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RAPID METHOD FOR THE SIMULTANEOUS ANALYSIS OF HYDROCORTISONE AND CLIOQUINOL IN TOPICAL PREPARATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Reversed-phase high performance liquid chromatographic methods for the analysis of ointments containing hydrocortisone and clioquinol have been investigated. A successful method using a C_{18} column and methanol–0.05 *M* phosphoric acid (80:20) as eluting solvent has been developed which allows both compounds to be determined simultaneously. The high-performance liquid chromatographic procedure is rapid and sensitive whereas the assay described in the 1980 BP involves a different method for the analysis of each component of the ointment. The method has also been further applied to the analysis of ointments containing hydrocortisone combined with other halogenated hydroxyquinolines.

INTRODUCTION

The use of the anti-inflammatory drug hydrocortisone together with the antibacterial compound clioquinol (5-chloro-7-iodo-8-quinolinol) is a well recognised regime for the treatment of skin disorders. It has been demonstrated in a number of studies that the combined use of these two drugs is more effective in treating patients with bacterial or fungal infections than either of the components alone[1–3]. Hydrocortisone is also marketed for topical treatment in conjunction with other halogenated hydroxyquinolines, in particular, 5,7-diiodo-8-quinolinol and 5,7-dichloroquinaldol. Most of these preparations contain 1% hydrocortisone and 3% halogenated hydroxyquinoline in an ointment or cream base.

High-performance liquid chromatography (HPLC) has been used by several workers in both adsorption and partition modes to analyse hydrocortisone and its acetate salt in topical preparations. Lea *et al.*[4] described a method using a silica column with isopropanol–cyclohexane as the mobile phase whereas Orr *et al.*[5] successfully used a C_{18}-bonded stationary phase with methanol–water as eluting solvent for the analysis of hydrocortisone ointment. The 1980 BP assay for the quantitative determination of the steroid in hydrocortisone–clioquinol ointment[6] is based on an HPLC method although the assay for hydrocortisone acetate ointment[7] still relies on the oxidation of the α-ketol side chain by a tetrazolium salt.

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In contrast to hydrocortisone there are very few reports on the use of HPLC for the assay of clioquinol in the literature. Electron-capture gas–liquid chromatography has often been used for the determination of halogenated hydroxyquinolines but it necessitates the time-consuming preparation of volatile derivatives[8,9]. A recent report by Ezzedeen *et al.*[10] describes a reversed-phase system for the determination of clioquinol in plasma. This involves using a C_{18}-bonded stationary phase in conjunction with a methanol–phosphoric acid mobile phase and is capable of measuring 1–15 μg/ml in plasma. As well as being very sensitive this assay is rapid and reproducible.

The present study describes the analysis of hydrocortisone and clioquinol-containing ointment using a reversed-phase partition system. The HPLC procedure which involves only a simple extraction step allows both compounds to be assayed simultaneously and can also be used for the analysis of hydrocortisone preparations containing clioquinol congeners.

EXPERIMENTAL

Materials

Hydrocortisone was obtained from Sigma and both clioquinol and Vioform-HC ointment were purchased from Ciba Labs. Chlorquinaldol and 5,7-diiodo-8-quinolinol were supplied by Geigy Pharmaceutical and Searle, respectively. Bromobenzene, 2-methoxyethanol, 2,2,4-trimethylpentane and 5,7-dichloro-8-quinolinol (Aldrich) were all analytical-reagent grade and the methanol was HPLC grade.

The solvents for chromatography were either degassed under vacuum (methanol) or by boiling (water) followed by filtration using a Sartorius 0.4-μm filter.

High-performance liquid chromatography

All HPLC work was carried out with a DuPont 810 pump combined with a fixed volume (20 μl) Rheodyne injection loop and a DuPont variable-wavelength UV detector. The wavelength used to monitor the separation was either 240 nm (λ_{max} for hydrocortisone) or 256 nm (λ_{max} for clioquinol). The reversed-phase columns used in this investigation were a 25 cm × 4 mm I.D. Hypersil-MOS column (Shandon), a 25 cm × 4 mm I.D. Zorbax-ODS column (DuPont) and a 22.5 cm × 4 mm I.D. Hypersil-ODS column (Shandon). The columns were flushed at the end of the day with methanol. Varying concentrations of methanol–water or methanol–0.05 *M* phosphoric acid used as the mobile phase which was run at a flow-rate of 2 ml/min. All chromatography was carried out at ambient temperature.

