography (HPLC)[1] (15–22).

The methods for determining hydrocortisone products in the last four revisions of the United States Pharmacopeia (USP) (2, 23–25) have employed the blue tetrazolium reaction as the final determinative step. This is preceded by extraction or thin layer chromatographic (TLC) isolation of the active ingredient. Interferences and critical parameters of the reaction have been reported (6–9, 26).

HPLC is rapidly becoming the method of choice for the analysis of many drugs, and numerous applications of this technique to hydrocortisone are reported in the literature. The majority of published methods utilize reversed-phase systems (15–20). Recent reports, however, demonstrate that normal-phase adsorption chromatography offers greater selectivity for closely related corticosteroid structures[2] (21–22).

This study was undertaken to develop an HPLC system suitable for the analysis of hydrocortisone products and to compare the relative advantages of the USP and HPLC methods. Accuracy, precision, specificity, indication of

[1] E. Bunch, Food and Drug Administration, Seattle, Wash., unpublished work (1975).

[2] M. J. Walters, Food and Drug Administration, Detroit, Mich., unpublished work, presented at the 6th Annual Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies (1979).

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product stability, and applicability to most products on the commercial market were the criteria considered necessary for a suitable HPLC method. Of particular concern was the separation of hydrocortisone (I) from the major degradation products 11β,17-dihydroxyandrost-4-ene-3-one-20-oic acid (II) and 11β-hydroxyandrost-4-ene-3,17-dione (III) (6, 27–29), the common synthesis precursor, hydrocortisone acetate (IV), and the other closely related steroids, cortisone acetate (V), cortisone (VI), prednisone (VII), and prednisolone (VIII).

A silica microparticulate column with a mobile phase of ethylene dichloride containing methanol as modifier with small, controlled amounts of water and acetic acid met all of the listed requirements for an HPLC system. This system was incorporated into the assay procedures developed for hydrocortisone drug substance, tablets, and enema.

EXPERIMENTAL

Reagents and Materials—All solvents used were HPLC grade[3,4]. Glacial acetic acid was analytical reagent grade[5]. USP Reference Standard I, the degradation products II and III[6], acetaminophen[7], and corticosteroids[8] were used without further treatment. The TLC plates were purchased precoated with a 250-μm layer of silica gel with a fluorescent indicator[9]. Polytetrafluoroethylene 0.5-μm porosity membrane filters[10] were used to filter HPLC solvents and samples.

HPLC Determination of I—*Internal Standard Solution*—Two hundred milligrams of acetaminophen (IX) was dissolved in 4 ml of methanol and diluted to 200 ml with ethylene dichloride. The solution was kept in a tightly stoppered flask protected from light.

Standard Solutions—Solution A was prepared by accurately weighing and dissolving ~10 mg of I in 2 ml of methanol in a 50-ml volumetric flask. The internal standard solution (4 ml) was added, and the solution was diluted to volume with methylene chloride.

Solution B was prepared by accurately weighing and dissolving ~8 mg of I in 4 ml of methanol in a 100-ml volumetric flask. Internal standard solution (2 ml) was added and the solution was diluted to volume with chloroform. Solution A was used in the drug substance and tablet assays. Solution B was used to assay the enema.

HPLC System—The liquid chromatograph[11] was equipped with an automatic injector[12] having a 10-μl loop, a 25-cm × 4.6-mm i.d. column packed with spherical, 5–6 μm diameter, porous silica microparticles[13], a 254-nm UV detector[14], and a 10-mv span recorder[15]. The HPLC system was interfaced with a data system[16] for tracking peak areas and performing calculations. The detector was set at 0.5 absorbance units full scale (aufs) which produced peak heights of ~50% of full scale for both I and the internal standard in a 10-μl injection of standard solution A. Mobile solvent A was prepared by mixing 55 ml of a 5% water in methanol solution with 1.0 ml of acetic acid and diluting to 1 liter with ethylene dichloride. The flow rate was 1.5 ml/min and the methanol content was adjusted when needed to obtain a retention time of ~7.5 min for I. An alternate mobile solvent B consisted of 45 ml of 5% water in methanol solution mixed with 1.0 ml of acetic acid and diluted to 1 liter with methylene chloride.

