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Report

Project		P 585-05 Kratom BSC HESI			
Related documents		[1] Indonesian FDA, in-house method for analysis of Kratom [2] Alkemist: Mitragyna speciosa 21251EJK_1_BSC HPTLC (002).pdf			
Customer		HESI			
Project objective		Identification of a Kratom extract			
Date	19.07.2022	Laboratory	CAMAG, Muttenz	Analyst	ER

Summary

1. Because no Kratom samples were available, identification of the **extract** (LotRK-3-25-1-MS) received for this study was attempted with mitragynine (a marker for Kratom) and 7-OH-mitragynin (a mitragynin metabolite usually not present in Kratom). An in-house method [1] provided by the Indonesian FDA was employed after some modifications (Figure 1, Test 1). This method is suitable for detecting both markers and separates the **extract** (tracks 4-7) sufficiently.

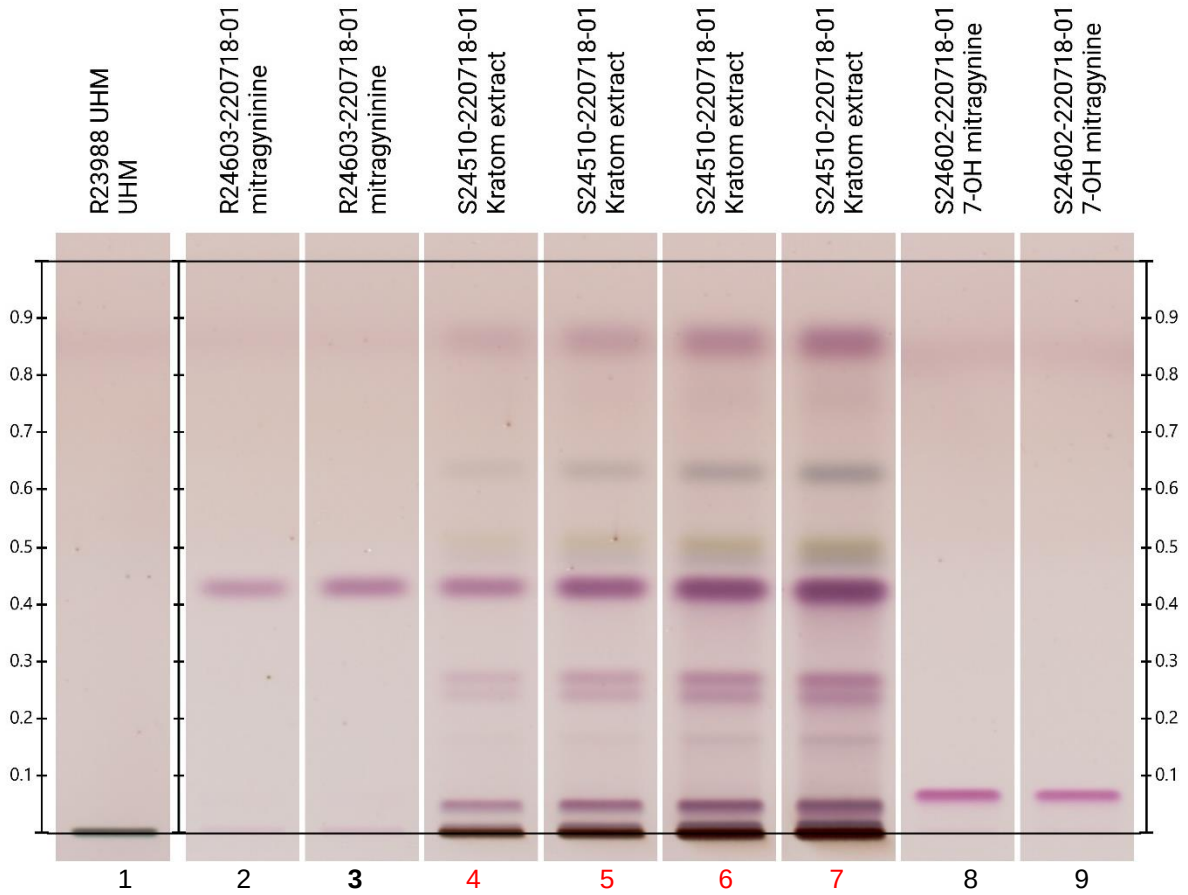

Figure 1: Fingerprints of Kratom extract, track 4: 1.0 uL, track 5: 2.0 uL, track 6: 4.0 uL; track 7: 6.0 uL; modified from [1]; white light RT after derivatization with anisaldehyde reagent. UHM: track 1; mitragynine - track 2: 5.0 uL, track 3: 7.0 uL; 7-OH mitragynine - track 8: 5.0 uL, Track 9: 7.0 uL

