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Report

Project		P585-03 Blue cohosh extract BSC HESI			
Related documents		[1] *Caulophyllum thalictroides*_Root and rhizome_HPTLC Association_AHP_V1.pdf/ AHP method			
Customer		HESI			
Project objective		Identification of a Blue chosh extract			
Date	15.08.2022	Laboratory	CAMAG, Muttenz	Analyst	ER

Summary

1. The **extract** (Lot RK-3-21-1-CT) received for this study was compared to several samples of Blue cohosh root and rhizome using the developing solvent of the AHP method for identification of *Caulophyllum thalictroides* (Figure 1) [1]. Samples and **extract** were prepared with methanol, water 8:2 (*v/v*). For details see Test 1.
2. The fingerprint of the **extract** (track 4) is generally similar to those of the plant samples. The markers cauloside C and cauloside D are clearly seen in all samples. A faint zone (red arrow) is seen in the **extract** but not in the samples. This zone is seen in all detection modes but shortwave UV (see Test 1). Some samples show this zone in longwave UV prior to derivatization in Test 2.

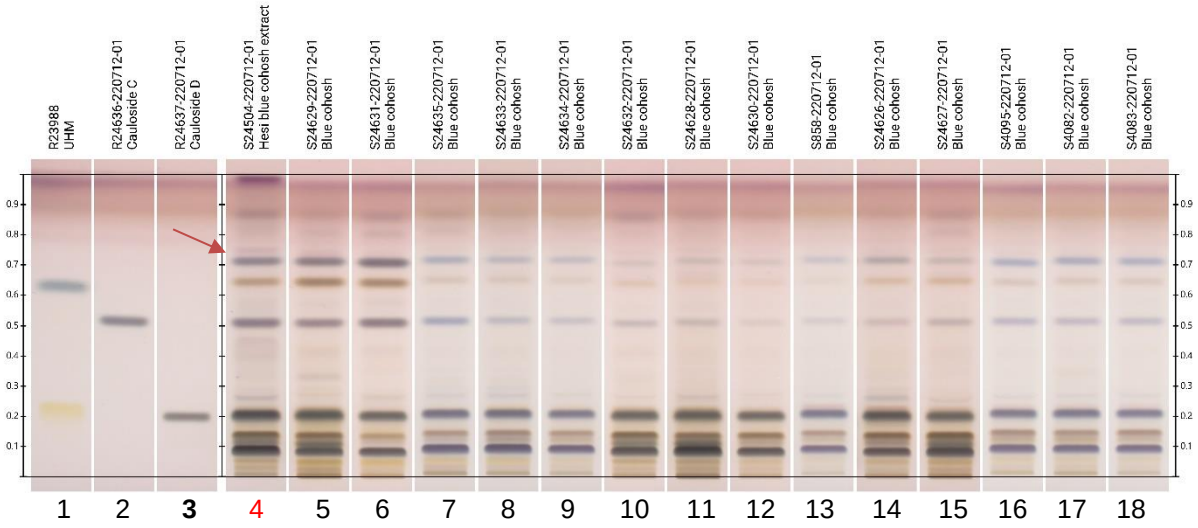

Figure 1
Fingerprints of Blue cohosh, derivatized with anisaldehyde reagent, white light RT

3. In Figure 2, an additional detection mode (UV 350 nm broadband) reveals, that there is considerable variability in the fingerprints of the plant samples. When compared to an electronically averaged fingerprint of all plant samples (Figure 2, Track 2), the **extract** (track 1) shows a distinct zones (yellow arrows) which are not present in the samples and might be process related.

