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The determination of fluocinolone acetonide in formulated products

F. BAILEY, A. HOLBROOK AND R. J. MILLER

A chromatographic procedure for the determination of fluocinolone acetonide is described. The method employs a hexane : dioxan : water partition system supported on Celite. The progress of the chromatogram is followed by measurement of the ultraviolet absorption of eluate fractions at 238 mμ. The application of the method to the determination of fluocinolone acetonide in cream, ointment and lotion formulations is described.

SYNTHETIC corticosteroids containing both a dihydroxyacetone side-chain and an α,β-unsaturated carbonyl function have been in use for many years for the treatment of inflammatory conditions. Published procedures (B.P. 1958; Mader & Buck, 1952; Umberger, 1955) for the determination of these substances in creams, lotions and ointments have depended almost exclusively on either the reducing properties of the former grouping or the chromophoric nature of the latter. The high activity of fluocinolone acetonide enables the use of preparations containing but a fraction of the steroid content of those formerly employed and difficulty in applying established corticosteroid assay techniques was encountered. This is due in part to complexity of formulation, and in certain instances to the presence of antibiotics.

Experimental

Preliminary experiments confirmed that techniques involving oxidation of the dihydroxy acetone side-chain by tetrazolium salts (Mader & Buck, 1952), ultraviolet light absorption or polarographic measurement (Kolthoff & Lingane, 1952) of the α,β-unsaturated carbonyl function could be applied to the parent compound whereas methods based on condensation with isonicotinic acid hydrazide (Umberger, 1955) failed to give stoichiometric results.

All the above methods suffered severe interference from excipients when applied to cream, lotion and ointment formulations, and classical solvent extraction methods failed to provide extracts sufficiently free from interfering material for any to be applied with success.

It was established, however, that fluocinolone acetonide was adsorbed from chloroform solution by both activated alumina and silica gel. Elution from the former was accomplished only by using the most polar solvents, and recovery of the steroid was never complete. The latter adsorbent was suitable only for extracts from the simplest formulation, as the degree of adsorption was markedly influenced by traces of extracted dispersing agent. A partition system similar to that used for triamcinolone (Smith, Foell, de Maio & Halwer, 1959) was therefore explored. An equilibrated mixture of hexane - dioxan - water (100:40:5) was used

From the Pharmaceutical Department, Pharmaceuticals Division, Imperial Chemical Industries Limited, Macclesfield, Cheshire.

as a solvent; the lower layer incorporated with Celite formed the stationary phase, and the upper layer was used as developing solvent. The progress of the chromatogram was followed by measuring the absorbance at 238 mμ* of successive fractions of column eluate. The relationship between absorbance and volume of eluate for a purified sample of fluocinolone acetonide is illustrated in Fig. 1.

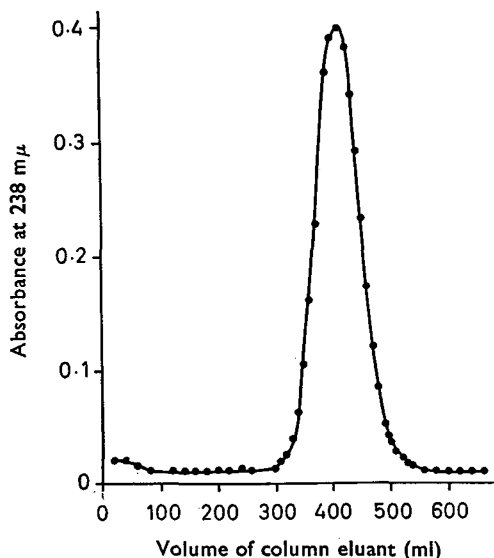

FIG. 1. Curve relating absorbance with volume of eluate for pure fluocinolone acetonide.

METHOD

Reagents: Prepared Celite. Stir Celite 545 (500 g) intermittently for 12 hr with concentrated hydrochloric acid (2 litres). Decant the bulk of the hydrochloric acid, and suspend the residue in about 1 litre of water. Filter through a Buchner funnel and wash with water until free from acid. Wash with methanol (500 ml) and finally with methanol-ethyl acetate (1 : 1) (1 litre). Dry in an oven at 100° and transfer to well stoppered jars. *Hexane.* Commercial samples of n-hexane, b.p. 67–69°, having an absorbance against air of 0·7 or less at 238 mμ do not normally require pretreatment. Poor samples can be rendered satisfactory by oleum washing followed by distillation.

