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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF BETA-METHASONE 17-VALERATE AND ITS DEGRADATION PRODUCTS

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## SUMMARY

A high-performance liquid chromatographic assay of betamethasone 17-valerate is described. The procedure may be use for quantitative assay of the degradation products, betamethasone 21-valerate and betamethasone, and the application to the analysis of ointments is described. The method is also suitable for the determination of the kinetics of decomposition from one experimental run, and the determination of rate constants from a four-compartment sequential reaction is described.

The procedure is also applicable to other corticosteroids, and hydrocortisone 17-butyrate, hydrocortisone 21-butyrate, and hydrocortisone may similarly be determined without modification to the method.

## INTRODUCTION

The esters of corticosteroids are a widely prescribed group of drugs. Esterification usually involves the $C_{21}$ hydroxyl group, but betamethasone is unusual, being esterified instead at $C_{17}$. The derivative most widely used for topical application is betamethasone 17-valerate (Fig. 1, I), which has fifteen times the activity of the 21-isomer (II)[1]. The 21-valerate derivative is the thermodynamically more stable compound, and although stable in properly formulated dosage forms, the 17-valerate may rapidly rearrange to this less active form under non-ideal conditions. Further decomposition to the parent steroid (III) may also occur[2]. One possible source for non-ideal conditions existing in a formulated system is the widespread use of dilutions of proprietary systems[3]. We have recently shown that such preparations may have half-lives as short as 40 min for the active isomer[4]. Traditionally, assays have been based upon the tetrazolium blue colour reaction[5], and although degradation may be followed by thin-layer chromatography (TLC) and densitometry[4], the procedure is tedious and needs a large number of standards for acceptable results. Several reports have now appeared on the application of high-performance liquid chromatography (HPLC) to corticosteroid analysis[6–8], including a recent paper on betamethasone sodium phosphate[9]. In view of the advantages of these techniques over the colorimetric procedures, we wish to report an HPLC procedure for the assay of betamethasone
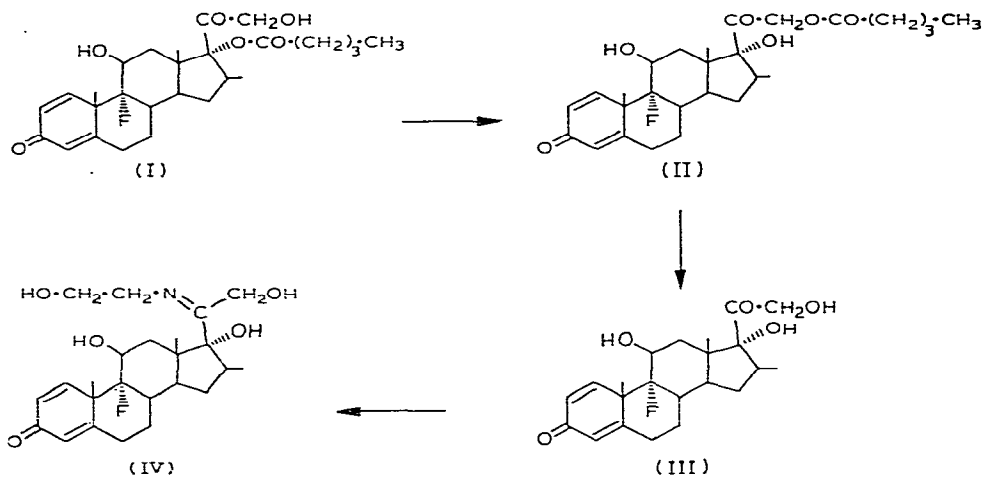
Fig. 1. Decomposition of betamethasone 17-valerate with ethanolamine. I = betamethasone, 17-valerate; II = betamethasone 21-valerate; III = betamethasone; IV = betamethasone 21-hydroxy-ethylimine.

