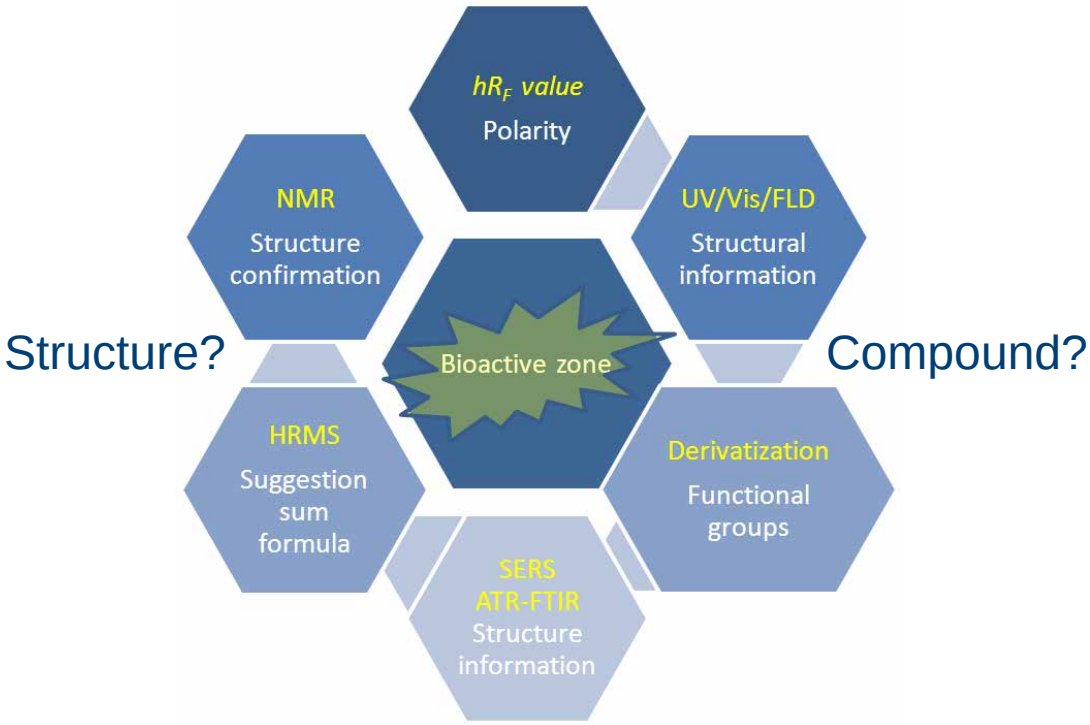
Latest developments in bioassays combined with planar chromatography

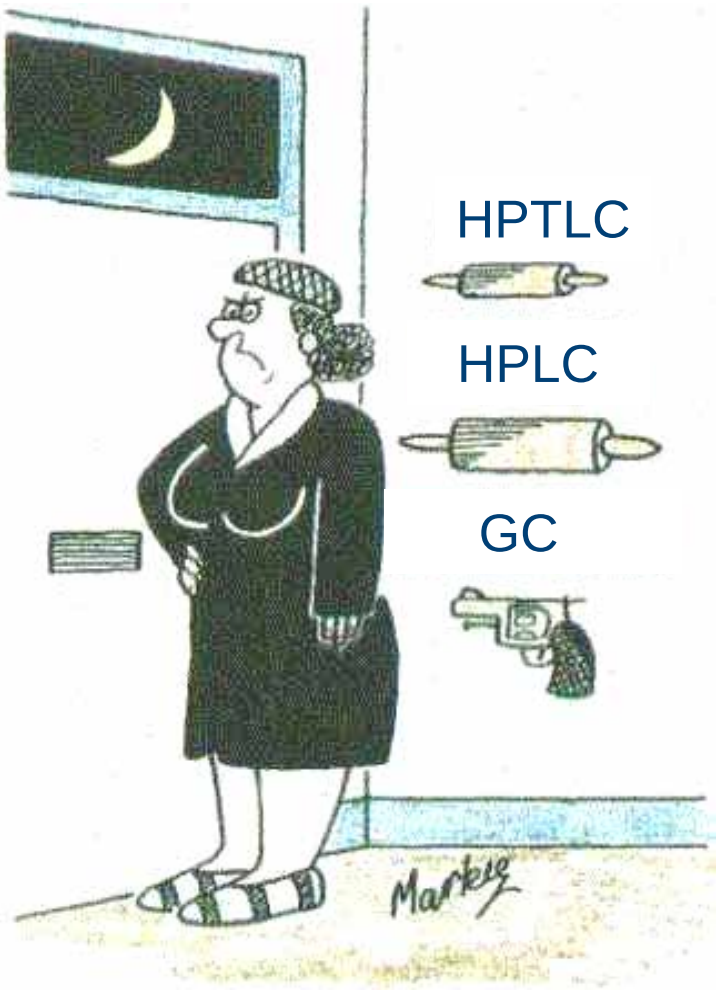
HPTLC coupled to (bio)assays



Gertrud Morlock, Chair of Food Science
Justus Liebig University Giessen

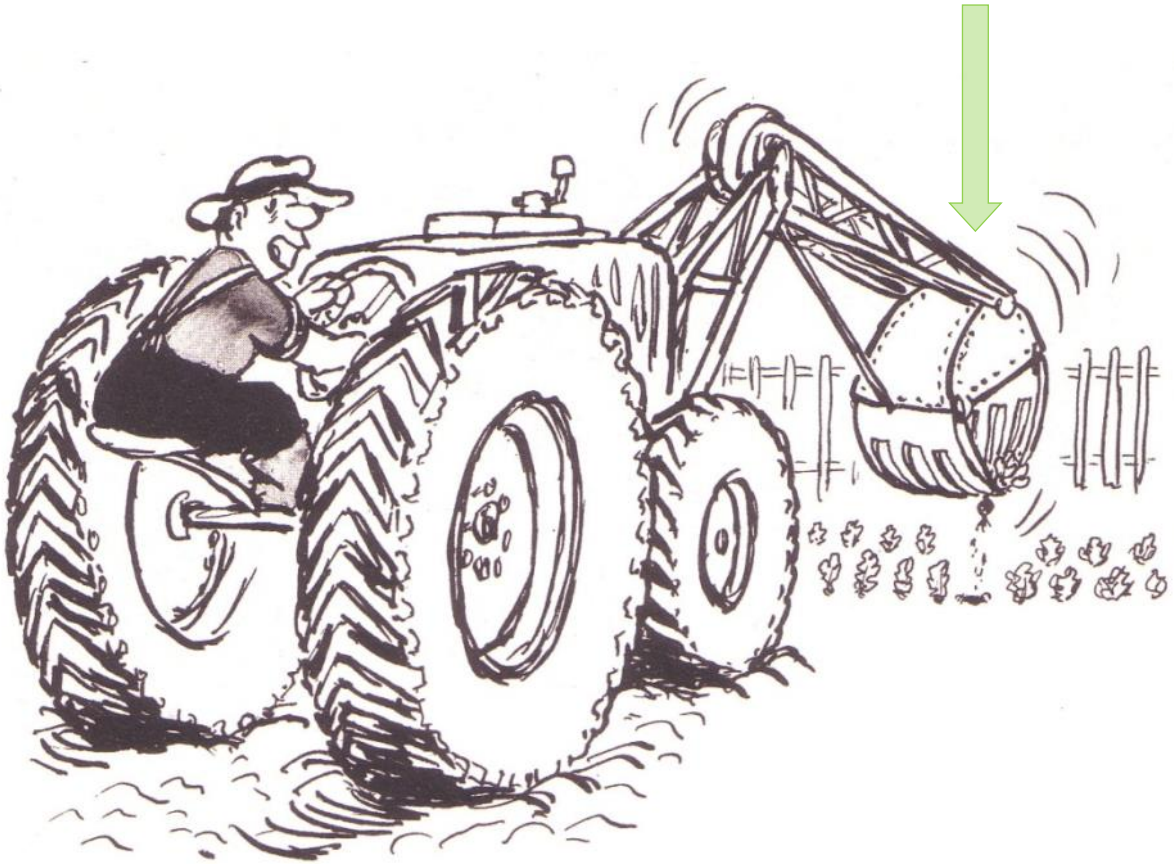
Use the best method…

Use the best method for the given task!



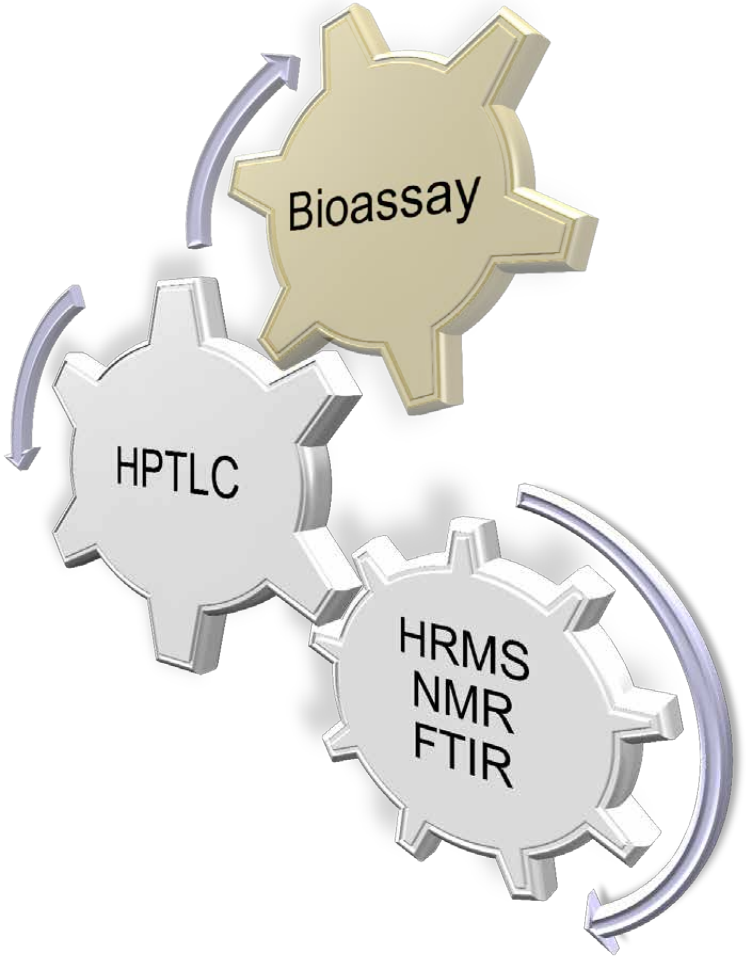
…not to end like this!

Radish harvest

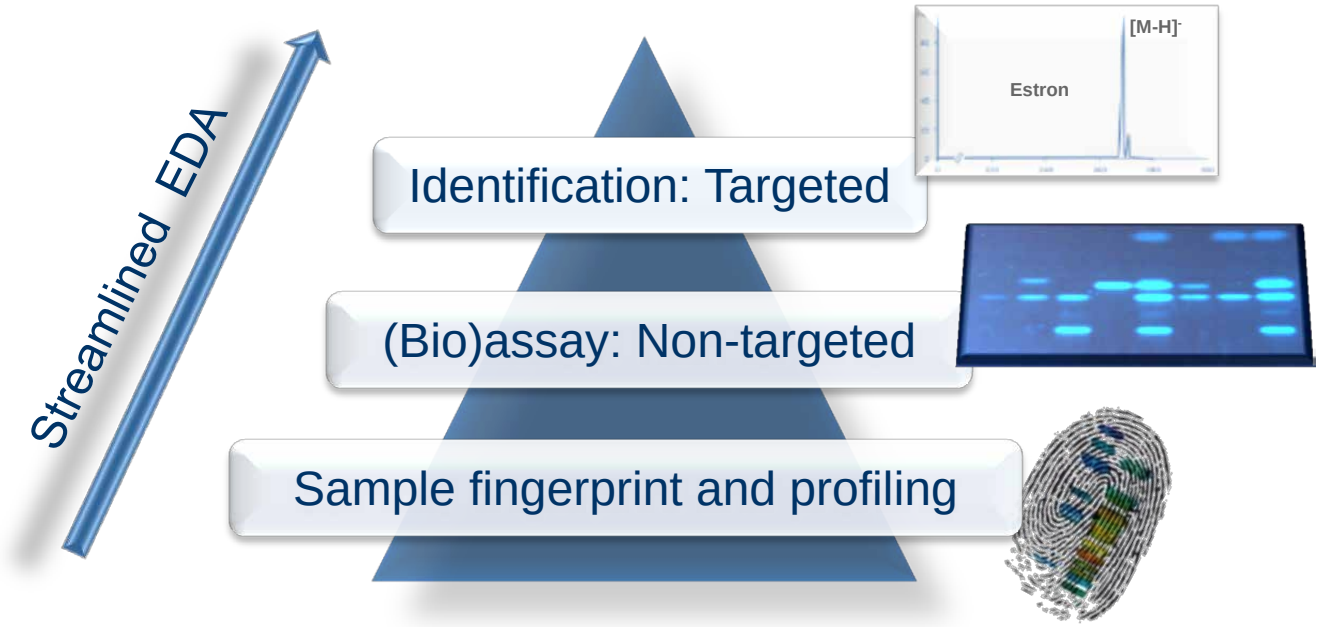


Loriot`s Grosser Ratgeber, 1968

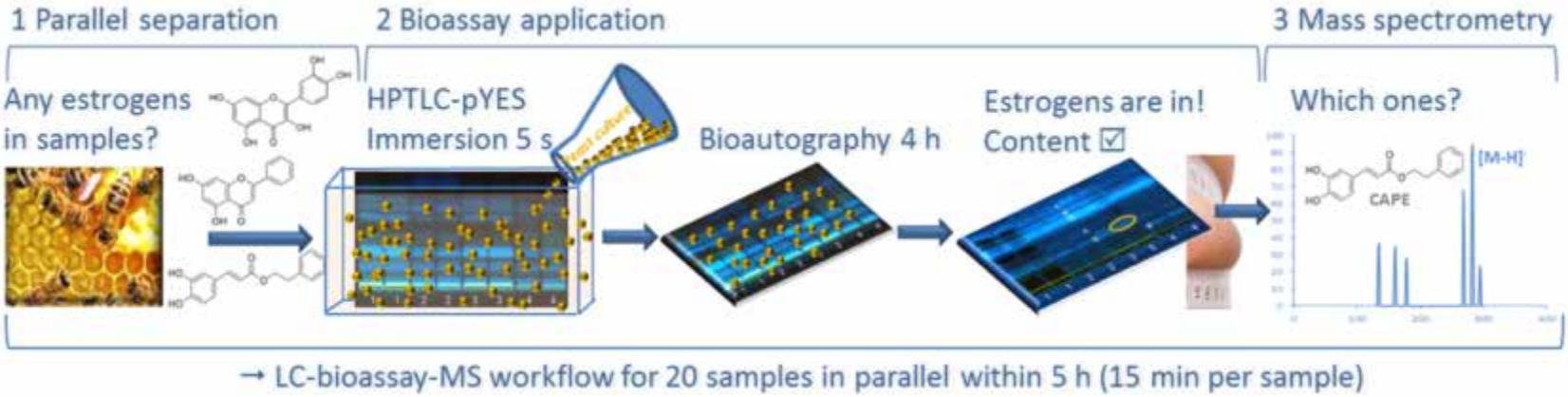
HPTLC-bioassay-HRMS/NMR/FTIR

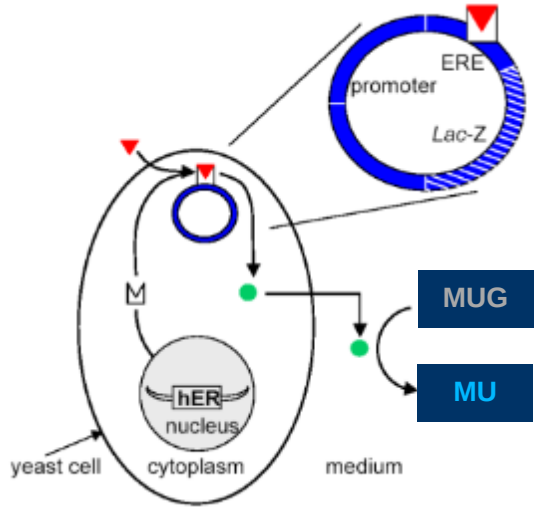


Workflow


Streamlined EDA
Identification: Targeted
(Bio)assay: Non-targeted
Sample fingerprint and profiling
Estron
[M-H]-

Detection of estrogen-effective compounds





Modified from draft of pYES expert group

Planar yeast estrogen screen (pYES)

→ using human estrogen receptor hERα

→ in *Saccharomyces cerevisiae*

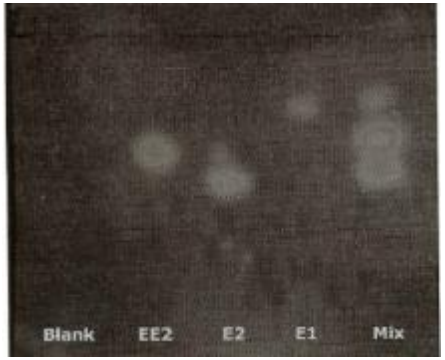
1. Routledge & Sumpter, Environ. Toxicol. Chem. 15 (1996) 241
2. McDonnell *et al.*, J. Steroid Biochem. Mol. Biol. 39 (1991) 291

→ blue fluorescent 4-methylumbelliferone

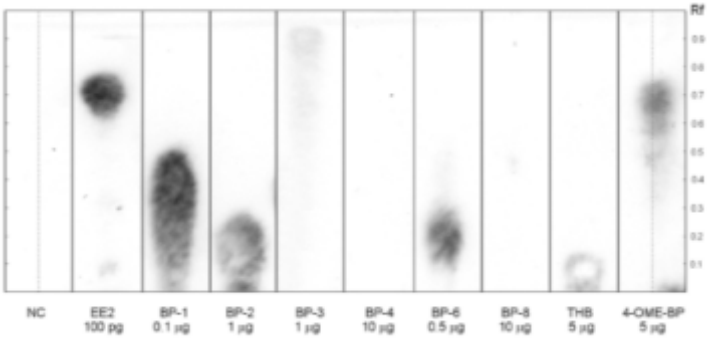
Challenge: avoid zone broadening

Goodall & Levi, *Nature* 158, 675–676 (1946)

