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**ADVANCES IN THE DETERMINATION OF NON-PROTEIN AMINO ACIDS  
IN FOODS AND BIOLOGICAL SAMPLES BY CAPILLARY  
ELECTROPHORESIS**

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## **ABSTRACT**

There are hundreds of non-protein amino acids whose importance in food and biological matrices is still unknown. Many of these compounds mainly exist in food as products formed during food processing, as metabolic intermediates or as additives to increase nutritional and functional properties of food. Moreover, they have also demonstrated to play an important role in the pharmaceutical and clinical fields since they may be used therapeutically in the treatment of some pathologies and their levels may be related with some diseases. For this reason, the analysis of non-protein amino acids may provide relevant information in the food and biological fields.

This article reviews the most recent advances in the development of analytical methodologies employing capillary electrophoresis for the achiral and chiral analysis of non-protein amino acids in food and biological samples. With this aim, the most relevant information concerning the separation and detection of these compounds by capillary electrophoresis is discussed and detailed experimental conditions under which their determination was achieved in food and biological samples are given covering the period of time from 2015 to 2018.

**Keywords:** Capillary electrophoresis, non-protein amino acids, food, biological samples.

## INTRODUCTION

Hundreds of amino acids are known, but only 20 of them are part of proteins. These 20 proteinogenic amino acids have been widely studied; however, there are other amino acids that are not found in protein main chain either for lack of a specific transfer RNA and codon triplet or because they do not arise from protein amino acids by post-translational modifications [1, 2]. Many of these non-protein amino acids (NPAAs) present an unknown origin and function, so it is difficult to attribute them a direct effect in the organism. Others have demonstrated to play an important role in the pharmaceutical and clinical fields since they may be used therapeutically for the treatment of some pathologies or have been related with some diseases. For instance, dihydroxyphenylalanine is used in Parkinson's disease treatment, norleucine is related with the oxidative stress associated with Alzheimer's disease (AD) and other amino acids such as  $\gamma$ -aminobutyric acid (GABA) and taurine have demonstrated to act as neurotransmitters that regulate synaptic transmission and memory [3, 4, 5]. Moreover, NPAAs can provide relevant information on food quality and safety since some of them are present in food as products formed during processing or as additives in foods to increase their nutritional value [6, 7]. Therefore, the determination of NPAAs constitutes an interesting tool to obtain information useful in the food, pharmaceutical and clinical fields. Consequently, there is a need to develop analytical methods capable to accurately determine NPAAs in real samples. Numerous works employing different techniques have been published reporting the determination of NPAAs. The most employed analytical techniques to face this challenge are High Performance Liquid Chromatography, Gas Chromatography and Capillary Electrophoresis. Among these techniques, capillary electrophoresis (CE) has emerged in the last decades as a powerful separation technique due to its versatility, high efficiency and the low reagent and

sample consumption required, among other advantages. In addition, CE has already demonstrated its potential in the analysis of NPAAAs. The most employed CE modes to analyze NPAAAs are Capillary Zone Electrophoresis (CZE) (based on the different mobility of the analyte in a conductive solution under the application of an electric field) and Micellar Electrokinetic Chromatography (MEKC) (whose separation is based on the different mobility of the analytes in a conductive solution that contains a micelle). Moreover, Electrokinetic Chromatography (EKC) and Capillary Electrochromatography (CEC) are the most employed modes to carry out the enantioseparation of chiral NPAAAs since they are based on the interaction of each enantiomer with a chiral selector present in the mobile phase (EKC) or with a chiral stationary phase (CEC). Recently, the use of microchip electrophoresis (MCE) in the analysis of NPAAAs has also become attractive. It presents some advantages over conventional systems such as the automatization, the lower sample and reagent consumption and its high efficiency [5].

The most common detection approach used in CE is the UV-Vis detector, although it requires a derivatization step since most amino acids do not have sufficient UV absorption to be detected [8]. Fluorescence detection has also been widely employed for the analysis of NPAAAs due to its high sensitivity; however, a derivatization procedure is also needed due to the lack of fluorescence of most amino acids. Many derivatization reagents such as 2,3-naphthalenedicarboxaldehyde (NDA) [9, 10, 11, 12, 13, 14], 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) [15, 16, 17, 18], fluorescein isothiocyanate (FITC) [19], 9-fluorenylmethyl chloroformate (FMOC-Cl) [20], benzoyl chloride, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), dansyl chloride (DNS-Cl) [21] and o-phthalaldehyde (OPA) [16] have been employed. An interesting alternative to these detection approaches is mass spectrometry (MS) that presents higher

sensitivity and selectivity than other systems [5] and provides structural information being not necessary a derivatization step although sometimes it is also employed.

The present article reviews the most recent advances achieved in the development of analytical methodologies for the determination of NPAAAs in foods and biological samples by CE (including CZE, MEKC, EKC, and CEC modes). Articles published in the period of time comprised between 2015 and 2018 have been considered following the previous review of the authors on the CE determination of NPAAAs in food [22]. **Tables 1 and 2** summarize the main characteristics (including CE mode, separation and detection conditions, sample treatment, LODs, and applications) of the different CE approaches developed for the analysis of NPAAAs in different samples.

## **DETERMINATION OF NON-PROTEIN AMINO ACIDS IN FOODS BY CE**

The determination of NPAAAs in food provides relevant information about food quality and safety. Different works have demonstrated the importance of analyzing NPAAAs in food to detect adulterations [21], to evaluate nutritional quality of foods [24, 26] or to detect toxic effects [15, 19], among others. When the NPAAAs of interest are chiral, their enantiomeric determination can also be a powerful tool to obtain valuable information on the effects of food processing or storage or on the presence of adulterations [42]. **Table 1** shows that a wide variety of food matrices were analyzed including beverages (juice, milk, beer, water or functional drinks), vegetables, fermented products or shellfish. The analysis of NPAAAs was mainly achieved using CZE and MEKC, although CEC and MCE were also employed. The detection systems most frequently used were UV and LIF detectors, despite of being necessary the use of a derivatization step. Other detectors less employed were mass spectrometry (MS) and capacitively coupled contactless conductivity (C<sup>4</sup>D). Li et al. [21] developed a MEKC methodology enabling

to carry out the simultaneous determination of hydroxyproline and hydroxylysine in different food samples (see **Table 1**). Both NPAAAs are relevant components of protein collagen and they may be present in numerous food products. The developed MEKC methodology was applied to find out differences between authentic and fake plastron-derived functional food product based on their amino acids profiles since this product can be adulterated with some low-priced materials. The MEKC-UV method based on the use of Dns-Cl as labeling reagent and 20 mM sodium tetraborate (pH 8.7) containing 0.1 M SDS and 6% methanol as BGE enabled the simultaneous separation of 18 protein amino acids, hydroxyproline and hydroxylysine, and their by-products formed during derivatization. Also, by using principal component analysis (PCA), hydroxyproline and hydroxylysine were selected as markers to discriminate between the authentic plastron and the adulterated one since these amino acids were not present in the other low-priced materials as it can be seen in **Figure 1** [21]. Moreover, the determination of hydroxyproline along with the 20 protein amino acids and cysteine in passion fruit juices was also performed by Passos et al. [20]. In this case, they proposed a MEKC approach with UV detection, using a 60 mM sodium tetraborate buffer (pH 10.1) containing 30 mM SDS and 5 % methanol, and FMOC as labeling reagent. The combination of the electrophoretic approach with PCA allowed to provide the characterization of different types of juices showing the potential to detect adulterations on industrial juice samples.

Other group of NPAAAs analyzed by CE in the last years are betaines. Betaines are a group of amino acids derivatives whose structure presents a quaternary ammonium group with a permanent positive charge and a carboxylic group. These compounds are known to present osmoregulating properties in many plants to protect them from the environmental stress [43]. L-carnitine and its main ester, acetyl-L-carnitine were the

betaines analyzed in the period covered by this review. They are found in different mammalian tissues, plants and microorganisms and they play an important role in fatty acid metabolism. Carnitine is produced in low levels in humans, so it may be supplied from diet [23]. Therefore, the development of analytical strategies capable of determine the content of these compounds in foods is required. Kong et al. [23] developed a new CZE method with indirect UV detection using 3.0 mM melamine and 10 % MeOH (pH 2.1) as BGE to quantify L-carnitine and acetyl-L-carnitine in liquid milk samples. An orthogonal experimental design ( $5^3$ ) was employed to investigate and optimize the BGE pH and composition (melamine concentration and percentage of methanol). The LODs achieved for carnitine and acetyl-L-carnitine were 3.0 y 5.0  $\mu\text{M}$ , respectively. As it can be seen in **Figure 2** the method was applied to the analysis of milk using the indirect UV detection since under normal CZE conditions with direct UV detection, carnitine cannot be detected. Thus, 14 kinds of milks were analyzed showing carnitine contents from 43.6 to 121.5  $\mu\text{M}$  and acetyl-L-carnitine contents from 17.5 to 68.5  $\mu\text{M}$ .

In the period of time covered by this review, only one work reported the use of CE to determine GABA and citrulline in food samples. Besides being an important neurotransmitter in mammals, GABA has also demonstrated to present other physiological functions as regulator of cells, hormones and blood pressure, among others [44]. Citrulline is precursor of the protein amino acid arginine and it is known to be involved in urea and NO cycles [45]. As it can be seen in **Table 1**, a MEKC methodology with LIF detection was developed by Qingfu et al. [9] using a flow-gated CE coupled with alternate injections (electrokinetic injection -5kV for 0.3 s) in a micro-fabricated switch to carry out the simultaneous determination of 17 protein amino acids, GABA, citrulline, phosphoryl ethanolamine (PEA) and ethanolamine (ETA) in beers. The use of NDA as labeling reagent and 40 mM sodium tetraborate containing 60 mM



SDS and 2 mM HP- $\beta$ -CD as running buffer (pH 9.2) allowed a high separation efficiency for all these compounds within 90 s using a capillary length of 10 cm. The LODs obtained for amino acids, PEA and ETA with the proposed methodology were from 2.0 to 5.0 nM. The quantitative results obtained in eight different brands of beer showed that GABA, alanine and valine were the most abundant amino acids in all samples whereas citrulline, glutamine and methionine were the less abundant (indeed, the content of citrulline was lower than 40  $\mu$ M in all beer brands analyzed). These differences in amino acids composition were in agreement with the differences among the characteristics of the samples (i.e. differences in flavor, raw materials, processing or enzyme activity) [9].

Sacristán et al. [24] developed a CZE methodology to analyze homoarginine and  $\beta$ -N-Oxalyl-L- $\alpha,\beta$ -diaminopropionic acid, that are the main NPAAAs in grass pea seeds (*Lathyrus* species). *Lathyrus* species are a rich source of proteins and are cultivated for human consumption. However, a high consumption of these species may produce a disease known as “lathyrism” responsible for humans and animal’s paralysis. The scientific committee of the Spanish Agency for Food safety and Nutrition recommends an occasional consumption of *Lathyrus* being the safe consumption lower than 1.5 mg/g for humans, but further research needs to be performed to ensure these safety values [46]. The developed CZE method was based on the use of a BGE (pH 9.2) containing 25 mM sodium borate and 5 mM sodium sulfate, and UV detection, and enabled the simultaneous determination and quantification of homoarginine and  $\beta$ -N-Oxalyl-L- $\alpha,\beta$ -diaminopropionic acid in *L. sativus* (grass pea) and *L.cicera* (red pea). Sample preparation was carried out by two different extraction protocols based on the use of a rotating shaker (24 h) and an Ultra-Turrax (1 min) with ethanol:water (60:40 v/v) as extraction solvent. Despite of the fact that no significant differences were found

between the two extraction protocols, the Ultra-Turrax method, which is simpler and faster, and demonstrated to provide higher yield results than the rotating shaking method, was selected to analyze all samples. Different *Lathyrus cicero* and *Lathyrus sativus* species were analyzed to evaluate the levels of homoarginine and  $\beta$ -N-Oxalyl-L- $\alpha,\beta$ -diaminopropionic acid showing that homoarginine contents (from 8.08 mg/g to 12.44 mg/g) were higher than the contents of  $\beta$ -N-Oxalyl-L- $\alpha,\beta$ -diaminopropionic acid (from 0.79 to 5.05 mg/g) in all samples. Moreover, the results obtained showed that  $\beta$ -N-Oxalyl-L- $\alpha,\beta$ -diaminopropionic acid levels for *L.cicera* species were lower than the recommended ones but this was not the case for *L.sativus* species, whose values exceeded those recommended [24].

