Journal of Pharmaceutical and Biomedical Analysis 98 (2014) 120–129

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

High performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight–mass spectrometry as a powerful analytical strategy for systematic analysis and improved characterization of the major bioactive constituents from Radix Dipsaci

Yun Ling[a,b,*], Keyue Liu[b], Qing Zhang[b], Liang Liao[b], Yunhua Lu[a]

[a] College of Life Sciences, Huzhou Teachers College, Huzhou 313000, People's Republic of China
[b] College of Life Sciences, Jiujiang University, Jiujiang 332005, People's Republic of China

ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 7 May 2014
Accepted 9 May 2014
Available online 16 May 2014

Keywords:
Radix Dipsaci
Constituents identification
HPLC-ESI-QTOF–MS/MS
Dipsacus saponins
Iridoid glycosides
Caffeoyl quinic acid

ABSTRACT

Radix Dipsaci (RD), the dried root of *Dipsacus asper*, is commonly used as a traditional Chinese medicine for the treatment of bone diseases and functions in strengthening bone and healing bone fractures. Nevertheless, the high polarity, non chromophores and low abundance of multiple compounds in this plant bring difficulty for their isolation and structural determination by traditional chromatographic and spectroscopic techniques, which hindered the use of RD in clinical practice and retarded the process of RD modernization. In this work, a sensitive and rapid high-performance liquid chromatography coupled with electrospray time-of-flight–mass spectrometry (HPLC-ESI-QTOF–MS/MS) was employed to rapidly separate and identify the multiple minor constituents in RD. Separation was performed an Agilent poroshell 120 EC-C18 column (2.1 mm × 100 mm, i.d., 2.7 μm) with 0.1% formic acid aqueous solution and acetonitrile as the mobile phase under gradient conditions. As a result, 36 major constituents including dipsacus saponins, iridoid glycosides and caffeoyl quinic acid derivatives were identified or tentatively characterized from the RD, 11 of which had not been previously reported to the best of our knowledge. In conclusion, the HPLC-ESI-QTOF–MS/MS is feasible and credible technique to separate and identify the constituents in complex matrices of traditional Chinese medicines.

1. Introduction

In the past decade, there has been a growing interest in the use of traditional Chinese medicine (TCM) due to the increasing awareness of people in their health benefits. TCM is usually composed of multi-components responsible for their efficacies. Therefore, systematic analysis and identification of the chemical components in TCM is important for revealing the material basis of their therapeutic effects and ensuring the safety. However, due to difficulties of isolation and preparation, it is unrealistic and unpractical to base all studies on the conventional phytochemical means, especially for those high polarity, non-chromophores and low abundance compounds. Therefore, there is an urgent need to establish a feasible and sensitive method for full-scale qualitative analysis of the major bioactive constituents of TCM.

Recently, high performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight–mass spectrometry (HPLC-ESI-QTOF–MS/MS) covering an extensive range of chemical constituents irrespective of their ultraviolet absorption have been demonstrated to be a good choice for elucidating known and unknown compounds in complex systems such as TCM research [1–3]. In addition, the inherent characteristics of QTOF–MS, such as the accurate mass measurements, elevated sensitivity and a high mass resolving power, make it one of the most desirable detection methods.

Radix Dipsaci (RD), derived from the root of *Dipsacus asper*, has been used in traditional Chinese medicine for hundreds of years as an antiosteoporosis, tonic and antiaging agent for the therapy of low back pain, traumatic hematoma, threatened abortion and bone fractures [4] and is officially documented in the Chinese Pharmacopoeia under the name "Xu Duan". Previous studies had been

* Corresponding author at: College of Life Sciences, Jiujiang University, Jiujiang 332005, People's Republic of China.
E-mail address: lymanuxt@126.com (Y. Ling).

http://dx.doi.org/10.1016/j.jpba.2014.05.006
0731-7085/

performed on the constituents of RD, which indicated that triterpene glycosides, iridoids glycosides and caffeoyl quinic acid were the major chemical components [5–7]. Recent studies have confirmed that RD extract can increase bone density and alter bone histomorphology in mice [8] and has an osteoprotective effect in ovariectomized mice [9]. Despite the popular use of RD, there is no report on systematic analysis of its chemical constituents, especially for those minor unknown compounds.

Therefore, the objective of the present study was to develop a HPLC-ESI-QTOF–MS/MS method for systematical analysis of the chemical profile of RD. The results of the present study will assist in better understanding the mechanism of action of this TCM and discovery of potential novel drug candidates.

2. Experimental

2.1. Materials and reagents

Radix Dipsaci was purchased from Bozhou, Anhui Province, PR China, in September 2013.

The six reference compounds of loganic acid, chlorogenic acid, sweroside, loganin, caffeic acid and akebia saponin D were purchased from the Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China) or Chengdu Mann Stewart Biological Technology Co., Ltd. (Chengdu, China).

