

ChromaDex[®]

Application Note

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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

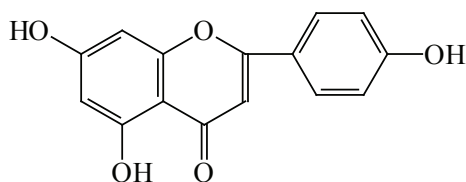
Uses: As an antispasmodic, anti-inflammatory, or sedative.

Modes of Action:

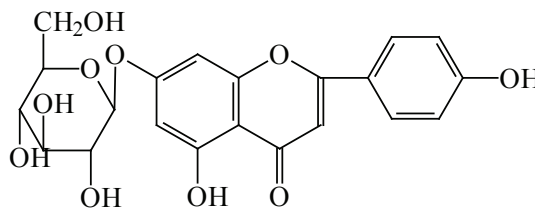
Several clinical trials have been performed on chamomile, and this herb has been proven effective for treating respiratory, neurological, and dermatological conditions. The apigenin-type flavonoids and the volatile oil components (chamazulene and α -bisabolol) were found to be responsible in part for 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"-monoacetates and 2",3"-, 3",4"-diacetates) were found to be the major flavonoids in the flowers; other flavonoids include luteolin glycosides, quercetin glycosides, and isorhamnetin 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



Apigenin-7-glucoside

Methods of Analysis:

Apigenin-types flavonoids are usually analyzed by HPLC because of their strong UV absorptions.

Method 1:

The method of Repcak and Martonfi² was used.

Sample Preparation:

Extract the sample with methanol.

Chromatography:

Column: SGX C18, 7 µm, 150 × 3.0 mm, 5 µm.

Mobile phase: Solvent A = acetonitrile–water–phosphoric acid (19:40:1), solvent B = acetonitrile–methanol–phosphoric acid (59:40:1).

Gradient:

Time (minutes)	%A	%B
0	76	24
5	70	30
10	62	38
15	46	54
21	30	70
25	15	85
30	76	24

Flow rate: 1 mL/minute
Injection volume: 20 µL
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 × 150 mm.

Mobile phase: Solvent A = acetonitrile–water–acetic acid (15:83:2), solvent B = acetonitrile–water–acetic acid (60:38:2).

Gradient: A to B in 25 minutes.
 Injection volume: 5 μ L
 Detection wavelength: 335 nm
 Flow rate: 1 mL/minute

Validation Data:

Not available.

Method 3:

The method of Schulz and Albroscheit⁵ 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 m, 100 \times 2.1 mm.

Mobile phase: Solvent A = water (adjusted to pH 2.8 with phosphoric acid), solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	97.5	2.5
9.9	97.5	2.5
10	80	20
14.9	80	20
15	65	35
16.9	65	35
17	0	100
20	0	100

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

References:

1. Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. Paris: Lavoisier Publishing; 1995:455–7.
2. Repcak M, Martonfi P. The variability pattern of apigenin glucosides in *Chamomilla recutita* diploid and tetraploid cultivars. *Pharmazie*. 1995;50:696–9.
3. Weglarz Z, Roslon W. Individual variability of chamomile [*Chamomilla recutita* (L.) Rausch.] in respect of the content and chemical composition of essential oil. *Herba Polonica*. 2002;48(4):169–73.
4. Redaelli C, Formentini L, Santaniello E. Reversed-phase high-performance liquid chromatography analysis of apigenin and its glucosides in flowers of *Matricaria chamomilla* and chamomile extracts. *Planta Med*. 1981;42:293–5.
5. Schulz H, Albroscheit G. High-performance liquid chromatographic characterization of some medical plant extracts used in cosmetic formulas. *J Chromatogr A*. 1988;442:353–61.