Taurine is the only sulfur-containing amino acid analyzed in food by CE in the reviewed period. This NPAA can be found in mammalian tissues in high concentration levels and it presents important physiological and therapeutic functions such as bile acid conjugation, maintenance of calcium homeostasis, [47], liver protection, and treatment of low blood pressure [48]. Taurine is the most employed component in the formulation of energy and sport drinks that have gained popularity among athletes as a consequence of their energetic properties. However, the consumption of these beverages is not recommended for people with heart diseases or high blood pressure, and high levels of taurine intake may produce undesirable effects also in healthy people [49]. Therefore, the development of analytical strategies enabling the determination of taurine in foods is needed. As it can be seen in **Table 1**, taurine was determined using different modes of CE such as CZE, MEKC and MCE. A CZE method with  $C^4D$  was employed to determine taurine in energy drinks. The results obtained with an instrument with a coaxial flow-gating interface (FGI) were compared with those obtained with an Agilent commercial equipment showing similar LODs (14.4 mg/mL and 8.2 mg/mL,

respectively). Both methods were able to determine lower percentages of taurine than the declared value (4000 mg/L). The coaxial FGI presents some characteristics comparable with common commercial CE instrument such as repeatable sample injection and improved total analysis time (73 s and 225 s, respectively). As **Table 1** shows, the separation was achieved using 20 mM CHES and 10 mM NaOH (pH 9.5) as separation buffer and only a 40-fold dilution step of samples was needed to analyze them by CE [26]. Wu et al. [27] developed a methodology using MCE with LIF detection to determine two amino acids (taurine and lysine) and vitamin B<sub>3</sub> in functional drinks. The use of an on-line preconcentration strategy combining field-amplified sample stacking (FASS) and reverse-field stacking allowed to improve the sensitivity and the separation efficiency in comparison with conventional MCE-LIF method. After optimizing different electrophoretic and derivatization variables, the use of 100 mM sodium borate (pH 9.88) as running buffer and sulfoindocyanine succinimidyl ester (Cy5) as derivatization reagent enabled the quantification of lysine, taurine and vitamin B<sub>3</sub> (within 4 min) in eight functional drinks showing a lower concentration of taurine in one of them than the values declared in the label.

Pyroglutamic acid is an interesting cyclical NPAA that may be produced as an intermediate in amino acid metabolic and transport pathways, or during protein biosynthesis during which it becomes the amino-terminal residue of many biologically peptides and proteins [50] and it can be found as a free acid or bound at the N terminal group of proteins and peptides [51]. This NPAA is usually found in urine, plasma, bones and other tissues, and it can also be present naturally in food or can be employed in beauty or dietary formulations [52]. The only article published in the reviewed period reporting the separation of pyroglutamic acid by CE was aimed to determine lactic acid and its organic impurities in fermented products. Among these impurities, taurine and

pyroglutamic acid were the NPPAs identified [25]. The MEKC methodology developed consisted of using a 25 mM sodium tetraborate buffer containing 50 mM SDS (pH 9.1) and UV detection (200 nm). Sample treatment including an enzyme-assisted extraction procedure and a fermentation process was accomplished. Thus, ten organic acids, thirteen protein amino acids, cysteine, tryptamine, taurine and pyroglutamic acid were identified and separated from lactic acid in fermentation broth of different renewable resources. It was observed a major unknown component before the lactic acid peak in some samples and it was identified as pyroglutamic acid using MS spectra followed by the standard confirmation. The methodology enabled to detect 0.3 ppm of pyroglutamic acid in presence of 718,400 ppm of lactic acid [25].

The NPAA  $\beta$ -N-methylamino-L-alanine is a toxin present in nature which is related to many neurodegenerative pathologies such as the amyotrophic lateral sclerosis, Alzheimer's dementia, or Parkinson's disease [53]. This NPAA presents some relevant structural isomers. Three of them, namely 2,4-diaminobutyric acid (2,4-DAB), N-2(aminoethyl)glycine (AEG) and  $\beta$ -amino-N-methyl-alanine have been found in food matrices (e.g. microalgae and mollusks). The major exposure pathway to  $\beta$ -N-methylamino-L-alanine is the dietary intake so the development of high selective methods able to separate the isomers of this NPAA is crucial [54]. However,  $\beta$ -N-methylamino-L-alanine analysis may be a hard task since all its isomers present the same monoisotopic mass and similar physicochemical properties making difficult their discrimination. Recently, two different methodologies based on the use of CZE with UV and MS detection were developed by Kerrin et al. [28] enabling the separation of  $\beta$ -N-methylamino-L-alanine and four of its isomers in a mussel tissue reference material. A simple sample treatment based on protein hydrolysis in acid conditions followed by Oasis-MCX cartridge cleanup procedure without any derivatization step was employed.

To develop the CZE-UV methodology, the effect of different separation variables, such as the running buffer composition, buffer concentration, organic modifiers and pH, and some instrumental CE parameters, such as temperature and voltage, were evaluated. Under the optimized conditions (see **Table 1**)  $\beta$ -N-methylamino-L-alanine and its isomers could be separated. However, the LOD (20 mg/g, dry mass) obtained for  $\beta$ -N-methylamino-L-alanine using this method was much higher than the reported content in cyanobacteria and mussels (300  $\mu$ g/g and 10  $\mu$ g/g, respectively). Afterwards, in order to improve the sensitivity, these authors developed a new methodology by CZE-MS. First, to select compatible CE-MS conditions, the phosphate BGE was replaced by 5 M formic acid containing 10 % acetonitrile and a custom interface was built with a straight tube enclosing the CE capillary which eliminated the plugging problems previously obtained. A 50 % aqueous MeOH containing 0.1 % formic acid was used as sheath liquid. Before the analysis by CZE-MS, a strong cation exchange solid-phase extraction (SPE) sample cleanup procedure to lower the conductivity of the extract enabling FASS was achieved with a final step of redissolution in a low conductivity solvent. This approach allowed to achieve a LOD of 16 ng/g (dry mass) for  $\beta$ -N-methylamino-L-alanine enabling the quantification of this NPAA in real samples (cycad leaves, lobster tail meat and lobster tomalley) [28].

Domoic acid is other neurotoxic water soluble tricarboxylic acid which is present in numerous types of shellfish and seafoods usually consumed as part of the human diet. The consumption of high levels of domoic acid may be responsible for Amnesic Shellfish Poisoning (ASP), a disease whose symptoms are cardiac arrhythmias, abdominal cramps and neurological dysfunction, among others. Therefore, analytical methods are needed to assess a safe content of this compound in food [55]. A CEC method, based on the use of a packed capillary column with octadecyl silica (ODS)

particles (using a supplementary pressure) and LIF detection, was employed to analyze traces of domoic acid in shellfish samples. As it can be seen in **Table 1**, this is the only work in which CEC was applied to the analysis of NPAAAs in the period of time reviewed in this article. A solid-liquid extraction followed by a clean-up procedure and a derivatization step with NBD-F was achieved before CEC analysis that was carried out in positive and negative voltage using 5 mM phosphate buffer containing 60 % acetonitrile (pH 2.5), enabling a LOD for domoic acid as low as 10 ng/mL [15]. The developed methodology was compared with a HPLC-MS/MS method showing similar LODs and RSD results, and better recoveries in the case of CEC. MCE was also employed to determine domoic acid in shellfish tissues. The use of a 5 mM sodium tetraborate buffer (pH 9.2), FITC as derivatizing reagent, and LIF detection, allowed the determination of domoic acid within 60 s with a LOD of  $2.8 \times 10^{-10}$  M which enabled to assess the accomplishment of the official regulatory limit of 20  $\mu\text{g}$  domoic acid /g wet tissue. The method constitutes a powerful alternative for toxin detection since it presents some advantages such as simplicity, sensitivity and high separation speed [19].

Chiral analysis of NPAAAs in food is of high interest to guarantee food quality, authenticity and safety. Although the L-enantiomer is the natural form, D-enantiomers of NPAAAs can be found in food as a consequence of racemization during food processing, microbiological processes, or the fraudulent addition of racemic mixtures in the particular case of supplemented foodstuffs [56], for which regulations establish the use of the L-enantiomer. Due to the different properties and biological activity that the enantiomers may have, their individual determination in foods present a high interest. In fact, the enantioselective determination of NPAAAs has demonstrated to play an important role to detect food adulterations [42] or to evaluate manufacturing processes [57]. During the period of time covered by this review, only one work has been

published reporting the enantiomeric separation of NPAAAs by CE in food [29]. New analytical methodologies were developed enabling the enantiomeric separation of eight NPAAAs by EKC. After FMOC derivatization, the optimized separation conditions consisted of the use of a 100 mM formate buffer (pH 2.0) and an anionic cyclodextrin (sulfated- $\alpha$ -CD or sulfated- $\gamma$ -CD depending on the amino acid). **Figure 3** shows the electropherograms corresponding to the enantiomeric separation of the NPAAAs investigated under the optimized conditions. The analytical characteristics of the developed method were shown to be adequate for the determination of L-citrulline and its enantiomeric impurity in food supplements. LODs of  $2.1 \times 10^{-7}$  M and  $1.8 \times 10^{-7}$  M were achieved for D- and L-citrulline, respectively. L-citrulline was quantified in six samples (three new and three submitted to a long storage time) where D-citrulline was not detected in any case. No racemization process took place in samples submitted to a long storage time.

## **DETERMINATION OF NON-PROTEIN AMINO ACIDS IN BIOLOGICAL SAMPLES BY CE**

From a biological point of view, the determination of NPAAAs has a special relevance since many of them are key compounds in metabolic pathways or are related with different pathologies. In fact, several diseases have shown to be related with metabolic dysfunctions which provide abnormal quantities of amino acids in body fluids. Thus, the determination of NPAAAs in different biological fluids can be used for the early detection of different cancer types [58, 34], as diagnostic tool to inspect vesicoureteral reflux samples [31], to detect an immature enzymatic system in preterm neonates [32], as indicator of ocular surface diseases [59], to assess the embryo viability in assisted reproduction [11, 10], as indicator of pathologies such as coronary artery disease,

diabetes renal insufficiency, or Alzheimer's disease [60], or even for clinical toxicology laboratory diagnostics [39]. These examples show the relevance of the determination of NPAAAs in biological fluids and the imperative need to develop high sensitive methodologies able to detect these compounds at the low levels at which they are present in biological samples. **Table 2** summarizes the main characteristics of the CE methodologies developed for the analysis of NPAAAs in the period of time reviewed in this article. As it can be observed in this table, the preferred detection mode was LIF followed by MS<sup>2</sup> and UV. The CE approaches developed have been applied to analyze a broad range of samples: urine, plasma, serum, tear fluid, saliva, human embryos, or human colon cancer and breast cells, among others (see **Table 2**).

Some of the developed CE methodologies have been applied to the simultaneous analysis of different NPAAAs, being CE coupled to LIF the approach mainly used in this kind of analysis [30, 18, 14]. For instance, Liang *et al.*, developed a MEKC-LIF methodology to achieve the simultaneous determination of homocysteine, homoarginine and five related metabolites (including ornithine and citrulline) after derivatization with 5-carboxyfluorescein succinimidyl ester (CFSE) [30]. Complete baseline separation was possible in 10 min using as BGE a 50 mM borate buffer at pH 9.5 containing 30 mM SDS and 30 % MeOH. LODs reached were between 0.12 and 1.70 nM which are much lower than other previously reported in the literature for the determination of some of the analyzed compounds by fluorescence (sensitivity was improved from 5 to 600 fold times). This methodology was fully validated using plasma and urine samples from type 2 diabetics with peptic ulcer bleeding. Also, an interesting CE-LIF method for the high-speed monitoring of branched chain amino acids uptake in 3T3-L1 cells was developed by Harstad and Bowser [18]. The interest in the measurement of these amino acids and their downstream metabolites (where GABA, ornithine, citrulline and taurine can be



included) is related to the fact that they play key roles in the tricarboxylic acid cycle and adipocyte lipogenesis. To carry out the analysis, analytes were sampled using microdialysis, on-line derivatized with a fluorescent reagent, separated by CE and detected by LIF using the device shown in **Figure 4A**. Under optimal conditions, the separation was achieved in less than 30 s as it can be seen in **Figures 4B** and **4C**. Other CE-LIF methodology developed within the time covered in this review has been proposed to achieve the simultaneous analysis of different NPAAAs (ornithine, citrulline, norvaline and norleucine) along with 17 protein amino acids in plasma [14]. This was a polymer-based separation method in the presence of mixed micelles and the analytes were derivatized with naphthalene-2,3-dicarboxaldehyde (NDA). In spite of the fact that the analysis time was too high (180 min), the high level of resolution obtained using this methodology allowed the accurate quantitation of amino acids in plasma without the need for protein filtration. Taking in mind that it has been demonstrated that free amino acids in plasma could be used for the early detection of different cancer types [59], this methodology will be suitable for clinical diagnosis using amino acids as biomarkers.

