Analysis of Corticosteroid Creams and Ointments by High Pressure Liquid Chromatography

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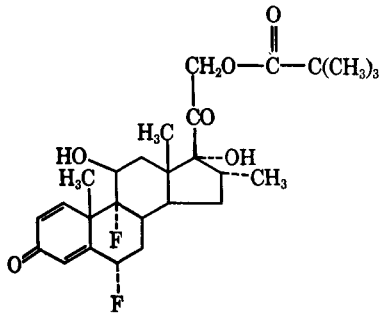
Abstract □ High pressure liquid chromatography is shown to be applicable to the specific and rapid assay of corticosteroid creams and ointments. The method is illustrated through the quantitative determination of flumethasone pivalate cream and ointment. The optimum solvent system for the analysis was 5% ethyl acetate–0.2% acetonitrile in hexane, with β,β′-oxydipropionitrile on Zipax as the support. With this system, flumethasone pivalate could be eluted in 7 min. with a column efficiency of 600 theoretical plates. The chromatography of other steroids including dexamethasone, prednisolone, and hydrocortisone is also shown.

Keyphrases □ Corticosteroid creams and ointments—analysis, high pressure liquid chromatography □ Flumethasone pivalate cream and ointment—analysis, high pressure liquid chromatography □ Hydrocortisone cream—analysis, high pressure liquid chromatography □ High pressure liquid chromatography—analysis, corticosteroid creams and ointments

Flumethasone pivalate[1] formulations can be assayed *via* the blue tetrazolium procedure (1), the official procedure in the compendia for all corticosteroid creams and ointments (2, 3). Differences in methodology appear in the extraction and preliminary purification of the steroid. Although this is a satisfactory method, it is subject to many variables and interferences, not the least of which is the quality of the reagents. Another colorimetric procedure (4) utilizes 4-aminoantipyrine as the color reagent. This method is sensitive and precise, but it is not specific for flumethasone pivalate and hence is not suitable as a stability method. Although both colorimetric procedures have been applied to the analysis of flumethasone, they are time consuming and tedious and lack specificity; therefore, a faster, more specific, and easier method was sought.

Because of the sensitivity (determination of flumethasone pivalate at concentration levels of 0.01–0.03% in creams and ointments) and specificity needed for a stability method, a chromatographic technique appeared to be a logical approach. Numerous publications on the analysis of steroids by GLC have appeared (5–9). Because most steroids cannot be chromatographed directly, derivatives must be formed prior to analysis by GLC; however, because of the complexity of these molecules, the formation of only a single derivative is difficult to obtain.



flumethasone pivalate

Several derivation techniques were attempted with flumethasone pivalate because the underivatized compound degraded on the GLC column [1% SE-30 on Gas Chrom Q, 100–120; column, 205–314° (15°/min.); detector, 340°; injector, 320°] and could not be successfully chromatographed. Flumethasone pivalate was reacted with several derivatizing agents and combinations[2] in an attempt to obtain a more easily chromatographed compound. The time required for formation of these derivatives ranged from 15 min. to 8 hr.; in every case, more than one peak resulted, even after an extensive clean-up procedure. Because the time required for derivative formation was lengthy, these procedures offered no advantage in that respect, and since several peaks were obtained, the specificity was questionable.

High speed liquid chromatography[3] has been applied to the analysis of several drugs and their metabolites including benzodiazepines (10), phenacetin (11), barbiturates (12), and *p*-hydroxyacetanilide (13). But although the manufacturers of high speed liquid chromatographs illustrate the use of high pressure chromatography for the analysis of steroids in their bulletins[4], only one paper has appeared on the analysis of steroids. Siggia and Dishman (14) reported on the separation of androgens, estrogens, progestins, and corticosteroids on several columns.

In this article, the use of high pressure liquid chromatography for the analysis of flumethasone pivalate cream and ointment and hydrocortisone cream is reported. The procedures and methods developed for flumethasone should be applicable with little or no modification to other corticosteroid creams and ointments.

EXPERIMENTAL

A liquid chromatograph[5] equipped with a UV photometric detector was used throughout the study. Columns were 1 m. × 2.1 mm. i.d., and stationary phases were Carbowax 4000 and β,β′-

[1] Locorten, CIBA.

[2] Including BSA, *N,O*-bis-(trimethylsilyl)-acetamide (Pierce Chemical Co., Rockford, IL 61105); Trisil BT, a mixture of five parts BSA and one part trimethylchlorosilane (Pierce Chemical Co.); Trisil TBT, a mixture of three parts trimethylsilylimidazole, three parts BSA, and two parts trimethylchlorosilane (Pierce Chemical Co.); and MOX, a 2% solution of methoxyamine hydrochloride in pyridine (Pierce Chemical Co.).

