Hyphenations in HPTLC – HPTLC-MS and EDA applications

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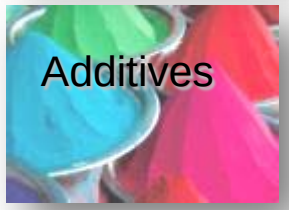
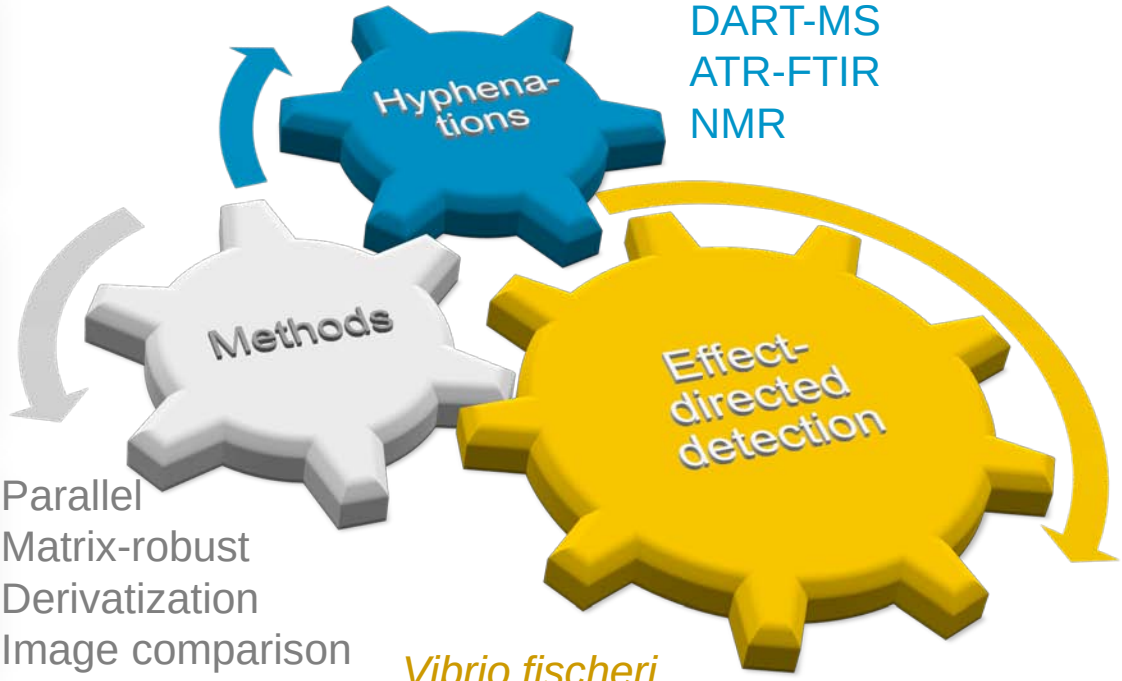
GIESSEN
Food Science

IFZ Interdisziplinäres Forschungszentrum Giessen Research Centre for BioSystems, Land Use and Nutrition

Modern effective platform



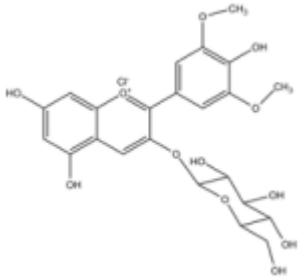


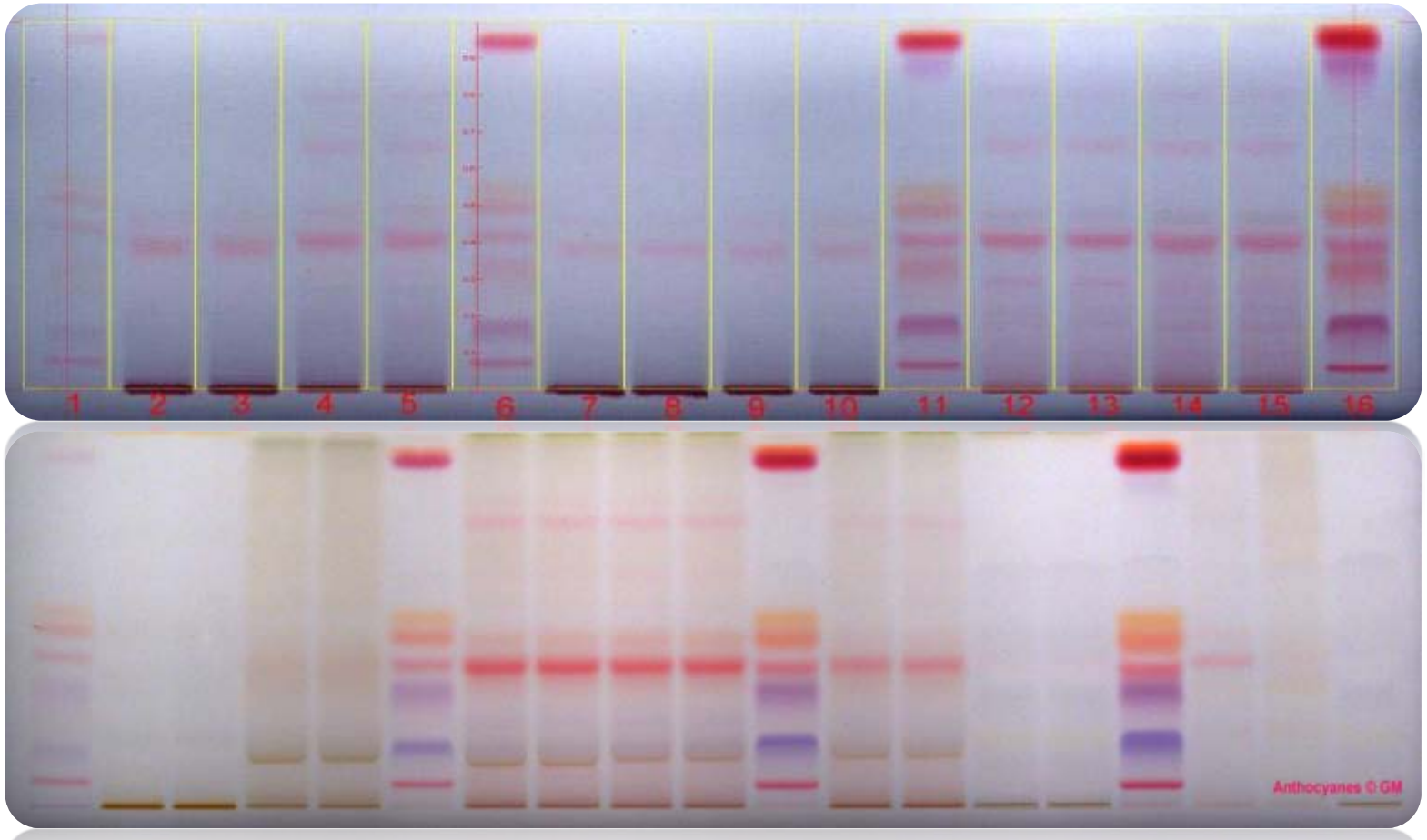
ESI-MS
MALDI-MS
DART-MS
ATR-FTIR
NMR

Parallel
Matrix-robust
Derivatization
Image comparison
Modular use

Vibrio fischeri
Bacillus subtilis
Planar-YES
Glucosidase inhibiting compounds
Esterase inhibiting compounds
Photosynthesis inhibiting compounds
Antioxidants or radical scavengers

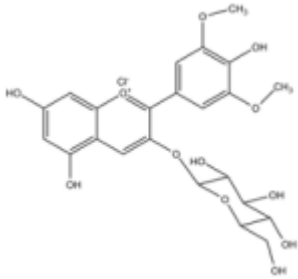
Anthocyanes in feed, pomace, juice and wine

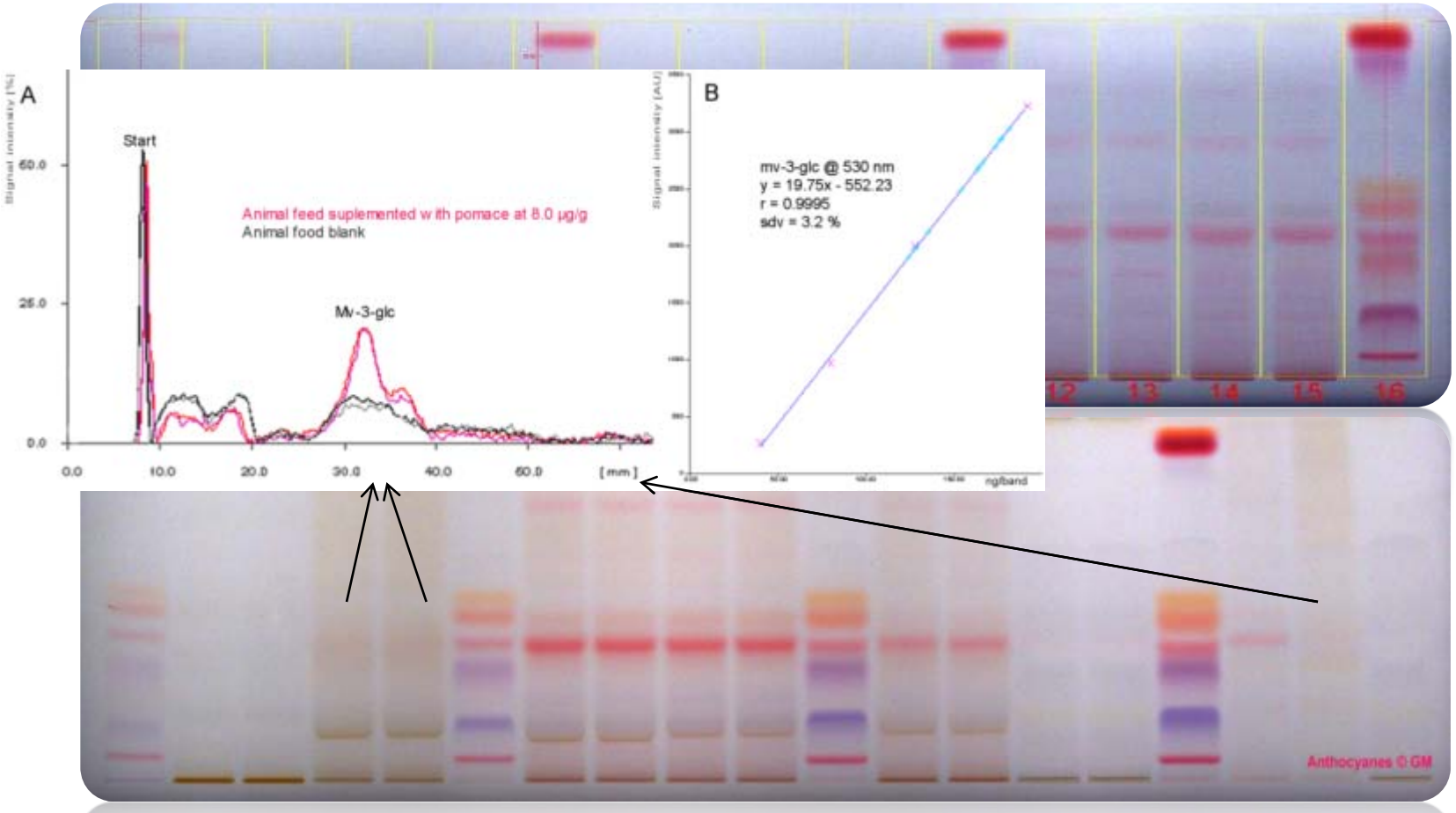




S. Krüger, O. Urmann, G. Morlock, *J Chromatogr A* 1289 (2013) 105-118

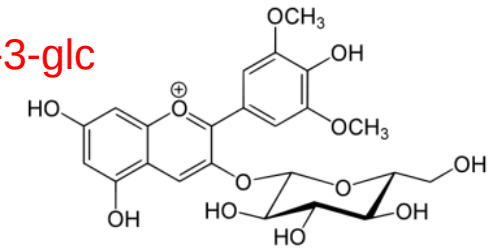
Anthocyanes in feed, pomace, juice and wine



