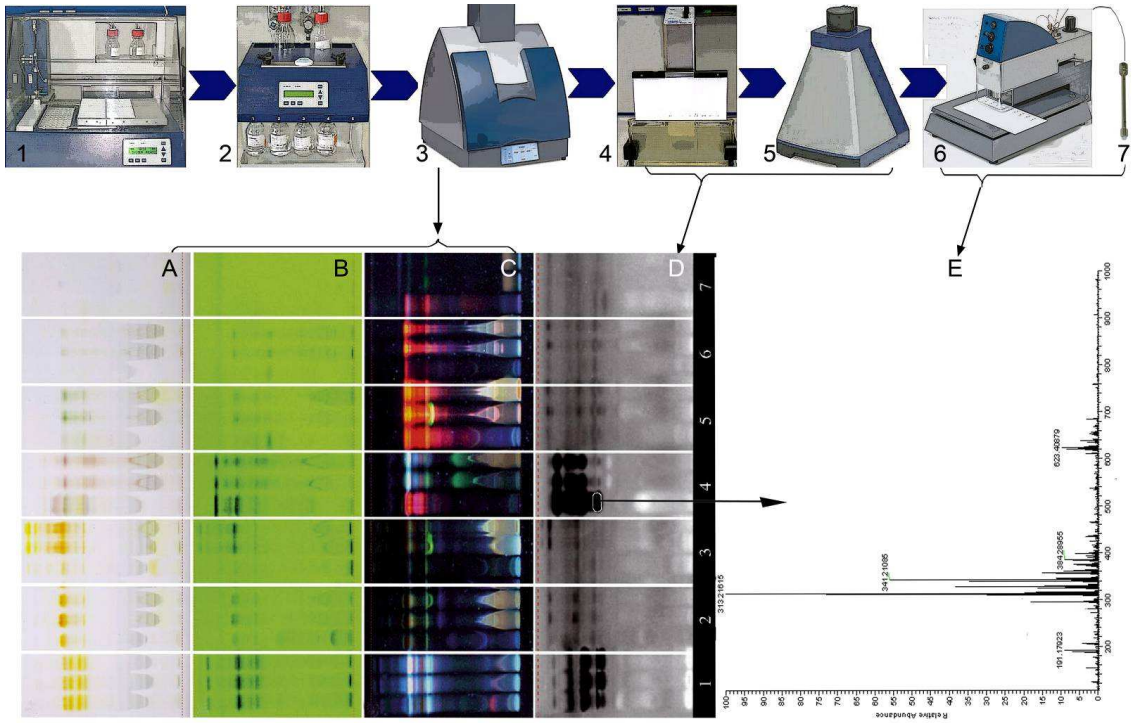
Hyphenated HPTLC methods for identification and structure elucidation



PD Dr. Gertrud Morlock,
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Hyphenation

- 1980: term hyphenation by Hirschfeld
- comprises the different approaches to combine mainly spectrometers with chromatographic systems to get further information about the sample
- hyphen (-) symbolizes this attempt of combination, which did not reach its stage of full maturity so far
- slash (/) is found for hyphenated methods at a maturate state
- 2007: term “hypernation“ (super-hyphenation) by Wilson and Brinkman
  → to place all of the required spectrometers into a single system
  so that all of the spectroscopic information is obtained in a single run

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Hyphenation

Problems associated with column-based hypernations are:

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

All these problems are less challenging in HPTLC-based hypernations:

- Open system is highly adaptive to different sensitivities
- Cost-effective by modular instrumentation
- Generating less data due to targeted access to points-of-care on the plate
- Directly accessible for the respective optimal solvent

=> The main difference:

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

HPTLC: solvent evaporated; after separation → still on the plate.

G Model
CHROMA-351018; No. of Pages 10

ARTICLE IN PRESS

Journal of Chromatography A, xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Review

Hyphenations in planar chromatography

- HPTLC-UV/Vis/FLD-MS [13,14],
- HPTLC-UV/Vis/FLD-bioactivity-HRMS [15],
- HPTLC-UV-FTIR [16,17],
- HPTLC-UV/Vis/FLD-FTIR ATR [18],
- TLC-Vis-SERS [12].

Gertrud Morlock*, Wolfgang Schwack

University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany

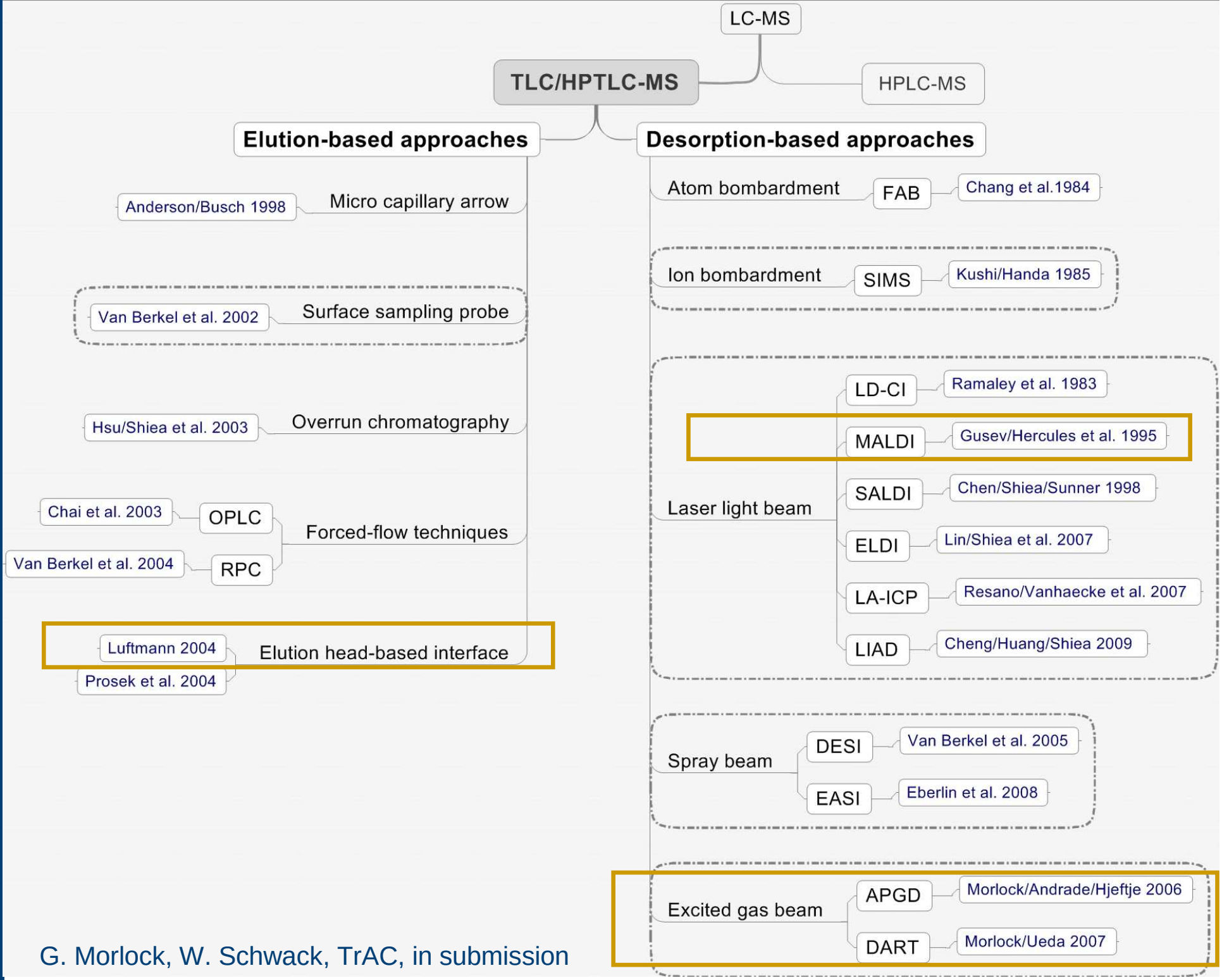
ARTICLE INFO

Article history:
Available online xxx

Keywords:
Mass spectrometry
High-performance thin-layer chromatography
Effect-directed analysis
Bioassays
Cost-effective analysis
High-throughput system

ABSTRACT

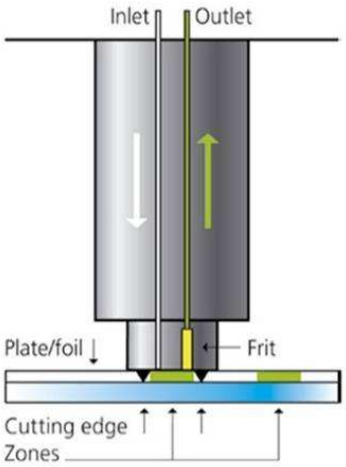
This review is focused on planar chromatography and especially on its most important subcategory high-performance thin-layer chromatography (HPTLC). The image-giving format of the open, planar stationary phase and the post-chromatographic evaporation of the mobile phase ease the performance of various kinds of hyphenations and even super-hyphenations. Examples in the field of natural product search, food and lipid analysis are demonstrated, which point out the hyphenation with effect-directed analysis (EDA) and mass spectrometry and illustrate the efficiency gain. Depending on the task at hand, hyphenations can readily be selected as required to reach the relevant information about the sample, and at the same time, information is obtained for many samples in parallel. The flexibility and the unrivalled features through the planar format valuably assist separation scientists.

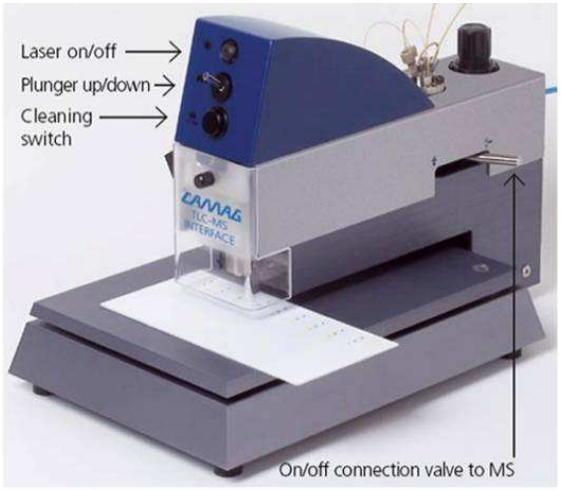


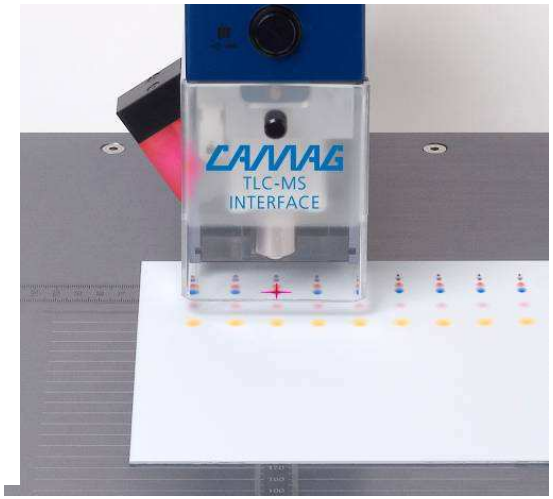
G. Morlock, W. Schwack, TrAC, in submission

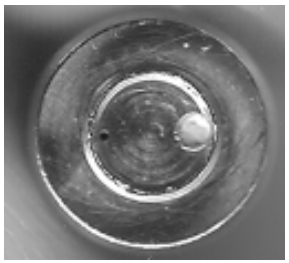
Institute of Food Chemistry
University of Hohenheim, Stuttgart

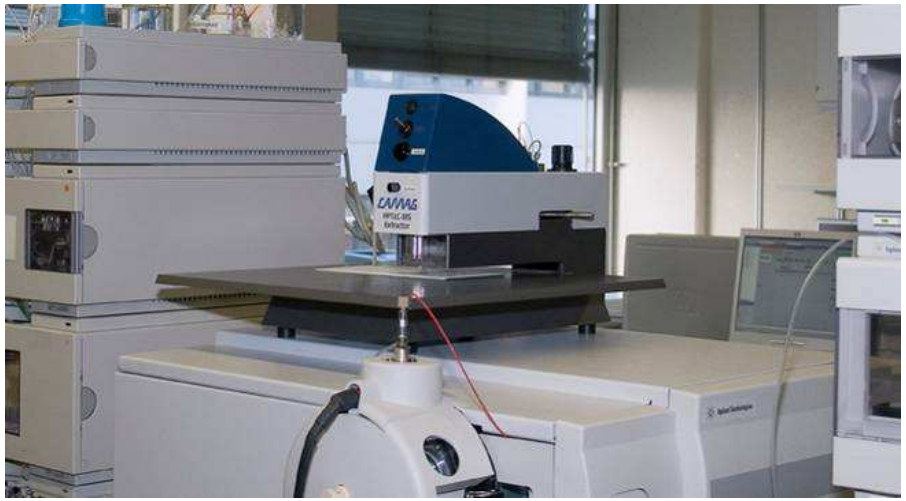
Elution head-based HPTLC-MS → TLC-MS Interface









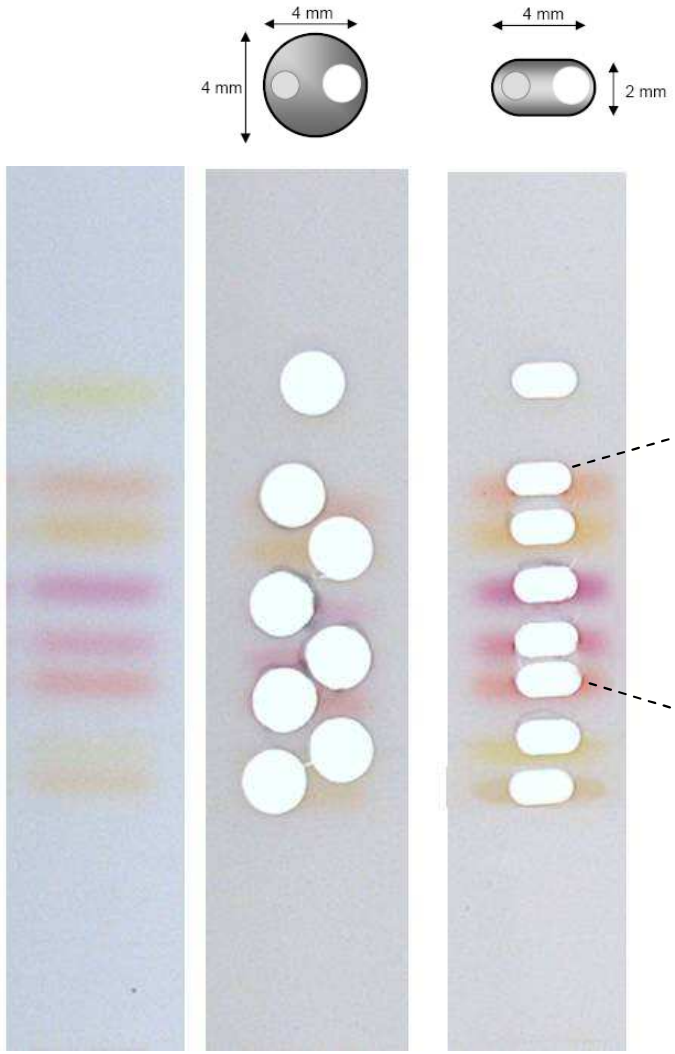


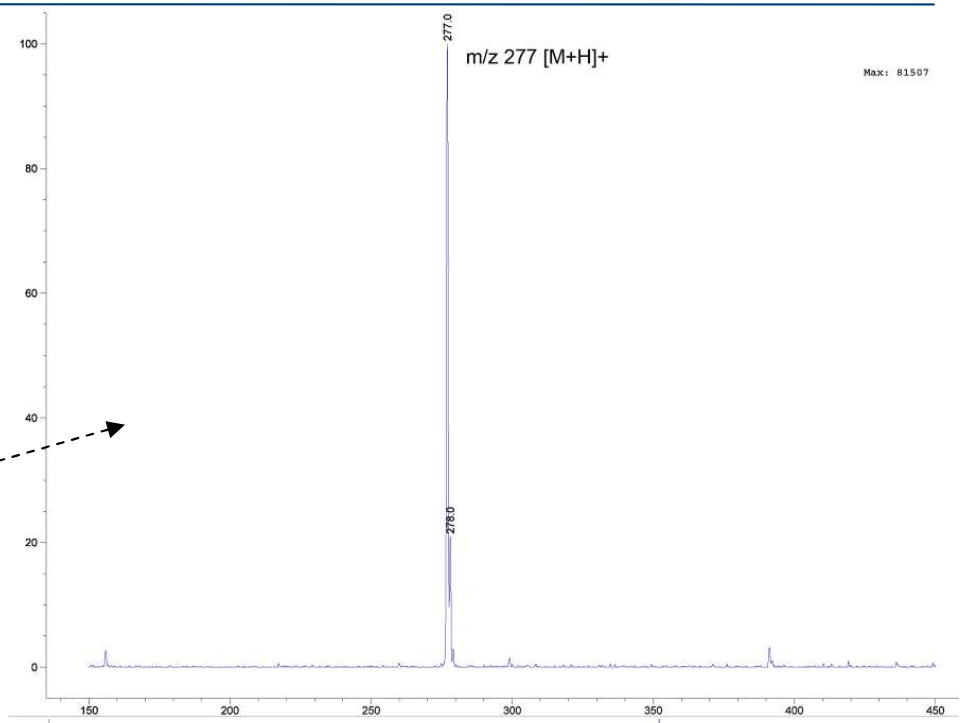
H. Luftmann, Anal Bioanal Chem 378 (2004) 964-968

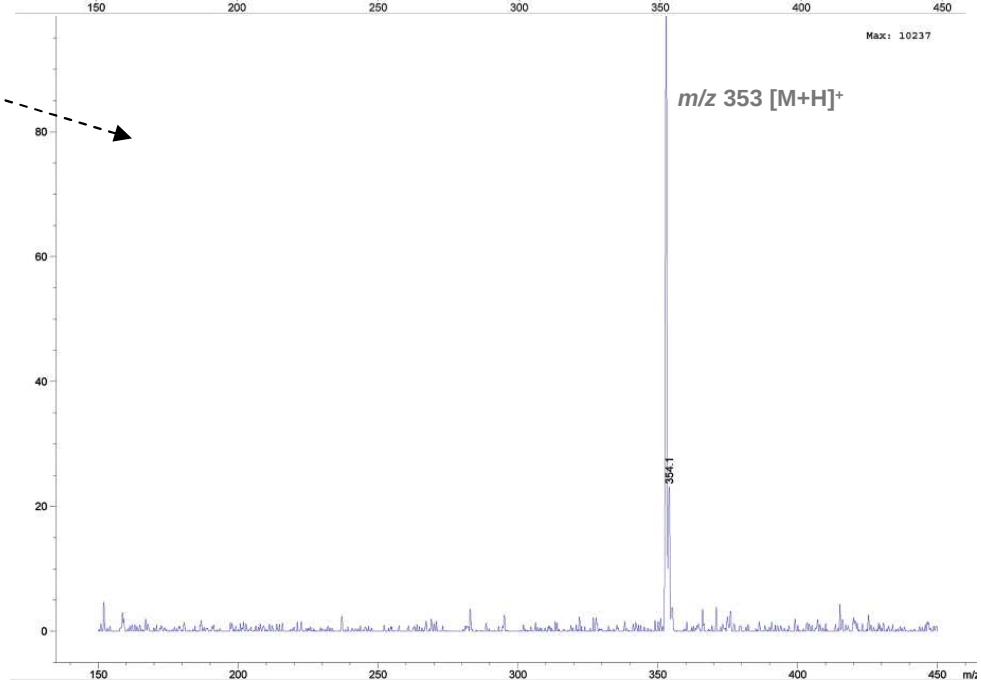
A. Alpmann, G. Morlock, Anal Bioanal Chem 386 (2006) 1543-1551

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Elution head-based HPTLC-MS





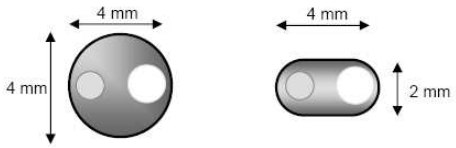


G. Morlock , CBS 103 (2009) 16

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Elution head

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

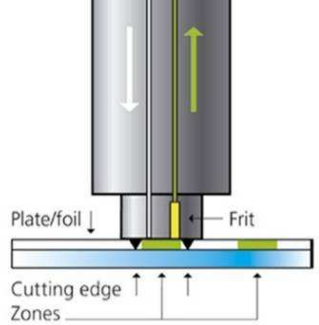


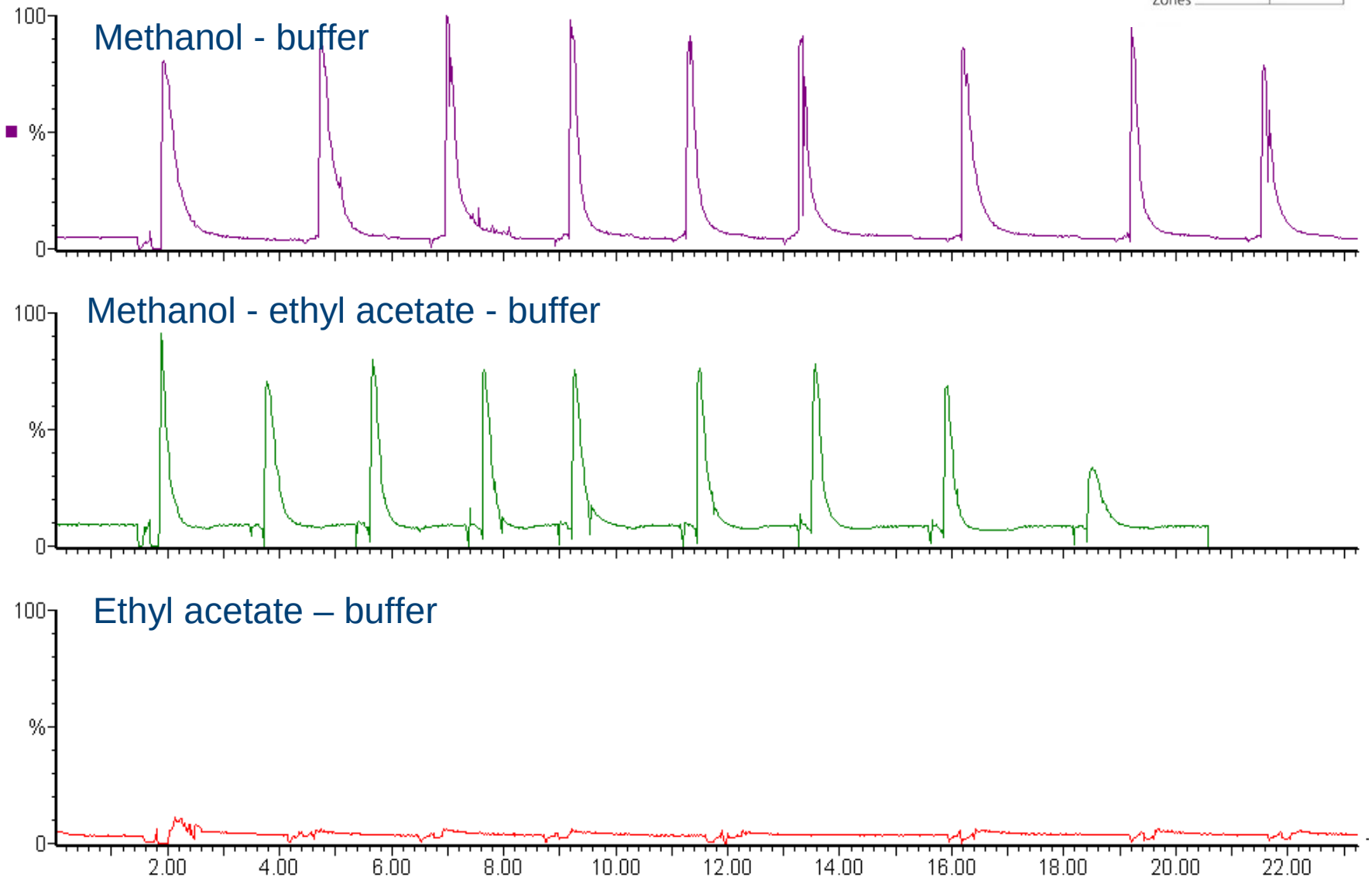
Cutting edge height

- 0.2 mm for standard layers → CAMAG Bibliography Service CBS 102 (2009)
- 0.1 mm for extra thin layers → U. Jautz, G. Morlock, Anal Bioanal Chem 387 (2007) 1083
- 0.5 mm for preparative layers → E. Dytkiewitz, G. Morlock, J AOAC Int 91 (2008) 1237

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Elution profiles with different solvents

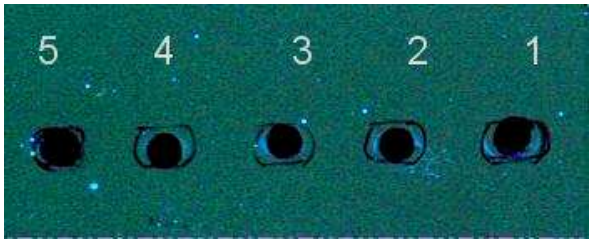




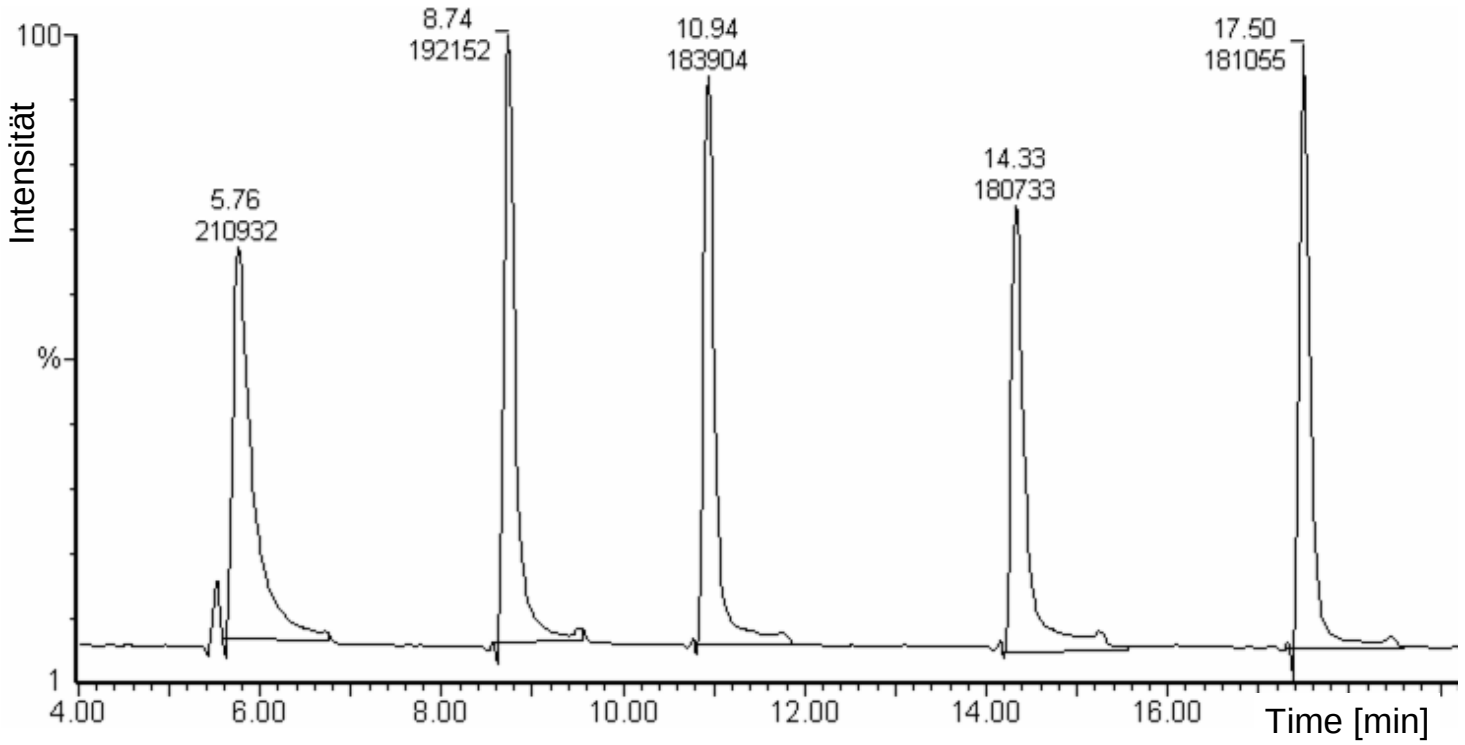
A. Alpmann, G. Morlock, Anal Bioanal Chem 386 (2006) 1543-1551

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Repeatability of the extraction



→ *%RSD* = 6.7 % (6 ng/zone, *n* = 5)



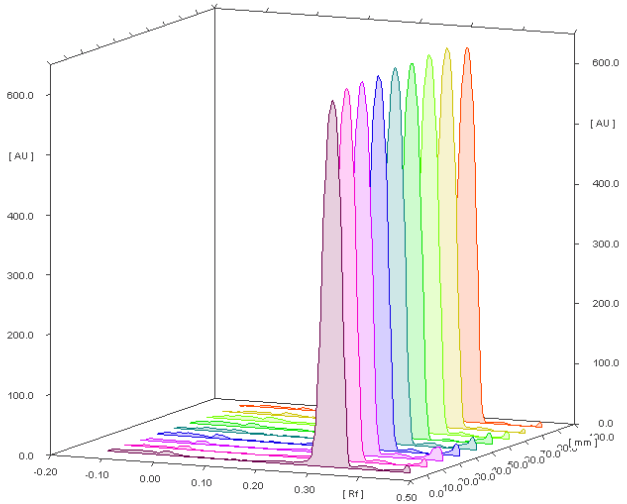
SIM elution profile of ITX @ m/z 255 $[M+H]^+$ and 277 $[M+Na]^+$

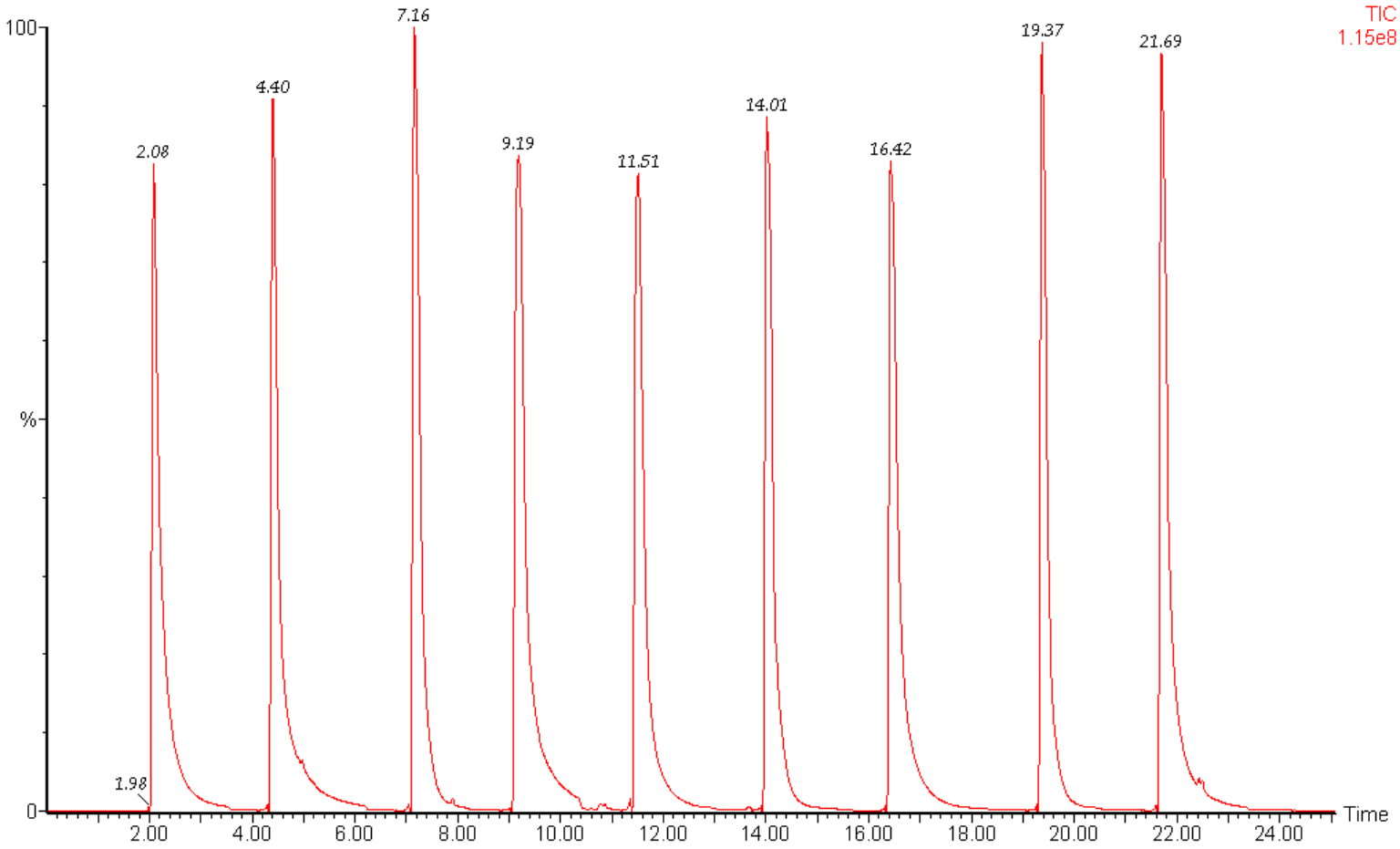
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Repeatability of extraction

SIM @ *m/z* 329 $[M+Na]^+$

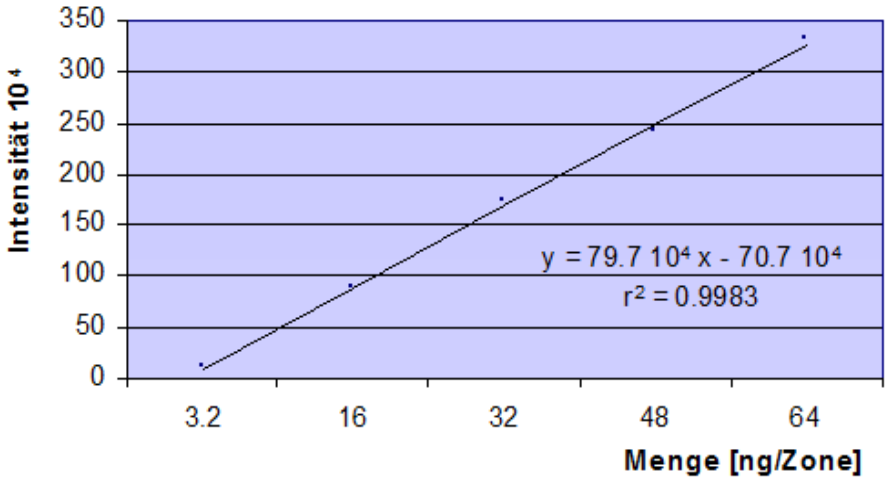
Repeatability (*%RSD*, *n* = 9): 7 %

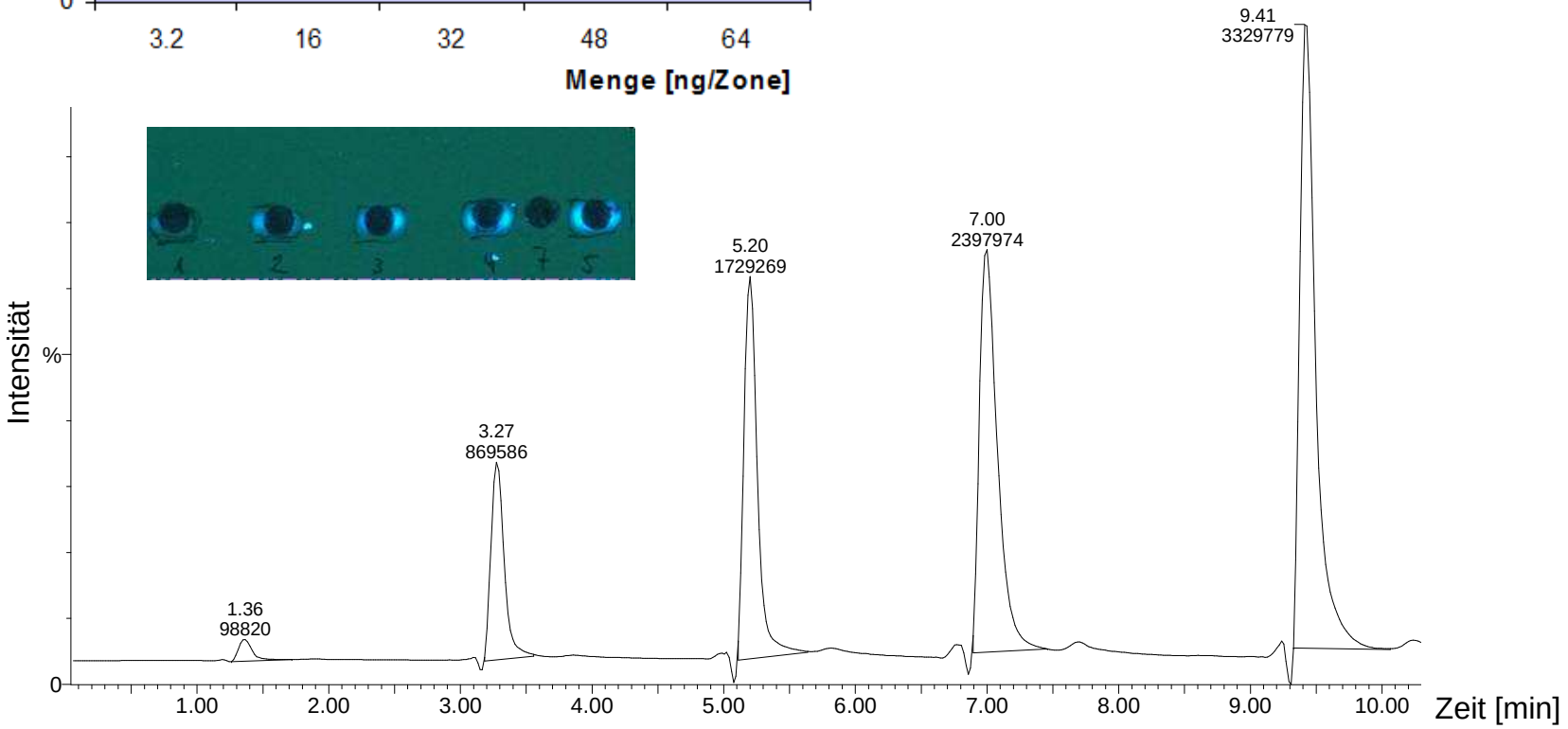




Institute of Food Chemistry
University of Hohenheim, Stuttgart

Analytical response





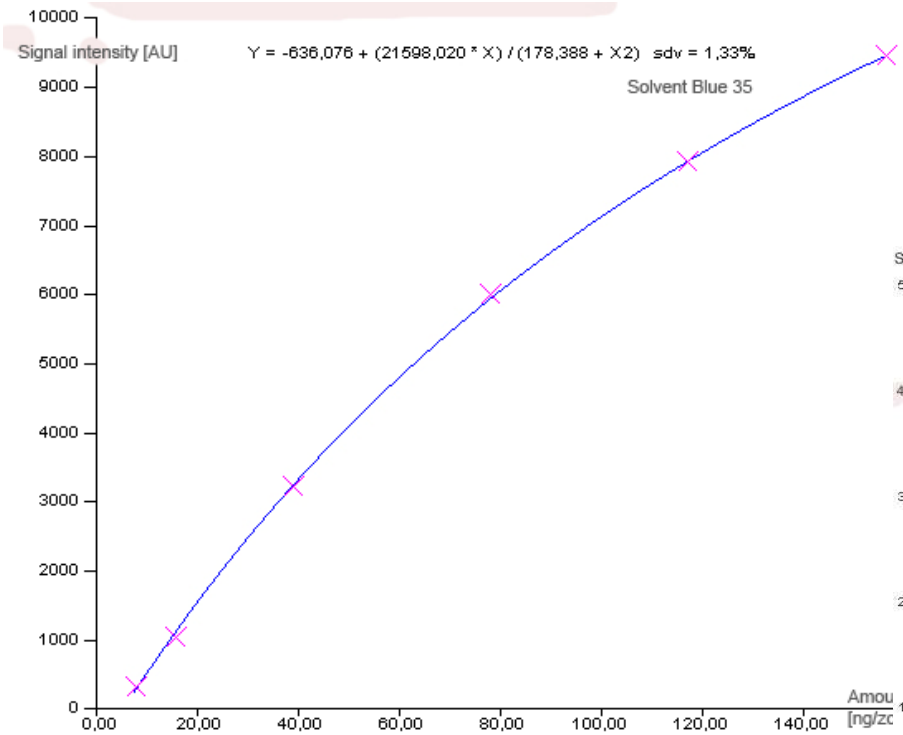
SIM elution profile of ITX @ m/z 255 $[M+H]^+$ and 277 $[M+Na]^+$

