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On-line HPLC method for clean-up and analysis of hydrocortisone and sulconazole nitrate in a cream

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Summary

The application of a new on-line high-performance liquid chromatographic method for the clean-up and analysis of drugs in semi-solid formulations was investigated. A short column of a chemically bonded stationary phase (10 μm octadecylsilane) mounted into the sample loop position of the high pressure injection valve was used as the primary clean-up step. The elution behavior of commonly used cream excipients on the loop column as a function of various combinations of water, methanol and THF was studied using a refractive index detector. Typically, samples of the cream were dissolved in methanol–THF and injected onto the loop column. The drug substances were eluted onto the analytical column with predetermined volumes of the mobile phase. The relatively non-polar cream excipients were retained on the loop column. At this time the injection valve was switched, isolating the loop column and the retained excipients were eluted off to waste with a strong solvent (methanol–THF). Loss of efficiency due to the insertion of the loop column was negligible. This technique was applied to the simultaneous analysis of a corticosteroid (hydrocortisone) and a hydrophobic amine (sulconazole nitrate) in a topical cream formulation.

Introduction

Quantitative extraction of drug substances from a complex matrix usually has involved the classical techniques of liquid–liquid extraction, thin-layer chromatography and column chromatography. These procedures are laborious and time consuming. In addition, liquid–liquid extraction involves the use of and exposure to large

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amounts of relatively toxic solvents. High-performance exclusion chromatography has been proposed (Majors and Johnson, 1978) for the analysis and clean-up of semisolid dosage forms: a low molecular weight steroid was separated from excipients via size exclusion and monitored directly as it eluted from the gel permeation column. However, this method lacks selectivity because of the inability of the column to resolve compounds in the same molecular weight range. These might include most of the degradation products and structurally related components present in the matrix.

Recently developed techniques have attempted to reduce sample handling and solvent use prior to the high-performance liquid chromatographic (HPLC) analysis. Commercially available cartridges packed with reversed-phase microparticulate material (Sep-Pak C_{18}) were used (Whitney, and Thaler, 1980) to separate hydrophobic drugs from biological fluids and aqueous solutions. However, the unit is not compatible with strong solvents such as tetrahydrofuran (THF) which can be used for the complete dissolution of cream samples. Mini-columns of granular Kieselgur (Extrelut) have also been used (Bamberg et al., 1980) for the extraction of steroids from plasma. However, the sample clean-up still involved multisolvent elution, fraction collection and concentration. Since these sample clean-up steps are outside the HPLC system, the potential for their automation is lacking.

There has been an increasing interest in multidimensional column chromatography or column switching for the separation and analysis of multicomponent samples. These have been reviewed by Majors (1980) and Freeman (1981). In this technique, fractions from one column are selectively transferred to one or more secondary columns for further separation. This technique can be carried out off-line or on-line. The on-line mode is normally preferred for convenience and its potential for automation. Such on-line liquid chromatographic/liquid chromatographic (LC/LC) techniques have been applied to the separation and analysis of pesticides and herbicides in nutritional products (Dolphin et al., 1976; Apffel et al., 1981) and drugs and their metabolites in biological samples (Apffel et al., 1981). These separations were primarily done with long precolumns and often encountered band broadening due to sample injection volume.

The aim of this study was to extend the application of on-line multidimensional HPLC to the separation and analysis of drug substances in semisolid dosage forms. In this work a short (3 cm × 4.6 mm i.d.) reversed-phase precolumn in the sample loop of a 6-port injection valve was intended to be utilized as the primary clean-up column coupled to a reversed-phase analytical column. Similar loop columns have previously been used for trace enrichment applications (Koch and Kissinger, 1980; Burns, 1981). This paper describes a rationale for the selection of parameters for a simple clean-up procedure and simultaneous HPLC analysis of hydrocortisone and sulconazole nitrate, two drugs of very different properties, in a topical cream formulation.