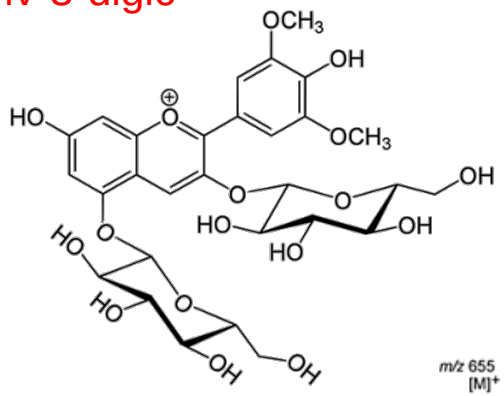


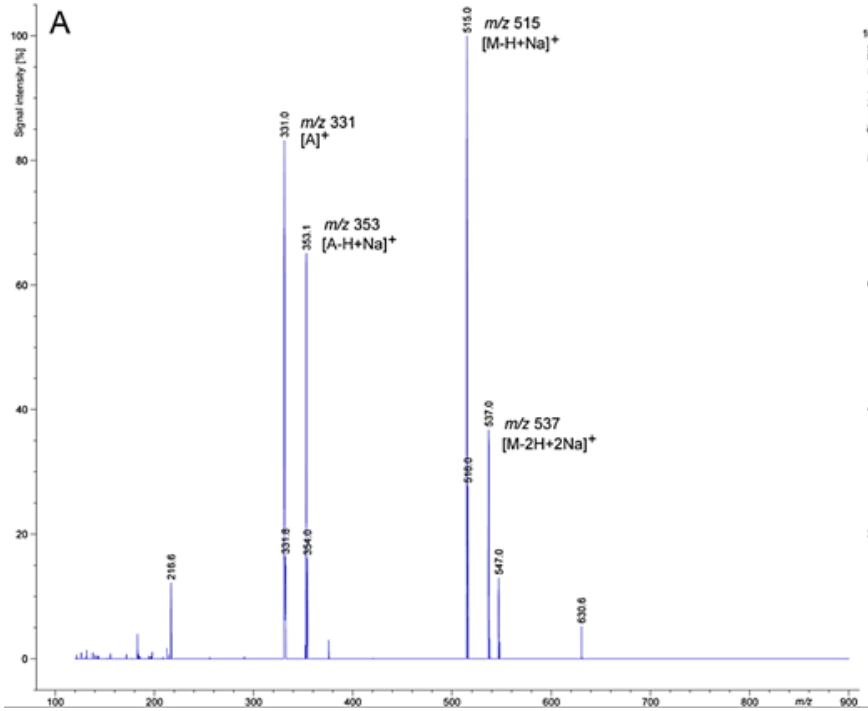
Mass spectra of anthocyanins

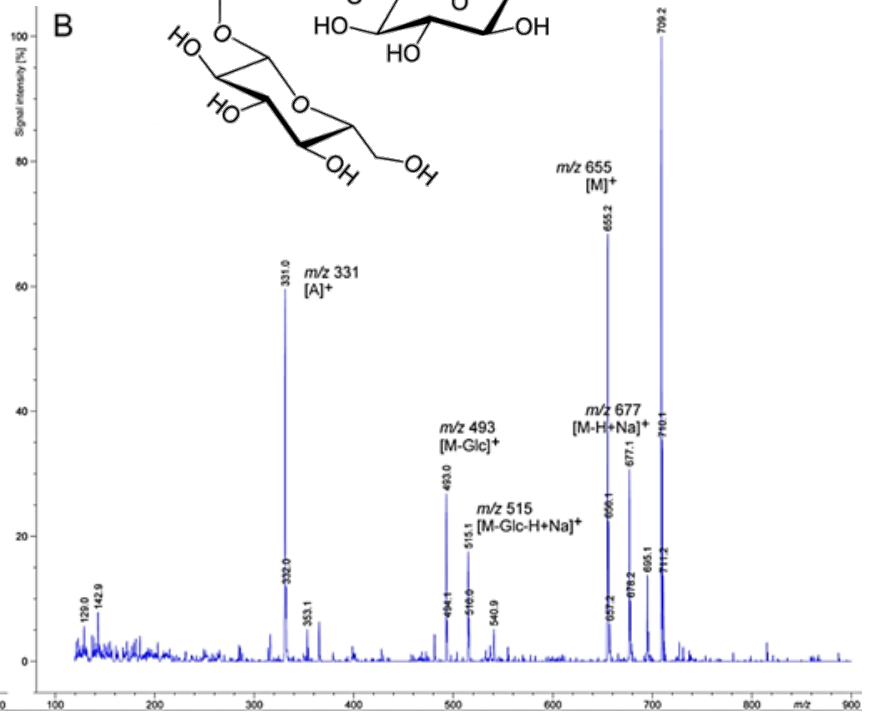
Mv-3-glc



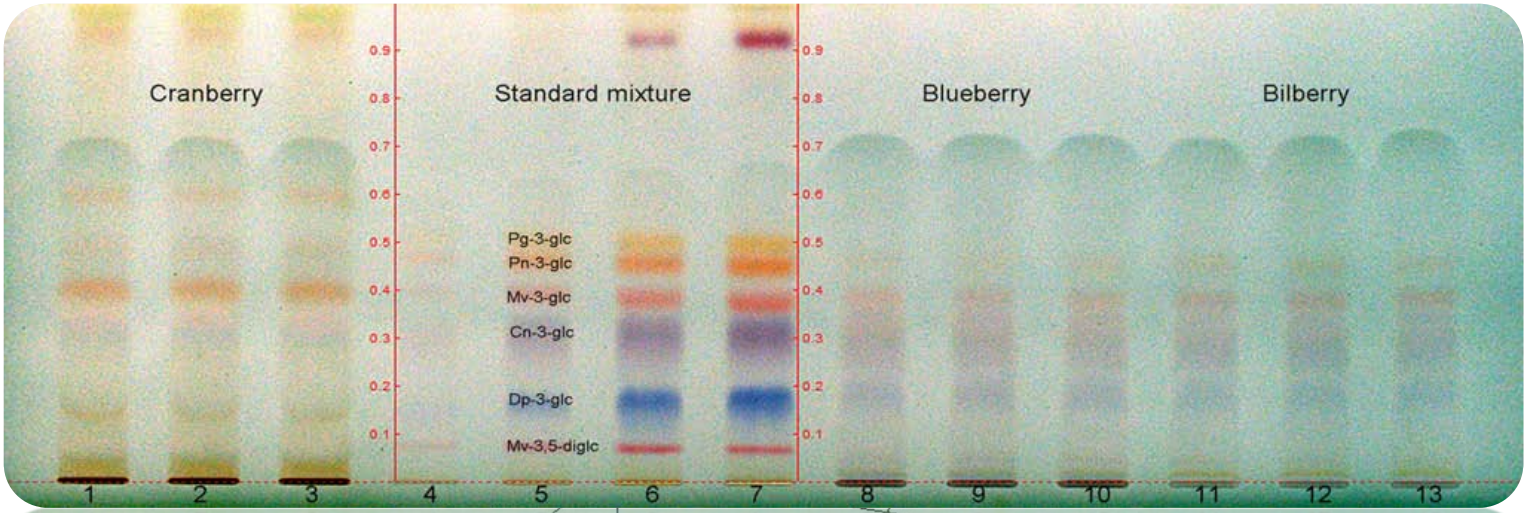
Mv-3-diglc

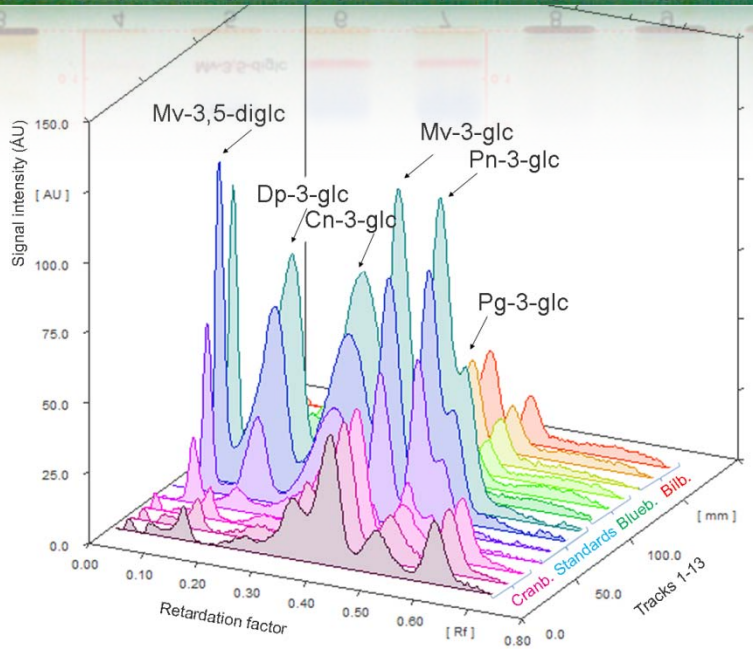






Anthocyanes in berry extracts

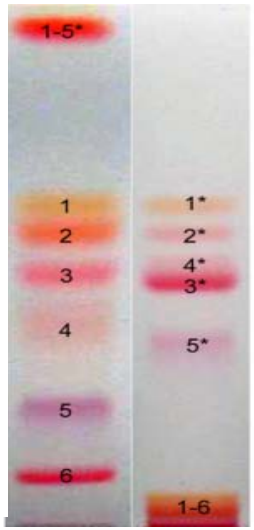


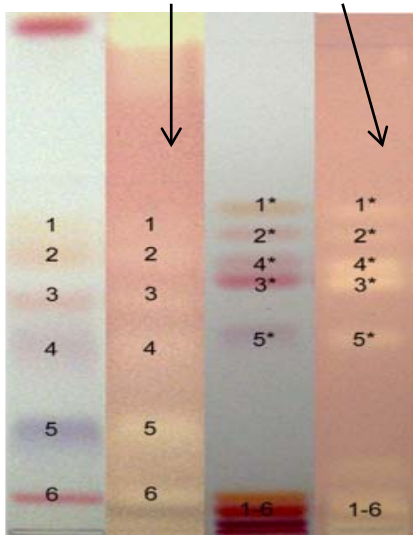


G. Cretu, G. Morlock, in preparation

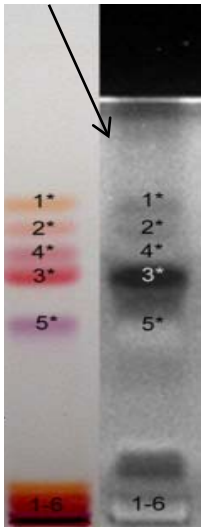
Effect-directed link to the compound

Radical scavenging property

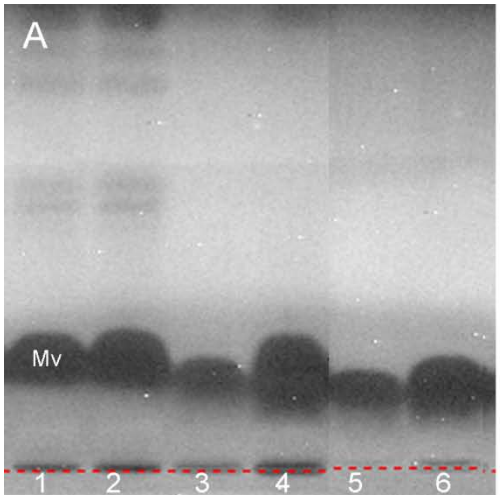


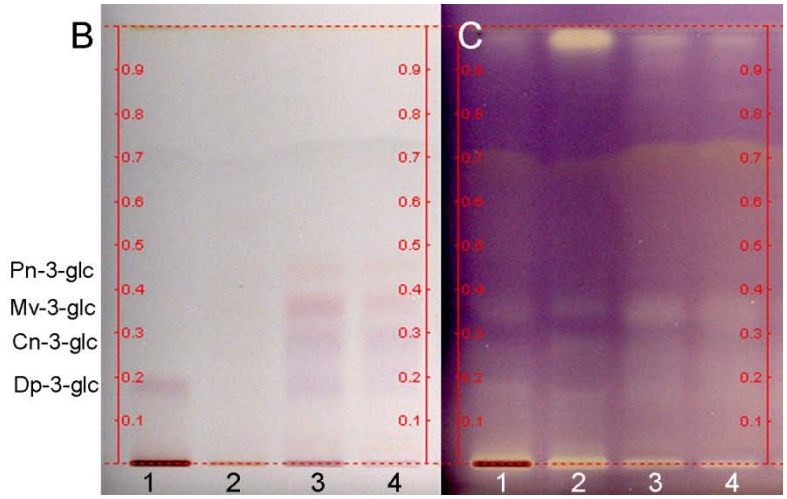


Vibrio fischeri bioactivity

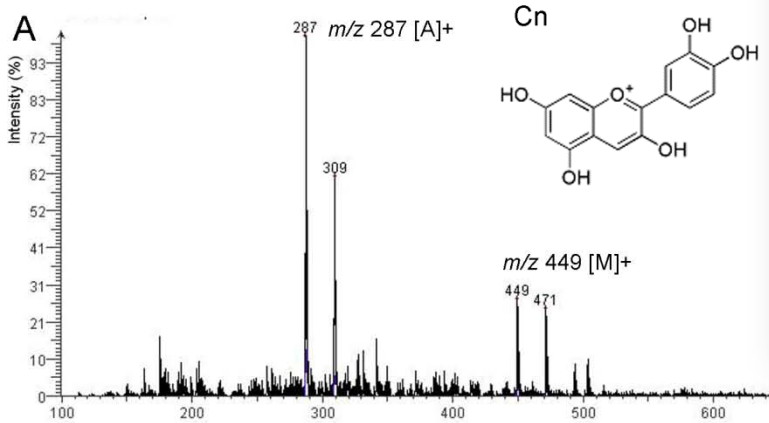


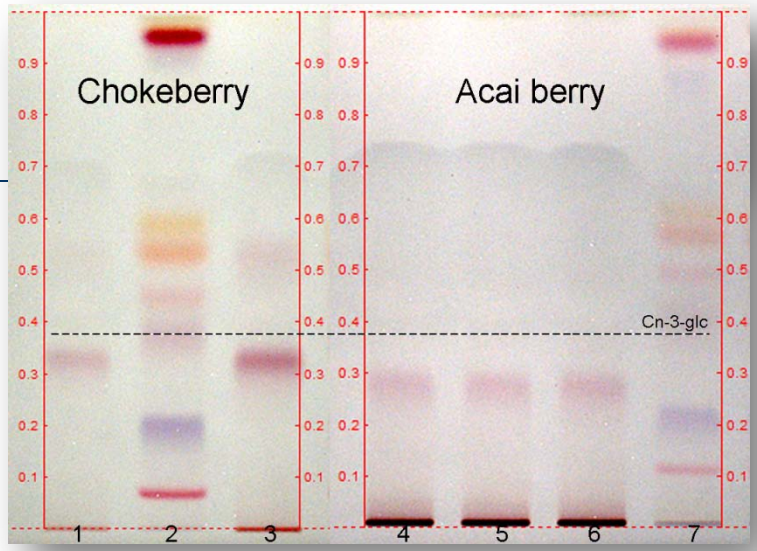
Powdered berry samples

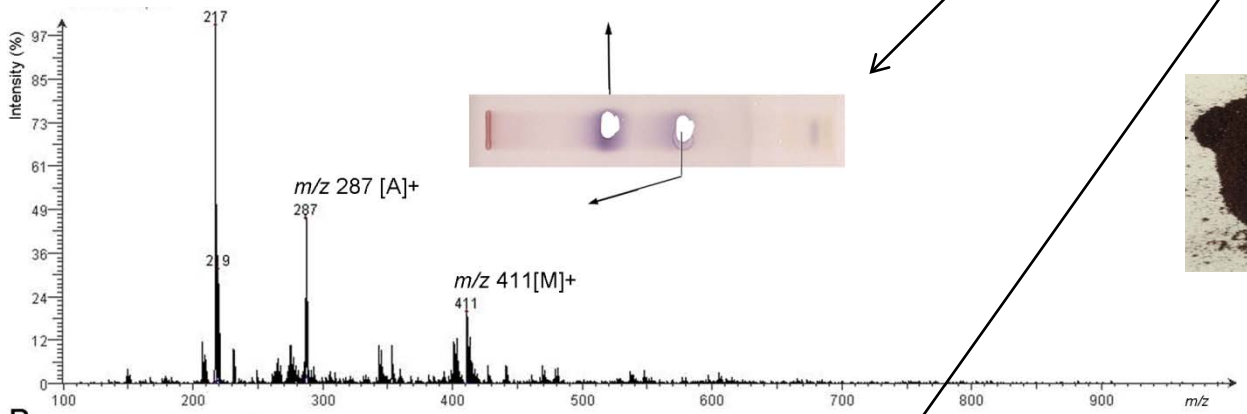


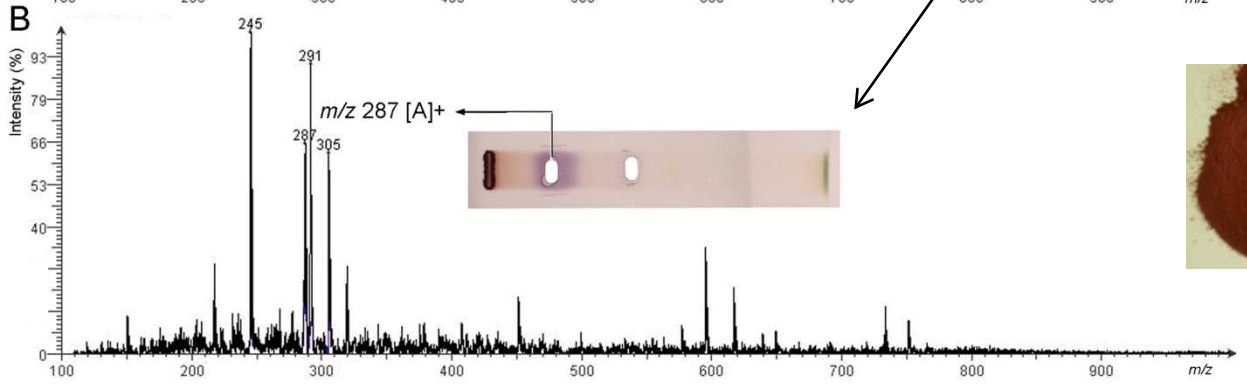


Mass spectra of unknowns









Quantitation of tanshinons in Chinese salvia

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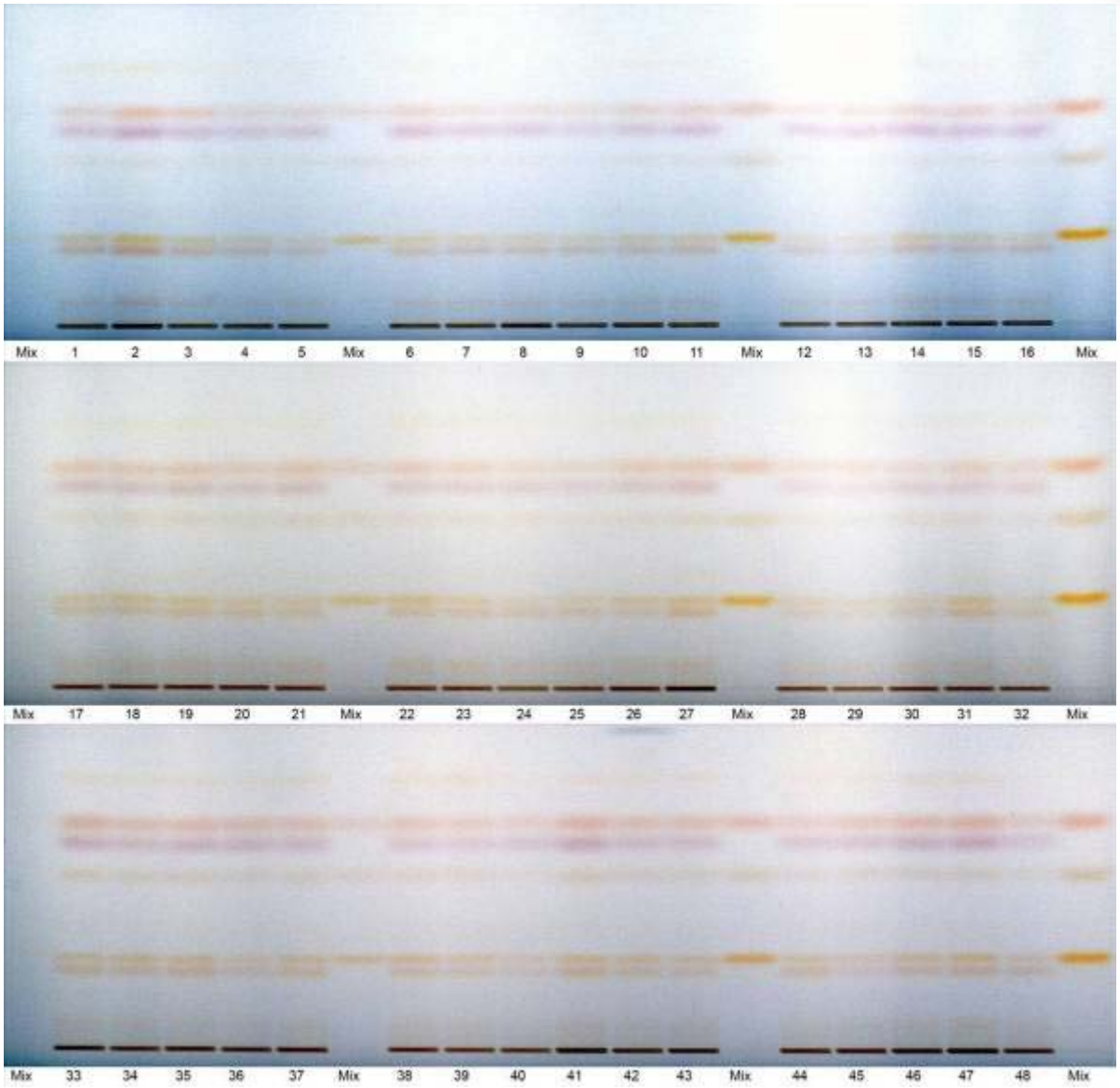
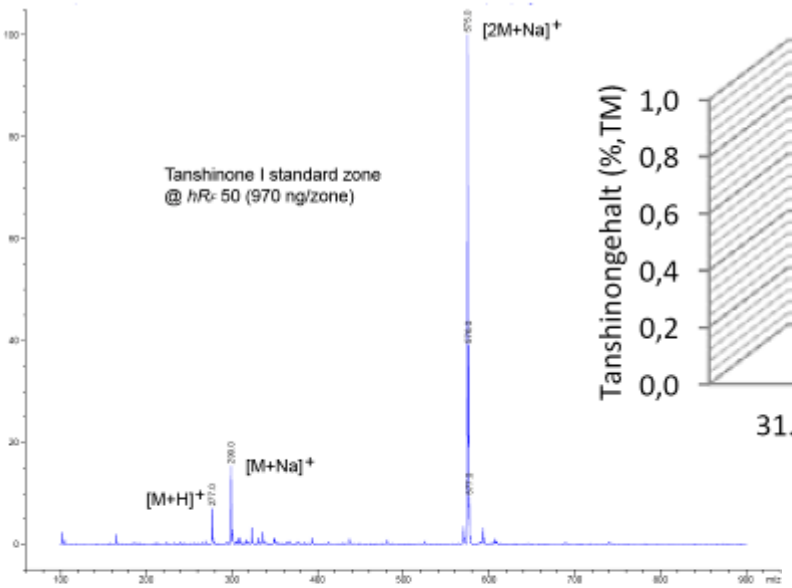
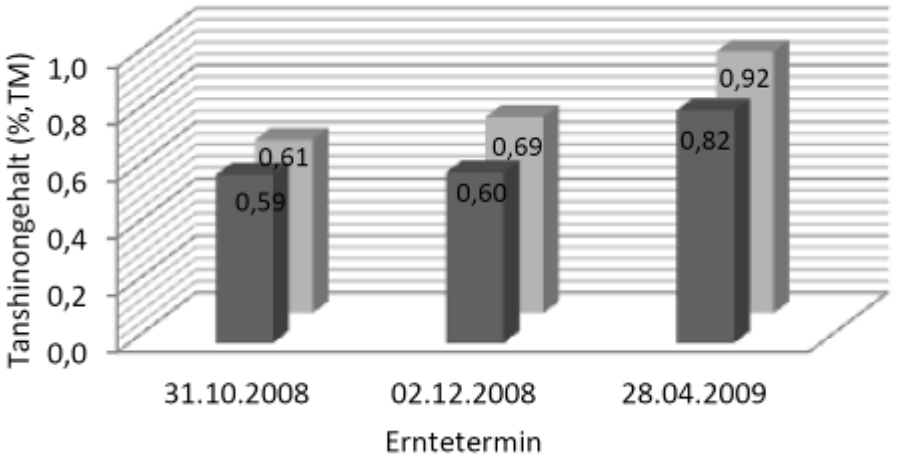
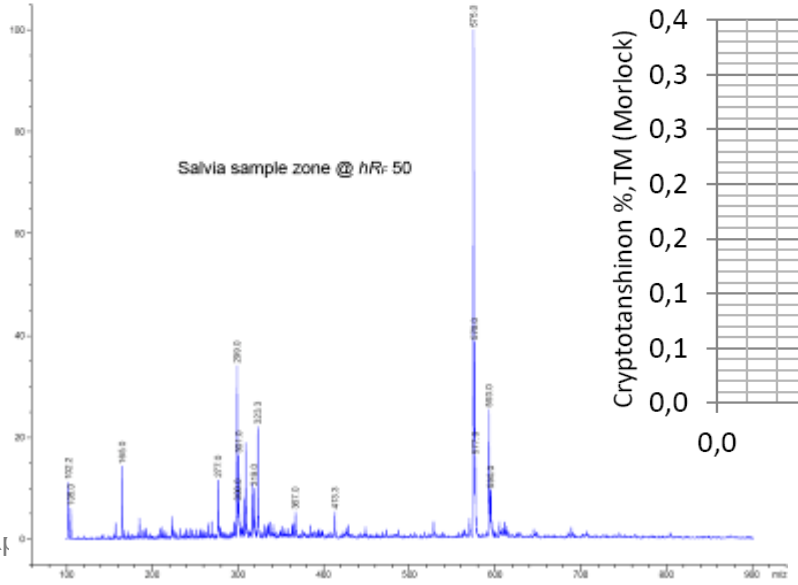
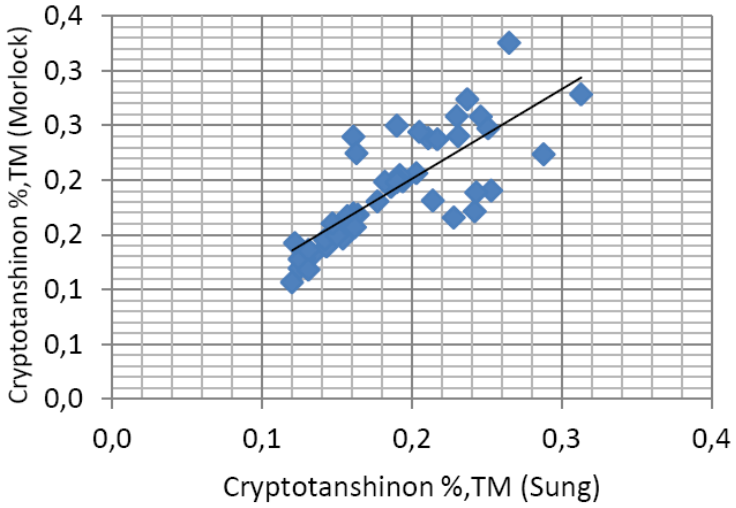


Confirmation by MS and method comparison



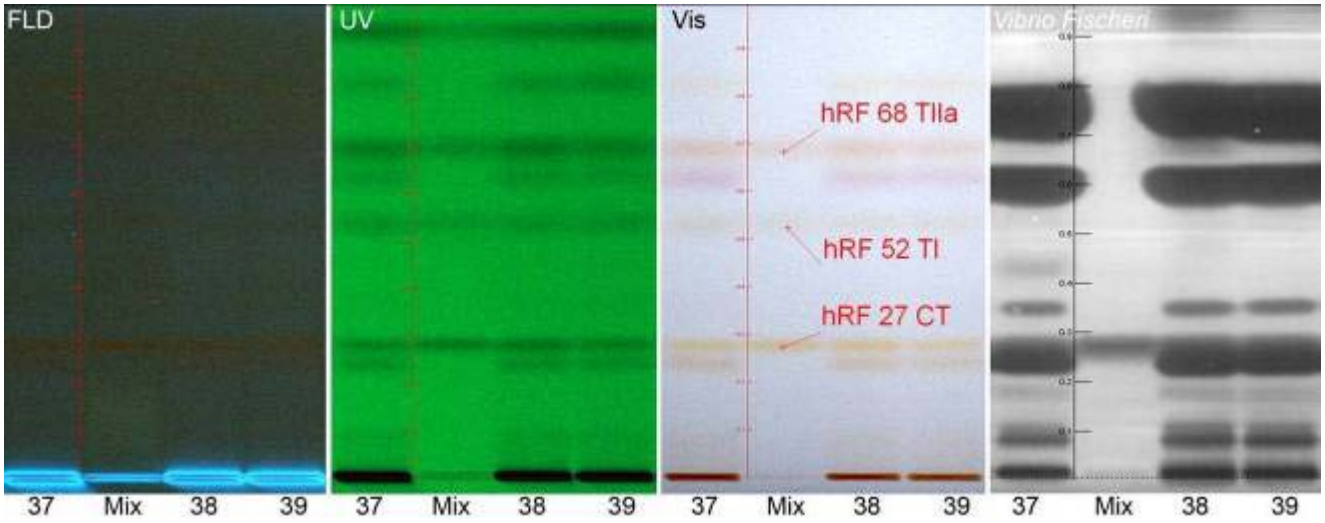


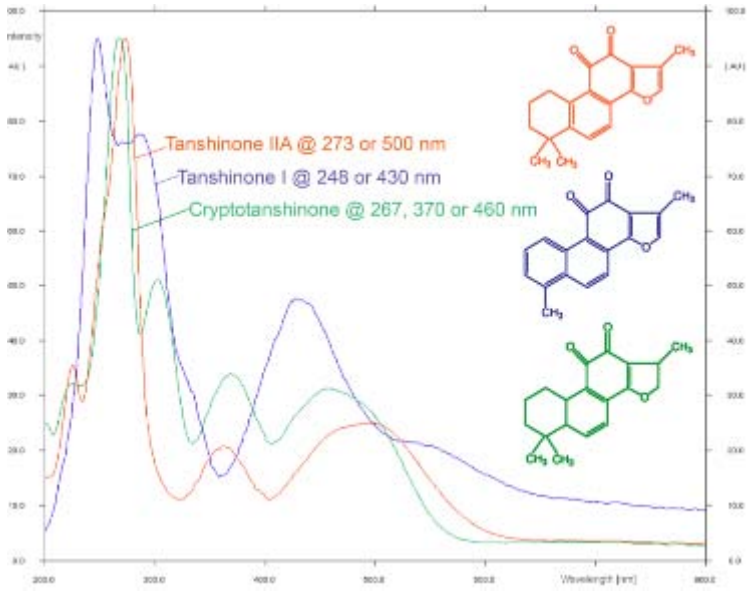




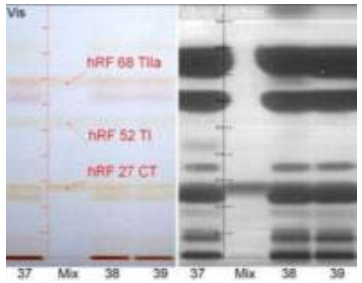
G. Morlock, Y. Sung, B. Honermeier, in preparation

Bioactivity of single compounds

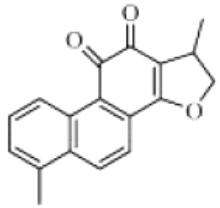




Unknown bioactive compound (below CT)

