ELSEVIER

Journal of Chromatography B, 754 (2001) 217–228

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Separation of free amino acids in human plasma by capillary electrophoresis with laser induced fluorescence: potential for emergency diagnosis of inborn errors of metabolism

Olivier Boulat[a,*], David G. McLaren[a], Edgar A. Arriaga[b], David D.Y. Chen[a,1]

[a]*Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1*
[b]*Department of Chemistry, University of Minnesota, Minneapolis, MN 55455-0431, USA*

Received 4 July 2000; received in revised form 23 November 2000; accepted 30 November 2000

Abstract

Free amino acids (AAs) in human plasma are derivatized with 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA) and analyzed by capillary electrophoresis (CE) with laser induced fluorescence (LIF) detection. The labeling procedure is significantly improved over results reported previously. Derivatization can be completed in 40 min, with concentrations as low as 4×10^{-8} *M* successfully labeled in favourable cases. Twenty-nine AAs (including 2 internal standards) are identified and can be reproducibly separated in 70 min. Migration time RSD values for 23 of these AAs were calculated and found in the range from 0.5 to 4%. The rapid derivatization procedure and the resolution obtained in the separation are sufficient for a semi-quantitative, emergency diagnosis of several inborn errors of metabolism (IEM). Amino acid profiles for both normal donor plasma samples and plasma samples of patients suffering from phenylketonuria, tyrosinemia, maple syrup urinary disease, hyperornithinemia, and citrullinemia are studied.

Keywords: Inborn errors; Amino acids

1. Introduction

Obtaining timely and reliable information on the amino acid (AA), organic acid (OA) and orotic acid (ORA) content of human biological samples (plasma, urine and cerebrospinal fluid (CSF)) is essential in the diagnosis of inborn errors of metabolism (IEM). Some IEM presenting in the neonatal period (i.e. neonatal metabolic distresses) are considered pediatric emergencies and are classified according to clinical types. More than 65% of neonatal metabolic distresses afflicting patients are due to one of the following diseases: maple syrup urinary disease (MSUD), organic acidurias (methylmalonic, propionic, isovaleric, glutaric type II, etc), urea cycle defects, β-oxidation defects, non-ketotic hyperglycinemia or tyrosinosis type I. These diseases

*Corresponding author. Permanent address: Clinical Chemistry Laboratory (LCC), Centre Hospitalier Universitaire Vaudois (CHUV), 1011 Lausanne, Switzerland. Tel.: +41-21-314-4150; fax: +41-21-314-4288.

E-mail addresses: olivier.boulat@chuv.hospvd.ch (O. Boulat), chen@chem.ubc.ca (D.D.Y. Chen).

[1]Corresponding author. Tel.: +1-604-822-0878; fax: +1-604-822-2847.

0378-4347/01/$ – see front matter
PII: S0378-4347(00)00611-3

are best diagnosed by AA, OA, ORA and acylcarnitine analysis [1].

At present, the biochemical diagnosis of IEM involves highly sophisticated methods utilizing tandem mass spectrometry (MS) (for acylcarnitine analysis), gas chromatography with MS detection (for OA and AA determination), ion-exchange chromatography (IEC) and HPLC (for AA and ORA analysis). However, simple, timely and reliable analysis procedures that can cope with the demands of medically emergent IEM cases are still needed. Ideally, methods developed to meet these needs should be designed so that they may be easily carried out on existing equipment in clinical laboratories to allow for a faster, more accurate and perhaps more frequent diagnosis of these pediatric emergencies.

Capillary electrophoresis (CE) is one of the most powerful separation techniques known to date, because both (electric) field and chemical equilibria are used in the separation process [2]. Methods have been developed for the determination of some OAs and ORA in urine using CE [3–6], and assays for acylcarnitine analysis are currently under investigation [7,8]. CE has also been used for the diagnosis of various metabolic diseases [4–10] and although these procedures could still be improved upon, they have the advantages of having short analysis times and the ability to be performed on standard apparatus (i.e. CE with UV detection). Although amino acids were among the first groups of compounds investigated by CE, this technique has not been widely applied to AA analysis in biological fluids because of the problems associated with detection and reproducibility. Furthermore, up to fifty AAs or AA conjugates must be routinely monitored in procedures designed for the diagnosis and follow up treatment of metabolic disorders [11,12]. Currently, only a few CE protocols have been established for biological AA analysis and these are limited to the quantification of particular sets of sulfur containing AAs [13,14]

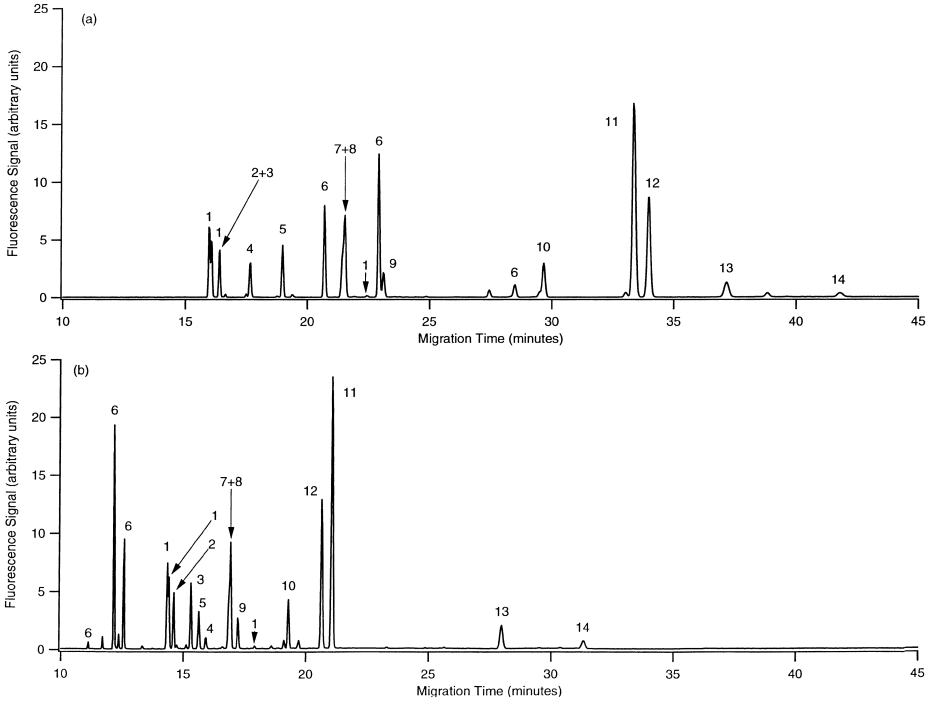

Fig. 1. Effect of γ-CD on the separation of a standard mix of 14 AAs. Peak identification: (1) Met; (2) Gln; (3) 3-CH_3-His; (4) Ser; (5) Tyr; (6) Lys; (7) Gly; (8) Ala; (9) Val; (10) Ile; (11) Leu; (12) Phe; (13) Glu; (14) Asp. CE parameters: BGE: 160 m*M* borate, 130 m*M* SDS, pH 9.5; Part (a) without γ-CD; Part (b) with 7.5 m*M* γ-CD. Capillary: 67 cm (60 cm to detector), 50 μm I.D. 30 kV.