Materials and methods

Reagents and chemicals

All chromatographic solvents were glass-distilled and spectral grade (Burdick and Jackson, U.S.A.). Water purified with the Barnstead Nanopure (U.S.A.) system was used. All mobile phases were filtered, brought to ambient temperature and degassed. Potassium perchlorate was purchased from J.T. Baker Chemicals (U.S.A.). Ion-pairing reagents were obtained from Eastman Kodak (U.S.A.). All other reagents were obtained from Mallinckrodt (U.S.A.). The formulation excipients were USP grade. Hydrocortisone was obtained from Upjohn (U.S.A.) and sulconazole nitrate was obtained from the Institute of Organic Chemistry. Syntex Research (U.S.A.).

Apparatus

The modular HPLC consisted of a dual piston pump (Constametric IIG, Laboratory Data Control, U.S.A.), a syringe-load 6-port injector (Model 7125, Rheodyne, U.S.A.) equipped with a loop column (MPLC 3 cm or 10 cm × 4.6 mm i.d., RP-18 packed with Lichrosorb or Partisil-10-ODS-3, Brownlee Labs, U.S.A.), a variable wavelength detector (SpectroMonitor III, Laboratory Data Control, U.S.A.) or a refractive index detector (Model 771, Micromeritics, U.S.A.) and a dual pen recorder (Model 585, Linear, U.S.A.). Two microparticulate octadecylsilane (ODS) 10 μm analytical columns and a microparticulate trimethylsilane (TMS) 7 μm column were obtained commercially. These included a μBondapak C_{18} (30 cm × 4 mm, Waters Associates, U.S.A.), a Partisil-10-ODS-3 (25 cm × 4.6 mm, Whatman, U.S.A.) and a Zorbax TMS (25 cm × 4.6 mm, DuPont, U.S.A.), respectively. A guard column (7.0 cm × 2.1 mm) dry packed with Co:Pell ODS (Whatman, U.S.A.) was coupled to the analytical column.

Elution behavior of excipients and drug substances on loop columns

This study was performed with the loop column on-line as a small column between the injector and detector and also as a loop in the injector with forward flushing of the elution solvent. No other column was present in the system. The retention volumes of corticoids and cream excipients were examined using each of these configurations. Injection solutions were prepared by separately agitating cream excipients in methanol to obtain saturated solutions which were then clarified by centrifugation at 1500 rpm for 10 min when necessary. Methanol containing THF was used to prepare solutions of mineral oil. Approximately 20 μl of each excipient solution was injected onto the loop column. Volumes of corticoids solution injected were 10–15 μl. The compounds were eluted using various compositions of methanol–water and methanol–THF as mobile phase at a flow rate of 1.0 ml/min. A refractive index detector at 5×10^{-5} RI units and a variable wavelength UV detector at 254 nm with a sensitivity of 0.04 AUFS arranged in series were used for detection.

HPLC conditions for cream assay

In selecting a mobile phase for the simultaneous chromatography of hydrocorti-

sone and sulconazole nitrate, the composition of mobile phase was varied and the corresponding change in capacity factor (k') was monitored. The final composition of the mobile phase was methanol–water–0.04 N HCl (63 : 36 : 1 v/v/v) containing 0.005 M sodium pentanesulfonate. The apparent pH of the mobile phase was approximately 3.7. The flow rate was 1.2 ml/min. The detector wavelength and sensitivity were 218 nm and 0.01 AUFS, respectively.

Preparation of calibration standard solutions

Standard stock solutions of hydrocortisone and sulconazole nitrate were prepared in methanol (75, 100 and 125 μg/ml). A 100 μg/ml solution of the internal standard (norethindrone) in methanol was also prepared. The calibration standard solutions were prepared by diluting 5 ml aliquots of each of the reference standard stock solutions and the internal standard solution to 25 ml with methanol. This resulted in final concentrations of 15, 20 and 25 μl/ml of the active drugs and 20 μg/ml of the internal standard.

