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Report

Project		P585-01 Green tea extract BSC HESI			
Related documents		[**1**] *Camellia sinensis* – decaffeinated extract_HPTLC Association_USP DSC_V2: [**2**] Reich *et. al*.; HPTLC methods for identification of green tea and green tea extract; Journal of LC&RT, DOI: 10.1080/15512160600760293 [**3**] Indena; 84650-60-2_20S0293200_CoA.pdf [**4**] Do *et al*: Development of the first universal mixture for use in system suitability tests for High-Performance Thin Layer Chromatography; https://doi.org/10.1016/j.chroma.2020.461830			
Customer		HESI			
Project objective		Identification of a green tea extract			
Date	18.07.2022	Laboratory	CAMAG, Muttenz	Analyst	ER

Summary

1. The **extract** (Lot 20S0293200) received for this study is labelled “green tea extract”, which may be misleading because in the CoA [**3**] the material is identified as “green tea dry decaffeinated extract polyphenols”.
2. Samples of various types of green tea leaf, green tea extracts, oolong and black tea as well as several standards were used for comparison.
3. When analyzed in **TEST 1** (see result section) with the method for identification of decaffeinated green tea extract [**1**], the polyphenol profile of the **extract** is typical for green tea (Figure 1, track 3). Due to the production mode (water extract) chlorophylls are not present (page 7). However, the zone seen R_F 0.62 in all detection modes (pages 7, 8) is not typical for *Camellia sinensis* leaf but also seen in the other two extracts (Figure 1, black arrow).

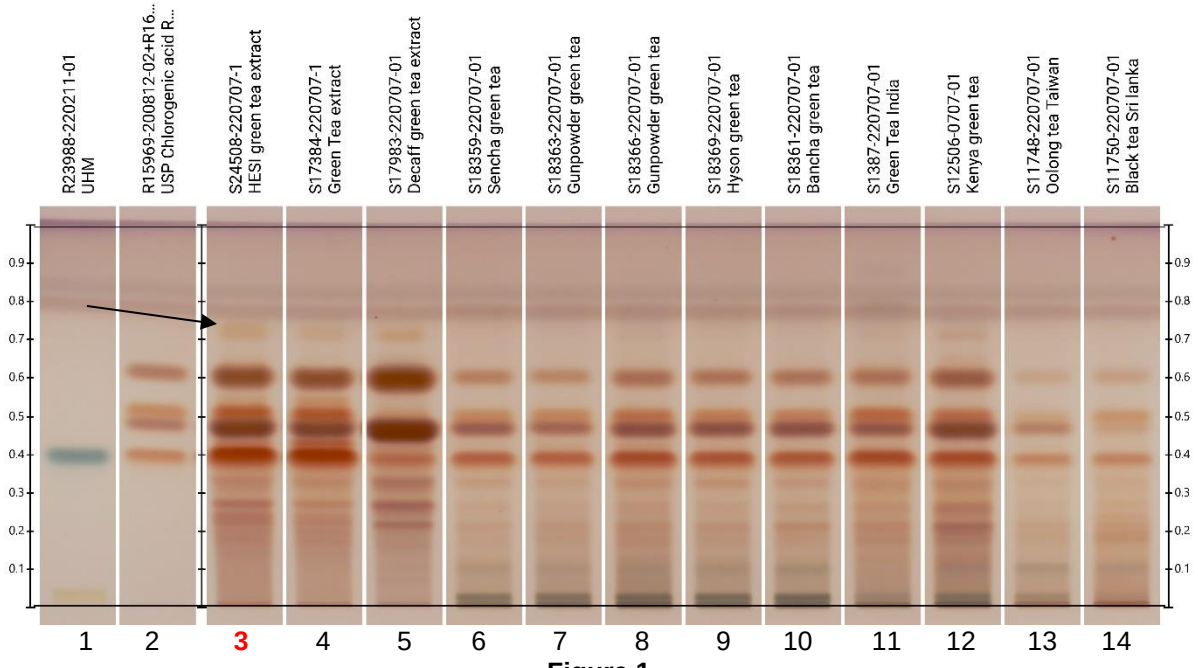

Figure 1

Polyphenol profile obtained with [**1**] after derivatization with NP+ AS reagents, white light RT; track 2: epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin with increasing R_F.

4. In short wave UV (254 nm) the absence of caffeine in the **extract** is observed (Figure 2, red arrow).

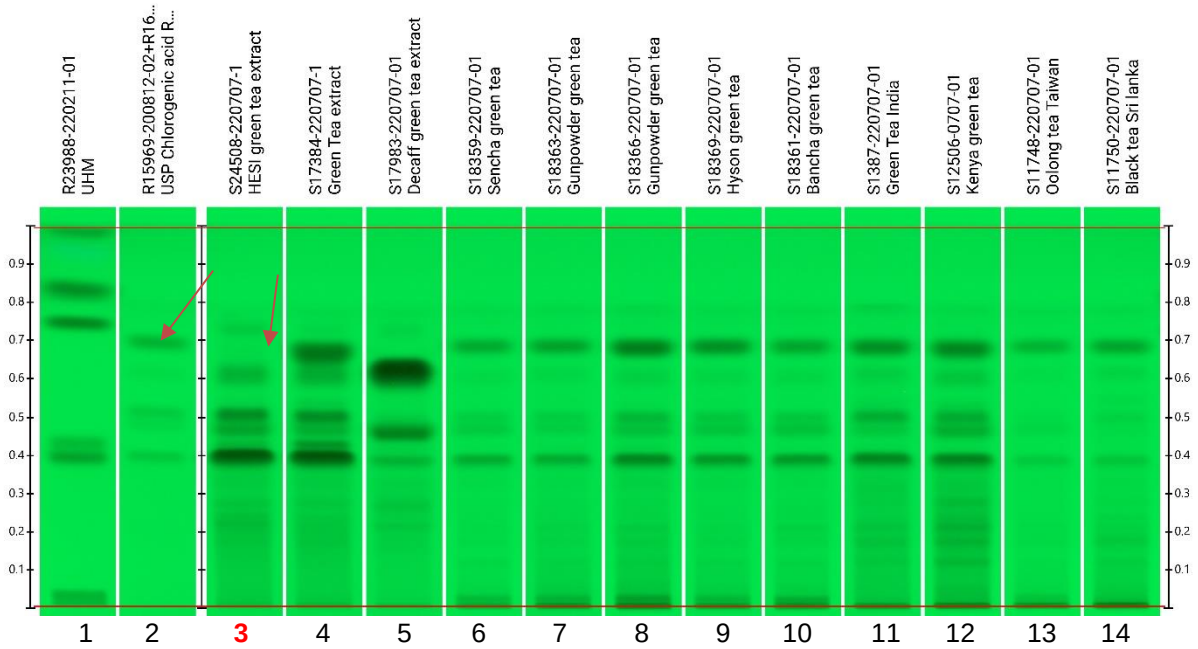

Figure 2

Fingerprints obtained with [**1**] in short wave UV (254 nm); track 2: epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin (faint) caffeine (red arrow) with increasing R_F.