System Suitability Test—The HPLC system was equilibrated by passing mobile solvent through the column for ~0.5 hr. Portions of the standard solution (10 μl) were then introduced. The system was considered suitable when (*a*) the retention times for I and IX were ~7.5 and ~10 min, respectively; (*b*) the resolution[17] R between I and IX was not less than 2.5, the column efficiency[18] N calculated using the I peak was not less than 5000 theoretical plates; and (*c*) the relative standard deviation of the response ratios of the I peak relative to the internal standard IX peak for six consecutive injections did not exceed 1%.

Table I—HPLC Retention of Hydrocortisone and Related Compounds

Compound	Retention Volume, ml	Capacity Factor, k'
Carbon tetrachloride	2.85	0
Benzene	2.85	0
Propylparaben	4.37	0.53
Methylparaben	4.67	0.61
11β-Hydroxyandrost-4-ene-3,17-dione	4.82	0.69
Cortisone acetate	4.88	0.71
Hydrocortisone acetate	5.55	0.95
Methylprednisolone acetate	6.02	1.11
Prednisolone acetate	6.32	1.22
Triamcinolone acetonide	6.89	1.42
Cortisone	6.96	1.44
Prednisone	7.74	1.72
Dexamethasone	10.28	2.60
Hydrocortisone	11.07	2.88
Methylprednisolone	12.84	3.51
Prednisolone	13.58	3.76
Acetaminophen	14.10	3.95
11β,17-Dihydroxyandrost-4-ene-3-one-20-oic acid	15.17	4.32
Triamcinolone	18.42	5.46

Sample Preparation—*Drug Substance*—Approximately 50 mg of sample, previously dried for 3 hr at 105°, was weighed into a 250-ml volumetric flask. Ten milliliters of methanol and 20.0 ml of internal standard solution were added and the solution was diluted to volume with methylene chloride.

Tablet Composite Assay—An accurately weighed portion of a 20-tablet composite equivalent to one tablet was transferred to a volumetric flask of an appropriate size to yield a final I concentration of ~0.2 mg/ml. Two milliliters of methanol per 10 mg of declared I was added and the flask placed in an ultrasonic bath for 2 min. Methylene chloride was then added until the flask was about half full and the flask was returned to the ultrasonic bath for 1 min. An accurately measured volume of internal standard solution equivalent to 4 ml/10 mg of declared I was added. The sample was diluted to volume with methylene chloride and a portion was filtered for HPLC assay.

Tablet Content Uniformity Determination—Each tablet was placed in an Erlenmeyer or volumetric flask of a size selected to yield a final I concentration of ~0.2 mg/ml. The tablet was softened by placing 100 μl of water/10 mg of declared I directly on the tablet and allowing it to soak in for 0.5 hr. Methanol (2 ml/10 mg of declared I) was added and the flask was placed in an ultrasonic bath for 10 min or until the tablet disintegrated. The procedure for tablet composite assay was then followed.

Enema—A sample equivalent to 8 mg of I was accurately weighed by difference into a separatory funnel and I was extracted with four 20-ml portions of chloroform, each portion filtered through chloroform-washed cotton into a 100-ml volumetric flask.

Methanol (4 ml) and internal standard solution (2 ml) were added, the sample was diluted to volume with chloroform, and a portion was filtered for HPLC assay.

Procedure—The HPLC system was allowed to equilibrate by passing the mobile solvent through the column for ~0.5 hr. Standard solution (10-μl portions) were introduced and the response ratios (R_s) of the I peak relative to the internal standard IX were calculated. When the R_s for three consecutive injections agreed within 1%, 10 μl of the sample preparation was injected. The quantity of I in the portion of the sample taken was calculated by the formula I (mg) = $V_u W_s R_u / V_s R_s$, where W_s is milligrams of I in the standard solution, V_s and V_u are the volumes (ml) of internal standard solution in the standard and sample solutions, respectively, and R_u is the response ratio of the peaks (I/IX) in the sample chromatogram.

