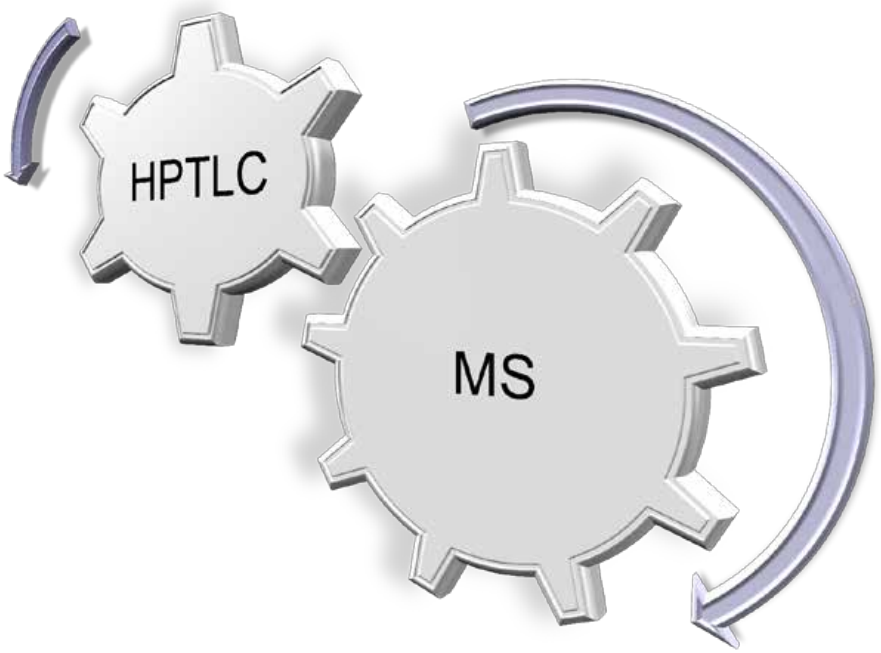
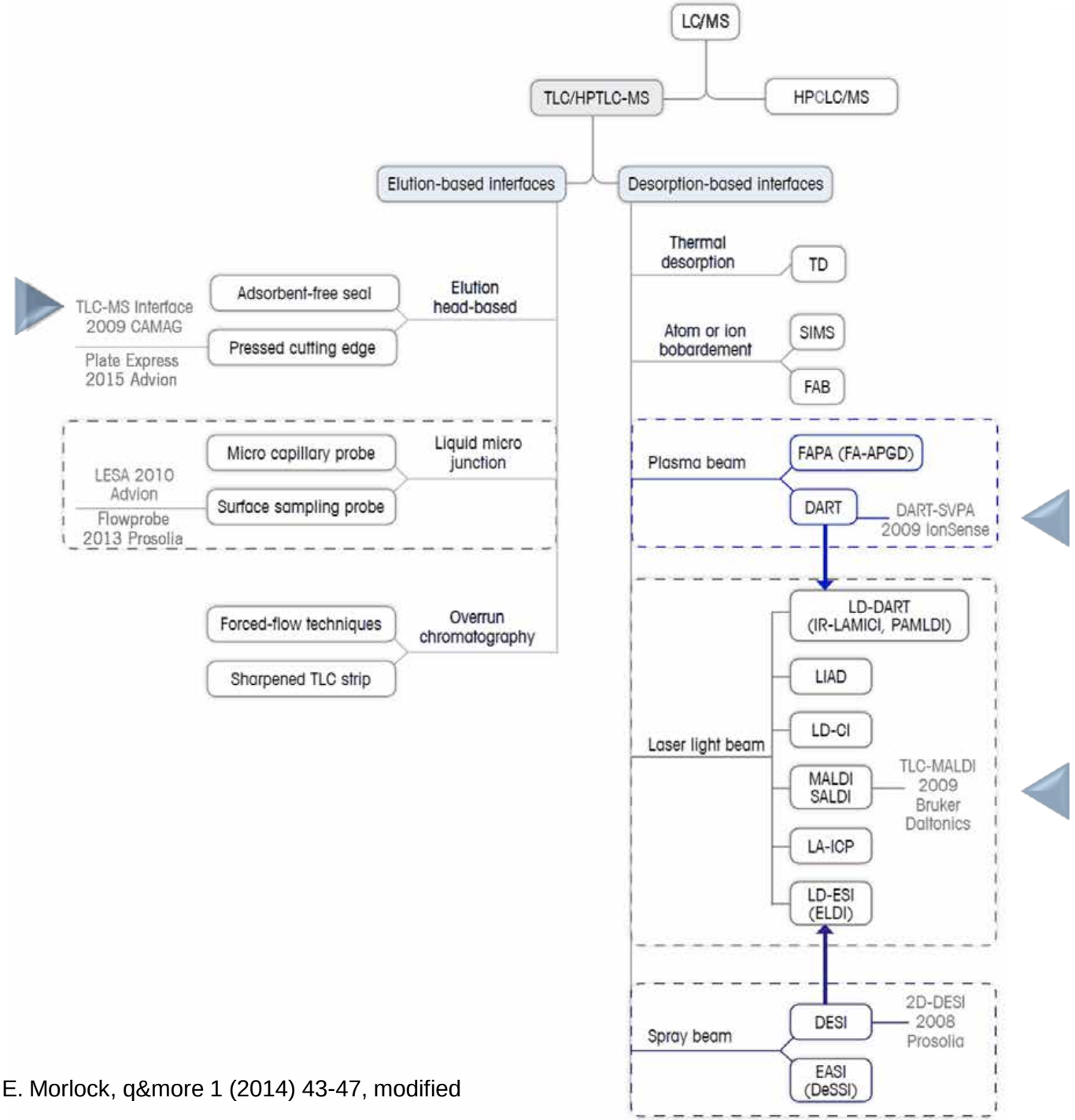
HPTLC-MS

Overview, quantitation and comparison

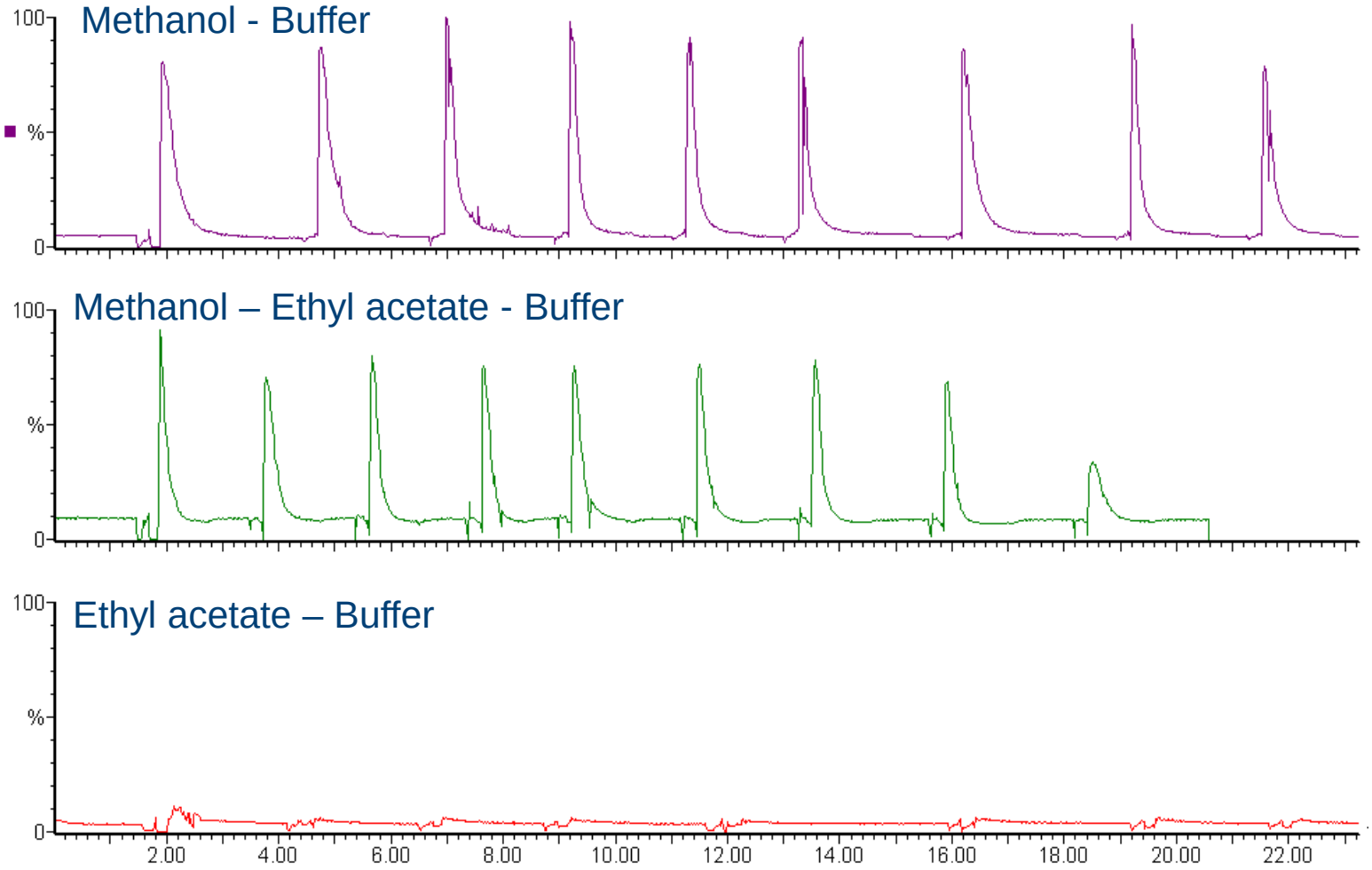


Gertrud Morlock, Chair of Food Science
Justus Liebig University Giessen



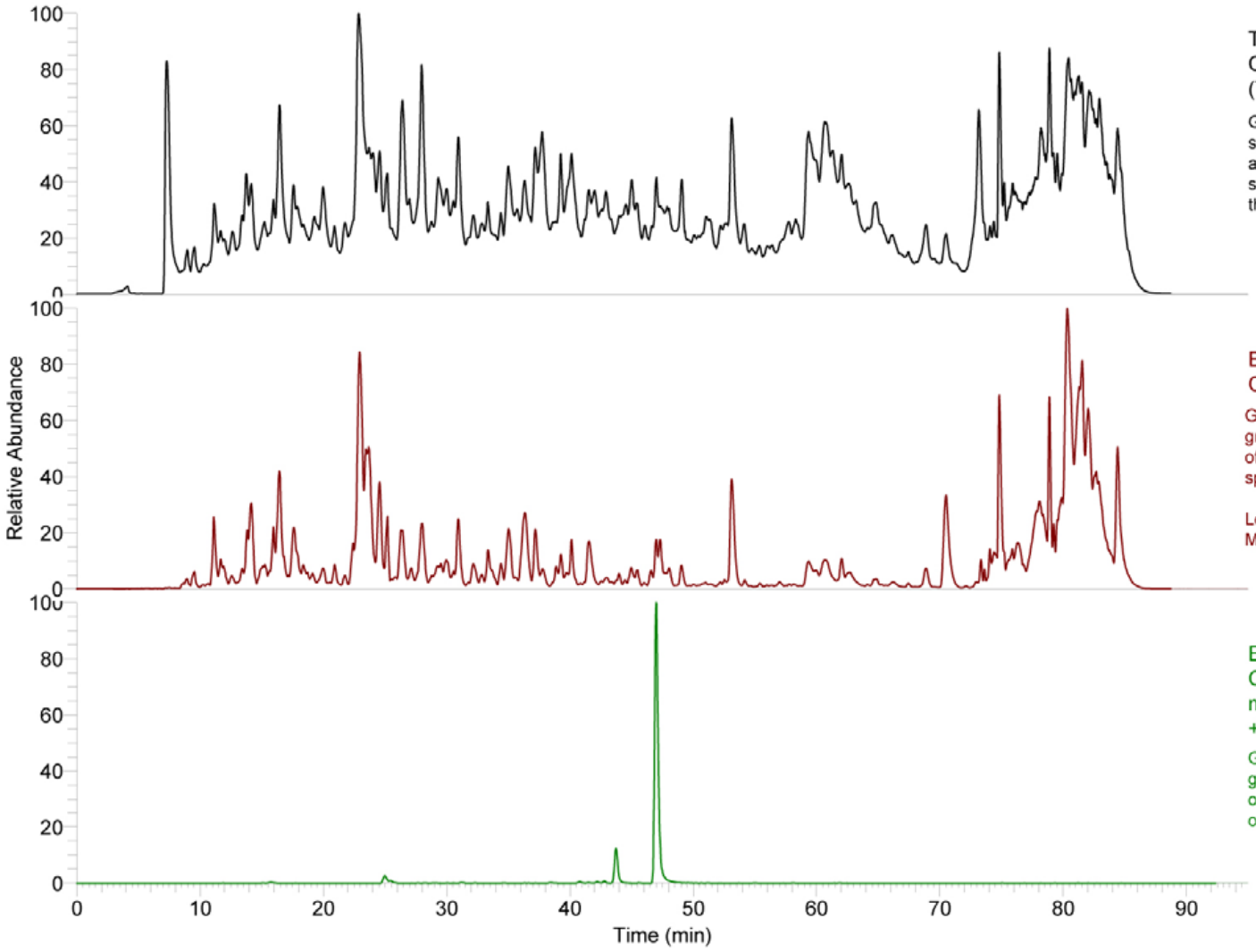
G.E. Morlock, q&more 1 (2014) 43-47, modified

Solubility *versus* elution power



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

MS chromatograms→ chronograms

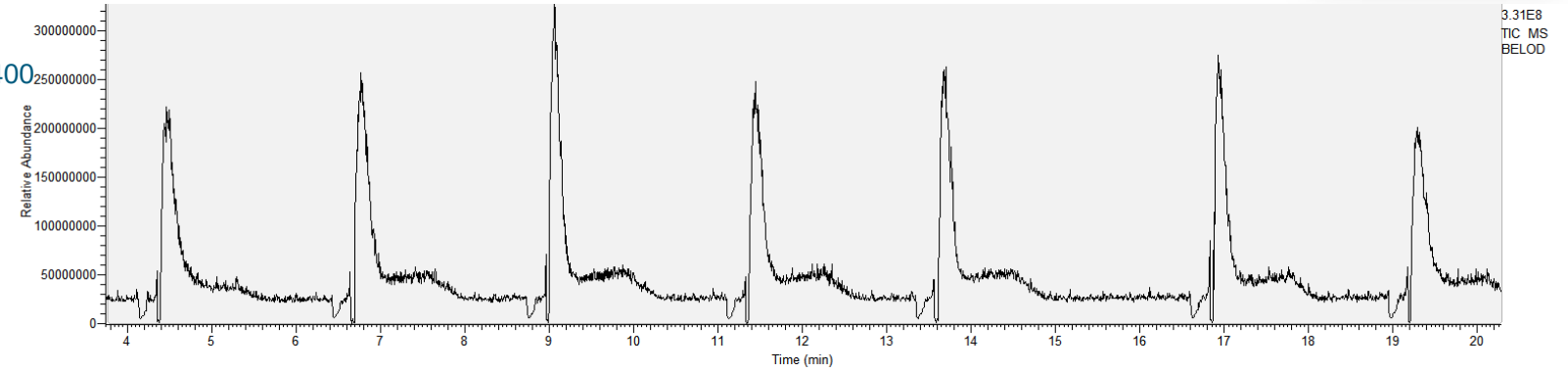


HPTLC-HRMS (Q Exactive plus)

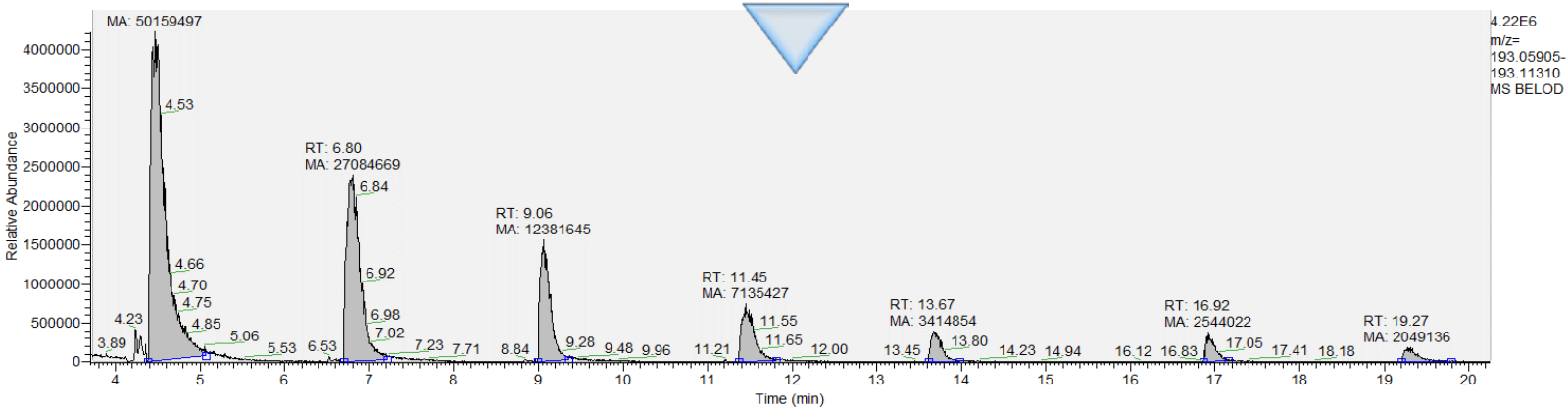
JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

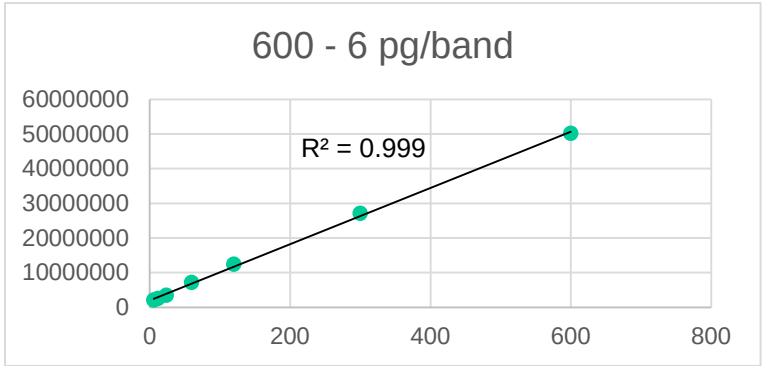
TIC

m/z 100-400



EIC



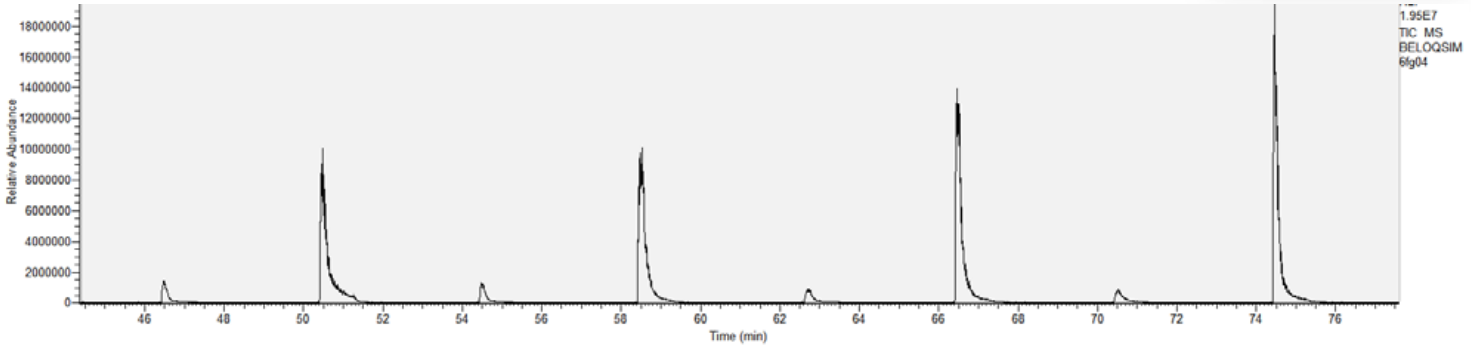


HPTLC-HRMS (Q Exactive plus)

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

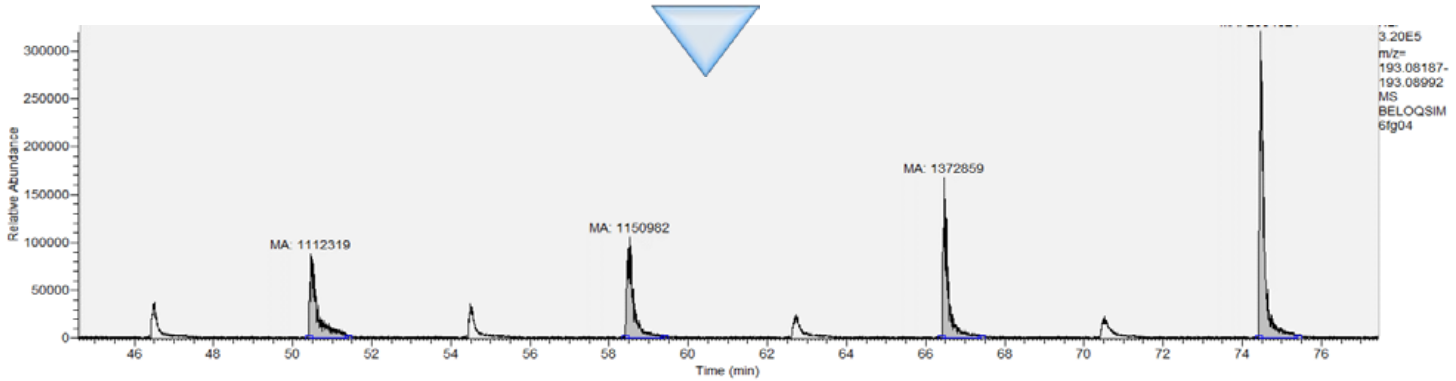
SIM

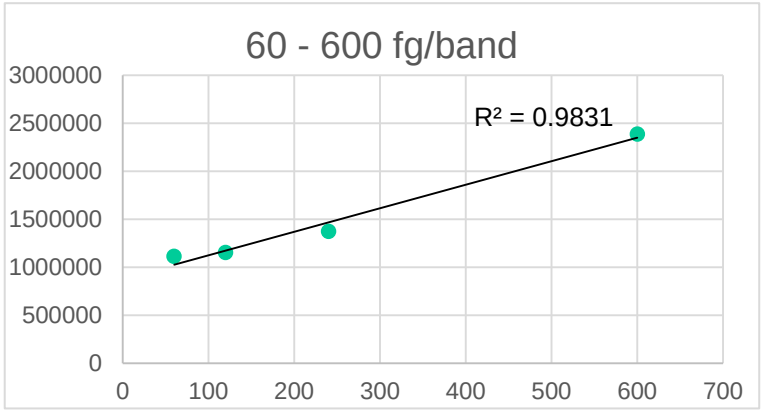
m/z 193.0861
± *m/z* 1.0



EIC

193.08187
- 193.08992





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JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

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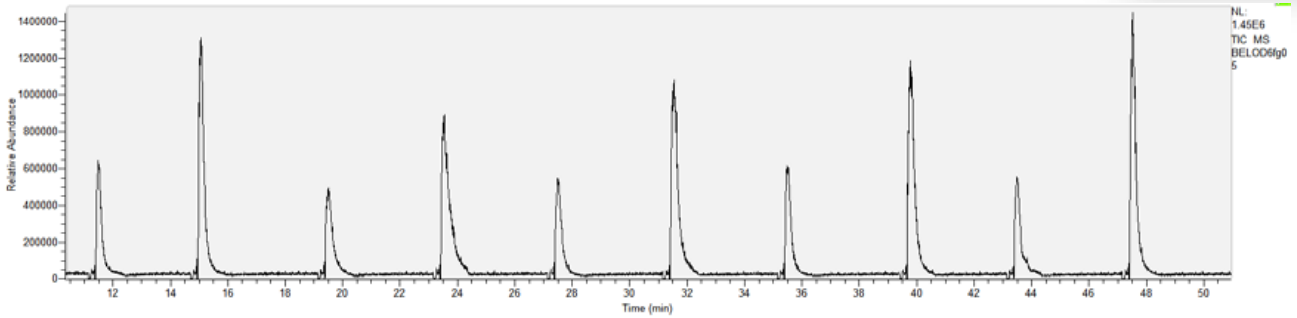
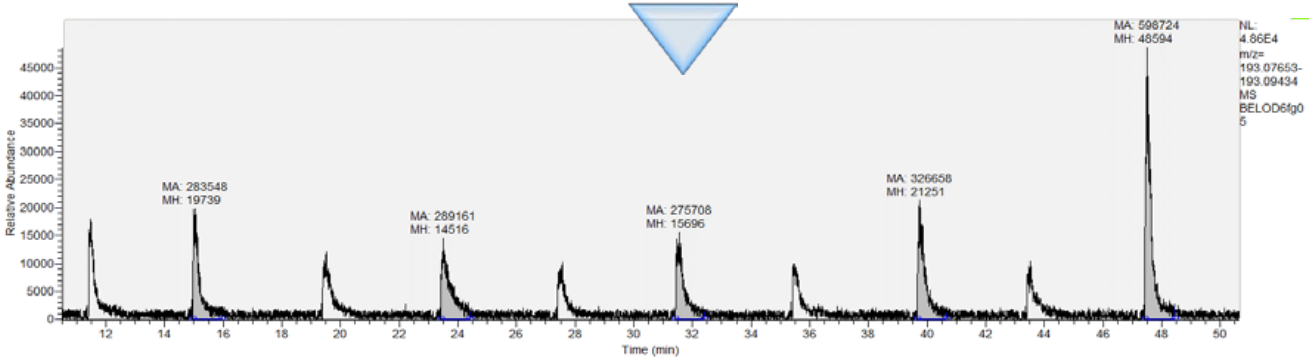


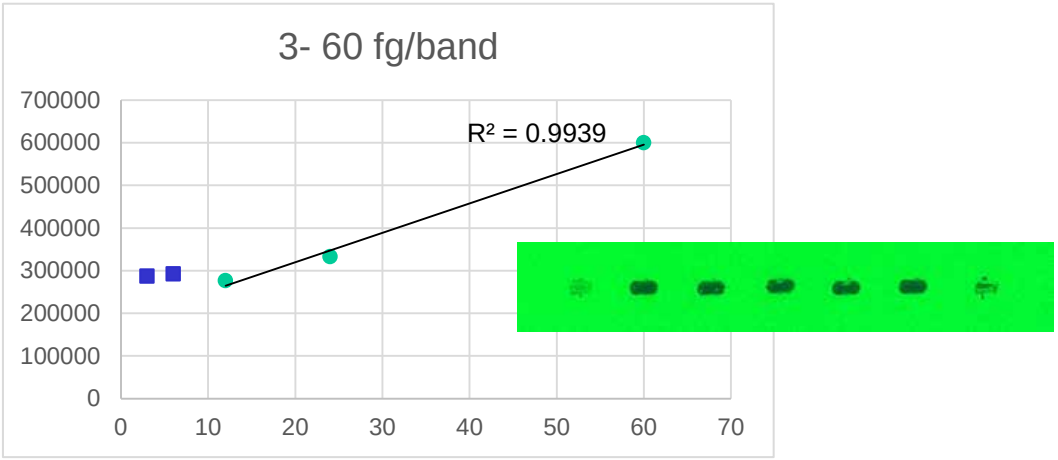
EIC

193.076
- 193.096



LOD of 24 fg/band
for butyl paraben

If 100 µL sample
volume applied:
→ LOD of 0.24 ng/L



HPTLC-MS

Can we just compare figures

like limit of detection (LOD)?

LOD 100 pg/band?

On what does LOD depend on?

