In a previous paper [4], cholesterol concentrations were measured with the enzymatic and the LB reagent in split aliquots of hexane extracts from several individual sera. Statistically the enzymatic values showed a small systematic constant negative bias vs the LB values (−0.052 mmol/L, corresponding to 0.9% at 6.0 mmol/L). Assuming equivalent interference by the noncholesterol sterols in the two reactions, it is tempting to speculate that the enzymatic reaction is less sensitive to the remaining unidentified sources of interference [2], thus being in position to generate results closer to the definitive isotope dilution mass spectrometric method. Again, this appears to have a negligible practical impact.

References


Determination of Organic Acids by Capillary Electrophoresis in Screening of Organic Acidurias, Carlas M. Farrégo and Angel Hernanz* (Servicio de Bioquimica, Hospital La Paz, Castellana 261, 28046 Madrid, Spain; *author for correspondence: fax 34-1-3582733)

Pathologic alterations of normal catabolism of amino acids, fatty acids, carbohydrates, cholesterol, biogenic amines, and steroids frequently result in abnormal excretion of urinary organic acids. These metabolic disorders manifest themselves either by excretion of abnormal organic acids in the urine that are usually not detected in healthy individuals or by excretion of massive amounts of certain acids that are normally present in the urine in small amounts [1,2]. Neonatal screening of urine for inherited and acquired organic acidurias is important for preventing severe neurologic disease, mental retardation, and death [3,4]. Organic acids are quantitated in urine by gas chromatography in combination with mass spectrometry [2,5–7]. This method is accurate and precise but is slow and requires expensive equipment. We propose a simple, fast, and reliable capillary electrophoresis method for detecting and measuring 10 organic acid markers—methylmalonate, glutarate, 3-methylglutarate, N-acetylaspartate, 2-aminoacidipate, propionate, lactate, 2-oxoisovalerate, isovalerate, and homogentisate—in the screening of organic acidurias. The method comprises direct diluted urine application, electrophoretic separation at 29 kV, and ultraviolet absorption at 185 nm. Complete separation was achieved within 10 min. This method is applicable for use in routine clinical chemistry laboratories.

Upon approval by the Ethics Committee of La Paz Hospital, urine samples were collected from 65 control children ranging from 2 months to 10 years old and from two children with glutaric aciduria type I. Samples were filtered through Ultrafree Millipore (Bedford, MA) filters (M, 10,000), centrifuged 15 min at 2000g, and then diluted with MilliQ water (Millipore) to obtain a creatinine concentration ~1 mmol/L.

The organic acid separation was performed on a Waters AccuSep polyimide-coated 100 cm × 75 μm fused-silica capillary using a CIA® Capillary Electrophoresis instrument (Waters, Milford, MA) equipped with a Millennium® Chromatography Manager (Waters). The electrolyte was a solution of 20
mmol/L sodium sulfate, 0.35 mmol/L calcium chloride, and 25 mL/L CIA-Pak OFM anion-OH (Waters), prepared daily, filtered, and degassed. Stock solutions were prepared by dissolving the sodium salts of methylmalonic, glutaric, 3-methylglutaric, N-acetylaspartic, 2-aminoacidic, propionic, lactic, 2-oxoisovaleric, isovaleric, and homogentisic acids (ICN Biomedicals, Costa Mesa, CA). The working calibrator was prepared by diluting the stock solutions to 1000, 750, 500, 250, 125, 50, and 25 μmol/L to obtain a calibration curve. The injection mode used was hydrostatic for 30 s, and the electrophoretic separation was carried out at 29 kV and thermostated at 25 °C. Ultraviolet direct absorption at 185 nm was used as detection mode.

Figure 1A shows the electropherogram obtained from a standard mixture of 250 μmol/L of each of the 10 organic acids. Electrolyte composition, working temperature, voltage applied, and wavelength detection were optimized to obtain baseline resolution of all organic acids. Some critical organic acids were difficult to separate. Thus, to obtain baseline separation of glutarate and 3-methylglutarate we found that the optimal concentration of sodium sulfate was 20 mmol/L. Separation of lactate, propionate, and oxoisovalerate was accomplished by optimizing the amount of calcium chloride added to the electrolyte. Concentrations between 25 and 1000 μmol/L for all organic acids gave a linear calibration curve (r >0.980). Within-run CVs were 0.9–1.3% (n = 20) and between-run were <5% (n = 15). Recovery of standard organic acids added to urine samples (n = 10) was 95–102% for all organic acids. The lower limit of detection for each standard organic acid added to urine was 5 μmol/L for methylmalonic, glutaric, 3-methylglutaric, N-acetylaspartic, 2-aminoacidic, and homogentisic acids, and 15 μmol/L for propionic, lactic, 2-oxoisovaleric, and isovaleric acids. This detection limit was defined as signal-to-noise ratio and determined by that ratio >3.1.

Figure 1B shows the electropherogram of a normal urine sample, and Fig. 1C shows the electropherogram of a patient with glutaric aciduria type 1, showing the high amount of glutarate in the urine sample.

In conclusion, capillary electrophoresis is a new, simple, reliable, fast, and low-cost method for detecting and measuring organic acids in the screening of organic acidurias.

References