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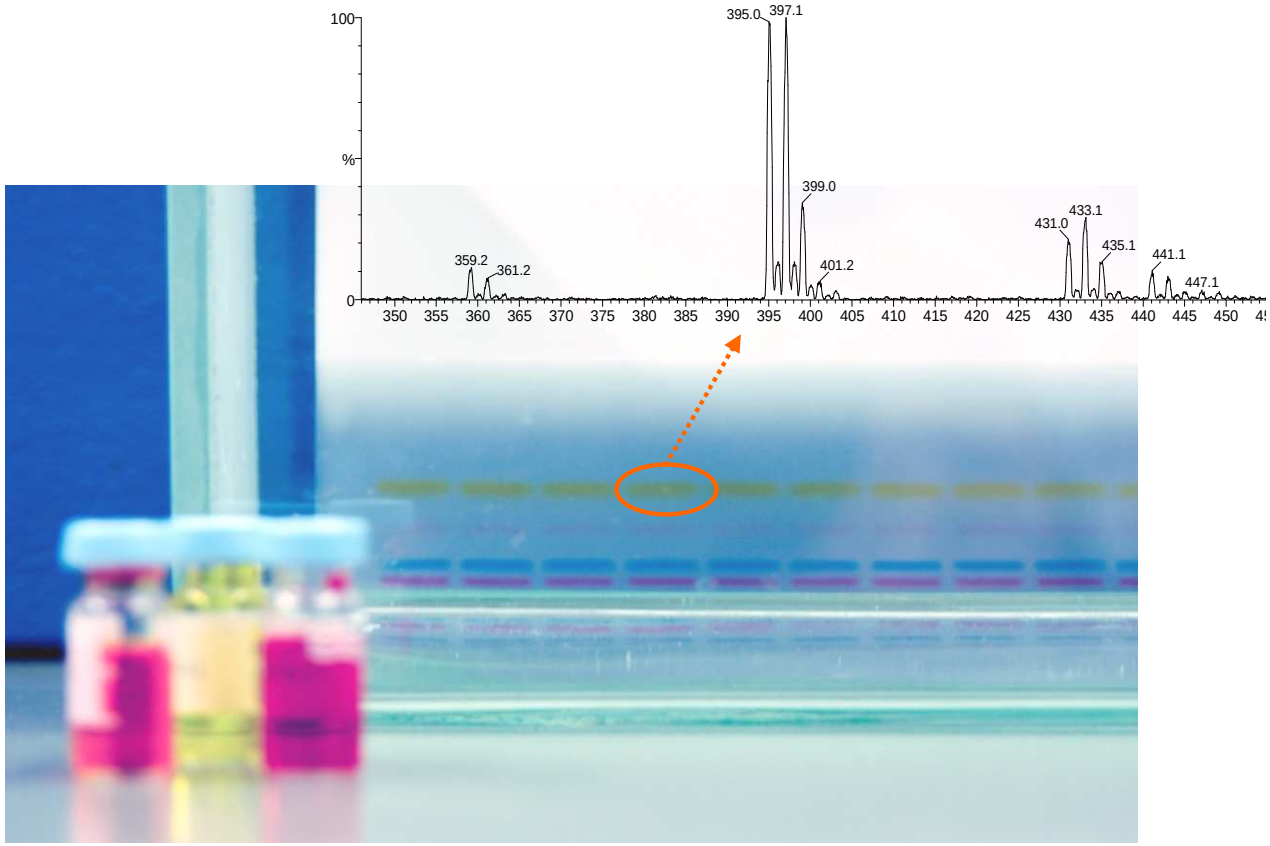
20 Reasons to use HPTLC
in the age of ultra-rapid separations



PD Dr. habil. Gertrud Morlock
Institut für Lebensmittelchemie
Universität Hohenheim, Stuttgart

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Use the most reasonable method!

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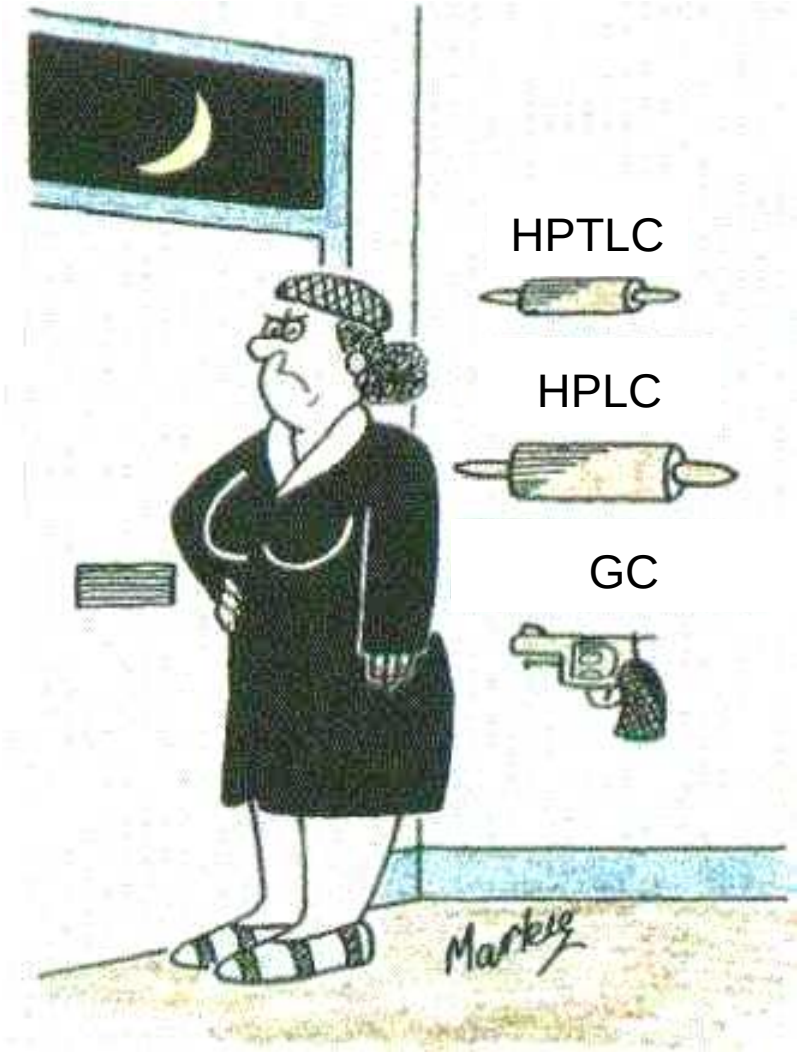
Use the most reasonable method!



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20 reasons to use HPTLC

Reasons 1-10 (presented 2009):

1. Reduced sample preparation (saves money!)
2. Parallel chromatography under identical conditions
3. High throughput (1000 runs per day) with minimal costs
4. Selective, simultan derivatisations
5. Multi-detection (UV/Vis, FLD, derivatisation, MS…)
6. More information about unknowns
7. Flexible working station
8. Concentration during application (factor 10.000)
9. Effect-directed analysis
10. Directed, cost-effective mass spectrometry (MS-independend mobile phase)

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20 reasons to use HPTLC

Reasons 11-20:

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Planar Chromatography

→ 1938 TLC

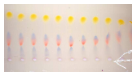
Thin-layer chromatography

→ 1975 HPTLC

High-performance TLC

→ 2001 UTLC

UltraTLC



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

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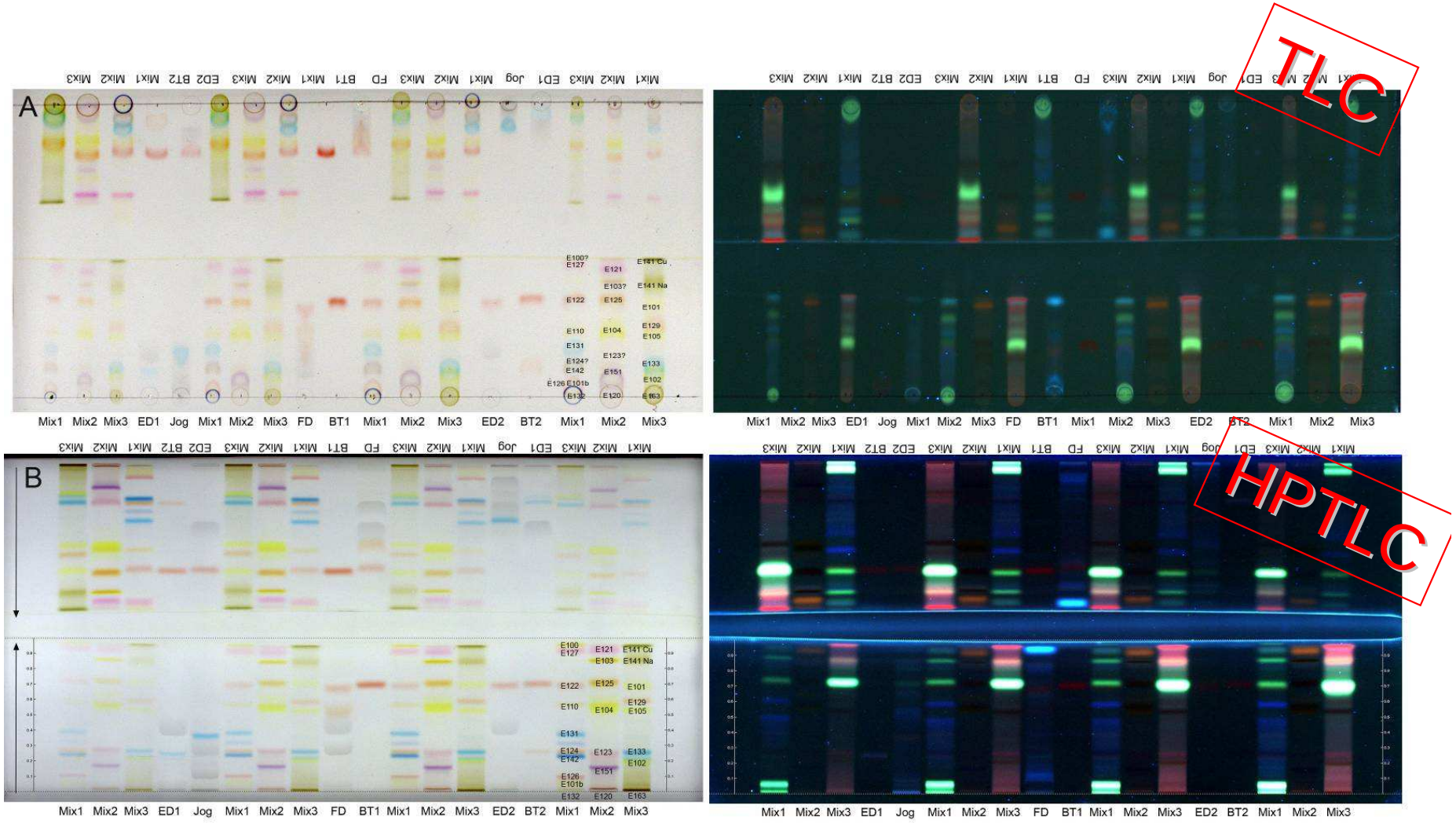
Dye analysis



→ No resonable calibration function was obtained by TLC.

→ For quantification, just HPTLC is reliable.

Dye analysis

G. Krämer, Die Aktuelle Wochenschau der GDCh, Woche 17 (2009)

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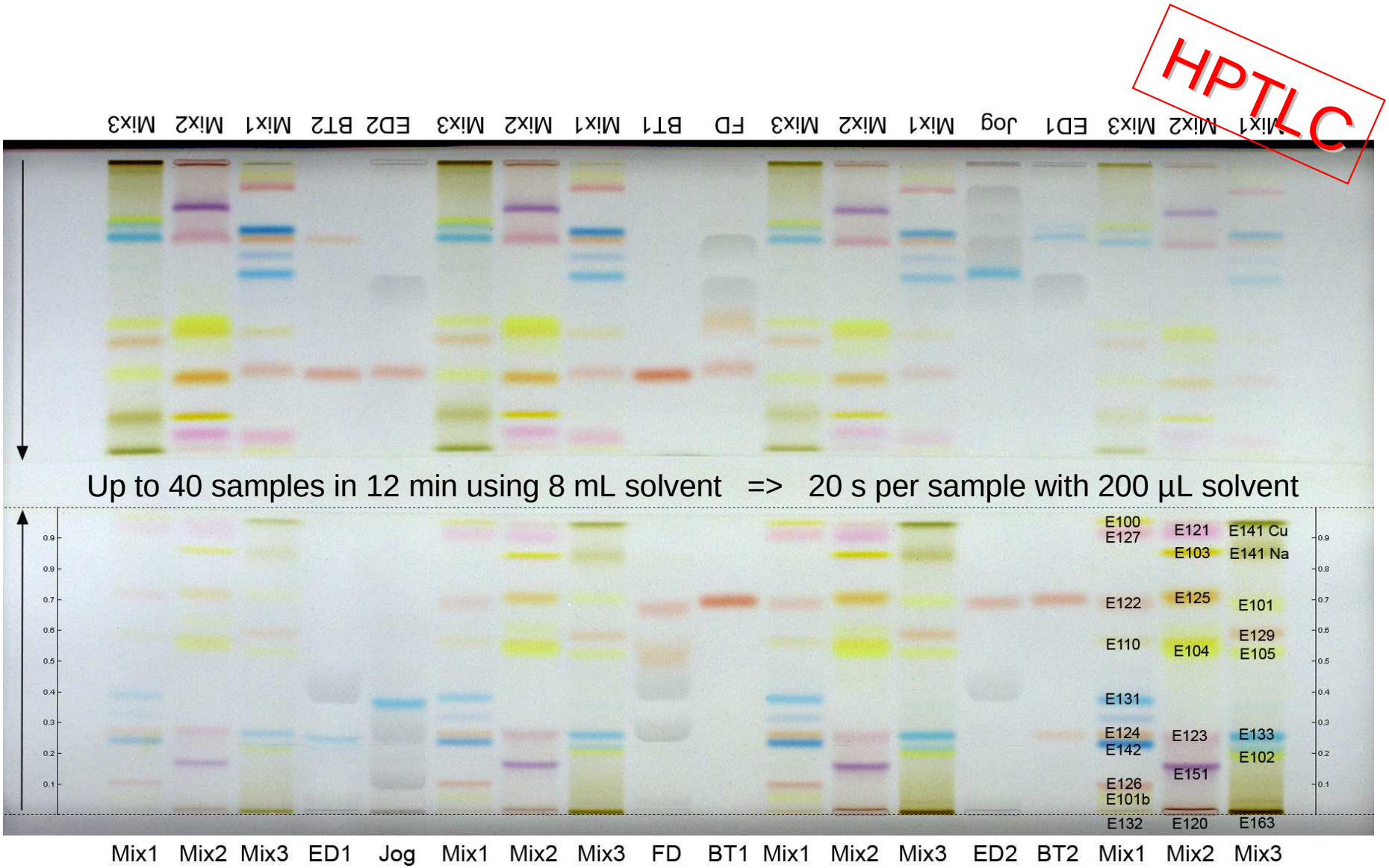
Dye analysis



G. Morlock, C. Oellig, J AOAC Int 92 (2009) 547-554
and CBS 103 (2009) 5-9

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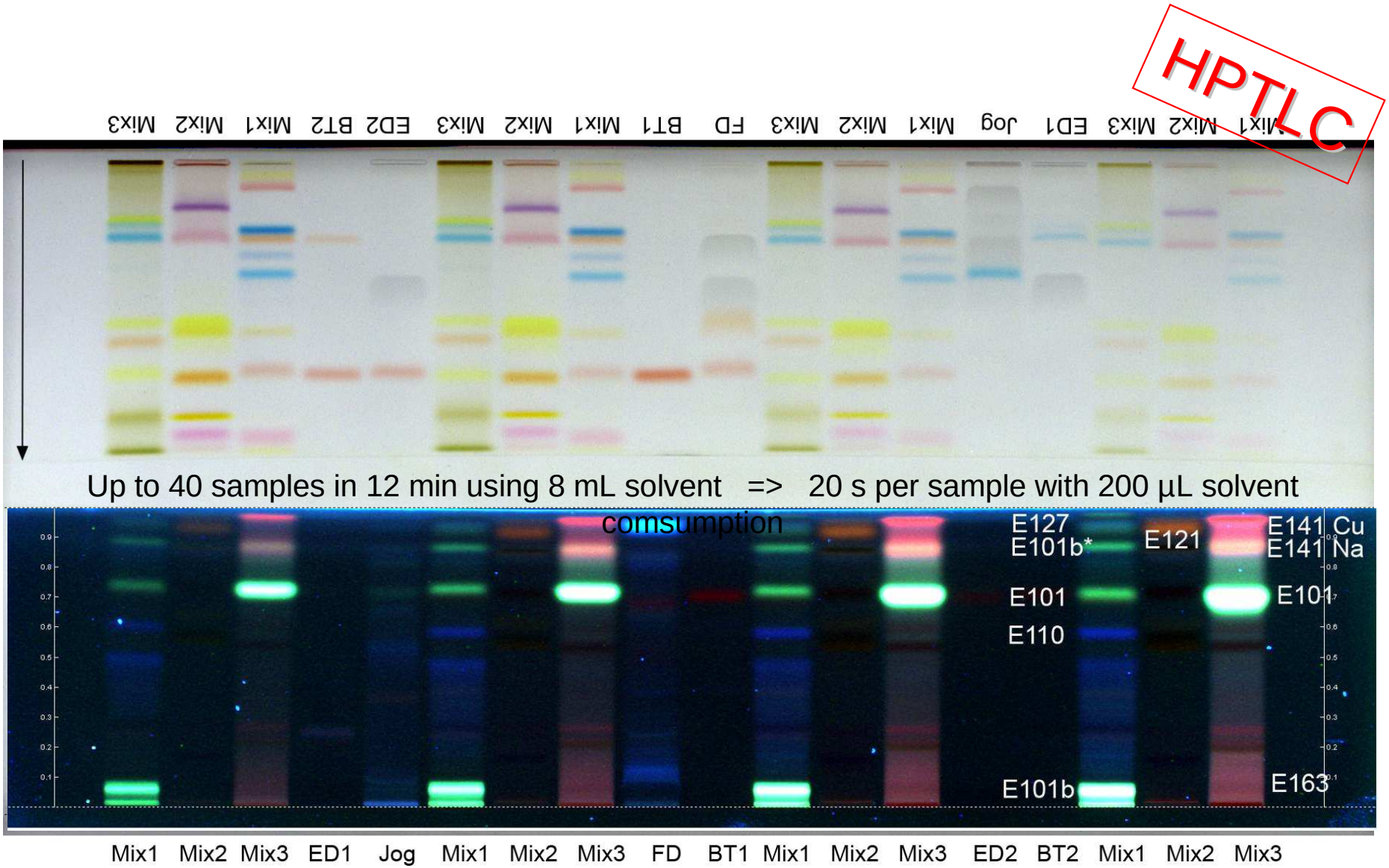
Dye analysis



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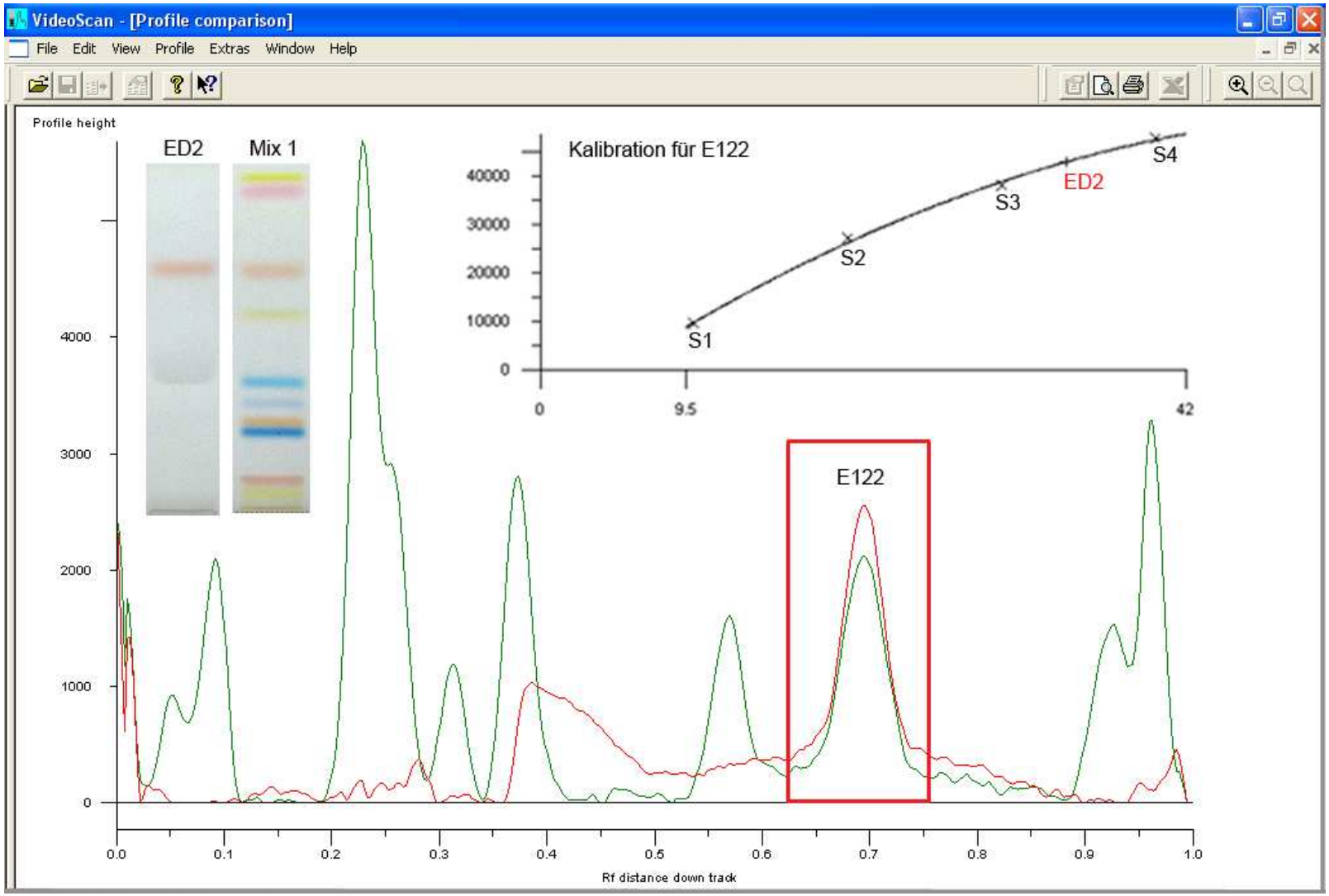
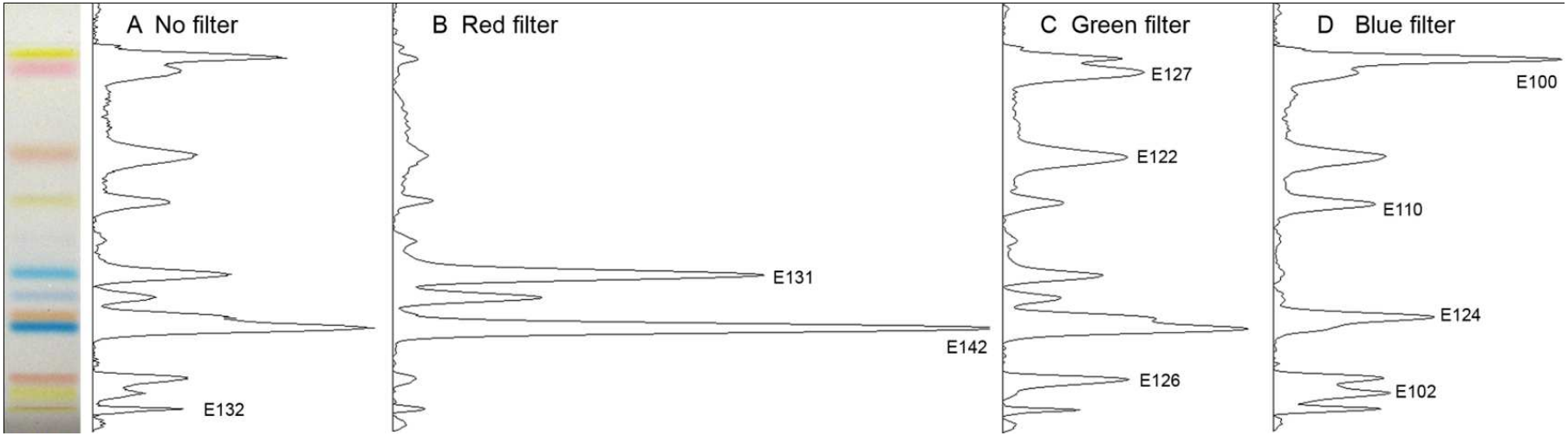
Digital quantification



Digital filters



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

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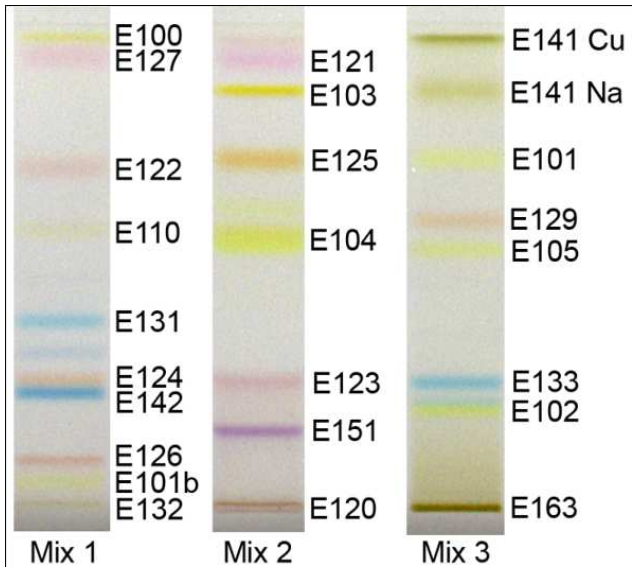
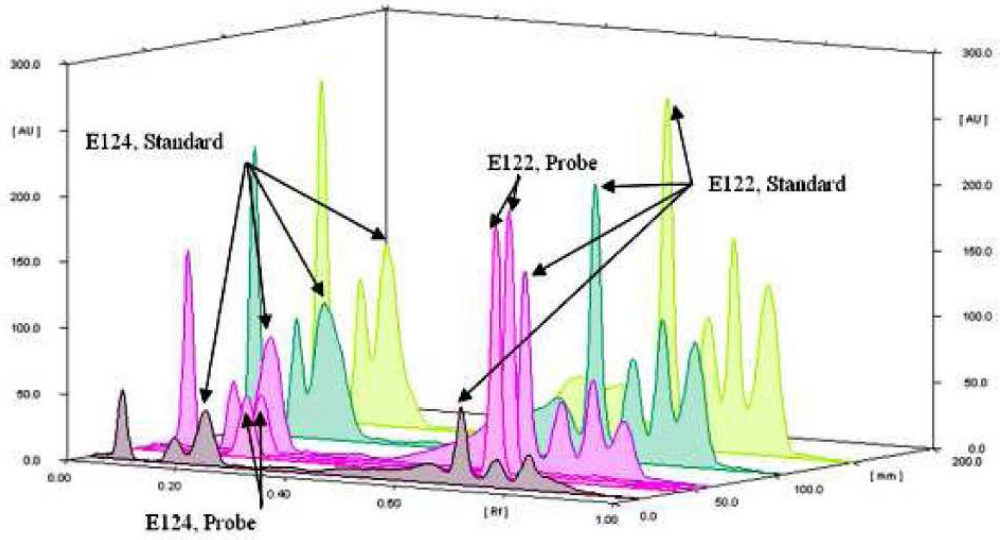
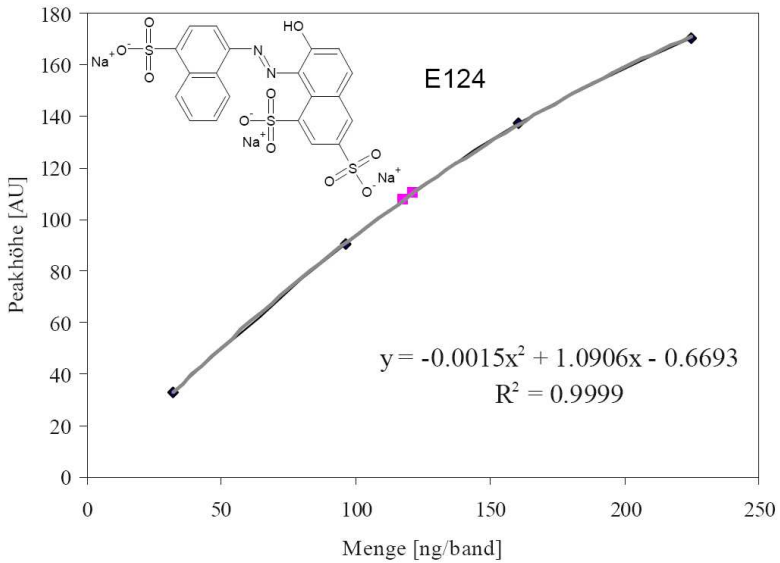
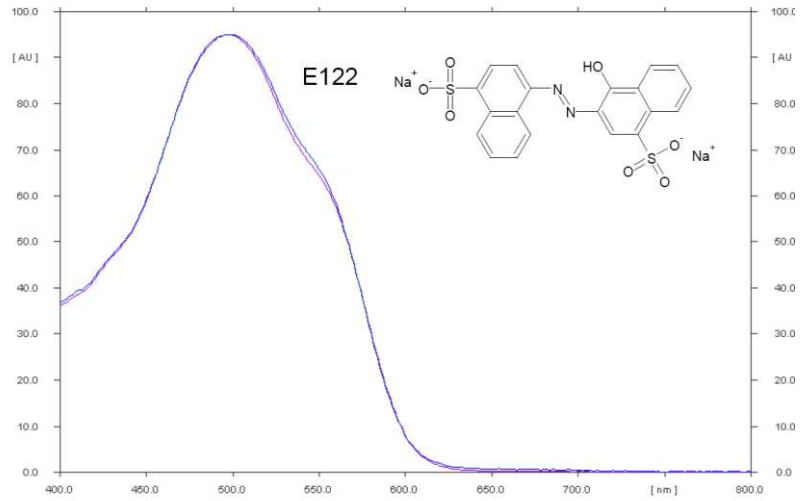
Dye analysis









