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# The quantitative determination of fluocinolone acetonide and acetonide acetate in formulated products by high pressure liquid chromatography

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A chromatographic procedure for the determination of fluocinolone acetonide and acetate is described. The method employs high speed liquid chromatography through a Permaphase ODS column with an aqueous methanol eluant phase. The application of the method to the determination of fluocinolone acetonide and acetate in cream, and ointment formulations, is described.

The assay of fluocinolone acetonide formulations in current use requires chromatography of a sample extract in a partition system, the stationary phase of which is supported on Celite (Bailey, Holbrook & Miller, 1966). The procedure is accurate and selective but has the disadvantage that the time required for a single determinaton approaches 3 h.

The application of high pressure techniques appeared to offer the advantages of reduced time for chromatography and also of high sensitivity which might permit the use of smaller samples and a simplified extraction process.

## MATERIALS AND METHODS

### *Choice of solvent system*

Using a Du-Pont 820 liquid chromatograph we sought a suitable solvent system that would achieve clear separation of active principle from any impurities or degradation products therein, and from residual excipient material present in the sample extract.

Non-aqueous systems were first tried using hexane/chloroform and hexane/dioxan as eluting solvents on a Carbowax 4000 stationary phase absorbed on Zipax (Du-Pont) but these were subsequently rejected in favour of an aqueous: methanol eluting system on a Permaphase ODS column pack. The progress of the chromatogram was followed at 254 nm on a low volume (9 $\mu$l) ultra sensitive ultraviolet detector and a suitable recorder.

Typical chromatograms are shown in Fig. 1A (fluocinolone acetonide standard) and B (fluocinolone acetonide acetate standard); comparison with Fig. 1C,D,E illustrates the separation of the acetonide from methyl- and propyl-*p*-hydroxy benzoates (which themselves separated), of the acetonide from the ointment and the acetate from a formulation, demonstrating the degree of selectivity obtainable.

The linearity of the response with changes of concentration was checked over the range 10–1000 ng, using solution of the steroid in methanol containing toluene as an internal reference standard injected onto the column; straight lines proving the

excellent correlation were obtained. We also found that the published extraction procedures (Bailey & others, 1966) could be simplified. Our recommendation for the various types of formulation likely to be encountered are now detailed.

*Reagents.* Eluting solvent: (*a*) fluocinolone acetate–water–methanol (80 : 20);

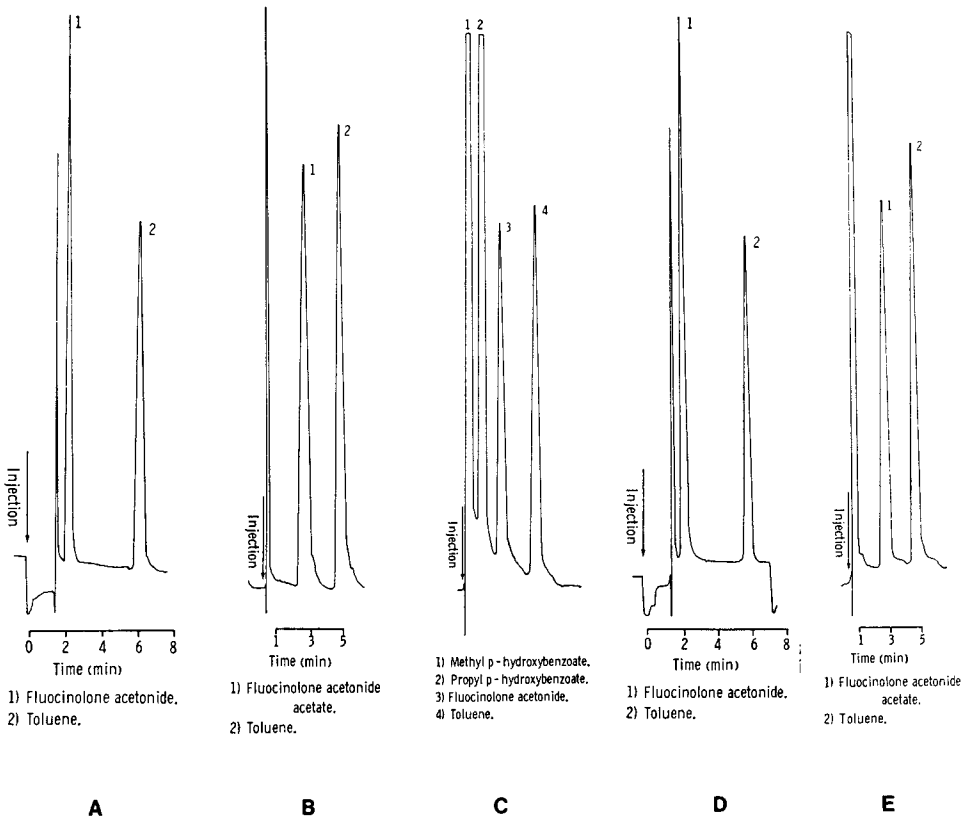


FIG. 1. Typical chromatograms. A, Fluocinolone acetonide standard; B, fluocinolone acetonide acetetate standard compared with separation of the acetonide from methyl- and propyl-*p*-hydroxybenzoates (which themselves separate) from the cream (C), ointment (D), and of the acetate from a formulation (E).

