HPLC Analysis of Six Active Components of Caulis Lonicerae Using a Phenyl-1 Column

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Application Note 299

Key Words
Pharmacopoeia of the People's Republic of China (PPRC), Traditional Chinese Medicine, Natural Iridoid Glycosides, Natural Organic Acids, Flavonoids

Introduction

Caulis Lonicerae (Figure 1), the dried rattan of *Lonicera japonica* (Caprifoliaceae or honeysuckle family), is an important traditional Chinese medicine used for the treatment of such ailments as acute fever, headache, respiratory infection, and epidermic diseases. The major active components in Caulis Lonicerae are loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin.[1] Their structures are shown in Figure 2. The Pharmacopoeia of the People's Republic of China (PPRC) 2010 regulates Caulis Lonicerae with different high-performance liquid chromatography (HPLC) methods for the determination of loganin (using a phenyl stationary phase) and chlorogenic acid (using a C18 phase), respectively.[2] Therefore, the PPRC quality control (QC) protocols for Caulis Lonicerae are inconvenient (requiring two methods) and inadequate (determining only two target components). Although there are other methods to determine sweroside, caffeic acid, rutin, galuteolin, and up to three other minor active components of Caulis Lonicerae on a C18 stationary phase using HPLC,[3–5] these methods require long separation times (≥ 25 min) and have insufficient peak resolution between loganin and sweroside.

Goal

To develop an efficient and comprehensive HPLC QC method for the analysis of Caulis Lonicerae. This method must separate the six main active components (loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin).

Figure 1. *Caulis Lonicerae*: A) *Lonicera japonica*, and B) dried rattan.

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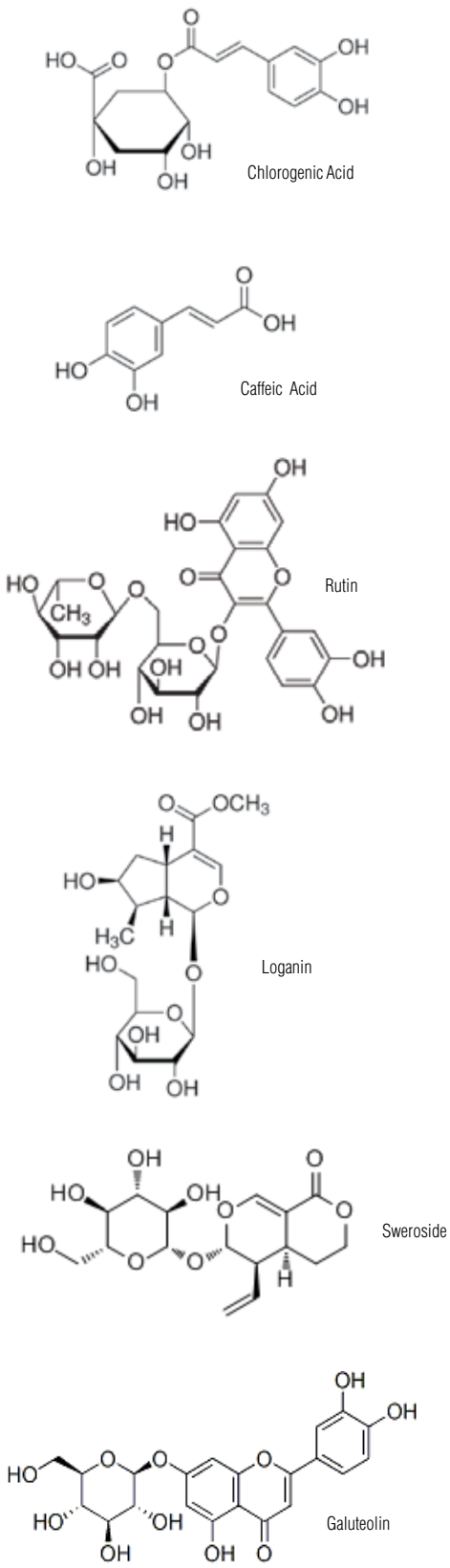

Figure 2. Structures of the six major active components of Caulis Lonicerae.

