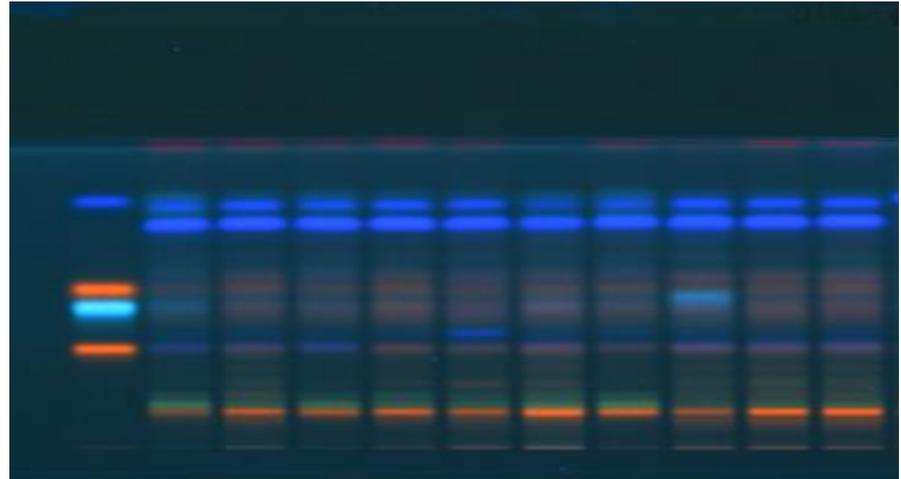


HPTLC HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

Ing. Aneta Hartmanová
DONAU LAB, s.r.o.

Why Thin Layer Chromatography ?

- Clearly visual & documented
- Rapid
- Cost efficient
- Small application volumes
- Less sample preparation
- Parallel analysis for standards and samples under identical conditions
- No solvent limitations, no carry-over
- Ideal method for complex matrices (plant materials, lipids, high sugar content samples)





