International Journal of Mass Spectrometry 392 (2015) 1–6

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

A novel method for detecting amino acids derivatized with phenyl isothiocyanate by high-performance liquid chromatography–electrospray ionization mass spectrometry

Guihua Zheng[a], Wenwen Jin[b], Pei Fan[b], Xiaonan Feng[a], Yun Bai[b], Tao Tao[a,**], Longjiang Yu[b,*]

[a] School of Environmental Science and Engineering, Huazhong University of Science and Technology, Wuhan 430074, China
[b] Institute of Resource Biology and Biotechnology, Department of Biotechnology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form 5 August 2015
Accepted 7 August 2015
Available online 17 August 2015

Keywords:
Amino acids
Derivatization
Electrospray ionization mass spectrometry
High-performance liquid chromatography
Phenyl isothiocyanate

ABSTRACT

High-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI-MS) is an important technique for amino acid analysis. As a common derivatization reagent, phenyl isothiocyanate (PITC) is widely used in this field. However, researchers have long faced the critical problem of coupling efficient separation with simultaneous, sensitive MS detection. In this work, a novel HPLC–ESI-MS method based on PITC derivatization was established for detecting amino acids. Complete separation of 15 amino acid derivatives was achieved with an Agilent Eclipse plus C18 column maintained at 30 °C in 29 min. The mobile phase was 0.05% formic acid aqueous solution (A) and 70:30 (*v*/*v*) acetonitrile–water (B). The spectral data of PITC-amino acids are indicative of losses of 17 Da (NH_3), 18 Da (H_2O) and/or 46 Da ($CO + H_2O$) from the parent protonated molecules. Assessment of this method revealed that response curves were linear within the range of 630–20,156 μmol L^{-1}. LOD and LOQ ranged within 111–816 and 311–2721 pmol μL^{-1}, respectively. The method was validated with precision values of 0.924–6.578% (intraday, $n = 6$) and 3.327–8.962% (interday, $n = 6$ in 3 d). Therefore, the proposed method is a simple, robust alternative to current methods of detecting amino acids.

1. Introduction

Amino acid detection is important in various fields, such as the food industry, medical science, and ecological environment. Foods rich in essential amino acids are considered highly nutritious [1]. Quantifying plasma amino acid together with organic acids is primarily performed in clinical laboratories for the diagnosis and monitoring of metabolic inborn errors in pathologies [2]. For the ecological environment, determining amino acids in the soil and tracing their transformation processes are powerful tools for characterizing N turnover in soil [3].

Among the various methods that have been applied to quantify amino acids, high-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI-MS) shows promise as an analytical technique with high selectivity and low detection limits. This technique presents notable potential for investigating compound–special isotope enrichment [4,5]. In theory, separating target compounds is unnecessary in MS detection, but certain limitations mainly occur in the following three cases: (1) the isomass compounds cannot be separated by mass; (2) to a lesser extent, the presence of isotopes can interfere with other molecules, especially with in ion trap mass difference of only 1 or 2 [6]; and (3) "chemical suppression" phenomenon occurs in the ion trap when several molecules are co-eluted and ionized together [3].

Abbreviations: PITC, phenyl isothiocyanate; His, histidine; Arg, arginine; Ser, serine; Gly, glycine; Pro, proline; Glu, glutamic acid; Asp, asparagine; Thr, threonine; Ala, alanine; Met, methionine; Tyr, tyrosine; Lys, lysine; Val, valine; Leu, leucine; Phe, phenylalanine; TEA, triethylamine; DNS, dansyl chloride; FMOC-Cl, 9-fluorenylmethyl chloroformate; DEEMM, diethyl ethoxymethylenemalonate; TAHS, p-N,N,N-trimethylammonioanilyl N′-hydroxysuccinimidyl carbamateiodide; FOSF, 2,5-dioxopyrrolidin-1-yl N-tri(pyrrolidino) phosphoranylideneamino carbamate; HPLC–ESI-MS, high-performance liquid chromatography–electrospray ionization mass spectrometry.

* Corresponding author at: College of Life Science and Technology, Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan 430074, China. Tel.: +86 27 8779 2265; fax: +86 27 8779 2265.
** Corresponding author at: School of Environmental Science and Engineering, Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan 430074, China. Fax: +86 27 8779 2101.
E-mail addresses: taocaiye@vip.sina.com (T. Tao), yulongjiang@hust.edu.cn (L. Yu).

http://dx.doi.org/10.1016/j.ijms.2015.08.004
1387-3806/

Therefore, good chromatography separation remains essential for sensitive MS analysis. Highly sensitive MS analysis is indispensable for the determination of underivatized amino acids.

However, most amino acids do not possess ultraviolet or fluorescence absorption capacities. Thus, derivatization is being explored for a more sensitive and selective analysis of amino acids. As a typical post-column derivatization reagent [7], ninhydrin is inappropriate for highly demanding applications because its reaction with amino acids has low sensitivity and is thus rarely reproducible [8]. The relatively high salt concentration of modifier also severely affects the sensitivity of mass spectrometry [3]. According to the previous study, methods which were applied for the analysis of underivatized amino acids contained electrochemical detection, indirect UV–fluorimetric detection and direct low-wavelength UV detection. Compared with the derivatization ones, these methods have not gained wide acceptance due to low sensitivity, baseline drift, potential instability, incompatibility with gradient elution mode and inability of analyzing complex matrices [9]. Moreover, the detection of underivatized amino acids is a problem as most of them are very weak chromophores in the UV–vis region and possess no native fluorescence, etc. [6].