Validation of HPLC Procedures—*Specificity*—Test solutions

[3] Omnisolv grade methanol, MCB Manufacturing Chemists, Cincinnati, OH 45212.
[4] Distilled in glass methylene chloride, chloroform, and ethylene dichloride Burdick & Jackson Laboratories, Muskegon, MI 49442. The ethylene dichloride purchased from Burdick & Jackson was found to be suitable for use without further treatment; other brands required water washing to remove impurities.
[5] Mallinckrodt, Inc., St. Louis, MO 63147.
[6] Received from Robert E. Graham, Food and Drug Administration, Dallas, TX 75204.
[7] Eastman Kodak, Co., Rochester, NY 14650.
[8] K & K Laboratories, Plainview, NY 11803.
[9] "Redi/Plate," Analtech Inc., distributed by Fisher Scientific Co., Pittsburgh, PA 15238.
[10] TE 36, Schleicher & Schuell, Inc., Keene, NH 03431.
[11] Series 2/1, Perkin-Elmer Corp., Norwalk, CT 06856.
[12] Model 725, Micromeritics Instruments Corp., Norcross, GA 30071.
[13] Zorbax Sil, Dupont Co., Wilmington, DE 19898.
[14] Model 440, Waters Associates, Milford, MA 01757.
[15] Model SR-204, Heath Co., Benton Harbor, MI 49022.
[16] PEP 2, Perkin-Elmer Corp., Norwalk, CT 06856.

[17] $R = 2(t_{r\,IX} - t_{r\,I})/(W_{IX} + W_I)$, where t_r and W are the retention times and peak widths at baseline measured in mm for compounds IX and I.
[18] $N = 16(t_r)^2/W$

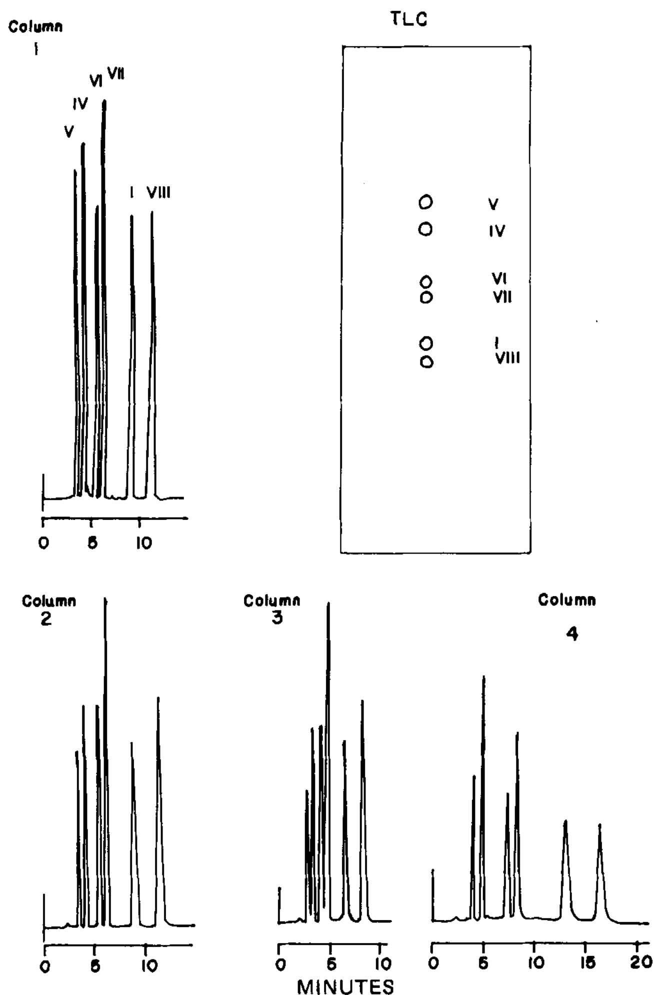

Figure 1—*Chromatographic separation of corticosteroids by four different HPLC columns and by TLC. Key: I, hydrocortisone; V, cortisone acetate; IV, hydrocortisone acetate; VI, cortisone; VII, prednisone; VIII, prednisolone; column 1, 25 cm × 4.6-mm i.d. packed with 5–6 μm diameter spherical silica particles; column 2, 25 cm × 4.6-mm i.d. packed with 5 μm irregular shaped silica; column 3, 30 cm × 3.9-mm i.d. packed with 10 μm, irregular shaped silica; column 4, 10 cm × 8-mm i.d. pressurized cartridge packed with 10 μm diameter, spherical silica particles. All chromatograms were obtained using the same mixture of compounds; the order of elution was the same for all columns.*

containing 0.04–0.2 mg/ml of the compounds listed in Table I, prepared in methylene chloride–methanol (98:2), were introduced into the HPLC system and their respective capacity factors[19] (k') were calculated. A model mixture containing 0.1 mg/ml each of I and IV–VIII in methylene chloride–methanol (98:2) was used to compare the performance of HPLC columns, mobile solvents, and HPLC to TLC.