2. In the fingerprint of the **extract** (green) the presence of mitragynine (red) is confirmed by its UV spectrum.

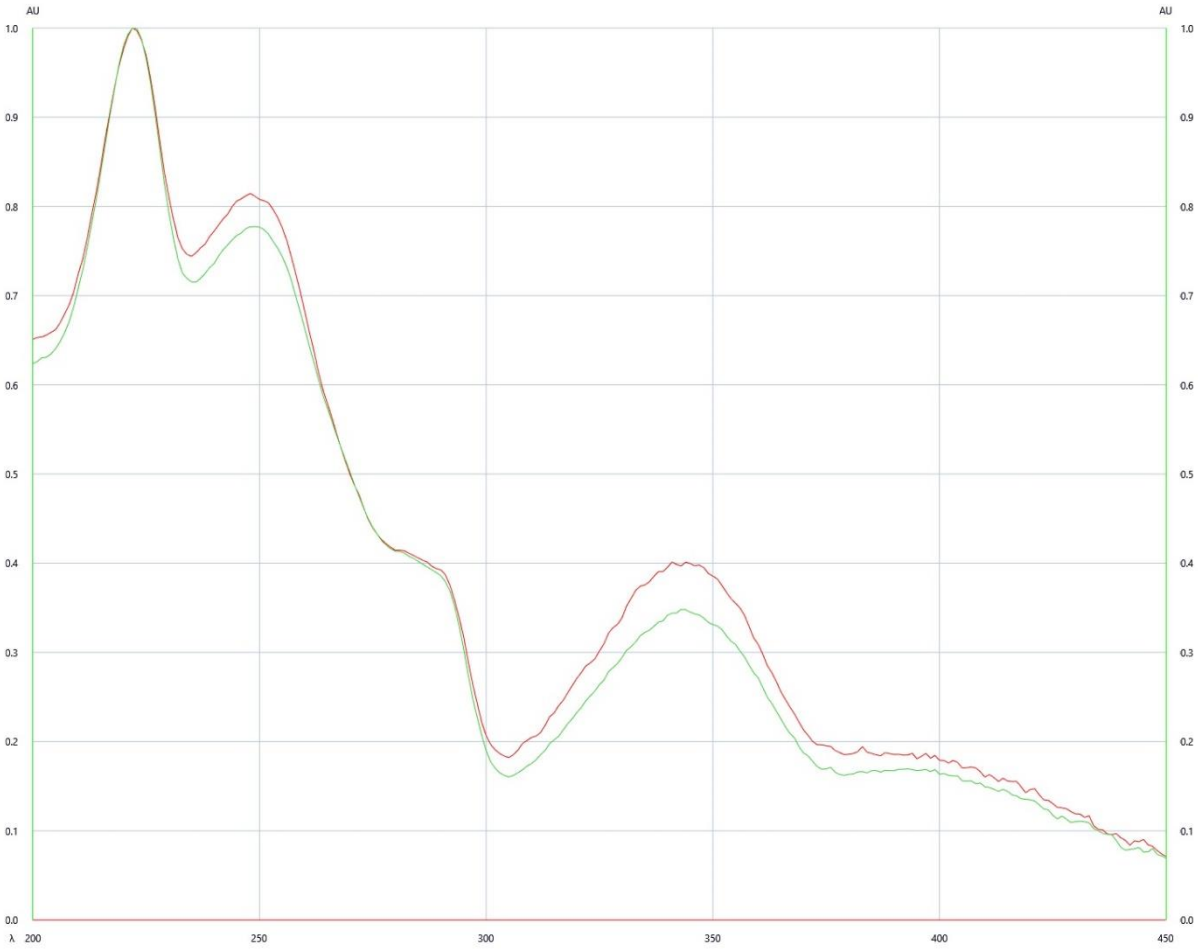

Figure 2
UV sprectra of a mitragynine reference (red) and the correspondinz zone in the extract

