

0.1 $\mu\text{g/L}$ on the same specimens. In the remaining three specimens, the *Vitros* DGXN method reported digoxin at 0.4, 0.5, and 0.6 $\mu\text{g/L}$; the Roche OnLine reported the samples as 0.0, 0.1, and 0.1 $\mu\text{g/L}$; and the Abbott Digoxin II reported the samples as 0.3, 0.3, and 0.3 $\mu\text{g/L}$, respectively.

The potential sensitivity of the DGXN assay to interference from DLIFs was further assessed by adding digoxin to serum samples from both newborns (which probably contain DLIFs) and adults not under digoxin therapy (at 0.40 $\mu\text{g/L}$ and 0.70 $\mu\text{g/L}$, respectively). This approach allowed for evaluation of bidirectional interference. The samples from newborns ($n = 20$) produced values of 0.41 ± 0.1 $\mu\text{g/L}$ digoxin when assayed with the Roche OnLine method and 0.7 ± 0.2 $\mu\text{g/L}$ when assayed with the *Vitros* DGXN digoxin method. The samples from adults ($n = 6$) produced values of 0.7 ± 0.0 $\mu\text{g/L}$ digoxin when assayed with the Roche OnLine method and 0.8 ± 0.1 $\mu\text{g/L}$ when assayed with the *Vitros* DGXN digoxin method.

The data taken as a whole suggest that the positive bias observed in the *Vitros* DGXN assay in comparison with the Roche OnLine assay of neonatal specimens is probably caused, in part, by the presence of DLIFs, which were to a slight degree detected as digoxin, and to a small analytical positive bias observed in comparisons of adult specimens and in recovery experiments. By subtracting the difference between the methods for adult specimens (0.13 $\mu\text{g/L}$) from the difference for neonatal specimens

(0.24 $\mu\text{g/L}$), the net apparent digoxin in neonatal specimens (0.11 $\mu\text{g/L}$) in the *Vitros* DGXN digoxin method is probably caused by co-measurement of DLIFs.

The *Vitros* DGXN digoxin immunoassay was sufficiently sensitive and precise for clinical applications and was not influenced by hemolysis. The *Vitros* method produced results with a small overall positive bias compared with the Roche OnLine method, which has been reported to be free of DLIF interference (5). In 3 of 44 neonatal samples, there was a difference in the measurement of digoxin that could be attributed to the positive bias of the *Vitros* DGXN method plus the detection of some DLIFs. The extent of the differences (average difference, 0.24 $\mu\text{g/L}$; maximum difference, 0.5 $\mu\text{g/L}$) may influence clinical decision-making. We found no evidence, however, of the systematic detection of large amounts of DLIFs by the *Vitros* DGXN method. We conclude that DLIFs interfere slightly with the *Vitros* DGXN method, and the resulting small positive bias may cause occasional discrepant results.

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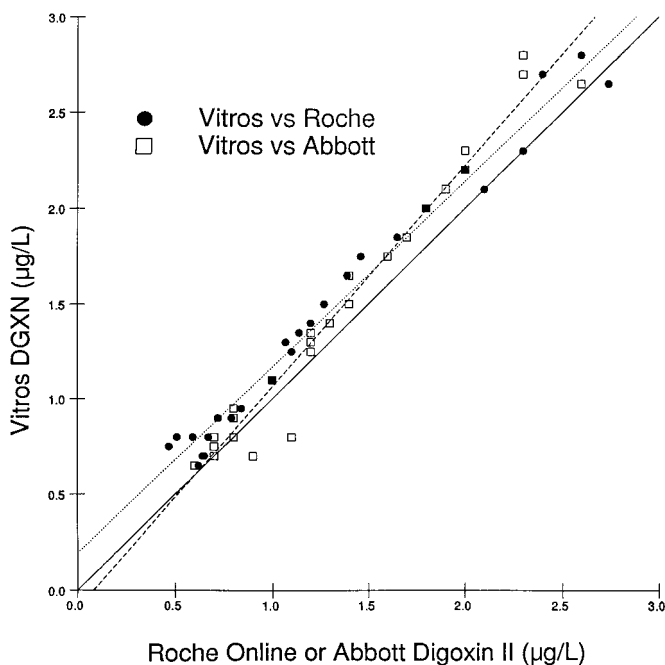


Fig. 1. Apparent digoxin values, measured with the Roche OnLine, Abbott Digoxin II, and *Vitros* DGXN immunoassays, for adults receiving digoxin.

