



Article

Electrophoretic Determination of L-Carnosine in Health Supplements Using an Integrated Lab-on-a-Chip Platform with Contactless Conductivity Detection

Iva Pukleš^{1,2,3}, Csilla Páger⁴ , Nikola Sakač^{5,*}, Bojan Šarkanj⁶ , Brunislav Matasović¹ , Mirela Samardžić^{1,7}, Mateja Budetić^{1,7}, Dean Marković⁸ and Marija Jozanović^{1,2,7,*}

- ¹ Department of Chemistry, Josip Juraj Strossmayer University of Osijek, Cara Hadrijana 8, 31000 Osijek, Croatia; jcf9b6@pte.hu (I.P.); brunislav.matasovic@kemija.unios.hr (B.M.); mirelas@kemija.unios.hr (M.S.); mbudetic@kemija.unios.hr (M.B.)
 - ² Doctoral School of Chemistry, University of Pécs, Ifjúság útja, 7624 Pécs, Hungary
 - ³ Department of Analytical and Environmental Chemistry, Faculty of Sciences, University of Pécs, Ifjúság Útja, 7624 Pécs, Hungary
 - ⁴ Institute of Bioanalysis, Medical School, Szentágotthai Research Center, University of Pécs, Honvéd Utca 1, 7624 Pécs, Hungary; csilla.pager@aok.pte.hu
 - ⁵ Faculty of Geotechnical Engineering, University of Zagreb, Hallerova 7, 42000 Varaždin, Croatia
 - ⁶ Department of Food Technology, University North, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia; bsarkanj@unin.hr
 - ⁷ Scientific Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek, Trg Svetog Trojstva 3, 31000 Osijek, Croatia
 - ⁸ Department of Biotechnology, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia; dean.markovic@biotech.uniri.hr
- * Correspondence: nsakac@gfv.unizg.hr (N.S.); mhorvat2@kemija.unios.hr (M.J.)



Citation: Pukleš, I.; Páger, C.; Sakač, N.; Šarkanj, B.; Matasović, B.; Samardžić, M.; Budetić, M.; Marković, D.; Jozanović, M. Electrophoretic Determination of L-Carnosine in Health Supplements Using an Integrated Lab-on-a-Chip Platform with Contactless Conductivity Detection. *Int. J. Mol. Sci.* **2023**, *24*, 14705. <https://doi.org/10.3390/ijms241914705>

Academic Editors: Rossana Morabito and Alessia Remigante

Received: 5 September 2023

Revised: 22 September 2023

Accepted: 27 September 2023

Published: 28 September 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The health supplement industry is one of the fastest growing industries in the world, but there is a lack of suitable analytical methods for the determination of active compounds in health supplements such as peptides. The present work describes an implementation of contactless conductivity detection on microchip technology as a new strategy for the electrophoretic determination of L-carnosine in complex health supplement formulations without pre-concentration and derivatization steps. The best results were obtained in the case of +1.00 kV applied for 20 s for injection and +2.75 kV applied for 260 s for the separation step. Under the selected conditions, a linear detector response of 5×10^{-6} to 5×10^{-5} M was achieved. L-carnosine retention time was 61 s. The excellent reproducibility of both migration time and detector response confirmed the high precision of the method. The applicability of the method was demonstrated by the determination of L-carnosine in three different samples of health supplements. The recoveries ranged from 91 to 105%. Subsequent analysis of the samples by CE-UV-VIS and HPLC-DAD confirmed the accuracy of the obtained results.

Keywords: L-carnosine; capacitively coupled contactless conductivity detection; green analytical chemistry; lab-on-a-chip; microchip electrophoresis

1. Introduction

The dipeptide L-carnosine is a vital biomolecule due to its contribution to numerous regular activities in the excitable tissues of vertebrates. The production of carnosine occurs in the liver through the condensation of amino acids β -alanine and L-histidine. In the human diet, exogenous L-carnosine is consumed through the consumption of foods of animal origin, mainly meat. The L-carnosine content of meat varies considerably between different animal species and different meat parts. Therefore, the amount of exogenous L-carnosine depends primarily on personal dietary choices [1,2].

The health benefits of L-carnosine to prevent and treat various medical conditions have been demonstrated in a large number of studies. It has been shown that L-carnosine has potent antioxidant and buffering effects, as well as strong chelating and neuroprotective properties [3–5]. L-carnosine treatment can regulate blood glucose levels and prevent drug-induced cardiotoxicity and neurotoxicity [6]. Moreover, L-carnosine showed beneficial effects on neurodegenerative diseases such as Alzheimer's disease [7,8]. Due to all the above listed beneficial properties, L-carnosine is widely used in the form of health supplements. In addition to its health purposes, L-carnosine containing health supplements are also used by athletes for the enhancement of ergogenic effects during intense training. Polaperzinc is a chelated form of zinc and L-carnosine. It is an approved zinc-containing drug used to clinically treat gastric ulcers and to reduce toxicity caused by radiation therapy [9].

To avoid the adverse effects of taking supplements, the recommended daily dose should be specified, and labels should indicate the exact amount of active ingredients. For instance, excessive blood pressure lowering may occur if L-carnosine is taken at the same time as medications to lower blood pressure. Consequently, poor quality control and deficient legal regulation that is trotting behind the commercial boom of the health supplements market may lead to potential health problems for consumers or the ingestion of an ineffective dose. Therefore, the need for the development of reliable and standardized analytical methods for health supplements has been widely recognized.

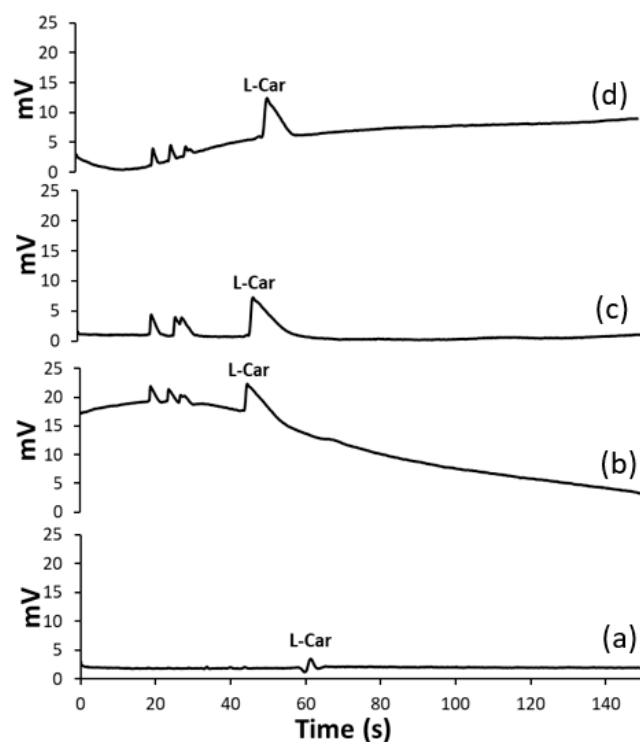
Existing methods for the determination of small peptides, although accurate and precise, are often complex, expensive, and require a high consumption of toxic organic solvents. Currently, high-performance liquid chromatography (HPLC) with integrated fluorescent [10] or mass spectrometry (MS) [11] detection is the most widely used method for the peptide analysis of foods. Ultraviolet (UV) detection can also be employed, but dipeptides have low UV absorbance, which requires a time-consuming derivatization process. Gatti et al. reported a reverse-phase HPLC-UV method for the analysis of L-carnosine in health supplements that employs the tedious derivatization step with 2,4-dinitrofluorobenzene [12]. Furthermore, nuclear magnetic resonance spectroscopy (NMR) was used to determine L-carnosine in foods [13]. Capillary electrophoresis (CE) is a green alternative to HPLC that can also be used for the routine analysis of pharmaceutical and biopharmaceutical substances. The main advantages of CE are the higher separation efficiency and the absence of toxic organic solvents. Some reports describe the use of CE for the analysis of amino acids and dipeptides in health supplements, but to our knowledge, there is no publication on the analysis of L-carnosine in health supplements [14–16]. The available studies report the use of mass spectrometry [17], amperometry [18], laser-induced fluorescence [19], and UV detection [20] for the CE analysis of L-carnosine in various biological and food samples. Microchip electrophoresis (MCE), as a miniaturized form of CE, is an even more environmentally friendly method that allows for a short separation time and the use of less expensive equipment. Zhao et al. reported a highly sensitive MCE method for the determination of L-carnosine and related peptides in biological samples. To convert the species to a detectable form, they used a time-consuming chemiluminescence reaction between hydrogen peroxide and N-(4-aminobutyl)-N-ethylisoluminol-labelled peptides in the presence of adenine and Co^{2+} [21]. Fresta et al. reported the use of fluorogenic reagent naphthalene-2,3-dicarboxaldehyde to quantify intracellular carnosine in macrophage cell lysates [22], and Chen et al. analyzed 4-fluoro-7-nitro-2,1,3-benzoxadiazole labeled L-carnosine and niacinamide in cosmetics [23]. Additional simplification of MCE analysis was achieved by integrated capacitively coupled contactless conductivity detection (C^4D). Until now, MCE- C^4D analysis was reported for diverse analytes, moreover, Tůma et al. summarized its usage in clinical samples, pharmaceuticals, drinking water, beverages, and foodstuffs [24,25]. Jozanović et al. reported the use of homemade MCE- C^4D devices for the simultaneous determination of L-carnosine and L-anserine in meat samples. The study demonstrates the suitability of C^4D for microfluidic analysis, considering portable devices and particularly low manufacturing costs. The major contribution of C^4D is its ability to analyze samples with complex compositions [26,27]. Difficulties in the analysis of

health supplements usually emerge due to the complex and non-specific composition of health supplements, which often contain various adulterants and different herbal extracts in addition to excipients. The herbal extract contents are usually listed only in percentages on the declaration, but the composition of the extract is not indicated. Such, unknown and possibly interfering components can be a particular problem in analysis, especially in optical detection. This report describes the development of a method for the determination of L-carnosine in health supplements using microchip electrophoresis. The objective was to develop a low-cost, rapid, simple, portable, and environmentally friendly method for the analysis of health supplements. Therefore, the development of the method was focused on avoiding the derivatization of the analyte. For this purpose, the MCE technology was conjugated with the C^4D system. The results obtained with the developed method were compared with the results obtained with CE-UV-VIS and HPLC conjugated with a diode array detector (DAD).

