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Report

Project		P585-02 Hydrastis extract BSC HESI			
Related documents		[1] *Hydrastis canadensis* – root and rhizome_HPTLC Association_USP 41-NF36_V2: [2] PhEur Monograph 1831 Goldenseal rhizome			
Customer		HESI			
Project objective		Identification of a goldenseal extract			
Date	19.07.2022	Laboratory	CAMAG, Muttenz	Analyst	ER

Summary

1. The **extract** (Lot RK-3-30-1-HC) received for this study was compared to several samples of Goldenseal root and rhizome using the developing solvents of the USP [1] (Figure 1, Test 1) and the PhEur [2] (Figure 2, Test 2) monographs on goldenseal rhizome
2. The fingerprint of the **extract** (track 3) is similar to those of the plant samples in both methods (Figures 1 and 2).
3. Additional derivatization with ninhydrin reagent (Figure 3) reveals the absence of at least two zones in the lower part of the fingerprint. Those zones are present in most samples of Godenseal root and rhizome (black arrows) but they are faint in one sample (Track 4).

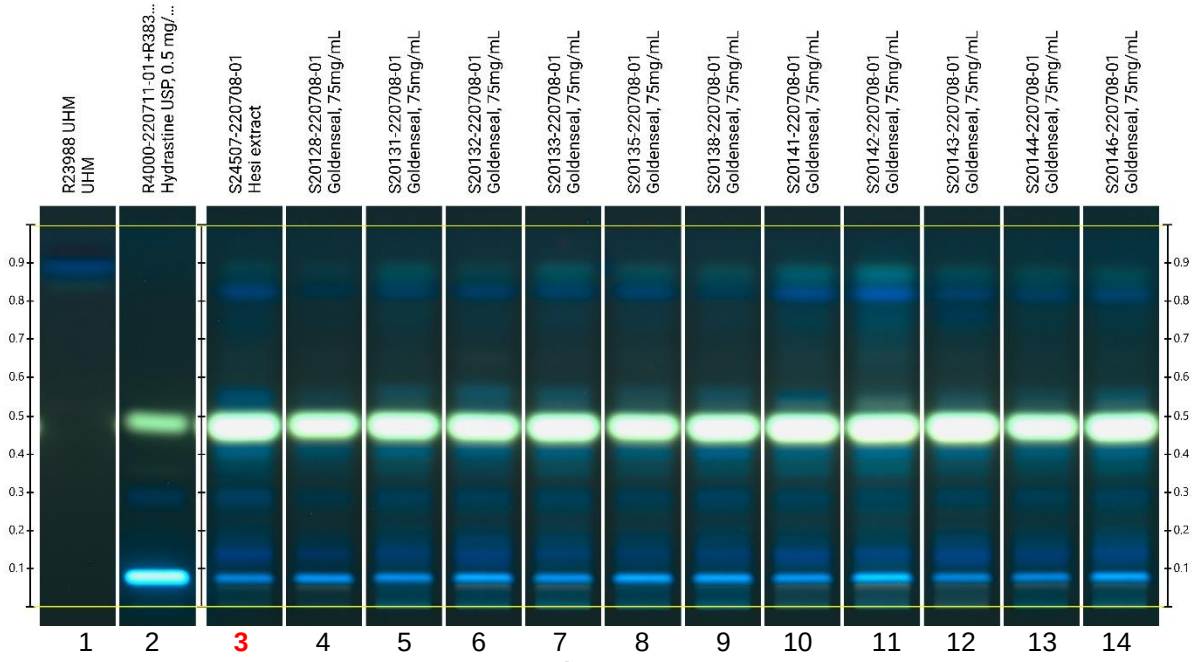


Figure 1

Fingerprints of Goldenseal with the USP method [1], long wave UV (350 nm broadband); track 2: hydrastinine, hydrastine, berberine (with increasing R_F)

