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A DIFFERENTIAL KINETIC METHOD FOR THE DETERMINATION OF BETAMETHASONE-17-VALERATE IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

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SUMMARY

A differential kinetic method is described for the selective determination of betamethasone-17-valerate in the presence of its degradation products including betamethasone-21-valerate and betamethasone. The method involves oxidation of the steroid 21-hydroxy group with methanolic cupric acetate to an aldehyde function and subsequent condensation of this with 3-methyl-benzothiazol-2-one hydrazone (MBTH) in alkaline solution to form a highly absorbing azine with λ_{max} at 394 nm. The selective spectrophotometric assay is based on the different rates of reaction of oxidized betamethasone-17-valerate and betamethasone with the MBTH reagent and makes use of the method of proportional equations, permitting simultaneous determination of betamethasone and its 17-valerate ester. The accuracy and precision of the procedure were evaluated and its applicability for assessing the stability of the 17-valerate ester in aqueous solutions was demonstrated.

INTRODUCTION

Corticosteroid-17-α-monoesters are unstable and in the presence of acid and base, they may undergo a rearrangement to the corresponding, and more stable, 21-monoesters (Gardi et al., 1963; Vitali and Gardi, 1972). It has recently been shown that isomerization of betamethasone-17-valerate to the 21-valerate ester may take place very easily in some ointment preparations (Yip and Li Wan Po, 1979) as well as in propylene glycol solutions containing ethanolamine (Li Wan Po et al., 1979). In aqueous solutions at pH 0.5–8 the overall degradation of betamethasone-17-valerate was shown to proceed entirely through a rearrangement of the 17-valerate ester to the 21-valerate ester followed by hydrolysis of

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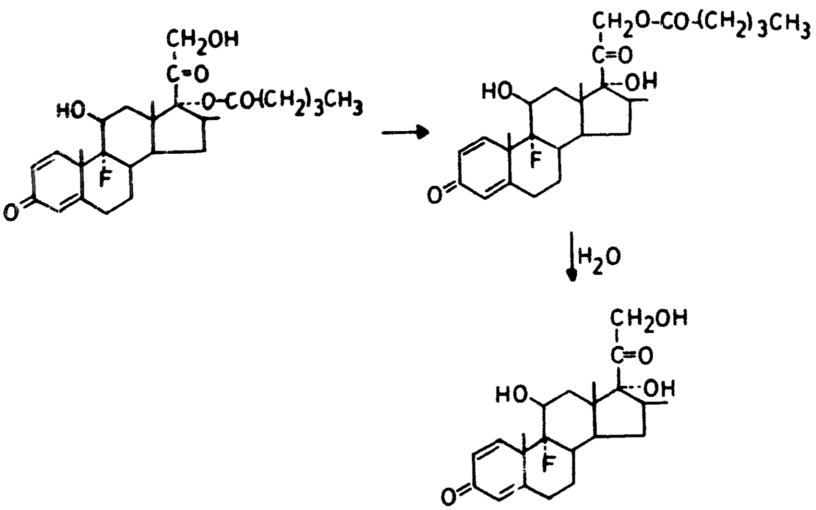
the latter to yield betamethasone (Scheme 1) (Bundgaard and Hansen, 1981).



Scheme 1

For the selective and quantitative determination of betamethasone-17-valerate in the presence of its degradation products, procedures based on thin-layer chromatography (Yip and Li Wan Po, 1979) and high-performance liquid chromatography (Li Wan Po et al., 1979; Bundgaard and Hansen, 1981) have been reported. An alternative approach of achieving selectivity which does not require any separation procedure is to utilize a differential reaction rate method. Various types of kinetically based analytical methods have been described for the in situ simultaneous analysis of closely related mixtures (Mark and Rechnitz, 1968), yet their potential for solving analytical problems in pharmaceutical fields has hitherto only been little recognized (e.g. Guttman, 1966; Bundgaard, 1979a, b, 1980b).

In the present work a differential kinetic method for the quantitative determination of betamethasone-17-valerate in the presence of its degradation products has been developed. The method is based on a spectrophotometric assay previously described (Bundgaard, 1978) and it permits simultaneous quantitation of both the 17-valerate ester and free betamethasone. The utility of the kinetic method for assessing the stability of betamethasone-17-valerate is demonstrated and some rate data are included in the paper.

