J Ginseng Res 40 (2016) 220–228

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Rapid separation and identification of 31 major saponins in Shizhu ginseng by ultra-high performance liquid chromatography–electron spray ionization–MS/MS

Ting-Ting Sun, Xin-Lei Liang, He-Yun Zhu, Xu-Ling Peng, Xing-Jie Guo*, Long-Shan Zhao

School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, PR China

ARTICLE INFO

Article history:
Received 7 April 2015
Received in Revised form
24 July 2015
Accepted 28 July 2015
Available online 5 August 2015

Keywords:
identification
saponins
separation
Shizhu ginseng
ultra-high performance liquid chromatography coupled with MS/MS

ABSTRACT

Background: Among the various ginseng strains, Shizhu ginseng is endemic to China, mainly distributed in Kuandian Manchu Autonomous County (Liaoning Province, China); however, not much is known about the compounds (especially saponins) in Shizhu ginseng.
Methods: A rapid, sensitive, and reliable ultra-high performance liquid chromatography coupled with MS/MS (UHPLC–MS/MS) method was developed to separate and identify saponins in Shizhu ginseng.
Results: The separation was carried out on a Waters ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm) with acetonitrile and 0.1% formic acid aqueous solution as the mobile phase under a gradient elution at 40°C. The detection was performed on a Micromass Quattro Micro API mass spectrometer equipped with electrospray ionization source in both positive and negative modes. Under the optimized conditions, a total of 31 saponins were identified or tentatively characterized by comparing retention time and MS data with related literatures and reference substances.
Conclusion: The developed UHPLC–MS/MS method was suitable for identifying and characterizing the chemical constituents in Shizhu ginseng, which provided a helpful chemical basis for further research on Shizhu ginseng.

1. Introduction

Panax ginseng Meyer is an important Chinese herbal medicine that has served as a rich source of natural nourishment for a long time and has become increasingly popular in China and Korea in recent years [1–3]. Shizhu ginseng as one of the excellent artificial cultivation herbs for more than 400 years of cultivation history [4,5], derived from the dry root and rhizome of *P. ginseng* Meyer and is mainly cultivated in the northeastern region of China [6]. Shizhu ginseng has the same morphological characteristics and medicinal value as wild ginseng; additionally, it also contains many different constituents such as amino acids, vitamins, ginseng polysaccharides, and dozens of saponins. Studies have shown that saponins in Shizhu ginseng play important roles in the treatment and prevention of diabetes, aging, and arteriosclerosis, etc. [7–9]. In particular, saponins such as Rg1, Re, Rf, Rg2, Rb1 are active ingredients used in the treatment of various tumors and cancers. In this paper, we attempt to separate and characterize a variety of saponins in Shizhu ginseng.

In recent years, various methods for determination of saponins in *P. ginseng*, including micellar electrokinetic capillary chromatography, HPLC, high-performance thin-layer chromatography, liquid chromatography coupled with MS (LC–MS), and LC–MS/MS (LC–MS/MS), have been well described in the literature [10–12]. Among these methods, LC–MS had been increasingly applied in studies on natural products. Compared with the common methods, application of ultra-high performance LC coupled with MS/MS (UHPLC–MS/MS) can effectively resist the interference caused by multicomponent samples, improve accuracy on the quantitative detection, and have the advantage of higher sensitivity and selectivity, which is suitable not only for the structural confirmation of known ingredients but also for the rapid identification of unknown ingredients in traditional Chinese medicine [13]. Because of these advantages, UHPLC–MS/MS has become

* Corresponding author. School of Pharmacy, Shenyang Pharmaceutical University, No. 103 Wenhua Road, Shenhe District, Shenyang 110016, Liaoning Province, PR China.
E-mail address: gxjhyz@aliyun.com (X.-J. Guo).

p1226-8453 e2093-4947/$ – see front matter
http://dx.doi.org/10.1016/j.jgr.2015.07.008

