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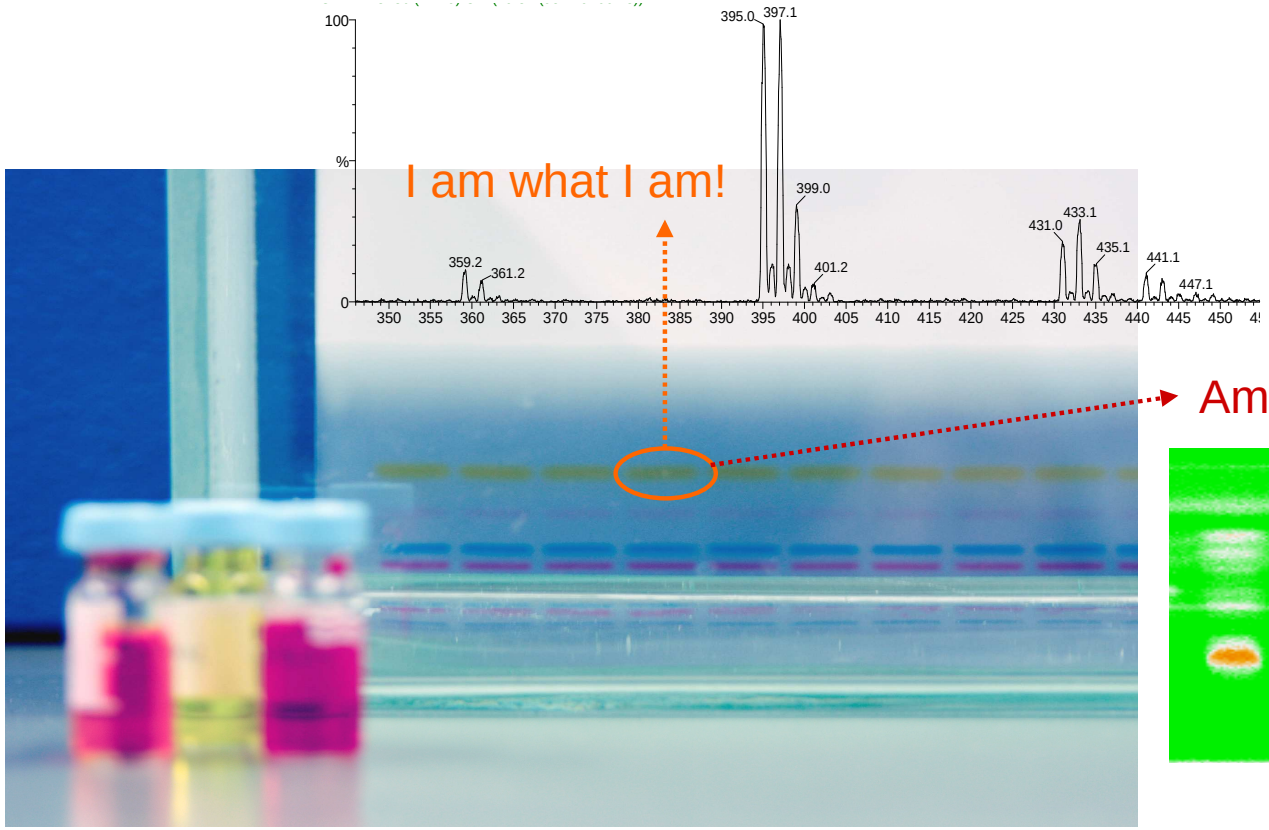
Why choosing HPTLC?



G. Morlock, Institute of Food Chemistry
University of Hohenheim, Stuttgart

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Where TLC is…

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HPTLC → Part of modern quantitative analysis

