Original article

Systematic screening and structural characterization of dipeptides using offline 2D LC-LTQ-Orbitrap MS: A case study of *Cordyceps sinensis*

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**Article Info**

**Abstract**

*Cordyceps sinensis* (C. sinensis) is a widely used and highly valuable traditional Chinese medicine. Several dipeptides have been detected in *C. sinensis*, but current scientific knowledge of its chemical makeup remains limited. In this study, an improved approach that integrates offline two-dimensional liquid chromatography (2D LC) separation, precursor ion list, library screening, and diagnostic ion filtering was established to systematically screen and characterize dipeptides in *C. sinensis*. Offline 2D LC integrating hydrophilic interaction LC and reverse phase separations was established to eliminate interference and identify the target dipeptides. A library containing the potential 400 dipeptides was created, and a precursor ion list with all theoretical precursor ions was adopted to trigger the MS/MS scan with high sensitivity. To identify dipeptides, the type and connection sequence of amino acids were determined according to the product ions. Ile and Leu residues were differentiated for the first time according to the characteristic ion at m/z 69.07. Ultimately, 170 dipeptides were identified or tentatively characterized from *C. sinensis*, and most are reported for the first time in this species herein. In addition, the identified dipeptides were also applied for discrimination among the three *Cordyceps* species, and 11 markers were identified. The obtained results provide a deeper understanding of the chemical basis of *C. sinensis*.

1. Introduction

Dipeptides comprise two amino acids linked by a peptide bond, and as many as 400 dipeptides can be constructed from 20 proteinogenic amino acids. They are widely present in human biofluids, cellular extracts, and fermented foods. These dipeptides have been attributed to the products of protein degradation. Recently, they have attracted broad attention owing to their important physiological functions. Some dipeptides exhibit prominent bioactivity, such as Tyr-Tyr (anti-tumor effect), Tyr-Leu (antidepressant-like effect), and Try-Tyr (memory improving effect). Some dipeptides have been found to serve as disease biomarkers. For instance, higher levels of certain dipeptides have been found in chronic myelogenous leukemia stem cells and non-small cell lung cancer tissues.

Given the importance of dipeptides, many efforts have been made in terms of qualification and quantification analyses. However, obstacles must be overcome when analyzing dipeptides, including the poor retention of hydrophilic dipeptides on conventional C18 columns, differentiation between opposite amino acid binding orders and isomeric amino acids (Ile and Leu), and background noise during MS detection. Analytical methods with different separation mechanisms, including reverse phase liquid chromatography (LC), hydrophilic interaction liquid chromatography (HILIC), and capillary electrophoresis (CE) [13], have been used to separate dipeptides. Recently, a method to profile and quantify 335 dipeptides, which was developed on an analytical platform, integrates two sets of instruments, namely, CE tandem mass spectrometry (MS) and LC-MS. However, to achieve full...
and unambiguous identification, 361 standard reagents are involved, which are quite costly. Taking full advantage of MS fragment information may facilitate the differentiation and identification of dipeptides.

Cordyceps sinensis (C. sinensis), a highly reputable and valuable traditional Chinese medicine, is widely used as a functional food in many regions. Although it has been reported to process a wide spectrum of biological functions, such as anti-aging, anti-tumor, antioxidant and reparative ones, and immune stimulation [14], the related active substances remain ambiguous. The authors identified a wide distribution of dipeptides in the aqueous extract of C. sinensis in addition to nucleobases, nucleosides, and amino acids [15]. Taking the potential activities of dipeptides into consideration, this study aimed to establish an approach to the systematic screening and characterization of dipeptides in C. sinensis, which can also be applied in the analysis of dipeptides in other foods or natural medicines.

