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Liquid Chromatographic Assay for Diflorasone Diacetate in Cream and Ointment Formulations

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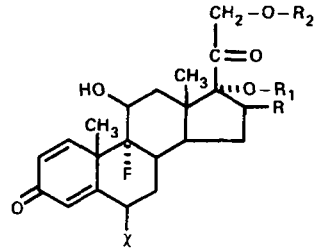
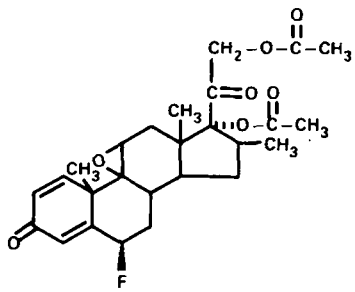
Abstract □ A high-performance liquid chromatographic assay for quantitating diflorasone diacetate (I) in cream and ointment formulations and in bulk form is described. Isoflupredone acetate (II) was used as the internal standard and a 3-μm silica gel column with a mobile phase comprised of water-saturated butyl chloride–water-saturated methylene chloride–tetrahydrofuran–acetic acid (350:125:10:15) led to an efficient separation. The method gave accurate results for four formulations, two creams and two ointments, as well as the bulk drug. The assay has an *RSD* of ~1.8% for the cream formulations, 1.3% for the ointment formulations, and 1.0% for the bulk drug. The method is specific for I and capable of resolving structurally related compounds.

Keyphrases □ Diflorasone diacetate - HPLC, cream, ointment, bulk drug □ HPLC—diflorasone diacetate, cream, ointment, bulk drug

Diflorasone diacetate (I) in topical formulations is indicated for the relief of inflammatory manifestations of acute and chronic corticosteroid-responsive dermatoses (1). It is available as a 0.05% cream- or ointment-based topically applied product[1]. The high-performance liquid chromatographic (HPLC) analysis of similar topical anti-inflammatory steroids has appeared (2). This report describes a new normal-phase HPLC assay that is specific for diflorasone diacetate in two cream and two ointment formulations. The assay is also capable of separating several structurally related compounds: the 9,11-epoxide (III), 6β-fluoro analogue (IV), 6-defluoro analogue (V), the 17-OH,21-OAc analogue (VI), and the 17-OAc,21-OH analogue (VII) of I.



I R=CH_3, R_1=Ac, R_2=Ac, X=α-F
II R=H, R_1=H, R_2=Ac, X=H
IV R=CH_3, R_1=Ac, R_2=Ac, X=β-F
V R=CH_3, R_1=Ac, R_2=Ac, X=H
VI R=CH_3, R_1=H, R_2=Ac, X=α-F
VII R=CH_3, R_1=Ac, R_2=H, X=α-F



III

EXPERIMENTAL SECTION

Materials—Diflorasone diacetate[2] (I), isoflupredone acetate[2] (II), and the related compounds III–VII[2] (3–7) were obtained in pure form and dissolved in water-saturated chloroform for chromatography and spectroscopy. Solvents and reagents were commercial analytical grade. Placebo materials were obtained in-house[2].

Chromatographic Conditions—A modular liquid chromatograph including pump[3], fixed-loop injector[4], fixed-wavelength detector[5] (254 nm), and a minicomputer[6] were used. Commercial 3-μm[7] silica gel columns (10 cm × 4.6 mm i.d.) were used at ambient temperature. The mobile phase, deaerated under vacuum sonication prior to use, consisted of water-saturated butyl

[1] Florone Cream, Florone Ointment; The Upjohn Company, Kalamazoo, Mich.
[2] The Upjohn Company.
[3] Model 110A; Altex Scientific Inc., Berkeley, Calif.
[4] Model AH60 equipped with a 10-μL fixed volume loop; Valco Instruments Co., Houston, Tex.
[5] Model 1203; LDC Corp., Riviera Beach, Fla.
[6] PDP 11-40; Digital Equipment Corp., Marlborough, Mass.
[7] Part #0258-1500; Perkin-Elmer Corp., Norwalk, Conn.

