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Note

Simple method for measuring valproate (Epilim) in biological fluids

S. WILLOX and SUSAN E. FOOTE

Section of Clinical Pharmacology, Department of Medicine, School of Medicine, University of Auckland, Private Bag, Auckland (New Zealand)

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Sodium valproate (sodium di-*n*-propylacetate) has proved a useful anticonvulsant in epilepsy, particularly in cases of petit mal epilepsy and where conventional anticonvulsant therapy has not achieved control[1,2]. In order to provide an analytical service for a clinical trial of sodium valproate at present underway in Auckland Hospital a simple method for measuring the drug in plasma and urine was required. The methods in the literature were either too complex and time consuming for routine use[3] or in our hands proved unreliable. On-column methylation has provided a quick and simple method for estimating this drug in biological fluids. Plasma levels likely to be achieved by therapeutic doses of sodium valproate fall within the range of 20–250 μg/ml and the method described here has been shown to be linear over this range.

METHODS AND MATERIALS

Sodium valproate was provided by Reckitt & Colman (New Zealand). Cyclohexane carboxylic acid, the internal standard, was purchased from Chem Service (West Chester, Pa., U.S.A.) and Methelute (trimethylanilinium hydroxide 0.2 *M* in methanol) was purchased from Pierce (Rockford, Ill., U.S.A.). Trimethylphenylanilinium hydroxide (0.1 *M* in methanol) was an Eastman-Kodak (Rochester, N.Y., U.S.A.) product. Diethylether was redistilled before use.

A Pye 104 gas chromatograph fitted with a flame ionisation detector was used for the analysis. A glass column 1.5 m × 6 mm I.D. was packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q. The detector and injection port temperatures were 220° and the oven temperature was 93°. The carrier gas was nitrogen flowing at 45 ml/min. Standard curves were prepared using blank human plasma or urine. Peak areas were measured with a Minilab electronic integrator and the peak area ratio of the drug to the internal standard was plotted against valproate concentration.

Sample preparation

Plasma. To 1 ml of plasma in a 10-ml glass centrifuge tube was added 25 μg (in 50 μl) of cyclohexane carboxylic acid and 50 μl of 6 *N* sulphuric acid. After addition of 4 ml of ether the tubes were rotated on a Heidolph mixer for 10 min. The mixture was centrifuged at 3000 *g* for 5 min and the organic layer transferred to a 3-

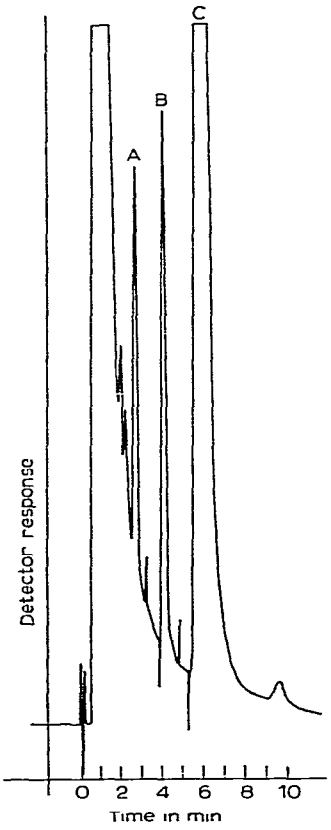

Fig. 1. Chromatograph of methylvalproate (A) and the internal standard (B) extracted from plasma. Peak C: see text.

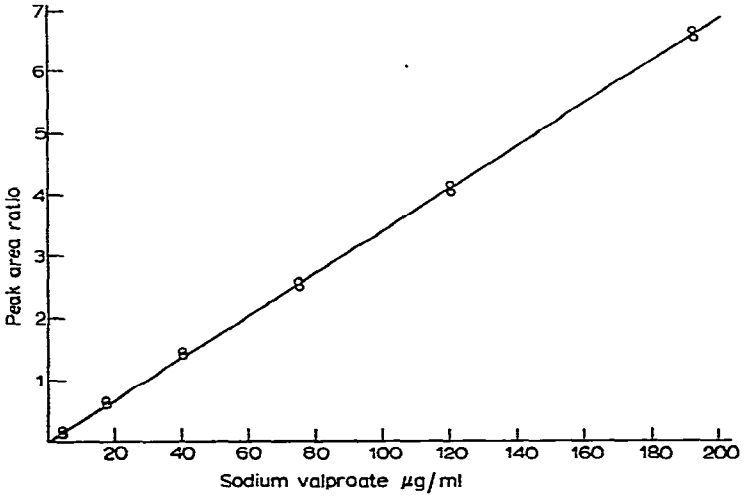

Fig. 2. Standard curve for sodium valproate in plasma or urine.