The simultaneous analysis of several NPAAAs not only was performed by CE with LIF detection but also using the hyphenation of CE with MS. Thus, the targeted assessment of amino acids in urine, including 20 protein amino acids,  $\beta$ -alanine, the dipeptide carnosine and 5 NPAAAs (GABA, ornithine, citrulline, hydroxyproline and alloisoleucine) was carried out by CE-MS<sup>2</sup> [31]. After optimizing the experimental parameters related to the CE-MS interface, BGE and MS settings, the method, based on the use of 0.8 M formic acid at pH 1.96 containing 15 % MeOH and a pH stacking procedure implemented to enhance the sensitivity (a plug of 12.5 % ammonium solution was injected before the sample), enabled the separation of the 27 analyzed compounds in less than 30 min with LODs ranging from 0.63 to 29  $\mu$ M. Once the method was

validated according to FDA and ICH guidelines, its feasibility was demonstrated by analyzing urine samples from children with vesicoureteral reflux which proved that the developed CE-MS<sup>2</sup> method could be considered as a potential auxiliary diagnostic tool to inspect vesicoureteral reflux samples.

Even though it is possible to find in the literature different works in which CE was used for the simultaneous analysis of several NPAAAs (as it has been described till now), the truth is that in most of the cases, CE analysis was focused on the determination of one or two NPAAAs, normally along with other protein amino acids. In this line, citrulline and arginine levels were determined by CE-LIF in blood samples from preterm newborns and mature neonates [32]. Based on the levels measured under the best separation conditions (see **Table 2**), it was possible to differentiate both groups of samples since significantly lower levels of both NPAAAs were found in preterm neonates which implies an immature enzymatic system in these neonates [32]. Citrulline has also been used along with taurine and other four neuroactive amines commonly found in brain dialysate samples as model compounds to design a portable microchip electrophoresis (MCE) with LIF detection [12]. The LODs achieved ranged from 250 nM to 1.3  $\mu$ M (being 0.36 and 0.42  $\mu$ M for citrulline and taurine, respectively) and were adequate for the detection of the investigated analytes at physiologically relevant concentrations.

The relation of taurine levels in biological fluids with different diseases has been pointed out by different works. For instance, it was found that it has a close relationship with the ocular surface disease so that it can be used as a useful indicator of this pathology [59]. For this reason, Du *et al.*, optimized a CE method with indirect amperometric detection to carry out the determination of taurine in tear fluid [33]. The methodology was based on the use of a 20 mM phosphate buffer containing 20 mM

SDS (pH 10.0) as BGE and a serial dual-electrode to conduct the detection. In this indirect detection mode, bromide is oxidized to bromine which reacts quantitatively and rapidly with taurine, so that the taurine concentration can be determined by the decrease of the current for bromine. Once optimized the principal experimental parameters governing the analytical performance (bromine concentration, dual-electrode potentials, and CE separation conditions), taurine was baseline separated from other interfering amino acids within 18 min. The LOD obtained for taurine by the proposed methodology (0.18  $\mu\text{M}$ ) was compared with that obtained by other detection modes, being lower than the LOD obtained by pulse amperometric detection, comparative to direct amperometric detection, and higher than the LOD obtained by LIF detection (but it requires a derivatization step which makes the process more labor-intensive) [33]. The successful application of the developed method to the determination of taurine in tear fluids makes the device attractive for clinical and biomedical applications. An interesting work recently published proposes an on-line microdialysis (MD)-CE method with LIF detection to measure the *in-vivo* dynamics of amino acids (taurine, GABA, and 10 proteinogenic amino acids) biomarkers of metabolism in adipose tissue [17]. Microdialysis probes were implanted into the inguinal adipose tissue depot of mice and the MD-CE assay enabled to monitor small molecules dynamics in near real time (22 s). The LOD obtained for taurine (which represents the concentration of taurine outside the probe and prior to the labeling reaction with 4-fluoro-7-nitrobenzofurazan (NBD-F)) was 2.7  $\mu\text{M}$ . To demonstrate the potential of the developed strategy, *in vivo* changes were assessed after administering an insulin stimulation. In this way, it could be observed that taurine, alanine and valine levels increased within 5 min of insulin delivering before return to an elevated baseline level.

In addition to the two afore-mentioned works, in which CE strategies were employed to measure the level of taurine in biological fluids, there are other three articles in which taurine, among other compounds, is analyzed by CE. On the one hand, the use of a CE-MS platform for the metabolomics analysis of saliva samples from patients with oral squamous cell carcinoma and healthy controls enabled to propose taurine along with other 24 metabolites as oral cancer specific markers [34], and on the other hand, MEKC-LIF was employed to obtain the amino acids profiles in the culture media used in embryo cultivation after *in vitro* fertilization in order to assess the embryo viability in assisted reproduction [10, 11]. In a first attempt, Celá *et al.*, used the transverse diffusion of laminar flow profiles (TDLFP) methodology to achieve the on-line derivatization of amino acids with NDA [11]. Using a BGE composed of 35 mM borate, 55 mM SDS, 2.7 M urea, 1 mM BIS-TRIS propane and 23 mM NaOH, the derivatives of 18 protein amino acids, taurine, cysteine and the dipeptide Ala-Gln were baseline resolved in 50 min. However, due to the limitations of this methodology, the method was subsequently modified to provide better separation conditions in terms of analysis time [10]. First, the BGE was changed to avoid urea and to decrease the pH of 9.8 since both effects contribute to the dissolution of carbon dioxide giving rise to a modification of the ionic strength of the BGE which increases the migration times due to an effect on the micelle-analyte distribution equilibrium. The optimum BGE, based on the use of 73 mM SDS, 6.7 % 1-propanol (v/v) + 0.5 mM HP- $\beta$ -CD + 135 mM boric acid/NaOH (pH 9.0), enabled the baseline resolution of the analytes in 46 min. Regarding the on-line derivatization, it was accomplished using electrophoretically mediated microanalysis (EMMA) which improved the LODs (a LOD of 12 nM was reached for taurine). Thus, the improved MEKC-LIF methodology was applied to the non-invasive targeted metabolomics of human embryos to find a correlation between the embryo's

developmental potential and amino acids turnover. Statistical analysis of the data showed that the discrimination between successfully and unsuccessfully implanted embryos was partial probably due to the small number of statistically significant samples [10].

Homocysteine is a low molecular weight aminothiols of high relevance in biological processes since higher levels in plasma or serum have been related with different pathologies such as coronary artery disease, diabetes renal insufficiency, or Alzheimer's disease, among others [60]. Along with homocysteine, it is also relevant to take into consideration the proteinogenic amino acid cysteine because the ratio cysteine/homocysteine reflects the bioavailability of homocysteine [61]. Ivanov *et al.*, developed a CE-UV approach based on the use of 1,1'-thiocarbonyldiimidazole (TCDI) as derivatizing reagent for determining the levels of homocysteine and cysteine in plasma [35]. In this work, the use of an electrokinetic injection with pH mediated stacking enabled to reach a LOD for homocysteine of 0.8  $\mu\text{M}$ . Subsequently, the authors improved the methodology introducing several modifications which enabled to achieve a LOD of 0.2  $\mu\text{M}$  [36]. These modifications included the use of a liquid-liquid extraction with chloroform-ACN to purify the sample and determine homocysteine and cysteine levels in urine (the previous approach was not suitable for determining both analytes in matrices like urine in which salt levels vary considerable), a different composition of the running buffer (see **Table 2**), and an in-capillary preconcentration step based on the use of field amplified sample stacking and pH mediated stacking. In this way, homocysteine and cysteine levels were determined in human plasma and urine samples from healthy subjects and patients with kidney disorders (see **Figure 5**), observing a decrease in the homocysteine levels in urine from patients with kidney disorders [36]. Other detection modes different from UV were also hyphenated with CE

to perform the determination of homocysteine in biological samples. Thus, LIF was used as detection mode in a high-sensitive CE method developed for chemical cytometry of homocysteine and other thiol compounds (cysteine, glutathione, and  $\gamma$ -glutamylcysteine) within human colon cancer (HCT-29) and breast (MCF-10A) single cells [37]. Here, 1,3,5,7-tetramethyl-8-phenyl-(2-maleimide)-difluoroboradiaza-s-indacene (TMPAB-o-M) was employed as fluorogenic probe in a post-column sheath flow cuvette. On the other hand, MS detection has demonstrated to be a powerful technique to carry out the analysis of homocysteine, cysteine, methionine, and glutamic acid in plasma by CE [38]. Since the levels of these compounds are altered in plasma of amyotrophic lateral sclerosis (ALS) patients, their determination is relevant because they could be pointed out as potential biomarkers of this disease. Prior to analyze these compounds by CE-MS<sup>2</sup>, the protein depletion of plasma samples was performed using DTT and cold acetone, and IAA was added to the sample to protect the thiol groups against oxidation. After validating the CE-MS<sup>2</sup> methodology, it was applied to the analysis of plasma samples from healthy subjects and patients with ALS, showing a significantly higher concentration of glutamic acid and cysteine in the latter group.

The analysis of other NPAAAs such as GABA, hydroxyproline, pyroglutamic acid and betaine by CE was also performed. To analyze the first one, Wang *et al.*, developed a MEKC-LIF method for measuring amino acid secretion from islets of Langerhans, an endocrine portion of pancreas responsible for helping to maintain glucose homeostasis, since they seem to play a critical role in cell functionality [13]. Under the optimized conditions for NDA derivatization and CZE separation (25 mM phosphate buffer containing 30 mM SDS at pH 8.3), GABA and other 17 proteinogenic amino acids were analyzed in 21 min allowing the quantitation of 14 of them with LODs ranging from 0.2 to 7 nM (namely the LOD obtained for GABA was 3 nM). The developed methodology

permitted to quantify the secretion amounts of amino acids from islets incubated in low or high glucose. The effect of glucose and 2,4-dinitrophenol (a pharmacological agent) in these secretions was tested observing a suppression effect of glucose on GABA release likely acting through ATP inactivation of glutamate decarboxylase [13]. 4-hydroxyproline and prolyl hydroxyproline in urine were suggested as biomarkers for bone turnover and osteoporosis, reason for why Zhang et al., proposed a flow-gated CE-LIF method to carry out the rapid determination of 4-hydroxyproline and prolyl dipeptides in urine samples. The developed methodology included the treatment of urine samples with OPA to block primary amines followed by the derivatization of the secondary ones with 4-fluoro-7-nitro-2,1,3 benzoxadiazole. Then, using a mixture of borate, cholate and deoxycholate at 40 mM each (pH 9.2) as running buffer, proline, 4-hydroxyproline and 4 propyl dipeptides were separated in just 30 s achieving LODs at the nM level [16]. An interesting investigation accomplished by Holzek et al. demonstrated the suitability of CE for clinical toxicology laboratory diagnostic [39]. High anion gap metabolic acidosis habitually complicates paracetamol poisoning and is normally attributed to lactic acidosis, renal failure or compromised hepatic function. But, it can also be produced by the accumulation of pyroglutamic acid (or 5-oxoproline). Therefore, 5-oxoprolinemia could be considered to the diagnosis of patients presenting acidosis after acute paracetamol overdose. Then, to determine paracetamol and pyroglutamic acid levels in serum samples from patients after either intentional paracetamol ingestion or therapeutic misadventures, these authors developed a CE-UV method based on a simple sample treatment and the employ of 40 mM CHES/sodium hydroxide at pH 10.2 as BGE. By using this methodology, it was possible to carry out the quantification of pyroglutamic acid in case of paracetamol overdose. Finally, Forteschi *et al.*, designed an isotope dilution CE-MS<sup>2</sup> method to

detect, for the first time, betaine, choline and dimethylglycine simultaneously in plasma samples, since they provide relevant information related to the flow of methyl groups in key biological processes, particularly in folate deficiency stages [40]. Under the experimental conditions detailed in **Table 2**, the compounds were detected in 22 min achieving LODs of 0.43, 0.62, and 0.31  $\mu\text{M}$  for choline, betaine and dimethylglycine, respectively. Based on the concentration of the three analytes measured by the application of the developed CE-MS<sup>2</sup>, it was possible to find differences between the plasma samples of healthy controls and patients with chronic kidney disease.

