Journal of Chromatography B 1086 (2018) 11–22

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Review

Rapid characterization of the chemical constituents of Sijunzi decoction by UHPLC coupled with Fourier transform ion cyclotron resonance mass spectrometry

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ARTICLE INFO

Keywords:
Sijunzi decoction
Poria
Atractylodis Macrocephalae Rhizoma
Fourier transform ion cyclotron resonance
Chemical constituents

ABSTRACT

Sijunzi decoction, a renowned Chinese prescription has long been utilized to treat gastrointestinal problems. In the context of this research work, the use of Ultra high performance liquid chromatography combined with Fourier transform ion cyclotron resonance mass spectrometry was made to separate and characterize the components of Sijunzi decoction. The performance of Liquid chromatography was carried out on a C8 column (150 mm × 2.1 mm, 1.8 μm); moreover, the mobile phase were consisted of 0.2% formic acid (A) and acetonitrile (B). In accordance with the findings, characterization of 120 chemical compounds was performed by liquid chromatography with mass spectrometry. The key constituents among them included ginsenosides (in Radix Ginseng), 16 triterpene carboxylic acids (in Poria), sesquiterpenes (in Rhizoma Atractylodis Macrocephalae), triterpenesaponins (in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle) as well as flavonoids (in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle) in Sijunzi decoction. This research developed the bases for prospective research associated with Sijunzi decoction, together with being expected to be useful to rapidly extract and characterize the constituents in other Traditional Chinese herbal formulations.

1. Introduction

An extensive use of Traditional Chinese herbal formulation (TCMF) has been made across China, in addition to its neighbouring nations in the lab practices owing to its broad effectiveness, along with some adverse impacts [1]. The chemical constitutes of TCMFs usually possess complexity for the interaction impacts of the constituting herbs found in them. Moreover, the synergistic action of intricate chemical ingredients constitutes the substantial basis for their pharmacological impacts. Accordingly, developing a fast and efficient analysis approach to separate and identify intricate constitutions in TCMFs is quite necessary for the substantial basis of pharmacological research.

Sijunzi decoction (SJZD) is termed as a renowned Chinese prescription, which was primarily recordation in the Song Dynasty. Four conventional herbal medicines, including Ginseng Radix et Rhizoma, Poria, Atractylodis Macrocephalae Rhizoma and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle make its key ingredients. In China, the use of SJZD has a long history to treat gastrointestinal issues, in addition to being capable of fighting nausea, vomiting, and diarrhoea in an effective manner. Currently, it has been revealed by the lab researches that SJZD, in combination with chemotherapy medicines, is quite effective to treat and oesophagus cancer [1–3].

Nowadays, the bases of constituents' research of SJZD have been developed by some relative research works. Yang Liu et al. had performed the identification and characterization of ginsenoside, flavonoid and triterpenoid in SJZD with the help of high-performance liquid chromatography coupled with tandem mass spectrometry (LC/MS^n) [2]. Nevertheless, the detection range, together with sensitivity of ion-trap mass spectrometer, limited both the scope and precision of the findings. UHPLC coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) brings forth an enormous benefit for quantitatively identifying intricate composition for its elevated responsiveness and precision. By usage of this technology, Yinan Wang et al. characterized 33 chemical compounds in Cortex Fraxini [4] and Ting Liu et al. characterized 174 chemical compounds in Gegenqinlian decoction as well as 107 prototypes and 67 metabolites in rats [5]. The reports demonstrated the efficient and practical of this method in chemical compounds and metabolites detection. Furthermore, it is capable of providing information about the fragmentation patterns of chemical compounds which could extend help to speculate the structure of other similar chemical constituents.

In the current research work, a fast as well as efficient methodology

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https://doi.org/10.1016/j.jchromb.2018.04.009
Received 17 December 2017; Received in revised form 30 March 2018; Accepted 6 April 2018
Available online 07 April 2018
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was developed to systematically characterize the chemical ingredients in SJZD with the help of UHPLC-FT-ICR-MS. The research is capable of laying the substantial bases, together with providing considerable information for the pharmacological research of SJZD.

2. Material and methods

2.1. Chemicals and materials

The purchase of key ingredients including, Ginseng Radix et Rhizoma (batch number: 20,151,012; source: Jilin China), Poria (batch number: 13,120,201; source: Hunan China), Atractylodis Macrocephalae Rhizoma (batch number: 13,102,101; source: Zhejiang China) and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle (batch number: 13,112,501; source: Gansu China) was made from Guoda pharmacy (Shenyang, China), which followed identification by Professor Jingming Jia (Department of TCM, Shenyang Pharmaceutical University, Shenyang, China). The key source of the reference compounds (purity > 98%), including atractyloside A, schaftoside, ononin and glycyrrhizic acid was Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China); ginsenoside Re, ginsenoside Rb1, formononetin, atractylenolide III and atractylenolide II were attained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). In addition to that, Acetonitrile of HPLC grade, together with formic acid of LC-MS grade was attained from Fisher Scientific (Fair Lawn, NJ, USA), followed by attaining the purified water from Wahaha (Hangzhou, China).

