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DETERMINATION OF VALPROIC ACID (DIPROPYLACETIC ACID) IN PLASMA BY GAS–LIQUID CHROMATOGRAPHY

D. J. BERRY and L. A. CLARKE

Poisons Unit, New Cross Hospital, Avonley Road, London SE14 5ER (Great Britain)

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SUMMARY

A specific and sensitive procedure for the determination of valproic acid at therapeutic concentrations in human plasma has been developed. The method involves a microscale extraction of the drug from acidified plasma into chloroform. Caproic acid is added as internal standard. The extract is subsequently analysed on a gas chromatograph fitted with a flame ionisation detector, and quantitation achieved by the relative-peak-height technique.

INTRODUCTION

Sodium valproate (Epilim) is an antiepileptic drug which has recently been introduced in Great Britain. Its anticonvulsant properties have been demonstrated in animals[1] and man[2,3]. It is used in management of generalised epilepsy, and is particularly effective for treatment of children having petit mal absence seizures. The drug is normally well tolerated although some gastro-intestinal disorders such as diarrhoea, nausea and vomiting have been reported. Hair loss has also been reported but these effects were not usually frequent or severe. Drowsiness is often temporarily associated with high doses of sodium valproate[4] or with concomitant barbiturate medication[5,6]. The latter is consistent with the increase in serum barbiturate concentrations reported when Epilim was added to the drug regime of patients already prescribed phenobarbitone[7,8]. The interaction of sodium valproate with phenytoin is less certain since both elevation[9] and depression[7] of plasma phenytoin levels have been reported.

Many gas chromatographic methods have been published for the determination of valproate concentrations in body fluids[10–16]; the plethora of methods demonstrates the inadequacy of any one to meet the various needs, and many have been quite long and complicated. The method of Meijer and Hessing-Brand[10] incorporates a tedious micro-diffusion technique while that of Dusci and Hackett[11] involves a large-scale extraction in a separating funnel, followed by a back extraction to clean up the specimen. The method of Jensen and Gugler[14] also employs a back extraction and is designed to measure the low plasma concentrations encountered when undertaking single-dose pharmacokinetic studies. All three procedures require 1 ml plasma

or serum and, because they are time consuming, would not be ideal for a large routine workload. Another method[12] is complicated by the need to form a derivative prior to chromatography. The method reported by Schultz and Toseland[15] uses a smaller sample and, although it includes an evaporation step to concentrate the residue, avoids loss of the volatile free acid by fixing it as the potassium salt.

The following method describes a rapid and specific gas chromatographic procedure for the determination of valproic acid in a small volume of plasma without derivative formation. In common with some other cited methods[14–16] it employs a chromatography phase recommended for free fatty-acid analysis. Since this technique was introduced into our laboratory, similar micro-scale methods for underivatised valproic acid have been reported elsewhere[13,16].

METHOD

Principle

Valproic acid is extracted directly into chloroform, from acidified plasma, to which an internal standard has been added. The extract is injected onto a gas chromatograph and quantitated against a range of water standards which are carried through the procedure.

Reagents

The reagents used are: chloroform (AnalaR grade), tested to ensure that it did not contain any extraneous peaks when examined by the gas–liquid chromatographic (GLC) system; hydrochloric acid (concentrated AnalaR grade) and caproic acid (*n*-hexanoic acid) (Sigma, St. Louis, Mo., U.S.A.), diluted with water to a concentration of 60 mg/l, stored in a dark bottle at 4° and used as the internal standard solution.

Instrumentation

A Pye 104 Model 24 dual-column gas chromatograph equipped with flame ionisation detectors, was used throughout in conjunction with a Hitachi Model 56 recorder (1mV f.s.d.). The column was a 1.5 m × 4 mm I.D. coiled glass tube which had been silanised with a 5% solution of dimethyldichlorosilane in toluene. Glass wool is silanised in the same solution. After rinsing with methanol and drying at 100° this column was packed with 1.5% SP 1000 (Applied Science Labs.,State College, Pa., U.S.A.) on 60–80 mesh Gas-Chrom Q (Applied Science Labs.). This packing was prepared by our standard evaporation technique as described elsewhere[17] and conditioned overnight at 180° with the nitrogen carrier gas flow-rate at 60 ml/min.

