Monitoring Serum Valproic Acid by Gas Chromatography with Electron-Capture Detection

S. C. Chan

I have developed a procedure to monitor, by gas chromatography with electron-capture detection, the concentration of valproic acid in serum. The valproic acid is derivatized into its phenacyl ester. The assay is very sensitive, measuring as little as 100 pg of valproic acid easily and accurately. Solvent extraction and concentration of the drug are not necessary; instead, one only has to precipitate the serum proteins. n-Octanoic acid is used as the internal standard. The peak-height ratio of phenacyl valproate to phenacyl octanoate is linearly related to concentration up to 200 mg of valproic acid per liter.

Additional Keyphrases: anticonvulzant drugs • pediatric chemistry

Valproic acid is a new member of the antiepileptic drugs in the North American market. Although a therapeutic range has been established—55 to 100 mg/L (1, 2)—this drug is not as widely monitored as some others: of 384 laboratories that participated in the Therapeutic Drug Monitoring Program of the American Association for Clinical Chemistry in November 1979, only 134 monitored valproate.

Valproic acid is a simple fatty acid, chemically unrelated to any other antiepileptic drug. Most of the methods for its assay in serum are gas-chromatographic. Simple fatty acids can be analyzed in the undervatized form if the column-packaging materials are carefully deactivated, and several methods for measuring valproate based on this have been proposed (3-5). Usually the serum is acidified and the drug extracted into an organic solvent. For further clean-up, the drug can be back-extracted into an alkaline solution; after re-acidification the drug is again extracted into an organic solvent, then injected into the chromatograph. Because valproic acid is volatile, one must be careful if attempting to increase the drug concentration by evaporating the solvent. On-column and pre-column derivatization have also been used to improve the gas-chromatographic characteristics of the compound (6, 7).

Pre-column formation of phenacyl esters of fatty acids via crown ether catalyst for enhanced ultraviolet detection in liquid chromatography was proposed by Gruhka and co-workers (8). This derivatization procedure was adapted for use in assaying valproic acid in serum (9). Phenacyl ester formation was also used to monitor serum valproate by gas chromatography with flame-ionization detection (10). I observed that phenacyl valproate has extremely high electron-capture activity: as little as 100 pg can be measured easily and accurately. Consequently, I have devised a procedure for monitoring this drug in as little as 100 μL of serum, using a gas chromatograph equipped with an electron-capture detector. Because this method is so highly sensitive, possible interference by serum components or other drugs can be eliminated by suitable dilution. The serum proteins are simply precipitated, and the valproic acid in the supernate is derivatized; solvent extraction and concentration are not necessary.

Materials and Methods

Reagents and Standards

Stock valproic acid solutions. Valproic acid (rel. density 0.905) was a gift from the Abbott Labs., Ltd., Montreal, P.Q., H3C 3K6, Canada. I prepared four stock solutions (0.905, 2.262, 4.525, and 9.05 g/L) by transferring 0.1, 0.25, 0.5, and 1.0 mL, respectively, of pure valproic acid into 100-mL volumetric flasks and diluting to the mark with methanol.

Serum standards. I prepared four serum-based standards by adding 1.96 mL of drug-free serum to 40 μL of each stock solution; concentrations of these standards were 18.1, 45.3, 90.5, and 181.1 mg/L, respectively. These sera are stored at 4 °C and are stable for three months.

Precipitating solution. n-Octanoic acid solution, 18.2 mg/L of acetonitrile, was used to precipitate the serum proteins. Using a microfilter syringe, I transferred 5 μL of n-octanoic acid (rel. density 0.91; Gold Label; Aldrich Chemical Co., Milwaukee, WI 53201), to a 250-mL volumetric flask, and diluted with acetonitrile to the mark. Octanoic acid was used as the internal standard.

Derivatizing reagent. Phenacyl-8 [phenacyl bromide (0.1 mol/L) and crown ether (5 mmol/L) in acetonitrile; Pierce Chemical Co., Rockford, IL 61105] was diluted 500-fold with acetonitrile.

Equipment

I used a gas chromatograph (Model 5713A; Hewlett-Packard Co., Avondale, PA 19311) fitted with a 63Ni electron-capture detector. The coiled glass column, 3 mm i.d. × 1.8 m, was packed with 3% PC 3210 Ultraphase on Chromosorb W(HP), 80/100 mesh (Pierce Chemical Co.). The oven temperature was 210 °C, and the flow rate of the carrier gas (50 mL of methane per liter of argon; Canadian Liquid Air Ltd., Montreal, P.Q., Canada) was 30 mL/min. The injection port and detector temperatures were 250 and 300 °C, respectively.

