

Chaste Tree

Chaste Tree consists of the dried ripe fruits of *Vitex agnus-castus* L. (Fam. Verbenaceae). It contains not less than 0.05 percent of agnuside and not less than 0.08 percent of casticin, calculated on the dried basis.

Packaging and storage— Preserve in a well-closed container, and store at controlled room temperature.

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

USP Powdered Chaste Tree Extract RS.

Botanic characteristics—

Macroscopic— Mature chaste tree fruits are spherical to ovoid, 2 to 4 mm in diameter, very hard, usually with a short pedicel. The fruit is reddish brown to black, slightly rough, and covered with glandular hairs. There are four grooves perpendicular to one another, and a slight depression on the apex, more evident on large fruits. The internal appearance of the fruit is yellowish. The internal structure of the fruit includes four compartments, each containing an oblong seed with a high fat content. A group of up to six spongy, light tan, immature fruits may also accompany mature fruits. The fruit is often covered by a tubular, greenish-gray, fine tomentous calyx, which is persistent and has five teeth.

Microscopic— The exocarp is brown and narrow, consisting of parenchymatous cells with thin walls and partially lignified cells with many pitted thickenings on the inside. In surface view, the exocarp shows an epidermis of polygonal cells with irregular thickenings and glandular hairs, each with a short single-celled stalk and a four-celled head containing essential oil. The outer mesocarp consists of several layers of brown, isodiametric parenchyma cells. The inner mesocarp consists of finely pitted sclerenchymatous cells, some with moderately thickened walls, others consisting of isodiametric stone cells with small lumen. The endocarp consists of a layer of small brown sclereid cells. The seeds are small, having large cotyledons surrounded by thin-walled, large parenchymatous cells that have ribbed thickenings. The nutritive tissue and the cells of the germ contain aleuron grains and oil globules. Starch is absent. The outer epidermis of calyx is composed of polygonal cells, covered by abundant unicellular or multicellular curved trichomes.

The inner epidermis of calyx is glabrous and composed of rectangular, elongated cells with slightly wavy walls.

Identification—

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

Test solution— Transfer about 1 g of the powdered plant material to a screw-capped centrifuge tube. Add 10 mL of methanol, and heat in a water bath at 60 °C for 10 to 15 minutes, cool, and filter. Apply 60 µL to the plate in bands that are 2 cm in length.

Standard solution— Transfer about 100 mg of USP Powdered Chaste Tree Extract RS to a screw-capped centrifuge tube. Add 1 mL of methanol, and heat in a water bath at 60 °C for 10 minutes. Centrifuge, and use the clear supernatant. Apply 90 µL to the plate.

Developing solvent system— Use a mixture of ethyl acetate, methanol, and water (77:15:8).

Spray reagent— Prepare a solution of p-dimethylaminobenzaldehyde in 1 N hydrochloric acid containing 10 mg per mL.

Procedure— Develop the chromatogram to a length of not less than 12 cm, and dry the plate in a current of air. Spray the plate with Spray reagent, and heat for 10 minutes at 120 °C. The chromatogram obtained from the Test solution shows the following: a blue zone (at an RF value of about 0.21) that is due to the presence of aucubin and that corresponds in color and RF value to a similar zone in the chromatogram of the Standard solution; a blue zone (at an RF value of about 0.44) as a result of the presence of agnuside that corresponds in color and RF value to a similar zone in the chromatogram of the Standard solution; and one broad zone, violet in the middle, that is near the solvent front and that corresponds in color and RF value to a similar zone in the chromatogram of the Standard solution. Other colored zones of varying intensities may be observed in the chromatogram of the Test solution.

B: The chromatogram of the Test solution in the test for Content of casticin shows a peak at the retention time corresponding to the casticin peak in the chromatogram of the Standard solution.

[Microbial enumeration](#) 2021 — It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*. The total aerobic microbial count does not exceed 10⁶

cfu per g, the total combined molds and yeast count does not exceed 104 cfu per g, and the enterobacterial count does not exceed 103 cfu.

[Loss on drying](#) [731](#) — Dry 1 g at 105 °C for 2 hours. It loses not more than 10.0%.

[Foreign organic matter](#) [561](#) : not more than 3.0%.

[Total ash](#) [561](#) : not more than 8.0%.

[Acid-insoluble ash](#) [561](#) : not more than 2.0%.

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

Heavy metals, [Method III](#) [231](#) : not more than 20 µg per g.

Content of casticin—

Solution A— Use filtered and degassed methanol.