HPTLC-pYES

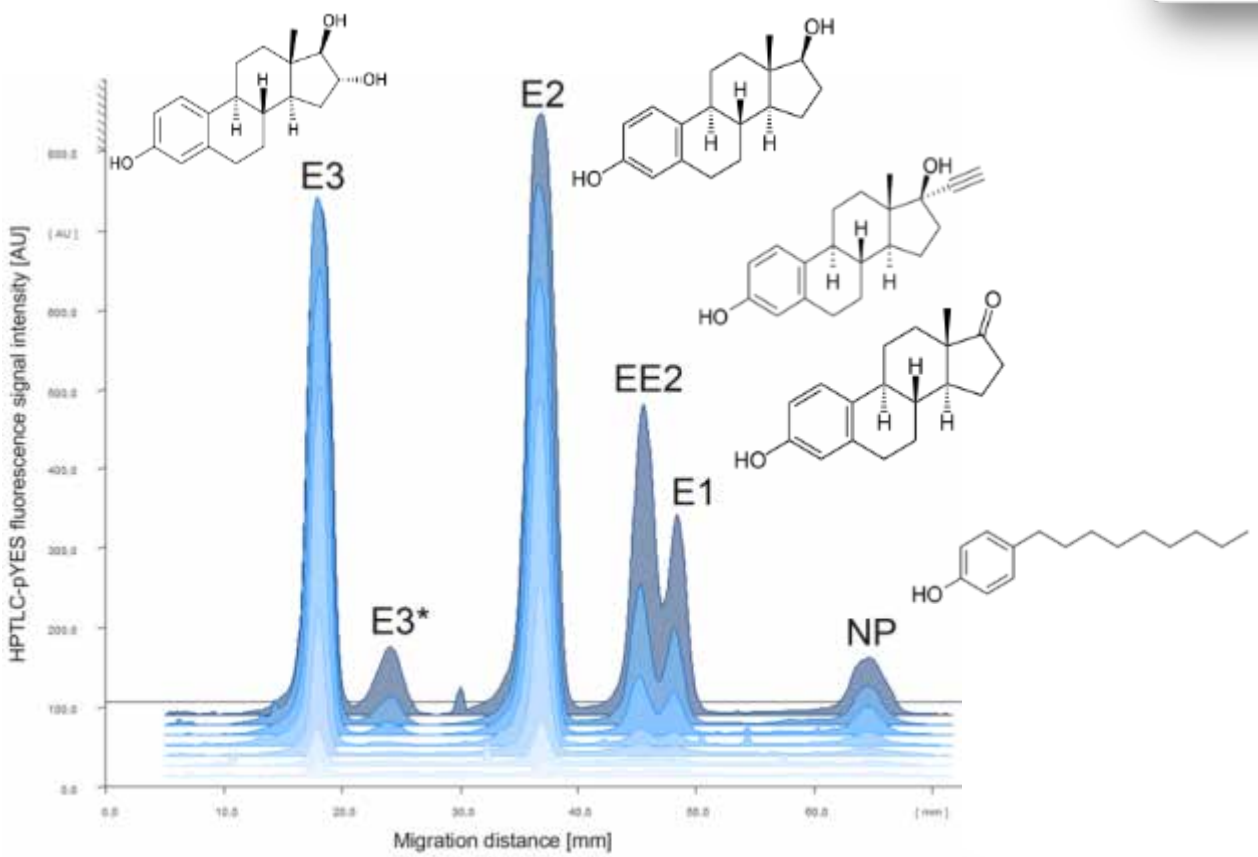


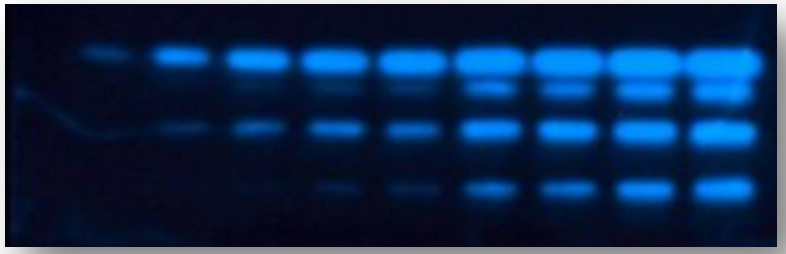
A. Schönborn, A. Grimmer, *J Planar Chromatogr* 26 (2013) 402-408



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

Biological detection



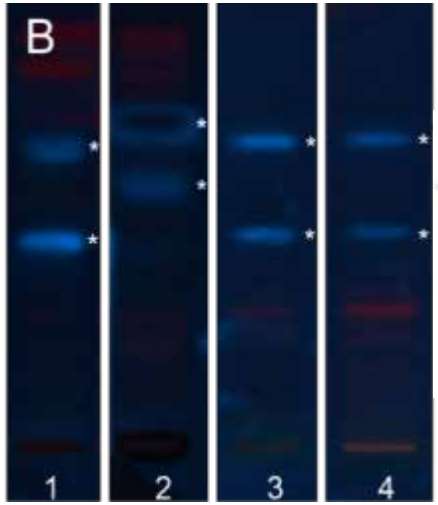


	pg/band
E1	25 – 1000
EE2	0.5 – 20
E2	0.5 – 20
E3	25 – 1000

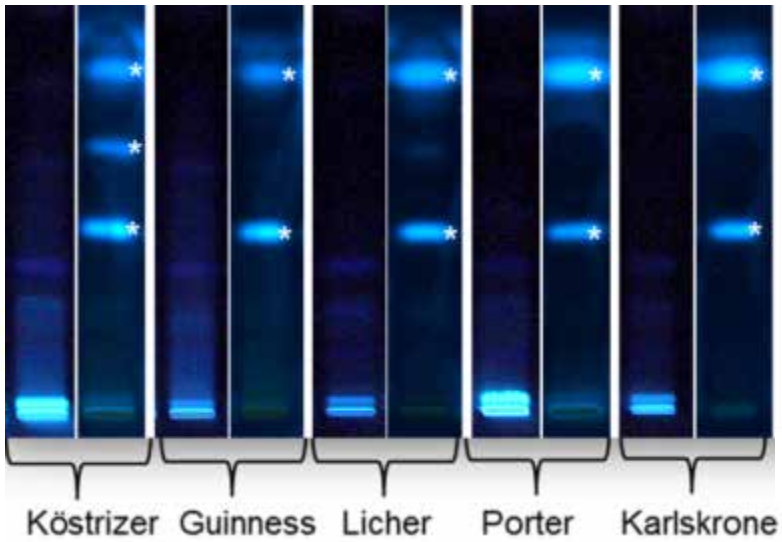
I. Klingelhöfer, G. Morlock, *J Chromatogr A* 1360 (2014) 288-295

Discovery in food

→ Spices



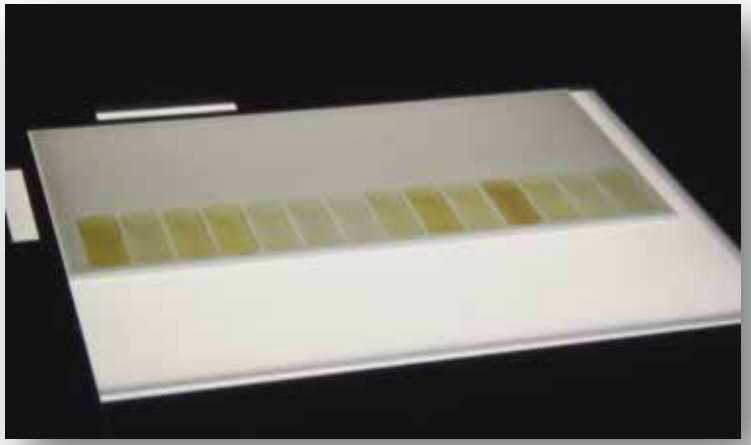
→ Beer samples

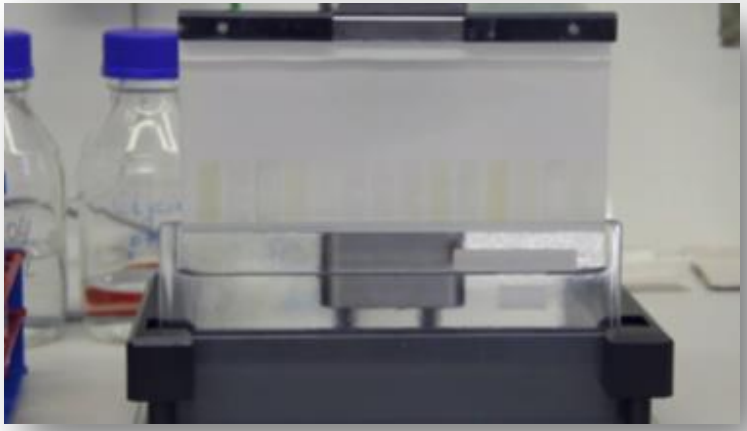


I. Klingelhöfer, G. Morlock, *J Chromatogr A* 1360 (2014) 288-295

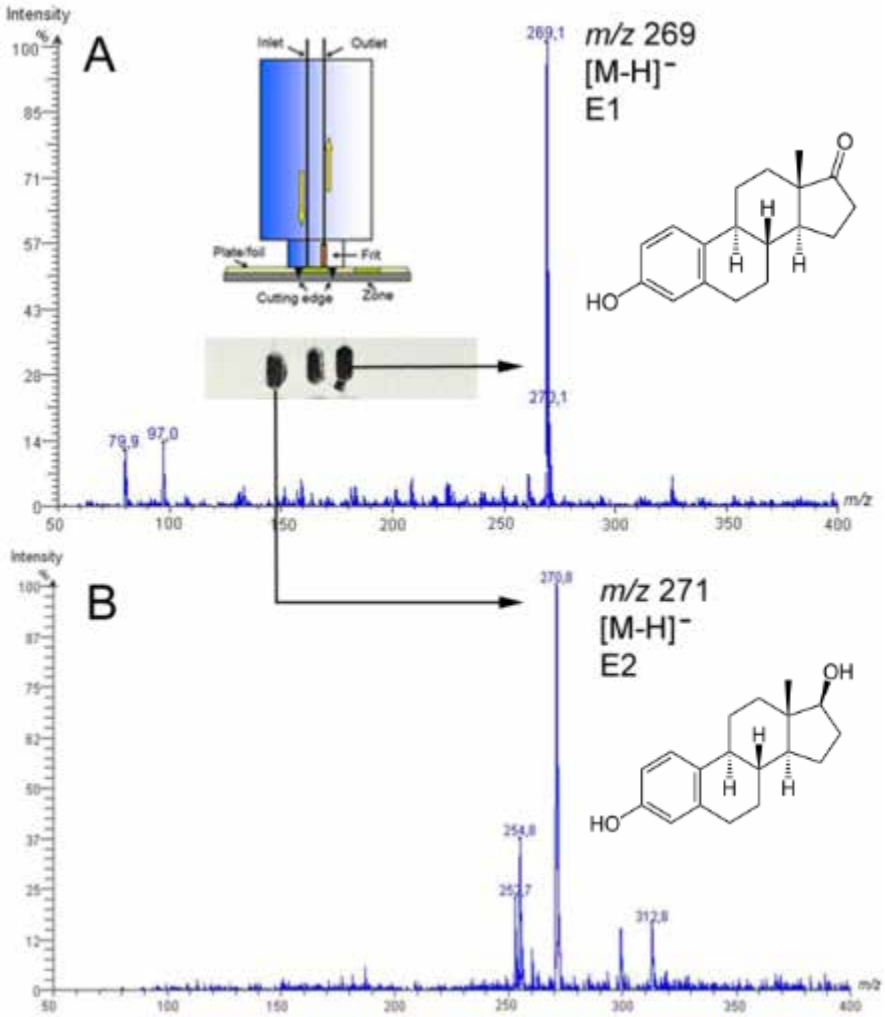
Estrogen-effective compounds in beer?

Video https://youtu.be/Q7AGuljcFvQ

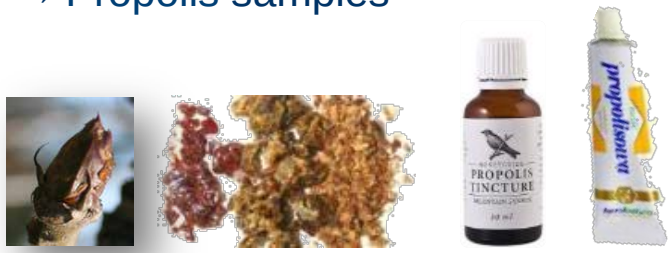


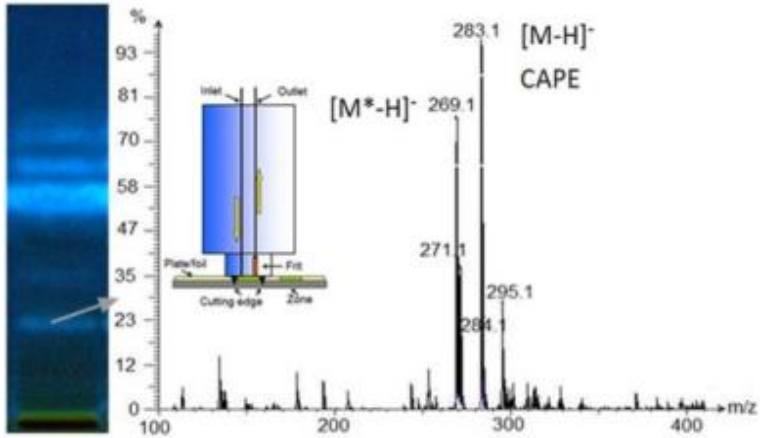


Confirmation by MS



→ Propolis samples

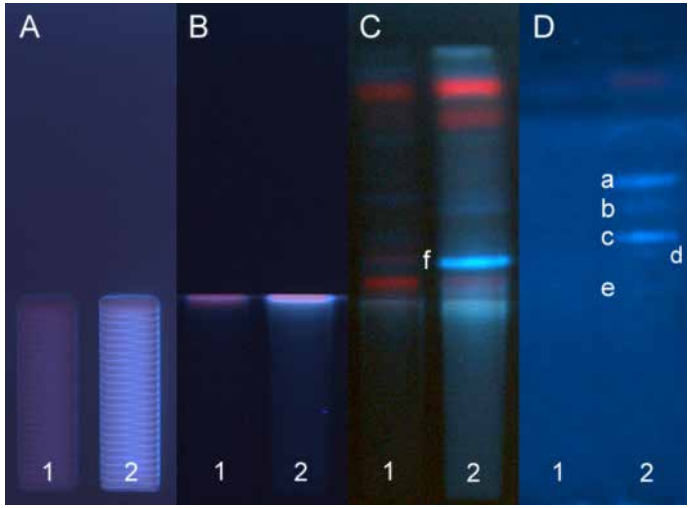


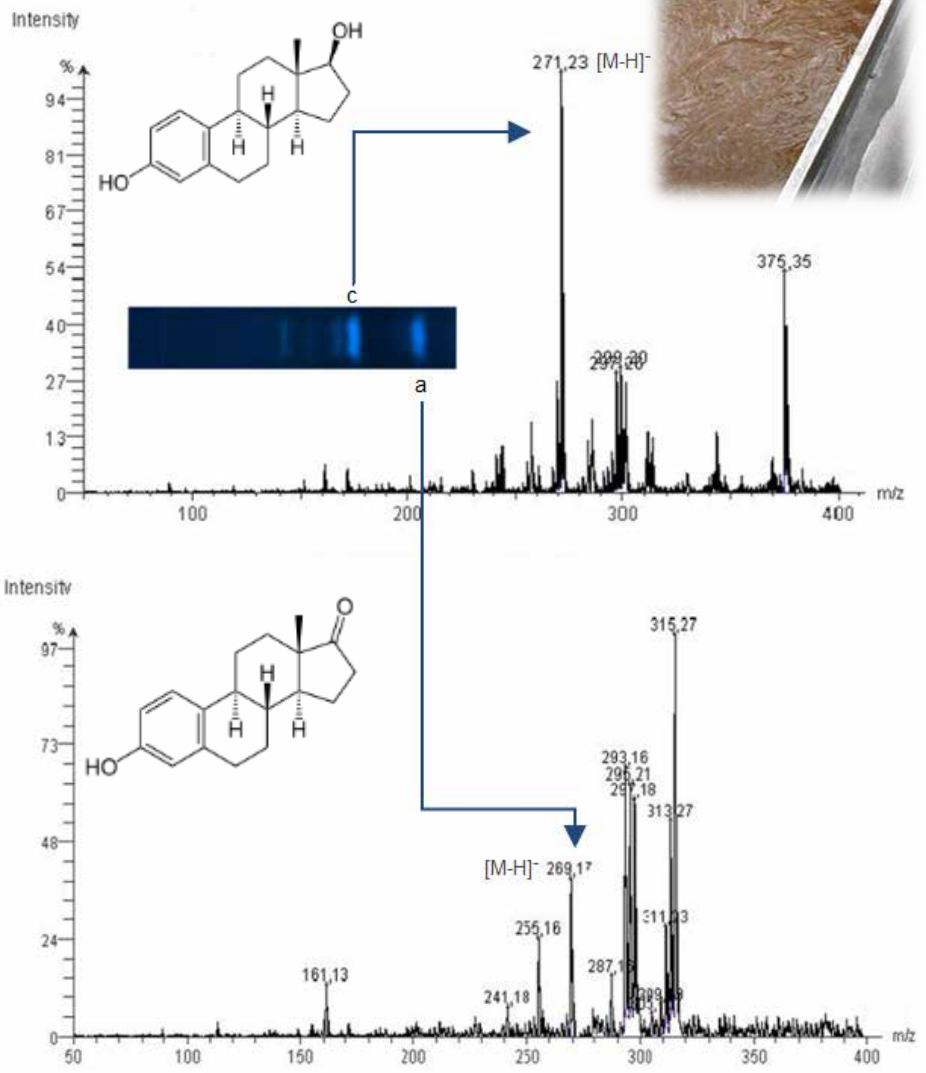


I. Klingelhöfer, G. Morlock, *J Chromatogr A* 1360 (2014) 288-295

G. Morlock, I. Klingelhöfer, *Anal Chem* 86 (2014) 8289–8295

Discovery in surface/waste water





Substance	LOD [ng/L]	LOQ [ng/L]
E2	1.0	2.5
EE2	2.5	5.0
E1	4.3	15.0
E3	75.0	250.0
BPA	1.6×10^3	5.0×10^3
NP	15.0×10^3	65.0×10^3

