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Capillary Electrophoresis for the Determination of Organic Acidurias in Body Fluids: A Review

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A systematic review of the literature on capillary electrophoresis applied to short chain organic acid analysis in body fluids has been conducted with special interest on those acids related to inborn errors of metabolism. The technique is briefly described, as well as the choice of the main analytical parameters: sample pre-treatment, polarity, capillary type, background electrolyte, and detection. The applications described in the literature are listed and the main features of the technique are discussed. Clin Chem Lab Med 2003; 41(6):000–000

Key words: Inborn errors; Capillary electrophoresis; Organic acids.

Abbreviations: BGE, ###; CE, capillary electrophoresis; CZE, capillary zone electrophoresis; GC-MS, gas chromatography-mass spectrometry; IEM, inborn errors of metabolism; ITP, isotachophoresis; μef, electrophoretic mobility; μeo; electro-osmotic mobility.

Introduction

Organic acids are water-soluble compounds containing one or more carboxyl groups as well as other functional groups (-keto, -hydroxyl). Some nitrogen-containing compounds are included, such as pyroglutamate or amino conjugates like hippurate (benzoylglycine). Organic acids are intermediate metabolites of all major groups of organic cellular components: amino acids, lipids, carbohydrates, nucleic acids, and steroids (1).

Organic acidurias are a biochemically heterogeneous group of inborn errors of metabolism. They are characterized biochemically by the accumulation of organic acids, metabolites that are not present under physiological conditions, produced by the activation of alternative pathways in response to the loss of function of a specific gene product (enzyme), or by the accumulation of pathological amounts of normal metabolites. These disorders share a common natural history, which is the occurrence of either acute life-threatening illness in early infancy or unexplained developmental delay with intercurrent episodes of metabolic decompensation in later childhood (2). Central nervous system diseases, neuroblastoma, nephrolithiasis, and other pathologies are also related to an increase in organic acids in body fluids.

The best fluid to analyze for organic acids is urine because: i) organic acids are concentrated in the urine so that most of them are present in the urine in much higher concentration than in the blood; ii) the virtual lack of protein facilitates the analysis of the sample; iii) a specimen consisting of a simple voiding is easy to obtain and is adequate for analysis (3). In rare instances and/or for follow-up or prenatal diagnostic purposes, quantitation of specific metabolites in other physiological fluid specimens is appropriate.

Gas chromatography-mass spectrometry (GC-MS) has been used routinely as a screening method for the analysis of patient urine for the diagnosis of metabolic disorders (4, 5). Although out of the scope of the present work, a recent publication of Kuhara (6) establishes a practical yet comprehensive diagnostic procedure for inborn errors of metabolism (IEM). This procedure involves the use of urine or eluates from urine on filter paper, stable isotope dilution, and GS-MS. This procedure not only offers reliable and quantitative evidence for diagnosing, understanding, and monitoring the diseases but also provides evidence for the diagnosis of new kinds of IEM.

In spite of its unquestionable sensitivity, selectivity, and identification ability, two significant drawbacks of the GC-MS technique are the length of time required for sample preparation and analysis and the need for trained personnel. That has impeded its use for general screening purposes, and often its use is unnecessary for the monitoring of diagnosed diseases.

Capillary electrophoresis (CE), a relatively new analytical technique, has been accepted as a powerful tool for diagnostic applications (7–10) suitable for detecting important changes in the metabolic profiles of body fluids. It is easy to include in routine analytical systems and it can offer a quick and simple alternative to other techniques.

The main features of the technique relevant to the problem are: i) the ability to separate small molecules in complex matrices without sample pre-treatment; ii) the small sample volume employed, which is of particular interest in such body fluids as tears, cerebrospinal fluid, or even blood in newborns; iii) the possibility of measuring the absorbance in UV at 200 nm or below, where the carboxylic group absorbance presents its maximum, because it works in aqueous media; iv) the low consumption of reactives: a few milliliters of aqueous buffers are enough for one day; iv) the high separation efficiency.

The main drawbacks are related to the detection systems. Since the sample volume employed is very small

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(nanoliters), the limits of detection in UV are not the best feature of the technique. It can be improved by a factor of around 103-fold with laser-induced fluorescence detectors, but then derivatization is needed, with all the associated problems. Finally, MS could give the identification capability that GC-MS presents, but the coupling is still at the beginning.