Application volume, MS analyzer, zone shape

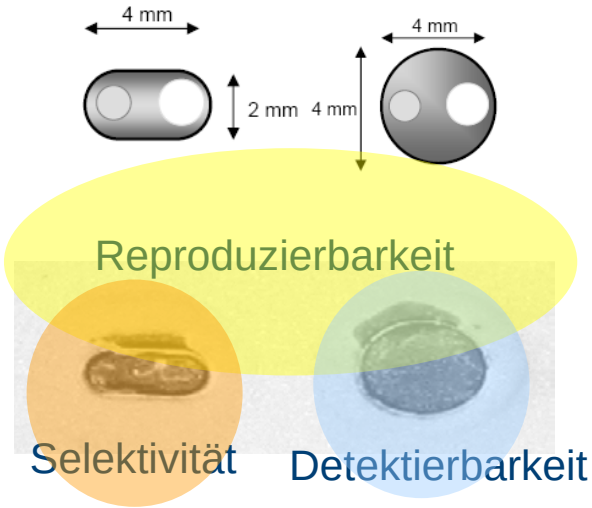
Elution head – cutting edge

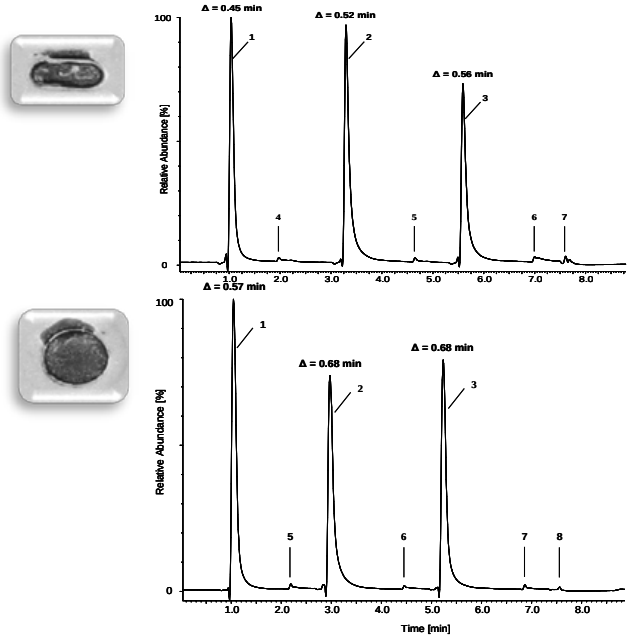
Höhe

- 0.2 mm für Standardschichten → G. Morlock, W. Schwack, *Anal Bioanal Chem* 385 (2006) 586 - 595
- 0.1 mm für extra dünne Schichten → U. Jautz, G. Morlock, *Anal Bioanal Chem* 387 (2007) 1083 - 1093
- 0.5 mm für preparative Schichten → E. Dytkiewitz, G. Morlock, *J AOAC Int* 91 (2008) 1237 - 1244

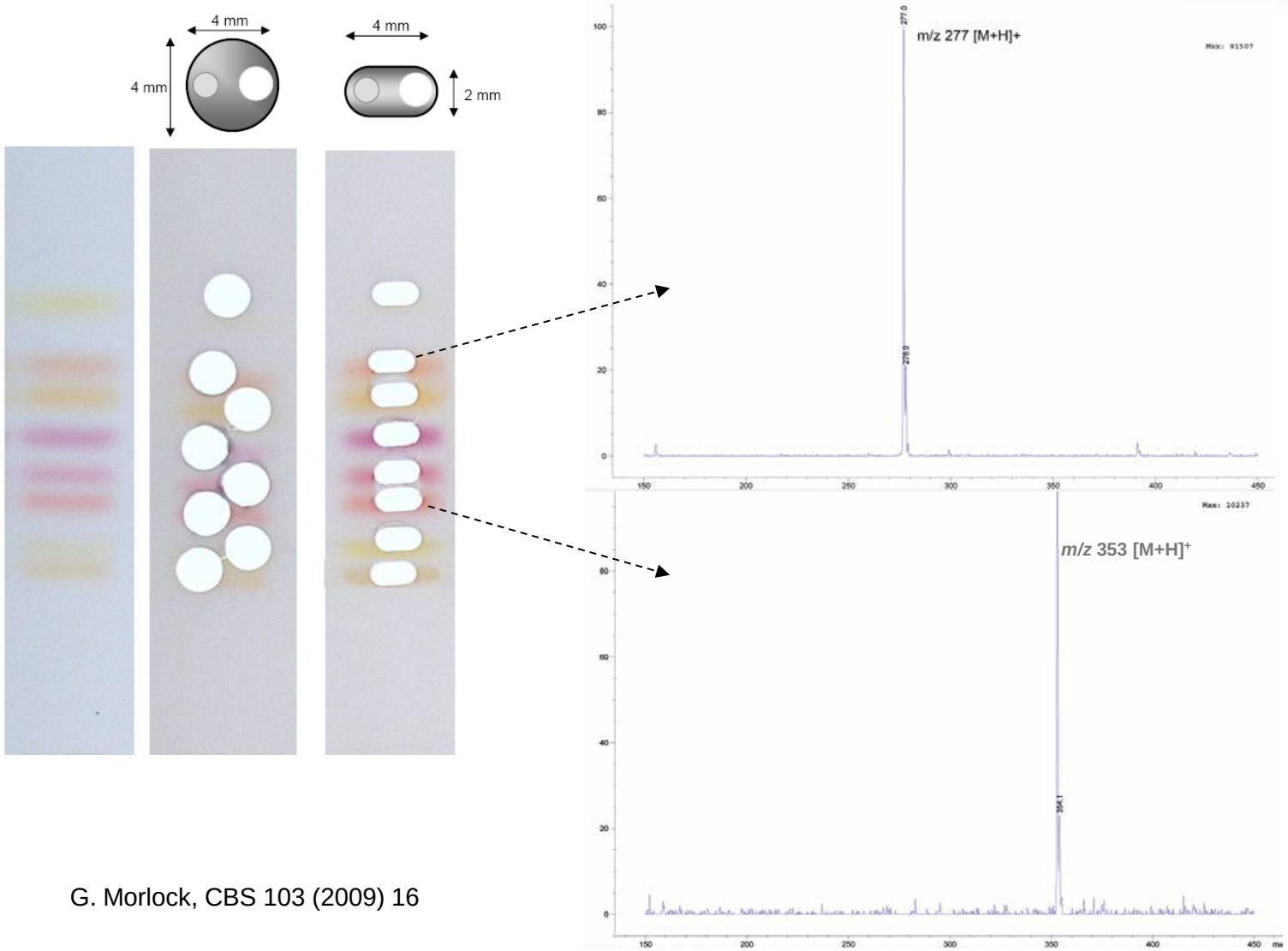
Geometrie

Circular *versus* oval → U. Jautz, G. Morlock, *J Planar Chromatogr* 21 (2008) 367 - 371





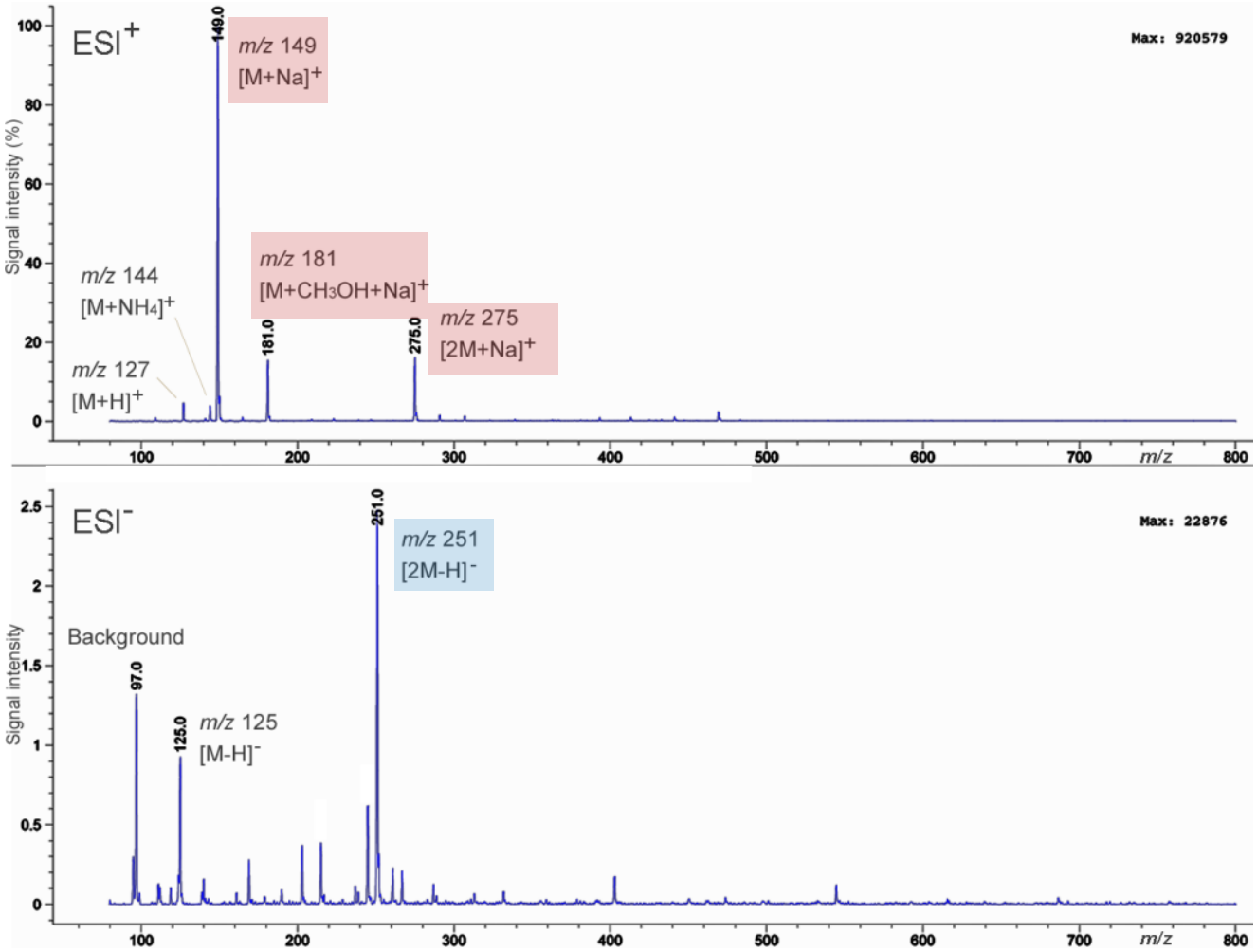
Elution head – cutting edge



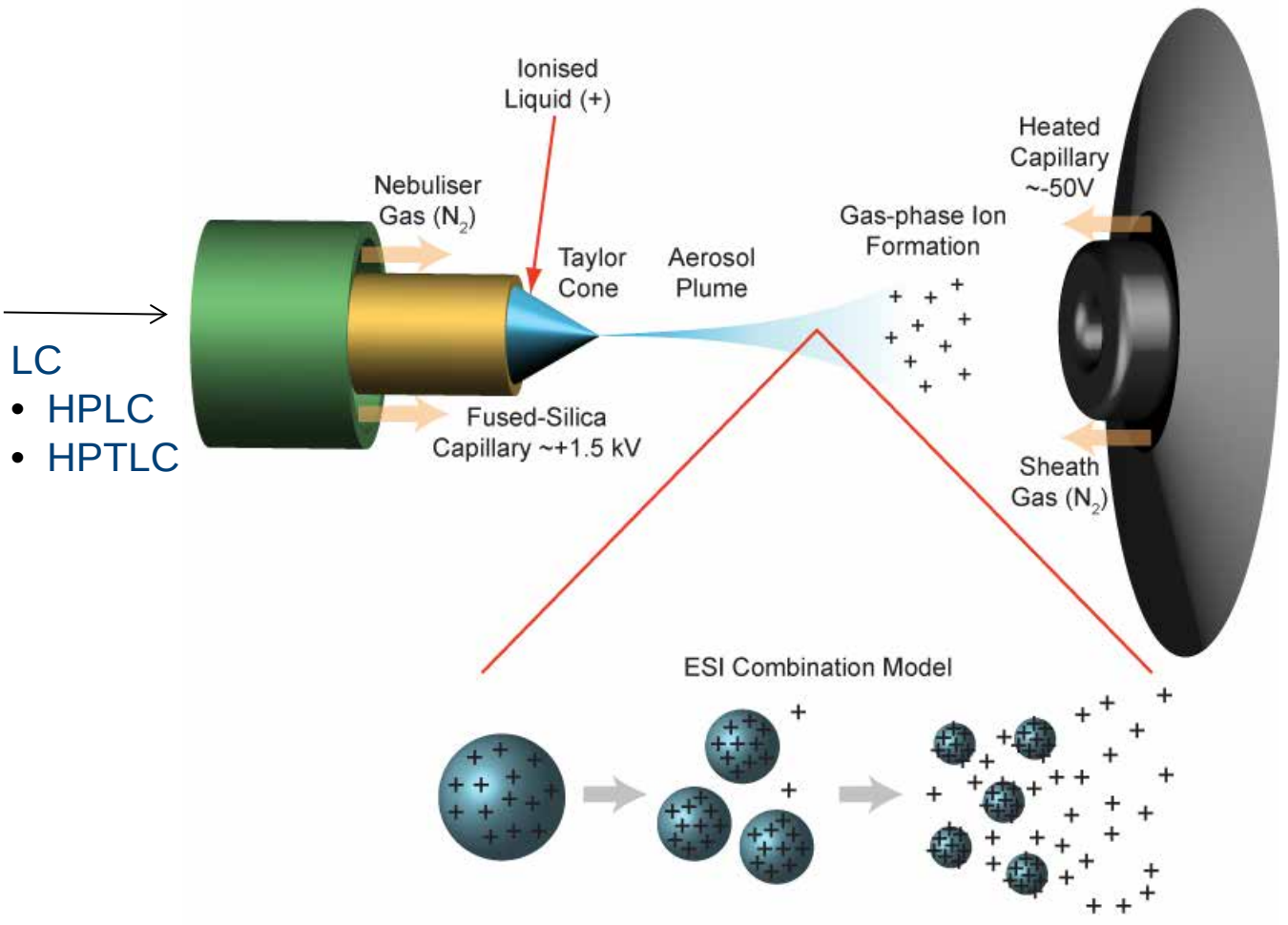
G. Morlock, CBS 103 (2009) 16

G. Morlock Food Science JLU Gießen

Adducts



Electrospray ionization (ESI)



LC
- HPLC
- HPTLC

MS Eingang
(Orifice)

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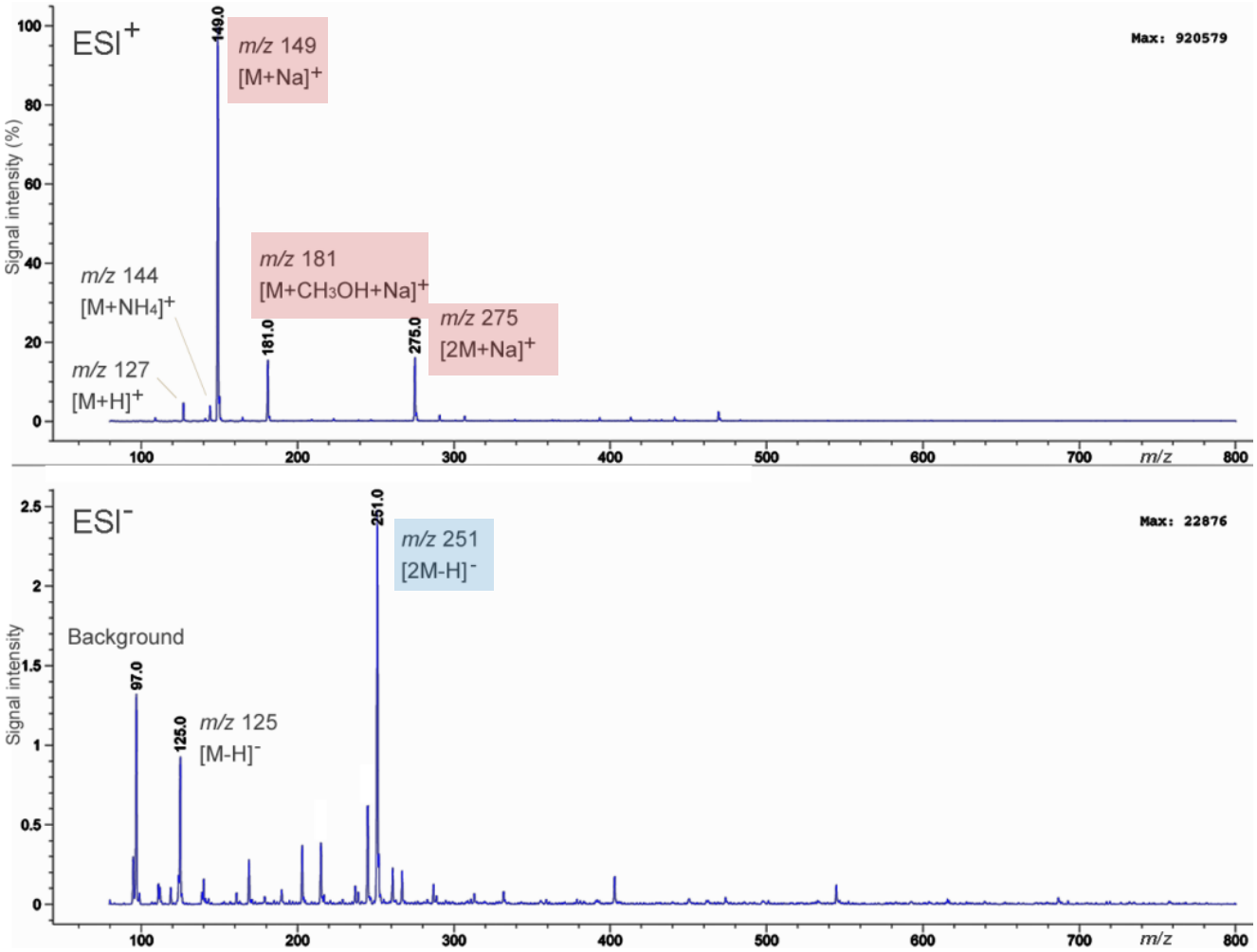
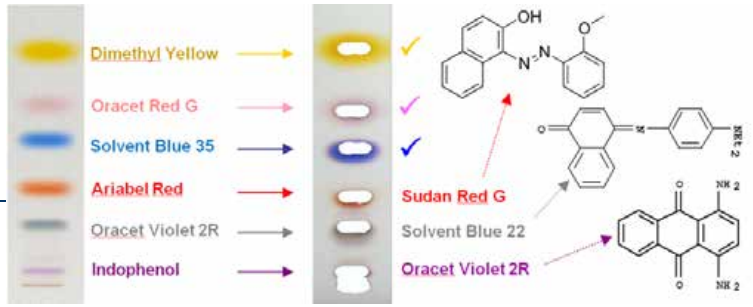
Adducts

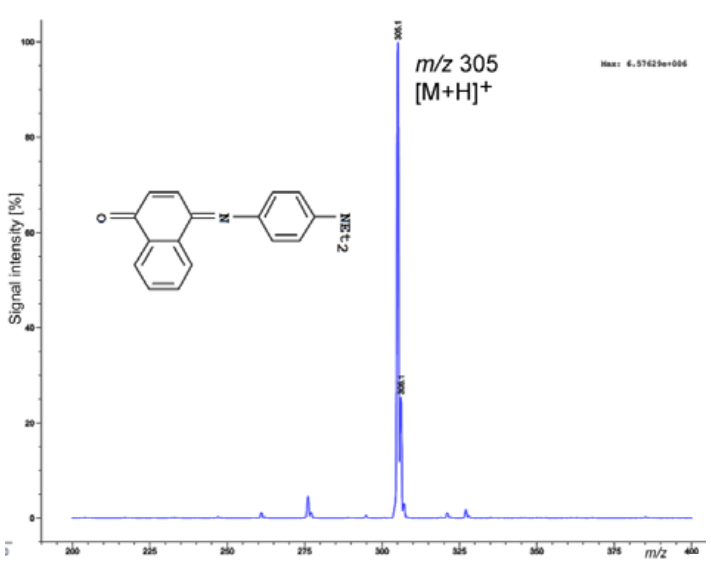


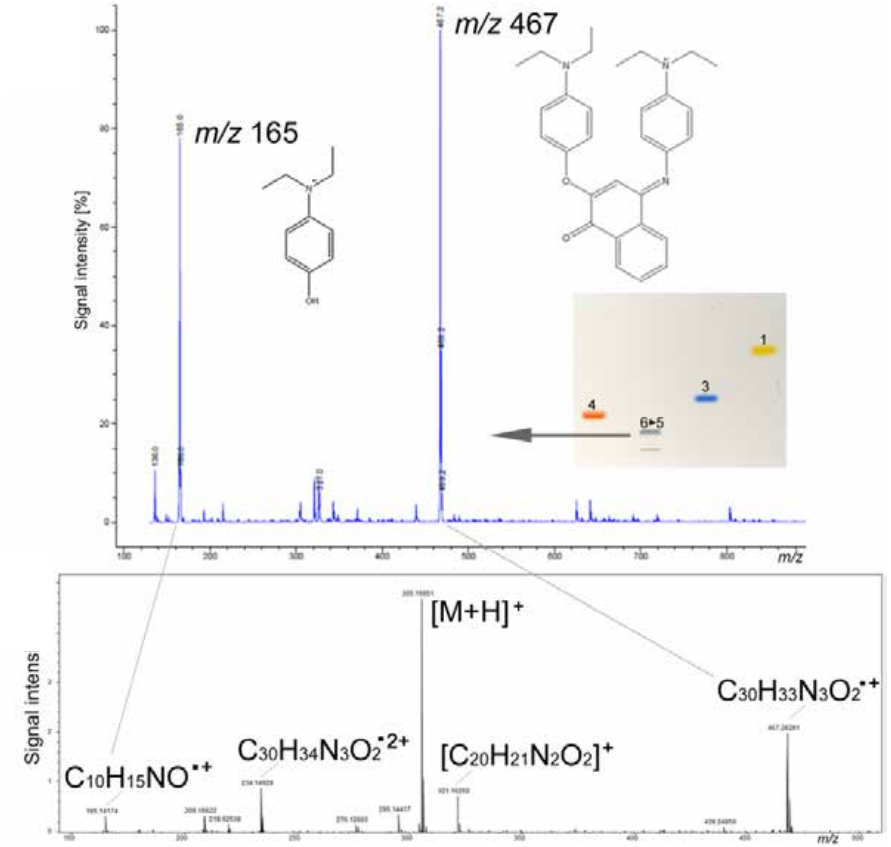
Adducts in ESI$^+$

Difference of 5 Da?	M + 23 Da Na M - 18 Da H_20	$[M+Na-H_20]^+$
No difference in Da?	M + 18 Da NH_4 M - 18 Da H_20	$[M+NH_4-H_20]^+$
Difference of 17 Da?	M + 1 Da H M - 18 Da H_20	$[M+H-H_20]^+$
Difference of 7 Da?		$[M+Li]^+$
Difference of 39 Da?		$[M+K]^+$
Difference of 45 Da?	M + 46 Da 2 Na M - 1 Da H	$[M+2Na-H]^+$

Photooxidation effects?







Morlock, G.E., Brett, N., J Chromatogr A 1390 (2015) 103-111

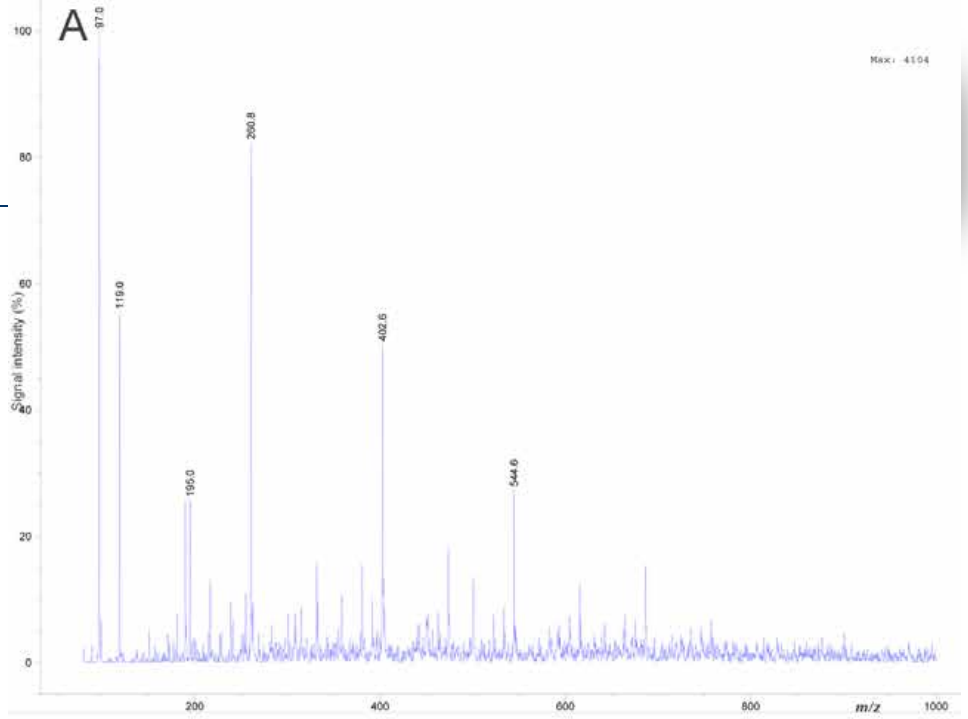
HPTLC-MS

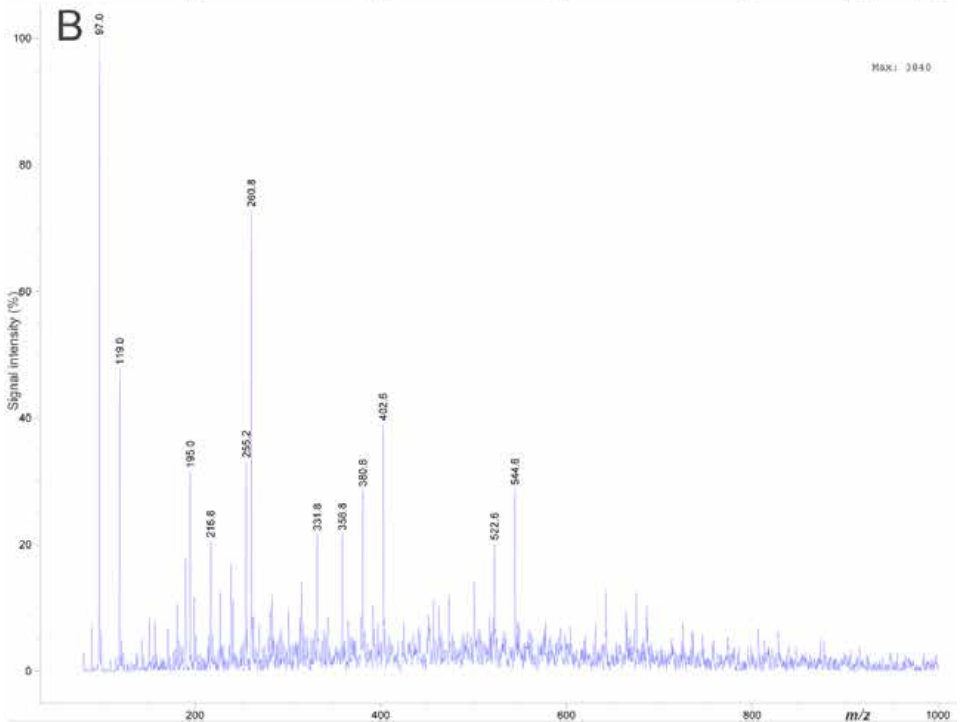
Other signals?

Fluorescence indicator

with F_{254}

without F_{254}





G. Morlock J. Liq. Chromatogr.
Relat. Technol. 37 (2014) 2892–2914

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	Main Background Mass Signals m/z (Intensity of Base Peak)	
HPTLC-ESI-MS	Negative Ion Mode	Positive Ion Mode
System signals		
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Dibutylphthalate and its potassium adduct		279 $[M+H]^+$ and 317 $[M+K]^+$
Diisooctyl phthalate and its sodium adduct		391 $[M+H]^+$ and 413 $[M+Na]^+$
1-Ethyl-3-methylimidazolium		*111*M^+
Trifluoroacetic acid or formate dimer	113 $[M-H]^-$ or $[2M+Na\text{-}2H]^-$	
Plate used directly	97.0 and cluster with $\Delta m/z$ 142: 119.0, 260.8 (5094), 402.8, 544.6	111.2, 391.2, 413.2 (52303), 419.2, 441.2
Plate pre-washed with neutral solvents and solvent mixtures (all v/v)		
Ethyl acetate	97.0 and cluster with $\Delta m/z$ 142: 119.0, 260.8 (5704), 402.6, 544.6	164.8, 301.0 (30595), 306.8
MeOH	97.0 and cluster with $\Delta m/z$ 142: 119.0, 260.8 (5943), 402.6, 544.6	111.2 (65390)
MeOH - H_2O 1:1	Very low: 255.0 (747)	413.2 (88052), 441.2
MeOH - H_2O 1:3	Very low: 97.0, 119.0, 123.0, 255.2, 281.2 (554), 501.0	391.2, 413.2 (64202)
MeOH - H_2O 3:1	Very low: 97.0 (816), 119.0, 123.0, 255.2, 281.2, 501.0	81.2, 111.2 (27139), 152.2, 301.0
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MeOH - NH_3 100:1	97.0 and cluster with $\Delta m/z$ 142: 260.8 (4102), 402.6, 544.6	81.2, 111.2 (25.741), 152.2, 301.0
MeOH - CH_3COOH 100:1	Cluster with $\Delta m/z$ 82: 141.0, 223.0, 305.0, 387.0 (12599), 468.8, 550.8, 632.8, 714.8, 796.8	111.2 (80743) and cluster with $\Delta m/z$ 82: 105.0, 187.0, 269.0, 351.0, 432.8, 514.8, 596.8
MeOH - HCOOH 100:5	97.0, 119.0 and cluster with $\Delta m/z$ 68: 181.0, 248.8, 316.8, 328.8, 384.8 (1635), 452.8, 520.8, 588.6, 656.6	111.2 (46844), 152.2 and cluster with $\Delta m/z$ 68: 91.0, 159.0, 227.0, 294.8, 362.8, 430.8, 498.8, 566.8, 634.6

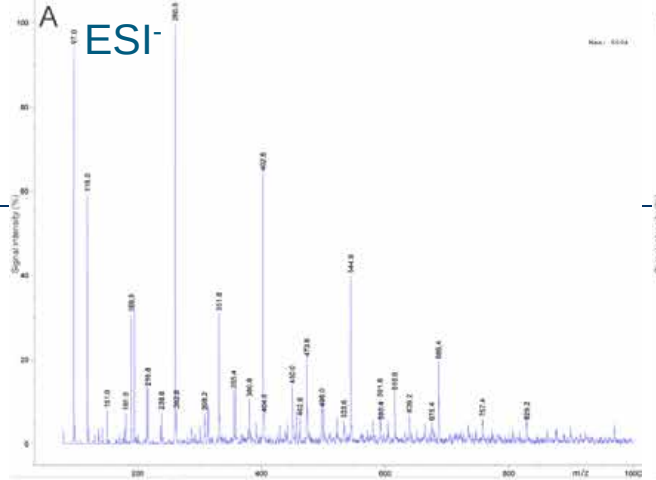
Eluted with methanol by TLC-MS Interface; assignments preliminary.