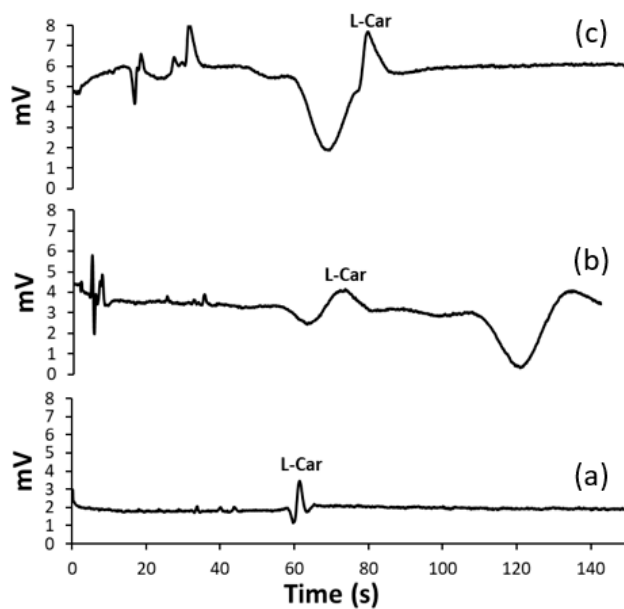
2. Results and Discussion

2.1. Method Optimization

The response of the detector to L-carnosine was studied to determine the optimal operating frequency, amplitude, and excitation voltage, using a buffer solution consisting of 0.5 M acetic acid (HAc). Frequency studies ranged from 250 to 800 Hz and the amplitude from 10 to 60%. The best detectability was achieved by applying an excitation sine wave of 400 kHz with an amplitude of 40%. These parameters were used for subsequent optimization of the electrophoretic measurement. In order to use a BGE characterized by a sufficiently large difference in conductivity and as similar in mobility as possible with respect to the analyte, a variety of different BGE were tested in this study. The MCE system had a normal polarity, and accordingly, an acidic pH was considered in the selection of the BGE to ensure the cationic form of L-carnosine and its migration to the cathode. Therefore, the following organic acids and their mixtures were tested: 0.5 M HAc (pH 2.52), 7.5×10^{-4} M iminodiacetic acid (pH 2.5), 2.4×10^{-9} M tartaric acid (pH 5.8), 1.41×10^{-3} M citric acid (pH 3), 0.01 M 2-hydroxyisobutyric acid + 2×10^{-4} M sodium acetate + 0.4 M HAc (pH 2.36), and 0.01 M 2-hydroxyisobutyric acid + 2×10^{-4} M rubidium acetate + 0.4 M HAc (pH 2.36). Baseline drift can be observed in Figure 1A for curves (b) and (c), which correspond to BGE consisting of iminodiacetic acid and citric acid, respectively. It is assumed that the high conductivity of these acids contributed to the generating Joule heating and therefore caused unstable trends. Hence, these acids were excluded from further research in BGE optimization. Using BGE consisting of tartaric acid, presented with curves (c), resulted in broad and unsymmetrical peaks, therefore they were also eliminated from further investigation. Only curve (a), which represents HAc, shows a stable trend, and symmetrical and sharp peaks, thus it was chosen for further investigation. The mixtures containing HAc, its conjugated salts, and 2-hydroxyisobutyric acid were further tested. These systems also did not yield useful results, as can be seen from the electropherograms shown in Figure 1B(b,c). The best results were obtained with 0.5 M HAc (Figure 1B(a)), which can be explained by the fact that it had the lowest conductivity and consequently the background noise was minimized. In this way, more intense peaks and a better readable signal were obtained. The suitability of BGE consisting of HAc for C^4D detection of AAs and related biomolecules is also described by Peter Tůma [24]. In this study, the BGE pH 2.52 caused complete protonation of the L-carnosine molecule, the β -alanine amino group, and the L-histidine imidazole ring, as well as protonation of the L-histidine carboxyl group. Under these conditions, the dominant form of L-carnosine was charged with +2 and therefore migrated to the cathode under the influence of the electric field (Figure 2). The pK_a of the L-histidine carboxyl group in L-carnosine is 2.64, which means that faster migration of L-carnosine is achieved if the pH of the BGE is lowered below this value, resulting in sharp and high peaks. Thus, the use of the proposed BGE ensures high ion-mobility and peak symmetry, so further lowering of the pH was not necessary. Moreover, very acidic media could cause serious damage to polymer chips such as PMMA chips.



(A)



(B)

Figure 1. The MCE-C⁴D electropherograms of 5×10^{-5} M L-carnosine standard solutions in (A) (a) 0.5 M Hac (pH 2.52), (b) 7.5×10^{-4} iminodiacetic acid (pH 2.5), (c) 2.4×10^{-9} M tartaric acid (pH 5.8), and (d) 1.41×10^{-3} M citric acid (pH 3); (B) (a) 0.5 M HAc (pH 2.52), (b) 0.01 M 2-hydroxyisobutyric acid + 2×10^{-4} M sodium acetate + 0.4 M acetic acid (pH 2.36), and (c) 0.01 M 2-hydroxyisobutyric acid + 2×10^{-4} M rubidium acetate + 0.4 M HAc (pH 2.36).

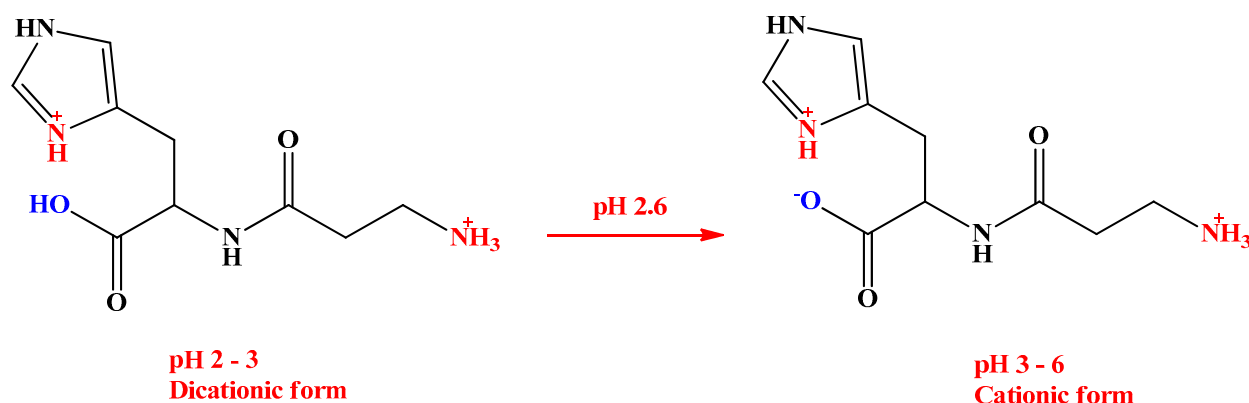


Figure 2. Schematic layout of ionic forms of L-carnosine in acidic media.

In order to achieve fast and efficient separation, another focus of the study was to optimize the injection and separation parameters. The influence of injection voltage was tested with a 0.20 kV steps increment in the range of +1.00 to +1.50 kV. The most symmetrical peak shape was obtained at an applied injection voltage of +1.00 kV. Injection times were evaluated from 12 to 25 s, with peak broadening being noted at times greater than 20 s. Moreover, lower reproducibility was recorded for injection times greater than 20 s. As a result, a 20 s injection time and +1.00 kV injection voltage were selected for further method development. The separation voltage was evaluated in the range from +2.00 to +2.75 kV, in 0.20 kV steps. A value of +2.75 kV was chosen due to the rapid analysis and maximum peak area. Since the migration time for L-carnosine was 61 ± 0.42 s, separation windows of 120 s, 180 s, and 240 s were tested. Baseline drift occurred when a separation time of 120 or 180 s was applied, so a separation time of 240 s was used in the experiments.

2.2. Analytical Features of the Method

The statistical parameters used to evaluate the method are summarized in Table 1. They represent the quality and consistency of the results in terms of the repeatability and mean precision of the data, as well as the sensitivity and linearity achieved. In this study, the linearity of the detector response was determined from the peak height calibration curve, which showed more analytically acceptable results than the peak area curve. For L-carnosine standard solutions ranging from 5×10^{-6} to 5×10^{-5} M, the standard deviation (SD) and relative standard deviation (RSD) of peak height varied from 1.3% to 12%, and from 3% to 19%, respectively. For the peak area, the values ranged from 4% to 63%, and from 4% to 51%, respectively. In the tested concentration range of 5×10^{-5} to 5×10^{-6} M, the correlation coefficient of 0.9976 indicated a good linear behavior of the C⁴D response to L-carnosine. Therefore, the analytical dependence of peak height on L-carnosine concentration was considered suitable to achieve satisfactory stability of the detector response. In this study, an achieved L-carnosine detection limit (LOD) of 2.5×10^{-6} M was obtained experimentally, which is comparable to the previously reported values [26,27]. The experimental limit of quantification (LOQ) was 5×10^{-6} M, thus allowing for reliable quantification of L-carnosine in commercial health supplements. LOD and LOQ were also determined by analyzing different solutions of L-carnosine and measuring the signal-to-noise ratio. The limit of detection (LOD) was the concentration giving a signal-to-noise ratio of 3:1, and the limit of quantitation (LOQ) was the concentration giving a signal-to-noise ratio of about 10:1, with an RSD of less than 10% with triplicate analysis. Thus, the calculated LOD and LOQ are 0.12, and 0.39 μ M, respectively. The repeatability and reproducibility of the migration time were calculated by using five series of five consecutive injections. RSD values ranged from 0.5% to 2%, confirming the excellent repeatability and reproducibility of the method.

