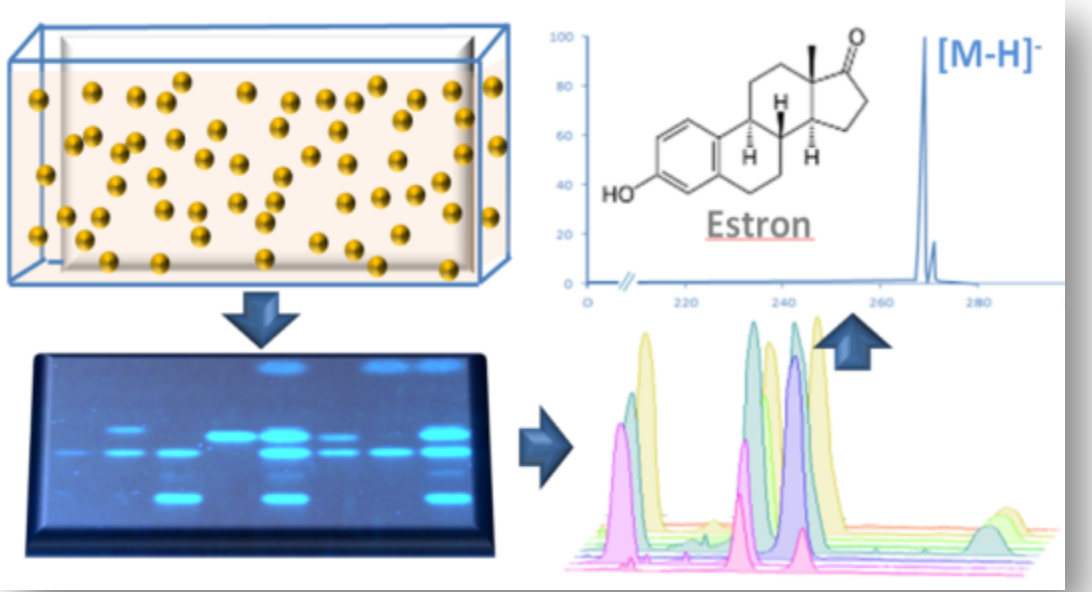
G. Morlock Food Science JLU Gießen

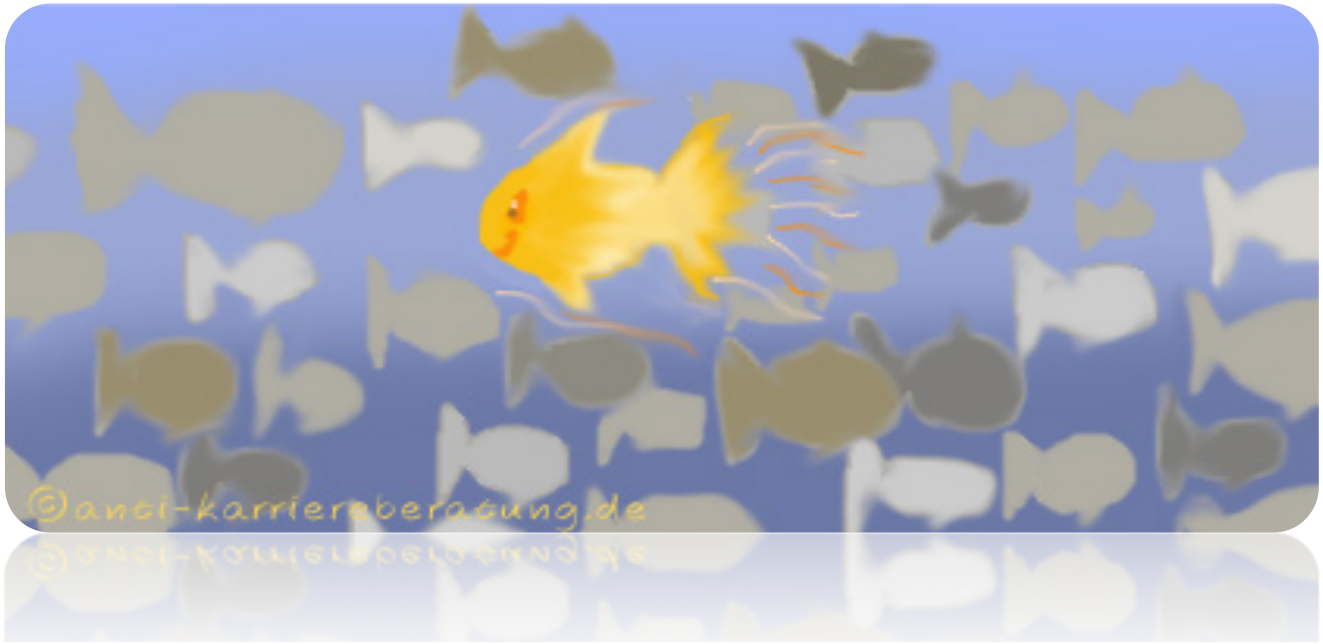
HPTLC-bioassays for effect-directed analysis



Gertrud Morlock
Chair of Food Science
Justus Liebig University Giessen

JLU

Research in HPTLC is…

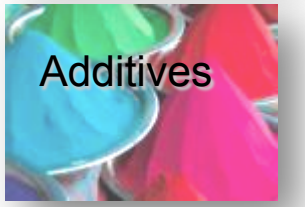


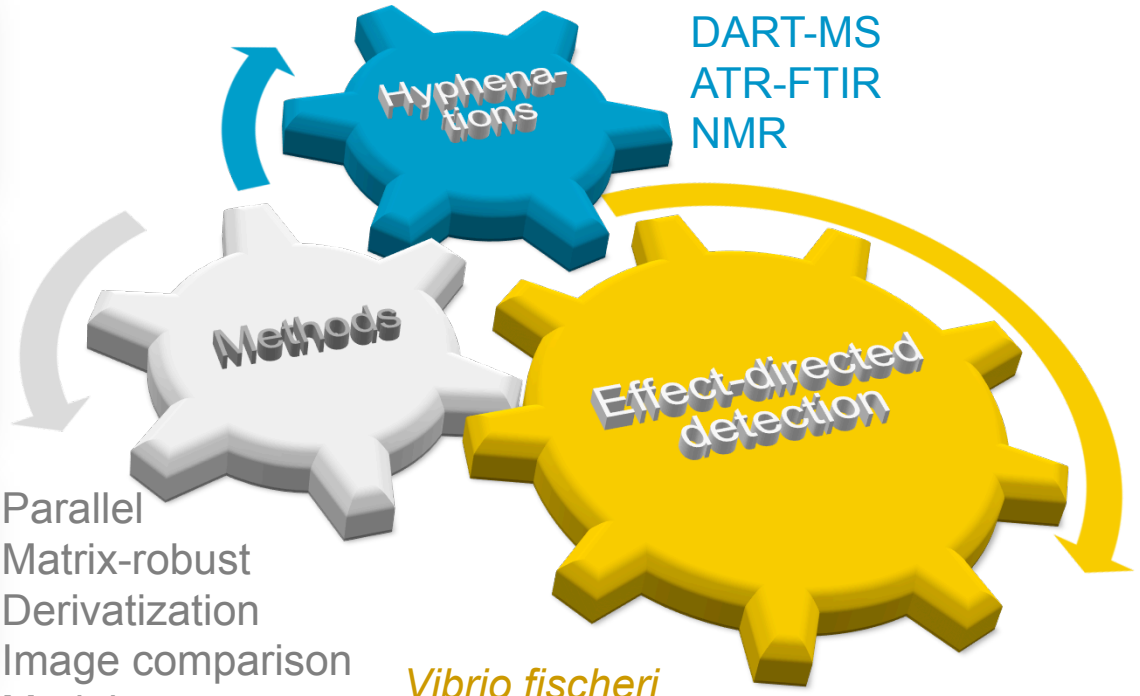
To reach the water source, you have to swim **against mainstream**.

Konfuzius

G. Morlock Food Science JLU Gießen

… we have something unique!



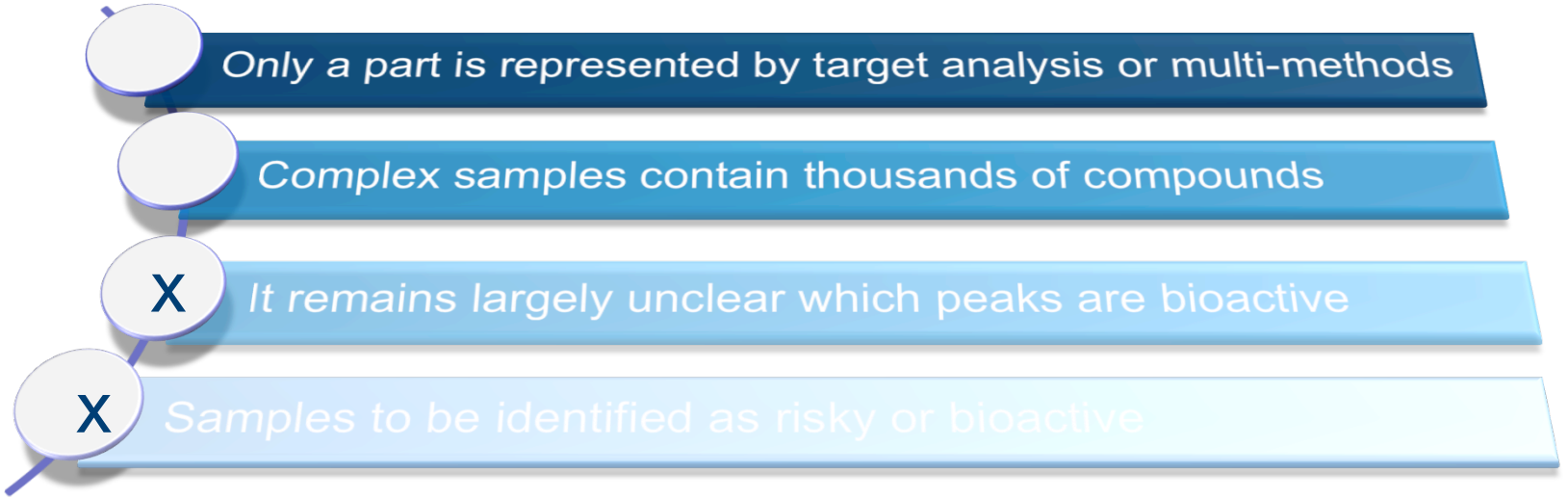


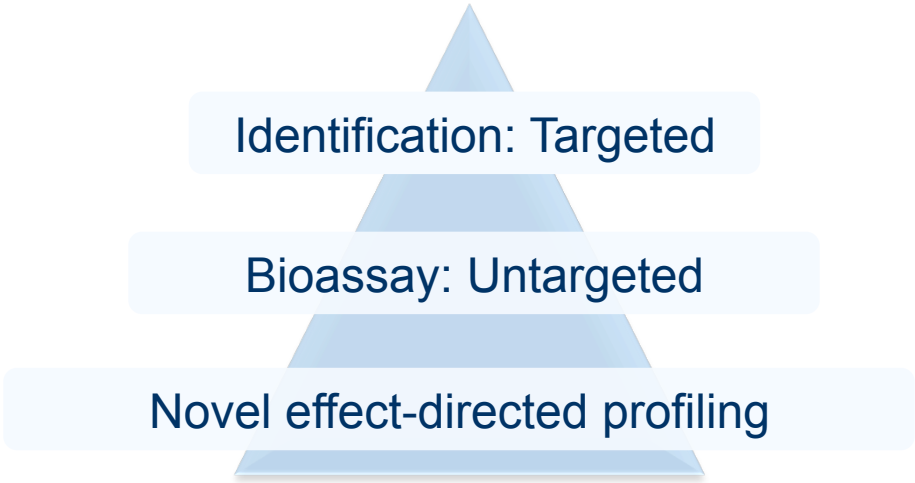
ESI-MS
MALDI-MS
DART-MS
ATR-FTIR
NMR

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

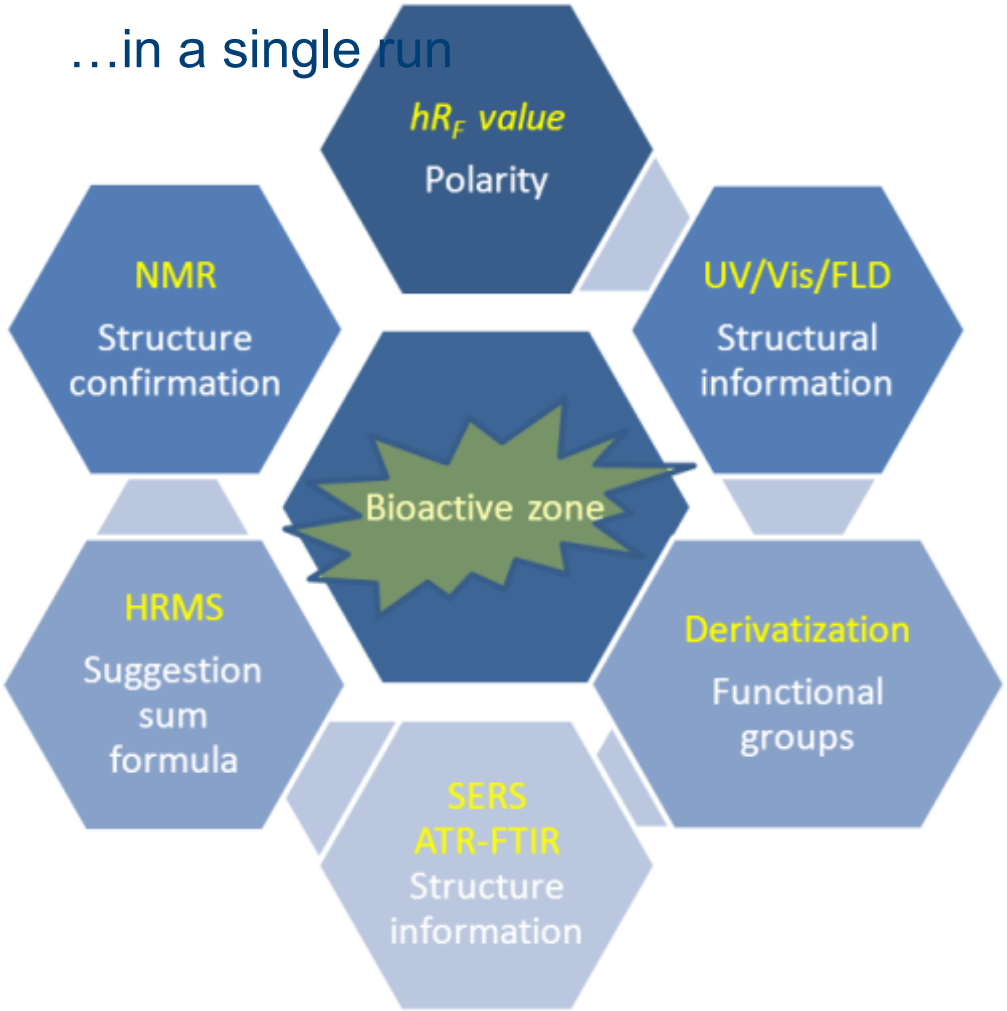
Impact?


Only a part is represented by target analysis or multi-methods
Complex samples contain thousands of compounds
X
It remains largely unclear which peaks are bioactive
X
Samples to be identified as risky or bioactive


Identification: Targeted
Bioassay: Untargeted
Novel effect-directed profiling

From bioactive zone to sum formula

…in a single run



G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, in print

G. Morlock Food Science JLU Gießen

JLU

Effect-directed link to the compound

Session 4
Poster P49-64

Goodall, R. R.; Levi, A. A. *Nature* **1946**, *158*, 675–676

- Microchemical derivatization
  - DPPH* reagent - radical scavenging test
  - ABTS reagent - radical scavenging test
  - Bleaching of ß-carotene - antioxidant test
- Microbiological detection
  - Biochemical assays
    - Enzymatic assays
      - Tyrosinase oxidase assay
      - Xanthine oxidase assay
      - (Cholin)esterase assay
      - α/ß-Glucosidase assay
    - Immuno staining
  - Biological assays
    - Algae
      - *Pseudokirchneriella subcapitata*
    - Fungi
      - *Candida albicans*
      - *Trichophyton rubrum*
      - *Cryptococcus neoformans*
      - *Cladosporium cucmerinum*
      - *Apergillus niger*
      - *Colletotrichum acutatum/gloeosporioides/fragaria*
    - Bacteria
      - *Pseudomonas savastanoi*
      - *Bacillus subtilis*
      - *Aliivibrio fischeri*
      - *Escherichia coli*
      - *Pseudomonas syringae*
      - *Xanthomonas campestris*
      - *Erwinia carotovora/herbicola*
      - *Cryptococcus neoformans*
    - Yeast
      - Planar yeast estrogen screen (pYES)

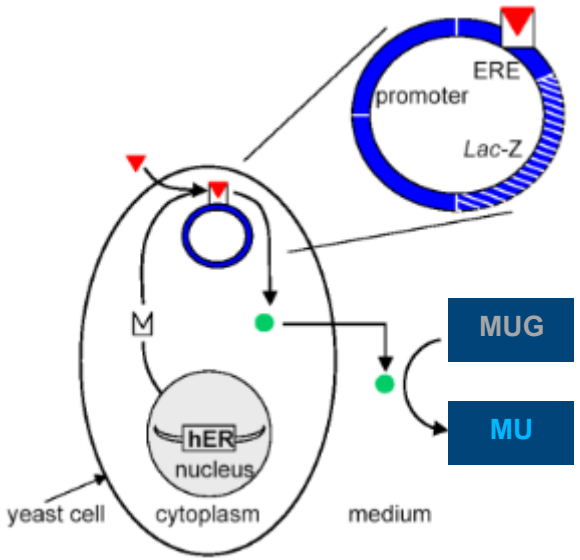
G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, in print

G. Morlock Food Science JLU Gießen

Liquid Yeast Estrogen Screen (L-YES)

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

Screening for endocrine disrupting compounds (EDCs)



Modified from draft of German expert working group for pYES assay

→ using the human estrogen receptor hERα

→ in yeast cells *Saccharomyces cerevisiae*

→ blue fluorescent zones (4-methylumbelliferone)

E. J. Routledge, J. P. Sumpter, Environ. Toxicol. Chem. 15 (1996) 241
McDonnell, *et al.* J. Steroid Biochem. Mol. Biol. 39 (1991) 291

