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What stands HPTLC for?

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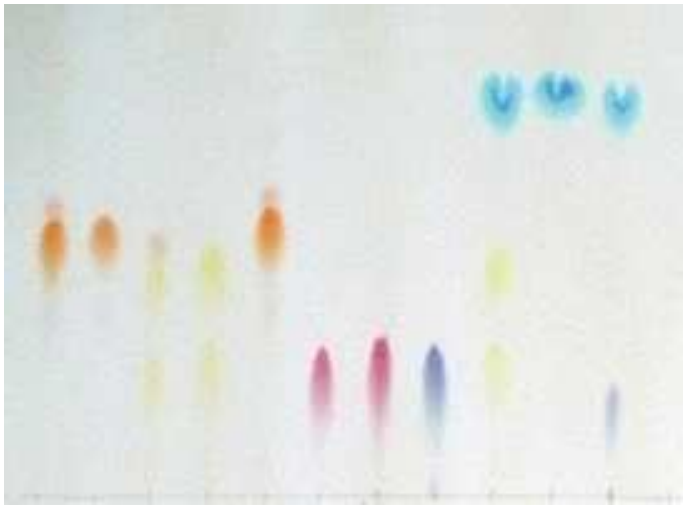
Planar Chromatography

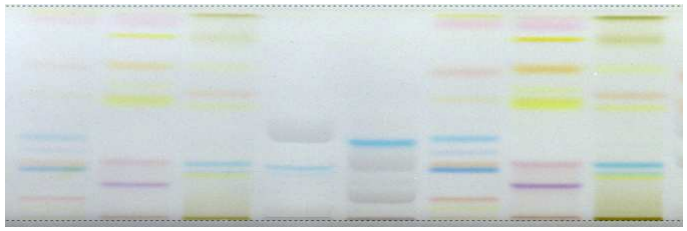
→ 1938 TLC

Thin-layer chromatography



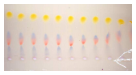
→ 1975 HPTLC

High-performance TLC



→ 2001 UTLC

UltraTLC



J. Sherma, G. Morlock, J. Planar Chromatogr. 21 (2008) 471-477

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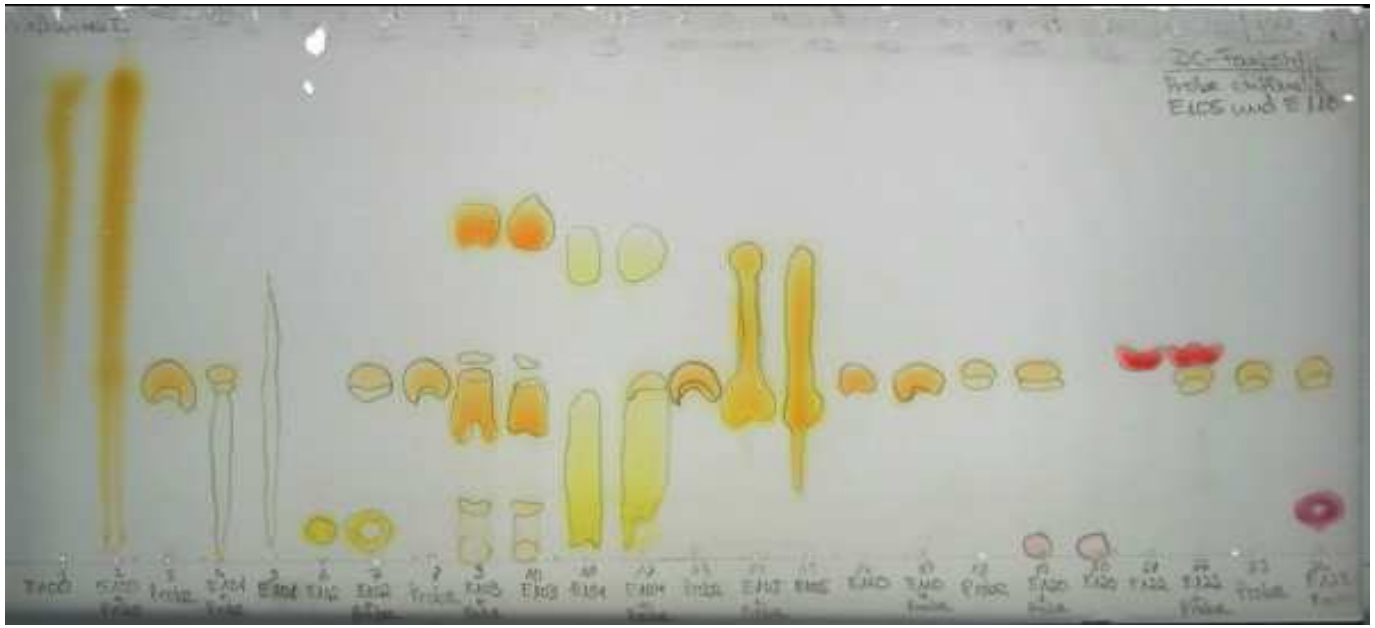
Students are trained in TLC – that´s it!



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HPTLC versus TLC

TLC is …. making LC like this

- No instrumentation!
- Large particle size!

Definition of HPTLC:

- Using modern instrumentation and
- Using more efficient layers with reduced particle size (~ 5 µm)

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HPTLC stands for instrumentation!

Institute of Food Chemistry, University of Hohenheim in Stuttgart, Germany

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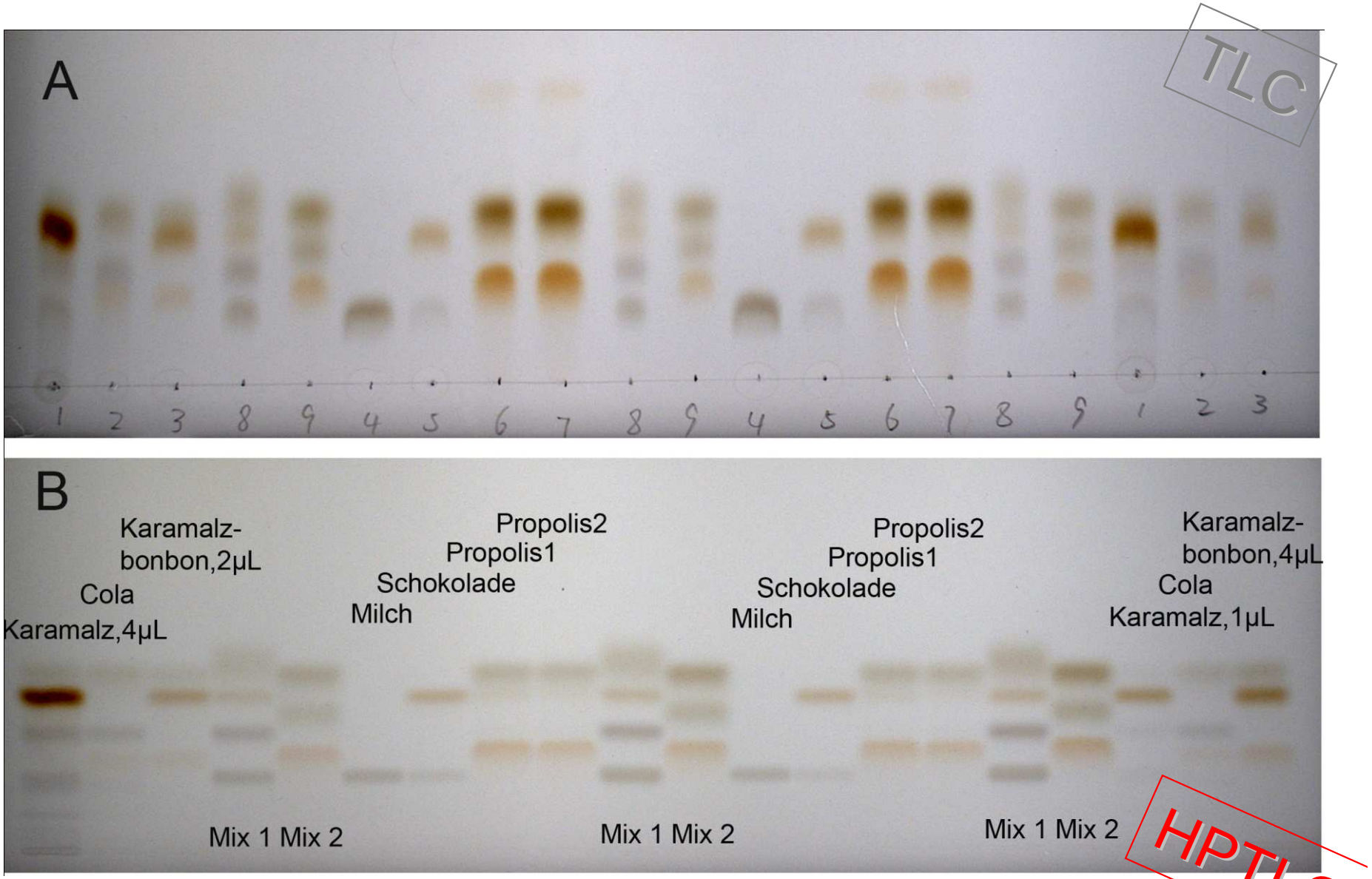
Analysis of sugars in various samples



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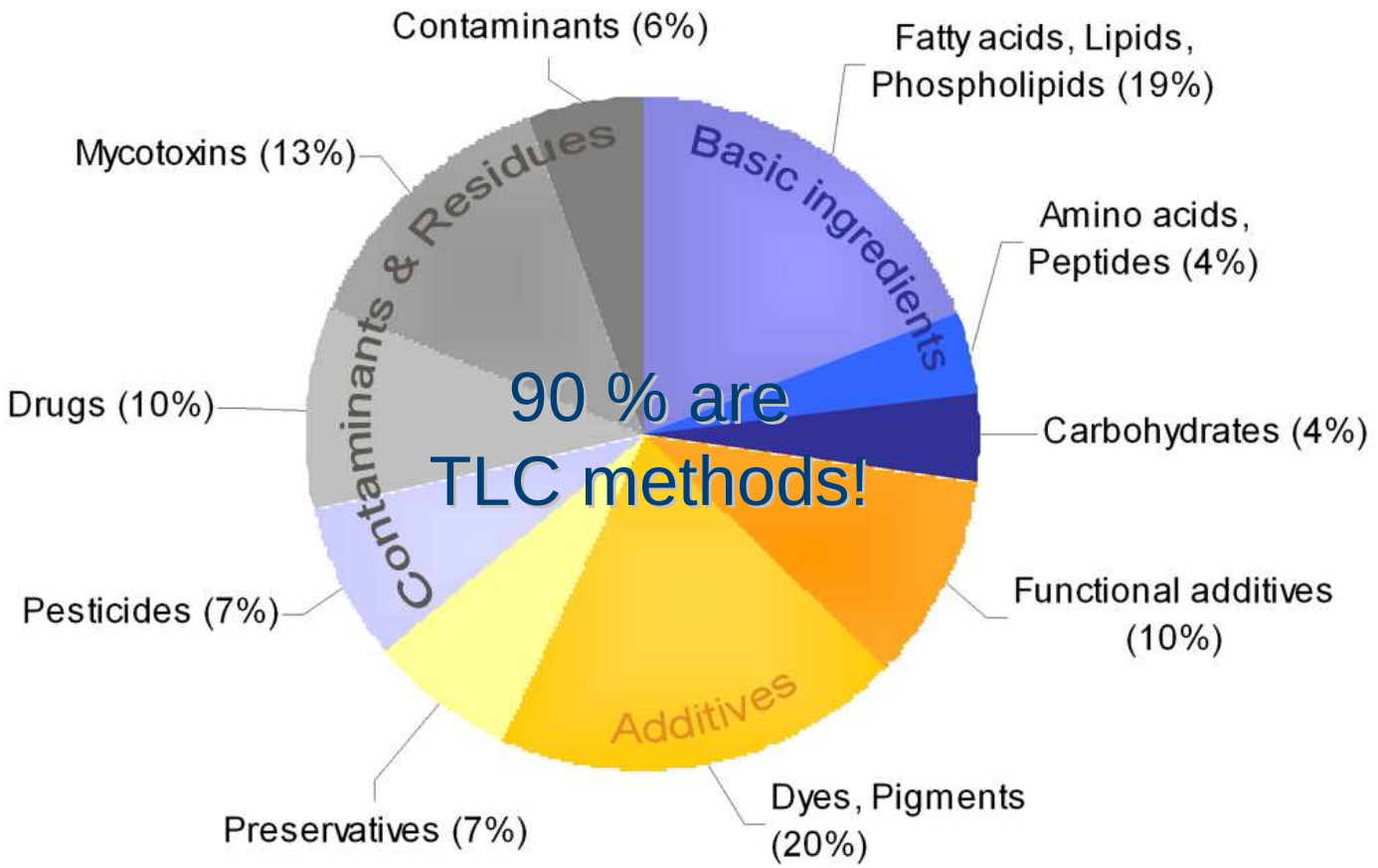
Planar Chromatography

Food analysis 1987-2007



G. Morlock, W. Schwack, J Planar Chromatogr 20 (2007) 399-407

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Many examples for TLC

… because students are just trained in TLC

Analysis in an official German Laboratory in 2009

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Rare examples for HPTLC

Why stop here?

Sample	Dyes found
Bakery ink formulation	122 124
Energy drink 1	133
Energy drink 2	122

Rare examples for HPTLC

From the same plate further data can be obtained:

Sample	Dyes found	Concentration calculated	%RSD (n = 2)	Identity: Spectra correlation (400–800 nm) of standard and sample	Identity: Mass signal(s) (full scan, *m/z* 100 – 900)
Bakery ink formulation	122	66.4 g/L	0.0	≥ 0.99996	228 $[M-2Na]^{2-}$
	124	13.3 g/L	2.1	≥ 0.99957	279 $[M-2Na]^{2-}$
					178 $[M-3Na]^{3-}$
Energy drink 1	133	9.1 mg/L	0.1	≥ 0.99964	373 $[M-2Na]^{2-}$
Energy drink 2	122	76.2 mg/L	3.6	≥ 0.99958	228 $[M-2Na]^{2-}$

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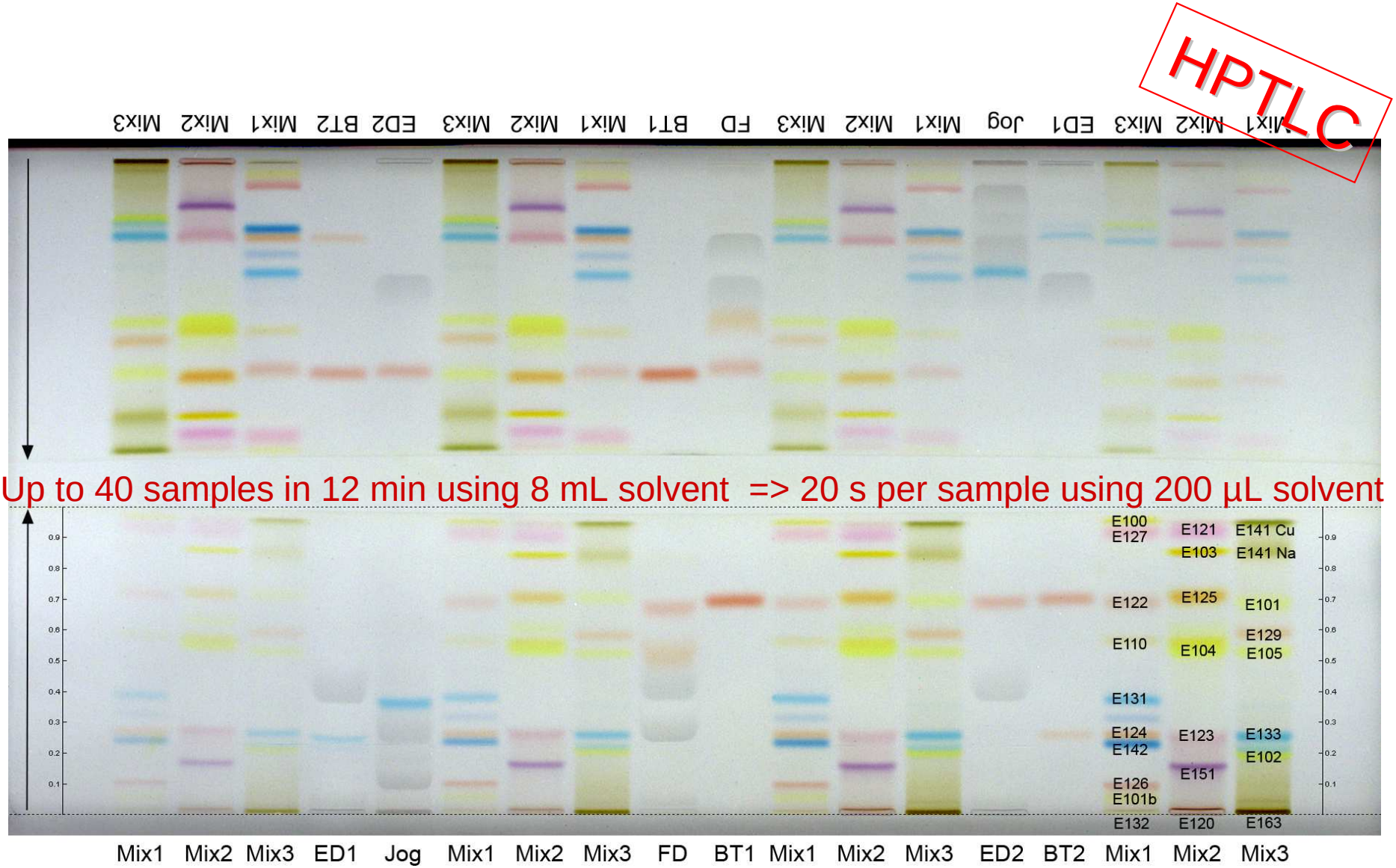
Analysis of water-soluble dyes