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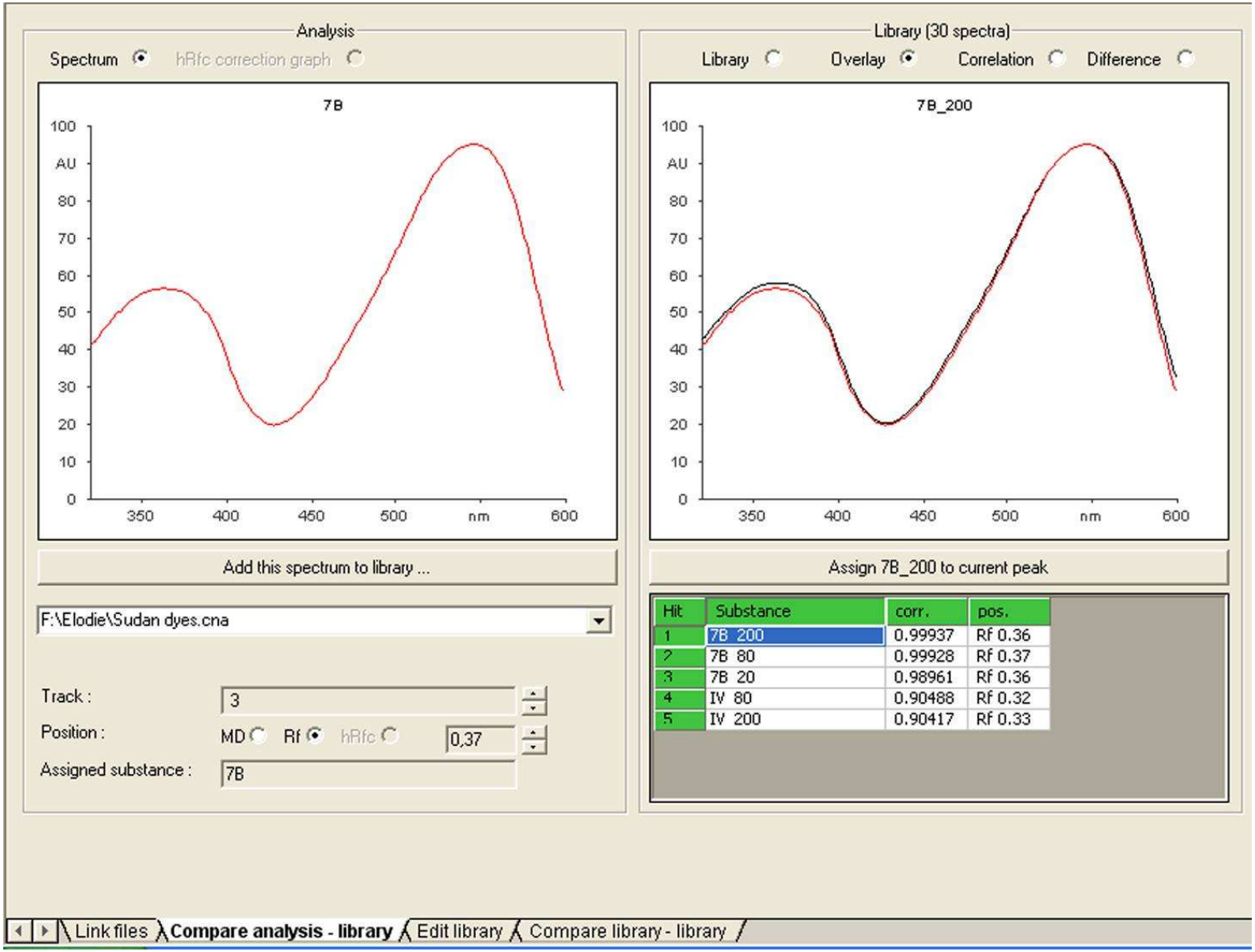
Search in spectra library



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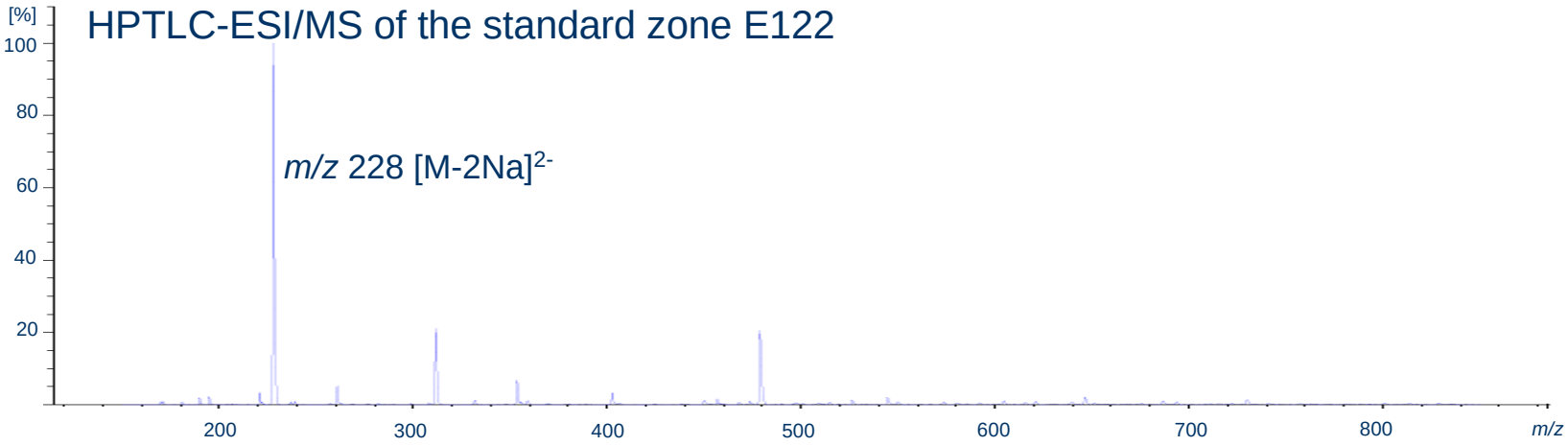
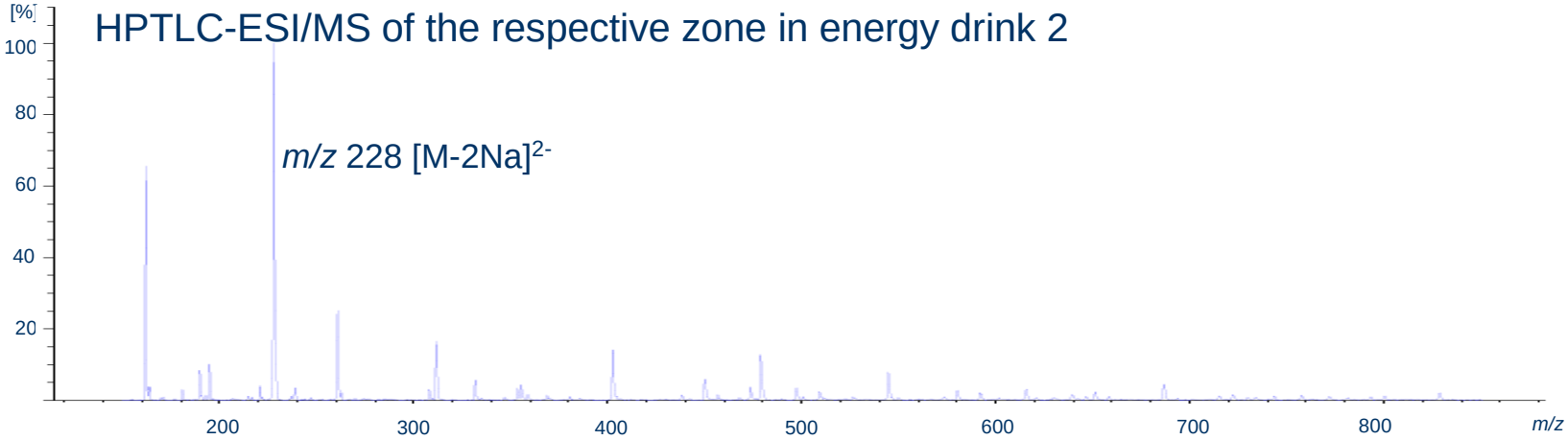
Confirmation by mass spectra

HPTLC-ESI/MS of the standard zone E122



HPTLC-ESI/MS of the respective zone in energy drink 2



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

Why stop here?

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

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…more information can be obtained from a single plate

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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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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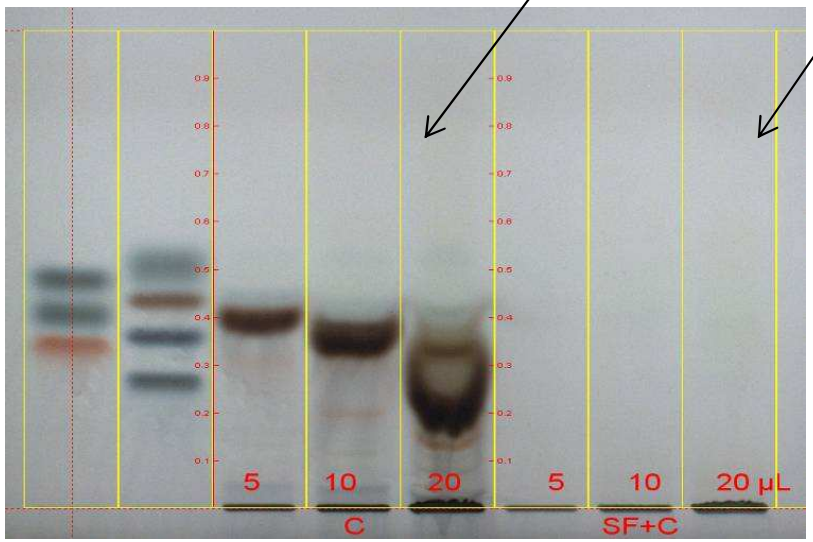
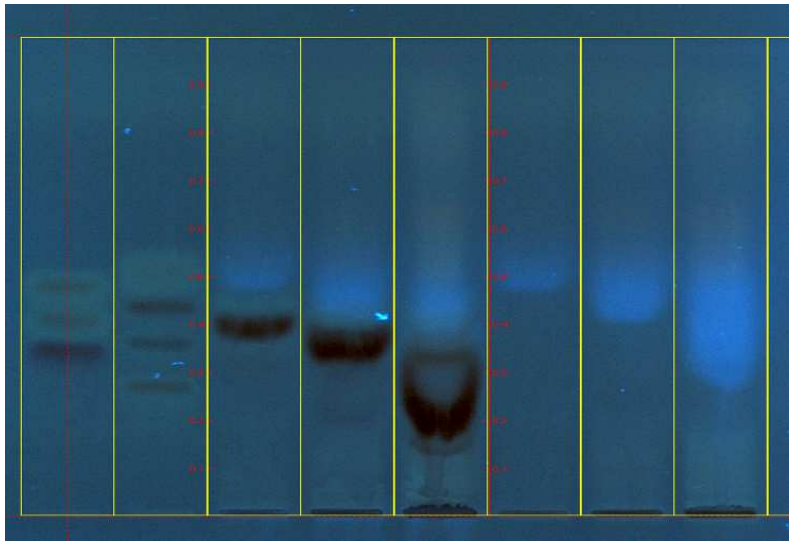
The utmost matrix-tolerant method

Are carbohydrates

produced by the algae?





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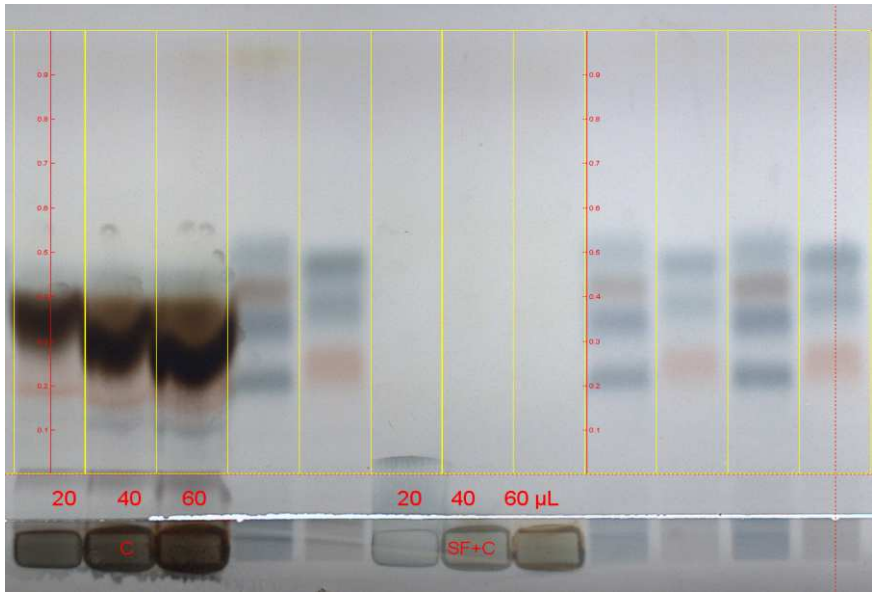
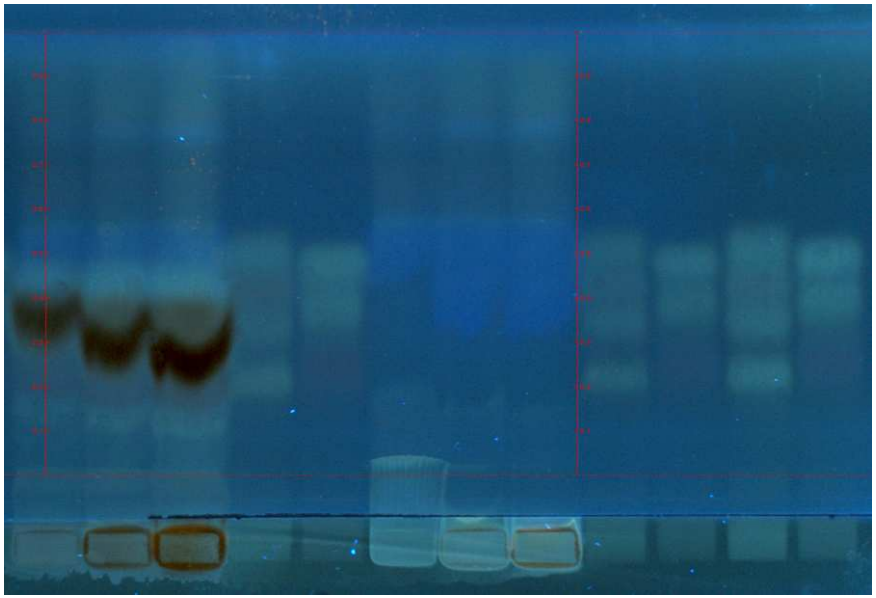
The utmost matrix-tolerant method





Steril filtrated culture medium of algae

→ centrifuged

→ no relevant concentrations of carbohydrates (< 0.00010 %)

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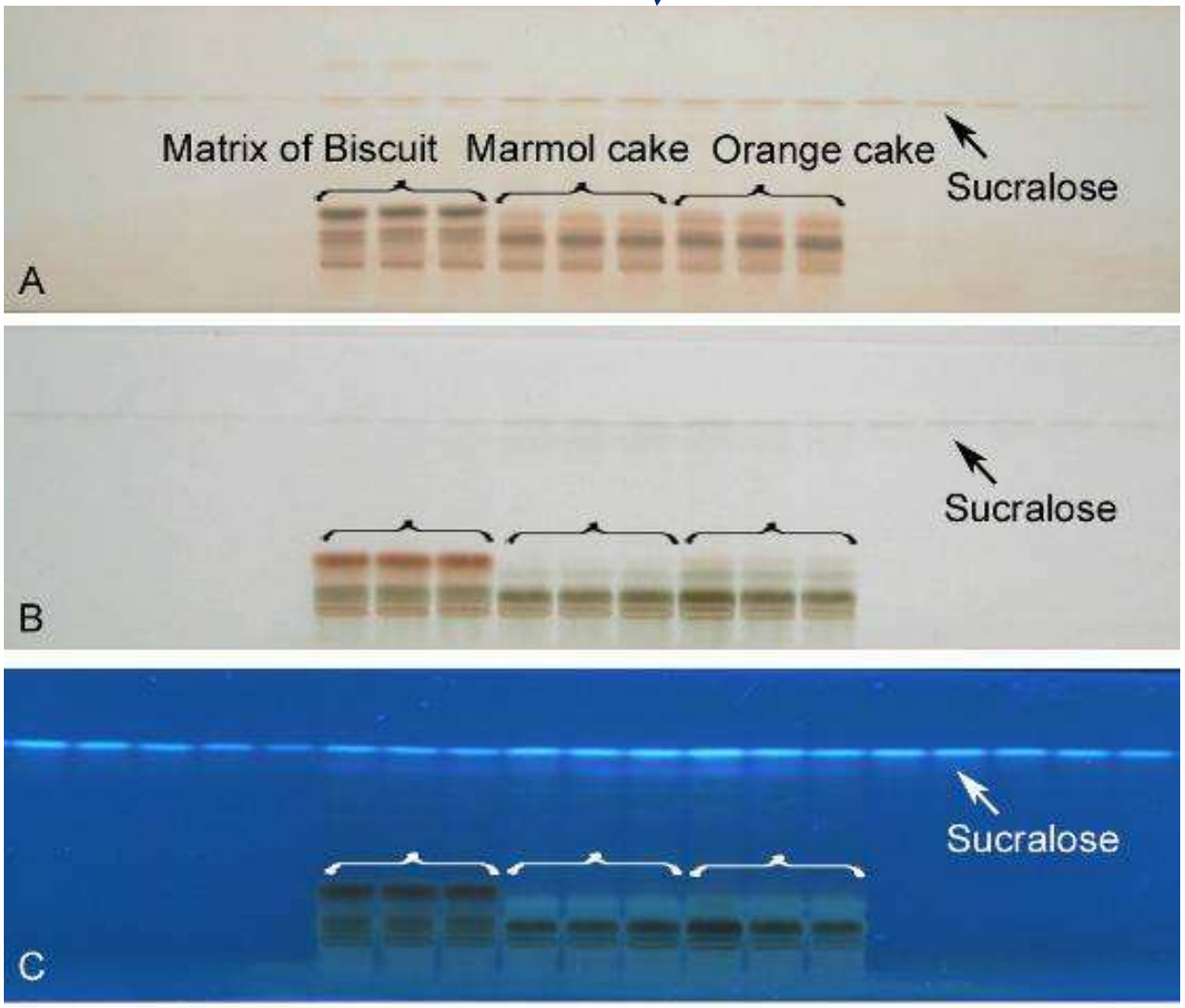
Analysis of sucralose in cakes

extracted
in MeOH, filtered



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

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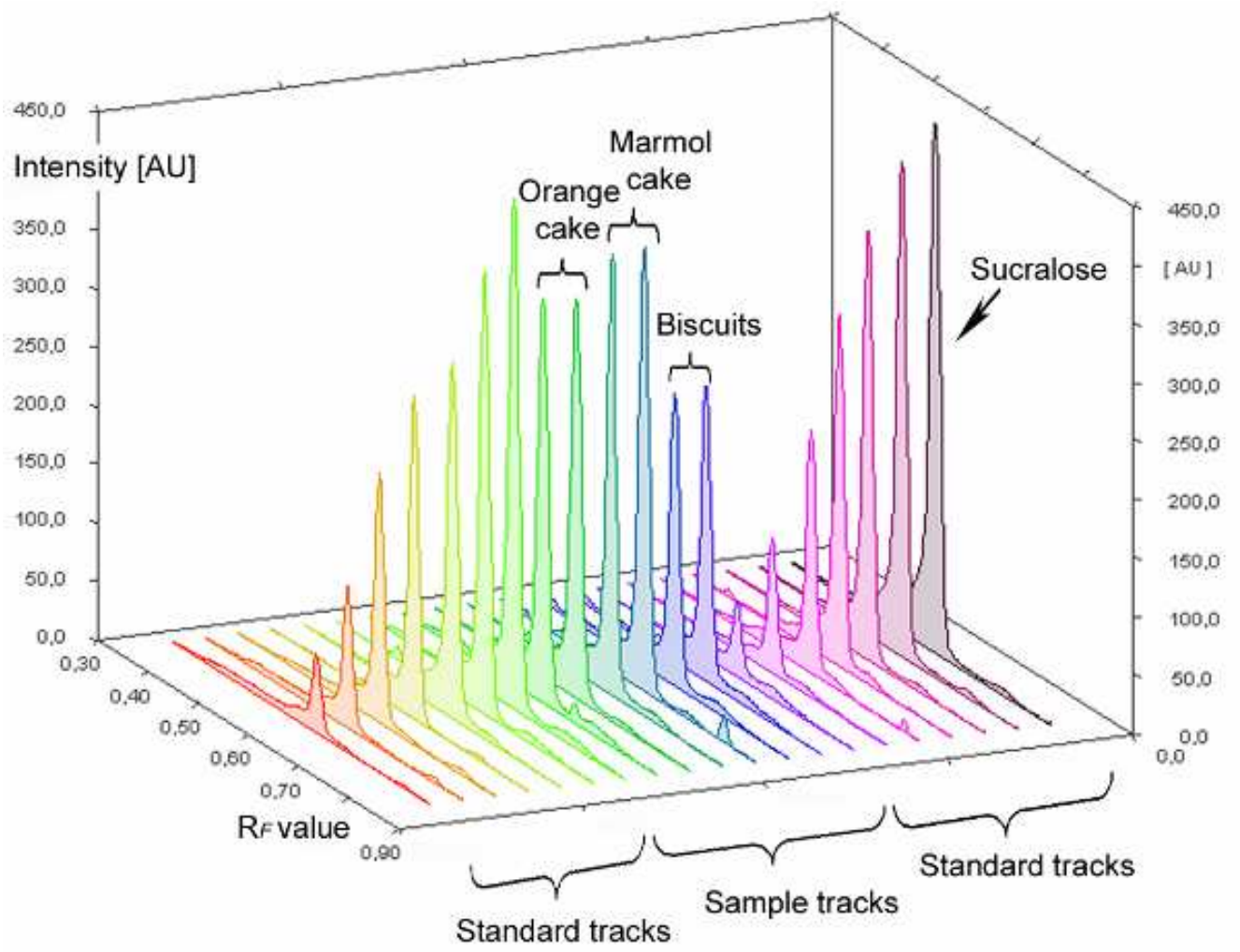
Quantification of sucralose in cakes



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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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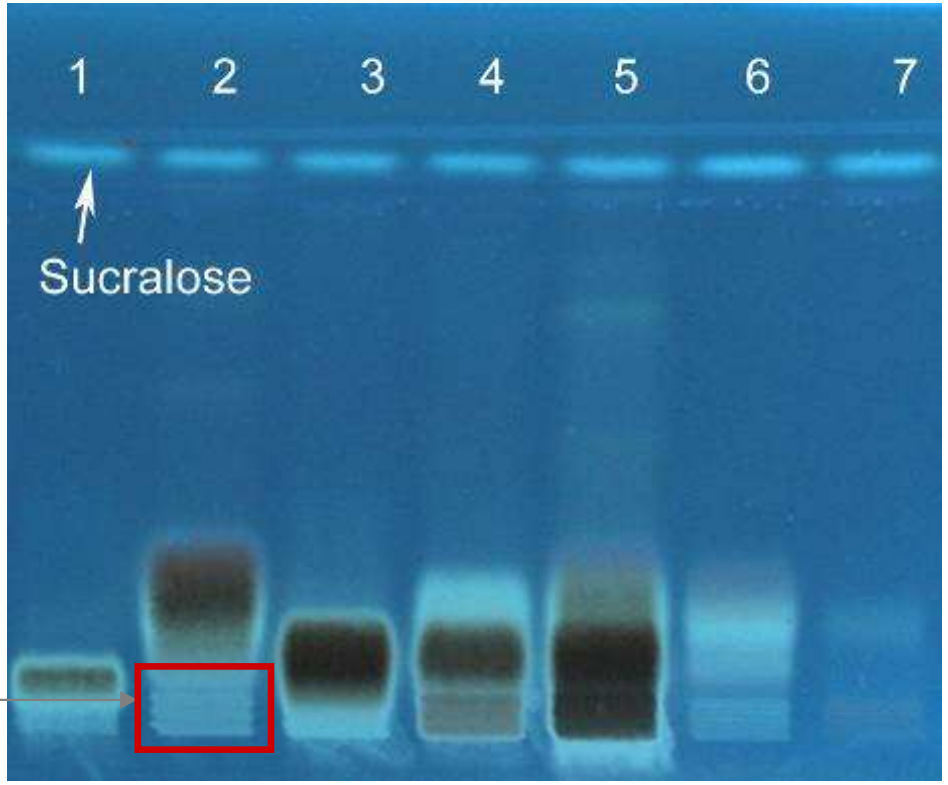
Sucralose in different food matrices



Area application is crucial!

