Chapter 14

Bioanalytical Application of Amino Acid Detection by Capillary Electrophoresis

Daniela Fico, Antonio Pennetta, and Giuseppe E. De Benedetto

Abstract

This chapter illustrates the usefulness of capillary electrophoresis (CE) for the analysis of amino acids, and both normal and chiral separations are covered. In order to provide a general description of the main results and challenges in the biomedical field, some relevant applications and reviews on CE of amino acids are tabulated. Furthermore, some detailed experimental procedures are shown, regarding the CE analysis of amino acids in body fluids, in microdialysate, and released upon hydrolysis of proteins. In particular, the protocols will deal with the following compounds: (1) underivatized aminoacids in blood; (2) γ-Aminobutyric acid, glutamate, and L-Aspartate derivatized with Naphthalene-2,3-dicarboxaldehyde; (3) hydrolysate from bovine serum albumine derivatized with phenylisothiocyanate. By examining these applications on real matrices, the capillary electrophoresis efficiency as tool for Amino Acid analysis can be ascertained.

Key words Capillary zone electrophoresis, Micellar electrokinetic chromatography, Amino acids, Chiral separations, Bioanalytical applications

Abbreviations

ANDA	7-Amino-1,3-naphthalene disulfonic acid
APC	1-(9-Anthryl)-2-propyl chloroformate
BMIC	1-Butyl-3-methyl imidazolium chloride
BrBQCA	3-(4-Bromobenzoyl)-2-quinoline carboxaldehyde
C4D	Capacitively coupled contactless conductivity detection
CAPS	3-Cycloexylamino-1-propanesulfonic acid
CBQCA	3-(4-Carboxy benzoyl) quinoline-2-carboxaldehyde
CC	2,4,6-Trichloro-1,3,5-triazine
CFSE	5-Carboxy-fluorescein succinimidyl ester
ClBQCA	3-(4-Chlorobenzoyl)-2-quinoline carboxaldehyde
DMAB	4-(*N,N'*-dimethylamino)benzoic acid
Dns	5-(Dimethylamino) naphthelene-1-sulfonyl chloride
DTAF	5-(4 6-Dichloro-s-triazin-2-yl amino) fluorescein
FITC	Fluorescein isothiocyanate

Philippe Schmitt-Kopplin (ed.), *Capillary Electrophoresis: Methods and Protocols*, Methods in Molecular Biology, vol. 1483,
DOI 10.1007/978-1-4939-6403-1_14, © Springer Science+Business Media New York 2016

FMAC	*N*-fluorenylmethoxycarbonyl-l-alanyl *N*-carboxyanhydride
FNBDA	4-Fluoro-7-nitro-2,1,3-benzoxadiazole
GABA	γ-Amino-n-butyric acid
HM-β-CD	Heptakis(2,6-di-O-methyl)-β-CD
HP-β-CD	Hydroxypropyl-β-cyclodextrin
IAF	5-Iodoacetamidofluoresceine
MBA	4-Methylbenzylamine
MDMA-β-CD	3-Monodeoxy-3-monoamino-β-CD
MDP-β-CDCl	Mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-cyclodextrin chloride
MGA	*N*-methyl-d-glucamine
MHP-β-CD	Mono-6-deoxy-6-mono(3-hydroxy) propylamino-β-CD
MMI-β-CDCl	Mono-(3-methylimidazolium)-β-CD chloride
NBD-F	4-Fluoro-7-nitro-2,1,3-benzoxadiazole
NDA	Naphthalene-2,3-dicarboxaldehyde
OPA	*o*-Phthaldehyde
PITC	Phenyl isothiocyanate
SAMF	6-Oxy-(*N*-succinimidyl acetate)-9-(2′-methoxycarbonyl) fluorescein
THSBE	1,3,5,7-Tetramethyl-8-(*N*-hydroxy succinimidyl butyric ester) difluoro boradiaza-S-indacene

1 Introduction

One of the primary goals of biomedical research is the study of new methods for the early diagnosis of a disease, able to follow its progression and to evaluate the therapeutic efficacy of a treatment. Many diseases, however, have a complex and multifactorial behaviour and their diagnosis and understanding cannot be based only on the evaluation of a single molecular marker. Among the whole small molecules produced by metabolism encompassed in metabolomics, amino acids have a key role. The protein "building blocks" are also essential precursors of important biomolecules including nucleotides and nucleotide coenzymes such as heme, a variety of hormones and neurotransmitters; therefore, given the important role in metabolism, it is possible, by carrying out the analysis of the amino acid profile, to get useful indications on various metabolic processes and the functioning of liver, kidneys, heart, or immune system. Amino acids differing from basal levels, in fact, are often symptoms of diseases, as in phenylketonuria and cystinuria. As a result, amino acids are an important target for the "metabolic profiling," being frequently present in biological matrices and several methods have been developed to identify the presence and measure the concentration with the aim to perform screening of various diseases. From the analytic point of view, on considering that AAs have different behavior (acidic, neutral, or basic) and many of them lack of a strong chromophore, their determination is both interesting and intriguing and has been widely pursued by many authors with the issue of determining

more amino acids in a single run with lower detection limits. Another related boosting analytical field is the chiral separation of amino acids: the structure and symmetry of organic molecules plays a crucial role in different biochemical processes but many methods validated for the quantification of amino acids are not stereoselective. For some time the free amino acids in biological matrices were typically quantified as the sum of their enantiomers, because in terrestrial organisms they exist in the form of stereoisomers L. The amino acids of the series D had been identified only in few peptides, such as those of the cell wall of the bacteria. With the increased understanding of the biological significance of the D-amino acids, interest in enantioselective quantification is increased and more applications of chiral CE to real problems of biomedical importance find attention.

The wealth of papers continuously published on amino acids analysis is continuously reviewed and a review of the reviews could be useful to evidence the peculiar aspects each time tracked. Presently, it is important to mention the general reviews published every 2 years in Electrophoresis [1–3].

Aiming at having a general overview, few significant methods, mainly related to biomedical applications and set up to separate and detect AAs, have been collected together with the most relevant experimental information in two tables handling normal [4–66] and enantiomeric [67–94] separations, respectively. Within each table both capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) have been described. All the referred methods also share the properties of being carried out on simple, commercial instruments, and, as a rule, they could be transferred on every CE system.

Different matrices, as far as the biomedical applications are concerned, have been analyzed: physiological fluids like urine, saliva, or plasma; cells like lymphocyte or erythrocyte; neurotransmitters and different hydrolysates (see column "Matrix" in Tables 1 and 2). Moreover, the hyphenation of microdialysis with the CE apparatus deserves attention as it allows both continuous and in vivo analysis: a fraction of the analytes can diffuse through the membrane dialysis and depending on them, temperature and probe characteristics, a definite recovery is attainable and quantitative measurements can be accomplished. Also fast monitoring of AAs is carried out by hyphenating microdialysis with CE: in particular if LIF is used as detector, thanks to the high separation efficiency and low volume sample requirement of CE and the very low detection limits of LIF detector, the sampling rate has been increased to levels not attainable by other analytical common techniques, like HPLC, and short-lasting changes in AAs concentration could be recorded. Moreover, to decrease the overall cost of the detector, the use of LED sources for fluorescence detectors is possible [24, 35, 90, 91].

Table 1
Selected applications of CZE and MEKC to biomedical applications

Analytes	Matrix	Labeling	BGE	Detection	Separation method	Capillary (i.d., effective/ total lenght)	Injection	Run time (min)	L.O.D.	Ref.
Lys, Arg, putrescine, cadaverine, spermidine, ornithine (Orn)	Saliva	FITC	12 g/l PSS, 3 mM CTAB, 80 mM borate buffer, pH 12.35	LIF, 483 nm	CZE, 22 kV, 25 °C	25 µm, 90 cm	Electrokinetic, 22 kV, 8 s	48	0.0072–0.26 nM	[4]
Creatinine, His	Urine	No	10 mM NaCl, 0.03 % (w/v) HPMC, pH 2.5 with 1 M HCl	UV, 200 nm	CZE, 20 kV, 25 °C	50 µm, 50/52.4 cm	Electrokinetic, 10 kV, 100 s	10	4.8 nM (creatinine), 9.0 nM (His)	[5]
Gaba, Ala, Gln, Gly, Tau, Glu, Asp	Brain cortex or spinal cord tissue (mouse)	THSBE	5.5 mM CTAB, 70 mM PBS, pH 4/20 % ACN	LIF, 488 nm	MEKC, −22.5 kV, 25 °C	75 µm, 50/60 cm	Hydrodynamic, 0.5 psi, 5 s	20	0.2–1.4 nM	[6]
Cys, Arg, Orn, Lys	Urine	No	1.0 M formic acid/10 % MeOH	ESI-MS	CZE, 21 kV, 25 °C	50 µm, 70 cm	Hydrodynamic, 2 psi, 10 s	11	30.7–114.2 µM	[7]
Phe, Tyr	Blood	No	3 mM ammonium acetate buffer, pH 10.7	ESI-MS	CZE, 25 kV, 25 °C	50 µm, 40 cm	Electrokinetic, 25 kV, 3 s	3	0.03 mg/l (Phe), 0.07 mg/l (Tyr)	[8]
Ala, Glu, Asp, Ser, Tau, Gly	Serum	NDA/CN or OPA	50 mM borate buffer, pH 9.2	Amperometric, 0.75 V	CZE, 15 kV, 25 °C	25 µm, 65 cm	Electrokinetic, 10 s, 15 kV	30	0.55–2.8 µM	[9]
Asn, Ser, Thr, Tyr, Gly, Glu, Asp, Ala, Tau, GABA, NE, Val, DA, Ile, Leu, Phe, Arg, Lys	Cells	ClBQCA	38.5 mM SDS, 120 mM boric acid, pH 9.2/17 % ACN	LIF, 488 nm	MEKC, 22.5 kV, 25 °C	75 µm, 50 cm	Hydrodynamic, 0.5 psi, 5 s	32	1.4–100 nM	[10]
28 Biogenic amino acids	Blood plasma, urine, saliva, and CSF	No	0.5–8 M acetic acid	C^4D	CZE, 20 kV, 25 °C	25 µm, 18/33 cm	Hydrodynamic, 50 mbar, 16 s	6	0.1–1.7 µM	[11]
Arg, Lys, Trp, Gaba, Ser, Ala, Tau, Gly, Glu and Asp	Brain microdialysate (rat)	FITC	70 mM SDS, 17.5 mM HP-β -CD, 5 mM DM-β-CD, 15 mM borate buffer, pH 10.2 /5% MeOH	LIF, 488 nm	MEKC, 22.5 kV, 25 °C	75 µm, 50/57 cm	Hydrodynamic, 0.5 psi, 5 s	20	0.10–1.00 nM	[12]

