0035 - Chamomile for Flavonoids by HPLC

Botanical Name: Matricaria recutita L.; Chamomilla recutita (L.) Rausch. 
Matricaria chamomilla L.

Common Names: German chamomile, Hungarian chamomile

Parts of Plant Used: Flowering tops, flowers

Uses: As an antispasmodic, anti-inflammatory, or sedative.

Modes of Action:
Several clinical trials have been performed on chamomile, and this herb has been proven effective for treating respiratory, neurological, and dermatological conditions. The apigenin-type flavonoids and the volatile oil components (chamazulene and α-bisabolol) were found to be responsible in part for its activity.

Chemistry and Chemical Markers for Quality Control:
Chamomile is well known to contain flavonoids, coumarins, sesquiterpenoid lactones, and phenolic acids. Apigenin, apigenin-7-glucoside and its acetylated derivatives (2”-, 3”-, 4”-, 6”-monoaacetates and 2”-,3”-, 3”-,4”-diacetates) were found to be the major flavonoids in the flowers; other flavonoids include luteolin glycosides, quercetin glycosides, andisorhamnetin glycosides.1,2 Chamomile also contains 0.3% to 1.5% oils. The major oil constituents were bisabolol oxide A and B, chamazulene, spiroether, farnesene, and spathulenol.3 Chamomile oil usually has a blue color, owing to chamazulene, which is produced from decomposition of the sesquiterpenoid lactone matricin.1 Apigenin and apigenin-7-glucoside are related flavonoids used as marker compounds for quality control of chamomile extracts.

![Apigenin and Apigenin-7-glucoside](image-url)
Methods of Analysis:
Apigenin-types flavonoids are usually analyzed by HPLC because of their strong UV absorptions.

Method 1:
The method of Repcak and Martonfi\(^2\) was used.

Sample Preparation:
Extract the sample with methanol.

Chromatography:
Column: SGX C18, 7 \(\mu\)m, 150 x 3.0 mm, 5 \(\mu\)m.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
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<tr>
<td>15</td>
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<td>21</td>
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<tr>
<td>25</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>30</td>
<td>76</td>
<td>24</td>
</tr>
</tbody>
</table>

Flow rate: 1 mL/minute
Injection volume: 20 \(\mu\)L
Detection wavelength: 335 nm

Validation Data:
Not available.

Method 2:
The method of Redaelli et al.\(^4\) was used.

Sample Preparation:
Reflux 100 to 120 mg of sample with 80 mL of methanol for 1 hour. Filter, concentrate the extraction solution, and adjust to volume (10 mL).

Chromatography:
Column: PerkinElmer HC-ODS Sil-X reversed-phase, 2.6 x 150 mm.
Gradient: A to B in 25 minutes.
Injection volume: 5 \( \mu L \)
Detection wavelength: 335 nm
Flow rate: 1 mL/minute

**Validation Data:**
Not available.

**Method 3:**
The method of Schulz and Albroscheit\(^5\) was used.

**Sample Preparation:**
Dissolve about 150 mg of plant extract in 5 mL of distilled water and load the solution onto a 3-mL Bakerbond C18 SPE cartridge, first washed by 30 mL of distilled water, then washed by 10 mL of methanol containing 0.2 mL of 25% ammonia solution. Evaporate the methanol solution to dryness and dissolve in 1 mL of methanol.

**Chromatography:**
Column: Hewlett-Packard Hypersil ODS microbore, 5 \( \mu m \), 100 \( mm \) 2.1 mm.
Mobile phase: Solvent A = water (adjusted to pH 2.8 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>
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<td>20</td>
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<td>0</td>
<td>100</td>
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<tr>
<td>20</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Detection wavelength: 337 nm
Flow rate: 0.5 mL/minute
Injection volume: 2 to 10 \( \mu L \)
Column temperature: 40°C

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