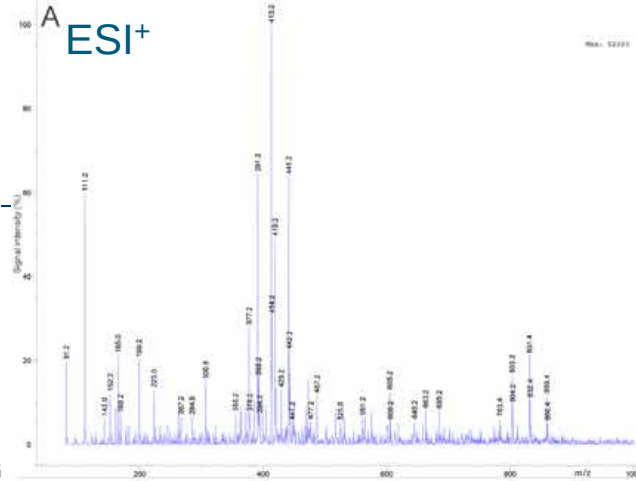
Pre-washing

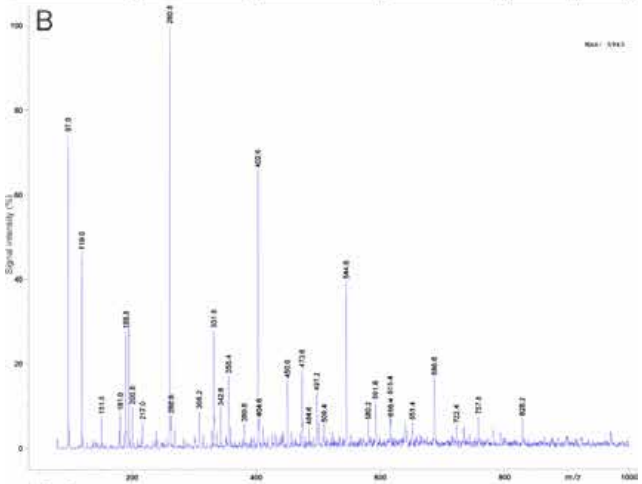
directly used

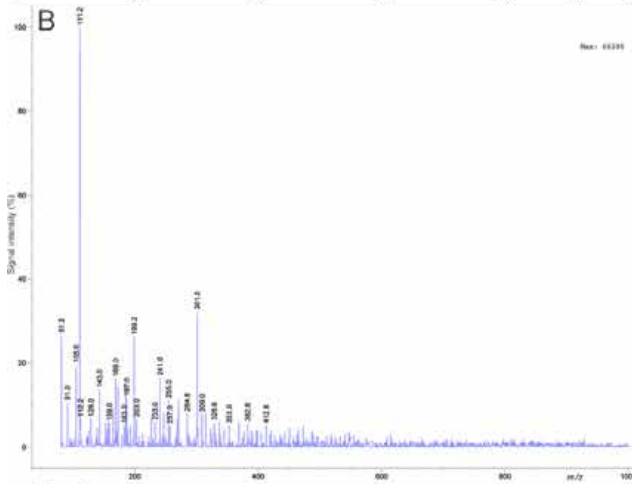
methanol

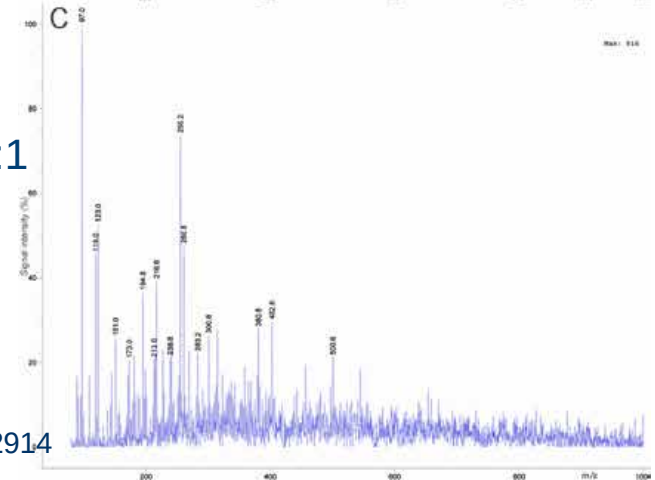
methanol–water 3:1

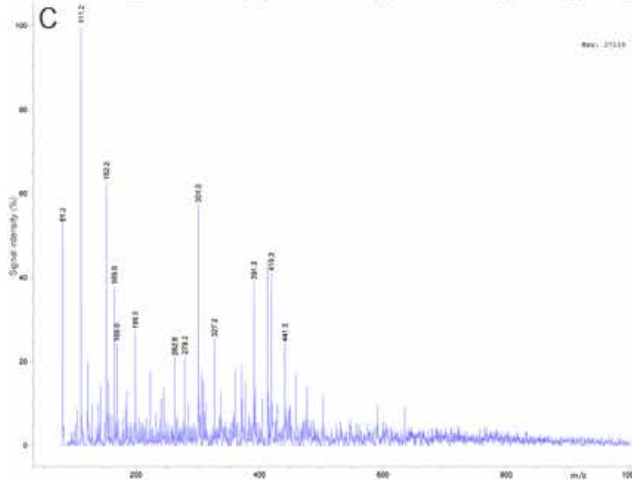












G. Morlock J. Liq. Chromatogr. Relat. Technol. 37 (2014) 2892–2914

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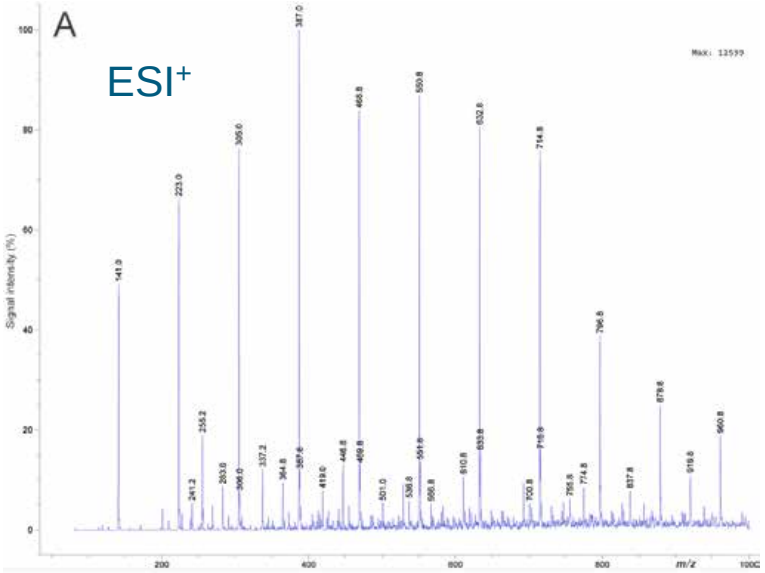
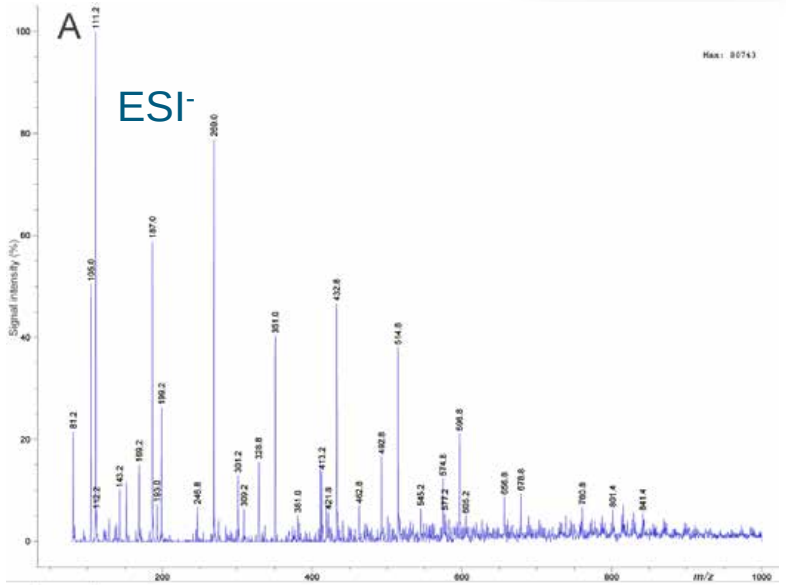
Eluted with methanol by TLC-MS Interface; assignments preliminary.

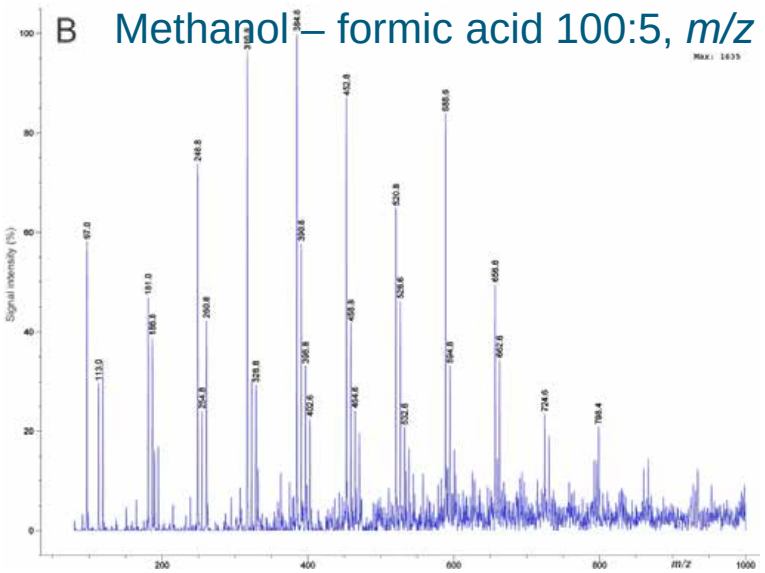
Acidic solvents

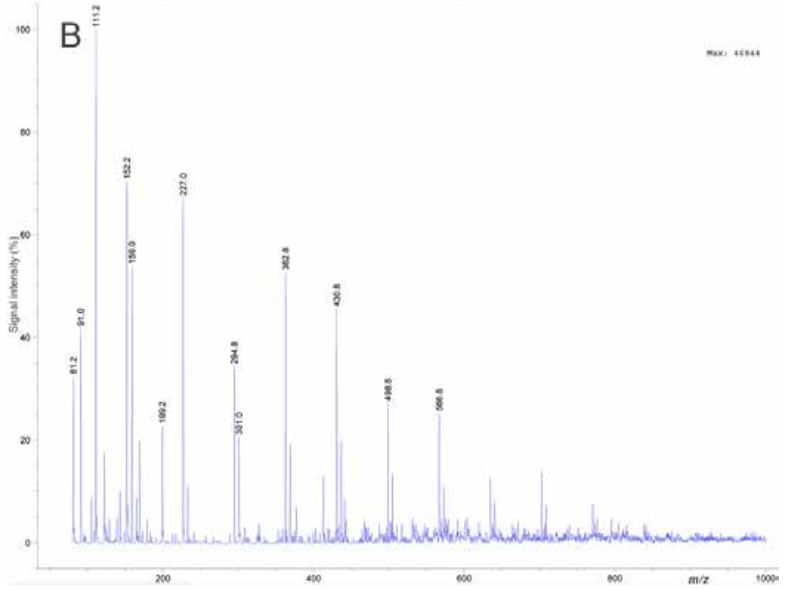
Methanol – acetic acid 100:1, *m/z* 82



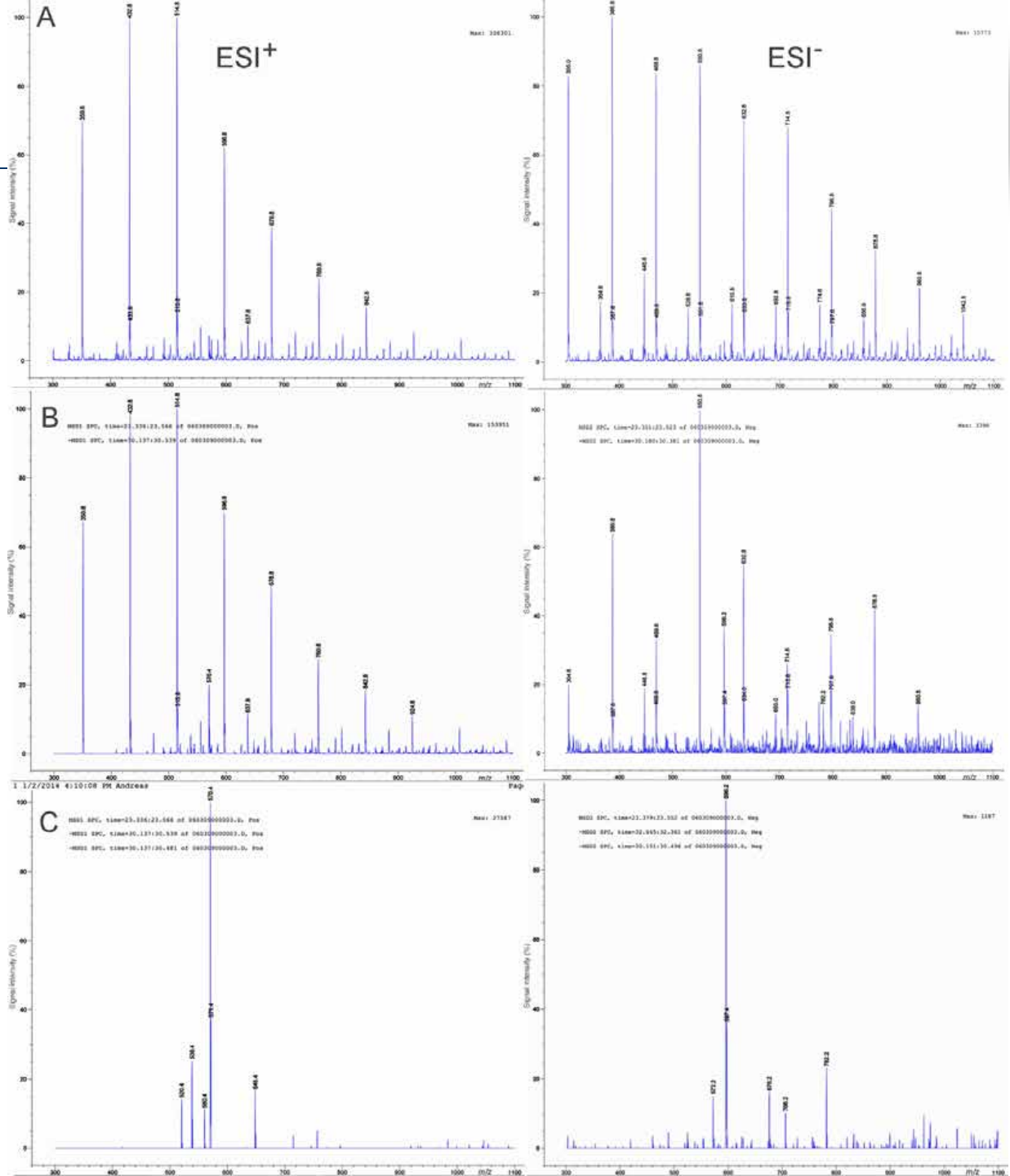


B Methanol – formic acid 100:5, *m/z* 68





Background subtraction



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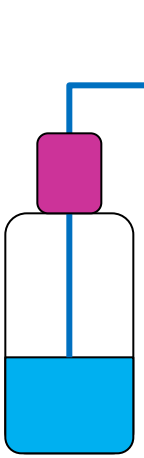
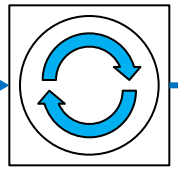
MeOH - NH_3 100:1	97.0 and cluster with $\Delta m/z$ 142: 260.8 (4102), 402.6, 544.6	81.2, 111.2 (25.741), 152.2, 301.0
MeOH - CH_3COOH 100:1	Cluster with $\Delta m/z$ 82: 141.0, 223.0, 305.0, 387.0 (12599), 468.8, 550.8, 632.8, 714.8, 796.8	111.2 (80743) and cluster with $\Delta m/z$ 82: 105.0, 187.0, 269.0, 351.0, 432.8, 514.8, 596.8
MeOH - HCOOH 100:5	97.0, 119.0 and cluster with $\Delta m/z$ 68: 181.0, 248.8, 316.8, 328.8, 384.8 (1635), 452.8, 520.8, 588.6, 656.6	111.2 (46844), 152.2 and cluster with $\Delta m/z$ 68: 91.0, 159.0, 227.0, 294.8, 362.8, 430.8, 498.8, 566.8, 634.6

Eluted with methanol by TLC-MS Interface; assignments preliminary.

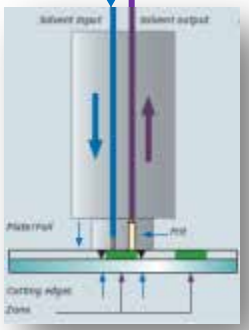
Workflow



Eluent
Methanol/ammonia formiate buffer (10 mM, pH 4) 19:1 (v/v)

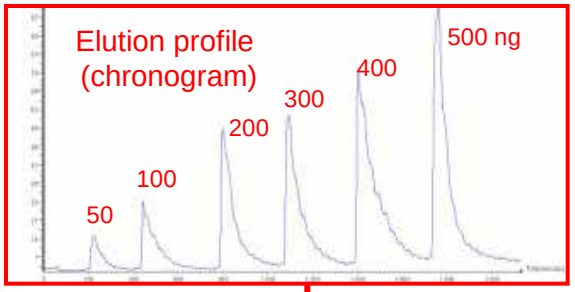


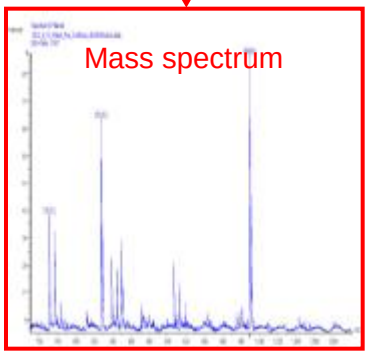
Pump
100-200 µL/min



Elution
Zone selection and elution

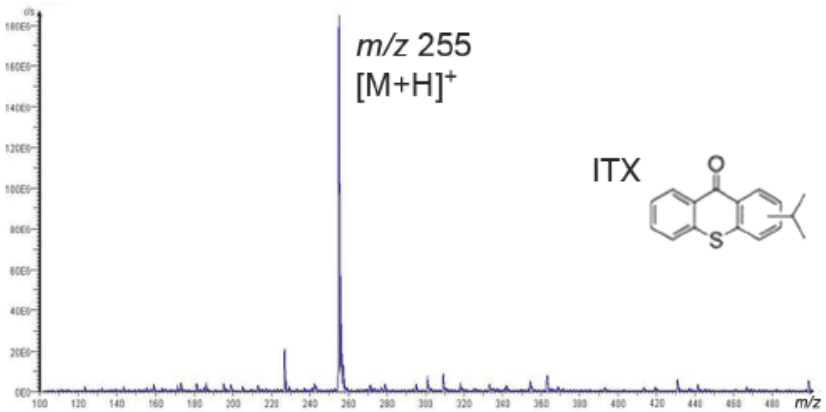
Mass signal
Online analysis of eluted analytes

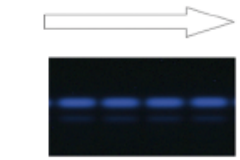


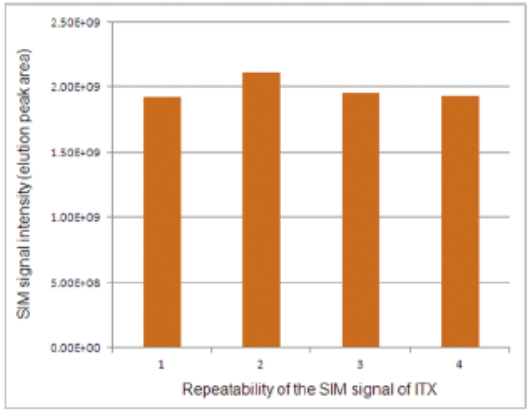


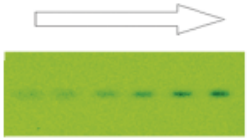
Data evaluation
→ Structure confirmation
→ Structure elucidation
→ Sum formula

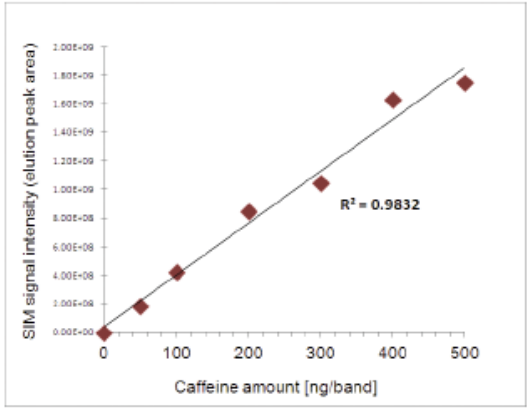
Performance





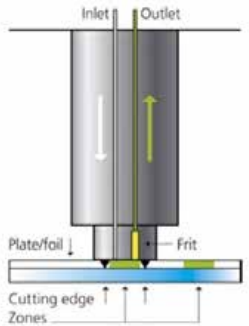






G. Morlock, F. Porbeck, A. Wiesner, I. Klingelhöfer, CBS 110 (2013) 9

Performance data →TLC-MS Interface



→ highly reliable hyphenation

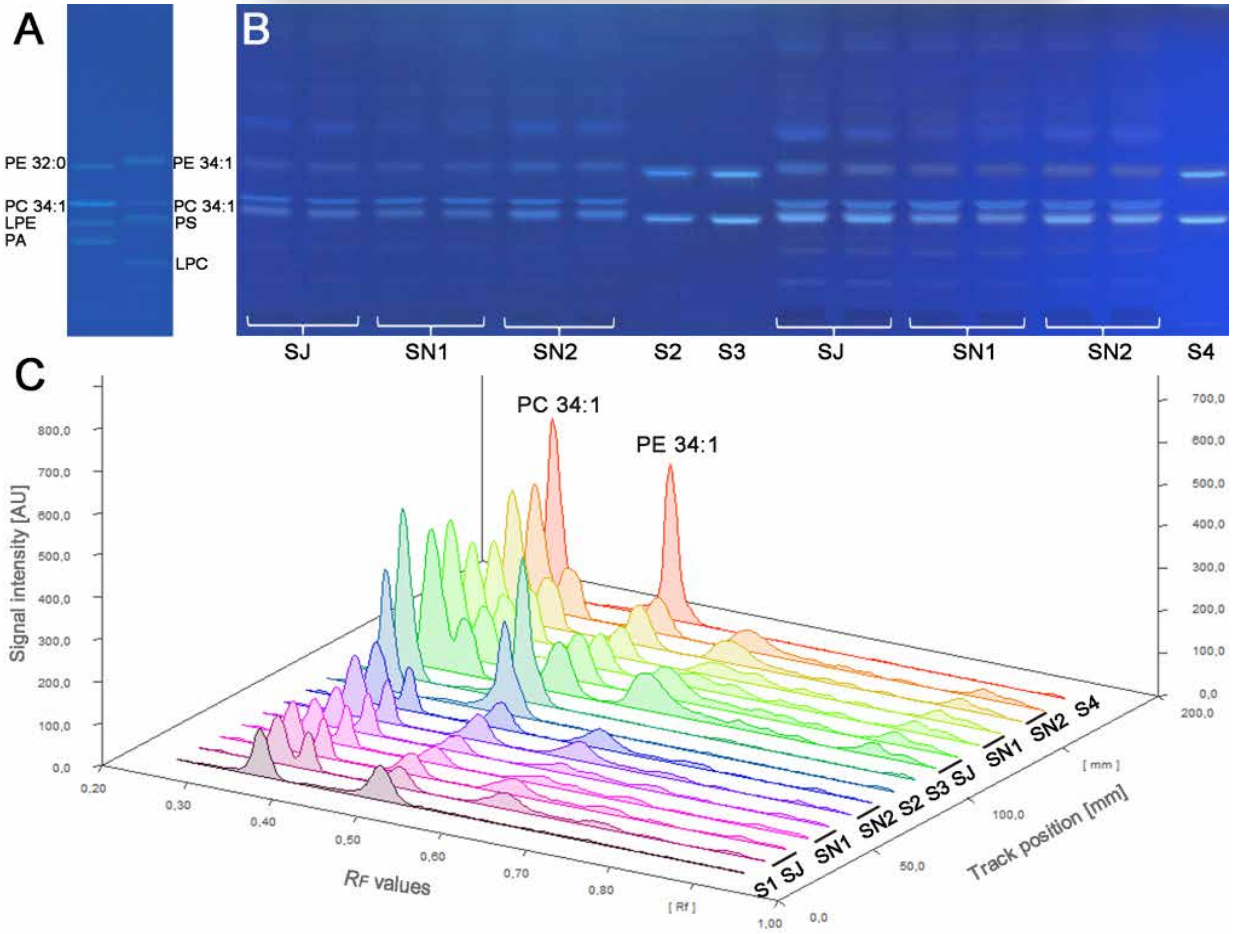
→ highly targeted

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

G. Morlock, N. Brett, J Chromatogr A 1390 (2015) 103-111

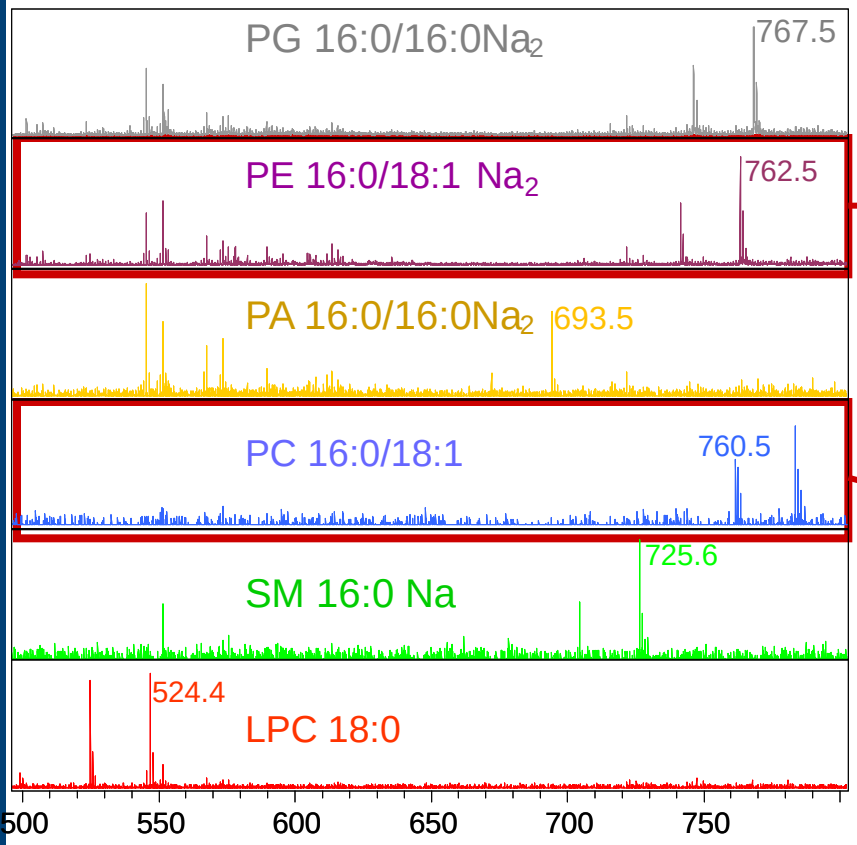
JLU

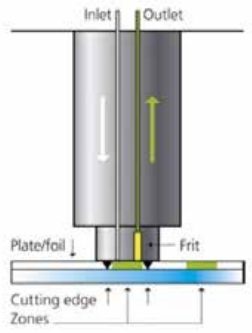
Analysis of phospholipids in lecithins

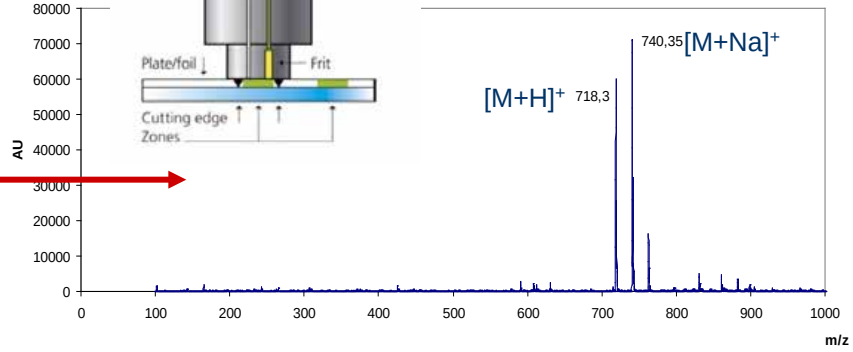


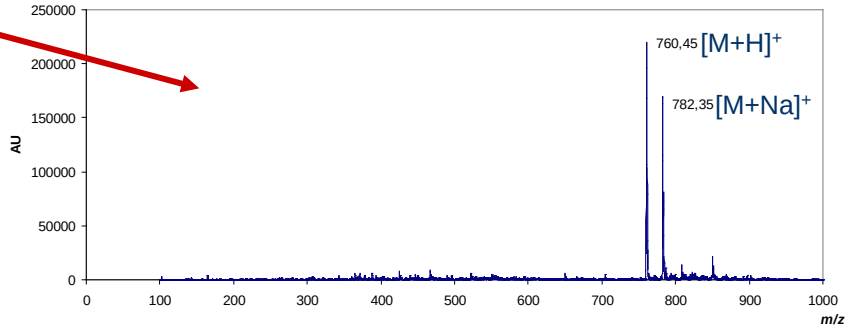
S. Krüger, L. Bürmann, G. Morlock, J Agric Food Chem 63 (2015) 2893–2901

Comparison of mass spectra

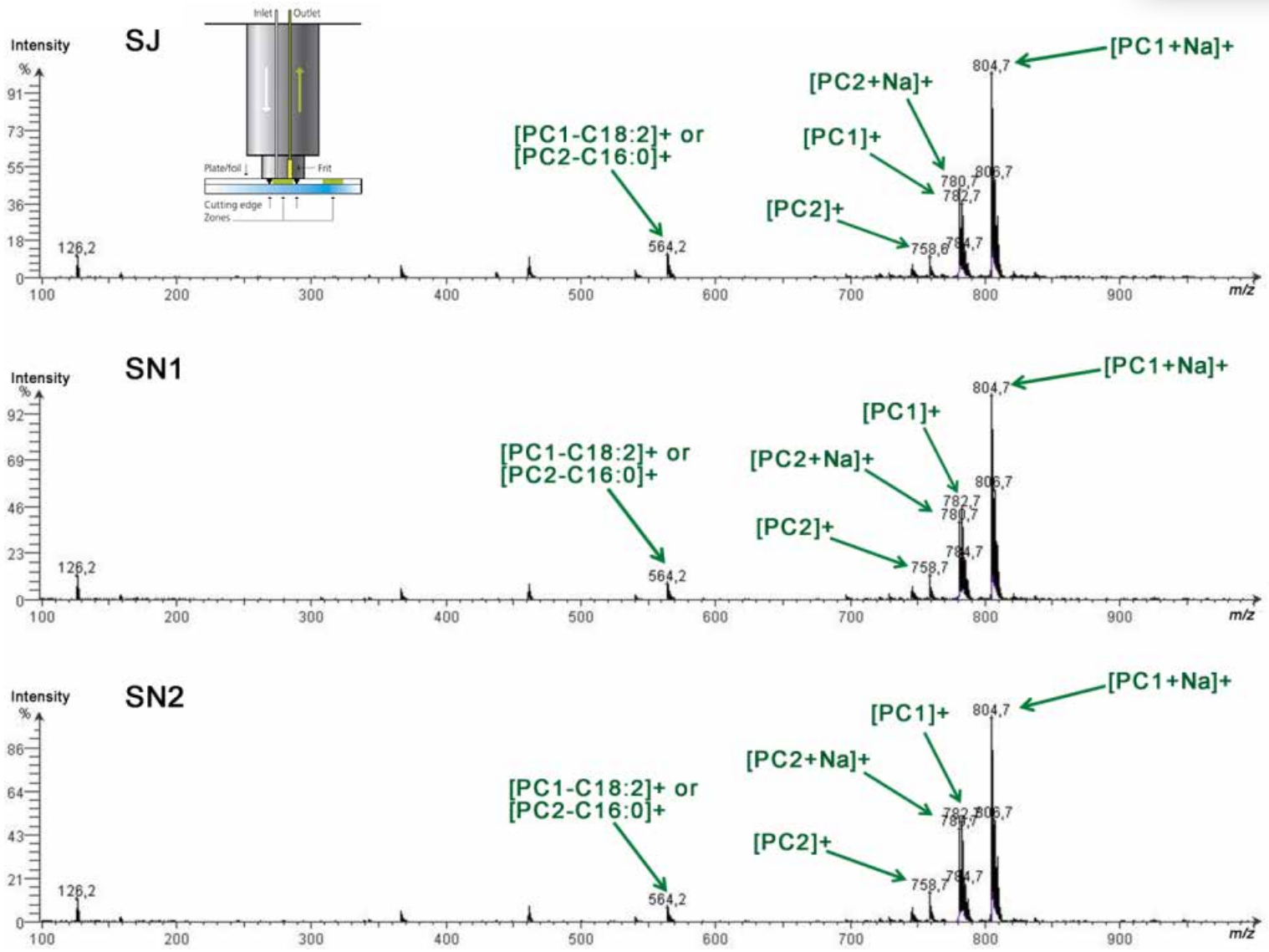






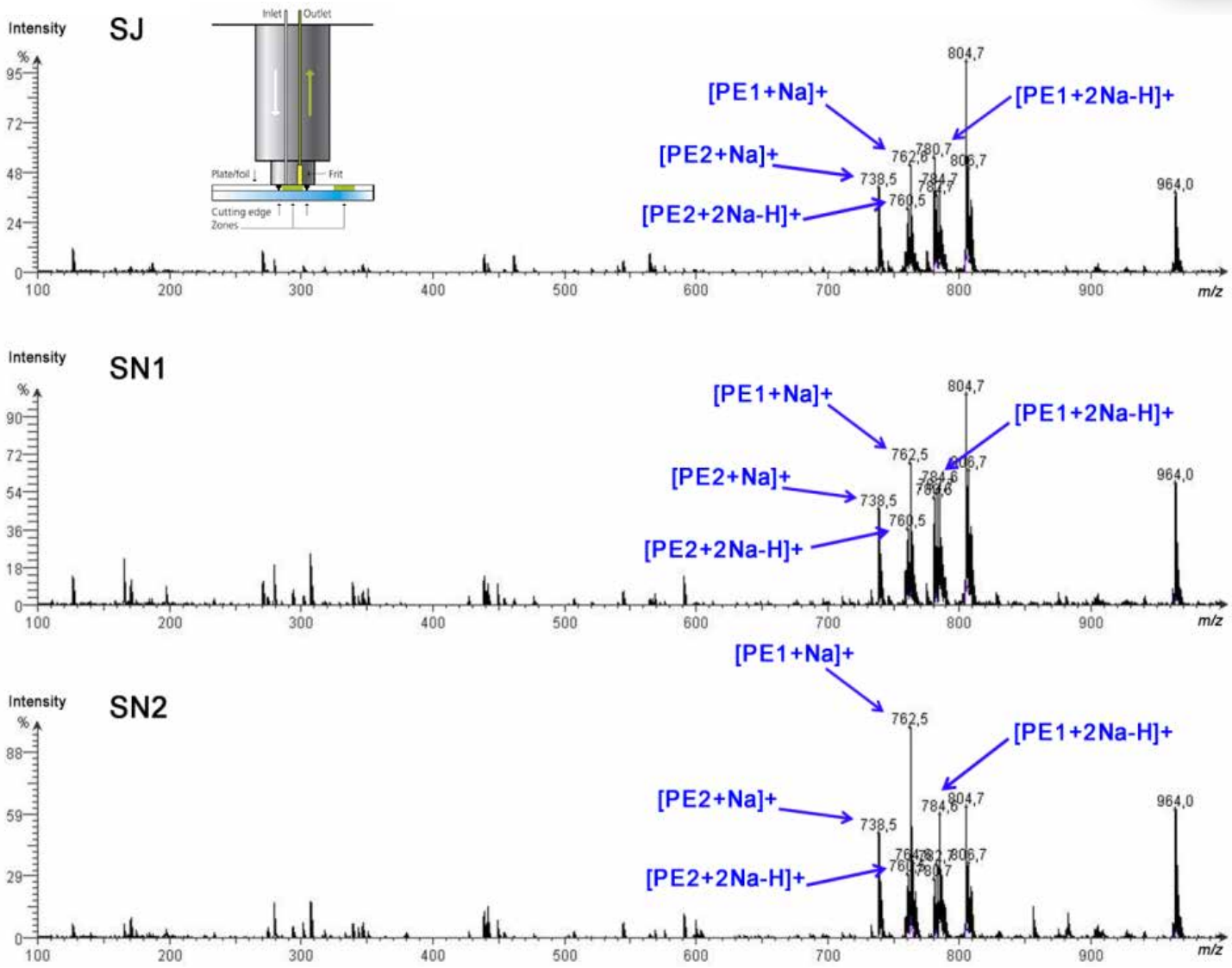


Characterization: soy bean *vs.* sunflower lecithin



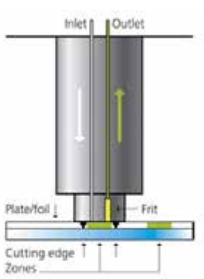
S. Krüger, L. Bürmann, G. Morlock, J Agric Food Chem 63 (2015) 2893–2901

