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Spectrophotometric determination of desoximetasone in ointment using 1,4-dihydrazinophthalazine[1]

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Abstract

The proposed method is based on coloured hydrazone formation with 1,4-dihydrazinophthalazine as a reagent. Heating at 85°C for 2 h was found necessary to ensure optimal hydrazone formation in the presence of hydrochloric acid. The yellow hydrazone product has an absorption maximum at 380 nm. A linear relationship between absorbance and concentration was established in the concentration range 3.19×10^{-6}–3.19×10^{-5} mol l^{-1} (the regression equation was $y = 0.013\ 167\ 3 + 0.019\ 025\ 9x$; correlation coefficient $r = 0.9991$; $n = 6$). The detection limit was 1.2 μg ml^{-1} (molar absorptivity found was 1.97×10^4 l mol^{-1} cm^{-1}). The reliability of the proposed method was checked at three different concentrations; the relative standard deviation (RSD) varied from 1.03 to 2.01%. The described method applied to the determination of desoximetasone in ointment gave precise and reproducible results; the recovery was 98.55% with RSD = 2.40% ($n = 10$).

Keywords: Desoximetasone; 1,4-Dihydrazinophthalazine; Ointment; Spectrophotometry

1. Introduction

Desoximetasone (9α-fluoro-11β,21-dihydroxy-16-methylpregna-1,4-diene-3,20-dione) is a corticosteroid used topically in the treatment of various skin disorders. It is usually employed as a cream, gel of ointment in the concentration range 0.05–0.25%.

There are numerous reports of the determination of desoximetasone in human biological material and dosage forms using UV-densitometry [1], TLC [2], HPLC [3,4], NMR [5] and HPLC-mass spectrometry [6]. Various derivatisation agents have been used to improve the sensitivity and selectivity of steroid determination in biological fluids and pharmaceutical formulations.

The condensation reactions of keto steroids with aromatic hydrazine derivatives are important in the quantitative analysis of steroid hormones. Different hydrazine derivatives have been used in the preparative analysis of steroids, forming stable

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coloured hydrazones. The effects of concentration of the reagent, temperature and reaction time on the formation of steroid hydrazones, such as 4-nitrophenylhydrazones [7], 2,4-dinitrophenylhydrazones [8,9] and isonicotinoylhydrazones [10,11], have been widely investigated.

The present paper describes a sensitive and reproducible spectrophotometric method for the determination of desoximetasone in ointment. The method is based on the formation of a yellow desoximetasone dihydralazinohydrazone with 1,4-dihydrazinophthalazine (1,4-DHPHT) as a reagent.

2. Experimental

2.1. Apparatus

A Specord M40 spectrophotometer (Carl Zeiss, Jena, Germany) equipped with 10 mm glass cells was used.

2.2. Reagents and solvents

Desoximetasone (Shering, Berlin, Germany) was used as the working standard. 1,4-Dihydrazinophthalazine sulphate (Ciba-Geigy, Basle, Switzerland) was used as the reagent. 1-Propanol, concentrated hydrochloric acid (both from Merck, Darmstadt, Germany) and methanol (Alkaloid, Skopje, Macedonia) were used as solvents. All solvents and reagents were of analytical grade.

2.3. Dosage form

Esperson M ointment (1 g of ointment containing 2.5 mg of desoximetasone) was obtained from Jugoremedija–Hoechst (Zrenjanin, Yugoslavia and Frankfurt/Main, Germany).

2.4. Solutions

2.4.1. Reagent solution

An accurately weighed amount of 1,4-DHPHT sulphate (50 mg) was transferred into a 25 ml calibrated flask and dissolved in methanol containing 1 ml of concentrated hydrochloric acid.

2.4.2. Standard solutions

2.4.2.1. Desoximetasone stock standard solution. An accurately weighted amount of desoximetasone (10 mg) was dissolved in 1-propanol in a 100 ml calibrated flask.

2.4.2.2. Standard solution A. A 2 ml volume of desoximetasone stock standard solution was transferred into a 5 ml calibrated flask and diluted with 1-propanol. The concentration of this final solution was 1.06×10^{-4} M.

2.4.3. Sample solution

A 1 g amount of accurately weighed ointment was extracted with 1-propanol (1×10 and 2×5 ml), after heating in a water-bath for 15 min at 50°C. After cooling on ice and centrifugation, the extracts were filtered into a calibrated flask and diluted to 25 ml with 1-propanol; 2 ml of this solution were transferred into a 5 ml calibrated flask and diluted with 1-propanol.