MATERIALS AND METHODS

Apparatus

A Zeiss PMQ II spectrophotometer equipped with a potentiometric recorder and a Radiometer Model PHM 62 pH meter were used for the measurements.

Materials and reagents

Samples of betamethasone-17-valerate and betamethasone-21-valerate were kindly supplied by Glaxo, Middlesex, U.K. Betamethasone was purchased from Sigma Chemicals, St. Louis. 3-Methyl-benzothiazol-2-one hydrazone hydrochloride (MBTH) was obtained from Fluka AG, Switzerland. All other chemicals and solvents used were of reagent grade.

Cupric acetate solution. Cupric acetate monohydrate (200 mg) dissolved in 100 ml of methanol. This solution is stable for at least one month.

MBTH reagent. Dissolve 200 mg of MBTH in 25 ml of water, add 1 ml of a 0.01 M disodium edetate solution and 50 ml of methanol and then dilute with 0.2 M aqueous carbonate buffer solution of pH 10.3 ± 0.1 to 100 ml. The reagent is stable for 3 days.

Disodium edetate solution (0.1 M). Disodium edetate (3.8 g) dissolved in 100 ml of water.

Procedure for betamethasone-17-valerate alone

Prepare a solution in methanol of betamethasone-17-valerate at a concentration of about 50 $\mu g\ ml^{-1}$. Pipette two equal samples of 500 μl, A and B, of this solution into separate test tubes. To A, add 500 μl of the cupric acetate solution, mix and let stand at room temperature for 25 min. To sample B, add 500 μl of methanol. Add 200 μl of 0.1 M disodium edetate solution and then 2500 μl of the MBTH reagent to solutions A and B. Let stand at room temperature for 25 min and measure the absorbance difference between solutions A and B in 1 cm cells at 394 nm. Determine the corticosteroid concentration of the original sample by reference to a standard curve.

Kinetic procedure for betamethasone-17-valerate and betamethasone

Prepare a solution in methanol of the test sample at a concentration of about 50 μg ml^{-1} total corticosteroid. Pipette 500 μl into a test tube, add 500 μl of the cupric acetate solution, mix and let stand at room temperature for 25 min. Add 200 μl of 0.1 M disodium edetate solution and then 2500 μl of the MBTH reagent. Transfer the solution to a spectrophotometer cuvette and measure the absorbance exactly 1.5 and 5 min after the addition of the MBTH reagent using a mixture of 500 μl test solution, 500 μl of methanol, 200 μl of 0.1 M disodium edetate solution and 2500 μl of MBTH reagent as reference solution. Calculation of the concentration of betamethasone and its C-17 ester in the test sample is done as described below using Eqns. 3 and 4.

The solutions and spectrophotometer were at ambient temperature (23 ± 0.5°C).

Determination of degradation course of betamethasone-17-valerate

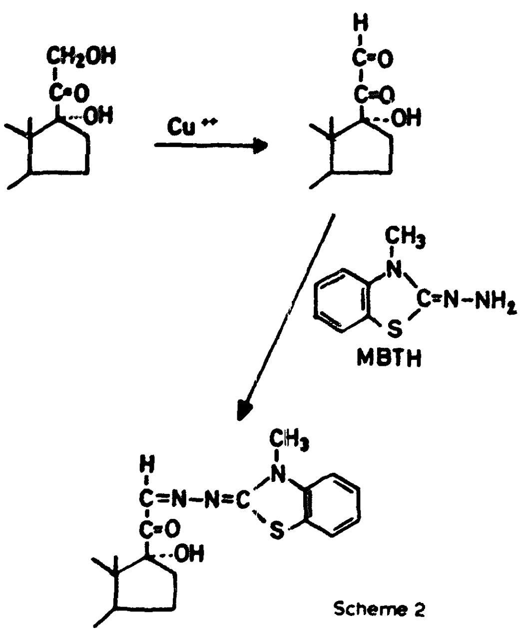
The degradation of betamethasone-17-valerate was studied in 0.5 M hydrochloric acid and in 0.05 M phosphate buffer solution (μ = 0.5 with potassium chloride) of pH 7.50 at 60°C. The reactions were initiated by adding 1000 μl of a solution of betamethasone-17-valerate in ethanol (0.80 mg ml^{-1}) to 25.00 ml of the buffer solutions pre-equilibrated in a water bath at 60°C. At appropriate times 2500 μl aliquots were withdrawn and added to 2500 μl of ethyl acetate in a test tube. The mixture was shaken thoroughly and after separation of the two phases 1500 μl of the ethyl acetate layer were transferred to a test tube and evaporated to dryness at ambient temperature using a mild air stream. The residual material was redissolved in 500 μl of methanol and subjected to the kinetic analytical procedure described above.