3. The extract was analyzed with the method provides in [2] and modified derivatization (anisaldheyde reagent instead of sulfuric acid reagent). Results are comparable (Figure 3, Test 2)

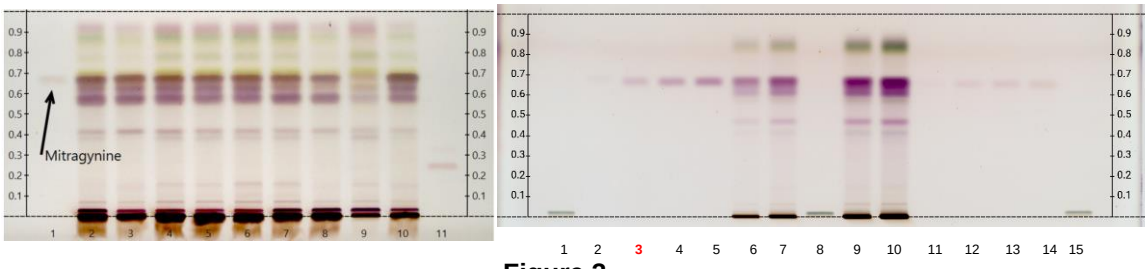

Figure 3
Fingerprints of Kratom with method [2]. Data from [2] left, data from Test 2 right

Conclusion

The **extract** (Lot RK-3-25-1-MS) is identified as an extract Kratom.

Experimental details

Samples (S) and reference materials

S24510	Kratom extract (*Mitragyna speciosa*)	Ethos Natural Medic. LLC via Alkemist Labs, LotRK-3-25-1-MS
R23988	UHM	In-house - 2202211
R24602	7-OH-mitragynine	Sigma-Aldrich; Supelco, Art. No: H-099; Lot FN04142103
R24603	mitragynine	Sigma-Aldrich; Supelco, Art. No: M-152; Lot FN03172008

Chemicals

Name	Manufacturer	Purity/quality	Batch
Toluene	Acros	99+ %	2101782
Ethyl acetate	Acros	99.5%	271888
Cyclohexane	Acros	99+ %	2185747
Diethyl amine	Fisher	99+ %	1725305
Ammonia 28%	Fisher	-	1733339
Anisaldehyde	Acros	99%	A0381986
Sulfuric acid	Acros	96%	A0419337
Acetic acid	Acros	99.5%	A0427447
Methanol	Roth	Rotisolv	0002001863

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:	25 mg/mL of extract in methanol. Sonicate for 10 min, centrifuge and use the supernatant.
Standard solutions:	Standards were obtained in methanolic solution at 1.0 mg/mL
Plate:	HPTLC, Si 60 F_{254} (Merck); HX87944542

TEST 1

Application

Instrument: ATS4
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	UHM	2.0	D1	Reference	☐
2	R24603-220718-01	mitragyninine	1.0	D2	Reference	☐
3	R24603-220718-01	mitragyninine	3.0	D2	Reference	☐
4	R24603-220718-01	mitragyninine	5.0	D2	Reference	☐
5	R24603-220718-01	mitragyninine	7.0	D2	Reference	☐
6	S24510-220718-01	Kratom extract	1.0	D3	Sample	☐
7	S24510-220718-01	Kratom extract	2.0	D3	Sample	☐
8	R23988 UHM	UHM	2.0	D1	Reference	☐
9	S24510-220718-01	Kratom extract	4.0	D3	Sample	☐
10	S24510-220718-01	Kratom extract	6.0	D3	Sample	☐
11	S24602-220718-01	7-OH mitragynine	1.0	D4	Reference	☐
12	S24602-220718-01	7-OH mitragynine	3.0	D4	Reference	☐
13	S24602-220718-01	7-OH mitragynine	5.0	D4	Reference	☐
14	S24602-220718-01	7-OH mitragynine	7.0	D4	Reference	☐
15	R23988 UHM	UHM	2.0	D1	Reference	☐

Development

Lab temperature (before chromatography):31°C
Lab relative humidity (before chromatography): 42%
End relative humidity (achieved by ADC2): 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: cyclohexane, ethyl acetate, 28% ammonia 30:15:1 (v/v)
Developing time: 11 min
Plate drying: 5 min with cold air in ADC2

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 ml of anisaldehyde.
Reagent use: spray with 3 ml of reagent (Derivatizer, blue nozzle, level: 3). Heat the plate at 100°C for 3 min.