Botanical



Pharma



Food & Feed



Cosmetics

HPTLC Application fields



Clinical



Forensic



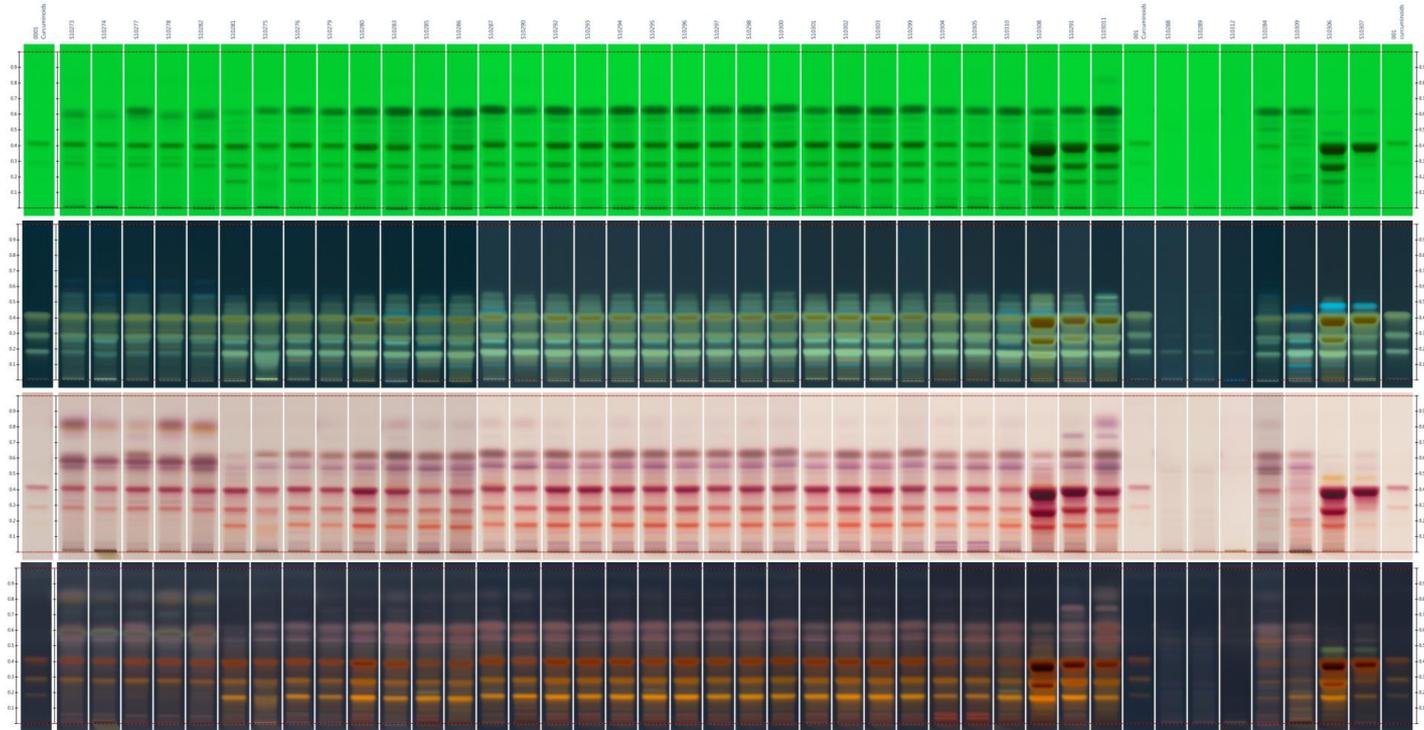
Industrial



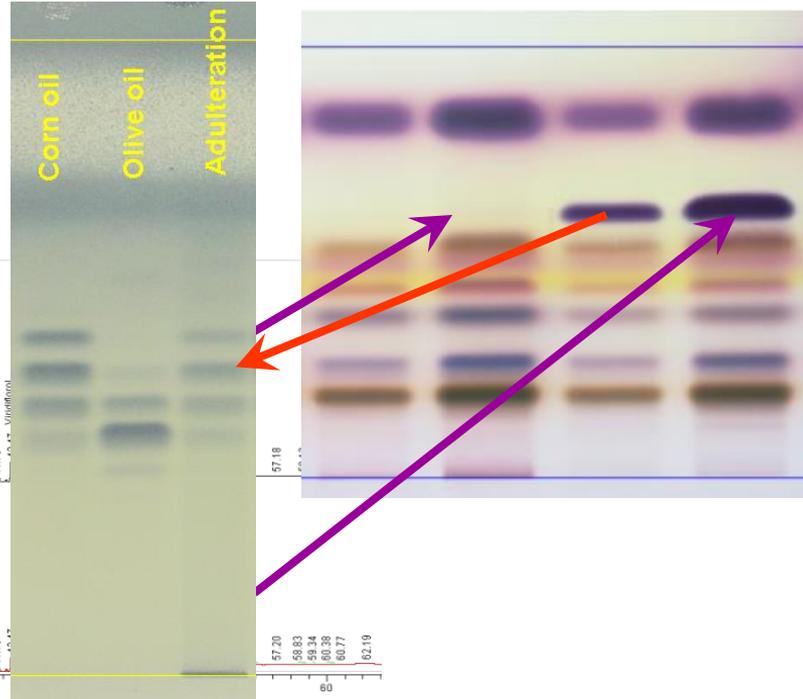
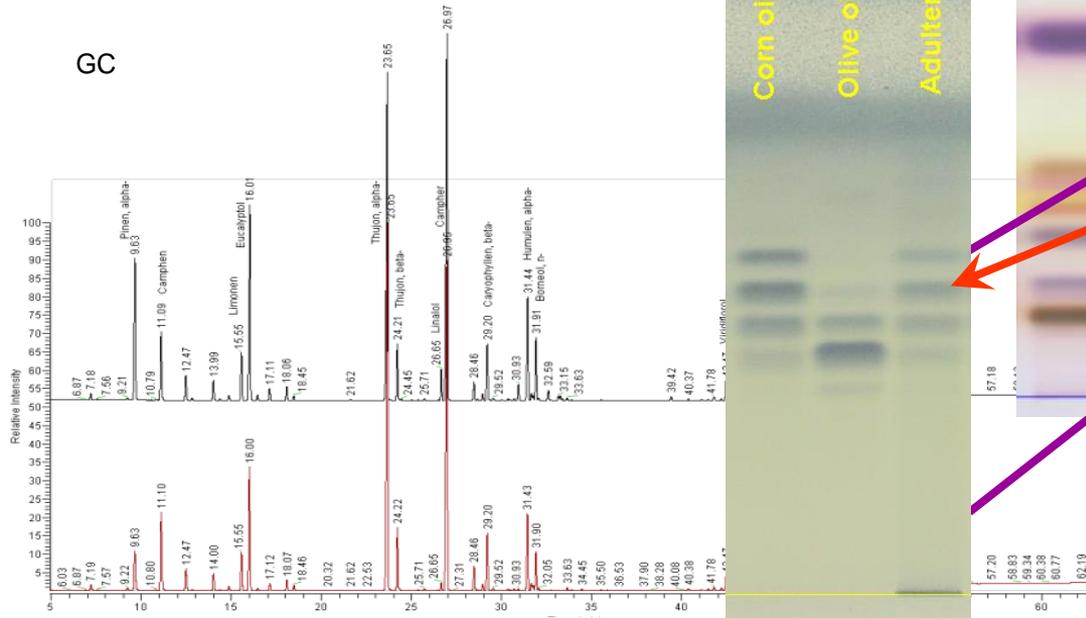
Environmental