5. The absence of caffeine is further proven in a densitometric scan at 273 nm (Figure 3).

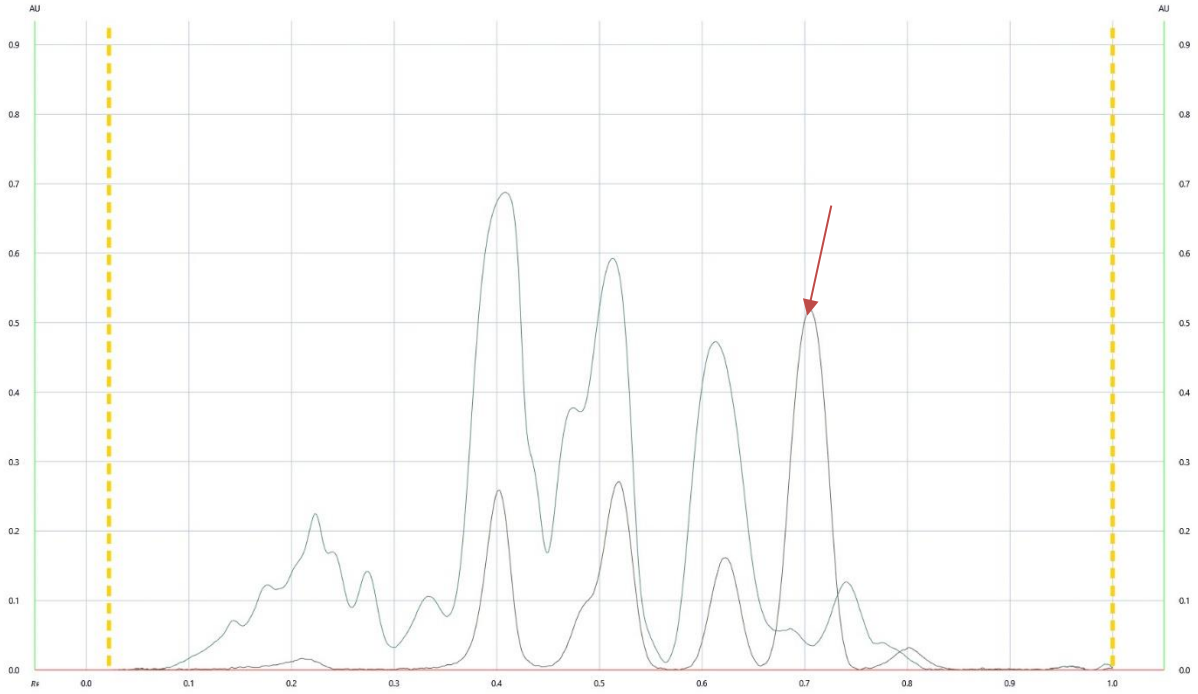

Figure 3

Densitogram at 273 nm (absorption) of **extract** (black) and standards (green); caffeine red arrow.

6. After derivatization with NP reagent in longwave UV light (350 nm broadband), the **extract** lacks the typical flavonoid zones of green tea (Figure 4, yellow bracket).

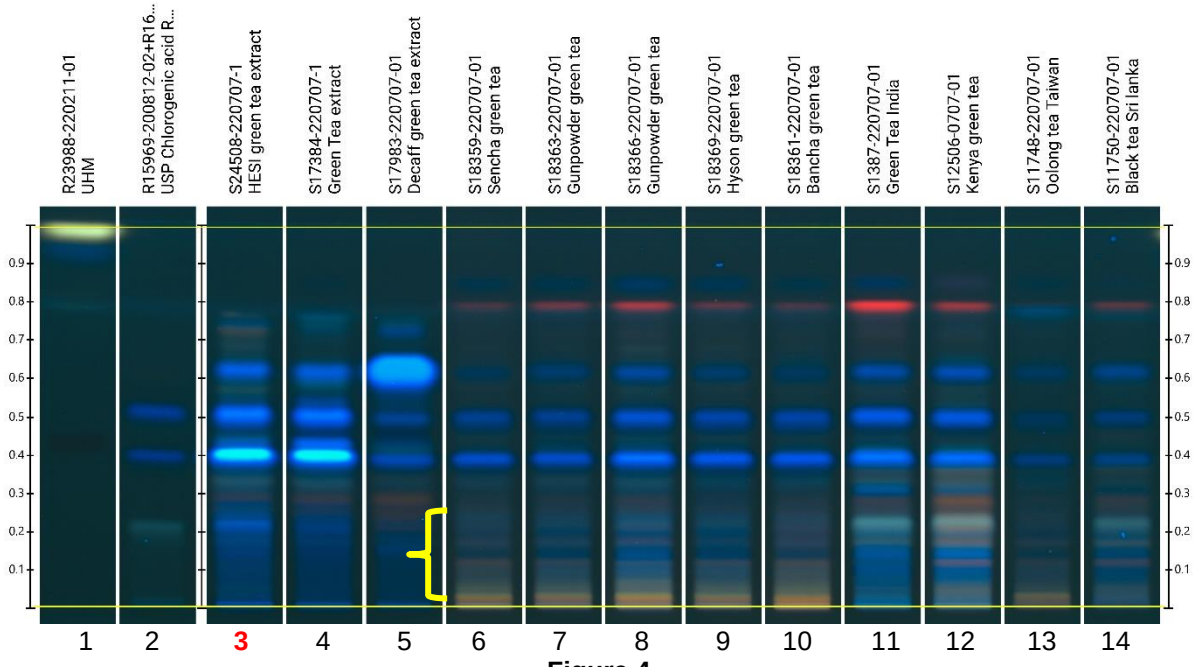

Figure 4

Fingerprints obtained with [**1**] in long wave UV (350 nm broadband) after derivatization with NP reagent; track 2: chlorogenic acid,epigallocatechin gallate, epicatechin gallate with increasing R_F.

7. The absence of higher glycosidated flavonoids is confirmed in **TEST 2** with the method for analysis of flavonoids (Figure 5 yellow bracket).