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Table I—Response Factors for Related Compounds

Compound	Mean Response Factor[a]
I	1.00
III	1.64
IV	1.04
V	1.23
VI	1.04
VII	0.96

[a] Mean of nine determinations, peak area response. Results calculated as in the *Experimental Section.*

chloride-water-saturated methylene chloride-tetrahydrofuran-acetic acid (350:125:10:15). The flow rate was adjusted to 2.5 mL/min.

Internal Standard—A solution of water-saturated chloroform containing 0.04 mg/mL of isoflupredone acetate (II) was used. About 3 mg of diflorasone diacetate (I) reference standard[2] was weighed accurately and 100.0 mL of internal standard solution was added.

Sample Preparations—*Creams*—Cream containing 1 mg of diflorasone diacetate was accurately weighed into a 35-mL screw-cap disposable vial[8], 30.0 mL of internal standard solution was added, and the solution was shaken for 30 min. The vial was then centrifuged at 2000 rpm for 15 min. The top layer (excipient) was then removed by aspiration leaving a clear lower chloroform layer.

Ointments—Ointment containing 0.5 mg diflorasone diacetate was accurately weighed into a 35-mL screw-cap disposable vial[8], 15.0 mL of internal standard solution was added, and the sample treated as in the cream assay.

Bulk Drug—About 1.5 mg of diflorasone diacetate was dissolved in 50.0 mL of internal standard solution.

Procedure—Aliquots of sample or standard preparation were injected using a 10-μL fixed-loop injector. The resulting chromatograms were recorded and analyzed by computer integration. Two reference standard weighings, weight normalized for response, were run at the beginning and end of the run and at every 6-8 samples. The detector attenuation was set at 0.032 absorbance units.

The diflorasone diacetate (I) content, expressed in milligrams per gram for creams and ointments and in percent for bulk drug, was calculated using the internal standard method and peak area response(8).

Recovery studies were performed by adding 50-150% of the labeled amount of diflorasone diacetate (I) to each of the two types of cream and ointment placebos. The samples were then treated as described under *Sample Preparations.*

The relative response of the structurally related compounds III-VII were determined by comparing the weight-normalized response for accurately prepared water-saturated chloroform solutions of each (~0.05 mg/mL) with a similar preparation of diflorasone diacetate (I). The response factors were calculated using peak area response (Table I).

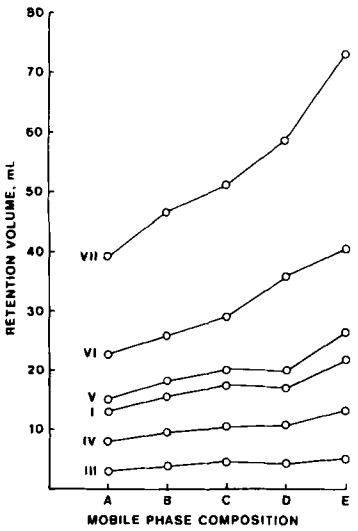

Figure 1—*Plot of retention volume* versus *mobile phase composition of water-saturated butyl chloride-water-saturated methylene chloride-tetrahydrofuran-acetic acid in a ratio of 325:150:10:15 (A), 350:125:10:15 (B), 375:100:10:15 (C), 350:125:5:15 (D), and 350:125:10:5 (E).*

[8] Part #225290; Wheaton Laboratory Products, Millville, N.J.

Table II—Diflorasone Diacetate Recovery from Placebo Cream and Ointment Formulations Using Peak Area Ratio Response

Formulation	Average Recovery, %[a]	*RSD*, %
Cream		
A	99.4	1.5
B	100.1	0.6
Ointment		
C	100.2	0.6
D	100.8	0.9

[a] Six point recovery curve from 50-150% of recommended assay concentration.

