amide with TLC on Silica Gel G in chloroform–dioxan (94:6)[5], the isolation of most C_{19}-steroid 2,4-dinitrophenylhydrazones becomes feasible, facilitating the estimation of these compounds in biological extracts[5,6]. At the same time, individual derivatives in discrete spots may be quantitated after TLC on polyamide by means of direct densitometry with a spectrodensitometer (Model SD 3000, Schoeffel Instr. Corp., Westwood, N.J., U.S.A.). The estimation of C_{19}-steroid 2,4-dinitrophenylhydrazones by this procedure, the sensitivity of which approximates 10 ng, as well as its application to the analysis of C_{19}-steroids in biological material, will be presented in a forthcoming communication.

Abteilung für Experimentelle Endokrinologie,
Universitäts-Frauenklinik,
65 Mainz (G.F.R.)

L. PENZES
P. MENZEL
G. W. OERTEL

1 H. REICH, D. H. NELSON AND A. ZAFFARONI, *J. Biol. Chem.*, 187 (1950) 411.
2 L. TREIBER AND G. W. OERTEL, *Z. Klin. Chem.*, 5 (1967) 83.
3 R. STUPNICKI AND E. STUPNICKA, *Nature*, 200 (1963) 165.
4 W. R. STARNES, A. H. RHODES AND R. H. LINDSAY, *J. Clin. Endocrinol. Metab.*, 26 (1966) 1245.
5 L. TREIBER AND G. W. OERTEL, *Clin. Chim. Acta*, 17 (1967) 81.
6 L. TREIBER AND G. W. OERTEL, *Z. Klin. Chem.*, 6 (1968) 367.

Received July 17th, 1969

J. Chromatog., 44 (1969) 189–190

CHROM. 4245

Rapid quantitation of Δ^4-3-ketosteroids by thin-layer densitometry

Thin-layer densitometry of steroids has recently received increased attention. Such methods are based either on staining of free substances after chromatography[1] or the formation of coloured derivatives[2–4]. The present paper describes the quantitation of Δ^4-3-ketosteroids by use of their quench effect upon the fluorescence at 254 mμ provided by a suitable dye in the adsorbent.

Methods

For chromatography Analtech (Wilmington, Del., U.S.A.) plates with a 250 μ thick layer were used. The coating material was Silica Gel GF_{254} with fluorescence at 254 mμ. In a special scoring device (Schoeffel Instr. Corp., Westwood, N.J., U.S.A.) the thin layer was divided into lanes of 1 cm width. Because of the double beam operating system of the densitometer only alternate lanes were loaded with a mixture of Δ^4-3-ketosteroids. The blank lanes served as reference for the instrument. The plates were then developed in a suitable solvent system such as chloroform–dioxane (94:6) yielding adequate separation of various steroids.

Direct quantitation was performed by means of a Schoeffel spectrodensitometer, Model SD 3000 (Schoeffel Instr. Corp., Westwood, N.J., U.S.A.). A quartz

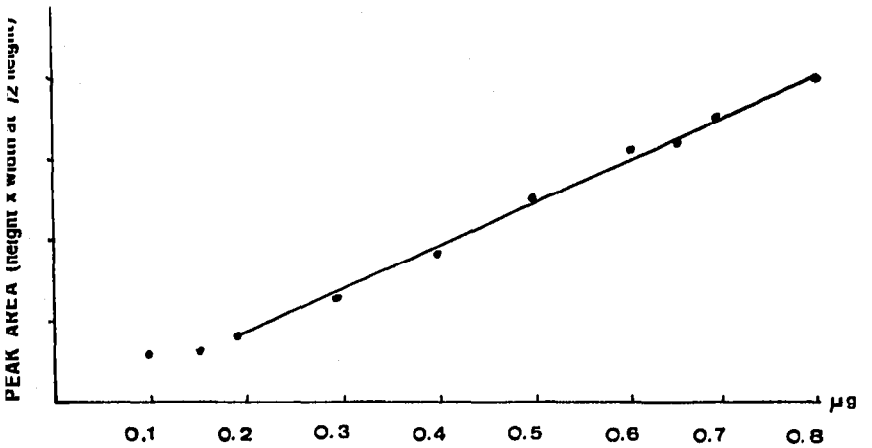

Fig. 1. Calibration curve of the densitometer (wavelength 254 mμ) with recorder full scale at optical density 0.5.

nonochromator provided UV light of 254 mμ wavelength that passed through two lits. The reference and the sample lane of the plate moved through the UV light beams and the emitted light was registered in an optical density computer, Model SDC 300 (Schoeffel Instr. Corp.) connected with an integrating 10 in. strip recorder, Model SDR 303 (Schoeffel Instr. Corp.). Whereas free areas of the sample lane appeared as a high positive base line the steroid spots became visible as negative peaks (Fig. 3). Peak areas were calculated by triangulation (height × width at 1/2 height) and evaluated for standard curves.

