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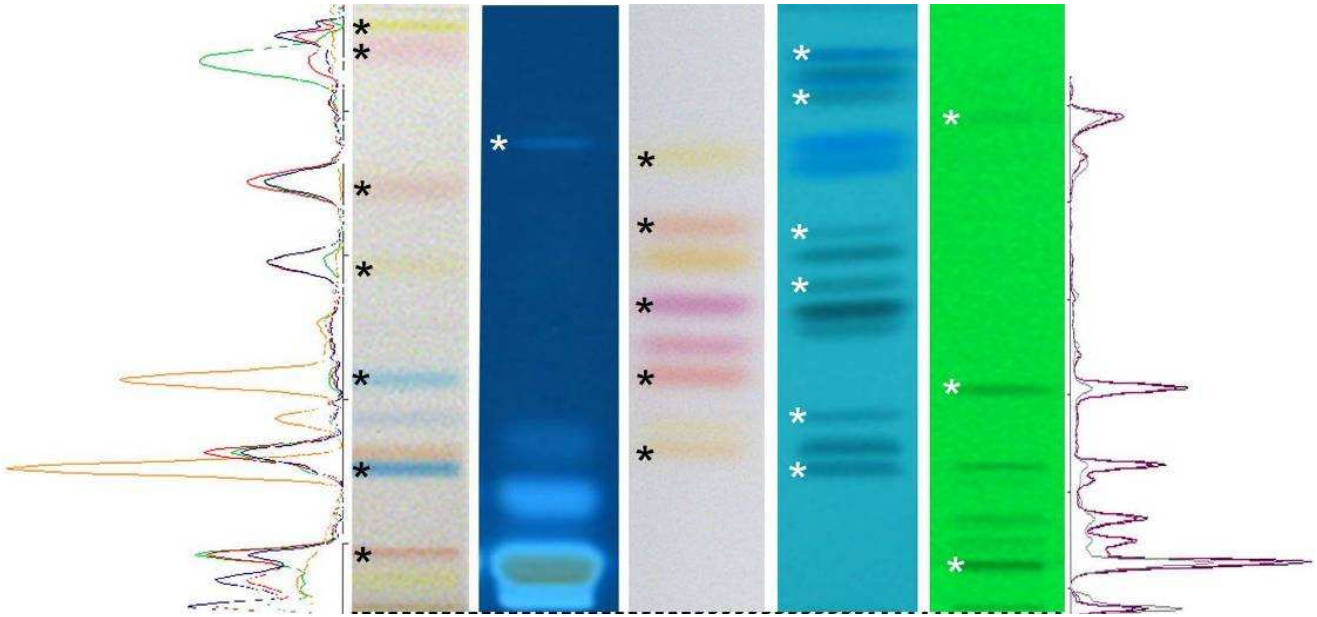
Hyphenations in HPTLC with UV/Vis/FLD, MS, FTIR, NMR and bioassays



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Institute of Nutritional Science, Justus-Liebig-University of Gießen

Institute of Food Chemistry, University of Hohenheim, Stuttgart

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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

Nobody takes care about it!

- 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

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

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
- Directly accessible for the respective optimal solvent

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Hyphenation

Poster 3a

→ The main difference

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

HPTLC: solvent evaporated; after separation → sample on plate

Journal of Chromatography A, 1217 (2010) 6600–6609

Contents lists available at ScienceDirect

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].

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ARTICLE INFO

Article history:
Available online 20 May 2010

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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Content

Hyphenations with

1. UV/VIS/FLD/derivatizations
2. MS
3. FTIR
4. NMR
5. Bioassays

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Trends in Analytical Chemistry, Vol. 29, No. 10, 2010, 1157-1171 Trends

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 greatly 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.
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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

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Germany

1. Introduction

Planar chromatography comprises all chromatographic techniques that 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 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.

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Hyphenation with MS

Journal of Chromatography A, 1218 (2011) 2700–2711

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Review

Thin layer chromatography/mass spectrometry

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[b] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan
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[d] Cancer Research Center, Kaohsiung Medical University, Kaohsiung 80424, Taiwan

ARTICLE INFO

Article history:
Available online 2 February 2011

Keywords:
TLC–MS
Ambient ionization
Vacuum-based ionization
Desorption/ionization
Direct sampling

ABSTRACT

Thin layer chromatography (TLC)—a simple, cost-effective, and easy-to-operate planar chromatographic technique—has been used in general chemistry laboratories for several decades to routinely separate chemical and biochemical compounds. Traditionally, chemical and optical methods are employed to visualize the analyte spots on the TLC plate. Because direct identification and structural characterization of the analytes on the TLC plate through these methods are not possible, there has been long-held interest in the development of interfaces that allow TLC to be combined with mass spectrometry (MS)—one of the most efficient analytical tools for structural elucidation. So far, many different TLC–MS techniques have been reported in the literature; some are commercially available. According to differences in their

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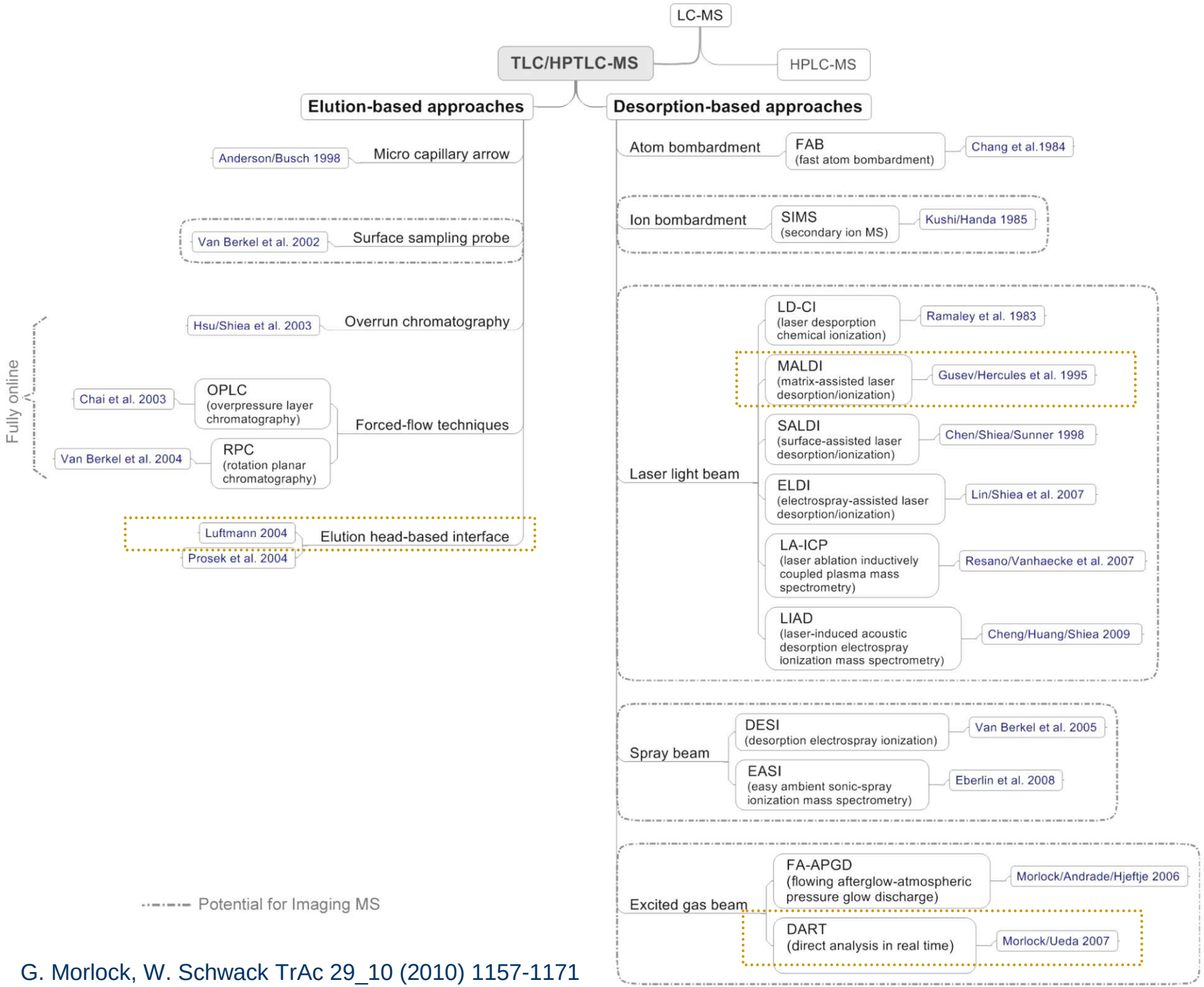

G. Morlock, W. Schwack TrAc 29_10 (2010) 1157-1171

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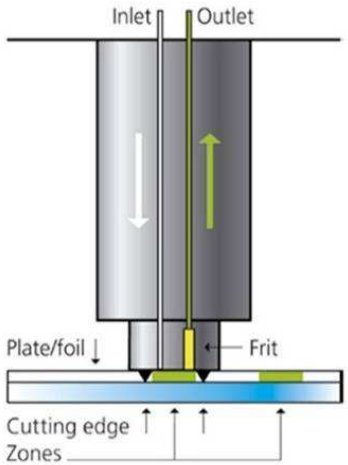
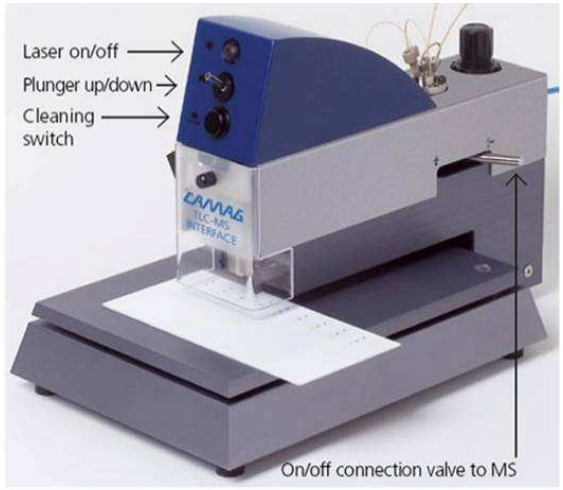
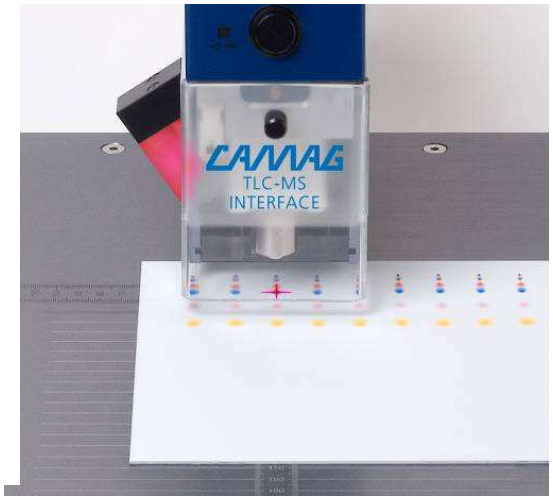
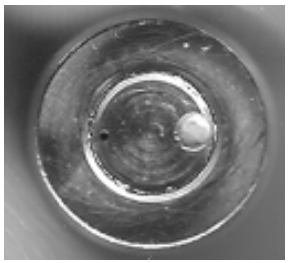
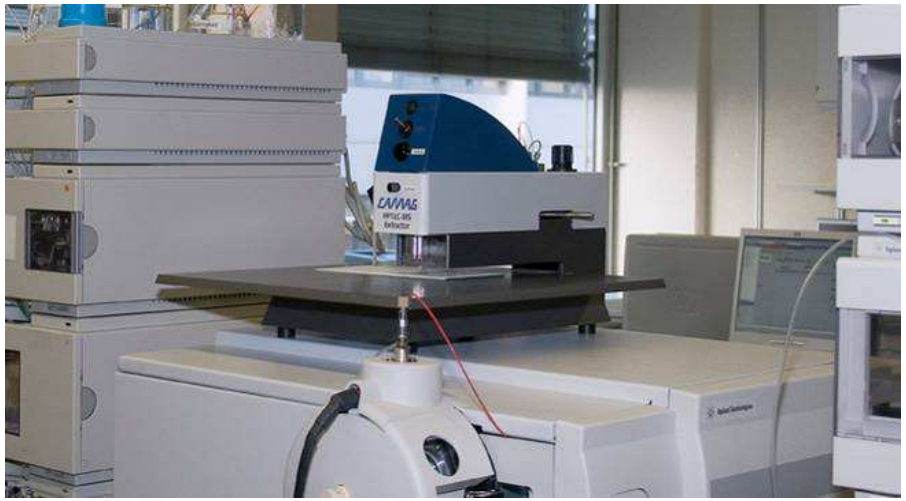
Elution head-based HPTLC-MS → TLC-MS Interface











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

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

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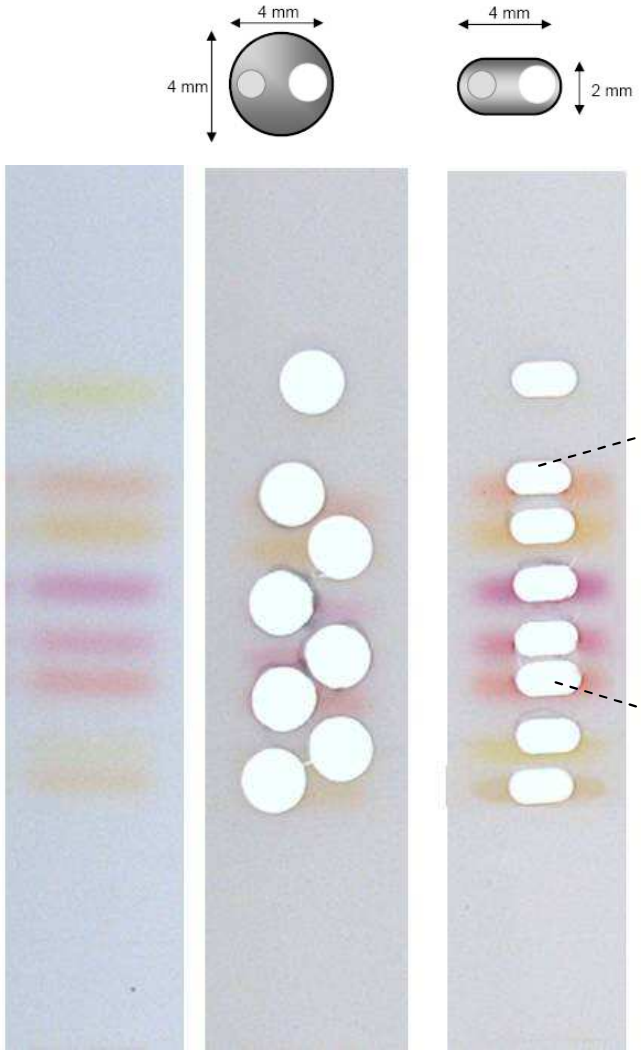
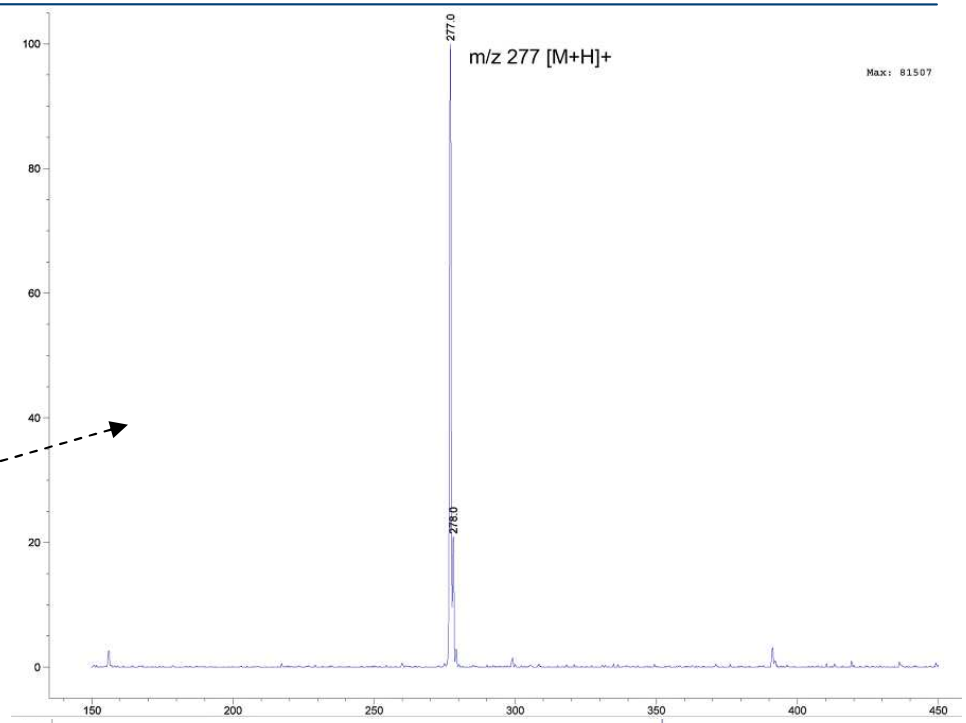
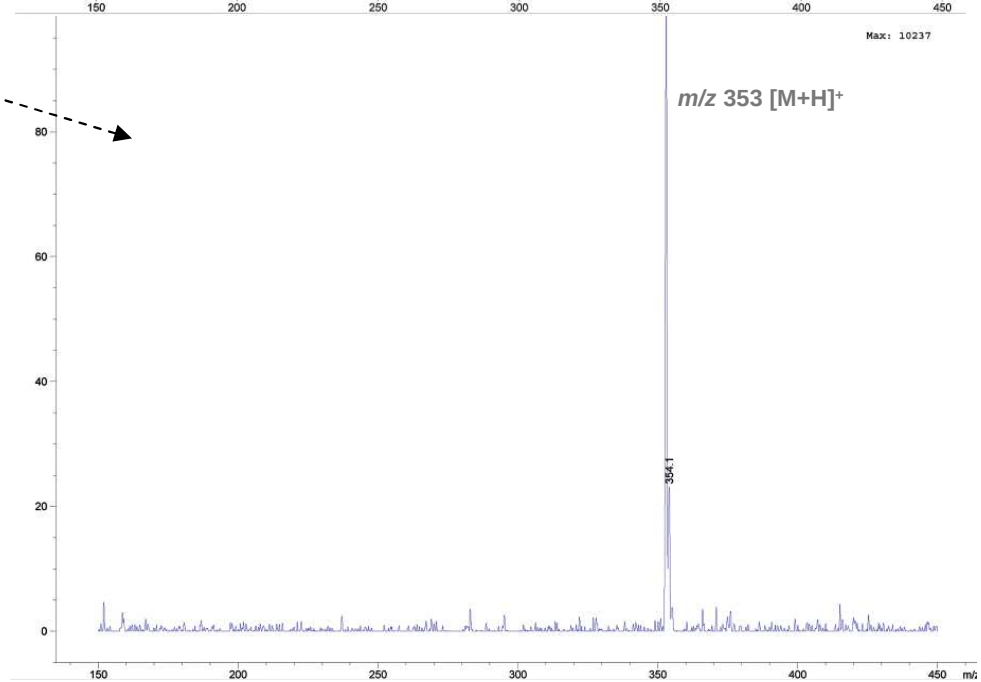
Elution head-based HPTLC-MS







G. Morlock, CBS 103 (2009) 16

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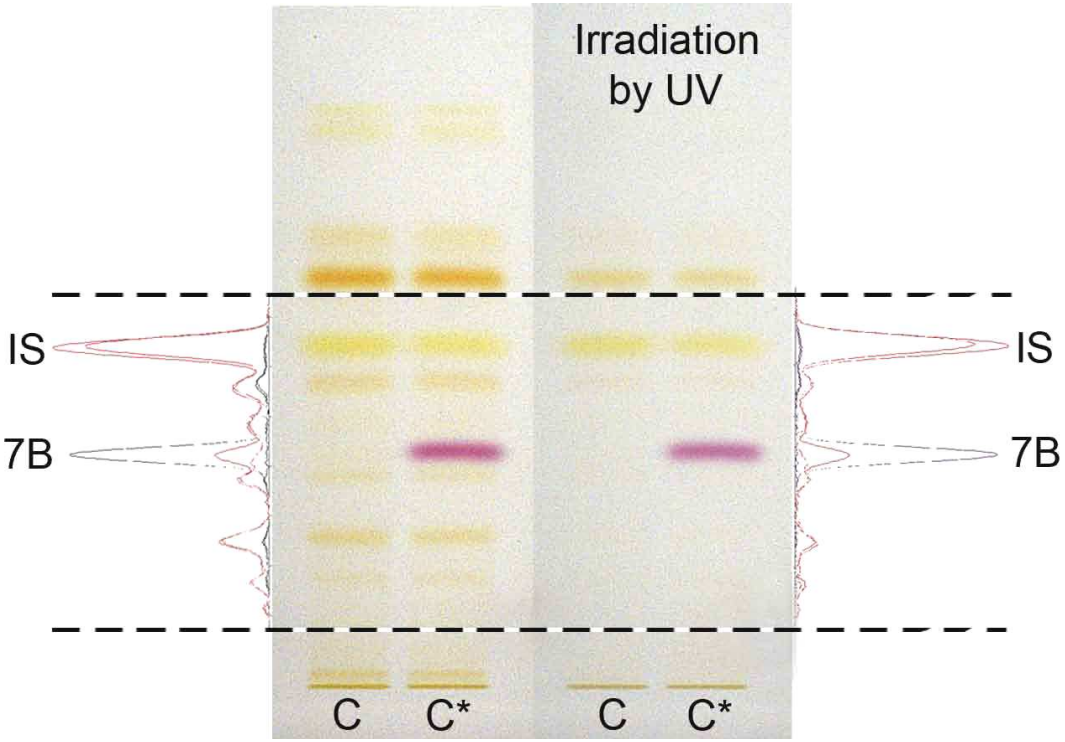
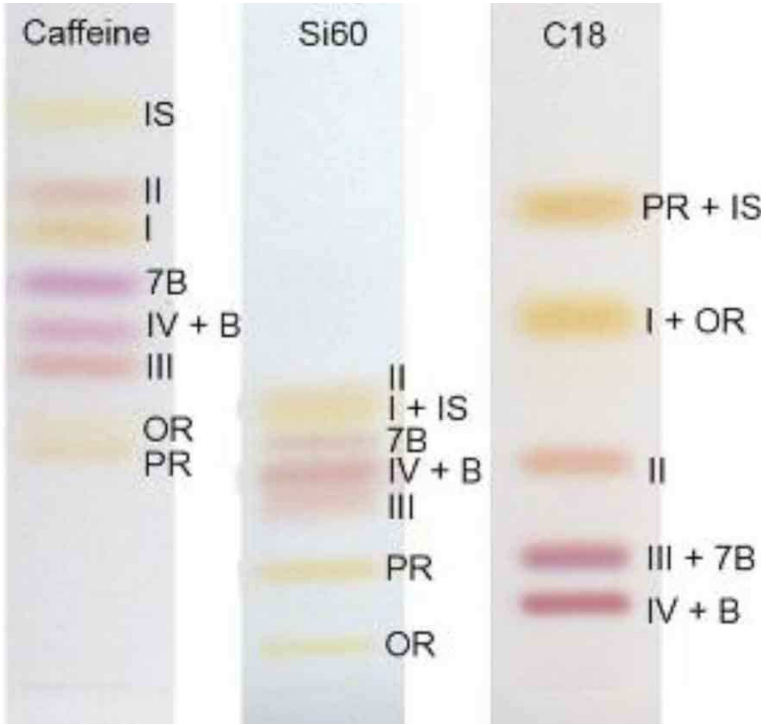
Falsification of food color with unauthorized Sudan dyes

Reactions on the plate



The utmost selectivity change



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

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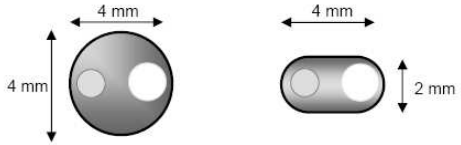
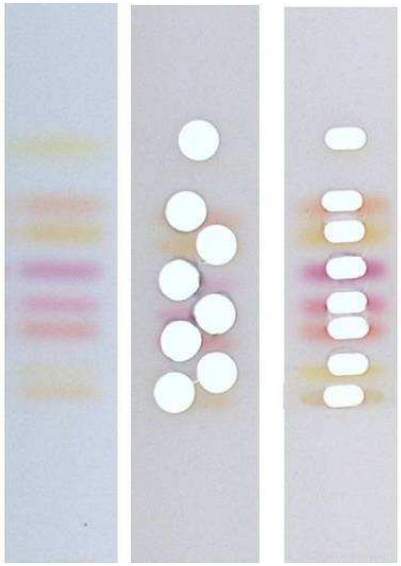
Elution head

Cutting edge geometry

→ U. Jautz, G. Morlock, J Planar Chromatogr 21 (2008) 367

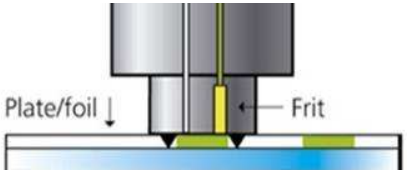
→ G. Morlock , CBS 103 (2009) 16





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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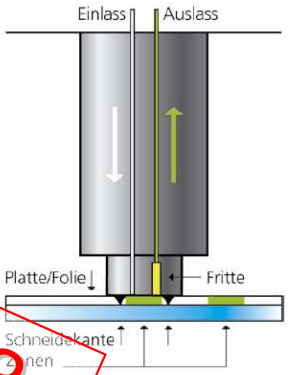
Performance data →TLC-MS Interface



Poster 3c

→ highly reliable hyphenation

→ highly targeted

HPTLC-ESI-MS (SIM, peak area)		Linearity		Precision	
Dyes	hR_F-value	Calibration range (ng/band)	Determination coefficient	Conc. (ng/band)	*%RSD,* n = 5
Dimethyl Yellow	65	12 – 234	0.9943	1125	8.1
Oracet Red G	50	2 – 39	0.9950	189	11.0
Solvent Blue 35	41	10 – 52	0.9931	750	4.6
Sudan Red G	27	6 – 117	0.9984	564	8.8
Solvent Blue 22	17	21 – 78	0.9976	750	3.8
Oracet Violet 2R	4	8 – 156	0.9752	1500	11.6
Mean			0.9923		8.0

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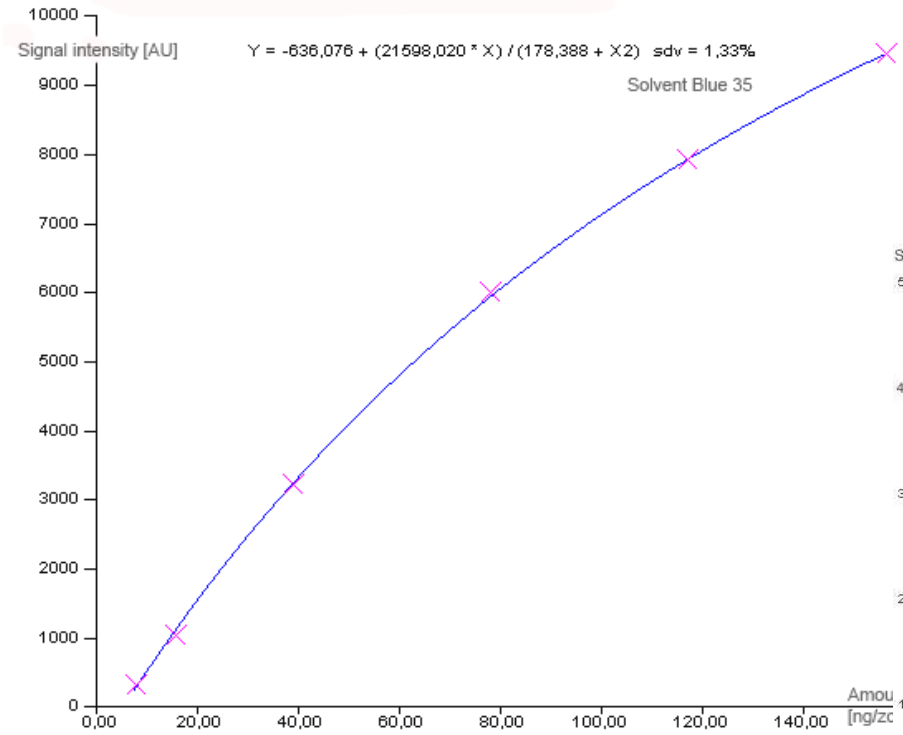
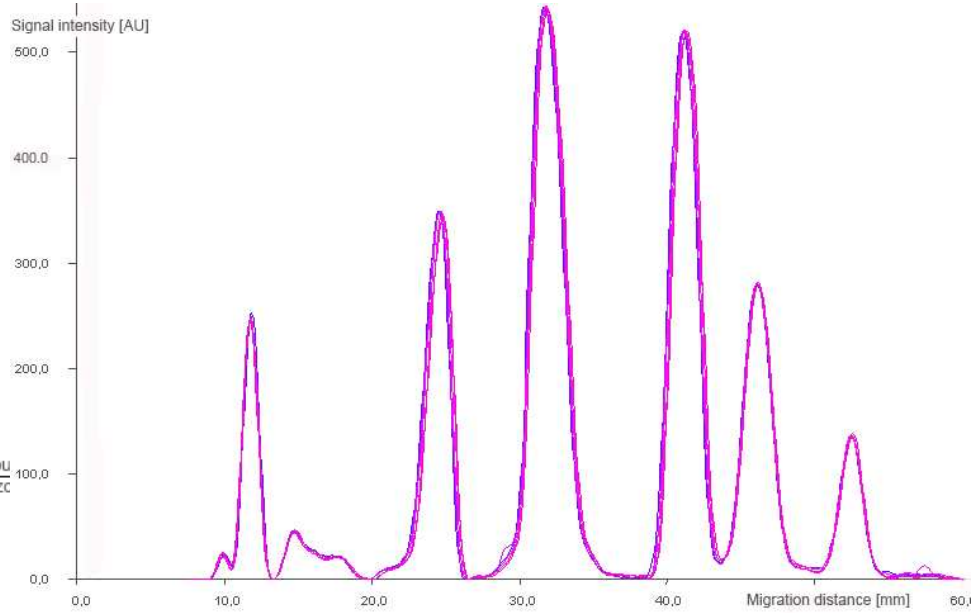
Performance data obtained with the TLC-MS interface

⇒ before: check of performance data by HPTLC-Vis

Calibration for Solvent Blue 35 *(%RSD* = 1.3%)



Overlay of 5 analog curves *(%RSD* ≤ 1.3%)