Asn, Ser, Thr, Tyr, Gly, Glu, Asp, Ala, Cys-Cys, Orn, Val, Gln, Phe, Arg, Lys, Cys, Pro	Urine	No	3.0 % formic acid/5.0 % MeOH	ESI-MS	CZE, 20 kV, 25 °C	50 μm, 60 cm	Hydrodynamic, 50 mbar, 40 s	11	0.0116–2.38 mg/l	[13]
Gly, Pro, Glu, and Ser	Saliva	No	50 mM $CuSO_4$, 0.05 % acetic acid, pH 4.5	UV, 254 nm	CZE, 15 kV, 25 °C	50 μm, 57/65 cm	Gravity, 15 cm, 727 s	24	0.1–0.5 μM	[14]
Phe, Val, Gln, Pro, Gly, Ser, Ala	Urine	FITC	45 mM α-CD, 80 mM borate buffer, pH 9.2	LIF, 488 nm	CZE, –15 kV, 25 °C	50 μm, 50/60 cm	Gravity	15	160–330 nM	[15]
Trp, Tyr	CSF	No	LE: 250 mM acetate, pH 9.5 with ETA; TE: 0.1 M NaOH, 0.1 % (v/v) ampholyte	UV, 280 nm	GEITP	30 μm, 6/15 cm	–	6	51 nM (Trp). 215 nM (Tyr)	[16]
21 Aas	Protein hydrolysate	No	0.8 % formic acid/20 % MeOH	ESI-MS (IT)	CZE, 30 kV, 25 °C	50 μm, 80 cm	Hydrodynamic, 1 psi, 10 s	13	15.9–172 μM	[17]
Adma, Sdma, Arg	Plasma	No	50 mM Tris/phosphate buffer, pH 2.30	UV, 190–200 nm	CZE,15 kV, 15 °C	75 μm, 50/60.2 cm	Hydrodynamic, 0.5 psi, 10 s	15	0.03 μM (ADMA, SDMA), 0.06 μM (Arg)	[18]
Arg, Glu	Gingival crevicular fluid	FITC	20 mM Carbonate buffer, pH 9.5	LIF, 488 nm	CZE, 21 kV, 25 °C	25 μm, 60 cm	Hydrodynamic, –19 psi, 0.2 s	–	–	[19]
Arg, Glu, Tau	Hemolymphe (Drosophila)	Fluorescamine	30 mM CsCl, 70 mM SDS, 20 mM borate buffer, pH 9.1	LIF, 405 nm	MEKC, 19 kV, 25 °C	50 μm, 36/50 cm,	Gravity, 15 cm, 15 s	10	–	[20]
Arg, Cit, and Argsuc	Neurons (Aplysia Californica)	Fluorescamine	30 mM borate buffer, pH 9.8	LIF, 350 nm	CZE, 20 kV, 20 °C	50 μm, 80 cm	Electrokinetic, 8 kV, 8 s	20	5.1 nM (Arg), 3.8 nM (Cit), 3.8 nM (ArgSuc)	[21]
Glu, Asp, Gaba, Gly, Tau, Gln	CSF, saliva	*N*-hydroxysuccinimidyl fluorescein-O-acetate	100 mM SDS, 100 mM borate buffer pH 9.6/8 % MeOH	LIF, 488 nm	MEKC, 20 kV, 25 °C	75 μm, 50/60.2 cm	Hydrodynamic, 0.5 psi, 10 s	42	0.06–0.1 nM	[22]
Arg, Gln, Glu, Asp	Brain microdialysate (rat)	CBQCA	50 mM SDS, 55 mM β-CD, 20 mM borate buffer, pH 9.3	LIF, 488 nm	MEKC, 21.5 kV, 25 °C	50 μm, 30/38 cm	Gravity, 8 cm, 10 s	3	–	[23]
Gly, Leu, Glu, Asp	CSF microdialysate	In capillary, NDA/CN	75 mM borate buffer, pH 9.2	LED-IF, 410 nm	CZE, 25 kV, 30 °C	75 μm, 56/70 cm	Electrokinetic, 5 kV, 2.5 s	12	11–94 nM	[24]

(continued)

Table 1
(continued)

Analytes	Matrix	Labeling	BGE	Detection	Separation method	Capillary (i.d., effective/ total lenght)	Injection	Run time (min)	L.O.D.	Ref.
His, Hcyss, Trp, Phe, Tyr, Cyss, Gsh, Gssg	Blood	No	10 mM PBS, pH 2.9	UV, 200 nm	CZE, 15 kV, 18 °C	75 μm, 40/47 cm	Hydrodynamic, 0.5 psi, 10 s	30	0.9–36.6 μM	[25]
Trp and Trp Metabolites	CSF	No	5 mM ammonium acetate, pH 9.7/5 % ACN	ESI-MS (TOF)	CZE, −30 kV, 25 °C	25 μm, 70 cm	Hydrodynamic, 50 mbar, 10–60 s	5	20 nM	[26]
Asn, Ser, Thr, Tyr, Gly, Glu, Asp, Ala, Ile, Leu, Val, Gln, Phe, Arg, Hlys, Lys, HPro, Pro	Tissue	No	1 M formic acid, pH 1.8	ESI-MS^2 (IT)	CZE, 25 kV, 20 °C	50 μm, 80 cm	Hydrodynamic, 0.8 psi, 60 s	20	0.05–2 mM (LLOQ)	[27]
Lys, Arg, Ala, Val, Met, Glu, Phe, Tyr	CSF	No	1 M formic acid, pH 1.8	ESI-MS (TOF)	CZE, 30 kV, 25 °C	PB-PVS coated, 50 μm, 130 cm	Hydrodynamic, 35 mbar, 10 s	13	85–225 nM	[28]
Lys, Arg, Ala, Val, Met, Glu, Phe, Tyr	Urine	No	1 M formic acid, pH 1.8	ESI-MS (TOF)	CZE, 30 kV, 25 °C	PB-PVS coated, 50 μm, 130 cm	Hydrodynamic, 90 mbar, 90 s	20	85–225 nM	[29]
His	Urine	No	Cathode: 1.0 mM NaCl, 50 mM PBS, pH 6.0; anode: 3 mM $NiCl_2$ 1.0 mM NaCl, 50 mM PBS, pH 6.0	UV, 208 nm	MAB-ACE, 25 °C, 30 kV	75 μm, 44/53 cm	Hydrodynamic, full of sample	8	43 ng/ml	[30]
Asn, Ser, Thr, Tyr, Gly, Glu, Asp, Ala, Tau, GABA, NE, Val, DA, Ile, Leu, Phe, Arg, Lys	Plasma and vitreous perfusate	BrBQCA	120 mM boric acid, pH 9.1, 38.5 mM SDS/19 % ACN	LIF, 488 nm	MEKC, 22.5 kV, 25 °C	75 μm, 50/60.2 cm	Hydrodynamic, 0.5 psi, 5 s	33	0,65–5 nM (Aas), 58–73 nM (catecholamine)	[31]
Histidine, 1- and 3-methylhistidine	Urine	FITC	22 mM sodium tetraborate, pH 10.5/32 % ACN	LIF, 488 nm	CZE, 25 kV, 25 °C	75 μm, 40/50.2 cm	Hydrodynamic, 0.5 psi, 3 s	30	0.023 ng/ml (His), 0.023 ng/ml (1-MH), 0.034 ng/ml (3-MH)	[32]

Pro, tetracaine, and enoxacin	Urine	No	70 mM phosphate, pH 8	ECL, 1.15 mV (5 mM $Ru(bpy)_3Cl_2$, 50 mM PBS, pH 9.6)	CZE, 15 kV, 25 °C	25 µm, 50 cm	Electrokinetic, 10 kV, 10 s	10	0.08 (Tetracaine), 0.06 (Pro), and 0.02(enoxacin) µg/ml	[33]
Ala, Glu, Asp, Ser, Tau, Gly	Cultured cells, CSF, saliva, vitreous humor	FITC	18 mM phosphate, pH 11.6	LIF, 488 nm	CZE, 28 kV, 23 °C	75 µm, 50/57 cm	Hydrodynamic, 0.5 psi, 3 s	12	0.075–0.2 µM	[34]
Tyr, Trp, DA, NE, 5-HT	Breast cancer cell lysate	NDA/CN	30 mM SDS, 0.1% PEO, 0.5 M Tris, pH 10.2	LED-IF 405 nm	MEKC, 15 kV, 25 °C	75 µm, 55 cm	Gravity, 31 cm, 10 s	16	2.06–19.17 nM	[35]
5-HTP, Trp, TA, 5-HT, and 5-HIAA	Urine and serum	No	2% Dextran sulfate, 100 mM Tris, pH 9.0	LIF, 266 nm	CZE, 15 kV, 25 °C	75 µm, 10 cm	Gravity, 20 cm, 20 s	20	4.1–366.0 pM	[36]
Trp, Phe, Tyr, tyramine, tryptamine	Mammalian decomposition fluids	No	70 mM boric acid, pH 9.5/32% MeOH	UV, 200 nm	CZE, 30 kV, 25 °C	75 µm, 56 cm	Hydrodynamic, 50 mbar, 2.5 s	12	5.8–8.6 mg/l	[37]
Phe, 4-hydroxyphenylacetic acid, phenylpyruvic acid, 2-hydroxyphenylacetic acid	Urine	No	35 mM SDS, 60 mM borate buffer, pH 8.2	C^4D and amperometric	MEKC, 16 kV, 25 °C	25 µm, 86.8 cm	Electrokinetic, 16 kV, 6 s	23	0.064–6.6 mg/l	[38]
Arg, monomethyl- and (symmetric and asymmetric) dimethylarginines	Plasma	No	1.5 M formic acid	ESI-MS^2 (IT)	CZE, 23 kV, 25 °C	50 µm, 79.5 cm	Hydrodynamic, 50 mbar, 10–20 s	12	20 nM (ADMA), 30 nM (MMA) and 10 nM (SDMA)	[39]
Gly, His, Ser, Ala, Glu, Trp, Asn, Tyr, Asp, Val, Pro, Orn, Ile, Phe, Gln, Lys, Leu, Met, Arg, Thr, and 4hyp	Latent fingerprints	No	1 M formic acid, pH 1.90 (by addition of ammonium formate) /5% ACN	ESI-MS	CZE, 28 kV, 23 °C	50 µm, 100 cm	Hydrodynamic, 50 mbar, 60 s	30	20–180 ng Deposited on the Mylar R substrate	[40]
Pro, pyroglutamic acid	Urine	No	25 mM TEA, pH 11.7/50% MeOH	ESI-MS	CZE, 30 kV, 20 °C	50 µm, 100 cm	Hydrodynamic, 0.5 psi, 30 s	20	2.6 µM (Pro); 3.6 µM (pyroglutamic acid)	[41]

(continued)

Table 1 (continued)