Institute of Food Chemistry
University of Hohenheim, Stuttgart

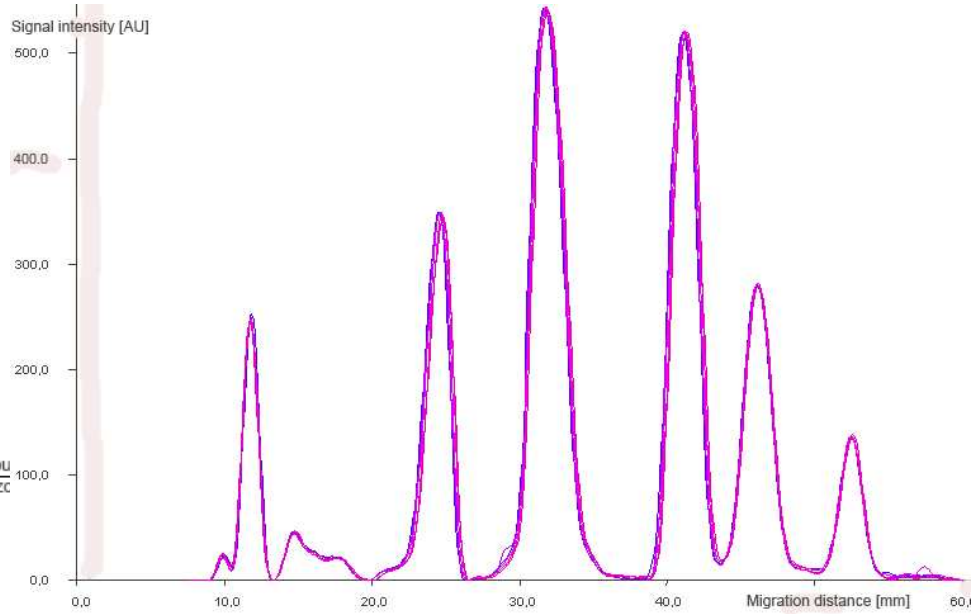
Performance data obtained with the TLC-MS interface

⇒ before: check of performance data by HPTLC-Vis

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



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



G. Morlock, W. Schwack, N. Brett, in preparation

Institute of Food Chemistry
University of Hohenheim, Stuttgart

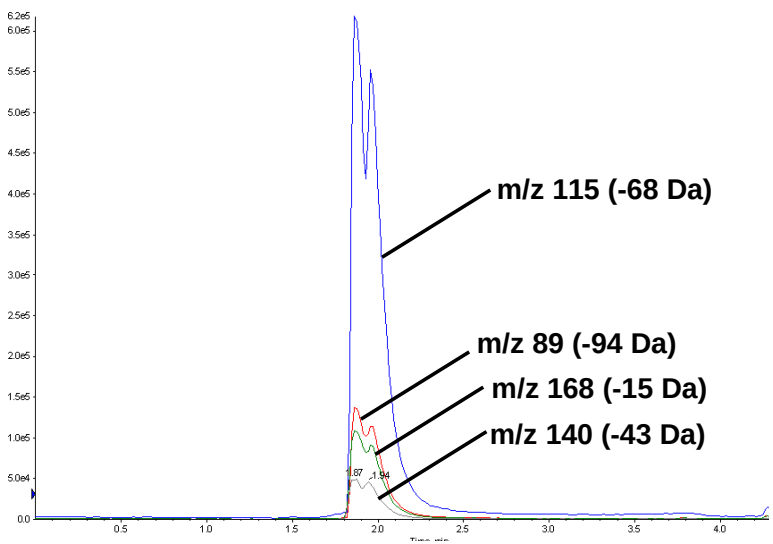
Performance data obtained with the TLC-MS interface

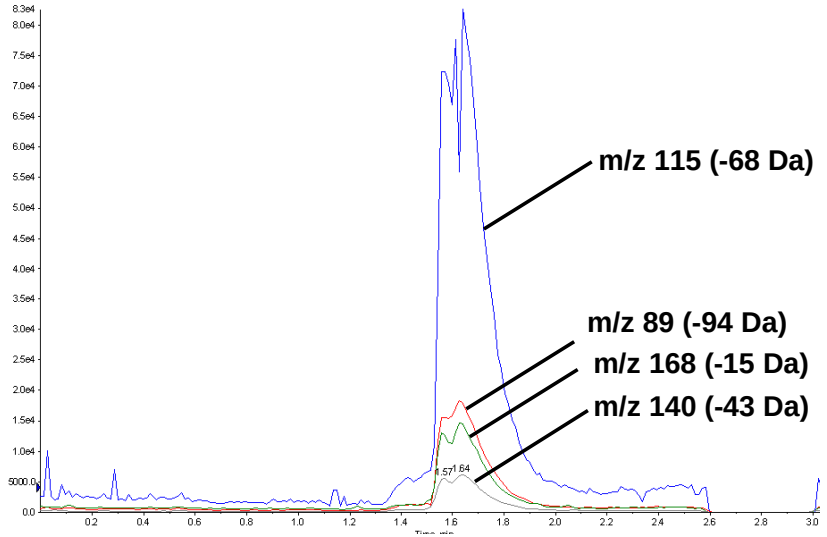
HPTLC-ESI-MS (SIM, peak area)		Linearity		Precision	
Dyes	hR_F-value	Calibration range (ng/band)	Determination coefficient	Conc. (ng/band)	n = 5, *%RSD*
Dimethyl Yellow	65	12 – 234	0.9943	1125	8.1
Oracet Red G	50	2 – 39	0.9950	189	11.0
Solvent Blue 35	41	10 – 52	0.9931	750	4.6
Sudan Red G	27	6 – 117	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

G. Morlock, W. Schwack, N. Brett, in preparation

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Detectability by HPTLC-ESI-MS/MS





200 pg/zone harman → 20 pg/zone harman

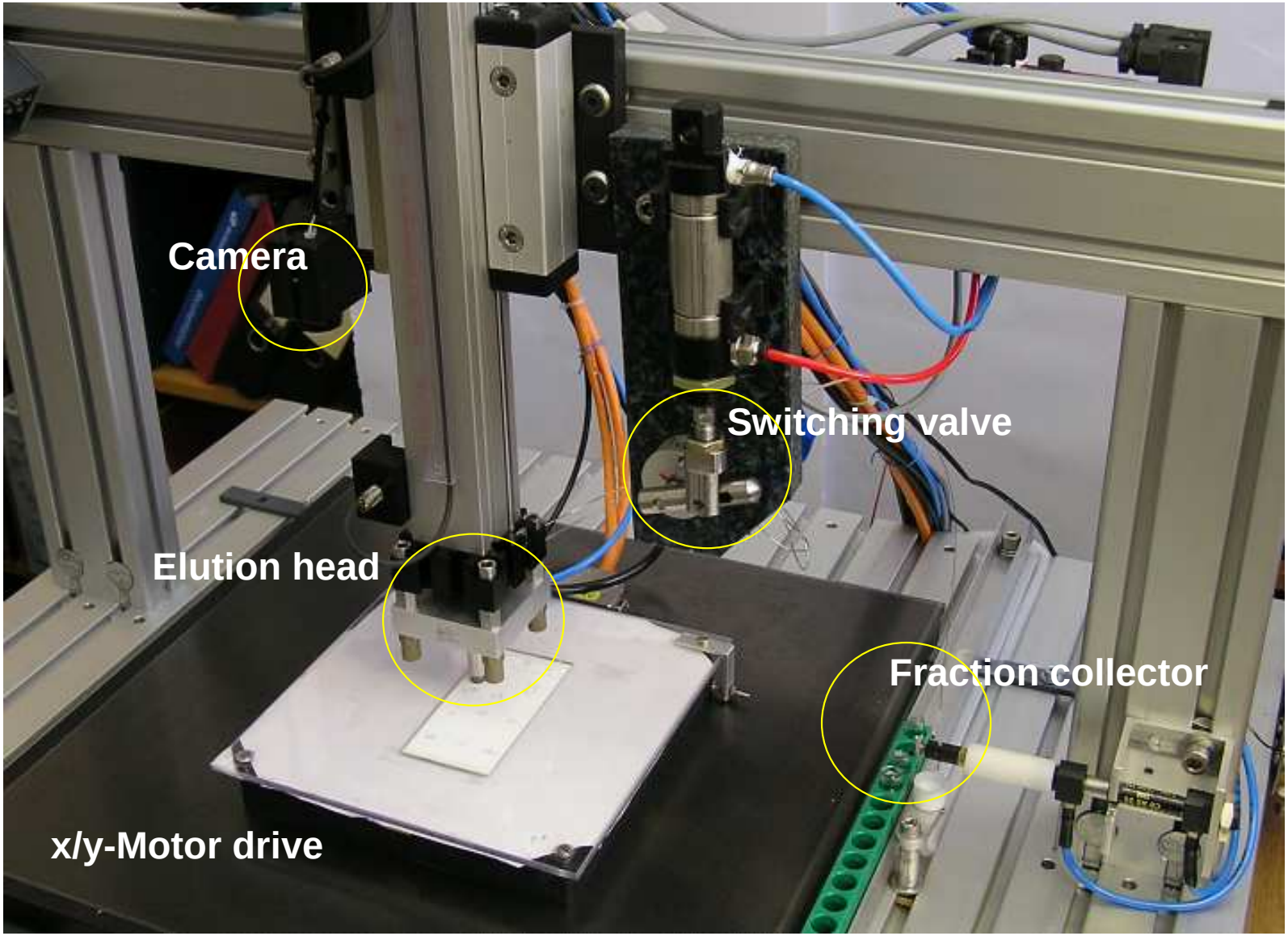
→ LOQ better than 20 pg/zone harman (S/N 20)

→ Detectability comparable to HPLC/MS

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

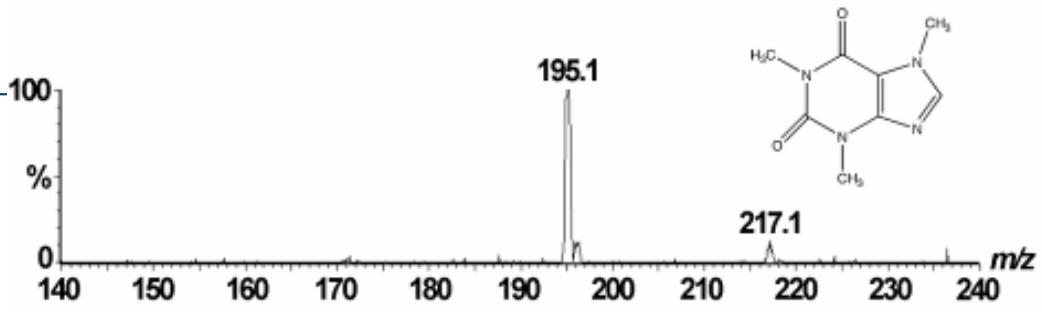
Institute of Food Chemistry
University of Hohenheim, Stuttgart

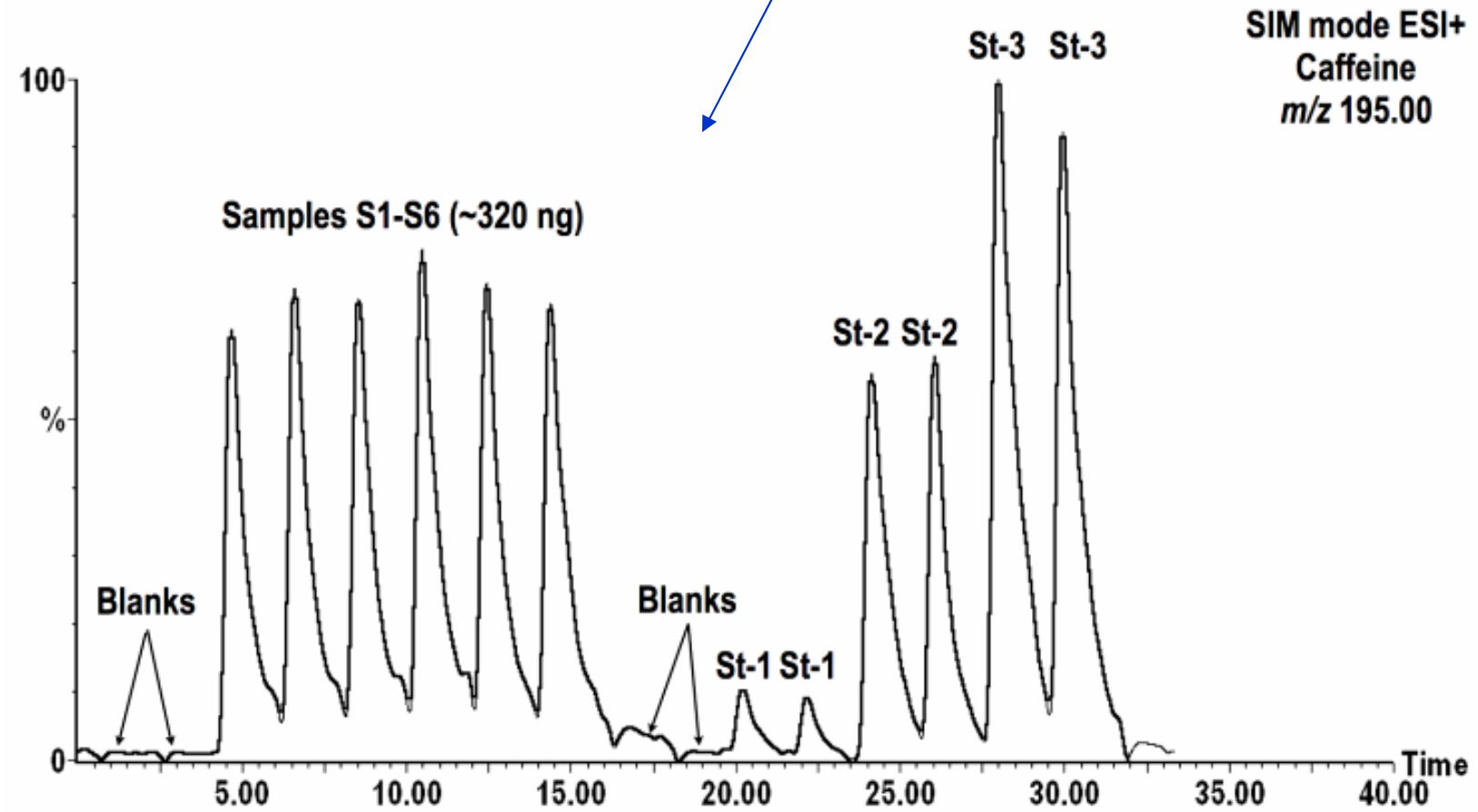
Hands-free interface called ‘R3D3’



Institute of Food Chemistry
University of Hohenheim, Stuttgart

Elution profiles



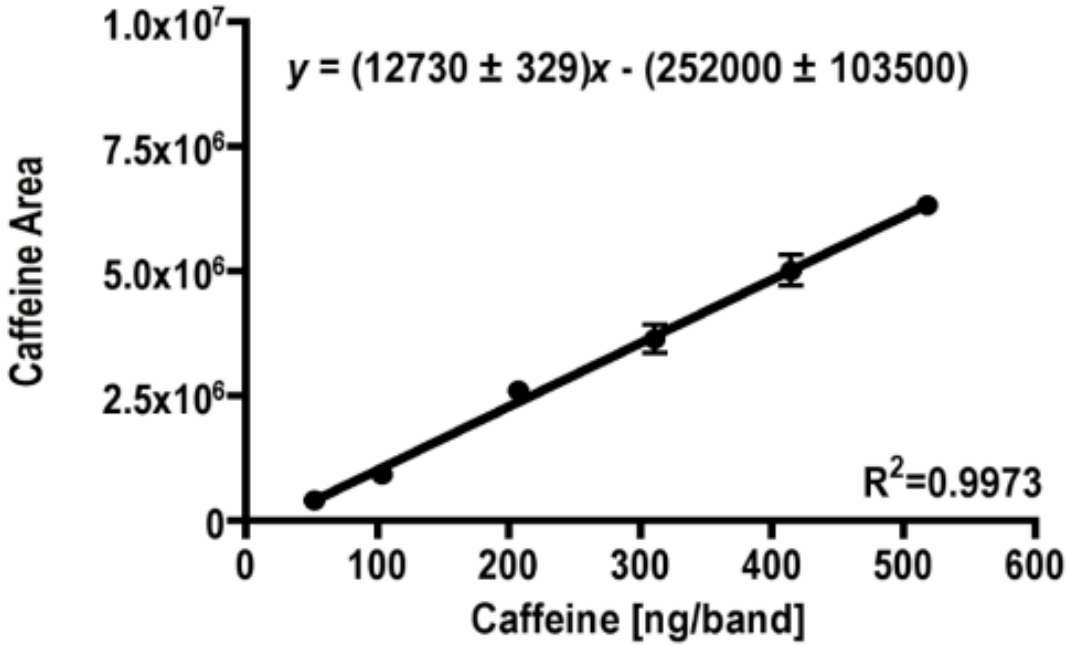


Institute of Food Chemistry
University of Hohenheim, Stuttgart

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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

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)

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Comparison of automated interfaces

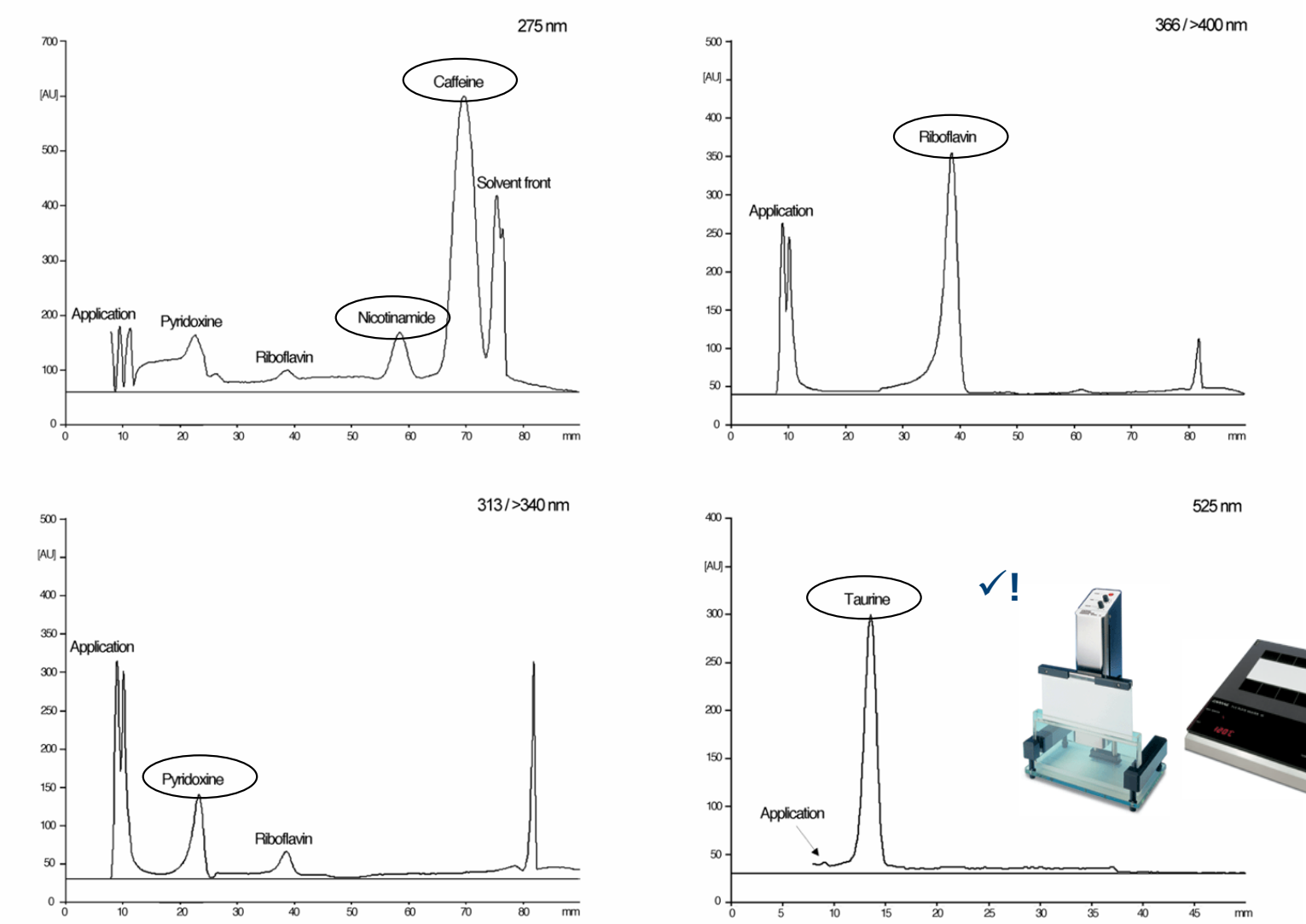
Parameter	Precision *%RSD*	Linear Response r^2
Quantification **without** internal standard		
Elution head (autom.)	≤ 5.6 %	0.9973
DESI	≤ 16.8 %	0.95 - 0.98
MALDI	10 %	-
LA-ICP	17 – 41 %	≥ 0.90
Quantification **with** internal standard		
Micro-junction ESI	≤ 4.4 %	0.9999
SALDI/APCI	7 %	0.9991
MALDI	≤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

G. Morlock, W. Schwack, TrAC, in submission

Institute of Food Chemistry
University of Hohenheim, Stuttgart

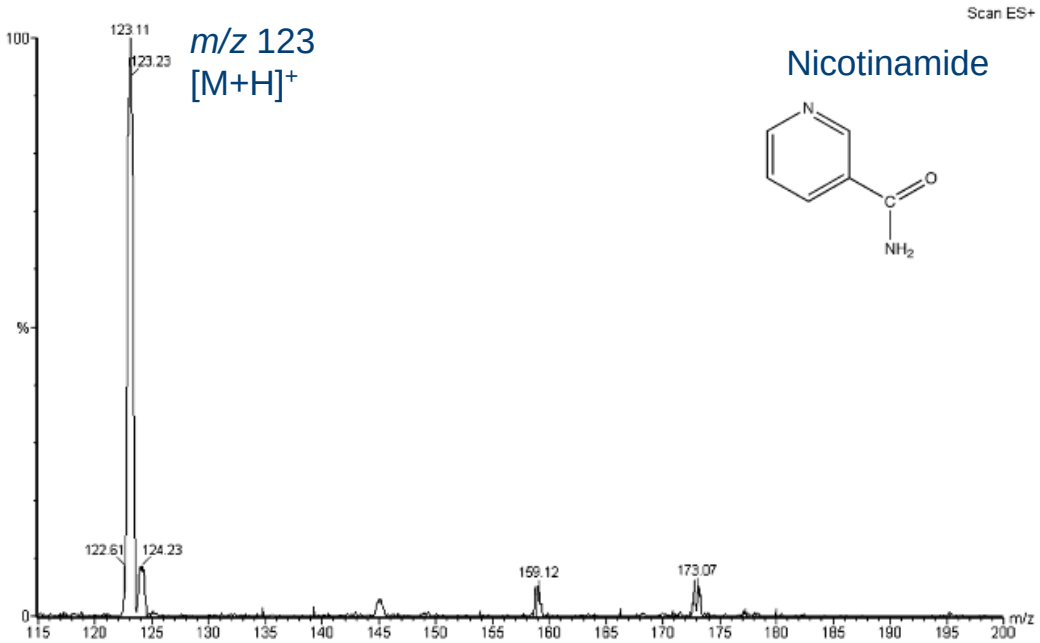
Active ingredients in energy drinks

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



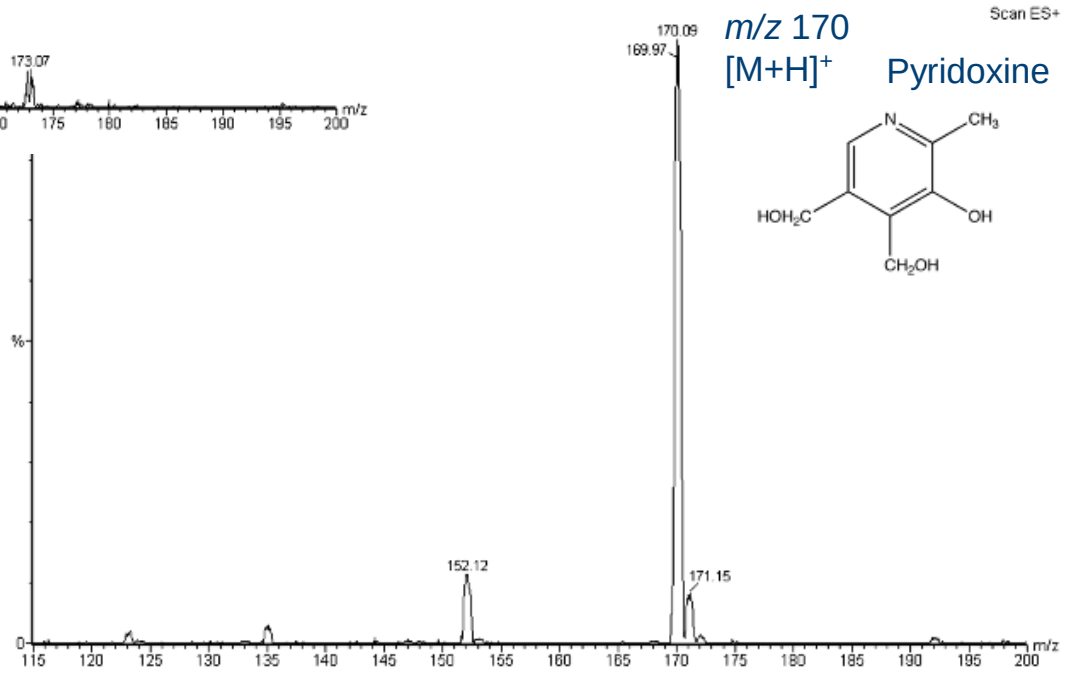
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Confirmation by MS



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

Nicotinamide



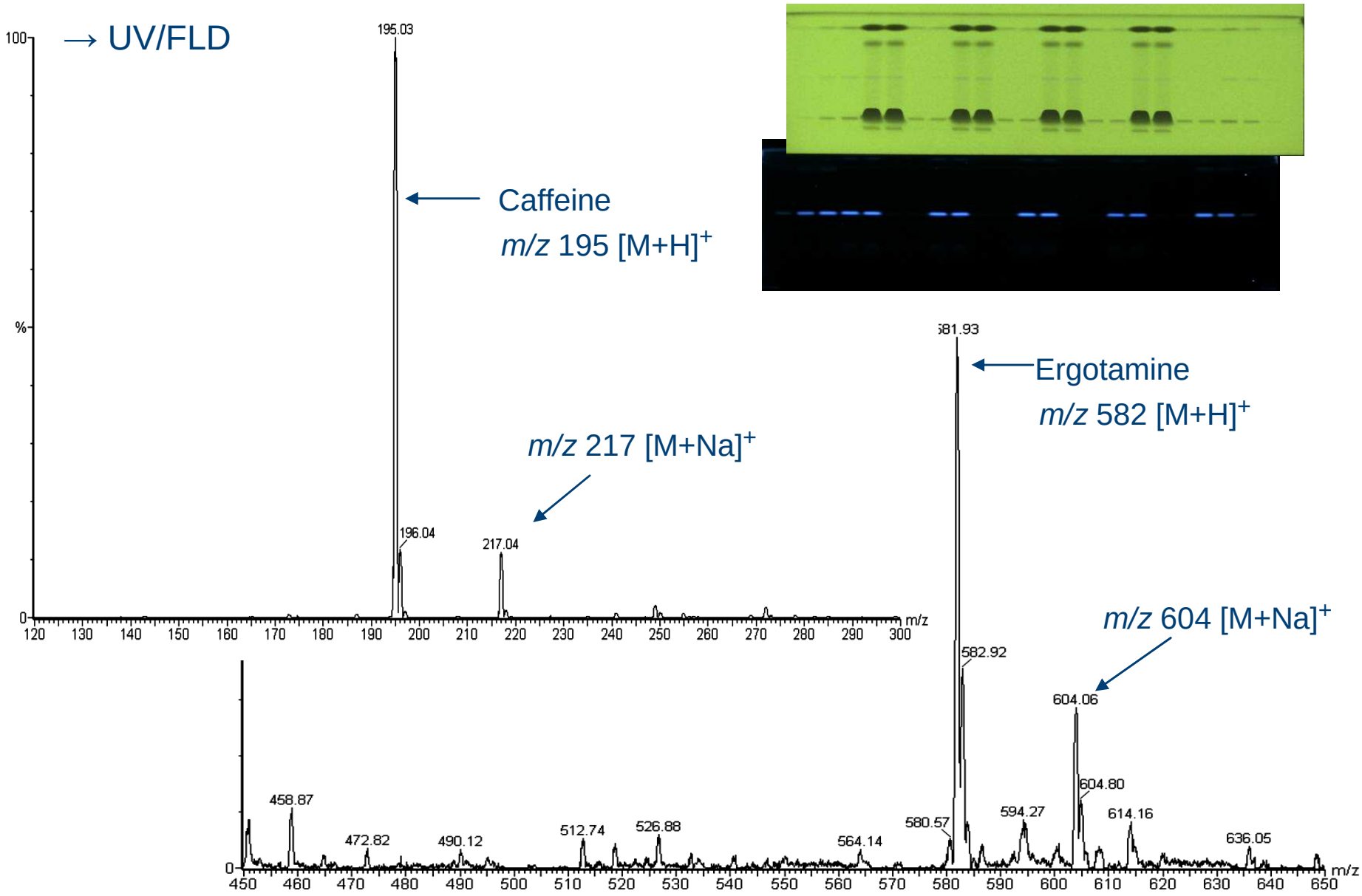
m/z 170
$[M+H]^+$

Pyridoxine

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

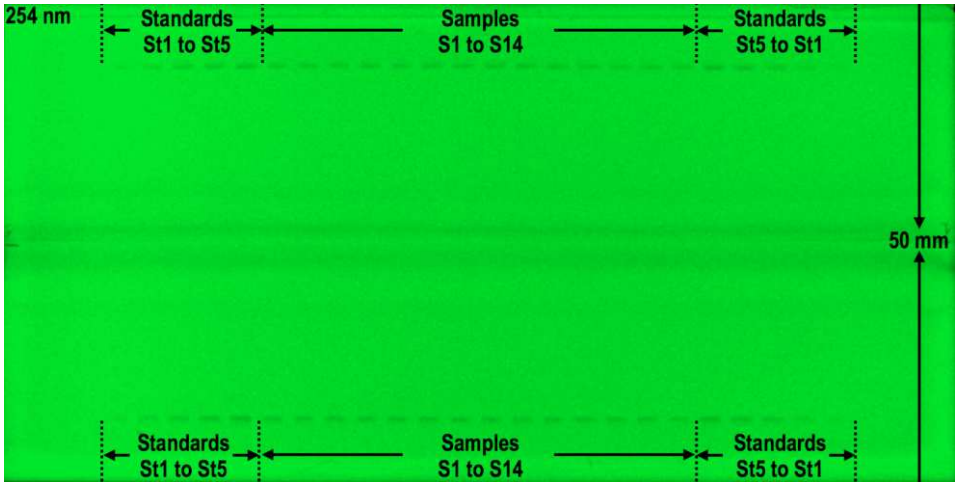
Institute of Food Chemistry
University of Hohenheim, Stuttgart

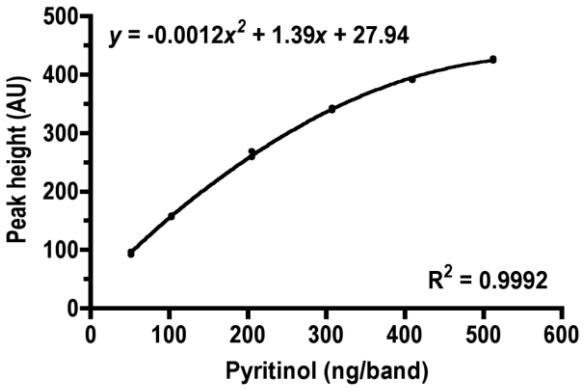
Caffeine, ergotamine and metamizol in tablets

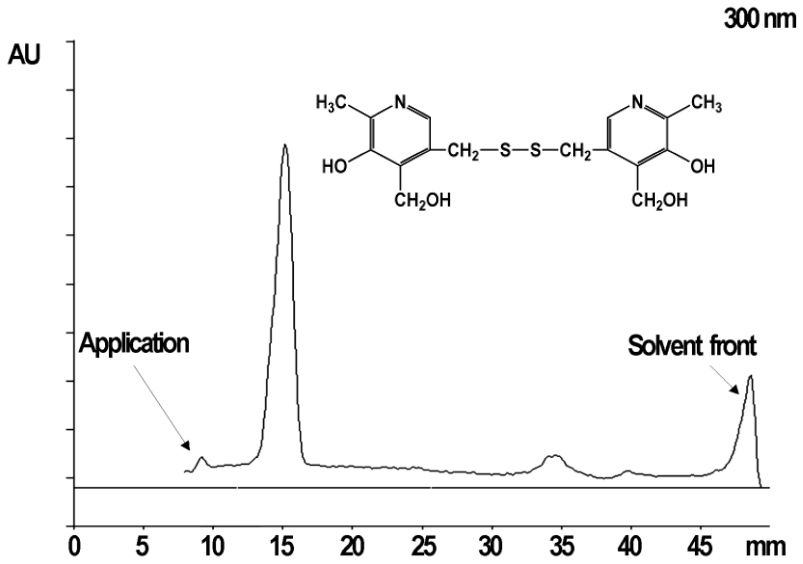


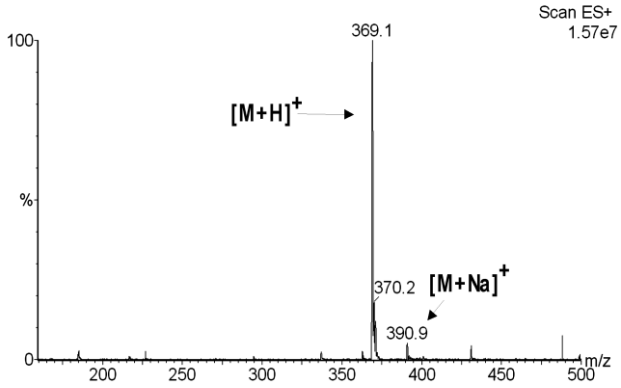
M. Aranda, G. Morlock, J Chromatogr Sci 45 (2007) 251-255

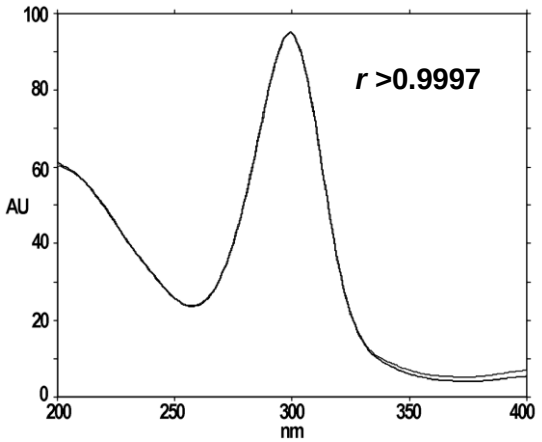
Pyridinol in tablets











M. Aranda, G. Morlock, J Liq Relat Technol, in press

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Institute of Food Chemistry
University of Hohenheim, Stuttgart

…no need for a higher separation power…

→ Repeatability (*%RSD*, n = 6) in matrix: 0.4 %

→ Intermediate precision (*%RSD*, n = 3) in matrix: 2.95 %

→ Recoveries of spiked samples (three levels): 98.5 - 101.9 % (± 3.6 - 4.7%)

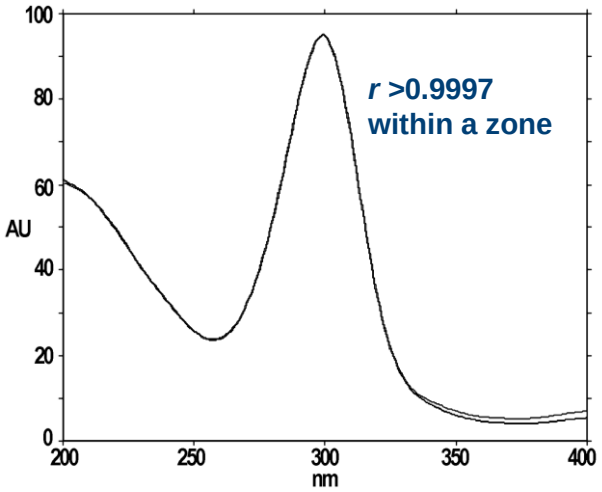
→ LOD/LOQ: 0.6/2.0 µg/mL (6/20 ng/band)

→ Up to 17 times less mobile phase consumption

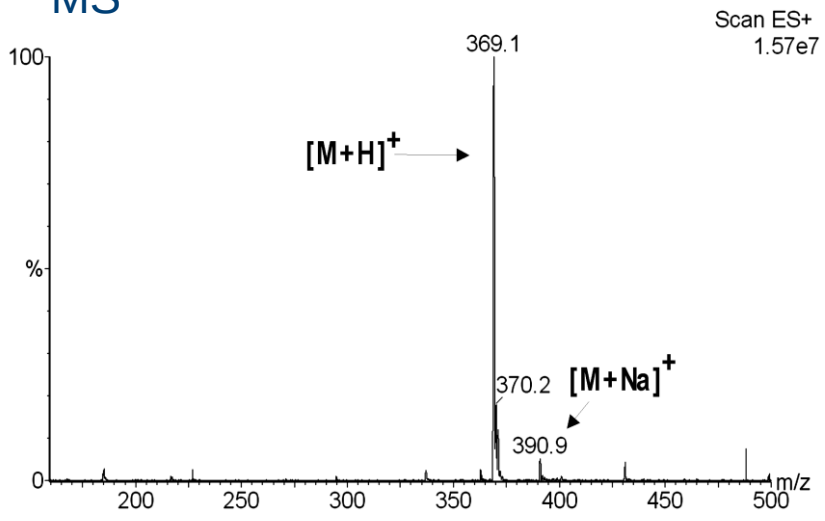
→ Up to 8 times faster

→ Selectivity proven by spectral purity

UV



MS



Effect-directed analysis → sum parameter!