2. Materials and methods

2.1. Chemicals and reagents

Six dipeptides (purity >98 %), namely, Ile-Glu, Leu-Glu, Ile-Thr, Leu-Thr, Thr-Ile, and Thr-Leu (Shanghai Aptide Co., Ltd., Shanghai, China), were used as reference standards. The chemical structures of these reference standards are shown in Fig. 1. HPLC-grade acetonitrile, methanol (Merck KGaA, Darmstadt, Germany), formic acid (ROE Scientific Inc., Newark, DE, USA), and ultra-pure water (18.2 MΩ cm at 25 °C) prepared using a Millipore Alpha-Q water purification system (Millipore, Bedford, MA, USA), were used. The samples (Table S1), including five batches of wild C. sinensis, five batches of cultured C. sinensis, five batches of C. cicadae, and five batches of C. militaris, were either provided by Sunfire Lake Pharma Co., Ltd. (Dongguan, China) or collected from markets. After collection, all specimens were deposited at Shanghai Institute of Materia Medica (Shanghai, China).

2.2. Sample preparation

Samples were pulverized into fine powder using a JXFSTPRP-48 ball mill (Jinxin, Shanghai, China) set at 60 Hz for 30 s and repeated four times. An aliquot of 0.2 g of fine Cordyceps powder was accurately weighed and ultrasonically extracted in 2 mL of ultra-pure water in a water bath at room temperature for 30 min. The samples were centrifuged at 14,000 r/min for 10 min, and the supernatant was stored at 4 °C prior to analysis. Quality control (QC) sample was prepared by mixing all test samples equally.

2.3. Comprehensive acquisition using offline two-dimensional (2D) LC-LTQ-Orbitrap

2D LC separation based on HILIC × RP was conducted following the protocols described in the literature [16–18]. The aqueous extract was separated in HILIC mode on an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) using a Waters Xbridge Amide column (4.6 mm × 150 mm, 3.5 μm). A binary mobile phase consisting of acetonitrile (A) and 0.1 % aqueous formic acid (B) was used at a flow rate of 1.0 mL/min following a gradient elution program: 0–5 min, 95 % (A); 5–25 min, 95–70 % (A); 25–35 min, 70 % (A). The column temperature was set to 30 °C. The variable wavelength detector was set at 252 nm to monitor the dipeptides. C. sinensis solution (20 μL) was injected into the column for chromatographic separation. Eluate collection was based on time-dependent fractionation, and 15 fractions were obtained from 1 to 31 min (2 min for each fraction) with 8 replicates. The pooled eluate from each fraction was dried under a steady flow of nitrogen (N2) at ambient temperature. The dried residues of 15 fractions were separately reconstituted in 1 mL of ultra-pure water and filtered through a 0.22 μm polytetrafluoroethylene filter. The fractions were separated on an Ultimate 3000 UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a vacuum degasser, binary pump, autosampler, diode array detector, and column compartment. An RP mode UHPLC column, Waters ACQUITY HSS T3 column (2.1 mm × 100 mm, 1.8 μm) maintained at 30 °C, was used and eluted by the mobile phase containing 0.1 % formic acid (A) and acetonitrile (B) in an 8 min gradient program: 0–1 min, 1 % B; 1–4 min, 1 %–3 % B; 4–6 min, 3 %–30 % B; 6–8 min, 30 % B. The diode array detector recorded UV signals at 252 nm and between 190 and 400 nm. The sample injection volume was 2 μL.

High-accuracy mass spectrometric analysis of dipeptides was performed on an LTQ-Orbitrap Velos Pro hybrid mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) in positive mode under the following conditions: spray voltage, 3.8 kV; capillary temperature, 350 °C; source heater temperature, 300 °C; sheath gas (N2), 40 arbitrary units; and auxiliary gas (N2), 10 arbitrary units. Source fragmentation was disabled. The Orbitrap analyzer scanned over a mass range of m/z 100–1000 at a resolution of 30000 (full width at half maximum at m/z 400) in the MS1 scan. The parent ion list representing all potential dipeptides was imported first. The most intense ion from the parent ion list was selected to trigger MS2 collision-induced dissociation (CID) fragmentation, and when the product ion at m/z 132.10 was detected in the MS2 scan, further MS3 CID fragmentation was activated. The normalized collision energy at 35 % was set for both MS2 and MS3 fragmentation.