Results

Amounts of 0.1 μg Δ^4-3-ketosteroid could still be clearly detected. At a full scale deflection of the recorder at different optical density values a satisfactory linearity of standard curves was achieved for concentrations between 0.2 and 8.0 μg

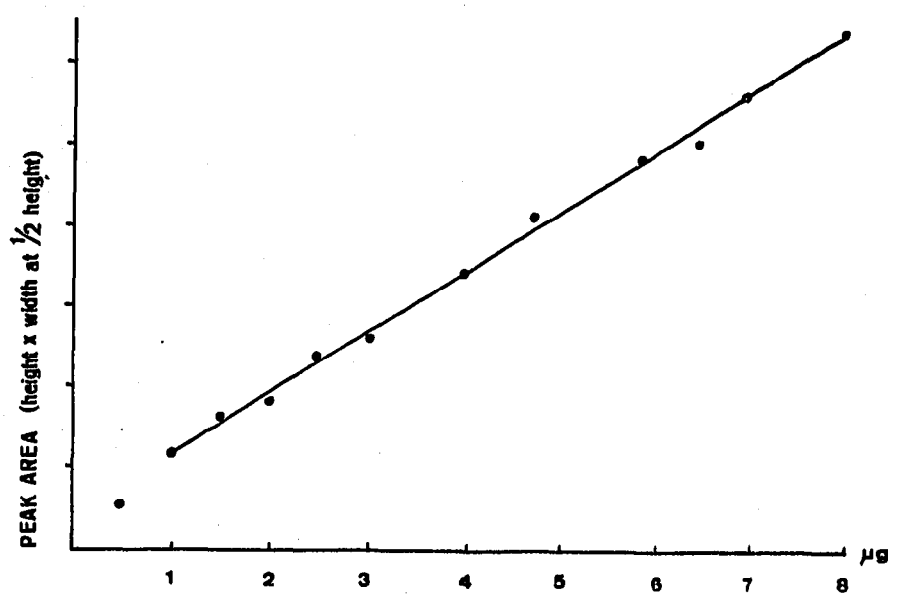

Fig. 2. Calibration curve of the densitometer with recorder full scale at optical density 1.0.

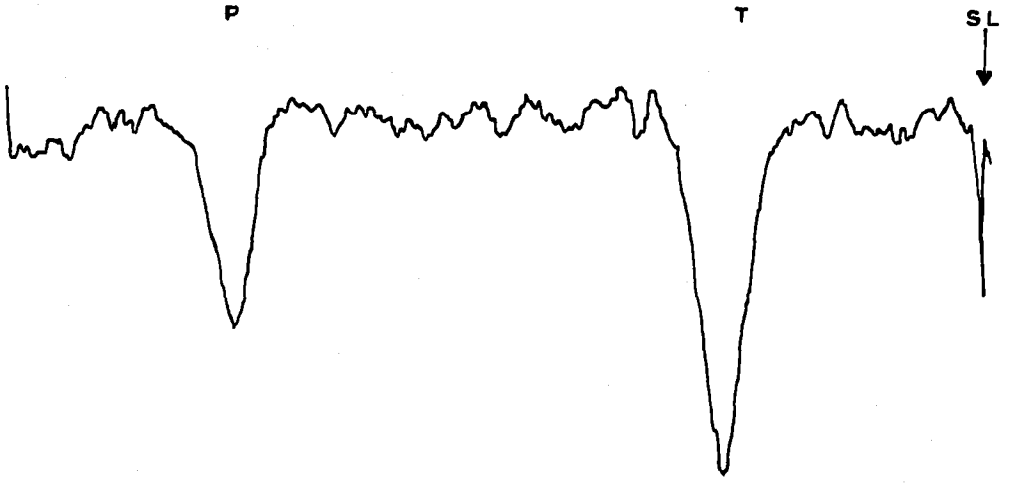

Fig. 3. Recording of 2 μg testosterone (T) and 1 μg progesterone (P) after chromatography in chloroform–dioxane (94:6), SL = starting line.

(Figs. 1 and 2). Fig. 3 demonstrates the recording of a chromatogram with 1 μg progesterone and 2 μg testosterone after development in chloroform–dioxane (94:6).

The present method was adopted for rapid estimation of free progesterone in peripheral plasma of pregnant women. Results of this investigation will be published in a forthcoming paper.

This work was supported by a grant of the Deutsche Forschungsgemeinschaft, Bad Godesberg, Germany, and National Institute of Health, Washington D.C., U.S.A., grants HD-01199 and AMK-14013.

Abteilung für Experimentelle Endokrinologie,
Universitäts-Frauenklinik,
Mainz (G.F.R.) and
Steroid Laboratory,
Department of Obstetrics and Gynecology,
School of Medicine,
University of Pennsylvania,
Philadelphia, Pa. (U.S.A.)

P. KNAPSTEIN
J. C. TOUCHSTONE
P. MENZEL
G. W. OERTEL

1 C. H. SHACKLETON AND F. H. MICHELL, *Steroids*, 10 (1967) 359.
2 P. KNAPSTEIN, L. TREIBER AND J. C. TOUCHSTONE, *Steroids*, 11 (1968) 915.
3 P. KNAPSTEIN AND J. C. TOUCHSTONE, *J. Chromatog.*, 37 (1968) 83.
4 J. C. TOUCHSTONE, A. BAILY AND P. KNAPSTEIN, *Steroids*, 13 (1969) 115.

Received May 14th, 1969