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Quantification of underivatized amino acids in solid beverages using high-performance liquid chromatography and a potentiometric detector

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ABSTRACT

The simultaneous quantification of amino acids (AAs) in solid beverages without prior derivatization was explored by high-performance liquid chromatography (HPLC) coupled to a potentiometric detector. Included were threonine, leucine, methionine, phenylalanine, and histidine. The potentiometric detector was made consisting of a copper(II)-selective electrode based on a polyvinyl chloride (PVC) membrane, and the potential changes in the detector were determined according to the coordination interactions between cupric copper ions released from the inner filling solution of the electrode and AAs. Conditions were optimized for effective separation and sensitive detection. Fundamental characteristics such as linearity, limits of detection, limits of quantitation, accuracy, precision, and robustness were validated experimentally. The calibration curves showed a linear relationship between peak heights and the injection concentrations of the AAs. The detection limits down to the sub-micromolar range were achieved under isocratic conditions, outperforming ultraviolet detection. The copper(II)-selective electrode had a minimum lifetime of one month. Some real samples were examined to further demonstrate the feasibility of the proposed approach. The measurement results obtained by the present method were in good agreement with those obtained by the HPLC-mass spectrometry (MS), indicating that the combined HPLC-potentiometric method is a potential option for quantifying AAs.

1. Introduction

Amino acids (AAs) are well known as building blocks of proteins and energy sources in living organisms. AAs from foods and drinks are essential nutritional supplements for people. Cooking, processing, and storage may change the amount of free AAs in foods and drinks, reducing their nutritional values [1,2]. There is, therefore, a need for the accurate and reliable determination of free AAs in foodstuffs to monitor their quality and manage the preparation process.

A variety of analytical methods have been described for the quantitation of AAs over the years, including high-performance liquid chromatography (HPLC) [3,4], ion chromatography (IC) [5], capillary electrophoresis (CE) [6,7], thin layer chromatography (TLC) [8], gas chromatography (GC) [9] and near-infrared spectroscopy (NIR) [10]. HPLC [11,12] and IC [13–19] coupled to either an ultraviolet detector (UVD) or fluorescence detector (FLD) are frequently used due to their excellent performance. Due to the high polarity and lack of significant chromophores in most AAs, a chemical derivatization step is necessary. Unfortunately, all of the current derivative methods suffer from reagent toxicity, by-product interference, and time-consuming sample preparation [20]. The use of HPLC combined with mass spectrometry (MS) or tandem mass spectrometry (HPLC-MS/MS) allows for the direct and sensitive analysis of AAs but at a high-cost expense [21,22]. Above all, persuasive attempts thus are made to use novel un-derivation methods with less expensive instruments.

Potentiometric detection based on ion-selective electrodes (ISEs) is versatile, affordable, and easy to miniaturize in comparison to other instrumental technologies [20]. It has been used as a detector in IC, LC, and CE since the 1970s [23–25], and a wide variety of substances, including inorganic ions [23], carbohydrates [26], organic acids [27], aliphatic amines [28], phosphate esters [29], nucleotides [30], cocaine [31], and steroids [32], have been detected, even in complex matrices. Alexander et al. [33] made the first attempts to demonstrate the ability of a potentiometric detector in HPLC to detect AAs using a copper tubular electrode (CTE). Although the sensitivity and response time were comparable to UVD, CTE was far more selective and did not require any sample pretreatment. The potentiometric response of CTE was dependent on

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the concentration of cupric ions at the electrode surface, which was altered by coordination interactions with AAs to form complexes $[Cu(AA)_n]^{2+}$. Another case in point was the determination of di- and tripe-tides containing histidine using a metallic copper electrode, which yielded low detection limits at the sub-ppb level [34]. Although potentiometry with a CTE or a metallic copper electrode provided particularly sensitive detections of AAs, some serious drawbacks must be addressed, such as a limited lifetime (rapid poisoning by complexing agents), insufficient response time at low concentrations, and poor baseline stability [35].

The introduction of polyvinyl chloride (PVC) membranes based on various ion exchangers or ionophores led to the development of new potentiometric detectors in LC, such as those used to measure monovalent cations, inorganic acids, and organic acids [20]. The detection limits of the detector were at least 20 times better than those obtained by indirect UV determination without sample pre-treatment [20]. PVC and other polymeric membrane-based ISEs have not yet been employed to detect AAs in HPLC, to the best of our knowledge.

Qin's group demonstrated that the PVC membrane ISE can be used as a reagent-free technology to measure a wide variety of analytes in bulk solution [36–38]. For these ISEs, indicator ions were released from the inner filling solution of the working electrodes under zero-current conditions or driven by an external current. This not only avoided the need for the addition of indicator ions but also automatically refreshed the membrane surfaces. They further applied, for the first time, this new strategy as a potentiometric detector in a flow injection analysis (FIA) system and achieved good analytical performance [35,39,40]. The detector can be tailored to serve a specific purpose, so here we present a fundamental exploration of such a potentiometric detector in HPLC and evaluate its capability as an alternative for detecting AAs in complex mixtures. In the current work, a PVC membrane copper(II)-selective electrode was used, which improved sensitivity and greatly overcame the disadvantages of CTE and the metallic copper electrode mentioned previously.

2. Material and methods

2.1. Chemicals and reagents

The copper(II) ionophore IV (N, N, N′, N′-tetra cyclohexyl-2,2′-thioacetamide), PVC of high molecular weight, tetradodecylammonium tetrakis(4-chlorophenyl)borate (ETH 500), sodium tetrakis[3,5-*bis*(*trifluoromethyl*)*phenyl*]borate (NaBARF), *o*-nitrophenyl octyl ether (*o*-NPOE), dioctyl phthalate (DOP), dibutyl phthalate (DBP), and dioctyl sebacate (DOS) were purchased from Sigma Aldrich (St. Louis, USA). AAs analytical standards, threonine (Thr, ≥98%), leucine (Leu, ≥98%), methionine (Met, ≥98%), phenylalanine (Phe, ≥98%), and histamine (His, ≥98%) were obtained from J&K Scientific (Beijing, China). Tetrahydrofuran (THF) was obtained from Fluka (Bucks, Switzerland), and 3-(N-Morpholino) propane sulfuric acid (MOPS, ≥99.5%) was purchased from Aladdin (Shanghai, China). Other chemicals were of analytical reagent grade. Real samples used to confirm the protocol were purchased from a local market in China. Deionized water with a specific resistance of 18.2 MΩ, generated by a Pall Cascada laboratory water system, was used throughout the experiments.

