0.1 \mu 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 g/L; the Roche OnLine reported the samples as 0.0, 0.1, and 0.1 \mu g/L; and the Abbott Digoxin II reported the samples as 0.3, 0.3, and 0.3 \mu 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 g/L and 0.70 \mu 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 g/L digoxin when assayed with the Roche OnLine method and 0.7 ± 0.2 \mu g/L when assayed with the Vitros DGXN digoxin method. The samples from adults (n = 6) produced values of 0.7 ± 0.0 \mu g/L digoxin when assayed with the Roche OnLine method and 0.8 ± 0.1 \mu 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 g/L) from the difference for neonatal specimens (0.24 \mu g/L), the net apparent digoxin in neonatal specimens (0.11 \mu 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 g/L; maximum difference, 0.5 \mu 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.

**References**


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**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 Montepríncipe 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, methymalonic, 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 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 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 concentration and migration time repetitiveness, because the latter is a key parameter for peak assignment.
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 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 μmol/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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References