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A Rapid and Simple Gas-Liquid Chromatographic Determination of Valproic Acid (α-Propyl-Valeric Acid) in Serum

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Summary: A simple and rapid gas-liquid chromatographic method for the determination of valproic acid in serum is described. Valproic acid is extracted from acidified serum into chloroform and analyzed by gas-liquid chromatography using an FFAP column.

The sensitivity of the method is 5 μmol/l and the within-run and day-to-day precisions (CV) are 4.5% or better. Analytical recovery is estimated to exceed 98% and no interference from other drugs or constituents of serum has been observed.

The method was used to study the half-life of valproic acid in five healthy persons receiving a single oral dose of the drug, and the technique is well adapted to the routine monitoring of epileptic patients. In routine analysis, the determination of 50 serum samples may be performed by one technician during a normal working day.

Schnelle und einfache gaschromatographische Bestimmung von Valproinsäure (α-Propylvaleriansäure) im Serum

Zusammenfassung: Eine einfache und schnelle gaschromatographische Methode zur Bestimmung von Valproinsäure im Serum wird beschrieben. Valproinsäure wird aus angesäuertem Serum in Chloroform extrahiert und gaschromatographisch mit einer FFAP-Säule analysiert. Die Empfindlichkeit der Methode beträgt 5 μmol/l, die Präzision in der Serie und von Tag zu Tag 4,5% oder weniger. Die Wiederfindung beträgt mehr als 98%; Störungen durch andere Arzneimittel oder Serumbestandteile wurden nicht beobachtet. Die Methode wurde zur Untersuchung der Halbwertszeit von Valproinsäure bei 5 gesunden Probanden nach einmaliger oraler Gabe eingesetzt und ist geeignet zur Routinekontrolle von Epileptikern. Von einer Mitarbeiterin kann an einem normalen Werktag die Bestimmung in 50 Serumproben durchgeführt werden.

Introduction

Sodium valproate (α-propyl-valeric acid) is widely used in the treatment of convulsive disorders. The quantitative determination of the drug is important both for monitoring and optimizing blood levels of patients receiving therapeutic doses, as well as the study of the pharmacokinetic properties. Several gas-liquid chromatographic methods are available for measuring the circulating concentrations of valproic acid (1–9). Although the methods appear to be specific and accurate, they tend to be tedious and lengthy, and thus not suitable for the rapid manual analysis of multiple samples.

This report describes a simple, rapid, and accurate gas-liquid chromatographic determination of valproic acid, using an FFAP column, and caproic acid as the internal standard. Parts of this work have been presented as a congress abstract (10).

Materials and Methods

Reagents

Chloroform (Merck No. 2445, E. Merck, D-6100 Darmstadt, F.R.G.) was distilled before use.

Hydrochloric acid (Merck No. 319) 6 mol/l aqueous.

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Internal standard solution: caproic acid (Fluka No. 21529, Fluka AG, CH-9470 Buchs, Switzerland) 1 g/l in absolute ethanol.

Stock valproic acid standard solution, 0.100 mol/l: dissolve 1.442 g of α-propyl-valeric acid (kindly supplied by Orion Pharmaceutical Co., PL 19, SF-00101 Helsinki, Finland) in 100 ml of absolute ethanol.

Working valproic acid standard, 1000 μmol/l, 500 μmol/l and 250 μmol/l in pooled, drug free serum. Store in aliquots at -20 °C.

Other drugs: tripropylacetamide (as a gift from Orion Pharmaceutical Co.), 1 g/l and dipropylacetamide (generous gift from Labaz, 39. Avenue Pierre 1. De Serbie, F-75008 Paris, France), 1 g/l, were dissolved in acetone (Merck No. 14). Ethosuximide (Orion Pharmaceutical Co.), 1 g/l, was dissolved in ethanol.

Gas chromatographic conditions

A Carlo Erba 2301 AG gas chromatograph was used with a flame ionization detector fitted with a 1 m × 3 mm i. d. glass column. The column contained a liquid phase of 5% FFAP (Varian Aerograph, Walnut Creek, Calif., U.S.A.) on Chromosorb G AW DMCS 80/100 mesh (Applied Science Laboratories Inc., State College, PA. 16801, U.S.A.). The oven and injector temperatures were 190 °C and 225 °C, respectively, and nitrogen was used as the carrier gas. The gas chromatograph was connected to a Varian Aerograph CDS 220/240 chromatography data system.

Procedure

To a serum sample (0.5 ml) in a stoppered, narrow pointed centrifuge tube is added 0.05 ml of internal standard solution, and 0.05 ml of hydrochloric acid (6 mol/l) to make the solution acidic. After mixing, the sample is extracted with 1.0 ml of chloroform by mixing for 30 seconds on a Vortex mixer, followed by centrifugation at 2000 *g* for 5 min. The upper water layer is removed and the clear chloroform phase transferred into a stoppered glass tube, lifting the protein and lipid layers with a Pasteur pipette. One microliter of this chloroform solution is injected into the gas chromatograph. A calibration curve was prepared by subjecting working valproic acid standards to the above procedure. Peak area ratios of valproic acid and the internal standard (by chromatography data processor) were plotted as a function of valproic acid concentration. It was also verified that the peak height ratio calculation gave sufficient accuracy. The relationship between peak height ratio and concentration was linear at least up to 2 mmol/l.