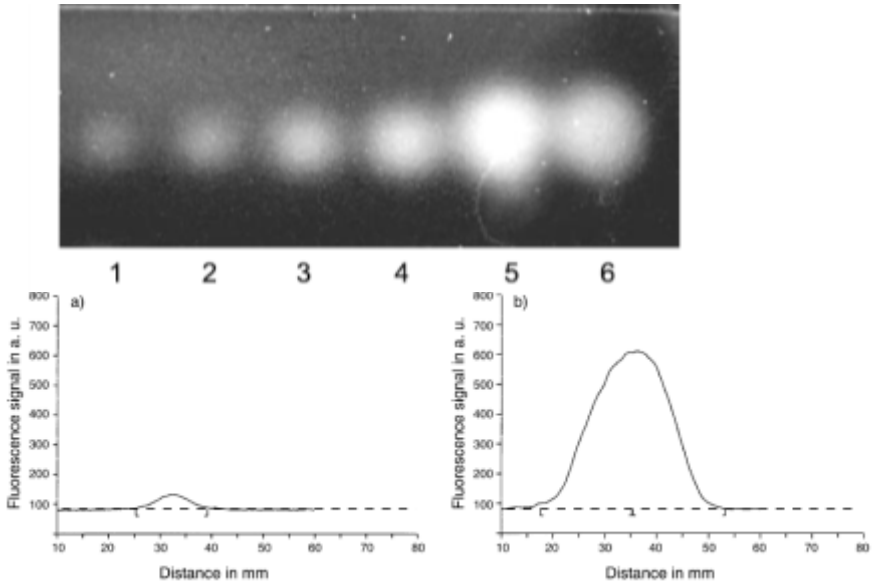
Hyphenation with chromatography (planar YES, pYES)

→ Reduced matrix interference

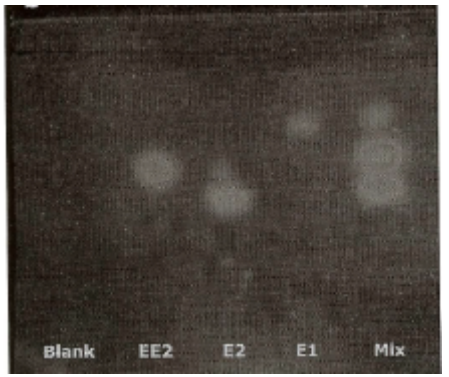
→ Improved capability of detection

→ Assignment of single bioactive compounds

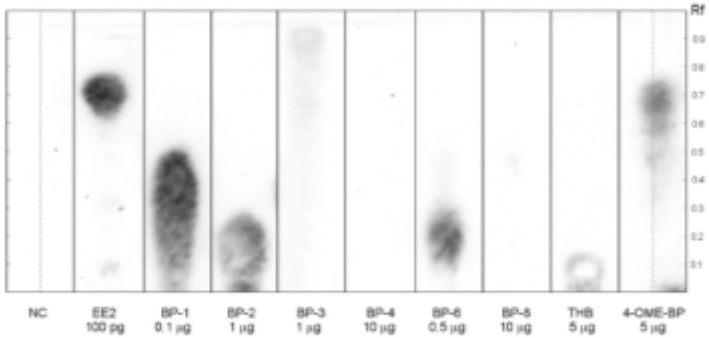
Challenge: Avoid zone broadening



Mueller, M.B., al. *Chromatographia*. 60, 207-211 (2004)



Schönborn, A. & Grimmer, A. A.
J. Planar Chromatogr. 26, 402-408 (2013)



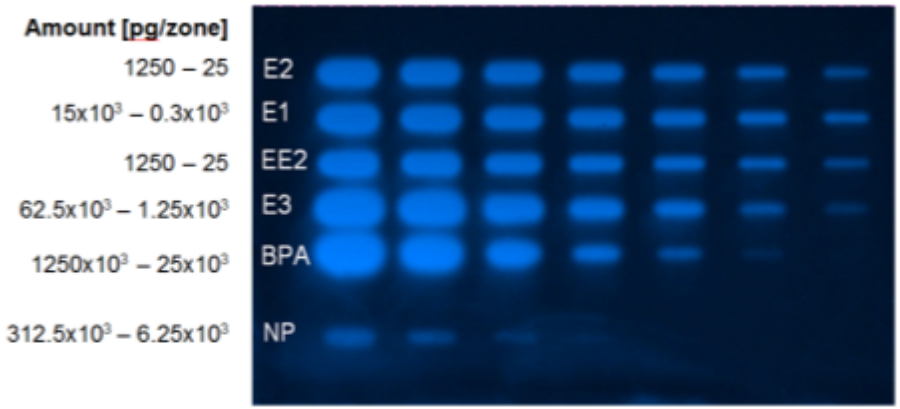
Buchinger, S. *et al.*
Anal. Chem. 85, 7248-7256 (2013)

G. Morlock Food Science JLU Gießen

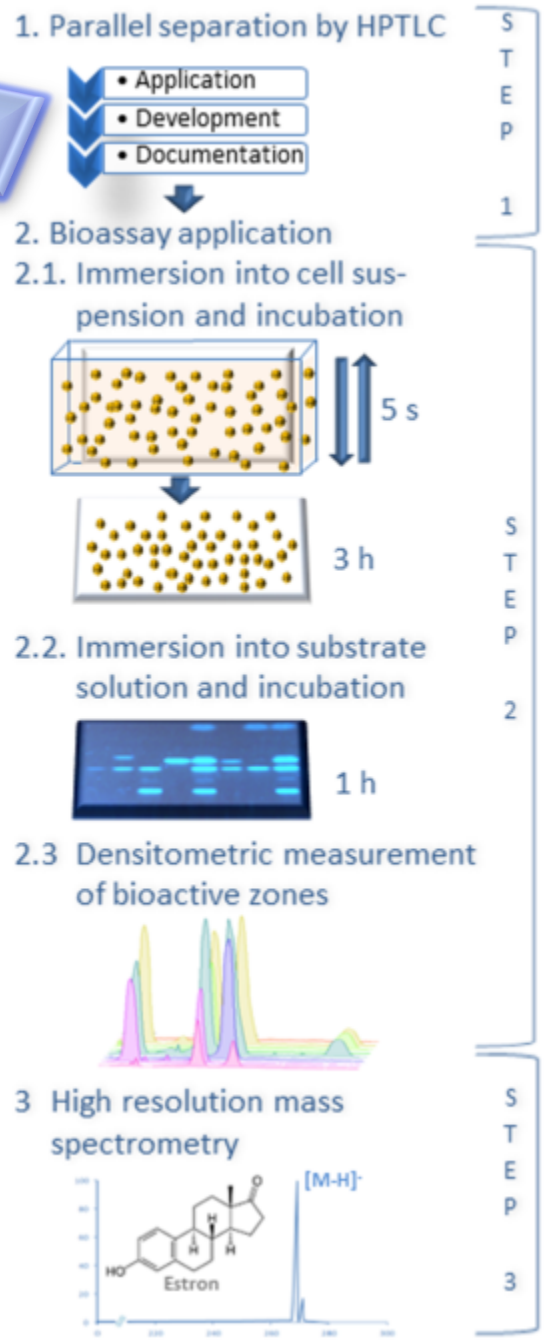
Poster P-54

Sharply bounded zones

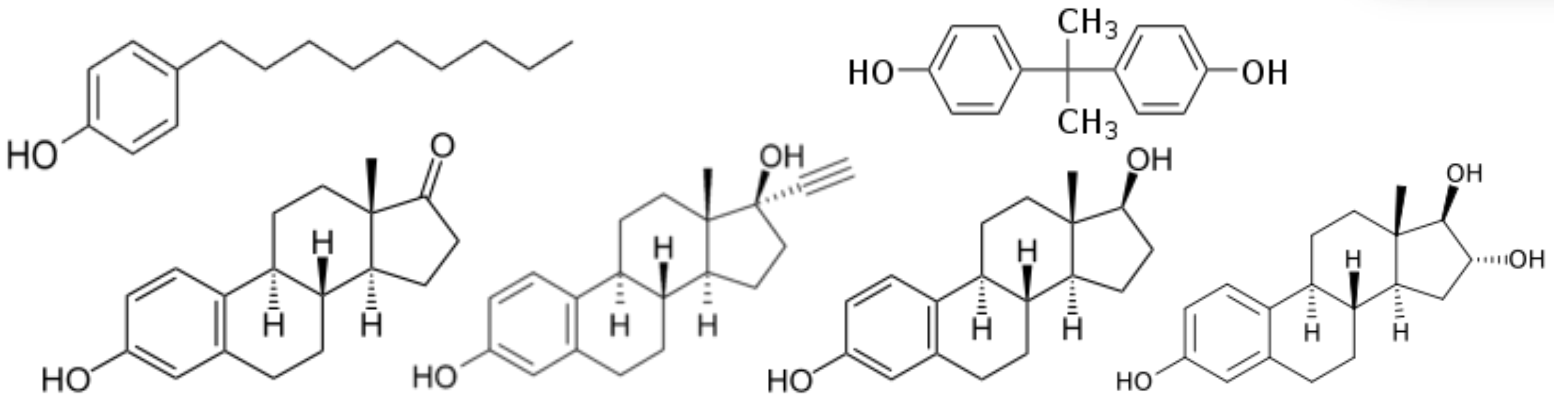
With optimized workflow
… even after aqueous incubation for 24 h



First proof of cultivation of cells on the plate



Detection of estrogenic compounds

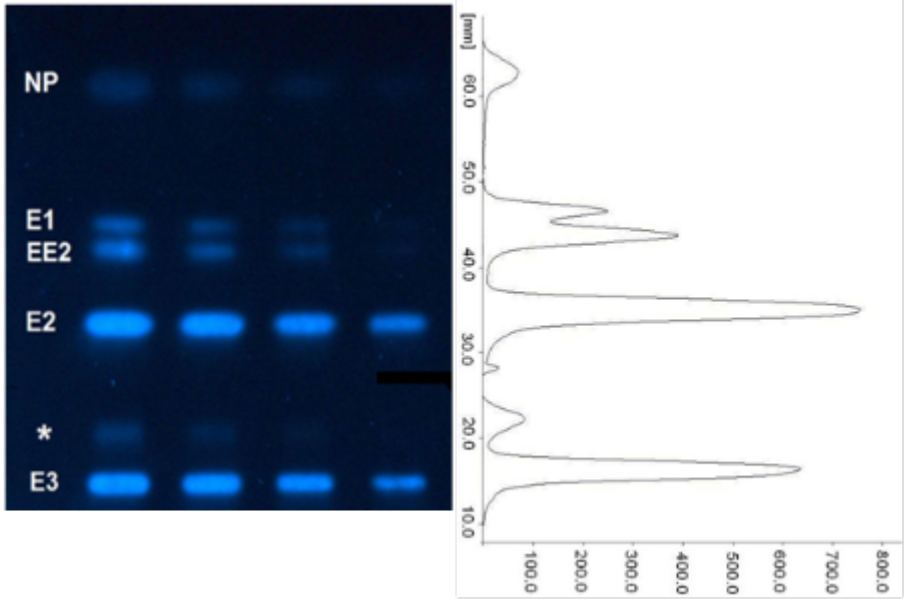


Amount [pg/zone]
$100x10^3 – 25x10^3$

500 – 100
50 – 5

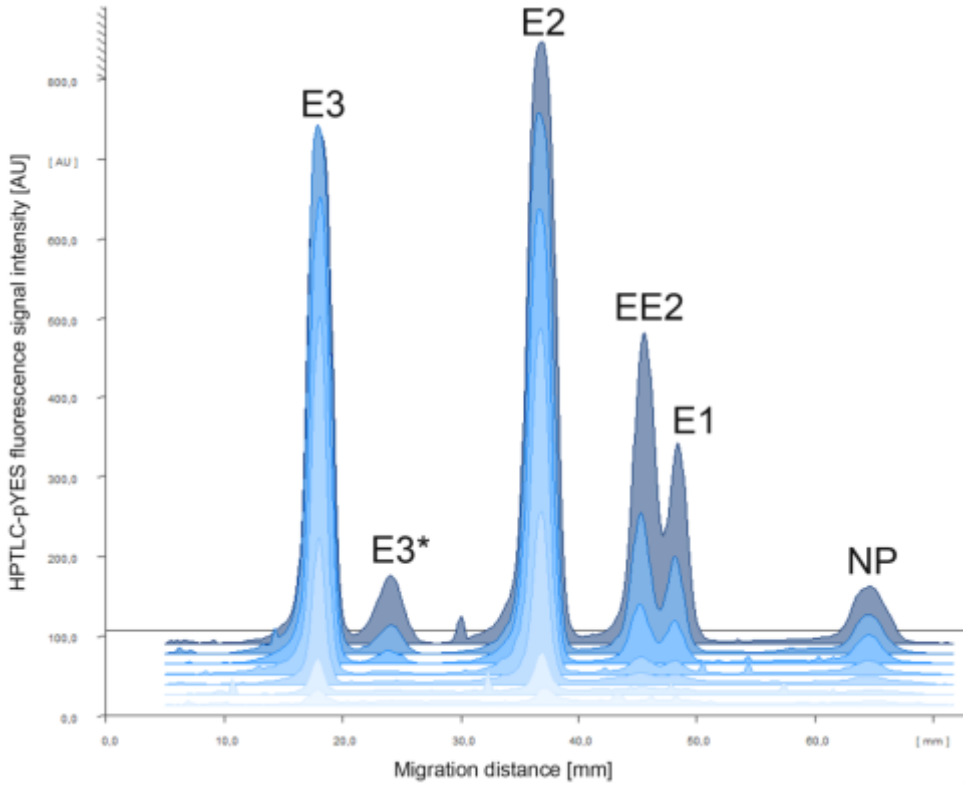
25 - 2.5

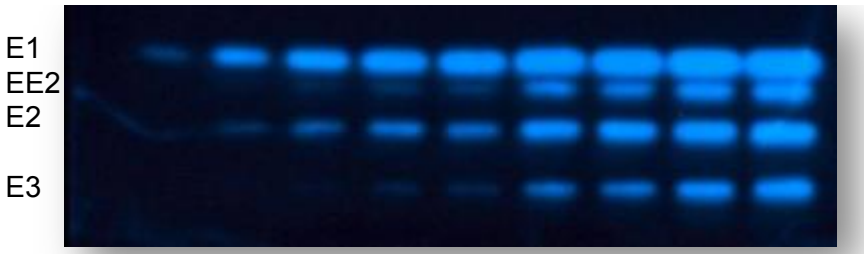
E3 impurity
$12.5x10^3 – 2.5x10^3$



G. Morlock Food Science JLU Gießen

Detection of estrogenic compounds

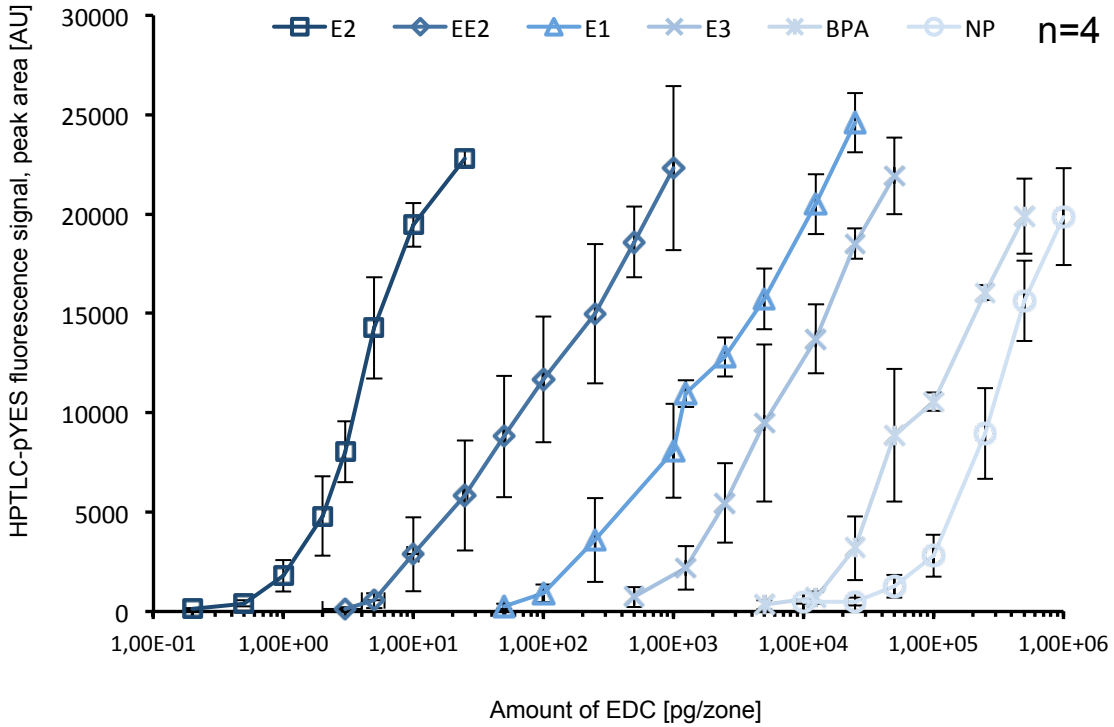




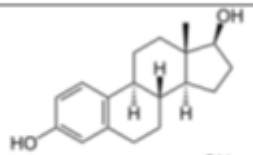
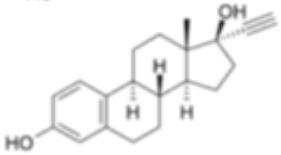
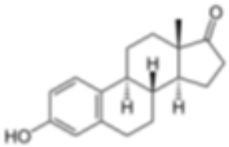
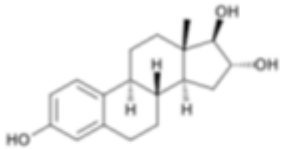
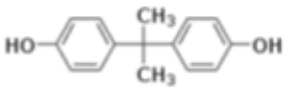
E1/E3: 25 – 1000 pg/band
E2/EE2: 0.5 – 20 pg/band

G. Morlock Food Science JLU Gießen

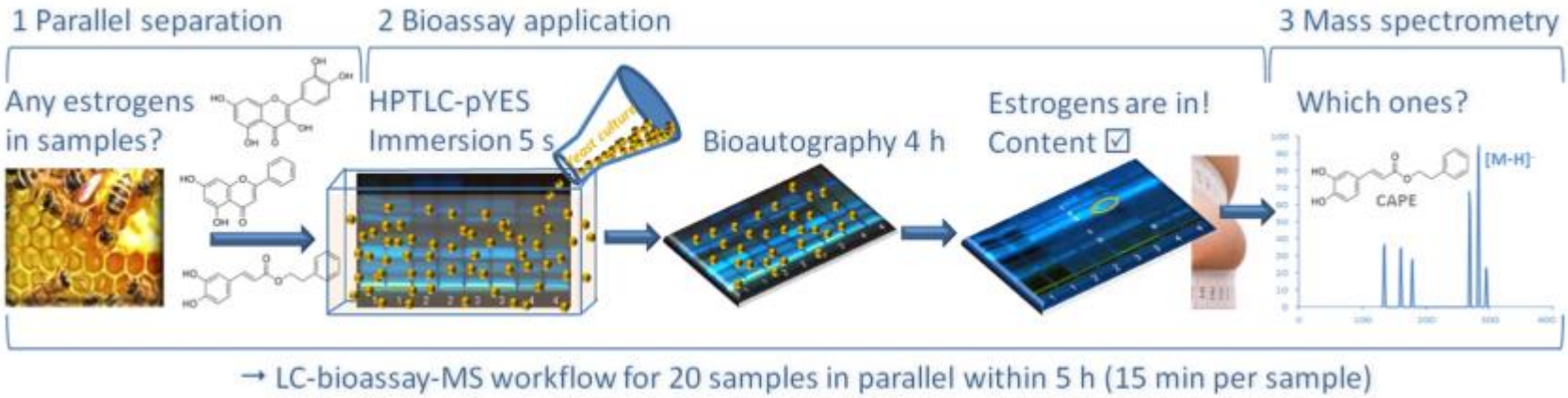
HPTLC-pYES: dose-response curves



HPTLC-pYES: enlarged working range

Substance (hR_F)	Structure formula[1]	Maximal working range [pg/zone] R^2 range (mean R^2, n=4)	E2Eq	LOD [pg/zone] (S/N 3, n=3	LOQ [pg/zone] (S/N 10, n=3)
E2 (15)		0.5 - 50 0.929 - 0.991 (0.96)	1	0.5	1
EE2 (24)		2 - $1x10^3$ 0.924 - 0.991 (0.99)	0.3	2	5
E1 (29)		25 - $25x10^3$ 0.930 - 0.999 (0.98)	$1.5x10^{-2}$	25	50
E3 (2)		$5x10^2$ - $50x10^3$ 0.996 - 0.997 (0.98)	$4.2x10^{-3}$	n.d.	500
BPA (22)		$62.5x10^2$ - $1x10^6$ 0.960 - 0.993 (0.97)	$3.3x10^{-4}$	$62.5x10^2$	$12.5x10^3$
NP (66)		$25x10^3$ - $1x10^6$ 0.986 - 0.991 (0.94)	$2.3x10^{-4}$	$25x10^3$	$50x10^3$

Untargeted → targeted link to effective comp.