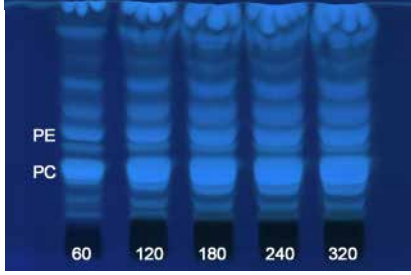
Characterization: soy bean *vs.* sunflower lecithin

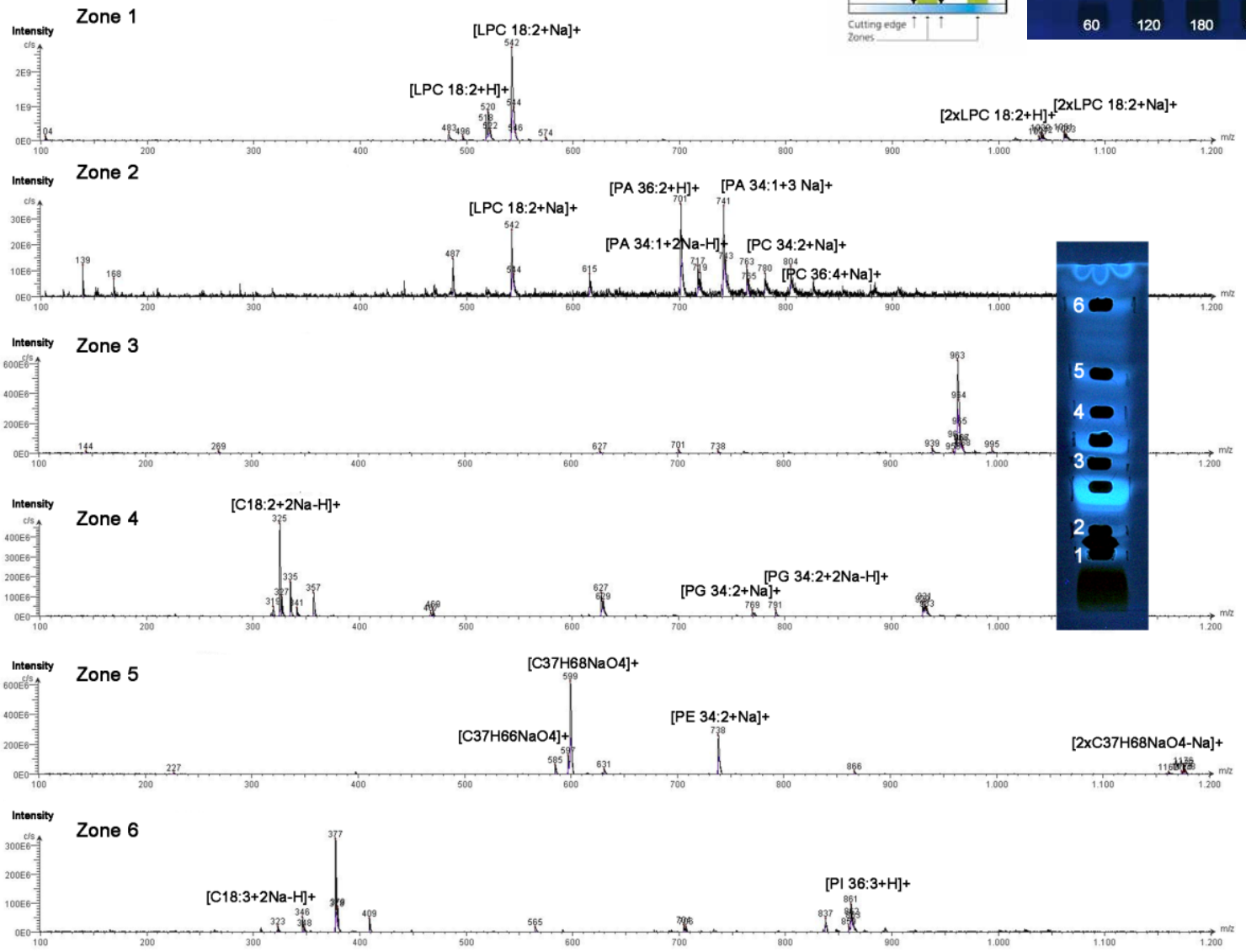


S. Krüger, L. Bürmann, G. Morlock, J Agric Food Chem 63 (2015) 2893–2901

Minor phospholipids?



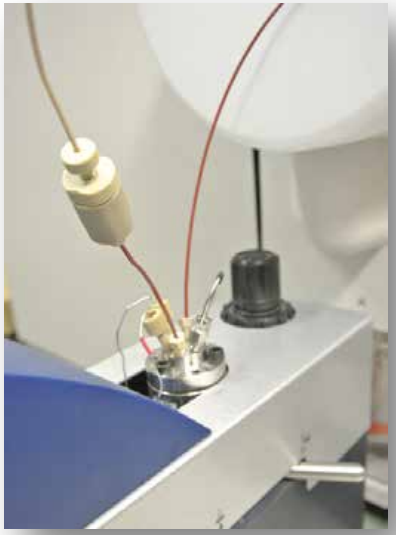


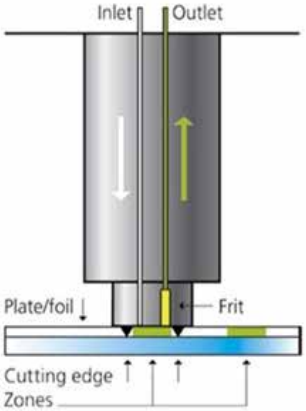


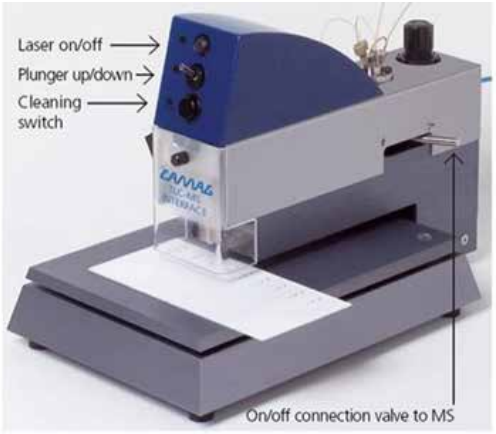
S. Krüger, L. Bürmann, G. Morlock, J Agric Food Chem 63 (2015) 2893–2901

Pressure increase

Integrated online filter





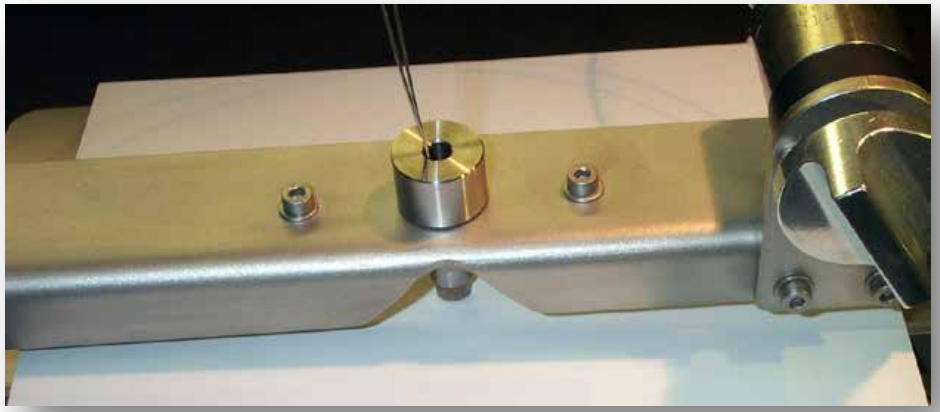


- Substance amount too high: the solubility balance can be reached and nonsoluble, solid particles can block the filter
- Forward sequence and backward sequence let forget the intermediate cleaning step

→ Full automatization is the next step

G. Morlock J. Liq. Chromatogr. Relat. Technol. 37 (2014) 2892–2914

ChromeXtractor

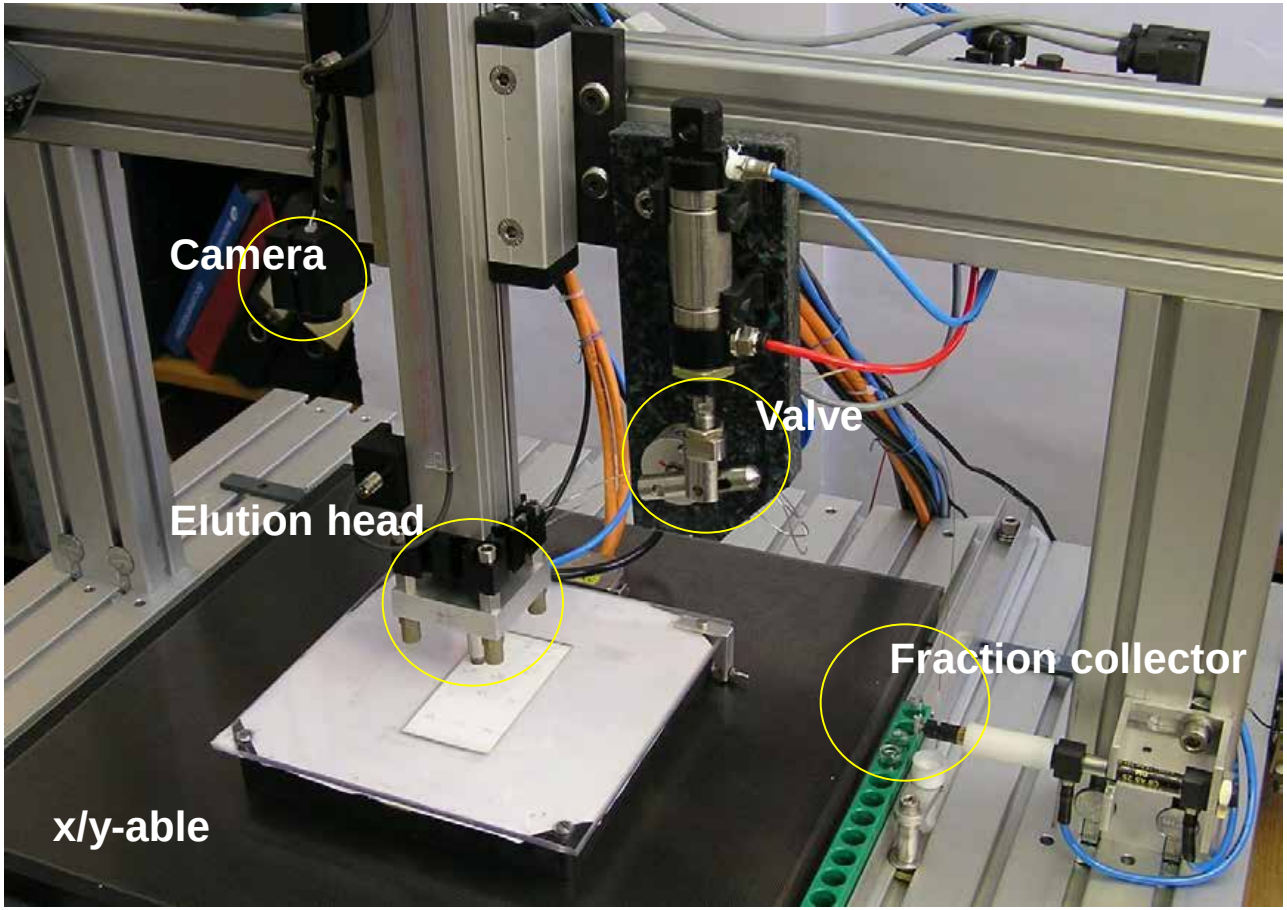


H. Luftmann, Anal Bioanal Chem 378 (2004) 964-968

→ Modification of the elution head for its use on glass plates

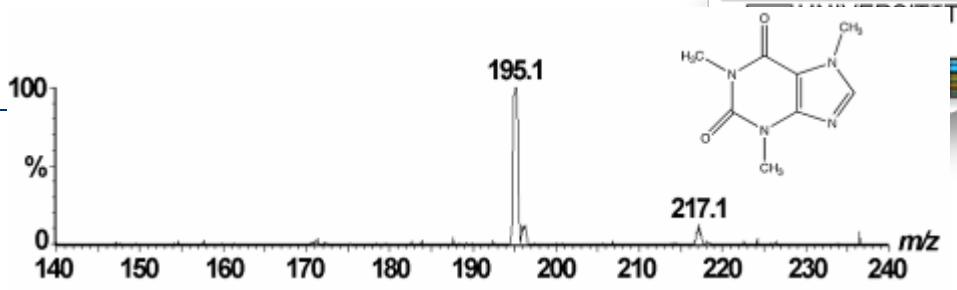
A. Alpmann, G. Morlock, Anal Bioanal Chem 386 (2006) 1543-1551

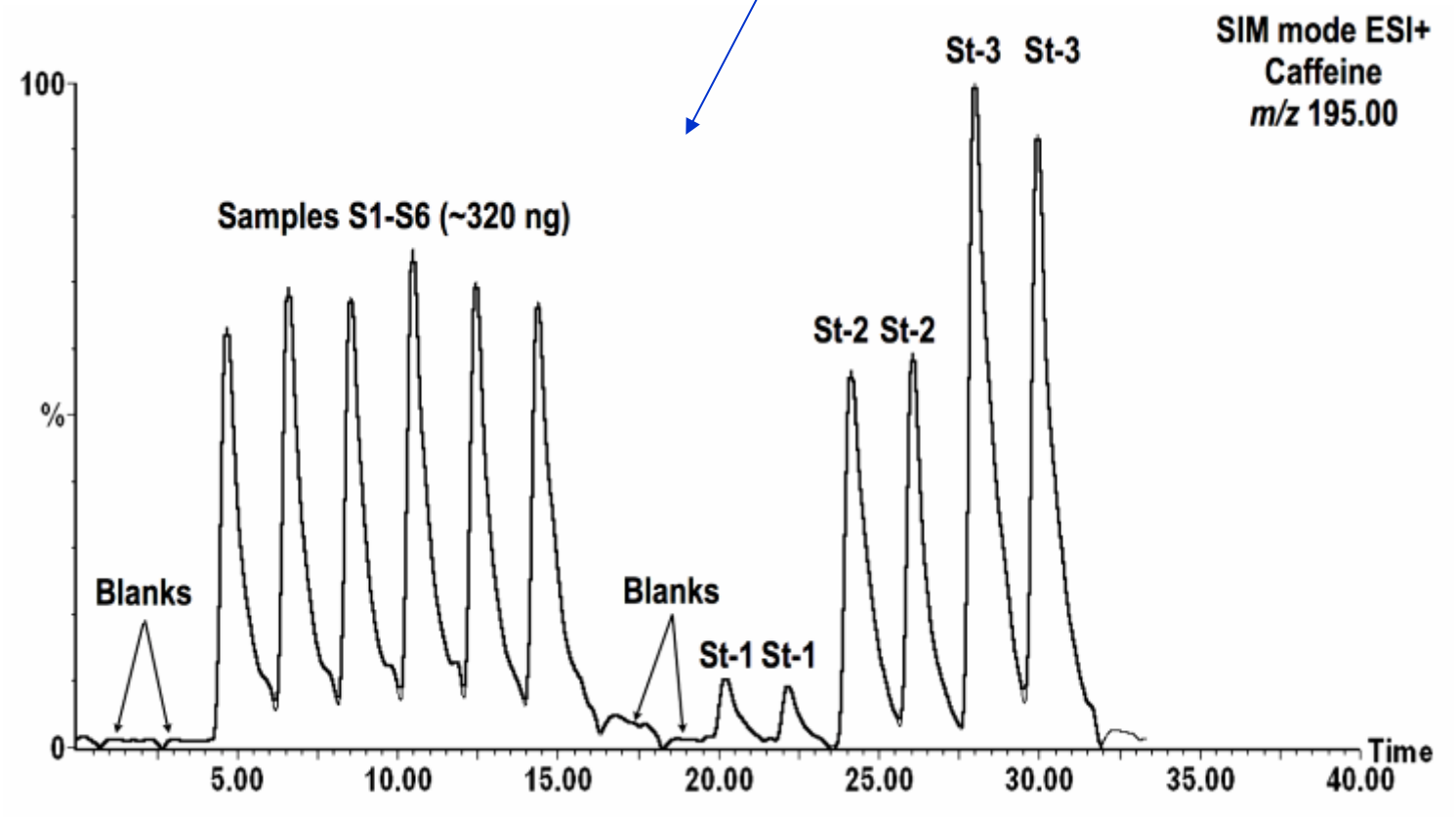
Automated elution head-based interface



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

Elution profiles



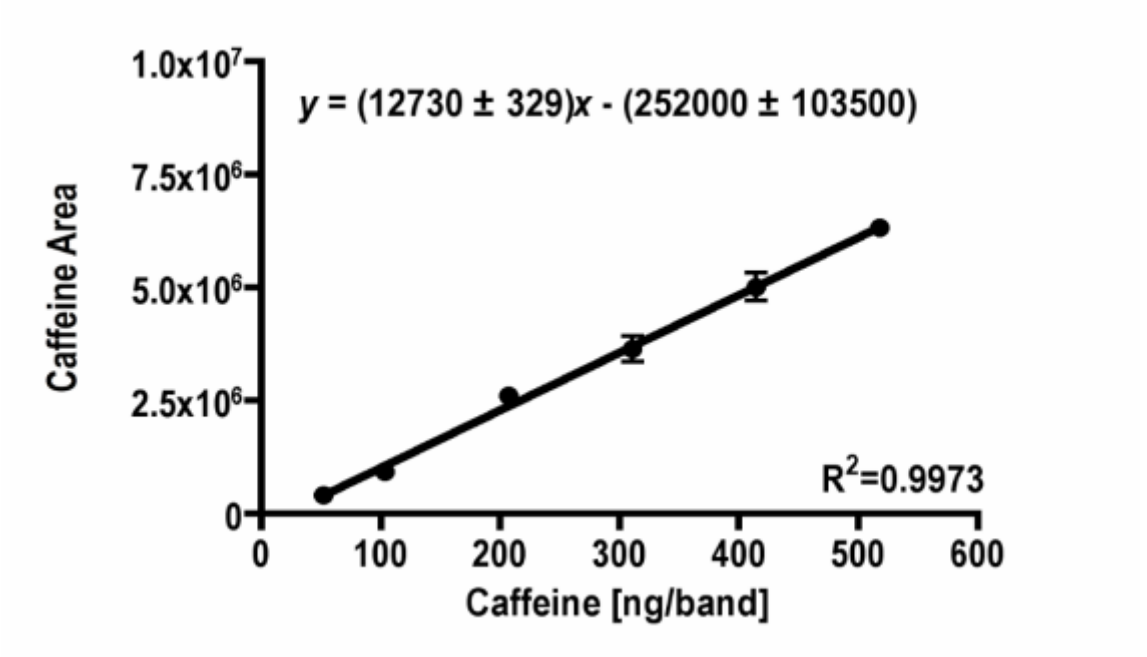


H. Luftmann, M. Aranda, G. Morlock, Rapid Commun Mass Spectrom 21 (2007) 3772-3776

Data of validation without IS

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

→ Linearity R^2: 0.9973



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

Analysis of samples containing caffeine

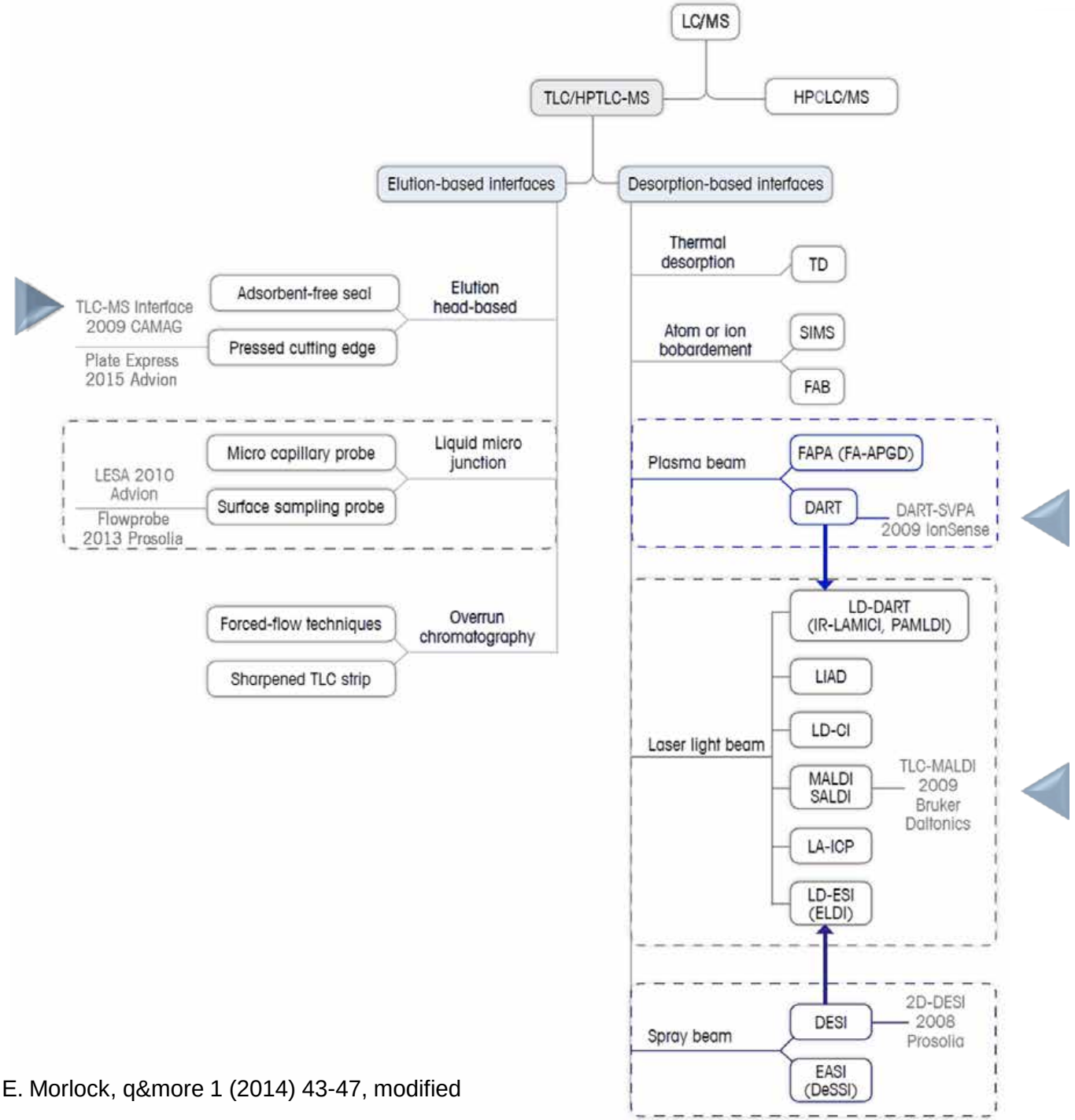
Sample	Pharmaceutical mean ± SD (mg/tablet)	Energy drink mean ± SD (mg/100 mL)
HPTLC/ESI-MS RSD (%, n = 6)	102.09 ± 5.76 (5.6)	32.91 ± 1.60 (4.9)
HPTLC/UV RSD (%, n = 5)	101.98 ± 2.30 (2.3)	33.71 ± 0.96 (2.8)
Label	100	32

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

Comparison of automated interfaces

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

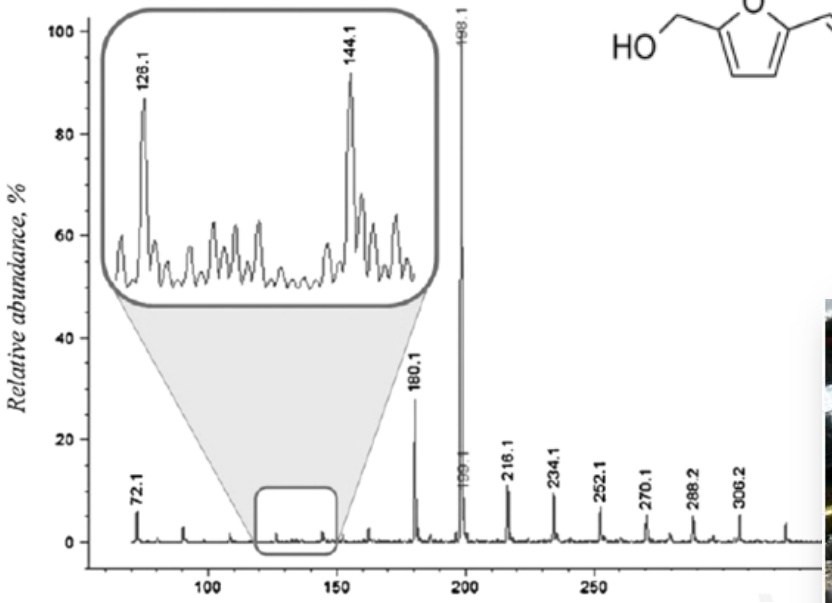
G. Morlock, W. Schwack TrAc 29 (2010) 1157-1171

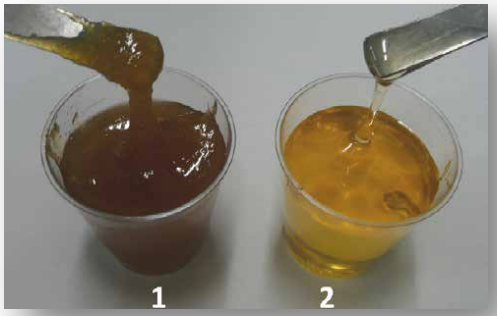


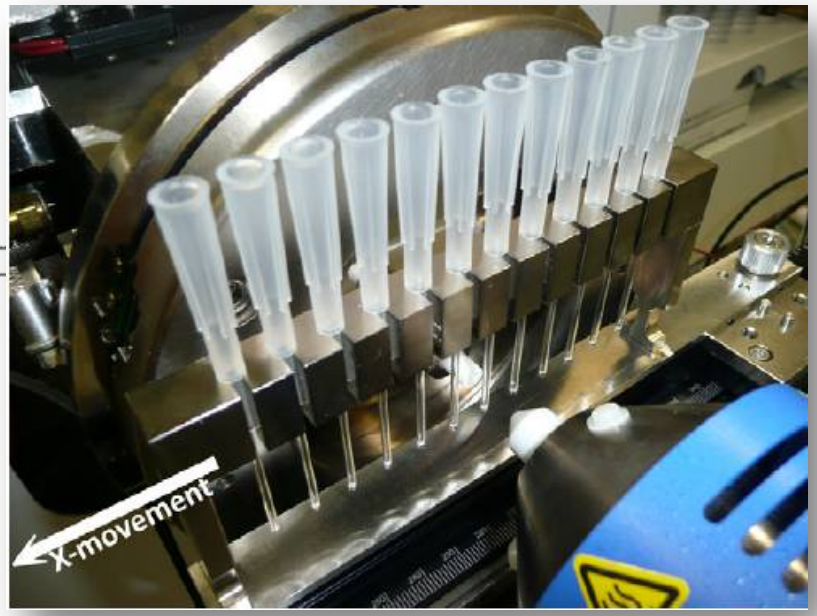
G.E. Morlock, q&more 1 (2014) 43-47, modified

DIP-it®-DART-MS

Direct Analysis in Real Time Mass Spectrometry





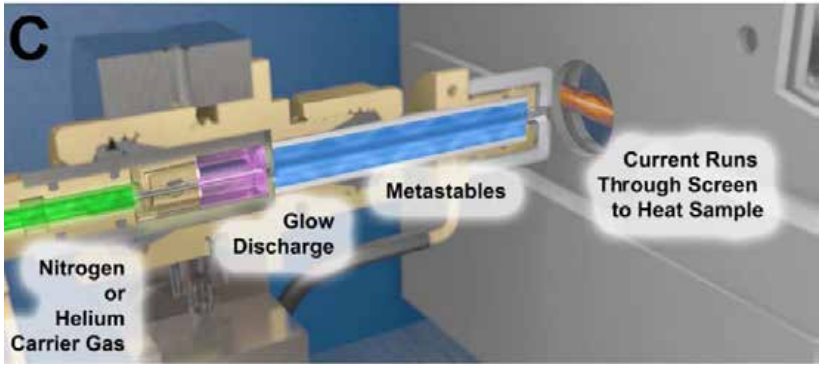


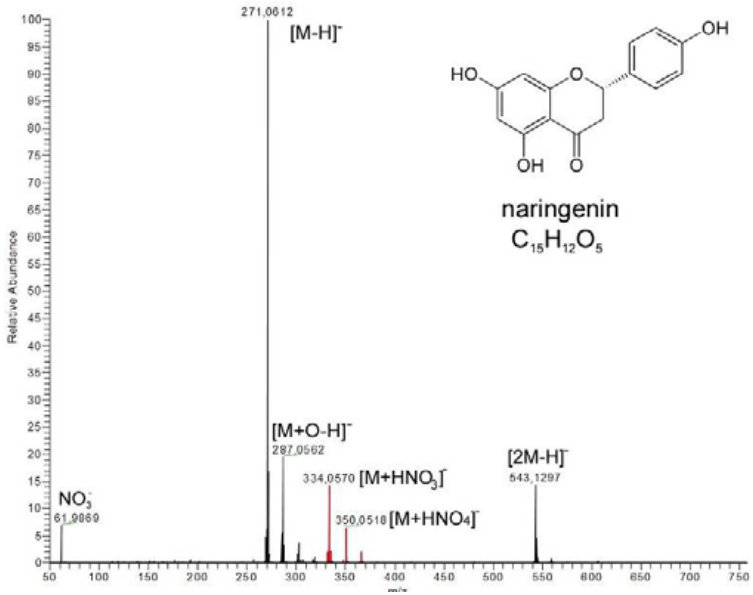
E. Chernetsova, G. Morlock, Int J Mass Spectrom 314 (2012) 22-32