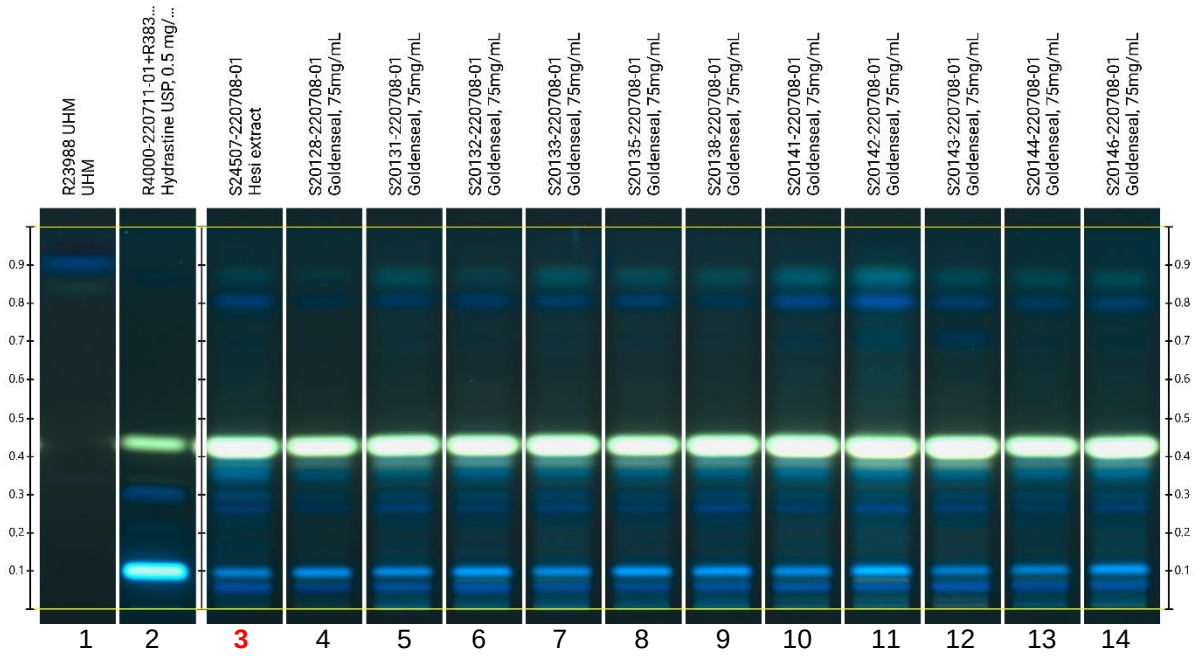


Figure 2

Fingerprints of Goldenseal with the PhEur method [2] long wave UV (350 nm broadband); track 2: hydrastinine, hydrastine, berberine (with increasing R_F)

