

# ChromaDex®

## Application Note

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### 0044 - Epimedium for Icariin by HPLC

**Botanical Name:** *Epimedium bravicornum* Maxim.; *Epimedium sagittatum* (Sieb.et Zucc.); *Epimedium koreanum* Nakai; *Epimedium pubescens* Maxim.; *Epimedium wushanense* T.S. Ying. These five species are listed in the Chinese Pharmacopeia as Epimedium.

**Common Names:** Herba Epimedii, horny goat weed, Ying-Yang-Huo.

**Parts of Plant Used:** Dried aerial parts

**Uses:** Treatment of osteoporosis and sexual dysfunctions such as impotence and seminal emission.



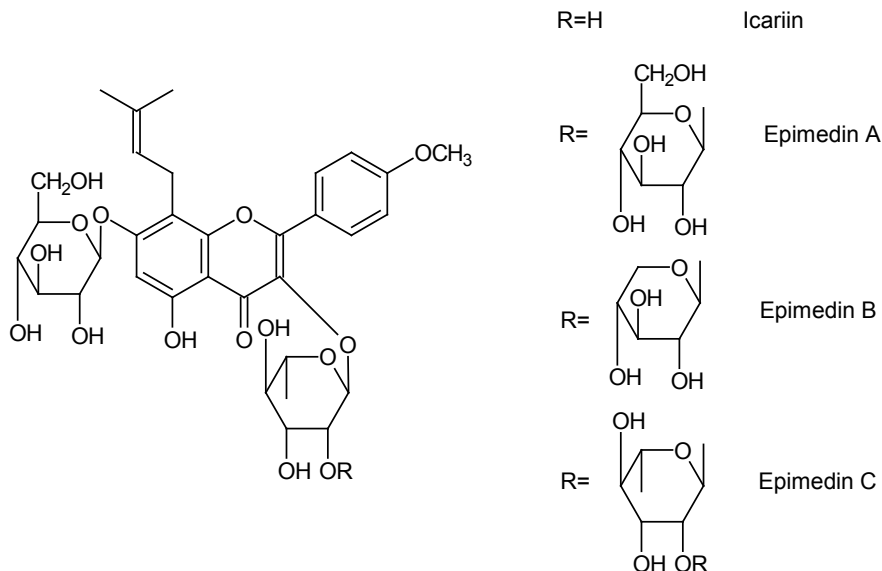
#### Modes of Action:

Few clinical trials support the claims for epimedium. Any type of activity that epimedium might have was attributed to the icariin-type flavonoids. The total flavonoids extract of epimedium was found in vivo to prevent and to treat bone loss in the castrate osteoporosis rat model and to increase mineral content and to promote bone formation in mice.<sup>1,2</sup> Icariin, one of the major flavonoid in Epimedium species, was found to increase the weight of the immature mouse epididymis and seminal vesicles.<sup>3</sup>

#### Chemical Markers:

Icariin-type flavonoids are believed to be main chemical components of *Epimedium*, and the major icariins are icariin, epimedin A, epimedin B, and epimedin C. Other flavonoids purified from *Epimedium* include quercetin, kaempferol-3,7-O- $\alpha$ -L-dirhamnoside, anhydroicaritin, ginkgetin, isoginkgetin, bilobetin, hyperoside, and 5-hydroxy-6,7-dimethyl-2,4-methylenedioxyflavone. Others compounds identified in *Epimedium* include lignans (acuminatin; icarisides E4, E5, E6, and E7; icariols A1 and A2), sterol ( $\beta$ -sitosterol, daucosterol, -sitosterol glucoside), phenylethanoids (icariside, salidroside), phenylpropanoids (icariside H1), and ionone derivatives (icariside B8, icariside B9).<sup>4-15</sup> As icariins are the bioactive components in *Epimedium*, they are used as marker compounds for quality control; usually, four flavonoids are analyzed: icariin and epimedins A, B, and C

## Major flavonoids in *Epimedium*:



## Methods of Analysis

Icariins as flavonoids are easy to detect by UV spectrophotometry. The only challenge for the analysis of icariins is that the structures of epimedins A, B, and C are similar and it is difficult to get baseline separation.

Extraction is key for accurate analysis of icariins. Various extraction solvents have been tried including methanol, water–acetonitrile (73:27), methanol–water (50:50), ethanol–water (70:30), ethanol–water (50:50), and water combined with various extraction techniques (e.g., water bath or ultrasonication). One method uses 50% methanol as the extraction solvent, the sample is sonicated for 30 minutes (300 mg of herbal powders in 50 mL of extraction solvent).

## Method 1:

The HPLC method of Ito et al.<sup>16</sup> was used to determine four major icariins in *Epimedium* systematically.

### Sample Preparation:

Place about 0.5 g of dried herb powder into 25 mL of water–acetonitrile (73:27) and reflux in a water bath at 85°C for 30 minutes. Dilute the resulting extract to 50 mL with the same solvent.

