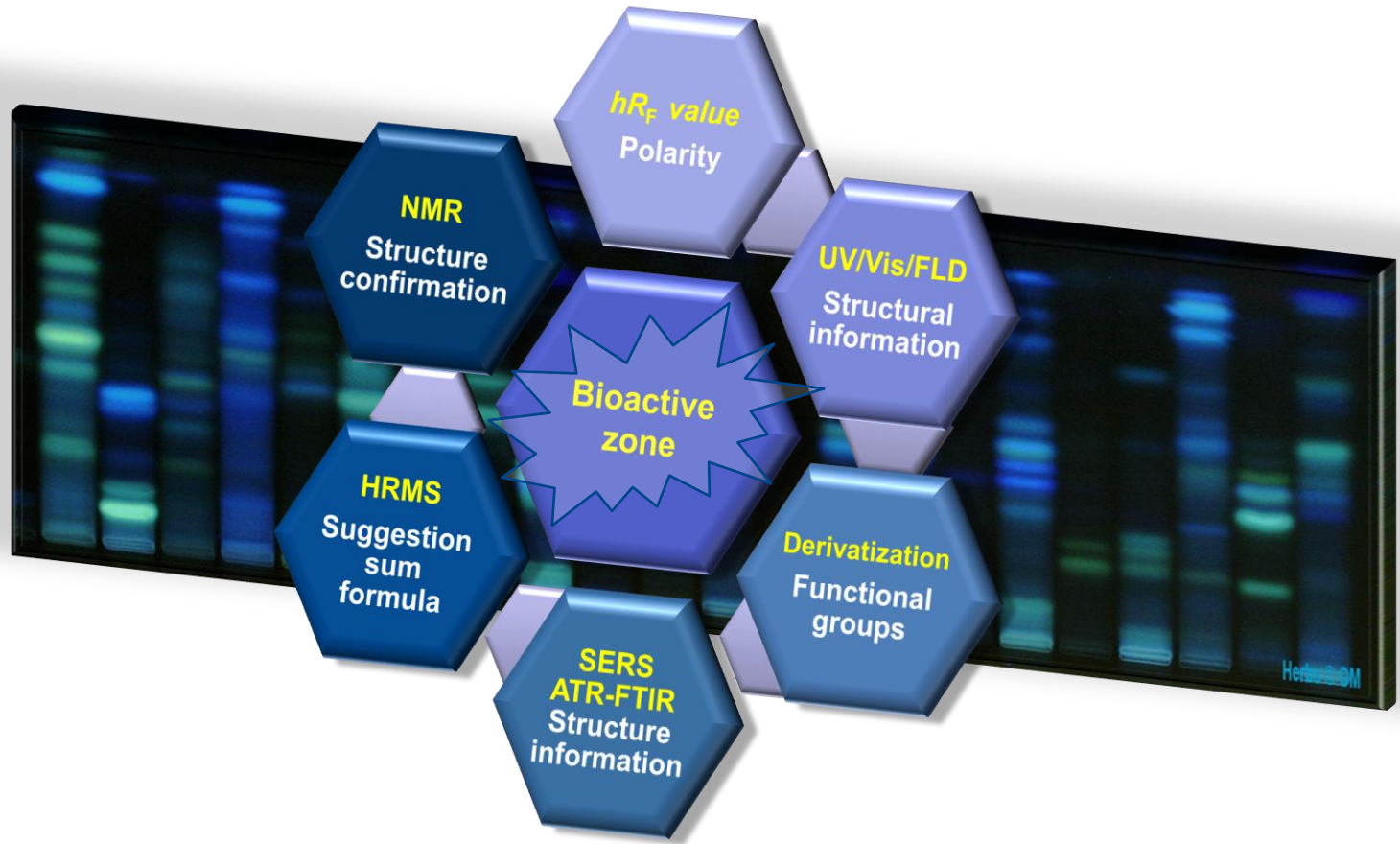


# Quantification après différentes détections

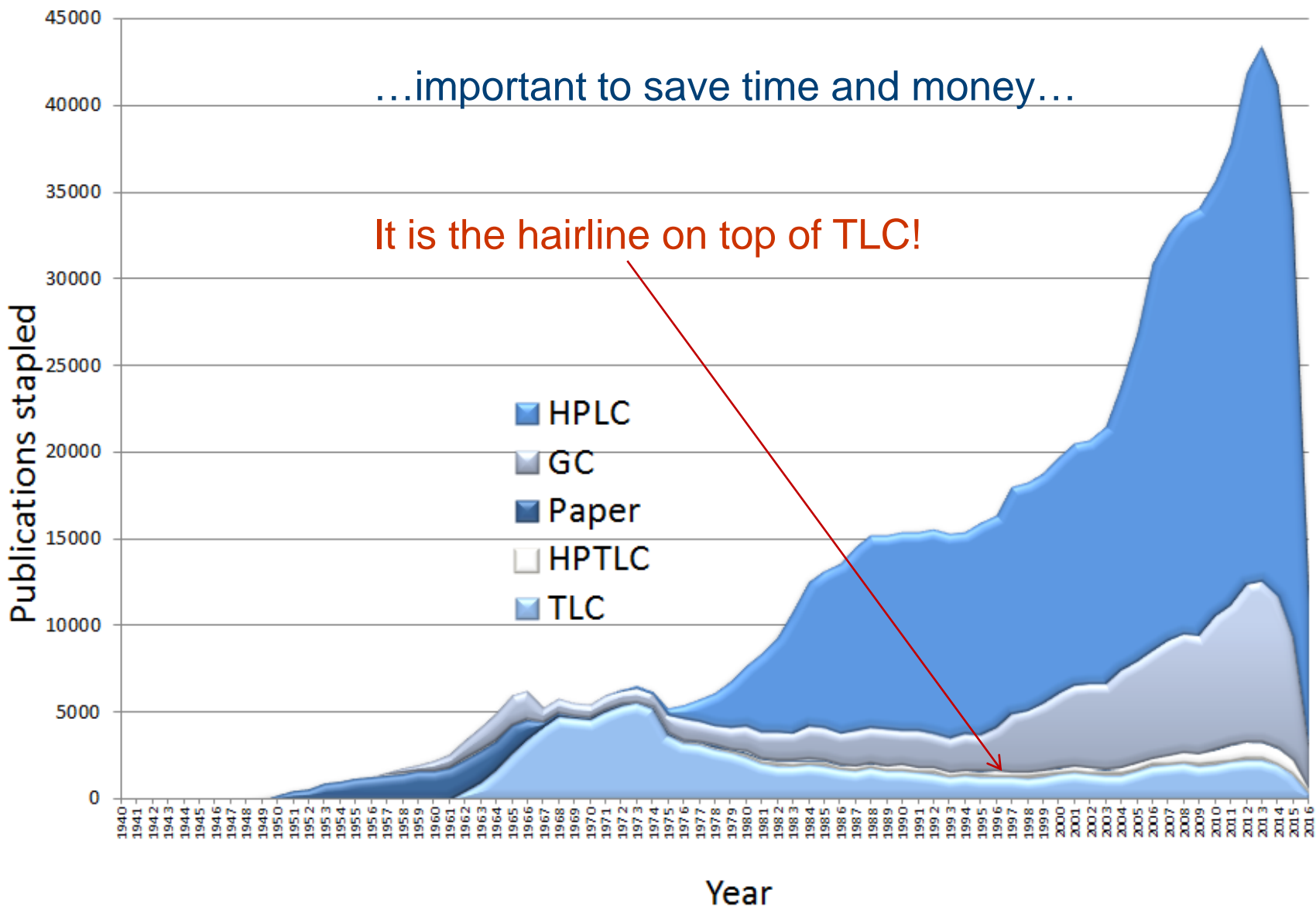


Gertrud Morlock, Chair of Food Science



Justus Liebig University Giessen

# Why CCCM is so important!



...not to end like this!

Justification of instruments? What an effort for the radish harvest!

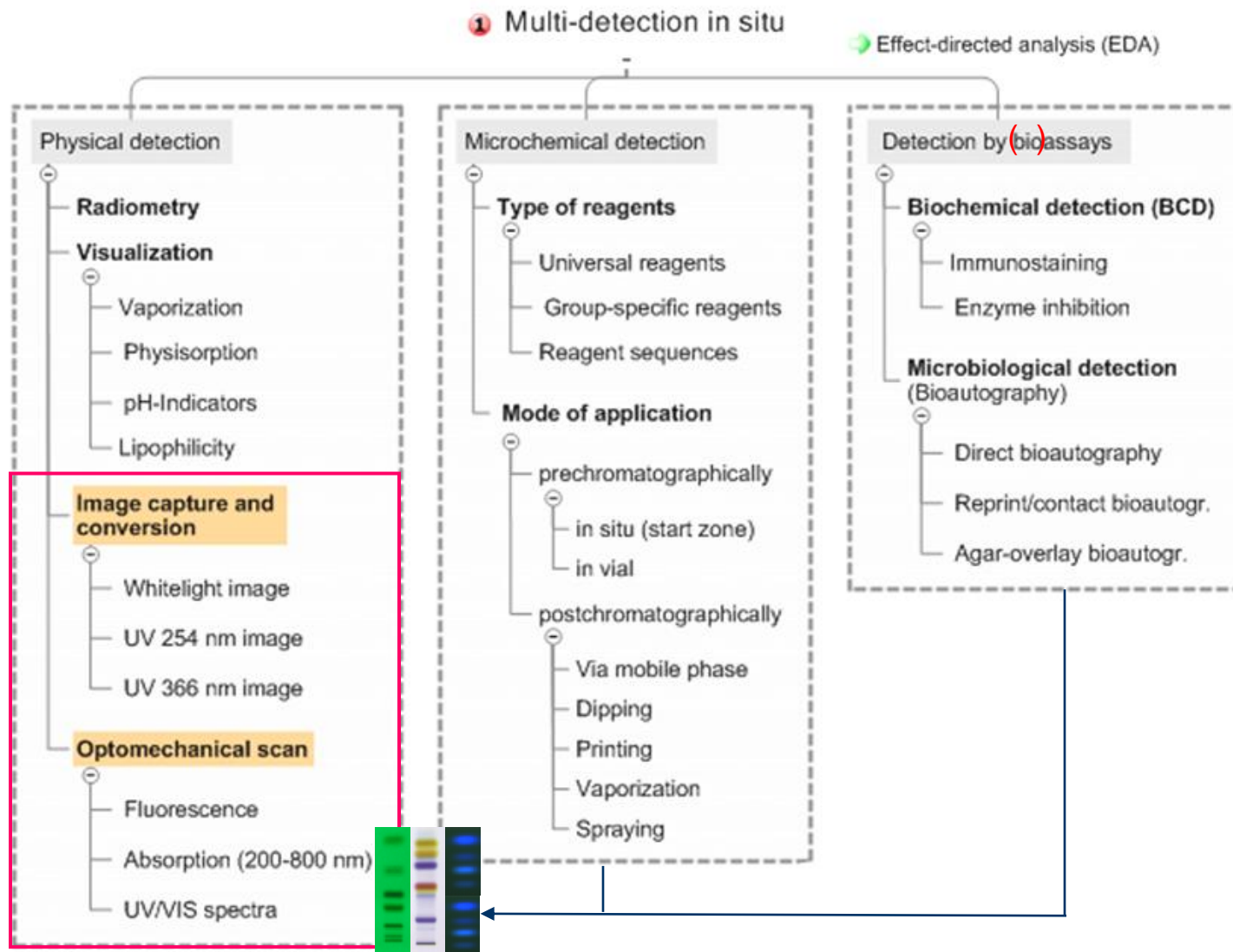


Loriot's Grosser Ratgeber, 1968

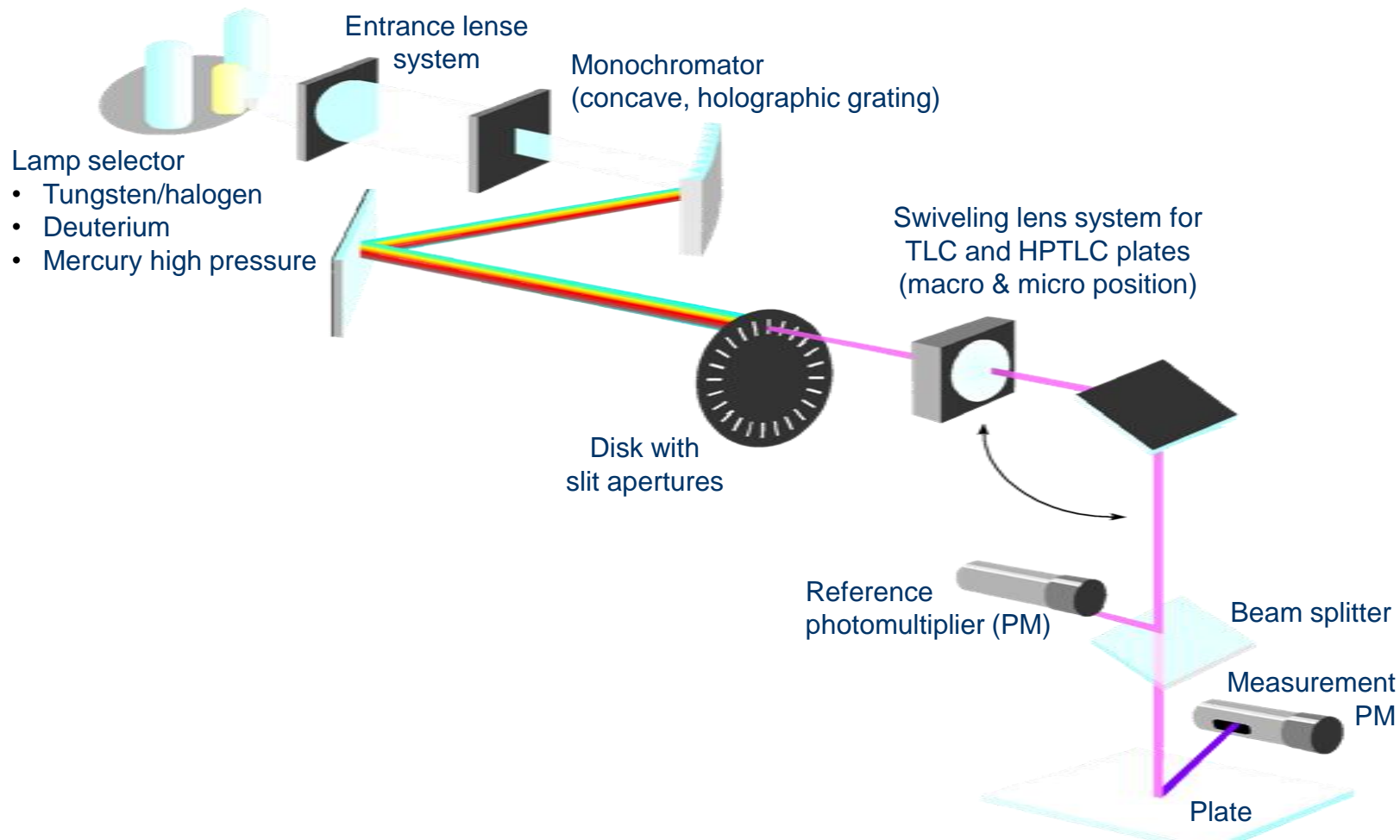
Lucky you, to have HPTLC  
as option!

If all you have is a hammer, everything looks like a nail. A.H. Maslow

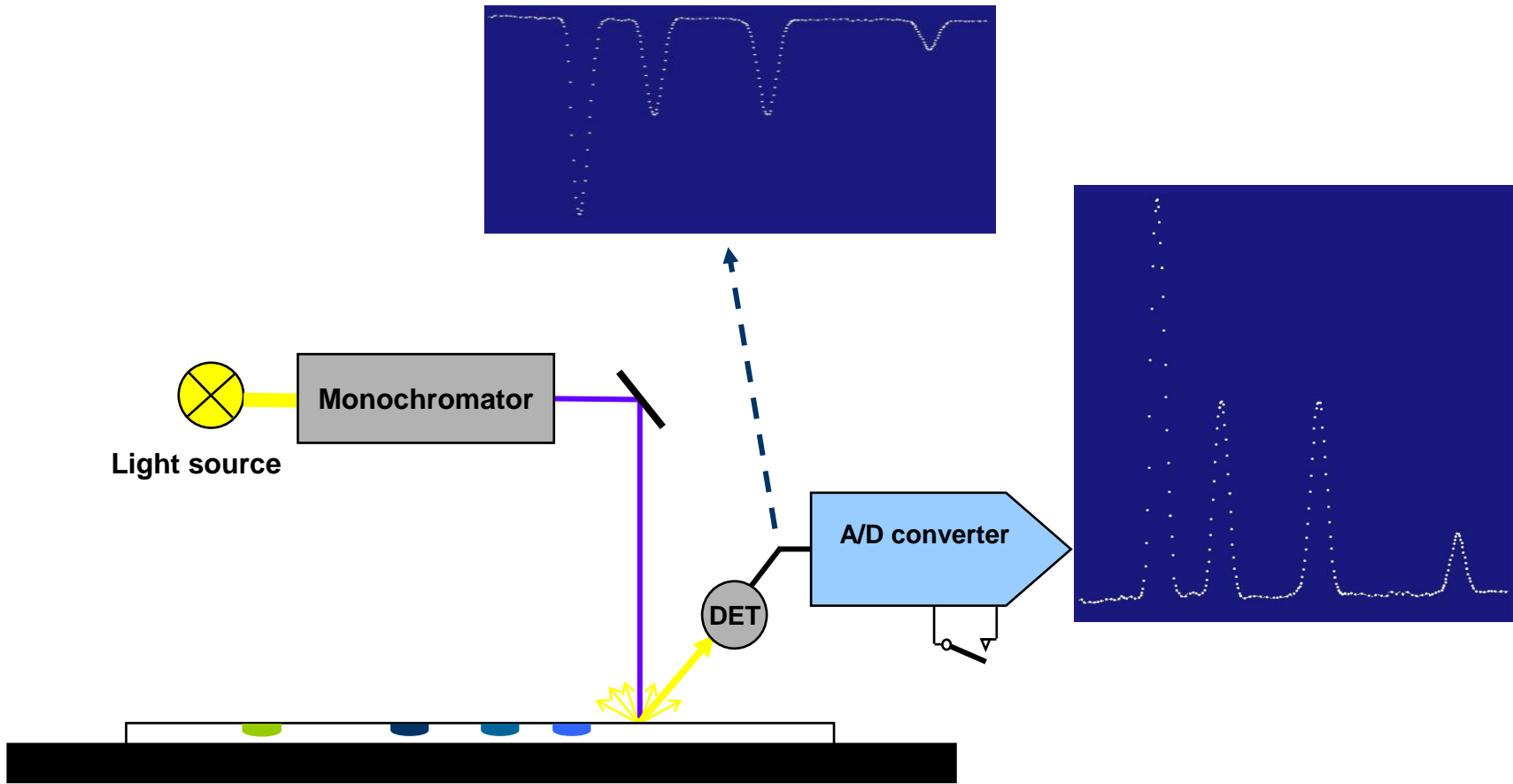
# Strong feature of HPTLC: multi-detection



