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Note

Rapid determination of 4-hydroxybutyric acid (Gamma OH) and 2-propyl pentanoate (Depakine) in human plasma by means of gas–liquid chromatography

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The routine laboratory of the Department of Clinical Pharmacy, responsible for the determination of plasma concentrations of various drugs used in the hospital, has to cope with large numbers of samples.

The drug 4-hydroxybutyric acid (Gamma OH) used as an anaesthetic for surgery and as a hypnotic, shows a zero-order elimination similar to ethanol ($T_{\frac{1}{2}}$ = 5 h–30 min). For this reason, concentration profiles of patients based on a large number of samples are required in order to derive pharmacokinetic parameters of the drug.

The short half-life ($T_{\frac{1}{2}}$ = 5–8 h) of 2-propyl pentanoate (di-*n*-propyl acetate, Depakine), used in the treatment of epilepsy, results in large fluctuations of the plasma concentration during chronic and intermittant treatment. One plasma sample taken at an uncontrolled time after intake of the drug is of little value, as frequent sampling is a prerequisite in drug therapy control. The gas chromatographic system described in this note permits a very fast analysis and almost instant therapy control.

EXPERIMENTAL

4-Hydroxybutyric acid

A 1-ml volume of blood is centrifuged for 10 min at *ca.* 16000 *g* in an Eppendorf tube (Model B-20A centrifuge, International Equipment Co.), then 200 μl of plasma are added to 200 μl of 12 *N* sulphuric acid and 30 μg of internal standard (cyclohexanecarboxylic acid; Merck, Darmstadt, G.F.R.) in 200 μl of chloroform in a nipple-tube. The solution is mixed for 15 sec on a Vortex mixer and centrifuged for 10 min at *ca.* 2600 *g* (Hereaus Christ centrifuge). Three layers appear to be present in the tube: an acidic aqueous layer and a denaturated protein layer in the wide tube, and a clear solution of chloroform in which is butyrolactone, the reaction product of 4-hydroxybutyric acid and 12 *N* sulphuric acid, and the internal standard in the nipple. A 2-μl volume of the chloroform layer is injected into the gas chromatograph.

2- Propyl pentanoate

A 1-ml volume of blood is centrifuged for 10 min at 7000 rpm in an Eppendorf tube as above, then 200 μl of plasma are added to 20 μl of 12 *N* sulphuric acid and 30 μg of internal standard (cyclohexanecarboxylic acid) in 200 μl of chloroform in a nipple-tube. The solution is mixed for 15 sec on a Vortex mixer and centrifuged for 10 min at *ca.* 2600 *g* as above. A 2-μl volume of the clear chloroform layer containing 2-propyl pentanoate and internal standard is injected into the gas chromatograph.

Gas chromatography

A Hewlett-Packard 402 gas chromatograph is used, equipped with a flame-ionization detector. The column, 1.80 m × 3 mm I.D., is packed with 5% (w/w) FFAP on Gas-Chrom Q, 60–80 mesh (Applied Science Labs., State College, Pa., U.S.A.). FFAP, free fatty acid phase, substance SP 1000, was obtained from Chrompack (Middelburg, The Netherlands). The temperatures used were: column, 150° for 4-hydroxybutyric acid and 170° for 2-propyl pentanoate, injector 200° and detector 225°; carrier gas flow-rates: nitrogen 30 ml/min, hydrogen 30 ml/min and air 150 ml/min; recorder, Yokogawa 1 mV and Honeywell 1 mV.

A calibration graph was prepared for both compounds, which revealed a correction factor of 0.80 ± 0.05 for 2-propyl pentanoate and 3.90 ± 0.10 for 4-hydroxybutyric acid (ratio of peak areas = correction factor × ratio of weight of compound to internal standard).

RESULTS

Table I gives the retention times of 4-hydroxybutyric acid, 2-propyl pentanoate and analogous compounds relative to cyclohexanecarboxylic acid as internal standard. It can be seen that there is a large difference in retention time between butyrolactone, formed from 4-hydroxybutyric acid, and the internal standard. This setback can be overcome by injecting three times and then waiting for the three peaks of the internal standard, as demonstrated in Fig. 1.