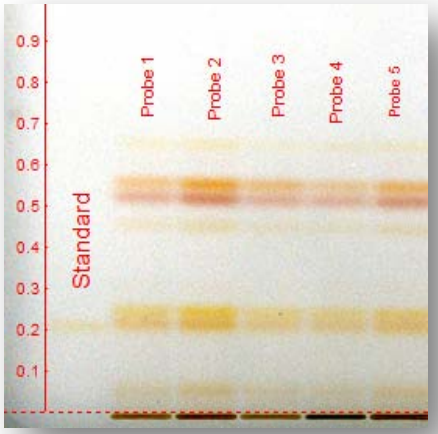


279.10208 $[M+H]^+$



Based on exact mass and chromatographic data:
15,16-Dihydrotanshinon I

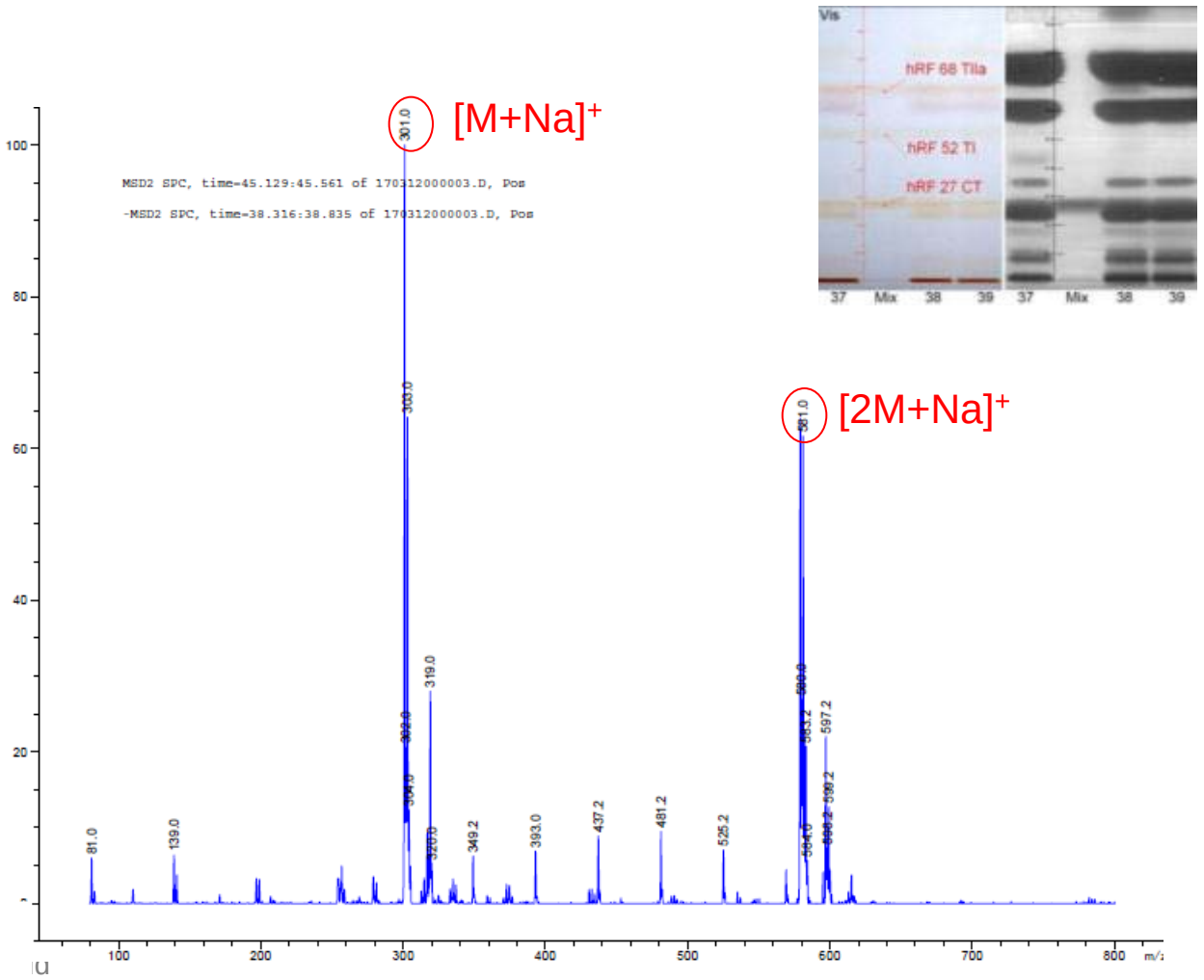
Standard found and bought:



G. Morlock, T. Sung, B. Honermeier, in preparation

Unknown bioactive compound (below CT)

Mass spectra recorded after detection with bioassay → salt adducts are pronounced!



Fingerprint of phenolic compounds in propolis

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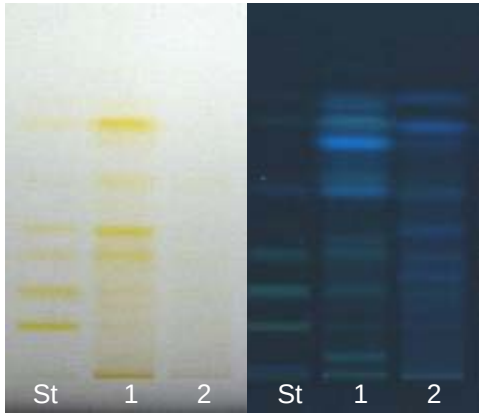
Fast characterization of samples by HPTLC

Visible zones

Native fluorescent zones (366 nm)



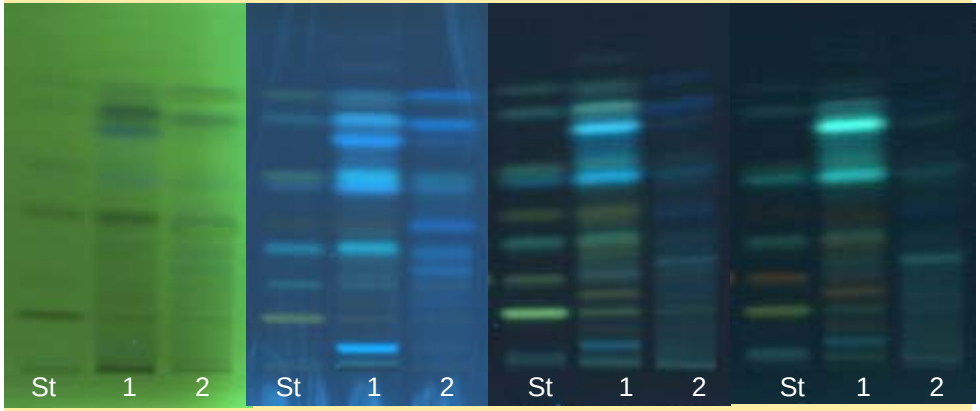
Selective derivatizations

Lipophilic zones → berberine (366 nm)

Flavonoids → $AlCl_3$ (366 nm)

Flavonoids → Neu (366 nm)

Flavonoids → Neu/PEG (366 nm)

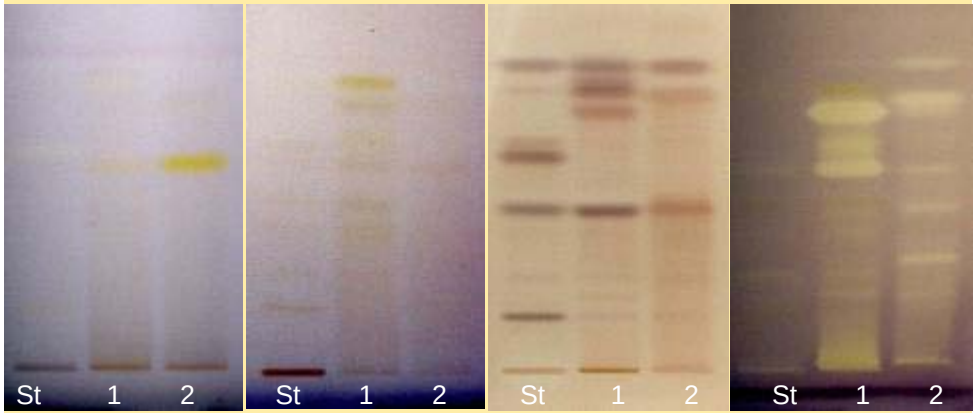


Sugars → DPA

Amino acids → Ninhydrin

Phenolics → Fast blue salt

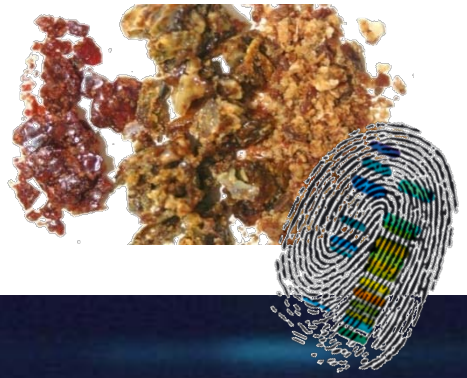
Antioxidatives → DPPH*

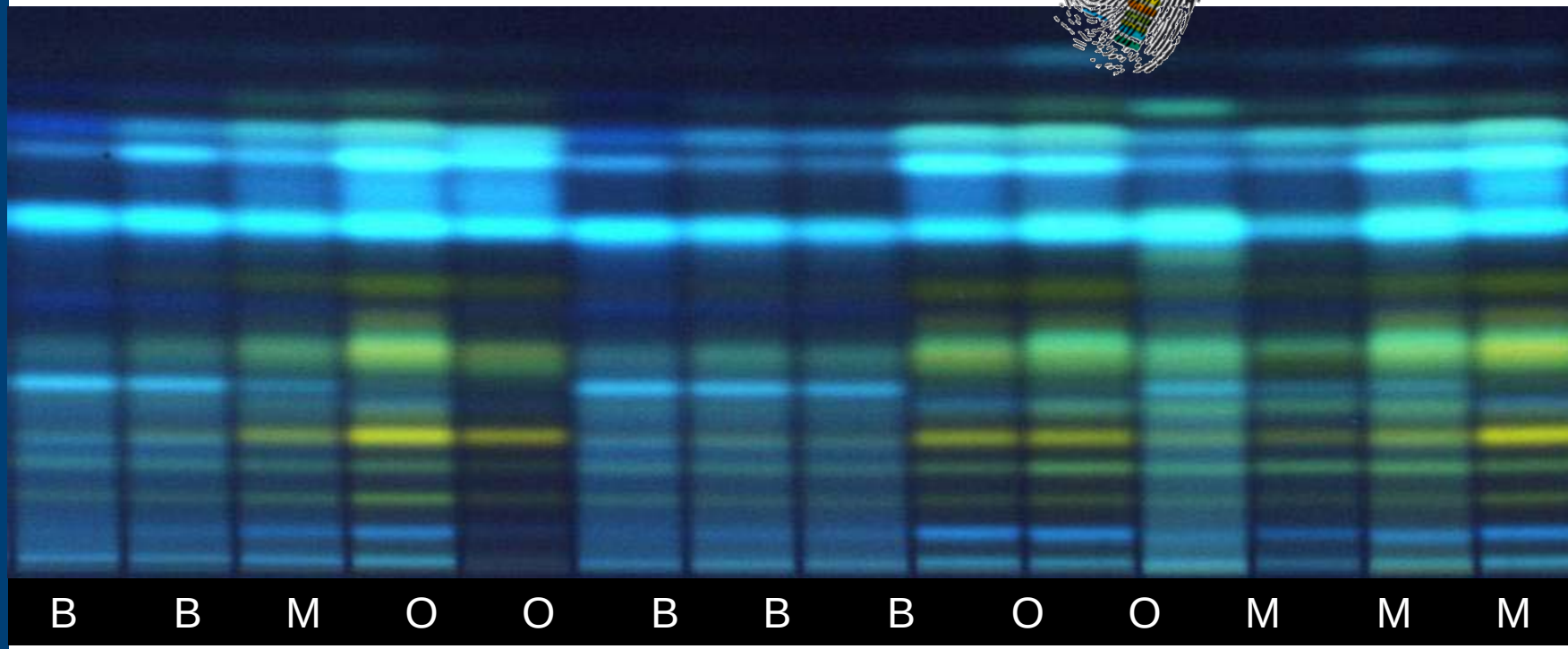


Fingerprint of phenolic compounds in propolis

→ Screening of >100 samples showed characteristic marker compounds

→ Mainly 2 types of German propolis

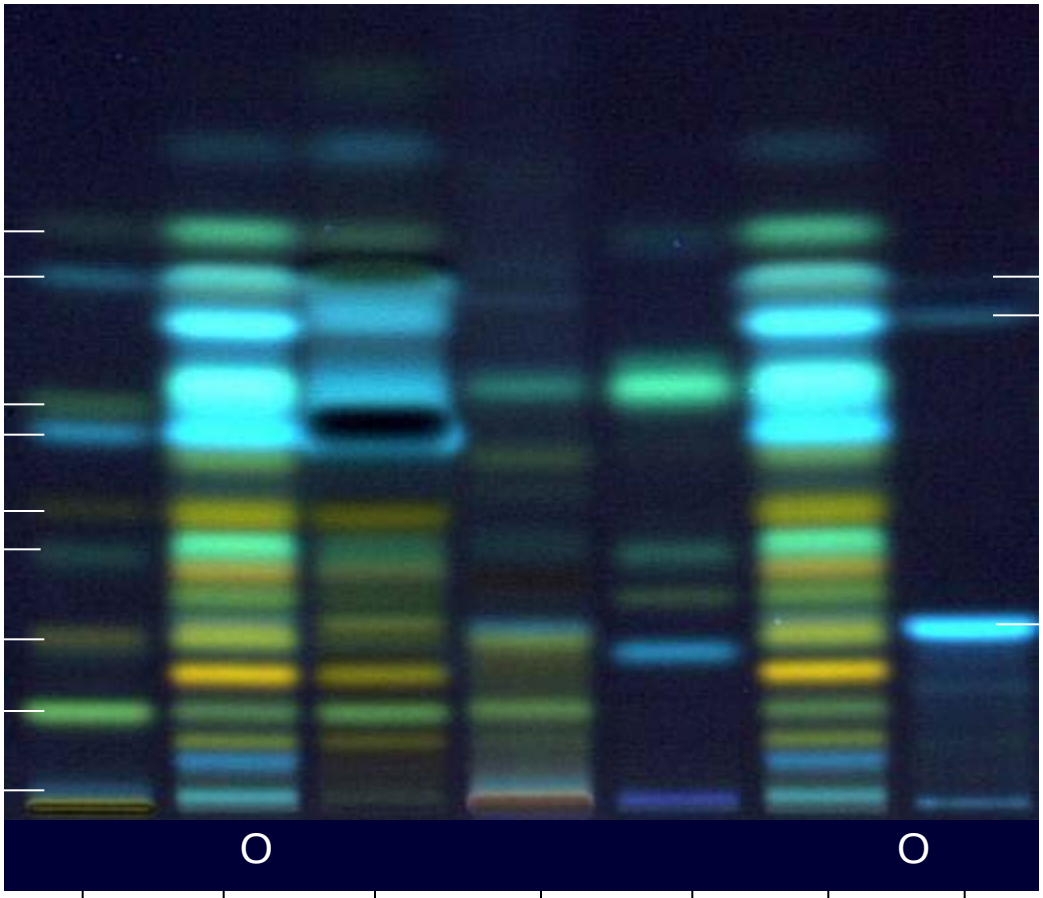




G. Morlock *et al.*, in preparation

Cooperation with Wala and Apicultural State Institute, Stuttgart

Plant origin of O-type?



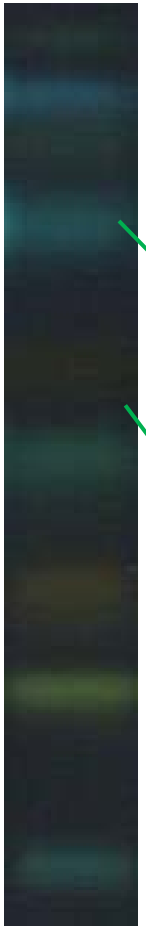
Confirmation of marker compounds by MS

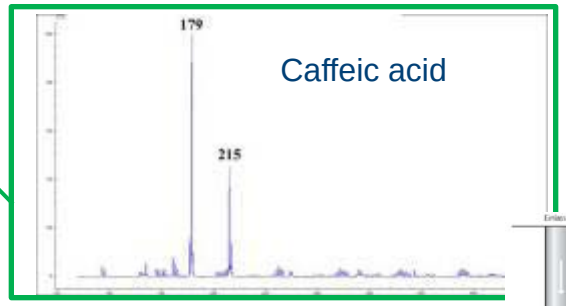
ESI-MS full scan spectra

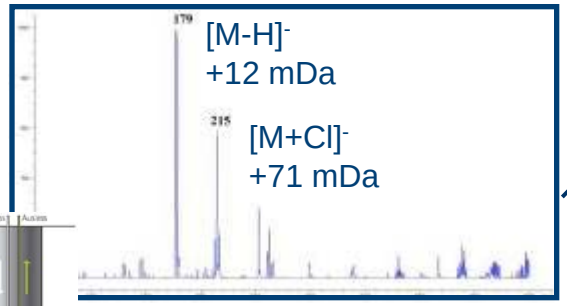
EIC of DART-MS

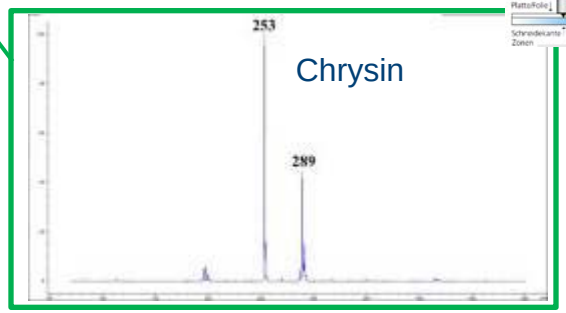
Spectra of standard

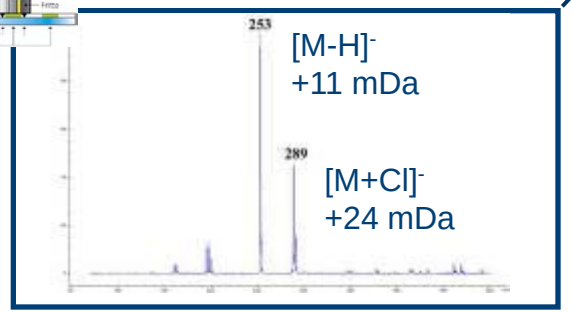
Spectra of sample

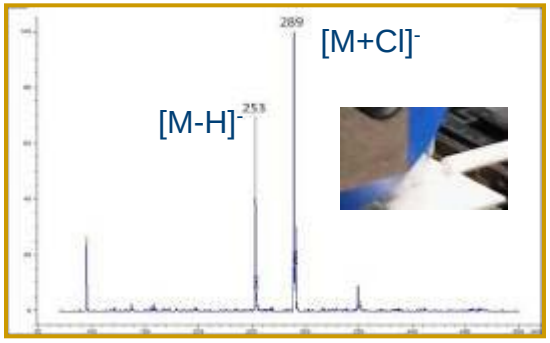


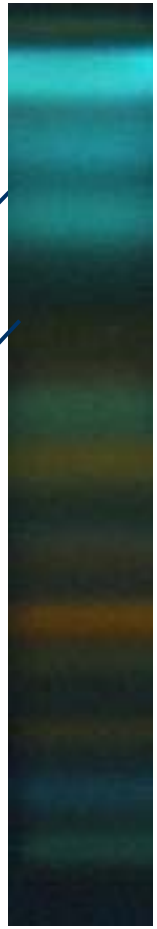




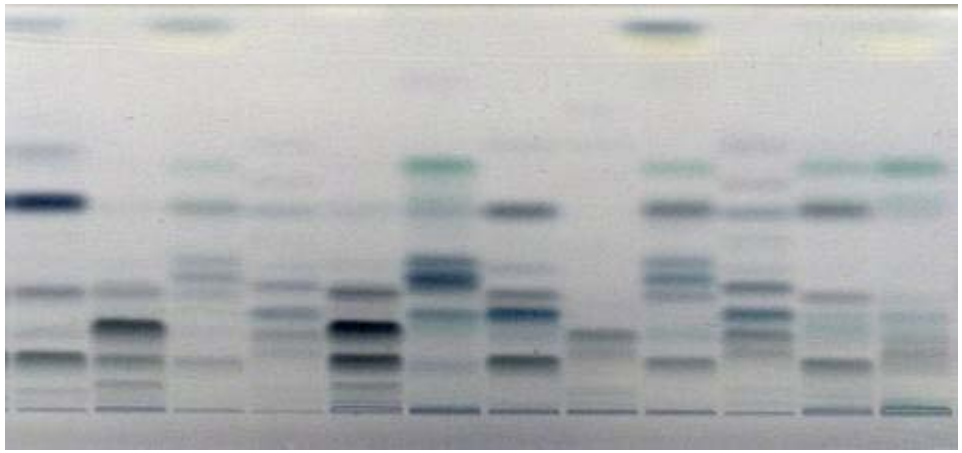




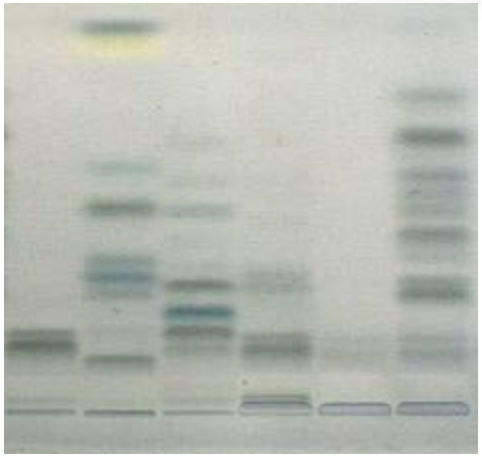




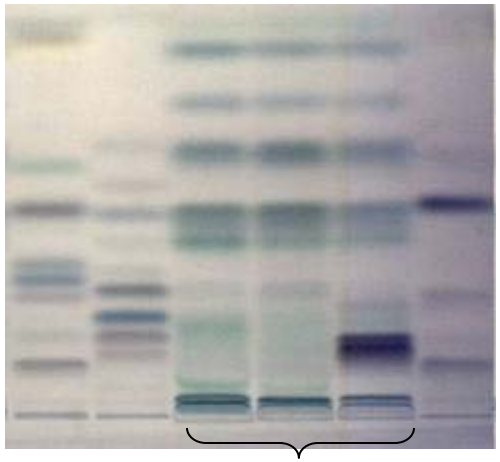
Analysis of biopolymers → monomeric units



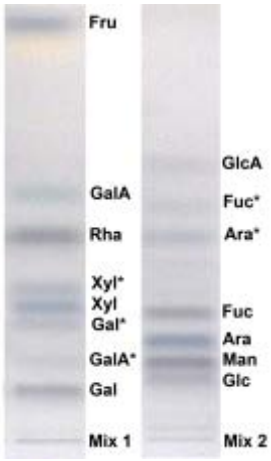
Carr JBKM Mix 1 Mix 2 Guar Trag Gum Xan Mix 1 Mix 2 Kara Pect



