Journal of Chromatography A, 1502 (2017) 38–50

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A robust platform based on ultra-high performance liquid chromatography quadrupole time of flight tandem mass spectrometry with a two-step data mining strategy in the investigation, classification, and identification of chlorogenic acids in *Ainsliaea fragrans* Champ

Hui Ouyang[a,1], Junmao Li[a,1], Bei Wu[b], Xiaoyong Zhang[a], Yan Li[a], Shilin Yang[a,c], Mingzhen He[a,*], Yulin Feng[a,c,*]

[a] *Jiangxi University of Traditional Chinese Medicine, No. 818 Yunwan Road, Nanchang 330002, PR China*
[b] *Nanchang Insitute for Food and Drug Control, No. 299 Diezihu Road, Nanchang 330038, PR China*
[c] *State Key Laboratory of Innovative Drug and Efficient Energy-Saving Pharmaceutical Equipment, No. 56 Yangming Road, Nanchang 330006, PR China*

ARTICLE INFO

Article history:
Received 21 January 2017
Received in revised form 25 April 2017
Accepted 25 April 2017
Available online 26 April 2017

Keywords:
A two-step date mining strategy
UHPLC–QTOF–MS/MS
Ainsliaea fragrans Champ
CGAs

ABSTRACT

The chlorogenic acids are the major bioactive constituents of the whole plant of *Ainsliaea fragrans* Champ. (Xingxiang Tuerfeng). These compounds are usually present as isomeric forms in Xingxiang Tuerfeng. Therefore, an efficient approach should be developed for the rapid discovery and identification of chlorogenic acids isomers through the fragmentation pathway and rules. In this study, the collision induced dissociation tandem mass spectrometry (CID-MS/MS) fragmentation routes of chlorogenic acids were systematically investigated by UHPLC–QTOF–MS/MS in the negative ion mode using eight chlorogenic acids standards. As a result, diagnostic product ions for rapid discovery and classification of chlorogenic acids isomers were determined according to their MS/MS fragmentation patterns and intensity analysis. Based on these findings, a novel two-step data mining strategy was established. The first key step was to screen different kinds of substitution and the skeleton of the quinic acid using the characteristic product ions and neutral losses. The second key step was to screen and classify different types of chlorogenic acids using their diagnostic product ions. It was apply to the rapid investigation, classification, and identification of chlorogenic acids. And the same carbon skeletons from a complex extract of *Ainsliaea fragrans* Champ. were effectively identified. 88 constituents, including 14 chlorogenic acids types, were rapidly discovered and identified, and in particular, 12 types of chlorogenic acids, including *p*-CoQC, FQA, BQC, CQA-Glu, CFQA, *p*-Co-CQC, *di*-*p*-CoQC, BCQA, *di*-CQA-Glu, PCQA, *tri*-QCA, and P-*di*-CQA, were first discovered in *Ainsliaea fragrans* Champ. In conclusion, UHPLC-QTOF-MS/MS method together with a systematic two-step data mining strategy was established as a feasible, effective, and rational technique for analyzing chlorogenic acids. Additionally, this study laid a foundation for the study of the active substances and quality control of *Ainsliaea fragrans* Champ.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Ainsliaea fragrans Champ. (AFC) is a folk herbal medicine referred to as'Xingxiang Tuerfeng' in south China that has a long history of medicinal practice. Xingxiang Tuerfeng has been identified as playing a role in heat-clearing, detoxifying, eliminating masses, relieving swelling, cooling blood, and stopping a cough [1]. Recently, AFC has attracted considerable attention to numerous researchers due to its antibacterial and anti-inflammatory effects [2–4]. Furthermore, the whole AFC plant was used as one of the predominant ingredients in preparing the 'Xingxiang Tuerfeng tablet' and 'Compound Xingxiang Tuerfeng granule,' which significantly aids the clinical treatment of gynecological diseases, such as cervicitis, ometritis, and pelvic inflammation [5,6]. As common over-the-counter (OTC) medicines in China, these preparations are the first-line treatments for combatting gynecological diseases. At