Application field: Botanical & Pharma – Comparison of Curcuma samples

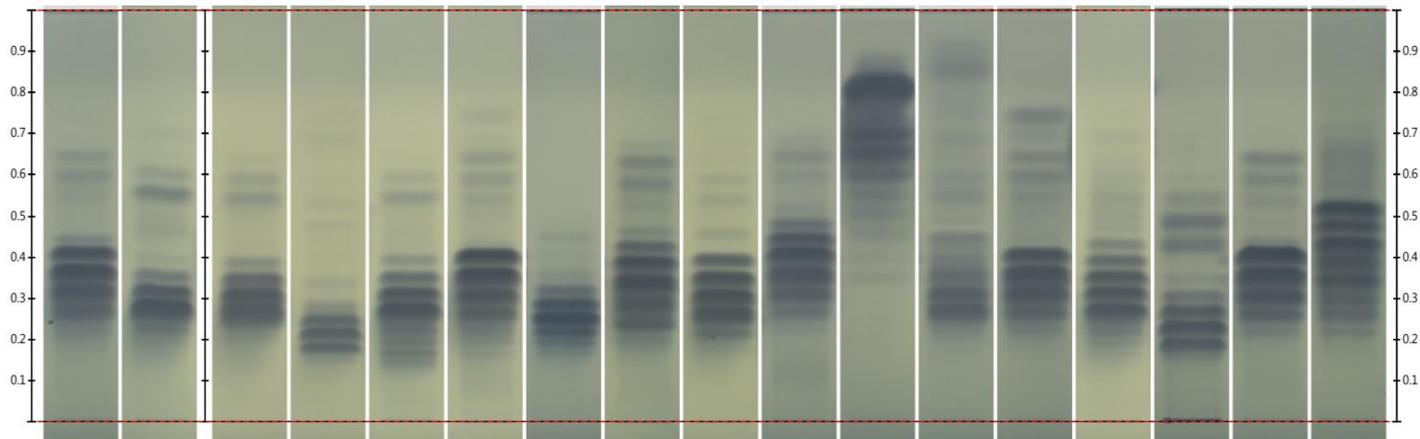
One separation & four ways to analyse



Application field: Botanical & Pharma – Falsification of Sage oil



Application field: Food & Feed – Identification of fatty oils



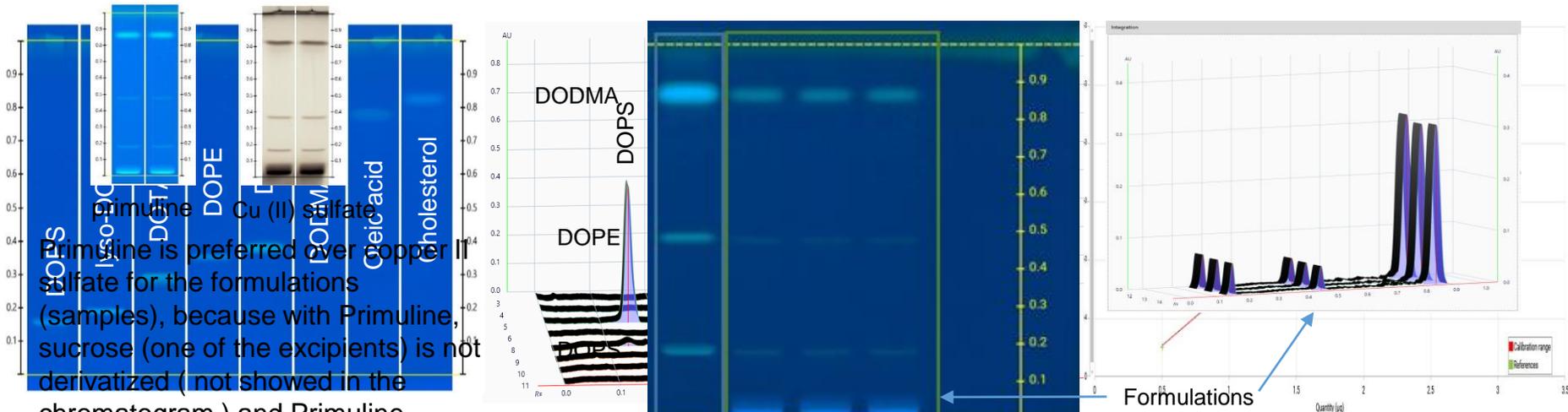
Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Corn oil (SST)	10	2 µL	Walnut oil
2	2 µL	Olive oil –USP (SST)	11	2 µL	Castor oil
3	2 µL	Avocado oil	12	2 µL	Laurel oil
4	2 µL	Cacao oil	13	2 µL	Nigella oil
5	2 µL	Henbane oil	14	2 µL	Rape seed oil
6	2 µL	Hypericum oil	15	2 µL	Shea butter
7	2 µL	Jojoba oil	16	2 µL	Grape seed oil
8	2 µL	Weat germ oil	17	2 µL	Linseed oil
9	2 µL	Argan oil			

USP general chapter <203>
Identification of Fixed oils by Thin-
Layer Chromatography.

Application field: Botanical & Pharma, Cosmetics – Analysis of lipid-based formulations

HPTLC allows an easy, rapid and robust high-throughput testing of complex lipid-containing samples

The liposome based formulations (samples) contain PEK liposomes (DODMA, DOPE, DOPS), and excipients (m, Metg and, Water 65:25:4 (V/V/V) as Primuline reagent for derivatization.



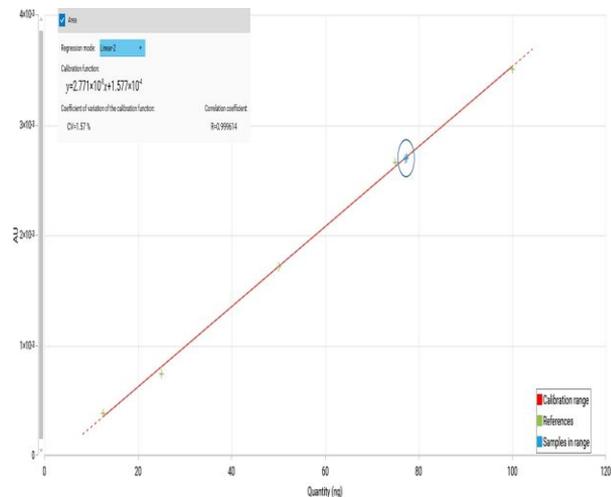
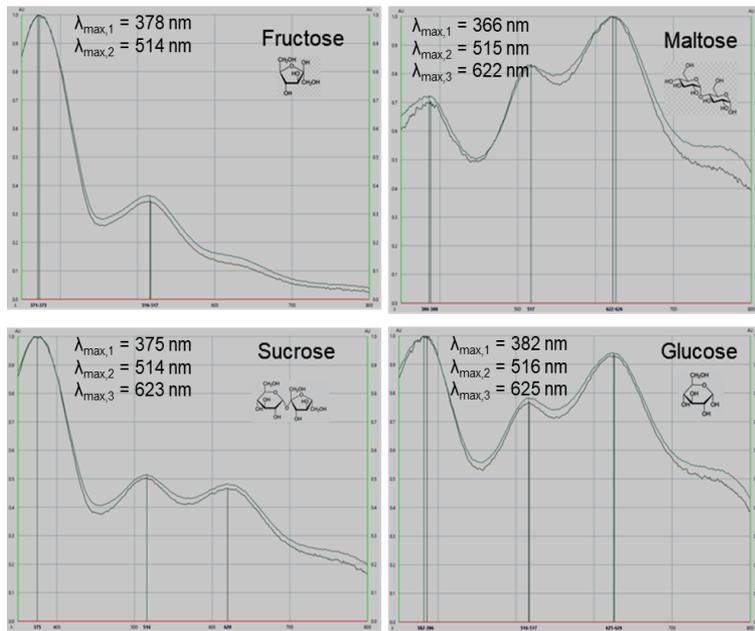
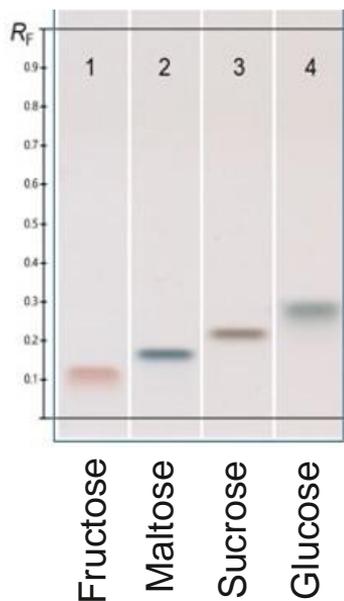
Primuline is preferred over copper II sulfate for the formulations (samples), because with Primuline, sucrose (one of the excipients) is not derivatized (not showed in the chromatogram) and Primuline allows a detection in fluorescence mode (better sensitivity, good S/N ratio and linear working range)

