Determination of Triterpenes in *Centella asiatica* (Gotu Kola) by HPLC-CAD

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Introduction

Centella asiatica (commonly called gotu kola) is a small herbaceous annual plant native to India, Sri Lanka, Northern Australia, other parts of Asia, and the Western Pacific. It is used as a medicinal herb in Ayurvedic medicine and in traditional Chinese medicine to treat a wide variety of conditions, such as improving memory, blood flow, as a wound-healing agent, and as a topical application for skin conditions such as ulcers, wounds, and eczema.

The chemical compounds of interest in gotu kola are usually considered to be the ursane- and oleanane-type triterpenes and the triterpene glycosides including asiaticoside, madecassoside (asiaticoside A), asiaticoside B, madecassic acid, asiatic acid, and terminolic acid (Figures 1–2).

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

The Thermo Scientific Dionex Corona™ CAD™ Charged Aerosol Detector offers an alternative to low-wavelength UV detection to improve the baseline and reduce interferences. Here, chromatograms generated using UV and Corona CAD detection are compared, and the assay of triterpene content in a *Centella asiatica* extract sample using the Corona CAD detector is shown.

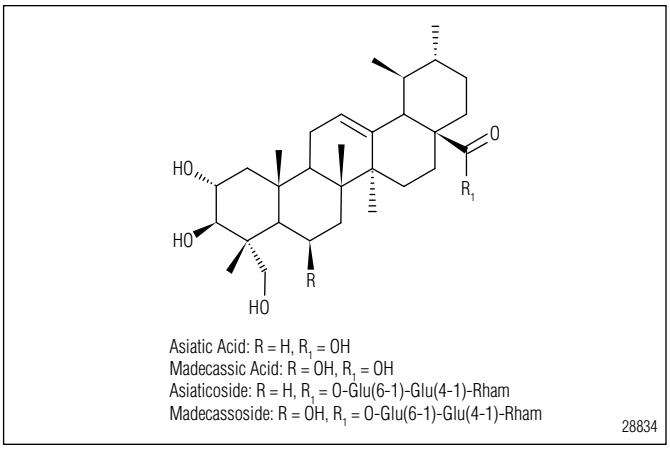

Figure 1. Structures of ursane-type triterpenes.

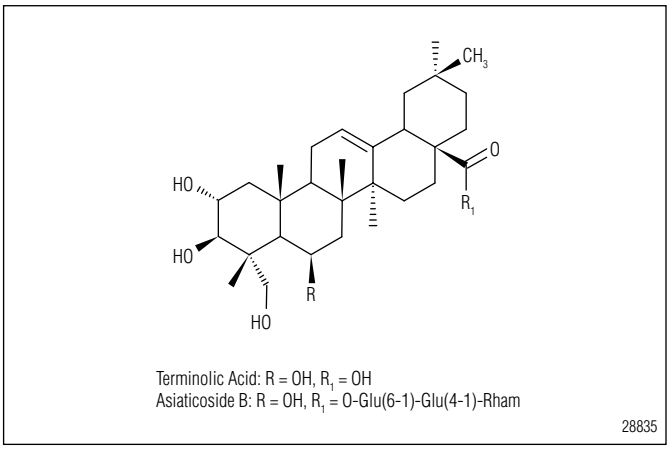

Figure 2. Structures of oleanane-type triterpenes.

Equipment

A Thermo Scientific Dionex Summit* HPLC system including:
- P680 pump
- ASI-100 autosampler
- TCC-100 column compartment

Corona CAD detector
Fused-Core® C18 HPLC column, 3.0 × 100 mm, 2.7 µm particle size
Sonication bath
Polytetrafluoroethylene (PTFE) syringe filters, 0.22 µm
**Company Note: The Dionex Summit system has been discontinued, so the company would recommend using a Thermo Scientific Dionex UltiMate™ 3000 system.*

Standards

Asiatic acid - LKT Laboratories
Madecassic acid - LKT Laboratories
Asiaticoside - PhytoLab
Madecassoside - PhytoLab

Sample

Centella asiatica powdered extract

Solvents and Reagents

Methanol
Water
Acetonitrile
Formic acid
Note: All solvents/reagents must be of HPLC-grade quality

Calibration Solutions Preparation

Weigh and transfer 10 mg (±1 mg) of each of the triterpene standards into a 50 mL volumetric flask. Dissolve the standard in methanol with sonication, and dilute to volume. Prepare serial dilutions from this stock standard solution in methanol as follows:

Calibration Solution	Volume of Stock Solution (mL)	Final Volume (mL)	Approx. Conc. of 27-Deoxyactein (µg/mL)
Stock #1	NA	NA	200
Stock #2	5	10	100
Stock #3	5	25	40
Stock #4	5	50	20
Stock #5	2	50	8

Sample Solution Preparation

Weigh and transfer ~250 mg of sample extract into a 100 mL volumetric flask. Add ~70 mL of methanol and sonicate the flask for 10 min with occasional shaking. Sonication will naturally heat the sample. After cooling to room temperature, fill the flask to volume with methanol and mix well. Filter a portion of the solution through a 0.2 µm polytetrafluoroethylene (PTFE) syringe filter into an HPLC autosampler vial.

Chromatographic Conditions

Column:	Fused-Core C18 HPLC, 3.0 × 100 mm, 2.7 µm particle size
Mobile Phase:	A: 0.1% Formic acid in water
	B: Acetonitrile
Gradient:	18% B to 22% B in 8 min
	22% B to 45% B from 8–17 min
	045% B to 80% B from 17–23 min
Flow Rate:	0.64 mL/min
Inj. Volume:	5 µL
Column Temp.:	35 °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

The quadratic calibration curve was used for all the triterpenes. Asiaticoside B was quantified against madecassoside, and terminolic acid was quantified against madecassic acid. Figure 3 shows the calibration curve for madecassic acid on the Corona CAD detector.