Analytes	Matrix	Labeling	BGE	Detection	Separation method	Capillary (i.d., effective/ total lenght)	Injection	Run time (min)	L.O.D.	Ref.
Ala, Phe, Pro, Tyr, Val	Serum	No	5 mM ammonium acetate, pH 10.8	ESI-MS	CZE, 25 kV, 25 °C	50 μm, 95 cm	Electrokinetic, 25 kV, 3 s	15	50–810 nM	[42]
Creatinine, Lys, Arg, His, Gly, Ala, Val, Ile, Leu, Thr, Asn, Met, Trp, Citruline, Phe, Pro, Tyr	Whole blood, serum, urine, plasma	No	2.5 M acetic acid, pH 2.0	C^4D	CZE, 30 kV, 25 °C	25 μm, 35/50 cm	Hydrodynamic, 50 mbar, 20 s	12	0.15–10 μM	[43]
Trp, Phe, Tyr, homophenylalanine	Urine	No	100 mM PBS, pH 2.0	UV, 200 nm	CZE, 20 kV, 25 °C	40 μm, 50/60 cm	Hydrodynamic, 0.5 psi, 10 s	14	70–500 nM	[44]
Ala, Asp, Gly, His, Leu, Lys, Phe, Ser, Tyr, Val	Brain (mouse)	No	1 % formic acid	ESI-MS (TOF)	CZE, 20 kV, 25 °C	40 μm, 90 cm	Hydrodynamic	–	–	[45]
Trp, kynurenine	Plasma	No	100 mM Bis-Tris propane pH 2.15; 1 M phosphoric acid pH 2.15	UV, 226 nm	CZE, 12 kV, 20 °C	75 μm, 20/30 cm	Hydrodynamic, –0.5 psi, 10 s	9	400 nM (Trp), 150 nM (kynurenine)	[46]
Phe, Tyr	Urine	No	1 M formic acid, pH 1.8	UV, 200 nm	CZE, 30 kV, 25 °C	50 μm, 91/100 cm	Hydrodynamic, 50 mbar, 7 s (2.5 % NH_3), 228 s (samples/ BGE, 1:1)	7.5	36 nM (Phe), 49 nM (Tyr)	[47]
Ala, Gly, Leu, D-L Orn, Val	Serum (rat)	No	1 M formic acid	ESI-MSMS	CZE, 25 kV, 25 °C	50 μm, 80 cm	Hydrodynamic, 0.8 psi, 60 s	–	0.5–10 μM	[48]
Orn, citrulline, alloleucine, β-alanine, GABA, pyroglutamic Acid	Serum (from patients with liver diseases)	No	0.1 μM formic acid, pH 2	ESI-MS	CZE, 25 kV, 25 °C	50 μm, 60 cm	–	–	0.04–0.19 mg/l	[49]
Ala, Asp, Gly, His, Leu, Lys, Phe, Ser, Tyr, Val, Trp, Cys, Thr, Arg, Gln, Pro, Met, Asn, Glu, Ile	Tissues (lung and prostate)	no	1 M formic acid, pH 2.2	ESI-MS (TOF)	CZE, 30 kV, 25 °C	50 μm, 80 cm	Hydrodynamic, 50 mbar, 10 s	–	–	[50]

Arg, Trp, Leu, Ile, Gln, Val, Thr, Pro, Ser, Ala, taurine, Gly, Tyr, His, Lys, Orn, Glu, Asp, Cys	Serum, urine, and saliva	CC, ANDA	20 mM borate buffer, pH 10.1	UV, 214 nm	CZE, 15 kV, 23 °C	50 µm, 40/47 cm	Hydrodynamic, 0.5 psi, 2 s	25	0.52–1.7 mg/l	[51]
Ala, Ser, Gly, taurine, Glu, Asp, homocysteic acid	Plasma, urine, blood	FITC	18 mM Na3PO4, pH 11.8	LIF, 488 nm	CZE, 28 kV, 25 °C	75 µm, 50/57 cm	Hydrodynamic, 0.5 psi, 5 s	12	–	[52]
Homocystein, Cys	Plasma	IAF	30 mM sodium phosphate, 33 mM boric acid, 75 mM *N*-methyl-d-glucamine, pH 11.3	LIF, 488 nm	CZE, 18 kV, 25 °C	75 µm, 50/57 cm	Hydrodynamic, 0.5 psi, 5 s	10	1.5 nM	[53]
Orn, His, Lys, Arg	Sweat	no	10 mM MBA, 3.5 mM citric acid, pH 4.05/25 % methanol	UV, 214 nm	CZE, 30 kV, 25 °C	75 µm, 100 cm	Hydrodynamic, 50 kPa, 3 s	13	0.27–0.79 µM	[54]
GABA, Gln, Ala, Gly, Tau, Glu, Asp	Lymphocytes	SAMF	40 mM sodium acetate, 2 mM Cu^{2+}, pH 6.0	LIF, 488 nm	CZE, 15 kV, 25 °C	75 µm, 50/57 cm	Hydrodynamic, 34.5 mbar, 5 s	35	0.1–0.2 nM	[55]
Glu, GABA, Gly, Tau, D-Ser	In vivo microdialysis (rat striatum)	NBD-F	100 mM borate, 20 mM HP-β-CD, pH 10.5	LIF, 488 nm	CZE, 22 kV, 25 °C	5 µm, 6.7 cm	Electrokinetic, –20 kV, 1 s	0.5	5.1–85 nM	[56]
Leu, Ile, Val	Ascites	NDA/CN	1.5 % m/v PEO, 10 mM borate buffer, pH 9.3	LED-IF	CZE, 20 kV, 25 °C	75 µm, 50/60 cm	Gravity, 30 cm, 5 s	7	10.6–10.9 nM	[57]
Glycine, taurine, D-serine, and glutamate	Astrocytes in vitro microdialysis	NBD-F	100 mM borate, 20 mM HP-β-CD, pH 10.5	LIF, 488 nm	CZE, 20 kV, 25 °C	40 µm, 50/60 cm	Electrokinetic, 18 kV, 0.7 s	0.5	100–250 nM	[58]
Tyr, Phe, Val, Pro, Gly, pyroglutamic acid, sarcosine	Urine	FITC	130 mM 18-crown-6, 80 mM borate buffer, pH 8.70	LIF, 488 nm	CZE, 24 kV, 25 °C	25 µm, 70/90 cm	Electrokinetic, 24 kV, 10 s	45	5–10 nM	[59]
Arg, Lys, Trp, Phe, Gln, Gaba, Asn, Pro, Ser, Ala, Tau, Gly, Glu, Asp	Dialysate of hypothalamus extracellular fluid (rats)	DTAF	5 mM HP-β-CD, 5 mM DM-β-CD, 100 mM SDS, 15 mM borate, pH 9.0/4 % isopropanol	LIF, 488 nm	MEKC, 17.5 kV, 25 °C	75 µm, 50/57 cm	Hydrodynamic, 0.5 psi, 5 s.	24	0.12–0.54 nM	[60]

(continued)

Table 1
(continued)

Analytes	Matrix	Labeling	BGE	Detection	Separation method	Capillary (i.d., effective/ total lenght)	Injection	Run time (min)	L.O.D.	Ref.
Arg, His, Lys, Met, Phe, Val, Ser, GABA, Tyr, Ala, Gly, Glu, Asp, taurine	Diabetic vitreous	CBQCA	0.6% PEO, 20 mM NaCl, 75 mM SDS, 55 mM β-CD, 20 mM borate buffer, pH 9.3	LIF, 488 nm	MEKC, 17 kV, 25 °C	50 µm, 30/40 cm	Gravity, 30 cm, 5 s	6	–	[61]
Ala, Pro, Gly, Val, Ile, Leu, Tyr, Gln, Trp, His, Met, Ser, Thr, Phe, Asn, Lys, Cys, Glu, Asp, Arg	Urine	No	150 mM ammonium perfluorooctanoate, pH 9.0	ESI-MS	MEKC, –22.5 kV, 25 °C	50 µm, 90 cm	Hydrodynamic, 30 mbar, 5 s	20	9–26 µg/l	[62]
Gly, Ser	Brain microdialysate (rat)	On-line, OPA	40 mM Borate, 0.9 mM HP-b-CD	LIF, 351 nm	CZE, 25 °C	–	–	15	–	[63]
Gly, Ala, Val, Leu, Ile, Met, Phe, Tyr, Trp, Ser, Pro, Thr, Cys, Asn, Gln, Lys, His, Arg, Asp, Glu	Blood	No	1 M formic acid	ESI-MS	CZE, 30 kV, 20 °C	20 µm, 115 cm	Hydrodynamic, 50 mbar, 6 s	35	1.0–14 fmol inj	[64]
Pro, Thr, Ser, Tyr, Ala, Val, Gly, Met, His, Ile, Leu, Phe, Glu, Lys, Asp, Arg	Protein hydrolysate	PITC	168.3 mM SDS, 29 mM PBS, pH 7.4	UV, 200 nm	MEKC, 25 kV, 26 °C	75 µm, 80/87 cm	Hydrodynamic, 0.6 psi, 10 s	30	100 fmol inj	[65]
Glu, Asp, GABA	Spinal dorsal horn microdialysate	NDA/CN	70 mM SDS, 10 mM HP-b-CD, 75 mM borate buffer, pH 9.2	LIF, 442 nm	MEKC, 25 kV, 25 °C	50 µm, 52/63 cm	Hydrodynamic, 0.6 psi, 10 s	10	1–3.7 nM	[66]

Table 2
Chiral separation of AAs enantiomers by CZE and MEKC

Analytes (D/L)	Matrix	Labeling	a. BGE b. Chiral selector	Detection	Separation method	Capillary (i.d., effective/total lenght)	Injection	Run time (min)	a. L.O.D. b. Resolution of AAs enantiomeric pairs	Ref.
Ser, Ile, Met	Standards	Dns-Cl	a. 5.0 mM NH_4Ac, 100.0 mM Tris/borate buffer, pH 8.2 b. 3 mM $ZnSO_4$, 6 mM L-Arg, 20 mM BMIC	UV, 254 nm	CLE-CE, –20 kV, 25 °C	50 µm, 50/65 cm	Gravity, 15 cm, 10 s	60	b. 4.18 (Ser), 6.44 (Ile), 4.18 (Met)	[67]
Trp, Tyr, Phe	Enzyme catalytic activity	No	a. 5.0 mM NH_4Ac, 100.0 mM boric acid, pH 8.2 b. 3 mM $ZnSO_4$, 6 mM L-Orn	UV, 254 nm	CLE-CE, –20 kV, 20 °C	50 µm, 50/57 cm	Gravity, 15 cm, 5 s	25	a. 6.5 µg/ml (D,L-Trp) b. 2.52 (Trp), 1.01 (Tyr), 3.62 (Phe)	[68]
Ala, Asn, Asp, Ile, Met, Ser, Phe, Thr, Tyr	Standards	Dns-Cl	a. 25 mM Cu(Ac)2, pH 4.0/20% Methanol b. 50 mM [L-Pro-CF3COO]	UV, 254 nm	CLE-CE, 20 kV, 25 °C	75 µm, 40/60 cm	Gravity, 15 cm, 8 s	60	b. 0.93 (Ser)—6.72 (Asp)	[69]
Ala, Asn, Asp, Cys, Glu, Ile, Leu, Lys, Met, Orn, Phe, Ser, Thr, Trp, Tyr, Val	Serum samples	Dns-Cl	a. 100 mM boric acid, 5 mM NH_4Ac, pH 8.4 b. 3 mM $ZnSO_4$, 6 mM L-Arg	UV, 214 nm	CLE-CE, –20 kV, 25 °C	50 µm, 50/57 cm	Hydrodynamic, 0.5 psi, 10 s	24	a. 9 µM b. 0.9 (Val)—5.1 (Cys)	[70]
Ala, Arg, Asn, Ile, Met, Ser, Phe, Thr, Tyr, Cys, Leu, Pro, Trp, Val	L-amino acid oxidase kinetic	No/Dns-Cl	a. 100 mM boric acid, 5 mM NH_4Ac, pH 8.1 b. 4 mM β-CD, 4 mM $ZnSO_4$, 8 mM L-valine	UV, 254 nm	CLE-CE, 21 kV, 20 °C	50 µm, 50/65 cm	Gravity, 15 cm, 5 s	70	a. 8 mg/l b. 1.05 (Tyr), 1.57 (Trp), 2.24 (Phe); 0.6 (Dns-Arg)—2.16 (Dns-Thr)	[71]
Asn, Ile, Met	L-amino acid oxidase kinetic	Dns-Cl	a. 100 mM boric acid, 5 mM NH_4Ac, pH 8.4 b. 6 mM [1-ethylpyridinium] [l-lysine], 3 mM $ZnSO_4$	UV, 254 nm	CLE-CE, –21 kV, 25 °C	75 µm, 50/65 cm	Gravity, 15 cm, 8 s	50	a. 13.40 µM (D,L-Met) b. 3.0 (D,L-Asn), 3.9 (D,L-Ile), 2.4 (D,L-Met)	[72]