HPTLC derivatization with primuline reagent

sucrose and mRNA are not migrating (stay at the application line) and no degradation products can be seen in the sample

(A-138.1)

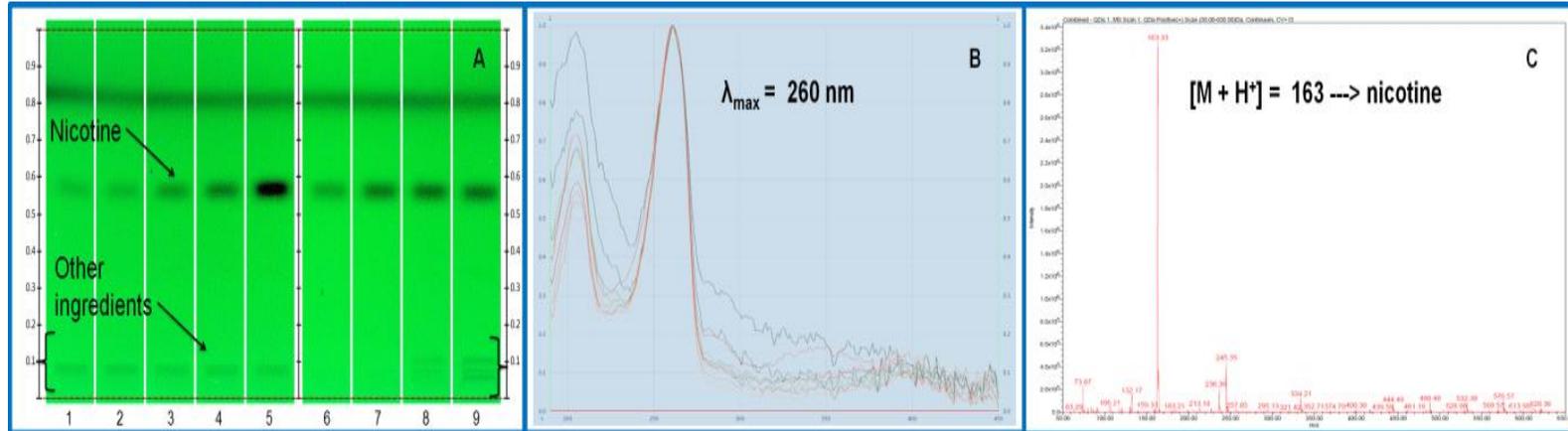
Application field: Food & Feed – Quantification of sugar in honey



Calibration curve of sucrose

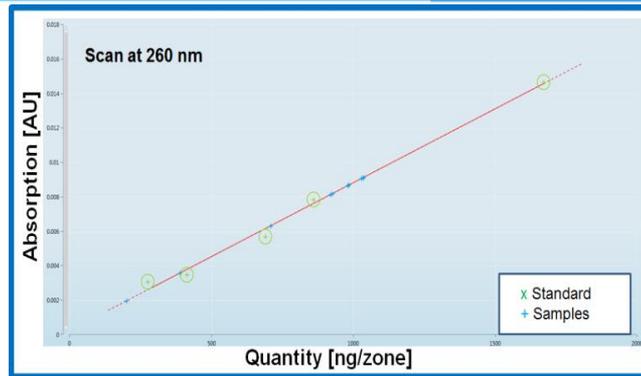
Mobile phase: 1-butanol, 2-propanol, aqueous boric acid (5 mg/mL) 30:50:10 (v/v/v) in ADC 2
Derivatization with ADPA reagent (aniline-diphenylamine-phosphoric acid)

Application field: Food & Feed – Semi-Quantification of nicotine in e-liquid



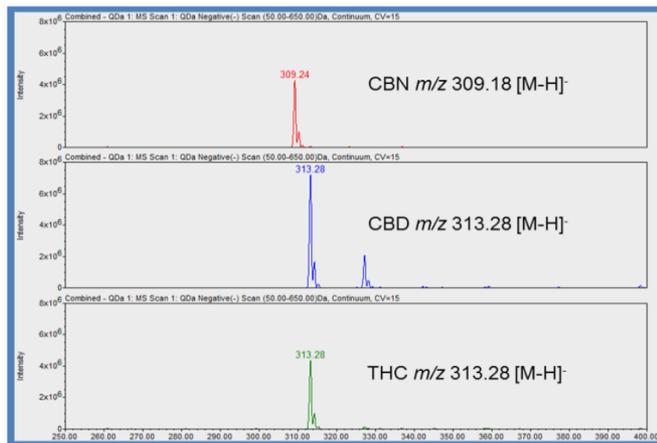
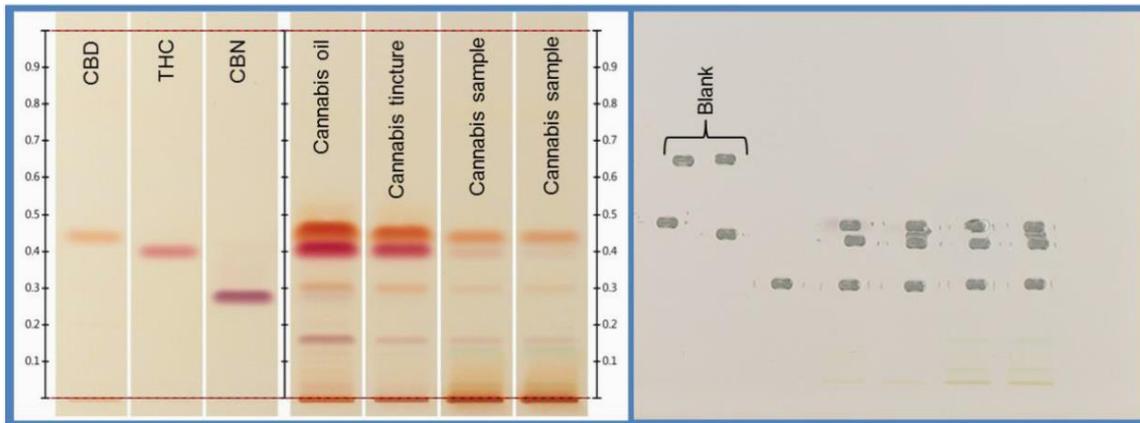
Tracks 1–5: reference solutions
Tracks 6–7: samples without flavor
Tracks 8–9: samples with flavor

