

HPTLC Application for *Passiflora incarnata*

Tiên Do, Scientific Support Specialist / Deputy head of laboratory (CAMAG), tien.do@camag.com

Melanie Broszat, Scientific Business Development Manager (CAMAG), melanie.broszat@camag.com

Matthias Nold, Global Product Manager Reference Materials, matthias.nold@sial.com



In previous articles we presented HPTLC (High-Performance Thin-Layer Chromatography) applications for a series of medicinal plants, such as Ginkgo (Analytix 5/2016), Hypericum (Analytix 1/2017) or Ginseng (Analytix Reporter Issue 2/2018). With this article, we continue this series with an application note for passion flower, to further demonstrate the effectiveness of HPTLC for fingerprints of botanicals. Our comprehensive offering of analytical reagents and standards includes all consumables (TLC/HPTLC plates, solvents, analytical

standards and extract reference materials) used for this application.

Passiflora incarnata (passion flower) has been familiar to native Latin Americans for many centuries. The Spanish physician Monardes discovered it in 1569 in Peru. In Europe, passion flower was long used as an ornamental plant. In the second half of the last century, passion flower entered the field of homeopathy. In low potencies it is used as a sedative, and also has a cardiogenic and nerve calming effect. Passion flower is primarily found in the tropical rainforest, and for production of the herbal drug, it is mainly grown in India, US (Florida), Italy and Spain.

TLC fingerprint analysis is stipulated for identification of herbal drugs in most pharmacopoeias. In the passion flower herb, several glycosylated flavonoids are present. After derivatization with Natural Product reagent, they can sensitively be detected via the HPTLC fingerprint analysis shown in this article.

Results & Discussion

With the demonstrated method according to the HPTLC Association¹, a rapid identification of *Passiflora incarnata* by HPTLC fingerprint analysis is possible. Reference

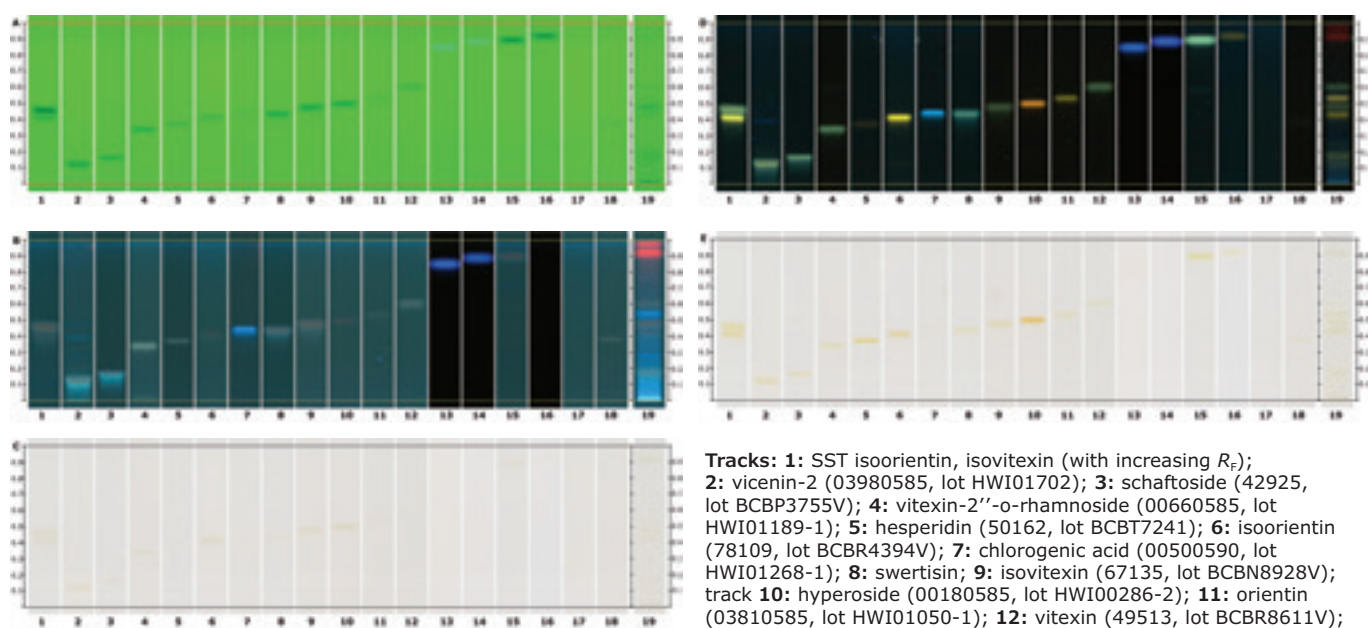


Figure 1: Chromatograms under UV 254 nm (A), UV 366 nm (B) and white light (C) prior to derivatization, and under UV 366 nm after NP/PEG derivatization (D), and white light after NP/PEG derivatization (E).