HPLC grade acetonitrile, methanol and formic acid were purchased from Dikma Company (Dikma, USA). Water was eionized and double distilled. All other analytical grade reagents were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample preparation

An aliquot of 1.0 g RD drug powder was accurately weighed and extracted with 10 mL of MeOH:water (70:30, v/v) by ultrasonication for 30 min. The solution was centrifuged at 12,000 rpm for 10 min, and the supernatant was filtered through a 0.22 μm filter. A volume of 2 μL was injected into the HPLC-ESI-QTOF–MS/MS system for the analysis.

2.3. HPLC conditions

The chromatography analytical procedures were performed on an Agilent 1260 Series (Agilent, Santa Clara, CA, USA) LC system equipped with a binary pump, an online degasser, an auto plate-sampler, and a thermostatically controlled column compartment. The columns were maintained at 30 °C. The separation was carried out on an Agilent poroshell 120 EC-C18 column (2.1 mm × 100 mm, i.d., 2.7 μm), preceded by a C18 guard column (4.00 mm × 2.00 mm; Agilent, CA, USA). The mobile phase consisted of water containing 0.1% (v/v) formic acid (A) and acetonitrile (B). A gradient program was used as follows: 0–10 min, 5–20% B; 10–25 min, 20–40% B; 25–40 min, 40–90% B. The composition was then held at 90% B for 5 min and returned to initial conditions and maintained 10 min for equilibration. The flow rate was 0.35 mL/min and sample injection volume was 2 μL.

2.4. Mass spectrometry conditions

Mass spectrometry was performed using an Agilent 6530 QTOF mass spectrometer (Agilent, Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) interface, and was operated in negative ion mode with parameters set as follows: capillary voltage, 4000 V; fragmentor, 130 V; skimmer, 65 V; OCT 1 RF Vpp, 750 V; pressure of nebulizer, 35 psi; drying gas temperature, 300 °C; sheath gas temperature, 350 °C. Nitrogen was used as sheath and drying gas at a flow rate of 8.0 and 11.0 L/min, respectively. The collision energy (CE) was set 45 V and the mass range recorded *m*/*z* 100–2000. The accurate-mass capability of the TOF analyzer allowed reliable confirmation of the identity of the detected metabolites, normally with mass errors below 5 ppm in routine analysis, which was sufficient to verify the chemical constituents in RD. An external calibration solution (Agilent calibration solution A) was continuously sprayed in the ESI source of the QTOF system, employing the ions with *m*/*z* 112.9855 (TFA anion) and 1033.9881 [HP-0921 (TFA adduct)] to recalibrate the mass axis, ensuring mass accuracy and reproducibility throughout the chromatographic run. All operations, acquisition, and analysis of data were monitored by Agilent HPLC-ESI-QTOF–MS/MS MassHunter Acquisition Software Version A.01.00 (Agilent Technologies) and operated under MassHunter Acquisition Software Version B.06.00 (Agilent Technologies).

3. Results and discussion

3.1. Optimization of LC and MS conditions

In order to obtain reliable chromatographic results and appropriate ionization, the mobile phase system, the parameters of flow rate of gas, gas pressure, spray voltage, capillary temperature and voltage of entrance potential were optimized. For the ESI-QTOF–MS/MS conditions, positive and negative ionization modes were evaluated. Finally, the conditions were obtained from the ESI negative ionization mode, which could achieve a higher response and provided more information for all compounds than the ESI positive ionization mode in MS spectra. The base peak chromatograms (BPC) of RD extract in negative ion mode are shown in Fig. 1.

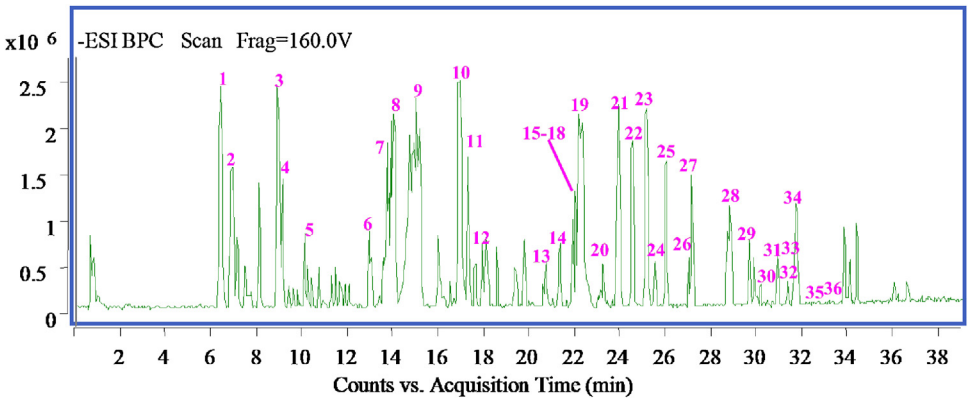

Fig. 1. The base peak chromatograms (BPC) of RD extract.