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Note

Gas chromatographic determination of valproate in minute serum samples after extractive methylation

OLLE GYLLENHAAL and AGNETA ALBINSSON
Section of Toxicology, Department of Clinical Chemistry, General Hospital, S-214 01 Malmö (Sweden)
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The anticonvulsant effect of valproate (Depakine®) has been known for several years[1], and efficient control of seizures in epilepsy therapy requires the monitoring of serum levels in patients. Determination of valproate has mainly been performed by gas–liquid chromatography (GLC) of the corresponding acid, the method usually being based on extraction of the acidified serum sample with a small volume of organic solvent[2]; an aliquot is then used for GLC.

Silylation[3,4] and methylation[5] procedures have been used to improve the chromatographic properties of valproic acid. Extractive alkylation is a convenient derivatisation technique for organic acids before GLC[6]; it combines extraction with derivatisation, and has been used in analysis for a wide range of drugs and related compounds[7–10].

The method described here for the determination of valproate was developed to improve the chromatographic properties and to reduce the volume of serum required for analysis. It involves partition of valproate, as an ion-pair with tetrabutylammonium as counter-ion, into dichloromethane containing iodomethane. After concentrating the organic phase, separation and quantitation are performed by GLC, with flame ionization detection.

EXPERIMENTAL

Apparatus

Gas chromatography. A Varian 2700 instrument, with flame ionization detector, was used. The glass column (180 × 0.2 cm I.D.) was filled with 2% of SP-1000 on Supelcoport (100–120 mesh) and was operated at 80°, with the injector at 180° and the detector at 240°. The flow-rate of the nitrogen carrier gas was 20 ml/min.

Mass spectrometry. The methyl derivatives were identified in an LKB 9000 instrument after separation on a glass column filled with 5% of OV-17 on Gas-Chrom Q (80–100 mesh). The ionisation potential was 70 eV.

Reagents and chemicals

Sodium valproate (Orion-Yhtymä, Espoo, Finland), tetrabutylammonium hydrogen sulphate (Labkemi, Gothenburg, Sweden), iodomethane (E. Merck, Darmstadt, G.F.R.) and heptanoic acid (Merck) were used. The solvents were of AnalaR quality (BDH, Poole, Great Britain).

Methods

Evaluation of reaction conditions. Serum (100 μl) was mixed with 500 μl of 0.2 *M* tetrabutylammonium hydrogen sulphate in 0.2 *M* sodium hydroxide and 0.1 *M* phosphate buffer of pH 8, and 500 μl of 1.6 *M* iodomethane in dichloromethane. To the mixture were added 50 μl of 0.1 *M* sodium valproate, 0.6 μl of heptanoic acid and 0.6 μl of bromobenzene (internal standard), and the mixture was shaken at 24°; 25-μl aliquots of the organic phase were withdrawn and mixed with 100 μl of 0.05 *M* sulphuric acid in order to quench the reaction, and 1 μl of each resulting organic phase was injected into the chromatograph.

Synthesis of methyl valproate. A solution of sodium valproate (300 mg) in dimethyl sulphoxide (20 ml) was mixed with iodomethane (200 μl)[11], and the mixture was allowed to stand overnight. After dilution with water (50 ml), the solution was extracted with dichloromethane (2 × 20 ml), and the combined extracts were washed once with the buffer solution of pH 8 and six times with water, then evaporated; the purity of the remaining oil was checked by GLC.

Determination of valproate in serum. Serum (100 μl) was mixed with 500 μl of 0.2 *M* tetrabutylammonium hydrogen sulphate in phosphate buffer solution of pH 8 and 500 μl of 1.6 *M* iodomethane in dichloromethane containing 22.5 μg/ml of heptanoic acid (internal standard). The tube was shaken for 40 min and centrifuged. About 300 μl of the organic phase was withdrawn, transferred to a tapered tube and evaporated to dryness (4–5 min), the residue was dissolved in 20 μl of carbon tetrachloride, and 1 μl of the organic phase was injected into the chromatograph.

RESULTS AND DISCUSSION

The time course for the methylation of valproate is illustrated in Fig. 1; after 35 min, a constant yield of methyl valproate was obtained.

Under our conditions, 80% of the valproate was present as an ion-pair with tetrabutylammonium in the dichloromethane phase (no iodomethane present).

