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A stability indicating high-performance liquid chromatography determination of Triple Corticoid Integrated System in a cream

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Summary

A reversed-phase high-performance liquid chromatographic method for Triple Corticoid Integrated System in a cream formulation was developed. This system includes acetate (fluocinonide), propionate (procinonide) and cyclopropyl carboxylate (ciprocinonide) esters of the common alcohol fluocinolone acetonide. The method was demonstrated to be stability-specific as well as linear, accurate (between 70 and 120% of labeled strength) and precise (RSD = 0.9–2.2%). The chromatography utilized a ternary system of THF–acetonitrile–water as the mobile phase with RP-18 column kept at 45°C. A study of the effects of the organic modifiers concentrations on the capacity factors suggested that only partition phenomena were responsible for the retention of the steroids. In addition the effect of column temperature on the capacity factors was evaluated. It was found that increase in temperature reduced retention times with some decrease in resolution capability of the system.

Introduction

The Triple Corticoid Integrated System (TCIS) consists of a mixture of three 21-esters of fluocinolone acetonide (I). The three esters are fluocinonide (II), procinonide (III) and ciprocinonide (IV), containing an α,β-unsaturated carbonyl

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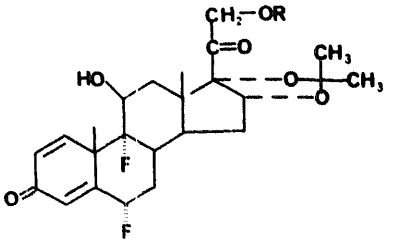
group as well as a derivitized dihydroxyacetone side-chain.



R = H, fluocinolone acetonide (I)
= $O{=}CCH_3$, fluocinonide (II)
= $O{=}CC_2H_5$, procinonide (III)
= O=C—, ciprocinonide (IV)

Poulsen et al. (1978) have shown that the mixture of the esters penetrated excised human skin independently. They found that the solubility, partition and diffusion behavior of the individual esters provided their mixture a mechanism of enhancing total percutaneous absorption. The mixture flux rate through the skin was demonstrated to exceed that attained by any of the steroid esters at an equal concentration The concentrations of the 3 steroids, II, III, and IV (0.00925%, 0.00365% and 0.0021%, respectively), in the TCIS cream formulation were based on the best estimate of their saturation solubilities in this formulation.

This report describes a new reversed-phase high pressure liquid chromatography (HPLC) analytical method that is specific for compounds I, II, III and IV and is suitable for following the stability of the three steroid esters in the cream formulation.

Background

Up to 15 years ago published procedures for the determination of corticosteroids containing both a dihydroxyacetone side-chain and α,β-unsaturated carbonyl group had depended almost exclusively on the reducing or chromophoric properties of these groups (Maderd and Buck, 1952; Kolthoff and Lingane, 1952; Umberger, 1955; Leung and Tam, 1981).

Gas chromatography (GC) was also used to determine such corticosteroids. The fact that steroids possessing the α-ketal moiety are thermally unstable made GC unattractive. However, oxidation prior to chromatography of the side-chain to the corresponding 17β-carboxylic acid was utilized (Oesterling, 1978) to overcome this problem. Other means, such as formation of derivatives which are stable under normal GC conditions were also applied (Oesterling, 1978; Bailey, 1967; Cosi and Bichi, 1969).

Fluocinonide (II) as well as its parent alcohol (I) are described in the USP XX,

and the pharmacopoea outlines HPLC assays for these two steroids (USP, 1980). There are only a few reports describing HPLC (Bailey and Brittain, 1972; Landgraf and Jennings, 1973) and open column (Bailey, 1966) chromatography procedures to determine I and II. A drug substance structurally similar to II, fluclocortisone acetate, was quantified (Ast and Abdou, 1979) by HPLC. Kirschbaum et al. (1980) have recently described an HPLC procedure to chromatograph II as well as triamcinolone acetonide and diflorasone diacetate.

