

Chamomile

Chamomile consists of the dried flower heads of *Matricaria recutita* Linn é (*Matricaria chamomilla* Linn é *Matricaria chamomilla* Linn é var. *courrantiana*, *Chamomilla recutita* Linn é) Rauschert (Fam. Asteraceae alt. Compositae). It contains not less than 0.4 percent of blue volatile oil, not less than 0.3 percent of apigenin-7-glucoside, and not less than 0.15 percent of bisabolane derivatives, calculated as levomenol.

Packaging and storage— Preserve in well-closed containers, protected from light.

Labeling— The label states the Latin binomial and, following the official name, the part of the plant contained in the article.

USP Levomenol RS.

Botanic characteristics—

Macroscopic— Flower head is hemispherical, about 6 mm in diameter, composed of a few ray florets and numerous disk florets (distinction from *Matricaria discoidea*, which has disk florets only), carried on a receptacle surrounded by an involucre. Involucre is green, formed of two to three rows of lanceolate, glabrous, and imbricated bracts with blunt apices and scarious whitish edges. Ray florets, which usually have fallen off, have 10 to 20 pistils; corolla is ligulate, white, but darkens at a length of 6 mm and a width of 2 mm, 3-toothed, and traversed by four main veins. Disk florets are yellow, perfect, about 2 mm in length; corolla is tubular with five teeth; five stamens are epipetalous and syngenesious. Receptacle is hollow (distinction from *Chrysanthemum* and *Anthemis* species), hemispherical in the young and conical in the old flower head, 3 to 10 mm in width, and lacking paleae. Achene is ovoid and has three to five longitudinal ribs.

Microscopic— Separate the capitulum into its parts and examine under a microscope. The outer, abaxial epidermis of the involucre bracts shows a scarious margin with a single layer of radially elongated cells and a central part made up of chlorophyll tissue covered by elongated epidermal cells with sinuous lateral walls, stomata, and secretory trichomes. The vascular bundles are surrounded by numerous elongated, pitted sclereids with fairly large lumens. In surface view, ligulate and tubular corollas show isodiametric or elongated cells with more or less wavy walls and a few glandular trichomes. The outer part of the epidermis of the ligulate florets consists of papillary cells with cuticular striations radiating from their tips. In the mesophyll, very small clusters of calcium oxalate are sometimes seen. Four main veins run lengthwise through the entire

mesophyll, sometimes accompanied by one or two other veins, which are shorter and run parallel to the main veins. Each of the two main median veins splits into two near the tip and, with the lateral veins, anastomose two by two to form three arcs at the three terminal teeth of the ligule. The ovaries, oval to spherical, of both kinds of florets have at their base a sclerous ring consisting of a single row of cells. The epidermis of the ovary is made up of elongated cells with sinuous walls between which secretory trichomes are situated. The ovaries contain numerous very small clusters of calcium oxalate. In the tubular florets, the low part of each stamen filament is surrounded by thick-walled cells. The ends of the two stigmata have papillose epidermal cells. The pollen grains have a diameter of about 30 μm and are rounded and triangular, with three germinal pores and a spiny exine.

Identification—

A: [Thin-Layer Chromatographic Identification Test](#) 201 —

Adsorbent: 0.25-mm layer of chromatographic silica gel.

Test solution— Reduce about 1.0 g of Chamomile to a coarse powder, using a porcelain pestle and mortar. Transfer to a 1.5-cm \times 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 and evaporate the extract to dryness. Dissolve the residue in 0.5 mL of toluene.

Standard solution— Prepare a solution of borneol, bornyl acetate, and guaiazulene in toluene containing 1.0 mg per mL, 2.0 mg per mL, and 0.4 mg per mL, respectively.

Developing solvent: chloroform.

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

Procedure— Separately apply, as 3-mm \times 20-mm bands, equal volumes (about 10 μL) of the Test solution and the Standard solution, and proceed as directed in the chapter. Examine the plate under short-wavelength UV light: the chromatogram of the Test 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 chromatogram of 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 to 105 for 5 to 10 minutes. 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. The chromatogram of the Test solution exhibits a blue zone due to matricin near the starting point; several violet-red zones, one of which is due to bisabolol, at RF values between those of borneol and bornyl acetate; a brownish zone, due to en-yne-dicycloether, at an RF value corresponding to that of bornyl acetate; red zones, due to terpenes, at RF values similar to that of guaiazulene; and other zones that appear in the middle and lower parts of the chromatogram.

B: 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 test solution, prepared as directed for Identification test A, to a test tube. Heat on a water bath for 2 minutes, and allow to cool. Add 5 mL of solvent hexane, and shake. The aqueous layer has a distinct greenish blue or blue color.

Broken flowers— Not more than 25% passes through a No. 25 standard-mesh sieve (see [Particle Size Distribution Estimation by Analytical Sieving](#) 786).

[Foreign organic matter](#) 561 : not more than 2.0%.

[Total ash](#) 561 : not more than 13.0%, determined on 1.0 g of powdered Chamomile.

[Microbial enumeration](#) 2021 — The total bacterial count does not exceed 10⁵ cfu per g, the total combined mold and yeast count does not exceed 10³ cfu per g, and the bile-tolerant Gram-negative bacterial count does not exceed 10³ cfu per g.