Table 1. Analytical characteristics of the MCE-C⁴D methods.

Characteristics	MCE-C ⁴ D
Migration time (s)	61 ± 0.42
R ²	0.9976
Linear range (M)	5 × 10 ⁻⁶ – 5 × 10 ⁻⁵
Slope	27,567
Intercept *	0.1852
LOD (µM)	2.5 × 10 ⁻⁶
LOQ (µM)	5 × 10 ⁻⁶

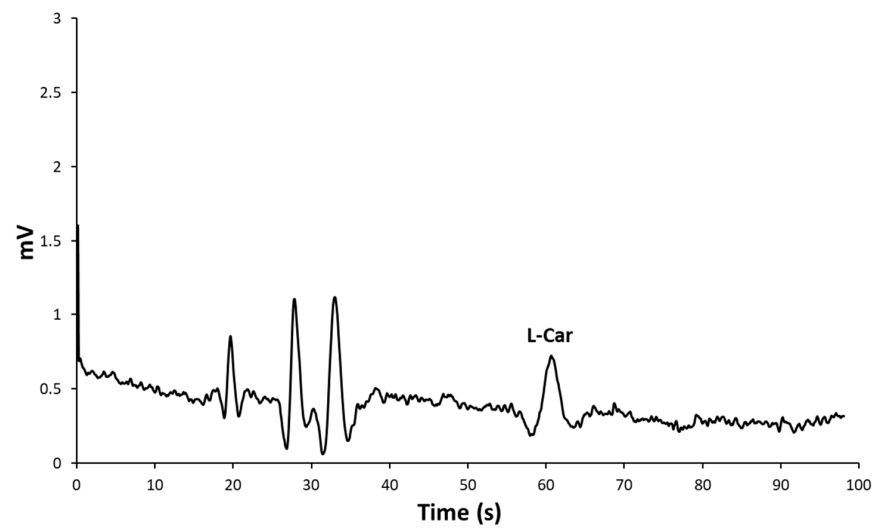
* Mean ± standard deviations (n = 3). Abbreviations: LOD, limit of detection, LOD; limit of quantification, R², coefficient of determination.

2.3. Determination of L-Carnosine in Real Samples

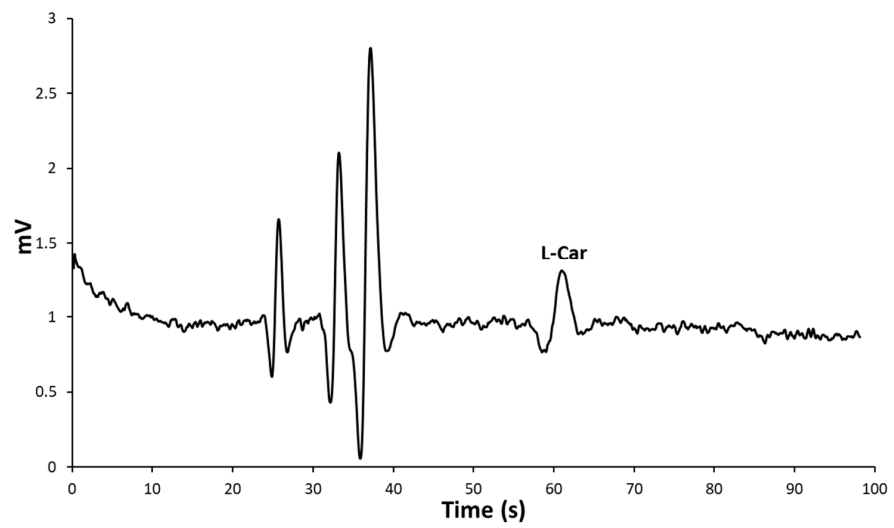
The developed electrophoretic method applicability was demonstrated using the selected experimental protocols and instrumental parameters for the quantification of L-carnosine in three different multicomponent health supplements. The procedure did not involve any sample preconcentration or derivatization of the analyte which are commonly used in peptide analysis. The electropherograms of the samples are shown in Figure 3. In all three electropherograms, the peaks corresponding to L-carnosine are well-defined, symmetrical, and completely separated from the other sample components. It should be noted that the migration times between L-carnosine in the case of the standard solution and those of the sample's solutions did not differ notably, which indicates the acceptable specificity of the method. The recorded values were 61 ± 0.42 s, 61.88 ± 0.59 s, 62.28 ± 0.6 s, and 63.35 ± 1.2 s for the standard solution, Sample 1, Sample 2, and Sample 3, respectively. The method specificity was further confirmed by spiking the samples with L-carnosine standard solutions.

The results summarized in Table 2 show that all three samples had a slightly higher content of L-carnosine than declared. These minor differences may be attributed to the capsule form of the supplement, as the highest deviations were observed in the capsule samples and the lowest in the powder samples [28]. In this study, the L-carnosine concentrations obtained were 106.03–115.90% of the reported value, which was within the tolerance variation. The highest variation was observed in Sample 3, which contained a concentration of L-carnosine five times higher than the other two samples. Sample 1 and Sample 2 contained a similar declared value for L-carnosine, so the deviations from the declared value were also similar: +7.65 and +6.03 for Sample 1 and Sample 2, respectively, raising the suspicion that the deviation from the declared values increases with an increasing amount of the active ingredient content. However, more samples are needed to confirm these claims. For the time being, however, the focus of the study was on developing an accurate and precise method for evaluating L-carnosine content.

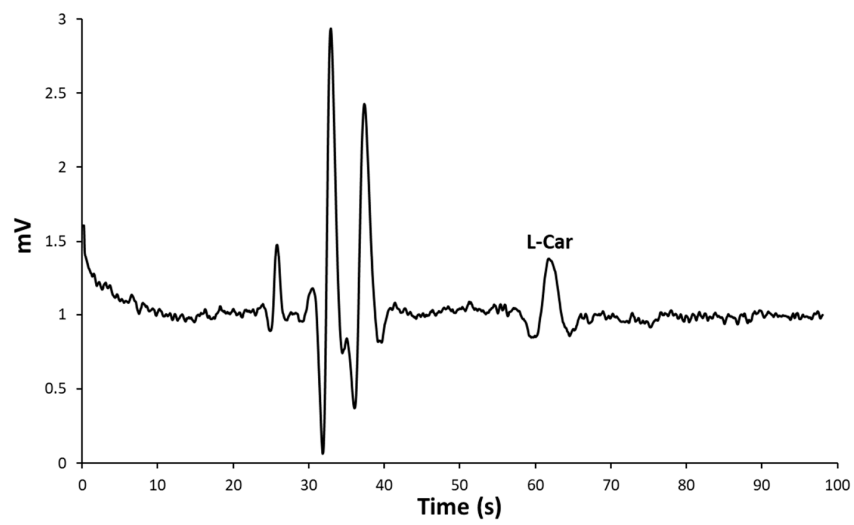
Recovery experiments were performed with two different concentrations to confirm the accuracy of the method and to exclude the influence of the matrix on L-carnosine analysis. Each sample was spiked with L-carnosine to obtain concentrations 20% and 40% higher than those already detected. The recovery values ranged from 94.46 to 112% and detailed information is given in Table 3. As another approach to assessing the method's accuracy, CE-UV-VIS and an HPLC-DAD were employed for further analysis of the samples. Table 2 shows the comparable results for L-carnosine concentration with all the methods used. By applying the *t*-test (n = 3, α = 0.05, *t*_{crit} = 4.303), it was found that there were no statistically significant differences between the precision of the concentrations of MCE-C⁴D and CE-UV-VIS. The calculated *t*-values were 0.70, 2.05, and 0.52 for Samples 1, 2, and 3, respectively. However, the *t*-test did not show statistically significant differences, even when comparing the proposed method and HPLC-DAD. The calculated *t*-values were 3.1620, 0.5424, and 0.1835 for Samples 1, 2, and 3, respectively. A very good correlation between these methods indicates a reasonable accuracy of the developed method for the determination of L-carnosine in health supplements.



(A)



(B)



(C)

Figure 3. Cont.