useful in the assessment of homocysteine as a risk factor for cardiovascular diseases. To date, no CE procedures have been published for AA analysis in plasma or urine that are feasible for the determination of any of the IEM emergency cases (which include such ailments as MSUD, urea cycle defects, non-ketotic hyperglycinemia and tyrosinosis) [15].

In order to detect AAs in the levels that are present in biological fluids, very sensitive detection methods such as laser-induced fluorescence (LIF) are required. A large number of fluorescent labels are available for use in tagging free AAs [16–19] and a judicious choice can help minimize complications associated with excess labeling reagent and slow reaction rates. 3-(4-Carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA) is a commercially available fluorogenic reagent, which produces stable isoindole derivatives when reacted with primary amines. CBQCA labeling is advantageous when used for the analysis of AAs and peptides because of the short reaction times and the fact that unreacted CBQCA is non-fluorescent. CBQCA labeled amino acids (CBQCA-AAs) can be readily excited by either the 488 nm line of an Ar ion laser [20] or the 442 line of a He/Cd laser [21], with the maximum excitation of CBQCA-glycine having been found to be 466 nm [20]. Although CBQCA cannot be used to label secondary amines, its ability to tag primary amines makes it extremely well suited to the analysis of free AAs in biological fluids [20,21] and CE-LIF analysis of AAs in CSF utilizing a 2-h CBQCA derivatization process has been reported [22].

In this paper we describe the separation of CBQCA-labeled AAs in human plasma using CE

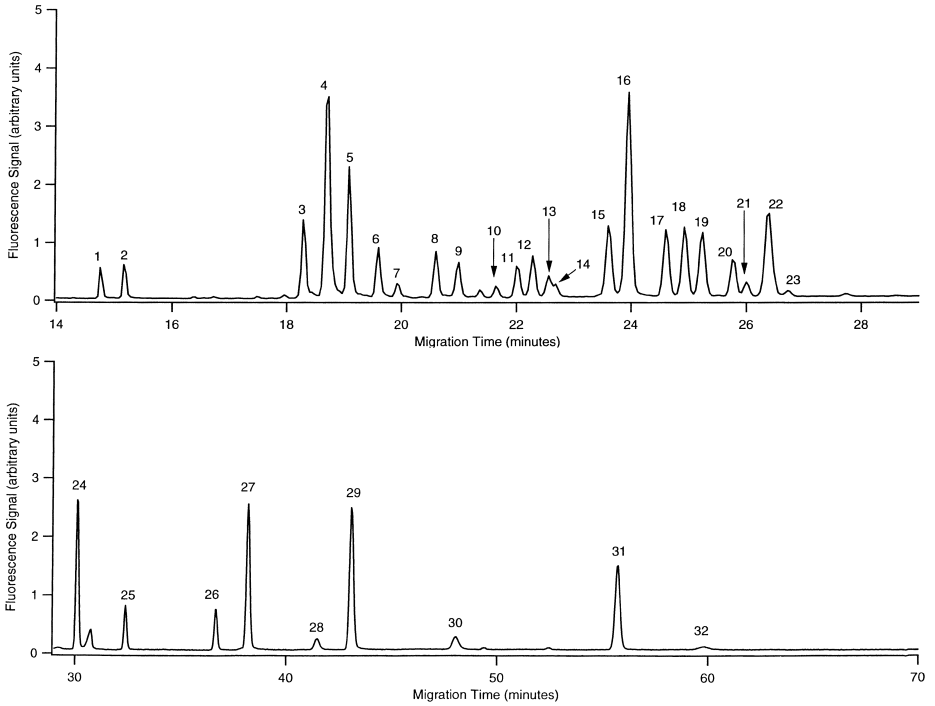

Fig. 2. Separation of 29 AAs in a mix of AAs standards. All AAs are at 100 μ*M* except *=30 μ*M*, **=60 μ*M* and ***=200 μ*M*. Peak identification: (1 and 2): Lys; (3): 1-CH_3-His: (4): Cit; (5): Gln; (6): Ans*; (7): Asn**; (8): 3-CH_3-His; (9): Tyr; (10): Orn; (11): β-Ala*; (12): Car*; (13): $(Hcy)_2$; (14): Ser; (15): Gly**; (16): Ala; (17): D-Abu; (18): Tau**; (19): L-Abu; (20): Val; (21): $(Hcy)_2$; (22): Met; (23): Thr; (24): N-Val; (25): Ile; (26): Phe; (27): Leu; (28): Aad; (29): N-Leu; (30): Glu***; (31): Arg***; (32): Asp***. CE Parameters: BGE:160 m*M* borate, 130 m*M* SDS, 7.5 m*M* γ-CD and 20 m*M* NaCl at a pH of 9.5; capillary: 67 cm length (60 cm to detector) and 50 μm I.D. 30 kV. Current: ca. 150 μA.

with LIF detection and report on the feasibility of applying this type of technique to the emergency diagnosis of inborn errors of metabolism.

2. Experimental

2.1. Chemicals

Borax, sodium dodecyl sulfate (SDS), γ-cyclodextrin (γ-CD) and HPLC grade methanol were purchased from Sigma (Sigma–Aldrich, Oakville ON, Canada); NaOH and HCl were from BDH Chemicals (Toronto, ON, Canada); sulfosalicylic acid (SSA) from Aldrich (Sigma Aldrich, Buchs, Switzerland); 3-(4-carboxybenzoyl)quinoline-2-carboxyaldehyde (CBQCA) and potassium cyanide (KCN) were obtained from Molecular Probes (Molecular Probes, Eugene OR, USA).