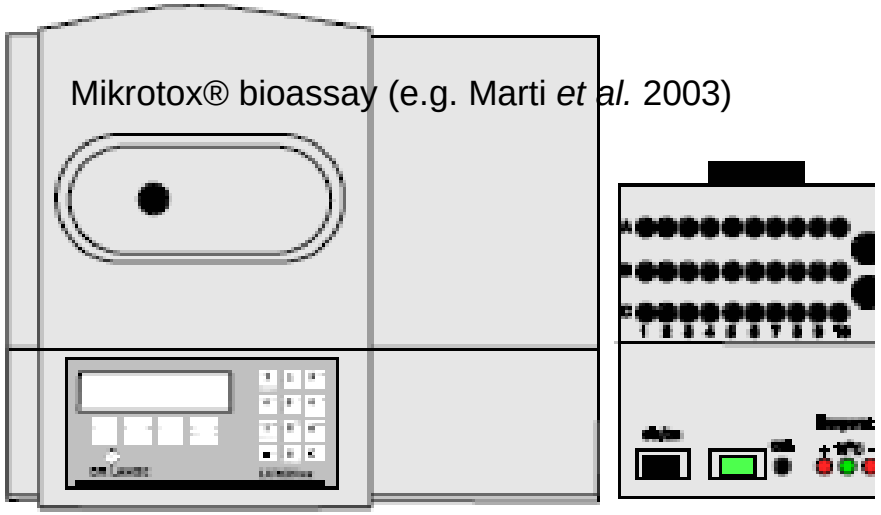
DEUTSCHE NORM Mai 2009

DIN EN ISO 11348-2 DIN

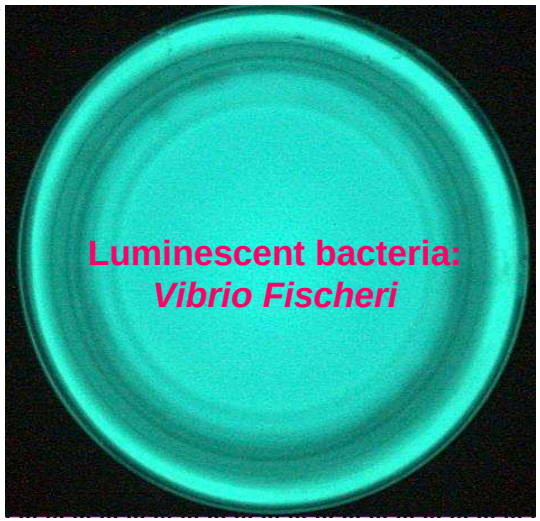
ICS 13.060.70

Ersatz für
DIN EN ISO 11348-2:1999-04

**Wasserbeschaffenheit –
Bestimmung der Hemmwirkung von Wasserproben auf die Lichtemission von *Vibrio fischeri* (Leuchtbakterientest) –
Teil 2: Verfahren mit flüssig getrockneten Bakterien (ISO 11348-2:2007);
Deutsche Fassung EN ISO 11348-2:2008**

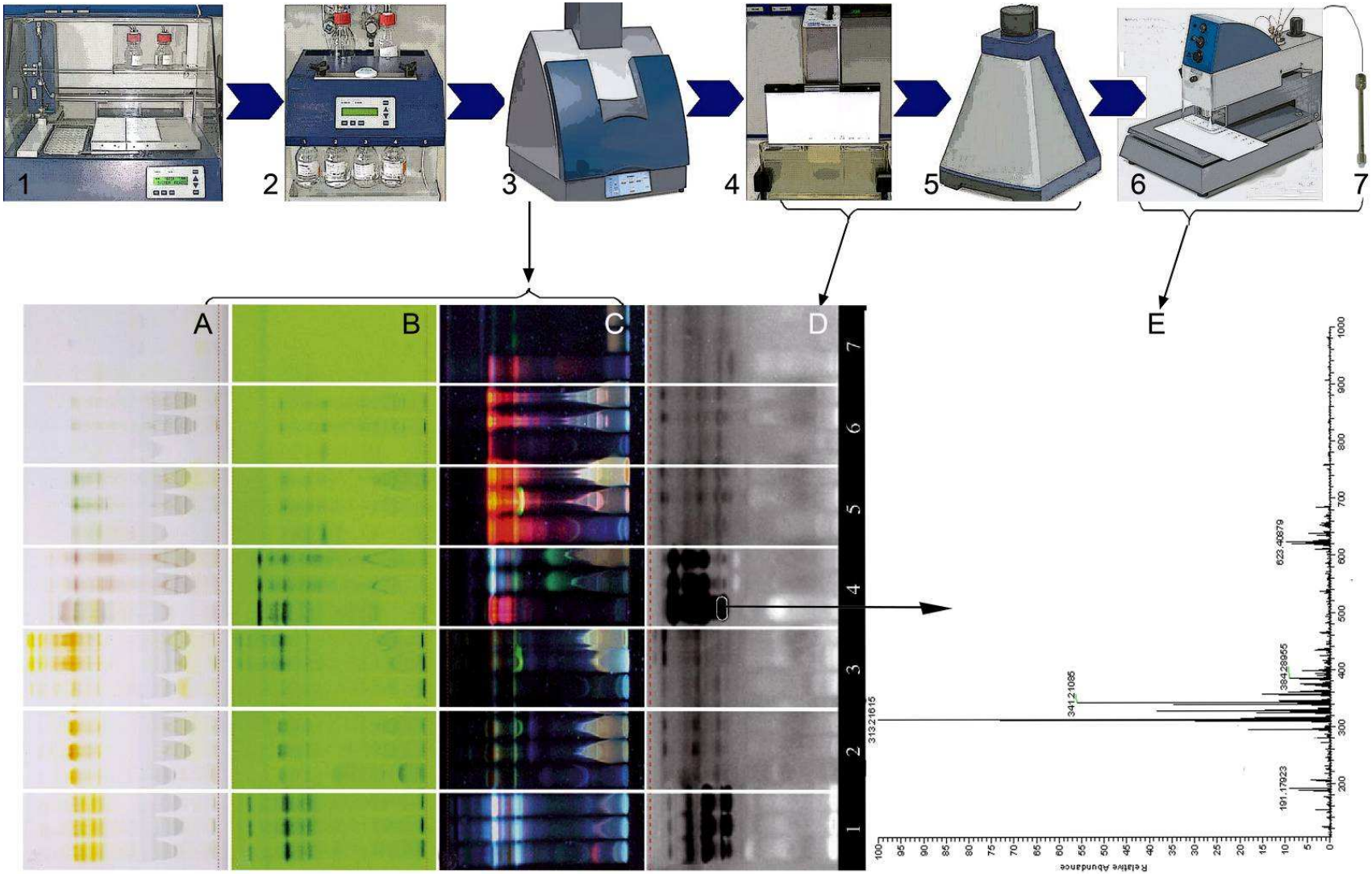


Mikrotox® bioassay (e.g. Marti *et al.* 2003)



Institute of Food Chemistry
University of Hohenheim, Stuttgart

HPTLC-VIS/UV/FLD-EDA-HRMS

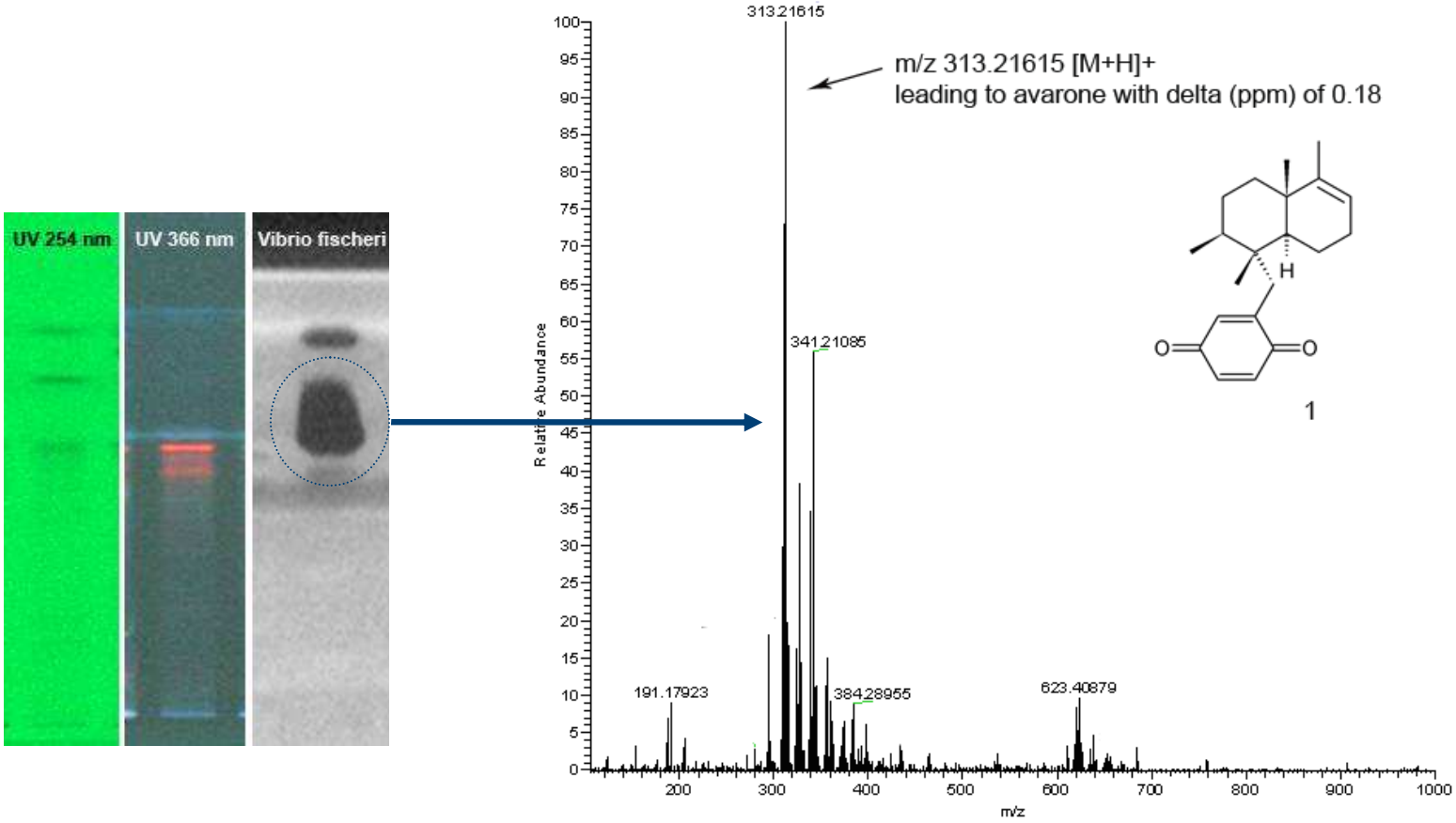


G. Morlock, W. Schwack LCGC Eur July (2008) 366-371

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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

What is it? → HRMS



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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Case study: Structure elucidation

Correct substance assignment of a dye mixture?

Dimethyl Yellow

Oracet Red G

Solvent Blue 35

Ariabel Red

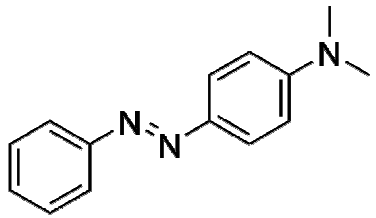
Oracet Violet 2R

Indophenol

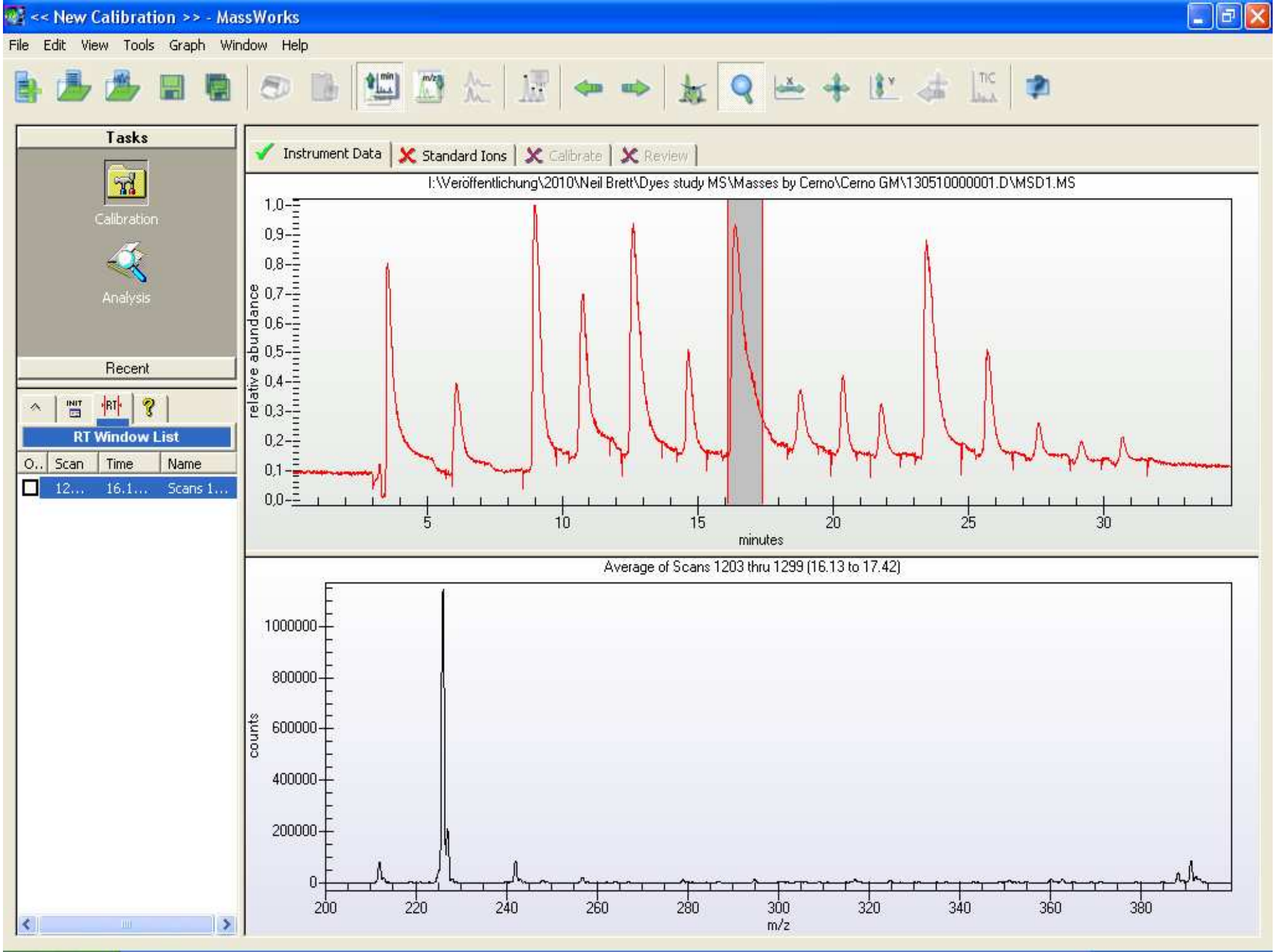
Improved mass assignment

... from a single quadrupole MS → MassWorks software (Cerno)
www.cernobioscience.com

Dye	hR_F-value	Monoisotopic mass measured	Theoretical monoisotopic mass of the proposed ion	Δ (ppm)	Spectral accuracy	Double bond equivalent	Protonated molecular formula	Assigned to
Dimethyl Yellow ✓	65	226.1402	226.1344	-25,5482	*98.5341*	*8.5*	$C_{14}H_{16}N_3$	$[M+H]^+$



Dimethyl Yellow


<< New Calibration >> - MassWorks
File Edit View Tools Graph Window Help
Tasks
Calibration
Analysis
Recent
RT Window List
O.. Scan Time Name
12... 16.1... Scans 1...
Instrument Data
Standard Ions
Calibrate
Review
I:\Veröffentlichung\2010\Neil Brett\Dyes study MS\Masses by Cerno\Cerno GM\130510000001.D\MSD1.MS
relative abundance
minutes
Average of Scans 1203 thru 1299 (16.13 to 17.42)
counts
m/z

I:\Veröffentlichung\2010\Neil Brett\Dyes study MS\Masses by Cerno\Cerno GM\Cerno Sudan Red G and Dimethyl yellow test.mwc - MassWorks

File Edit View Tools Graph Window Help

Tasks

Calibration

Analysis

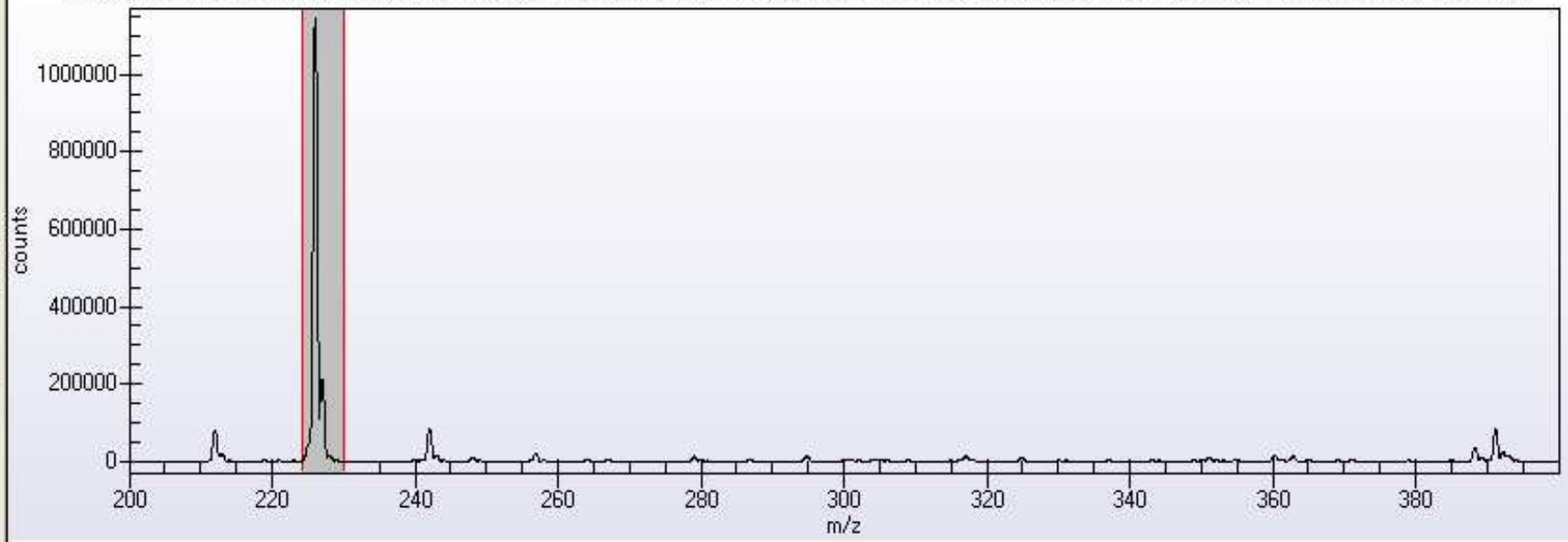
Recent

RT Window List

O.	Scan	Time	Name
☐	1...	16....	Scans...

Instrument Data | Standard Ions | Calibrate | Review

Average of Scans 1203 thru 1299 (16.13 to 17.42) - I:\Veröffentlichung\2010\Neil Brett\Dyes study MS\Masses by Cerno\Cerno GM\130510000001.D\MSD1.MS



	RT Window	Name	Formula Entry	Net Formula	Charge	Mono Isotope	Centroid	Left Edge	Right Edge
1	0: Scans 1203-1299		C14H16N3	C14H16N3	+1	226,1344	226,2976	224,134	230,134
2	0: Scans 1203-1299								

<< New Calibration >> - MassWorks

File Edit View Tools Graph Window Help

Tasks

Calibration

Analysis

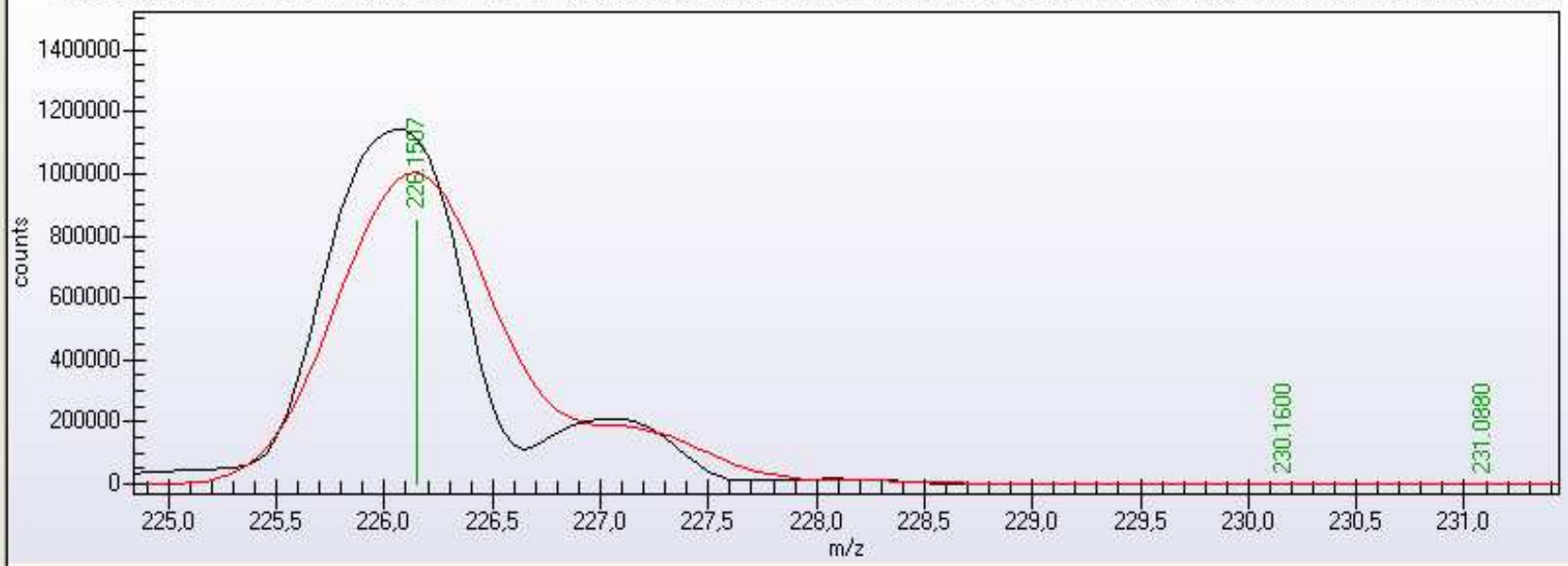
Recent

RT Window List

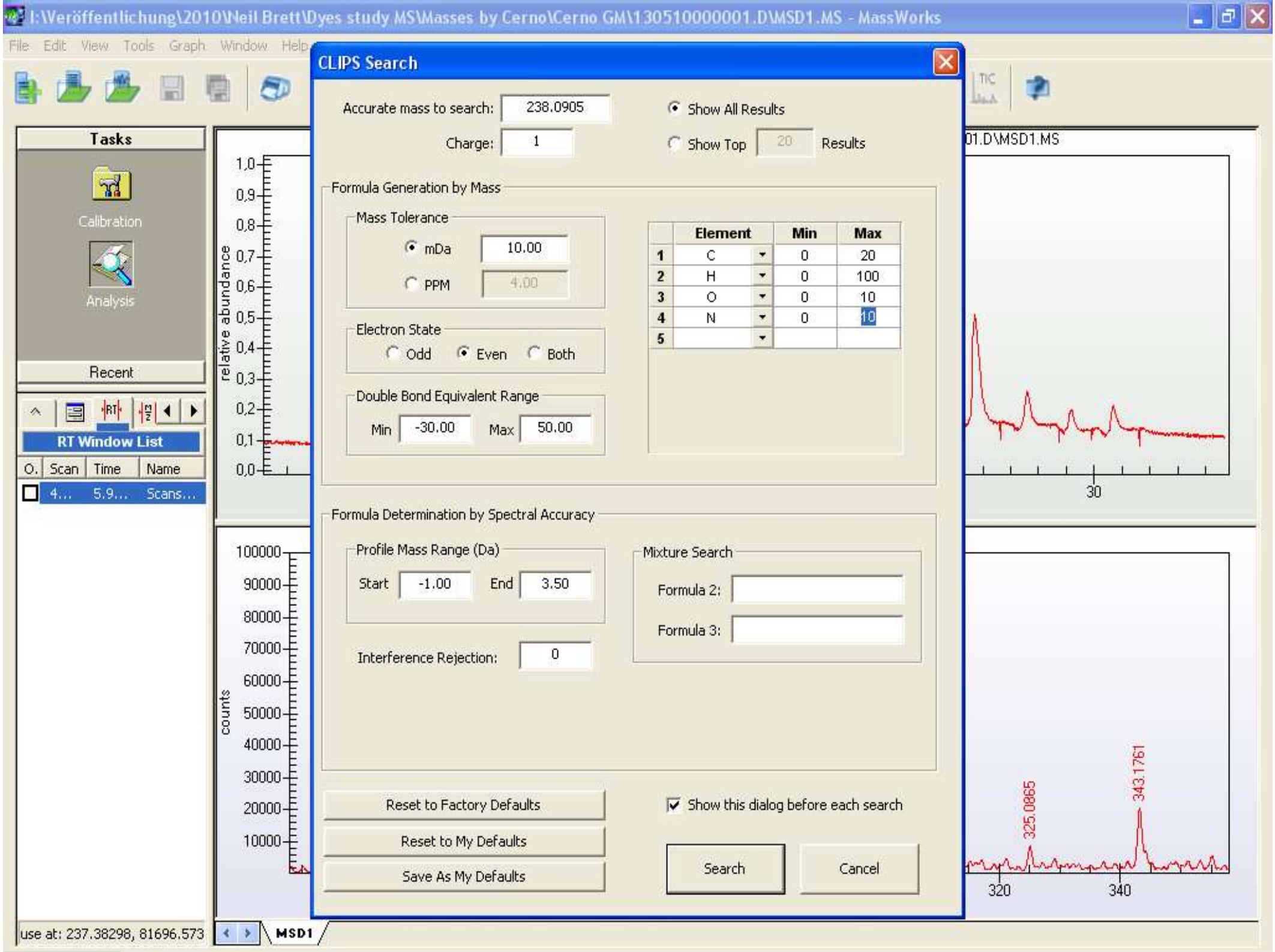
O..	Scan	Time	Name
☐	12...	16.1...	Scans 1...

Instrument Data | Standard Ions | Calibrate | Review

Average of Scans 1203 thru 1299 (16.13 to 17.42) - I:\Veröffentlichung\2010\Neil Brett\Dyes study MS\Masses by Cerno\Cerno GM\130510000001.D\MSD1.MS



	RT Window	Name	Net Formula	Mono Mass	Closest Centroid	ΔM (mDa)	ΔM (ppm)	Spectral Accuracy	RMSE
1	0: Scans 1203-1299		C14H16N3	226,1344	226,1507	16,3500	72,3	99,1	3.107,36


I:\Veröffentlichung\2010\Neil Brett\Dyes study MS\Masses by Cerno\Cerno GM\130510000001.D\MSD1.MS - MassWorks
File Edit View Tools Graph Window Help
Tasks
Calibration
Analysis
Recent
RT Window List
O. Scan Time Name
4... 5.9... Scans...
use at: 237.38298, 81696.573
MSD1
CLIPS Search
Accurate mass to search: 238.0905
Charge: 1
Show All Results
Show Top 20 Results
Formula Generation by Mass
Mass Tolerance
mDa 10.00
PPM 4.00
Electron State
Odd Even Both
Double Bond Equivalent Range
Min -30.00 Max 50.00
Element Min Max
1 C 0 20
2 H 0 100
3 O 0 10
4 N 0 10
5
Formula Determination by Spectral Accuracy
Profile Mass Range (Da)
Start -1.00 End 3.50
Interference Rejection: 0
Mixture Search
Formula 2:
Formula 3:
Reset to Factory Defaults
Reset to My Defaults
Save As My Defaults
Show this dialog before each search
Search
Cancel
relative abundance
counts
01.D\MSD1.MS
325.0865
343.1761

I:\Veröffentlichung\2010\Neil Brett\Dyes study MS\Masses by Cerno\Cerno GM\130510000001.D\MSD1.MS - MassWorks

File Edit View Tools Graph Window Help

Tasks

Calibration

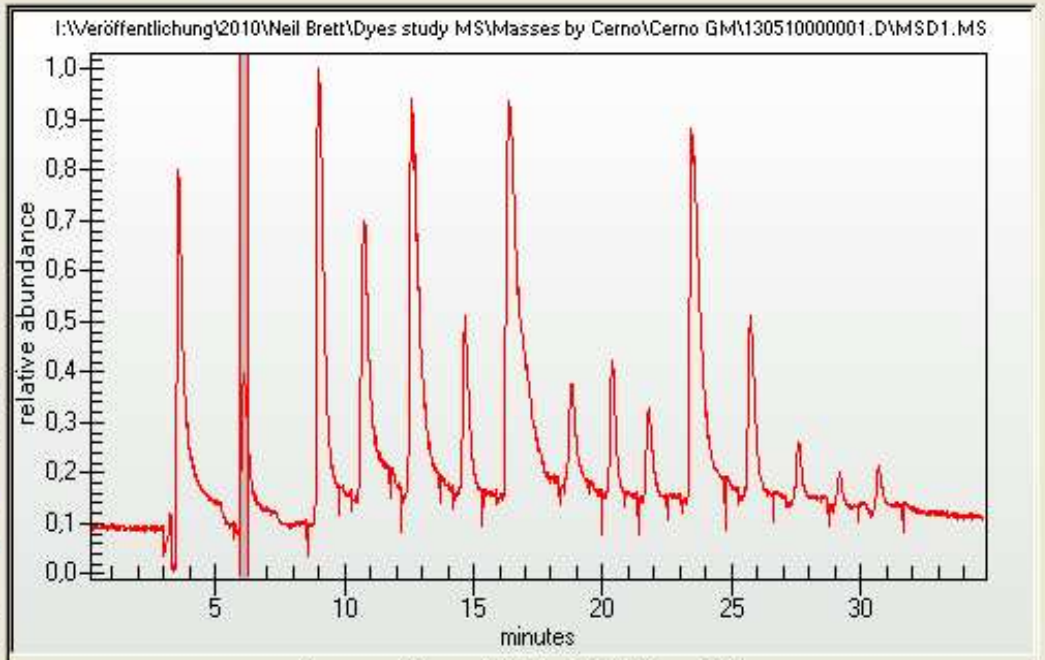
Analysis

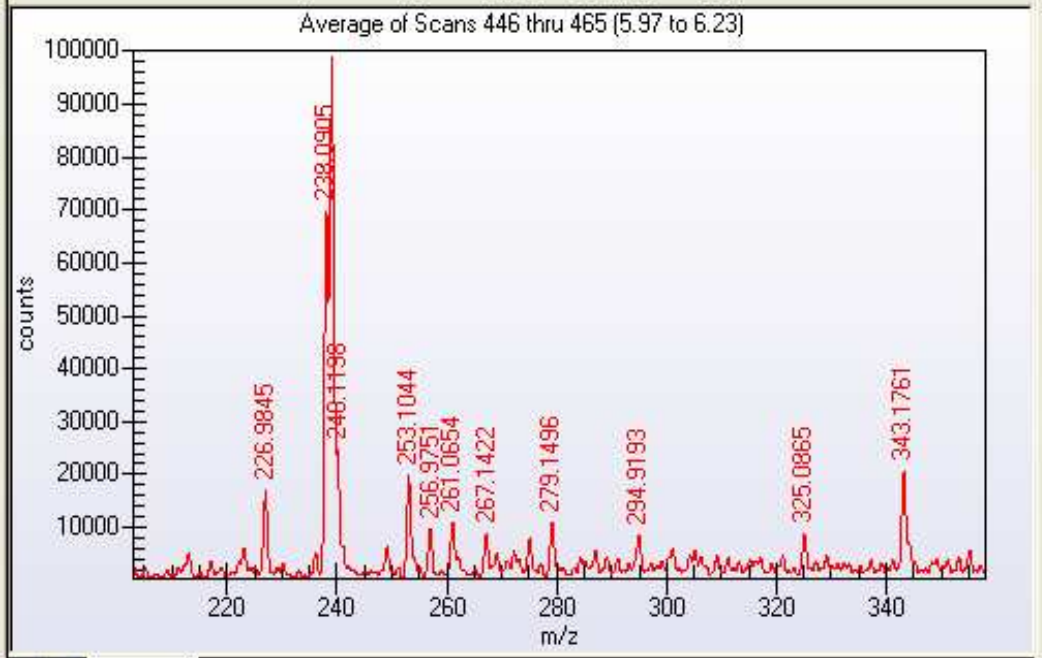
Recent

CLIPS Results

	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C15H12O2N	238,0868	-3,6964	-15,5250	45,5841	25.459	10,5
2	C14H12ON3	238,0980	7,5370	31,6561	45,7740	25.370	10,5
3	C10H12O4N3	238,0828	-7,7191	-32,4209	43,8702	26.261	6,5
4	C9H12O3N5	238,0940	3,5143	14,7603	44,1377	26.136	6,5
5	C8H16O7N	238,0927	2,1769	9,1431	43,3133	26.521	1,5
6	C3H16O9N3	238,0887	-1,8459	-7,7528	41,6101	27.318	-2,5
7	C2H16O8N5	238,0999	9,3875	39,4284	41,7678	27.245	-2,5
8	CH20O12N	238,0985	8,0501	33,8111	41,0559	27.578	-7,5

Mouse at: 180.92042, 71947.77524



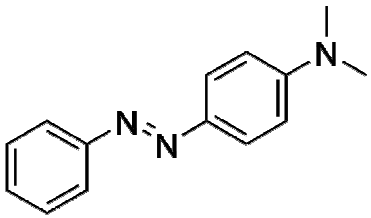


MSD1

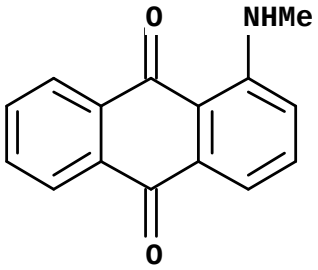
Improved mass assignment

... from a single quadrupole MS → MassWorks software (Cerno)
www.cernobioscience.com

Dye	hR_F-value	Monoisotopic mass measured	Theoretical monoisotopic mass of the proposed ion	Δ (ppm)	Spectral accuracy	Double bond equivalent	Protonated molecular formula	Assigned to
Dimethyl Yellow ✓	65	226.1402	226.1344	-25,5482	*98.5341*	*8.5*	$C_{14}H_{16}N_3$	$[M+H]^+$
Oracet Red G ✓	50	238.0893	238.0868	*-10.4850*	91.1892	*10.5*	$C_{15}H_{12}NO_2$	$[M+H]^+$



Dimethyl Yellow

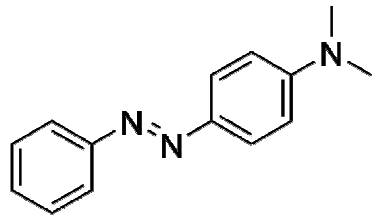


Oracet Red G

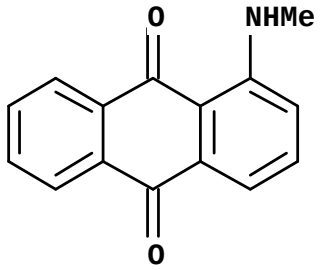
Improved mass assignment

... from a single quadrupole MS → MassWorks software (Cerno)
www.cernobioscience.com

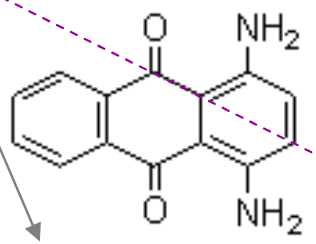
Dye	hR_F-value	Monoisotopic mass measured	Theoretical monoisotopic mass of the proposed ion	Δ (ppm)	Spectral accuracy	Double bond equivalent	Protonated molecular formula	Assigned to
Dimethyl Yellow ✓	65	226.1402	226.1344	-25,5482	*98.5341*	*8.5*	$C_{14}H_{16}N_3$	$[M+H]^+$
Oracet Red G ✓	50	238.0893	238.0868	*-10.4850*	91.1892	*10.5*	$C_{15}H_{12}NO_2$	$[M+H]^+$
Oracet Violet 2R ?	17	305.1610	305.1654	14.3803	98.*0042*	*11.5*	$C_{20}H_{21}N_2O$	$[M+H]^+$
Indophenol ?	4	239.0839	239.0821	*7.7270*	*85.4156*	*10.5*	$C_{14}H_{11}N_2O_2$	$[M+H]^+$



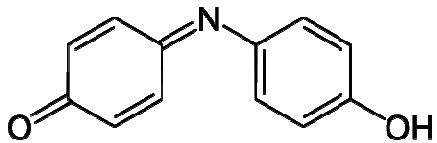
Dimethyl Yellow



Oracet Red G



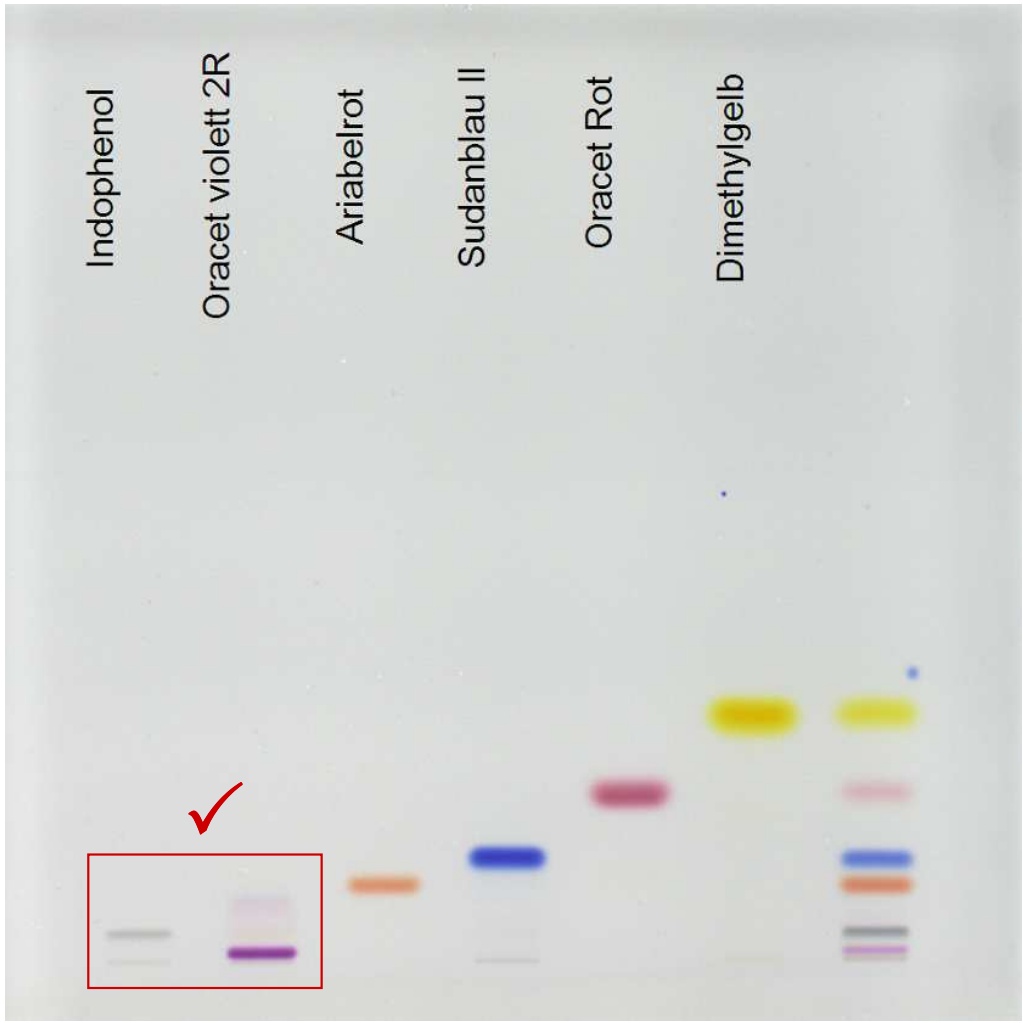
Oracet Violet 2R
238 Da



Indophenol
199 Da

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Request to supplier for single compounds



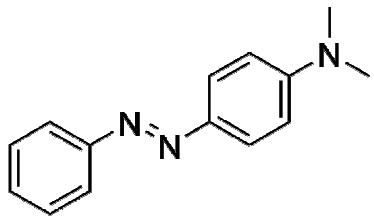
→ The elution order was wrongly assigned.

