Table 1. Inter-Run Precision for Serum and Plasmaa

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Mean b</th>
<th>SD b</th>
<th>CV, %</th>
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<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
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<tr>
<td>PEG</td>
<td>19</td>
<td>491</td>
<td>27</td>
<td>5.5</td>
</tr>
<tr>
<td>Mg2+·Phos</td>
<td>15</td>
<td>567</td>
<td>45</td>
<td>8.0</td>
</tr>
<tr>
<td>ISO-Poly</td>
<td>8</td>
<td>592</td>
<td>31</td>
<td>5.2</td>
</tr>
<tr>
<td>Hep-Mn2+ (enzymic)</td>
<td>8</td>
<td>583</td>
<td>27</td>
<td>4.6</td>
</tr>
<tr>
<td>Hep-Mn2+ c</td>
<td>11</td>
<td>456</td>
<td>13</td>
<td>2.8</td>
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</table>

(Liebermann–Burchard)

<table>
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<th>Plasma</th>
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<tbody>
<tr>
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<td>11</td>
<td>474</td>
<td>17</td>
<td>3.7</td>
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<tr>
<td>Mg2+·Phos</td>
<td>13</td>
<td>494</td>
<td>32</td>
<td>6.5</td>
</tr>
<tr>
<td>ISO-Poly</td>
<td>6</td>
<td>482</td>
<td>16</td>
<td>3.3</td>
</tr>
<tr>
<td>Hep-Mn2+ (enzymic)</td>
<td>11</td>
<td>558</td>
<td>79</td>
<td>14.2</td>
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<tr>
<td>Hep-Mn2+ c</td>
<td>14</td>
<td>496</td>
<td>12</td>
<td>2.3</td>
</tr>
</tbody>
</table>

(Liebermann–Burchard)

a All precipitation procedures evaluated the same serum or plasma pool. The Helena electrophoresis control provided with the kit was used for electrophoresis evaluation.

b mg HDL-C per liter, except for electrophoresis, which is percent HDL-C.

c Analyzed at the Northwest Lipid Research Clinic, Harborview Medical Center, Seattle, WA 98104, by methods yielding values closely approximating the Abel-Kendall procedure (see text), in accordance with the Lipid Research Clinics Protocol.

We had both pools evaluated at another laboratory by a continuous-flow (AutoAnalyzer II) method involving the Liebermann–Burchard determination of cholesterol after Mn2+·Hep (92 mmol Mn2+ per liter) precipitation of lipoproteins (3, 6). Values obtained for HDL-C in serum with Mg2+·Phos, Iso-poly, and Mn2+·Hep, by use of the one enzymic procedure on the KDA, all exhibited significant positive bias as compared to the Liebermann–Burchard (AutoAnalyzerII) method (Table 1). For both serum and plasma, the Mn2+·Hep enzymically determined HDL-C reflected strong positive and variable interference relative to the Liebermann–Burchard (AutoAnalyzer II) method (Table 1). Similar positive interference was noted by Steele et al. (7) and Liedtke et al. (8), using enzymic determination of HDL-C and Mn2+·Hep precipitation of lipoproteins. We were not able to correct for this interference by including ethylenediaminetetraacetate in the KDA reagent diluent. The PEG procedure yielded serum HDL-C values closest to those obtained with the comparison procedure; the Mg2+·Phos procedure gave values closest to those for plasma HDL-C by the comparison procedure. Differences among results after various methods of precipitation (Table 1) may reflect interference with the KDA enzymatic method rather than real differences in HDL-C.

The overall CV for percent HDL (electrophoresis) was quite high: 14%. This agrees with the data first reported by Cobb and Sanders (9) but disagrees with a later report by Gamblin (10), who quoted CVs of about 5 and 3%. The CV for our densitometer scanning was 4%.

Patient evaluation. Using Mg2+·Phos and PEG, we compared HDL-C of 25 patients’ serum samples. The Mg2+·Phos procedure yielded significantly higher results, averaging 19 mg/dl higher (p < 0.01, r = 0.95). We favor the PEG procedure for use with the KDA. Variable interferences were encountered with most of the methods tested and clinical-decision values must be carefully determined.

References

1. HDL Cholesterol precipitation reagent, Pierce Chemical Co., Rockford, IL 61105 (June 1978).

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Misleading Results in Cases of Coexisting Acetaminophen and Salicylate Overdose

To the Editor:

Acetaminophen has been aggressively promoted recently in the U.S.A. as being safer than aspirin. When ingested in excess, however, the drug may produce hepatic-cell necrosis. Indeed, the increased use of acetaminophen in Great Britain in the past decade has resulted in the recognition of acetaminophen poisoning as one of the commonest causes of liver failure in that country (1). As U.S. manufacturers of salicylates appreciate loss of their share of the analgesic-antipyretic market to acetaminophen-containing products, it is likely that many new products containing acetaminophen will appear in the American marketplace and, consequently, the potential for abuse of this drug will increase. Between 1973 and 1975, acetaminophen overdose cases in the U.S.A. reportedly doubled (2).