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

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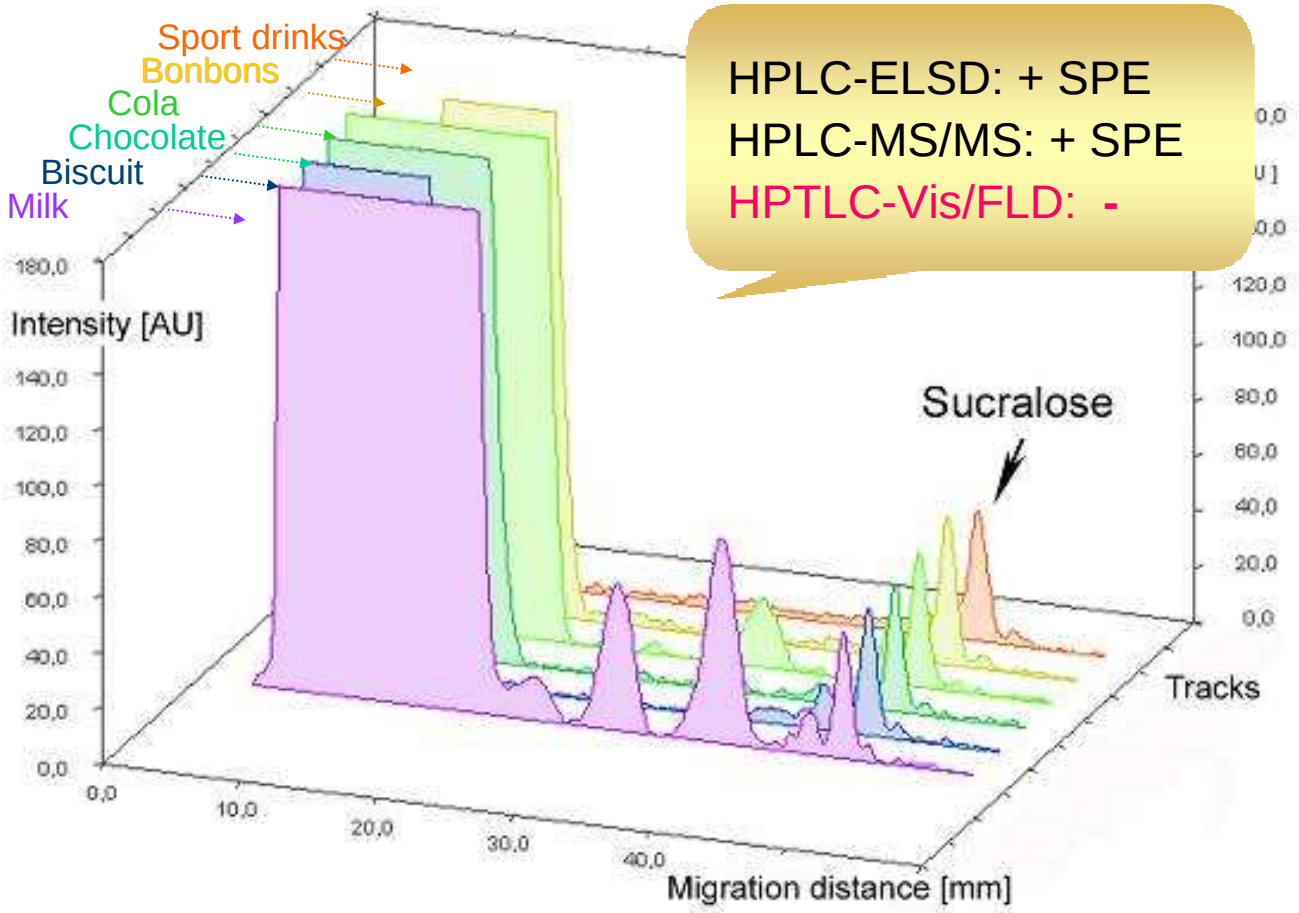
Sample preparation and chromatography



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

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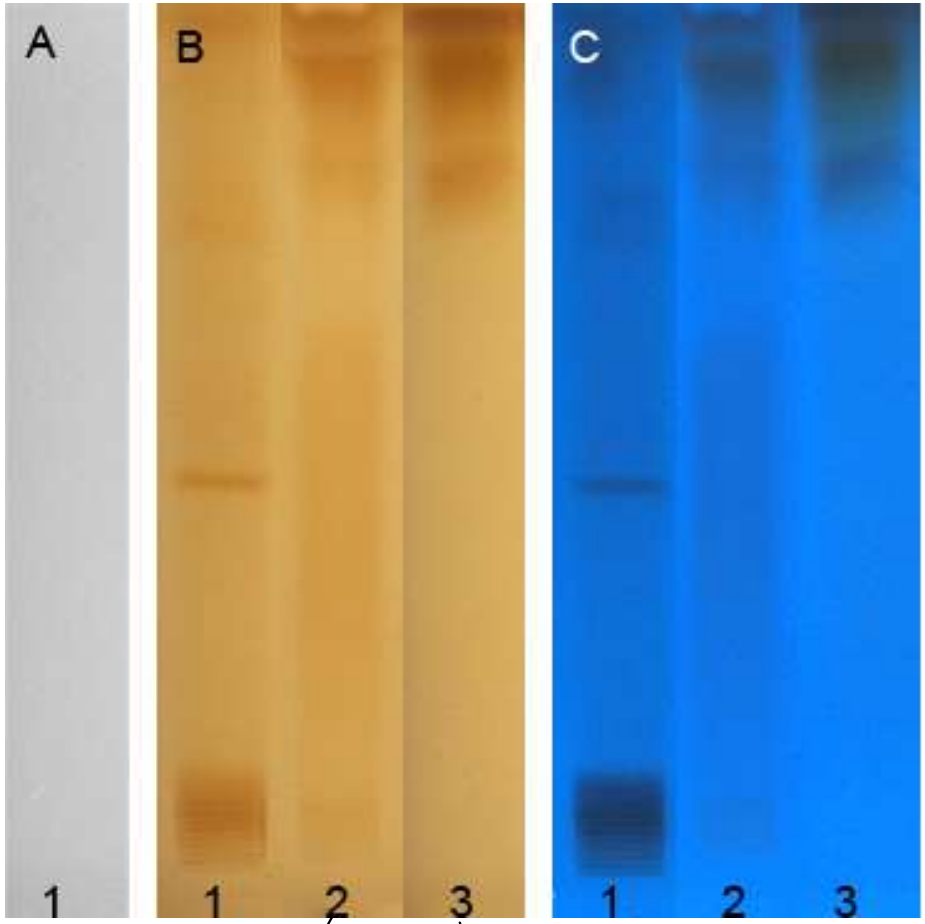
Re-use of the stationary phase

Analysis of sewage effluent (1)



After chromatography, plate washing with MeOH MeOH - H_2O 4:1
followed by derivatization

→ Test to clarify sufficient re-conditioning of HPLC columns

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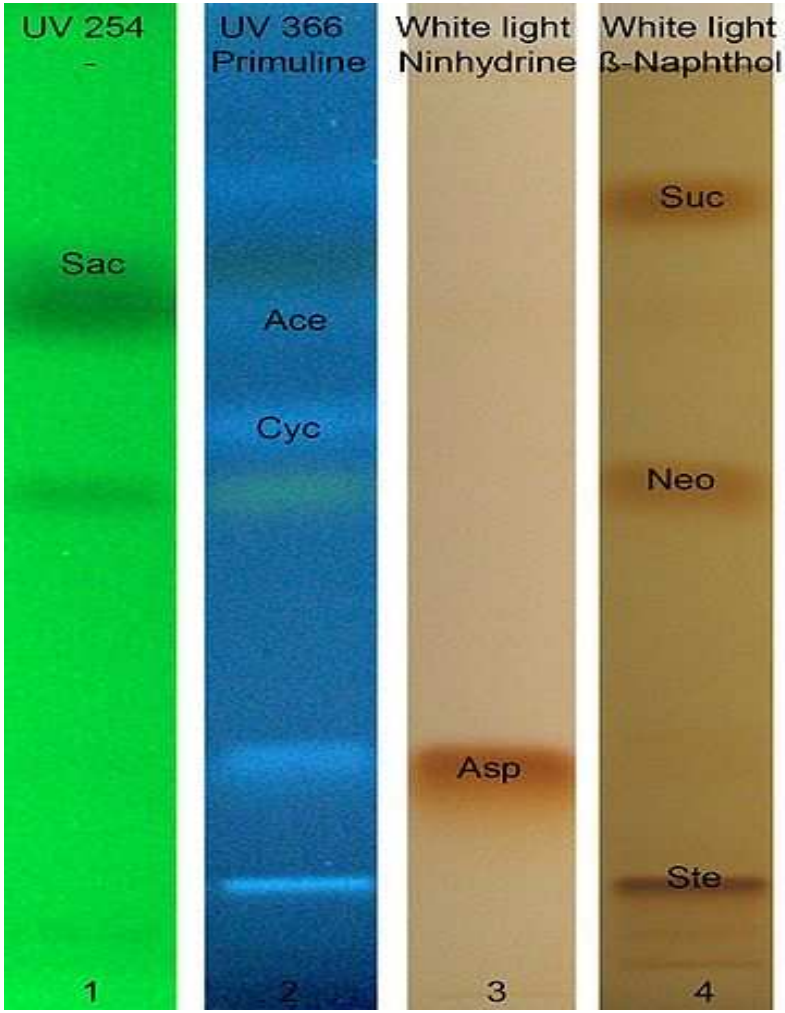
Benefit of reagent sequences

Detection of sweeteners allowed in EU



G. Morlock, S. Shabier, in submission 2010

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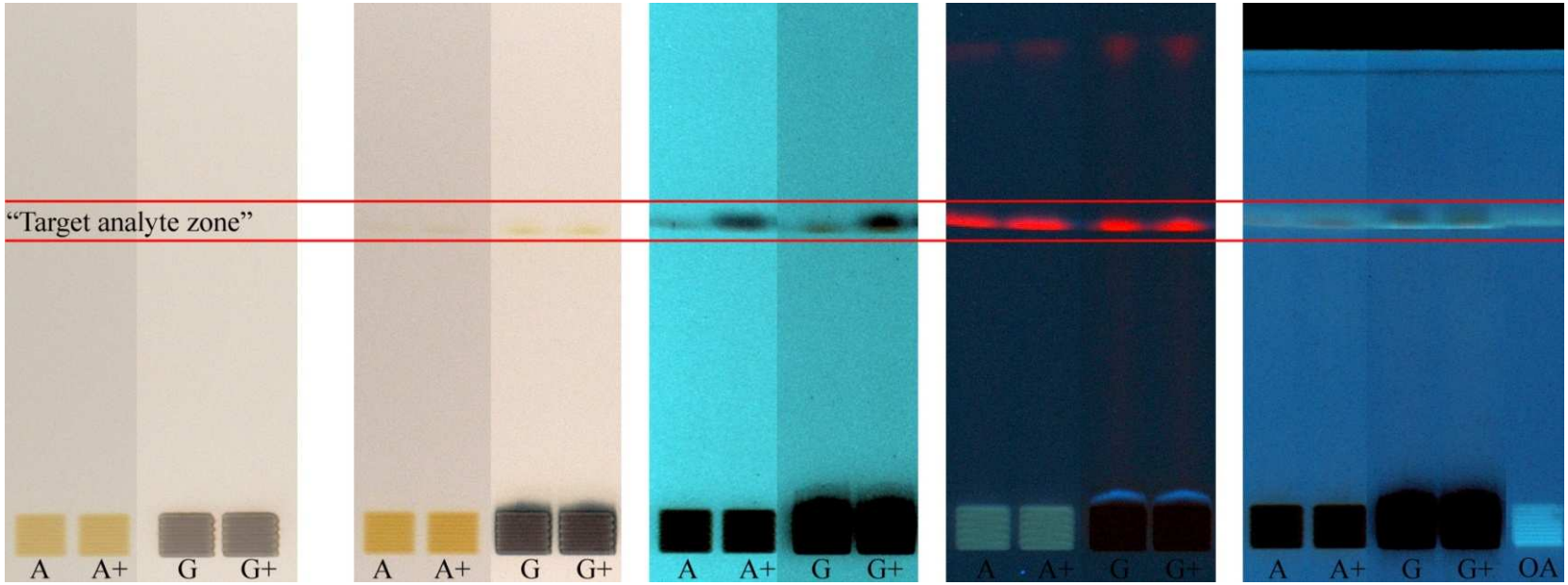
Sample preparation for many samples at one go

… and at low costs



C. Oellig, W. Schwack, Poster PA 085, EPRW Strasbourg, 2010

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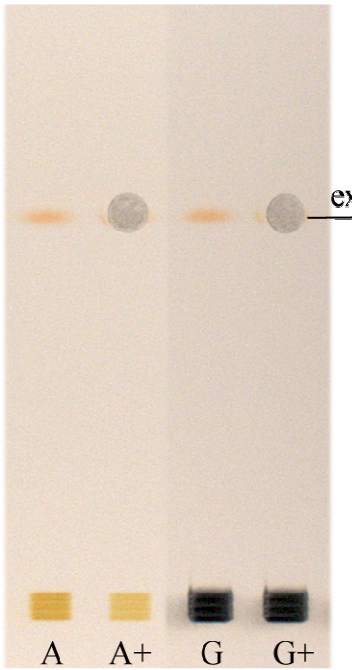
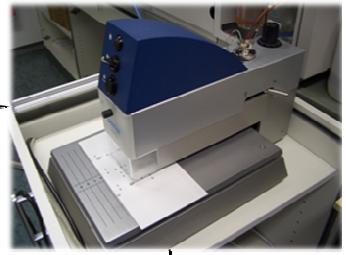
Sample preparation for many samples at one go…

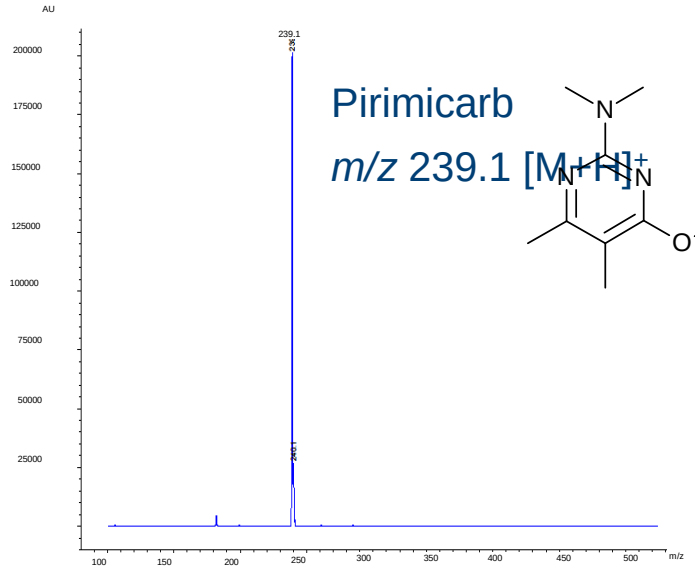
Grapes spiked with a mixture of pesticides of various substance classes



extraction



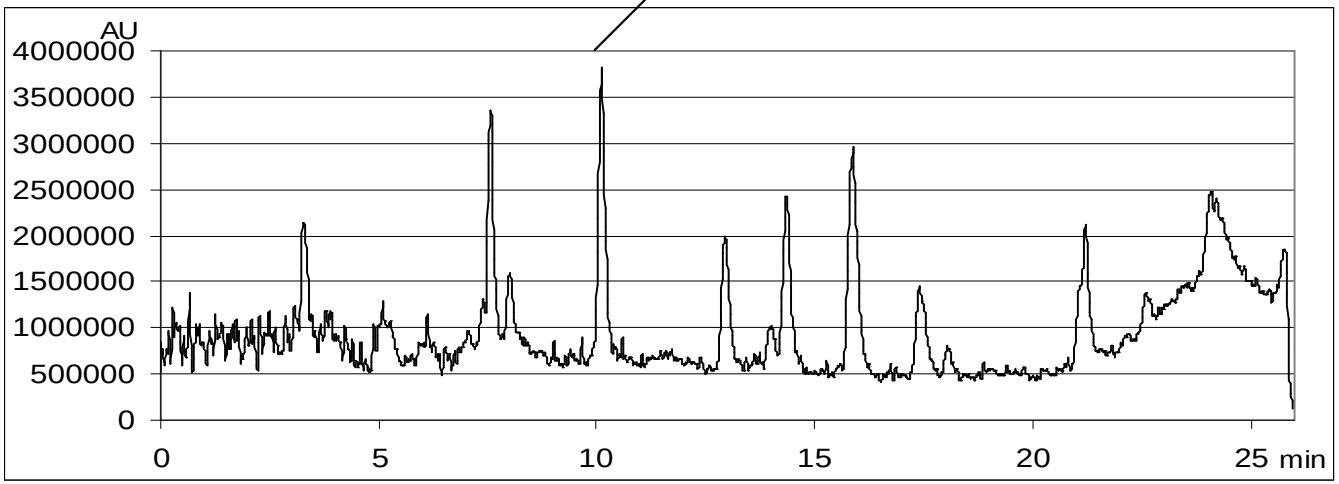
HPLC/ESI-MS



Pirimicarb

m/z 239.1 $[M+H]^+$

HPTLC-HPLC-MS (TIC)



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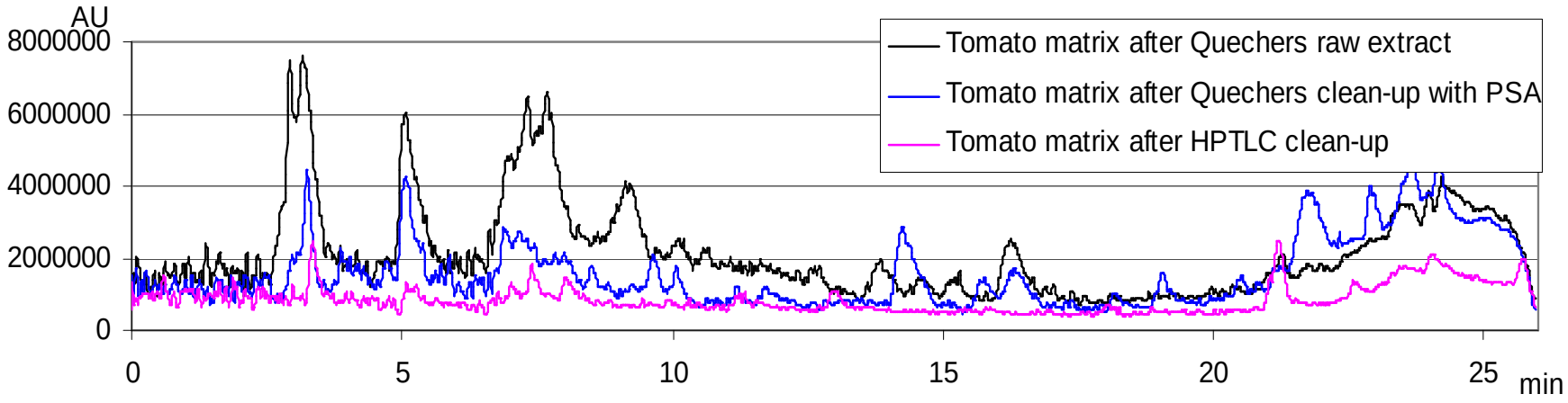
Sample preparation for many samples at one go…



- Solvent consumption of only 1 mL/sample
- Parallel chromatographic clean-up of over 20 extracts in 20 min
- Improved purification of the samples compared to existing methods

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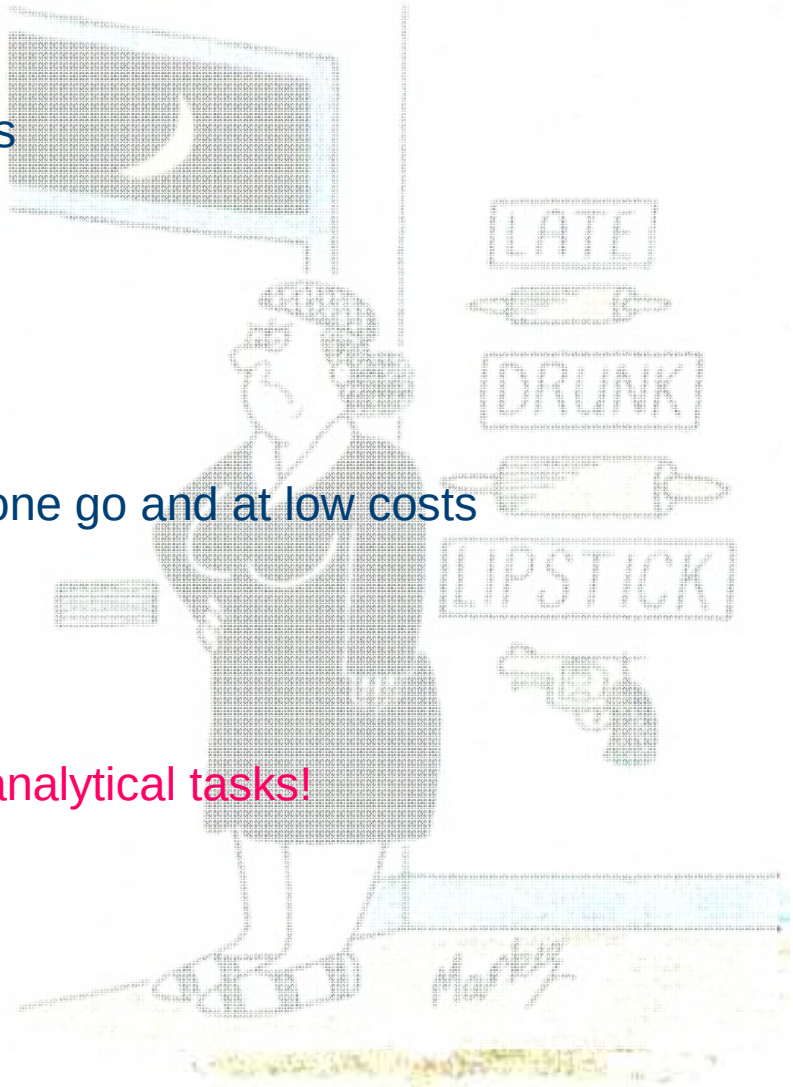

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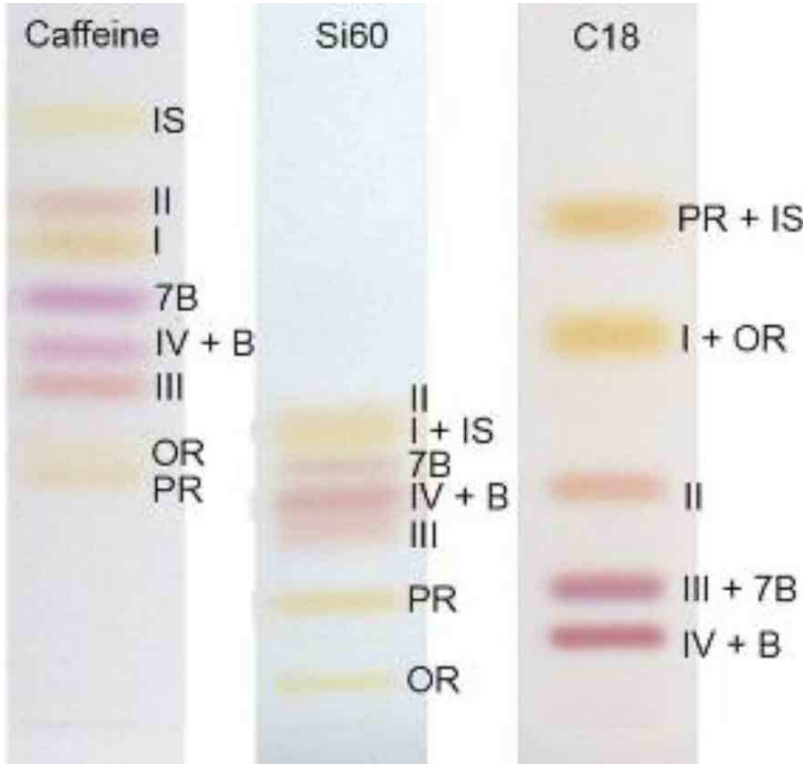
The utmost selectivity change is possible

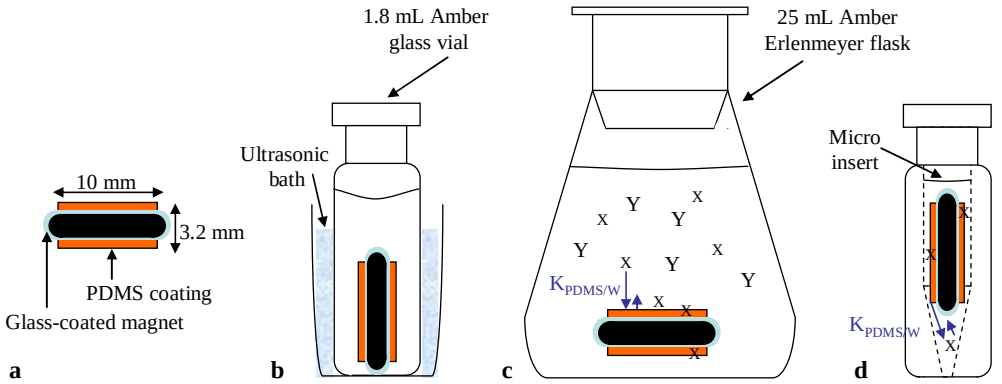


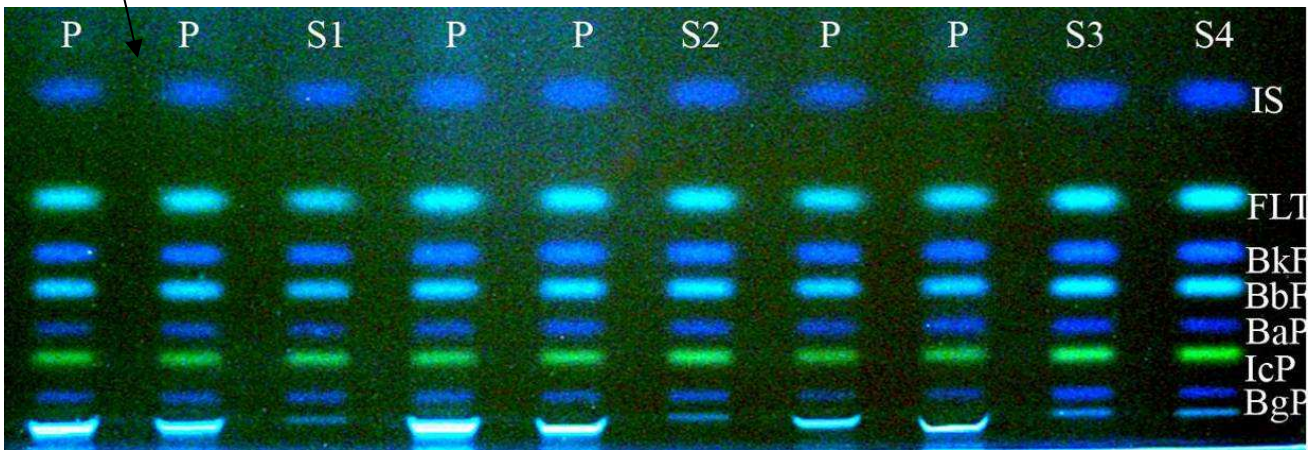
E. Pellissier, W. Schwack, CBS 103 (2009) 13-15

The utmost selectivity change is possible

Analysis of polyaromatic hydrocarbons (PAHs) in drinking water

Caffeine-impregnated plate



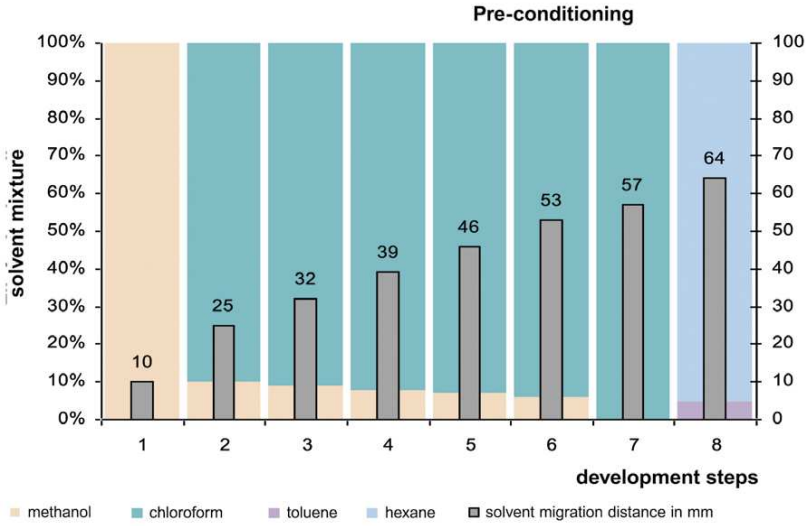


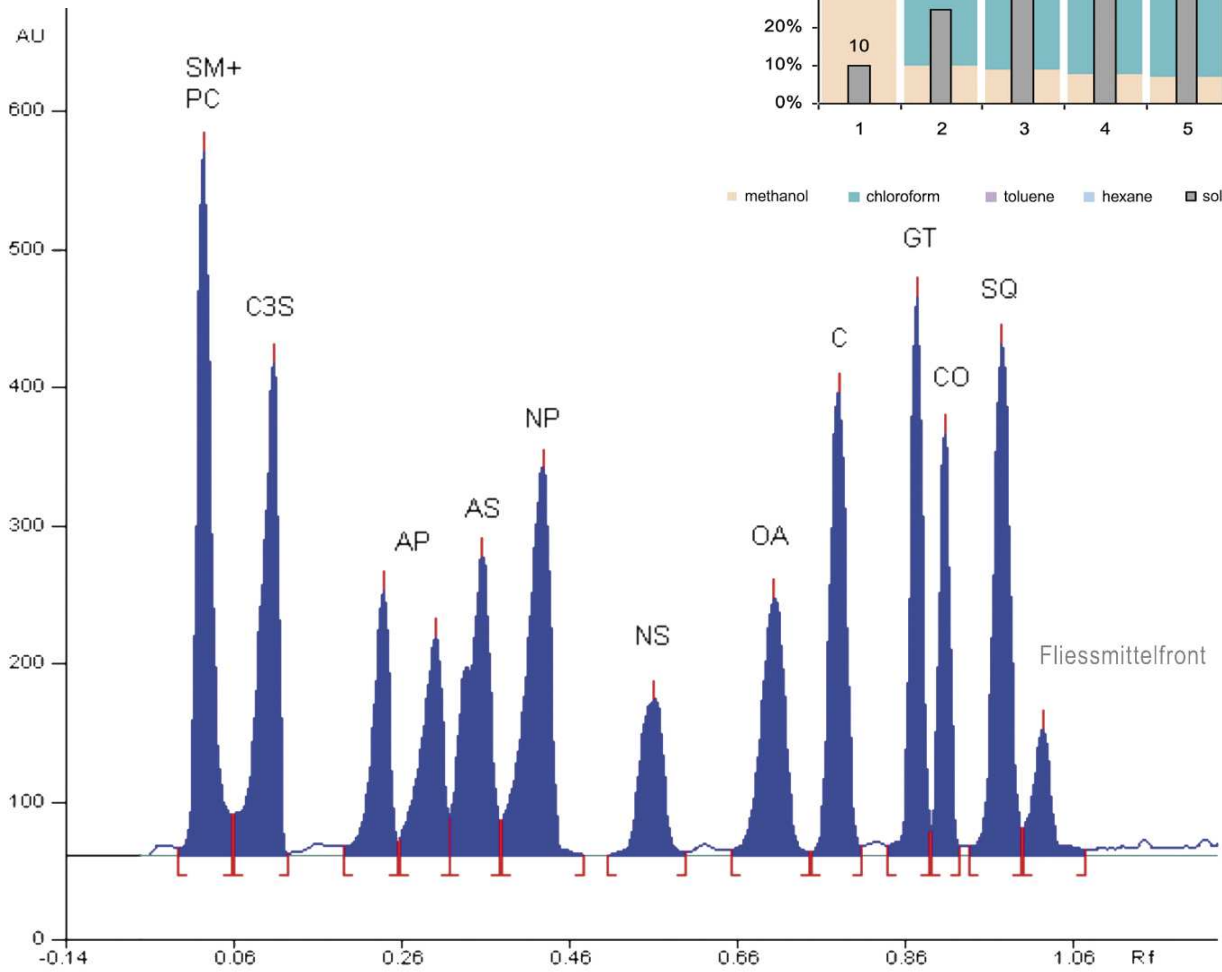
G. Morlock, S. Kopacz, J Liq Chromatogr Relat Technol 31 (2008) 1925-1942

The utmost selectivity change is possible

Ceramide analysis

AMD2 gradient with 8 steps → 1.5 h





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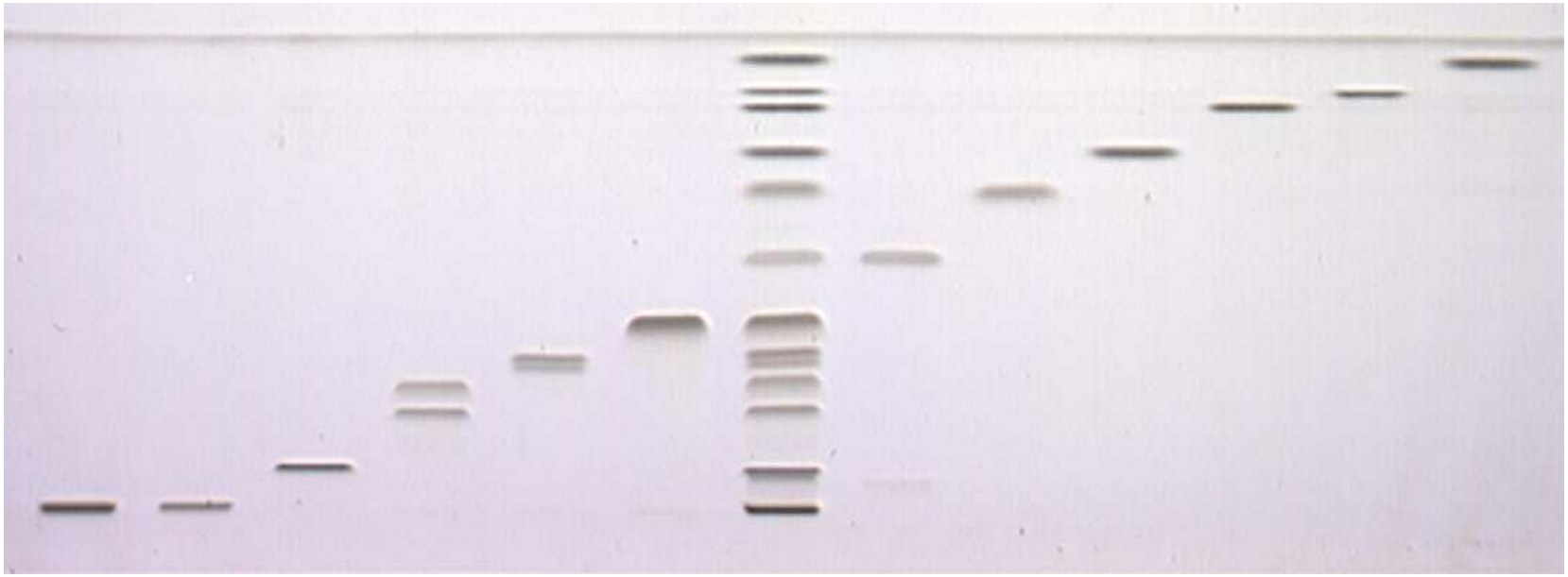
The utmost selectivity change is possible

with AMD!



