Chaparral leaf (*Larrea tridentata*)

1. Scope

This method identifies dried Chaparral leaf (*Larrea tridentata* (Sessé & Moç. Ex DC.) Coville, syn. *Larrea mexicana* Moric) based on its HPTLC fingerprint of flavonoids. An additional HPTLC method for the detection of nordihydroguaiaretic acid (NDGA) is provided.

2. Source of method

Starwest Botanicals/CAMAG Laboratory

3. Procedure

A) Method for flavonoids

Test solution:	Mix 0.5 g of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant / filtrate as test solution.
Reference solution:	Individually dissolve 1 mg of rutin and 5 mg of hyperoside in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of reference solutions, 4 µL of test solution
Mobile phase:	Ethyl acetate, acetic acid, formic acid, water 100:11:11:26 (v/v/v/v)
Development:	- Saturated chamber - Developing distance 70 mm from lower edge - Relative humidity 33%
Derivatization reagent:	1.) Natural products reagent (NP reagent) Preparation: dissolve 1 g of 2-aminoethyl diphenyl-borinate in 200 mL of ethyl acetate. 2.) Polyethylene glycol reagent (PEG reagent) Preparation: dissolve 10 g of polyethylene glycol 400 in 200 mL of dichloromethane. Use: heat plate at 100°C for 3 min, dip (time 0, speed 5) the warm plate first in NP reagent, dry and dip in PEG reagent.
Documentation:	NP and PEG reagents, UV 366 nm

B) Method for detection of nordihydroguaiaretic acid

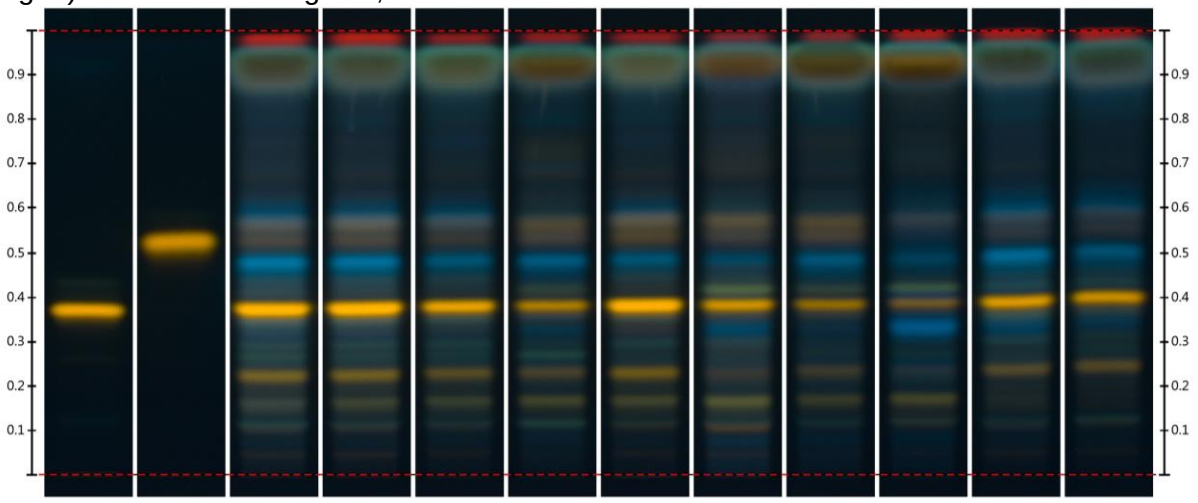
Test solution:	Mix 0.5 g of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant / filtrate as test solution.
Reference solution:	Dissolve 1 mg of nordihydroguaiaretic acid (NDGA) in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of reference solution, 2 µL of test solution
Mobile phase:	Dichloromethane, methanol 70:9.2 (v/v)
Development:	- Saturated chamber - Developing distance 70 mm from lower edge - Relative humidity 33%
Derivatization reagent:	Fast Blue salt B reagent Preparation: dissolve 2 g of Fast Blue salt B reagent in 200 mL of water. Use: dip the plate (time 1, speed 5) and dry under a stream of cold air.
Documentation:	Fast Blue salt B reagent, white light 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.