G. Morlock *et al.*, in preparation

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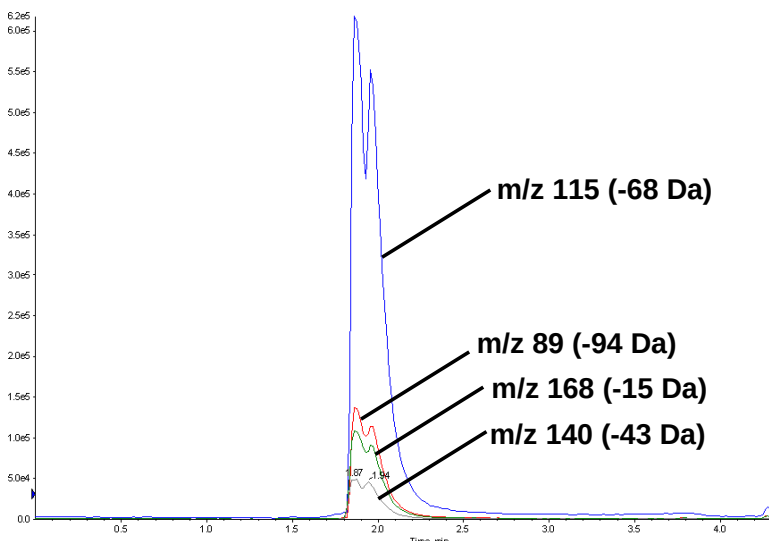
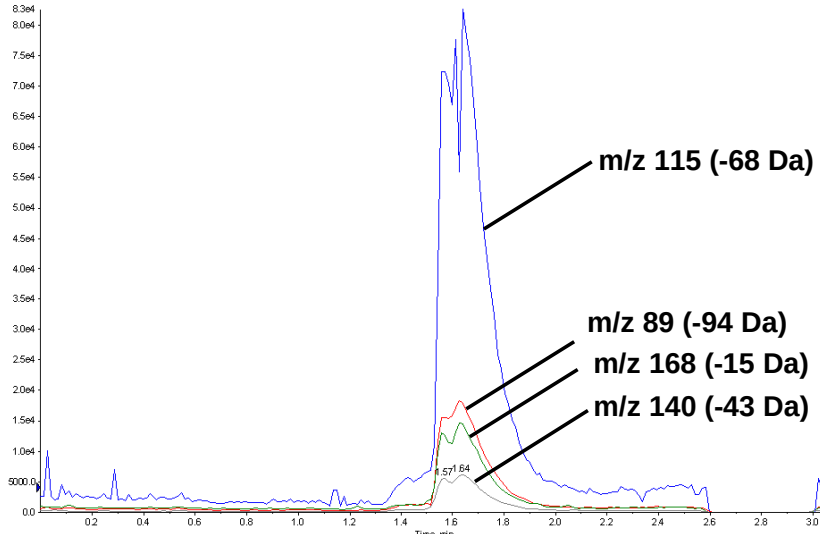
Detectability by HPTLC-ESI-MS/MS





200 pg/zone harman → 20 pg/zone harman

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

→ Detectability comparable to HPLC/MS

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

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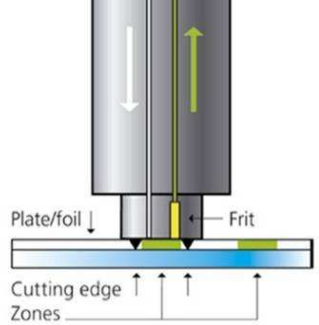
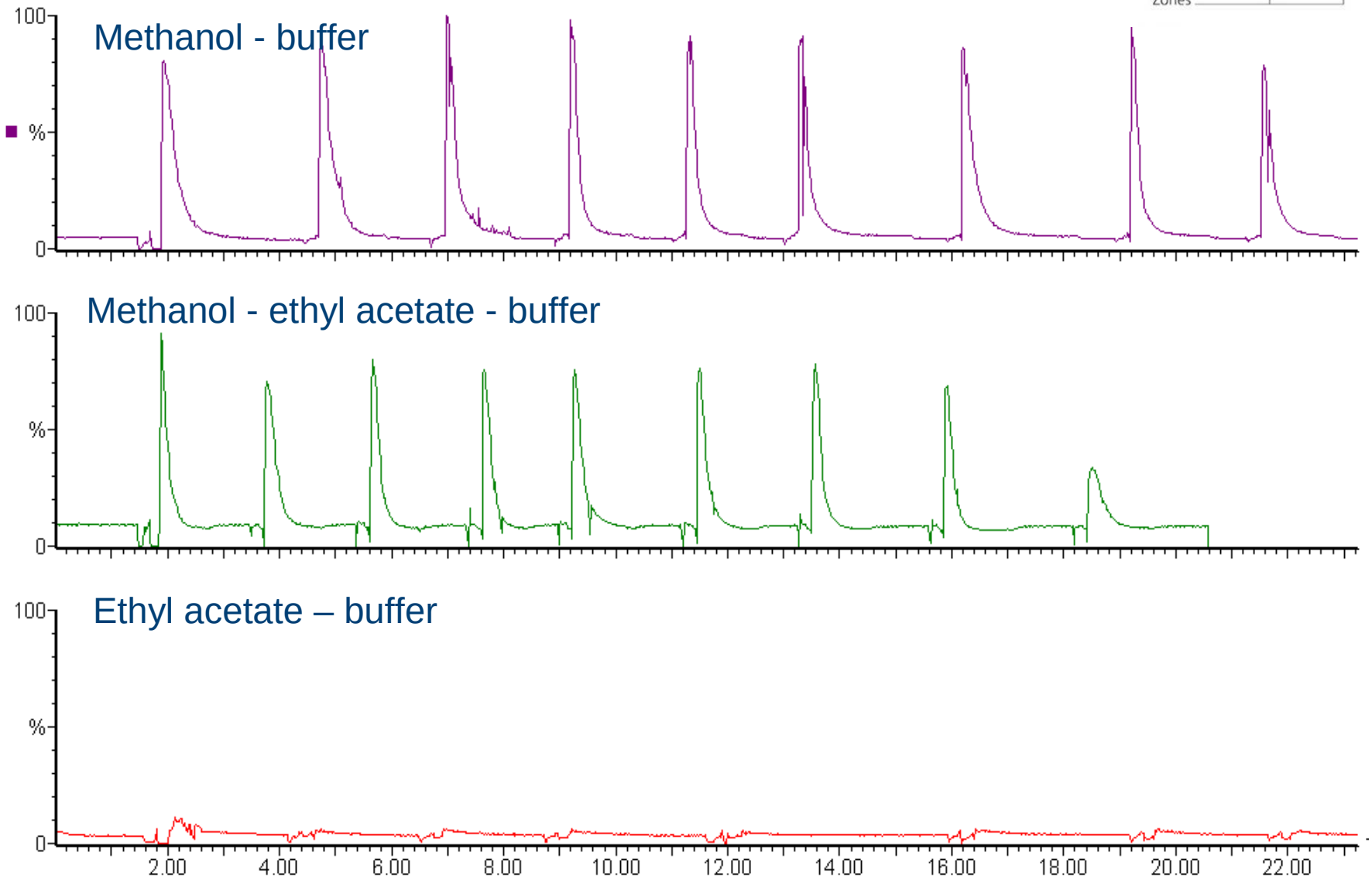
Elution profiles with different solvents





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

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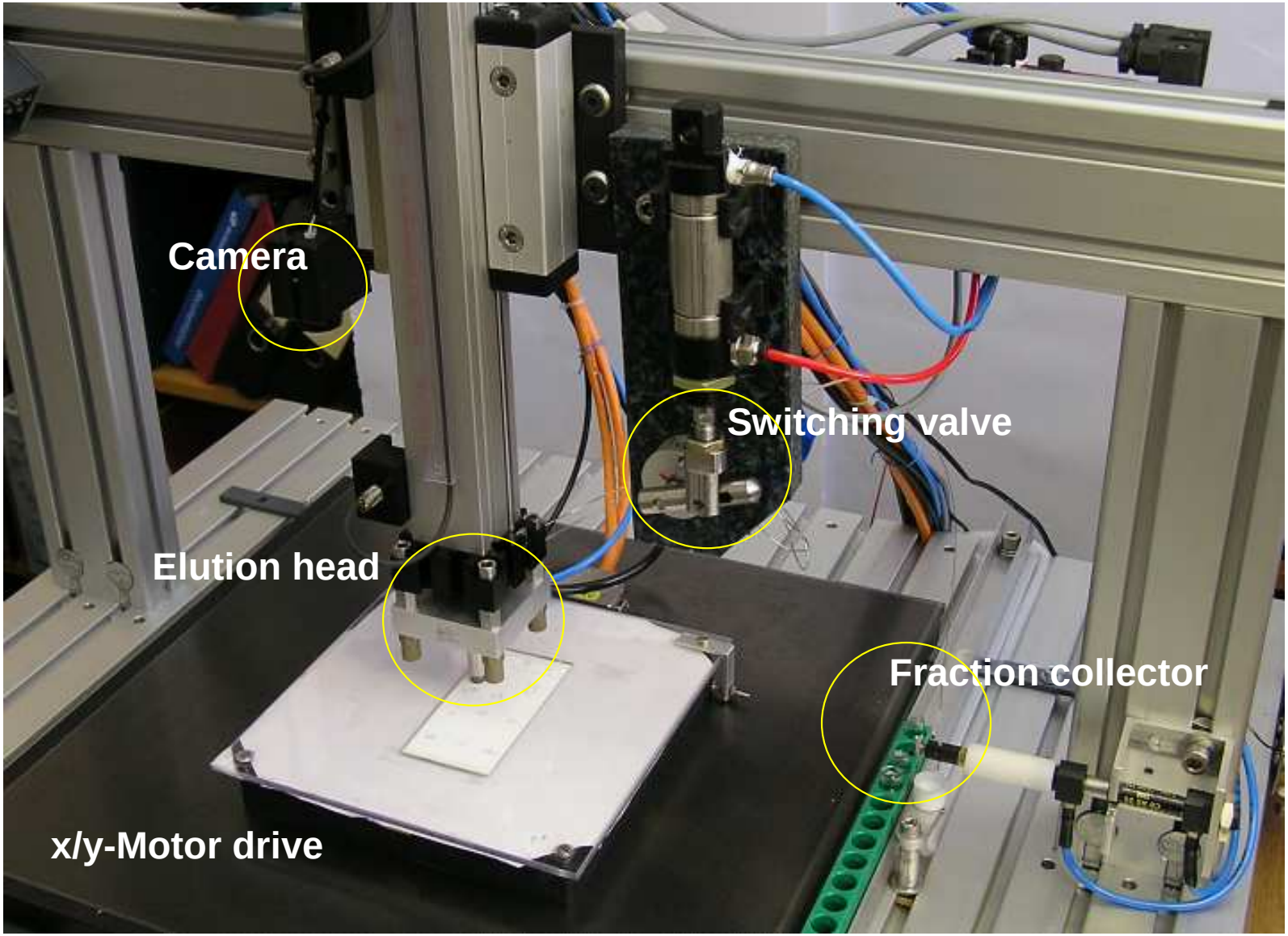
Hands-free interface called ‘R3D3’



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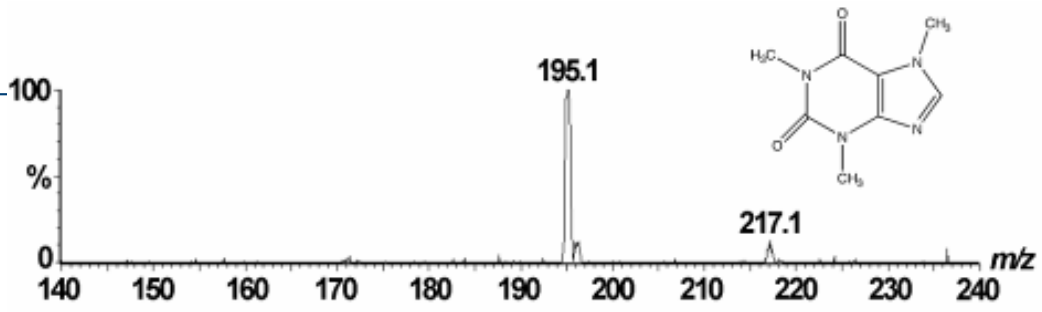
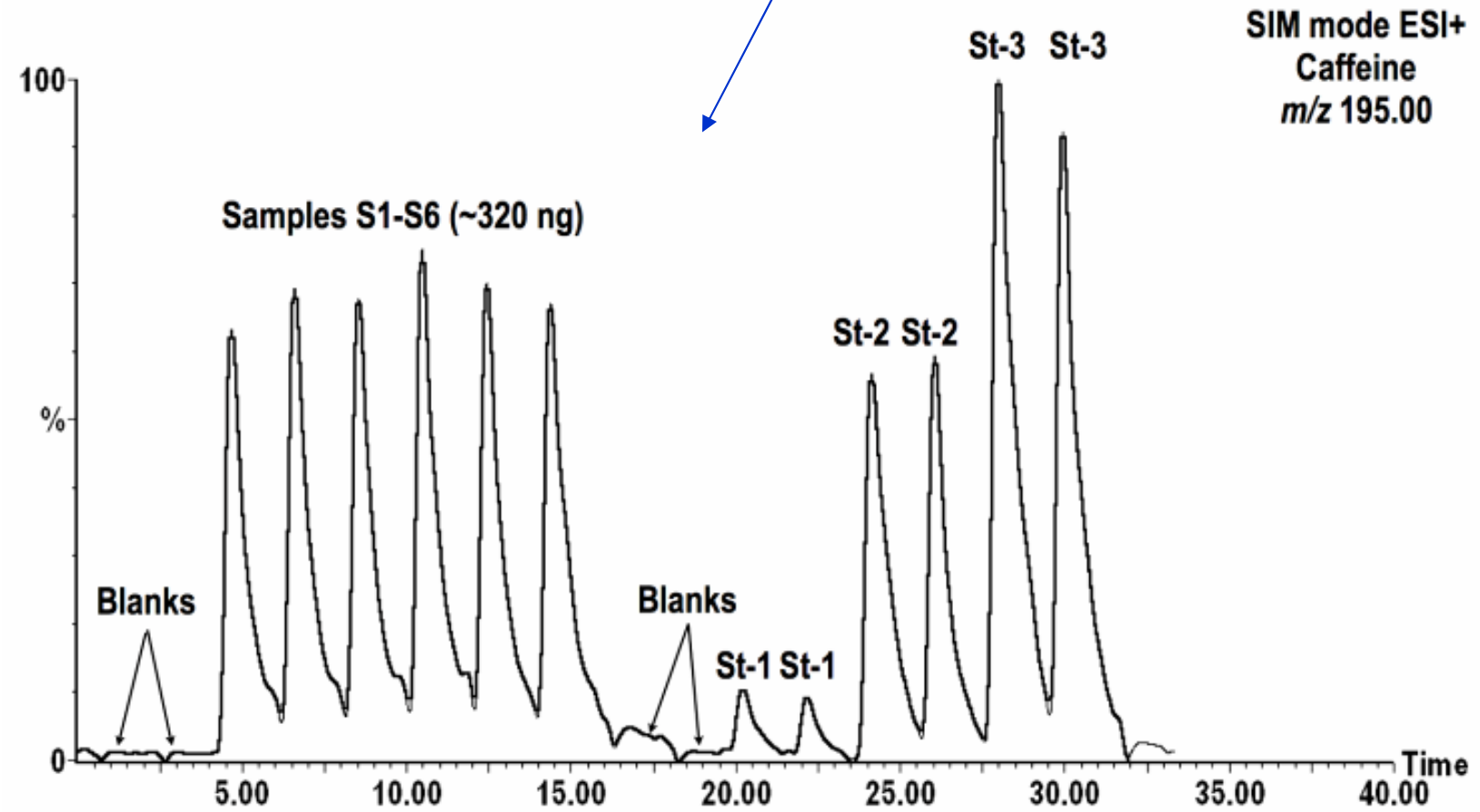
Elution profiles





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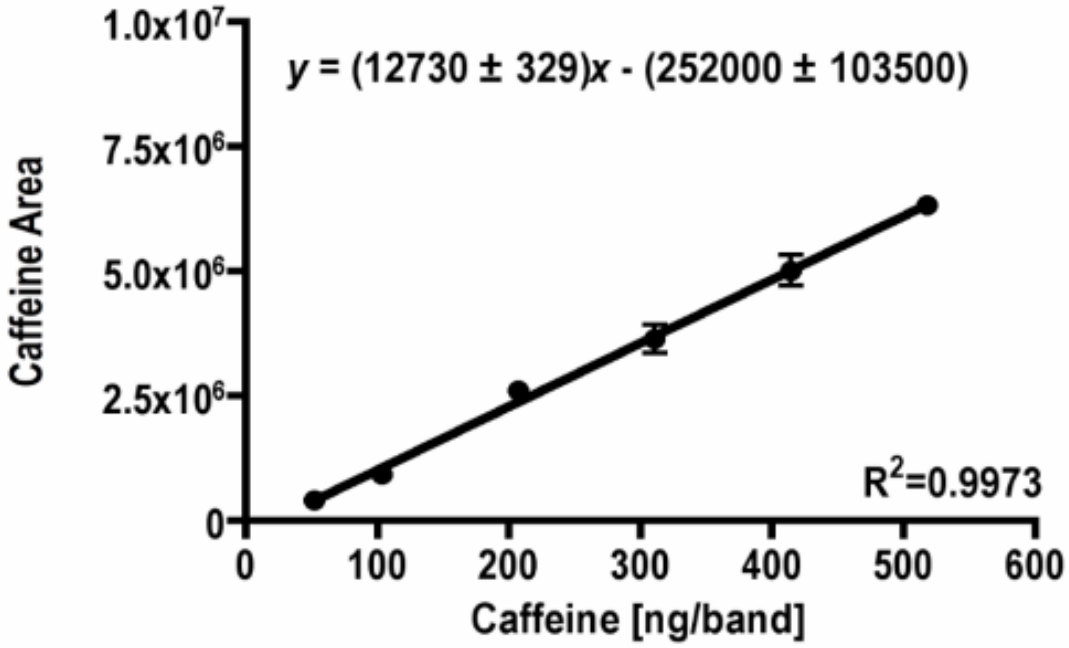
Data of validation without IS

→ Repeatability (*%RSD, n* = 6) in matrix: 5.6 %

→ Linearity R^2: 0.9973



H. Luftmann, M. Aranda, G. Morlock, Rapid Commun Mass Spectrom 21 (2007) 3772-3776

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Analysis of samples containing caffeine

Sample	Pharmaceutical mean ± SD (mg/tablet)	Energy drink mean ± SD (mg/100 mL)
HPTLC/ESI-MS RSD (%, n = 6)	102.09 ± 5.76 (5.6)	32.91 ± 1.60 (4.9)
HPTLC/UV RSD (%, n = 5)	101.98 ± 2.30 (2.3)	33.71 ± 0.96 (2.8)
Label	100	32

→ Comparable findings to validated HPTLC/UV methods (F-test, t-test)

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Comparison of automated interfaces

Parameter	Precision *%RSD*	Linear Response r^2
Quantification **without** internal standard		
Elution head (autom.)	≤ 5.6 %	0.9973
DESI	≤ 16.8 %	0.95 - 0.98
MALDI	10 %	-
LA-ICP	17 – 41 %	≥ 0.90
Quantification **with** internal standard		
Micro-junction ESI	≤ 4.4 %	0.9999
SALDI/APCI	7 %	0.9991
MALDI	≤ 8.9 %	0.9969
LA-ICP	3 – 40 %	≥ 0.98

G. Morlock, W. Schwack TrAc 29_10 (2010) 1157-1171

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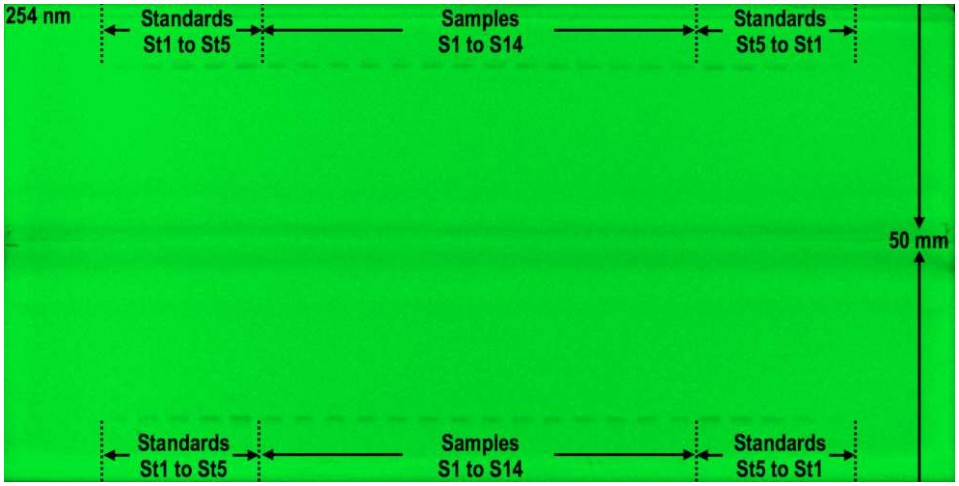
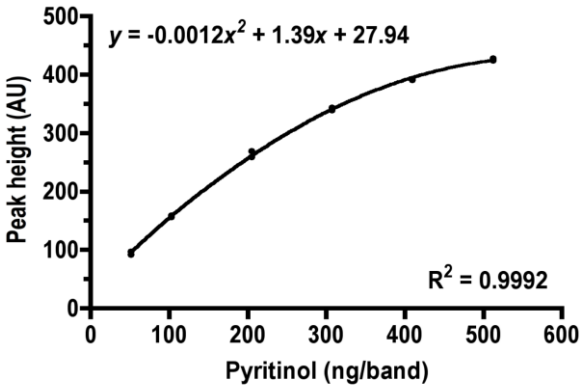
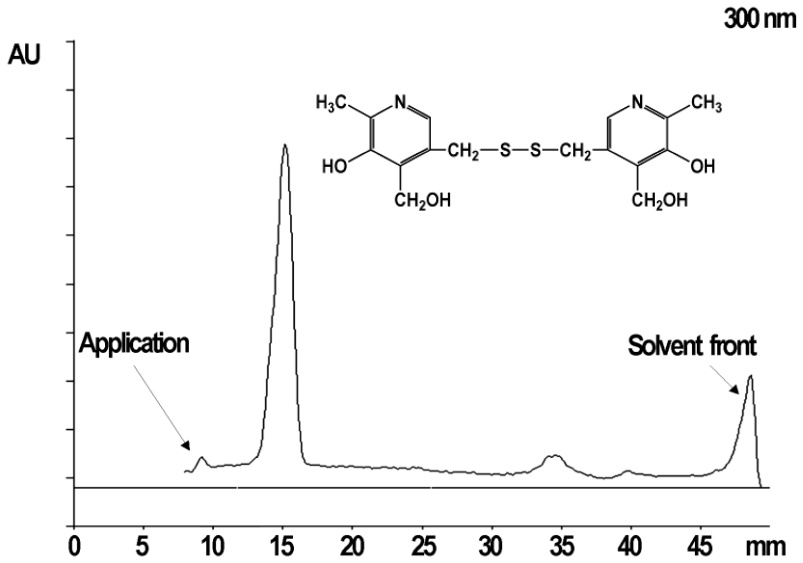
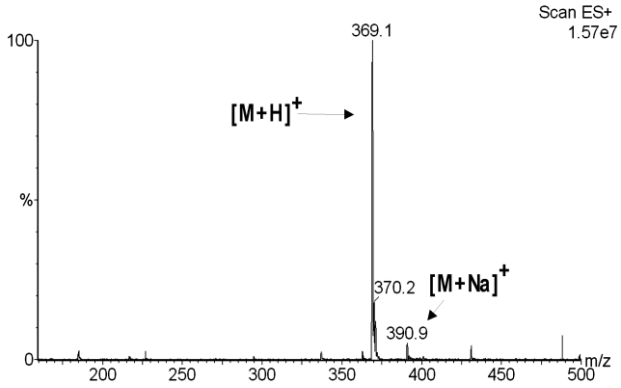
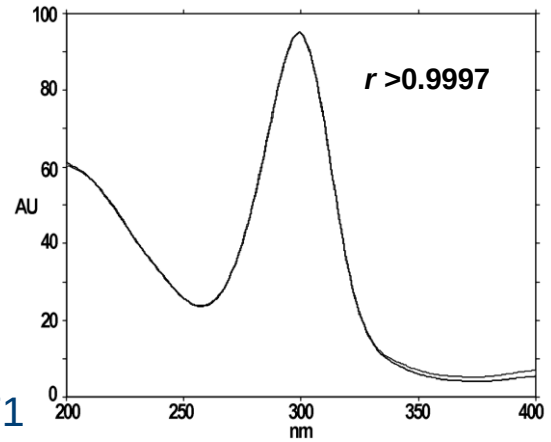
Pyridinol in tablets











M. Aranda, G. Morlock, J Liq Relat Technol 33 (2010) 957-971

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…no need for a higher separation power…

→ 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%)

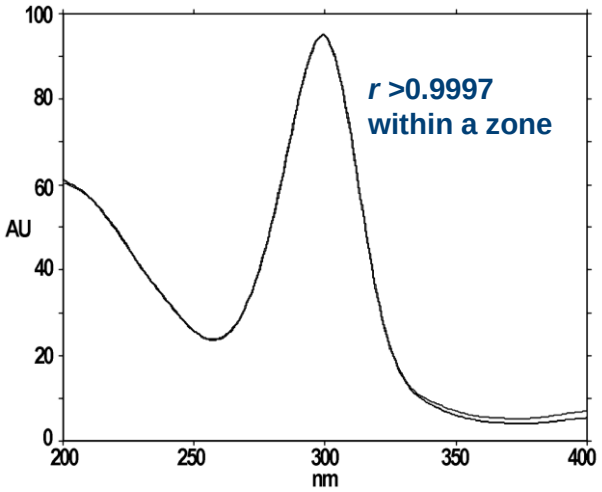
→ LOD/LOQ: 0.6/2.0 µg/mL (6/20 ng/band)

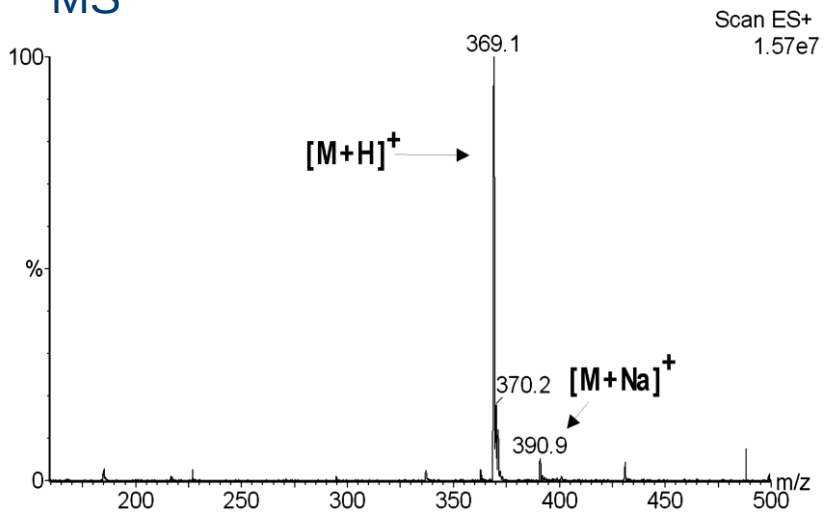
→ Up to 17 times less mobile phase consumption

→ Up to 8 times faster

→ Selectivity proven by spectral purity

UV MS





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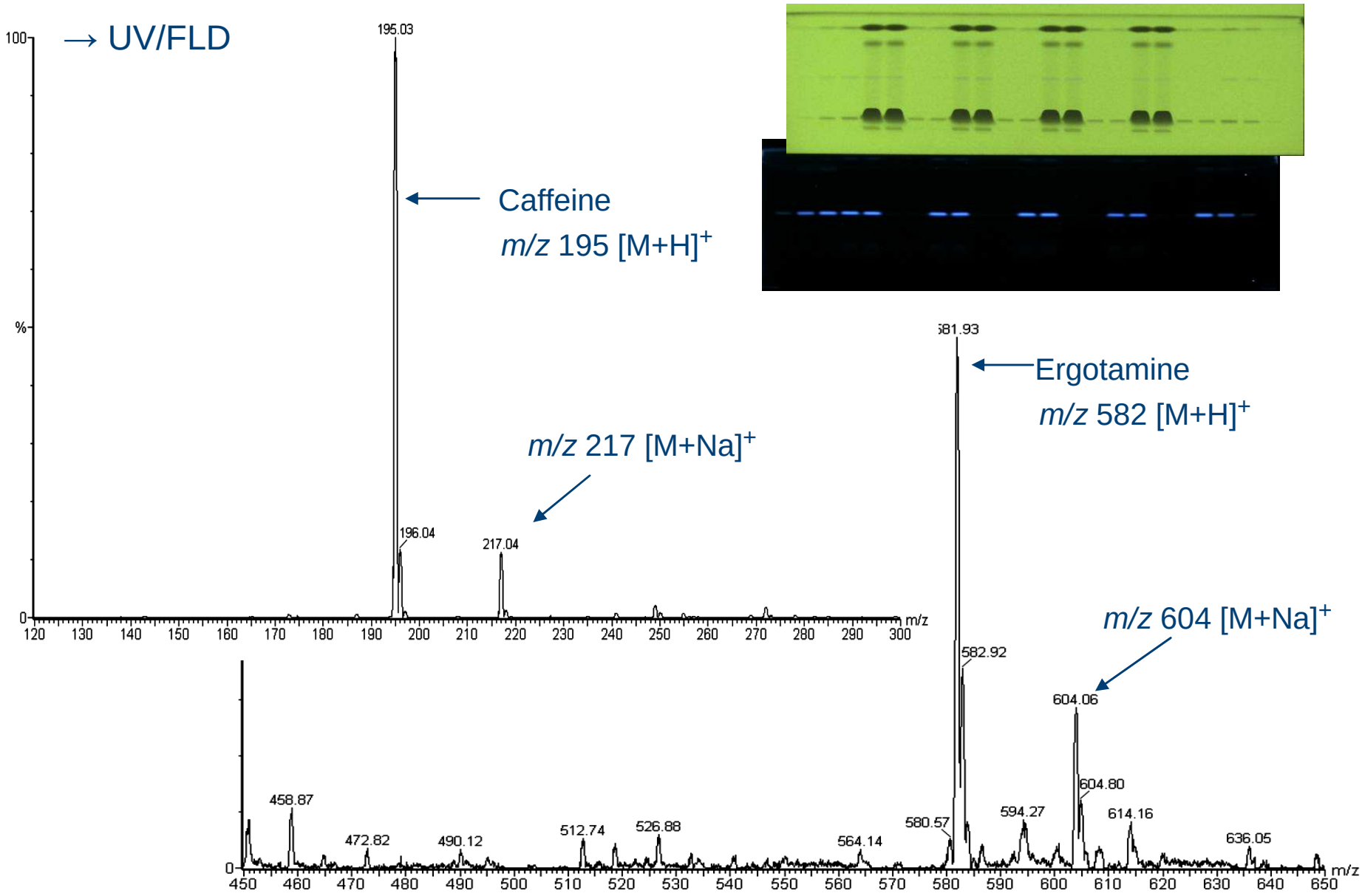
Caffeine, ergotamine and metamizol in tablets



