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Determination of fluocortolone pivalate and fluocortolone hexanoate in suppositories using reverse-phase HPLC

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Abstract

Fluocortolone and its esters are synthetic corticosteroids used topically in the treatment of various skin disorders. A method that can be successfully used for the separation and determination of fluocortolone pivalate and fluocortolone hexanoate in suppositories was developed. This method is based on reverse-phase HPLC on Supelcosil LC-18 (25 cm × 4.6 mm, 5 μm), using methanol–acetonitrile–water–glacial acetic acid (17:46:37:0.4 v/v/v/v) as mobile phase at a flow rate of 3.0 ml/min. Detection was carried out using a UV detector at 238 nm. The method developed was validated, and calibration curves were established dependent on peak area. The validated ranges for fluocortolone pivalate and fluocortolone hexanoate are 15–305 μg/ml ($r = 0.9995$) and 15–315 μg/ml ($r = 0.9996$), respectively. The limits of detection and the limits of quantification for both esters were also determined.

Keywords: Reverse-phase HPLC; Fluocortolone pivalate; Fluocortolone hexanoate

1. Introduction

Fluocortolone (6α-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione) and its esters fluocortolone pivalate (FP) and fluocortolone hexanoate (FH) are synthetic corticosteroids used in the treatment of various skin disorders. Fluocortolone hexanoate has a longer duration of action than the free alcohol or pivalate ester. They are usually employed as a cream or ointment: concentrations usually used are 0.1 or 0.25% of either the free alcohol or fluocortolone esters. There are numerous references in the literature describing the determination of fluocortolone and its esters in human biological material and dosage forms using TLC [1], UV-densitometry [2,3], HPTLC [4], HPLC [5], HPLC–MS [6], Raman spectroscopy [7] and differential pulsed polarography [8]. The British Pharmacopoeia gives an assay for fluocortolone cream and fluocortolone ointment [9].

The present paper describes a sensitive and simple reverse-phase HPLC method with UV detection, which can be successfully used for identification and determination of fluocortolone pivalate and fluocortolone hexanoate in suppositories.

2. Experimental

2.1. Reagents and solvents

Fluocortolone pivalate (LOT AS 1580) and fluocortolone hexanoate (LOT AS 1579) manufactured by Schering AG/PhyBE were used as the working standards. Methanol, acetonitrile, glacial acetic acid (Merck, Darmstadt, Germany) and doubly distilled water were used to prepare the mobile phase. All the solvents used for the preparation of the mobile phase were of HPLC grade of purity and the mixture was filtered and degassed before used. Ultraproct suppositories were manufactured by Schering AG D-13342, Berlin. One suppository contains 0.61 mg fluocor-

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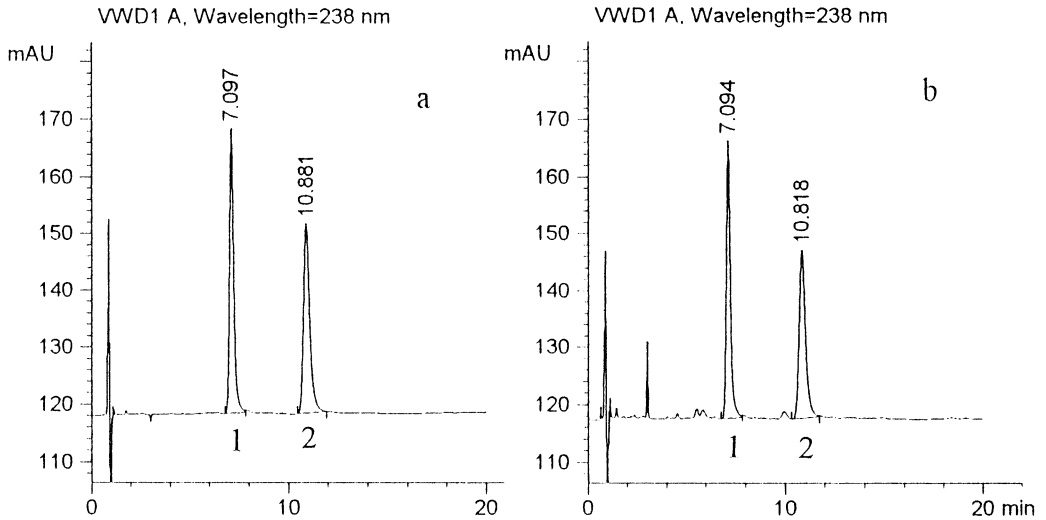

Fig. 1. Chromatograms of 1-fluocortolone pivalate and 2-fluocortolone hexanoate: (a) standard; (b) suppositories. Chromatographic conditions: mobile phase: methanol–acetonitrile–water–glacial acetic acid (17:46:37:0.4 v/v/v/v); flow rate: 3 ml/min; column: Supelcosil LC-18.

tolone-21-pivalate and 0.63 mg fluocortolone-21-hexanoate.