2.4. Software-aided identification and statistical analysis

A library containing 400 dipeptides was then constructed. First, the simplified molecular-input line-entry system (SMILES) structures of 400 dipeptides were generated based on the SMILES structures of 20 amino acids through all possible pairwise combinations. Then, the SMILES structures were converted to the sdf format. The acquired data were imported into Progenesis QI software, and the library was loaded for comprehensive screening of dipeptides.

Multiple batches of samples acquired with the above RP-LTQ-Orbitrap method with the MS2 and MS3 scans were disabled. The acquired data were imported into Progenesis QI for data preprocessing, involving multiple steps, such as alignment, peak picking, and deconvolution. The intensity of all features within a sample was normalized. A data matrix containing all metabolic features, including retention time, m/z, and normalized peak area, was generated, and the metabolic features of the dipeptides were
filtered out. The obtained data matrix was exported into SIMCA-P software (version 13.0; Umertrics, Umea, Sweden) for pattern recognition chemometric analysis. Pareto scaling was also performed. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis were employed to visualize the differences among different groups.

3. Results and discussion

3.1. Optimization of the chromatographic and MS conditions

In an attempt to achieve better chromatographic separation of the dipeptides, different columns, additives, gradients, and column temperatures were compared and optimized (Figs. S1–S4) for both dimensional separations. It was found that the T3 column provided stronger retention abilities than the other C18 columns, especially for polar dipeptides. Formic acid, as an additive in the second dimension, greatly improved the peak shapes and MS responses. The gradient elution program was further optimized, and an 8 min gradient was adopted. Representative HILIC (1D) and RP (2D) chromatograms are shown in Figs. S5 and S6.

To precisely identify dipeptide mixtures, it is crucial to discriminate the binding order of amino acids and isomeric amino acids (Leu and Ile). Standard reagents facilitate high analytic certainty; however, they are expensive for a large number of dipeptides. Derivatization is an alternative method, but the procedure complicates experimental operation, chromatographic elution orders, and MS spectra [15]. In this experiment, high-resolution MS/MS spectra were adopted for the precise identification of the reversed analogues. To enhance the detection of target components, a precursor ion list (PIL) method was adopted based on the established library. To clarify the C-terminal Leu and Ile residues, further fragmentation of the unique ion at m/z 132.10, in MS² was compared using three different fragmentation mechanisms, namely, CID, high-energy C-trap dissociation, and pulsed-Q dissociation, at different energies. A relatively higher characteristic product ion for Ile was produced at m/z 69.07 in MS³, which was speculated to correlate with the carbocation rearrangement of Ile ion (Fig. 2). The characteristic ion at m/z 69.07 was also exclusively observed for N-terminal Ile residues in MS². Therefore, a full MS-PIL-CID-MS²-CID-MS³ method was established.

