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Analysis of Fludrocortisone Acetate and Its Solid Dosage Forms by High-Performance Liquid Chromatography

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Abstract □ A newly developed reversed-phase high-performance liquid chromatographic assay and test method for determining content uniformity are described for fludrocortisone acetate. The method is stability indicating and separates most known degradation products and impurities. In addition, the method is simple, sensitive, accurate, and relatively free of interferences. The coefficient of variation for multiple weight assays is between 0.3 and 1.8%.

Keyphrases □ Fludrocortisone acetate—and solid dosage forms, high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, fludrocortisone acetate and solid dosage forms □ Steroids—fludrocortisone acetate and solid dosage forms, high-performance liquid chromatographic analysis

Fludrocortisone acetate (9-fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-acetate) (I) is a powerful synthetic adrenocortical steroid. As an unsaturated 3-keto steroid, it may be analyzed by the isoniazid method (1) or with 4-aminoantipyridine hydrochloride reagent (2). The latter is used in the USP procedure for content uniformity. As a 17α-ketol steroid, fludrocortisone acetate is analyzed by the blue tetrazolium method (1), the official USP assay. A colorimetric assay in fermentation broths (3) and an automated colorimetric method for assaying single tablets of fludrocortisone acetate (4) also were reported.

Since none of these methods is selective for fludrocortisone acetate, it was the purpose of this study to develop a specific, stability-indicating, high-performance liquid chromatographic (HPLC) assay that would circumvent any excipient interferences. The HPLC method described here is capable of separating fludrocortisone acetate from its known degradation products and possible impurities. Norethindrone was used as an internal standard. The HPLC data were in excellent agreement with results obtained by the isoniazid assay. The method is simple, selective, and highly reliable.

EXPERIMENTAL

Reagents and Materials—Fludrocortisone acetate and norethindrone USP reference standards and acetonitrile[1] (UV grade and distilled in glass) were used. The mobile phase was 42 ± 2% acetonitrile in water.

The internal standard solution was prepared by dissolving norethindrone in acetonitrile to concentrations of 65 μg/ml for assay and 10 μg/ml for content uniformity analysis.

Standard Solutions and Calibration Curves—Fludrocortisone acetate solutions were prepared to contain 50, 70, 100, and 120 μg of fludrocortisone acetate/ml for assay and 15, 20 and 25 μg/ml for content uniformity analysis.

Sample Preparation—*Assay*—A portion of ground tablets equivalent to 2.5 mg of fludrocortisone acetate was transferred to a 50-ml low actinic centrifuge tube, and 5 ml of distilled water was added by pipet. After the suspension was mixed[2] for 1 min, 20 ml of internal standard solution was added, and the resulting mixture was transferred to a mechanical shaker for 40 min. The tablet extract was then centrifuged, and the clear liquid was injected into the HPLC system. The ratio of the peak heights of fludrocortisone acetate to the internal standard was determined, and the quantity of fludrocortisone acetate in the sample was calculated using the assay calibration curve.

Content Uniformity Analysis—One tablet (containing 0.1 mg of fludrocortisone acetate) was transferred to a 10-ml low actinic centrifuge tube, and 1 ml of distilled water was added. A vortex mixer[2] was used to effect complete disintegration, and then 4 ml of the internal standard solution was added. The mixture was then treated as in the assay.

Apparatus and Operating Conditions—The liquid chromatograph consisted of a reciprocating pump[3], a 20-ml loop injector[4], and a UV detector[3] equipped with a 254-nm filter. The analytical column was 30-cm × 4-mm i.d. stainless steel, packed with porous siliceous microbeads to which a stationary phase of octadecyltrichlorosilane was chemically bonded[3]. A flow rate of 1.8–2.0 ml/min, maintained at about 800–1000 psi, was used at ambient temperature. The output of the detector was displayed on a recorder having a full-scale range of 10 mv[5].