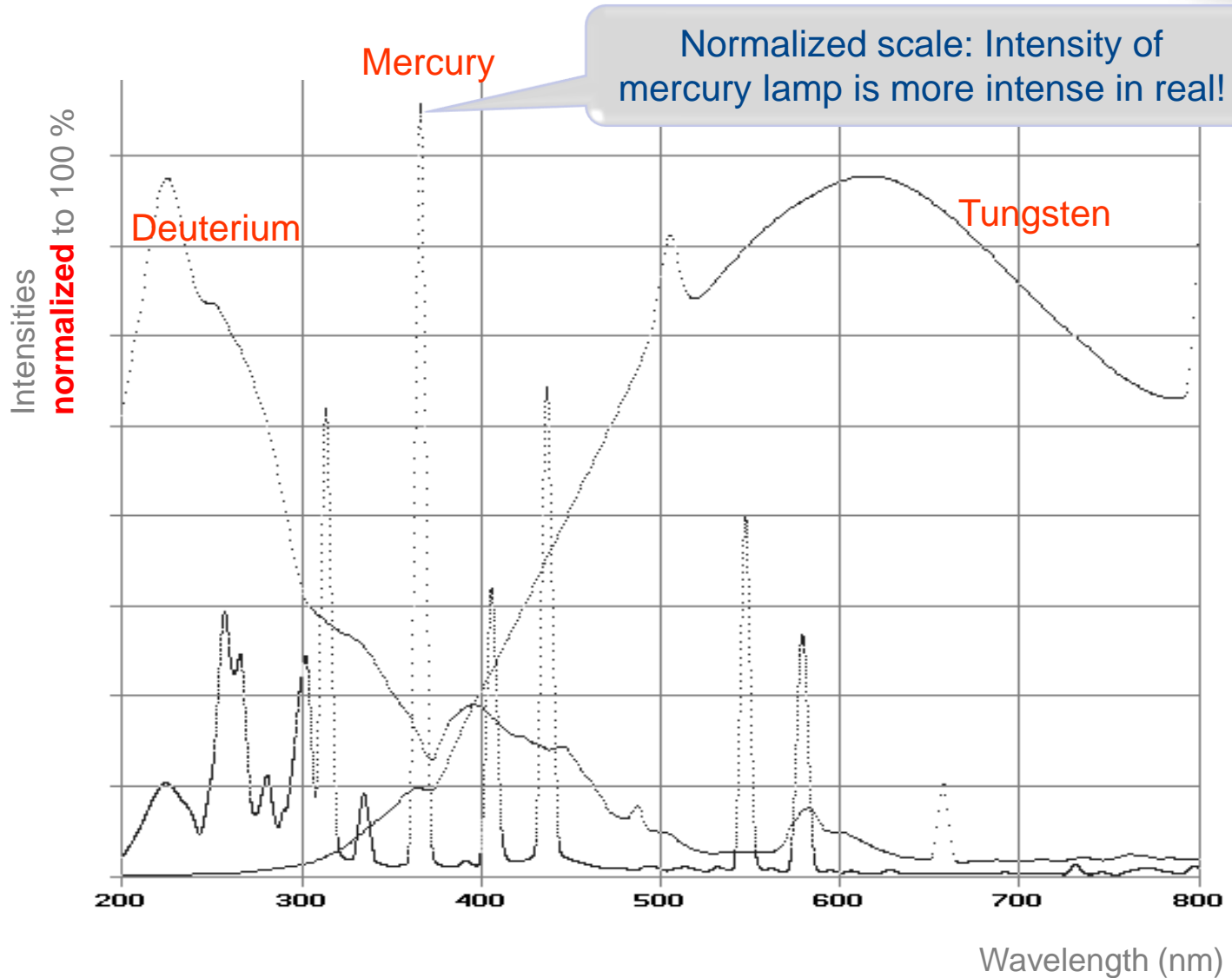
# Absorption measurement



# Absorption measurement → indirect signal



# Emission spectra of the lamps

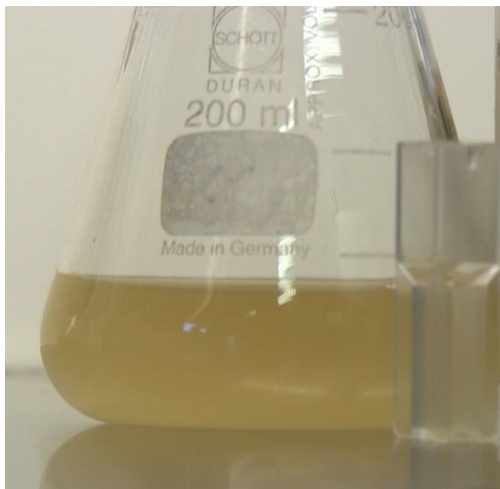
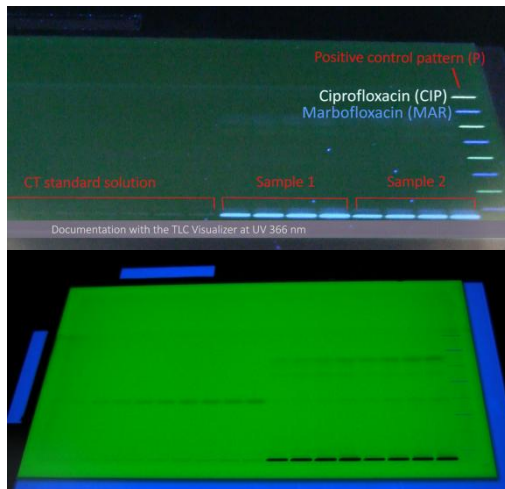
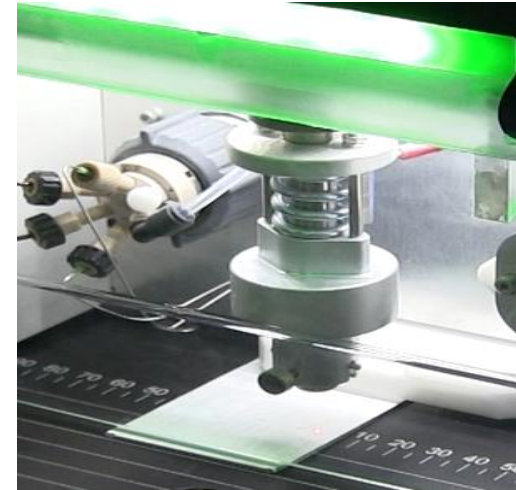
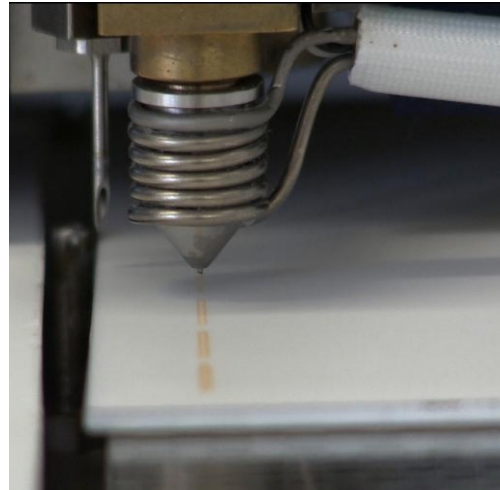
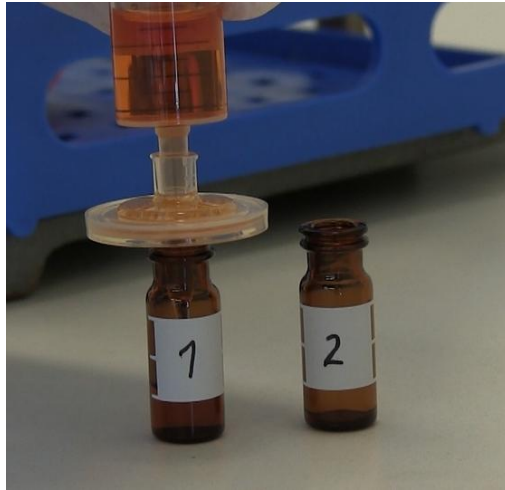


Bioquantification: Bacterial response is measured  
Absorption measurement at 546 nm (mercury lamp)

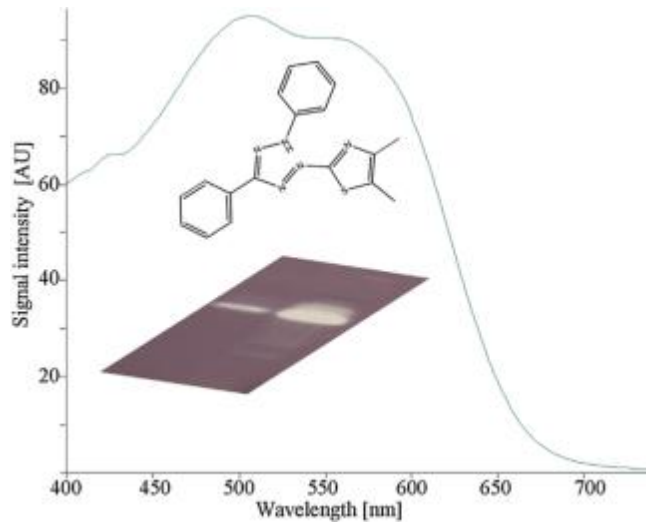
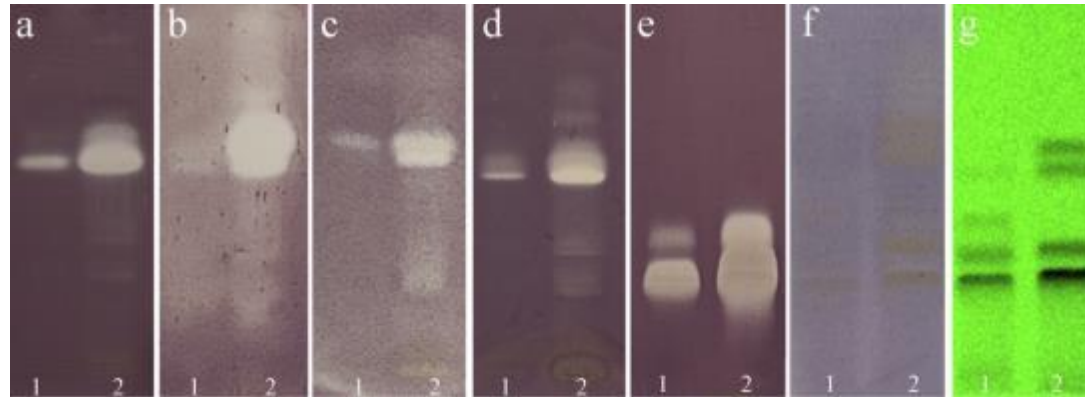


# Discover natural antibiotics!

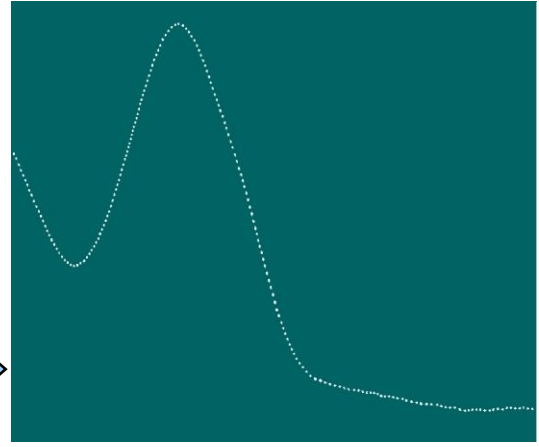
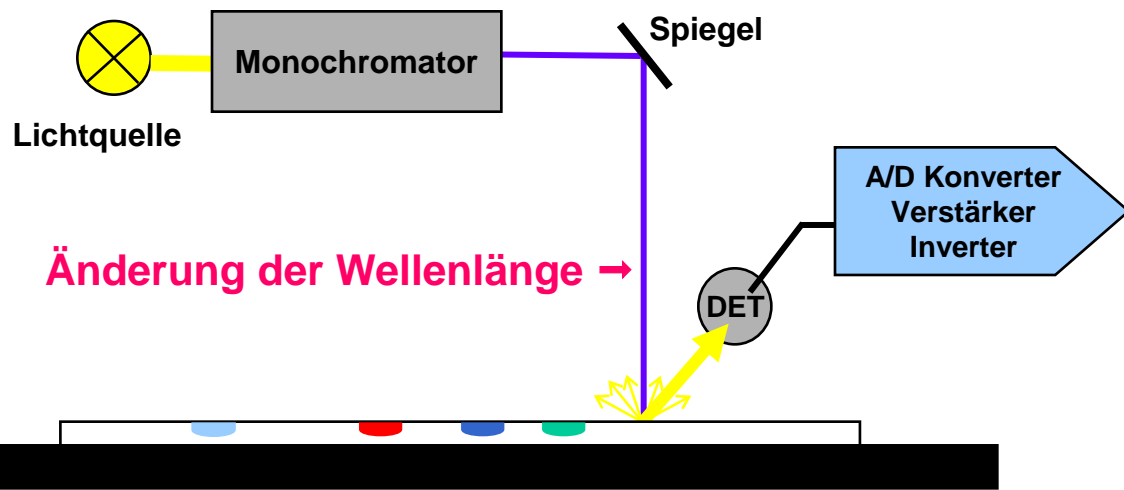
The YouTube link is coming soon [here](#)



# Optimization of *Bacillus subtilis* bioassay

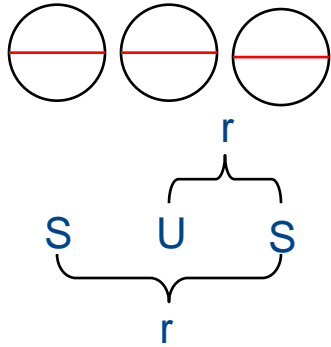


# Spectra recording

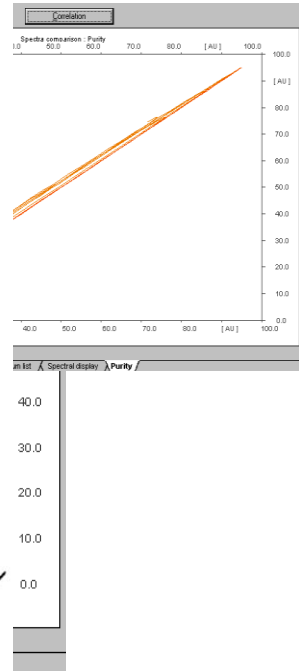
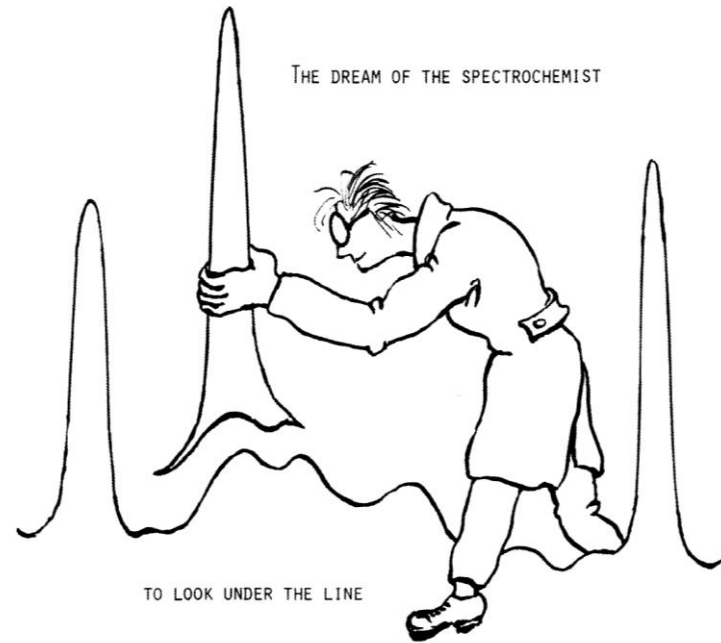
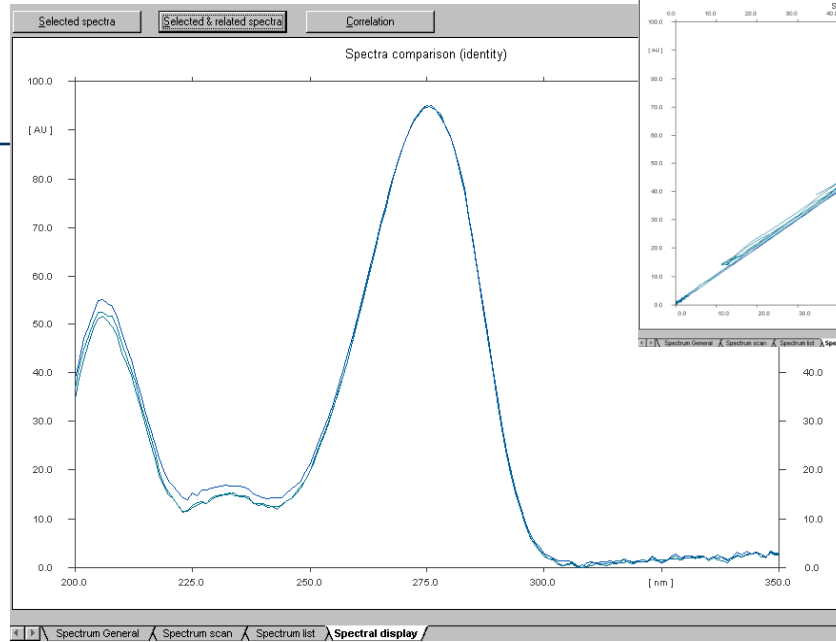
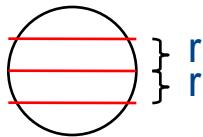


# Two modes

## Identity



## Purity



# Spectra recording

---

$$\lambda_{\text{Sample corr.}} = \lambda_{\text{Sample}} - \lambda_{\text{Lamp}} - \lambda_{\text{Background}}$$

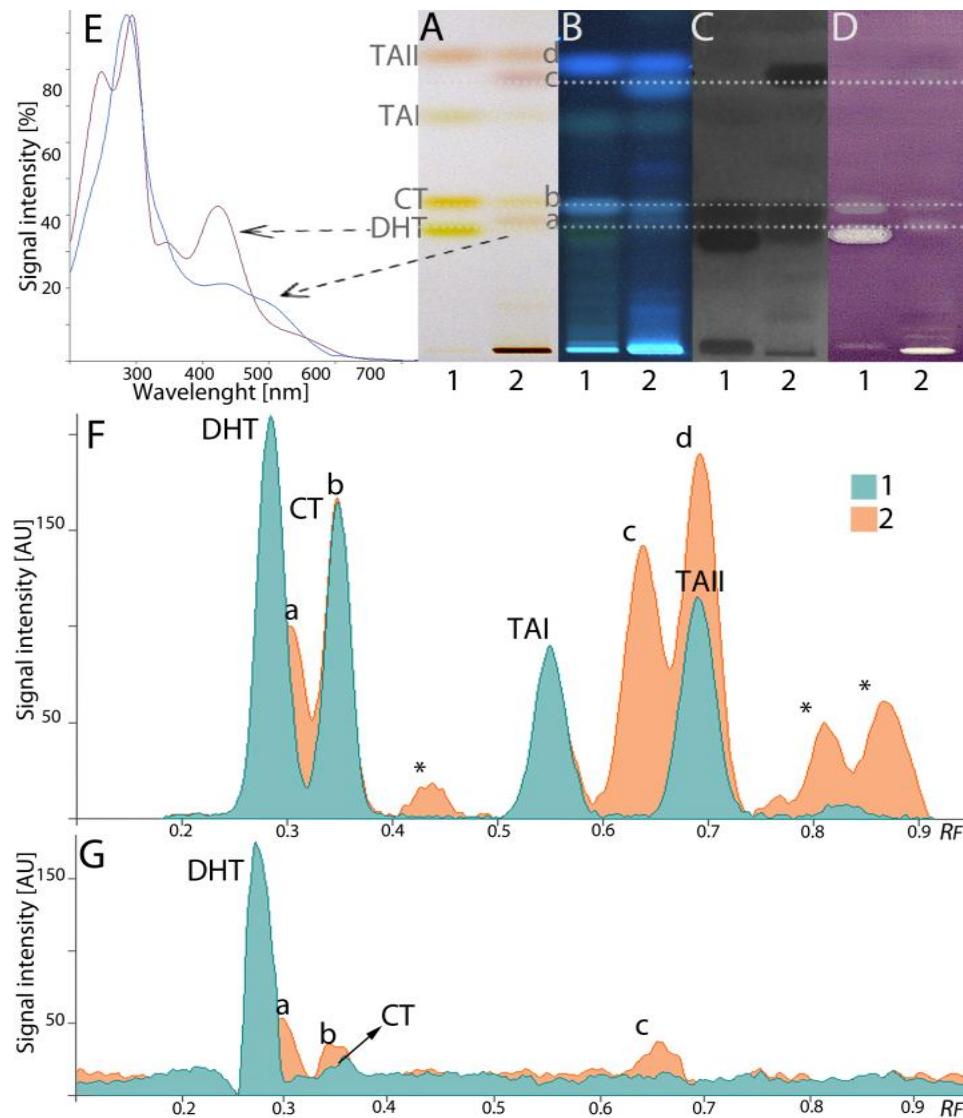
Difference to spectra in solution

- no solvent
- adsorbed on the layer

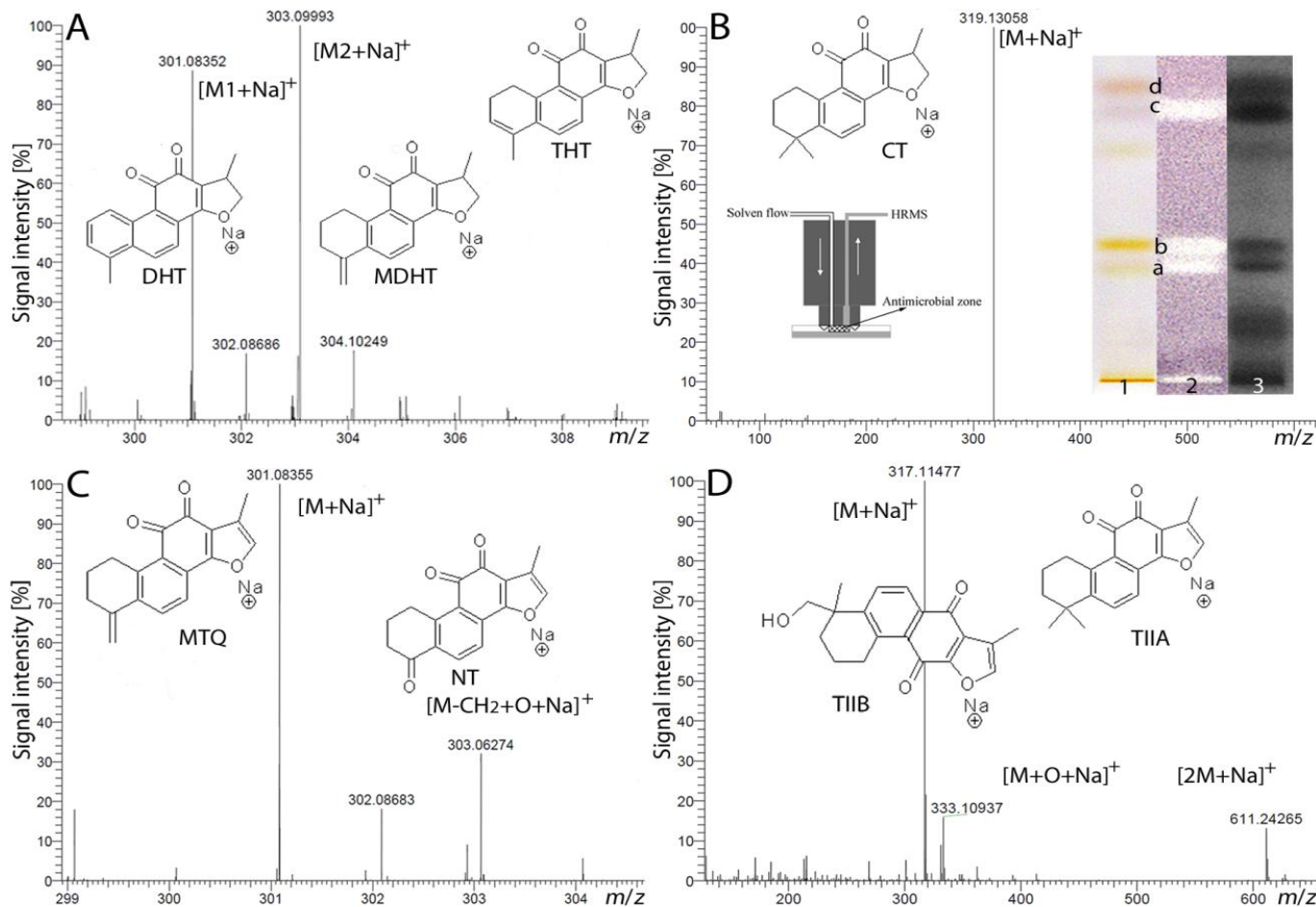
Note

- compare spectra at similar concentrations!
- dependend on SP (RP, NP)
- dependend on MP and pH

