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Short communication

Simultaneous analysis method for polar and non-polar ginsenosides in red ginseng by reversed-phase HPLC-PAD

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ABSTRACT

The paper describes the development of a simultaneous determination method for polar and non-polar ginsenosides in red ginseng with a reversed-phase high-performance liquid chromatography-pulsed amperometric detection method. This method could be applied directly without any pretreatment steps and enabled the performance of highly sensitive analysis within 1 h. The detection ($S/N = 3$) and quantification ($S/N = 10$) limits for the ginsenosides ranged 0.02–0.10 ng and 0.1–0.3 ng, respectively. The linear regression coefficients ranged 0.9975–0.9998. Intra- and inter-day precisions were <9.91%. The mean recoveries ranged 98.08–103.06%. The total amount of ginsenosides in the hairy root of red ginseng was higher than that in the main root.

1. Introduction

Ginseng, the root of *Panax ginseng*, is widely used in Chinese traditional medicine and contains diverse polar ginsenosides (triterpene saponins) as active ingredients. Red ginseng is generated from ginseng through an additional steaming and drying step. During the steaming process, some of the polar ginsenosides are transformed into non-polar ginsenosides by hydrolysis of the sugar moieties. Consequently, non-polar ginsenosides are newly produced from polar ginsenosides and exist only in red ginseng. As such, the name provided by the Korean Pharmacopoeia (IX) for red ginseng (Ginseng Radix Rubra) is different from that for ginseng (Ginseng Radix Alba).

Polar and non-polar ginsenosides have diverse biologically beneficial activities, such as neuroprotective (G-Rb_1, G-Rd, G-Rg_1, G-Rg_2, G-Rg_3) [1–4], anti-cancer (G-Rb_2, G-Rg_3, G-Rh_2) [5–8], cardio-protective (G-Rb_3) [9], anti-diabetic (G-Rc) [10], anti-nociception (G-Rf) [11], anti-oxidant (G-Re, G-Rg_3, G-Rh_1, G-Rh_2) [12–15], vasodilating (G-Rg_3) [16], and hepatoprotective effects (G-Rg_3, G-Rh_2) [17]. Red ginseng contains typical non-polar ginsenosides such as ginsenoside Rg_2 (G-Rg_2), G-Rg_3, G-Rh_1, and G-Rh_2, as well as polar ginsenosides such as G-Rb_1, G-Rb_2, G-Rb_3, G-Rc, G-Rd, G-Rf, G-Rg_1, and G-Re [18,19]. The chemical structures of these various ginsenosides are shown in Fig. 1.

The separation of ginsenosides on various functional columns was unsuccessful because of the significant difference in polarity between polar and non-polar ginsenosides. Therefore, analytical methods for ginsenosides have mainly been developed specifically for polar [20–23] or non-polar ginsenosides [24]. HPLC-evaporate light scattering detection (HPLC-ELSD) is a general method for detecting both polar and non-polar ginsenosides [25]. However, it could not be used to detect the small quantity of non-polar ginsenosides in red ginseng because of its limited detectability (0.3 μg for G-Rg_2, 0.3 μg for G-Rh_1, 0.1 μg for G-Rh_2). Charged aerosol detection (CAD) method introduced as an alternative to ELSD, had better sensitivity, wider dynamic range, ease of use, and constancy of response factors than ELSD method [26]. And there was enzyme-linked immunosorbent assay (ELISA) method as a way to detect ginsenosides [27,28]. However, they could not also solve the problem, such as limited detectability for non-polar ginsenosides. A sensitive and quantitative method to simultaneously identify polar and non-polar ginsenosides in red ginseng is therefore needed.

Pulsed amperometric detection (PAD) is an electrochemical detection method that measures the positive potential produced by carbohydrate oxidation on a gold electrode, allowing the direct detection of carbohydrates at low pico-mol levels [29]. High-performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) has been used to quantify carbohydrates in plant resources [30–32] because of its strong

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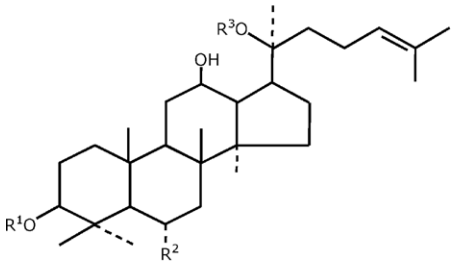
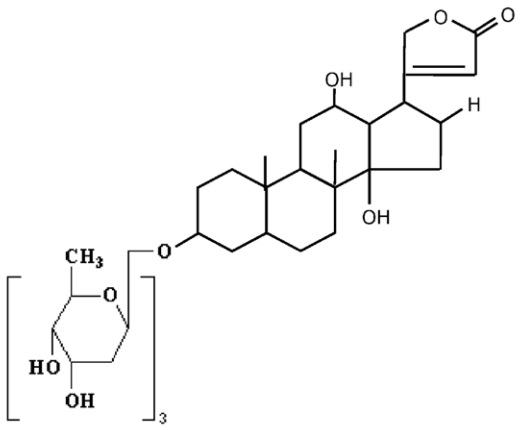