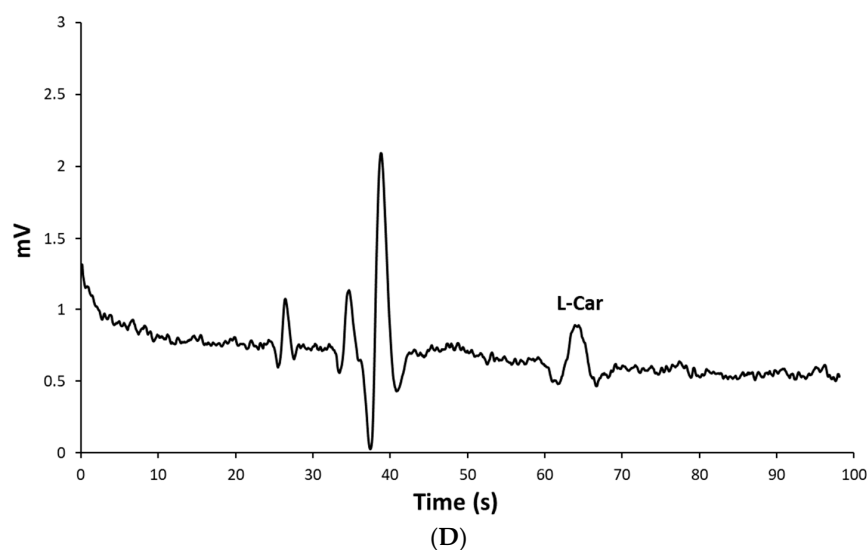


Figure 3. MCE-C⁴D electropherogram for L-carnosine (A) 5×10^{-5} M standard solutions, (B) Sample 1, (C) Sample 2, and (D) Sample 3. Used conditions: BGE consisted of 0.5 mol L⁻¹ (pH 2.52), 20 s electrokinetic injection, and +2.75 kV applied separation voltage.

Table 2. Results of the quantitative determinations of L-carnosine in health supplement samples.

		Sample 1	Sample 2	Sample 3
MCE-C ⁴ D analysis	Declared Value (mg/g Sample)	171.42	172.50	833.33
	Results (mg/g sample)	184.53 ± 8.28	182.91 ± 15.95	965.79 ± 130.50
	RSD (%)	4.49	8.72	13.51
	% Deviation from the declared value	+7.65 ± 4.83	+6.03 ± 9.24	+15.95 ± 15.66
CE-UV-VIS analysis	Results (mg/g sample)	178.60 ± 6.39	167.7 ± 4.57	927.72 ± 3.45
	RSD (%)	3.58	2.72	0.37
	% Deviation from the declared value	+4.19 ± 3.73	-2.78 ± 2.65	+11.33 ± 0.42
HPLC-DAD analysis	Results (mg/g sample)	198.01 ± 9.80	177.4 ± 8.48	980.63 ± 47.10
	RSD (%)	5	5	5
	% Deviation from the declared value	+15.51 ± 5.71	+2.84 ± 4.92	+17.68 ± 5.65

A comparison of the analytical characteristics of all methods used is presented in Table 4. It is noteworthy that the coefficient of determination was higher in the case of the MCE-C⁴D analysis than in the CE-UV-VIS analysis. Furthermore, observing the CE electropherograms of the samples (Figure 4), it is evident that the analysis time was significantly longer using CE-UV-VIS than MCE-C⁴D. The recorded retention time of L-carnosine on the CE was 807.72 ± 2.49 s, while the migration time on the MCE was only 61 ± 0.42 s.

Table 3. MCE-C⁴D results of recovery experiment for spiked health supplement sample.

	Unspiked Sample (μM)	Added Amount (μM)	Spiked Sample (μM)	Recovery (%)
* Recovery experiment 1				
Sample 1	11.01 ± 0.04	2.20	13.09 ± 0.07	96.66 ± 1.90
Sample 2	10.19 ± 0.10	2.18	13.09 ± 0.07	99.73 ± 3.32
Sample 3	8.53 ± 0.010	1.70	10.28 ± 0.08	102.36 ± 4.91
* Recovery experiment 2				
Sample 1	11.01 ± 0.04	4.40	15.76 ± 0.20	108.00 ± 4.54
Sample 2	10.19 ± 0.10	4.36	15.22 ± 0.23	98.62 ± 5.29
Sample 3	8.53 ± 0.010	3.41	12.12 ± 0.25	108.73 ± 3.20

Recovery experiment 1—L-carnosine was added to obtain a 20% higher value than an unspiked sample. Recovery experiment 2—L-carnosine was added to obtain a 40% higher value than an unspiked sample * Number of measurements = 3.

Table 4. Analytical characteristics of the MCE-C⁴D, CE-UV-VIS, and HPLC-DAD methods.

Characteristics	ME-C ⁴ D	CE-UV-VIS	HPLC-DAD
R ²	0.9976	0.9905	0.9996
Measured concentration range	5 × 10 ⁻⁶ –5 × 10 ⁻⁵	4.4 × 10 ⁻⁴ –4.4 × 10 ⁻³	5 × 10 ⁻⁶ –2.5 × 10 ⁻⁴
Slope	27,567	64.022	3.72 × 10 ⁻⁷
Intercept *	0.1852	-3.938	2.43 × 10 ⁻⁶

* Mean ± standard deviations (n = 3).

A lot of the available literature deals with the MCE analysis of proteins, polypeptides, and their digestion products, still, fewer papers describe the MCE analysis of small peptides, especially dipeptides. An overview of the MCE methods for the analysis of peptides containing less than 20 AAs residues is presented in Table 5. To date, a few methods, each of similar sensitivity, have been reported on the MCE of dipeptides. As in this study, micromolar concentrations were analyzed, however, slightly lower LODs were achieved using optical detectors, which is expected. For example, reported LODs for the chemiluminescence detection of L-carnosine, L-homocarnosine, and L-anserine were 0.030 μM, 0.028 μM, and 0.034 μM, respectively [21], while laser-induced fluorescence (LIF) detection was performed with LODs of 0.050 μM for Gly-Gly and Gly-Leu [29], and 0.065 μM for L-carnosine [22]. MCE was utilized in the CE-fluorescence analysis as a preconcentration method for Phe-Ala, which enabled for concentration enrichment from 2 μM to 0.02 μM [30]. Considering the linear detection range of our method was sufficient for the analysis of food supplements, and it is assumed to be appropriate for the other types of samples, it can be said that the slightly higher detection limit of 2.5 μM is a reasonable compensation for avoiding expensive instrumentation and the use of derivatization agents. Studies using a homemade MCE-C⁴D unit reached somewhat better sensitivity; LODs were below 1 μM for L-carnosine and L-anserine [26,27]. However, in this case, complex samples' pre-concentration was necessary since the meat samples contain a large amount of proteins and fats. Unlike for the HPLC analysis of meat samples, the prepared samples' aliquots needed to be further deproteinized and concentrated, which included several extra steps: dilution by methanol, shaking, storing at 4 °C, centrifugation, filtration, and supernatant evaporation to dryness. The solid residues were then supplemented with an extremely small water volume, shaken, heated, and finally sonicated. In contrast, in this study, method simplicity and rapidness are emphasized. MCE-C⁴D analysis of the L-carnosine in capsule samples required only two simple steps since the concentration was significantly higher than those in meat samples. Therefore, there was no demand for deproteinization, sample enrichment, or extensive BGE optimization in order to lower the

detection limit. This indicates the huge potential of MCE-C⁴D for the analysis of samples such as health supplements, dietary supplements, or different medicaments.

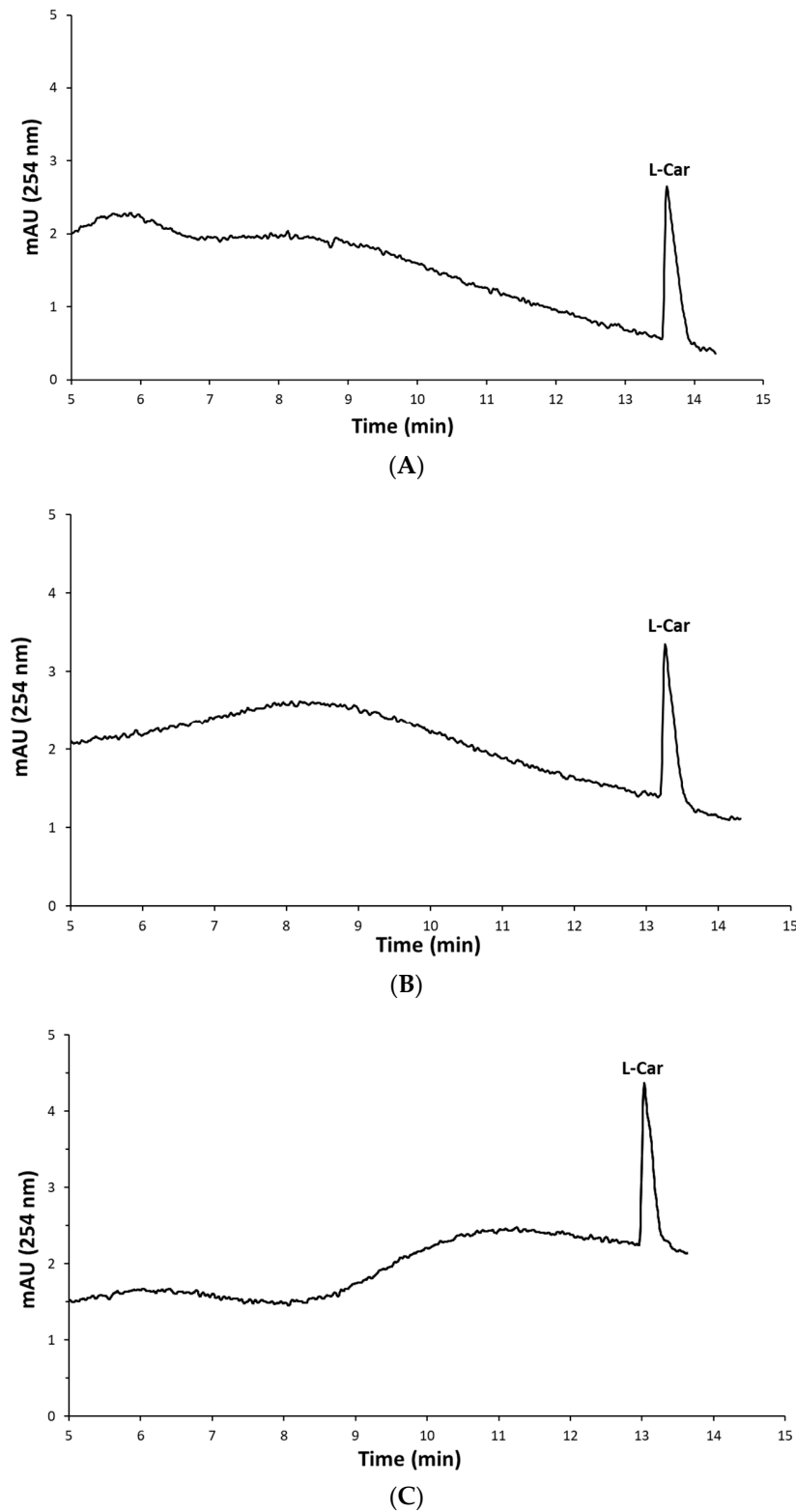


Figure 4. CE-UV-VIS electropherogram of (A) Sample 1, (B) Sample 2, and (C) Sample 3. CE conditions: 50 mM CuSO₄ + 0.05% HAc electrophoretic buffer (pH 4.5), hydrodynamic injection at 50 mbar for 5 s, and +15 kV separation voltage.