It was separately shown that this extraction procedure afforded a quantitative extraction of the steroids from the aqueous buffer solutions.

RESULTS AND DISCUSSION

Equilibrium procedure for betamethasone-17-valerate

The analytical procedure given is almost identical to that previously described for various 21-hydroxy corticosteroids (Bundgaard, 1978). The method is based on quantitative oxidation of 21-hydroxy steroids with methanolic cupric acetate to the corresponding 21-dehydro derivatives (steroid-glyoxals) and subsequent condensation of these with 3-methylbenzothiazol-2-one hydrazone (MBTH) in alkaline solution to form highly absorbing azines with λ_{max} at 394 nm (Scheme 2). For betamethasone-17-valerate the



Scheme 2

oxidation to the corresponding glyoxal was found to proceed rather slowly as compared with the reaction of unesterified corticosteroids but this problem was simply solved by using a 10-fold higher concentration of cupric acetate in the oxidation step. Under these conditions the glyoxal formation is complete after 20–25 min.

The time course of the condensation of the glyoxal derived from betamethasone-17-valerate with MBTH under the previously selected assay conditions (Bundgaard, 1978) is shown in Fig. 1 along with the time course for betamethasone. For the ester 25 min at room temperature are needed to complete the reaction whereas only about 5 min are needed for betamethasone, the difference in reactivity most probably being due to the steric hindrance of the 17-valerate group.

A straight-line relation between absorbance and concentration of betamethasone-17-valerate was observed within the range of 0–150 $\mu g\ ml^{-1}$ of the steroid in the test solution, the molar absorptivity being 20.0×10^3. For betamethasone a molar absorptivity of 19.8×10^3 was determined.

The precision of this equilibrium procedure for betamethasone-17-valerate was evaluated by making 10 determinations on the same methanolic solution of the ester. A relative standard deviation of 0.7% was obtained.

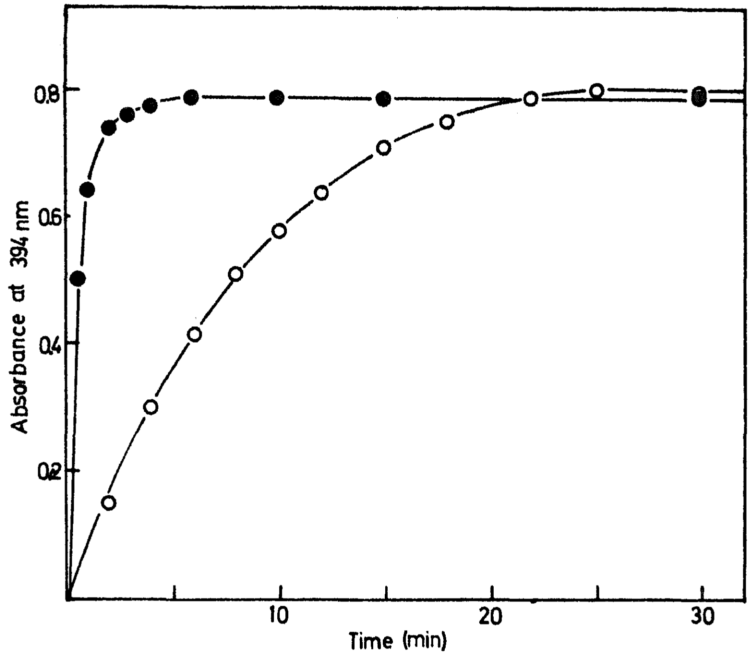


Fig. 1. Time courses of the condensation of betamethasone-17-valerate (o) and betamethasone (•), pretreated with the cupric acetate solution, with the MBTH reagent at 23°C. The concentrations of the compounds in the final reaction solutions were 4.0×10^{-5} M.