The aforementioned problems can generally be solved by pre-column derivatization. Widely used reagents for pre-column derivatization include ortho-phthalaldehyde [10], dansyl chloride (DNS), 9-fluorenylmethyl chloroformate (FMOC-Cl) [11], diethyl ethoxymethylenemalonate (DEEMM), phenyl isothiocyanate (PITC) [12], p-N,N,N-trimethylammonioanilyl N′-hydroxysuccinimidyl carbamateiodide (TAHS) [13], and an in-house synthesized reagent 2,5-dioxopyrrolidin-1-yl N-tri(pyrrolidino) phosphoranylideneamino carbamate (FOSF) [14]. Among these reagents, PITC is a common pre-column derivatization reagent that has been extensively used for more than 30 years for detecting amino acids in orange juices [15], wine [16–18], vinegar [19], phytosiderophores [20], green beans [21], edible seaweeds [22], human brain tumors [23], and sea urchins [24]. PITC has higher sensitivity than derivatization reagents for detecting amino acids, especially proline (Pro) and hydroxyproline, and this sensitivity is an important feature of the PITC derivatization technique [23]. The reaction of PITC with amino acids only takes 10–20 min, after which, excess reagents can be readily removed without interfering with detection sensitivity. PITC-amino acids are produced under mild conditions, and the dried matter could be stable for 4 weeks at −20 °C [1]. For these reasons, PITC was chosen for the derivatization of amino acids in the present study.

HPLC–ESI-MS technique has been successfully applied in the analysis of amino acids derived with DNS, FMOC-Cl, and DEEMM. Moreover, novel amino acid derivatization reagents (TAHS and APDS) have been specially designed for HPLC–ESI-MS [14]. However, no study has been reported on the identification and quantification of PITC-amino acid using HPLC–ESI-MS. PITC-amino acids are still identified by comparing their retention times with those of amino acid standards, and previous studies extensively used sodium acetate buffer for the chromatographic separation of PITC derivatives [1,22]. Sodium acetate is salted out when mixed with an organic solvent in an improper proportion because it is non-volatile. The solid salt will block the LC–MS system components (column, pipeline, and sealing washer), thereby seriously affecting the system's performance. Consequently, a relatively high concentration of salt buffer is not suitable for sensitive MS analysis.

Optimizing the mobile phase is essential for simultaneously achieving both good separation and high sensitivity of amino acid identification and quantification if PITC-derivatization technique is applied. Therefore, our objective is to develop a new HPLC–ESI-MS method to provide a new alternative for detecting amino acids.

2. Materials and methods

2.1. Chemicals and materials

The amino acid standards and mixed solution of these standards were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). These standards include arginine (Arg), histidine (His), glycine (Gly), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), threonine (Thr), Pro, alanine (Ala), methionine (Met), valine (Val), phenylalanine (Phe), leucine (Leu), lysine (Lys), and tyrosine (Tyr). The concentration of the mixed solution was 2.5 mmol L^{-1} for each standard previously mentioned. Triethylamine (TEA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The derivatization reagent PITC (98%) was procured from Aladdin Industrial Corporation (Shanghai, China). Acetonitrile and methanol were purchased from Tedia Company (USA). Formic acid (98–100%) was purchased from Tianjin Guangfu Fine Chemical Research Institute (China). All reagents were of analytical grade except acetonitrile and methanol, which are chromatographically pure.

2.2. Preparation of standard amino acid solutions

The amino acid standard was dissolved and diluted to the required concentration with 0.1 mol L^{-1} HCl aqueous solution prepared with ultrapure water using Millipore Simplicity 165 (Millipore, USA). The solution was prepared well and kept at 4 °C.

2.3. Derivatization procedure

The derivatization procedure was a modification of the method of González-Castro [25]. PITC solution was prepared about half an hour earlier, and this preparation approach is the general practice for the preparation of FMOC-Cl, DNS, FOSF, and TAHS [14], although studies on PITC have not reported this fact [15–18]. About 10 μL of amino acid solution was pipetted into a 1.5 mL centrifuge tube and vacuum dried at 65 °C for 2 h. The dried sample was mixed with 20 μL of methanol–water–TEA (2:2:1, *v*/*v*/*v*) and re-dried in a vacuum at 65 °C for 30 min. The sample was then mixed with 20 μL of methanol–water–TEA–PITC (7:1:1:1, *v*/*v*/*v*/*v*) and vigorously vortexed for 5–10 s. The PITC derivatization of the amino acids was performed at 25 °C for 20 min, after which, the excess reagent was removed by vacuum drying at 65 °C for 30 min. The dried samples were dissolved with 12 μL of 60% acetonitrile aqueous solution and 113 μL of 0.05% formic acid aqueous solution. The mixture was vortexed, centrifuged at 11,000 × *g* for 5 min, and filtered through a 0.45 μm Nylon 66 membrane filter. 10 μL of sample was transferred into a sample vial for LC–MS analysis. All amino acid samples mentioned were treated according to the above procedure.

2.4. HPLC and MS conditions

Agilent 1100 LC/MSD Trap (Santa-Clara, USA), equipped with four pumps, was used to analyze the amino acids. Detection photodiode array detector and XCT type ion trap mass spectrometer with ESI source (Agilent micromesh ZQ4000, USA) were used. Data analysis was carried out with a LC/MSD Trap software (version 5.2). The chromatographic analysis of the amino acid derivatives was conducted using an Agilent Eclipse plus C18 column (4.6 mm × 250 mm × 5 μm) maintained at 30 °C. The wavelength was set at 254 nm, and the analysis time was 30 min. The HPLC conditions for the PITC derivatives were as follows: mobile phase A, 0.05% formic acid aqueous solution; mobile phase B, 70:30 (*v*/*v*) acetonitrile–water. Each mobile phase was filtered through a 0.45 μm Nylon 66 membrane filter before use. The derivatives of the samples were infused into the ESI-MS system at a flow rate of