Materials and methods

HPLC instrument and column

A Spectra Physics Model 3500 (Spectra Physics, U.S.A.) HPLC unit equipped with an oven, a Valco loop injector (Valco, U.S.A.) and a Spectro Monitor III (Laboratory Data Control, U.S.A.) or Spectra Physics Model 8300 UV detectors capable of detection at 254 nm was generally used. In addition, for evaluating mobile phase and column temperature effects on capacity factor, the HPLC unit consisted of two Model 6000A and one Model M-45 pumps (Waters, U.S.A.), WISP autoinjector (Waters), Model 730 Data Module (Waters) and Model 720 System Controller (Waters) as well as UV detector and an oven. The column was stainless steel (25 cm × 4.6 mm i.d.) packed with porous microparticular silica, permanently bound to a monomolecular layer of octadecylsilane (Spherisorb ODS, Spectra Physics). A guard column (7 cm × 2.1 mm i.d.) with Co:Pell ODS (Whatman, U.S.A.) packing material was used as well.

Solvents and chemicals

The solvents used for the mobile phase, tetrahydrofuran (THF), and acetonitrile, were glass-distilled and spectral grade (Burdick and Jackson, U.S.A.). The water was deionized and glass-distilled, or filtered through Nanopure system (Barnstead, U.S.A.). The usual precautions of filtering and degassing were taken in preparing the mobile phases. The extraction solvent 2,2,4-trimethylpentane (iso-octane), was reagent grade, A.R. (Mallinckrodt, U.S.A.). All steroids used were obtained from the Institute of Organic Chemistry (Syntex Research, U.S.A.).

HPLC conditions

The composition of the mobile phase was THF–acetonitrile–water (1:3:6 v/v/v). The flow rate was 1.2 ml/min and the detector sensitivity was set at 0.02 a.u.f.s. The column was kept at 45°C or at other temperatures as appropriate.

Assay procedures

Standard solutions. A stock standard solution of the mixture of fluocinonide, procinonide and ciprocinonide (462.5, 182.5 and 105 μg/ml, respectively) was prepared in acetonitrile. The internal standard, flucloronide (190 μg/ml), and parent alcohol, fluocinolone acetonide (100 μg/ml), were also dissolved in acetonitrile. From these 3 solutions a calibration standard solution was prepared by pipetting

two parts each of the triple corticoid mixture solution and flucinolone acetonide, and 5 parts of the internal standard solution into a flask containing 41 parts of acetonitrile and 50 parts water. This resulted in steroids concentrations of 9.25, 3.65, 2.1, 2.0 and 9.5 μg/ml of fluocinonide, procinonide, ciprocinonide, fluocinolone acetonide and flucloronide, respectively, in a mixture of acetonitrile–water (1 : 1 v/v).

Sample preparation. Approximately 2 g of cream was transferred to a 250 ml separator and partitioned between 75 ml iso-octane and 25 ml acetonitrile containing 1 ml of the internal standard solution. After vigorous shaking for 1 min, part of the excipients were partitioned into iso-octane while the steroids were extracted into acetonitrile. The extraction was repeated 3–4 times collecting always the lower acetonitrile layer into a 150 ml inverted conical flask. The acetonitrile solutions in the conical flasks were evaporated almost to dryness (leaving about 1 ml liquid residue) using a steam bath and the help of a stream of nitrogen. The residue was dissolved in 10 ml of acetonitrile and to the solution 10 ml of water were added. This precipitated those excipients which had been extracted by acetonitrile. After a few minutes the lower portion became clear. Using a Pasteur pipette, an aliquot of this solution was clarified further by centrifugation for 10 min (3000 rpm). The clear solution was injected (50 μl) into the HPLC unit.

Results and discussion

Stability specificity

The purpose of this study was to develop a stability-indicating method for TCIS in a cream formulation. An analytical method is stability-indicating if the measured response is due to the compound of interest and not to other compounds present in the sample. Thus, to establish specificity of an analytical method, one has to demonstrate the ability of the method to resolve the intact compound from potential interfering compounds.

As the 3 active steroids (II, III, IV) in the cream are esters of a common alcohol (I), it was expected that under hydrolytic or solvolytic conditions the major degradation product would be I. It was shown (Thompson and Chan, 1980) that compounds II, III and IV hydrolize to the alcohol I. Fig. 1 shows that under the chromatographic conditions of the analytical method I did not interfere with the analytical responses of the three esters.