2.2. Running buffer and standard solutions

MOPS buffer solution at 0.01 mol/L was prepared by dissolving 2.19 g solid in 0.8 L of deionized water and the pH was adjusted to pH 7.4 by 1 N sodium hydroxide. Then the volume was completed to 1 L with deionized water. The running buffer was prepared daily, filtered through a 0.22-μm filter, and degassed by ultrasonic for more than 30 min before use.

The stock solutions of each AA at a concentration of 20 mmol/L were prepared in deionized water and stored at 4°C. Then the mixed AAs stock solution was prepared at a concentration of 0.2–5 mmol/L in the running buffer. All the working solutions in the range of 2–50 μmol/L, except for His (0.2–2 μmol/L), were obtained by diluting the mixed solution with running buffer and filtering (0.22 μm) before injection.

2.3. Sample preparation

The appropriate amount of the solid drinks was weighed as a homogenized sub-sample into a 50 mL centrifuge tube, and 10 mL of 10% (*v*/*v*) trichloroacetic acid was then added. Following 10 min of ultrasonic treatment, the mixture was centrifuged at 15 000 rpm for 10 min. A 0.1 mL aliquot of the supernatant was transferred to a glass vial and dried under a gentle stream of nitrogen gas. The residue was finally reconstituted with either 2.0 mL of running buffer (for HPLC-potentiometric analysis) or 2.0 mL of mobile phase (for HPLC-MS analysis) and vortexed for 1 min.

2.4. Preparation of the copper(II)-selective electrode

Copper(II)-selective membranes consisted of 1.0% ionophore, 32.3% PVC, 64.7% *o*-NPOE as a plasticizer, 1.0% NaBARF as an ionic additive, and 1.0% ETH 500 as ionic additive expressed as a weight percentage. About 360 mg mass of this membrane cocktail was dissolved in 2.5 mL of THF. The mixture was then poured into a glass ring of 36 mm i.d. fixed on a glass plate and let the solvent evaporate sufficiently at room temperature. Finally, a uniform polymeric liquid membrane with a thickness of about 210 μm was obtained, which was visually measured by a CX31–32C02 microscope (Olympus, Japan). Disks 5 mm in diameter were cut from the parent membrane and glued on the bottom of electrode bodies (PVC tubes, 3 mm i.d., 5 mm o.d.) with THF. Upon drying up of THF, electrodes were filled and soaked in 0.1 mmol/L copper chloride ($CuCl_2$) for one day, then complimented with an internal silver-silver chloride (Ag/AgCl) reference electrode. When not in use, the electrodes were kept in 0.1 mmol/L $CuCl_2$.

2.5. Fabrication of the potentiometric detector

The potentiometric detector was a homemade wall-jet-type flow cell machined from bulk material of Perspex, as illustrated in Fig. 1. Two holes, 5 mm and 2 mm in diameter were drilled from the top side of the Perspex block, into which the copper(II)-selective electrode and an Ag/AgCl external reference electrodes were placed respectively. To guarantee there would be no leaks, the perforations were sealed using polytetrafluoroethylene (PTFE) tape. Another hole was made across the copper(II)-selective electrode to fix the polyether ether ketone (PEEK) tube from the column outlet with a PEEK fitting, allowing effluent to inject vertically toward the membrane of the copper(II)-selective electrode. To avoid peak broadening brought on by dead volume, a 15-cm-long PEEK tube (0.25-mm i.d., 1-mm o.d.) was used to connect the column outlet to the potentiometric detector. The distance between the tube outlet and the membrane, as well as the diameter of the flow channel in the Perspex body, were both around 0.8 mm, and the volume of the detection chamber was calculated to be 2.5 μL (calculated as $3.14159 \times (0.4\ mm)^2 \times 5\ mm$) as a result. A platinum (Pt) wire with a diameter of 0.2 mm was also inserted into the detecting chamber as a counter electrode.

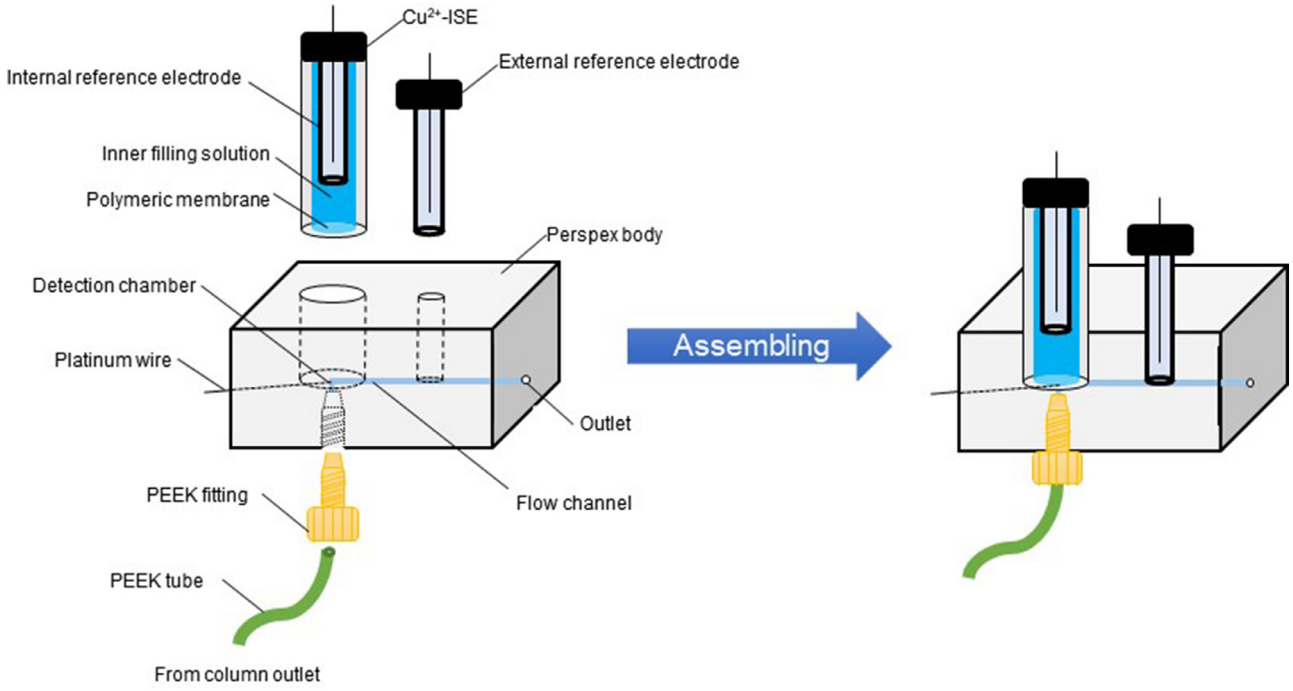

Fig. 1. Schematic representation of the potentiometric detector in high-performance liquid chromatography.