Serum samples from 26 adults were split into three aliquots and assayed for digoxin by all three methods. (\square), Abbott Digoxin II vs *Vitros* DGXN; (\bullet), Roche OnLine vs *Vitros* DGXN; (dashed line), *Vitros* DGXN vs Abbott Digoxin II; (dotted line), *Vitros* DGXN vs Roche OnLine; (solid line), $x = y$.

Quantitative Determination of Short-Chain Organic Acids in Urine by Capillary Electrophoresis, Coral Barbas,* Natalia Adeva, Rosa Aguilar, Marta Rosillo, Teresa Rubio, and Mario Castro (Facultad de CC Experimentales y Técnicas, Universidad S. Pablo-CEU, Urbanización Montepíncipe Ctra. Boadilla del Monte, km 5,3-28668 Madrid, Spain; * author for correspondence: fax (91) 3510475, e-mail cbarbas@ceu.es)

Analysis of organic acids is a powerful technique in the diagnosis of inborn errors of metabolism characterized by organic aciduria, either by the excretion of excessive amounts of urinary organic acids ordinarily expressed or by the presence of organic acids rarely present in urine. The development of more reliable and reproducible analytical methods has shown that ill neonates and children who have primary disorders of organic acid metabolism are more frequent than previously thought (1), and early

diagnosis and treatment may prevent life-threatening episodes or later physical and mental handicaps in some disorders.

Gas chromatography-mass spectroscopy is the technique more widely used (2), but it is a sophisticated, expensive technique with time-consuming sample pretreatment; therefore, it is not suitable in all settings. Moreover, some analytes, such as propionic acid, may be missed because of loss during sample preparation or because of elution with the solvent peak.

Capillary electrophoresis is a modern analytical technique that permits both a rapid and efficient separation of charged compounds present in small sample volumes. When adequate conditions are chosen, the separation can often be achieved directly in aqueous media, without sample pretreatment, giving simple, fast, reliable, and easily automated methods.

Because a large number of organic acids are found in urine (3), we selected an homogeneous group of analytes that are specific markers of organic acidurias (2, 4–8) to develop the methodology. Fumarate, malate, methylmalonate, citrate, pyruvate, acetoacetate, propionate, lactate, and 3-hydroxybutyrate are all short-chain organic acids, with no primary amino groups that might react with ninhydrin.

The diagnosis of several metabolic disorders in urine by capillary electrophoresis has already been described (9–12). Previous studies to determine urinary organic acids by capillary electrophoresis (13, 14) focused on 10 substances, including organic acids of different types and some amino acids. The conditions proposed by Shirao et al. (13) did not separate our acids, whereas Jariego and Hernanz (14) did not describe the complete chemical composition of the buffer used.

In the present study, a simple, fast, quantitative, and reliable method to separate, identify, and measure the short-chain organic acids mentioned above in urine has been developed, and analytical conditions have been validated.

We used organic acids as calibrators from Sigma Chemical, phosphoric acid and sodium hydroxide from Panreac, and methanol from Scharlau.

The calibration urine for method development and validation was from a 3-month-old healthy infant, taken at various times of the day, pooled together, and stored at -20°C until analysis. The calibrators at the concentrations indicated below were added to this urine to obtain the unknown sample.

