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Note

Simple, rapid procedure for the determination of valproate and ethosuximide in plasma by gas—liquid chromatography

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Therapeutic monitoring of the anticonvulsants ethosuximide (Zarontin, Parke Davis, Sydney, Australia) and valproate (Epilim, Reckitts, Sydney, Australia) is a routine aid in the clinical management of epileptic patients treated with these drugs. With the exception of a recently developed enzyme immunoassay for valproate [1], gas—liquid chromatography (GLC) is the only technique available for the measurement of plasma valproate and it also remains an attractive alternative for the determination of ethosuximide. Since requests for plasma ethosuximide estimations are infrequent in this department, and consequently uneconomic to perform by the enzyme multiplied immunoassay technique, we therefore examined a range of conditions which would be suitable for the gas chromatographic (GC) determination of both valproate and ethosuximide.

Although GC conditions suitable for the determination of valproate and ethosuximide have been reported [2], sample preparation for the assay included evaporation of the plasma extract which, due to the volatility of valproic acid, may result in loss of this drug. Furthermore, precision data were not reported. While valproate may be analysed by GLC following the direct injection of diluted plasma [3], use of this method results in accumulation of denatured proteins in the column which may necessitate frequent replacement of the stationary phase. A number of simple procedures which require only a single extraction from plasma have been described [4, 5] for the analysis of valproate by GLC. Similarly, ethosuximide in plasma may be assayed by GLC after a single extraction [6—8], although such methods generally employ an evaporation step to concentrate the sample. This report describes a rapid GC procedure for the simultaneous analysis of valproate and ethosuximide using the stationary phase Carbowax 20M—terephthalic acid. The method employs a

single extraction from plasma but does not require evaporation of the extract or derivatisation of the sample. The assay is reproducible and applicable to the routine therapeutic monitoring of both adult and paediatric patients.

EXPERIMENTAL

Gas chromatography

Analyses were performed on a Hewlett-Packard Model 5720A gas chromatograph fitted with a flame ionisation detector. The following chromatographic conditions were employed; a glass column (1.6 m × 2 mm I.D.) containing 10% Carbowax 20M—terephthalic acid on Chromosorb W HP, 80—100 mesh (Applied Science Labs, State College, Pa., U.S.A.), column temperature 180°, injector port and detector temperatures 250°, and carrier gas (nitrogen) flow-rate 40 ml/min.

Reagents and standards

Pure samples of ethosuximide and methsuximide were donated by Parke Davis. Sodium valproate was donated by Reckitts and octanoic acid was supplied by Sigma (St. Louis, Mo., U.S.A.). Other reagents and solvents were of analytical grade.

Standard solutions of sodium valproate and ethosuximide were prepared containing 50, 250, 500, 750 and 1000 mg/l in distilled water.

A 0.1-ml aliquot of valproate was combined with a 0.1-ml aliquot of the appropriate ethosuximide standard and then diluted with 0.3 ml of drug-free plasma to give final concentrations of 10, 50, 100, 150 and 200 mg/l. The internal standards, octanoic acid for valproate determination and methsuximide for ethosuximide determination, were prepared by dissolving 60 mg of the compounds in 100 ml of methanol. For analytical samples containing a single drug, only the appropriate standard and a single internal standard need be used.

Extraction

To 0.5 ml of analytical sample and standards in a conical tip glass tube were added 0.1 ml of the internal standard solution, 0.4 ml of saturated potassium dihydrogen phosphate and 0.5 ml of chloroform. The mixture was vortexed for 1 min and then centrifuged at 1500 *g* for 3 min. An aliquot (1—2 μl) of the organic phase was then injected into the gas chromatograph.

For paediatric samples, 0.1 ml of the analytical sample and standards were extracted by the above procedure using 1/5th of the reagent and solvent volumes described. The extractions were performed in 1.5-ml Eppendorf microtubes and the organic phase was separated during 1 min on an Eppendorf Model 5412 high-speed centrifuge at 6500 *g*.

Unknown concentrations were determined by comparison of the valproate/octanoic acid and ethosuximide/methsuximide peak height ratios with those of the calibration curve. A factor of 0.87 must be employed in the calculation of valproate concentration since standards were prepared from the sodium salt.

RESULTS AND DISCUSSION

Fig. 1B shows a chromatogram obtained following the procedure for a patient plasma sample containing valproate (45 mg/l) and ethosuximide (55 mg/l). Sharp, symmetrical peaks with retention times of 45, 90, 180 and 360 sec are obtained for valproic acid, octanoic acid, ethosuximide and methsuximide respectively. Drug-free plasma gave no interfering peaks under the chromatography conditions described (Fig. 1A).

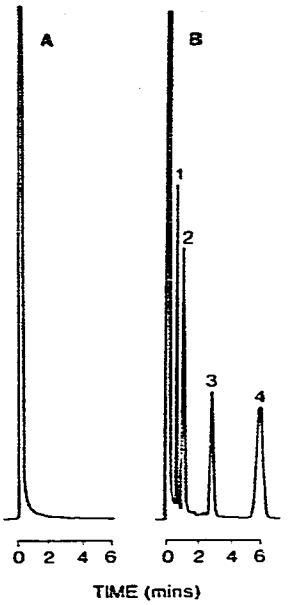

Fig. 1. Chromatograms of plasma extracts (attenuation 2^6). (A) Drug-free plasma; (B) patient sample containing valproate (45 mg/l) and ethosuximide (55 mg/l). Peaks: 1, valproate; 2, octanoic acid; 3, ethosuximide; 4, methsuximide.

Linear calibration curves passing through the origin were obtained for plots of valproate and ethosuximide to the appropriate internal standard peak height ratio versus concentration in the range 10—200 mg/l. The mean slopes of the linear responses were 0.0239 ± 0.0012 and 0.0219 ± 0.0017 (n = 14) for valproate and ethosuximide respectively. Analyses of repetitive samples (n = 20) at concentrations of 25 and 100 mg/l had coefficients of variation, respectively, of 2.8 and 3.7% for valproate, and 8.5 and 5.0% for ethosuximide. The mean recoveries of valproate and ethosuximide from plasma were 86 ± 3 and 73 ± 4% over the range 10—200 mg/l.

The anticonvulsants phenytoin, phenobarbitone, primidone and carbamazepine, as well as salicylate and paracetamol, do not interfere with the determination of valproate or ethosuximide. Moreover, in over a year's operation no other drugs, metabolites or endogenous plasma constituents have been found to interfere with the analysis.

In summary, a simple and rapid GC procedure for the estimation of valproate and ethosuximide in plasma has been developed. The procedure is reproducible and has been applied to the routine analysis of both paediatric and adult patients.

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