Precision—Precision of the HPLC system was tested by injecting 36 portions of standard solution A, determining the R_s values for the peaks (I/IX) based on areas as well as heights, and calculating the relative standard deviation. The precision of the respective assay methods was tested by subjecting 10 portions of each of the appropriate sample composites to the entire assay procedure and determining the relative standard deviation of the results.

Accuracy—The accuracy of the assay procedures was tested using synthetic samples. The product formulations were categorized by excipient formulation. Typical formulations were selected for five tablet and two enema products, and placebo mixtures were prepared by combining all ingredients except I. Five portions of each placebo were taken and an accurately weighed amount of I, equivalent to the declared product potency, was added to each portion. These were analyzed by the appropriate HPLC assay procedures and the recovery of I was calculated.

Confirmation of Impurities—(*A*) *Separation of II–VII from I*—Drug substance I (~80 μg) was spotted on a TLC plate previously impregnated with 15% formamide in acetone (30). The chromatogram was developed three times using chloroform as the mobile solvent. The spots were detected by examining in short wavelength (254 nm) UV light followed by spraying with 25% H_2SO_4 in methanol, heating 5 min at 120°, and observing characteristically colored corticosteroid spots in long wavelength (366 nm) UV light. Compound I with added II–VIII at a level of 0.5% each was spotted and developed using the above system. Compounds II–VII were resolved and detected, while VIII was not separated from I at this level.

(*B*) *Separation of VIII from I*—Concentrated solutions (10 mg/ml) of I samples suspected to contain VIII were injected into the HPLC system and eluate portions corresponding to VIII retention were collected. After concentrating by evaporation, the eluate was spotted on a TLC plate and developed with methylene chloride–methanol–water (180:15:1) mobile solvent (2). Prednisolone was detected as in the TLC system described in A.

[19] $k' = (t_r - t_0)/t_0$, where t_0 is the time required to elute unretained component through the system.

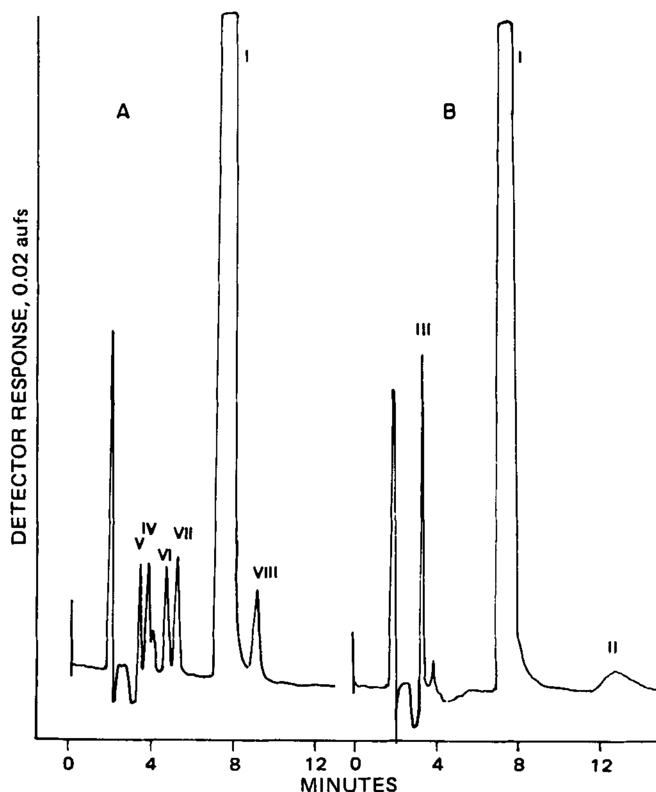

Figure 2—*Chromatograms of hydrocortisone (I) with added (0.5% of each) corticosteroids IV–VIII(A) and degradation products II and III (B).*