Results

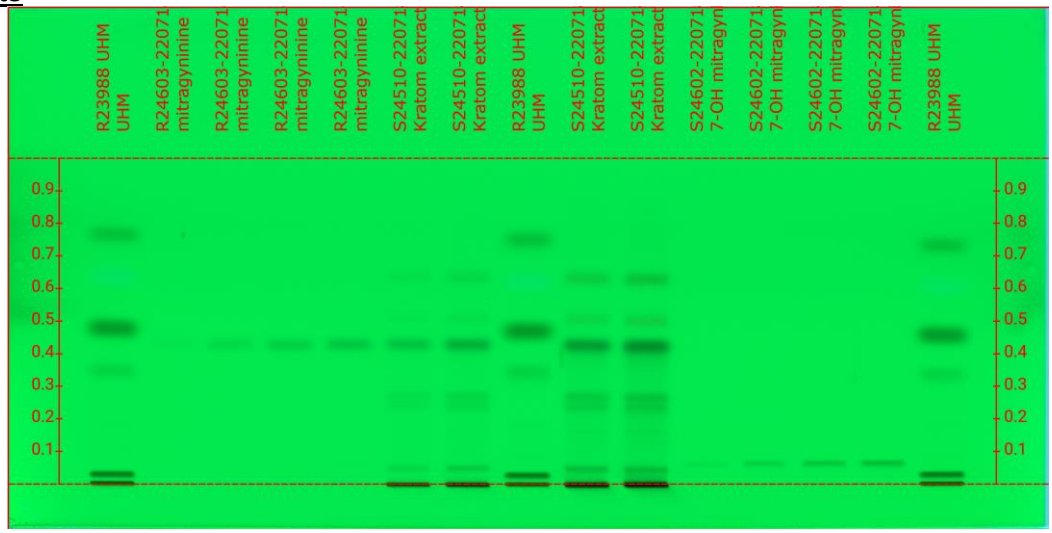

Image in short wave UV (254 nm)

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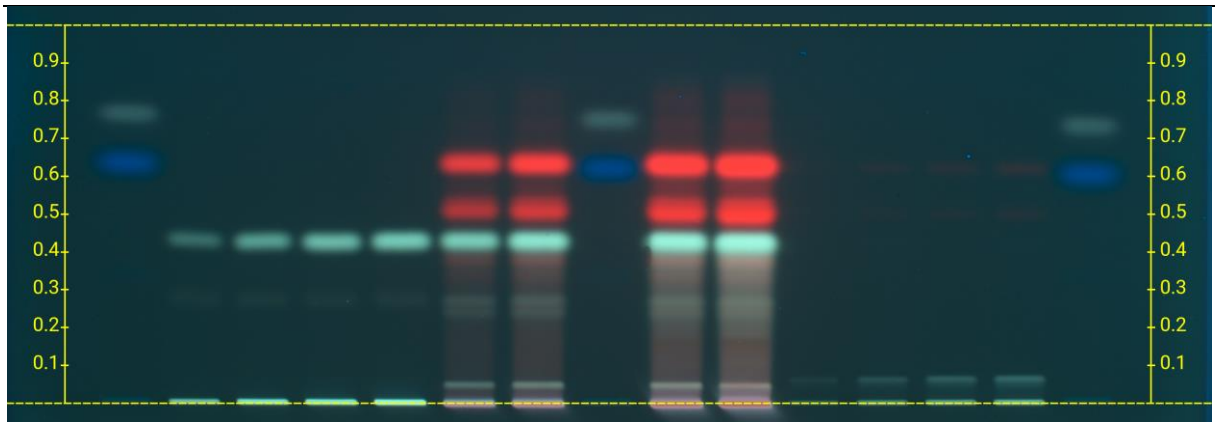