Absence of specified microorganisms 2022 — It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

[Pesticide residues](#) 561 : meets the requirements.

[Volatile oil content](#) 561 — Proceed as directed, except to use 60 g of coarsely powdered Chamomile as the test specimen, a 2-L round-bottom flask, 300 mL of water as distillation liquid,

and 0.5 mL of xylene in the graduated tube. Distill for 4 hours at a rate of 3 to 4 mL per minute: not less than 0.4% of blue volatile oil is found.

Note—Retain the volatile oils for use in the test for Content of bisabolane derivatives.

Content of apigenin-7-glucoside—

Dilute phosphoric acid— Transfer 5.0 mL of phosphoric acid to a 100-mL volumetric flask containing about 50 mL of water, dilute with water to volume, and mix.

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

Solution B— Prepare a mixture of acetonitrile and methanol (65:35).

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for

Chromatographic system

Standard solution— Dissolve accurately weighed quantities of [USP Apigenin-7-glucoside RS](#) and 7-methoxycoumarin in methanol and water (1:1) to obtain a solution having known concentrations of about 25.0 µg per mL of [USP Apigenin-7-glucoside RS](#) and 10.0 µg per mL of 7-methoxycoumarin.

Test solution— Transfer about 1.0 g of Chamomile, accurately weighed, 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 hour. Cool the flask to room temperature, pass the extract through filter paper, and collect the filtrate in a 100-mL volumetric flask. Rinse the extraction 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 solution to a suitable 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 minutes. Cool the flask, and adjust the solution with hydrochloric acid to a pH of 5.0 to 6.2. Quantitatively transfer the solution to a 50-mL volumetric flask, dilute with methanol to volume, mix, and filter, discarding the first 10 mL of the filtrate.

Chromatographic system— The liquid chromatograph is equipped with a 335-nm detector and a 4-mm × 12.5-cm column that contains packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows:

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–3	74	26	isocratic
3–22	74→15	26→85	linear gradient
22–27	15→74	85→26	linear gradient
27–30	74	26	isocratic

Make adjustments, if necessary, to obtain relative retention times of about 0.63 and 1.0 for apigenin-7-glucoside and 7-methoxycoumarin, respectively. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the resolution, *R*, between apigenin-7-glucoside and 7-methoxycoumarin is not less than 3.5; and the relative standard deviation for the apigenin-7-glucoside peak for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 15 µL) of the Standard solution and the Test solution into the chromatograph, and allow the Test solution to elute for not less than six times the retention time of apigenin-7-glucoside. Record the chromatograms, and measure the responses for all the peaks observed in the chromatogram of the Test solution: the approximate relative retention times are about 0.63, 1.0, 1.2, 1.6, and 1.8 for apigenin-7-glucoside, 7-methoxycoumarin, apigenin, trans-spiroether, and cis-spiroether, respectively. Calculate the percentage of apigenin-7-glucoside in the portion of Chamomile taken by the formula:

$$20(C/W)(rU / rS)$$

in which *C* is the concentration, in mg per mL, of [USP Apigenin-7-glucoside RS](#) in the Standard solution; *W* is the weight, in g, of Chamomile taken for the Test solution; and *rU* and *rS* are the apigenin-7-glucoside peak responses obtained from the Test solution and the Standard solution, respectively: not less than 0.3% is found.

Content of bisabolane derivatives—

Standard solution— Prepare a solution of USP Levomenol RS in cyclohexane having a known concentration of about 1 mg per mL.

Test solution— Transfer the volatile oils obtained in the test for Volatile oil content 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— The gas chromatograph is equipped with a flame-ionization detector and a 0.32-mm × 30-m fused-silica capillary column coated with a 0.25-µm film of phase G16.

The carrier gas is helium, flowing at a rate of about 1.0 mL per minute. The chromatograph is programmed as follows. Initially the column temperature is maintained at 70 , then it is increased at a rate of 4 per minute to 230 , and maintained at 230 for 10 minutes. The detector is maintained at a temperature of 250 , and the injection port is maintained at 220 . Chromatograph the Standard solution, and record the peak areas as directed for Procedure: the tailing factor for levomenol is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 1 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak areas. Identify the peaks due to levomenol, bisabol oxide B, bisabol oxide, and bisabol oxide A in the Test solution using the retention time of levomenol in the Standard solution and the approximate relative retention times of 0.89, 0.97, and 1.1 for bisabol oxide B, bisabol oxide, and bisabol oxide A, respectively, with reference to the levomenol peak. Calculate the percentage of the bisabolane derivatives in the portion of Chamomile taken by the formula:

$$2500 (rU / rS)(CS / W)$$

in which rU is the sum of the peak areas for bisabol oxide B, bisabol oxide, levomenol, and bisabol oxide A obtained from the Test solution; rS is the levomenol peak area obtained from the Standard solution; CS is the concentration, in mg per mL, of USP Levomenol RS in the Standard solution; and W is the weight, in mg, of Chamomile used in the test for Volatile oil content: not less than 0.15% of bisabolane derivatives is found.