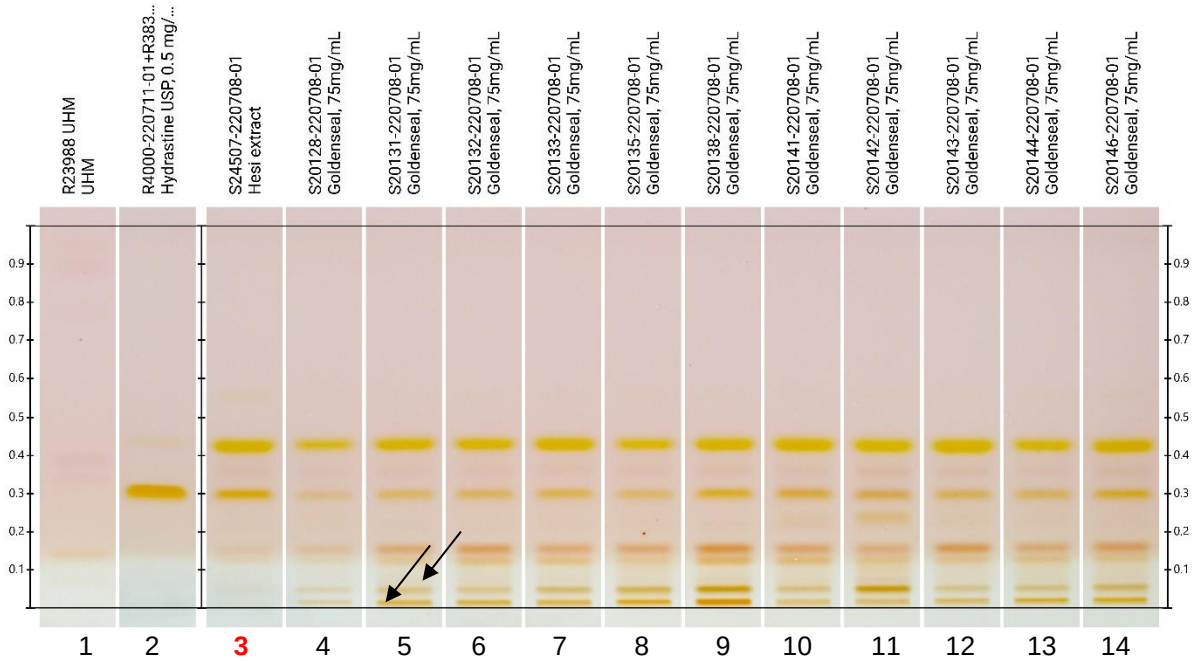


Figure 3

Fingerprints of Goldenseal with the PhEur method [2], derivatization with ninhydrin reagent, white light RT; track 2: hydrastinine, hydrastine, berberine (with increasing R_F)

Conclusion

The **extract** (Lot RK-3-30-1-HC) is identified as an extract from Goldenseal. It lacks a few zones of alkaloids or amino acids, which are found in most of the investigated samples of Goldenseal root and rhizome.

Experimental details

Samples (S) and reference materials

S24507	Goldenseal extract	MRIGlobal, Supplier: Uni Mississippi, Lot RK-3-30-1-HC
S20128	Goldenseal root and rhizome	CAMAG
S20131	Goldenseal root and rhizome	CAMAG
S20132	Goldenseal root and rhizome	CAMAG
S20133	Goldenseal root and rhizome	CAMAG
S20135	Goldenseal root and rhizome	CAMAG
S20138	Goldenseal root and rhizome	CAMAG
S20141	Goldenseal root and rhizome	CAMAG
S20142	Goldenseal root and rhizome	CAMAG
S20143	Goldenseal root and rhizome	CAMAG
S20144	Goldenseal root and rhizome	CAMAG
S20146	Goldenseal root and rhizome	CAMAG
R23988	UHM	In-house - 2202211
R4000	Hydrastine HCl	USP Lot F0E204
R383	Hydrastinine HCl	Sigma 95F0155
R22072	Berberine HCl	BP batch 3504

Chemicals

Name	Manufacturer	Purity/quality	Batch
Methanol	Roth	Rotisolv	0002001863
Ethyl acetate	Acros	99.5%	271888
Formic acid	Thermo Scientific	98+ %	A0438424
Acetic acid	Acros	99.5%	A0427447
Water	inhouse	De-ionized	-
Ninhydrin	Fluka	p.a.	SZBA2910
Acetone	Acros	99+ %	2196727
Na_2HPO_4 x 2 H_2O	MERCK	99+ %	389320/1
NaH_2PO_4 x H_2O	Sigma	99+ %	BCBH7651V

Equipment

Name, article	Manufacturer
Automatic TLC Sampler 4	CAMAG
TLC Plate Heater III	CAMAG
Automatic Development Chamber ADC 2	CAMAG
Visualizer	CAMAG
Derivatizer	CAMAG
Filter paper for chamber saturation	CAMAG
Tube Mill control	IKA
Centrifuge EBA21	Hettich
Ultrasonic Bath SW 3H	Sono Swiss
Analytical Balance MS 205 DU	Mettler-Toledo
Pioneer Balance PA4120C	Ohaus

Sample preparation

Sample solutions:	75.0 mg/mL of powdered Goldenseal; 25.0 mg/mL of extract in methanol – water 8:2 (v/v). Sonicate for 10 min, centrifuge and use the supernatant.
Standard solutions:	Standards were prepared in methanol, 0.50 mg/mL hydrastine HCl and hydrastinine HCl; 0.025 mg/mL berberin HCl
Plate:	HPTLC, Si 60 F_{254} (Merck); HX87944542

TEST 1

Application

Instrument: ATS 4
Band length: 8.0 mm, Distance between tracks: 11.4 mm, Application position X: 20.0 mm Y: 8.0 mm

A special wash solution (methanol, acetone, phosphate buffer (1 mM) 7.5:1.5:1 (*v/v*)) was applied on Track 1 after each sample, to prevent cross contamination with berberin.

