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Powdered Chaste Tree

DEFINITION

Powdered Chaste Tree is Chaste Tree reduced to a powder or a very fine powder. It contains NLT 0.05% of agnuside and NLT 0.08% of casticin, calculated on the dried basis.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 100 mg of [USP Powdered Chaste Tree Extract RS](#) in 1 mL of methanol. Heat in a water bath at 60° for 10 min. Centrifuge, and use the clear supernatant.

Sample solution: Transfer about 1 g of Powdered Chaste Tree to a screw-capped centrifuge tube. Add 10 mL of methanol, heat in a water bath at 60° for 10–15 min, cool, and filter.

Adsorbent: Chromatographic silica gel with an average particle size of 10–15 µm (TLC plates)

Application volume: 90 µL, *Standard solution*; 60 µL, *Sample solution*; in bands that are 2 cm in length

Developing solvent system: Ethyl acetate, methanol, and water (77:15:8)

Derivatization reagent: 10 mg/mL of *p*-dimethylaminobenzaldehyde in 1 N hydrochloric acid

Analysis

Samples: *Standard solution* and *Sample solution*

Develop to a length of NLT 12 cm, and dry the plate in a current of air. Treat the plate with *Derivatization reagent*, and heat for 10 min at 120°.

Acceptance criteria: The *Sample solution* shows the following: a blue zone (at an *R_F* value of about 0.21) due to the presence of aucubin and that corresponds in color and *R_F* value to a similar zone for the *Standard solution*; a blue zone (at an *R_F* value of about 0.44) as a result of the presence of agnuside that corresponds in color and *R_F* value to a similar zone for the *Standard solution*; and one broad zone, violet in the middle, near the solvent front and that corresponds in color and *R_F* value to a similar zone for the *Standard solution*. Other colored zones of varying intensities may be observed for the *Sample solution*.

• **B.** In the test for *Content of Casticin*, the chromatogram of the *Sample solution* shows a peak at the retention time corresponding to the casticin peak in the chromatogram of the *Standard solution*.

COMPOSITION

• **CONTENT OF CASTICIN**

Standard solution: About 0.05 mg/mL of [USP Casticin RS](#) in methanol, with sonication. Pass through a cellulose membrane filter of 0.45-µm or finer pore size.

Sample solution: Place about 1000 mg of Powdered Chaste Tree in a container with a stopper. Extract twice with 40 mL of methanol, using a hand homogenizer at 19,000 rpm for 2 min. 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. Pass through a cellulose membrane filter of 0.45-µm or finer pore size.

Solution A: Methanol

Solution B: 5.88 g/L of phosphoric acid in water

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
0	50	50
13	65	35
18	100	0

Time (min)	Solution A (%)	Solution B (%)
23	50	50

Chromatographic system(See [Chromatography \(621\), System Suitability](#).)**Mode:** LC**Detector:** UV 348 nm**Column:** 3.1-mm × 12.5-cm; 5-µm packing L1**Column temperature:** 25°**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0 for the casticin peak**Relative standard deviation:** NMT 2.0% for the casticin peak, in repeated injections**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of casticin in the portion of Powdered Chaste Tree taken:

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

 r_U = peak response of casticin from the *Sample solution* r_S = peak response of casticin from the *Standard solution* C_S = concentration of [USP Casticin RS](#) in the *Standard solution* (mg/mL) C_U = concentration of Powdered Chaste Tree in the *Sample solution* (mg/mL)**Acceptance criteria:** NLT 0.08% of casticin on the dried basis**• CONTENT OF AGNUSIDE****Solvent:** Methanol and water (1:19)**Standard solution:** Dissolve a quantity of [USP Agnuside RS](#) in *Solvent*, with sonication. Dilute with methanol to obtain a concentration of about 0.125 mg/mL. Pass through a cellulose membrane filter of 0.45-µm or finer pore size.**Sample solution:** Place about 1000 mg of Powdered Chaste Tree in a container with a stopper. Extract twice with 40 mL of methanol, using a hand homogenizer at 19,000 rpm for 2 min. 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*, 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.**Solution A:** Acetonitrile**Solution B:** 5.88 g/L of phosphoric acid in water**Mobile phase:** See [Table 2](#).**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	7	93
0.6	10	90
5	10	90
7	14	86
13	15	95