Image in long wave UV (350 nm broadband)

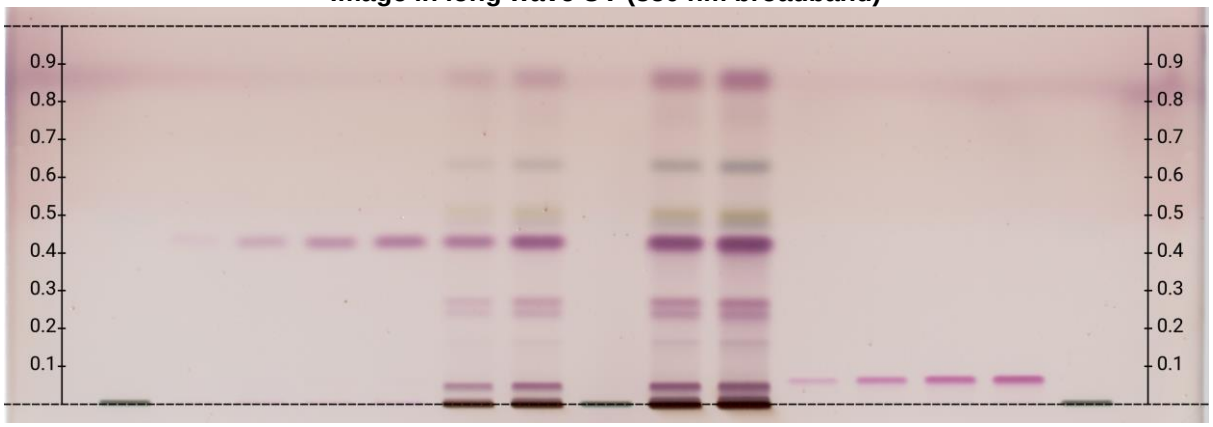

Image of derivatized plate in white light RT

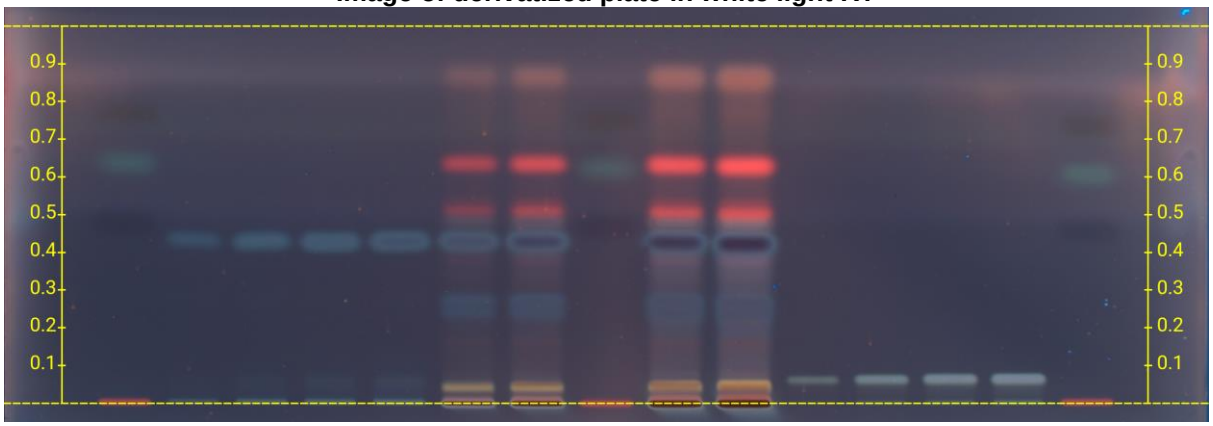

Image of derivatized plate long wave UV (350 nm broadband)

TEST 2

Evaluation of method [2]

Application

As in TEST 1

Development

Lab temperature (before chromatography): 22°C
Lab relative humidity (before chromatography): 46%
End relative humidity (achieved by ADC 2): 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: toluene, ethyl acetate, diethylamine 7:2:1 (v/v)
Developing time: 11 min
Plate drying: 5 min with cold air in ADC 2

Derivatization reagent: (Deviation from [2])
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 of reagent (Derivatizer, blue nozzle, spraying level: 3). Heat the plate at 100°C for 3 min.