20(S)-protopanaxadiol type
R^2=H

	R^1	R^3
Rb_1	-Glc^2-Glc	-Glc^6-Glc
Rb_2	-Glc^2-Glc	-Glc^6-Ara(p)
Rb_3	-Glc^2-Glc	-Glc^6-Xyl
Rc	-Glc^2-Glc	-Glc^6-Ara(f)
Rd	-Glc^2-Glc	-Glc
Rg_3	-Glc^2-Glc	-H
Rh_2	-Glc	-H

20(S)-protopanaxatriol type
R^1= -H, R^2= -O-R^4

	R^3	R^4
Re	-Glc	-Glc^2-Rha
Rf	-H	-Glc^2-Glc
Rg_1	-Glc	-Glc
Rg_2	-H	-Glc^2-Rha
Rh_1	-H	-Glc



Digoxin

Fig. 1. Chemical structures of ginsenosides and digoxin (I.S.).

anion-exchange properties that efficiently separate carbohydrates. However, HPAEC-PAD has rarely been applied to the analysis of glycosides because of the difficulty in separating glycosides from sugars on an anion exchange column.

We have developed two different reversed-phase methods of HPLC-PAD (RP-HPLC-PAD) for polar [33] or non-polar ginsenosides [24]. However, simultaneous separation of the polar and non-polar ginsenosides remains difficult. Such separations result in a chromatogram with various problems, such as severely overlapping ginsenoside peaks and long analysis times when changes in the eluent compositions or gradient system are applied. The present study describes a simultaneous determination method for polar and non-polar ginsenosides in red ginseng through the optimization of several analytical conditions. This new method could be applied directly without pretreatment or extraction steps and enabled highly sensitive analysis within 1 h.

2. Experimental

2.1. Materials

Crude drugs were purchased from the *Kyungdong* Market (Seoul, South Korea) in accordance with the standards stipulated in Korea Pharmacopoeia (VIII). The main roots of 6-year-old red ginseng (MR-6) and the hairy roots of 6-year-old red ginseng (HR-6) were purchased from a local drug store. HPLC-grade acetonitrile and 50% sodium hydroxide were purchased from Fisher Scientific (Fairlawn, NJ, USA). All other reagents and solvents used were of guaranteed or analytical grade.

G-Rg_3, G-Rh_2, G-Rg_2, G-Rh_1, G-Rb_2, G-Rb_3, and G-R_f were purchased from ChromaDex (Santa Ana, CA, USA). G-Rb_1, G-Rc, G-Rd, G-Re, and G-Rg_1 were purchased from Wako (Tokyo, Japan). Digoxin was purchased from Toronto Research Chemicals (Toronto, Canada). A Millipore membrane filter (type HA, pore size 0.45 μm) was used for solvent filtration. All samples were filtered through disposable syringe filters (Hydrophobic PTFE, pore size 0.20 μm, Advantec MFS, Tokyo, Japan) before injection. Standard solutions, sample solutions, and mobile phase were prepared using 18 MΩ purified water produced by an Automatic Aquarius AW-1001 water purification system (Top Trading, Seoul, South Korea).

2.2. Apparatus and high-performance liquid chromatography

A PAD system from the ICS-3000 series Dionex (Sunnyvale, CA, USA) was equipped with an Au-Flowcell containing a gold working electrode and a solvent-compatible cell containing an Ag/AgCl reference electrode. The gold electrode was cleaned by rubbing the surface twice a week with the pink eraser (Dionex P/N. 049721) provided in the polishing kit. HPLC equipment, consisting of a Model Nanospace SI-2/3201 pump and a 3004 column oven, was purchased from Shiseido (Tokyo, Japan). The Nanospace SI-2/3201 pump has a metal-free head made of polyetheretherketone (PEEK) resin, which resists aggressive chemicals such as alkaline solutions.

Chromatographic separation was performed using a Kinetex C-18 column (100 mm × 2.1 mm I.D.; 2.6 μm, Phenomenex, Torrance CA) or a Unison UK-C-18 column (150 mm × 2.0 mm I.D.; 3 μm, Imtakt, Kyoto, Japan). The potential waveform was as follows: E1 = −0.2 V (from 0.00 to 0.04 s); E2 = 0 V (from 0.05 to 0.21 s); E3 = +0.22 V (from 0.22 to 0.46 s); E4 = 0 V (from 0.47 to 0.56 s); E5 = −2 V (from 0.57 to 0.58 s); and E6 = +0.6 V (0.59 s). The mobile phase consisted of 10% (v/v) acetonitrile (solvent A) and 85% (v/v) acetonitrile (solvent B).