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ANALYSIS OF HYDROCORTISONE ACETATE OINTMENTS AND CREAMS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

High-performance liquid chromatographic (HPLC) methods for the analysis of hydrocortisone containing ointments and creams have been investigated. A method which uses a silica column and involves a minimum of sample pre-treatment has been shown to compare favourably with the triphenyltetrazo. ım chloride method of the British Pharmacopoeia. For hydrocortisone ointments the HPLC procedure provides results of equivalent precision and has advantages with respect to the time taken for each analysis and specificity. Application of the method to the analysis of hydrocortisone creams has been explored and the deviation between the HPLC and colorimetric method requires further investigation.

INTRODUCTION

Hydrocortisone acetate is used as an anti-inflammatory agent in topical formulations. The present pharmacopoeial methods of assay for hydrocortisone acetate creams and ointments are of limited specificity and depend on the oxidation of the α-ketol side chain by either triphenyltetrazolium chloride or blue tetrazolium. Base concentration, water, air, solvent and light are known[1] to interfere with the colour formation in the tetrazolium reaction. Graham *et al.*[2] have shown that many excipients interfere with the colour formation including sorbitan monostearate, sorbitan monooleate, lanolin, polysorbate 60 and stearic acid.

The authors[2] also quote the concentration of excipient necessary to give a 4% level of interference. The present official methods in Australia for hydrocortisone ointments and creams are the requirements of the British Pharmacopoeia (B.P.)[3] and the British Pharmaceutical Codex,[4] respectively. Both these methods involve the formation of the red tetrazolium formazan. The work reported here was initiated as a result of problems with the official method in respect to the selectivity, reproducibility, time of analysis and the necessity for extreme caution when carrying out the assays.

Several workers[5–9] have published papers on the high-performance liquid

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chromatography (HPLC) of corticosteroids in topical creams and ointments. Olsen[10] described a method using reversed-phase chromatography on a cyanoethylsilicone column as well as a bonded octadecylsilane column. The mobile phase used in both cases was methanol–water (1:99) while the injecting solvent was ethanol. It is felt, after reviewing our own difficulties with the reversed-phase method, that these conditions could lead to precipitation of excipients in the injector with subsequent inlet filter blockages resulting. The authors experienced some difficulty with interference from excipients in the formulations examined. The behaviour of C_{18}-bonded phase systems when using injecting solvents which are markedly different from the eluting phase has been investigated by Tseng and Rogers[11] who found considerable peak distortion and observed the appearance of distinct shoulders in some instances.

A method for flumethasone pivalate was developed by Mollica and Strusz[12] using a liquid–liquid system with β,β-oxydipropionitrile on Zipax and this method was applied to the analysis of hydrocortisone creams. The method of extraction was similar to the method used by the B.P. tetrazolium method except the B.P. extraction is performed three additional times using twice as much solvent on each occasion.

Korpi *et al.*[13] adopted an ion-paired reversed-phase approach to the analysis of mixtures of hydrocortisone and hydrocorisone phosphate. A reversed-phase HPLC procedure has been reported[14] for the determination of hydrocortisone hemisuccinate together with hydrocotrisone and a favourable comparison with the United States Pharmacopoeia blue tetrazolium method obtained. Cavina *et al.*[15] employed a silica column with methanol gradients in chloroform to separate complex corticosteroid mixes in biological matrices. It was felt that the comparatively simple pharmaceutical matrices dealt with in this study could be adequately handled by an isocratic mobile phase. In addition the choice of an optically clear mobile phase was seen to offer a flexibility advantage should it be found necessary to analyse low concentration samples by using short wavelength UV detection.

Our laboratory had developed a reversed-phase method for the analysis of a number of corticosteroids in topical formulations. Consequently the applicability of this method for the analysis of hydrocortisone acetate creams and ointments was investigated. However, when this method was attempted quantitatively, non-reproducible data resulted. This problem showed up as non-repetitive integrals for the steroid and internal standards. The peaks, when broadened by a much faster chart speed, could not be superimposed upon each other. This effect was due in most part to fouling of the column inlet frit, possibly from precipitation of excipients in the injector. The silica gel method described below was adopted for this survey as it possessed the advantage of being compatible with the sample matrix, the paraffin base being readily eluted from the column. Details of the reversed-phase method are included for comparison.

