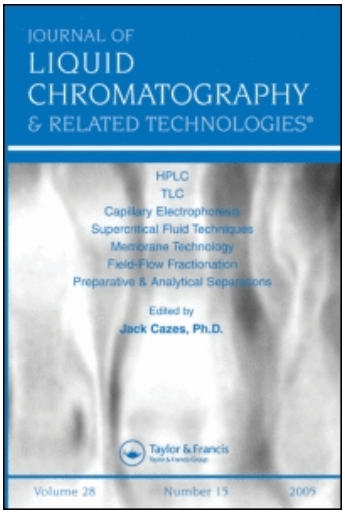

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HPLC DETERMINATION OF CLOBETASOL PROPIONATE IN COSMETIC PRODUCTS

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ABSTRACT

A simple and rapid HPLC method for the determination of clobetasol 17-propionate in cosmetic formulations, with antidranduff and antiseborrheic activity, has been developed. The chromatographic separation was carried out on a 5 μm Purospher-Lichrocart column using water-acetonitrile (40:60, v/v) as the mobile phase and UV detection at 237 nm. The extraction procedure has been validated analysing samples spiked with known amounts of the active principle. The recoveries were greater than 94% and the reproducibility was within 3.0%.

INTRODUCTION

Recently, some cosmetic products with antidranduff and antiseborrheic activity have appeared on the European market. Such products, described in USA. and Russia as preparations containing zinc pyrithione as the only active principle, have been proved very effective for the treatment of psoriasis and scalp seborrhea.[1-4] The fact that they are sold, in Italy, with the indication "for the hydration of dry and desquamated skin" has led us to verify their adherence to the EEC Directive 76/768, taken into account by the Italian law 713/86.

Particularly, our attention has been devoted to the search, in the cosmetic samples of interest, of the synthetic corticosteroids with antipsoriasis activity, which are forbidden in the cosmetic field as stated by the above mentioned law, enclosure II.

Among them, it has been pointed out the presence of clobetasol 17-propionate (I) [pregna-1, 4-diene-3, 20-dione, 21-chloro-9-fluoro-11β-hydroxy-16β-methyl-17- (1-oxopropoxy)], a steroid derivative for dermatological use which is employed in topical preparations for the therapy of different pathologies, particularly for the treatment of psoriasis.[5-7]

The literature reports some methods for the determination of (I) in pharmaceutical forms, based on spectrophotometry,[8,9] TLC coupled with densitometry[10,11] and HPLC with UV diode array detector or particle-beam-mass detector,[12] but no report deals with analysis of this substance in cosmetic formulations.

The aim of this work was to develop a simple analytical method for extracting and determining (I), by HPLC, in those cosmetics, which are suspected to be not in conformity with legislation.

EXPERIMENTAL

Standards and Reagents

All reagents were of analytical-reagent grade and were used as obtained. The pure principle clobetasol 17-propionate was purchased by Glaxo Wellcome (Greenford, UK). Acetonitrile and methanol were of HPLC grade.

Water was deionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a Millipore filter (pore size 0.45 μm) and vacuum degassed, by an ultrasound treatment, before use.

Apparatus

Chromatography was performed with a Varian 9010 pump (Varian, Zug, Switzerland), equipped with a Rheodyne injector valve, fitted with a 10 µL sample loop, and a Hewlett Packard 1050 photodiode-array detector, connected to a Vectra HP 486 computer for the elaboration of the chromatographic data. The analytical column was of stainless steel (250 mm x 4.0 mm I.D.) packed with 5 µm Purospher-Lichrocart (Merck,Darmstadt, Germany).

HPLC Conditions

The adopted operating conditions were as follows: mobile phase, water-acetonitrile in the ratio 40:60 (v/v); flow-rate, 1.0 mL · min^{-1}; injection volume, 10 µL; column temperature, 25°C; detection wavelength, 237 nm. The range of wavelengths examined by the photodiode-array detector was 200-400 nm.

Calibration Standard Solutions

A stock solution of (I) was prepared by dissolving a weighed amount (about 50 mg) of the pure compound in 100 mL of methanol. A set of working standard solutions was prepared by diluting aliquots of the stock solution to give concentrations ranging from 1 to 100 µg · mL^{-1}. The peak areas, obtained at the optimum wavelength of detection, were plotted against the corresponding amounts (ng) injected to obtain the calibration graph.

Sample Preparation

1-g aliquots of the cosmetic sample were exactly weighed, transferred to a 10 mL volumetric flask and taken to volume with methanol. The dispersion was submitted to ultrasonic treatment for 10 min, and filtered through a nylon filter (0.45 µm) before injecting into the liquid chromatograph.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a standard solution containing 100 µg mL^{-1} of clobetasol propionate and Table 1 reports its chromatographic parameters. The retention time was reproducible under the experimental conditions used, the coefficient of variation (C. V.) ranging from 0.8 to 1.3 for within-day, and from 1.2 to 2.0% for between-day studies.