RESULTS AND DISCUSSION

The USP (2) single steroid assay (included in the I drug substance and tablet monographs) requires a TLC separation, quantitative transfer of portions of the silica layer, extraction, and final quantitation by the blue tetrazolium colorimetric procedure. The USP assay for hydrocortisone enema (2) involves a chloroform extraction followed by blue tetrazolium quantitation. This reaction is not specific for I and because I is not isolated from related steroids in the enema assay the results would include total corticosteroids as well as other compounds which react with this reagent (26). The USP methods for hydrocortisone were found to be tedious and time-consuming, and presented difficulties with the sample preparation of some commercial products[23]. Therefore, it was considered advantageous to develop alternate methods capable of providing equal specificity without these limitations.

HPLC was selected as the technique offering the greatest potential with respect to specificity, speed, and convenience. Adsorption HPLC was utilized due to its capabilities to separate closely related structures. The desired separation of I from its degradation products, precursor (IV), and related steroids (V–VIII) was achieved on a silica column with two equivalent mobile solvent systems (A and B). Though both mobile solvents have the same retention and selectivity for I–VIII, A is less volatile

Table II—Analysis of Hydrocortisone Drug Substance

Sample	Hydrocortisone Found, %		Impurities Found, %[b]			
	HPLC[a]	USP[a]	Cortisone	Prednisolone	Hydrocortisone Acetate	Unidentified
22	98.6 (0.0)	98.2 (1.3)	0.13	—	—	Trace
23	98.5 (0.4)	98.2 (0.9)	0.26	0.53	0.1	Trace
24	98.8 (0.3)	98.7 (0.4)	0.13	0.57	0.1	Trace
25	97.2 (0.2)	98.2 (1.2)	0.20	—	—	Trace
26	99.1 (0.0)	102.4 (1.1)	0.16	0.31	—	Trace

[a] % by weight on dried basis, average of two determinations (% difference between duplicates). [b] % by weight, trace was estimated to be less than 0.1% based on UV response equivalent to cortisone.

Table III—HPLC Analysis of Synthetic Samples

Product	Formulation Type	HPLC Assay: Number of Determinations	Added I, mg[a]	Added I found, %: Average	Range	RSD^{1}
Tablets	A	5	20	99.4	98.6–98.8	0.48
Tablets	B	5	20	99.0	98.6–99.4	0.30
Tablets	C	5	10	99.7	99.1–100.6	0.56
Tablets	D	5	10	101.2	99.7–101.8	0.84
Tablets	H	5	20	100.2	100.1–100.5	0.21
Average				99.9		
Enema	K	5	8	99.9	99.5–100.4	0.38
Enema	L	5	8	99.1	98.9–99.5	0.24
Average				99.5		

[a] Approximate amount of accurately weighed hydrocortisone (I) added to each portion of placebo.

resulting in a more rugged system and was therefore incorporated into the assay procedures. The selectivity of this HPLC system is illustrated in Table I, which shows the capacity factors (k') for compounds of interest. A good separation was obtained for corticosteroid pairs I and VIII, and VI and VII, which are difficult to resolve by reversed-phase systems (20). Corticosteroid acetates, which eluted close together when mobile solvent A was used, were found to have higher k' values and better resolution when the methanol content of the mobile solvent was decreased to 3.5%. Thus, the same column with slight modifications in the mobile solvent shows a potential for application to corticosteroid products other than those reported here.

A model mixture of I and IV–VIII was used to investigate four silica columns, using resolution (R) between VI and VII as well as efficiency (N) (calculated as theoretical plates based on the I peak) as the criteria for comparison. Columns 1 and 2 were both 25 cm × 4.6-mm i.d. in size. Column 1 was packed with 5-6 μm diameter, spherical silica[13], while column 2 contained irregular-shaped 5-μm silica particles[20]. Column 3 was 30 cm × 3.9-mm i.d. in size packed with 10-μm, irregular-shaped silica[21]. Column 4 was packed with 10-μm spherical silica particles in a 10 cm × 8-mm i.d. plastic cartridge[22] maintained under pressure. The test compounds eluted in the same order through all four columns. Differences were observed in the total elution times and resolution (Fig. 1). The highest efficiency (N = 8300) and resolution (R = 1.56) were obtained with column 1. The performance of column 2 was very similar to 1, while the differences were more apparent with columns 3 and 4 which had 3400 and 3700 theoretical plates, respectively. Compound I was completely resolved on all four columns. Column 1 was used for all analyses reported in this paper.