G. Morlock, C. Oellig, CBS 103 (2009) 5-9

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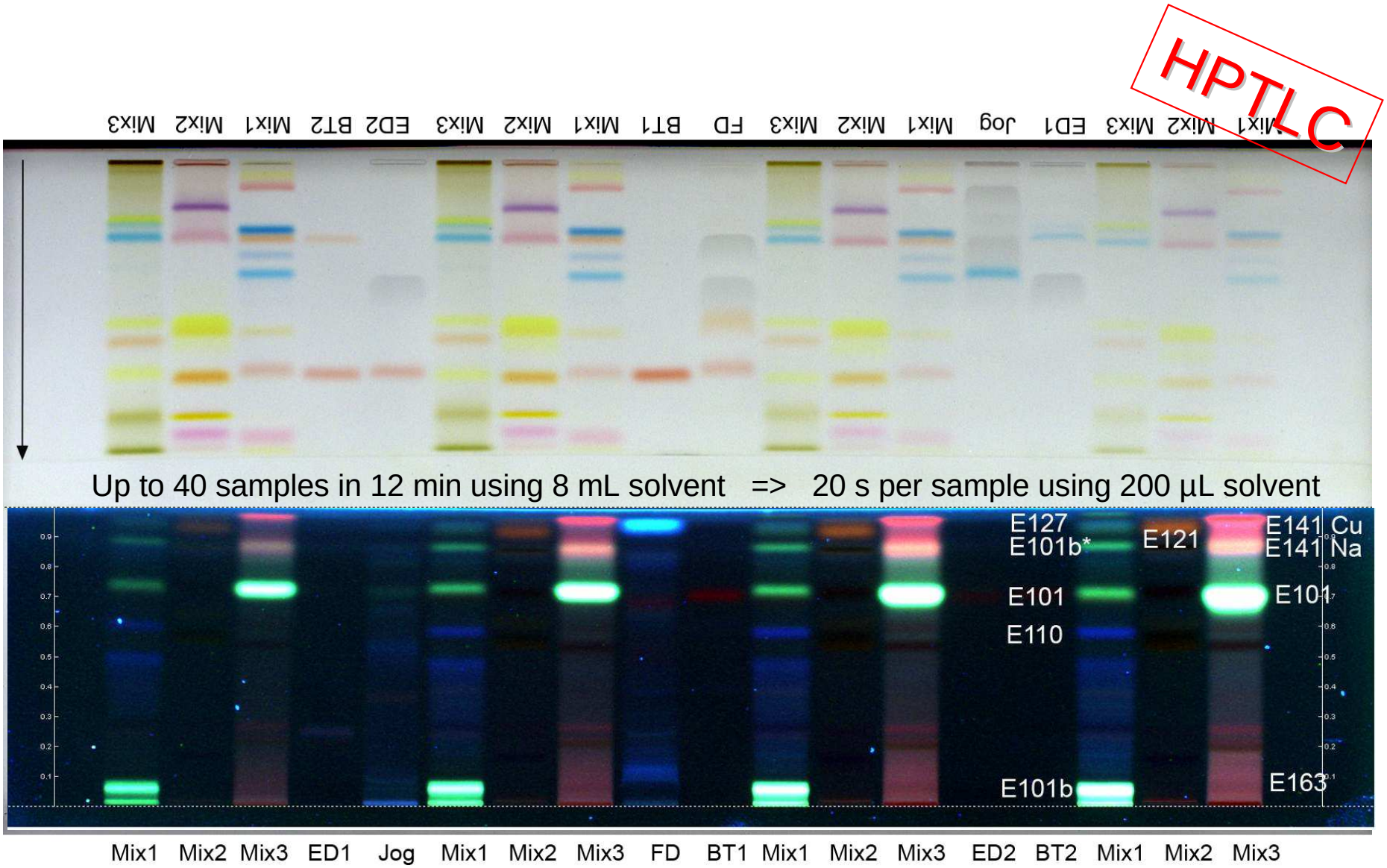
…just another view



G. Morlock, C. Oellig, CBS 103 (2009) 5-9

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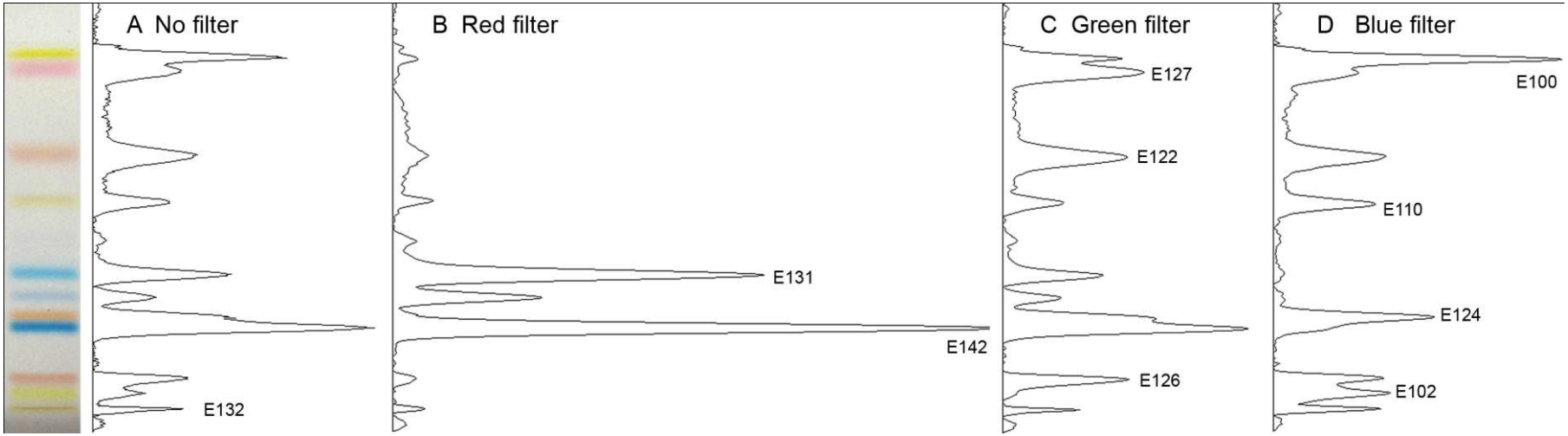
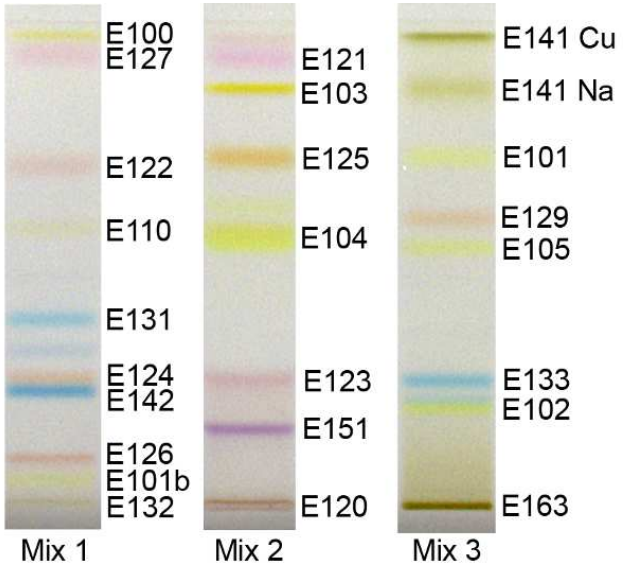
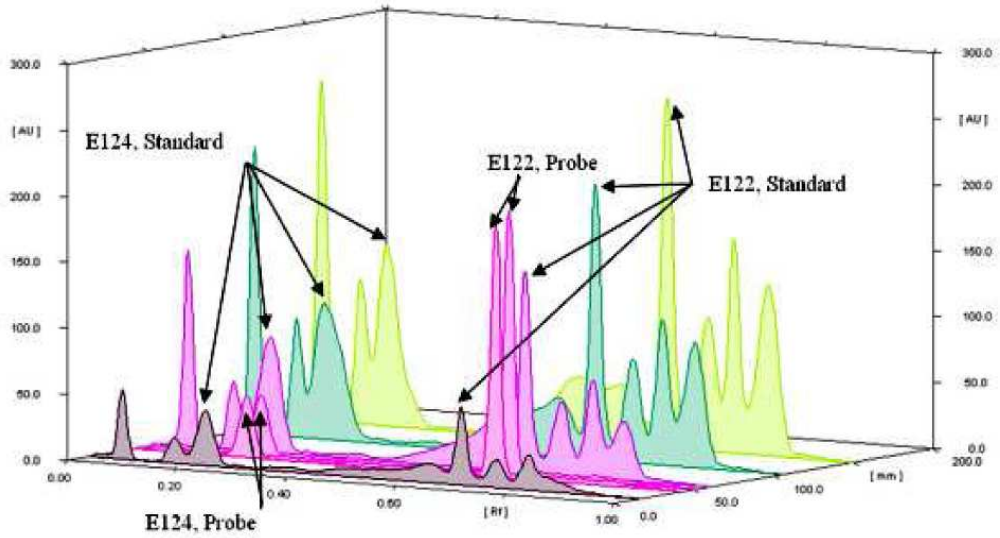
Digital quantification using electronic filters

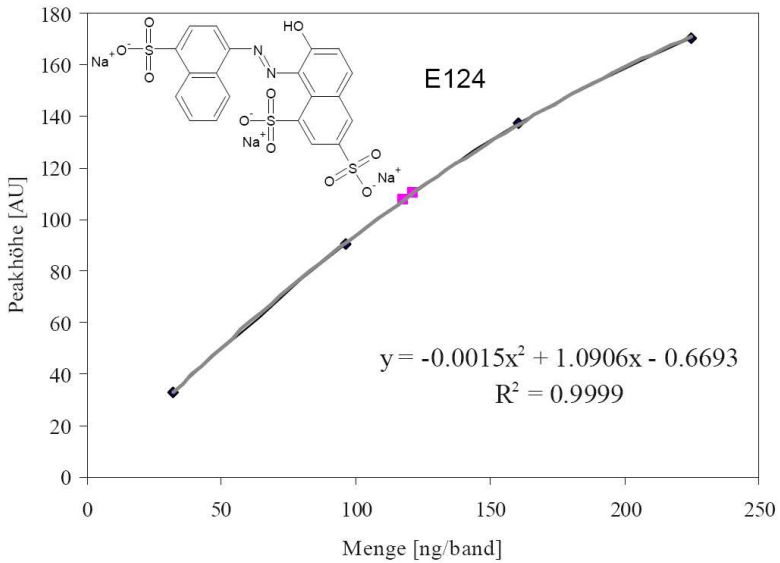


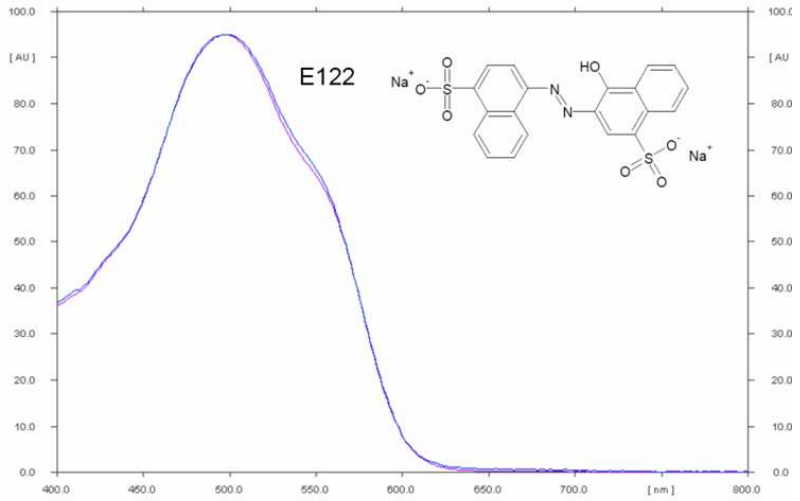
G. Morlock, W. Schwack, Die Aktuelle Wochenschau der GDCh,
Woche 26 (2009), www.aktuelle-wochenschau.de

… confirmation in case of need









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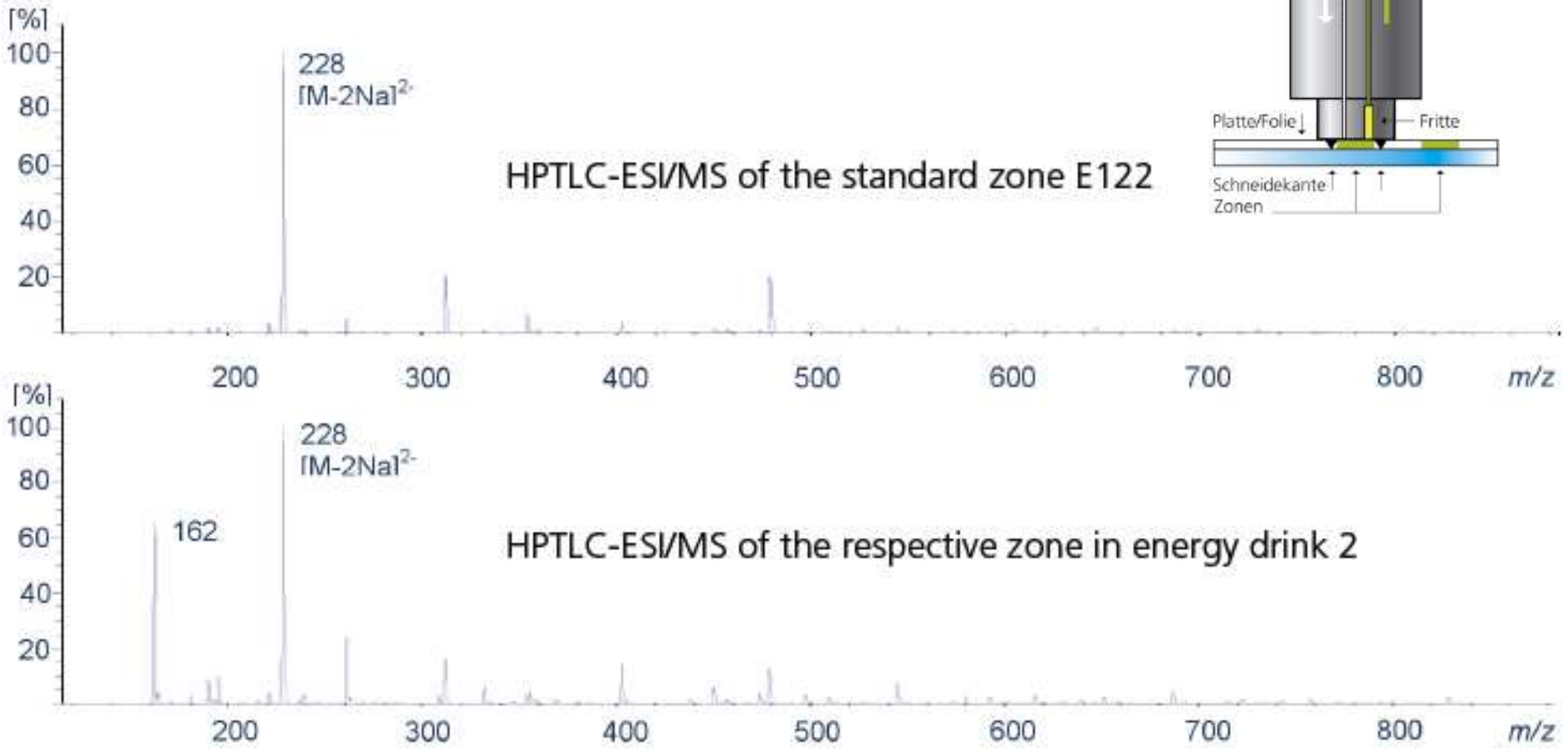
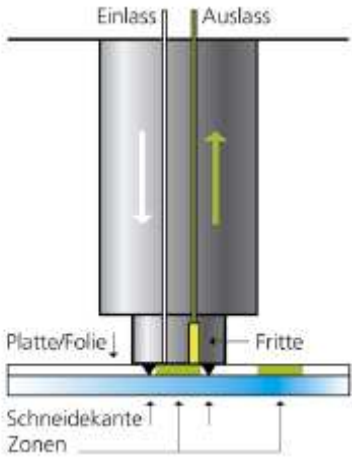
Mass spectra recording





G. Morlock, C. Oellig, J AOAC Int 92 (2009) 547-554

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Cost comparison

[1]K. Minioti et al., Anal Chim Acta 583 (2007) 103–110
[2]G. Morlock, C. Oellig, J AOAC Int 92 (2009) 547-554

Operating costs/run (€)	**HPLC[1]**	**HPTLC[2]**
Mobile phase	0,58	0,003
Stationary phase	0,64	0,11
Disposal	0,04	0,0001
Sum	**1,26**	**0,11**

=> 11 x lower

Time/run (min)	**HPLC**	**HPTLC**
Application/Injection		0,50
Run time	43	0,20
Detection		0,10
Sum	**43**	**0,80**

=> 54 x faster

thereof labor time/40 runs	none	5 min

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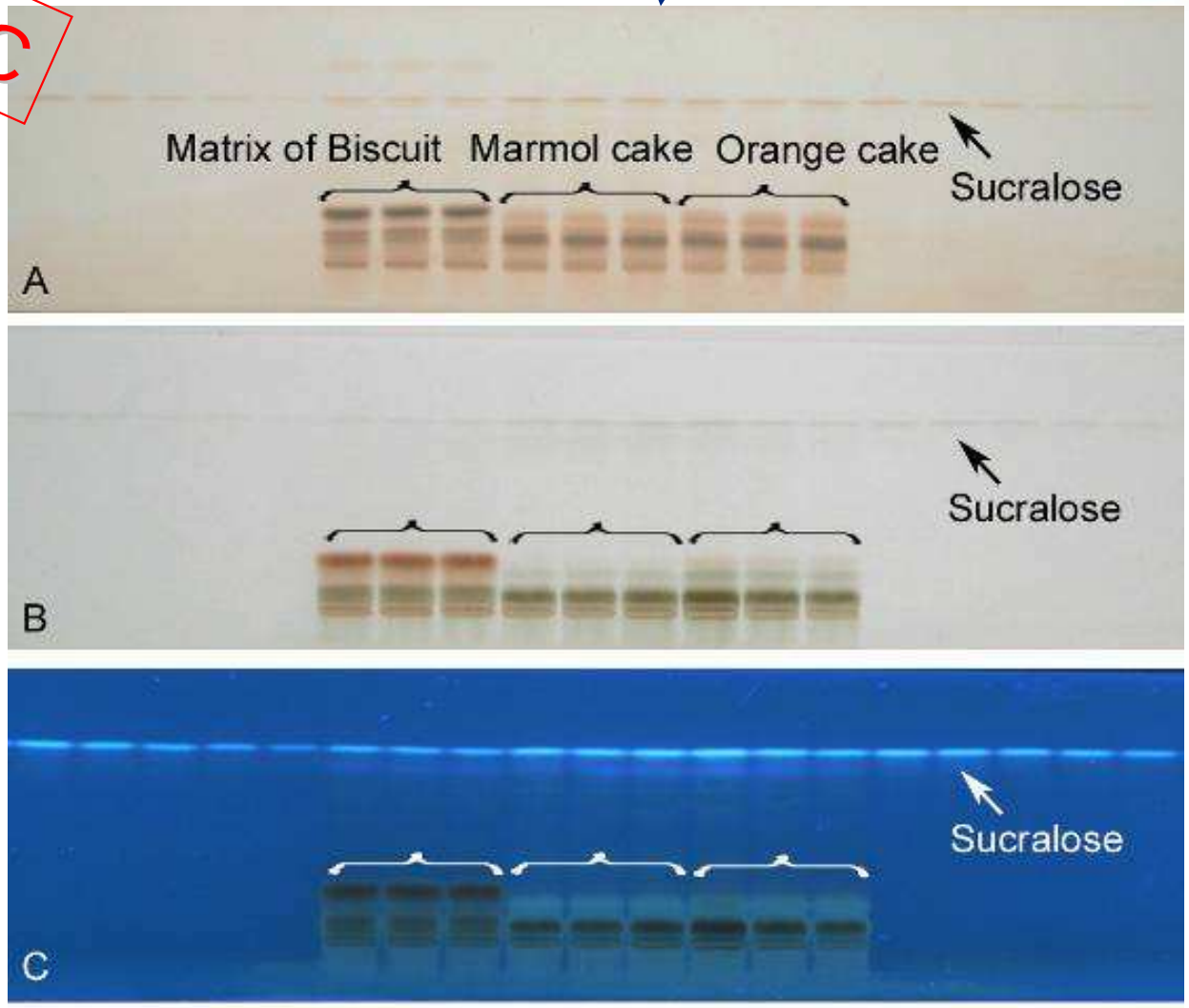
Analysis of sucralose in cakes

extracted
in MeOH, filtered

HPTLC

Highly matrix-tolerant!



