

### 0036 - Chaste Tree for Flavonoids by HPLC

**Botanical Name:** *Vitex agnus-castus L.*

**Common Names:** Agnus-castus, Monk's pepper, Vitex

**Parts of Plant Used:** Berries

**Uses:** Relief of premenstrual syndrome and painful menstruation; treatment of corpus luteum insufficiency.

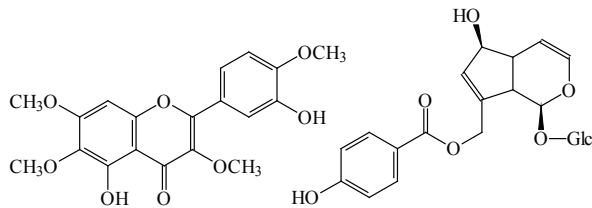
#### Modes of Action:

Several clinical trials have demonstrated positive effects of chaste tree for treating corpus luteum abnormalities, premenstrual syndrome, and menstrual cycle abnormalities. However, the compounds responsible for chaste tree berry's activities are unknown, although some diterpenes showed dopaminergic properties and were found to bind to recombinant DA<sub>2</sub>-receptor protein and to suppress prolactin release, which may result in improving premenstrual mastodynia and possibly other symptoms of the premenstrual syndrome.<sup>1-3</sup>

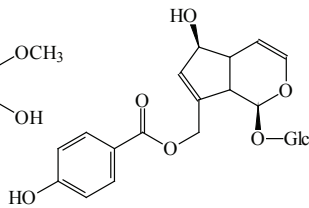


#### Chemistry and Chemical Markers for Quality Control:

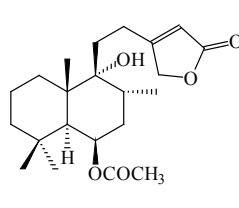
Several groups of natural products have been identified in chaste tree berries including flavonoids with casticin as the major lipophilic flavone, iridoid glycosides with agnuside as the major one, diterpenes such as rotundifuran, vitexilactone, vitetrifolin D, and vitexilactum A.<sup>4-6</sup> Other compounds purified from flowering tops and root barks of chaste tree include agnucastolide A, B, and C; mussaenosidic acid; 6'-O-p-hydroxybenzoylmussaenosidic acid; myzodendrone; luteolin 6-C-(4"-methyl-6"-O-trans-caffeoylglucoside); luteolin-6-C-(6"-trans-caffeoylglucoside); luteolin 7-O-(6"-p-benzoylglucoside); luteolin; artemetin; and isorhamnetin.<sup>7,8</sup> Dried chaste tree berries also contain 0.15% to 1% essential oil with sabinene, 1,8-cineole, (E)- $\beta$ -farnesene,  $\alpha$ -pinene, and  $\beta$ -caryophyllene as the major volatiles.<sup>9</sup> Currently in the market, casticin and agnuside are used as marker compounds for quality control of chaste tree berries.



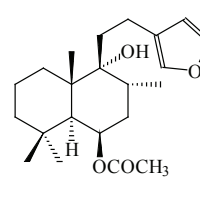
Casticin



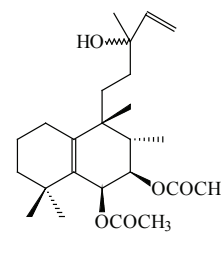
Agnuside



Vitexilactone



Rotundifuran



Vitetrifolin D

## Methods of Analysis

HPLC is the most accepted method for quality control of chaste tree extract. Several HPLC methods have been developed to analyze casticin, agnuside, and diterpenes in the berries.

### Method 1:

The method of Hoberg et al.<sup>10</sup> can be used for the analysis of casticin.

#### *Sample Preparation:*

Extract 1 g of sample twice by turbo extraction at 19,000 rpm with 40 mL of methanol for 2 minutes. Rinse the residues using a small amount of methanol. Combine the resulting solutions, evaporate to dryness, and dissolve the residue in 20 mL of methanol.

#### *Chromatography:*

Column: Hypersil ODS, 5  $\mu$ m, 125  $\times$  3.1 mm.

Mobile phase: Solvent A = water (0.5% phosphoric acid), solvent B = methanol.

Gradient:

Time (minutes)	%A	%B
0	50	50
13	35	65
13.1	0	100
18	0	100
18.1	50	50
25	50	50

Flow rate: 1.0 mL/minute

Injection volume: 10  $\mu$ L

Detection wavelength: 258 nm

Column temperature: 25°C

#### *Validation Data:*

Linearity: 25 to 125 mcg/mL with a correlation coefficient of 0.996.

Accuracy: The extract was spiked with 30%, 50%, and 80% of the expected amount of casticin; the percent recovery was 98.2 (2.2% RSD).

Precision: 2.6% RSD (six determinations)

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

### Method 2:

The method of Hoberg et al.<sup>11</sup> was used for the determination of agnuside.

### Sample Preparation:

Extract 1 g of sample twice by turbo extraction at 19,000 rpm with 40 mL of methanol for 2 minutes. Rinse the residues using a small amount of methanol. Combine the resulting solutions and evaporate to dryness. Dissolve the residue in 2 mL of water–methanol (95:5) and subject this solution to solid-phase extraction over neutral aluminum oxide. Adjust the final solution volume to 10 mL with water–methanol (95:5).

### Chromatography:

Column: Hypersil ODS, 5  $\mu$ m, 125 x 3.1 mm.

