Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1004 (2003) 29–37
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

# Capillary electrophoresis with laser-induced fluorescence detection for laboratory diagnosis of galactosemia

Christopher J. Easley[a], Lian Ji Jin[a,1], Katja B. Presto Elgstoen[b], Egil Jellum[b], James P. Landers[a,c], Jerome P. Ferrance[a,*]

[a]*University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904, USA*
[b]*Institute of Clinical Biochemistry, Rikshospitalet, 0027 Oslo, Norway*
[c]*University of Virginia Health Science Center, Department of Pathology, Charlottesville, VA 22908, USA*

**Abstract**

Galactosemia, a metabolic disorder associated with the intolerance to dietary galactose due to an inherited enzymatic deficiency, is indicated by heightened levels of galactose in urine (galactosuria). In this report, capillary electrophoresis (CE) with laser-induced fluorescence detection was evaluated for its ability to screen urinary carbohydrates, particularly galactose. Neonatal urine samples with normal and abnormal levels of galactose were analyzed with galactose concentrations quantitated relative to urinary creatinine concentrations to account for variable urinary dilution. Analysis of nine samples by CE in a single-blind manner defined four as negative (normal) and five as positive for galactosuria with galactose levels as high as 146.8±5.9 m*M*. Galactosuria was correlated with clinical galactosemia diagnoses for four of the positive samples, while the remaining positive was associated with a patient diagnosed with Hurler's syndrome.


*Keywords:* Galactosemia; Aminopyrene trisulfonate; Galactose; Carbohydrates

## 1. Introduction

### 1.1. Galactosemia

The toxic syndrome, galactosemia, is associated with an intolerance to dietary galactose as a result of certain enzymatic deficiencies. Malfunctions in the following three enzymes, which participate in the normal metabolism of galactose (Fig. 1), are known to be causes of galactosemia: galactose-1-phosphate uridyltransferase (GALT) (3), galactokinase (2), and uridine diphosphate-galactose-4-epimerase (4) [1]. In-born errors of metabolism result in defects in these enzymes that are categorized as Type I (classical), Type II, and Type III galactosemias, respectively (Table 1). Galactose-1-phosphate a metabolite derived from ingestion of galactose, is considered to be toxic in several tissues—particularly the liver, brain, and renal tubules [2]. Provided the disease goes unrecognized, galactose-1-phosphate accumulates within the cells of these tissues upon ingestion of milk or other sources of galactose. A toxic syndrome develops which leads to failure to thrive, liver disease, cataracts, mental retardation, or death. Additionally, females may exhibit ovarian failure in later stages. It is critical that diagnosis of this disease

*Corresponding author. Tel.: +1-434-243-8801; fax: +1-434-982-3048.
*E-mail address:* jpf3p@virginia.edu (J.P. Ferrance).
[1]Present address: Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221, USA.

0021-9673/03/$ – see front matter 
doi:10.1016/S0021-9673(03)00767-2

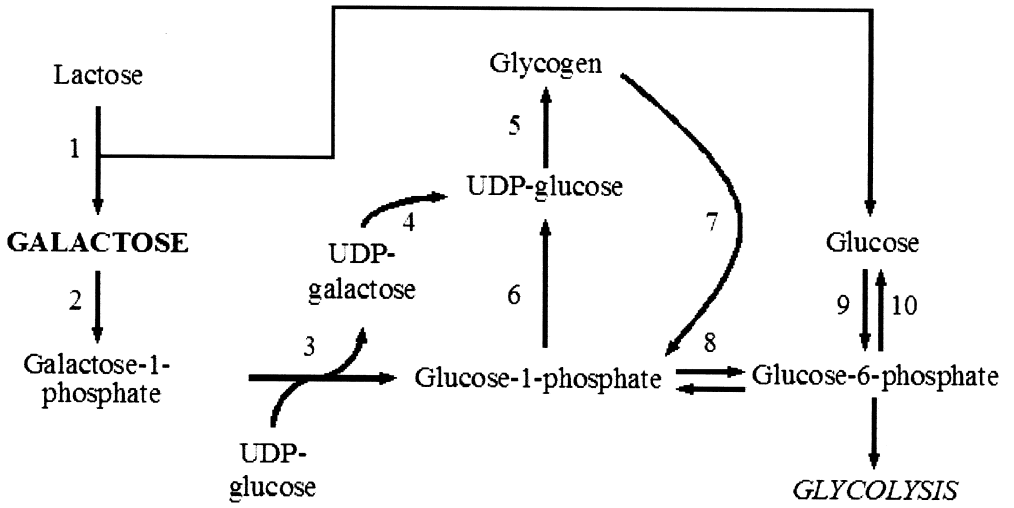


Fig. 1. Major steps in the intermediary metabolism of galactose [1]. The numbers shown refer to the following enzymes: β-galactosidase (1), galactokinase (2), galactose-1-phosphate-uridyl transferase (GALT) (3), UDP-galactose-4-epimerase (4), glycogen synthetase (5), UDP-glucose pyrophosphorylase (6), glycogen phosphorylase (7), phosphoglucomutase (8), glucokinase/hexokinase (9), glucose-6-phosphatase (10). The enzymes associated with galactosemia are specified in Table 1.