Sample preparation and assay procedure

Accurately weighed samples (2.0 g) of cream were dissolved in 20 ml of THF by agitation in an ultrasonic bath. The THF solution was diluted to 200 ml with methanol. A 5 ml aliquot of this methanol–THF solution and 5 ml of the internal

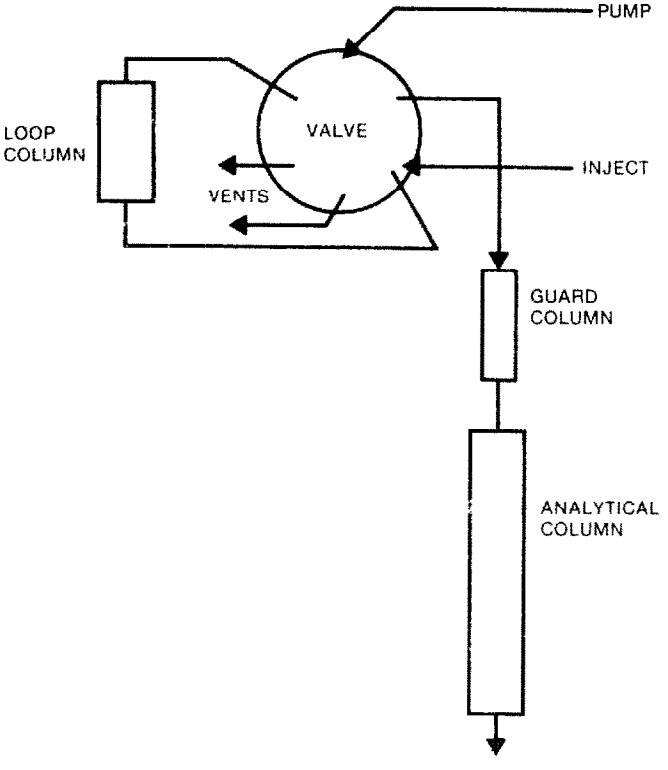

Fig. 1. Schematic diagram of the HPLC system for the on-line clean-up and analysis.

standard solution were diluted to 25 ml with methanol.

A 10 μl volume of sample or calibration standard solution was injected onto the system (Fig. 1) with the loop column in the 'load' position. The valve was returned to the 'inject' position placing the loop column on-line with the analytical column. The drug substances were allowed to elute from the loop column with mobile phase for 5 min. The valve was then returned to the 'load' position and the loop column was forward-flushed with 1 ml THF to remove the trapped excipients. When all peaks of interest had eluted from the analytical column, the valve was switched to the 'inject' position to allow the loop column, guard column and analytical column to equilibrate with mobile phase prior to the next injection.

Results and discussion

Sample clean-up and assay procedure

It was important to determine which excipients are retained on the loop column

TABLE 1

RETENTION VOLUMES (ml) OF CORTICOSTEROIDS AND EXCIPIENTS ON 3 cm LOOP COLUMN

Configuration	Sample	Methanol–water				Methanol–THF
		70:30	80:20	90:10	100:0	75:25
In place of analytical column	Norethindrone	a	a	a		
	Polysorbate 60	b	6.8	2.4	2.0	
	Sorbitan monostearate		4.0, [c] 8.0	2.0	2.0	
	Stearyl alcohol		23.2	3.2	a	
	Ascorbyl palmitate	28	4.4	a	a	
	Isopropyl myristate	b	12.4	a		
	Glyceryl stearate with PEG-100 stearate	b	4.8, [c] 11.6	a		
	Mineral oil		b	b	6.8	2.0
As loop	Norethindrone		a	a		
	Polysorbate 60	b	6.0	2.8	2.0	
	Sorbitan monostearate	b	2.0	3.2	2.0, [c] 2.8	
	Stearyl alcohol		24.0	4.0	a	
	Ascorbyl palmitate	b	4.0	a	a	
	Isopropyl myristate		12.0	a	a	
	Glyceryl stearate with PEG-100 stearate	b	4.8, [c] 8.0, 11.2	2.6, [c] 3.2	a	
	Mineral oil		b	b	7.0	

a = Eluted with solvent front. b = Not eluted within 20 min. c = More than one peak observed in most cases.