Track no.5 is **the 20 mg/ml limit**
which is the maximum
concentration allowed in Europe



(A-106.1)

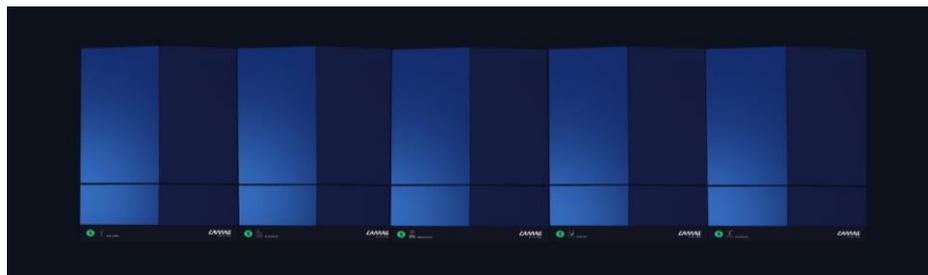
Application field: Forensics – Cannabinoids in different Cannabis samples



(A-98.1)



CAMAG instruments & tools portfolio



CAMAG instruments & tools portfolio

TLC line – **Basic kit**



CAMAG instruments & tools portfolio

HPTLC line – **Sample Application: semi automatic**

LINOMAT 5



- Applies samples by spray technique on **20x20, 20x10 & 10x10 cm plates & sheets**
 - Manual :
 - Syringe cleaning
 - Syringe filling
 - replacing the syringe
 - Automatic sample spraying in homogeneous bands or spots and in exact preprogrammed positions
- 
- **Stand-alone** or by visionCATS software operation
 - Syringes : **Standard 100 µL** , optional 500 µL

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HPTLC line – **Sample Application: automatic**

AUTOMATIC TLC SAMPLER 4



- Applies bands and spots by spray and by contact technique on **20x20, 20x10 & 10x10 cm plates & sheets**
 - Fully automatic sample application and syringe cleaning
 - standard rack of **66 positions**
 - Automatic sample spraying in homogeneous bands or spots and in exact preprogrammed positions
- 
- The image shows two examples of TLC results. On the left, a horizontal band of dark spots is visible on a TLC plate, representing a homogeneous band application. On the right, five distinct, circular spots are visible, representing individual spot applications.
- **Fully controlled** by visionCATS software
 - Syringes : **Standard 25 μL** , optional 10 μL & 100 μL

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HPTLC line – Plate Development

Automatic Developing Chamber ADC2



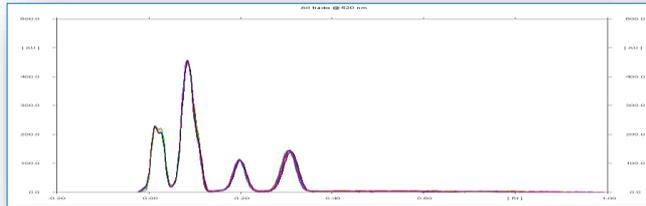
- Fully automatic development of only **HPTLC plates 20x10 cm**
- Saturation, preconditioning & humidity are controlled which leads to unsurpassed **reproducible RFs**
- **Stand-alone** or via visionCATS software operation

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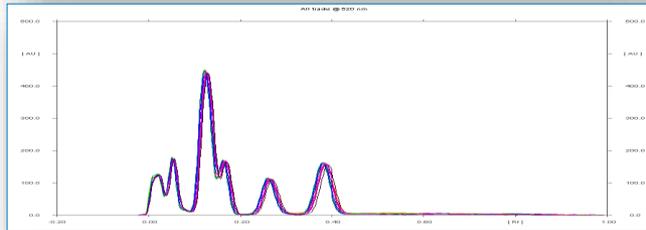
HPTLC line – Plate Development

Humidity effect on RF (test dye)

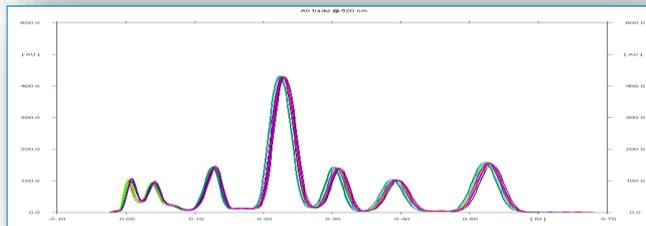
17% RH.



47% RH.