Tr.	Vial ID	Description	Vol. (µl)	Position	Type	SST
1	Wash 1	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	A5	Sample	☐
+	Wash 2	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	A7	Sample	
+	Wash 3	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	A9	Sample	
+	Wash 4	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	A11	Sample	
+	wash 5	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	B2	Sample	
+	wash 6	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	B4	Sample	
+	wash 7	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	B6	Sample	
+	wash 8	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	B8	Sample	
+	wash 9	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	B10	Sample	
+	wash 10	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	C1	Sample	
+	wash 11	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	C3	Sample	
+	wash 12	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	C5	Sample	
+	wash 13	MeOH: Acet: phosphate buffer (7.5: 1.5: 1)	5.0	C7	Sample	
2	R4000-220711-01	Hydrastine USP, 0.5 mg/mL	10.0	A2	Reference	☑
+	R383-220708-02	hydrastinin	1.0	A3	Reference	
+	R22072-220708-01	Berberine chloride USP, 0.025 mg/mL	10.0	A4	Reference	
3	S24507-220708-01	Hesi extract	5.0	A6	Sample	☐
4	S20128-220708-01	Goldenseal, 75mg/mL	5.0	A8	Sample	☐
5	S20131-220708-01	Goldenseal, 75mg/mL	5.0	A10	Sample	☐
6	S20132-220708-01	Goldenseal, 75mg/mL	5.0	B1	Sample	☐
7	S20133-220708-01	Goldenseal, 75mg/mL	5.0	B3	Sample	☐
8	R23988 UHM	UHM	5.0	A1	Sample	☑
9	S20135-220708-01	Goldenseal, 75mg/mL	5.0	B5	Sample	☐
10	S20138-220708-01	Goldenseal, 75mg/mL	5.0	B7	Sample	☐
11	S20141-220708-01	Goldenseal, 75mg/mL	5.0	B9	Sample	☐
12	S20142-220708-01	Goldenseal, 75mg/mL	5.0	B11	Sample	☐
13	S20143-220708-01	Goldenseal, 75mg/mL	5.0	C2	Sample	☐
14	S20144-220708-01	Goldenseal, 75mg/mL	5.0	C4	Sample	☐
15	S20146-220708-01	Goldenseal, 75mg/mL	5.0	C6	Sample	☐

Development

Lab temperature (before chromatography): 23°C
Lab relative humidity (before chromatography): 41%
End relative humidity (achieved by ADC2): 37 %
Chamber: ADC 2
Humidity control: $MgCl_2$
Saturation: 20 min, saturation pad
Developing distance from application position/lower edge: 62/70 mm
Developing solvent: **ethyl acetate, methanol, formic acid, water 50:10:6:3 (v/v)**
Developing time: 19 min
Plate drying: 5 min with cold air in ADC2

Derivatization reagent

Reagent name: Ninhydrin reagent
Reagent preparation: 100 mg of ninhydrin are dissolved in 50 mL of ethanol 96%. 1.5 mL of acetic acid are added.
Reagent use: Spray with 3.0 mL of reagent (Derivatizer, blue nozzle, spraying level: 3) and then heat the plate at 100°C for 3 min