The perfect solution for complex separations.

I. Schellenberg, K. Kabrodt, CBS 105 (2010) 5-7

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20 reasons to use HPTLC

Reasons 11-20:

11. Analytical workflow adjusted to the findings
12. The utmost matrix-tolerant method
13. Re-use of the stationary phase
14. Benefit of reagent sequences
15. Sample preparation for many samples at one go and at low costs
16. The utmost selectivity change is possible
17. The ease of super-hyphenations
18. The ever possible best solution for some analytical tasks!
19. Reactions on the plate
20. The creativity offer – unlimited

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Hyphenation

- 1980: term hyphenation by Hirschfeld
- comprises the different approaches to combine mainly spectrometers with chromatographic systems to get further information about the sample
- hyphen (-) symbolizes this attempt of combination, which did not reach its stage of full maturity so far
- slash (/) is found for hyphenated methods at a maturate state
- 2007: term “hypernation“ (super-hyphenation) by Wilson and Brinkman
  → to place all of the required spectrometers into a single system
  so that all of the spectroscopic information is obtained in a single run

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Hyphenation

Problems associated with column-based hypernations are:

- Capital cost and strategies for dealing with the large amounts of data produced by such systems.
- Complexity of the instrumentation increases → difficult to operate in routine
- A single eluent (→ optimal for all detectors) is difficult to obtain.
- Differences in sensitivity are challenging.

All these problems are less challenging in HPTLC-based hypernations:

- Open system is highly adaptive to different sensitivities
- Cost-effective by modular instrumentation
- Generating less data due to targeted access to points-of-care on the plate
- Directly accessible for the respective optimal solvent

=> The main difference:

HPLC: sample in solvent; after separation → sample in the waste

HPTLC: solvent evaporated; after separation → still on the plate & concentrated

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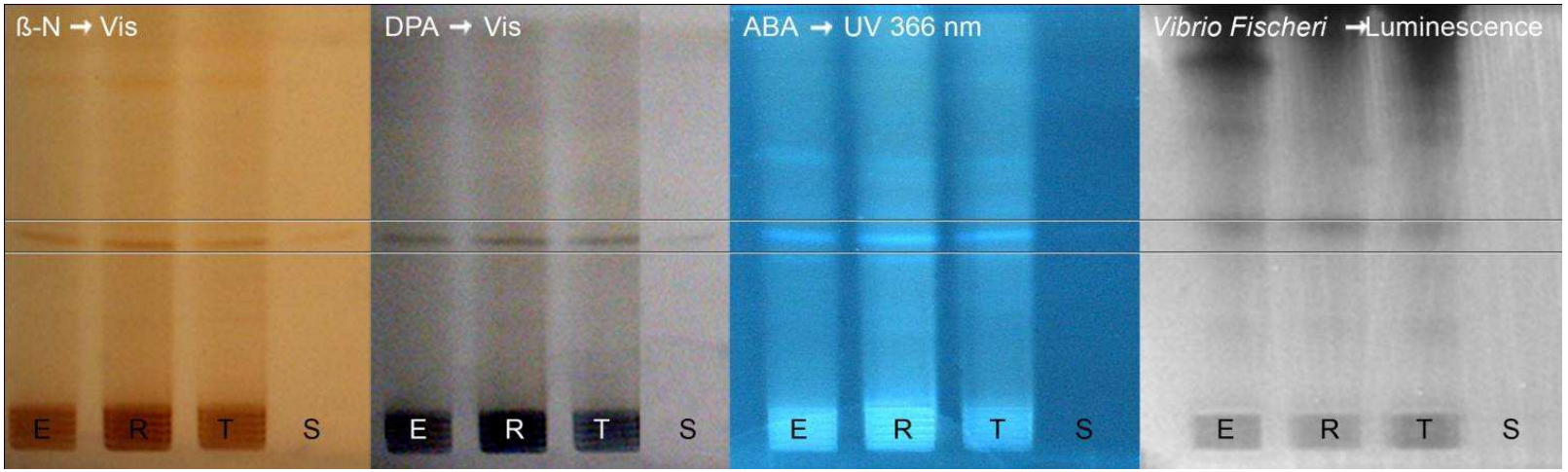
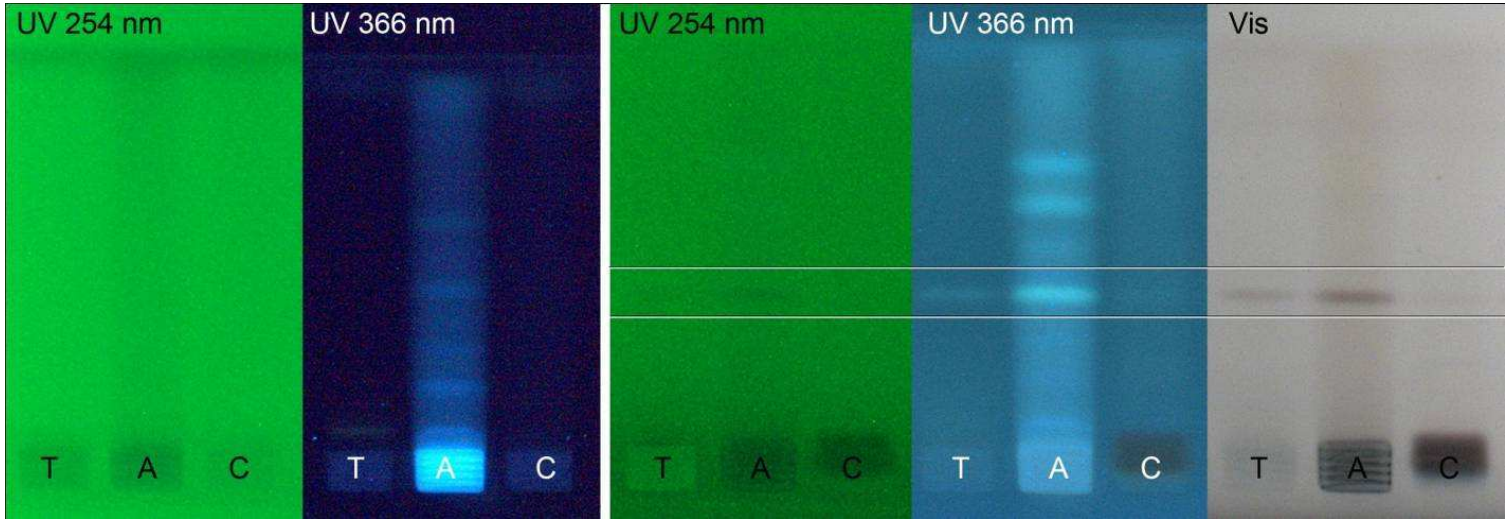
Multi detection

The choice of different derivatization reagents and bioassays



The open planar chromatogram allows multi detection (on the same plate!)



G Model
CHROMA-351018; No. of Pages 10

ARTICLE IN PRESS

Journal of Chromatography A, xxx (2010) xxx–xxx

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Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Hyphenations in planar chromatography

- HPTLC-UV/Vis/FLD-MS [13,14],
- HPTLC-UV/Vis/FLD-bioactivity-HRMS [15],
- HPTLC-UV-FTIR [16,17],
- HPTLC-UV/Vis/FLD-FTIR ATR [18],
- TLC-Vis-SERS [12].

Gertrud Morlock*, Wolfgang Schwack

University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Mass spectrometry
High-performance thin-layer chromatography
Effect-directed analysis
Bioassays
Cost-effective analysis
High-throughput system

ABSTRACT

This review is focused on planar chromatography and especially on its most important subcategory high-performance thin-layer chromatography (HPTLC). The image-giving format of the open, planar stationary phase and the post-chromatographic evaporation of the mobile phase ease the performance of various kinds of hyphenations and even super-hyphenations. Examples in the field of natural product search, food and lipid analysis are demonstrated, which point out the hyphenation with effect-directed analysis (EDA) and mass spectrometry and illustrate the efficiency gain. Depending on the task at hand, hyphenations can readily be selected as required to reach the relevant information about the sample, and at the same time, information is obtained for many samples in parallel. The flexibility and the unrivalled features through the planar format valuably assist separation scientists.

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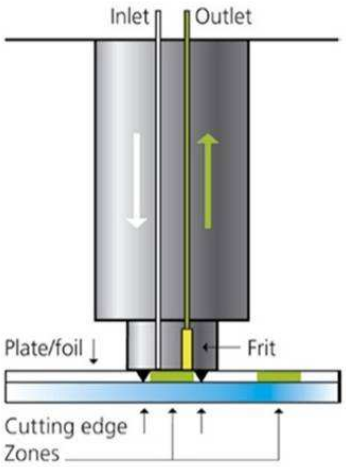
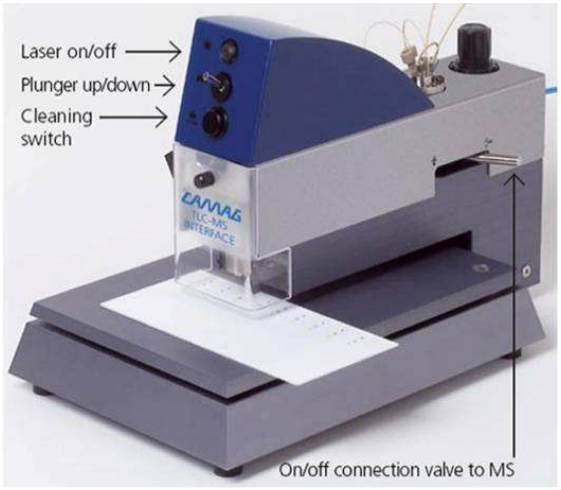
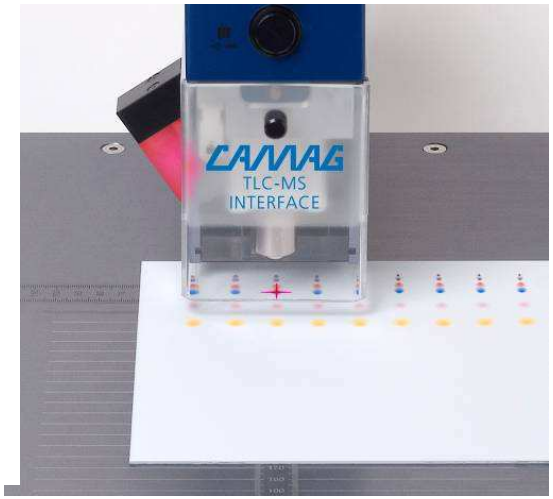
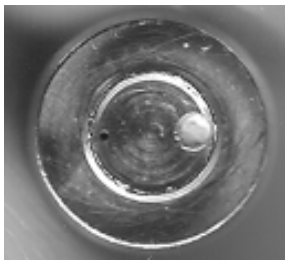
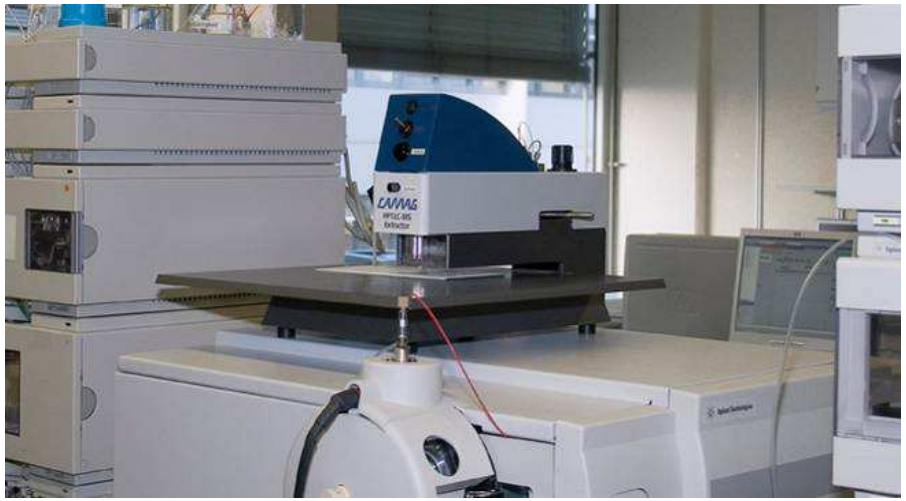
Elution head-based HPTLC-MS → TLC-MS Interface











H. Luftmann, Anal Bioanal Chem 378 (2004) 964-968

A. Alpmann, G. Morlock, Anal Bioanal Chem 386 (2006) 1543-1551

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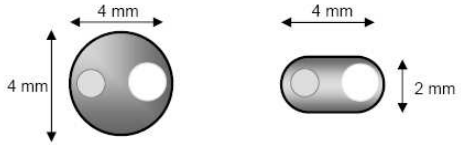
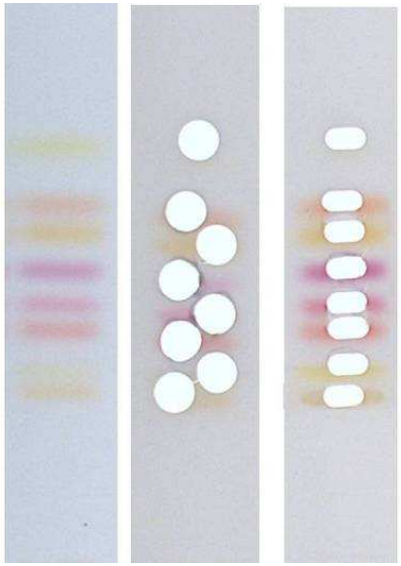
Elution head

Cutting edge geometry

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





Cutting edge height

- 0.2 mm for standard layers → CAMAG Bibliography Service CBS 102 (2009)
- 0.1 mm for extra thin layers → U. Jautz, G. Morlock, Anal Bioanal Chem 387 (2007) 1083
- 0.5 mm for preparative layers → E. Dytkiewitz, G. Morlock, J AOAC Int 91 (2008) 1237

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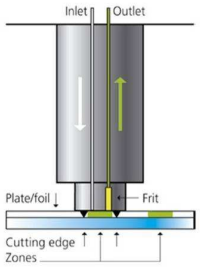
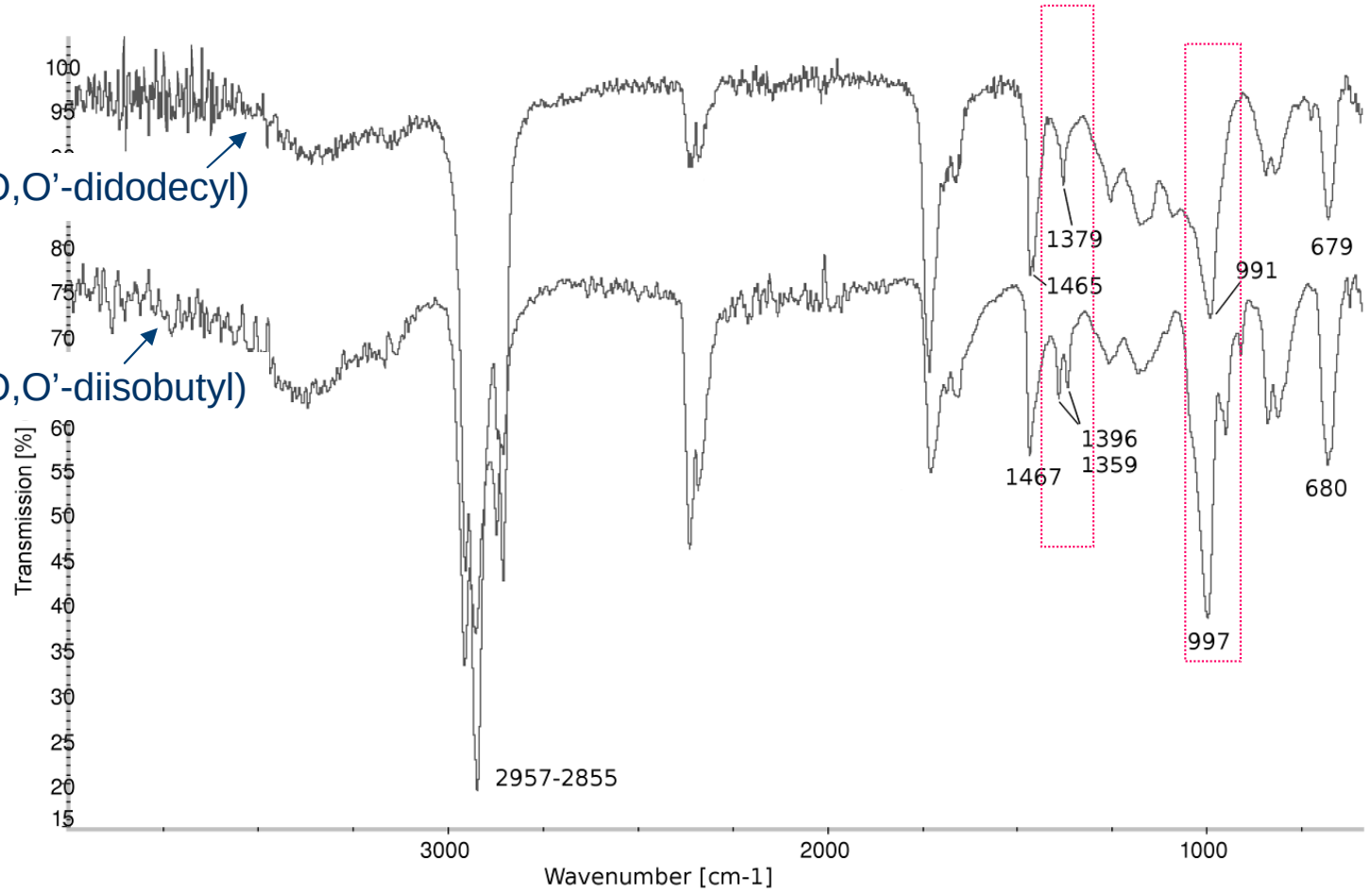
HPTLC/ATR-FTIR spectra using the TLC-MS Interface



Additives in mineral oil

Zink bis(O,O'-didodecyl)

Zink bis(O,O'-diisobutyl)



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

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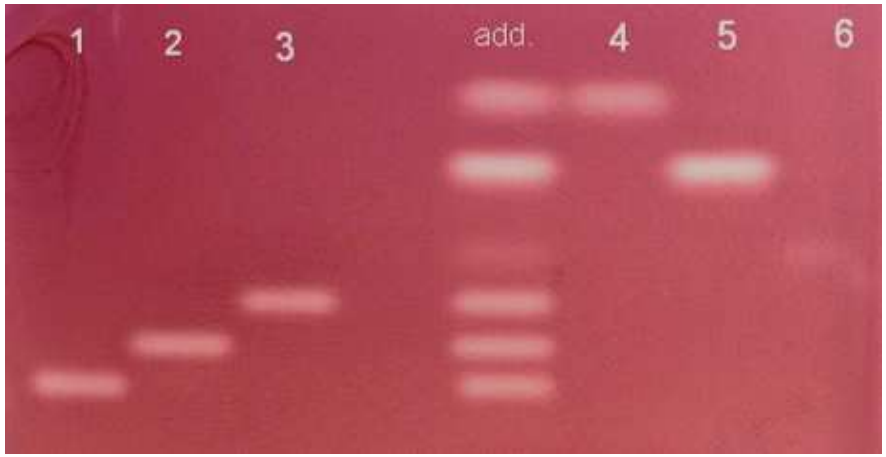
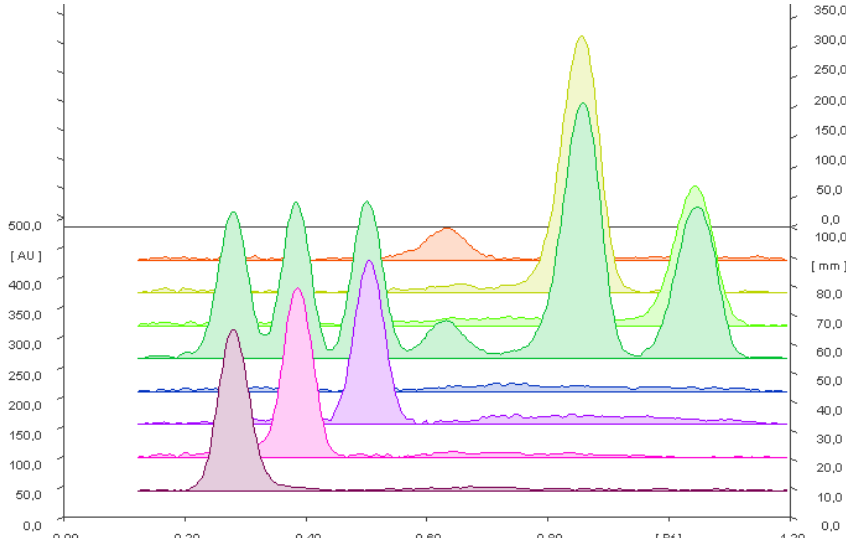
HPTLC-VIS/UV/FLD-EDA

Cholinesterase inhibiting pesticides by esterases

→ detectability down to 2 pg/zone
→ using an esterase and substrate (1-naphthylacetate/fast blue salt B) solution
→ white zones on a pink background



1. Paraoxon-methyl, 2. malaoxon, 3. paraoxon,
4. ethiofencarb, 5. chlorfenvinfos, 6. dichlorvos



Absorption measurement at 533 nm

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

Effect-directed analysis → sum parameter!

DEUTSCHE NORM Mai 2009

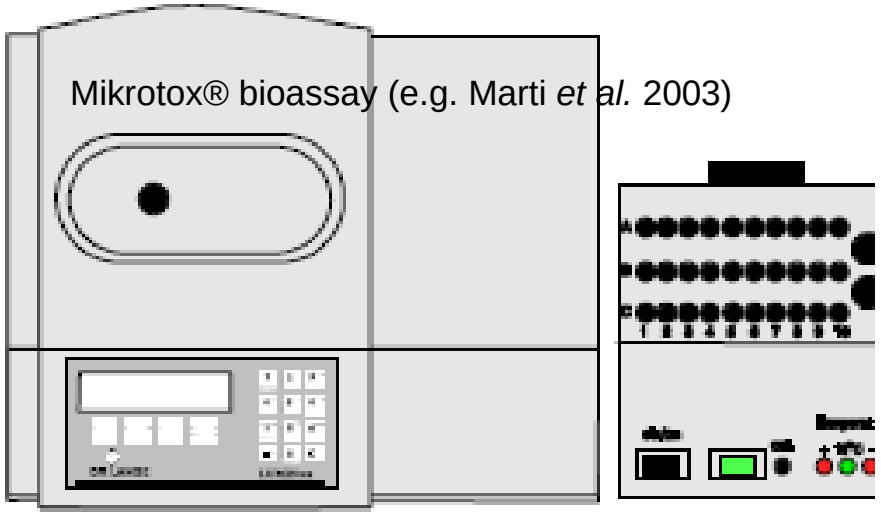
DIN EN ISO 11348-2

DIN

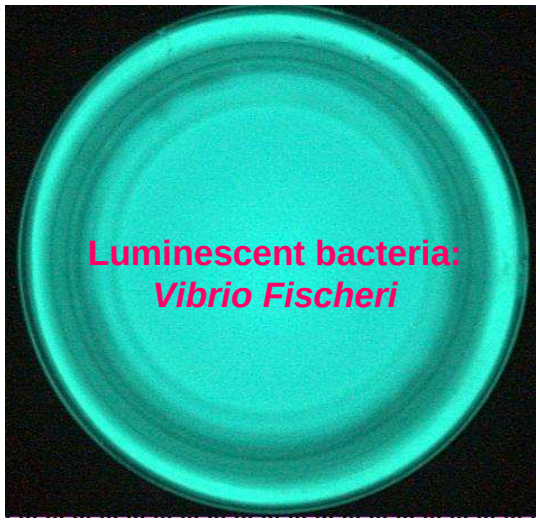
ICS 13.060.70

Ersatz für
DIN EN ISO 11348-2:1999-04

**Wasserbeschaffenheit –
Bestimmung der Hemmwirkung von Wasserproben auf die Lichtemission von *Vibrio fischeri* (Leuchtbakterientest) –
Teil 2: Verfahren mit flüssig getrockneten Bakterien (ISO 11348-2:2007);
Deutsche Fassung EN ISO 11348-2:2008**



Mikrotox® bioassay (e.g. Marti *et al.* 2003)



**Luminescent bacteria:
*Vibrio Fischeri***

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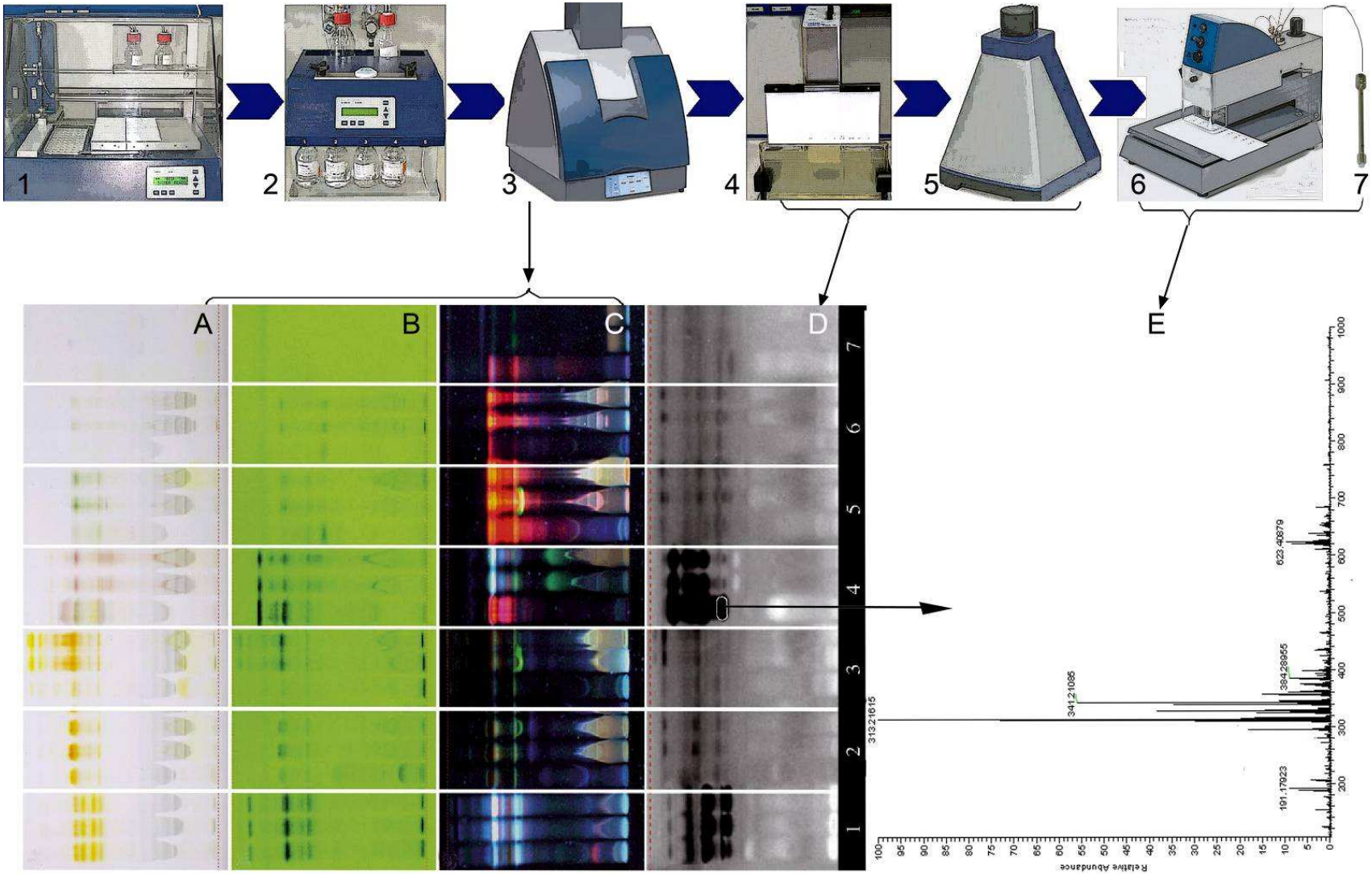
HPTLC-VIS/UV/FLD-EDA-HRMS