17-valerate. This method may also be used to follow the rearrangement (betamethasone 21-valerate) and hydrolysis (betamethasone) products and to obtain a full kinetic profile of the degradation of betamethasone 17-valerate. The method is suitable for other corticosteroids and may be used for the analysis of the hydrocortisone 17-butyrate→hydrocortisone 21-butyrate→hydrocritisone system without modification.

EXPERIMENTAL

*Apparatus and conditions*

Analyses were performed with a high-performance liquid chromatograph, constructed from an Altex 100A constant-flow solvent-metering pump, a Rheodyne 7120 injector fitted with a 20-$\mu$l loop, and a Pye LC3 variable-wavelength ultraviolet monitor, equipped with an 8-$\mu$l flow-cell and operated at 260 nm with a sensitivity of 0.32 a.u.f.s. Chromatography was performed with a 25 cm × 4.6 mm I.D. column of Spherisorb (5 $\mu$m spherical, totally porous silica) and a mobile phase of ethyl acetate–chloroform–methanol (71:28:1) saturated with water and delivered at a flow-rate of 1 ml/min under a pressure of 60 bar.

*Materials and methods*

*Calibration.* Standard solutions of betamethasone 17-valerate, betamethasone 21-valerate and betamethasone were prepared, either separately or in mixture, in dimethyl sulphoxide over a concentration range of 20–120 ng ml$^{-1}$. The standard solutions (20 $\mu$l) were chromatographed and calibration lines were constructed on the basis of peak-height measurement.

*Ointments.* Synthetic ointments containing 0.1 % (w/w) of betamethasone 17-valerate were prepared by dissolving the steroid in propylene glycol (5 %) ultra-sonically and dispersing this into a white soft paraffin base (95 %) by trituration on a glass slab. Commercial Betnovate ointment (Glazo, Greenford, Great Britain) nomi-

nally containing 0.1 % (w/w) of betamethasone 17-valarate, was also used. The steroid content of these ointments was determined by weighing 1 g into glass-stoppered test tubes and partitioning the mixture between hexane (10 ml) and dimethyl sulphoxide (10 ml). The dimethyl sulphoxide extract (20 $\mu$l) was chromatographed and the peak heights were used for quantitative analysis.

*Kinetics.* A solution of betamethasone 17-valerate (0.01 % w/v) was prepared in propylene glycol containing ethanolamine (0.128 %). The solution was maintained at 60°, and 20-$\mu$l aliquots were injected directly into the chromatograph at intervals of up to 80 h.

*Hydrocortisone.* Hydrocortisone 17-butyrate, hydrocortisone 21-butyrate and hydrocortisone were dissolved in dimethyl sulphoxide (20–100 ng ml$^{-1}$) and were treated in a manner identical to that of the betamethasone solutions.

RESULTS AND DISCUSSION

The chromatograms obtained from betamethasone and its 17- and 21-valerate derivatives are in Fig. 2 and those from hydrocortisone and its 17- and 21-butyrate esters are shown in Fig. 3. The retention parameters are recorded in Table I. In each case the order of elution is 21-ester, 17-ester and parent alcohol. This, and the relative retention of betamethasone and hydrocortisone, are paralleled by the TLC behaviour of these compounds[4].

Quantitative analysis was readily achieved. A convenient range of concentrations for the assay of betamethasone 17-valerate in pharmaceuticals is 20–120 ng ml$^{-1}$ and over this range both the 17- and 21-esters and betamethasone yielded linear calibration plots. Reproducibility was satisfactorily controlled by the loop injector, and an internal standard was not required. Peak-height measurements allowed rapid and accurate analysis and yielded the following regression equations ($x$ in ng ml$^{-1}$, $y$ in mm):

$$\text{betamethasone 17-valerate:}\quad y = 376.7x - 0.2 \quad (r^2 = 99.90\%)$$
$$\text{betamethasone 21-valerate:}\quad y = 413.6x + 0.1 \quad (r^2 = 99.95\%)$$
$$\text{betamethasone:}\quad\qquad\qquad y = 292.7x \qquad\qquad (r^2 = 99.92\%)$$

The difference in slope mainly reflects the increasing half-peak width with longer retention times. These data may be used directly to assay solid or solution forms of the three compounds, either alone, upon admixture, or as degraded samples, by dilution to approximately 100 ng ml$^{-1}$, followed by immediate chromatography.

