Acetaminophen Metabolite Interferes in Analysis for Amino Acids

To the Editor:

Metabolic screening by chromatographic analysis for amino acids and organic acids in blood and urine is now commonly used as a diagnostic test in patients with neurological abnormalities and metabolic acidosis. The most frequent cause of artifacts is medication such as antibiotics (1), mucolitic agents (1), and anticonvulsants (2). Recently, we encountered another drug metabolite that gave a false-positive amino acid screening result.

Acetaminophen has become increasingly popular as a pain reliever. We have observed a ninyhydrin-reactive compound in urine specimens from patients who took this drug before urine collection. This compound migrated to a location near dihydroxyphenylalanine (DOPA) in the system of two-dimensional separation of amino acids by high voltage electrophoresis at pH 1.6 (6% formic acid) followed by ascending solvent chromatography in butanol/acetic acid/water (12:3:5). It gave a purple color with ninhydrin reagent, indicating the presence of an alpha-amino group, and a white spot with iodoplatinate reagent, indicating that this amino acid contains sulfur and is probably a thioester. This unknown was eluted from a cation-exchange column (Beckman 119CL Amino Acid Analyzer) approximately 2 min before phenylalanine and partly overlapping it. This compound co-chromatographed in all the above-mentioned systems with authentic acetaminophen—cytochrome conjugate, a known metabolite of acetaminophen (a generous gift from McNeil Consumer Products), and has the same staining characteristics as the standard.

In the two-dimensional paper-chromatographic system, this metabolite can be differentiated from dihydroxyphenylalanine by its positive reaction to iodoplatinate reagent. It can also be distinguished from two other sulfur-containing compounds, which migrate to nearby locations, N-acetylcySTEINE-cysteine disulfide (from a mucolytic inhalant (1) and β-mercaptoactate-cysteine disulfide (excreted by patients with β-mercapto-pyruvate sulfur transferase deficiency (1)), by a negative cyanide–nitroprusside test for these disulfides. When urinary amino acids are measured by an analyzer, it is important that this drug metabolite be distinguished from phenylalanine, which is excreted by patients with phenylketonuria.

References

Vivian E. Shih
Victor Nikiforov
Miriam M. Carney

Massachusetts General Hosp.
Boston, MA 02114

Automated Dry-Film Method for Phosphorus in Serum Evaluated

To the Editor:

Many methods for measuring inorganic phosphate in serum are based on the reaction of phosphate with molybdate to form a phosphomolybdate complex that subsequently is reduced. Although the absorbance of the unreduced phosphomolybdate complex may be measured at 340 nm (1), such methods require removal of serum protein to prevent large interferences in icteric or hemolyzed samples (2). Variations among these methods arise from the use of different reducing agents and different methods of protein removal (dialysis, ultrafiltration, or precipitation).

I evaluate here a method for measurement of serum phosphorus based on use of multilayer dry-film reagents (Ektachem PHOS clinical chemistry slide; Eastman Kodak, Rochester, NY 14650). Phosphate reacts with ammonium molybdate and the complex formed is reduced with p-methylaminophenol sulfate. After incubation, light is reflected off the slide and measured at 680 nm. To evaluate this method, I compared results with those by the manual Selected Method (3), which involves protein precipitation and semidine·HCl as the reducing agent, and also studied the between-day precision and stability of calibration.

The dry-film slides are used with an Ektachem 400 analyzer (Eastman Kodak Co.), which aspirates about 25 μL of sample, depositing 10 μL on the spreading layer of the slide. The reagents on the slide are arranged in layers, as in other Ektachem products (e.g., ref. 4).

I calibrated the methods independently by using the serum-based calibrators from Eastman Kodak (14, 51, and 153 mg of phosphorus per liter) or aqueous standards prepared in house (0, 20, 40, 60, 80, 100 mg/L). The quality-control sera were Monitrol ES Level I and ES Level II (Dade, Miami, FL 33152).

The manufacturer claims that absorbance varies linearly with concentration to 130 mg/L, and initially recommended diluting samples of a higher concentration with a 70 g/L albumin solution. I found that samples containing up to 140 mg of phosphorus per liter needed no dilution and that the use of distilled water gave results nearly identical to those from samples diluted with the albumin solution. The manufacturer now recommends that distilled water be used as diluent.

I calibrated the Ektachem 400 analyzer once weekly with freshly prepared calibrators, as recommended. The small variations of the absolute reflectance densities at each concentration indicate the stability of the system was excellent (CV ≤1.1%, for 15 calibrations done during 100 days). The manufacturer has recently recommended calibration at 90-day intervals.

Between-day precision was determined from the first analysis each day of quality-control material. In all, analyses were done on 67 days during a 108-day period. The precision (CV) was

Zhor El-Mokhef
Joseph A. Duivier
Guy G. Plomteux
Jacques E. Gielen

Lab. de Chim. Méd.
Institut de Pathol., B 23
Université de Liège
4000 Sart Tilman
Liège 1, Belgium

1 Author for correspondence.
better than 2% for both control sera (33.4 ± 0.58 and 82.9 ± 1.54 mg/L, mean ± SD).

I selected serum samples from different patients to represent a variety of clinical conditions, or to obtain samples that were icteric, hemolyzed, or lipemic. All samples were analyzed within 24 h of collection, and by the Ektachem method and the manual method (3) within 4 h each other. Linear regression of the 119 results by the Ektachem Analyzer (EK) and the Selected Method (SM), for phosphorus concentrations ranging from 7 to 147 mg/L, yielded the equation EK = (1.03 ± 0.006) SM − (1.0 ± 0.3) mg/L, r = 0.998, S, = 1.87 mg/L.