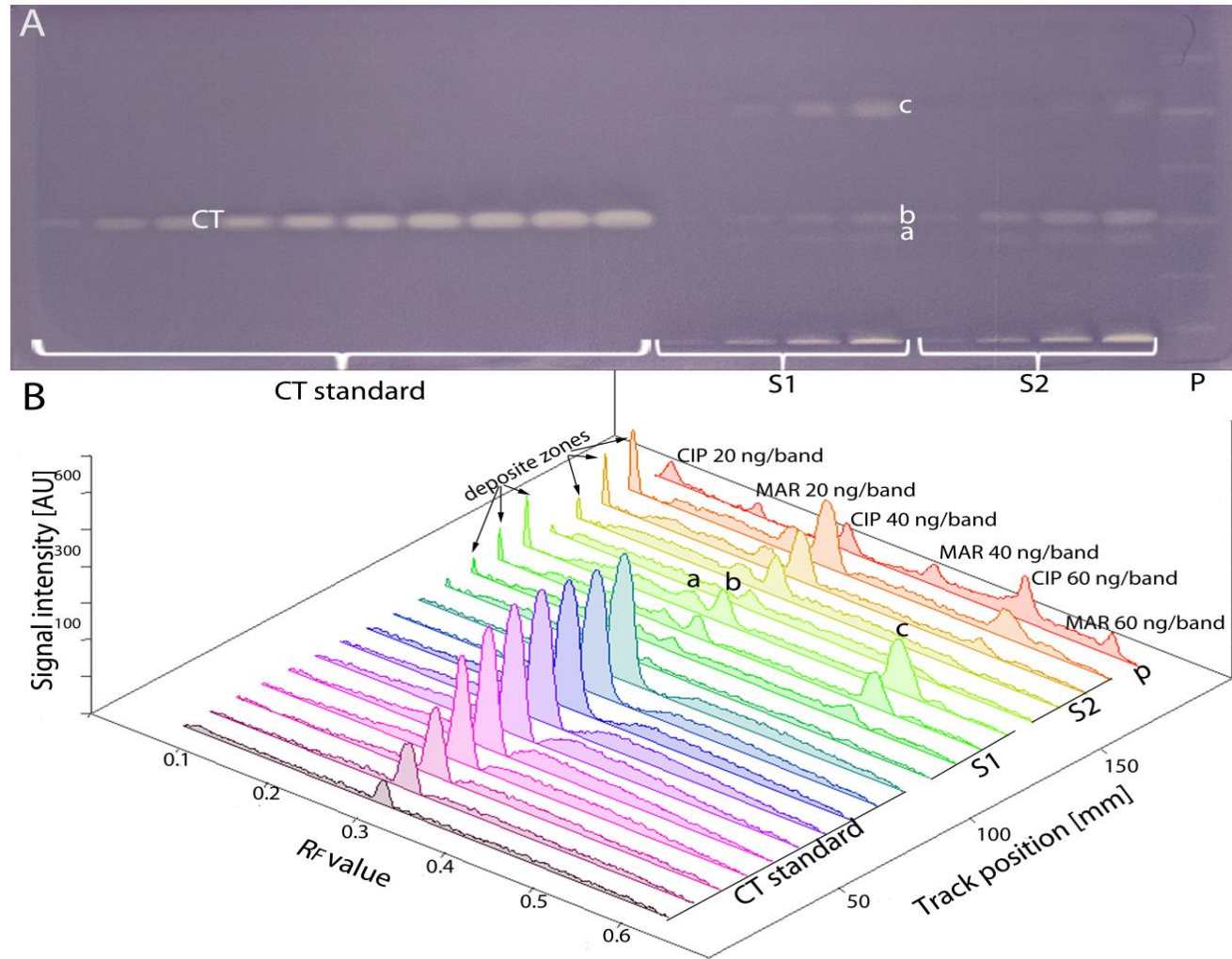
# HPTLC-*Bacillus subtilis*: antibiotics in *Salvia*



# HPTLC-*Bacillus subtilis*: antibiotics in *Salvia*



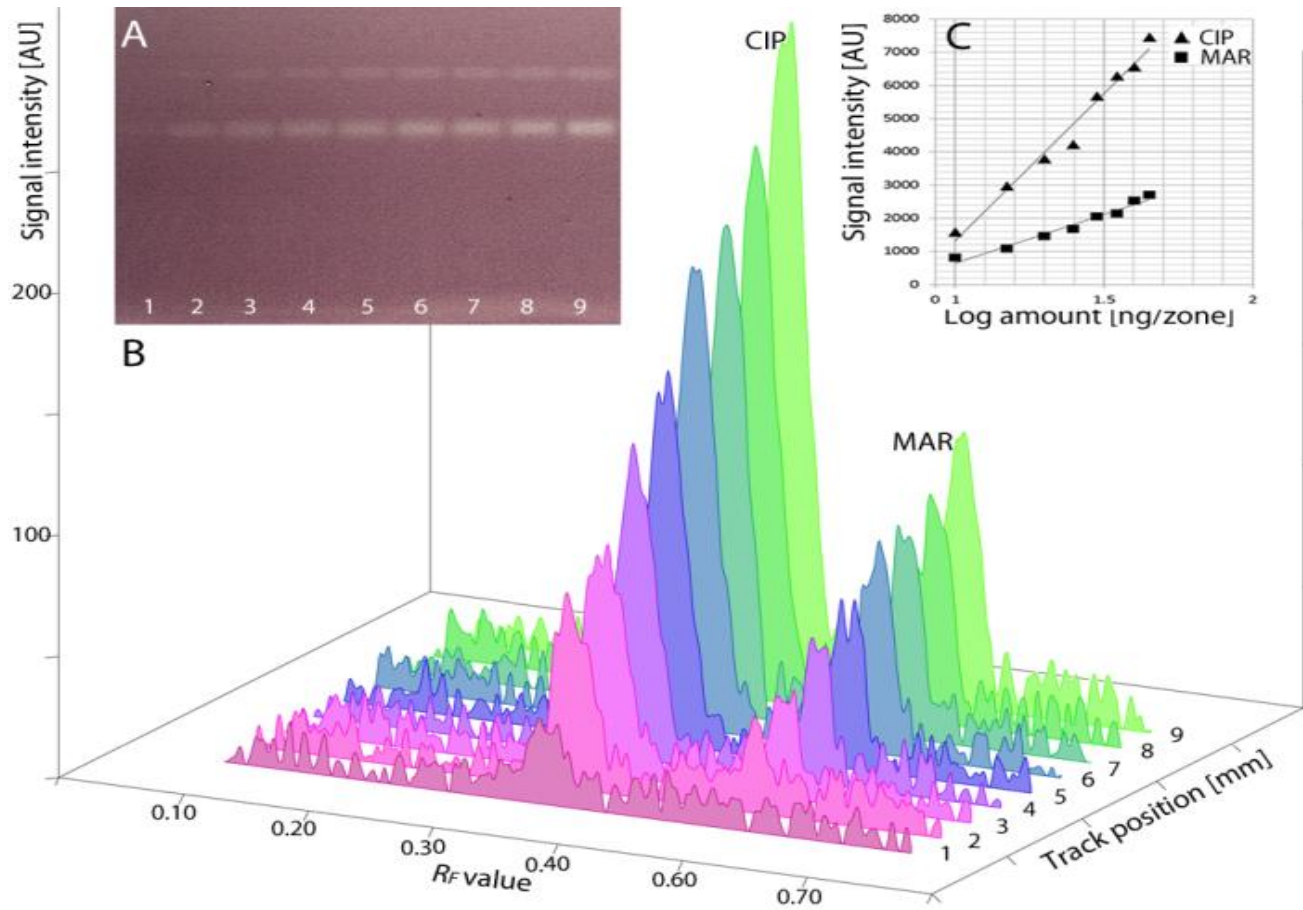
# HPTLC-*Bacillus subtilis*: antibiotics in *Salvia*



IC<sub>50</sub> of CT: 65 ng/zone → 447 ng S1 and 246 ng S2 root powder (%RSD 7-9%, n = 4)

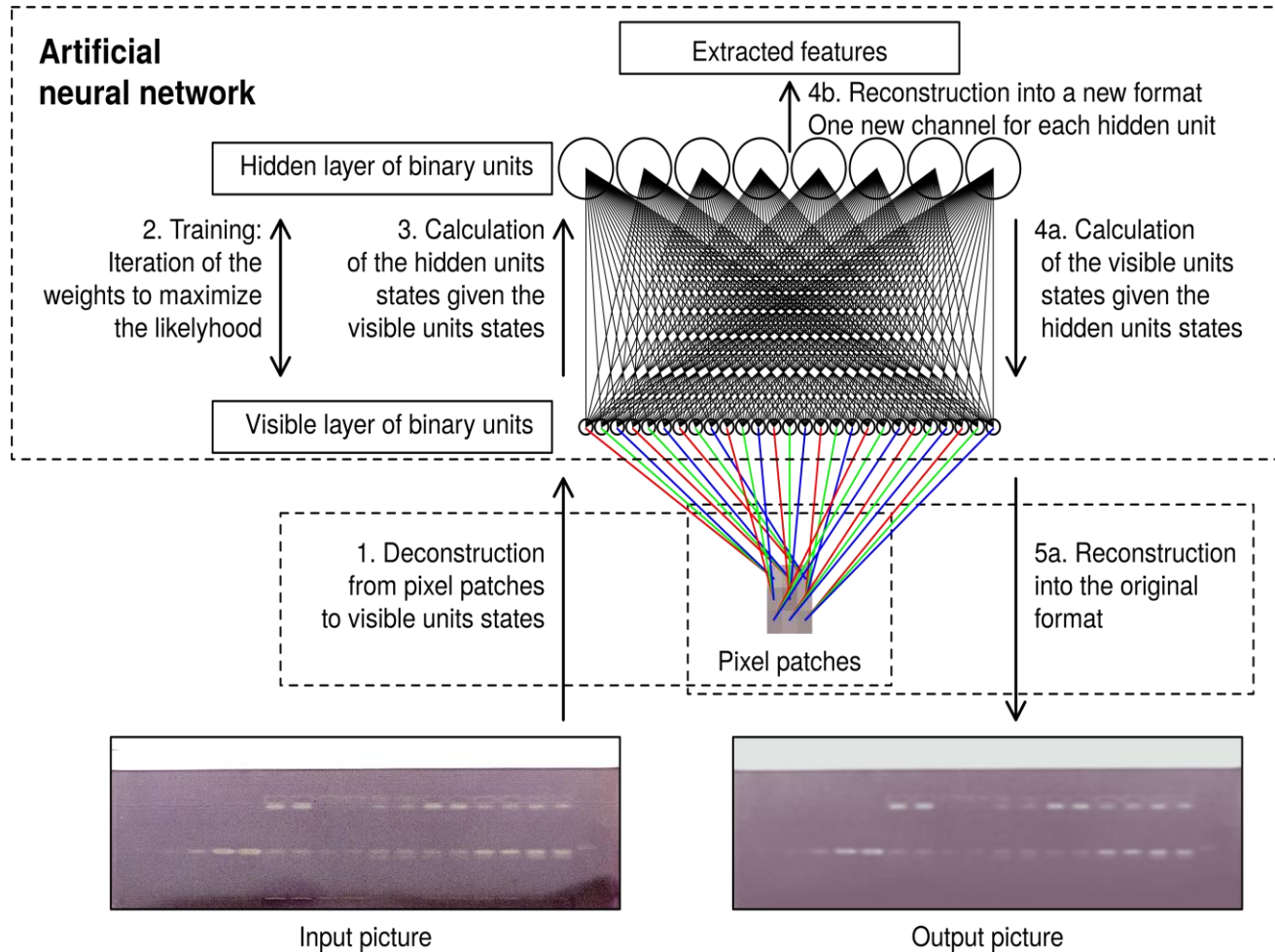
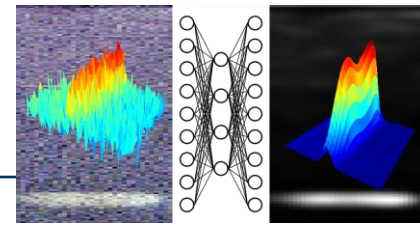


# HPTLC-*Bacillus subtilis*: antibiotics in *Salvia*

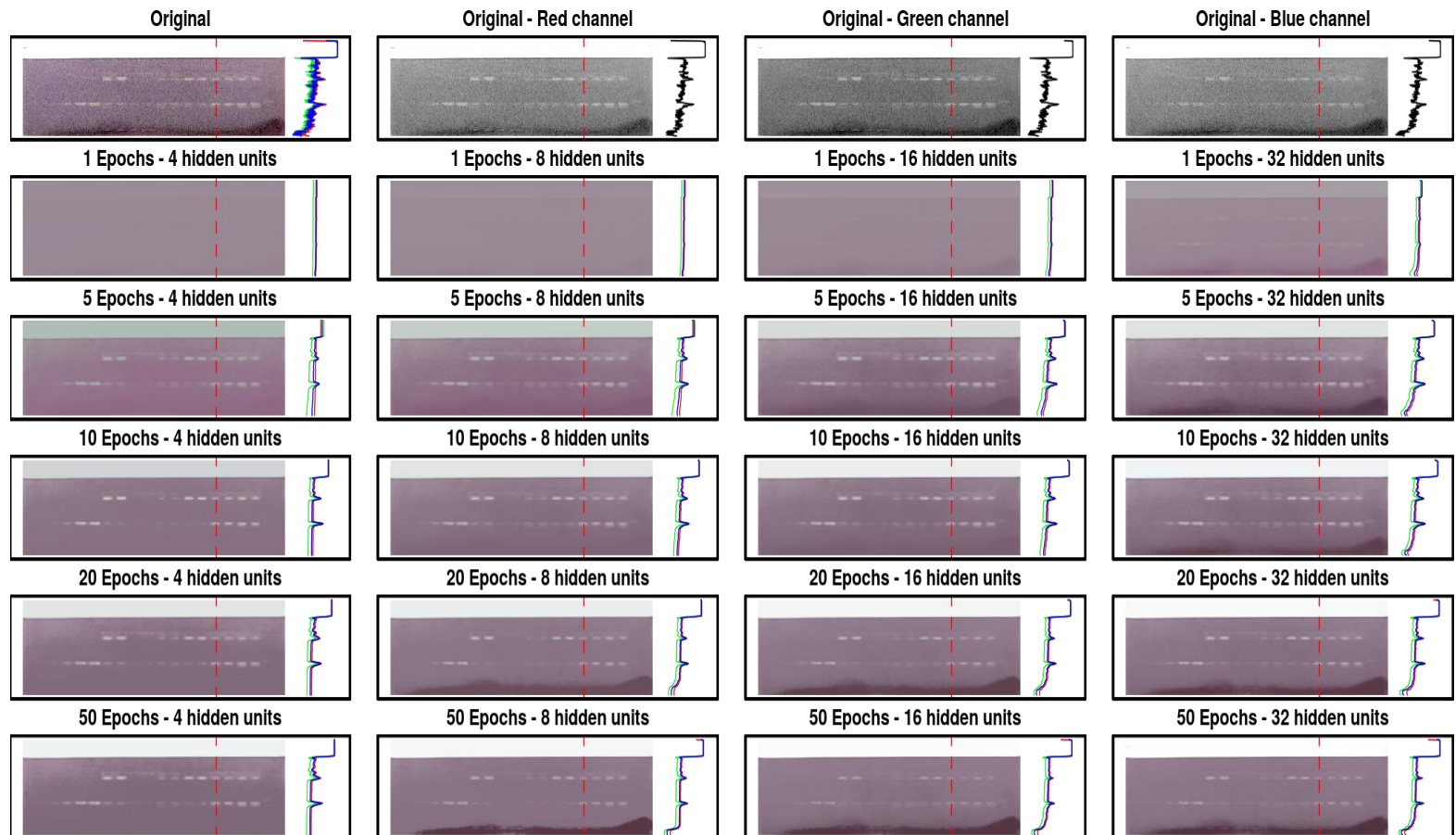


ANN helps to improve quantitative data!