Starch Mix 1 Mix 2 CMC Cell HPMC

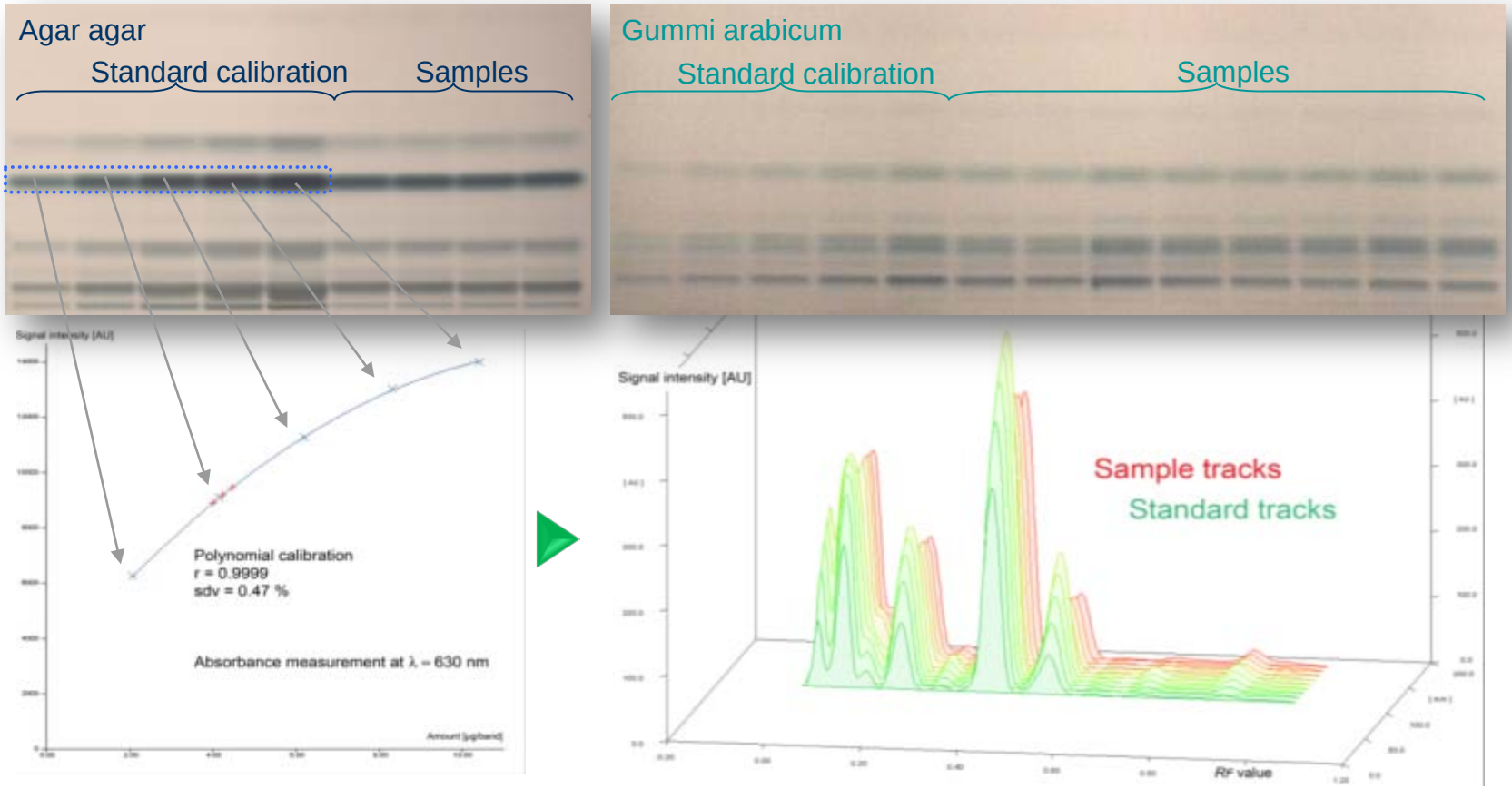


Mix 1 Mix 2 Alginate Agar



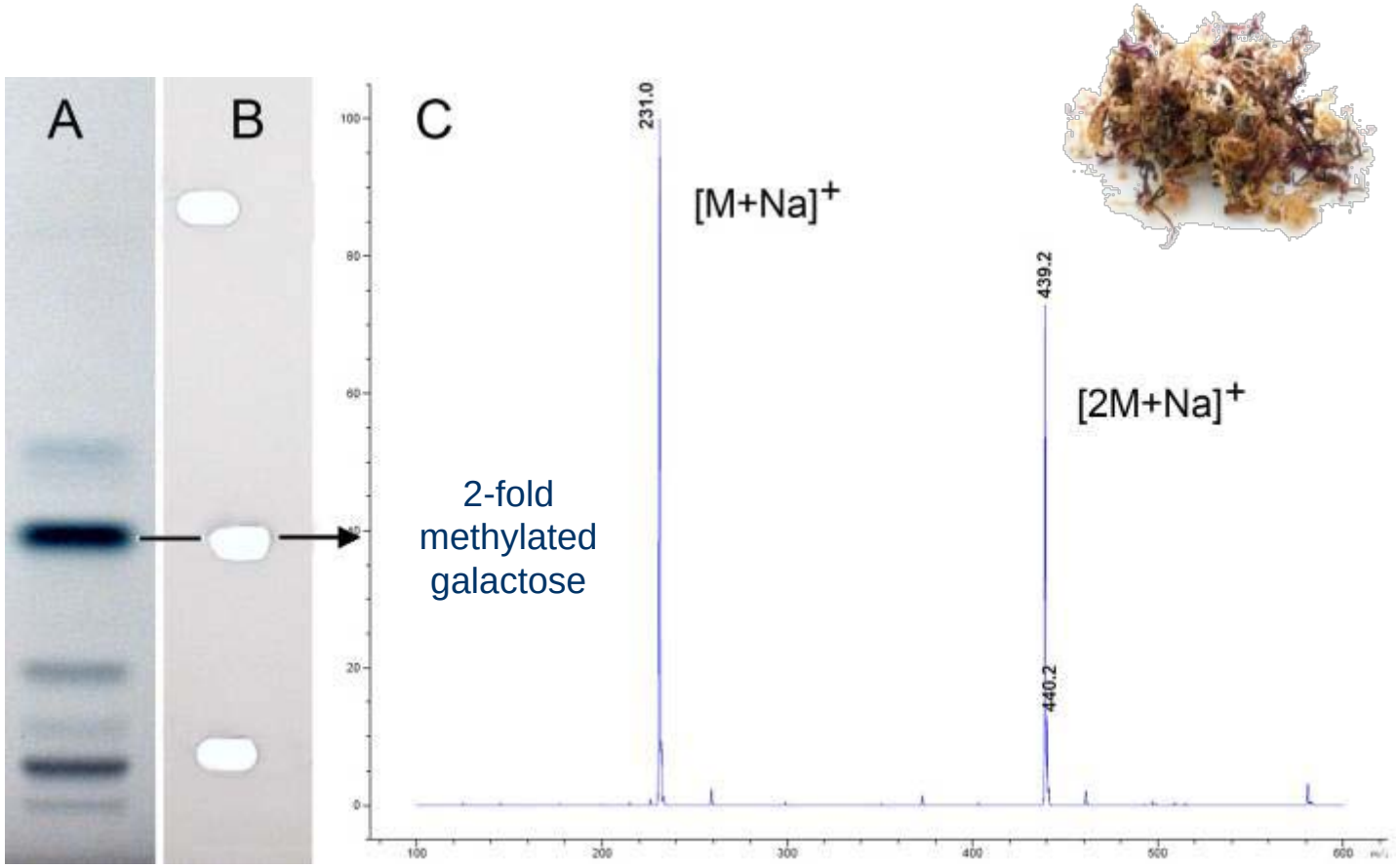
G. Morlock, F. Gamlich, *J Planar Chromatogr* 25 (2012) 244-250

Quantitation

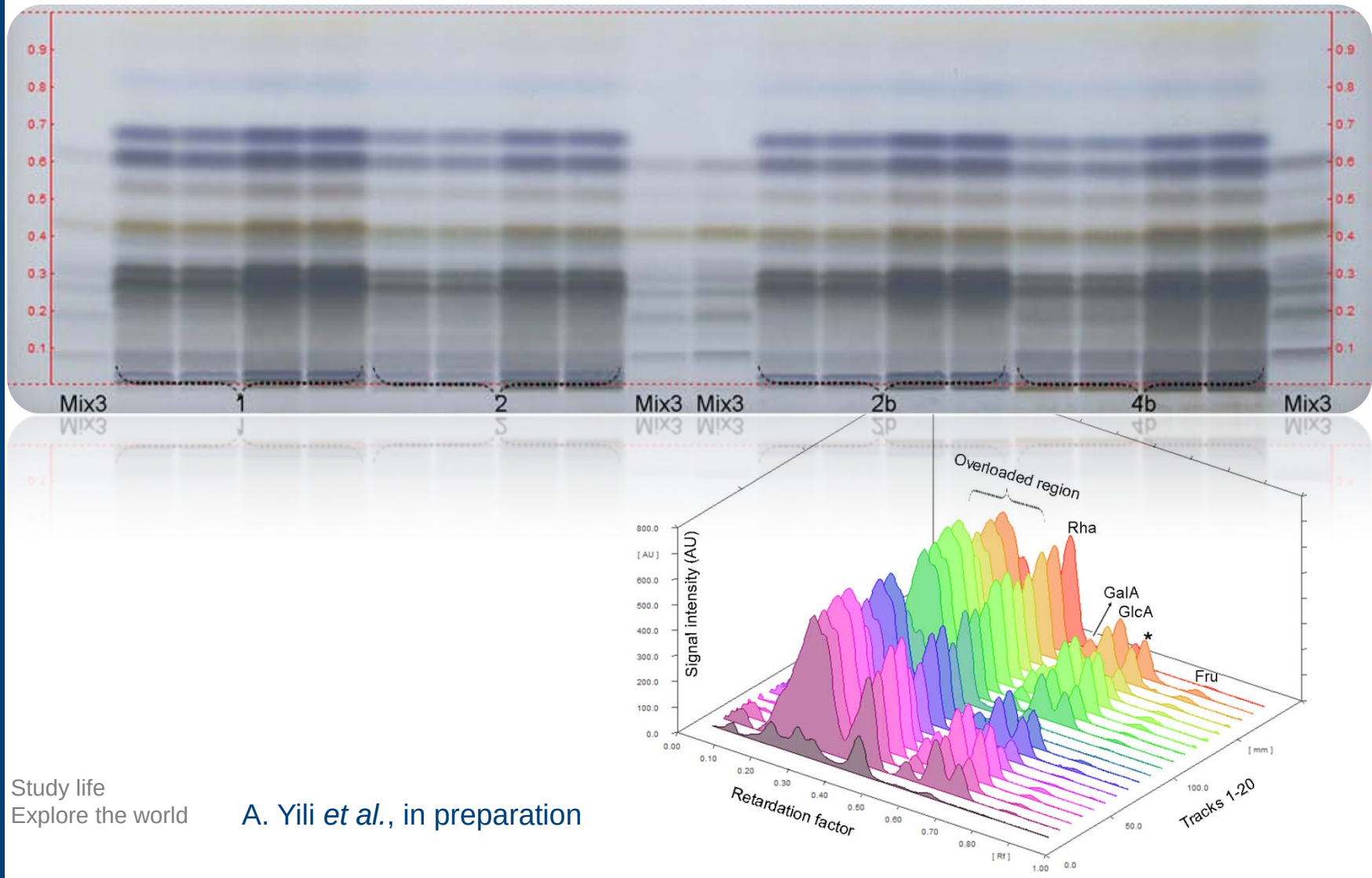


G. Morlock, D. Schick, W. Schwack, in preparation

Carrageen



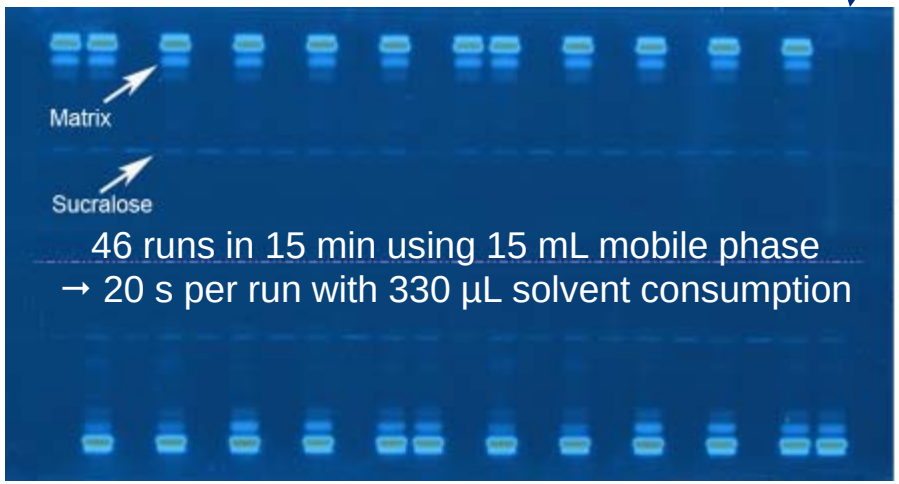
Analysis of *Ocimum basilicum* → use in TCM

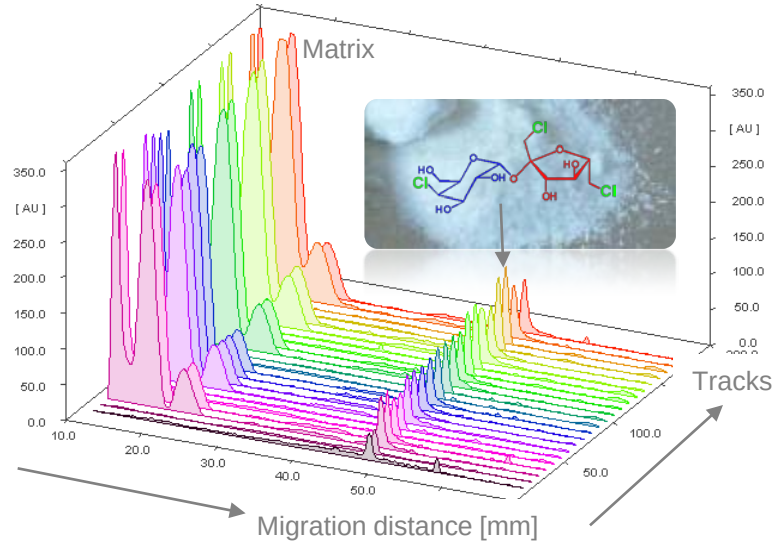


A. Yili *et al.*, in preparation

Sucralose in milk-based confection (*Burfi*)

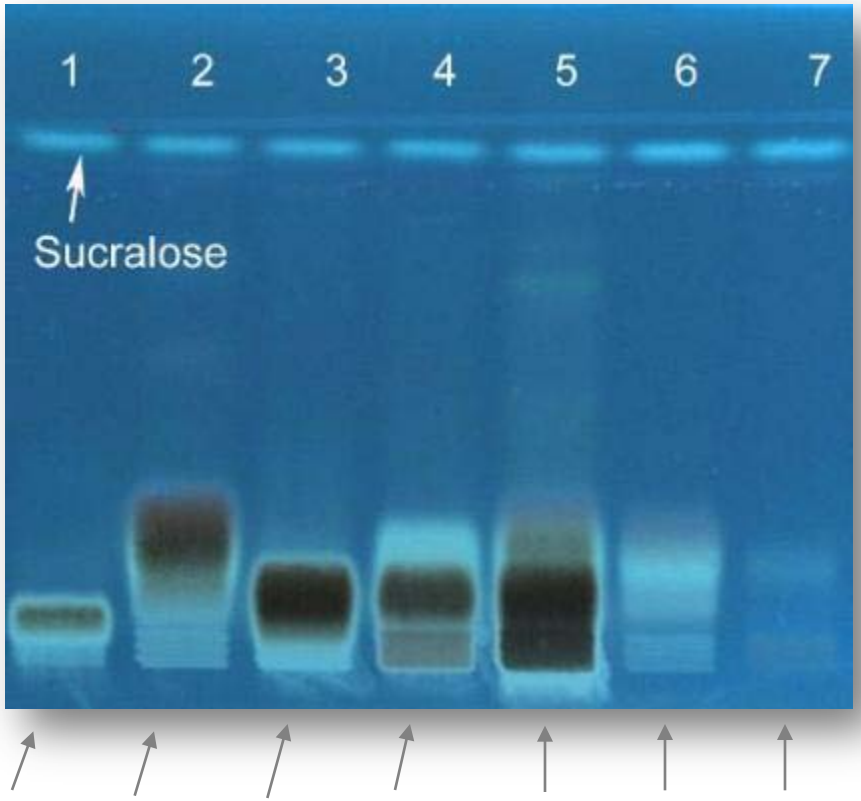
extracted in MeOH, filtered





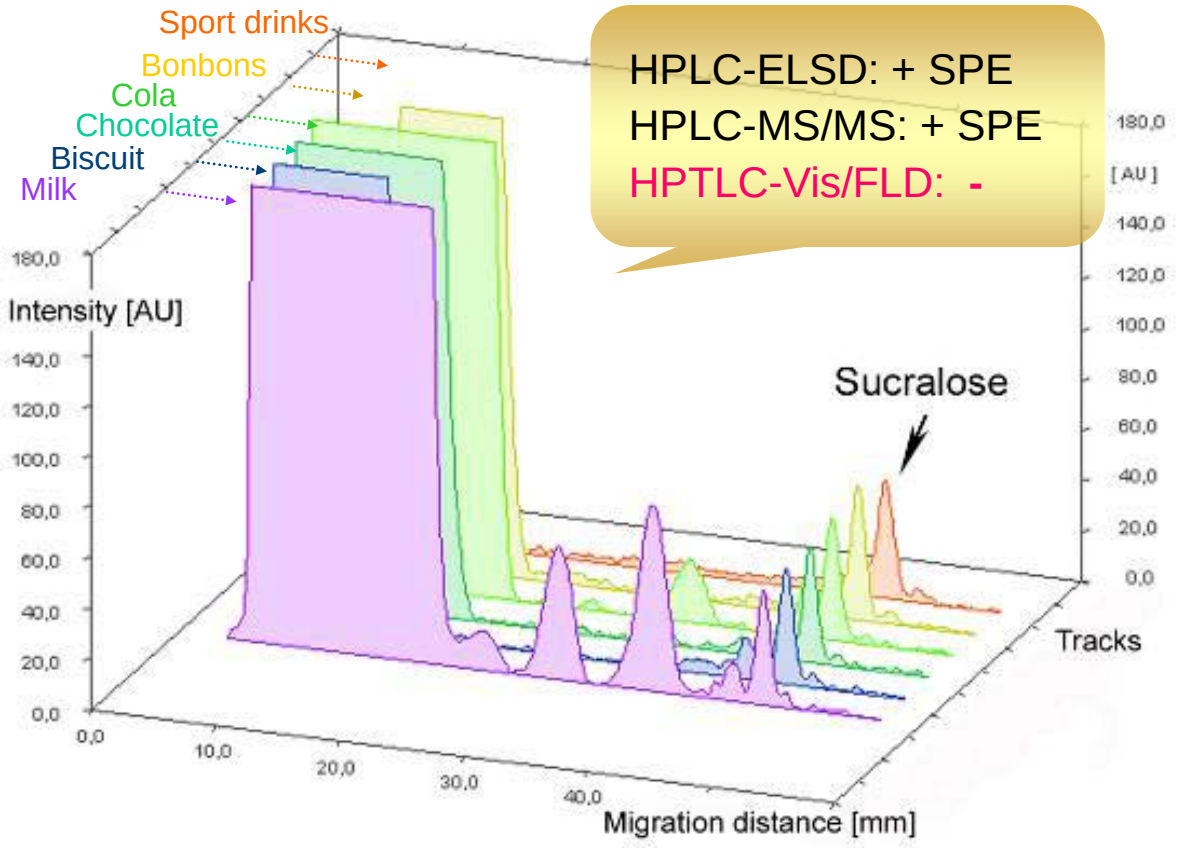
G. Morlock, S. Prabha, *J Agric Food Chem* 55 (2007) 7217-7223

… in further matrices



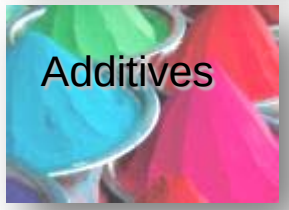
Milk, biscuit, chocolate, cola, bonbons, energy/sport drinks

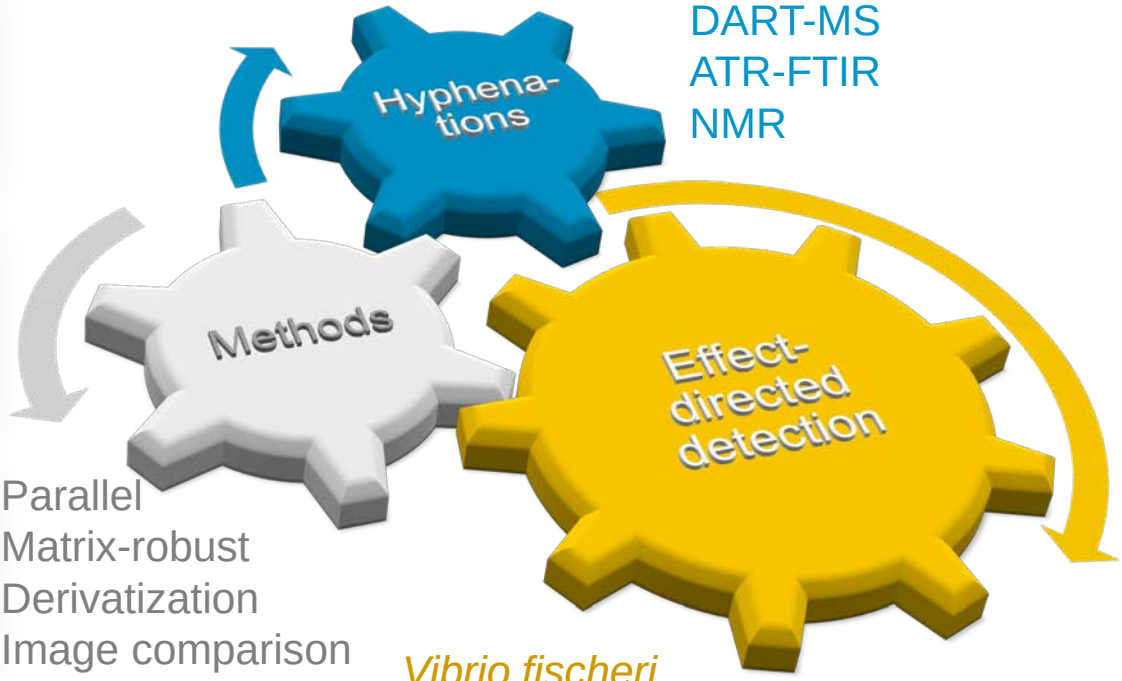
Sample preparation and chromatography



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

Modern effective platform





ESI-MS
MALDI-MS
DART-MS
ATR-FTIR
NMR

Parallel
Matrix-robust
Derivatization
Image comparison
Modular use

Vibrio fischeri
Bacillus subtilis
Planar-YES
Glucosidase inhibiting compounds
Esterase inhibiting compounds
Photosynthesis inhibiting compounds
Antioxidants or radical scavengers