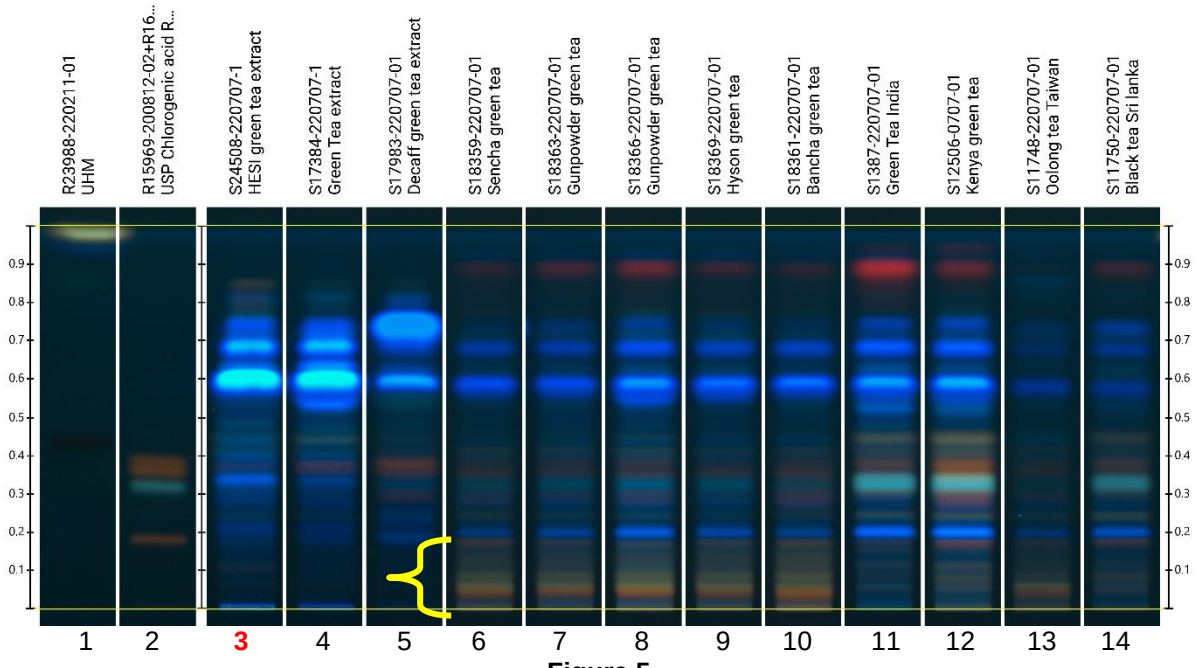

Figure 5

Fingerprints obtained with the flavonoid method of [**2**] after derivatization with NP+PEG reagents in long wave UV (350 nm broadband); track 2: rutin, chlorogenic acid, hyperosid, isoquercitrin with increasing R_F.

8. With the amino acid method of [**2**] (**TEST 3**) the absence of amino acids, particularly that of theanin, in the **extract** is noticed.

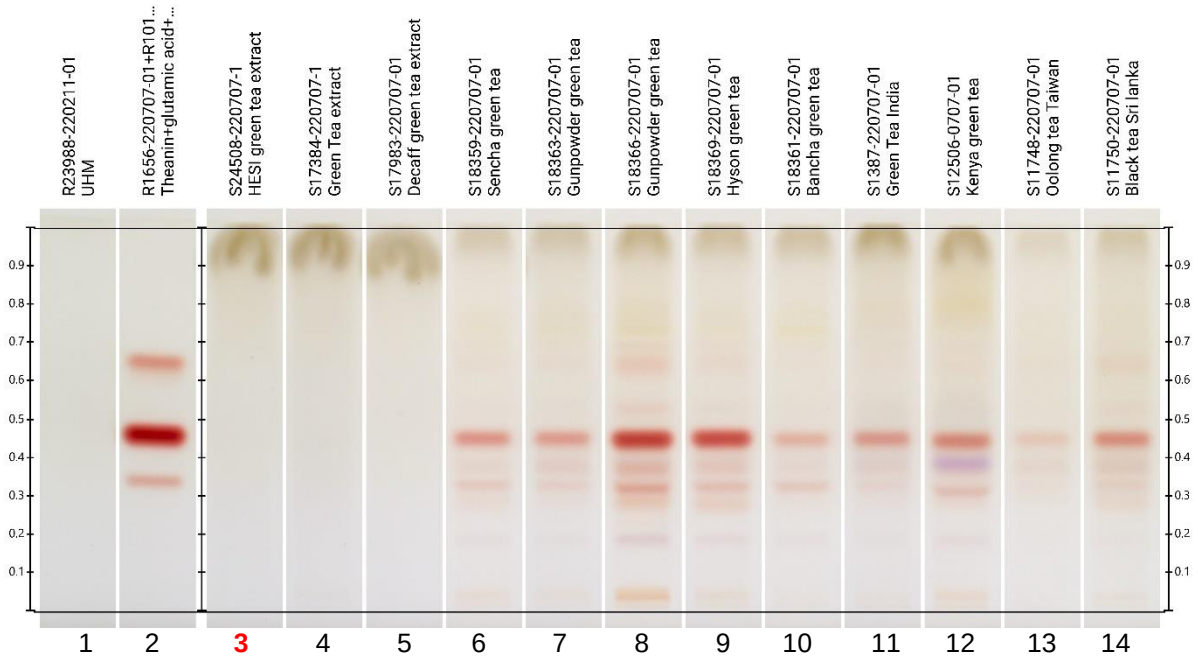

Figure 6

Fingerprints obtained with the amino acid method of [**2**] after derivatization with ninhydrin reagent in white light (transmission only). Track 2: glutamic acid, theanine, tyrosine with increasing R_F

Conclusion

The **extract** (Lot 20S0293200) is identified as a decaffeinated extract from green tea. It lacks amino acids and higher glycosidated flavonoids, which are typical constituents of green tea leaf.