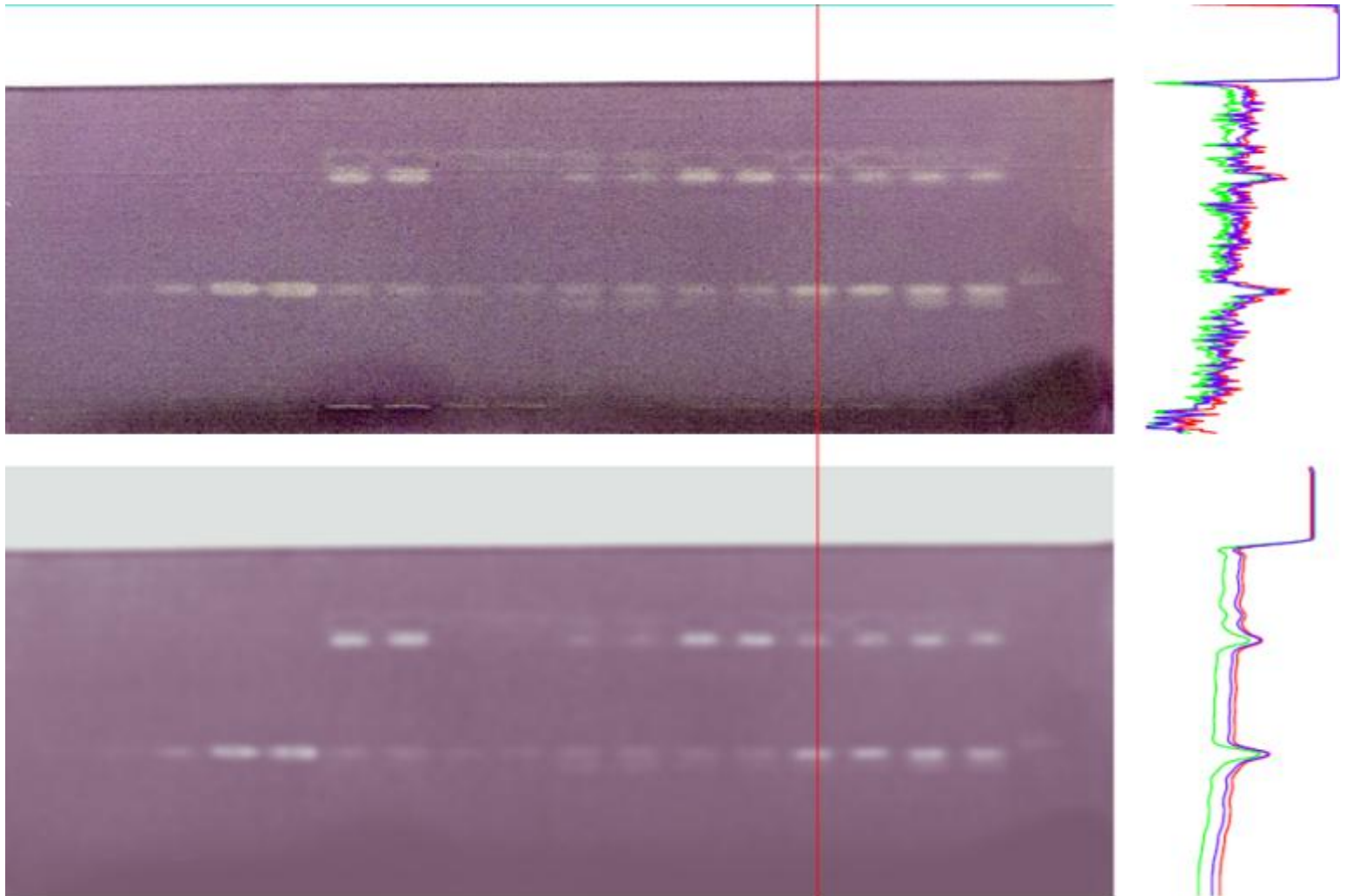
# Artificial neural network → HPTLC



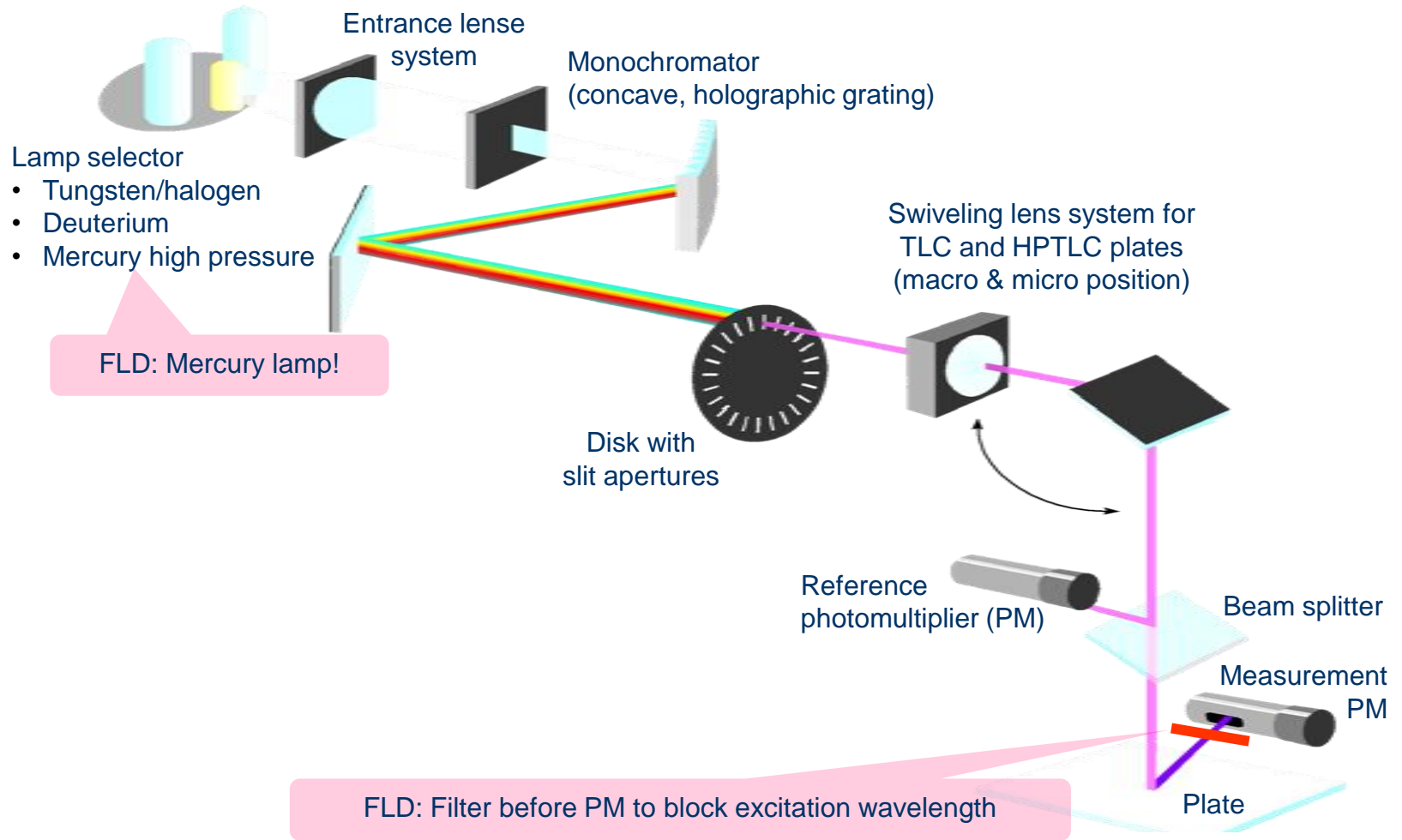
# Artificial neural network → HPTLC



# Artificial neural network → HPTLC



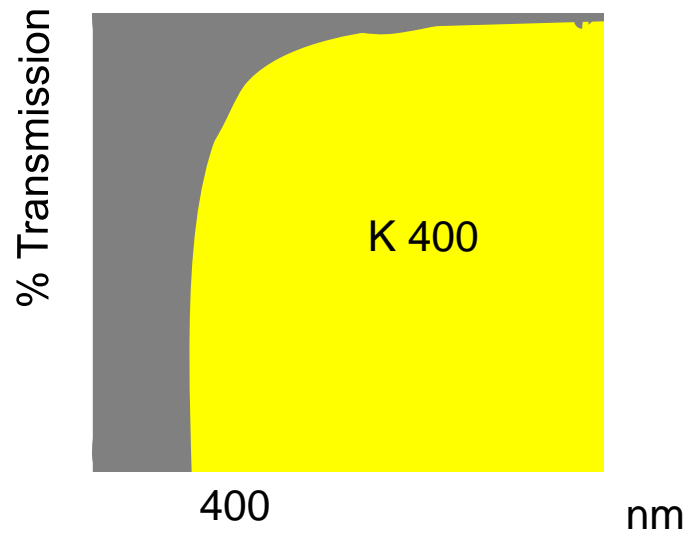
# Fluorescence measurement



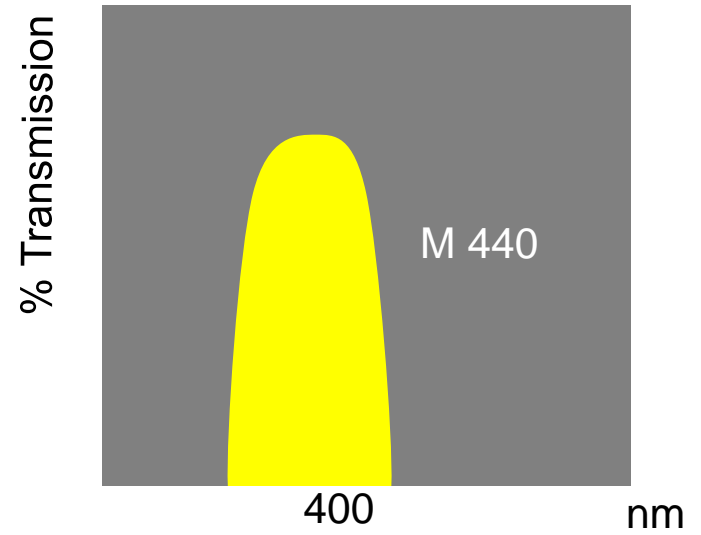
# Fluorescence – Filter types



Edge filter



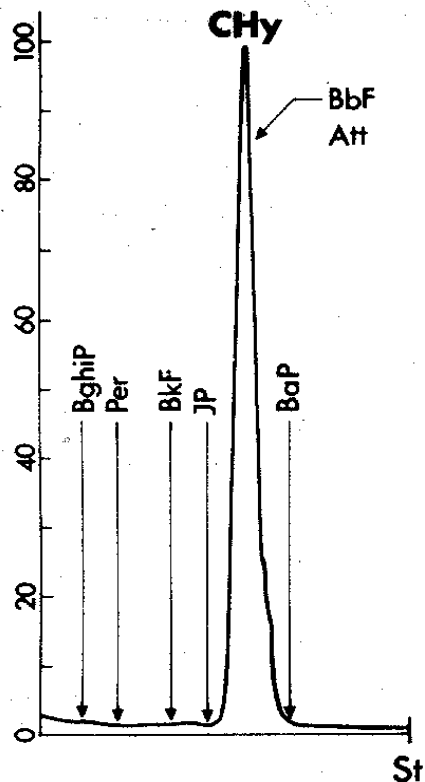
Monochromate filter



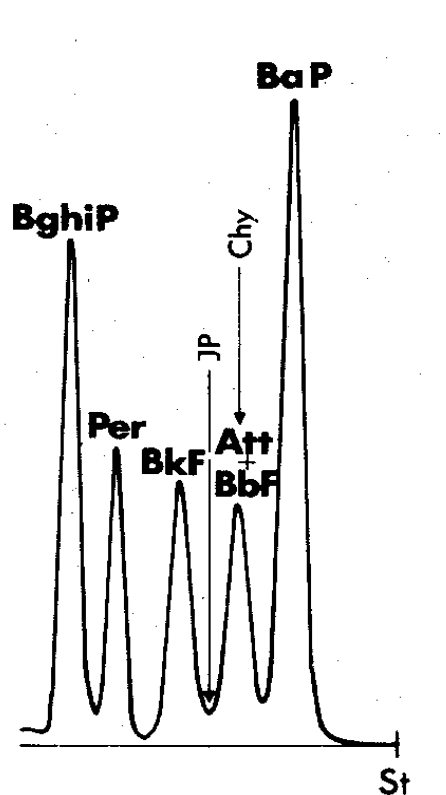
# Wavelength combination



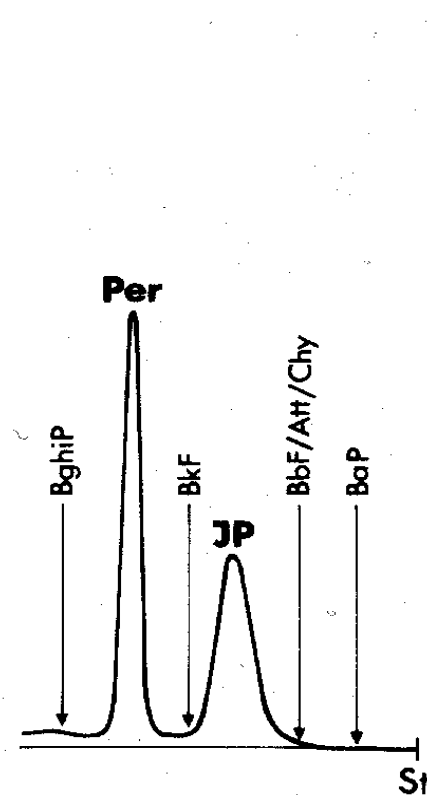
266/M365 nm



365/M436 nm



436/M578 nm



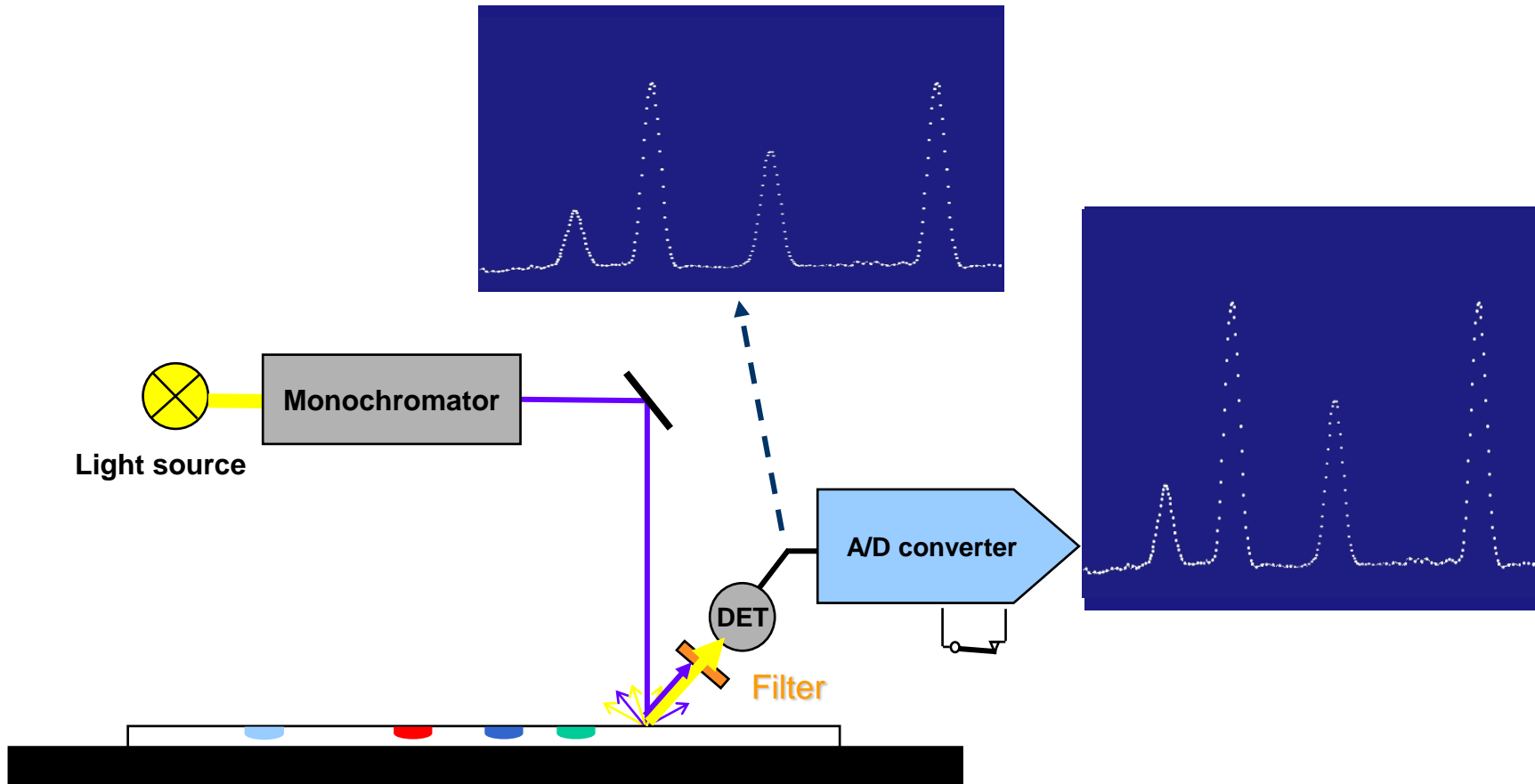
366/>400 nm



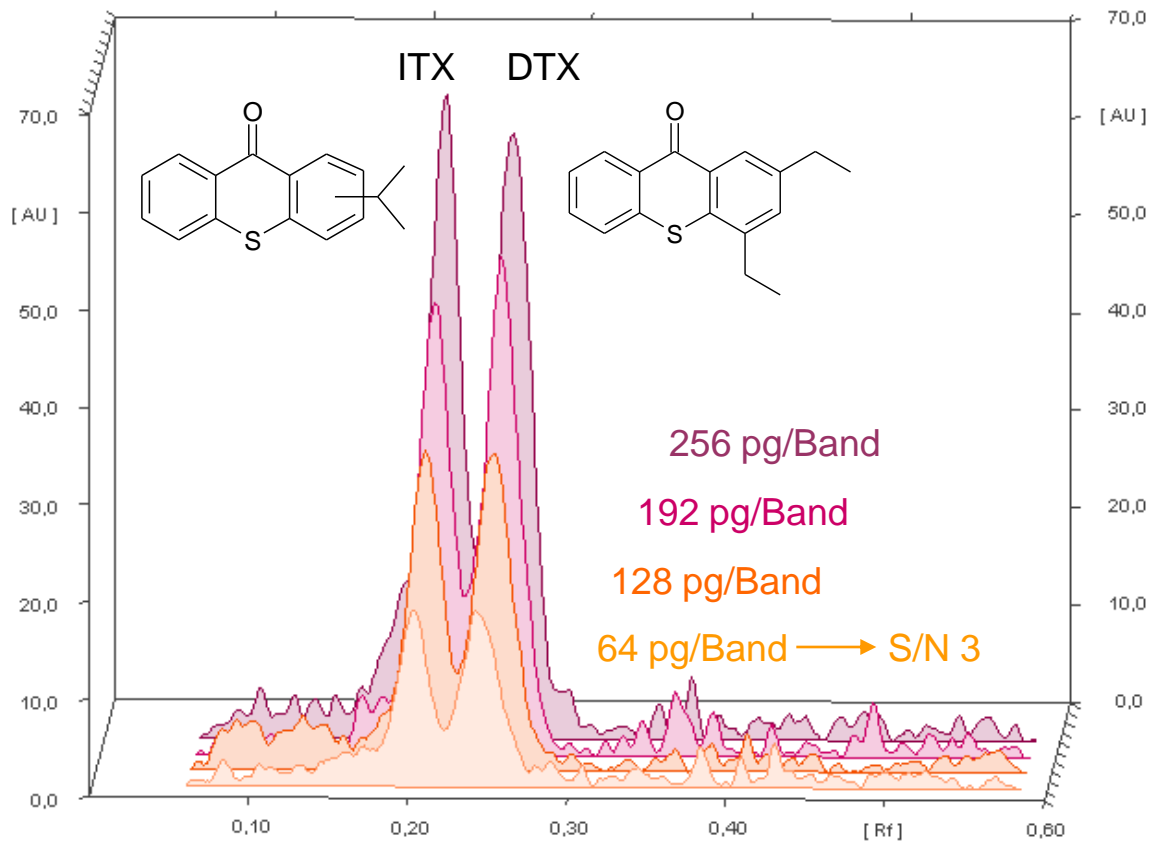
H. Jork, W. Funk, W. Fischer, H. Wimmer: Thin-Layer Chromatography, volume 1a and b, VCH Weinheim 1990 and 1994.