Table 5. An overview of the MCE methods for the analysis of oligopeptides containing less than 20 AAs residues.

Analyte/Sample	BGE	Chip Material	Linear Range/Tested Range	LOD (Analyzed Conc.) (μM)	Detector	Ref.
β A-H (carnosine)/health supplements	0.5 M HAc	PMMA	5–50	2.5	C ⁴ D	This work
β A-H (carnosine), homocarnosine (GABA-H) anserine (β A-methyl-H)/human cerebrospinal fluid and canine plasma	15 mM borate buffer (pH 9.8) + 1.0 mM Co ²⁺ + 1.0 mM adenine +35 mM SDS	glass/PDMS	0.06–15	0.030 0.028 0.034	CL	[21]
β A-H (carnosine) β A-methyl-H (anserine)/breast muscle	200 mM HAc + 10 mM HIBA + 0.3 mM KOAc (pH 2.7)	PMMA	0–200	0.10 0.16	C ⁴ D	[26]
β A-H (carnosine)/macrophage cells	20 mM borate (pH 9.2)	glass	0.025–5	0.065	LIF	[22]
β A-H (carnosine), β A-methyl-H (anserine)/meat	250 Mm HAc + 10 mM 2-OH-n-butyric acid + 0.3 mM RbOAc (pH 2.6)	PMMA	0–88.42	0.038 0.069	C ⁴ D	[27]
β A-H (carnosine)/cosmetics	0.1 mM borax with + 0.03% w/w SDS	BSA-COC	0.38–226.24 mg/kg	0.17 mg/kg, (0.10, 10)	LIF	[23]
GG, GL, RPP, KPV, VKK WYD, YWS/ anode chamber of microbial fuel	10 mM borax-H ₃ BO ₃ buffer + 20 mM SDS (pH = 9.0)	ITO-glass	0.625–10 0.625–10 0.31–10 1.25–20 1.25–20 0.625–10 0.625–10	0.050 0.050 0.025 0.625 0.100 0.050 0.050	LIF	[29]
GF isomers/ model solution	20 mM PBS (pH 7.17)	PDA/GO/BSA-PDMS	N/A	N/A (4000)	Amperometry	[31]
FA, YGGFL (leucine enkephalin), GGYR/ model solution	10 mM Na ₂ CO ₃ + 0.5% HPC (w/v) (pH 9.7)	HPC-PDMS	N/A 1–10 N/A	N/A (0.02)	Fluorescence	[30]
GGGG GGGGG GGGGG/ model solution	20 mM Na ₂ B ₄ O ₇ (pH 10.02)	glass	N/A	N/A (10)	Fluorescence	[32]
GCE (glutathione)/ rat liver cells	20 mM Na ₃ PO ₄ + 1.5 mM luminol (pH 9.6)	glass	3–600	0.96	CL	[33]
XLYENKPRRPYIL (neurote-nsin), CYIQNCPLG (oxytocin), YGGFL (leucine enkephalin), EADPNK + FYGLM (tryptic digestion of physalaemin)/ model solution	Ampholyte mixture (pH 4–7)	acrylate-glass	N/A	N/A (100, 100, 100, 966)	Fluorescence	[34]
pI markers: GCDDD (pI 3.64), GCHQHQQHQ (pI 53) * GCQHHR (pI 7.58) * GCYYYKK (pI 9.56)/ model solution	2.5% (v/v) Pharmalyte 3–10	glass	N/A	3 × 10 ⁻⁶ for the pI 5.53 (0.0025)	LIF	[35]
β -casomorphins: YPFPG, TPFPG, YPFPGPI, TPFPG, PFPGPI/cheese	15 mM H ₃ BO ₃ -Na ₂ H ₂₀ B ₄ O ₁₇ (pH 10.5)	glass	0.01–1 0.05–1 0.05–1 0.03–1 0.05–1	0.075 0.042 0.024 0.019 0.019	LIF	[36]
DRVYIHPFHL (Angiotensin I), DRVYIHPF (Angiotensin II), RVYIHPF (Angiotensin III), RPPGF (Bradykinin fragment 1–5)/ model solution	30 mM NH ₄ Ac + 30% MeOH	PDMS	N/A	N/A (100)	ESI/MS	[37]
RPPGFSP (Bradykinin fragment 1–7), SYSMEHFRW, GKPVGKKR (ACTH fragment 1–17)/ model solution	ACN: 10 mmol/L Tris buffer (pH 8.2)/80:20	NPs -silicon	N/A	N/A (6)	MALDI-TOF-MS	[38]

Table 5. Cont.

Analyte/Sample	BGE	Chip Material	Linear Range/Tested Range	LOD (Analyzed Conc.) (μM)	Detector	Ref.
Vancomycin (7 modified AAs); (NLEU)-(HDPY)-(ASN)-p-(DPYG)-(DHPG)-HDPY-(LPGH)/human plasma	300 mM HAc	glass	5.18–4.86	0.83	C ⁴ D	[39]
Ac-R-[CMLNRVYRPC]/model solution	25 mM phosphate buffer (pH 7)	PEG- glass	N/A	N/A (100)	Fluorescence	[40]
PO ₄ -glycogen synthase (PLSRTLSSVS)/model solution	100 mM Tris phosphate buffer (pH 7.0)/10 mM EDTA	PMMA	0.001–0.025	0.001	Fluorescence	[41]
PO ₄ -peptides: DHTGFLpTEYVATR, DHTGFLTEpYVATR, DHTGFLpTEpYVATR, Non-PO ₄ -peptide: DHTGFLTEYVATR	25 mM borax + 2.8% NH ₄ OH (pH 11.5)	glass	0.04–1.34	0.015 for PO ₄ -peptides (0.31)	Fluorescence	[42]
Insulin receptor peptide: Non-PO ₄ -peptides: TRDIYETDYYRK PO ₄ -peptides: TRDIpYETDYYRK TRDIYETDpYYRK TRDIpYETDpYpYRK	40 H ₂ O/60 MeOH (<i>v/v</i>) + 30 mM NH ₄ -Ac (pH 7.4)	PDMS	N/A	N/A (125, 250)	ESI-MS	[43]

Abbreviations: Ac-R-[CMLNRVYRPC], Acetyl-D-Arg-[Cys-Met-Leu-Asn-ArgVal-Tyr-Arg-Pro-Cys]-NH₂; BS, bovine serum albumin; CL, chemiluminescence; COC, olefin copolymermicrochips; ESI-MS; electrospray ionization-mass spectrometry; GABA, Gamma-aminobutyric acid; HAc, acetic acid; HPC, Hydroxypropyl cellulose; ITO, indium tin oxide; MALDI-TOF-MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; (NLEU)-(HDPY)-(ASN)-p-(DPYG)-(DHPG)-HDPY-(LPGH), N-methyl-D-leucine-m-chloro-bhydroxy-D-tyrosine-asparagine-p-(2-[a4-L-epi-vancosaminyl]-b-1-D-glucosyl)-D-phenylglycine-p-hydroxy-D-phenylglycine-HDPY-m,m-dihydroxy-L phenyl-glycine; Non-PO₄-peptides, nonphosphorylated peptides; (NPs, nanoparticles; PDA/GO/BSA; bovine serum albumin conjugated polydopamine-graphene oxide nanocomposites, PO₄-peptides, phosphorylated; SDS, sodium dodecyl sulfate.

3. Materials and Methods

3.1. Chemicals and Materials

Acetic acid (Panreac Quimica Ltd., Barcelona, Spain), citric acid (Sigma-Aldrich, Saint Louis, MO, USA), glycolic acid (Sigma-Aldrich, Saint Louis, MO, USA), iminodiacetic acid (Sigma-Aldrich, Saint Louis, MO, USA), tartaric acid (Sigma-Aldrich, Saint Louis, MO, USA), rubidium acetate (Sigma-Aldrich, Saint Louis, MO, USA), sodium acetate (Sigma-Aldrich, Saint Louis, MO, USA), and 2-hydroxyisobutyric acid (Sigma-Aldrich, Saint Louis, MO, USA) were tested as background electrolyte (BGE) components. L-carnosine of analytical grade (Sigma-Aldrich, Saint Louis, MO, USA) was used for standard solutions. Copper (II) sulfate (T.T.T. Ltd., Novaki, Croatia) was used in UV-VIS detection. All chemicals were of analytical reagent grade. The health supplements were obtained from web shops. They were all in the form of capsules. The declared mass of the capsule in Sample 1 was 583 mg and the mass of L-carnosine was 100 mg. The Sample 2 declaration noted capsules of 361.45 mg of mass that contained 62.35 mg of L-carnosine and 84 mg of zinc. The declared mass of Sample 3 capsules was 600 mg with 500 mg of L-carnosine. The capsules contained various additional ingredients; chromium, calcium carbonate, silicon dioxide, talc, magnesium stearate, and hydroxypropyl methylcellulose. In addition, the samples contained complex mixtures of unknown ingredients due to the dry extract of Chinese cinnamon and rice powder. Before analysis, cellulose syringe filters (0.22 μm pore diameter, Labex Ltd., Marrickville, NSW, Australia) were used to filter the sample solutions.