The six-steroid model mixture was also used to compare the specificity of HPLC to that of the TLC system used in the USP single steroid assay, and Fig. 1 shows that HPLC provides equal or better selectivity for related steroids. Figure 2 demonstrates the system's capability to separate I from its major degradation products, II and III, as well as from its immediate synthesis precursor, IV.

Acetaminophen (IX) was incorporated into the procedures as an internal standard to compensate for possible injection variability and solution volume changes. The relative standard deviation of the response ratios (R_s) for the peaks (I/IX) of 36 replicate injections of standard solution was found to be 0.19% based on peak areas and 0.60% using manually measured peak heights. While the computerized area calculations are preferred due to better precision and a savings of time and effort, manual peak height calculations also gave acceptable results.

[20] LiChrosorb Si60, E. M. Reagents, Cincinnati, OH 45212.
[21] μ Porasil, Waters Associates, Milford, MA 01757.
[22] RCM Cartridge B, Waters Associates, Milford, MA 01757.
[23] M. J. Walters, Food and Drug Administration, Detroit, Mich., unpublished work (1980).

The response for I was found to be linear in the tested range of 0.8–3.4 μg, equivalent to 40–170% of the injection amount specified in the method. The internal standard (IX) produced a linear response as did the response ratios for the I peak relative to the internal standard peak over the range of 50–150% of the specified concentrations.

The HPLC assay results for five I drug substance samples (Table II) show reasonable agreement with those obtained by the USP assay. Duplicate assays by the HPLC method agree within <0.5% (average = 0.2%) while the USP duplicates range from 0.4 to 1.3% (average = 1%). An additional portion from each sample was prepared and chromatographed

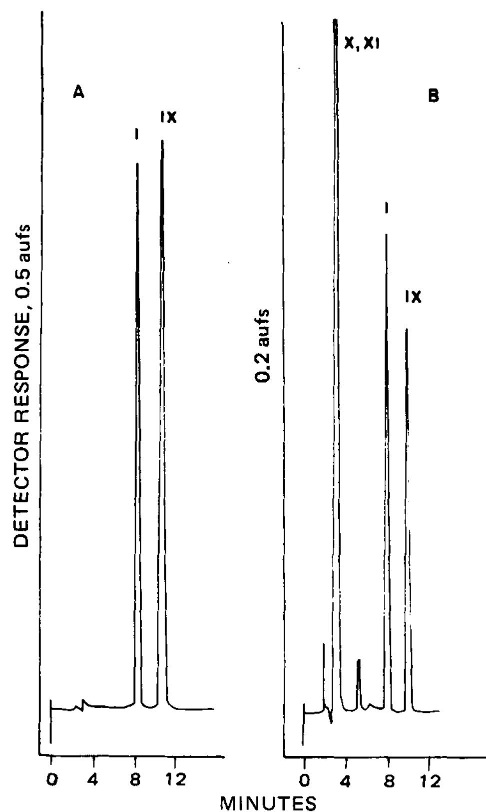

Figure 3—*Typical hydrocortisone (I) assay chromatograms. Key: A, tablets; B, enema; IX, internal standard; X, methylparaben; XI, propylparaben.*

