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Chamomile

DEFINITION

Chamomile consists of the dried flower heads of *Matricaria recutita* L. (*Matricaria chamomilla* L., *Matricaria chamomilla* L. var. *courrantiana*, *Chamomilla recutita* L.) Rauschert (Fam. Asteraceae alt. Compositae). It contains NLT 0.4% of blue volatile oil, NLT 0.3% of apigenin-7-glucoside, and NLT 0.15% of bisabolol derivatives, calculated as levomenol.

IDENTIFICATION

• **A. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST](#)**

Standard solution: 1.0 mg/mL of borneol, 2.0 mg/mL of bornyl acetate, and 0.4 mg/mL of guaiazulene in toluene

Sample solution: Reduce 1.0 g of Chamomile to a coarse powder, using a porcelain pestle and mortar. Transfer to a 1.5-cm × 15-cm chromatographic column, and tamp lightly with a short length of rubber hose. Rinse the pestle and mortar twice, each time with 10 mL of methylene chloride. Pour the rinsings into the column. Collect the percolate in a flask with a long, narrow neck, and remove the solvent by evaporation on a water bath. Dissolve the residue in 0.5 mL of toluene.

Adsorbent: 0.25-mm layer of chromatographic silica gel

Developing solvent: Chloroform

Spray reagent: Mix anisaldehyde, glacial acetic acid, and methanol (0.5:10:85). Then carefully add 5 mL of sulfuric acid to this solution.

Application volume: 10 µL, as 3-mm × 20-mm bands

Analysis

Samples: *Standard solution* and *Sample solution*

Examine the plate under short-wavelength UV light: the *Sample solution* exhibits a number of quenching areas, the largest of which is due to en-yne-dicycloether and has the same R_F value as the band due to bornyl acetate in the *Standard solution*. There is also a band due to matricin near the line of application. Spray the plate evenly with the *Spray reagent*. Examine the plate in daylight while heating at 100°–105° for 5–10 min. The chromatogram obtained from the *Standard solution* shows in the lower third a brownish yellow zone that becomes violet-gray after a few hours and is due to borneol; in the middle a yellowish brown to gray zone due to bornyl acetate; and in the upper third a deep red zone with a blue edge due to guaiazulene.

Acceptance criteria: The *Sample solution* exhibits a blue zone due to matricin near the starting point; several violet-red zones, one of which is due to bisabolol, at R_F values between those of borneol and bornyl acetate; a brownish zone, due to en-yne-dicycloether, at an R_F value corresponding to that of bornyl acetate; red zones, due to terpenes, at R_F values similar to those of guaiazulene; and other zones that appear in the middle and lower parts of the chromatogram.

• **B.**

Analysis: Dissolve 0.25 g of dimethylaminobenzaldehyde in a mixture of 5 mL of phosphoric acid, 45 mL of acetic acid, and 45 mL of water. Transfer 2.5 mL of this solution and 0.1 mL of the *Sample solution*, prepared as directed for *Identification* test A, to a test tube. Heat on a water bath for 2 min, and allow to cool. Add 5 mL of solvent hexane, and shake.

Acceptance criteria: The aqueous layer has a distinct greenish blue or blue color.

COMPOSITION

• **CONTENT OF APIGENIN-7-GLUCOSIDE**

Dilute phosphoric acid: Mix 5.0 mL of phosphoric acid in 50 mL of water. Dilute with water to 100 mL.

Solution A: 0.005 M solution of monobasic potassium phosphate. Adjust with *Dilute phosphoric acid* to a pH of 2.55 ± 0.05.

Solution B: Acetonitrile and methanol (13:7)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	74	26
3	74	26
22	15	85

Time (min)	Solution A (%)	Solution B (%)
27	74	26
30	74	26

Standard solution: 25.0 µg/mL of [USP Apigenin-7-glucoside RS](#) and 10.0 µg/mL of 7-methoxycoumarin in methanol and water (1:1)

Sample solution: Transfer 1.0 g of Chamomile to a suitable flask fitted with a reflux condenser and a stirrer. Add 80.0 mL of methanol, and reflux the mixture with stirring for 1 h. Cool the flask to room temperature, pass the extract through a folded filter paper, and collect the filtrate in a 100-mL volumetric flask. Rinse the flask with 3 mL of methanol, pour the methanolic rinsings through the filter paper, and add the filtrate to the volumetric flask. Dilute with methanol to volume, and mix. Transfer 25.0 mL of the filtered solution to a round-bottom flask fitted with a reflux condenser and a stirrer; add 5.0 mL of sodium hydroxide solution, prepared by dissolving 0.4 g of sodium hydroxide in 5.0 mL of water; and reflux the mixture for 25 min. Cool the flask, and adjust the solution with hydrochloric acid to a pH of 5.0–6.2. Quantitatively transfer the solution to a 50-mL volumetric flask, dilute with methanol to volume, and filter, discarding the first 10 mL of the filtrate.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 335 nm

Column: 4-mm × 12.5-cm; packing L1

Flow rate: 1 mL/min

[NOTE—Make adjustments, if necessary, to obtain relative retention times of 0.63 for apigenin-7-glucoside and 1.0 for 7-methoxycoumarin.]

Injection size: 15 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for apigenin-7-glucoside, 7-methoxycoumarin, apigenin, *trans*-spiroether, and *cis*-spiroether are about 0.63, 1.0, 1.2, 1.6, and 1.8, respectively.]

Suitability requirements

Resolution: NLT 3.5 between apigenin-7-glucoside and 7-methoxycoumarin

Relative standard deviation: NMT 2.0% for apigenin-7-glucoside

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Allow the *Sample solution* to elute for NLT 6 times the retention time of apigenin-7-glucoside.]

Calculate the percentage of apigenin-7-glucoside in the portion of Chamomile taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak response of apigenin-7-glucoside from the *Sample solution*

r_S = peak response of apigenin-7-glucoside from the *Standard solution*

C_S = concentration of [USP Apigenin-7-glucoside RS](#) in the *Standard solution* (mg/mL)

V = volume of *Sample solution* (mL)

W = weight of Chamomile taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 0.3%

• CONTENT OF BISABOLANE DERIVATIVES

Standard solution: 1 mg/mL of [USP Levomenol RS](#) in cyclohexane

Sample solution: Transfer the volatile oils obtained in the test for [Articles of Botanical Origin \(561\), Volatile Oil Determination](#) to a 25-mL volumetric flask, rinse the graduated tube of the apparatus with a small portion of cyclohexane, transfer the rinsing to the 25-mL volumetric flask, add cyclohexane to volume, and mix.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused-silica capillary; coated with a 0.25-µm film of phase G16

Temperature

Column: See [Table 2](#).

Table 2