3.2. Real Samples Preparation for MCE-C⁴D Analysis

The sample preparation procedure was identical for all three supplements but differed in dilution. The powder was poured from 10 capsules and crushed in a mortar. Then, 100 mg of the homogenized powders was diluted in 10 mL of ultrapure water (Veolia Water Technologies, Paris, France). The prepared solutions were further diluted. The

dilution factors depended on the L-carnosine mass fraction needed to obtain an appropriate concentration of the sample solution. In the case of Sample 1 and Sample 2, the dilution factor was 1:750, and in the case of Sample 3 dilution was 1:5000. Before the analysis, the solutions were filtered using CHROMAFIL Xtra RC-20/25 syringe filters with a pore size of 0.2 μm (Macherey-Nagel GmbH and Co. KG., North Rhine-Westphalia, Germany) and placed in an ultrasonic bath for 10 min before analysis.

3.3. MCE- C^4D Analysis

MCE analyses were performed on a double-T poly(methyl methacrylate) chip (Chip-Shop GmbH). The dimensions of the chip were 1.6 cm \times 9.5 cm. The separation channel had a full length of 8.7 cm and a cross-section of 50 \times 50 μm . The chip was placed on the MCE model ET121 (eDAQ) device (Figure 5B), which is connected to an ER430 High Voltage Sequencer (HVS) and an ER225 C^4D system (Figure 6A). The detection electrodes are located at the ET121 platform, separated from the microchip. Indeed, the ET121 platform contained four 2 mm \times 1 mm gold electrodes (Figures 6C and 5A), two of which are used for detection. Before the measurement, the microchip was positioned on the platform in such a manner that the electrodes were located at the end of the separation microchannel. The electrodes touched only the microchip outside and were still separated from the microchannel and BGE. A high-frequency alternating current was applied on the transmitter electrode, and thereupon was capacitively coupled into the electrolyte and then in the receiver electrode (see Figure 5A). The signal decreased due to the passing through the BGE and the size of the received signal depended on the electrical conductivity of the sample. Due to the coupling of the detection electrodes, stray capacitance would be generated, however, in a four-electrode configuration it was efficiently eliminated. The pairing of the other two electrodes also produced a stray capacitance that annulled the stray capacitance created by the first pair of electrodes. In this way, the negative influence of stray capacitance on the signal was significantly reduced. Preparation of the chip for analysis involved a simple rinse of the microchannel, first with ultrapure water and then with BGE. To test the current flow and gauge the stability of the measuring device and microchannels permeability, 50 μL of BGE and high voltage electrodes were added to each chip reservoir and a blank test was performed. If the baseline was suitable, a sample was recorded. The sample solution was placed in reservoir 1 (R1) and BGE in reservoirs 2, 3, and 4 (R2, R3, R4). Only electrodes 1 and 3 were controlled, during floating injection by applying 1000 mV on R1 and ground on R3 for 20 s (illustrated in Figure 5B(I)). The resulting current was 40 μA due to microchannels double-T geometry, in which the distance between R1 and R3 was only 1 cm. This injection microchannel crossed over an 8.7 cm length separation channel at 0.5 cm of length. At 21 s, the sample was at the intersection of the two microchannels and the separation was started. At this moment the electrodes in R1 and R3 were disconnected from the power supply and 2750 mV was applied to R2 and ground on R4 for 260 s (illustrated in Figure 5B(II)). Thus, the sample migrated to R4, and just before reaching it, the sample was detected on the C^4D detector placed on the MCE platform (outside the microchip). A signal was detected using an excitation sine wave of 400 kHz with an amplitude of 40%. Each sample was recorded in three series, with one series comprising five consecutive measurements. Between each series of measurements, the microchannels were flushed with BGE in order to achieve satisfactory reproducibility. Upon the end of the experiments, the microchannels were rinsed with ultrapure water. The obtained C^4D electropherograms were analyzed using PowerChrom (eDAQ, Version 2.7.8, Denistone East, NSW, Australia). In the used high-conductivity acidic BGE, the obtained signals were negative and therefore inverted.

3.4. CE-UV-VIS Analysis

Agilent Technologies 7100 CE instrument (Waldbronn, Germany) and fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with an effective length of 41.6 cm \times 50 μm ID were used for CE analysis. The 3D-CE ChemStation software, Agilent Technologies (B.

04.00. and Version 7.01, Waldbronn, Germany) was used for UV detection data processing. Measurements were performed using a running buffer consisting of 5×10^{-5} M CuSO_4 and 0.05% HAc (pH 4.5) as previously reported in the work of Jiang et al. [44]. Direct analysis of the underivatized L-carnosine was possible due to the complex formation between Cu^{2+} and L-carnosine which absorbs at a wavelength of 254 nm.

In this work, the optimized hydrodynamic injection at 50 mbar for 5 s was applied. The electrophoretic separations were performed with a voltage of +15.00 kV. Before analysis, the capillary was rinsed with aqueous solutions in the following order: (1) ultrapure water for 5 min; (2) 0.1 M NaOH for 5 min; (3) ultrapure water for 5 min; (4) running buffer for 5 min. Between each run, the capillary was rinsed with a running buffer for 5 min. At the end of each day, the capillary was rinsed in the following order: (1) ultrapure water for 5 min; (2) 0.1 M NaOH for 5 min; (3) ultrapure water for 10 min.

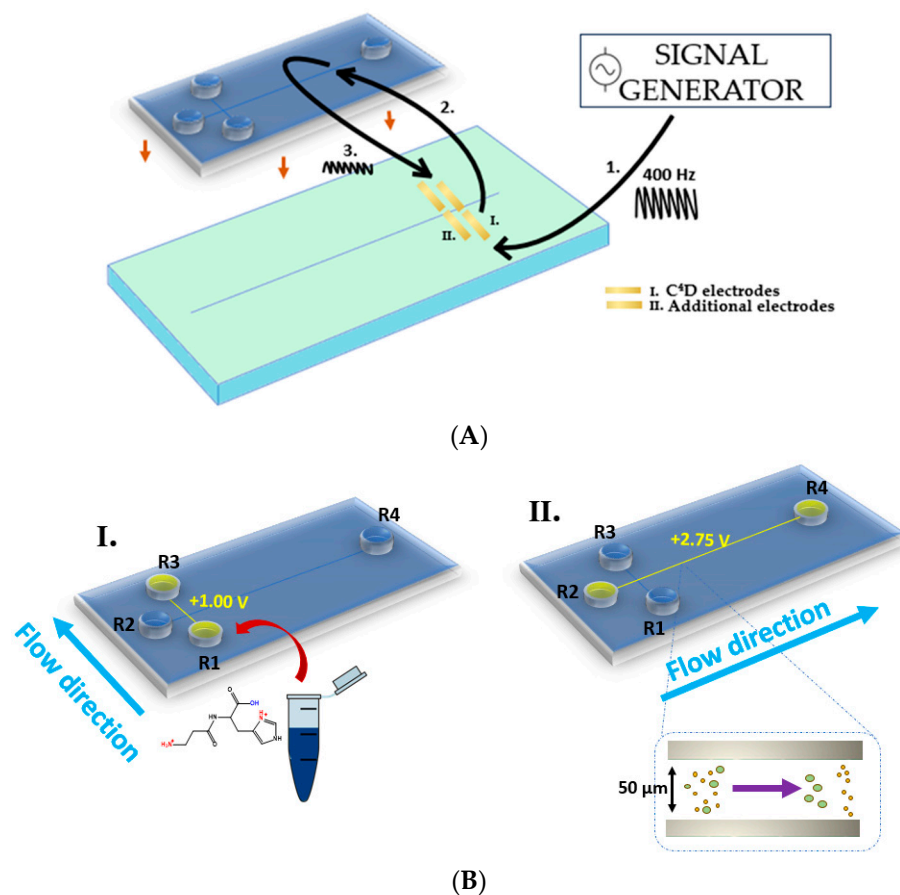


Figure 5. Layout of the assay design for (A) integrated MCE-C⁴D analysis, (B) MCE sample injection (I) and separation processes (II)