The glyoxal formation requires a free 21-hydroxy group and like other corticosteroid 21-esters (Bundgaard, 1978) betamethasone-21-valerate was shown to be non-responsive in the assay.

Differential kinetic procedure

By the equilibrium procedure it is not possible to determine selectively betamethasone-17-valerate in the presence of free betamethasone. By making use of the difference in the rate of the condensation step with MBTH for the compounds (cf. Fig. 1) it is, on the other hand, possible to design a kinetic procedure permitting a selective determination of the 17-valerate ester or, when both ester and free betamethasone are present in admixture, a simultaneous determination of the components.

Among the most commonly used differential kinetic methods for analyzing closely related compounds in admixture, the method of proportional equations or the double-point method (Garmon and Reilley, 1962) appeared most suitable for the present purpose. The method consists of making measurements at two different times, substituting these values into a pair of simultaneous equations, and solving for the concentrations of both components. The equations are:

$$P_t = K_A[A]_0 + K_B[B]_0 \tag{1}$$

$$P'_t = K'_A[A]_0 + K'_B[B]_0 \tag{2}$$

where P_t and P'_t are parameters proportional to concentration (in this case absorbance) measured at times t and t', respectively. The initial concentrations of the components are expressed as $[A]_0$ and $[B]_0$, and K_A, K_B, K'_A and K'_B are constants corresponding to the slopes of plots of P_t and P'_t vs concentrations at times t and t', respectively.

Solving Eqns. 1 and 2 simultaneously for $[A]_0$ and $[B]_0$, respectively, the following expressions are obtained:

$$[A]_0 = \frac{P_t - P'_t(K_B/K'_B)}{K_A - K_B(K'_A/K'_B)} \tag{3}$$

$$[B]_0 = \frac{P_t - P'_t(K_A/K'_A)}{K_B - K_A(K'_B/K'_A)} \tag{4}$$

For the analysis of mixtures of betamethasone and the 17-valerate ester, the longer reaction time (t') was chosen to be 5 min at which time the reaction of betamethasone is completed and the 17-valerate ester reaction is approximately 45% completed (cf. Fig. 1). A shorter reaction time, t, of 1.5 min was found to be optimum using the graphical approach to time selection described by Garmon and Reilley (1962).

The absorbances produced upon reaction of the two compounds after these reaction times were shown to be directly proportional to the initial concentrations. The values of the proportionality constants calculated from these absorbance–concentration plots are listed in Table 1.

To evaluate the accuracy and precision of the method, binary mixtures (methanolic solutions) of betamethasone-17-valerate and betamethasone in different proportions were prepared and analyzed. The results obtained are given in Table 2. The minimum concentrations of the components that can be analyzed in mixtures within tolerable limits of error are about 5%.

Application of the kinetic method to assessing stability of betamethasone-17-valerate

The applicability of the kinetic method for assessing the stability of betamethasone-17-valerate was investigated by studying the degradation kinetics of the steroid ester in 0.5 M hydrochloric acid and in a 0.05 M phosphate buffer solution, pH 7.50 at 60°C.

TABLE 1

EXPERIMENTAL PROPORTIONALITY CONSTANTS OF BETAMETHASONE AND BETAMETHASONE-17-VALERATE FOR THE REACTION TIMES t = 1.5 MIN (K) AND t' = 5 MIN (K')[a]

Compound	K (M^{-1})	K' (M^{-1})
Betamethasone	17.5×10^3	19.9×10^3
Betamethasone-17-valerate	2.5×10^3	9.5×10^3

[a] The K-values were determined from the slopes of linear plots of absorbance at the appropriate reaction time vs molar concentration in the reaction solution.

TABLE 2

ANALYSIS OF MIXTURES OF BETAMETHASONE-17-VALERATE AND BETAMETHASONE

Betamethasone-17-valerate ($\mu g\ ml^{-1}$)		Betamethasone ($\mu g\ ml^{-1}$)	
Taken	Found	Taken	Found
200.0	198.7 ± 3.8 [a]	0	0
190.0	191.4	10.0	8.5
180.0	176.3	20.0	21.0
150.0	147.8	50.0	52.7
100.0	101.4 ± 3.0 [a]	100.0	98.8 ± 4.1 [a]

[a] Mean values ± S.D. of 8 analyses. All other results are averages of two determinations.