Table IV—Analysis of Hydrocortisone Tablets

Sample Number	Formulation Category	Declared Potency, mg/tablet	Results, % of Declared Potency: USP Assay[a]	HPLC Assay[a]	Content Uniformity of Individual Tablets by HPLC: Average[b]	RSD, %
1	A	20	91.6	92.5	95.0	2.5
2	B	20	99.3	104.9	105.6	1.4
3	B	20	99.2	101.8	102.2	1.4
4	B	10	95.2	94.8	95.9	1.0
5	B	20	91.8	95.8	95.1	4.6
6	B	20	98.8	100.2	100.4	1.0
7	C	20	90.4	96.6	96.9	1.0
8	C	10	95.8	96.8	96.4	1.5
9	D	20	95.5	97.1	95.4	3.6
10	D	10	97.7	97.7	98.9	3.0
11	E	20	93.7	96.6	96.8	2.1
12	F	20	95.2	95.4	96.5	0.82
13	G	20	96.5	94.4	93.0	2.7
14	H	10	99.6	98.0	97.8	2.3
15	H	20	100.8	100.2	103.0	3.1
16	I	20	92.9	94.6	95.4	1.4
17	I	10	93.8	96.1	96.7	1.6
18	[c]	20	92.6	95.0	96.5	1.1
19	J	5	95.2	100.6	99.9	0.63
20	J	10	100.8	99.2	95.5	0.58
21	J	20	100.4	99.0	99.0	0.67

[a] Average of duplicate determinations. [b] Average of 10 individual tablet assays. [c] Not known.

without the internal standard, and none of the chromatograms showed interfering peaks at the retention corresponding to the internal standard. These solutions were also used to test for the presence of impurities by increasing the detector sensitivity 25-fold, from 0.5 to 0.02 aufs. Chromatograms of reference standard I with degradation products II, III, and the related steroids IV–VIII, each added at a level of 0.5% of I, showed that these impurities are detectable at this level by this procedure (Fig 2). An estimated 0.1–0.3% of cortisone was found in all five samples and 0.3–0.6% of prednisolone was found in three samples of I drug substance (Table II). The presence of cortisone and prednisolone was confirmed by TLC, the latter after subjecting concentrated solutions to HPLC, collecting, and concentrating the appropriate portions of the eluate. Traces of hydrocortisone acetate and unidentified impurities were also detected. No degradation products were found. The total amount of detected impurities was $<1\%$ in all samples.

The HPLC system was applied to the analysis of I tablets by introducing a sample preparation step and performing additional validation experiments. The dilutions specified in the sample preparation for tablets result in the same final concentration of I regardless of the dosage level, which ranges from 5 to 20 mg/tablet. Thus, the same standard solution and detector sensitivity can be used.

The recovery of I through the method and the influence of excipients was checked by the synthetic sample approach. Five typical product formulations prepared in the laboratory were assayed. The recovery results (Table III) ranged from 98.6 to 101.8% and the overall mean for a total of 25 determinations was 99.9% with a relative standard deviation of 0.91%. No bias in the method is indicated. The precision for commercial products was checked by assaying 10 composite portions for each of five samples. The results showed good precision with the relative standard deviation for the five samples ranging from 0.27 to 0.78%.

The tablet content uniformity procedure is essentially identical to the assay, the only difference being in the initial sample preparation required to disintegrate the intact tablet. A small amount of water was needed to soften the tablet and enhance the disintegration. The volumes of water and methanol were limited to the specified low levels in order to maintain the equilibrium of the chromatographic system.

Twenty-one commercial tablet products manufactured by 12 different firms were subjected to the HPLC assay and content uniformity procedures. The results are summarized in Table IV along with those obtained by the USP assay. While all the results are within USP limits, 14 of the 21 USP assay results are lower than the HPLC assay with an average difference of 1.45%, suggesting a negative bias in the USP assay. The average difference between results of duplicate determinations was 1.1% for the USP method and 0.49% for the HPLC assay method. The HPLC composite assay results show good agreement with the respective average content uniformity results for 10 individual tablets.

A typical chromatogram is shown in Fig. 3A. A portion of each composite chromatographed without internal standard showed no interferences at the retention volume of IX.

The same HPLC system was applied to the assay of hydrocortisone enema after introducing an appropriate sample preparation step. Since the adsorption HPLC system requires that the water content be maintained at a low and closely controlled level, aqueous enema samples could not be introduced without eliminating the water. An extraction of I into chloroform was found to overcome this problem. Methyl- and propylparabens, present as preservatives, were also extracted into chloroform but were well resolved from I by HPLC (Fig. 3B).