Results

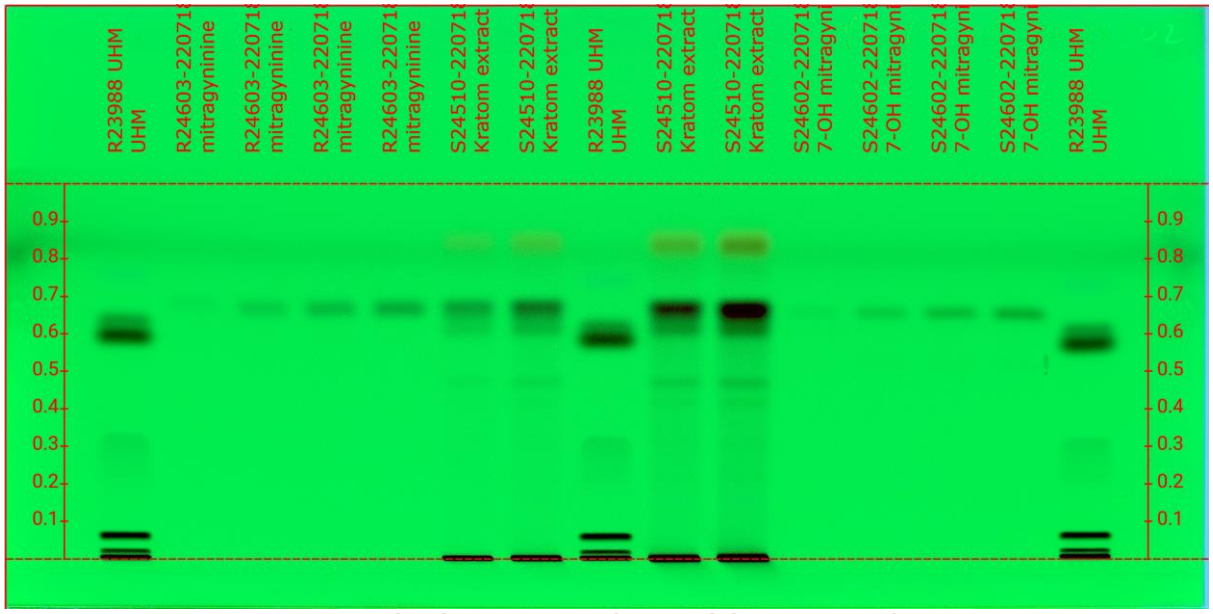

Image in shortwave UV (254 nm) (contrast 1.5)

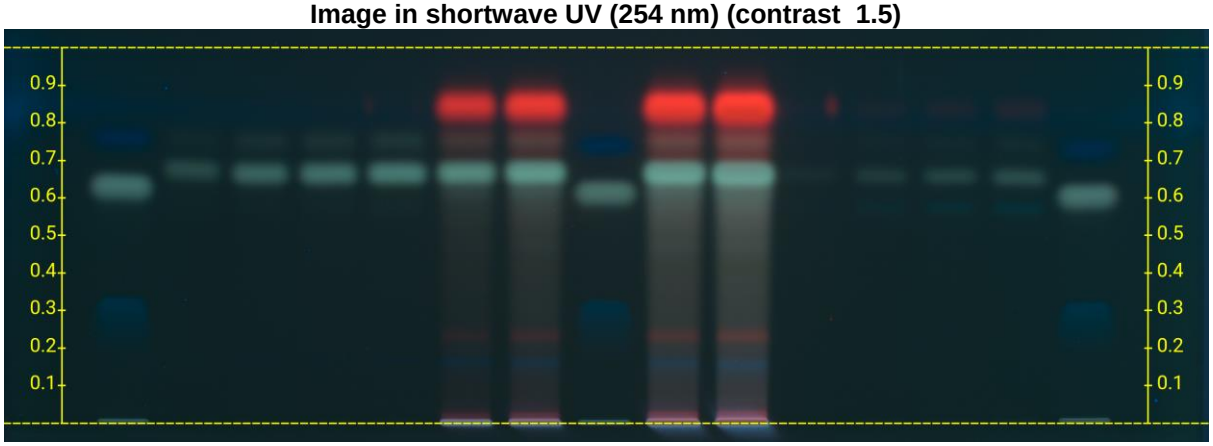

Image in longwave UV (350 nm broadband)