Experimental details

Samples (S) and reference materials (R)

S24508	HESI green tea extract	MRIGlobal, Supplier: Indena via Battelle Memorial Inst., Lot 20S0293200
S17384	Green tea extract	CAMAG
S17983	Green tea extract decaffeinated	CAMAG
S18363	Green tea, Gunpowder	CAMAG
S1387	Green tea, India	CAMAG
S18369	Green tea, Hunan	CAMAG
S18359	Green tea, Sencha	CAMAG
S18366	Green tea, Gunpowder	CAMAG
S18361	Green tea, Bancha	CAMAG
S12506	Green tea, Kenya	CAMAG
S11748	Oolong, Taiwan	CAMAG
S11750	Black tea, Sri Lanka	CAMAG
R23988	Universal HPTLC Mixture	In-house - 2202211
R15969	chlorogenic acid	Extrasynthese, Batch 08 ID0511
R16427	(-)-epigallocatechin	Sigma 68H0844
R14344	caffeine	USP Lot K0K210
R17705	epicatechin	Sigma, Lot BCBT1189
R14343	epigallocatechin gallate	USP Lot G0L208
R19724	epicatechin gallate	Extrasynthese lot 11030807
R21660	rutin	USP; Lot R054J0
R20195	hyperoside	Roth; Charge: 418270974
R23059	isoquercitrin	PhytoLab, Charge: 14052
R1656	theanin	Sigma 127H1206
R10161	glutamic acid	Lesaffre
R24625	tyrosin	Sigma BCBJ9279V

Chemicals

Name	Manufacturer	Purity/quality	Batch
Methanol	Roth	Rotisolv	0002001863
Ethyl acetate	Acros	99.5%	271888
Ethyl formate	Acros	98+ %	A0398171
Formic acid	Thermo Scientific	98+ %	A0438424
Acetic acid	Acros	99.5%	A0427447
Toluene	Acros	99+ %	2101782
Acetone	Acros	99+ %	2196727
n-butanol	Acros	99%	A0433326
water	inhouse	De-ionized	
Ninhydrin	Fluka	p.a.	SZBA2910
Natural products reagent	Sigma	97%	BCCF3928
PEG 400	Aldrich		MKBG7718V
Anisaldehyde	Acros	99%	A0381986
Sulfuric acid	Acros	96%	A0419337

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:	100 mg/mL of powdered tea leaf; 50 mg/mL of extract in methanol – water 7:3 (*v/v*). Sonicate for 10 min, centrifuge and use the supernatant
Standard solutions:	Standards were prepared in methanol, at 0.5 mg/mL for catechins, caffeine and chlorogenic acid, 1.0 mg/ml for flavonoids and amino acids
Plate:	HPTLC glass plates, 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

r.	Vial ID	Description	Vol. (µl)	Position	Type	SST
1	R23988-220211-01	UHM	2.0	D1	Reference	☐
2	R15969-200812-02	USP Chlorogenic acid RS	2.0	D2	Reference	☑
+	R16427-170727-01	(-)-Epigallocatechin	2.0	D3	Reference	
+	R14344-171110-01	USP Caffeine RS	2.0	D7	Reference	
+	R17705-220707-01	Epicatechin	2.0	D6	Reference	
+	R14343-220707-01	epigallocatechin gallate	2.0	D5	Reference	
+	R19724-220707-01	Epicatechingallate	2.0	D4	Reference	
3	S24508-220707-1	HESI green tea extract	2.0	A1	Sample	☐
4	S17384-220707-1	Green Tea extract	2.0	A2	Sample	☐
5	S17983-220707-01	Decaff green tea extract	2.0	A3	Sample	☐
6	S18363-220707-01	Gunpowder green tea	2.0	A4	Sample	☐
7	S1387-220707-01	Green Tea India	2.0	A5	Sample	☐
8	S18369-220707-01	Hyson green tea	2.0	A6	Sample	☐
9	S18359-220707-01	Sencha green tea	2.0	A7	Sample	☐
10	S18366-220707-01	Gunpowder green tea	2.0	A8	Sample	☐
11	S18361-220707-01	Bancha green tea	2.0	A9	Sample	☐
12	S12506-0707-01	Kenya green tea	2.0	A10	Sample	☐
13	S11748-220707-01	Oolong tea Taiwan	2.0	A11	Sample	☐
14	S11750-220707-01	Black tea Sri lanka	2.0	B1	Reference	☐
15	R23988-220211-01	UHM	2.0	D1	Reference	☐

Development

Lab temperature (before chromatography): 26°C
Lab relative humidity (before chromatography): 39%
End relative humidity (achieved by ADC 2): 39%
Chamber: ADC 2
Humidity control: $MgCl_2$
Saturation: **unsaturated**
Developing distance from application position/lower edge: 62/70 mm
Developing solvent: toluene, acetone, formic acid 9:9:2 (v/v)
Developing time: 18 min
Plate drying: 5 min with cold air in ADC 2

Derivatization reagent 1:

Reagent name: NP reagent
Reagent preparation: 1.0 g of diphenylborinic acid aminoethyl ester is dissolved in 100 mL of methanol.
Reagent use: Heat plate at 100°C for 3 min and cool down to room temperature for 3 min, spray with 3.0 mL of reagent (Derivatizer, green nozzle, level: 3) and let dry for 2 min.

Derivatization reagent 2:

Reagent name: Anisaldehyde reagent (AS)
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, level: 3). Heat the plate at 100°C for 3 min.

Results

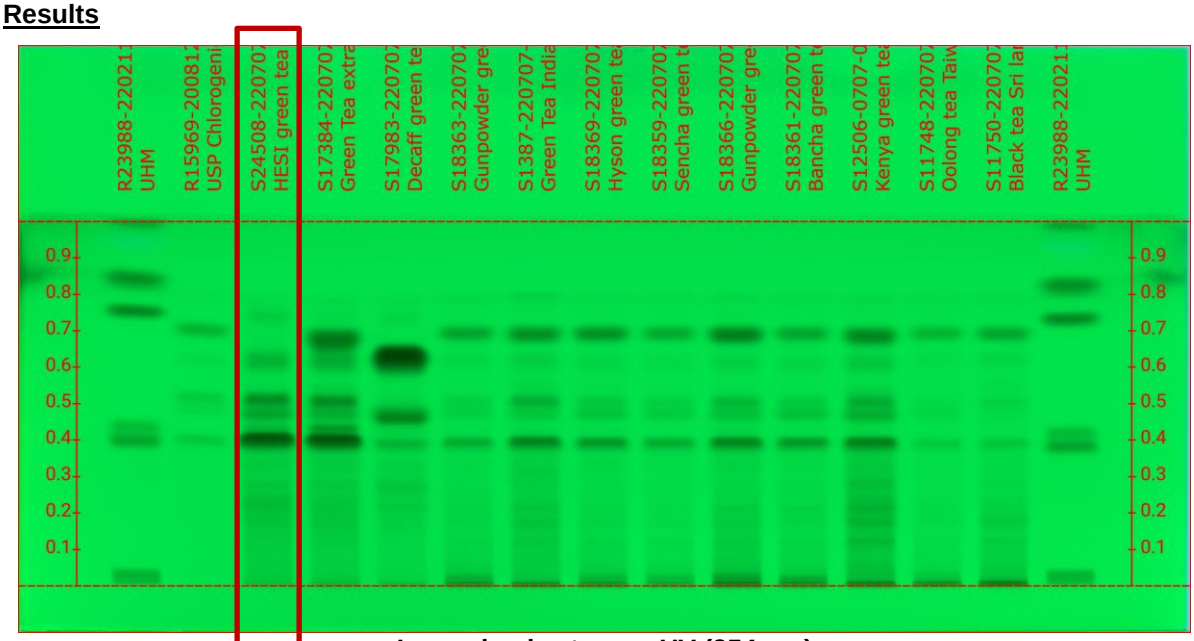

Image in short wave UV (254 nm)

