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Note

Gas chromatographic analysis of sodium di-*n*-propylacetate in human plasma

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The anticonvulsant properties of sodium di-*n*-propylacetate were first reported in 1963 by Meunier *et al.*[1]. For some years, this drug has been used in primary generalized epilepsy. The optimal therapeutic levels are believed to be *ca.* 60 μg/ml of plasma. For clinical studies a rapid and accurate determination of the drug plasma level is required.

The drug represents a challenge to the normal gas chromatographic (GC) procedures since it is not possible to concentrate solvent extracts containing the free acid because of its high volatility. Using this property, Meyer and Hessing-Brand[2] described a method using a micro-diffusion technique of the Conway type. This is, however, a very time-consuming procedure. Other gas–liquid methods have also been described, but the sodium di-*n*-propylacetate levels were difficult to measure, due to peak tailing of the drug on the conventional liquid phases used[3–5]. The injection of whole plasma as described by Dacremont and Cocquyt[6] results in a considerable column contamination.

Using a new stationary phase (AT-1000), mainly intended for determinations of free fatty acids, very symmetric peaks were obtained. This paper describes the deproteinization and solvent extraction of the drug with small volumes of carbon disulphide, followed by direct injection into the GC column.

EXPERIMENTAL

Chemicals

Sodium di-*n*-propylacetate was obtained from Labaz (Brussels, Belgium), 2-ethyl-2-methylcaproic acid from Fluka (Buchs, Switzerland) and analytical grade carbon disulphide, perchloric acid and sodium hydroxide from E. Merck (Darmstadt, G.F.R.). AT-1000 was from Alltech (Arlington Heights, Ill., U.S.A.), Gas-Chrom Q (100–120 mesh) from Applied Science Labs. (State College, Pa., U.S.A.).

Reagents

Solutions of sodium di-*n*-propylacetate (200 mg/l) in water and of 2-ethyl-2-methylcaproic acid (2 g/l) in 1 *M* sodium hydroxide were kept at 4°. A standard solution of the free di-*n*-propylacetic acid was prepared by acidification of an aliquot of sodium di-*n*-propylacetate and extraction with diethyl ether. The ether was gently removed at room temperature and the free acid was dried in a vacuum desiccator. The standard solution contained 200 mg/l in ethanol and was stored at —22°.

Gas chromatography

All the analyses were performed with a Model 5710A gas chromatograph with dual flame-ionization detectors and a Model 3380A recorder-integrator (both from Hewlett-Packard, Avondale, Pa., U.S.A.). Columns were glass (150 cm × 3 mm I.D.), packed with 10% AT-1000 on Gas-chrom Q (100–120 mesh). Before use, the columns were conditioned at 250° for 24 h. The carrier gas (nitrogen) flow-rate was 22 ml/min; air, 240 ml/min and hydrogen, 20 ml/min. The column temperature was maintained at 200°. The injector block and the flame-ionization detector were kept at 250°.

Method

Into a 2.5-ml vial were pipetted 0.5 ml of plasma and 10 μl of the internal standard solution. After rapid mixing, 150 μl of carbon disulphide and 100 μl of a 10% perchloric acid solution in water were added mixed for 1 min with a vortex-type mixer. The mixture was then centrifuged for 5 min at *ca.* 3000 *g*. The upper layer was removed by suction and subsequently lifting the protein cloth with a Pasteur pipette. A 1-μl volume of the organic layer was injected into the gas chromatograph.

