Emergency Determination of Acetaminophen

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I describe a sensitive colorimetric micromethod in which a pH 11.0 carbonate buffer is used to induce acetaminophen to react with Folin–Ciocalteau reagent at room temperature. These reagents and a simple solvent extraction favor formation of a stable indophenol dye with acetaminophen, giving this procedure a good degree of specificity. Results correlate well with those by liquid-chromatographic procedures, and day-to-day precision is <8%.

**Additional Keyphrases:** emergency methods · toxicology · methods for the small laboratory · drug assay · drug monitoring

A fast, simple, accurate procedure is needed for detecting and monitoring acetaminophen in blood after overdoses. This drug is associated with hepatotoxicity and renal failure (1, 2) and, being a very common medication, overdoses are seen often in hospital emergency rooms. Early treatment with sulfhydryl compounds effectively counters acetaminophen toxicity (3).

The numerous high-performance liquid-chromatographic (HPLC) procedures (4–7) require expensive instrumentation that is not available in many hospitals. Other authors describe colorimetric methods requiring acid hydrolysis of acetaminophen to p-aminophenol (8, 9) and reaction with nitrous acid to form 2-nitro-4-acetamidophenol (10, 11). These laborious procedures are not adaptable to routine or emergency situations. Furthermore, drugs such as salicylate, ascorbic acid, and methyl-3,4-dihydroxyphenylalanine (Aldomet, Merck Sharp, & Dohme) interfere positively.

A recent gas-chromatographic procedure (12) requires use of trimethylsilyl hydroxide to derivatize acetaminophen, and there is significant interference from salicylamide.

The procedure I describe meets the requirements for monitoring cases of drug overdose and does not necessitate expensive and highly specialized equipment, a spectrophotometer being the only instrument required. Unconjugated acetaminophen is measured.

**Materials and Methods**

**Reagents**

*Carbonate buffer, pH 11.0.* Dissolve 21 g of sodium carbonate and 420 mg of sodium bicarbonate in 700 ml of water. Adjust the pH to 11.0 ± 0.1 with 2 mol/liter NaOH or 2 mol/liter HCl. Dilute to 1 liter with water. Keep refrigerated.

*Phenol reagent, 2 mol/liter solution* (Folin–Ciocalteau; cat. no. 0175-F; MSP Reference Standard, The United States Pharmacopeia, Rockville, Md. 20852). Prepare this by dissolving 10 mg of the drug in 100 ml of methanol.


1. **Procedure**

   *Extraction.* Transfer 0.1 ml serum and 0.1 ml of each working standard to 10 × 75 mm disposable glass tubes (Kimble no. 73500). Add 0.1 ml of the pH 5.0 acetate buffer and 1.0 ml of mixed solvent to each tube. Vortex mix for 1 min (a vortex-type mixer is used for this and all subsequent mixing). Centrifuge the tubes for 5 min. Remove the protein portion and transfer 0.5-ml aliquots of the solvent to 10 × 75 mm round Coleman cuvettes. To another cuvette add 0.5 ml of mixed solvent; this will be used as a reagent blank. Evaporate the contents of all tubes at room temperature under a stream of compressed air.

   *Color development and measurement.* Pipet 1.5 ml of carbonate buffer into each cuvette. Dissolve all residue by thorough mixing. Add 20 μl of Folin–Ciocalteau phenol reagent to each cuvette. Mix and let stand at room temperature for 25 min. The color is stable for at least 2 h.

   Measure the absorbance at 660 nm vs. the blank (we use a Coleman Jr. II A Spectrophotometer).

   *Calculations.* Plot the absorbances of the standards vs. their concentrations to construct a standard curve. If the absorbance of a sample extract exceeds that of the 120 mg/liter standard the analysis should be repeated on 50 μl of serum or less.

2. **Results**

The absorbance at 660 nm correlates linearly with serum acetaminophen concentrations from 5 to 120 mg/liter. The absorbance for a 120 mg/liter standard is 0.230 when a 10 × 75 mm cuvette is used. Drug-free sera always yield absorbances less than that of a 5 mg/liter acetaminophen solution.

Table 1 summarizes recovery of known amounts of acetaminophen in methanol added to drug-free sera.

Within-run precision was evaluated by processing samples of serum containing acetaminophen at two concentrations. Day-to-day precision (20 days) was evaluated for the higher
Table 1. Analytical Recovery of Acetaminophen
Added to Serum*

<table>
<thead>
<tr>
<th>Added mg/liter</th>
<th>Found mg/liter</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>45</td>
<td>90</td>
</tr>
<tr>
<td>100</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>120</td>
<td>107</td>
<td>89</td>
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* Five sera at each concentration of drug.

Table 2. Reproducibility of Acetaminophen Analysis (n = 20)

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<thead>
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<th>Acetaminophen concn., mg/liter</th>
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<th>Day-to-day</th>
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<tr>
<td></td>
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<td>SD</td>
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Comparison with Liquid Chromatography

To assess this colorimetric procedure I compared it to two high-performance liquid-chromatographic methods (5, 7). The one used in our laboratory, used on 11 samples, yielded a correlation coefficient of 0.9968 with y = 0.944x + 3.32. The other, in use at the University of Colorado Medical Center, gave a correlation coefficient of 0.9932 for 11 samples, with y = 0.893x + 2.80.

Discussion

This colorimetric technique for acetaminophen is accurate and particularly well-suited to cases involving overdose. The extraction step eliminates interference from common drugs and endogenous compounds. The reagents are readily available and stable. Because no expensive and complicated instrumentation is needed, this analysis is available to any laboratory that has a colorimeter that can measure light absorption at 660 nm.

I thank Michelle Krone, Rita Roussel, Mary Alice Pasanen, and Catherine Brito for valuable technical assistance.

References