ID-CUBE-DART-MS

→ Phenolic compounds of *Bergenia crassifolia* leaves

#	$m/z_{exp.}$	δm, mmu	elemental formula, ion	ion composition	supposed compound	number of citations in literature for presence in herbal extracts
1	115.0024	-0.2	$C_4H_3O_4$	$[M-H]^-$	fumaric acid	43
2	125.023	-0.3	$C_6H_5O_3$	$[M-H]^-$	pyrogallol	20
3	133.0128	-0.3	$C_4H_5O_5$	$[M-H]^-$	malic acid	108
4	191.0551	0.2	$C_7H_{11}O_6$	$[M-H]^-$	quinic acid	38
5	193.0133	0.2	$C_9H_5O_5$	$[M-H]^-$	trihydroxycoumarin	2

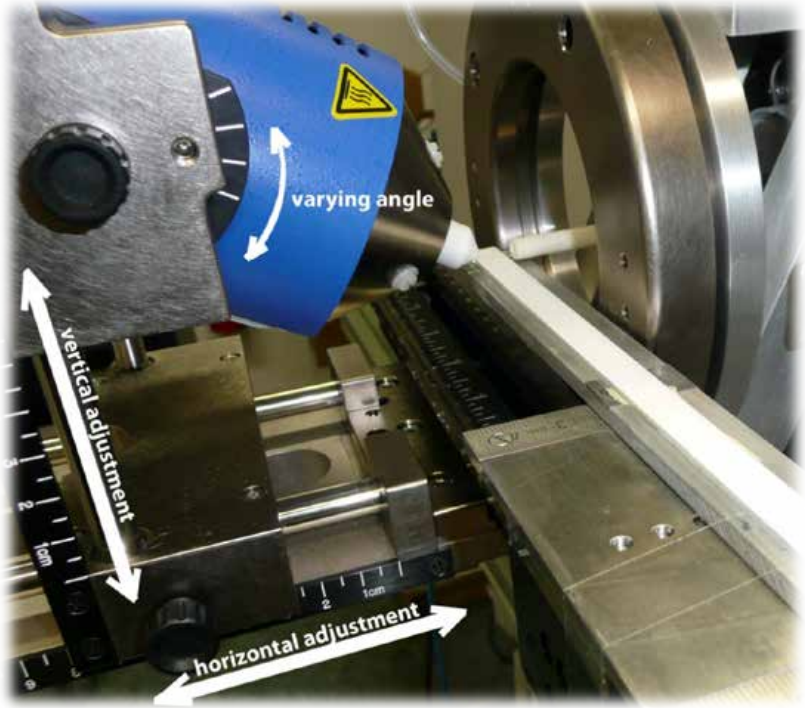


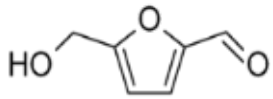


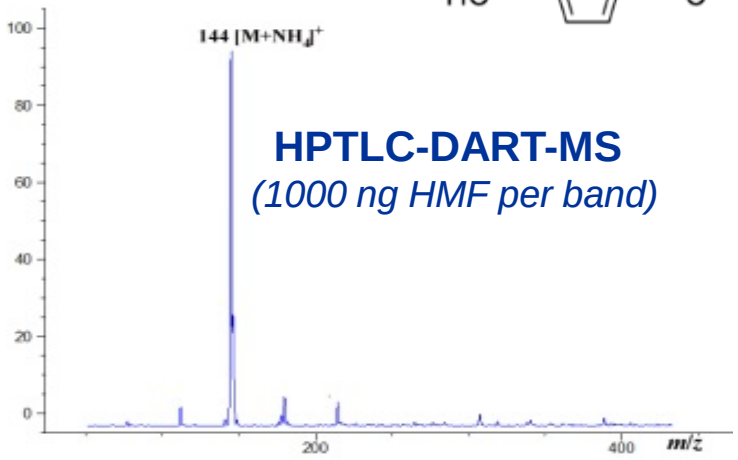
E.S. Chernetsova et al. Rapid Commun Mass Spectrom 26 (2012) 1329–1337

HPTLC-DART-SVPA-MS

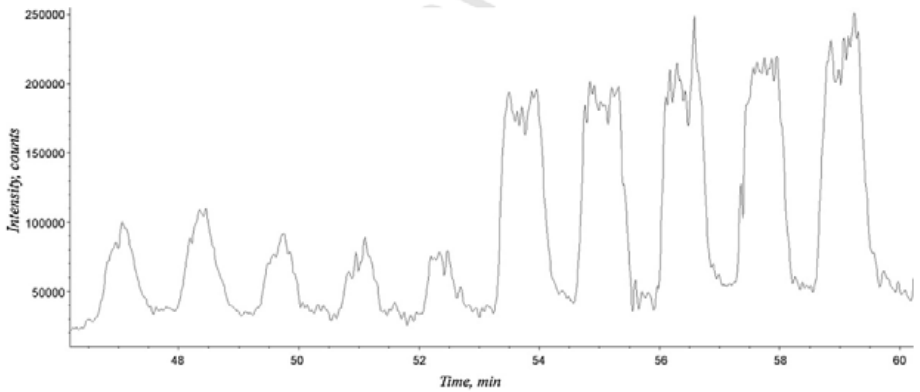
2006 ↔ 2011





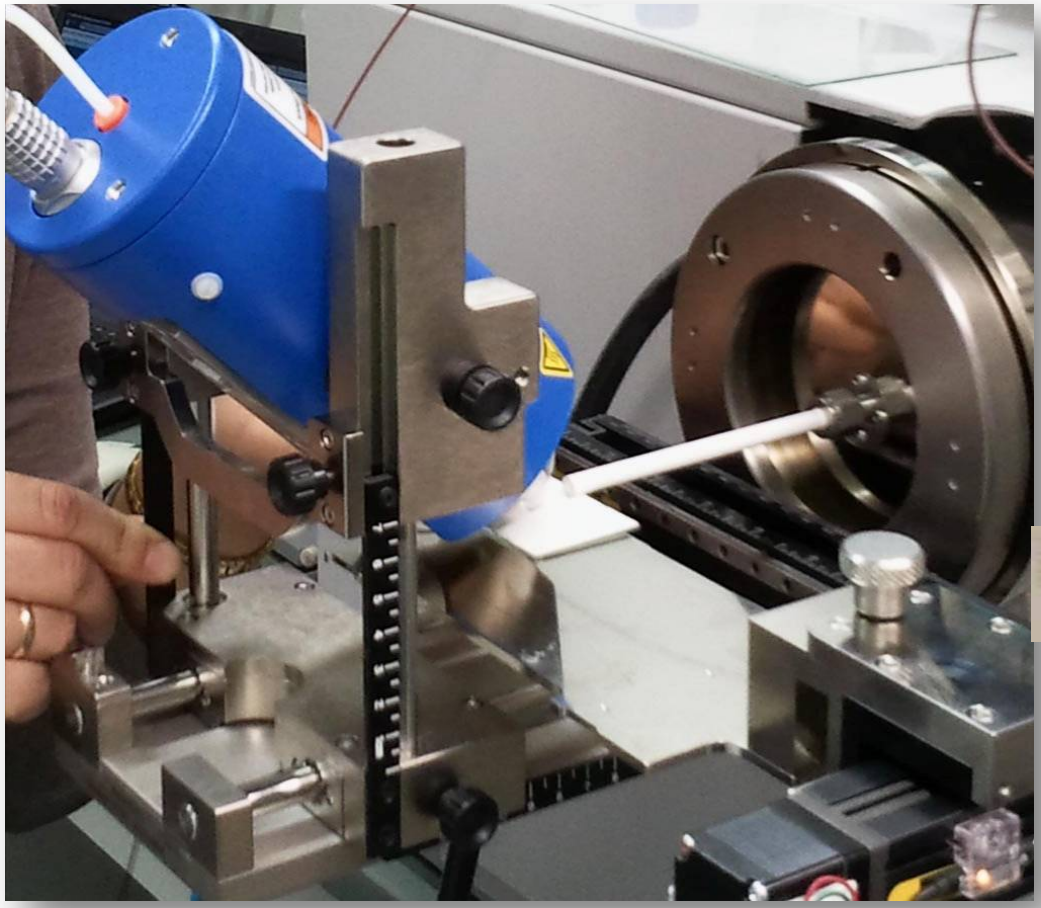


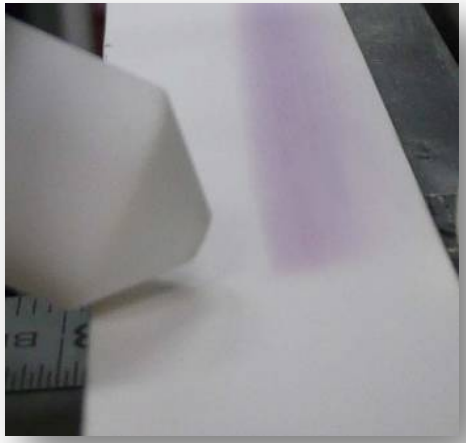
HPTLC-DART-MS
(1000 ng HMF per band)

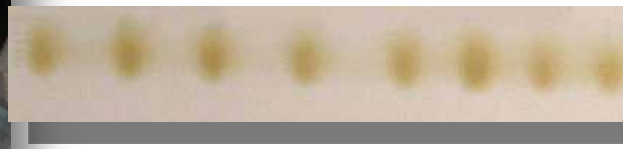


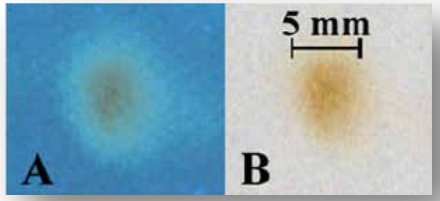
E. Chernetsova, G. Morlock, Int J Mass Spectrom 314 (2012) 22-32

HPTLC-DART-SVPA-MS



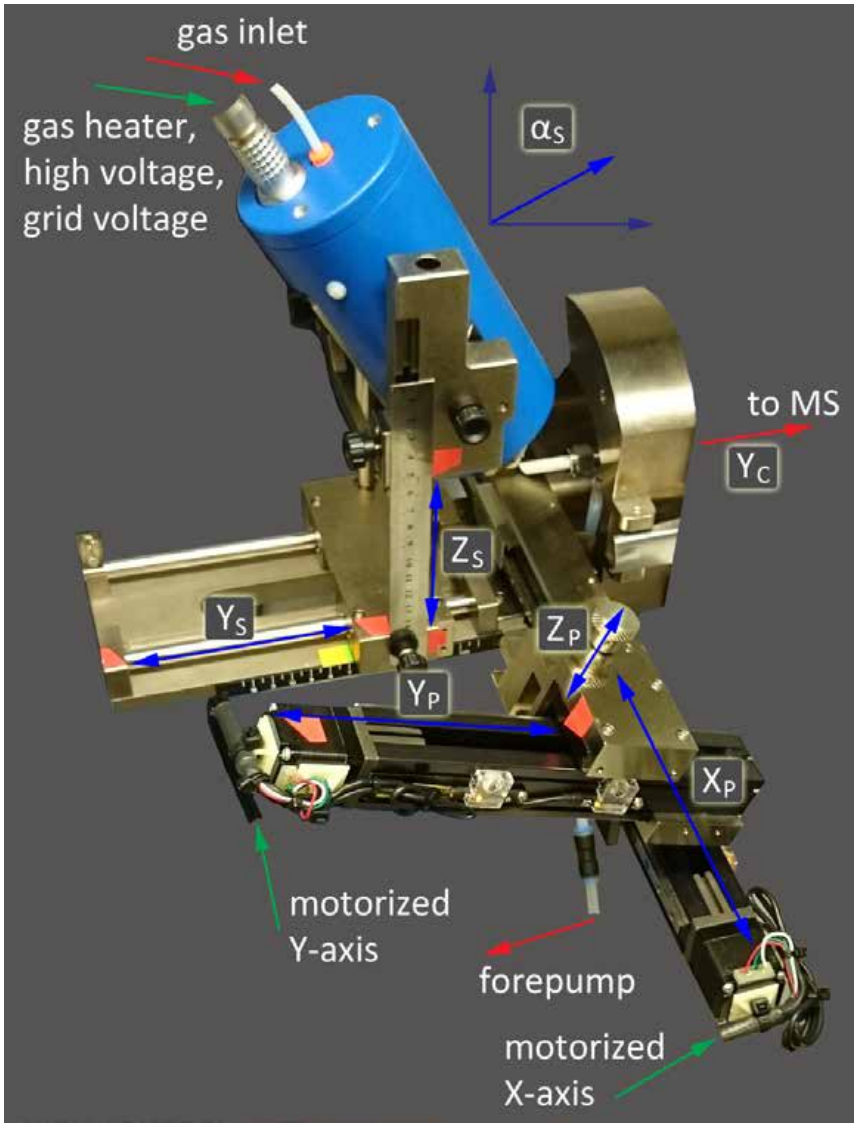






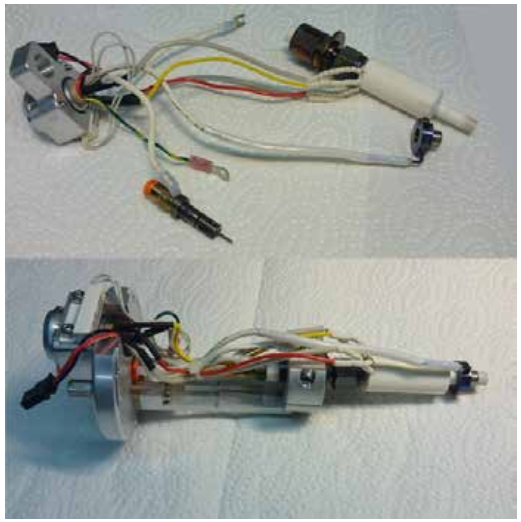
E. Chernetsova, A. Revelsky, G. Morlock, Rapid Commun Mass Spectrom 25 (2011) 2275-2282

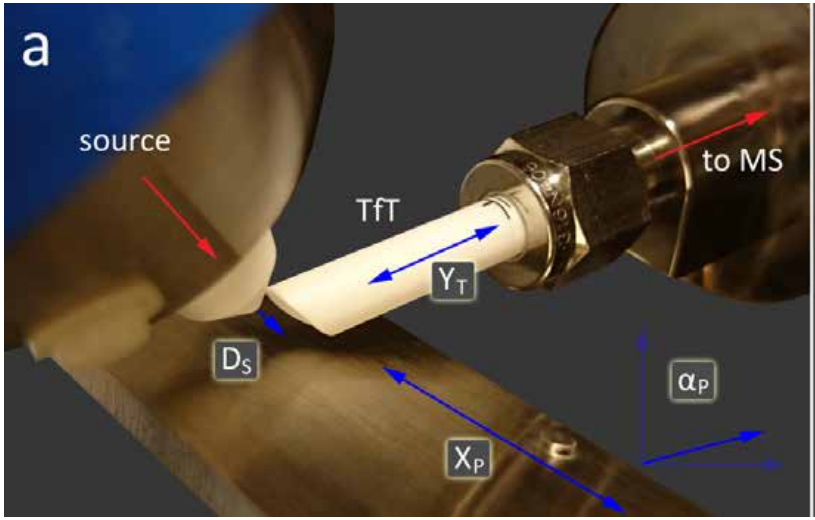
Optimization of HPTLC-DART-SVPA-MS

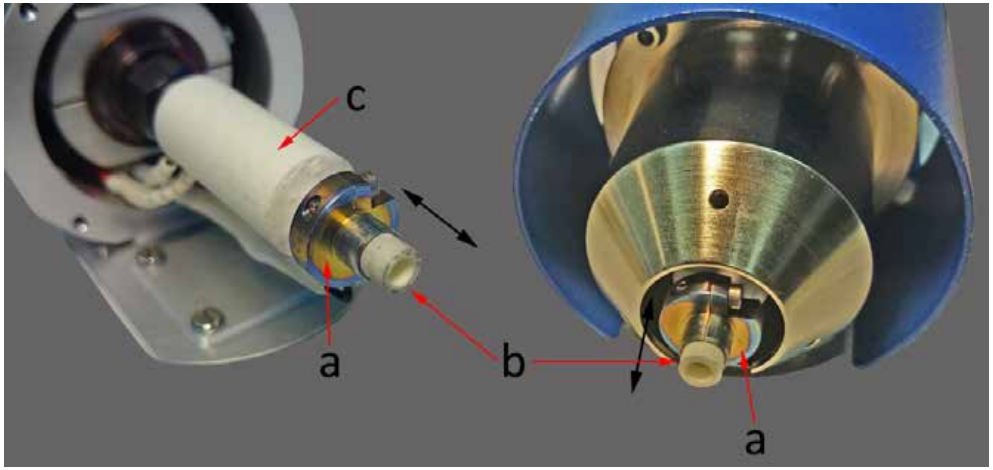


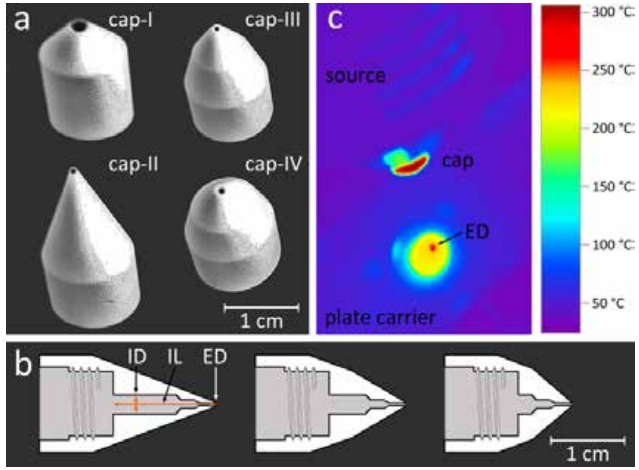
T. Häbe, G. Morlock, Rapid Commun Mass Spectrom 30 (2016) 321–332

Optimization of HPTLC-DART-SVPA-MS



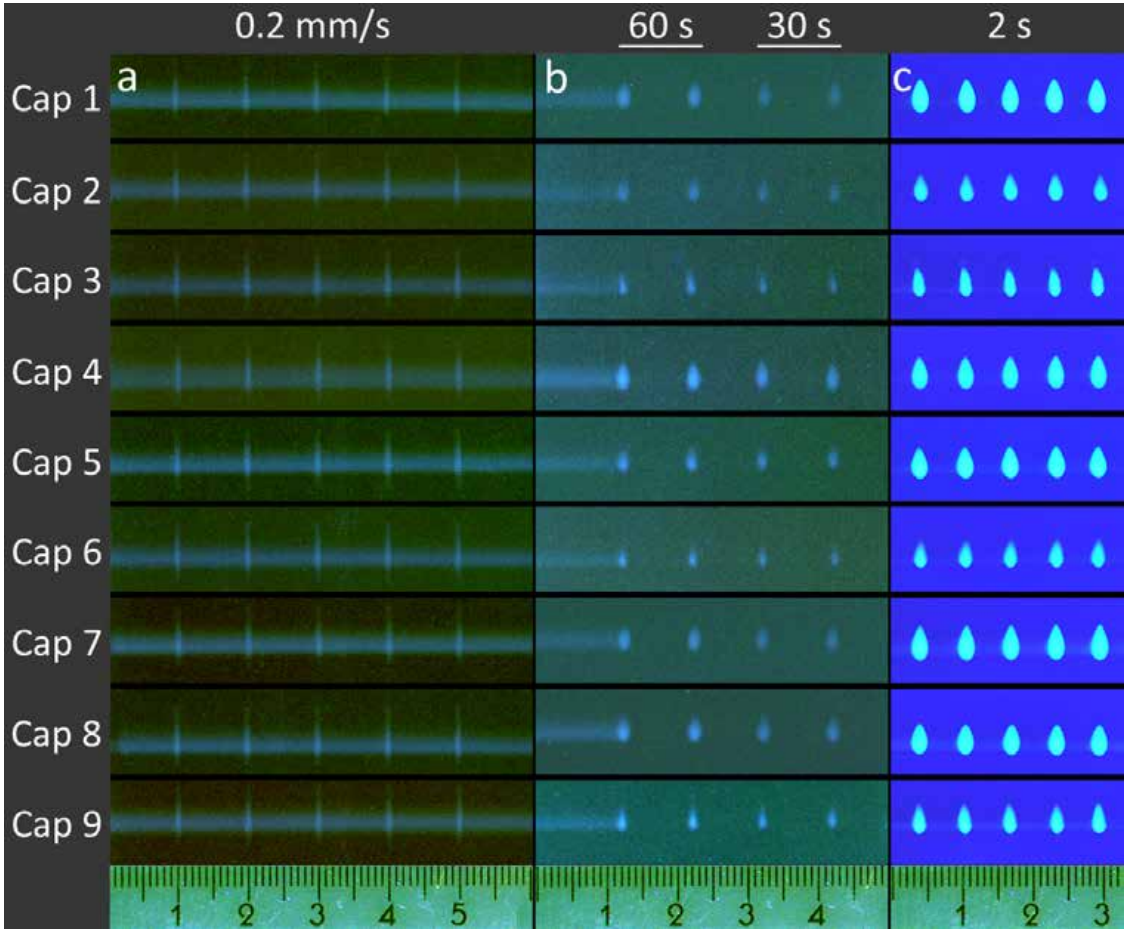






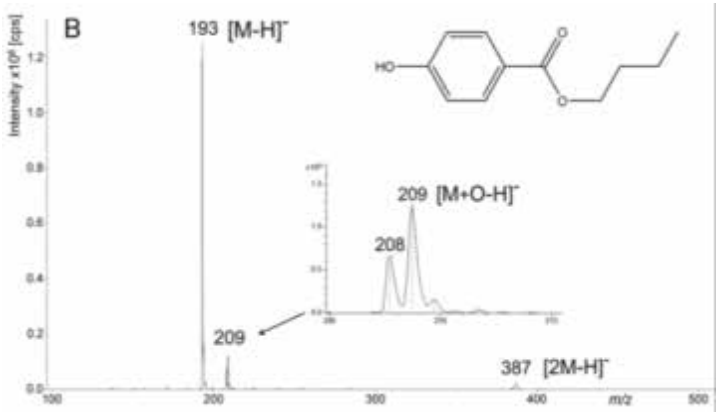
T. Häbe, G. Morlock, Rapid Commun Mass Spectrom 30 (2016) 321–332

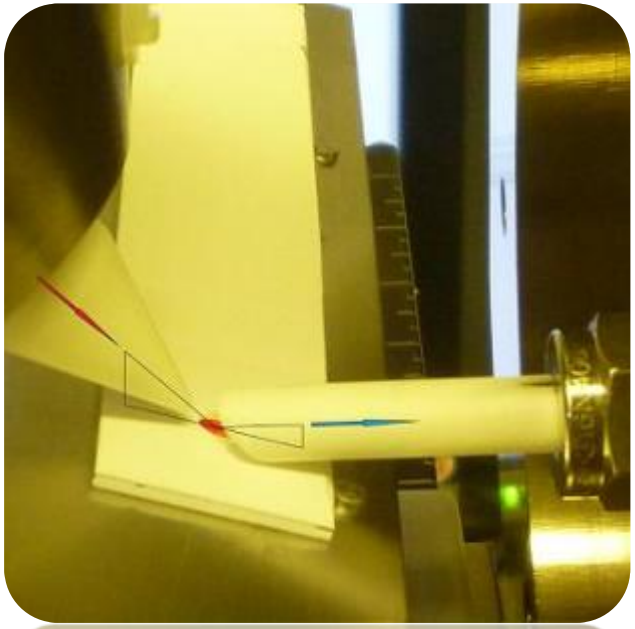
Optimization of HPTLC-DART-SVPA-MS



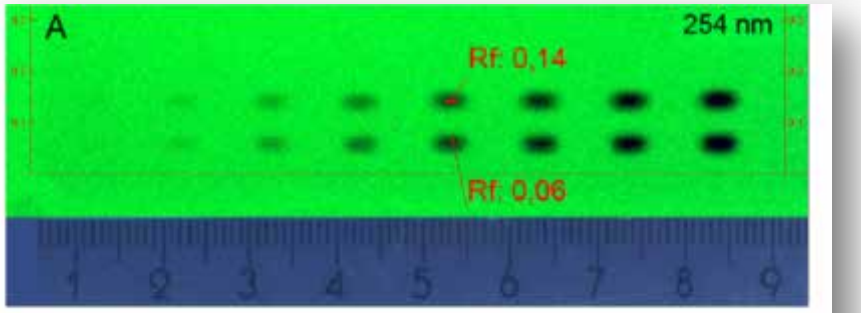
T. Häbe, G. Morlock, Rapid Commun Mass Spectrom 30 (2016) 321–332

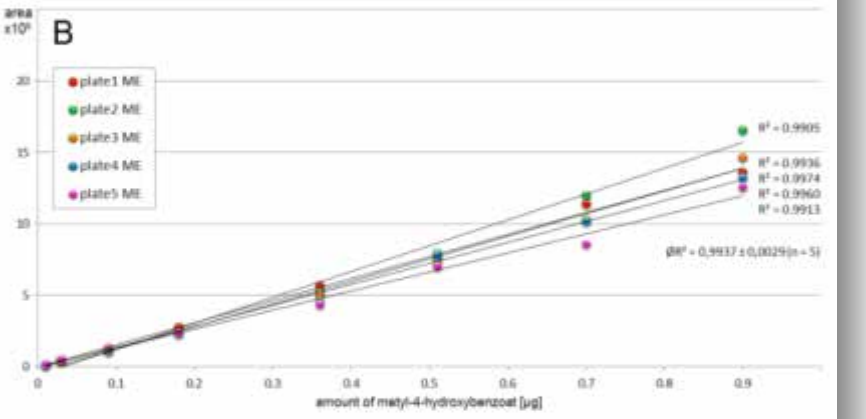
HPTLC-DART-SVPA-MS of preservatives

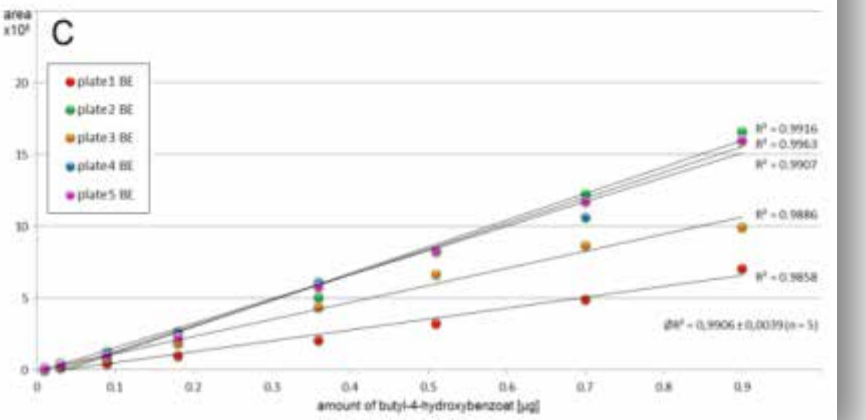




T. Häbe, G. Morlock, Rapid Commun Mass Spectrom 29 (2015) 474–484

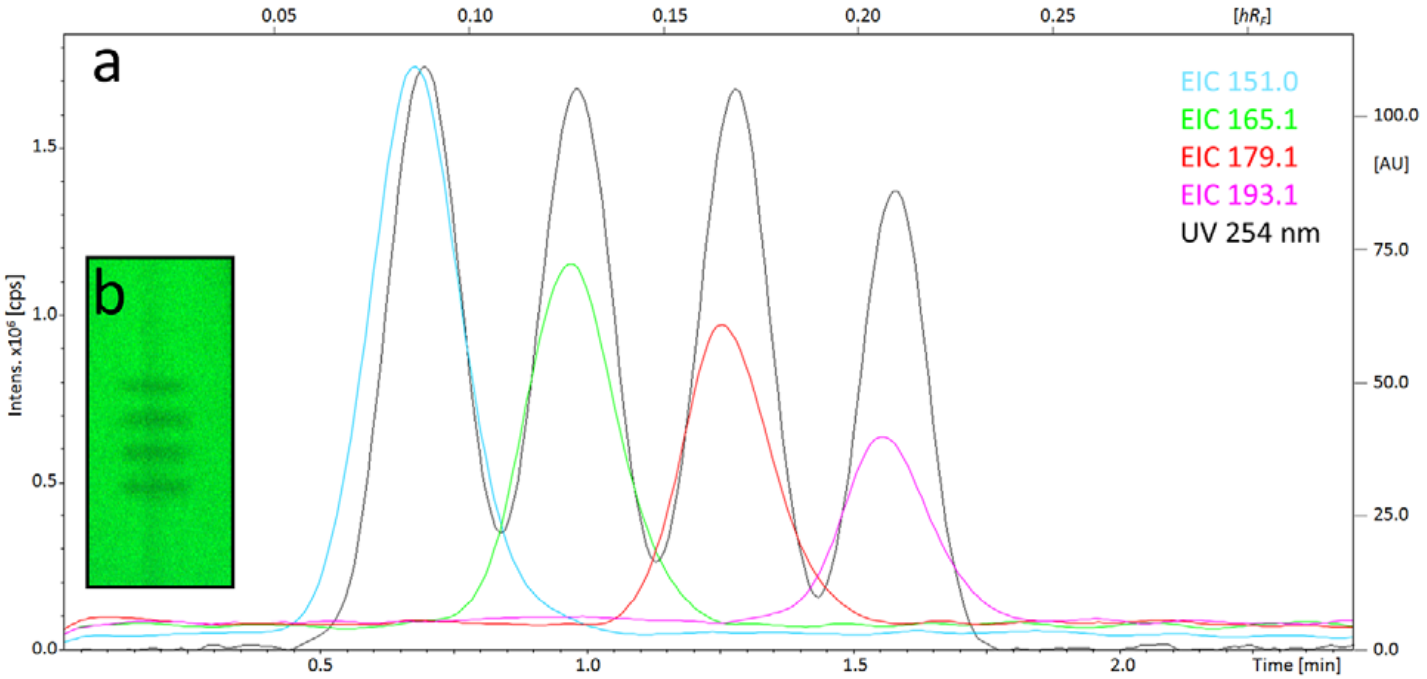






HPTLC-UV *versus* scanning HPTLC-DART-MS

Four separated parabens (each 120 ng/band) detected via densitometry and DART-MS



T. Häbe, G. Morlock, in preparation

Comparison of the approaches

DART/APGD

→ dry desorption technique ⟷ DESI

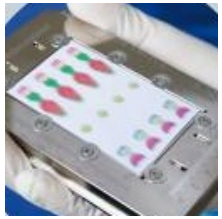
→ no plate preparation etc. ⟷ SALDI, MALDI