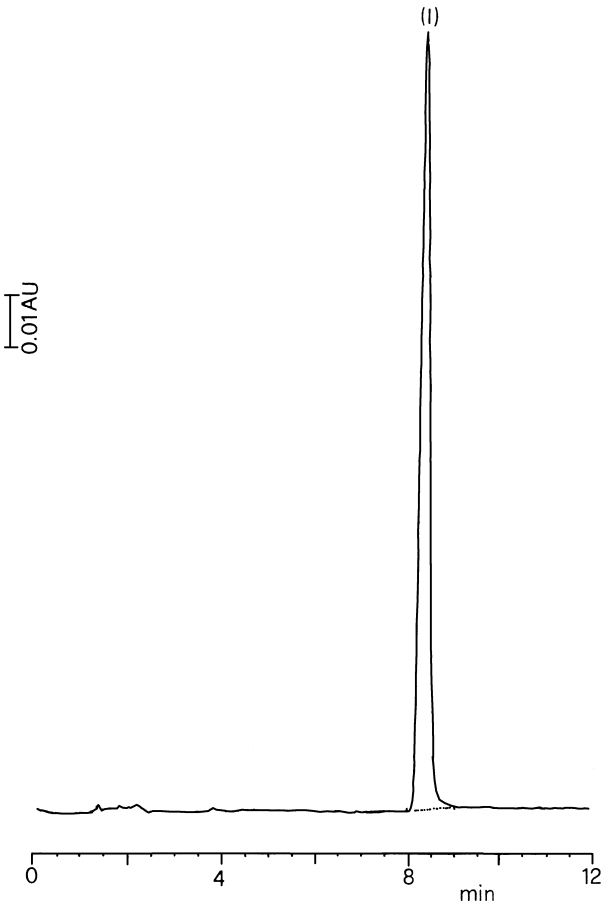

Figure 1. Typical chromatogram obtained at 237 nm for a standard solution containing 100 μg mL^{-1} of clobetasol propionate.

The calibration graph for (I) was constructed from five consecutive injections, and was rectilinear over the studied range of concentrations. The equation obtained by least-squares regression fit was: $y = (156.8 \pm 1.2)\ x + (4.1 \pm 1.7)$ where y is the peak area and x is the amount of (I) injected (ng). The corresponding correlation coefficient was 0.9998. The limit of determination (LOD), defined as the concentration giving a signal-to-noise ratio of 3 at the optimum detection wavelength, was 2 ng injected. Within-day and between-day precisions, as indicated by the C.V. values of the peak areas obtained from replicate analyses (n=10) of clobetasol propionate standard solutions (50 μg mL^{-1}) were satisfactory, C.V. values resulting 1.2 and 1.5%, respectively.

Table 1

Retention Parameters

Compound	Retention Volume (mL)	Capacity Factor	Peak Asymmetry Factor
Clobetasol Proprionate	8.30 (0.06)	2.45 (0.02)	0.95 (0.03)
Chlorocresol	4.20 (0.05)	0.75 (0.02)	0.92 (0.03)
Methyl p-Hydroxybenzoate	2.60 (0.03)	0.088 (0.011)	0.94 (0.02)
Ethyl p-Hydroxybenzoate	3.10 (0.03)	0.30 (0.02)	0.93 (0.04)
n-Propyl p-Hydroxybenzoate	3.90 (0.04)	0.63 (0.03)	0.93 (0.02)

Table 2

Analysis of Cosmetic and Pharmaceutical Samples and Recovery Studies*

Commercial Formulation	Amount Found (%, w/w)	Amount Added (%, w/w)	Recovery
Shampoo 1	4.64 (0.15) E-2	----	----
Lotion 1	2.25 (0.11) E-2	----	----
Cream 1	5.1 (0.14) E-2	----	----
Cream 2	ND	5.00 E-2	94.5 (2.6)
Cream 3	ND	5.00 %-2	96.7 (2.3)
Shampoo 2	ND	5.00 E-2	98.2 (2.1)
Shampoo 3	ND	5.00 E-2	98.4 (2.3)
Shampoo 4	ND	5.00 E-2	100.1 (1.9)
Pharmaceutical Cream	5.08 (0.26) E-2	----	----
Pharmaceutical Ointment	4.92 (0.19) E-2	----	----

* Mean of 5 determinations; ND; not detected; SD in parenthesis (Tables 1,2).

