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Detection of saponins in extract of *Panax notoginseng* by liquid chromatography–electrospray ionisation-mass spectrometry

Li Li[a], Rong Tsao[b], Jianpeng Dou[a], Fengrui Song[a], Zhiqiang Liu[a,*], Shuying Liu[a,*]

[a] *Laboratory of New Drug Research and Mass Spectrometry, Changchun Institute of Applied Chemistry, Graduate School of the Chinese Academy of Science, Changchun 130022, PR China*
[b] *Food Research Program, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ont., Canada N1G 5C9*

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Abstract

The liquid chromatography–electrospray ionisation-mass spectrometry (LC–ESI-MS) method was developed for the analyses and identification of saponins in plant extract from the root of *Panax notoginseng* (Burk.) F.H. Chen. The HPLC experiments were proceeded by means of a reversed-phase C18 column and a binary mobile phase system consisting of 0.2% acetic acid and acetonitrile under gradient elution conditions. Eight major peaks were separated and detected using both evaporative light scattering and MS detectors. The mass spectrometer was operated in the negative ion mode using electrospray ionization. The molecular ions, $[M-H]^-$ and the adduct ions $[M+AcO]^-$ of saponins were observed, and from which the molecular weights were obtained. A collision-induced dissociation (CID) experiment was carried out to aid the identification of the backbone and glycosidic linkage sites of the saponins. The identification of the saponins (peaks 1–7) in the extract of *P. notoginseng* was based on matching their retention times, the detection of the saponin molecular ions, and the fragment ions of the molecular ion obtained in the CID experiments with those of the authentic standards and data reported in the literature. The molecular structure of peak 8 was elucidated according to the fragmentation patterns and the literature reports.

Keywords: *Panax notoginseng*; Saponins; LC–ESI-MS; CID

1. Introduction

Panax notoginseng (Burk.) F.H. Chen (Chinese name: SanQi or TianQi) is a traditional Chinese medicine (TCM) that has been used to treat cardiovascular diseases, different pains, bruise, and hemostasia [1–3] in China. The major bioactive constituents of *P. notoginseng* are dammarane saponins such as ginsenosides and notoginsenosides [4,5], which are usually obtained from the roots of this plant. The structures of saponins from *P. notoginseng* studied in this investigation are showed in Fig. 1.

The profile of the saponins in *P. notoginseng* was similar to those in *Panax ginseng* and *Panax quinquefolium* [4,5]; however, compared to the many reported methods on *P. ginseng* and *P. quinquefolium*, analytical methods using high performance liquid chromatography (LC) for *P. notoginseng* are scarce. UV has been the detector of choice for the detection of saponins in *P. notoginseng* in reported LC methods, however due to poor absorbance of these compounds in the UV region, the detectors are often set at 198–205 nm, which greatly increases the baseline noise and lowers the sensitivity of the detection [6–8]. Some methods using LC–evaporative light scattering detection (LC–ELSD) have been found to provide a stable baseline even with a gradient elution and have been successfully applied to the analysis of saponins in *P. ginseng* [9–11]. Only one paper has reported on the analysis of saponins from *P. notoginseng* using LC–ELSD [12].

* Corresponding authors. Tel.: +86 431 526 2613 (Z. Liu); fax: +86 431 526 2236 (Z. Liu).
E-mail address: liuzq@ciac.jl.cn (Z. Liu).

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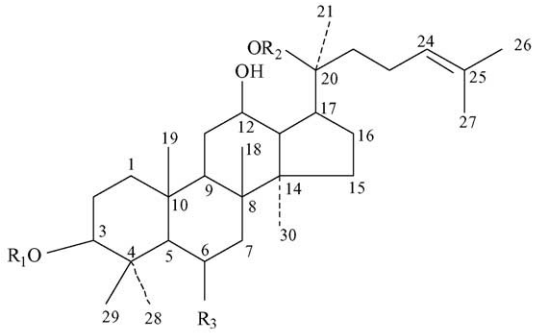

saponins	R1	R2	R3	Molecular mass
Notoginsenoside R1	-H	-Glc	-O-Glc $\frac{2\text{-}1}{}$ Xyl	932
Ginsenoside Rg1	-H	-Glc	-O-Glc	800
Ginsenoside Re	-H	-Glc	-O-Glc $\frac{2\text{-}1}{}$ Rha	946
Ginsenoside Rb1	-Glc $\frac{2\text{-}1}{}$ Glc	-Glc $\frac{6\text{-}1}{}$ Glc	-H	1108
Ginsenoside Rc	-Glc $\frac{2\text{-}1}{}$ Glc	-Glc $\frac{6\text{-}1}{}$ Ara(f)	-H	1078
Ginsenoside Rb2	-Glc $\frac{2\text{-}1}{}$ Glc	-Glc $\frac{6\text{-}1}{}$ Ara(p)	-H	1078
Ginsenoside Rd	-Glc $\frac{2\text{-}1}{}$ Glc	-Glc	-H	946
Notoginsenoside K	-Glc $\frac{6\text{-}1}{}$ Glc	-Glc	-H	946

Abbreviations: Glc, β-D-glucose; Ara(p), arabinose in pyranose form; Ara(f), arabinose in furanose form; Rha, α-L-rhamnose; Xyl, β-D-xylopyranosyl

Fig. 1. Structures of saponins in *P. notoginseng* identified in this study.

Among various methods that have been applied to the analysis and identification of ginsenosides from the extract of *P. ginseng* and *P. quinquefolium*, LC–MS appears to be most favorable and capable [10,13–15]. Only one paper reported the analysis the saponins in *P. notoginseng* using LC–MS in the positive ion mode, however, the method provided only limited structural information of saponins [6].

In the present paper, a simple, direct and reliable LC–ESI-MS method for the identification of saponins in the crude extract from the root of *P. notoginseng* was reported. Structural information of the saponins was obtained by using the collision-induced dissociation (CID) technique.

2. Experimental

2.1. Reagents and materials

Acetonitrile and methanol were of HPLC grade from Fisher Chemicals (USA), the other reagents were of analytical grade from Beijing Chemicals (China). Water was purified using a Milli-Q water purification system (Millipore, France). Ginsenosides Re, Rg1, Rb1, Rb2, Rc, Rd and notoginsenoside R1 were purchased from Jilin University (China). *P. notoginseng* root powder was purchased from Beijing TongRenTang Medicinal Store (China).

2.2. Preparation of samples

P. notoginseng root powder (1.0062 g) was immersed in 50 mL of methanol and extracted using ultrasonication for 1 h at room temperature. The mixture was filtered through a Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England), and the filtrate was evaporated to dryness using a rotary evaporator at <40 °C. The residue was then dissolved in 10 mL methanol and filtered through a 0.45 μm membrane before being used for the LC–ESI-MS analyses.