Using an HPLC procedure the overall degradation of the drug under such conditions has been shown to proceed entirely through an intramolecular migration of the valeryl group from C-17 to C-21 followed by a slow hydrolysis of the 21-valerate ester to yield free betamethasone (Bundgaard and Hansen, 1981).

In the phosphate buffer solution the pseudo-first-order rate constant for the disappearance of betamethasone-17-valerate was determined to be 1.1 h^{-1} which is in good agreement with the value (1.2 h^{-1}) obtained by using an HPLC procedure (Bundgaard and Hansen, 1981). In agreement with the study using HPLC, formation of free betamethasone was detected only after almost complete disappearance of the 17-valerate ester.

In the acidic reaction solution the formation of betamethasone is more marked. The time courses for the 17-valerate ester and betamethasone, as obtained by the kinetic analytical procedure, are shown in Fig. 2 and compared with those determined under the same conditions by means of HPLC (Bundgaard and Hansen, 1981). In the latter study the intermediate formation of betamethasone-21-valerate in the overall degradation was directly proved. The excellent agreement observed between the results obtained by HPLC and the kinetic procedure demonstrates the capability of the latter method of accurately quantitating the 17-valerate ester and free betamethasone in the degraded solutions. From Fig. 2 the pseudo-first-order rate constant for the overall degradation of the 17-valerate ester was determined to be 0.078 h^{-1} whereas the rate constant for the formation of betamethasone from the intermediate 21-valerate ester was found to be 0.30 h^{-1}.

Under oxidative conditions as well as in acidic solutions steroid-glyoxals may be important degradation products of 21-hydroxy corticosteroids (Bundgaard and Hansen, 1979; 1980; Bundgaard, 1980a; Hansen and Bundgaard, 1980a and b) and thus of betamethasone-17-valerate directly or via the secondary product of degradation, betamethasone. Therefore, both the equilibrium procedure and the kinetic method described have been designed to eliminate the interference of such products in that the blank included accounts for any preformed steroid-glyoxal (cf. Bundgaard, 1978).

In summary, it appears that the differential kinetic method described permits a convenient and selective determination of betamethasone-17-valerate in the presence of its degradation products including betamethasone and at the same time makes it possible to quantitate this product.

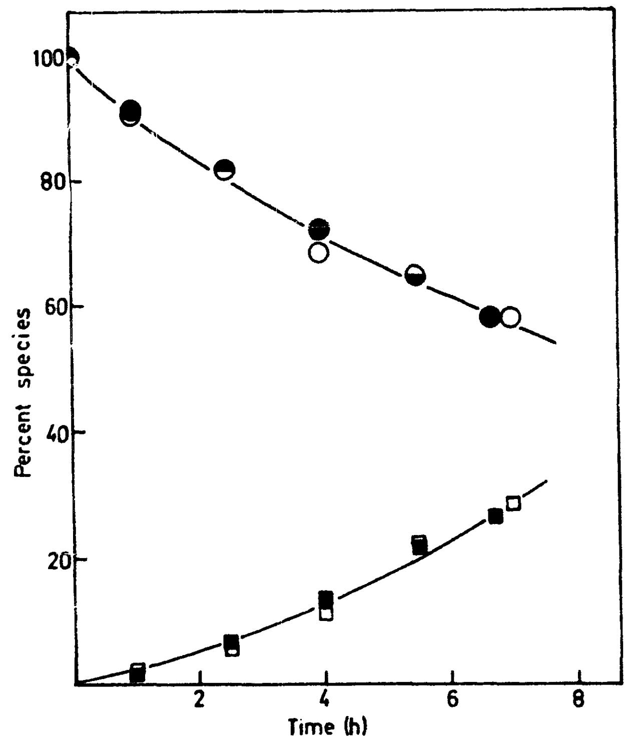


Fig. 2. Time courses for betamethasone-17-valerate (o) and betamethasone (□) in the degradation of betamethasone-17-valerate in 0.5 M hydrochloric acid at 60°C. The concentrations at various times, expressed as mol% in relation to the initial 17-ester concentration, were determined by the differential kinetic method described as well as by HPLC (filled circles and squares).

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