* Corresponding authors at: Jiangxi University of Traditional Chinese Medicine, No. 818 Yunwan Road, Nanchang 330002, PR China.
E-mail addresses: hmz20170118@163.com (M. He), fengyulin2003@126.com (Y. Feng).
[1] These authors contributed equally to this article.

http://dx.doi.org/10.1016/j.chroma.2017.04.051
0021-9673/© 2017 Elsevier B.V. All rights reserved.

present, there's not sufficient research on the chemical constituents of chlorogenic acids (CGAs) in AFC. CGAs are some of the key ingredients in phenolic compounds and the major bioactive constituents of AFC [7,8]. Previous phytochemical studies have found that AFC primarily contains triterpenoids, flavonoids, sesquiterpenes, and phenolic acids [9–12]. Notably, it has been reported that CGAs have various biological activities [13–16], such as antibacterial activities that can be used to treat gynecological diseases.

CGAs are a large family of esters formed between a common skeleton of quinic acid and certain cinnamic acids, most commonly caffeic, *p*-coumaric, and ferulic acids [17,18] and sometimes benzenepropanoic acid, β,3,4-trihydroxy, caffeic acid glucoside, and propanedioic acid [19–21]. CGAs are usually present in the form of isomers due to the different substituted positions of the same substituent group on the quinic acid. However, due to insufficient reference standards and structural similarity, it is difficult to identify and differentiate CGAs from isomers. Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) is a very powerful method for the rapid qualitative analysis of botanic extracts and crude material of traditional Chinese medicines (TCMs) [22,23]. To date, these methods can only provide a quasi-molecular ion and fragments information yielded by multistage cleavage, but cannot provide an accurate mass measurement of precursor and product ions, or elemental composition information. Therefore, there is an urgent need for establishing a reliable strategy for screening and identifying CGAs at main and trace levels in addition to summarizing CGAs characterization in complex samples.

Based on the above requirement, a rapid and sensitive method that can thoroughly detect the main and trace amounts of CGAs is desirable. In this study, an ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight tandem mass spectrometry (UHPLC–QTOF–MS/MS) was used. This technique can provide the excellent resolution of chromatography separation with an accurate mass measurement of precursor and product ions for individual compounds. It is the first time that LC–MS/MS technology was used for the identification of CGAs from AFC. The CID-MS/MS fragmentation routes of eight CGAs standards were systematically studied. Based on the CID-MS/MS fragmentation patterns and the routes of these compounds, a two-step date mining strategy was established for the rapid investigation, classification, and identification of CGAs. An appropriate UHPLC–QTOF–MS/MS method coupled with an effective two-step data mining strategy was developed as a feasible, rational, and systematic technique for analyzing CGAs that possess the same carbon skeletons from a complex extract of AFC.

2. Materials and methods

2.1. Chemicals and reagents

Pure distilled water was purchased from Watsons (Hong Kong, China). Analytical grade formic acid and ammonium formate were purchased from Sinopharm Chemical Reagent Co., LTD (Shanghai, China). HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The eight reference standards of CGAs, including 3-CQA, 4-CQA, 5-CQA, 1,3-*di*-CQA, 1,5-*di*-CQA, 3,4-*di*-CQA, 3,5-*di*-CQA, and 4,5-*di*-CQA, were purchased from Chengdu Must Bio-technology Co., Ltd. (Sichuan, China) (Fig. 1). The purities of their reference standards were greater than 98% as determined by HPLC-UV. The chemical structures of all reference standards were characterized by spectroscopic data (^{1}H, ^{13}C NMR, and HR-MS) and were compared to those found in the literature [24].