[3] For general discussions of high speed liquid chromatography, see papers by Kirkland, Horvath, Lipsky, Waters, Giddings, Scott, and others, particularly in recent years' issues of *Analytical Chemistry* and *Journal of Chromatographic Science*.

[4] Manufacturer's literature by Du Pont, Waters, and Pye-Unicam.

[5] Du Pont model 820.

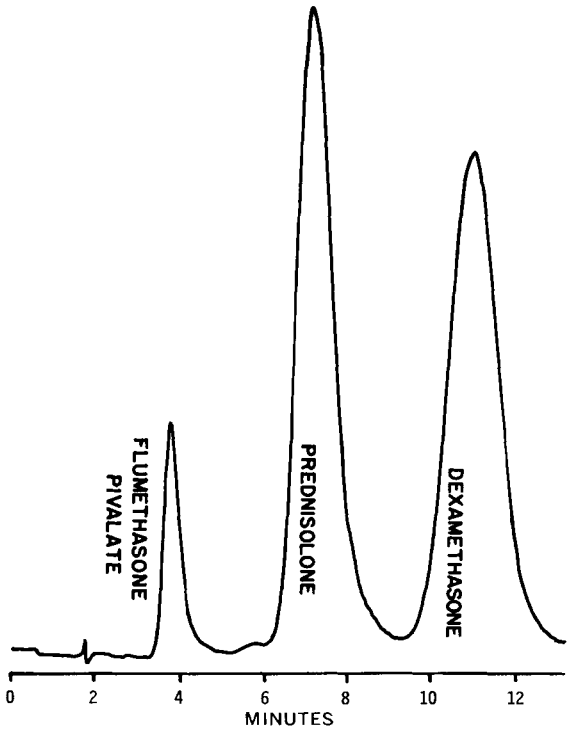


Figure 1—*Chromatogram of flumethasone pivalate, prednisolone, and dexamethasone on β,β'-oxydipropionitrile. Mobile phase: 1% ethanol in hexane; pressure: 500 lb.; flow rate: 0.8 ml./min.; and temperature: ambient.*

oxydipropionitrile coated on Zipax[6]. A precolumn, 5 mm. × 60 cm., was employed with both stationary phases; in all cases, mobile phases were presaturated with the stationary liquid. An evaporator[7] was used to evaporate solvents. Peak areas were determined with an electronic integrator[8] as well as by triangulation. Samples were injected using standard syringes or with a 3-μl. sampling valve[9].

The flumethasone pivalate, 6α,9α-difluoro-11β,17α-dihydroxy-16α-methyl-21-trimethylacetoxy-1,4-pregnadiene-3,20-dione, used in this study was 99.0% pure by phase solubility analysis. The hydrocortisone, prednisolone, and dexamethasone were USP grade and used directly. All other chemicals were of reagent grade and were used without further purification.

Procedures for the creams and ointments are given as follows.

Flumethasone Pivalate Cream, 0.03%—Accurately weigh 5 g. of cream and add 20 ml. of 0.36 *N* H_2SO_4 solution. Add 5 ml. of reagent grade ethyl acetate and shake for 15 min. on a mechanical shaker. If the sample is not completely dissolved, warm gently on a steam bath to complete the dissolution. If necessary, centrifuge to aid separation of the phases. Remove the organic layer and repeat the extraction with two additional 5-ml. portions of ethyl acetate. Evaporate the combined extracts on the evaporator under a stream of dry nitrogen to 2.0 ml. and inject a 3-μl. aliquot into the liquid chromatograph. Elute with 5% ethyl acetate–0.2% acetonitrile in hexane, with β,β'-oxydipropionitrile coated on Zipax as the support.

Flumethasone Pivalate Cream, 0.01%—Accurately weigh 10 g. of cream and add 50 ml. of 0.36 *N* H_2SO_4 solution. Add 10 ml. of reagent grade ethyl acetate and shake for 15 min. on a mechanical shaker. Warm gently on a steam bath if necessary to complete dissolution. If necessary, centrifuge to aid separation of the phases. Remove the organic layer and repeat the extraction with two additional 5-ml. portions of ethyl acetate. Evaporate the combined extracts to exactly 2.0 ml. on the evaporator with a stream of dry nitrogen. Inject a 3-μl. aliquot into the liquid chromatograph and elute with 5% ethyl acetate–0.2% acetonitrile in hexane, with β,β'-oxydipropionitrile coated on Zipax as the support.

