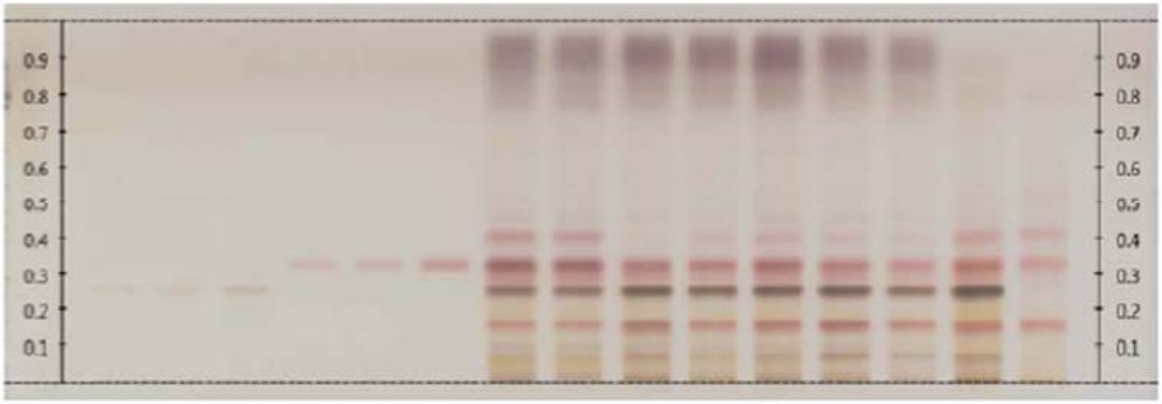
Vitex negundo Leaf – Identification

Thin-Layer Chromatography



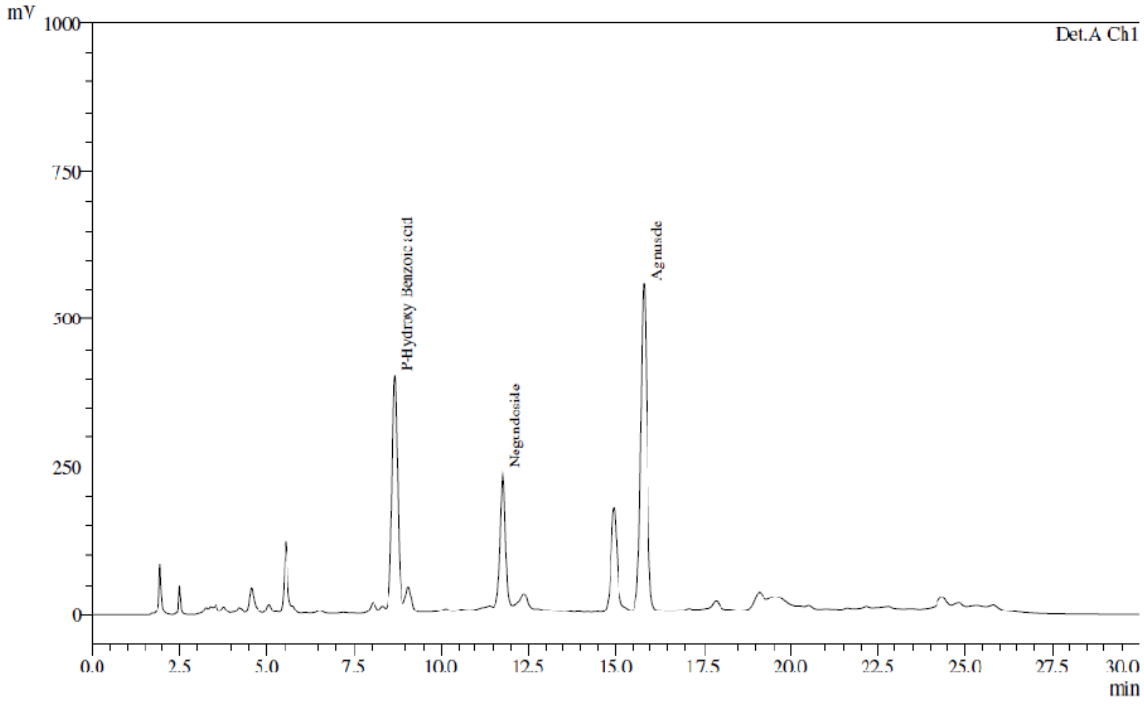
Typical HPTLC Chromatogram

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_{254}, 5 µm
Saturation time:	20 minutes
Application volume:	2 µL, as 8-mm bands
Relative Humidity:	about 33%
Developing solvent system:	ethyl acetate, glacial acetic acid, and water (80:10:5)
Developing distance:	6 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

Time (min)	Solution A (%)	Solution B (%)
0.01	90	10
10	85	15
20	80	20
23	80	20
25	85	15
30	90	10
35	90	10