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Note

NOTE

Spectrophotometric determination of fluocortolone in tablets using 1,4-dihydrazinophthalazine

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The proposed method for the determination of fluocortolone is based on the formation of a stable, yellow coloured hydrazone product with 1,4-dihydrazinophthalazine as the reagent. Heating at 85 ºC for 2 h was found to be necessary to ensure optimal hydrazone formation in acidified 1-propanol as the solvent. The detection limit was 1.2 μg/ml. This method when applied for the determination of fluocortolone in pharmaceutical formulations gave precise and reproducible results.

Keywords: fluocortolone, 1,4-dihydrazinophthalazine, tablets, spectrophotometry.

INTRODUCTION

Fluocortolone (6α-fluoro-11β,21-dihydroxy-16α-methylpregna- 1,4-diene-3,20-dione) is a synthetic fluor-containing corticosteroid used in oral therapy and, topically, in the treatement of various skin disorders.

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] HPTLC,[3] HPLC,[4] HPLC-mass spectrometry[5] and differential pulse polarography.[6]

The aim of the present work was to optimize a method using 1,4-dihydrazinophthalazine (1,4-DHPHT) as the reagent for the spectrophotometric determination of fluocortolone in the investigated pharmaceutical formulations.

EXPERIMENTAL

Apparatus

A Specord M 40 UV-VIS spectrophotometer (Carl Zeiss, Jena, Germany) equipped with 10 mm glass cells was used.

Solutions

Reagent solution: 50 mg of 1,4-DHPHT sulphate was dissolved in methanol containing 1 ml of concentrated hydrochloric acid. The resulting solution was diluted with the same solvent to 25 ml.

Standard solution: 10 mg of fluocortolone was dissolved in 1-propanol to 100 ml (standard solution A). 2 ml of fluocortolone standard solution A was diluted with 1-propanol to 5 ml (standard solution B, 1.06×10^{-4} mol/l).

Sample solution: Ten tablets were weighed and triturated. An accurately weighed sample of powder equivalent to 5 mg of fluocortolone was diluted with 1-propanol to 50 ml and mixed in an ultrasonic bath (15–20 min). 2 ml of this solution was filtered off quantitatively and then diluted with 1-propanol to 5 ml.

General procedure for the colour development

To each standard solution and sample solution 1 ml of reagent solution was added; the solutions were mixed well and heated in a water bath at 85 °C for 2 h. After cooling, 1-propanol was added up to the mark of each calibrated flask and the absorbance of the solution was measured at 380 nm against the reagent blank (fluocortolone-free sample).

RESULTS AND DISCUSSION

The nucleophilic addition reactions of 3-keto steroids with aromatic hydrazine derivatives are very important in the determination of steroid hormones.[7,8] The influence of the solvent on the formation of fluocortolone-1,4-DHPHT hydrazone was investigated and it was found that this reaction was quantitative in 1-propanol. The reaction was carried out with different concentrations of the reagent, at different temperatures (Fig. 1) and different heating intervales (Fig. 2).

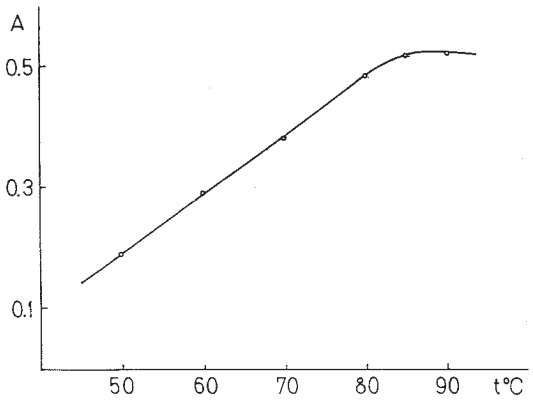

Fig. 1. The influence of the temperature on the formation of fluocortolone-1,4-DHPHT hydrazone. Fluocortolone, $c = 2.76\times10^{-5}$ mol/dm^3. 1,4-DHPHT, $c = 1.39\times10^{-3}$ mol/dm^3.

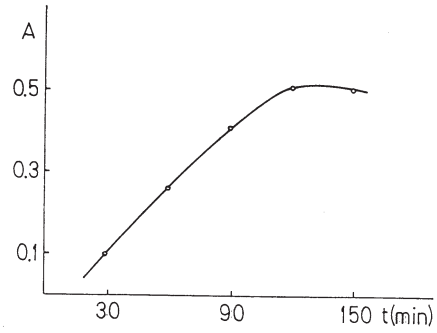

Fig. 2. The influence of the heating interval (heated at 85 °C) on the formation of fluocortolone-1,4-DHPHT-hydrazone. Fuocortolone, $c = 2.76\times10^{-5}$ mol/dm^3. 1,4-DHPHT, $c = 1.39\times10^{-3}$ mol/dm^3.

