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Note

Analysis of flumethasone pivalate formulations by high-performance liquid chromatography

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Flumethasone pivalate (6α,9α-difluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-pivalate) is a synthetic, anti-inflammatory corticosteroid used in topical preparations at low dosage. In developing a general method for the analysis of corticosteroidal preparations[1], it had been our intention to include the analysis of flumethasone pivalate in the method; however, it proved to be not amenable chromatographically to the inclusion in the general method, because the time of analysis would have been excessively long. Therefore the procedure described in this publication was developed. It should be noted that a high-performance liquid chromatographic (HPLC) method for flumethasone pivalate formulations was reported some time ago by Mollica and Strusz[2] using the relatively inefficient β,β'-oxydipropionitrile on Zipax, one of the early non-bonded systems used for the analysis of corticosteroids. The current U.S.P. assay[3] is a relatively non-specific colorimetric procedure.

EXPERIMENTAL

Materials

Flumethasone pivalate was U.S.P. reference standard. The methanol was HPLC-grade (J. T. Baker, Phillipsburgh, NJ, U.S.A.), the tetrahydrofuran was glass-distilled (BDH) and the water was double-distilled in glass.

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) HPLC system equipped with a Model 440 absorbance detector, set at 254 nm (0.5 a.u.f.s.), and a U6K injector were used. Peak heights were determined with an Hewlett-Packard (Missisauga, Ontario, Canada) 3370B integrator.

Column

A C_8 reversed-phase column (25 cm × 4.6 mm I.D.) (Brownlee Labs., Santa Clara, CA, U.S.A.) was used.

Mobile phase

A solution of tetrahydrofuran (THF)–methanol–water (30:30:40, v/v/v) was prepared and the flow-rate set at 1.5 ml/min.

Sample preparation —ointment

To an accurately weighed amount of ointment (1.0–1.5 g) was added 10 ml of chloroform–hexane (1:1). The solution was warmed on a water bath until the ointment was dissolved. With the aid of a multiple extraction unit (J. T. Baker), the solution was filtered through a silica Sep-Pak column (Waters Assoc.) fitted with 3-ml disposable syringes. The flask was rinsed with 10 ml of chloroform–hexane (1:1) and the washings were filtered through the column (the chloroform–hexane solution was discarded). The flask was then carefully rinsed with 5 ml of methanol and the methanolic solution was filtered through the column into a 25-ml volumetric flask.

The flumethasone pivalate was eluted from the column with 15 ml of methanol into the same flask and made up to volume with methanol. Volumes of 25 μl of this solution were injected into the chromatograph.

Sample preparation —cream

A 1-g sample of cream was accurately weighed and treated with 10 ml of methanol in a scintillation vial. The vial was shaken for 5 min, and then sonicated for a further 5 min, after which time the contents were filtered. This solution (10 μl) was injected into the chromatograph.

RESULTS AND DISCUSSION

A standard curve was obtained by injecting, in quadruplicate (C.V. on replicates $< 1\%$) amounts of flumethasone pivalate corresponding to 0.15, 0.30, 0.45, 0.60, 0.75, 0.90 and 1.05 μg, and measuring the corresponding peak heights. The response was linear (Fig. 1) (slope = 7.64, intercept = 0.0229, coefficient of correlation = 0.9997).

Percentage assays (Table I) were calculated by comparing peak heights of the samples with peak heights of a standard solution. Analyses were performed in duplicate on at least three samples of each formulation.

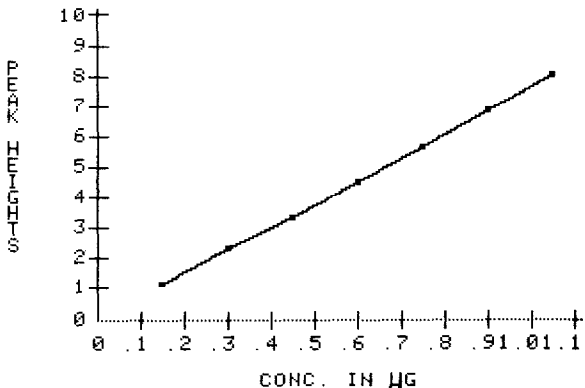

Fig. 1. Standard curve for flumethasone pivalate (peak height *vs.* amount injected).

TABLE I

ANALYSIS OF FORMULATION

Sample	*Assay (%)*	*Relative standard deviation*
Cream	101.8	1.35
Ointment	101.5	0.57

Complete elution of flumethasone pivalate was verified by adding methanol to the Sep-Pak column and injecting a portion of the eluate into the chromatograph. No flumethasone pivalate could be detected, thus confirming complete recovery.

To the chloroform–hexane fraction was added 10 ml of methanol. The precipitate obtained was filtered. The solvent was removed by evaporation and the residue was dissolved in a minimum amount of methanol. No flumethasone pivalate could be detected in this solution by HPLC, thus confirming no loss in the washings.

A typical chromatogram is shown in Fig. 2 (retention time of flumethasone pivalate = 8.4 min).

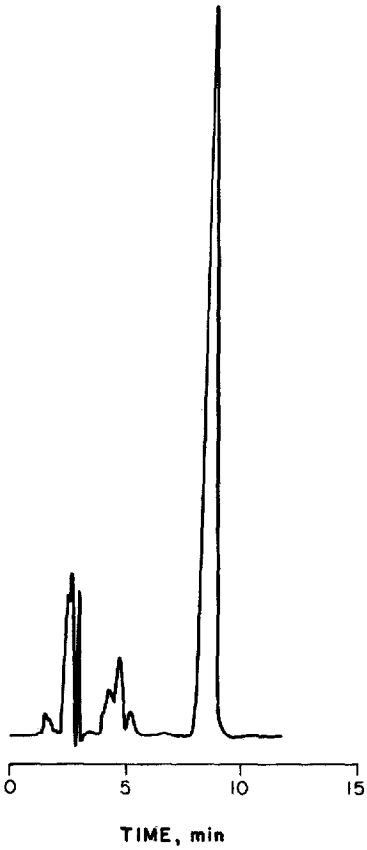

Fig. 2. Sample chromatogram (flumethasone pivalate, 0.03% ointment, retention time 8.4 min).

No related steroids were found in either the cream or the ointment; other peaks in the chromatogram are due to excipients.

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REFERENCES

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