The lowest plasma concentration that can be measured is 5 μg/ml for 4-hydroxybutyric acid and 1 μg/ml for 2-propyl pentanoate. The FFAP column is able to withstand more than 500 injections of the chloroform extract before it deteriorates.

TABLE I

RETENTION TIMES OF BUTYROLACTONE, 2-PROPYL PENTANOATE AND ANALOGOUS COMPOUNDS ON A COLUMN OF 5% (w/w) FFAP AT 150°C, RELATIVE TO CYCLOHEXANECARBOXYLIC ACID

Compound	*Relative retention time*
2-Propyl pentanoate ethyl ester	0.04
α-Methylbutyrolactone	0.16
Butyrolactone (4-hydroxybutyric acid)	0.18
Valerolactone (5-hydroxyvaleric acid)	0.28
ε-Caprolactone	0.48
2-Propyl pentanoate	0.54
Cyclohexanecarboxylic acid	1.00
Cycloheptanecarboxylic acid	2.00

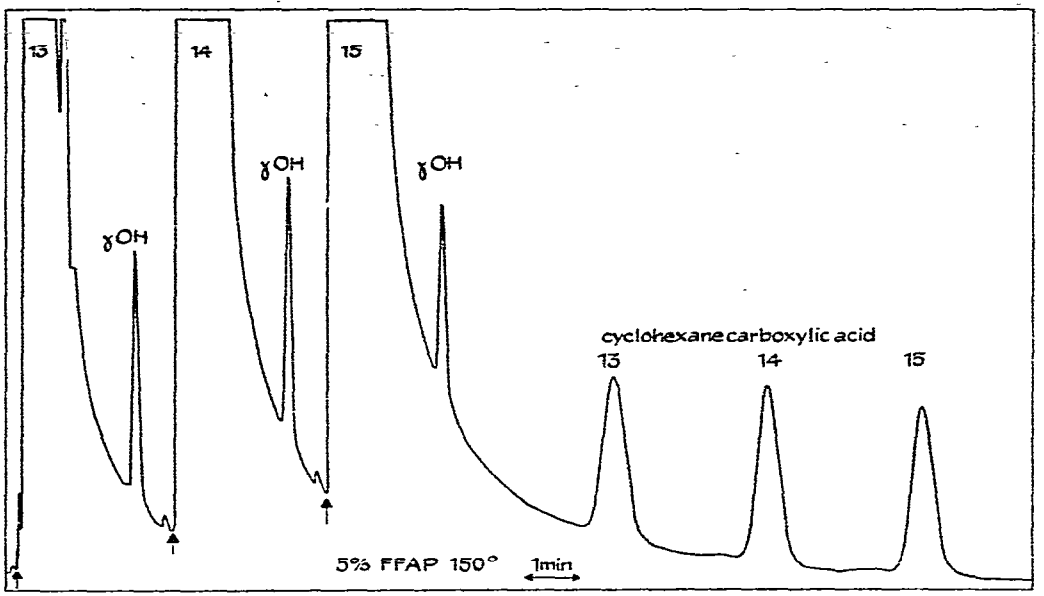

Fig. 1. Gas chromatogram of three injections of butyrolactone (4-hydroxybutyric acid, Gamma OH) extracted from human plasma samples numbered 13, 14 and 15.

DISCUSSION

The FFAP column has a higher stability than the diethylene glycol succinate columns used in earlier assay methods based on almost the same technique[1–4]. The sensitivity that is obtained with this method for both drugs is acceptable, as the plasma level of 4-hydroxybutyric acid for anaesthesia is greater than 100 μg/ml, for induction of sleep about 100 μg/ml and for sedation about 50 μg/ml. The therapeutic level of 2-propyl pentanoate is between 60 and 80 μg/ml.

The gas chromatographic analysis of 2-propyl pentanoate and 4-hydroxybutyric acid takes about 5 min, while gas chromatography plus extraction takes no longer than 15 min. Hence this column for acidic drugs with cyclohexanecarboxylic acid as internal standard has a very high capacity in handling plasma samples for drug monitoring and therapy control.

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