accounts for the large variations noted (Figure 1), especially in those patients with no stainable marrow iron.

A diagnostic role for the serum ferritin assay is becoming apparent. It can provide information similar to that obtained by the invasive bone-marrow aspirate stained for iron (12). The two techniques, serum ferritin seems more routinely applicable because of the convenience of sampling. The assay requires 50 µL of serum, and quantitation of serum ferritin is considerably more objective and reproducible than is the assessment of bone-marrow iron. Serum ferritin also seems to be a more sensitive and specific index of declining iron stores than other measures of iron deficiency. In contrast to indices such as percentage transferrin saturation and quantitation of erythrocyte protoporphyrin, serum ferritin concentration becomes abnormal long before mobilizable iron stores are exhausted and before onset of clinically apparent anemia. Thus this assay is clinically valuable because of its sensitivity and convenience for assessing the status of physiological iron stores.

References

Measurement of Sodium Valproate in Serum by Direct-Insertion Chemical-Ionization/Mass Spectrometry

Gary Martin Schler, Ignatius Eng Tho Gan, Berthold Halpern, and John Korth

To quantitatively determine sodium valproate, we use a stable isotope-labeled internal standard and chemical-ionization/mass spectrometry, without prior chromatographic separation. The technique is rapid, simple, sensitive, and provides for the routine analysis of 100 µL of serum with good within-day and day-to-day precision. The technique also allows for the simultaneous determination of phenobarbital, mephobarbital, carbamazepine, primidone, and phenytoin.

Additional Keyphrases: anticonvulsant drugs • phenobarbital • mephobarbital • carbamazepine • primidone • phenytoin

Therapeutic monitoring of the amounts of anticonvulsant drugs in blood is now an established procedure in the treatment of epilepsy, and there is an increasing demand for the development of reliable, rapid, and convenient methods of estimation of these drugs. The anticonvulsant properties of sodium valproate were not discovered until 1963, by Meurier et al. (1). Since then, it has been found most effective in patients with typical absence seizures, simple and complex (petit mal).

Sodium valproate differs chemically from the other commonly used anticonvulsants by its low relative molecular mass and the absence of nitrogen and aromatic rings. Hence nitrogen-specific or ultraviolet absorbance detectors cannot be used, and simultaneous analysis of sodium valproate with the other anticonvulsants by gas–liquid chromatography (GLC) and “high-pressure” liquid chromatography is difficult.

Established methods for the estimation of sodium valproate in serum have been based on procedures for analysis of short-chain fatty acids by GLC. In these procedures, after extraction from serum, valproic acid is assayed by GLC as the free acid (2–5), its silyl derivative (5), or its methyl ester (6). In this work we have applied to the analysis of sodium valproate isotopic dilution and chemical-ionization/mass spectrometry without prior chromatographic separation. The new procedure allows for the simultaneous analysis of sodium valproate with other common anticonvulsant drugs (7).

Materials and Methods

Reagents

Drug samples were obtained as follows: sodium valproate

CLINICAL CHEMISTRY, Vol. 26, No. 1, 1980 147
from Reckitts and Coleman Pty. Ltd., West Ryde, New South Wales 2114; phenobarbital from Frosanna Laboratories Pty. Ltd., Enfield, N.S.W. 2212; carbamazepine from W. Warner Co. Pty. Ltd., M187 Sydney Mail Exchange, N.S.W. 2012; mepobarbital from Ciba-Geigy, Lane Cove, N.S.W. 2116; and phenytoin from Parke Davis, Caringbah, N.S.W. 2229.

The stable isotopic-labeled standards [1,3,15N2,2-13C]-phenytoin; [5-2H5]ethylidihydro-5-phenyl-4,6-(1H,5H)-pyrimidinedione (15-2H5) primidone; and sodium [1,2-13C2]2-propylpentanoate were kindly supplied by Dr. M. G. Horning, Texas Medical Center, Baylor College of Medicine, Houston, TX 77030. 5-Ethyl-[1-2H3]methyl-5-phenylbarbituric acid ([1-2H3]mepobarbital); [1,3-15N2]phenobarbital; and [2H3]carbamazepine were kindly supplied by Dr. R. Summons, Research School of Chemistry, Australian National University, Canberra, A.C.T. 2600, Australia.

The 13C-labeled internal standard for sodium valproate was prepared as a 5 mmol/L solution in ethanol (7.25 mg in 10 mL). The labeled internal standards for the other drugs were prepared as 1 mmol/L solutions in ethanol as follows: phenobarbital (2.3 mg in 10 mL), mepobarbital (2.2 mg in 10 mL), carbamazepine (2.4 mg in 10 mL), primidone (2.2 mg in 10 mL), and phenytoin (2.5 mg in 10 mL). In addition, a working solution containing all the drugs was prepared to the following concentration, per liter: phenobarbital, 0.90 mmol; mepobarbital, 0.30 mmol; carbamazepine, 0.18 mmol; primidone, 0.36 mmol; phenytoin, 0.90 mmol.

Standard solutions of sodium valproate in serum were prepared from a 2 mmol/L stock solution of sodium valproate (16.8 mg in 5 mL of ethanol) by dilution of the stock solution with ethanol to give 20, 15, 10, 5, and 2.5 mmol/L solutions. To 2 mL of serum we added 100 μL of the standard solutions, giving sodium valproate concentrations of 1000, 750, 500, 250, and 125 μmol/L, respectively.