# Fluorescence measurement → direct signal

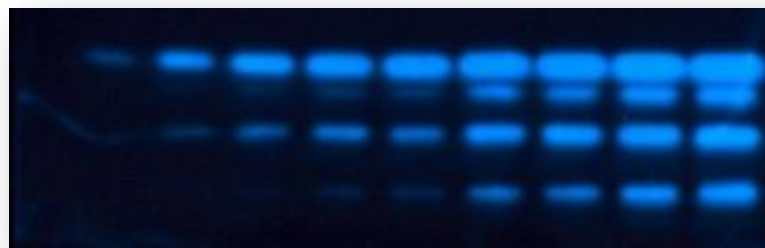
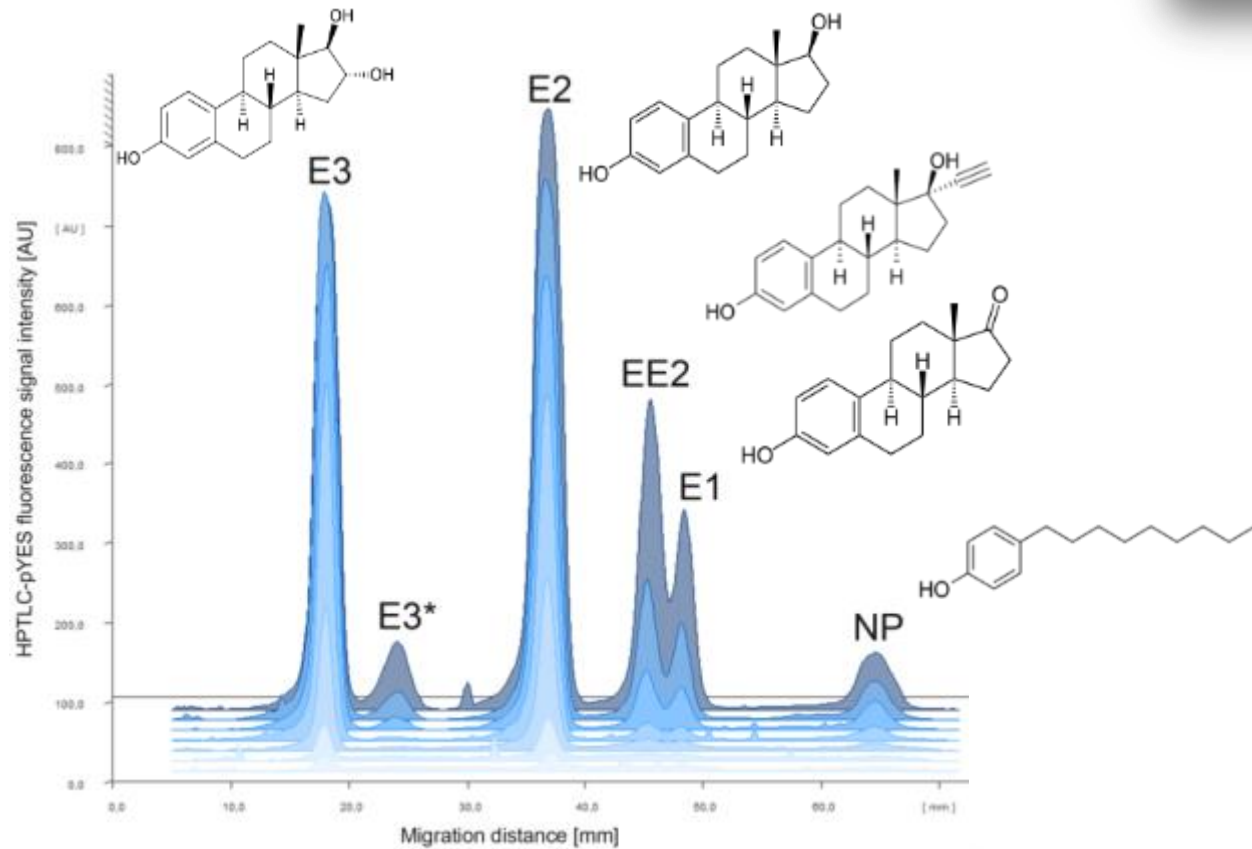


# HPTLC-FLD → high detectability



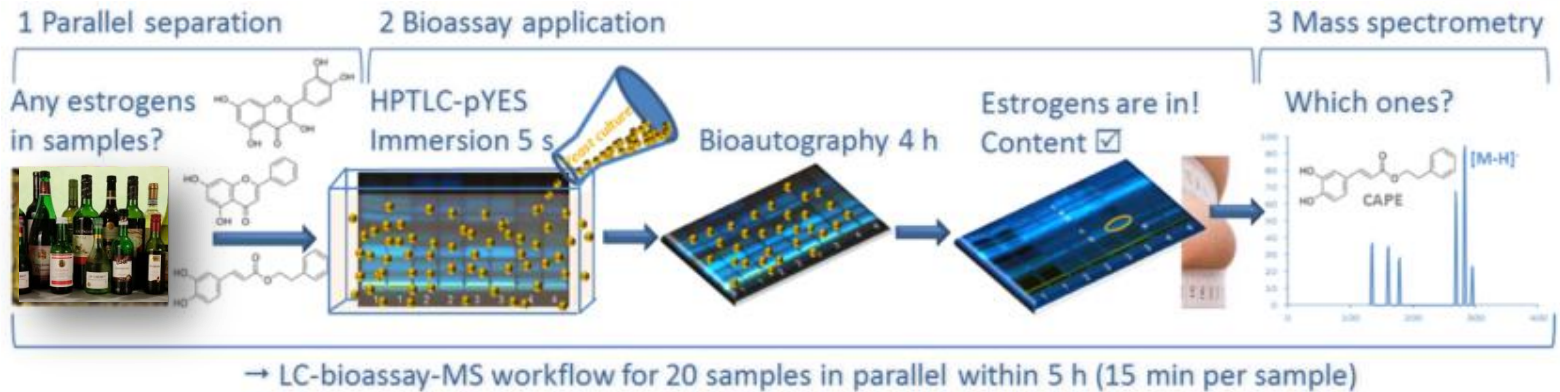
Bioquantification: Yeasts response is measured  
Fluorescence measurement at 366/>400 nm  
(mercury lamp)

# Detection of estrogen-effective compounds

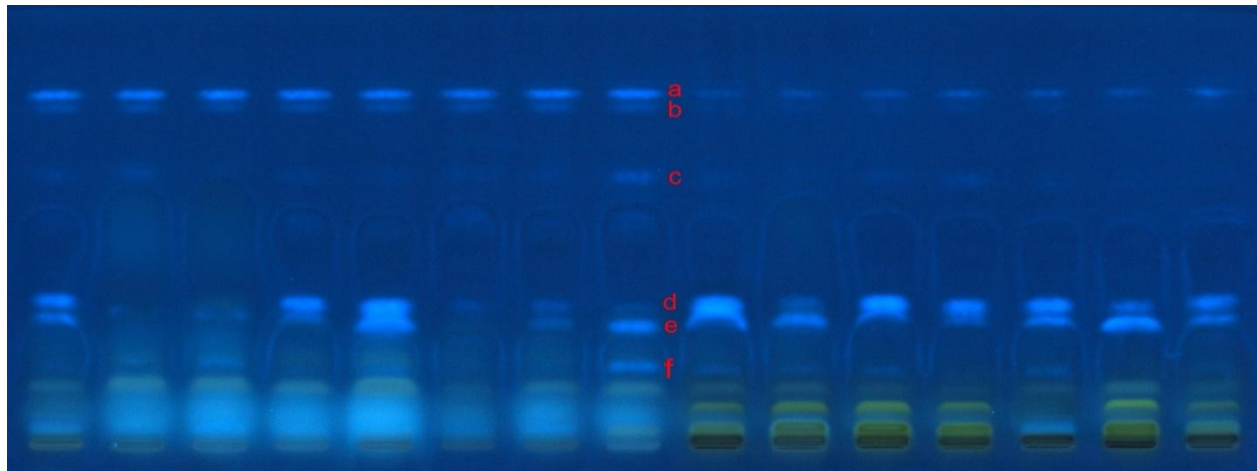


	pg/band
E1	25 – 1000
EE2	0.5 – 20
E2	0.5 – 20
E3	25 – 1000

# Discovery of estrogen-effective compounds



→ Wine



# Discovery of estrogen-effective compounds

→ Spices



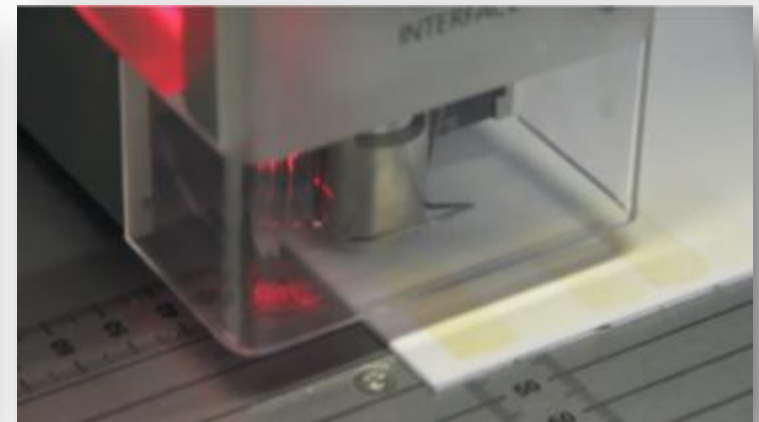
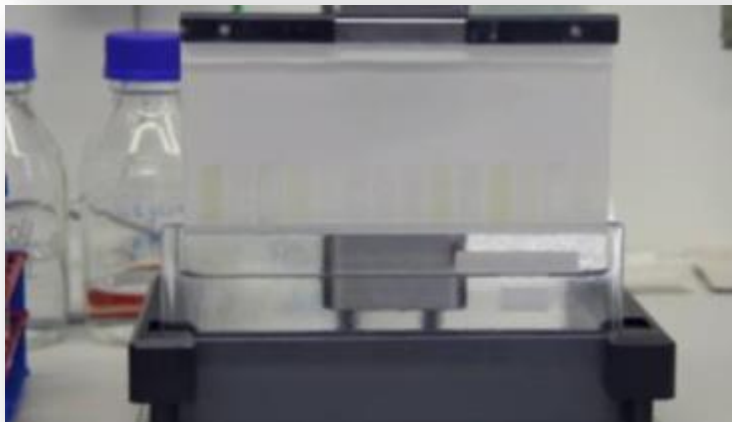
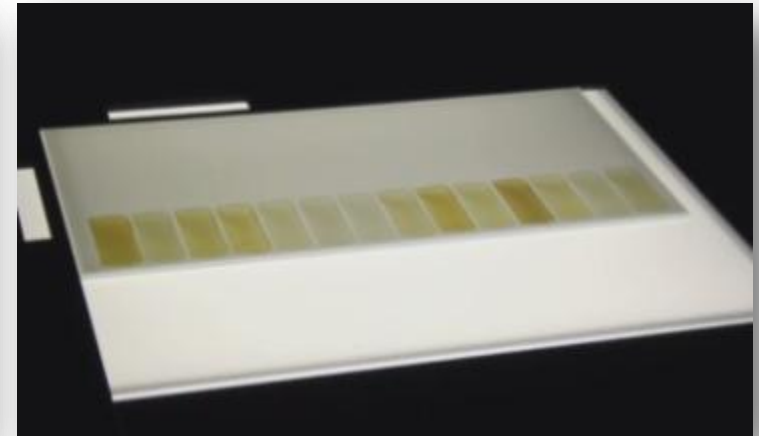
→ Beer samples



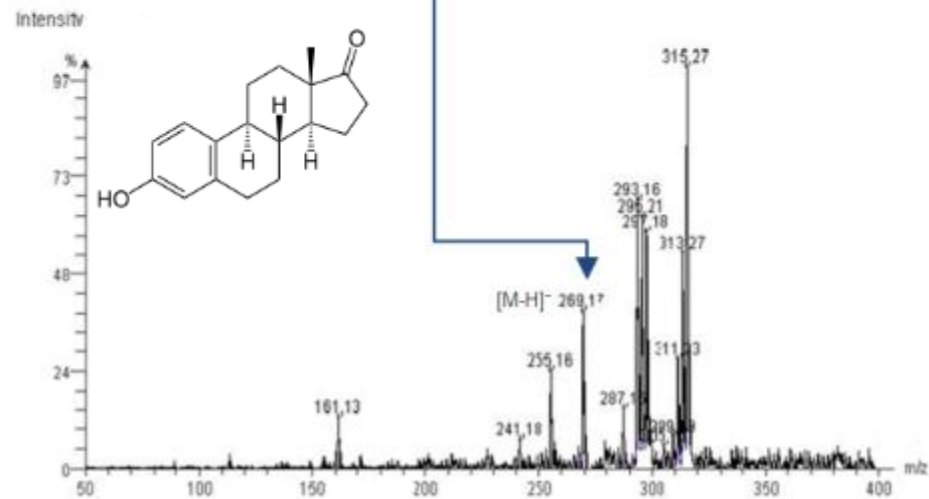
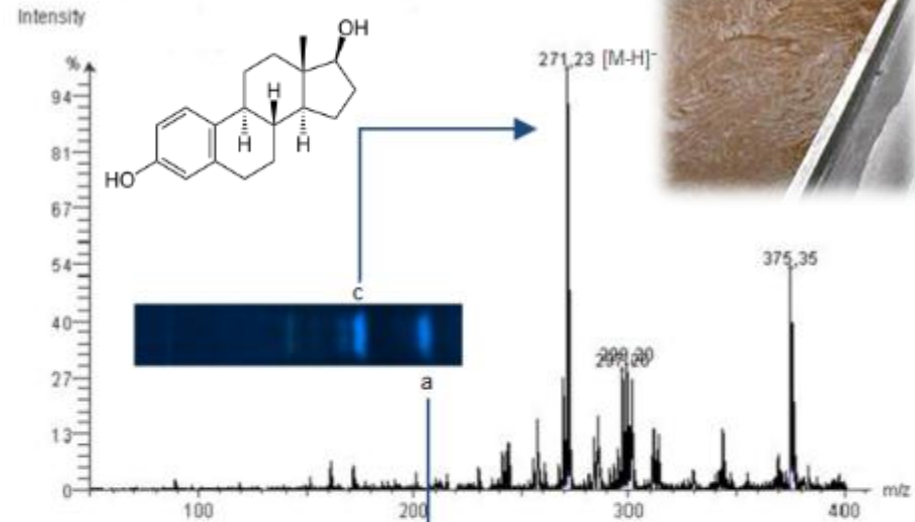
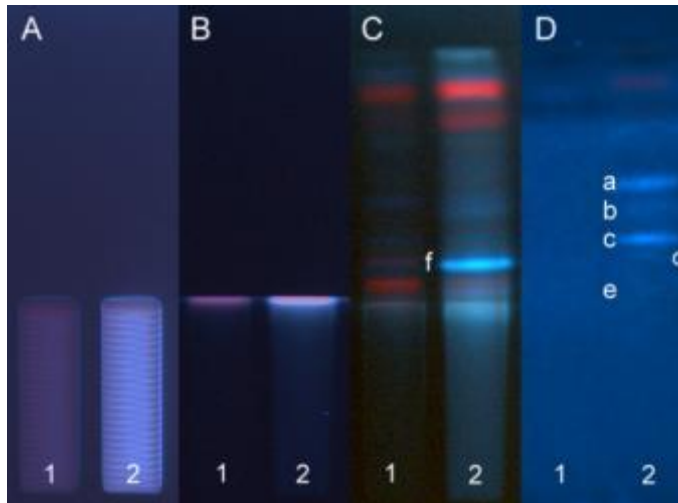
# Estrogen-effective compounds in beer?



Workflow <https://youtu.be/Q7AGuljcFvQ>



# Discovery in surface/waste water



Substance	LOD [ng/L]	LOQ [ng/L]
E2	1.0	2.5
EE2	2.5	5.0
E1	4.3	15.0
E3	75.0	250.0
BPA	$1.6 \times 10^3$	$5.0 \times 10^3$
NP	$15.0 \times 10^3$	$65.0 \times 10^3$

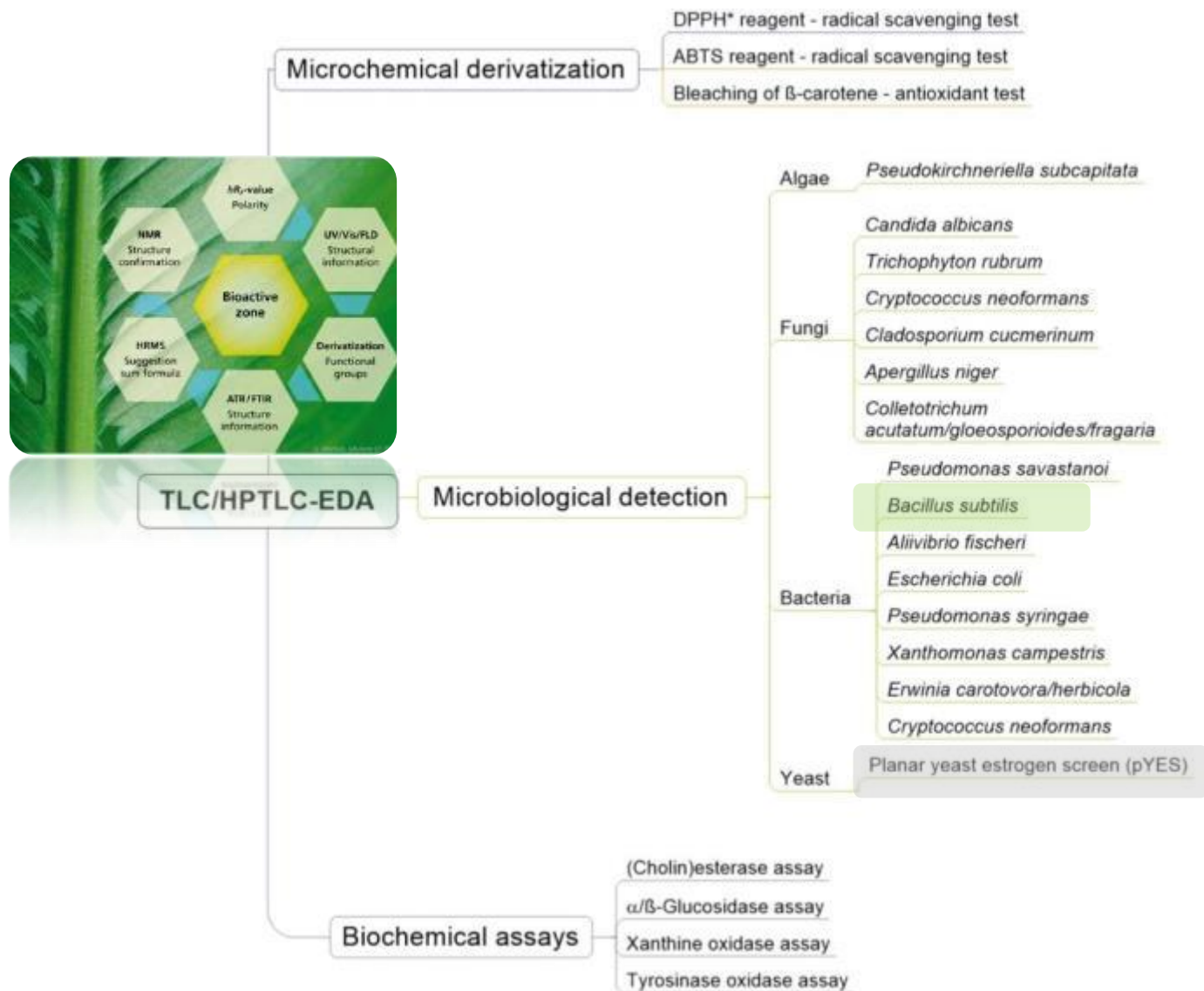


# Bioquantitation (by microorganisms' response)



Content [ng/L]	E2	E1	E3
STP <u>influent</u>	10.1	40.5	98
	6.7	17.6	<u>nd</u>
	3.1	12.4	<u>nd</u>
	4.9	36.4	150
	12.6	49.7	210
STP <u>effluent</u> (x5)	<u>nd</u>	<u>nd</u>	<u>nd</u>
<u>Lücke</u> bach	1.6	20.5	<u>nd</u>
	8.3	12.7	<u>nd</u>
Flachs <u>ba</u> ch	6.6	16.6	<u>nd</u>
	<u>nd</u>	<u>nd</u>	<u>nd</u>
Wetter (x2)	<u>nd</u>	<u>nd</u>	<u>nd</u>
Weid <u>gr</u> aben	2.1	11.6	<u>nd</u>
	**	4.9	<u>nd</u>
Wiese <u>ck</u> (x2)	<u>nd</u>	<u>nd</u>	<u>nd</u>

# Effect-directed link to the compound

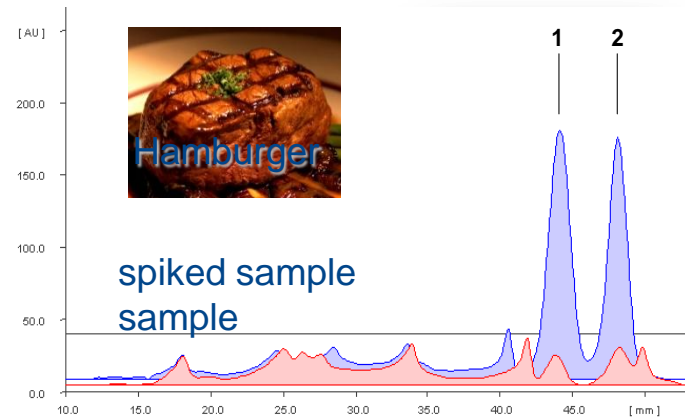
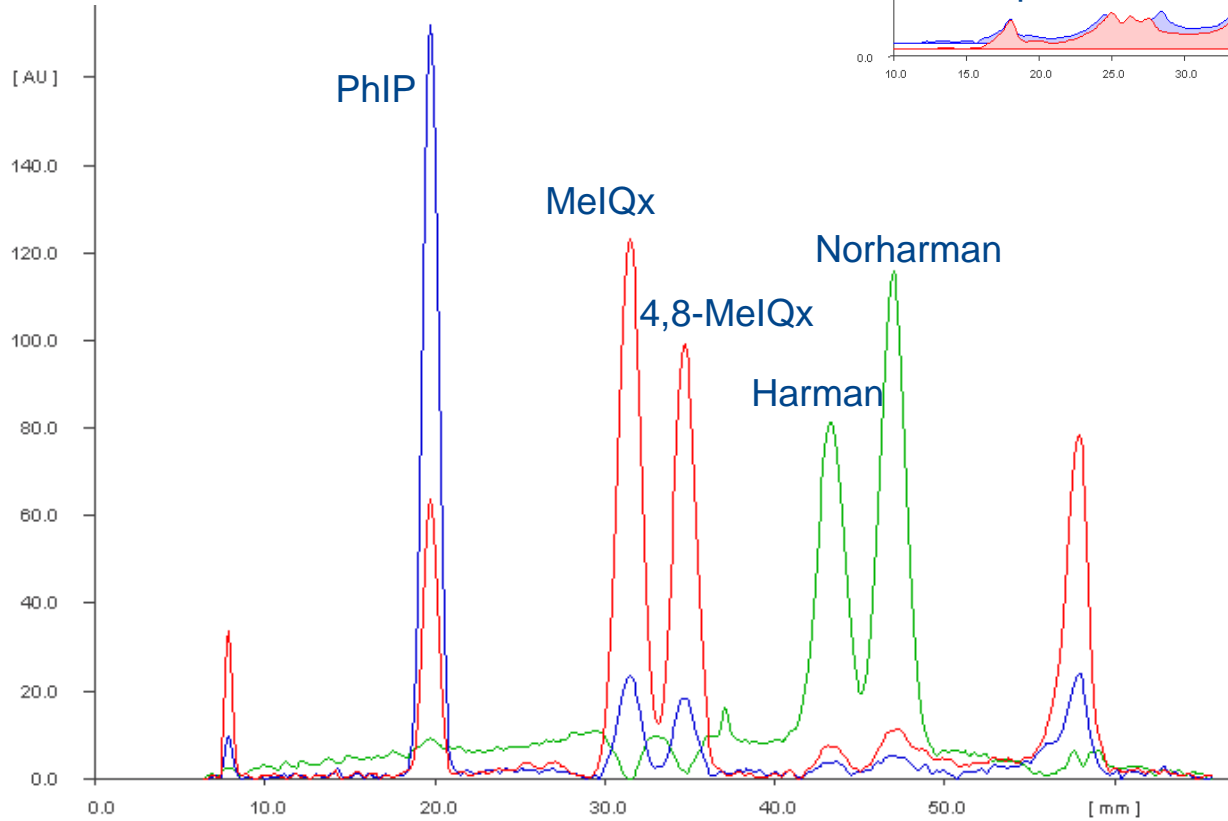


MWL with both modes (absorption and fluorescence)  
for evaluation of a track?

