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Note

Gas chromatographic determination of valproic acid in human plasma

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Valproic acid is an anti-epileptic drug. A high-performance liquid chromatographic method [1] and many gas chromatographic (GC) methods have been described for its determination in body fluids. In some GC methods, valproic acid is derivatized [2—6].

In most of the published assays, however, no derivatization is used, and the usual procedure entails only one extraction step. Different solvents were used for this extraction step: heptane [7], carbon tetrachloride [8, 9], diethyl ether [10—13], toluene [14], chloroform [13, 15—20], dichloromethane [21, 22] and carbon disulphide [23]. In two procedures [24, 25], no extraction is required. One method [26] involves a large-scale extraction in a separating funnel, followed by back-extraction. Another method [27] also employs back-extraction, and is designed to measure low plasma concentrations of valproic acid down to 1 μg/ml. The other methods cited above have a sensitivity between 5 and 20 μg/ml.

A number of problems are encountered in the estimation of valproic acid. The free acid is volatile and is hence lost on evaporation; it also has a tendency to be adsorbed on the column.

In the method described below there is no evaporation or derivatization; after a one-step extraction a very polar stationary phase is used, and the technique is suitable for the analysis of valproic acid down to a concentration of 1 μg/ml.

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EXPERIMENTAL

Chemicals

Sodium valproate was stored in a desiccator under vacuum. The aqueous solutions of sodium valproate and octanoic acid (Aldrich 15 375-3; Aldrich, Milwaukee, Wisc., U.S.A.) are stable at +4° for more than three months.

Equipment

A gas chromatograph (Carlo Erba, Fractovap 2400T) equipped with a flame-ionization detector was used. The glass column (2 m × 2.8 mm I.D.) is operated at 175° and the injector at 200°, with a nitrogen flow-rate of 35 ml/min, an air flow-rate of 300 ml/min and a hydrogen flow-rate of 30 ml/min. The column packing is 10% SP 216 PS on 100—120 mesh Supelcoport (Supelco 1 1879; Supelco, Bellefonte, Pa., U.S.A.).

Extraction

To 1 ml of plasma in a 4-ml stoppered glass tube are added 0.3 ml of the internal standard solution (19.1 μg of octanoic acid), 0.5 ml of water and 0.25 ml of 4 *N* hydrochloric acid. After gentle shaking on a Vortex, 0.5 ml of carbon disulphide is added; the mixture is again gently shaken a further 2 min and centrifuged for 10 min. The upper layer is removed. (It may be necessary to stir the carbon disulphide with a Pasteur pipette to break the emulsion.) The organic phase is then transferred to another tube.

Gas chromatography

A 3-μl portion of the organic layer is injected into the gas chromatograph.

The sodium valproate concentration is calculated from the peak-area ratio by reference to a calibration curve. This curve is obtained by extraction of plasma spiked with increasing amounts of sodium valproate (from 1 to 100 μg/ml) and a constant amount of internal standard (19.1 μg per ml of plasma).

RESULTS

Sensitivity, reproducibility and accuracy

Table I gives the results obtained when the described procedure was applied to spiked plasma samples. The coefficients of variation are calculated on the basis of six replicate analyses at each concentration. Concentrations down to 1 μg of sodium valproate per ml of plasma can be accurately determined. At lower concentrations there is no detectable chromatographic peak.

Plasma interference

Fig. 1 shows the chromatograms of an extract of human plasma and of the same plasma spiked with 10 μg of sodium valproate. There is no interference from the normal constituents of plasma.

DISCUSSION AND CONCLUSION

An inter-laboratory variability test of the results of valproate determinations

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF SODIUM VALPROATE APPLIED TO SPIKED PLASMA SAMPLES

Amount of sodium valproate added (μg/ml)	Amount of sodium valproate found (μg/ml)	Mean (μg/ml)	Precision/ reproducibility (C.V., %)	Recovery/ accuracy (%)
1	0.98			98
	0.96			96
	0.96	0.96	5.8	96
	0.96			96
	0.86			86
	1.03			103
2	2.02			101
	2.00			100
	1.90	1.96	2.4	95
	1.95			97
	1.99			100
	1.92			96
10	9.0			90
	9.1			91
	10.3	9.9	6.8	103
	10.7			107
	9.9			99
	10.2			102
20	19.7			98
	22.1			111
	18.7	20.0	6.8	94
	20.8			104
	18.4			92
	20.1			100
100	98			98
	96			96
	99	103	5.5	99
	108			108
	107			107
	109			109
			Mean ± S.D.:	99 ± 5.9

