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**Note**

# High-performance liquid chromatography of the topical anti-inflammatory steroid halcinonide*

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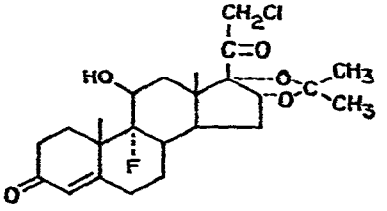
Halcinonide is a topical anti-inflammatory agent[1] with the systematic name 9α-fluoro-21-chloro-11β,16α,17α-trihydroxypregn-4-ene-3,20-dione, cyclic 16,17 acetal with acetone (E. R. Squibb, New Brunswick, N.J., U.S.A.). Colorimetric assays for steroids[2] depend on the reactivity of only a portion of the molecule such as the 4-ene-3-one conjugated system[3]. An assay that is based on the integrity of the entire molecule would best indicate the stability of the drug. High-performance liquid chromatography (HPLC), which can separate closely related compounds on the basis of weak competitive interactions of the molecule between liquid mobile phase and the stationary column packing material, is the basis of the stability-indicating assay described below.




## EXPERIMENTAL

### *Apparatus and reagents*

The liquid chromatograph is assembled from commercially available, interchangeable components. Either a constant volume pump (Altex or Waters Assoc.) or a precision, constant pressure gas regulator (Hoke) from a tank of nitrogen to a 500-ml reservoir (Varian) provides pressurized mobile phase. A precision loop injector (Chromatronix or Rheodyne) with a nominal capacity of 20 μl, is used. The column is an octadecylsilane, reversed-phase (RP-18), prepacked column [Partisil (Whatman), μBondapak (Waters Assoc.) or Micropak (Varian)]; U.S.P. designation L-1. Detection is usually with a fixed wavelength detector (Altex or Chromatronix)

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at 254 nm, with the attenuation adjusted to give peak heights at least 40% of the chart width of the 25-cm recorder (Linear Instruments). Since the absorption maximum of halcinonide is at 239 nm, a variable wavelength detector (Schoeffel Instrument) may have greater sensitivity, depending on the optical system. The mobile phase is pesticide or HPLC-grade acetonitrile–double distilled water (60:40), adjusted to give a retention time of approximately 6 min for halcinonide. If necessary, increasing the proportion of acetonitrile decreases the retention time.

*Procedure*

Halcinonide and related compounds, at a concentration of *ca.* 20 μg/ml mobile phase, are chromatographed with the option of using progesterone (*ca.* 24 μg/ml) as internal standard. A 20-ml volume of hexanes followed by acetonitrile–water (2:1, saturated with HPLC-grade hexanes) is added to 0.5 to 2 g (0.1 to 0.025%) of cream or ointment in a test tube. The test tube is gently heated over a steam bath for *ca.* 1 min (to dissolve the lipophilic matrix in hexanes), shaken and then ultrasonicated for about 20 min. After centrifugation at 1100 *g* for 10 min, the lower acetonitrile–water layer, containing steroid, is drawn off with a syringe and a cannula. The contents are quantitatively transferred to a 25-ml volumetric flask. A 5-volume of acetonitrile–water is added to the test tube. After shaking the tube for *ca.* 1 min, and recentrifugation, the lower layer is transferred to the volumetric flask. A third extraction is performed with 5 ml of acetonitrile–water. The contents of the flask are diluted to volume using acetonitrile–water that had been used to rinse the syringe and cannula. Solutions containing halcinonide are diluted with aqueous acetonitrile to 25 ml and then injected into the chromatograph. Either peak heights or areas are used for quantitation.

## RESULTS AND DISCUSSION

A typical chromatogram is shown in Fig. 1. Chromatograms of various concentrations of halcinonide, using the recommended procedure, gave linear responses passing through the origin. The relative standard deviations (coefficients of variation) of 20 sets of standards, with concentrations of 16 to 24 μg/ml, ranged from 0.2 to 1.2%. Halcinonide can be separated from its synthetic precursors by HPLC. The relative retention time, based on halcinonide, of the starting compound in its synthesis, 9α-fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione, is 0.18. The acetonide, 9α-fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione, cyclic 16, 17 acetal with acetone, has a relative retention time of 0.32. The acetonide-21-mesylate, produced by reacting the acetonide with mesyl chloride, has a relative retention time of 0.72. Reacting the acetonide-21-mesylate with lithium chloride in dimethylformamide gives halcinonide; relative retention time = 1.00.

Formulated or unformulated $^{14}$C-labeled halcinonide in hexanes is quantitatively extracted into acetonitrile–water (2:1), as shown by the absence of radioactivity in the hexanes layer. This acetonitrile–water–hexanes system is an extension of a *n*-heptane–acetonitrile–hexanes medium[4] used in the column chromatography of corticosteroids. Aqueous acetonitrile has also been used to extract a related steroid, triamcinolone acetonide, from a formulation[5]. Internal standards are optional in this HPLC assay for halcinonide in creams and ointments, because the final volume is fixed at 25 ml and a precision loop injector is used. The average content of hal-

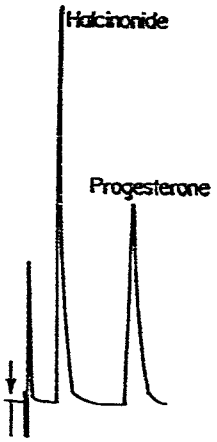


Fig. 1. Reversed-phase HPLC of halcinonide, using the option of progesterone as internal standard. Absorbance units full scale is 0.08. Halcinonide elutes in *ca.* 6 min, progesterone in *ca.* 8 min.