Procedure

Add 1 mL of precipitating solution to 100 μL of serum, patient's sample, or standard. Vortex-mix for 10 s and centrifuge at about 2500 × g for 5 min. Transfer 100 μL of the supernatant liquid to a 1-mL Reacti-vial (Pierce Chemical Co.) fitted with a Teflon-lined screw cap. Add 200 μL of the derivatizing reagent and 20 μL of saturated aqueous sodium bicarbonate solution. Tightly cap the vial, and heat it in a dry heating block at 80 °C for 20 min. Vortex-mix the reaction mixture for a few seconds, and then inject 2 to 3 μL of it into the chromatograph.

Plot the peak-height ratio between phenacyl valproate and phenacyl octanoate vs the concentration of the drug, to construct a calibration curve, using the results from the serum standards and the serum blank. Drug concentrations in patient's serum can then be calculated from this graph.
Results and Discussion

Figure 1 shows chromatograms of the blank serum with the proteins precipitated by pure acetonitrile, and a serum with 48.6 mg of valproic acid per liter. The retention times of the phenacyl esters of valproic acid and n-octanoic acid are 2.35 and 3.25 min, respectively.

Figure 2 demonstrates the linearity of the calibration curve from 0 to 181.1 mg/L. The linearity range conceivably extends beyond this value; however, since the upper limit of the therapeutic “window” is 100 mg/L, this range will most likely cover all clinical situations.

The within-run precision (CV) of the procedure for two concentrations of valproate (n = 10 each) was 1.5% for 46.9 (SD 0.9) mg/L and 3.0% for 97.9 (SD 2.9) mg/L. Between-run CVs were 3.7% (mean concn 48.6, SD 1.8, mg/L) and 3.6% (mean concn 99.4, SD 3.6, mg/L), respectively (n = 10 each).

My laboratory participates in the Therapeutic Drug Monitoring Program sponsored by the American Association for Clinical Chemistry; Table 1 shows that our results are fairly accurate and compare favorably with the overall performance of the participating laboratories.

We have analyzed serum from patients who were receiving various drugs, including anti-epileptics (carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, mephenytoin, diazepam, and clonazepam) and psychotropic drugs (amitriptyline, chlorpromazine, haloperidol, mesoridazine, and pericyazine). We have encountered no interference with the present analysis. As a matter of fact, the blank serum in Chromatogram A of Figure 1 was our in-house quality-control serum, and contained 66.5, 5.3, 22.0, 13.5, and 9.0 mg/L, respectively, of ethosuximide, primidone, phenobarbital, phenytoin, and carbamazepine. The absence of interference from these drugs is clearly demonstrated in the chromatogram.

Under our experimental conditions—namely, 500-fold dilution of the phenacyl-8 reagent and a temperature of 80°C—the reaction is in a steady state in 20 min. Increasing the concentration of the reagent or the reaction temperature would increase the yield, but there are drawbacks. Use of a more concentrated reagent would produce by-products that interfere with the chromatographic analysis. Use of a higher reaction temperature would increase evaporation from the mixture. I think that the present set of conditions is a good compromise. The packing PC 3210 was used, but some other common packings would probably perform just as well. For example, on a 3% OV 101 on Chromosorb W(HP) column (Pierce Chemical Co.), 2 mm i.d. X 1.8 m, oven temperature 210 °C, and a flow rate of 20 mL/min, the retention times of the phenacyl esters of valproic acid and octanoic acid are 2.3 and 3.15 min, respectively. The reaction mixture is a clear solution with some white precipitate at the bottom of the vial. Column performance is not affected by repeated injections.

The basis of this procedure is the high sensitivity towards electron-capture detection of the phenacyl ester, and because of this we have been able to do away with tedious extraction and concentration. All the steps are simple, and the procedure

Table 1. Valproate Results by This Method Compared with Those of Other Laboratories Participating in the AACC TDM Program

<table>
<thead>
<tr>
<th>Target value mg/L</th>
<th>This method mean ± SD</th>
<th>All labs. mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.0</td>
<td>60.0</td>
<td>61.1, 17.7 (n = 134)</td>
</tr>
<tr>
<td>115.0</td>
<td>118.8</td>
<td>112.5, 29.1 (n = 141)</td>
</tr>
<tr>
<td>86.0</td>
<td>89.4</td>
<td>84.7, 17.8 (n = 138)</td>
</tr>
<tr>
<td>42.0</td>
<td>39.8</td>
<td>42.8, 6.1 (n = 148)</td>
</tr>
</tbody>
</table>
can easily be mastered by trained laboratory personnel. It only takes 5 min to perform the chromatographic analysis, and as many as 50 samples can be processed in one working day.

Because only 100 μL of serum is needed for an analysis, this procedure can be modified for use with pediatric samples without much difficulty.

I thank Abbott Laboratories, Ltd., Montreal, for providing the authentic valproic acid.

References