Simplified Simultaneous Determination of Valproic Acid and Ethosuximide in Serum by Gas-Liquid Chromatography

To the Editor:

Valproic acid (VPA) and ethosuximide (ES) are currently monitored in the serum of epileptic patients being so treated (1, 2). Two gas-chromatographic methods have been proposed for simultaneously measuring these two drugs (3, 4); in them, the organic extract must be concentrated by evaporation before chromatography. This incurs some loss of these relatively volatile compounds, a disadvantage that can be overcome if isoamyl acetate is added to the organic extract (3).

Van der Kleijn et al. (5) proposed that the ES be extracted into a small volume of solvent, such that no concentration would be required. Sufficient extraction efficiency is achieved by adding saturated KH$_2$PO$_4$ solution to the sample.

We have found that this suggestion could easily be applied to other drugs, such as VPA, paramethadione, trimethadione, dimethadione, and ethylphenacimide. Here we report the gas-liquid chromatographic determination of VPA and ES after NaH$_2$PO$_4$ is added to the sample and it is extracted into a small volume of chloroform.

Prepare the following internal-standards solution: 200 µL of 2-methyl-2-ethylcaproic acid (not commercially available; kindly supplied by Dr. J. Meijer, Heemstede, The Netherlands) and 150 µg of α,α-dimethyl-β-methylsuxcinimide (Aldrich Chem. Co., Beezse, Belgium 2340; cat. no. 16350-3) per liter of reagent-grade chloroform. This solution, stored refrigerated, is stable for at least a month.

Mix 200 µL of serum and 200 µL of a saturated NaH$_2$PO$_4$ solution in a conical glass centrifuge tube. Add 75 µL of the internal-standards solution. Shake the tube on a vortex-type mixer for 30 s and centrifuge for 5 min at 4000 x g. Inject 1.5 µL of the organic extract into the chromatograph (prior removal of the aqueous upper layer is unnecessary), but take care not to aspirate any of the protein phase.

We use an Intersmat (Chelles les Coutreux, France) IGC 120 DFL gas-chromatograph equipped with a flame-ionization detector. The glass column is 1.8 m x 2 mm i.d., packed with 10% SP-1000 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA 16823). Injector temperature: 260 °C; oven: 220 °C; detector: 250 °C; carrier gas (nitrogen) flow rate: 35 mL/min; attenuation: $4 \times 10^{-10}$ A/mV.

For quantitation, calculate the two peak-area ratios VPA/2-methyl-2-ethylcaproic acid and ES/α,α-dimethyl-β-methylsuxcinimide. Evaluate the concentration of VPA and ES in the unknown sample by comparing the ratios obtained for it to those obtained for a calibration serum containing, per liter, 50 µg each of VPA and ES. Prepare this calibration serum by mixing 19 volumes of a drug-free serum pool with one volume of water containing, per liter, 1153 mg of sodium valproate (Labaz) and 1000 mg of ES (Parke-Davis). This calibration serum is divided into 1-mL aliquots, which are stable for at least six months when stored at -30 °C.

A chromatogram of serum from a patient being treated with VPA and ES will show baseline separation of the peaks corresponding to the two drugs and the two internal standards. Retention times are: VPA 100, 2-methyl-2-ethylcaproic acid 127, α,α-dimethyl-β-methylsuxcinimide 296, and ES 362 seconds. Calibration curves are linear to at least 200 mg VPA or ES per liter of serum. Detection limits for VPA and ES are 1 and 3 µg/mL, respectively.

The within-day CV estimated on patients' samples assayed in duplicate was 5.1% for VPA (2n = 78) and 4.1% for ES (2n = 32). The between-days CV of a single sample assayed on separate days was 7.9% for VPA (n = 33) and 6.5% for ES (n = 12).

For the several hundred sera so analyzed in our laboratory, no interferences from other drugs have been found. Trimethadione has a retention time of 55, paramethadione 63, dimethadione 504, ethylphenacimide 783, and other antiepileptics >900 s. Moreover, chromatograms of drug-free sera never show interfering peaks from serum constituents.

References

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Dimenhydrinate Interferes with Radioimmunoassay of Theophylline

To the Editor:

I wish to report the positive interference of dimenhydrinate with a theophylline radioimmunoassay (RIA).

A 44-year-old woman, admitted to the hospital with steroid-dependent asthma, had been treated as an outpatient with prednisone, theophylline, and dimenhydrinate (Dramamine, Searle). She was treated with, and responded to, intravenous theophylline and methylprednisolone sodium succinate.