75% RH.



saturated salt solution	% relative humidity
NaCl	75
KSCN	47
MgCl₂	33
KCOOH	20
(Molecular sieve)	0-5

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HPTLC line – Plate Development

AUTOMATED MULTIPLE DEVELOPMENT AMD 2



- Multiple development using a solvent gradient of only **HPTLC plates 20x10 cm**
- Between runs, the solvent is **completely removed** from the developing chamber and the layer is dried under vacuum.
- Each following run uses a **solvent of lower elution strength** than that of the one used before. Separation power **improved** over regular HPTLC development
- Fully controlled by visionCATS software

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HPTLC line – Derivatization

Chromatogram Immersion Device III

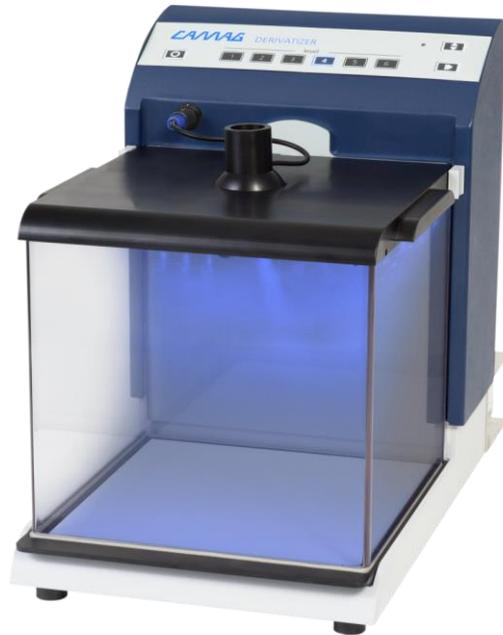


- Derivatization by **dipping**
- Selectable **immersion time** and **vertical speed**
- Uniform reagent transfer
- Dip tanks for **20x20 & 20x10 cm plates & sheets**
- It takes **200 ml** to fill the glass dip tank

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HPTLC line – Derivatization

DERIVATIZER



- Derivatization by **spraying**
- Reproducible & homogeneous reagent thanks to ``**Micro droplet**`` spraying technology
- Low reagent consumption (**2 - 4 ml**)
- **Standalone** instrument with small footprint
- Suitable for **plates & sheets up to 20x20 cm**
- Environment & user friendly thanks to the **closed hood** and the **wash bottle aspiration system**



CAMAG instruments & tools portfolio

HPTLC line – Derivatization

TLC PLATE HEATER III



- Derivatization by **thermal activation**
- Heating the plate after spaying or after dipping in chemical reagent
- Temperature of heating surface is **programmable** from ambient to 200° Celsius and continuously displayed
- Suitable for **plates & sheets up to 20x20 cm**

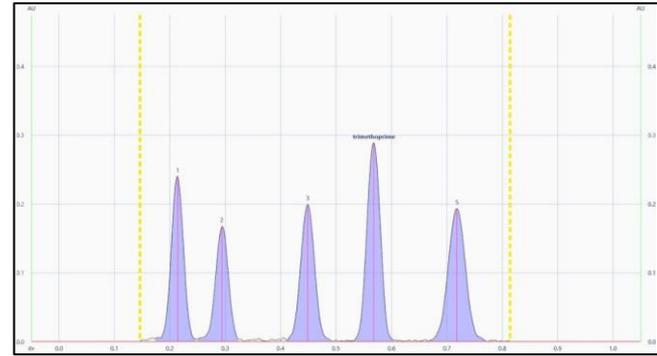
CAMAG instruments & tools portfolio

HPTLC line – Detection

TLC SCANNER 4



- Detection by scanning **plates & sheets up to 20x20 cm** under specific wavelength in the UV or visible range



- Works in **absorption, fluorescence or spectrum** mode
- Spectral range from **190 to 900 nm**
- Fully controlled by visionCATS software

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HPTLC line – Detection

visionCATS by CAMAG® - Licensed to CAMAG with SN 100151

Explorer Example Analysis... scan [read-only]

1 HPTLC Steps 2 Chromatography 3 Data 4 Spectrum 5 SST

Remarks Report Log

Spectrum Steps

Positioning Execution Spectrum data Correlation data

Overview

Step Substance

Developed 1c

Spectrum correlation data

Export correlation (CSV)

Step	Substance	Track	Rf	r(s,m)	r(e,m)	Ref. spectrum	Correlation
Developed 1c	sulfamerazin	1	0.349			Tr. 3, Rf 0.345, Sub. sulfamerazin	0.999726
Developed 1c	sulfamerazin	2	0.348			Tr. 3, Rf 0.345, Sub. sulfamerazin	0.999522
Developed 1c	sulfamerazin	3	0.345			Tr. 6, Rf 0.345, Sub. sulfamerazin	0.999757
Developed 1c	sulfamerazin	4	0.345			Tr. 3, Rf 0.345, Sub. sulfamerazin	0.999773
Developed 1c	sulfamerazin	5	0.345			Tr. 6, Rf 0.345, Sub. sulfamerazin	0.999844
Developed 1c	sulfamerazin	6	0.345			Tr. 3, Rf 0.345, Sub. sulfamerazin	0.999757
Developed 1c	sulfamerazin	7	0.345			Tr. 6, Rf 0.345, Sub. sulfamerazin	0.999715
Developed 1c	sulfamerazin	8	0.346			Tr. 9, Rf 0.346, Sub. sulfamerazin	0.999857
Developed 1c	sulfamerazin	9	0.346			Tr. 6, Rf 0.345, Sub. sulfamerazin	0.999749
Developed 1c	sulfamerazin	10	0.346			Tr. 9, Rf 0.346, Sub. sulfamerazin	0.999839
Developed 1c	sulfamerazin	11	0.345			Tr. 12, Rf 0.343, Sub. sulfamerazin	0.999853
Developed 1c	sulfamerazin	12	0.343			Tr. 9, Rf 0.346, Sub. sulfamerazin	0.999668
Developed 1c	sulfamerazin	13	0.345			Tr. 12, Rf 0.343, Sub. sulfamerazin	0.999789
Developed 1c	sulfamerazin	14	0.346			Tr. 15, Rf 0.349, Sub. sulfamerazin	0.999855
Developed 1c	sulfamerazin	15	0.349			Tr. 12, Rf 0.343, Sub. sulfamerazin	0.999605