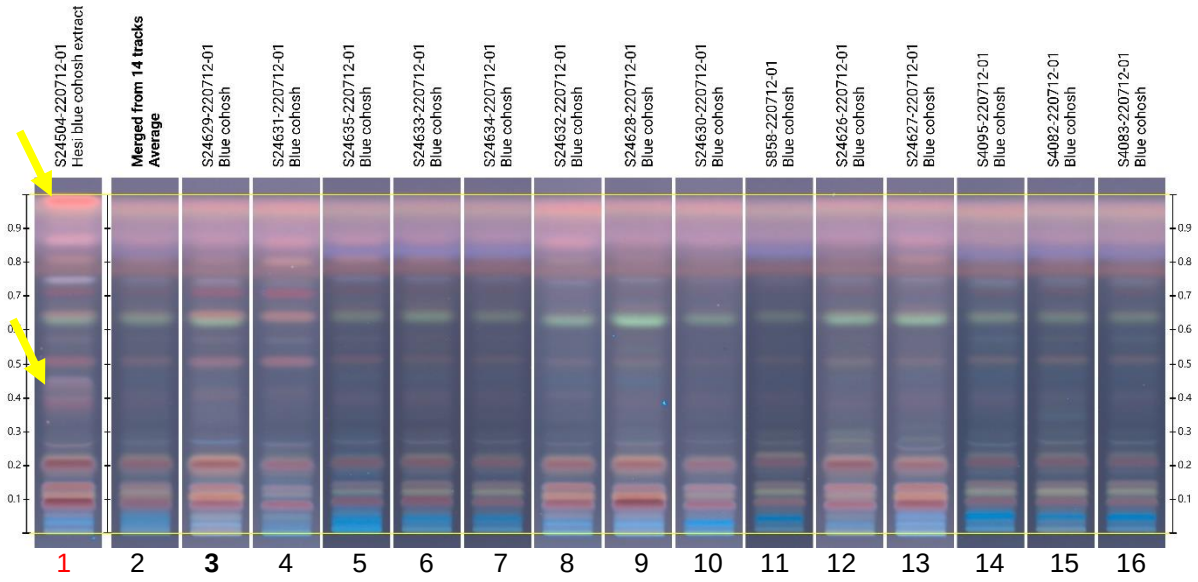

Figure 2

Fingerprints of Blue cohosh (track 1) compared to average fingerprint (track 2) of 14 samples (tracks 3-16), derivatized with anisaldehyde reagent, long wave UV (350 nm broadband).

Conclusion

The **extract** (Lot RK-3-21-1-CT) is identified as an extract from Blue cohosh. It shows two zones genererally not seen in samples of *Caulophyllum thalictroides*.

Experimental details

Samples (S) and reference materials

Sample no.	Sample description	Source, batch, date received
S24504	Blue cohosh extract	MRIGlobal, Supplier: Uni Mississippi, Lot RK-3-21-1-CT
S858	Blue cohosh	CAMAG
S4082	Blue cohosh cut	CAMAG
S4083	Blue cohosh capsule	CAMAG
S4095	Blue cohosh powder	CAMAG
S24626	Blue cohosh rhizome	CAMAG
S24627	Blue cohosh rhizome	CAMAG
S24628	Blue cohosh root	CAMAG
S24629	Blue cohosh root	CAMAG
S24630	Blue cohosh root	CAMAG
S24631	Blue cohosh root	CAMAG
S24632	Blue cohosh root, cultivated	CAMAG
S24633	Blue cohosh root (cut, sifted)	CAMAG
S24634	Blue cohosh root (cut sifted)	CAMAG
S24635	Blue cohosh root (cut sifted)	CAMAG
R23988	UHM	In-house - 2202211
R24636	Cauloside C	Universal Biologicals (UK); ChemScene LLC (USA);#CS-0092099, LOT 155389
R24637	Cauloside D	Universal Biologicals (UK); ChemScene LLC (USA);#CS-0100467 LOT 1114996

Chemicals

Name	Manufacturer	Purity/quality	Batch
Methanol	Roth	Rotisolv	0002001863
Dichloromethane	Acros	99.8% + amylen	2100396
Anisaldehyde	Acros	99%	A0381986
Sulfuric acid	Acros	96%	A0419337
Acetic acid	Acros	99.5%	A0427447
Water	inhouse	De-ionized	

Equipment

Name, article	Manufacturer
Automatic TLC Sampler 4	CAMAG
TLC Plate Heater III	CAMAG
Automatic Development Chamber ADC 2	CAMAG
Visualizer	CAMAG
TLC Scanner	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:	50 mg/mL of powdered Blue cohosh; 25 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, 1.0 mg/mL cauloside C and cauloside D
Plate:	HPTLC glass plate, Si 60 F_{254} (Merck); HX87944542

TEST 1

Analysis with method [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

Tr.	Vial ID	Description	Vol. (µl)	Position	Type	SST
1	R23988	UHM	2.0	A1	Reference	☐
2	R24636-220712-01	Cauloside C	2.0	A2	Reference	☐
3	R24637-220712-01	Cauloside D	2.0	A3	Reference	☐
4	S24504-220712-01	Hesi blue cohosh extract	2.0	A4	Sample	☐
5	S24504-220712-01	Hesi blue cohosh extract	4.0	A4	Sample	☐
6	S24504-220712-01	Hesi blue cohosh extract	6.0	A4	Sample	☐
7	S24626-220712-01	Blue cohosh	2.0	A5	Sample	☐
8	R23988	UHM	2.0	A1	Reference	☐
9	S24627-220712-01	Blue cohosh	2.0	A6	Sample	☐
10	S24628-220712-01	Blue cohosh	2.0	A7	Sample	☐
11	S24629-220712-01	Blue cohosh	2.0	A8	Sample	☐
12	S24630-220712-01	Blue cohosh	2.0	A9	Sample	☐
13	S24631-220712-01	Blue cohosh	2.0	A10	Sample	☐
14	S24632-220712-01	Blue cohosh	2.0	A11	Sample	☐
15	R23988	UHM	2.0	A1	Reference	☐

Development

Lab temperature (before chromatography): 24°C
Lab relative humidity (before chromatography): 45%
End relative humidity (achieved by ADC 2): 36%
Chamber: ADC 2
Humidity control: $MgCl_2$

Saturation: 20 min, saturation pad
Developing distance from application position/lower edge: 62/70 mm
Developing solvent: dichloromethane, methanol, water 70:30:4 (*v/v*)
Developing time: 16 min
Plate drying: 5 min with cold air in ADC 2

Derivatization reagent

Reagent name: Anisaldehyde reagent
Reagent preparation: Slowly and carefully mix 170 mL of ice-cooled methanol with 20 mL of acetic acid and 10 mL of sulfuric acid. Allow the mixture to cool to room temperature and then add 1.0 mLof anisaldehyde.
Reagent use: spray with 3.0 mL (Derivatizer, blue nozzle, level: 3). Heat the plate at 100°C for 3 min.

Results

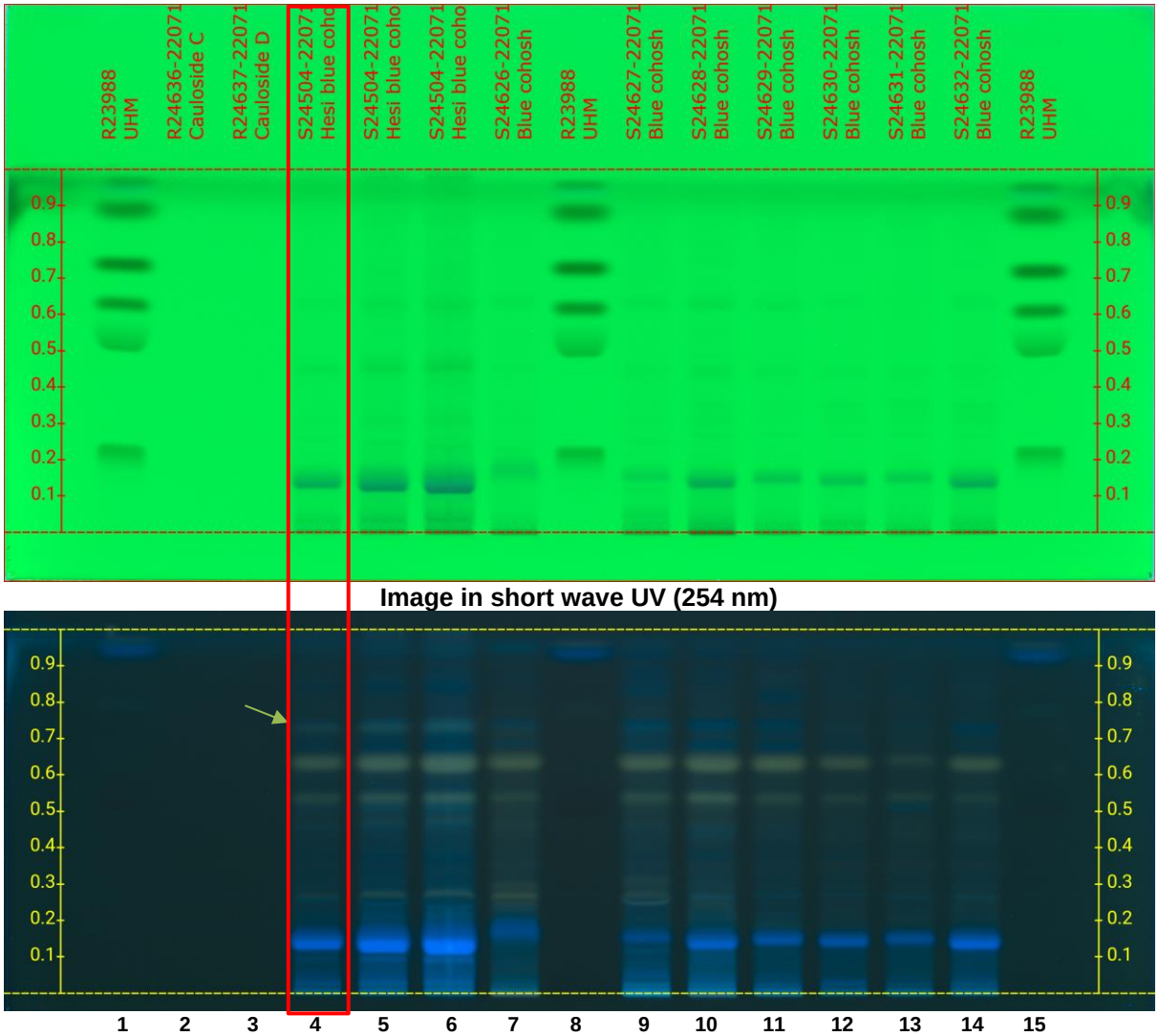

Image in short wave UV (254 nm)

Image in long wave UV (350 nm broadband); normalized on track 4

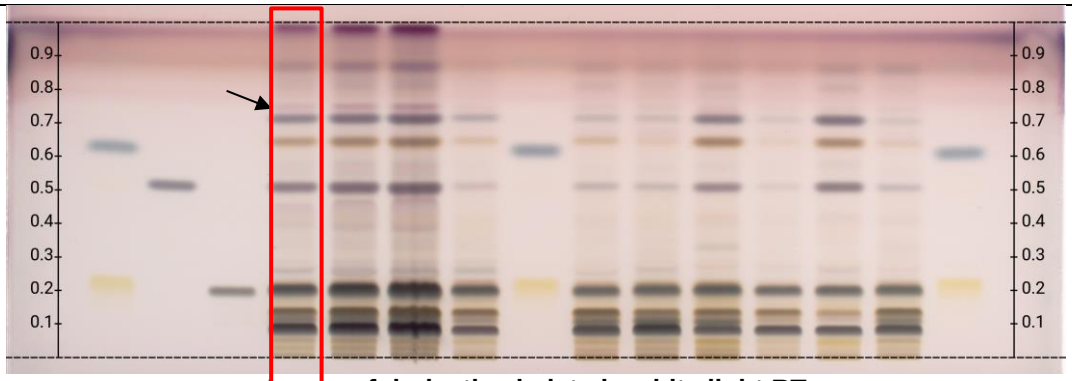

Image of derivatized plate in white light RT

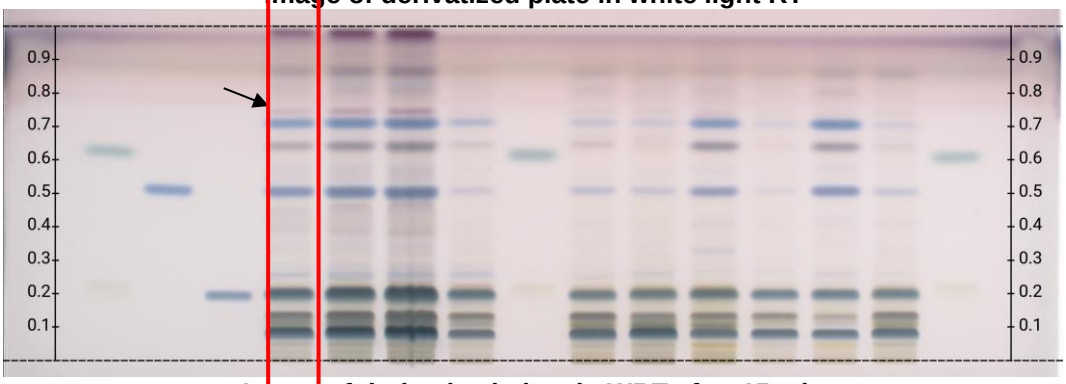

Image of derivatized plate in WRT after 15 min

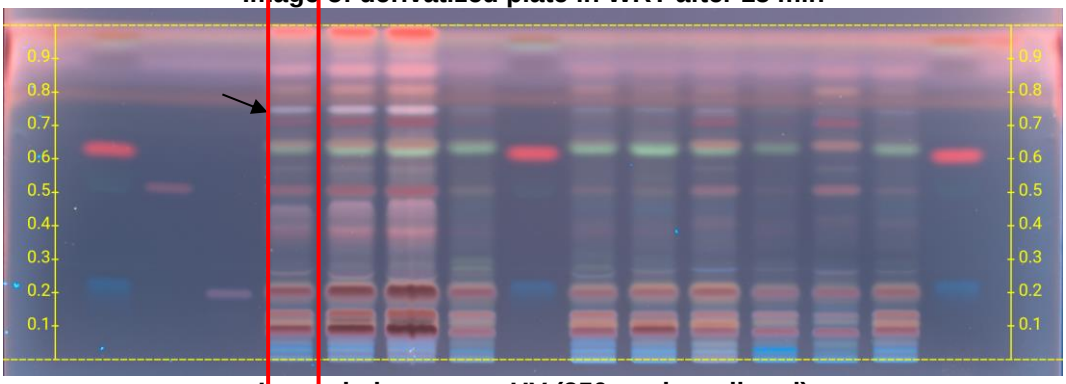

Image in long wave UV (350 nm broadband)

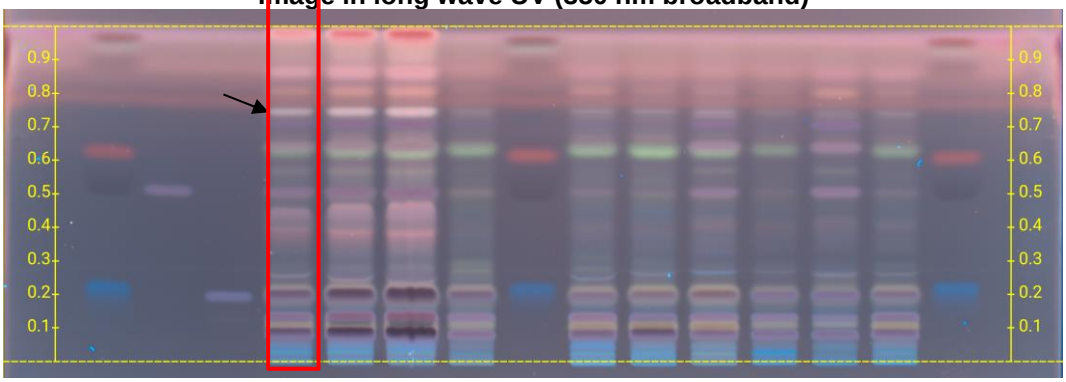

Image in long wave UV (350 nm broadband) after 15 min

There is a zone in the extract (arrow) not seen in the samples. An application volume of 2.0 µL for the extract is suitable for comparison.

TEST 2

Analyis of additional samples

Application

Tr.	Vial ID	Description	Vol. (µl)	Position
1	R23988	UHM	2.0	A1
2	R24636-220712-01	Cauloside C	2.0	A2
3	R24637-220712-01	Cauloside D	2.0	A3
4	S24504-220712-01	Hesi blue cohosh extract	2.0	A4
5	S24628-220712-01	Blue cohosh	4.0	A5
6	S24504-220712-01	Hesi blue cohosh extract	1.0	A4
7	S24633-220712-01	Blue cohosh	2.0	B1
8	R23988	UHM	2.0	A1
9	S24634-220712-01	Blue cohosh	2.0	B2
10	S24635-220712-01	Blue cohosh	2.0	B3
11	S858-220712-01	Blue cohosh	2.0	B4
12	S4082-220712-01	Blue cohosh	2.0	B5
13	S4083-220712-01	Blue cohosh	2.0	B6
14	S4095-220712-01	Blue cohosh	2.0	B7
15	R23988	UHM	2.0	A1

Development

Lab temperature (before chromatography): 24°C
Lab relative humidity (before chromatography): 41%
End relative humidity (achieved by ADC 2): 36%
Chamber: ADC 2
Humidity control: $MgCl_2$
Saturation: 20 min, saturation pad
Developing distance from application position/lower edge: 62/70 mm
Developing solvent: dichloromethane, methanol, water 70:30:4 (v/v)
Developing time: 15 min
Plate drying: 5 min with cold air in ADC 2

Derivatization reagent

Reagent name: Anisaldehyde reagent
Reagent preparation: Slowly and carefully mix 170 mL of ice-cooled methanol with 20 mL of acetic acid and 10 mL of sulfuric acid. Allow the mixture to cool to room temperature and then add 1.0 mL of anisaldehyde.
Reagent use: spray with 3.0 mL (Derivatizer, blue nozzle, spraying level: 3). Heat the plate at 100°C for 3 min.

Results

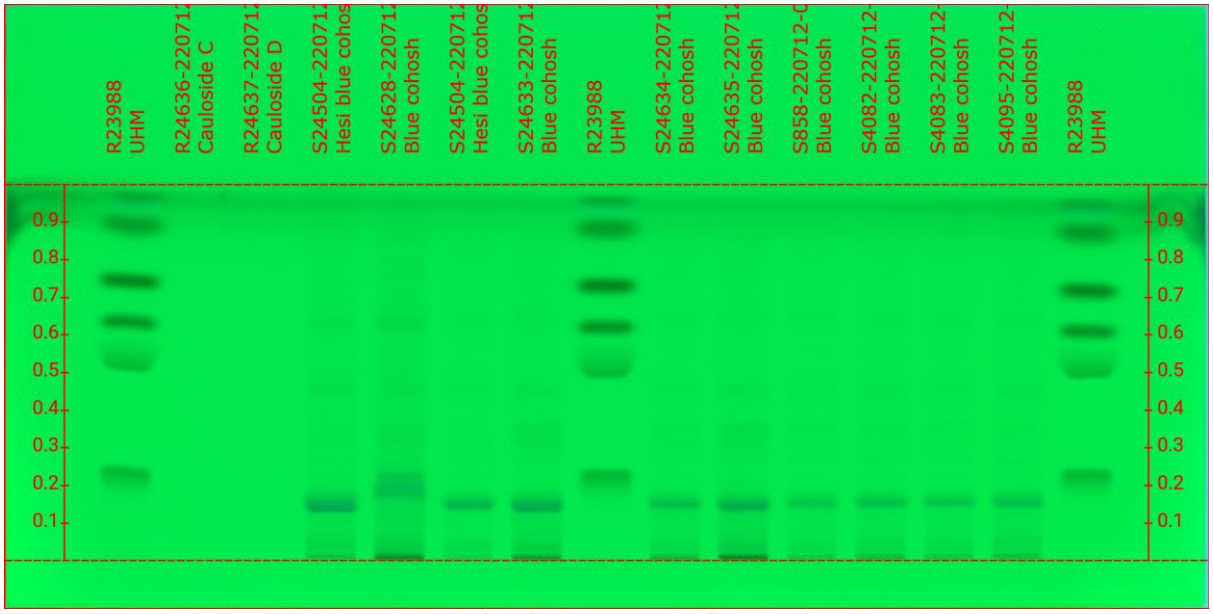

Image in short wave UV (254 nm)

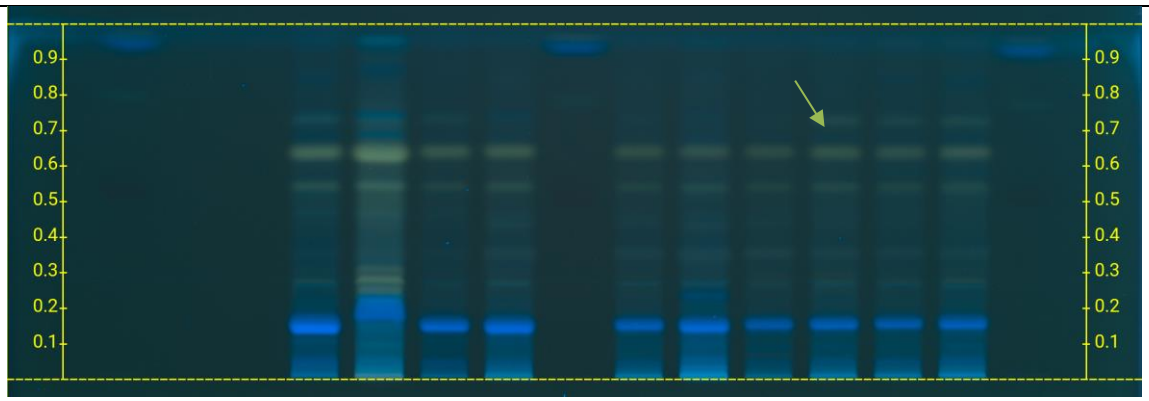

Image in long wave UV (350 nm broadband)

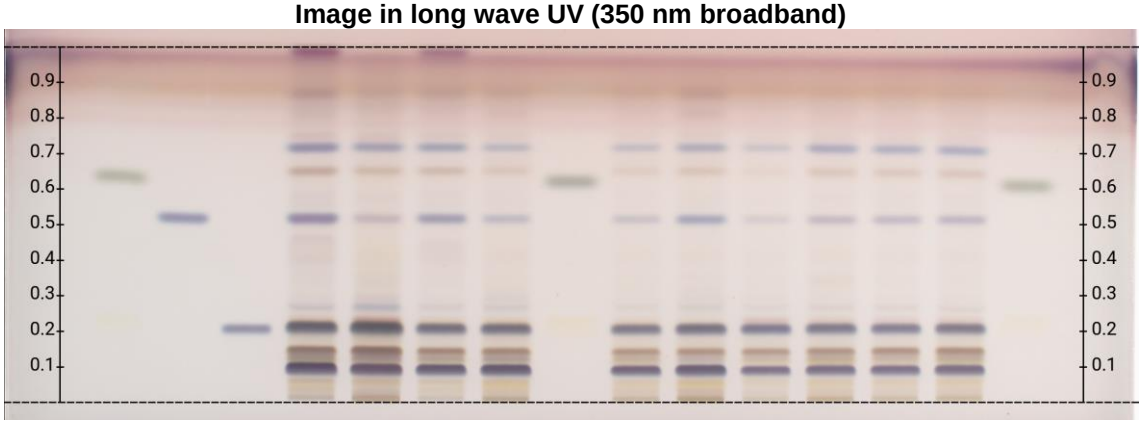

Image of derivatized plate WRT

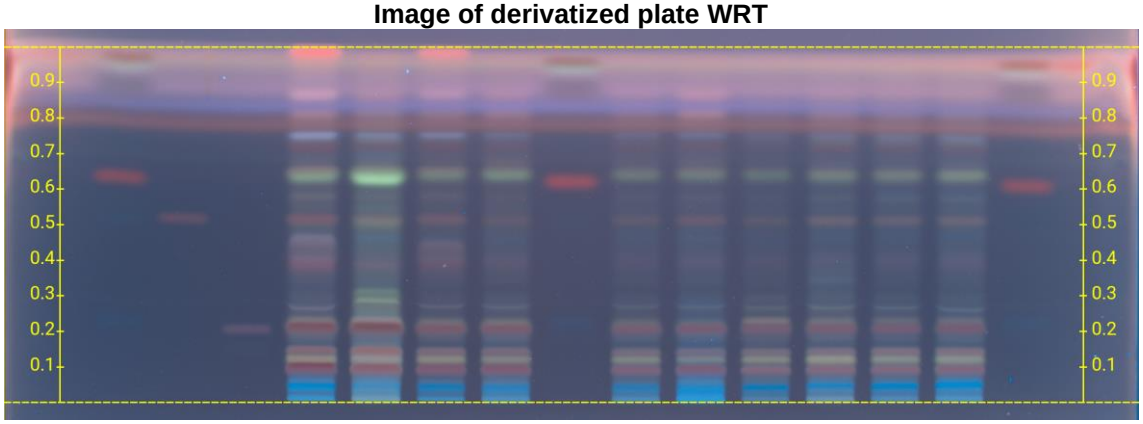

Image of derivatized plate (350 nm broadband)

The questionable zone is also seen under long wave UV in three samples. An application volume of 1.0 µL extract provides sufficient intensity in the fingerprint.

Additional experimental details are available upon request.

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

Disclaimer

Statements and interpretations provided in this report are the opinion of CAMAG Laboratory. They do not represent a declaration of conformity with respect to inspection or product certification. Test results correspond to the listed samples only and may not be generalized.