Improved mass assignment

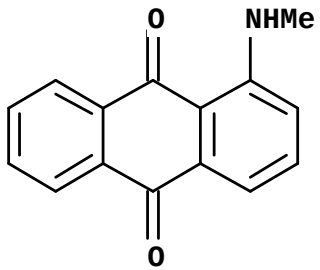
... from a single quadrupole MS → MassWorks software (Cerno)

Dye	hR_F-value	Monoisotopic mass measured	Theoretical monoisotopic mass of the proposed ion	Δ (ppm)	Spectral accuracy	Double bond equivalent	Protonated molecular formula	Assigned to
Dimethyl Yellow ✓	65	226.1402	226.1344	-25,5482	*98.5341*	*8.5*	$C_{14}H_{16}N_3$	$[M+H]^+$
Oracet Red G ✓	50	238.0893	238.0868	*-10.4850*	91.1892	*10.5*	$C_{15}H_{12}NO_2$	$[M+H]^+$
Indophenol ?	17	305.1610	305.1654	14.3803	98.*0042*	*11.5*	$C_{20}H_{21}N_2O$	$[M+H]^+$
Oracet Violet 2R ✓	4	239.0839	239.0821	*7.7270*	*85.4156*	*10.5*	$C_{14}H_{11}N_2O_2$	$[M+H]^+$

≠

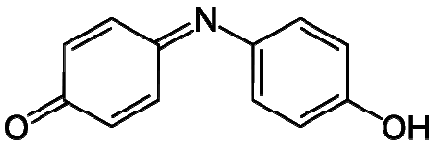


Dimethyl Yellow

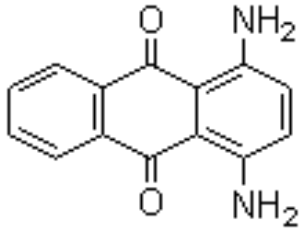


Oracet Red G

?



Indophenol
199 Da

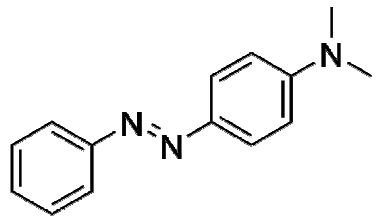


Oracet Violet 2R

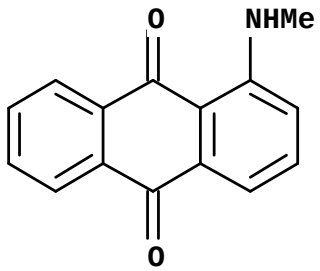
Improved mass assignment

... from a single quadrupole MS → MassWorks software (Cerno)

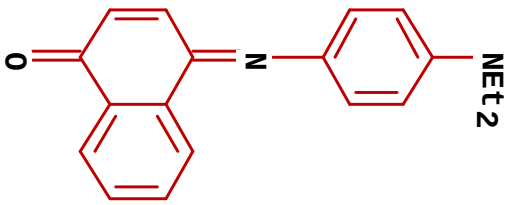
Dye	hR_F-value	Monoisotopic mass measured	Theoretical monoisotopic mass of the proposed ion	Δ (ppm)	Spectral accuracy	Double bond equivalent	Protonated molecular formula	Assigned to
Dimethyl Yellow ✓	65	226.1402	226.1344	-25,5482	*98.5341*	*8.5*	$C_{14}H_{16}N_3$	$[M+H]^+$
Oracet Red G ✓	50	238.0893	238.0868	*-10.4850*	91.1892	*10.5*	$C_{15}H_{12}NO_2$	$[M+H]^+$
Solvent Blue 22 ✓	17	305.1610	305.1654	14.3803	98.*0042*	*11.5*	$C_{20}H_{21}N_2O$	$[M+H]^+$
Oracet Violet 2R ✓	4	239.0839	239.0821	*7.7270*	*85.4156*	*10.5*	$C_{14}H_{11}N_2O_2$	$[M+H]^+$



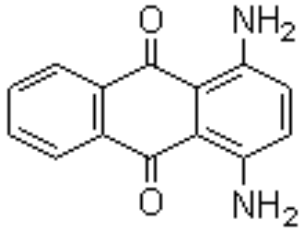
Dimethyl Yellow



Oracet Red G



Chinoneanil



Oracet Violet 2R

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Case study: Structure elucidation

Correct substance assignment of a dye mixture?

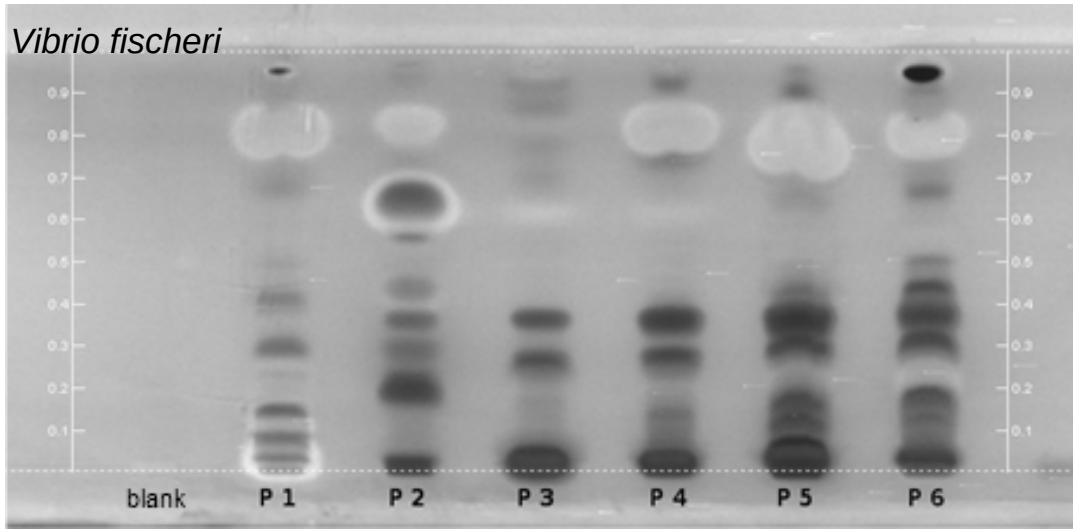
Just partly:

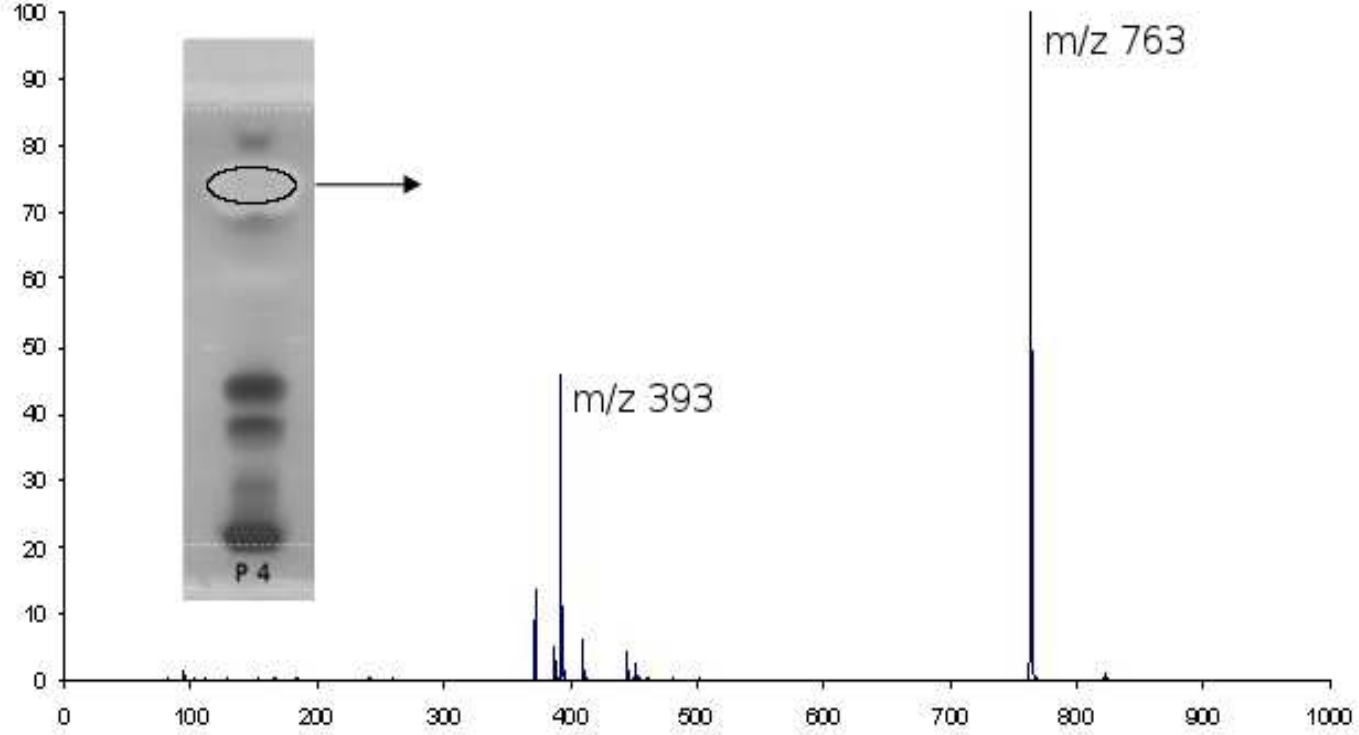
1. Ariabel red is Sudan red G.
2. The elution order was incorrectly assigned.
3. One compound was not the one intended to be: The quinoneanil *Solvent Blue 22* was incorrectly labeled as *indophenol*.
4. Two manufacturers with incorrect label.

G. Morlock, W. Schwack, N. Brett, in preparation

Institute of Food Chemistry
University of Hohenheim, Stuttgart

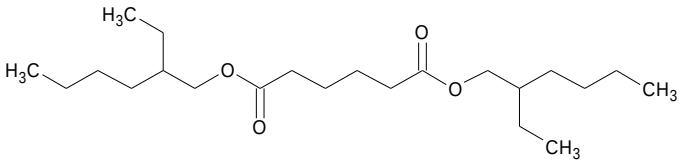
Additives in food packaging foils





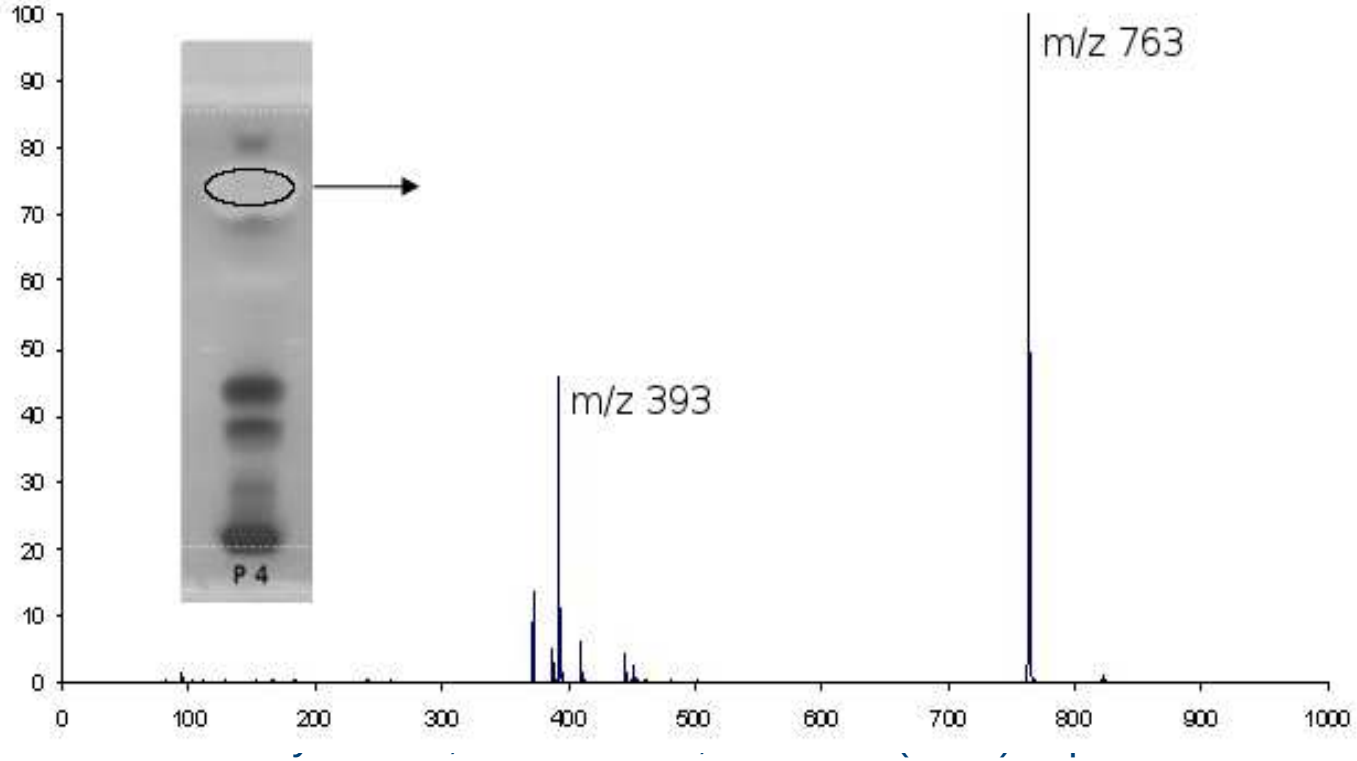
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Detection of additives in polymer packaging foils



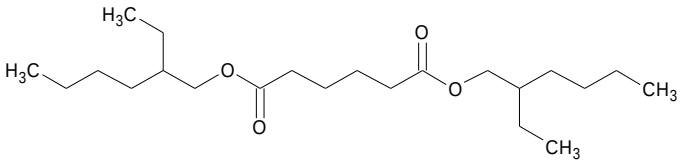
Bis-2-ethylhexyladipate

MS signals of	Mass determined	Mass theoretical	Δ (ppm)	Sum formula	Assignment
HPTLC zone	393,2985	393,2981	-1,0691	$C_{22}H_{42}O_4Na$	$[M+Na]^+$
	763,6077	763,6064	-1,7164	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$



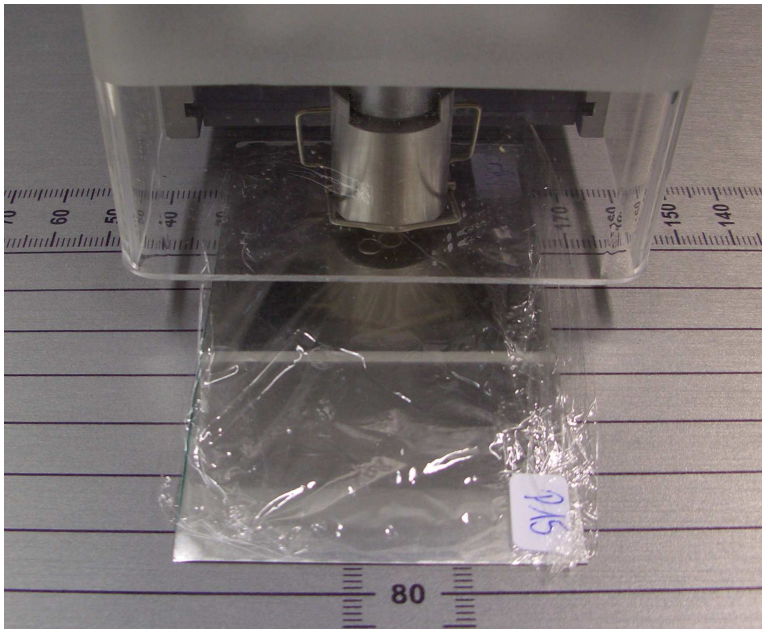
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Detection of additives in polymer packaging foils



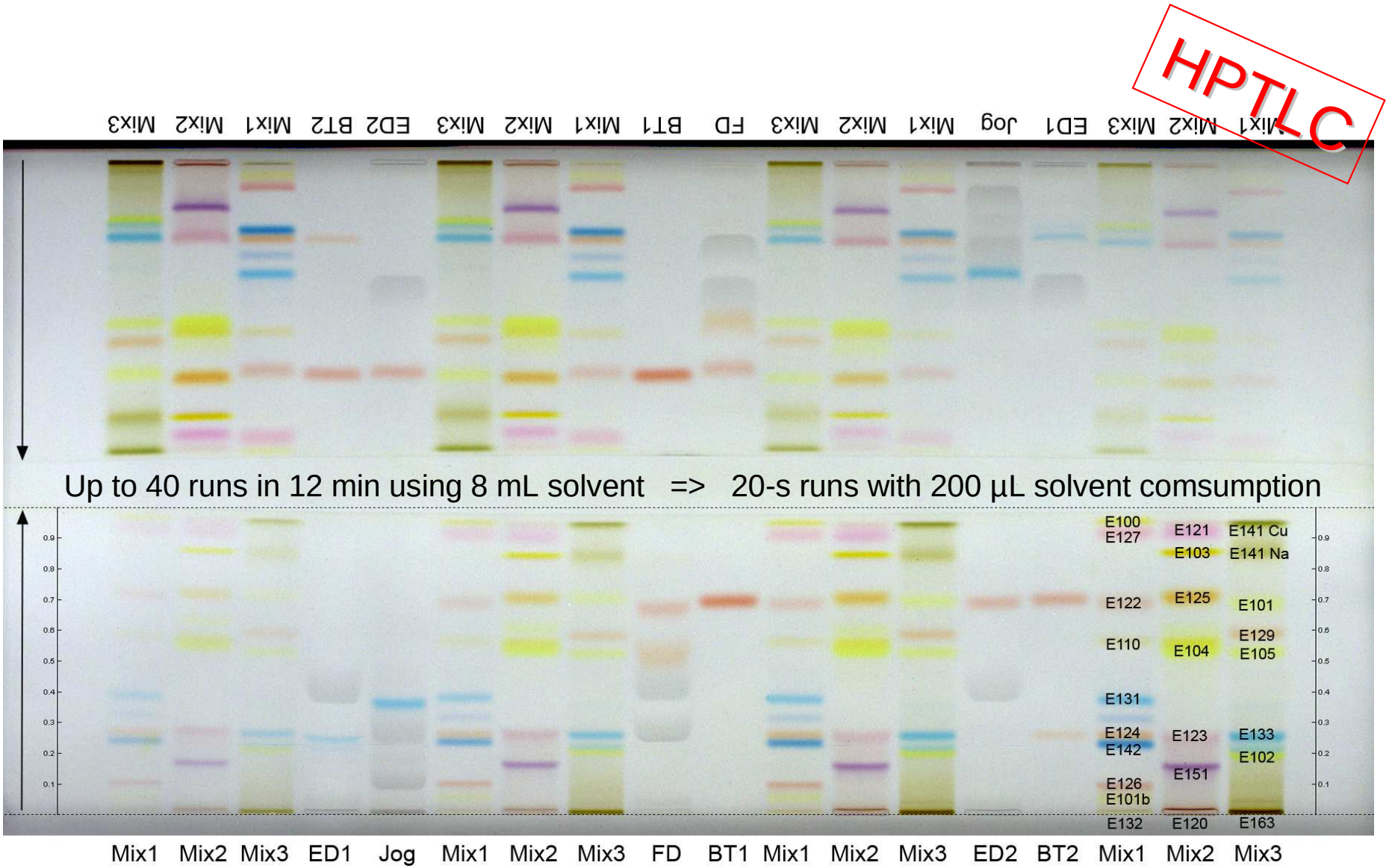
Bis-2-ethylhexyladipate

MS signal of	Mass determined	Mass theoretical	Δ (ppm)	Sum formula	Assignment
Plastic foil	**371,3174**	**371,3161**	**-3,4071**	**$C_{22}H_{43}O_4$**	**$[M+H]^+$**
HPTLC zone	393,2985	393,2981	-1,0691	$C_{22}H_{42}O_4Na$	$[M+Na]^+$
	763,6077	763,6064	-1,7164	$C_{44}H_{84}O_8Na$	$[2M+Na]^+$



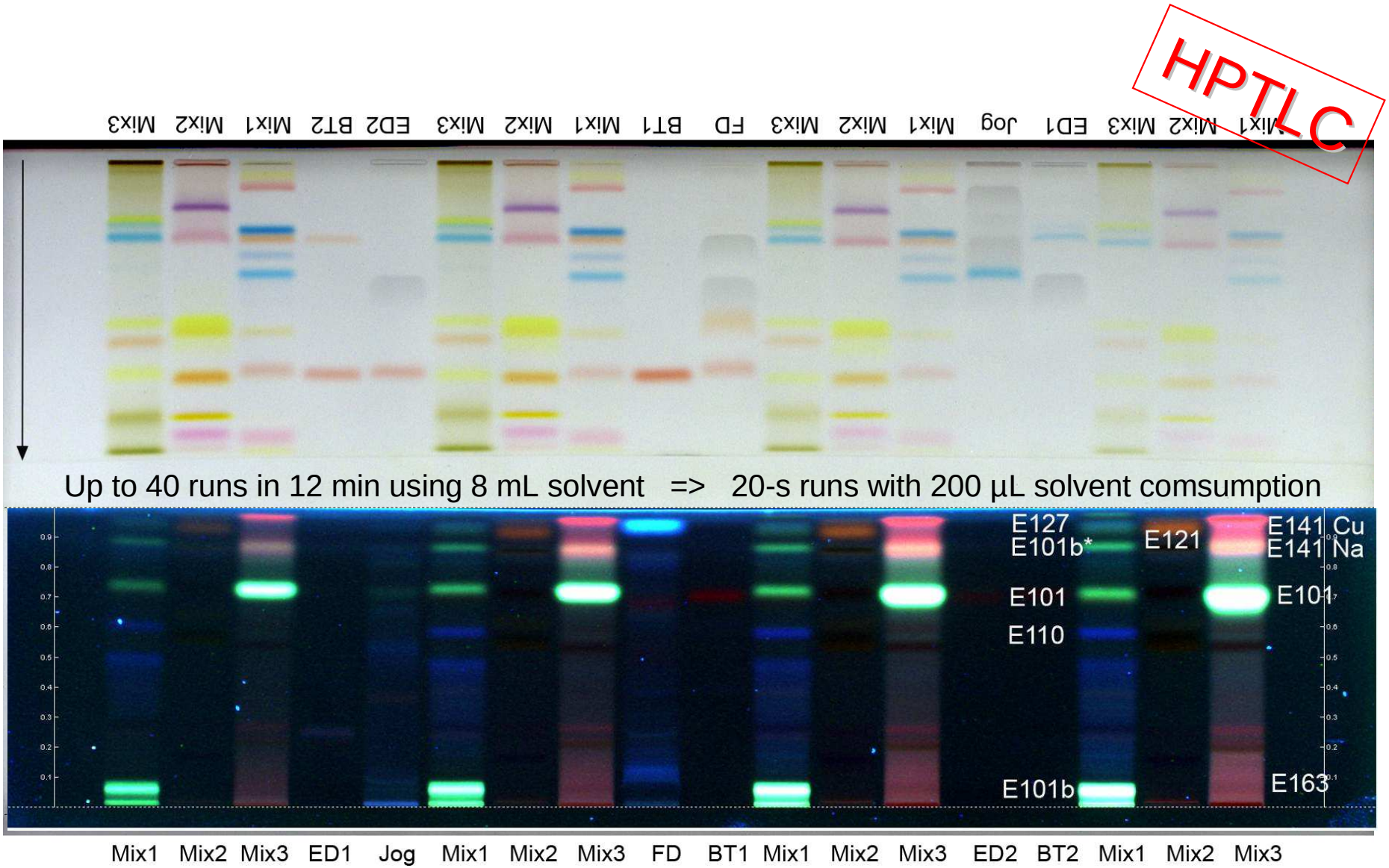
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Dye analysis



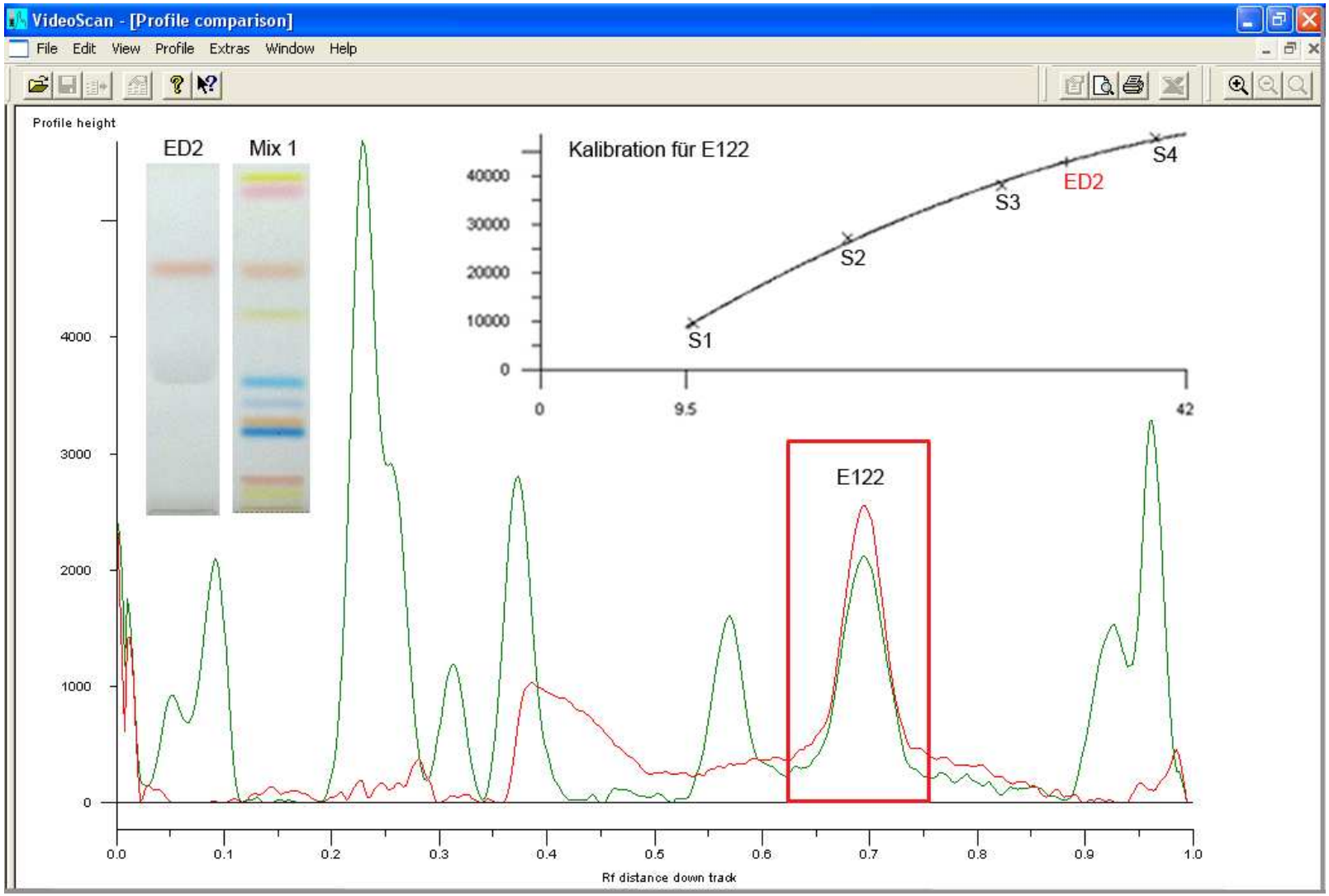
G. Morlock, C. Oellig, J AOAC Int 92 (2009) 547-554

Dye analysis



Institute of Food Chemistry
University of Hohenheim, Stuttgart

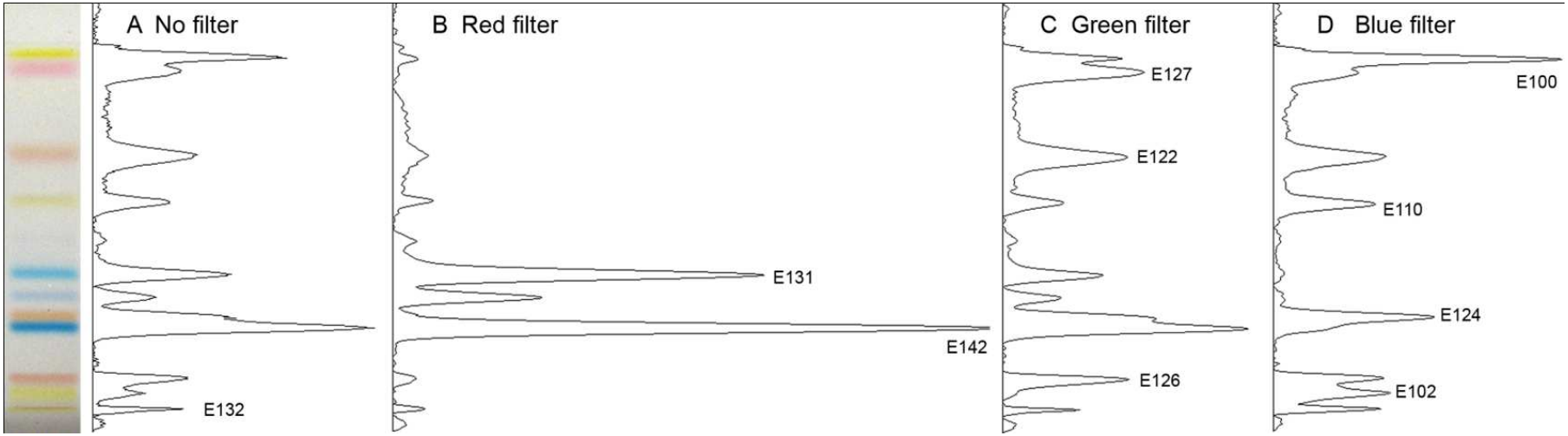
Digital quantification



Institute of Food Chemistry
University of Hohenheim, Stuttgart

Institute of Food Chemistry
University of Hohenheim, Stuttgart

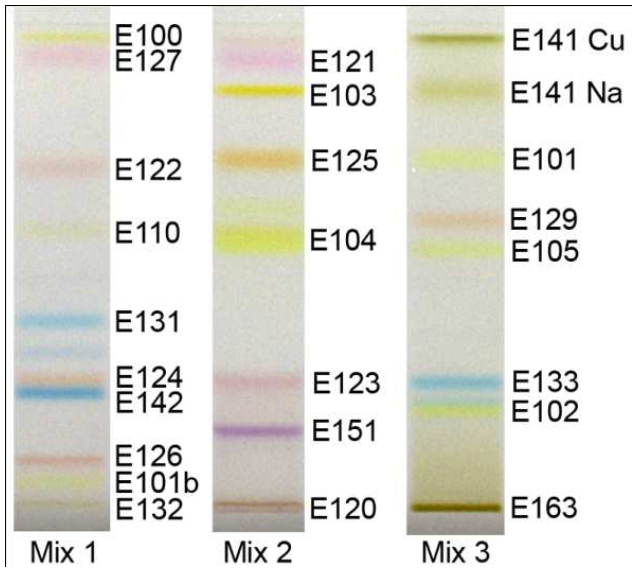
Digital filters

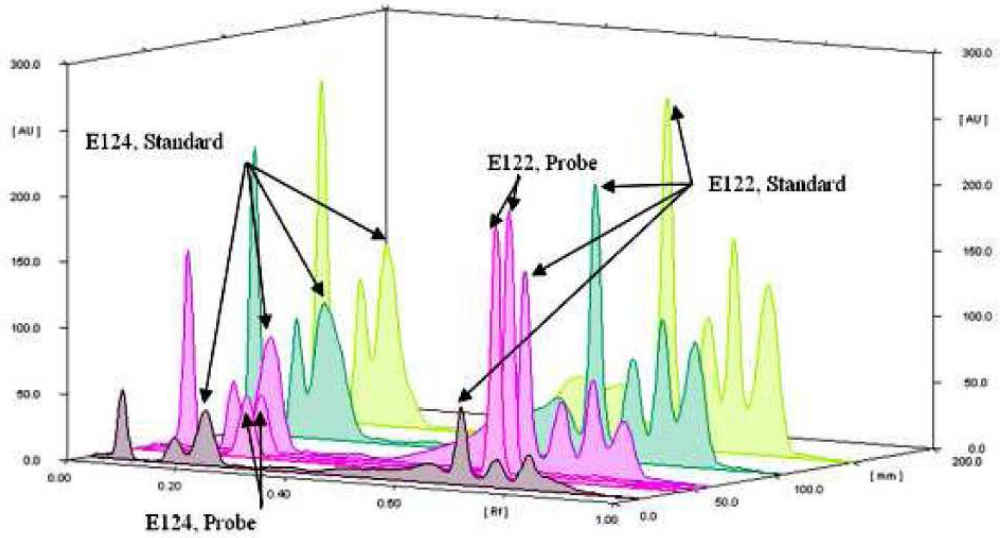


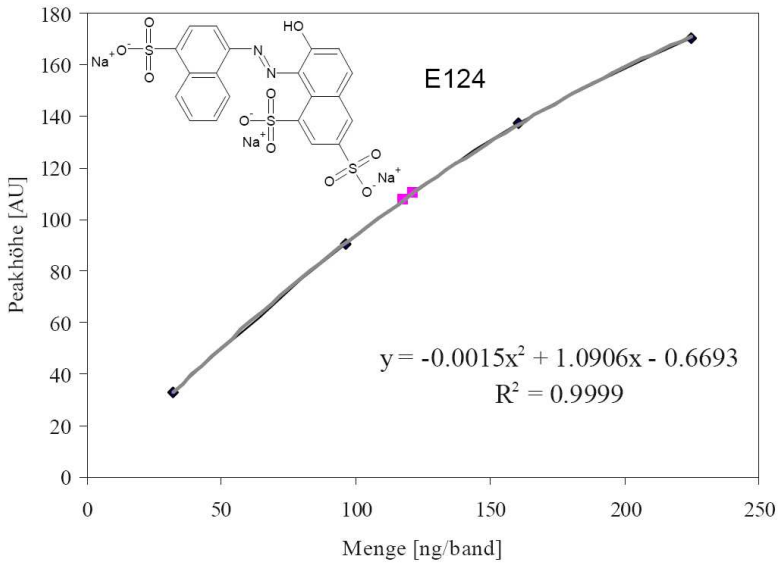
G. Morlock, W. Schwack, Die Aktuelle Wochenschau der GDCh,
Woche 26 (2009), www.aktuelle-wochenschau.de/2009/index09.htm

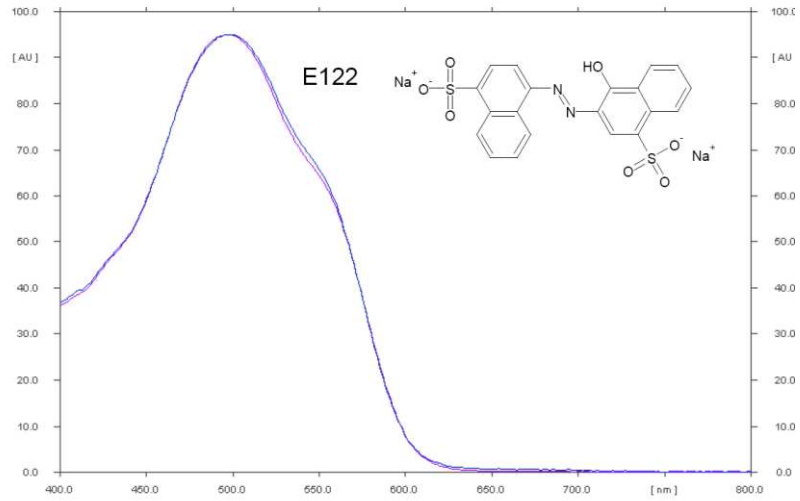
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Dye analysis



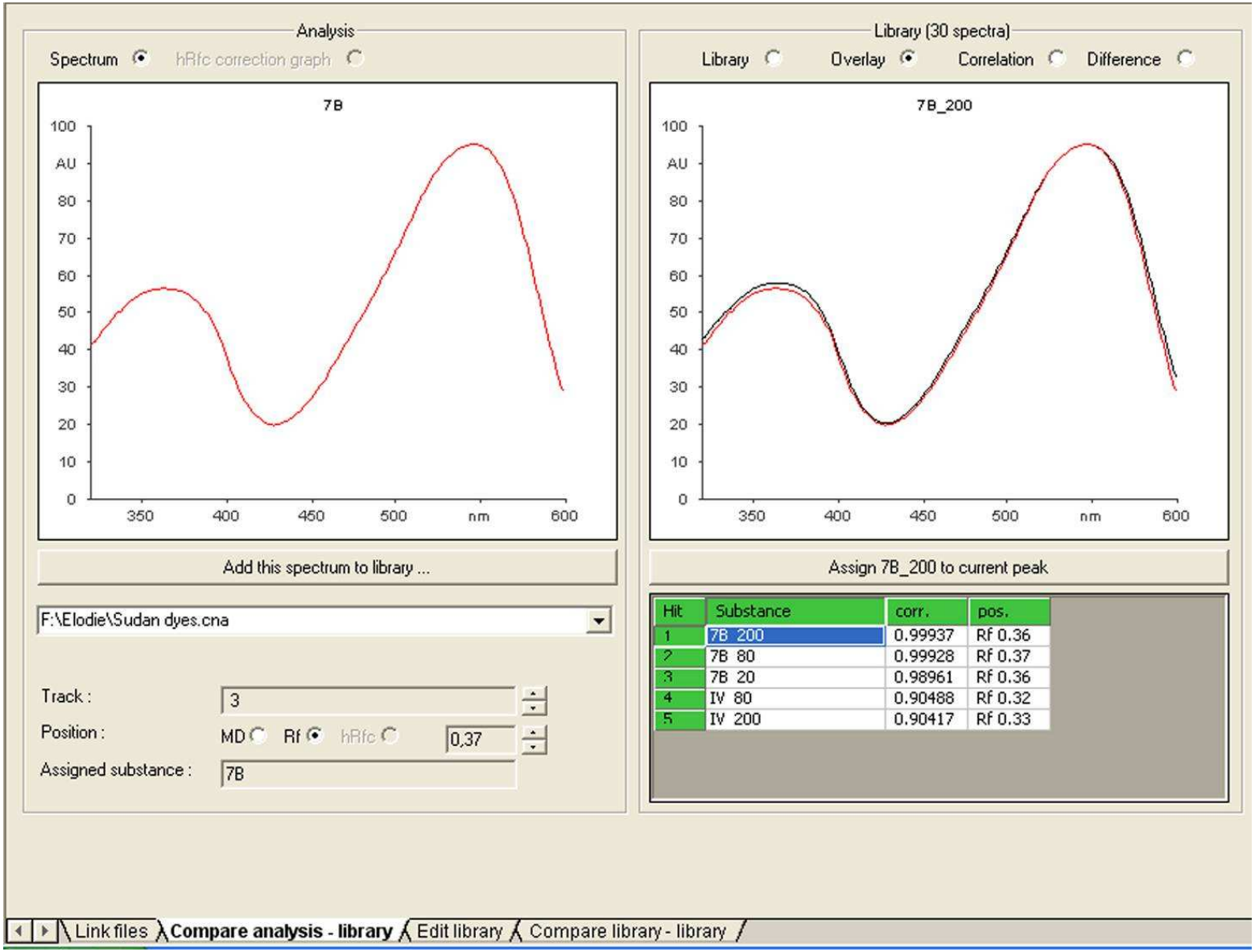






Institute of Food Chemistry
University of Hohenheim, Stuttgart

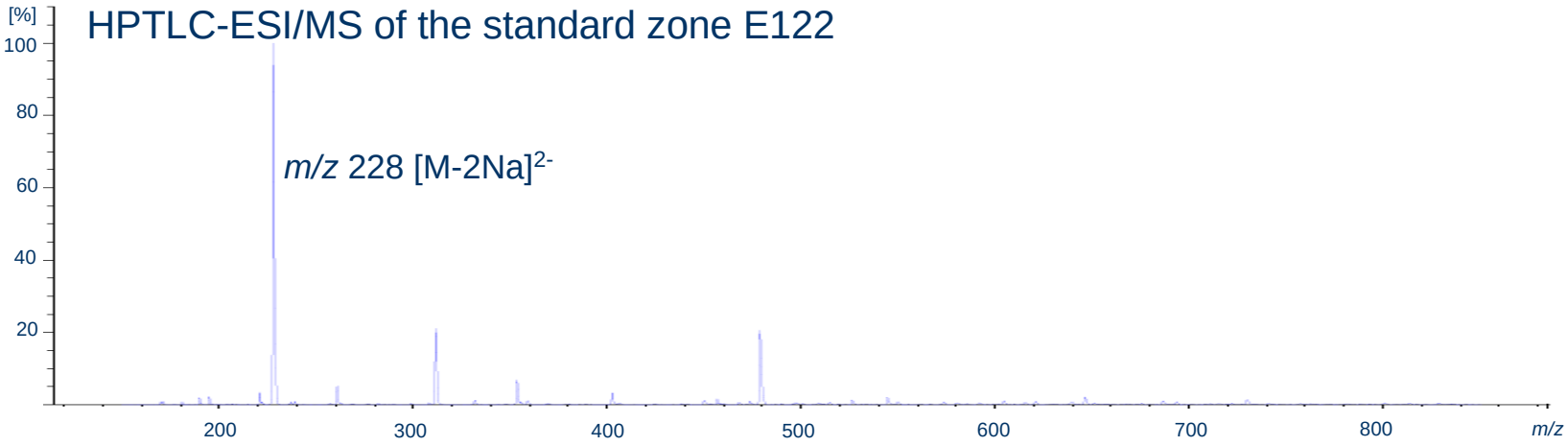
Search in spectra library



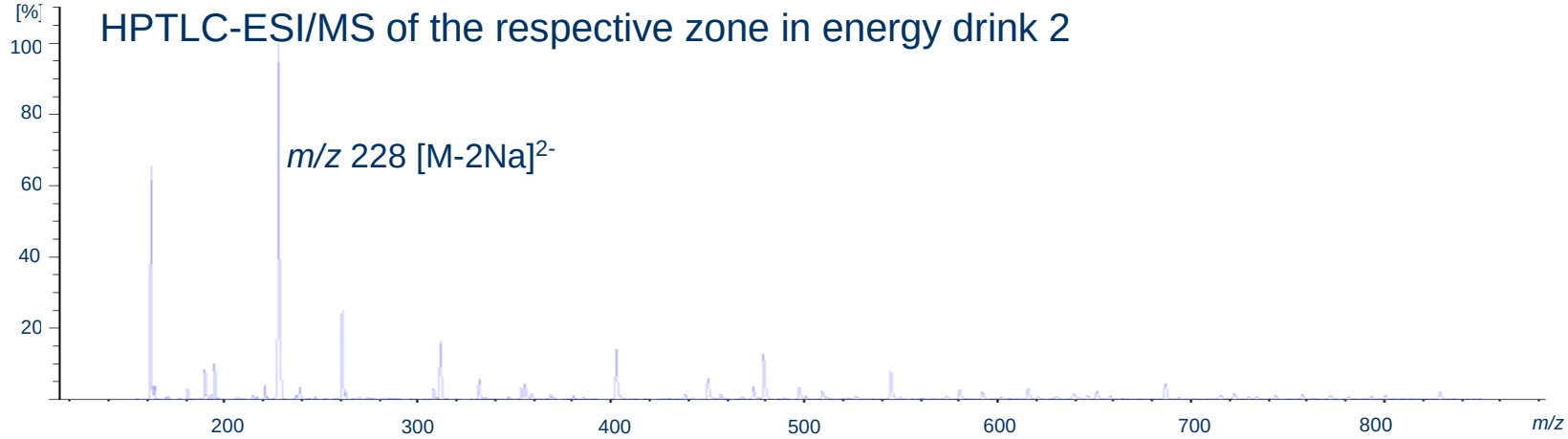
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Confirmation by mass spectra

HPTLC-ESI/MS of the standard zone E122



HPTLC-ESI/MS of the respective zone in energy drink 2



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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Rare examples for HPTLC

Why stop here?