EXPERIMENTAL

Materials

Hydrocortisone acetate was obtained from Ascot Pharmaceuticals (North Sydney, Australia) and hydrocortisone was obtained from Sigma (St. Louis, MO, U.S.A.). Chloroform and methanol were analytical reagent grade (Merck, Darmstadt, G.F.R.) and the isopropanol and cyclohexane were of spectroscopy grade (Ajax

Chemicals, Sydney, Australia). The triphenyltetrazolium chloride and the tetramethylammonium hydroxide were both obtained from BDH (Poole, Great Britain). Water used for the HPLC mobile phase was freshly distilled.

Apparatus

A Varian 4100 Series high-performance liquid chromatograph was used for this survey. The injector used was an Altex high-pressure loop injector fitted with 20-μl loop. The detector was a Varian 635D spectrophotometer which was equipped with an 8-μl flow cell, the wavelength used throughout this study was 240 nm. A Hewlett-Packard 3370A digital integrator was used to determine the peak areas and also attenuated the signal to the Varian A25 recorder.

The columns used in the survey were a 25 cm × 2 mm. Varian SI-10 column and a 30 cm × 3.9 mm μBondapak C_{18} column (Waters Assoc.).

Sampling

The hydrocortisone creams and ointments were each assayed six times using HPLC, two consecutive samples from each of three tubes were taken. The tetrazolium assays were performed four times by taking two consecutive samples from each of two tubes.

Steroid assay procedure by adsorption chromatography

Hydrocortisone acetate ointments. Weigh accurately 0.5 g ointment into a 25-ml conical flask and add 10 ml chloroform. Transfer this solution with washing into a 25-ml volumetric flask and make up to volume with chloroform. Inject 20 μl onto the column.

Hydrocortisone acetate creams. Weigh accurately 0.5 g into a 25-ml conical flask and place in a vacuum desiccator which contains silica gel. Evacuate and then heat the dessicator to 60°C for 2–4 h. Allow the desiccator and contents to cool and then dissolve the residue in 10 ml of chloroform. Quantitatively transfer the chloroform with the aid of washing into a 25-ml volumetric flask and make up to volume with chloroform. Inject 20 μl onto the column.

Chromatography conditions. Column, Varian SI-10 25 cm × 2 mm; mobile phase, isopropanol–cyclohexane (1:9); flow-rate, 60 ml/h; pressure, 50 kg/cm^2.

Steroid assays by reversed-phase chromatography. Weigh accurately a quantity of sample equivalent to 10 mg of active ingredient, into a separating funnel. Add 20 ml warm methanol–water (4:1) and shake vigorously. Extract the aqueous methanol with 20 ml *n*-hexane; then remove the aqueous methanol and extract the hexane with a further 2 × 10 ml methanol–water (4:1). Combine the aqueous extracts and make up to 50 ml with methanol–water (4:1) and inject 20 μl.

Chromatography conditions. Column 30 cm × 3.9 mm. μBondapak C_{18} (Waters Assoc.); mobile phase, methanol–water (7:3); flow-rate, 60 ml/h; pressure 150 kg/cm^2.

Colorimetric method of assay. The hydrocorisone acetate ointments and creams were assayed in accordance with the B.P. method. Precautions were taken to prevent any interference from light and air.

RESULTS AND DISCUSSION

The assay method required a total of 15 min per sample, including sample preparation. Throughout the study no appreciable change in activity of the silica column was observed. A typical chromatogram of an ointment extract is shown in Fig. 1.

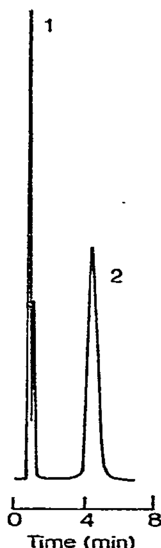

Fig. 1. Analysis of hydrocortisone cream. 1 = Chloroform and excipients, 2 = hydrocortisone acetate.