Urine samples, both unaffected and pathologic, were kindly provided from infants and children who were patients in Hospital La Paz (2 days to 13 years of age) and Hospital Virgen del Rocío and in agreement with the current revision of the Helsinki Declaration of 1975.

Before injection, samples were only filtered through (M_r 30 000) Ultrafugue filters (Scharlau) and centrifuged at 10 000g for 5 min.

The separation was performed on a capillary electrophoresis P/ACE (Beckman) with UV detection at 200 nm. The injection was by pressure for 5 s, and the remaining

conditions were optimized during method development. Previous studies (13, 14) performed the separation in a fused-silica capillary with cationic surfactants to avoid electroosmotic flow; however, in the neutral capillary proposed in this method, the surfactant is not necessary. The neutral coated capillary was 37 cm long, with an internal diameter of $0.75\ \mu\text{m}$, and was operated at $-10\ \text{kV}$ potential. The electrolyte used in the separation was a pH 6 sodium phosphate buffer (200 mmol/L) with 100 mL/L methanol added. The organic modifier permitted the resolution of methylmalonate, propionate, and lactate.

Fig. 1 shows electrophoregrams of a standard mixture (0.8 mmol/L) of each of the organic acids under study. The broad peak corresponding to pyruvic acid (Fig. 1A) may be due to the tautomeric keto-enolic equilibrium of this acid at the working pH. Fig. 1B shows an unaffected urine sample, and Fig. 1C shows a methylmalonic aciduria disease sample with the corresponding methylmalonic acid increase (peak number 3).

Among the 10 components selected at the beginning of the study were the 9 acids named before and butyric acid. The latter was considered as a possible internal standard, but it interfered with an unidentified peak in samples; therefore, it was not used.

Individual stock solutions of each organic acid (200 mmol/L) in water were prepared and stored at -20°C ; with this concentration, acids could be added to samples without appreciable dilution. On the day of the analysis, aliquots of the stock solutions were mixed, and the mixture was diluted as needed.

Linearity of response for calibrators was tested by assaying in duplicate four concentrations of organic acids, from unaffected to medium pathological, ranging between 0.16 and 1.6 mmol/L. Linearity of response for samples was tested in the same way but with urine replacing water.

Recovery was estimated by comparing the values obtained in the linearity of the calibration urine with the linearity of the calibrators, taking into account the endogenous urinary concentrations, which had been measured previously.

Within-day precision was tested to check both the constancy of instrumental response to a given analyte and the concentration and migration time repetitiveness, because the latter is a key parameter for peak assignment. For this purpose, the assay was performed with six calibrators and six samples, in the mid-range of the calibration curve (0.8 mmol/L), for all of the compounds.

The calibrators fit the linear model ($r > 0.99$) for all of the organic acids, and no bias was found because the confidence limits of the intercept include the zero value. Dilution of samples also showed a good linearity ($r > 0.99$). We attribute an apparent bias in some of the acids, in which the zero value is not included within the limits of confidence, to the usual presence of these acids in the sample, mainly fumaric acid and citric acid.

Except for pyruvic acid, recoveries ranged from 86% to 110%; for methylmalonic, citric, propionic, lactic, and

