

Customer Application Note

Determination of Ginsenosides in *Panax ginseng* by HPLC-CAD

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Introduction

Asian ginseng (*Panax ginseng*) traditionally is used as a tonic to reduce the effects of stress, counteract fatigue, and increase stamina. The main bioactive ingredients found in *Panax ginseng* and a related species, *Panax quinquefolius* (American ginseng), are triterpene saponins, commonly referred to as ginsenosides. There are seven major ginsenosides present in *Panax ginseng*: the protopanaxatriols (Rg1, Re, and Rf) and the protopanaxadiols (Rb1, Rc, Rb2, and Rd). *Panax quinquefolius* contains the same ginsenosides, with the exception of Rf.

Most methods for the analysis of ginsenosides use gradient elution reversed-phase high-performance liquid chromatography (RP-HPLC) with low-wavelength UV detection (203–205 nm) because the ginsenosides do not strongly absorb above 205 nm.¹ This often results in strongly sloping baselines that complicate integration, and interferences from minor components that have stronger UV chromophores than the ginsenosides.

The Thermo Scientific Dionex Corona™ CAD™ Charged Aerosol Detector offers an alternative to low-wavelength UV detection. Charged aerosol detection does not overrespond to strong chromophores, reducing interferences. Baseline slopes are not as pronounced or as variable, making peak area determinations more reliable.

Equipment

A Thermo Scientific Dionex Summit* HPLC system including:

- P680 pump
- ASI-100 autosampler
- TCC-100 column compartment

*Company Note: The Dionex Summit system has been discontinued, so the company would recommend using a Thermo Scientific Dionex UltiMate™ 3000 system.

- Corona CAD detector
- Fused-Core® C18 HPLC column, 3.0 × 100 mm, 2.7 μm particle size
- Sonication bath
- Polyvinylidene fluoride (PVDF) syringe filters, 0.22 μm

Standards

Ginsenosides Rb1 and Rg1 – Cogon Bio-tech Co., Ltd.
Ginsenosides Rb2, Rc, Rd, and Re – INDOFINE Chemical Company, Inc

Sample

Panax ginseng powdered extract

Solvents and Reagents

Methanol
Water
Acetonitrile
Diluent: 30:70 (v/v) methanol: water

Note: All solvents/reagents must be of HPLC-grade quality

Calibration Solutions Preparation

Weigh and transfer 5 mg (±0.5 mg) of each ginsenoside standard into a 25 mL volumetric flask. Dissolve the standard in the diluent with sonication, and dilute to volume. Prepare serial dilutions from this stock standard solution in the diluent as follows:

Calibration Solution	Volume of Stock Solution (mL)	Final Volume (mL)	Approx. Conc. Each Ginsenoside (μg/mL)
Stock #1	NA	NA	200
Stock #2	5	10	100
Stock #3	2	25	16
Stock #4	2	50	8
Stock #5	1	50	4

Sample Solution Preparation

Weigh and transfer ~400 mg of sample extract into a 100 mL volumetric flask. Add 15 mL of methanol and sonicate the flask for 10 min with occasional shaking. Add ~60 mL of water and sonicate the flask for an additional 10 min. Sonication will naturally heat the sample, so allow to cool to room temperature. Fill the flask to volume with water and mix well. Filter a portion of the solution through a 0.2 μm PVDF syringe filter into an HPLC autosampler vial.



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Chromatographic Conditions

Column: Fused-Core C18 HPLC, 3.0 × 100 mm,
2.7 µm particle size
Mobile Phase: A: Water
B: Acetonitrile
Gradient: 15% B to 35% B in 30 min
Flow Rate: 0.67 mL/min
Inj. Volume: 20 µL
Column Temp.: 30 °C
Detection: CAD

Procedure

Inject each calibration solution, then follow with duplicate injections of the sample solution. Inject each calibration solution again after the sample solution injections.

