Results by the BioRad HbA1c method correlated well with those by the BioRad HbA1 method. The increased specificity of the HbA1c method offers theoretical advantages when other Hb adducts may be encountered (e.g., in alcoholic subjects, patients with renal failure). The additional 30 min of incubation needed to achieve this increased specificity is, however, a significant disadvantage.

Reference

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Buflomedil Concentrations in Blood and Viscera in a Case of Fatal Intoxication

To the Editor:

Buflomedil hydrochloride, 4-(1-pyrrolidiny)-1-(2,4,6-trimethoxyphenyl)-1-butanone hydrochloride, is a recently introduced vasoactive agent used as a peripheral vasodilator (1). Commercially it is sold as 150-mg tablets, as injectable 10 g/L solutions, and as 150 g/L drops ("Loftyl," Abbott Laboratories; "Fonzylane," Lafon Laboratories).

Little information exists on the toxicity of this compound and we were unable to find any publication regarding fatal intoxications.

We report here analytical data for the drug in blood, urine, and viscera in a case of suicidal acute intoxication with buflomedil.

An 19-year-old girl was found 1.5 h after she had taken a bottle of Loftyl drops containing a total of 3 g of buflomedil. The usual maximum daily therapeutic dose is 600 mg. The girl developed epileptic convulsions, pulmonary edema, and ventricular fibrillation. Attempts of resuscitation were unsuccessful. Gastric washout was also performed, but despite all efforts the girl died 2.5 h after ingesting the drug.

A middle-grade cyanosis and extensive congestion of the internal organs were seen at autopsy.

Toxicological analysis by spectrophotometry and chromatography (2) showed the presence of buflomedil in the following concentrations:

<table>
<thead>
<tr>
<th>Drug concn</th>
<th>In blood, mg/L</th>
<th>63.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In urine, mg/L</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>In liver, mg/kg</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>In kidney, mg/kg</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Buflomedil is absorbed rapidly from the gastrointestinal tract (concentrations in serum are greatest about 2 to 3 h after administration), and it has a relatively short biological half-life (1.91–3.65 h) in plasma. Metabolized extensively (a low percentage, 12–28%, of intact drug is excreted in urine within 48 h), it is extensively distributed throughout body-fluid compartments and tissues (apparent volume of distribution, about 80–100 L) (1).

References

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Kinetic Glucose Dehydrogenase Method for Glucose Measurement with a Discrete Kinetic Analyzer Overcomes Interference by Ascorbate

To the Editor:

Even low concentrations of ascorbate in cerebrospinal fluid of neonates can markedly interfere with glucose estimations by a kinetic glucose oxidase/peroxidase method as used in the IL 919 discrete kinetic analyzer (Instrumentation Laboratory, Lexington, MA) (1). This interference can be overcome by using a kinetic glucose dehydrogenase procedure based on those described by Banauch et al. (2) and Price and Spencer (3).

Prepare the kinetic glucose dehydrogenase reagent by adding to the contents of one 85-mL bottle of endpoint glucose dehydrogenase reagent (Mercotest glucose Gluc DH method, cat. no. 2524419; BDH Chemicals Ltd., Poole, U.K.) 765 mL of sodium phosphate buffer (pH 7.4, 120 mmol/L, containing 150 mmol of sodium chloride per liter) and 1 g of NAD° (Sigma Chemical Co., Poole, U.K.). This reagent contains, per liter, 1 kU of glucose dehydrogenase, 0.021 kU of mutarotase, and 1.8 mmol of NAD°, in addition to the 120 mmol of sodium phosphate and 150 mmol of sodium chloride. Substitute a 340-nm filter for the 520-nm filter in the glucose channel of the IL 919. Calibrate and analyze patients' samples according to instructions supplied with the IL 919.

"Expected" and "found" values are linearly related up to 40 mmol/L, and the underestimate at 50 mmol/L is only 2%. Within- and between-batch precision at three concentrations of glucose was satisfactory:

<table>
<thead>
<tr>
<th>Glucose concn, mmol/L</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within batch (n = 20 each)</td>
<td>2.62</td>
<td>0.07</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>10.53</td>
<td>0.21</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>16.48</td>
<td>0.13</td>
<td>0.8</td>
</tr>
<tr>
<td>Between batch (n = 10 each)</td>
<td>2.60</td>
<td>0.13</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>11.24</td>
<td>0.36</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>16.9</td>
<td>0.42</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The following substances (at the concentrations in the sample as listed within parentheses) do not interfere when added either to aqueous solutions, patients' samples, or control material at the three glucose concentrations: ascorbic acid (10 mmol/L), bilirubin (400 μmol/L), creatinine (884 μmol/L), bovine hemoglobin (0.25 g/L), lactic acid (20 mmol/L, in the presence of 1000 U of lactate dehydrogenase per liter), or uric acid (1 mmol/L). Addition
of hemolysate to give a concentration of hemoglobin of about 0.5 g/L (3% hemolysate by vol) was also without effect, but greater contrived hemolysis in certain patients' samples showed a positive interference. In one, 10% (by vol) hemolysate increased the apparent glucose concentration from 5.1 to 6.9 mmol/L. Lipemia (triglyceride concentration >35 mmol/L) did not interfere as judged by analytical recovery of glucose added to lipemic samples and the linear relationship between dilution of lipemic samples and measured glucose concentrations.