Standard solutions

Stock solutions of clioquinol and hydrocortisone in methanol at a concentration of 1 mg/ml were prepared daily.

Extraction of clioquinol and hydrocortisone from the ointment

Standard hydrocortisone (1%) and clioquinol (3%) ointment was prepared by dispersing a mixture of the powders in white soft paraffin BP. This was stored at 4°C and used within 3 days.

Extraction of both compounds from either a standard or proprietary preparation of the ointment was carried out by three different methods.

(a) Aliquots (1 g) of ointment (containing 10 mg hydrocortisone and 30 mg clioquinol) were warmed on a water bath with 30 ml of trimethylpentane until the ointment had melted. 10 ml of a 4% solution of internal standard (bromobenzene) in methanol–water (80:20) was added to the contents and the warm mixture was extracted with successive quantities of 30 ml, then 2 × 20 ml of methanol–water (80:20); the extracts were combined, cooled and made up to 100 ml with the same solvent.

(b) Method (a) was repeated using methanol–0.05 *M* phosphoric acid (80:20) to extract.

(c) 80 ml of a hot mixture of methoxyethanol–water (80:20) was added to 1 g of ointment and heated on a water bath with frequent stirring. The mixture was cooled in ice for 10 min to allow the paraffin to solidify, then allowed to warm to room temperature and diluted to 100 ml with the methoxyethanol mixture. The solution was mixed and filtered.

RESULTS AND DISCUSSION

Two chromatographic systems were found that gave satisfactory separation of hydrocortisone, clioquinol and the internal standard bromobenzene. In both cases the eluting solvent was methanol–0.05 *M* phosphoric acid (80:20) and the stationary phase was either a C_8-Hypersil column or a C_{18}-bonded Hypersil column. The method described in the 1980 BP[6] for the analysis of hydrocortisone uses a C_{18} column in conjunction with methanol–water (80:20) as the eluent, however this mobile phase was found to be unsuitable for the present assay as clioquinol was retained on the column.

Typical chromatograms of standard solutions containing hydrocortisone, clioquinol and bromobenzene are shown in Fig. 1. It was found that repeated injections of the ointment extract resulted in reduced efficiency of the C_8 column in system I, this was particularly noticeable with the clioquinol peak. The performance of the column was restored by prolonged flushing with hexane and was thought to be due to retention of the ointment base on the column. The efficiency of the C_{18} column was not altered by repeated injections of the ointment extract therefore this column was chosen as being the most suitable one for the assay.

Two different proprietary makes of ODS packing were tested in this assay; Zorbax-ODS which is a highly retentive reversed-phase packing with a percentage loading of 15% and Hypersil-ODS which is a spherical packing with only 9% of the silica bonded to C_{18} groups. It was found that the ODS packing with the higher percentage loading resulted in excessive retention of the clioquinol peak and therefore in this particular assay it is desirable to use a packing with a relatively low loading of C_{18} on the silica.

The UV absorbance spectrum of hydrocortisone in methanol exhibits a λ_{max} at 240 nm ($E_1^{1\%}{}_{cm} = 435$) whereas that of clioquinol is at 256 nm ($E_1^{1\%}{}_{cm} = 1070$). All the proprietary preparations of the ointment contain more than three times as much clioquinol as hydrocortisone therefore the decreased sensitivity towards clioquinol at 240 nm is compensated for by it being present at a higher concentration in the ointment. The optimum wavelength was therefore chosen to be 240 nm ($E_1^{1\%}{}_{cm}$ for clioquinol = 270).

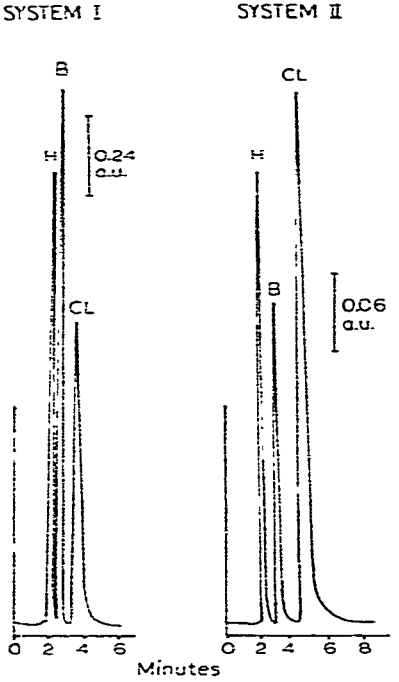

Fig. 1. Chromatograms of a mixture of hydrocortisone (H), clioquinol (CL) and bromobenzene (B). Mobile phase, methanol–0.05 *M* phosphoric acid (80:20) (flow-rate = 2 ml/min). System I: C_8 column; system II: C_{18} column.