**Table 1. Comparison of Results for Sodium Valproate by the Mass-Spectrometric Method with Results by GLC of the Bartscontrol Quality-Control Scheme a**

<table>
<thead>
<tr>
<th>Mass spectrometry mean, μmol/L (n = 10)</th>
<th>GLC</th>
<th>No. GLC labs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>μmol/L</td>
<td></td>
</tr>
<tr>
<td>274.4</td>
<td>278.30</td>
<td>48.40</td>
</tr>
<tr>
<td>283.3</td>
<td>287.49</td>
<td>39.18</td>
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<tr>
<td>466.9</td>
<td>468.48</td>
<td>57.67</td>
</tr>
<tr>
<td>876.0</td>
<td>866.11</td>
<td>172.68</td>
</tr>
<tr>
<td>510.2</td>
<td>508.42</td>
<td>56.28</td>
</tr>
<tr>
<td>135.5</td>
<td>139.43</td>
<td>20.59</td>
</tr>
</tbody>
</table>

* "Bartscontrol Quality-Control Scheme" of Dr. A. Richens, Clinical Pharmacology Unit, The Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG.

Standard solution is added to the serum before the extraction.

**Direct Chemical-Ionization/Mass-Spectrometric Analysis**

Mass spectra were recorded with a Varian MAT-44 Quadrupole mass spectrometer (Varian MAT GmbH, Bremen, F.R.G.) operated in the chemical-ionization mode, with isobutane at 450 μb (0.45 Pa). The source temperature was maintained at 140 °C; the electron energy was 1600 eV, and emission current was 0.2 mA.

Samples were introduced in a capillary with a stainless-steel insertion probe. Scanning began immediately after the insertion of the sample into the ion source. The sample was heated at the rate of 10 °C/min and the initial temperature of the probe was 20 °C.

The mass spectrometer was programmed in the direct-insertion multiple-ion scanning mode of operation to monitor the ions at m/e 145.1 and 147.1 for the protonated ions of sodium valproate and its 1,2-13C2-labeled internal standard, respectively.

**Results and Discussion**

**Linearity**

To establish linearity of the results of assay method, we analyzed duplicate aliquots of serum standards with concentrations of 125, 250, 500, 750, and 1000 μmol of sodium valproate per liter. The internal standard was 500 μmol/L. Analysis of the results by the least-squares method allowed determination of the line of best fit, together with the slope (0.002), intercept (0.011), and correlation coefficient with a straight line (0.999). In this concentration range, a linear relationship exists between the average ion-intensity ratio of unlabeled to labeled drug and the concentration of sodium valproate in serum.

**Precision**

We determined within-day precision by analyzing 10 100-μL aliquots of a serum pool containing 1000 μmol of sodium valproate per liter and an internal standard at approximately 500 μmol/L. The calculated mean was 999.8 μmol/L, the standard deviation was 5.62 μmol/L, and the coefficient of variation was 0.56%. Similarly, aliquots of the same serum pool were analyzed daily for 10 days to determine day-to-day precision. The calculated mean was 995.6 μmol/L, the standard deviation 12.85 μmol/L, and the coefficient of variation 1.29%.

**Accuracy and Sensitivity**

We compared results of the chemical-ionization/mass-spectrometric method with the results of six sodium valproate-containing reference sera that had been analyzed by GLC in the Bartscontrol Quality-Control Scheme (Table 1). Our results were in close agreement with these: by the least-squares method, the line of best fit had slope 1.021, intercept −9.092, and correlation coefficient 0.999. Similarly, values for routine patients' samples analyzed for sodium valproate by GLC (5) and chemical-ionization/mass spectrometry in our laboratory had a correlation coefficient of 0.999.

Sodium valproate can be estimated at concentrations greater than 1.5 μmol/L, allowing for a signal-to-noise ratio of 50 to 1. The detection limit and sensitivity can be enhanced 30-fold by increasing the electron energy to 2500 eV. However, for normal monitoring of patients this increased sensitivity (to 50 μmol/L) is not required.

**Interference**

Several drug-free sera were extracted and examined for ions
at m/e 145 and 147. We did not detect any ions exceeding background values, indicating that serum does not contain any interfering compounds with ions at m/e 145 and 147.

Other commonly used drugs added to drug-free sera were mephobarbital, phenobarbital, salicylic acid, acetylsalicylic acid, sulfadiazine, acetaminophen, benzyl penicillin, saccharin, carbamazepine, primidone, and phenytoin at 100 μmol/L, and ethosuximide at 500 μmol/L. We examined the mass spectra of the chloroform extracts from these additions for ions at m/e 145 and 147 and detected no interfering signals from these drugs. Similarly, to exclude possible interference from drug metabolites, we also analyzed sera from 10 patients taking each of the other anticonvulsant drugs. Again, no interference could be detected. Therefore this procedure can be used for the simultaneous quantitation of sodium valproate in the presence of the other anticonvulsants (Figure 1) and other acidic drugs.

The assay we describe requires only 100 μL of serum, is simple to perform, and requires no derivatization or chromatographic separation. This assay for sodium valproate can be carried out in the presence of the other anticonvulsant drugs, and conversely, the other anticonvulsants can be determined in the presence of sodium valproate. The precision and accuracy of the method are excellent, which makes this procedure ideally suited for the quantitative estimation of sodium valproate in serum.

The analysis of sodium valproate by chemical ionization/mass spectrometry has the advantage over previously published methods of being able to process 40 samples per hour through the mass spectrometer; it can also be used for the simultaneous determination of the other anticonvulsants. These advantages are particularly important for specialized laboratories such as regional drug-monitoring centers. Routine application of this method for analysis of other drugs will depend on the availability of reliable automated instrumentation.

References