CHROM. 6000

Qualitative analysis of solvents in steroid hormones by means of gas–liquid chromatography and solid injection

Many steroids contain solvents bound in the crystal lattice or exhibit a tendency to form crystalline solvates. The presence of solvents may be noticed as loss on drying or by examining the crystals thermomicroscopically. Some information about the identity of the solvents present has been obtained by infrared spectrophotometry[1–3]. No sensitive and rapid method for a qualitative detection of solvents in steroid hormones has been available until now. We found it of interest to try to solve the problem by means of gas chromatography combined with solid injection of the steroid hormones.

The solid sample injection device used in this investigation is the same as that previously described for studies of essential oil constituents in plant material, barbiturates in urine and cannabinoids in cannabis[4–8]. In the present investigation the solvents in two steroid hormones, estradiol benzoate and cortisone acetate, have been analyzed. The concentration of solvents was in both cases less than 0.01%.

Experimental

The following operating conditions were used:

System 1. Gas chromatograph, Becker GC oven Model 1452 D (Becker-Delft, The Netherlands); detector, FID; column, 2-m long glass coil, I.D. 2 mm, filled with Porapak Q; carrier gas, nitrogen, 30 ml/min; temperature, oven: 175°; injector: 230°.

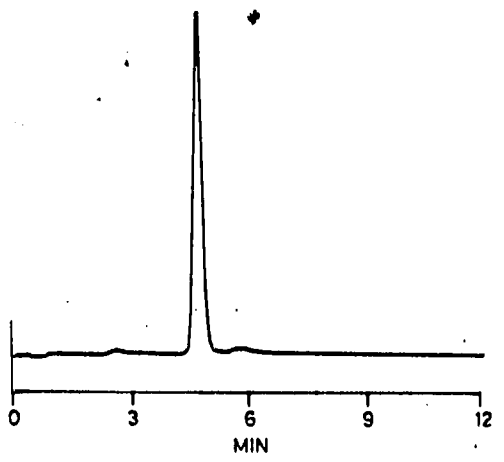
System 2. Gas chromatograph, the top of a Becker GC oven Model 1452 D inclusive of injection and detector units. The oven was replaced with a Lauda ultrathermostat water bath; detector, FID; column, 2-m long glass coil, I.D. 2 mm, filled with 5% polyethylene glycol 400, on Chromosorb W, AW, 60–80 mesh; carrier gas, nitrogen; 30 ml/min; temperature, column: 30°; injector: 230°.

Solid sample injection device as described previously[4–8].

5–10 mg steroid hormone were introduced into a thin-walled glass tube closed at one end (2.5 cm long, I.D. 1.5 mm). A thin film of PTFE ("Crosslite tape") was put around the upper part of the glass tube to fasten it to the holder of the injection device. This was connected to the flash heater of the gas chromatograph. The glass tube with the steroid hormone was moved into the flash heater and withdrawn after 5–10 sec.

Results and discussion

On heating, desolvation of the solvate crystals begins at a temperature far below the melting point of the compound. For cortisone acetate the process starts at 70–90° depending upon the type of solvent present[1]. For our gas chromatographic investigations, however, we preferred to use a temperature of the flash heater equal to or higher than the melting point of the steroid hormone. Desolvation then occurs rapidly and the solvent is brought into the column by the carrier gas. The identification was carried out by comparison of retention times on two different columns (Porapak Q and polyethylene glycol 400). Figs. 1 and 2 show chromatograms of the solvents in estradiol benzoate (acetone) and cortisone acetate (ethanol) on the



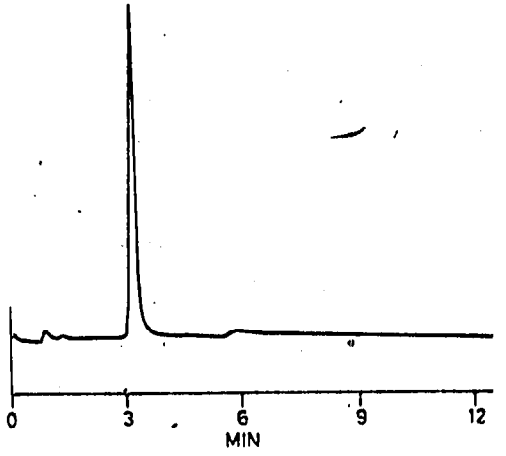


Fig. 1. Acetone in estradiol benzoate. Column, Porapak Q, attenuation 3×10^2.
Fig. 2. Ethanol in cortisone acetate. Column, Porapak Q, attenuation 1×10^2.