The focus of this review is to familiarize clinical scientists with the different CE methods employed for organic acid analysis in body fluids, mainly related to inborn errors. These compounds present similar CE behavior, linked to their chemical structure, independently of the disease to which they are related. This is why heterogeneous pathologies are included in the present paper and why physiological compounds not associated with a pathological increase, but which appear in the electrophoretic profile under analysis, are often also included. Table 1 summarizes the main groups of short chain organic acids analyzed in body fluids.

Table 1 Groups of short chain organic acids analyzed in body fluids by CE.

Organic acids	Reference
Formic, succinic, acetic, lactic and propionic acids	(11)
Acetic, lactic, citric, tartaric, malic and succinic acids	(12)
It doesn't work with fumaric and orotic	
Lactate and pyruvate	(13)
Oxalic, formic, methylmalonic, fumaric, succinic, 2-ketoglutaric... (n = 12).	(14)
Oxalic, formic, propionic, fumaric, and others acids (n = 14).	(15)
It does not work with oxalic acid.	
Methylmalonic and short chain dicarboxylic acids (n = 6)	(16)
Uric, hippuric, and others acids	(27)
Oxalate and citrate	(18)
Pyruvic, citric, malic, acetoacetic, and lactic acids	(19)
Organic and inorganic acids (n = 13)	(20)
Methylmalonic, citric, 2-ketoglutaric, succinic acids	(21)
Methylmalonic acid	(22)
Short chain organic acids (n = 14)	(23)
Aliphatic (formic and tartaric) and aromatic acids	(24)
Methylmalonic, glutaric, N-acetylaspartic, aminoadipic, propionic acids (n = 10)	(25)
Oxalic, malonic, maleic, succinic, pyruvic, lactic, 3-hydroxybutyric, and hippuric acids	(26)
Orotic acid	(27)
Orotic acid	(28)
Mevalonic, glutaric, glyceric, and methylmalonic acids	(7)
Short chain organic acids	(29)
Short and medium chain organic acids (n = 9)	(30)
Short and medium chain organic acids (n = 27)	(31)
Oxalic, ascorbic and uric acids	(32)
Orotic acid	(33)
Succinic, maleic, malonic, and glutaric acids	(34)
Homogentisic, pyroglutamic acids, and others compounds	(35)
Oxalic, citric, glyoxylic, and glyceric acids	(36)
Propionic, benzoic, homogentisic, homovanillic, vanillyl mandelic, glyceric, orotic, and more organic acids	(37)
D- and L-Lactic acid	(38)

Mechanism of Separation

There are various CE modes, but small ions are separated by the more simple mechanism of capillary zone electrophoresis (CZE). Two main forces control ions moving in CZE electro-osmotic mobility (μeo) and electrophoretic mobility (μef).

In the capillaries without an internal neutral coating to eliminate the effect of the negative charges of the capillary, and therefore μeo = 0, both electrophoretic and electro-osmotic migration are simultaneous in a sample component, and usually the electro-osmotic mobility due to the capillary wall is higher than the electrophoretic mobility of the analytes. Thus, the velocity that the substances are going to adopt inside the capillary will be the addition or subtraction of these factors according to whether they go in the same or in the opposite sense, depending on the electric charge of the substances. In conventional CE, a specimen is injected on the anodic electrode and detection is performed on the cathodic electrode, as electro-osmotic flow goes from the anode to the cathode.

Very small anions usually have higher μef than μeo, and therefore they would migrate toward the injection end (anode) without passing through the detector, unless the electro-osmotic flow is suppressed and/or the polarity inverted.

Sample Pre-Treatment

One of the advantages of CE is the ability to separate small molecules in complex matrices without sample pre-treatment. This is due to the fact that once the analytes pass through the detector the capillary can be emptied and washed, and it is ready for a new analysis.

That is why many authors measure organic acids in urine without any sample treatment other than dilution and filtration or centrifugation to eliminate solid matter (7, 9, 23, 25, 30–32). Organic acids have also been measured in human saliva after only sample dilution (11).

Nevertheless, although protein content in urine is low, when uncoated capillaries are employed, proteins can interfere because they get adsorbed on the capillary wall and must be eliminated prior to the analysis. This can be done with solid-phase extraction (SPE) on C18 cartridges (14, 27), although Willetts *et al.* detected selective retention of certain organic acids such as lactate in the cartridges (26), or with cationic exchange resins for orotic acid in urine (28); by liquid-liquid extraction with ethyl acetate (21); or by acidification followed by thermic treatment (18). Deproteinization with acetonitrile (two volumes of acetonitrile to one volume of sample) seems to offer a simultaneous method of stacking for small molecules, which is a mechanism of analyte concentration on the capillary itself (39).

