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A stability-indicating HPLC assay with on-line clean-up for betamethasone 17-valerate in topical dosage forms

E.W. Smith, J.M. Haigh and I. Kanfer
School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140 (South Africa)

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Summary

A stability-indicating high-performance liquid chromatographic method with on-line clean-up has been developed for the analysis of betamethasone 17-valerate in topical dosage forms. A short pre-column containing 10 μm octadecylsilane mounted into the sample loop position of an injection valve was used as the primary clean-up step. The utilization of a diode-array UV detector allowed the quantitative analysis of betamethasone 17-valerate together with its degradation product, betamethasone 21-valerate, as well as the qualitative analysis of these compounds, relevant internal standards and the preservatives chlorocresol and methyl hydroxybenzoate contained in the cream and lotion formulations, respectively. Typically, cream and lotion dosage forms were dissolved in acetonitrile and ointments in tetrahydrofuran, internal standards added and aliquots injected onto the analytical system. Dosage form excipients were retained on the loop column and back-flushed to waste with the aid of a second solvent pump while components of interest were allowed to transfer to the analytical column for quantitative analysis. The method is accurate, precise and stability indicating and permits the rapid on-line analysis of betamethasone 17-valerate from complex topical formulation matrices without prior extractions.

Correspondence: I. Kanfer, School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140, South Africa.

Introduction

A stability-indicating high-performance liquid chromatographic (HPLC) assay for betamethasone 17-valerate and its degradation product, betamethasone 21-valerate, in cream, lotion and ointment formulations was developed using an on-line sample clean-up approach. Other assay methods previously reported for betamethasone 17-valerate topical dosage forms involve a liquid–liquid extraction procedure (Bailey and Brittain, 1972; Burgess, 1978; Li Wan Po et al., 1979; Mehta et al., 1982), alcoholic extraction (United States Pharmacopeia, 1980) or silica gel column separation (Tokunaga et al., 1984) prior to chromatography.

A major problem encountered in topical dosage form analysis is the interference due to formulation adjuvants and preservatives present in these relatively complex formulations. Generally, only one or two components require quantitative analysis and these must be adequately separated from the other formulation excipients which may interfere with the assay process. Prior to chromatographic analysis an extraction or clean-up step is normally required. These manipulations are laborious, time consuming and generally require large samples. The use of an on-line sample clean-up technique prior to chromatography as reported here has resulted in a decrease in sample size and handling with a concomitant increase in accuracy and precision as well as a reduction in solvent usage, cost and analysis time.

Various techniques have been reported (Mollica and Strusz, 1972; Burgess, 1978; Gagne et al., 1980; Williams and Biehl, 1981; Rego and Nelson, 1982; Lake et al., 1983) where samples of creams or ointments have been totally or partially dissolved in various solvent systems and aliquots injected directly onto the analytical HPLC column without any clean-up procedure. In most of these reports no mention is made of column integrity after repeated sample injections although intermediate column washes with methanol are often recommended (Van de Vaart et al., 1982). Conversely, Olson (1973) has reported some loss of chromatographic resolution due to cream excipients passing through the column. It is therefore assumed that some form of sample clean-up is necessary to limit the amount of lipophilic material, especially liquid paraffin (Lake et al., 1983), entering the column.

Column switching procedures are highly suitable for the separation and analysis of multi-component samples, especially those formulated in complex matrices (Benjamin and Conley, 1983; Conley and Benjamin, 1983). Using this technique the sample is dissolved or dispersed in a suitable solvent and an aliquot injected onto the HPLC system. A front-cutting technique (Majors, 1980; Little et al., 1984) has been utilized in which only the first part of the chromatogram eluting from a pre-column is selectively transferred to the analytical column. The non-polar excipients remaining on the pre-column are then isolated from the analytical column using a switching valve and these constituents are back-flushed to waste using a different solvent system. Multi-column HPLC is thus a highly suitable procedure as it is more convenient, precise and less time consuming than classical clean-up procedures (Majors, 1980). It was on the basis of these reports that this versatile and rapid stability-indicating method for betamethasone 17-valerate in topical dosage forms was developed.