A) Method for flavonoids

Fig. 1) NP and PEG reagents, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Rutin	**7**	**4 µL**	**Chaparral leaf 5**
2	2 µL	Hyperoside	**8**	**4 µL**	**Chaparral leaf 6**
3	**4 µL**	**Chaparral leaf 1**	**9**	**4 µL**	**Chaparral leaf 7**
4	**4 µL**	**Chaparral leaf 2**	**10**	**4 µL**	**Chaparral leaf 8**
5	**4 µL**	**Chaparral leaf 3**	**11**	**4 µL**	**Chaparral leaf 9**
6	**4 µL**	**Chaparral leaf 4**	**12**	**4 µL**	**Chaparral leaf 10**

System suitability test

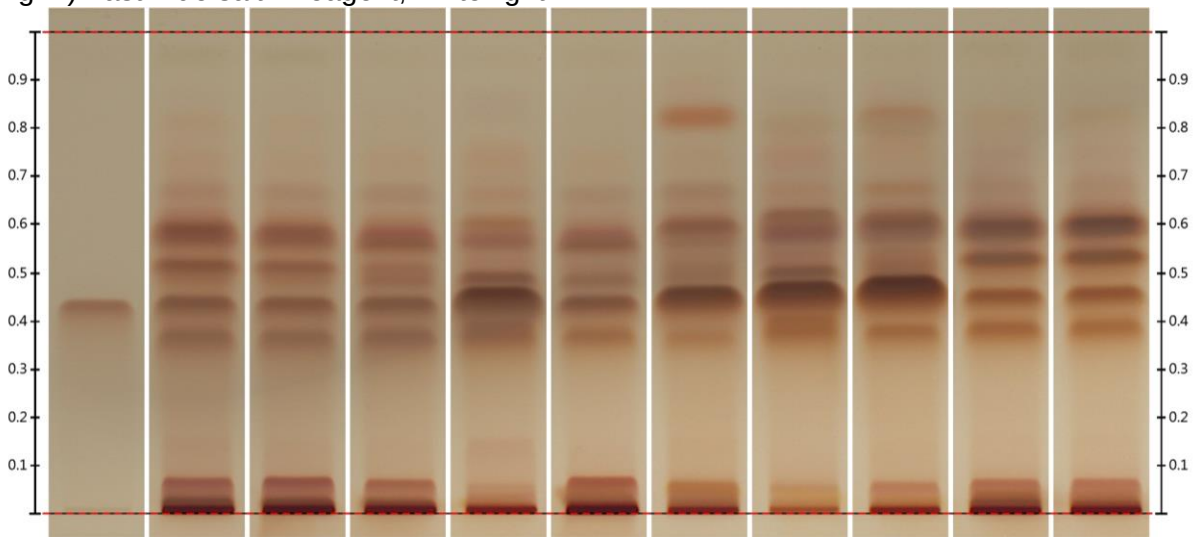
Rutin: an orange fluorescent zone at R_F ~ 0.37
Hyperoside: an orange fluorescent zone at R_F ~ 0.53

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.
Under UV 366 nm the derivatized chromatogram of the test solution shows an intense orange fluorescent zone corresponding in color and position to reference solution rutin. In the lower third there are two to three faint fluorescent yellowish zones. There is a blue fluorescent zone between the position of the references rutin and hyperoside. Right above this zone there is a cluster of two diffuse orange and one blue fluorescent zone. Below the solvent front there is a broad greenish brown zone and a red zone (corresponding to chlorophyll).

B) Method for detection of nordihydroguaiaretic acid

Fig. 2) Fast Blue salt B reagent, white light RT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Nordihydroguaiaretic acid	**7**	**2 µL**	**Chaparral leaf 6**
2	**2 µL**	**Chaparral leaf 1**	**8**	**2 µL**	**Chaparral leaf 7**
3	**2 µL**	**Chaparral leaf 2**	**9**	**2 µL**	**Chaparral leaf 8**
4	**2 µL**	**Chaparral leaf 3**	**10**	**2 µL**	**Chaparral leaf 9**
5	**2 µL**	**Chaparral leaf 4**	**11**	**2 µL**	**Chaparral leaf 10**
6	**2 µL**	**Chaparral leaf 5**			

System suitability test

Nordihydroguaiaretic acid: a brown zone at $R_F \sim 0.43$

Identification

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.

Under white light the derivatized chromatogram of the test solution shows a brown zone corresponding to reference nordihydroguaiaretic acid. There is one brown zone below and two to three brown zones right above nordihydroguaiaretic acid. Other, possibly faint, brown zones may be seen right above the application position and in the upper third of the chromatogram.