PHCOG MAG: Analysis of Plant Drugs HPTLC Identification of Chaste tree fruits (*Vitex agnus-castus*)

Courtesy : CAMAG

Method: HPTLC
Sample for Identification: Hydrophilic flavonoids

Sample preparation:
0.75 g of powdered drug (fruits) is extracted for 2 min with 30 mL methanol in a turbo-extractor (polytron). After centrifuging for 5 min the supernatant is removed and set aside. The sediment is again extracted for 2 min with 30 mL methanol in the polytron, then centrifuged for 5 min. The combined supernatants are evaporated to dryness and reconstituted with 5 mL methanol. This is the test solution.

Standards (optional) preparation:
1 mg chlorogenic acid and 2 mg of iso-orientin are dissolved in 5 mL methanol.

Derivatization reagents:
Natural Products reagent (NP reagent): 1 g of diphenylborinic acid aminoethylester is dissolved in 200 mL ethyl acetate.
Macrogol reagent: 10 g of polyethylene glycol 400 (macrogol) are dissolved in 200 mL dichloromethane.

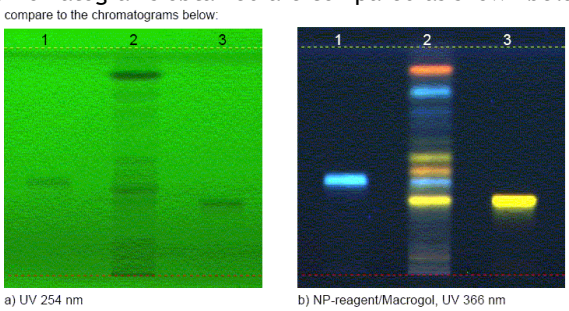
Chromatographic conditions:
Stationary phase:
HPTLC plates silica gel 60 F254 (Merck), 10x10 cm or 20x10 cm.

Mobile Phase:
Tetrahydrofurane, toluene, formic acid, water (8:4:1:0.5)

Sample application:
2 µL test solution and standard are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.
Development:
CAMAG 10x10 cm or 20x10 cm Horizontal Developing Chamber (HDC), unsaturated, 2 mL (respectively 5 mL) developing solvent.
Developing distance 60 mm from lower edge of plate. The plate is then dried with a hair dryer (warm air) for 5 min.

Detection:
a) UV 254 nm
b) NP/Macrogol reagent: the plate is heated at 100°C for 3 min, then dipped while still hot in NP reagent, dried in a stream of cold air, then dipped in Macrogol reagent. Examination under UV 366 nm.

Results:
Chromatograms obtained are compared as shown below:



Track 1: Chlorogenic acid,
Track 2: *Vitex agnus castus* (Croatia),
Track 3: iso-orientin

a) UV 254 nm
The reference solution shows a dark band for iso-orientin at Rf = 0.31 and a dark band for
chlorogenic acid at Rf = 0.41. The test solution shows strong dark bands at Rf = 0.31 (isoorientin), Rf = 0.36, and at Rf = 0.88. There is a faint band at the position of chlorogenic acid and for additional weak bands between Rf = 0.45 and Rf = 0.8.

b) NP-reagent/Macrogol, UV 366 nm

The reference solution shows a strong yellow band for iso-orientin at Rf = 0.31 and a light blue band for chlorogenic acid at Rf = 0.41. The test solution shows bands matching those of iso-orientin and chlorogenic acid in color and Rf. There is a faint band at Rf = 0.07, a yellow brown band at Rf = 0.45, a olive green band at Rf = 0.51, a dark blue band at Rf = 0.70, a light blue band a Rf = 0.78 and an orange band at Rf = 0.88

NOTE: The flavonoid pattern of various samples of *Vitex agnus castus* does not vary significantly.

However, the content of flavonoids of some samples may be very low.

COURTESY : CAMAG APPLICATION NOTES

PHCOG MAG. is thankful to Dr. Marcel Hug, Camag, Switzerland for permission to use Application Notes.