Purification has also been developed in line by isotacophoresis (33) prior to CZE. On the condition that a suitable electrolyte system is selected for the isotachophoresis (ITP) step, performed in the first pre-separation capillary of a higher internal diameter, sample components create correct and stable isotacophoretic zones with sharp boundaries. Only a well-defined fraction of the sample containing the stacked analyte is transferred into the second analytical capillary and analyzed by CE.

Serum, with higher protein content than urine, is usually deproteinized because proteins can precipitate in the capillary or get adsorbed to the wall in uncoated capillaries and migration times, in such cases, vary broadly. This has been done by ultrafiltration (17) not only in serum but also in cerebrospinal liquid (13); or by precipitation with cold methanol (23). However, some authors have succeeded in measuring directly in serum with polyacrylamide coated capillaries and a careful selection of the background electrolyte components (19).

Derivatization including a group that facilitates detection is another way of sample pre-treatment used by some authors, but it will be described in more detail in the section on detection.

Polarity

Polarity is the first choice in developing an analytical method for short chain organic acids. Since their electrophoretic mobility toward the anode is usually higher than the electro-osmotic flow toward the cathode, as previously mentioned, the most common mode of analysis is the mode called inverted polarity, which means that the injection is performed at the negative end (cathode) while the detector is placed in the positive end (anode) (14, 26, 13, 15, 19).

Normal polarity has only been employed for derivatized acids (16, 29), because the resulting ions are larger and therefore with a decreased μef or medium-size organic acids (35, 40, 41); nitrogen containing compounds (27, 33, 17).

Capillaries

Inverted polarity has involved the use of coated capillaries or a surfactant in the electrolyte acting as dynamic coating to suppress or even reverse the electro-osmotic flow.

Fourteen short chain organic acids were studied by CE with indirect UV detection in three different capillary conditions: polyacrylamide coated, myristyltrimethylammonium bromide dynamically coated, and uncoated capillary. The best performance in terms of precision in migration time, highest column efficiency, and better limits of detection were obtained by using the polyacrylamide coated capillary. Nevertheless, when the method was applied to clinical urine samples, several interferences appeared and the authors recognized that this method needs further study on real samples (23). Our experience, as much with standards as with many different biological samples, is also that the performance of polyacrylamide coated capillaries, with regard to reproducibility, is the best.

Orotic acid was measured in capillaries coated with polyvinylalcohol (27). According to the authors, these capillaries performed well, were stable, and required little conditioning to give reproducible migration times. However, the necessity to employ relatively complex specimen preparation steps to achieve good assay precision eliminates the major advantage of the technique.

Fluorinated ethylene-propylene copolymer (FEP) and fused silica were employed for the ITP-CZE measurement of orotic acid in the urine of children (33). Other authors used uncoated capillaries (11–18, 20, 21, 23, 25, 26, 28, 29, 33–35, 37, 40, 42) that are less expensive, but the adsorption of compounds to the walls make reproducibility poorer.

Background Electrolyte

Short chain organic acid separation in CE relies on differences in electrophoretic mobility between analyte anions. Harrold *et al.* (20) demonstrated the ability to modify electrophoretic mobility and selectivity as a function of temperature and electrolyte ionic strength for inorganic and short chain organic acids. Although once again this is work was developed only for standards and focused on the application of a particular mode of detection, it provides strategies to be applied for the separation of these compounds. It is well known

that as electrolyte ionic strength decreases, the inherent electrophoretic mobility of the anions toward the anode decreases, while the electro-osmotic flow toward the cathode increases, resulting in an overall runtime decrease. The important observation is that the electrophoretic mobility of the anions changes at different rates, and that permits the selectivity to be manipulated.

The direct addition of metal ions to the mobile phase produces changes in the ionization of a series of organic compounds that determine a significant variation in their electrophoretic mobility. This provides an efficient and rapid means of manipulating selectivity (24). Willetts *et al.* (26) employed 50 mM Borax, 0.4 mM Ca^{2+}, and 1 ml of 3-hydroxybutyric acid adjusted to pH 10 using NaOH.