Effect-directed profiling (owed to sharply-bounded zones)

→ comprehensive information taking **raw** samples

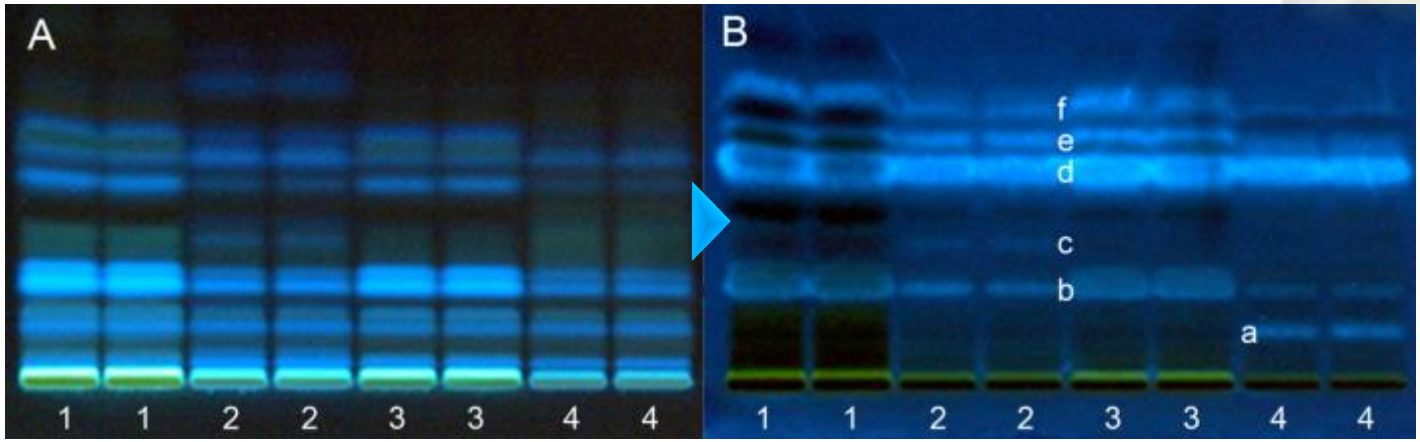
→ identifying **all** compounds generating the effect

→ support decisions

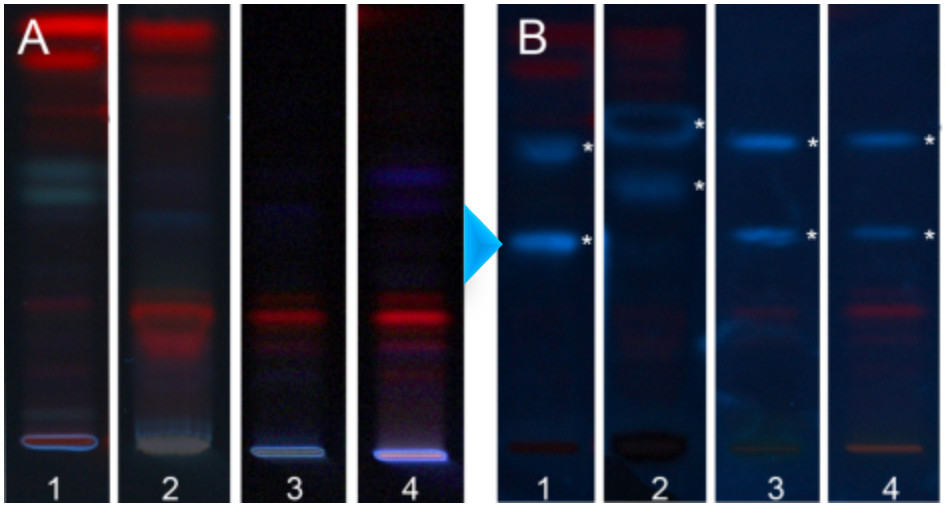
→ join the international pYES expert group (contact C. Weins)

Detection of EDCs (estrogenic activity)

→ Propolis samples



→ Spices/tea

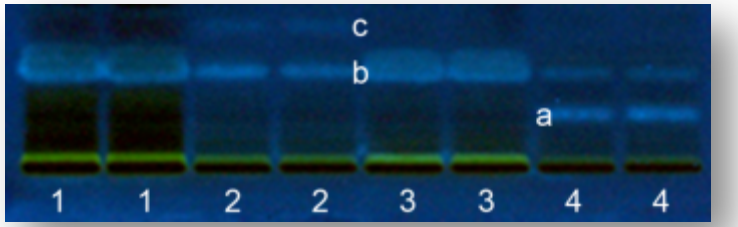


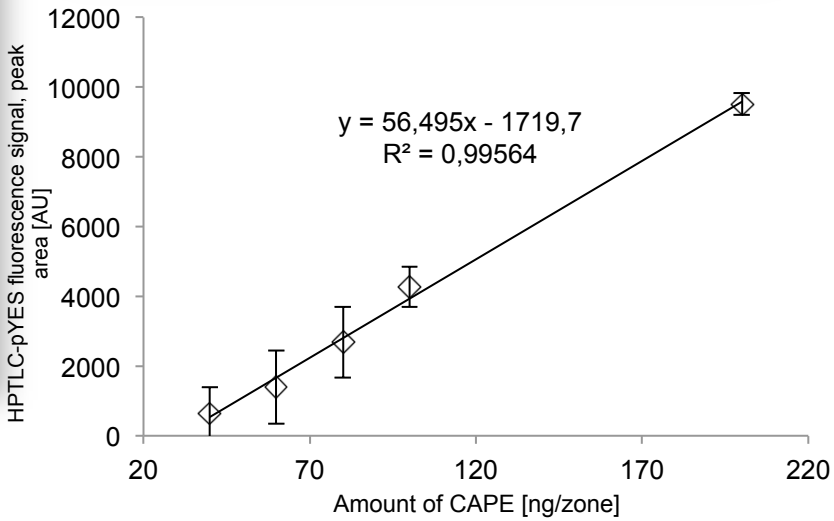
G. Morlock Food Science JLU Gießen

Quantitation of CAPE

Propolis sample	CAPE content in sample [µg/mL]	CAPE content [µg/g] referred to propolis weight (n=2)
P1 (30 %)	481	2028
P2 (30 %)	476	2009
P3 (25 %)	471	2387
P4 (62 %)	348	710
P5 (not specified)	380	380[3]
P6 (250 mg/capsule)	359[1]	1435
P7 (30 mg/lozenge)	22[2]	1089

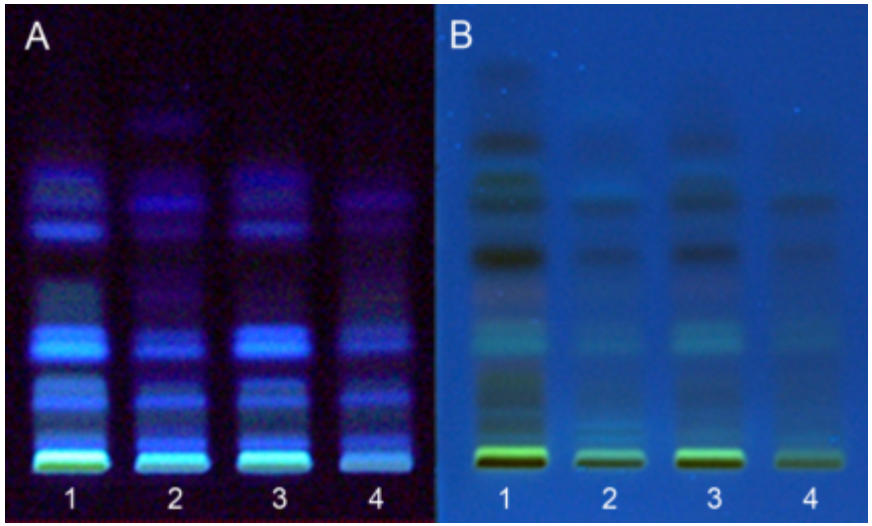
[1]µg/capsule, [2]µg/pastille, [3]µg/mL



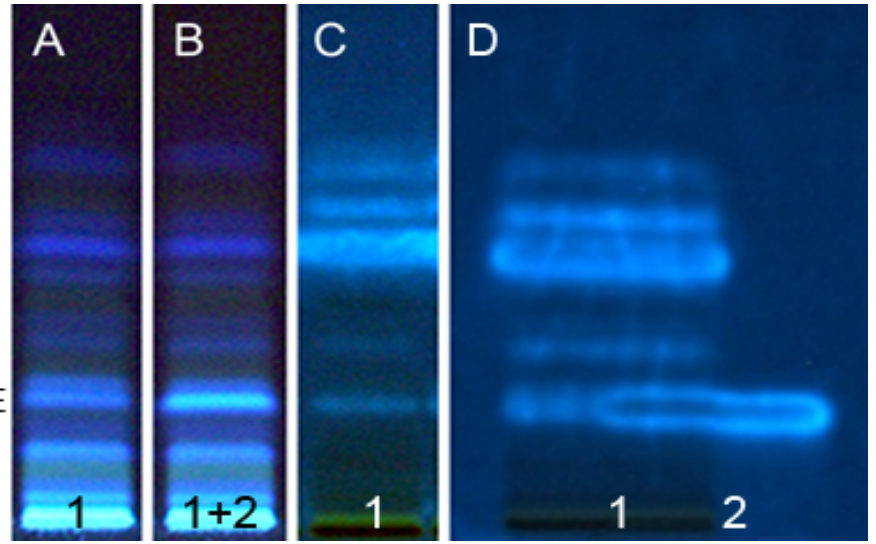


Confirmation

Negative control:
A: UV 366 nm
B: pYES, but without the yeast cells in medium

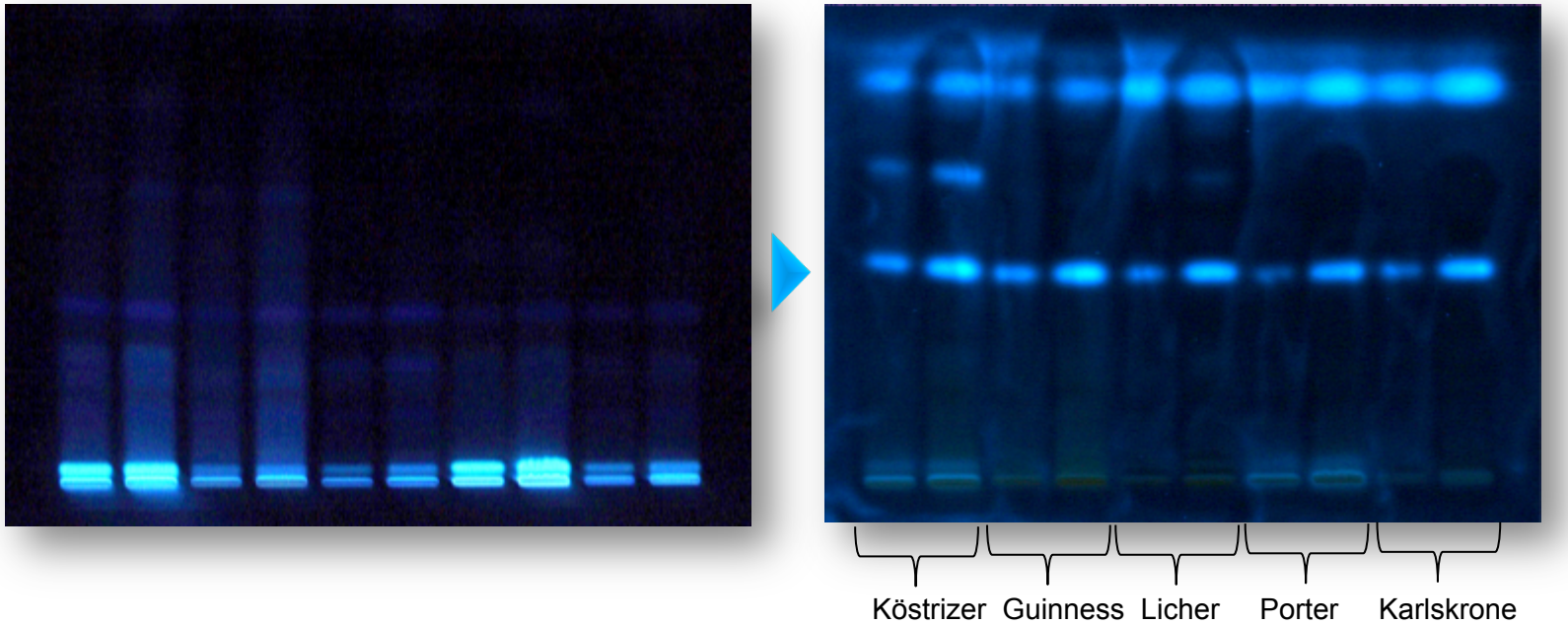


A-C: Oversprayed application
D: Overlapped application

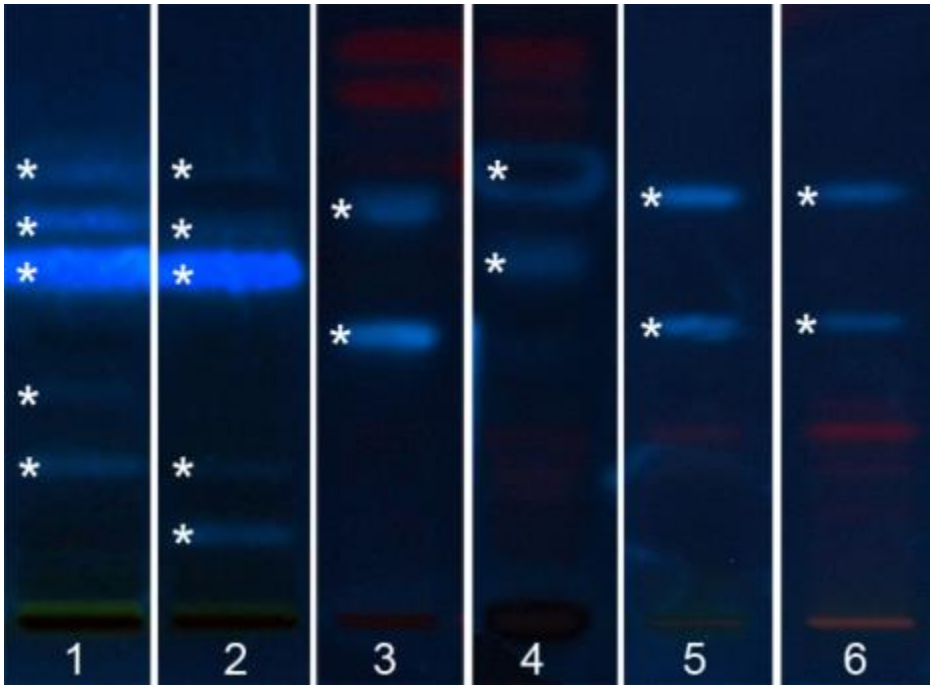


Detection of EDCs (estrogenic activity)

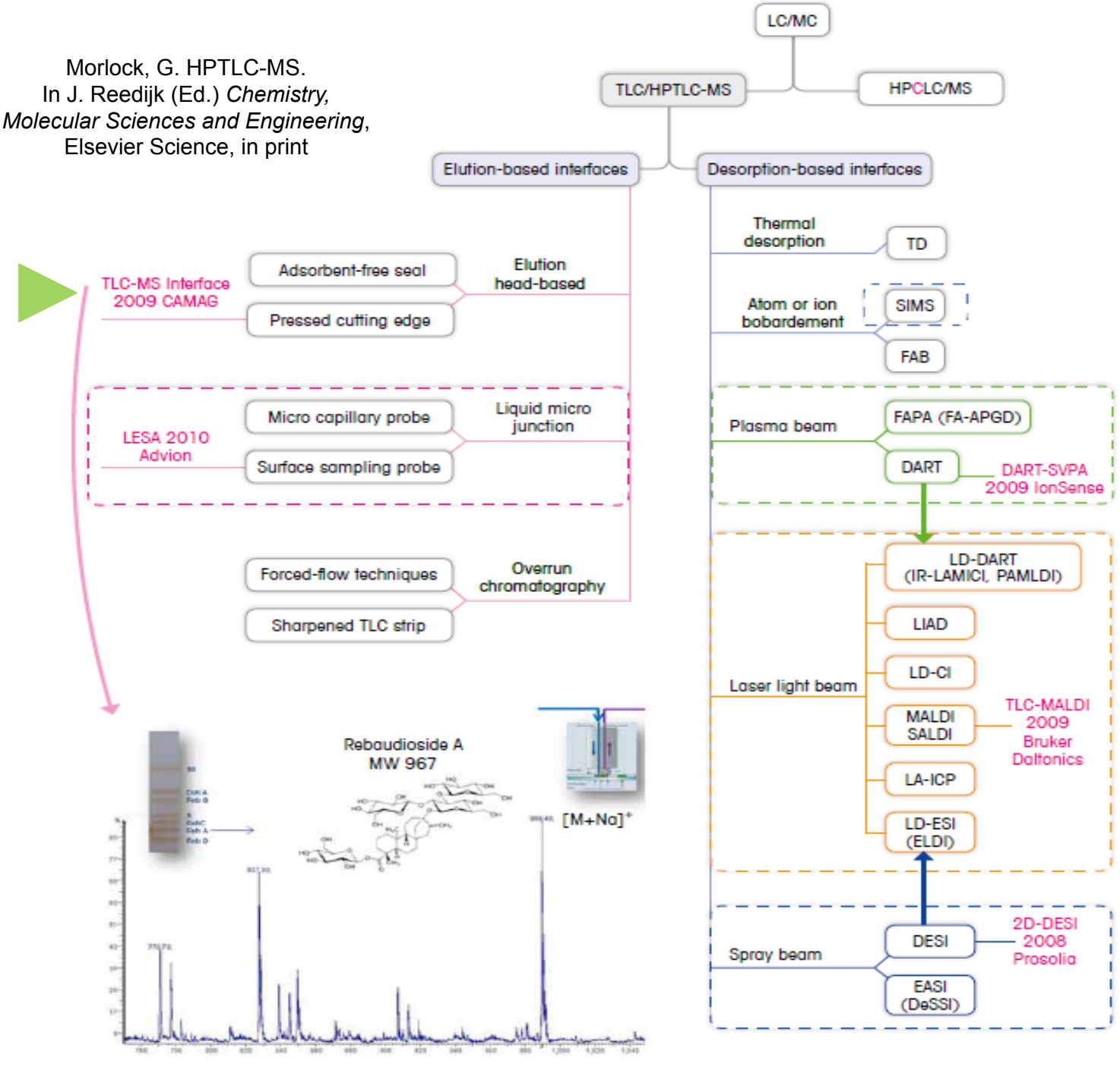
→ Beer samples



What is it?