→ ambient conditions, no high voltage ⟷ micro junction

→ simple spectra ⟷ MALDI, SIMS

→ **quantitativ *with* internal standard → scan function**

MALDI

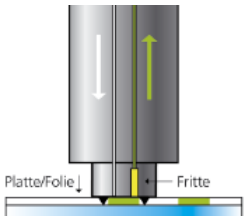


✓ strict protocol for plate preparation

✓ complex spectra

✓ quantitativ *with* internal standard → scan function

Elution-head based Interface



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

✓ plug & play interface (without adjustments or modifications)

✓ whole plate (no cut)

✓ all carriers ând all layers ⟷ micro junction

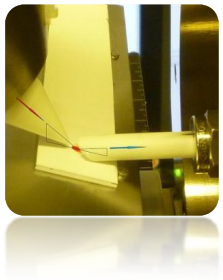
✓ whole zone incl. depth profile ⟶ high detectabilities

✓ quantitativ *without* internal standard ⟷ desorption techniques

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

Comparison of the approaches

DART/FAPA



→ dry desorption technique ⟷ DESI

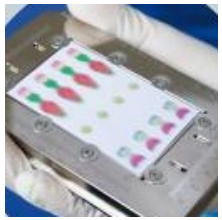
→ no plate preparation etc. ⟷ SALDI, MALDI

→ ambient conditions, no high voltage ⟷ micro junction

→ simple spectra ⟷ MALDI, SIMS

→ **quantitativ → scanning MS……. discriminative MS**

MALDI

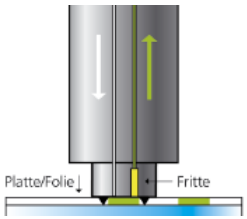


✓ strict protocol for plate preparation

✓ complex spectra

✓ quantitativ *with* internal standard → scan function

Elution-head based Interface



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

✓ plug & play interface (without adjustments or modifications)

✓ whole plate (no cut)

✓ all carriers ând all layers ⟷ micro junction

✓ whole zone incl. depth profile ⟶ high detectabilities

✓ quantitativ *without* internal standard ⟷ desorption techniques

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

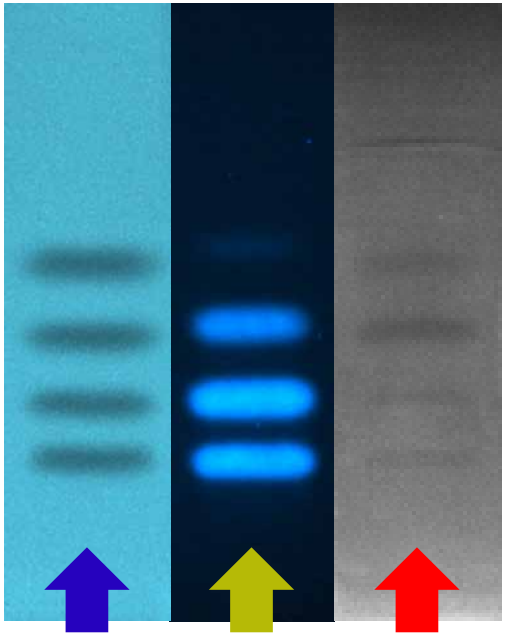
HPTLC-DART-MS

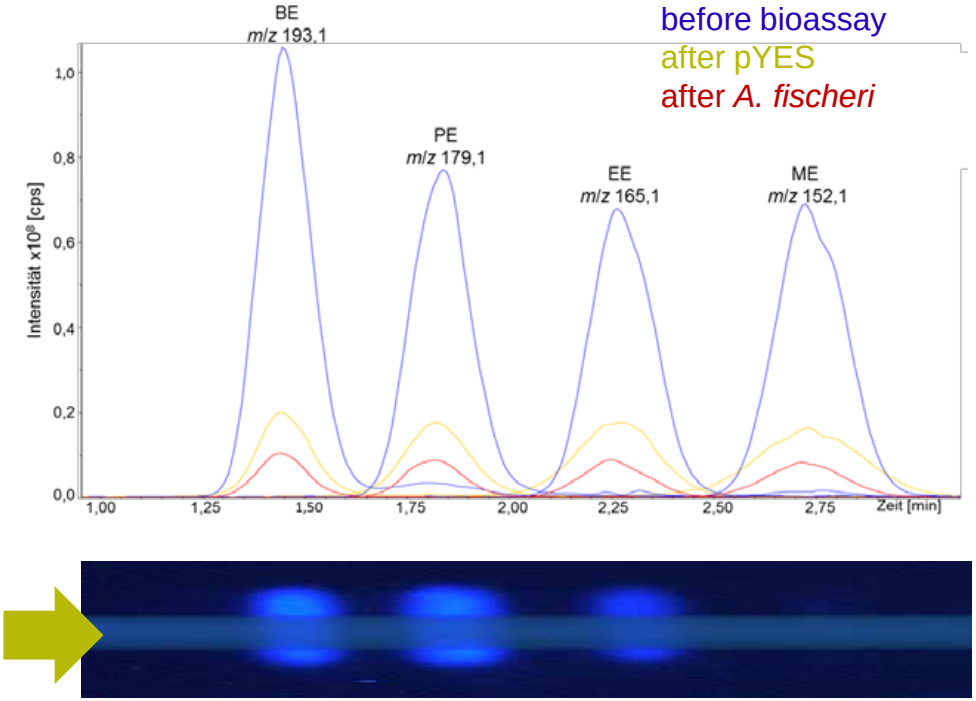
Can we apply DART-MS

directly after the bioassay?

Still on the same plate?

Direct Bioautography hyphenated to DART-MS





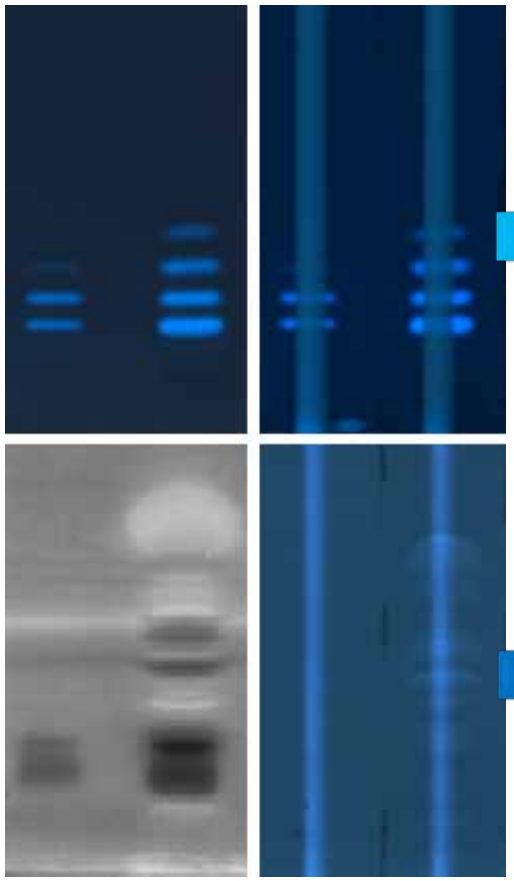
EICs of 4 separated parabens (each 600 ng/band)

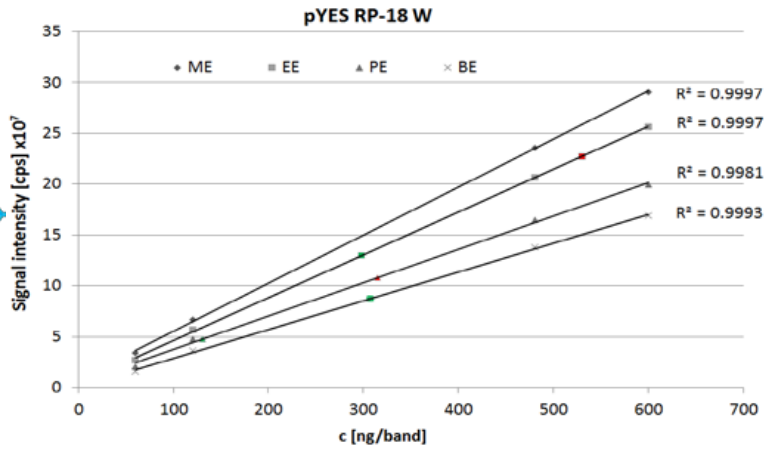
- Signal decay *ca.* 90% for *A. fischeri* and 65-81% for pYES
- Sensitivity still sufficient for quantitation

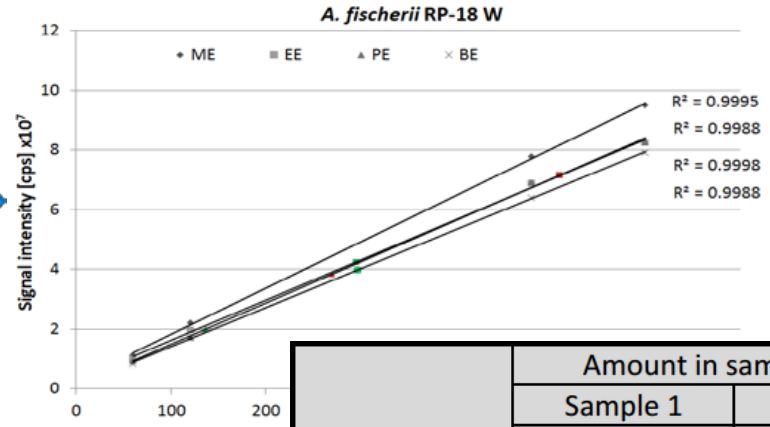
	Signal decay [%]	
	A. fischeri	pYES
ME	88	65
EE	89	67
PE	90	76
BE	91	81

T. Häbe, G. Morlock, in preparation

Sample quantitation after direct bioautography







- Mean detemination coefficients of 0.9992
- Mean *RSD* of 4.6% between both methods.
- Reliable quantitation was possible after direct bioautography on normal and reversed phase layers

T. Häbe, G. Morlock, in preparation

		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

HPTLC-DART-MS

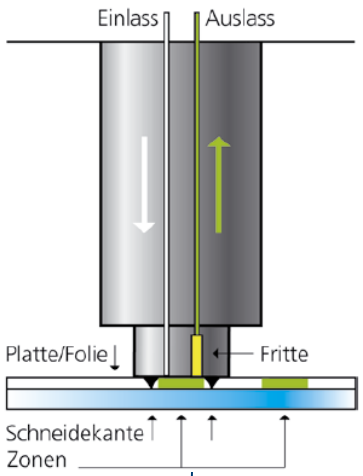
Can we apply DART-MS

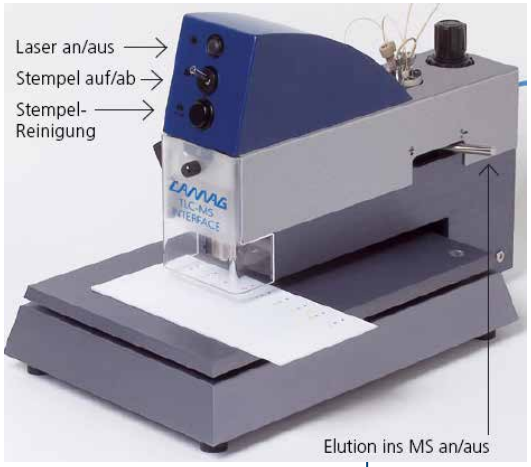
directly after the bioassay?

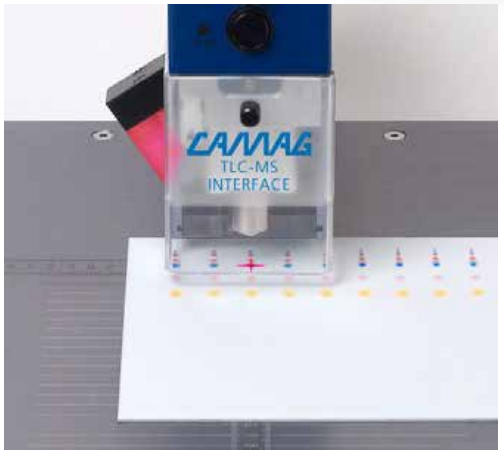
Still on the same plate?

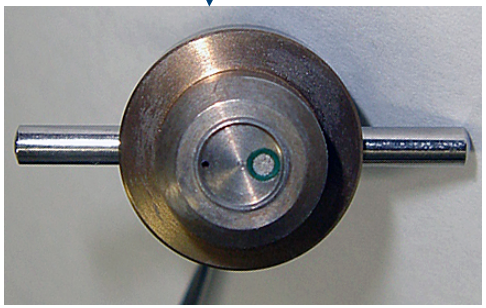
Yes, seems to work.

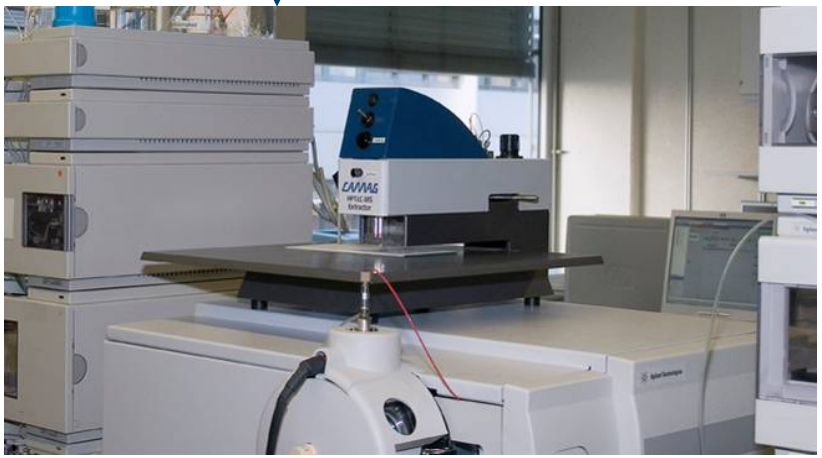
… and for elution head-based HPTLC-MS?







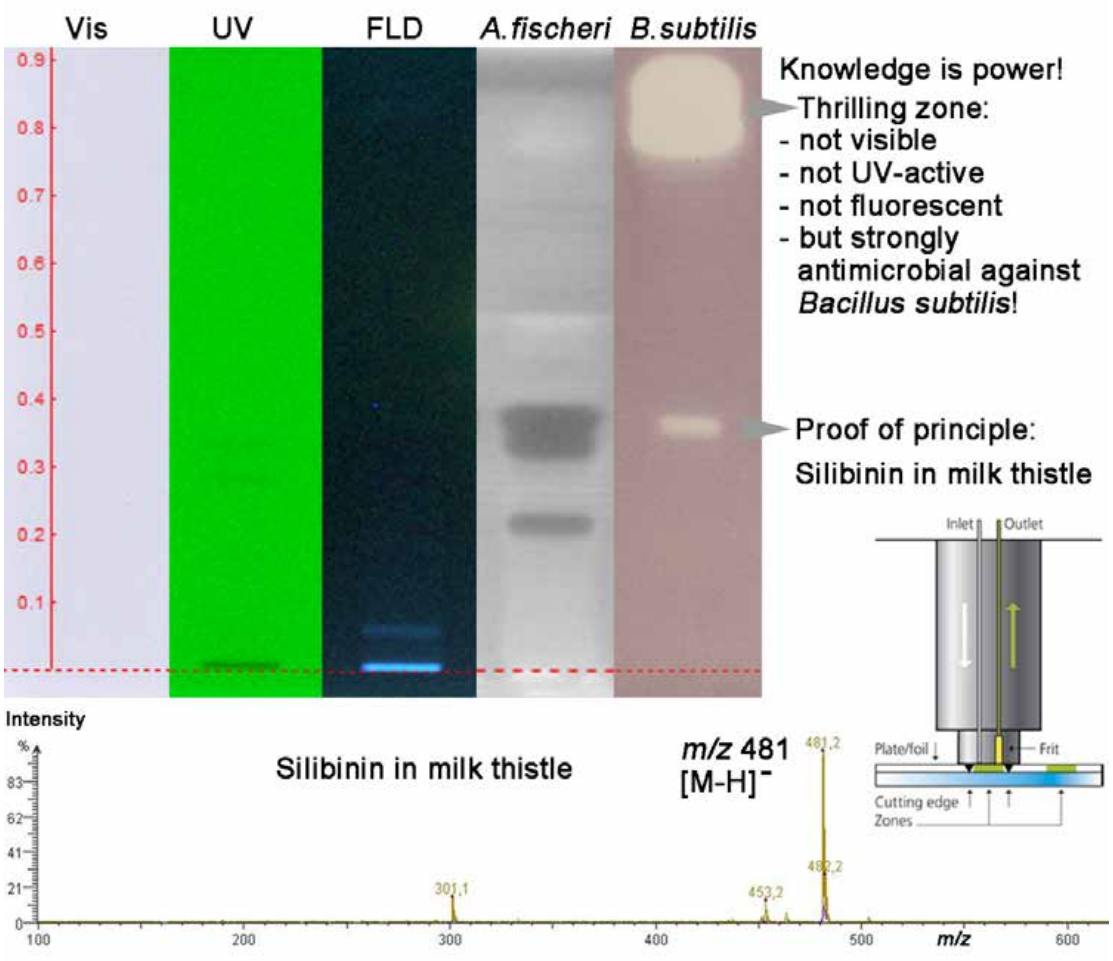




H. Luftmann, Anal Bioanal Chem 378 (2004) 964-968

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

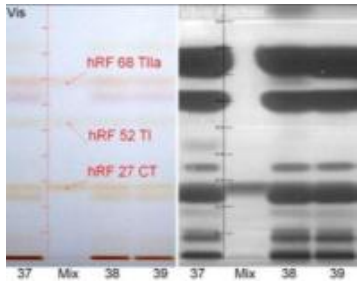
Directly from bioassay plate?



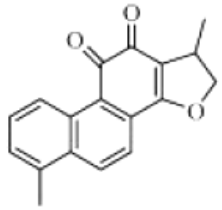
G. Morlock, The Analytical Scientist 27 (2015) 42-43

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

Unknown bioactive compound (below CT)

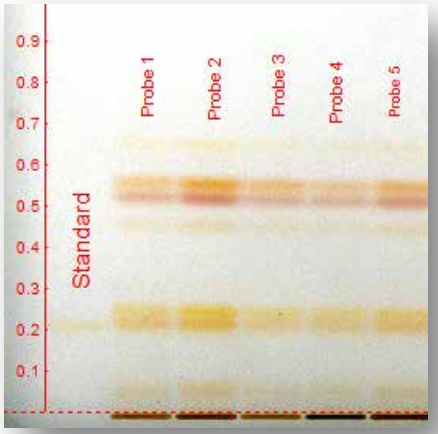


279.10208 $[M+H]^+$



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

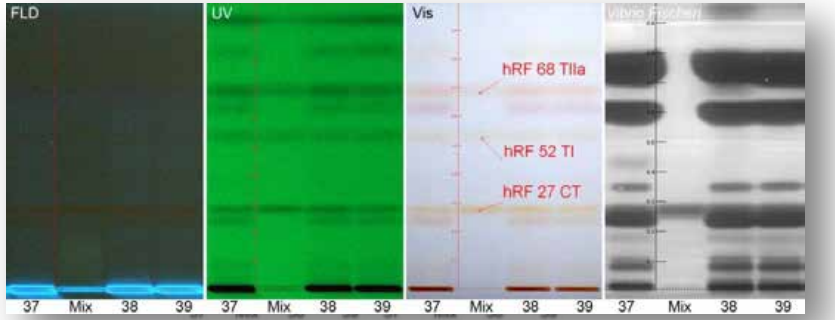
Standard found and bought:

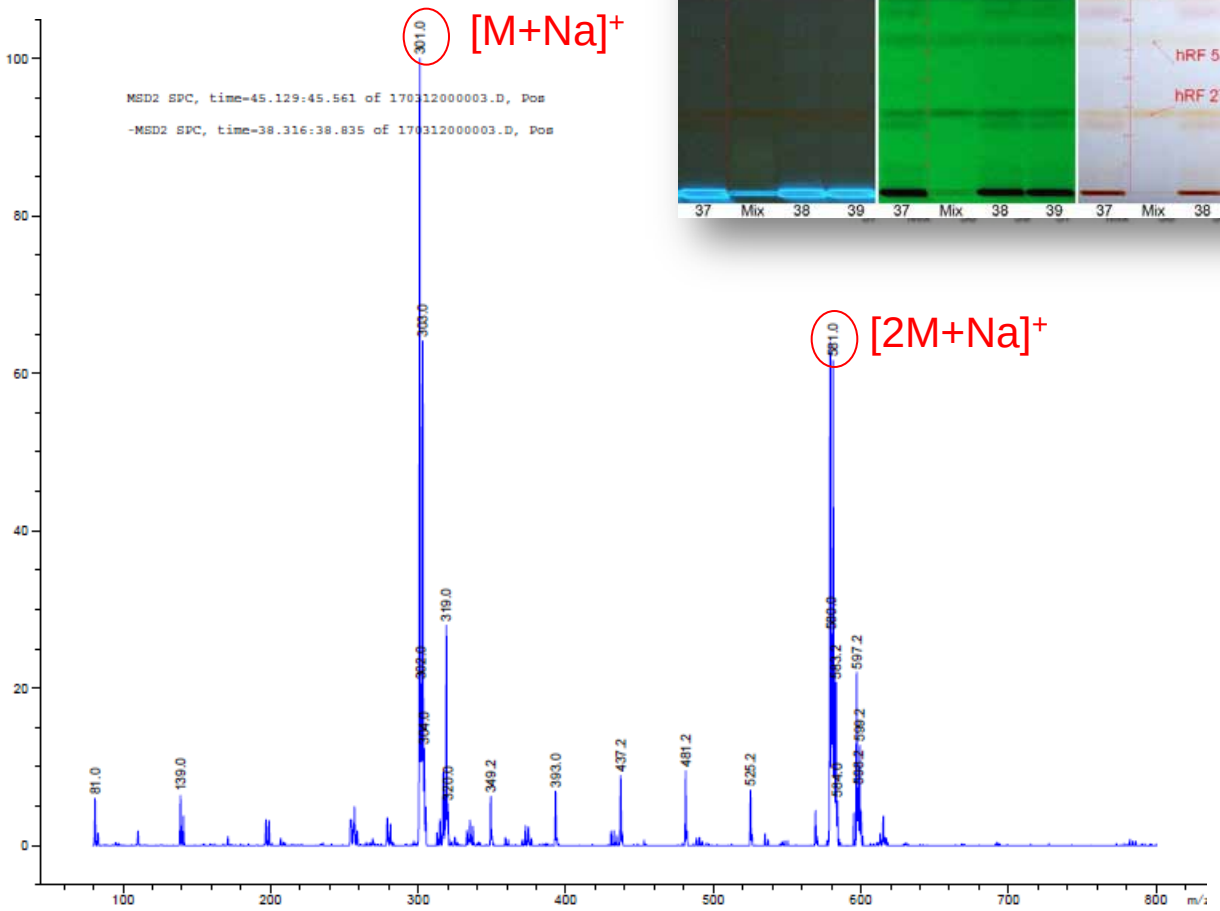


G. Morlock, T. Sung, B. Honermeier, in preparation

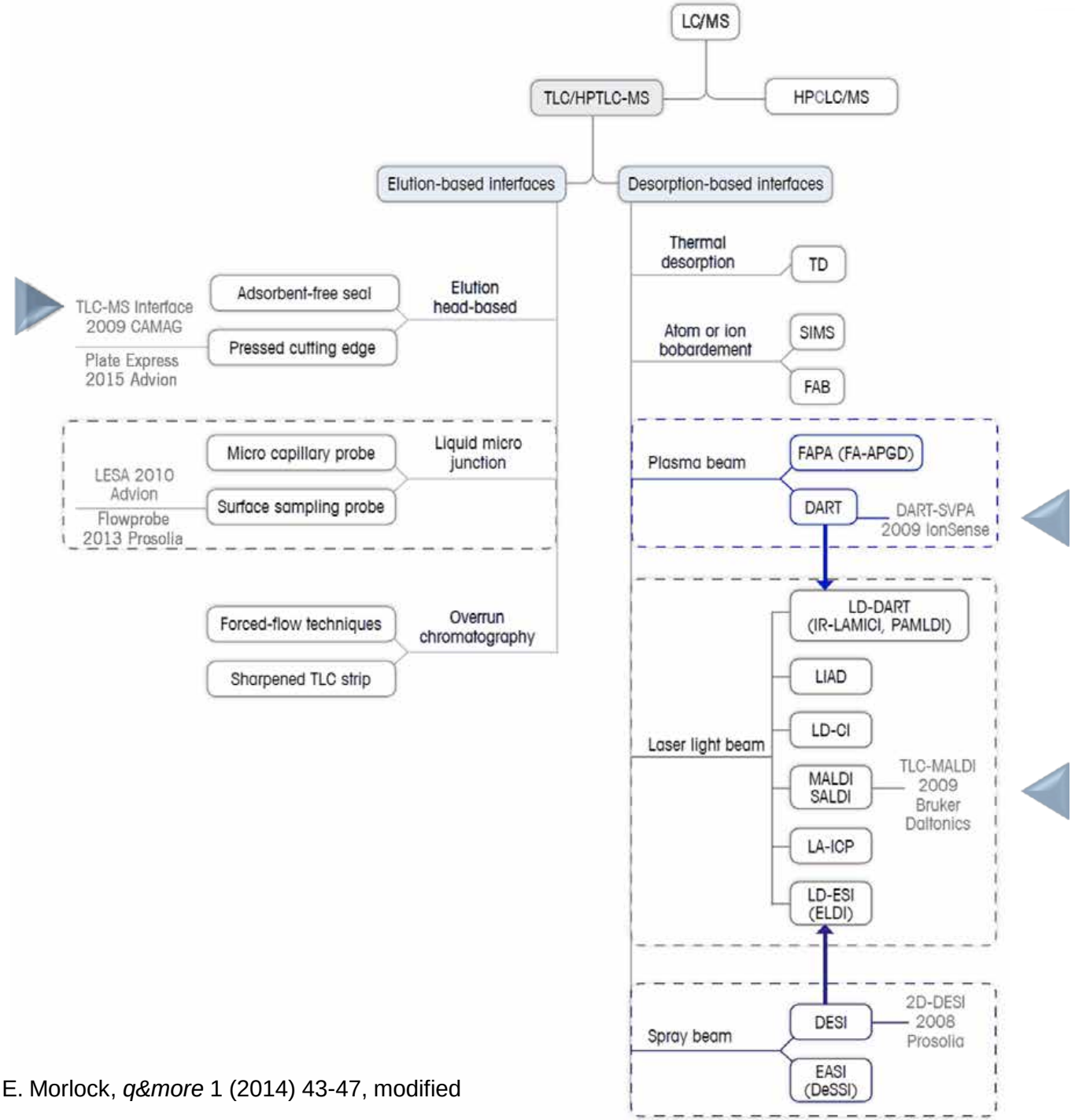
HPTLC-bioassay-ESI-MS

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



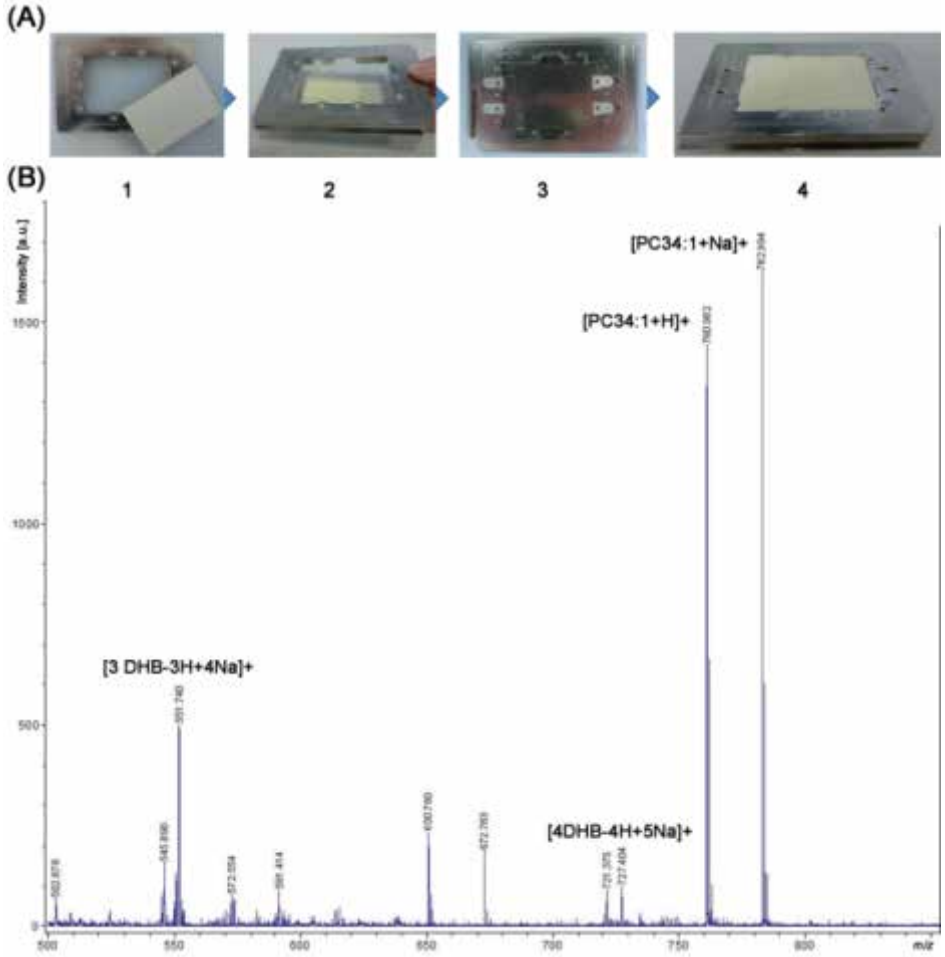


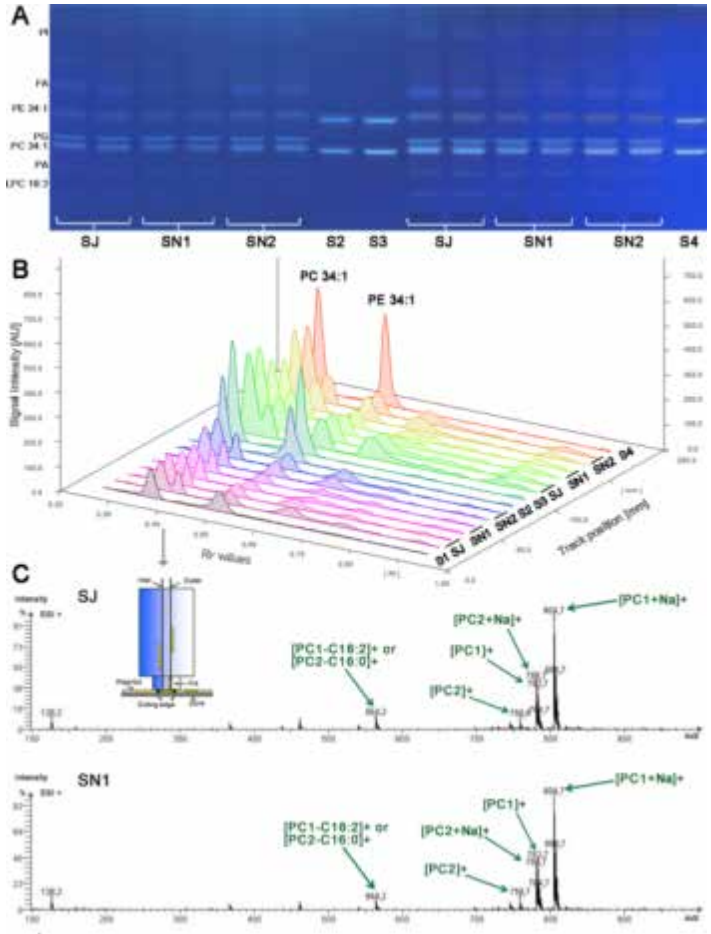
G. Morlock *et al.*, in preparation



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

HPTLC-MALDI-TOF MS of phospholipids

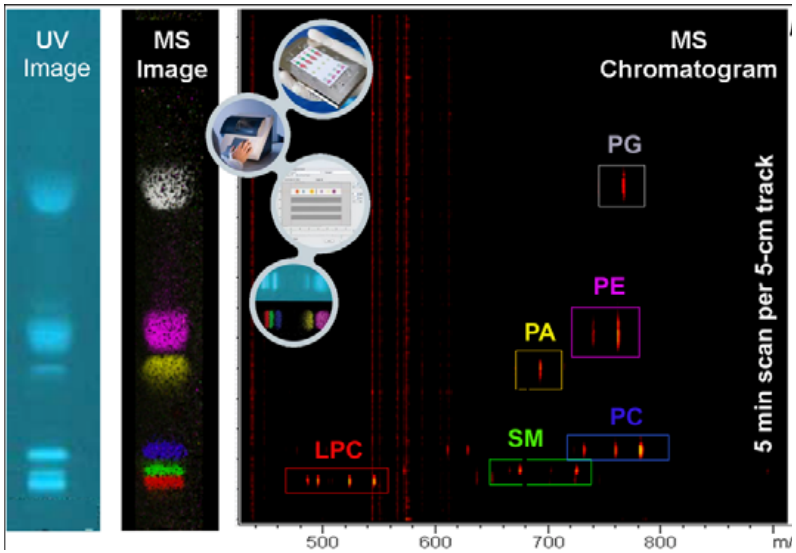


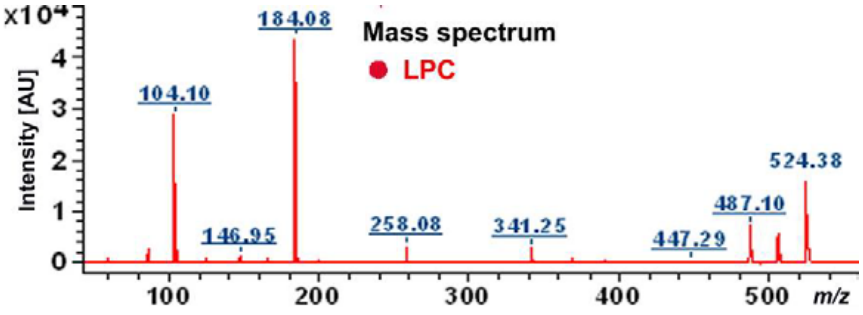


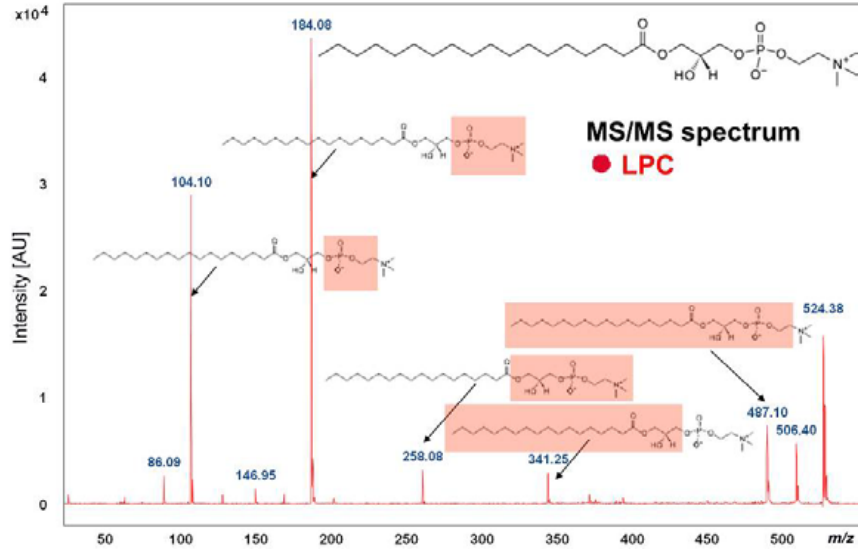
S. Krüger, L. Bürmann, G. Morlock, J Agric Food Chem 63 (2015) 2893–2901