M. Aranda, G. Morlock, J Chromatogr Sci 45 (2007) 251-255

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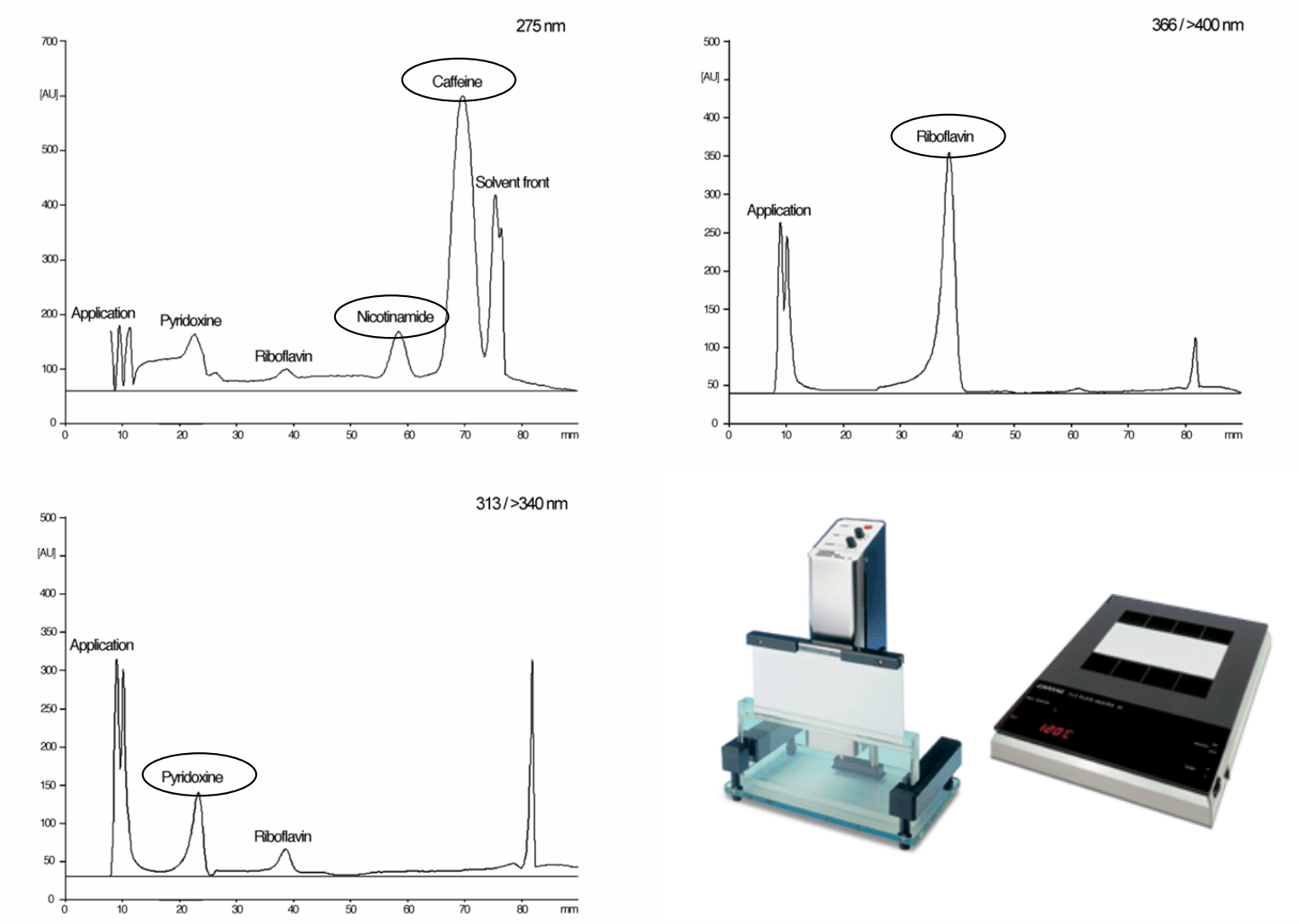
Active ingredients in energy drinks

Simultaneous determination by MWL scan (UV/FLD) → Derivatization → Vis



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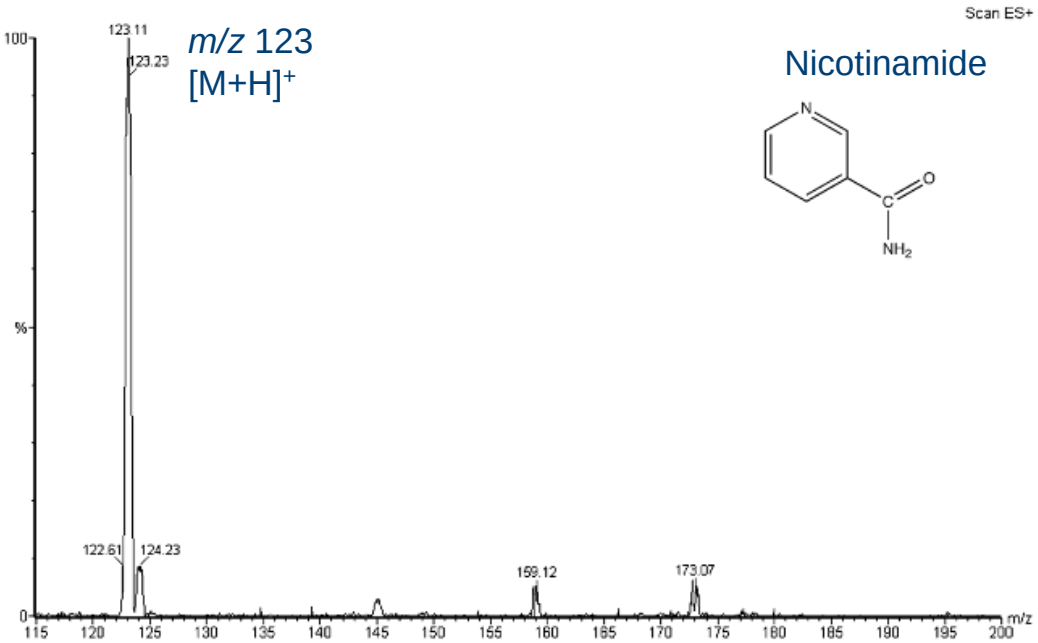
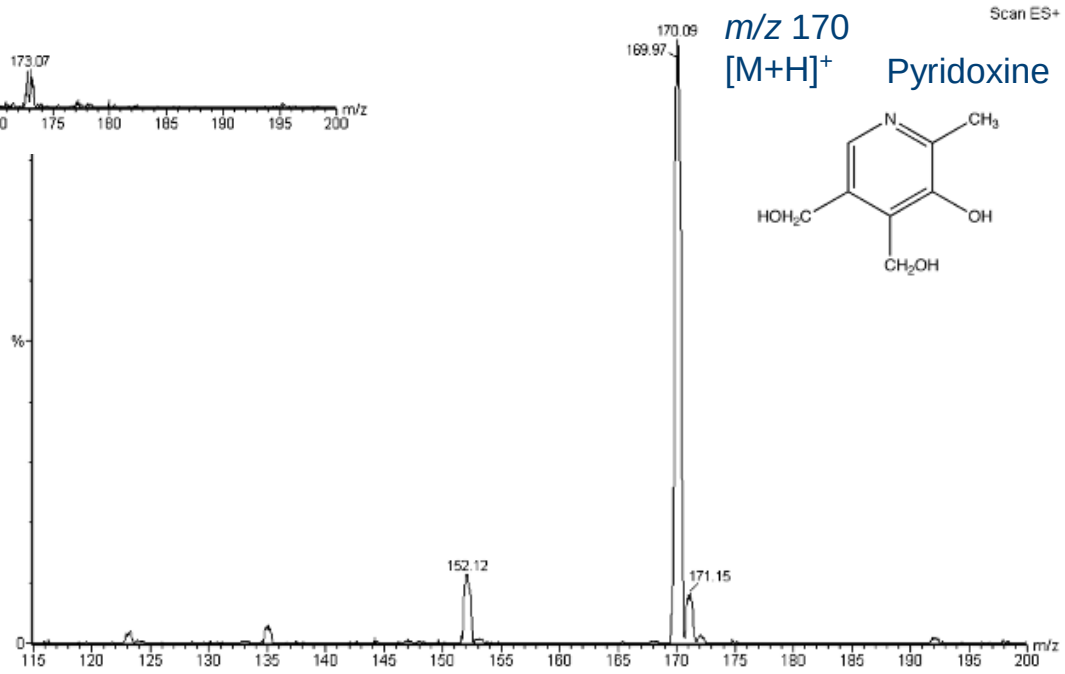
Confirmation by MS



m/z 123
$[M+H]^+$

Nicotinamide



m/z 170
$[M+H]^+$

Pyridoxine

M. Aranda, G. Morlock, J Chromatogr A 1131 (2006) 253-260

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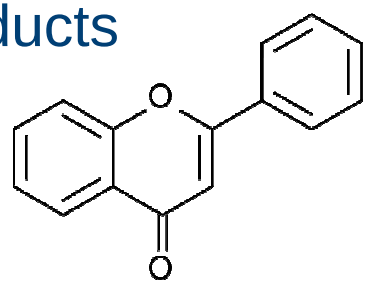
Are there different types of German propolis?

→ Use as food supplement and for cosmetic products



→ Flavonoid/phenolic acid profil

Resinated buds, *e.g.* of poplar and horse chestnut

German propolis

Cooperation with Wala and Apicultural State Institute, Stuttgart

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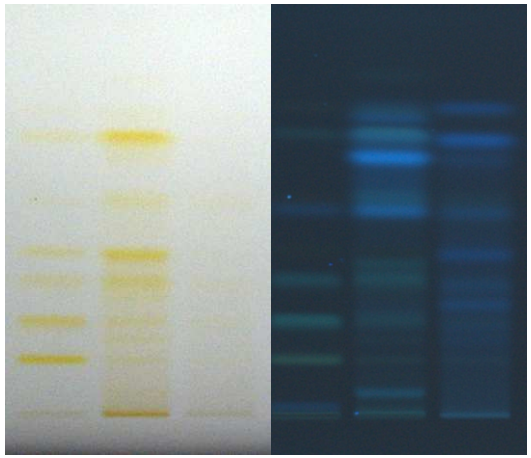
Information obtained from one plate

Fast characterization of samples by HPTLC

Visible zones

Native fluorescent zones (366 nm)



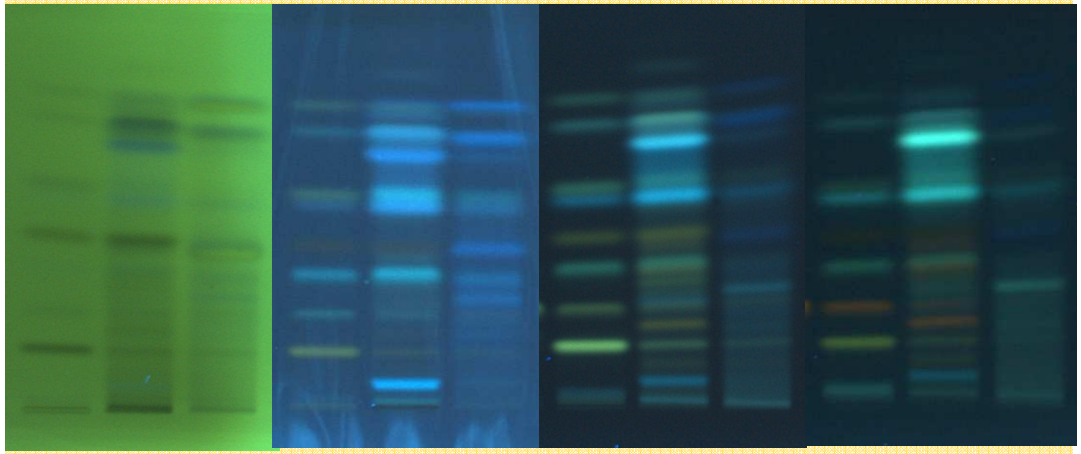
Selective derivatizations

Lipophilic zones → berberine (366 nm)

Flavonoids → $AlCl_3$ (366 nm)

Flavonoids → Neu (366 nm)

Flavonoids → Neu/PEG (366 nm)

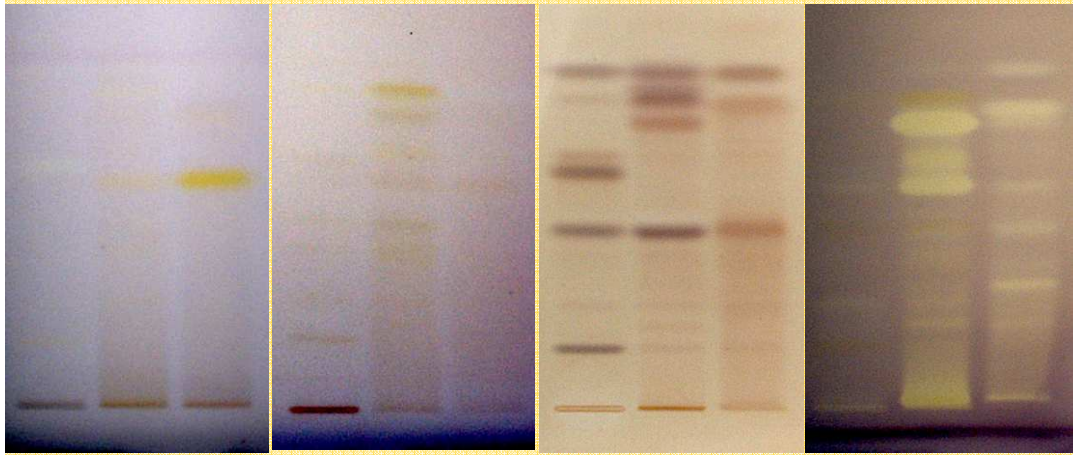


Sugars → DPA

Amino acids → Ninhydrin

Phenolics → Fast blue salt

Antioxidatives → DPPH*



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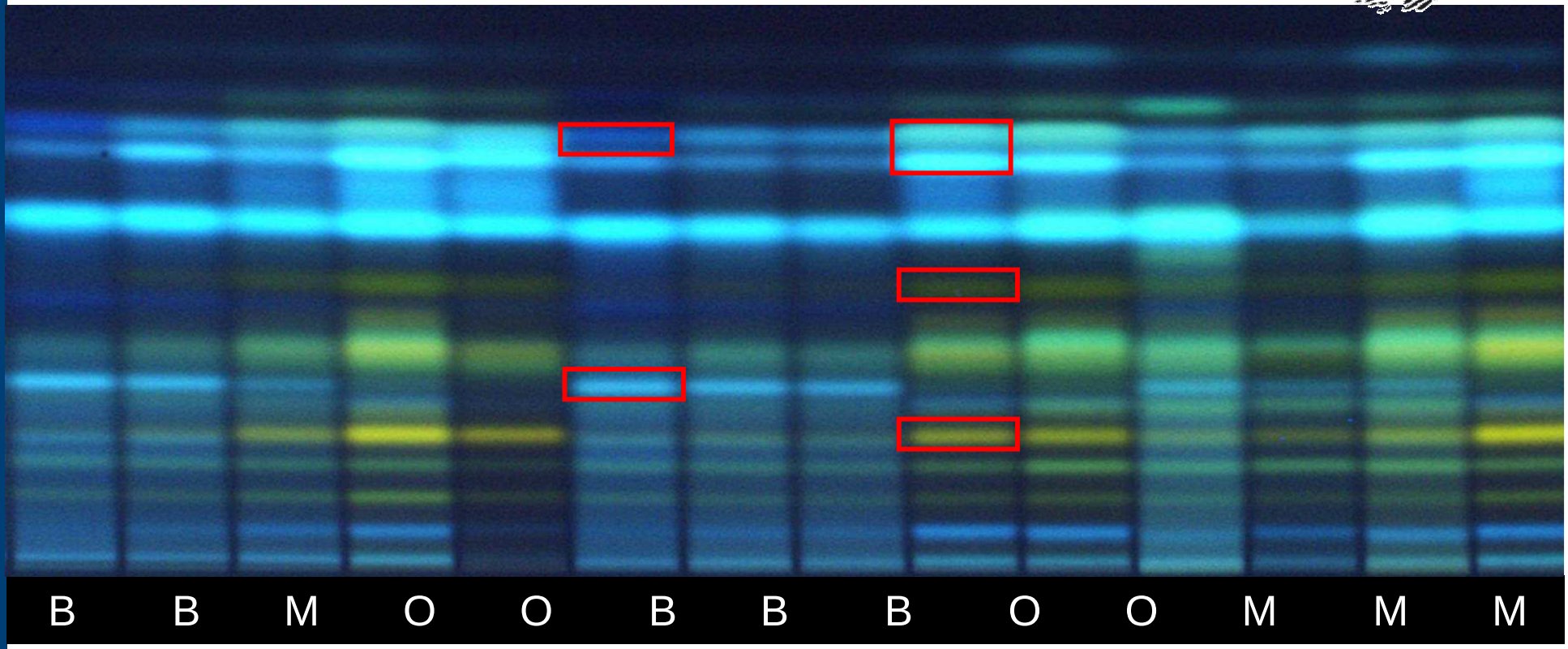
Flavonoid/phenolic acid profil

→ German propolis



→ Screening of 100 samples showed characteristic marker compounds

→ 2 types of propolis

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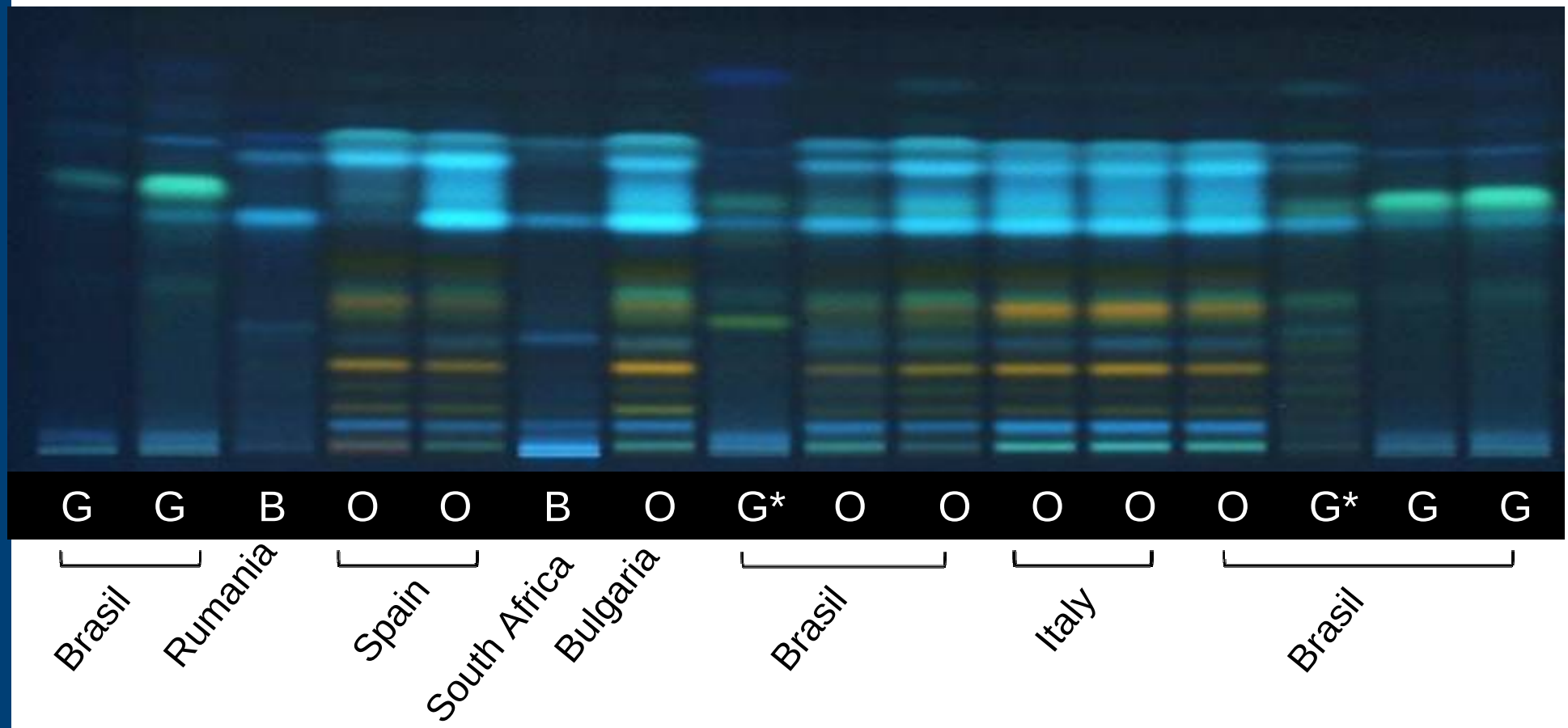
Flavonoid/phenolic acid profil

→ Foreign propolis: additional green type



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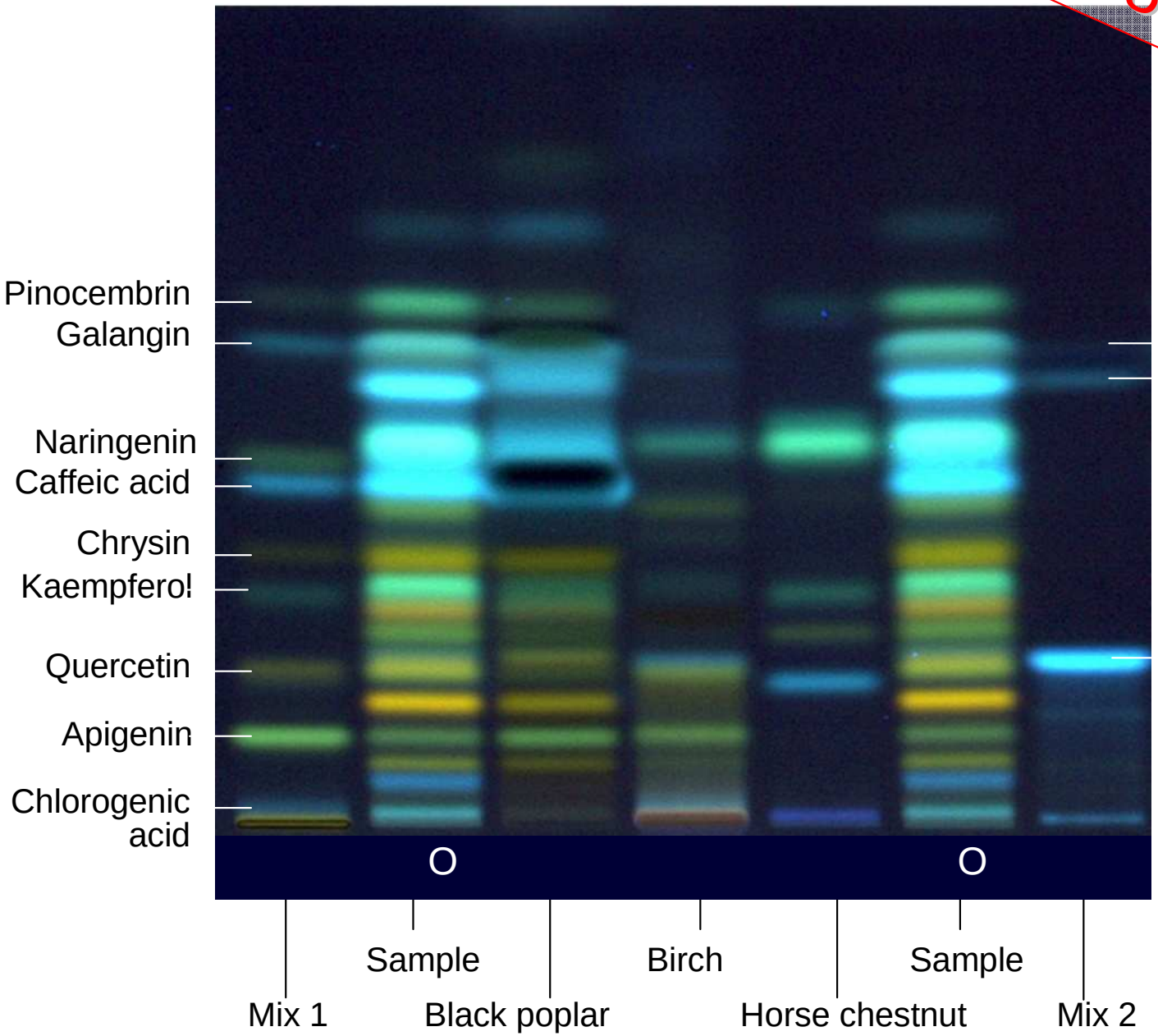
Plant origin in type O?

Poster 8f



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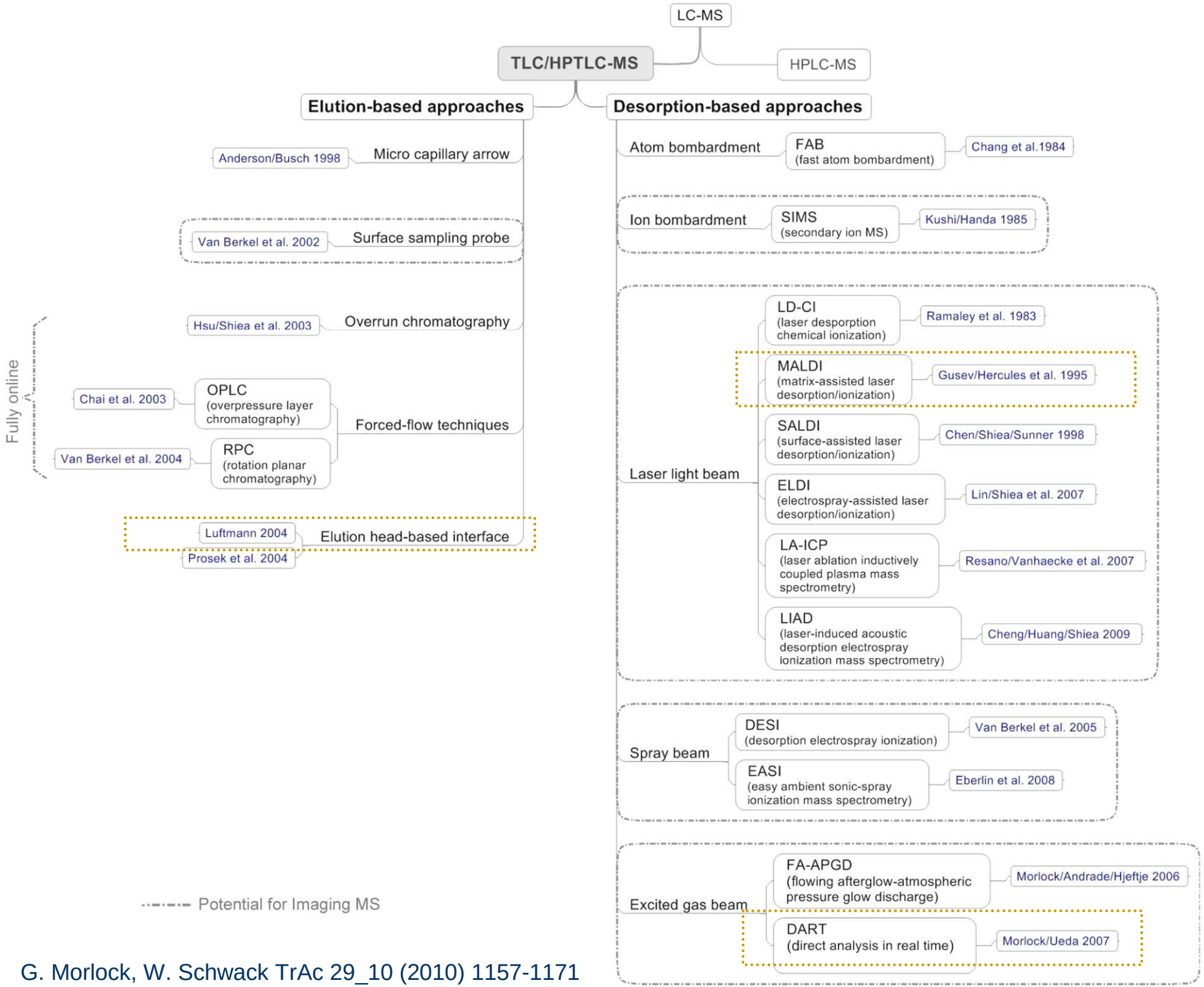

G. Morlock, W. Schwack TrAc 29_10 (2010) 1157-1171

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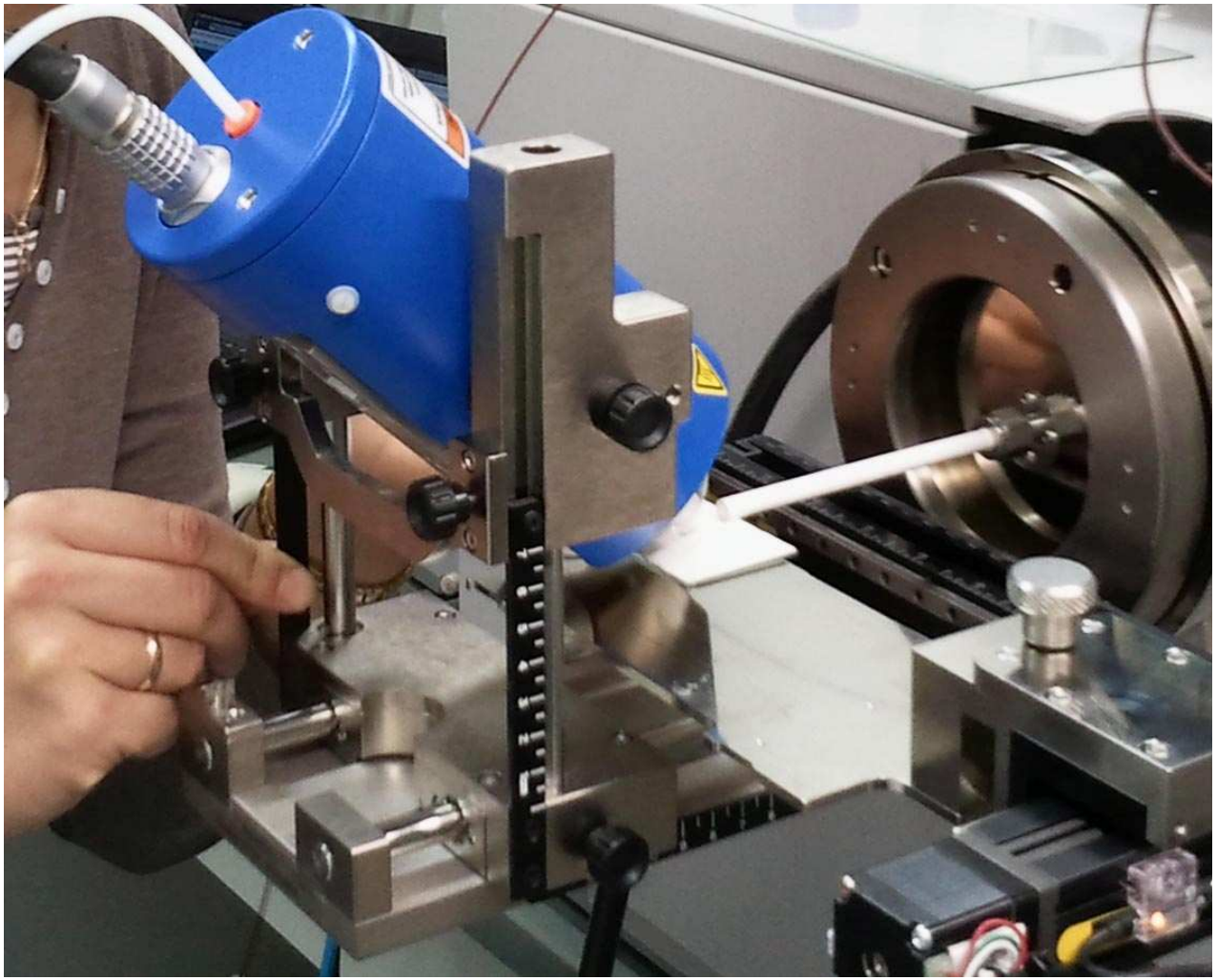
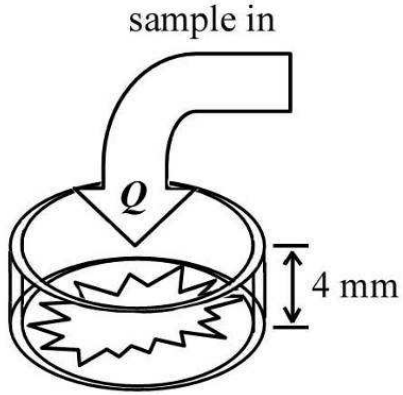
Desorption-based HPTLC-MS → HPTLC-DART-MS

Direct Analysis in Real Time Mass Spectrometry





E. Chernetsova, G. Morlock, *Rapid Commun Mass Spectrom,* in print

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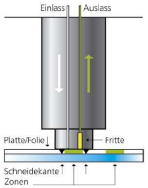
Confirmation of marker compounds by MS