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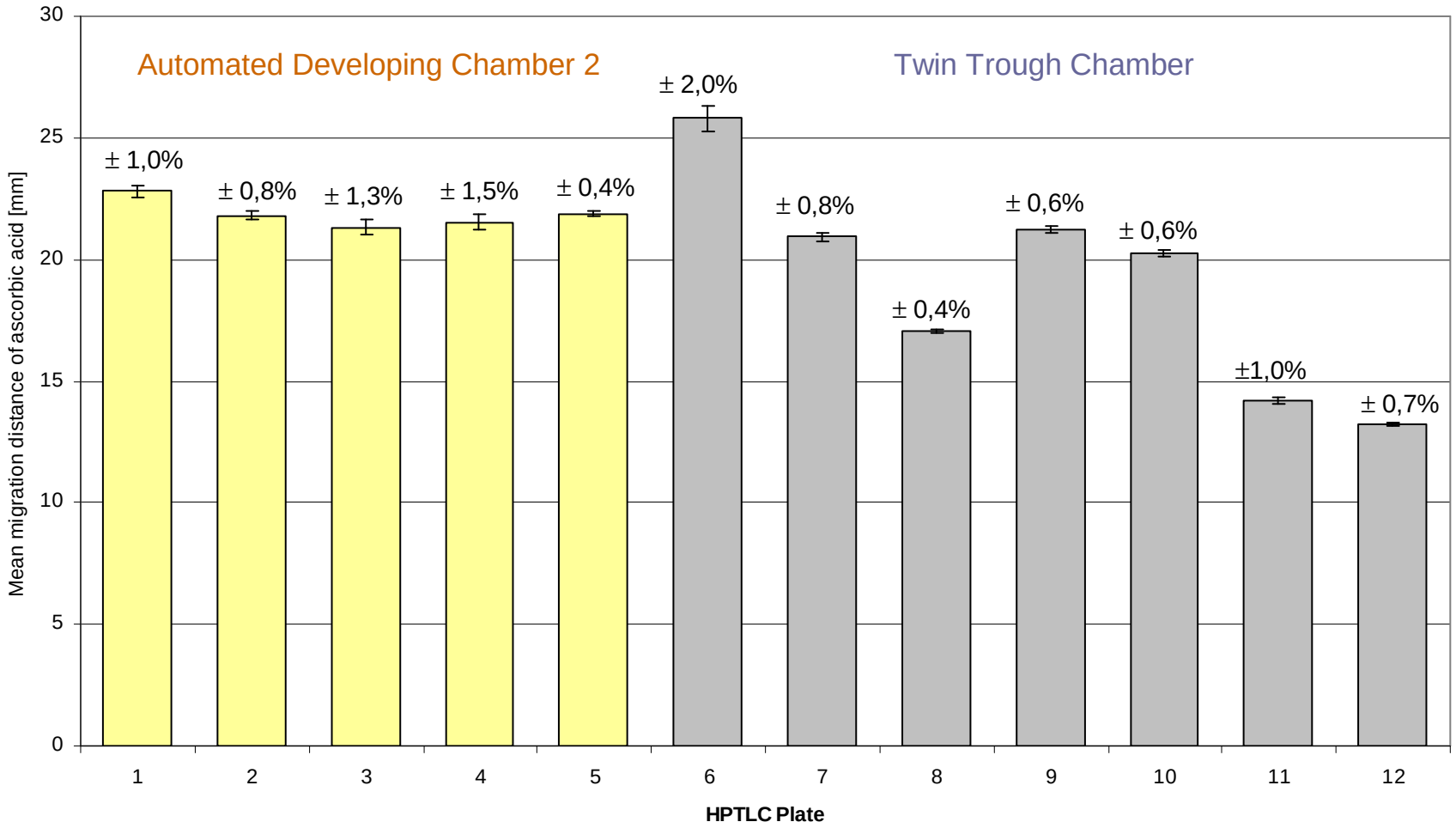
HPTLC → Automated equipment per step