HPTLC-FLD-MALDI-TOF MS

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

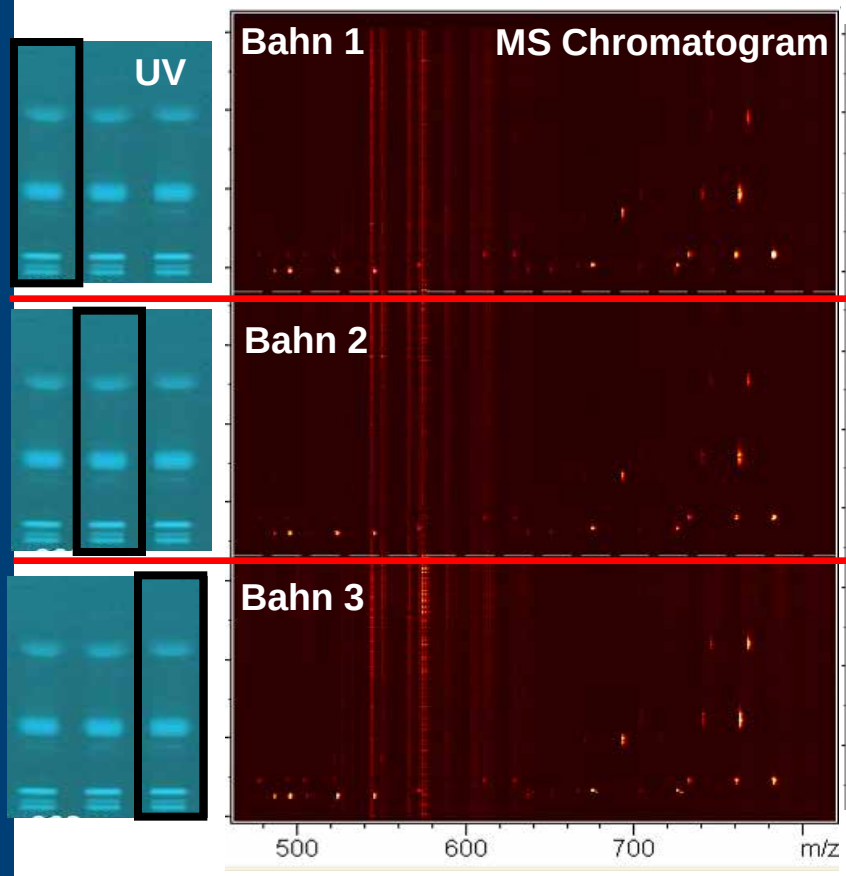




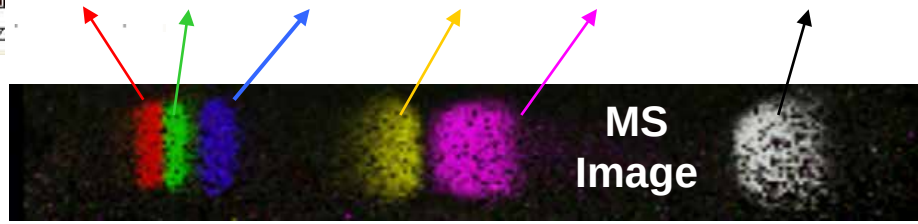


Bruker Daltonics Application Note MT-101

Quantification?

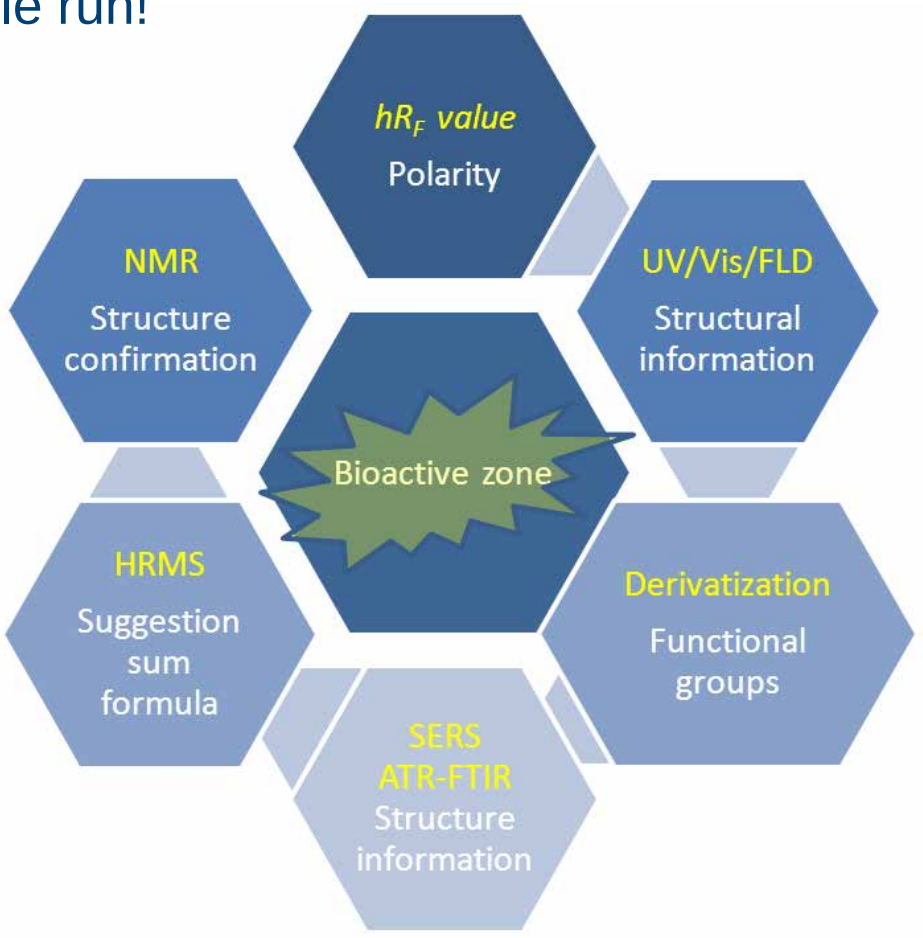


Extracted Ion Chromatograms (EIC)



Goal: From bioactive zone to sum formula

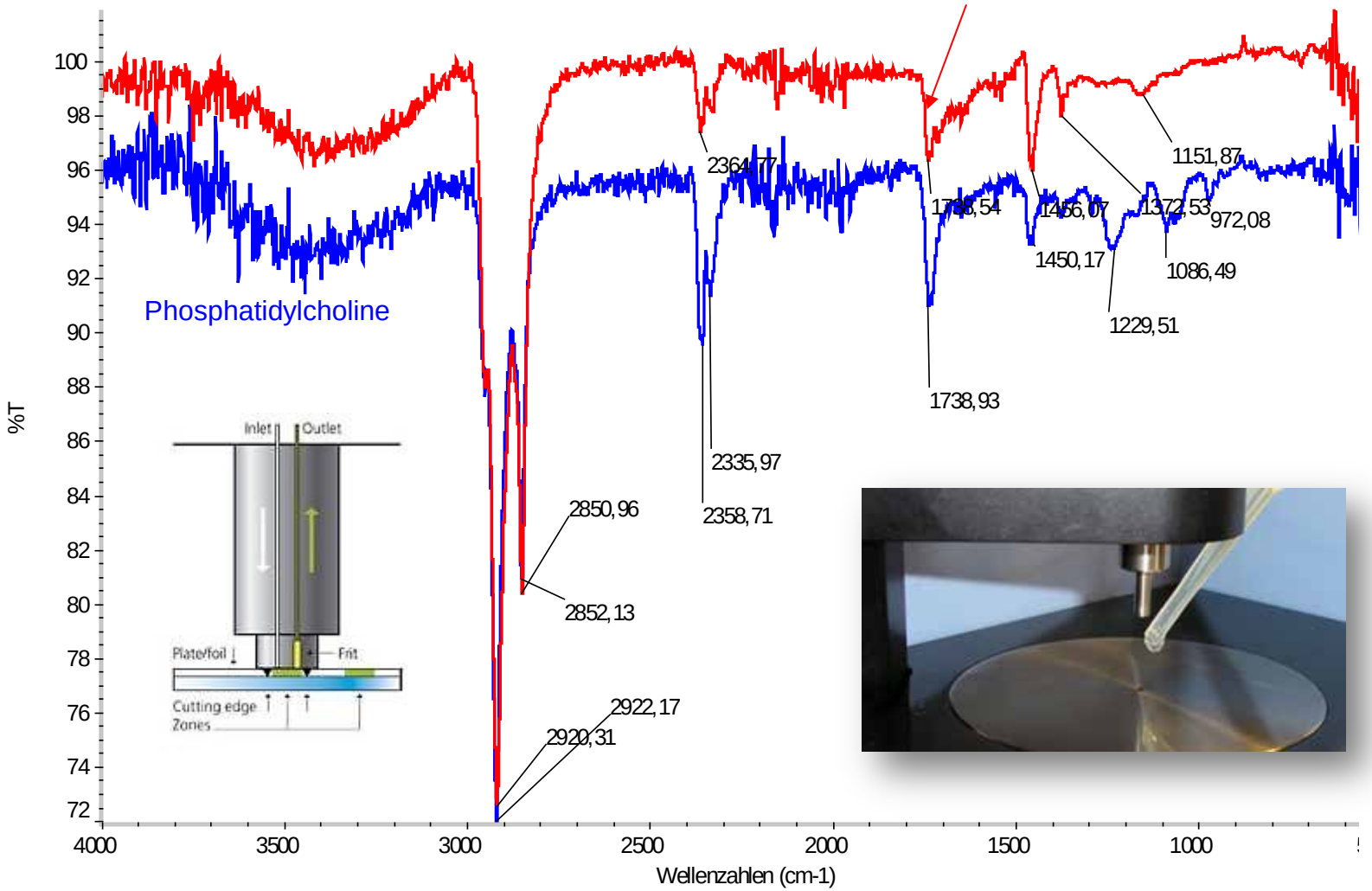
…in a single run!



G. Morlock, in *Instrumental Methods for the Analysis of Bioactive Molecules*, B. S. Patil, G. K. Jayaprakasha, F. Pellati, Eds., ACS Books Publishing, 2014

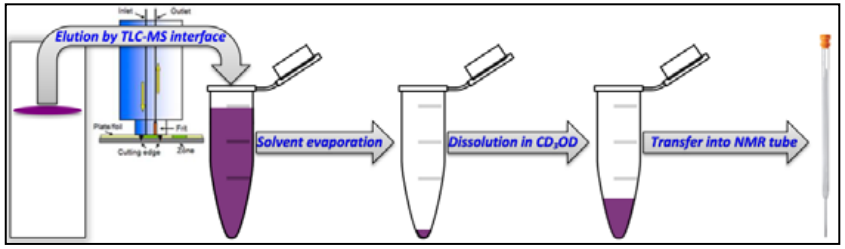
HPTLC-ATR FTIR

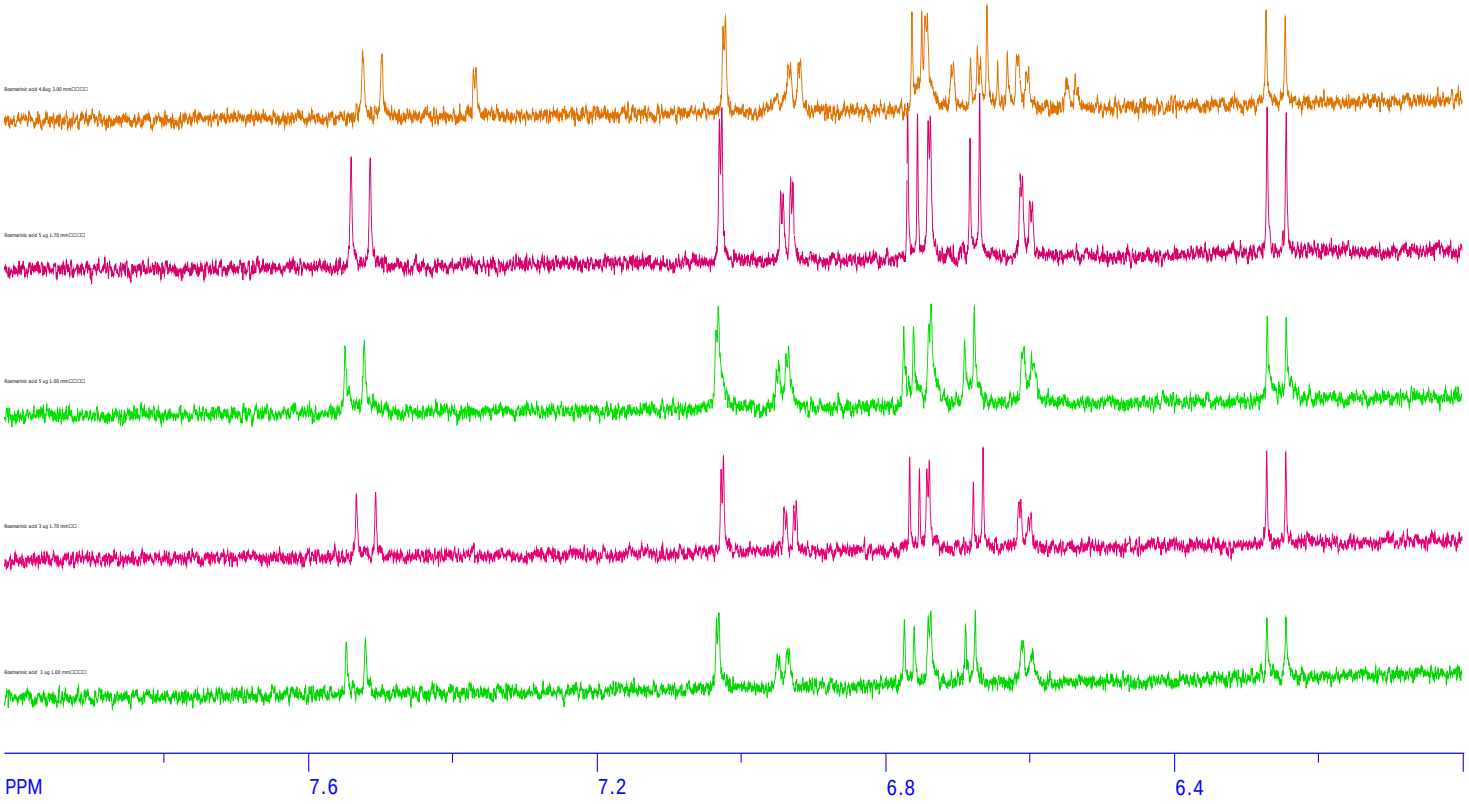
Anti-inflammatoric compound isolated from *Lactobacillus fermentum*



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

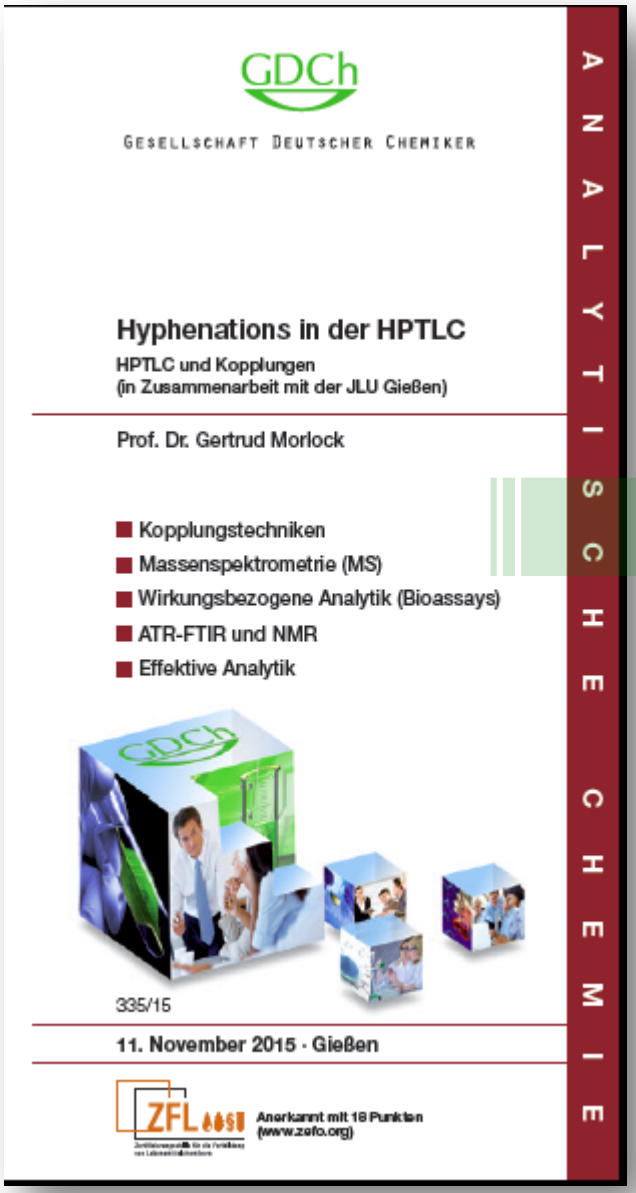
HPTLC-NMR





E. Azadniya, G. Morlock, in preparation

GDCh course 335/16



PROGRAMM

Mittwoch, 11. November 2015

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)

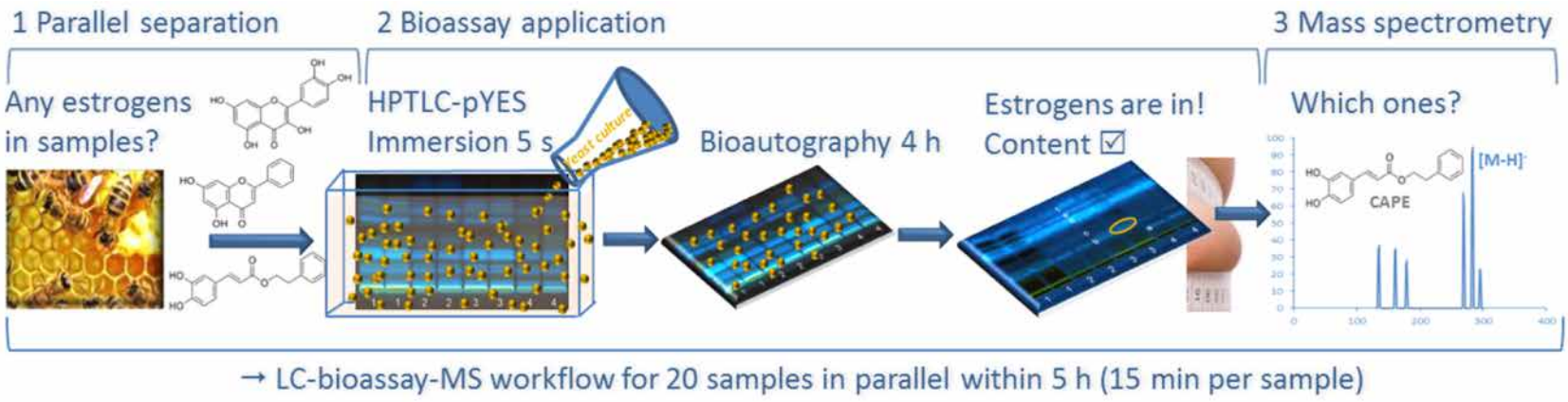
16.00 Gruppe 1: Experiment HPTLC-UV/Vis/FLD-DART-MS (Häbe, Krüger)
Gruppe 2: Experiment HPTLC-UV/Vis/FLD-DESI-MS (Kirchert, Stiefel)

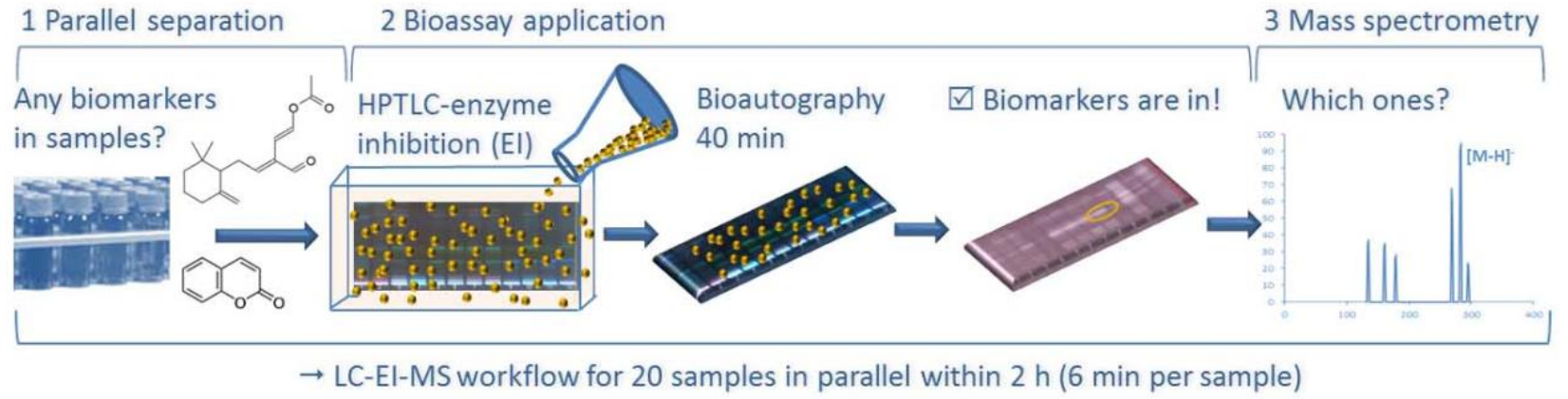
16.15 Gruppe 1: Experiment HPTLC-UV/Vis/FLD-DESI-MS (Kirchert, Stiefel)
Gruppe 2: Experiment HPTLC-UV/Vis/FLD-DART-MS (Häbe, Krüger)

16.30 Diskussion (Morlock)

17.00 Voraussichtliches Ende der Veranstaltung

HPTLC-(bio)assay-MS





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GDCh

Gesellschaft Deutscher Chemiker

ANALYTISCHE CHEMIE

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

12. November 2015 · Gießen

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

PROGRAMM

Donnerstag, 12 November 2015

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

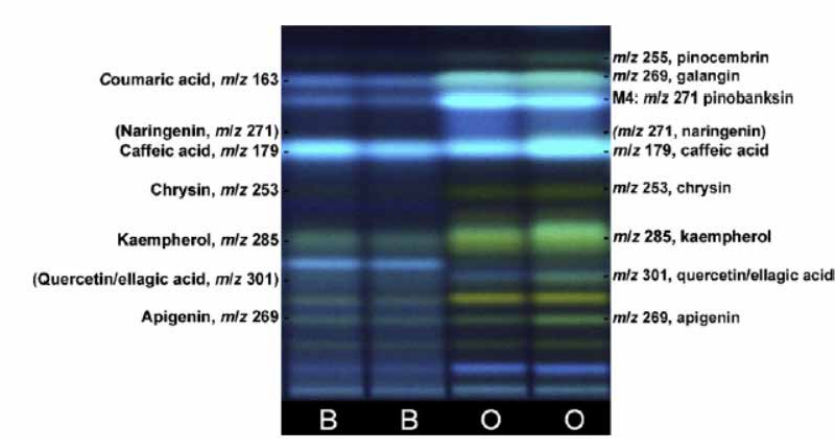
15.00 Gruppe 1: EDA von Tyrosinase- und Xanthinoxidasehemmer: Experiment HPTLC-UV/Vis/FLD-Enzym-(HPLC-)ESI-HRMS sowie Experiment HPTLC-UV/Vis/FLD-DPPH•-ESI-HRMS (Hage, Xingmei)

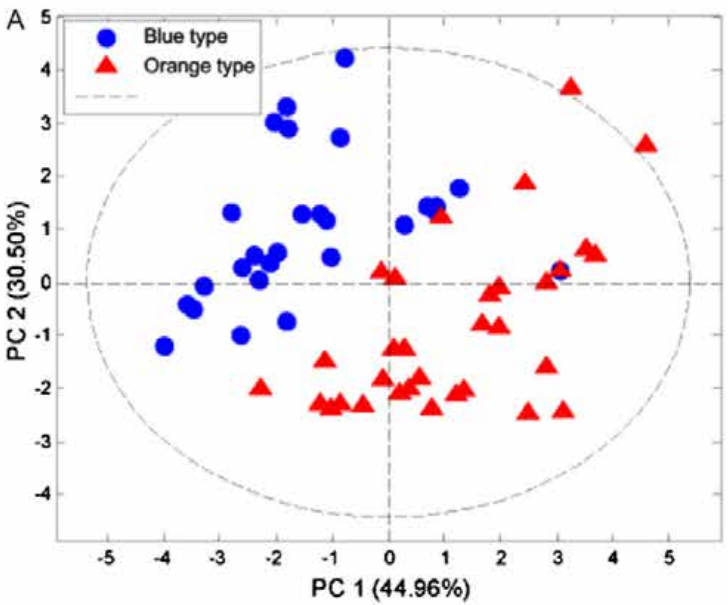
Gruppe 2: EDA von bioaktiven Verbindungen (genereller Hinweis auf Bioaktivität): Experiment HPTLC-UV/Vis/FLD-*Alivibrio fischeri*-DART-HRMS (Krüger/Häbe)

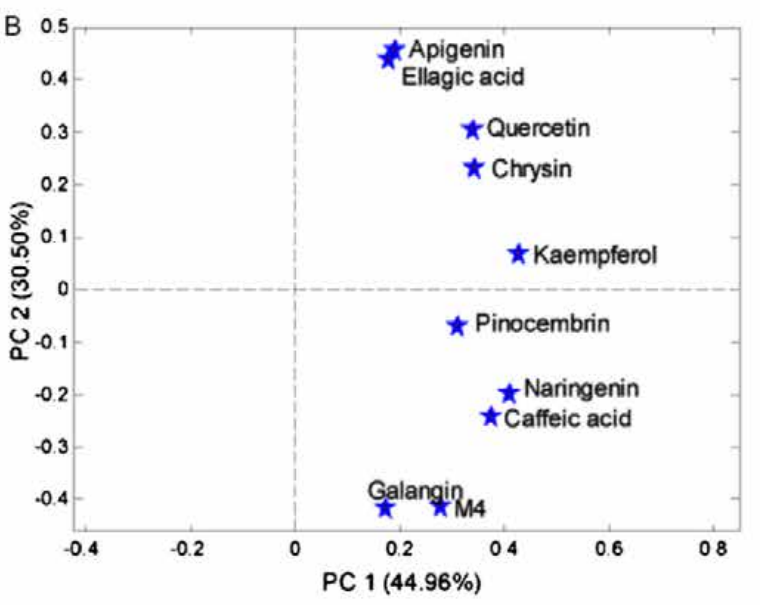
16.15 Zusammenfassung und Diskussion der unterschiedlichen Bioassays

17.00 Voraussichtliches Ende der Veranstaltung

Multivariate data analysis: HPTLC and MS



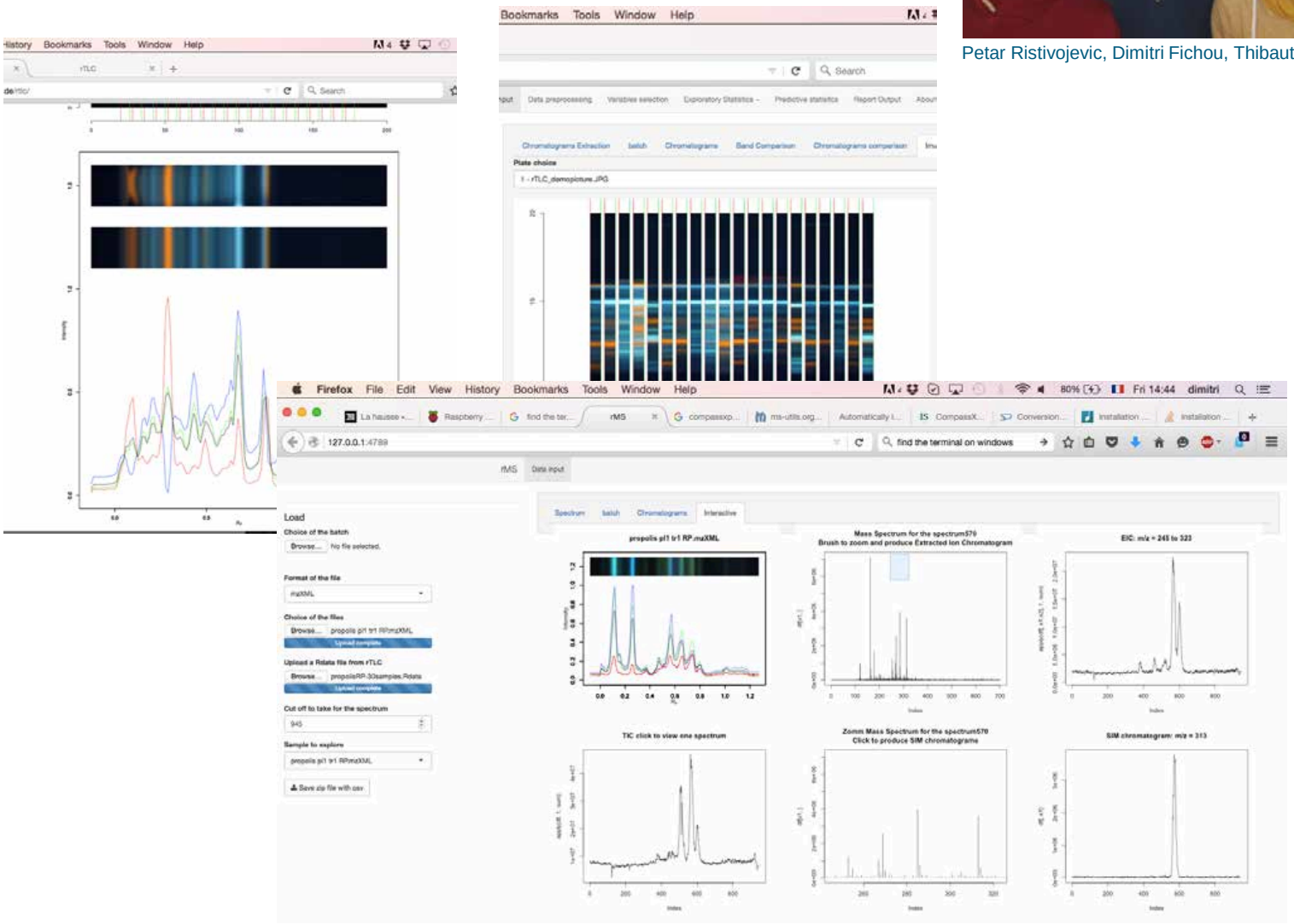




G.E. Morlock *et al.* J Chromatogr A 1328 (2014) 104– 112

Multivariate data analysis

Petar Ristivojevic, Dimitri Fichou, Thibaut Chasset



D. Fichou *et al.*, in preparation

Use the best method for the given task!