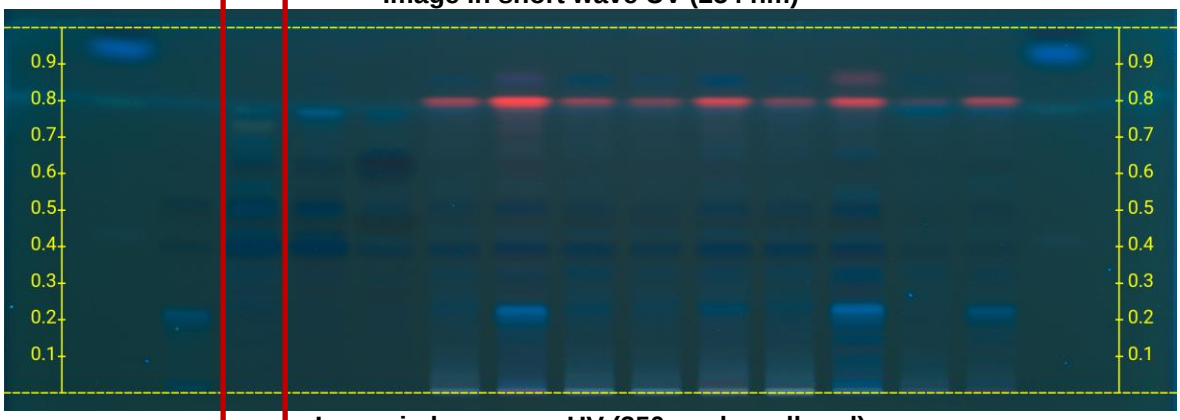

Image in long wave UV (350 nm broadband)

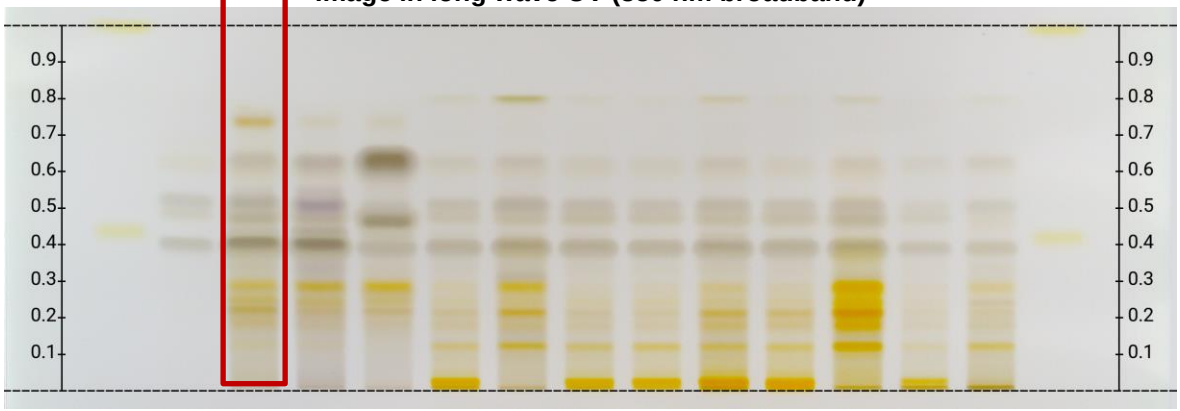

Image of derivatized plate (NP) WRT (enhanced, contrast 2)

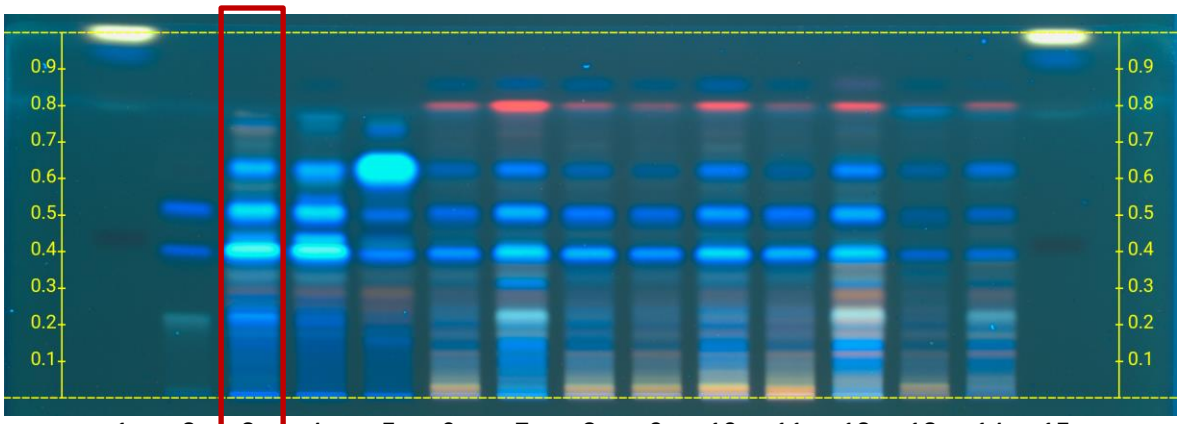

Image of derivatized plate (NP) longwave UV (350 nm broadband) (normalized on track 2)