2.6. Apparatus

HPLC was performed with an e2695 system (Waters, USA) outfitted with a pump, a degasser, and an autosampler. The outlet of the reverse phase column was connected to the potentiometric detector using PEEK tubing.

All potentiometric measurements were carried out using a CHI 760C electrochemical workstation (Chenhua, China). Electromotive force (E) was measured in the following galvanic cell: Ag/AgCl/ 3 mol/L KCl // sample solution / ISE membrane / inner filling solution /AgCl/Ag. All E values were corrected for the liquid-junction potential according to the Henderson equation and the activity coefficients were calculated by the Debye–Hückel approximation.

An UltiMate 3000 UPLC system (Thermo Fisher Scientific, San Jose, CA, USA) coupled with a QExactive Orbitrap MS (Thermo Fisher Bremen, Germany) was used to obtain the MS result.

2.7. Chromatographic conditions

The separation was performed using an Atlantis T3 column (4.6 × 250 mm, 5 µm) (Waters, USA) with isocratic elution. The flow rate was set at 0.5 mL/min. A sample of 10 µL was auto-injected into the HPLC system.

After assembling the potentiometric detector, a fixed current of 0.1 µA was applied on the copper(II)-selective electrode and the electrode was equilibrated by running buffer in the HPLC system for at least 30 min until a stable baseline was obtained.

MS detection used electrospray ionization in positive mode (ESI+), according to the method ascribed in the reference [21].

2.8. Method validation

The linear range, the limit of detection (LOD), the limit of quantification (LOQ), precision, accuracy, and stability of this method were all validated. To generate the calibration curves for the linearity investigation, mixed standards solutions of AAs at five distinct concentration levels were determined in triplicate. The LODs and LOQs were calculated by multiplying the signal-to-noise ratio (S/N) of 3 and 10, respectively. The intra-day repeatability as relative standard deviation (RSD%) for retention time and peak height was used to assess precision. On the same day, six consecutive injections of the AAs standards mixture were performed. The selected beverage samples with known AAs concentration were spiked with mixed AAs standards at a concentration level of 5.0 µmol/L for the recovery study.

2.9. Data analysis

Statistical analyses were performed using SPSS.

3. Results and discussion

3.1. Optimization of the membrane composition

The potential response of PVC-based ISE depends upon the property of the plasticizer and the amount of ionophore and ion exchanger. The optimum membrane composition for the copper(II)-selective electrode was determined by varying the parameters individually while keeping the other parameters constant and observing the linear range, slope, and selectivity to Na^+ in this work. In a previous study, the PVC membrane ISE performed much better when the plasticizer/PVC mass ratio was 2:1 [41], so this ratio was maintained when other components were optimized. First, several plasticizers of *o*-NPOE, DOP, DBP, and DOS with different dielectric constants (ε_r = 24.0, 5.1, 6.4, and 3.9) were used to investigate their influences on the electrochemical characteristics of the electrodes (No.1–4), and the findings are shown in Table 1. *o*-NPOE, with the highest dielectric constant and polarity, gave the best results. Additionally, copper(II)-selective electrodes fabricated with different amounts of ionophore were tested. As indicated in Table 1, the electrode with a small amount of ionophore (No.5) did not provide a sufficient linear range. The electrode prepared with 1.0% ionophore (No.1) had the widest working range from 3.0×10^{-6} mo/L to 1.0×10^{-2} mol/L and an appropriate slope of 29.1 ± 0.2 mV per decade of cupric ion activity. Super-Nernstian was observed when the amount of the ionophore

Table 1
Characteristics of copper(II)-selective electrodes with different membrane compositions ($n = 3$).

Electrode No.	PVC (%,)	Plasticizer (%)				Ionophore (%)	NaBARF (%)	ETH 500 (%)	Linear range (mol/L)	Slope (mV/decade)	lg $K^{pot}_{Cu,Na}$
		o-NPOE	DOS	DOP	DBP						
1	32.3	64.7				1.0	1.0	1.0	3.0×10^{-6}–1.0×10^{-2}	29.1 ± 0.2	−9.1 ± 0.1
2	32.3		64.7			1.0	1.0	1.0	1.0×10^{-5}–1.0×10^{-2}	25.2 ± 0.2	−4.5 ± 0.3
3	32.3			64.7		1.0	1.0	1.0	1.0×10^{-4}–1.0×10^{-2}	19.1 ± 0.5	−7.5 ± 0.2
4	32.3				64.7	1.0	1.0	1.0	1.0×10^{-4}–1.0×10^{-2}	23.2 ± 0.3	−6.2 ± 0.3
5	32.7	65.3				0.5	0.5	1.0	1.0×10^{-4}–1.0×10^{-2}	27.2 ± 0.2	−8.7 ± 0.1
6	32.0	64.0				1.5	1.5	1.0	1.0×10^{-5}–1.0×10^{-2}	40.7 ± 0.7	−8.7 ± 0.5
7	32.0	64.0				1.0	2.0	1.0	3.0×10^{-5}–1.0×10^{-2}	20.4 ± 0.4	−7.5 ± 0.2
8	32.4	64.9				1.0	0.7	1.0	1.0×10^{-5}–1.0×10^{-2}	22.4 ± 0.2	−8.2 ± 0.2

Table 2
Potentiometric selectivity coefficients for the copper(II)-selective electrodes ($n = 3$).