be accomplished early, since postponing treatment beyond the first few weeks of life can lead to rapid manifestation of the disease [3]. The only treatment available is to simply eliminate galactose from the patient's diet at an early age (neonatal period) and continue this diet for the remainder of the patient's life. This allows for relatively normal development of the diseased subject [4] and helps to prevent irreversible changes [1]. The incidence of the disorder is approximately 1 in 60 000 [5,6].

Galactose-1-phosphate uridyltransferase (EC 2.7.7.12) catalyzes the conversion of galactose-1-phosphate and UDP-glucose to UDP-galactose and glucose-1-phosphate. GALT deficiencies (Type I galactosemia) result in elevation of blood galactose, high tissue levels of toxic galactose-1-phosphate, and increased urine galactose excretion [7]. As with most inborn errors in carbohydrate metabolism, this disorder is inherited as an autosomal recessive trait [1]. Lactose, the disaccharide found in milk, is metabolized to glucose and galactose in the body, thus neonates with this form of the disorder exhibit vomiting and signs of liver disease a few days after milk ingestion.

Table 1
Inborn errors of galactose metabolism. Information taken from Burtis and Ashwood [1]

| Enzyme deficiency | Disease state |
|---|---|
| Galactose-1-phosphate-uridyl transferase | Type I Galactosemia |
| Galactokinase | Type II Galactosemia |
| UDP-Galactose-4-epimerase | Type III Galactosemia |

Galactokinase (EC 2.7.1.6) deficiency (Type II) is manifested predominantly by cataracts due to galactitol deposits in the lens and is a milder condition than Type I [1]. This enzyme catalyzes the transfer of phosphate from ATP to galactose. Type II is analogous to Type I in that it is an inherited autosomal recessive disorder and can be identified by an increased excretion of galactose in urine [7]. Uridine diphosphate-galactose-4-epimerase (EC 5.1.3.2) deficiency (Type III) is a rare and usually benign form of the disease that—in most cases—is also associated with increased levels of urinary galactose. This enzyme catalyzes conversion of UDP-glucose to UDP-galactose [7].

### *1.2. Current neonatal screening*

Currently, the most common methods for galactosemia screening utilize blood samples dried onto filter paper. The Beutler enzyme spot test is a traditional method that monitors GALT activity with

the aid of phosphoglucomutase and glucose-6-phosphate dehydrogenase with visualization of the fluorescence of reduced $NADP^+$ under ultraviolet light [8]. The Beutler test is independent of galactose exposure and substrate accumulation, but deactivation of GALT in dried blood spots due to humidity or temperature typically leads to false positive diagnoses [9,10]. Furthermore, this test is specific for the Type I form of the disease, and visual evaluation is necessary. Another popular screening technique, described by Paigen et al. [11], is quantitation of galactose and galactose-1-phosphate by a microbiological assay. This method is sensitive to antibiotic treatment of newborns or their mothers and is not suitable for automation [3]. A combination of the Beutler and Paigen assays is currently used by a number of neonatal screening laboratories [12]. The National Newborn Screening and Genetics Resource Center (NNSGRC) reports that most states in the USA, with parental consent, perform galactosemia screening of all newborns as a routine procedure. The screening protocols, as administered by each individual state, typically use the fluorescent testing procedure, and may include supplementary microbiological techniques [13].

The third frequently used method for galatosemia diagnosis is an alkaline phosphatase-galactose dehydrogenase assay [14–18], which quantifies galactose and galactose-1-phosphate in blood spots. This approach is time-consuming and requires multiple reagents in large quantities, however, and it often yields a high number of false positive results [19]. A number of new methodologies for galactosemia screening through blood have recently been investigated. These include a stable-isotope dilution method [20], a modified Beutler enzyme spot test for newborn mass screening using a fluorometric microplate reader [21], tandem mass spectrometry detection of total hexose monophosphates as a marker of galactose-1-phosphate [3], and gas chromatography–mass spectrometry (GC–MS) monitoring of D-galactose-1-phosphate in erythrocytes of patients [22].