3.2. Structure identification and discrimination of reverse analogue dipeptides

Amino acids were expressed in three-letter abbreviations, and the nomenclature for peptide ions proposed by Roepstorff and Fohlman [20] was adopted. We first examined the MS and MS/MS spectra of the positional isomers with reference standards (Ile-Thr, Leu-Thr, Thr-Ile, and Thr-Leu) (Fig. 3). The positive ion ESI mass spectra all showed abundant [M+H]⁺ ions at m/z 233.15. Ile-Thr and Leu-Thr displayed spectra similar to those of main product ions at m/z 215.14 [M+H+H₂O]⁺, 120.07 (y₁⁺: [Thr+H]⁺), and 86.10 (a₁⁺: [Ile/Leu+H+H₂O+CO]⁺), while Thr-Ile and Thr-Leu showed main product ions at m/z 215.14 [M+H+H₂O]⁺, 187.15 [M+H+H₂O+CO]⁺, 132.10 (y₁⁺: [Ile/Leu+H]⁺), and 74.06 (a₁⁺: [Thr+H+H₂O+CO]⁺). Accordingly, a₁⁺ and y₁⁺ ions were indicative of the N-terminal and C-terminal residues in the dipeptides, respectively. Careful comparison between the MS/MS spectra of Ile-Thr and Leu-Thr led to a minor product ion at m/z 69.07, diagnostic for N-terminal Ile residue. This was also evidenced by two other reference standards, Leu-Glu and Ile-Glu. Further fragmentation of m/z 132.10 in Thr-Ile
and Thr-Leu provided differential MS/MS spectra with relatively high ions at m/z 69.07 for Thr-Ile. Therefore, the isomeric Ile and Leu in the dipeptides could be precisely identified. According to the results of the present study, only MS3 allowed precise identification of C-terminal Ile/Leu residues, whereas MS2 was sufficient for the identification of N-terminal Ile/Leu residues. In addition, for the three pairs of isomers involving Ile and Leu, consistent chromatographic elution orders were observed on RP columns, with isoleucyl peptides eluted earlier (Ile-Glu vs. Leu-Glu: 4.43 vs. 4.88 min, Thr-Ile vs. Thr-Leu: 6.72 vs. 6.95 min, Ile-Thr vs. Leu-Thr: 2.94 vs. 3.45 min). The elution orders were also consistent with those of the amino acids of Ile and Leu, which can be partially explained by the ClogP (Ile: 1.757 and Leu: 1.667, calculated using ChemBioDraw Ultra 12.0). Consequently, elution orders may contribute to the differentiation of isomeric dipeptides. Among the 170 identified dipeptides in this work, 50 contained Ile and Leu moieties, indicating the importance of differentiating Ile and Leu. To the best of our knowledge, this is the first report on the differentiation of Ile and Leu in peptides without chemical derivatization.

Due to the limits of dipeptide standards, accessible databanks (e.g., human metabolome database, massbank, and METLIN) do not contain global dipeptide information. Therefore, a home-made dipeptide library containing all 400 items was established and imported into the Progenesis QI for comprehensive screening. In this way, 170 features corresponding to the dipeptides were filtered. However, one extracted feature might be identified as several isomers, and one identity might be attributed to several features at different retention time. For precise identification, a three-step strategy was adopted. 1) The types of amino acids (except Ile and Leu) were determined according to the corresponding product ions; 2) the C-terminal (such as a1+) and N-terminal (such as y1+)–related fragments were analyzed in detail to

Fig. 3. MS2 spectra of reference standards (A) Ile-Thr and (B) Leu-Thr and along with MS2 and MS3 spectra of reference standards (C) Thr-Ile and (D) Thr-Leu.
clarify the binding order of dipeptides; and 3) Ile and Leu were differentiated according to the characteristic ion at m/z 69.07. Taking two isomeric compounds 79 and 140 with identical protonated parent ions at m/z 246.1446 as an example (Fig. 4), there were six options (Asn-Ile, Asn-Leu, Gln-Val, Ile-Asn, Leu-Asn, and Val-Gln) in the library. In the first step, for compound 79, characteristic product ions at m/z 147.08 and 129.07, corresponding to Gln and m/z 118.09 and 72.08 corresponding to Val, respectively, were detected; therefore, compound 79 was deduced to contain Gln and Val. For compound 140, characteristic product ions at m/z 132.10 and 86.10 (corresponding to Ile and Leu), and 115.05 (corresponding to Asn) indicated the composition of Ile/Leu and Asn. In the next step, the MS/MS spectrum of compound 79 led to precise identification of the C-terminal Val residue, based on the intense product ion of y1+: m/z 118.09 ([Val+H]+) and N-terminal Gln residue based on the intense product ion of a1+: m/z 101.07 ([Gln+H-H2O-CO]+). In addition, y2a2+ ion at m/z 72.08 ([Val-H-H2O-CO]+) and b1+ ion at m/z 129.07 ([Gln+H-H2O]+) also support the binding order. Accordingly, compound 79 was deduced to be Gln-Val. Similarly, compound 140 was inferred to be Asn-Ile/Leu, with product y1+ ion at m/z 132.10 ([Ile/Leu+H]+) and b1+ ion at m/z 115.05 ([Asn+H-H2O]+), along with the y2a2+ ion at m/z 86.10 ([Ile/Leu+H-H2O-CO]+). The third step was applied in the differentiation of Ile and Leu for compound 140, as further fragmentation of m/z 132.10 did not produce characteristic ion m/z 69.07, and compound 140 was thus characterized as Asn-Leu.