Interference Ions	lg $K^{pot}_{Cu,J}$	Interference Ions	lg $K^{pot}_{Cu,J}$
K^+	−8.72±0.11	Na^+	−9.10±0.11
Mg^{2+}	−7.88±0.05	Ni^{2+}	−5.76±0.08
Ca^{2+}	−6.31±0.08	Zn^{2+}	−6.29±0.04
Mn^{2+}	−5.47±0.06	Pb^{2+}	−0.31±0.03
NH_4^+	−7.18±0.16	Ag^+	−6.55±0.09
Fe^{2+}	−7.06±0.10	Fe^{3+}	−7.22±0.13
Al^{3+}	−5.75±0.15	Ba^{2+}	−6.93±0.08
Hg^{2+}	−5.85±0.12	Cd^{2+}	−5.28±0.07
Cr^{3+}	−4.13±0.07	Sn^{2+}	−5.66±0.10

(No.6) was further increased. Finally, research into the impact of ion exchanger level on characteristics of copper(II)-selective electrodes (No.1, No.7, and No.8) revealed that 1.0% was the ideal level. In summary, electrode No.1 was selected for the following experiments.

3.2. Interferences

The influences of the discriminated ions in the running buffer were described by the selectivity coefficient using the method which is termed the “strong interference” method introduced by Bakker to eliminate the influence of the inherent sensitivity limit on the response toward discriminated ions [42]. Table 2 shows the resulting logarithmic Nikolskii coefficients (lg $K^{pot}_{Cu,J}$). Except for Pb^{2+}, the potentiometric detector had strong discrimination against K^+, Na^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, NH_4^+, Ag^+, Hg^{2+}, Fe^{2+}, Fe^{3+}, Cd^{2+}, Cr^{3+}, Sn^{2+}, Ba^{2+}, and Al^{3+}, allowing it to be used in a variety of running buffers with little background noise.

3.3. Optimization of the potentiometric detection conditions

The complex interaction between AAs and cupric ions released at the surface of the copper(II)-selective electrode served as the foundation for the potentiometric detector. Thus, the sensitivity of the method was significantly influenced by the amount of cupric ions released at the electrode surface. A low concentration of cupric ions may not be enough to complex with all the AAs, but if there are too many cupric ions, the potential change may be not discerned after complexation because relatively few cupric ions are consumed. Several variables, including the thickness of the membrane, the concentration of $CuCl_2$ in the filling solution, and the external current would affect the amount of cupric ions released to the electrode surface, which can be calculated based on the intersection of the potential value in the running buffer and the line with the potential values in 0.1, 0.3, and 1.0 mmol/L $CuCl_2$, respectively. As shown in Fig. 2, a thicker membrane, larger current, and more $CuCl_2$ in the filling solution could cause a great flux of cupric ions from the inner filling solution of the electrode, resulting in a higher concentration of cupric ions at the surface. Experiments revealed that the detection sensitivities of the five AAs tested were at their highest when the amount of cupric ions released at the membrane surface was in the range of 2–5 μmol/L. To account for these parameters, a 210 μm membrane thickness, 0.1 mmol/L $CuCl_2$ in the inner filling solution, and 0.1 μA current were used. A lower current took a long time for the detector to recover, resulting in peak tailing and poor separation, whereas a higher current and too-thin membrane amplified the noise (Fig. S1). The concentration of cupric ions produced at the electrode surface was calculated to be 3.6 μmol/L.

The running buffer and its pH are critical to the sensitivity of the potentiometric detector because they affect the ionization of AAs. The influence of pH on the potentiometric response of copper(II)-selective electrode to the AAs tested was investigated by varying the pH values of 0.01 mol/L sodium chloride with sodium hydroxide and hydrochloric acid, and the results are displayed in Fig. 3A. As the pH increased from 4 to 7, so did the potential responses. This is because only negatively charged AAs can combine with positively cupric ions. On the other hand, the copper(II)-selective electrode lost its responsiveness to AAs due to the hydrolysis of cupric ions at a pH above 8. Experiments revealed that the ideal pH range was 7–8. In addition, several buffer solutions were tried and 0.01 mol/L MOPS was selected because of its low hydrophilicity.

3.4. Potentiometric detector performance in static conditions

The potentiometric responses for the five AAs were evaluated using the running buffer to mimic the detection conditions in HPLC, and the potentiometric responses for each AA are displayed in Fig. 3B. The detection limits for these AAs were in the following order: Thr < Phe < Leu < Met < His. It was strongly dependent on their capacity to complex under the conditions of the current work [43].

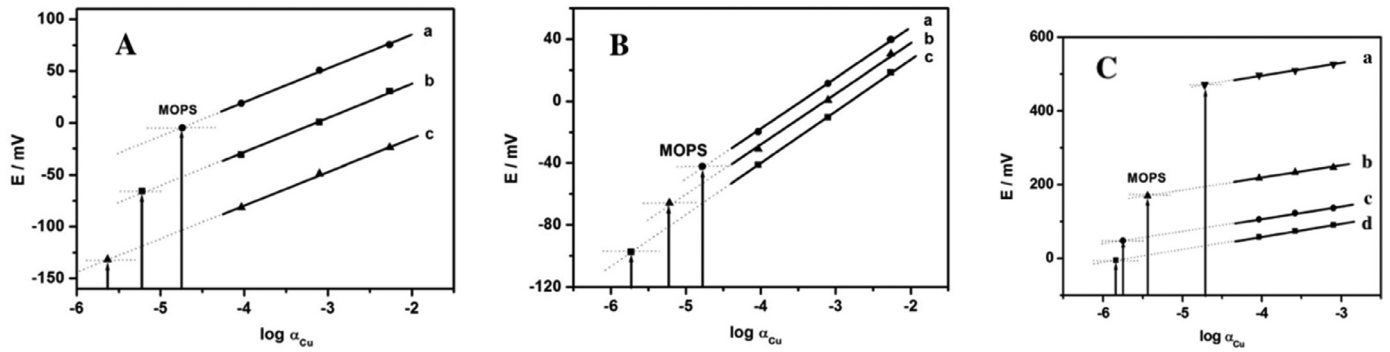

Fig. 2. (A) Fluxes of cupric ions from the inner filling solution of copper(II)-electrode with membrane thicknesses of 130 μm (a), 210 μm (b), and 300 μm (c). (B) Fluxes of cupric ions with $CuCl_2$ concentrations in the filling solution of 0.01 mmol/L (a), 0.1 mmol/L (b), and 1 mol/L (c). (C) Fluxes of cupric ions under an external current of 0 μA (a), 0.01 μA (b), 0.1 μA (c), and1.0 μA (d).