Analysis Example Analysis 6 spectrum scan loaded

HPTLC PRO SYSTEM User: visionCATSuser Server: CM426ED-W10.camag.ch Used memory: 663 MB

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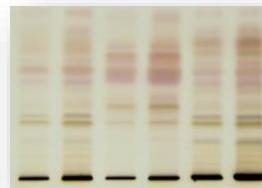
HPTLC line – Documentation

TLC VISUALIZER 2

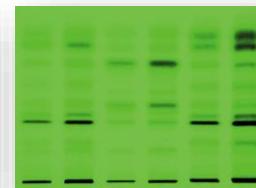


- Completely dark compartment with large **sliding door** and small **viewing window**
- Three types of illumination

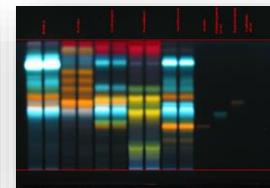
White light



UV 254



UV 366

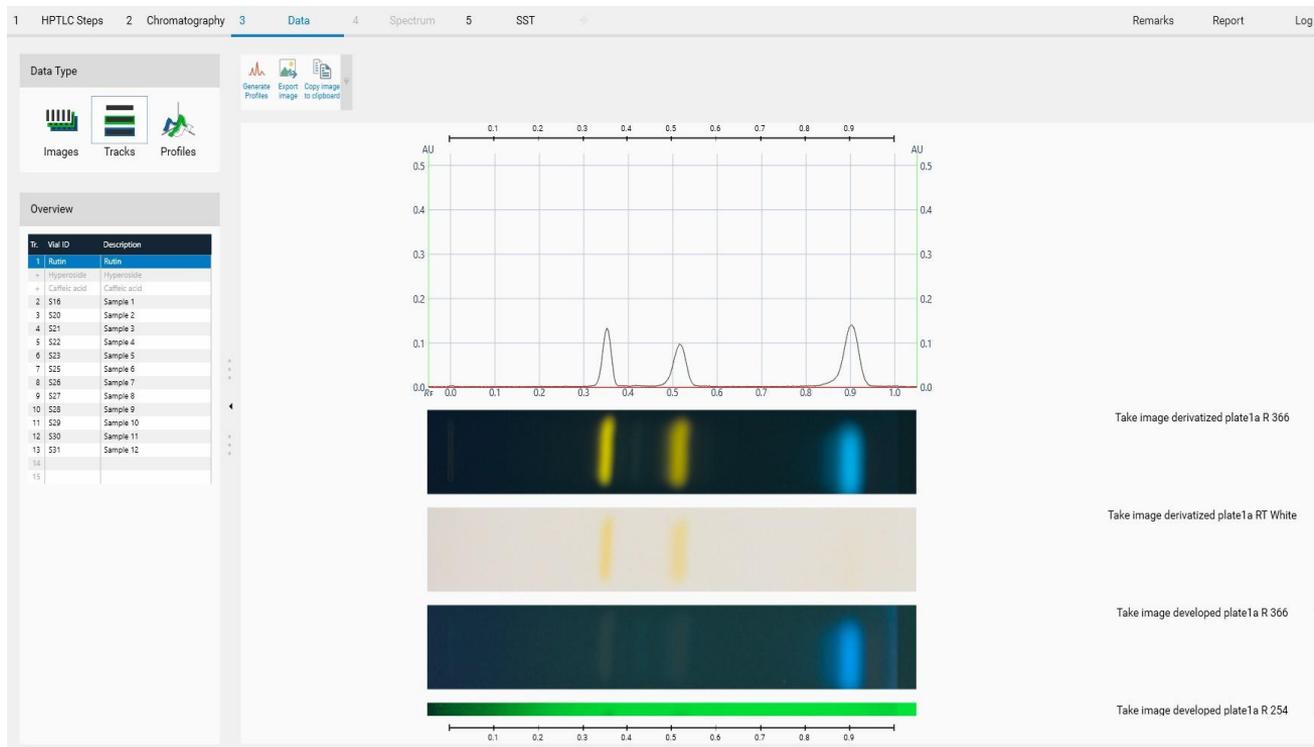


- Professional industrial digital camera
- Suitable for **plates & sheets up to 20x20 cm**
- Fully controlled by visionCATS software