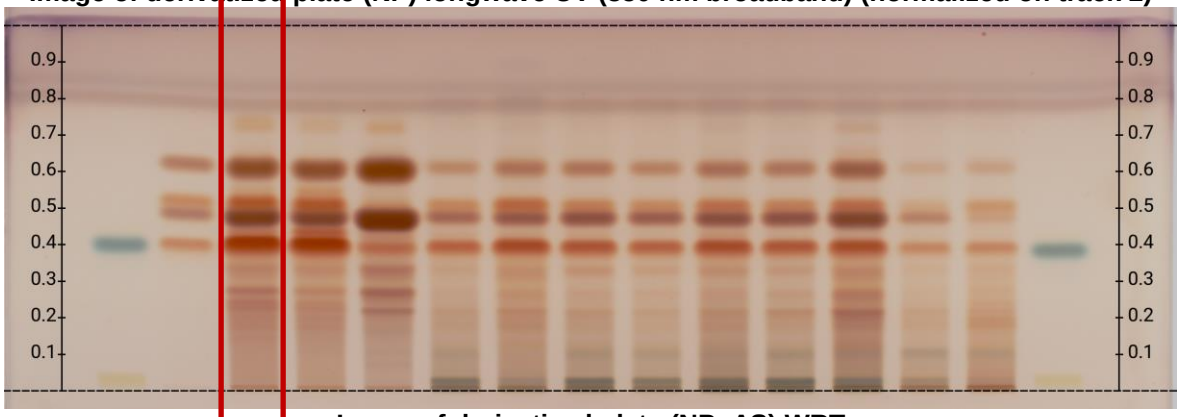

Image of derivatized plate (NP+AS) WRT

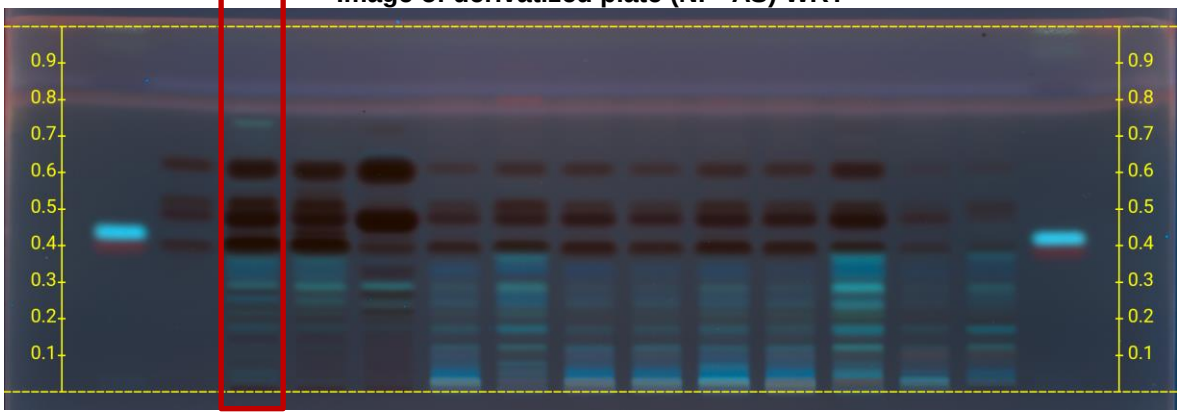

Image of derivatized plate (NP+AS) longwave UV (normalized on Track 1 UHM)

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

Tr.	Vial ID	Description	Vol. (µl)	Position	Type	SST
1	R23988-220211-01	UHM	2.0	C1	Reference	☐
2	R15969-200812-02	USP Chlorogenic acid RS	2.0	C2	Reference	☑
+	R16427-170727-01	(-)-Epigallocatechin	2.0	C3	Reference	
+	R14344-171110-01	USP Caffeine RS	2.0	C4	Reference	
+	Rxxxx10-190305	Rutin, hyperoside, isoquercitrin	2.0	C5	Reference	
3	S24508-220707-1	HESI green tea extract	2.0	A1	Sample	☐
4	S17384-220707-1	Green Tea extract	2.0	A2	Sample	☐
5	S17983-220707-01	Decaff green tea extract	2.0	A3	Sample	☐
6	S18363-220707-01	Gunpowder green tea	2.0	A4	Sample	☐
7	S1387-220707-01	Green Tea India	2.0	A5	Sample	☐
8	S18369-220707-01	Hyson green tea	2.0	A6	Sample	☐
9	S18359-220707-01	Sencha green tea	2.0	A7	Sample	☐
10	S18366-220707-01	Gunpowder green tea	2.0	A8	Sample	☐
11	S18361-220707-01	Bancha green tea	2.0	A9	Sample	☐
12	S12506-0707-01	Kenya green tea	2.0	A10	Sample	☐
13	S11748-220707-01	Oolong tea Taiwan	2.0	A11	Sample	☐
14	S11750-220707-01	Black tea Sri lanka	2.0	B1	Reference	☐
15	R23988-220211-01	UHM	2.0	C1	Reference	☐

Development

Lab temperature (before chromatography): 27°C
Lab relative humidity (before chromatography): 38%
End relative humidity (achieved by ADC 2): 38%
Chamber: ADC 2
Humidity control: $MgCl_2$
Saturation: 20 min with filter paper
Developing distance from application position/lower edge: 62/70 mm
Developing solvent: ethyl formate, water, toluene, formic acid 60:6:3:8 (v/v)
Developing time: 18 min
Plate drying: 5 min with cold air in ADC 2

Derivatization

Reagent name: NP / PEG reagent
Reagent preparation: Mix 1 part of NP reagent with 1 part of 5.0 g of polyethylene glycol 400 in 100 mL of ethanol.
Reagent use: heat the plate at 100°C for 3 min and let cool down to room temperature for 3 min. Then spray 3 mL of reagent (Derivatizer, green nozzle, spraying level: 3).

Results

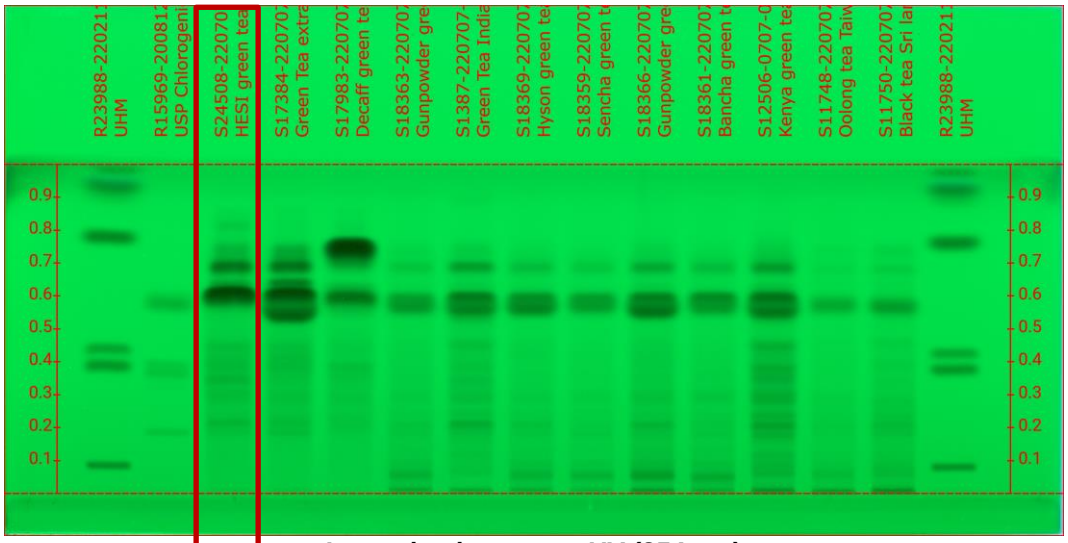

Image in short wave UV (254 nm)