System Suitability Test—The standard preparation, 100 μg/ml, was chromatographed five or six times, and the peak response was measured as the ratio of resulting peak heights of fludrocortisone acetate to the internal standard. The relative standard deviation was less than 2%. The resolution factor between fludrocortisone acetate and the internal standard was more than three. For a particular column, resolution may be increased by decreasing the amount of acetonitrile in the mobile phase.

Placebo Analysis—A placebo of the sample commercial tablets used in this study was prepared and extracted in parallel with the sample. The placebo chromatogram was checked for interfering peaks near fludrocortisone acetate or norethindrone.

Recovery Studies—The efficiency of the recovery was checked by

[1] Burdick & Jackson Laboratories, Muskegon, Mich.

[2] Vortex-Genie mixer, Scientific Industries, Springfield, Mass.
[3] μBondapak-C_{18}, Waters Associates, Milford, Mass.
[4] Chromatronix HPSV-20, Spectra-Physics, Santa Clara, Calif.
[5] Model 410, Pharmacia Fine Chemicals, Piscataway, N.J.

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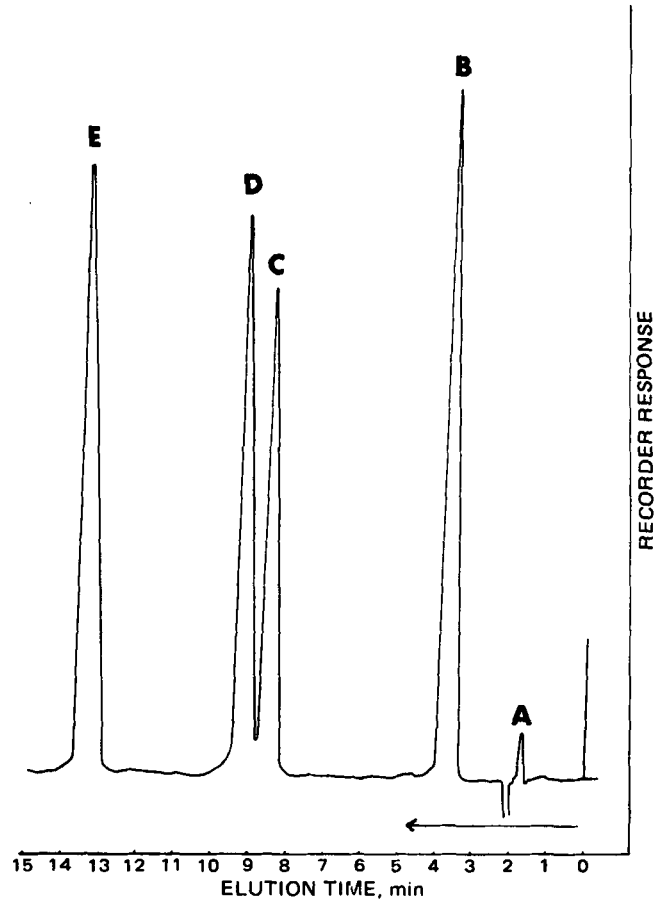


Figure 1—*HPLC separation of fludrocortisone acetate (I) and its impurities and degradation products. Key: A, solvent front; B, II and IV; C, III; D, I; and E, norethindrone acetate (internal standard).*