RESULTS

Chromatography and Specificity—The selectivity of the chromatographic conditions for I is demonstrated by its ability to resolve compound IV, which differs only in the spatial orientation of the fluorine atom at the 6-position. In addition compound V, which lacks a fluorine atom at the 6-position, is also resolved from I.

The development of chromatographic conditions appropriate for the analysis of I in bulk form and in cream and ointment formulations was based in part on the work of Beyer (9). In addition to the microparticulate column[9] described by Beyer, other 5- and 10-μm silica columns[10] were tried, but with limited success.

Experimentation with mobile phases containing butyl chloride and methylene chloride in addition to polar modifiers (tetrahydrofuran, methanol, acetic acid) revealed that the proportion of methylene chloride was important in column selectivity. Figure 1 depicts the effect on retention volume of selective changes in a mobile phase containing water-saturated methylene chloride. These data were obtained using a small-particle short silica column offering resolution of all peaks of interest, a reasonable analysis time, reproducible chromatography, and lack of placebo interference[7]. The final mobile phase condition chosen (Fig. 1, condition B) was judged to offer the most satisfactory results from the standpoint of resolution and analysis time. The chromatographic separation of compounds I-VII, using condition B, is shown in Fig. 2.

Recovery and Linearity—Recovery of I from either cream- or ointment-spiked placebos was tested by adding 50-150% of the labeled amount of I (0.5 mg of I/g) to each of the cream or ointment placebo formulations. The average recovery of I, calculated using peak area response, for the two cream formulations, "A" and "B," and ointment formulations, "C" and "D," are shown in Table II. A plot of the amount of I added *versus* the amount of I recovered indicated that the slope was within experimental error of the expected slope of I, and the *y*-intercept was 0 for all the formulations tested. The coefficients of correlation, *r*, for the recovery plots were >0.999, indicating that the assay is linear for the cream or ointment formulations tested (50-150% of labeled amount of I, 0.5 mg of I/g).

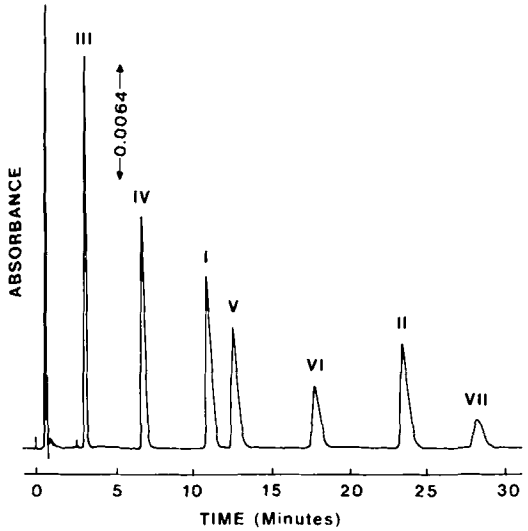

Figure 2—*Chromatogram of diflorasone diacetate and related compounds.*

[9] Part #27477; Waters Associates, Millford, Mass.

[10] Part #850952-701; E.I. DuPont, Wilmington, Del. and Part 8634989; IBM Instruments, Danbury, Conn.

Table III—Diflorasone Diacetate (I) Assay Results for Cream and Ointment Formulations Using Peak Area Ratio Response

Formulation[a]	I Found, mg/g[b]	Percent of Label, %
Cream		
A	0.494–0.505	98.8–101.0
B	0.483 0.510	96.6–102.0
Ointment		
C	0.488–0.498	97.6–99.6
D	0.451–0.485	90.2–97.0

[a] Product (A and C) and experimental (B and D) formulations. [b] Range of assay results obtained after assaying four lots per formulation.

Quantitation of III–VII was examined to determine if the responses of these structurally related compounds were linear over the 0.1–1.0% (w/w) range. These data were obtained to test the applicability of the method for potential impurities. The individual slopes for the calibration curves using peak area percent for III–VII were all 0.96–1.00 and the y-intercepts were not significantly different from zero. The coefficients of correlation, r, for plots of related compounds added *versus* amount found were all >0.999.