G. Morlock, M. Vega, J Planar Chromatogr 20 (2007) 411-417

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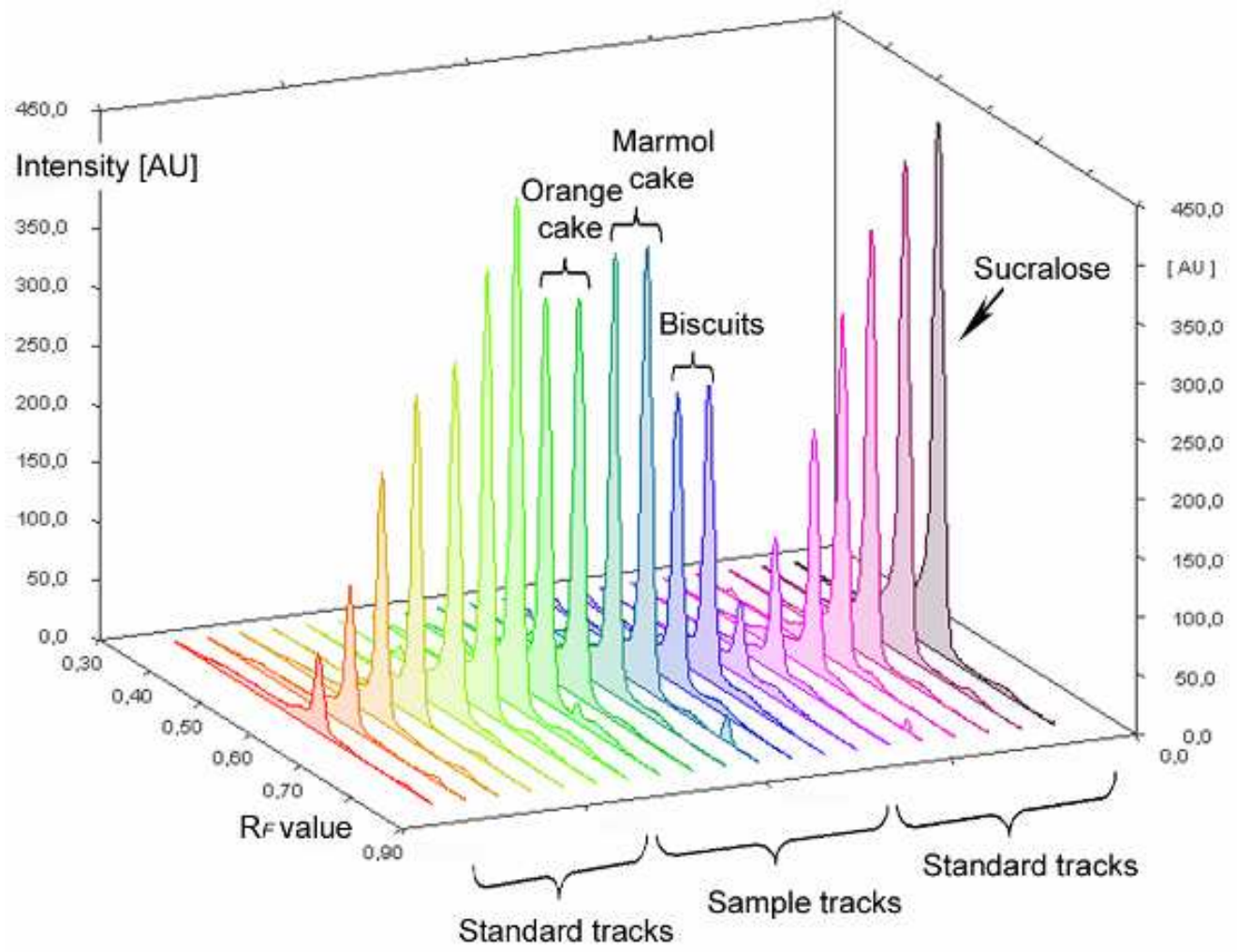
Quantification of sucralose in cakes



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Quantification of sucralose in cakes

Mode A Reagent 1 @ 500 nm				
Samples	hR_F	**Sucralose found (mg/100 g)**	*%RSD* *n* = 3	Sucralose labeled (mg/100g)
Biscuits	57	**27.7**	2.4	24.8
Marmol cake	57	**48.0**	2.0	45.3
Orange cake	56	**43.9**	0.6	45.3
Mode B Reagent 2 @ 405 nm				
Biscuits	56	**27.9**	1.5	24.8
Marmol cake	56	**47.4**	0.5	45.3
Orange cake	56	**44.2**	1.6	45.3
Mode C Reagent 2 @ UV 366/>400 nm				
Biscuits	56	**27.1**	0.9	24.8
Marmol cake	57	**44.8**	4.2	45.3
Orange cake	56	**41.6**	3.0	45.3

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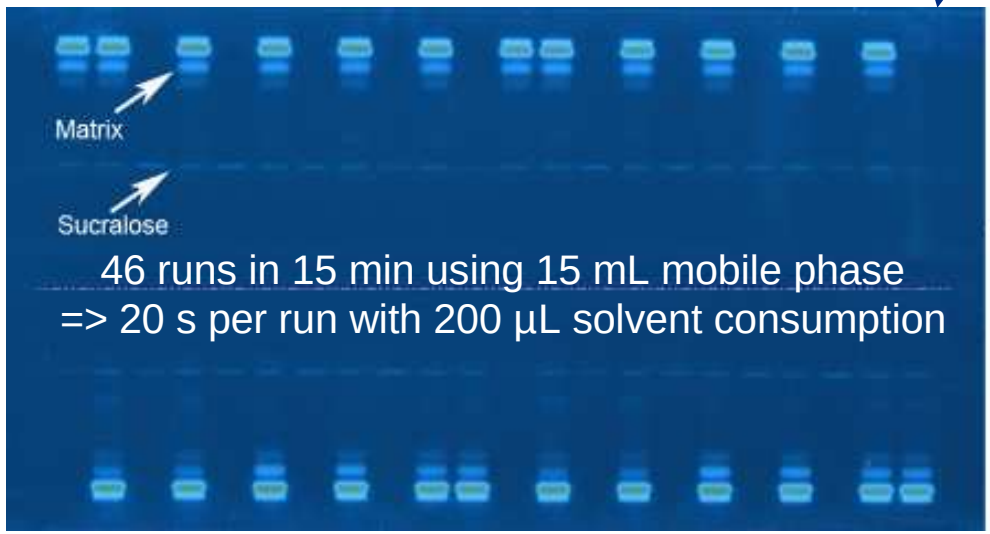
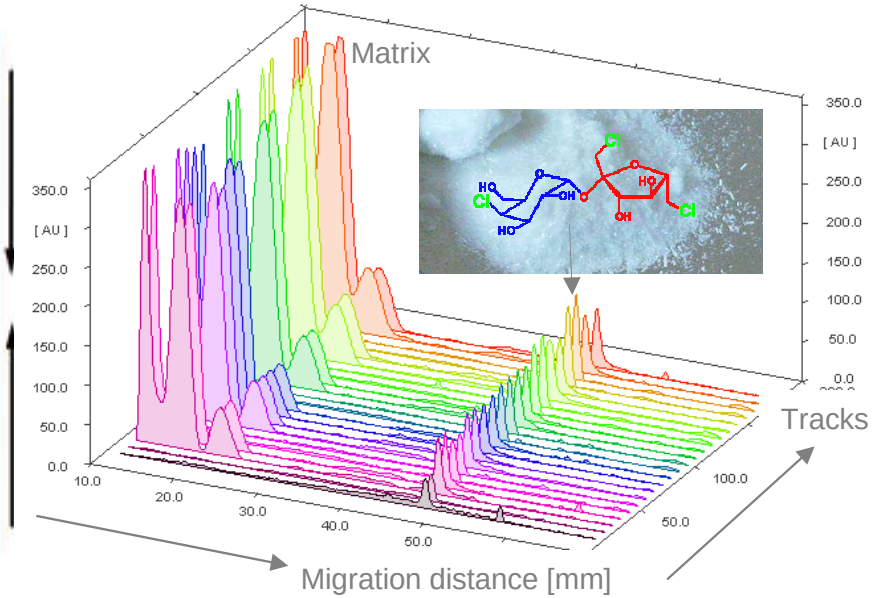
… in milk-based confection (Burfi)

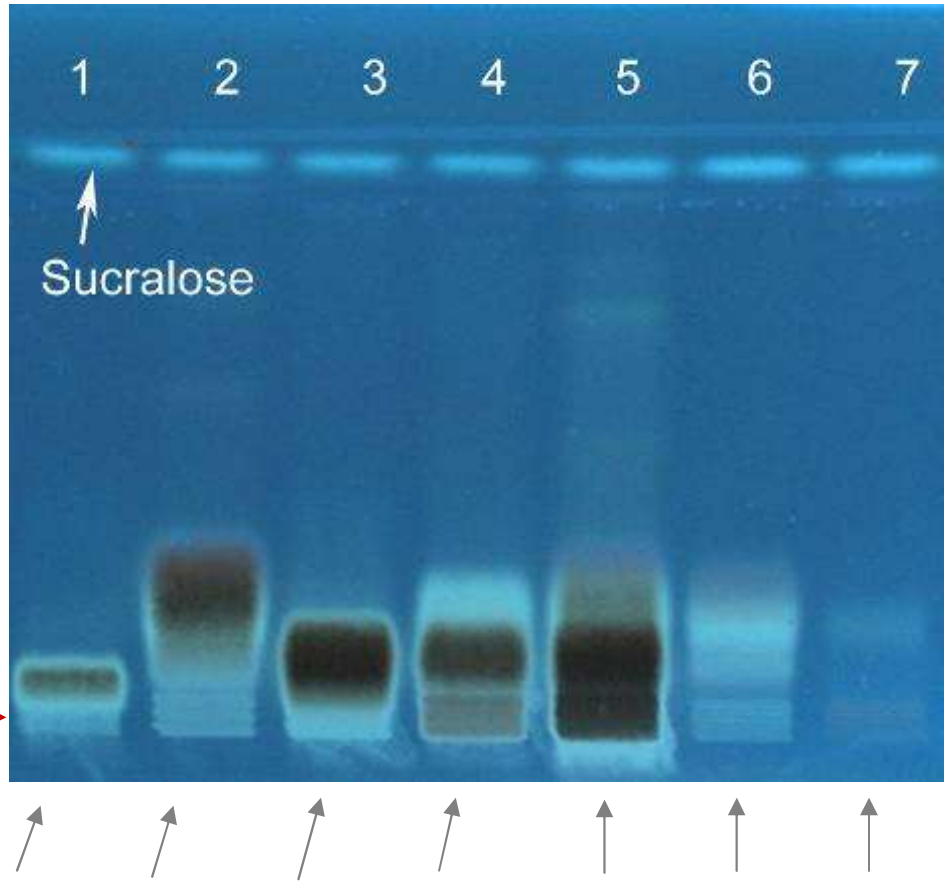
extracted in MeOH, filtered





G. Morlock, S. Prabha, J Agric Food Chem 55 (2007) 7217-7223

Sample preparation and chromatography



Area application!

Milk, biscuit, chocolate, cola, bonbons, energy/sport drinks

G. Morlock, S. Prabha, J Agric Food Chem 55 (2007) 7217-7223

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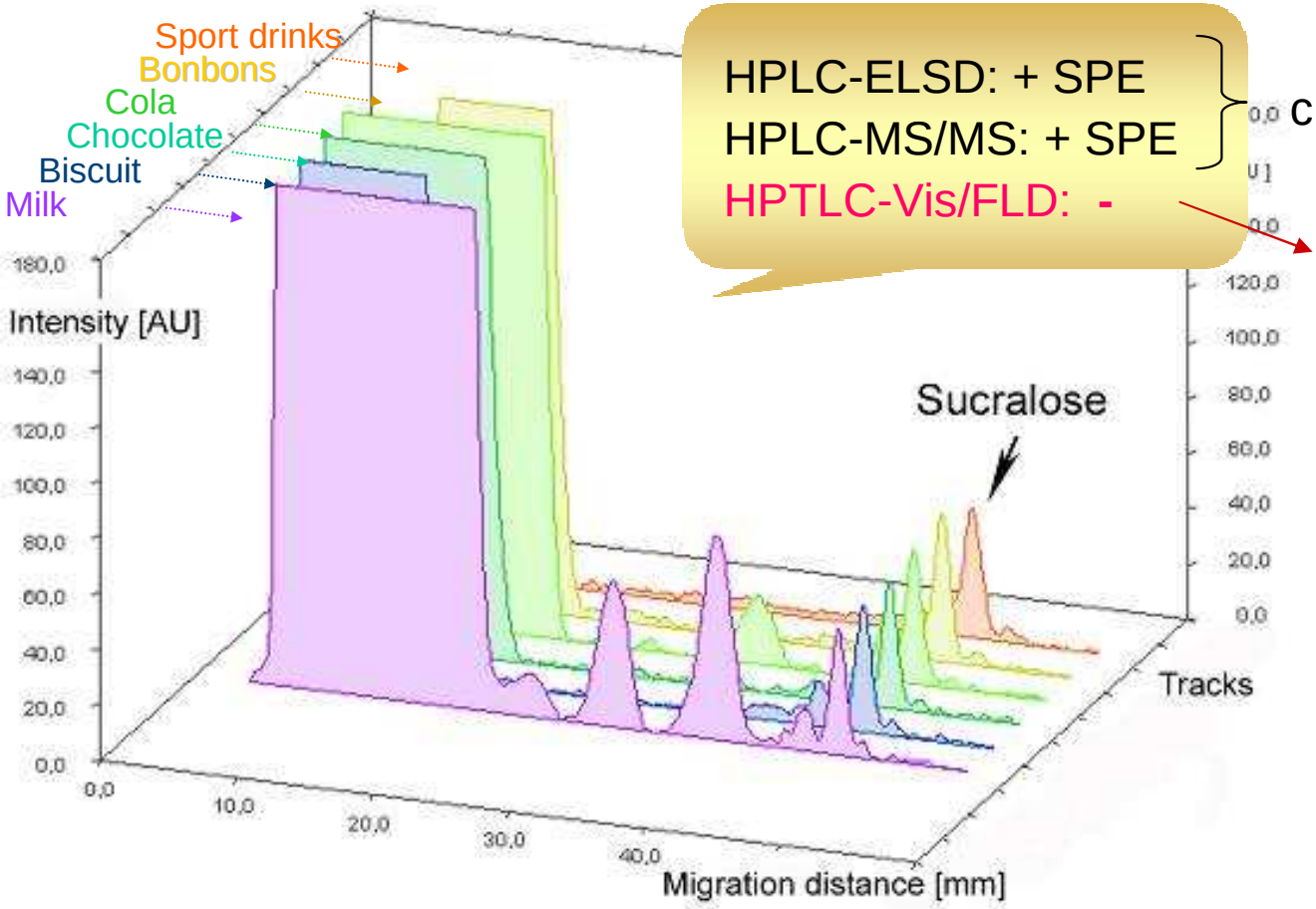
Sample preparation and chromatography



HPLC-ELSD: + SPE
HPLC-MS/MS: + SPE
HPTLC-Vis/FLD: -

Both options choose analysts of today!

Why not HPTLC?

… as they are not trained!

G. Morlock, M. Vega, J Planar Chromatogr 20 (2007) 411-417

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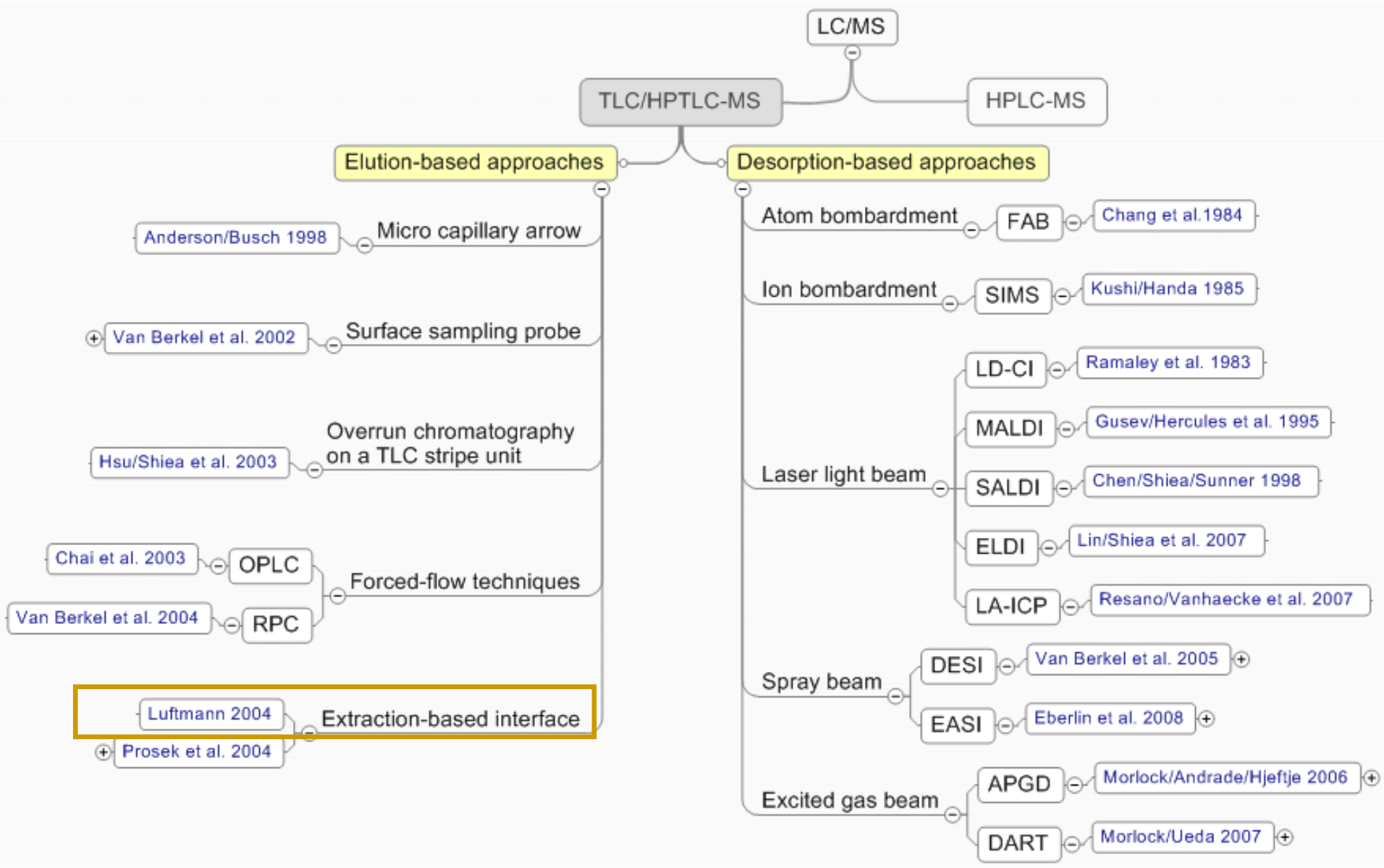
Hyphenation HPTLC-MS