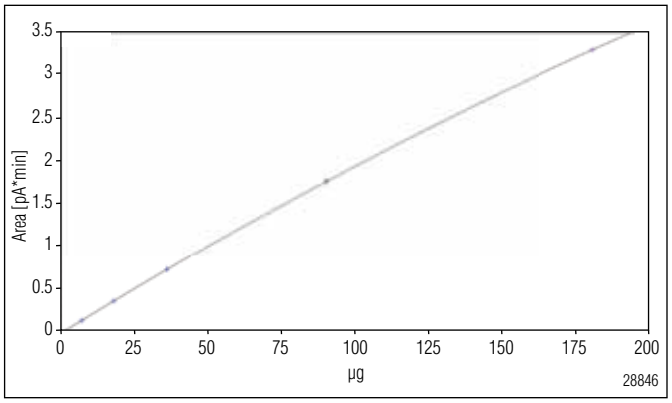

Figure 3. Madecassic acid calibration curve using the Corona CAD detector.

Figures 4 and 5 show the calibration standard and sample solution chromatograms, respectively. Figure 6 shows overlay chromatograms of the gotu kola sample solution obtained using the Corona CAD detector and UV detection. Minor components with stronger UV chromophores coelute with many of the triterpines, especially asiaticoside B, madecassoside, madecassic acid, and terminolic acid, reducing the accuracy of quantitation (chromatogram B). These interferences were minimized when using the Corona CAD detector (chromatogram A). The changing slope of the UV baseline can also adversely affect quantitation. For example, between 10 and 15 min, the rising UV baseline will complicate quantitation of asiaticoside, whereas the Corona CAD baseline is more level in that region.

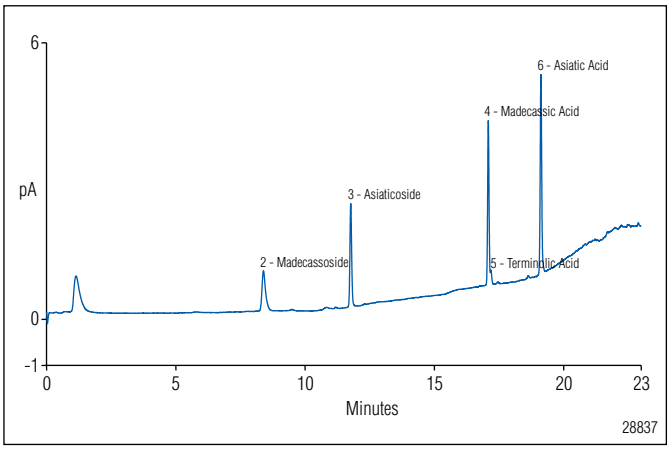

Figure 4. Calibration Solution #5 chromatogram (~8 µg/mL of each triterpene).

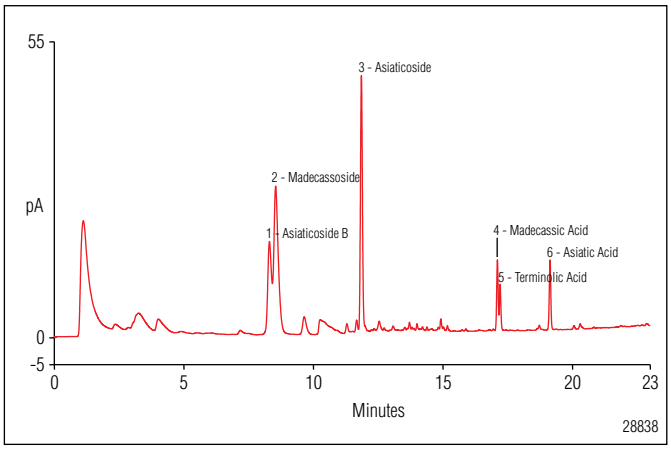

Figure 5. Centella asiatica *sample chromatogram.*

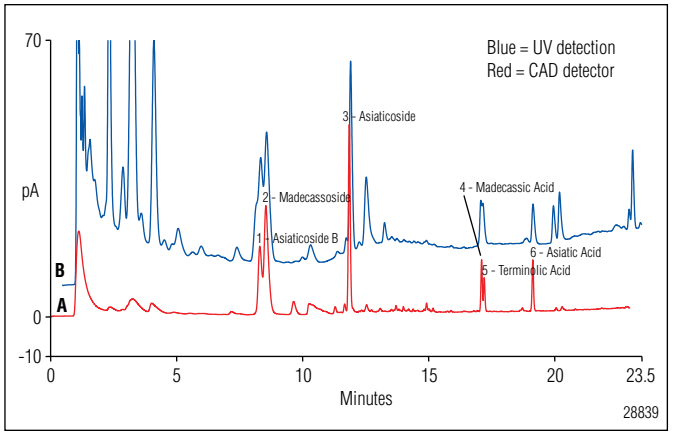

Figure 6. Overlay of chromatograms using the A) Corona CAD detector and B) UV detection.

Table 1 shows the results of the triterpene analysis.

Table 1. Results of the Triterpene Analysis

Triterpene	Amount (%)
Asiaticoside B	5.67
Madecassoside	13.4
Asiaticoside	10.7
Madecassic Acid	1.147
Terminolic Acid	0.744
Asiatic Acid	0.908
TOTAL	32.6

Conclusion

Low-wavelength UV detection commonly used for triterpene 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, as shown in Figure 6, enabling improved quantitation of triterpene content in gotu kola.