Flumethasone Pivalate Ointment, 0.03%—Accurately weigh 5 g. of ointment and add 5 ml. of 3A anhydrous ethanol; heat on a steam bath until two liquid layers are formed. Allow to cool until the lower layer solidifies and then filter the ethanol layer through medium porosity filter paper. Repeat the extraction with two additional 5-ml. portions of alcohol and evaporate the combined extracts to 2.0 ml. utilizing the evaporator. Inject a 3-μl. aliquot into the liquid chromatograph, using either 5% ethyl acetate–0.2% acetonitrile in hexane or 1% ethanol in hexane as the mobile phase, with β,β'-oxydipropionitrile on Zipax as the support.

Hydrocortisone Cream, 1%—Accurately weigh 1 g. of cream, and add 5 ml. of 3A anhydrous ethanol; heat gently on a steam bath to aid dissolution. Mix well, allow to cool, and dilute to 10.0 ml. with anhydrous ethanol. Inject a 3-μl. aliquot into the liquid chro-

Figure 2—*Chromatogram of placebo cream on β,β'-oxydipropionitrile. Mobile phase: 5% ethyl acetate–0.2% acetonitrile in hexane; pressure: 800 lb.; temperature: ambient; and flow rate: 1 ml./min.*

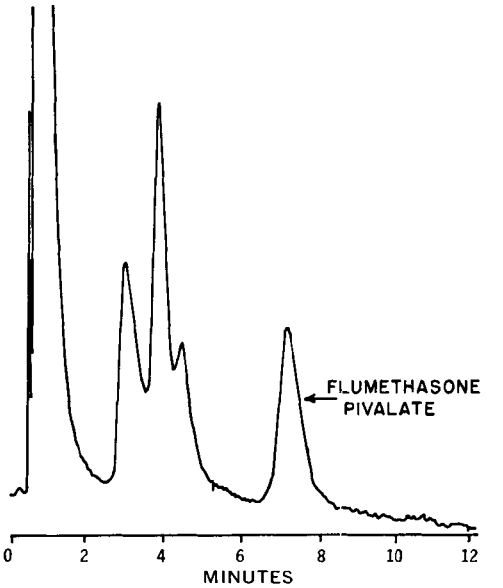


Figure 3—*Chromatogram of flumethasone pivalate cream, 0.03%, under the same conditions as the placebo in Fig. 2. The steroid is completely separated from excipients. Temperature: ambient; and flow rate: 1 ml./min.*

[6] E. I. DuPont de Nemours & Co., Instrument Products Div. Wilmington, DE 19898

[7] N-Evap, Organomation Associates, Shrewsbury, MA 01545

[8] Model CRS-115B/41, Infotronics Corp., Houston, TX 77042

[9] Supplied by Du Pont.

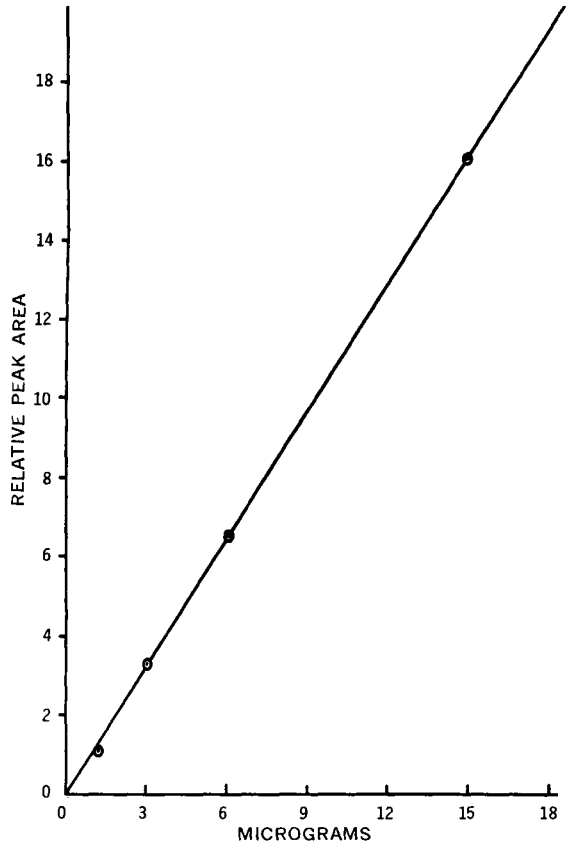


Figure 4—*Plot indicating linearity of response of the UV detector to flumethasone pivalate.*

matograph using 1% ethanol in hexane as the solvent system, with β,β′-oxydipropionitrile on Zipax as the support.

