J. Pharm. Pharmac., 1971, **23**, *Suppl.*, 176S–180S *Received December* 18, 1970

A polarographic method for the determination of flurandrenolone

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A simple fast scan polarographic method has been developed for the determination of the Δ^4-3 ketosteroid flurandrenolone in pharmaceutical formulations. The polarographic peak current due to steroid reduction is measured after ointments have been subjected to a preliminary extraction procedure and creams have been treated with tannic acid to precipitate interfering excipients. The method enables concentrations down to 0·01% w/w of the steroid to be measured. Neomycin sulphate does not interfere and clioquinol is readily removed using an ion exchange resin. Evidence is presented to show that the carbon-fluorine bond rather than the enone is primarily reduced. The method is faster than a colorimetric method using tetrazolium blue and has a similar accuracy and precision.

Flurandrenolone, 6α-fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione-16,17-acetonide is used for the topical treatment of local inflammatory conditions and because of the low concentrations employed in formulations, previously described analytical methods meet problems owing to interference from excipients. Lengthy extraction or chromatographic procedures are often necessary to remove this interference (Jakovljevic, Hartsaw & Drummond, 1965; Bailey, Holbrook & Miller, 1966; Görög, 1968).

The polarographic behaviour of Δ^4-3-ketosteroids has been well documented (Milner, 1957; Brezina & Zuman, 1958; Kabasakalian & McGlotten, 1962; Cohen, 1963; Zuman, 1967), but there are few references to the use of this technique in the quantitative analysis of formulated products. Gantés & Juhasz (1966) have described a polarographic method for the determination of hydrocortisone in ointments, but their method applies only to concentrations much in excess of those likely to be encountered using fluorinated steroids.

MATERIALS AND METHODS

Apparatus

Because of the low levels of fluorinated steroid involved, a cathode ray polarograph type A1660 manufactured by Southern Analytical Ltd., Camberley, Surrey, was used. Two synchronized dropping mercury electrodes (subtractive polarography) were found necessary for the determination of flurandrenolone in creams. In all cases measurements were made with a mercury pool as the reference electrode.

Reagents

Unless otherwise stated, all chemicals are of analytical reagent grade.

n-Heptane—M & B laboratory reagent. 50% v/v aqueous methanol containing 0·1% v/v concentrated hydrochloric acid. 50% v/v aqueous methanol containing 10% tannic acid (B.D.H. laboratory reagent).

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Supporting electrolyte. 50% aqueous methanol 0·5M with respect to potassium chloride and M with respect to hydrochloric acid.

Stock standard solution. Accurately weigh about 50 mg of flurandrenolone analytical standard and dissolve in 50·0 ml of methanol.

Working standards 1 and 2. Solutions containing 0·1 mg and 0·2 mg/ml of flurandrenolone (analytical standard) in 50% v/v aqueous methanol.

Preparation of samples for polarography

Ointments containing 0·0125% w/w of flurandrenolone. Disperse the ointment (4·0 g) in n-heptane at about 40° and extract the flurandrenolone with 20 ml, then 15 ml and finally 5 ml methanol-hydrochloric acid (see reagents): add supporting electrolyte (5·0 ml) and dilute to 50·0 ml with methanol-hydrochloric acid.

To 20·0 ml of the above solution add working standard 1 (2·0 ml).

Ointments containing 0·05% w/w of flurandrenolone. These are prepared as above but a 2·0 g sample of ointment is used and working standard 2 (2·0 ml) added to the final extract.

Creams containing 0·0125% w/w flurandrenolone. Dissolve 8·0 g of cream sample in a stoppered erlenmeyer flask containing methanol (25 ml) at 50°, add water (20 ml) mix well and cool to room temperature. Add tannic acid solution (10 ml) and shake vigorously (1 min). Add celite 545 (filter aid) (4 g) and again shake then filter the sample through a porosity 3 sintered funnel (low vacuum) into a 100 ml volumetric flask containing supporting electrolyte (10·0 ml). Wash through and dilute to volume with several small amounts of 50% v/v aqueous methanol.

Using 8·0 g of cream sample without steroid prepare a blank solution in an identical manner.