Results

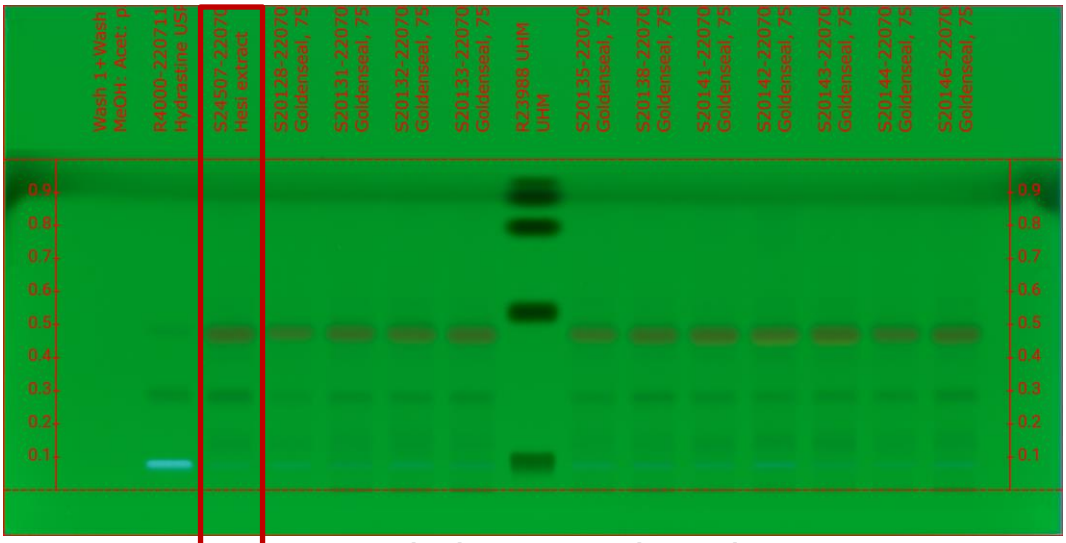


Image in short wave UV (254 nm)

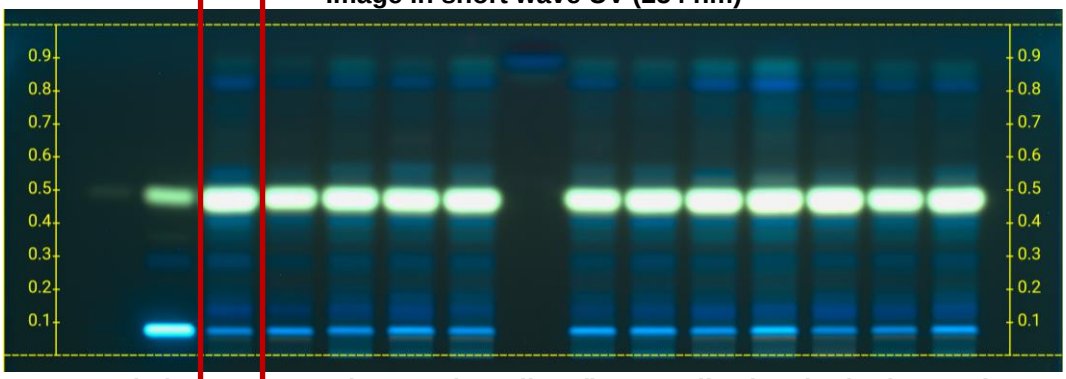


Image in long wave UV (350 nm broadband); normalized on berberine track 2

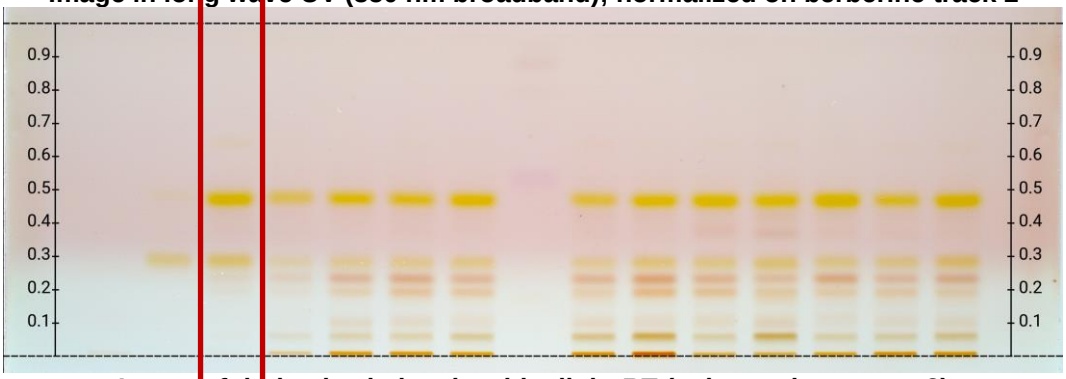
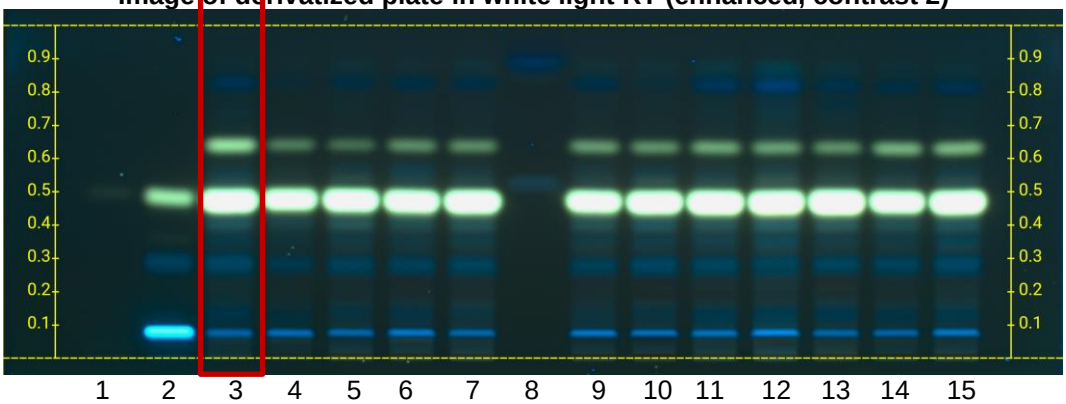