Results and Discussion

Due to the broad concentration range of the calibration standards (almost two orders of magnitude), a quadratic calibration curve was used for all of the ginsenosides. The six ginsenosides present in the standard solution were quantified in the extract sample. Figure 1 shows the calibration curve for ginsenoside Rb1 on the Corona CAD detector. Figures 2 and 3 show the calibration standard and sample solution chromatograms, respectively. The lowest calibration standard solution (~4 µg/mL each ginsenoside) showed excellent signal-to noise ratios. There was virtually no baseline drift in the chromatograms due to mobile phase gradient. In addition, minor components with stronger UV chromophores that cause interferences that affect the quantitation of ginsenosides Rb1 and Rc when using UV detection were minimized when using the Corona CAD detector.

Table 1 shows the results of the ginsenoside analysis.

Table 1. Results of the Ginsenoside Analysis	
Ginsenoside	Amount (%)
Rg1	0.0895
Re	1.16
Rb1	2.36
Rc	0.402
Rb2	0.0505
Rd	0.618
Total	4.68

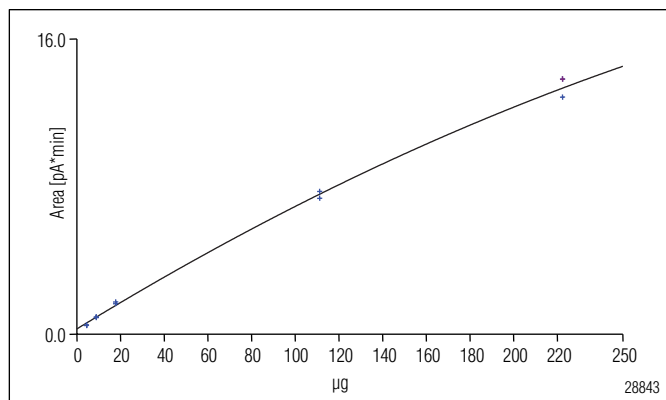


Figure 1. Ginsenoside Rb1 calibration curve using the Corona CAD detector.

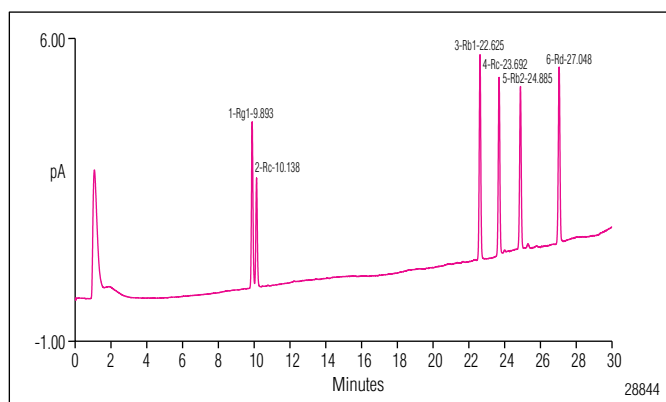


Figure 2. Calibration Solution #5 chromatogram (~4 µg/mL of each ginsenoside).

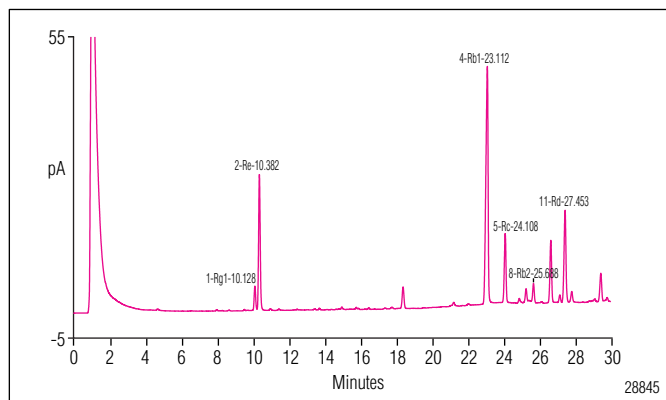


Figure 3. Panax ginseng sample chromatogram.

Conclusion

Low-wavelength UV detection commonly used for ginsenoside analysis often results in strongly sloping baselines, and interferences from minor components can adversely affect quantitation. Charged aerosol detection minimizes these interferences and improves the baseline, improving quantitation of ginsenoside content in ginseng extracts and preparations.

Reference

1. Thermo Fisher Scientific. Dionex Application Note 192: Rapid Analysis of Ginseng Using Accelerated Solvent Extraction and High Performance Liquid Chromatography. LPN 1965, Sunnyvale, CA, 2007.

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