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Note

Determination of valproic acid in human serum by gas—liquid chromatography on OV-17 using nitrogen-specific detection

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The determination of valproic acid (di-*n*-propylacetic acid) in serum or plasma is an important part of the clinical management of epileptic patients receiving that drug. Although high-performance liquid chromatographic (HPLC) [1—4], enzyme immunoassay [5, 6], isotachophoresis [7] and direct insertion mass spectrometry [8] methods have been reported, gas—liquid chromatographic (GLC) methods have been the most extensively used.

Due to the volatility of valproic acid, concentration by evaporation is avoided in several GLC methods which employ extraction into a small amount of organic solvent, followed by chromatography without derivatization [9—32]. However, these methods require specific and frequently unstable stationary phases (e.g. FFAP, SP-1000), and large sample volumes — a significant disadvantage since this drug is commonly used with pediatric patients. Procedures involving derivatization decrease the volatility of valproic acid and allow the use of more stable stationary phases. The GLC of valproic acid as its methyl [33—37], butyl [38], propyl [39] and phenacyl [40, 41] esters have been reported.

GLC using the stationary phase OV-17, coupled with nitrogen-specific detection, is extensively employed in the analysis of several other antiepileptic drugs [42]. We investigated the determination of valproic acid as its *p*-nitrophenacyl ester since Gupta et al. [40] previously noted that this nitrogen-containing derivative showed good chromatographic properties on OV-17. This paper reports a sensitive, simple and precise method for the determination of valproic acid in human serum employing derivatization with α-bromo-*p*-nitroacetophenone followed by chromatography on OV-17 using a gas chromatograph equipped with a nitrogen—phosphorus specific detector.

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EXPERIMENTAL

Materials

The internal standard, cyclohexane carboxylic acid, and triethylamine (99%) were supplied by Aldrich (Milwaukee, WI, U.S.A.). α-Bromo-*p*-nitroacetophenone was obtained from Pfaltz and Bauer (Stanford, CT, U.S.A.), *n*-hexane 98% (rein) from Merck (Darmstadt, G.F.R.) and sodium valproate from Reckitt and Colman (Avondale, New Zealand). All materials were used without further purification.

Extraction and derivatization

To 100 μl of internal standard solution (0.02% cyclohexane carboxylic acid in 0.5 *M* ammonium hydroxide, prepared fresh weekly and stored at 4°C) in a 12-ml glass-stoppered conical tube were added 100 μl of serum and 100 μl of 1 *M* hydrochloric acid. The mixture was vortex-mixed and then extracted with 4 ml *n*-hexane by vigorous mixing on a platform shaker for 10 min. The organic layer was transferred to a 75 × 12 mm glass tube and 20 μl of α-bromo-*p*-nitroacetophenone solution (0.5% in acetonitrile prepared monthly and stored at 4°C) and 20 μl of triethylamine were added. This was vortex-mixed, placed in a heating block (65°C), allowed to react for 15 min and then taken to dryness under a stream of air. The residue was taken up in 50 μl of *n*-hexane and a 2-μl aliquot was injected into the gas chromatograph.

Chromatography

A Hewlett-Packard 5840A gas chromatograph, equipped with a nitrogen—phosphorus detector, was used. The 2 m × 2 mm glass column was silanized before packing with 2% OV-17 on Chromosorb W HP, 100—120 mesh. The operating conditions were: injection port, 280°C; column, 230°C; and detector 280°C. The flow-rate of the carrier gas (nitrogen) was 24 ml/min.

Quantitation

The concentration of valproic acid in serum samples was calculated with the aid of standards prepared by adding sodium valproate to blank serum to give concentrations of 10—150 μg/ml valproic acid. These were analysed by the described procedure and calibration curves were prepared by plotting the ratio of the peak area of valproic acid to that of the internal standard against the known concentrations of valproic acid. Analyses were also carried out using 50-μl and 20-μl aliquots of the standards (using 100-μl aliquots of a 2-fold and 5-fold dilution of the internal standard solution, respectively).

RESULTS

A typical chromatogram of an extract of drug-free serum and that of serum containing valproic acid (75 μg/ml) are shown in Fig. 1. The retention time of the *p*-nitrophenacyl ester of valproic acid is 3.6 min, while that of the ester of cyclohexane carboxylic acid is 6.0 min. *n*-Hexane was used to reconstitute the final residue since the use of more polar solvents, such as methanol, led to the appearance of extraneous peaks in the chromatogram.

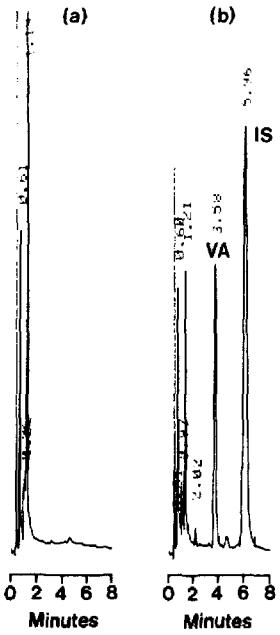

Fig. 1. Elution profiles of (a) an extract of drug-free serum and (b) an extract of serum containing 75 μg/ml valproic acid (VA) and internal standard (IS).