Calculation—Determine the peak area utilizing either an electronic integrator or the method of peak height × peak width at one-half the peak height. Compute the average area for three injections. Compare the area with that produced by a standard peak under similar conditions.

RESULTS AND DISCUSSION

The initial column selected was Carbowax 4000 on Zipax. Hexane modified with 1–10% of ethanol, chloroform, acetonitrile, isopropanol, and *tert*-butanol was used as the mobile phase. Several problems were encountered with these systems, including column bleed and too much tailing.

Attention was then directed toward β,β′-oxydipropionitrile on Zipax. With alcohol in hexane, the flumethasone pivalate was eluted relatively rapidly as a symmetrical peak. With 1% ethanol in hexane

Table I—Reproducibility of Peak Area for Flumethasone Pivalate (c = 1 mg./ml.) Using the 3-μl. Injection Valve

Sample Number	Peak Area	
	Electronic Integrator	Triangulation
1	59833	7.831
2	57098	8.022
3	59795	7.776
4	57001	7.817
5	61284	8.061
6	58207	7.790
7	59235	7.885
8	59413	8.148
9	58363	—
Mean	58913	7.916
Standard deviation	±1387	±0.148
Coefficient of variation	2.37%	1.87%

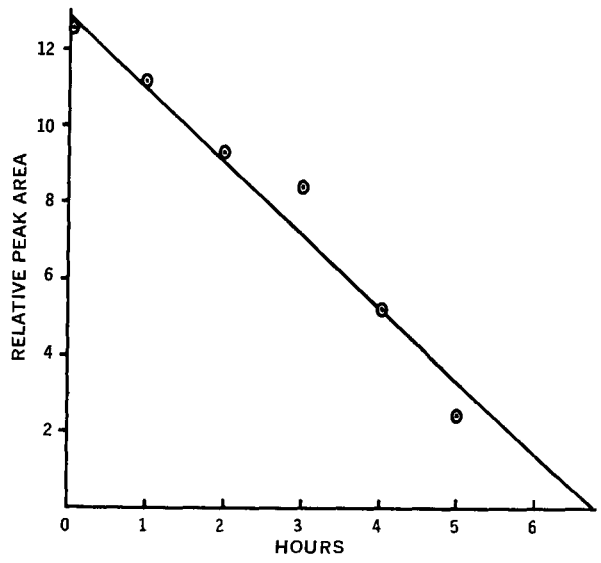


Figure 5—*Plot showing disappearance of flumethasone pivalate in cream as a function of time (see text for details).*

at 500 p.s.i., a mixture of flumethasone pivalate, dexamethasone, and prednisolone was separated (Fig. 1). (The latter two compounds are potential internal standards for use in the analysis of flumethasone pivalate.) When a sample of flumethasone pivalate, 0.03%, was run using the alcohol–hexane solvent system, interferences from some of the cream excipients were encountered. Good separation could not be effected by varying the pressure or changing the ethanol concentration in the solvent system. An alumina column clean-up procedure was incorporated without success as a preseparation step in an attempt to remove these interferences. The addition of acetonitrile to the solvent system did effect slight separation of the interfering peaks from the flumethasone

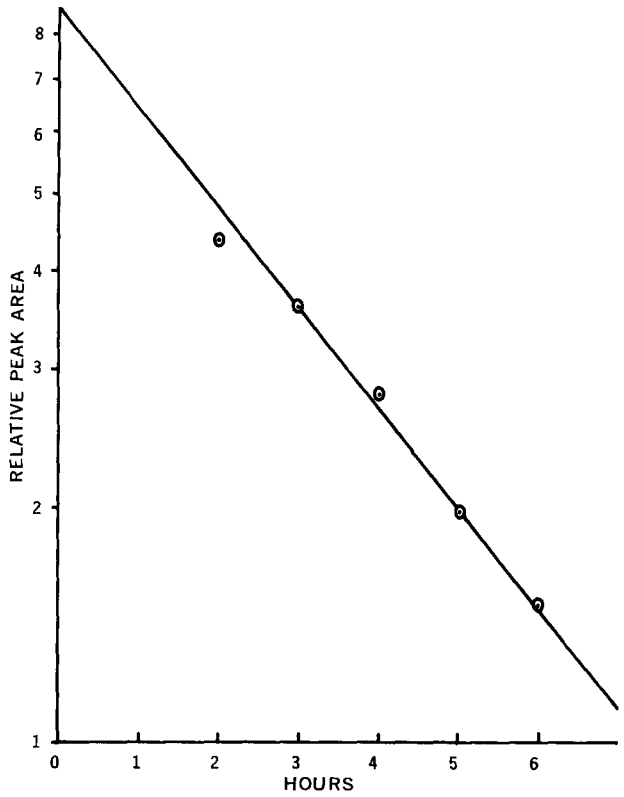


Figure 6—*Plot showing disappearance of flumethasone pivalate in alkaline propylene glycol solution as a function of time (see text for discussion).*

pivalate, but it was not considered satisfactory for quantitative purposes.