How to use track subtraction?

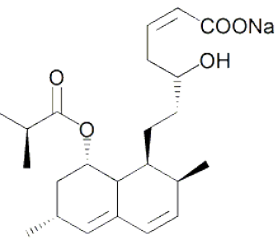
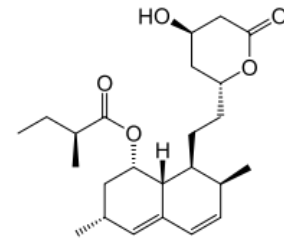
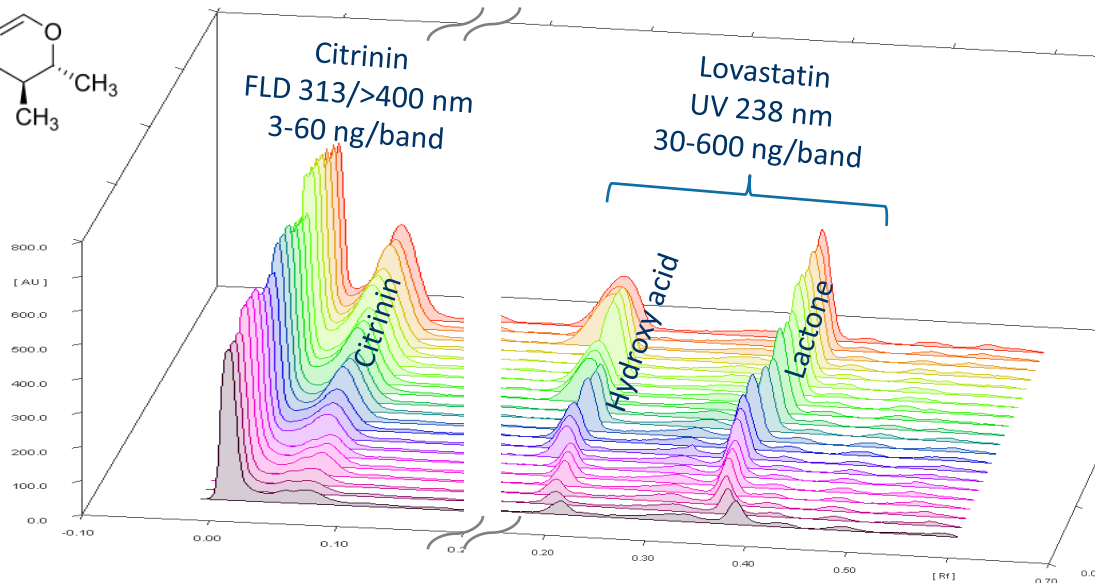
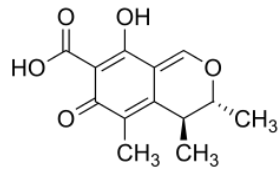
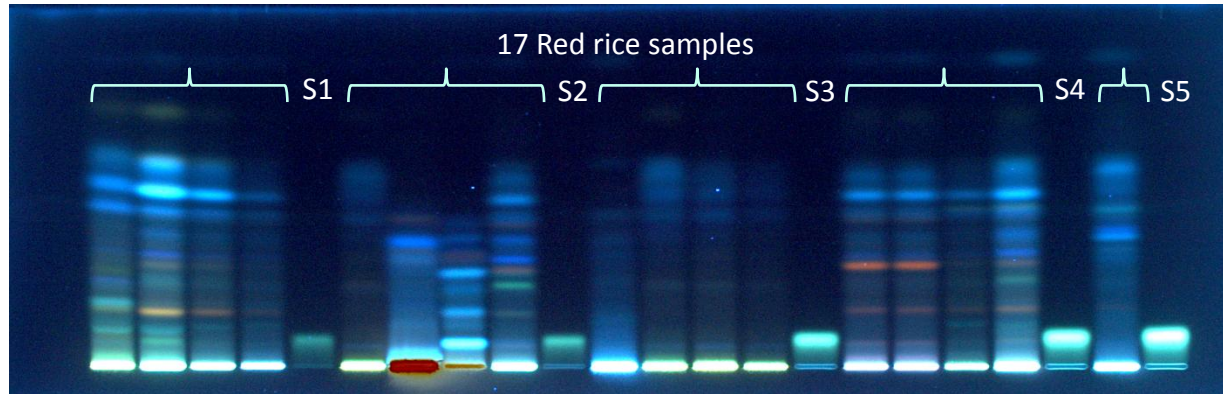


# HAAAs in meat



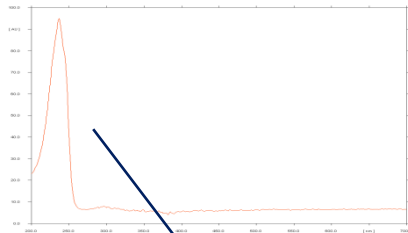
U. Jautz, G. Morlock, Proceedings of 51<sup>th</sup> ICoMST Baltimore (2005) 1342-1348  
 U. Jautz, M. Gibis, G. Morlock, J Agric Food Chem 56 (2008) 4311-4319

# Two-wavelength scan to subtract background

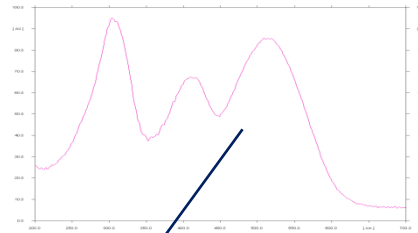


# Two-wavelength scan to subtract background

Lovastatin acid



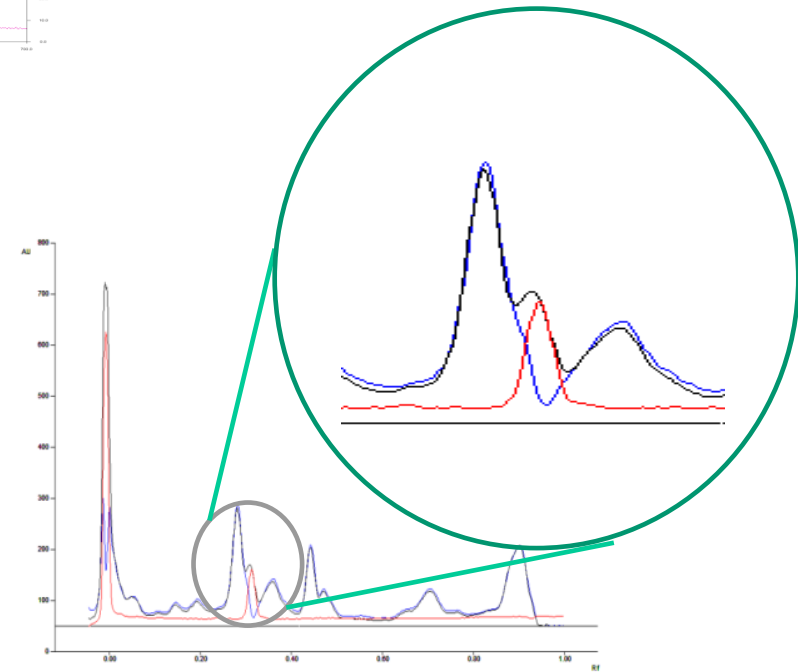
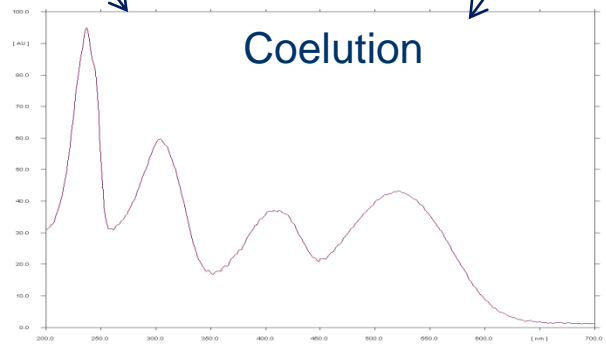
Rubropunctamin



Measurement wavelength 238 nm

Background wavelength 588 nm

→ subtracted result



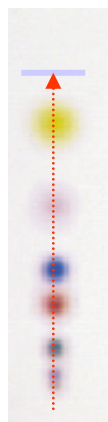
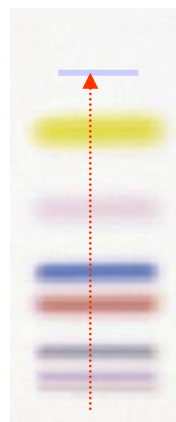
# Two-wavelength scan to subtract background

	Determination coefficient $R^2$ (n = 5, ng/band)	Amount (ng/band)	Recovery (L = 3, J = 5) (%)	Reproducibility (%RSD, L = 3)	Lab precision (%RSD, J = 5)
Lovastatin acid	0,9998 (25– 500)	80	109,3 ± 2,8	0,7	2,6
		200	105,7 ± 5,8	3,0	5,5
		400	102,9 ± 3,8	2,4	3,7
Lovastatin	0,9999 (25– 350)	80	114,7 ± 5,2	1,6	4,5
		200	111,3 ± 7,7	2,7	6,9
		400	110,6 ± 4,9	3,3	4,4
Citrinin	0,9989 (2,5– 50)	8	113,5 ± 11,1	2,2	9,8
		20	120,0 ± 6,8	3,3	5,7
		40	101,4 ± 2,9	2,2	2,9



## Influence of measurement parameters?

## Geometry of the slit



Slit length:

Bands 70 - 80 %

Spots 120 %



Slit width:

the higher, the better the light intensity,  
but the worse resolution

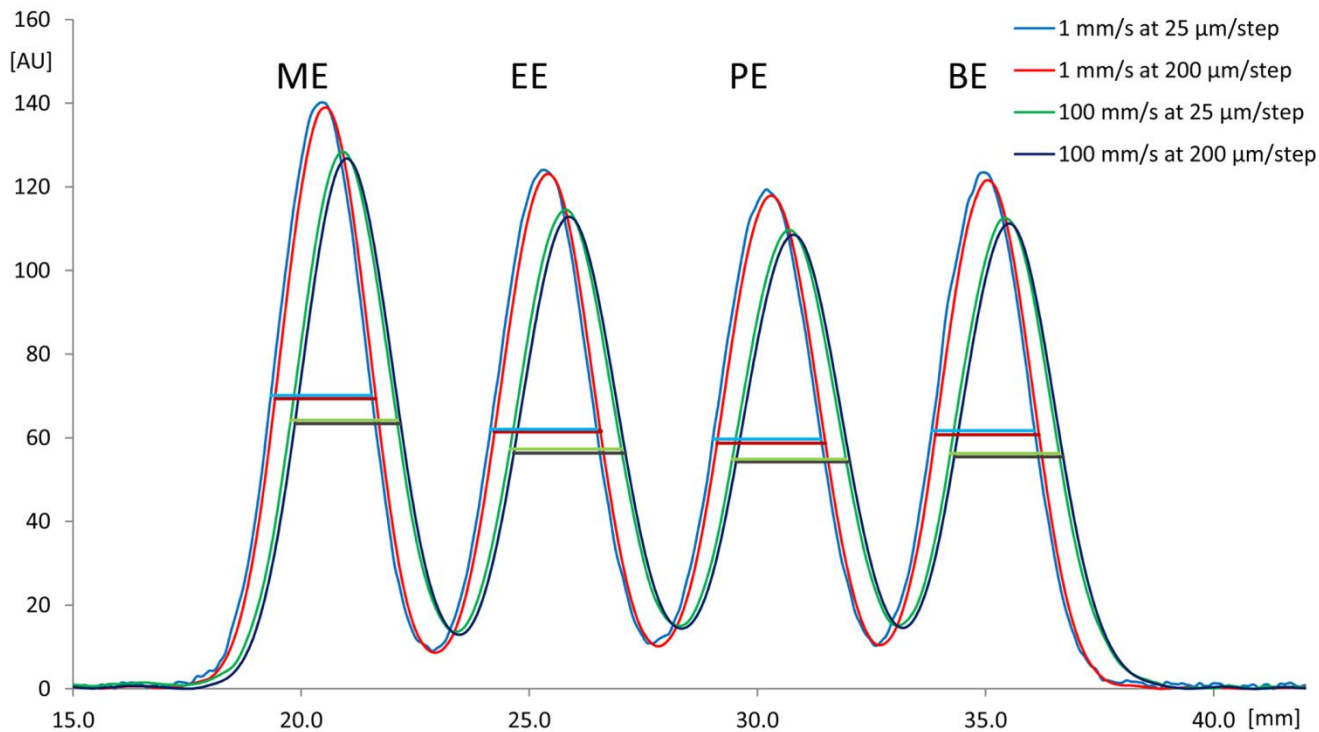
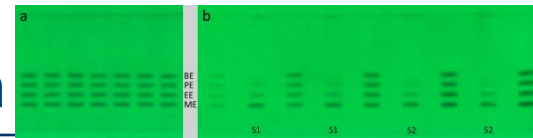
**Interested in latest results?**

T. Häbe, G. Morlock: Challenges in quantitative HPTLC – Part 1:

Influence of densitometric settings on the result,

J. Planar Chromatogr. 28 (2015) 426–435

# Influence of scan speed and resolution



	Scan speed at 1 mm/s		Scan speed at 100 mm/s		FWHM Increase [%] from 1 to 100 mm/s		Peak Shift [mm] from 1 to 100 mm/s	
	25	200	25	200	25	200	25	200
Resolution [μm/step]	25	200	25	200	25	200	25	200
FWHM pHB-ME [mm]	2.2	2.2	2.3	2.3	5.4	5.0	0.5	0.4
FWHM pHB-EE [mm]	2.3	2.3	2.4	2.5	4.7	5.6	0.5	0.4
FWHM pHB-PE [mm]	2.4	2.4	2.5	2.5	5.1	5.0	0.5	0.4
FWHM pHB-BE [mm]	2.3	2.3	2.4	2.4	6.2	4.8	0.5	0.6
Mean values	2.3 mm	2.3 mm	2.4 mm	2.4 mm	5.4%	5.1%	0.5 mm	0.5 mm

## Recommendations for TLC Scanner 4

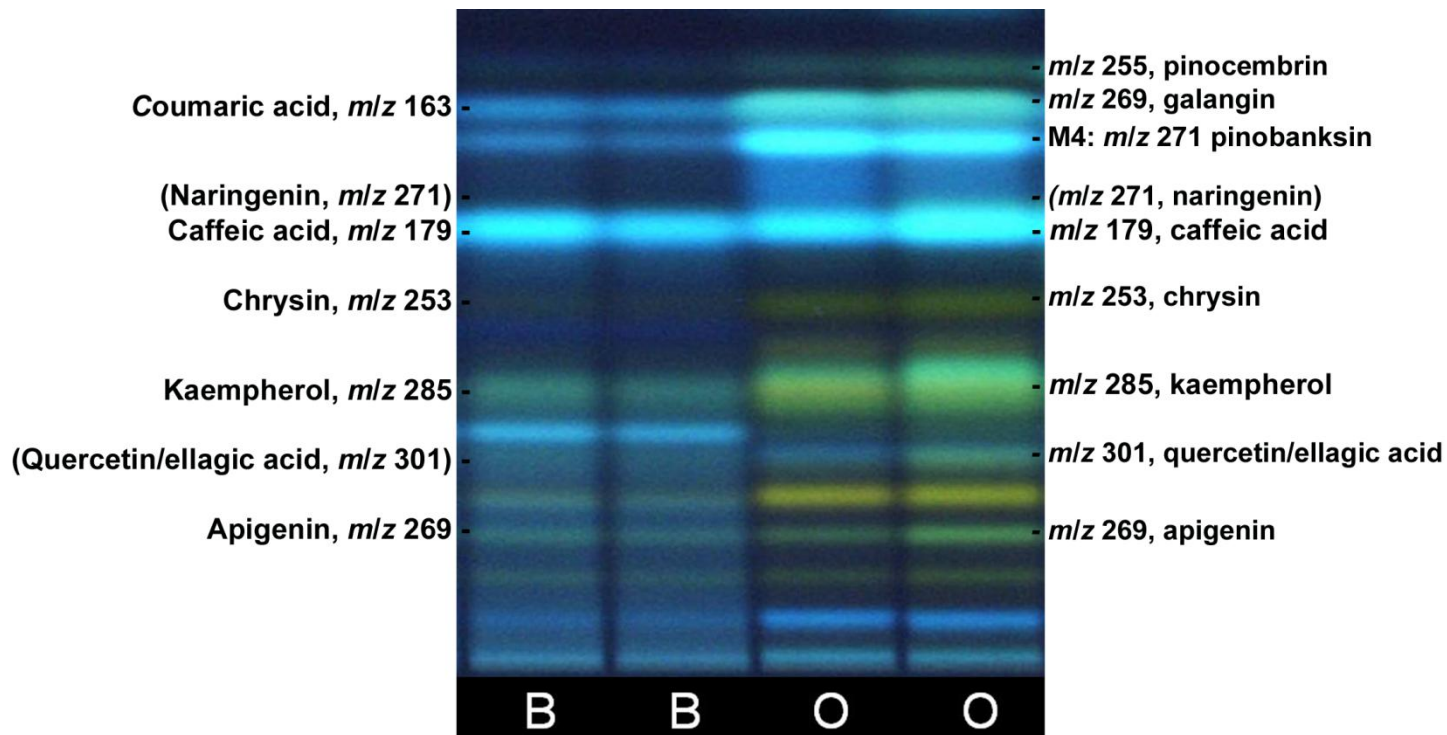
---

- Higher signal intensities in *resolution* mode of the optical system compared to *light* mode
- Peak broadening for higher scan speeds whereby signal height decreased significantly
- Peak area evaluations more robust with regard to changed settings
- Moderate scan speeds of 5 or 10 mm s<sup>-1</sup>
- Data resolution of 25 or 50 μm step<sup>-1</sup>

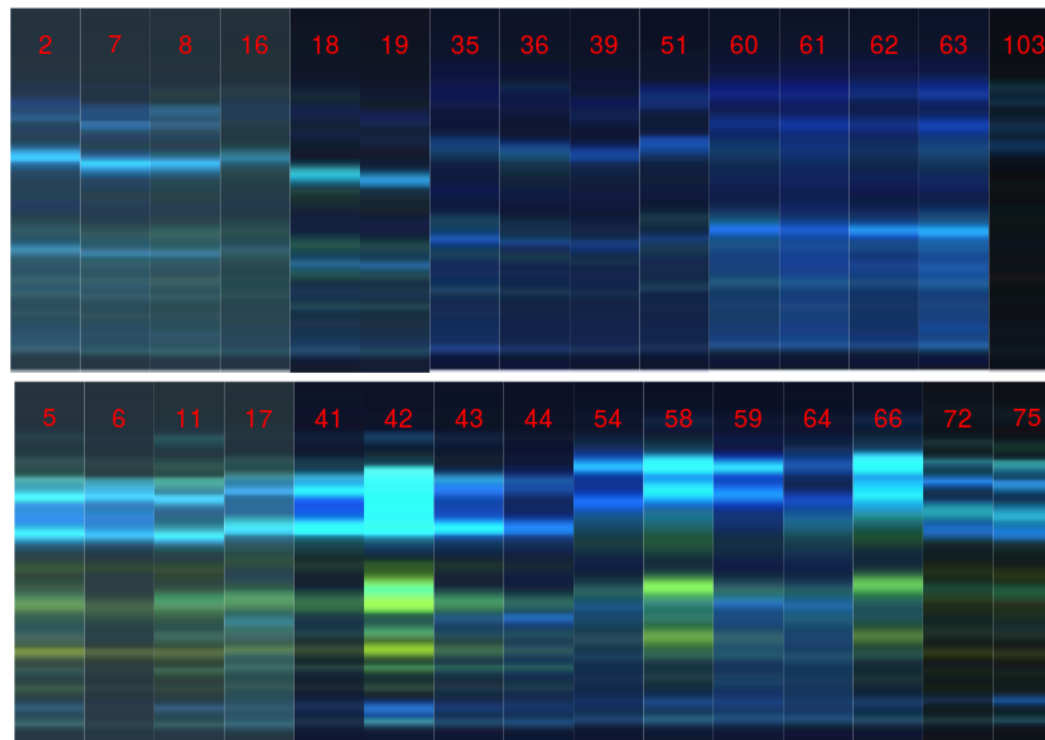
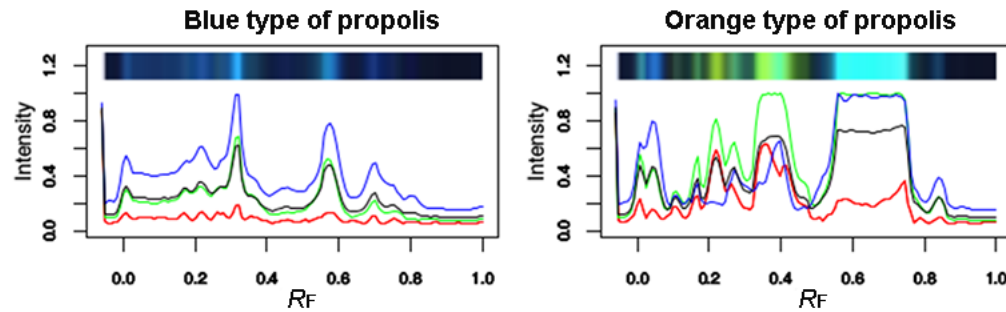
Of course, minor impact!