1-methylhistidine (1-CH_3-His), 3-methylhistidine (3-CH_3-His), L-alanine (Ala), β-alanine (β-Ala), L-alloisoleucine (Aile), L-α-aminoadipic acid (Aad), DL-β-aminoisobutyric acid (Baib), L-anserine (Ans), L-arginine (Arg), Argininosuccinate (ASA), L-asparagine (Asn), L-aspartic acid (Asp), L-cysteine ($(Cys)_2$), γ-aminobutyric acid (GABA), d-glucosaminic acid (d-gluc), L-glutamic acid (Glu), L-glutamine (Gln), L-glycine (Gly), L-histidine (His), L-homoarginine (Har), L-homocarnosine (Hcar), hydroxyproline (OH-Pro), L-isoleucine (Ile), kynurenine (Kyn), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-norleucine (N-Leu), L-norvaline (N-Val), L-ornithine (Orn), L-phenylalanine (Phe), phosphoethanolamine (Pea), DL-pipecolic acid (Pip), L-proline (Pro), L-serine (Ser), sarcosine (Sar), L-serine (Ser), taurine (Tau), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr) and L-valine (Val) were all purchased from Sigma (Sigma Chemie

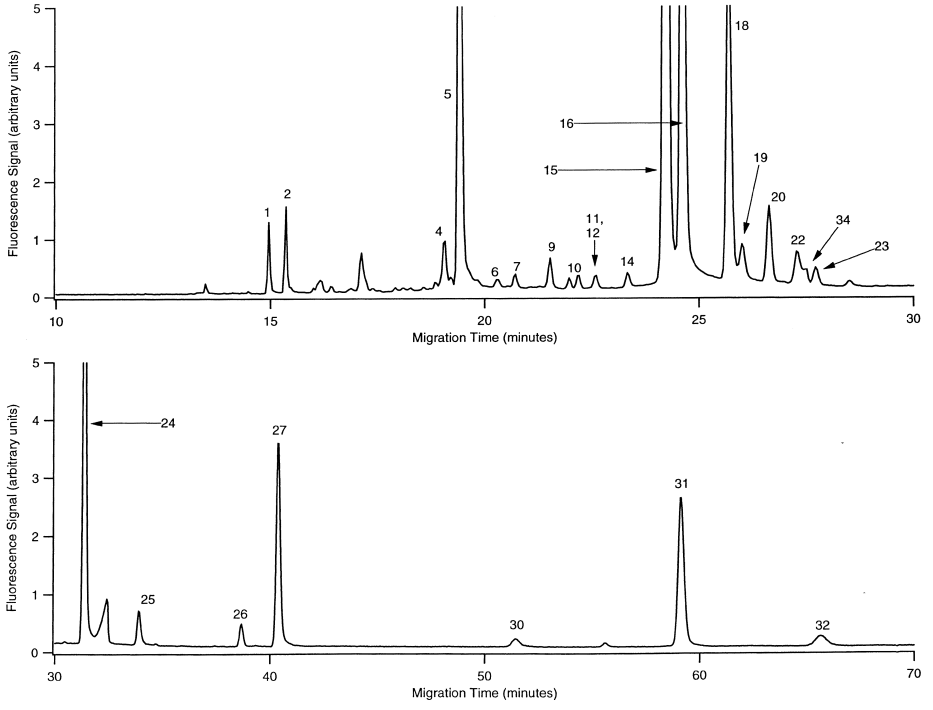

Fig. 3. Separation of CBQCA-AAs in a plasma sample of a child not afflicted with IEM. The sample has been spiked with 200 μ*M* Arg and 400 μ*M* Asp. Peak identification and CE parameters as in Fig. 2. Concentrations (μ*M*) in plasma as measured by IEC: Lys: 184; Cit: 18; Gln: 530; Asn: 155; Tyr: 57; Orn: 97; Ser: 121; Gly: 255; Ala: 513; Tau: 87; L-Abu: 23; Val: 205; Met: 29; Thr: 182; Ile: 71; Leu: 123; Glu: 49; Arg: 53; Asp: 7.

Switzerland) and used without further purification. L-homocystine ($(Hcy)_2$), L-citrulline (Cit), carnosine (Car) were obtained from Fluka (Sigma Aldrich, Buchs, Switzerland); DL-α-amino-butyric acid (ABU) from Aldrich (Sigma Aldrich, Buchs, Switzerland), L-homocitrulline (Hci) from ICN Biomedicals (Aurora, Ohio, USA).

An aqueous background electrolyte (BGE), consisting of 160 m*M* Borate (40 m*M* Borax), 130 m*M* SDS, 20 m*M* NaCl, and 7.5 m*M* γ-CD, was used in the separations. The pH of the BGE was adjusted before the addition of NaCl and γ-CD. The final solution was then ultrasonicated for 10 min using a Transsonic bath (model 310, Mandel Scientific Company, Ltd., Guelph, ON, Canada) and filtered through a Millex-HV 0.45 μm syringe end filter (Millipore, Bedford, USA) before use.

Standard amino acid solutions were prepared as 10 m*M* stock solutions in 10 m*M* borate. The medium was alkalinized with 5 *M* NaOH for dissolution of $(Cys)_2$, $(Hcy)_2$ and Tyr, while Kyn, Sac and Hcy(Ala) were dissolved after acidification with 5 *M* HCl. All stock solutions were stored at +4°C except for Asn and Gln, which were made fresh or stored for less than 3 weeks at −20°C.

Working solutions of a mixture of AAs were prepared by mixing defined volumes of the stock solutions. The final concentrations of the AA mixtures were adjusted to the desired values with 10 m*M* Borate.