Flumethasone pivalate was extracted from the cream in these studies according to the following procedure. An accurately weighed portion of the cream is dried overnight over silica gel under vacuum. The dried sample is then partitioned between acetonitrile and isooctane; the steroid remains in the acetonitrile phase. The sample is then concentrated with the aid of a stream of nitrogen.

Subsequently, the cream was extracted directly into ethyl acetate from an acidic solution as described previously.

Ethyl acetate, at concentrations greater than 5% in hexane, eluted flumethasone pivalate from the β,β'-oxydipropionitrile column. With a 10% ethyl acetate–hexane solvent system, the flumethasone pivalate was eluted in about 3 min.; with a 5% ethyl acetate–hexane solvent system, the elution time was 15 min. Since the addition of acetonitrile, which is an excellent solvent for flumethasone pivalate, to the alcohol systems aided the separation of flumethasone pivalate from excipient interferences, 0.2% acetonitrile was added to the 5% ethyl acetate–hexane. The optimum solvent system for the analysis of flumethasone pivalate cream is then 5% ethyl acetate and 0.2% acetonitrile in hexane. With this system, flumethasone pivalate elutes in approximately 7 min., with a column efficiency of 600 theoretical plates, and is completely separated from interferences (Figs. 2 and 3). Figure 4 shows the linearity of response over the concentration range of 1–15 mcg. of flumethasone pivalate. A placebo cream to which 0.03% of flumethasone pivalate had been added gave recoveries of 99.3, 100.7, 98.7, and 98.7%.

A standard solution of flumethasone pivalate was injected nine times through a 3-μl. sampling valve to evaluate the reproducibility of injection. Areas were calculated using peak height $\times$ the width at half the peak height and also using the electronic integrator. Statistical analysis of the data is shown in Table I. The coefficient of variation is slightly greater using the electronic integrator. This is probably due to the necessity of supplying high filtering to mask the cycling of the pump and air compressor. The applicability of electronic integration is still under investigation.

Flumethasone pivalate ointment and hydrocortisone cream presented no problems in analysis. The ointment is a simple preparation in a petrolatum base and, hence, relatively free of interferences. Hydrocortisone cream is 30–100 times more concentrated than the flumethasone pivalate, and there were no significant inteferences at this level of concentration.

To ascertain its suitability as a stability-indicating method, two studies were initiated. In the first study, six samples of the 0.03 % cream were weighed into 40-ml. centrifuge tubes and 1 ml. of 1 *N* NaOH solution was added to accelerate decomposition. Tubes were placed in a water bath, and samples were removed at appropriate time intervals for analysis. Figure 5 shows the loss of flumethasone pivalate over 5 hr. The data are plotted in a linear fashion and appear to follow pseudo-zero-order kinetics. This is not unexpected for a heterogeneous system. In the second study, under greater control, 50 mg. of flumethasone pivalate was weighed into a 100-ml. round-bottom flask. To this was added 50 ml. of propylene glycol to solubilize the drug and 50 ml. of 0.1 *N* NaOH solution. The flask was placed in a constant-temperature bath, and samples were withdrawn at appropriate intervals for analysis. After 2 hr. the samples followed an apparent first-order degradation process (Fig. 6). TLC of the flumethasone pivalate on silica gel GF, using ethyl acetate–benzene (40:60) as a solvent system, confirmed the disappearance of the flumethasone pivalate. On the TLC plate, a spot appeared at the origin and at R_f 0.17 while the flumethasone pivalate spot at R_f 0.61 decreased over the 6-hr. span.

Although the degradation of a steroid such as flumethasone pivalate is complex and involves hydrolysis, oxidation, and possibly, to a lesser extent, transesterification, the intact drug remaining can be determined. Since the disappearance of the flumethasone pivalate peak can be followed in the cream as well as in the standard solution, this method appears suitable as a stability method for this product and the authors believe it would be suitable as a stability method for other corticosteroid formulations.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 17, 1971, from the *Research and Development Department, Pharmaceuticals Division, CIBA-Geigy Corporation, Summit, NJ 07901*

Accepted for publication December 15, 1971.

A preliminary account of this work was reported at the Symposium on High Speed Liquid Chromatography, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, March 1971.

The authors thank Mr. J. B. Smith for his helpful discussions and Dr. C. R. Rehm for his support of this work.

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