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Selective derivatization **post**-chromatographically compensates the low separation power!

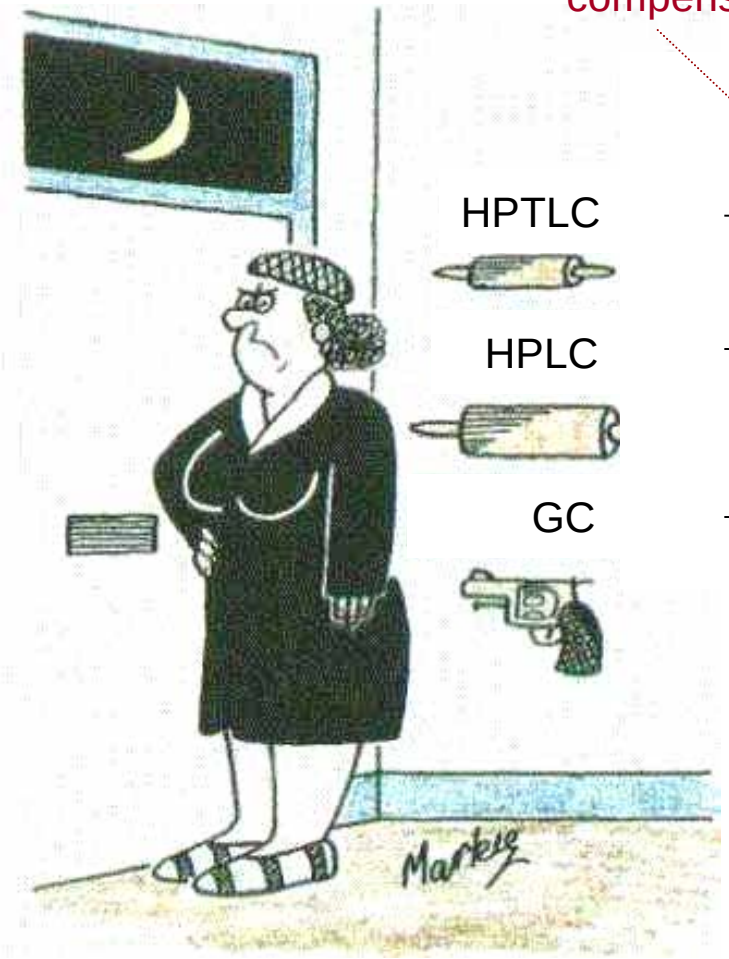


Plate number N

HPTLC → 8.000 ✓

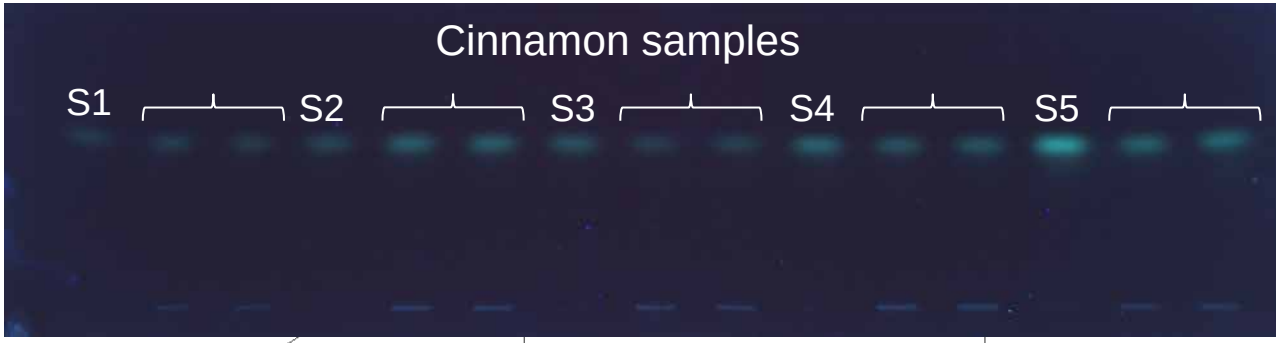
HPLC → 18.000

180.000 / m
9.000 / 5 cm

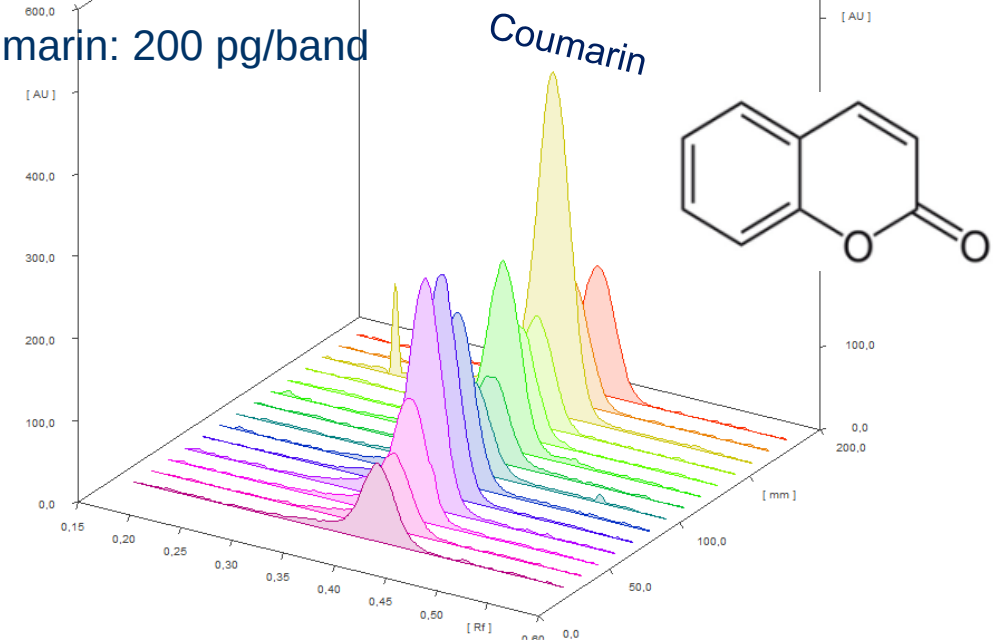
GC → 80.000

Analysis of coumarin

5 different cinnamom spice samples containing coumarin



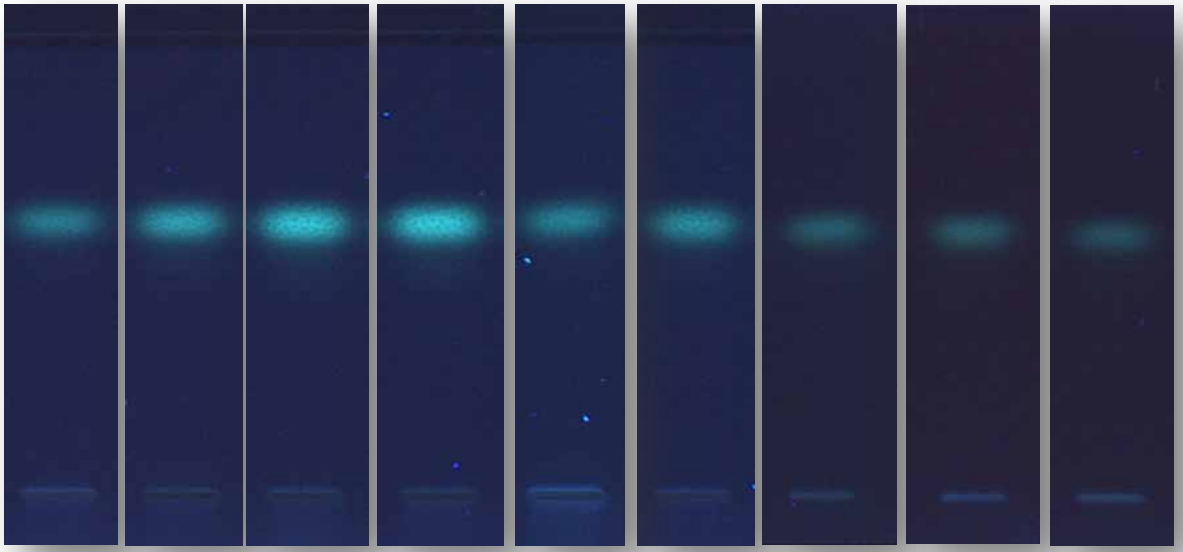
LOD of coumarin: 200 pg/band

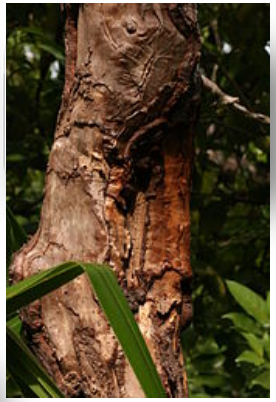


L. Winheim, S. Krüger, G. Morlock, in preparation

Analysis of coumarin

9 different food samples containing coumarin





www.wikipedia.de

www.colourbox.de

www.deutsche-wirtschafts-nachrichten.de

www.institut-fresenius.de

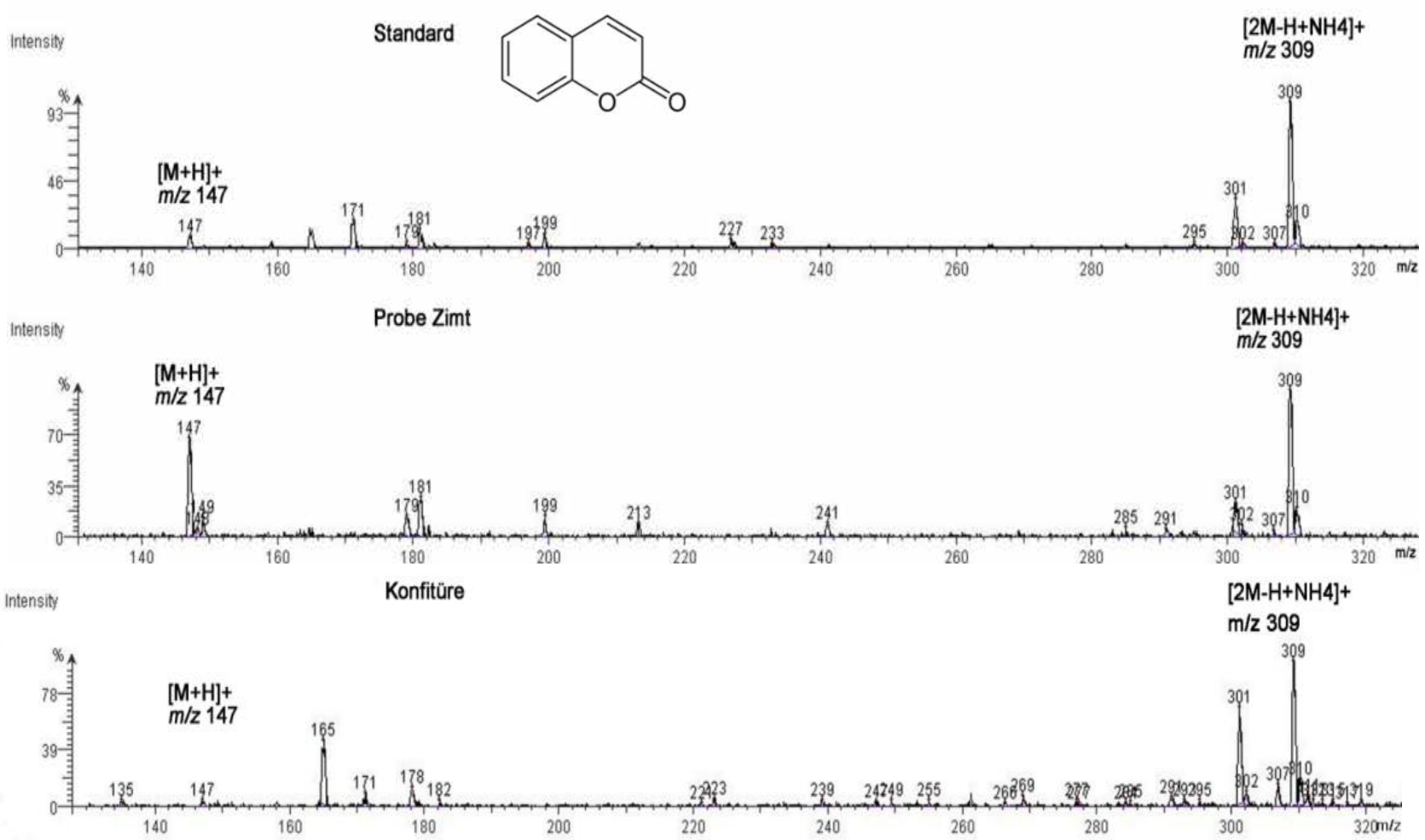
L. Winheim, S. Krüger, G. Morlock, in preparation

Coumarin content

Sample	Manufacturer	Coumarin (mg/kg)	Repeatabilities *(%RSD, n=2)*	Reproducibility *(%RSD, n=2)*
Spice	Sonnentor - gemahlen	3732	0.4/1.2	1.0
	Lidl Kania - gemahlen	1615	0.4/0.3	2.4
Tea	YogiTea - Frauenpower	19	1.4/3.2	4.5
	TeeGeschwendner – Kaminfeuer	22	3.3/2.0	0.5
Cereals	Aldi Knusperone - Zimt Chips	1	3.8/5.9	0.4
Cinnamon bun	IKEA - Kanelbullar	16	1.4/4.5	6.6
Jam	Grafschafter Konfitüre – Winterzauber	4	1.6/1.6	1.6

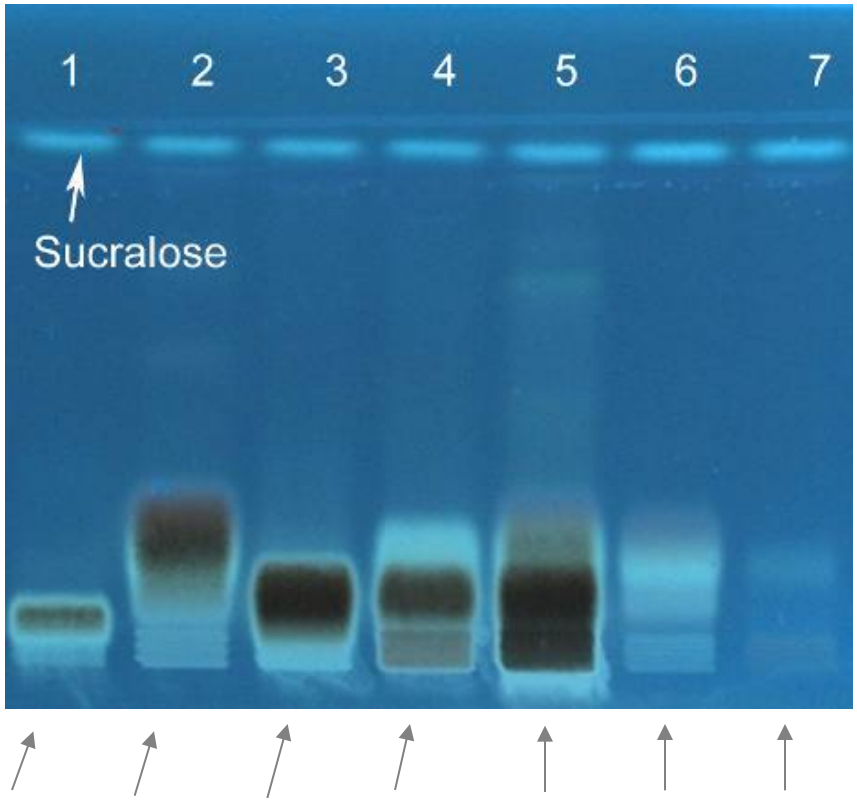
L. Winheim, S. Krüger, G. Morlock, in preparation

Confirmation by HPTLC-ESI-MS



L. Winheim, S. Krüger, G. Morlock, in preparation

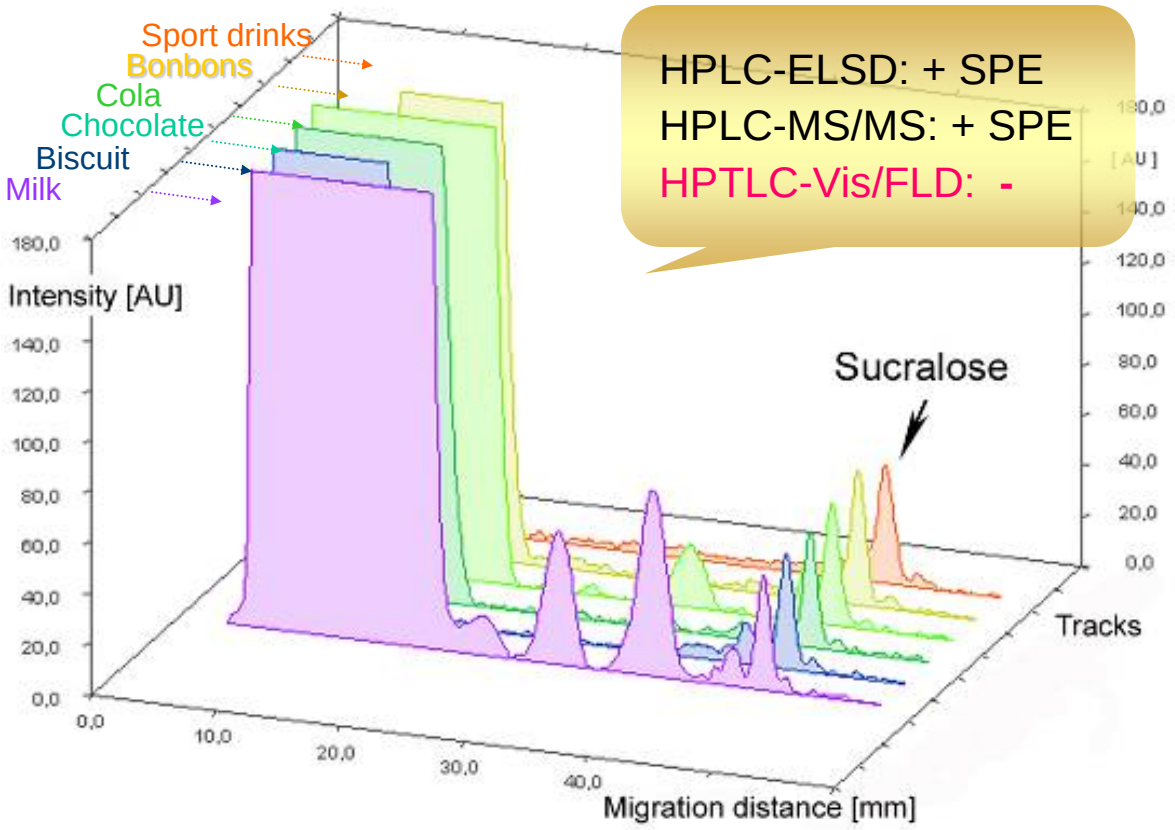
HPTLC is worth to keep in mind



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

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

Sample preparation and chromatography



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

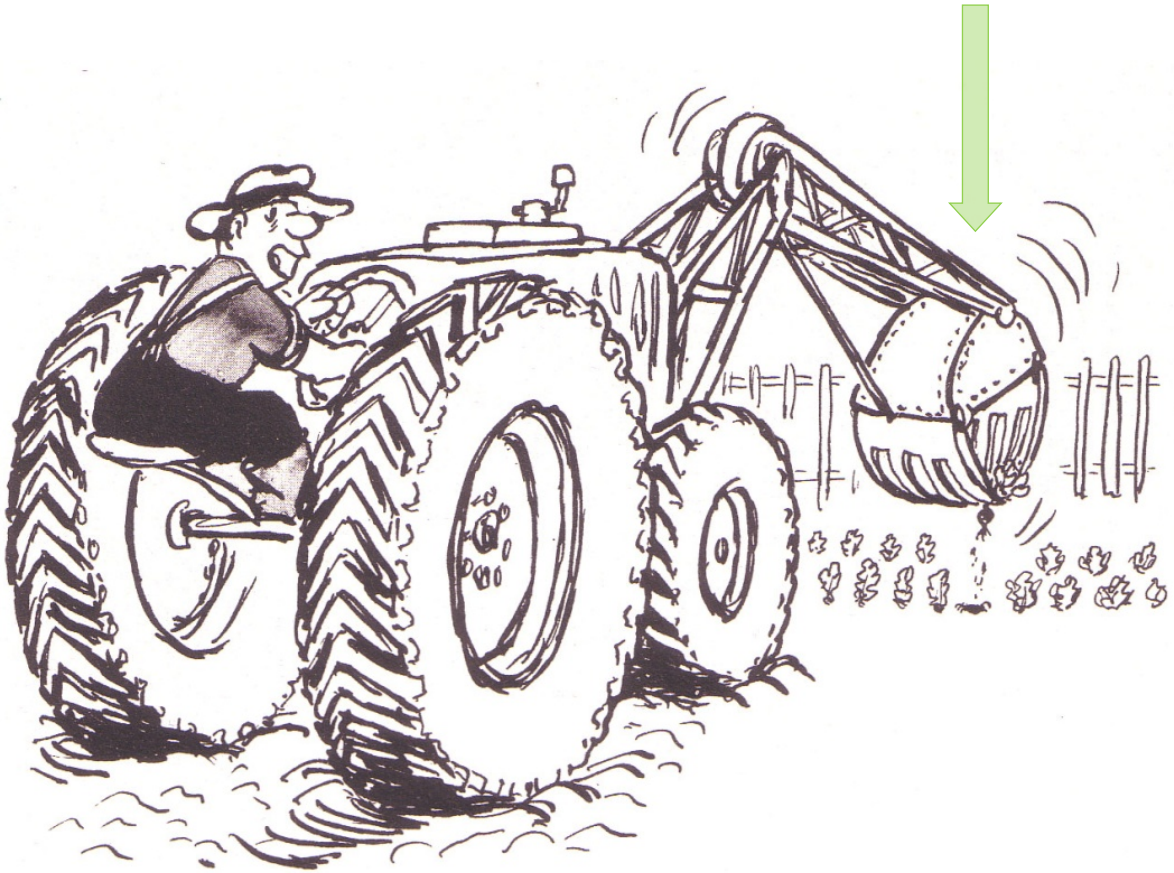
Facts for sucralose analysis

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

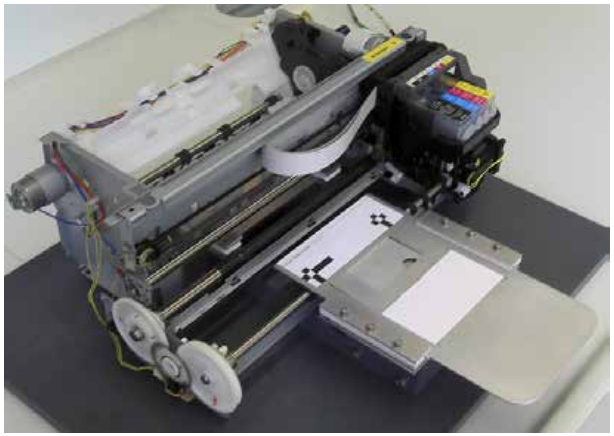
- Ultra-rapid HPLC with 2 min gradient: 720 runs/24-h day
- Sample preparation: Need of SPE for MS or ELSD as detector

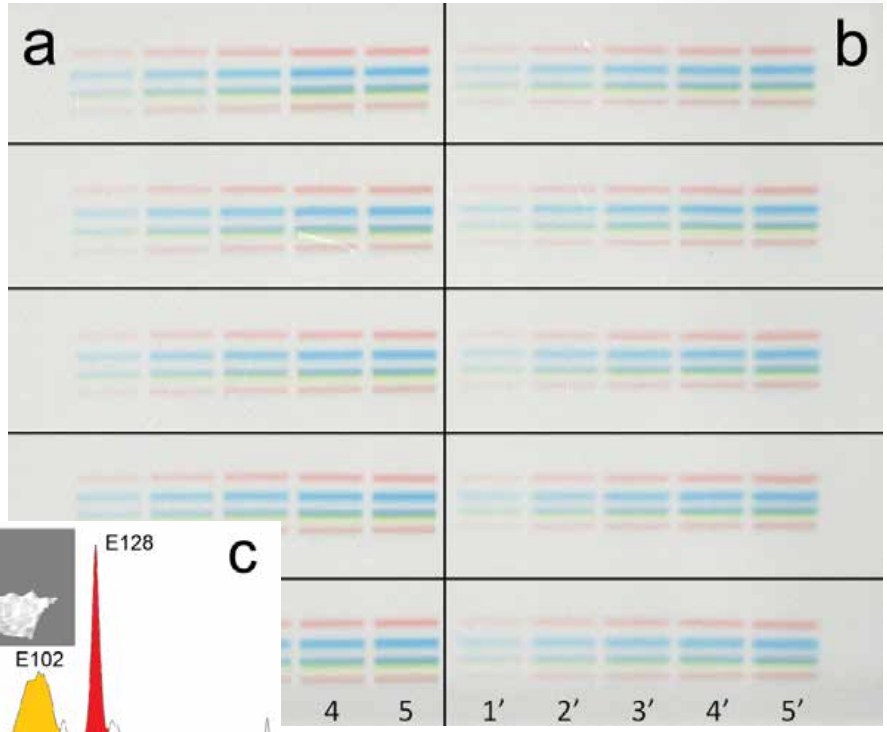
G. Morlock, W. Schwack, LCGC Eur July (2008) 366-371

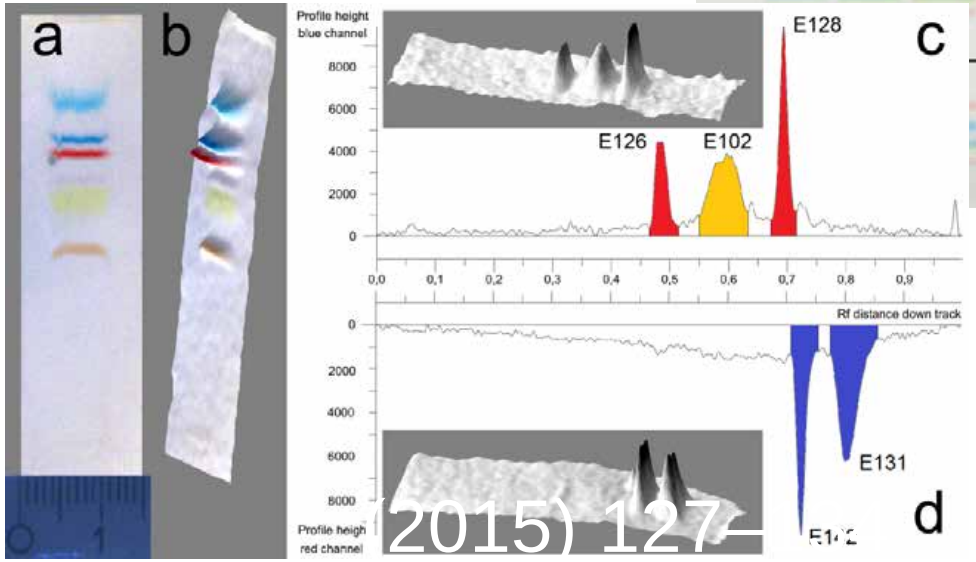
…not to end like this

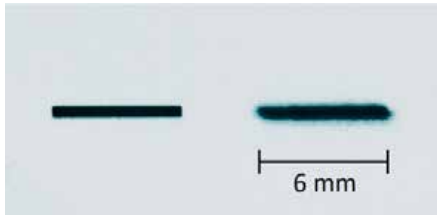


Office chromatography









(2015) 127–

T. Häbe, G. Morlock, J Chromatogr A 1413 (2015) 127–134

Thank you!

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