Gas-Chromatographic Method for Plasma Acetate Analysis in Acetate-Intolerance Studies

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We describe a modified gas-chromatographic method for acetate in serum or plasma, intended for use in the investigation of acetate intolerance in hemodialysis patients. The assay may be adapted for use with a single-column gas chromatograph equipped with a flame ionization detector. The analysis, made isothermally, requires only 0.5 ml of plasma or serum. Only one deproteinizing step is required to prepare the sample for analysis. Additionally, we present preliminary findings of an ongoing acetate-intolerance study.

Uremic acidosis among hemodialysis patients has been previously described in the literature (1-5). Patients suffering from chronic renal failure are unable to excrete H+ ions and incur a buffer deficit, resulting in acidosis and decreased plasma CO₂ concentrations, presumably secondary to hyperventilation (1).

One function of hemodialysis is to correct the buffer deficit, restoring the buffer to normal. To accomplish this, sodium bicarbonate was added to the dialysate bath to increase the plasma bicarbonate concentration. Formation of calcium carbonate precipitate required that sodium acetate be substituted for sodium bicarbonate (5). Most of the acetate is metabolized via the Krebs cycle to the bicarbonate ion on a mole-to-mole basis, thus correcting uremic acidosis (1, 2, 4, 5).

In some hemodialysis patients, however, the addition of acetate in the dialysate bath does not correct uremic acidosis. This phenomenon is termed "acetate intolerance" and is characterized by high concentrations of plasma acetate with insufficient elevation in plasma bicarbonate (1-3). The etiology of this condition is obscure; however, several mechanisms have been proposed (1-3): (a) acetate is diffused into the patient too rapidly, (b) circulatory insufficiency restricts acetate from reaching active metabolic sites within the body, and (c) there may be a defect in the acetate metabolic pathway.

Acetate intolerance has been observed in several of our hemodialysis patients, and we believed the phenomenon warranted further study. At that time our medical facility had no analytical method established for plasma acetate analysis. Various acetate methods appearing in the literature, both enzymatic (1, 6) and gas-chromatographic (3, 7-11) were evaluated. We were interested in an assay that afforded the sensitivity and accuracy of gas chromatography but which did not require special extractions, distillations, or conversion of acetate to various derivatives. The method of Wadke and Lowenstein (8) appeared to suit our needs and was evaluated in our laboratory, but we found it insufficiently sensitive and accurate.

Here, we present the gas chromatographic method for analysis of plasma acetate adopted in our laboratory. It is a modification of the method of Wadke and Lowenstein (8), and requires no special sample processing other than a single deproteinization step. The method, as presented, is suitable for acetate-intolerance investigations among hemodialysis patients.

Preliminary findings in our acetate intolerance study are also presented.

Materials and Method

Apparatus and Instrumental Technique

A Varian Model 2740 Dual-Channel Gas Chromatograph equipped with Flame Ionization Detectors and a 1-mV recorder were used. Only one channel is required and the column is operated isothermally.

Column preparation. A glass column, 1.8 m × 2 mm i.d., was packed with "10% SP-1200 plus 1% H₃PO₄ on Chromosorb W AW," 80-100 mesh (Supelco Inc., Bellefonte, Pa. 16823) under reduced pressure, with gentle tapping. Silanized glass wool was used to plug the column's ends, and the column was installed in the injection port to permit on-column injection. The column was preconditioned overnight at 185 °C with a carrier gas flow-rate of 40 ml/min.

Operational Variables. During analysis the column was operated isothermally at 95 °C. Between assay runs the column was maintained at 150 °C. The injection port and detector oven were maintained at 200 °C. The carrier gas was nitrogen, at a continual flow rate of 40 ml/min. The flame ionization detector utilized compressed air and hydrogen gas at flow rates of 300 and 30 ml/min, respectively. The chromatograph was operated at a range of 10⁻¹¹ mA/V and attenuated at 8X.

Shut-down procedure. To increase column life and sensitivity we made, at the end of each assay run, four water injections at 1-min intervals (50 μl each), at a column temperature of 170 °C. This temperature was maintained for 15 min to clear the column of water. The column was then allowed to cool to the standby temperature, 150 °C. Column temperature was stabilized at 95 °C before beginning an assay run.