Experimental

A Thermo Scientific Acclaim Phenyl-1 4.6 × 150 mm, 3 µm column (P/N 071969) was chosen for this separation because its phenyl groups are structurally similar to the phenyl groups and aromatic structures contained in the compounds of interest. The separation of a Caulis Lonicerae sample can be completed within 10 min, and the resolution between loganin and sweroside in 5.5 min using a 0.4% formic acid/acetonitrile mobile phase.

Equipment

- Thermo Scientific Dionex UltiMate 3000 HPLC system including:
  - DGP-3600 Dual Ternary Rapid Separation (RS) Pump System
  - SRD-3600 Integrated Solvent and Degasser Rack
  - WPS-3000TRS Well Plate Sampler, Thermostatted
  - TCC-3000RS Thermostatted Column Compartment
  - DAD-3000RS Diode Array Detector (with a 5 µL semianalytical flow cell)
- Thermo Scientific Dionex Chromeleon 6.80 SR9 Chromatography Data System (CDS) software or higher
- Kudos SK3200LH Ultrasonic Generator, Kudos Ultrasonic Instrumental Co., Shanghai, China

Reagents and Standards

Deionized (DI) water, 18.2 MΩ–cm resistivity

Acetonitrile (CH_3CN), HPLC grade, (Cat.#AC610010040), Fisher Chemical

Methanol (CH_3OH), HPLC grade, Cat.# AC610090040), Fisher Chemical

Formic acid (FA), analytical grade, SCRC, China

Loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin, purity ≥ 98%, Research Center of Standardization of CTM, Shanghai, China

Sample Preparation

The Caulis Lonicerae sample was purchased from the Research Center of Standardization of CTM, Shanghai, China.

Accurately weigh 0.2 g of sample powder and place in a 10 mL volumetric flask. Add 10 mL methanol/water (1:1, v/v). After 45 min in an ultrasonic bath, cool to room temperature. Filter it through a 0.22 µm membrane (Millex-LH®) prior to injection.

Results and Discussion

Effect of Column Temperature on the Separation of Caulis Lonicerae Active Components

The effect of column temperature was evaluated for the separation of loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin using an Acclaim™ Phenyl-1 column. Figure 3A shows the separation of the standards on the column, with temperature changing from 25–45 °C in five degree steps. As expected, increasing the column temperature decreased retention time, and baseline separations of the standards were achieved in the temperature range. However, the investigation of samples shown in Figure 3B demonstrated that changing column temperature could cause some other compounds to interfere with detection of the analytes. For example, increasing column temperature could improve the resolution of loganin (Peak 1), and decrease that of sweroside (Peak 2). Therefore, an operation temperature at 30 °C was chosen as a compromise between speed and resolution. Table 1 lists the calculated peak purity match factors (the corresponding value for 100% purity is 1000) for loganin, sweroside, chlorogenic acid, and caffeic acid (the four compounds found in the sample extract), demonstrating the good separation for the sample at a column temperature of 30 °C.

Method Performance (Reproducibility, Linearity, and Detection Limits)

The method reproducibility was estimated by making consecutive injections of a Caulis Lonicerae sample mixed with loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin standards. Excellent RSDs for retention time and peak area were obtained, as shown in Table 2.

Column: Acclaim Phenyl-1 (4.6 × 150 mm, 3 µm, P/N 071969)
Mobile Phase: A - 0.4% formic acid aqueous (v/v), B - acetonitrile
Gradient: -2–0 min, 17% B; 0–4 min, 17–30% B; 4–6 min, 30–45% B; 6–10 min, 45% B
Flow Rate: 1.0 mL/min
Inj. Volume: 5 µL
Detection: UV, 236 nm
Scans at Different Column Temperature: a. 25 °C, b. 30 °C, c. 35 °C, d. 40 °C, e. 45 °C

Peaks: 1. loganin, 2. sweroside, 3. chlorogenic acid, 4. caffeic acid, 5. rutin, 6. galuteolin