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

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

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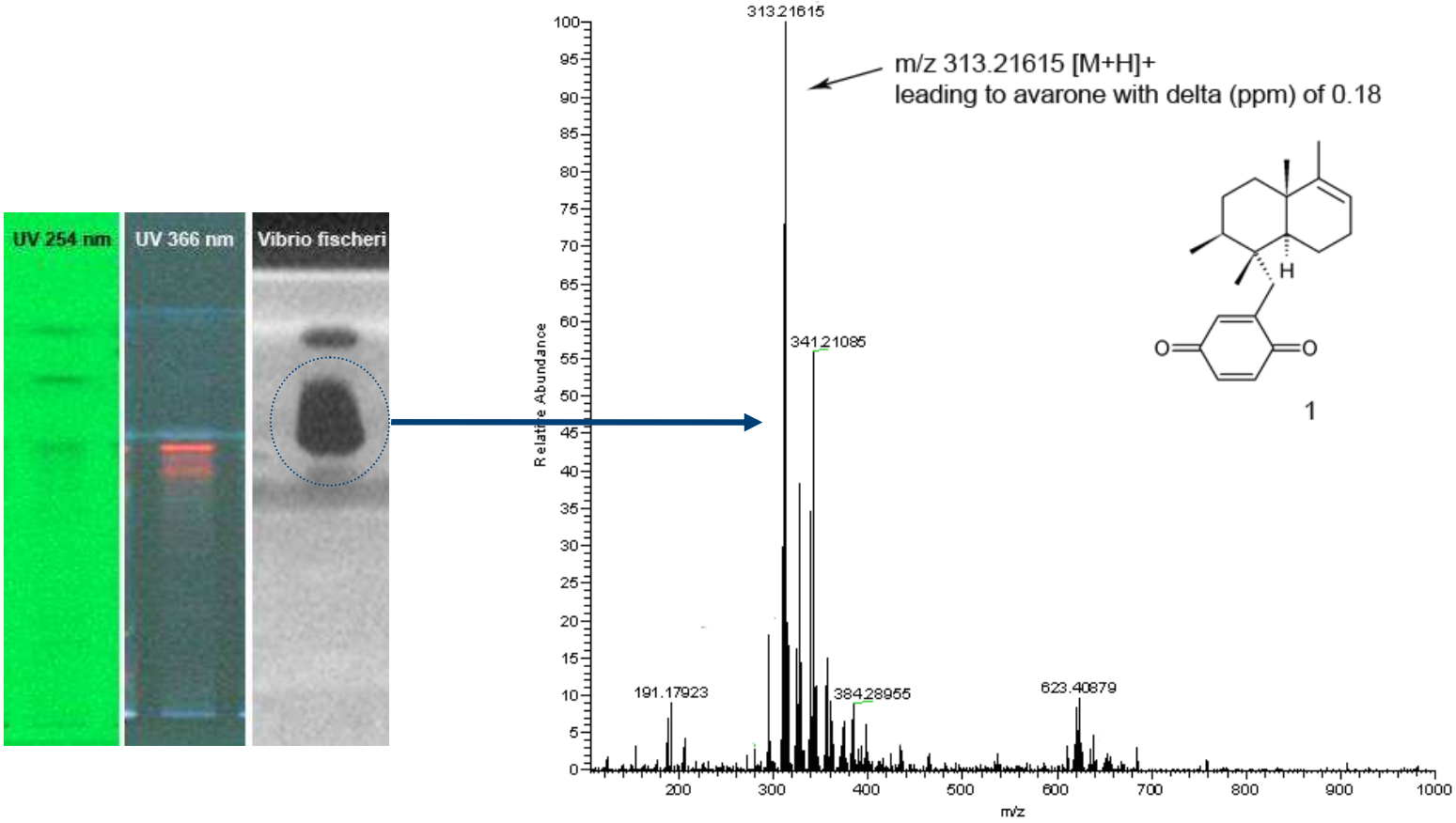
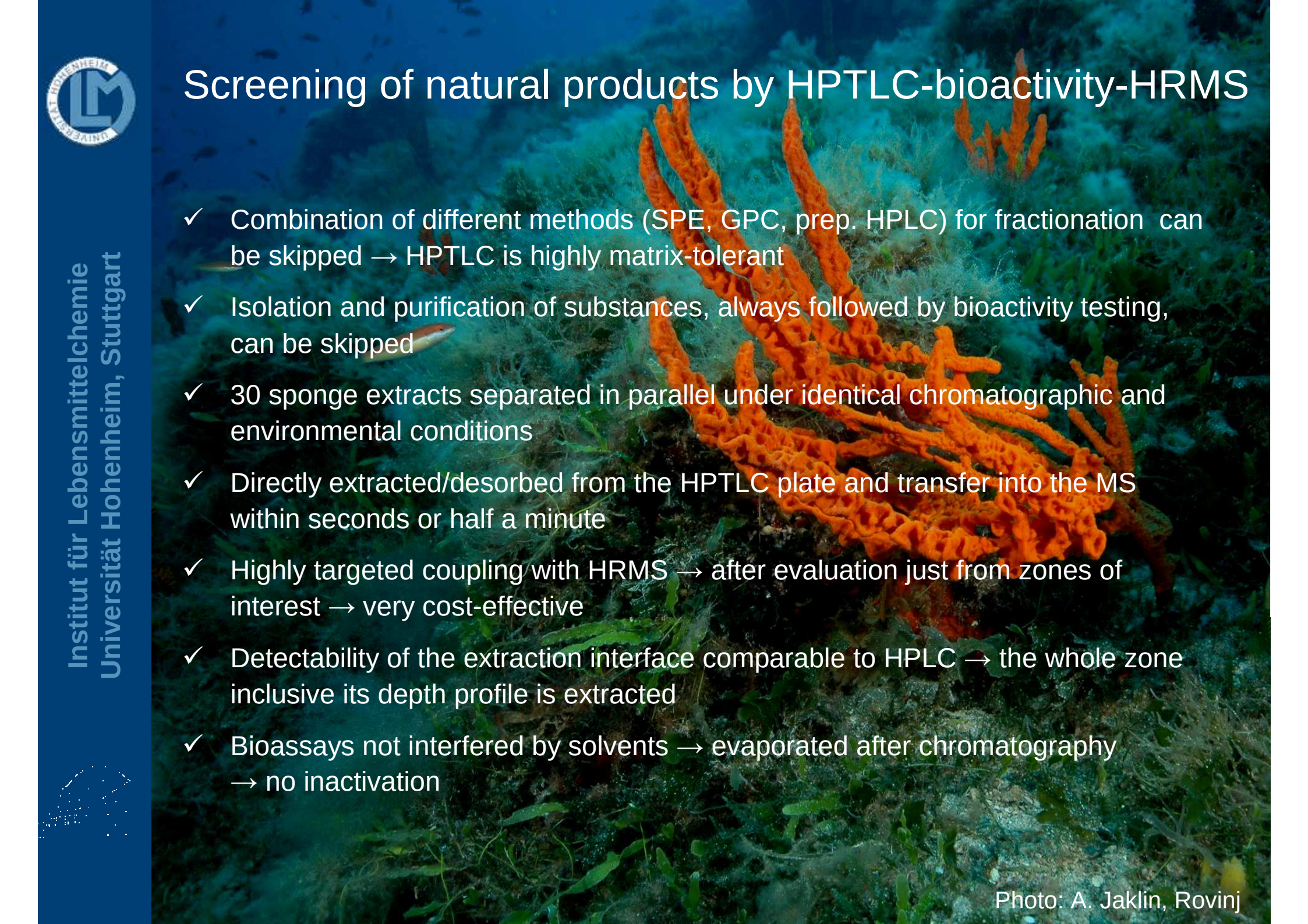
What is it? → HRMS



G. Morlock, W. Schwack LCGC Eur 21 (2008) 366-371



Screening of natural products by HPTLC-bioactivity-HRMS

- ✓ Combination of different methods (SPE, GPC, prep. HPLC) for fractionation can be skipped → HPTLC is highly matrix-tolerant
- ✓ Isolation and purification of substances, always followed by bioactivity testing, can be skipped
- ✓ 30 sponge extracts separated in parallel under identical chromatographic and environmental conditions
- ✓ Directly extracted/desorbed from the HPTLC plate and transfer into the MS within seconds or half a minute
- ✓ Highly targeted coupling with HRMS → after evaluation just from zones of interest → very cost-effective
- ✓ Detectability of the extraction interface comparable to HPLC → the whole zone inclusive its depth profile is extracted
- ✓ Bioassays not interfered by solvents → evaporated after chromatography → no inactivation

Photo: A. Jaklin, Rovinj

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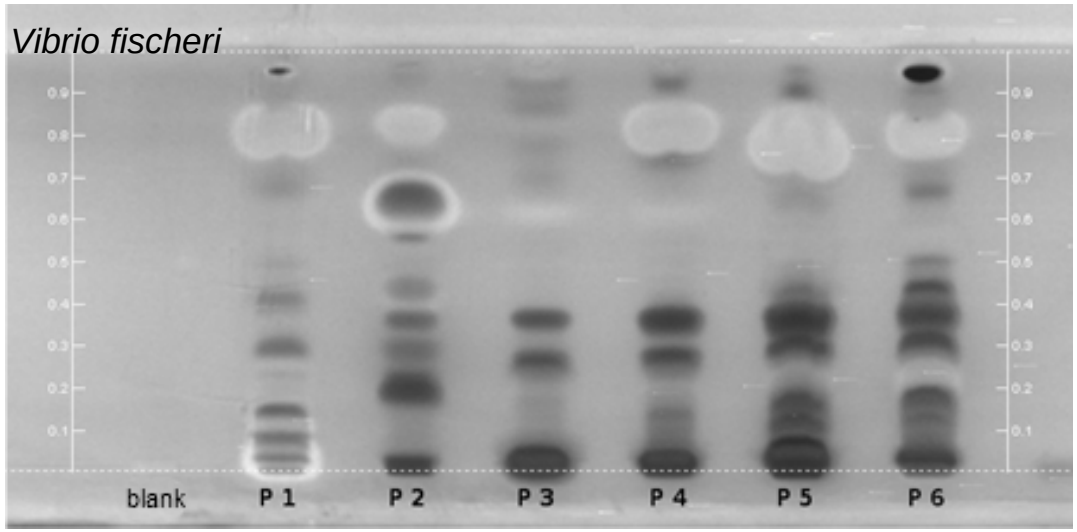
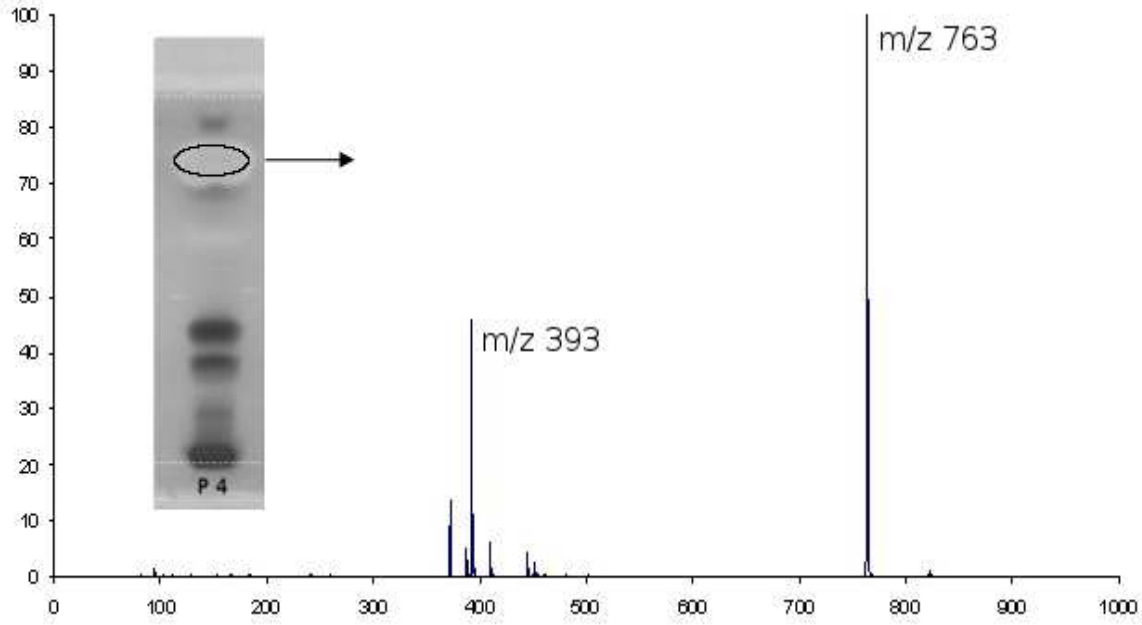
Additives in food packaging foils





E. Dytkiewitz, W. Schwack, CBS 105 (2010) 12-15

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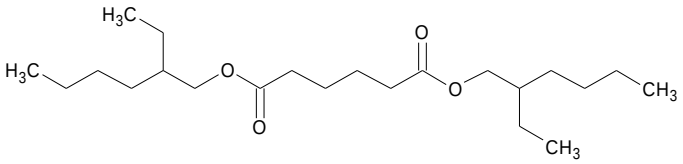
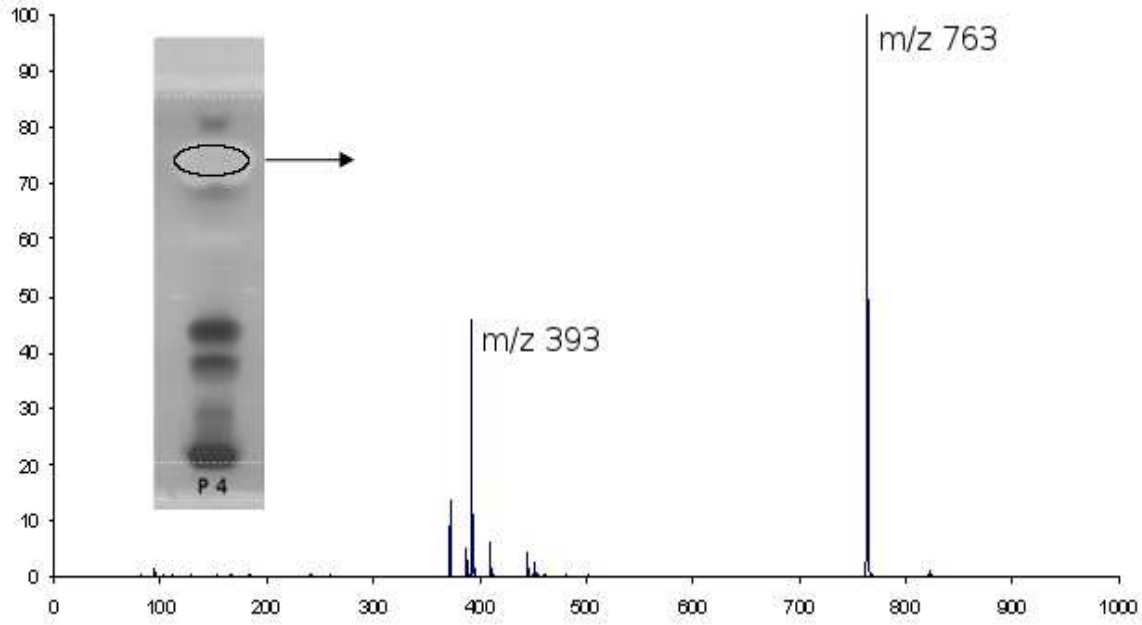
HPTLC-VIS/UV/FLD-EDA-MS



Bis-2-ethylhexyladipate

MS signals of	Mass determined	Mass theoretical	Δ (ppm)	Sum formula	Assignment
HPTLC zone	393,2985	393,2981	-1,0691	$C_{22}H_{42}O_4Na$	$[M+Na]^+$
	763,6077	763,6064	-1,7164	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$



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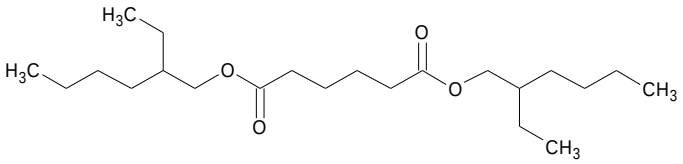
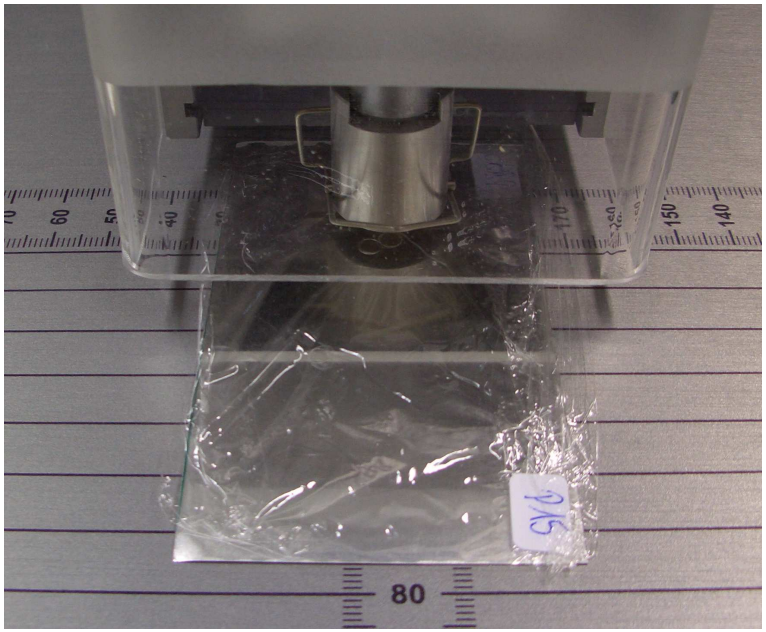
HPTLC-VIS/UV/FLD-EDA-MS



Bis-2-ethylhexyladipate

MS signal of	Mass determined	Mass theoretical	Δ (ppm)	Sum formula	Assignment
Plastic foil	**371,3174**	**371,3161**	**-3,4071**	**$C_{22}H_{43}O_4$**	**$[M+H]^+$**
HPTLC zone	393,2985	393,2981	-1,0691	$C_{22}H_{42}O_4Na$	$[M+Na]^+$
	763,6077	763,6064	-1,7164	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$



Coupling of planar chromatography to mass spectrometry

Gertrud Morlock, Wolfgang Schwack

Coupling of planar chromatography to mass spectrometry (MS) and especially ambient MS is a relatively new field of great interest. The direct sample access at ambient conditions and the feasibility to obtain mass spectra free of contamination within a minute or even within seconds highly contributes to the progress of planar chromatography. Targeted recording of mass spectra on zones of interest is performed after evaluation of the chromatogram, thus providing high efficiency. Reported approaches for coupling are divided into elution-based and desorption-based techniques. Devices of both categories are commercially available. As a consequence of increasing importance, a rethink of the terminology of liquid chromatography with MS has to be considered.

Keywords: Ambient mass spectrometry; Cost-effective analysis; Coupling to mass spectrometry; Desorption-based technique; Elution-based technique; High-performance thin-layer chromatography; HPTLC-MS; Planar chromatography; Thin-layer chromatography

Gertrud Morlock*, Wolfgang Schwack
University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany

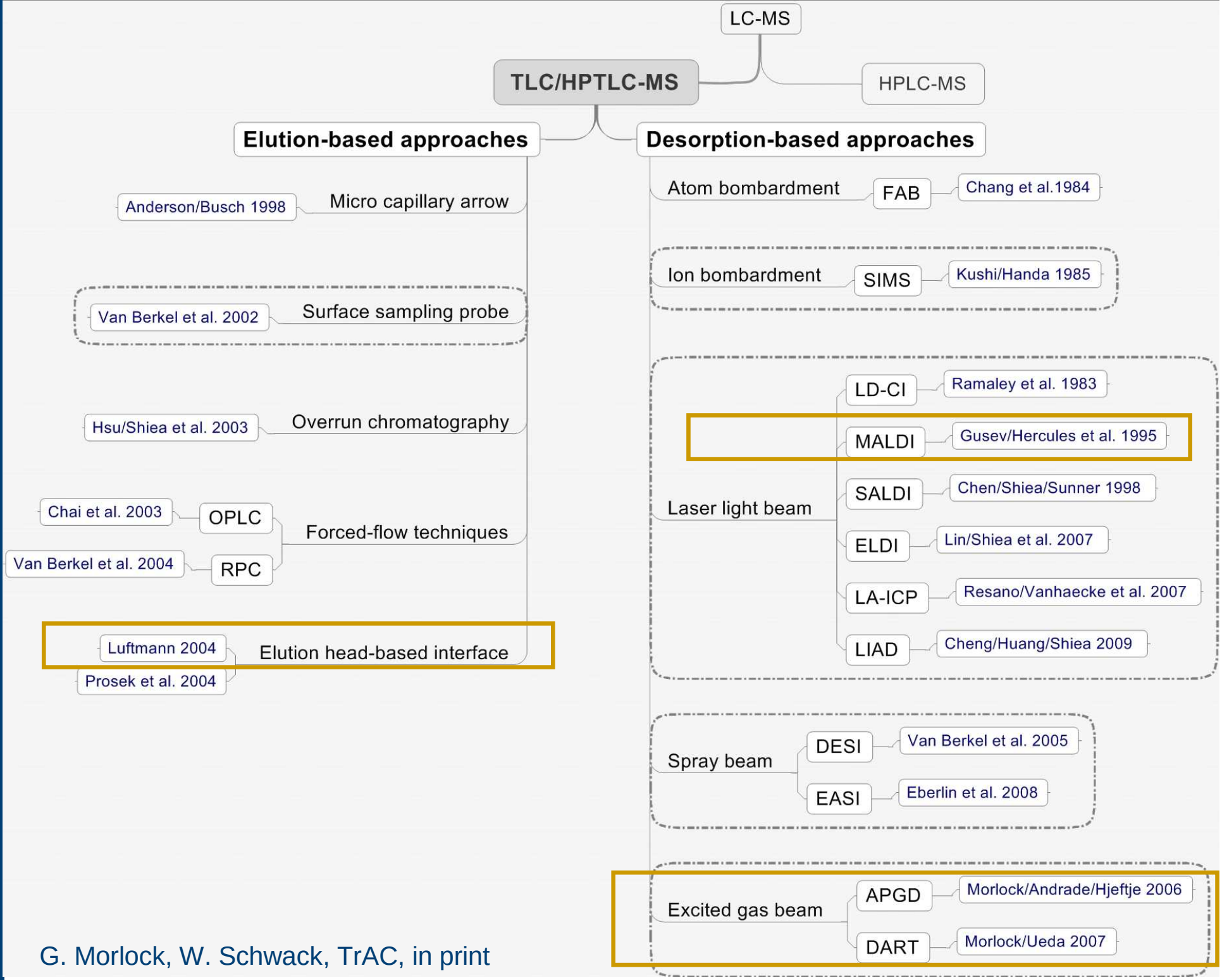
1. Introduction

Planar chromatography comprises all chromatographic techniques, which have an open planar stationary phase present as or on a plane [1]. Therein, simple thin-layer chromatography (TLC) is the most widespread chromatographic technique; whereas high-performance TLC (HPTLC) is considered as the most efficient and powerful planar chromatographic technique, with optimized coating material (lower particle size and narrower particle-size distribution) combined with advanced instrumentation for most of the steps of the chromatographic process [2]. Paper chromatography is not used very much at present. Since 1970, comprehensive reviews of planar chromatographic publications have been reported biennially by Sherma [3,4].

the MS by means of a specially-built inlet probe [6].

Since that time, the spectrometry market has continued to grow and column chromatography has been brought forward, but coupling of an open planar system with MS required more effort than column-derived techniques. Although reviews about TLC-MS were regularly reported by Busch [7–10] or Wilson [11–13], it was not until the past decade that it attracted interest because of several successful approaches and the invention of ion sources working under ambient conditions and atmospheric pressure, which enormously eased the introduction of a planar object.