The standard curve for hydrocortisone using peak areas is presented in Fig. 2 and that for clioquinol in Fig. 3. Each point on the graph represents the mean, and the bars represent the standard deviation of the mean for three injections. The calibration curves were found to be linear over a range of 10–400 μg/ml for hydrocortisone and 50–700 μg/ml for clioquinol. The detection limit was 0.5 μg/ml in the case of hydrocortisone and 5 μg/ml for clioquinol.

Three different extraction techniques were investigated which were based on the method described in the 1980 BP assay for hydrocortisone and clioquinol ointment[6]. The results are presented in Table I. Method (a), which is that recommended for the extraction of hydrocortisone, using water–methanol as solvent resulted in slightly less than 100% extraction of hydrocortisone but only 68% recovery of clioquinol from the ointment. This is not unexpected in view of the low solubility of clioquinol in water. Alteration of the extracting solvent to 0.05 *M* phosphoric acid–methanol produced complete recovery of both compounds from the ointment (Method b). Method (c) was found to be unsatisfactory for the extraction of either compound, in particular only 40% clioquinol was detected in the methoxyethanol–water extract. Two further extractions did not yield complete recovery of clioquinol from the ointment. This is surprising as it is this method that is recommended by the 1980 BP specifically for the extraction of clioquinol but the reason for the discrepancy is not clear.

The extraction was therefore carried out as described in the experimental sec-

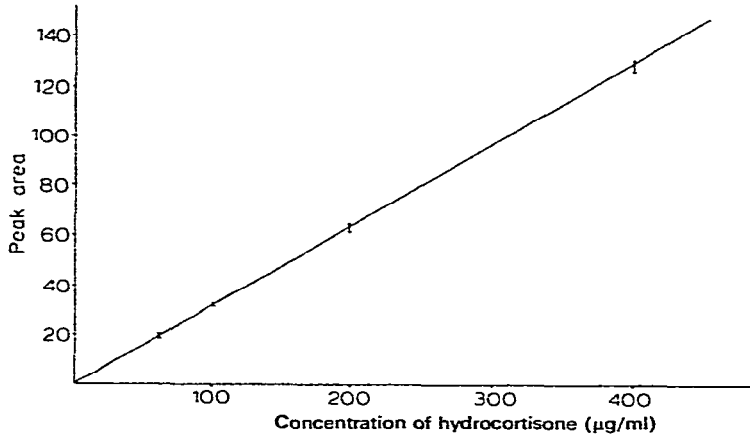

Fig. 2. Standard curve of hydrocortisone showing linear range.

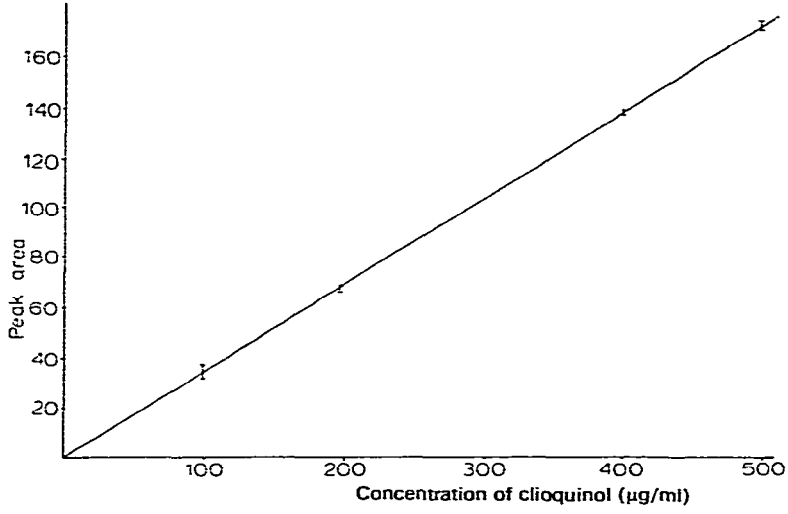

Fig. 3. Standard curve of clioquinol showing linear range.