The results obtained for the hydrocortisone acetate ointments are shown in Tables I and II. The results shown in Table I are the means obtained (left hand figure) with the standard deviation alongside. The figure on the extreme right is the difference between the means obtained. The results shown in Table II are the assay figures obtained and arranged so that each pair represents the replicate results from each tube.

The standard deviations are high in some instances for both methods. This was in many cases because of irregular assay results as demonstrated by the data for sample K, Table II. As these irregular results can be found in the data from both assay procedures, the implication is that these results reflect homogeneity problems in the sample. The difference between the two methods as shown in Table I appear to be randomly distributed about zero difference. Analysis of these differences gives a mean difference of —0.623 with a standard deviation of 2.99. By examining all the results obtained by both methods the colorimetric methods was found to have a standard error of 2.3% while the HPLC method possessed a standard error of 2.0% and no significant difference between the methods was detected ($F_{1,12} = 0.6$).

The results in Table III for the hydrocortisone acetate creams show a marked bias, the HPLC method giving consistently lower results. The standard deviations for the results by HPLC are generally lower than those for the results obtained by the tetrazolium method. Statistical analysis indicates that significant differences

TABLE I

ASSAY OF HYDROCORTISONE ACETATE OINTMENTS

Results are given in percentages ± S.D. Each value represents the mean of replicate results. Individual values are given in Table II.

Sample	*HPLC assay (%)*	*Colorimetric assay (%)*	*Difference (%)*
A	95.0 ± 1.2	97.1 ± 2.2	−2.1
B	91.3 ± 2.3	93.9 ± 0.8	−2.6
C	98.7 ± 3.3	102.5 ± 3.0	−3.8
D	98.0 ± 5.2	92.9 ± 1.8	+5.1
E	101.3 ± 4.0	99.7 ± 0.6	+1.6
F	96.9 ± 0.8	100.3 ± 2.5	−3.4
G	100.7 ± 1.0	95.9 ± 2.0	+4.8
H	101.6 ± 1.4	105.3 ± 1.9	−3.7
I	98.2 ± 1.3	100.5 ± 2.5	−2.3
J	98.3 ± 0.5	98.5 ± 1.4	−0.2
K	98.9 ± 3.6	98.3 ± 3.9	+0.6
L	98.4 ± 1.3	98.3 ± 1.3	+0.1
M	99.8 ± 1.2	102.0 ± 2.4	−2.2

exist between the two sets of results. The possibility that the lower HPLC results were derived from the dehydration step used in the sample preparation was investigated by using the reversed-phase separation. The reversed-phase system was chosen as the

TABLE II

ASSAY OF HYDROCORTISONE ACETATE OINTMENTS

Sample	*Colorimetric assay (%)*	*HPLC method (%)*
A	97.3, 99.9	93.0, 94.1, 95.2
	94.8, 96.2	96.1, 95.9, 95.2
B	92.9, 94.4	87.9, 90.6, 91.9
	94.6, 94.0	89.9, 92.9, 94.4
C	106.0, 100.0	97.8, 104.3, 94.8
	104.7, 100.0	98.4, 100.1, 96.7
D	95.3, 91.1	93.9, 94.1, 96.1
	92.3, 92.7	95.2, 97.6, —
E	100.6, 99.4	98.1, 99.4, 103.9
	100.0, 99.6	98.6, 99.4, —
F	97.0, 103.0	97.0, 98.4, 96.2
	101.0, 100.0	96.5, 96.9, 96.3
G	98.8, 95.0	100.7, 99.0, 101.2
	94.5, 95.0	100.1, 101.4, 101.9
H	108.0, 103.7	103.3, 100.1, 100.1
	104.7, 104.6	102.4, 102.7, 100.9
I	103.0, 101.0	98.6, 97.4, 99.0
	97.0, 101.0	100.2, 97.1, 97.1
J	97.3, 99.1	97.6, 98.7, 99.0
	100.3, 97.4	98.7, 97.7, 97.1
K	95.0, 97.0	105.7, 96.6, 95.6
	97.0, 104.6	99.1, 97.0, 99.1
L	97.0, 98.0	96.3, 98.0, 98.4
	100.0, 98.0	98.3, 99.6, 100.0
M	102.0, 99.0	98.0, 101.8, 100.0
	105.0, 102.0	100.0, 95.5, 99.3