During the period of time covered in this review just a research work described the enantioselective determination of a chiral NPPA in a biological sample. Namely, Sánchez-Lopéz et al., developed a CE-MS<sup>2</sup> methodology for the simultaneous enantioseparation of all the chiral constituents of the phenylalanine-tyrosine metabolic pathway; the protein amino acids phenylalanine and tyrosine, the catecholamines dopamine, norepinephrine and epinephrine, and the NPPA 3,4-dihydroxyphenylalanine (DOPA)) [41]. The method, consisting of the use of 180 mM methyl- $\beta$ -cyclodextrin plus 40 mM 2-hydroxypropyl- $\beta$ -CD in 2 M formic acid (pH 1.2) as BGE and a large volume sample stacking as in-capillary preconcentration step, enabled for the first time, the simultaneous enantiomeric separation of all the chiral compounds involved in this metabolic pathway, in 90 min with LODs from 40 to 150 nM. The applicability of this methodology was demonstrated through the successful analysis of some of the compounds investigated in rat plasma samples (**Figure 6**).

## **CONCLUDING REMARKS**

This article reviews the works dealing with the determination of non-protein amino acids in food and biological samples published from 2015 to 2018. Non-protein amino



acids are related with the quality and safety of food and they have also been considered as biomarkers of some pathologies. For this reason, the determination of these compounds in real samples is relevant. During the period of time covered by this review, the analysis of non-protein amino acids by CE in food and biological samples was mainly achieved using MEKC and CZE modes. The most employed detection systems include direct and indirect UV absorption, LIF, capacitively coupled contactless conductivity, electrochemical detection and mass spectrometry. In most cases, a derivatization step is required to provide chromophore and fluorophore groups into non-protein amino acids in order to enable their determination or to improve the sensitivity. Thus, many labeling reagents were employed such as NDA, NBD-F, FITC, FMOC-Cl, AQC, DNS-Cl, or OPA. CE and MCE are attractive strategies providing good sensitivity and selectivity to carry out the analysis of a great number of complex food samples such as beverages, vegetables, fermented products or shellfish and biological samples such as urine, plasma, serum, tear fluid, saliva, etc. Moreover, the chiral separation of non-protein amino acids in food and biological samples has also demonstrated to provide relevant information of these samples, but the number of publications in the last years is scarce. The use of a chiral selector in the separation buffer or a chiral stationary phase allow the separation of the enantiomers of non-protein amino acids giving information about food quality and safety or about the diagnostic or treatment of some pathologies. Due to the high number of non-protein amino acids whose function has not been investigated yet and whose presence in real samples is still unknown, the interest of the development of analytical methodologies capable to analyze these compounds in real samples is of high interest for scientists.

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## REFERENCES

- [1] Vranova, V.; Rejsek, K.; Skene, K. R.; Formanek, P. Non-protein amino acids: plant, soil and ecosystem interactions. *Plant soil* **2011**, *342*, 31-48.
- [2] Hunt, S. The non-protein amino acids. In: *Chemistry and Biotechnology of Amino Acids*; Barret, G.C., Ed.; Oxford Polytechnic, 1985; p. 55.
- [3] Yuan, B.; Wua, H.; Sanders, T.; McCullum, C.; Zheng, Y.; Tchounwou, P. B.; Liu, Y. M. Chiral capillary electrophoresis-mass spectrometry of 3,4-dihydroxyphenylalanine: evidence for its enantioselective metabolism in PC.12 nerve cells. *Anal. Biochem.* **2011**, *416*, 191-195.
- [4] Francisco-Marquez, M.; Galano, A. Role of the sulfur atom on the reactivity of methionine toward OH radicals: comparison with norleucine. *J. Phys. Chem. B* **2009**, *113*, 4947-4952.
- [5] Song, Y.; Xu, C.; Kuroki, H.; Liao, Y.; Tsunod, M. Recent trend in analytical methods for the determination of amino acids in biological samples. *J. Pharm. Biomed. Anal.* **2018**, *147*, 35-49.
- [6] Kvasnička, F. Capillary electrophoresis in food authenticity. *J. Sep. Sci.* **2005**, *28*, 813-825.
- [7] Peace, R.W.; Gilani, G.S. Chromatographic determination of amino acids in foods. *J. AOAC Int.* **2005**, *88*, 877-887.
- [8] Omar, M. M. A.; Elbashir, A. A.; Schmitz, O. J. Capillary electrophoresis method with UV-detection for analysis of free amino acids concentrations in food. *Food Chem.* **2017**, *214*, 300-307.

- [9] Zhu, Q.; Zhang, N.; Gong, M. Rapid amino acid analysis of beers using flow-gated capillary electrophoresis coupled with side-by-side calibration. *Anal. Methods*. **2017**, *9*, 4520-4526.
- [10] Celá, A.; Mádr, A.; Glatz, Z. Electrophoretically mediated microanalysis for simultaneous on-capillary derivatization of standard amino acids followed by micellar electrokinetic capillary chromatography with laser-induced fluorescence detection. *J. Chromatogr. A* **2017**, *1499*, 203-210.
- [11] Celá, A.; Mádr, A.; Děnová, T.; Pelcová, M.; Ješeta, M.; Žáková, J.; Crha, I.; Glatz, Z. MEKC-LIF method for analysis of amino acids after on-capillary derivatization by transverse diffusion of laminar flow profiles mixing of reactants for assessing developmental capacity of human embryos after in vitro fertilization. *Electrophoresis* **2016**, *37*, 2305-2312.
- [12] Oborny, N. J.; Costa, E. E. M.; Suntornsuk, L.; Abreu, F. C.; Lunte, S. M. Evaluation of a portable microchip electrophoresis fluorescence detection system for the analysis of amino acid neurotransmitters in brain dialysis samples. *Anal. Sci.* **2016**, *32*, 35-40.
- [13] Wang, X.; Yi, L.; Guillo, C.; Roper, M. G. Micellar electrokinetic chromatography method for measuring amino acid secretions from islets of Langerhans. *Electrophoresis* **2015**, *36*, 1172-1178.
- [14] Chen, Y-C.; Chang, P-L. Baseline separations of amino acid biomarkers of hepatocellular carcinoma by polyvinylpyrrolidone-filled capillary electrophoresis with light-emitting diode-induced fluorescence in the presence of mixed micelles. *Analyst* **2015**, *140*, 847-53.

- [15] Chen, Q.; Deng, L.; Chi, J.; Liu, M.; Lin, X.; Xie, Z. Sensitive profiling of trace neurotoxin domoic acid by pressurized capillary electrochromatography with laser-induced fluorescence detection. *RSC Adv.* **2017**, *7*, 53778-53784.
- [16] Zhang, N.; Zhu, Q.; Gong, M. Rapid determination of free prolyl dipeptides and 4-hydroxyproline in urine using flow-gated capillary electrophoresis. *Anal. Bioanal. Chem.* **2017**, *409*, 7077-7085.
- [17] Weisenberger, M. M.; Bowser, M. T. In vivo monitoring of amino acid biomarkers from inguinal adipose tissue using online microdialysis-capillary electrophoresis. *Anal. Chem.* **2017**, *89*, 1009-1014.
- [18] Harstad, R. K.; Bowser, M. T. High-speed microdialysis-capillary electrophoresis assays for measuring branched chain amino acid uptake in 3T3-L1 cells. *Anal. Chem.* **2016**, *88*, 8115-8122.
- [19] Cheng, Y.; Guo, C.; Zhao, B.; Yang, L. Fast analysis of domoic acid using microchip electrophoresis with laser-induced fluorescence detection. *J. Sep. Sci.* **2017**, *40*, 1583-1588.
- [20] Passos, H. M.; Cieslarova, Z.; Simionato, A. V. CE-UV for the characterization of passion fruit juices provenance by amino acids profile with the aid of chemometric tools. *Electrophoresis* **2016**, *37*, 1923-1929.
- [21] Li, L-Q.; Baibado, J. T.; Shen, Q.; Cheung, H-Y. Determination of the authenticity of plastron-derived functional foods based on amino acid profiles analysed by MEKC. *J. Chromatogr. B* **2017**, *1070*, 23-30.

- [22] Pérez-Míguez, R.; Marina, M. L.; Castro-Puyana, M. Capillary electrophoresis determination of non-protein amino acids as quality markers in foods. *J. Chromatogr. A* **2016**, *1428*, 97-114.
- [23] Kong, Y.; Yang, G.; Chen, S.; Hou, Z.; Du, X.; Li, H.; Kong, L. Rapid and sensitive determination of L-carnitine and acetyl-L-carnitine in liquid milk samples with capillary zone electrophoresis using indirect UV detection. *Food Anal. Methods* **2018**, *11*, 170-177.
- [24] Sacristán, M.; Varela, A.; Pedrosa, M.M.; Burbano, C.; Cuadrado, C.; Legaz, M. E.; Muzquiz, M. Determination of  $\beta$ -N-oxalyl-L- $\alpha$ ,  $\beta$ -diaminopropionic acid and homoarginine in *Lathyrus sativus* and *Lathyrus cicera* by capillary zone electrophoresis. *J. Sci. Food Agric.* **2015**, *38*, 1414-1420.
- [25] Laube, H.; Boden, J.; Schneider, R. Capillary electrophoresis method for the analysis of organic acids and amino acids in the presence of strongly alternating concentrations of aqueous lactic acid. *Bioprocess Biosyst. Eng.* **2017**, *40*, 981-988.
- [26] Openkar, F.; Tũma, P. Coaxial flow-gating interface for capillary electrophoresis. *J. Sep. Sci.* **2017**, *00*, 1-6.
- [27] Wu, M.; Gao, F.; Zhang, Y.; Wang, Q.; Li, H. Sensitive analysis of amino acids and vitamin B<sub>3</sub> in functional drinks via field-amplified stacking with reversed-field stacking in microchip electrophoresis. *Talanta* **2015**, *131*, 624-631.
- [28] Kerrin, E. S.; White, R. L.; Quilliam, M. A. Quantitative determination of the neurotoxin  $\beta$ -N-methylamino-L-alanine (BMAA) by capillary electrophoresis-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2017**, *409*, 1481-1491.

- [29] Pérez-Míguez, R.; Marina, M. L.; Castro-Puyana, M. Enantiomeric separation of non-protein amino acids by electrokinetic chromatography. *J. Chromatogr. A* **2016**, *1467*, 409-416.
- [30] Liang, Q.; Chen, H.; Li, F.; Du, X. Simultaneous sensitive MEKC-LIF determination of homocysteine, homoarginine, and six arginine metabolic derivatives in fluids from Type 2 diabetics with peptic ulcer bleeding. *Chromatographia* **2015**, *78*, 1049-1056.
- [31] Rodrigues, K. T.; Mekahli, D.; Tavares, M. F. M.; Schepdael, A. V. Development and validation of a CE-MS method for the targeted assessment of amino acids in urine. *Electrophoresis* **2016**, *37*, 1039-1047.
- [32] Contreras, M.T.; Gallardo, M.J.; Betancourt, L. R.; Rada, P. V.; Ceballos, G. A.; Hernandez, L.E.; Hernandez, L.F. Correlation between plasma levels of arginine and citrulline in preterm and full-term neonates: Therapeutical implications. *J. Clin. Lab. Anal.* **2017**, *31*, e22134.
- [33] Du, F.; Zhao, W.; Cao, S.; Fung, Y-S. Determination of taurine in human tear fluid by capillary electrophoresis with indirect amperometric detection based on electrogenerated bromine. *J. Sep. Sci.* **2015**, *38*, 3271-3278.
- [34] Ohshima, M.; Sugahara, K.; Kasahara, K.; Katakura, A. Metabolomic analysis of the saliva of Japanese patients with oral squamous cell carcinoma. *Oncol. Rep.* **2017**, *37*, 2727-2734.
- [35] Ivanov, A. V.; Virus, E. D.; Luzyanin, B. P.; Kubatiev, A. A. Capillary electrophoresis coupled with 1,1-thiocarbonyldiimidazole derivatization for the rapid detection of total homocysteine and cysteine in human plasma. *J. Chromatogr. B* **2015**, *1004*, 30-36.

