Vitex negundo Leaf – Identification

Thin-Layer Chromatography

![Typical HPTLC Chromatogram](image)

*These chromatograms are supplied for information only*

**Track assignment:** 1-3) Agnuside (3 mg/mL); 4-6) Negundoside (3 mg/mL); 7-13) Vitex negundo Leaf, commercial samples; 14-15) Vitex negundo Leaf Dry Extracts, commercial samples (30 mg/mL).

**Sample solutions:** according to the monograph

**Standard solutions:** in methanol

**Plate:** HPTLC, Silica gel 60 F<sub>254</sub>, 5 µm

**Saturation time:** 20 minutes

**Application volume:** 5 µL, as 8-mm bands

**Relative Humidity:** about 33%

**Developing solvent system:** ethyl acetate, glacial acetic acid, and water (80:10:5)

**Developing distance:** 7 cm

**Derivatization reagent:** anisaldehyde-sulfuric acid reagent (a mixture of 170 mL of ice cold methanol with 20 mL of glacial acetic acid, 10 mL sulfuric acid, and 1 mL anisaldehyde)

**Detection:** derivatize, heat at 100°C for 3 min, and examine under visible light.
HPLC (Negundoside)

Representative chromatogram of Content of Iridoid Glycosides in *Vitex negundo* Leaf

*This chromatogram is supplied for information only*

**Solution preparation:** according to the monograph

**Mode:** HPLC

**Detector:** UV, 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1 (Similar to Lichrospher 100 RP 18)

**Column temperature:** 25°±1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**Solution A:** dissolve 0.14 g of potassium phosphate monobasic in 900 mL of water, add 0.5 mL of o-phosphoric acid, complete to 1 L with water

**Solution B:** acetonitrile

**Mobile phase:** see *Table 1*
Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
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