2.5. General procedure

Standard solution A and sample solution were transferred into 5 ml calibrated flasks. After adding 1 ml of reagent to each volumetric flask, the solutions were mixed well and heated in a

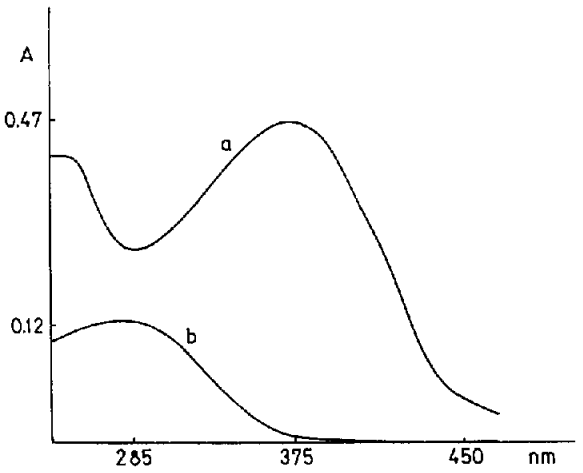

Fig. 1. UV spectrum of desoximetasone 1,4-DHPHT from 250 to 450 nm; (a) $c = 2.1 \times 10^{-5}$ M, UV spectrum of the blank reagent; (b) $c = 1.4 \times 10^{-3}$ M.

water-bath for 2 h at 85°C. After cooling, each solution was diluted to volume with 1-propanol and the absorbance was measured at 380 nm against a reagent blank (desoximetasone-free sample).

2.6. Calibration curve

A series of six solutions containing 0.15, 0.30, 0.70, 1.00, 1.30 and 1.50 ml of standard solution A were treated by the described procedure. For each concentration three experiments were performed and the absorbance was measured at 380 nm against a reagent blank.

3. Results and discussion

The nucleophilic addition reactions of 3-keto steroids with aromatic hydrazine derivatives are very important in the determination of steroid hormones. 1,4-DHPHT was used as a reagent for the first time in the spectrophotometric determination of methandienone (17β-hydroxy-17α-methylandrosta-1,4-dien-3-one) [12]. The influence of the solvent on the formation of desoximetasone 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 and heating intervals. A concentration of the reagent of 2 mg ml^{-1} was found to be optimal; higher reagent concentrations interfere with the UV adsorption spectra of the reaction product formed. The optimal experimental conditions for the formation of 1,4-DHPHT hydrazone are heating time 2 h and temperature 85°C. The reaction of desoximethasone with 1,4-DHPHT was found to be more sensitive than that reported data for the reaction of 1,4-diene-3-keto steroids with isonicotinoyl hydrazide [13], which is the most frequently used standard method for keto steroids. The protonated form of the condensation product of 4-ene-3-keto steroids treated with INH has a molar absorptivity of 11 000–12 000 l mol^{-1} cm^{-1}. The reactivity of 1,4-diene-3-ketones is lower with a detection limit of 10 μg ml^{-1} [11].

Table 1
Spectrophotometric determination of desoximetasone with 1,4-DHPHT

Sample No. ($n = 10$)	Taken (μg)	Found (μg)	Recovery (%)	RSD (%)
1	28.00	27.68	98.86	1.50
2	39.00	40.23	103.15	2.01
3	52.00	51.45	98.94	1.03

The absorption spectrum of the yellow product formed, desoximetasone dihydralazinohydrazone, under the optimal reaction conditions is presented in Fig. 1.

The composition of the condensation product, desoximetasone 1,4-DHPHT was determined by the Bent–French method of logarithmic absorbance (A) analysis [14]. It was found that log A varied linearly with $-\log C$(1,4-DHPHT) for the range of investigated concentrations from 2×10^{-6} to 3.4×10^{-5} mol l^{-1}. The Bent–French equation was $y = -3.75 + 0.9259x$ ($r = 0.9981$), and the slope of the straight line was 0.9259, which means that only one molecule of 1,4-DHPHT takes part in the formation of the investigated product, so the molar ratio of desoximetasone to 1,4-DHPHT is 1:1.

A linear relationship between absorbance and concentration was established in the range 3.19×10^{-6}–3.19×10^{-5} M. Beer's law was obeyed up to 12 μg ml^{-1}. The regression equation was $y = 0.013\ 167\ 3 + 0.019\ 025\ 9x$, the correlating coefficient being $r = 0.9991$ for $n = 6$, indicating excellent linearity. The molar absorptivity and the detection limit were $1.97 - 10^4$ l mol^{-1} cm^{-1} and 1.2 μg ml^{-1}, respectively.

The reliability of the proposed method was checked at three different concentrations of desoximetasone; the relative standard deviation ($n = 10$) varied from 1.03 to 2.01% for concentrations of 1.49×10^{-5} M (sample 1), 2.10×10^{-5} M (sample 2) and 2.76×10^{-5} M (sample 3) (Table 1).

The proposed method was applied to the detemination of desoximetasone in the investigated

Table 2
Determination of desoximetasone in ointment

Sample ($n = 10$)	Taken (mg)	Found (mg)	Recovery (%)	RSD (%)
Desoximetasone[a]	2.50	2.48	99.20	1.98
Esperson M	2.50	2.46	98.55	2.40

[a] Laboratory-made sample.

ointment and a laboratory-made sample. The proposed spectrophotometric method gave precise and reproducible results; the recovery was 98.55% with RSD = 2.40% ($n = 10$).(Table 2).

4. Conclusion

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

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