Materials and Methods

Reagents and chemicals

All chromatographic solvents were glass-distilled and spectral grade (Burdick and Jackson, U.S.A.). The HPLC grade water used in the mobile phase was purified through a Milli-Q system (Millipore, U.S.A.). The mobile phase was filtered through a 0.45 μm membrane filter (type BD, Millipore, U.S.A.) and simultaneously degassed under vacuum at ambient temperature. All chemicals used were analytical reagent grade. The betamethasone 17-valerate was obtained from Glaxo (South Africa), the norethisterone from Ethnor (South Africa), and the betamethasone 21-valerate and hydrocortisone were authentic specimens (British Pharmacopoeia Commission, U.K.).

Apparatus

The HPLC system consisted of a solvent delivery system (Model M45, Waters, U.S.A.), a syringe loading sample injector (Model 7125, Rheodyne, U.S.A.) equipped with a loop column (MPLC 3 cm × 4.6 mm i.d., RP-18 packed with Lichrosorb, Brownlee Labs., U.S.A.) and a diode-array detector (Model 1040A, Hewlett Packard, U.S.A.) coupled to either a strip-chart recorder (Model 100A, Perkin Elmer, U.S.A.) or an integrator (Model 3390A, Hewlett Packard, U.S.A.). A second solvent delivery system (Model 6000A, Waters, U.S.A.), capable of solvent switching, was connected to the injector for loop column back-flushing. The analytical column (25 cm × 4.6 mm i.d.) was custom-packed with Techsil 10 μm octadecylsilane packing material (HPLC Technology, U.K.).

HPLC conditions

The mobile phase was prepared by adding 45 parts by volume of water to 55 parts by volume of acetonitrile in a stoppered flask and allowing the mixture to equilibrate to room temperature prior to filtering and degassing. The flow rate was 1.5 ml/min and all chromatography was carried out at ambient temperature. The detector wavelength used was 239 nm, bandwidth 30 nm, with a reference wavelength of 330 nm, bandwidth 30 nm.

Preparation of calibration standard solutions

Standard stock solutions of betamethasone 17-valerate were prepared in acetonitrile (2.5, 5.0, 10.0 12.5 and 20.0 μg/ml) each containing either 3.0 μg/ml hydrocortisone as the internal standard for creams or 3.5 μg/ml norethisterone as the internal standard for the ointment and lotion determinations. Similarly, in order to quantitate the degradation product, betamethasone 21-valerate solutions were prepared in acetonitrile (0.5, 1.0, 1.5 and 2.0 μg/ml) each containing the relevant internal standard (hydrocortisone 3.0 μg/ml or norethisterone 3.5 μg/ml).

Sample preparation

Accurately weighed samples of cream or lotion (250 mg) were dissolved in 10 ml of acetonitrile in a 25 ml volumetric flask. In the case of ointments, tetrahydrofuran was used as the solvent. Solution was aided by swirling in a stream of hot air. The flasks were then allowed to equilibrate to room temperature and an aliquot of the appropriate internal standard solution was added. Cream and lotion solutions were made up to volume with acetonitrile and the ointment solutions were made up to volume with tetrahydrofuran. Samples were filtered (Millex SR, 0.45 μm, Millipore, U.S.A.) prior to injection into the HPLC system. Based on a label concentration of 0.1% betamethasone 17-valerate, sample preparation as outlined above produced a final betamethasone 17-valerate injection solution concentration of approximately 10 μg/ml (equivalent to a column loading of 100 ng) together with either 3.0 μg/ml of hydrocortisone or 3.5 μg/ml norethisterone as internal standard.

Injection procedure

A 10 μl volume of sample or calibration standard solution was injected into the valve (Fig. 1) placed in the "load" position (off-line). Without removing the syringe the injector valve was turned to the "inject" position, thus introducing the sample aliquot into the chromatographic system (on-line). The syringe was then withdrawn and the valve was left in the "inject" position for 3 min to allow all components of interest to pass through the loop column and transfer to the analytical column. The injector valve was then turned back to the "load" position. In the case of sample injection this trapped formulation excipients on the loop column. These excipients were back-flushed off the loop column using 1 ml of methanol followed by 5 ml of mobile phase which was pumped using the second solvent delivery system at a flow rate of 2.0 ml/min. In this way no re-equilibration of the loop column was required prior to the next injection. Injections of samples were alternated with calibration standard solutions until each sample had been injected at least three times. Peak height ratios of injected samples could be compared with calibration standard solutions to assign a value to the mass of steroid present in the sample aliquots.