2.2. Apparatus

Separations were performed on a Beckman P/ACE 5000 automated capillary electrophoresis system equipped with argon ion laser induced fluorescence detection (Beckman Instruments Inc., Missisauga, ON, Canada). Uncoated capillaries (Polymicro Technologies, Phoenix, AZ) 67 cm in length (60 cm to detector) and 50 μm I.D. were used. New capillaries were first rinsed with 0.1 *M* NaOH (15 min at 20 p.s.i.), followed by rinsing with the separation BGE

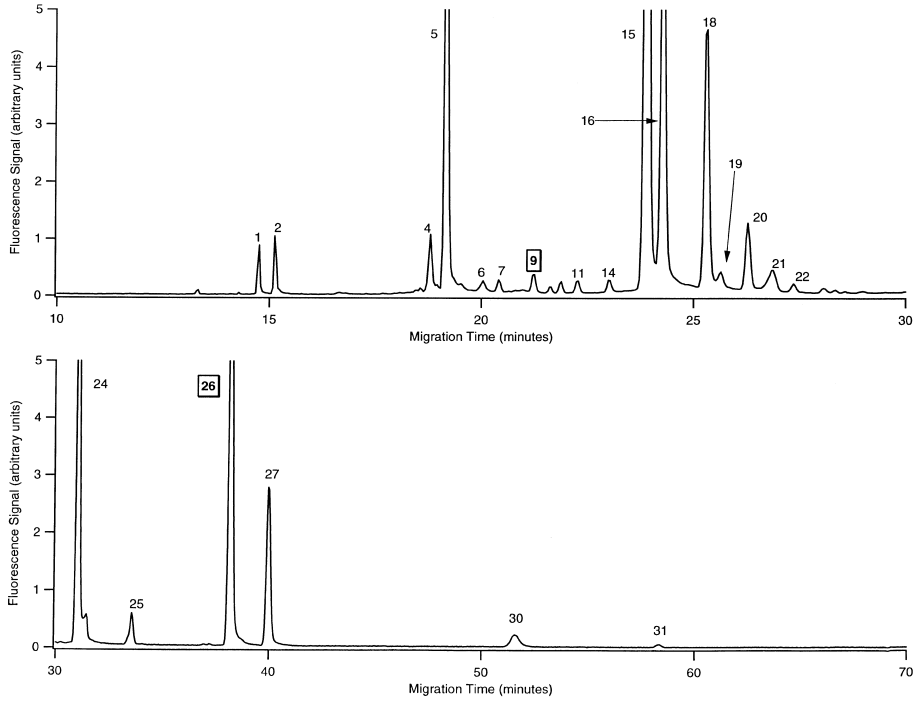

Fig. 4. Phenylketonuria: Aminogram of a plasma sample. The levels of Tyr and Phe as measured by IEC were 1089 and 47 μmol/l, respectively. Peak identification and CE parameters as in Fig. 2.

(60 min at 20 p.s.i.). The capillary was then left to equilibrate overnight in the separation BGE prior to use. Each separation was preceded by a 1 min, 20 p.s.i. rinse with 0.1 *M* NaOH, followed by a 3 min, 20 p.s.i. rinse with the separation BGE. Samples were introduced into the capillary using a 5 second, 0.5 p.s.i. injection (approximately 6.5-nL volume injected). Separations were carried out for 30–80 min at 30 kV (normal polarity) at an ambient temperature of ca. 25°C. Fluorescence was induced with the 488-nm line of a 4-mW argon ion laser (Beckman P/ACE system Laser Module 488). Emission was monitored at 520 nm with a Beckman fluorescence detector. All data were collected and processed using System Gold software (GOLDV810, Beckman). Migration time RSD values were calculated from separations performed on a Beckman P/ACE system MDQ with the accompanying Beckman system Laser Module 488 using a 4 ml BGE volume. All other experimental conditions were identical to those carried out using the P/ACE system. Quantitative data of the AA content of patient plasma samples were obtained previously using a Beckman 6300 AA analyzer (Beckman Instruments, Los Angeles, CA) with ninhydrin detection [12].

2.3. Biological samples

Pools of plasma were obtained, after anonymisation, both from normal donors and from patients suffering from various IEM. The samples were then centrifuged, and stored at −20 or −80°C until shipping on dry ice. Upon receipt, these samples were stored at −20°C until analysis (no more than 6 weeks). Before analysis, the plasma used was deproteinized by adding 25 μl of a 160 g/l sulfosalicylic acid solution (containing 200 μmol/l of N-Val as an internal standard) to 100 μl of sample [11,12]. After mixing for 30 s, the samples were left to stand for 10 min and then centrifuged at 13 000 *g* for 5 min. Two to 10 μl of 5 *M* NaOH were then added to the supernatant obtained after centrifuging in order to bring the solution to a pH above 9.0, as

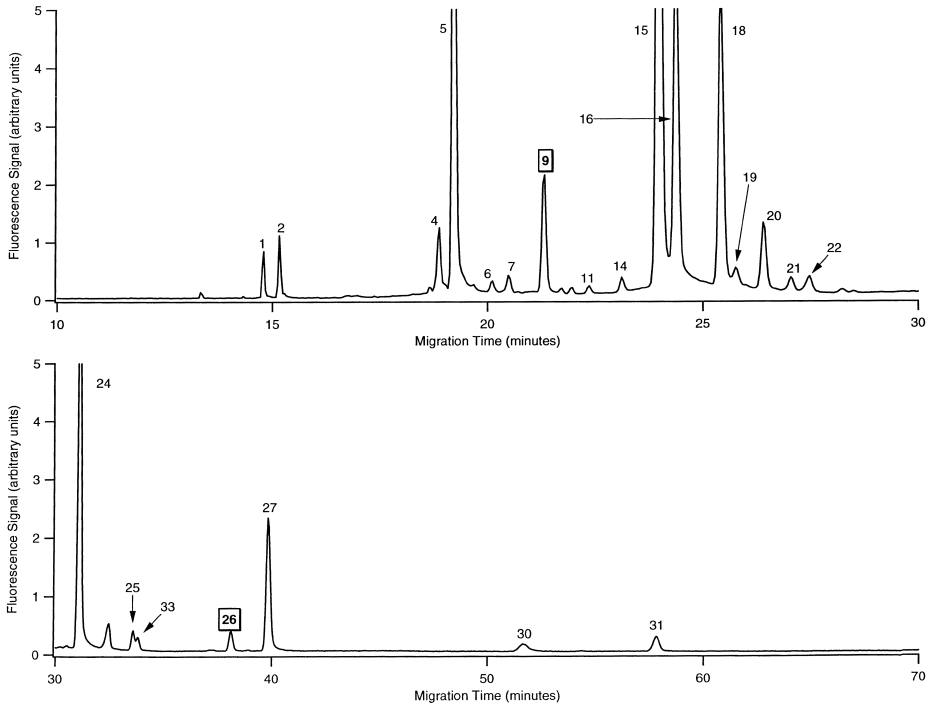

Fig. 5. Tyrosinemia: Aminogram of a plasma sample. The levels of Tyr and Phe as measured by IEC were 341 and 56 μmol/l, respectively. Peak identification and CE parameters as in Fig. 2.

checked with pH paper (Duotest®, pH 1–12, Machery Nagel, Duren, Germany).