Morlock, G. HPTLC-MS.
In J. Reedijk (Ed.) *Chemistry, Molecular Sciences and Engineering*, Elsevier Science, in print



JLU Gießen Food Science G. Morlock

JLU

GDCh course 335/14

NEU

GDCh

Gesellschaft Deutscher Chemiker

ANALYTISCHE CHEMIE

Hyphenations in der HPTLC

HPTLC und Kopplungen
(in Zusammenarbeit mit der JLU Gießen)

Prof. Dr. Gertrud Morlock

- Kopplungstechniken
- Massenspektrometrie (MS)
- Wirkungsbezogene Analytik (Bioassays)
- ATR-FTIR und NMR
- Effektive Analytik

335/14

12. November 2014 · Gießen

ZFL

Anerkannt mit 18 Punkten
(www.zefo.org)

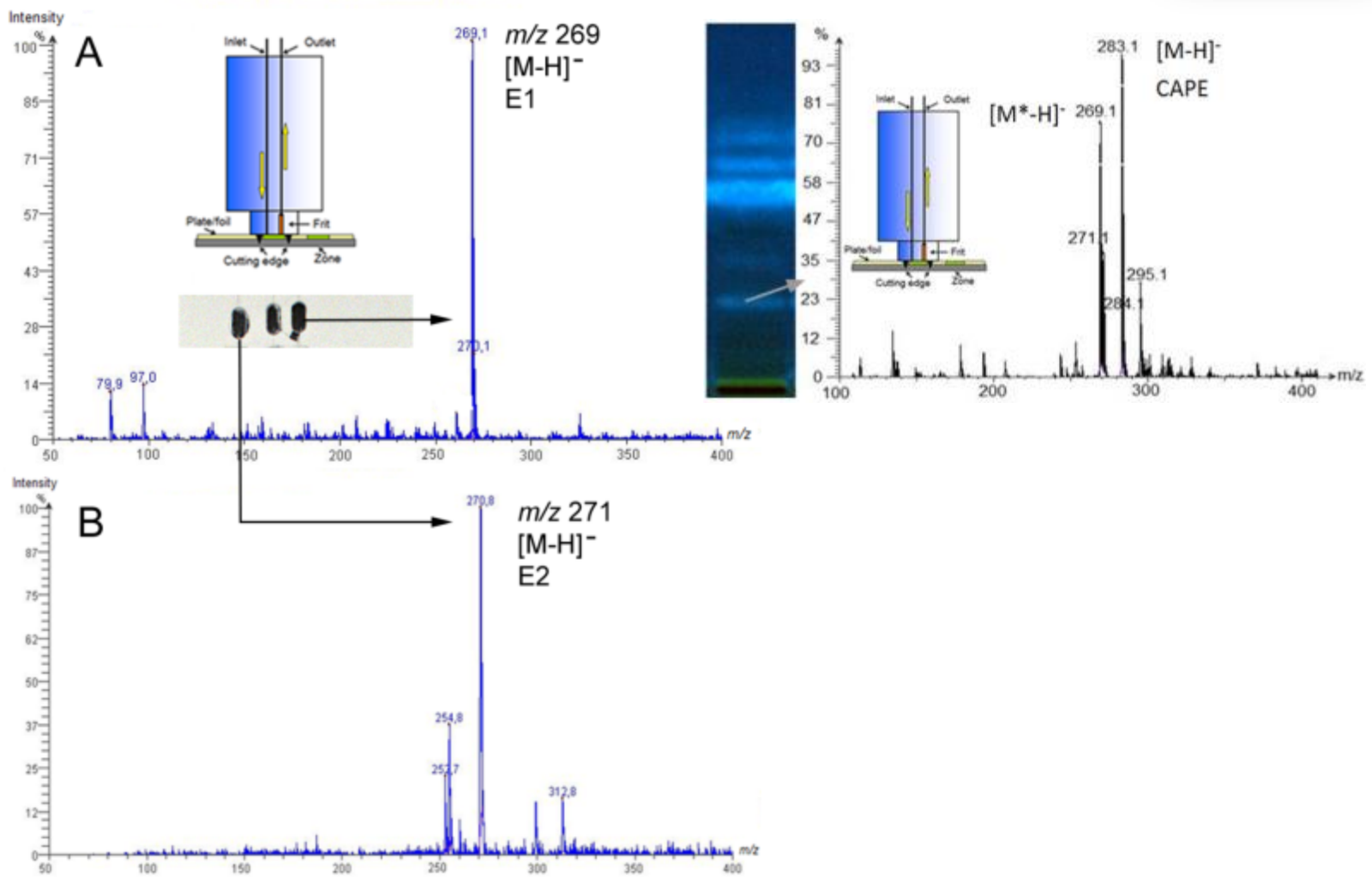
PROGRAMM

Mittwoch, 12. November 2014

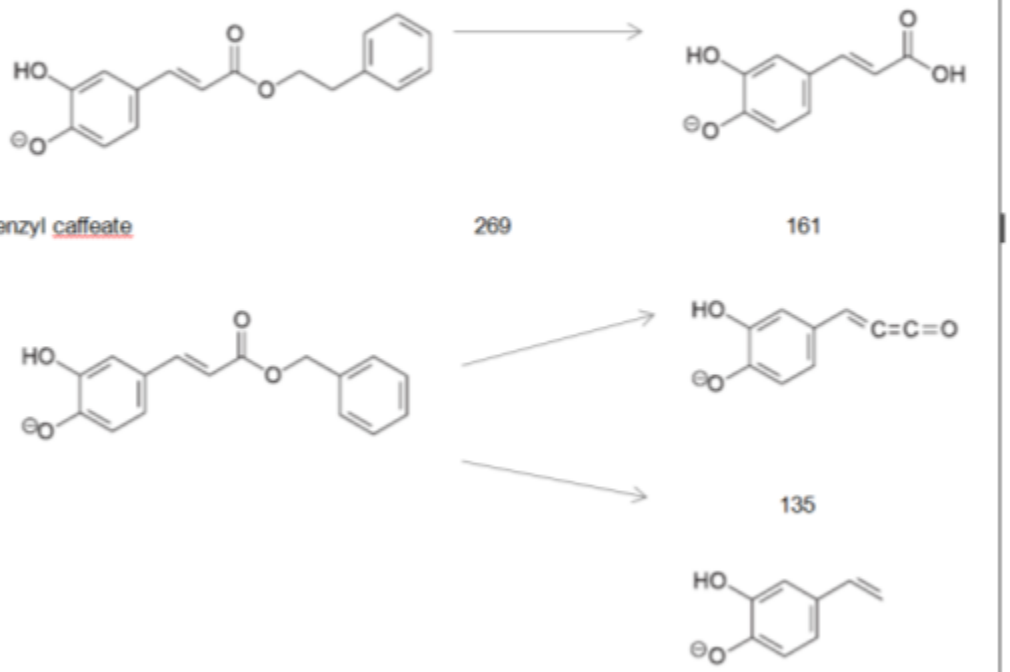
9.00	Begrüßung und Einführung in die HPTLC (Morlock)
9.45	HPTLC erfahren – Experimente (Häbe, Klingelhöfer)
10.45	Kaffeepause
11.00	Hyphenations in der Planar-Chromatographie – Teil 1 (Morlock, Schwack)
11.45	Gruppe 1: Experiment DC-HPLC/DAD-ESI MS (Oellig, Schwack)
	Gruppe 2: Experiment HPTLC-UV/Vis/FLD-MALDI-TOF MS/MS (Lochnit, Krüger)
12.30	Gruppe 1: Experiment HPTLC-UV/Vis/FLD-MALDI-TOF MS/MS (Lochnit, Krüger)
	Gruppe 2: Experiment DC-HPLC/DAD-ESI MS (Oellig, Schwack)
13.15	Mittagspause
13.45	Hyphenations in der Planar-Chromatographie – Teil 2 (Morlock)
14.00	Gruppe 1: Experiment HPTLC-UV/Vis/FLD-ATR FTIR (Klingelhöfer, Reisenauer)
	Gruppe 2: Experiment HPTLC-UV/Vis/FLD-Bioassay-ESI MS (Krüger, Kirchert)
14.45	Gruppe 1: Experiment HPTLC-UV/Vis/FLD-Bioassay-ESI MS (Krüger, Kirchert)
	Gruppe 2: Experiment HPTLC-UV/Vis/FLD-ATR FTIR (Klingelhöfer, Reisenauer)
15.30	Kaffeepause
15.45	Hyphenations in der Planar-Chromatographie – Teil 3 (Morlock)
16.00	Gruppe 1: Experiment HPTLC-UV/Vis/FLD-DART-MS (Häbe, Krüger)
	Gruppe 2: Experiment HPTLC-UV/Vis/FLD-DESI-MS (Kirchert, Morlock)
16.15	Gruppe 1: Experiment HPTLC-UV/Vis/FLD-DESI-MS (Kirchert, Morlock)
	Gruppe 2: Experiment HPTLC-UV/Vis/FLD-DART-MS (Häbe, Krüger)
16.30	Diskussion (Morlock)
17.00	Voraussichtliches Ende der Veranstaltung

G. Morlock Food Science JLU Gießen

Confirmation by MS

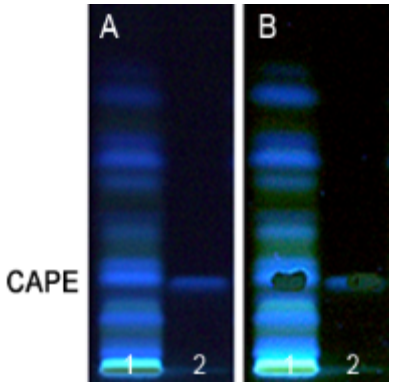


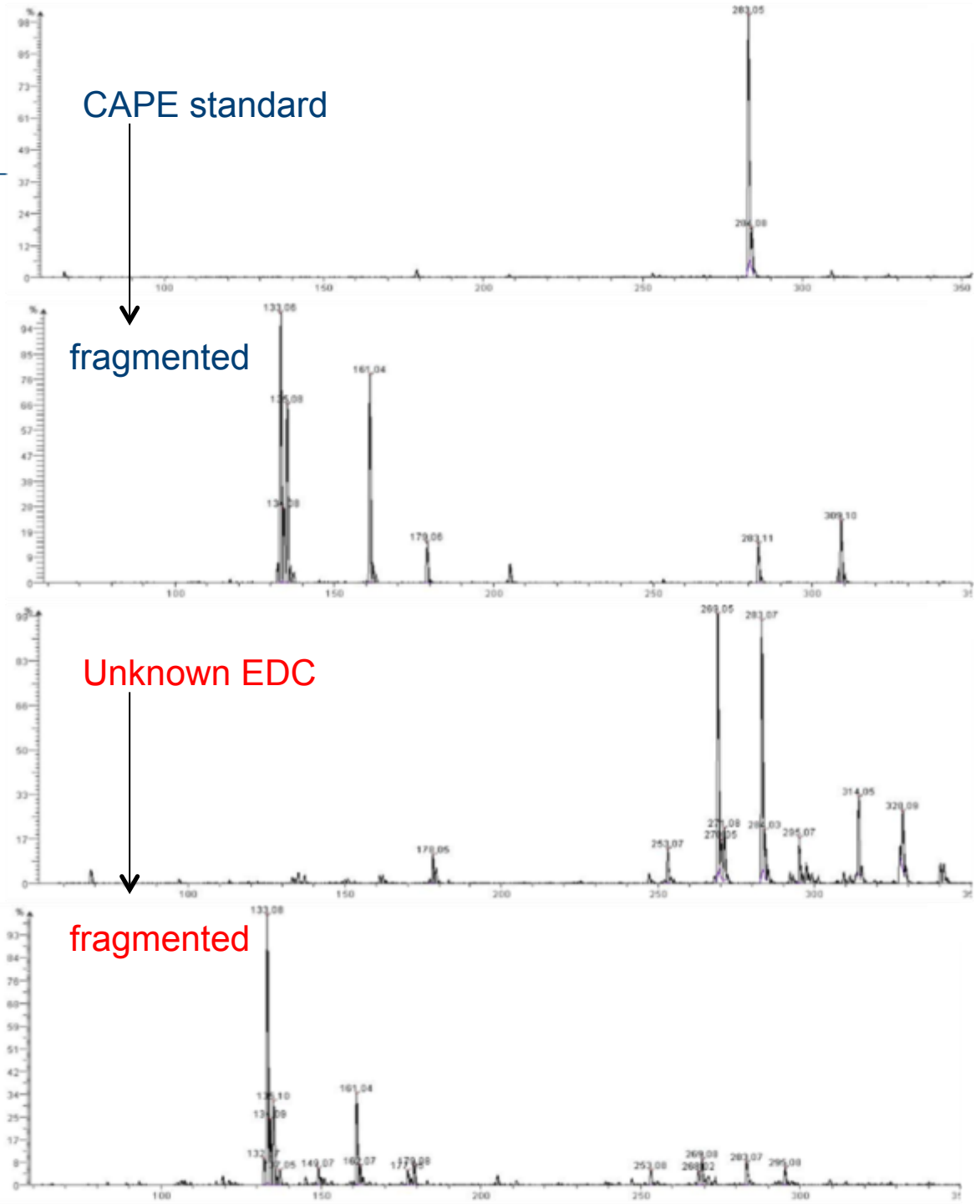
Bioactive compounds	$[M-H]^-$	Fragmentation
Caffeic acid phenethyl ester (CAPE)	283	179
Benzyl caffeate	269	161
		135



G. Morlock Food Science JLU Gießen

CAPE?





Effect-directed link to the compound

Goodall, R. R.; Levi, A. A. *Nature* **1946**, *158*, 675–676

- Microchemical derivatization
  - DPPH* reagent - radical scavenging test
  - ABTS reagent - radical scavenging test
  - Bleaching of ß-carotene - antioxidant test
- Microbiological detection
  - Biochemical assays
    - Enzymatic assays
      - Tyrosinase oxidase assay
      - Xanthine oxidase assay
      - (Cholin)esterase assay
      - α/ß-Glucosidase assay
    - Immuno staining
  - Biological assays
    - Algae
      - *Pseudokirchneriella subcapitata*
    - Fungi
      - *Candida albicans*
      - *Trichophyton rubrum*
      - *Cryptococcus neoformans*
      - *Cladosporium cucmerinum*
      - *Apergillus niger*
      - *Colletotrichum acutatum/gloeosporioides/fragaria*
    - Bacteria
      - *Pseudomonas savastanoi*
      - *Bacillus subtilis*
      - *Aliivibrio fischeri*
      - *Escherichia coli*
      - *Pseudomonas syringae*
      - *Xanthomonas campestris*
      - *Erwinia carotovora/herbicola*
      - *Cryptococcus neoformans*
    - Yeast
      - Planar yeast estrogen screen (pYES)

G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, in print

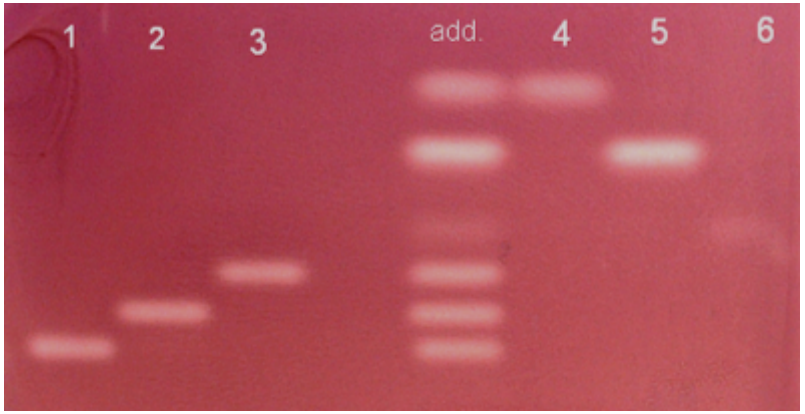
Detection of esterase inhibitors

Cholinesterase inhibiting pesticides by esterases

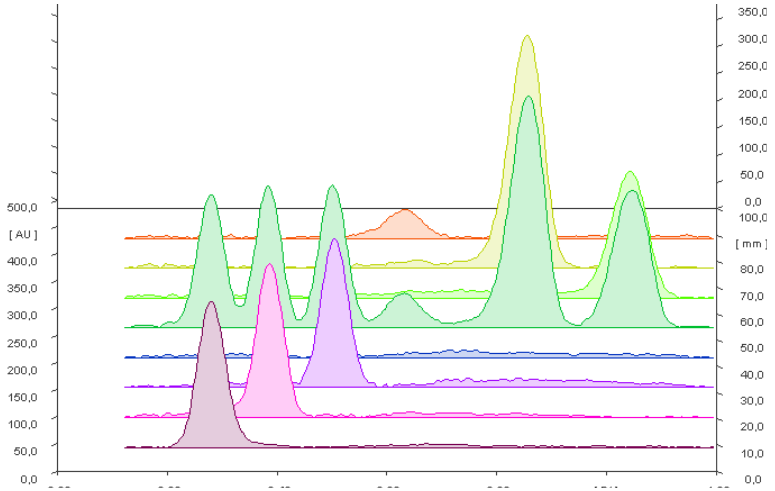
→ detectability down to 2 pg/zone

→ using an esterase and substrate (1-naphthylacetate/fast blue salt B) solution

→ white zones on a pink background



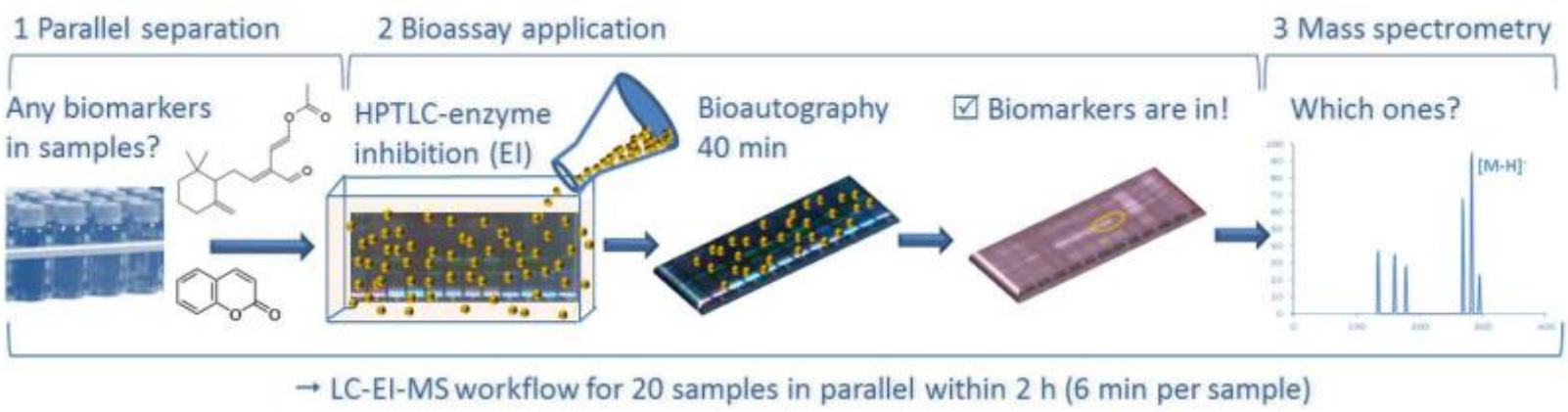
1. Paraoxon-methyl, 2. malaoxon, 3. paraoxon,
4. ethiofencarb, 5. chlorfenvinfos, 6. dichlorvos