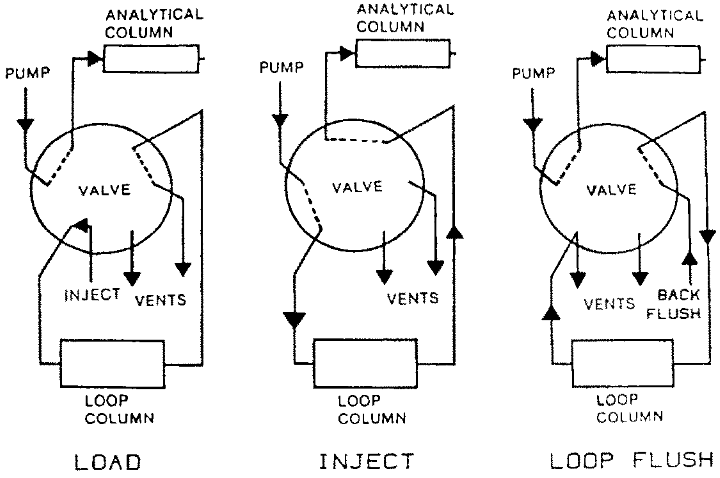

Fig. 1. Sequential schematic diagram of injection valve positioning during assay showing solvent elution direction at each stage.

Results and Discussion

Possible elution interferences were due to preservatives in the formulations; the creams investigated contained chlorocresol and the lotions contained methyl hydroxybenzoate, both of which eluted prior to the betamethasone 17-valerate. The ointment dosage forms investigated contained no preservatives. Optimal choice of internal standard and mobile phase allowed all the relevant peaks to be adequately resolved.

Fig. 2 is a typical chromatogram of a sample of betamethasone 17-valerate cream including the internal standard, hydrocortisone, whilst Fig. 3 depicts the elution profile of the lotion sample showing the relevant internal standard, norethisterone. Since the ointments investigated did not contain any preservative, their chromatograms resembled those obtained for the lotion samples.

Specificity studies

To ensure that formulation ingredients did not contribute to the betamethasone 17-valerate sample peak, a UV spectrum was recorded at half second intervals as the corticosteroid eluted from the analytical column. Additional UV spectra were similarly obtained for all other components of interest (as depicted in Figs. 2 and 3) to confirm peak homogeneity. The HPLC method thus detailed enables specific resolution of betamethasone 17-valerate from betamethasone 21-valerate, all other formulation co-ingredients and added internal standards.

Linearity

Calibration curves constructed on the basis of the peak height ratios of

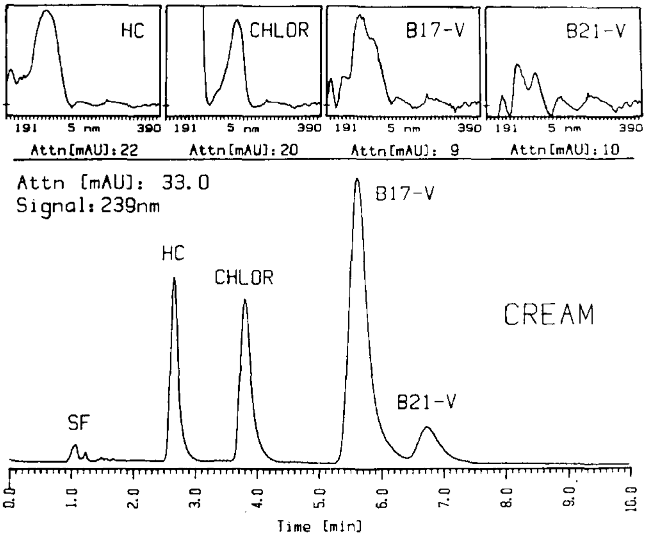

Fig. 2. Typical HPLC chromatogram of a betamethasone 17-valerate cream sample including UV spectra of all components of interest.