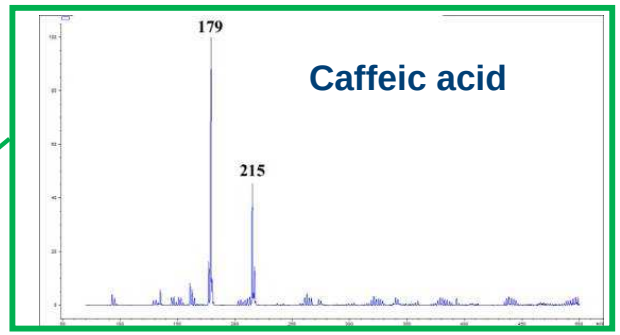
Lecture 9b

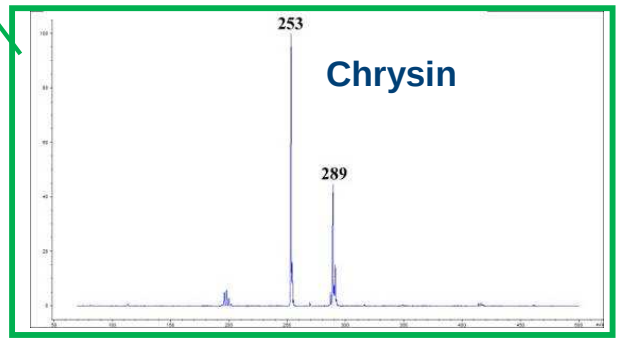
ESI-MS full scan spectra



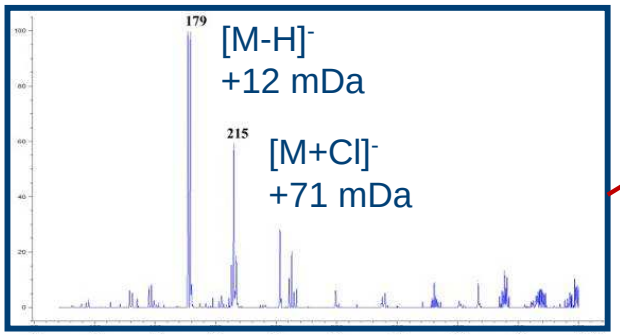
EIC of DART-MS

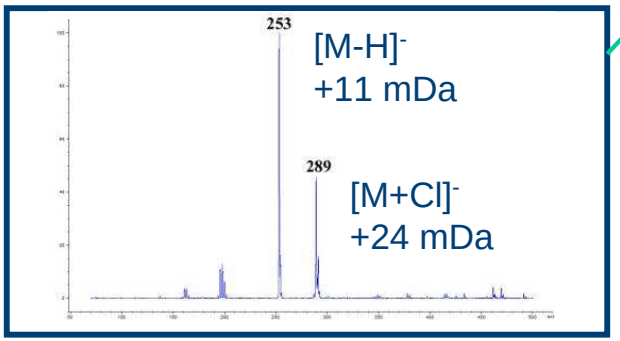
Spectra of standard





Spectra of sample





G. Morlock, W. Schwack, *TrAc* 2910 (2010) 1157-1171
E. Chernetsova et al. *Mass Spectrom Rev* 2011, in print

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Confirmation of marker compounds by MS

Lecture 9c

TLC-MS VERSUS TLC-LC-MS FINGERPRINTS OF HERBAL EXTRACTS. PART II. PHENOLIC ACIDS AND FLAVONOIDS

Mieczysław Sajewicz,[1] Dorota Staszek,[1] Maja Natić,[1,2] Łukasz Wojtal,[1] Monika Waksmundzka-Hajnos,[3] and Teresa Kowalska[1]

[1] *Institute of Chemistry, University of Silesia, Katowice, Poland*
[2] *Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*
[3] *Department of Inorganic Chemistry, Medical University of Lublin, Lublin, Poland*

□ *In the previous paper from this series, we proposed mass spectrometric fingerprinting of a complex and volatile botanical sample upon an example of the essential oil derived from* Salvia lavandulifolia. *In that paper, we compared two variants of fractionation of such a mixture. A simpler one-dimensional variant consisted of the low-temperature thin-layer chromatographic fractionation coupled with mass spectrometric fingerprinting of each separated fraction (1D LT TLC-MS). A more sophisticated variant was the two-dimensional liquid chromatographic system*

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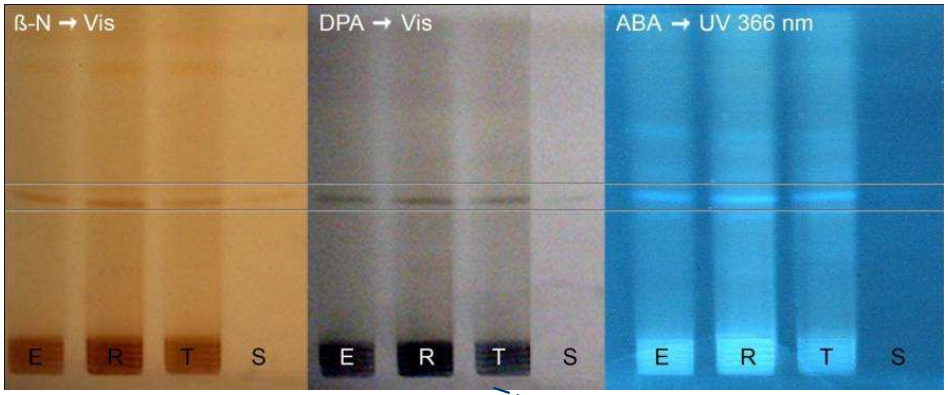
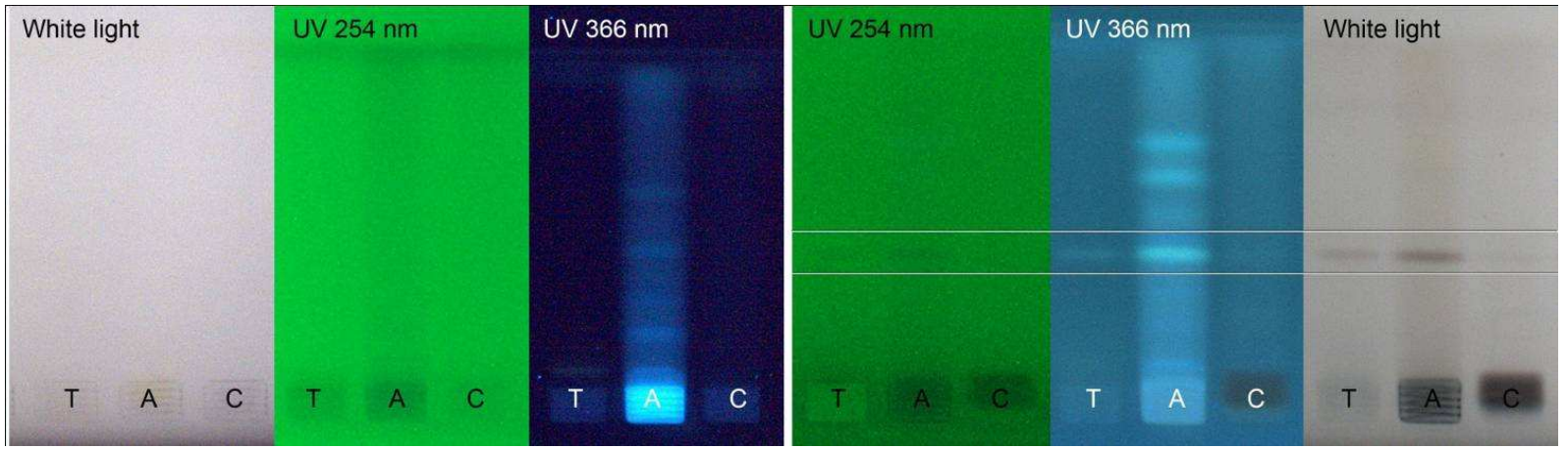
More information about unknown samples

Sucralose in sewage and waste water

Variety of derivatization options





→ MS

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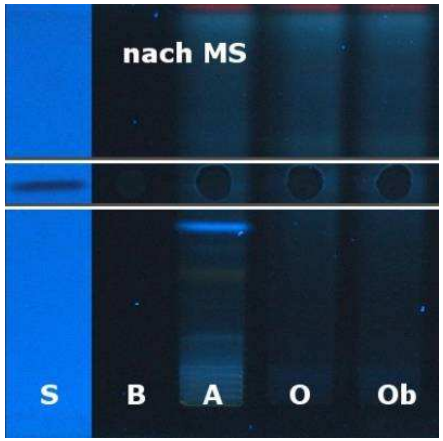
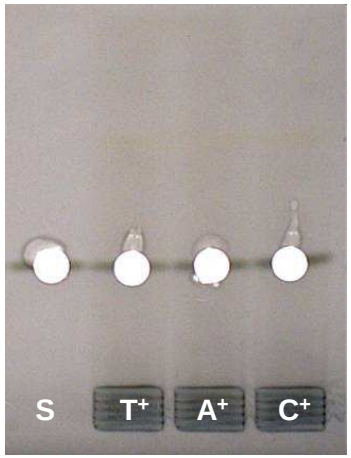
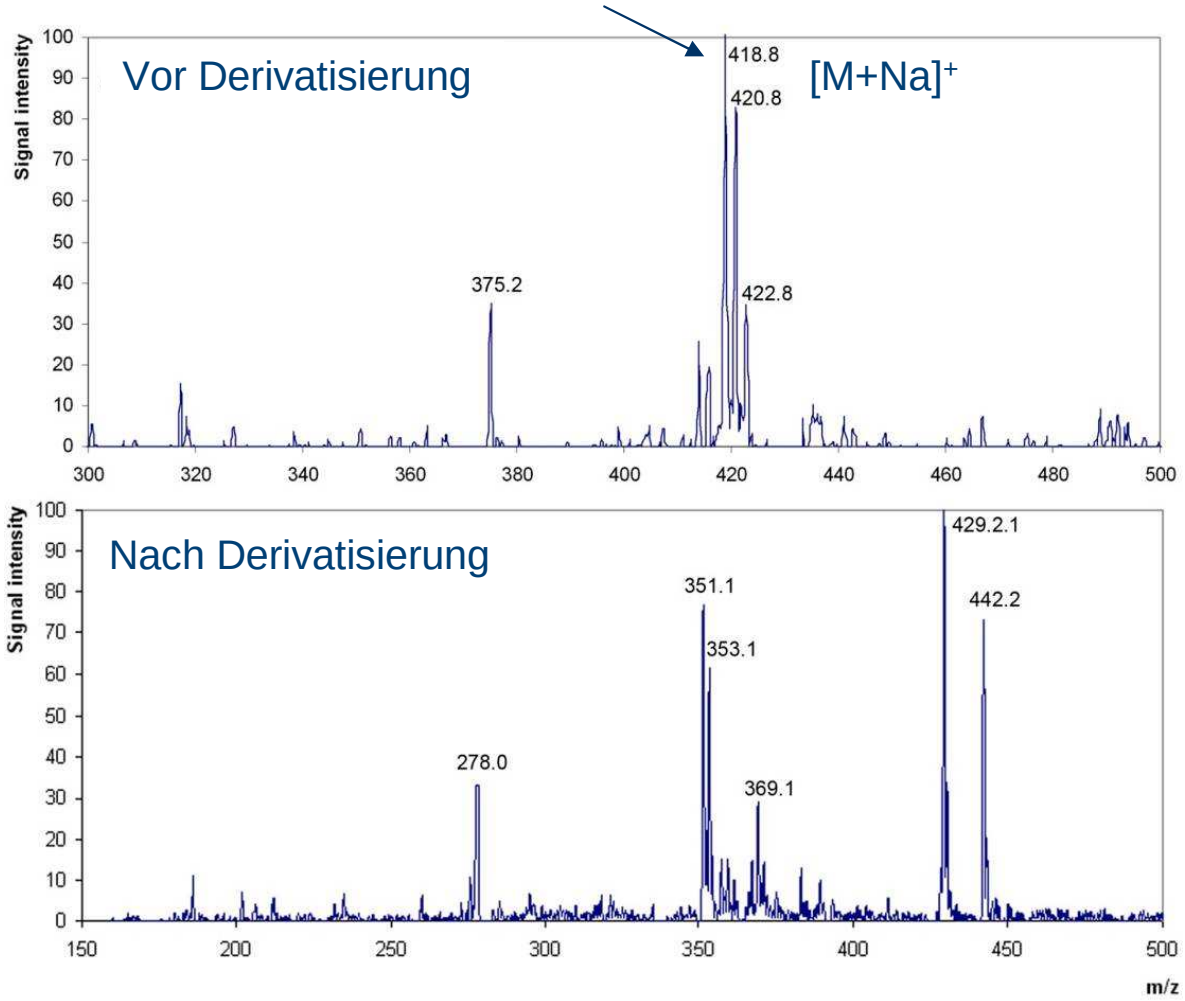
Confirmation by HPTLC-MS







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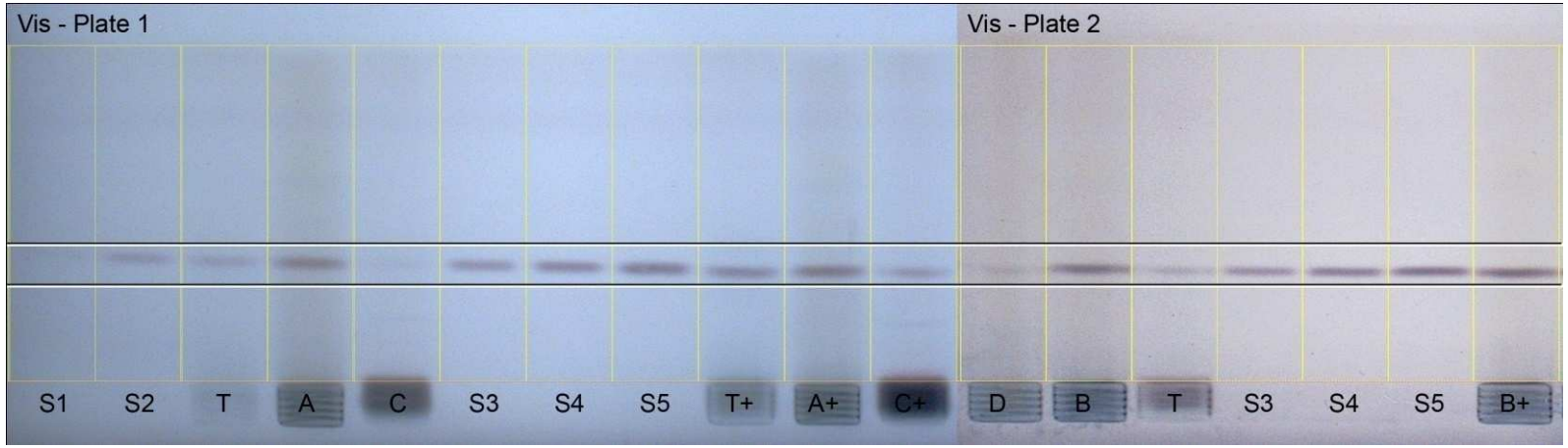
Analysis of sewage effluent and river water

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

…or HPTLC-Vis?



Mean value (ng/L)	Sample A	Sample B	Sample C	Sample D
HPLC-TOF or -MS/MS (n = 6 laboratories)	5869	7302	186	200
HPTLC-Vis (n = 2)	5863	7034	247	218

G. Morlock, S. Grashorn, L. Schuele, *J Chromatogr A* 1218 (2011) 2745–2753

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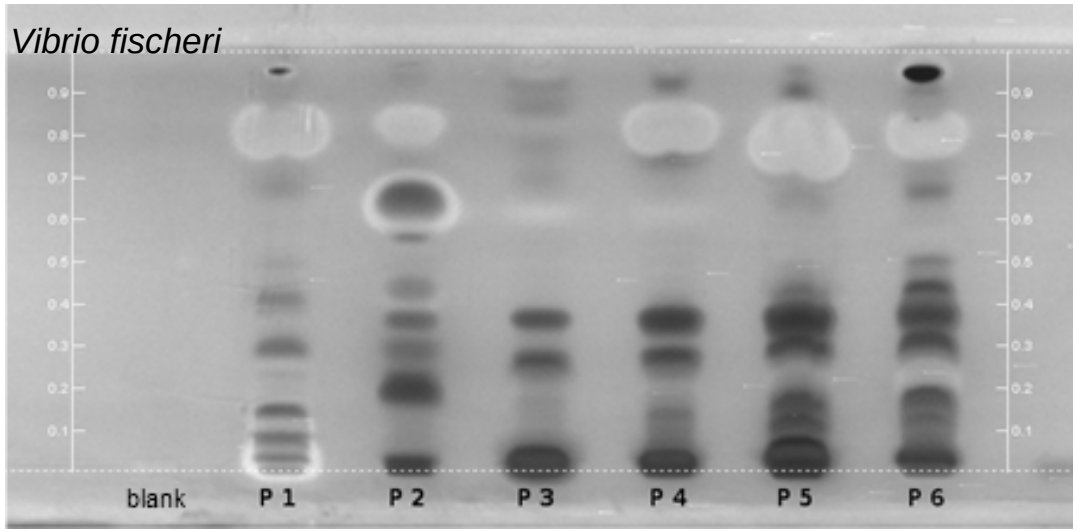
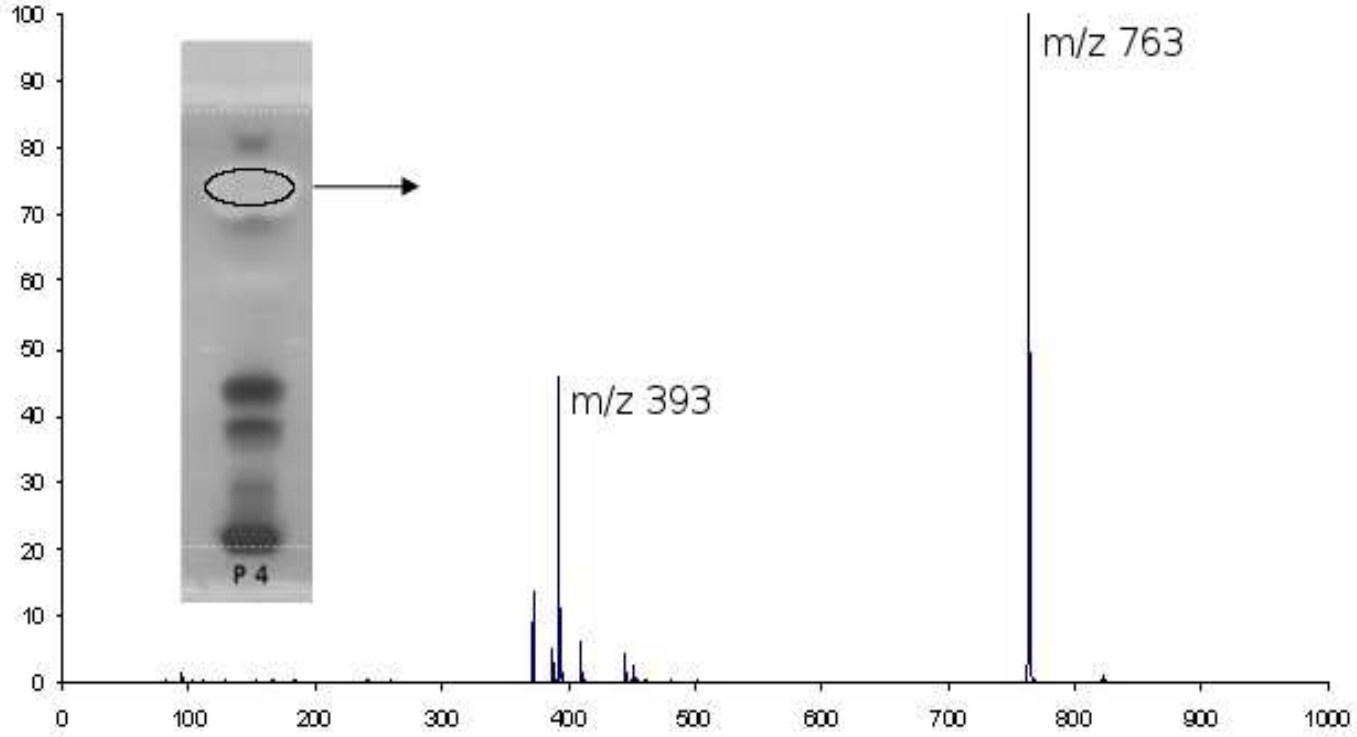
Additives in food packaging foils

Poster 8f





E. Dytkiewitz, W. Schwack, CBS 105 (2010) 13-15

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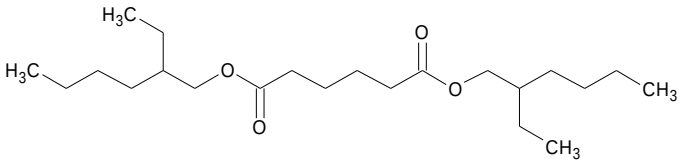
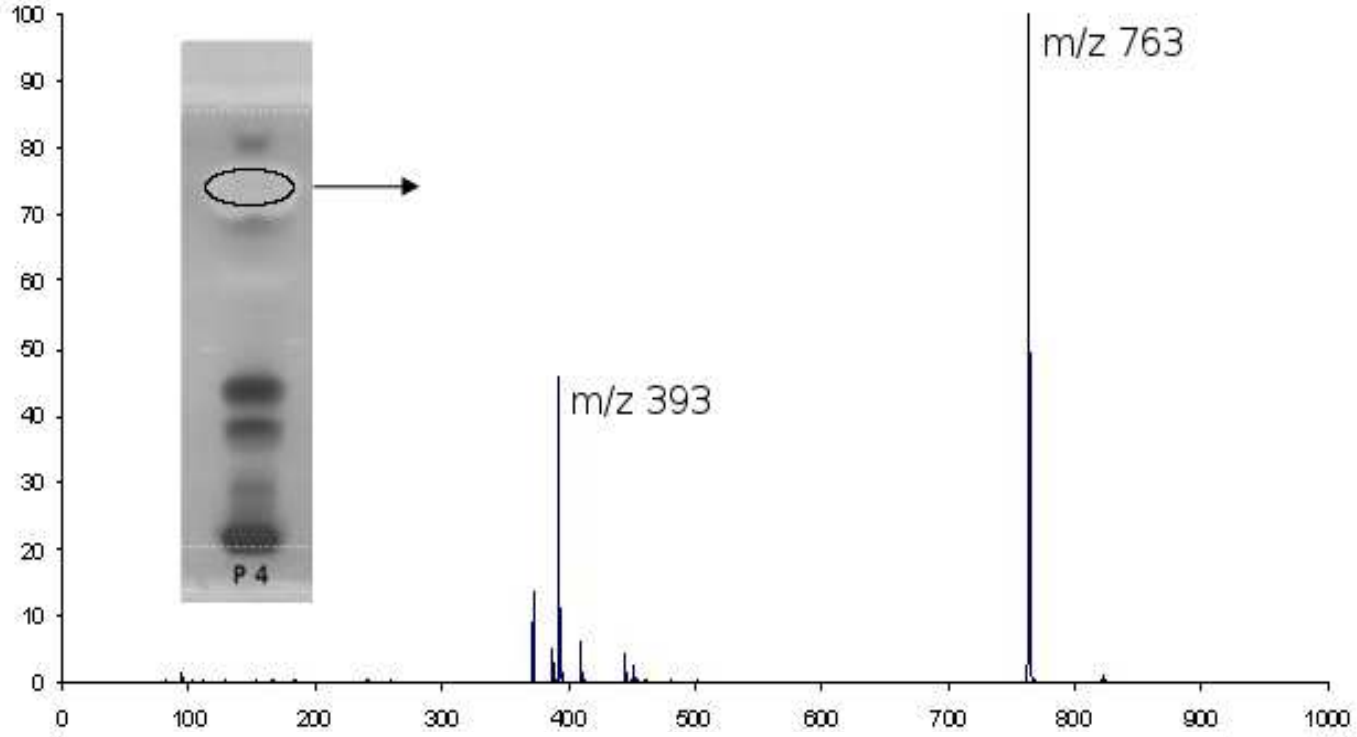
Detection of additives in polymer packaging foils



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	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$



E. Dytkiewitz, W. Schwack, CBS 105 (2010) 13-15

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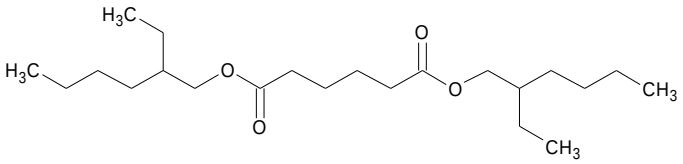
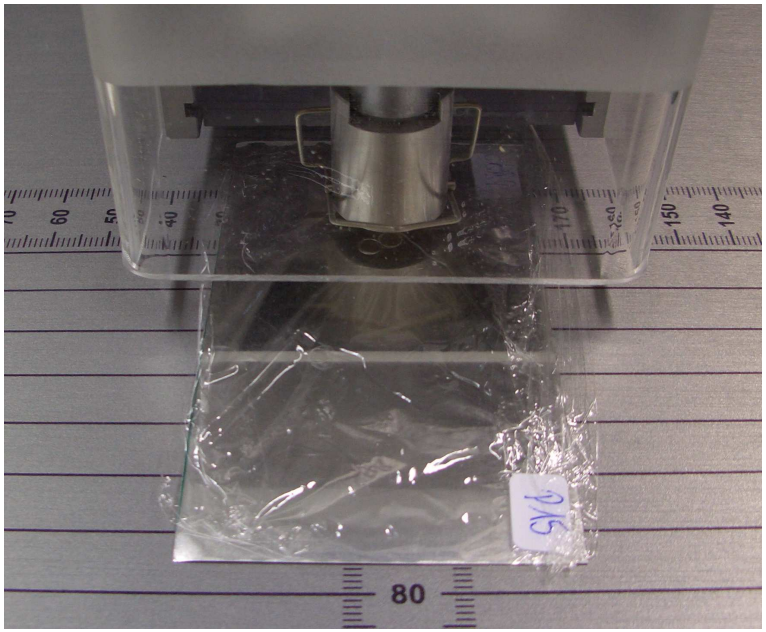
Detection of additives in polymer packaging foils



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	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$



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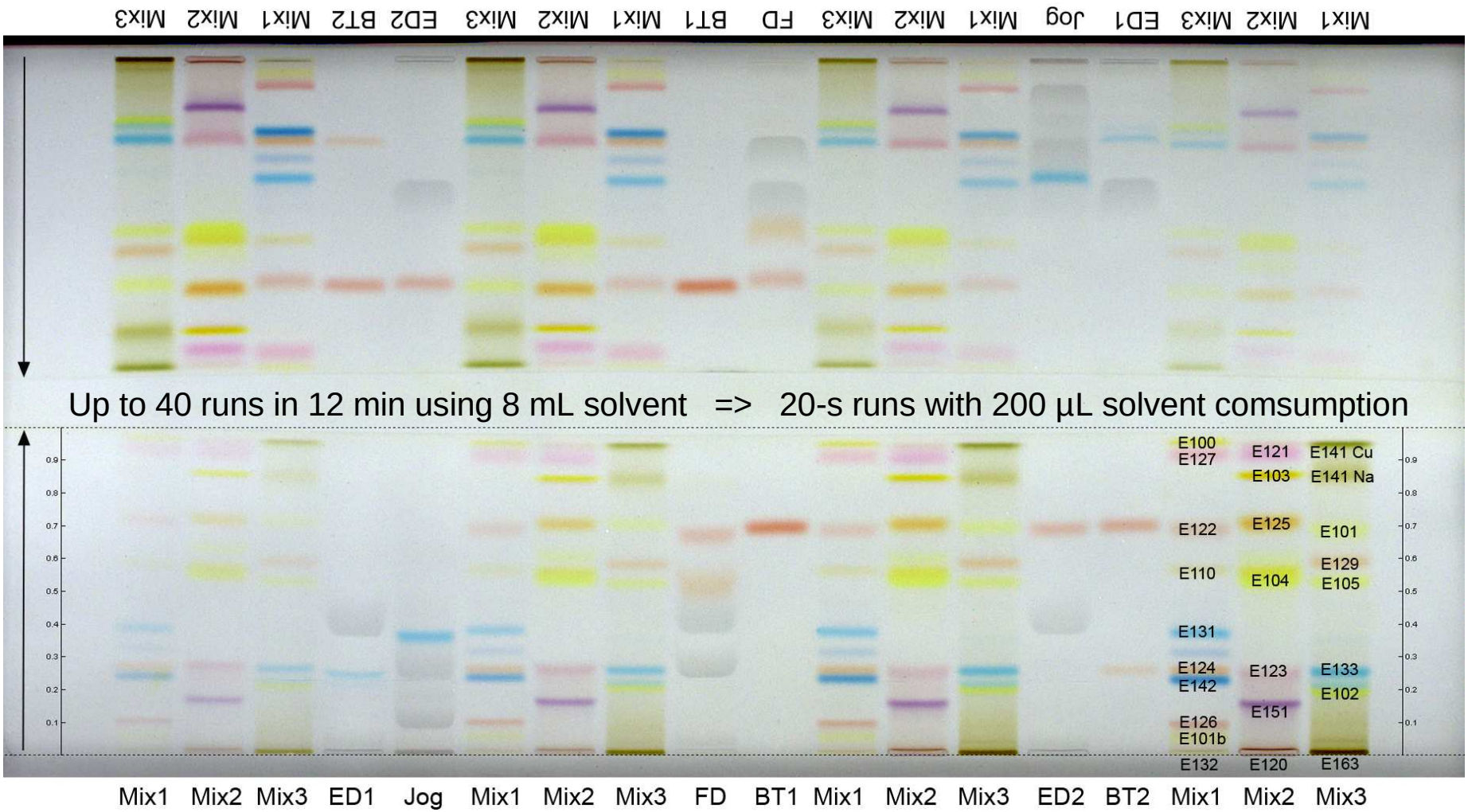
Dye analysis



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

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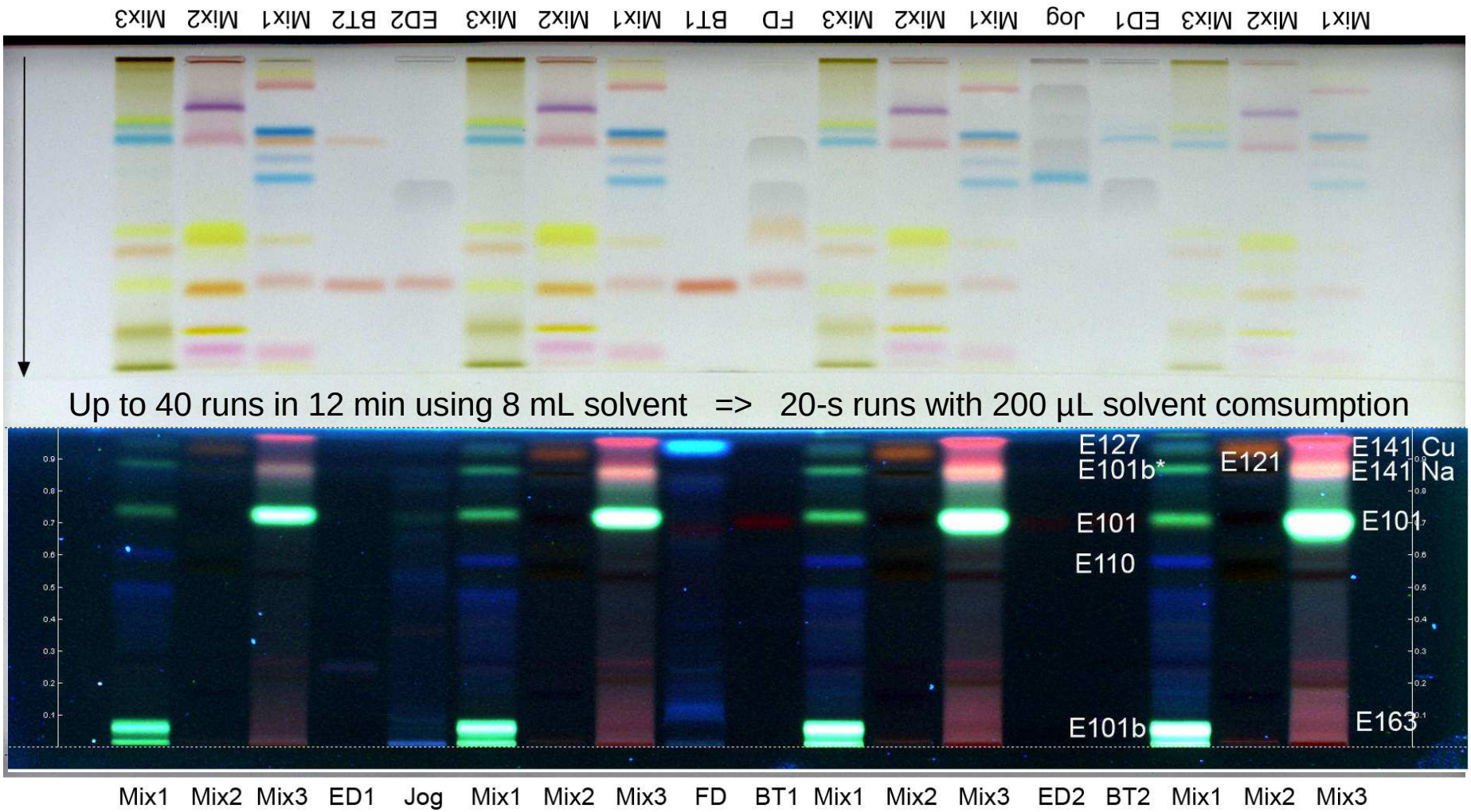
Dye analysis