Hyphenation

Problems associated with column-based hyphenations

- Capital cost and strategies for dealing with large amounts of data
- 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 hyphenations

- 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

Hyphenation

→ 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

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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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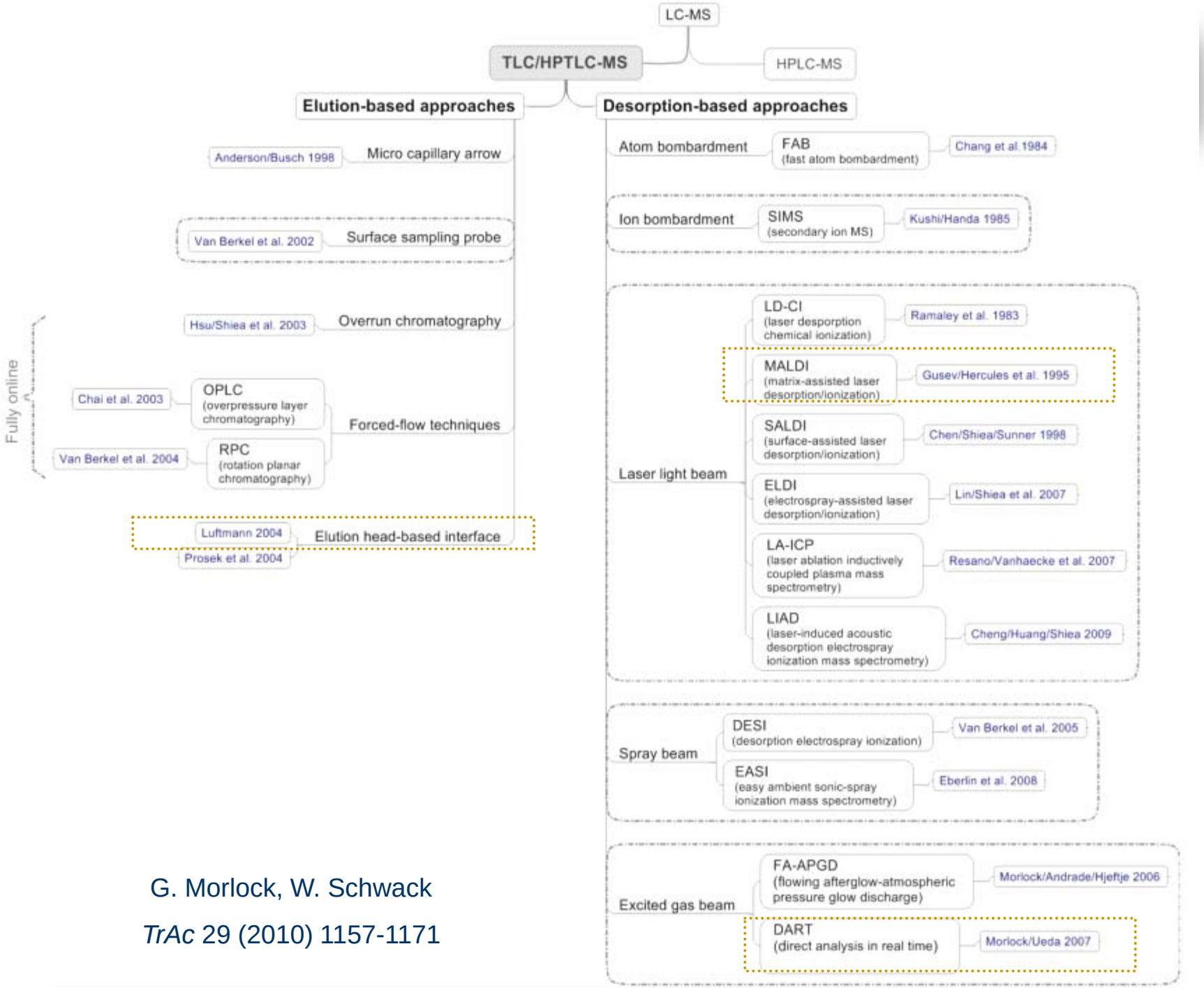
High-performance thin-layer chromatography
Effect-directed analysis
Bioassays
Cost-effective analysis
High-throughput system

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.

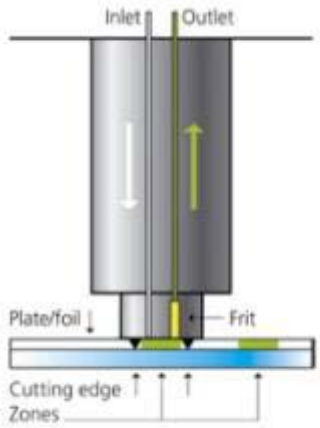


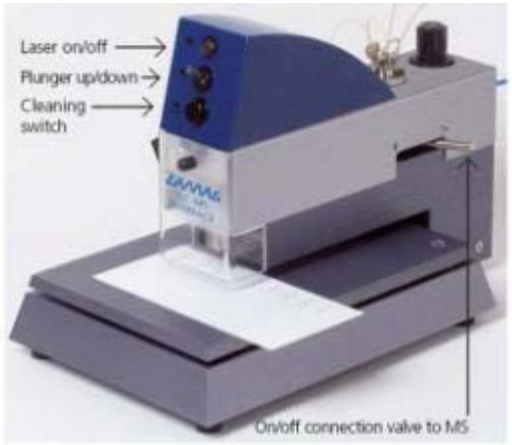
G. Morlock, W. Schwack

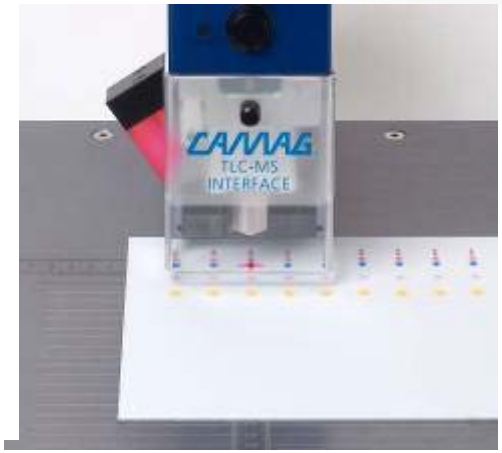
TrAc 29 (2010) 1157-1171

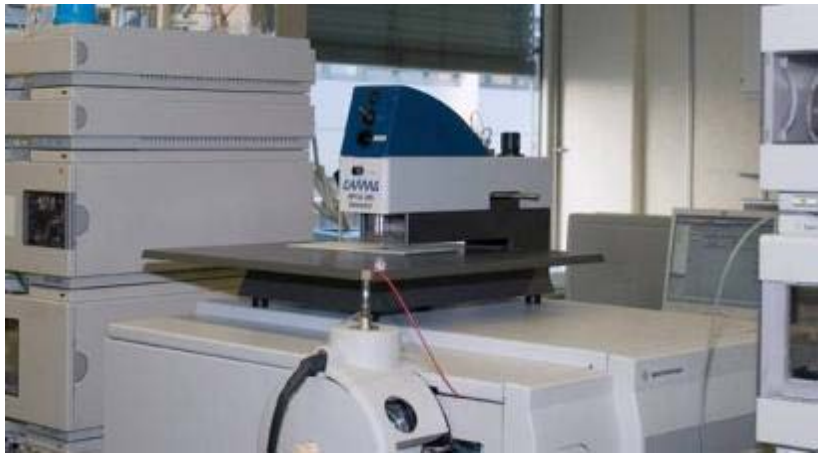
JLU

Elution head-based → TLC-MS Interface





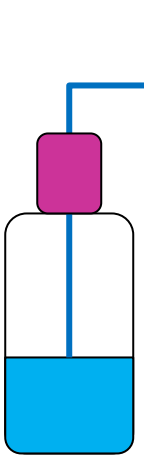




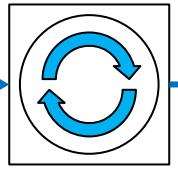
H. Luftmann, Anal Bioanal Chem 378 (2004) 964-968

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

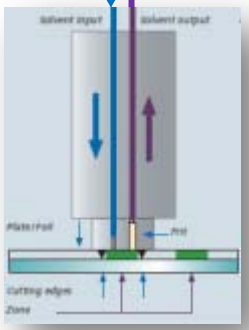
Scheme of operation



Eluent
Methanol/ammonium formate buffer
(10 mM, pH 4)
19:1 (v/v)

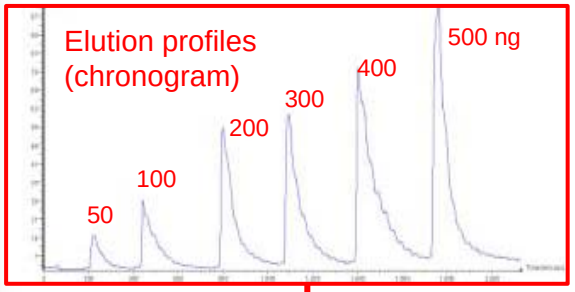


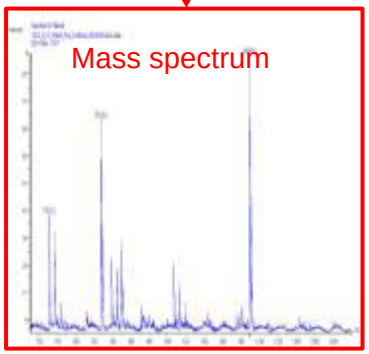
Pump
100-200 µL/min



Elution
Zone selection and elution

Mass Signals
Online analysis of eluted analytes

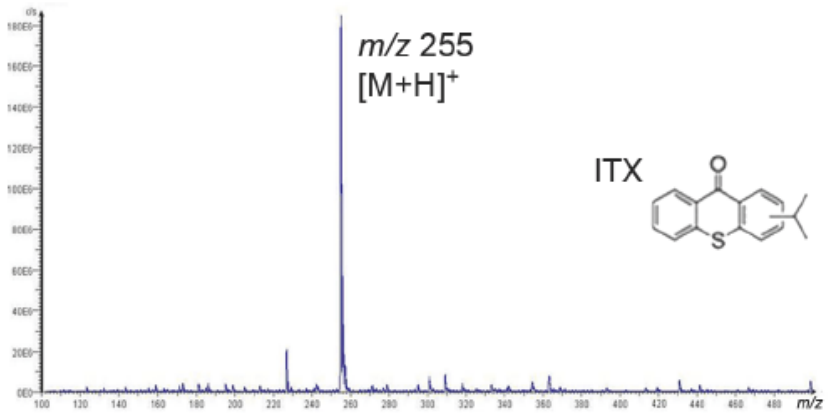


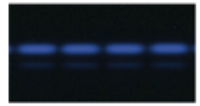


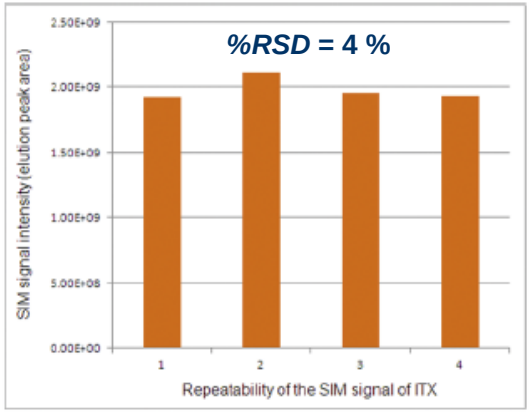
Data Evaluation
for structural confirmation or impurity control or search for sum formula

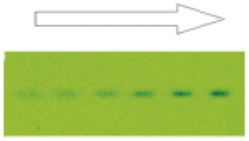
New HPTLC-MS system setup

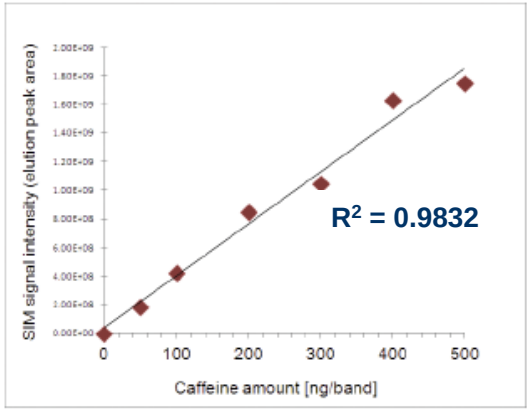
Performance data of expression CMS







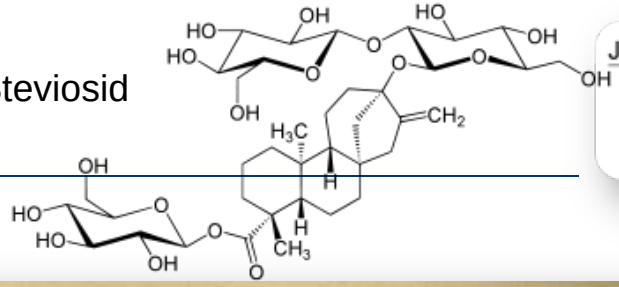




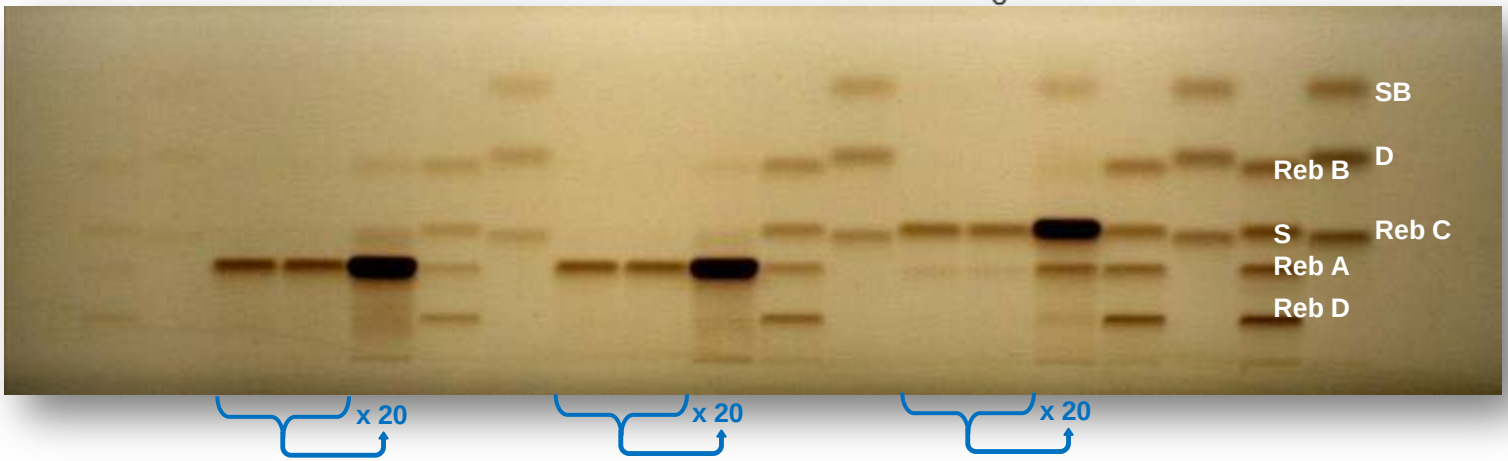
G. Morlock, F. Porbeck, A. Wiesner, I. Klingelhöfer, *CBS* 110 (2013) 9