To the sample solution (20 ml), add 2·0 ml of working standard 1. This is the sample plus standard solution.

Creams containing 0·05% w/w flurandrenolone. The method is as for the 0·0125% cream but only 4·0 g of sample and blank are used with working standard 2 (2·0 ml).

Polarography of samples

Ointments. Polarograph the solutions of sample alone and sample plus standard at 20 ± 0·1° and determine the flurandrenolone peak currents at about −0·9 V for both solutions. All solutions should be degassed with oxygen-free nitrogen (saturated with 50% aqueous methanol).

Creams. Place the sample solution and blank solution under each of two synchronized dropping mercury electrodes. Polarograph and determine the flurandrenolone peak current at about −1·0 V with the instrument in the subtractive mode. Determine the sample plus standard peak current in the same manner.

$$\% \text{ flurandrenolone} = \frac{\text{Pa}}{1{\cdot}1\ \text{Pas} - \text{Pa}} \times \frac{\text{Ws}}{\text{Wa}} \times \text{F}$$

Where Pa = peak current of sample; Pas = peak current of sample plus standard; Ws = weight of standard (mg) in 50 ml of stock standard; Wa = weight of sample (mg); F = dilution factor = 1 for 0·0125% ointments, 2 for 0·0125% creams and 0·05% ointments and 4 for 0·05% creams.

RESULTS

The results obtained using the polarographic procedures for the analysis of cream and ointment samples, together with comparative results obtained by the colorimetric method using tetrazolium blue are summarized in Table 1.

Table 1. *Results for the analysis of cream and ointment samples using the polarographic and colorimetric assays.*

Flurandrenolone content claimed (mg/g)	No. of determinations	Flurandrenolone found (mg/g)* Polarographic assay	Colorimetric assay
Ointment (0·5)	12	0·515 ± 0·005	0·517 ± 0·010
Ointment (0·125)	12	0·130 ± 0·003	0·127 ± 0·005
Cream (0·5)	9	0·482 ± 0·007	0·484 ± 0·008
Cream (0·125)	12	0·125 ± 0·004	0·125 ± 0·003

* Average ± standard deviation.

DISCUSSION

Cathode ray polarography shows that flurandrenolone in 50% aqueous methanol is reduced in a variety of buffered systems having pH values within the range of 1–11. In acid solutions the major reduction peak is sharp and well defined but becomes broader at higher pH values indicating a slower reduction. The most satisfactory polarograms are obtained at pH 1 using a supporting electrolyte of potassium chloride and hydrochloric acid. At this pH, the flurandrenolone peak occurs at −0·79 V versus the mercury pool anode and is most probably due to reduction of the fluorine atom rather than to the enone system in ring A. This was shown by controlled potential electrolysis (−0·8 V for 48 h) after which the flurandrenolone solution still retained the typical absorption of an $\alpha\beta$-unsaturated ketone in λ_{max} from 238 nm to 242 nm The infrared spectrum (KBr disc) of the residue obtained from this experiment shows little change in the carbonyl stretching region (1700 cm^{-1}) but an absence of the C–F stretching frequency present in flurandrenolone itself. Reduction of the carbonyl in addition to the C–F group can be accomplished by working with a more negative potential (−1·22 V) at which a second poorly defined reduction peak occurs. At this potential a large drop in absorbance of the ≈240 nm band is observed.

Calibration graphs of flurandrenolone at concentrations from 0 to 100 ppm against peak current on the cathode ray tube show deviation from linearity above 20 ppm. This has been tentatively ascribed to adsorption of the flurandrenolone on the surface of the mercury cathode drop (McIver & Rooney, 1962). Such adsorption effects, common in organic polarography, can be prevented by the addition of a small amount of a non-ionic surface-active agent to the solution to be electrolysed.

In flurandrenolone ointments which do not contain surfactants, therefore, the polarographic peak current at −0·79 V for such ointment samples is best compared directly with that of a standard in the region of linearity (curve A, Fig. 1).