(continued)

Table 2
(continued)

Analytes (D/L)	Matrix	Labeling	a. BGE b. Chiral selector	Detection	Separation method	Capillary (i.d., effective/total lenght)	Injection	Run time (min)	a. L.O.D. b. Resolution of AAs enantiomeric pairs	Ref.
Ser, Ile, Met, Asn	Standards	Dns-Cl	a. 5.0 mM NH_4Ac, 100.0 mM Tris/borate buffer, pH 8.2 b. 4 mM $ZnSO_4$, 8 mM L-phenylalaninamide	UV, 254 nm	CLE-CE, −23 kV, 25 °C	50 μm, 50/57 cm	Gravity, 15 cm, 10 s	50	b. 1.9 (Ile), 1.7 (Met), 2.3 (Ser), 1.1 (Asn)	[73]
His, Phe, Trp, and Tyr	Standards	No	a. 15 mM Cu(Ac)2, pH 4.0/30 % MeOH b. 30 mM 1-hexyl-3-methylimidazolium L-proline, 15 mM Cu(Ac)2	UV, 200 nm	CLE-CE, 30 kV, 25 °C	50 μm, 40/50 cm	Hydrodynamic, 0.5 psi, 5 s	22	b. 1.34 (Hys)—4.27 (Tyr)	[74]
Ser, Ala, Ile, Leu, Glu, Asp	Cells (human and rat)	FITC	a. 80 mM Borate, pH 9.3 b. 0.5–1 mM HP-β-CD	LIF, 488 nm	CZE, 15 kV (0–44 min) then 22 kV	50 μm, 50/60.2 cm	Hydrodynamic, 0.5 psi, 10 s	76	a. 0.1 μM b. 1.03 (Ile)—1.85 (Ser)	[75]
AAs	Standards	FMOC	a. 50 mM Tris-H_3PO_4 buffer, pH 6.0 b. 2 mM vancomycin	UV, 214 or 254 nm.	CZE, 15 kV, 20 °C	PDMA-coated, 50 μm, 39.2 cm	Hydrodynamic, 0.2 psi, 5 s	4.2	b. 2 (Ser)—8 (Ala)	[76]
Ala, Aba, Nva, Val, Nle, Leu, Aca, Phe, Ser, Thr, Asp, Glu	Standards	Dns-Cl	a. 50 mM PBS, pH 8.0 b. 3 mM MMI-β-CDCl	UV, 214, 254, and 280 nm	CZE, 15 kV, 25 °C	50 μm, 59.2 cm	Hydrodynamic, 0.5 psi, 5 s	30	a. 0.8 mg/l (D-Ala), 1.5 mg/l (D-Ser) b. 0.82 (Ser)—3.02 (Phe)	[77]
D-Ser	Retinal ganglion cells (mouse)	FNBDA	a. 165 mM borate, pH 10.2 b. 34 mM HP-β-CD	LIF, 488 nm	CZE, −15 kV, 25 °C	50 μm, 30/40.2 cm	Hydrodynamic, 0.5 psi, 5 s	30		[78]
Ser, Thr, Met, Asp Val, Glu, norvaline	Standards	Dns-Cl	a. 50 mM PBS, pH 6.0 b. MDP-β-CDCl	UV, 214, 254 and 280 nm	CZE, 15 kV, 25 °C	50 μm, 40/50 cm	Hydrodynamic, 0.5 psi, 4 s	11	b. 1.85 (Thr), 1.30 (Met), 0.88 (Ser), 1.97 (Val), 2.69 (Asp), 1.00(Glu), 3.55 (norvaline)	[79]
Ala, Asn, Asp, Cit, Gln, Glu, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val	Standards	FMOC-Cl	a. 50 mM NH_4OOCH buffer, pH 7.0 b. 10 mM vancomycin	ESI-MS/MS2	CZE, −20 kV, 25 °C	HDB-coated, 50 μm, 100 cm;	Hydrodynamic, 50 mbar, 15 s	22	a. 0.1 μM (Trp)—3.1 μM (Tyr) b. 0.8 (Thr)—3.5 (Phe, Ala)	[80]

L-Pro, L-Phe, L-Leu, L-Ile, L/D-Orn, L-Gln, L-Ala, L-Thr, Gly, L/D-Ser, Tau and L-Glu	Plasma	NBD-F	a. 175 mM borate buffer, pH 10.25 b. 12.5 mM β-CD	LIF, 488 nm	CZE, 21 kV, 17 °C	75 μm, 60 cm	Hydrodynamic, 0.5 psi, 10 s	22	a. 69.5 nM (D-Ser), 38.3–433 nM (L- Aas)	[81]
Gly, L/D-Ser and taurine in urine. L-Gln,Gly, L-Glu and L-Asp in hippocampus tissue	Urine, hippocampus tissue	NBD-F	a. 90 mM borate buffer, pH 10.25 b. 12.5 mM β-CD	LIF, 488 nm	CZE, 21 kV, 25 °C	75 μm, 50/60 cm	Hydrodynamic, 0.5 psi, 10 s	20	a. 99–263 nM (human urine samples); 34.9–163 nM (hippocampus extracts)	[82]
L-Glu, L-Asp, D-Ser, L-Thr, L-Gln	Hippocampus slices (rats)	NDA/CN	a. 25 mM PBS, pH 2.15 b. 20 g/l sulfated β-CD	LIF, 420 nm	CZE, –21 kV, 25 °C	25 μm, 45/48 cm	Hydrodynamic, 380 mbar, 1 s	6	a. 14.7 nM (L-Glu), 16.0 nM (L-Asp), 19.3 nM (D-Ser), 26.2 nM (L-Thr), 12.5 nM (L-Gln)	[83]
Ser	Microdialysate (extract during ischemia/ reperfusion in rats)	FITC	a. 15 mM borate (pH 10.2) containing 5 % (v/v) methanol, 70 mM SDS b. 17.5 mM HP-β-CD and 5 mM DM-β-CD	LIF, 488 nm	CZE, 22.5 kV, 25 °C	75 μm, 50/57 cm	Hydrodynamic, 0.5 psi, 5 s	20		[84]
Asp, Glu	Brain (chicken)	4-Fluoro-7-nitro-2,1,3-benzoxadiazole	a. 100 mM Borate, pH 8 b. 8 mM HDM-β-CD, 5 mM MHP-β-CD	LIF, 488 nm	CZE, –24 kV, 25 °C	Polyacrylamide coated, 75 μm, 50/60 cm	Hydrodynamic, 1 psi, 20 s	10	a. 17 nM (D-Asp), 9 nM (D-Glu)	[85]
Glu and Asp	Standards	5-(4,6-Dichloro-s-triazin-2--ylamino) fluorescein	a. 8 mM sodium borate, pH 8.9/10 % methanol (Asp); 10 mM sodium borate, pH 9.1/5 % methanol (Glu) b. 12 mM cholate, 0.8–1.6 % human serum albumine	LIF, 488 nm	CZE, –25 kV, 25 °C	50 μm, 50/60.2 cm	Hydrodynamic, 0.5 psi, 5 s	10.5	a. 36 mg/l (D-Asp), 38 mg/l (L-Asp), 22 mg/l (D-Glu), 23 mg/l (L-Glu) b. 2.8 (Asp), 1.8 (Glu)	[86]

(continued)

Table 2
(continued)

Analytes (D/L)	Matrix	Labeling	a. BGE b. Chiral selector	Detection	Separation method	Capillary (i.d., effective/total lenght)	Injection	Run time (min)	a. L.O.D. b. Resolution of AAs enantiomeric pairs	Ref.
Ala, His, Leu, Trp, Tyr	Standards	FITC	a. 20 mM NH_4Ac, pH 6.4 b. 0.7 mM MDMA-β-CD	UV, 200 nm	CZE, 25 kV, 25 °C	75 μm, 50/60 cm	Hydrodynamic, 0.6 psi, 8 s	12	b. 5.00 (Ala), 2.07 (His), 3.06 (Leu), 2.69 (Trp), 3.28 (Tyr)	[87]
AAs	Standards	3,5-Dichloro-benzoyl chloride	a. 25 mM PBS, pH 7.0 b. 15 mM bromobalhimycin	UV, 214 nm	CZE, −25 kV, 25 °C	eCap capillary, 50 μm, 31.5/40 cm	Electrokinetic, −3 kV, 5 s	18	b. 5.37 (Ala), 2.35 (Leu), 4.13 (Met), 1.75 (Thr)	[88]
DOPA, Phe, Tyr	Nerve cells	No	a. 200 mM formic acid b. 5 mM Sulfated β-CD	ESI-MSMS	CZE, 30 kV, 20 °C	75 μm, 80 cm	Hydrodynamic, 50 mbar, 12 s	12	a. 510 nM (D-DOPA), 480 nM (L-DOPA)	[89]
Val, Leu, Ile	Urine, plasma	NDA/CN	a. 150 mM SDS, 0.5% PEO, 20 mM Tris-borate, pH 9.0; in capillary: 100 mM Tris-borate, 150 mM SDS b. 50 mM HP-β-CD	LED-IF, 410 nm	MEKC, 10 kV, 25 °C	50 μm, 40 cm	Gravity, 20 cm, 180 s	27	a. 0.18–0.22 nM b. 1.1 (Val), 1.3 (Leu), 3 (Ile)	[90]
Ile, Thr, Leu, His, Val, Asp	CSF	NDA/CN	a. 0.6% PEO, 150 mM SDS, 150 mM Tris/borate buffer, pH 9.0; in capillary: 150 mM SDS, 150 mM Tris/borate buffer, pH 9.0 b. 60 mM HP-β-CD	LED-IF, 410 nm	MEKC, 8 kV, 25 °C	75 μm, 50/60 cm	Gravity, 20 cm, 10 s	30	a. 24 nM (D-Asp), 25 nM (L-Asp)	[91]
Asp	Cerebral ganglia	NDA/CN	a. 50 mM SDS, 50 mM borate, pH 9.4 b. 20 mM β-CD	LIF, 457.9 nm	MEKC, 20 kV, 25 °C	50 μm, 50/86 cm	-	15		[92]
Arg, Ser, Leu, Ala, Gln, Glu, Lys, Asp	CSF	FITC	a. 100 mM sodium tetraborate, 80 mM SDS, pH 10 b. 20 mM β-CD	LIF, 488 nm	MEKC, 20 kV, 30 °C	50 μm, 50/57 cm	Hydrodynamic, 0.5 psi, 3 s	45	a. 0.7 nM (L-Leu), 16.5 nM (L-Asp) b. 2.6 (Arg)—9.5 (Glu)	[93]

As already pointed out, detection is a general concern, common to all the different separation schemes. Indeed, nonaromatic nonderivatized amino acids can only be efficiently detected by means of indirect methods; upon derivatization instead, the selected dye/tag determines the appropriate or most useful detector. Derivatization can be effectively employed to overcome both the lack of a chromophore on many AAs and the interferences caused by extraneous compounds in real samples: it results in both improved detection sensitivity and selectivity. Hence the choice of a derivatization reagent is of crucial importance and high demands are therefore put on its properties. Different approaches have been devised: precapillary and in-capillary. Precapillary derivatization is time consuming as it requires batch procedures but it is affordable and widely diffused (*see* tables). In-capillary (or on-column) derivatization [24, 63] is classified into either "on-site in-capillary derivatization" or "throughout in-capillary derivatization." In the former the inlet of a separation capillary is used as a reaction chamber and the reaction is performed by introducing an analyte into the capillary between two plugs of labeling reagent. In the latter, the separations and derivatizations of analytes are performed simultaneously during the electromigration of native analytes in a separation capillary tube filled with a run buffer containing a derivatization reagent.