DISCUSSION

Presented in Table III are the range of results obtained using the procedure for cream and ointment product formulations A and C and cream and ointment experimental formulations B and D. The precision of the method was determined by replicate analyses of lots from each formulation. The day-to-day assay precision, expressed as *RSD*, was ~1.8% for cream formulations A and B and ~1.3% for ointment formulations C and D.

In addition to the determination of I in cream and ointment formulations, three lots of bulk drug were assayed for purity on the "as is" basis (Table IV). The *RSD* for the purity determination of I was ~1.0% using peak area ratio response.

The chromatographic method described is an accurate and precise method for the determination of diflorasone diacetate (I) in creams, ointments, and bulk drug. The method was found to be reproducible as well as rugged, making it suitable for use as a routine assay. The ability of the method to separate structurally related compounds makes the method useful for monitoring bulk drug and product stability.

Table IV—Diflorasone Diacetate Assay Results for Bulk Drug Using Peak Area Ratio Response

Lot	Assay[a] %	RSD, %
I1	99.3	1.1
I2	99.2	0.9
I3	99.7	0.7

[a] Calculated on the "as is" basis; the results are averages of six determinations over 2 d.

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(5) U.S. Patent No. 3,312,590 (Formula III).

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Effect of Nitroglycerin-Soluble Additives on the Stability of Molded Nitroglycerin Tablets

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Received June 22, 1983, from *Eli Lilly and Company, Indianapolis, IN 46285.* Accepted for publication February 1, 1984.

Abstract □ Nitroglycerin vapor pressures at 25°C were determined for additive–nitroglycerin systems over the additive–nitroglycerin weight ratio range of 0.5 to 3.0 for 16 additives exhibiting solubility in nitroglycerin. The effects of the additives on nitroglycerin chemical stability at 25°C and 50°C were also studied. Tablet stability characteristics, *i.e.*, content uniformity, open-dish stability, and chemical decomposition were evaluated for selected tablet formulations. Most additives lowered the vapor pressure of nitroglycerin sufficiently to stabilize content uniformity when used at additive–nitroglycerin weight ratios near 1. Higher additive levels are needed for significant potency stabilization in open-dish stability tests, but these levels normally decrease the chemical stability of nitroglycerin. However, stabilization of content uniformity, a twofold reduction of potency loss in an open-dish stability test, and chemical stability are possible with at least three of the additives studied.

Keyphrases □ Nitroglycerin—vapor pressure in sublingual molded tablets, intertablet migration, effect of additives □ Tablet stability—nitroglycerin sublingual molded tablets, vapor pressure, intertablet migration, effect of additives

A number of workers have emphasized both the physical instability of molded nitroglycerin tablets arising from vaporization, and subsequent migration of nitroglycerin, and the improvement in stability obtained by incorporating a nitroglycerin-soluble additive in the formulation (1–7). The additive lowers the vapor pressure of nitroglycerin which results in: (*a*) a reduction of the evaporation rate of nitroglycerin from tablets exposed to ambient air currents (1); (*b*) a marked decrease in losses due to absorption in packaging material (7); (*c*) stabilized content uniformity, in that most of the intertablet nitroglycerin migration which occurs in conventional (unstabilized) tablets is thermodynamically impossible in a closed system of stabilized tablets (4). A modest vapor pressure reduction is sufficient to stabilize the content uniformity within a sample of tablets (4). However, both evaporation rate and package absorption are least for the formulation of lowest vapor pressure.

Although there have been several reports of chemical instability in nitroglycerin tablets (8, 9), chemical decomposition is normally not a problem in conventional nitroglycerin tablets. However, the agent employed to stabilize content uniformity may have an adverse effect on chemical stability. Several studies (7, 8, 10) suggest that both povidone (7) and polyeth-

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