Theophylline in serum was assayed by an RIA kit procedure (Clinical Assays, unmodified), by enzyme-multiplied immunoassay (EMIT, Syva), and by liquid chromatography, with the following results:

<table>
<thead>
<tr>
<th>Apparent theophylline, µg/mL</th>
<th>RIA</th>
<th>EMIT</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>59.7</td>
<td>21.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Day 2</td>
<td>57.2</td>
<td>25.6</td>
<td>24.2</td>
</tr>
</tbody>
</table>

Though the patient had apparent toxic concentrations of theophylline by RIA, there was no clinical evidence of...
serious toxicity other than some nausea. Continued treatment with intravenous theophylline resulted in compensation of her respiratory difficulties.

In its literature, Clinical Assays mentions that 8-chlorotheophylline may interfere. Dimenhydrinate is the 8-chlorotheophylline salt of the antihistaminic diphenhydramine. In addition to the theophylline peak on chromatography, there was another peak present, which was consistent with 8-chlorotheophylline.

Evidently, the 8-chlorotheophylline in dimenhydrinate interferes positively with the Clinical Assays theophylline assay. It is surprising that this interference is not more commonly seen, because dimenhydrinate is often prescribed for nausea, a symptom of mild theophylline toxicity.

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Modified Acetonitrile Protein-Precipitation Method of Sample Preparation for Drug Assay by Liquid Chromatography

To the Editor:

Serum samples are commonly prepared for drug assay by liquid chromatography by mixing equal volumes of serum and acetonitrile to precipitate the proteins, and centrifuging. The supernate is injected directly onto the analytical column (1, 2). Proteins may be incompletely precipitated, and some (3, 4) have recommended that the relative volume of acetonitrile be doubled, and a larger sample volume be injected.

We find that by adding electrolyte to serum–acetonitrile mixtures a phase separation occurs, yielding an upper layer that is primarily acetonitrile. It occurred to us that this might be an efficient means of extracting drugs from serum. Dilution of the specimen could be avoided and an even cleaner solution would be available for analysis. This approach has now been successfully tested in our laboratory for several analgesic and anti-convulsant drugs, in two different analytical procedures.

The analgesic procedure was designed to analyze for acetaminophen, salicylic acid, and acetylsalicylic acid. The anti-convulsant procedure was essentially that of Kabra et al. (2) and the drugs tested were ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine, and 5-ethyl-5-p-tolybarbituric acid (internal standard). We prepare specimens for analysis by mixing 1 mL of serum with 1 mL of acetonitrile containing the internal standard. To this we add about 0.4 g of a finely powdered mixture of sodium bisulfate - H2O and sodium chloride (1/4 by wt), mix vigorously on a rotary mixer for 1 min, centrifuge, and inject the clear upper phase directly onto the analytical column for analysis. We made sodium bisulfate part of the electrolyte mixture to yield a sufficiently acid pH during the phase separation to permit quantitative recovery of salicylic acid. Linearities and recoveries for the above drugs have been examined and are quite acceptable. Within-run reproducibility for the anticonvulsants gave CVs ranging from 1 to 2%.

This procedure modification is rapid and convenient. If necessary, the pH in the phase separation is easily controlled by substituting appropriate buffering salts for the sodium bisulfate. The upper phase is mostly acetonitrile, so the sample is easily concentrated by evaporation.

This general technique appears applicable wherever the drugs involved are lipophobic enough to yield favorable and reproducible partition coefficients between the two phases, if the drug concentrations are adequate for detection.

References

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IgD-λ Myeloma with Separate Heavy- and Light-Chain M-Components

To the Editor:

We read the interesting Letter (1) about a case of IgD paraproteinemia, in which one serum M-component reacted only with anti-δ but not with anti-light chain antisera, and the other monoclonal protein fraction consisted of free light chains. The possibility of δ heavy chain disease (HCD) reportedly was excluded by ultracentrifugation and by immunoelectrophoresis in sodium dodecyl sulfate. We have recently discovered a case of δ HCD, in whom the absence of a light chain moiety was documented by several techniques (2). We present here a new case of IgD-λ myeloma, which shows similar paraprotein characteristics to those described by Vladutiu (1). In particular, these kinds of cases necessitate discussion of the basic classification and criteria of HCD.

Our patient, a 68-year-old man, presented with a typical myeloma with multiple osseous lesions, heavy marrow infiltrations of pathological plasmacytoid cells, serum IgD M-component, decreased concentrations of polyclonal immunoglobulins (IgG, IgA, IgM), and light-chain-uria as well. Despite cytostatic chemotherapy, the disease proved fatal in nine months.

In repeated serum protein electrophoresis a peculiar pattern was recorded. A prominent M-component was seen at the anodic end of γ-region and a weaker one in the β1-region (Figure 1).