Standards and controls. Aqueous sodium acetate solutions, 1.0, 3.0, 7.0, 10.0, and 15.0 mmol/liter, were used to provide data for construction of the calibration curve. As internal standard we used an aqueous 10 mmol/liter sodium butyrate solution.

Control sera of two acetate concentrations (3.5 and 12.5 mmol/liter) were prepared by adding weighed amounts of

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sodium acetate to commercial control serum, and 0.5-ml aliquots were stored at \(-20^\circ\text{C}\) for future use. An aliquot of each control pool was analyzed in every assay run.

**Procedure**

Place 0.5 ml of heparinized plasma, serum, or aqueous standard in a 10 \(\times\) 75 mm test tube (one per patient, standard, and control).

Add 100 \(\mu\)l of internal standard (10.0 mmol/liter sodium butyrate) to each tube. Mix well.

Add 100 \(\mu\)l of trichloroacetic acid solution (200 g/liter) to each tube and seal with Parafilm.

Mix the contents of all tubes vigorously for 15 s with a vortex-type mixer. Allow the tubes to stand for 2 min, then repeat the vortex mixing.

Centrifuge all tubes for 10 min at 1200 \(\times\) g, then carefully remove the supernatant fluid, and inject 1.0 \(\mu\)l of it into the injection port (Hamilton 10-\(\mu\)l syringe, No. 399965, Standard Scientific). Samples with acetate concentrations exceeding 6.0 mmol/liter may cause carry-over to the following sample. In this case, inject 1.0 \(\mu\)l of water and allow 10 min before resuming the analysis.

**Calculations.** The acetate/butyrate (A/B) ratios of the standards are calculated and plotted on graph paper as a function of acetate concentration. The A/B ratios of the unknowns are read from the standard curve to determine acetate concentrations. The calibration curve must be reconstructed at the beginning of each assay run.

**Results**

A typical gas chromatogram of a 7.0 mmol/liter acetate plasma specimen, showing relative column retention times, is shown in Figure 1, and a typical calibration curve demonstrating the method’s linearity up to 15.0 mmol/liter and sensitivity to 0.5 mmol/liter is provided in Figure 2.

**Precision**

**Within-run:** The method showed a standard deviation (SD) of 0.15 mmol/liter and a coefficient of variation (CV) of 4.2\% for a concentration of 3.5 mmol/liter \((n = 10)\); at 12.5 mmol/liter the SD was 0.09 mmol/liter and the CV 0.7\% \((n = 10)\).

**Day-to-day:** At 3.5 mmol/liter acetate concentration, the SD was 0.26 mmol/liter, with a CV of 7.4\% \((n = 33)\); for a 12.5 mmol/liter concentration the SD was 0.62 mmol/liter, with a CV of 4.9\% \((n = 33)\). No outlier values—i.e., values exceeding \(\pm 3\) SD of the means—were encountered.

**Accuracy and Analytical Recovery**

Since there is no reference method for acetate, the assessment of accuracy was based on recovery experiments in which specimens were fortified with known amounts of acetate before analysis. Recovery was calculated as follows:

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\text{% recovery} = 100 \times \left( \frac{\text{recovered value} - \text{actual value}}{\text{actual value}} \right)
\]

The recovery after mixing equal volumes of the two control serum pools \((x = 7.8 \text{ mmol/liter})\) was 100\%. When 10.0 mmol/liter of sodium acetate was added to a serum specimen with no detectable acetate, the recovery was 97\% (positive bias). The same technique was used to formulate a 2.5 mmol/liter specimen, 3.0 mmol/liter was recovered (80\%, with positive bias). The 80\% recovery may be misleading because the original serum could contain as much as 0.4 mmol of acetate per liter, which would be undetectable by the assay method because the lower limit of sensitivity is 0.5 mmol/liter. The overall mean recovery for the method over the useful range was 92\%.

**Sensitivity and Specificity**

Acetate concentrations down to 0.5 mmol/liter were detectable, but the concentrations near this value were difficult to quantitate accurately. We only measured acetate concentrations of 1.0 mmol/liter or greater. The assay is specific for acetate, which emerges from the column shortly after tri-chloroacetate, well before the internal standard. No other compound appeared near the acetate peak.