Image of derivatized plate in white light RT (enhanced, contrast 2)



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Image of derivatized plate long wave UV (350 nm broadband); normalized on berberine track 2

TEST 2

Application

Instrument: ATS 4
Band length: 8.0 mm, Distance between tracks: 11.4 mm, Application position X: 20.0 mm Y: 8.0 mm

A special wash solution (methanol, acetone, phosphate buffer (1 mM) 7.5:1.5:1 (*v/v*)) was applied on Track 1 after each sample, to prevent cross contamination with berberin.

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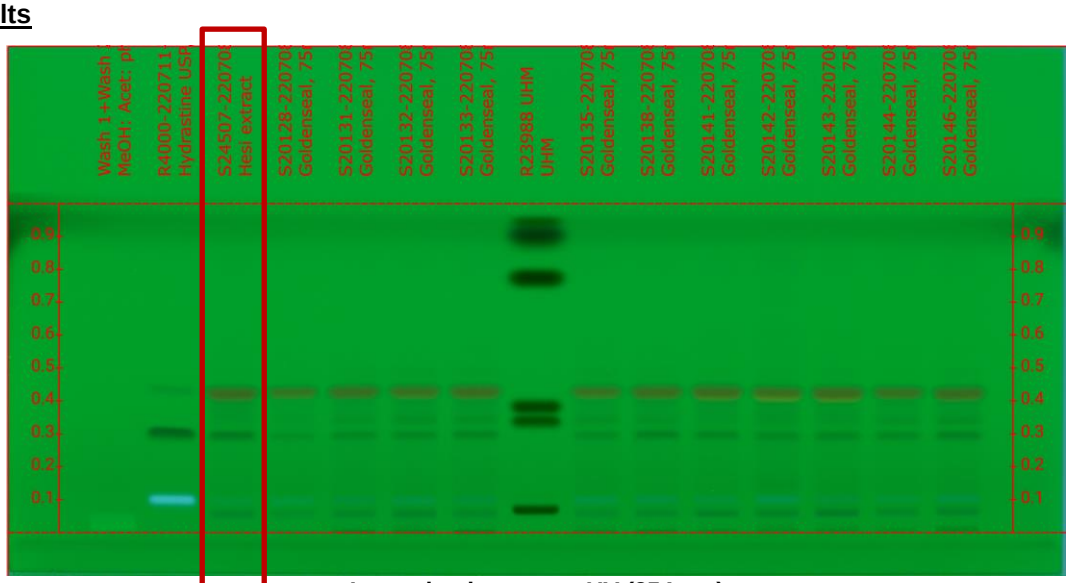