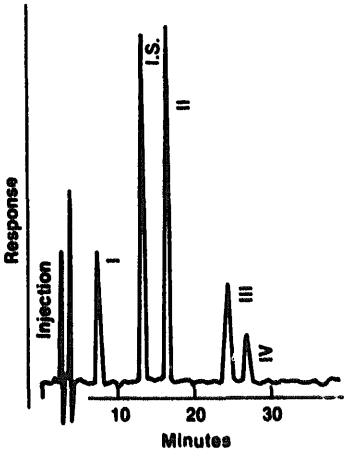
I itself being a 21-hydroxy corticosteroid is capable of undergoing degradation which results from reactions of the C_{17}-side chain (Hansen and Bundgaard, 1980a). For example, the major degradation products of hydrocortisone observed in aqueous buffer solutions were the 17-deoxy glyoxal derivative (Hansen and Bundgaard, 1980a and b), 21-dehydrocortisone (Hansen and Bundgaard, 1980a and b) etianic acid and glycolic acid derivatives (Hansen and Bundgaard, 1980a). Furthermore, triamcinolone acetonide, a compound with a similar chemical structure to I, was oxidized (Smith et al., 1960) to the corresponding etianic acid acetonide. Thus, it is reasonable to assume that the α-ketal-side chain of I might degrade under similar conditions to its glyoxal (V) and etianic acid (VI) derivatives.

In addition to these possible degradation products, the cyclic ketal group of I can be cleaved to yield VII and acetone, as reported for the hydrolysis of triamcinolone acetonide by a variety of acids (Smith et al., 1960).

The decomposition of flunisolide, the 9-defluoro derivative of I, in water was shown (Thompson, 1980) to produce degradation products structurally related to V, VI and VII. In this same study another degradation product, lactone type, was found in basic solutions. This indicated that I might also degrade to a similar degradation product, VIII.

To test further the specificity of the analytical method for TCIS, standard solutions of I and its possible degradation products (V, VI, VII, VIII) were chromatographed separately and as a mixture with the three intact ester steroids (II, III, IV). Fig. 2 shows the excellent separation of the active components of the formulation (II, III, and IV) and the internal standard from all these possible degradation products.

The major degradation product detected during the stability studies of TCIS cream was I. It was tested further whether I might degrade in the cream base and resulting degradation products would interfere with the specificity of the method: samples of I in the cream base formulation aged for 6 months at room temperature, 45°C and 60°C were analyzed.



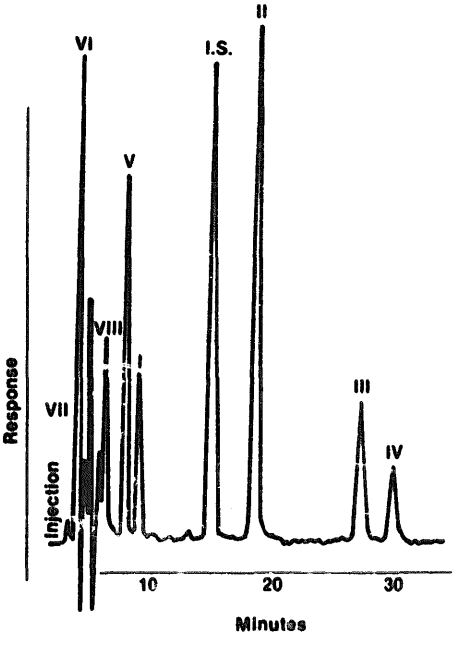
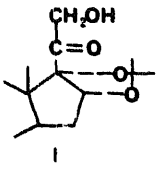


Fig. 1. Chromatographic tracing of a typical calibration standard solution containing fluocinolone acetonide (I), fluocinonide (II), procinonide (III), ciprocinonide (IV) and the internal standard flucloronide (I.S.).

Fig. 2. Chromatographic tracing of a mixture of standard solutions of fluocinolone acetonide (I), fluocinonide (II), procinonide (III), ciprocinonide (IV), the internal standard flucloronide (I.S.) and possible degradation products (V, VI, VII, VIII).