The analysis of pharmaceutical preparations is more complex. Betamethasone 17-valerate is extensively used in dermatology, formulated as an ointment or a cream. In these systems a large quantity of excipient is present, which must be removed before a reliable assay may be undertaken. Typically, an ointment consists of propylene glycol (5 %), sufficient to dissolve the steroid, dispersed in a soft paraffin base (95 %). A useful preliminary treatment of these systems involves the partitioning of the ointment between two immiscible organic solvents[10]. The partition coefficient for betamethasone 17-valerate between dimethyl sulphoxide and hexane exceeds 1500 suggesting that this solvent system should yield adequate recoveries for assay purposes. Partitioning of synthetic ointments between dimethyl sulphoxide and hexane enables
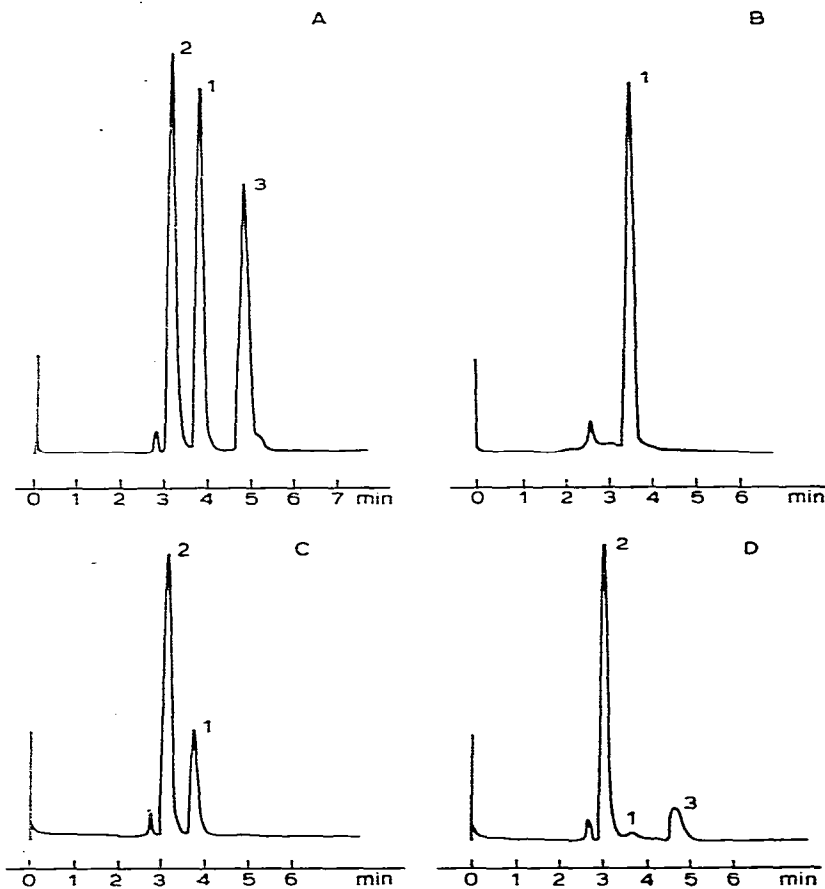
Fig. 2. HPLC of betamethasone 17-valerate and degradation products. Peaks: 1 = betamethasone 17-valerate; 2 = betamethasone 21-valerate; 3 = betamethasone. (A) Standard solutions each 100 ng ml$^{-1}$; (B) betamethasone 17-valerate in propylene glycol–ethanolamine (100 ng ml$^{-1}$) at 0 min; (C) after 30 min; (D) after 230 min.

the removal of the hydrocarbon fraction by the hexane, and chromatography of the lower dimethyl sulphoxide phase allowed the ready estimation of the betamethasone 17-valerate level. Recovery studies for these ointments showed levels of 99.80 $\pm$ 1.56% ($P$ = 0.95) of the prepared strength (0.1%). The commercial samples also showed satisfactory reproducibility but were found to contain 97.20 $\pm$ 0.52% ($P$ = 0.95) of the nominal content (0.1%) of betamethasone 17-valerate. This may reflect the disparate storage conditions for this batch of samples, but formulation differences cannot be excluded.