I. Klingelhöfer, G. Morlock, *Anal Chem* 87 (2015) 11098−11104

Bioquantitation (by microorganisms´ response)

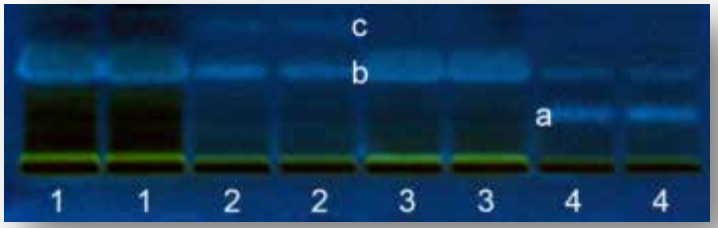
Content [ng/L]	E2	E1	E3
STP influent	10.1	40.5	98
	6.7	17.6	nd
	3.1	12.4	nd
	4.9	36.4	150
	12.6	49.7	210
STP effluent (x5)	nd	nd	nd
Lückebach	1.6	20.5	nd
	8.3	12.7	nd
Flachsbach	6.6	16.6	nd
	nd	nd	nd
Wetter (x2)	nd	nd	nd
Weidgraben	2.1	11.6	nd
	**	4.9	nd
Wieseck (x2)	nd	nd	nd

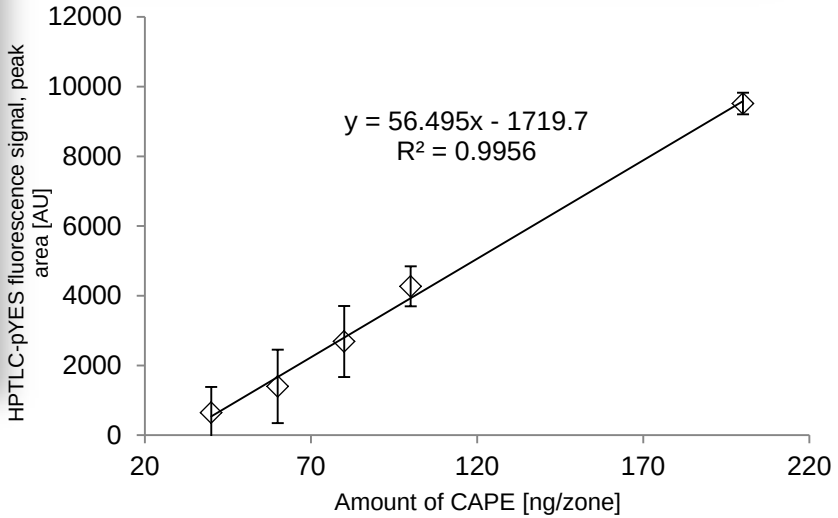
I. Klingelhöfer, G. Morlock, *Anal Chem* 87 (2015) 11098−11104

Bioquantitation of CAPE in propolis

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

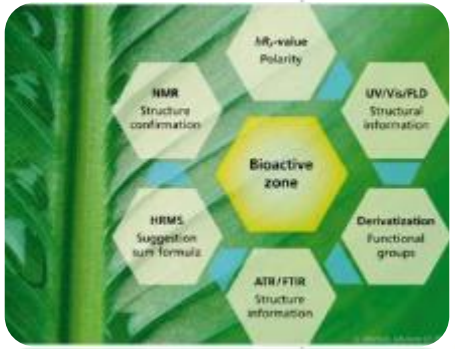
[1]µg/capsule, [2]µg/pastille, [3]µg/mL





G. Morlock, I. Klingelhöfer, *Anal. Chem*. 86 (2014) 8289–8295

Effect-directed link to the compound



TLC/HPTLC-EDA

- Microchemical derivatization
  - DPPH* reagent - radical scavenging test
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  - Bleaching of ß-carotene - antioxidant test
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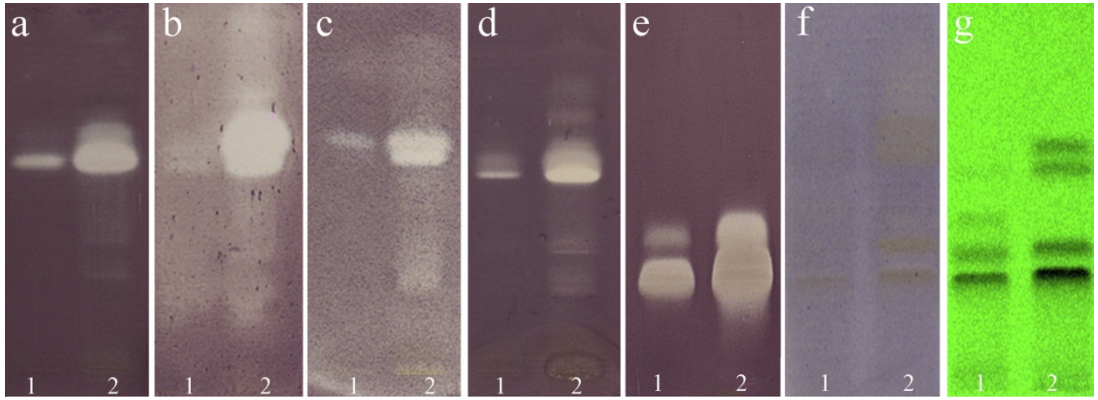
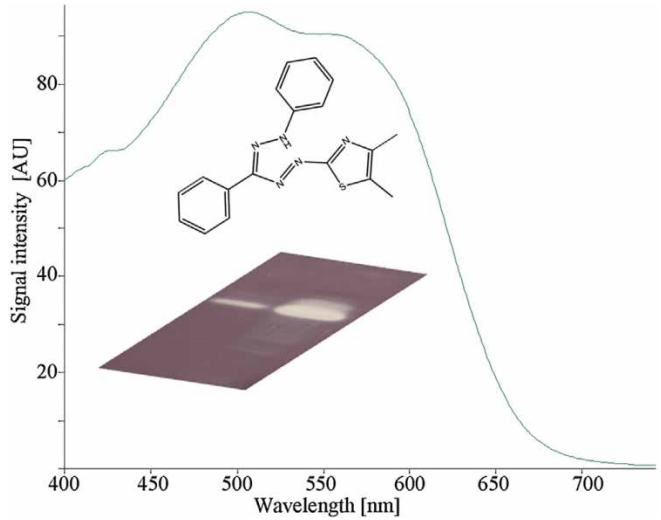
  - Tyrosinase oxidase assay

G. Morlock, *ACS Symposium Series* 1185 (2013) 101-121

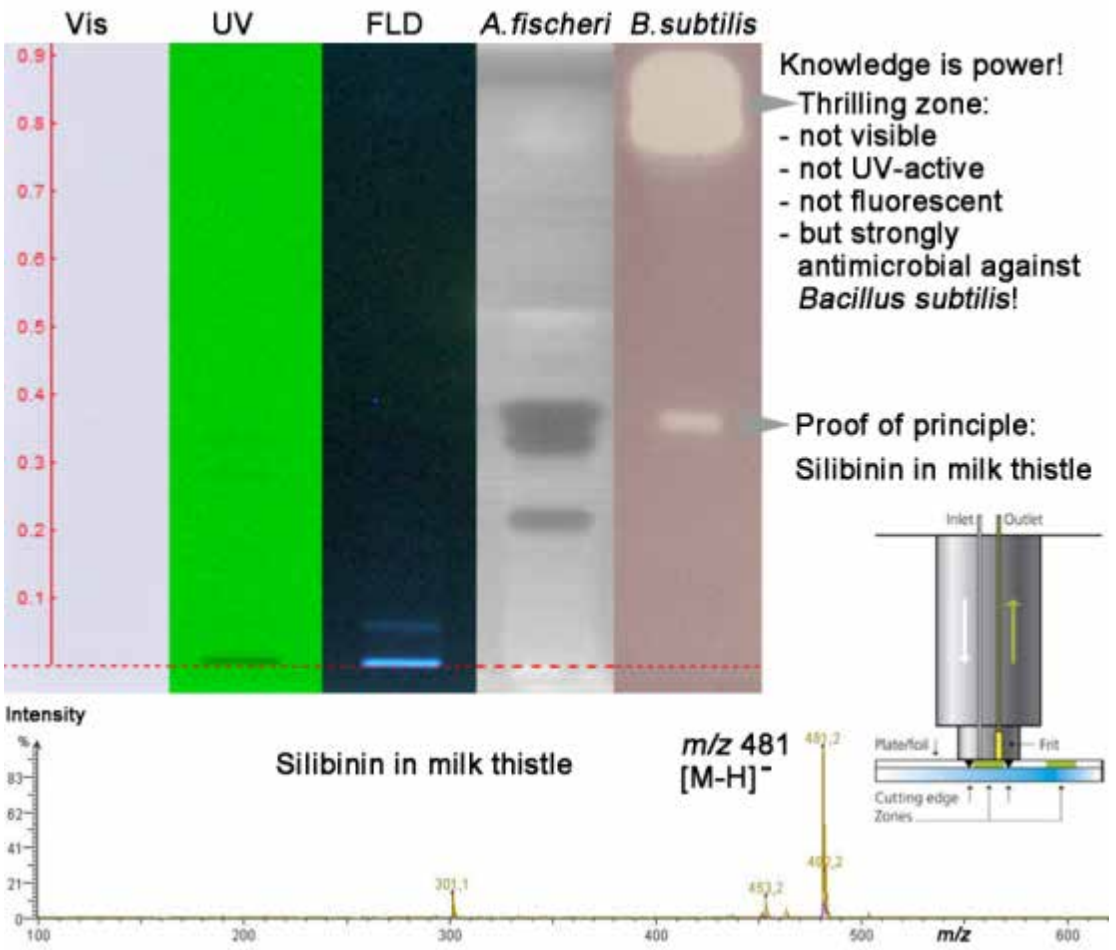
Optimization of *Bacillus subtilis* bioassay





M. Jamshidi, G. Morlock, J Chromatogr A 1420 (2015) 110–118

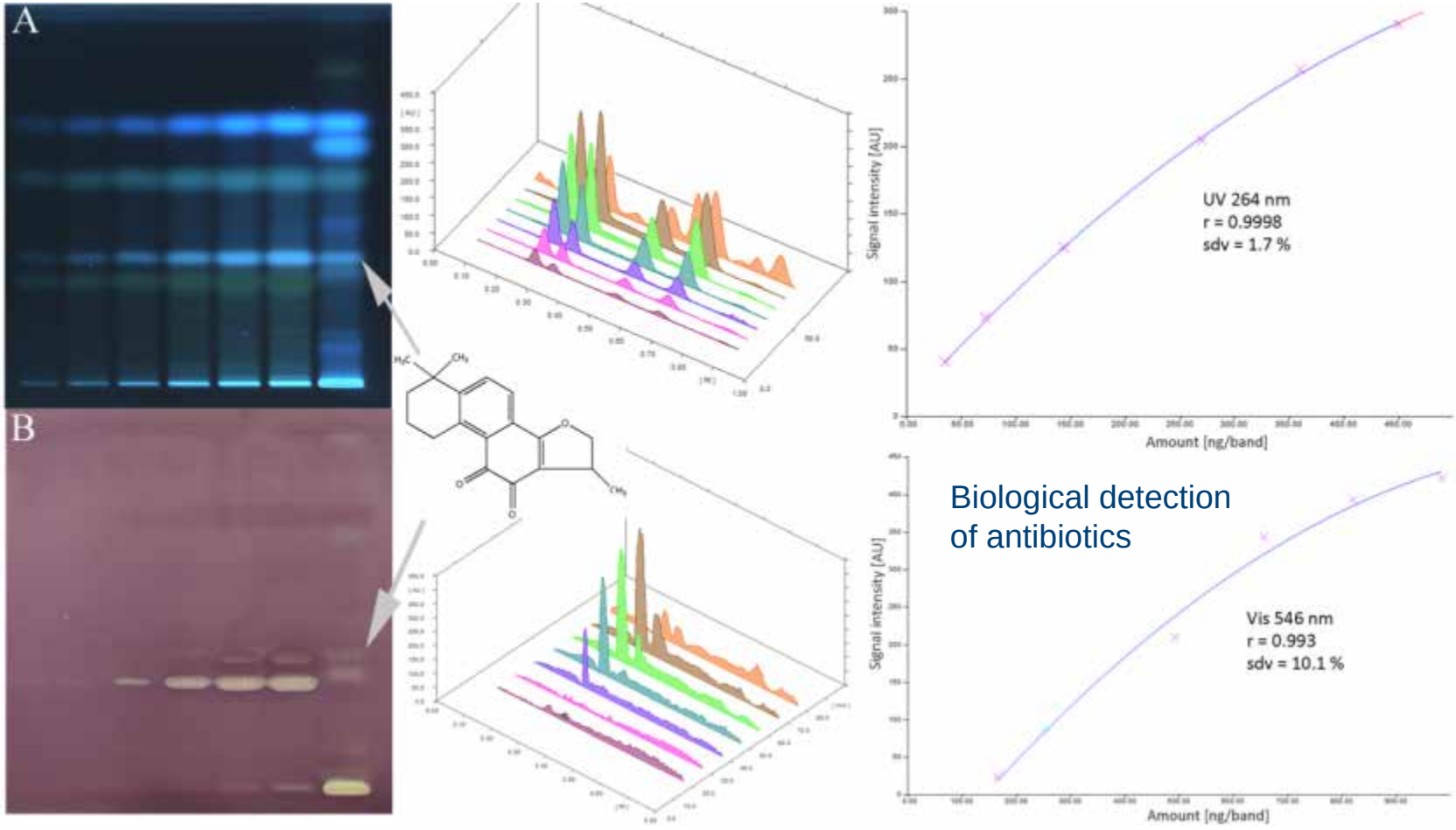
Antibiotic compound in stored milk thistle



G. Morlock, *The Analytical Scientist*, 27-04 (2015) 42-43

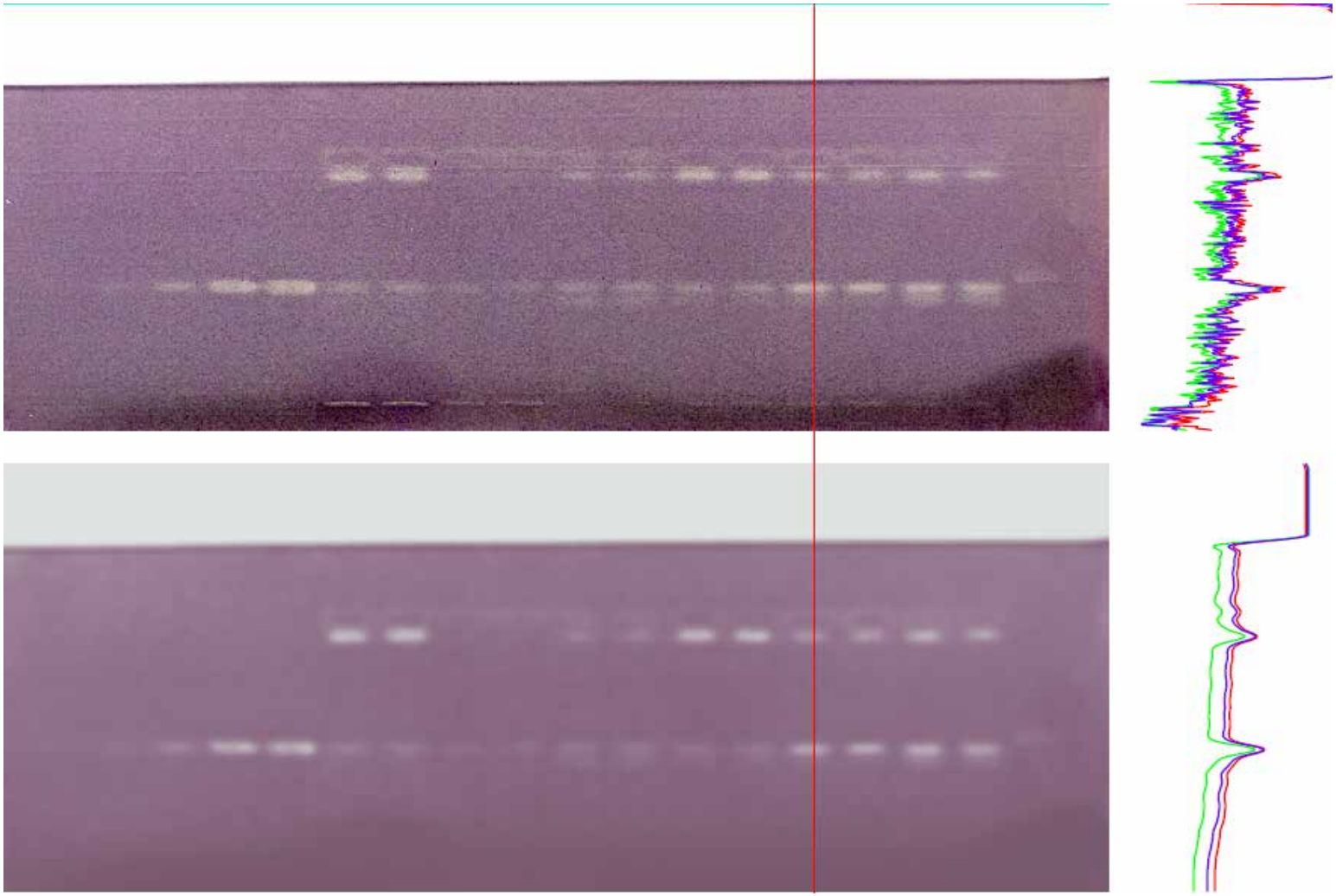
M.T. Taha, M.B. Krawinkel, G.E. Morlock, *J Chromatogr A* 1394 (2015) 137-147