TABLE I

HPLC DETERMINATIONS OF HALCINONIDE CONTENT IN VARIOUS 0.100% FORMULATIONS

| *Formulation* | *Actual content (%)* | *Number of determinations* | *Relative standard deviation (%)* |
|---|---|---|---|
| Ointment* | 0.0993 | 12 | 1.2 |
| Cream** | 0.0997 | 6 | 1.5 |
| Solution*** | 0.0999 | 9 | 0.4 |

* Formulated in mainly plasticized hydrocarbon gel and polyethylene glycols.
** Formulated in mainly castor oil, promulgen, petrolatum, silicone fluid and water.
*** Formulated in mainly polyethylene glycols and water.

TABLE II

HPLC OF HALCINONIDE IN 0.105% SOLUTIONS STORED FOR 27 MONTHS AT VARIOUS TEMPERATURES AND ANALYZED BY TWO ANALYSTS USING COLUMNS FROM TWO DIFFERENT VENDORS

| *Sample* | *Temperature (°C)* | *Partisil column* | | *Micropak column* | |
|---|---|---|---|---|---|
| | | *Analyst* | | *Analyst* | |
| | | *I* | *II* | *I* | *II* |
| I | Ambient | 0.103 | 0.104 | 0.104 | 0.103 |
| | 33 | 0.101 | 0.102 | 0.102 | 0.102 |
| | 40 | 0.098 | 0.099 | 0.100 | 0.099 |
| II | Ambient | 0.102 | 0.102 | 0.102 | 0.099 |
| | 33 | 0.102 | 0.103 | 0.103 | 0.100 |
| | 40 | 0.100 | 0.099 | 0.100 | 0.099 |
| III | Ambient | 0.104 | 0.103 | 0.105 | 0.102 |
| | 33 | 0.103 | 0.102 | 0.105 | 0.101 |
| | 40 | 0.101 | 0.097 | 0.100 | 0.100 |

TABLE III

HPLC DETERMINATION OF TRIAMCINOLONE ACETONIDE, FLUOCINONIDE AND DIFLORASONE DIACETATE, USING THE EXTRACTION SYSTEM RECOMMENDED FOR HALCINONIDE

| *Steroid* | *Retention time (min)* | *HPLC Assay of bulk steroid* | | | | *Content of formulated steroid* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Repetitive injections* | | | *Linearity (16–24 μg/ml)–relative standard deviation of corr. response (%)* | *Theory (%)* | *Found* | | |
| | | *Peak height[1] (cm)* | *n* | *Relative standard deviation (%)* | | | *Mean* | *n* | *Relative standard deviation* |
| Triamcinolone acetonide* | 6 | 18.42 | 6 | 0.6 | 0.8 | 0.050 | 0.0505 | 2 | 1.4 |
| Fluocinonide** | 7 | 22.47 | 4 | 0.8 | 0.8 | 0.050 | 0.0515 | 2 | 2.8 |
| Diflorasone diacetate*** | 6 | 23.30 | 5 | 0.3 | 1.0 | 0.100 | 0.0997 | 6 | 0.6 |

* Squibb; formulated in propylene glycol, octyl alcohol, glycerol monostearate, spermaceti, isopropyl palmitate, polysorbate 60 and water (Kenalog).

** Syntex; formulated in stearyl alcohol, cetyl alcohol, mineral oil, propylene glycol, sorbitan monostearate, polysorbate 60, citric acid and water (Lidex-E).

*** Upjohn; formulated in stearic acid, sorbitan monooleate, sorbic acid, sorbitan monostearate, polyoxyethylene 20 sorbitan monostearate, citric acid, propylene glycol and water (Florone).

[1] Concentration of 20.0 μg/ml.

cinonide found in 0.100% formulations are summarized in Table I. Two analysts, using HPLC columns from two different vendors, analyzed 0.105% halcinonide solutions stored for 27 months at various temperatures. The results, Table II, are similar within experimental error. The data summarized in Tables I and II indicate both the stability of halcinonide in the formulation and the good precision, accuracy and ruggedness of the assay.

As seen in Table III, triamcinolone acetonide, fluocinonide and diflorasone diacetate can also be chromatographed using the recommended HPLC procedure. All standard curves are linear and pass through the origin. The responses of the various standards are corrected for dilution to calculate relative standard deviations. Extractions of steroids from various commercial formulations follow the recommended procedure for halcinonide.

In summary, bulk and formulated halcinonide can be rapidly and specifically quantitated by HPLC. Since the drug can be separated from such closely related compounds as its synthetic precursors, this assay indicates stability. The recommended extraction and chromatographic procedure can be used for triamcinolone acetonide, fluocinonide and diflorasone diacetate, and may be applicable to other steroids.

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