The proposed method of extraction and quantitation of clobetasol propionate, has been applied to the analysis of some commercial formulations, commercially available on the European market. In particular, we have examined eight cosmetic samples and two medicinal specialties containing (I) at a concentration of 0.05%. Three cosmetic samples were found to contain clobetasol propionate at a level practically equal to that of the pharmaceutical

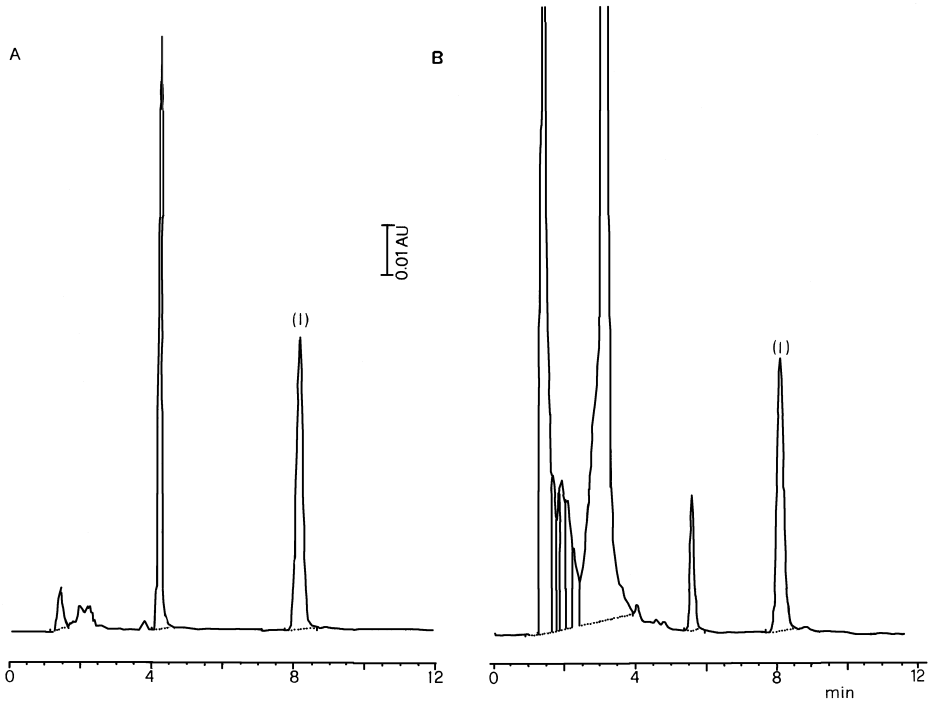

Figure 2. Chromatograms obtained at 237 nm following the injection of (a) 10 μL of the extract relative to the pharmaceutical cream and (b) 10 μL of the extract deriving from shampoo 1 (see Table 2).

formulations, as shown in Table 2. It is evident that these cosmetics are not in compliance with the current legislation. Moreover, as stated by Lubach[13], their use can result in momentaneous improvements of the pathologies, but followed by dramatic relapses if the treatment is interrupted.

The other cosmetic products, for which the absence of any peaks at the retention volume corresponding to (I) had been verified, were used to perform recovery tests. They were spiked with weighed amounts of clobetasol propionate and analysed according to the present method. The recoveries obtained, as well as the standard deviations, were good, as shown in Table 2.

Figures 2a and 2b show the chromatograms obtained for two of the analysed samples, namely pharmaceutical cream and shampoo 1 in Table 2. The chromatogram of the extract deriving from the pharmaceutical cream is very clean at the detection wavelength and the resulting concentration of clobetasol propionate is in agreement with the value claimed by the manufacturer. In addition to the

peak relative to (I), the chromatogram 2a displays another peak which was ascribed to chlorocresol, added to the sample as an antimicrobial agent. In the chromatogram 2b a very big peak close to the solvent peak, which resulted due to ethyl p-hydroxybenzoate is present.

Since preservatives are always added to pharmaceutical and cosmetic formulations to improve their stability, we have also submitted to HPLC analysis the compounds listed in Table 1, which reports their chromatographic characteristics besides those relevant to clobetasol propionate. The calibration graphs, obtained at 237 nm, for the preservatives tested allowed us to quantitate their levels in the products where their presence was confirmed. Our results have demonstrated that ethyl p-hydroxybenzoate was present in the samples named as shampoo 1, lotion 1 and cream 1 at the concentration of 0.77, 0.48, and 0.61%, respectively. Moreover, the above mentioned sample of pharmaceutical cream contained chlorocresol at a level equal to 0.05%.

The photodiode-array detector allowed the estimation of the peak purity, which was calculated over the range 200-400 nm and resulted greater than 0.95 for all the compounds tested, so confirming the identity of the active principle.

Due to the rapidity and good recovery of the sample processing, the HPLC method described here, for determining clobetasol propionate, is a valuable tool for quality control analyses, especially in verifying the adherence of a cosmetic sample to the EEC Directive 76/768.

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