We measured glucose in six cerebrospinal fluids and in plasmas from 51 venous and 43 capillary bloods by both the kinetic glucose oxidase/peroxidase method (x) currently used with the kinetic analyzer and the present method (y), with excellent agreement (y = 0.266 + 0.979x, r = 0.9985, n = 99; Deming method (d)). For one specimen of cerebrospinal fluid from a neonate, our method gave a higher result for glucose than did the kinetic glucose oxidase/peroxidase method, which was attributable to ascorbate interference in the latter.

Reagent costs and assay characteristics for the present method as adapted for the IL 919 discrete analyzer were similar to those for the kinetic glucose oxidase/peroxidase method. Its significant advantage is that it is free from interference by ascorbate. Thus, in cases where this is likely to be a problem, as in cerebrospinal fluid samples from neonates (1), use of the kinetic glucose dehydrogenase method is preferable.

**Assay for Amitriptyline and Nortriptyline Facilitated by Use of “Toxi-Tubes” for Extraction**

**To the Editor:**

Various methods for measuring tricyclic antidepressant drugs have been described since 1960 (1). Most involve a three-step extraction procedure before analysis; extraction of the drug from 3 to 5 mL of alkaline serum into an organic solvent, back extraction into acid, and a further extraction into organic solvent. Such procedures necessitate relatively large sample volumes, can require more than an hour for a single analysis, and usually result in analytical recoveries as low as 50–70% (1).

We describe a one-step extraction procedure for the determination of amitriptyline and nortriptyline that is rapid, simple, and reproducible. It involves the use of “Toxi-Tubes” (Analytical Systems, Inc., Laguna Hills, CA 92653), which are part of a commercially available thin-layer chromatographic system. After extraction, the drugs are measured by gas–liquid chromatography on a two-phase column (2). The whole analysis is complete within 10 min.

All glassware used in the procedure was silanized by soaking overnight in a solution of dichlorodimethylsilane/toluene (1/20 by vol) and dried in an oven at 120 °C. After addition of 1 mL of serum and 4 mL of distilled water to a “Toxi-Tube A” (pH 9), each sample was extracted by continually inverting the tube by hand for 1 min. After centrifugation (3000 rpm, 3 min), the upper layer was transferred to a small glass vial and evaporated to dryness at 55 °C under a slow stream of dry nitrogen. The residue was redissolved in absolute ethanol and injected into the gas chromatograph.

Figure 1 (left) shows a chromatogram of the extract obtained from the serum of a patient known to be taking 250 mg of amitriptyline daily. As Figure 1 (right) indicates, this procedure is able to distinguish between the tricyclics and some other commonly used drugs that will be co-extracted with no matter which extraction procedure is used.

To determine the efficiency of the extraction, we added 0.18, 0.36, 0.72, 0.89, and 1.44 μmol of amitriptyline and nortriptyline hydrochlorides per liter of drug-free serum before analysis. Analytical recoveries were 95% (SD 6%) for amitriptyline and 90% (SD 5%) for nortriptyline. The within-run precision was determined by analyzing 10 replicate samples (0.27 μmol/L) on the same day. Day-to-day precision was determined by analyzing a similar sample 12 times over three weeks. Coefficients of variation for amitriptyline and nortriptyline were, respectively, 3.4% and 6.5% (within run) and 5.2% and 8.6% (day to day).

**References**

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**Plasma Lipid and Lipoprotein Concentrations Are Normal in Congenital Hypothyroidism**

**To the Editor:**

Both hypercholesterolemia and hypertriglyceridemia have been observed in adult hypothyroidism, and often very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) increase simultaneously (1). Results for high-density lipoproteins (HDL) in hypothyroidism are contradictory: their concentration is either slightly increased (2) or decreased (3). Similarly, both normal and above-normal concentrations of plasma cholesterol have been observed in hypothyroid newborns (4). To our knowledge, no systematic data are available on plasma lipids and lipoproteins in primary congenital hypothyroidism, even though atherosclerosis has its genesis in child-

References


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