# Open source web application for multivariate analysis

# rTLC software: open-source web application

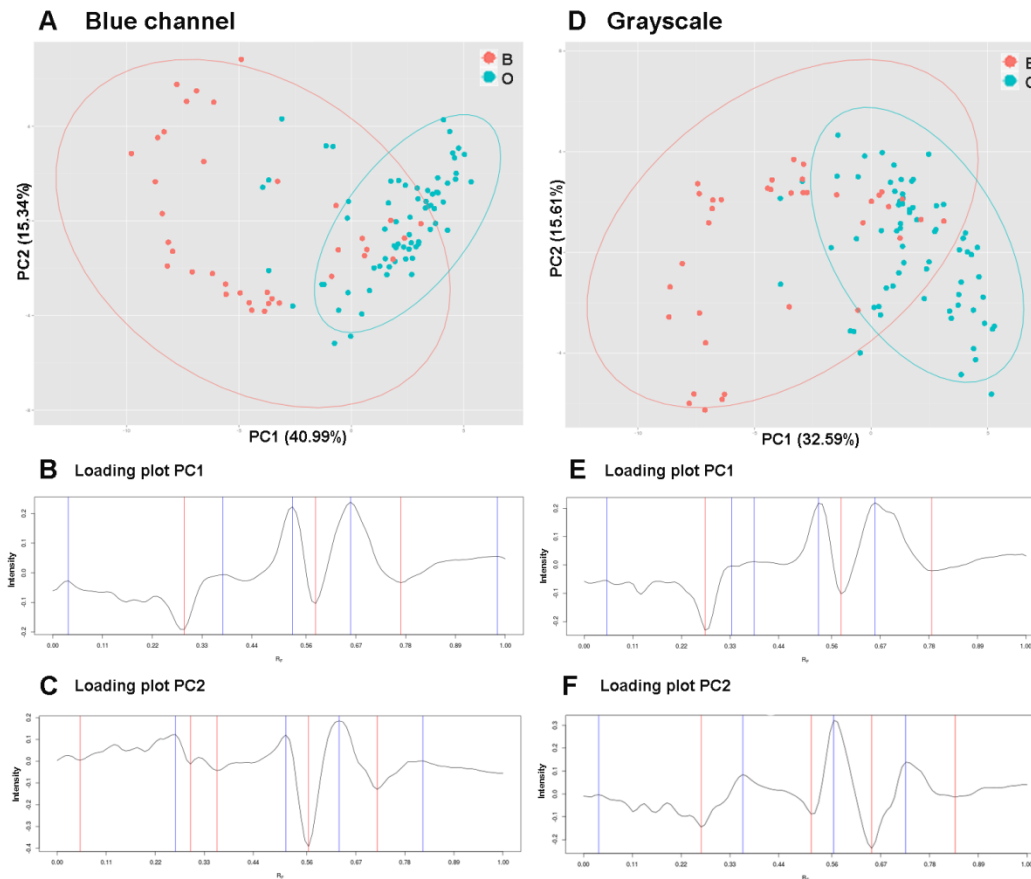


# rTLC software: open-source web application



# rTLC software: open-source web application

The rTLC link is coming soon [here](#)

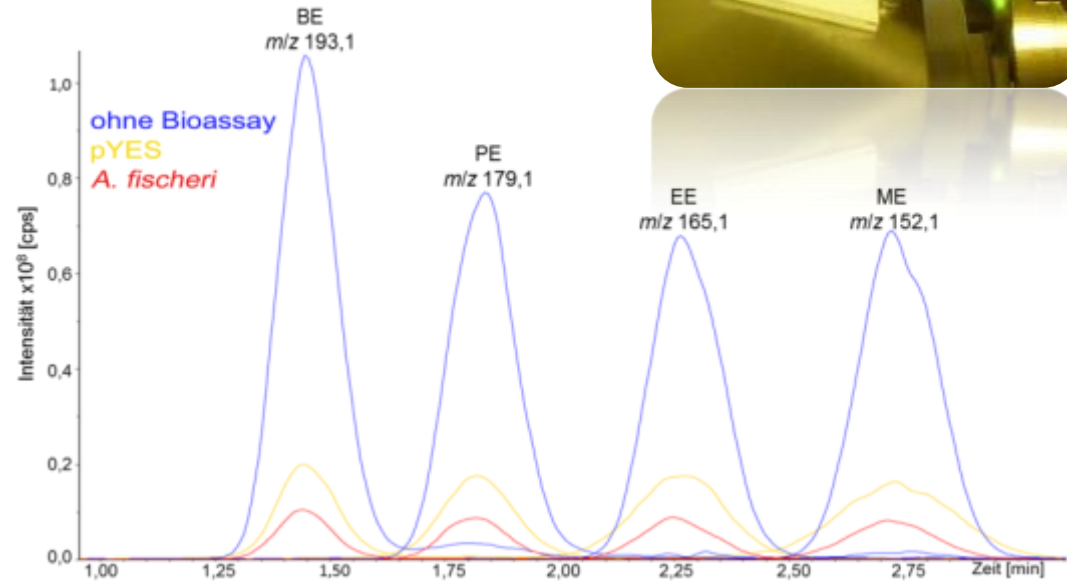
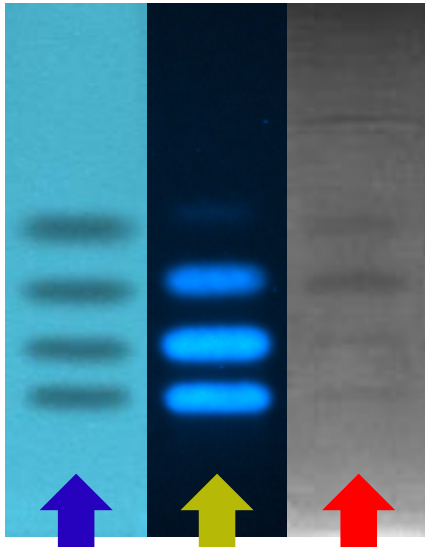
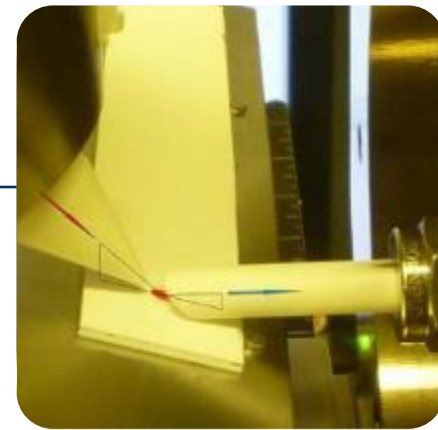




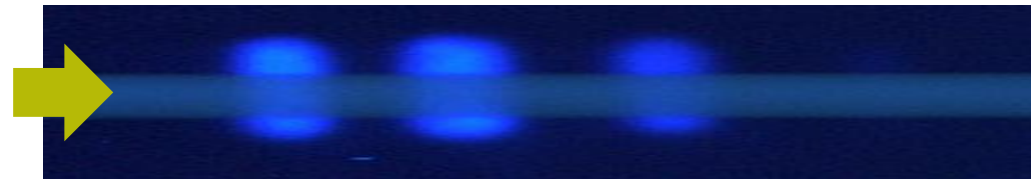
## Quantification after direct bioautography (DB)

...bioassay medium, substrate and cells are present

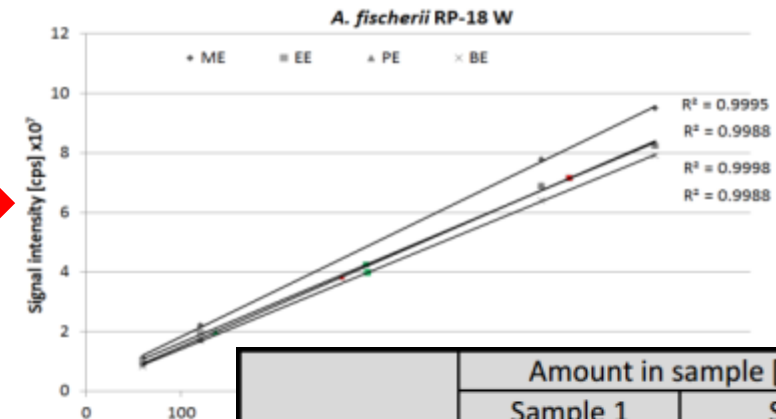
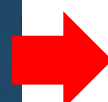
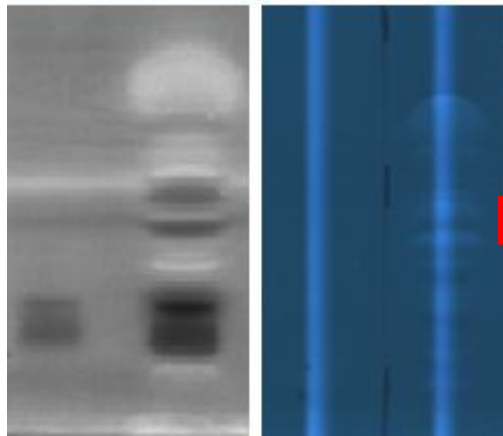
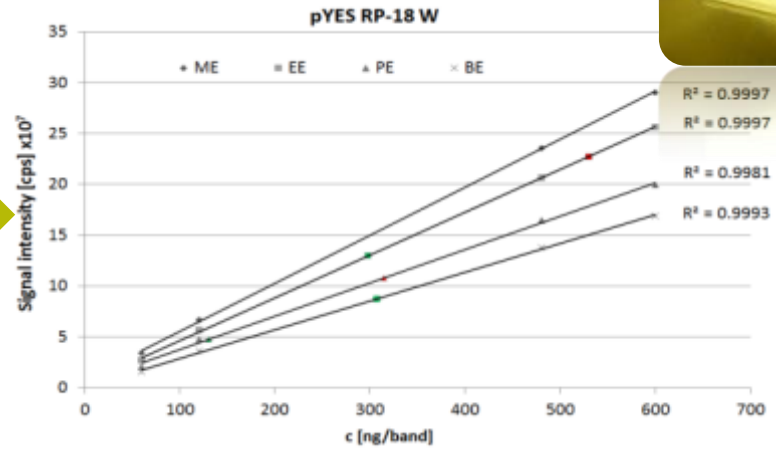
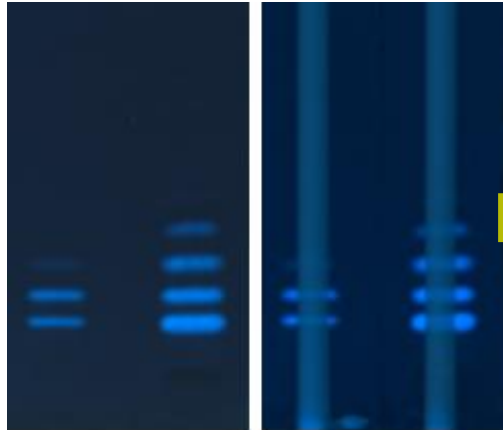
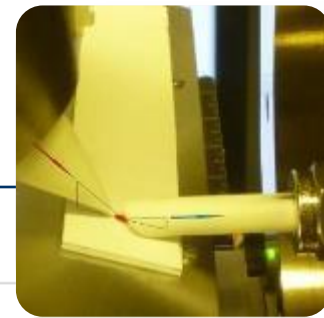
# DB-DART-MS



	Signal decay [%]	
	<i>A. fischeri</i>	pYES
ME	88	65
EE	89	67
PE	90	76
BE	91	81



# DB-DART-MS

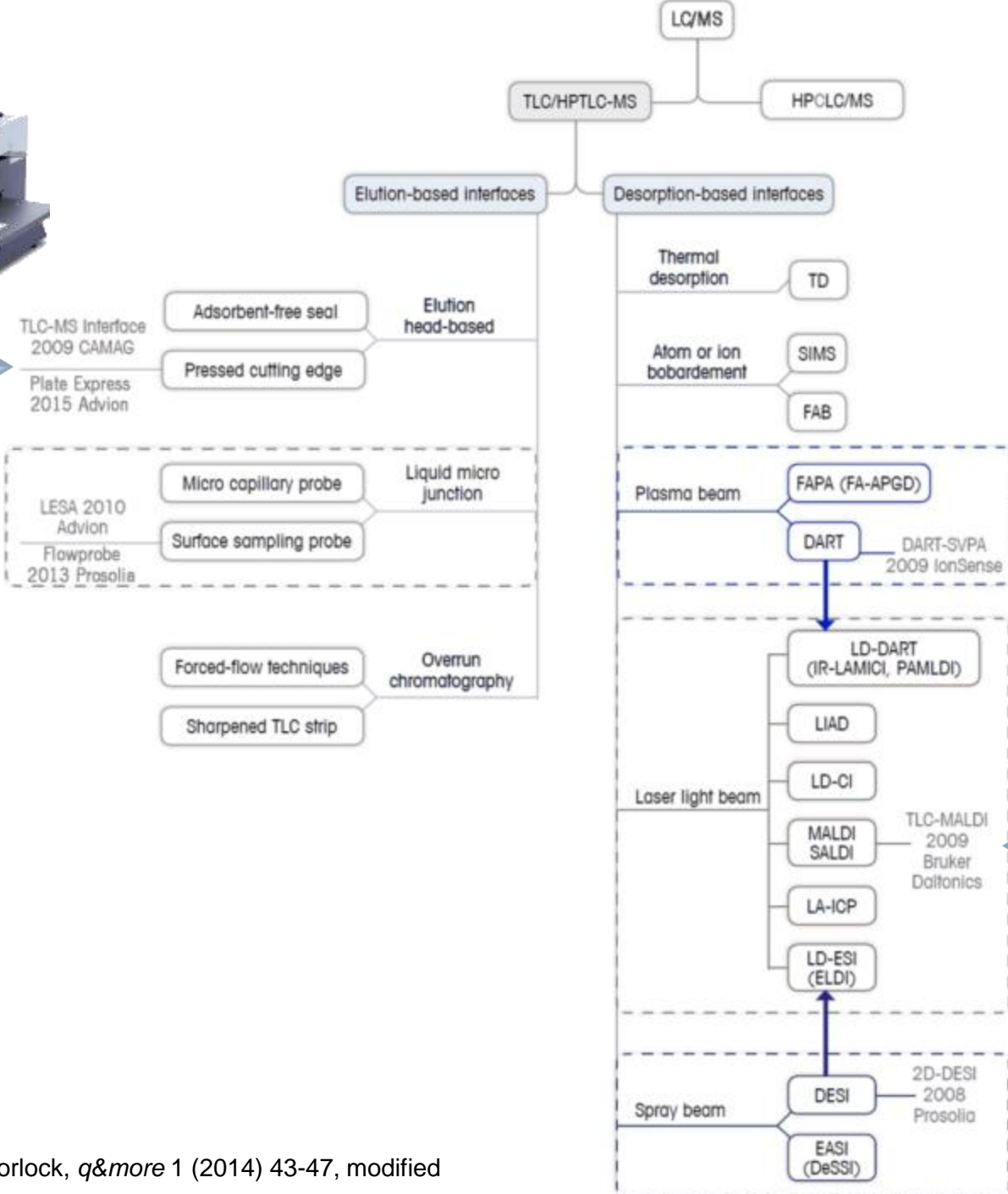


T. Häbe, G. Morlock, in preparation

		Amount in sample [mg/100g]							
		Sample 1			Sample 2				
		ME	EE	PE	ME	EE	PE	BE	
without	NP	103	56	30	165	75	37	65	
BioAssay	RP	97	59	34	147	69	30	67	
<i>A. fischerii</i>	NP	96	51	27	173	69	24	53	
	RP	101	51	27	157	59	27	59	
pYES	RP	111	53	31	170	60	26	62	

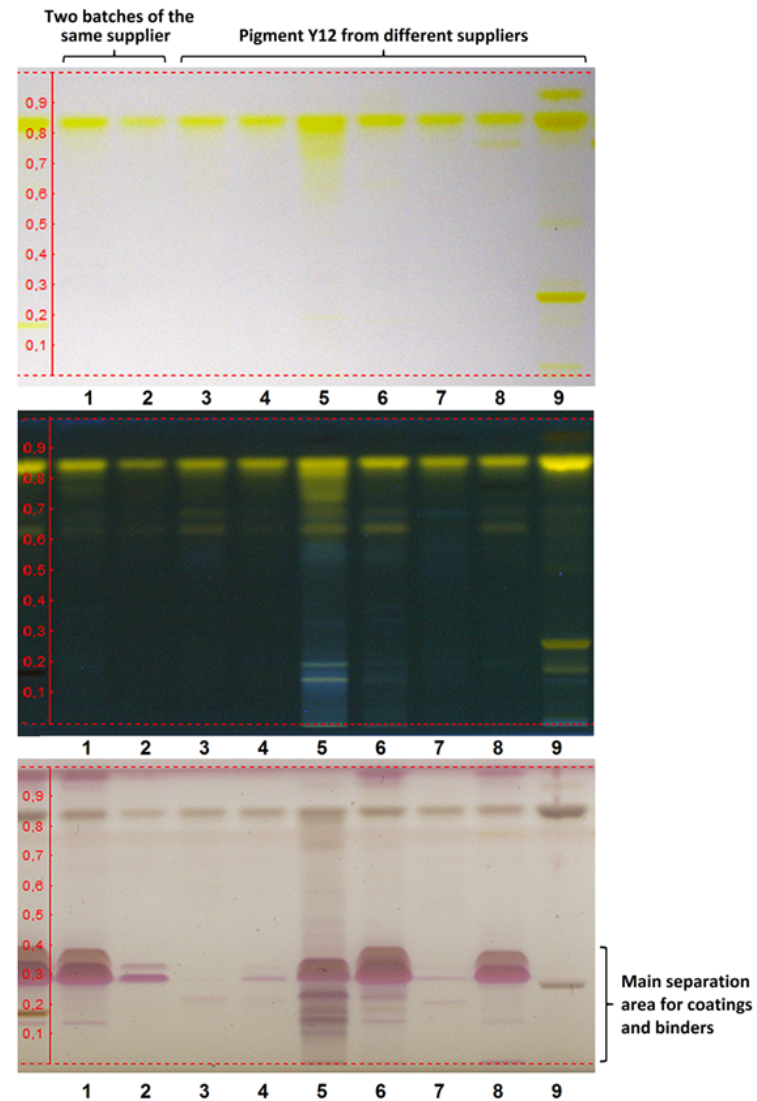
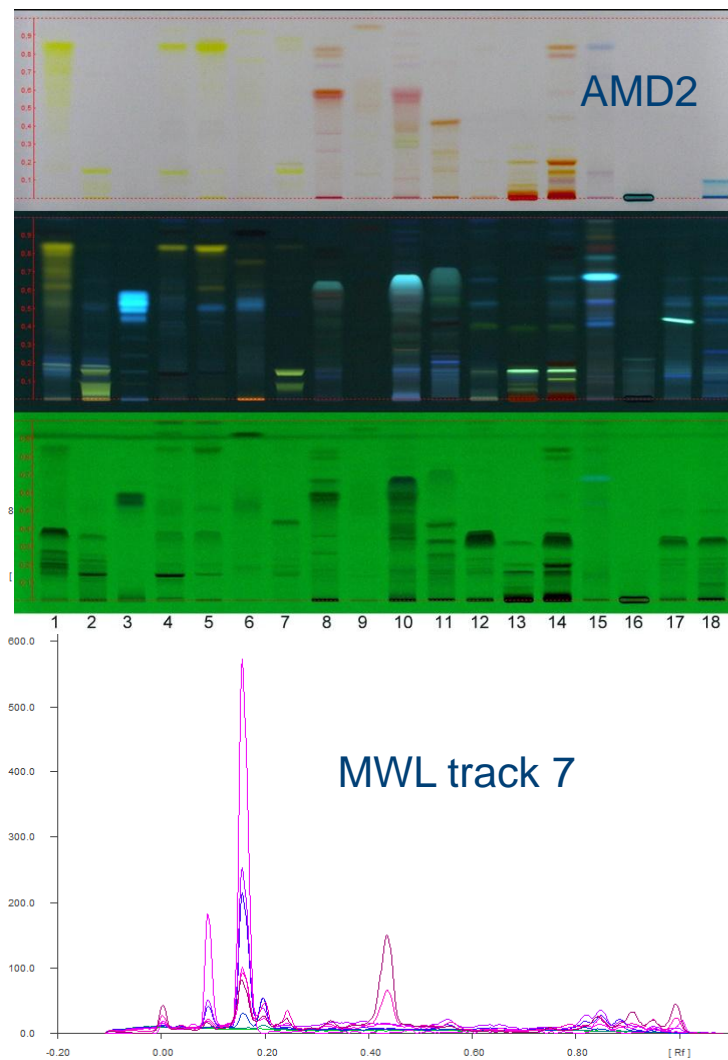