In the last few years, however, two detection systems have been acknowledged as valuable: contactless conductivity and above all mass spectrometry detection. Both allow detection of free amino acids without derivatization, the former is universal and does not interact with the analytes or separation system, the latter is expensive but offer great selectivity. MS detection for CE is viewed, indeed, as more universal than UV or electrochemical detection. The selectivity and specificity of MS compensate for variations in migration times of the analytes, provides molecular weight and structural information. Most important, it adds a second dimension in separation selectivity for coeluting molecules having different fragmentation patterns. This is of great importance in chiral separation of AAs where this possibility greatly enhances the capability of the technique [80, 89].

As to the background electrolyte, an impressing variety respect to the pH (from about 2 up to 12) or the nature (from acetic or formic acid to borate or phosphate buffers) is found. The electrolyte modification with organic, which improves the separation possibly because of a decrease of EOF, lower solute adsorption to the capillary and Joule heating, or cyclodextrins is often reported and specific example can be found in the tables herein.

Chiral separations of amino acids are achieved by using mainly different and differently derivatizated cyclodextrins and selected antibiotics like vancomycin. Blends of chiral selector demonstrated useful in selected applications [84–86] as well. However, application

of ligand exchange CE, the separation of two enantiomer analytes due to the difference in complex stability constants of the two ternary diastereomeric mixed complexes formed by a metal ion (often Zinc or Copper), a chiral selector (one of the L-amino acids), and the analyte is increasing [67–74].

The protocols described in the following paragraphs represent different approaches to the AA analysis and all are related to possible application to biomedical problems.

2 Materials

2.1 Analysis of the Amino Acid Standards and the Blood Samples

1. 48% Hydrogen fluoride (Merck, Darmstadt, Germany).
2. Background electrolyte (BGE): 1 M formic acid solution: dilute 1.90 ml of 98–100% Suprapur formic acid (Merck) to 50 ml with water in a volumetric flask. Store at room temperature (*see* **Note 1**).
3. Sheath liquid: 5 mM ammonium acetate (Merck) in methanol/water (50:50, v/v).
4. Preparation of the blood sample: soak a 5 mm diameter blood spot on filter paper in 100 μl of water for 10 min. Then take a 20 μl aliquot of this solution and dilute to 200 μl with a solution of acetonitrile/water/formic acid (49.9/49.9/0.2; v/v).
5. High performance capillary electrophoresis/mass spectrometry system.
6. Uncoated 115 cm long, 20 μm i.d., 150 μm o.d. fused silica capillary (Polymicro Technologies, Inc., Phoenix, AZ).

2.2 Capillary Electrophoresis Combined with Microdialysis: Analysis of Trace Amino Acids Neurotransmitters

1. Ringer solution: 140.0 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM $NaHCO_3$ at pH 7.4 (*see* **Note 1**).
2. Prepare the 1 mM γ-Aminobutyric acid (GABA), glutamate (Glu), and L-Aspartate (L-Asp) (all from Sigma-Aldrich) standard solutions in 0.1 M hydrogen chloride (prepared from 30% Suprapur hydrogen chloride, Merck) and store at 4 °C.
3. NDA solution: 3.0 mM Naphthalene-2,3-dicarboxaldehyde (NDA) (Buchs, Switzerland) in acetonitrile (hypergrade LiChrosolv, Merck)/water 50:50 v/v
4. Borate/NaCN solution: 0.5 M borate buffer pH 9.2/87 mM NaCN in water (100:20 v/v).
5. Internal standard: 0.1 mM cysteic acid in 0.1 M hydrogen chloride.
6. BGE: 75 mM sodium borate, 10 mM hydroxypropyl-β-cyclodextrin (HP-β-CD), 70 mM sodium dodecyl sulfate (SDS) buffer (pH 9.20). (Sigma-Aldrich) (*see* **Note 2**).

7. A microdialysis apparatus composed by a microinfusion pump and a microdialysis probe equipped with a polycarbonate ether dialysis membrane having a molecular weight cutoff of 20000 D.
8. High performance capillary electrophoresis equipped with a laser-induced fluorescence detector and Helium Cadmium laser (8 mW, 442 nm).
9. Uncoated 63 cm long, 50 μm i.d. fused silica capillary (Polymicro Technologies, Inc.). Effective length 52 cm.

2.3 Analysis of Protein Hydrolysates

1. Hydrochloric acid solution: 6 M HCl (Suprapur, Merck) containing 0.5 % (w/v) phenol (Merck) (*see* **Note 1**).
2. Triethylamine solution: mix 2 ml of 99.5 % ethanol, 2 ml of water, and 1 ml triethylamine.
3. PITC solution: mix 70 μl of 99.5 % ethanol, 20 μl of triethylamine, and 10 μl of phenylisothiocyanate (Sigma-Aldrich).
4. Bovine serum albumin (BSA, 607 residues) was obtained from Sigma-Aldrich.
5. BGE: 29 mM phosphate buffer, pH 7.4, 168.3 mM SDS (Sigma-Aldrich) (*see* **Note 3**).
6. Glass tubes for hydrolysis and derivatization.
7. High performance capillary electrophoresis with UV–vis detection.
8. Uncoated 57 cm long, 50 μm i.d. fused silica (Polymicro Technologies). The length to the detector is 50 cm.

3 Methods

The methods described herein outline the use of different electrophoretic techniques to separate and detect AAs in biomedical applications. In the first example a CE–MS system is effectively used to detect phenylketonuria and tyrosinemia, two metabolic diseases, in blood samples. A sheath-flow interface is used due to its easy and reproducible setup. It also poses less constraints on buffer used in the separation. Pressure-assisted CE also minimizes loss of resolution due to the diffusion of counter ion from the sheath liquid back into the capillary. This hyphenation, as already observed, deserves great attention: the results are interesting and the methods can be further improved, for example, separating AAs after derivatization. MS, indeed, has a greater sensitivity when higher molecular weight compounds are detected, and a simpler tuning of the spectrometer is feasible if the tag represents the main part of the molecule.

CE–LIF demonstrated the method of choice for monitoring simultaneously neurotransmitters. Its sensitivity and the low

injected volume, typical of CE, make it an ideal technique for the analysis of biological samples, such as microdialysate from discrete brain areas, whose absolute amounts are very small. No cleanup procedures are required as the dialysis membrane is not crossed by high molecular weight substances like the proteins. By selecting the proper membrane cutoff, different real samples can be analyzed without time-consuming purification procedures. Also, if the perfusate is compatible with the derivatization mixture, the derivatized AAs can be collected and promptly analyzed avoiding batch operation.

The microdialysis-CE-LIF experiment, herein described, permitted to monitor the extracellular concentration of neurotransmitters which have a key role in the understanding of human chemical, physiological, and behavioral events.

The last protocol provides a rapid and sensitive tool for analysis of amino acids in polypeptide or protein hydrolysates, which can find application in different fields, from protein analysis to glue identification. The agreement with the conventional methods and the better sensitivity (the needed amounts are 100–1000 times lower than those used for the ninhydrin-based determinations) made the method valuable for real samples.

3.1 CE-ESI-MS

1. Electrophoresis sample to sample holder SI:A1. In some instruments for electrical reasons, the outlet terminal in normal mode becomes the inlet terminal with the external adapter.
2. BGE (2.0 ml) in sample holder position BI:A1.
3. 1 M NaOH solution (2.0 ml) in sample holder position B1:D1 and water (2.0 ml) in sample holder position BI:E1; place an empty vial in sample holders BI:C1.
4. Fill the syringe with the sheath liquid solution and place it in its holder on mass spectrometer.
5. Before the run, rinse at high forward pressure (20 psi) the capillary sequentially with NaOH (1 min), water (1 min), and electrophoresis buffer (4 min) (*see* **Note 4**)
6. CE programmed to inject electrophoresis sample for 5 s at low pressure (0.5 psi, 3.45 kPa).
7. The conditions used in the CE were as follows: voltage 30 kV, temperature 25 ± 0.5 °C, pneumatic assistance to classical electrophoretic driving force, 10 psi (*see* **Note 5**).
8. 1.5 kV were applied to the CE outlet/ESI electrode and the heated capillary used in these measurements is kept at 200 °C. The source temperature is maintained at 80 °C and nitrogen is used for both nebulizing (35 l/h) and drying (100 l/h). The sheath liquid flow at a flow rate of 5 μl/min is provided by the mass spectrometer controlled syringe pump (*see* **Note 6**).

9. Set up the mass spectrometer detector to scan the m/z range between 74 and 250 amu under positive ionization mode at unit mass resolution to monitor free AAs.
10. The UV detector, located 20 cm from the capillary injection end, can be operated continuously at 200 nm for coarse control of analyte migration.
11. Figure 1 shows the electropherograms of blood sample of both healthy and afflicted individuals.