2. Categorization of TLC/HPTLC-MS approaches



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

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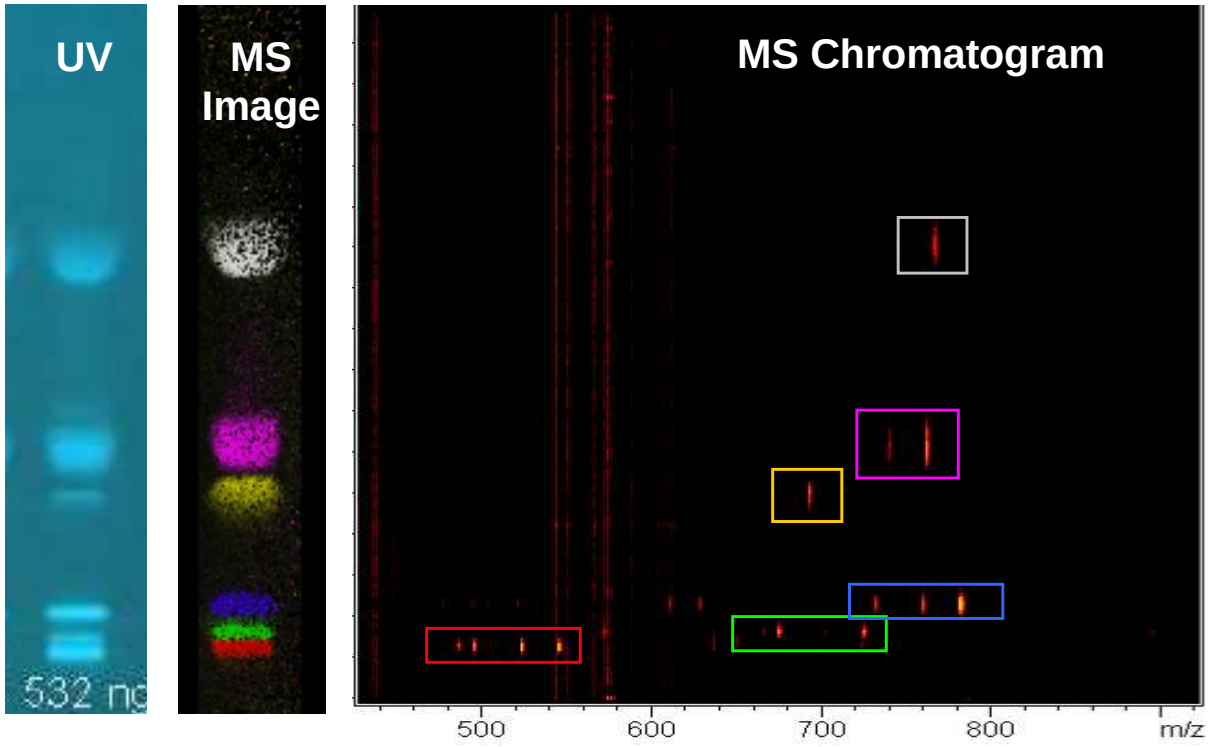
HPTLC-FLD-MALDI-TOF MS



M. Schuerenberg *et al.,* IMSC 2009, Bremen, Poster PMM 386

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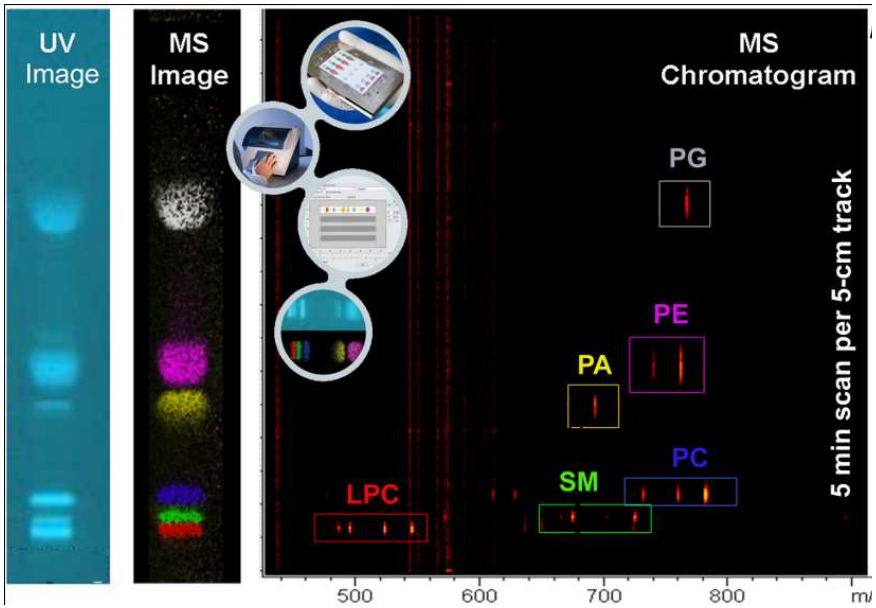
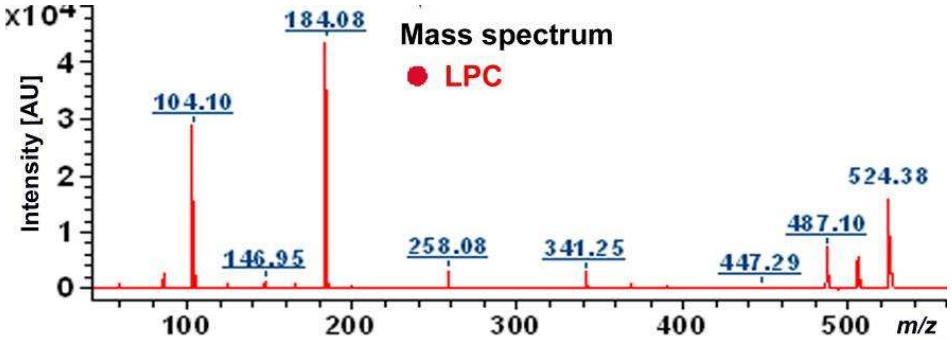
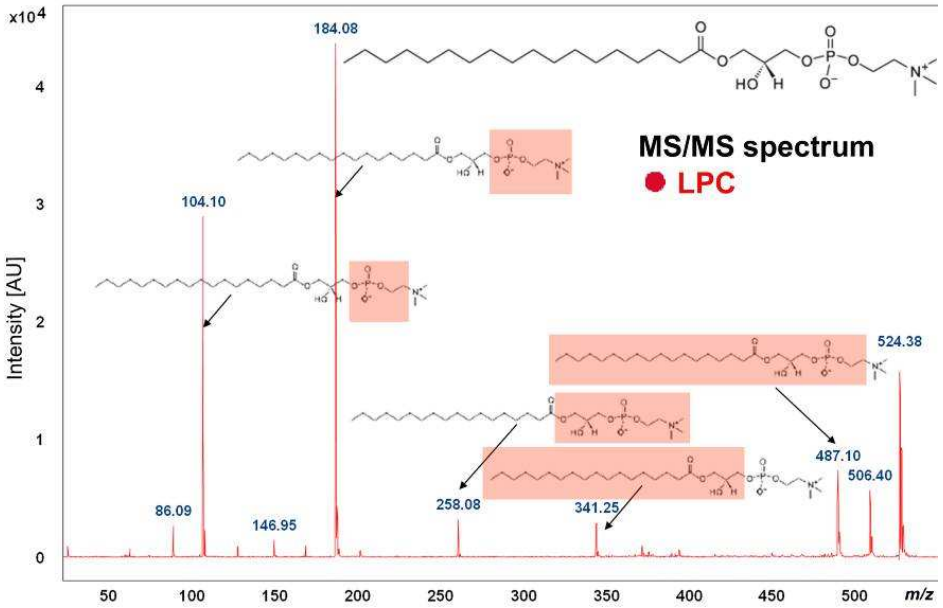
HPTLC-FLD-MALDI-TOF MS







Bruker Daltonics Application Note MT-101

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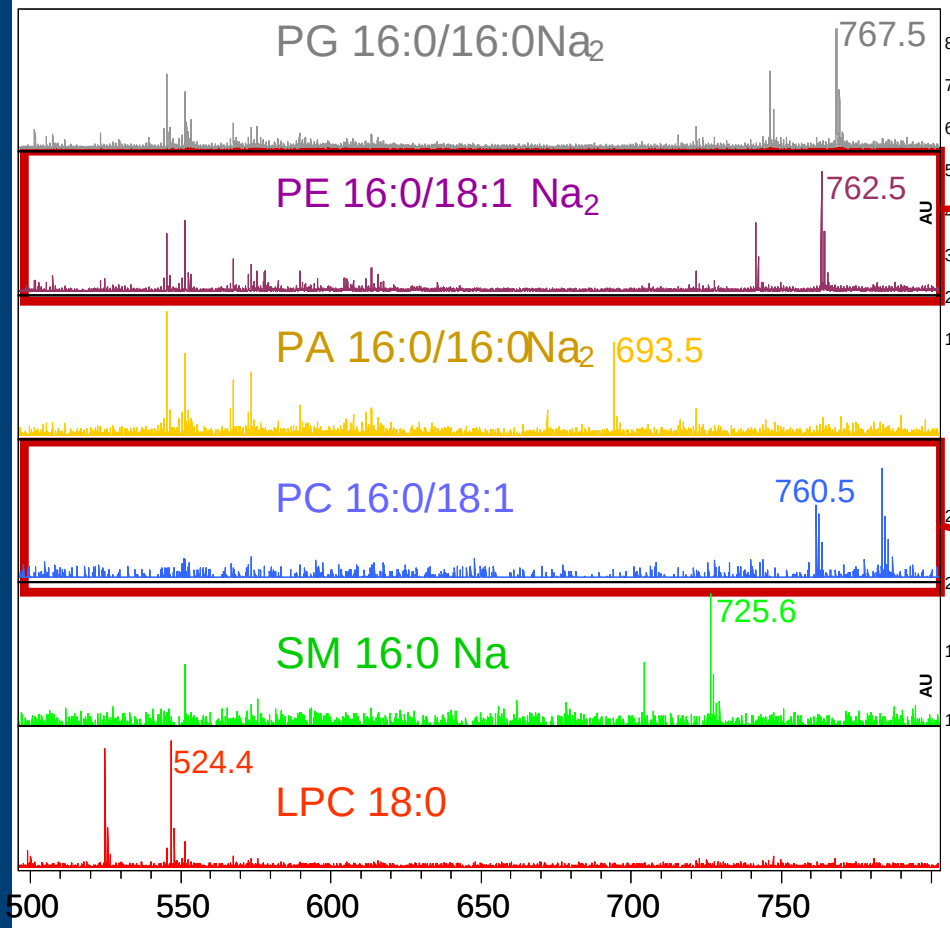
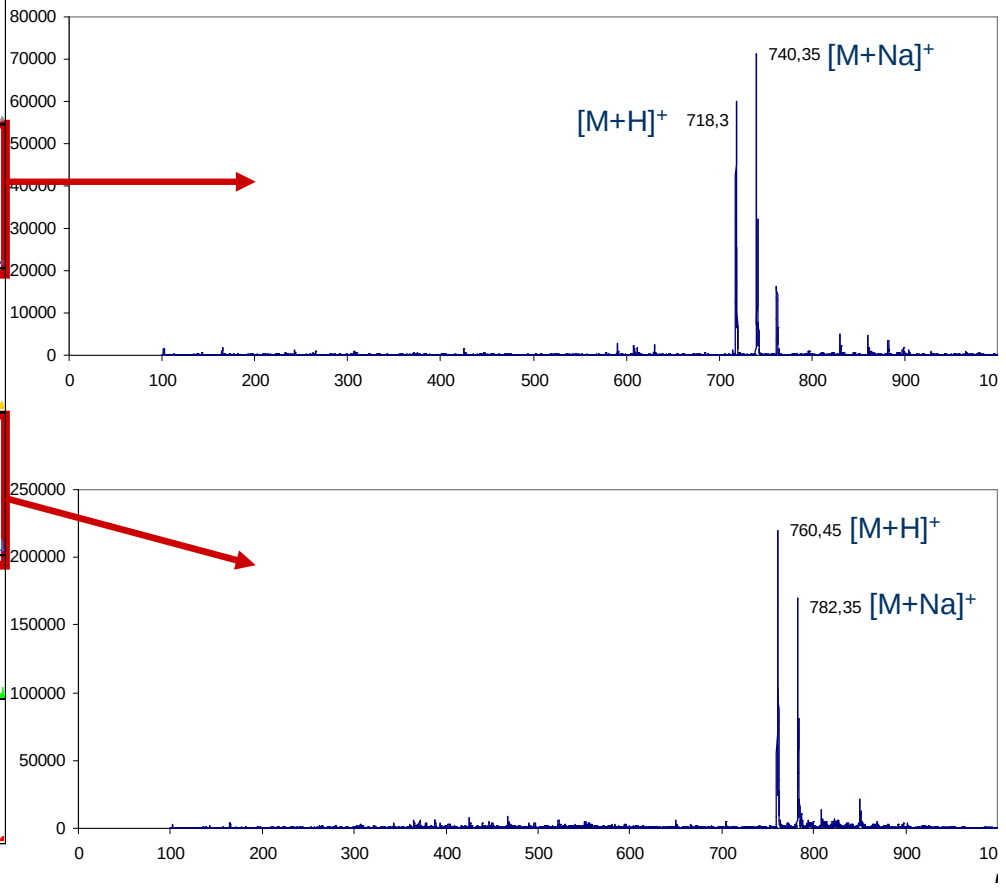
Comparison of mass spectra

HPTLC-FLD-MALDI-TOF MS



HPTLC-FLD-ESI-MS



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20 reasons to use HPTLC

Reasons 11-20:

11. Analytical workflow adjusted to the findings
12. The utmost matrix-tolerant method
13. Re-use of the stationary phase
14. Benefit of reagent sequences
15. Sample preparation for many samples at one go and at low costs
16. The utmost selectivity change is possible
17. The ease of super-hyphenations
18. The ever possible best solution for some analytical tasks!
19. Reactions on the plate
20. The creativity offer – unlimited

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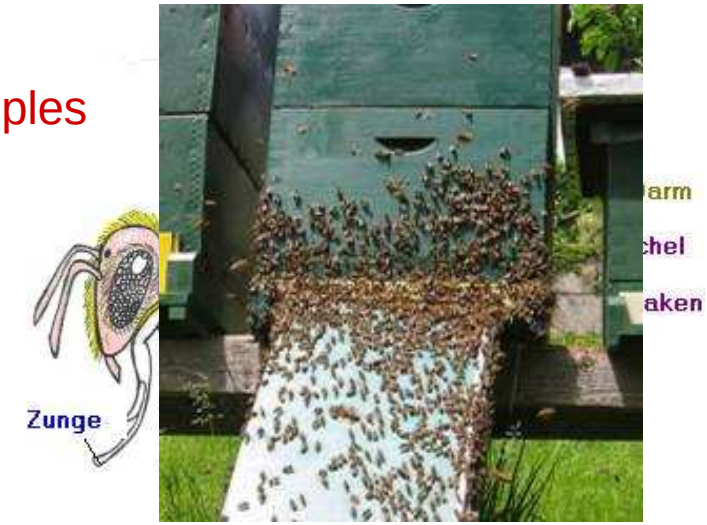
Carbohydrates in bee's honey cyst?

→ Differentiation between nectar- and honey-collecting bees

- Number of samples 700/batch
- Analysis of 4 batches

} 2800 samples



HPLC-ELSD!

- 10,-/sample => 28.000,-
  15 min/sample => 725 h => Instumentation is 5 weeks blocked!

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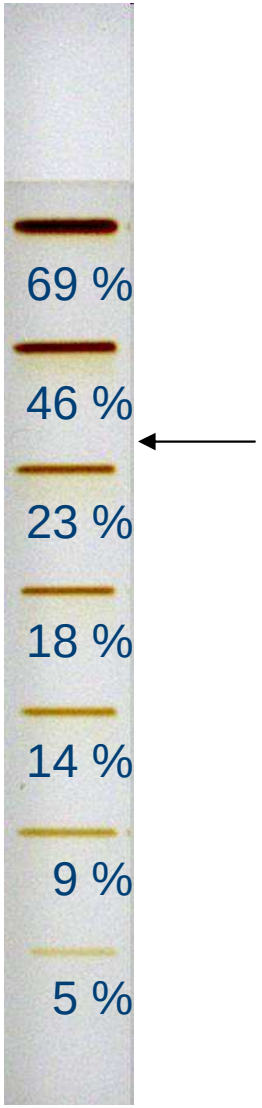
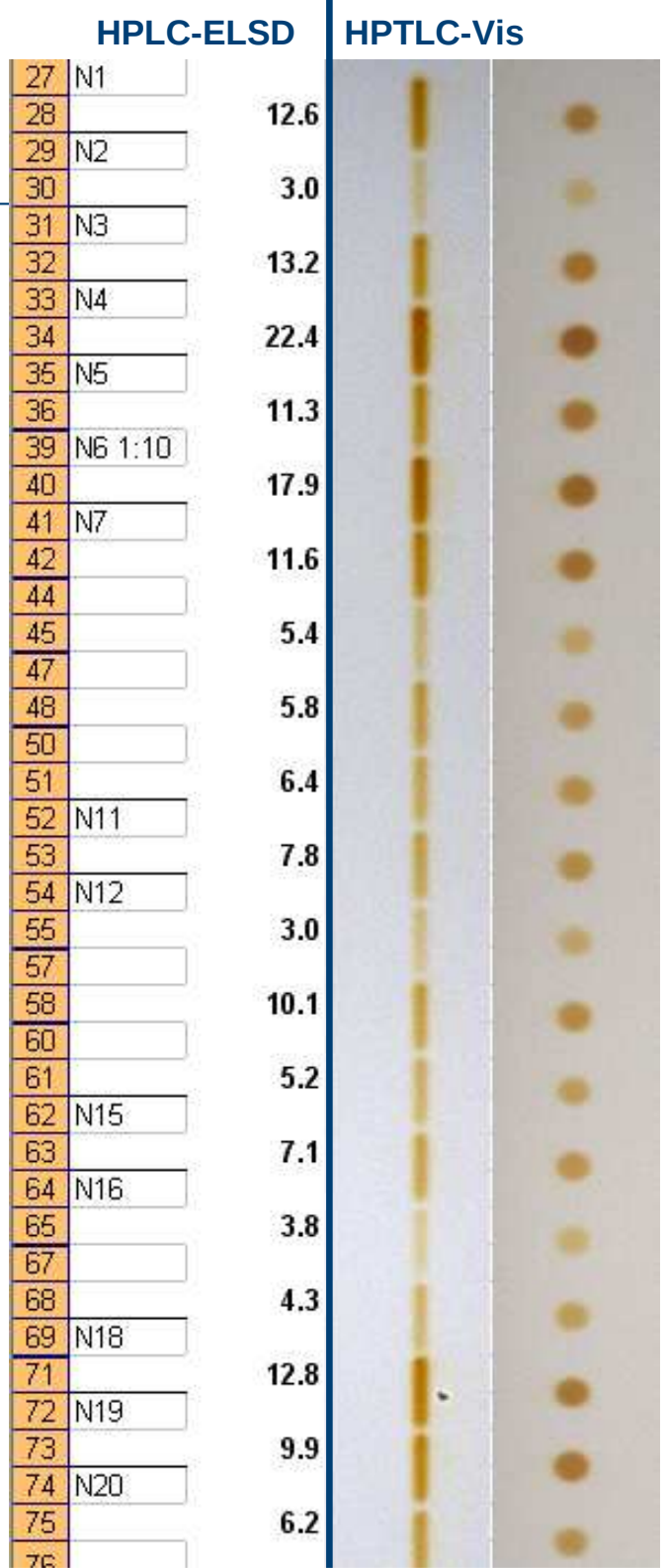
Universität Hohenheim, Stuttgart

HPTLC-Vis!

80 s/sample → 15.5 h/ 700

For 4 batches:
=> 1.3 weeks instead of 6 weeks





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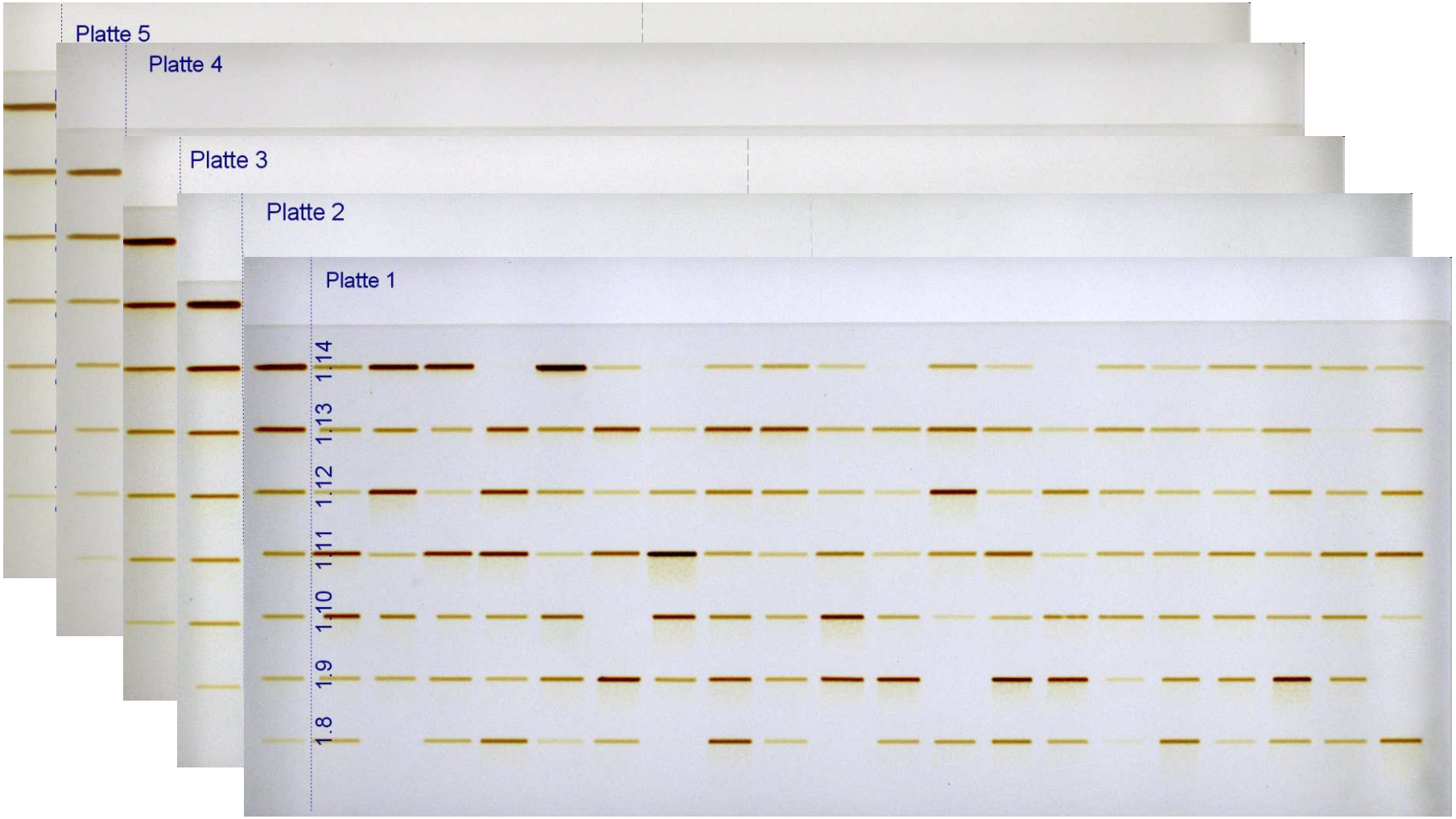
The ever possible best solution for some analytical tasks!

Per batch (700 samples) ca. 1.5 days



→ Advantage: selective derivatization & image evaluation

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The ever possible best solution for some analytical tasks!

Without HPTLC?

- Longer time for evaluation
- HPLC-ELSD: 1.17 Euro/sample versus
  HPTLC-Vis: 0.04 Euro/sample
  → no additional holidays:
  3160,- Euro materials costs is not saved within 6 days
- Analysis time is 1 month longer → instrumentation occupied & blocked for other tasks!

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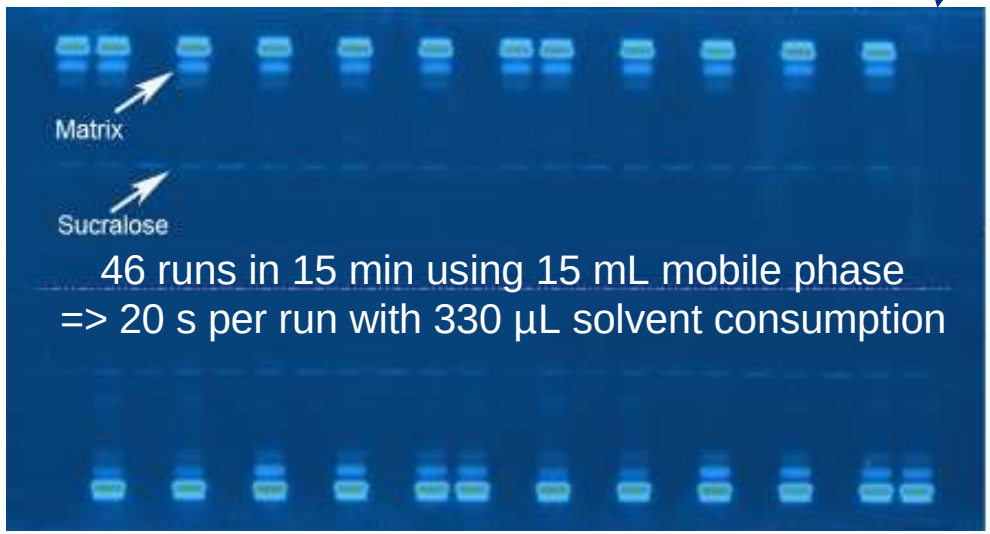
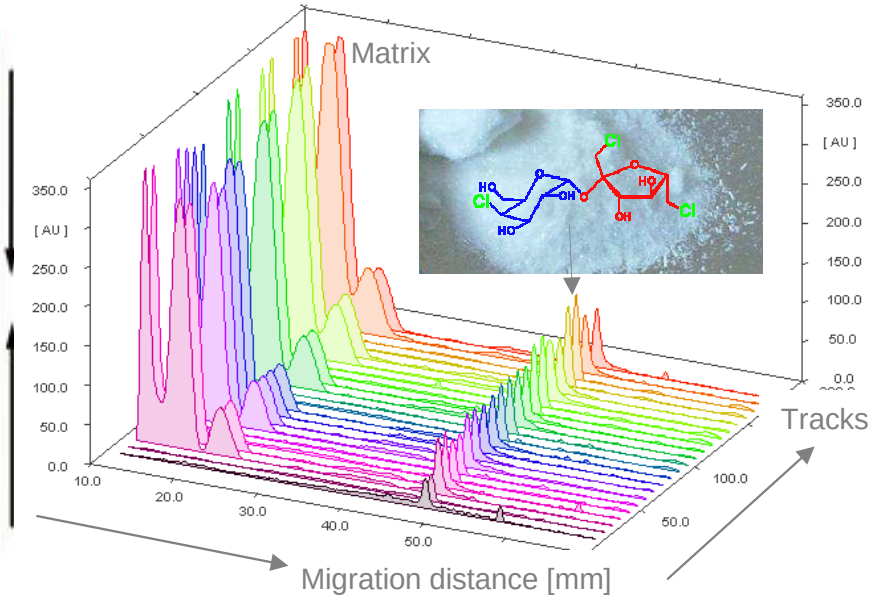
Sucralose in milk-based confection (Burfi)

extracted in MeOH, filtered





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

Institut für Lebensmittelchemie
Universität Hohenheim, Stuttgart

Facts for sucralose analysis

- High throughput (46 runs in 15 min by (anti-)parallel development, 15 min-staggered offline system) → 1000 samples/8h-day
- Resulting in 20 s per sample with 330 µL solvent consumption
- Almost no disposal costs < 0.01 Cent/sample
- Selective derivatization → compensates low separation power
- Reduced sample preparation: no SPE
- Analysis without acetonitrile!

- Ultra-rapid HPLC with 2 min gradient: 720 runs/24-h day
- Sample preparation: Need of SPE for MS or ELSD as detector

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

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Universität Hohenheim, Stuttgart

Sucralose in saliva samples

Release study of sucralose nano-encpsulated in chewing gum

- Number of samples 6 x 2= 12/batch
- Analysis of 11 batches

} 132 samples

HPLC-MSD!

- 50.-/sample → 6.600,-
- 30 min/sample → 70 h → 3 days

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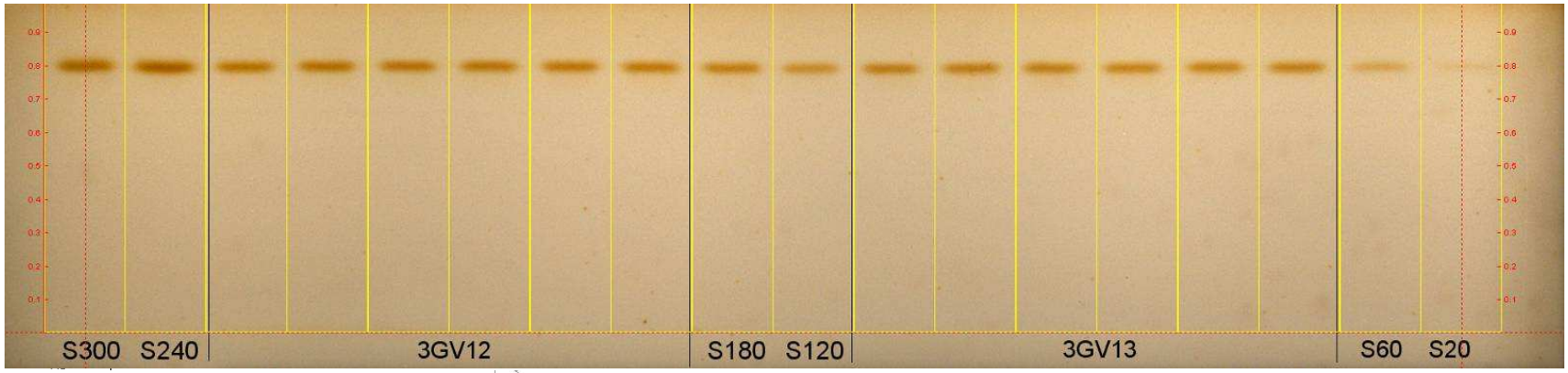
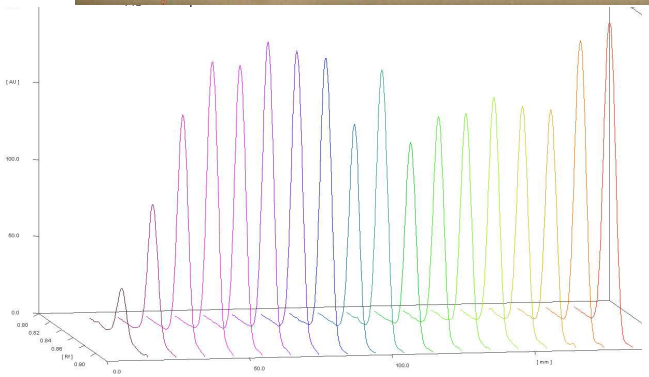
HPTLC-Vis!





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The ever possible best solution for some analytical tasks!

Without HPTLC?

- HPLC-MSD: 3.20 Euro/sample versus
  HPTLC-Vis: 0.21 Euro/sample
  → no nice one-star-dinner at night:
  384,- Euro materials costs is not saved within 0.5 days
- HPLC-MSD: 3 days versus
  HPTLC-Vis: 0.5 days
  → Analysis time is 2.5 days longer → instrumentation occupied & blocked for other tasks!