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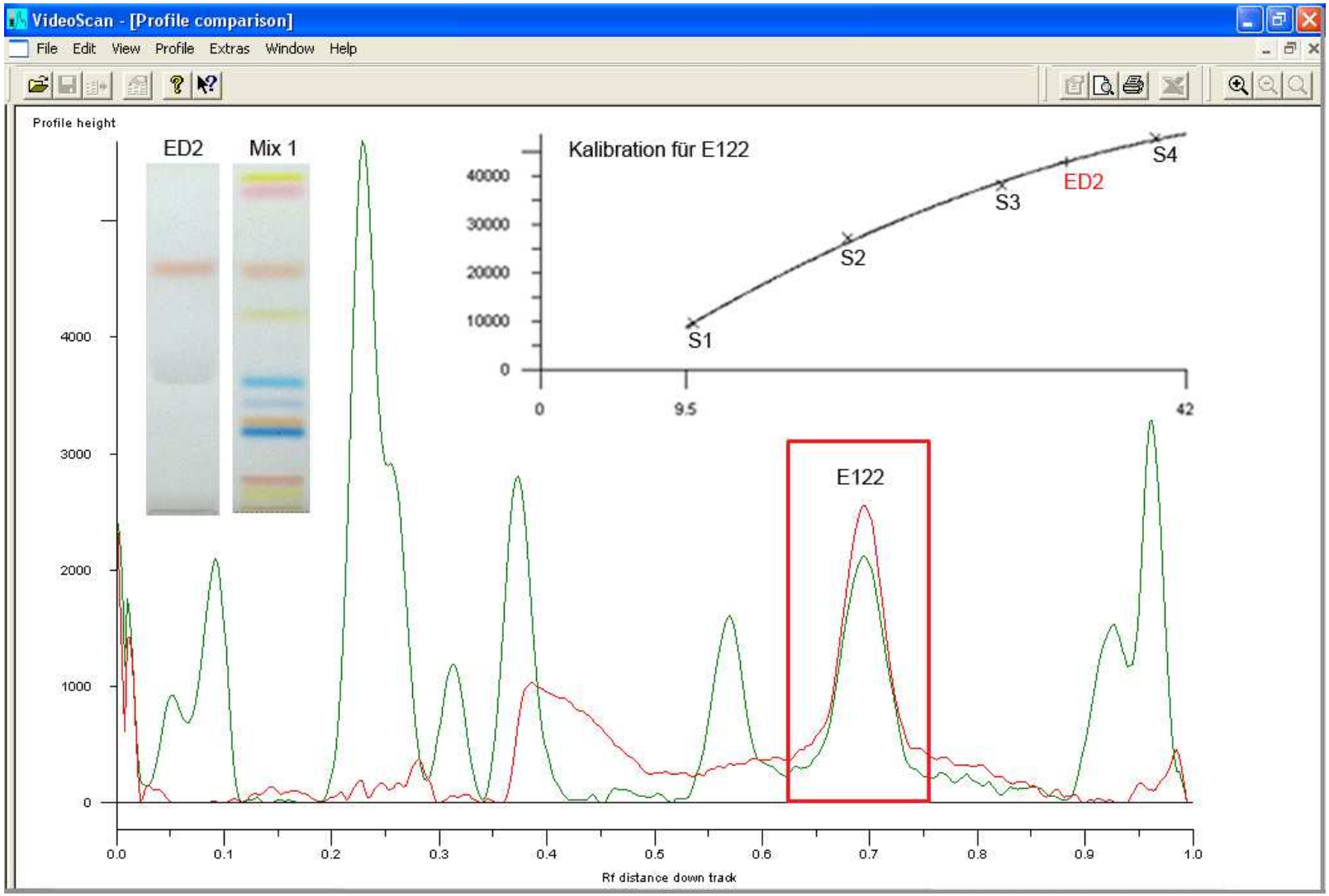
Digital quantification



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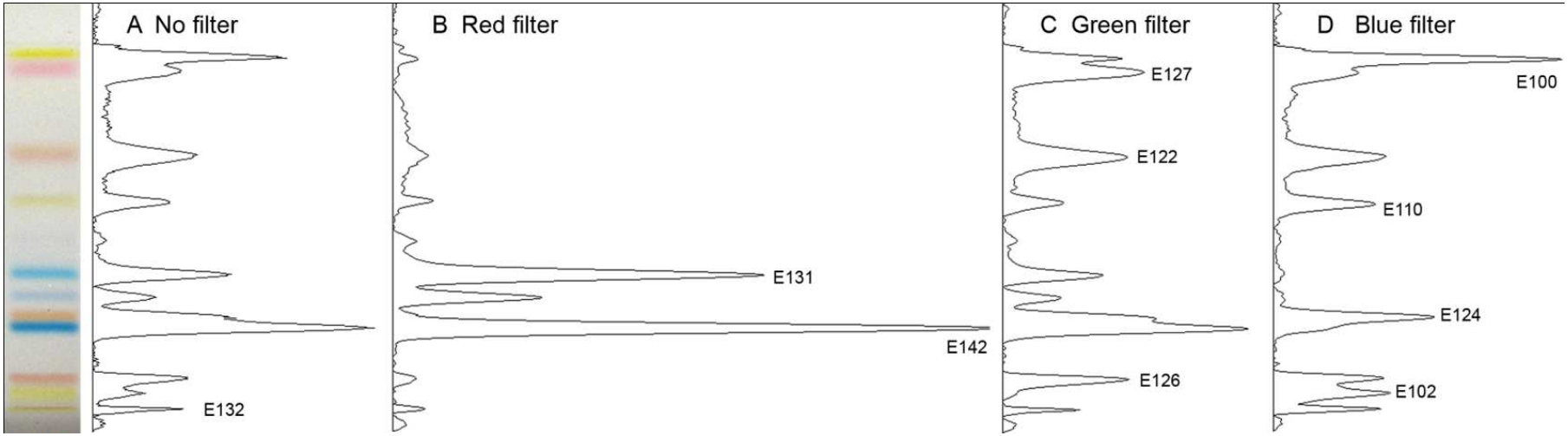
Digital filters



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

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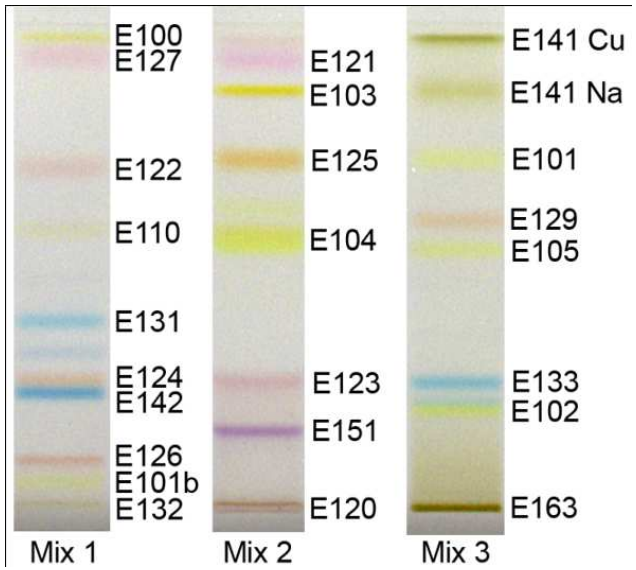
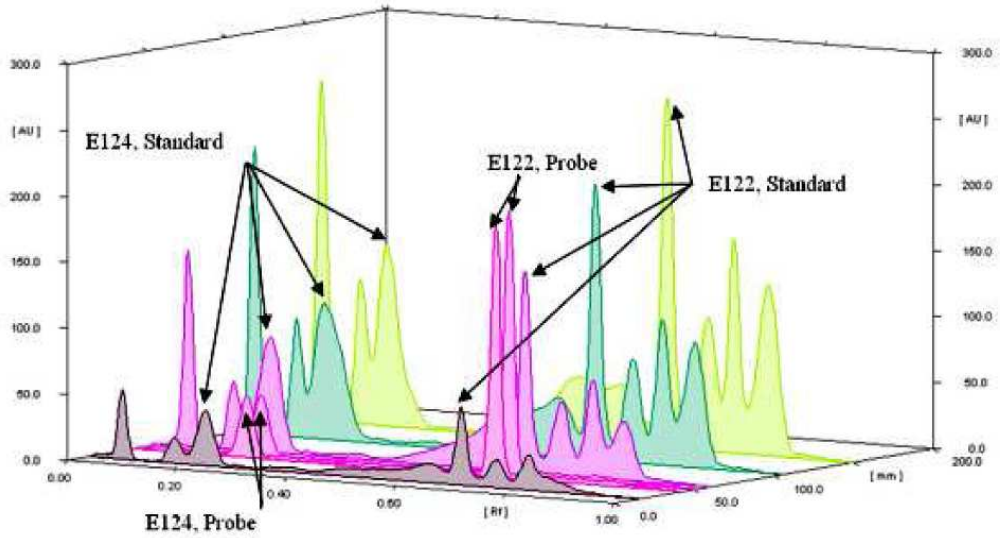
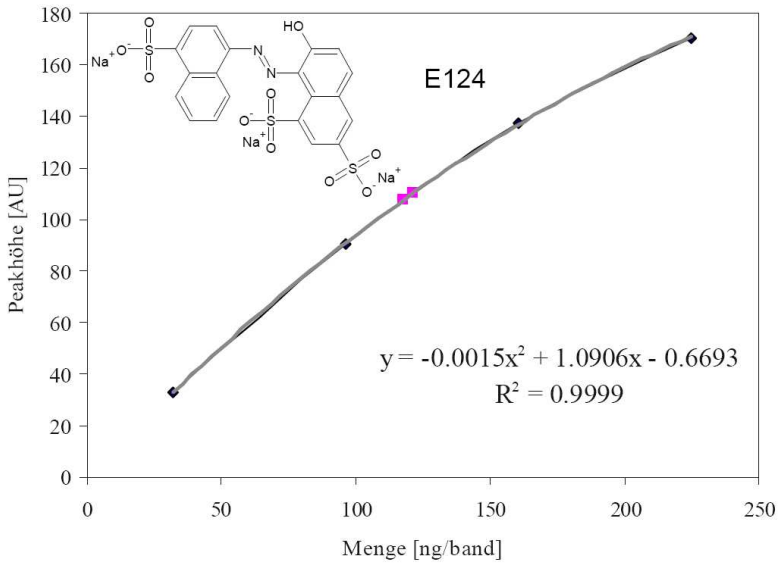
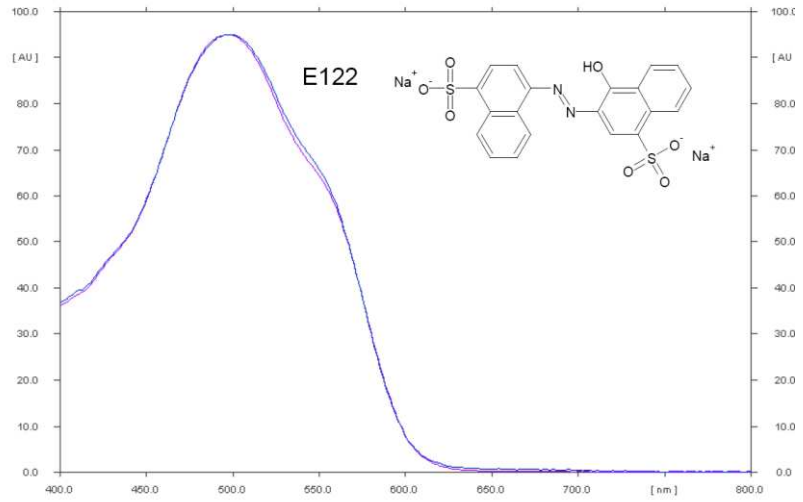
Dye analysis









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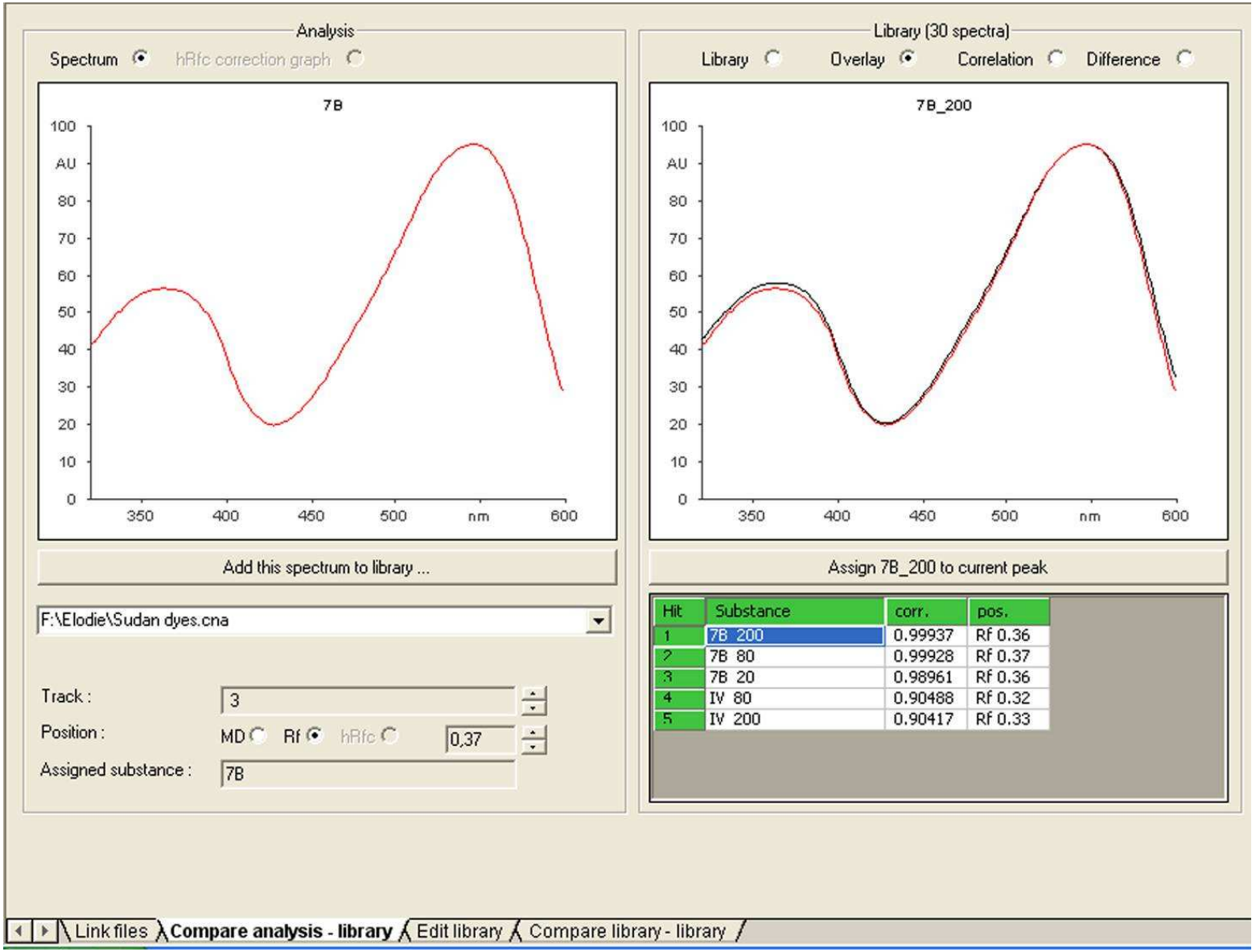
Search in spectra library



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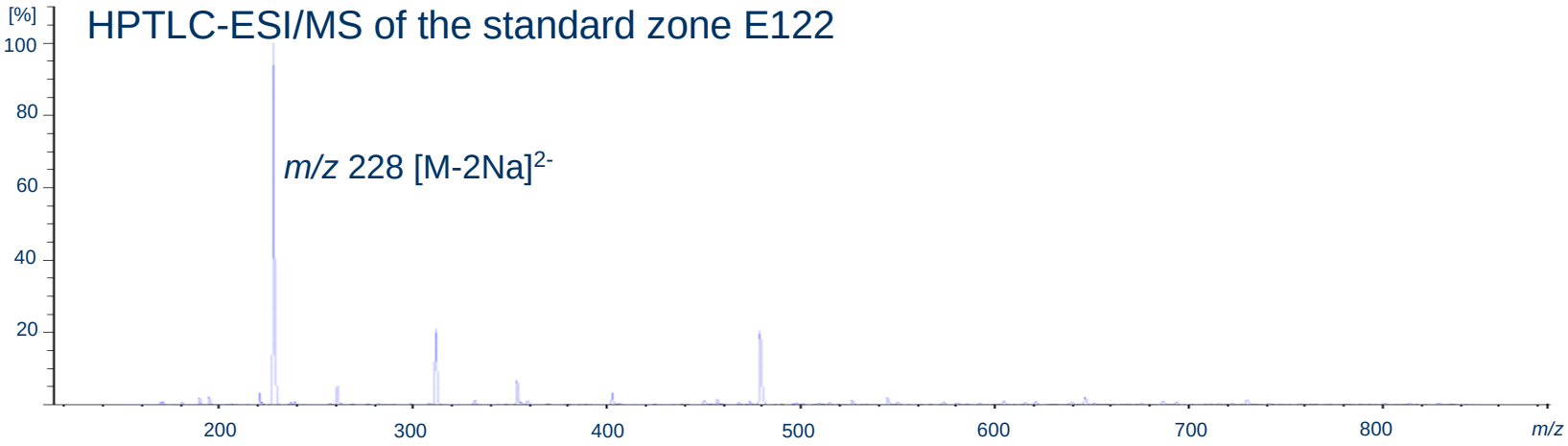
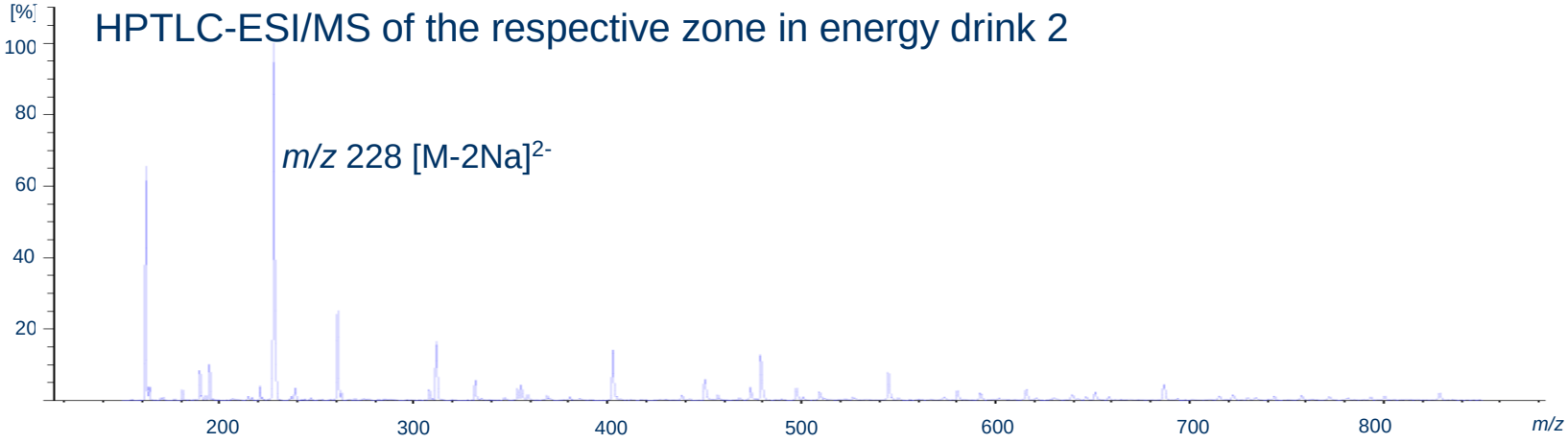
Confirmation by mass spectra

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

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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

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Rare examples for HPTLC

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

Cost comparison

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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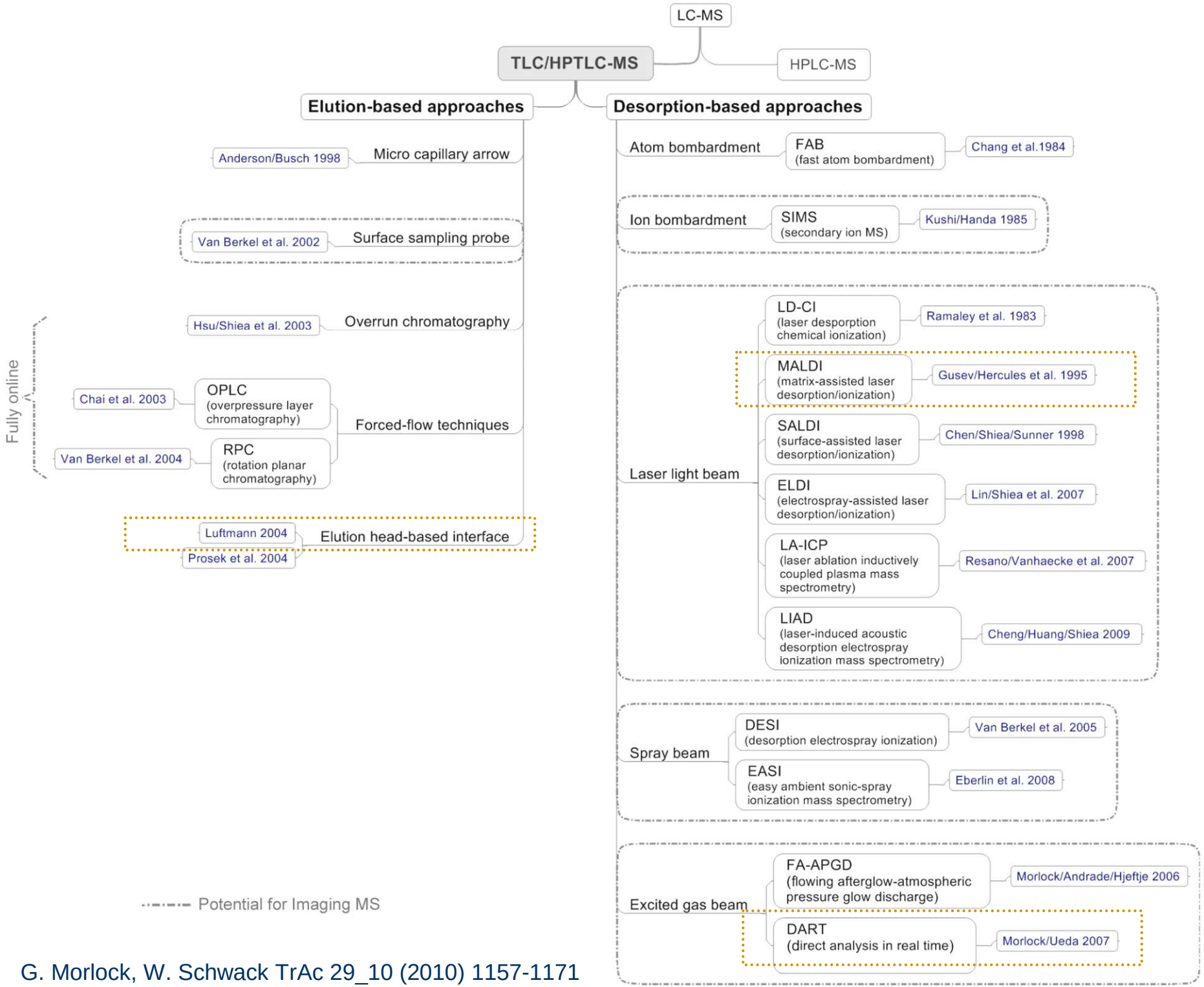

G. Morlock, W. Schwack TrAc 29_10 (2010) 1157-1171

apl. Prof. Dr. Gertrud Morlock
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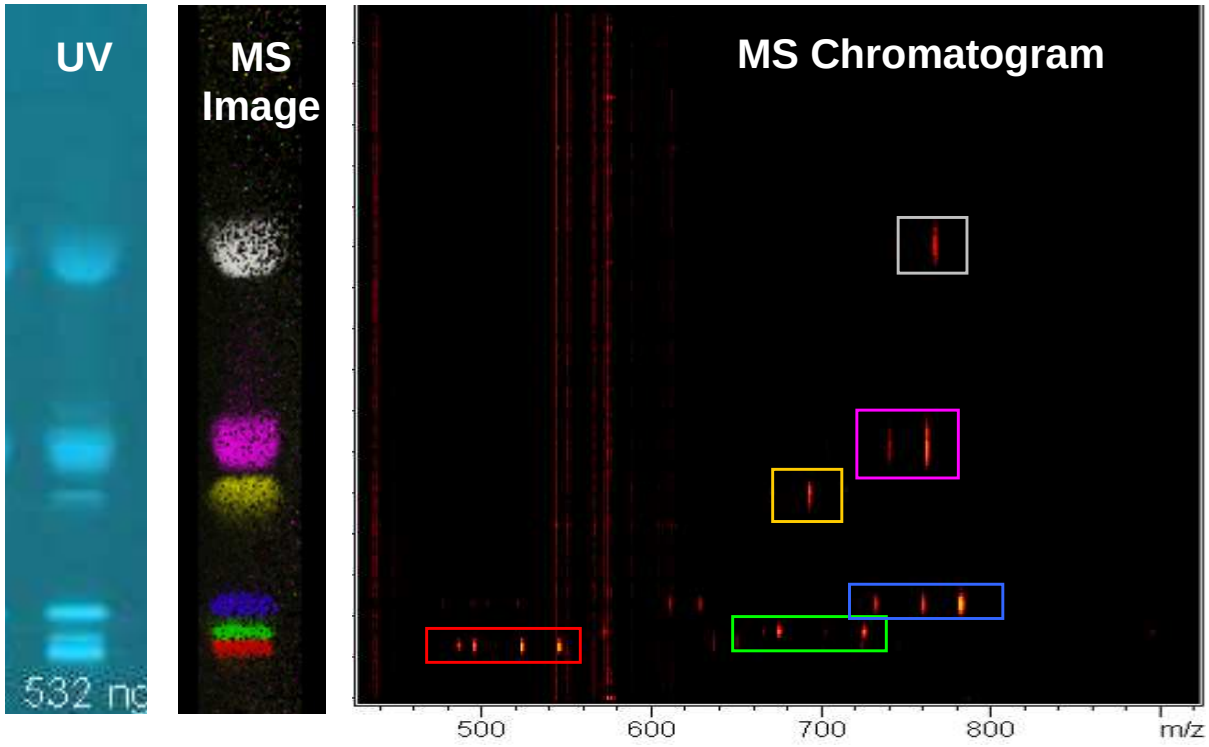
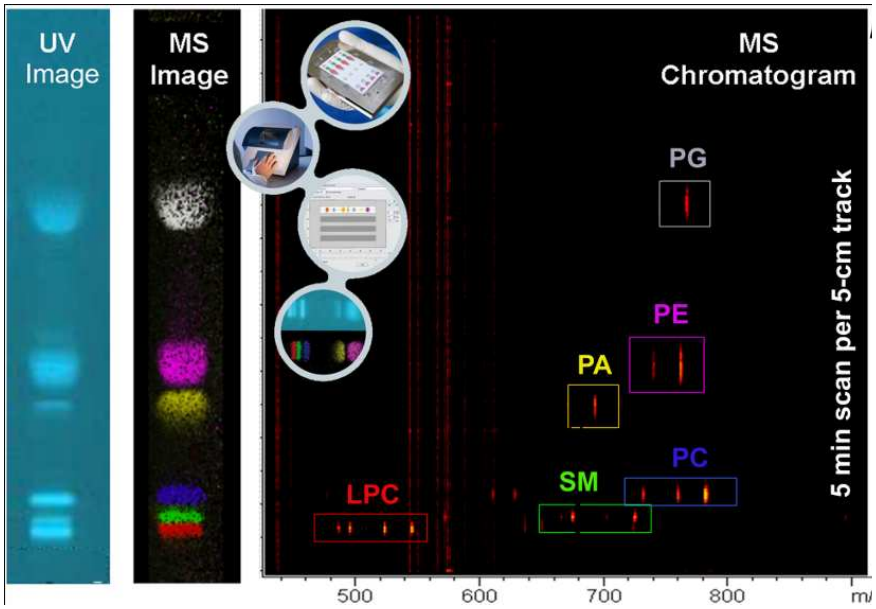
HPTLC-FLD-MALDI-TOF MS