One further point of interest in the study of the stability of betamethasone and its esters, is the elucidation of the kinetic parameters for the degradation. The method described here also enables the calculation of all rate constants to be achieved during one kinetic run. To illustrate this application, the decomposition of betamethasone 17-valerate in propylene glycol solution catalysed by ethanolamine was undertaken. The reaction sequence in this system is recorded in Fig. 1. It involves rearrangement (I → II), hydrolysis (II → III) and the loss of betamethasone possibly via condensation
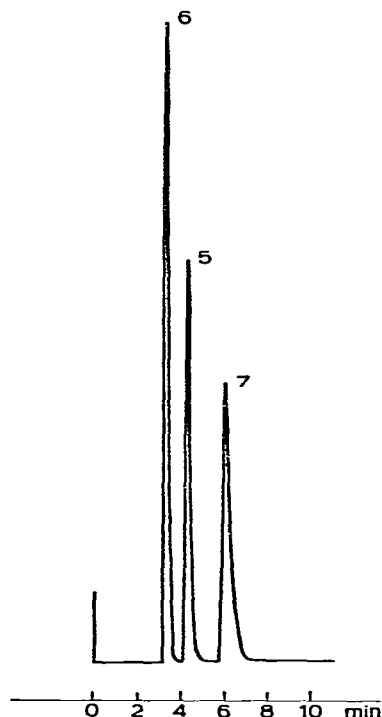
Fig. 3. HPLC of hydrocortisone 17-butyrate and degradation products. Peaks: 5 = hydrocortisone 17-butyrate; 6 = hydrocortisone 21-butyrate; 7 — hydrocortisone; each 100 ng ml$^{-1}$.

TABLE I

RETENTION DATA FOR BETAMETHASONE AND HYDROCORTISONE AND THEIR ESTERS

| Steroid | Retention time (min) | Capacity ratio | Resolution |
|---------|----------------------|----------------|------------|
| Betamethasone 21-valerate | 3.1 | 0.11 | |
| Betamethasone 17-valerate | 3.7 | 0.32 | 1.20 |
| Betamethasone | 4.8 | 0.71 | 1.69 |
| Hydrocortisone 21-butyrate | 3.2 | 0.14 | |
| Hydrocortisone 17-butyrate | 4.1 | 0.46 | 1.29 |
| Hydrocortisone | 5.9 | 1.11 | 1.80 |

(III→IV) and follows the kinetics of an A→B→C→D sequential reaction. The relevant expressions allowing the calculation of the composition of the mixture at time $t$ are shown in Table II. Typical chromatograms showing the progress of the reaction are shown in Fig. 2 (B–D) and illustrate the rapid disappearance of the 17-valerate and the slower appearance of betamethasone. The reaction profile is displayed in Fig. 4 and yields the kinetic parameters of Table III. Although the final product (IV) is not transmitted by the column under the conditions of this analysis, owing to the increased polarity of this compound, the appearance profile may be calculated from eqn. 4 (Table II). The stability of the betamethasone 17-valerate in this system parallels

TABLE II

CALCULATION OF THE COMPOSITION OF BETAMETHASONE 17-VALERATE SOLUTION DURING THE ETHANOLAMINE-CATALYSED DEGRADATION IN PROPYLENE GLYCOL

$$A \xrightarrow{K_1} B \xrightarrow{K_2} C \xrightarrow{K_3} D$$

(I)      (II)      (III)      (IV)
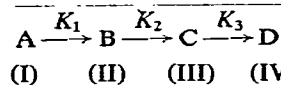
(1) $[A]_t = [A]_0 e^{-K_1 t}$