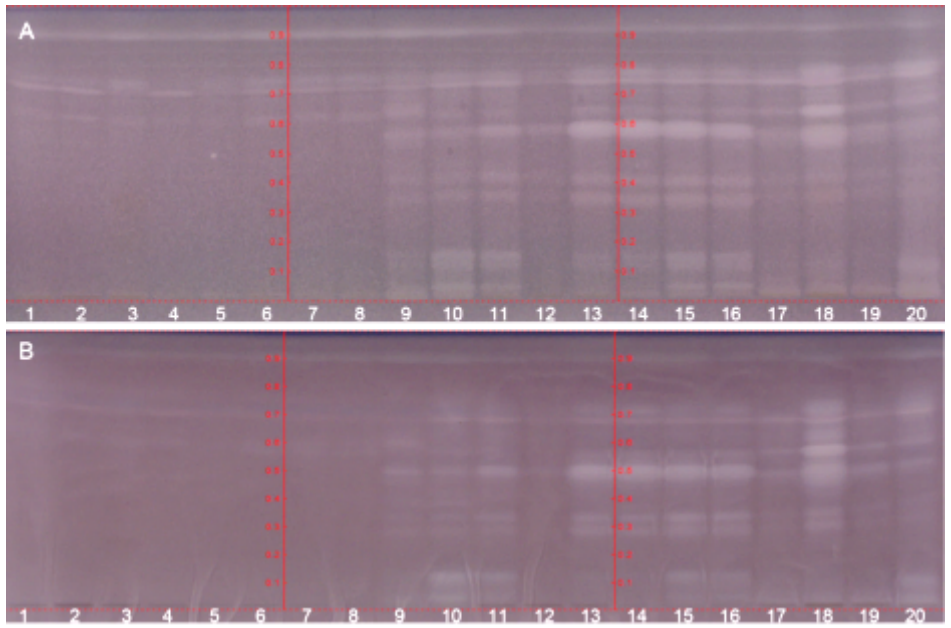


Absorption measurement at 533 nm

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

Detection of esterase inhibitors in *Rosaceae*





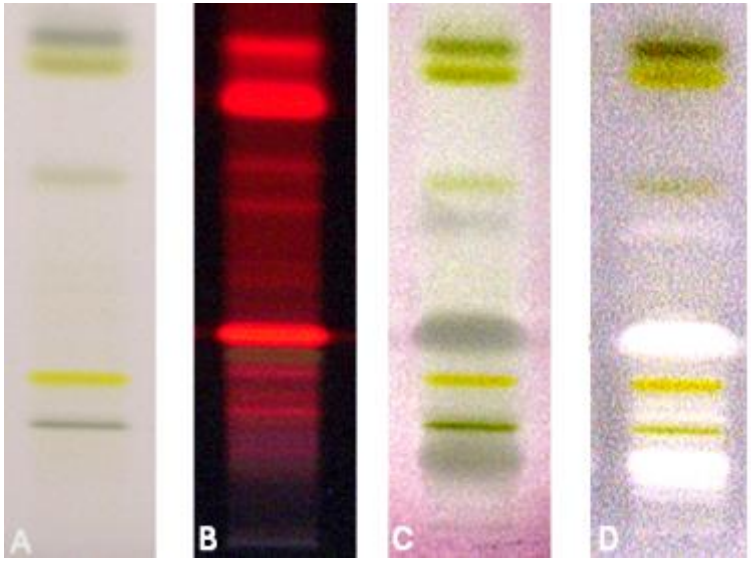
S. Hage, G. Morlock, in preparation

G. Morlock Food Science JLU Gießen

Detection of α- and β-glucosidase inhibitors

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

Extract of leaves of *Annona cherimola*



Vis UV366 nm α-/β-glucosidase

Effect-directed link to the compound

Goodall, R. R.; Levi, A. A. *Nature* **1946**, *158*, 675–676

- Microchemical derivatization
  - DPPH* reagent - radical scavenging test
  - ABTS reagent - radical scavenging test
  - Bleaching of ß-carotene - antioxidant test
- Microbiological detection
  - Biochemical assays
    - Enzymatic assays
      - Tyrosinase oxidase assay
      - Xanthine oxidase assay
      - (Cholin)esterase assay
      - α/ß-Glucosidase assay
    - Immuno staining
  - Biological assays
    - Algae
      - *Pseudokirchneriella subcapitata*
    - Fungi
      - *Candida albicans*
      - *Trichophyton rubrum*
      - *Cryptococcus neoformans*
      - *Cladosporium cucmerinum*
      - *Apergillus niger*
      - *Colletotrichum acutatum/gloeosporioides/fragaria*
    - Bacteria
      - *Pseudomonas savastanoi*
      - *Bacillus subtilis*
      - *Aliivibrio fischeri*
      - *Escherichia coli*
      - *Pseudomonas syringae*
      - *Xanthomonas campestris*
      - *Erwinia carotovora/herbicola*
      - *Cryptococcus neoformans*
    - Yeast
      - Planar yeast estrogen screen (pYES)

G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, in print

G. Morlock Food Science JLU Gießen

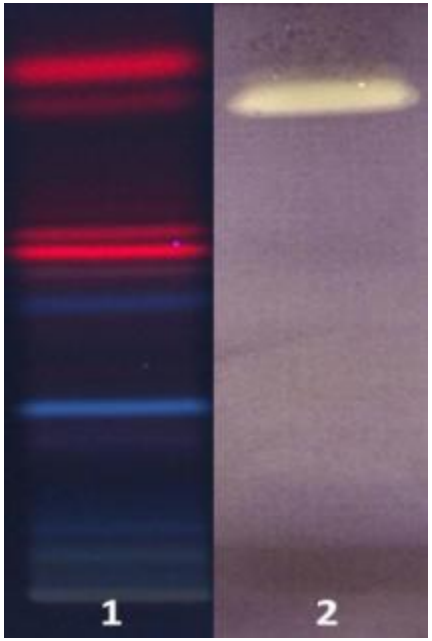
Detection of antibiotics with *Bacillus subtilis*

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

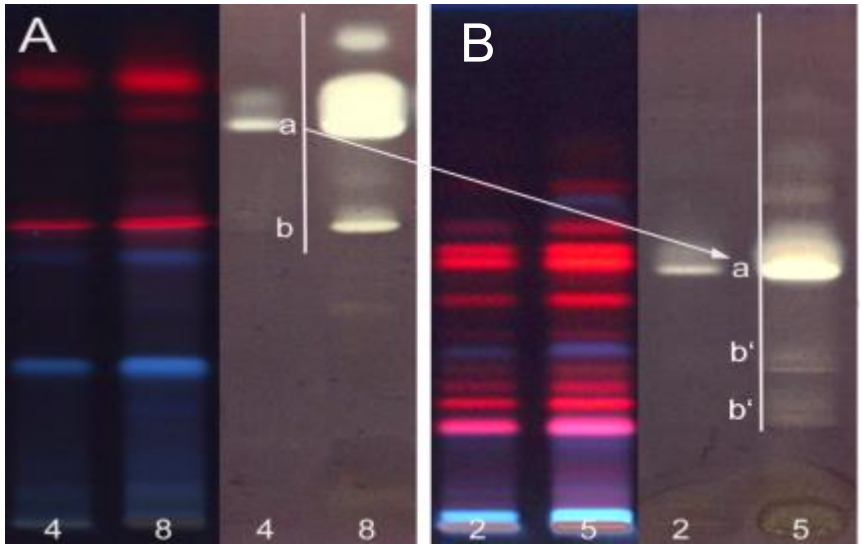
Poster P-55

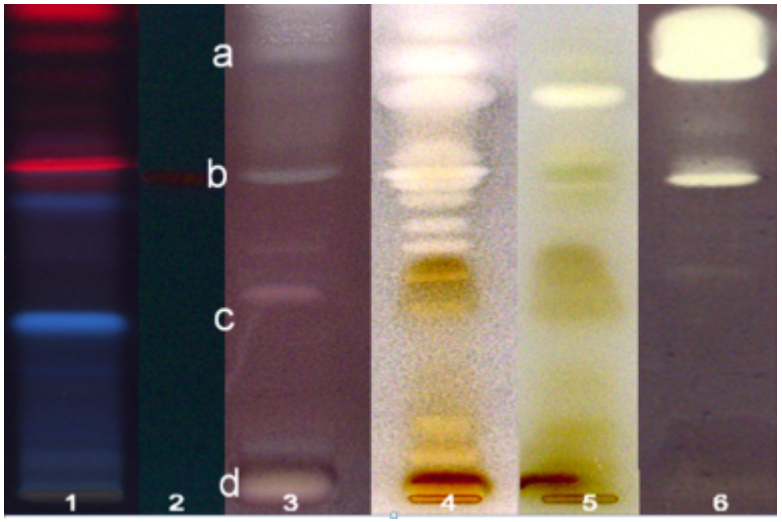
Antibiotics in plant extracts

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



Effect-directed analysis of *Salvia officinalis*



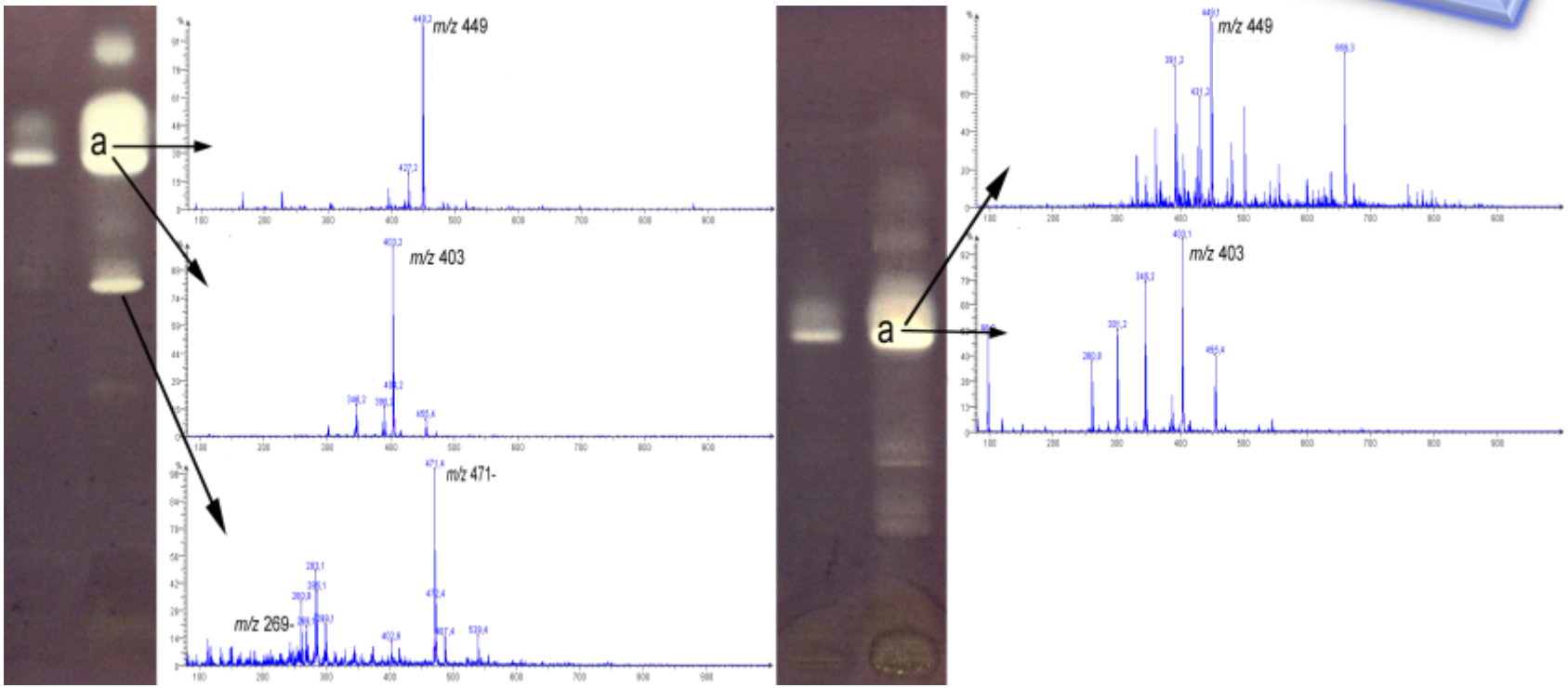


C. Stenfert Kroese, M. Jamshidi, G. Morlock, in preparation

G. Morlock Food Science JLU Gießen

Effect-directed analysis of *Salvia officinalis*

Poster P-56



C. Stenfert Kroese et al., in preparation

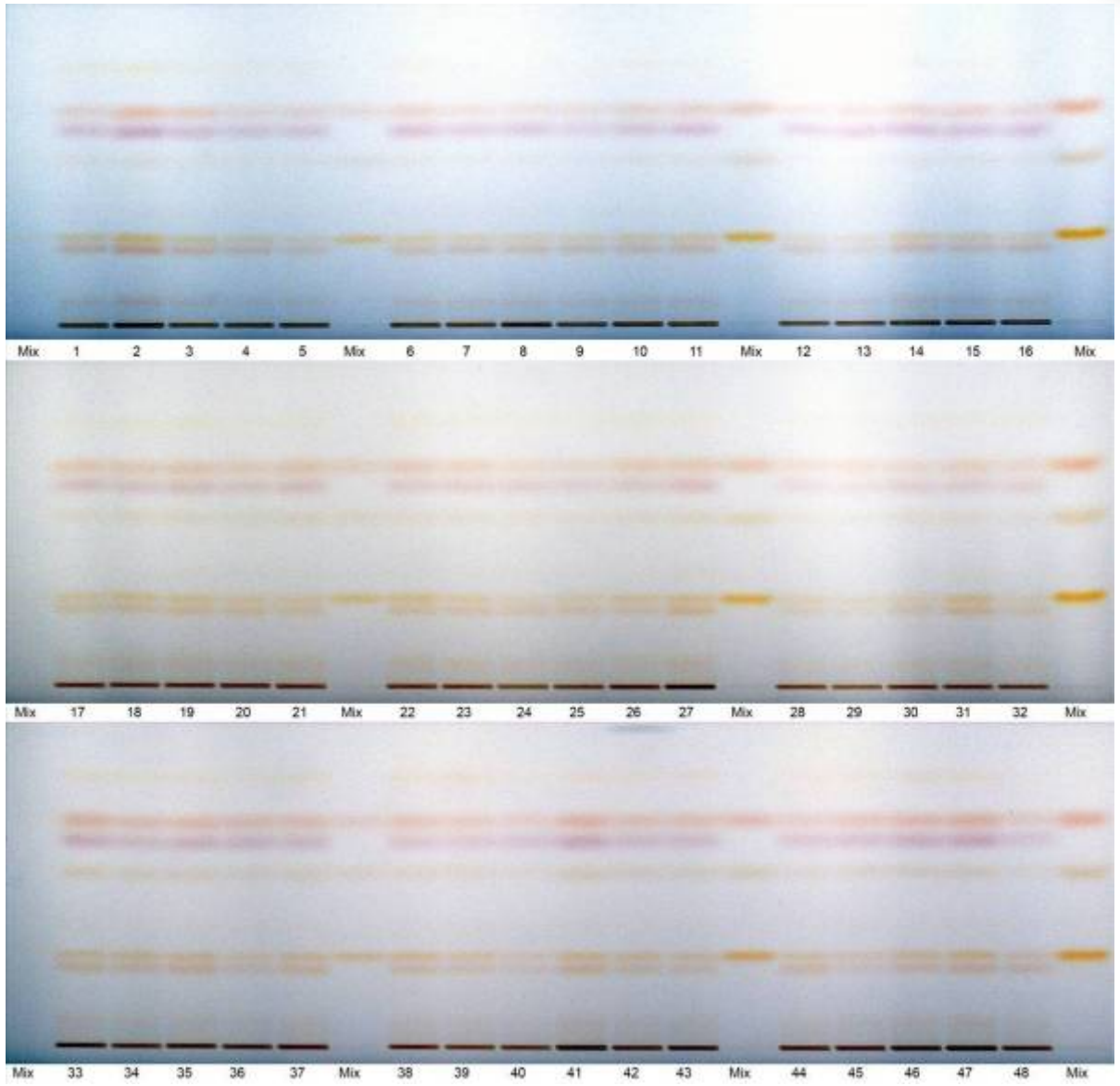
Effect-directed link to the compound

Goodall, R. R.; Levi, A. A. *Nature* **1946**, *158*, 675–676

- Microchemical derivatization
  - DPPH* reagent - radical scavenging test
  - ABTS reagent - radical scavenging test
  - Bleaching of ß-carotene - antioxidant test
- Microbiological detection
  - Biochemical assays
    - Enzymatic assays
      - Tyrosinase oxidase assay
      - Xanthine oxidase assay
      - (Cholin)esterase assay
      - α/ß-Glucosidase assay
    - Immuno staining
  - Biological assays
    - Algae
      - *Pseudokirchneriella subcapitata*
    - Fungi
      - *Candida albicans*
      - *Trichophyton rubrum*
      - *Cryptococcus neoformans*
      - *Cladosporium cucmerinum*
      - *Apergillus niger*
      - *Colletotrichum acutatum/gloeosporioides/fragaria*
    - Bacteria
      - *Pseudomonas savastanoi*
      - *Bacillus subtilis*
      - *Aliivibrio fischeri*
      - *Escherichia coli*
      - *Pseudomonas syringae*
      - *Xanthomonas campestris*
      - *Erwinia carotovora/herbicola*
      - *Cryptococcus neoformans*
    - Yeast
      - Planar yeast estrogen screen (pYES)