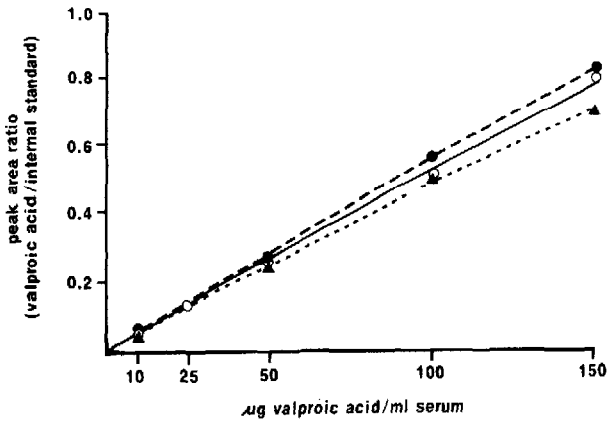

Fig. 2. Calibration graphs for analyses using 100-μl (○), 50-μl (▲) and 20-μl (●) aliquots of serum containing valproic acid.

When samples containing 10—15 μg/ml valproic acid were analysed using 100-, 50- and 20-μl aliquots the resulting calibration curves were linear with correlation coefficients of 0.9994, 0.9991 and 0.9998, respectively (Fig. 2). Samples containing valproic acid concentrations of 0.5—10 μg/ml similarly gave a linear calibration curve (correlation coefficient of 0.9998) when 100-μl aliquots were assayed.

The intra-batch and inter-batch coefficients of variation (C.V.), determined using the paired-sample technique [43], were 3.1% (n = 18 pairs, range 26.1—148.9 μg/ml) and 3.5% (n = 37 pairs, range 23.8—153.9 μg/ml), respectively.

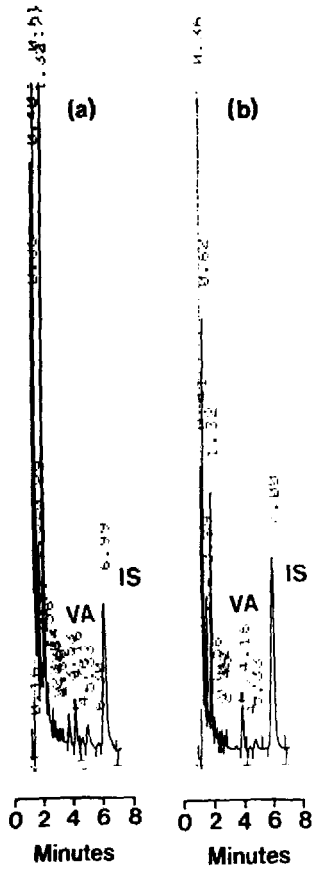

Fig. 3. Elution profiles of (a) serum spiked to 2 μg/ml and (b) patient serum containing 2 μg/ml valproic acid (VA, arrowed). The internal standard (IS) added was a 12.5-fold dilution of the solution used in the standard assay. Oven temperature was 225°C.

The repeated analysis of a single sample over a 6-month period gave an inter-batch C.V. of 3.7% (n = 50, mean = 78.1 μg/ml, S.D. = 2.87). The repeated analysis of a single sample spiked to 2 μg/ml, using 100 μl serum and 100 μl 12.5-fold diluted internal solution, gave an intra-batch C.V. of 6.3% (n = 10, mean = 1.87 μg/ml) (Fig. 3).

Mean absolute recoveries, determined by adding an increment of valproic acid after hexane extraction, were 72.1, 79.5, 73.5 and 73.5% at serum valproic acid concentrations of 2, 40, 80 and 120 μg/ml, respectively (n = 5 in each case).

The following drugs were added to blank sera to 50 mg/l and the sera were analysed as for valproic acid: amitriptyline, beclamide, benzhexol, benztropine, betazole, chloral hydrate, chlorpromazine, danthron, diazepam, doxapram, ethosuximide, fluphenazine, haloperidol, methylprednisolone, naproxen, nitrazepam, nortriptyline, oxazepam, pericyazine, phenobarbitone, phenytoin, poloxamer, primidone, prochlorperazine, prothiaden, thioridazine, triazolam and trifluoperazine. None interfered with valproic acid quantitation. Haemolysed, icteric and lipaemic samples similarly did not interfere with quantitation.

Serum which had been in prolonged contact with the red, grey or green stoppers of Vacutainer brand (Becton Dickinson, Rutherford, NJ, U.S.A.) blood collection tubes manufactured before 1980 gave an extra peak eluting between valproic acid and the internal standard. This is possibly the plasticizer tributoxyethylphosphate which has been eliminated from the most recent formulation of the stoppers. The extra peak was not seen when Venoject brand (Terumo Medical, Elkton, MD, U.S.A.) blood collection tubes were used.

DISCUSSION

The formation of the *p*-nitrophenacyl ester of valproic acid permitted the analysis of small samples of serum containing concentrations of valproic acid as low as 2.0 μg/ml. At this concentration each 2-μl injection represented the equivalent of 8 ng of valproic acid. This sensitivity was achieved because (i) the ester is less volatile than the parent drug and thus could be concentrated by removal of the extracting solvent and (ii) the nitro-group on the ester permitted the use of a nitrogen-sensitive detector. An important additional factor was the use of *n*-hexane as the extracting solvent and as the final reconstituting solvent since the resulting chromatograms contained far fewer extraneous peaks than when more polar solvents were used. The recovery of valproic acid from serum using *n*-hexane was similar to that obtained by Gupta et al. [40] using pentane.

A significant advantage of the method described over many other methods is that the commonly used stationary phase, OV-17, is employed. This phase is more stable than polar phases such as FFAP and SP-1000 which are commonly used in the analysis of fatty acids. It is likely that the method presented here could be readily extended to the analysis of other fatty acids.

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