The optimal experimental conditions for the formation of 1,4-DHPHT hydrazone are: heating time 2 h, temperature 85 °C. The yellow product has an absorption maximum at 380 nm. The reaction of fluocortolone with 1,4-DHPHT was found to be more sensitive than the reported data for the reaction of 1,4-diene-3-keto steroids with isonicotinoylhydrazide INH (detection limit 10 μg/ml), which is the most frequently used standard method for the determination of keto steroids.[9]

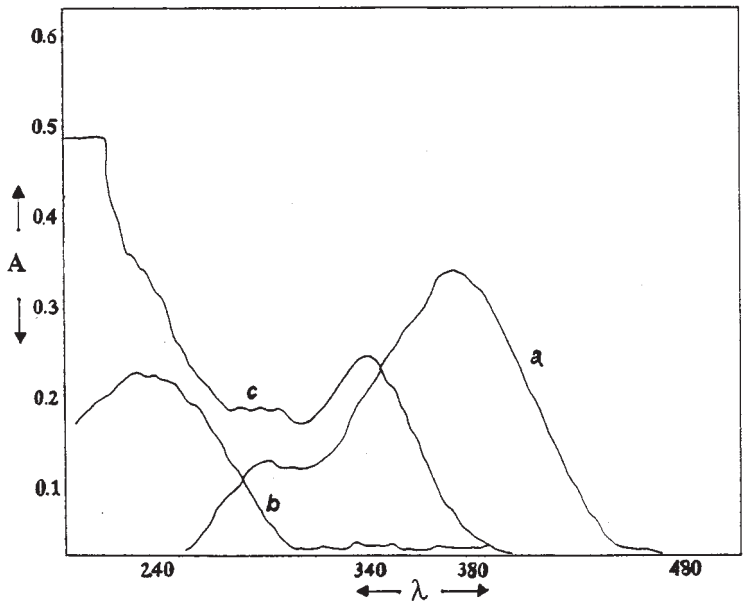

Fig. 3. UV spectrum of fluocortolone-1,4-DHPHT-hydrazone from 200 to 480 nm (curve a); UV spectrum of fluocortolone (curve b); UV spectrum of 1,4-DHPHT (curve c).

The absorption specta of fluocortolone, 1,4-DHPHT and fluocortolone-dihydrazinohydrazone, formed under optimal reaction conditions, are presented in Fig. 3.

A linear relationship between absorbance (λ_{max} 380 nm) and concentration was established in the range $3.19 \times 10^{-6} - 3.61 \times 10^{-5}$ mol/l. The regression equation was $y = 0.0136797 + 0.0171\,x$, the correlation coefficient being $r = 0.9978$ for $n = 7$, indicating good linearity. The molar absorptivity and the detection limit were 1.78×10^4 dm^3/mol cm and 1.2 μg/ml, respectively.

The reliability of the proposed method was checked at three different concentrations and the results are presented in Table I, the RSD ($n = 10$) varied from 0.60 to 2.90 % for concentrations: 4.00 μg/ml (sample 1), 8.00 μg/ml (sample 2) and 12.00 μg/ml (sample 3).

TABLE I. Spectrophotometric determination of fluocortolone with 1,4-DHPHT

Sample (n = 10)	Taken/μg	Found/μg	RSD/%
1	4.00	3.50	2.90
2	8.00	7.50	1.85
3	12.00	12.11	0.60

The proposed method was applied to the determination of fluocortolone in tablets and a laboratory made ointment. The proposed spectrophotometric method gave precise and reproducible results: the recovery was 98.54 % with a RSD = 2.07 % ($n = 10$). These results are presented in Table II.

TABLE II. The determination of fluocortolone in pharmaceutical formulations

Sample (n = 10)	Taken/mg	Found/mg	Recovery/%	RSD/%
Fluocortolone*	5.00	4.81	96.46	3.04
Ultralan®-oral-5	5.00	4.93	98.54	2.07

*Laboratory-made ointment (excipient mixtures spiked with a determined quantity of fluocortolone)

The obtained results suggest that because of its sensitivity and reproducibility, the proposed reagent may be used for the determination of fluocortolone in pharmaceutical formulations.

И З В О Д

СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ ФЛУОКОРТОЛОНА У ТАБЛЕТАМА СА РЕАГЕНСОМ 1,4-ДИХИДРАЗИНОФТАЛАЗИНОМ

ОЛИВЕРА ЧУДИНА, СОТЕ ВЛАДИМИРОВ, ДОБРИЛА ЖИВАНОВ-СТАКИЋ и ДАНИЦА АГБАБА

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Предложена спектрофотометријска метода одређивања флуокортолона се заснива на грађењу жуто обојеног производа типа хидразона са 1,4-дихидразинофталазином као реагенсом. Метода је репродуктивна када се реакција изводи из 1-пропанола коме је додата концентрована киселина, уз загревање на 85 °C у току 2 сата. Лимит детекције износи 1,2 μg/ml. Предложена спектрофотометријска метода је примењена за одређивање садржаја флуокортолона у фармацеутским облицима.

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