The demand exists for a rapid, high throughput neonatal screening method due to the early on-set and permanently damaging effects of galactosemia. As described, several methods have been developed for galactosemia screening of blood samples, but these current methods typically consume large amounts of time and reagents and are not always reliable. As mentioned above, excess galactose in human urine is indicative of galactosemia but urinary screening of this disorder is not common practice. Galactosuria will be present in all types of galactosemia, thus galactose detection in urine could be used as a general screening method, with further analysis performed for specific disease diagnosis. In addition, other disease states which result in elevated levels of galactose in urine could be detected using a method for urine analysis. Furthermore, the use of urine instead of blood samples from neonates provides a less invasive route for both screening and continued monitoring of the disease. It is important to mention, however, that the screening proposed here must be carried out after the first few days of milk feeding in order to reliably diagnose galactosuria.

### *1.3. Screening with capillary electrophoresis (CE)*

CE has been reported as a viable method for urinary diagnoses of metabolic disorders [23,24] and has enormous potential for rapid, high-throughput testing. The utility of CE for laboratory analyses of biological matrices (e.g., blood and urine) relies on its inherent simplicity, small sample requirements, speed, and low reagent consumption. In addition, a number of CE methods for carbohydrate analysis have been reported [25,26]. Progress is also being made in the field of microfluidics providing further increases in the speed of analysis and the potential for integration of sample handling steps [27,28]. Recent applications of microchip CE for carbohydrate analysis have already been reported [29,30].

A CE method using laser-induced fluorescence (LIF) detection for the screening of urinary carbohydrates, including D-galactose, has been demonstrated by Jin and Li [25], as an improvement over previously used thin-layer and paper chromatographic methods. This method relied on fluorescent labeling of carbohydrates using 9-aminopyrene-1,4,6-trisulfonate (APTS), and showed separation of mono- and disaccharide carbohydrate standards of interest for urinary carbohydrate analysis within 40 min. Carbohydrates detected in urine from a normal subject with this method all fell within the acceptable ranges.

This report details a further investigation of this

CE methodology for the screening of carbohydrates in urine, particularly D-galactose. Fluorescently-labeled carbohydrates were separated on shorter capillaries than previously investigated [25], and neonatal urine samples were analyzed and evaluated to demonstrate the utility of the method. The approach was found to be effective in identifying galactosuria and correlated well with previous galactosemia diagnoses. Again, this screening method should be advantageous since all three varieties of galactosemia manifest increased D-galactose in urine.

## 2. Experimental

### 2.1. Chemicals

Carbohydrates were purchased either from Fluka (Buchs, Switzerland) or Sigma (St. Louis, MO, USA). APTS was obtained from Lambda Fluoreszenztechnologie (Graz, Austria). Sodium cyanoborohydride ($NaBH_3CN$) (1.0 *M* solution in tetrahydrofuran) was acquired from Aldrich (Milwaukee, WI, USA). Sodium borate ($Na_2B_4O_7{\cdot}10H_2O$) obtained from Fisher (Fair Lawn, NJ, USA) was used to prepare a 135 m*M* borate buffer, adjusted to pH 10.2 using NaOH, and used for all separations unless otherwise indicated. Carbohydrates were dissolved in water at 20 m*M* and stored in small aliquots at −20 °C. Urine samples were obtained in a single-blind fashion from the Institute of Clinical Biochemistry at the National University Hospital (Rikshospitalet), University of Oslo, Norway.

### 2.2. CE separations

A Beckman P/ACE 2100 (Beckman Instrument, Fullerton, CA, USA) equipped with an LIF detector employing an argon-ion laser was used for CE with fluorescence detection (excitation 488 nm/emission 520 nm). A bare silica capillary of 20 μm I.D. was purchased from Polymicro Technologies (Phoenix, AZ, USA) and cut to 27 cm lengths (20 cm effective length). The new capillary was conditioned using a high-pressure rinse (20 p.s.i.; 1 p.s.i.=6894.76 Pa) of 1.0 *M* NaOH for 15 min followed by separation buffer for 30 min. Prior to each separation, a 2.0 min NaOH rinse was performed followed by a 4.0-min rinse of separation buffer. Each sample was injected into the capillary with a 5 s low-pressure injection (0.5 p.s.i.) followed by electrophoretic separation at 25 kV.