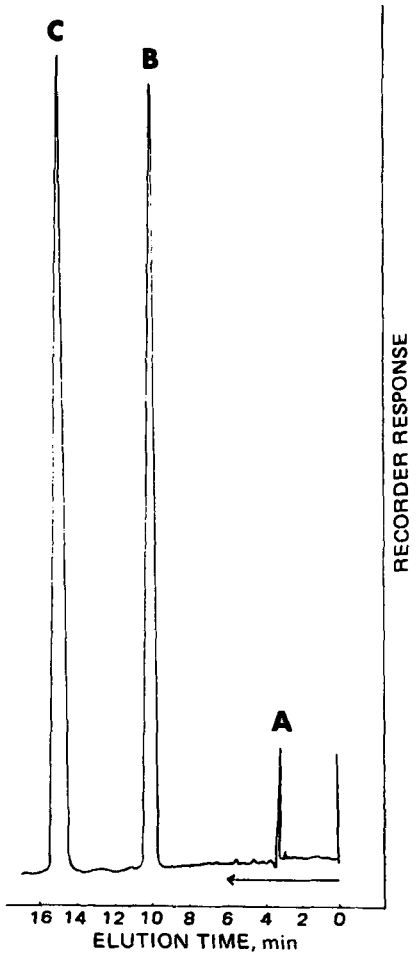


Figure 2—*HPLC assay of fludrocortisone acetate "tablet extract." Key: A, solvent front and tablet excipients; B, fludrocortisone acetate; and C, norethindrone acetate (internal standard).*

spiking both the placebo and tablets with known amounts of fludrocortisone acetate and norethindrone reference standards. The spiked samples were extracted and chromatographed in parallel with the sample.

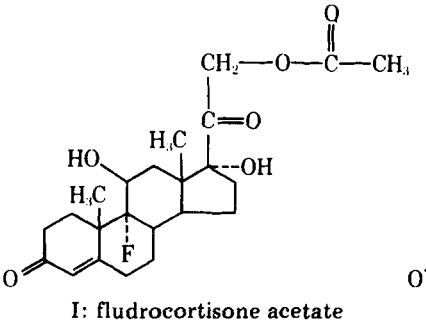
Stress Test—To demonstrate the stability-indicating ability of the HPLC procedure, fludrocortisone acetate tablets were assayed by HPLC and then were intentionally degraded by elevated temperature for an extended period. The fludrocortisone acetate content was then determined by both HPLC and the blue tetrazolium procedures (compendial assay).

RESULTS AND DISCUSSION

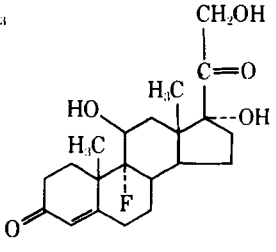
Fludrocortisone acetate (I) is stable as a solid, especially when stored in closed brown bottles (5). However, II–IV may be present either as impurities or as degradation products. The presence of $\Delta^{1,4}$-diene system (III and IV) can be attributed to the fact that it is a possible precursor in the preparation of fludrocortisone acetate. The presence of the 21-hydroxyl analogs (I and II) are expected as hydrolytic products of the acetate esters, especially in hydrolytic solvents. Moreover, these hydrolytic degradation products can be formed on storage for long periods. The reliability of the HPLC method is best shown by its ability to resolve fludrocortisone acetate from all of II–IV (Fig. 1). Furthermore, no interfering peaks were noticed in the placebo chromatogram.

The linearity of both fludrocortisone acetate and norethindrone chromatographic responses was checked by a separate calibration curve for each compound. The correlation coefficient for a straight-line equation was higher than 0.999 (concentration range of 45–120 μg/ml) in both cases. A representative chromatogram for the fludrocortisone acetate commercial tablet is shown in Fig. 2. Recoveries of fludrocortisone acetate and norethindrone were 100.7 and 99.1%, respectively. There was no significant difference between recoveries from spiked placebo or spiked tablets.

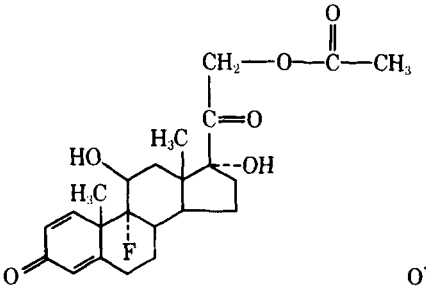
The stability-indicating ability of the HPLC procedure was demonstrated by the results of the stress test. Fludrocortisone acetate tablets, which assayed at 95.2% of "label claim" by HPLC before being subjected to elevated temperatures, assayed at only 72.2% of label claim, and 10.1% of the 21-hydroxyl analog (II) was found. However, no $\Delta^{1,4}$-diene derivatives (III and IV) were detected. When the same degraded sample was analyzed by the blue tetrazolium procedure (compendial assay), the