Solution B— Use a filtered and degassed solution of 5.88 g of phosphoric acid in 1000 mL of water.

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary.

Standard solution— Dissolve an accurately weighed quantity of [USP Casticin RS](#) in methanol, with sonication. Dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.05 mg per mL. Filter through a cellulose membrane having a 0.45-µm or finer porosity.

Test solution— Accurately weigh approximately 1000 mg of ground plant material, and place in a suitable container with a stopper. Extract twice with 40 mL of methanol, using a hand homogenizer at 19,000 rpm for 2 minutes. Filter each supernatant, and transfer to a 250-mL round-bottom flask. Rinse the residue with methanol, and filter the resulting solution into the flask. Evaporate the combined extract to dryness. Dissolve the residue in methanol, quantitatively transfer to a 20-mL volumetric flask, and dilute with methanol to volume. Filter through a cellulose membrane having a 0.45-µm or finer porosity.

Chromatographic system— The liquid chromatograph is equipped with a 348-nm detector and a 3.1-mm × 12.5-cm column that contains 5-µm packing L1. The column temperature is maintained at 25 . The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	50	50	equilibration
0–13	50→65	50→35	linear gradient
13–18	65→100	35→0	linear gradient
18–23	100→50	0→50	linear gradient

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the tailing factor for casticin is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas of the analyte peaks. Identify the retention time of the peak corresponding to casticin in the Test solution by comparison with the chromatogram of the Standard solution. Calculate the percentage of casticin in the portion of Chaste Tree taken by the formula:

$$2000(C/W)(rU / rS),$$

in which C is the concentration, in mg per mL, of [USP Casticin RS](#) in the Standard solution; W is the weight, in mg, of the Chaste Tree taken to prepare the Test solution; and rU and rS are the peak responses of casticin obtained from the Test solution and the Standard solution, respectively.

Content of agnuside—

Solvent: a mixture of water and methanol (95:5).

Solution A— Use filtered and degassed acetonitrile.

Solution B— Use a filtered and degassed solution of 5.88 g of phosphoric acid in 1000 mL of water.

Standard solution— Dissolve an accurately weighed quantity of [USP Agnuside RS](#) in Solvent, with sonication. Dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.125 mg per mL. Filter through a cellulose membrane having a 0.45-µm or finer porosity.

Test solution— Accurately weigh approximately 1000 mg of ground plant material, and place in a suitable container with a stopper. Extract twice with 40 mL of methanol, using a hand homogenizer at 19,000 rpm for 2 minutes. Centrifuge, and transfer each supernatant to a 250-mL round-bottom flask. Rinse the residue with methanol, and filter the resulting solution into the flask. Evaporate the combined extract to dryness, and dissolve the residue in 2 mL of Solvent.

Quantitatively transfer the solution to a solid-phase extraction cartridge packed with neutral aluminum oxide previously conditioned with 5 mL of Solvent. Connect the cartridge to a vacuum pressure not exceeding 300 mbar, and collect the eluate. Rinse the round-bottom flask with 2 mL of Solvent, and pass this solution through the cartridge, apply the vacuum, and collect the eluate. Rinse the cartridge with 4 mL of Solvent, and collect the eluate. Combine the eluates from the cartridge, transfer to a 10-mL volumetric flask, and dilute with Solvent to volume.

Chromatographic system— The liquid chromatograph is equipped with a 258-nm detector and a 3.1-mm × 12.5-cm column that contains 5- μ m packing L1. The column temperature is maintained at 25 . The flow rate is about 1.3 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	7	93	equilibration
0.6–5	10	90	isocratic
5–7	10→14	90→86	linear gradient
7–13	14→15	86→85	linear gradient
13–13.1	15→100	85→0	linear gradient
13.1–18	100	0	isocratic
18–18.1	100→7	0→93	linear gradient
18.1–23	7	93	re-equilibration

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the tailing factor for agnuside is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 10 μ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas for the analyte peaks. Identify the retention time of the peak corresponding to agnuside in the Test solution by

comparison with the chromatogram obtained from the Standard solution. Calculate the percentage of agnuside in the portion of Chaste Tree taken by the formula:

$$1000(C/W)(rU / rS),$$

in which C is the concentration, in mg per mL, of [USP Agnuside RS](#) in the Standard solution; W is the weight, in mg, of the Chaste Tree taken to prepare the Test solution; and rU and rS are the peak responses of agnuside obtained from the Test solution and the Standard solution, respectively.