2.2. Plant materials and sample preparation

The raw material of AFC plants that has been grown in two different geographic areas (Jiangxi Province and Jiangsu Province in China) were purchased from herbal medicine markets located in Zhangshu City, Jiangxi Province and Anguo City, Hebei Province, respectively. These samples were identified by Vice Director of Pharmacists Bei Wang, Nanchang Institute for Drug Control. The voucher specimens were deposited at the herbarium of the Jiangxi University of Traditional Chinese Medicine. The raw materials of AFC from different geographic areas were separately pulverized into powders for the extraction preparation.

The extraction method referenced to our previous study and was set as follow [25]: accurately weighed powder (1.0 g) of each location sample was placed into a 50 mL flask, and each sample was extracted with 30 mL of an ethanol: water solution (70:30, *v/v*) in an ultrasonic water bath at room temperature for 45 min. The extraction solutions of these samples were separately centrifuged for 10 min at 13000 rpm and then filtered through a 0.22 μm membrane. Finally, 3 μL of the two individual AFC filtered supernatants were injected for UHPLC–QTOF–MS/MS analyses.

As for stock solutions, the eight reference standards of CGAs were dissolved in methanol at a concentration of 1.00 mg/mL, respectively. The stock solutions of the eight reference standards were mixed with methanol to obtain a concentration of 0.2 μg/mL mixture solution. Finally, 2 μL of the mixture solution was injected for UHPLC-QTOF-MS/MS analyses.

2.3. UHPLC-QTOF-MS/MS analyses conditions

The UHPLC analyses were carried out on a Shimadzu system (Kyoto, Japan), equipped with a LC-3AD solvent delivery system, a SIL-30ACXR auto-sampler, a CTO-30AC column oven, a DGU-20A3 degasser, and a CBM-20A controller. The analytical column was a Waters ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm). The column oven temperature was set at 40 °C. The mobile phases consisted of water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and acetonitrile (solvent B). The flow rate was set at 0.3 mL/min. The binary gradient was applied with linear interpolation as follows: 0.01 min, 5% B; 3.0 min, 10% B; 12.0 min, 17.5% B; 20.0 min, 18% B; 27.0 min, 30% B; 34.0 min; 60% B; 44.0 min, 95% B; 47.0 min, 95% B; 47.1 min, 5% B; 50.0 min, 5% B.

The UHPLC-QTOF-MS/MS detection was conducted on a Triple TOF™ 5600+ system with a Duo Spray source in the negative electrospray ion mode (AB SCIEX, Foster City, CA, USA). The electrospray ionization was applied in the negative mode with the following parameters: ion spray voltage, −4500 V; ion source temperature, 500 °C; curtain gas, 25 psi; nebulizer gas (GS 1), 50 psi; heater gas (GS 2), 50 psi; and declustering potential (DP), −100 V. The mass ranges were set at *m/z* 50–1250 Da for the TOF-MS scan and 50–1250 Da for the TOF MS/MS experiments. In the IDA-MS/MS experiment, the collision energy (CE) was set at 35 eV, and the collision energy spread (CES) was (±)15 eV for the UHPLC-QTOF-MS/MS detection. The most intensive 8 ions from each TOF-MS scan were selected as MS/MS fragmentation. Dynamic background subtraction (DBS) was applied to match the information dependent acquisition (IDA) tests for UHPLC–QTOF–MS/MS detection. The LC–MS/MS data was analyzed using PeakView® 1.2 software (AB SCIEX, Foster City, CA, USA)

3. Result and discussion

3.1. Optimization of LC–MS/MS conditions

In order to achieve massive fragment ions for the identification of CGAs in AFC, the ESI–MS and CID-MS/MS conditions were optimized, and the reference standards of CQA and *di*-CQA were applied to obtain a satisfactory ESI–MS response for detection. The MS response of these substances in the negative ion mode was higher than that in the positive ion mode. As a result, all param-

Download English Version:

https://daneshyari.com/en/article/5135478

Download Persian Version:

https://daneshyari.com/article/5135478

Daneshyari.com