Sample	Dyes found
Bakery ink formulation	122 124
Energy drink 1	133
Energy drink 2	122

Rare examples for HPTLC

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Information obtained from a single plate

Sample	Dyes found	Concentration calculated	*%RSD* (n = 2)	Identity: Spectra correlation (400–800 nm) of standard and sample	Identity: Mass signal(s) (full scan, *m/z* 100 – 900)
Bakery ink formulation	122	66.4 g/L	0.0	≥ 0.99996	228 $[M-2Na]^{2-}$
	124	13.3 g/L	2.1	≥ 0.99957	279 $[M-2Na]^{2-}$
					178 $[M-3Na]^{3-}$
Energy drink 1	133	9.1 mg/L	0.1	≥ 0.99964	373 $[M-2Na]^{2-}$
Energy drink 2	122	76.2 mg/L	3.6	≥ 0.99958	228 $[M-2Na]^{2-}$

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Cost comparison

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

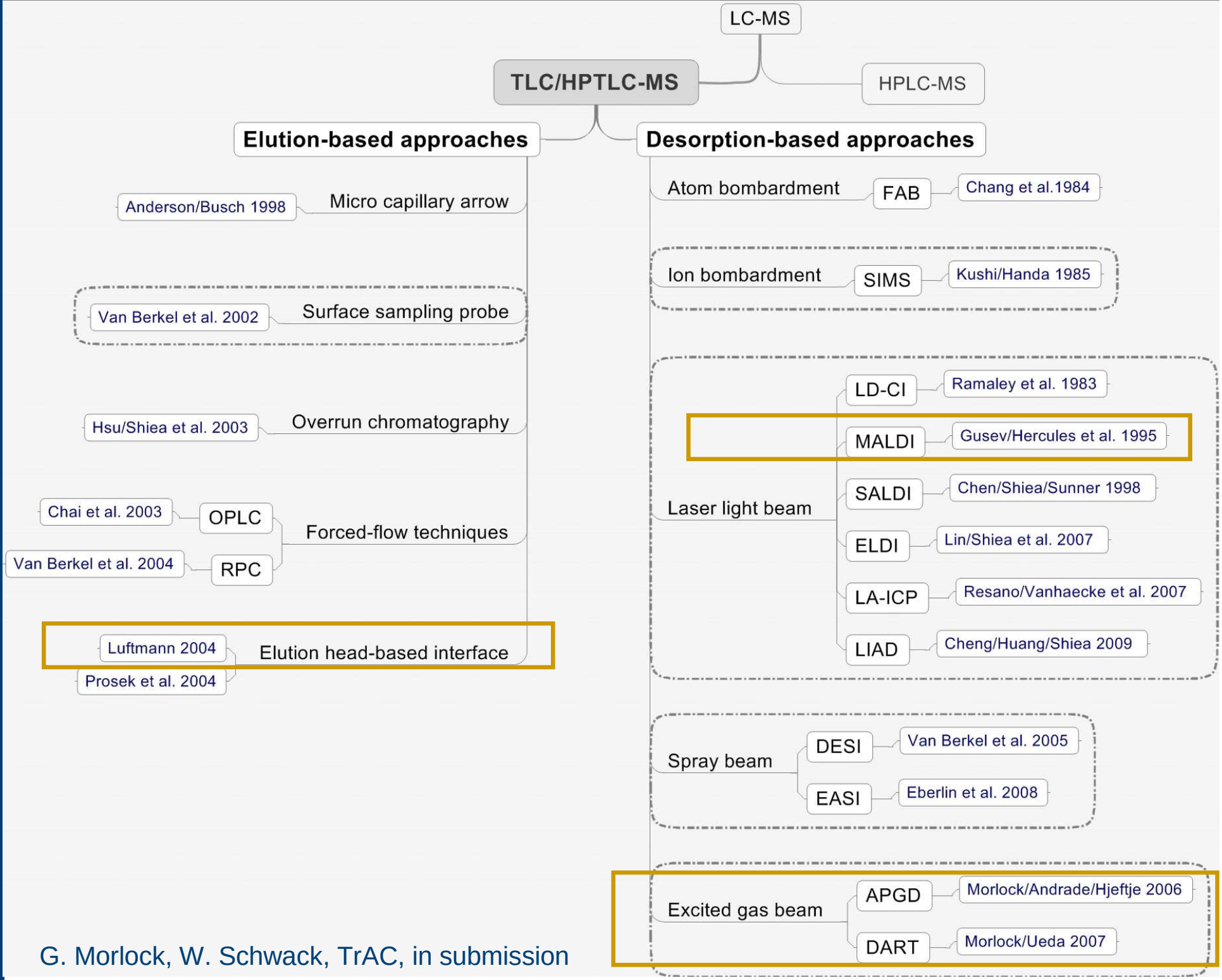
Operating costs/run (€)	**HPLC[1]**	**HPTLC[2]**
Mobile phase	0,58	0,003
Stationary phase	0,64	0,11
Disposal	0,04	0,0001
Sum	**1,26**	**0,11**

=> 11 x lower

Time/run (min)	**HPLC**	**HPTLC**
Application/Injection		0,50
Run time	43	0,20
Detection		0,10
Sum	**43**	**0,80**

=> 54 x faster

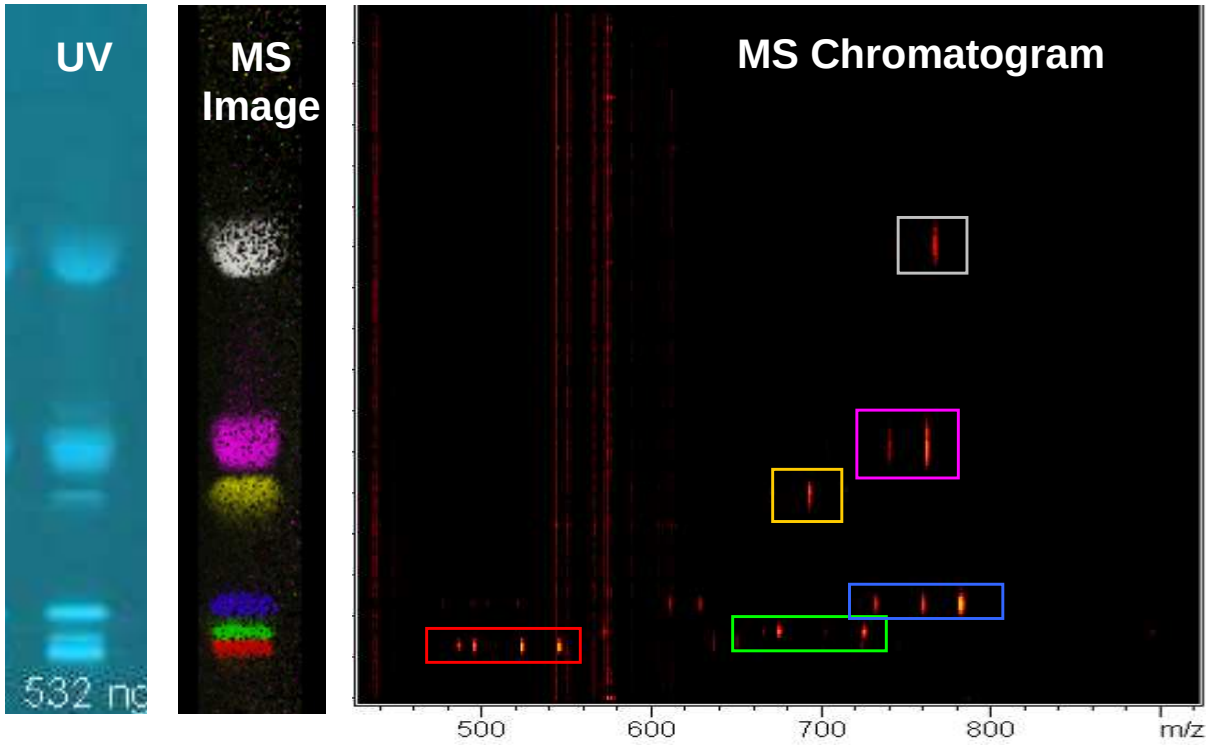
thereof labor time/40 runs	none	5 min



G. Morlock, W. Schwack, TrAC, in submission

Institute of Food Chemistry
University of Hohenheim, Stuttgart

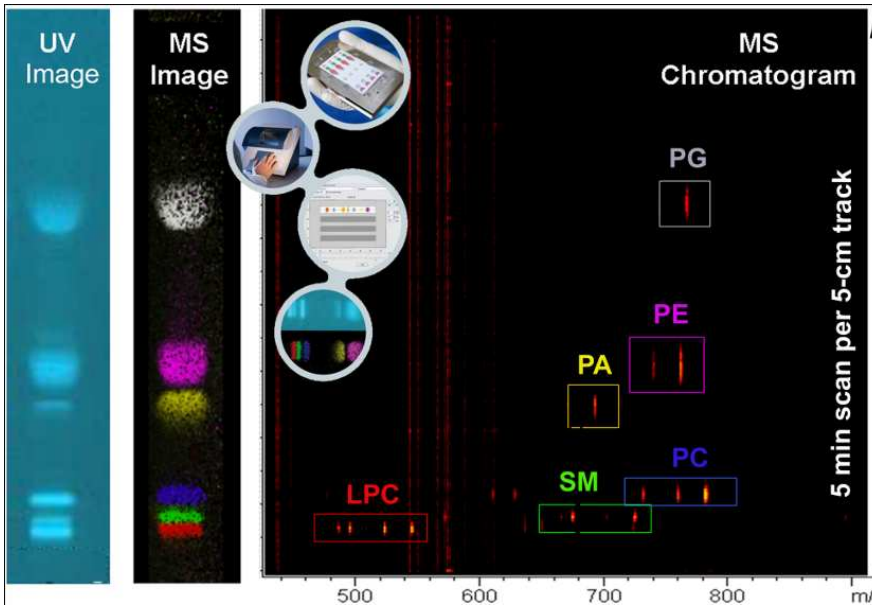
HPTLC-FLD-MALDI-TOF MS

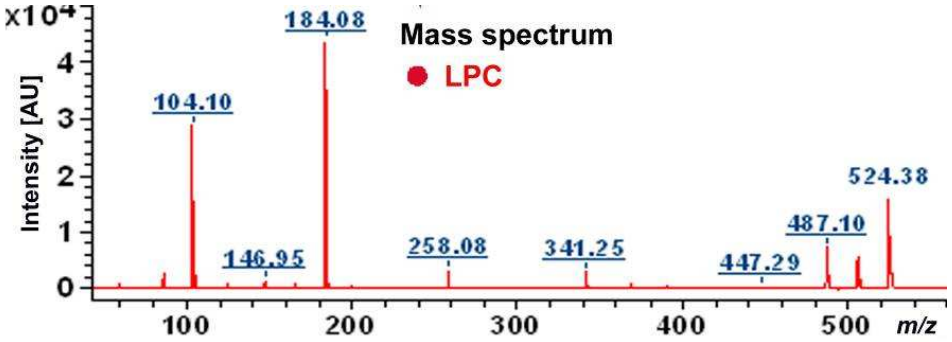


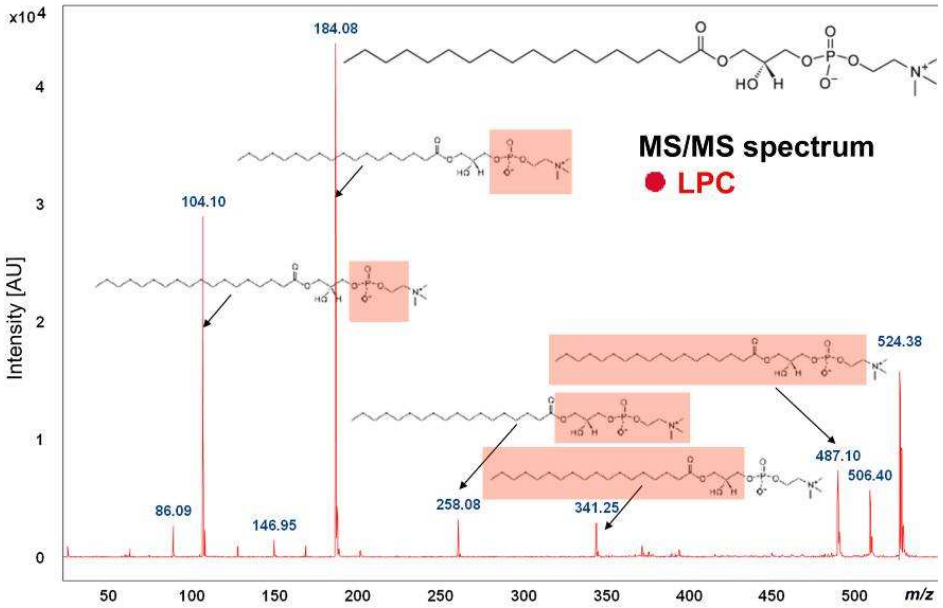
M. Schuerenberg *et al.,* IMSC 2009, Bremen, Poster PMM 386

Institute of Food Chemistry
University of Hohenheim, Stuttgart

HPTLC-FLD-MALDI-TOF MS



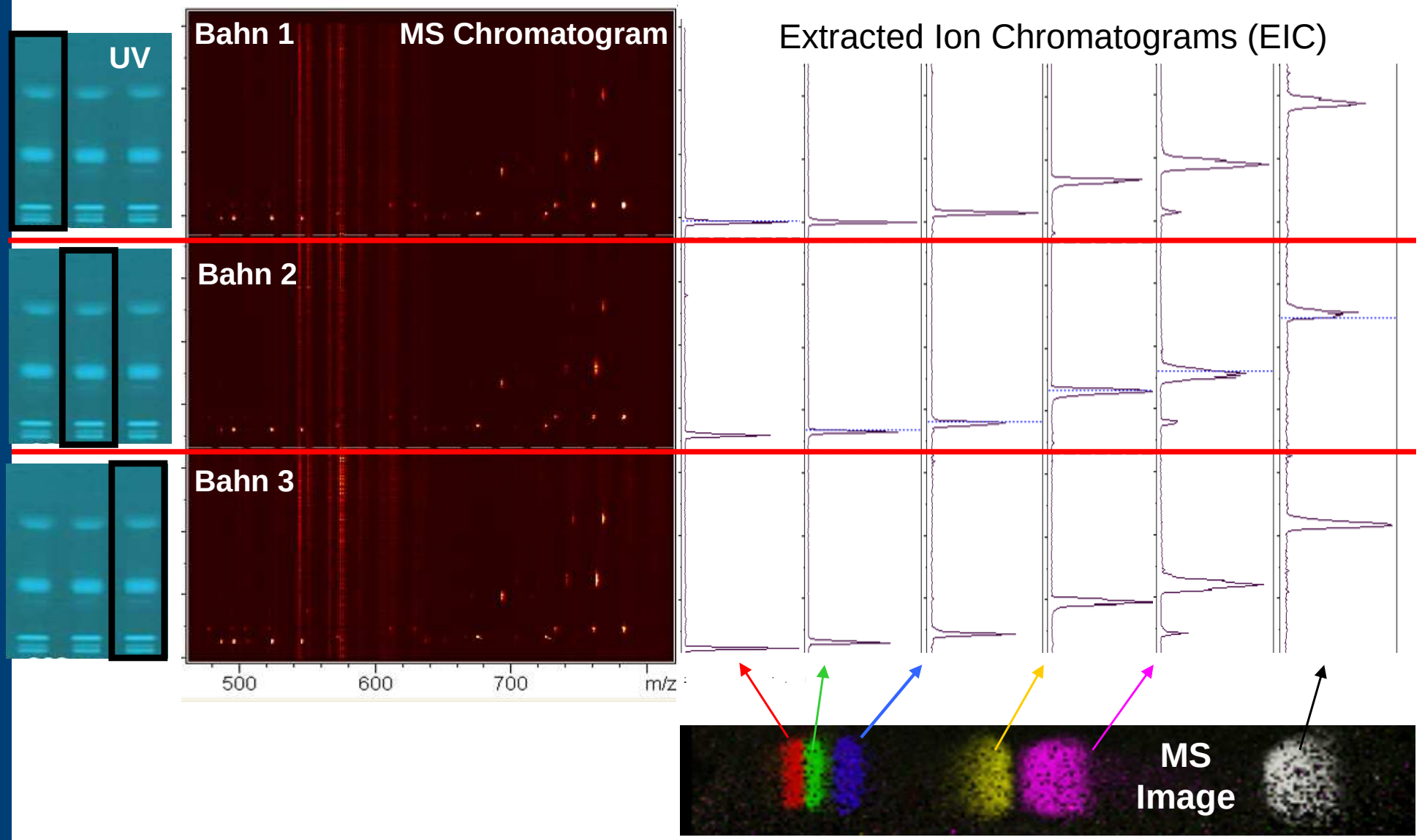




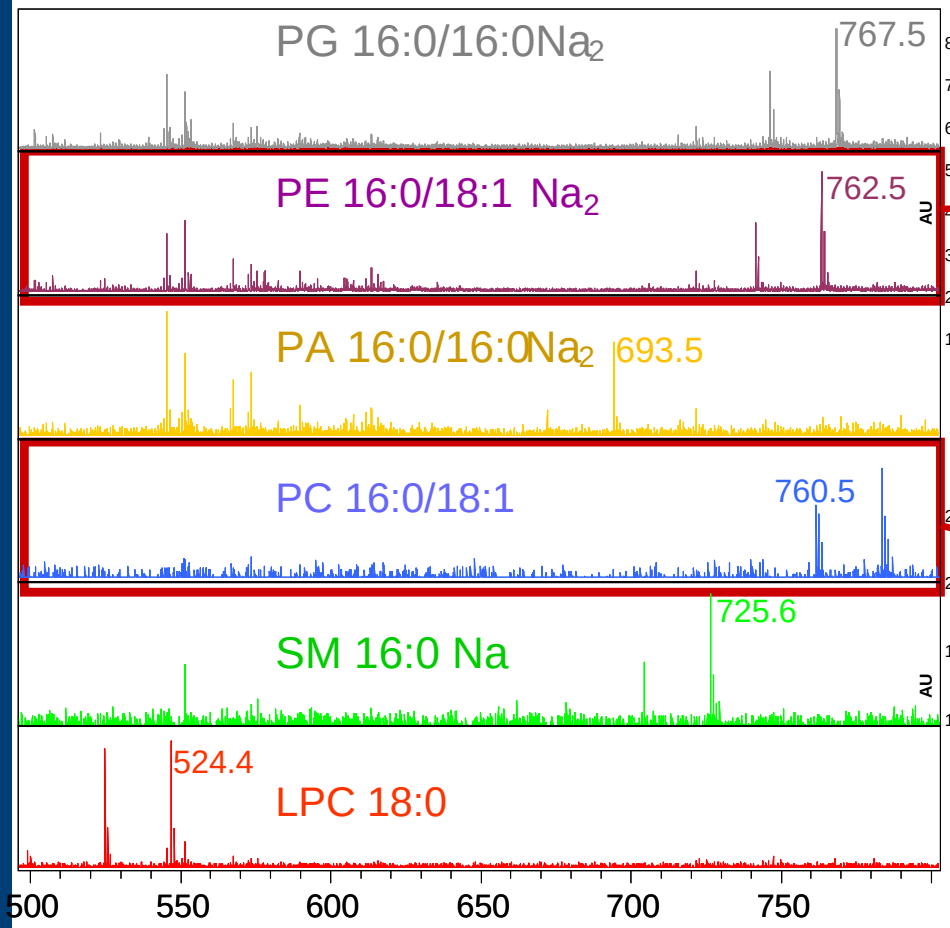
Bruker Daltonics Application Note MT-101

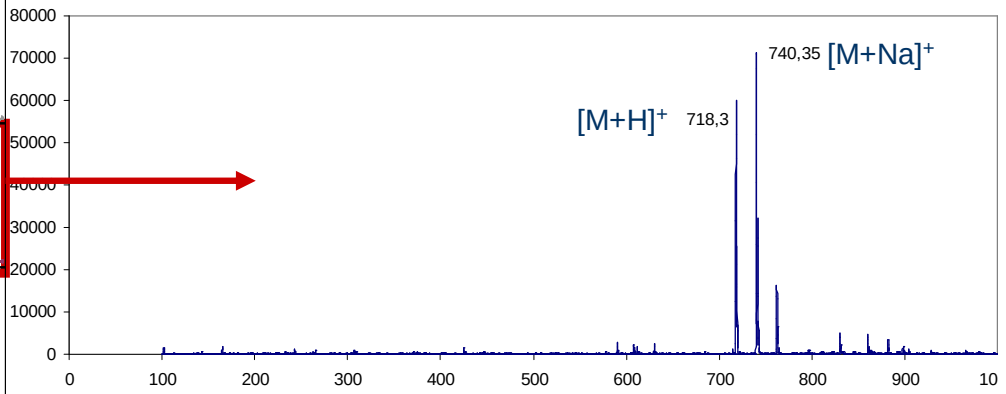
Institute of Food Chemistry
University of Hohenheim, Stuttgart

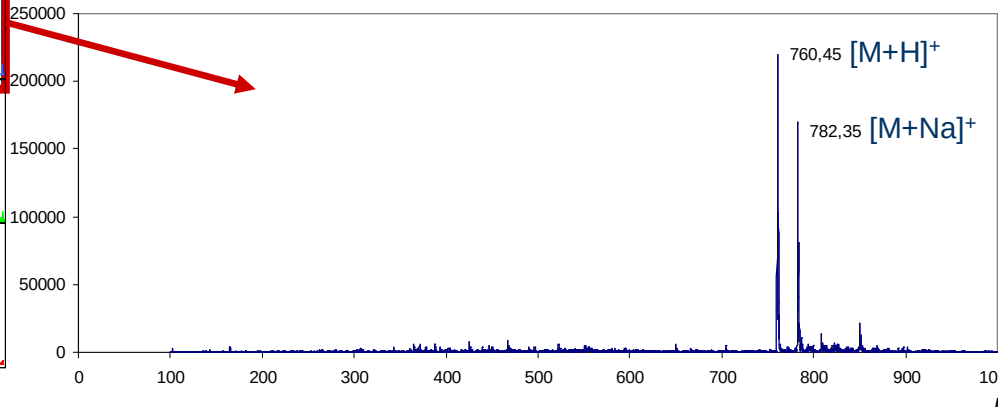
Quantification?

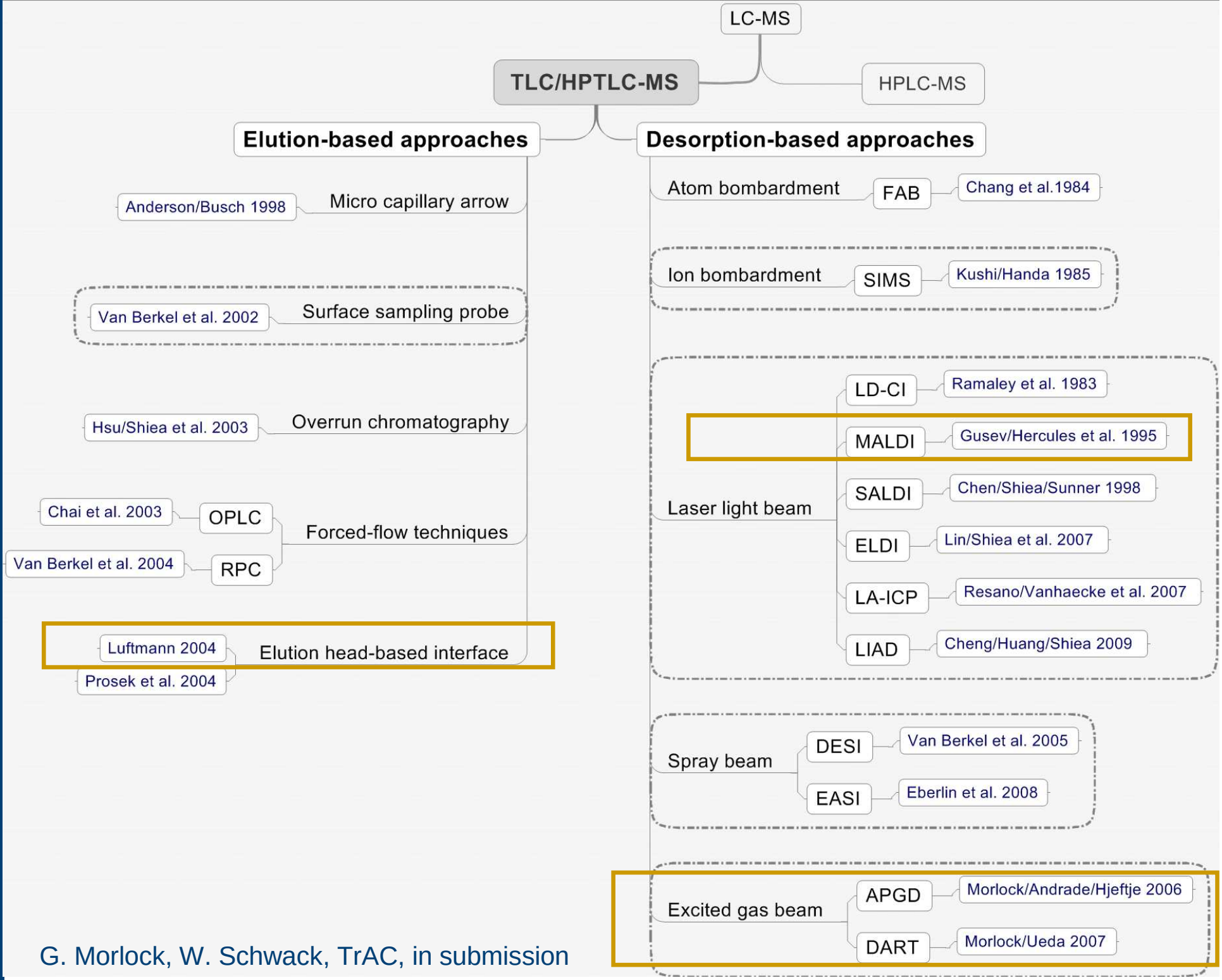


Comparison of mass spectra





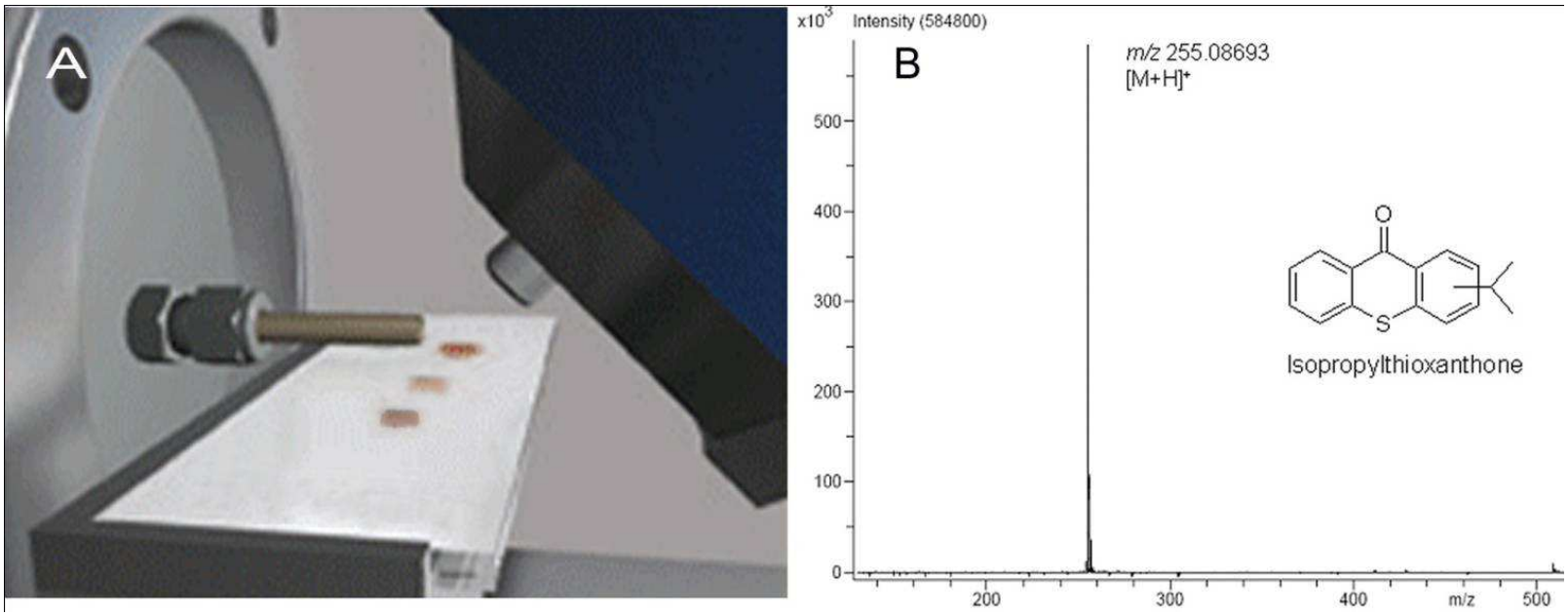




G. Morlock, W. Schwack, TrAC, in submission

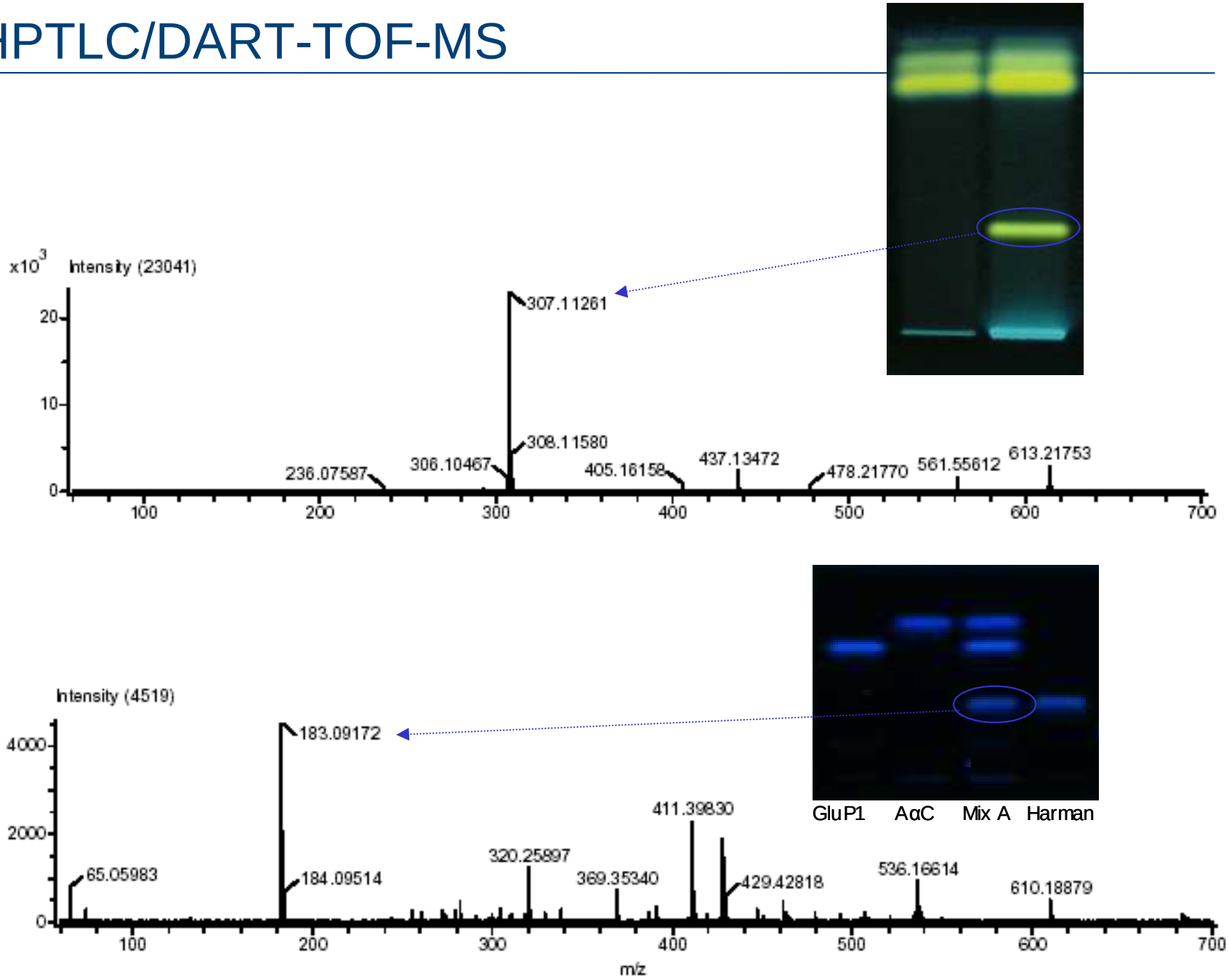
Institute of Food Chemistry
University of Hohenheim, Stuttgart