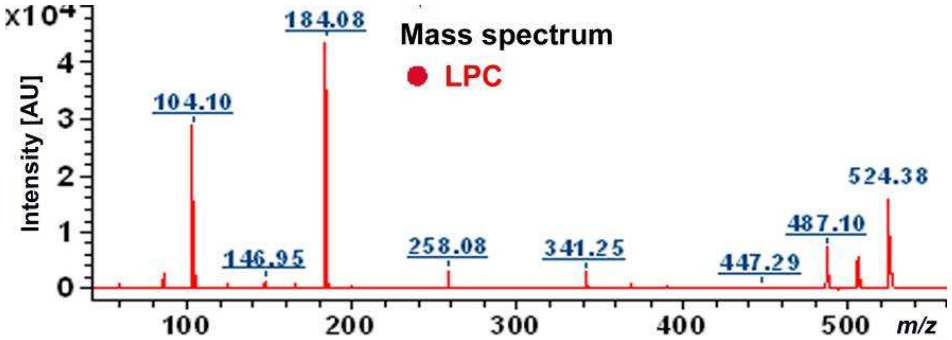


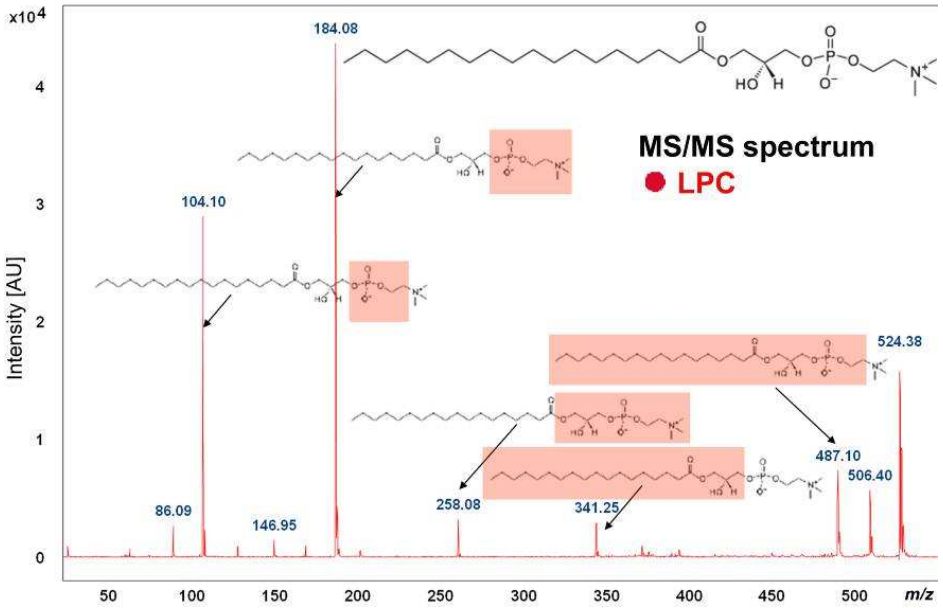
M. Schuerenberg *et al.,* IMSC 2009, Bremen, Poster PMM 386

apl. Prof. Dr. Gertrud Morlock
Universities of Hohenheim and Gießen

HPTLC-FLD-MALDI-TOF MS







Bruker Daltonics Application Note MT-101

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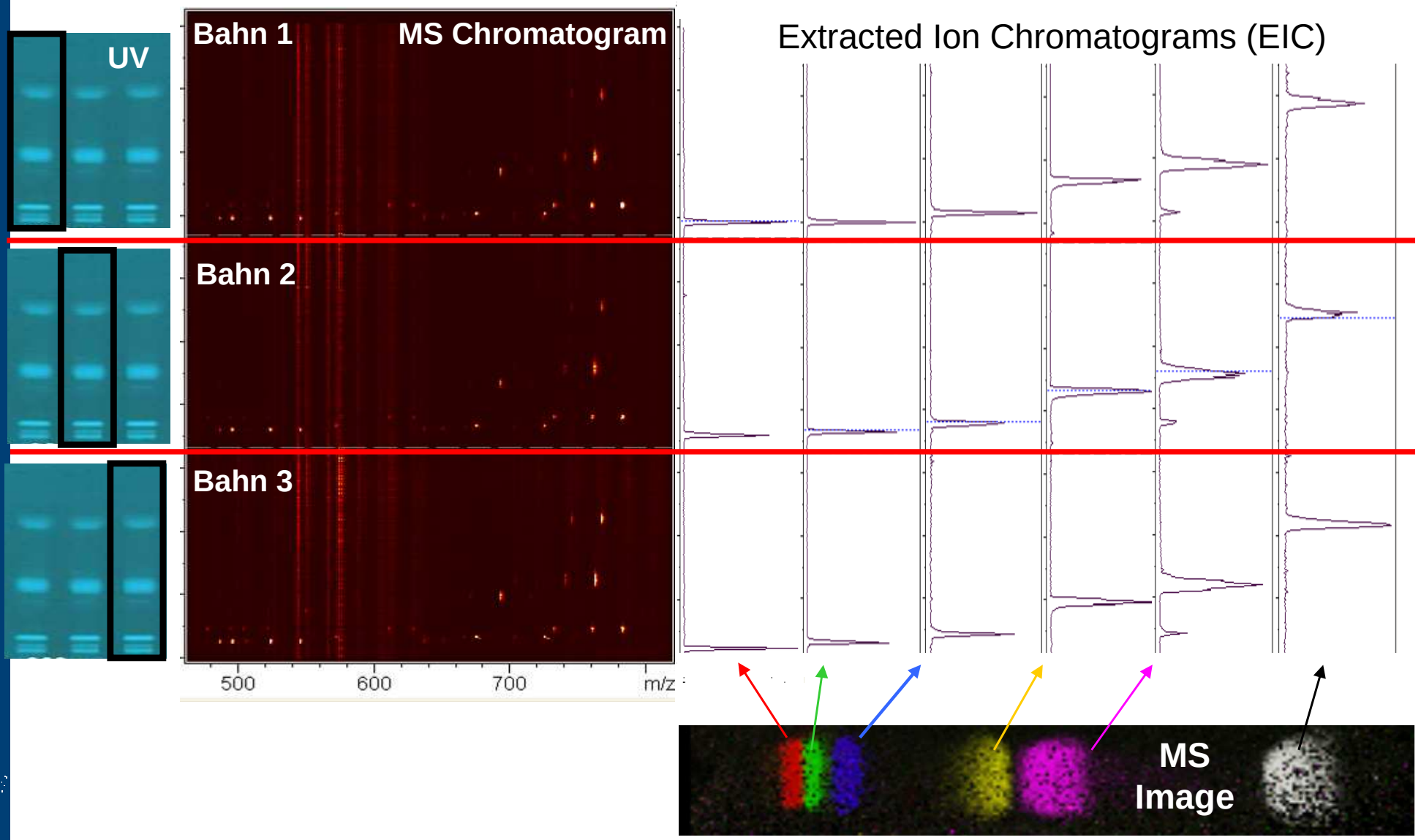
Quantification?



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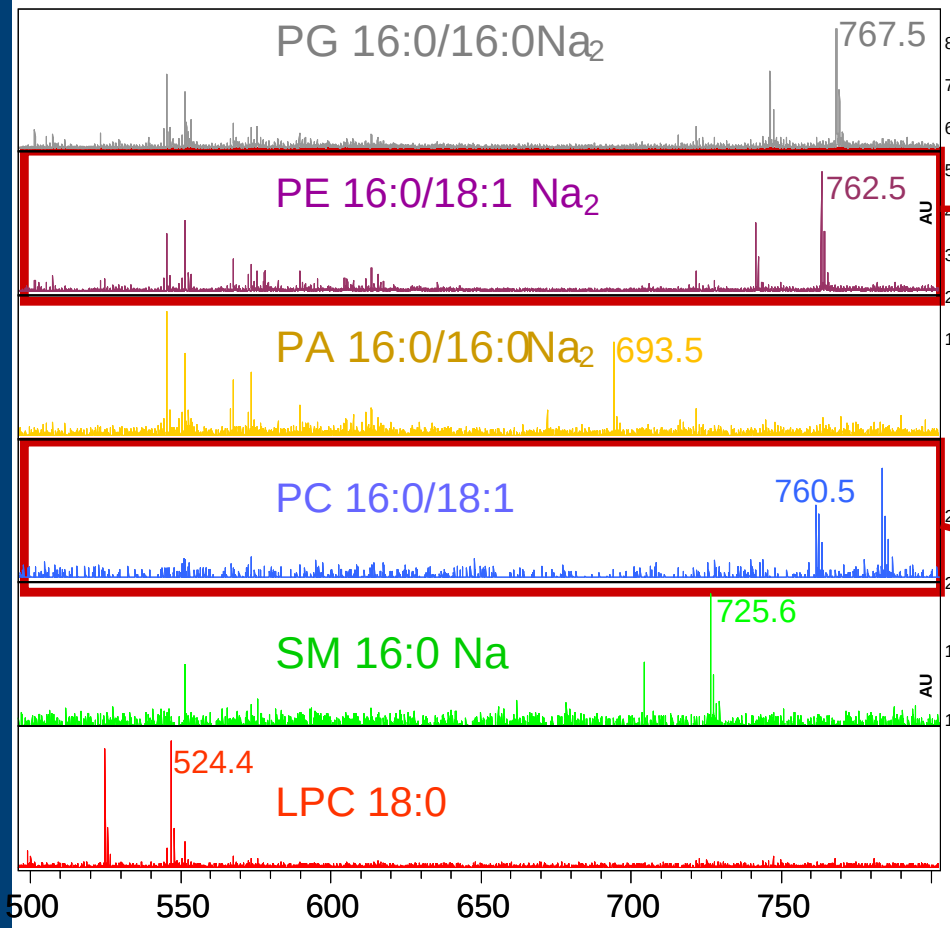
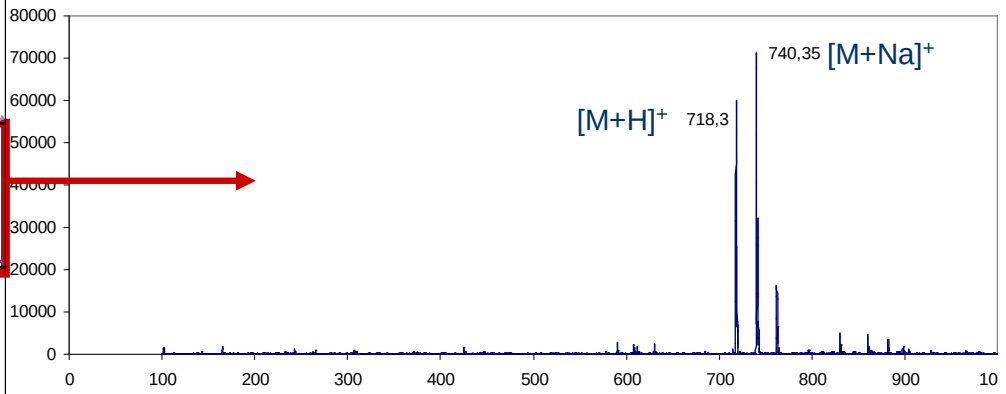
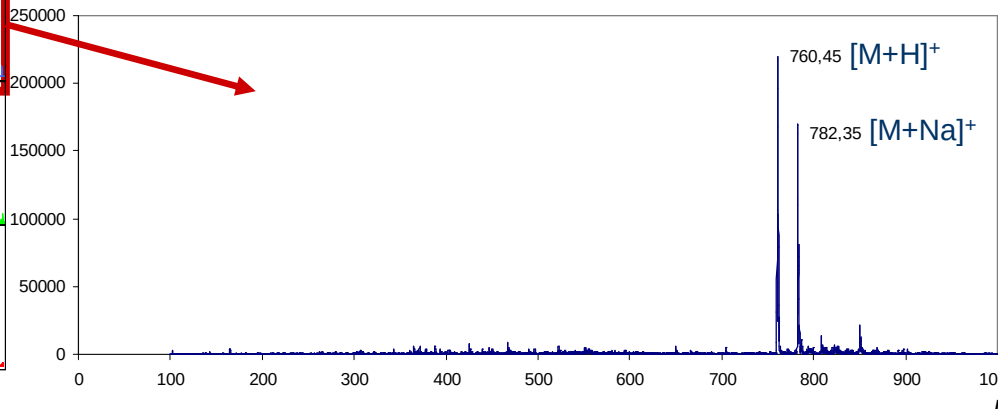
Comparison of mass spectra







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Comparison of the approaches

DART/APGD →

→ dry desorption technique ⟷ DESI

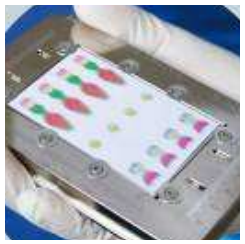
→ no plate preparation etc. ⟷ SALDI, MALDI

→ ambient conditions, no high voltage ⟷ micro junction

→ simple spectra ⟷ MALDI, SIMS

→ quantitativ *with* internal standard → scan function

MALDI

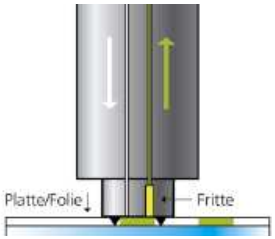


✓ strict protocol for plate preparation

✓ complex spectra

✓ quantitativ *with* internal standard → scan function

Elution-head based Interface



✓ *universally* connectable to any LC-MS system given

✓ plug & play interface (without adjustments or modifications)

✓ whole plate (no cut)

✓ all carriers on mostly all layers ⟷ micro junction

✓ whole zone incl. depth profile ⟶ high detectabilities

✓ quantitativ *without* internal standard ⟷ desorption techniques

✓ targeted recording ⟶ cost-effective, but *no* scan function

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Content

Hyphenations with

1. UV/VIS/FLD/derivatizations
2. MS
3. **FTIR**
4. NMR
5. Bioassays

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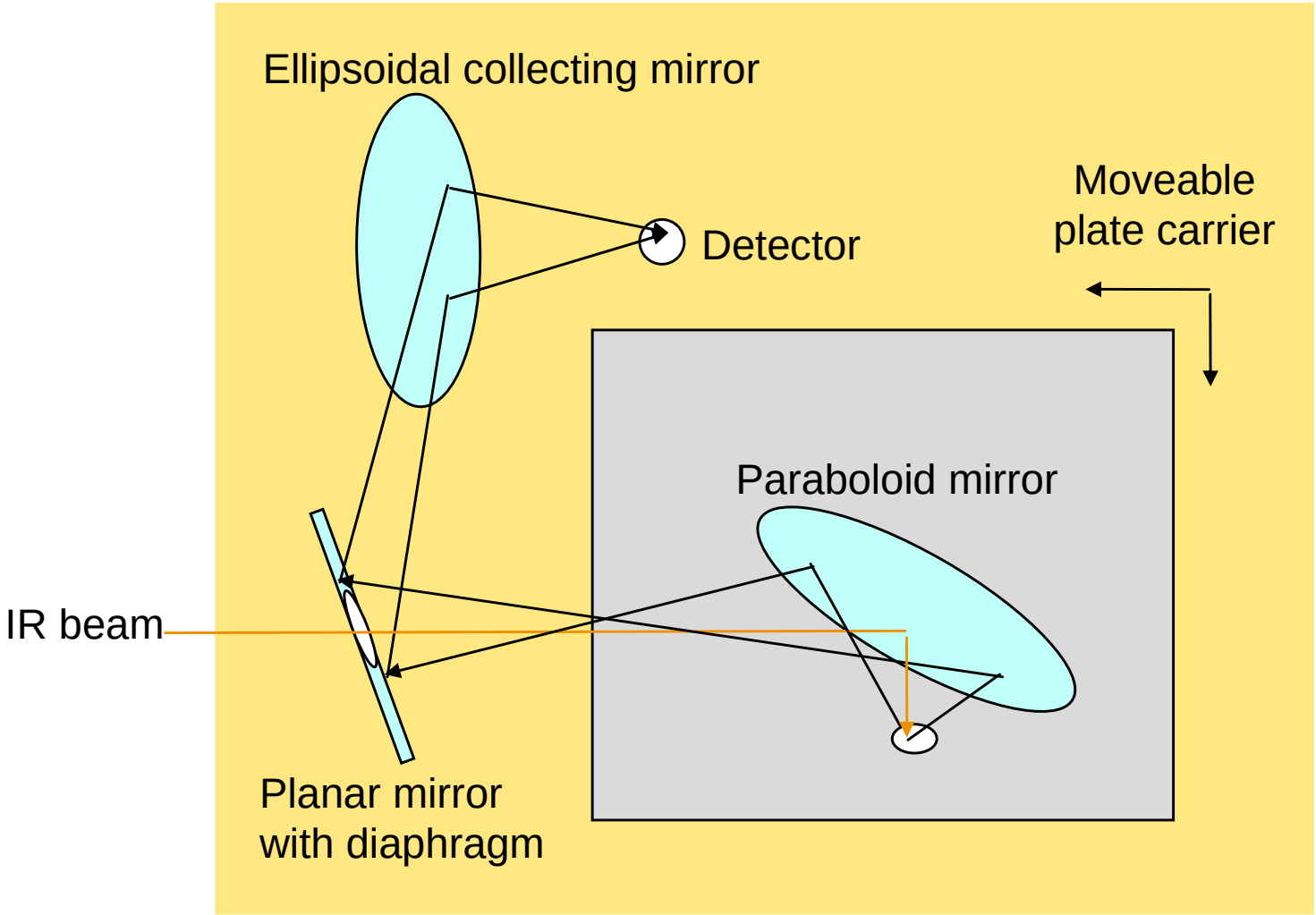
HPTLC-DRIFT



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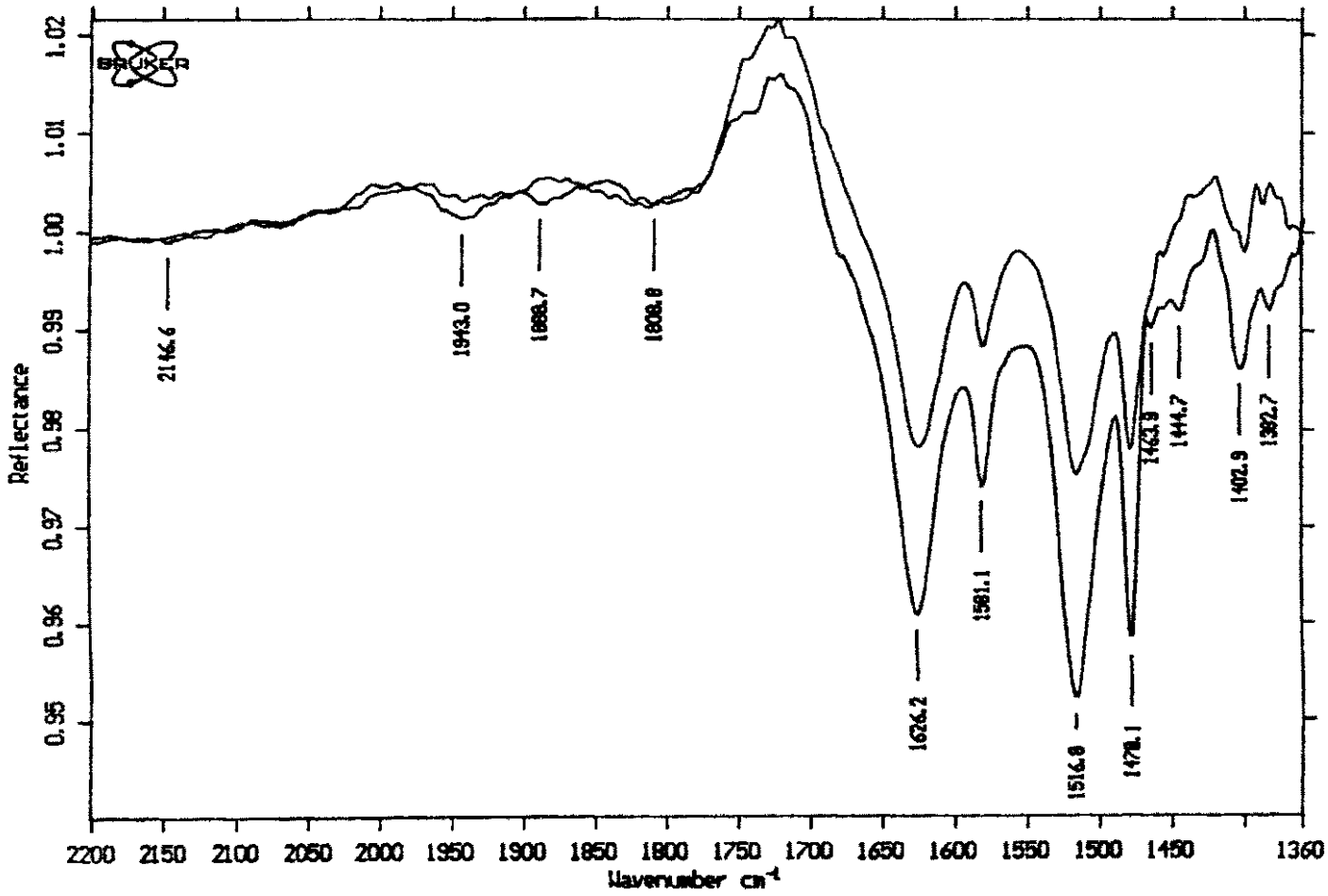
FTIR spectrum of neburon in drinking water



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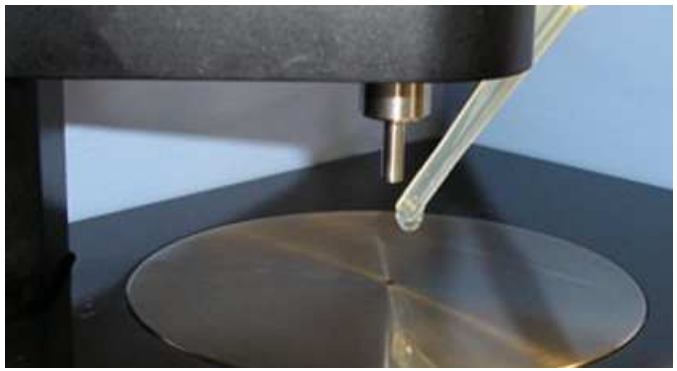
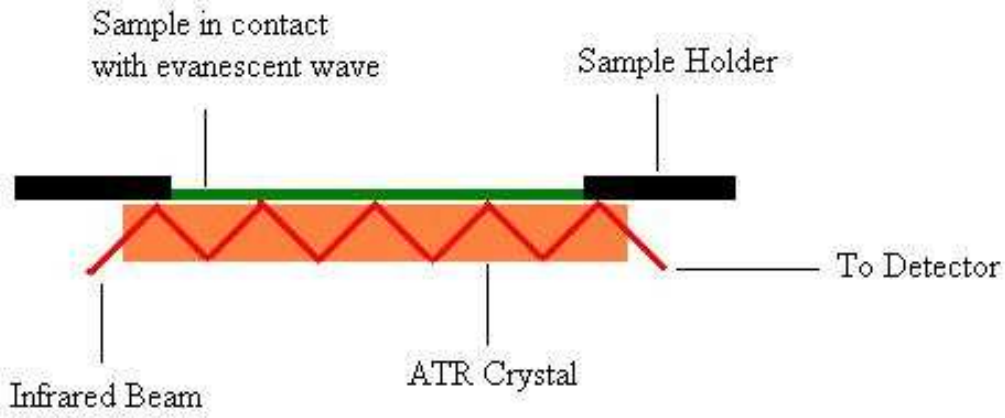
HPTLC-ATR FTIR

- attenuated total reflection infrared (ATR IR) spectroscopy
- samples examined directly (solid or liquid) without any preparation





- zone eluted via TLC-MS Interface in 100 µL
- drop is directly applied and solvent rapidly evaporats

☺ fast protocol

☺ µg-amount per zone

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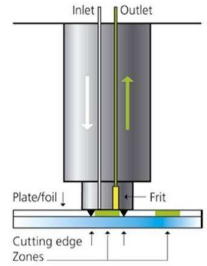
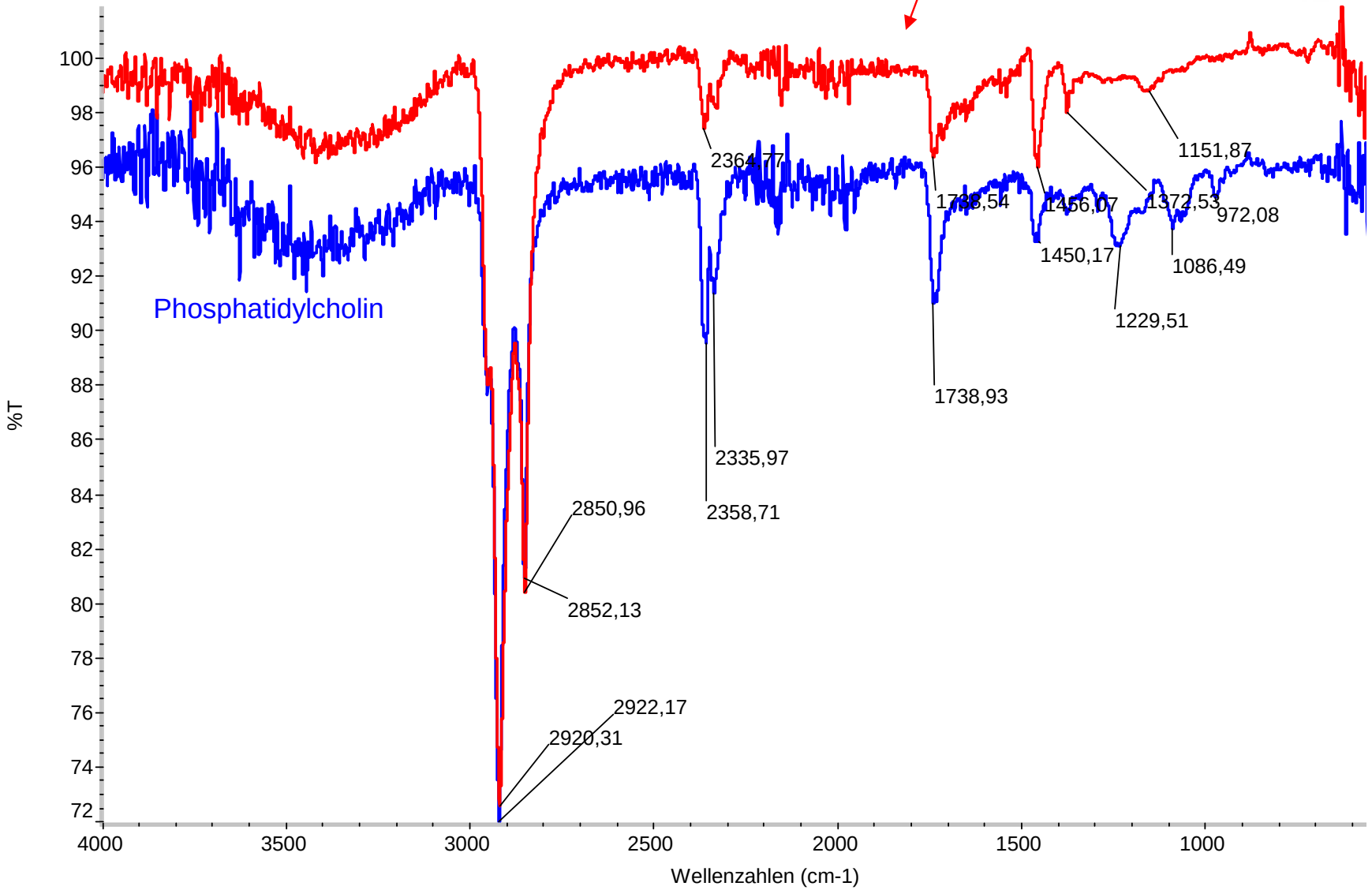
HPTLC-ATR FTIR of an anti-inflammatoric compound

Isolated from *Lactobacillus fermentum*





G. Morlock *et al.*, in preparation

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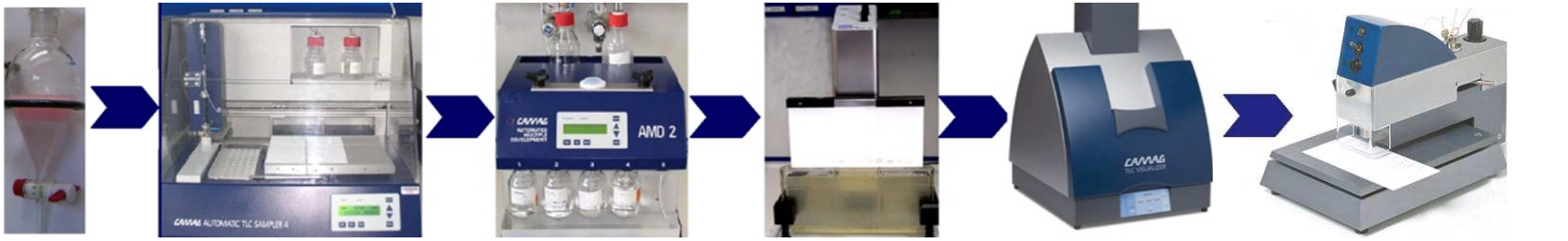
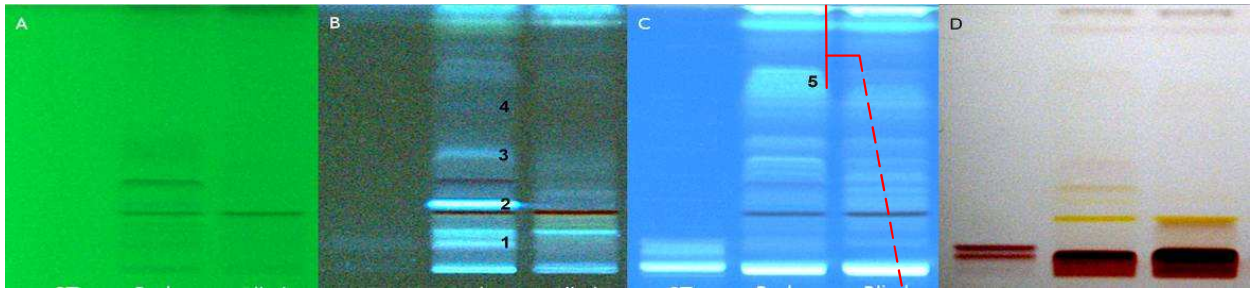
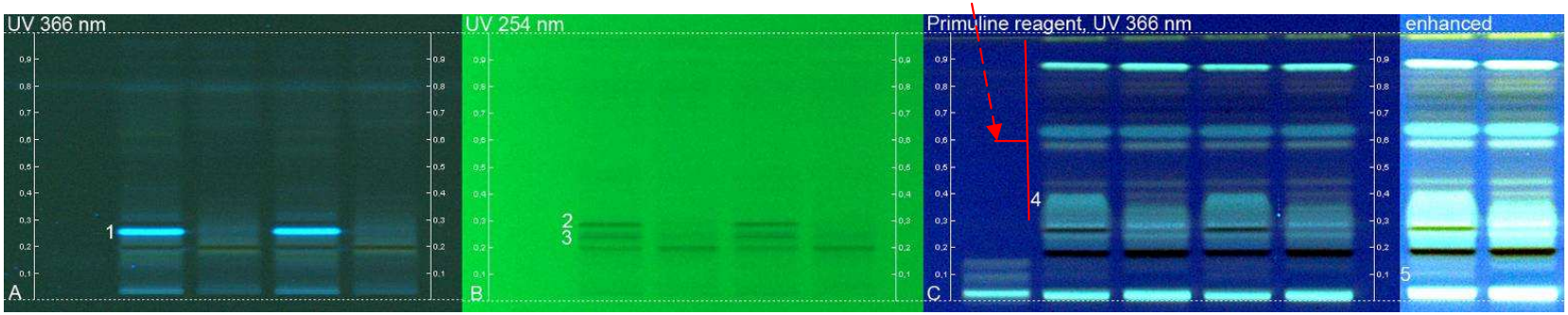
Active compounds of *Lactobacillus fermentum*



LLE → Application → Gradient development → Derivatization → Vis/UV/FLD → HRMS, NMR, FTIR