2.4. Fluorescent labeling

Labeling reactions were performed according to the procedure established by Arriaga et al [21], with certain modifications. A stock solution of 30 m*M* CBQCA was prepared in HPLC grade methanol and sonicated for 45 min. At the end of the sonication, the temperature of the sonicating bath was measured to be ca. 41°C. Once prepared, the solution was stored at −20°C and protected from light. A 0.2 *M* cyanide stock solution was prepared by dissolving KCN in deionized water. A working cyanide solution was prepared by dilution of the stock to a final concentration of 75 m*M* and was kept in aliquots at −20°C. Thirty μl of the deproteinized sample supernatant was transferred to an eppendorf tube containing 30 μl of the 30 m*M* CBQCA solution. The amount of CBQCA added to the sample solution was chosen so that the final concentration of the probe would be in ca. 10-fold molar excess to the total plasma AA concentration [23]. After mixing and being left to stand for 5 min, the derivatization reaction was initiated by adding 6 μl of the 75 m*M* KCN solution. The reaction was allowed to proceed for 30 min in a 65°C water bath and was subsequently quenched by dilution with 434 μl of a 10 m*M* Borate solution at ambient temperature. Standard AA solutions were derivatized following the same procedure.

3. Results and discussion

3.1. Labeling reaction

Single AA standard solutions were derivatized in order to characterize the labeling reaction for individual AAs. The labeling procedure was tested on a number of AAs and was found to be suitable for 1-CH_3-His, 3-CH_3-His, Aad, Abu, Ala, ASA, Aile, Ans, Arg, Asn, Asp, β-Ala, Baib, $(Cys)_2$, Car, Cit,

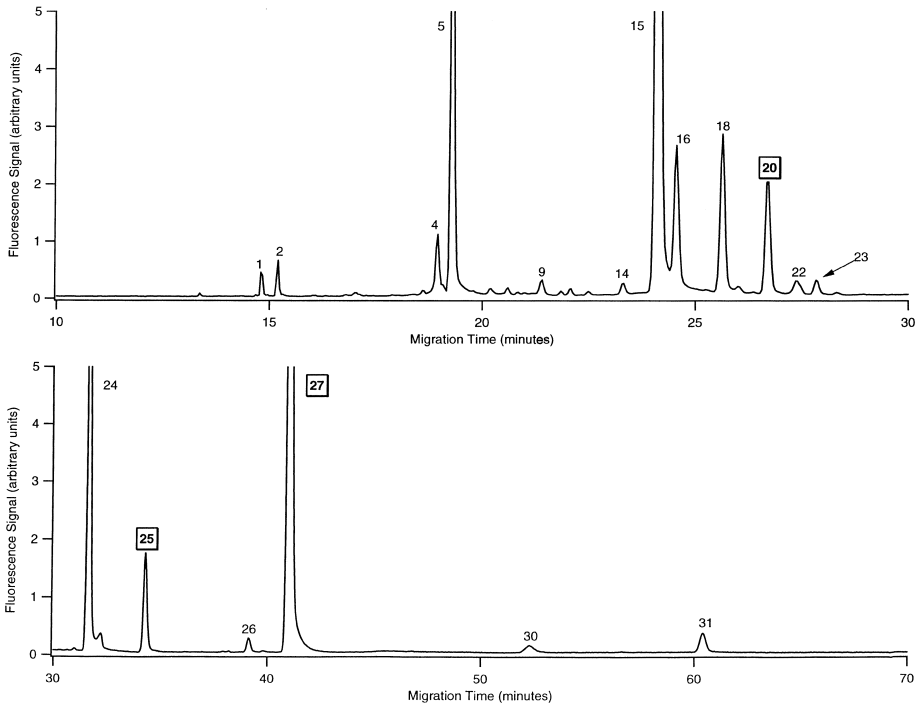

Fig. 6. MSUD: Aminogram of a plasma sample. The AA levels of interest, as measured by IEC, were 879, 402 and 292 μmol/l for Val, Ile, and Leu, respectively. Aile (157 μmol/l by IEC) co-migrates with Ile. Peak identification and CE parameters as in Fig. 2.

EA, GABA, Glu, D-Gluc, Gln, Gly, His, $(Hcy)_2$, Ile, Kyn, Leu, Lys, N-Leu, N-Val, Met, Orn, Phe, PEA, Ser, Tau, Thr, Trp, Tyr and Val. As expected, secondary AAs (Pro, OH-Pro, Sar) gave no peaks. More than one peak was obtained for some AAs/conjugates that contained only one primary amine (derivatization of Met yields 2 peaks, for example), illustrating the complexity of the derivatization process. Additionally, side reactions can further complicate the analysis. It is apparent that much work is still needed to properly characterize the CBQCA derivatives of specific AAs, but such an endeavour was beyond the scope of this investigation.

To determine the optimal reaction time for maximum fluorescent response, the labeling procedure was tested on 3 AAs (Gly (neutral), Arg (basic) and Asp (acidic)). Time curve studies with Arg (5, 10, 20, 30, 60, 120, 180 min) indicated that a plateau was reached at 30 min when the temperature was set at 65°C. This cuts the reaction time nearly in half as when derivatization is carried out at 50°C [21]. Under these conditions, the yield of the reaction was found to be very low for Asp as compared to the yield observed for Gly or Arg.

In order to determine the concentration limit of detection (CLOD) for AAs derivatized using this method, a sensitivity study was performed in which the concentration of one AA (Arg) was decreased while the concentrations of the two others (Gly, Asp) were maintained at 500 μ*M*. The CLOD for Arg was calculated to be 4.0×10^{-8} *M* based on the method described by Knoll [24]. Using similar procedures, the CLOD for Gly and Asp were determined to be 1.0×10^{-7} *M* and 8.0×10^{-7} *M*, respectively. At a concentration of 0.1 μ*M*, Arg was found to give a signal-to-noise ratio of more than 20. Unfortunately, detection below 1 μ*M* for Gly and Asp was not achieved due to the presence of interfering peaks, which are thought to be fluorescent products from side reactions between CBQCA and cyanide [21]. However, the ability to detect 1 μ*M* of AA in the presence of high levels of other components makes this method suitable for the study of AAs in biological fluids [25].