Bacillus subtilis bioassay: antibiotics in *Salvia*



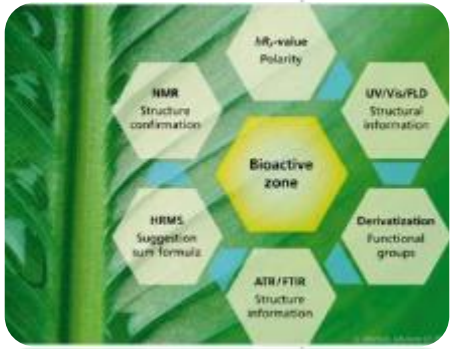
M. Jamshidi *et al.*, in preparation

Artificial neural network (ANN) for HPTLC



D. Fichou, G. Morlock, in preparation

Effect-directed link to the compound



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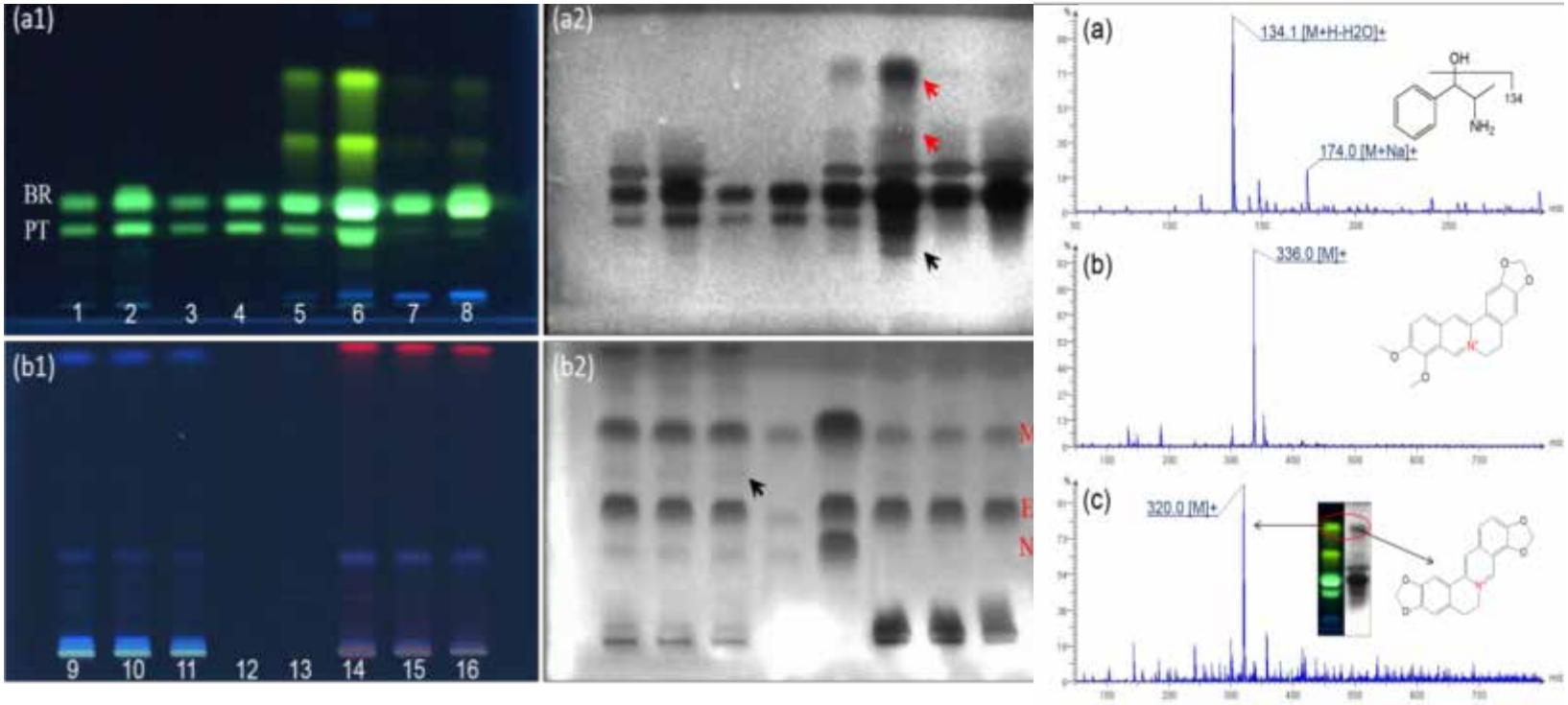
G. Morlock, *ACS Symposium Series* 1185 (2013) 101-121

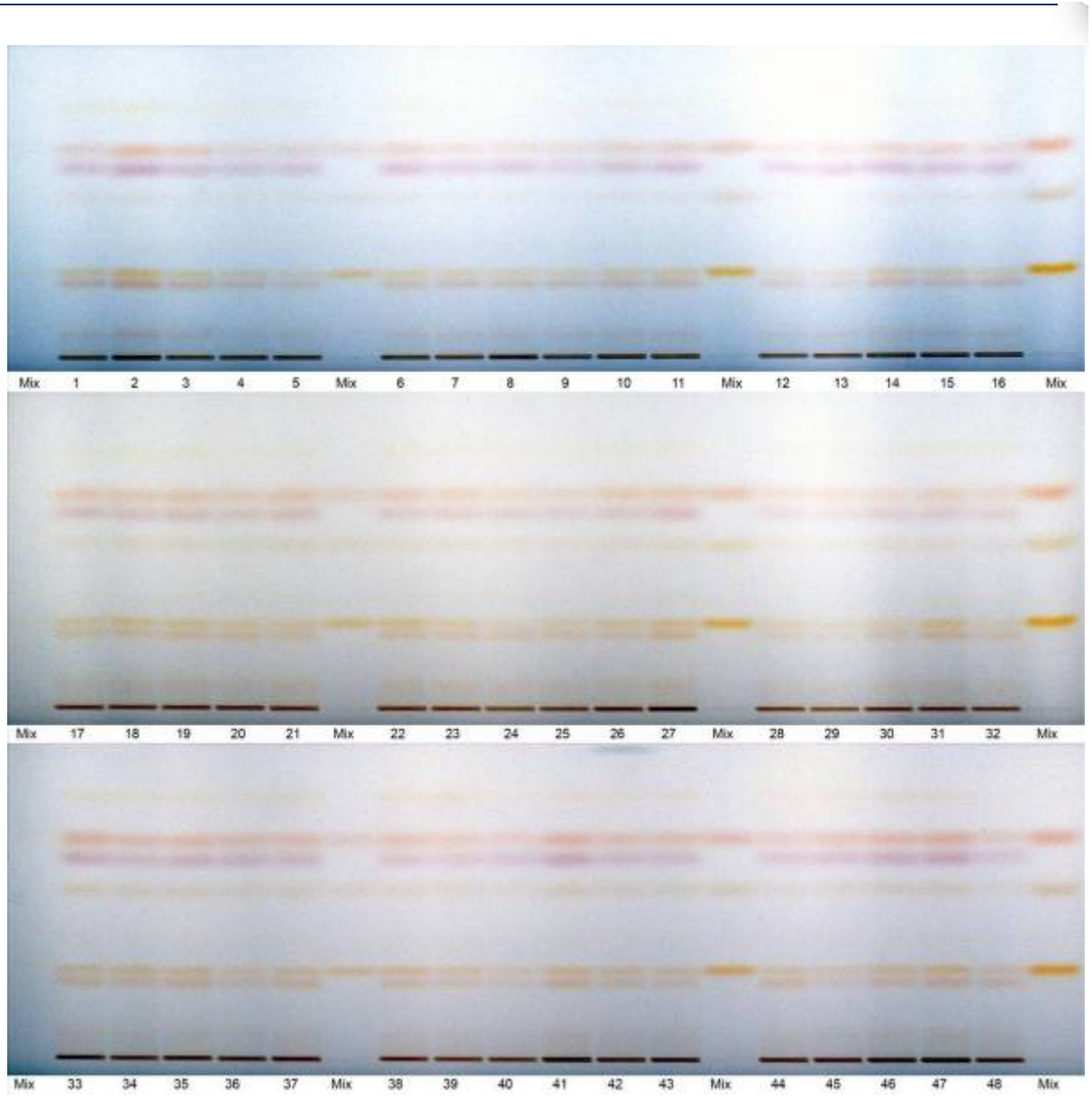
Protoberberines and ephedrines in herbal drugs

… Phelodendron, Coptis, Tinospora and Ephedra

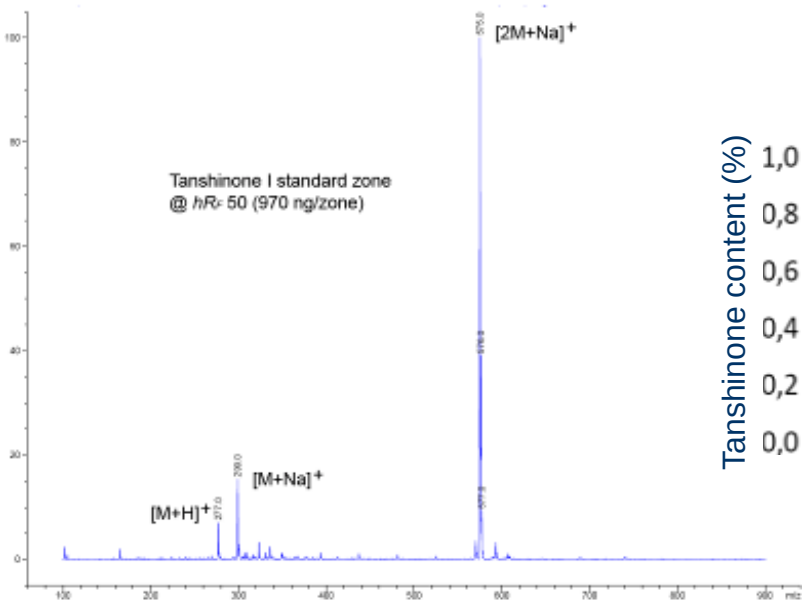


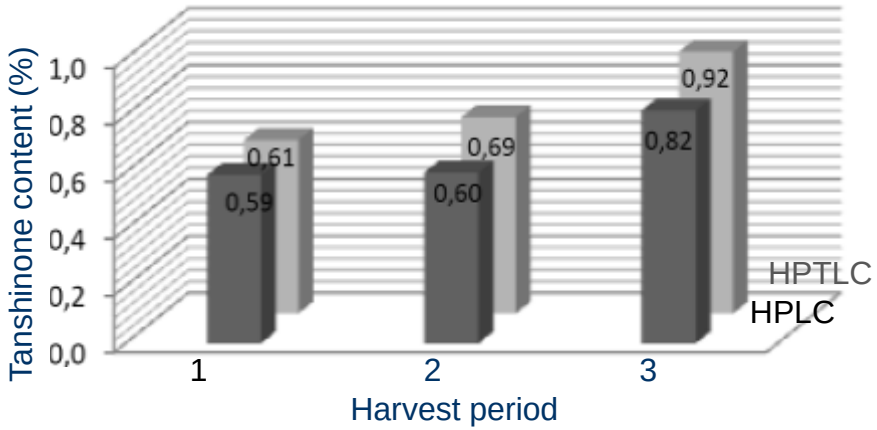
Y. Chen, G. Morlock, *Chromatographia* 79 (2015) 89-96

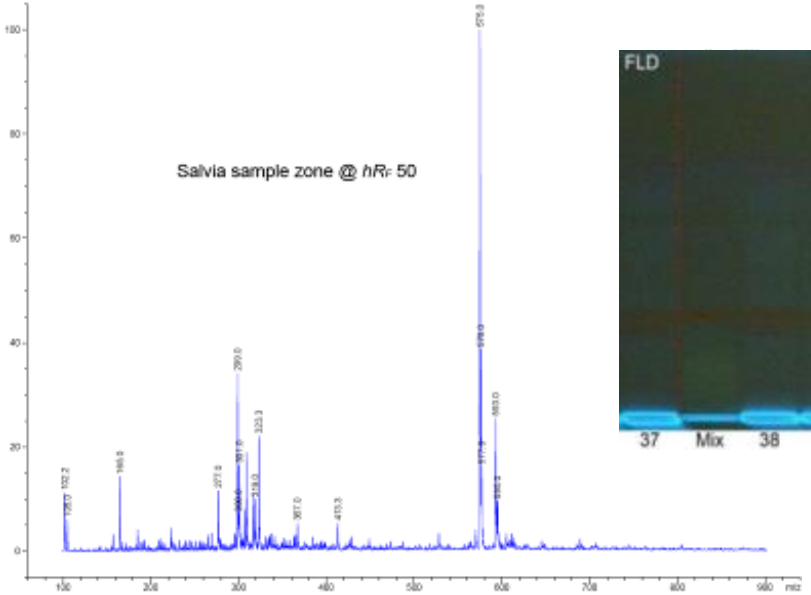
Quantitation of tanshinons in Chinese *Salvia*

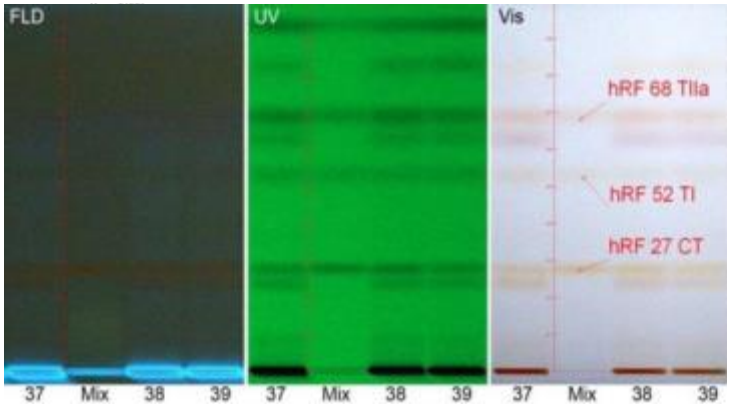


Confirmation, method comparison – more?





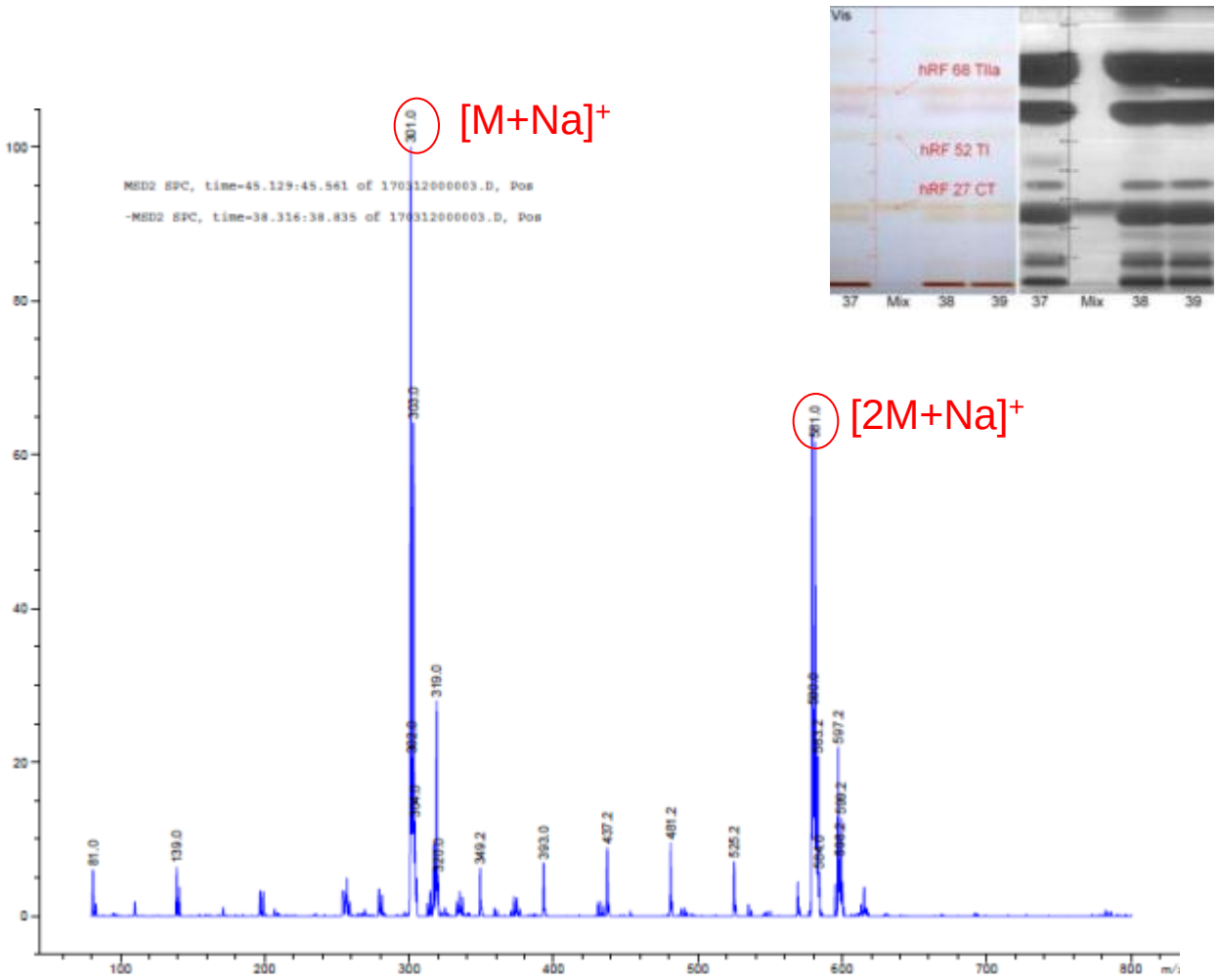




G. Morlock *et al.*, in preparation

Detection of bioactive compounds

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



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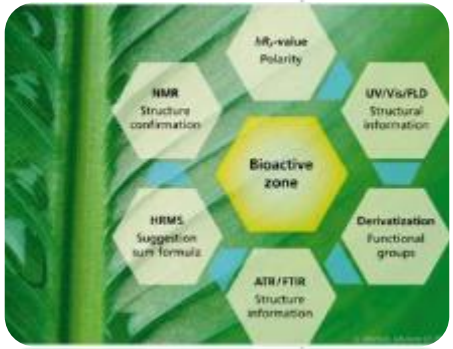

