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Note

Direct determination of valproate in minute whole blood samples

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Monitoring of valproate in blood is of importance in the treatment of epilepsy. Chromatographic procedures are most commonly used for analysis of this drug. Many methods include solvent extraction followed by gas chromatography (GC) without derivatization. The shortcomings of these methods are the use of relatively large sample volumes, the formation of emulsion causing loss of analyte, low sensitivity because of the volatility of valproic acid thus precluding solvent evaporation, use of unsuitable chromatographic columns for the accurate quantification of valproic acid. To overcome these last two drawbacks, some authors [1—4] proposed the conversion of valproic acid to its esters. Recently, a procedure has been described employing solvent extraction, esterification and quantification by GC with a nitrogen detector to analyse valproate in plasma [5]. More recently, another method couples a non-extractive preparative procedure with liquid chromatography of valproic acid as its phenacyl ester [6]. This approach is simple and original, but appears to be insufficiently accurate and precise for serum valproate levels lower than 50 mg/l.

Graphitized carbon black (Carbopack) is very effective both as packing material in GC for the separation of acidic compounds [7—10] and as an adsorbing material for the extraction of very polar compounds from biological liquids [8—10]. In this paper, we describe its use for isolating valproate directly from blood and quantifying it by GC. This method requires only 10 μl of blood, is simple and accurate even at the lowest therapeutic level.

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MATERIALS AND METHODS

Reagents

The following analytical grade reagents (Fluka, Buchs, Switzerland) were used: heptanoic acid, 1,3,5-tricarboxybenzene (trimesic acid), polyethylene glycol (PEG) 20 M. Stock solutions of sodium valproate (Labaz, Maassluis, The Netherlands) were prepared by dissolving the salt in water to give concentrations of 6.4, 2.4, 0.8, 0.4, and 0.2 mmol/l. Blood standards were prepared by adding 10 μl of the stock solutions to 100 μl of drug-free blood. The working internal standard was prepared by diluting heptanoic acid with 0.01 mol/l hydrochloric acid to give a concentration of 0.10 μg/ml. A second internal standard solution was prepared by dissolving octanoic acid in methanol to give a concentration of 30 μg/ml. Carbopack B and C (Supelco, Bellefonte, PA, U.S.A.) were 80—100 mesh.

Procedure

A 10-μl freshly collected blood sample is placed in a glass tube containing 1 ml of 0.01 mol/l hydrochloric acid and 0.1 μg/ml heptanoic acid. The mixture is well agitated and applied to a glass column filled with 0.25 g of Carbopack B. The bed (3 × 0.6 cm) of Carbopack B is prepared by suspending the material in water and introducing the suspension into a 15 × 0.6 cm glass column with a small flock of glass wool in the bottom. After the sample is passed, the column is washed with 10 ml of 0.05 mol/l hydrochloric acid followed by 1 ml of water—methanol (50:50, v/v) and 0.01 mol/l hydrochloric acid. Valproic acid is eluted by applying to the top of the column 2 ml of methanol, discarding the first 0.5 ml of the effluent, which is the residue of the previous washing phase, and subsequently collecting 1.5 ml. The collected fraction is made alkaline with 20 μl of methanol, 1.5 mol/l potassium hydroxide and evaporated under nitrogen at 50° C. The residue is dissolved with 40 μl of 0.5 mol/l oxalic acid and 1.6 μl are injected into the gas chromatograph.

GC was performed on a Carlo Erba (Milan, Italy) Model 4200 gas chromatograph, equipped with a flame ionization detector. The GC column adopted for quantification was glass, 1.2 m × 2 mm I.D. packed with Carbopack C coated with 0.2% trimesic acid and 0.4% PEG 20 M, which are added to Carbopack C from methanol—methylene chloride (50:50, v/v). Other details concerning the preparation of the packing material and the column packing procedure were described previously [11]. The GC column was conditioned overnight at 230° C with nitrogen. For quantification, the column was operated at 160° C with a dead time of 9 sec. The injector and detector temperatures were both 170° C.

To ensure that the column packing was totally conditioned to water, 1.6 μl of 0.5 mol/l oxalic acid were injected four times into the GC column. This operation was repeated whenever the column had been left unused for some hours.