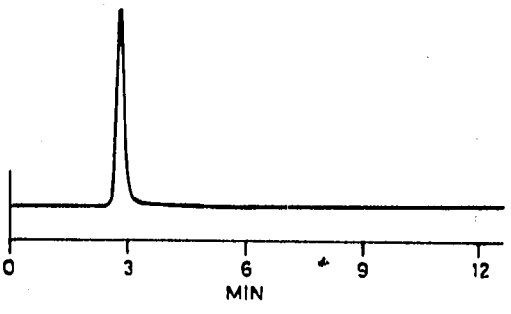


Fig. 3

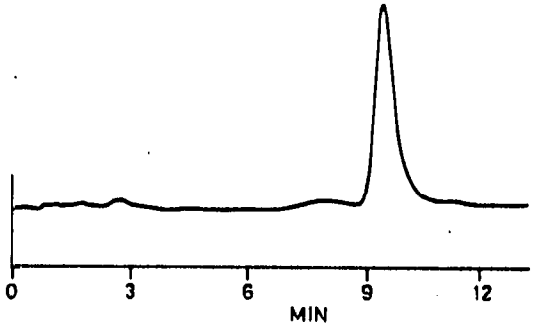


Fig. 4

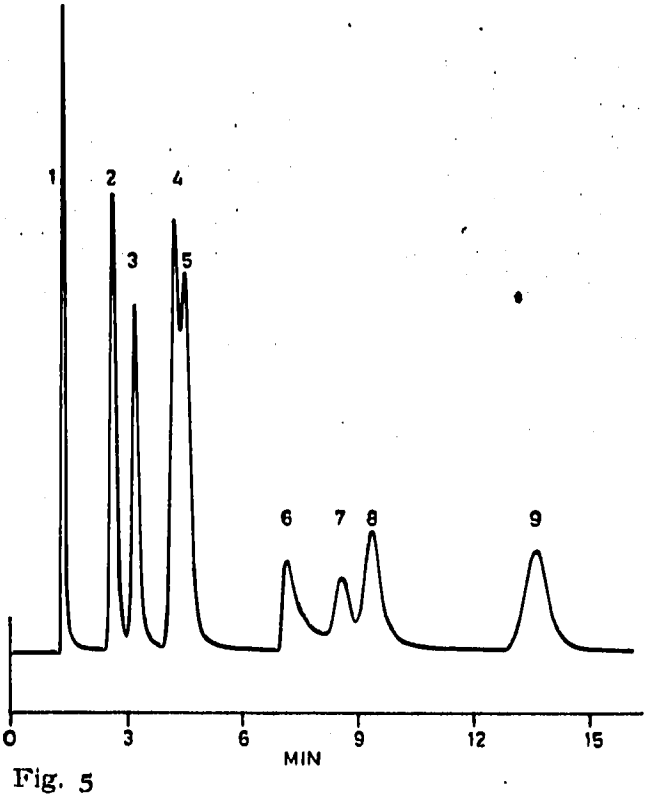


Fig. 5

Fig. 3. Acetone in estradiol benzoate. Column, 5% polyethylene glycol, attenuation 3×10^2.
Fig. 4. Ethanol in cortisone acetate. Column, 5% polyethylene glycol, attenuation 1×10^2.
Fig. 5. Usual solvents separated on a 5% polyethylene glycol 400 column. 1, Cyclohexane; 2, acetone; 3, tetrahydrofuran; 4, methylene chloride; 5, benzene; 6, methanol; 7, chloroform; 8, ethanol; 9, dioxane.

Porapak column. Figs. 3 and 4 show the chromatograms of the solvents in the hormones mentioned on the polyethylene glycol 400 column.

Since a column temperature of 30° can hardly be obtained with a normal gas chromatographic oven when a flash heater temperature of 230° has to be used, we preferred to use an ultra-thermostat water bath for this purpose. An excellent tem-

perature stability was obtained in this way. At 30° the usual solvents are separated on a polyethylene glycol 400 column as shown in Fig. 5. At column temperatures below approximately 30° the alcohol peaks are not eluted as Gaussian peaks, but appear negatively skewed on the chromatogram, probably due to condensation in the column.

At high flash heater temperatures and relatively long injection time, pyrolysis of the steroid hormone may take place. If cortisone acetate is heated for 30 sec at 230° a compound is eluted having the same retention time as acetic acid on the Porapak Q column. By repeating the injection of the sample the peak area of the pyrolysis product, in contrast to those of the solvents, will not decrease. This could be shown for cortisone acetate by repeated injection at 230° for 30 sec.

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