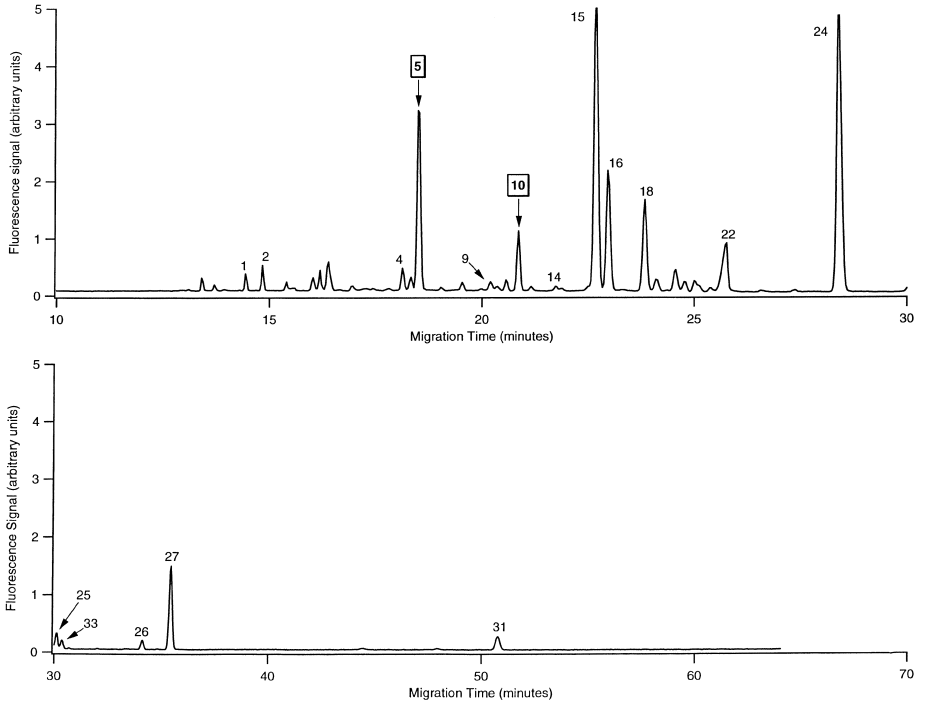

Fig. 7. Hyperornithinemia: Aminogram of a plasma sample. The levels as measured by IEC were 865 and 455 μmol/l for Orn and Gln, respectively. Peak identification and CE parameters as in Fig. 2.

3.2. Deproteinization

Free AA analysis in plasma requires that all proteins be removed from the sample to avoid precipitation inside the capillary and competition for the fluorescent probe during the labeling reaction [25]. Precipitation with sulfosalicylic acid (SSA) is one of the preferred techniques for deproteinization [11] because it is simple, fast and effective. To investigate the effects of deproteinization with SSA on our labeling procedure, 100 μl of standard solution containing 500 μ*M* each of Gly, Arg and Asp was treated with 25 μl of an SSA solution (160 g/l) and derivatized by taking 30 μl of the supernatant. The yield of the reaction was found to be less than when performed without the protein precipitation procedure. The ratios of the peak areas for samples treated with SSA to those without (taking into account the dilution of the sample that occurs as a result of the protein precipitation procedure) were found to be 0.5, 0.18 and 0.02 for Gly, Arg and Asp respectively.

3.3. Separation of AAs

The BGE was selected based on experimenting with various concentrations of borate (50, 80, 100, 120, 160 m*M*) and SDS (7, 30, 50, 70, 100, 120, 130, 150 m*M*) at various pH conditions (8.0, 8.5, 9.0, 9.5 and 10.0). It was found that the BGE of 130 m*M* SDS in 160 m*M* borate at a pH of 9.5 gives the best separation. Under these conditions, it was noted that the migration times of the labeled AAs were longer than was desirable and certain AA pairs (most notably Gly and Ala and Cit and 1-CH_3-His) still co-migrate. A series of additives were tested in the BGE in attempts to reduce the migration times of the labeled AAs and improve the separation. It was found that γ-CD at a concentration of 7.5 m*M* was effective in reducing the overall migration times of the CBQCA-AAs and this effect can be seen for a standard mixture of 14 AAs in Fig. 1. At the running pH of 9.5, the probe moiety of the labeled AAs will bear a negative charge due to deprotonation of the benzoic acid substituent. This negative charge will

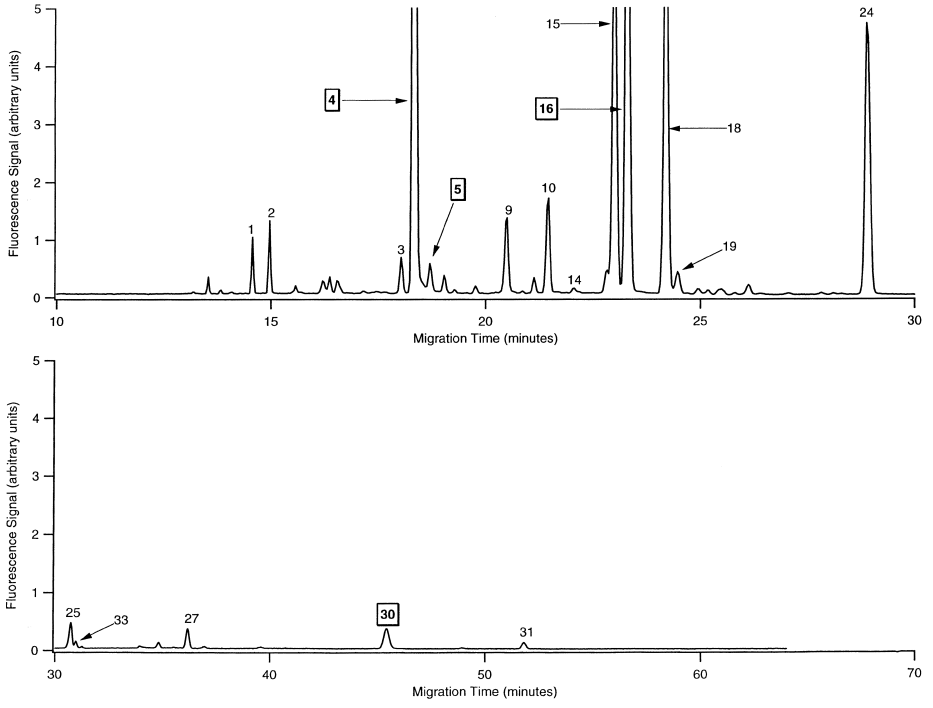

Fig. 8. Citrullinemia: Aminogram of a plasma sample. The levels as measured by IEC were 3940, 1640 and 1750 μmol/l for Cit, Ala and Glu, respectively. Peak identification and CE parameters as in Fig. 2.

