Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1893–1896, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Short Communication

Spectrophotometric determination of mesterolone in tablets using 3-acetylaminobenzaldehydethiosemicarbazone*

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Keywords: *Mesterolone; colorimetry; 3-acetylaminobenzaldehydethiosemicarbazone; Proviron tablets.*

Introduction

Condensation reactions of keto steroids with R—NH_2 reagents are important in the quantitative analysis of steroid hormones [1]. Certain saturated 3-keto steroids in an acidic medium react quantitatively with 2,4-dinitrophenylhydrazine to form coloured hydrazones; this reaction can be used for the colorimetric determination of such steroids [2]. However, there are no published reports on the colorimetric determination of mesterolone (17β-hydroxy-1α-methyl-5-androstan-3-one), a steroid that lacks significant UV absorption [3]. The aim of the present work was to devise a colorimetric method for the determination of mesterolone in tablets using 3-acetylaminobenzaldehydethiosemicarbazone as the reagent.

Experimental

Apparatus

A Gilford 2600 spectrophotometer was used with 10-mm glass cells.

Materials

Mesterolone was obtained from Schering AG (Berlin) and was used as the working standard. Proviron tablets, each containing 25 mg of mesterolone, were obtained from Alkaloid (Skopje). The reagent, 3-acetylaminobenzaldehydethiosemicarbazone, was purchased from Fluka (Buchs, Switzerland).

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
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Solutions

Mesterolone standard solutions. An accurately weighed amount of mesterolone (20 mg) was dissolved in methanol in a 50-ml calibrated flask. 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4-ml portions of this solution were transferred into separate tubes (150 × 20.0 mm) and diluted to 0.5 ml with methanol. These six standard solutions were used for establishing the calibration graph.

Reagent solution. 3-Acetylaminobenzaldehydethiosemicarbazone (150 mg) was dissolved in concentrated sulphuric acid in a 25-ml calibrated flask. The reagent solution was used 24–72 h after preparation.

Sample solution. A quantity of powdered tablets containing 25 mg of mesterolone was transferred to a 50-ml calibrated flask and 30 ml methanol was added; the mixture was shaken for 10 min, diluted to 50 ml with methanol and filtered. A 0.1-ml portion of this solution was transferred to a tube (150 × 20.0 mm) and diluted to 0.5 ml with methanol.

Colour development

To 0.5 ml of 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 80°C for 15 min. After cooling, 3 ml of methanol was added to each tube and the absorbance was measured at 544 nm against the reagent blank (mesterolone-free).

Results and Discussion

The absorption spectra of the condensation product (curve 1) and the blank reagent (curve 2) were recorded against a reagent blank and methanol, respectively (Fig. 1). The absorption maximum of the coloured condensation product was observed at 544 nm; the

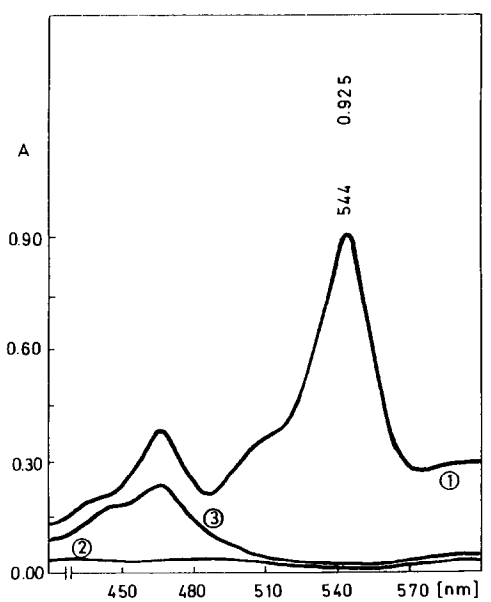

Figure 1
Absorption spectra of the condensation product of mesterolone, 35.5 μg ml^{-1} (1); the reagent blank (2); and mesterolone treated with sulphuric acid (3).

absorption maximum at about 465 nm originated from the solution of mesterolone treated with reagent-free sulphuric acid, as shown by curve 3 in Fig. 1.

The effects of temperature, reaction time and concentration of the reagent on the intensity of colour developed were investigated. It was shown that heating at 80°C produced maximum colour intensity after 15 min; maximum intensity of colour was also obtained using a solution containing 6 mg ml^{-1} of the reagent (Fig. 2). Under the reaction conditions employed, a solution of the coloured condensation product was stable at room temperature for about 180 min after colour development. A calibration graph was plotted of the absorbance at 544 nm versus concentration of mesterolone. The regression equation was $y = 0.0267x - 0.025$; the correlation coefficient (r) was 0.998 ($n = 5$), indicating excellent linearity. Beer's law was obeyed up to a concentration of 35.5 μg ml^{-1} of mesterolone.

The precision of the method was determined using four different concentrations (Table 1). The relative standard deviation, RSD ($n = 10$) was 0.46–2.31% for concentrations of mesterolone of 8.88–35.52 μg ml^{-1}.

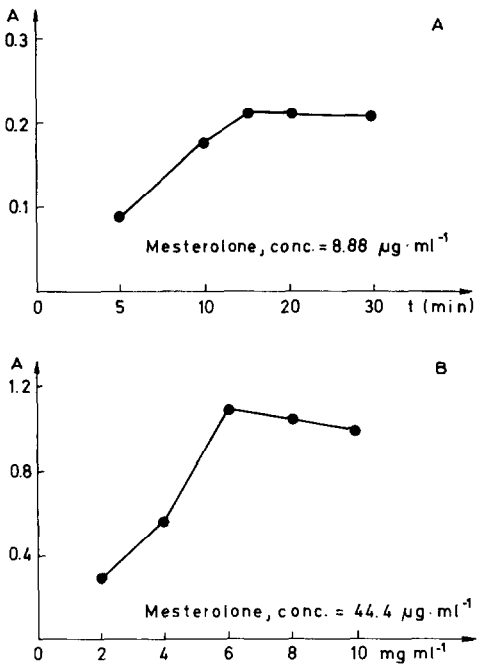

Figure 2
Effects of: heating time at 80°C on the intensity of colour (A); and reagent concentration on the intensity of colour after heating at 80°C for 15 min (B). The absorbance was measured at 544 nm.

Table 1
Spectrophotometric determination of mesterolone with 3-acetylaminobenzaldehydethiosemicarbazone ($n = 10$)

Concentration added (μg ml^{-1})	Mean concentration found (μg ml^{-1})	Standard deviation (μg ml^{-1})	RSD (%)
8.88	8.30	0.192	2.31
17.76	18.03	0.090	0.51
26.64	27.39	0.252	0.92
35.52	35.03	0.161	0.46

Table 2
Assay of Proviron tablets

Declared (mg/tablet)	Found* (mg/tablet)	RSD (%)	Recovery (%)
25	24.89	0.64	99.56

*Mean (n = 10).

The method was applied to the determination of mesterolone in Proviron tablets. The recovery was 99.6% (n = 10) in respect of the labelled drug content of the tablets; the RSD was 0.64%. The results suggest that because of its sensitivity and reproducibility, the proposed method may be suitable for the routine analysis of mesterolone in dosage forms.

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[*Received for review 16 May 1989; revised manuscript received 2 June 1989*]