- [36] Ivanov, A. V.; Bulgakova, P. O.; Virus, E. D.; Kruglova, M. P.; Alexandrin, V. V.; Gadieva, V. A.; Luzyanin, B. P.; Kushlinskii, N. E.; Fedoseev, A. N.; Kubatiev, A. A. Capillary electrophoresis coupled with chloroform-acetonitrile extraction for rapid and highly selective determination of cysteine levels in human blood plasma and urine. *Electrophoresis* **2017**, *38*, 2646-2653.
- [37] Guo, X-F.; Arceo, J.; Hüge, B. J.; Ludwig, K. R.; Dovichi, N. J. Chemical cytometry of thiols using capillary zone electrophoresis-laser induced fluorescence and TMPAB-o-M, an improved fluorogenic reagent. *Analyst*. **2016**, *141*, 1325-1330.
- [38] Cieslarova, Z.; Lopes, F. S.; do Lago, C. L.; Franca, M. C.; Simionato, A. V. C. Capillary electrophoresis tandem mass spectrometry determination of glutamic acid and homocysteine's metabolites: Potential biomarkers of amyotrophic lateral sclerosis. *Talanta* **2017**, *170*, 63-68.
- [39] Hložek, T.; Křížek, T.; Tůma, P.; Bursová, M.; Coufal, P.; Čabala, R. Quantification of paracetamol and 5-oxoproline in serum by capillary electrophoresis: implication for clinical toxicology. *J. Pharm. Biomed. Anal.* **2017**, *145*, 616-620.
- [40] Forteschi, M.; Zinellu, A.; Assaretti, S.; Mangoni, A. A.; Pintus, G.; Carru, C.; Sotgia, S. An isotope dilution capillary electrophoresis/tandem mass spectrometry (CE-MS/MS) method for the simultaneous measurement of choline, betaine, and dimethylglycine concentrations in human plasma. *Anal. Bioanal. Chem.* **2016**, *408*, 7505-7512.
- [41] Sánchez-López, E.; Marcos, A.; Ambrosio, E.; Marina, M. L.; Crego, A. L. Enantioseparation of the constituents involved in the phenylalanine-tyrosine metabolic pathway by capillary electrophoresis tandem mass spectrometry. *J. Chromatogr. A* **2016**, *1467*, 372-382.



- [42] Simó, C.; Rizzi, A.; Barbas, C.; Cifuentes, A. Chiral capillary electrophoresis-mass spectrometry of amino acids in foods. *Electrohoresis* **2005**, *26*, 1432-1441.
- [43] Zhang, J.; Nishimura, N.; Okubo, A.; Yamazaki, S. Development of an analytical method for the determination of betaines in higher plants by capillary electrophoresis at low pH. *Phytochem. Anal.* **2002**, *13*, 189-194.
- [44] Diana, M.; Quílez, J.; Rafecas, M. Gamma-aminobutyric acid as a bioactive compound in foods: a review. *J. Funct. Foods* **2014**, *10*, 407-420.
- [45] Curis, E.; Nicolis, I.; Moinard, C.; Osowska, S.; Zerrouk, N.; Bénazeth, S.; Cynober, L. Almost all about citrulline in mammals. *Amino Acids* **2005**, *29*, 177-205.
- [46] Anadón-Navarro, A.; Cacho-Palomar, J.F.; Ortega-Hernandez, T.; Palou-Oliver, A.; Grupo de Trabajo. Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) sobre el consume humano ocasional de almortas (*Lathyrus sativus*). *Revista de Comité Científico de la AESAN* **2010**, *11*, 9-19.
- [47] Yu, Y-R.; Liu, X-C.; Zhang, J-S.; Ji, C-Y, Qi, Y-F, Taurine drinking attenuates the burden of intestinal adult worms and muscle larvae in mice with *Trichinella spiralis* infection. *Parasitol. Res.* **2003**, *112*, 3457-3463.
- [48] El Idrisi, A.; Okeke, E.; Yan, X.; Sidime, F.; Neuwirth, L. S. Taurine regulation of blood pressure and vasoactivity. *Adv. Exp. Med. Biol.* **2013**, *775*, 407-425.
- [49] Arandas, M.; Morlock, G. Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization. *J. Chromatogr. A* **2006**, *1131*, 253-260.

- [50] Abraham, G. N.; Podell, D. N. Pyroglutamic acid: Non-metabolic formation, function in proteins and peptides, and characteristics of the enzymes effecting its removal. *Mol. Cell. Biochem.* **1981**, *38*, 181-190.
- [51] Mucchetti, G.; Locci, F.; Gatti, M.; Neviani, E.; Addeo, F.; Dossena, A.; Marchelli, R. Pyroglutamic acid in cheese: presence, origin, and correlation with ripening time of Grana Padano cheese. *J. Dairy Sci.* **2000**, *83*, 659-665.
- [52] Salisbury, J. J.; Li, M.; Boyd, A. Validation of an enantioselective analysis for (L)-pidolic acid by chiral gas chromatography with derivatization. *J. Pharm. Biomed. Anal.* **2016**, *120*, 79-83.
- [53] Zhou, X.; Escala, W.; Papapetropoulos, S.; Zhai, G.  $\beta$ -N-Methylamino-L-Alanine induces neurological deficits and shortened life span in *Drosophila*. *Toxins* **2010**, *2*, 2663-2679.
- [54] Regueiro, J.; Negreira, N.; Carreira-Casais, A.; Pérez-Lamela, C.; Simal-Gándara, J. Dietary exposure and neurotoxicity of the environmental free and bound toxin  $\beta$ -N-methylamino-L-alanine. *Food Res. Int.* **2017**, *100*, 1-13.
- [55] Pulido, O. M. Domoic acid toxicologic pathology: a review. *Mar. Drugs* **2008**, *6*, 180-219.
- [56] Sánchez-Hernández, L.; García-Ruiz, C.; Crego, A. L.; Marina, M. L. Sensitive determination of D-carnitine as enantiomeric impurity of levo-carnitine in pharmaceutical formulations by capillary electrophoresis-tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2010**, *53*, 1217-1223.
- [57] Martínez-Girón, A. B.; Domínguez-Vega, E.; García-Ruiz, C.; Crego, A. L.; Marina, M. L. Enantiomeric separation of ornithine in complex mixtures of amino acids

by EKC with off-line derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *J. Chromatogr. B* **2008**, *875*, 254-259.

[58] Miyagi, Y.; Higashiyama, M.; Gochi, A.; Akaike, M.; Ishikawa, T.; Miura, T.; Saruki, N.; Bando, E.; Kimura, H.; Imamura, F.; et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. *PLoS One* **2011**, *6*, e24143.

[59] Nakatsukasa, M.; Sotozono, C.; Shimbo, K.; Ono, N.; Minavo, H.; Okano, A.; Hamuro, J.; Kinoshita, S. Amino acid profile in human tear fluids analyzed by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. *Am. J. Ophthalmol.* **2011**, *151*, 799-808.

[60] Wierzbicki, A.S. Homocysteine and cardiovascular disease: a review of the evidence. *Diab. Vasc. Dis. Res.* **2007**, *4*, 143-150.

[61] Hortin, G.L.; Sullivan, P.; Csako, G. Relationships among plasma homocysteine, cysteine, and albumin concentrations: potential utility of assessing the cysteine/homocysteine ratio. *Clin. Chem.* **2001**, *47*, 1121-1124.

## FIGURE CAPTIONS

**Figure 1.** Comparison of electropherograms of AAs profile of plastron with other amino acid-containing materials. Experimental conditions: BGE, 20 mM borate and phosphate containing 0.1 M SDS and 6% methanol (pH 8.74); voltage, 25 kV; temperature, 25°C; injection, 9 kV for 9 s at 25°C; UV detection at 214 nm.

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**Figure 2.** Electropherograms of milk sample and standard solution with established CZE indirect UV method. Sample information: **A-B)** real milk samples, **C)** standard mixture of carnitine (carn) and acetyl-carnitine (a-carn) (0.5 mmol/L), **D)** CZE with direct UV detection for sample (B) spiked with carnitine and acetyl-carnitine (4.0 mmol/L). Experimental conditions: pH 2.1, 3.0 mmol/L of melamine solution (in 10% methanol), voltage 10 kV.

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**Figure 3.** **A)** Electropherograms corresponding to the enantiomeric separation of different FMOC-NPAAs (0.2 mM) obtained under the best separation conditions. Experimental conditions: BGE, 10 mM of the corresponding CD in 100 mM formate buffer (pH 2.0); uncoated fused-silica capillary, 58.5 cm (50 cm to the detector window) x 50  $\mu$ m ID; UV detection at 210 nm (except for Pyro which was detected at 200 nm); voltage, -20 kV; injection by pressure in the cathodic end, 50 mbar for 4 s. \* Indicates the derivatizing reagent (FMOC). **B)** Electropherogram obtained for (a) DL-FMOC-citrulline standard (0.025 mM) and two food supplements at 0.2 mM L-Citrulline (b) FS1, spiked with D-citrulline and non spiked and (c) FS6, spiked with D-citrulline and non spiked. Experimental conditions: BGE, 10 mM sulfated  $\gamma$ -CD in 100 mM formate buffer (pH 3.0); uncoated fused-silica capillary, 48.5 cm (40 cm to the detector window)

x 50  $\mu\text{m}$  ID; UV detection at 210 nm, applied voltage, -20 kV; temperature, 25  $^{\circ}\text{C}$ ; injection by pressure in the cathodic end, 50 mbar for 15 s.

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**Figure 4.** **A)** Schematic on the online MD-CE system. **B)** Full electropherogram and **C)** expanded one from an online analysis of CE analysis of 3T3-L1 cells supernatant after 30 min of incubation with Ringer's solution, glucose, isoleucine, leucine and valine. Experimental conditions: BGE, 90 mM borate containing 35 mM  $\alpha$ -CD (pH 9.8); capillary, 50  $\mu\text{m}$  x 30 cm; voltage, 21 kV. Peaks: (1) lysine, (2) isoleucine, (3) leucine, (4) ornithine, (5) methionine, (6) phenylalanine, (7) valine, (8) ornithine, (9) GABA, (10) glutamine, (11) alanine, (12) threonine, (13)  $\beta$ -alanine, (14) glycine, (15) NBDOH, (16) taurine, (17) internal standard, (18) glutamate, and (19) aspartate.

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**Figure 5.** Electropherograms corresponding to the analysis of homocysteine and cysteine in blood plasma and urine by CE-UV. **A)** Plasma without additions, **B)** spiked with internal standard (penicillamine), **C)** spiked with internal standard, homocysteine and cysteine, **D)** urine. Experimental conditions: BGE, 0.1 M phosphate containing 30 mM TEA, 25 M CTAB, 2.5 M SDS and 2.5% PGE-600 (pH 2); capillary, 50  $\mu\text{m}$  x 23.5 cm; voltage, -17 kV; temperature, 30  $^{\circ}\text{C}$ ; injection: 2250 mbar\*s. Peaks: (1) cysteine, (2) penicillamine, (3) homocysteine.

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**Figure 6.** Extracted Ion Electropherograms obtained by CE-MS<sup>2</sup> method of **A)** a rat plasma sample (the inserts show the MS<sup>2</sup> spectra of L-Phe and L-Tyr peaks), and **B)** a spiked rat plasma sample. Experimental conditions: BGE, 2 M formic acid containing 180 mM M- $\beta$ -CD and 40 mM HP- $\beta$ -CD (pH 1.2); capillary, 50  $\mu\text{m}$  x 120 cm; voltage, 30 kV; temperature, 15  $^{\circ}\text{C}$ ; injection, 50 mbar x 250 s.

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**Table 1.** Characteristics of the analytical methodologies developed for the determination of NPAAAs in foods by CE.

