

0065 - Skullcap for Flavonoids by HPLC

Botanical Name: *Scutellaria baicalensis* Georgi

Common Names: Chinese skullcap, Huang-Qin

Parts of Plant Used: Dried roots

Uses: Treatment of upper respiratory infections, as an anti-inflammatory agent.

Modes of Action:

Few clinical trials have involved the herb skullcap alone. However, clinical trials have tested herbal formulas that included skullcap as one of the main ingredients. The total flavonoids, especially baicalin, baicalein, wogonin, and wogonin 7-O-glucuronide, are believed to be the active ingredients in skullcap and various in vitro and in vivo experiments have established the activity of these compounds.¹⁻³

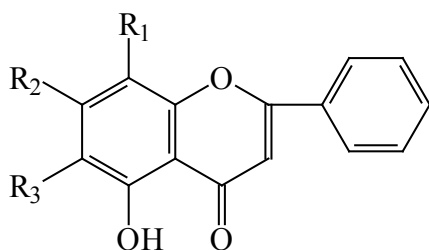
Chemical Markers:

Skullcap contains high amounts of flavonoids in the roots. Four flavones (baicalin, baicalein, wogonin, wogonin 7-O-glucuronide) are the major components. Other identified flavones include apigenin, apigenin 7-O-glucuronide, baicalin methyl ester, chrysin 6-C- β -D-glucopyranoside, chrysin 8-C- β -D-glucopyranoside, chrysin 7-O- β -D-glucuronopyranoside, chrysin 6-C- β -D-glucoside-8-C- α -L-arabinoside, isoscutellarein, isoscutellarein 8-O-glucuronide, 6,2'-dihydroxy-5,7,8,6'-tetramethoxyflavone, oroxylin A, oroxylin A glucuronide, 3,5,7,2',6'-pentahydroxyflavanone, salvigenin, scutellarein, skullcapflavone II, 5,7,2',6'-tetrahydroxyflavone, 5,7,2',5'-tetrahydroxyflavone, 5,7,2',5'-tetrahydroxy-8,6'-dimethoxyflavone, 5,2',6'-trihydroxy-6,7-dimethoxyflavone 2'-O-glucoside, 5,7,2'-trihydroxy-6-methoxyflavone 7-O- β -D-glucuronopyranoside, and 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone 2'-O-glucoside.⁴⁻⁶

Flavanones (hydrobaicalin, carthamidin 7-O-glucuronide, isocarthamidin 7-O-glucuronide), lignans [(+)-5,5'-dimethoxylariciresinol, hedyotol C-4 -O- β -glucopyranoside, hedyotol D-4 -O- β -glucopyranoside, erythroguaiacylglycerol- β -syringaresinol ether 4"-O- β -glucopyranoside], phenylethanoids, phenylpropanoids, and sterols also were isolated from skullcap.⁵⁻⁹ Usually, the four major flavonoids (baicalin, baicalein, wogonin, wogonin 7-O-glucuronide) are used as marker compounds for quality control of skullcap.



Major Flavonoids in Skullcap



R1=H, R2=OH, R3=OH, baicalein
 R1=H, R2=O-glucuronyl, R3=OH, baicalin
 R1=OCH3, R2=OH, R3=H, wogonin
 R1=OCH3, R2=O-glucuronyl, R3=H, wogonin-7-glucuronide
 R1=H, R2=OH, R3=OCH3, oroxylin A
 R1=H, R2=O-glucuronyl, R3=OCH3, oroxylin A-7-glucuronide

Methods of Analysis

Various HPLC methods have been published for analysis of the flavonoids in skullcap and related species.¹⁰⁻¹³

Colorimetric methods and micellar electrokinetic chromatography also have been used to analyze the flavonoids in skullcap.¹⁴ The flavonoids in skullcap can be extracted easily with aqueous alcohol-acetonitrile solution.

Method 1:

The method of Takino et al.¹⁵ provides clear separation for six flavonoids (baicalin, baicalein, wogonin, wogonin 7-O-glucuronide, oroxylin, and oroxylin 7-glucuronide.)

Sample Preparation:

Extract about 2.5 g of root powder with 50 mL of 30% acetonitrile for 1 hour at 60 to 70°C and repeat the procedure three times. Adjust the volume to 250 mL with 30% aqueous acetonitrile solution.

Chromatography:

Column: Waters NovaPak C18 Radial-pak, 8.0 × 100 mm.