TABLE I

INVESTIGATION OF THE EXTRACTION CONDITIONS FOR THE OINTMENT

See experimental section for the description of methods (a), (b) and (c). N.D. = Not determined.

Method	*Solvent*	*Recovery (%)*	
		Hydrocortisone	*Clioquinol*
(a)	Methanol–water (80:20)	100	68
(b)	Methanol–phosphoric acid (80:20)	100	100
(c)	Methoxyethanol–water (80:20)	N.D.	40

tion under method (b) and 20-μl aliquots were injected onto the column. In both cases the concentration of the components in the ointment extract (hydrocortisone, 100 μg/ml, clioquinol, 300 μg/ml) lie within the linear portion of the calibration graph.

Aliquots of a proprietary preparation of the ointment were found to contain 95–99% of the stated amount of clioquinol and 102–106% hydrocortisone. The 1980 BP defines the limits for clioquinol as 90–110% and 92.5–107.5% for hydrocortisone therefore it can be concluded that the described method is suitable for the analysis of combined preparations of hydrocortisone and clioquinol.

In addition it was shown that 5,7-dichloro-8-quinolinol, 5,7-diiodo-8-quinolinol and 5,7-dichloro-8-quinaldol could each be separated from hydrocortisone under the conditions described above (Fig. 4). Thus the use of this assay is not restricted to analysing hydrocortisone–clioquinol preparations but could also be of value in looking at products containing hydrocortisone combined with other halogenated quinolinols such as Steroxin HC (chloroquinaldol 3%, hydrocortisone 1%) or Cor-Tar-Quin (diiodoquinolinol 1%, hydrocortisone 0.5%). Furthermore this assay can also be used to distinguish between the different congeners of clioquinol, in fact in the present investigation it was found that there were impurities in some commercial preparations of clioquinol. These were shown to be 5,7-dichloro- and 5,7-diiodo-quinolinol which are intermediates in the synthesis of clioquinol.

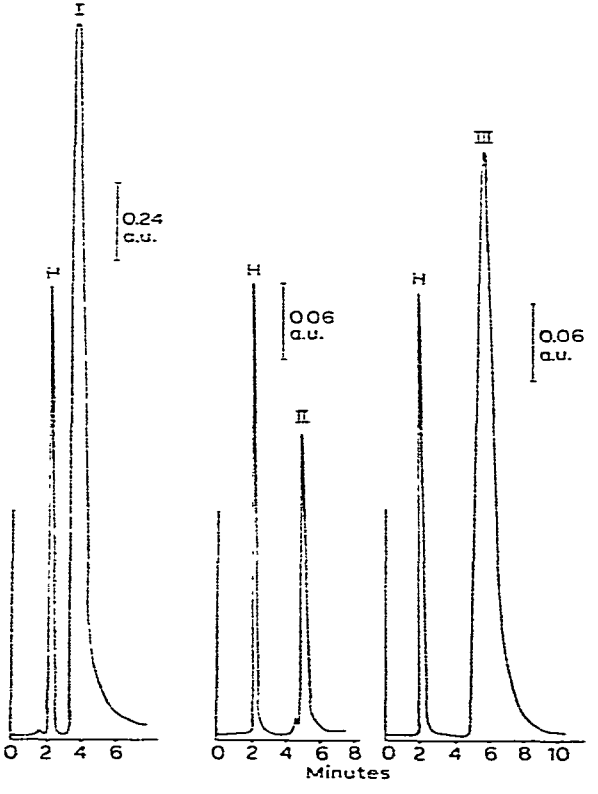

Fig. 4. Separation of clioquinol congeners from hydrocortisone (H). I = 5,7-Dichloro-8-quinolinol; II = 5,7-diiodo-8-quinolinol; III = 5,7-dichloro-8-quinaldol.

CONCLUSION

The HPLC procedure described in the present communication provides a rapid, sensitive and reproducible method for the combined analysis of hydrocortisone and clioquinol preparations. The use of a single analytical technique to monitor both compounds is an improvement over the 1980 BP method which describes two separate assays. Furthermore it would appear that the extraction method suggested in the 1980 BP for clioquinol does not always produce reproducible results.

This simple HPLC method can also be adapted for the analysis of preparations containing clioquinol congeners.

ACKNOWLEDGEMENT

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