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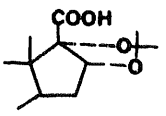

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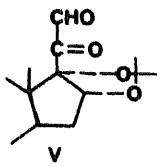

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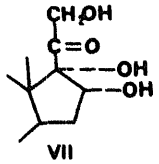
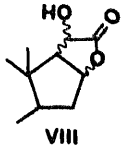


VII



VIII

It was found that I is quite stable in the TCIS cream base. A few peaks eluting earlier than I were detected in the samples aged at the accelerated temperatures. However, none of them interfered with the quantitation of the three active drug substances (II, III, IV) or even I.

The analytical method was used to evaluate the stability of TCIS in the cream formulation. The assay results of samples kept at 45°C, 37°C and 22°C were analyzed statistically assuming first order kinetics. The natural log of percent remaining for every one of the TCIS steroids were plotted versus time. Table 1 lists the calculated rate constants from such plots as well as other statistical parameters. The data indicate that the relative chemical stability of the steroids in the cream is ciprocinonide > procinonide > fluocinonide. This is in agreement with the reported (Thompson and Chan, 1980) chemical reactivities of these steroids in aqueous solutions.

Recovery linearity and precision

Known amounts of II, III and IV were added to samples of placebo cream corresponding to 0, 70, 80, 90, 100, 110 and 120% of labeled strength. These samples were analyzed by the analytical procedure (Table 2). A plot of the amount of each steroid added versus the amount recovered indicated that statistically the slopes and intercepts were not significantly different from 1 and 0, respectively. These data and

TABLE 1

FIRST-ORDER RATE CONSTANTS[a] FOR THE DEGRADATION OF THE STEROIDS IN THE TCIS CREAM FORMULATION

Steroid	Temperature		
	22°C	37°C	45°C
Fluocinonide (II)	4.3 ± 4.7 (1.1)	32.3 ± 10.2 (1.4)	81.0 ± 13.0 (1.1)
Procinonide (iII)	0.5 ± 5.5 (1.3)	15.4 ± 11.1 (1.5)	44.6 ± 13.7 (1.2)
Ciprocinonide (IV)	2.7 ± 9.8 (2.3)	5.0 ± 20.0 (2.7)	6.5 ± 28.4 (2.5)

[a] Rate constant ± 90% CI × 10^4 $months^{-1}$ (standard error of regression × 10^2).

TABLE 2

RECOVERIES AND LINEARITY OF THE ASSAY PROCEDURES FOR FLUOCINONIDE, PROCINONIDE AND CIPROCINONIDE

Sample (% LS[a])	Amount added (mg)	Amount recovered (mg)	Recovery (%)
Fluocinonide (II)			
70%	0.129	0.130	100.8
80%	0.148	0.149	100.7
90%	0.166	0.167	100.6
100%	0.185	0.186	100.5
110%	0.203	0.204	100.5
120%	0.222	0.223	100.4
mean			100.6
RSD			0.1
Procinonide (III)			
70%	0.050	0.049	98.0
80%	0.057	0.056	98.2
90%	0.064	0.063	98.4
100%	0.071	0.071	100.0
110% LS	0.078	0.078	100.0
120% LS	0.085	0.084	98.8
mean			98.9
RSD			0.9
Ciprocinonide (IV)			
70%	0.029	0.029	100.0
80%	0.034	0.034	100.0
90%	0.038	0.037	97.4
100%	0.042	0.041	97.6
110%	0.046	0.045	97.8
120%	0.050	0.050	100.0
mean			98.8
RSD			1.3

[a] LS = labeled strength.

complete recoveries show that the assay is linear and accurate between 70 and 120% of label strength.

The precision of the method was tested by assaying a lot of TCIS cream which was 31 months old. The assays were performed by two analysts on two different days. The results (Table 3) indicate that the method is precise.