For amino acids with complex side chains, such as nitrogen-containing side chains (i.e., Arg, His, Lys, Try, and Asp) and

Fig. 4. Interpretation of (A and B) the MS2 spectra of compound 79, and (C and D) the MS2 and MS3 spectra of compound 140.

Fig. 5. (A) A two-dimensional (2D) scatter plot of all the characterized dipeptides. Triangle facing left represents N-terminal amino acids, while triangle facing right represents C-terminal amino acids. (B) The numbers of occurrence of amino acids in the identified dipeptides.
carboxyl side chains (i.e., Glu and Asp), the reactive side chains might lead to complex conjugates. Compound 118 was characterized as an example. The protonated parent ion at \(m/z\) 322.1870 could provide the molecular formula \(C_{15}H_{23}N_5O_3\). The MS/MS product ions at \(m/z\) 305.16, 287.15, 263.14, 190.10, 175.12, 157.11, and 120.08 were ascribed to \([M+H]^+\), \([M+H\cdot\text{NH}_3]^+\), \([M+H\cdot\text{CH}_4N_3]^+\), \([M+H\cdot\text{C}_5H_12O_2N_2]^+\), \([\text{Arg}+H]^+\), \([\text{Arg}+H\cdot\text{H}_2O]^+\), and \([\text{Phe}+H\cdot\text{H}_2O\cdot\text{CO}]^+\). Therefore, compound 118 was deduced to be Phe-Arg. Notably, after careful comparison, several compounds might be tentatively annotated as the same compound (compounds 83, 118, and 128) due to the multiple conjugation sites and the potential non-proteinogenic amino acids. Similarly, in total of 59 dipeptides with isomeric structures were only tentatively identified. As a result, a total of 170 dipeptides (Table S2 and Fig. 5) were characterized, about 4.3 times more than that in the previous report with only RP separation [15], and the characterization confidence was greatly improved.

3.3. Differentiation of three cordyceps species based on dipeptides

The substitution or adulteration of inferior Cordyceps species, such as \(C.\) militaris and \(C.\) cicadae, has been a major concern in the QC of \(C.\) sinensis. However, the differentiation of these species based on nonspecific nucleotides and amino acids is difficult [21,22]. Some attempts have adopted the duplex PCR method to identify \(C.\) sinensis; however, the PCR method is limited to the raw material and primary products [23]. To uncover the differences in the composition of dipeptides among different Cordyceps species and discover effective dipeptide markers to differentiate authentic \(C.\) sinensis and their substitutes, a targeted metabolomics strategy based on dipeptides was adopted in this study. A total of 20 samples (involving five batches of wild \(C.\) sinensis, five cultured \(C.\) sinensis, five \(C.\) cicadae, and five \(C.\) militaris) were analyzed. The data of all samples were first processed by Progenesis QI according to previously reported procedures [24], and 145 dipeptides were extracted from the QC sample.

According to the PCA score plots (Fig. 6A), ten batches of \(C.\) sinensis (including wild and cultured samples) could be completely discriminated from \(C.\) cicadae and \(C.\) militaris, while the difference between the wild and cultured \(C.\) sinensis was relatively insignificant. Therefore, the wild and cultured samples were combined for further analysis. Supervised PLS-DA and variable importance plot (VIP) graphs were employed to discriminate the three species. The PLS-DA model was validated using a permutation test (Fig. S7). As shown in Fig. 6B, the three species were well gathered and segregated into three different groups within the 95 % Hotelling T2 ellipse. Potential markers with VIP values > 2.0 were filtered (Fig. 6C), leading to the discovery of 11 potential markers, i.e., Ala-Leu, Leu-Pro, Val-Leu, Leu-Ile, Val-Phe, Glu-Gln, Phe-Leu, Val-Tyr, Ile-Arg, Leu-Arg, and Tyr-Arg, among which Leu-Pro and Glu-Gln were much higher in \(C.\) sinensis. The variations of these potential dipeptide markers among the three Cordyceps species can be intuitively exhibited by a two-way hierarchical clustering analysis heat map (Fig. 6D).