→ Chamber climate control enables reproducibility



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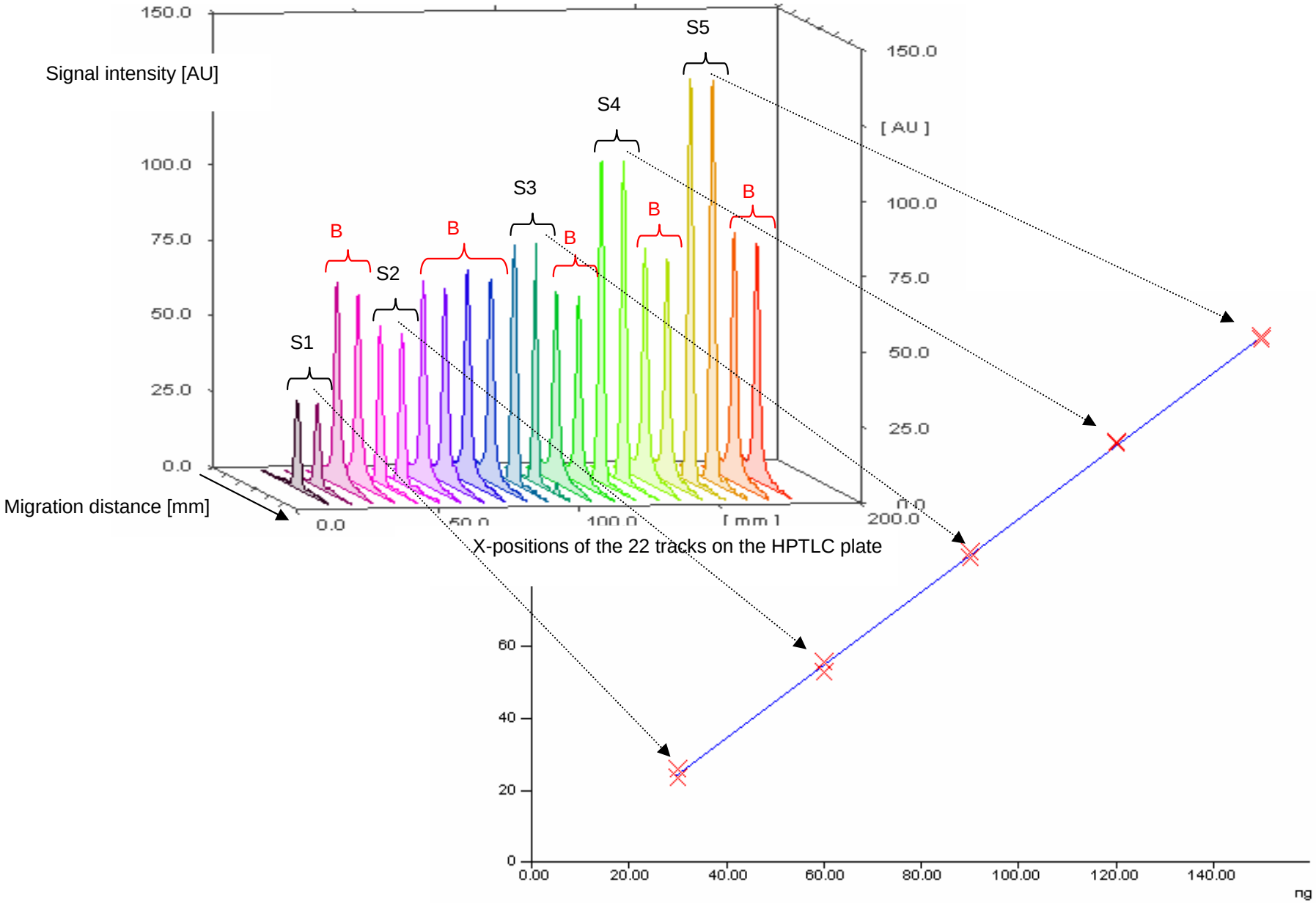
HPTLC → Quantitative method



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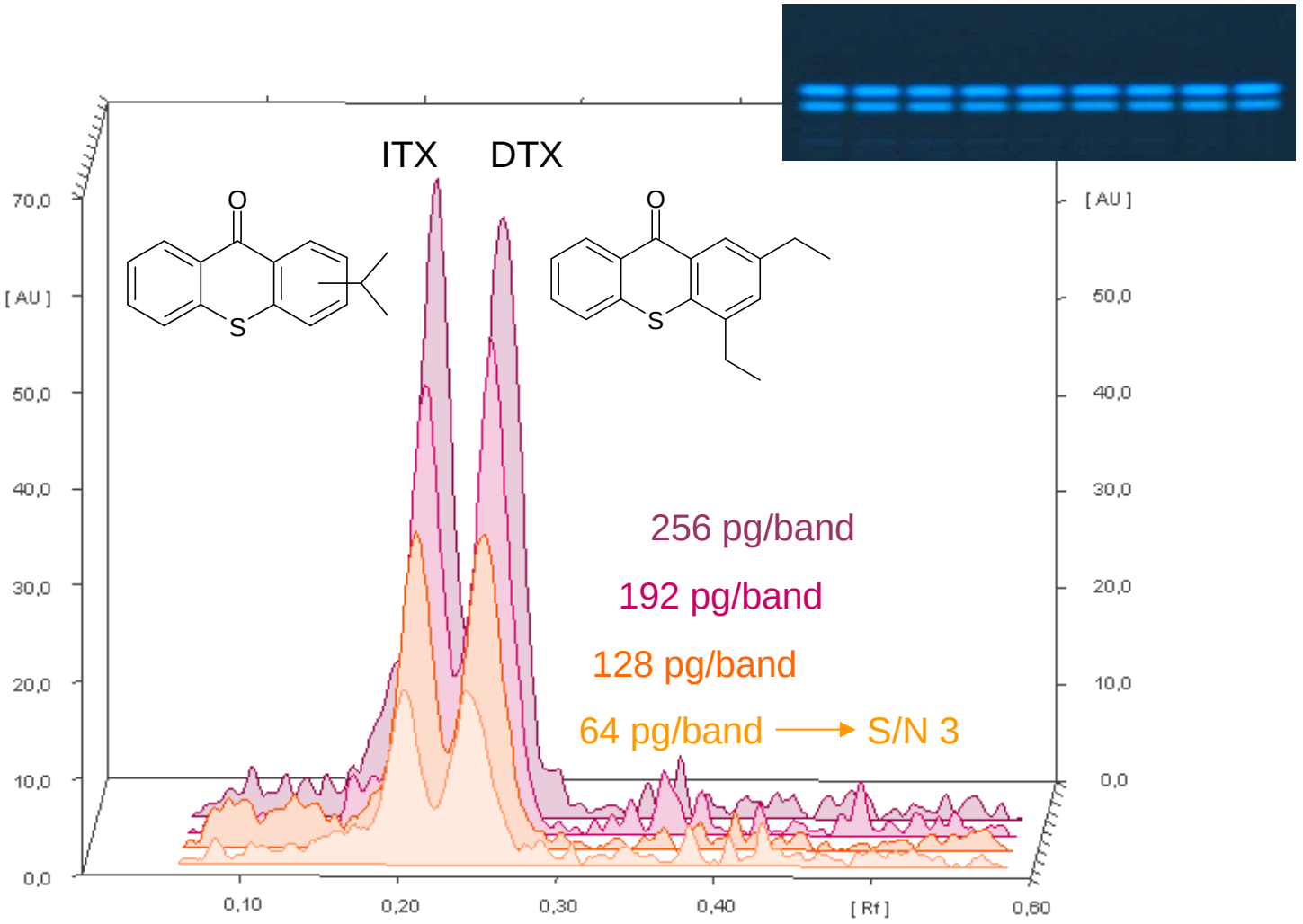
HPTLC → Sensitive method (detectability)