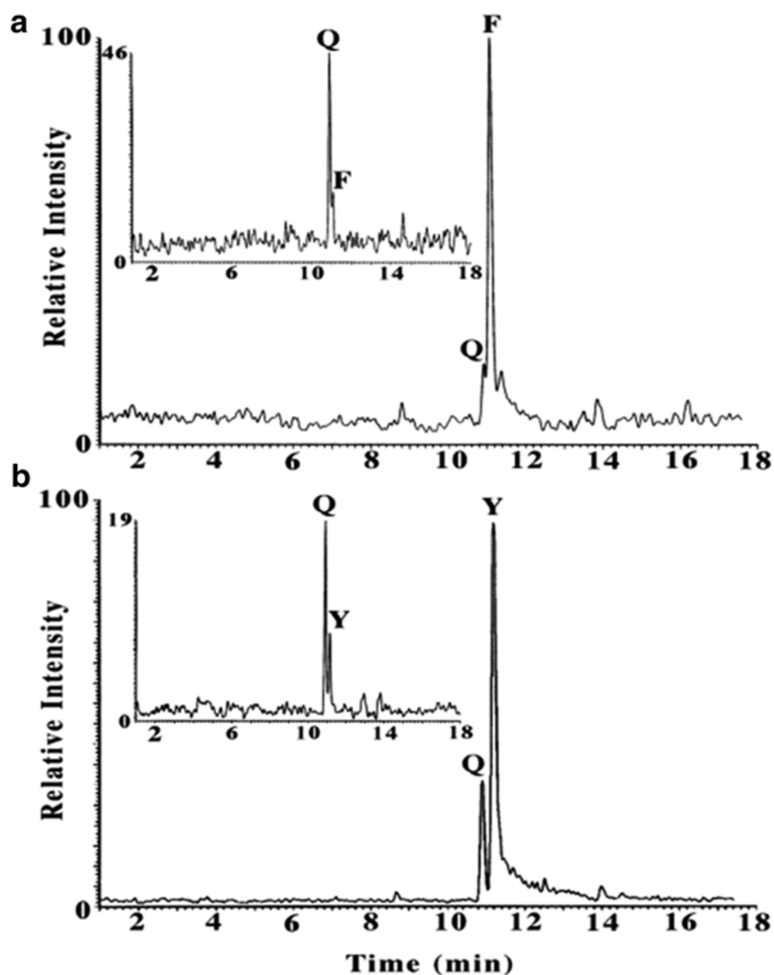

Fig. 1 Analysis by CE–MS. (**a**) Amino acid analysis of the blood of an individual afflicted with PKU and its comparison to that of a healthy one (inset). (**b**) Amino acid analysis of the blood of an infant afflicted with tyrosinemia. The *inset* contains the electropherogram of the blood of a healthy individual. Reprinted with permission from [64]. Copyright 2003 American Chemical Society

3.2 CE-LIF

1. Perfuse the microdialysis probe with the Ringer's solution at high flow rate (10 μl/min) for 1 h, then lower the flow rate to 2 μl/min and implant the probe. Monitor the basal level of the analytes for at least 30 min before stimulus.
2. Collect the perfusate fraction in microvials every 1 min (2 μl sample volume) and immediately store each of them at −40 °C before derivatization. Stop the fraction collection 30 min after the stimulus.
3. After recovery to room temperature, derivatize the microdialysate as follows: add 0.2 μl of the internal standard, 0.4 μl of the borate/NaCN, and 0.2 μl of the NDA solutions to the sample (2 μl). Let the mixture react for about 1 h (*see* **Note 7**).
4. Electrophoresis buffer (2 × 2.0 ml) in sample holder position BI:A1 and BO:A1. Electrophoresis sample in sample holder SI:A1. Standard solution in sample holder SI:B1.
5. 0.25 M NaOH solution (2.0 ml) in sample holder position B1:D1 and water (2.0 ml) in sample holder position BI:E1; place empty vials in sample holders BI:C1 and BO:B1.
6. CE programmed to inject electrophoresis sample for 10 s at 0.5 psi.
7. The conditions used in the CE were as follows: voltage, 25 kV, temperature 25 ± 0.5 °C. The excitation was performed with a Helium Cadmium laser (8 mW, 442 nm) whereas the fluorescence emission intensity was recorded at 490 nm.
8. Between runs, rinse at high pressure (20 psi) the capillary sequentially with 0.25 NaOH (30 s), water (1 min), and electrophoresis buffer (1 min).
9. Figure 2 shows the electropherograms relevant to the analysis of a microdialysate obtained from the spinal dorsal horn, a standard solution, and a brain dialysate from a rat striatum.

3.3 CE-UV

1. Hydrolysis of Proteins and Peptides: vacuum dry the solution of proteins or peptides in 5 × 35-mm glass tubes. Then add to each tube 40 μl of hydrochloric solution. Evacuate and flame seal the tubes. Put the tubes in an oven at 110 °C for 24 h. After opening of the tubes, dry with a gentle nitrogen flow (*see* **Note 8**).
2. Derivatization with phenylisothiocyanate: add to each tube 40 μl of triethylamine solution, vortex shortly and evaporate (*see* **Note 9**). Then add 3 μl 50% ethanol to each tube followed by subsequent addition of 7 μl of PITC solution. Vortex and incubate the samples for 30 min at room temperature. Dry the derivatized samples under vacuum overnight in a desiccator. Dissolve the PITC-AAs in water before CE analysis (*see* **Note 10**).

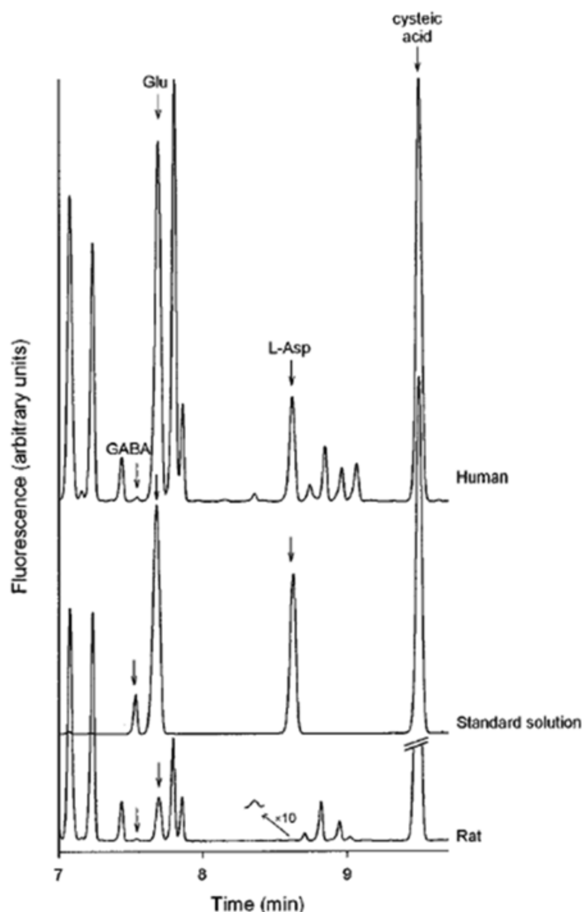

Fig. 2 Analysis by CE-LIF. Typical electropherograms of a microdialysate obtained from the spinal dorsal horn in a patient with chronic pain (*top*), a standard solution (*middle*) containing 5×10^{-7} mol/l GABA, 5×10^{-6} mol/l Glu/L-Asp compared to a brain dialysate obtained from rat striatum (*bottom*). Cysteic acid is the internal standard. Reprinted with permission from [66]. Copyright © 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

3. BGE (2 × 2.0 ml) in sample holder position BI:A1 and BO:A1. Electrophoresis sample to sample holder SI:A1.
4. CE programmed to inject electrophoresis sample for 5 s at 0.5 psi.
5. The conditions used in the CE were as follows: voltage, 27 kV, temperature 24 ± 0.5 °C. The online UV detector, located 7 cm from the capillary end, is operated continuously at 200 nm for control of analyte migration.
6. Change the BGE after each run and wash the capillary with the fresh electrolyte at least 5 min.
7. Figure 3 shows the electropherograms of a hydrolysate of BSA.

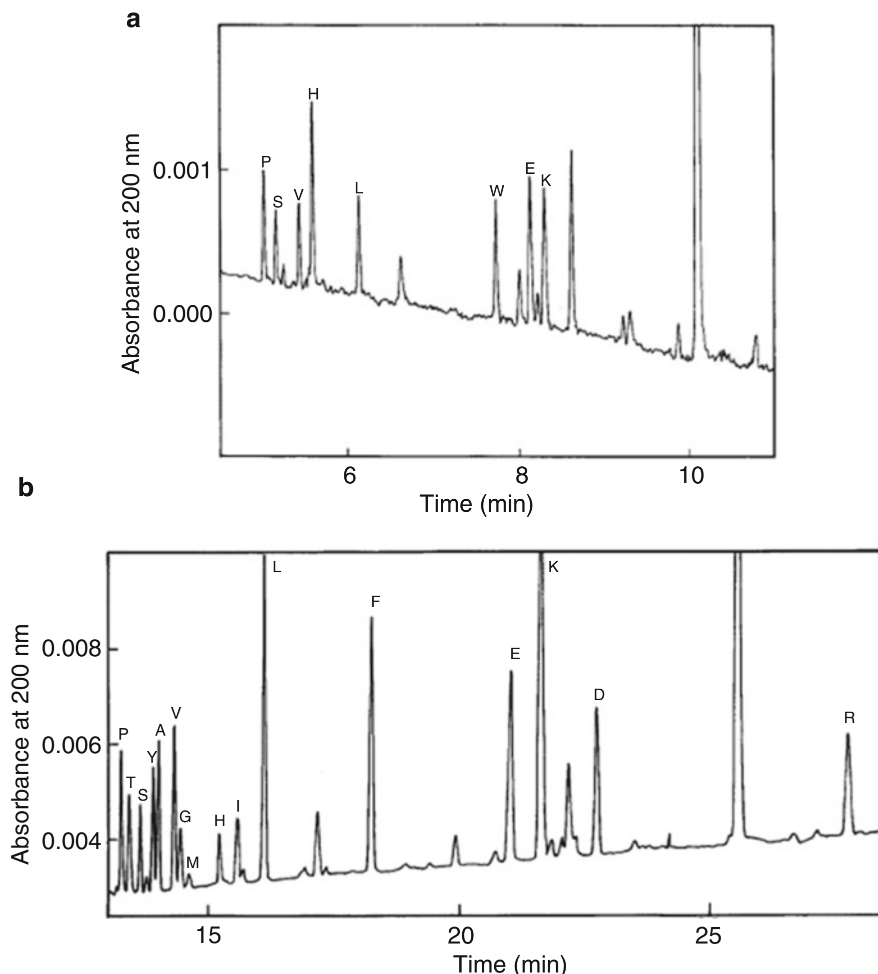

Fig. 3 Analysis by CE-UV. Capillary electrophoresis of PTC-derivatized hydrolysate amino acids. (**a**) 0.58 pmol of the 10-residue peptide SA-2, (**b**) 0.50 pmol of the 607-residue protein BSA. Reprinted from [65], Copyright 2000, with permission from Elsevier

4 Notes

1. All solutions were prepared in water that has a resistivity of 18.2 MΩ cm and total organic content of less than 5 ppb. An UltraClear system (SG Water, Hamburg, Germany) equipped with an UV lamp was used.
2. Filter the BGE through a 0.2 μm filter to prevent blockage of the CE capillary and for degassing.
3. The running buffer may conveniently be prepared by titration of phosphoric acid (Merck) with NaOH (Sigma-Aldrich).

Then dissolve the SDS and filter through 0.2-μm membranes before use. It could be stored at room temperature for at least 6 months.