RESULTS AND DISCUSSION

Analytical recovery and precison of the method were determined by analysing blood samples spiked with various known amounts of valproic acid. Absolute recovery was assessed by adding 10 μl of the octanoic acid solution to the collected fraction. Analytical and absolute recoveries were calculated by measuring the peak height of valproate relative, respectively, to those of heptanoic acid and octanoic acid and comparing them to those of a reference standard. Results are given in Table I. Relative and absolute recoveries in the concentration range considered average 99.2% and 98.5%, respectively. The fact that absolute recovery is not dependent upon the valproate concentration demonstrates that, under the experimental conditions selected, neither chemisorption nor saturation phenomena are present when the sample is passed through the Carbopack B column. Day-to-day coefficient of variation ranged from 5.6% at 19.5 μmol/l to 1.8% at 624 μmol/l. The limit of sensitivity of our method was about 8 μmol/l of blood. At this concentration, a well defined chromatographic peak for valproate could still be obtained and the coefficient of variation of the measurement was 7.2%. Fig. 1 shows typical chromatograms.

TABLE I

RESULTS OF SIX REPLICATE ANALYSES OF SODIUM VALPROATE IN SPIKED BLOOD SPECIMENS

Added (μmol/l)	Found (μmol/l, mean ± S.D.)	C.V. (%)	Analytical recovery (%)	Absolute recovery (%)
19.5	19.2 ± 1.0	5.3	98.4	98.3
39.0	38.4 ± 1.5	3.8	99.5	98.5
78.0	76.9 ± 1.8	2.4	99.2	98.6
254	249 ± 4	1.8	99.2	98.2
624	616 ± 11	1.8	99.0	98.7

Under the GC conditions selected, lactic acid among the endogenous compounds and ethosuximide among the exogenous ones were taken into consideration as possible interfering substances in the valproate determination. Although lactic acid is eluted by the GC column with a broadened peak whose retention time is close to that of valproic acid, it has been reported [9] that lactic acid is scarcely retained by the extraction column and a few millilitres of acidic water are sufficient to eliminate any trace of the hydroxy acid considered. A blood sample supplemented with ethosuximide at level of 100 μg/ml was carried through the procedure described. It was found that the drug was almost completely lost during the washing step with the water—methanol mixture and the last vestiges of ethosuximide produced a GC peak with a retention time of 10 min.

A stability test was performed by adding a blood sample spiked with valproic acid to 0.01 mol/l hydrochloric acid and assaying aliquots in duplicate on the day of collection and after 1, 3, 5, and 10 days' storage at 4°C. No significant deterioration of the sample occurred during the storage (Table II).