In general terms, most of the buffers were chosen to give a pH where the carboxylic acids were charged, that is over 4. Near pKa, small pH variations produce large migration changes, which produce important changes in selectivity but also a low reproducibility if buffers are not accurately prepared. On the other hand, once the acids are fully ionized, the influence of pH is negligible.

Special mention must be made of CE-MS buffers, which should contain mainly volatile compounds to work at an optimum level. Thus, ammonium bicarbonate (35) or an aqueous solution of naphthalene disulfonate, pyromellitic acid, and methanol with diethylene triamine as electro-osmotic flow modifier have been employed (34).

Compounds such as orotic acid with a protonable nitrogen can also be measured at low pH, positively charged (27).

Detection

UV absorbance is the most common mode of detection in commercial CE equipment. As previously described, organic acids can be separated in aqueous buffers and if non-absorbing electrolytes are employed, direct measurement at 200 nm or below is a good option for the carboxylic group (13, 14, 17, 24, 26, 27, 30, 31, 40, 43).Obviously, when organic acids with a characteristic spectra are measured, different wavelengths can be employed, for example orotic acid was measured at 280 nm (28).

Indirect detection can be employed adding an absorbing substance at the BGE and detecting the less-absorbing carboxylic group as a negative peak. Generally it is considered a more sensible mode than direct detection and it can be the case for standards, but when dirty or complex samples have to be measured, such as biological fluids, high dilution rates have to be employed to avoid very noisy baselines and overlapping peaks, and in that case the result is not so good. The choice of the indirect chromophore is dictated by the mobility of the ions to be analyzed, since the best resolution occurs when the mobility of the anion in the buffer is close to that of the sample ions.

Wu *et al.* (44) investigated and discussed the suitability of several absorbance providers, additives, and pH, affecting the selectivity and resolution of CE for mono-, di-, and tri-carboxylic acids, as well as for hydroxyl acids. Although their work was only applied to standards, the information can be very useful when working with indirect detection of short chain organic acids. Several compounds have been employed as background absorbing additives: 4-hydroxybenzoate and detection at 254 nm (15); ε-aminocaproic and phenylhydroxyacetic or mandelic acids at 220 nm (19); phthalate at 254 nm (11), at 210 nm (21) and at 230 nm (23); benzoic acid and Tris at 220 nm (29); glutamic acid plus spermine at 254 nm and 280 nm (33); chromate at 254 nm (18), and some authors employed a commercial BGE of undescribed composition (11).

Fluorescence has also been employed after derivatizing the carboxylic acids, but derivatization of short chain organic acids in aqueous solution is the most challenging because of the low reactivity of the carboxylic group in water. Usually it requires several reaction steps. Nevertheless, some derivatizing agents have been employed in biological samples: 5-bromofluorescein for C_8 to C_{11} carboxylic acids to be detected with the Argon laser at 488 nm (29); 1-pirenyldiazomethane for dicarboxylic acids to be detected with He-Cd laser (16, 22).

Methods of describing limits of detection are usually very confusing because many authors report absolute masses in the capillary and it must be borne in mind that there are only a few nanoliters of sample in the capillary. Other authors give concentration in the vial, but samples have to be diluted during treatment. Schneede *et al.* (16) clearly describe 40 nM for the methylmalonic-pirenyldiazomethane derivative and under 1 μM of methylmalonic acid in human serum.

An exhaustive study of parameters that influence separation was developed by these authors, but there are no data about the quantitativity or reproducibility of the derivatization reaction.

Finally, electrochemical detection has also been employed in some cases, but the problem of measuring very small currents at the end of a capillary with high voltage is well known, as are the problems related to the extreme potentials needed for obtaining a redox response from carboxylic acids. Fu *et al.* (32) employed a graphite paste electrode modified with cobalt phtalocianine, but they only measured oxalate, ascorbate, and uric acids, those with known electrochemical properties. On the other hand, a conductivity detector was employed, for standards and in a wine sample. Although methods developed for organic acids in food have not generally been included, this last one is interesting due to the detector. The authors considered that, since conductivity detection shows a direct relationship between retention time and peak area, the use of an internal standard allows accurate determination of absolute concentrations in a mixture without separate calibration for each component. Nevertheless, the work was developed in 1989 with homemade equipment, and currently available commercial equipment does not yet implement this type of detector.