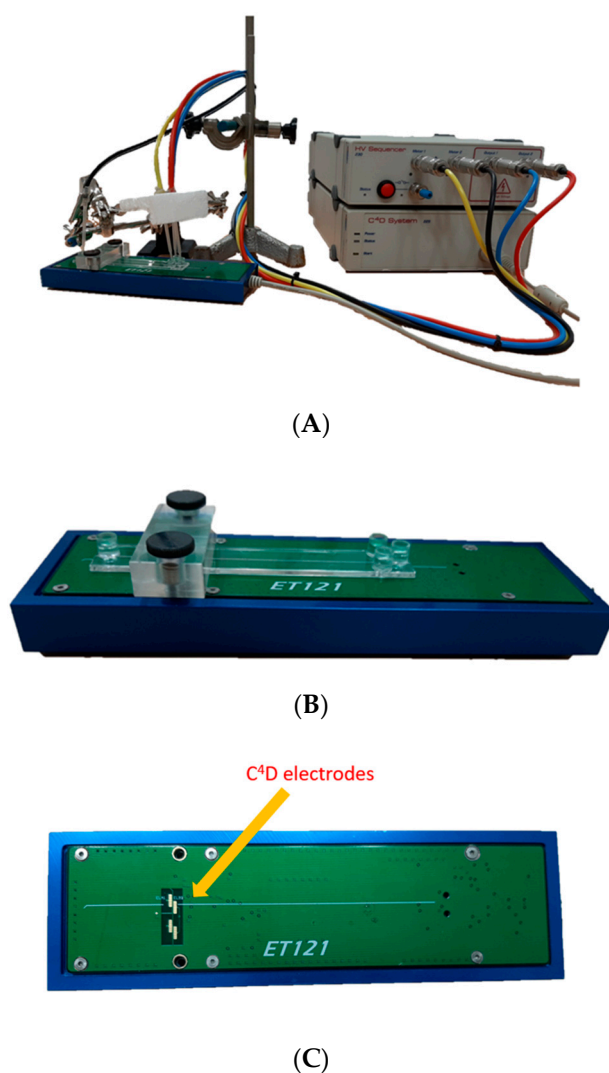


Figure 6. (A) Integrated instrumentation of ET121 microchip platform, ER430 High Voltage Sequencer, and ER225 C⁴D system; (B) a double-T poly(methyl methacrylate) chip on the ET121 microchip platform; (C) display of ET121 microchip platform with C⁴D electrodes.

3.5. HPLC-DAD Analysis

Additionally, method evaluation was performed using HPLC-DAD analysis, according to the official amino acid analysis methodology of Rita Steed [45]. The used instrumentation was an Agilent 1260 Infinity II HPLC system (Agilent, Santa Clara, CA, USA) containing a quaternary pump system, autosampler, and column department. For the acquisition and processing of the data, Agilent OpenLAB CDS software (Version 2.6, Agilent, Santa Clara, CA, USA) was employed. The separations were performed on the Poroshell 120 EC-C18, at 2.7 μm , 4.6 \times 100 mm, sustained at 40 $^{\circ}\text{C}$ (exit side at 35 $^{\circ}\text{C}$). Before the measurement, all the samples were derivatized with phthalaldehyde for 2 min. Two different mobile phases were used for gradient elution; MP A (10 mM Na₂HPO₄–10 mM Na₂B₄O₇, pH 8.2) and MP B (45 ACN:45 MeOH:10 H₂O by volume). The ratio of mobile phase B changed as follows: 2% in the start time, 2.00% in 1 min, 57.00% in 7 min, 100.00% in 7.1 min, 100.00% in 8.4 min, and 2.00% in 8.6 min. In 0.00 min, the L-carnosine was measured at bandwidths 338 nm and 10 nm (Ref 390 nm and 20 nm). Thereafter, it was measured in 5.53 min at bandwidths 262 and 16 nm (Ref 324 and 8 nm).

4. Conclusions

A simple electrophoretic method for the determination of L-carnosine with a short analysis time was developed using the integrated system of a C⁴D detector and microchip platform. The main advantages of the presented method are its low price and simple operating procedure without a derivatization process. Analytical parameters such as LOD, LOQ, linear range, RDS, and recovery values confirmed the suitability of the proposed method for the analysis of health supplements containing L-carnosine. The accuracy of the method was confirmed by the standard addition method and the comparative sample analyses at CE-UV and HPLC-DAD. Thus, this study supports the potential of using MCE-C⁴D methods for quality control in the health supplement industry.

Author Contributions: Conceptualization, M.J., N.S. and D.M.; methodology, I.P. and M.J.; software, B.M.; validation, I.P., C.P. and B.Š.; formal analysis, I.P. and C.P.; investigation, I.P. and M.J.; resources, M.J., N.S., B.M. and B.Š.; data curation, I.P., C.P., N.S., B.Š. and M.J.; writing—original draft preparation, I.P. and M.J.; writing—review and editing, C.P., N.S., M.J., B.Š. and D.M.; visualization, I.P., C.P., N.S., M.S., M.B. and M.J.; supervision, M.J.; project administration, M.J.; funding acquisition, M.J., N.S. and B.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This study is supported by the European Structural and Investment Funds grant for the Croatian National Scientific Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek (grant #KK.01.1.1.01.0010).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available by contacting the authors.

Conflicts of Interest: The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper. The authors declare no conflict of interest.

References

1. Sharma, R.; Sehrawat, R.; Ahlawat, S.; Sharma, V.; Parmar, A.; Thakur, M.S.; Mishra, A.K.; Tania, M.S. An attempt to valorize the only black meat chicken breed of India by delineating superior functional attributes of its meat. *Sci. Rep.* **2022**, *12*, 3555. [CrossRef] [PubMed]
2. Decker, C.; Krapf, R.; Kuballa, T.; Bunzel, M. Differentiation of meat species of raw and processed meat based on polar metabolites using 1H NMR spectroscopy combined with multivariate data analysis. *Front. Nutr.* **2022**, *30*, 985797. [CrossRef] [PubMed]
3. Aiello, G.; Rescigno, F.; Meloni, M.; Baron, G.; Aldini, G.; Carini, M.; D'Amato, A. Oxidative Stress Modulation by Carnosine in Scaffold Free Human Dermis Spheroids Model: A Proteomic Study. *Int. J. Mol. Sci.* **2022**, *23*, 1468. [CrossRef] [PubMed]
4. Hasanein, P.; Felegari, Z. Chelating effects of carnosine in ameliorating nickel-induced nephrotoxicity in rats. *Can. J. Physiol. Pharmacol.* **2017**, *95*, 1426–1432. [CrossRef] [PubMed]
5. Jain, S.; Kim, E.-S.; Kim, D.; Burrows, D.; De Felice, M.; Kim, M.; Baek, S.-H.; Ali, A.; Redgrave, J.; Doepfner, T.R.; et al. Comparative Cerebroprotective Potential of D- and L-Carnosine Following Ischemic Stroke in Mice. *Int. J. Mol. Sci.* **2020**, *21*, 3053. [CrossRef] [PubMed]
6. Hariharan, R.; Cameron, J.; Menon, K.; Mesinovic, J.; Jansons, P.; Scott, D.; Lu, Z.X.; de Courten, M.; Feehan, J.; de Courten, B.; et al. Carnosine Supplementation Improves Glucose Control in Adults with Pre-Diabetes and Type 2 Diabetes: A Randomised Controlled Trial. Available online: <https://www.medrxiv.org/content/10.1101/2023.03.18.23287432v1> (accessed on 22 September 2023).
7. Ansari, F.A.; Khan, A.A.; Mahmood, R. Ameliorative effect of carnosine and N-acetylcysteine against sodium nitrite induced nephrotoxicity in rats. *J. Cell. Biochem.* **2019**, *120*, 7032–7044. [CrossRef]
8. Kawahara, M.; Sadakane, Y.; Mizuno, K.; Kato-Negishi, M.; Tanaka, K. Carnosine as a Possible Drug for Zinc-Induced Neurotoxicity and Vascular Dementia. *Int. J. Mol. Sci.* **2020**, *21*, 2570. [CrossRef]
9. Hewlings, S.; Kalman, D. A review of zinc-L-carnosine and its positive effects on oral mucositis, taste disorders, and gastrointestinal disorders. *Nutrients* **2020**, *12*, 665. [CrossRef]
10. Kim, S.K.; Kwon, D.; Kwon, D.A.; Paik, I.K.; Auh, J.H. Optimizing Carnosine Containing Extract Preparation from Chicken Breast for Anti-glycating agents. *Korean J. Food Sci.* **2014**, *34*, 127–132. [CrossRef]
11. Cao, M.; Han, Q.; Zhang, J.; Zhang, R.; Wang, J.; Gu, W.; Kang, W.; Lian, K.; Ai, L. Meat differentiation between pasture-fed and concentrate-fed sheep/goats by liquid chromatography quadrupole time-of-flight mass spectrometry combined with metabolomic and lipidomic profiling. *J. Chromatogr. A* **2020**, *1610*, 460553. [CrossRef]