HPTLC-DART-MS



Institute of Food Chemistry
University of Hohenheim, Stuttgart

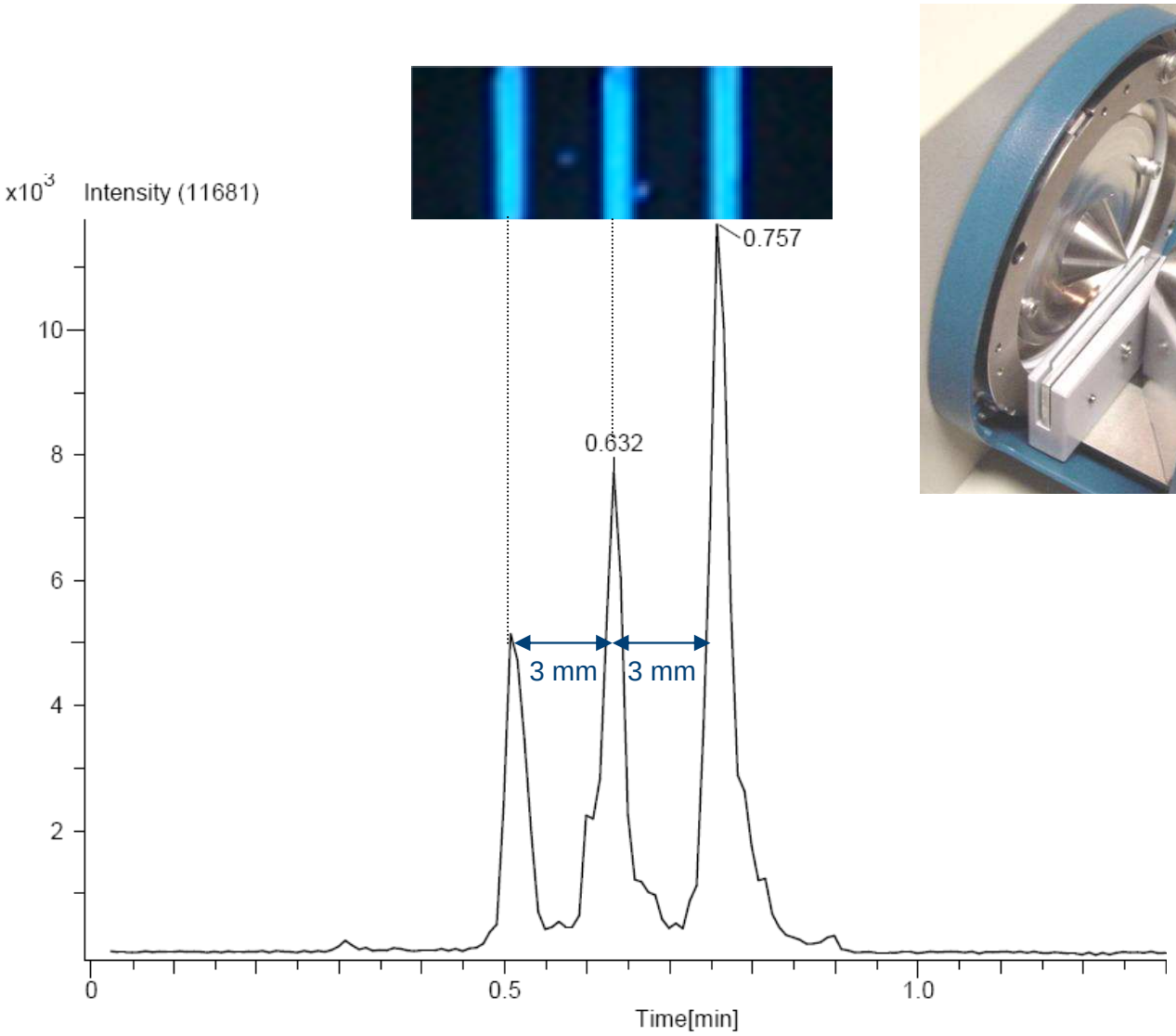
HPTLC/DART-TOF-MS



G. Morlock, Y. Ueda, J Chromatgr A 1143 (2007) 243-251

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Spatial resolution of DART

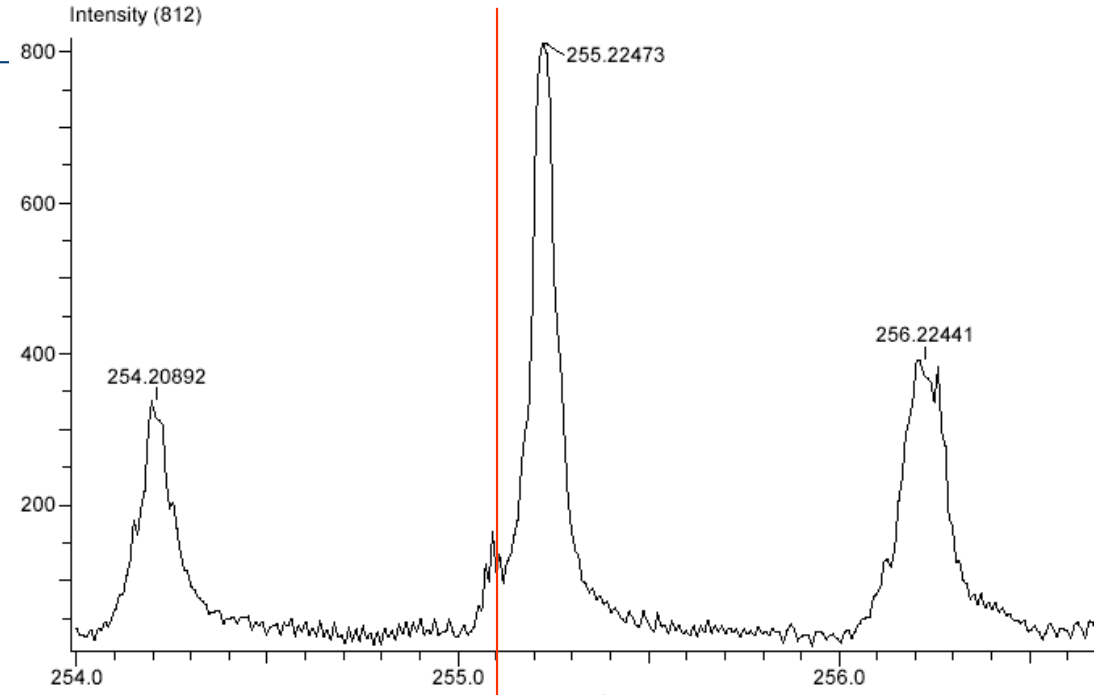


Speed: ~2 mm/10s

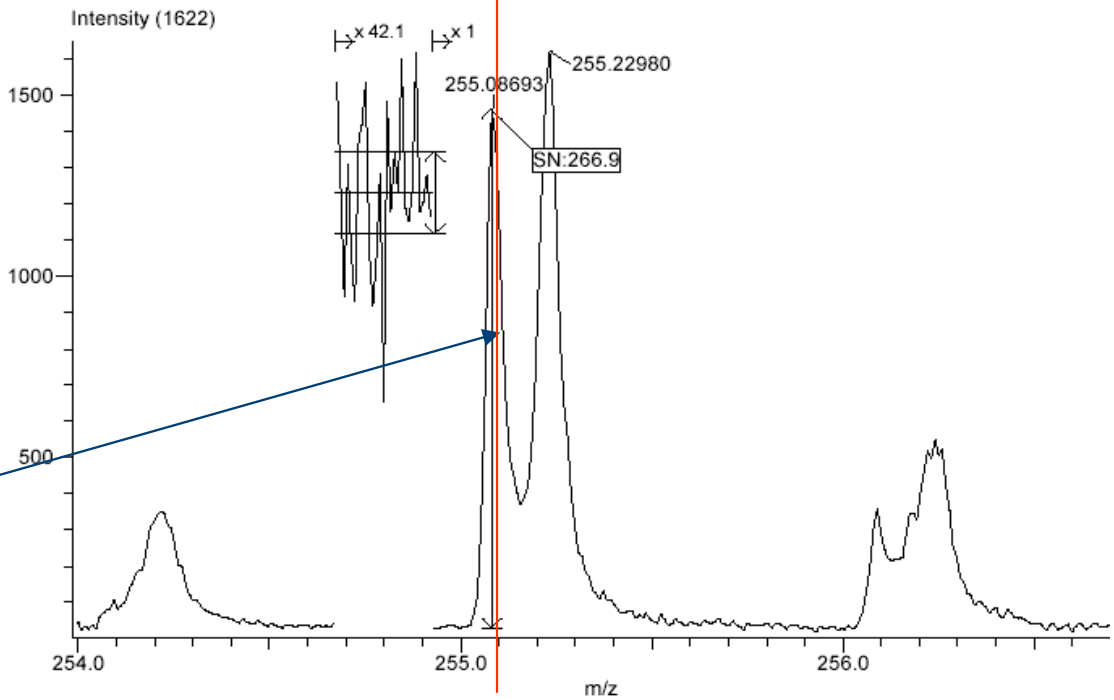
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Detectability

Blank
m/z 255.08693



3 ng ITX
m/z 255.08693
S/N of 267

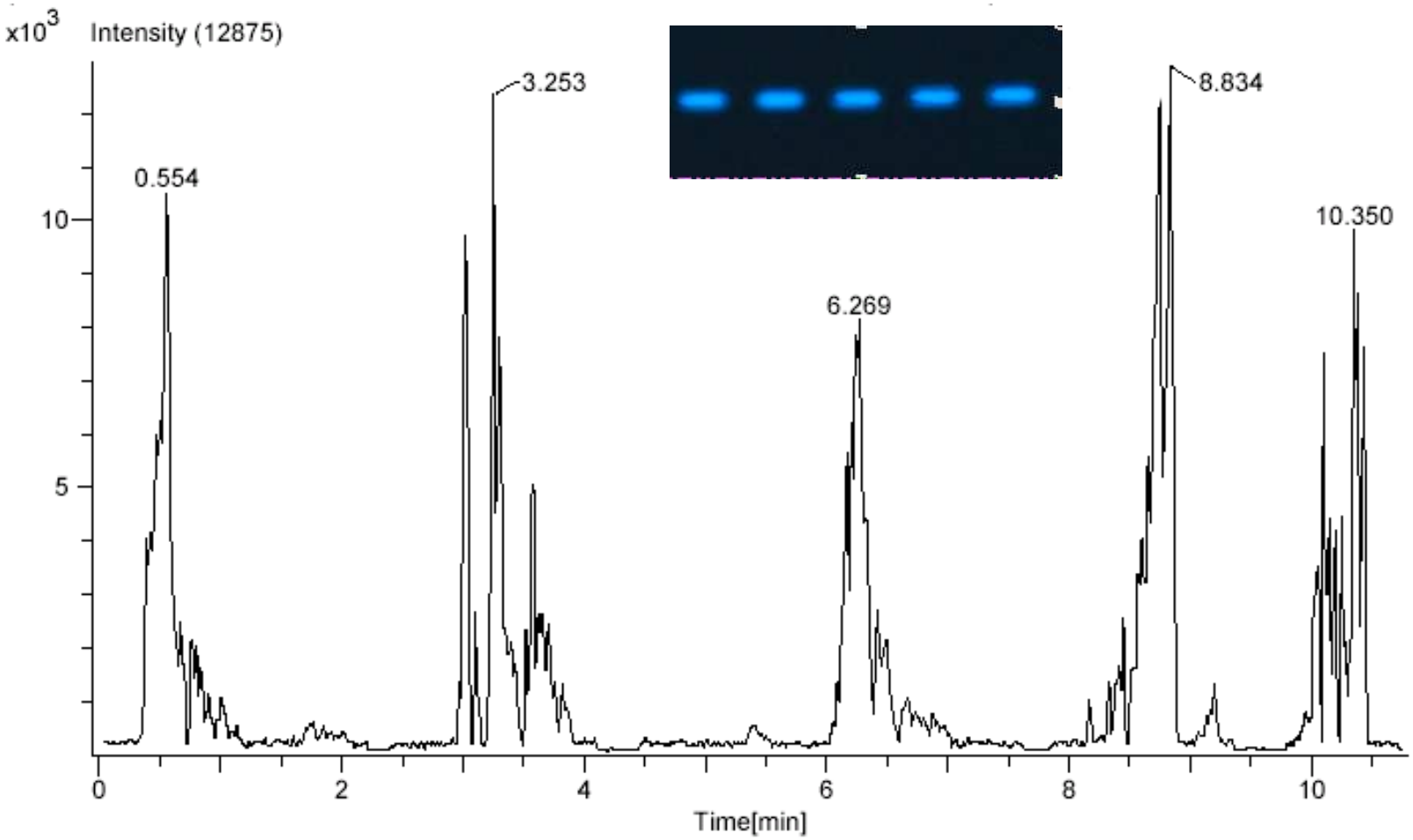


Institute of Food Chemistry
University of Hohenheim, Stuttgart

Repeatability

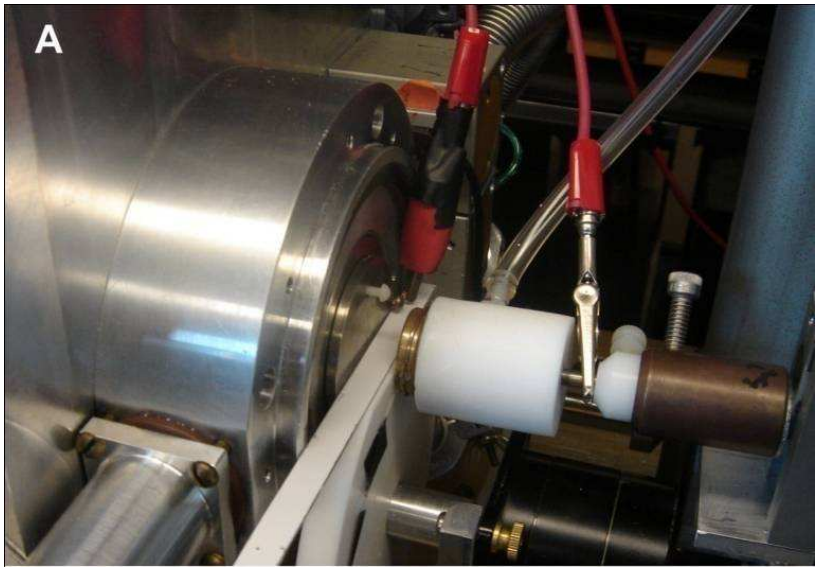
Mass chromatogram of ITX @ *m/z* 255.087 $[M+H]^+$

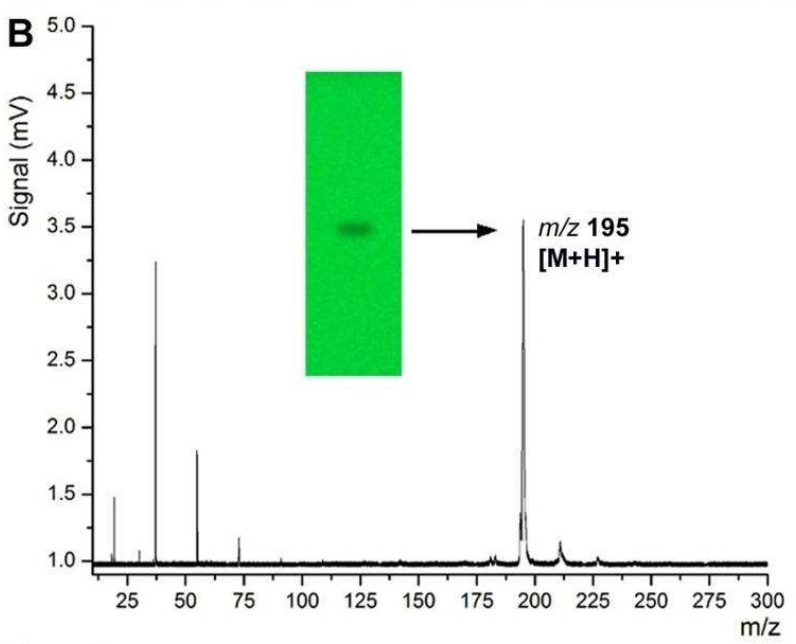
→ *%RSD* = 71 % (32 ng/zone, *n* = 5)

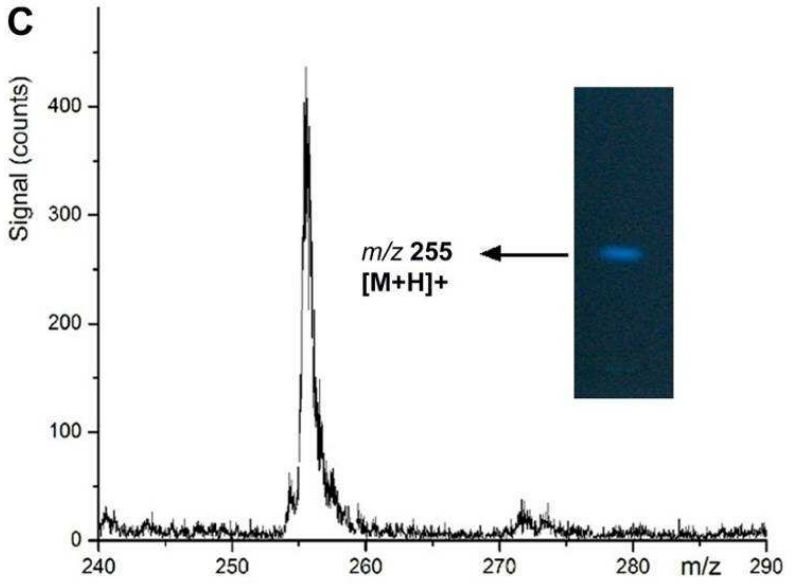


Institute of Food Chemistry
University of Hohenheim, Stuttgart

APGD-HPTLC-TOF MS







G. Morlock, W. Schwack, TrAC, in submission

Institute of Food Chemistry
University of Hohenheim, Stuttgart

G. Morlock, W. Schwack, TrAC, in submission

Comparison of the approaches

DART/APGD →

→ dry desorption technique ⟷ DESI

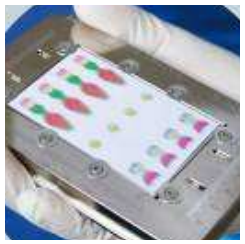
→ no plate preparation etc. ⟷ SALDI, MALDI

→ ambient conditions, no high voltage ⟷ micro junction

→ simple spectra ⟷ MALDI, SIMS

→ quantitativ *with* internal standard → scan function

MALDI

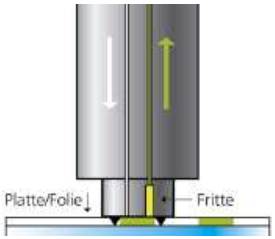


✓ strict protocol for plate preparation

✓ complex spectra

✓ quantitativ *with* internal standard → scan function

Elution-head based Interface



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

✓ plug & play interface (without adjustments or modifications)

✓ whole plate (no cut)

✓ all carriers on mostly all layers ⟷ micro junction

✓ whole zone incl. depth profile ⟶ high detectabilities

✓ quantitativ *without* internal standard ⟷ desorption techniques

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

G Model
CHROMA-351018; No. of Pages 10

ARTICLE IN PRESS

Journal of Chromatography A, xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Review

Hyphenations in planar chromatography

- HPTLC-UV/Vis/FLD-MS [13,14],
- HPTLC-UV/Vis/FLD-bioactivity-HRMS [15],
- HPTLC-UV-FTIR [16,17],
- HPTLC-UV/Vis/FLD-FTIR ATR [18],
- TLC-Vis-SERS [12].

Gertrud Morlock*, Wolfgang Schwack

University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Available online xxx

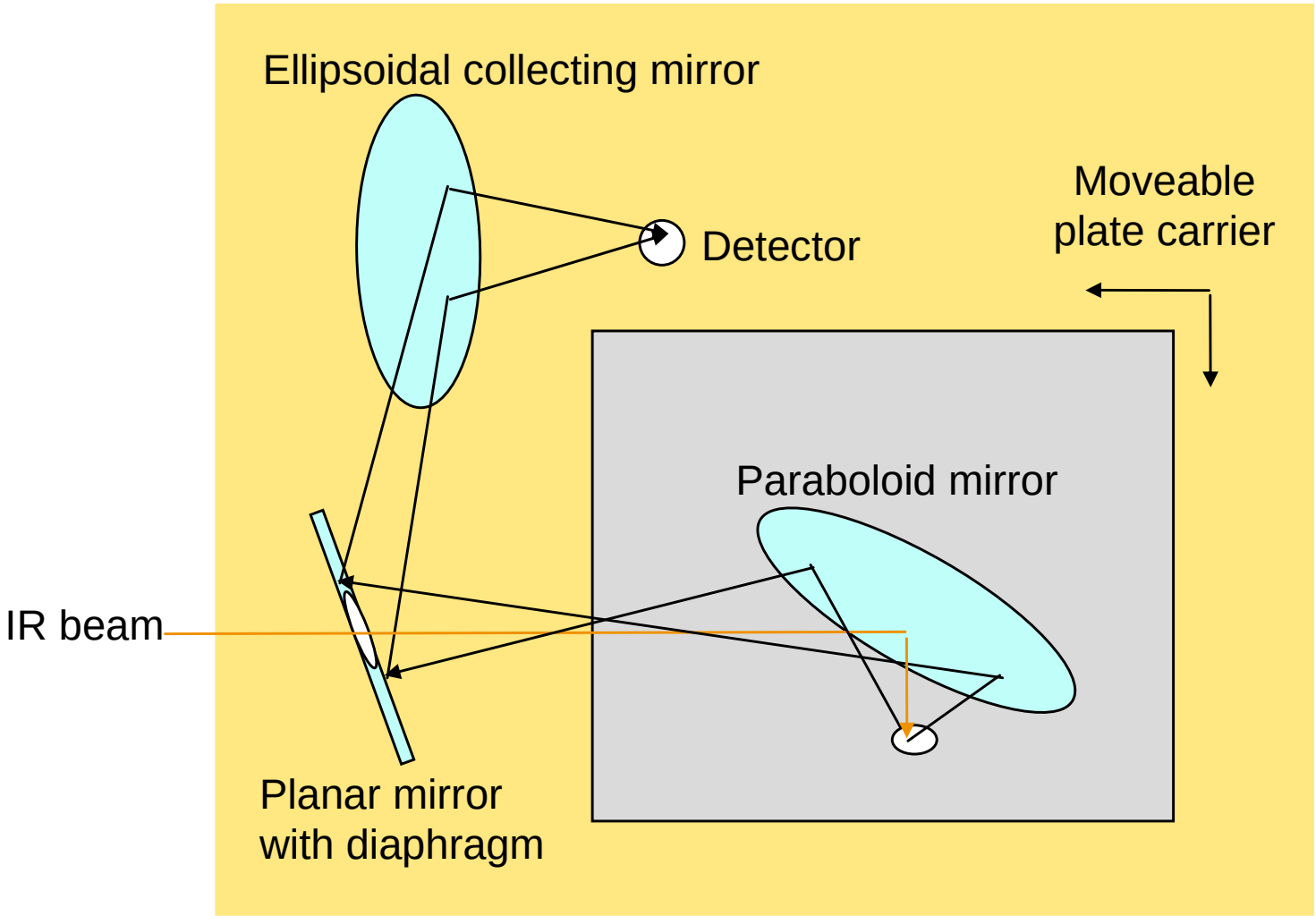
Keywords:
Mass spectrometry
High-performance thin-layer chromatography
Effect-directed analysis
Bioassays
Cost-effective analysis
High-throughput system

ABSTRACT

This review is focused on planar chromatography and especially on its most important subcategory high-performance thin-layer chromatography (HPTLC). The image-giving format of the open, planar stationary phase and the post-chromatographic evaporation of the mobile phase ease the performance of various kinds of hyphenations and even super-hyphenations. Examples in the field of natural product search, food and lipid analysis are demonstrated, which point out the hyphenation with effect-directed analysis (EDA) and mass spectrometry and illustrate the efficiency gain. Depending on the task at hand, hyphenations can readily be selected as required to reach the relevant information about the sample, and at the same time, information is obtained for many samples in parallel. The flexibility and the unrivalled features through the planar format valuably assist separation scientists.

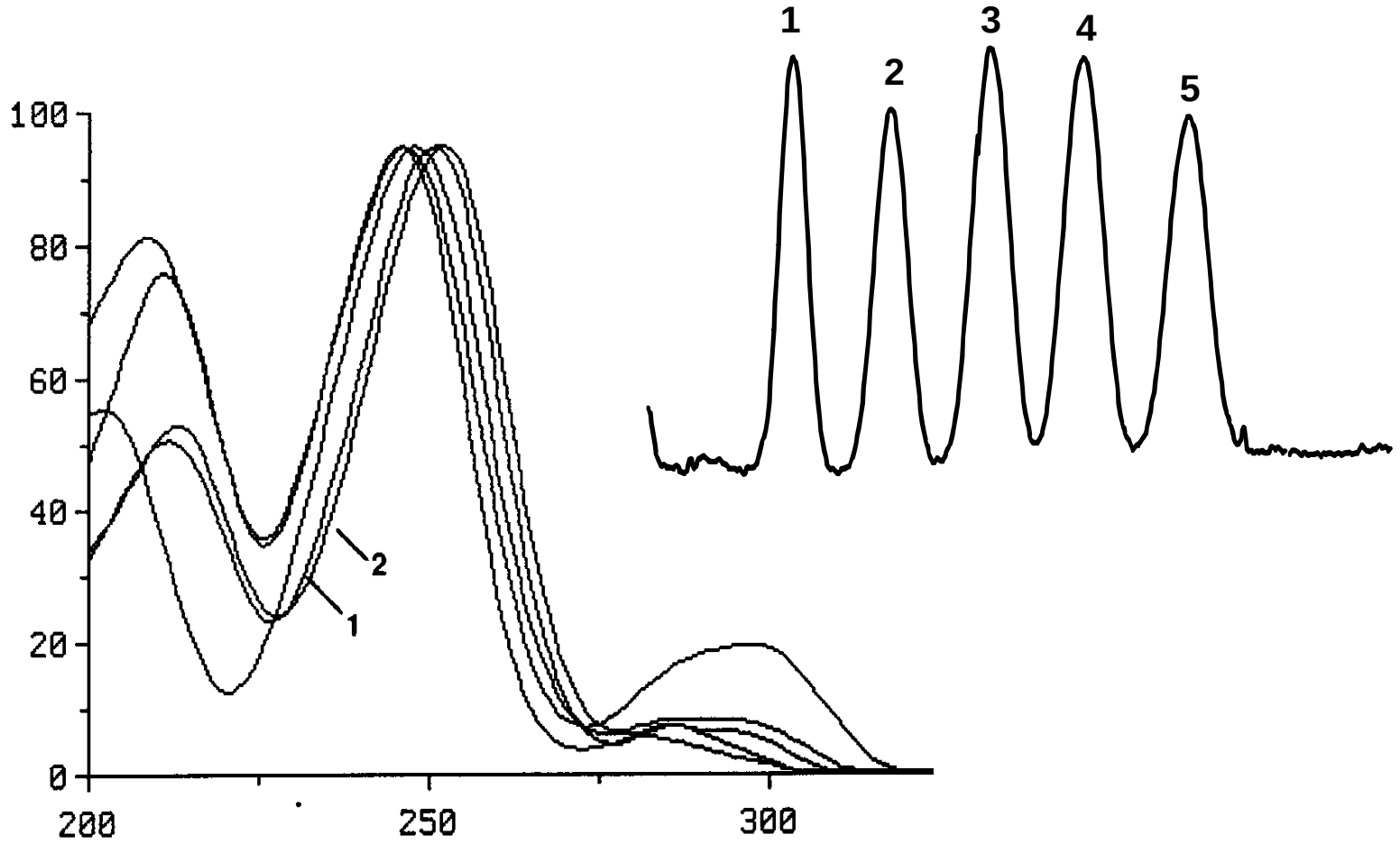
Institute of Food Chemistry
University of Hohenheim, Stuttgart

HPTLC-DRIFT



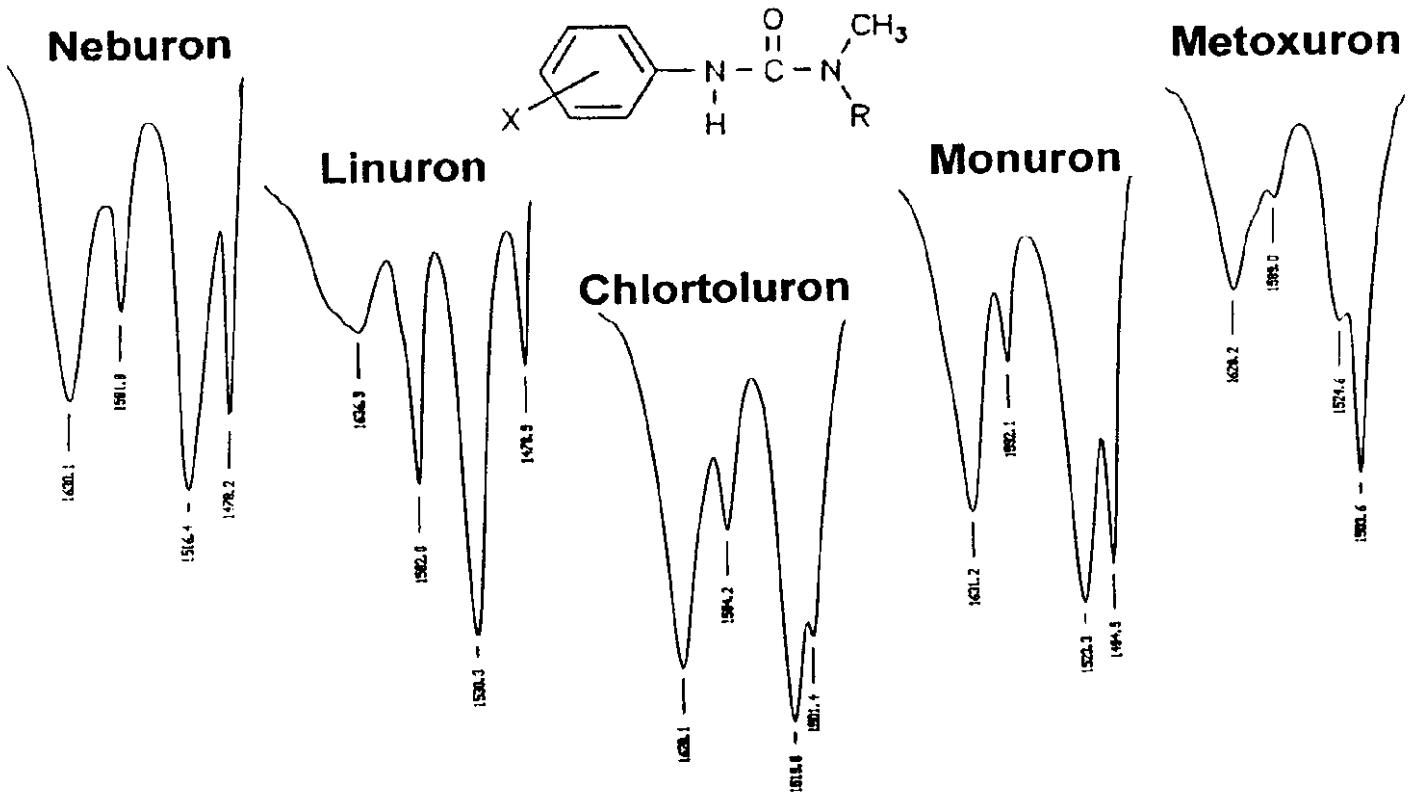
Institute of Food Chemistry
University of Hohenheim, Stuttgart

UV-spectra of 5 phenyl urea herbicides



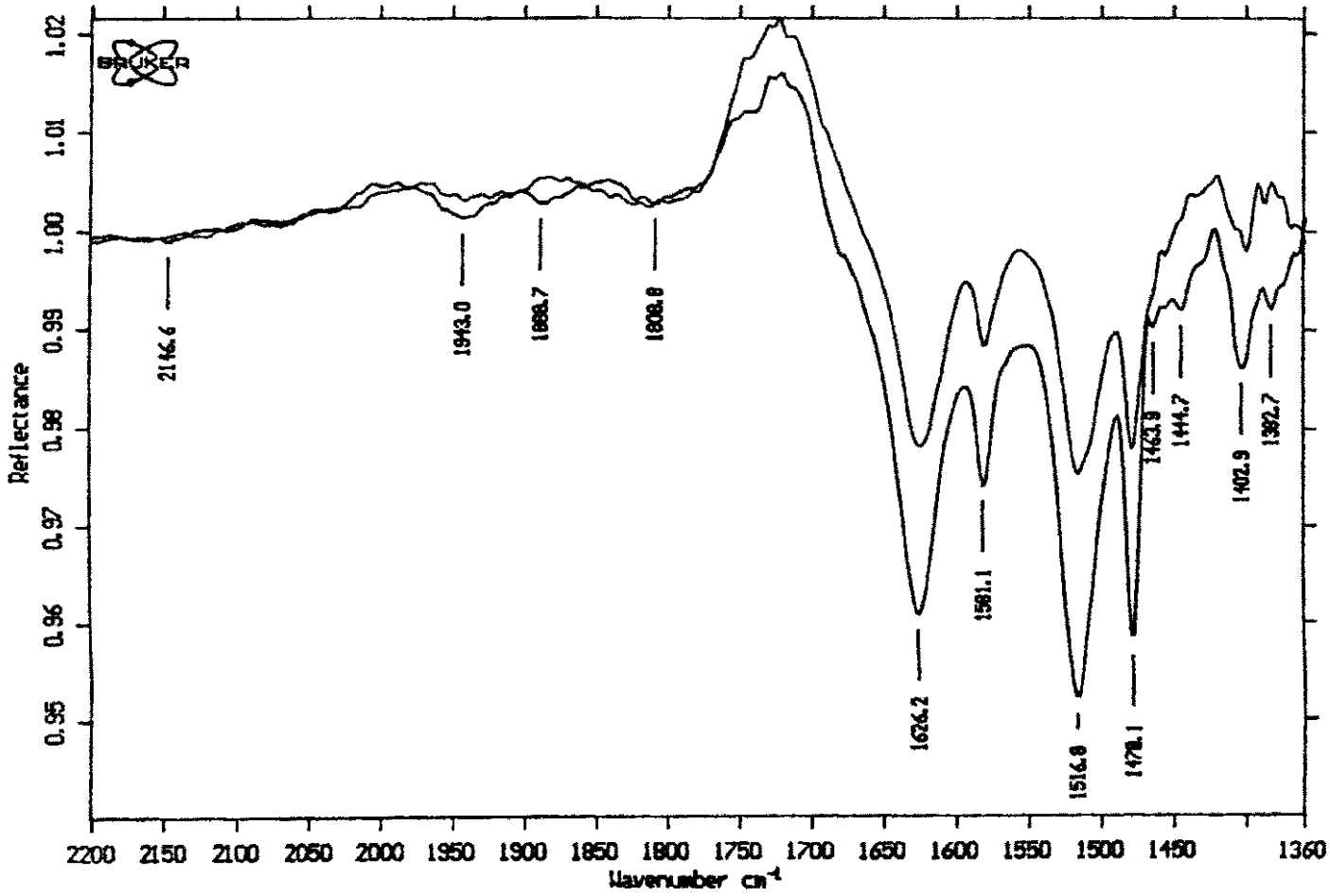
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Characteristic FTIR bands



Institute of Food Chemistry
University of Hohenheim, Stuttgart

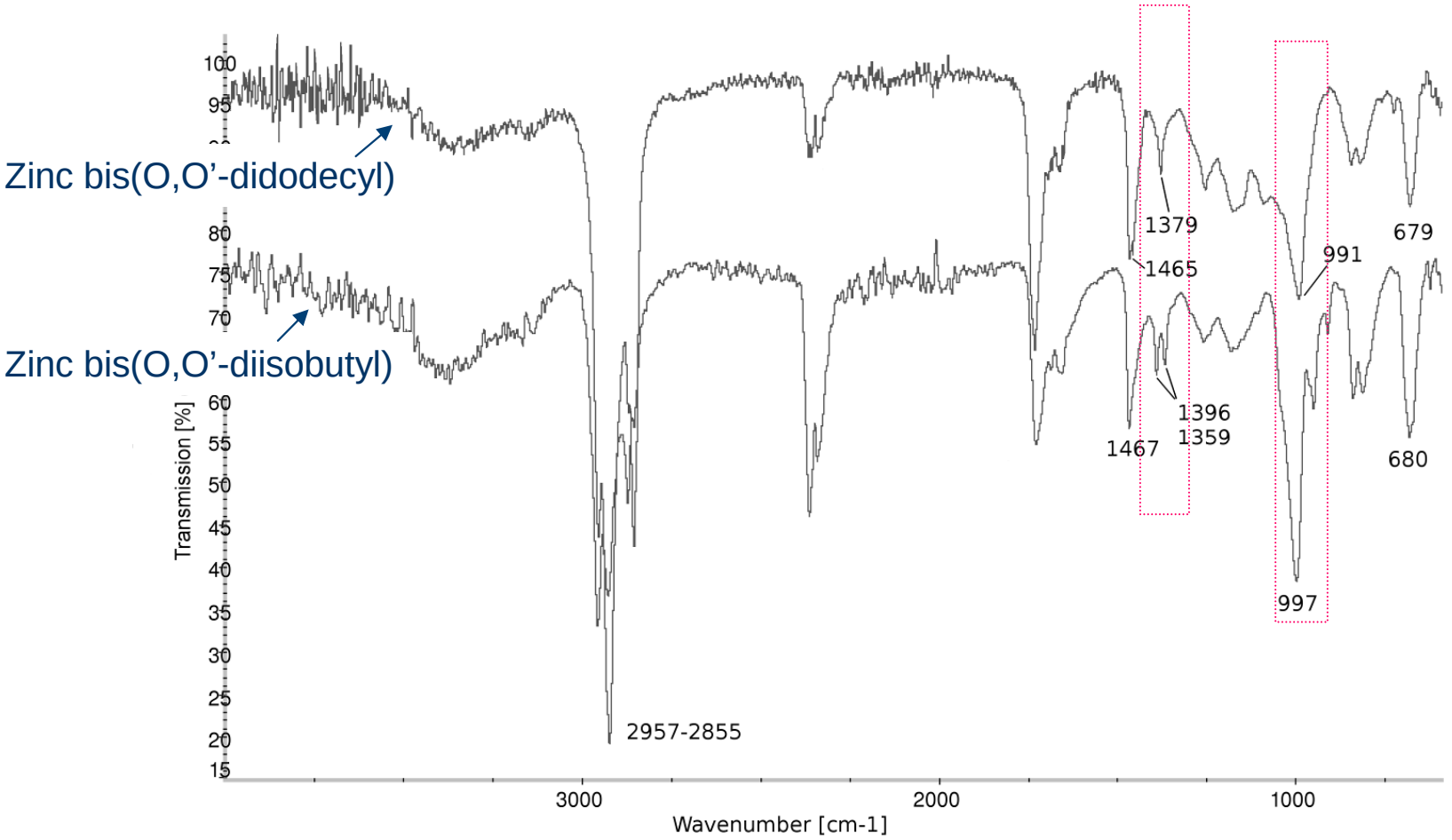
FTIR spectrum of neburon in drinking water



Institute of Food Chemistry
University of Hohenheim, Stuttgart

HPTLC/ATR-IR spectra via the interface

Dithiophosphate additives in mineral oil



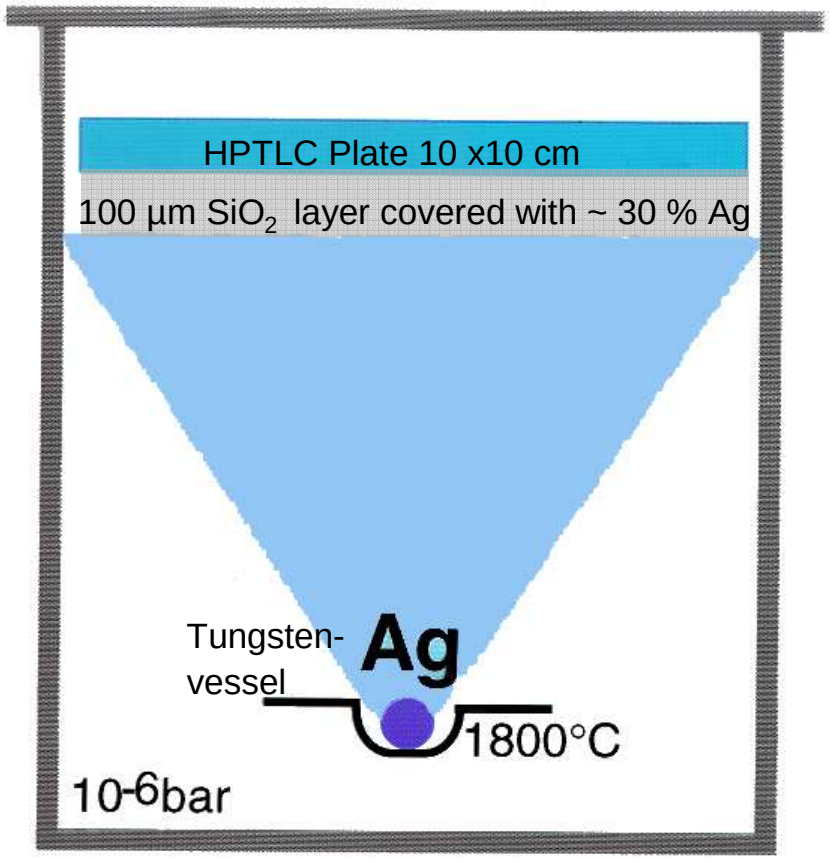
E. Dytkiewitz, G. Morlock, J AOAC Int 91 (2008) 1237-1244