On the other hand, where creams and ointments do contain non-ionic surface-active agents, calibration graphs show linearity up to at least 100 ppm of flurandrenolone. Some of the surface active agent is carried through in the method of analysis to the final solution to be electrolysed and probably prevents adsorption

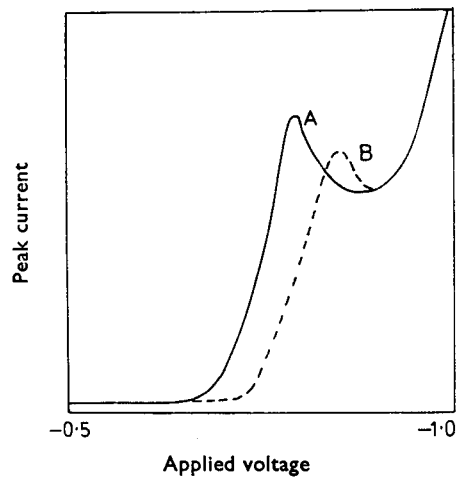

FIG. 1. Polarographic peak displayed by flurandrenolone after extraction from an ointment not containing surface-active agents (curve A), and one containing surface-active agents (curve B).

effects from taking place. In ointments of this type, however, the presence of non-ionic surface active agent, and possibly other excipient, in the final solution to be electrolysed, results in a shift of peak potential for flurandrenolone from −0·79 V to approximately −0·88 V. As no adsorption of flurandrenolone occurs on the mercury drop surface, a reduction in peak current is also apparent (curve B, Fig. 1). These two effects make the direct comparison of sample and standard peak current values impossible and the technique of standard addition to the sample may be adopted.

The non-ionic surface active agents (and possibly other excipients) in creams, which remain in solution after tannic acid precipitation, shift the peak potential for flurandrenolone very close to that of the hydrogen ion reduction wave (Fig. 2, curve

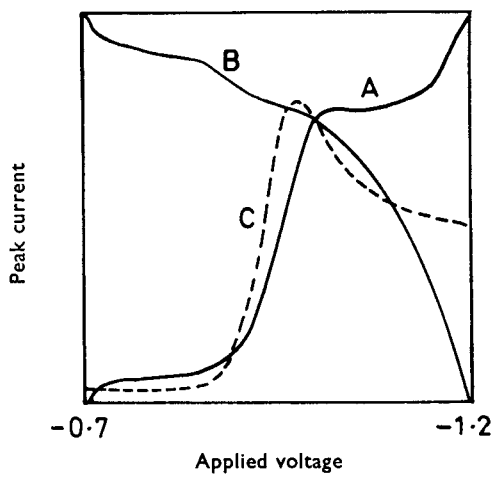

FIG. 2. Curve A—single wave displayed for flurandrenolone after extraction from cream excipients. Curve B—inverted single cell wave displayed by a solution resulting from treating a blank cream in the same manner as the sample. Curve C—the resultant wave obtained by subtraction of B from A.

A). Although the flurandrenolone peak and hydrogen wave can be resolved using derivative polarography, great loss in instrument sensitivity occurs and a more satisfactory result is achieved using subtractive polarography. Fig. 2, curve C shows the resultant wave which is the subtraction of the wave for the blank solution (Fig. 2, curve B) from the wave for the sample solution. Standard readings for creams are obtained using the technique of standard addition to the sample.

Neomycin sulphate which may be present in some formulations only gives a reduction wave in 50% v/v aqueous methanol when the pH is greater than 3. Consequently, no interference occurs due to the presence of this antibiotic under the conditions for determining flurandrenolone.

Clioquinol gives a reduction peak on the polarogram which completely masks that of flurandrenolone. The interference can be overcome by removal of the clioquinol from 50% v/v aqueous methanol solutions using Dowex 50W-X12 (a sulphonated ion exchange resin).

Comparison of the polarographic with the colorimetric assay (tetrazolium blue) shows that the accuracy and precision is similar in both (Table 1). The main advantage of the polarographic method lies in the speed at which a particular determination can be made. This makes it particularly suitable for the rapid checking of content and homogeneity of production samples.

Acknowledgements

The authors thank Mr. R. Rooney and Mr. C. Pratt of Southern Analytical for their helpful discussions and technical advice. They also wish to thank Mr. D. Tompkins for technical assistance.

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