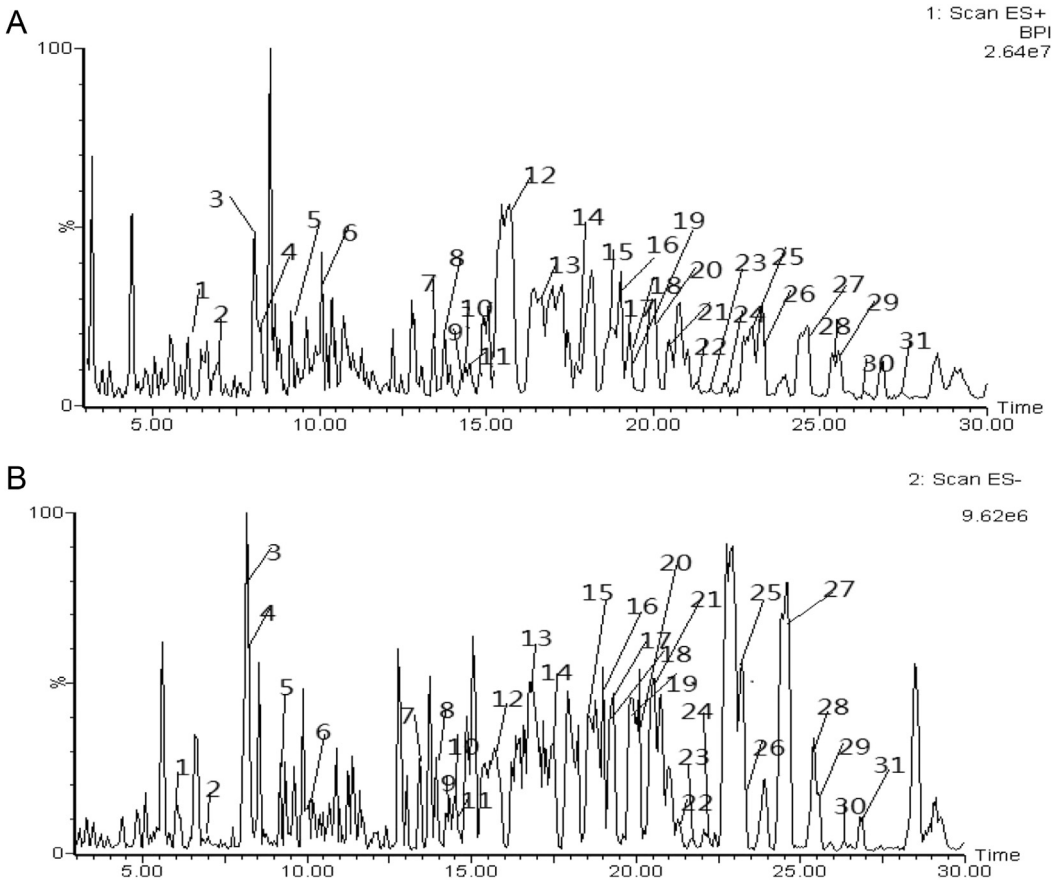

Fig. 1. Total ion current chromatogram of Shizhu Ginseng at (A) positive ionization and (B) negative ionization modes.

one of the effective technologies used for determination of saponins in ginseng. In is also widely used method for pharmaceutical analysis [14,15]. Therefore, in this paper, a UHPLC–MS/MS system equipped with an electrospray ionization source in both positive and negative modes was chosen to separate and identify saponins in Shizhu ginseng.

The aim of our study was to develop a direct and rapid UHPLC–MS/MS method for simultaneously identifying 31 saponins in Shizhu ginseng. In the full-scan mode, the structure of mother nucleuses and the molecular weight of 31 major saponins were established by analyzing the mass-to-charge ratios from $[M+Na]^+$ ions produced in the electron spray ionization-positive (ESI^+) mode and from $[M-H]^-$ ions produced in the ESI^- spectra. The structures of 29 saponins in Shizhu ginseng were determined by comparing them with reference substances, analyzing the MS/MS spectra data, and comparing the fragmentation pattern with related literatures. Our study results show that the analytic method adopted here can be used as a valuable tool for further research on Shizhu ginseng.

2. Materials and methods

2.1. Chemicals and materials

Shizhu ginseng specimens were collected from Kuandian, Liaoning. The plant was identified by Professor Jincai Lu of Shenyang Pharmaceutical University (Shenyang, Liaoning, China). The reference standards of ginseng saponins Rg1, Re, and Rb1 were purchased from the National Institute for Food and Drug Control (Beijing, China). Ginseng saponins Rb2, Rb3, Rc, Rd, Rf, Rg2, Rh1, and notoginsenoside R1 were obtained from College of Pharmacy, Jilin University (Changchun, Jilin Province, China). Acetonitrile and formic acid of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and HPLC-grade ammonium acetate was purchased from Sigma (Saint Louis, MO, USA). Other reagents were of analytical grade. Deionized water was purified using a Milli-Q system (Millipore, USA).

2.2. Instrumentation and analytical conditions

Chromatographic analysis was carried out on an ACQUITY ultra-high performance liquid chromatography column (Waters, USA) equipped with a quaternary pump, a vacuum degasser, an auto-sampler, and a diode array detector. Separation was achieved on an ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm; Waters, USA) with the column temperature maintained at 40°C. The mobile phase consisted of acetonitrile (A) and 0.1% formic acid in water (B), with a flow rate of 0.25 mL/min. The gradient elution program was as follows: 0–5.0 min, 20–20% A; 5–10 min, from 20% to 30% A; 10–30 min, from 30% to 35% A. The Photo-Diode Array (PDA) spectrum was recorded from 200 nm to 400 nm.

Mass spectrometric detection was performed on a Micromass Quattro Micro API mass spectrometer equipped with an ESI source operating in both positive- and negative-ion modes. The optimal MS parameters were as follows: capillary voltage, 2.9 kV; cone voltage, 50 V; cone gas flow, 300 L/h; ion-source temperature,