NPAA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Hydroxyproline Hydroxylysine	MEKC-UV (214 nm)	20 mM sodium tetraborate and sodium phosphate + 0.1M SDS + 6% MeOH (pH 8.7); capillary, 50 µm x 50 cm; 25 kV, 25°C	18 protein amino acids	Plastron, fish skin, pig skin, chicken tendon, calf tendon, pork, chicken and fish: dried, pulverized, dilution, alkali digestion, filtration and neutralization with HCl before derivatization with Dns-Cl Egg and milk: acid hydrolysis, filtration, lyophilization, dilution, alkali digestion, filtration and neutralization with HCl before derivatization with Dns-Cl	Potential of amino acids as markers of adulterations of plastron-derived functional foods	Hydroxyproline: 1.57 x 10 <sup>-4</sup> mg/mL  Hydroxylysine: 6.65 x 10 <sup>-4</sup> mg/mL	[21]
Hydroxyproline	MEKC-UV (265 nm)	60 mM sodium borate + 30 mM SDS + 5% MeOH (pH 10.1); capillary, 50 µm x 72 cm; 25 kV, 23°C	20 protein amino acids and Cystine	10-Fold water dilution (previously centrifuged and filtered), and derivatization with FMOC	Characterization and quantification of amino acid profile in passion fruit juices	-	[20]
Carnitine Acetyl-L-carnitine	CZE-indirect UV (200 nm)	3.0 mM melamine + 10 % MeOH (pH 2.1); capillary, 75 µm x 39 cm; 10 kV, 20°C	-	Extraction with ACN:MeOH (4:1 v/v), centrifugation, evaporation and dilution in water	Determination of L-Carnitine and Acetyl-L-carnitine in liquid milk samples	L-carnitine: 3.0 µM  Acetyl-L-carnitine: 5.0 µM	[23]
γ-Aminobutyric acid  Citrulline	MEKC-LIF (λ <sub>ex</sub> 442 nm; λ <sub>em</sub> 485 nm)	40 mM sodium tetraborate + 60 mM SDS + 2 mM HP-β-CD (pH 9.2); capillary, 10 µm x 10 cm; -25 kV, 25°C	17 protein amino acids, phosphoryl ethanolamine and ethanolamine	50-Fold dilution with water (previously degassed in ultrasonic bath) followed by NDA derivatization in presence of cyanide	Quantitation of 19 amino acids, phosphoryl ethanolamine and ethanolamine in beers	γ-Aminobutyric acid and Citrulline: 2.0-5.0 nM	[9]

**Table 1.** Continued

NPAA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Homoarginine β-N-Oxalyl-L- α,β- diaminopropionic acid	CZE-UV (195 nm)	25 mM sodium borate + 5 mM sodium sulfate (pH 9.2); capillary, 75 μm x 50 cm; 21 kV, 22°C	-	Extraction with MeOH:water (60:40 v/v) (under Ultra-Turrax), centrifugation, evaporation and dilution in sample buffer (10 mM sodium borate + 5 mM sodium sulfate + 12.84 mM hippuric acid), filtration prior to the CE analysis. Extraction with ethanol:water (60:40 v/v) (under rotating shaking), centrifugation, evaporation and dilution in the sample buffer (10 mM sodium borate + 5 mM sodium sulfate + 12.84 mM hippuric acid), filtration before CE analysis.	Simultaneous analysis of β-N-Oxalyl-L-α,β-diaminopropionic acid and Homoarginine in <i>Lathyrus</i> species	-	[24]
Pyroglutamic acid Taurine	MEKC-UV (200 nm)	25 mM sodium tetraborate + 50 mM SDS (pH 9.1); capillary, 50 μm x 56 cm; 30 kV, 35°C	Organic acids, 13 protein amino acids, Cystine and Tryptamine	Water solution, basic hydrolysis (with α-amylase), thermal sterilization, microfiltration and fermentation (tapioca starch and yeast) before CE analysis	Simultaneous determination of lactic acid and its organic impurities in fermentatively products	-	[25]
Taurine	CZE- C <sup>4</sup> D	Commercial equipment: 20 mM CHES + 10 mM NaOH (pH 9.5); capillary, 25 μm x 11.5 cm; 10 kV  Instrument with a coaxial flow-gating interface: 20 mM CHES + 10 mM NaOH (pH 9.5); capillary, 25 μm x 18 cm; 20 kV, 25°C	Citrate and Carbonate	Sonication (to remove dissolved gases) and 40-fold dilution for energy drink	Determination of Taurine in energy drink	14.4 mg/L  8.2 mg/L	[26]
	MCE-LIF (λ <sub>ex</sub> 635 nm; λ <sub>em</sub> 495 nm)	100 mM sodium borate (pH 9.9); glass microchip with a simple cross channel design; separation channel, (60 mm x 25 μm x 70 μm (length x depth x width)), 45 mm from injection to the detector	Lysine and Vitamine B <sub>3</sub>	2-Fold dilution with 40 mM sodium borate, pH adjustment (8.60), derivatization with Cy5 and dilution with 10 mM sodium borate (pH 9.88)	Analysis of amino acids (Lysine and Taurine) and vitamin B <sub>3</sub> (NA) in functional drinks	0.50 nM	[27]



**Table 1.** Continued

<b>NPAA</b>	<b>CE-mode/detection</b>	<b>Separation conditions*</b>	<b>Separation from:</b>	<b>Sample treatment</b>	<b>Application</b>	<b>LOD**</b>	<b>Ref</b>
β-N-Methylamino-L-alanine	CZE-UV (192 nm)	250 mM sodium phosphate (pH 3.0); capillary, 50 μm x 46 cm; 25 kV, 17°C	Four β-N-Methylamino-L-alanine isomers	Acid hydrolysis, drying and dilution with HCl, clean-up, drying and re-dilution with HCl prior to CE analysis	Separation of five β-N-Methylamino-L-alanine isomers and quantification of β-N-Methylamino-L-alanine in cycad, mussel and lobster samples	0.25 μg/mL 20 mg/g <sup>a</sup>	[28]
	CZE-MS <sup>2</sup>	5 M formic acid + 10 % (v/v) ACN (pH 1.55); capillary, 50 μm x 100 cm; 20 kV, 17°C				0.8 ng/mL 16 ng/g <sup>a</sup>	
Domoic acid	MCE-LIF (λ <sub>ex</sub> 475 nm; λ <sub>em</sub> 535 nm)	5 mM sodium tetraborate (pH 9.2); glass microchipcross-channel design; separation channel, (49 mmx 30 μm x 80 μm (length x depth x width)), 27 mm from injection to the detector; 400 V/cm	-	Extraction with MeOH:water (1:1 v/v), centrifugation, filtration and derivatization with FITC	Determination of Domoic acid in shellfish tissues	0.28 nM	[19]
	pCEC-LIF (λ <sub>ex</sub> 473 nm; λ <sub>em</sub> 530 nm)	5 mM phosphate:ACN (40:60 v/v) (pH 2.5) at a flow rate of 1.2 μL/min; packed capillary column, 100 μm x 55 cm (total length of which 20 cm was packed with ODS particles); 6 kV, 25°C; supplementary pressure 7.2 MPa	-	Extraction with MeOH:water (1:1 v/v), centrifugation, filtration, clean-up, purification and derivatization with NBD-F before CEC analysis	Quantification of Domoic acid in shellfishsamples (oyster, <i>mytilus edulis</i> and <i>ruditapes</i> )	10 ng/mL <sup>c</sup> 15 ng/g <sup>b</sup>	[15]

**Table 1.** Continued

<b>NPAA</b>	<b>CE-mode/detection</b>	<b>Separation conditions*</b>	<b>Separation from:</b>	<b>Sample treatment</b>	<b>Application</b>	<b>LOD**</b>	<b>Ref</b>
Norvaline Norleucine 2-Amino adipic acid Seleno-methionine 3,4-Dihydroxy-phenylalanine Pyroglutamic acid Pipelicolic acid Citrulline	EKC-UV (210 nm)	10 mM sulfated-CDs in 100 mM formate (pH 2.0); capillary, 50 $\mu$ m x 40 cm; -20 kV, 15-25°C	-	Water solution (sonication), centrifugation, filtration, and derivatization with FMOc before CE analysis	Individual enantiomeric separation of the 8 NPAAAs studied and development of a method for the quantitation of L- and D-Citrulline in food supplements	D-Citrulline: 0.21 $\mu$ M  L-Citrulline: 0.18 $\mu$ M	[29]

ACN, acetonitrile; C<sup>4</sup>D, capacitively coupled contactless conductivity; CHES, 2-(N-cyclohexylamino)ethane sulfonic acid; Cy5, Sulfoindocyanine succinimidyl ester; CZE, capillary zone electrophoresis; Dns-Cl, dansyl chloride; FITC, fluorescein isothiocyanate; FMOc, 9-fluorenyl-methyloxycarbonyl; HP- $\beta$ -CD, 2-hydroxypropyl- $\beta$ -cyclodextrin; LIF, laser-induced fluorescence; MCE, microchip capillary electrophoresis; MEKC, micellar electrokinetic chromatography; MeOH: methanol; MS<sup>2</sup>, tandem mass spectrometry; NBD-F, 4-Fluoro-7-nitro-2,1,3-benzoxadiazole; NDA, 2,3-naphthalenedicarboxaldehyde; ODS, octadecyl silica; pCEC, pressurized capillary electrochromatography; SDS, sodium dodecyl sulfate.

\*Capillary dimensions expressed as internal diameter x effective length (cm to the detector).

\*\*LODs units expressed as in the original work. These LODs are referred to the concentration of injected standard solutions except for a) LODs referred to the dry sample mass, b) LODs referred to the wet sample weight, and c) LOD referred to the concentration of injected real sample solutions extracts.

**Table 2.** Characteristics of the analytical methodologies developed for the determination of NPAAs in biological samples by CE.

NPPA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Homocysteine Homoarginine Ornithine Citrulline	MEKC-LIF ( $\lambda_{ex}$ 488 nm; $\lambda_{em}$ 520 nm)	50 mM borate + 30 mM SDS + 30 % MeOH (pH 9.5); capillary, 50 $\mu$ m x 50 cm; 21 kV, 21 °C	Arginine, asymmetric dimethyl-L-arginine, symmetric dimethyl-L-arginine, and Monomethyl-L-arginine	Protein precipitation with 5-sulfosalicylic, evaporation to dryness under vacuum, redissolution in BGE, and derivatization with CFSE before CE analysis.	Determination of Homoarginine, Homocysteine and Arginine metabolic derivatives in fluids from Type 2 diabetics with peptic ulcer bleeding	Ornithine: 1.70 nM Citrulline: 1.67 nM Homoarginine: 0.88 nM Homocysteine: 0.12 nM	[30]
$\gamma$ -Aminobutyric acid Ornithine Citrulline Taurine	EKC-LIF ( $\lambda_{ex}$ 488 nm; $\lambda_{em}$ 520 nm)	90 mM borate + 35 mM $\alpha$ -CD (pH 9.8); capillary, 50 $\mu$ m x 30 cm; 21 kV	12 protein amino acids	On-line derivatization with 20 mM NBD-F/250 $\mu$ M HCl in 50% MeOH	Measurement of branched chain amino acid uptake in 3T3-L1 cells	-	[18]
Ornithine Citrulline Norvaline Norleucine	CZE-LEDIF ( $\lambda_{ex}$ 405 nm; $\lambda_{em}$ 486 nm)	1% PVP + 10 mM HEPES (pH 7.0); PVP coated capillary, 75 $\mu$ m x 28 cm; -20 kV	17 protein amino acids	Protein precipitation by heating, derivatization with NDA before CE injection.	Separation of amino acids in human plasma	-	[14]
$\gamma$ -Aminobutyric acid Ornithine Citrulline Hydroxyproline Alloisoleucine	CZE-(IT)MS <sup>2</sup>	0.8 M formic acid + 15 % MeOH (pH 1.96); capillary, 50 $\mu$ m x 85 cm; 30 kV, 20°C	20 protein amino acids, Carnosine	Centrifugation and dilution prior to CE analysis	Determination of amino acids in urine samples	$\gamma$ -Aminobutyric acid: 4 $\mu$ M Ornithine: 4.8 $\mu$ M Citrulline: 7.7 $\mu$ M Hydroxyproline: 3.7 $\mu$ M Alloisoleucine: 2.5 $\mu$ M	[31]