reduce the forward mobility of the labeled AAs and thus increase the time required for the analytes to migrate past the detector. The reduction in negative charge to size ratio upon complexation with γ-CD through hydrophobic interaction [26] decreases the analytes' negative mobility and thus shortens the overall analysis time. Additionally, NaCl at a concentration of 20 m*M* was found to improve resolution for the comigrating pairs Gly and Ala and Cit and 1-CH_3-His. Because of these benefits, 7.5 m*M* γ-CD and 20 m*M* NaCl were included in the final BGE used to analyze the patient plasma samples.

During the optimization of the separation conditions, AAs and related compounds were identified by spiking. The AA spike was added to the sample prior to the deproteinization and derivatization steps. Fig. 2 shows the identification of 29 AAs in a standard solution. His and Trp could not be identified (although these AAs are readily detected when derivatizing individual standard solutions), indicating that either the deproteinization or SSA neutralization procedure (or a combination of both) has a deleterious effect on the production of these labeled AAs.

3.4. AAs in human plasma

An aminogram of a normal human plasma sample (spiked with 200 μ*M* Arg and 400 μ*M* Asp) is shown in Fig. 3. Most AAs were readily identifiable using the same spiking procedure developed for the standard mixtures. Asp, though well separated in the sample, is found to give a very low signal.

The spiking experiments indicate that the labeling reaction is influenced by the total AA concentration present in the plasma sample. For example, reductions in Leu and Phe peak areas were noted in a plasma sample spiked first with 400 μ*M* Arg and then with 400 μ*M* of both Arg and Glu. Because of this and other factors (including co-migration of certain AA pairs), it was determined that absolute quantification would be problematic, as it would be for all labeling reactions that involve more than one analyte. However, in many cases, analysis of the plasma samples had been previously performed using IEC methodologies and the AA concentrations obtained by IEC are indicated in the captions where appropriate. Although quantitation of the AAs was not pursued in this study, qualitative and semi-quantitative analysis can be accomplished based on the general migration order and relative peak areas. The migration profile obtained under fixed conditions is quite reproducible with migration time RSD values for 23 AAs ranging from 0.5 to 4.0% as listed in Table 1. These RSD values are sufficient to allow identification of most of the AA peaks in plasma on the basis of their migration times.

3.5. Diagnosis of inborn errors of metabolism

Plasma samples from patients suffering from a number of inborn metabolic diseases were analyzed using the method established for normal plasma samples. In each case, the disease can be identified by comparing the aminograms to the profile of a healthy patient, shown in Fig. 3, and noting the increased levels of the specific amino acid(s) accompanying each individual disease.

Phenylketonuria (PKU) is a disorder of phenylalanine metabolism, which is caused primarily by a deficiency of the hepatic apoenzyme phenylalanine-

Table 1
Migration time RSD values for a standard mixture of 23 CBQCA-labeled amino acids

Amino Acid	Mean ($n=6$) migration time (min)	RSD (%)
Lys (peak 1)	16.61	0.5
Lys (peak 2)	17.07	0.5
1-CH_3-His	20.59	0.7
Cit	21.15	0.7
Gln	21.51	0.7
Asn	21.67	0.7
3-CH_3-His	23.31	0.9
Tyr	23.92	0.8
Orn	24.55	0.9
Ser	25.69	1.0
Gly	26.72	1.0
Ala	27.18	1.1
Tau	28.45	1.2
Abu	28.72	1.2
Val	29.38	1.3
Met	30.26	1.4
Thr	30.60	1.4
N-Val	34.66	1.7
Ile	37.37	1.9
Phe	43.08	2.2
Leu	44.69	2.4
N-Leu	50.81	2.8
Arg	70.82	4.0

4-hydroxylase and is characterized by a marked increase in the free Phe levels present in the plasma of those afflicted [27]. Hyperphenylalaninemia, as evidenced by an increase in the area of the Phe peak, is shown in Fig. 4.

Tyrosinemia is clinically heterogeneous and presents as either acute or chronic forms both of which are characterized by a marked increase in the free Tyr levels present in the plasma of those afflicted [28]. Fig. 5 shows an aminogram of a well-controlled type-2 tyrosinemic patient.

Maple syrup urine disease (MSUD), also known as branched-chain α-ketoacid dehydrogenase complex deficiency, is characterized by an increase of Leu, Ile and Val in the blood with increased Aile levels also being observed [29]. A MSUD profile of a patient under control is shown in Fig. 6 (Unfortunately, Aile is not separated from Ile).

Citrullinemia, (CITR) and hyperornithinemia–hyperammonemia–homocitrullinuria (HHH syndrome) are two representative entities of inherited hyperammonemia disorders [30]. The free AA content of plasma from a patient with controlled HHH syndrome and one with CITR are shown (with IEC quantifications) in Figs. 7 and 8 respectively. In this sample of citrullinemia, no increase of Gln could be demonstrated, though a value of 1670 μmol/l was measured by IEC for Gln in the same sample more than 10 years ago. In this particular case, the disappearance of Gln can be attributed to its known long-term instability. When the Gln concentration of a different sample from the same patient (stored under the same conditions) was measured by IEC, results indicated a significant decrease in the Gln concentration, from 2095 to 136 μ*M* after 11 years.