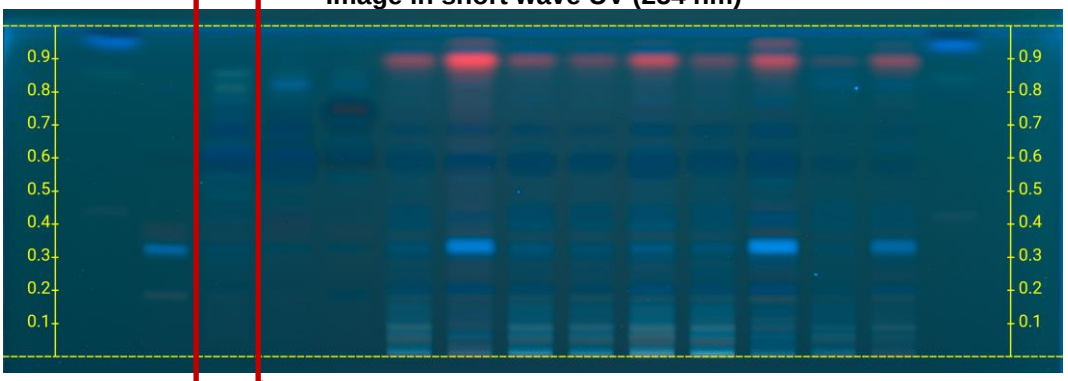

Image in long wave UV (350 nm broadband)

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

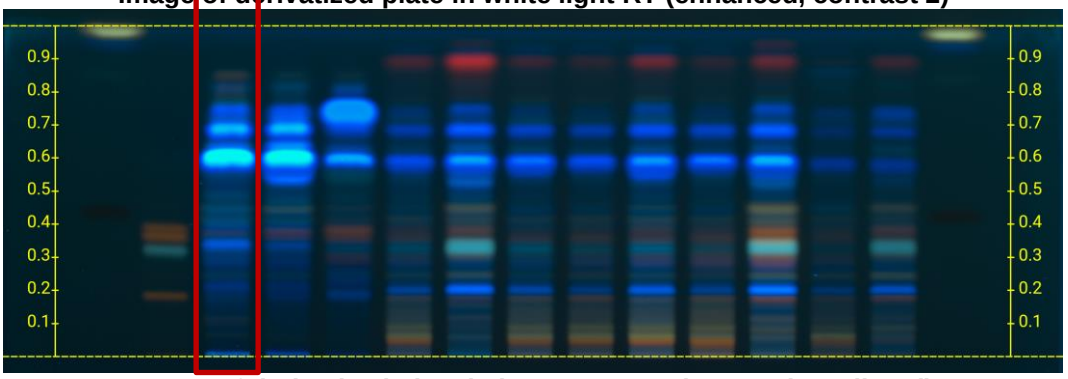

Image of derivatized plate in long wave UV (350 nm broadband)

TEST 3

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	R23988-220211-01	UHM	2.0	C1	Reference	☐
2	R1656-220707-01	Theanin	5.0	E1	Reference	☑
+	R10161-220707-01	glutamic acid	5.0	E2	Reference	
+	R14344-171110-01	USP Caffeine RS	2.0	E3	Reference	
+	Rxxxx	tyrosin	5.0	E4	Reference	
3	S24508-220707-1	HESI green tea extract	2.0	A1	Sample	☐
4	S17384-220707-1	Green Tea extract	2.0	A2	Sample	☐
5	S17983-220707-01	Decaff green tea extract	2.0	A3	Sample	☐
6	S18363-220707-01	Gunpowder green tea	2.0	A4	Sample	☐
7	S1387-220707-01	Green Tea India	2.0	A5	Sample	☐
8	S18369-220707-01	Hyson green tea	2.0	A6	Sample	☐
9	S18359-220707-01	Sencha green tea	2.0	A7	Sample	☐
10	S18366-220707-01	Gunpowder green tea	2.0	A8	Sample	☐
11	S18361-220707-01	Bancha green tea	2.0	A9	Sample	☐
12	S12506-0707-01	Kenya green tea	2.0	A10	Sample	☐
13	S11748-220707-01	Oolong tea Taiwan	2.0	A11	Sample	☐
14	S11750-220707-01	Black tea Sri lanka	2.0	B1	Reference	☐
15	R23988-220211-01	UHM	2.0	C1	Reference	☐

Development

Lab temperature (before chromatography): 27°C

Lab relative humidity (before chromatography): 36%

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

Chamber: ADC 2

Humidity control: $MgCl_2$

Saturation: 20 min with filter paper

Developing distance from application position/lower edge: 52/60 mm

Developing solvent: n-butanol, acetone, acetic acid, water 7:7:2:4 (v/v)

Developing time: 18 min

Plate drying: 5 min with cold air in ADC 2

Derivatization

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 the plate with 3.0 mL of reagent (Derivatizer, blue nozzle, spraying level: 3) and the heat at 100°C for 3 min.