Lower limit of detection

The ability of the method to detect the major degradation product (I) was evaluated by adding known amounts of this steroid to samples of placebo cream corresponding to 0–12% of total steroids label strength (0.015%). These samples were analyzed and the corresponding peak height of I was plotted versus the percent added (Fig. 3). The lower limit of detection was defined in this study as that

TABLE 3

ASSAY RESULTS FOR TCIS CREAM FORMULATION

Analyst/day	Recovery, % of labeled strength		
	Fluocinonide	Procinonide	Ciprocinonide
1/1	101.0	102.5	103.0
	101.3	102.0	106.6
	101.2	101.9	100.3
	101.6	102.5	101.9
1/2	100.4	103.1	103.0
	100.3	104.4	104.4
	100.9	102.1	100.7
2/1	101.0	102.2	100.6
	100.2	100.8	102.5
	99.5	99.4	103.7
	100.9	99.6	102.5
2/2	101.1	103.5	104.1
	99.5	100.3	99.1
	101.2	103.9	103.9
	98.2	98.4	97.3
mean	100.6	101.8	102.2
RSD	0.9	1.7	2.2

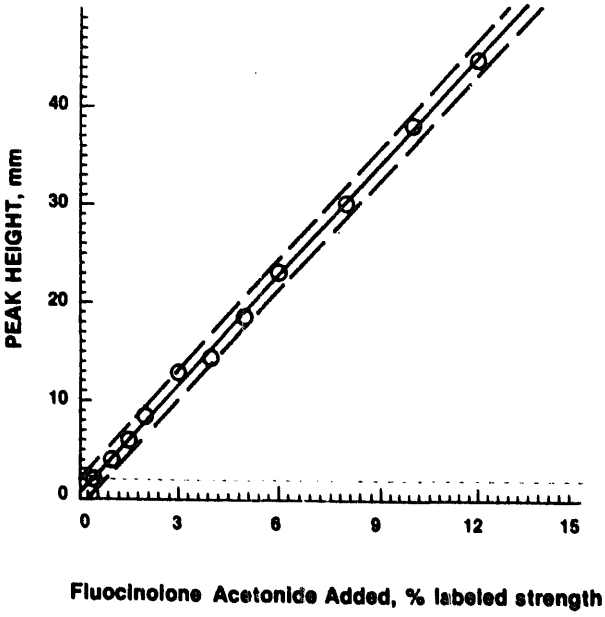


Fig. 3. Lower limit of detection for fluocinolone acetonide. Linear regression line (———), 95% confidence interval (— — —). Noise level was estimated to be 1 mm and the lower limit of detection 0.85% labeled strength (1.3 μg/g).

concentration which resulted in a signal to baseline noise ratio of 2. The recorder baseline noise level was estimated to be 1 mm. A conservative estimate of the lower limit for detecting I was based on the 95% lower confidence limit of individual points of the linearity data. Fig. 3 shows that I can be detected at a level of 0.85% (equivalent to 1.3 μg/g) of total steroids concentration using the analytical procedures described in this study.

Chromatography characteristics

To characterize further the chromatographic system, the effects of the 3 solvents composing the mobile phase on the capacity factors (k') of the steroids (at 45°C) was studied. The concentration of one of the solvents in the ternary mobile phase system was kept constant while the concentrations of the other two were varied. A semilogarithmic plot of k' versus acetonitrile concentration in mobile phase (% v/v) at a constant level of 10% v/v THF shows linear behavior (Fig. 4). Similar linear plots showing the effect of THF concentration on the capacity factors at constant level of water (60% v/v) and acetonitrile (30% v/v) are shown in Fig. 5 and Fig. 6, respectively. The excellent linear dependence of log k' on the concentrations of the organic modifiers in the mobile phase indicates that the retention of the steroids, in the reversed phase HPLC system, is due to partitioning of the analyte between the stationary and mobile phases. This is generally expected for non-polar steroids (Munson and Wilson, 1981). The retention of the steroids paralleled their expected polarity. While adequate resolution of I and II as well as of II and III and of II and