Dioxan. Analar grade material has always been found satisfactory but certain other selected batches of less expensive material have proved an acceptable substitute.

* This represents the wavelength of maximum absorption of fluocinolone acetonide in eluent phase.

PREPARATION OF SOLVENT SYSTEM

Shake together n-hexane (1000 ml), dioxan (400 ml), water (50 ml) and allow to separate. The upper layer is the eluent phase, the lower layer the stationary phase.

PREPARATION OF SAMPLE

(1) *Cream and lotion* 0·025%. Transfer an accurately weighed quantity of sample (about 10 g to a 250 ml separating funnel with methanol (50 ml). Add cyclohexane (100 ml) and shake vigorously (3 min). Transfer the lower layer to a 500 ml separating funnel containing water (140 ml), add chloroform (100 ml) and shake vigorously (3 min). Filter the lower layer through a No. 1 Whatman filter paper and evaporate 50·0 ml of the filtrate to dryness on a steam bath in a current of air. Dissolve the residue in stationary phase (1 ml) and reserve for the chromatographic stage.

(2) *Ointment* 0·025%. Proceed as described above using methanol - water (5 : 1) (60 ml) in place of methanol (50 ml) in the primary extraction stage.

(3) *Cream, lotion and ointment* 0·01%. Take 12–14 g sample and proceed as described under appropriate section above, using a 75·0 ml aliquot of the final chloroform filtrate.

(4) *Cream, lotion and ointment* 0·025% *and* 0·01% *containing neomycin sulphate*. Carry out the preliminary partition between cyclohexane and aqueous methanol as described under the appropriate fluocinolone acetonide preparation. Transfer the lower (aqueous methanol) layer as completely as possible to a 500 ml separating funnel taking care to exclude any flocculent precipitate of neomycin sulphate present at the interface. Add methanol - water (4 : 1) (25 ml) to the residual cyclohexane layer and shake for 3 min. Combine the lower layers, add water (215 ml), chloroform (100 ml) and proceed as described under the appropriate preparation without neomycin sulphate.

PREPARATION OF STANDARD

Dissolve an accurately weighed quantity of pure fluocinolone acetonide (about 20 mg) in chloroform (100 ml.)

PREPARATION AND STANDARDISATION OF CHROMATOGRAPHIC COLUMN

Mix prepared Celite (15 g) with stationary phase (7·5 ml) and pack into a chromatographic column (80 cm in length, 2·2 cm internal diameter, fitted with a sinter plate and a bottom glass tap) in portions of about 3 g: pack down firmly with a tamper between each addition. Evaporate standard fluocinolone acetonide solution 5 ml, to a dryness in a current of air. Dissolve the residue in stationary phase (1·0 ml), add prepared Celite (2 g), mix thoroughly and pack onto the top of the stationary phase. Complete the transfer of any material remaining with the aid of 50 ml of eluent phase, ensuring the minimum disturbance of the column packing. Add eluent phase carefully to the column to a depth of about 50 cm and maintain this level throughout the chromatogram, adjust the

flow rate to 8–10 ml of eluent per min and collect 60 successive fractions of eluate. Measure the absorbance at 238 mμ of each fraction against eluent phase in the reference cell.

Chromatography of sample. Add prepared Celite (2 g) to the sample residue dissolved in stationary phase (1 ml) and pack on a fresh stationary phase. Complete the chromatogram in the manner previously described.

The fluocinolone acetonide content of the sample $= \frac{\text{Ea} \times \text{Ws} \times 100}{\text{Es} \times \text{Wa} \times \text{V}} \times 100\%$ where Ea and Es are the sums of absorbance values under the sample and standard peaks respectively, after correction for any base line blank values. Wa = weight of sample (mg); Ws = weight of standard fluocinolone acetonide (mg) applied to column; V = volume of chloroform extract (m) evaporated to dryness before application to column.