Tracks: 1: SST isoorientin, isovitexin (with increasing R_f); 2: vicenin-2 (03980585, lot HWI01702); 3: schaftoside (42925, lot BCBP3755V); 4: vitexin-2''-o-rhamnoside (00660585, lot HWI01189-1); 5: hesperidin (50162, lot BCBT7241); 6: isoorientin (78109, lot BCBR4394V); 7: chlorogenic acid (00500590, lot HWI01268-1); 8: swertisin; 9: isovitexin (67135, lot BCBN8928V); track 10: hyperoside (00180585, lot HWI00286-2); 11: orientin (03810585, lot HWI01050-1); 12: vitexin (49513, lot BCBR8611V); 13: scopoletin (38332, lot BCBS2552V); 14: umbelliferone (54826, lot BCBR1694V); 15: apigenin (01760595, lot HWI00086-1); 16: chrysin (95082, lot BCBP9460V); 17: oleanolic acid (42515, lot BCBT0832); 18: α -terpineol (04899, lot BCBS7535V); 19: *Passiflora incarnata* Extract Reference Material (05085001, lot HWI01280).

substances are applied as a system suitability test to qualify the results (R_F values have to be in the specified tolerance window of ΔR_F 0.025). Furthermore, reference substances of glycosylated flavonoids present in *Passiflora incarnata* are applied.

System suitability test

(under UV 254 nm, ΔR_F 0.025; min_{height} 0.010 AU)

- Isoorientin (0.15 mg/mL): a quenching zone at $R_F \sim 0.041$
- Isovortexin (0.15 mg/mL): a quenching zone at $R_F \sim 0.046$

For our tests, a reference extract from HWI (track 19) was used. Zones present in the extract corresponded in color and position to those of the standards vicenin-2, schaftoside, isoorientin, isovortexin, orientin, and vitexin. The obtained HPTLC fingerprint was similar to the *Passiflora* herb chemotype isovortexin¹.

HPTLC fingerprint analysis allows a rapid identification of many samples in parallel without a time-consuming and cost-intensive sample preparation. Evaluation by visual comparison is simple and convenient. HPTLC can be considered the method of choice for identity testing of plant materials.

Reference

1. www.hptlc-association.org: Passion flower herb and passion flower dry extract identification (version 2018-05-07)

Chromatography according to USP <203>

Stationary phase	HPTLC Si 60 F ₂₅₄ , 20 x 10 cm
Sample application	Bandwise application with ATS 4, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 4 µL for test solutions and standards
Developing solvents	Ethyl acetate, formic acid, water, ethyl methyl ketone, (50:10:10:30; v/v/v/v)
Development	In the ADC 2 with chamber saturation (with filter paper) for 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride (MgCl ₂).
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the ADC 2
Derivatization	NP reagent: 5 mg/mL of 2-aminoethyl diphenylborinate in ethyl acetate; PEG reagent: 50 mg/mL of polyethylene glycol 400 in dichloromethane
For spraying, before the derivatization: mix NP reagent and PEG reagent (1:1, v/v)	Heat at 100 °C for 3 min, let the plate cool down, derivatize (spray with nozzle: green, spraying level: 3; spraying volume: 3 mL) with the mixture, dry the plate for 2 min in cold air flow and take picture

Featured Products

Description	Package Size	Cat. No.
Analytical Standards		
Chlorogenic acid*	25 mg	00500590
Vitexin 2-O-rhamnoside*	10 mg	00660585
Apigenin	10 mg	01760595
Chrysin	50 mg	95082
Hesperidin	10 mg	50162
Hyperoside*	25 mg	00180585
Isoorientin	10 mg	78109
Isoorientin*	10 mg	03820585
Isovortexin	5 mg	67135
Isovortexin*	10 mg	01120590
Oleanolic Acid	10 mg	42515
Oleanolic Acid*	10 mg	03920590
Orientin*	10 mg	03810585
Schaftoside	5 mg	42925
Scopoletin	10 mg	38332
α-Terpineol	250 mg	04899
α-Terpineol*	100 mg	03420590
Umbelliferone	50 mg	54826
Vicenin-2*	10 mg	03980585
Vitexin	10 mg	49513
Vitexin*	10 mg	00840595
Extract Reference Material		
<i>Passiflora incarnata</i> extract	500 mg	05085001
Quantified Components:		Isovortexin
Qualitatively Confirmed Components:		Vitexin, Orientin, Homoorientin, Isovortexin
TLC Plates		
HPTLC glass plate silica gel 60 F ₂₅₄ 20x10 cm	50 Plates	1.05642

*HWI reference standard

Find all available analytical standards for phytochemicals listed on [SigmaAldrich.com/medicinalplants](https://www.sigmaaldrich.com/medicinalplants)

An overview of all plant extract reference materials can be found at [SigmaAldrich.com/plantextracts](https://www.sigmaaldrich.com/plantextracts)

Learn more about the features of High Performance Thin Layer Chromatography plates at [SigmaAldrich.com/tlc](https://www.sigmaaldrich.com/tlc)