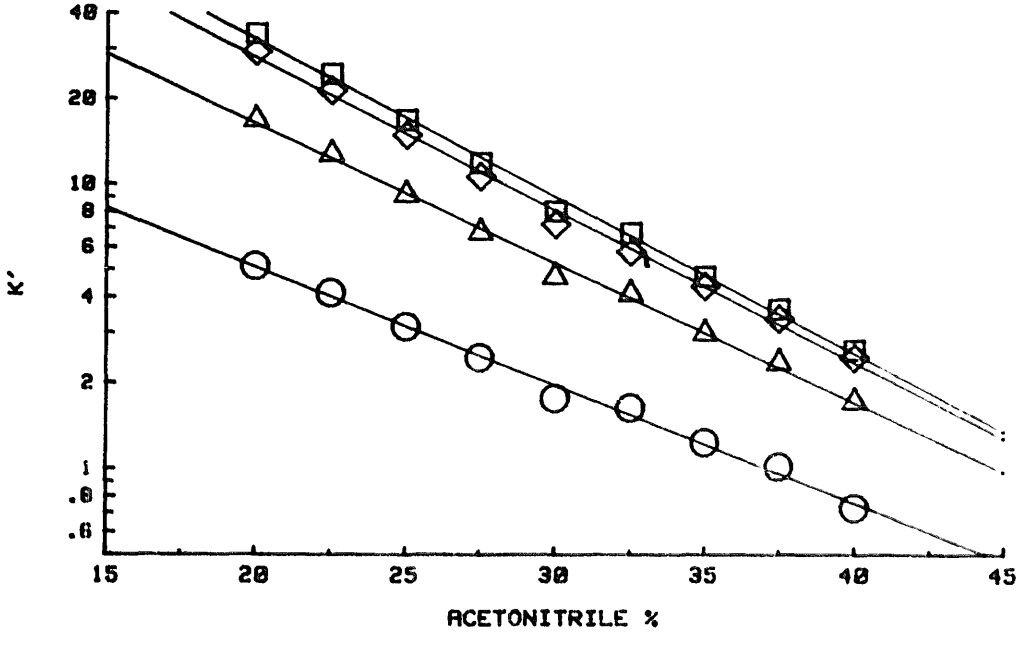


Fig. 4. Relationship of log k' (capacity factor) and acetonitrile concentration (% v/v) in water for fluocinolone acetonide (○), fluocinonide (△), procinonide (◇) and ciprocinonide (□). The mobile phase contains also 10% v/v THF.