Institute of Food Chemistry
University of Hohenheim, Stuttgart

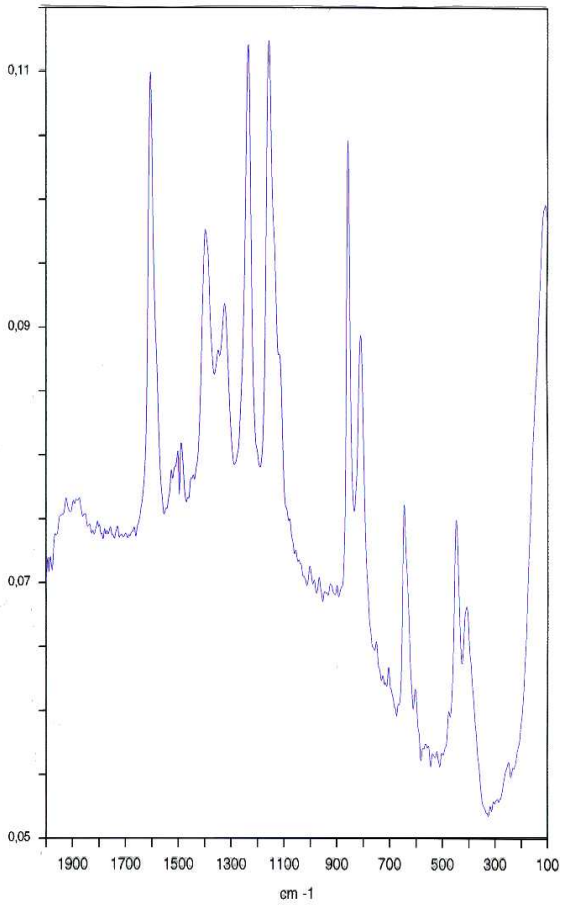
Raman: FT-SERS

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

Vacuum transfer



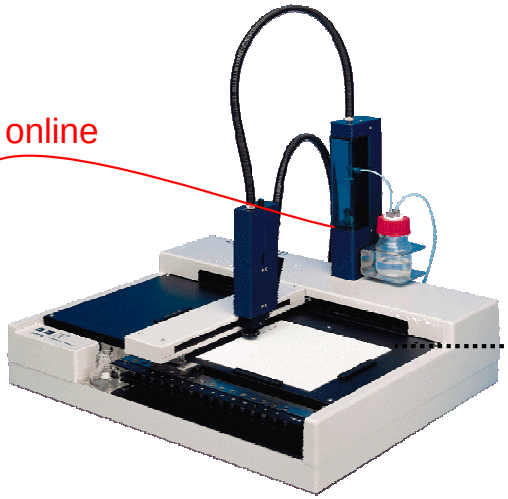
10 ng/zone p-nitrophenol



HPLC-HPTLC

Fractions (1-2 min each)
controlled by cutting standards
are applied as area

online

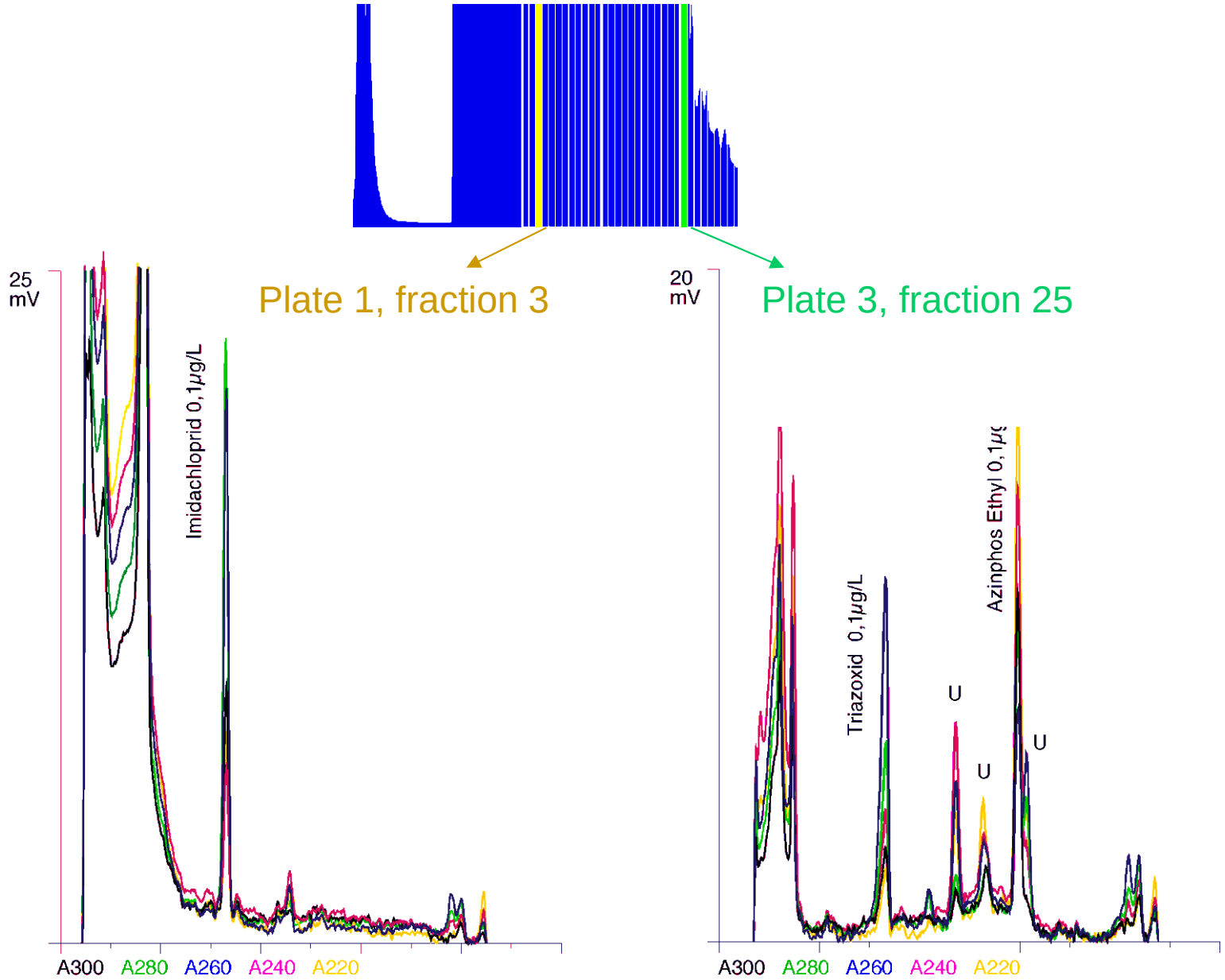


HPTLC analysis of 18 tracks (fractions) per plate

Microbore 2.1 x 100 mm
Flow rate 50-100 µL/min

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Surface water spiked with 50 pesticides



Institute of Food Chemistry
University of Hohenheim, Stuttgart

HPLC-HPTLC

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

→ predestinated for

☺ separation problems due to lack of separation power

☺ samples with varying matrix content (multi-method)

☺ peak purity testing of HPLC peaks

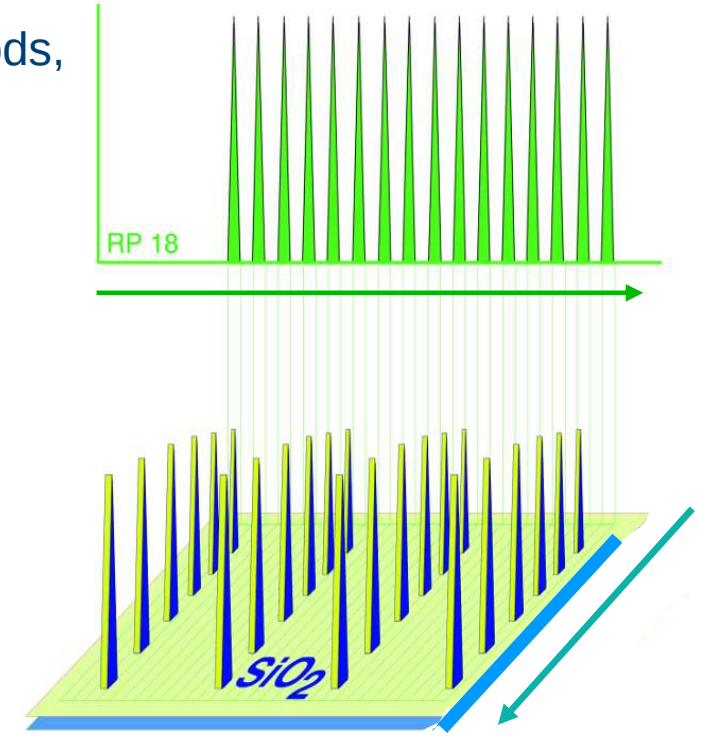
☺ problematic, time-consuming post-chromatographic derivatization in HPLC

→ results obtained by two independend methods,
i.e. two different separation mechanisms

→ gain in analytical quality

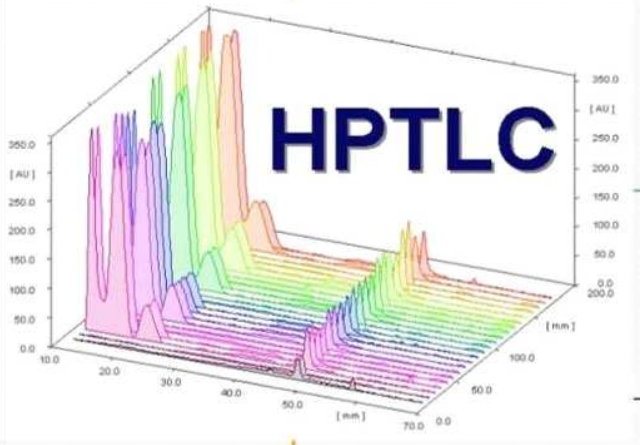
→ still in use by Andreas Kinast
andreas.kinast@currenta.de
www.currenta.de

CURRENTA
Performance for the chemical industry



Institute of Food Chemistry
University of Hohenheim, Stuttgart

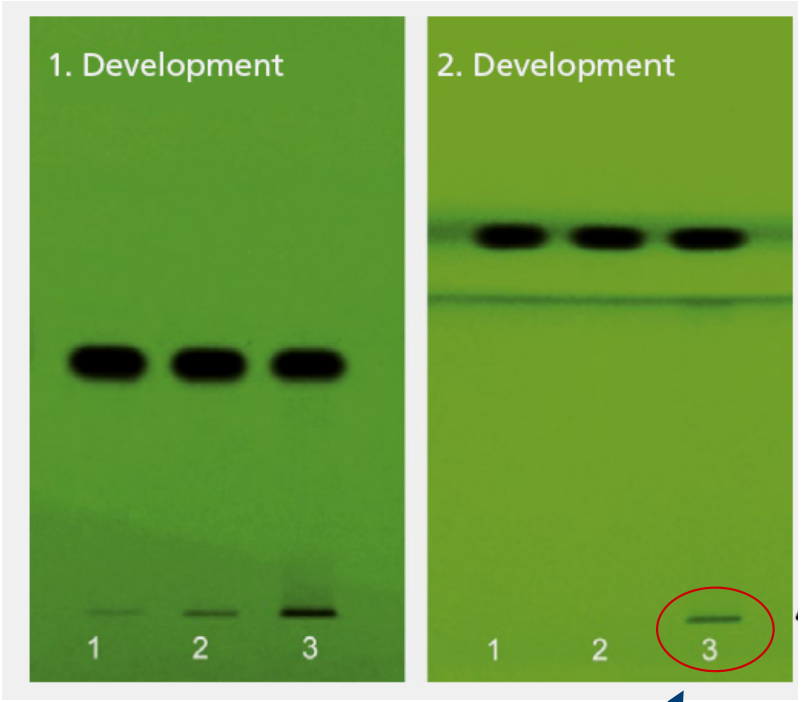
Benefits



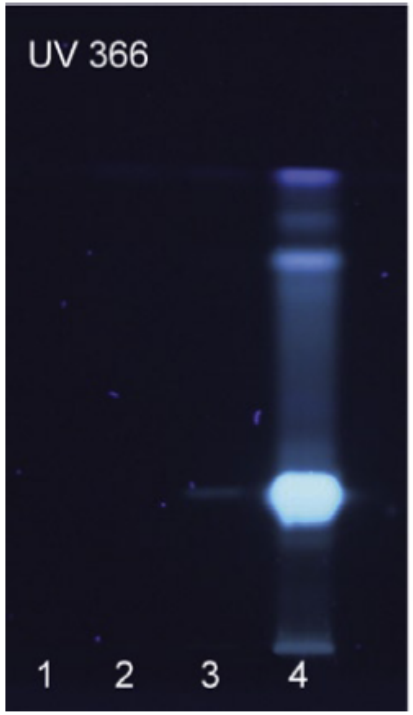
- The powerful image
  - A picture is worth a thousand words
  - Quick digital quantification
  - More information about an unknown
- Reduced costs
  - Parallel chromatography under identical conditions
  - Simplified sample preparation
  - Concentration 1:10.000 during application
- Detection
  - Effect-directed detection (bioactivity-based)
  - Cost-effective coupling to MS
  - Simultaneously for all (72 at one go)
  - Multiple detection & unlimited selectivity
- Step-automatization
  - Comparable results to other methods
  - Quantitative & sensitive method
- Flexible analysis
  - Robust system, no carry-over, no memory effects
  - Rapid change of stationary/mobil phases/detection principles
  - Staggered system allows throughput of 1000 analyses per day
  - Use of one HPTLC system for different projects

Institute of Food Chemistry
University of Hohenheim, Stuttgart

More information about unknowns



Mass imbalance caused by light degradation product



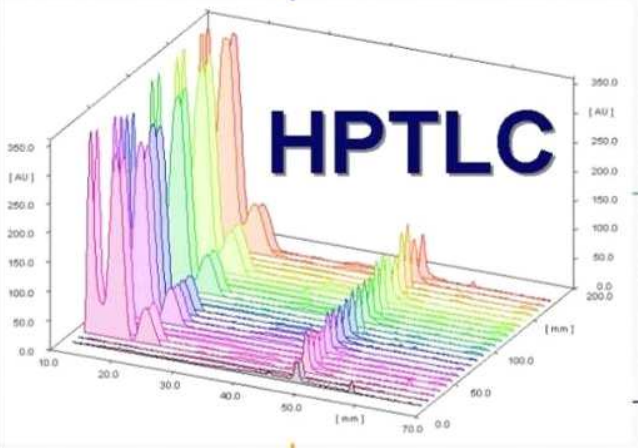
New yellow impurity caused color differences between batches of an API

C. McKinlay, CBS 101 (2008) 12-13

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Benefits

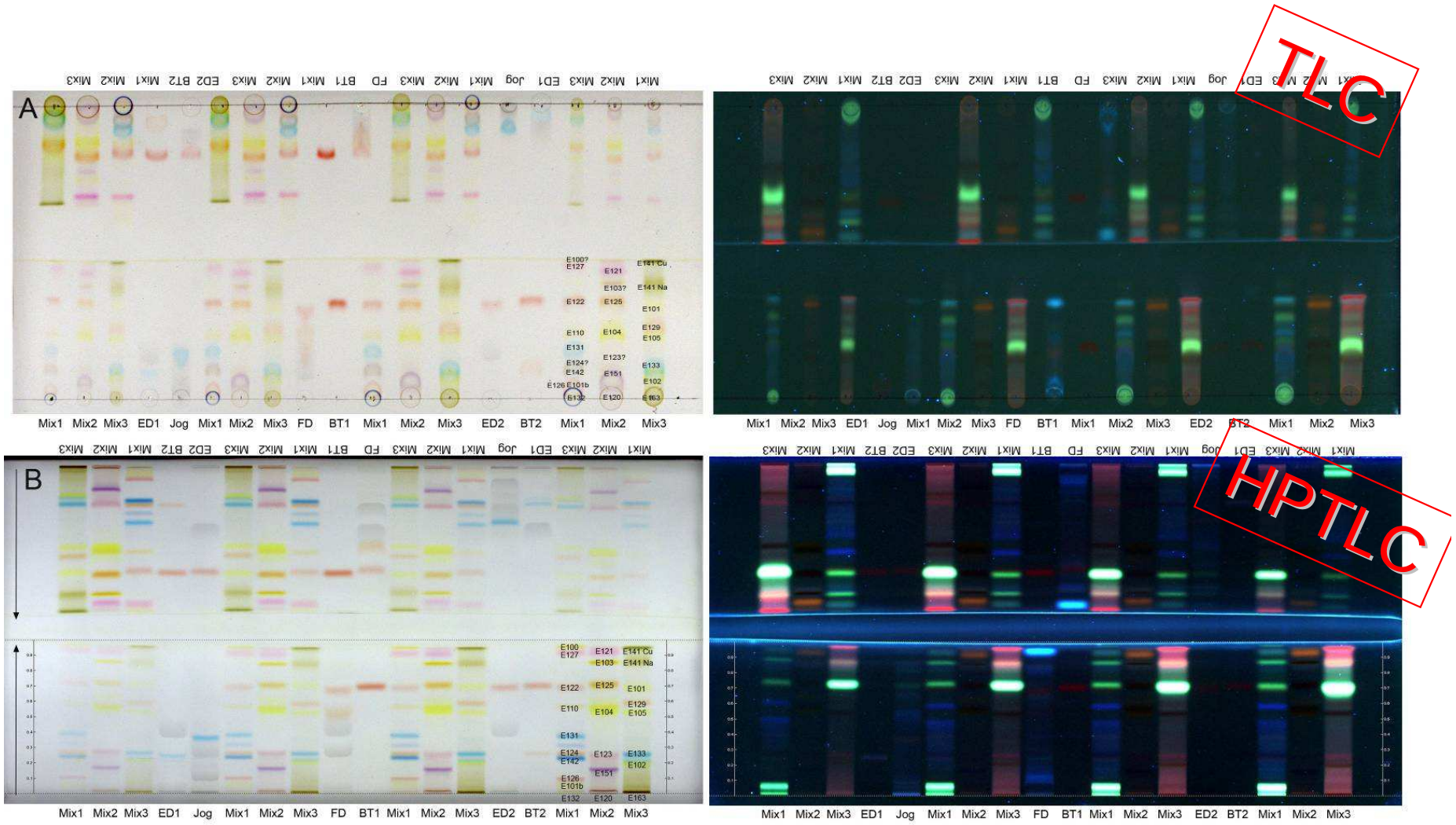
- The powerful image
  - A picture is worth a thousand words
  - Quick digital quantification
  - More information about an unknown
- Reduced costs
  - Parallel chromatography under identical conditions
  - Simplified sample preparation
  - Concentration 1:10.000 during application



- Detection
  - Effect-directed detection (bioactivity-based)
  - Cost-effective coupling to MS
  - Simultaneously for all (72 at one go)
  - Multiple detection & unlimited selectivity
- Step-automatization
  - Comparable results to other methods
  - Quantitative & sensitive method
- Flexible analysis
  - Robust system, no carry-over, no memory effects
  - Rapid change of stationary/mobil phases/detection principles
  - Staggered system allows throughput of 1000 analyses per day
  - Use of one HPTLC system for different projects

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Dye analysis



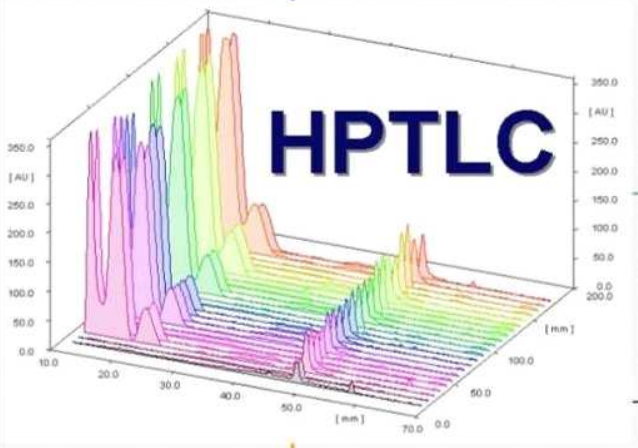
→ No resonable calibration function was obtained by TLC.

→ For quantification, just HPTLC is reliable.

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Benefits

- The powerful image
  - A picture is worth a thousand words
  - Quick digital quantification
  - More information about an unknown
- Reduced costs
  - Parallel chromatography under identical conditions
  - Simplified sample preparation
  - Concentration 1:10.000 during application



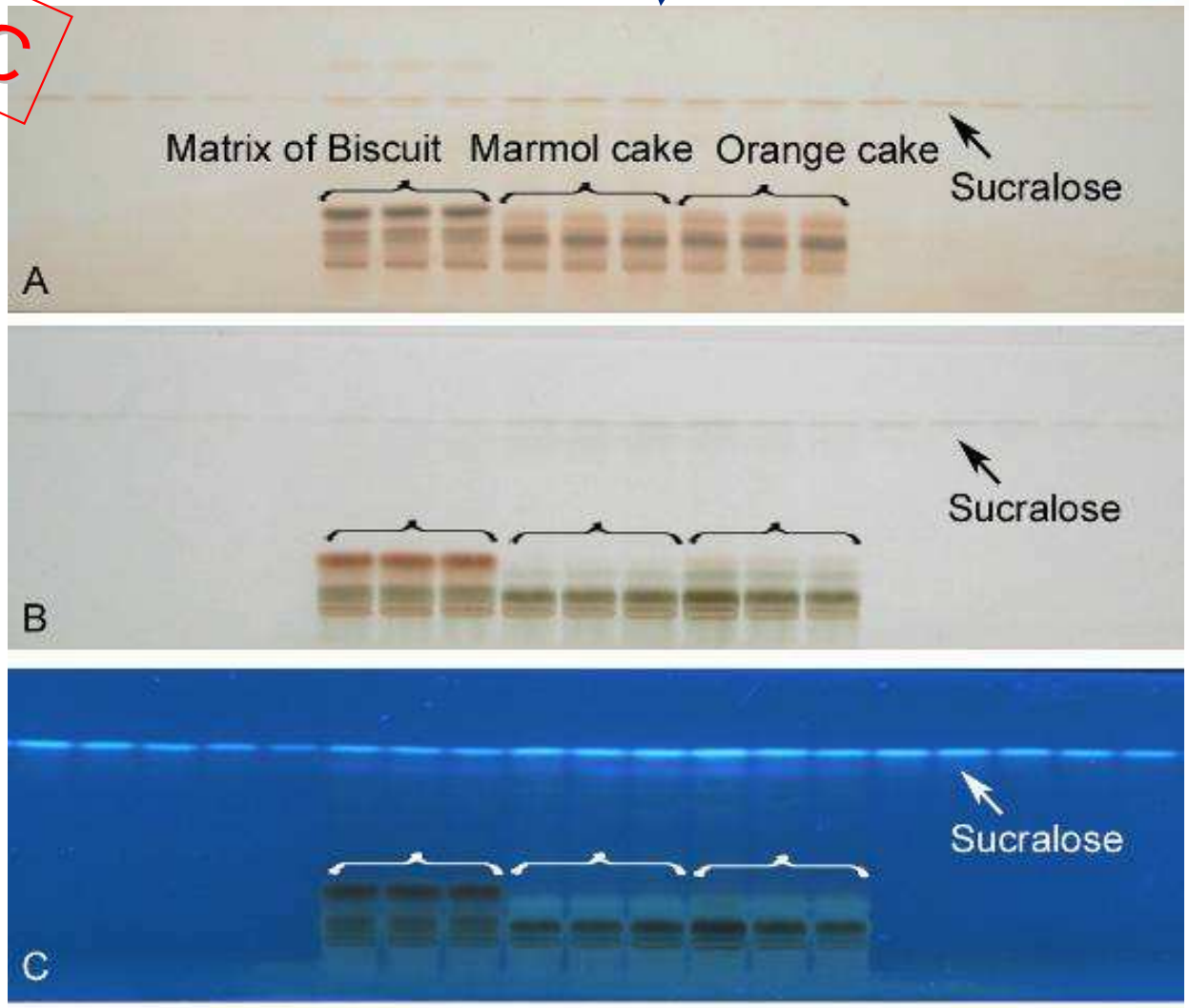
- Detection
  - Effect-directed detection (bioactivity-based)
  - Cost-effective coupling to MS
  - Simultaneously for all (72 at one go)
  - Multiple detection & unlimited selectivity
- Step-automatization
  - Comparable results to other methods
  - Quantitative & sensitive method
- Flexible analysis
  - Robust system, no carry-over, no memory effects
  - Rapid change of stationary/mobil phases/detection principles
  - Staggered system allows throughput of 1000 analyses per day
  - Use of one HPTLC system for different projects

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Analysis of sucralose in cakes

extracted
in MeOH, filtered

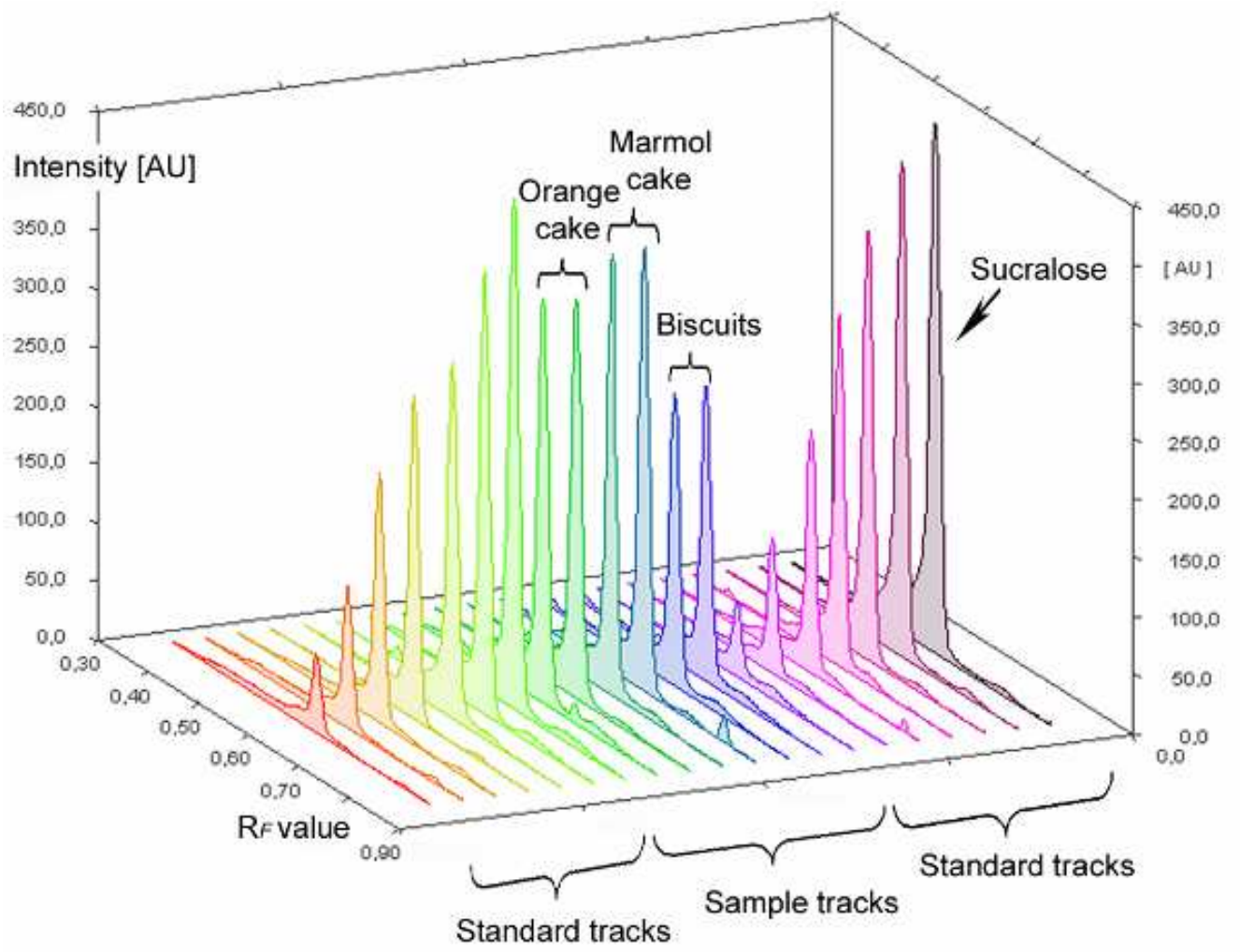
HPTLC



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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Quantification of sucralose in cakes



Institute of Food Chemistry
University of Hohenheim, Stuttgart

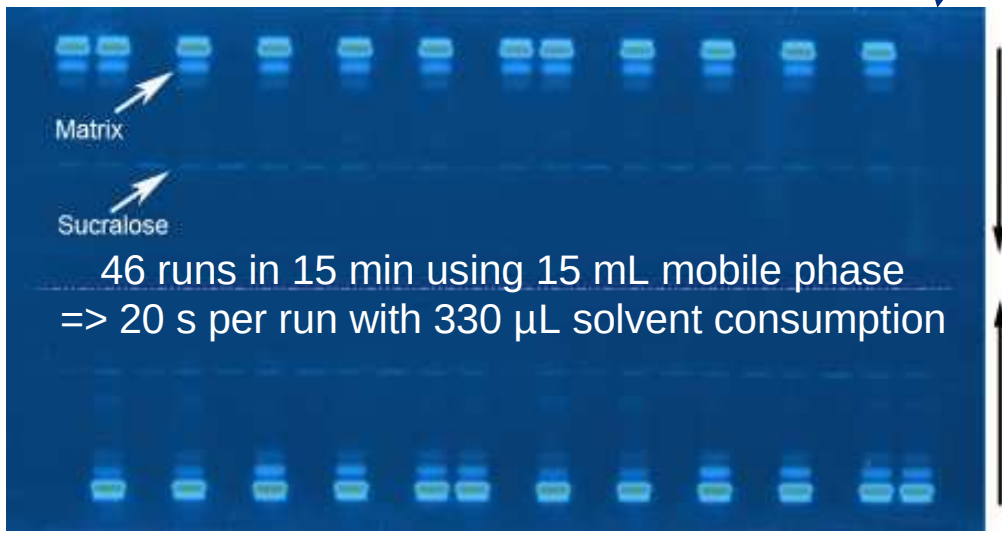
Quantification of sucralose in cakes

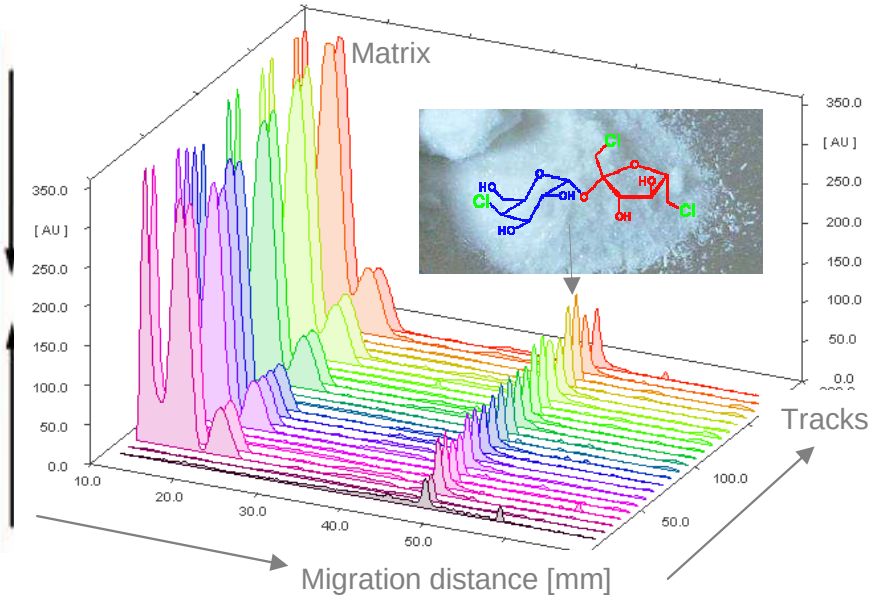
Mode A Reagent 1 @ 500 nm				
Samples	hR_F	**Sucralose found (mg/100 g)**	*%RSD* *n* = 3	Sucralose labeled (mg/100g)
Biscuits	57	**27.7**	2.4	24.8
Marmol cake	57	**48.0**	2.0	45.3
Orange cake	56	**43.9**	0.6	45.3
Mode B Reagent 2 @ 405 nm				
Biscuits	56	**27.9**	1.5	24.8
Marmol cake	56	**47.4**	0.5	45.3
Orange cake	56	**44.2**	1.6	45.3
Mode C Reagent 2 @ UV 366/>400 nm				
Biscuits	56	**27.1**	0.9	24.8
Marmol cake	57	**44.8**	4.2	45.3
Orange cake	56	**41.6**	3.0	45.3

Institute of Food Chemistry
University of Hohenheim, Stuttgart

… in milk-based confection (Burfi)

extracted in MeOH, filtered

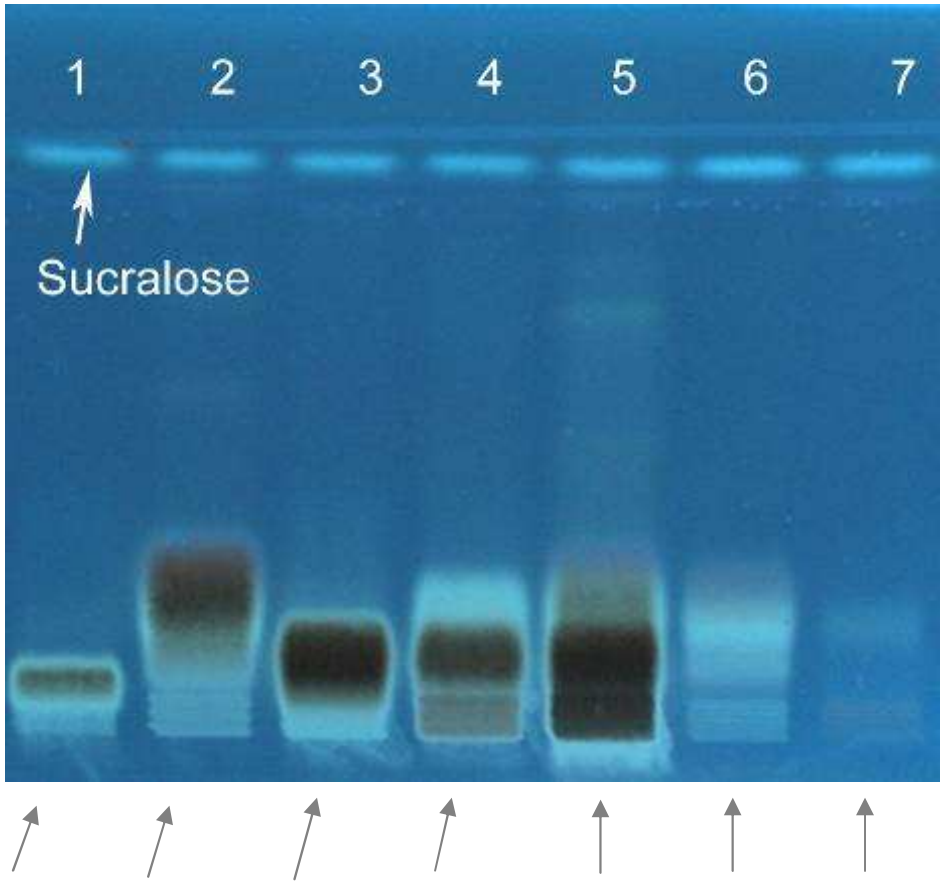




G. Morlock, S. Prabha, J Agric Food Chem 55 (2007) 7217-7223

Institute of Food Chemistry
University of Hohenheim, Stuttgart

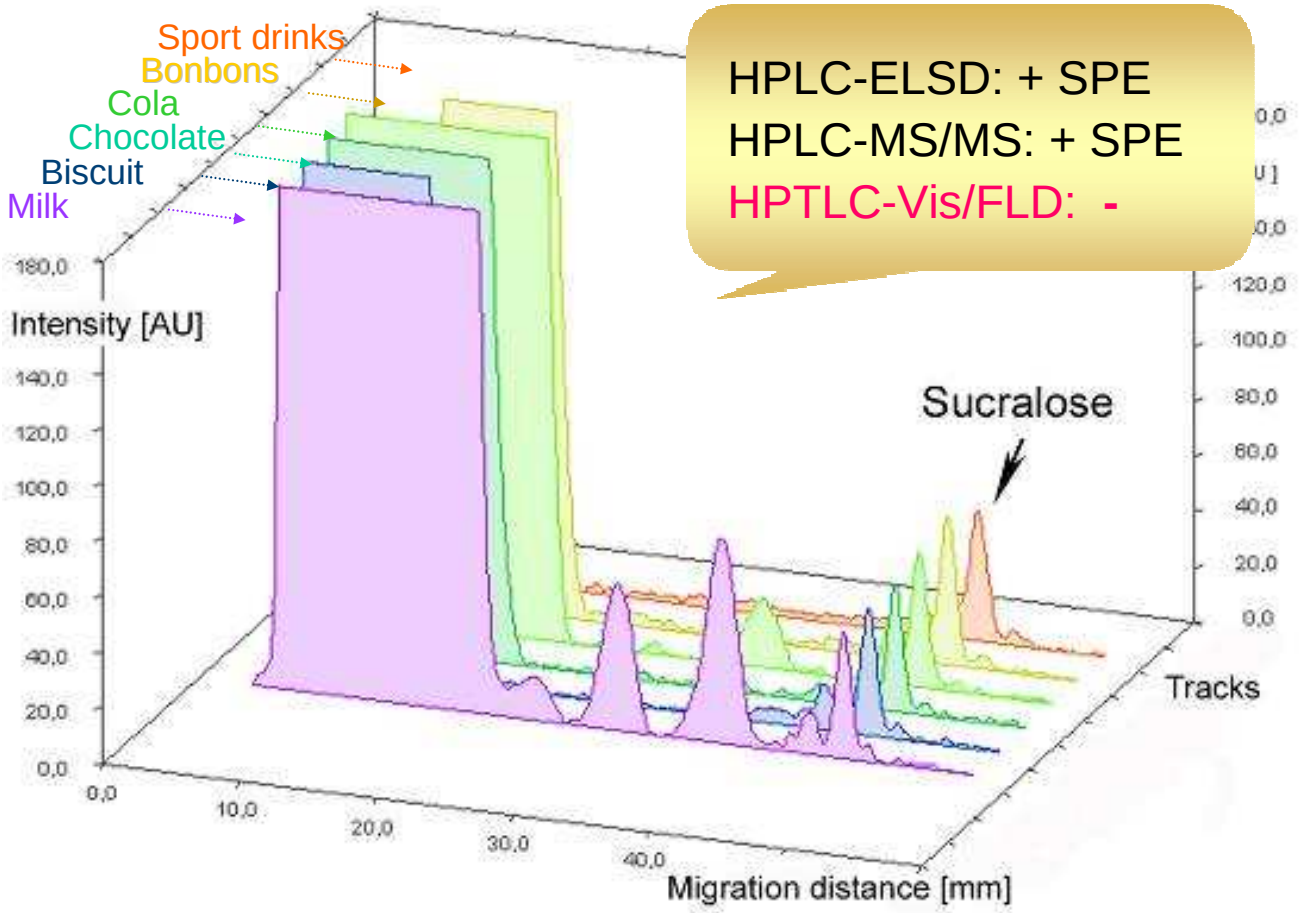
… in further matrices



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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Sample preparation and chromatography



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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Facts for sucralose analysis