To date only two works have been published dealing with the analysis of diagnostic metabolites by CE-MS. The first one includes glutathione, pyroglutamate, adenylosuccinate, ornithine, histidine, and homogentisic acid measurement in normal and spiked urine samples (35). The second one was developed by Jellum *et al.* and is devoted to the analysis of urine and blood samples from patients with known metabolic disorders (galactosemia, neuroblastoma, Zellweger syndrome, propionic academia, and alcaptonuria) by CE-tandem MS (37). The authors say that although the results are promising, there is still a long way to go. Libraries of urinary metabolites must be created for automated identification and the potential of these techniques must be further evaluated by running patient samples routinely.

Enantiomeric Resolution

Most biochemical reactions have enantiomeric selectivity. Different enantiomers of the same compound can activate different metabolic pathways (45). The enantiomeric ratio of chiral metabolites is an important parameter for understanding metabolic processes, and in many cases it can have diagnostic purposes.

From this point of view it is possible to determine the origin of several pathologies by an enantiomeric analysis of selected metabolites. D-enantiomers usually have a bacteriological origin while L-enantiomers have predominantly an endogenous one. CE has been demonstrated to be a good choice for enantiomeric resolutions, using chiral selectors in the separation buffer, which can provide very simple and automated method development (38). This very complex area goes beyond the scope of the present paper, except to mention that a comprehensive review on the subject is in the process of being published (38).

Conclusion

CE permits the development of fully automatic methods, with little or no sample pre-treatment, small sample volumes, important separation efficiency, and low cost. This provides an interesting tool for screening programs in newborns. For prenatal diagnosis or when the patient is acutely ill, the procedures utilized must be capable of providing not only accurate but also rapid results. With regard to organic acidurias, the CE method with a higher number of organic acids separated and identified permits analysis of 27 compounds in a 15 min run (31), out of nearly 200 that might exist. Figure 1 shows the separation obtained for 22 standards with this method. Although this is not many, it is important to consider that included in this list are some of the more frequent pathologies, such as methylmalonic acid, propionic acid, Canavan disease, pyroglutamic aciduria, hyperoxaluria, orotic acid, fumaric acid, isovaleric acid, alkaptonuria, lactic aciduria, ketosis, and even more importantly, glutaric aciduria. In the last instance, if it is detected and treated at an early stage, development can be normal. In the opinion of Seymour *et al.* (46), screening programs would be recommended for glutaric aciduria. This method has been evaluated to deal with filter paper collected urine samples and 20 organic acids can be detected (43).