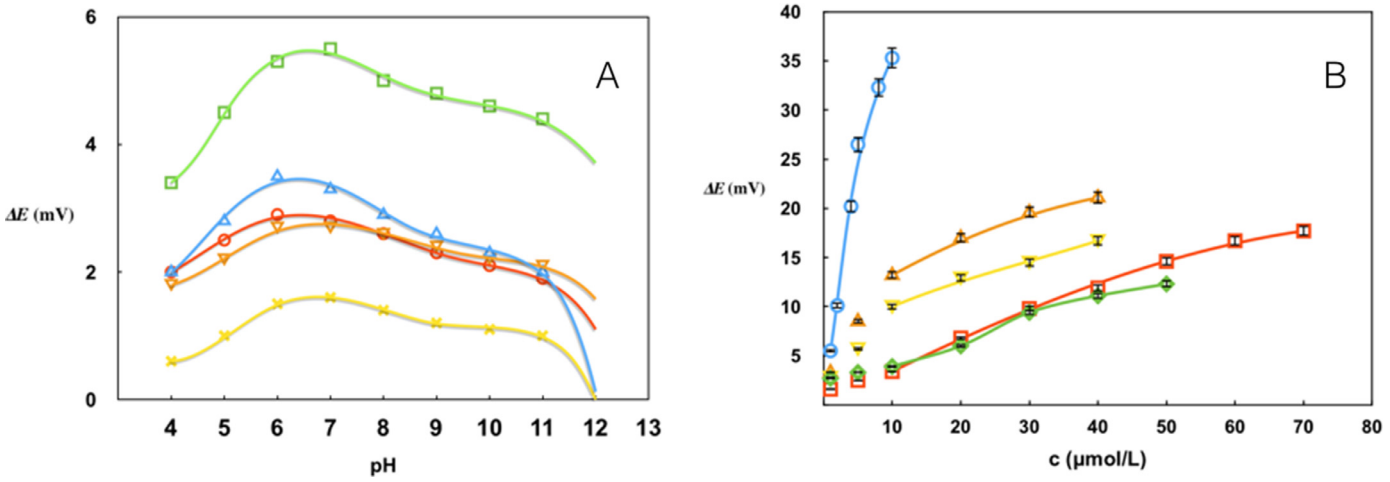

Fig. 3. (A) Potentiometric responses for Thr (✗), Leu (○), Met (△), Phe (▽), and His (□) in different pH solutions. (B) Potentiometric responses for Thr (◇), Leu (▽), Met (△), Phe (□), and His (○) in static conditions.

Table 3
Linear ranges, correlation coefficients (R^2), limits of detection (LODs), and limits of quantification (LOQs) of Thr, Leu, Met, Phe, and His-with the potentiometric detector in HPLC.

Amino Acids	Linear range (μmol/L)	R^2	LOQ (μmol/L)	LOD (μmol/L)
Thr	8–30	0.9958	0.79	0.25
Leu	5–50	0.9981	0.33	0.1
Met	2–20	0.9987	0.22	0.073
His	0.2–2	0.9992	0.021	0.005
Phe	5–50	0.9953	0.55	0.17

Table 4
Results of Thr, Leu, Met, Phe, and His-in real samples ($n = 3$).

Samples	Thr PD[a] (mg/g)	MSD[b] (mg/g)	Leu PD (mg/g)	MSD (mg/g)	His PD (mg/g)	MSD (mg/g)	Met PD (mg/g)	MSD (mg/g)	Phe PD (mg/g)	MSD (mg/g)
1	17.8 ± 0.6	16.9 ± 0.4	5.3 ± 0.2	5.5 ± 0.2	0.71±0.05	0.74±0.03	14.2 ± 0.11	14.5 ± 0.09	9.4 ± 0.2	10.7 ± 0.1
2	35.3 ± 1.2	33.4 ± 1.0	4.6 ± 0.3	5.0 ± 0.1	0.06±0.01	0.06±0.01	4.1 ± 0.1	4.3 ± 0.1	3.0 ± 0.1	2.8 ± 0.1
3	41.4 ± 0.6	39.5 ± 0.7	0.4 ± 0.1	0.5 ± 0.1	0.08±0.01	0.08±0.01	5.9 ± 0.1	5.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
4	55.1 ± 1.1	56.6 ± 1.2	2.4 ± 0.1	2.5 ± 0.2	0.62±0.02	0.58±0.01	3.6 ± 0.1	3.9 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
5	15.9 ± 0.4	17.1 ± 0.4	2.2 ± 0.1	1.9 ± 0.2	1.68±0.02	1.71±0.02	24.1 ± 0.3	24.6 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
6	13.1 ± 0.3	13.8 ± 0.2	4.0 ± 0.1	3.9 ± 0.1	0.28±0.04	3.15±0.03	29.5 ± 0.5	31.0 ± 0.3	7.6 ± 0.1	7.4 ± 0.1

[a] Detected by HPLC-potentiometric method.
[b] Detected by HPLC-MS method.

3.5. Optimization of chromatographic conditions

Iterations were carried out using a mixed standard of five AAs at a concentration of 5.0 µmol/L to optimize separation conditions. Two different types of reversed-phase columns were tested first of all. These columns were Waters C18 (4.6 × 250 mm, 5 µm) and Dikma C8 (4.6 × 250 mm, 5 µm). The separation of the test mixture obtained using a Dikma C8 column is shown in Fig. S2. It shows a shorter retention time for all AAs and very poor resolution between His-and Met. Satisfactory separation of this mixture was achieved using a non-polar C18 column.

Then, the effect of the pH of the running buffer (0.01 mol/L MOPS) on the separation of five AAs was examined over a pH range of 6.5 to 7.9, and the best separation was found at a buffer pH of 7.4, so it was chosen as the optimal pH for running buffer.

Finally, the influence of flow rate was studied in the range of 0.3 to 0.8 mL/min. The increased flow rate not only resulted in poor separation but also lower peak height due to the short complexation reaction time at the electrode surface between the AAs and cupric ions. A lower flow rate, on the other hand, could prolong the analysis period. The flow rate of 0.5 mL/min was chosen as a compromise between sensitivity and analytical efficiency.