RESULTS AND DISCUSSION

To prevent the formation of a thick emulsion during the extraction, deproteinization is essential. The effect of perchloric acid in various concentrations was studied, and it was found that the addition of 100 μl of a 10% perchloric acid solution per 0.5 ml plasma gave the best results. Carbon disulphide gives a very good extraction efficiency; chloroform, diethyl ether, hexane and carbon tetrachloride were less

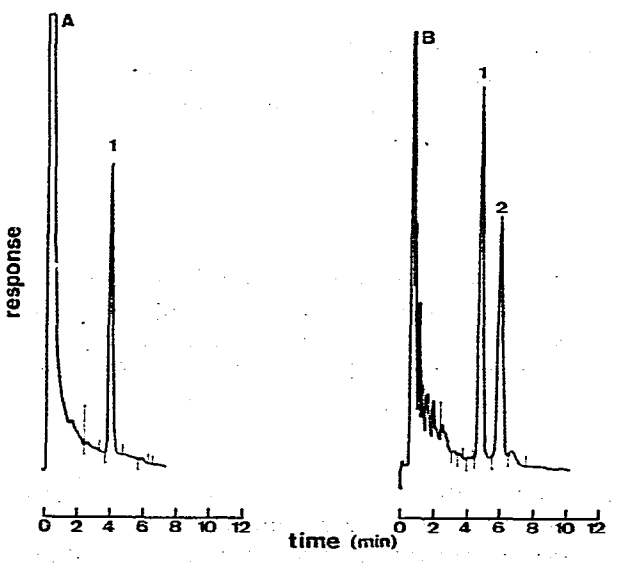

Fig. 1. Chromatogram of a plasma extract of a patient given sodium di-*n*-propylacetate medication. A, No internal standard added; B, quantitative estimation (plasma level = 45 μg/ml). Attenuation, × 32. Peaks: 1 = di-*n*-propylacetic acid, RRT = 0.92; 2 = 2-ethyl-2-methylcaproic acid, RRT = 1.00. RRT = Retention time relative to that of the internal standard.

efficient and, in terms of gas–liquid chromatography, gave more impurities and larger solvent peaks.

The stationary phase used, AT-1000, is an esterified modification of Carbowax 20M, recommended specially for the analysis of free fatty acids. Fig. 1 illustrates chromatograms obtained from plasma samples of patients receiving therapeutic doses of sodium di-*n*-propylacetate. For the determination of the extraction efficiency we used blank plasma samples containing known amounts of sodium di-*n*-propylacetate and blank plasma samples containing the internal standard only. To the latter extracts was added a known amount of the free acid in order to measure absolute recoveries. The average percentage extraction was 76.8% over a concentration range of 10–150 μg/ml.

Fig. 2 illustrates a typical standard curve obtained by our procedure. The ratio of the peak area of di-*n*-propylacetic acid to the peak area of the internal standard was linearly related to concentration up to 200 mg/l in plasma.

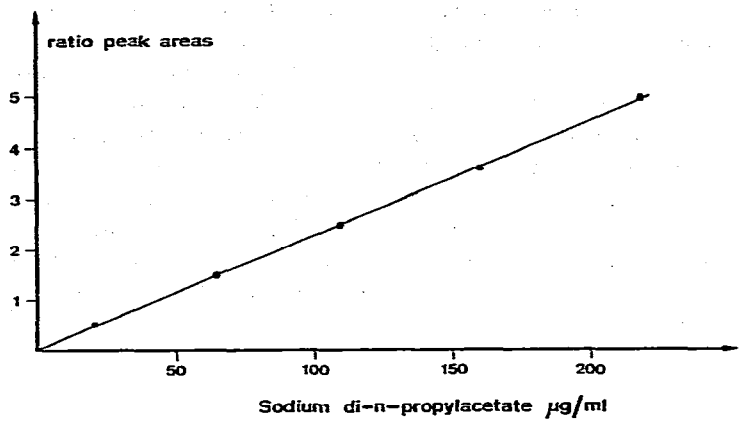

Fig. 2. Standard curve for sodium di-*n*-propylacetate from plasma.

For five analyses of each of three different samples the relative standard deviation was 1.8%. The analysis of drug-free pooled plasma by the proposed procedure showed no interfering peaks at the retention times of di-*n*-propylacetic acid and the internal standard.

The determination of plasma levels of sodium di-*n*-propylacetate with the above method is very simple and offers the possibility of a more accurate determination at low drug levels. An urgent determination can easily be performed within 30 min, while the routine analysis of 50 samples per day may be performed by only one technician.

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