G. Morlock, W. Schwack, TrAC, in submission

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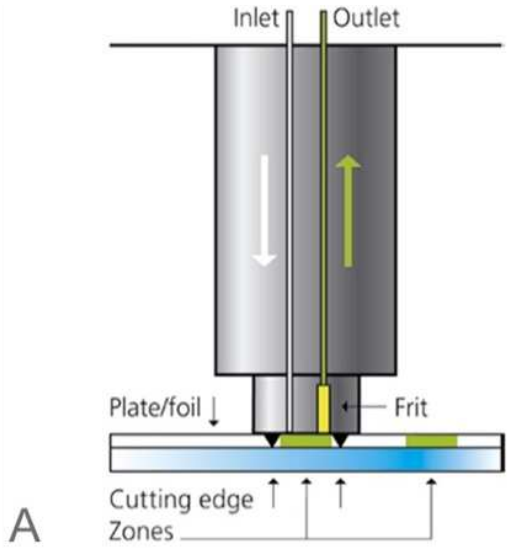
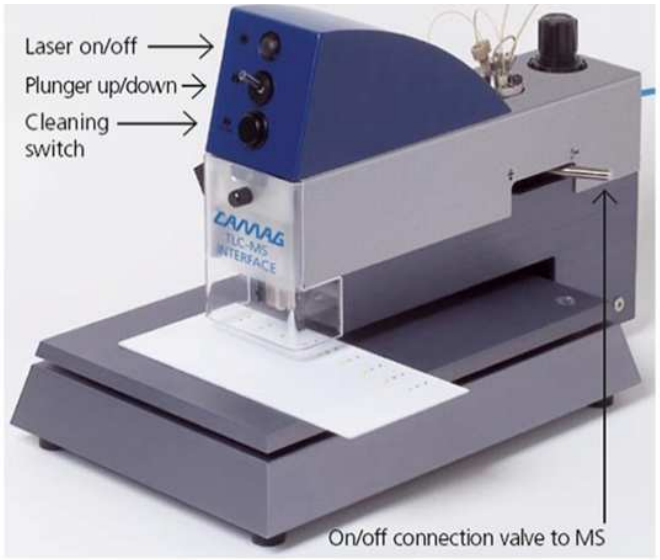
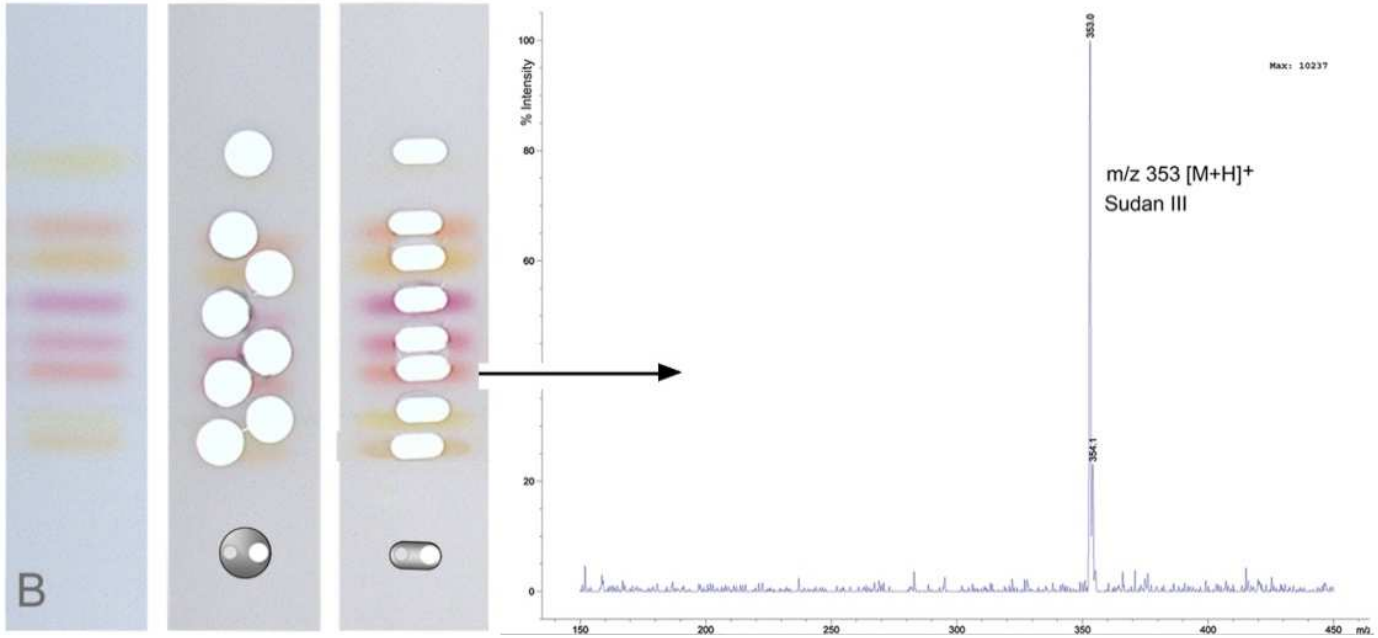
Elution head-based HPTLC-MS







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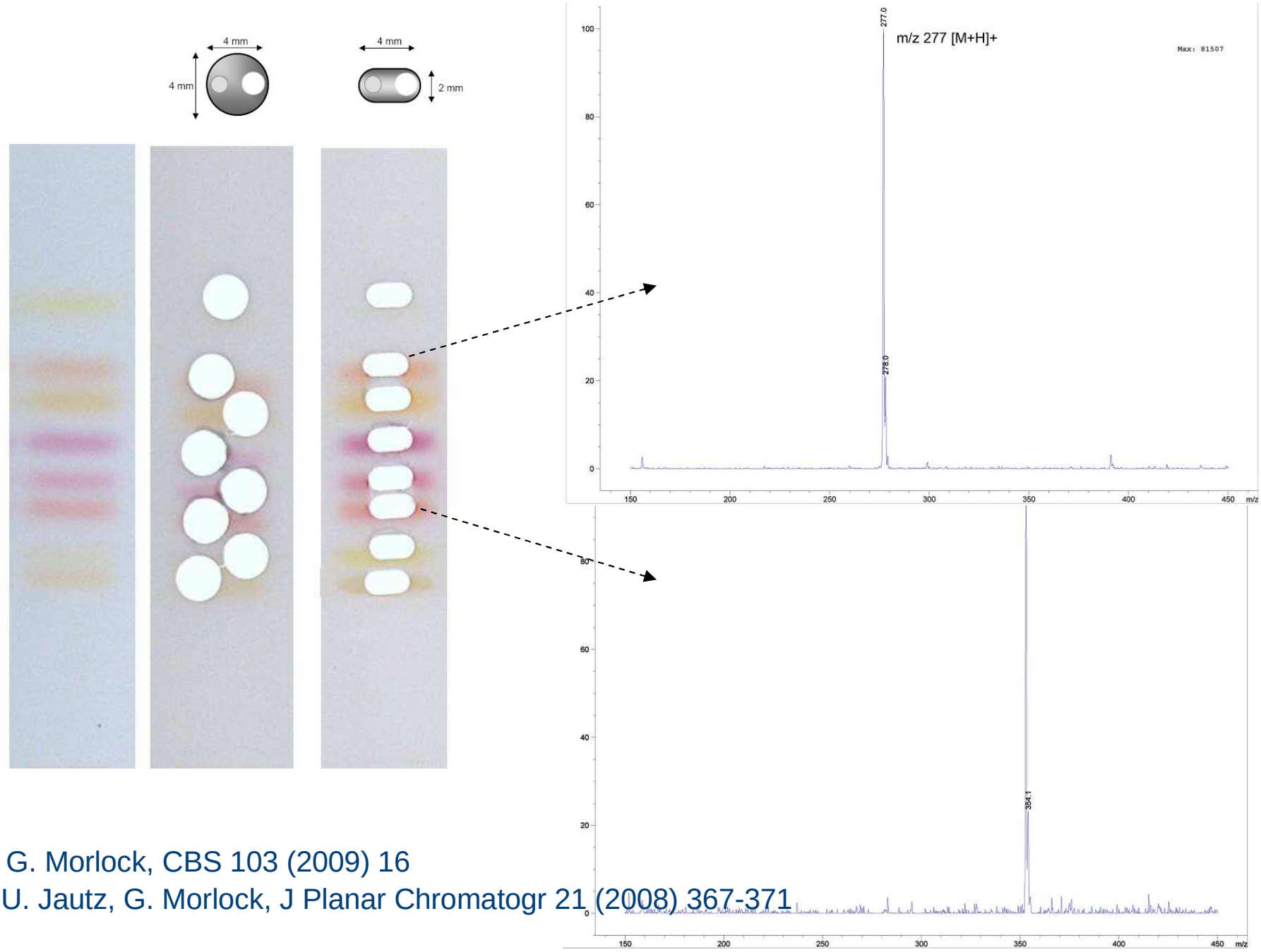
Elution head geometries



G. Morlock, CBS 103 (2009) 16
U. Jautz, G. Morlock, J Planar Chromatogr 21 (2008) 367-371

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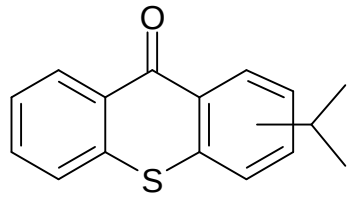
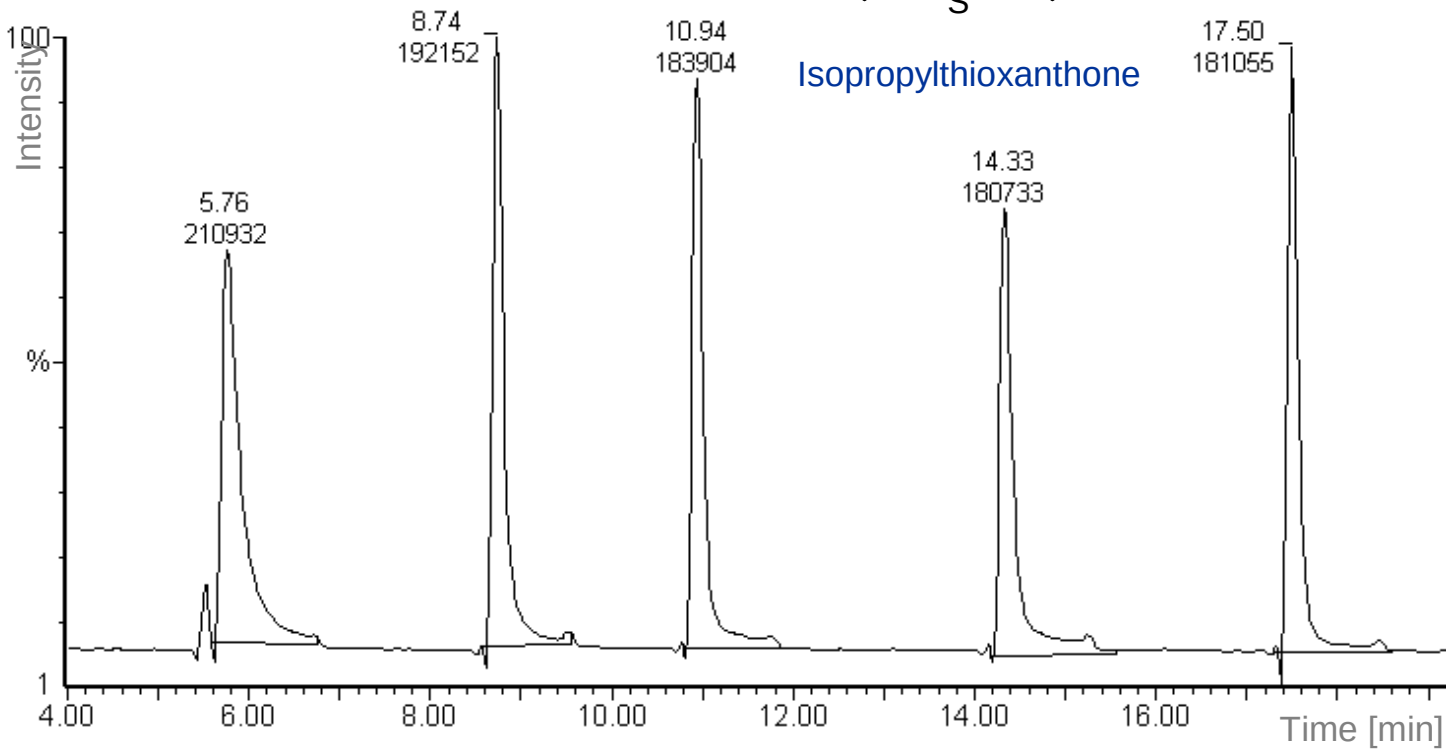
Repeatability of extraction

SIM elution profiles at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$

Repeatability (*%RSD, 6 ng/band, n* = 5): 6.7 %



Isopropylthioxanthone



G. Morlock, W. Schwack, Anal Bioanal Chem 385 (2006) 586-595

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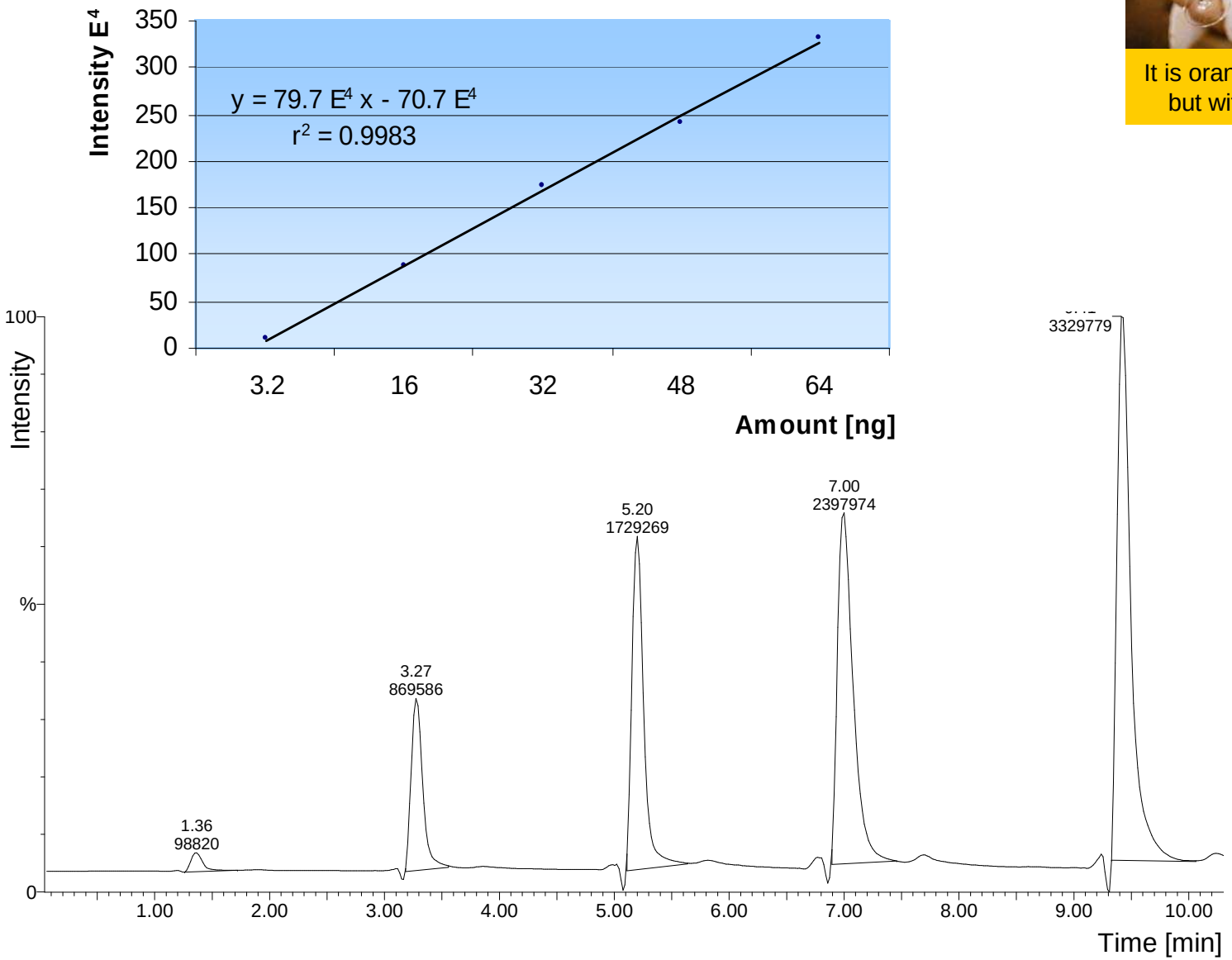
Analytical response

SIM elution profiles at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$

It is orange juice, but with ITX.



