Journal of Chromatography, 132 (1977) 145–147

CHROM. 9627

Note

Gas chromatographic determination of valproic acid in human plasma

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(Received August 9th, 1976)

Valproic acid (di-*n*-propylacetic acid) is the free acid form of the drug marketed under the names of Epilim, Sodium Valproate, and Depakine. Although valproic acid has been used for a number of years as an anti-epileptic drug, little has been published on analytical methods and plasma levels[1,2]. Several trials have recently been performed using this drug both in combination with other anti-epileptic drugs and by itself, illustrating its effectiveness in controlling epilepsy[2,3].

A number of problems are encountered in the estimation of valproic acid. Firstly, the acid is volatile and hence lost on evaporation. Secondly, the acid has a tendency to absorb on the column, making chromatography somewhat difficult. We have described a method where there is no evaporation or derivatization and which is suitable for the analysis of valproic acid in plasma down to levels of 5 mg/l.

EXPERIMENTAL AND RESULTS

Reagents

The extraction solvent was 5% isopropanol in dichloromethane (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.). The internal standard was a 226 mg/l solution of methyl myristate in chloroform.

Gas–liquid chromatography

A Packard Model 7400 Series gas chromatograph equipped with a flame ionization detector was used. The column was a 6 ft. × 4 mm I.D. coiled glass tube which had been silanized with a solution of 5% dichlorodimethylsilane in benzene, then rinsed with methanol, and dried. The packing consisted of 10% Carbowax 20M-TPA on Chromosorb W-AW, 80–100 mesh.

In the present work the instrument settings were as follows: column temperature, 200°; injection port temperature, 215°; detector temperature, 220°; carrier gas flow-rate, 45 ml/min; hydrogen flow-rate, 45 ml/min; air flow-rate, 425 ml/min. Under these conditions the retention times of valproic acid and methyl myristate were 3.5 and 4.5 min, respectively.

Extraction procedure

A 1.0-ml sample of serum was acidified with 1 ml of 1 *M* hydrochloric acid and extracted with 20 ml of the extraction solvent by shaking for 3 min in a separating

funnel. After settling, the solvent was filtered through a Whatman No. 4 filter paper. A 15-ml aliquot of the filtrate was transferred to a 25-ml centrifuge tube and extracted with 3 ml of 0.5 *M* sodium hydroxide. The tube was centrifuged and 2 ml of the 0.5 *M* sodium hydroxide was transferred to a 10-ml pointed glass centrifuge tube. The sample was acidified with 1.5 ml of 1 *M* hydrochloric acid and 200 μl of the internal standard solution were added. The tube was shaken vigorously by hand and then centrifuged. An aliquot of the organic solvent was injected directly onto the column.

Quantitation

To known amounts of valproic acid in pointed glass tubes, 200 μl of the internal standard solution was added; then each was examined by GLC as described above. Over a range of 0.3–2.0 μg of the drug, the ratio of the peak height of valproic acid to that of the internal standard was linear.

Recovery studies

Amounts of valproic acid ranging from 6–100 μg were added to 1 ml of blank plasma

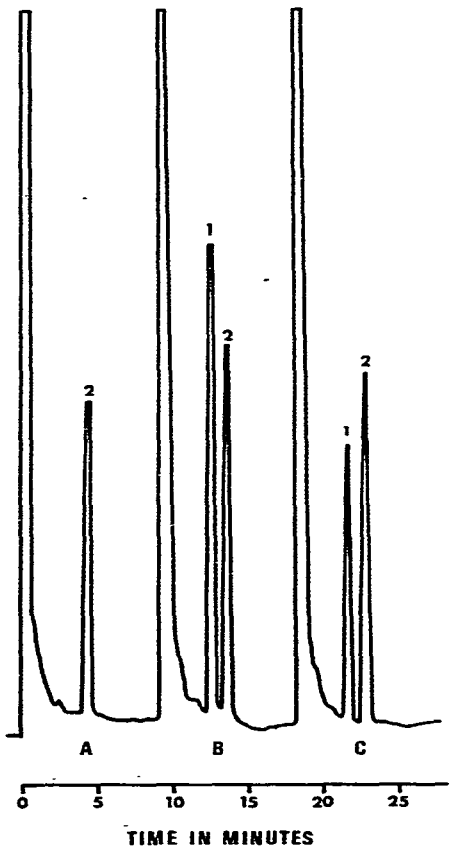

Fig. 1. (A) Chromatogram of a blank plasma extract. (B) Chromatogram of a plasma extract from a subject receiving Epilim. (C) Chromatogram of a plasma extract containing added valproic acid at a level of 20 mg/l. 1 = Valproic acid; 2 = methyl myristate (internal standard).

TABLE I

PLASMA LEVELS OF VALPROIC ACID FROM SUBJECTS RECEIVING EPILIM

Patient	*Weight (kg)*	*Daily medication (mg)*	*mg/kg*	*Plasma level (mg/l)*
1	23	1600	70	204
2	29	1000	35	83
3	19	800	42	86
4	28	1000	36	94
5	57	600	11	49
6	27	800	30	57
7	16	600	38	87
8	57	600	11	42
9	14	700	50	126
10	63	400	6	29
11	57	1000	18	44
12	63	200	3	12
13	52	800	15	57
14	39	600	15	46

in order to examine the efficiency of the extraction procedure. The mean recovery of ten spiked samples (6–100 mg/l) was 87 $\pm$ 8%.

Specificity

In all the plasma samples we examined, the gas chromatograms have been free from interfering peaks. In addition, none of the common acidic or anti-epileptic drugs were found to interfere with the assay (Fig. 1).

Application

The above method was performed on plasma from patients receiving treatment with sodium valproate. Some of the results obtained are shown in Table I.

DISCUSSION

A trial using sodium valproate by Richens and Ahmad[2], who studied plasma levels in four patients on a dose of 1200 mg/day, found levels ranging from 34–68 mg/l. No weights were given, however. Schobben *et al.*[1] cited a therapeutic range of 50–100 mg/l, though no prospective studies have been performed to establish such a range. In this paper, plasma levels were found to be 12–204 mg/l from subjects receiving between 3–70 mg/kg. The method outlined in this paper has proved satisfactory, reliable, and specific and should allow rapid and accurate analyses to be performed.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. P. Silberstein for providing the clinical samples and the Commissioner of Health for allowing the data to be published.

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