Chinese chastetree leaf, huang jing ye *(Vitex negundo)*

1. Scope
This method identifies dried leaves of Chinese chastetree (*Vitex negundo* L.) by HPTLC fingerprint.

2. Source of method
CAMAG Laboratory/USP Herbal Medicines Compendium

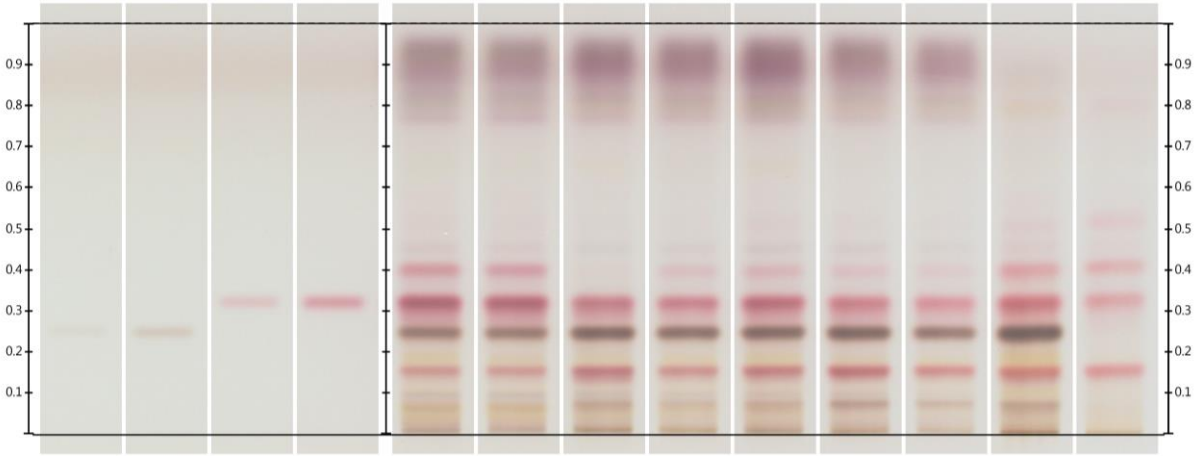
3. Procedure

Test solution:	Mix 0.5 g of powdered sample with 5 mL of methanol (or 0.1 g of extract with 3 mL of methanol) and sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant / filtrate as test solution.
Reference solution:	Dissolve 3 mg each of negundoside and agnuside in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of reference solutions and test solutions
Mobile phase:	Ethyl acetate, glacial acetic acid, water 80:10:5 (v/v/v)
Development:	- Saturated chamber - Developing distance 70 mm from lower edge - Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: Mix 170 mL of ice-cooled methanol with 20 mL of glacial acetic acid, 10 mL of sulfuric acid and 1 mL of p-anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Anisaldehyde reagent, white RT

4. Results

Note: These chromatographic fingerprints are representative of the samples used in this particular analysis. Fingerprints obtained may vary from sample to sample. Analysts must validate the most appropriate fingerprint for their identity standard.

Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Agnuside (3 mg/mL)	**8**	**2 µL**	**Chinese chastetree leaf 4**
2	6 µL	Agnuside (3 mg/mL)	**9**	**2 µL**	**Chinese chastetree leaf 5**
3	2 µL	Negundoside (3 mg/mL)	**10**	**2 µL**	**Chinese chastetree leaf 6**
4	6 µL	Negundoside (3 mg/mL)	**11**	**2 µL**	**Chinese chastetree leaf 7**
5	**2 µL**	**Chinese chastetree leaf 1**	**12**	**2 µL**	**Chinese chastetree leaf extract 1**
6	**2 µL**	**Chinese chastetree leaf 2**	**13**	**2 µL**	**Chinese chastetree leaf extract 2**
7	**2 µL**	**Chinese chastetree leaf 3**			

System suitability test

Agnuside: grey zone at R_F ~ 0.25
Negundoside: pink zone at R_F ~ 0.32

Identification

Compare result with reference images. The fingerprint of the test solution prepared from a sample is similar to those prepared from corresponding botanical reference samples (marked in bold). Additional weak zones may be present.
The chromatogram of the test solution shows an intense pink zone at R_F ~ 0.32, corresponding to reference negundoside and an intense brown zone at R_F ~ 0.25, corresponding to reference agnuside. There is a reddish zone below the position of agnuside and a (possibly faint) reddish zone above the position of negundoside. Samples of leaf also show a broad violet cluster of zones close to the solvent front.