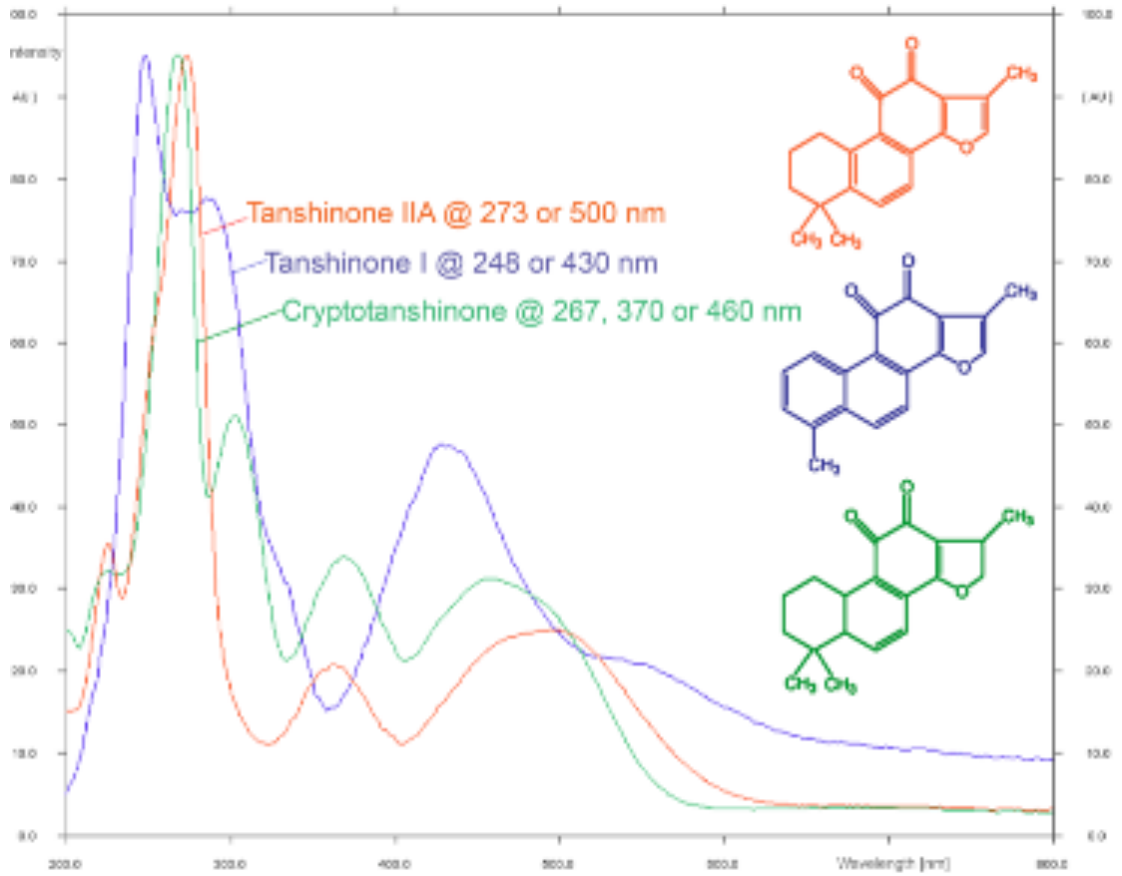
G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, in print

Quantitation of tanshinons in Chinese salvia



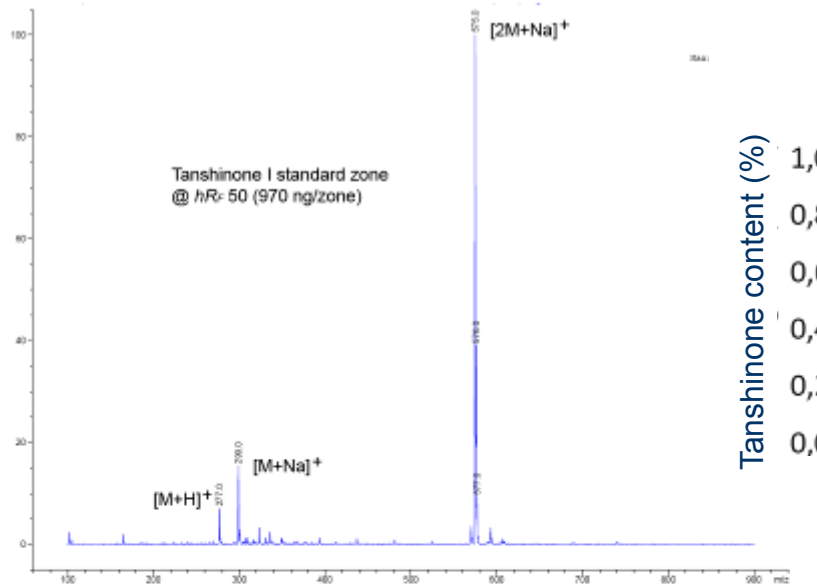
G. Morlock Food Science JLU Gießen

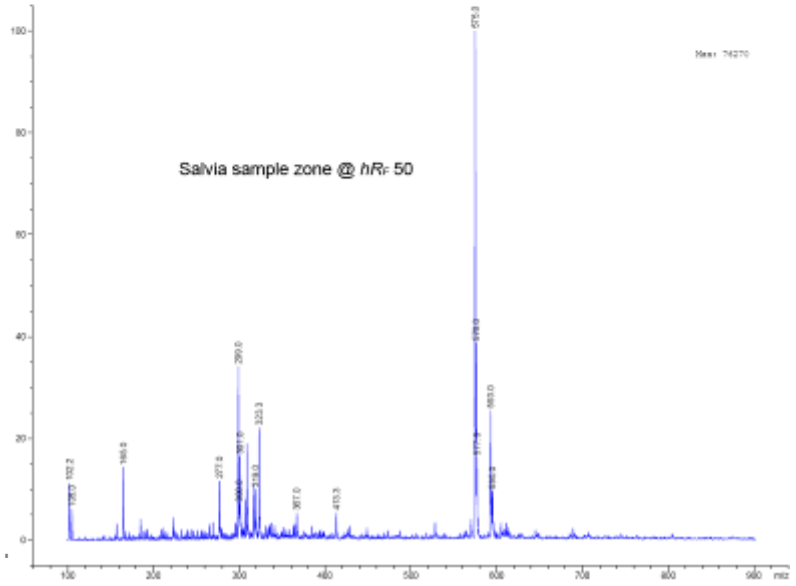
Quantitation of tanshinons in Chinese salvia