Results

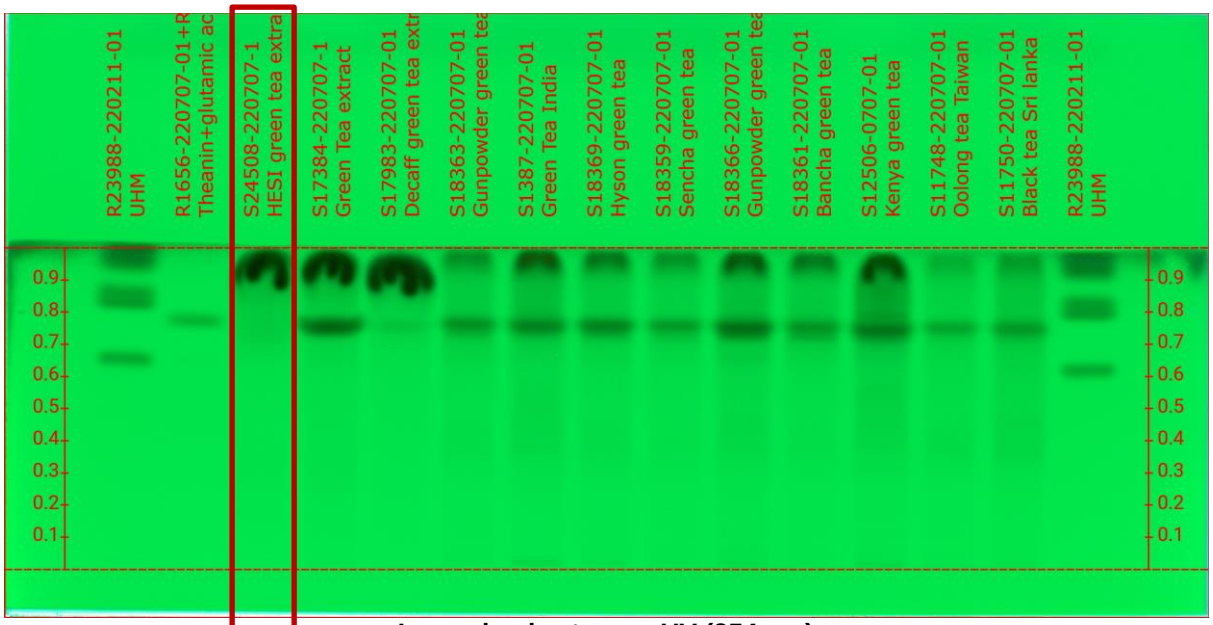

Image in short wave UV (254 nm)

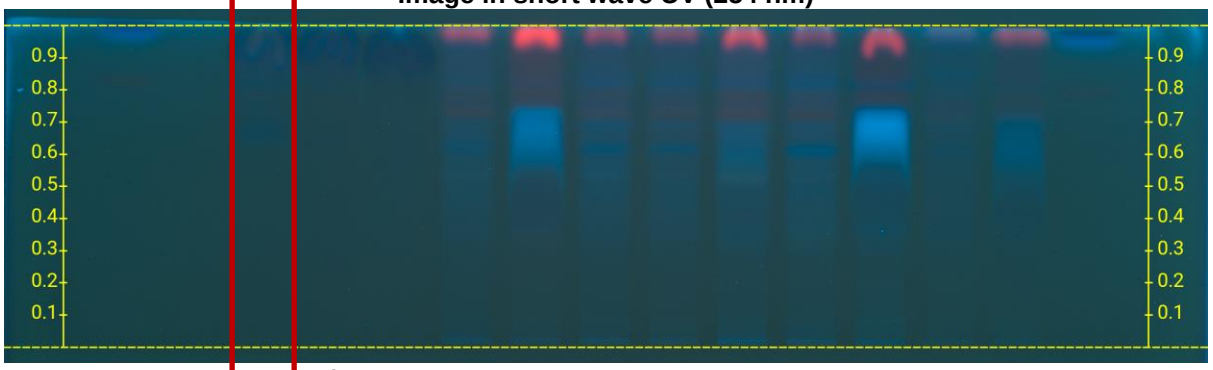

Image in long wave UV (350 nm broadband)

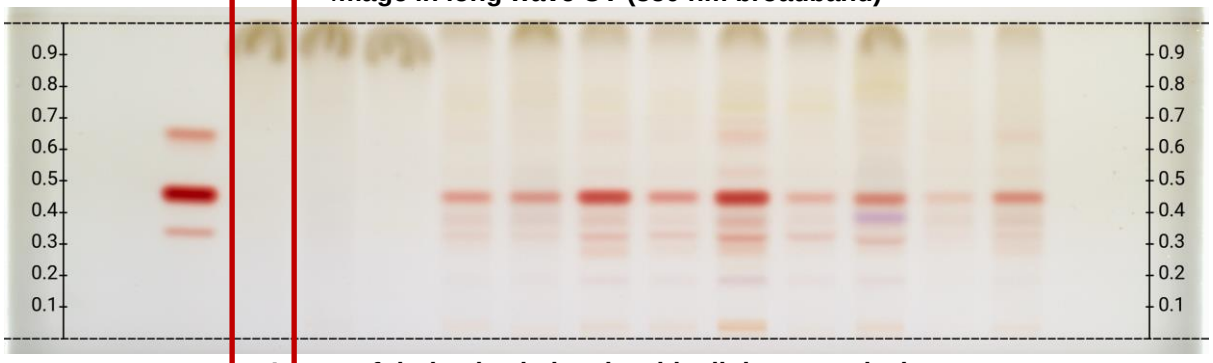

Image of derivatized plate in white light transmission

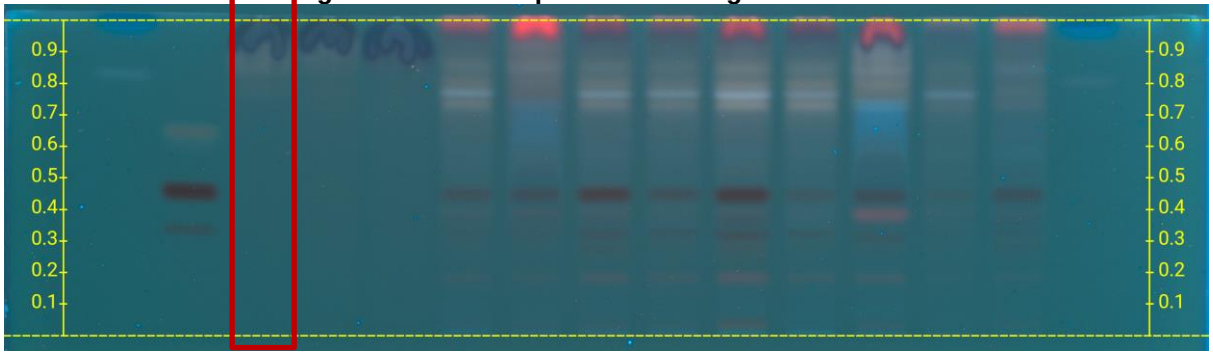

Image of derivatized plate in long wave UV (350 nm broadband)

Additional experimental details are available upon request.

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

Disclaimer

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.