**Stability**

We found acetate to be stable in serum, plasma, and in aqueous standard solutions for at least four working days at room temperature, for at least 10 days at 4 \(^\circ\text{C}\), and for at least three months at \(-20^\circ\text{C}\). Samples for acetate analysis may be frozen and thawed as many as five times or more without altering the stability.

**Interfering Factors**

We found no difference between serum and heparinized plasma in acetate assay characteristics. Moderate hemolysis does not interfere with the assay. Acetate in erythrocytes amounted to 40 to 80\% of the plasma acetate values. Lipemic serum also did not interfere with the results of the assay.
Reference Values

We assessed plasma acetate concentrations in 30 men and women, 17 to 78 years of age, both healthy and with disease states other than renal failure. All had acetate concentrations notably less than 1.0 mmol/liter.

Clinical Application

Preliminary studies of acetate intolerance have been done in our Dialysis Center. Two of 23 hemodialysis patients seemed unable to correct their metabolic acidosis in the appropriate way during dialysis. All patients were dialyzed for 4 to 6 h, with use of either a Scribner shunt or an arteriovenous Cimino–Brescia fistula with a single needle system. A single-pass dialysis system with a dialyzing fluid flow of 300 ml/min was used; the fluid contained, per liter, 35 mmol of sodium acetate and a total CO2 of 6.7 mmol.

Predialysis acetate concentrations in plasma were all less than 1 mmol/liter. The postdialysis values ranged from 1.0 to 23.2 mmol/liter, but the patients with known inability to correct their metabolic acidosis did not have the highest postdialysis acetate values (1.4 to 17.8 mmol/liter). Because of recirculation of blood in the fistula and in the single needle itself, only blood samples from a vein other than the fistula site give a true reading of the acetate and total CO2 concentrations.

The two patients with known acetate intolerance were further evaluated as to the rate of acetate metabolism. Blood samples were obtained immediately after dialysis and 15 min later from the same vein. Within the 15-min period, the acetate concentration declined from 5 to 1.0 mmol/liter, with an accompanying increase in CO2, indicating a very rapid acetate metabolism. Further work, directed to the more specific determination of acetate metabolism, is currently underway.

Discussion

This assay method is sufficiently precise and accurate for studies of acetate intolerance in hemodialysis patients, but is not suitable for measuring plasma acetate concentrations of less than 1.0 mmol/liter. The linearity of a method with sufficient sensitivity to quantitate normal plasma acetate values (<1.0 mmol/liter) would probably not extend to the acetate concentrations encountered in hemodialysis patients. The sensitivity of our method was continually verified by including a 0.5 mmol/liter acetate standard in each run.

Other investigators (3, 10) have presented data on normal plasma acetate concentrations as being from 25 ± 2 (SD) mmol/liter to 108 ± 9 mmol/liter, values that generally are in harmony with ours. We find normal plasma acetate concentrations to be considerably less than 1.0 mmol/liter. Renal-dialysis patients were found to have a near-normal acetate concentration before dialysis, but after dialysis the values ranged from 1.0 to 30.8 mmol/liter; values for most were less than 10.0 mmol/liter. These findings also agree with those of other investigators (1, 3, 10).

It has been reported that the gas-chromatographic response to acetic acid varies with the amount of water used as the solvent (12). This characteristic is not of concern when an internal standard and constant sample volumes are used.

For the determination of acetate/butyrate ratios, peak-area measurement would be preferred over peak-height measurements. However, as long as both the acetate peak and the internal standard peak have the same geometry, the peak height may be used as an accurate reflection of acetate concentration.

In this method an internal standard of sodium butyrate is used, but sodium propionate may be used alternatively. In our laboratory a phthalein contaminant in our distilled-water source emerged from the column at nearly the same time as did propionate. For this reason, we used butyrate as our internal standard. Trichloroacetic acid was used both as a protein precipitant and to maintain an acid pH. The pH of the sample was kept on the acid side of the pK of acetic acid, to increase the compound's volatility on the column. Possible acetate contaminant (if any) in the trichloroacetic acid will not contribute to assay error, because the same amount of trichloroacetic acid solution is added to all standards, controls, and unknowns.

Thus we found this method to be suitable for acetate intolerance studies. The time required for each sample analysis is about 7 min, after construction of the calibration curve and assaying the controls.

References