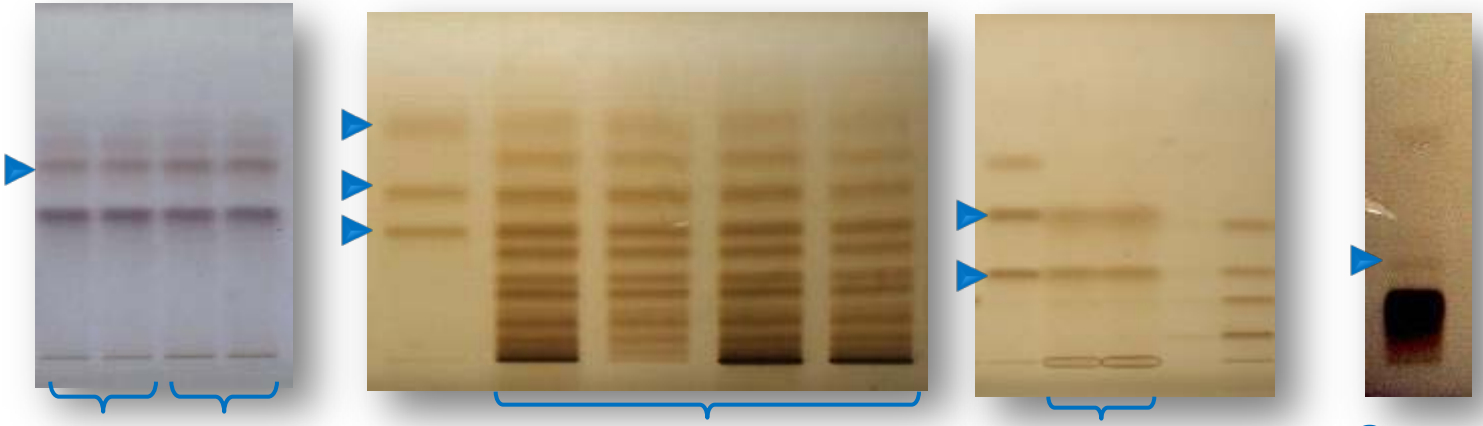
Steviol glycosides

Steviosid



JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science





Citron/strawberry joghurt

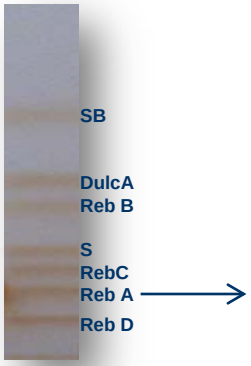
Stevia fluids

Tea

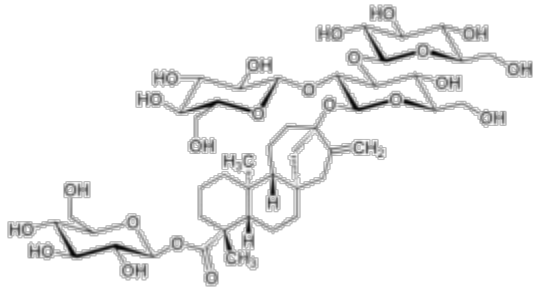
Sweety

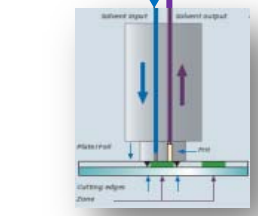
G. Morlock, S. Meyer, in preparation

ESI+ MS full scan

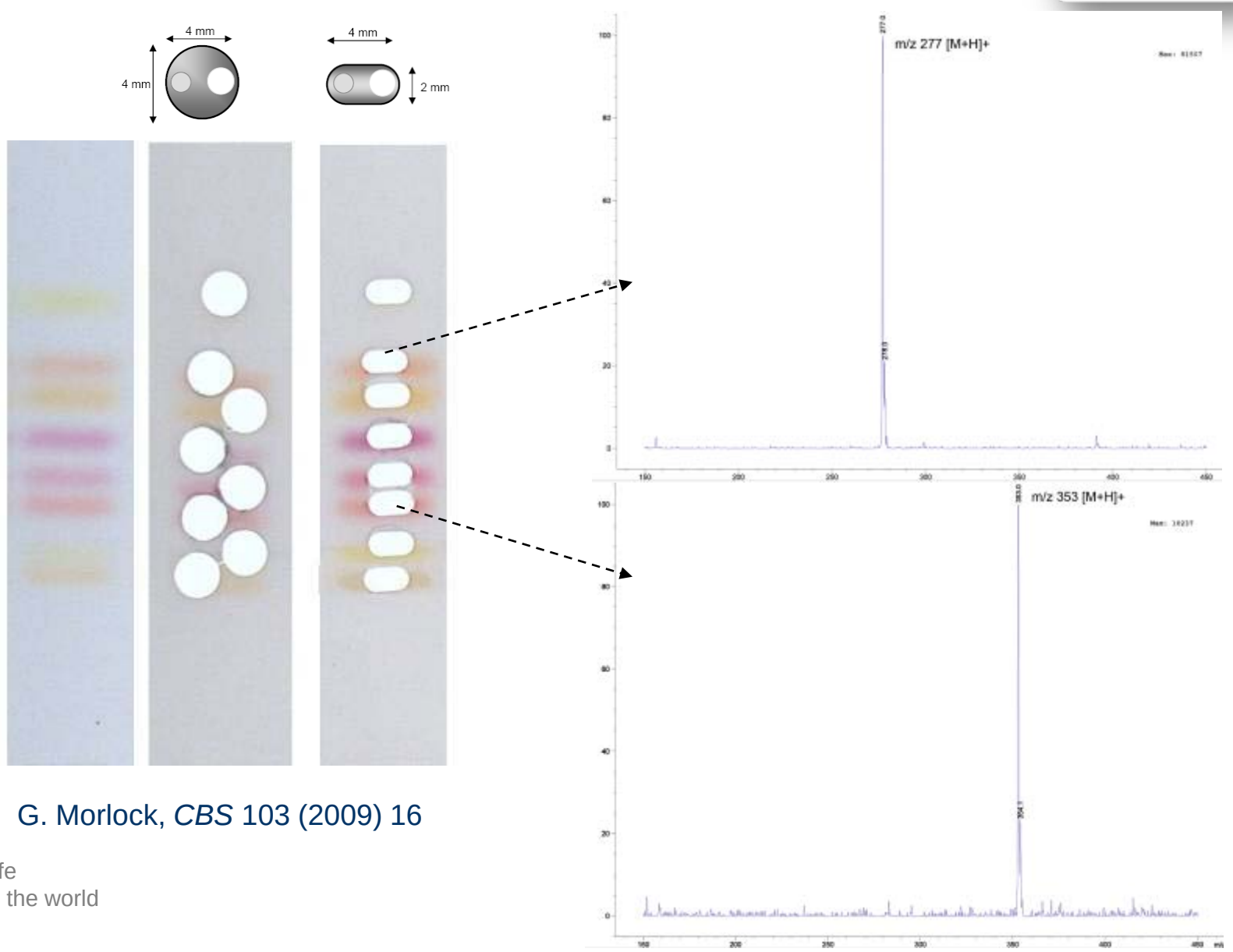


Rebaudioside A
MW 967



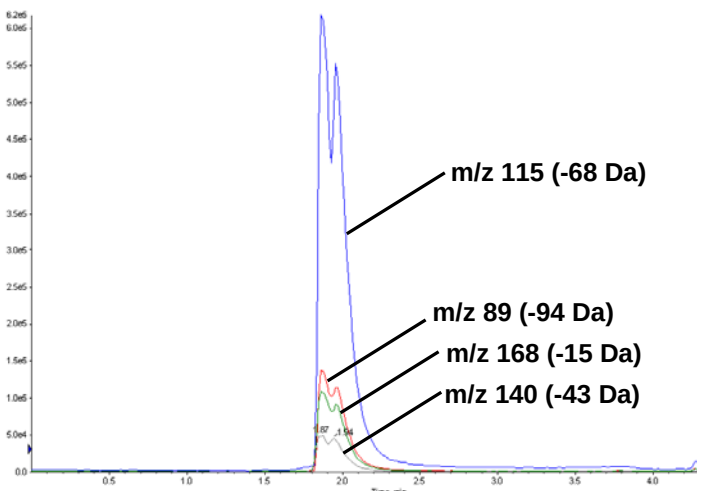


Elution head-based HPTLC-MS

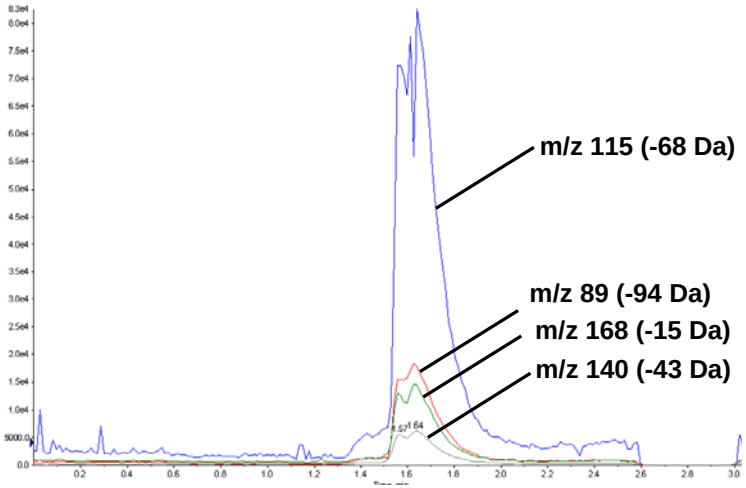


G. Morlock, *CBS* 103 (2009) 16

Detectability by HPTLC-ESI-MS/MS



200 pg/zone harman

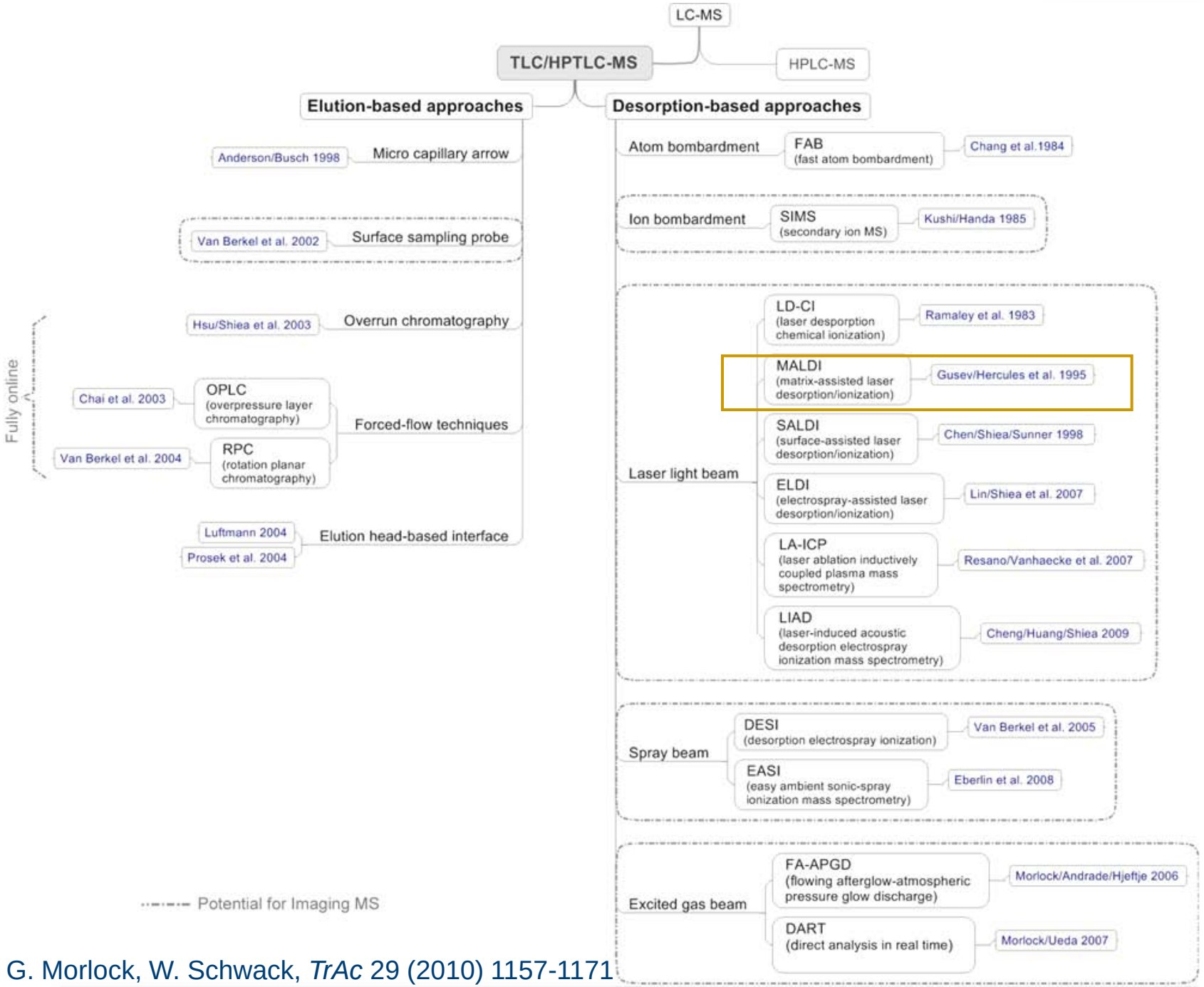


20 pg/zone harman

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

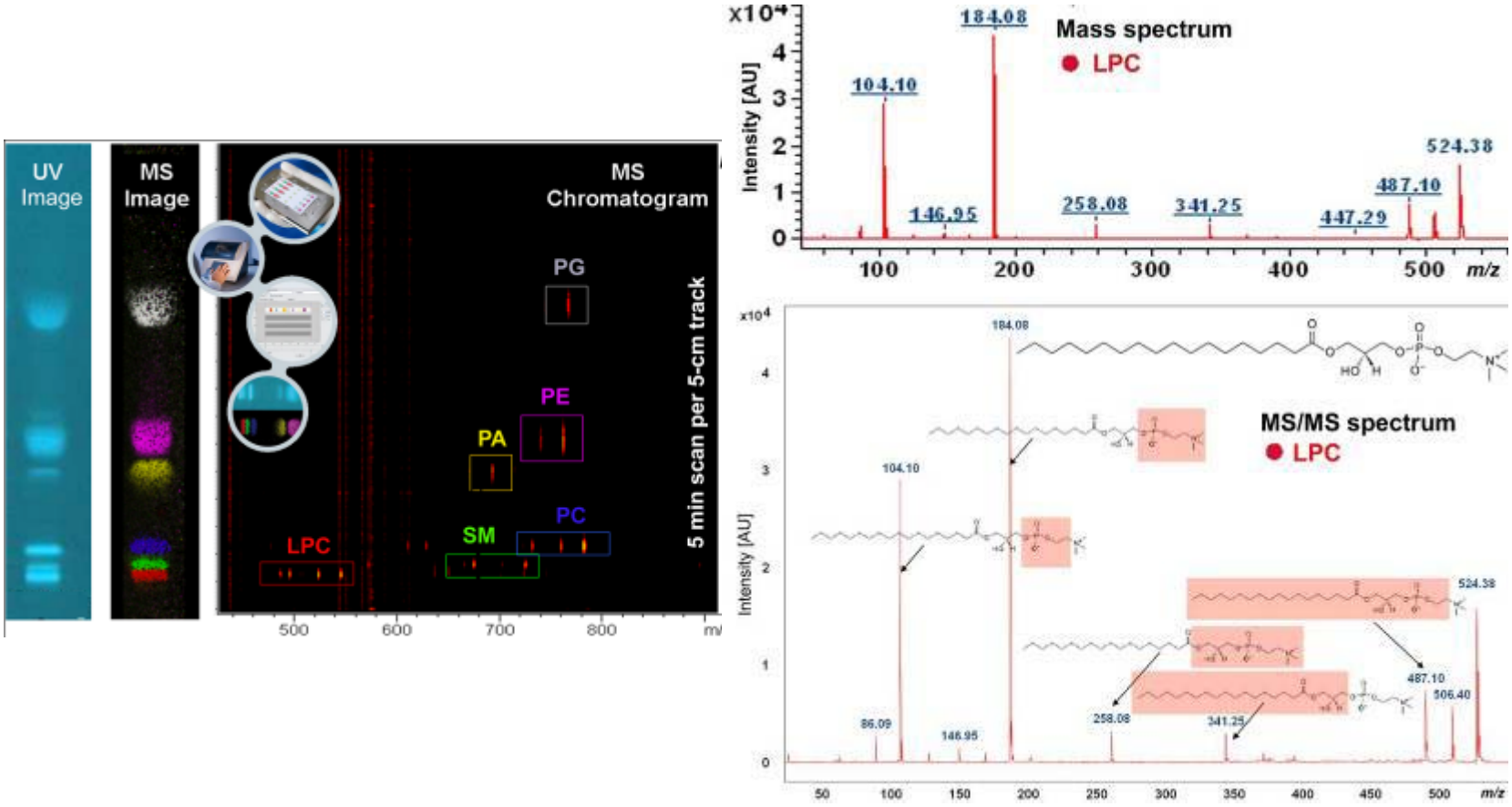
→ Detectability comparable to HPLC/MS

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



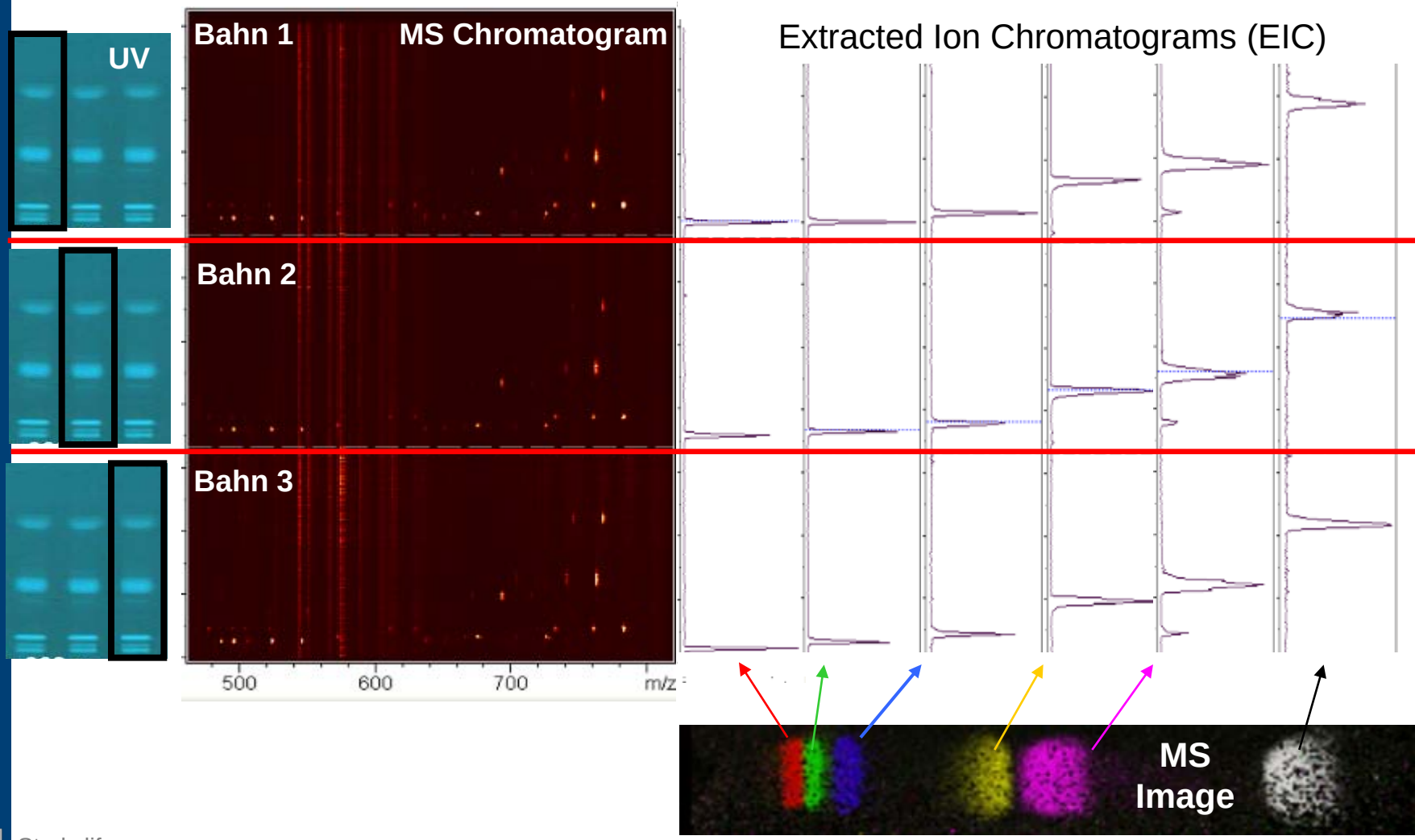
G. Morlock, W. Schwack, *TrAc* 29 (2010) 1157-1171

HPTLC-FLD-MALDI-TOF MS

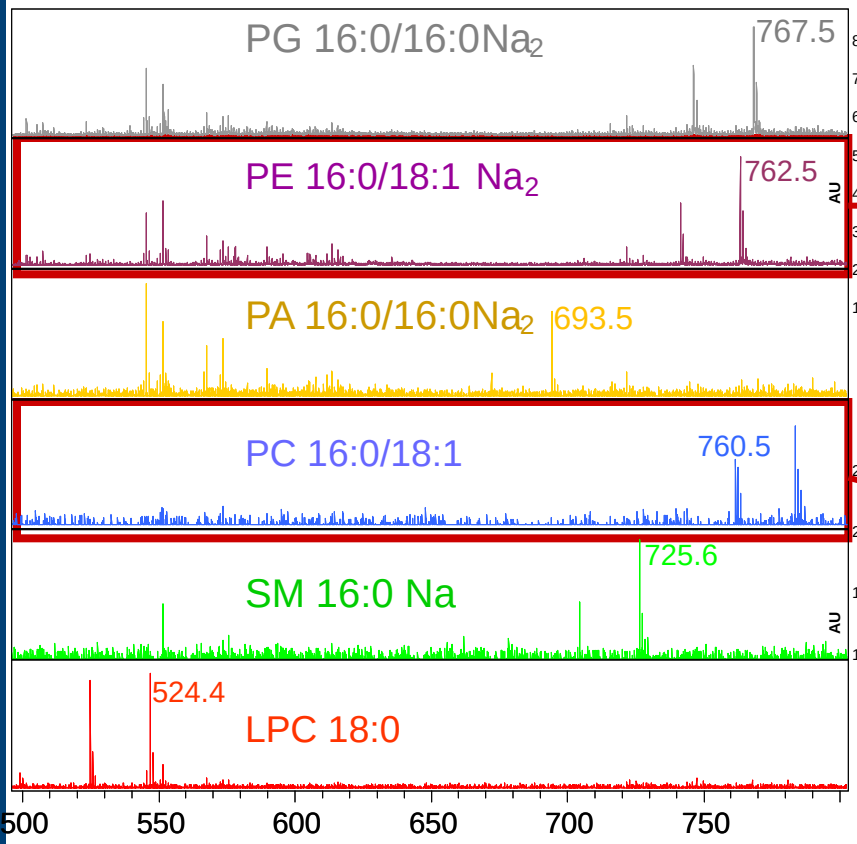


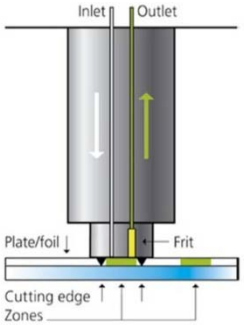
Bruker Daltonics, Application Note MT-101

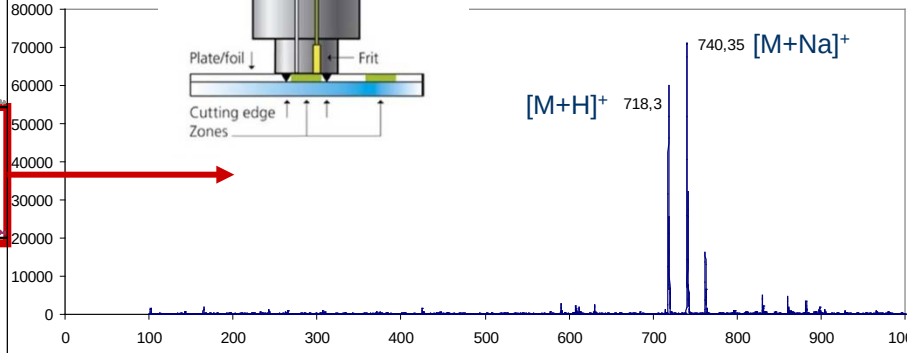
Quantitative?