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Goodall & Levi, Nature 158, 675–676 (1946)

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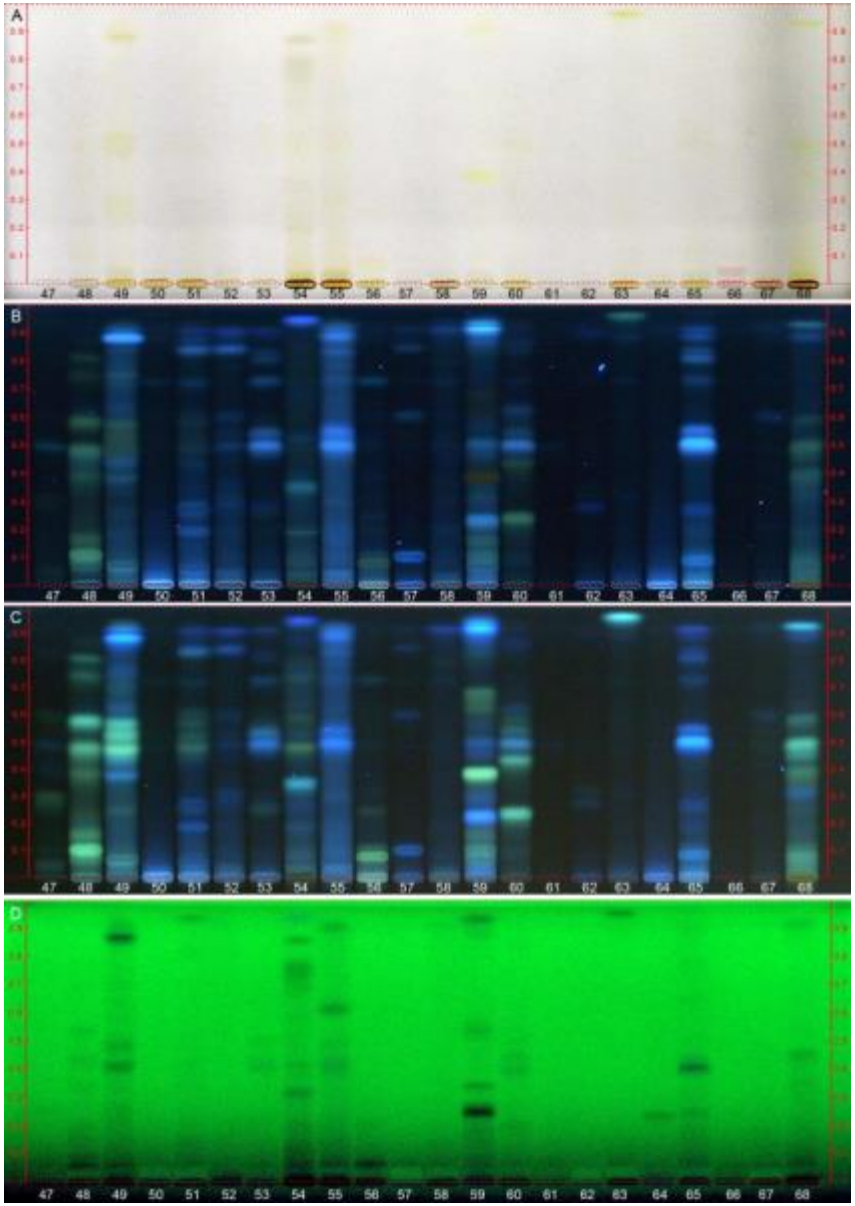
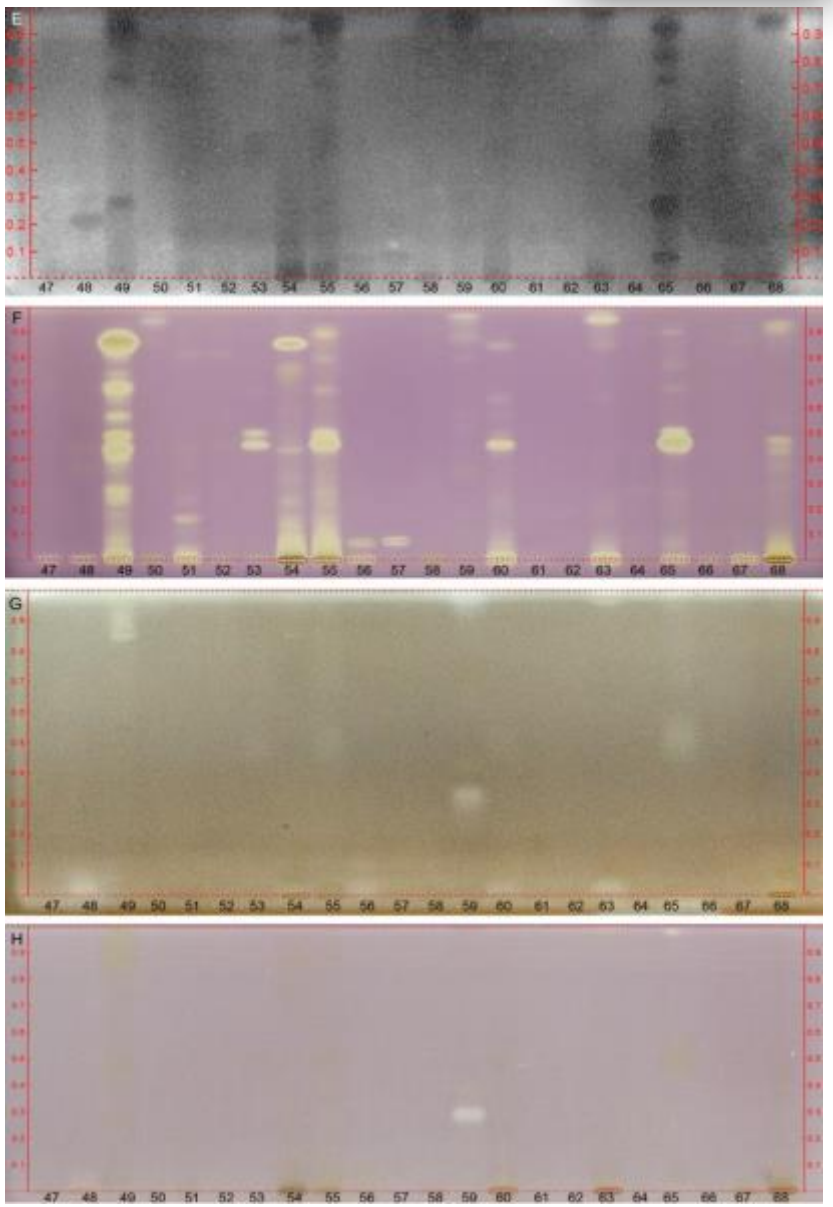
  - Tyrosinase oxidase assay

G. Morlock, *ACS Symposium Series* 1185 (2013) 101-121

EDA of 68 botanicals (#47-68)





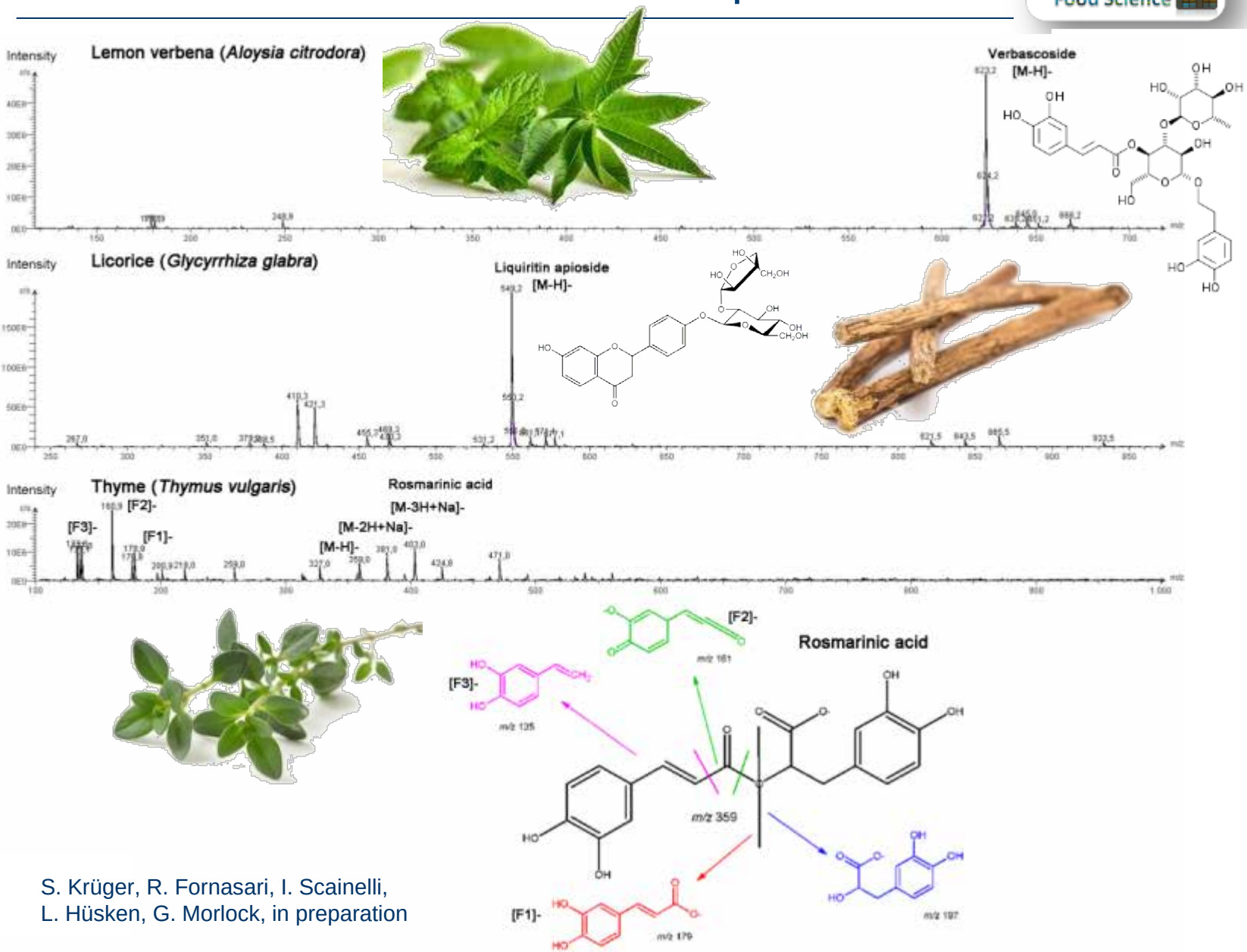
G. Morlock Food Science JLU Gießen

EDA of 68 botanicals

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

1. Ginkgo
2. Guarana
3. Kola
4. Pfefferminze
5. Oregano
6. Zichorie
7. Weinblätter
8. Kardamom
9. Zitronenschale
10. Hagebutte
11. Wacholderbeere
12. Honigbusch
13. Schafgarbe
14. Passionsblume
15. Hibiskus
16. Gelber Früchtetee
17. Brombeerblätter
18. Acerola
19. Holunderbeere
20. Grüner Matetee
21. Spitzwegerich
22. Schwarzes Johannis-beersaftkonzentrat
23. Weißdornblätter #1
24. Heidelbeere
25. Rosmarin
26. Lemon verbena
27. Eukalyptus
28. Melisse
29. Kümmel
30. Salbei
31. Hopfen
32. Majoran
33. Orangenschale
34. Liebstöckelwurzel
35. Traubenkerne
36. Fenchel
37. Ginseng
38. Holunderblüten
39. Roter Früchtetee
40. Zimtrinde
41. Schachtelhalm
42. Sternanis
43. Orangenblüten
44. Basilikum
45. Weißdornblätter Charge 2
46. Kamille
47. Sanddorn
48. Bockshornklee
49. Thyme
50. Ingwer
51. Andorn
52. Brennnessel
53. Artischocken
54. Nelken
55. Gerösteter Matetee
56. Jasmin
57. Sonnenhut
58. Koriander
59. Licorice
60. Weißdornextrakt
61. Apfelschalen
62. Sellerieknolle
63. Galgant
64. Knoblauch
65. Taigawurzel
66. Himbeersaftkonzentrat
67. Traubenschalen
68. Rooibos

Characterization of bioactive compounds



S. Krüger, R. Fornasari, I. Scainelli,
L. Hüsken, G. Morlock, in preparation

GDCh course 338/16

NEU

GDCh

Gesellschaft Deutscher Chemiker

Wirkungsbezogene Analytik mit HPTLC-Bioassay-HRMS

(in Zusammenarbeit mit der JLU Gießen)

Prof. Dr. Gertrud Morlock

- Direkter Link zur wirkenden Substanz
- Chromatographie verbunden mit Bioassay
- HPTLC-UV/Vis/FLD-bioassay-HRMS
- Non-target Analytik
- Effektive Analytik

338/15

17. Nov. 2016

ZFL Anerkannt mit 18 Punkten (www.zefo.org)

ANALYTISCHE CHEMIE

PROGRAMM

Donnerstag, 17. Nov. 2016

9.00 Begrüßung und Überblick über die wirkungsbezogene Analytik (effect-directed analysis, EDA) unter besonderer Berücksichtigung der planar-chromatographischen Möglichkeiten (Morlock)

Durchführung von Experimenten
(in 2 Gruppen parallel à 6-8 Personen)

9.15 Gruppe 1: EDA von antimikrobiell-wirkenden Inhaltsstoffen: Experiment HPTLC-UV/Vis/FLD-*Bacillus subtilis*-(HPLC-)ESI-HRMS (Jamshidi-Aidj/Stiefel)

Gruppe 2: EDA von estrogenartig-wirkenden Inhaltsstoffen mit dem planar Yeast Estrogen Screen (pYES): Experiment HPTLC-UV/Vis/FLD-pYES-(HPLC-)ESI-HRMS (Klingelhöfer)

10.15 Gruppe 1: EDA von α/β-Glucosidasehemmer: Experiment HPTLC-UV/Vis/FLD-Enzym-(HPLC-)ESI-HRMS (Jamshidi-Aidj/Kirchert)

Gruppe 2: EDA von Cholinesterasehemmer: Experiment HPTLC-UV/Vis/FLD-Enzym-(HPLC-)ESI-HRMS (Hage)

11.00 Kaffeepause

11.15 Fortführung des *Bacillus subtilis*-Bioassays und der Enzymassays

12.00 Mittagessen

13.00 pYES-Fortführung

13.30 HPTLC-(HPLC-)ESI-HRMS von bioaktiven Verbindungen (Stiefel, Häbe)

14.15 pYES-Fortführung

14.45 Kaffeepause

15.00 Gruppe 1: EDA von Tyrosinase- und Xanthinoxidasehemmer: Experiment HPTLC-UV/Vis/FLD-Enzym-(HPLC-)ESI-HRMS sowie Experiment HPTLC-UV/Vis/FLD-DPPH•-ESI-HRMS (Hage, Xingmei)

Gruppe 2: EDA von bioaktiven Verbindungen (genereller Hinweis auf Bioaktivität): Experiment HPTLC-UV/Vis/FLD-*Alivibrio fischeri*-DART-HRMS (Krüger/Häbe)

16.15 Zusammenfassung und Diskussion der unterschiedlichen Bioassays

17.00 Voraussichtliches Ende der Veranstaltung

JLU

GDCh course 335/16

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

335/15

16. Nov. 2016

ZFL Anerkannt mit 16 Punkten (www.zefo.org)

PROGRAMM

Mittwoch, 16. Nov. 2016

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, Gerbig)
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, Gerbig)

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, Stiefel)

16.15 Gruppe 1: Experiment HPTLC-UV/Vis/FLD-DESI-MS (Kirchert, Stiefel)
Gruppe 2: Experiment HPTLC-UV/Vis/FLD-DART-MS (Häbe, Krüger)