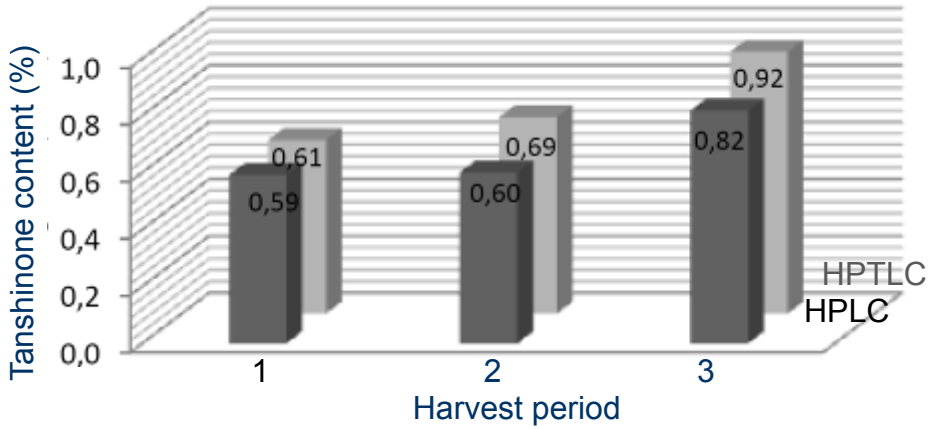


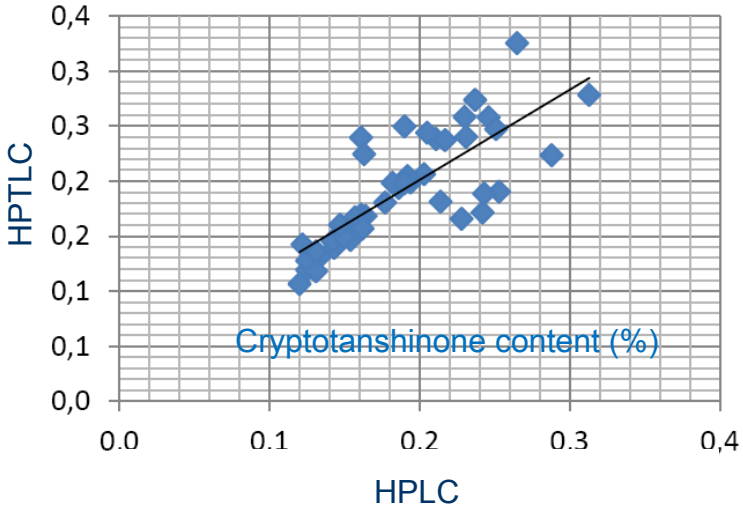
G. Morlock Food Science JLU Gießen

Confirmation by MS and method comparison

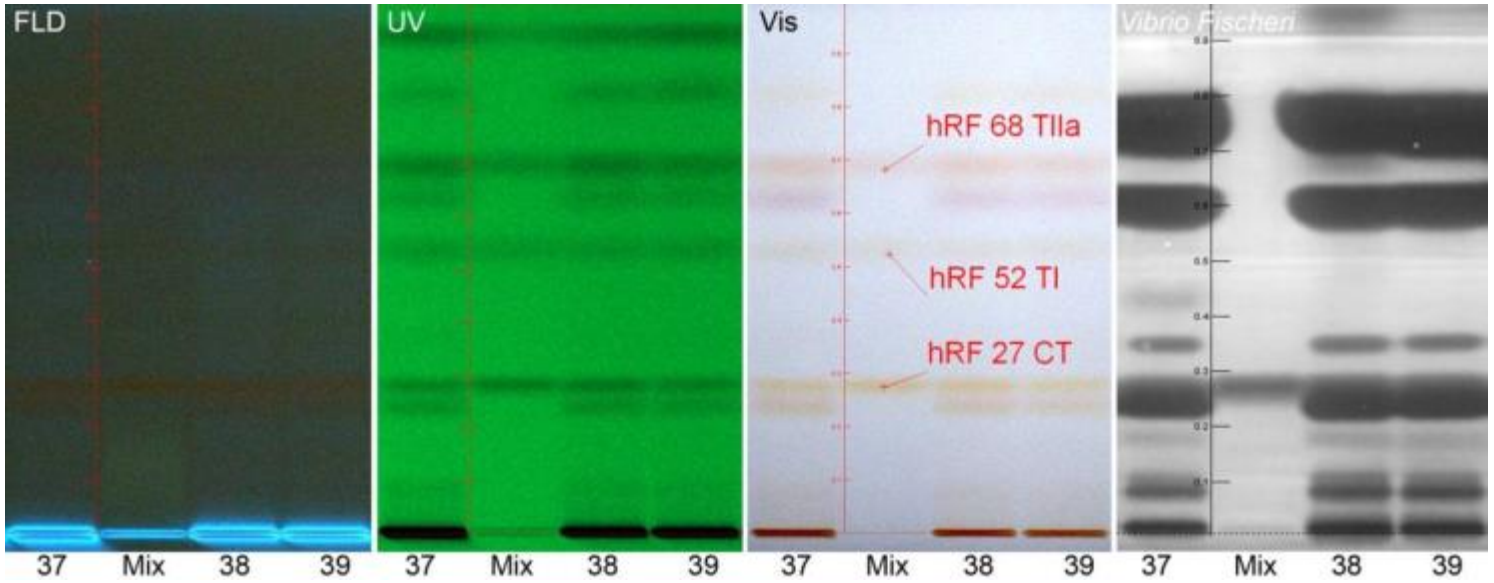






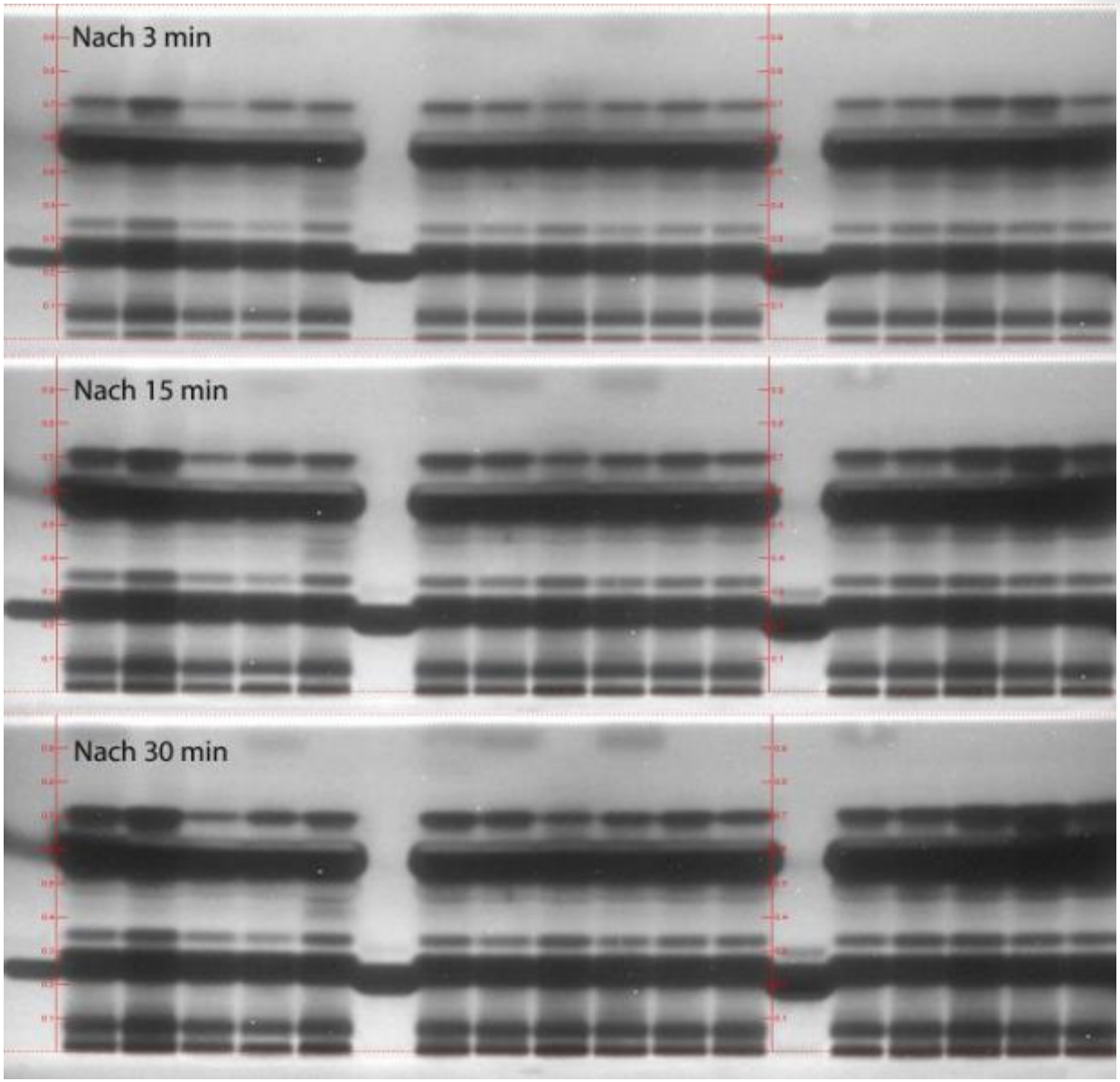


Bioactivity of single compounds

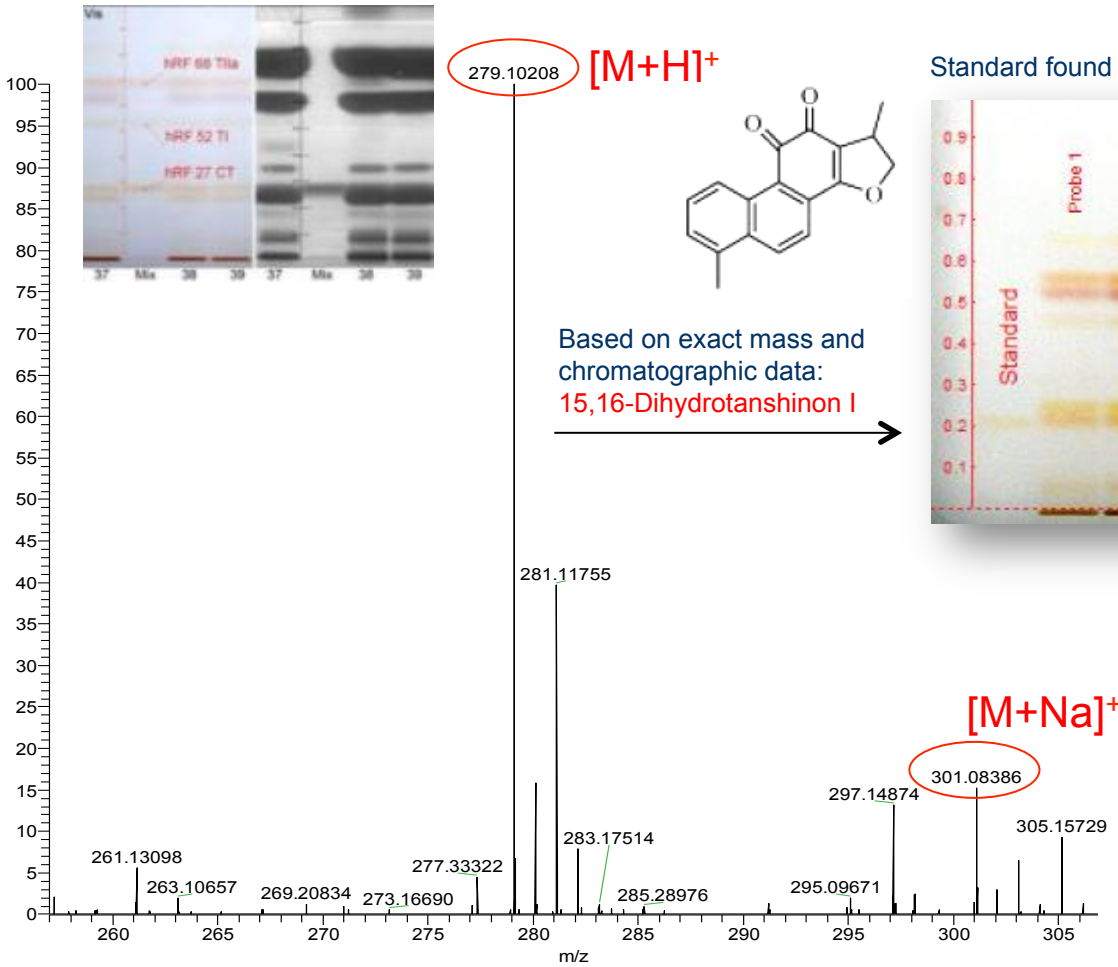


G. Morlock Food Science JLU Gießen

Bioactivity of single compounds



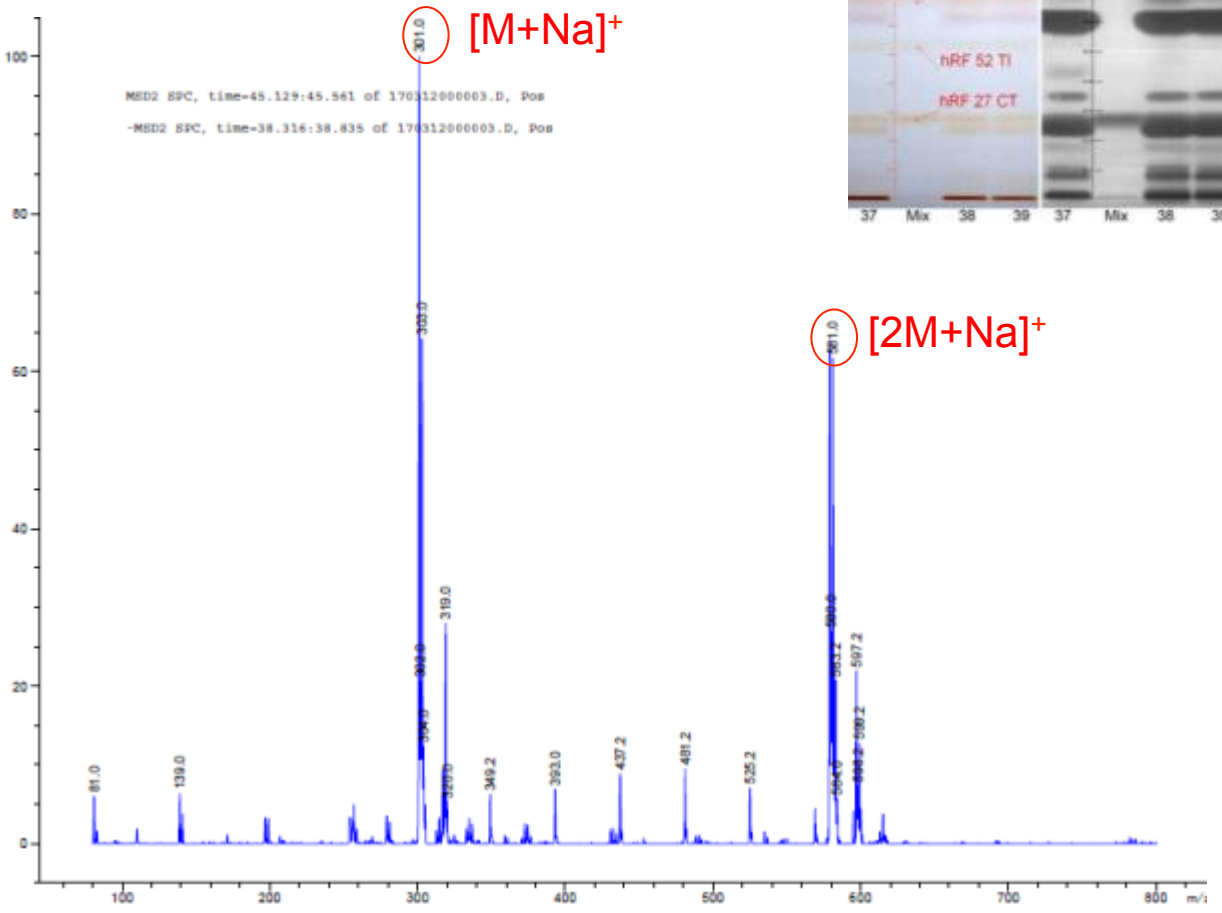
Unknown bioactive compound (below CT)



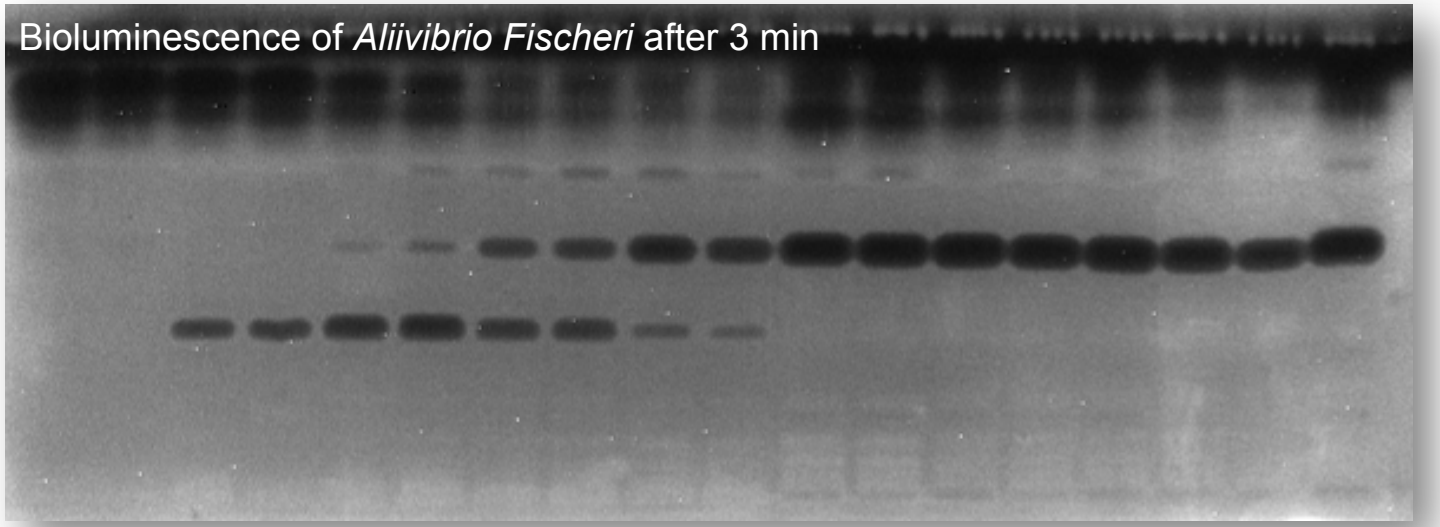
G. Morlock, T. Sung, B. Honermeier, in preparation

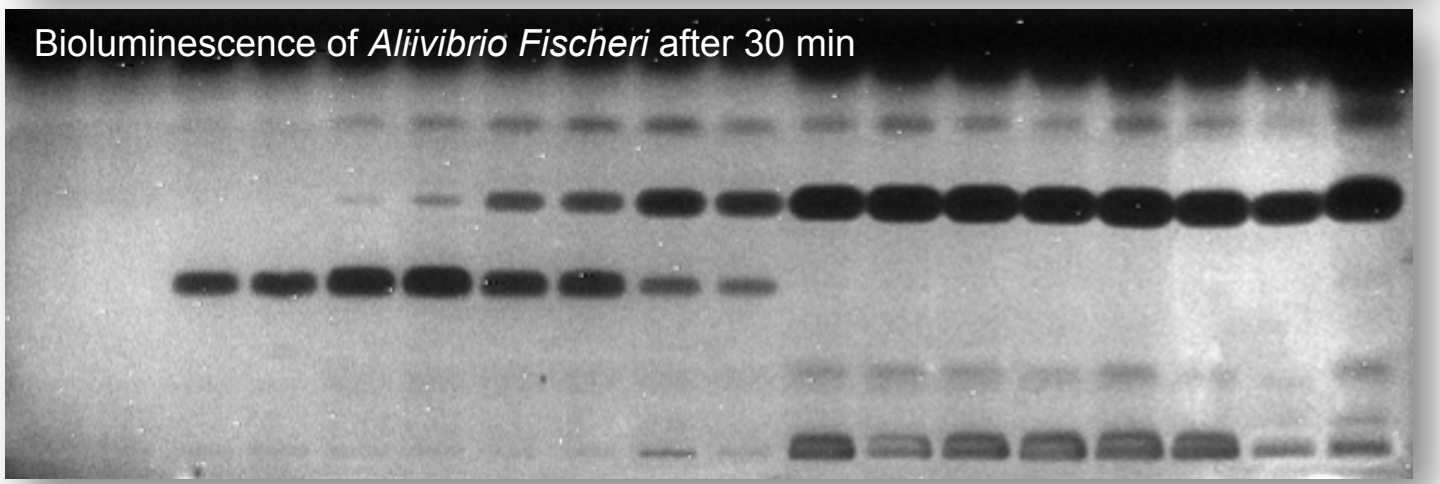
Unknown bioactive compound (below CT)

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



Bioactive compounds in *Basidomycetes*





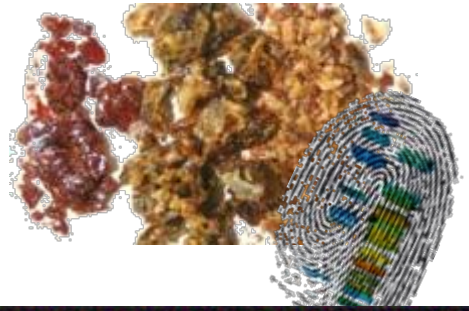
T. Shen, H. Zorn, G. Morlock, in preparation

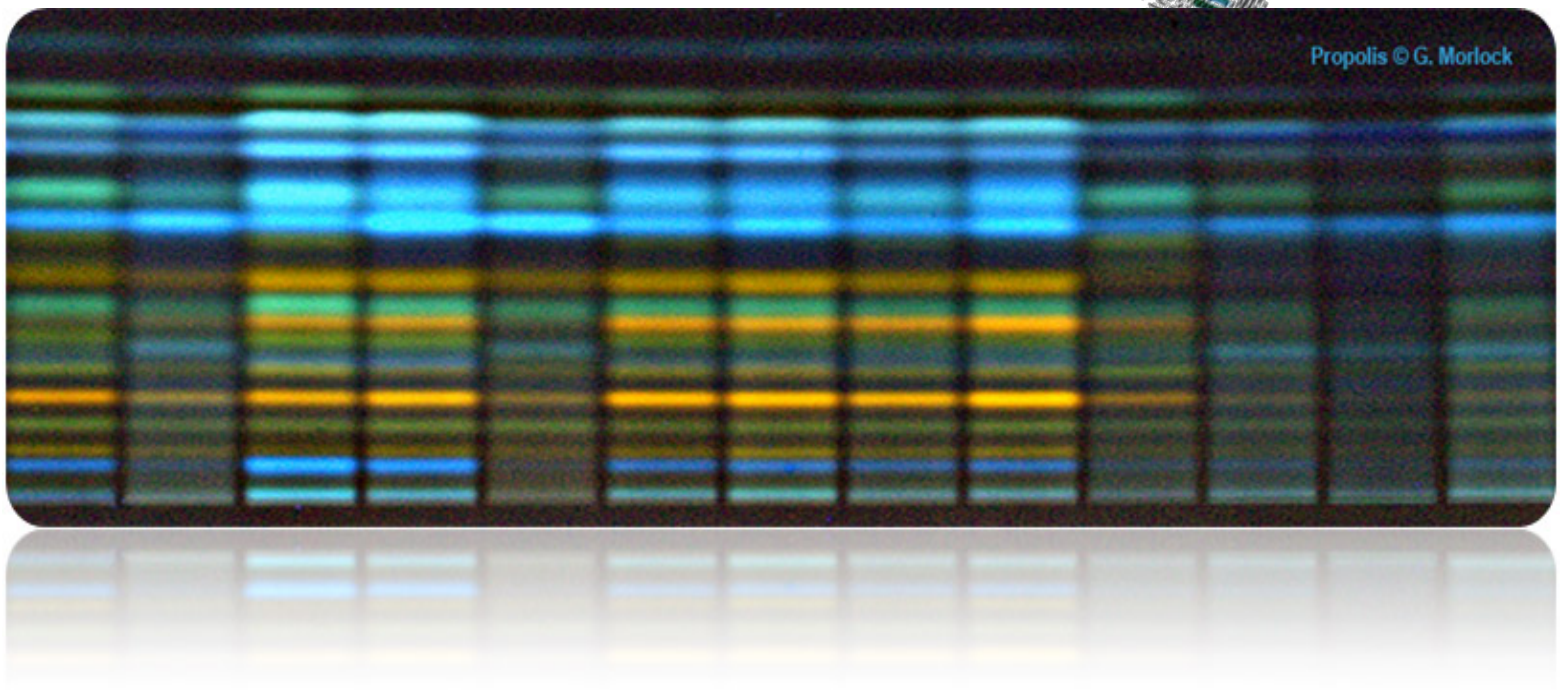
Fingerprint of phenolic compounds in propolis

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

→ 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

G. Morlock Food Science JLU Gießen

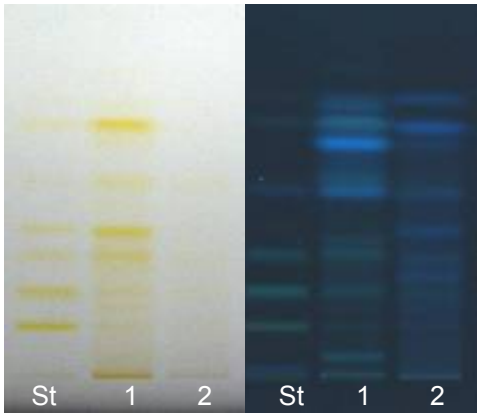
Fingerprint of phenolic compounds in propolis

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

Fast characterization of samples by HPTLC (chemical derivatizations)

Visible zones

Natively fluorescent zones (366 nm)



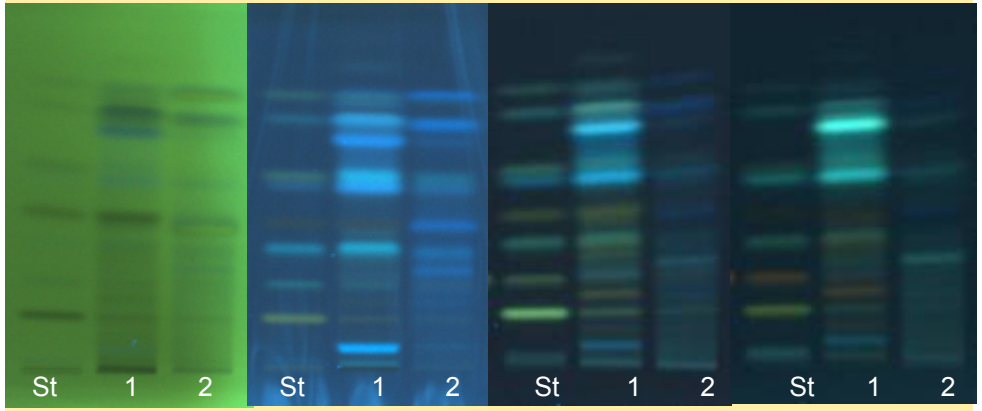
Selective derivatizations

Lipophilic zones → berberine (366 nm)

Flavonoids → $AlCl_3$ (366 nm)

Flavonoids → Neu (366 nm)

Flavonoids → Neu/PEG (366 nm)