Nowadays, when done, screening of inborn errors of

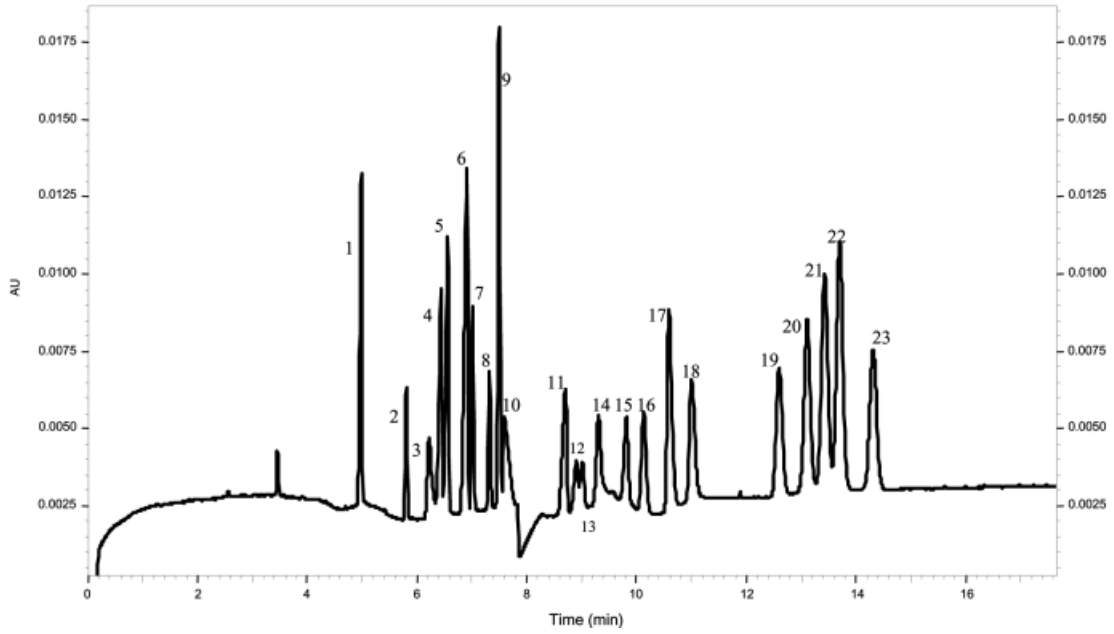

Figure 1 Separation obtained for 23 organic acids by CE with direct detection and reverted polarity, polyacrylamide-coated capillary, and phosphate buffer pH 6 with 10% methanol added (31).In order, peaks correspond to: 1: oxalic, 2: fumaric, 3: 2-ketoglutaric, 4: malic, 5: methylmalonic, 6: glutaric, 7: citric, 8: adipic, 9: N-acetylaspartic, 10: glycolic, 11: acetoacetic, 12: propionic, 13: lactic, 14: 2-ketoisovaleric, 15: glyceric, 16: 2-hydroxybutyric, 17: 3-hydroxybutyric, 18: 2-hydroxyisovaleric, 19: phenyllactic, 20: homogentisic, 21: hippuric, 22: uric, 23: p-hydroxyphenyllactic acids.

metabolism including phenylketonuria is developed by MS/MS in blood samples (47). Tandem mass spectrometry (MS/MS) has become a key technology in the fields of biochemical genetics and newborn screening. The development of electrospray ionization (ESI) and associated automation of sample handling and data manipulation have allowed the introduction of expanded newborn screening for disorders that feature accumulation of acylcarnitines and certain amino acids in a number of programs worldwide. A review from Carpenter *et al.* (48) covers some of the basic theory of MS/MS and focuses on the practical application of the technique.

Nevertheless, this is a very expensive technique and it measures carnitines and glycines more than organic acids. Thus, it does not allow to differentiate propionic from methylmalonic aciduria. Both derive from the metabolism of propionate, and the same conjugated compounds are increased, but methylmalonic acid is the essential marker of methylmalonic aciduria (49). In this case, CE could be a complementary diagnostic tool. Clinical management of methylmalonic aciduria is considered to be most critical during the early years of life (50).

On the other hand, CE, with a very different separation mechanism, can be also a complementary analytical tool for compounds such as propionic or oxalic acids poorly detected by GC-MS, due to their low recovery after sample pre-treatment, and for compounds such as pyroglutamic and pipecolic acids with the same masses, and therefore interfering.

Finally, CE would be the best option for monitoring previously diagnosed diseases, when the structural identification of unknown diagnostic metabolites is not necessary.

Moreover, CE may be very suitable as a fast and relatively simple screening method, in particular for disorders presenting episodes of acute life threatening illness, characterized by massive excretion of a limited number of metabolites, as well as for monitoring the response to treatment, or to determine the concentration of particular acids, poorly detected by GC-MS. It does not, however, offer the chromatographic resolution, sensitivity, and specificity needed to detect, identify and quantify the more than 400 organic acid metabolites that today can be found by automated GC-MS profiling of normal and pathological urine samples.

A possible role for CE in the routine system for metabolic disorders might be following the diagnosed samples and pre-testing urine samples as a complement to other screening techniques.