16.30 Diskussion (Morlock)

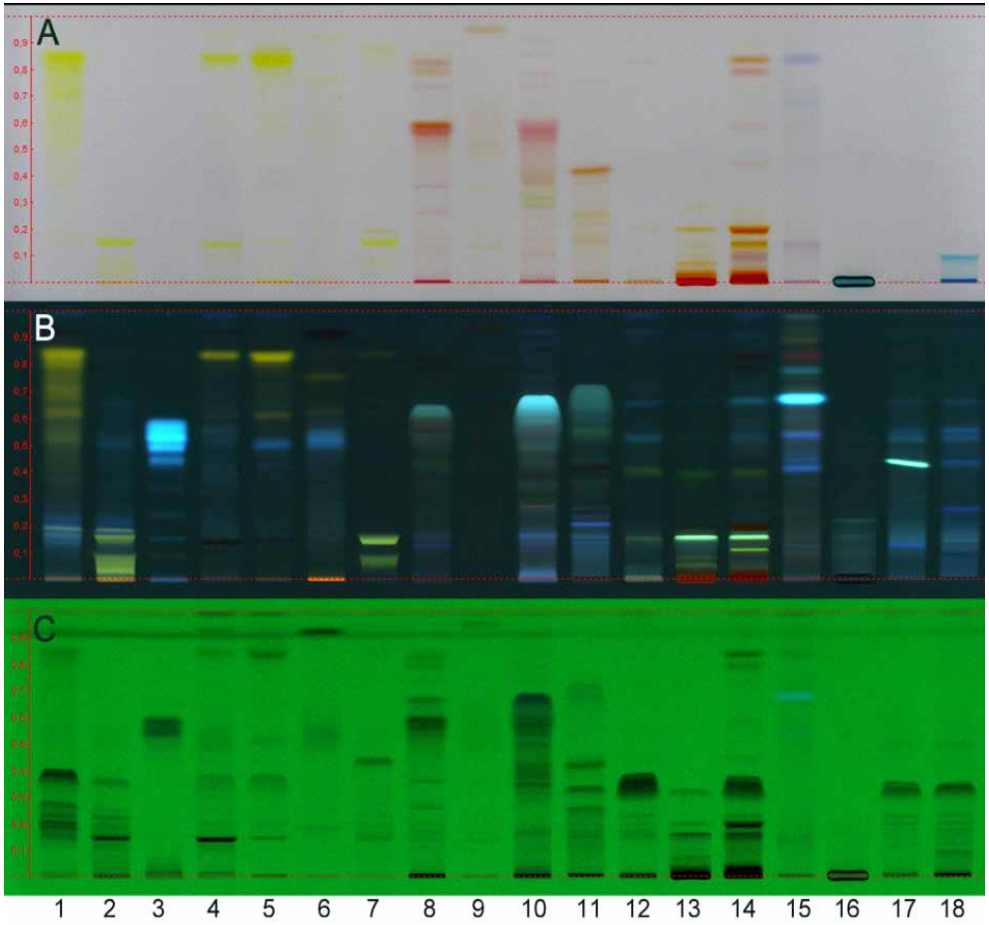
17.00 Voraussichtliches Ende der Veranstaltung

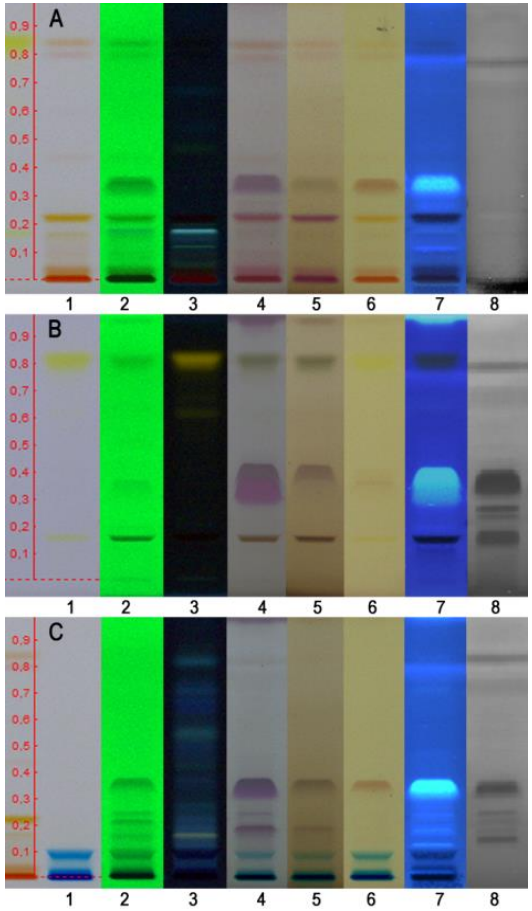
16. Nov. 2016

JLU

HPTLC profiling of organic pigments

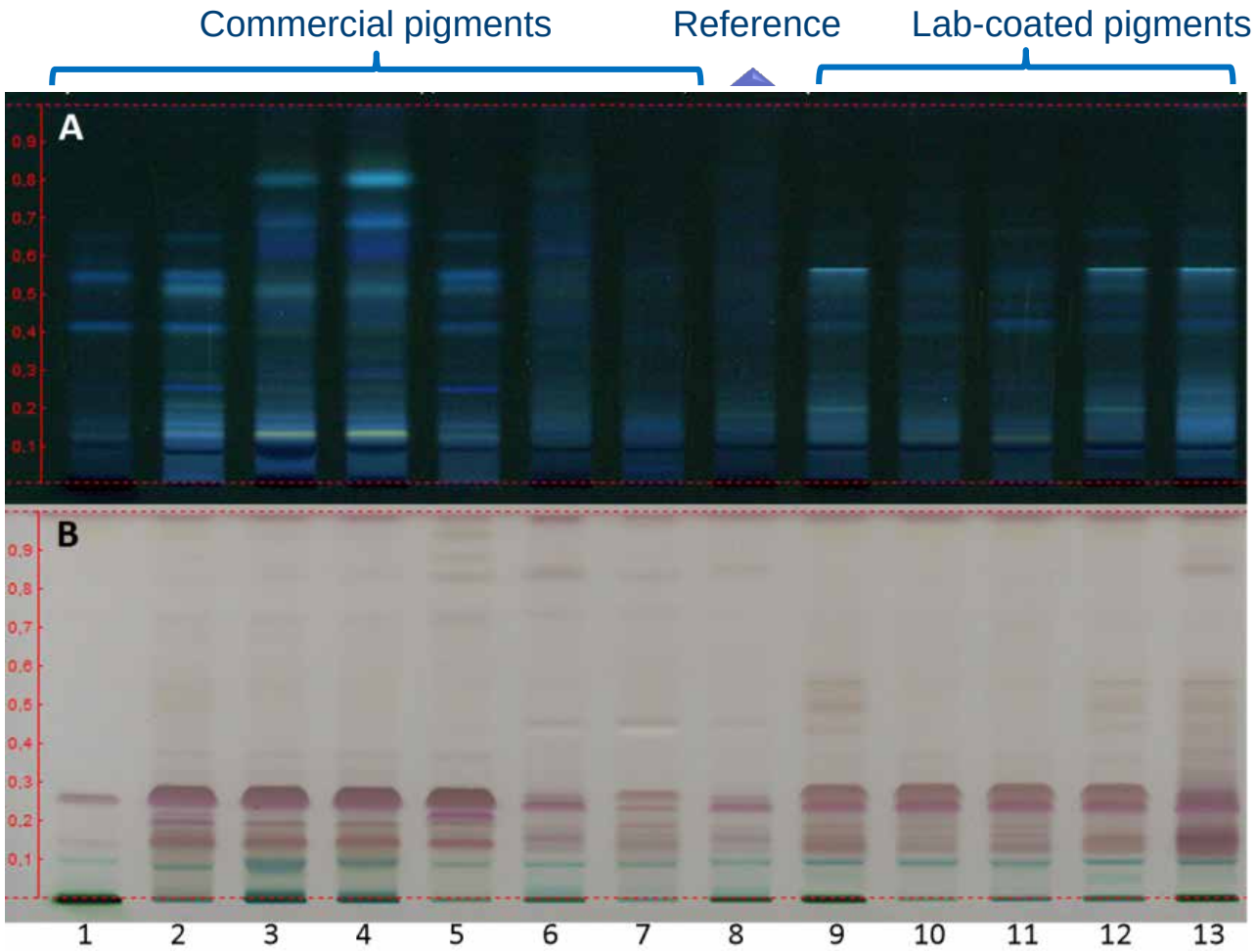
→ usable in packaging inks





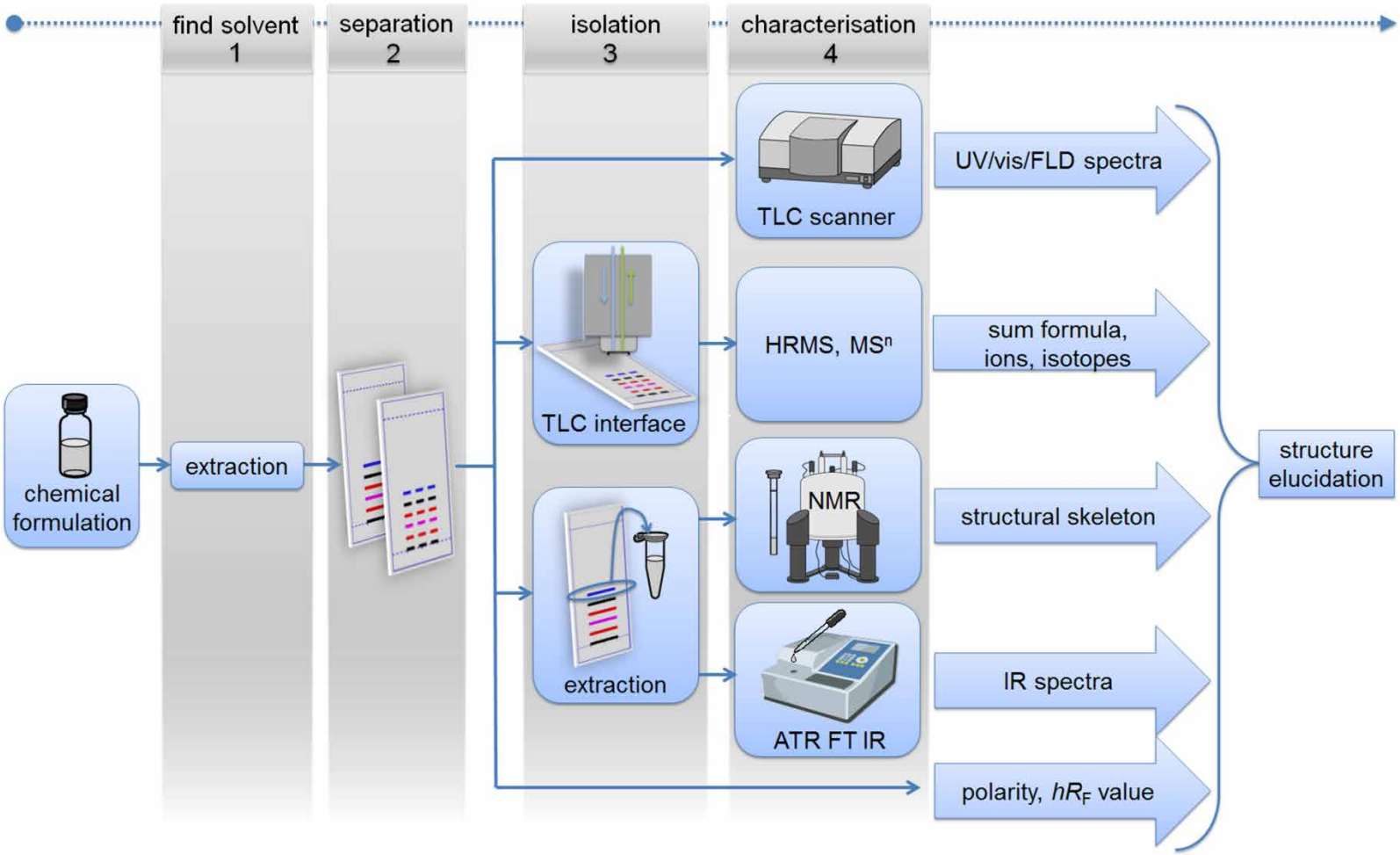
C. Stiefel, G. Morlock, in preparation
Cooperation with Siegwerk Druckfarben AG & Co. KGaA

HPTLC profiling of pigment PB 15:4



C. Stiefel, G. Morlock, in preparation
Cooperation with Siegwerk Druckfarben AG & Co. KGaA

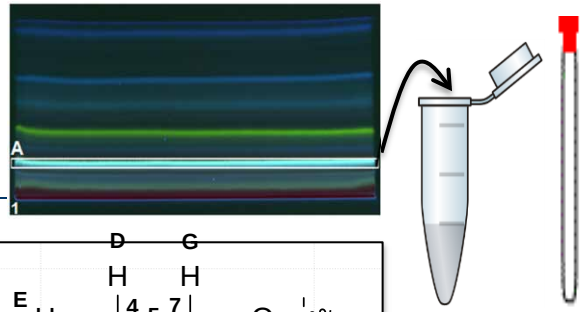
Fast structure elucidation using 2 plates