Due to the fact that quantification of the free AAs in plasma was determined to be problematic, this CE method still needs to be improved before it can be used as an alternative to AA analyzers. Specifically, much more work must be done to characterize the derivatization procedure for each of the particular amino acids. However, the real examples presented in this paper suggest that this CE method may be useful as a diagnostic tool in cases of emergency IEM determinations. MSUD, inherited hyperammonemias (including the urea cycle defects), tyrosinemias and hyperphenylalaninemias can all be easily recognized using this CE method. Furthermore, the times required for both derivatization and analysis of the sample using this method are well suited to cases of emergencies [12].

Our results on AA analysis in plasma and those of others on OA and ORA analysis [3–5,7–10] offer the potential to identify most emergencies of IEM (as discussed in the introduction [1]) using common CE instrumentation with various modes of detection. Although significant methodological problems remain to be resolved, the growing acceptance of CE in the clinical chemistry laboratories of more medical centers [15] make it reasonable to believe that CE will be an alternative for the preliminary identification (selective screening) of IEM emergencies in these centers in the near future at a minimum of extra cost.

4. Conclusions

The identification of most free AAs in human plasma by capillary electrophoresis with laser induced fluorescence detection has been demonstrated. The separation is completed in 70 min following a 40-min labeling procedure for primary amines using CBQCA. Most of the primary amino acids present in plasma are identified, and plasma AA profiles of patients suffering from a variety of IEM (phenylketonuria, tyrosinemia, MSUD, hyperornithinemia and citrullinemia) are presented. The number of AAs successfully separated and identified demonstrates the potential of CE AA analysis of real patient samples. However, the method suffers from limitations in quantification and presently is only suited to pattern recognition of some IEM neonatal emergencies.

Acknowledgements

This work is supported by the Centre Hospitalier Universitaire Vaudois and the Laboratoire Central de Chimie Clinique of the Centre Hospitalier Universitaire Vaudois (Head: Prof. C. Bachmann) and the Natural Science and Engineering Research Council of Canada. The IEC analyses were performed by F. Huppertz.

References

[1] J.-M. Saudubray, C. Charpentier, in: C. Scriver, A. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic Bases of Inherited Disease, Vol. 1, McGraw Hill, New York, 1995, p. 327.
[2] M. Bowser, G. Bebault, X. Peng, D.D.Y. Chen, Electrophoresis 18 (1997) 2928.
[3] A. Garcia, C. Barbas, R. Aguilar, M. Castro, Clin. Chem. 44 (1998) 1905.
[4] C. Barbas, N. Adeva, R. Aguilar, M. Rosillo, T. Rubio, M. Castro, Clin. Chem. 44 (1998) 1340.
[5] A. Procházková, L. Krivánková, P. Bocek, J. Chromatogr. A 838 (1999) 213.
[6] J. Sevcik, T. Adam, V. Sazel, Clin. Chim. Acta 259 (1997) 73.
[7] C. Mardones, N. Vizioli, C. Carducci, A. Rios, M. Valcarcel, Anal. Chim. Acta 382 (1999) 23.
[8] S. Kiessig, C. Vogt, J. Chromatogr. A 781 (1997) 475.
[9] E. Jellum, H. Dollekamp, C. Blessum, J. Chromatogr. B 683 (1996) 55.
[10] K.B.P. Elgstoen, E. Jellum, Electrophoresis 18 (1997) 1857.
[11] V. Walker, M.G. A, Ann. Clin. Biochem. 32 (1995) 28.
[12] R.H. Slocum, J.G. Cummings, Techniques in Diagnostic Human Biochemical Genetics: A Laboratory Manual, Wiley–Liss, 1991, Page 87.
[13] G. Vecchione, M. Margaglione, E. Grandone, D. Colaizzo, G. Cappucci, I. Fermo, A. D'Angelo, G. Di Minno, Electrophoresis 20 (1999) 569.
[14] E. Caussé, N. Siri, H. Bellet, S. Champagne, C. Bayle, P. Valdiguié, R. Salvayre, F. Couderc, Clin. Chem. 45 (1999) 412.
[15] D. Perett, Ann. Clin. Biochem. 36 (1999) 133.
[16] J.T. Smith, Electrophoresis 18 (1997) 2377.
[17] H.A. Bardelmeijer, J.C.M. Waterval, H. Lingeman, R. Vanthof, A. Bult, W.J.M. Underberg, Electrophoresis 18 (1997) 2214.
[18] H.J. Issaq, K.C. Chan, Electrophoresis 16 (1995) 467.
[19] J.T. Smith, Electrophoresis 20 (1999) 3078.
[20] J. Liu, Y.-Z. Hsieh, D. Wiesler, M. Novotny, Anal. Chem. 63 (1991) 408.
[21] E.A. Arriaga, Y. Zhang, N.J. Dovichi, Anal. Chim. Acta 299 (1995) 319.
[22] J. Bergquist, S.D. Gilman, A.G. Ewing, R. Ekman, Anal. Chem. 66 (1994) 3512.
[23] C. Mircher, A. Salabelle, M.A. Peeters, D. Rabier, P. Parvy, P. Kamoun, J. Lejeune, Arch. Pediatr. 4 (1997) 1093.
[24] J.E. Knoll, J. Chromatogr. Sci. 23 (1985) 422.
[25] V.E. Shih, in: M.D.N. Blau, M.E. Blaskovics (Eds.), Physician's Guide To the Laboratory Diagnosis of Metabolic Diseases, Chapman and Hall Medical, 1996, p. 13.
[26] I.R. Lian Ji Jin, Sam F.Y. Li, Electrophoresis 20 (1999) 1538.
[27] N. Blau, M.E. Blaskovics, in: M.D.N. Blau, M.E. Blaskovics (Eds.), Physician's Guide To the Laboratory Diagnosis of Metabolic Diseases, Chapman and Hall Medical, 1996, p. 65.
[28] R. Berger, in: M.D.N. Blau, M.E. Blaskovics (Eds.), Physician's Guide To the Laboratory Diagnosis of Metabolic Diseases, Chapman and Hall Medical, 1996, p. 107.
[29] K.M. Gibson, O.N. Elpeleg, R.S. Wappner, in: M.D.N. Blau, M.E. Blaskovics (Eds.), Physician's Guide To the Laboratory Diagnosis of Metabolic Diseases, Chapman and Hall Medical, 1996, p. 125.
[30] M. Tuchman, in: M.D.N. Blau, M.E. Blaskovics (Eds.), Physician's Guide To the Laboratory Diagnosis of Metabolic Diseases, Chapman and Hall Medical, 1996, p. 209.