TABLE 2

RETENTION VOLUMES (ml) OF CORTICOSTEROIDS AND EXCIPEINTS ON 10 cm LOOP COLUMN

Configuration	Sample	Methanol–water					Methanol–THF		
		60:40	70:30	80:20	90:10	100:0	95:5	85:15	75:25
In place of analytical column	Hydrocortisone	2.8			a	a			
	Polysorbate 60		b	4.0 [c], 8.8	2.8 [c], 4.4, 6.8	a			
	Sorbitan monostearate			14 [c], 26.8	2.8 [c], 4.4, 7.6	2.4 [c] 3.6, 4.8			
	Stearyl alcohol	b	b	62	9.2	1.2			
	Ascorbyl palmitate		b	13.2	3.2	a			
	Glyceryl stearate with PEG-100 stearate			19.2 [c], 26.0, 35.2	3.2 [c], 4.4, 6.4	a			
	Mineral oil						b	5.2	3.0
As loop	Norethindrone	13.6	4	a	a				
	Polysorbate 60		b	b	2.8 [c], 4.0, 6.4	a			
	Sorbitan monostearate			b	2.8 [c], 4.0, 6.8	3.2			
	Stearyl alcohol		b	63.0	8.4	3.0			
	Ascorbyl palmitate		b	12.0	2.4				
	Isopropyl myristate		b	43.2	7.2	2.0			
	Glyceryl stearate with PEG-100 stearate		b	14.4	2.0 [c], 3.0, 4.8	a			
	Mineral oil								2.8

a = Eluted with solvent front. b = Not eluted within 20 min. c = More than one peak observed in most cases.

with the mobile phase used in the assay and to select a solvent strong enough to elute most retained excipients from this column. Thus, the retention behavior of excipients and the corticosteroids on the loop column was examined as a function of mobile phase composition. Since most of the excipients do not possess UV absorbing chromophores, a refractive index detector was used. Table 1 lists the retention times of the cream excipients on the 3 cm ODS loop column as a function of loop column configuration and mobile phase composition. When the loop column was placed on-line (in place of the analytical column) or in the loop and forward-flushed, the non-polar excipients did not elute with the mobile phase used in the analytical method. It was possible to elute them by increasing the methanol concentration of the mobile phase. However, mineral oil did not elute until THF was added to methanol. A 1.0 ml loop column wash of pure THF was found to elute the excipients as well as a 2.0 ml mixture of methanol–THF (75 : 25).

To resolve early eluting excipient peaks from the solvent front, a 10 cm ODS loop column was substituted. Retentions of the excipients on the 10 cm column in the two configurations are shown in Table 2. The excipients were well resolved from the solvent front and their retention behavior relative to the steroids was about the same as found using the 3 cm column. The order of elution did not change whether the loop column was on-line (replacing the analytical column) or placed in the injection loop. Sulconazole nitrate was not injected in these experiments because the mobile phase did not contain acid or ion-pair reagent and under these conditions it would not elute. However, using the mobile phase of the analytical method containing ion-pair reagent, sulconazole nitrate eluted from the analytical column after the internal standard. Therefore it was assumed that the elution behavior of sulconazole nitrate on the loop column would be similar to the internal standard.

This assay procedure was validated by evaluating recoveries of spiked placebos corresponding to 100% of labeled strength (LS) (Table 3). Complete recoveries of both compounds were obtained using this procedure. The linearity was demon-

TABLE 3

RECOVERIES OF HYDROCORTISONE AND SULCONAZOLE NITRATE FROM PLACEBOS SPIKED AT 100% LABELED STRENGTH (LS)

Hydrocortisone			Sulconazole nitrate		
Mg added	Mg found	% recovered	Mg added	Mg found	% recovered
20.38	20.33	99.7	20.42	20.42	100.0
20.36	20.15	99.0	19.90	19.61	98.5
20.48	20.91	102.1	19.91	20.32	102.1
20.53	20.80	101.3	19.92	19.60	98.4
20.15	20.67	102.6	20.17	19.86	98.4
Mean recovery		100.9%			99.5%
Standard deviation		1.5%			1.6%
95% confidence limits		99.4–102.4%			97.9–101.1%

TABLE 4

RECOVERIES OF HYDROCORTISONE AND SULCONAZOLE NITRATE FROM PLACEBOS SPIKED AT 0 AND 60–120% LS [a] LEVEL

	Hydrocortisone		Sulconazole nitrate	
	% LS added	% LS found	% LS added	% LS found
	0	0	0	0
	61.9	61.3	62.4	61.2
	79.2	78.4	82.6	81.2
	90.4	90.7	92.8	87.5
	100.2	104.8	100.2	97.8
	117.7	115.4	120.9	122.8
Slope ± 95% confidence limits		1.003 ± .079		0.9941 ± .079
Intercept		−0.0058 ± 1.3		−0.1935 ± 1.4
Standard error of regression		0.525		0.538
Correlation coefficient		0.99839		0.99836

[a] Labeled strength.

strated by assaying samples spiked with each component at 0 and 60–120% LS (Table 4). A plot of percent LS added vs percent LS recovered indicated that the slopes and intercepts were not significantly different from one and zero, respectively.