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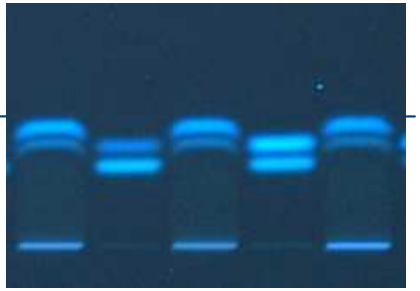
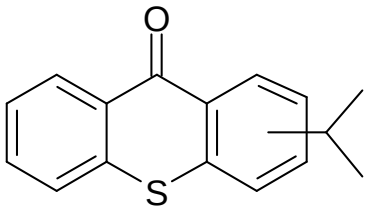
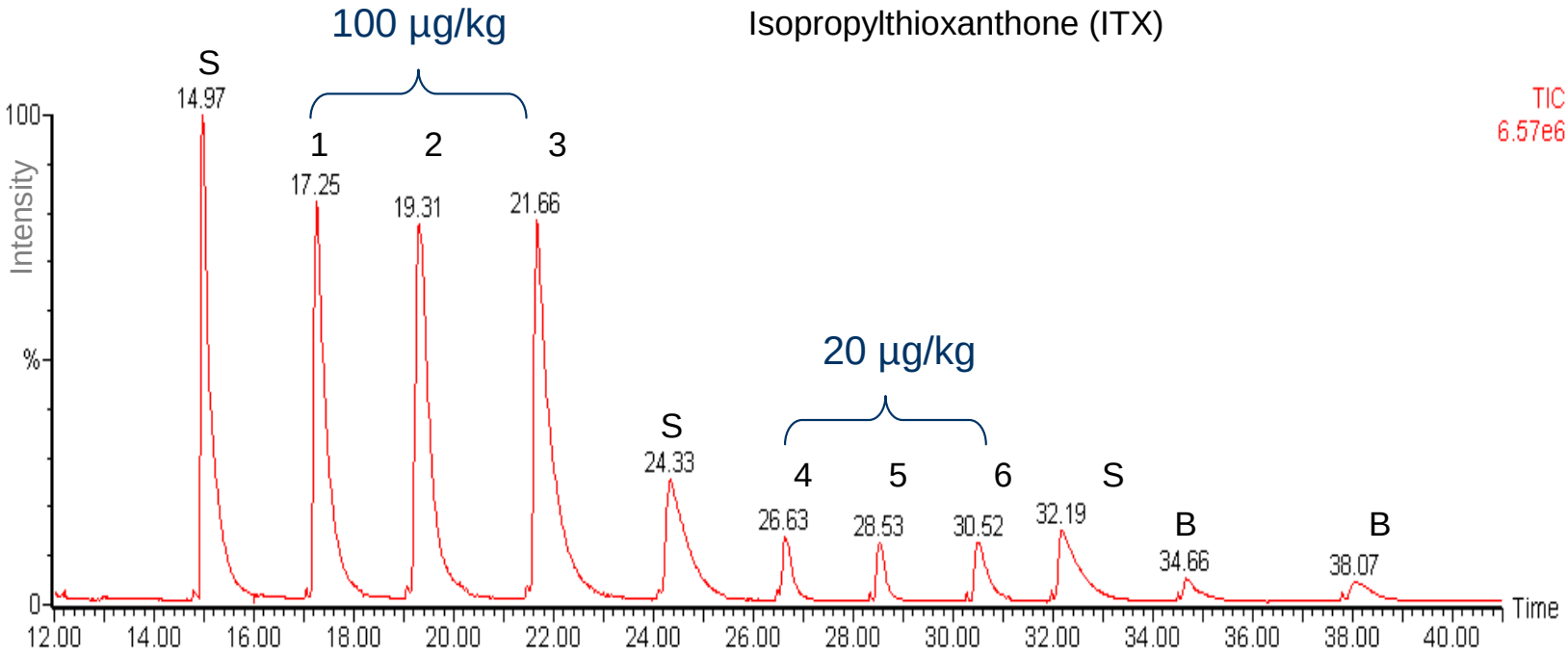
Confirmation by HPTLC/ESI-MS



TIC elution profiles of yoghurt samples spiked with ITX



Isopropylthioxanthone (ITX)



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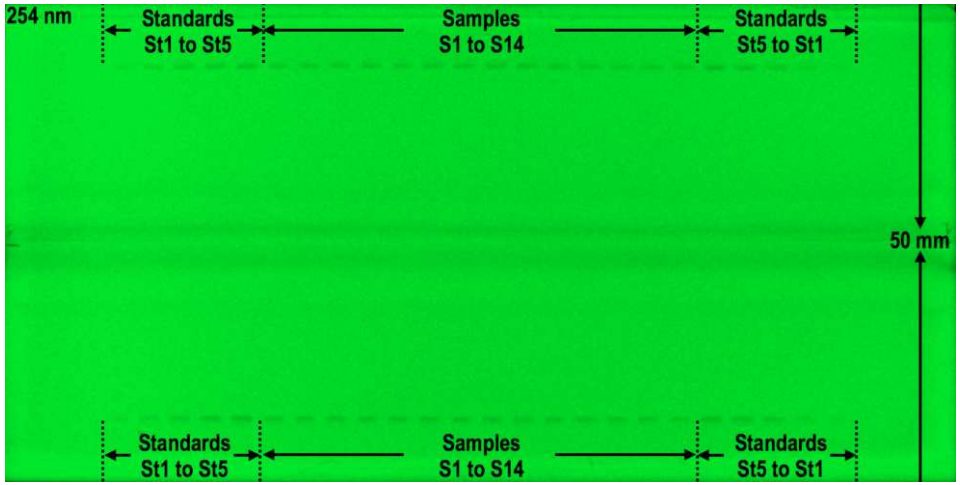
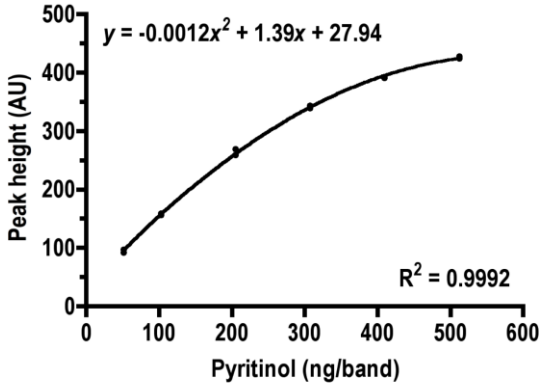
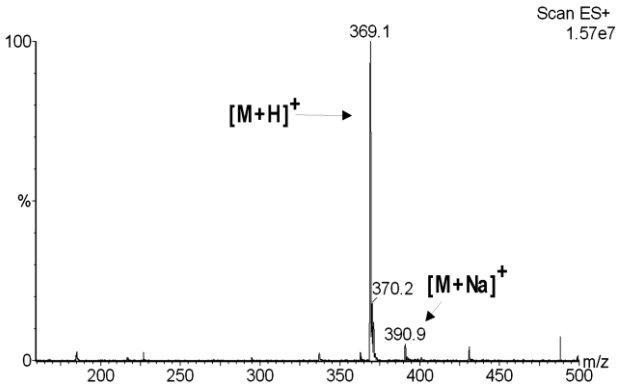
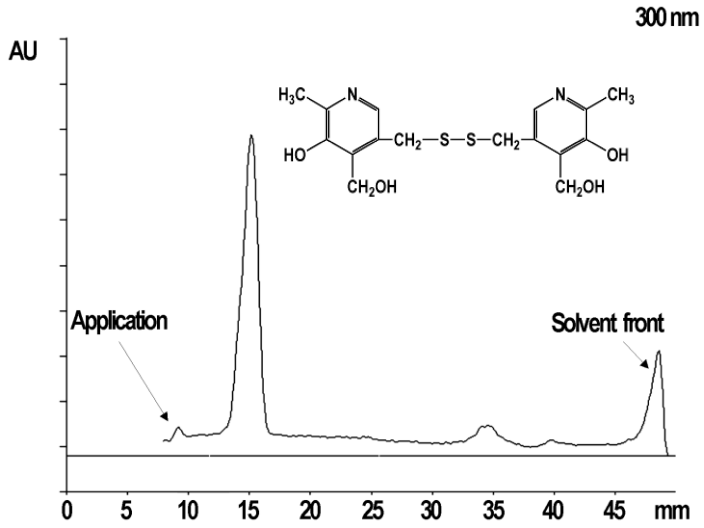
…no need for a higher separation power!

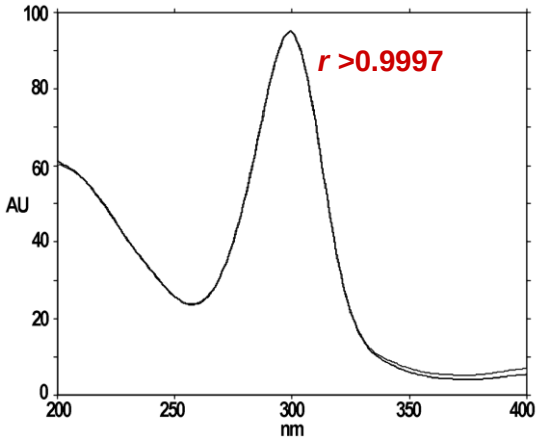
Pyridinol quantification in solid formulations











M. Aranda, G. Morlock, J. Liq. Chromatogr. Relat. Technol. 33 (2010) in print

…no need for a higher separation power!

Pyridinol quantification in solid formulations

→ Repeatability (*%RSD,* n = 6) in matrix: 0.4 %

→ Intermediate precision (*%RSD,* n = 3) in matrix: 2.95 %

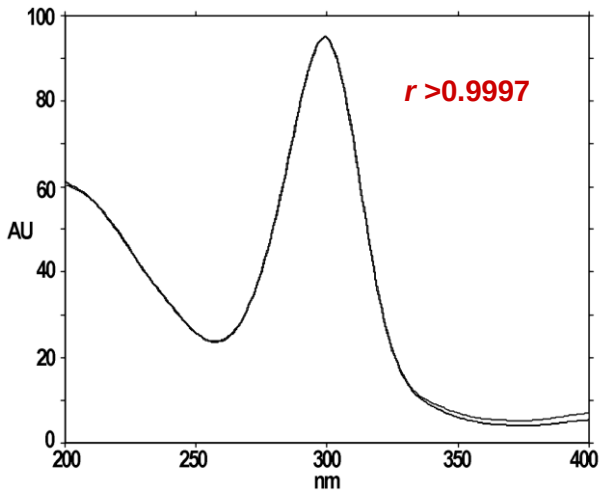
→ Recoveries of spiked samples (three levels): 98.5 - 101.9 % (± 3.6 - 4.7%)

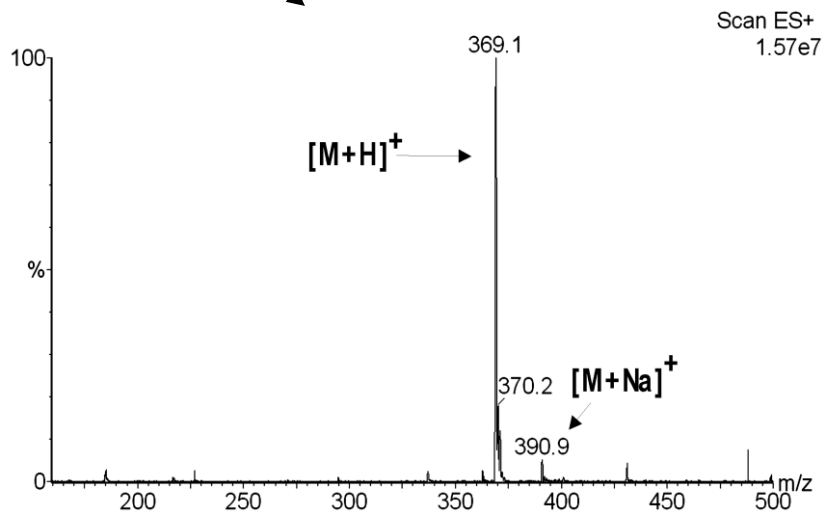
→ LOD/LOQ: 0.6/2.0 µg/mL (6/20 ng/band)

→ Up to 17 times less mobile phase consumption

→ At least 2 times faster (calculated for worst case: 10 x 10 cm plate, one side)

→ Selectivity proved by spectra purity and MS





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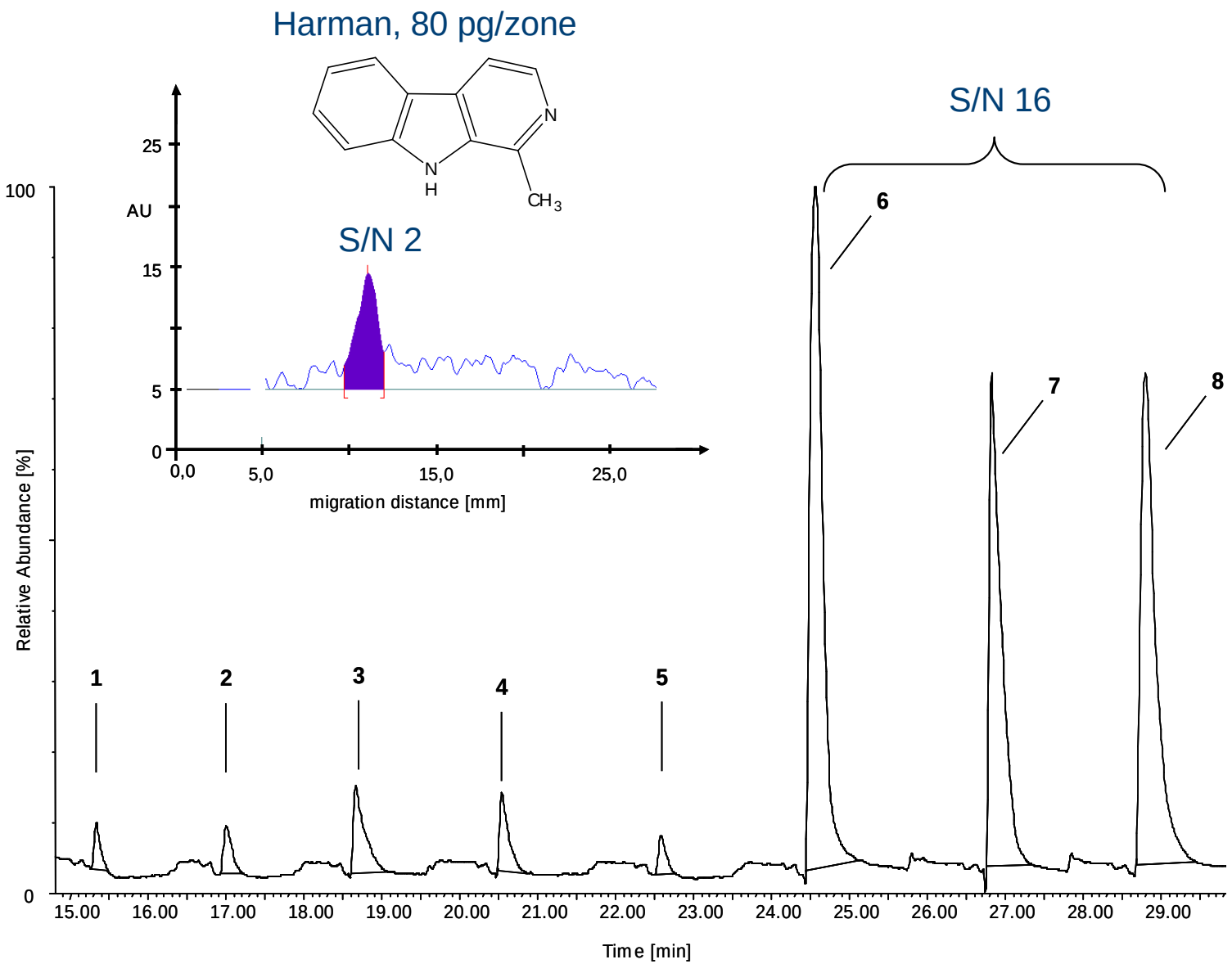
Detectability: FLD versus MSD



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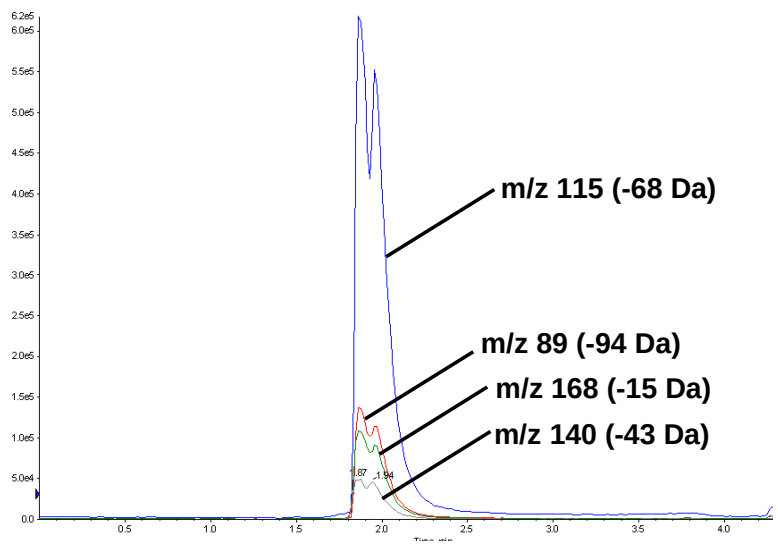
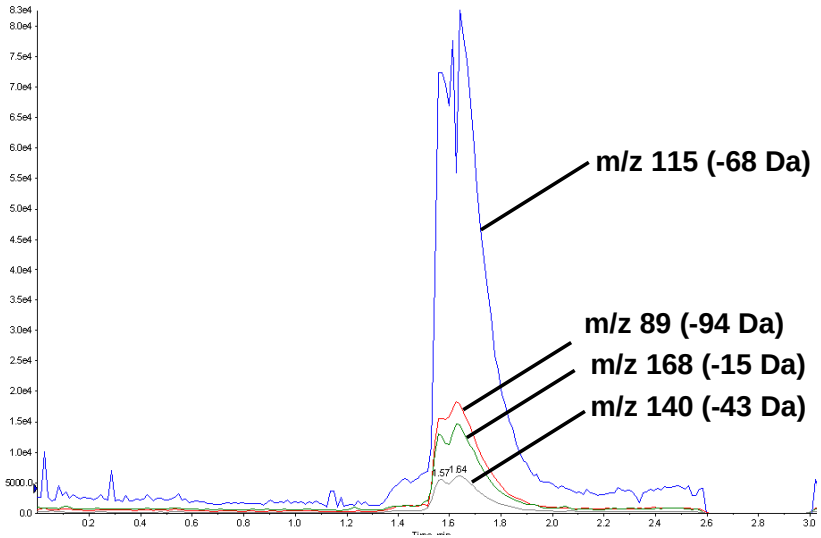
Detectability by HPTLC/ESI-MS-MS

→ LOQ better than 20 pg/zone Harman (S/N 20)

→ Detectability comparable to HPLC/MS





200 pg/zone Harman → 20 pg/zone Harman

U. Jautz, G. Morlock, J Chromatogr A 58 (2006) 244-250

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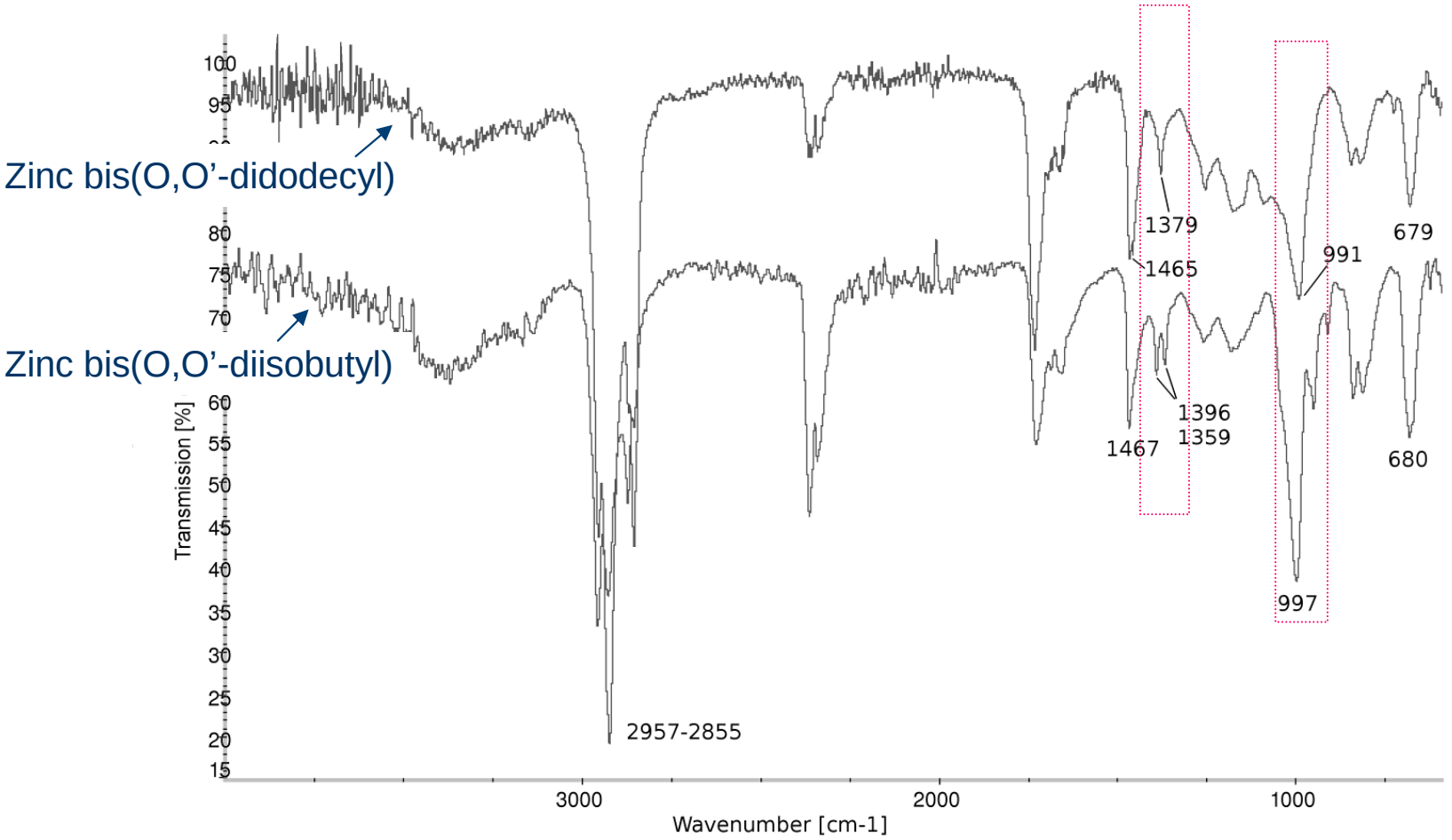
HPTLC/ATR-IR spectra via the interface