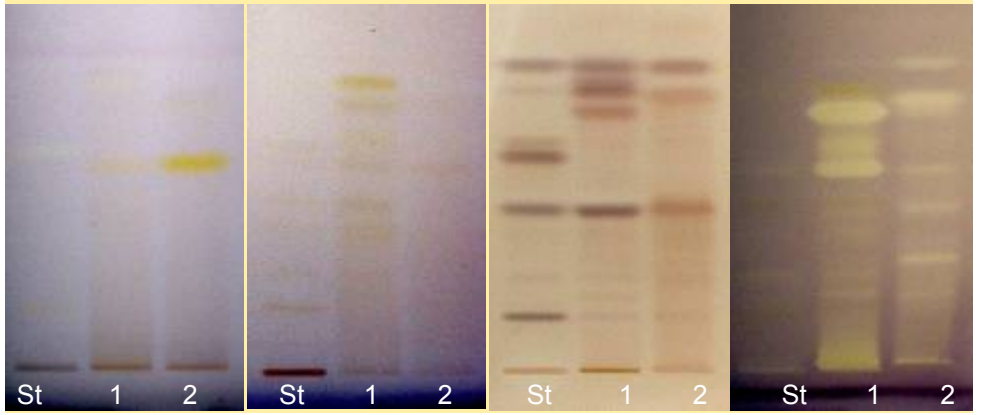


Carbohydrates → DPA

Amino acids → Ninhydrin

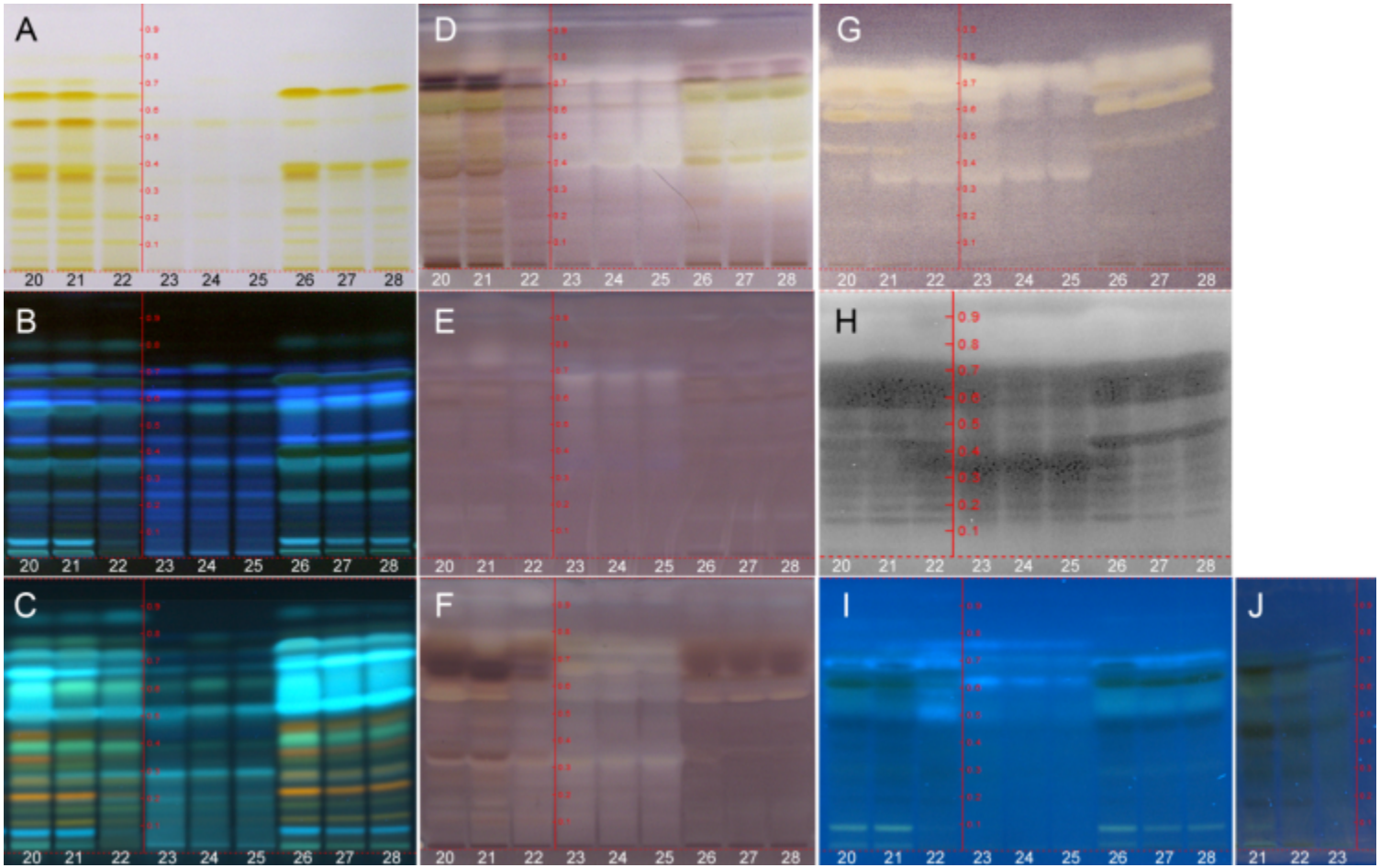
Phenolics → Fast blue salt

Antioxidatives → DPPH*



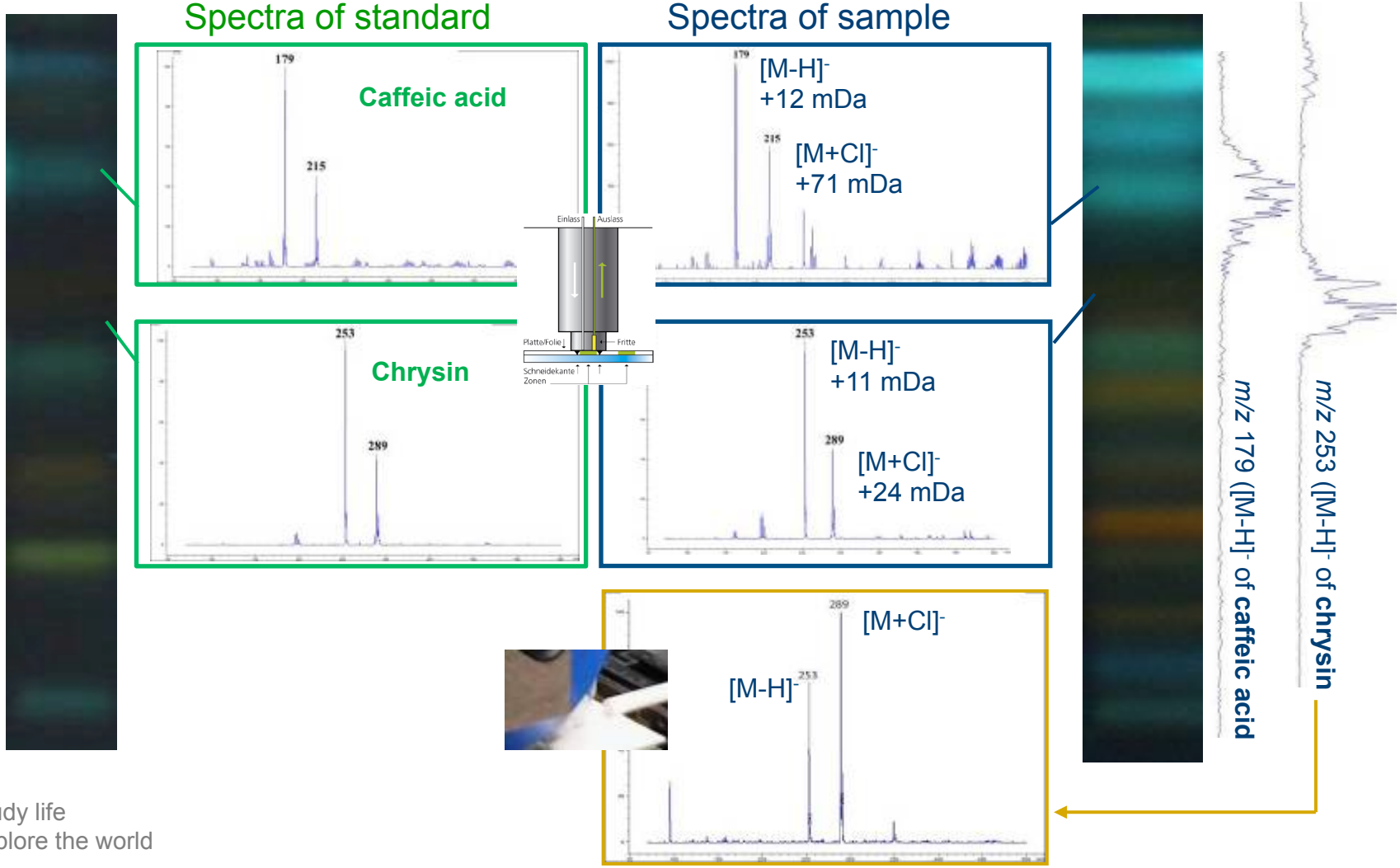
G. Morlock Food Science JLU Gießen

Effect-directed analysis of propolis

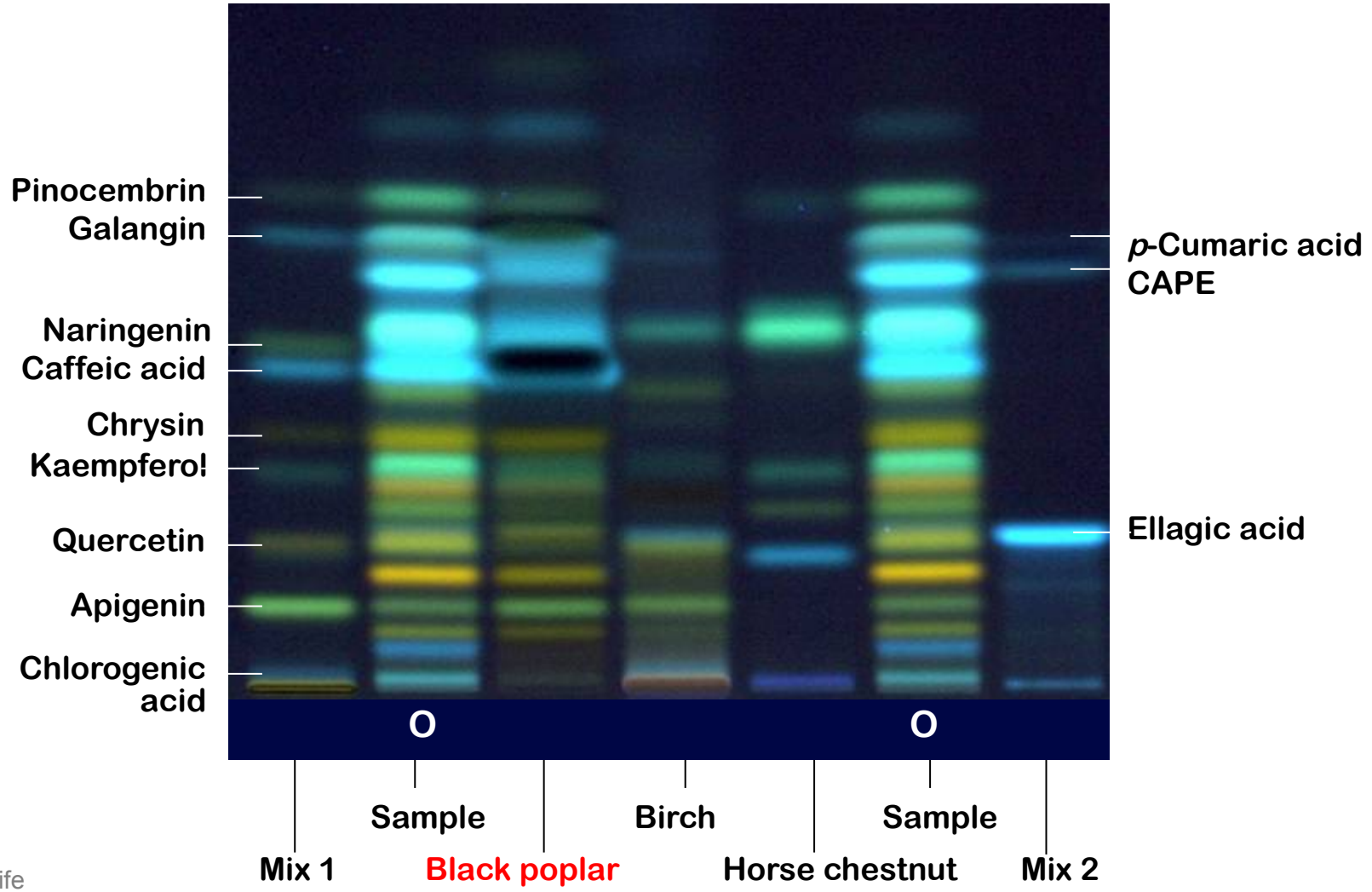


S. Hage, G. Morlock, in preparation

Confirmation of marker compounds by MS



Plant origin of O-type?

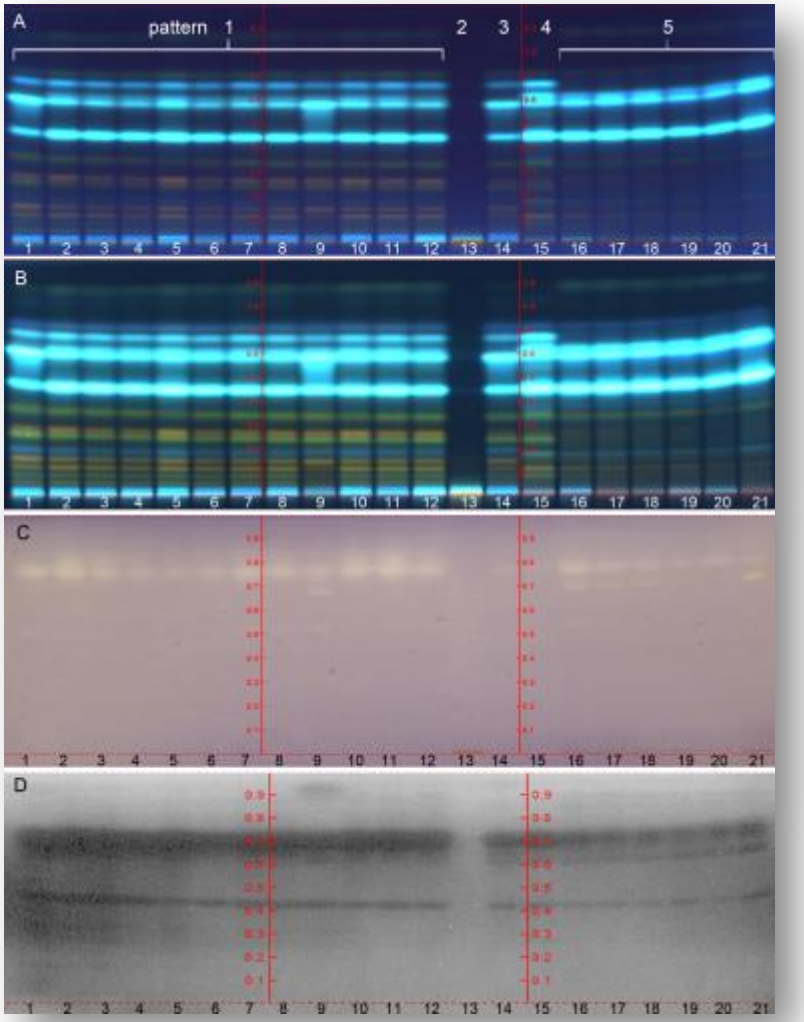


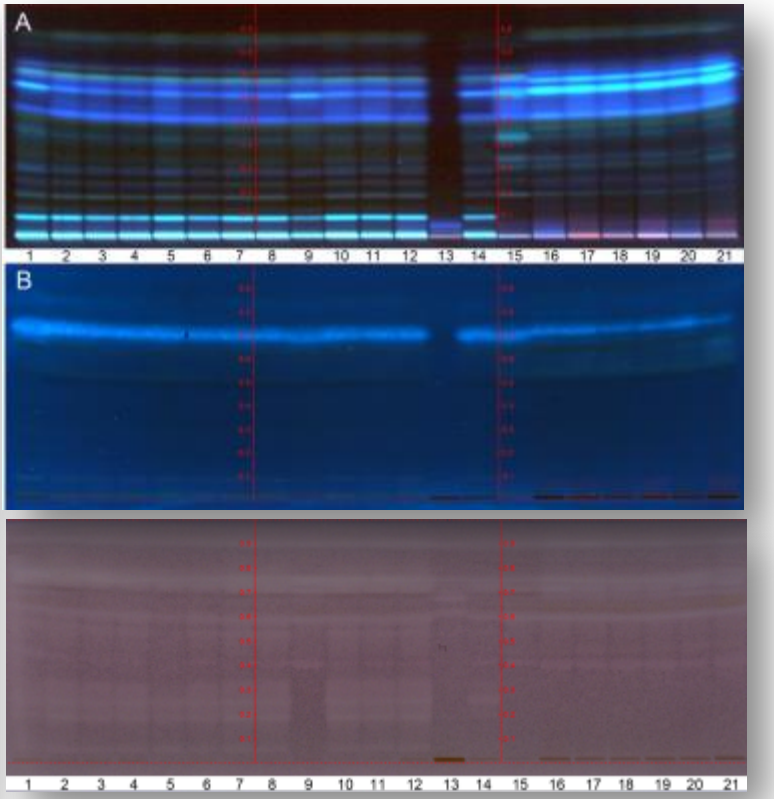
G. Morlock Food Science JLU Gießen

Salicaceae bud extract samples

Poster P-64

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

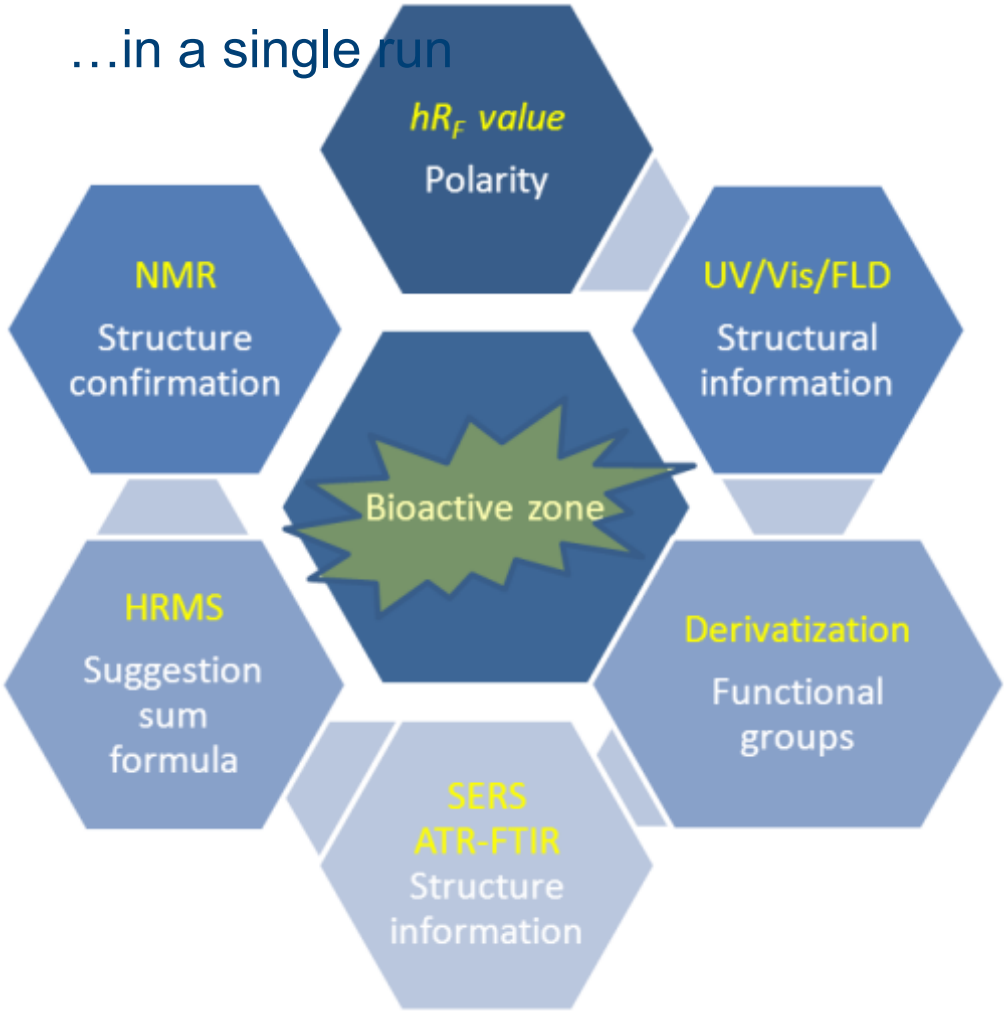




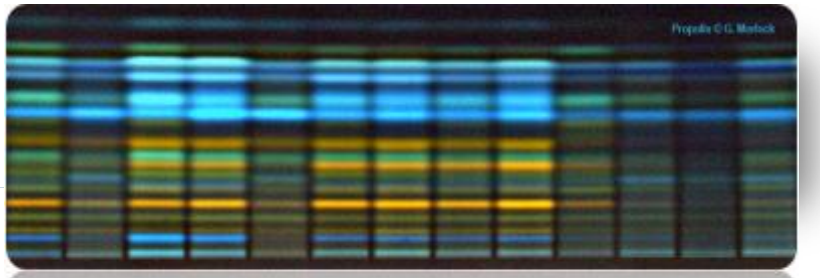
S. Hage, G. Morlock, in preparation

From bioactive zone to sum formula

…in a single run



G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, in print



JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

Thanks to
- CAMAG
- Merck Millipore
- Bruker
- IonSense
- KR Analytical
- Advion

Food Science