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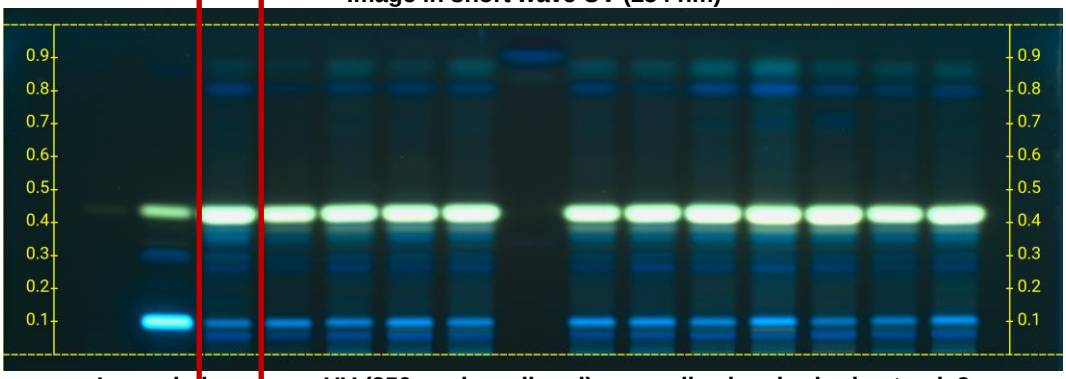

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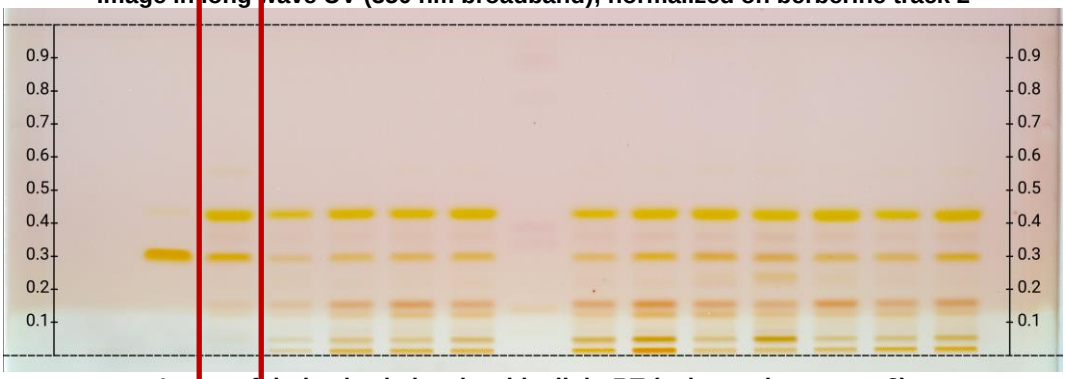

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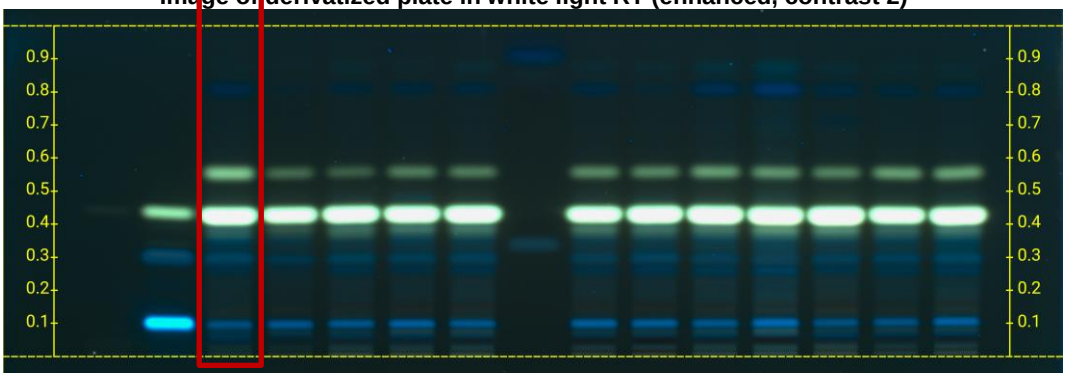

Image of derivatized plate long wave UV (350 nm broadband); normalized on berberine track 2

Additional experimental details are available upon request.

Date	19.07.2022	Date	23.08.2022
Author		Reviewed	
	Dr. Eike Reich		Dr. Tiên Do

Disclaimer

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