This clean-up and assay procedure was used to assay many cream samples without significant deterioration of the loop or analytical columns. Thus, this simple on-line HPLC method is feasible for clean-up and analysis of drug substances in cream formulations. The clean-up procedure is flexible and can be adapted to other mobile phase compositions. Excipients which elute with or before the active ingredient are not expected to accumulate on the analytical column. The excipients that are retained on the loop column can be eluted to waste using a THF wash.

Chromatographic conditions

Another aim of this study was to select a chromatography system which would enable a simultaneous determination of hydrocortisone and sulconazole nitrate. Chromatography of hydrophobic amines on reversed-phase packing material has been associated with asymmetric peak shapes and long retention times due to ion exchange or adsorption reactions of the solute amine with non-bonded silanol sites on the stationary phase (Sokolowski and Wahlund, 1980). Stationary phases which possess a maximum surface coverage by C_8 and C_{18} groups and which have been exhaustively end-capped have been shown (Knox and Jurand, 1977; Tanaka, 1978) to result in improved chromatography of such amines. Two C_{18} columns, μBondapack and Partisil-10-ODS-3, and one trimethylsilane column were tested. The Partisil-10-ODS-3 analytical column gave the best results probably due to its higher

coverage of silanol sites. The data presented in this study were generated using a Lichrosorb C_{18} loop column. Subsequent lots of these loop columns did not allow elution of sulconazole nitrate. However, it was found that a Partisil-10-ODS-3 loop column yielded superior chromatography for this amine.

Mobile phase containing 60% methanol in water resulted in a reasonable retention time for hydrocortisone. However, addition of an ion-pairing agent was necessary to elute sulconazole nitrate. Mobile phases containing perchlorate or methanesulfonate did not provide good resolution between hydrocortisone and one of the degradation products of sulconazole nitrate. Improved resolution was obtained using pentanesulfonate as the ion-pair reagent. The effects of changes in the pentanesulfonic acid content of the mobile phase on the capacity factor (k′) of the compounds of interest are shown in Fig. 2. The retention of hydrocortisone was not affected by the ion-pair reagent. However, the semilogarithmic plots of k′ vs pentanesulfonic acid concentration for sulconazole nitrate and its major degradation product were reasonably linear with positive slopes. This indicated that the retention of these two compounds is due to an ion-pairing phenomenon. The effect of pH and