3.6. Calibration curves, detection limits, and quantification limits

Under optimal chromatographic conditions, the potential peaks were identified as Thr, Leu, His, Met-and Phe, respectively, compared with AAs retention times with the single standard solutions (Fig. 4A). There was no doubt that these potential peaks were brought on by the consumption of cupric ions at the membrane surface, and not by the potentiometric responses AAs. Because the isoelectric points (IPs) of Thr, Leu, Met, Phe-and His-are respectively 6.18, 5.98, 5.74, 5.49 and 7.59, respectively, and they existed mainly as non-cations in the running buffer. Table 3 summarizes the performance of the proposed potentiometric detector for detecting Thr, Leu, His, Met-and Phe. Peak heights relative to AA concentrations with correlation coefficients (R^2) ≥ 0.9953 for the concentration range under consideration were plotted to create calibration curves. The limits of detection for the potentiometric detector appeared to be slightly lower than those obtained by derived UV-spectrophotometry [44,45].

3.7. Precision, accuracy, and reproducibility

The precision of the peak heights of Thr, Leu, His, Met-and Phe-at 5 µmol/L provided RSD% values (n = 6) of 5.6%, 3.9%, 3.0, 4.5% and 5.4%, respectively. For the retention time, RSD% values were less than 0.5%. The recovery percentage varied from 86.8 ± 2.4% to 109.2 ± 3.5%, with RSD% values less than 7.8%. Furthermore, there were no noticeable changes in the potentiometric responses for five AAs during a one-month period. One of the reasons for the high reproducibility may be the continuous refreshing of the membrane surface of the copper(II)-selective electrode by an external current, thus a lack of sample contamination.

3.8. Application to real samples

The proposed HPLC-potentiometric detector was applied for the determination of the free Thr, Phe, Leu, Met, and His-in real samples with complex composition. Several solid beverage samples, such as milk, dietary fiber, enzymes, and fruit extracts, were tested by potentiometric and MS detection. Potentiometric measurements were performed on each sample and AA concentrations were quantified according to the calibration curves. The results are shown in Table 4 and one of the representative chromatograms of the solid beverage is illustrated in Fig. 4B. As can be seen, the data obtained using the proposed potentiometric detector and the data obtained with the MS detector match very well, suggesting that the HPLC-potentiometric detector presented in this work has potential significant capacity for AAs analysis.

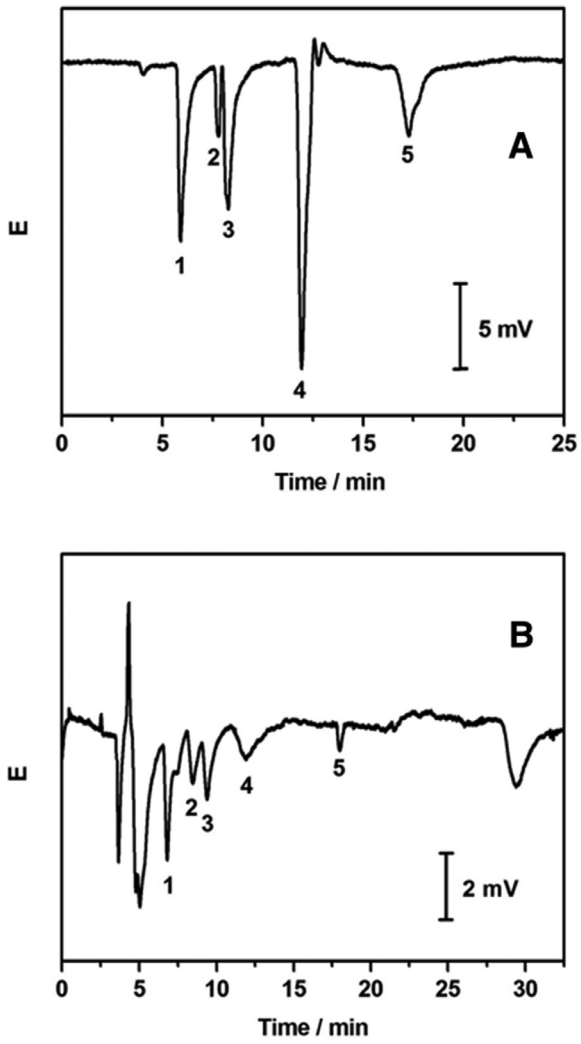

Fig. 4. Chromatograms of a mixed standard solution (A) and a sample solution (B) obtained by HPLC/potentiometric detector.
Peak identification: (1) Thr, (2) Leu, (3) His, (4) Met, (5) Phe.

4. Conclusions

This work demonstrates the feasibility of using a potentiometric detector based on a PVC membrane copper(II)-ISE to measure AAs in HPLC without derivatization. Under optimal chromatographic conditions, the proposed method attained excellent LODs in the range of 5–250 nmol/L. The detector was successfully validated and applied to the simultaneous quantitative analysis of Thr, Leu, Met, Phe-and His-in solid beverages, and the results were in good agreement with those obtained by HPLC-MS. The present study serves as a guide to establish an efficient and cost-effective approach based on potentiometric detectors for routine analysis.

Credit author statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2023.463986.