(2) $[B]_t = \dfrac{[A]_0 K_1}{(K_2 - K_1)} \{e^{-K_1 t} - e^{-K_2 t}\}$

(3) $[C]_t = [A]_0 \left[ \left\{ \dfrac{K_1 K_2}{(K_2 - K_1)(K_3 - K_1)} \right\} e^{-K_1 t} + \left\{ \dfrac{K_1 K_2}{(K_1 - K_2)(K_3 - K_2)} \right\} e^{-K_2 t} \right.$

$$\left. + \left\{ \dfrac{K_1 K_2}{(K_1 - K_3)(K_2 - K_3)} \right\} e^{-K_3 t} \right]$$

(4) $[D]_t = [A]_0 \left[ 1 - \left\{ \dfrac{K_2 K_3}{(K_2 - K_1)(K_3 - K_1)} \right\} e^{-K_1 t} - \left\{ \dfrac{K_1 K_3}{(K_1 - K_2)(K_3 - K_2)} \right\} e^{-K_2 t} \right.$

$$\left. - \left\{ \dfrac{K_1 K_2}{(K_1 - K_3)(K_2 - K_3)} \right\} e^{-K_3 t} \right]$$
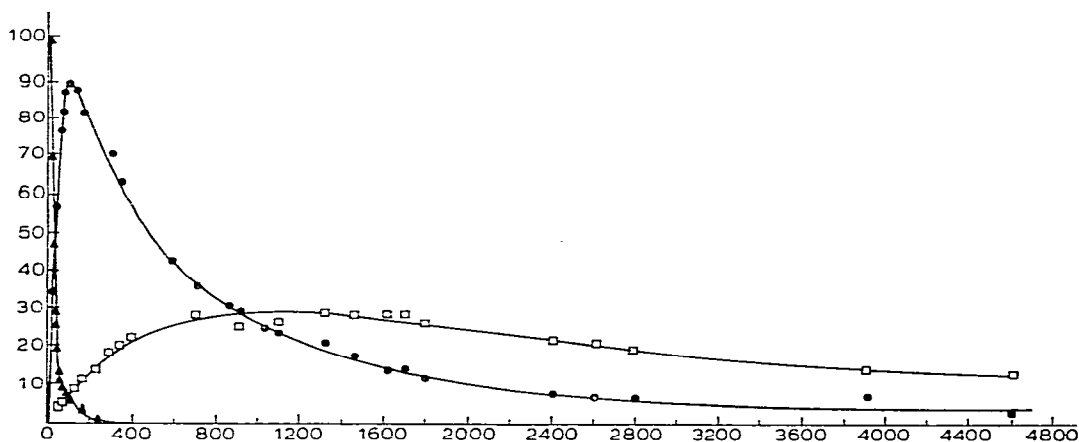


Fig. 4. Reaction profile of the ethanolamine-catalysed degradation of betamethasone 17-valerate in propylene glycol. ▲, Betamethasone 17-valerate; ●, Betamethasone 21-valerate; □, Betamethasone.

TABLE III

KINETIC PARAMETERS FOR THE ETHANOLAMINE-CATALYSED DEGRADATION OF BETAMETHASONE 17-VALERATE IN PROPYLENE GLYCOL

| Reaction | Rate constant $(h^{-1})$ |
|---|---|
| Rearrangement (I → II) | $K_1 = 2.4460$ |
| Hydrolysis (II → III) | $K_2 = 0.0859$ |
| Condensation (III → IV) | $K_3 = 0.0502$ |

that found in ointments[4], with the most rapid degradation being the isomerisation of the 17- to the 21-valerate, followed by a slower hydrolysis to yield betamethasone.

Clearly, the benefits afforded by this HPLC procedure, in particular the speed of assay and the precision of the results, are compelling advantages over current assay methods.

## ACKNOWLEDGEMENT

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