The instrument settings were as follows. Column oven temperature, 140°; nitrogen flow-rate, 60 ml/min; hydrogen flow-rate, 45 ml/min; air flow-rate, 500 ml/min; sensitivity, $2 \cdot 10^{-10}$ A; injection port setting, 0.

Extraction procedure

Eppendorff pipettes were used to dispense 100 µl plasma and 100 µl internal standard (Caproic acid 60 mg/l) into a Dreyer tube (Scientific Supplies, Vine Hill, London, Great Britain), which was used as the extraction vessel. After adding one drop of concentrated hydrochloric acid and 100 µl chloroform the tube was whirli-

mixed for 30 sec. It was best to add the solvent using a repeating Hamilton dispenser attached to a luer-fitting syringe and fitted with an Everett stainless-steel needle. After mixing, the tube was centrifuged at high speed to separate the layers before injecting 5 µl of the lower phase onto the GLC column. This was done by taking a small quantity of air into the syringe and passing it through the lipoprotein interface into the organic phase. After slowly expelling the air, 5 µl of solvent extract were taken into the syringe and the outside of the needle was wiped with a tissue prior to the injection of the extract onto the gas chromatographic column. The extraction was performed in duplicate and the mean result taken. If the difference between duplicates was greater than 10%, the analysis was repeated.

Quantitative analysis

Standard water solutions of sodium valproate were carried through the extraction procedure with each batch of samples.

A stock solution of sodium valproate was prepared by dissolving 115.3 mg in 100 ml distilled water (this is equivalent to 1 g/l valproic acid). The stock solution was diluted to 10 ml with water as described in Table I to give a range of standards containing 20–100 mg/l.

TABLE I

COMPOSITION OF STANDARD SOLUTIONS OF VALPROIC ACID OBTAINED BY DILUTING A STOCK SOLUTION OF SODIUM VALPROATE (115.3 mg per 100 ml) TO 10 ml WITH WATER

Volume stock solution (µl)	*Concentration of valproic acid (mg/l)*
200	20
400	40
600	60
800	80
1000	100

Of each standard extract 2–5 µl were injected onto the column and the peak heights measured from an extrapolated baseline. A calibration graph was prepared by plotting the ratio of the peak height of drug to peak height of internal standard against valproate concentration. The valproate concentration in a sample was calculated by comparing the peak-height ratio of the sample directly with the standard calibration graph.

Quality control

Sodium valproate was added to fresh, heparinised human plasma to give concentrations of 60 and 80 mg/l, in a way similar to that described above. A plasma control at each concentration was carried through the procedure with each batch of samples.

Reproducibility

Three plasma samples were each analysed twenty times to obtain the reproducibility of the method. The results are shown in Table II.

TABLE II

RESULTS OBTAINED WHEN THREE PLASMA SAMPLES WERE EACH ANALYSED TWENTY TIMES

Sample	*Mean concentration (mg/l)*	*Standard deviation (mg/l)*	*C.V. (%)*
1	38.2	1.43	3.7
2	63.9	2.27	3.6
3	76.8	2.80	3.7

RESULTS AND DISCUSSION

The SP 1000 phase chromatographs of the free, underivatised valproic acid are giving symmetrical peaks and adequate sensitivity (Fig. 1). The phase has been previously recommended for free fatty-acid analysis[17] and is similar to the FFAP advocated by Jensen and Gugler[14]. Another recent publication[16] recommends a new free fatty-acid phase, SP-216-PS for the analysis of underivatised valproic acid. By using these liquid phases one can avoid lengthy derivatisation procedures which are subject to interference.