References

[1] H. Ito, H. Kikuzaki, H. Ueno, Effects of cooking methods on free amino acid contents in vegetables, J. Nutr. Sci. Vitaminol. 65 (2019) 264–271, doi:10.3177/jnsv.65.264.
[2] R.F. Hurrell, P.A. Finot, Food processing and storage as a determinant of protein and amino acid availability, Experientia. Suppl. 44 (1983) 135–156, doi:10.1007/978-3-0348-6540-1_9.
[3] G. Jaudzems, C. Fuerer, Determination of total amino acids in infant formulas, adult nutritionals, dairy, and cereal matrixes by UHPLC-UV: interlaboratory validation study, Final. Action. 105 (2018) 1625–1639, doi:10.1093/jaoacint/qsac083.
[4] H. Vakili, Z. Talebpour, F. Haghighi, Development, validation, and uncertainty measurement of HPLC-DAD method for determination of some free amino acids in infant formula and medical food products for inborn errors of metabolism, Food Chem. 390 (2022) 133204, doi:10.1016/j.foodchem.2022.133204.
[5] H. Yu, Y.S. Ding, S.F. Mou, P. Jandik, J. Cheng, Simultaneous determination of amino acids and carbohydrates by anion-exchange chromatography with integrated pulsed amperometric detection, J. Chromatogr. A 966 (2002) 89–97, doi:10.1016/s0021-9673(02)00739-2.
[6] M.M.A. Omar, A.A. Elbashir, O.J. Schmitz, Capillary electrophoresis method with UV-detection for analysis of free amino acids concentrations in food, Food Chem. 214 (2017) 300–307, doi:10.1016/j.foodchem.2016.07.060.
[7] T.C. Yan, H.H. Zhang, J. Cao, L.H. Ye, Analysis of iodinated amino acids by microemulsion electrokinetic chromatography using aliphatic amine as a cosurfactant, J. Chromatogr. A 1685 (2022) 463644, doi:10.1016/j.chroma.2022.463644.
[8] S.A. Bhawani, M.N. Mohamad Ibrahim, O. Sulaiman, R. Hashim, A. Mohammad, S. Hena, Thin-layer chromatography of amino acids: a review, J. Liq. Chromatogr. Relat. Technol. 35 (2012) 1497, doi:10.1080/10826076.2011.619039.
[9] K. Ichihara, C. Kohsaka, Y. Yamamoto, Determination of proteinaceous free amino acids by gas chromatography, Anal. Biochem. 633 (2021) 114423, doi:10.1016/j.ab.2021.114423.
[10] L. Wang, Q. Wang, H. Liu, L. Liu, Y. Du, Determining the contents of protein and amino acids in peanuts using near-infrared reflectance spectroscopy, J. Sci. Food Agric. 93 (2013) 118–124, doi:10.1002/jsfa.5738.
[11] F. Wang, K. Zhang, N. Wang, N. Muhammad, S. Wu, Y. Zhu, Ultrahigh-performance liquid chromatography-ion chromatography system for the simultaneous determination of vanillin, ethyl vanillin, and inorganic anions in food samples, Food anal. methods 11 (2018) 243–250, doi:10.1007/s12161-017-0994-z.
[12] N. Muhammad, I. Hussian, A. Ali, T. Hussain, A. Intisar, I.U. Haq, Q. Subhani, M. Hedar, J.L. Zhong, M. Asif, D. Guo, H. Cui, Y. Zhu, A comprehensive review of liquid chromatography hyphenated to post-column photoinduced fluorescence detection system for determination of analytes, Arab. J. Chem. 15 (2022) 104091, doi:10.1016/j.arabjc.2022.104091.
[13] N. Muhammad, Q. Subhani, F. Wang, C. Lou, J. Liu, Y. Zhu, Simultaneous determination of two plant growth regulators in ten food samples using ion chromatography combined with QuEChERS extraction method (IC-QuEChERS) and coupled with fluorescence detector, Food Chem 241 (2018) 308–316, doi:10.1016/j.foodchem.2017.08.112.
[14] N. Muhammad, Q. Subhani, F. Wang, D. Guo, Q. Zhao, S. Wu, Y. Zhu, Application of a simple column-switching ion chromatography technique for removal of matrix interferences and sensitive fluorescence determination of acidic compounds (pharmaceutical drugs) in complex samples, J. Chromatogr. A 1515 (2017) 69–80, doi:10.1016/j.chroma.2017.07.007.
[15] N. Muhammad, F. Wang, Q. Subhani, Q. Zhao, M.A. Qadir, H. Cui, Y. Zhu, Comprehensive two-dimensional ion chromatography (2D-IC) coupled to a post-column photochemical fluorescence detection system for determination of neonicotinoids (imidacloprid and clothianidin) in food samples, RSC Adv 8 (2018) 9277–9286, doi:10.1039/c7ra12555k.
[16] N. Muhammad, Y. Zhang, W. Li, Y.G. Zhao, A. Ali, Q. Subhani, T. Mahmud, J. Liu, H. Cui, Y. Zhu, Determination of nitenpyram and 6-chloronicotinic acid in environmental samples by ion chromatography coupled with online photochemically induced fluorescence detector, J. Sep. Sci. 41 (2018) 4096–4104, doi:10.1002/jssc.201800612.
[17] N. Muhammad, Y. Zhang, M. Asif, M.F.S. Khan, A. Intisar, M. Ye, Q. Subhani, H. Cui, Y. Zhu, Feasibility of pyrohydrolysis and extended-steam distillation method for the extraction of two halides from zinc and lead concentrate samples followed by ion chromatography analysis, Microchem. J. 159 (2020) 105593, doi:10.1016/j.microc.2020.105593.
[18] N. Muhammad, M. Zia-ul-Haq, A. Ali, S. Naeem, A. Intisar, D. Han, H. Cui, Y. Zhu, J. Zhong, A. Rahman, B. Wei, Ion chromatography coupled with fluorescence/UV detector: a comprehensive review of its applications in pesticides and pharmaceutical drug analysis, Arab. J. Chem. 14 (2020) 102972, doi:10.1016/j.arabjc.2020.102972.
[19] N. Muhammad, A. Ali, I. Hussain, Q. Subhani, D. Guo, H. Cui, Y. Zhu, Determination of fluorine and chlorine in standard steel residues and zinc sulfide concentrates by ion chromatography-matrix interference study, Chinese J. Anal. Chem. 51 (2) (2023) 100147, doi:10.1016/j.cjac.2022.100147.
[20] R.L. Gil, C.G. Amorim, M.C.B.S.M. Montenegro, A.N. Araújo, Potentiometric detection in liquid chromatographic systems: an overview, J. Chromatogr. A 1602 (2019) 326–340, doi:10.1016/j.chroma.2019.06.006.
[21] A. Raimbault, A. Noireau, C. West, Analysis of free amino acids with unified chromatography-mass spectrometry-application to food supplements, J. Chromatogr. A 1616 (2020) 460772, doi:10.1016/j.chroma.2019.460772.
[22] B. Yuan, W. Lyu, F.F. Dinssa, J.E. Simon, Q. Wu, Free amino acids in African indigenous vegetables: analysis with improved hydrophilic interaction ultra-high performance liquid chromatography tandem mass spectrometry and interactive machine learning, J. Chromatogr. A 1637 (2021) 461733, doi:10.1016/j.chroma.2020.461733.
[23] F.A. Schultz, D.E. Mathis, Ion-selective electrode detector for ion-exchange liquid chromatography, Anal. Chem. 46 (1974) 2253–2255, doi:10.1021/ac60350a03.
[24] M.C. Franks, D.L. Pullen, A technique for the determination of trace anions by the combination of a potentiometric sensor and liquid chromatography, with particular reference to the determination of halides, Analyst 99 (1974) 503–514, doi:10.1039/AN9749900503.
[25] B.L. De Backer, L.J. Nagels, Potentiometric detection for capillary electrophoresis: determination of organic acids, Anal. Chem. 68 (1996) 4441–4445, doi:10.1021/ac9604494.
[26] Z.L. Chen, P.W. Alexander, P.R. Haddad, Liquid chromatography of carboxylic acids using potentiometric detection with a tungsten oxide electrode, Anal. Chim. Acta 338 (1997) 41–49, doi:10.1016/S0003-2670(96)00434-5.
[27] D. Zielinska, A. Gil, M. Pietraszkiewicz, O. Pietraszkiewicz, D. Van de Vijver, L.J. Nagels, Podand and macrocyclic amine receptors with urea functionalities for potentiometric detection of organic acids in HPLC, Anal. Chim. Acta 523 (2004) 177–184, doi:10.1016/j.aca.2004.08.008.
[28] Z.L. Chen, P.W. Alexander, Potentiometric detection of aliphatic amines by flow injection analysis and ion-interaction chromatography with a metallic copper electrode, J. Chromatogr. A 758 (1997) 227–233, doi:10.1016/S0021-9673(96)00741-8.
[29] S. Picioreanu, I. Poels, J. Frank, J.C. van Dam, G.W.K. van Dedem, L.J. Nagels, Potentiometric detection of carboxylic acids, phosphate esters, and nucleotides in liquid chromatography using anion-selective coated-wire electrodes, Anal. Chem. 72 (2000) 2029–2034, doi:10.1021/ac991294d.
[30] Y. Bao, J. Everaert, M. Pietraszkiewicz, O. Pietraszkiewicz, H. Bohets, H.J. Geise, B.X. Peng, L.J. Nagels, Behaviour of nucleotides and oligonucleotides in potentiometric HPLC detection, Anal. Chim. Acta 550 (2005) 130–136, doi:10.1016/j.aca.2005.06.049.
[31] D. Daems, A.L. van Nuijs, A. Covaci, E. Hamidi-Asl, G. Van Camp, L.J. Nagels, Potentiometric detection in UPLC as an easy alternative to determine cocaine in biological samples, Biomed. Chromatogr. 29 (2015) 1124–1129, doi:10.1002/bmc.3400.
[32] B. Vissers, J. Everaert, J. Sekula, A. Malak, H. Bohets, G. Bazylak, L.J. Nagels, Unique potentiometric detection systems for HPLC determination of some steroids in human urine, J. Sep. Sci. 32 (2009) 167–179, doi:10.1002/jssc.200800489.
[33] P.W. Alexander, P.R. Haddad, G.K.C. Low, C. Maitra, Application of a copper tubular electrode as a potentiometric detector in the determination of amino-acids by high-performance liquid-chromatography, J. Chromatogr. 209 (1981) 29–39, doi:10.1016/S0021-9673(00)80420-3.
[34] M. Trojanowicz, G.B. Martin, M.E. Meyerhoff, Reversed-phase HPLC of peptides with tetraphenylporphyrin-based stationary phase and potentiometric detection with a copper electrode, Chem. Anal. (Warsaw) 41 (1996) 521–530.
[35] W. Song, J. Ding, R. Liang, W. Qin, Potentiometric flow injection system for determination of reductants using a polymeric membrane permanganate ion-selective electrode based on current-controlled reagent delivery, Anal. Chim. Acta 704 (2011) 68–72, doi:10.1016/j.aca.2011.08.004.
[36] H.M. Guo, T.J. Yin, Q.M. Su, W. Qin, Potentiometric measurement of ascorbate by using a solvent polymeric membrane electrode, Talanta 75 (2008) 851–855, doi:10.1016/j.talanta.2007.12.023.