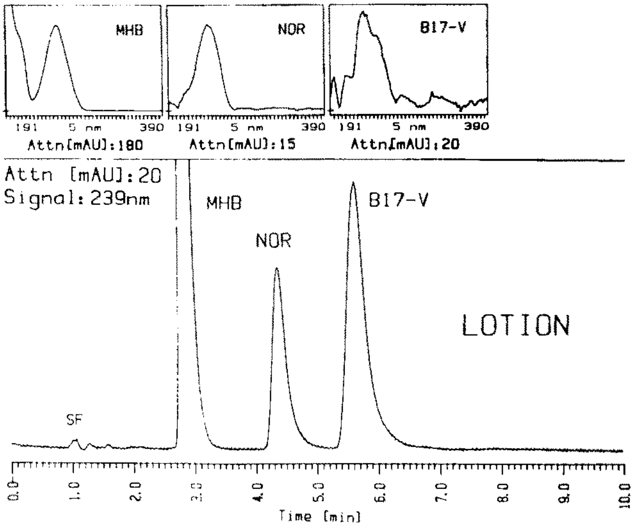

Fig. 3. Typical HPLC chromatogram of a betamethasone 17-valerate lotion sample including UV spectra of the components of interest.

betamethasone 17-valerate/internal standard versus betamethasone 17-valerate concentrations were linear over the concentration range studied. Similar results were obtained for betamethasone 21-valerate calibration curves (Table 1).

Precision and accuracy

The average percentage purities of six samples of betamethasone 17-valerate cream, lotion and ointment were found to be: 115.37% (S.D. = ±0.77), 120.53% (S.D. = ±2.45) and 124.79% (S.D. = ±1.73) respectively.

Recovery studies

To determine the accuracy of the method, samples of creams, lotions and ointments were initially assayed to determine the mass of betamethasone 17-valerate present in the dosage form. Samples of the same batch were spiked with known amounts of betamethasone 17-valerate and the spiked samples were subjected to the

TABLE 1

LINEARITY DATA; CALIBRATION CURVE CONSTRUCTION

Compound	Internal standard	Slope	Intercept	Correlation coefficient	n
B 17-V	HC	0.008951	0.01058	0.9998	21
B 17-V	NOR	0.01052	0.01844	0.9998	30
B 21-V	HC	0.005381	0.01164	0.9942	16

TABLE 2

RECOVERIES OF BETAMETHASONE 17-VALERATE FROM SPIKED COMMERCIAL SAMPLES

	μg added	μg found	% recovery
Creams	188.10	188.57	100.25
	187.35	189.09	100.93
	189.45	191.08	100.86
	181.87	181.20	99.63
	180.58	178.79	99.01
	Mean: 100.14%		
	S.D.: ±1.218%		
	95% confidence limit: 1.514%		
Lotions	187.35	196.10	104.67
	189.45	191.55	101.11
	181.87	180.00	98.97
	188.10	187.32	99.58
	180.58	180.02	99.69
	Mean: 100.80%		
	S.D.: ±2.056%		
	95% confidence limit: 2.557%		
Ointments	188.10	193.11	102.66
	187.35	193.47	103.26
	189.45	186.35	98.36
	181.87	180.33	99.15
	180.58	179.04	99.15
	Mean: 100.52%		
	S.D.: ±2.264%		
	95% confidence limit: 2.814%		

normal assay procedure. The results in Table 2 indicate that negligible loss of betamethasone 17-valerate is observed during sample preparation and that the method is, therefore, sufficiently accurate for analytical purposes.

On-line clean-up

The column switching technique was found to be highly suitable for the clean-up of topical corticosteroid formulations thus obviating the need for time-consuming sample preparation. Confirming reports in the literature (Benjamin and Conley, 1983; Conley and Benjamin, 1983), formulation excipients did not elute from the loop column and, therefore, did not interfere with the analysis. Moreover, no deterioration of analytical column performance was experienced even after several months of use.

Conclusions

The recovery, linearity and specificity data indicate that this method is suitable for the rapid and simple on-line clean-up and analysis of betamethasone 17-valerate from complex topical formulation matrices without conventional extractions. Although all injections and column switching were carried out manually in this study, the potential for fully automating these procedures has already been well established (Conley and Benjamin, 1983).

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