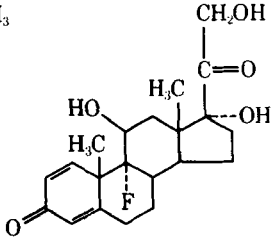
I: fludrocortisone acetate



II: fludrocortisone



III: $\Delta^{1,4}$-diene system of fludrocortisone acetate



IV: $\Delta^{1,4}$-diene system of fludrocortisone

Table I—HPLC Assay (Milligrams per Tablet) of 0.1-mg Fludrocortisone Acetate Tablets

Lot	First Weight: Average of Two Injections	First Weight: *RSD*, %	Second Weight: Average of Two Injections	Second Weight: *RSD*, %	Total Average: Total Average of Two Weights	Total Average: Overall *RSD*, %	Isoniazid Assay, mg/tablet
1	0.0948	0.1	0.0930	0.6	0.0939	1.8	0.094
2	0.0970	0.2	0.0976	1.0	0.0973	0.3	0.095
3	0.0952	0.4	0.0953	0.7	0.0953	0.4	0.0947
4	0.0908	0.6	0.0924	0.6	0.0916	0.9	0.094

Table II—HPLC Content Uniformity Analysis (Milligrams per Tablet) of 0.1-mg Fludrocortisone Acetate Tablets

	Lot 1	Lot 2
Tablet 1	0.0966	0.0975
Tablet 2	0.0933	0.1012
Tablet 3	0.0907	0.0946
Tablet 4	0.0875	0.0989
Tablet 5	0.0976	0.0951
Tablet 6	0.0952	0.0991
Tablet 7	0.0932	0.0994
Tablet 8	0.0927	0.0963
Tablet 9	0.0941	0.1009
Tablet 10	0.0928	0.0994
Average	0.0933	0.0982
RSD, %	0.3	2.3
HPLC assay	0.0939	0.0970
Isoniazid assay[a]	0.0940	0.0947
4-AAP content uniformity[b]	0.0966	0.0967

[a] Average of three sets of runs, 10 tablets each. [b] 4-Aminoantipyrine method, average of 10 tablets.

fludrocortisone acetate content was 88.3% of label value. This high result compared to that obtained by the HPLC method (72.2%) may be attributed to the fact that the blue tetrazolium method does not differentiate between the 21-hydroxyl analog and fludrocortisone acetate.

To identify other possible degradation products in the degraded sample, which may explain the other 6% loss unaccounted for by the HPLC procedure, a foreign peak that eluted just before III in the degraded sample chromatogram was collected from 40 runs. Mass spectral and NMR analyses showed that the compound was not a steroid but probably a fatty acid formed by hydrolysis of excipients. The UV spectrum of that foreign peak showed a maximum at 259 nm. Other foreign peaks eluted very close to the solvent front, and their separation and identification were not attempted.

Results of the HPLC analysis of four lots of a commercial tablet preparation are shown in Table I. Fludrocortisone acetate content varied between 91.6 and 97.3% of label claim. The average relative standard deviation for multiple weights of the same lot was 0.85% (range of 0.3–1.8%) while that for duplicate injections of the same weight was 0.5% (range of 0.1–1.0%). Table I also shows a comparison between the HPLC results and the isoniazid assay values for the same lots. No significant differences were found.

For content uniformity analysis, 10 individual tablets of two lots were analyzed by the HPLC method (Table II). The relative standard deviation between individual tablets of the same lot was 2–3% compared to 2–5% with the compendial content uniformity method. This result was probably due to the elimination of excipient interferences, especially from impurities present in lactose, which caused up to 7% bias on the high side with the official procedure.

In conclusion, the reversed-phase HPLC assay developed for fludrocortisone acetate is suitable for batching, content uniformity, and stability assays. The method is more selective than the blue tetrazolium and isoniazid assays because it separates most of the expected impurities and degradation products. The method is simple, sensitive, accurate, and virtually free from interferences.

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