12. Gatti, R.; Andreatta, P.; Boschetti, S. An high-performance liquid chromatographic method for the simultaneous analysis of acetylcarnitine taurinate, carnosine, asparagine and potassium aspartate and for the analysis of phosphoserine in alimentary supplements. *J. Chromatogr. A* **2013**, *1298*, 95–102. [[CrossRef](#)] [[PubMed](#)]
13. do Nascimento Fontes, J.E.; Rodriguez-Caturla, M.Y.; Sant'Ana, A.S.; Lopes, T.I.B.; Marsaioli, A.J. Foodomics and storage monitoring of three meat cuts by ¹H NMR. *Concepts Magn. Reason. Part. A Bridg. Educ. Res.* **2019**, *47A*, E21474. [[CrossRef](#)]
14. Zikmundová, J.; Tůma, P.; Opekar, F. A dual spectrophotometric/contactless conductivity detector for CE determination of incompletely separated amino acids. *J. Sep. Sci.* **2008**, *31*, 353–355. [[CrossRef](#)]
15. Duong, H.A.; Vu, M.T.; Nguyen, T.D.; Nguyen, M.H.; Mai, T.D. Determination of 10-hydroxy-2-decenoic acid and free amino acids in royal jelly supplements with purpose-made capillary electrophoresis coupled with contactless conductivity detection. *J. Food Compos. Anal.* **2020**, *87*, 103422. [[CrossRef](#)]
16. Pormsila, W.; Krähenbühl, S.; Hauser, P.C. Determination of carnitine in food and food supplements by capillary electrophoresis with contactless conductivity detection. *Electrophoresis* **2010**, *31*, 2186–2191. [[CrossRef](#)]
17. Staňová, A.; Marák, J.; Rezeli, M.; Páger, C.; Kilár, F.; Kaniansky, D. Analysis of therapeutic peptides in human urine by combination of capillary zone electrophoresis–electrospray mass spectrometry with preparative capillary isotachopheresis sample pretreatment. *J. Chromatogr. A* **2011**, *1218*, 8701–8707. [[CrossRef](#)]
18. Nardiello, D.; Cataldi, T.R.I. Determination of carnosine in feed and meat by high-performance anion-exchange chromatography with integrated pulsed amperometric detection. *J. Chromatogr. A* **2004**, *1035*, 285–289. [[CrossRef](#)]
19. Huang, Y.; Duan, J.; Chen, H.; Chen, M.; Chen, G. Separation and determination of carnosine-related peptides using capillary electrophoresis with laser-induced fluorescence detection. *Electrophoresis* **2005**, *26*, 593–599. [[CrossRef](#)]
20. He, L.; Xu, Z.; Hirokawa, T.; Shen, L. Simultaneous determination of aliphatic, aromatic and heterocyclic biogenic amines without derivatization by capillary electrophoresis and application in beer analysis. *J. Chromatogr. A* **2017**, *1482*, 109–114. [[CrossRef](#)]
21. Zhao, S.; Huang, Y.; Shi, M.; Huang, J.; Liu, Y.M. Quantification of carnosine-related peptides by microchip electrophoresis with chemiluminescence detection. *Anal. Biochem.* **2009**, *393*, 105–110. [[CrossRef](#)]
22. Fresta, C.G.; Hogard, M.L.; Caruso, G.; Melo Costa, E.E.; Lazzarino, G.; Lunte, S.M. Monitoring carnosine uptake by RAW 264.7 macrophage cells using microchip electrophoresis with fluorescence detection. *Anal. Methods* **2017**, *9*, 402–408. [[CrossRef](#)] [[PubMed](#)]
23. Chen, Y.; Xia, L.; Xiao, X.; Li, G. Enhanced capillary zone electrophoresis in cyclic olefin copolymer microchannels using the combination of dynamic and static coatings for rapid analysis of carnosine and niacinamide in cosmetics. *J. Sep. Sci.* **2022**, *45*, 2045–2054. [[CrossRef](#)] [[PubMed](#)]
24. Tůma, P.; Opekar, F.; Dlouhý, P. Capillary and microchip electrophoresis with contactless conductivity detection for analysis of foodstuffs and beverages. *Food Chem.* **2022**, *375*, 131858. [[CrossRef](#)] [[PubMed](#)]
25. Tůma, P. Monitoring of biologically active substances in clinical samples by capillary and microchip electrophoresis with contactless conductivity detection: A review. *Anal. Chim. Acta* **2022**, *1225*, 340161. [[CrossRef](#)] [[PubMed](#)]
26. Jožanović, M.; Hajduković, M.; Galović, O.; Kralik, G.; Kralik, Z.; Sakač, N.; Medvidović-Kosanović, M.; Sak-Bosnar, M. Determination of anti-oxidative histidine dipeptides in poultry by microchip capillary electrophoresis with contactless conductivity detection. *Food Chem.* **2017**, *221*, 1658–1665. [[CrossRef](#)]
27. Jožanović, M.; Sakač, N.; Sak-Bosnar, M.; Carrilho, E. A simple and reliable new microchip electrophoresis method for fast measurements of imidazole dipeptides in meat from different animal species. *Anal. Bioanal. Chem.* **2018**, *410*, 4359–4369. [[CrossRef](#)]
28. Domínguez-Vega, E.; Martínez-Girón, A.B.; Ruiz, C.G.; Crego, A.L.; Marina, M.L. Fast derivatization of the non-protein amino acid ornithine with FITC using an ultrasound probe prior to enantiomeric determination in food supplements by EKC. *Electrophoresis* **2009**, *30*, 1037–1045. [[CrossRef](#)]
29. Wang, W.; Wang, Z.; Lin, X.; Wen, Z.; Wang, F. Simultaneous analysis of seven oligopeptides in microbial fuel cell by micro-fluidic chip with reflux injection mode. *Talanta* **2012**, *100*, 338–343. [[CrossRef](#)]
30. Cong, Y.; Katipamula, S.; Geng, T.; Prost, S.A.; Tang, K.; Kelly, R.T. Electrokinetic sample preconcentration and hydrodynamic sample injection for microchip electrophoresis using a pneumatic microvalve. *Electrophoresis* **2016**, *37*, 455–462. [[CrossRef](#)]
31. Liang, R.P.; Wang, X.N.; Liu, C.M.; Meng, X.Y.; Qiu, J.D. Facile preparation of protein stationary phase based on poly-dopamine/graphene oxide platform for chip-based open tubular capillary electrochromatography enantioseparation. *J. Chromatogr. A* **2014**, *1323*, 135–142. [[CrossRef](#)]
32. Liaoa, T.; Guoa, Z.; Lia, J.; Liua, M.; Chen, Y. One-step packing of anti-voltage photonic crystals into microfluidic channels for ultra-fast separation of amino acids and peptides. *Lab. Chip* **2013**, *13*, 706–713. [[CrossRef](#)] [[PubMed](#)]
33. Shi, M.; Huang, H.; Zhao, J.; Li, S.; Liu, R.; Zhao, S. Quantification of glutathione in single cells from rat liver by microchip electrophoresis with chemiluminescence detection. *Talanta* **2018**, *179*, 466–471. [[CrossRef](#)] [[PubMed](#)]
34. Herzog, C.; Poehler, E.; Peretzki, A.J.; Borisov, S.M.; Aigner, D.; Mayr, T.; Nagl, S. Continuous on-chip fluorescence labelling, freeflow isoelectric focusing and marker-free isoelectric point determination of proteins and peptides. *Lab. Chip* **2016**, *16*, 1565–1572. [[CrossRef](#)] [[PubMed](#)]
35. Shimura, K.; Takahashi, K.; Koyama, Y.; Sato, K.; Kitamori, T. Isoelectric Focusing in a Microfluidically Defined Electrophoresis Channel. *Anal. Chem.* **2008**, *80*, 3818–3823. [[CrossRef](#)] [[PubMed](#)]

36. Wang, Z.; Wang, W.; Wang, W.; Xu, L.; Chen, G.; Fu, F. Separation and determination of β -casomorphins by using glass microfluidic chip electrophoresis together with laser-induced fluorescence detection. *J. Sep. Sci.* **2011**, *34*, 196–201. [[CrossRef](#)] [[PubMed](#)]
37. Nordman, N.; Lauren, S.; Kotiaho, T.; Franssila, S.; Kostianen, R.; Sikanen, T. Interfacing microchip isoelectric focusing with on-chip electrospray ionization mass spectrometry. *J. Chromatogr. A* **2015**, *1398*, 121–126. [[CrossRef](#)]
38. Wu, J.-K.; Yang, C.-S.; Wu, Y.-S.; Wang, P.-C.; Tseng, F.-G. Continuous affinity-gradient nano-stationary phase served as a column for reversed-phase electrochromatography and matrix carrier in time-of-flight mass spectrometry for protein analysis. *Anal. Chim. Acta* **2015**, *889*, 166–171. [[CrossRef](#)]
39. Chong, K.C.; Thang, L.Y.; Quirino, J.P.; See, H.H. Monitoring of vancomycin in human plasma via portable microchip electrophoresis with contactless conductivity detector and multi-stacking strategy. *J. Chromatogr. A* **2017**, *1485*, 142–146. [[CrossRef](#)]
40. Reschke, B.R.; Schiffbauer, J.; Edwards, B.F.; Timperman, A.T. Simultaneous separation and detection of cations and anions on a microfluidic device with suppressed electroosmotic flow and a single injection point. *Analyst* **2010**, *135*, 1351–1359. [[CrossRef](#)]
41. Yamamoto, S.; Himeno, M.; Kobayashi, M.; Akamatsu, M.; Satoh, R.; Kinoshita, M.; Sugiura, R.; Suzuki, S. Microchip electrophoresis utilizing an in situ photopolymerized Phos-tag binding polyacrylamide gel for specific entrapment and analysis of phosphorylated compounds. *Analyst* **2017**, *142*, 3416–3423. [[CrossRef](#)]
42. Araya-Fariast, M.; Dziombat, S.; Carbonnier, B.; Guerrouache, M.; Ayed, I.; Aboud, N.; Taverna, M.; Tran, N.T. A lab-on-a-chip for monolith-based preconcentration and electrophoresis separation of phosphopeptides. *Analyst* **2017**, *142*, 485–494. [[CrossRef](#)] [[PubMed](#)]
43. Ollikainen, E.; Bonabi, A.; Nordman, N.; Jokinen, V.; Kotiaho, T.; Kostianen, R.; Sikanen, T. Rapid separation of phosphopeptides by microchip electrophoresis–electrospray ionization mass spectrometry. *J. Chromatogr. A* **2016**, *1440*, 249–254. [[CrossRef](#)] [[PubMed](#)]
44. Jiang, X.; Xia, Z.; Wei, W.; Gou, Q. Direct UV detection of underivatized amino acids using capillary electrophoresis with online sweeping enrichment. *J. Sep. Sci.* **2009**, *32*, 1927–1933. [[CrossRef](#)] [[PubMed](#)]
45. Steed, R. *Manual of Analysis of Amino Acids by HPLC*; Agilent Technologies, Inc.: Santa Clara, CA, USA, 2010.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.