2.2. Standard solution

A 15.25 mg sample of fluocortolone-21-pivalate (305 μg/ml FP) and a 15.75 mg sample of fluocortolone-21-hexanoate (315 μg/ml FH) were transferred into a 50 ml calibrated flask and dissolved to the mark in methanol. This solution was used for preparing the calibration curves.

2.3. Sample preparation

One suppository was extracted with methanol after heating in an ultrasonic bath for 10 min at 45–50°C. After cooling, the extract was decanted. The procedure was repeated three times, all extracts were collected and transferred into a 10 ml calibrated flask and diluted with methanol. Prior to injection, the solution was filtered through a 20 μm Millipore filter (Millipore Co., Bedford, MA, USA).

2.4. Apparatus, mobile phase and chromatographic conditions

HPLC analyses were performed with an HP 1100 system equipped with an HP 1100 Diode-Array detector and a Rheodyne injector valve (20 μl sample loop). Separations were made on a Supelcosil LC-18 column (250 × 4.6 mm i.d., particle size 5 μm) using methanol–acetonitrile–water–glacial acetic acid (17:46:37:0.4 v/v/v/v) as the mobile phase. The detector wavelength was 238 nm. The elution was performed at a flow rate of 3.0 ml/min and the experiment conducted at 24°C.

2.5. HPLC procedure

A 20 μl aliquot of the analytical solutions was injected and triplicate injections were made for each solution. The standard solutions were chromatographed both at the beginning and the end of the daily analysis. The areas of the peaks were measured for the calibration curves.

2.6. Calibration curves

A series of eight solutions containing 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.0 and 10 ml of the standard solution were accurately transferred into 10 ml calibrated flasks and diluted to volume with methanol. Three injections (20 μl) of each of these solutions were made into the chromatographic system.

3. Results and discussion

A method that could be used for the separation and determination of fluocortolone pivalate (FP) and fluocortolone hexanoate (FH) in suppositories was developed. This method is based on reverse-phase HPLC on a Supelcosil LC-18 column using methanol–acetonitrile–water–glacial acetic acid as mobile phase at a flow rate of 3.0 ml/min. Fig. 1 presents experimental chromatograms showing separations of FP and FH in suppositories. Under the described experimental conditions FP and FH showed peaks with retention times of 7.1 and 10.9 min, respectively.

The Beer's law plots were found to be linear over the concentration ranges 15–305 μg/ml (FP) and 15–315 μg/ml (FH). Over these concentration ranges, linear

Table 1
Precision of assay for fluocortolone pivalate and fluocortolone hexanoate

	Fluocortolone pivalate bulk drug (μg/ml)			Fluocortolone hexanoate bulk drug (μg/ml)		
Taken	30.5	61.0	91.5	31.5	63	94.5
Found	30.16	62.57	87.11	33.11	65.92	90.35
SD	0.908	0.03	1.568	0.977	0.127	2.162
RSD (%)	3.01	0.05	1.80	2.95	0.19	2.39

Table 2
RP–HPLC determination of fluocortolone pivalate and fluocortolone hexanoate in suppositories

	Fluocortolone pivalate	Fluocortolone hexanoate
Taken (mg)	0.61	0.63
Found (mg)	0.602	0.593
x_{min} (mg)	0.58	0.58
x_{max} (mg)	0.619	0.615
SD	0.0159	0.0143
RSD (%)	2.62	2.37
Recovery (%)	98.75	94.2

regression analysis of peak area (y) versus concentration (x) yielded the equations $y = 11.844x + 8.37421$ ($r = 0.9995$) for FP and $y = 11.6078x - 16.419$ ($r = 0.9996$) for FH.

The precision of the method was determined with three different concentrations of FP and FH, as shown in Table 1.

The applicability of the method for the assay of sample dosage forms was verified by determining FP and FH in Ultraproct suppositories ($n = 10$). The results of quantitative determination of fluocortolone esters in dosage forms are presented in Table 2.

The limits of detection (LOD) and quantification were determined by fitting interday back-calculated standard deviations of each calibration standard. The LOD is defined as the lowest determinable quantity that indicates the presence of an analyte at a given statistical level of confidence (3 SD), and the LOQ is defined as the lowest measured quantity above which the analyte can be quantified at a given statistical level of confidence (10 SD). The LOD for FP and FH were found to be 9.96 and 9.11 μg/ml and the LOQs 33.19 and 30.37 μg/ml, respectively.

The good recoveries of fluocortolone pivalate and fluocortolone hexanoate in Ultraproct suppositories indicate that the method is suitable for the determination of fluocortolone esters in bulk drug and pharmaceutical formulations. The HPLC method provides microgram sensitivity and adequate linearity and repeatability. This method is rapid and simple, which is important for routine application.

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