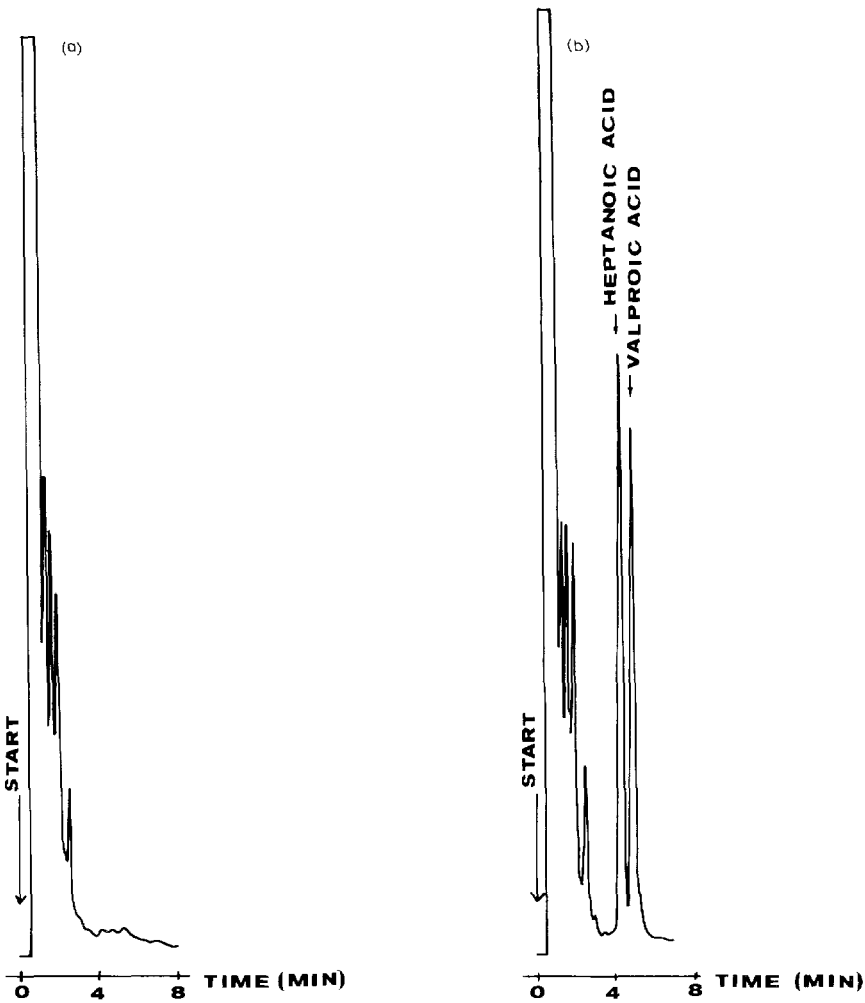

Fig. 1. Gas chromatograms obtained from blood specimens: (a) blank blood; (b) blood spiked with valproate (80 μmol/l) and heptanoic acid.

TABLE II

EFFECT OF STORAGE AT 4°C ON BLOOD VALPROATE CONCENTRATION

$n = 4$.

Days of storage	Concentration (μmol/l, mean ± S.D.)
0	76.8 ± 1.5
1	77.0 ± 1.9
3	77.9 ± 1.5
5	76.2 ± 1.7
10	75.3 ± 1.8

For rapid, accurate and simple valproate determination, analytical optimization studies were performed by changing sequentially the analytical conditions of the sample preparation procedure. The initial pH of the mixture of

blood and acidified water was increased by either increasing the blood volume or decreasing the hydrochloric acid concentration. Concurrently, a steady decrease in the absolute recovery of valproate was observed from 98.6% at pH 2.5 to 84.4% at pH 4.5. This loss can be explained on the basis that valproic acid is increasingly ionized as the pH increases and that some chemical heterogeneities present on the Carbopack surface are promptly rearranged in the presence of water to form salts able to exchange anions [12]. To avoid this undesirable effect, we found it useful to mix 1 ml of 0.01 mol/l hydrochloric acid with 10 μl of blood sample, the resulting pH being 2.3—2.5. When larger volumes of blood were analysed, we took care to increase the volume of 0.01 mol/l hydrochloric acid proportionally, letting other parameters of the analytical procedure remain unmodified.

The flow-rates at which the sample volume and the other liquids percolated through the Carbopack B bed were varied over the range 0.5—5 ml/min by varying the particle size of the Carbopack. No significant variation in the absolute recovery of valproate was noted.

A water bath temperature of 50°C was found to be the best compromise to avoid loss of sample and to shorten the evaporation time, which was about 15 min.

The reusability of the Carbopack B column for valproate determination in blood was studied by repeated extractions on the same column. After each extraction the column was regenerated with 5 ml of methanol and 5 ml of water, taking care to avoid formation of bubbles. After four extractions, the absolute recovery did not change in a significant way, while a 9.3% decrease of absolute recovery was observed after five extractions, even though the analytical recovery remained unaltered.

CONCLUSION

The procedure described here meets the important requirement of using very small blood samples; it is rapid, total analysis time being of about 30 min, as well as highly accurate and sensitive. The stability test has shown the possibility of day-to-day home monitoring of valproate in blood for patients affected by epilepsy.

REFERENCES

1 R.N. Gupta, F. Eng and M.L. Gupta, Clin. Chem., 25 (1979) 1303.
2 A. Hulshoff and H. Roseboom, Clin. Chim. Acta, 93 (1979) 9.
3 S.C. Chan, Clin. Chem., 26 (1980) 1528.
4 Y. Morita, T.I. Ruo, M.L. Lee and A.J. Atkinson, J. Ther. Drug Monit., 3 (1981) 193.
5 N.J. Cook and D.A. Jowett, J. Chromatogr., 272 (1983) 181.
6 L.P. Moody and S.M. Allan, Clin. Chim. Acta, 127 (1983) 263.
7 A. Di Corcia and R. Samperi, Anal. Chem., 46 (1974) 140.
8 G. Cosmi, A. Di Corcia, L. Ripani, R. Samperi and G. Vinci, Clin. Chim. Acta, 126 (1982) 101.
9 G. Cosmi, A. Di Corcia, R. Samperi and G. Vinci, Clin. Chem., 29 (1983) 319.
10 A. Di Gorcia, L. Ripani and R. Samperi, J. Chromatogr., 229 (1982) 365.
11 A. Di Corcia, A. Liberti and R. Samperi, J. Chromatogr., 122 (1976) 459.
12 L. Campanella, A. Di Corcia, R. Samperi and A. Gambacorta, Mater. Chem., 7 (1982) 429.