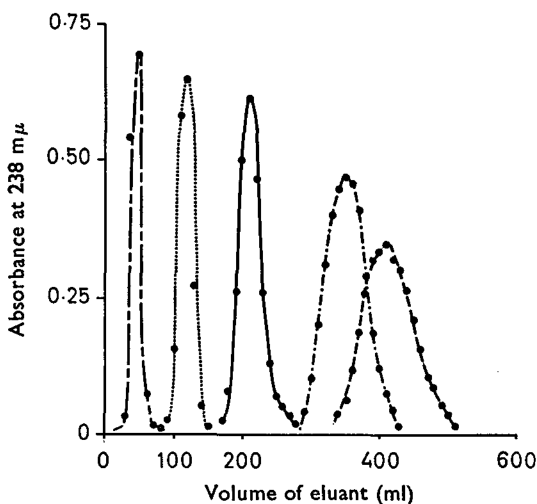

FIG. 2. Curves relating absorbance volume for fluocinolone acetonide and other currently available corticosteroids. – – — Betamethasone 17-valerate. · · · · Hydrocortisone acetate. ——— Prednisolone acetate. –·–·–·– Triamcinolone acetonide. — — — — Fluocinolone acetonide.

Results and discussion

Preservatives such as *p*-hydroxybenzoic and gallic acid esters or dispersing agents of the anionic and non-ionic variety do not interfere with the proposed assay for fluocinolone acetonide. Likewise the degradation products resulting from oxidation of the 11β-hydroxy group or rearrangement of the C(17) side-chain were without effect.

The method has been applied to samples of other anti-inflammatory corticosteroids including betamethasone 17-valerate, hydrocortisone, hydrocortisone acetate, prednisolone and prednisolone acetate and the plot of extinction at 238 mμ versus eluate volume is illustrated in Fig 2. With the exception of hydrocortisone and prednisone all the examples cited are

eluted in the first 600 ml and the degree of separation is such that confusion between individual members is unlikely. The retention volume of individual members can readily be adjusted to an optimum by variation of the dioxan to water ratio of the solvent system; this is particularly necessary for the two less polar members of the group. The system hexane-dioxan-methanol-water (100:30:5:5) gives a retention volume of 200 ml and has been used successfully for the analysis of betamethasone 17-valerate formulations after identical pretreatment to that described for the appropriate fluocinolone acetonide preparation.

TABLE 1. RESULTS OBTAINED BY THE PROPOSED METHOD ON LABORATORY PREPARED FLUOCINOLONE ACETONIDE FORMULATIONS

Sample		Fluocinolone acetonide % w/w		Sample		Fluocinolone acetonide % w/w	
		Added	Found			Added	Found
Fluocinolone	1.	0·025	0·025 0·025	Fluocinolone	16.	0·024	0·024 0·025 0·024
acetonide lotion	2.	0·024	0·024 0·024	acetonide cream	17.	0·025	0·025 0·025 0·025
	3.	0·024	0·024 0·024	with neomycin	18.	0·025	0·024
	4.	0·010	0·010 0·009		19.	0·025	0·025
	5.	0·010	0·010 0·010		20.	0·010	0·011
Fluocinolone	6.	0·025	0·024 0·025		21	0·009	01·00
acetonide lotion	7.	0·023	0·023		22.	0·009	0·009
with neomycin	8.	0·025	0·025 0·025	Fluocinolone	23.	0·025	0·024 0·024
	9.	0·024	0·025	acetonide ointment	24.	0·025	0·025 0·023
	10.	0·024	0·024		25.	0·025	0·025
	11.	0·011	0·011		26.	0·024	0·023
	12.	0·010	0·009		27.	0·010	0·010
Fluocinolone	13	0·024	0·024 0·024	Fluocinolone	28.	0·024	0·024
acetonide cream	14.	0·025	0·024	acetonide ointment	29.	0·024	0·023 0·023
	15.	0·025	0·025	with neomycin	30.	0·025	0·023 0·025

The proposed method has been applied to a comprehensive range of accurately compounded samples and the results given in Table 1 demonstrate the wide applicability of the procedure.

The technique incorporates a high degree of specificity and is equally suitable for the examination of samples from both routine manufacture and stability study experiments.

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