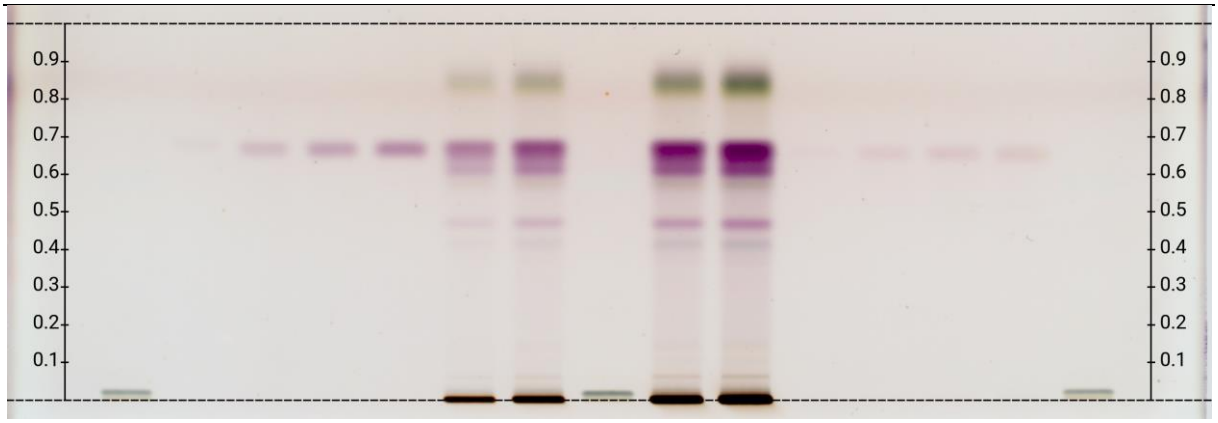

Image of derivatized plate in WRT contrast 1,4

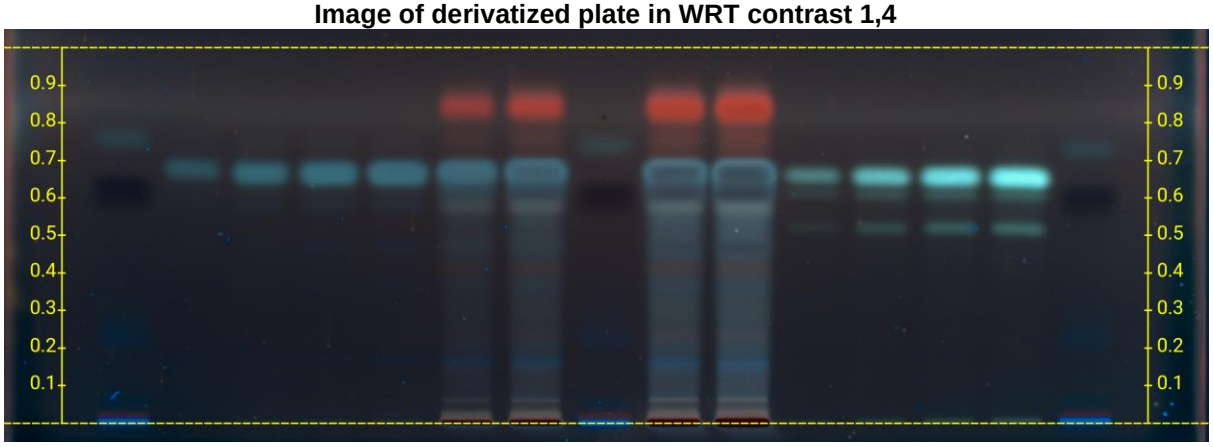

Image of derivatized plate long wave UV (350nm broadband)

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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