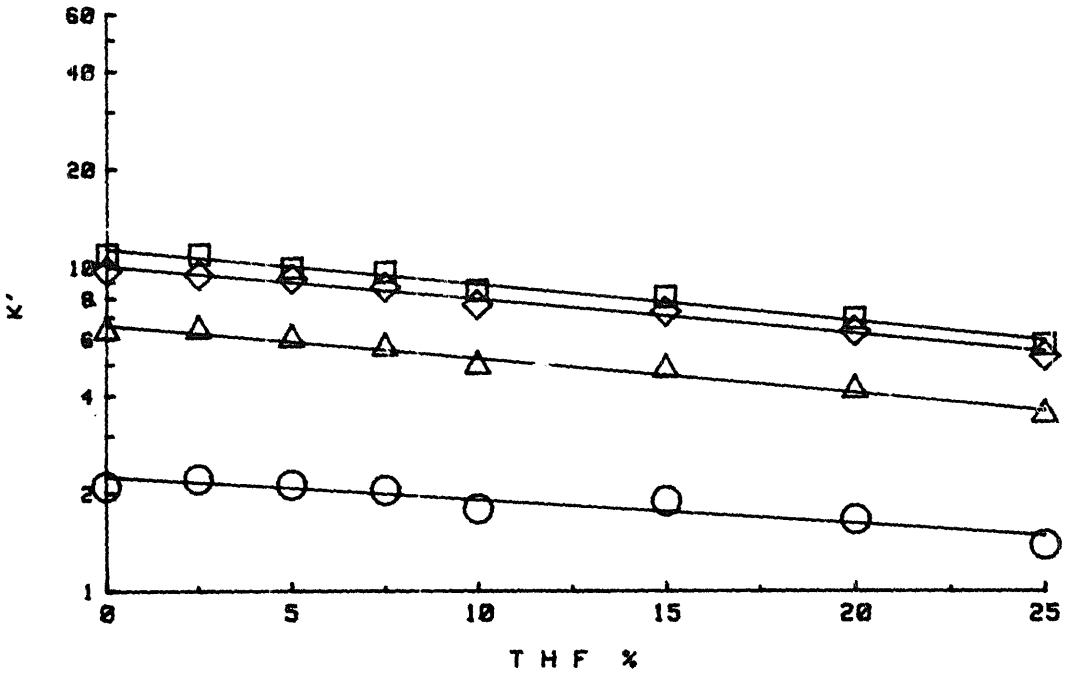


Fig. 5. Relationship of log k' (capacity factor) and THF concentration (% v/v) in acetonitrile for fluocinolone acetonide (○), fluocinonide (△), procinonide (◇) and ciprocinonide (□). The mobile phase contains also 60% v/v water.

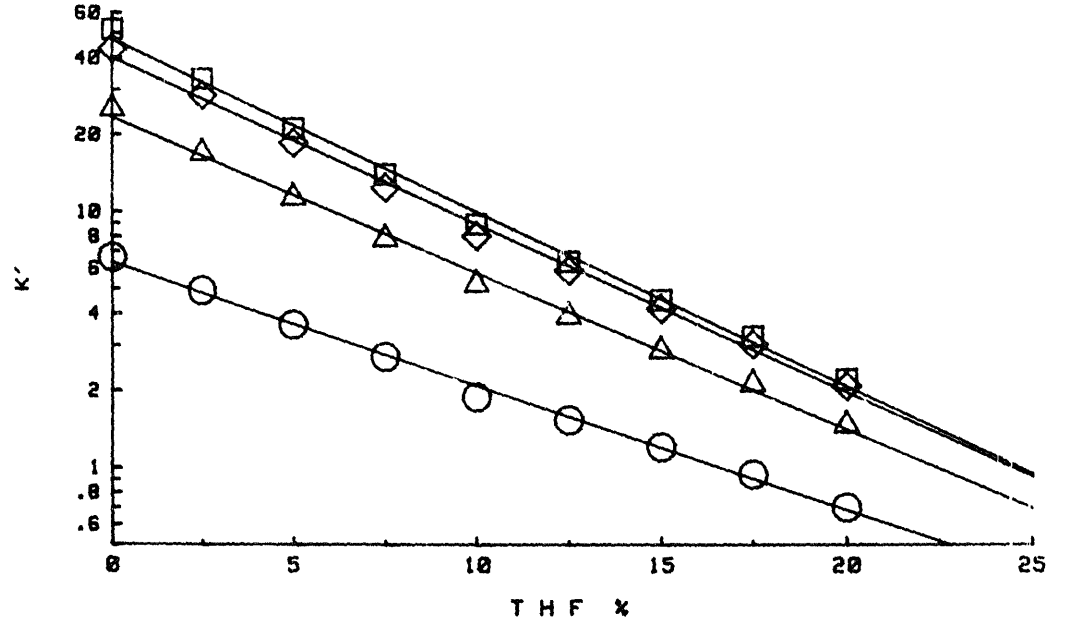


Fig. 6. Relationship of log k' (capacity factor) and THF concentration (% v/v) in water for fluocinolone acetonide (○), fluocinonide (△), procinonide (◇) and ciprocinonide (□). The mobile phase contains also 30% v/v acetonitrile.

IV could be achieved easily with all the compositions of the mobile phase (Figs. 4, 5 and 6), resolution of III and IV was less well defined. Cyclization of the additional carbon of IV (relative to III) reduces the difference in the polarity characteristics of these two compounds and thus, makes their resolution less evident.

THF showed the strongest eluting property. However, it did not affect dramatically the selectivity of the system (Fig. 6). This was also true with acetonitrile (Fig. 4). It is possible that the resolution of the steroids in this HPLC system is governed by their hydrophobic interactions with the apolar stationary phase in presence of water. The main role of the organic modifiers in this system was to reduce retention times and shortened assay period rather than improvement in the selectivity.