Upgrade in 2015

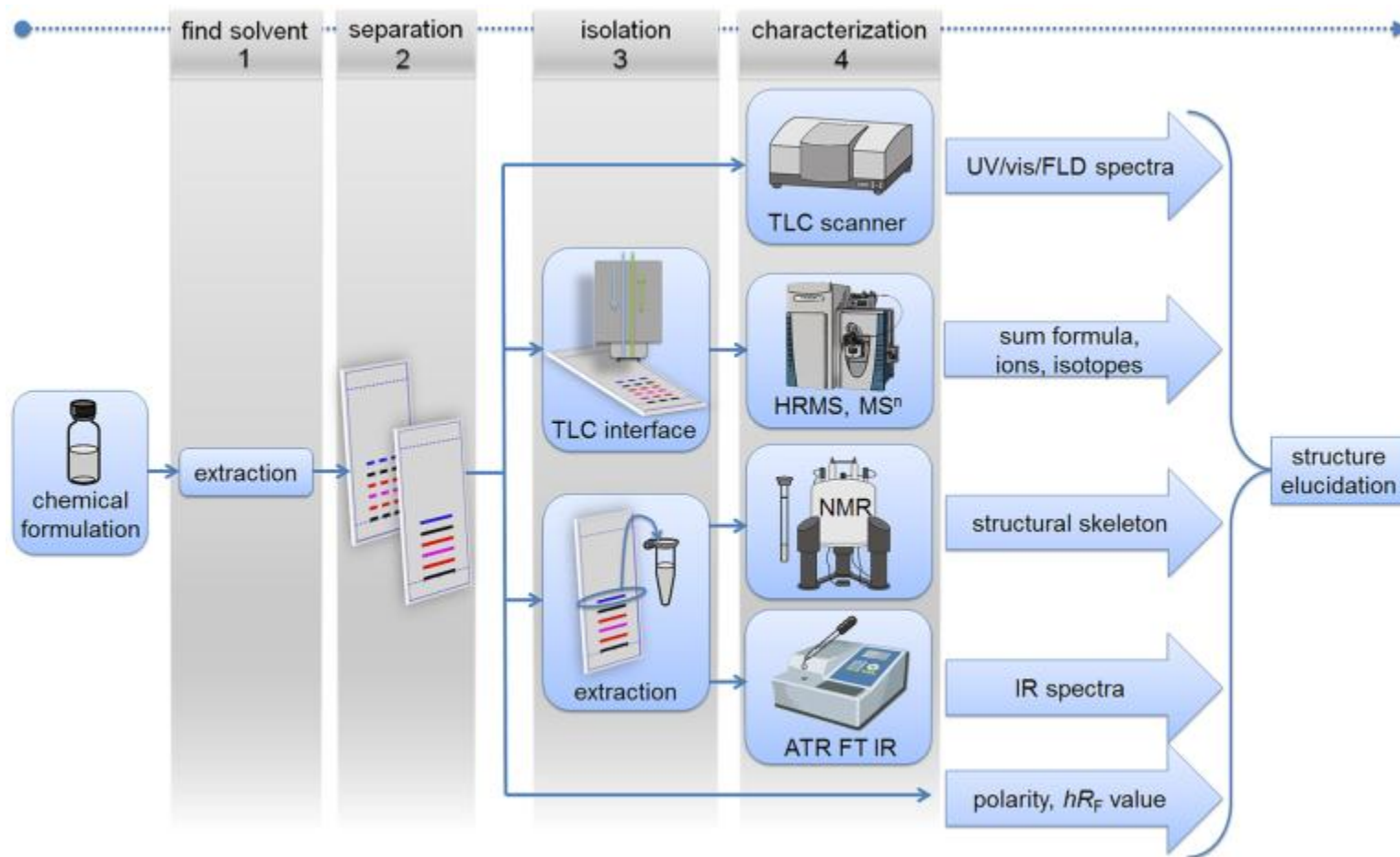


Latest news on structure elucidation...

# Quality control of pigment formulations

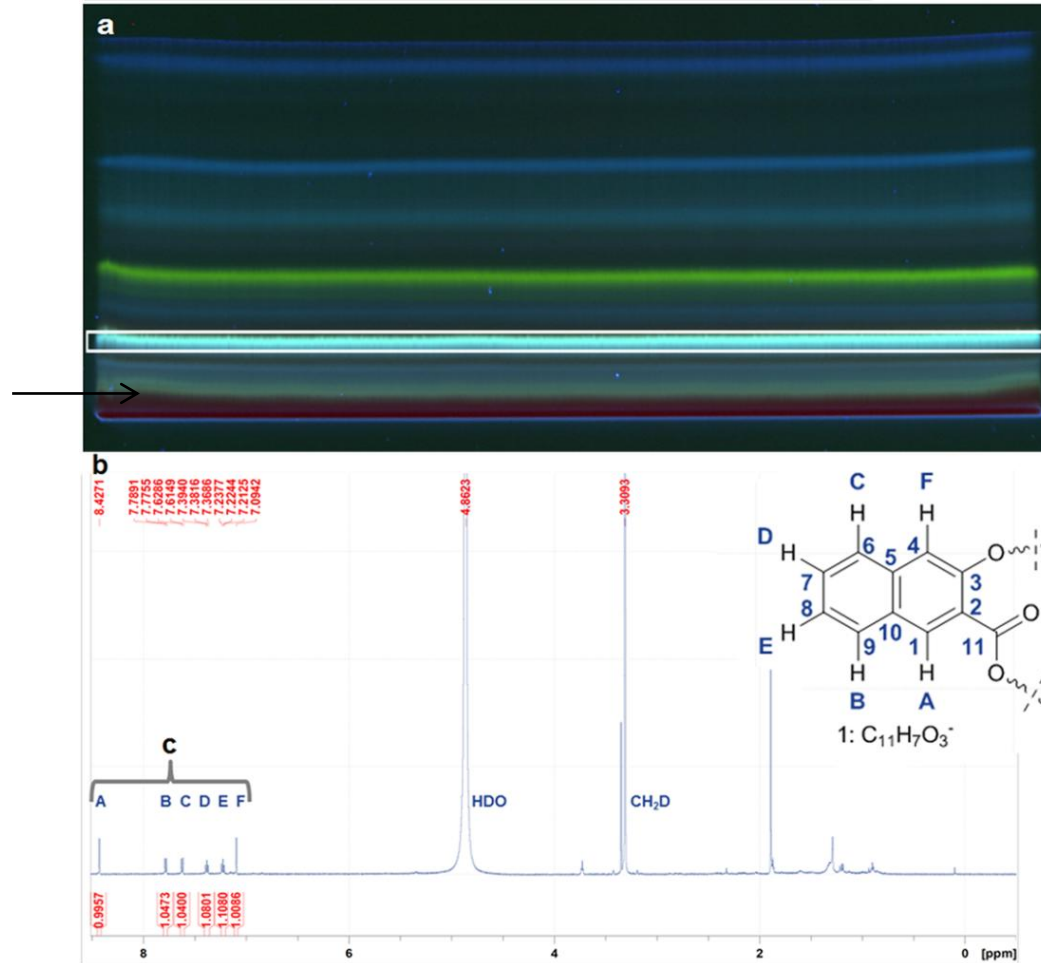


# Fast structure elucidation using 2 plates



# Fast structure elucidation using 2 plates

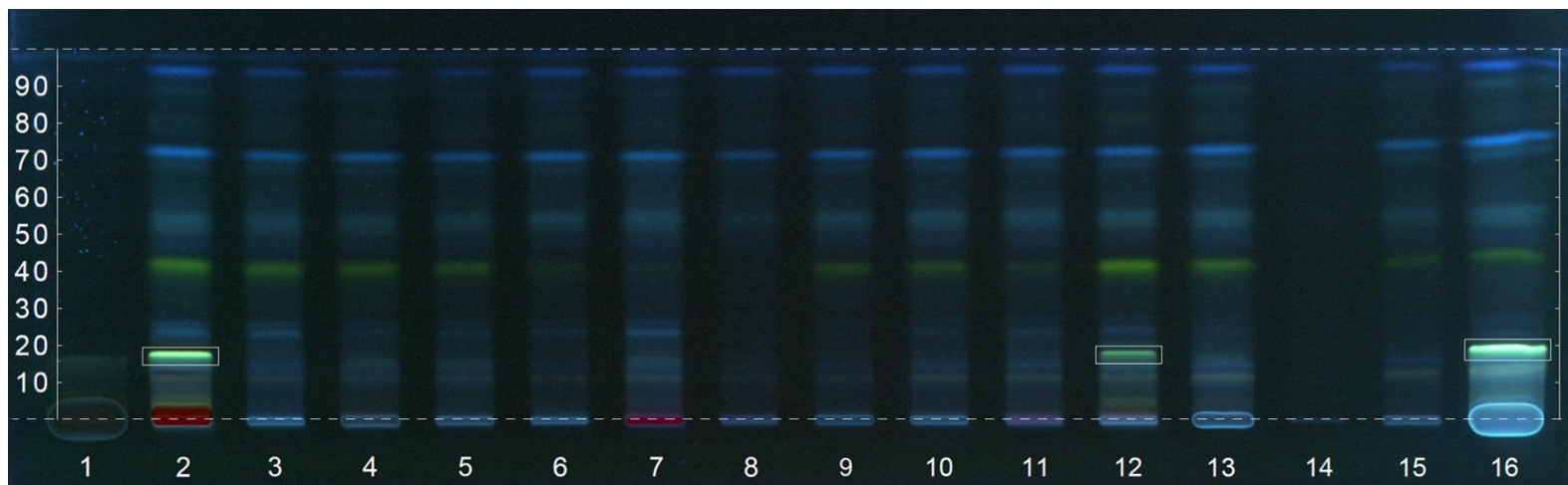
→ Focused band via AMD2 separation on 0.5 mm preparative plate



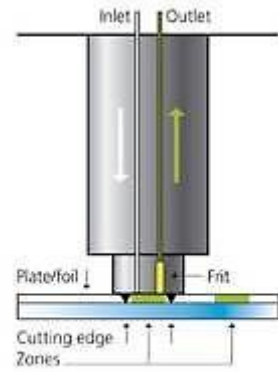


## Fast structure elucidation using 2 plates

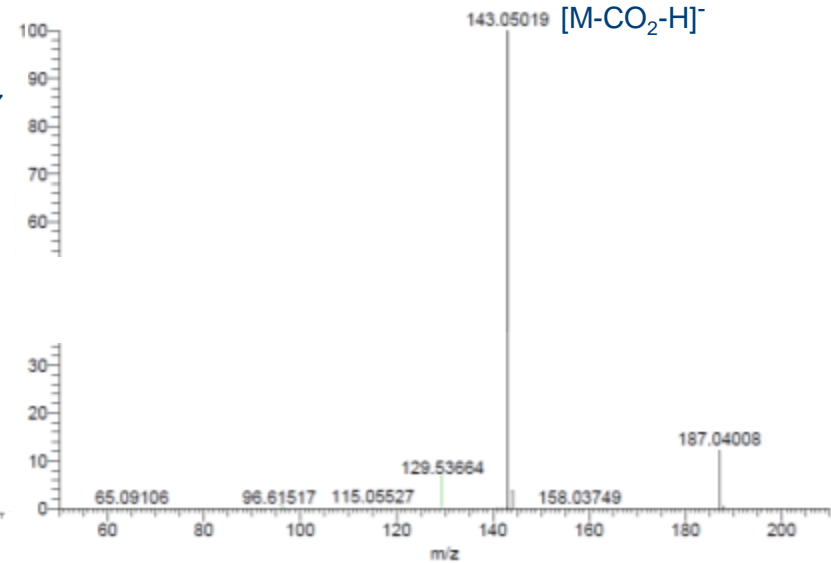
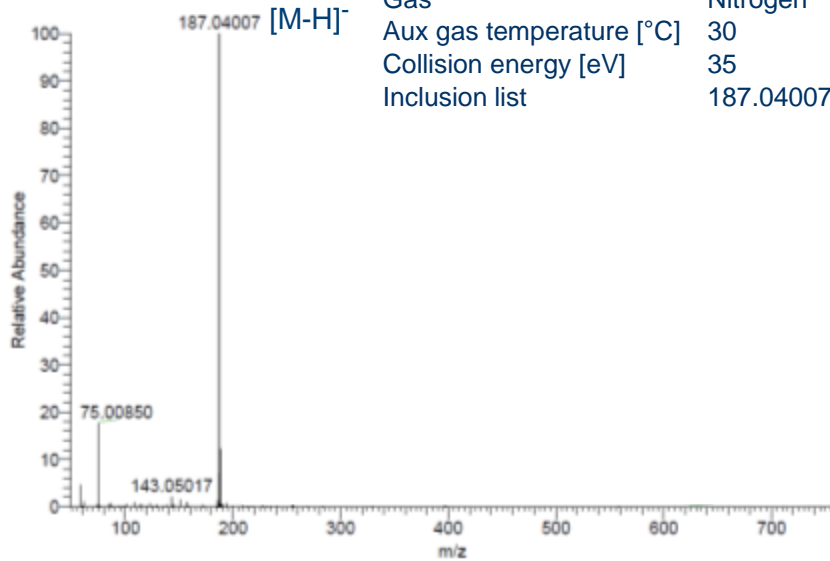
- Focused band via AMD2 separation on 0.5 mm preparative plate
- High target amount via selective solvent extraction (factor of 9)



# HPTLC-HRMS and -MS/MS of unknown



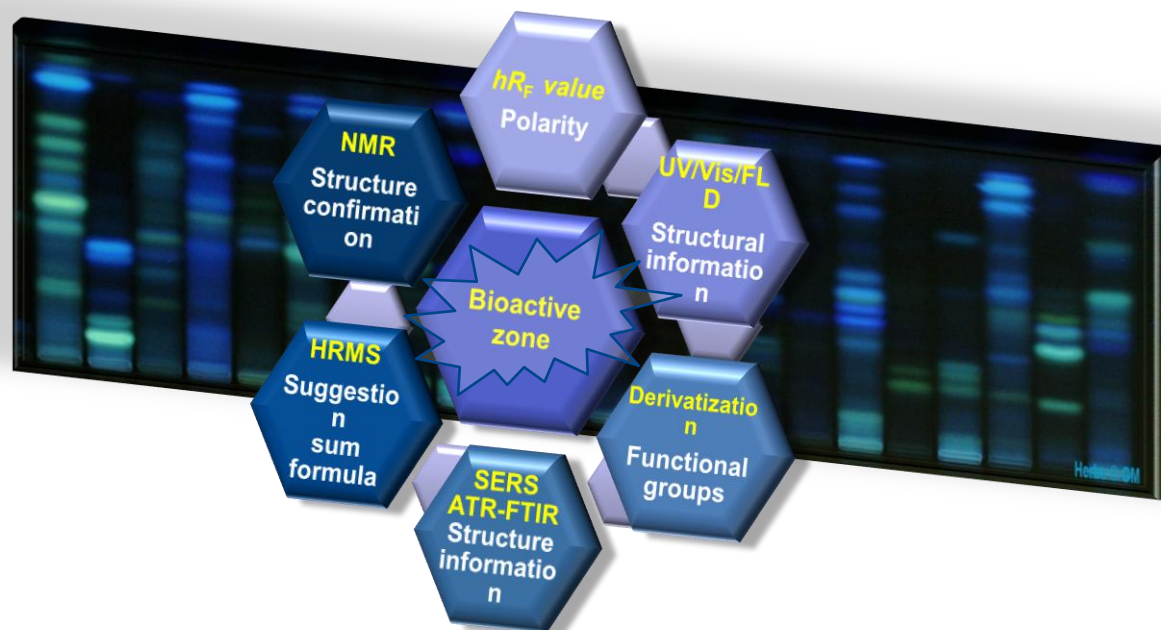
Polarity Negative  
 Measuring range [ $m/z$ ] 50-210  
 Spray voltage [kV] 3.3  
 Capillary temperature [°C] 320  
 Gas Nitrogen  
 Aux gas temperature [°C] 30  
 Collision energy [eV] 35  
 Inclusion list 187.04007



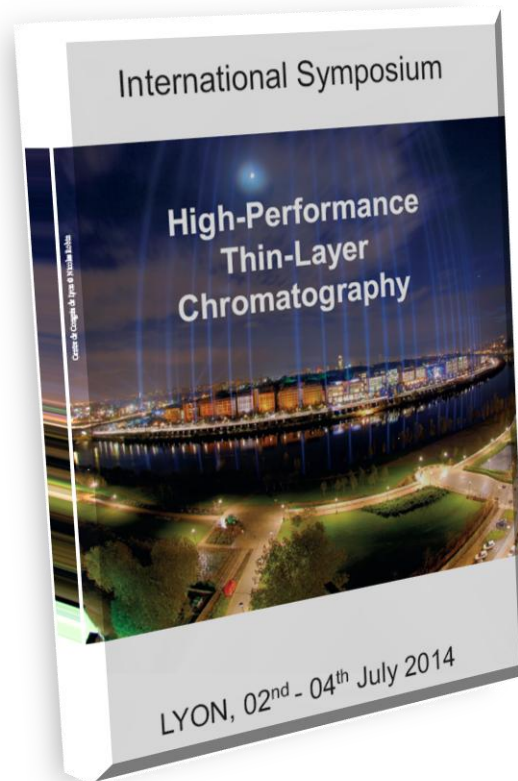
➔ 3-Hydroxy-2-naphthoic acid

## Take home - aspects of quantification

- Special feature: track subtraction
- Influence of parameter settings
- Bioautography → sharpness → quantitatively (bioquantification)
- Bioautogram-DART-MS → quantitatively
- Data treatment → artificial neural network improves quantification



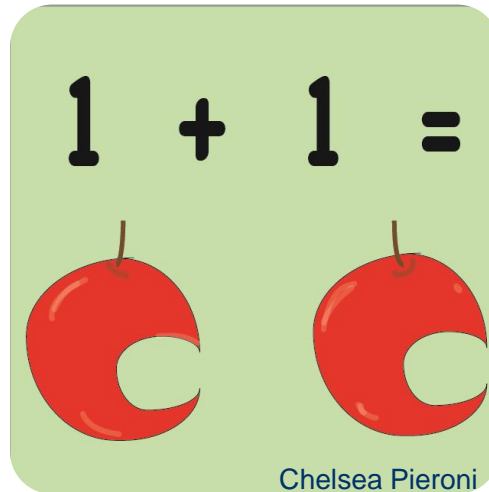
www.hptlc.com



# Steve Jobs

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Simple can be harder than complex:  
You have to work hard ...  
to make it simple.



But better...

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Instead of working hard,  
select the proper method  
to make analytical life easy.

Right decisions stop chains of troubles and costs  
at the very beginning.



**Thank you!**