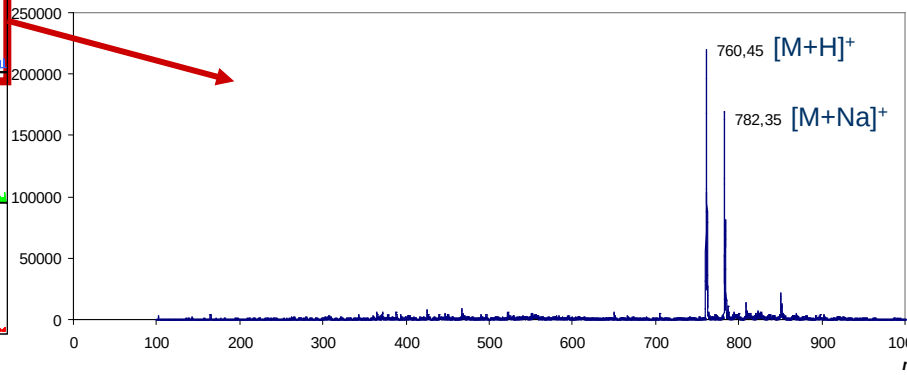


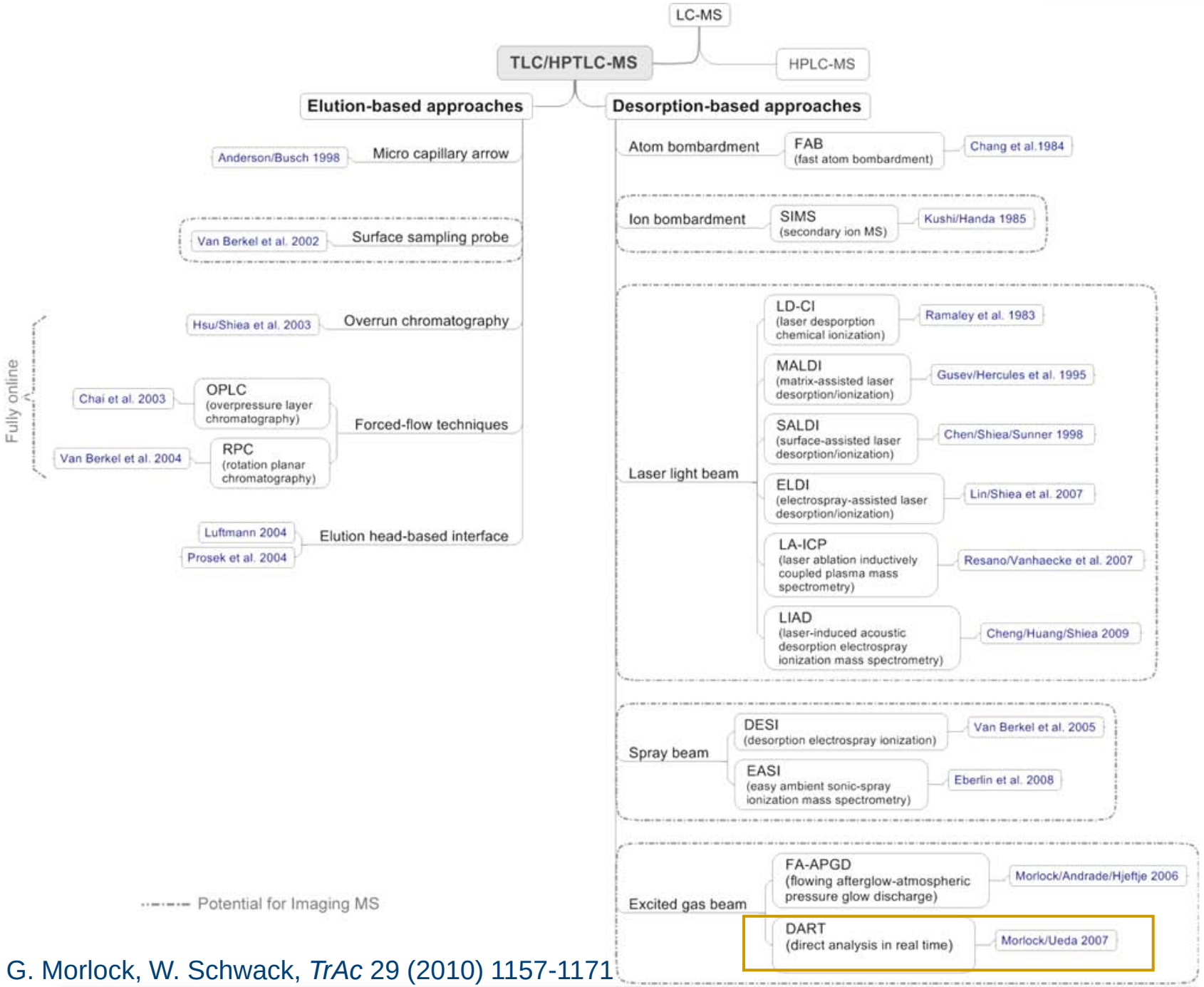
Comparison of mass spectra







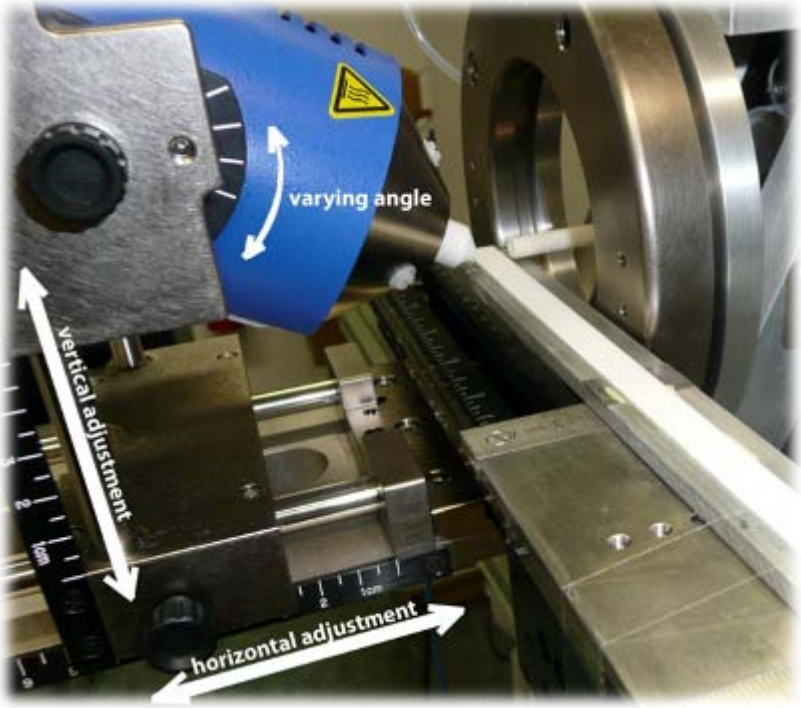


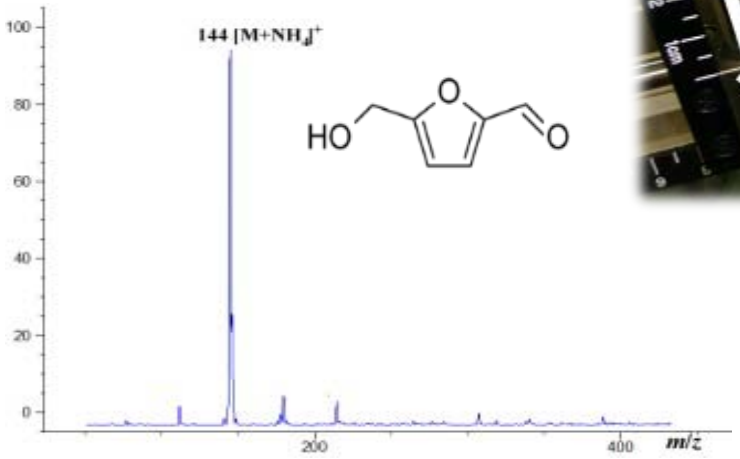


G. Morlock, W. Schwack, *TrAc* 29 (2010) 1157-1171

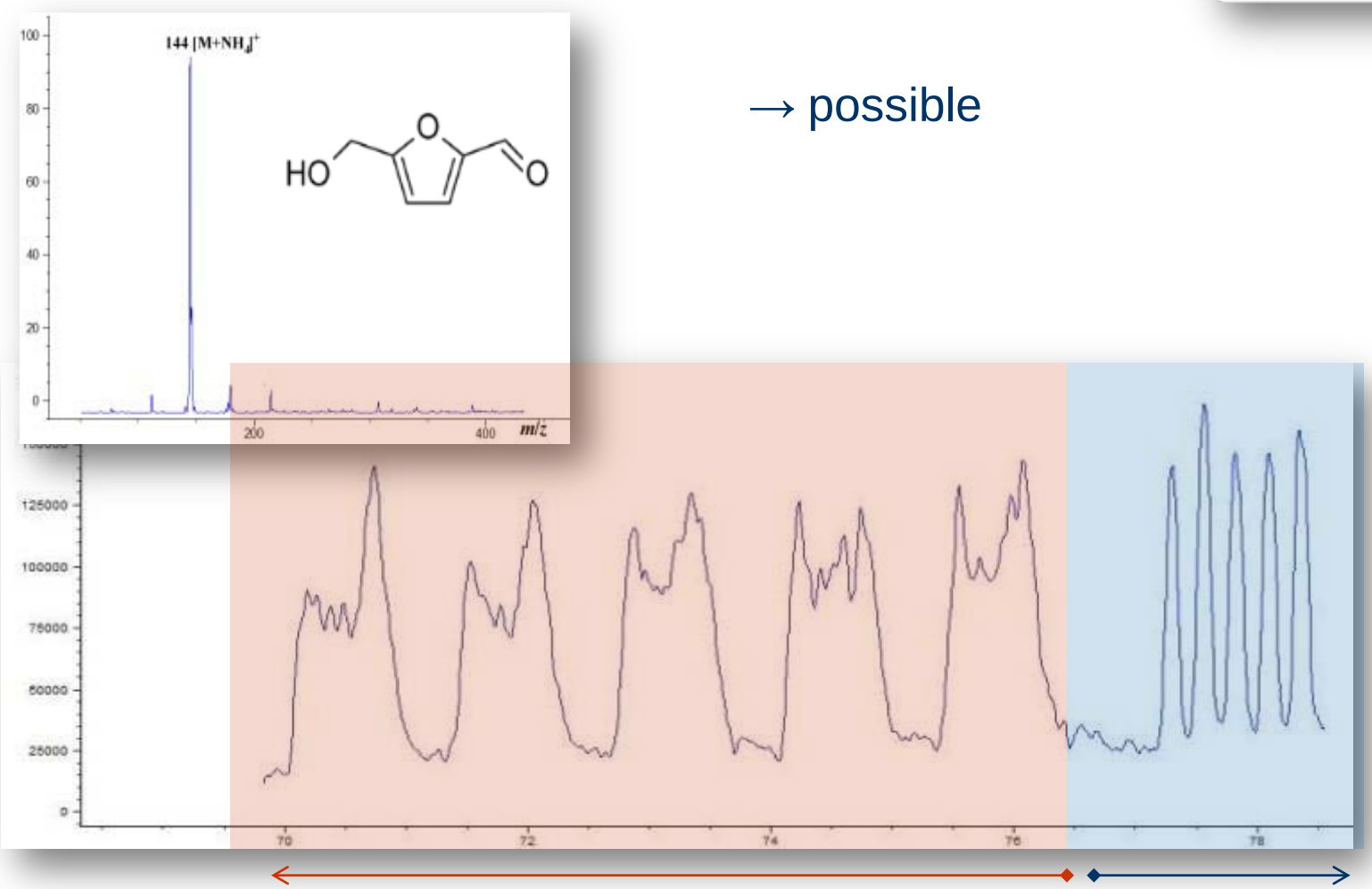
HPTLC-DART-SVPA-MS

2006 ↔ 2011



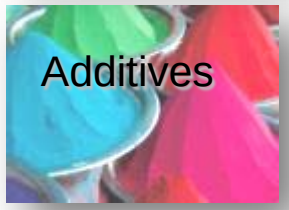


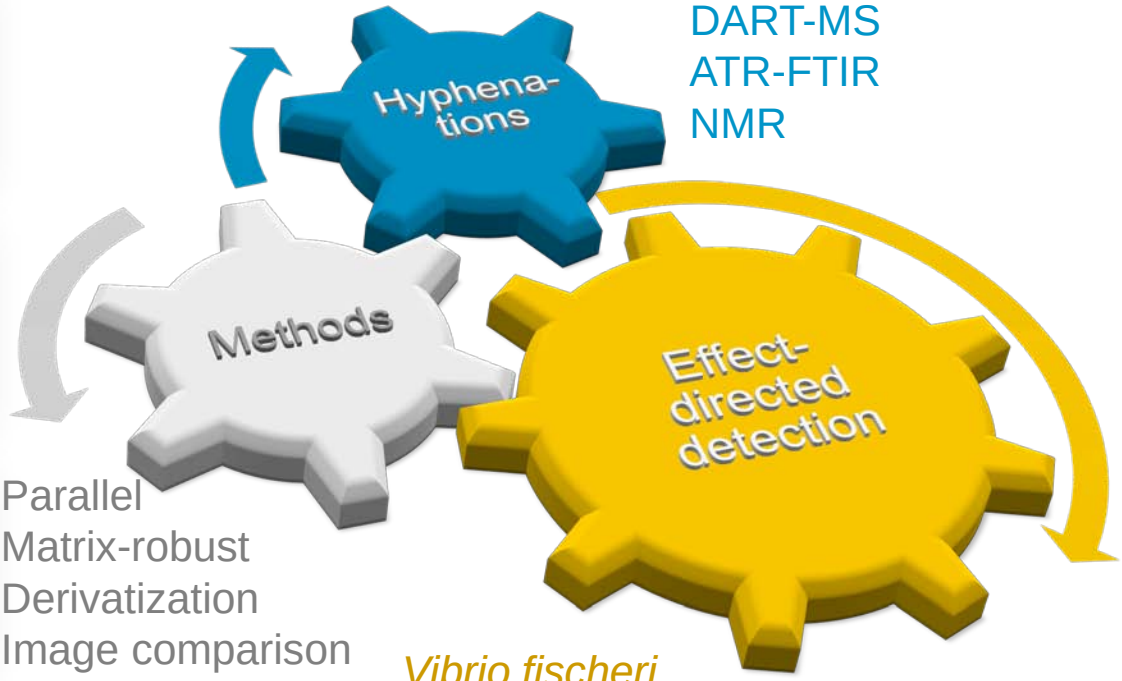
Repeated horizontal scanning?



→ possible

Modern effective platform





ESI-MS
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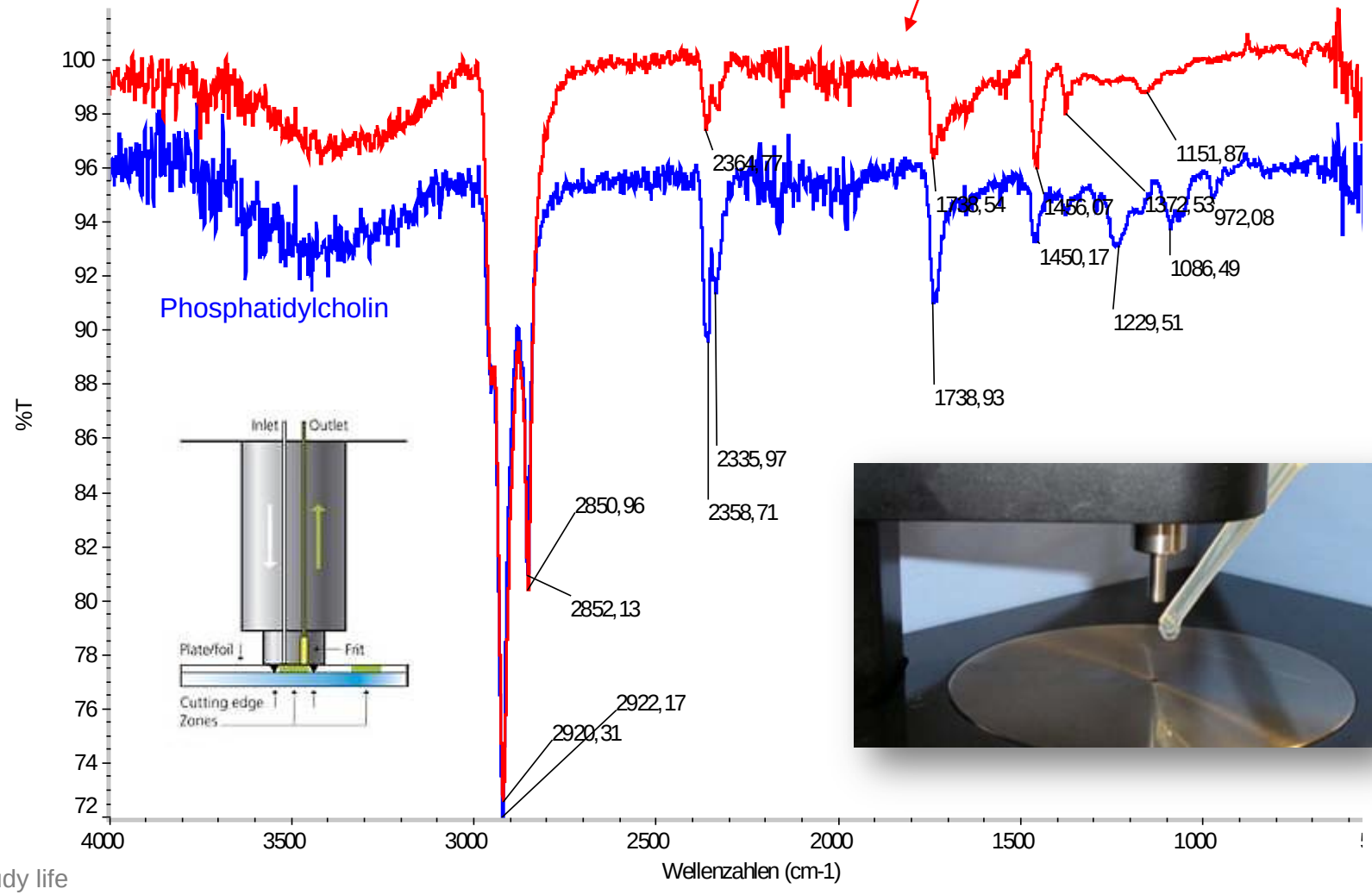
Modular use

Vibrio fischeri
Bacillus subtilis
Planar-YES
Glucosidase inhibiting compounds
Esterase inhibiting compounds
Photosynthesis inhibiting compounds
Antioxidants or radical scavengers

HPTLC-ATR FTIR of anti-inflammatoric compound

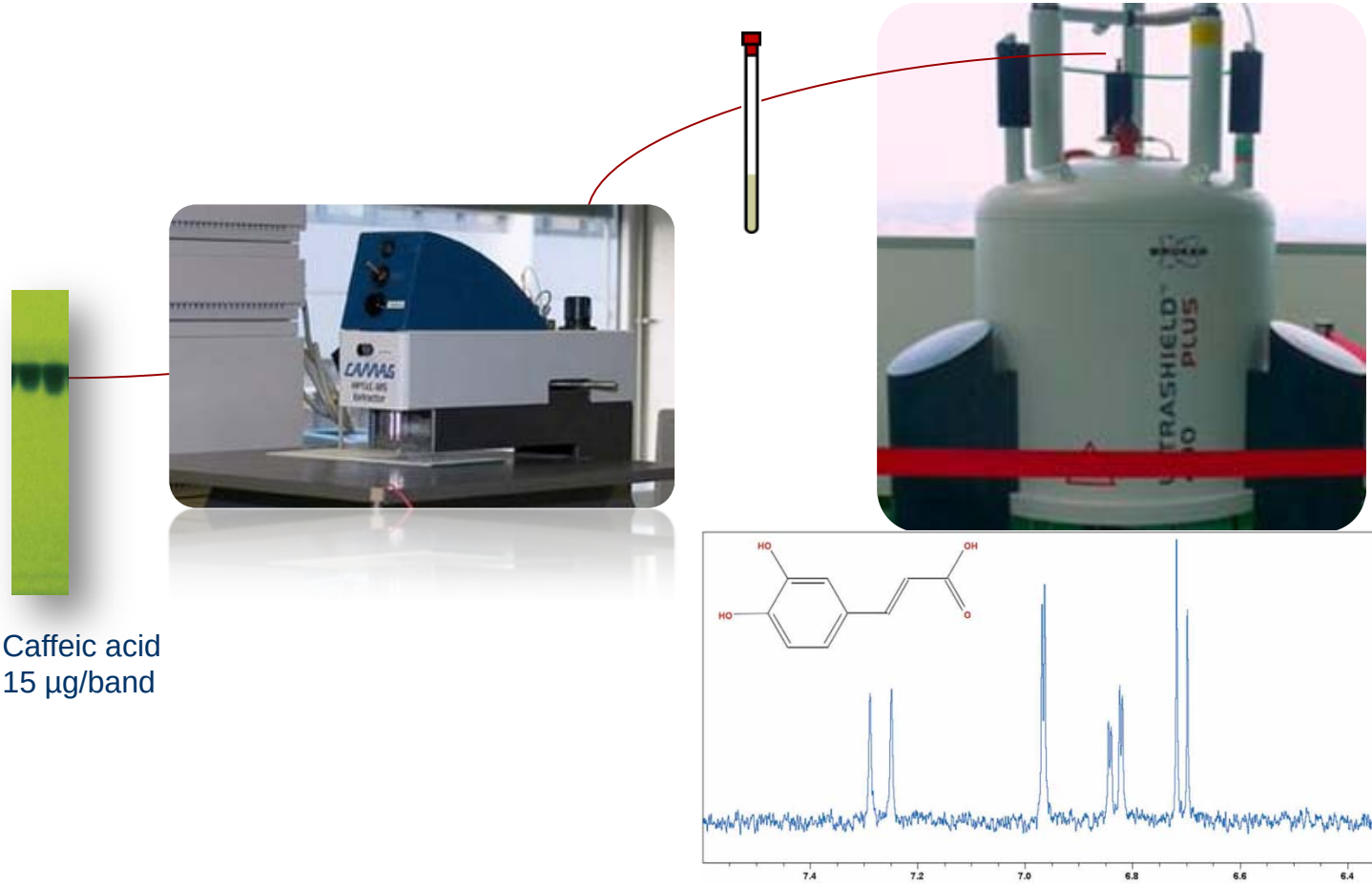
Isolated from *Lactobacillus fermentum*



G. Morlock *et al.*, in preparation

HPTLC-NMR

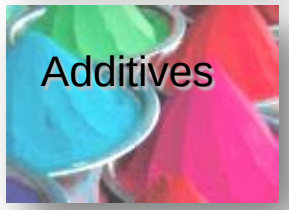
→ hyphenation of HPTLC with ^{1}H-NMR via TLC-MS Interface

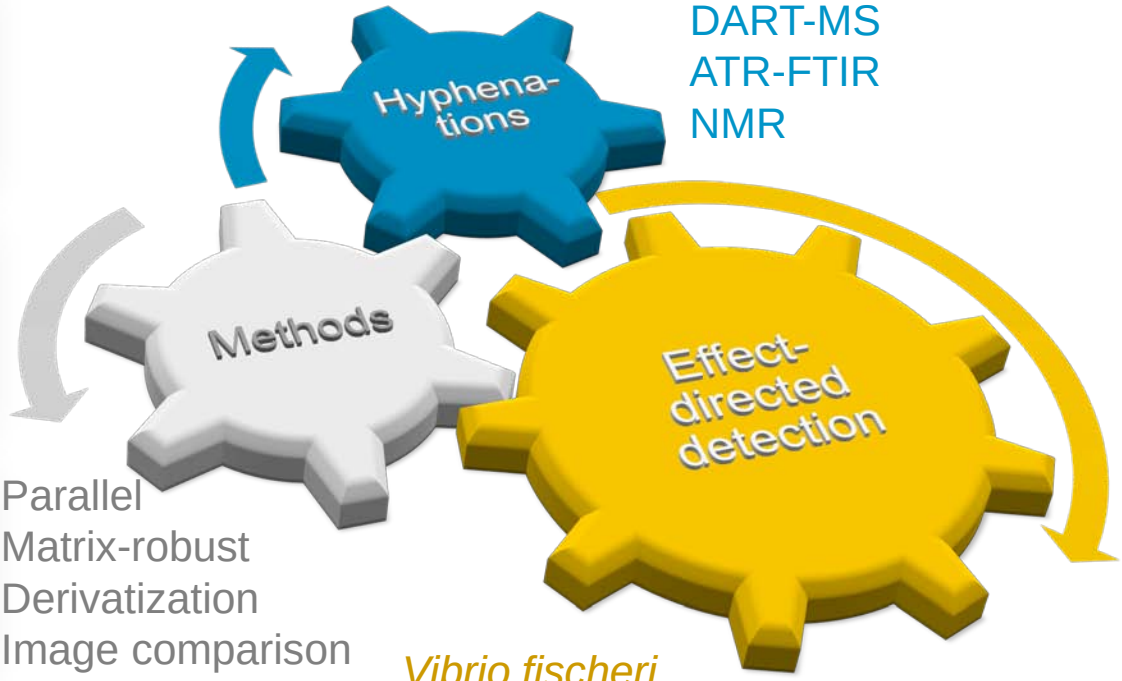


Caffeic acid
15 µg/band

A. Gössi, U. Scherer, G. Schlotterbeck, *Chimia* 66 (2012) 347

Modern effective platform



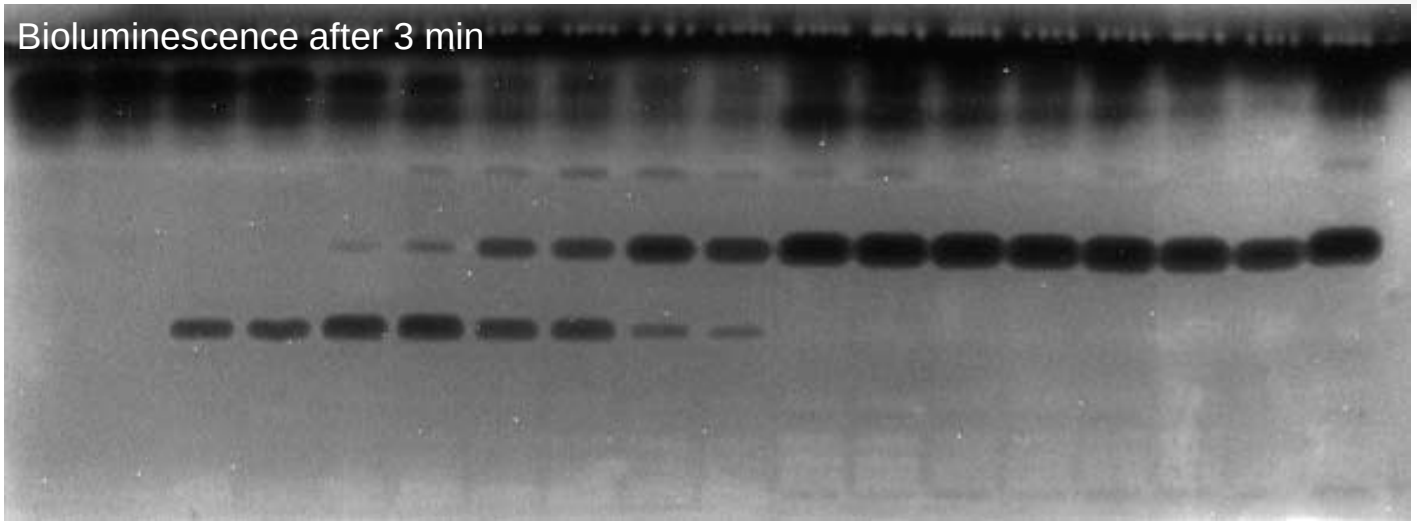


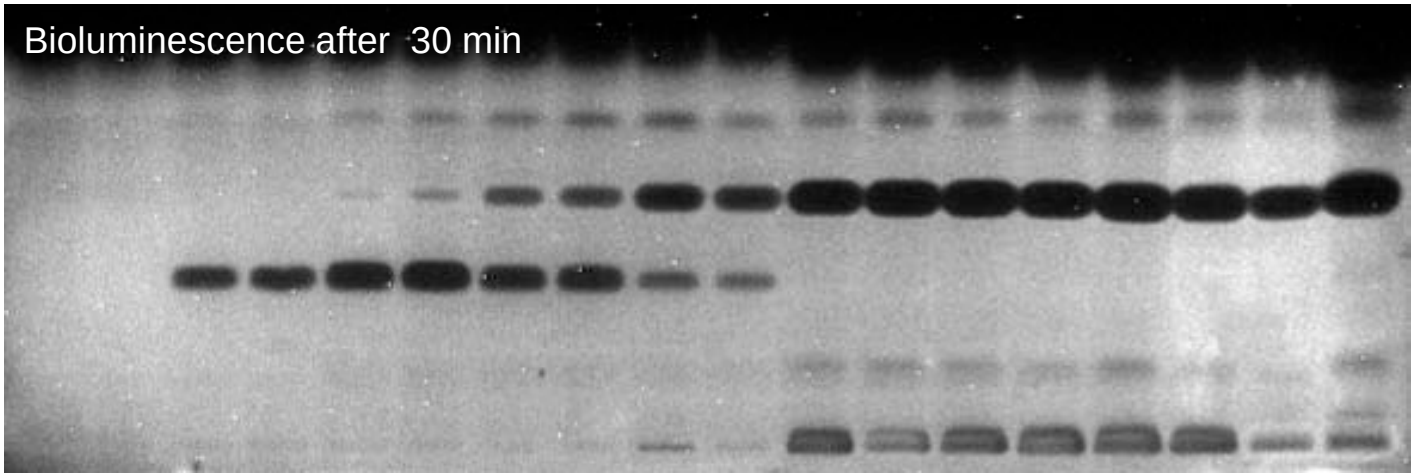
ESI-MS
MALDI-MS
DART-MS
ATR-FTIR
NMR

Parallel
Matrix-robust
Derivatization
Image comparison
Modular use

Vibrio fischeri
Bacillus subtilis
Planar-YES
Glucosidase inhibiting compounds
Esterase inhibiting compounds
Photosynthesis inhibiting compounds
Antioxidants or radical scavengers