by different methods [13] showed that the sodium valproate used by all the investigators as a standard may give deceptively high values, as it is hygroscopic. The salt must be dried and stored in a desiccator under vacuum, as recommended here. The use of the same solvent for extraction and chromatography also avoids the problem of loss on evaporation.

There is no mention in the literature of the presence of conjugated valproic acid in plasma. Approximately one quarter of an 800-mg dose is excreted as free and conjugated valproate in the urine [2]. The main metabolite of valproic acid is 3-keto-valproic acid, and 4- and 5-hydroxyvalproic acids are also present [3, 28, 29]. The method described here may be considered specific. Schäfer and Lührs [8] indicated that the three metabolites are unstable and non-volatile, so that they had to convert them into stable and easily volatilized derivatives. In the present method no such a treatment was applied.

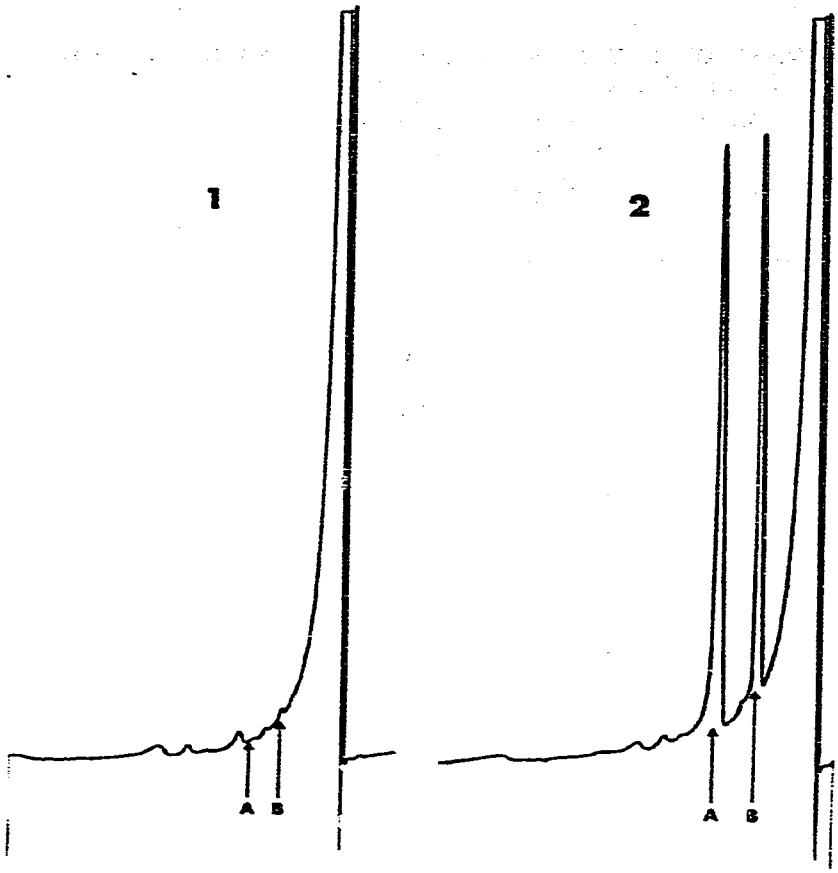

Fig. 1. Examples of chromatograms. (1) Human plasma blank. (2) 19.1 μg/ml of internal standard (A) and 10 μg/ml of sodium valproate (B) in human plasma.

According to what is known about the pharmacokinetics of valproic acid [30—34], the described method is convenient for measuring plasma concentrations until they decline to levels of ten to twenty times less than the peak concentration. Moreover, its sensitivity of around 1 μg of sodium valproate per ml of plasma permits analyses to be made in very small samples, which is useful because the drug is frequently given to children.

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