Table 1 summarizes the findings for 100 samples from patients for whom a diagnosis was available. Patients with chronic renal failure or on hemodialysis tended to have somewhat higher results by the Ektachem analyzer than by the Selected Method (which chiefly accounts for the slope of 1.03 in the above equation). For samples that appeared moderately to grossly icteric, hemolyzed, or lipemic, none differed by more than 3 mg/L between methods, but all the grossly hemolyzed samples had lower results by the Ektachem Analyzer. The Selected Method reportedly has a positive bias of about 7 mg/L for 5 g/L hemoglobin (3), so the lower results may indicate that the dry-film method is less affected by hemolysis than is the Selected Method.

The overall agreement of results between the two methods was excellent: All results below 60 mg/L differed between methods by no more than 3 mg/L. Of the 37 results above 60 mg/L, six differed by 5 to 7 mg/L, and in all cases results by the Ektachem analyzer were higher. Five of these six were from patients with renal disease: three on hemodialysis, one with chronic renal failure, and one a renal transplant recipient. While some of these samples may show a small positive bias by the Ektachem analyzer, differences at these increased values should not be clinically significant.

In summary, the accuracy compared well with that of the Selected Method, and exceeded between-day precision, long-term stability of calibration, relatively infrequent need for analyses of quality control samples, and fewer repeats on dilutions of high-value samples, along with the low sample volume requirement, 24 h per day availability, and speed, indicate that this dry-slide test methodology is well suited to general, emergency, and pediatric testing in the clinical laboratory.

I thank Eastman Kodak Co, for providing technical support, disposables, and use of the instrument.

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**Table 1. Method Biases for Serum Samples in Various Categories**

<table>
<thead>
<tr>
<th>Clinical status of patient</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>Hemodialysis</td>
<td>12</td>
<td>3.0</td>
<td>2.5</td>
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<tr>
<td>Renal disease</td>
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<td>2.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Malignancy, carcinoma</td>
<td>14</td>
<td>−0.4</td>
<td>1.5</td>
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<tr>
<td>Liver disease</td>
<td>7</td>
<td>0.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Renal transplant disease</td>
<td>7</td>
<td>1.1</td>
<td>2.2</td>
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<tr>
<td>Cardiac disease</td>
<td>12</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>GI disorders</td>
<td>7</td>
<td>1.8</td>
<td>2.1</td>
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<td>Sickle cell disease</td>
<td>4</td>
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<td>Pediatric patients</td>
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<tr>
<td>Ascites</td>
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<tr>
<td>Tuberculosis, lung disease</td>
<td>6</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Other than above</td>
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<td>0.3</td>
<td>0.9</td>
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**Confirmation of the Optimal pH for Measuring Renin Activity in Plasma**

To the Editor:

The optimal pH to be used during the incubation step in the determination of plasma renin activity (PRA) has been assessed in numerous methodological studies (1–12). Reported values range from 5.4 to 6.5. Most of them were obtained by analysis with a single plasma pool (1–9) or a small series of plasma samples (10–12), and with generally rather large increments between successive pH adjustments (about 0.5 pH unit in most studies). The different optima reported for the individual plasmas in the series of Cohen et al. (10) and Katz and Smith (11) suggest interindividual variation.

We have assessed the optimal pH within a narrow pH range, using a large number of plasmas, and have evaluated the results by analysis of variance.

A 15-mL sample of blood, drawn from 23 patients with essential hypertension or kidney diseases was collected in pre-chilled tubes containing 30 mg of K2 EDTA·2H2O placed in ice, and centrifuged within 30 min at 4 °C. Plasma samples were stored at −20 °C until determination. We placed ten 0.5-mL aliquots of each sample into tubes containing phenylmethylsulfonylfluoride (Merck, Darmstadt, F.R.G.), which had been added by evaporating 10 μL of a saturated solution in ethanol (final concentration in plasma, 7 mmol/L), then added 10 μL each of a 2.65 mmol/L solution of captoril (Squibb, Princeton, NJ) in water (final concentration 0.05 mmol/L) and a 265 mmol/L solution of 9-hydroxyquinoline hemihydrate (Sigma Chemical Co, St Louis, MO) in 1.8 mol/L HCl (final concentration 5 mmol/L). Duplicate samples, which were kept on ice throughout, were brought to pH 5.40, 5.55, 5.70, 5.85, and 6.00 with 1 mol/L HCl, as monitored with a Model 62 pH meter (Radiometer, Copenhagen, Denmark) equipped with a type C-1018 combined glass electrode (Russell, Helmstadt, F.R.G.). We then added 10 μL of a 1 mol/L citrate/citric acid buffer at the desired pH to stabilize the pH during incubation. After incubating the samples for 1 h at 37 °C, we deproteinized them by adding a mixture of 4 mol/L NH4OH and acetone (1/3, by vol) and centrifuging. Each pellet was washed with water/acetone (1/4 by vol), and the wash was combined with the supernate, then evaporated under a stream of air, at 37 °C. The residue was dissolved in 2.5 mL of Tris buffer (0.1 mol/L, pH 7.4). In our hands, 85 ± 1% of the angiotensin I in the samples is analyzed for in this procedure. We analyzed duplicate 50-μL aliquots of each set by radioimmunoassay for angiotensin I (limit of detection, 5 fmol/50 μL).

The logarithms of the PRA values were analyzed by one-way analysis of variance with a three-level hierarchical classification (13) in the order patient, pH, pH-duplication, RIA-duplication.

The mean logarithms of the PRA determinations at the five pH values were 2.782, 2.792, 2.807, 2.814, and 2.807, respectively (SD ranging from 0.561 to 0.576). The highest mean activity was obtained at pH 5.85, which was the optimal pH for about half the samples. The degree of kurtosis and skewness in the pH groups was not significant. Table 1 shows the analysis of variance of the five pH values. Because 90% of the plasmas had an opti-