Mobile phase: Solvent A = water (0.5% phosphoric acid), solvent B =acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	93	7
0.6	90	10
5	90	10
7	86	14
13	85	15
13.1	0	100
18	0	100
18.1	93	7
23	93	7

Flow rate: 1.3 mL/minute

Injection volume: 10  $\mu$ L

Detection wavelength: 258 nm

Column temperature: 25°C

Injection volume: 5  $\mu$ L

Detection wavelength: 335 nm

Flow rate: 1 mL/minute

### Validation Data:

Linearity: 1.3 to 422 mcg/mL with a correlation coefficient of 0.9998.

Accuracy: The extract was spiked with 30%, 50%, and 80% of the expected amount of casticin; the percent recovery was 98.3.

Precision: 3.22% RSD (six determinations)

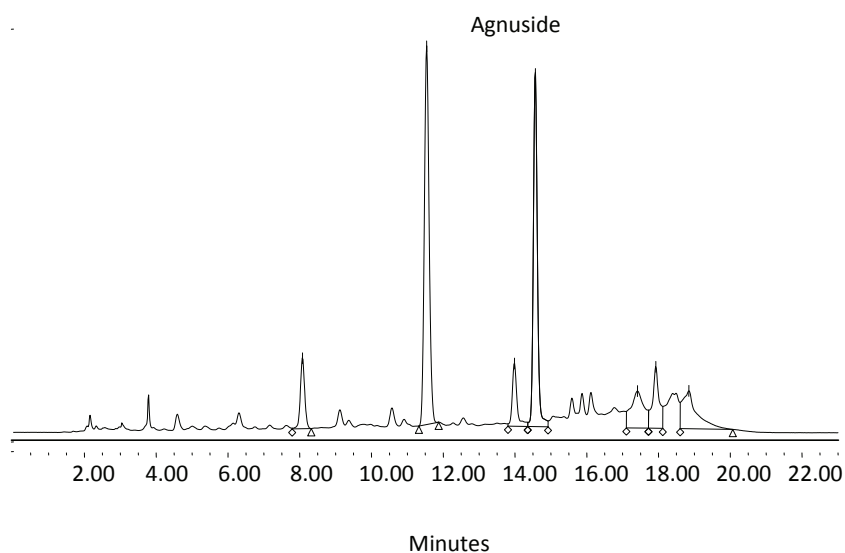
Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: Not specified

### Representative HPLC Chromatogram for Chaste Tree Berries Run by Method 2



#### Method 3:

The method of Hoberg et al.<sup>11</sup> was used for the determination of diterpenes, rotundifuran, vitexilactone, and 6 $\beta$ ,7 $\beta$ -diacetoxy-13-hydroxy-labda-8,14-diene (later on this compound was corrected to vitetrifolin D).

#### Sample Preparation:

For fruit powders, extract 1 g of fruits twice by turbo extraction at 19,000 rpm with 40 mL of methanol for 2 minutes, and rinse the residues using a small amount of methanol. Combine the resulting solutions and evaporate to dryness. Dissolve the residue in 10 mL of acetonitrile–water (4:1).

For extracts, dissolve 0.2 g of extract in 10 mL of acetonitrile–water (4:1).

#### Chromatography:

Column: Hypersil ODS, 5- $\mu$ m, 125  $\times$  3.1 mm.

Mobile phase: Solvent A = water, solvent B = acetonitrile.

Gradient:

Time (minutes)	%A	%B
0	60	40
5	52	48
30	52	48
30.01	0	100
35	0	100
35.01	60	40
45	60	40

Flow rate: 1.0 mL/minute

Injection volume: 20  $\mu$ L

Detection wavelength: 210 nm

Column temperature: 25°C

**Validation Data:**

Linearity: 31 to 155, 26 to 130, and 52 to 260 mcg/mL for vitexilactone, vitetrifolin D, and rotundifuran, respectively, with correlation coefficients greater than 0.996.

Accuracy: The percent recoveries were 105, 103, and 100 for vitexilactone, vitetrifolin, and rotundifuran, respectively.

Precision: RSD was less than 3.4% for three compounds (six determinations).

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD: 1.3 mcg for rotundifuran, 0.325 mcg for vitetrifolin D, and 0.069 mcg for vitexilactone.

**Method 4:**

The unpublished method of Mingfu Wang was used for the determination of agnuside.

**Sample Preparation:**

Weigh 400 mg of extract into a 50-mL volumetric flask. Add 35 mL of 70% methanol aqueous solution. Sonicate the sample for 30 minutes. Cool to room temperature and dilute to volume with 70% methanol.

**Chromatography:**

Column: Phenomenex Luna, 5 $\mu$ m, 4.6  $\times$  250 mm.

Mobile phase: Solvent A = water (0.1% phosphoric acid), solvent B = acetonitrile

Gradient:

Time (minutes)	%A	%B
0	90	10
10	80	20
15	70	30
20	50	50
21	90	10
30	90	10

Flow rate: 1 mL/minute

Detector: 258 nm

Injection volume: 10  $\mu$ L

**Validation Data:**

Not available.

## References:

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3. Loch EG, Selle H, Boblitz N. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing *Vitex agnus castus*. *J Women Health Gen Based Med*. 2000;9(3):315–20.
4. Hoberg E, Orjala J, Meier B, et al. Diterpenoids from the fruits of *Vitex agnus-castus*. *Phytochemistry*. 1999;52(8):1555–8.
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6. Li SH, Zhang HJ, Qiu SX, et al. Vitexlactam A, a novel labdane diterpene lactam from the fruits of *Vitex agnus-castus*. *Tetrahedron Lett*. 2002;43(29):5131–4.
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