### 2.3. Sample preparation

Derivatization of samples followed the procedure described previously [25]. A standard solution was prepared consisting of 2 m*M* of the following carbohydrates: maltose, lactose, D-mannose, D-glucose, D-ribose, D-xylose, L-arabinose, and D-galactose. In a nitrogen atmosphere maintained via a glove box, 10 μl of this standard (0.16 μmol of each carbohydrate) was derivatized by addition of 2 μl of 0.1 *M* APTS in 4.2 *M* acetic acid (0.2 μmol APTS) along with 4 μl of 1.0 *M* $NaBH_3CN$ solution in tetrahydrofuran (4.0 μmol $NaBH_3CN$). Reductive amination of the eight carbohydrates was allowed to proceed at 75 °C for 1 h using a water bath. To prevent oxygen exposure, Eppendorf Safe-Lock polypropylene vials (Fisher) were used for all labeling reactions and were sealed before removal from the nitrogen atmosphere. The product (16 μl volume, 1.25 m*M* carbohydrates) was then diluted appropriately depending on the desired concentration of standard for calibration. For the urine samples, proteins were first precipitated out using acetonitrile (1:1) followed by centrifugation at 1000 rpm for 1.5 min. The supernatant was saved for analysis, while the precipitate was discarded. Two 10-μl aliquots of the treated urine were then used in the analysis. One aliquot was spiked with 100 μ*M* D-galactose for reliable peak identification while the other was unaltered for quantitation. They were both derivatized with APTS in the same manner as the standard solutions for quantitation of D-galactose. To ease the handling of such small volumes (16 μl after labeling), each sample was diluted further with water to a total of 40 μl. The extraction (acetonitrile–sample, 1:1), labeling (APTS–$NaBH_3CN$–sample, 2:4:10), and further dilution (water–sample, 24:16) of the samples resulted in a total dilution of 8:1 (additives–sample) of the carbohydrates in the original urine; additional dilutions were carried out when necessary.

### 2.4. Calibration and sample quantitation

Calibration of D-galactose concentrations was obtained by fluorescent labeling of the standard solutions followed by separation under the conditions described above. The labeled standard solution was diluted to the desired concentration based on the 1.25 m*M* post-labeling carbohydrate concentrations. For each concentration a separation was executed and recorded in triplicate. The electropherograms were analyzed and galactose peak areas were determined using the Beckman software (System Gold V810). This data was used to generate a calibration curve.

Quantitation of galactose in neonatal urine samples was determined by correlating the galactose peak areas—obtained from the respective electropherograms—with the calibration curve. The creatinine level of each sample (provided with the urine samples) was determined using the classical Jaffe reaction.

## 3. Results and discussion

### 3.1. Separation of carbohydrate standards in short capillaries

Separation of eight clinically-important carbohydrates including (in order of migration) maltose, lactose, D-mannose, D-glucose, D-ribose, D-xylose, L-arabinose, and D-galactose was previously achieved with 130 m*M* borate buffer at pH 10.2 using a 59 cm (47 cm effective length)×25 μm I.D. capillary [25]. The migration order was determined by spiking experiments, but the electrophoresis was completed in what could be considered a lengthy analysis time by CE standards (40 min). To reduce the migration time, the use of shorter capillaries was proposed but not demonstrated due to instrument limitations. In this study, the use of a shorter—27 cm (20 cm effective length)—capillary was possible with the commercial instrumentation employed. In addition, the diameter of the capillary was reduced from 25 to 20 μm to allow higher field strengths.

The separation method relies on the tendency of borate to form complexes with *cis*-oriented hydroxyl groups as a logical approach for separating carbohydrates of similar or equal molecular mass using borate buffers [31]; this approach has been used for the resolution of both free and derivatized sugars [26]. The ionic strength of the borate buffer plays an important role in the resolution of all eight carbohydrates. In the previous work, 130 m*M* borate buffer effectively separated all of the standard peaks. With the shorter capillary, we found that the glucose and ribose peaks were not baseline resolved in 130 m*M* borate (pH 10.2), but an increase of ionic strength from 130 to 135 m*M* rendered the pair baseline-resolved. This buffer allowed analysis of the eight carbohydrates in the standard solution in approximately 10 min with an applied electric field of 926 V/cm. These optimized separation conditions were consistent with those previously published [32] and were used for all subsequent separations of calibration standards and clinical samples. A representative electropherogram showing separation of the standard carbohydrates is given in Fig. 2. It is of interest to note that the migration time could be reduced from 40 min to less than 10 min without compromising the resolution.