Bioactive compounds in *Basidomycetes*





T. Shen, H. Zorn, G. Morlock, in preparation

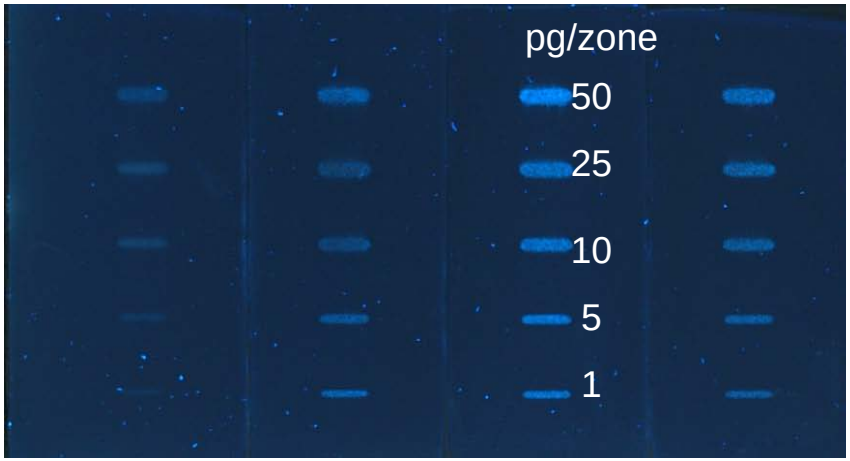
Detection of hormones

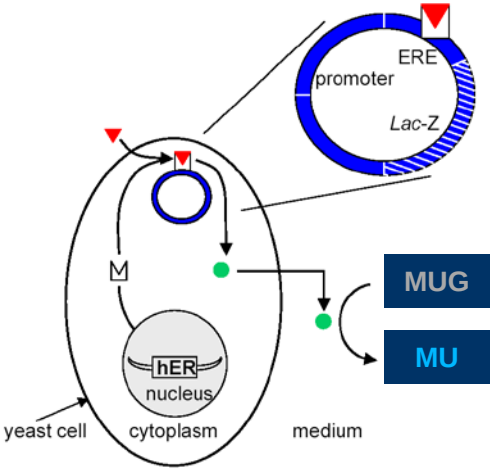
Planar yeast estrogen screen (p-YES)

→ detectability down to 1 pg/zone

→ using the human estrogen receptor expressed in *Saccharomyces cerevisiae* yeast cells

→ blue fluorescent zones (4-Methylumbelliferon)





I. Klingelhöfer, G. Morlock, in preparation

E. J. Routledge, J. P. Sumpter, Environ. Toxicol. Chem. 15 (1996) 241

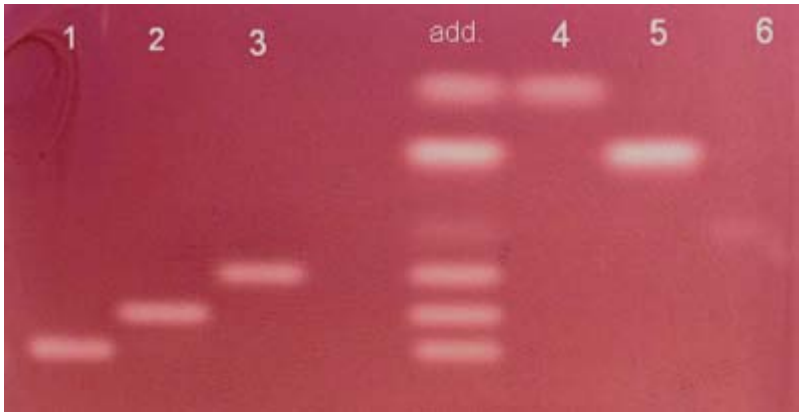
Detection of esterase inhibitors

Cholinesterase inhibiting pesticides by esterases

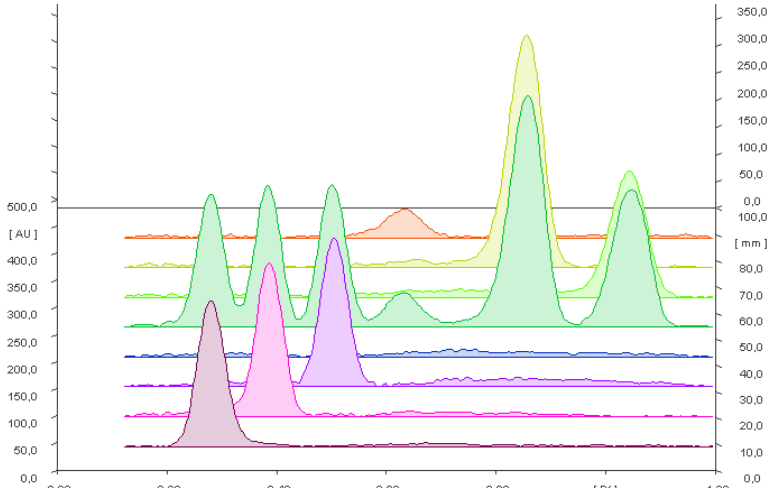
→ detectability down to 2 pg/zone

→ 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

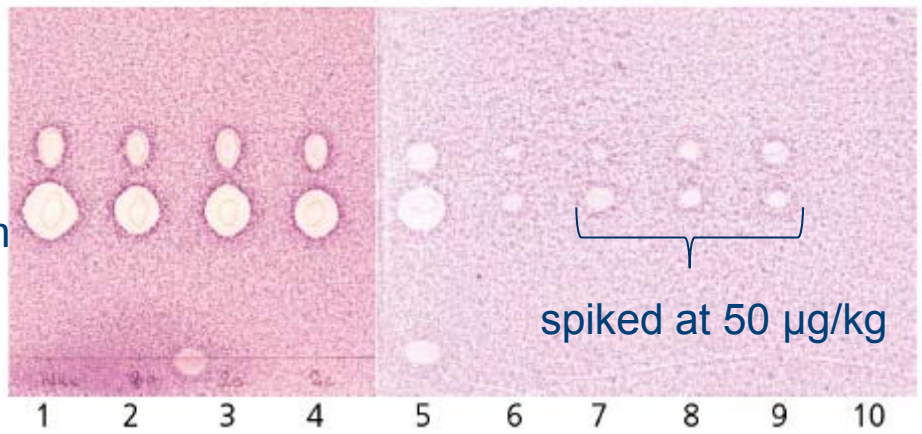
Detection of antibiotics with *Bacillus subtilis*

Antibiotics in milk extracts

→ dipping in *Bacillus subtilis* bacteria suspension and incubation
→ dipping in tetrazolium salt as substrate
→ white zones on a pink background

Enrofloxacin

Ciprofloxacin



I. Choma *et al.*, *CAMAG Bibliogr Service CBS* 106 (2011) 1-4

G. Morlock Food Science JLU Gießen

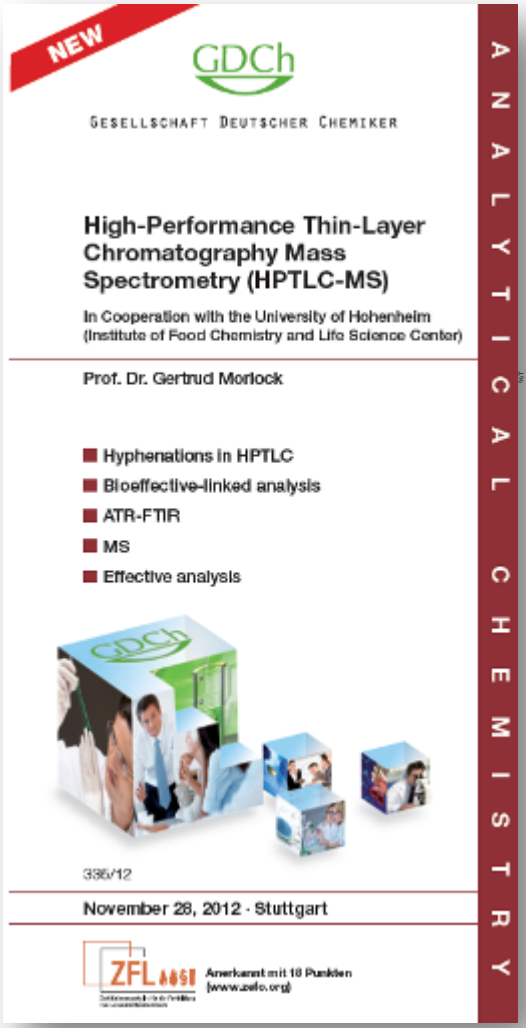
Effect-directed analysis by HPTLC-Bioactivity-HRMS

- ✓ Matrix-robust
  → combination of different methods (SPE, GPC, prep. HPLC) for fractionation, isolation and purification of substances, always followed by bioactivity testing, can be skipped
- ✓ Parallel
  → 30 extracts separated in parallel under identical chromatographic and environmental conditions
- ✓ Effect-directed detection
  → bioassays not interfered by solvents
- ✓ Modular
  → targeted coupling with HRMS → very cost-effective
- ✓ Image/derivatizations
  → additional helpful information

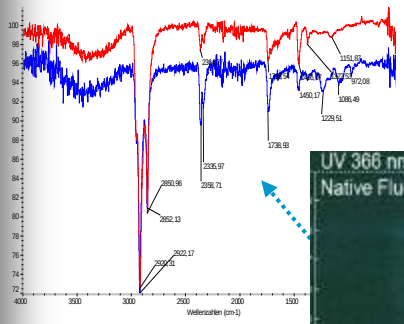
Photo: A. Jaklin, Rovinj

New GDCh course 335/13

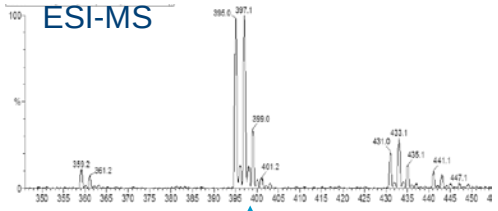
→ 13th November 2013 in Giessen

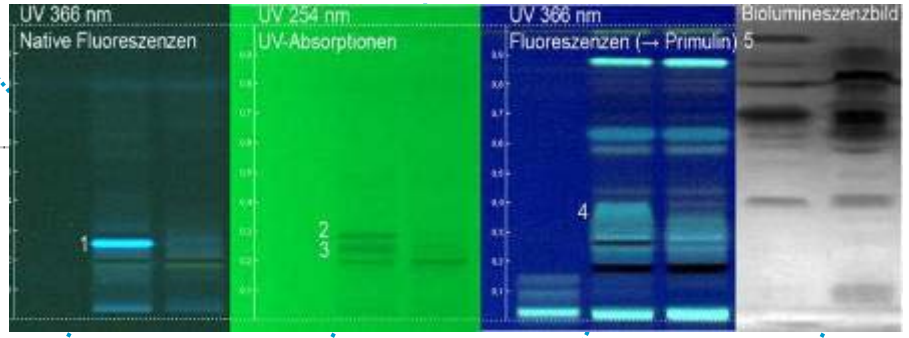


ATR-FTIR

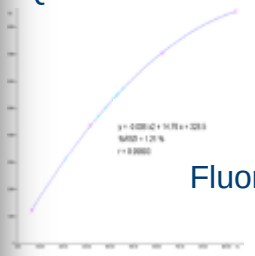


ESI-MS



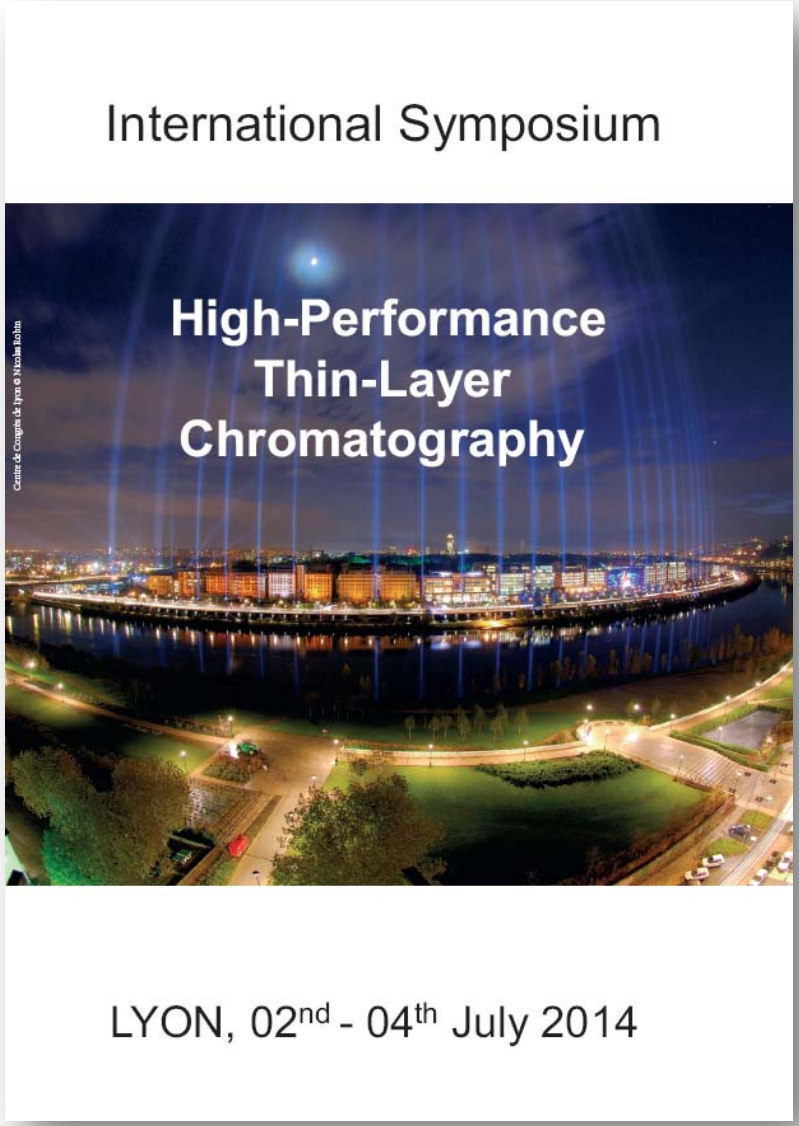


Quantitation



Fluorescence | UV/Vis absorption | Selective derivatization | Bioassay

www.hptlc.com



Merck Millipore

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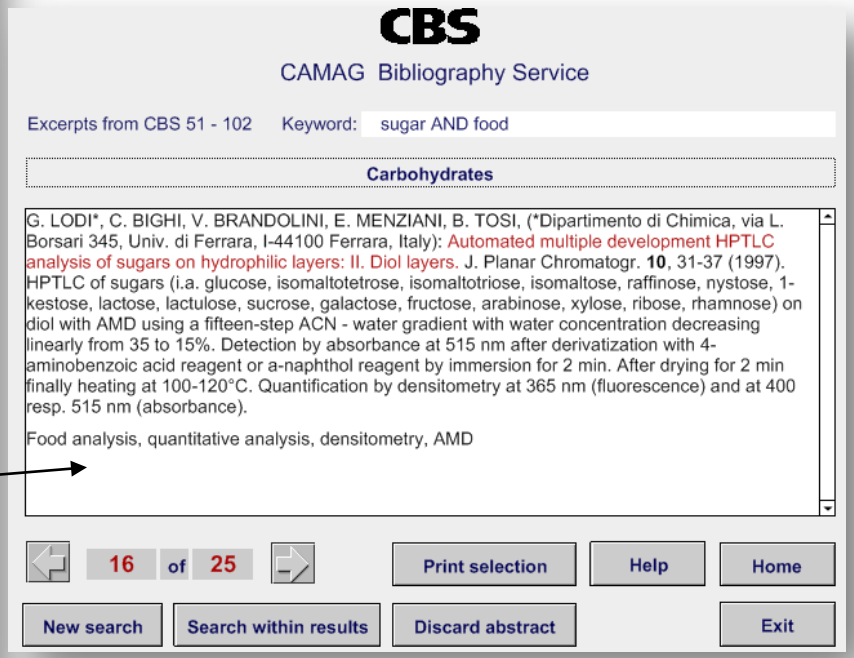
IonSense/KR Analytical

Food Science

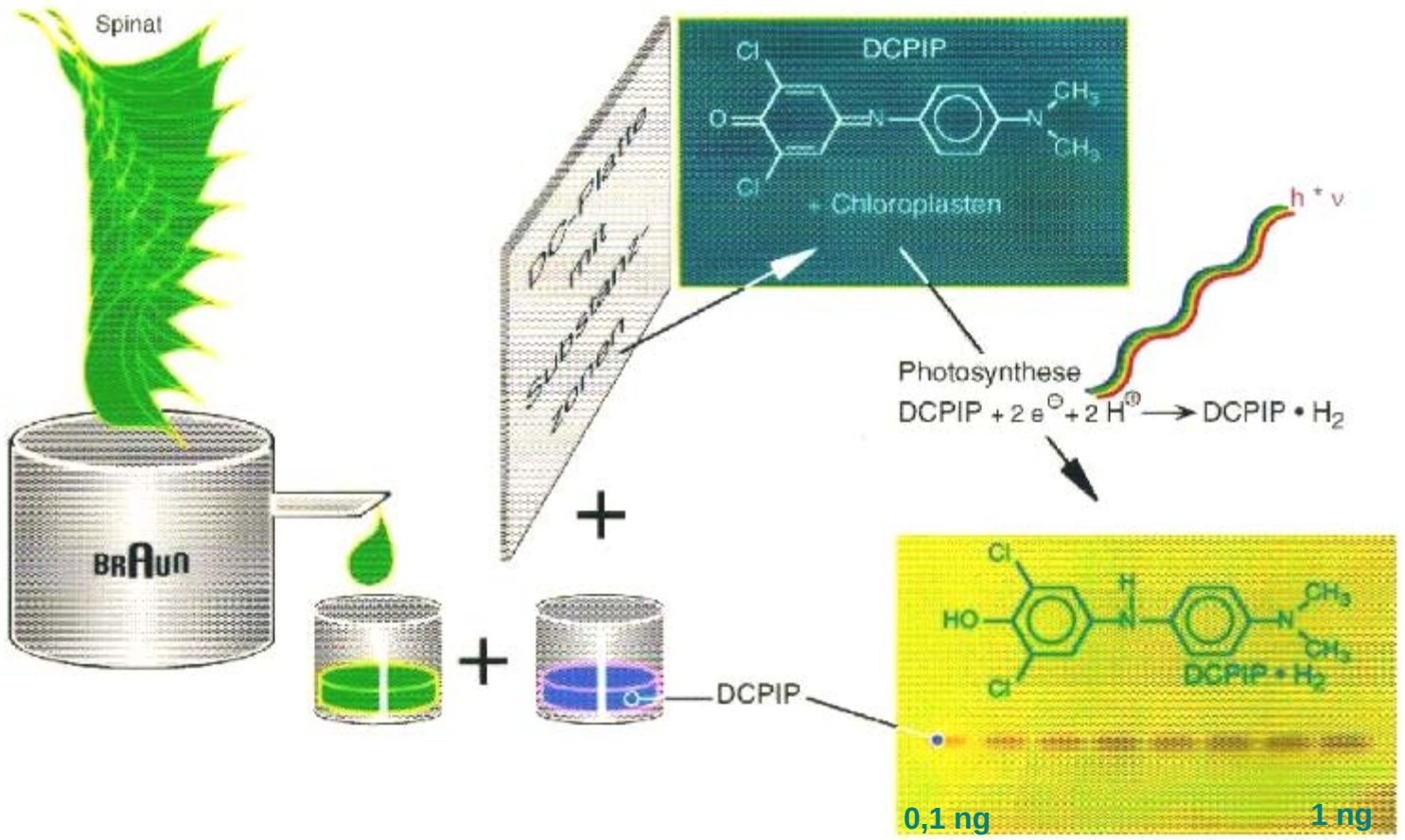
Database support for analysts



…over 10.000 abstracts

Detection with chloroplasts (spinach)

→ Photosynthesis inhibiting herbicides (→ 100 pg/zone)



K. Burger, Bayer AG, Dormagen