### Chromatography:

Column: TSK gel ODS-120 Å, 5 µm, 150 × 4 mm.

Mobile phase: Water–acetonitrile (73:27) isocratic.

Flow rate: 1.0 mL/minute

Injection volume: 10 µL

Detection wavelength: 270 nm

Column temperature: 50°C

### Validation Data:

Linearity: 2.5 to 215.0, 5.0 to 50.0, 5.0 to 50.0, and 12.5 to 125.2 mcg for epimedins C, B, and A, and icariin, respectively, with a correlation coefficient of 0.999 for all four compounds.

Accuracy: Not specified

Precision: Not specified

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: LOD for epimedins C, B, and A and icariin were 2.5, 3.0, 5.0, and 1.3 ng/mL, respectively, at a signal-to-noise ratio of 3:1 for the peak heights.

## Method 2:

The method of Ma et al.<sup>17</sup> was used.

### Sample Preparation:

Extract 50 mg of epimedium powder with 10 mL of methanol by sonicating for 30 minutes and shaking for 5 minutes.

### Chromatography:

Column: Phenomenex LiChrosorb RP-18, 5 µm, 250 × 4.6 mm.

Mobile phase: Solvent A = water, solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	80	20
20	78	22
23	75	25
35	70	30
50	50	50
55	40	60
60	0	100

Flow rate: 1.5 mL/minute

Injection volume: 10 µL

Detection wavelength: 203 nm; this method analyzes icariins and ginsenosides from ginseng at the same time.

Column temperature: 40°C

### Validation Data:

Linearity: Not specified

Accuracy: In the spike recovery tests, an average of 99.3% of a 1-mg icariin standard spiked into 0.25 g of epimedium extract in 10 mL of methanol was recovered.

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 300 mg of epimedium powder or 50 mg of epimedium extract to a 50-mL volumetric flask, add 35 mL of extraction solvent (50% methanol), and sonicate for 30 minutes. Cool to room temperature and fill to volume with

50% methanol.

### Chromatography:

Column: Phenomenex Prodigy ODS-3 100Å, 5 µm, 4.6 × 250 mm.

Mobile phase: Solvent A = water (0.2% phosphoric acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	74	26
16	74	26
24	20	80
25	74	26
35	74	26

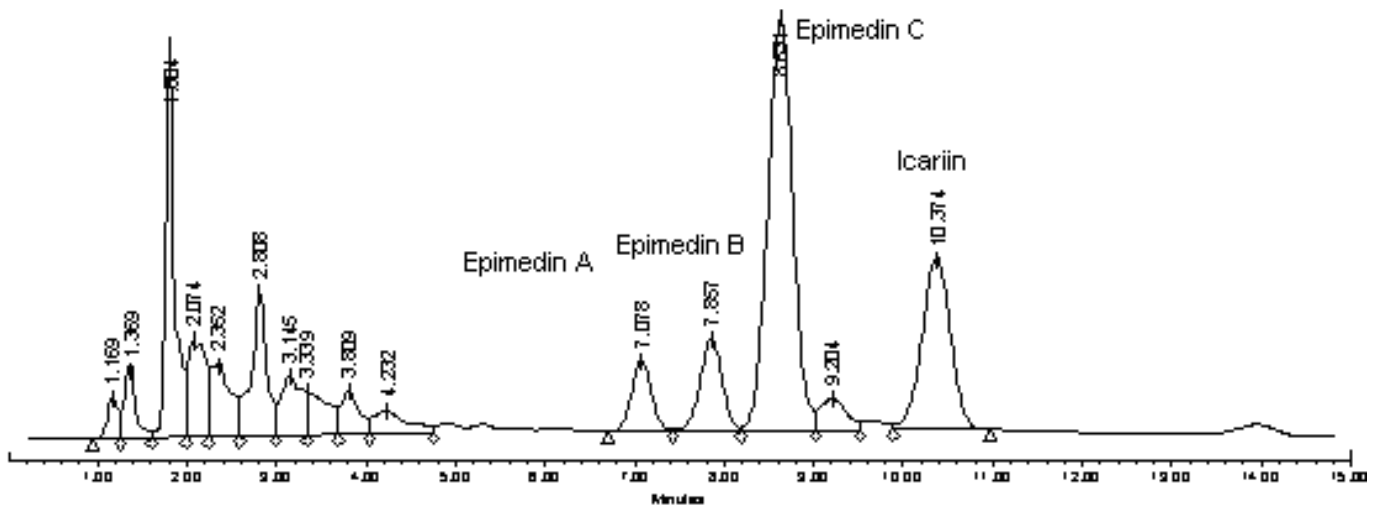
Flow rate: 1.2 mL/minute

Injection volume: 10 µL

Detection wavelength: 270 nm

Column temperature: Ambient

Representative HPLC chromatogram of *Epimedium koreanum* Nakai run by method 3.



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