

HPTLC Application for *Passiflora incarnata*

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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 cardiotonic 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 Association,¹ a rapid identification of *Passiflora incarnata* by HPTLC fingerprint analysis is possible. Reference 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

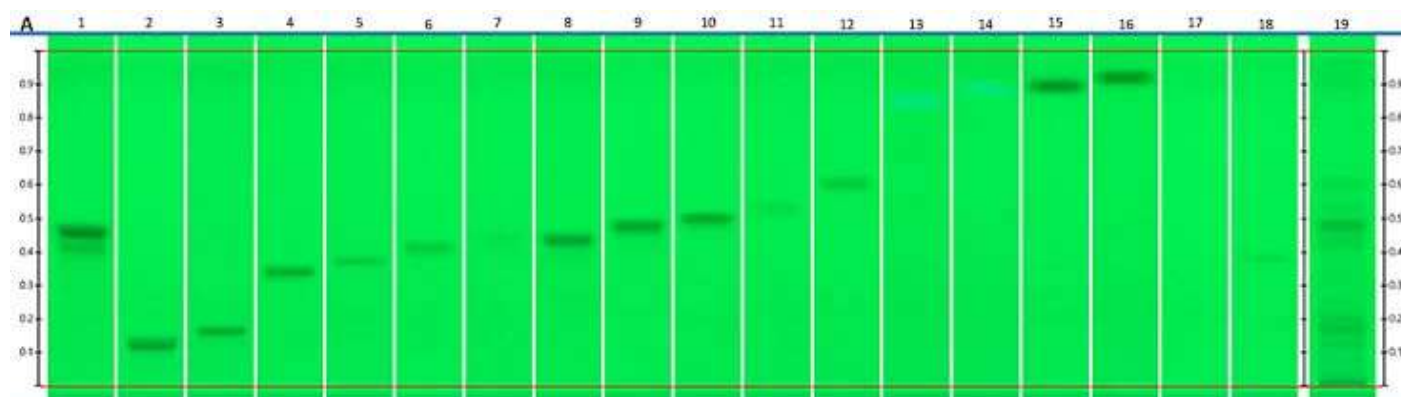
(under UV 254 nm, ΔR_F 0.025; \min_{height} 0.010 AU)

- Isoorientin (0.15 mg/mL): a quenching zone at $R_F \sim 0.041$
- Isovitexin (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 vicianin-2, schaftoside, isoorientin, isovitexin, orientin, and vitexin. The obtained HPTLC fingerprint was similar to the *Passiflora* herb chemotype isovitexin.¹

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.