Results and Discussion

Specificity

Representative chromatograms of serum specimens extracted according to our procedure are presented in figure 1. Chromatogram (a) is a pattern typical of the serum of a normal individual not receiving drugs. Chromatogram (b) is obtained from the serum of an individual receiving sodium valproate. The concentration of the drug in this sample was 380 μmol/l. No interfering peaks in the same region of either drug or internal standard have been encountered from constituents of lipemic or hemolytic sera. Dipropylacetamide, tripropylacetamide and ethosuximide do extract and chromatograph under these conditions; moreover, they have retention times of about 7, 10 and 16 min, respectively, compared to 3.5 min for valproic acid. In addition, no other interference has been found in the analysis of nearly 2000 serum samples from patients taking anticonvulsant drugs.

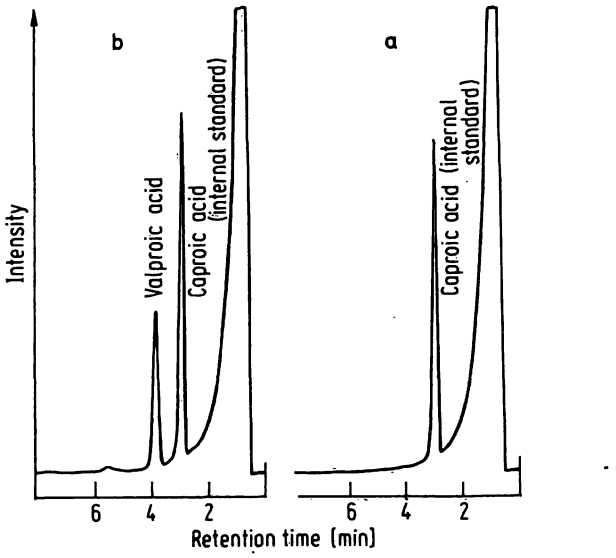

Fig. 1. Typical gas-chromatogram of: a) Serum blank (normal individual not receiving drugs). b) Serum from a patient treated with sodium valproate.

Sensitivity

In sensitivity studies a drug free serum sample was spiked with valproic acid to a concentration of 5 μmol/l and processed as described above. The mean peak area ratio was found to be 0.0089 (N = 6, CV = 10.8%) indicating that 5 μmol/l of valproic acid in serum can be determined with acceptable precision in 0.5 ml serum samples.

Recovery

Absolute recovery of valproic acid from serum was studied by adding known quantities of valproic acid to a serum known to be drug-free. These samples were processed as described above. A second (non-extracted) set of standards was prepared in chloroform at the same concentration. One microliter of each sample was chromatographed. Absolute recovery of valproic acid from serum was calculated by comparision of the ratio of the peak heights of the extracted serum samples with those of the non-extracted standards. The recovery of valproic acid determined in this way was 99.7% at a concentration of 1 mmol/l, and 100.4% at concentration of 2 mmol/l.

The relative recovery of valproic acid was also determined. Known amounts of valproic acid in ethanol were added to pooled patient serum to achieve the concentrations shown in table 1. The samples were processed as

Tab. 1. Relative recovery of valproic acid added to pooled patient sera (N = 5).

Initial concentration (μmol/l)	Calculated concentration after addition (μmol/l)	Measured concentration after addition (μmol/l)	Recovery %
302	602	598	98.7
302	902	897	99.2

described above. At least five samples were analyzed at both concentrations. Relative recoveries are shown in table 1.

Precision

Within-run precision was evaluated by processing aliquots of pooled patient sera containing valproic acid in the concentrations shown in table 2. Day-to-day precision was calculated from data obtained on samples analyzed over 6 months.

Tab. 2. Precision of assay for valproic acid in serum.

	Within-run			Day-to-day
Mean (μmol/l)	146.3	439.2	759.1	354.5
SD (μmol/l)	4.56	9.93	15.21	17.34
CV (%)	3.1	2.3	2.0	4.5
N	20	20	20	25

Clinical studies

Examination of five healthy volunteers showed that after an oral dose of 600 mg, sodium valproate is rapidly absorbed, reaching peak levels (436 ∓ 22 μmol/l; mean ± standard error of mean, N = 5) in the serum about one hour after ingestion. Serum levels of the drug then fell rapidly over 8 hours and more slowly after this time. The drug was still present 24 hours after a single dose. The half-life of valproic acid was about 10 hours (8–10 hours), which is in good agreement with the times reported in the literature (11–14).

In a study of 950 serum samples from epileptic patients receiving sodium valproate, the highest serum level was 1.1 mmol/l, 70% of the results falling in the range 210–700 μmol/l. This compares well with the results of others (1, 15).

Conclusion

For those laboratories in which a gas chromatograph with a flame ionization detector is available, the present method is fast and simple, while retaining excellent accuracy and precision. Complete analysis time for an urgent determination is 30 minutes, while in routine analysis the determination of 50 serum samples may be performed by one technician in a normal working day.

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