[37] J.W. Ding, W. Qin, Current-driven ion fluxes of polymeric membrane ion-selective electrode for potentiometric biosensing, J. Am. Chem. Soc. 131 (2009) 14640–14641, doi:10.1021/ja906723h.
[38] J.W. Ding, W. Qin, Polymeric membrane ion-selective electrode for butyrylcholinesterase based on controlled release of substrate, Electroanal. 21 (2009) 2030–2035, doi:10.1002/ELAN.200904635.
[39] J.H. Lei, J.W. Ding, Y. Chen, W. Qin, Potentiometric flow injection sensing system for determination of heparin based on current-controlled release of protamine, Anal. Chim. Acta 858 (2015) 60–65, doi:10.1016/j.aca.2014.12.018.
[40] J.H. Lei, J.W. Ding, W. Qin, A chronopotentiometric flow injection system for aptasensing of E. coli O157, Anal. Methods-UK 7 (2015) 825–829, doi:10.1016/j.aca.2014.12.018.
[41] E. Bakker, P. Bühlmann, E. Pretsch, Carrier-based ion-selective electrodes and bulk optodes. 1. general characteristics, Chem. Rev. 97 (1997) 3083–3132, doi:10.1021/cr940394a.
[42] E. Bakker, Determination of improved selectivity coefficients of polymer membrane ion-selective electrodes by conditioning with a discriminated ion, J. Electrochem. Soc. 143 (1996) L83–L85, doi:10.1351/pac197951091913.
[43] R.R. Khoury, G.J. Sutton, D. Ebrahimi, D.B. Hibbert, Formation constants of copper(II) complexes with tripeptides containing Glu, Gly, and His: potentiometric measurements and modeling by generalized multiplicative analysis of variance, Inorg. Chem. 53 (2014) 1278–1287, doi:10.1021/ic4009575.
[44] S.M. Eid, M.A. Farag, S. Bawazeer, Underivatized amino acid chromatographic separation: optimized conditions for HPLC-UV simultaneous quantification of isoleucine, leucine, lysine, threonine, histidine, valine, methionine, phenylalanine, tryptophan, and tyrosine in dietary supplements, ACS Omega 7 (2022) 31106–31114, doi:10.1021/acsomega.2c03228.
[45] J. Qiu, J. Wang, Z. Xu, H. Liu, J. Ren, Quantitation of underivatized branched-chain amino acids in sport nutritional supplements by capillary electrophoresis with direct or indirect UV absorbance detection, PLoS ONE 12 (2017) e0179892, doi:10.1371/journal.pone.0179892.