References

1. Lehotay DC. Chromatographic techniques in inborn errors of metabolism [review]. Biomed Chromatogr 1991; 5:113–21.
2. Chalmers RA. Current research in the organic acidurias. J Inherited Metab Dis 1989; 12:225–39.
3. Tuchman M, Ulstrom RA. Urinary organic acids in health and disease. ####: Year Book Medical Publishers, Inc., 1985:469–507.
4. Tanaka K, Hine D, West-Dull A, Lynn TB. Gas-chromatographic method for urinary organic acids. Retention indices for155 metabolically important compounds. Clin Chem 1980; 26:1839–46.
5. Tanaka K, Hine DG. Compilation of gas chromatographic retention indices of 165 metabolically important organic acids and their use in detection of patients with organic acidurias. J Chromatogr 1982; 239:301–22.
6. Kuhara T. Diagnosis and monitoring of inborn errors of metabolism using urease-pretreatment of urine, isotope dilution, and gas chromatography-mass spectrometry [review]. J Chromatogr B 2002; 781:497–517.
7. Jellum E, Dollekamp H, Brunsvig, A, Gislefoss R. Diagnostic applications of chromatography and capillary electroporesis. J Chromatogr B 1997; 689:155–64.
8. Jellum E, Thorsrud AK, Time E. Capillary electrophoresis for diagnosis and studies of human disease, particularly metabolic disorders. J Chromatogr 1991; 559:455–65.
9. Jellum E, Dollekamp H, Blessum C. Capillary electrophoresis for clinical problem solving: analysis of urinary diagnostic metabolites and serum proteins. J Chromatogr B 1996; 683:55–65.
10. Hong J, Baldwin RP. Profiling clinically important metabolites in human urine by capillary electrophoresis and electrochemical detection. J Capillary Electrophor 1997; 4:65–71.
11. Romano J, Jandik P, Jones WR, Jackson PE. Optimization of inorganic capillary electrophoresis for the analysis of anionic solutes in real samples. J Chromatogr 1991; 546: 411–21.
12. Kenney BF. Determination of organic acids in food samples by capillary electrophoresis. J Chromatogr 1991; 546:423–30.
13. Hiraoka A, Akai J, Tominaga, I, Hattori M, Sasaki H, Arato T. Capillary zone electrophoretic determination of oranic acids in cerebrospinal fluid from patients with central nervous system diseases. J Chromatogr A 1994; 680:243–6.
14. Shirao M, Furuta R, Suzuki S, Nakazawa H, Fujita S, Maruyama T. Determination of organic acids in urine by capillary zone electrophoresis. J Chromatogr A 1994; 680: 247–51.
15. Devêvre O, Putra DP, Botton B, Garbaye J. Sensitive and selective method for the separation of organic acids by capillary zone electrophoresis. J Chromatogr 1994; 679:349–57.
16. Schneede J, Mortensen JH, Kvalheim G, Ueland PM. Capillary zone electrophoresis with laser-induced fluorescence detection for analysis of methylmalonic acid and other short-chain dicarboxylic acids derivatized with 1-pyrenyldiazomethane. J Chromatogr 1994; 669:185–93.
17. Petucci CJ, Kantes HL, Strein TG, Veening H. Capillary electrophoresis as a clinical tool. Determination of organic anions in normal and uremic serum using photodiode-array detection. J Chromatog. B 1995668:241–51.
18. Holmes RP. Measurement of urinary oxalate and citrate by capillary electrophoresis and indirect ultraviolet absorbance. Clin Chem 1995; 41:1297–301.
19. Dolnik V, Dolnikova J. Capillary zone electrophoresis of organic acids in serum of critically ill children. J Chromatogr A 1995; 716:269–77.
20. Harrold M, Stillian J, Bao L, Rocklin R, Avdalovic N. Capillary electrophoresis of inorganic anions and organic acids using suppressed conductivity detection. Strategies for selectivity control. J Chromatogr A 1995; 717:371–83.
21. Marsh DB, Nuttall KL. Methylmalonic acid in clinical urine specimens by capillary zone electrophoresis using indirect photometric detection. J Capillary Electrophor 1995; 2: 63–7.