4. Either a chemical or a mechanical method can be used to sharpen the outlet tip of a new capillary, before mounting it in the cartridge. If nitrile gloves and a fume hood are available, the chemical etching in 49 % hydrofluoric acid could be accomplished by soaking 2–4 mm of the capillary end for 5 min while passing nitrogen through the capillary to minimize the etching of the inner wall of the capillary. Otherwise the tip could also be sharpened mechanically with fine emery paper: in this case pay attention to the debris, not to clog the capillary. Moreover before use new capillaries should be eluted with 1 M NaOH for 2–4 h under constant pressure. At the beginning of each day the capillary should be conditioned by flushing with 1 M NaOH solution (5 min), followed by 5 min flush of water and 30 min of electrolyte solution.
5. If available use an HPLC pump as generally the baseline noise is halved.
6. With different CE equipment the pneumatic assistance, which is used to shorten analysis time, could be not available.
7. NDA is a fluorescent tag not fluorescent itself (in contrast with fluorescein isothiocyanate, for instance) and rapidly reacts to give stable fluorescent derivatives. However, since the internal standard cysteic acid reacts less quickly than Glu, L-Asp, and GABA, a reaction time of 1 h at room temperature is necessary to complete the derivatization reaction.
8. To get rid of the metal ions eventually present in the sample, it is possible to extract the proteic fraction in 6 N NH_3 first, then to dry the extract and hydrolyze it. For biomedical applications the glass tube should be pyrolyzed (400 °C, 3–4 h) before use.
9. This step is essential to remove residual hydrolysis acid.
10. Reagent mixtures were made fresh daily, stock PITC was stored at about 20 °C under nitrogen. Triethylamine and 50 % ethanol were stored at +4 °C. PTC–amino acids were stored at –20 °C.

Acknowledgement

This work was partly funded by Italian MIUR through the project PON 254/Ric./2011 (Cod. PONa3_00334). Francesca Liuzzi is gratefully acknowledged for compiling tables.

References

1. Poinsot V, Gavard P, Feurer B (2010) Recent advances in amino acid analysis by CE. Electrophoresis 31:105–121
2. Poinsot V, Carpene M-A, Bouajila J et al (2012) Recent advances in amino acid analysis by capillary electrophoresis. Electrophoresis 33:14–35
3. Poinsot V, Ong-Meang V, Gavard P et al (2014) Recent advances in amino acid analysis by capillary electromigration methods, 2011–2013. Electrophoresis 35:50–68
4. Li Z, Zhang Y, Tong F-H et al (2014) Capillary electrophoresis with laser-induced fluorescence detection of main polyamines and precursor amino acids in saliva. Chin Chem Lett 25:640–644
5. Hattori T, Fukushi K, Hirokawa T (2014) Role of counter-ions in background electrolyte for the analysis of cationgenic weak electrolytes and amino acids in neutral aqueous solutions by capillary electrophoresis with electrokinetic injection. J Chromatogr A 1326:130–133
6. Xiong X-J, Guo X-F, Ge X-X et al (2013) Determination of neurotransmitter amino acids in mouse central nervous system by CE-LIF. J Sep Sci 36:3264–3269
7. Barbosa CG, Goncalves NS, Bechara EJH et al (2013) Potential diagnostic assay for cystinuria by capillary electrophoresis coupled to mass spectrometry. J Braz Chem Soc 24:534–540
8. Jeong J-S, Kim S-K, Park S-R (2013) Amino acid analysis of dried blood spots for diagnosis of phenylketonuria using capillary electrophoresis-mass spectrometry equipped with a sheathless electrospray ionization interface. Anal Bioanal Chem 405:8063–8072
9. Ge S, Wang H, Wang Z et al (2013) Simultaneous determination of neuroactive amino acids in serum by CZE Coupled with amperometric detection. Chromatographia 76:149–155
10. Zhang N, Guo X-F, Wang H et al (2011) Determination of amino acids and catecholamines derivatized with 3-(4-chlorobenzoyl)-2-quinolinecarboxaldehyde in PC12 and HEK293 cells by capillary electrophoresis with laser-induced fluorescence detection. Anal Bioanal Chem 401:297–304
11. Tuma P, Malkova K, Samcova E et al (2010) Rapid monitoring of arrays of amino acids in clinical samples using capillary electrophoresis with contactless conductivity detection. J Sep Sci 33:2394–2401
12. Li H, Li C, Yan Z et al (2010) Simultaneous monitoring multiple neurotransmitters and neuromodulators during cerebral ischemia/reperfusion in rats by microdialysis and capillary electrophoresis. J Neurosci Meth 189:162–168
13. Wang S, Yang P, Zhao X (2009) Amino acid profile determination in the urine of bladder cancer patients by CE-MS with On-Line pH-mediated stacking and pattern recognition. Chromatographia 70:1479–1484
14. Jiang X, Xia Z, Wei W et al (2009) Direct UV detection of underivatized amino acids using capillary electrophoresis with online sweeping enrichment. J Sep Sci 32:1927–1933
15. Kaneta T, Maeda H, Miyazaki M et al (2008) Determination of amino acids in urine by cyclodextrin-modified capillary electrophoresis-laser-induced fluorescence detection. J Chromatogr Sci 46:712–716
16. Mamunooru M, Jenkins RJ, Davis NI et al (2008) Gradient elution isotachophoresis with direct ultraviolet absorption detection for sensitive amino acid analysis. J Chromatogr A 1202:203–211
17. Simionato AVC, Moraes EP, Carrilho E et al (2008) Determination of amino acids by capillary electrophoresis-electrospray ionization-mass spectrometry: an evaluation of different protein hydrolysis procedures. Electrophoresis 29:2051–2058
18. Zinellu A, Sotgia S, Zinellu E et al (2007) High-throughput CZE-UV determination of arginine and dimethylated arginines in human plasma. Electrophoresis 28:1942–1948
19. Tellez N, Aguilera N, Quinonez B et al (2008) Arginine and glutamate levels in the gingival crevicular fluid from patients with chronic periodontitis. Braz Dent J 19:318–322
20. Piyankarage SC, Augustin H, Grosjean Y et al (2010) Hemolymph amino acid variations following behavioral and genetic changes in individual Drosophila larvae. Amino Acids 38:779–788
21. Ye X, Kim WS, Rubakhin SS et al (2007) Ubiquitous presence of argininosuccinate at millimolar levels in the central nervous system of Aplysia californica. J Neurochem 101:632–640
22. Deng YH, Wang H, Zhang HS (2008) Determination of amino acid neurotransmitters in human cerebrospinal fluid and saliva by capillary electrophoresis with laser-induced fluorescence detection. J Sep Sci 31:3088–3097
23. Thongkhao-on K, Wirtshafter D, Shippy SA (2008) Feeding specific glutamate surge in the rat lateral hypothalamus revealed by low-flow push–pull perfusion. Pharmacol Biochem Behav 89:591–597

24. Denoroy L, Parrot S, Renaud L et al (2008) In-capillary derivatization and capillary electrophoresis separation of amino acid neurotransmitters from brain microdialysis samples. J Chromatogr A 1205:144–149
25. Zunic G, Spasic S (2008) Capillary electrophoresis method optimized with a factorial design for the determination of glutathione and amino acid status using human capillary blood. J Chromatogr B 873:70–76
26. Arvidsson B, Johannesson N, Citterio A et al (2007) High throughput analysis of tryptophan metabolites in a complex matrix using capillary electrophoresis coupled to time-of-flight mass spectrometry. J Chromatogr A 1159:154–158
27. Shama N, Bai SW, Chung BC et al (2008) Quantitative analysis of 17 amino acids in the connective tissue of patients with pelvic organ prolapse using capillary electrophoresis–tandem mass spectrometry. J Chromatogr B 865:18–24
28. Ramautar R, Mayboroda OA, Deelder AM et al (2008) Metabolic analysis of body fluids by capillary electrophoresis using noncovalently coated capillaries. J Chromatogr B 871:370–374
29. Ramautar R, Mayboroda OA, Derks RJ et al (2008) Capillary electrophoresis-time of flight-mass spectrometry using noncovalently bilayer-coated capillaries for the analysis of amino acids in human urine. Electrophoresis 29:2714–2722
30. Meng J, Zhang W, Cao CX et al (2010) Moving affinity boundary electrophoresis and its selective isolation of histidine in urine. Analyst 135:1592–1599
31. Zhang N, Zhang HS, Wang H (2009) Separation of free amino acids and catecholamines in human plasma and rabbit vitreous samples using a new fluorogenic reagent 3-(4-bromobenzoyl)-2-quinolinecarboxaldehyde with CE-LIF detection. Electrophoresis 30:2258–2265
32. Zhou L, Yan N, Zhang H et al (2010) Microwave-accelerated derivatization for capillary electrophoresis with laser-induced fluorescence detection: a case study for determination of histidine, 1- and 3-methylhistidine in human urine. Talanta 82:72–77
33. Sun H, Su M, Li L (2010) Simultaneous determination of tetracaine, proline, and enoxacin in human urine by CE with ECL detection. J Chromatogr Sci 48:49–54
34. Zinellu A, Sotgia S, Pisanu E et al (2010) Quantification of neurotransmitter amino acids by capillary electrophoresis laser-induced fluorescence detection in biological fluids. Anal Bioanal Chem 398:1973–1978
35. Kao YY, Liu KT, Huang MF et al (2010) Analysis of amino acids and biogenic amines in breast cancer cells by capillary electrophoresis using polymer solutions containing sodium dodecyl sulfate. J Chromatogr A 1217:582–587
36. Li MD, Tseng WL, Cheng TL (2009) Ultrasensitive detection of indoleamines by combination of nanoparticle-based extraction with capillary electrophoresis/laser-induced native fluorescence. J Chromatogr A 1216:6451–6458
37. Swann LM, Forbes SL, Lewis SW (2010) A capillary electrophoresis method for the determination of selected biogenic amines and amino acids in mammalian decomposition fluid. Talanta 81:1697–1702
38. Zhang DL, Li WL, Zhang JB et al (2010) Determination of unconjugated aromatic acids in urine by capillary electrophoresis with dual electrochemical detection – potential application in fast diagnosis of phenylketonuria. Electrophoresis 31:2989–2996
39. Desiderio C, Rossetti DV, Messana I et al (2010) Analysis of arginine and methylated metabolites in human plasma by field amplified sample injection capillary electrophoresis tandem mass spectrometry. Electrophoresis 31:1894–1902
40. Atherton T, Croxton R, Baron M et al (2012) Analysis of amino acids in latent fingerprint residue by capillary electrophoresis-mass spectrometry. J Sep Sci 35:2994–2999
41. Kok MGM, de Jong GJ, Somsen GW (2011) Sensitivity enhancement in capillary electrophoresis-mass spectrometry of anionic metabolites using a triethylamine-containing background electrolyte and sheath liquid. Electrophoresis 32:3016–3024
42. Jeong JS, Kim SK, Park SR (2012) Capillary electrophoresis mass spectrometry with sheathless electrospray ionization for high sensitivity analysis of underivatized amino acids. Electrophoresis 33:2112–2121
43. Strieglerová L, Kubáň P, Boček P (2011) Electromembrane extraction of amino acids from body fluids followed by capillary electrophoresis with capacitively coupled contactless conductivity detection. J Chromatogr A 1218: 6248–6255
44. Choi J, Choi K, Kim J et al (2011) Sensitive analysis of amino acids with carrier-mediated single drop microextraction in-line coupled with capillary electrophoresis. J Chromatogr A 1218:7227–7233
45. Knolhoff AM, Nautiyal KM, Nemes P et al (2013) Combining small-volume metabolomic and transcriptomic approaches for assessing brain chemistry. Anal Chem 85:3136–3143