hydrolysis product hydrocortisone elutes before hydrocortisone acetate thus allowing ready quantitation. In the adsorption system the hydrocortisone eluted as a broad diffuse peak after the hydrocortisone acetate and presented problems in terms of quantitation.

TABLE II

ASSAY OF HYDROCORTISONE AND HYDROCORTISONE ACETATE CREAMS

Sample	*HPLC assay (%)*	*Colorimetric assay (%)*	*Difference (%)*
A	97.9 ± 1.3	103 5 ± 1.7	−5.6
B	99.0 ± 1.1	103.2 ± 5.6	−4.2
C	95.3 ± 1.2	102.5 ± 1.0	−7.2
D	92.4 ± 3.8	103.7 ± 3.3	−11.3
E	94.8 ± 2.7	94.6 ± 2.6	+0.2
F	99.4 ± 0.7	92.3 ± 3.4	+7.1
G	93.4 ± 2.5	98.2 ± 0.9	−4.8
H	99.8 ± 1.9	104.8 ± 2.3	−5.0
I	115.3 ± 0.8	114.1 ± 1.8	+1.2
J	100.3 ± 0.8	99.3 ± 1.5	+1.0

Three samples C, D and F were analysed by reversed phase both before and after the drying step. No decomposition was observed. The chromatograms of Sample D showed the presence of approximately 10% hydrocortisone in both the dried and undried samples. Whilst in this case the HPLC assay could be expected to

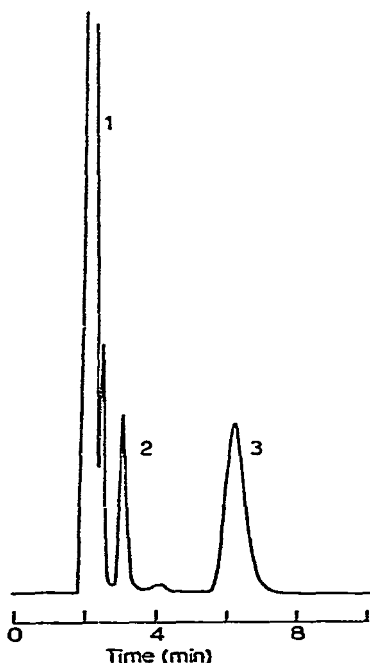

Fig. 2. Analysis of hydrocortisone acetate ointment with internal standard. 1 = Excipients and chloroform, 2 = dienestrol (internal standard), 3 = hydrocortisone acetate.

yield a lower assay than the colorimetric method (as the hydrocortisone would not be quantitated), no such explanation was available for the other samples.

The UV spectrum of one of the cream samples was recorded by using a stop-flow technique and the spectra compared with a standard similarly treated. No discernable spectral difference and hence interference was noted.

In conclusion the HPLC method for determining hydrocortisone acetate in ointment base is accurate and possesses the advantage of very rapid sample preparation coupled with a robust chromatographic quantitation. Another advantage of the adsorption method is that the spent mobile phase can be distilled to give an eluent that results in slightly larger elution volumes than the original. Throughout the study described the reproducibility of injection obtained was sufficiently high to obviate the use of an internal standard. However as the method is thought to be suitable for wider application, an internal standard was sought to improve the versatility of the procedure. Dienestrol has been tried as an internal standard and proved satisfactory, a typical chromatogram is shown in Fig. 2. While the method developed for the analysis of hydrocortisone acetate creams is simple it is time consuming and may be less suitable for use as an in-process control method. The results of the HPLC and colorimetric analysis are at variance and the source of this difference for the creams is currently being investigated.

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