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



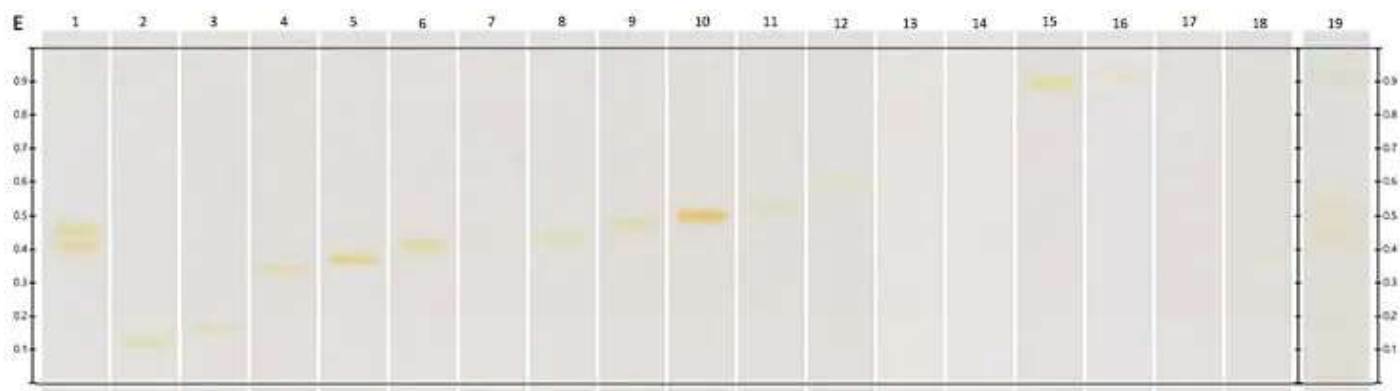
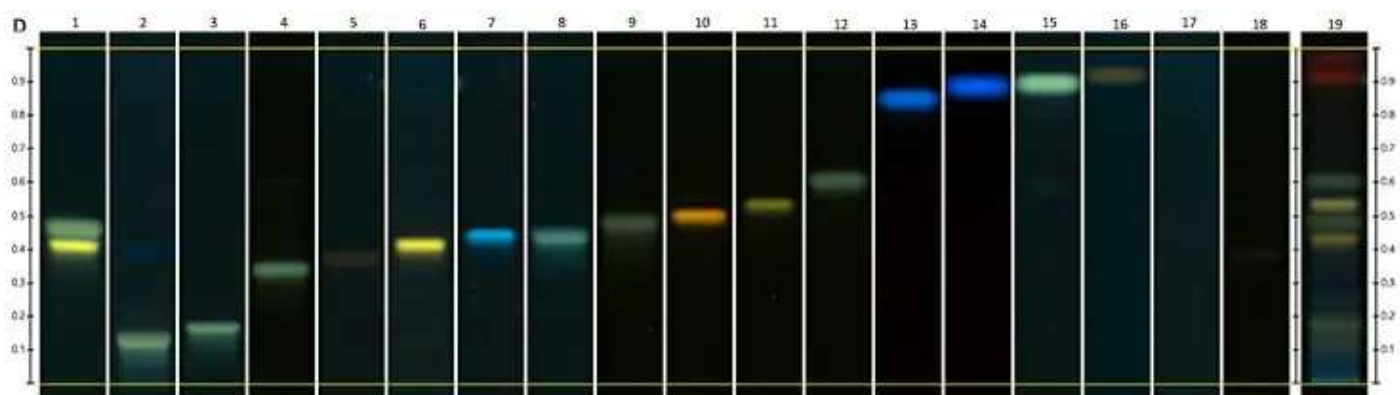
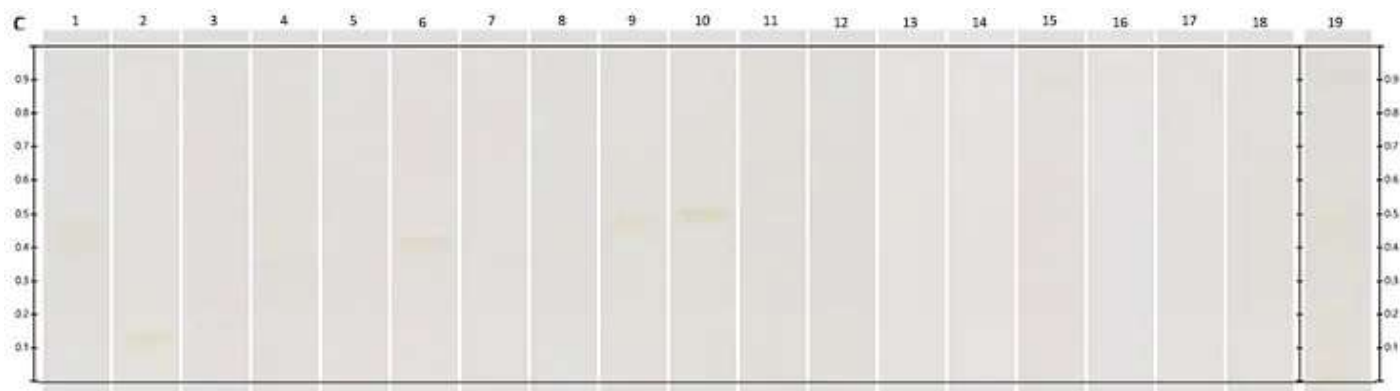
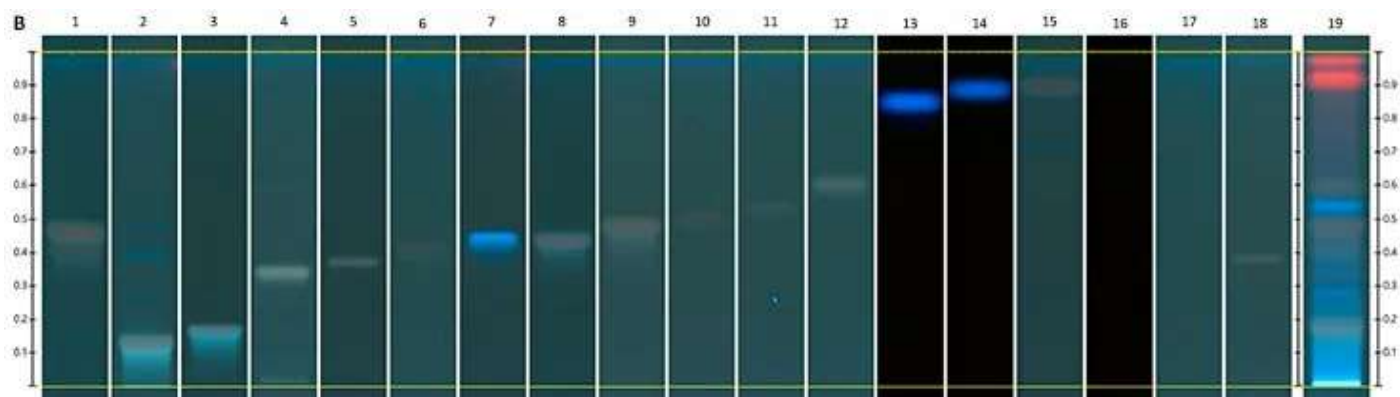