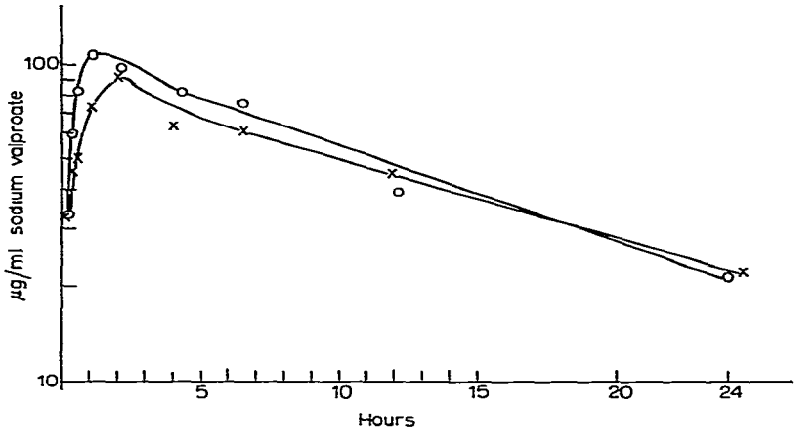

Fig. 3. Plasma decay curve of sodium valproate after a single 800-mg oral dose (two volunteers).

ml glass container where it was dried over sodium sulphate. The ether was evaporated on a heating block at about 55° and when dry 25 µl of either Methelute or trimethylphenylanilinium hydroxide was added. One microlitre was injected onto the gas chromatographic column.

Urine. To 1 ml of urine in a 2-ml glass ampoule was added 100 µl of 10 *N* HCl and 25 µg (in 50 µl) of internal standard. The ampoule was flushed with nitrogen, sealed and heated at 90° for 2 h. After cooling, the liquid was transferred to a 10-ml centrifuge tube and treated as described above. Unconjugated valproate was estimated in urine without hydrolysis using 1 ml of urine and proceeding as above.

RESULTS AND DISCUSSION

A chromatogram of methyl valproate and methylcyclohexanoate extracted from plasma is shown in Fig. 1. The retention times are 2.7 and 4.2 min, respectively.

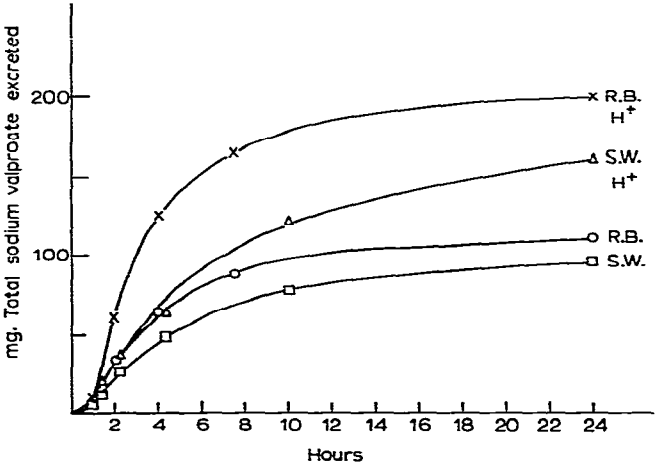

Fig. 4. Cumulative urinary excretion of sodium valproate for two volunteers (R.B. and S.W.) before and after acid hydrolysis (H^+) of the samples.

A third peak, C, occurs in all the blanks but does not interfere with the analysis. The standard curve was linear over the range 5–300 μg/ml and using the method of least squares the line of best fit had a correlation coefficient greater than 0.99 (Fig. 2).

We have used this method to measure routine plasma levels in epileptic patients and for simple pharmacokinetic measurements in volunteers. Typical plasma decay curves and urinary excretion curves are shown in Figs. 3 and 4. It is apparent from Fig. 4 that hydrolysis is required to release conjugated valproate in urine in order to measure total urinary excretion of the drug. Approximately one quarter of an 800-mg oral dose was excreted as free or conjugated valproate in the urine. In agreement with other workers[4] we found the plasma half-life of the drug to be about 10–12 h in normal volunteers.

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