CAMAG instruments & tools portfolio

HPTLC line – Documentation

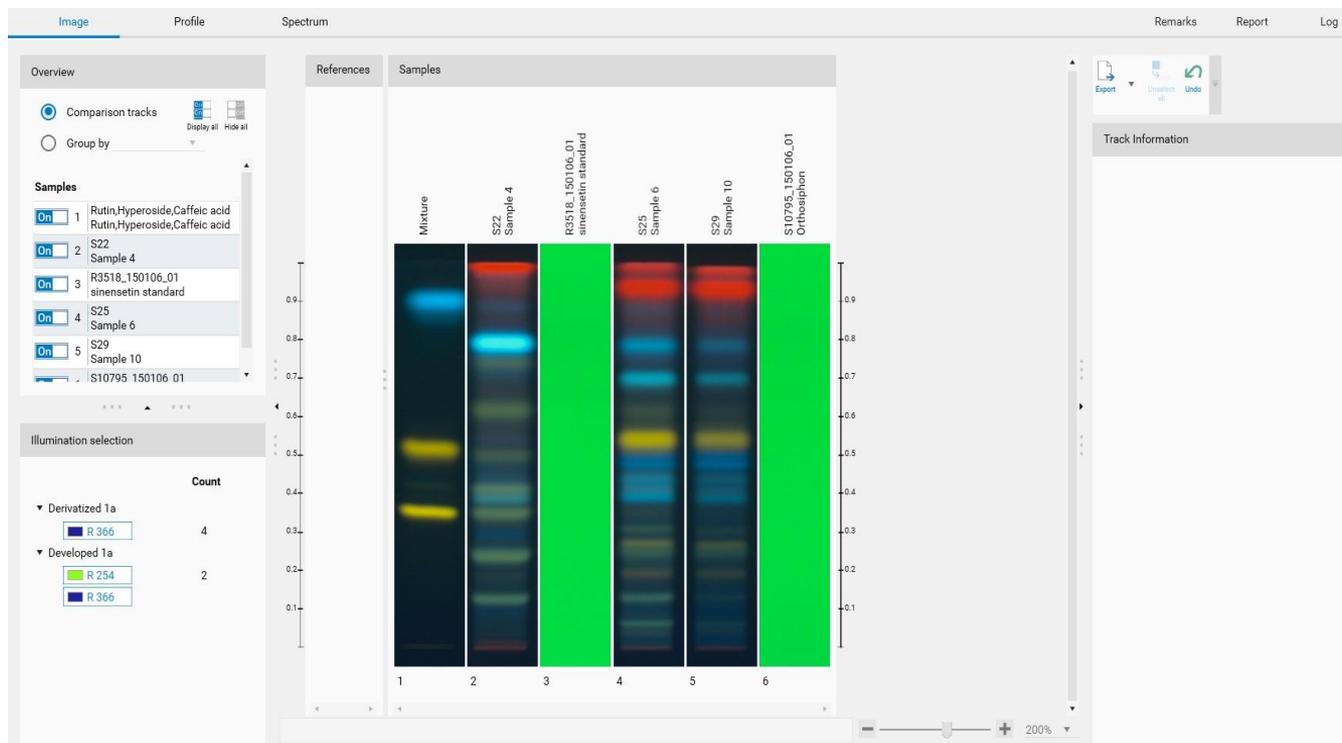
TLC Visualizer 2 with VisionCATS - Data view options



CAMAG instruments & tools portfolio

HPTLC line – Documentation

TLC Visualizer 2 with VisionCATS – Images comparison



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HPTLC line – Quantitative Evaluation

1 HPTLC Steps 2 Chromatography 3 Data 4 Spectrum 5 SST 6.1 Evaluation Remarks Report Log

Evaluation Steps

Definition Integration Subst. assign. Calibration **Results**

Overview

- Toggle selection for all substances
- Sinensetin @ 366 nm

Results

Sort: Track number

^ Sinensetin @ 366 nm (8 samples assigned)

^ Sample 'R3518_150106_02'		102.3 ng/ml	CV=0.36 %	(2 applications)	102.3 µg in 1.000 mg
^ Volume: 5.0 µl		102.3 ng/ml	CV=0.36 %	(2 replicas)	
Track 7	R _f 0.331	102.6 ng/ml	513.0 pg		
Track 15	R _f 0.330	102.1 ng/ml	510.3 pg		
^ Sample 'S10795_150106_01'		97.66 ng/ml	CV=0.82 %	(2 applications)	48.83 µg in 500.0 mg
^ Volume: 5.0 µl		97.66 ng/ml	CV=0.82 %	(2 replicas)	
Track 1	R _f 0.313	98.23 ng/ml	491.2 pg		
Track 9	R _f 0.334	97.10 ng/ml	485.5 pg		
^ Sample 'S10795_150106_02'		71.61 ng/ml	CV=0.34 %	(2 applications)	35.80 µg in 500.0 mg
^ Volume: 5.0 µl		71.61 ng/ml	CV=0.34 %	(2 replicas)	
Track 3	R _f 0.317	71.78 ng/ml	358.9 pg		
Track 11	R _f 0.334	71.44 ng/ml	357.2 pg		
^ Sample 'S10795_150106_03'		62.51 ng/ml	CV=0.16 %	(2 applications)	31.26 µg in 500.0 mg
^ Volume: 5.0 µl		62.51 ng/ml	CV=0.16 %	(2 replicas)	
Track 5	R _f 0.325	62.45 ng/ml	312.2 pg		
Track 13	R _f 0.333	62.58 ng/ml	312.9 pg		

[Lock evaluation](#)

CAMAG instruments & tools portfolio

HPTLC line – Software

visionCATS



- CAMAG software to control CAMAG instruments, create and store TLC/HPTLC methods and analysis, process TLC/HPTLC data and print analysis reports.
- **Microsoft® SQL Server® 2017 Express 64 bit** database
- Has **server/client** architecture
- **21 CFR Part 11** compatible

CAMAG instruments & tools portfolio

HPTLC line – Hyphenation (MS coupling)

TLC-MS Interface 2

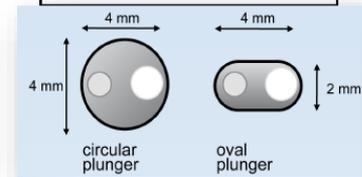
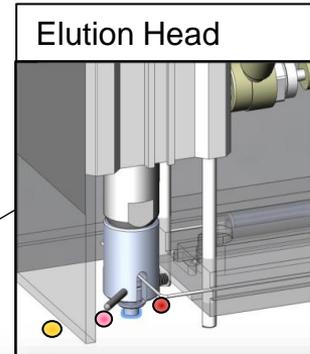


- Hyphenating TLC and mass spectrometry opens new application areas for both techniques
- The classical procedure is : scratch, dilute, filter - **Discontinuous** and prone to **contamination**
- TLC-MS Interface 2 is a **stand-alone**, **flexible**, **fast** and **easy to use** alternative
- Suitable for **plates & sheets up to 20x20 cm**

CAMAG instruments & tools portfolio

HPTLC line – Hyphenation (MS coupling)

External filter in a ferrule:
Easy cleaning, easy changing



Magnetic plate
positioner (ruler)

Scaled Table

Děkuji Vám za pozornost!

Stánek č. E 13