The average recovery for 10 portions of I through the enema assay was 100.2% with a relative standard deviation of 0.65%. The average of 10 recoveries for two synthetic enema formulations was 99.9 and 99.1% with a relative standard deviation of 0.38 and 0.24%, respectively (Table III). Replicate determinations of two commercial enema products resulted in a relative standard deviation of 1.0% in both cases, showing acceptable precision. One of the products was found to have satisfactory potency with good agreement between the HPLC and blue tetrazolium assays, 99.8 and 101.0%, respectively. The second product showed only 82.7% of the labeled I content by HPLC and 86.5% by blue tetrazolium. Since the blue tetrazolium reaction is not specific, it is possible that an impurity in the product may have caused false high results. This suspicion was further reinforced when a small peak at 5.6 min was observed in the sample chromatograms. This peak did not appear in the standard, synthetic sample or the other enema product chromatograms; did not correspond to I–IX; and remains unidentified. The low HPLC assay result was confirmed by subjecting the sample to a reversed-phase HPLC assay using an octyl-silane bonded column and a methanol–water (60:40) mobile solvent.

The presented HPLC methods for the analysis of I drug substance tablets and enema have been shown to be accurate, precise, specific, and applicable to products on the commercial market. It is anticipated that with appropriate sample preparation the same HPLC system will be applicable to other hydrocortisone products.

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NOTES

Systematic Error Associated with Apparatus 2 of the USP Dissolution Test I: Effects of Physical Alignment of the Dissolution Apparatus

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Abstract ☐ The physical alignment of the paddle and the vessel is critical in obtaining reproducible results from the USP dissolution test with Apparatus 2. Large variations in dissolution results were traced to minor variations in alignment of different apparatuses.

Keyphrases ☐ Dissolution—USP Apparatus 2, reproducibility of results ☐ USP—dissolution Apparatus 2, reproducibility of results ☐ Apparatus—USP dissolution Apparatus 2, reproducibility of results

This laboratory has been recently studying the systematic error associated with Apparatus 2 of the USP dissolution test (1). Collaborative studies conducted by the Academy of Pharmaceutical Sciences[1] and by the Food and Drug Administration (2) showed a wide variation in test results reported by different laboratories. The present report is the first of a series of papers describing sources of systematic error associated with the dissolution test.

The test method first appeared in the Fourth Supplement to USP XIX and NF XIV (3). The stirring element consisted of a shaft with a detachable paddle blade positioned on its side. In the Fifth Supplement of USP XIX and NF XIV, the stirring element was modified to its present configuration: the blade is now rigidly mounted through the diameter of the shaft. The data reported in this paper were collected prior to the modification of the apparatus.

EXPERIMENTAL

Two commercial samples of 5-mg prednisone tablets (referred to as Tablet 0 and Tablet 1) were used for the evaluation of six dissolution apparatuses. Dissolution data for the two samples from each apparatus were collected using a single set of six glass dissolution vessels[2] and uniform analytical technique. Two appartuses, designated A and B, were designed and built by the Food and Drug Administration. Four apparatuses, designated C, D, E, and F, were commercially available[3]. Each apparatus could test six tablets simultaneously.

The dissolution and analytical methodology is described in the Fourth Supplement to USP XIX and NF XIV (4).

RESULTS AND DISCUSSION

The data collected from the two samples with each apparatus are shown in Table I. The results for apparatuses E and F are considerably higher than those for the other four apparatuses. These discrepancies were traced to minor variations in the vertical alignment of the paddle shafts. The experiment pointed out two deficiencies in the dissolution methodology.

The first deficiency was that the equipment operator could not be certain that the USP alignment specifications were being met: the paddle shaft must be aligned so that its axis is not more than 0.2 cm from the vertical axis of the vessel at any point. Devices adequate to measure and adjust the equipment to meet this requirement were not available initially. Although the apparatus was adjusted to make the drive head parallel with the base, no conscientious effort was made to improve the precision with which a vessel was centered around its shaft.

The second deficiency lay in the design of the apparatus. The support

[1] Unpublished data, Dissolution Technology Committee, APhA Academy of Pharmaceutical Sciences.

[2] Kimble, Vineland, N.J.
[3] C: Model 72A, Hanson Research Corp., Northridge, Calif. D, E, and F: Three separate Hanson Model 72R apparatuses.

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