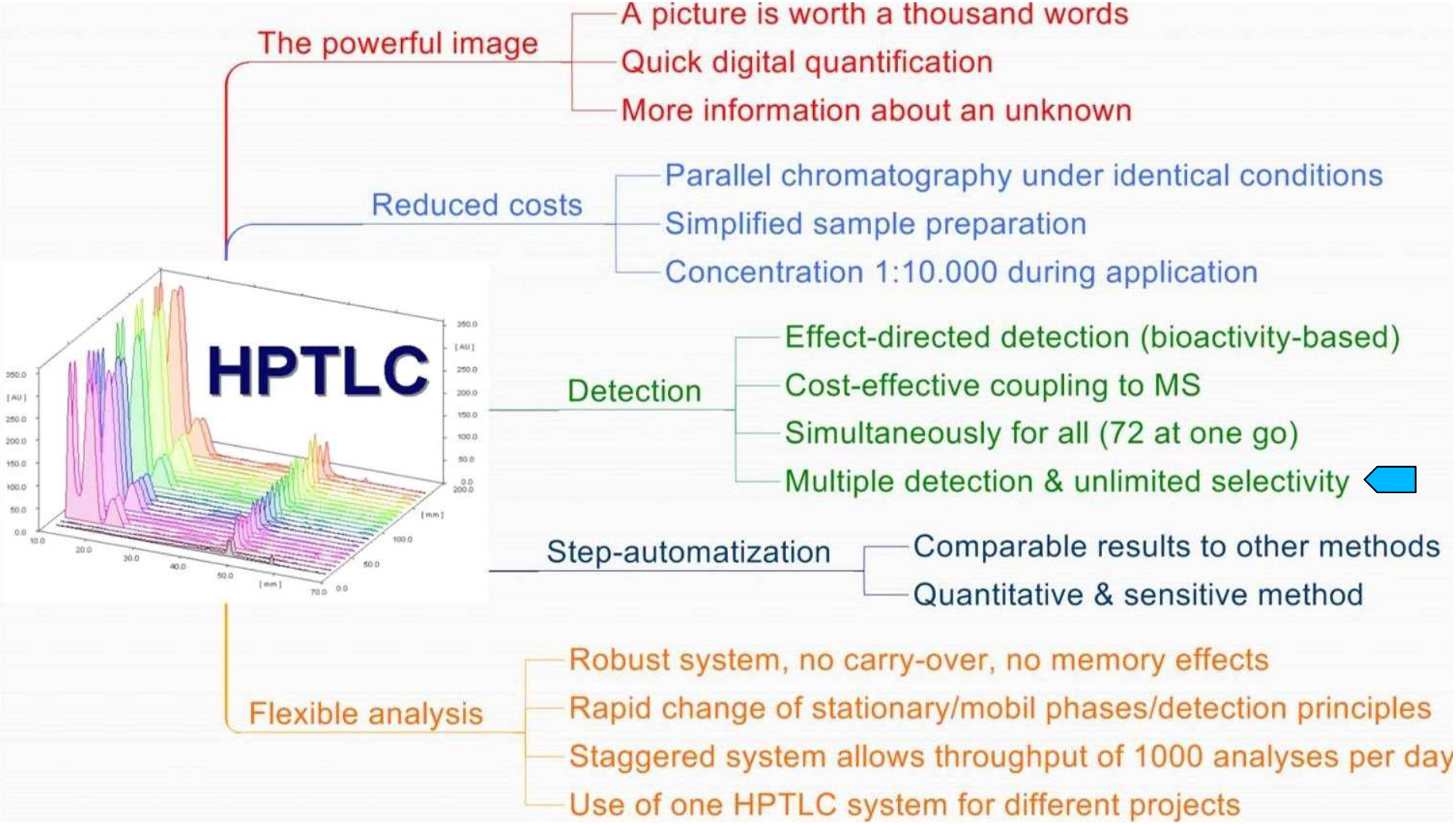
- High throughput (46 runs in 15 min by (anti-)parallel development, 15 min-staggered offline system) → 1000 runs/8h-day
- Resulting in 20-s runs with 330 µL solvent consumption
- Almost no disposal costs < 0.01 Cent/run
- Selective derivatization → compensates low separation power
- Reduced sample preparation: 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

Institute of Food Chemistry
University of Hohenheim, Stuttgart

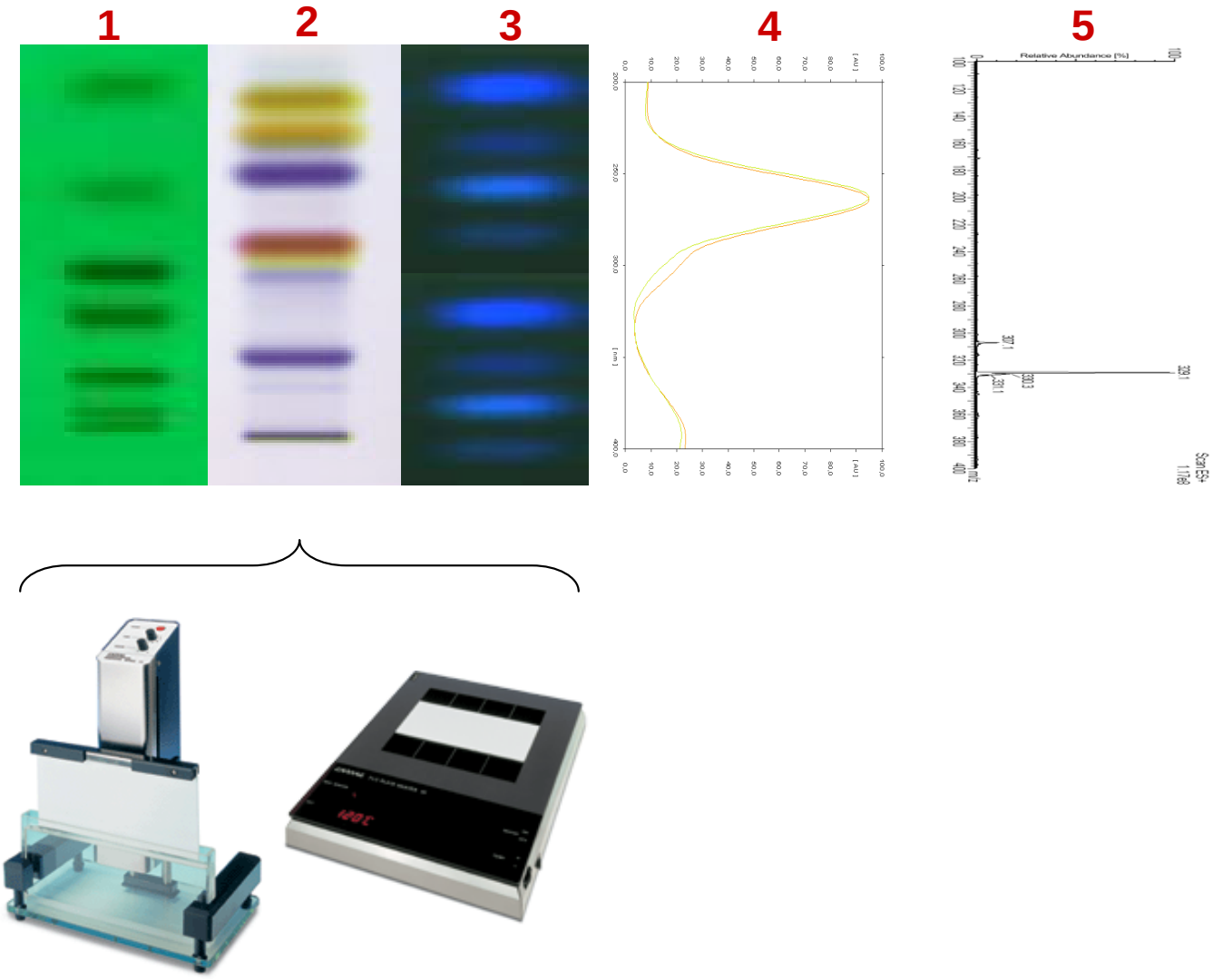
Benefits



Institute of Food Chemistry
University of Hohenheim, Stuttgart

Multiple detection on a single track/plate

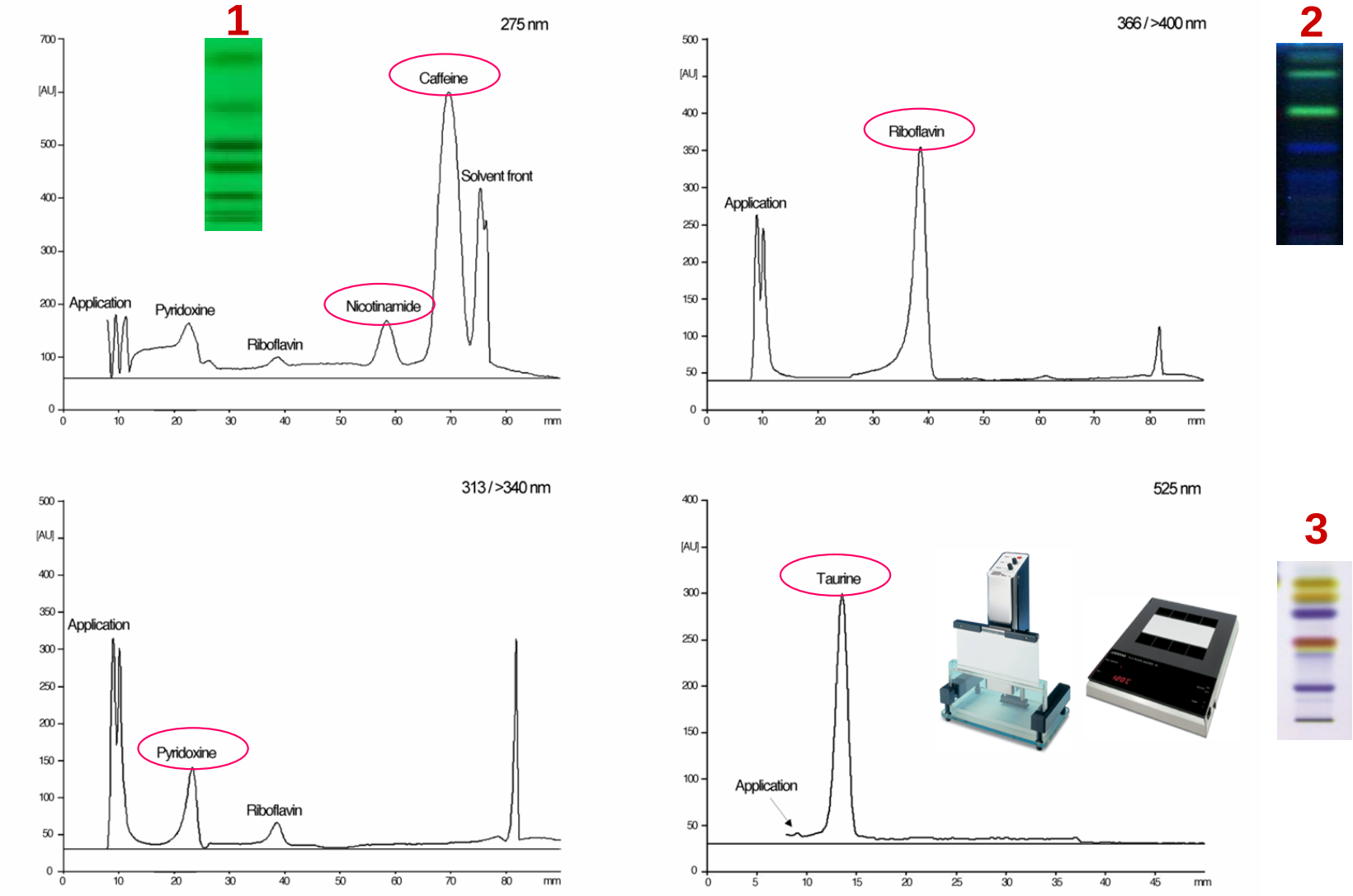
HPTLC-UV/Vis/FLD-MS



Institute of Food Chemistry
University of Hohenheim, Stuttgart

All active ingredients in energy drinks

Simultaneous determination by MWL scan for UV/FLD → derivatization → Vis…



Institute of Food Chemistry
University of Hohenheim, Stuttgart

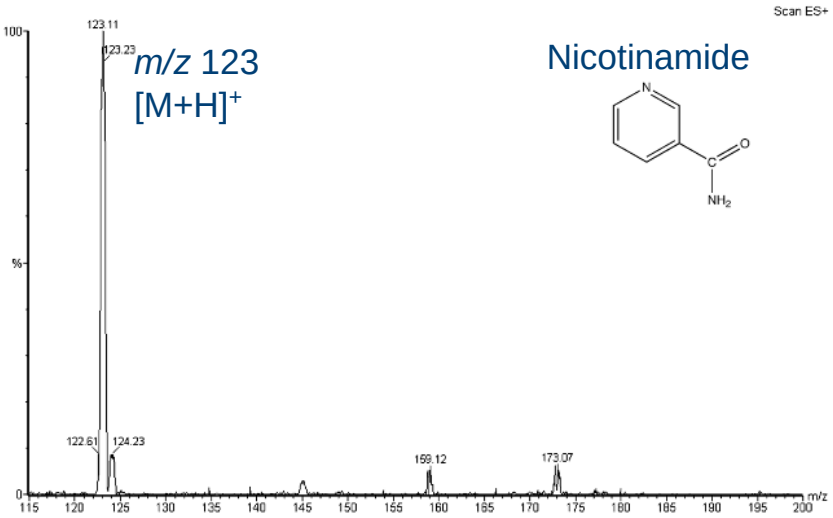
All active ingredients in energy drinks

→ Confirmation by MS or UV spectra

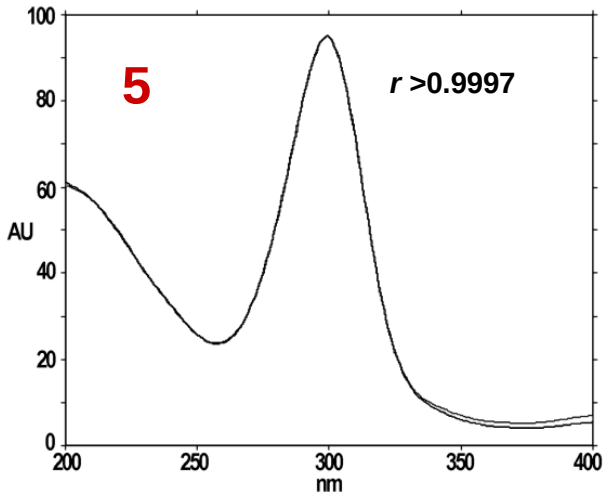
4

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

Nicotinamide

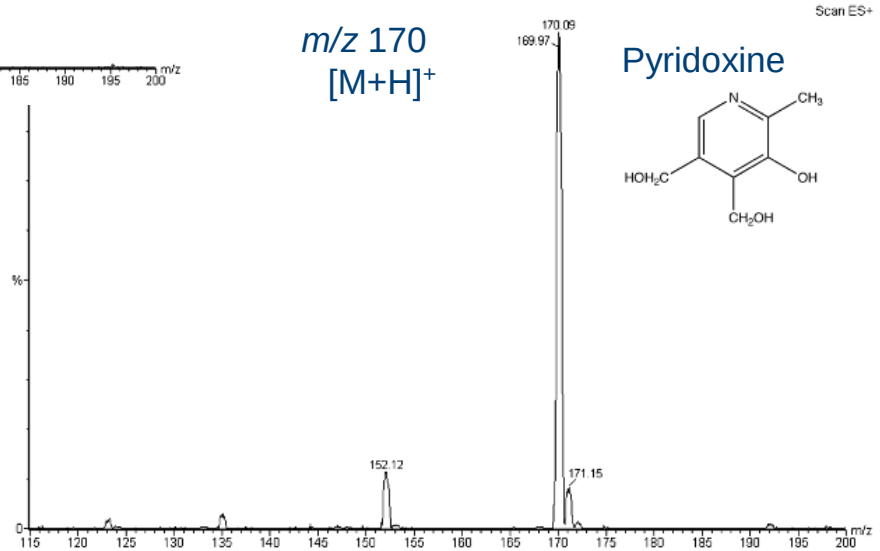


5



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

Pyridoxine

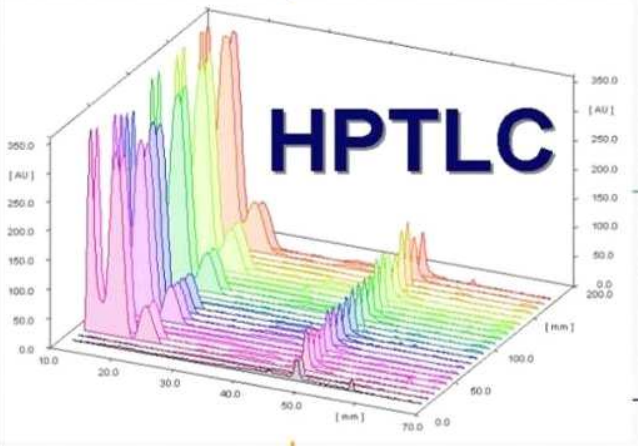


M. Aranda, G. Morlock, J Chromatogr A 1131 (2006) 253-260

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Benefits

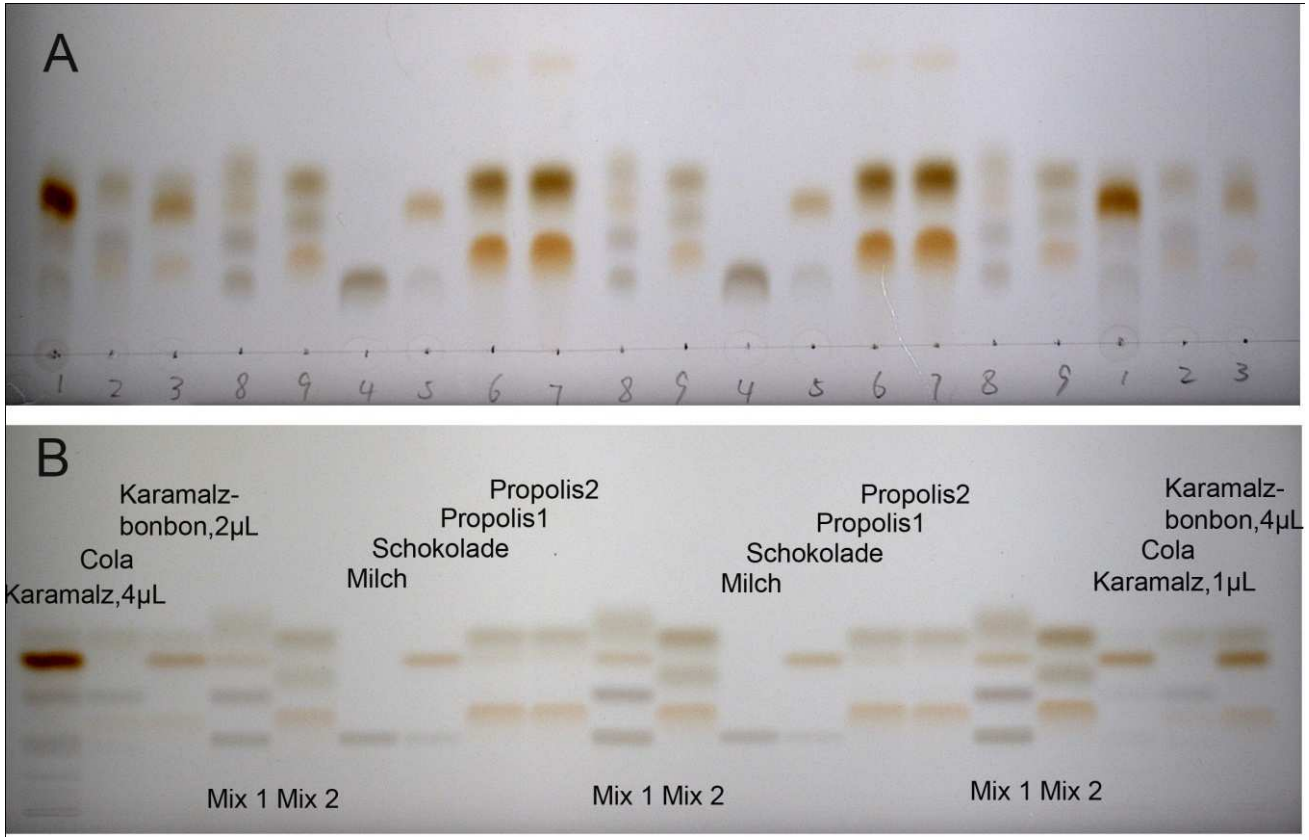
- The powerful image
  - A picture is worth a thousand words
  - Quick digital quantification
  - More information about an unknown
- Reduced costs
  - Parallel chromatography under identical conditions
  - Simplified sample preparation
  - Concentration 1:10.000 during application



- Detection
  - Effect-directed detection (bioactivity-based)
  - Cost-effective coupling to MS
  - Simultaneously for all (72 at one go)
  - Multiple detection & unlimited selectivity
- Step-automatization
  - Comparable results to other methods
  - Quantitative & sensitive method
- Flexible analysis
  - Robust system, no carry-over, no memory effects
  - Rapid change of stationary/mobil phases/detection principles
  - Staggered system allows throughput of 1000 analyses per day
  - Use of one HPTLC system for different projects

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Method comparison



Poor quantitative results

Good quantitative results

Comparable to HPLC-ELSD?

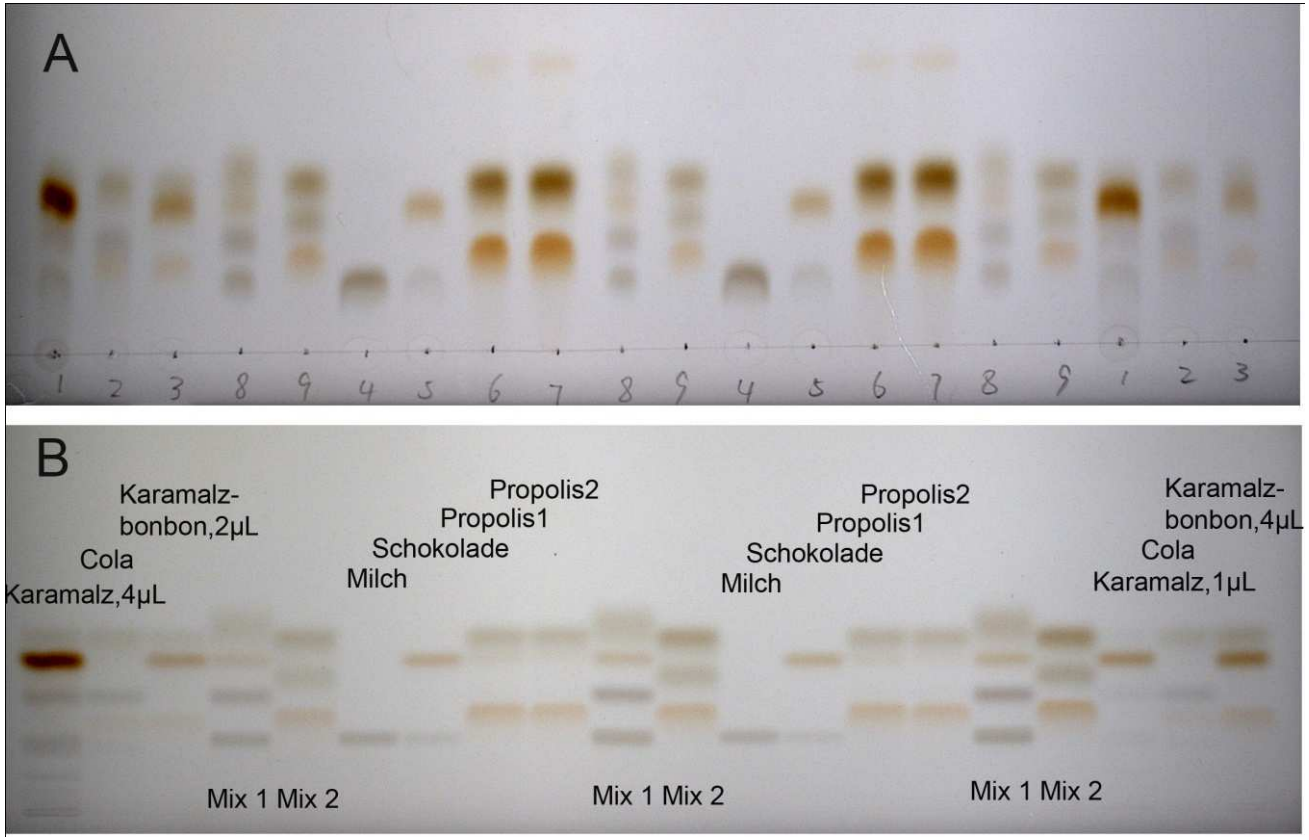
Institute of Food Chemistry
University of Hohenheim, Stuttgart

Method comparison

Sample	Sugar found (n = 2)	HPLC-ELSD		HPTLC-Vis	
		%	*%RSD*	%	*%RSD*
Cola	Sucrose	**12.0**	0.1	**12.5**	5.3
	Fructose	**1.1**	0.4	**1.1**	5.3
	Glucose	**1.3**	4.5	**-**	-
Milk	Lactose	**8.0***	5.6	**5.3**	1.8
Chocolate	Sucrose	**34.9**	0.5	**35.9**	0.8
	Lactose	**6.9**	12.9	**7.1**	10.0
Propolis	Glucose	**10.9**	9.3	**10.7**	1.1
	Fructose	**17.3**	0.0	**17.4**	6.0
	Sucrose	**4.4***	5.0	**7.3***	7.0
Karamalt	Glucose	**3.3**	2.8	**4.1**	3.9
	Fructose	**2.2**	0.7	**2.0**	1.6
	Maltose	**2.5**	2.5	**2.5**	6.5
Biscuits	Sucrose	**17.9***	1.2	**23.9***	1.8

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Method comparison



Poor quantitative results

Good quantitative results

Comparable to HPLC-ELSD? => YES

Analyses time

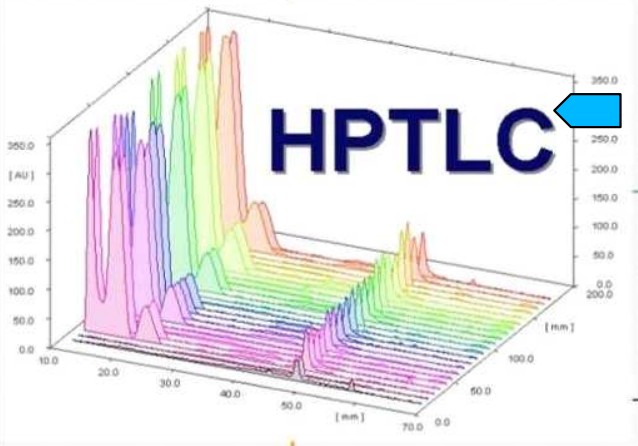
- HPTLC: 1 h => 3 min per sample
- HPLC: 5.3 h => 16 min per sample

G. Morlock, G. Shabier, in preparation

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Benefits

- The powerful image
  - A picture is worth a thousand words
  - Quick digital quantification
  - More information about an unknown
- Reduced costs
  - Parallel chromatography under identical conditions
  - Simplified sample preparation
  - Concentration 1:10.000 during application

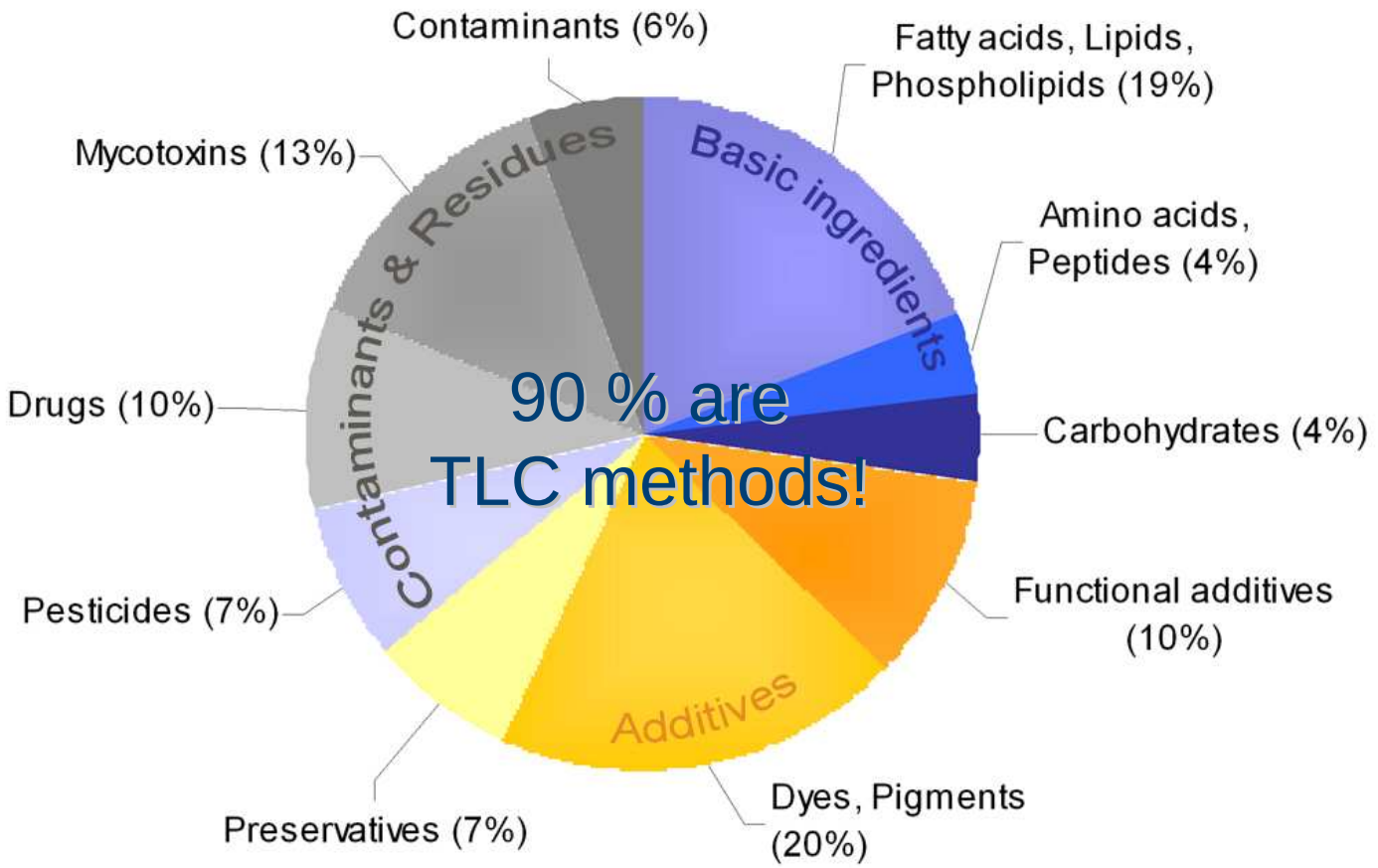


- Detection
  - Effect-directed detection (bioactivity-based)
  - Cost-effective coupling to MS
  - Simultaneously for all (72 at one go)
  - Multiple detection & unlimited selectivity
- Step-automatization
  - Comparable results to other methods
  - Quantitative & sensitive method
- Flexible analysis
  - Robust system, no carry-over, no memory effects
  - Rapid change of stationary/mobil phases/detection principles
  - Staggered system allows throughput of 1000 analyses per day
  - Use of one HPTLC system for different projects

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Planar Chromatography

Food analysis 1987-2007



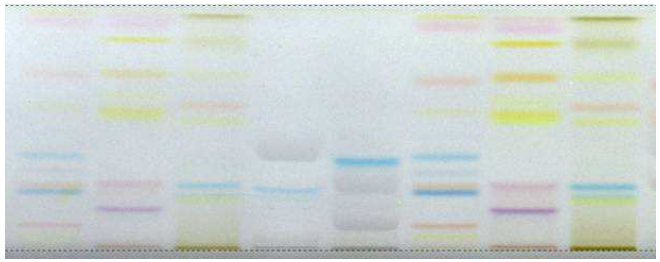
G. Morlock, W. Schwack, J Planar Chromatogr 20 (2007) 399-407

Institute of Food Chemistry
University of Hohenheim, Stuttgart

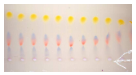
Planar Chromatography

- 1938 TLC
  Thin-layer chromatography

- 1975 HPTLC
  High-performance TLC



- 2001 UTLC
  UltraTLC

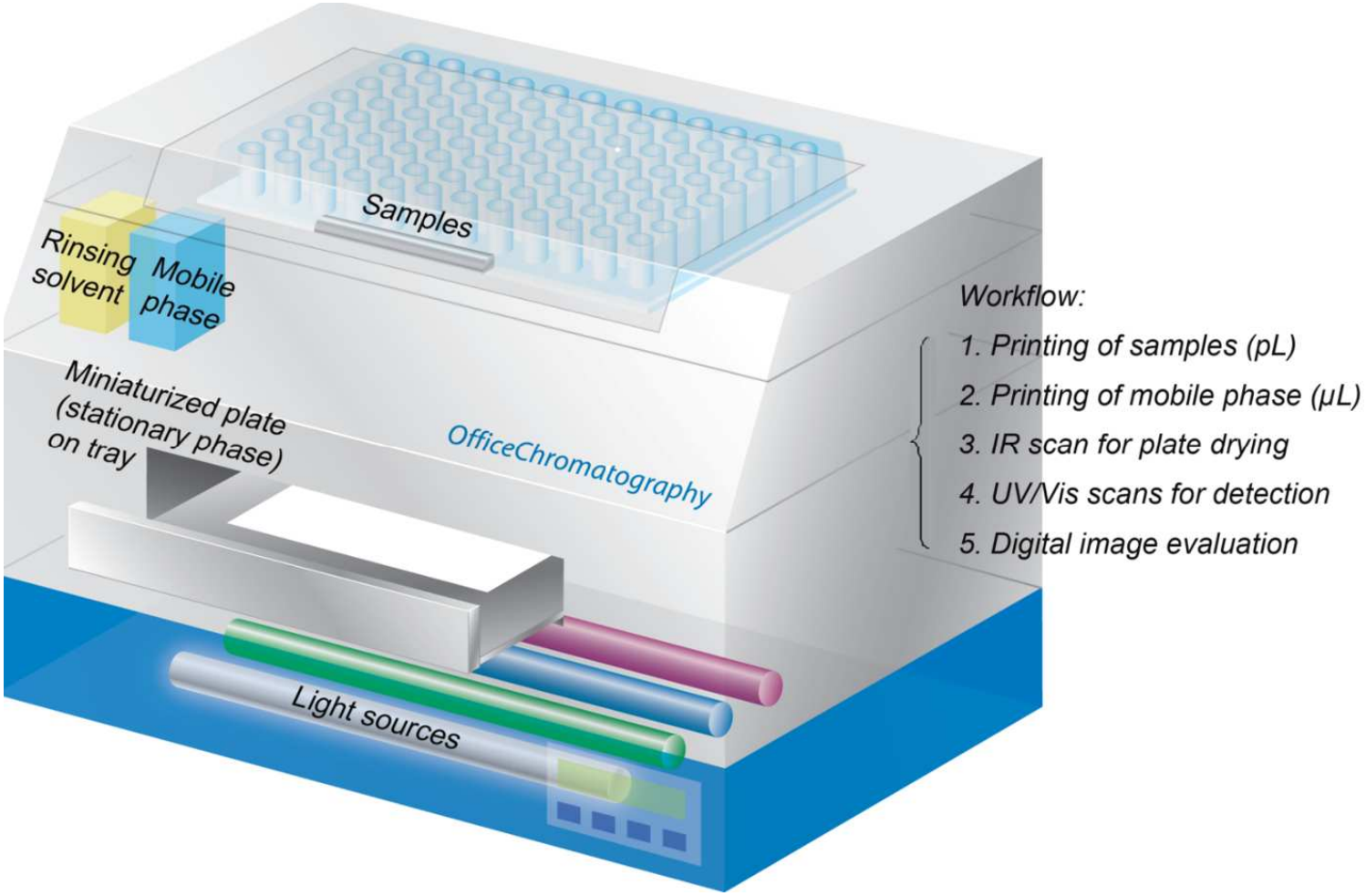


J. Sherma, G. Morlock, J. Planar Chromatogr. 21 (2008) 471-477

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Office Chromatography

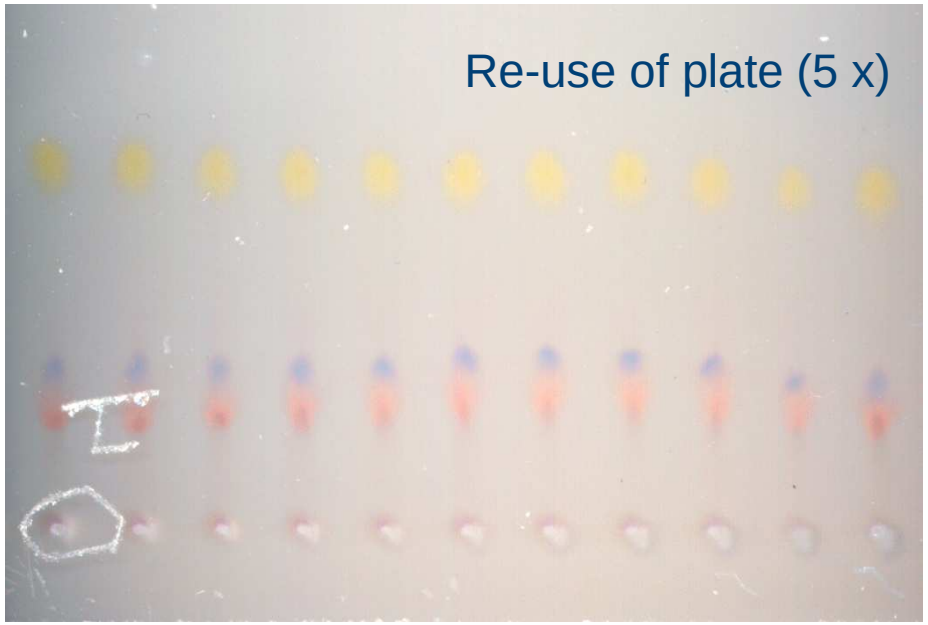
(Miniaturized) Planar chromatography using office peripherals

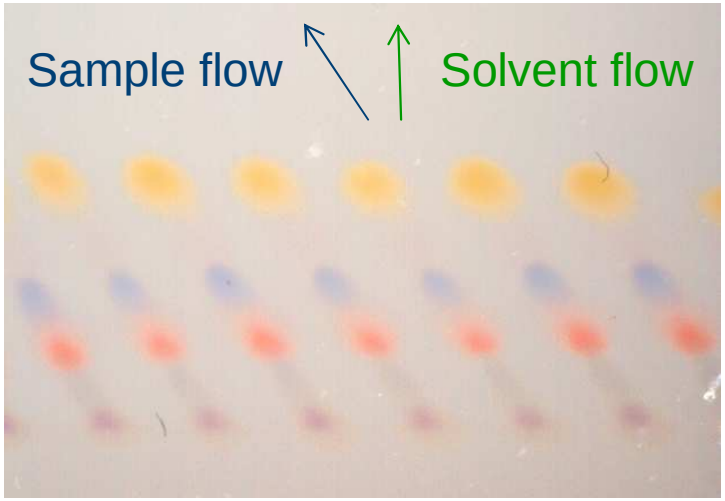


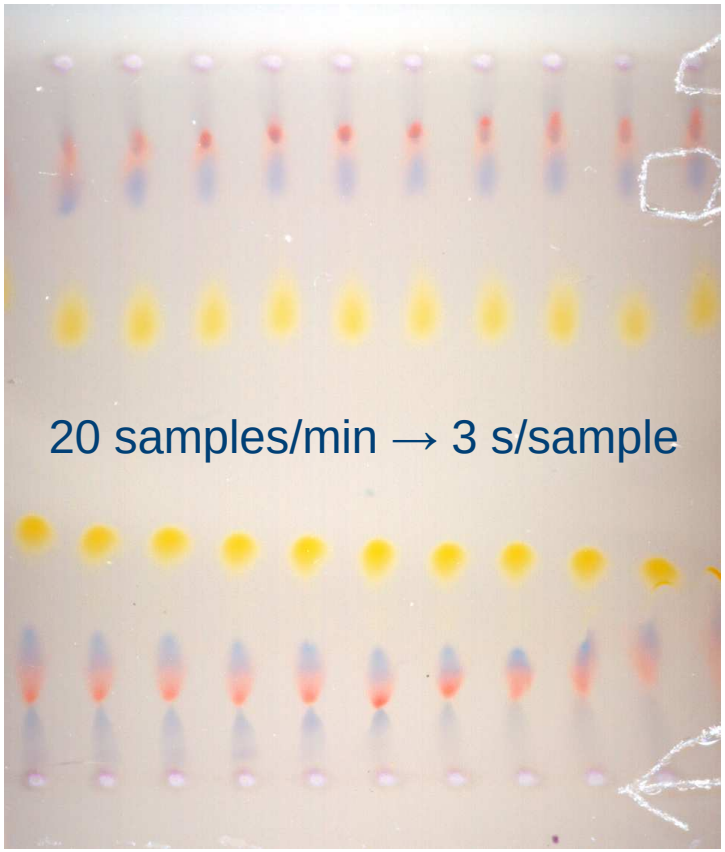
G. Morlock, C. Oellig, L. Bezuidenhout, M. Brett & W. Schwack,
Anal. Chem. 82 (2010) 2940-2946

Institute of Food Chemistry
University of Hohenheim, Stuttgart

Nanostructured UTLC plates







Institute of Food Chemistry
University of Hohenheim, Stuttgart

Ultrathin plate (UTLC)

S. Jim, M. Taschuk, G. Morlock, L. Bezuidenhout, W. Schwack, M. Brett
Anal. Chem. 82 (2010) in print

HPTLC 2011, Basel : 6th-8th July 2011 → www.hptlc.com