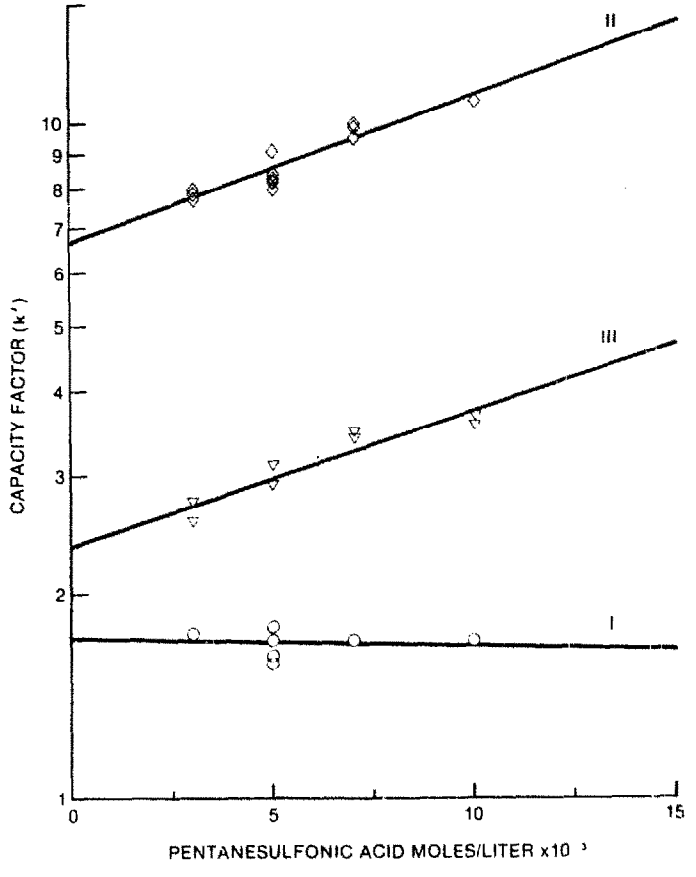

Fig. 2. Relationship of k′ (capacity factor) and pentanesulfonic acid concentration in the mobile phase for hydrocortisone (I), sulconazole nitrate (II) and the major degradation product of sulconazole nitrate (III). Mobile phase methanol–water (60:40) containing 1% acetic acid.

TABLE 5

EFFECT OF METHANOL AND ACID CONCENTRATIONS ON THE CAPACITY FACTOR (k')

Methanol [a] (%)	Acetic acid (%)	pH	k'		
			Hydrocortisone	Sulconazole nitrate	Degradation product
50	1.0	3.5	8.2	b	7.5
60	1.0	3.5	3.2	5.8	2.0
70	1.0	3.5	1.5	1.5	1.5
63	0.0	5.7	c	b	c
63	0.5	3.6	2.1	3.9	1.3
63	1.0	3.4	2.5	3.8	1.4
63	2.0	3.4	1.7	2.9	1.0

a = Mobile phase contained 0.001 M $KClO_4$ and various concentrations of water. b = Did not elute. c = Not injected.

methanol content of the mobile phase on the capacity factor of the components of the chromatogram were determined and are shown in Table 5. Sulconazole nitrate appeared to be relatively sensitive to changes in pH and methanol content. It did not elute when acid was eliminated from the mobile phase probably due to interaction between the hydrophobic moiety of the unionized amine and the stationary phase. A mobile phase of apparent pH below 4.0 yielded best results.

The concentrations of hydrocortisone and sulconazole nitrate are the same in the formulation. However, the former elutes much earlier than the latter and also has a higher extinction coefficient at conventional wavelengths of 254 or 280 nm resulting in disproportionate peak sizes for the compounds. Therefore an optimum wavelength of 218 nm was chosen to maximize the response of sulconazole nitrate and reduce the response of hydrocortisone. The detection at 218 nm made it possible to quantitate both compounds using the same detector sensitivity. Acetic acid in the mobile phase interfered with detection at this low wavelength. Use of hydrochloric acid to obtain the same apparent pH seemed to be satisfactory.

The effect of the loop column on the overall efficiency of the system was evaluated by chromatographing a calibration standard solution with and without the loop column and calculating the theoretical plate count (N). The data indicated that the loss in efficiency due to the loop column was not significant. However, the efficiency was low in both cases (n = 760–860). This could be due to band broadening caused by injecting samples in a stronger eluting solvent (methanol–THF) than the mobile phase (Tseng and Rogers, 1978).

Specificity of the method

A method is specific for a particular compound if the measured response is due to that compound and due to no other compound present in the sample. The specificity of the method for sulconazole nitrate was shown by chromatographing standard