4. Conclusion

An improved characterization approach developed by integrating offline 2D LC separation, precursor ion list, library screening, and diagnostic ion filtering was established to facilitate the thorough characterization of 170 dipeptides in \(C.\) sinensis. Dipeptides derived from Ile and Leu were differentiated for the first
time according to the characteristic ion at m/z 69.07. In addition, 11 markers for discrimination among the three Cordyceps species were identified using a targeted metabolomics method. Clarification of the dipeptides can improve our understanding of the therapeutic basis of C. sinensis. The marker dipeptides may be applied as qualitative indicators in the QC of C. sinensis, specifically for the adulteration detection of similar species. The improved approach is also promising for the characterization of other small peptides, such as tripeptides and tetrapeptides, from other sources.

CRediT author statement

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was financially supported by the National Key R&D Program of China (Grant Nos.: 2018YFC1707900, 2019YFC1711000, and 2019YFC1711400), Key-Area Research and Development Program of Guangdong Province (Grant No.: 2020B1111110007), the National Natural Science Foundation of China (Grant No.: 82003938), and Chief Scientist of Qi-Huang Project of National Traditional Chinese Medicine Inheritance and Innovation “One Hundred Million” Talent Project (2020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2021.07.007.

References

[1] Y. Tang, R. Li, G. Lin, et al., PEP search in MyCompoundID: detection and identification of dipeptides and tripeptides using dimethyl labeling and hydrophilic interaction liquid chromatography tandem mass spectrometry, Anal. Chem. 86 (2014) 3568–3574.
[2] S. Pievesana, C.M. Montone, C. Cavaliere, et al., Sensitive untargeted identification of short hydrophilic peptides by high performance liquid chromatography on porous graphic carbon coupled to high resolution mass spectrometry, J. Chromatogr. A 1590 (2019) 73–79.
[3] B.K. Ubhi, P.W. Davenport, M. Welch, et al., Analysis of chloroformate-derivatised amino acids, dipeptides and polypeptides by LC-MS/MS, J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 934 (2013) 79–88.
[4] K. Takahashi, M. Tokouka, H. Kohno, et al., Comprehensive analysis of dipeptides in alcoholic beverages by tag-based separation and determination using liquid chromatography/electrospray ionization tandem mass spectrometry and quadrupole-time-of-flight mass spectrometry, J. Chromatogr. A 1242 (2012) 17–25.
[5] S.N.S. Vasconcelos, J.M. Sciani, N.M. Lisboa, et al., Synthesis of a tyr-tyr dipeptide library and evaluation against tumor cells, Med. Chem. 14 (2018) 709–714.
[6] T. Mizushige, T. Uchida, K. Chinata, Dipeptide tyrosyl-leucine exhibits anti-depressant-like activity in mice, Sci. Rep. 10 (2020), 2257.
[7] Y. Ano, T. Ayabe, R. Ohyu, et al., Tryptophan-tyrosine dipeptide, the core sequence of β-lactolin, improves memory by modulating the dopamine system, Nutrients 11 (2019), 348.
[8] K. Naka, Y. Jomen, K. Ishihara, et al., Dipeptide species regulate p38MAPK-Smad3 signalling to maintain chronic myelogenous leukaemia stem cells, Nat. Commun. 6 (2015), 8039.
[9] M. Wu, Y. Xu, W.J. Fitch, et al., Liquid chromatography/mass spectrometry methods for measuring dipeptide abundance in non-small-cell lung cancer, Rapid Commun. Mass Spectrom. 27 (2013) 2091–2098.
