0002 - Artichoke for Phenolic Acids by HPLC

Botanical Name: Cynara scolymus L.

Common Names: Cynara, globe artichoke

Parts of Plant Used: Dried leaves

Uses: As a choleretic

Modes of Action:
Long known as an herbal medicine, the dried leaves of artichoke have been used for their choleretic properties since remote times. In various pharmacological test systems, artichoke leaf extracts have shown antibacterial, antioxidative, anti-HIV, bile-expelling, and hepatoprotective properties; urinative activity; and inhibition of cholesterol biosynthesis and LDL oxidation. The mono- and dicaffeoylquinic acid derivatives and flavonoids are believed to be bioactive compounds in artichoke, although there are no supporting data.

Chemistry and Chemical Markers for Quality Control:
Artichoke leaves have been studied extensively and found to be a rich source of polyphenolic compounds, with mono- and dicaffeoylquinic acids and flavonoids the major chemical components. The main caffeoylquinic acid derivatives in artichoke leaves are chlorogenic acid and cynarin (1,5-dicaffeoylquinic acid). Other compounds identified in artichoke include sesquiterpenoid lactones and flavonoids (e.g., luteolin-7-O-glycoside, luteolin-7-O-rutinoside). The caffeoylquinic derivatives are used as marker compounds for quality control of artichoke leaf extracts.

Major Phenolic Compounds Identified in Artichoke Leaves:

Chlorogenic acid

Cynarin (1,5-dicaffeoylquinic acid)

Cynaroside (luteolin-7-O-glucoside)

Luteolin-7-O-rutinoside
Methods of Analysis:
Various methods have been used to analyze the phenolic compounds in artichoke, including colorimetry, reversed-phase HPLC (RP-HPLC), micellar electrokinetic capillary chromatography, and TLC. HPLC is the most accepted analytical method for caffeoylquinic acid derivatives.

The extraction solvent is key in the analysis of caffeoylquinic acid derivatives. Various solvents have been tried for extracting compounds from artichoke leaves, with 60% methanol found to be best.

Method 1:
The method of Bilia et al. can be used to analyze chlorogenic acid, cynarin, isochlorogenic acid, luteolin-7-O-glucoside(cynaroside), and luteolin-7-O-rutinoside.

Sample Preparation:
Prepare samples in 60% and 40% methanol.

Chromatography:
Column: LiChrosorb RP-18, 5-μm, 250mm x 4 mm with LiChrosorb RP-18 guard column, 5 μm, 10 x 4 mm.
Mobile phase: Solvent A = water (adjusted to pH 3 with phosphoric acid), solvent B = acetonitrile.
Gradient:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%A</th>
<th>%B</th>
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<tbody>
<tr>
<td>0</td>
<td>88</td>
<td>12</td>
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<tr>
<td>10</td>
<td>82</td>
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<td>0</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>88</td>
<td>12</td>
</tr>
</tbody>
</table>

Flow rate: 1.3 mL/minute
Injection volume: 25 μL
Detection wavelength: 350 nm
Column temperature: 26°C

Validation Data:
Linearity: 0.10 to 2.5 mcg with a correlation coefficient greater than 0.99.
Accuracy: Not specified
Precision: Each of the seven compounds had an RSD that was less than 3.7%.
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: Not specified
Method 2:
The method of Wang et al.\textsuperscript{11} was used to analyze cynarin, 1-caffeoylquinic acid, chlorogenic acid, luteolin-7-O-glucoside, and luteolin-7-O-rutinoside in artichoke leaves and to analyze two additional compounds, narirutin (naringenin-7-O-rutinoside) and apigenin-7-O-rutinoside (isorhoifolin), in artichoke heads (flowers).

**Sample Preparation:**
Extract 500 mg of dried leaves with 70 mL of 60% methanol by sonicating for 25 minutes. Cool the sample to room temperature and dilute to 100 mL with 60% methanol.

**Chromatography:**
Column: Phenomenex Prodigy ODS(3), 5 μm, 150mm x 3.2mm.
Mobile phase: Solvent A = water (with 0.2% phosphoric acid), solvent B = acetonitrile.
Gradient: 6%B linear to 30%B in 20 minutes, hold at 30%B for an additional 5 minutes.
Flow rate: 1.2 mL/minute
Injection volume: 10 μL
Detection wavelength: 330 nm

**Validation Data:**
Linearity: 1.08 to 216 mcg/mL for chlorogenic acid, 1.1 to 220 mcg/mL for 1-caffeoylquinic acid, 0.92 to 184 mcg/mL for luteolin-7-O-rutinoside, 1.06 to 212 mcg/mL for luteolin-7-O-glycoside, and 1.24 to 248 mcg/mL for cynarin; the correlation coefficient for each compound was 1.
Accuracy: The percent recoveries were from 97.6 to 100.7 for these five compounds. Precision: Each compound had an RSD that was less than 2.33%.
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: Not specified

![Representative HPLC chromatogram run by method 2.](image-url)
References: