

Report

Project	P585-04 Aristolochia fangchi BSC HESI				
Related documents	[1] PhEur Screening for aristolochic acids in herbal drugs				
Customer	HESI				
Project objective	Identification of an Aristolochia fangchi extract				
Date	24.08.2022	Laboratory	CAMAG, Muttenz	Analyst	ER

Summary

1. The **extract** (Lot RK-3-27-1-AF) received for this study was compared to several samples of *Aristolochia fangchi* root and root samples of other *Aristolochia* species. **NOTE: the available samples were between 7 and 10 years old.** In general, the HPTLC method described in [1] was followed. The derivatization with stannous chloride reagent was followed by another derivatization step using 10% sulfuric acid in ethanol. Fingerprints were recorded in multiple detection modes. For experimental details see section TEST.
2. As seen in Figure 1, the fingerprint of the **extract (track 3)** is unique and does not fully match the fingerprints of *A. fangchi* considering all detection modes. The samples used for comparison show some variability but S3257(track 6*) is a pooled sample and might be considered representative for the drug. There are some similarities to fingerprints of other *Aristolochia* species in some detection modes. A possible explanation for the differences in composition may be the samples preparation with formic acid, water, methanol 1:9:40 (v/v). Another possibility is a mixture of species.

3. Conclusion

The **extract** (Lot RK-3-21-1-CT) cannot be confirmed as produced from *Aristolochia fanchi* based on the selected method. Further verification would require more information about the extraction process.

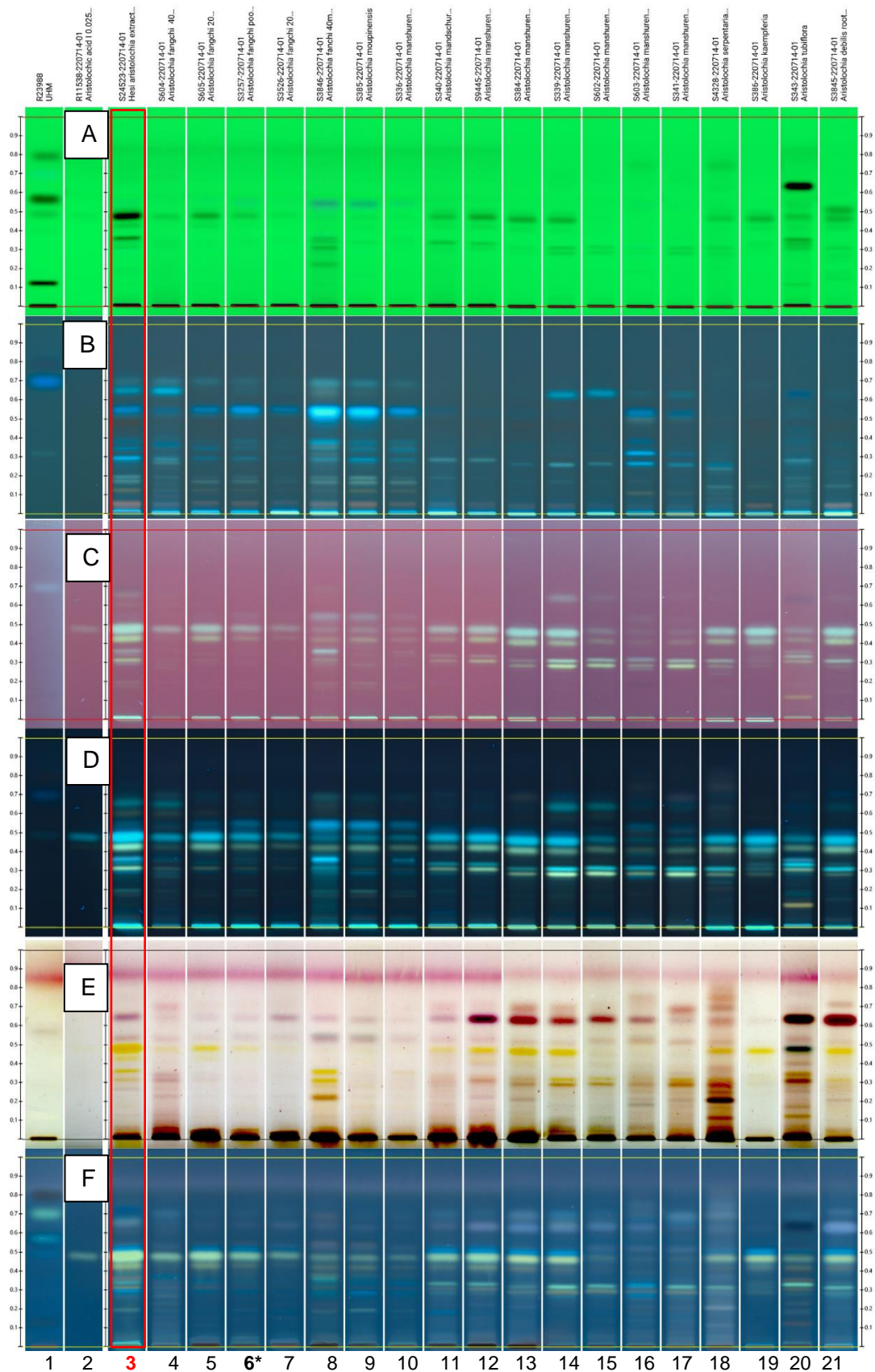


Figure 1

Fingerprints of *Aristolochia* species: *A. fangchi* (4-8), *A. moupinensis* (9), *A. mandshuriensis* (10-17), *A. serpentaria*, (18), *A. kaempferia* (19), *A. tubiflora* (20), *A. debilis* (21). Prior to derivatization, **A**: shortwave UV 254 nm, **B**: long wave UV (350 nm broadband); derivatized with stannous chloride reagent. **C**: shortwave UV 254 nm, **D**: long wave UV (350 nm broadband); subsequently derivatized with 10% sulfuric acid in ethanol, **E**: white light transmission **F**: long wave UV (350 nm broadband);

CAMAG LABORATORY

Experimental details

Samples (S) and reference (R) materials

Sample no.	Sample description	Source, batch, date received
S24503	<i>Aristolochia fangchi</i> extract	MRIGlobal, Uni Mississippi, Lot RK-3-27-1-AF
S336	<i>Aristolochia manshuriensis</i>	CAMAG
S339	<i>Aristolochia manshuriensis</i>	CAMAG
S340	<i>Aristolochia manshuriensis</i>	CAMAG
S341	<i>Aristolochia manshuriensis</i>	CAMAG
S343	<i>Aristolochia tubiflora</i>	CAMAG
S385	<i>Aristolochia moupinensis</i>	CAMAG
S384	<i>Aristolochia manshuriensis</i>	CAMAG
S386	<i>Aristolochia kaempferia</i>	CAMAG
S602	<i>Aristolochia manshuriensis</i>	CAMAG
S603	<i>Aristolochia manshuriensis</i>	CAMAG
S604	<i>Aristolochia fangchi</i>	CAMAG
S605	<i>Aristolochia fangchi</i>	CAMAG
S632	<i>Aristolochia debilis</i> fruit	CAMAG
S3257	<i>Aristolochia fangchi</i> blend	CAMAG
S3526	<i>Aristolochia fangchi</i>	CAMAG
S3845	<i>Aristolochia debilis</i>	CAMAG
S3846	<i>Aristolochia fangchi</i>	CAMAG
S4328	<i>Aristolochia serpentaria</i>	CAMAG
S6623	<i>Aristolochia debilis</i> , herb	CAMAG
S8056	Fructus Aristolochiae	CAMAG
S8057	Fructus Aristolochiae	CAMAG
S8058	Fructus Aristolochiae	CAMAG
S8059	Fructus Aristolochiae	CAMAG
S8060	Fructus Aristolochiae	CAMAG
S8061	Fructus Aristolochiae	CAMAG
S8062	Fructus Aristolochiae	CAMAG
S8063	Fructus Aristolochiae	CAMAG
S9445	<i>Aristolochiae manshuriensis</i>	CAMAG
R23988	UHM	In-house - 2202211
R11538	Aristolochic acid I (CRS)	EDQM, Code: Y0001185

Chemicals

Name	Manufacturer	Purity/quality	Batch
Methanol	Roth	Rotisolv	0002001863
Toluene	Acros	99+ %	2101782
Ethyl acetate	Acros	99.5%	271888
Sulfuric acid	Acros	96%	A0419337
Formic acid	Thermo Scientific	98+ %	A0438424
Water	inhouse	De-ionized	
SnCl ₂ x H ₂ O	Merck	p.a.	B275715 308

Equipment

Name, article	Manufacturer
Automatic TLC Sampler 4	CAMAG
TLC Plate Heater III	CAMAG

CAMAG LABORATORY

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:	20 or 40 mg/mL of powdered Aristolochia; 4 mg/mL of extract in formic acid, water, methanol 1:9:40 (v/v). Sonicate for 10 min, centrifuge and use the supernatant
Standard solutions:	0.024 mg/mL of aristolochic acid I in formic acid, water, methanol 1:9:40 (v/v)
Plate:	HPTLC glass plate, Si 60 F ₂₅₄ (Merck); HX87944542

TEST

Analysis with method [1]

Application (example)

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	C1	Reference	<input type="checkbox"/>
2	R11538-220714-01	Aristolochic acid I 0.025mg/ml	10.0	C2	Reference	<input type="checkbox"/>
3	S24523-220714-01	Hesi aristolochia extract 4mg/mL	4.0	C3	Sample	<input type="checkbox"/>
4	S602-220714-01	Aristolochia manshuriensis 40mg/mL	5.0	E1	Sample	<input type="checkbox"/>
5	S603-220714-01	Aristolochia manshuriensis 40mg/mL	5.0	E2	Sample	<input type="checkbox"/>
6	S384-220714-01	Aristolochia manshuriensis 40mg/mL	5.0	E3	Sample	<input type="checkbox"/>
7	S339-220714-01	Aristolochia manshuriensis 40mg/mL	5.0	E4	Sample	<input type="checkbox"/>
8	R23988	UHM	2.0	C1	Reference	<input type="checkbox"/>
9	S341-220714-01	Aristolochia manshuriensis 40mg/mL	5.0	E5	Sample	<input type="checkbox"/>
10	S386-220714-01	Aristolochia kaempferia	10.0	E6	Sample	<input type="checkbox"/>
11	S4328-220714-01	Aristolochia serpentaria root	10.0	E7	Sample	<input type="checkbox"/>
12	S3845-220714-01	Aristolochia debilis root 40mg/mL	5.0	E10	Sample	<input type="checkbox"/>
13	S385-220714-01	Aristolochia moupinensis	10.0	E8	Sample	<input type="checkbox"/>
14	S343-220714-01	Aristolochia tubiflora	10.0	E9	Sample	<input type="checkbox"/>
15	R23988	UHM	2.0	C1	Reference	<input type="checkbox"/>

Development

Lab temperature (before chromatography): 24°C

Lab relative humidity (before chromatography): 38%

End relative humidity (achieved by ADC 2): 37%

Chamber: ADC 2

Humidity control: MgCl₂

Saturation: 20 min, saturation pad

Developing distance from application position/lower edge: 62/70 mm

Developing solvent: Toluene, ethyl acetate, formic acid water 60:30:3.3 (v/v)

Developing time: 13 min

Plate drying: 5 min with cold air in ADC 2

Derivatization reagent 1

Reagent name: Tin (II) chloride reagent

Reagent preparation: 3.0 g of SnCl₂ are dissolved in a mixture of 24.0 mL of water and 4.5 mL of HCl 37%

Reagent use: spray plate until slightly wet, heat at 100°C for one min. Evaluate in longwave UV.

Derivatization reagent 2

Reagent name: sulfuric acid in ethanol

Reagent preparation: 10.0 mL of sulfuric acid are carefully added to 90.0 mL of ice-cooled ethanol

Reagent use: dip the plate (speed 5, time 0) into reagent. Heat the plate at 120°C for 5 min. Evaluate in white light (transmission) and longwave UV.

Results

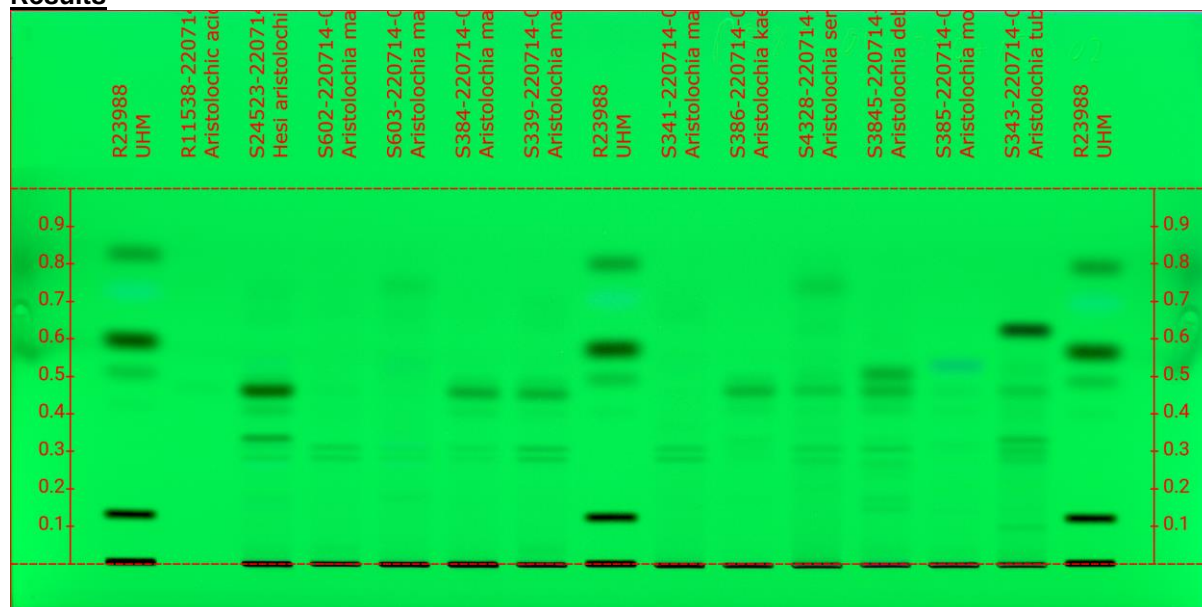


Image in short wave UV (254 nm) contrast 1,5

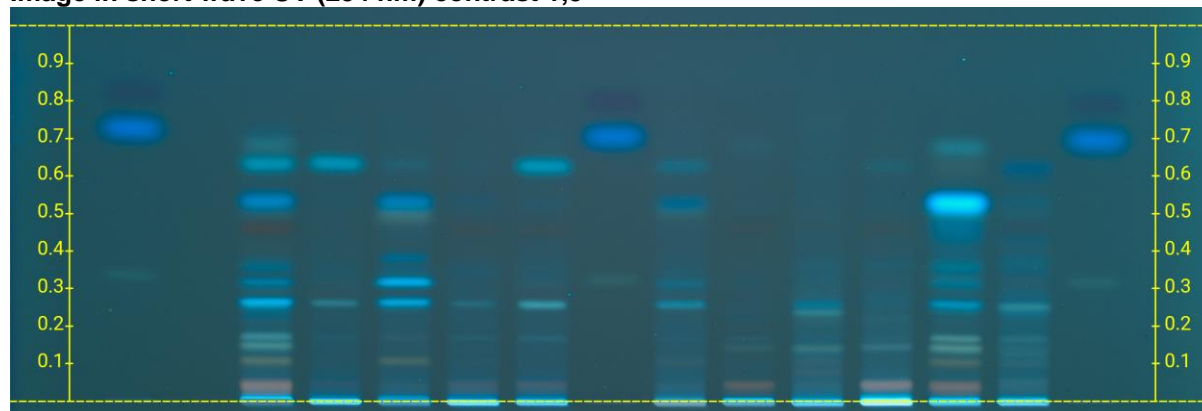


Image in longwave UV (350 nm broadband) (normalized on track 3)

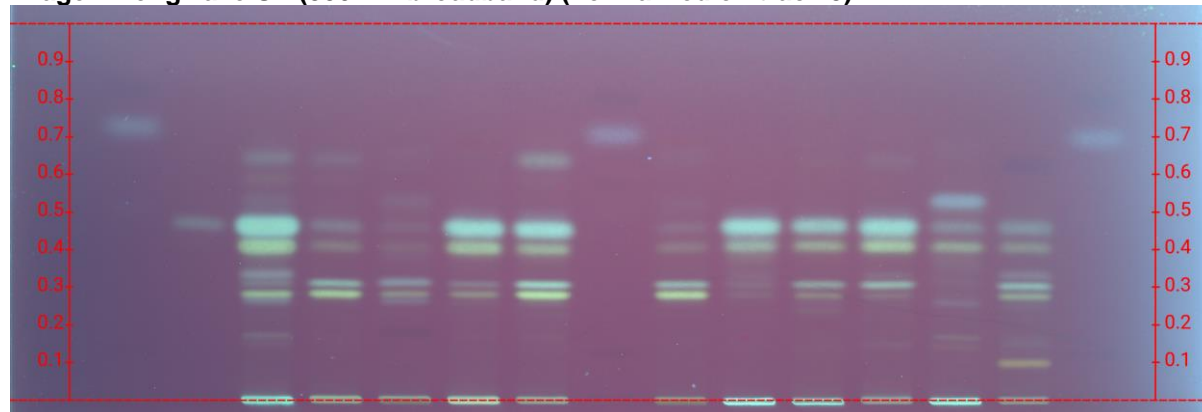


Image in short wave UV (254 nm) after derivatization

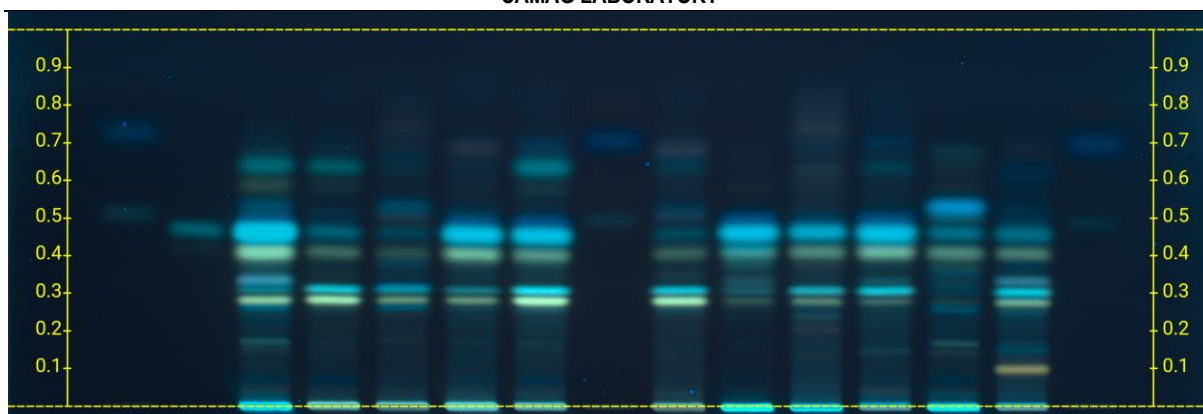


Image of derivatized plate in longwave UV (350 nm broadband) (normalized on track 3)

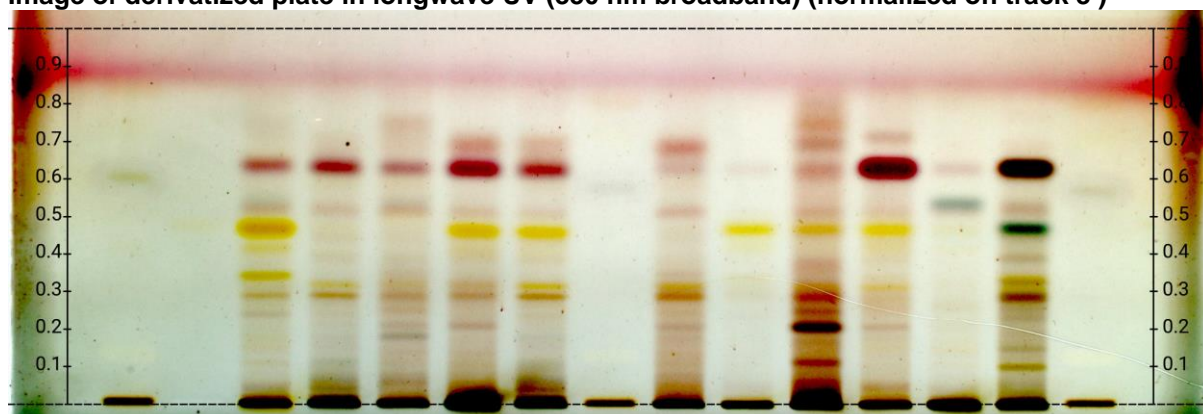


Image of derivatized (stannous chloride/sulfuric acid) plate in WT, contrast 2.8

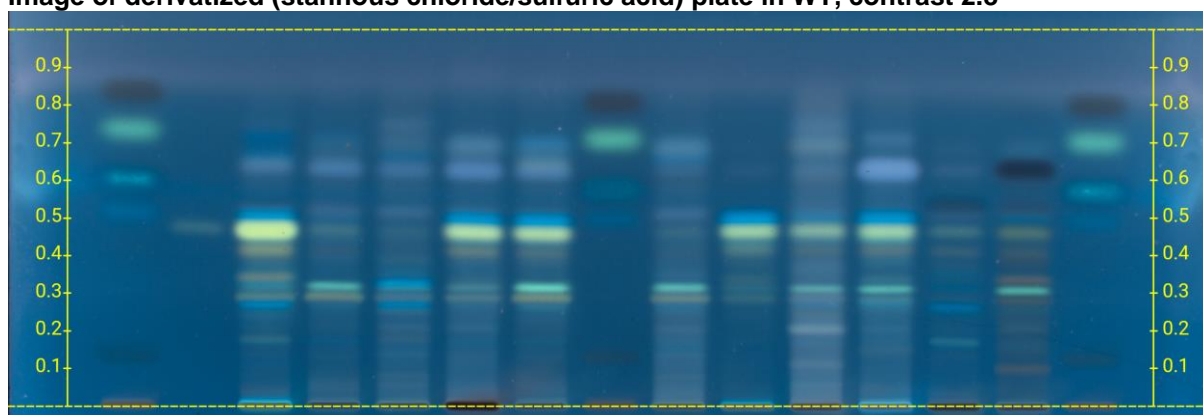


Image of derivatized (stannous chloride/sulfuric acid) longwave UV (350 nm broadband) (normalized on track 3)

Additional experimental details are available upon request.

Date	24.08.2022	Date	24.08.2022
Author	<i>Eike Reich</i>	Reviewed	<i>Tien Do</i>
	Dr. Eike Reich		Dr. Tien 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.