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## Quantitative determination of residual solvents in steroid hormones by means of gas–liquid chromatography and solid injection

We recently published a paper describing a gas–liquid chromatography (GLC) technique for the qualitative demonstration of residual solvent in some steroid hormones based on solid injection[1]. The purpose of the present investigation was to devise a method for the quantitative determination of such solvents and by means of this method to determine the content of residual solvents present in commercially available steroid hormones.

*Experimental*

The following operating conditions were used: gas chromatograph, Becker GC Oven Model 1452 (Becker, Delft, The Netherlands); flame ionization detector; column, 2-m long glass coil, I.D. 2 mm, filled with Porapak Q; carrier gas, nitrogen, 30 ml/min; temperature: oven, 175° and injector, 270°; integrator, Kent Chromalog 2 (Kent Instruments Ltd.); solid sample injection device as described previously[1–7].

*Calibration curves.* Standard solutions of acetone and methyl ethyl ketone were prepared in methanol, standard solutions of methanol, ethanol and ethyl acetate were prepared in benzene. Thin-walled glass tubes closed at one end (4-cm long, I.D. 1 mm) were placed over solid $CO_2$ and known volumes of the standard solutions were introduced into the glass tubes by means of a 10-$\mu$l Hamilton syringe. The open ends of the tubes were sealed in a flame. The glass tubes were fastened to the holder of the solid injector and this was connected to the injection port of the gas chromatograph. The glass tubes were moved into the flash heater and crushed there, as described

TABLE I

QUALITATIVE ANALYSES OF RESIDUAL SOLVENTS IN STEROID HORMONES

| *Steroid hormone* | *Acetone* | *Ethanol* | *Ethyl acetate* | *Methanol* | *Methyl ethyl ketone* |
|---|---|---|---|---|---|
| Betamethasone | | | | | |
| Betamethasone valerate | + | | | | |
| Cortisone acetate[a] | | + | | | |
| Deoxycorticosterone acetate | | | + | | |
| Estradiol[b] | | + | | | |
| Estradiol benzoate[b] | + | + | | | |
| Estrone | | + | | | |
| Ethynylestradiol | + | | | | |
| Hydrocortisone[b] | | | | | + |
| Hydrocortisone acetate[b] | + | | + | | |
| Methyltestosterone[b] | | | + | + | |
| Prednisolone[b] | + | | | | + |
| Prednisone[b] | + | | | + | |
| Progesterone[b] | | | | + | |
| Testosterone propionate | | | | | |

[a] One sample contained ethanol; in two other samples no solvent could be detected.
[b] Two samples analyzed.

previously[4,5]. The detector signals were registered by the electronic integrator and then plotted against the amount of solvent. The best straight lines were calculated by the method of least squares.

*Residual solvents in steroid hormones.* Amounts of 2–10 mg of steroid hormone were accurately weighed in thin-walled glass tubes closed at one end (2.5-cm long, I.D. 1.5 mm). A thin film of PTFE ("Crosslite tape") was fixed 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 30 sec. After the GC analysis was completed, the hormone was again moved into the flash heater for 30 sec for a new analysis to check if the evaporation of the residual solvent had been completed by the first injection. The cooling cap of the injector was filled with ice so as to cool the sample between the analyses. The detector signals were registered by the electronic integrator and the content of residual solvent calculated by means of the calibration curve.

*Results and discussion*

The results of the quantitative determinations are shown in Table I. In the samples of betamethasone, testosterone propionate and two samples of cortisone

TABLE II

QUANTITATIVE ANALYSES OF RESIDUAL SOLVENTS IN STEROID HORMONES

| *Steroid hormone* | *Solvent* | *Residual solvent (μg per mg of steroid hormone)* |
|---|---|---|
| Deoxycorticosterone acetate | Ethyl acetate | 0.16 |
| Estradiol | Ethanol | 0.18 |
| Estradiol benzoate | Ethanol | 0.62 |
| Hydrocortisone | Methyl ethyl ketone | 1.05 |
| Hydrocortisone acetate | Acetone | 0.12 |
| Methyltestosterone | Ethyl acetate | 0.66 |
| Prednisolone | Acetone | 1.84 |
| Prednisone[a] | Methanol | 0.62 |
| | Acetone | 2.02 |
| Progesterone | Methanol | 0.15 |

[a] Two samples analyzed.

acetate* investigated, no solvent could be detected. In some instances one mair solvent and traces of others were found. In Table I only the main solvents are tabulated

The introduction of known amounts of volatile liquids into the gas chromato graph is difficult. Encapsulation in glass capillaries[8], micro-pipettes[9] and indium[1] has been used. The introduction technique used in the present investigation gav satisfactory results. The dimensions of the glass tubes are, however, critical. To great an inner diameter makes the sealing difficult, and too small an inner diamete causes pushing back of the liquid along the syringe needle. Cooling over $CO_2$ and long glass tube prevents evaporation of the solvent before the sealing. Residues o

* Nomenclature according to the Merck Index, VIII.

crushed glass tubes in the injection port of the gas chromatograph have to be removed periodically, usually after 5–8 analyses.

For some steroid hormones, the evaporation of the residual solvent was not completed during the first 30 sec heating, and a repeated injection was necessary so as to obtain a complete evaporation. An injection time of 40–50 sec was, however, sufficient in all instances. As a relatively long injection time usually gives considerable peak broadening and may also lead to decomposition of sensitive hormones, we preferred to carry out the analyses by repeated injections. No difference could be observed in the quantitative results obtained by the two injection techniques.

The results of the quantitative analyses are shown in Table II. A random sample of ten steroid hormones was taken. The results are the mean values of four determinations per steroid hormone. The standard error of the determination of acetone in prednisone, the highest value that was found, was 5.1%. The standard error of the determination of acetone in hydrocortisone acetate, the lowest value that was found, was 16.3%.

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