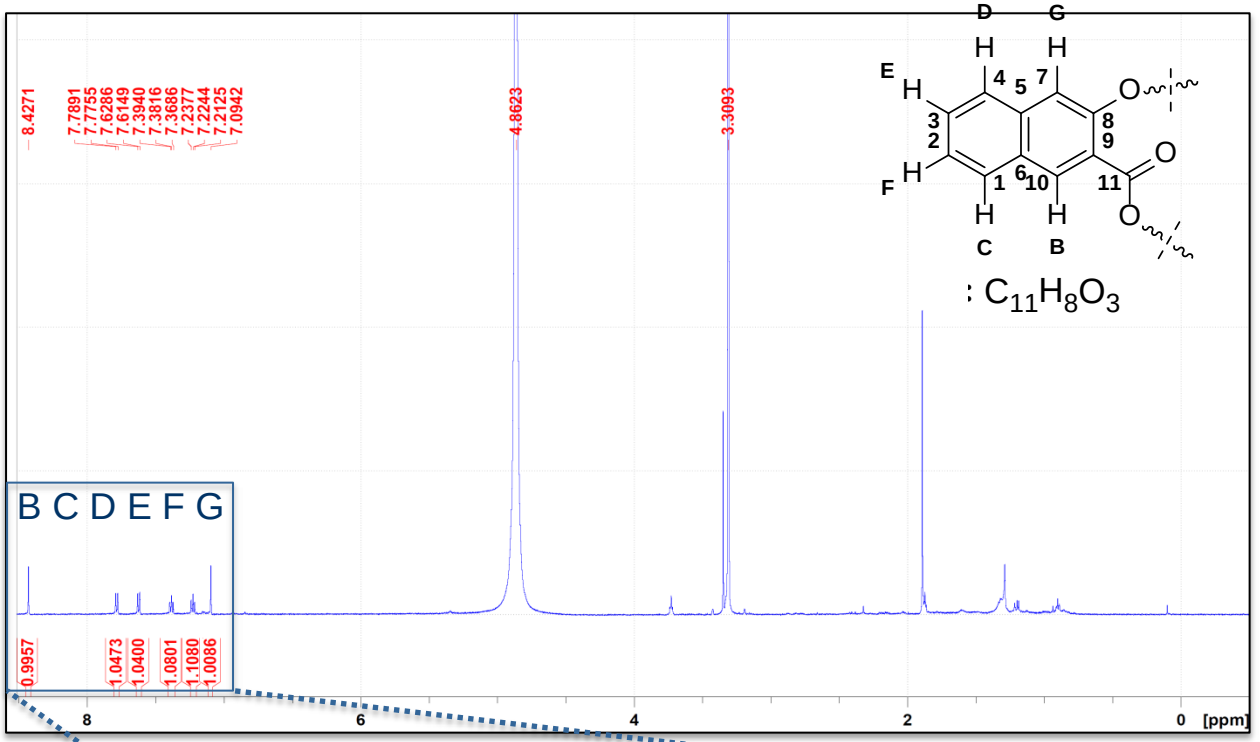


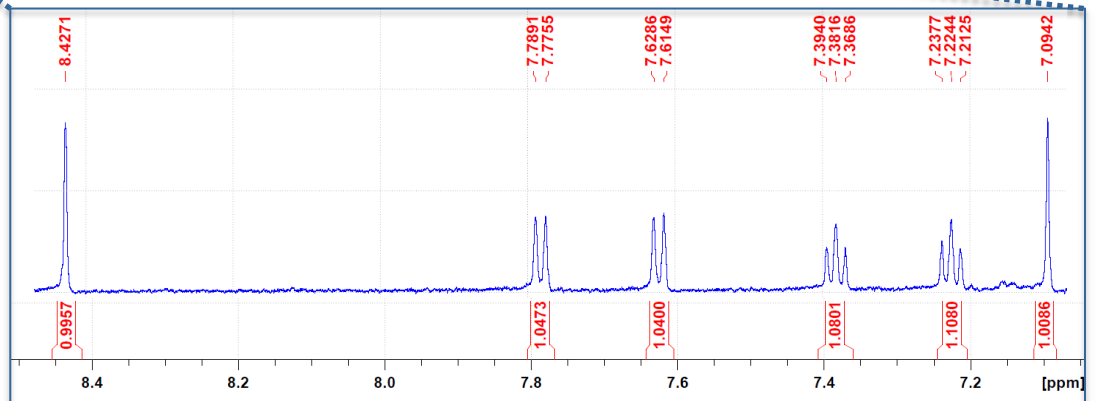
I. Yüce,G. Morlock, in preparation

G. Morlock Food Science JLU Gießen

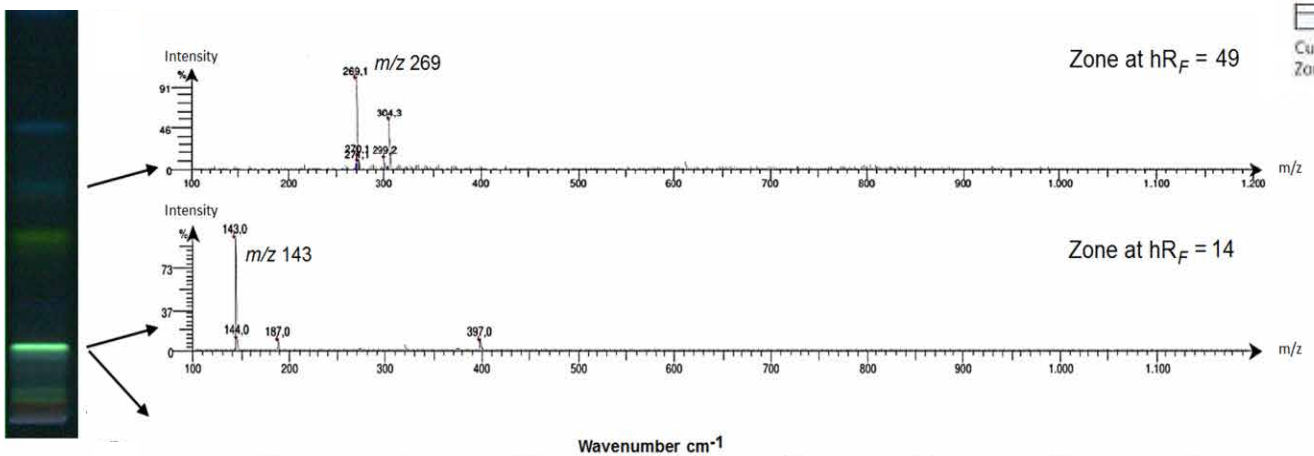
HPTLC-^{1}H NMR of unknown

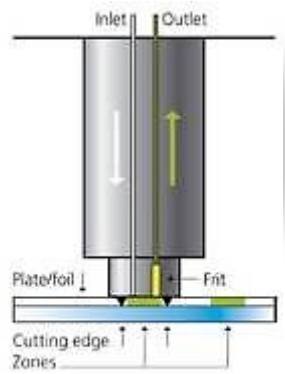


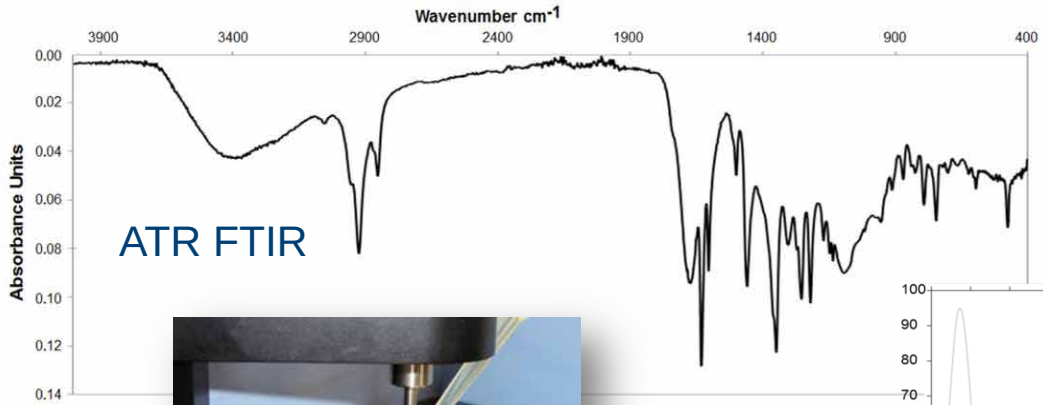


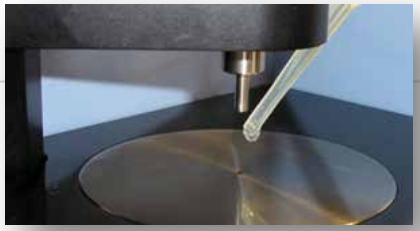


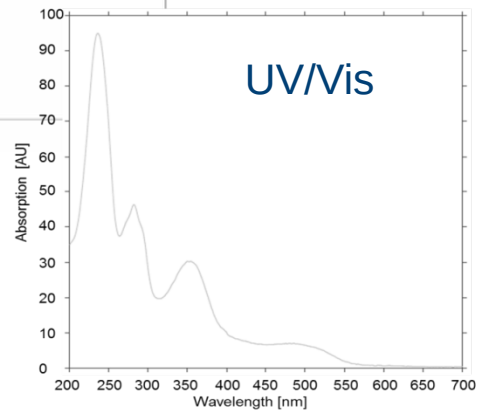
HPTLC-UV/Vis/ATR FTIR of unknown





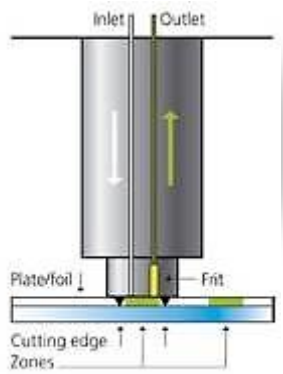




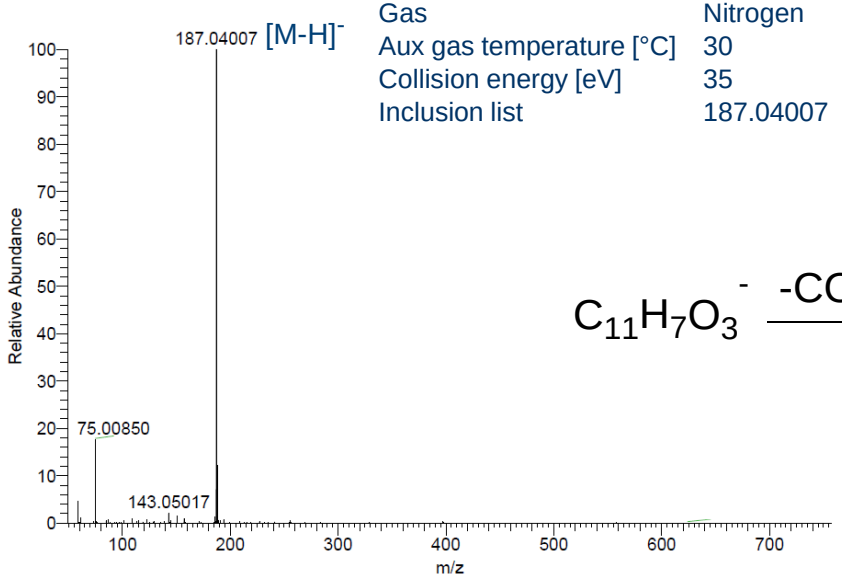


C. Stiefel, G. Morlock, in preparation

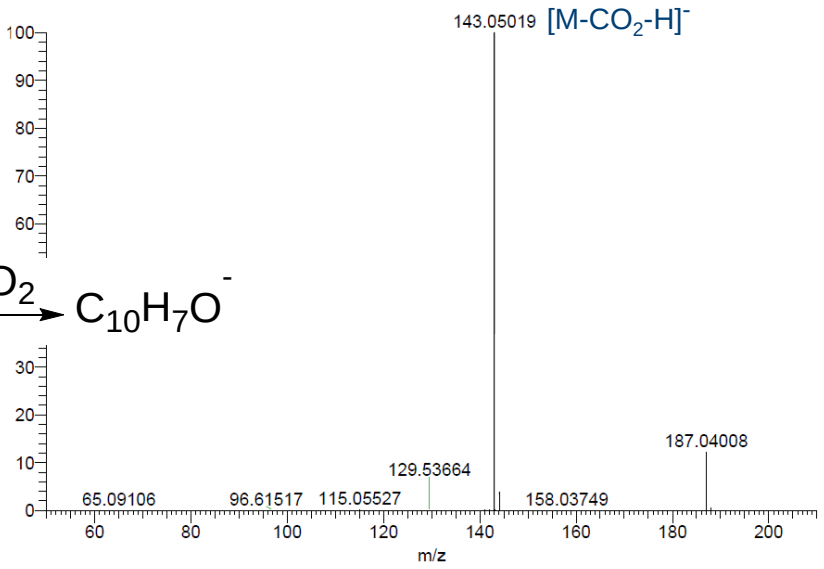
HPTLC-HRMS and -MS/MS of unknown



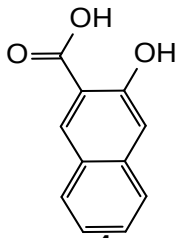
Polarity	Negative
Measuring range [*m/z*]	50-210
Spray voltage [kV]	3.3
Capillary temperature [°C]	320
Gas	Nitrogen
Aux gas temperature [°C]	30
Collision energy [eV]	35
Inclusion list	187.04007



$C_{11}H_7O_3^- \xrightarrow{-CO_2} C_{10}H_7O^-$

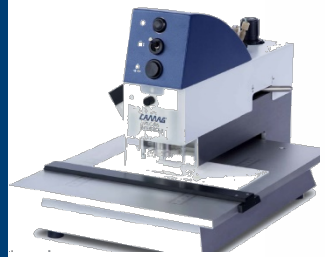


3-Hydroxy-2-naphthoic acid

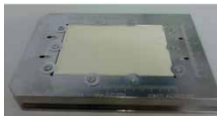


$C_{11}H_8O_3$
188.04734

I. Yüce, G. Morlock, in preparation



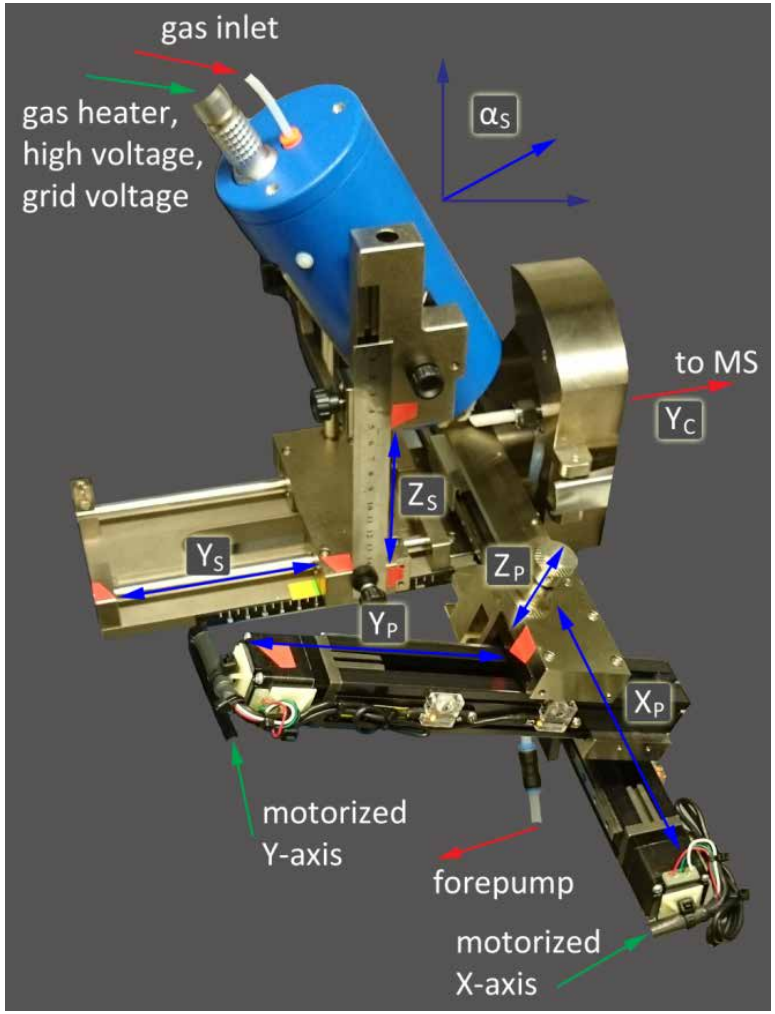
Upgrade in 2015



G.E. Morlock, *q&more* 1 (2014) 43-47, modified

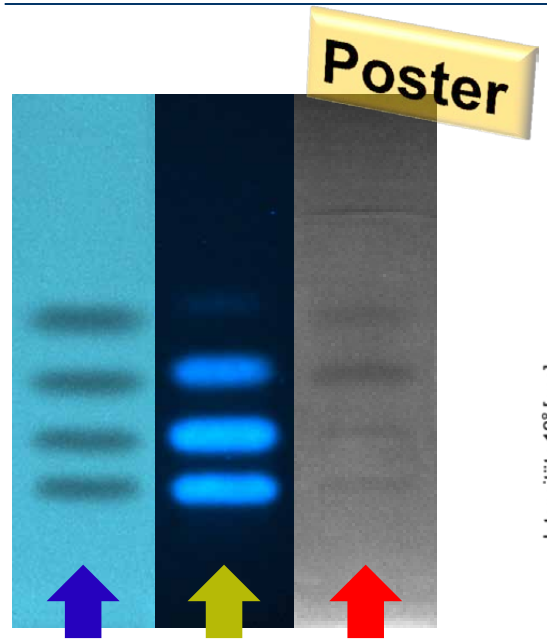
Optimization of HPTLC-DART-SVPA-MS

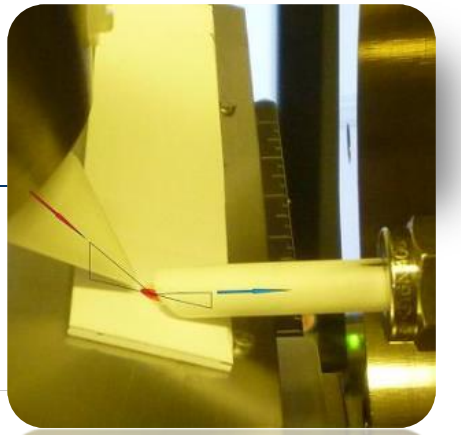
→ Ion signals increased by factor of 34

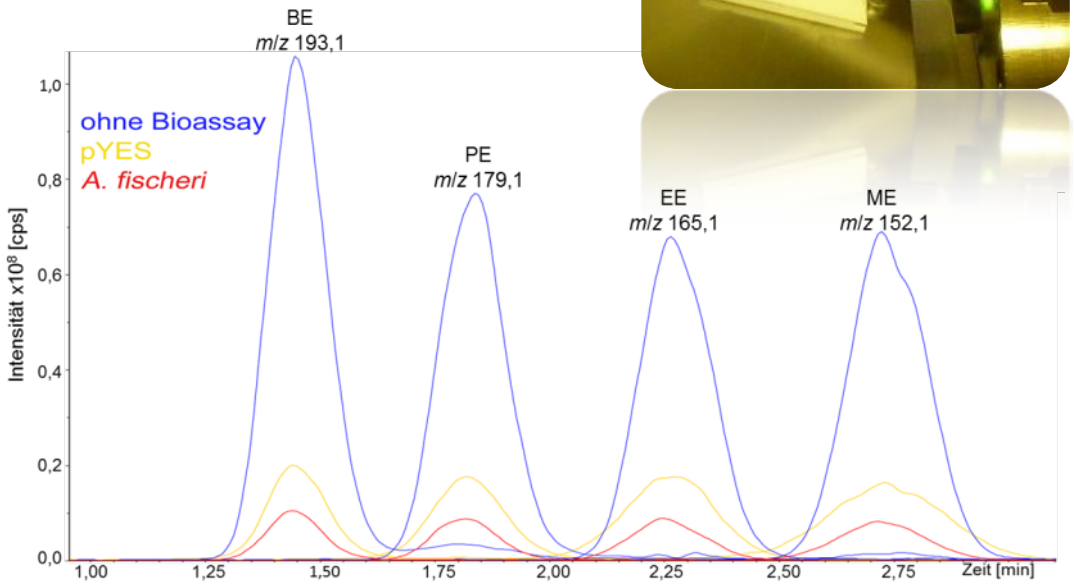


T. Häbe, G. Morlock, *Rapid Commun Mass Spectrom* 30 (2016) 321–332

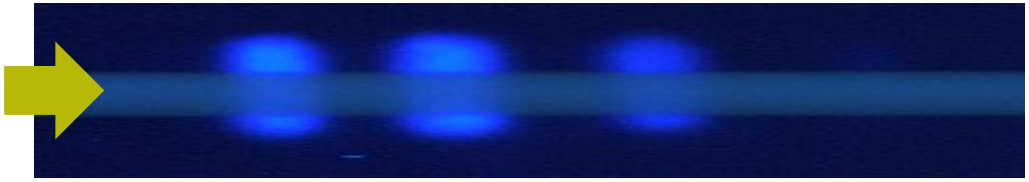
DB-DART-MS





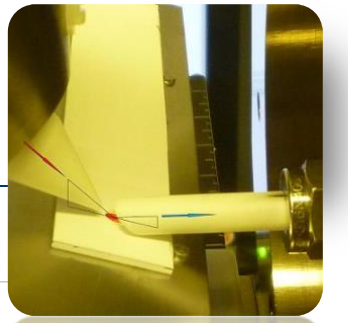


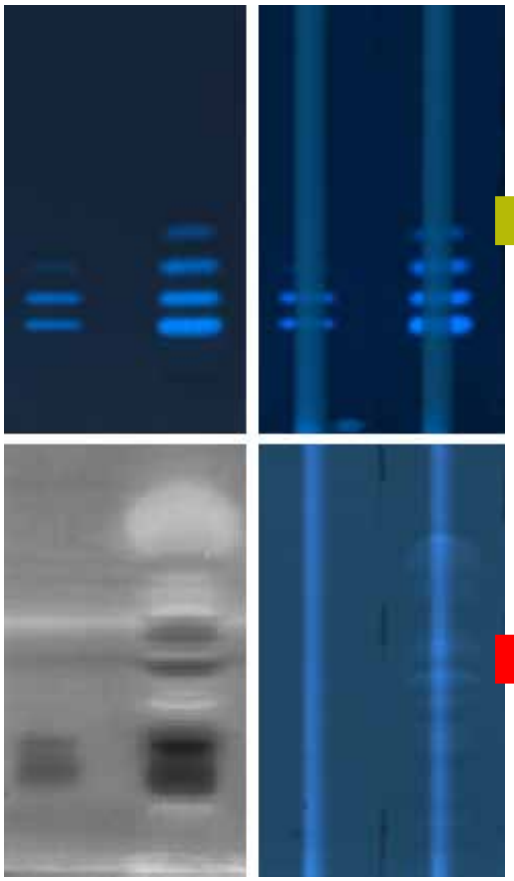
	Signal decay [%]	
	A. fischeri	pYES
ME	88	65
EE	89	67
PE	90	76
BE	91	81

