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Note

Rapid gas chromatographic determination of valproic acid in serum

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The anticonvulsant effect of valproic acid (VPA) has been known for some time. Determination of VPA in serum is of most importance in epilepsy therapy for efficient control of seizure.

VPA is a simple fatty acid chemically different from other commonly used anticonvulsants. Most of the methods for its determination in serum are gas chromatographic (GC), and have been based on procedures for analysis of short-chain fatty acids. Some problems need to be solved in its determination. In order to prevent severe tailing of the free acid peak, the column packing must be conveniently deactivated and, after extraction of acidified serum, VPA is assayed by GC in the underivatized form [1—5]. On-column and pre-column derivatization have also been used to improve the characteristics of the VPA separation [6—11]. The volatility of VPA is another problem to be tackled. In some methods the organic extract must be concentrated before chromatography is carried out, but the addition of isoamyl acetate can overcome this disadvantage [12].

In this report a new packing, 2% SP-2110/1% SP-2510 DA from Supelco, is used for the analysis of VPA; it is deactivated for acidic compounds allowing VPA analysis without derivatization. After extraction of acidified serum, the VPA is injected in free form avoiding any evaporation of the solvent.

MATERIALS AND METHOD

Reagents

Methylene chloride and sulphuric acid were pro analysis grade from E. Merck (Darmstadt, G.F.R.).

The internal standard solution was cyclohexane carboxylic acid, 100 μg/ml in methylene chloride (I.C.N. Pharmaceuticals, Cleveland, OH, U.S.A.). For the

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VPA solution, lyophilized sodium valproate is dissolved in water in a 1 mg/ml concentration (Labaz, Maassluis, The Netherlands). For the working VPA control, a 100 μg/ml solution is prepared in pooled drug-free serum, divided into aliquots and stored at −20°C.

Gas chromatographic conditions

A Perkin-Elmer 3920B gas chromatograph was used with a flame ionization detector, fitted with a 1 m × 2 mm I.D. glass column. The column packing is 2% SP-2110/1% SP-2510 DA on 100—120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The injector and detector temperatures were both 250°C, the oven temperature was 100°C, and the nitrogen flow-rate was 50 ml/min, the hydrogen flow-rate 34 ml/min and the air flow-rate 300 ml/min. A Minigrator M2 (Perkin-Elmer) was used to measure the area of the peaks.

Procedure

To 0.5 ml of serum sample poured into a PTFE-lined screw-capped extraction tube (Kimax 13 × 100 mm, 45066) is added 0.04 ml of 1 *N* sulphuric acid. After mixing, the sample is extracted with 0.5 ml of internal standard solution by placing it for 5 min at 25 rpm in a Vortex mixer followed by 5 min centrifugation at 1400 *g*. The aqueous layer is aspirated and discarded, and the organic layer is transferred to an 8-ml tube (Kimax 13 × 100 mm, 45066). A 2-μl aliquot of this methylene chloride extract is injected into the gas chromatograph. Calibration is made with the VPA aqueous solution. To 0.5 ml of drug-free serum are added 50 μl of aqueous solution and treated as described above. The working VPA control was used to estimate the between-days precision; 0.5 ml is processed following the described procedure.

VPA concentration is calculated by dividing the VPA area by the internal standard area, taking into account the standard quantity given above, and multiplied by the response factor obtained from the calibration.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of an extract of human drug-free serum and of a patient receiving sodium valproate. The retention times of VPA and cyclohexane carboxylic acid are 1.5 and 2 min, respectively. The chromatogram of drug-free serum does not show interfering peaks from serum constituents.

The range of linearity of the results obtained with this method of analysis was determined by analysing in duplicate serum spiked with 10, 20, 40, 60, 80, 100 and 160 μg/ml VPA and a constant quantity of internal standard (50 μg) by plotting the quotient of the peaks areas against VPA concentration, a straight line is obtained whose equation of linear regression is $y = 0.019\ x - 0.03$, with a linear correlation coefficient of 1.0 and a standard error of 0.015.

The within-run precision (C.V.) of the procedure for two concentrations of VPA ($n = 12$) was 3% for 60.4 μg per 0.5 ml (S.D. = 1.8) and 3.9% for 25.9 μg per 0.5 ml (S.D. = 1.0). The between-days precision ($n = 18$) was 5.6% (mean concentration 55.1 μg per 0.5 ml, S.D. = 3.1).

The range studied is sufficient to cover all clinical situations. Analysis of

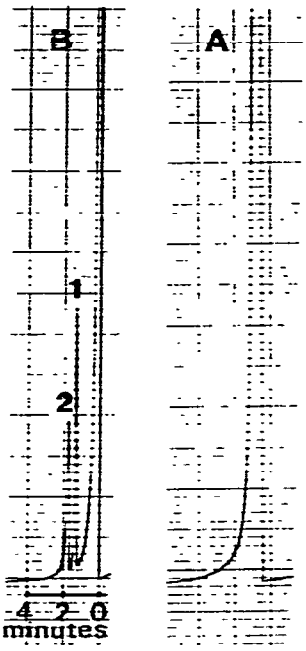

Fig. 1. Gas chromatogram of (A) blank serum and (B) serum from a patient receiving sodium valproate. Serum level = 104 μg/ml. Peaks: 1 = valproic acid; 2 = internal standard.

serum samples from the Antiepileptic Drug Level Control Program was performed. Serum samples from patients who were receiving ethosuximide, phenobarbital, carbamazepine, primidone and phenytoin were analyzed and no interference was found with either the VPA or the internal standard; they have longer retention times.

The detector sensitivity limit was studied in six replicates from a serum pool to which VPA was added. The chromatographic peak corresponding to 5 μg of VPA was perfectly measurable by the integrator, being the mean of the results of the division of the VPA peak area by the internal standard peak area equal to 0.085 (n = 6, S.D. 0.0077, C.V. 9.06%). This limit is correct to the clinic because it is clearly under therapeutic values, and on the other hand it also allows a reduction in the volume of pediatric serum samples.

Recently several techniques have been published for the determination of VPA in serum without derivatization. Different packing materials have been used [1—5], but with these packings nothing has been published on the analysis of the other anticonvulsant drugs in underivatized form. Routine work to determine all common anticonvulsant drugs is more rapid and easy if a change of column is not required in the analysis since many patients are treated with a diversity of anticonvulsant drugs.

The described method is simple and does not require derivatization or evaporation of solvent, and it could be a valuable tool in those laboratories having just one chromatograph. At the same time, the use of the same solvent for the extraction and chromatography avoids the problem of loss by evaporation.

This technique for VPA can be used in the presence of other anticonvulsant

drugs such as ethosuximide, phenobarbital, carbamazepine, primidone and phenytoin, and, conversely, the determination of these drugs can be realized in isothermic conditions with no derivatization and with a simple extraction method [13] using the same column as the one used for VPA.

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