Figure 1: Chromatograms under UV 254 nm (A), UV 366 nm (B) and white light (C) prior to derivatization, and under UV 366 nm after NP/PEG derivatization (D), and white light after NP/PEG derivatization (E).

Tracks: 1: SST isoorientin, isovitexin (with increasing *RF*); 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).

Materials

Product No.	Description	SDS	Pricing
01760595	Apigenin primary reference standard	↓	Expand ▾
00500590	Chlorogenic acid primary reference standard	↓	Expand ▾
95082	Chrysin analytical standard	↓	Expand ▾
50162	Hesperidin analytical standard	↓	Expand ▾
1.05642	HPTLC Silica gel 60 F₂₅₄ pkg of 50 plates, plate L × W 20 cm × 10 cm, glass support		Expand ▾
00180585	Hyperoside primary reference standard	↓	Expand ▾
03820585	Isoorientin primary reference standard	↓	Expand ▾
78109	Isoorientin analytical standard	↓	Expand ▾
01120590	Isovitexin primary reference standard	↓	Expand ▾
67135	Isovitexin analytical standard	↓	Expand ▾
42515	Oleanolic acid analytical standard	↓	Expand ▾

Product No.	Description	SDS	Pricing
03920590	Oleanolic acid primary reference standard	↓	Expand ▾
03810585	Orientin primary reference standard	↓	Expand ▾
05085001	Passiflora incarnata extract secondary reference standard		Expand ▾
42925	Schaftoside analytical standard	↓	Expand ▾
38332	Scopoletin analytical standard	↓	Expand ▾
04899	α-Terpineol analytical standard	↓	Expand ▾
03420590	α-Terpineol primary reference standard	↓	Expand ▾
54826	Umbelliferone analytical standard	↓	Expand ▾
03980585	Vicenin 2 primary reference standard	↓	Expand ▾

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Reference

1. *Passion flower herb and passion flower dry extract identification (version 2018-05-07)*. [Internet]. Available from: <https://www.hptlc-association.org/home.cfm>