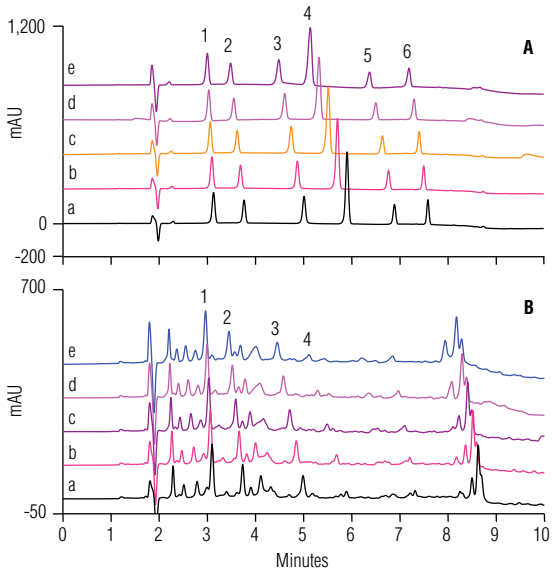

Figure 3. Chromatograms of A) a mixture of standards (10 µg/mL each), and B) a Caulis Lonicerae sample on an Acclaim Phenyl-1 column at different column temperatures.

Table 1. Calculated peak purity match factors.*

Analyte	10% Height of Peak Front	50% Height of Peak Front	50% Height of Peak Tail	10% Height of Peak Tail
Loganin	899	997	998	892
Sweroside	891	996	994	880
Chlorogenic Acid	824	996	987	781
Caffeic Acid	470	952	988	370

Note: * The corresponding value for 100% purity is 1000.

Table 2. Reproducibility for peak retention time and area.

Analyte	Retention Time RSD	Peak Area RSD
Loganin	0.136	0.335
Sweroside	0.087	0.407
Chlorogenic Acid	0.056	0.756
Caffeic acid	0.094	0.399
Rutin	0.054	0.376
Galuteolin	0.018	0.646

Calibration linearity for the six compounds was investigated by making five consecutive injections of a mixed standard prepared at seven different concentrations, 1.0, 2.0, 5.0, 10, 20, 50, and 100 mg/L. The external standard method was used to establish the calibration curve and to quantify the amounts of the six active components in samples. Table 3 reports the data from the calibration as calculated by the Chromeleon™ CDS software.

MDLs were calculated using the single-sided Student's test method (at the 99% confidence limit). Using seven consecutive injections of a Caulis Lonicerae sample mixed with loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin standards, the testing determined the standard deviation values for calculating MDLs, also reported in Table 3.

Table 3. Method linearity data and MDLs.

Analyte	Regression Equation	r	Range (mg/L)	MDL (mg/L)
Loganin	Y = 0.1309 X – 0.1541	0.9996	1.0–100.0	0.37
Sweroside	Y = 0.0950 X – 0.1179	0.9996		0.39
Chlorogenic Acid	Y = 0.1499 X – 0.2366	0.9992		0.81
Caffeic Acid	Y = 0.3463 X – 0.4300	0.9996		0.30
Rutin	Y = 0.0800 X – 0.1156	0.9993		0.35
Galuteolin	Y = 0.0887 X – 0.1525	0.9984		0.64