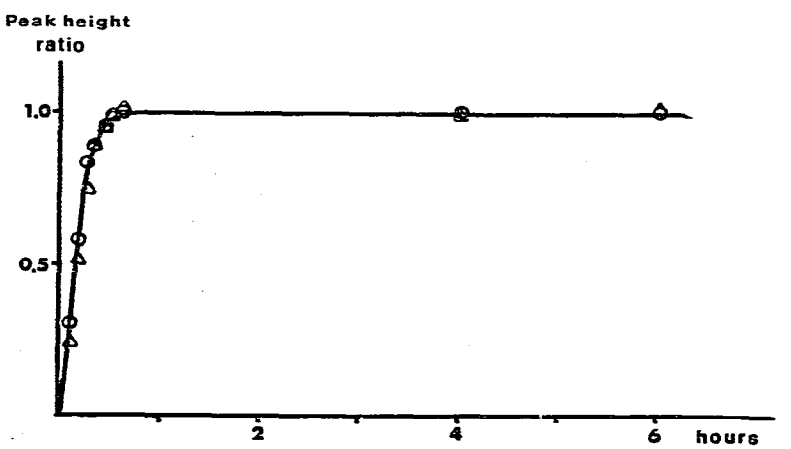

Fig. 1. Time course for methylation of valproate and heptanoate. Aqueous phase: 0.5 ml of 0.2 *M* tetrabutylammonium (pH 8), 100 μl of serum, 50 μl of 100 m*M* sodium valproate and 0.6 μl of heptanoic acid. Organic phase: 0.5 ml of dichloromethane containing 1.6 *M* iodomethane and 0.6 μl of bromobenzene (internal standard). △ = Methyl heptanoate; ○ = methyl valproate.

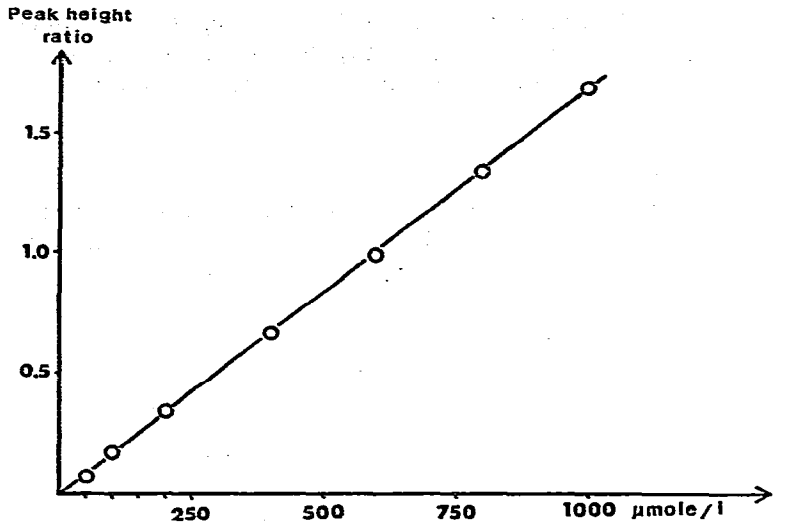

Fig. 2. Standard graph for determination of valproate in serum.

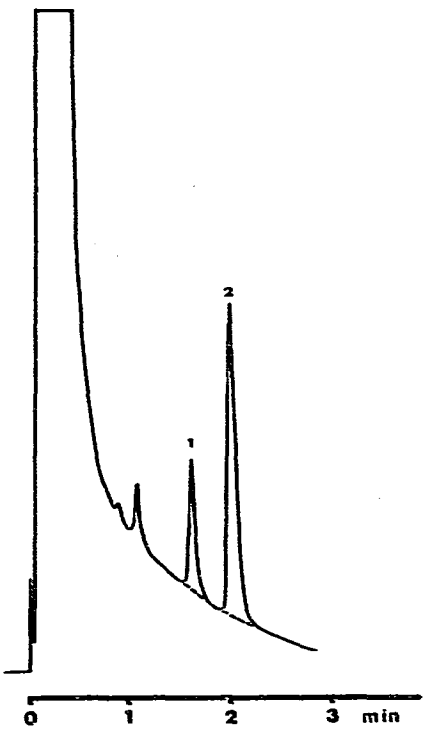

Fig. 3. Gas chromatogram from analysis for valproate in serum: Full line, serum from patient; dotted line, blank serum. Peaks: 1 = methyl valproate; 2 = methyl heptanoate (internal standard). Results: 265 µmoles of valproate per litre of serum.

Heptanoic acid was selected as internal standard because heptanoate reacted similarily to valproate (*cf.* Fig. 1) and was eluted as methyl heptanoate in the vicinity of methyl valproate (*cf.* Fig. 3). Methyl valproate and heptanoate were stable for at least 7 h prolonged reaction time.

Determination of valproate in serum

In order to determine small amounts of valproate in minute samples of serum, the organic phase was concentrated by evaporating the dichloromethane and iodomethane and reconstituting the residue in a small volume of carbon tetrachloride. This also reduced the solvent peak in the chromatograms compared with those obtained when dichloromethane was used as solvent.

A standard graph for the determination of valproate in serum at concentrations down to 50 μmole/l is shown in Fig. 2. The precision at the level of 200 μmole/l was 4.3% ($n = 6$), and the yield in relation to a solution of the pure methyl ester was 92%.

A chromatogram obtained by analysing serum from a patient receiving sodium valproate is shown in Fig. 3; the serum level was 265 μmole/l.

The proposed method has been in use for about a year, during which time we have observed no interference from such other antiepileptic drugs as carbamazepine, clonazepam, phenytoin and phenobarbitone.

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