### 3.2. Quantitation of D-galactose in neonatal urine samples

According to previous studies, the APTS labeling efficiency is essentially 100% when APTS and $NaBH_3CN$ are kept in excess of the target carbohydrates [33]. Therefore, it should be possible to obtain accurate quantitation based on peak areas to determine the amount of galactose present in each sample. A calibration curve generated using triplicate analyses of six galactose concentrations between 1 and 500 μ*M* indicated a sensitivity of 13.11 $\mu M^{-1}$ and a correlation coefficient of 0.9967. The response was found to be linear in the concentration range from 1 to 500 μ*M*; concentrations exceeding 500 μ*M* resulted in a loss of resolution. Resolving these higher concentrations demanded an increase in run buffer concentration and, consequently, an increase in total analysis time. Instead, samples with galactose concentrations above this limit were diluted to allow accurate quantitation using the established calibration curve. The limit of detection (LOD) for D-galactose in this analysis was determined by decreasing the D-galactose concentration until a peak having a $S/N$ greater than 3 was still detectable. The concentration

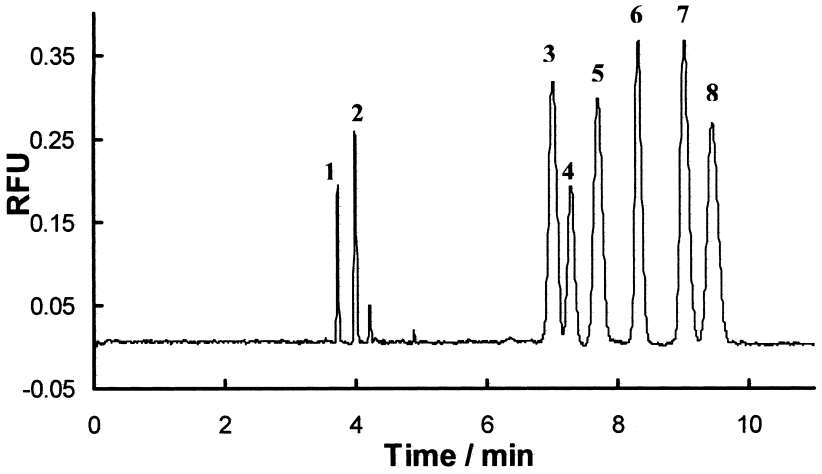


Fig. 2. Standard separation of eight clinically relevant carbohydrates—maltose (1), lactose (2), mannose (3), glucose (4), ribose (5), xylose (6), arabinose (7), and galactose (8)—all at concentrations of 200 μ*M*. Galactose (8), which migrated past the detector in less than 10 min, is the target peak for quantitation. Separation was performed in a 27 cm (20 cm effective length) fused-silica capillary using 135 m*M* borate buffer at 926 V/cm, with the current stabilizing at about 30 μA.

limit was found to be 100 n*M*, well below the concentrations in normal and abnormal clinical samples.

Quantitation of D-galactose in urine from nine subjects was carried out following protein precipitation and labeling of the supernatant. Only 5 μl of urine was required for a single run, although more was typically used to ease the sample handling. The galactose concentrations for the samples are given in Table 2. It is apparent that the galactose concentration in urine can vary over a wide range particularly in patients with galactosuria, and significant dilution of these samples was usually necessary to accurately quantitate the concentration. In addition, the variations reported for the concentrations indicate a difficulty in quantitating galactose concentration when low levels (<50 μ*M*) are present in urine. This is due to the migration times for the carbohydrates in the urine sample being different than those for the standard solutions, with the galactose peak in the majority of samples not being completely resolved from the arabinose peak (Fig. 3). This decreased resolution required the use of a spiking step to reliably differentiate between the arabinose and galactose peaks and contributed to the problem of low galactose level determination. Analysis of a

Table 2
Results of CE analysis and comparison with the conventional method

| Sample label | Galactose (μ*M*) | Creatinine (m*M*) | GAL/CRN ratio | CE diagnosis | Conventional diagnosis |
|---|---|---|---|---|---|
| 1K | 87.0±1.1 | 0.70 | 124.3 | − | − |
| 2K* | 253.2±15.7 | 0.42 | 604.4 | + | − |
| 3G | 47 360±260 | 0.60 | 78 930 | + | + |
| 89 | 146 800±5900 | 0.60 | 244 700 | + | + |
| 92 | 35 110±530 | 1.0 | 35 110 | + | + |
| 95 | 25.8±34.5 | 0.30 | 86.0 | − | − |
| 96A | 118.0±2.3 | 1.6 | 73.8 | − | − |
| 96B | 123.6±13.8 | 0.10 | 1236 | + | + |
| 96C | 1.6±64.4 | 12.2 | 0.13 | − | − |

All diagnoses matched with the exception of sample 2K, which had tested positive for Hurler's syndrome.
* Patient known to have Hurler's syndrome.