Control of the apolar part



Zentrum für Ernährungsmedizin, Project-No. 2A IV-08, 2010

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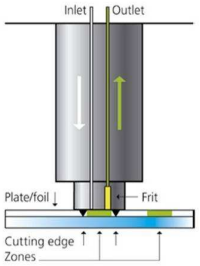
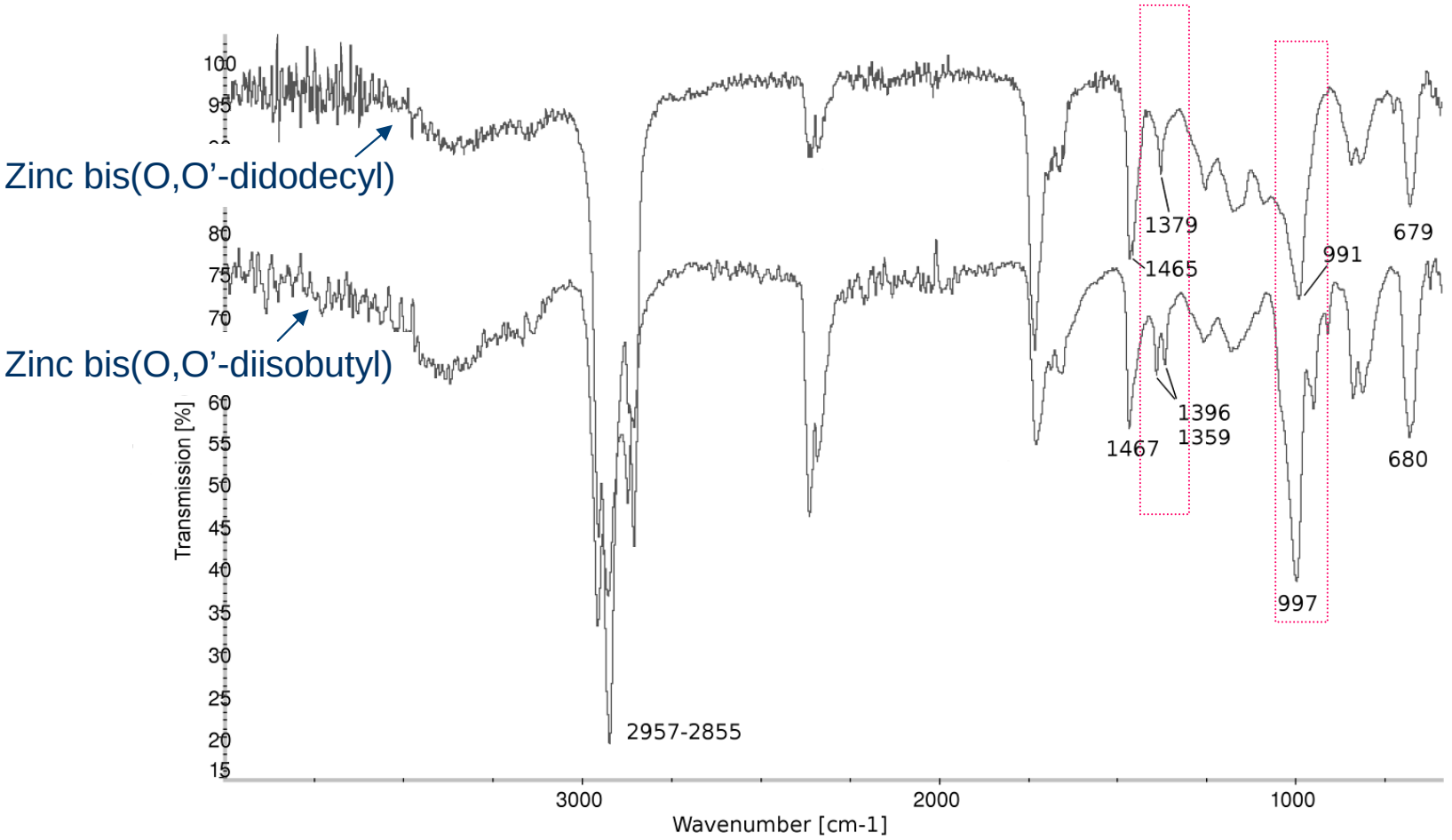
HPTLC/ATR-IR spectra



Dithiophosphate additives in mineral oil



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

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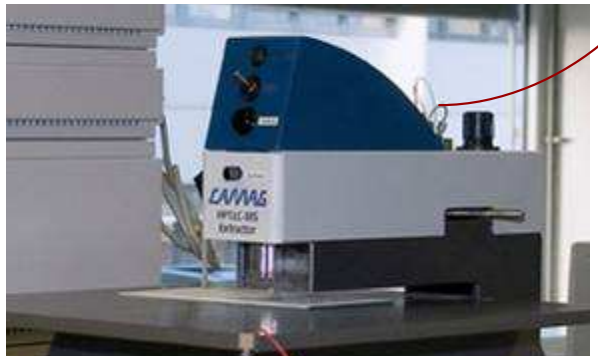
Hyphenation with NMR

→ direct and online hyphenation

→ limitations and possibilities

→ coming soon

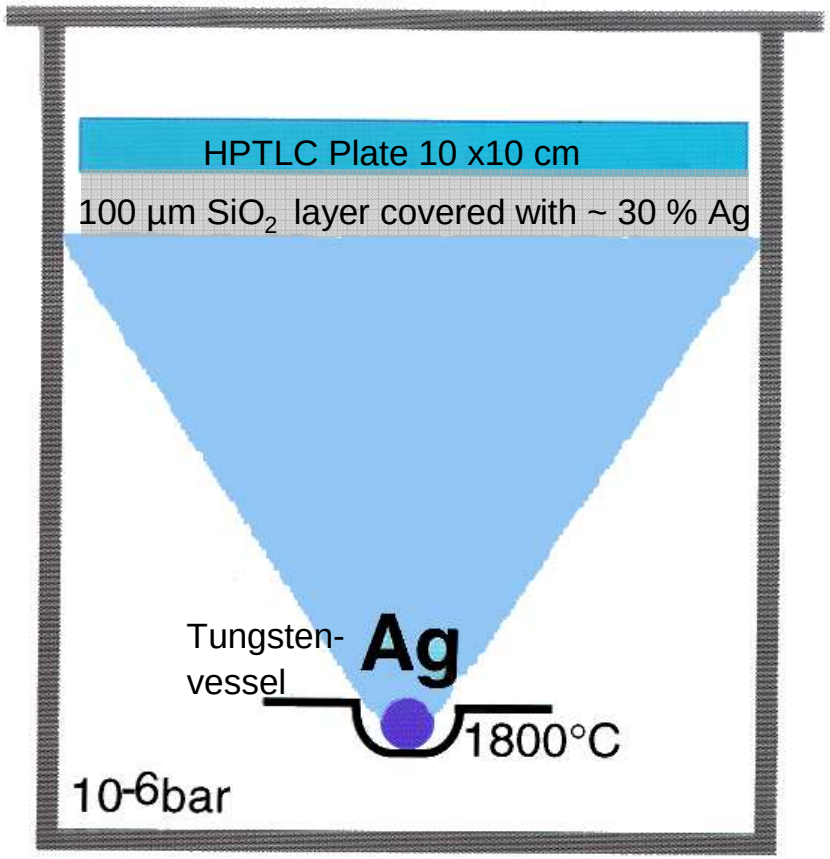
to NMR



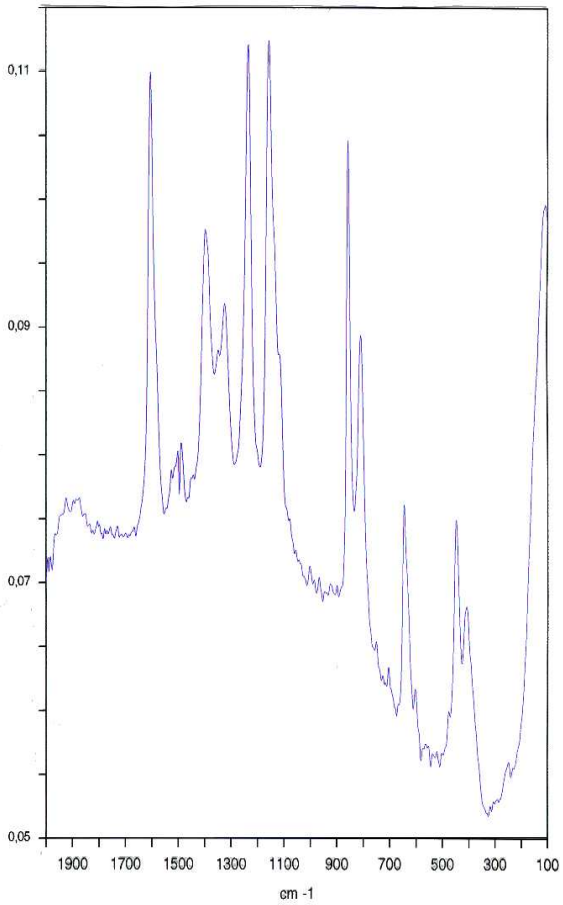
Raman: FT-SERS

→ based on the work of Dr. Klaus Burger†; Bayer Laboratories, Germany

Vacuum transfer



10 ng/zone p-nitrophenol



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5. **Bioassays**

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Effect-directed analysis → sum parameter!

DEUTSCHE NORM Mai 2009

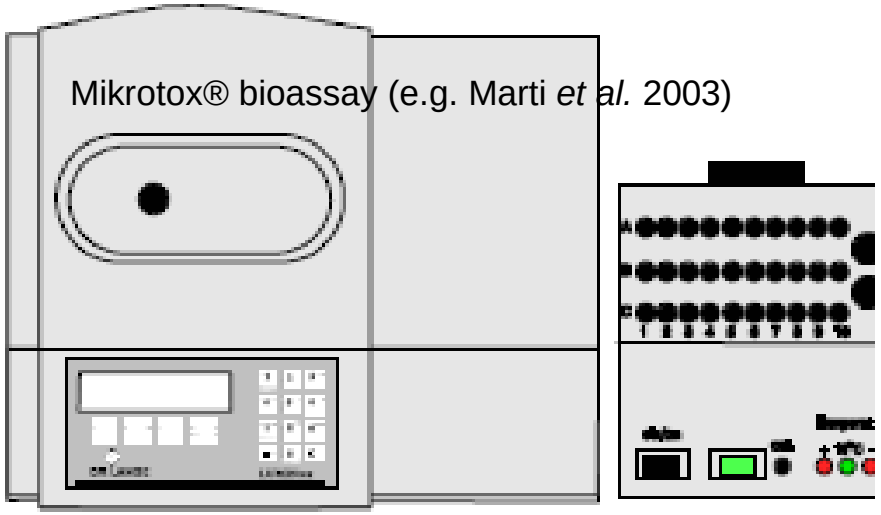
DIN EN ISO 11348-2

DIN

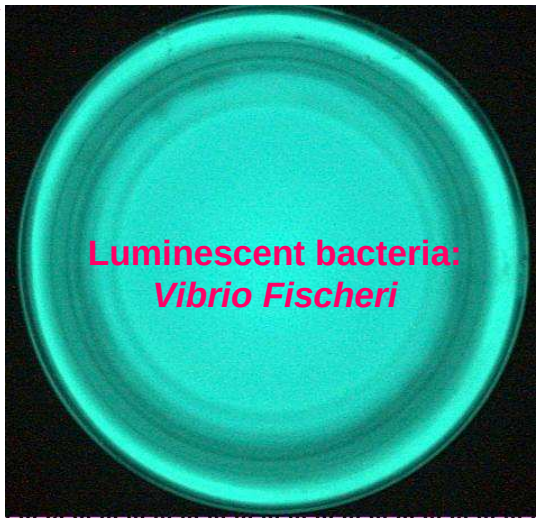
ICS 13.060.70

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)



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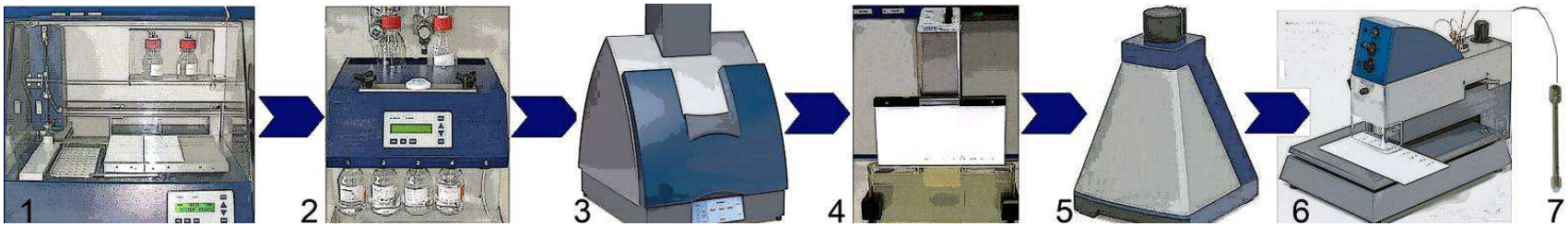
Chromatography-bioassay versus cuvette test



→ Sampe as original as possible
→ Assignment of single compounds
→ Bio-pattern is visible
→ Polarity is clear

Some days versus some months

DEUTSCHE NORM Mai 2009

DIN EN ISO 11348-2

DIN

ICS 13.060.70

Ersatz für
DIN EN ISO 11348-2:1999-04

…only sum parameter!

**Wasserbeschaffenheit –
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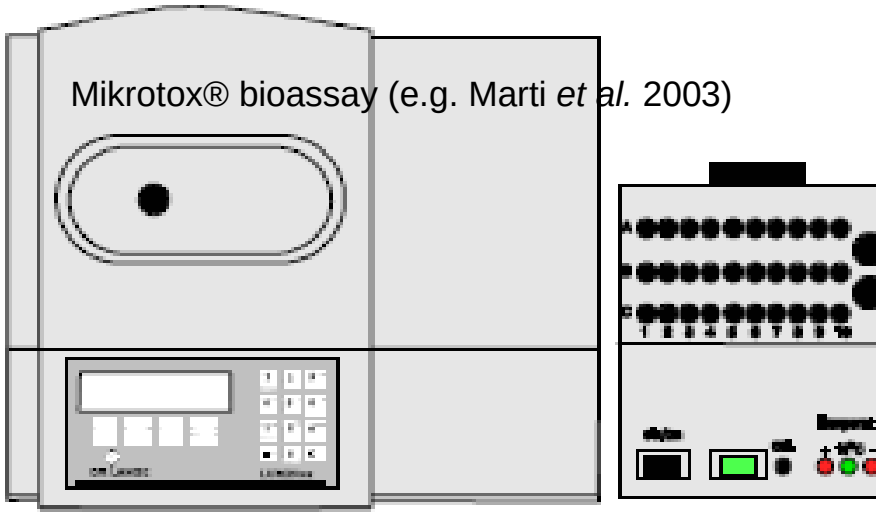

→ Schlesinger *et al., Crambe crambe* marine sponge extract targets pancreatic and prostate cancer stem-like characteristics, in submission

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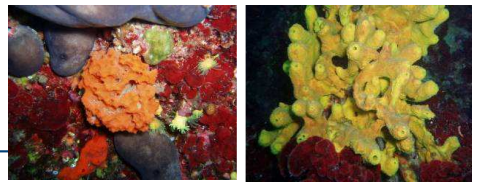
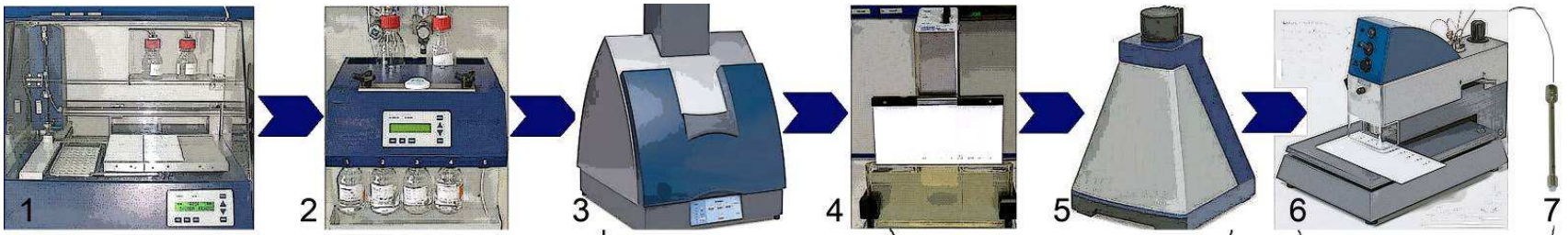
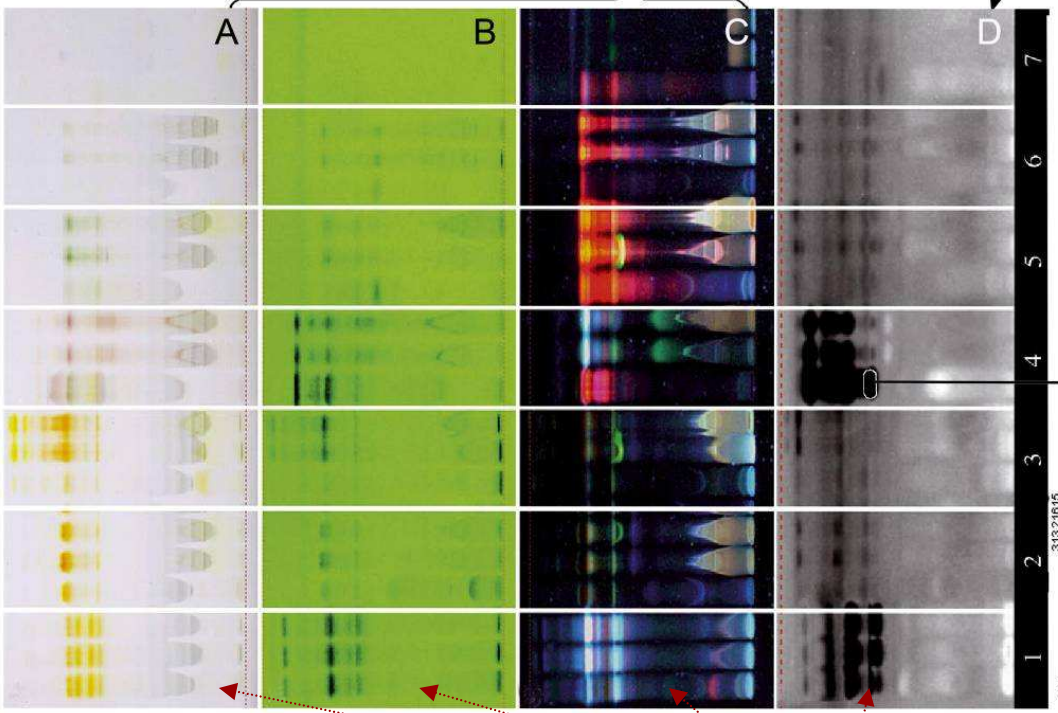
Bioactive products in marine sponges





Application | Gradient development | Vis/UV/FLD | Bioassay | → HRMS, NMR, FTIR



E

→ HPTLC-Vis/UV/FLD-EDA-HRMS

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

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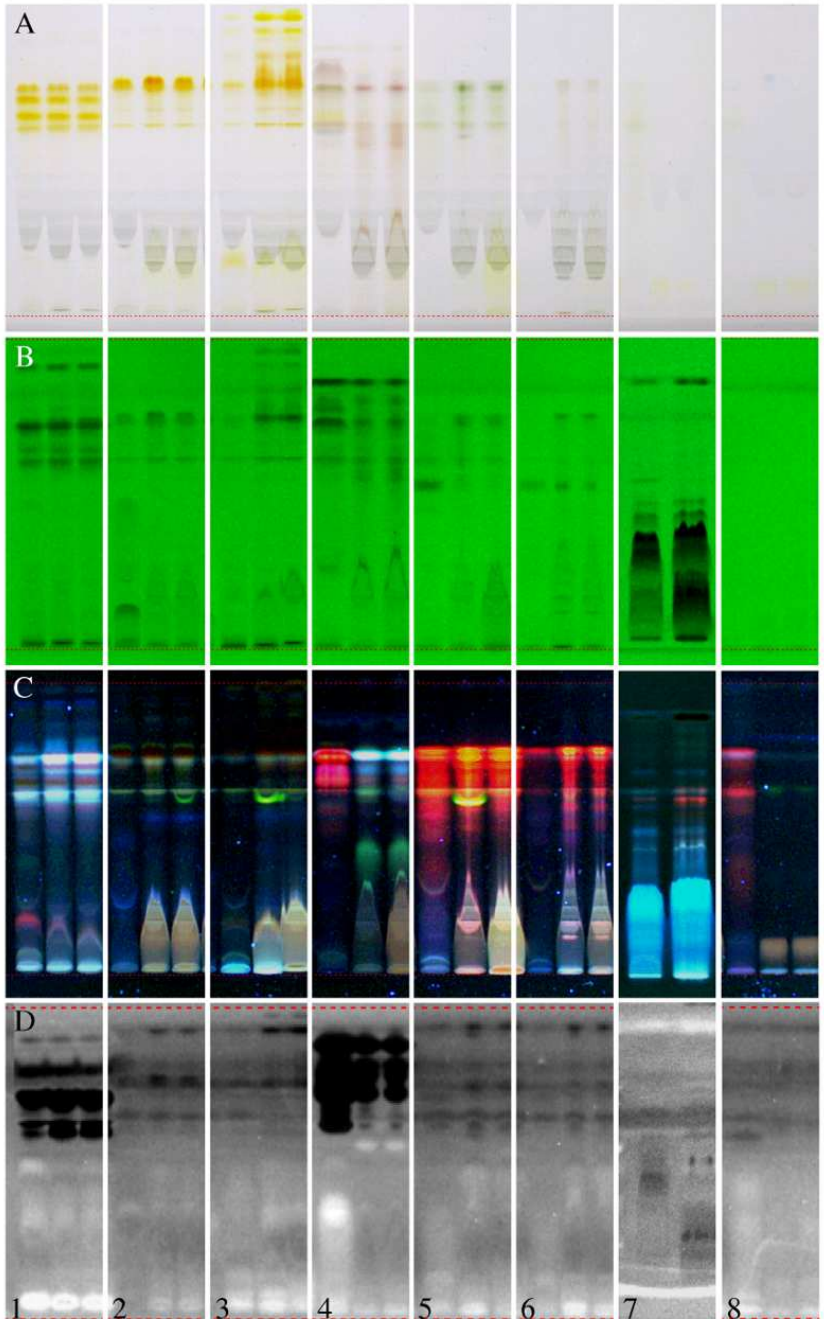
Primmorphe as bioreactor

Poster 3b



1 *Acanthella acuta*, very toxic

2 *Axinella polypoides*, primmorphs more toxic than *in situ* sponge

3 *Suberites domuncula*, primmorphs more toxic than *in situ* sponge

4 *Dysidea avara*, bioactive metabolites besides Avarol and Avarone, loss of toxic metabolite, synthesis of enhancing metabolite

5 *Petrosia ficiformis* with symbiotic cyanobacteria,
6 *Petrosia ficiformis* without symbiotic cyanobacteria, different pattern but equal toxicity

7 *Axinella damicornis*, synthesis of a new toxic metabolite in primmorphs

8 *Ephydatia fluviatilis*, first documentation of bioactivity, loss in primmorphs

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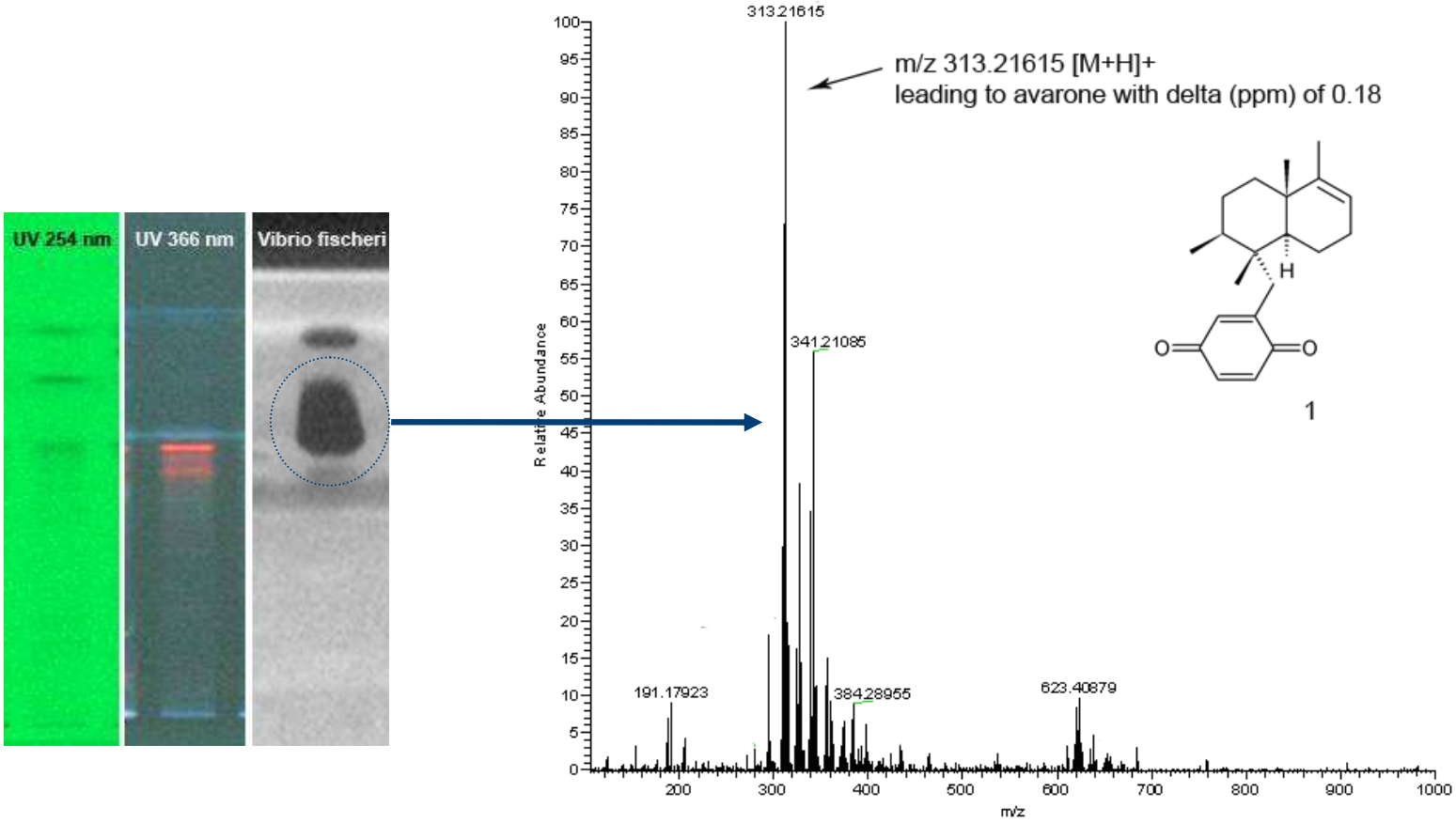
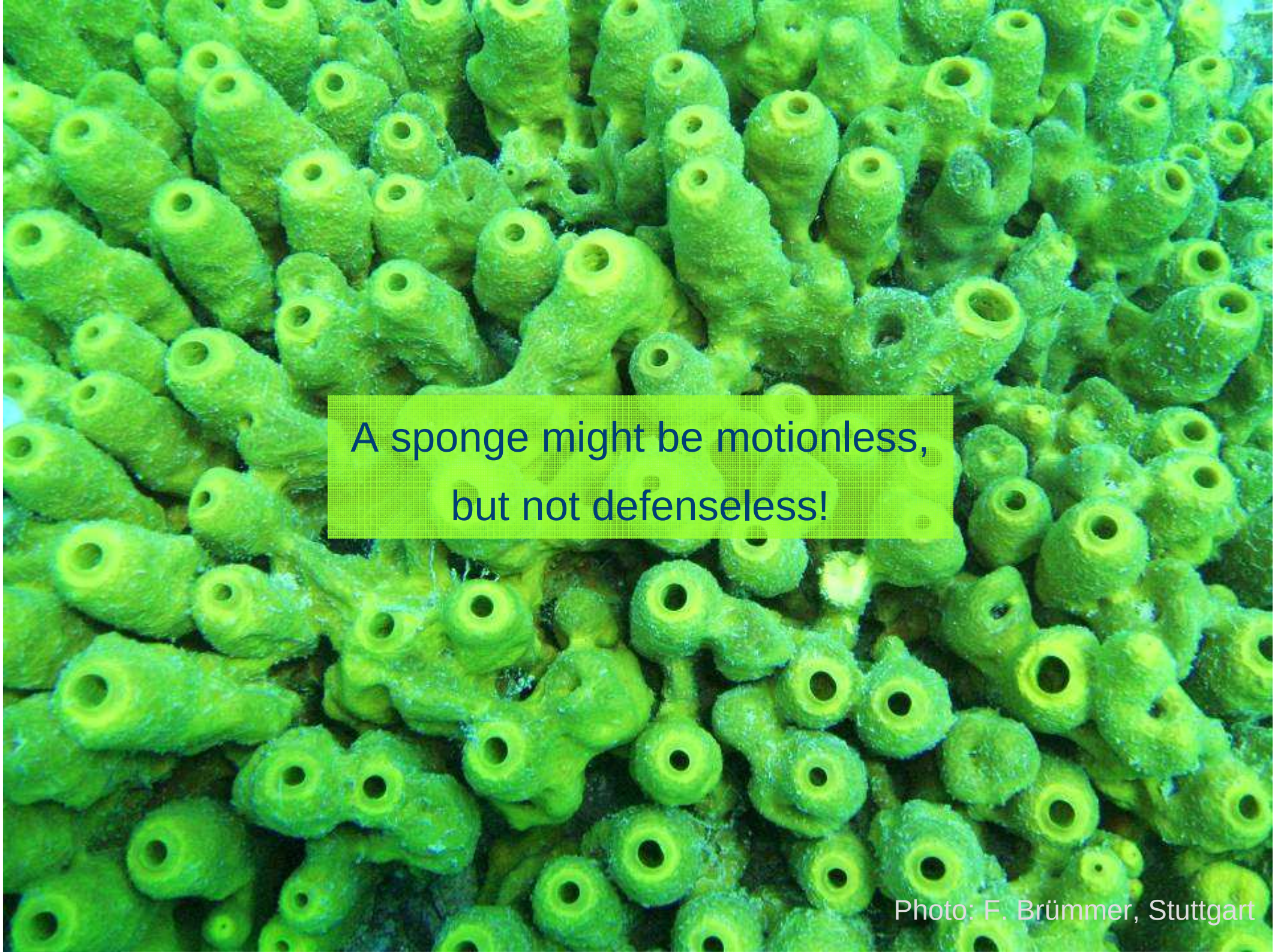
HPTLC-Bioactivity-HRMS



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


A sponge might be motionless,
but not defenseless!
Photo: F. Brümmer, Stuttgart

Screening of natural products by HPTLC-Bioactivity-HRMS

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Universities of Hohenheim and Gießen

- ✓ 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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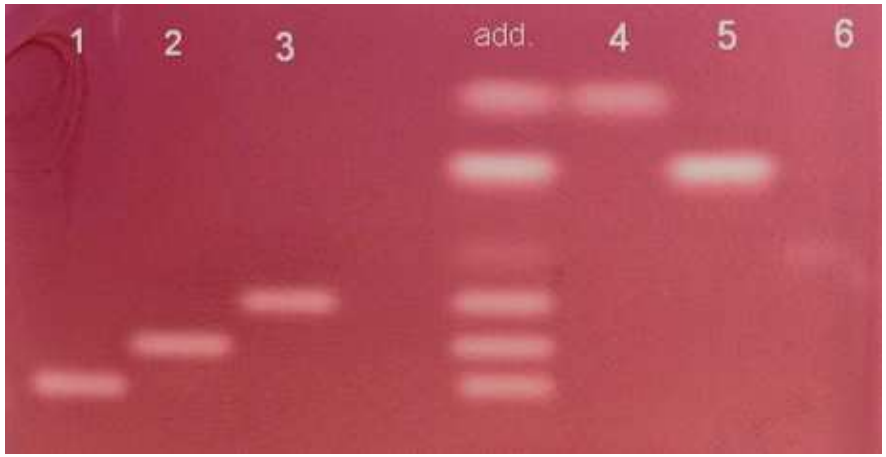
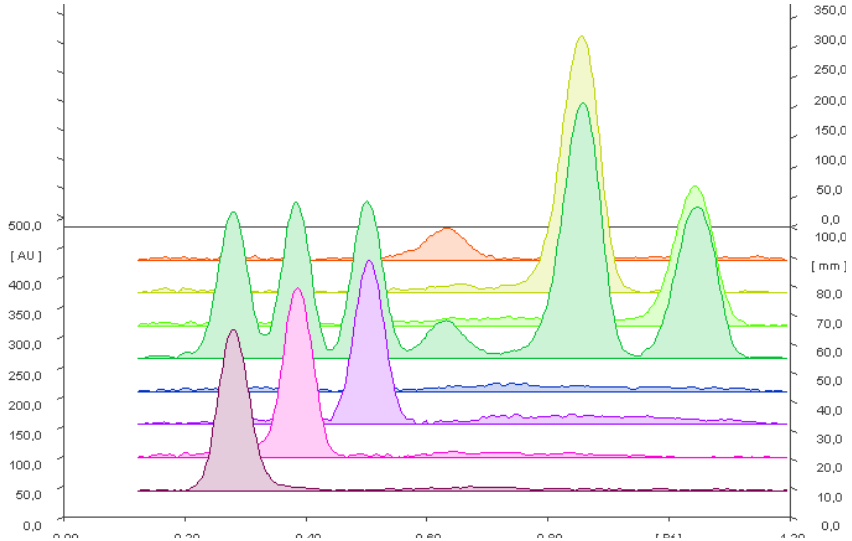
HPTLC-VIS/UV/FLD-EDA

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

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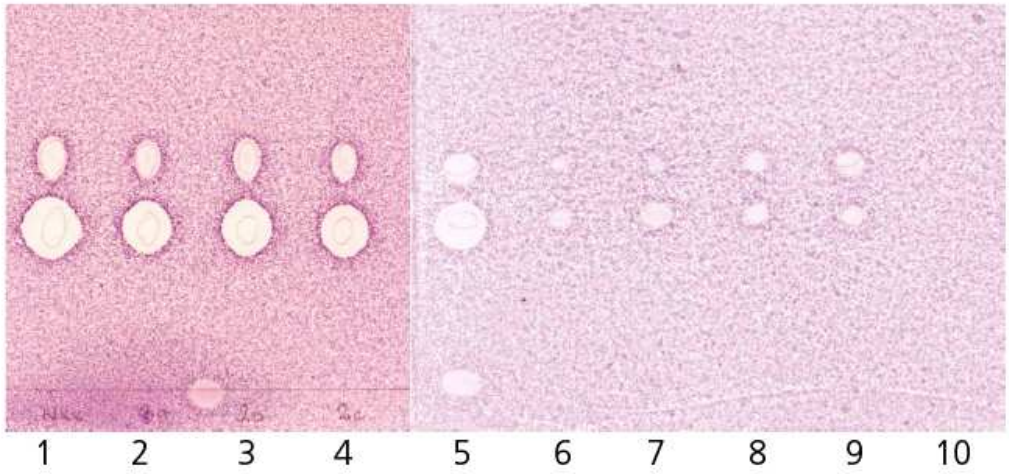
Detection of antibiotics with *Bacillus subtilis*

Determination of enrofloxacin and ciprofloxacin in milk by direct bioautography detection

From left: M.Sc. Wioleta Bąk, Dr. hab. Irena Choma, M.Sc. Edyta Grzelak, Dr. Karol Pilorz and Dr. hab. Barbara Majer-Dziedzic.