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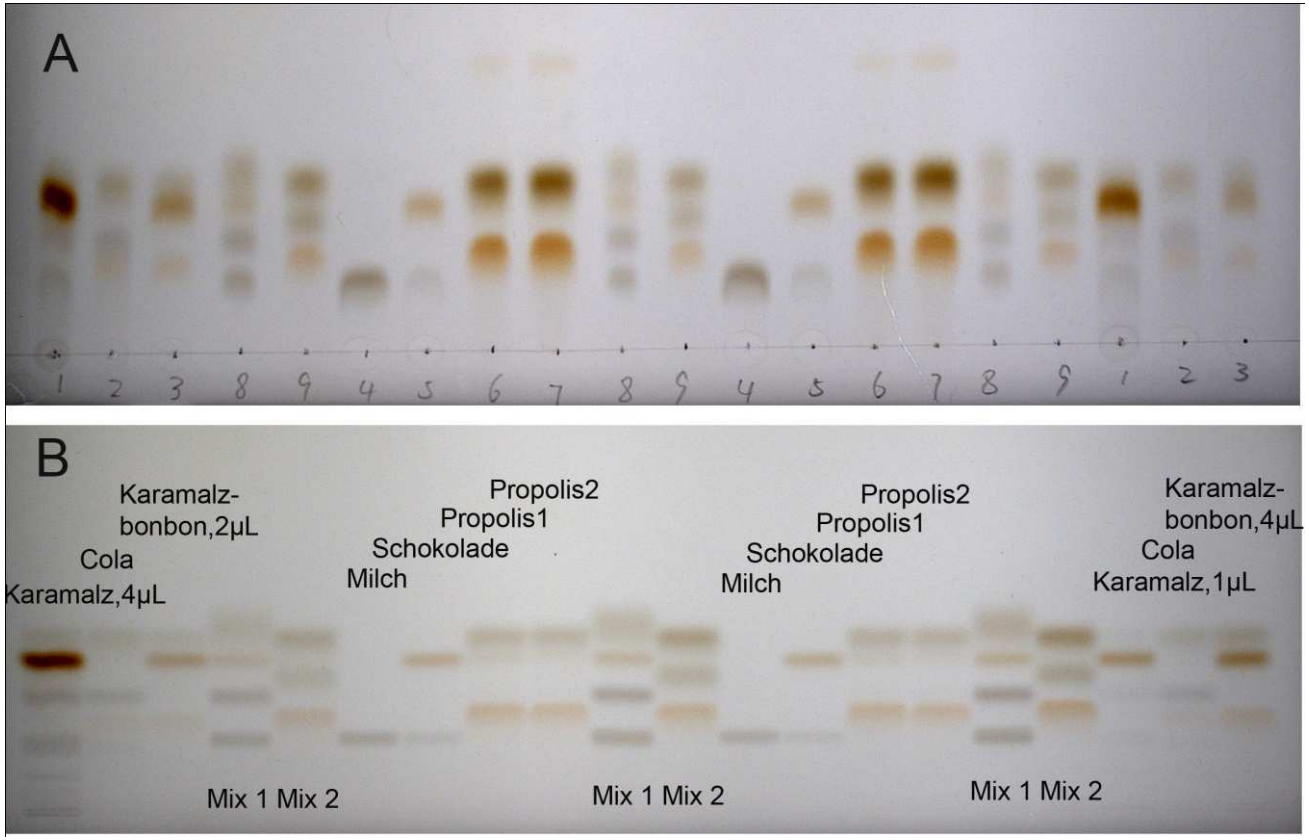
Method comparison



Poor quantitative results

Good quantitative results

Comparable to HPLC-ELSD?

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Method comparison

Sample	Sugar found (n = 2)	HPLC-ELSD		HPTLC-Vis	
		%	*%RSD*	%	*%RSD*
Cola	Sucrose	**12.0**	0.1	**12.5**	5.3
	Fructose	**1.1**	0.4	**1.1**	5.3
	Glucose	**1.3**	4.5	**-**	-
Milk	Lactose	**8.0***	5.6	**5.3**	1.8
Chocolate	Sucrose	**34.9**	0.5	**35.9**	0.8
	Lactose	**6.9**	12.9	**7.1**	10.0
Propolis	Glucose	**10.9**	9.3	**10.7**	1.1
	Fructose	**17.3**	0.0	**17.4**	6.0
	Sucrose	**4.4***	5.0	**7.3***	7.0
Karamalt	Glucose	**3.3**	2.8	**4.1**	3.9
	Fructose	**2.2**	0.7	**2.0**	1.6
	Maltose	**2.5**	2.5	**2.5**	6.5
Biscuits	Sucrose	**17.9***	1.2	**23.9***	1.8

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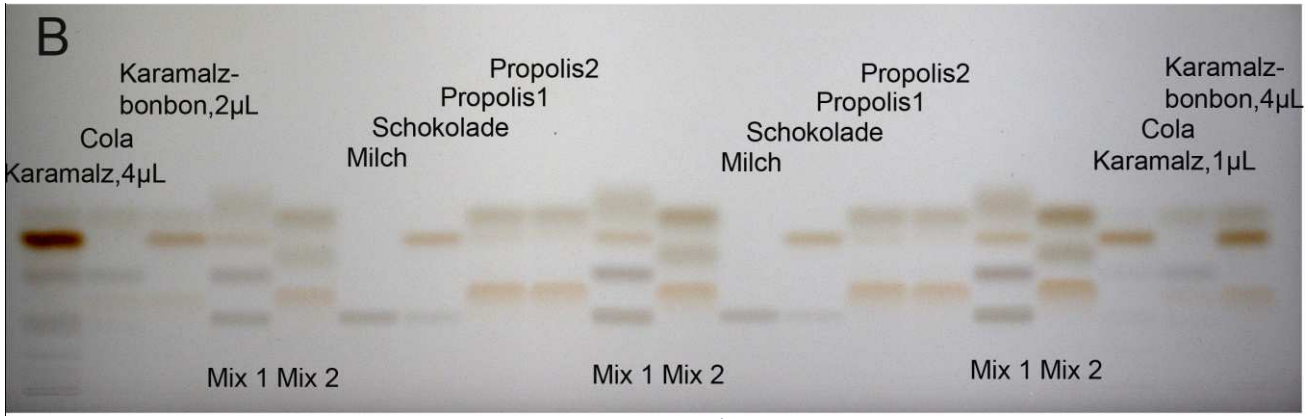
The ever possible best solution for some analytical tasks!



Comparable to HPLC-ELSD? → YES

Analyses time

- HPTLC: 1 h → 3 min per sample
- HPLC: 5.3 h → 16 min per sample

G. Morlock, G. Shabier, in submission

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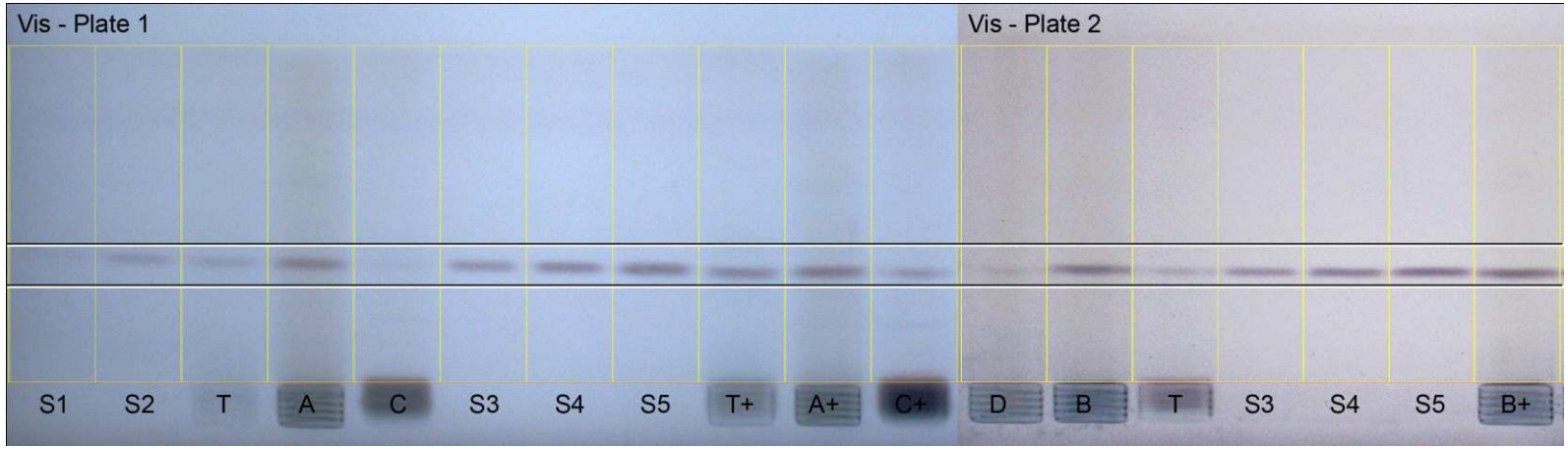
HPLC or HPTLC?

HPLC-TOF or -MS/MS with isotopically labeled standard

…or HPTLC-Vis?

Analysis of sewage effluent and river water



Mean values (ng/L)	Sample A	SampleB	SampleC	SampleD
HPLC-TOF//MS/MS (mean of 6 laboratories)	5869	7302	186	200
HPTLC-Vis (n=2)	5863	7034	247	218

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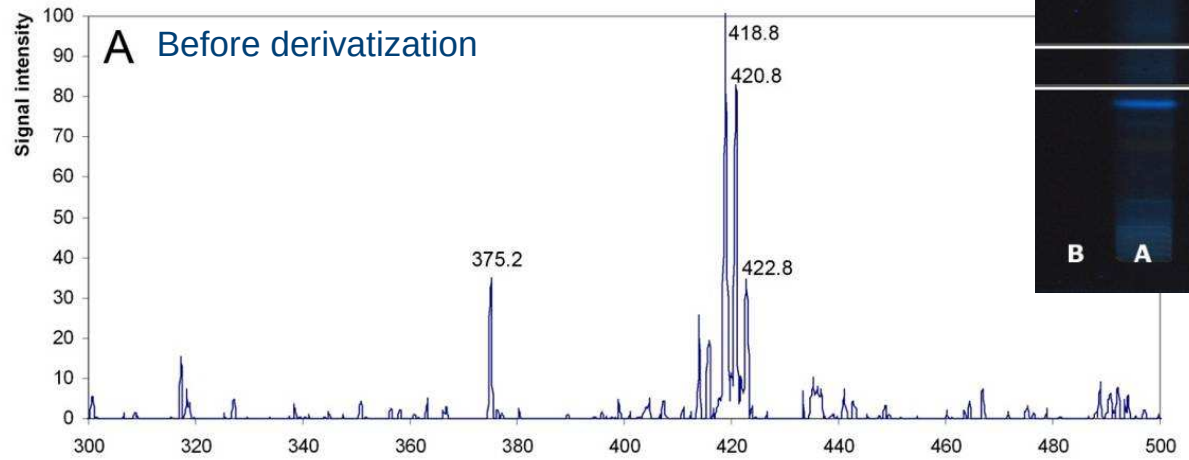

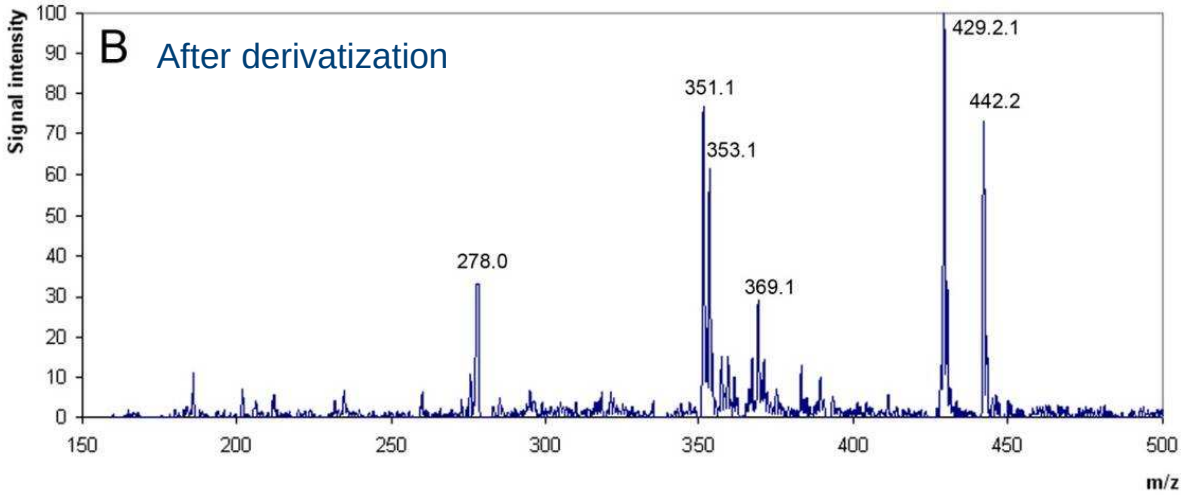

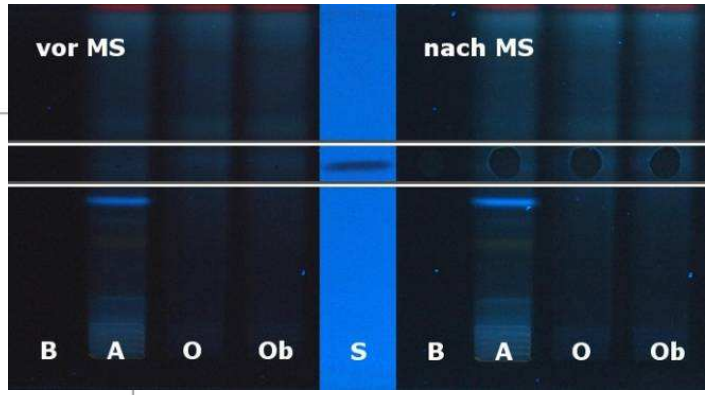

Positive ESI-Modus

- Typical isotope pattern
- Only confirmation of positive findings of higher concentration

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20 reasons to use HPTLC

Reasons 11-20:

11. Analytical workflow adjusted to the findings
12. The utmost matrix-tolerant method
13. Re-use of the stationary phase
14. Benefit of reagent sequences
15. Sample preparation for many samples at one go and at low costs
16. The utmost selectivity change is possible
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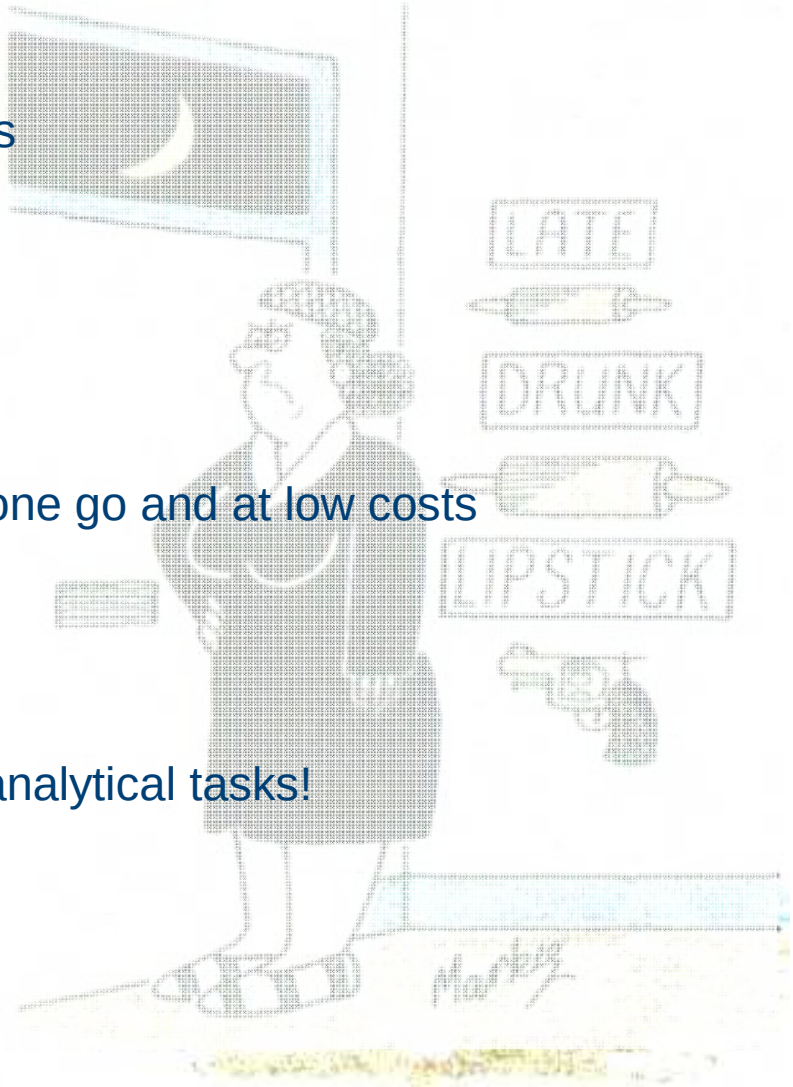

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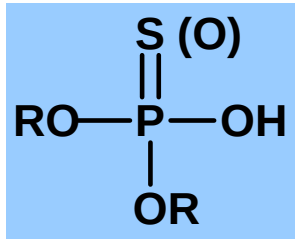
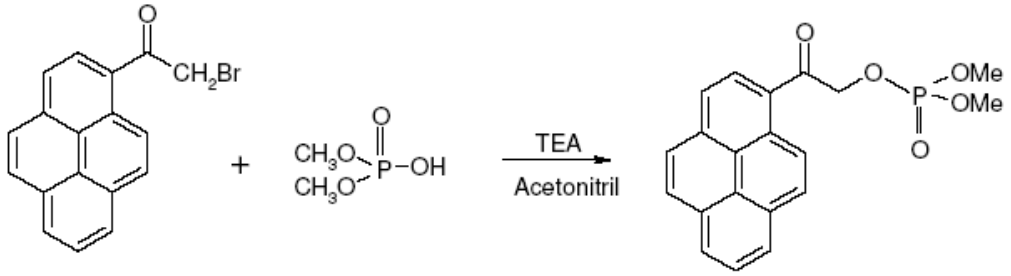
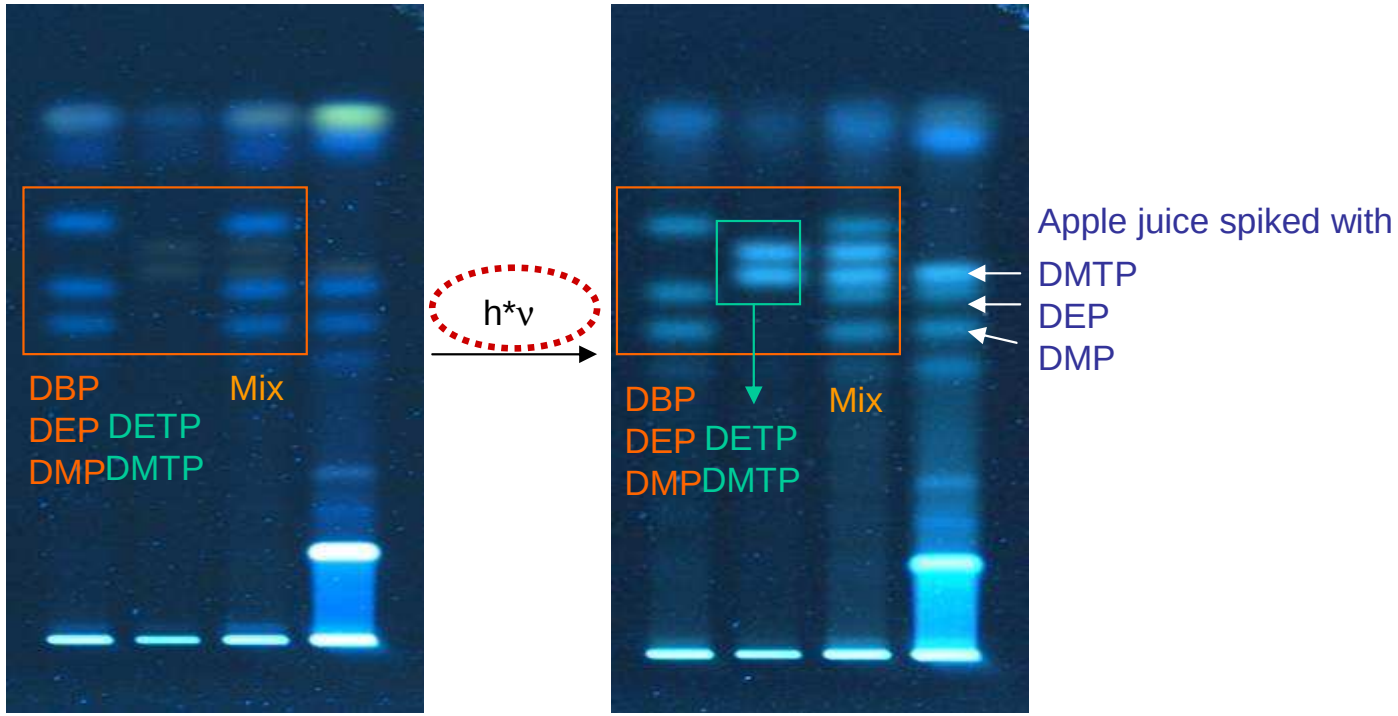
Reactions on the plate

→ Dialkyl phosphates as breakdown products during fruit juice processing







W. Schwack, T. Zeisler, C. Stiefel, J AOAC Internat 92 (2009) 691-698

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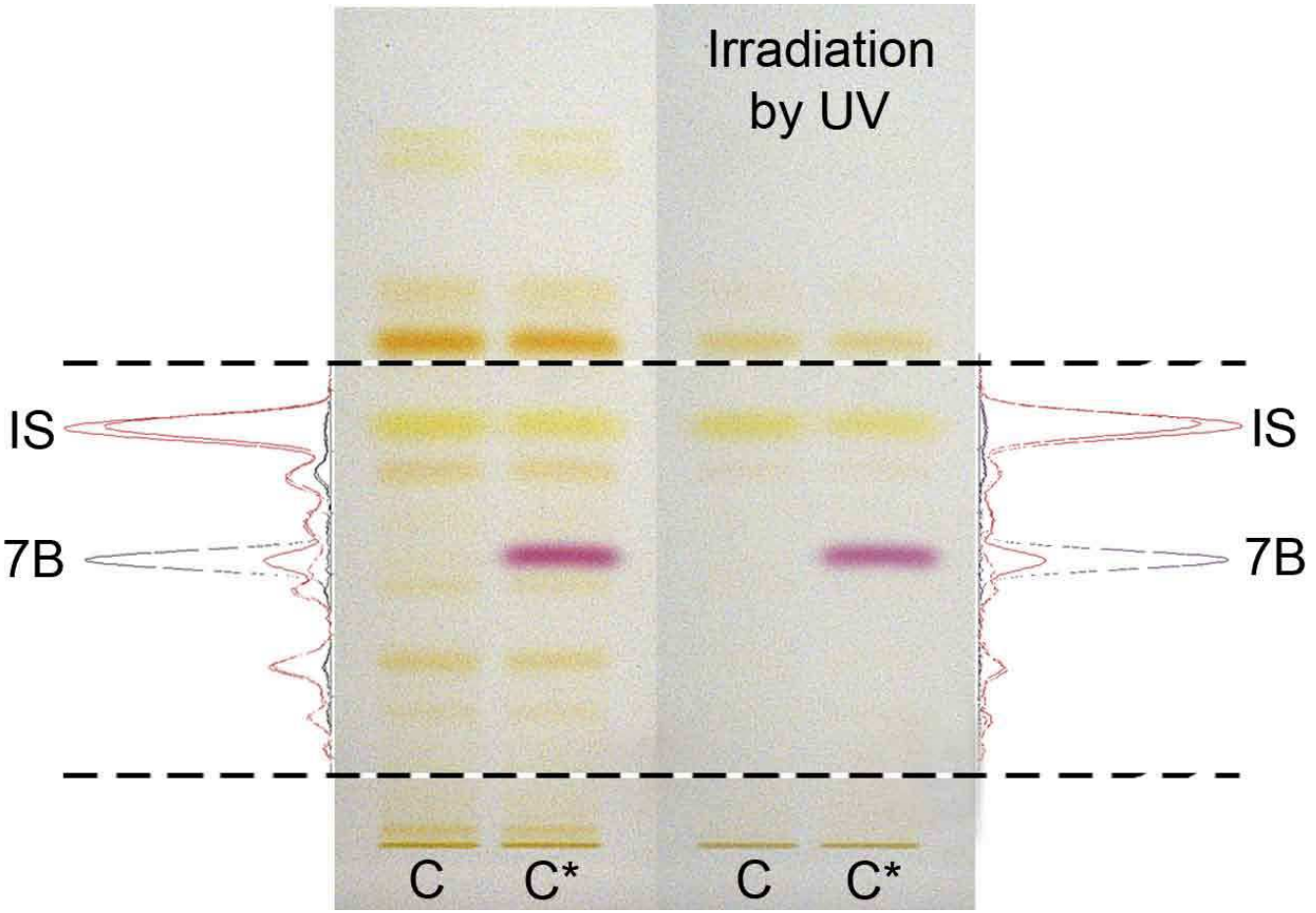
Reactions on the plate



E. Pellissier, W. Schwack, CBS 103 (2009) 13-15

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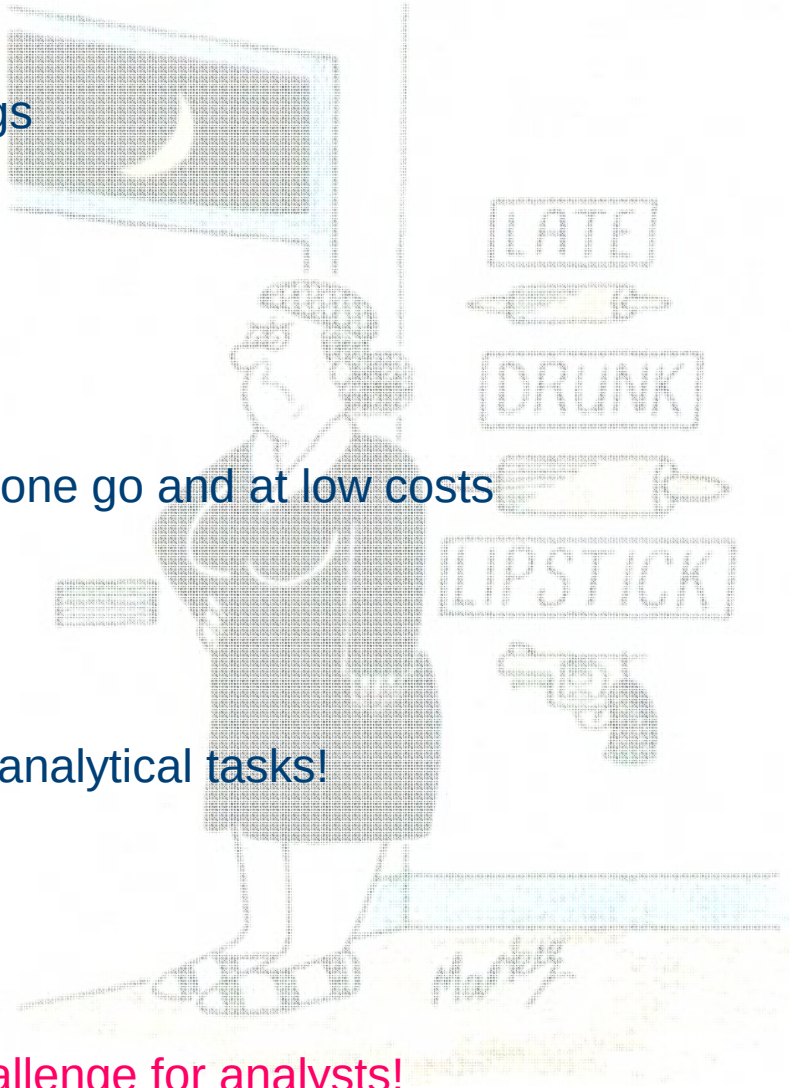

→ Unfortunately, the creativity potential is a challenge for analysts!

→ Unfortunately, analysts do not know HPTLC at all!

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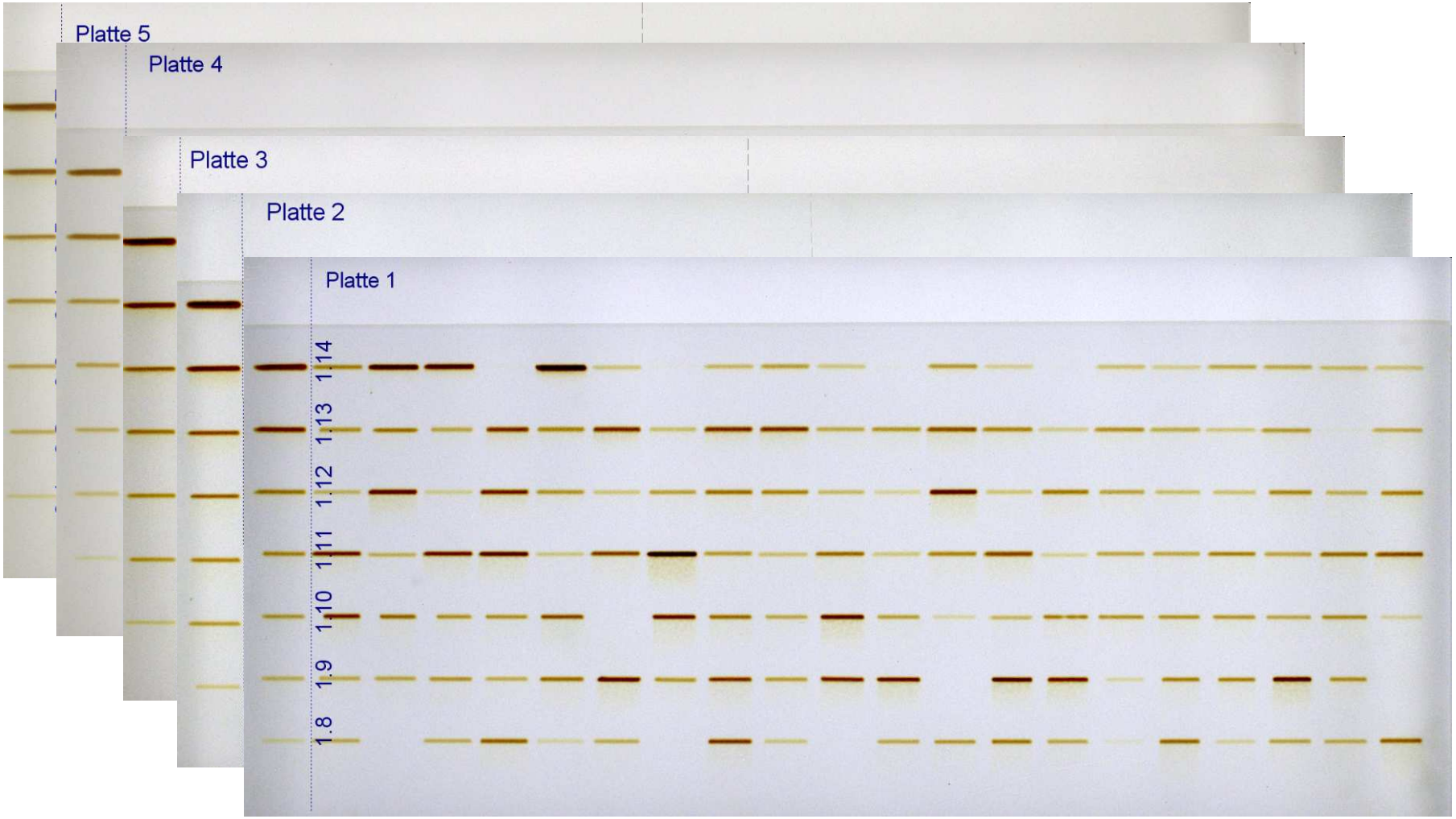
The ever possible best solution for some analytical tasks!