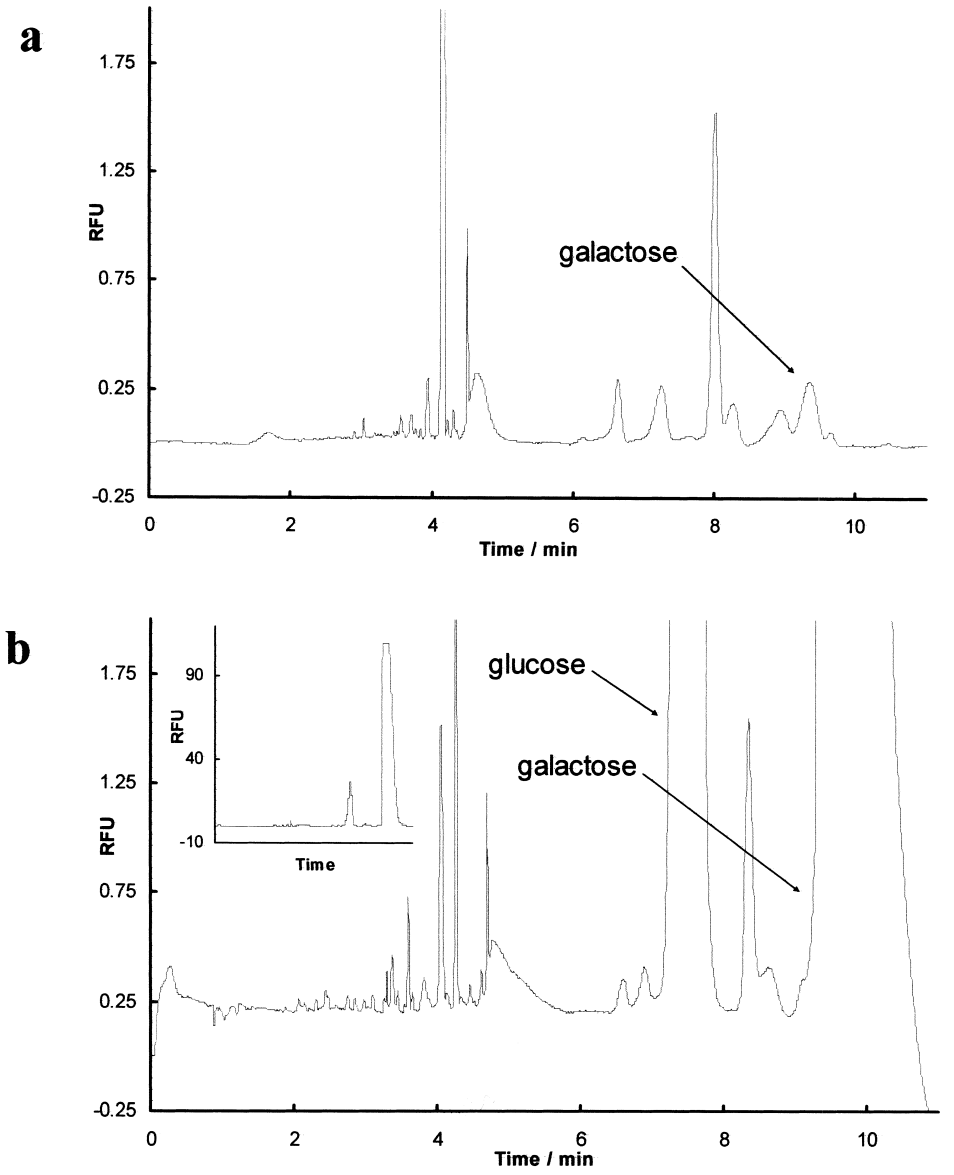


Fig. 3. Exemplary electropherograms of neonatal urine samples that were diagnosed as (a) negative sample (1K) and (b) positive sample (3G) for galactosemia based on the presence of galactosuria. These samples allow easy comparison as they have similar creatinine levels (0.7 and 0.6 m*M*, respectively). The inset shows the abnormally high level of urinary galactose in this sample. As seen from both the inset and the figure, this patient also showed elevated levels of urinary glucose.

spiked aliquot of each sample in addition to the original sample obviously increased the time of analysis, but ensured accurate selection of the galactose peak. The migration time changes and loss of resolution are attributed to variations in the concentrations of salts and other interfering components inherent to biological samples such as the urine analyzed here.

The urinary galactose concentrations listed in Table 2 are not clinically relevant alone. Dilution of urine is variable from person to person, thus diagnoses are made based on galactose/creatinine ratios.