Dithiophosphate additives in mineral oil



E. Dytkiewitz, G. Morlock, J AOAC Int 91 (2008) 1237-1244

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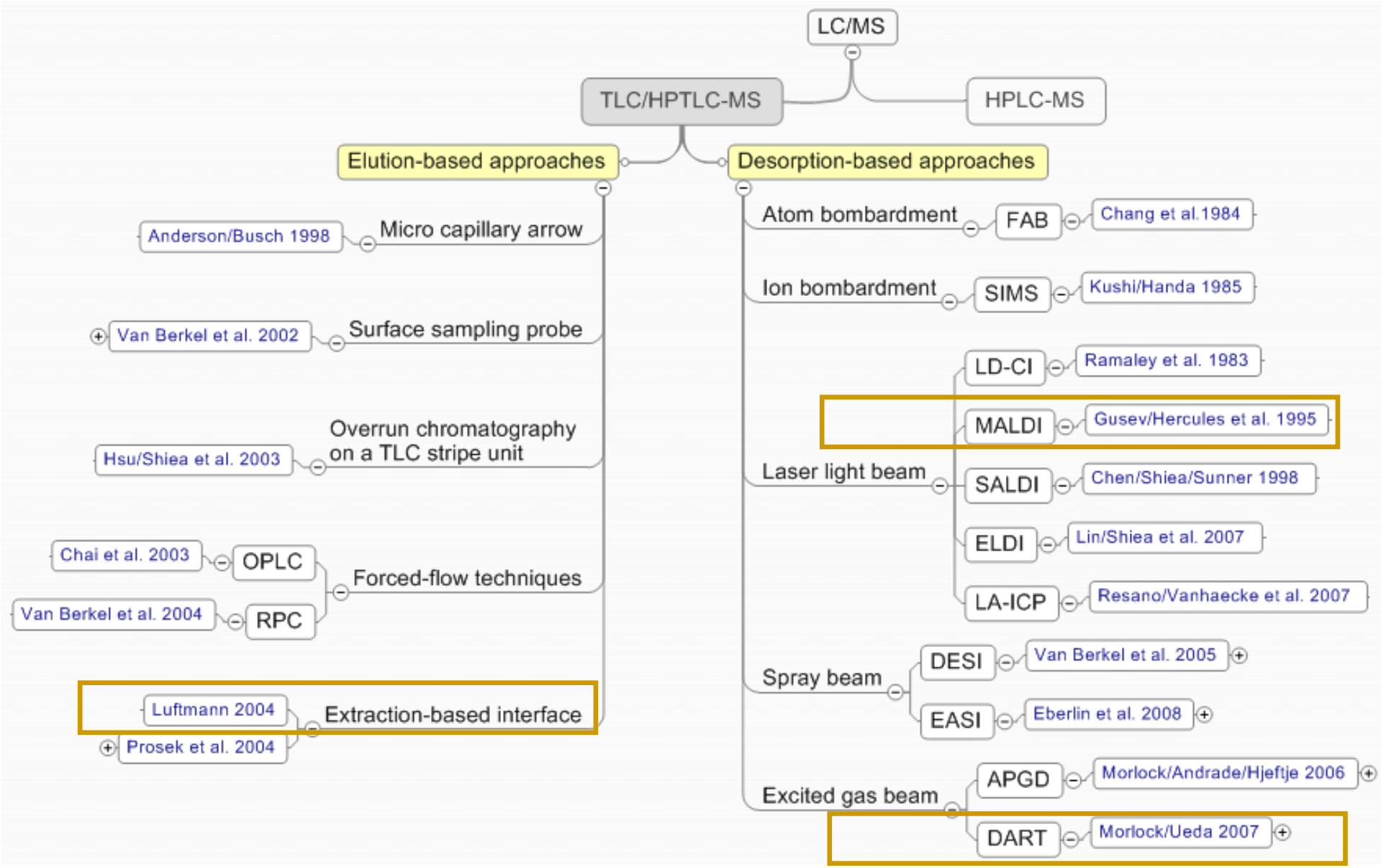
HPTLC-MS



G. Morlock, W. Schwack, TrAC, in submission

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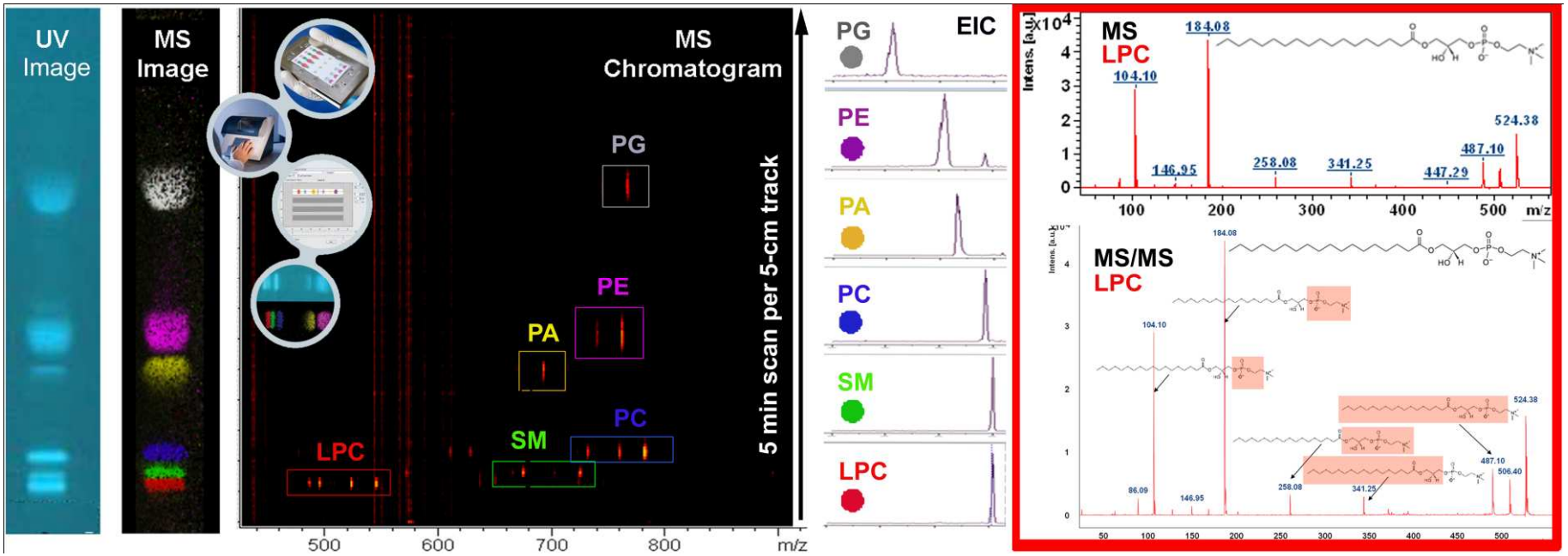
HPTLC-FLD-MALDI-TOF MS



IMSC 2009 Bremen, Poster PMM 386
→ Bruker Daltonics Application Note MT-101

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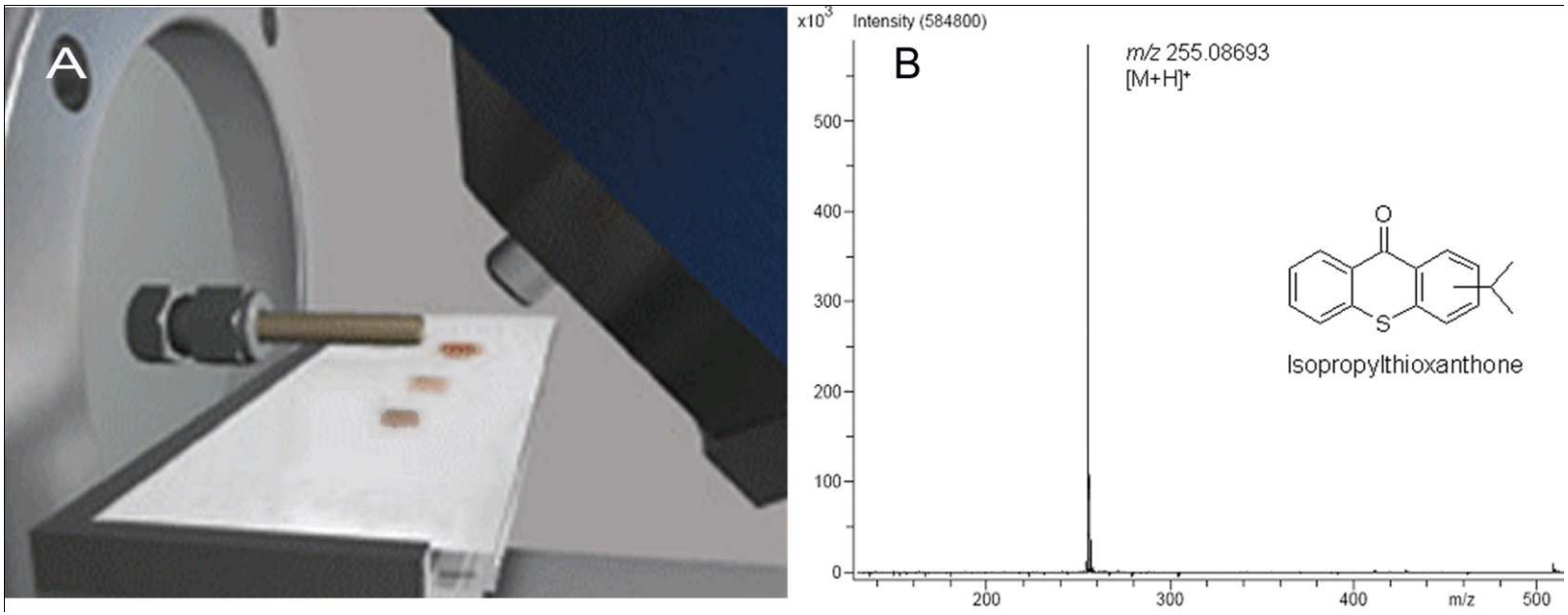
HPTLC-DART-ET-MS



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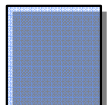
Future analysis

More than 30 million compounds registered in Chemical Abstracts

100 000 chemicals in daily use

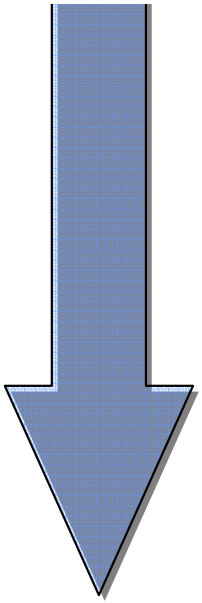
M.J.F. Suter, Anal Bioanal Chem 390 (2008) 1957



So far: target analysis

TOF better suited as MS/MS?

More information for unknowns by HPTLC?



We have to widen the focus!

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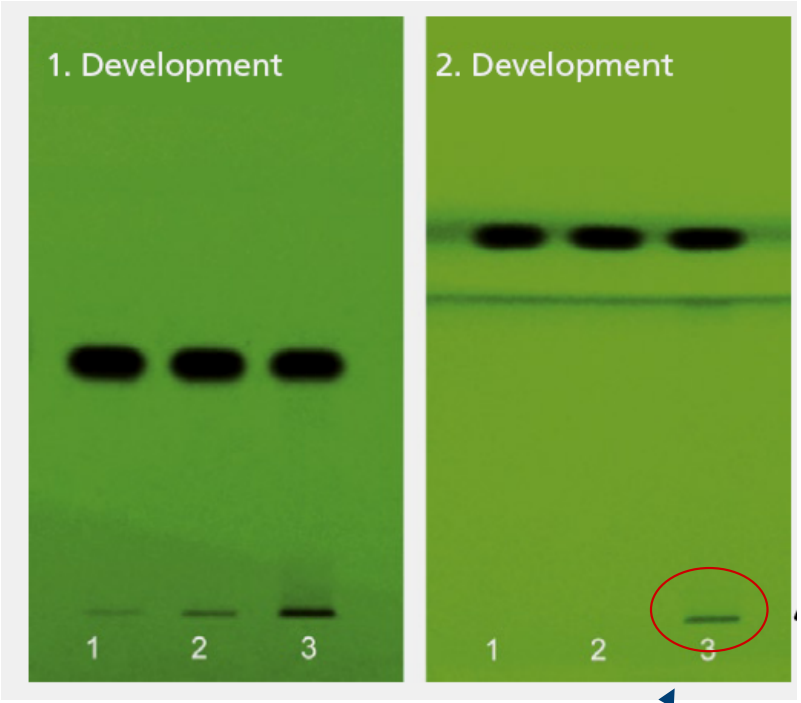
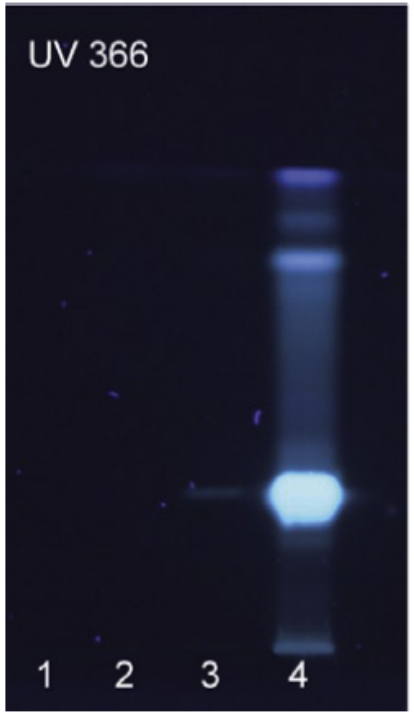
What´s left at the origin of the adsorbent?



Mass imbalance caused by
new degradation product



New yellow impurity caused
color difference in API batches

C. McKinlay, CBS 101 (2008) 12-13

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Future analysis

More than 30 million compounds registered in Chemical Abstracts

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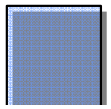
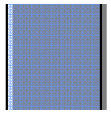
M.J.F. Suter, Anal Bioanal Chem 390 (2008) 1957



So far: target analysis

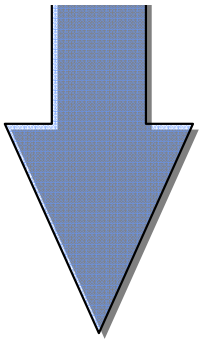
TOF better suited as MS/MS?

More information for unknowns by HPTLC?



Effect-directed analysis by

HPTLC better suited than by HPLC?



We have to widen the focus!

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Effect-directed or bioactivity-based detection

You detect everything what generates the effect:
Unknown contaminants, breakdown products, degradation products, metabolism products and compounds not in focus so far!

This is more efficient than any multi-method can be.

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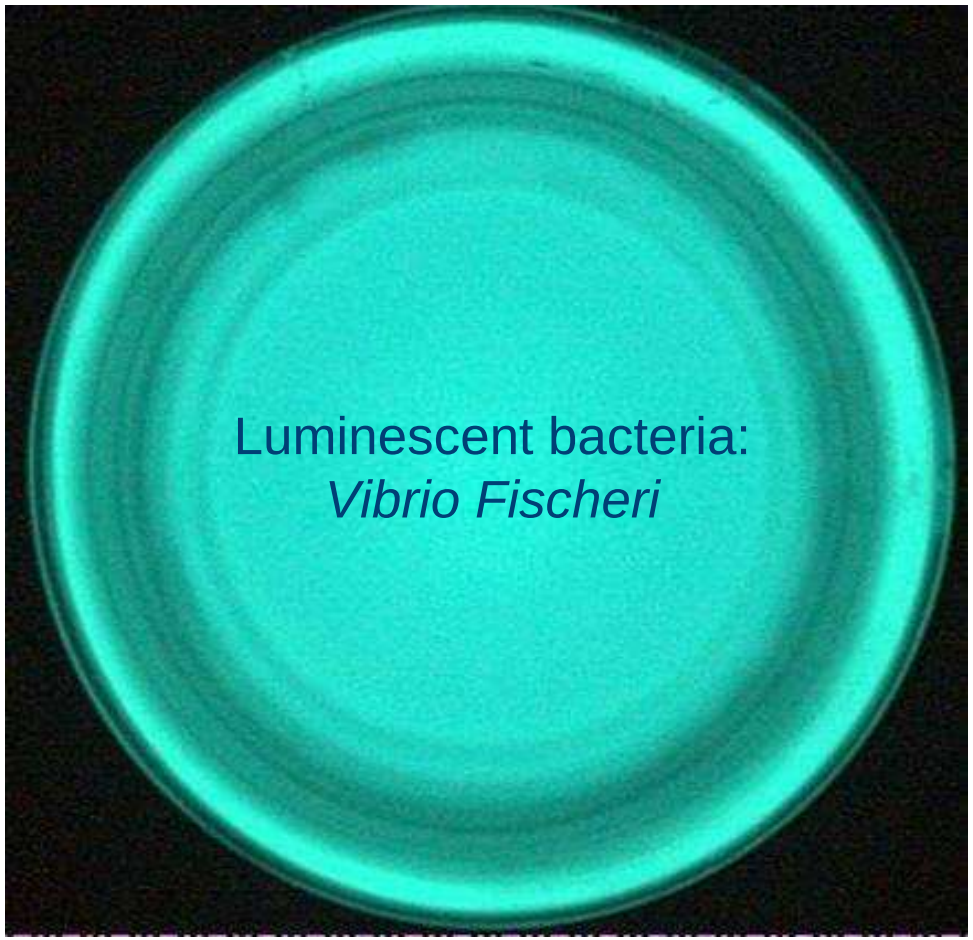
Bioactivity-based detection