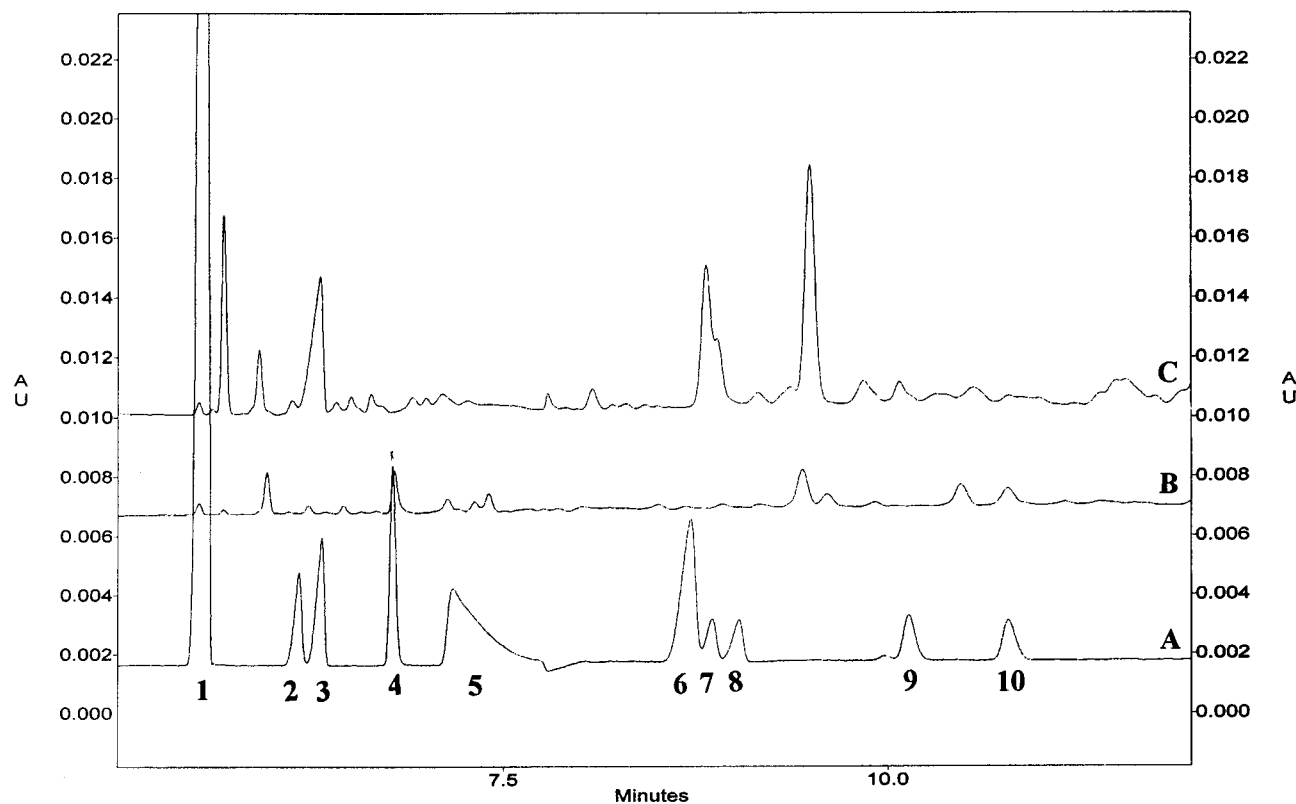


Fig. 1. Electrophoregrams.

(A) A standard mixture (0.8 mmol/L) of each of the following: 1, fumaric; 2, malic; 3, methylmalonic; 4, citric; 5, pyruvic; 6, acetoacetic; 7, propionic; 8, lactic; 9, butyric; and 10, 3-OH-butyric acids. A sample of unaffected urine (B) and urine from a patient with methylmalonic aciduria (C) are shown. In C, methylmalonic acid, peak number 3, is increased. AU, arbitrary unit.

3-hydroxybutyric acids, recoveries did not differ statistically from 100%.

When six assays were run per day of both calibrators and samples, the daily CV in migration time was $\approx 0.2\%$ for all analytes (range, 0.18–0.25%).

The within-day CV of calibrator peak areas was 0.9–3.6% for all calibrators, except for pyruvic acid, for which it was 8.5%.

The method was applied to the urine samples of infants and children to observe whether different profiles were found. The electrophoregrams of four samples were similar, with fumarate and citrate as the only quantifiable compounds, ranging between 3.7 and 10.0 $\mu\text{mol/L}$ for fumarate and between 0.27 and 4.0 mmol/L for citrate. The sample of methylmalonic aciduria disease (Fig. 1C) had increased values of this acid, reaching 10.51 mmol/L.

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