Since it is endogenously produced and released into bodily fluids at a constant rate [34], creatinine concentrations are often used as a reference value for urinary metabolites. The creatinine levels of each urine sample were obtained at the point of sample collection (University of Oslo) before being forwarded for galactose analysis. These were determined using the classical Jaffe reaction and are also presented in Table 2. The galactose concentration in each sample was then ''normalized'' to account for variable urine dilution using its respective creatinine concentration to generate the galactose/creatinine (GAL/CRN) ratio values listed in Table 2.

The expected ratio for infants not afflicted with galactosemia is 0–377 mmol galactose per mol creatinine. Any sample with a ratio outside this range based on the CE analysis indicated galactosuria and was considered ''positive'' for galactosemia. The diagnoses obtained using this method and the original diagnoses are included in Table 2. Of the nine samples analyzed, five were found to have abnormally high galactose levels, and thus were diagnosed as positive for galactosemia, while the other four were consistent with a negative diagnosis. This agreed with the conventional diagnoses for these samples except for urine sample 2K, obtained from a patient without galactosemia, but showing elevated galactose levels in our analysis.

This abnormal sample presented an interesting result in that the galactose concentration was only mildly elevated above the normal level, compared with the galactosemia patients whose levels were significantly elevated. It was revealed after the galactose analysis that this patient was previously diagnosed with Hurler's syndrome (mucopolysaccharidosis type 1 h), a lysosomal storage disease resulting from the deficiency of the enzyme α-L-iduronidase [1]. Several studies have indicated a link between this disease and heightened levels of galactose—and other monosaccharides—in bodily fluids, including urine [35,36]. The galactosuria observed in our analysis of sample 2K was, therefore, attributable to the Hurler's syndrome rather than galactosemia. This particular syndrome normally does not exhibit symptoms until 4 months after birth, while galactosemia can be diagnosed after the first few days of feeding. Additionally, the disease is generally manifested by symptoms dissimilar to galactosemia such as corneal opacities, coarse facies, stiff joints, and dwarfing [1]; and it results in amplified levels of dermatan and heparan sulfates in urine [5]. It is, therefore, improbable that galactosemia would be mistaken for Hurler's syndrome (or vice versa) in a hospital or clinical laboratory.

CE has also been shown to be a dependable and more rapid alternative for creatinine quantitation in urine using UV detection [24,37–39]. This imparts the possibility that CE could serve as the sole method for galactosemia screening based on the GAL/CRN ratio, eliminating the need for additional more complicated methods such as the Jaffe reaction. This also presents the possibility of moving both analyses to a microchip based method where a single step would allow accurate detection of elevated levels of galactose in urine. While this approach will not specifically indicate galactosemia, the requirement of additional testing can easily be based on this screening method.

## 4. Conclusion

We have shown that CE with LIF detection has the potential to serve as a rapid, high throughput technique with low sample requirements for neonatal screening of the metabolic disorder galactosemia. Particularly appealing advantages of CE over the current techniques is the speed of analysis and the potential throughput. Urinary analysis is less invasive than the typical heel prick required for the blood spot based analyses currently performed, and CE requires a minimal volume for a dependable diagnosis (5 μl of urine for a single run). The method presented significantly reduced the separation time of clinically-relevant carbohydrates and the technique could reliably be applied to clinical samples taken after the first few days of feeding. Out of nine neonatal urine samples analyzed, the only inconsistency with the original diagnoses came from a sample from a patient previously diagnosed with Hurler's syndrome. This shows the ability of this screening method to identify additional abnormalities which would then require further testing to confirm. These results indicate that CE has the quantitative capacity to serve as a more rapid and less costly

alternative for carbohydrate screening of urine for galactosemia diagnosis.

## Acknowledgements

The authors would like to express their thanks to Braden Giordano and Paul Mangold for their invaluable contributions to this work.