2.2. Preparation of SJZD for analysis

In accordance with the documentary records of SJZD, four key constituting herbs that included Radix Ginseng (100 g), Poria (100 g), Rhizoma Atractylodis Macrocephalae (100 g), and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle (50 g) were crushed and made to form small pieces, followed by mixing and decocting two times in 3500 mL water for a period of 1 h each time in a glass flask [2,6]. Subsequently, mixing of the ensuing solution was done that followed drying with the help of lyophilization. Prior to the analysis, dissolution of 0.4 g of dried powder was made using 5 mL menthol, followed by extraction with the help of ultrasonic with a period of 20 min.

2.3. Instrument and analytical conditions

The use of an Agilent 1260 UHPLC system (USA) was made in order to perform the chromatographic analysis. The use of a Universal XB C_8 column (150 mm × 2.1 mm, 1.8 μm; Kromat, USA) having the column temperature of 45 °C was made. The mobile phase comprised 0.2% formic acid (A) and acetonitrile (B), which was conducted with gradient condition as hereunder: 2–2% (B) in 0 to 3 min, 2–22% (B) in 3 to 13 min, 22–70% (B) in 13 to 45 min. The setting of the flow rate at 0.20 mL·min^{-1} was ensured, together the injection volume amounting to be 2 μL.

The use of a Bruker Solarix7.0 T FT-ICR-MS system (Bruker, Germany) as well as a Bruker Compass-Hystar workstation (Bruker, Germany) was made to carry out the mass spectra analysis. Ionization was carried out using positive as well as negative electrospray ionization (ESI) modes, followed by setting the optimized conditions as hereunder: nebulizer gas pressure, 4.0 bar; dry gas flow rate, 8 L·min^{-1}; dry gas temperature, 200 °C; ion accumulation time, 0.15 s; time of flight, 0.6 ms; capillary voltage, 4.5 kV; and end plate offset, 500 V. Recording of the Full-scan mass spectrum data was performed between *m/z* 100 and 3000 amu. In respect of auto MS/MS mode, selection of both MS/MS Boost and MS/MS Isolation was made; moreover, range of the collision power lied between 10 eV and 30 eV as regards the MS/MS experimentations.

3. Results and discussion

The Fig. 1 reveals the base peak ion chromatograms (BPC) of SJZD in positive as well as negative ion modes, together with the respective compounds. The extract ion chromatograms (EIC) of each molecule weight were correspondingly attained for detecting the associated compound followed by presenting in Supporting information Fig. S1. Among the compounds identified, accurate identification of nine was carried out with the help of comparison with the retention time (t_R) as well as the MS/MS data associated with the reference compounds in

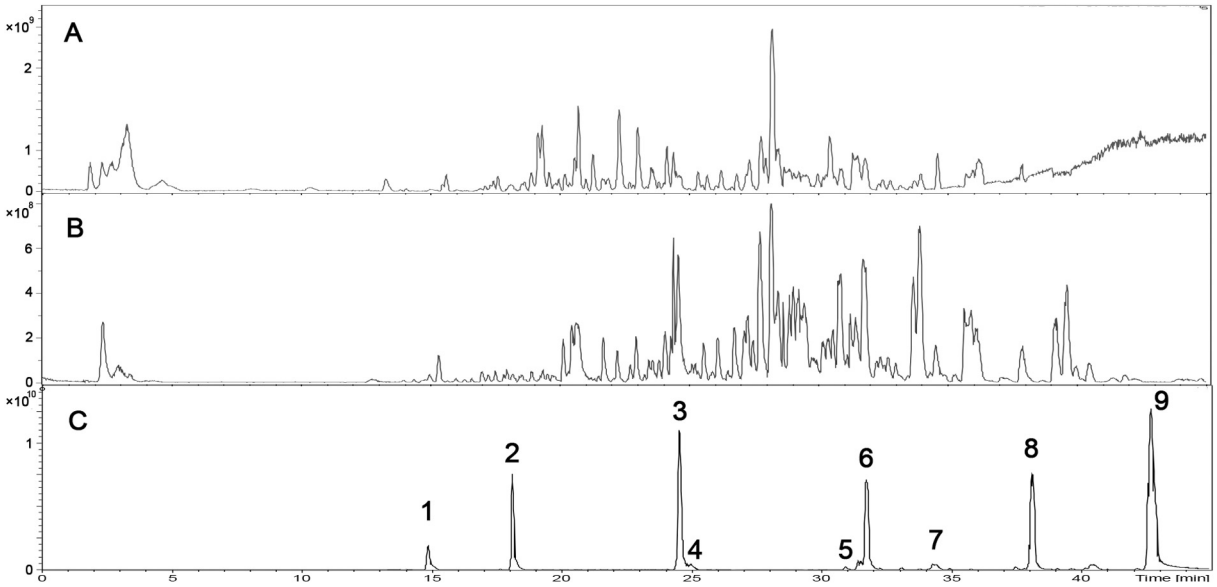

Fig. 1. The base peak ion chromatograms (BPC) of SJZD in both positive (A) and negative (B) ion modes and the corresponding compounds (C). 1-Atractyloside A, 2-schaftoside, 3-ononin, 4-ginsenoside Re, 5-ginsenoside Rb1, 6-formononetin, 7-glycyrrhizic acid, 8-atractylenolide II, 9-atractylenolide III.

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