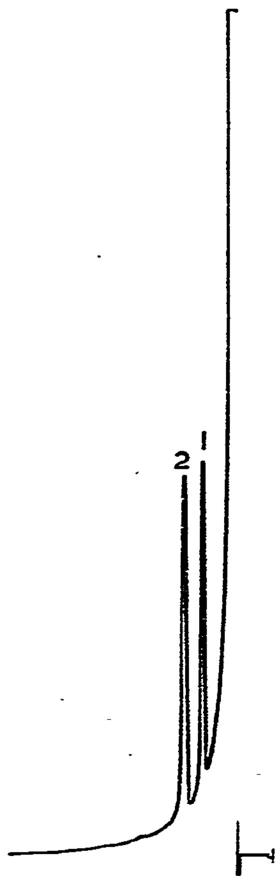

Fig. 1. Separation of valproic acid and caproic acid under the chromatographic conditions described. 1 = Caproic acid, 2 = valproic acid.

The internal standard, *n*-hexanoic acid, was recommended by Schultz and Toseland[15] who worked with WG11, a liquid phase very similar to SP 1000. *n*-Hexanoic acid separates from both the solvent front and valproic acid and, since it is added at the beginning of the assay, automatically compensates for any intersample differences in recovery. Fig. 2 demonstrates both that the calibration graphs are rectilinear, and that recovery of drug from water and plasma is essentially the same.

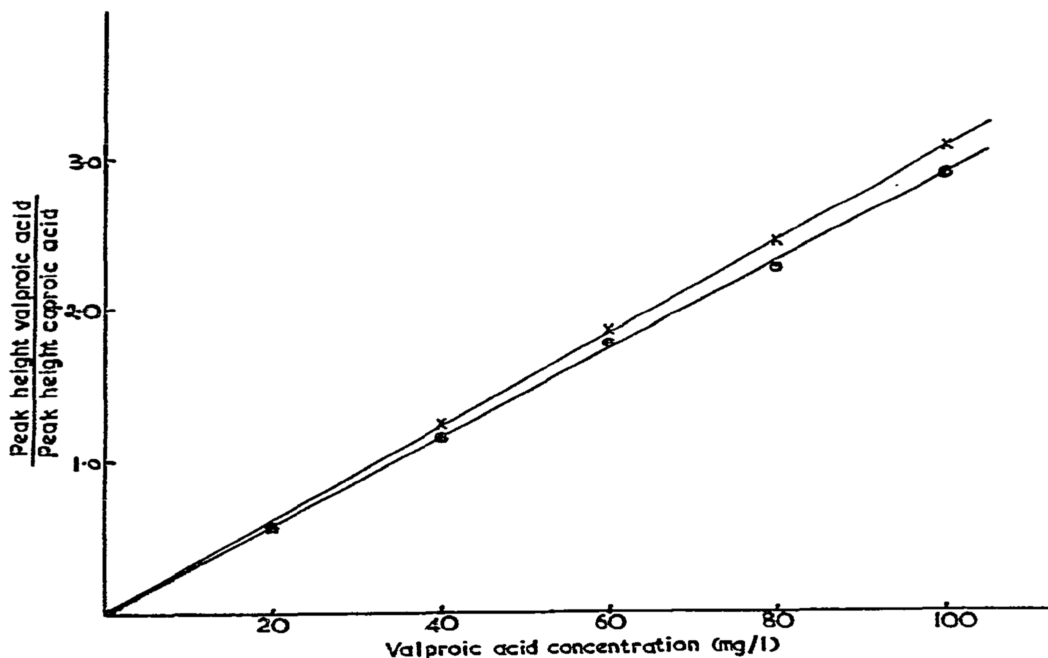

Fig. 2. Standard calibration graph relating the ratio of the peak heights of valproic acid and caproic acid to the concentration of valproic acid in the sample. × = Water standards, ⊙ = plasma standards.

The procedure has been shown to be specific for valproic acid. No interfering peaks in the same region as either drug or internal standard have been encountered from constituents of normal plasma (Figs. 3 and 4 show typical traces from patient samples). Ethosuximide does extract and chromatograph under these conditions, but has a retention time of about 12 min compared to 4.4 min for valproic acid. In addition, the peak shape for ethosuximide is poor so it never presents a problem during subsequent injections.