Mobile phase: Solvent A = methanol-acetic acid-water (5:5:90), solvent B = methanol-acetic acid-water (90:5:5).

Gradient: 40%B to 80%B in 40 minutes.

Flow rate: 1.0 mL/minute

Injection volume: 10 µL

Detection wavelength: 275 nm

Validation Data:

Not available

Method 2:

The method of Bochorakova et al.¹⁶ was used.

Sample Preparation:

Not available

Chromatography:

Column: Separon SGX C18, 5 μ m, 3.0 \times 150 mm.

Mobile phase: Solvent A = water–acetonitrile (20:80), solvent B = water–acetonitrile (40:60). The water contained 0.1 mole of triethylamine and the pH was adjusted to 2.5 with phosphoric acid.

Gradient:

Time (minutes)	%A	%B
0	70	30
1	70	30
16	0	100
25	0	100

Flow rate: 0.5 mL/minute

Injection volume: 10 μ L

Detection wavelength: 270 nm

Validation Data:

Linearity: 5 to 50 mg/L for wogonin 7-O-glucuronide with a correlation coefficient of 0.9971 and 5 to 50 mg/L for baicalin with a correlation coefficient of 0.9987.

Accuracy: Not specified

Precision: Not specified

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: Not specified

Method 3:

The unpublished method of Mingfu Wang was used.

Sample Preparation:

Transfer about 100 mg of skullcap powder to a 50-mL volumetric flask, add 35 mL of extraction solvent (60% methanol), and sonicate for 45 minutes. Cool to room temperature and fill to volume with 60% methanol.

Chromatography:

Column: Phenomenex Phenylhexyl, 3 μ m, 4.6 \times 150 mm with Phenomenex Phenylhexyl guard cartridge.

Mobile phase: Solvent A = water (0.1% formic acid by volume), solvent B = acetonitrile (0.1% formic acid by volume), solvent C = isopropanol.

Gradient:

Time (minutes)	%A	%B	%C
0	84	14	2
6	84	14	2
10	78	20	2
20	78	20	2
40	58	40	2
50	38	60	2

Flow rate: 1.0 mL/minute

Injection volume: 5 μ L

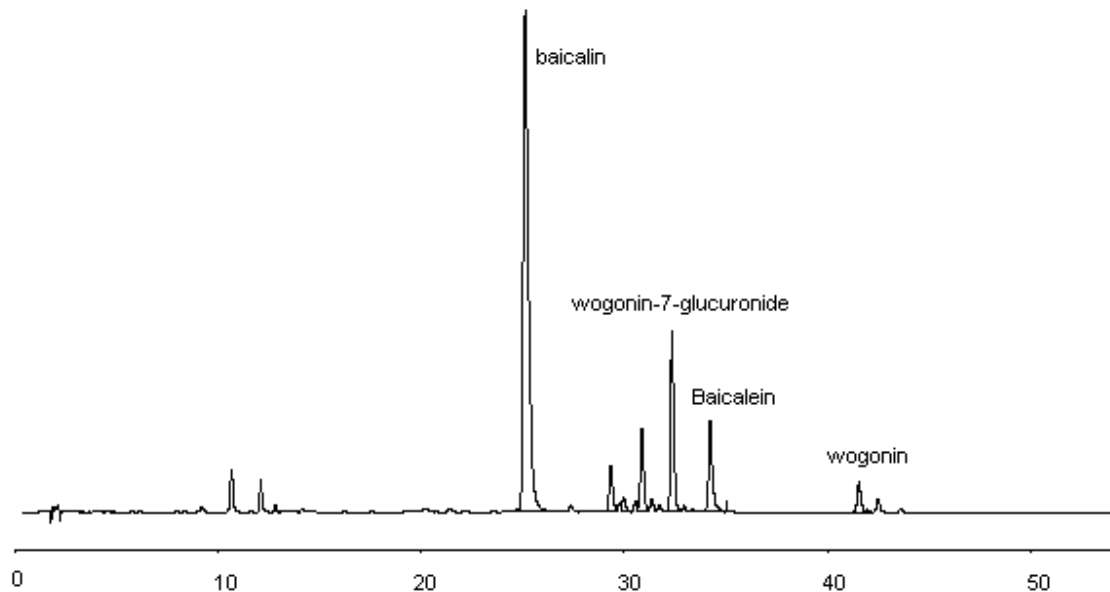
Detection wavelength: 275 nm

Column temperature: 40°C

Validation Data:

Not available

Representative HPLC Chromatogram of Skullcap by Method 3



References:

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