[10] H. Ozawa, A. Hirayama, T. Ishikawa, et al., Comprehensive dipeptide profiling and quantitation by capillary electrophoresis and liquid chromatography coupled with tandem mass spectrometry, Anal. Chem. 92 (2020) 9799–9806.
[11] C.L. Xie, S.S. Kang, C. Lu, et al., Quantification of multifunctional dipeptide YA from oyster hydrolysate for quality control and efficacy evaluation, Biomed. Res. Int. 2018 (2014), 843779.
[12] S. Le Maux, A.B. Nongonierma, B. Murray, et al., Identification of short peptide sequences in the nanofiltration permeate of a bioactive whey protein hydrolysate, Food Res. Int. 77 (2015) 534–539.
[13] X. Mu, L. Qi, J. Qiao, et al., Enantioseparation of dansyl amino acids and dipeptides by chiral ligand exchange capillary electrophoresis based on Zn(II)-L-hydroxyproline complexes coordinating with γ-cyclodextrins, Anal. Chim. Acta 846 (2014) 68–74.
[14] P.X. Chen, S. Wang, S. Nie, et al., Properties of Cordyceps sinensis: a review, J. Funct. Foods 3 (2015) 550–560.
[15] C.L. Yao, Z.M. Qian, W.S. Tian, et al., Profiling and identification of aqueous extract of Cordyceps sinensis by ultra-high performance liquid chromatogram tandem quadrupole-orbitrap mass spectrometry, J. Chin. Med. Ther. 17 (2019) 631–640.
[16] S. Qiu, W.Z. Yang, X.J. Shi, et al., A green protocol for efficient discovery of novel natural compounds: characterization of new ginsenosides from the stems and leaves of Panax ginseng as a case study, Anal. Chim. Acta 893 (2015) 65–76.
[17] C.L. Yao, W.Z. Yang, W. Si, et al., An enhanced targeted identification strategy for the selective identification of flavonoid O-glycosides from Carthamus tinctorius by integrating offline two-dimensional liquid chromatography-linear ion-trap-Orbitrap mass spectrometry, high-resolution diagnostic product ions/neutral loss filtering and liquid chromatography-solid phase extraction-nuclear magnetic resonance, J. Chromatogr. A 1491 (2017) 87–97.
[18] C.L. Yao, H.Q. Pan, H. Wang, et al., Global profiling combined with predicted metabolites screening for discovery of natural compounds: characterization of ginsenosides in the leaves of Panax notoginseng as a case study, J. Chromatogr. A 1538 (2018) 34–44.
[19] M. Tanaka, S.M. Hong, S. Akiyama, et al., Visualized absorption of anti-atherosclerotic dipeptide, Trp-His, in Sprague-Dawley rats by LC-MS and MALDI-MS imaging analyses, Mol. Nutr. Food Res. 59 (2015) 1541–1549.
[20] P. Roepstorff, J. Fohlman, Proposal for a common nomenclature for sequence ions in mass spectra of peptides, Biomed. Mass Spectrom. 11 (1984), 601.
[21] S.F. Li, F.Q. Yang, K.W.K. Trim, Quality control of Cordyceps sinensis, a valued traditional Chinese medicine, J. Pharm. Biomed. Anal. 41 (2006) 601–607.
[22] H. Hu, L. Xiao, B. Zheng, et al., Identification of chemical markers in Cordyceps sinensis by HPLC-MS/MS, Anal. Bioanal. Chem. 407 (2015) 8059–8066.
[23] F.L. Zhang, X.F. Yang, D. Wang, et al., A simple and effective method to discern the true commercial Chinese cordyceps from counterfeits, Sci. Rep. 10 (2020), 2974.
[24] H. Pan, C. Yao, S. Yao, et al., A metabolomics strategy for authentication of plant medicines with multiple botanical origins, a case study of Uncariae Rammulus Cum Uncis, J. Sep. Sci. 43 (2020) 1043–1050.