Direct extraction micro-procedures have been successfully applied within our laboratory to the analysis of several drugs in plasma[18–20]. The method is both rapid since it employs a short extraction and does not require a solvent concentration step, and economic in time, reagents and apparatus, requiring only one small glass tube for the whole extraction. This makes it particularly valuable when large numbers of samples are to be analysed. In addition, the sample requirements are small; this is important when several different assays are needed on the same sample which is commonly the case with anticonvulsant therapy.

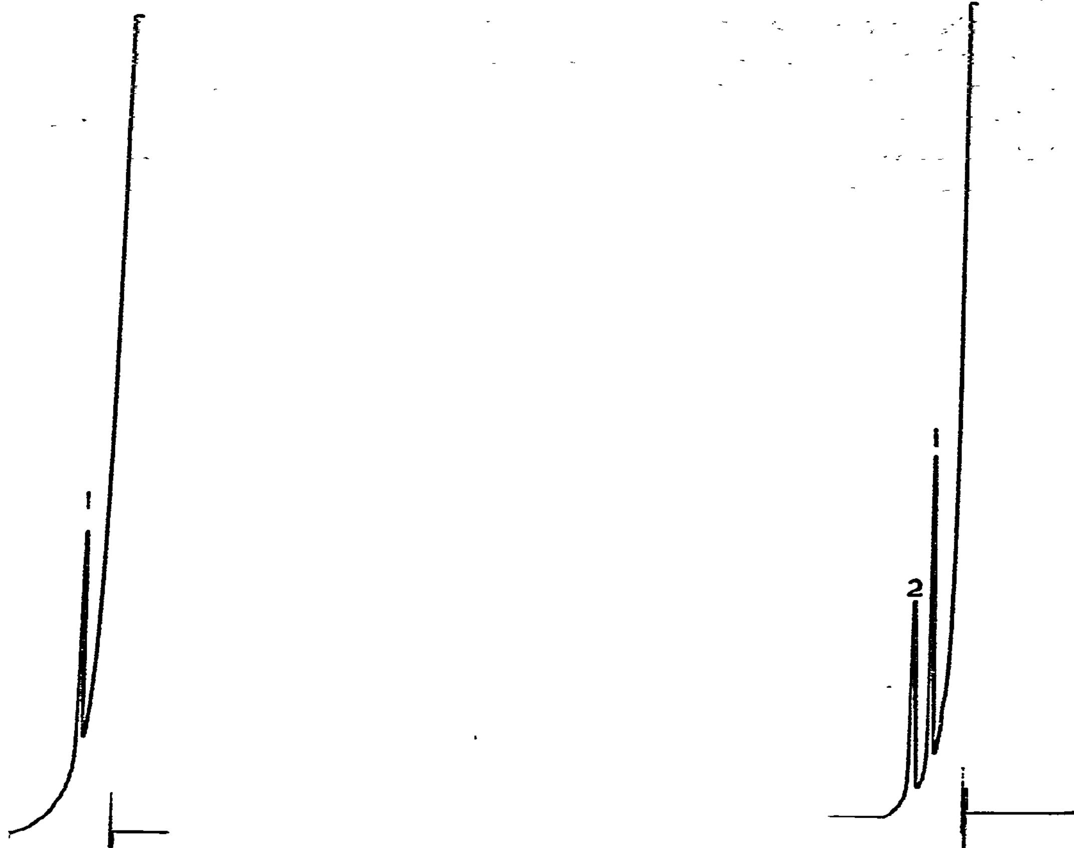

Fig. 3. Gas chromatogram of a normal plasma extract with addition of internal standard.

Fig. 4. Gas chromatogram of a plasma extract from an individual treated with sodium valproate.

During the past 18 months we have analysed 763 plasma samples from 493 patients to determine their valproic acid concentration. Of these 32% were in the normal range of 50–100 mg/l quoted by Schobben *et al.*[8], 61% were below 50 mg/l and 7% were above 100 mg/l. However, initially we did not specify a time interval between dose and blood sampling. Since plasma valproate half-lives are reported to be short leading to wide fluctuations in plasma concentrations throughout the day[12] this probably accounts for the high percentage of our results which were below 50 mg/l as this range refers to samples drawn 2 h after dosage.

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