Lecture 10a



CAMAG Bibliogr Service CBS 106 (2011) 1-4

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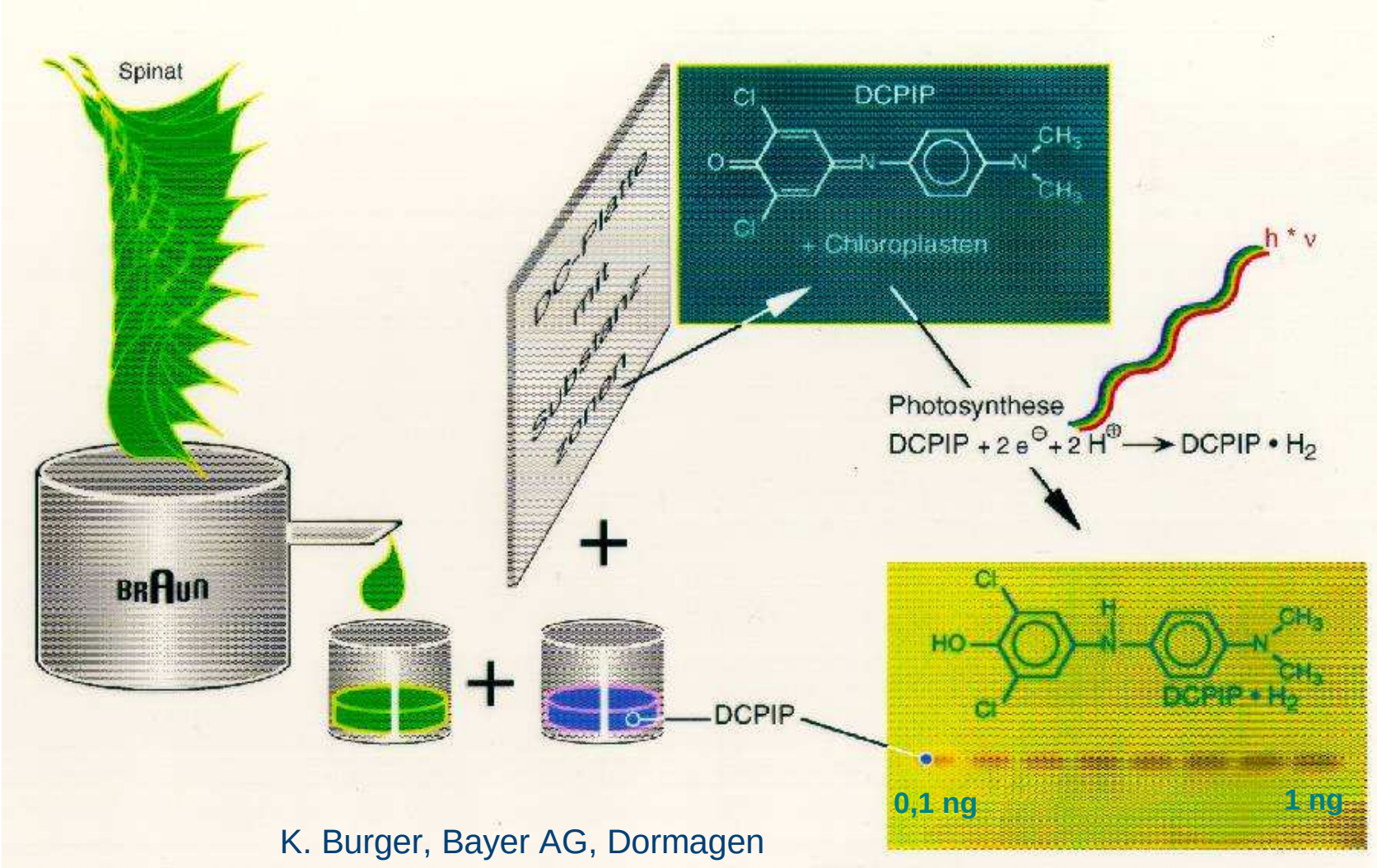
Detection with chloroplasts (spinach)

→ Photosynthesis inhibiting herbicides (→ 100 pg/zone)



K. Burger, Bayer AG, Dormagen

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Content

Hyphenations with

1. UV/VIS/FLD/derivatizations
2. MS
3. FTIR
4. NMR
5. Bioassays

Session 10

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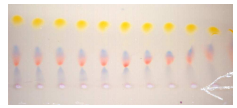
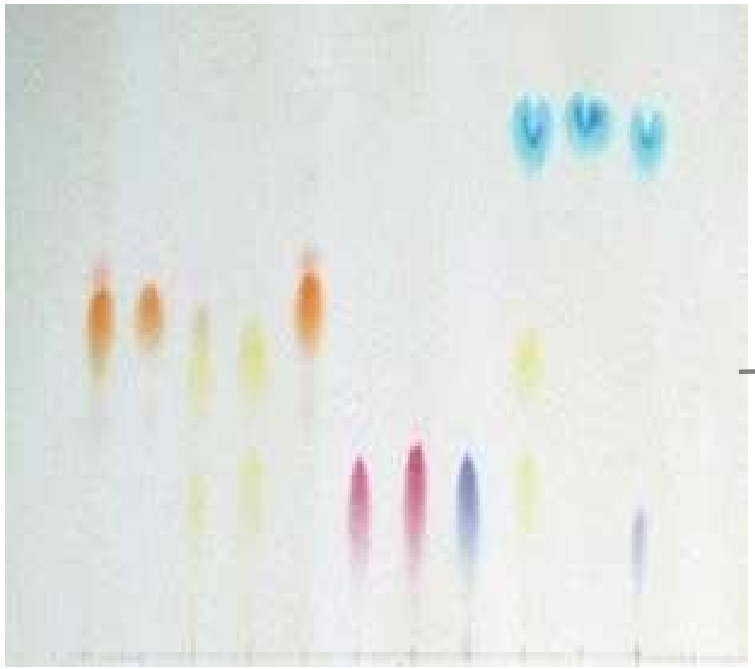
High-Performance Thin-Layer Chromatography

Planar Chromatography → UTLC
UltraTLC

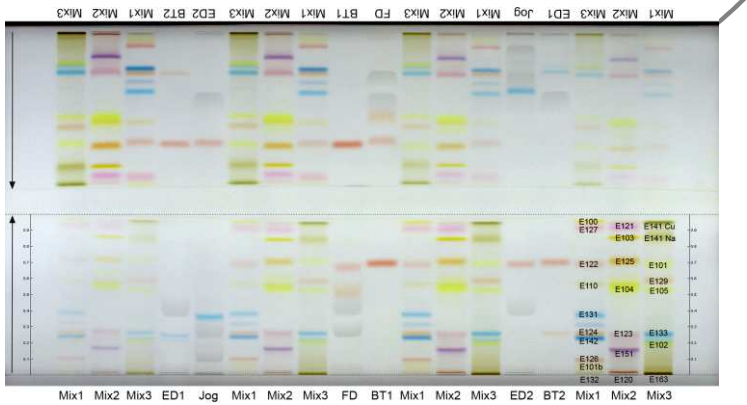


DC/TLC
Thin-layer chromatography



→ point of care
→ qualitative

HPTLC
High-performance TLC



→ standardized
→ reproducible
→ reliable
→ quantitative
→ adequate
→ compliant with regulations

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

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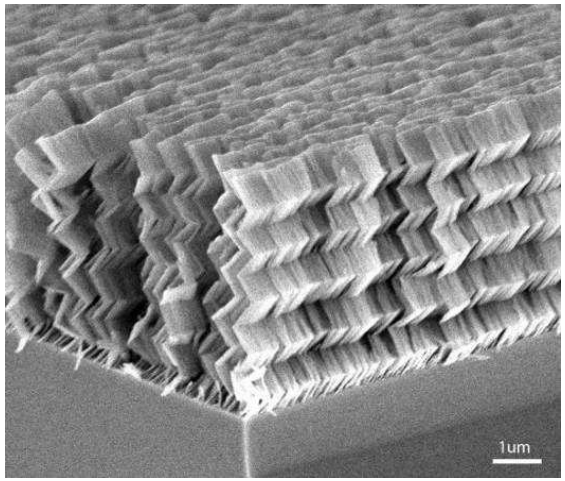
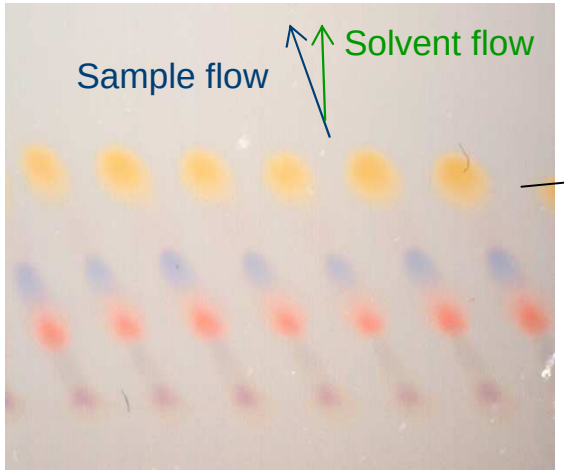
Office Chromatography

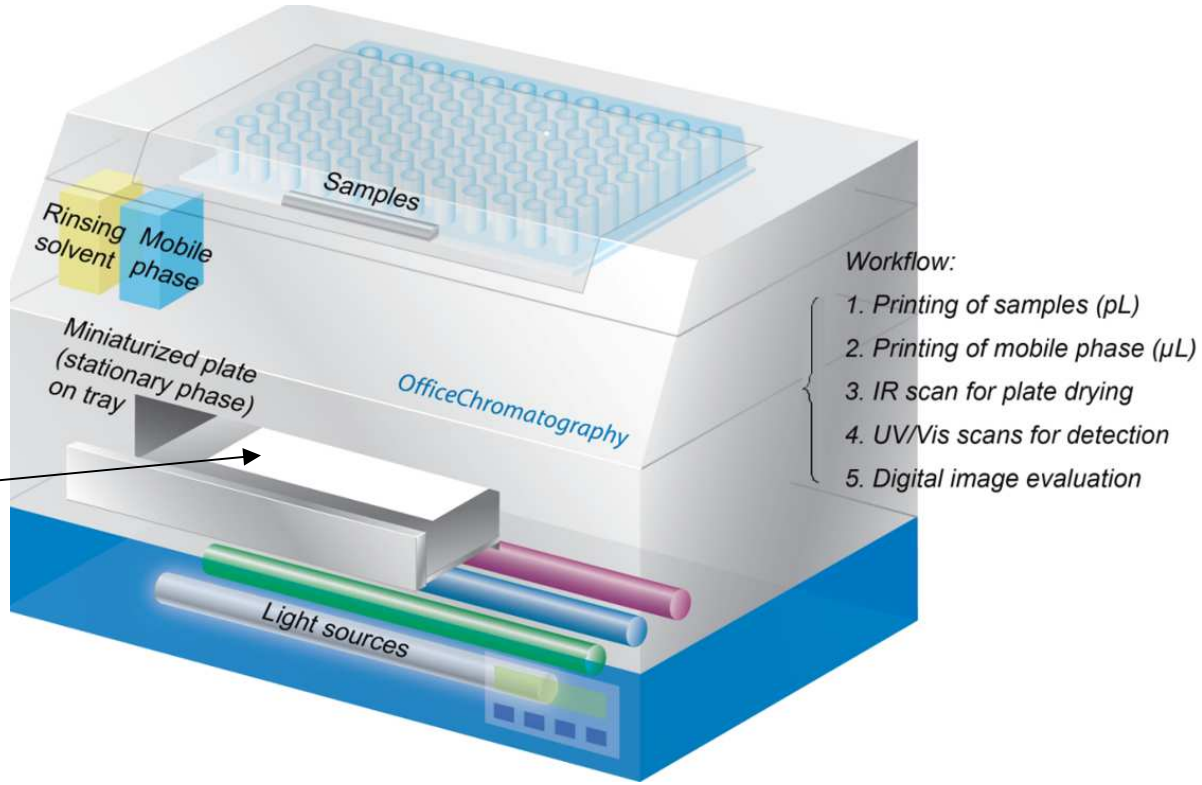
Lecture 2b

Print & Media Technologies
+
Chromatography

Nanostructured UTLC plates







G. Morlock, C. Oellig, L. Bezuidenhout, M. Brett & W. Schwack,
Anal. Chem. 82 (2010) 2940-2946

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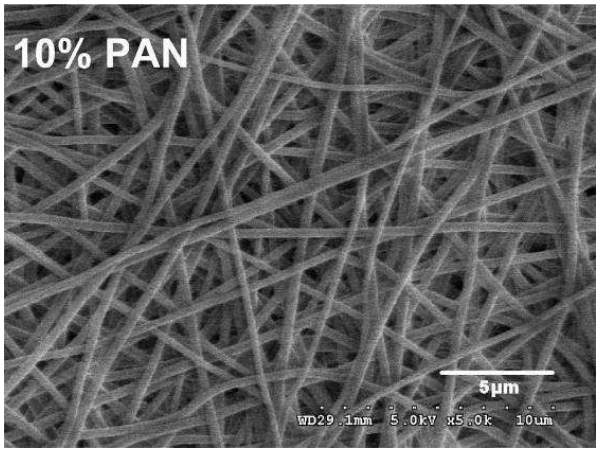
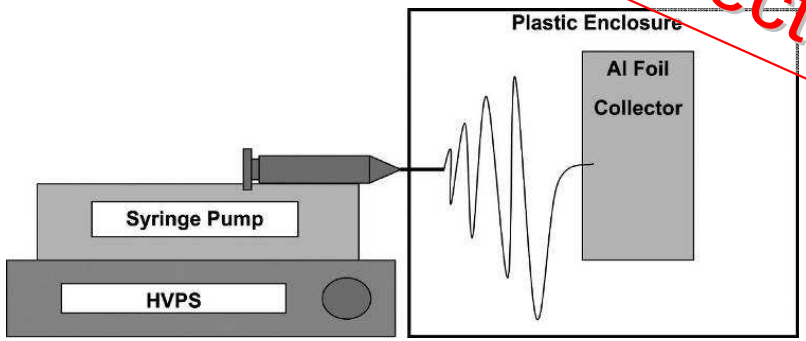
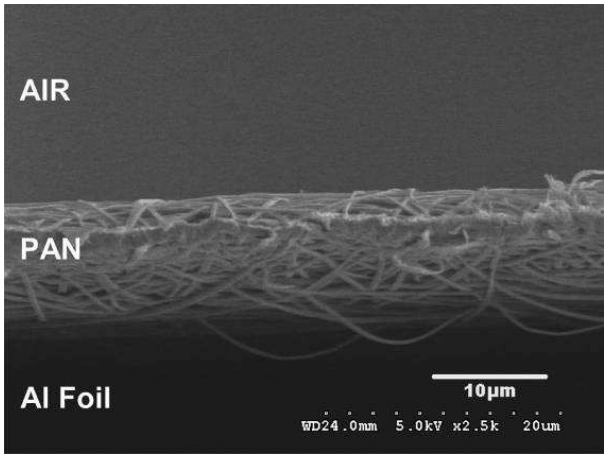
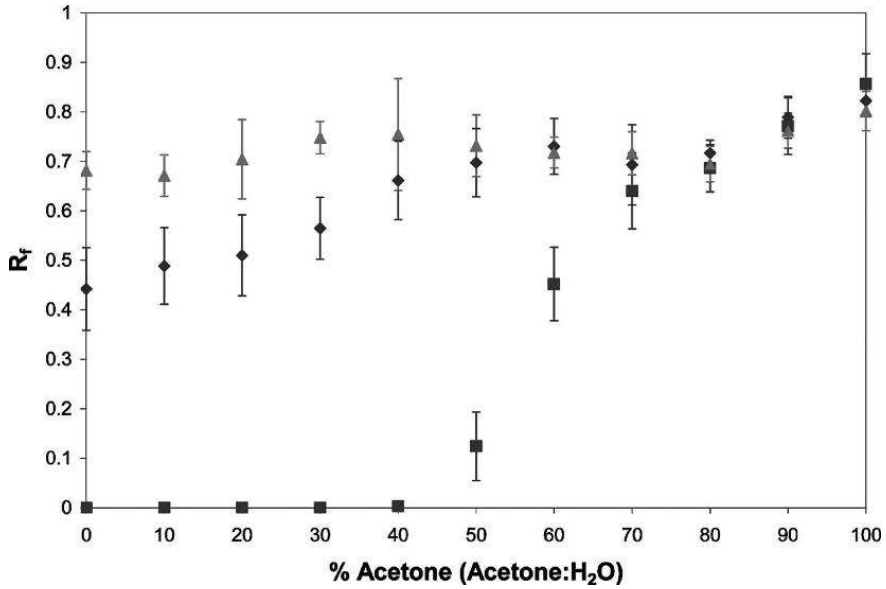
Electrospun UTLC

Lecture 2a









Jonathan E. Clark, Susan V. Olesik, Anal Chem 81 (2009) 4121–4129
and J Chromatogr A 1217 (2010) 4655–4662

Nanotube UTLC

Lecture 2c

Materials Views
www.MaterialsViews.com

ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Carbon-Nanotube-Templated Microfabrication of Porous Silicon-Carbon Materials with Application to Chemical Separations

*Jun Song, David S. Jensen, David N. Hutchison, Brendan Turner, Taylor Wood, Andrew Dadson, Michael A. Vail, Matthew R. Linford, Richard R. Vanfleet, and Robert C. Davis**

Carbon-nanotube-templated microfabrication (CNT-M) of porous materials is demonstrated. Partial chemical infiltration of 3D carbon-nanotube structures with silicon results in a mechanically robust material, structured from the 10 nm scale to the 100 μm scale. The nanoscale dimensions are determined by the diameter and spacing of the resulting silicon/carbon nanotubes, while the microscale dimensions are controlled by the lithographic patterning

recombination rates, and mobilities are strongly influenced by nanoscale structuring.[4] Often, multiple physical properties are coupled and are jointly influenced by nanoscale structuring, as is the case for strained silicon: nanoscale strain control is used to produce higher mobilities than achievable in the bulk.[5] Coupling

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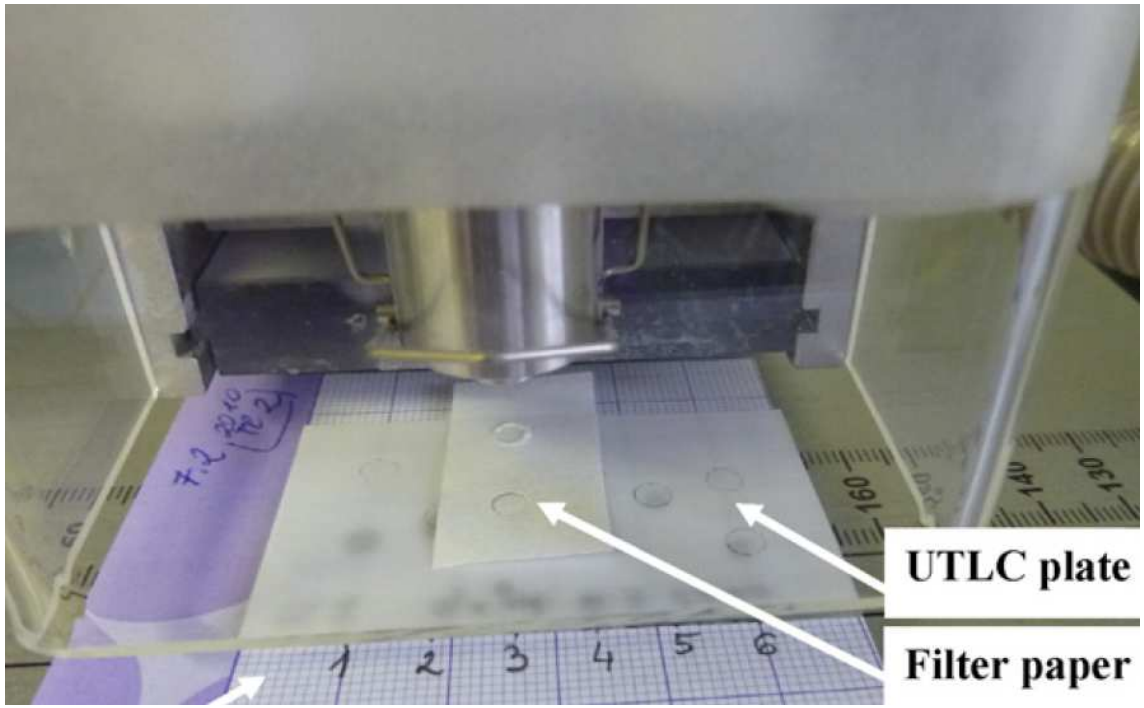
UTLC-MS



I. Vovk et al. J Chromatogr A 1218 (2011) 3089–3094

Lecture 3a

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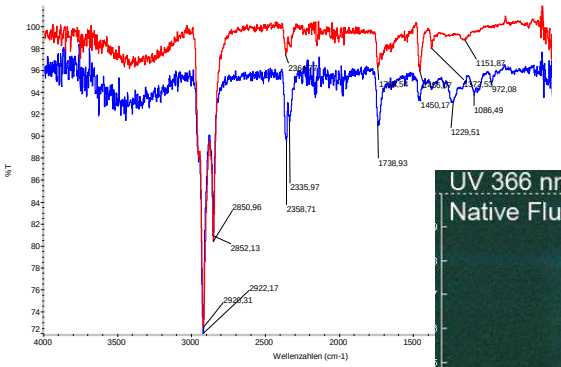
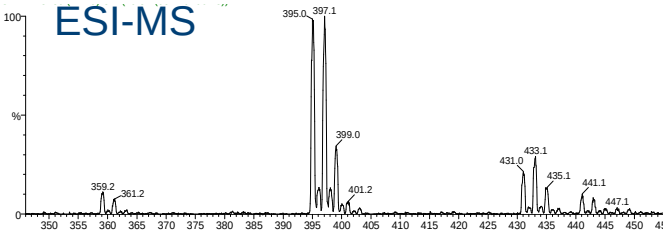
Find the difference!

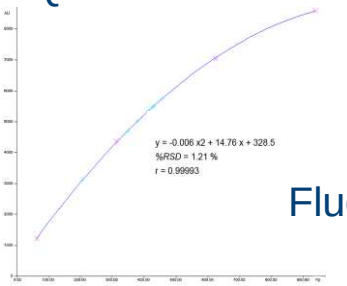
ATR-FTIR

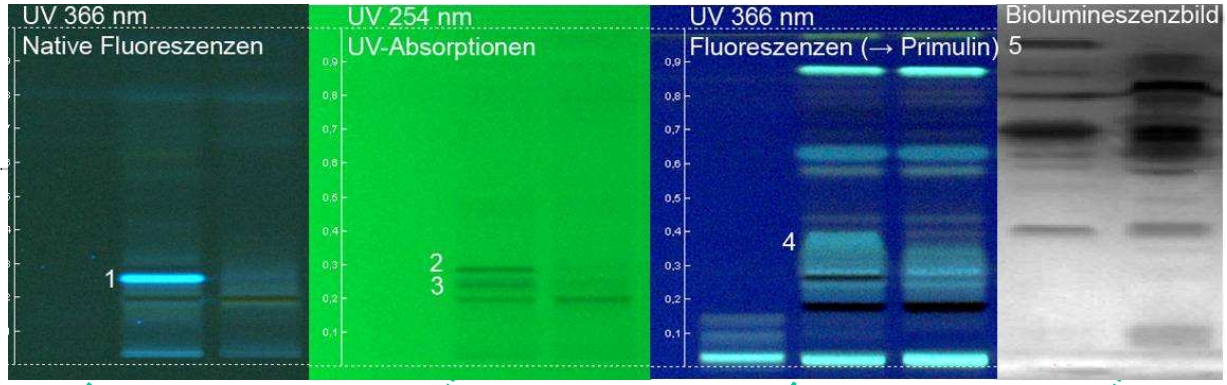


ESI-MS



Quantitation





Fluorescence UV/Vis absorption Selective derivatization Bioassay

→ HPTLC-UV/Vis/FLD-EDA-HRMS

→ New GDCh course 335/11

G. Morlock, W. Schwack, *J Chromatogr A* 1217 (2010) 6600-6609

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Excerpts from CBS 51 - 102 Keyword: sugar AND food

Carbohydrates

G. LODI*, C. BIGHI, V. BRANDOLINI, E. MENZIANI, B. TOSI, (*Dipartimento di Chimica, via L. Borsari 345, Univ. di Ferrara, I-44100 Ferrara, Italy): Automated multiple development HPTLC analysis of sugars on hydrophilic layers: II. Diol layers. J. Planar Chromatogr. **10**, 31-37 (1997). HPTLC of sugars (i.a. glucose, isomaltotetrose, isomaltotriose, isomaltose, raffinose, nystose, 1-kestose, lactose, lactulose, sucrose, galactose, fructose, arabinose, xylose, ribose, rhamnose) on diol with AMD using a fifteen-step ACN - water gradient with water concentration decreasing linearly from 35 to 15%. Detection by absorbance at 515 nm after derivatization with 4-aminobenzoic acid reagent or a-naphthol reagent by immersion for 2 min. After drying for 2 min finally heating at 100-120°C. Quantification by densitometry at 365 nm (fluorescence) and at 400 resp. 515 nm (absorbance).

Food analysis, quantitative analysis, densitometry, AMD

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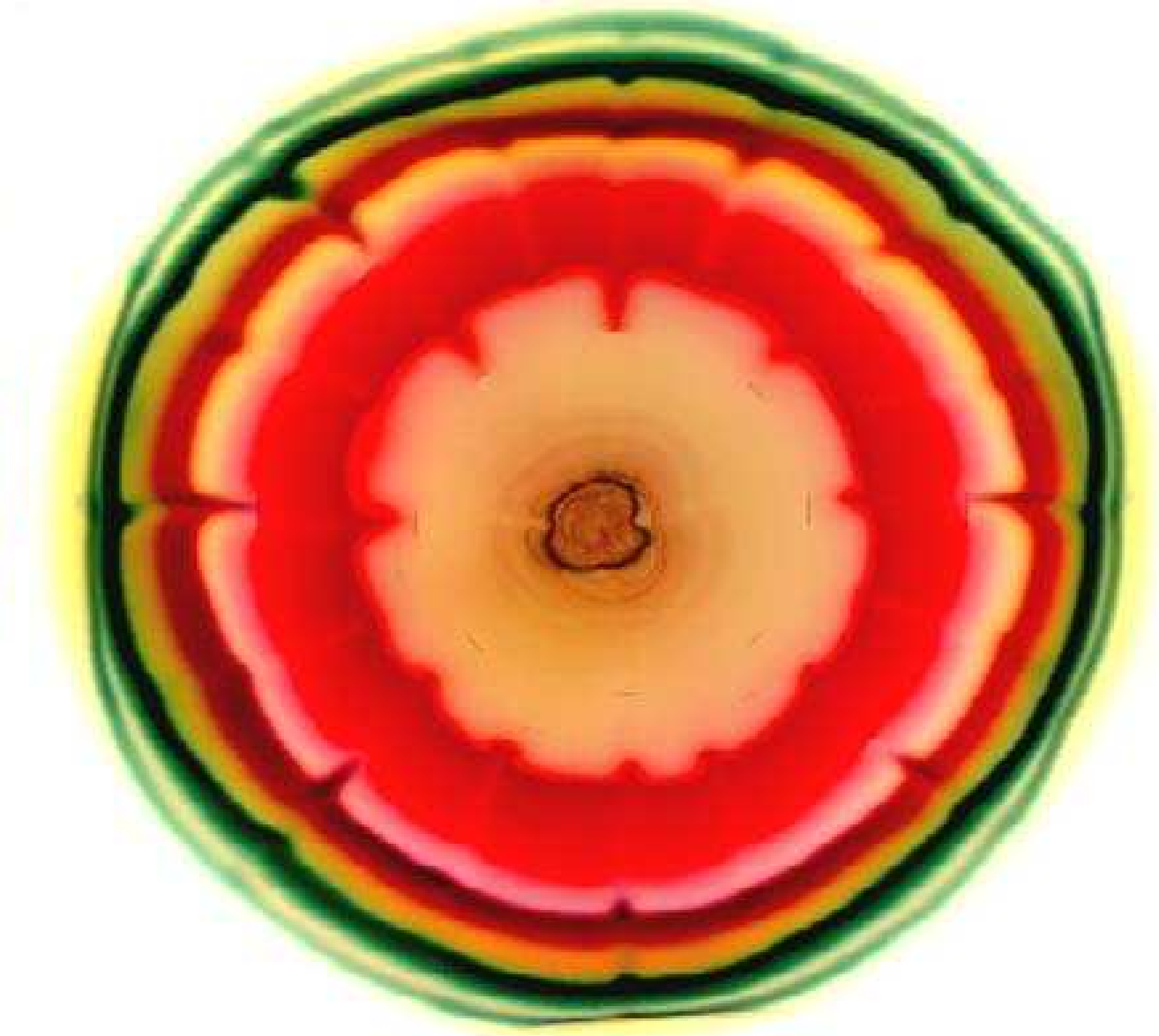
Planar chromatography being art!



CHROMart by Drs. Karla & Herbert Halpaap