**Table 2.** Continued

NPPA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Citrulline	CZE-LIF ( $\lambda_{\text{ex}}$ 488 nm; $\lambda_{\text{em}}$ 510 nm)	20 mM carbonate (pH 10.0); capillary, not indicated, 27 kV	Arginine	Protein precipitation with ACN, derivatization with FITC and dilution before CE analysis.	Determination of plasma levels of Arginine and Citrulline in preterm and full-term neonates	-	[32]
Citrulline + Taurine	MCE-LIF ( $\lambda_{\text{ex}}$ 445 nm; $\lambda_{\text{em}}$ 480 nm)	15 mM borate + 1.4 mM SBEC + 10 % DMSO (pH 9.2); 10 kV	Arginine, Glutamic acid, Aspartic acid and Histamine	Fluorogenic derivatization with NDA.	Analysis of amino acid neurotransmitters in brain dialysis samples	Citrulline: 0.36 $\mu\text{M}$ Taurine: 0.42 $\mu\text{M}$	[12]
Taurine	MEKC-SDED	20 mM phosphate + 20 mM SDS (pH 10.0); capillary, 25 $\mu\text{m}$ x 40 cm; 12 kV	20 protein amino acids	Extraction with water, protein precipitation with ACN, evaporation to dryness under nitrogen stream, redissolution in BGE.	Determination of Taurine in human tear fluid	0.18 $\mu\text{M}$	[33]
Taurine $\gamma$ -Aminobutyric acid	EKC-LIF ( $\lambda_{\text{ex}}$ 488 nm)	90 mM borate + 35 mM $\alpha$ -CD (pH 10.0); capillary, 5 $\mu\text{m}$ x 6.2 cm; -23 kV	10 protein amino acids	On-line derivatization with 20 mM NBD-F/250 $\mu\text{M}$ HCl in 50 % MeOH.	Monitoring the <i>in vivo</i> dynamics of amino acids biomarkers of metabolism in adipose tissue	2.7 $\mu\text{M}$	[17]

**Table 2.** Continued

NPPA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Taurine	CZE-(TOF)MS	Commercial electrophoresis buffer for anion and cation analysis; capillary, 50 $\mu\text{m}$ x 80 cm	24 metabolites including 5 protein amino acids	Centrifugation, filtration and dissolution in water.	Metabolome analysis of saliva samples	-	[34]
	MEKC-LIF ( $\lambda_{\text{ex}}$ 488 nm; $\lambda_{\text{em}}$ 515 nm)	35 mM borate + 55 mM SDS + 2.7 M urea + 1 mM BIS-TRIS propane + 23 mM NaOH (pH 9.8); capillary, 50 $\mu\text{m}$ x 50 cm; 30 kV, 24°C	18 protein amino acids, Alanine-Glutamine, Cystine	Protein precipitation with MeOH. On-capillary derivatization by TDLFP: sequential injection of 1.5 mM NDA in the reaction buffer with 50 % MeOH, sample, and 10 mM NaCN in the reaction buffer.	Assessing developmental capacity of human embryos after <i>in vitro</i> fertilization	0.02 $\mu\text{M}$	[11]
	MEKC-LIF ( $\lambda_{\text{ex}}$ 488 nm; $\lambda_{\text{em}}$ 515 nm)	73 mM SDS + 6.7 % (v/v) 1-propanol + 0.5 mM HP- $\beta$ -CD + 135 mM boric acid/NaOH (pH 9.00); capillary, 50 $\mu\text{m}$ x 45 cm; 30 kV, 25°C	18 protein amino acids Alanine-Glutamine, Cystine	Protein precipitation with MeOH:IPA (4:1) and dilution. On-capillary derivatization by EMMA: sequential injection of 1 mM NDA in the reaction buffer with a 12.5 % MeOH/IPA (4:1) mixture, sample, 1 mM NDA in the reaction buffer with a 12.5 % MeOH/IPA (4:1) + 2.5 mM NaCN. Reaction products were on capillary preconcentrated by sweeping.	Non-invasive targeted metabolomics of human embryos.	12 nM	[10]

**Table 2.** Continued

NPPA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Homocysteine	MEKC-UV ( $\lambda_{\text{abs}}$ 285 nm)	0.1 M TEA + 0.15 M formic acid + 50 $\mu$ M CTAB (pH 3.9); capillary, 50 $\mu$ m x 21.5 cm; -12 kV, 25°C	Cysteine, Cysteine-Glycine	Reduction with DTT before TCDI derivation.	Rapid detection of total Homocysteine and Cysteine in human plasma	0.8 $\mu$ M	[35]
	MEKC-UV ( $\lambda_{\text{abs}}$ 285 nm)	0.1 M phosphate + 30 mM TEA + 25 M CTAB + 2.5 M SDS + 2.5% PGE-600 (pH 2); capillary, 50 $\mu$ m x 23.5 cm; -17 kV, 30°C	Cysteine	Derivatized with TCDI, extraction with chloroform-ACN.	Determination of Homocysteine and Cysteine levels in human plasma and urine	0.2 $\mu$ M	[36]
	CZE-LIF ( $\lambda_{\text{ex}}$ 473 nm)	10 mM sodium citrate (pH 7.5); capillary, 50 $\mu$ m x 50 cm; 15 kV, room temperature	Glutation, Cysteine, $\gamma$ -glutamylcysteine	Cells lines were resuspended in PBS and incubated with NEM. Single cells were incubated with TMPAB-o-M and mixed with the runing buffer.	Chemical cytometry of thiols in human colon cancer and breast cells	13 pM	[37]
	CZE-(QqQ)MS <sup>2</sup>	5 M acetic acid; capillary, 50 $\mu$ m x 60 cm; 25 kV, 20°C	Glutamic acid, Cysteine, Methionine	Blood samples + EDTA, centrifugation. For analysis of aminothiols: Mix samples with DTT, IAA and ACN, centrifugation and analysis of the supernatant.	Determination of Homocysteine as potential biomarkers of amyotrophic lateral sclerosis	35 nM	[38]
$\gamma$ -Aminobutyric acid	MEKC-LIF ( $\lambda_{\text{ex}}$ 450 nm; $\lambda_{\text{em}}$ 480 nm)	25 mM phosphate + 30 mM SDS (pH 8.3); capillary, 25 $\mu$ m x 50 cm; 29 kV, 25°C	17 protein amino acids	Derivatization with NDA, cyanide, and internal standard (D-Nva) (9:1:1:1, sample:NDA:cyanide:D-norvaline)	Measuring amino acid secretions from islets of Langerhans	3 nM	[13]
Hydroxyproline	MEKC-LIF ( $\lambda_{\text{ex}}$ 492 nm; $\lambda_{\text{em}}$ 520 nm)	40 mM cholate + 40 mM deoxycholate + 40 mM tetraborate (pH 9.2); capillary, 10 $\mu$ m x 10 cm; -25 kV, room temperature	Proline, 4 propyl dipeptides	Acid Hydrolysis, 100-fold dilution, addition of EDTA, OPA and derivatization with NBD-F prior to CE analysis.	Rapid determination of free prolyl dipeptides and Hydroxyproline in urine	70 nM	[16]

**Table 2.** Continued

NPPA	CE-mode/detection	Separation conditions*	Separation from:	Sample treatment	Application	LOD**	Ref
Pyroglutamic acid	CZE-UV ( $\lambda_{\text{abs}}$ 200 nm)	40 mM CHES/NaOH (pH 10.2); capillary, 25 $\mu\text{m}$ x 23 cm; 30 kV, 25°C	Paracetamol	Protein precipitation with ACN and $\text{NH}_4\text{OH}$ , centrifugation and analysis of the supernatant.	Quantification of Paracetamol and Pyroglutamic acid in serum	1.3 $\mu\text{g/mL}$	[39]
Betaine	CZE-(QqQ)MS <sup>2</sup>	10 % MeOH v/v + 5 % formic acid v/v; capillary, 75 $\mu\text{m}$ x 120 cm; 28 kV, 20°C, pressure assistance: 40 mbar	Choline, dimethylglycine	Protein precipitation with ACN, evaporation to dryness under vacuum, redissolution in water.	Simultaneous quantification of Choline, Betaine, and Dimethylglycine in human plasma	0.62 $\mu\text{M}$	[40]
3,4-Dihydroxy-phenylalanine	EKC-ESI-MS <sup>2</sup>	180 mM M- $\beta$ -CD + 40 mM HP- $\beta$ -CD + 2 M formic acid (pH 1.2); capillary, 50 $\mu\text{m}$ x 120 cm; 30 kV, 15 °C	Phenylalanine, Tyrosine, Dopamine, Norepinephrine, and Epinephrine	Precipitation of proteins with ACN (plasma/ACN, 1:2), centrifugation, dilution of the supernatant with formic acid, sonication and filtration.	Simultaneous enantioseparation of all the chiral constituents of the Phenylalanine-Tyrosine metabolic pathway	L-3,4-Dihydroxy-phenylalanine: 54 nM	[41]

$\alpha$ -CD,  $\alpha$ -cyclodextrin; ACN, acetonitrile; BIS-TRIS propane, 1,3-bis[tris(hydroxymethyl)methylamino]propane; CFSE, 5-carboxyfluorescein succinimidyl ester; CHES, 2-(N-cyclohexylamino)ethane sulfonic acid; CTAB, hexadecyltrimethylammonium bromide; CZE, capillary zone electrophoresis; DMSO, dimethyl sulfoxide; DTT, dithiotreitol; EDTA, tetrasodium salt of ethylenediaminetetraacetic acid; EMMA, electrophoretically mediated microanalysis; FITC, fluorescein isothiocyanate; HP- $\beta$ -CD, 2-hydroxypropyl- $\beta$ -cyclodextrin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IAA, iodoacetic acid; IPA, 2-propanol; (IT)MS, ion trap; LEDIF, light emitting diode induced fluorescence; LIF, laser-induced fluorescence; M- $\beta$ -CD, methyl- $\beta$ -cyclodextrin; MEKC, micellar electrokinetic chromatography; MeOH, methanol; MS<sup>2</sup>, tandem mass spectrometry; NBD-F, 4-Fluoro-7-nitro-2,1,3-benzoxadiazole; NDA, 2,3-naphthalenedicarboxaldehyde; NEM, N-ethylmaleimide; ODS, octadecyl silica; OPA, o-phthalaldehyde; PBS, phosphate buffered saline; PGE-600, polyethylene glycol 600; PVP, polyvinylpyrrolidone; (QqQ)MS<sup>2</sup>, triple quadrupole mass spectrometry; SBEC, sulfobutylether- $\beta$ -cyclodextrin; SDED, serial dual-electrode detection; SDMA, symmetric dimethyl-L-arginine; SDS, sodium dodecyl sulfate; TCDI, 1,1-thiocarbonyldiimidazole; TDLFP, transverse diffusion of laminar flow profiles; TEA, triethanolamine; TMPAB-o-M, 1,3,5,7-tetramethyl-8-phenyl-(2-maleimide)-difluoroboradiazas-indacene; (TOF)MS, time-of-flight mass spectrometry; UV, ultraviolet.

\*Capillary dimensions expressed as internal diameter x effective length (cm to the detector).

\*\*LODs units expressed as in the original work.

Figure 1.