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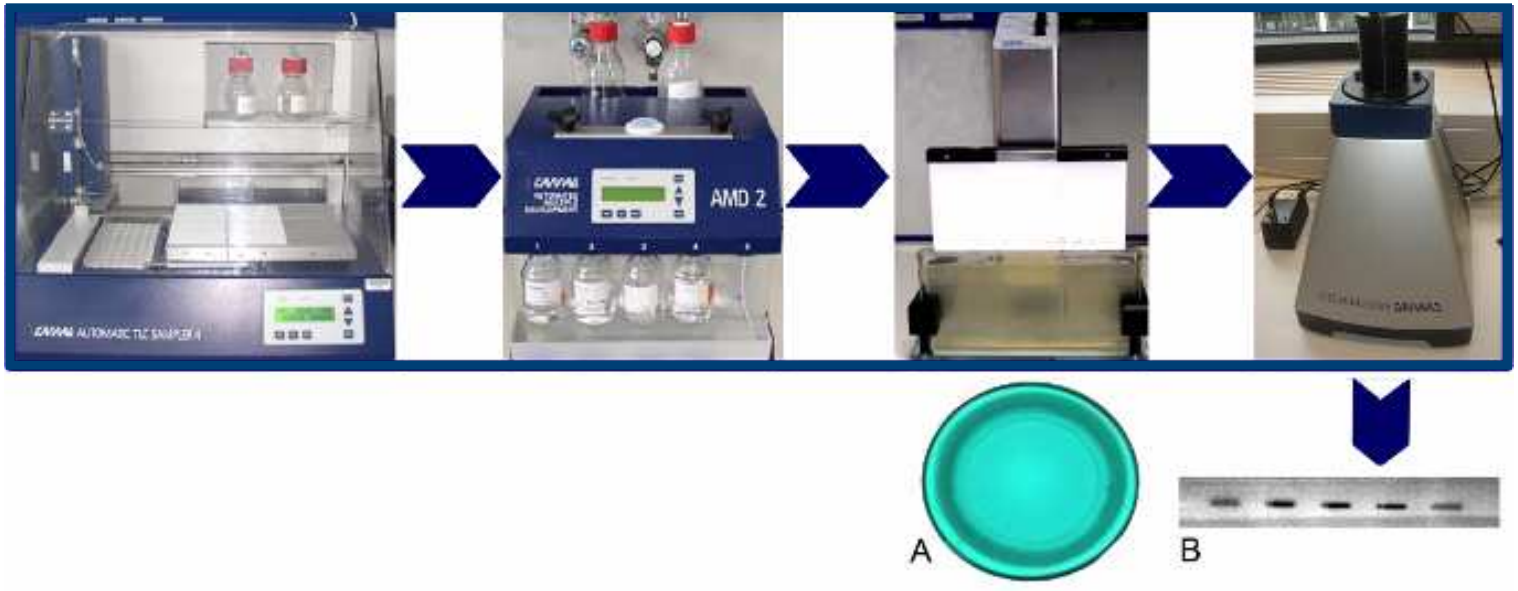
Protocol

Luminescent bacteria → **NEW**: combined with HPTLC

Coupling chromatography with a toxicity-directed detection system
→ effect-directed analysis ↔ different approach to target-analysis
→ detection of **single** toxic compounds ↔ Microtox cuvette test (sum parameter)



EP 0588 139 B1, ChromaDex, www.bioluminex.com/applications
Test Kit “BiolumineX“, Chroma Dex, Inc.

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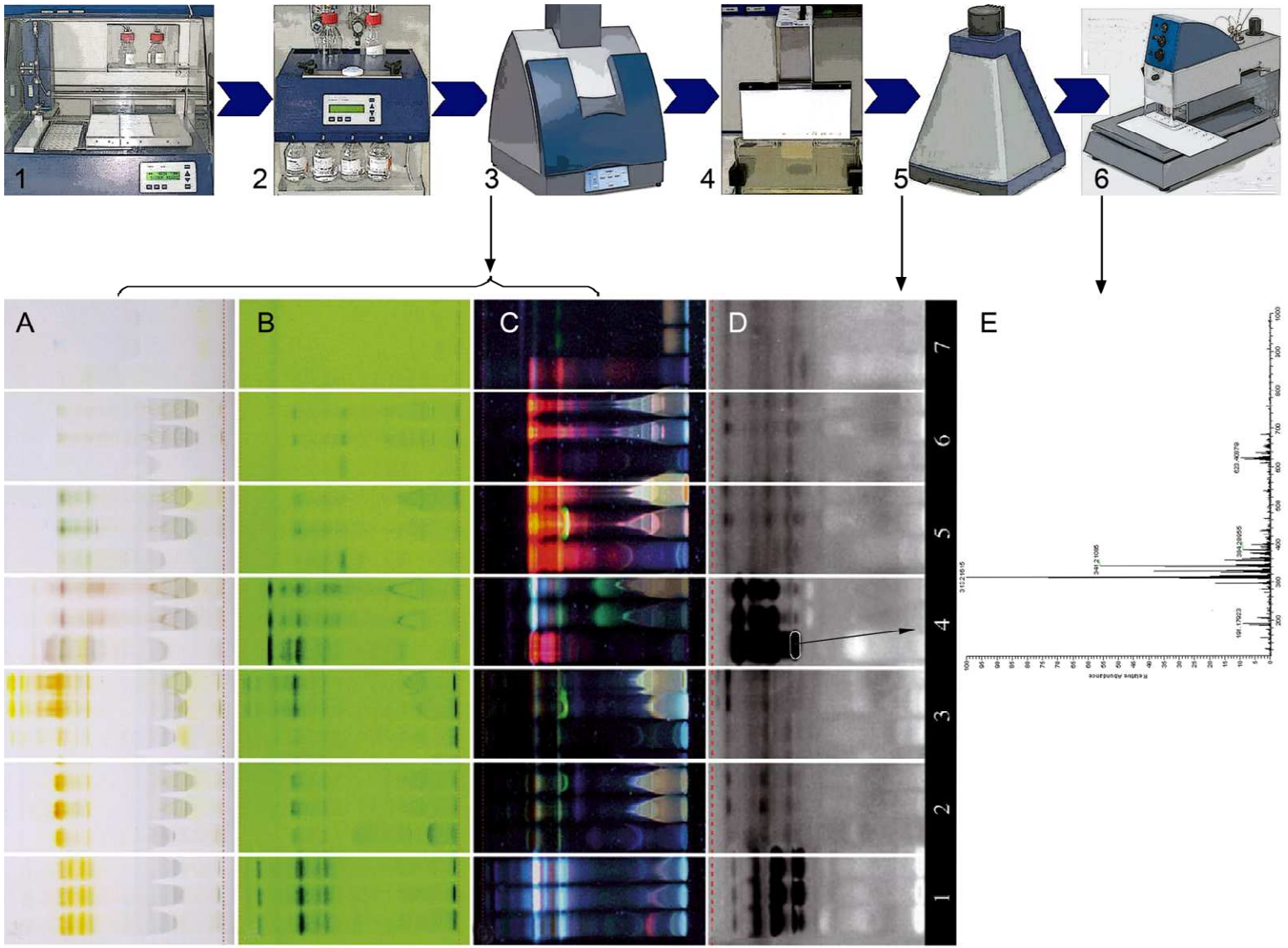
HPTLC-VIS/UV/FLD-EDA(bioactivity)-MS



A. Klöppel, W. Grasse, F. Brümmer, G. Morlock, J Planar Chromatogr 21 (2008) 431-436

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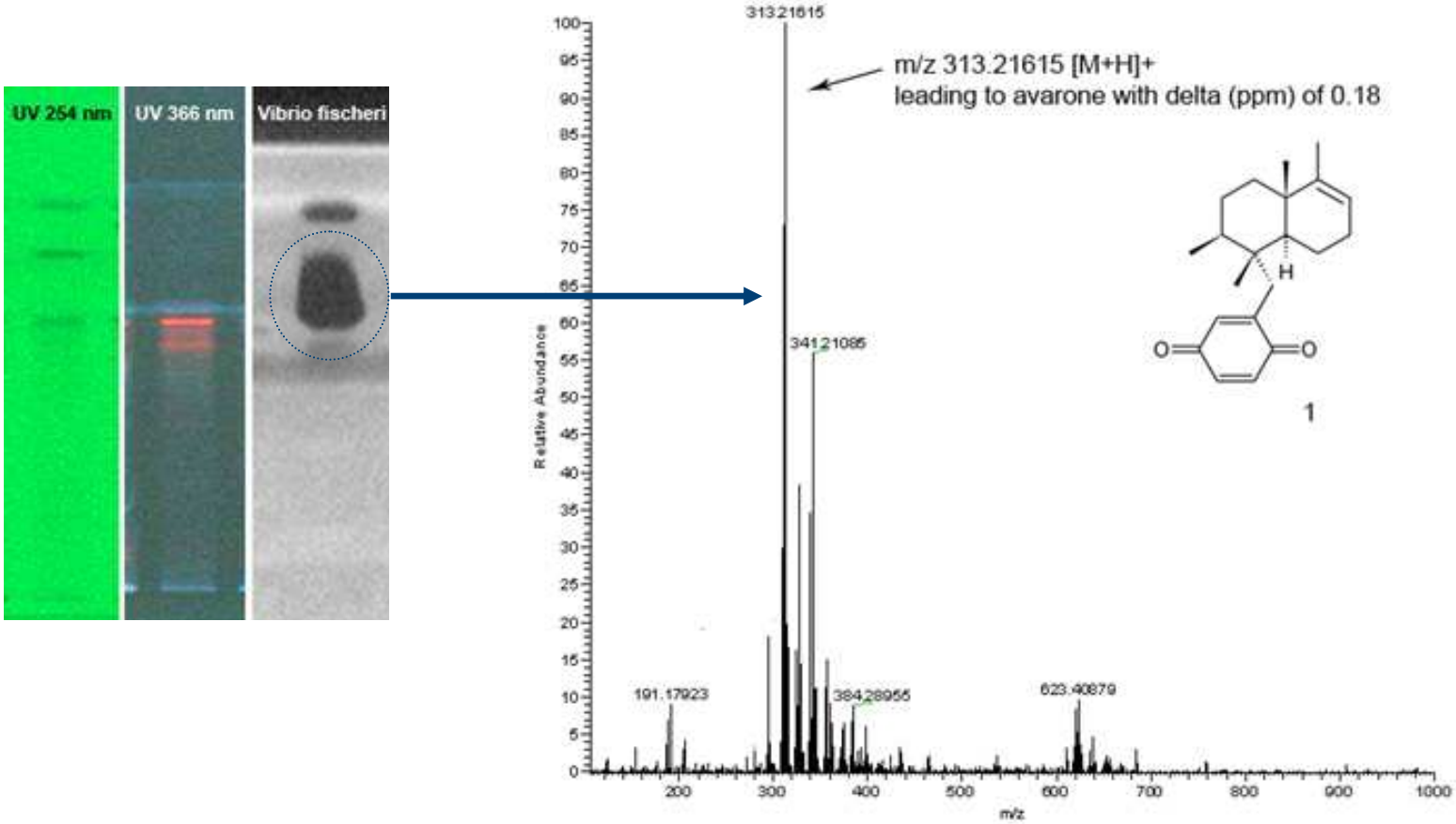
What is it?

HPTLC/MS → highly targeted recording

→ reduced costs and storage of data

→ separation solvent independent from mass spectrometry



G. Morlock, W. Schwack, LCGC Eur July (2008) 366-371

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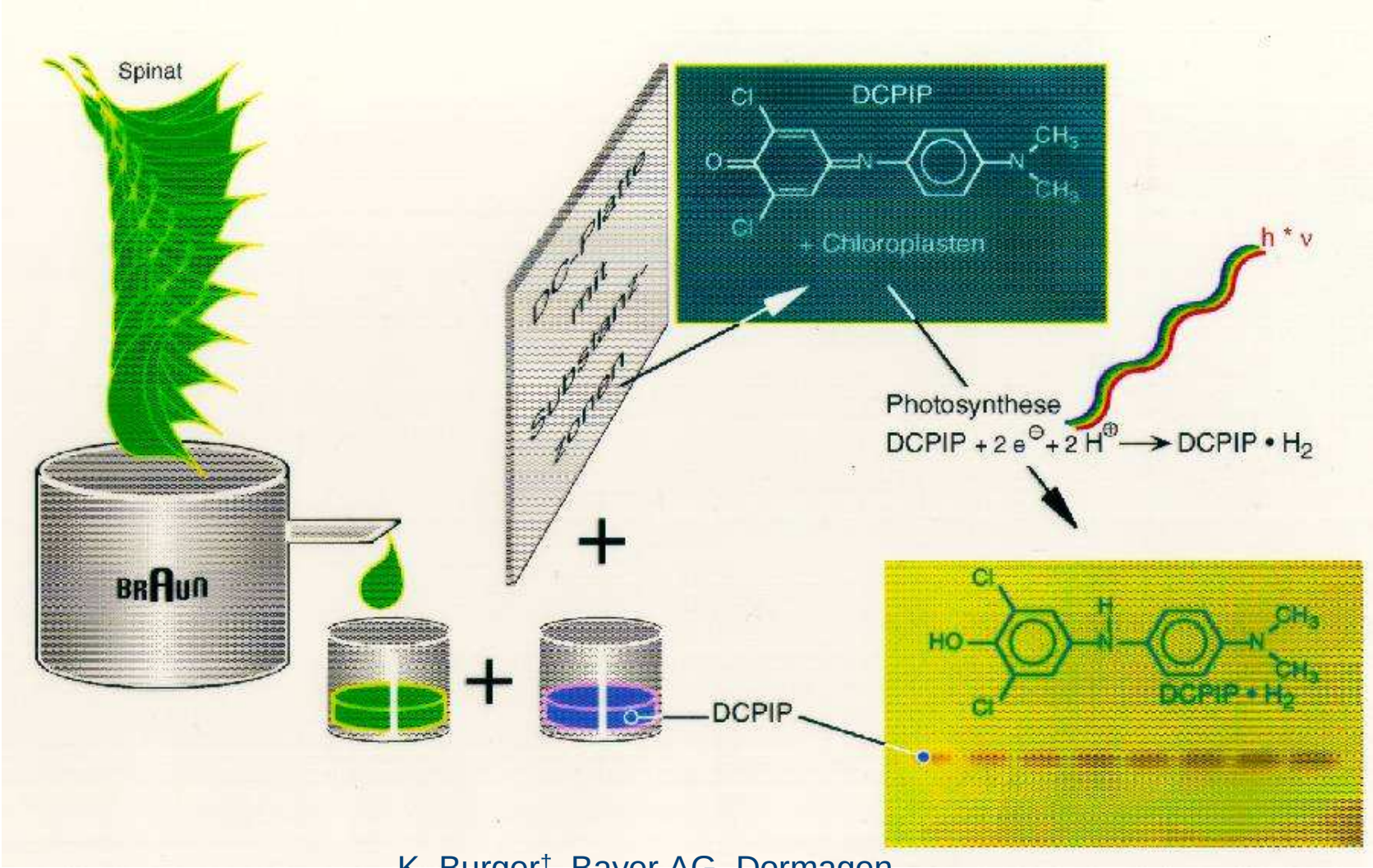
Effect-directed analysis using chloroplasts

→ detecting herbicides inhibiting the photosynthesis (down to 0,1 ng/zone) using spinach juice, dichlorophenolindophenol (electron acceptor) and light

→ green-bluish zones on a yellow (decoloured) background



K. Burger†, Bayer AG, Dormagen

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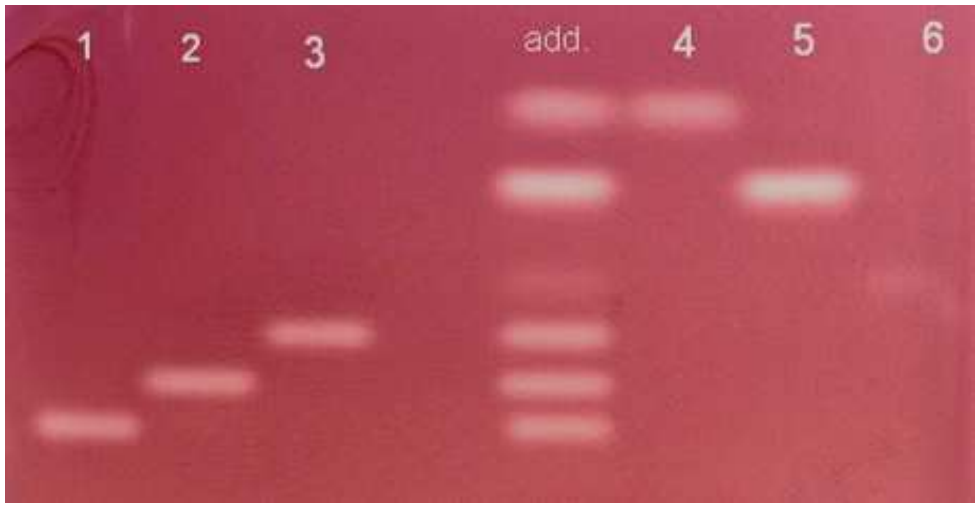
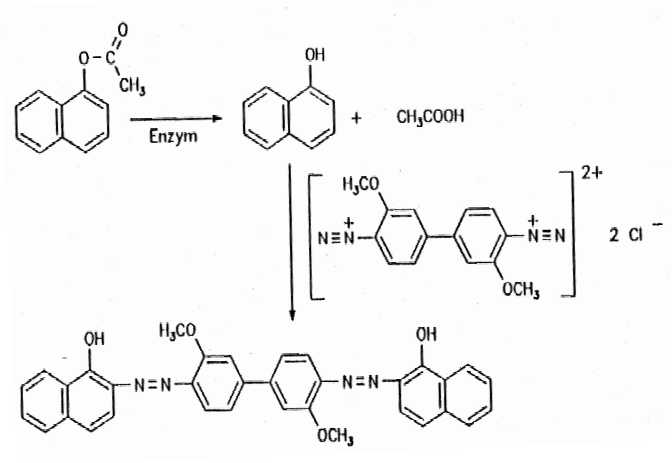
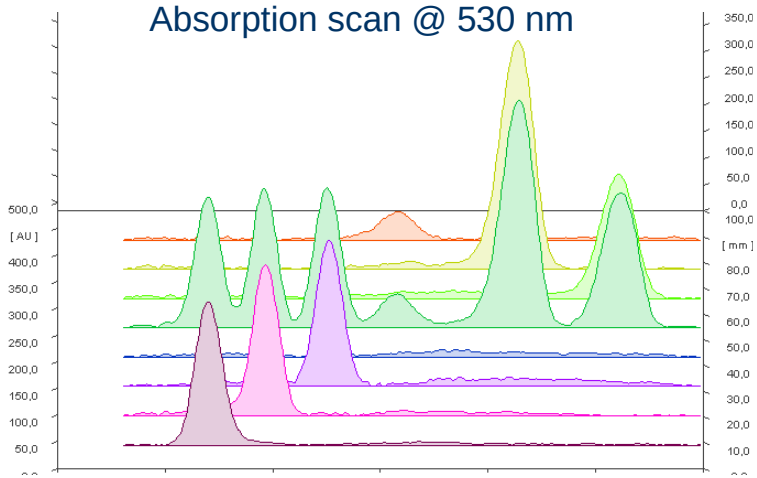
Effect-directed analysis using esterases

→ Cholinesterase inhibiting compounds (→ 2 pg/band)