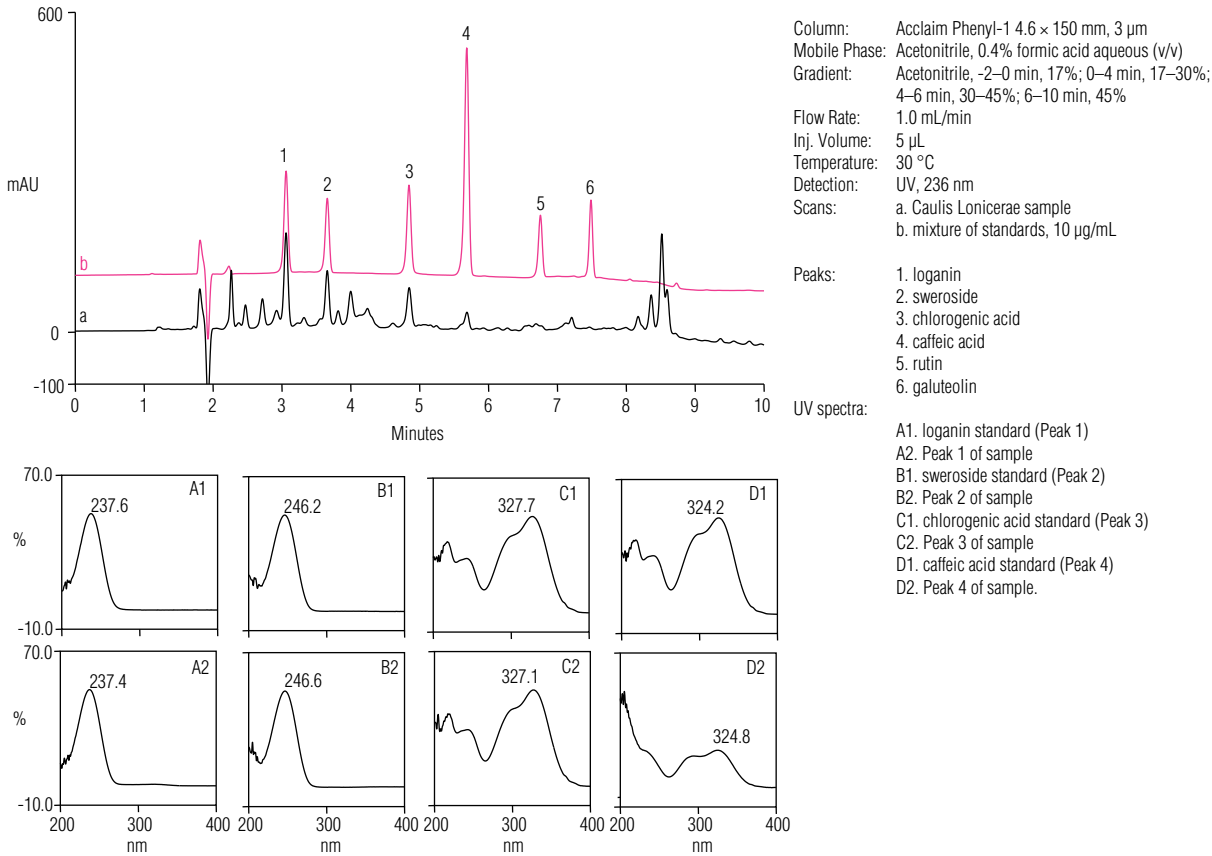

Figure 4. Chromatograms and UV spectra of a) a Caulis Lonicerae sample, and b) a mixture of standards (10 µg/mL each).

Sample Analysis

Chromatograms of a Caulis Lonicerae sample as well as the mixture of loganin, sweroside, chlorogenic acid, caffeic acid, rutin, and galuteolin standards are shown in Figure 4. Comparison of UV spectra and retention times allows the identification of loganin, sweroside, chlorogenic acid and caffeic acid in the Caulis Lonicerae sample. Recoveries for each standard in spiked Caulis Lonicerae sample ranged from 85–99%, suggesting that the analysis method is accurate. The results are shown in Table 4.

Conclusion

This work describes an HPLC method that baseline resolves six active components in Caulis Lonicerae using an Acclaim Phenyl-1, 4.6 × 150 mm, 3 µm column and a 0.4% formic acid/acetonitrile mobile phase. This method can be used for the quality control of Caulis Lonicerae, a common medicinal plant in China. It is superior to the PPRC method that measures only two of the purported active components of Caulis Lonicerae with two separate methods that require long separation times and have insufficient peak resolution between loganin and sweroside.

Table 4. Analysis results of the active components of Caulis Lonicerae

Analyte	Detected (mg/g)	Added (mg/g)	Found (mg/g)	Recovery (%)
Loganin	2.73	2.50	2.18	87
Sweroside	2.79	2.50	2.23	89
Chlorogenic acid	2.77	2.50	2.33	93
Caffeic acid	0.40	0.50	0.45	90
Rutin	Not Found	0.50	0.425	85
Galuteolin	Not Found	0.50	0.495	99

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