Per batch (700 samples) ca. 1.5 days



→ Advantage: selective derivatization & image evaluation

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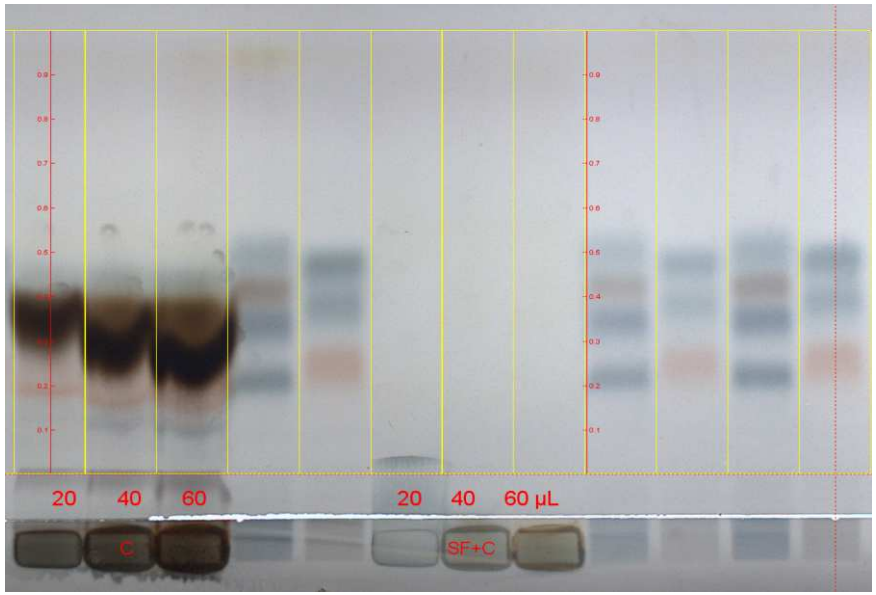
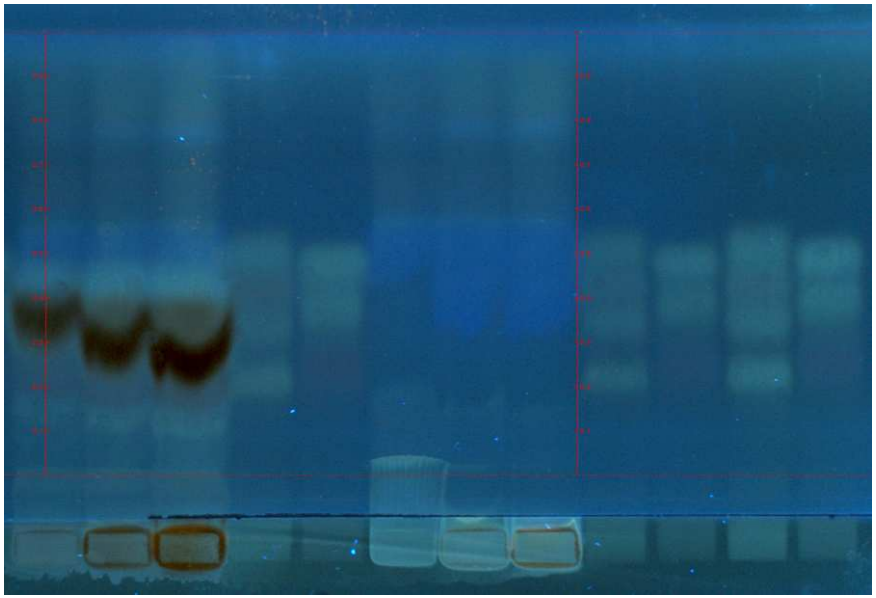
The utmost matrix-tolerant method





Steril filtrated culture medium of algae

→ centrifuged

→ no relevant concentrations of carbohydrates (< 0.00010 %)

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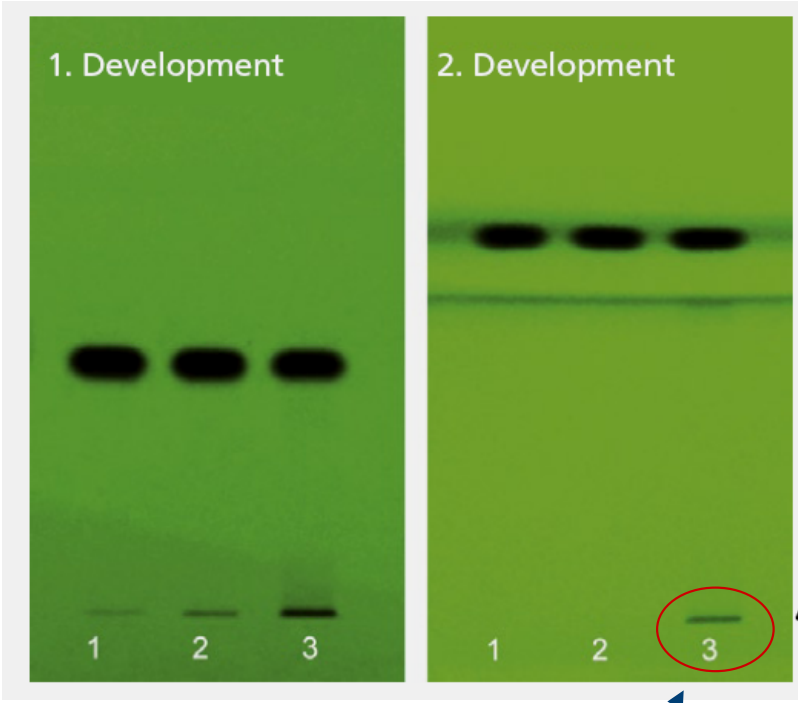
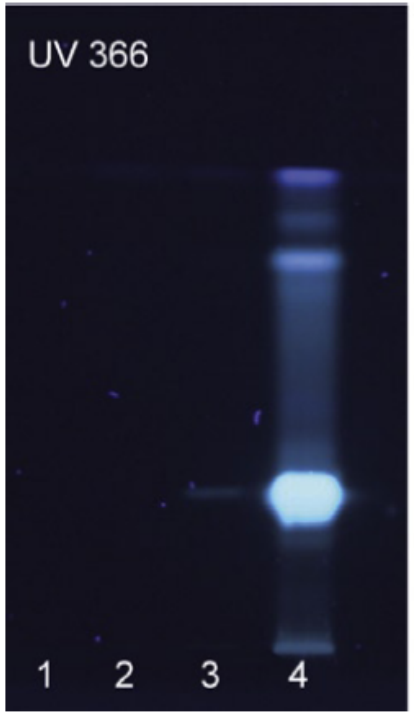
More information about unknowns



Mass imbalance caused by
light degradation product



New yellow impurity caused color differences
between batches of an API

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

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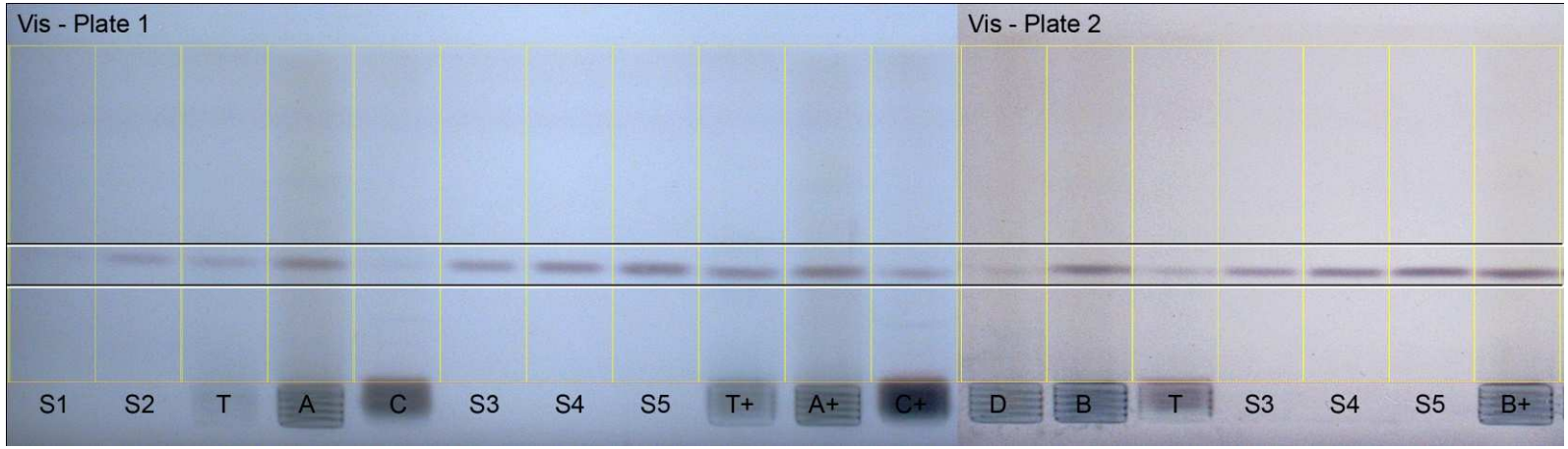
HPLC or HPTLC?

HPLC-TOF or -MS/MS with isotopically labeled standard

…or HPTLC-Vis?

Analysis of sewage effluent and river water



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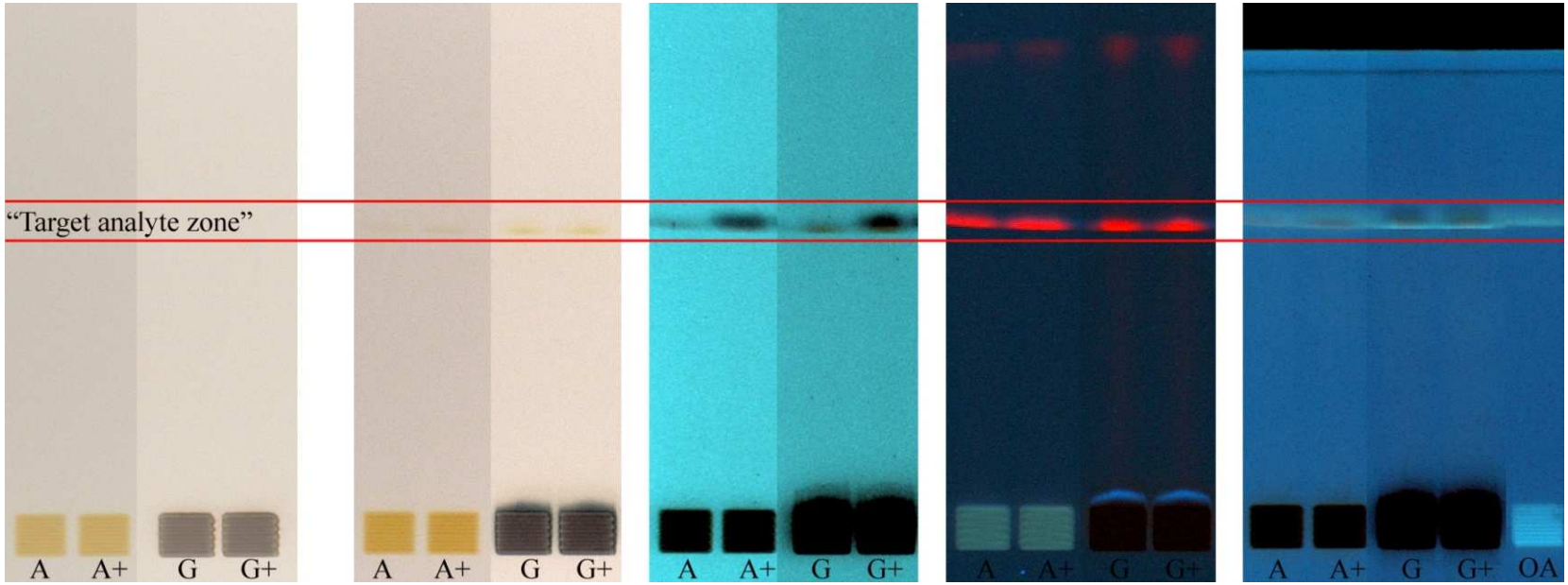
Sample preparation for many samples at one go

… and at low costs



C. Oellig, W. Schwack, Poster PA 085, EPRW Strasbourg, 2010

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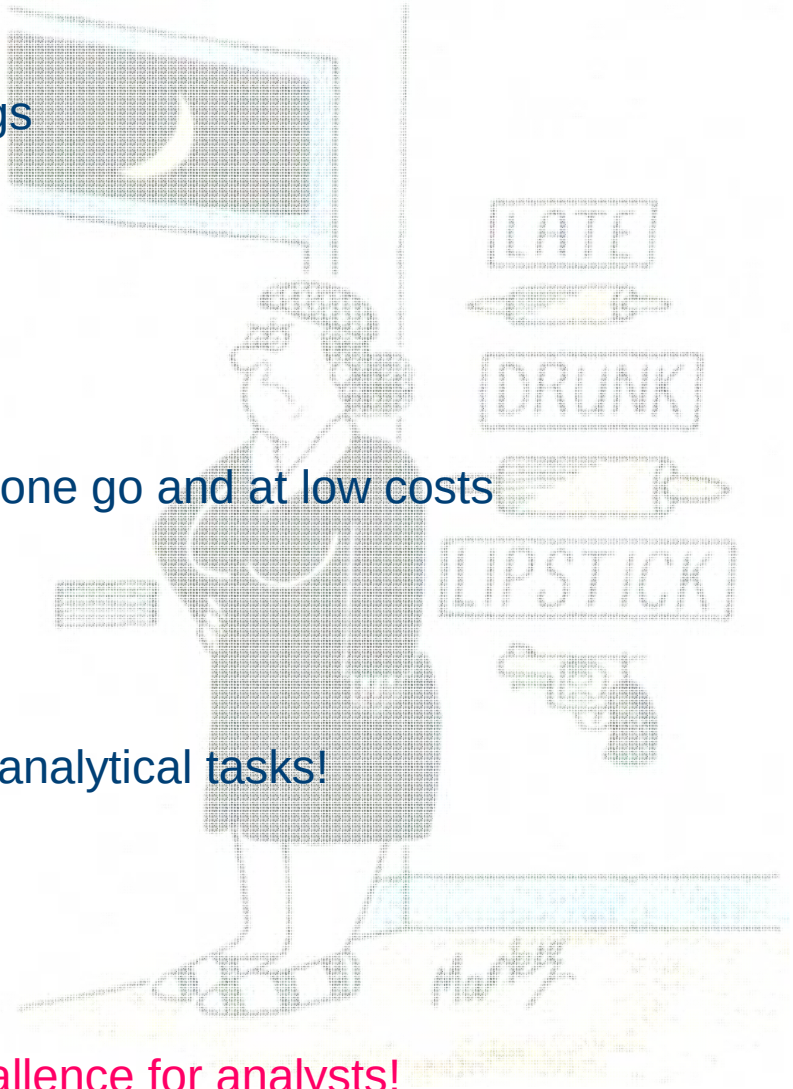

→ Unfortunately, the creativity potential is a challence for analysts!

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Just trained in TLC!

TLC is …. making HPLC 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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This is not HPTLC!

www.wikipedia.de → soon in English

Hochleistungs-Dünnschicht-Chromatographie

Die **Hochleistungs-Dünnschicht-Chromatographie** (engl. *high-performance thin-layer chromatography*, **HPTLC**) ist ein physikalisch-chemisches Trennverfahren und basiert auf der Dünnschicht-Chromatographie (DC).

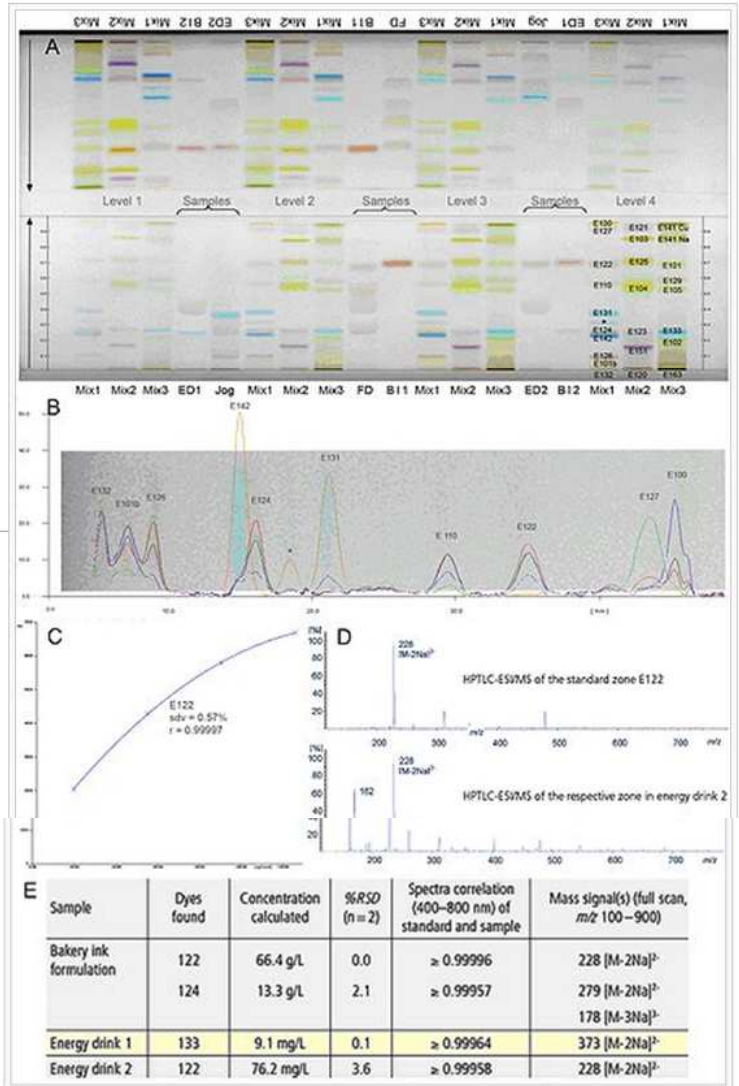
Inhaltsverzeichnis [Verbergen]

Geschichte [Bearbeiten]

1975 wurde der Begriff HPTLC eingeführt[2], der seitdem mit der bestmöglichen Trenneffizienz (Trennzahl max. 40), Präzision (typischerweise ≤ 2 %) und Detektierbarkeit (bis in den pg/Zone-Bereich) verbunden ist. Durch den Einsatz von - im Vergleich zur DC - leistungsstärkerem Trennmaterial (kleinere Korngröße von 5-7 µm, engere Korngrößenverteilung, homogene Schichtdicke), modernen automatisierten Geräten für die HPTLC-Schritte und verbesserten, standardisierten Methoden ist es mit der HPTLC nicht nur möglich eine qualitative, sondern auch eine schnelle quantitative Analyse von Proben aller Art durchzuführen (Abb. 1).

Bei hohem Probendurchsatz ist z.B. die Trennzeit pro Probe 20 sec bei einem Fließmittelverbrauch von 200 µL.[1] Unter den planar-chromatographischen Methoden (Papier-Chromatographie, DC, HPTLC, ultrathin-layer chromatography (UTLC)) ist die HPTLC derzeit die leistungsstärkste. Der Begriff HPTLC ist international nicht geschützt und wird in Ländern unterschiedlich gehandhabt. Um die volle Leistungsstärke der HPTLC zu erreichen, müssen sowohl entsprechende Geräte, als auch HPTLC-Schichten in Kombination verwendet werden. Unabhängig voneinander eingesetzt, ist der Begriff HPTLC nicht berechtigt.

Instrumentelle Entwicklung [Bearbeiten]



E

Sample	Dyes found	Concentration calculated	%RSD (n = 2)	Spectra correlation (400–800 nm) of standard and sample	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 $[M-2Na]^{2-}$

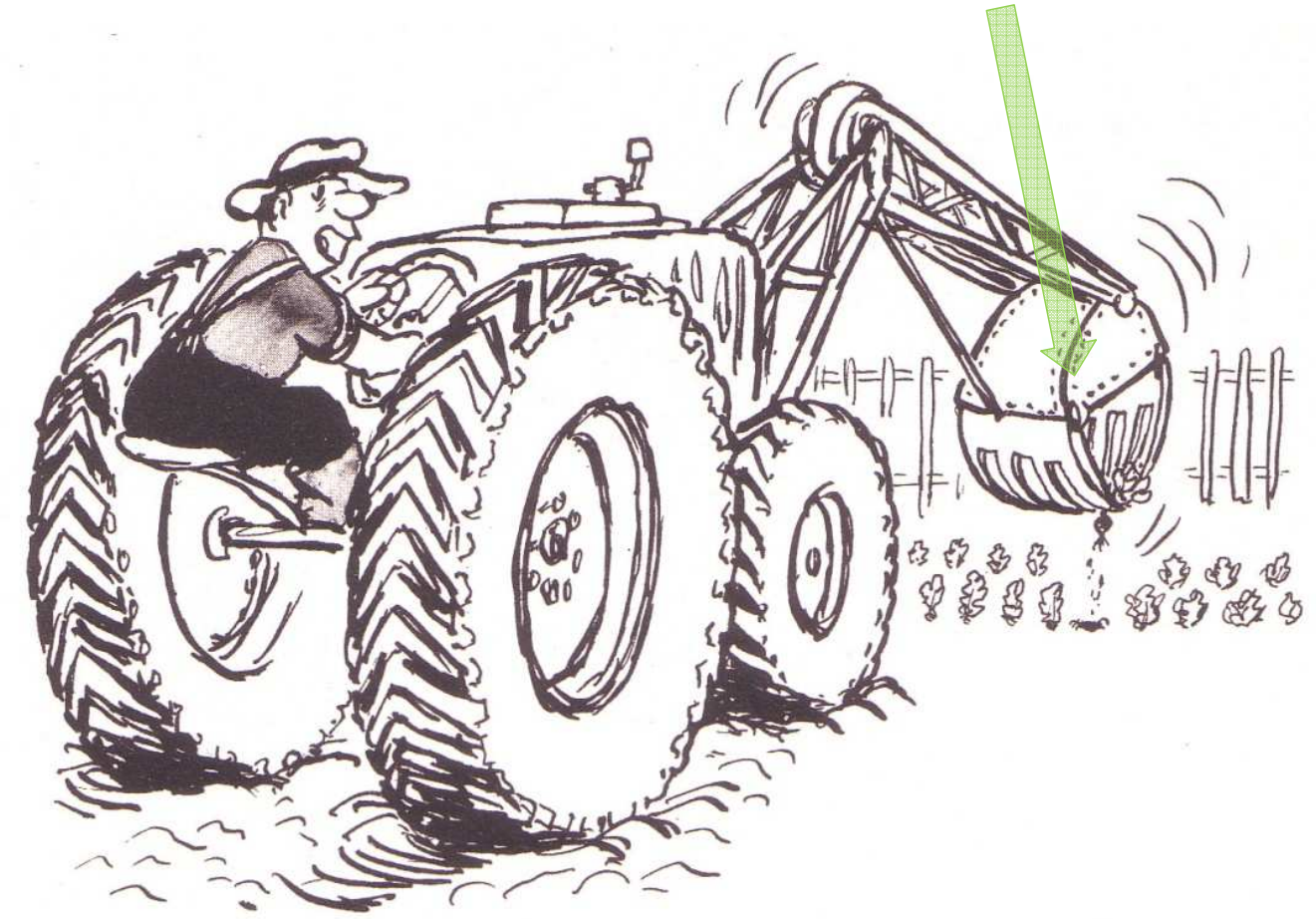
Abb. 1: Analytik von Lebensmittel-Farbstoffen in Proben: HPTLC-Chromatogramm von beiden Seiten entwickelt (A), Mehrwellenlängenscan von

Institut für Lebensmittelchemie
Universität Hohenheim, Stuttgart

Why HPTLC?

Reviewer 1

From the view point of resolution, efficiency, speed and sensitivity HPLC should be better than HPTLC even if the latter method proposed can give us the best in HPTLC and therefore why do we need to use HPTLC/MS rather than HPLC/MS for such easy analytical target.



My paper was rejected based on this short expert opinion.

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

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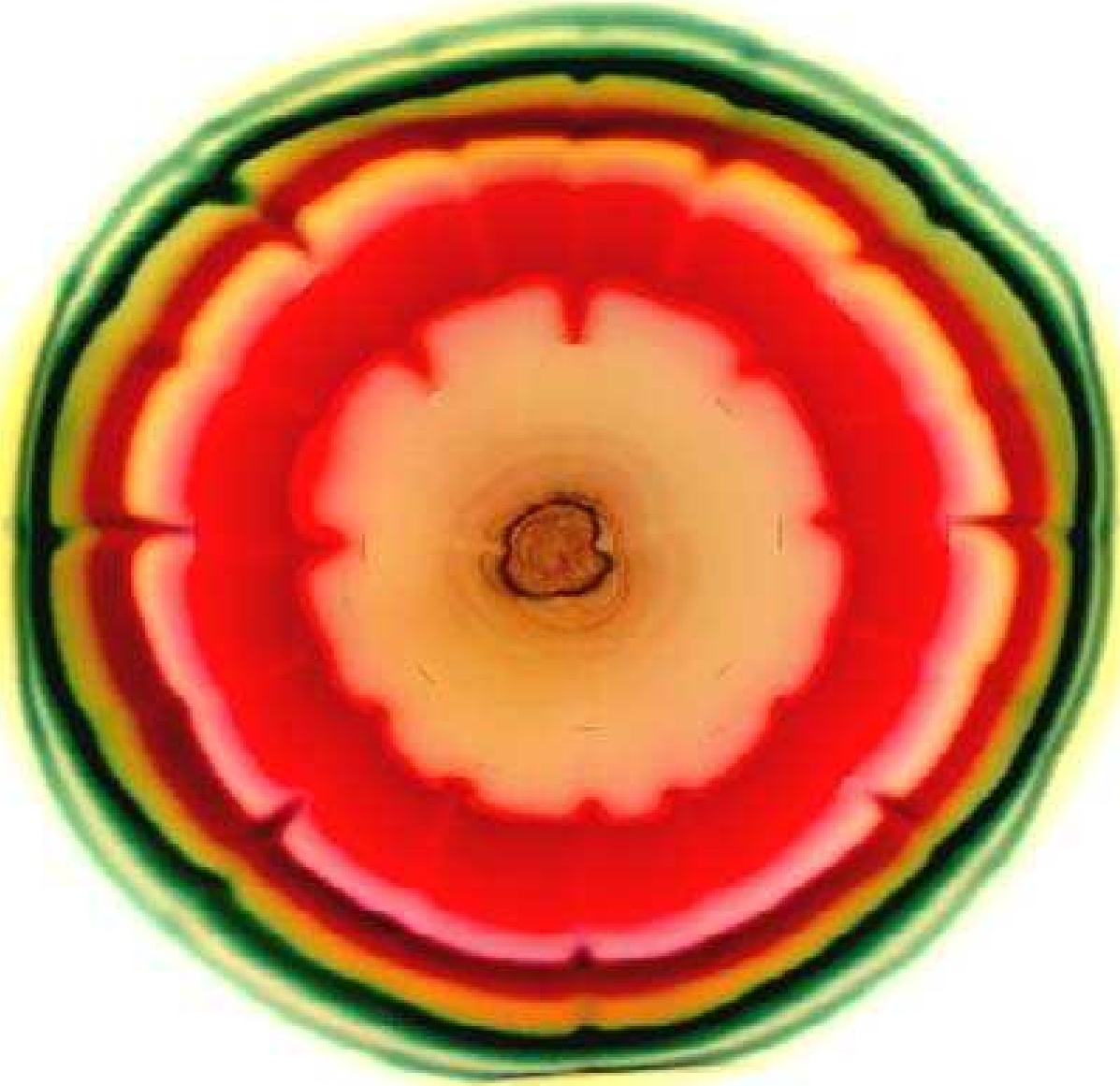
Planar chromatography is art!



CHROMart von Drs. Karla und Herbert Halpaap