46. Zinellu A, Sotgia S, Deiana L et al (2012) Simultaneous analysis of kynurenine and tryptophan in human plasma by capillary electrophoresis with UV detection. J Sep Sci 35:1146–1151
47. Tak YH, Somsen GW, de Jong GJ (2011) Optimization of dynamic pH junction for the sensitive determination of amino acids in urine by capillary electrophoresis. Anal Bioanal Chem 401:3275–3281
48. Bhowmik SK, Bong CC, Oh-Seung K et al (2012) Discovery of common urinary biomarkers for hepatotoxicity induced by carbon tetrachloride, acetaminophen and methotrexate by mass spectrometry-based metabolomics. J Appl Toxicol 32:505–520
49. Soga T, Sugimoto M, Honma M et al (2011) Serum metabolomics reveals γ-glutamyl dipeptides as biomarkers for discrimination among different forms of liver disease. J Hepatol 55:896–905
50. Kami K, Fujimori T, Sato H et al (2013) Metabolomic profiling of lung and prostate tumor tissues by capillary electrophoresis time-of-flight mass spectrometry. Metabolomics 9:444–453
51. Song L, Guo Z, Chen Y (2011) One-pot labeling-based capillary zone electrophoresis for separation of amino acid mixture and assay of biofluids. Anal Chim Acta 703:257–263
52. Zinellu A, Sotgia S, Deiana L (2013) Analysis of neurotransmitter amino acids by CE-LIF detection in biological fluids. Meth Mol Biol 919:35–42
53. Zinellu A, Sotgia S, Scanu B et al (2012) Low density lipoprotein S-homocysteinylation is increased in acute myocardial infarction patients. Clin Biochem 45:359–362
54. Hirokawa T, Okamoto H, Gosyo Y (2007) Simultaneous monitoring of inorganic cations, amines and amino acids in human sweat by capillary electrophoresis. Anal Chim Acta 581:83–88
55. Cao L, Zhang H, Hong W (2007) Analysis of amino acid neurotransmitters by capillary electrophoresis and laser-induced fluorescence using a new fluorescein-derived label. Microch Acta 158:361–368
56. Klinker CC, Bowser MT (2007) 4-Fluoro-7-nitro-2,1,3-benzoxadiazole as a fluorogenic labeling reagent for the in vivo analysis of amino acid neurotransmitters using online microdialysis-capillary electrophoresis. Anal Chem 79:8747–8754
57. Chang P-L, Chiu T-C, Wang T-E et al (2011) Quantitation of branched-chain amino acids in ascites by capillary electrophoresis with light-emitting diode-induced fluorescence detection. Electrophoresis 32:1080–1083
58. Hogerton AL, Bowser MT (2013) Monitoring neurochemical release from astrocytes using in vitro microdialysis coupled with high-speed capillary electrophoresis. Anal Chem 85:9070–9077
59. Zhang J-B, Li M-J, Li Z et al (2013) Study on urinary profile of inborn errors of metabolism by 18-crown-6 modified capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr B 929:102–106
60. Li H, Yan Z-Y (2010) Analysis of amino acid neurotransmitters in hypothalamus of rats during cerebral ischemia-reperfusion by microdialysis and capillary electrophoresis. Biomed Chromatogr 24:1185–1192
61. Lu M-J, Pulido JS, McCannel CA et al (2007) Detection of elevated signaling amino acids in human diabetic vitreous by rapid capillary electrophoresis. Experim Diab Res ID 39765
62. Moreno-Gonzalez D, Sastre-Torano J, Gamiz-Garcia L et al (2013) Micellar electrokinetic chromatography–electrospray ionization mass spectrometry employing a volatile surfactant for the analysis of amino acids in human urine. Electrophoresis 34:2615–2622
63. Li Z, Zharikova A, Bastian J et al (2008) High temporal resolution of amino acid levels in rat nucleus accumbens during operant ethanol self-administration: involvement of elevated glycine in anticipation. J Neurochem 106:170–181
64. Schultz CL, Moini M (2003) Analysis of underivatized amino acids and their D/L--enantiomers by sheathless capillary electrophoresis/electrospray ionization mass spectrometry. Anal Chem 75:1508–1513
65. Zahou E, Jornvall H, Bergman T (2000) Amino acid analysis by capillary electrophoresis after phenylthiocarbamylation. Anal Biochem 281:115–122
66. Parrot S, Sauvinet V, Xavier J-M et al (2004) Capillary electrophoresis combined with microdialysis in the human spinal cord: a new tool for monitoring rapid peroperative changes in amino acid neurotransmitters within the dorsal horn. Electrophoresis 25:1511–1517
67. Wagner Z, Tábi T, Jakó T et al (2012) Chiral separation and determination of excitatory amino acids in brain samples by CE-LIF using dual cyclodextrin system. Anal Bioanal Chem 404:2363–2368
68. Scanlan C, Shi T, Hatcher NG et al (2010) Synthesis, accumulation, and release of d-aspartate in the Aplysia californica CNS. J Neurochem 115:1234–1244
69. Lin KC, Hsieh MM, Chang CW et al (2010) Stacking and separation of aspartic acid enantiomers under discontinuous system by capillary electrophoresis with light-emitting

diode-induced fluorescence detection. Talanta 82:1912–1918
70. Samakashvili S, Ibáñez C, Simó C et al (2011) Analysis of chiral amino acids in cerebrospinal fluid samples linked to different stages of Alzheimer disease. Electrophoresis 32:2757–2764
71. Sullivan SJ, Miller RF (2010) AMPA receptor mediated D-Ser release from retinal glial cells. J Neurochem 115:1681–1689
72. Kirschner DL, Wilson AL, Drew KL et al (2009) Simultaneous efflux of endogenous D-Ser and L-Glu from single acute hippocampus slices during oxygen glucose deprivation. J Neurosci Res 87:2812–2820
73. Lorenzo MP, Villasenor A, Ramamoorthy A et al (2013) Optimization and validation of a capillary electrophoresis laser-induced fluorescence method for amino acids determination in human plasma: application to bipolar disorder study. Electrophoresis 34:1701–1709
74. Huang Y, Shi M, Zhao S et al (2011) Trace analysis of d-tyrosine in biological samples by microchip electrophoresis with laser induced fluorescence detection. J Chromatogr B 879:3203–3207
75. Li C, Yan Z, Yang J et al (2010) Neuroprotective effects of resveratrol on ischemic injury mediated by modulating the release of neurotransmitter and neuromodulator in rats. Neurochem Int 56:495–500
76. Yuan B, Wu H, Sanders T et al (2011) Chiral capillary electrophoresis–mass spectrometry of 3,4-dihydroxyphenylalanine: evidence for its enantioselective metabolism in PC-12 nerve cells. Anal Biochem 416:191–195
77. Singh NS, Paul RK, Sichler M et al (2012) Capillary electrophoresis–laser-induced fluorescence (CE-LIF) assay for measurement of intracellular D-serine and serine racemase activity. Anal Biochem 421:460–466
78. Qi L, Yang G (2009) On-column labeling technique and chiral ligand-exchange CE with zinc(II)-L-arginine complex as a chiral selector for assay of dansylated D, L-amino acids. Electrophoresis 30:2882–2889
79. Lorenzo MP, Navarrete A, Balderas C et al (2013) Optimization and validation of a CE-LIF method for amino acid determination in biological samples. J Pharm Biomed Anal 73:116–124
80. Tseng WL, Hsu CY, Wu TH et al (2009) Highly sensitive detection of chiral amino acids by CE based on on-line stacking techniques. Electrophoresis 30:2558–2564
81. Qi L, Qiao J, Yang G et al (2009) Chiral ligand-exchange CE assays for separation of amino acid enantiomers and determination of enzyme kinetic constant. Electrophoresis 30:2266–2272
82. Liu Q, Wu K, Tang F et al (2009) Amino acid ionic liquids as chiral ligands in ligand-exchange chiral separations. Chemistry 15:9889–9896
83. Wang Z, Wang J, Hu Z et al (2007) Enantioseparation by CE with vancomycin as chiral selector: improving the separation performance by dynamic coating of the capillary with poly(dimethylacrylamide). Electrophoresis 28:938–943
84. Sanchez-Hernandez L, Dominguez-Vega E, Montealegre C et al (2014) Potential of vancomycin for the enantiomeric resolution of FMOC-amino acids by capillary electrophoresis-ion-trap-mass spectrometry. Electrophoresis 35:1244–1250
85. Peng Y, Zhang T, Wang T et al (2013) Use of neutral capillaries for the enantioseparation of N-benzoylated amino acids by capillary electrophoresis with bromobalhimycin as chiral selector. J Sep Sci 36:1568–1574
86. Zhang H, Qi L, Mu X et al (2013) Influence of ionic liquids as electrolyte additives on chiral separation of dansylated amino acids by using Zn(II) complex mediated chiral ligand exchange CE. J Sep Sci 36:886–891
87. Giuffrida A, Caruso R, Messina M et al (2012) Chiral separation of amino acids derivatised with fluorescein isothiocyanate by single isomer derivatives 3-monodeoxy-3-monoamino-β- and γ-cyclodextrins: the effect of the cavity size. J Chromatogr A 1269:360–365
88. Qi L, Yang G (2009) Enantioseparation of dansyl amino acids by ligand-exchange capillary electrophoresis with zinc(II)-L-phenylalaninamide complex. J Sep Sci 32:3209–3214
89. Xiao Y, Ong T-T, Tan TTY et al (2009) Synthesis and application of a novel single-isomer mono-6-deoxy-6-(3R,4R-dihydroxypyrrolidine)-β-cyclodextrin chloride as a chiral selector in capillary electrophoresis. J Chromatogr A 1216:994–999
90. Tang W, Ong TT, Ng S-C (2007) Chiral separation of dansyl amino acids in capillary electrophoresis using mono-(3-methyl-imidazolium) -β-cyclodextrin chloride as selector. J Sep Sci 30:1343–1349
91. Qi L, Yang G, Zhang H et al (2010) A chiral ligand exchange CE essay with zinc(II)–L-valine complex for determining enzyme kinetic constant of L-amino acid oxidase. Talanta 81:1554–1559
92. Wang S, Fan L, Cui S (2009) CE-LIF chiral separation of aspartic acid and glutamic acid enantiomers using human serum albumin and

sodium cholate as dual selectors. J Sep Sci 32:3184–3190

93. Mu X, Qi L, Zhang H et al (2012) Ionic liquids with amino acids as cations: novel chiral ligands in chiral ligand-exchange capillary electrophoresis. Talanta 97:349–354

94. Sun B, Mu X, Qi L (2014) Development of new chiral ligand exchange capillary electrophoresis system with amino acid ionic liquids ligands and its application in studying the kinetics of L-amino acid oxidase. Anal Chim Acta 821:97–102