1. Paraoxon-methyl, 2. Malaoxon, 3. Paraoxon,
4. Ethiofencarb, 5. Chlorfenvinfos, 6. Dichlorvos





R. Akkad, W. Schwack, J Planar Chromatogr 21 (2008) 411-415

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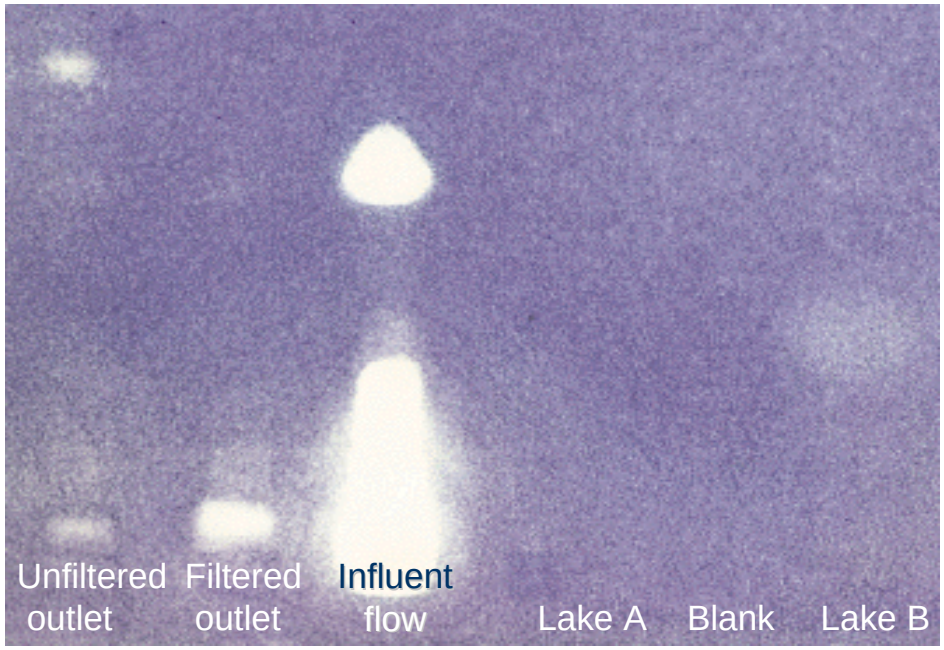
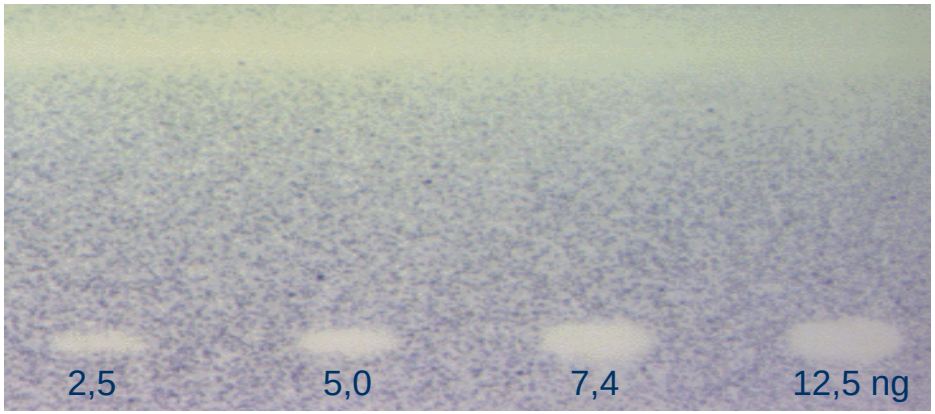
Effect-directed analysis using *Bacillus subtilis*

→ detecting antibiotics using the Bioautography Testkit *Chrom Biodip* (Merck)

...in waste water treatment facilities



...in Supracycline tablets (ng/zone)



CAMAG Bibliogr Service CBS 85 (2000) 10-12

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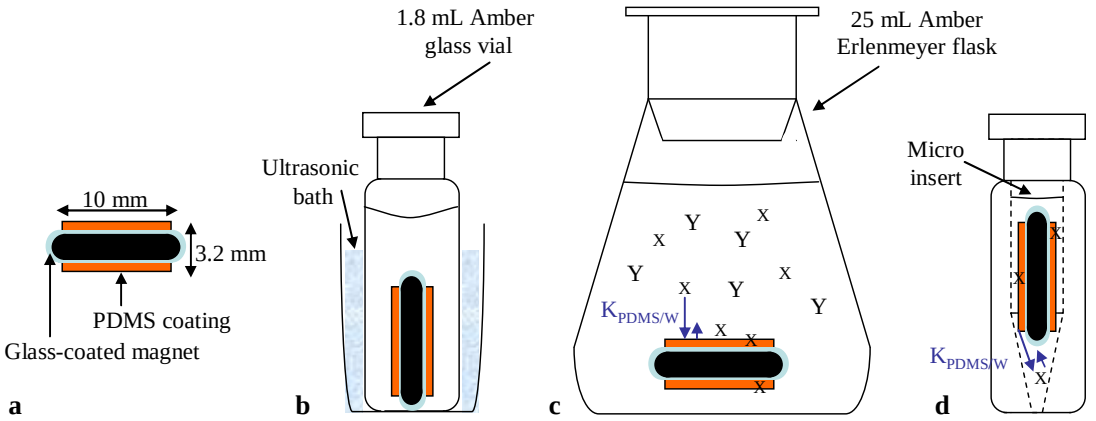
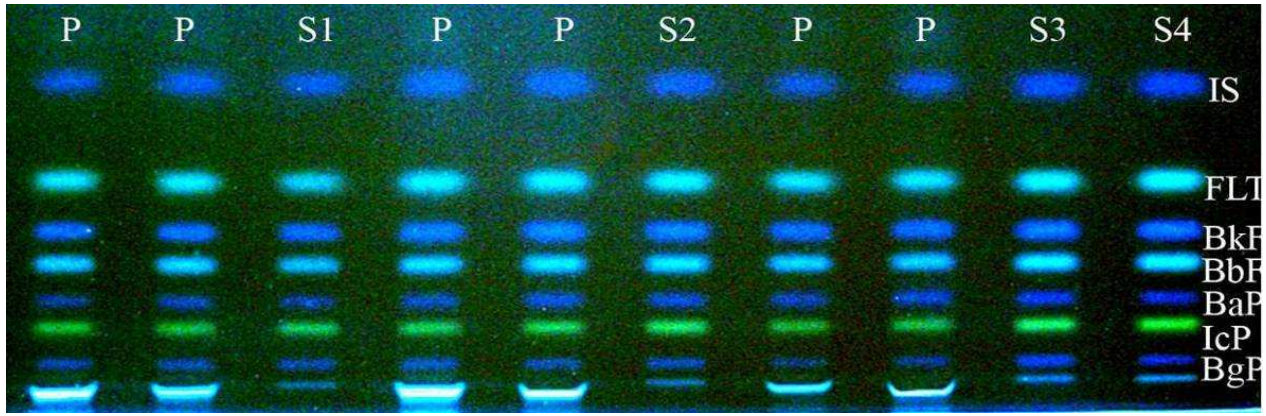
High throughput → PAH in drinking water

Analysis of 30 water samples per day incl. sample preparation





G. Morlock, S. Kopacz, J Liq Chromatogr Relat Technol 31 (2008) 1925-1942

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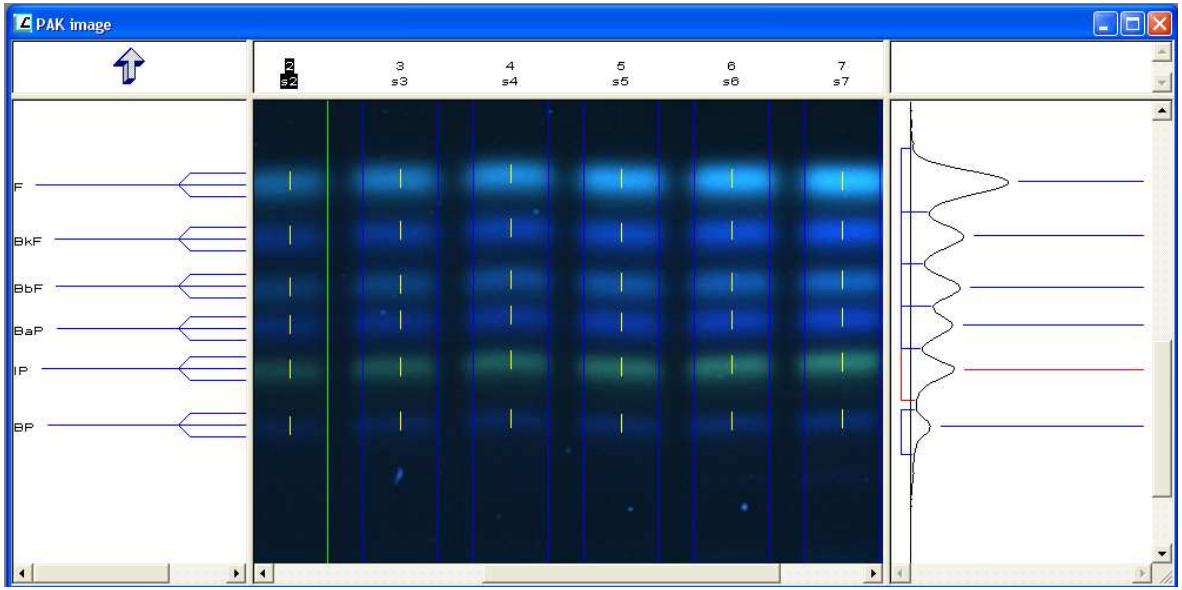
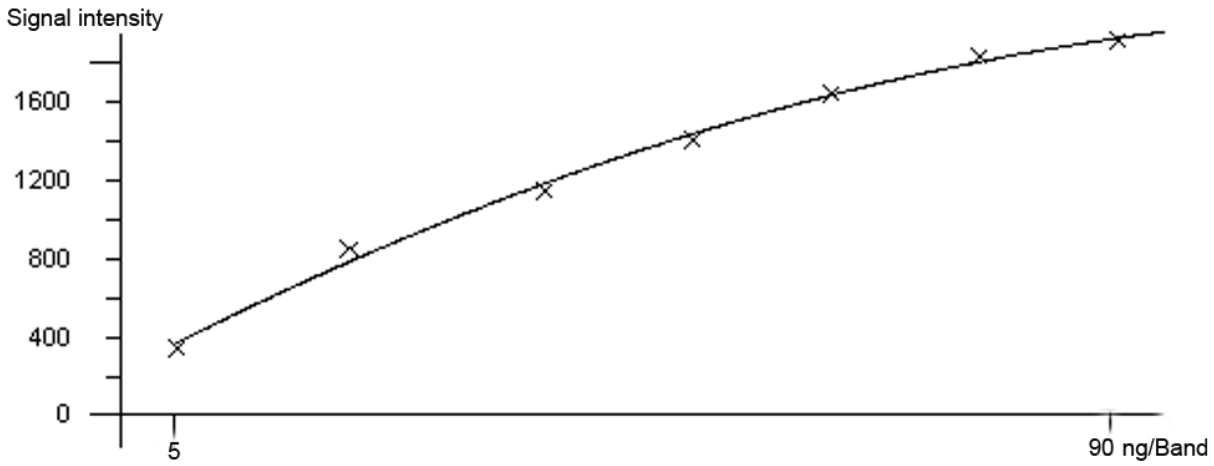
Digital evaluation





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HPTLC-Identification of Panax ginseng

CAMAG Applikation F-31

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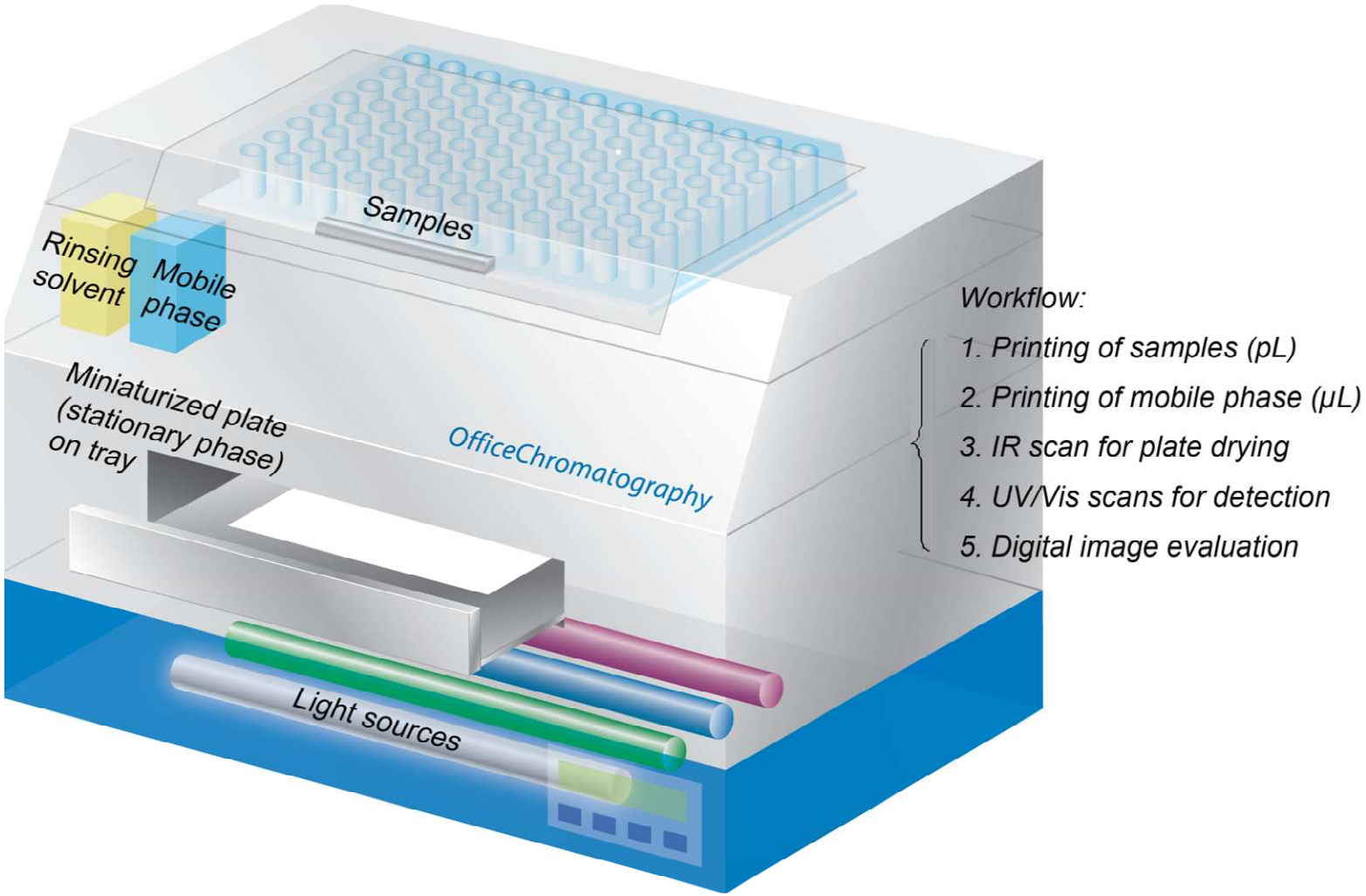
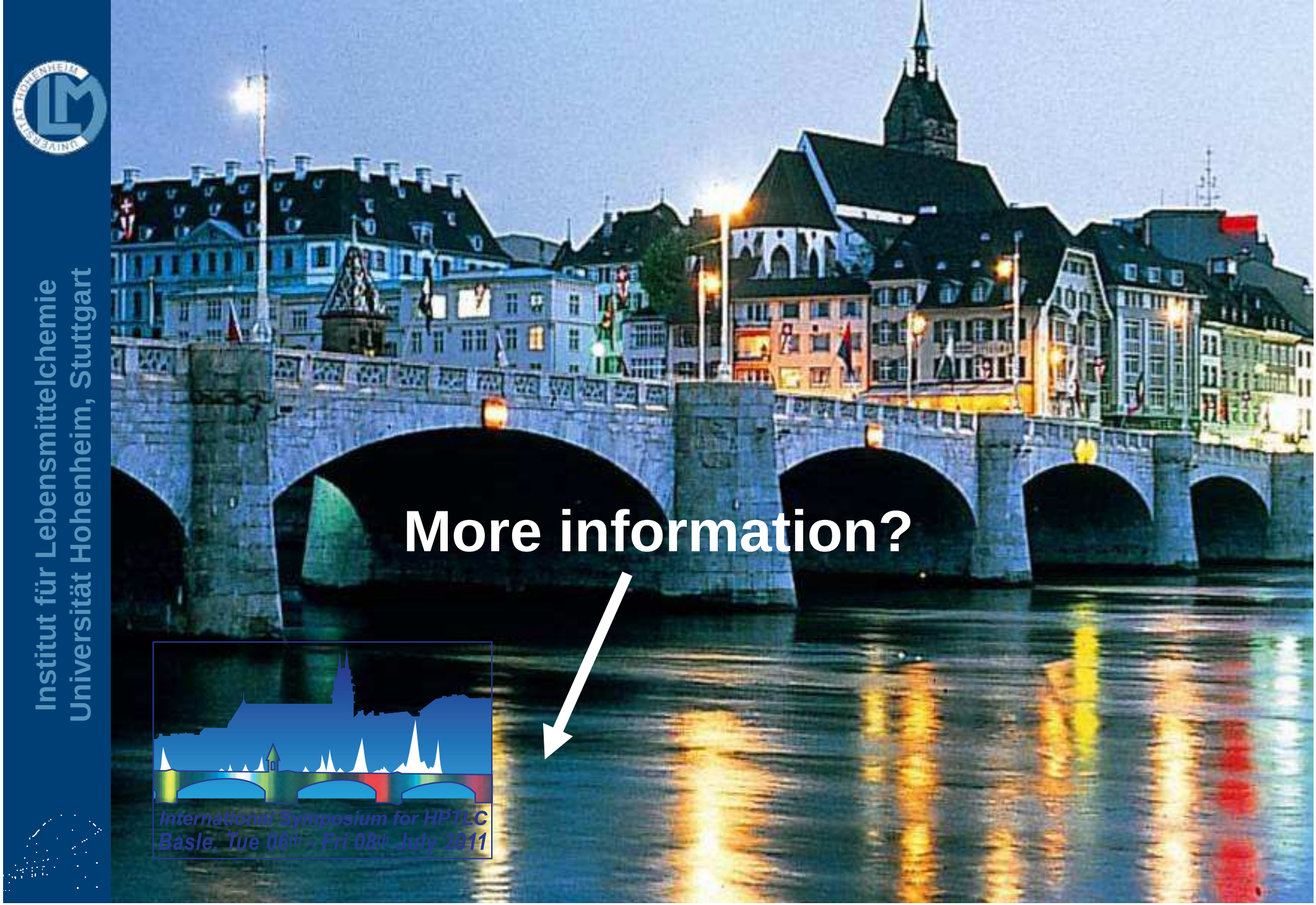
Future Potential: Office Chromatography



Miniaturized planar chromatography using office peripherals,
G. Morlock, C. Oellig, L. Bezuidenhout, M. Brett & W. Schwack, Anal. Chem. 2010 in print



HPTLC 2011, Basel : 6th-8th July 2011 → www.hptlc.com

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Ideal to understand chromatography…

... train the next generation in HPTLC!