(*b*) fluocinolone acetonide acetate–water–methanol (70 : 30). Internal standard: Analar toluene. Iso-octane-reagent grade.

*Apparatus.* Du-Pont 820 liquid chromatograph (Felton, 1969) fitted with a uv detector and Honeywell Brown recorder. Permaphase ODS chromatographic column, 1 m long, 2 mm i.d. (Du Pont Instruments Ltd.). A Hamilton syringe (50 $\mu$l).

## *Preparation of sample*

*Fluocinolone acetonide cream and lotion* 0·025%. Transfer an accurately weighed quantity of sample (10 g) to a 100 ml separating funnel with methanol (15 ml) shake until the cream is dispersed, add iso-octane (50 ml) and shake for a further 2 min. Allow the layers to separate and transfer the lower aqueous methanol layer quanti-

tatively to a 25 ml volumetric flask, add toluene (37·5 $\mu$l) and make up to volume with methanol reserved for chromatography.

*Fluocinolone acetonide ointment* 0·025%. Proceed as described above, using methanol–water (4 : 1) (20 ml) in place of methanol (15 ml) in the extraction.

*Fluocinolone acetonide ointment* 0·01%. Transfer an accurately weighed quantity of ointment (10 g) to a 100 ml separating funnel with methanol–water (4 : 1) (8 ml), add iso-octane (50 ml), warm gently on a steam bath until dispersed, shake for 2 min. Allow to stand until cool, run off the lower aqueous methanol phase into a 10 ml volumetric flask. Add toluene (15 $\mu$l) and make to volume with methanol.

*Fluocinolone acetonide acetate* 0·05%. Transfer an accurately weighed quantity of sample (5 g) to a 100 ml separating funnel with methanol–water (4 : 1) 20 ml, swirl gently to disperse the formulation, add iso-octane 50 ml and shake for two min. Run off the lower aqueous methanol phase into a 25 ml volumetric flask, add toluene (37·5 $\mu$l) make to volume with methanol.

*Standard fluocinolone acetonide and fluocinolone acetonide acetate.* Transfer an accurately weighed quantity of fluocinolone acetonide (10 mg) to a 100 ml volumetric flask with the aid of methanol–water (4 : 1), shake until solution is effected, add (150 $\mu$l) of toluene as internal standard, make to volume with methanol and water.

*Conditions for chromatography*

Fluocinolone acetonide: column, Permaphase ODS; solvent system water–methanol (80 : 20): pressure, 1500 p.s.i.; temp. 25°. flow rate, 1 ml/min; uv detector attenuation $4 \times 10^{-2}$ absorbance units full scale (A.U.F.S.). Retention time: fluocinolone acetonide = 160 s; toluene = 393 s.

*Fluocinolone acetonide acetate:* column, Permaphase ODS; solvent system, water–methanol (70 : 30); Pressure, 1500 p.s.i.; temp. 27°; flow rate, 1 ml/min; uv detector attenuation $4 \times 10^{-2}$ A.U.F.S. Retention time: fluocinolone acetonide acetate = 175 s; toluene = 285 s.

*Chromatography*

Inject 2, 4, 6, 8, and 10 $\mu$l (200–1000 ng) of standard to check the linearity of the detector over the range of concentration required.

*Sample:* Inject 5 $\mu$l (≡ 500 ng) of sample and observe the chromatogram on the recorder. The concentration of fluocinolone acetonide in the sample is calculated.

$$\frac{\text{Peak height of sample}}{\text{Peak height of standard}} \times \frac{\text{Peak height of toluene in std.}}{\text{Peak height of toluene in sample}} \times \frac{\text{Wt of std (mg)}}{\text{Wt of sample (mg)}} \times 100 = \% \text{ fluocinolone acetonide in sample.}$$

## RESULTS AND DISCUSSION

The method has been applied to a variety of samples, from formulation development, stability studies and routine manufacture and in all cases has given results indistinguishable from those obtained by the partition chromatographic method (all within 0·001% of sample; n = 3 or 5) for samples of fluocinolone acetonide ointment 0·01 and 0·025%, fluocinolone acentonide + clioquinol ointment 0·025%, fluocinolide acetonide + neomycin sulphate ointment, fluocinolone acetonide cream 0·025% and fluocinolone acetonide acetate 0·05%.

The method is unaffected by the presence of methyl and propyl hydroxybenzoates, neomycin sulphate and iodochlorohydroxyquinoline (clioquinol). The degradation products resulting from oxidation of the 11$\beta$-hydroxy group or oxidation of 21-OH group to 21 COOH or rearrangement of the side chain are all separated in this system.

With slight variation in the aqueous : methanol ratio the method has been applied to samples of other anti-inflammatory corticosteroids including betamethasone 17-valerate, hydrocortisone, hydrocortisone acetate, prednisolone, and prednisolone acetate.

The advantages of the method are the saving in time—6 min compared to 3 h by conventional partition chromatography—and in solvents—eluting phase, aqueous methanol in the high pressure system, compared with a litre of spectrosol n-hexane per assay by partition chromatography.

## REFERENCES

BAILEY, F., HOLBROOK, A. & MILLER, R. J. (1966). *J. Pharm. Pharmac.*, **18**, Suppl. 12S–16S.
FELTON, H. (1969). *J. chromatog. Sci.*, **7**, 13–16.