In acetaminophen overdose, the associated early symptoms are nonspecific and non-predictive of later serious complications, particularly liver injury. Further, liver-function test abnormalities do not become apparent until the second day or later after drug ingestion. Recent clinical experience indicates that the history of quantity of drug ingested and concentrations of acetaminophen in plasma, related to time of ingestion, are the most reliable early variables to predict liver damage and to select which patients should be treated with a recommended antidote, such as acetylcysteine (2, 3).

The American Academy of Pediatrics Committee on Drugs recently recommended a simple colorimetric method for determining acetaminophen concentration in plasma, which can easily be
set up in any hospital laboratory (4, 5). The case reported below indicates our recent experience with this recommendation:

Case report. The patient was a 25-year-old white woman, admitted to a suburban hospital 11 h after allegedly ingesting 32 g of aspirin and 8 to 12 g of acetaminophen (Tylentol). Her plasma salicylate concentration on admission was 580 mg/L, and the acetaminophen concentration in plasma on admission was later reported by a reference laboratory as 38 mg/L. The salicylate value continued to increase during the hospitalization, however, reaching 780 mg/L approximately 16 h after her admission to the hospital. She was then transferred to our hospital for further management. Her salicylate and acetaminophen concentrations on arrival at our hospital (approximately 30 h after drug ingestion) were 1200 and 181 mg/L, respectively, as measured in our laboratory. Acetaminophen was measured according to the method of Glynn and Kendal (4, 5). The patient died about 1 h later. The acetaminophen concentration in the blood sample drawn at our hospital was later measured at two independent reference laboratories, both of which reported values of <12 mg/L.

Salicylates are said not to interfere with this colorimetric method (4, 5). Subsequent review of the literature revealed, however, that toxic concentrations of salicylate do contribute a significant positive bias to this colorimetric procedure (6). In his review Dinwoodie (6) provides a correction factor for salicylate that we found to be inappropriate, because it was based on his own modification of the method of Glynn and Kendal. Initial studies in our laboratory indicate that, for the original Glynn and Kendal method (4, 5), an appropriate correction factor for toxic concentrations of salicylates would be:

\[ \text{Apparent} \text{ acetaminophen (mg/L)} = 14x, \text{where } x = \text{salicylates in mg/L}. \]

Because toxic overdose of acetaminophen and salicylate may coexist, laboratories measuring acetaminophen by the method of Glynn and Kendal should be aware of the potential interference described here. Furthermore, each laboratory should confirm the correction factor described above.

References

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Extraction of Underivatized Valproic Acid from Serum before Gas Chromatography

To the Editor:

We wished to measure underivatized serum valproic acid by gas chromatography on a multi-column instrument (Victoreen 4400) that is routinely used to screen volatiles at 135 °C.

According to Kupferberg (1), a column of 10% SP-1000 or 10% DEGS-PS could be used. Because the latter is used at a temperature closer to 135 °C than the SP-1000 is, we installed a stainless-steel column (155 cm × 2 mm) containing 10% DEGS-PS on Chromasorb-W-HP, 100/120 (Chromatographic Specialties Ltd., Brockville, Ontario).

Jakobs et al. (2) reported the very simple technique of injecting acidified serum directly onto the column, but we found this method to be unsatisfactory because of baseline noise. Solvent extractions requiring evaporation steps (1) were also unsatisfactory. Therefore, we developed a simple extraction procedure that requires no solvent evaporation, yet gives a clean sample for application to the column.

After testing injections of a variety of solvents, we found methylene chloride and chloroform to be most suitable injection solvents, and chose the latter for the extraction procedure.

We extracted valproic acid from supplemented standard sera and from control and patients’ sera as follows. We mixed 200 μL of serum and 40 μL of 85% phosphoric acid in a 100 × 13 mm test tube, to which we added 600 μL of chloroform containing the internal standard (cyclohexane carboxylic acid, 70 mg/L). We vigorously agitated (but did not vortex-mix) the mixture by touching the base of the tube to the lip of the well on a vortex-type mixer set at medium speed. After being mixed twice more, the tubes and contents were centrifuged for 5 min at 2500 rpm. After the phases were allowed to separate, 3 μL of the chloroform (lower) phase was injected into the column. The temperatures of the oven, injection block, and flame-ionization detector were 150, 190, and 190 °C, respectively; gas flow (N2) was 40 mL/min.

Figure 1 shows a typical chromatogram of an extracted standard serum. We made quadruplicate determinations of valproic acid in quality-control sera (Therapeutical Drug Monitoring Program) with weighed-in values of 37.4, 104, and 210 mg/L. The respective amounts calculated by peak-height ratios were 37.9 (SD 2.27), 104 (SD 2.2), and 210 (SD 7.2) mg/L. Although there was a contaminant in the control sera, its chromatographic peak (retention time = 2 min) was completely resolved from those of valproic acid and the internal standard.

The extraction procedure described here for use with our column has three advantages: it is simple, there is no loss of valproic acid in a solvent evaporation step, and evaporation of the extracting solvent on standing during an assay is prevented by the overlying aqueous phase.

References
2. Jakobs, C., Bolasch, M., and Hanefeld, F. New direct micro-method for determination of valproic acid in serum by gas chromatog-