The effect of column temperature on the chromatography characteristics was evaluated at a specific mobile phase composition. Fig. 7 shows Van 't Hoff plots of k' for the steroids determined in THF–acetonitrile–water system (10:30:60) at ambient (22°C), 38°, 45°, 55° and 65°C. In addition, separation parameters for III and IV such as resolution and selectivity were calculated as previously described (Snyder and Kirkland, 1974) and are listed in Table 4. The data indicate that while temperature had no significant effect on selectivity of the system it had some effect on the resolution. In general, increase in the temperature reduced the resolution of

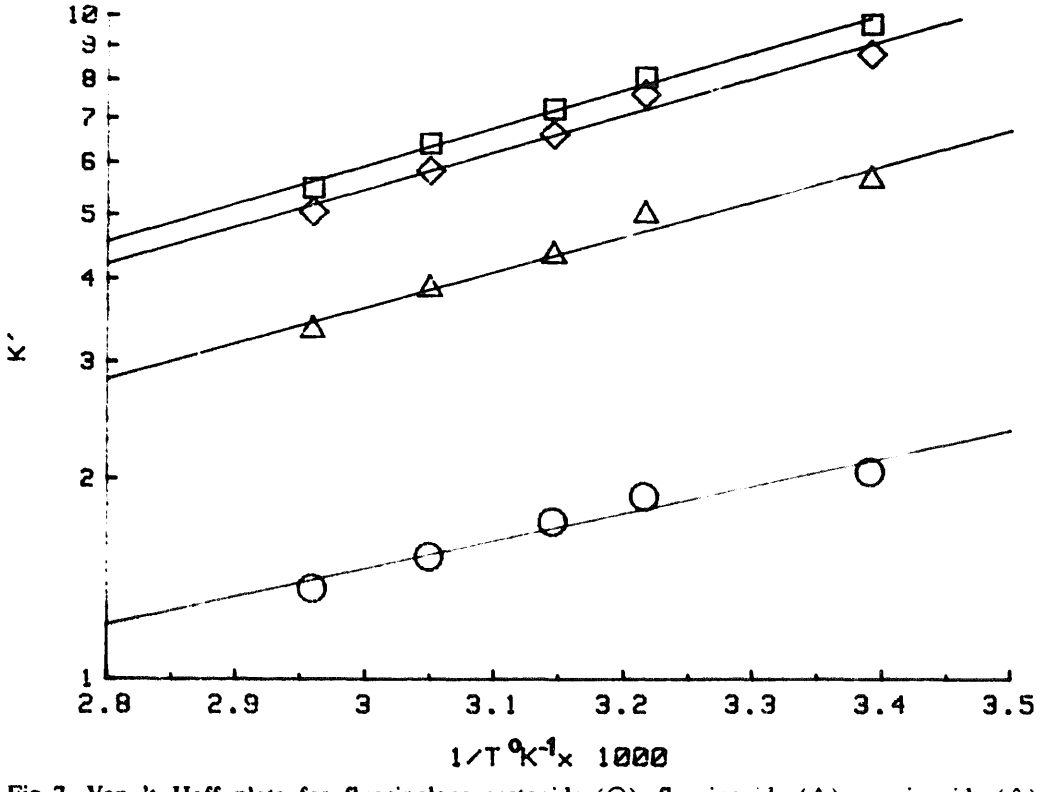


Fig. 7. Van 't Hoff plots for fluocinolone acetonide (○), fluocinonide (△), procinonide (◇) and ciprocinonide (□) capacity factors (k') determined in THF–acetonitrile–water (10:30:60) mobile phase and a RP-18 column.

TABLE 4

TEMPERATURE DEPENDENCE OF RESOLUTION (R) AND SELECTIVITY (α) OF PROCINONIDE (III) AND CIPROCINONIDE (IV) IN THF–ACETONITRILE–WATER (10:30:60) SYSTEM AND RP-18 COLUMN

Parameter	Temperature (°C)				
	22	38	45	55	65
$R_{III, IV}$	2.30	2.25	2.13	2.00	1.83
$\alpha_{III, IV}$	1.11	1.06	1.09	1.10	1.15

III and IV slightly. Thus, increasing the column temperature exerts the same effect on retention behavior as increasing the concentration of the organic modifiers in the mobile phase, namely a reduction in the retention times (Fig. 7). However, this also reduced to some extent the resolution capability of the system.

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