22. Schneede J, Ueland PM. Application of capillary electrophoresis with laser-induced fluorescence detection for routine determination of methylmalonic acid in human serum. Anal Chem 1995; 67:812–9.
23. Chen H, Xu Y, Van Lente F, Ip MP. Indirect ultraviolet detection of biologically relevant organic acids by capillary electrophoresis. J Chromatogr B 1996; 679:49–59.
24. Chiari M, Dell'Orto N, Casella L. Separation of organic acids by capillary zone electrophoresis in buffers containing divalent metal cations. J Chromatogr A 1996; 745:93–101.
25. Jariego CM, Hernanz A. Determination of organic acids by capillary electrophoresis in screening of organic acidurias. Clin Chem 1996; 42:477–8.
26. Willetts M, Clarkson P, Cooke M. The use of capillary zone electrophoresis to determine lactate, pyruvate and other organic acids in neonatal urine. Chromatographia 1996; 23:671–4.
27. Franke DR, Nuttall KL. Orotic acid in clinical urine specimens by capillary zone electrophoresis using polyvinyl alcohol coated capillaries. J Capillary Electrophor 1996; 3:309–12.
28. Sevcik J, Adam T, Sazel V. A rapid and simple screening method for detection of orotic aciduria by capillary zone electrophoresis. Clin Chim Acta 1997; 259:73–81.
29. Zuriguel V, Causse E, Bounery JD, Nouadje G, Simeon N, Nertz M, *et al.* Short chain fatty acids analysis by capillary electrophoresis and indirect UV detection or laser-induced fluorescence. J Chromatogr A 1997; 781:233–8.
30. Barbas C, Adeva N, Aguilar R, Rosillo M, Rubio T, Castro M. Quantitative determination of short-chain organic acids in urine by capillary electrophoresis. Clin Chem 1998; 44:1340–2.
31. Garcia A, Barbas C, Aguilar R, Castro M. Capillary electrophoresis for rapid profiling of organic acidurias. Clin Chem 1998; 44:1905–11.
32. Fu C, Wang L, Fang Y. Determination of oxalic acid in urine by co-electroosmotic capillary electrophoresis with amperometric detection. Talanta 1999; 50:953–8.
33. Procházková A, Krivánková L, Bocek P. Analysis of ortoric acid in human urine by on-line combination of capillary isotachophoresis and zone electrophoresis. J Chromatogr A 1999; 838:213–21.
34. Johnson SK, Houk LL, Johnson DC, Houk RS. Determination of small carboxylic acids by capillary electrophoresis with electrospray-mass spectrometry. Anal Chim Acta 1999; 389:1–8.
35. He T, Quinn D, Fu E, Wang YK. Analysis of diagnostic metabolites by capillary electrophoresis-mass spectrometry. J Chromatogr B 1999; 727:43–52.
36. García A, Muros M, Barbas C. Measurement of nephrolithiasis urinari markers by capillary electrophoresis. J Chromatogr B 2001; 755:287–95.
37. Barbas C, García A, Saavedra L, Muros M. Urinary analysis of nephrolithiasis markers. J Chromatogr B 2002; 781: 433–55.
38. Saavedra L, Barbas C. Optimization of the separation of lactic acid enantiomers in body fluids by capillary electrophoresis. J Chromatogr B 2002; 766:235–42.
39. Bongaerts G, Bakkeren J, Severijnen R, Sperl W, Willems H, Naber T, *et al.* Lactobacilli and acidosis in children with short small bowel. J Pediatr Gastroenterol Nutr 2000; 30:288–93.
40. Presto Elgstoen KB, Jellum E. Capillary electrophoresis for diagnosis of metabolic disease. Electrophoresis 1997; 18:1857–60.
41. Presto Elgstoen KB, Zhao JY, Anacleto JF, Jellum E. Potential of capillary electrophoresis, tandem mass spectrometry and coupled capillary electrophoresis-tandem mass spectrometry as diagnostic tools. J Chromatogr A 2001; 914:265–75.
42. Zunic G, Spasic S, Jelic-Ivanovic Z. Simple and rapid method for the measurement of nitrite and nitrate in human plasma and cerebrospinal fluid by capillary electrophoresis. J Chromatogr B 1999; 727:73–9.
43. Barbas C, García A, Miguel L, Simó C. Evaluation of filter paper collection of urine samples for detection and measurement of organic acidurias by capillary electrophoresis. J Chromatogr B 2002; 780:73–82.
44. Wu CH, Lo YS, Lee Y-H, Lin T-I. Capillary electrophoretic determination of organic acids with indirect detection. J Chromatogr A 1995; 716:291–301.
45. Lloyd DK, Aubry A-F, De Lorenzi E. Selectivity in capillary electrophoresis: the use of proteins. J Chromatogr A 1997; 792:349–69.
46. Seymour CA, Thomason MJ, Chalmers RA, Addison GM, Bain MD, Cockburn F, *et al.* Newborn screening for inborn errors of metabolism: a systematic review. Health Technol Assess 1997; 1:1–95.
47. Millington DS, Kodo N, Norwood DL, Roe CR. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. J Inherited Metab Dis 1990; 13:321–4.
48. Carpenter KH, Wiley V. Application of tandem mass spectrometry to biochemical genetics and newborn screening [review]. Clin Chim Acta 2002; 322:1–10.
49. Kuhara T, Shinka T, Inoue Y, Ohse M, Zhen-wei X, Yoshida I, *et al.* Pilot study of gas chromatographic-mass spectrometric screening of newborn urine for inborn errors of metabolism after treatment with urease. J Chromatogr B 1999; 731:141–7.
50. Van den Bergh FA, del Canho H, Duran M. Methylmalonic aciduria and sudden child death. J Inherit Metab Dis 1992; 15:897–8.

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