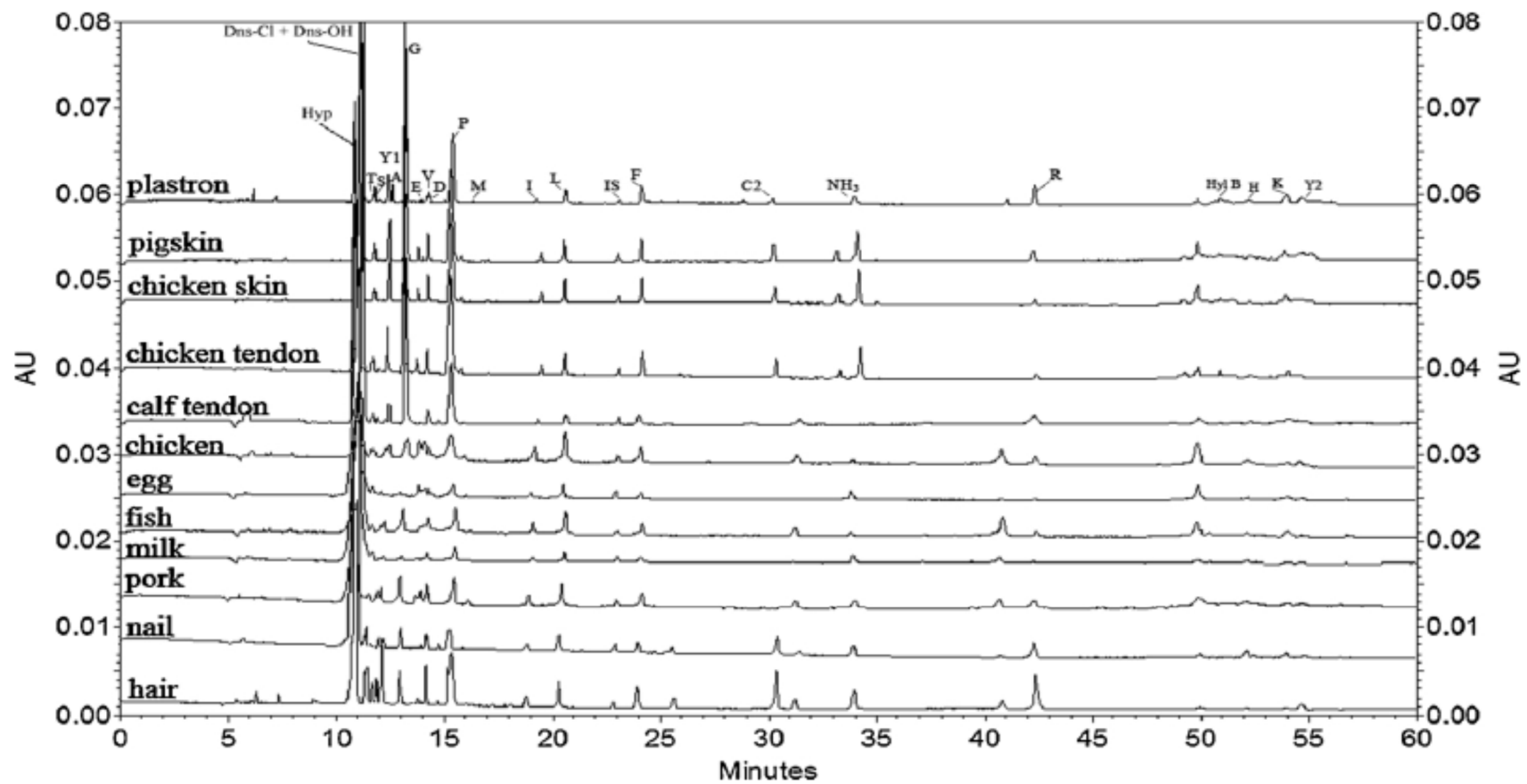
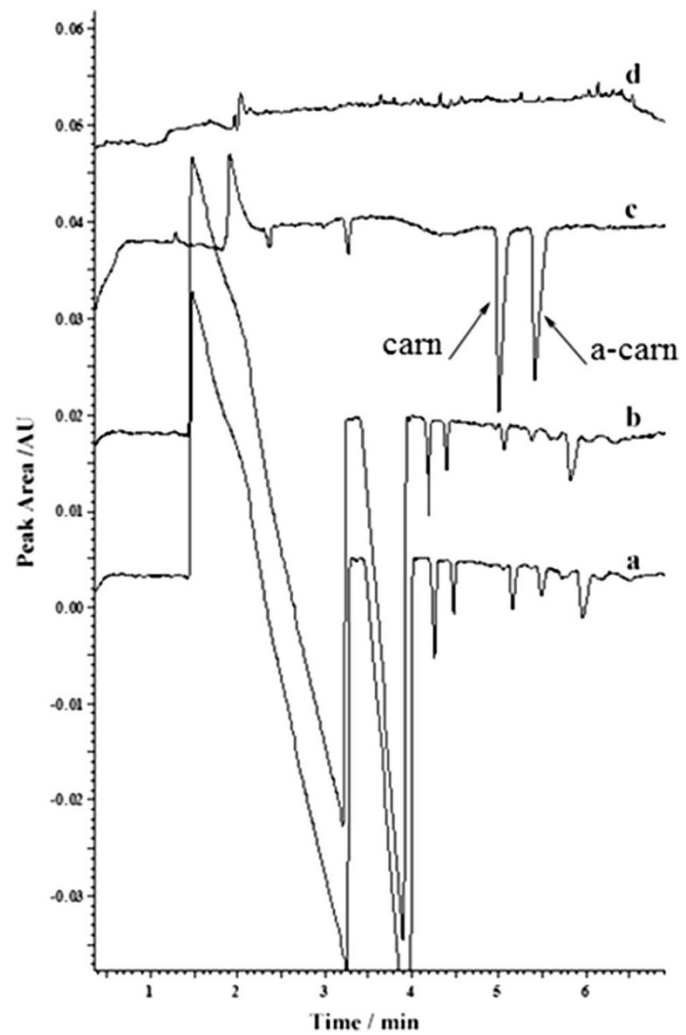
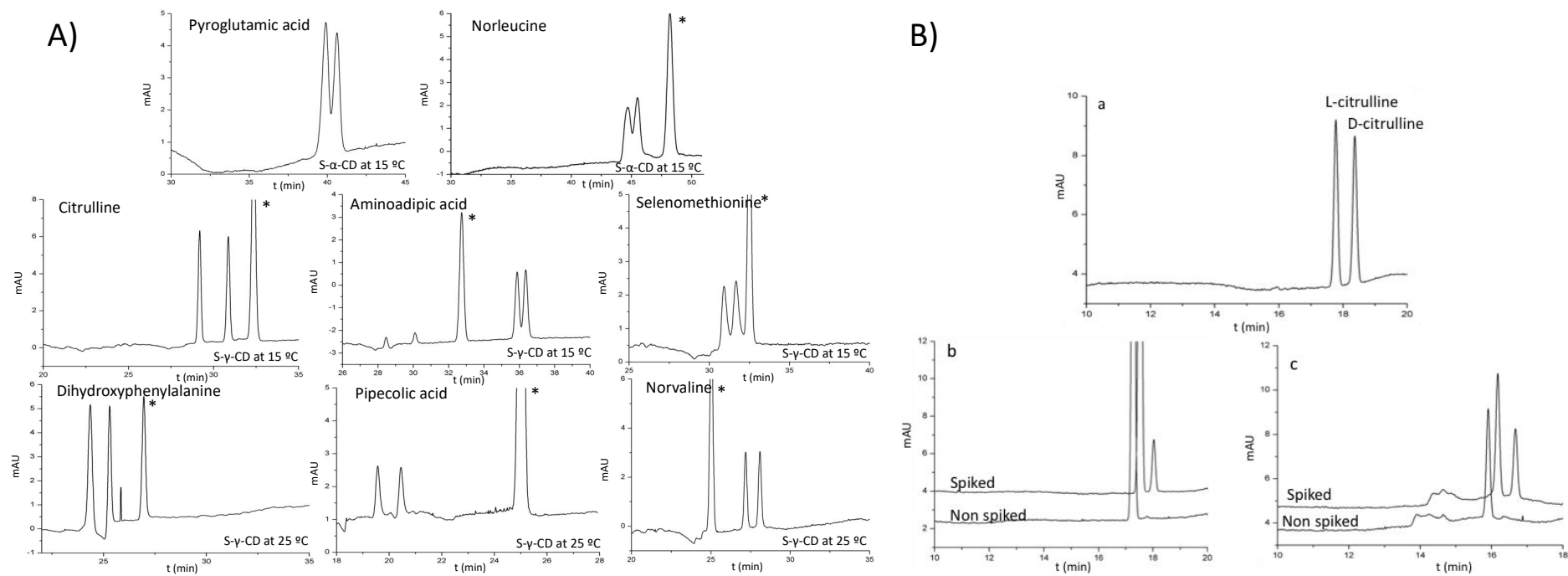




Figure 2.



**Figure 3.**



**Figure 4.**

**A)**

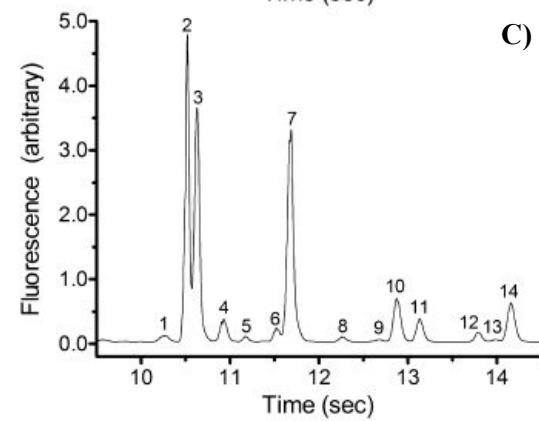
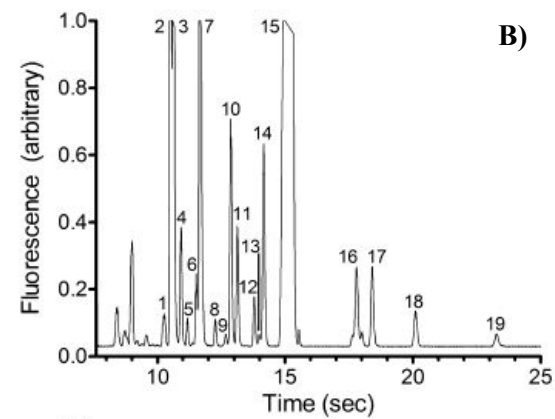
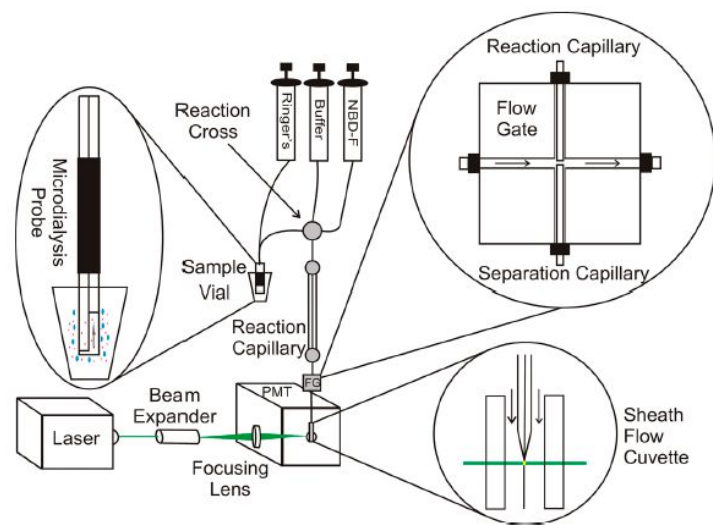


Figure 5.

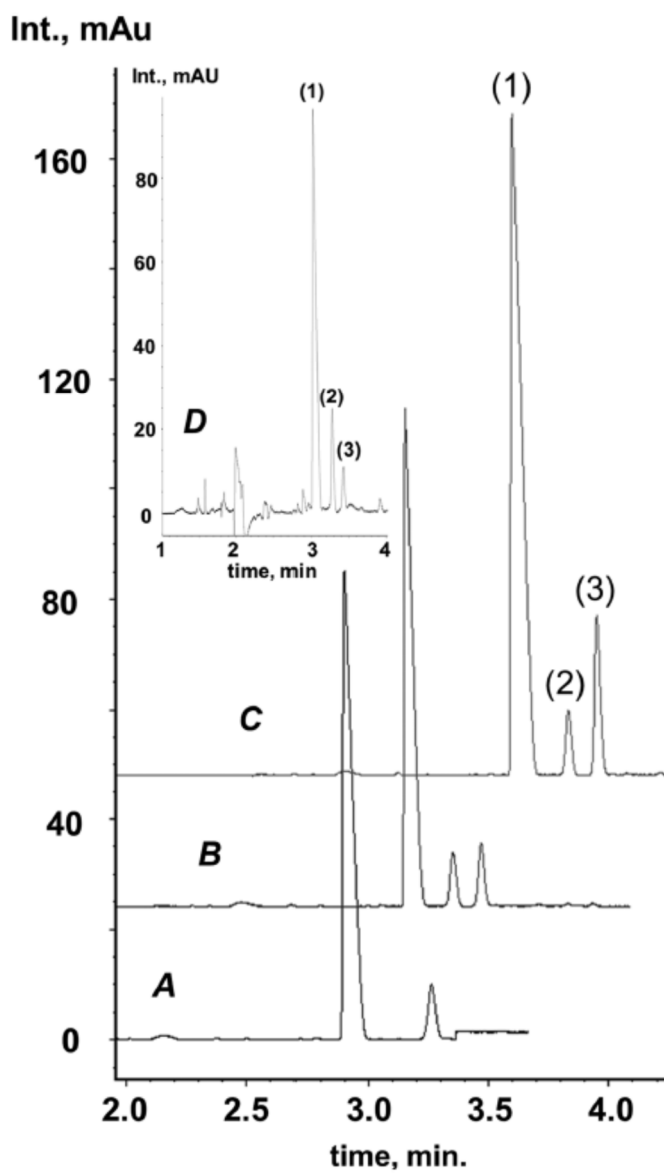


Figure 6.