## References

[1] C.A. Burtis, E.R. Ashwood, Tietz Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, PA, 1999.
[2] J.B. Holton, J.H. Walter, L.A. Tyfield, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001, p. 1553.
[3] U.G. Jensen, N.J. Brandt, E. Christensen, F. Skovby, B. Norgaard-Pedersen, H. Simonsen, Clin. Chem. 47 (2001) 1364.
[4] R. Koch, P. Acosta, G.N. Donnell, Clin. Pediatr. 4 (1965) 571.
[5] L.A. Kaplan, A.J. Pesce, in: L.A. Kaplan, A.J. Pesce (Eds.), Clinical Chemistry, C.V. Mosby, St. Louis, MO, 1984.
[6] J.R. Sobenes, J.E. Sherwin, in: R.C. Telton, A. Balows, D.C. Hohnade, R.F. Reks (Eds.), Clinical Laboratory Medicine, Mosby, London, 1992, p. 109.
[7] D. Webster, D. Allen, in: J. Bradford, L. Therrell (Eds.), Laboratory Methods for Neonatal Screening, American Public Health Association, Washington, DC, 1993, p. 77.
[8] E. Beutler, M.C. Baluda, J. Lab. Clin. Med. 68 (1966) 137.
[9] P. Frazier, G. Summer, J. Lab. Clin. Med. 83 (1974) 334.
[10] D.M. Frazier, E.H. Clemons, H.N. Kirkman, Biochem. Med. Metab. Biol. 48 (1992) 199.
[11] K. Paigen, F. Pacholec, H.L. Levy, J. Lab. Clin. Med. 99 (1982) 895.
[12] S. Schweitzer, Eur. J. Pediatr. 154 (1995) S37.
[13] National Newborn Screening and Genetics Resource Center, National Newborn Screening Report—2000, NNSGRC, Austin, TX, February 2003.
[14] A. Cantarow, B. Schepartz, Carbohydrate Metabolism, W.B. Saunders, Philadelphia, PA, 1957.
[15] G.A. Mason, G.K. Summer, H.H. Dutton, R.C. Schwaner, Clin. Chem. 13 (1977) 971.
[16] A. Grenier, A. Laberge, Clin. Chem. 19 (1973) 463.
[17] A.P. Orfanos, D.C. Jinks, R. Guthrie, Clin. Biochem. 19 (1986) 225.
[18] G.L. Hoffman, R.H. Laessig, D.J. Hassemer, E.R. Makowski, Clin. Chem. 30 (1984) 287.
[19] C. Item, B.P. Hagerty, A. Muhl, S. Greber-Platzer, S. Stockler-Ipsiroglu, W. Strobl, Pediatr. Res. 51 (2002) 511.
[20] P. Schadewaldt, H.-W. Hammen, K. Loganathan, A. Bodner-Leidecker, U. Wendel, Clin. Chem. 46 (2000) 612.
[21] A. Fujimoto, Y. Okano, T. Miyagi, G. Isshiki, T. Oura, Clin. Chem. 46 (2000) 806.
[22] J. Chen, C. Yager, R. Reynolds, M. Palmieri, S. Segal, Clin. Chem. 48 (2002) 604.
[23] E. Jellum, H. Dollekamp, A. Brunsvig, R. Gislefoss, J. Chromatogr. B 689 (1997) 155.
[24] K.B. Presto Elgstoen, E. Jellum, Electrophoresis 18 (1997) 1857.
[25] L.J. Jin, S.F.Y. Li, Electrophoresis 20 (1999) 3450.
[26] J.D. Olechno, J.A. Nolan, in: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, New York, 1997, p. 297.
[27] C.L. Colyer, T. Tang, N. Chiem, D.J. Harrison, Electrophoresis 18 (1997) 1733.
[28] L.J. Jin, J. Ferrance, J.P. Landers, BioTechniques 31 (2001) 1332.
[29] J.C. Fanguy, C.S. Henry, Analyst 127 (2002) 1021.
[30] S. Honda, S. Suzuki, A. Taga, J. Pharm. Biomed. Anal. 30 (2003) 1689.
[31] J.P. Landers, R.P. Oda, M.D. Schuchard, Anal. Chem. 64 (1992) 2846.
[32] F.T. Chen, R.A. Evangelista, Anal. Biochem. 230 (1995) 273.
[33] M.-S.L. Ramon, A. Evangelista, F.-T.A. Chen, Anal. Chem. 67 (1995) 2239.
[34] D.J. Newman, C.P. Price, in: C.A. Burtis, E.R. Ashwood (Eds.), Tietz Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, PA, 1999, p. 1241.
[35] W.V. Robertson, J. Harvey, Biochem. Med. 6 (1972) 246.
[36] T. Faraggiana, S. Shen, C. Childs, L. Strauss, J. Churg, Histochem. J. 14 (1982) 655.
[37] H. Shi, Y. Ma, Y. Ma, Anal. Chim. Acta 312 (1995) 79.
[38] D.G. Burke, P.G. MacLean, R.A. Walker, P.J. Dewar, T. Smith-Palmer, J. Chromatogr. B 732 (1999) 479.
[39] R. Gatti, V. Lazzarotto, C.B.D. Palo, E. Cappellin, P. Spinella, E.F.D. Palo, Electrophoresis 20 (1999) 2917.