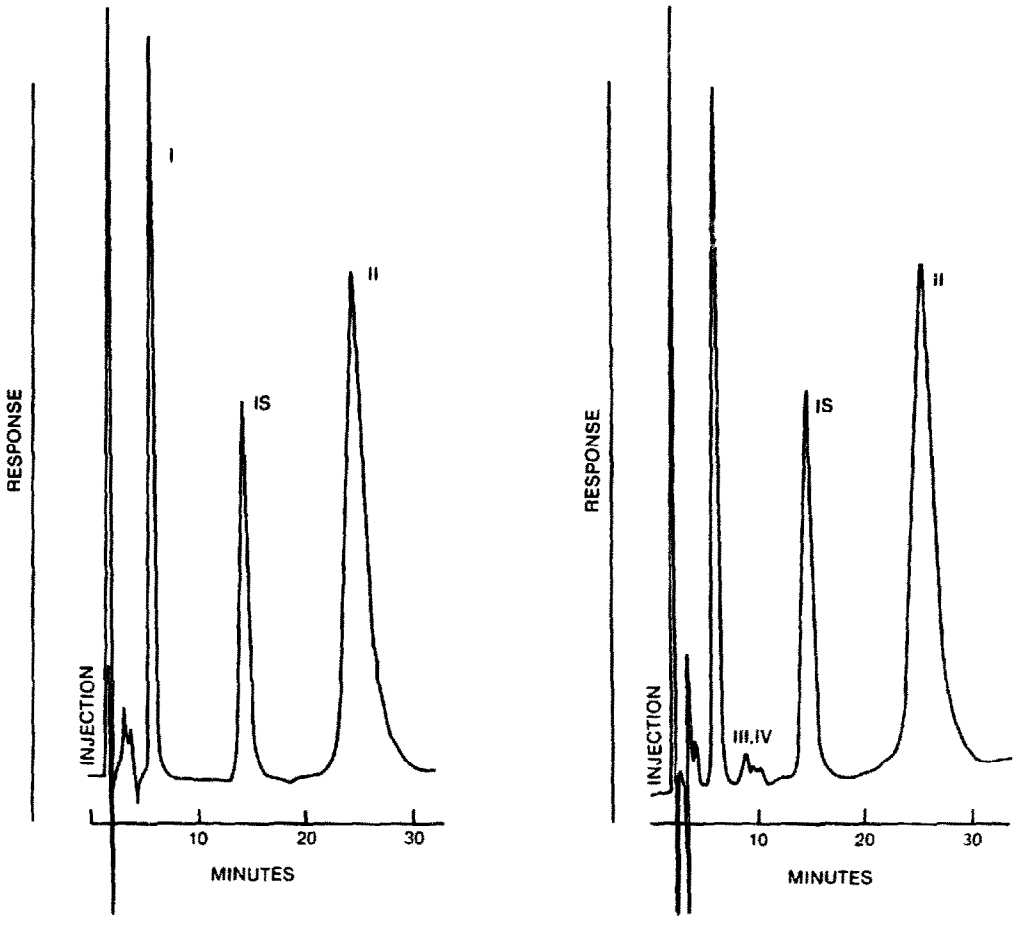

Fig. 3. Chromatographic tracing of a typical calibration standard solution containing hydrocortisone (I), sulconazole nitrate (II) and the internal standard (IS).

Fig. 4. Chromatographic tracing of a degraded cream sample showing the degradation products (III and IV).

TABLE 6

QUANTITATION OF HYDROCORTISONE IN DEGRADED SAMPLES

Sample [a]	Mobile phase A [b]	Mobile phase B [c]
1	95,92	95,92
2	90,87	94,91
3	85,84	86,82
4	108	109
5	100,98	102,97
6	84,85	86,84

[a] The storage conditions were different for each sample.

[b] 63:36:1 methanol–water–0.04 N HCl plus 0.005 M pentanesulfonic acid.

[c] 58:42 methanol–water.

solutions of sulconazole nitrate, its possible degradation products, internal standard and hydrocortisone using the HPLC conditions of the method. A chromatogram of the calibration standard is shown in Fig. 3 and a chromatogram of a degraded sample is illustrated in Fig. 4. In addition, overly stressed cream samples containing hydrocortisone and sulconazole nitrate in combination and each component by itself were chromatographed and checked for interference. Since hydrocortisone and its degradation products eluted close to the solvent front and the possible degradation products of sulconazole nitrate, its specificity was substantiated by re-assaying overly stressed samples of the combination cream employing a weaker eluting mobile phase of methanol–water (58 : 42) without ion-pair reagent. This trapped sulconazole nitrate and its possible degradation products on the loop column preventing any potential interference with the quantitation of the hydrocortisone. The results are presented in Table 6. Comparable recoveries using both mobile phases further support the specificity of the method for hydrocortisone.

Conclusions

Excellent recovery and linearity data for hydrocortisone and sulconazole nitrate from cream formulations indicate that this procedure is feasible for the rapid and simple on-line clean-up and analysis of compounds of varied polarities from a complex matrix without conventional extractions.

The major drawback of the present procedure is that the sample introduction into the injection port and switching of valves are performed manually. However, the concept has great potential for automation. The instrumentation and conditions for automated operation have been completed and are reported elsewhere (Conley and Benjamin, 1982).

Acknowledgements

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