Journal of Chromatography, 146 (1978) 494—497
Biomedical Applications

CHROMBIO. 195

Note

New direct micro-method for determination of valproic acid in serum by gas chromatography

CORNELIS JAKOBS, MONIKA BOJASCH and FOLKER HANEFELD

Department of Pediatrics, Free University of Berlin, Kaiserin Auguste Victoria Haus, Heubnerweg 6, D - 1000 Berlin 19 (G.F.R.)

(Received March 23rd, 1978)

The determination of anticonvulsant serum concentration is of great importance for correct treatment of epileptic patients, in particular when it can be measured in short intervals at the beginning of or after a change in therapy. It allows the monitoring of the serum concentration, which is not always dose dependent and therefore not always predictable. In recent years, valproic acid* (dipropylacetic acid, DPA) has found increasing use in the treatment of generalized epilepsy, especially of the petit mal type in children (for a review see refs. [1] and [2]). Specific and rapid methods for routine analyses have been developed to determine DPA-serum concentrations in epileptic patients. Schmidt et al. [3] have presented a good review of the main gas chromatographic (GC) methods available. A serious drawback of all known methods, however, is the relatively large volume of serum needed, which is of special importance for newborns and infants (the repeated drawing of venous blood is difficult for technical and psychological reasons) and the rather tedious procedure, involving solvent extraction followed by evaporation.

Therefore we have searched for a new method without the above mentioned disadvantages, that is, a method which requires less serum and does not require extraction steps. The method which we describe in this paper does not require more than 20 μl of serum obtained from blood drawn from the finger trip. A 1-μl volume of this serum is applied after acidifying directly into the gas chromatograph.

MATERIALS AND METHODS

Dipropylacetic acid (sodium salt) was used in the form of the commercial 30% solution (Ergenyl®, Labaz, Düsseldorf, F.F.R.). 2-Ethyl-2-methyl-caproic acid (internal standard) was obtained from Fluka (Neu Ulm, G.F.R.). Micro

*Other nomenclature: dipropylacetic acid, 2-propyl-valeric acid, 2-propyl-pentanoic acid.

hematocrit tubes were from Dade (Miami, Fla., U.S.A.) and a micro-capillary centrifuge from IEC (Boston, Mass., U.S.A.).

Preparation of samples

Serum may be obtained from venous or from capillary blood. In the latter case the blood from the finger tip is drawn into a micro hematocrit tube (40 μl) and centrifuged in a microcapillary centrifuge. After the capillary has been broken off, the serum part is blown into a small vial. To 20 μl of serum 10 μl of 1 *N* HCl containing the internal standard 2-ethyl-2-methyl-caproic acid (0.3 mg/ml) are added. After briefly mixing, 1 μl is injected directly into the gas chromatograph. A standard working curve is prepared by adding known amounts of DPA to normal sera. A 5-μl volume from different stock solutions of DPA, made up in distilled water, is added to 15 μl of serum to obtain the different concentrations (10—150 ug/ml). The standard sera are handled in the same way as described above. The peak area ratios of the internal standard relative to DPA are plotted against the corresponding drug concentrations.

Gas chromatography

A Varian 2740 gas chromatograph was used, equipped with a glass column (6 ft. × 1/4 in. O.D.) packed with 5% DEGS-PS on Supelcoport (100—120 mesh) (Supelcoport, Bellefonte, Pa., U.S.A.). The injection temperature was 200°, detector temperature 200° and column temperature 135° isothermal. Nitrogen flow-rate was 30 ml/min, hydrogen flow-rate 40 ml/min and air flow-rate 400 ml/min. The attenuation was $(4 \cdot 10^{-11}) - (8 \cdot 10^{-11})$.

RESULTS AND DISCUSSION

A typical gas chromatogram of a serum sample is shown in Fig. 1. The retention time for DPA is 2 min and for the internal standard ca. 2.5 min. The calibration curve is linear in the range 10—150 μg/ml serum (the assumed therapeutic range is within this range [4,5]), passing through the origin. The accuracy and reproducibility of the method was evaluated by ten replicate analyses of different serum samples containing known amounts of DPA, as shown in Table I. The coefficient of variation ranged from 2.7 to 4.2%, and therefore was less than 5% which is generally accepted for quantitative analyses of antiepileptic drugs. We first compared our new method on serum samples obtained from venous blood with the method previously used in our laboratory, which was based upon a modification of common extraction procedures [6,7]. The DPA-values from both methods correspond very closely to one another. We then compared the DPA concentrations in serum obtained from venous blood with serum from blood from the finger tip (both samples from the same patient at the same time).

We did not find any significant difference between the DPA-concentrations in serum obtained from venous blood and the blood drawn from the finger tip.

The serum proteins do not interfere but become denaturated by the high injection temperature and remain in the column. Repeated use of the column does not change the retention times and only after about 300 samples does the first ca. 10 cm of the column have to be renewed. We used a normal microliter

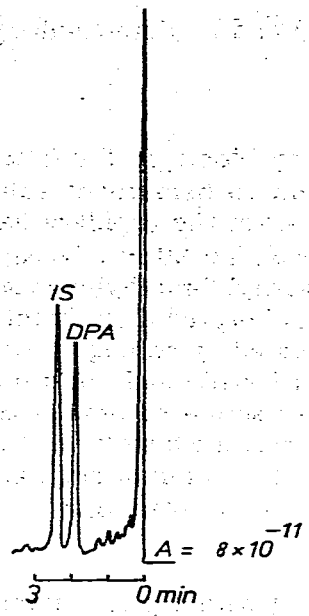

Fig. 1. Gas chromatogram of a serum sample containing DPA (76 μg/ml). Internal standard (IS) is 2-ethyl-2-methylcaproic acid; A = attenuation.

TABLE I

ACCURACY AND REPRODUCIBILITY OF THE GC DETERMINATION OF DPA

Sera contain different amounts of the drug (n=10 for each amount). S.D. = standard deviation; C.V. = coefficient of variance.

Added drug (μg/ml)	Mean	S.D.	C.V. (%)
25	25.4	1.1	4.2
50	51.7	2.9	3.8
75	76.8	2.5	3.2
100	98.9	2.6	2.7

syringe (Hamilton, 701), which is rinsed with distilled water after each sample to prevent plugging of the metal tip by the denaturated serum proteins. Normal serum constituents do not interfere, and the short chain fatty acids especially (which may accumulate as a result of an inborn error in amino acid metabolism) are eluted before DPA appears. Also there is no interference from other anti-consulvants such as phenytoin, carbamazepine, primidone, phenobarbital and ethosuximide, which we tested on patients with multitherapy.

The amount of serum required is actually not more than that which is needed for injection. To prevent too much dilution when the sample is acidified and the internal standard is added, and to retain good accuracy and reproducibility, we used 20 μl of serum.

Our method is rapid and easily practicable and is suitable for clinical routine analyses.

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