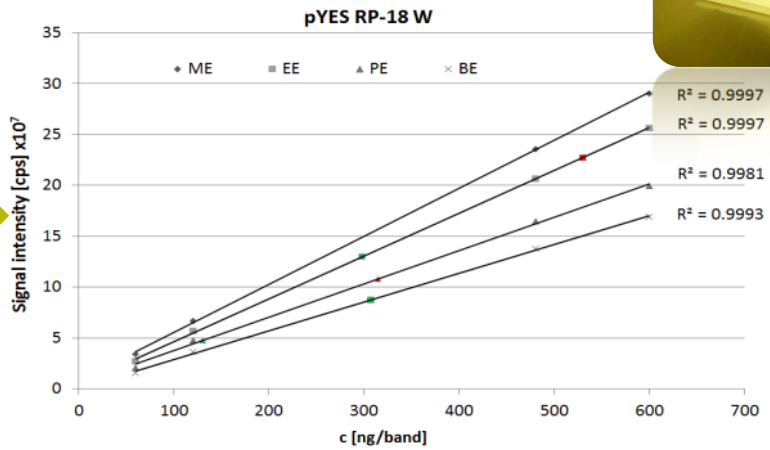


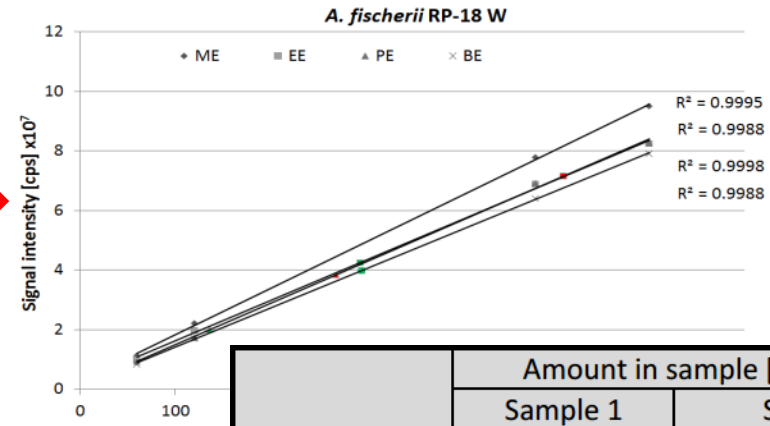
T. Häbe, G. Morlock, in preparation

DB-DART-MS





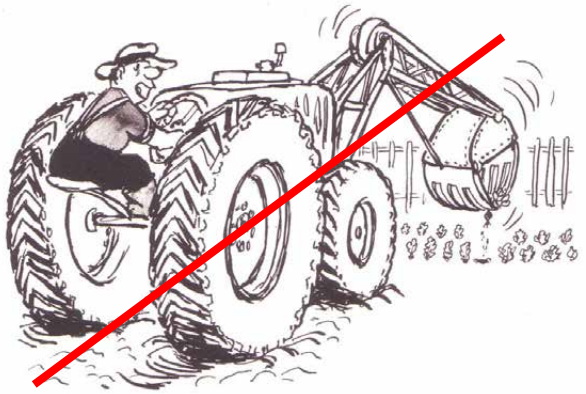




		Amount in sample [mg/100g]						
		Sample 1			Sample 2			
		ME	EE	PE	ME	EE	PE	BE
without BioAssay	NP	103	56	30	165	75	37	65
	RP	97	59	34	147	69	30	67
A. fischeri	NP	96	51	27	173	69	24	53
	RP	101	51	27	157	59	27	59
pYES	RP	111	53	31	170	60	26	62

T. Häbe, G. Morlock, in preparation

EDA by HPTLC



Single compound(s)
→ chromatography-(bio)assay

Parallel screening
→ mostly 20 extracts separated in parallel
under identical chromatographic and environmental conditions

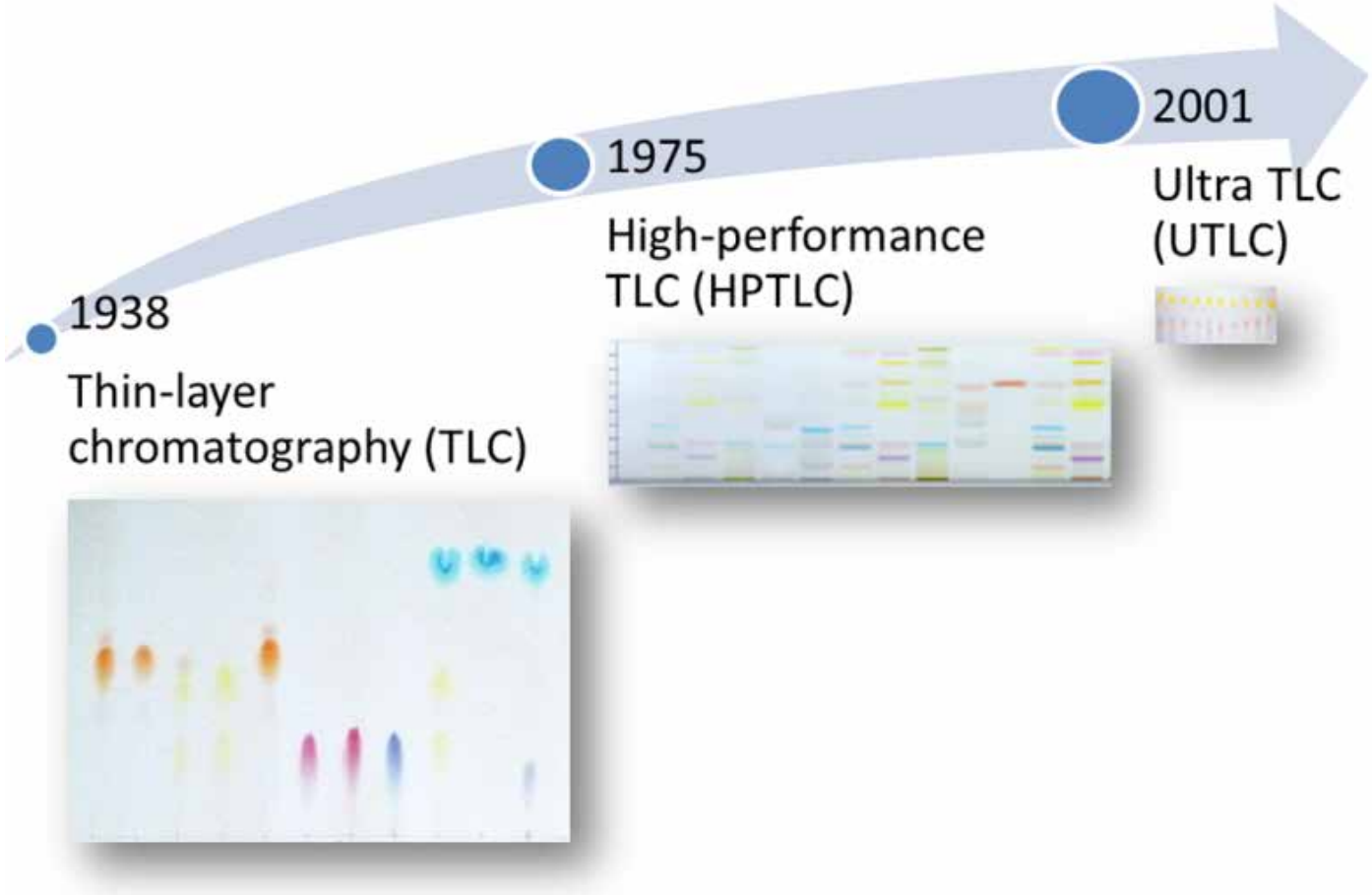
Streamlined analysis
→ skip all the different methods (SPE, GPC, prep. HPLC)
for fractionation, isolation and purification of substances,
always followed by the bioactivity test → cost-effective

Matrix-robust detection
→ (bio)assays not interfered by solvents and matrix

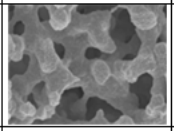
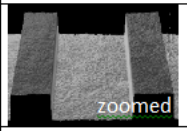
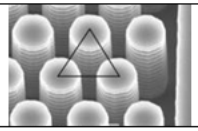
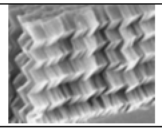
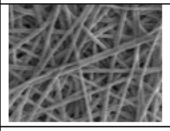
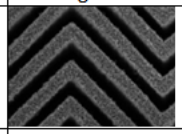
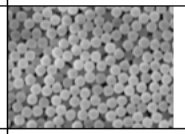
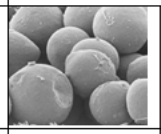
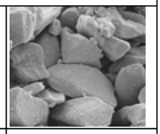
Modular hyphenations
→ targeted coupling with HRMS, NMR…

Image/derivatizations
→ additional helpful information

Miniaturization



Novel layers

	UTLC							HPTLC	
First report	**2001 [5]**	**2001 [47]**	**2007 [49]**	**2008 [28]**	**2009 [56]**	**2011 [60]**	**2011 [63]**	**1975 [3]**	
Layer type	Monolithic layer	Monolayer on channel bottom	Ordered (non)po-rous pillar arrays	Nanostructured layer	Electrospun mat	Carbon-nano-tube-templated microfabrication (CNT-M)	Submicrometer particulate layer with cross linked polymer brushes	Particulate layer	
Technique of fabrication	Polymerization on glass plate (sol-gel pro-cess) and opt. photografting	DRIE of Si-wafer surface and coating	Mid/Deep-UV lithography, DRIE of Si-wafer surface and coating	GLAD of inorganic oxides on glass plates	Electrospinning of (composite) polymer solutions on aluminium foil	Coating CNTs with silica by (pseudo) atomic layer deposition plus second coating	Slurry overlay on Si-wafer and brush coating by polymerization	Slurry overlay on various carriers (glass plate, aluminium or polymer foil) and coatings	
Layer icon									
Layer struc-ture	Monolithic texture with 1-2 µm macropores	Monolayered porous silicon bottom of a nanochannel (0.7 µm wide, 0.3 µm deep)	Monolayer coa-ting or monolithic silica shell of cylindrical pillars (Ø 4 µm, 10 µm high, spaced 0.3 - 1.7 µm) in a 70 µm nanochannel	Column array of verticals, posts, helices, zig-zags or blades (spaced 2 - 50 nm) with (an)isotropic structure	Spun mat of nanofibers (Ø 200-400 nm, cm to m long) forming cylindric channels	Silica coated (20-60 nm) herring-bone hedge array (3-4 µm wide, spaced 4-7 µm) forming channels (50-100 µm long)	Non-porous particles coated with a polymer brush layer	Spherical	Irregular
								particles of ∅ 5 - 7 µm	
Layer thick-nesses (µm)	10 - 50	Monolayer 0.05 – 0.3	Monolayer or 0.5 µm porous shell	1.3 - 7	15 - 25	50	15	50 - 200	
Adsorbent types	Silica gel, poly-(4-methyl-styrene-co-chloromethyl-styrene-co-divinylbenzene)	C8, C18	C8; C18 [50]	Silica, zirconia alumina, titania, C18	Glassy carbon, polyvinyl alcohol, polyacrylonitrile	Silica, amino	Polyacrylamide; poly(GMA-co-DEGDMA-NH_2) [64]	Silica, amino, cyano, diol, C2, C8, C18, cellulose, *etc.*	
Layer geome-try (mm)	60 × 36 or 30 x 33	0.7 × 20	0.14 x 40; 10 x 30 [50]	25 × 25 or 100 × 20	30 × 60, indivi-dually sliceable	12 x 60	25 × 25	200 × 100, individually sliceable	

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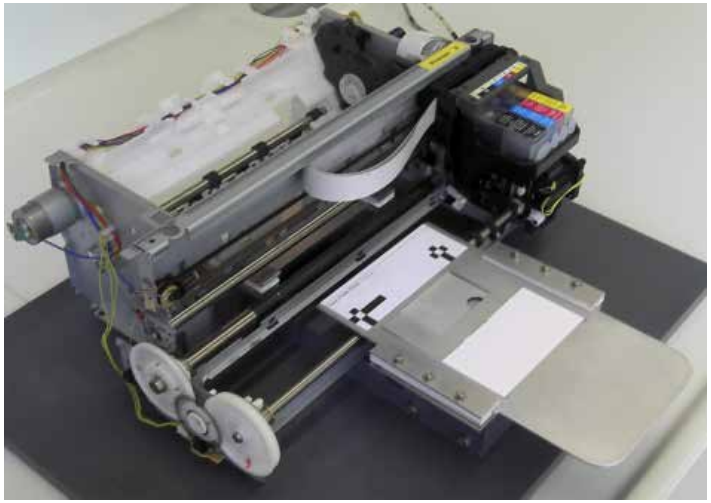

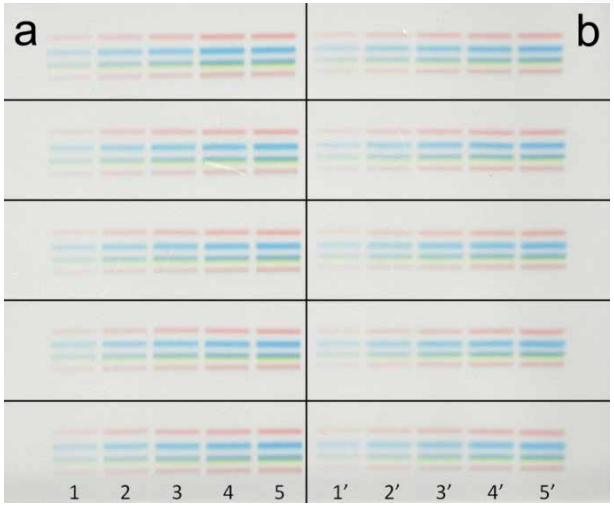

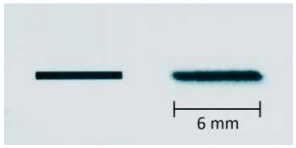

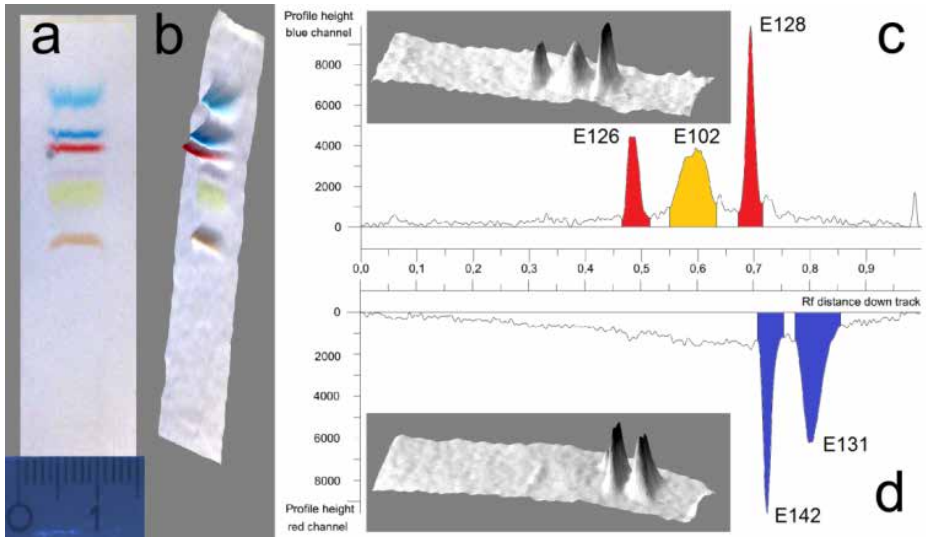

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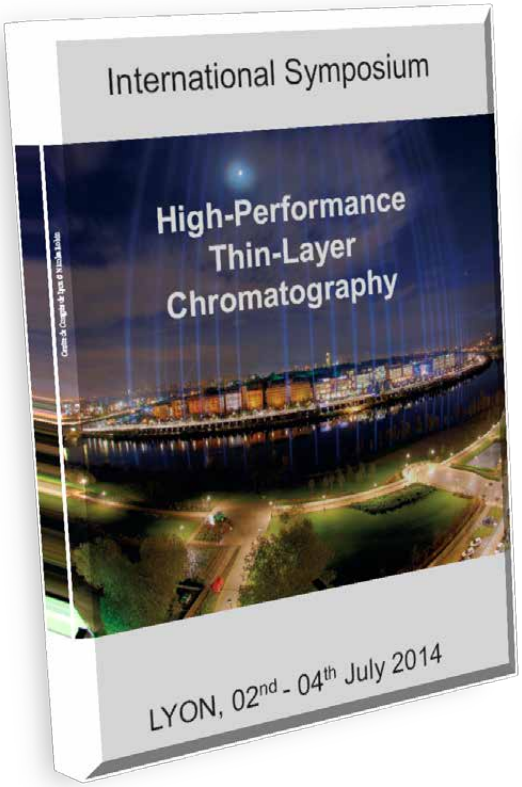

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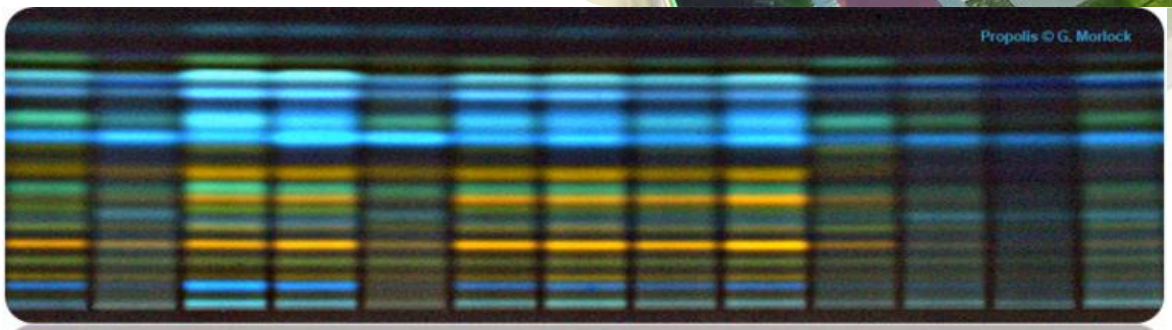
Thank you!