G. Morlock, W. Schwack, Anal. Bioanal. Chem 385 (2006) 586-595

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… but other methods as well → Why HPTLC?

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Plate heights of the different methods

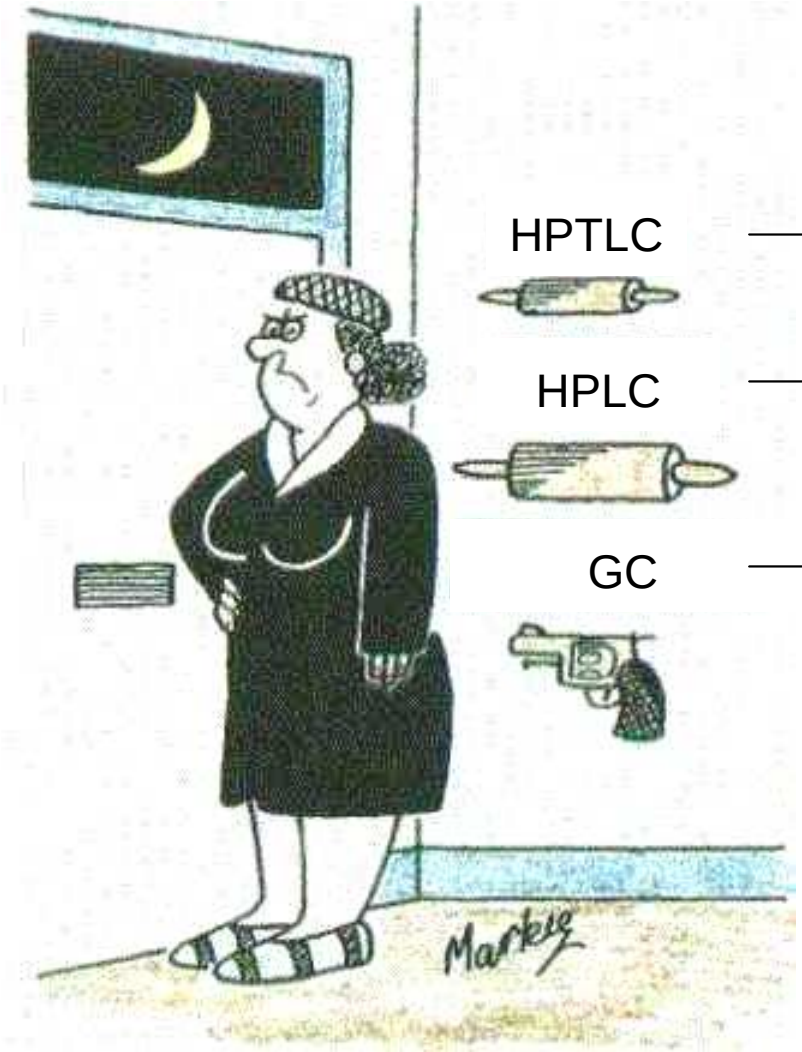

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Plate numbers → Why HPTLC?

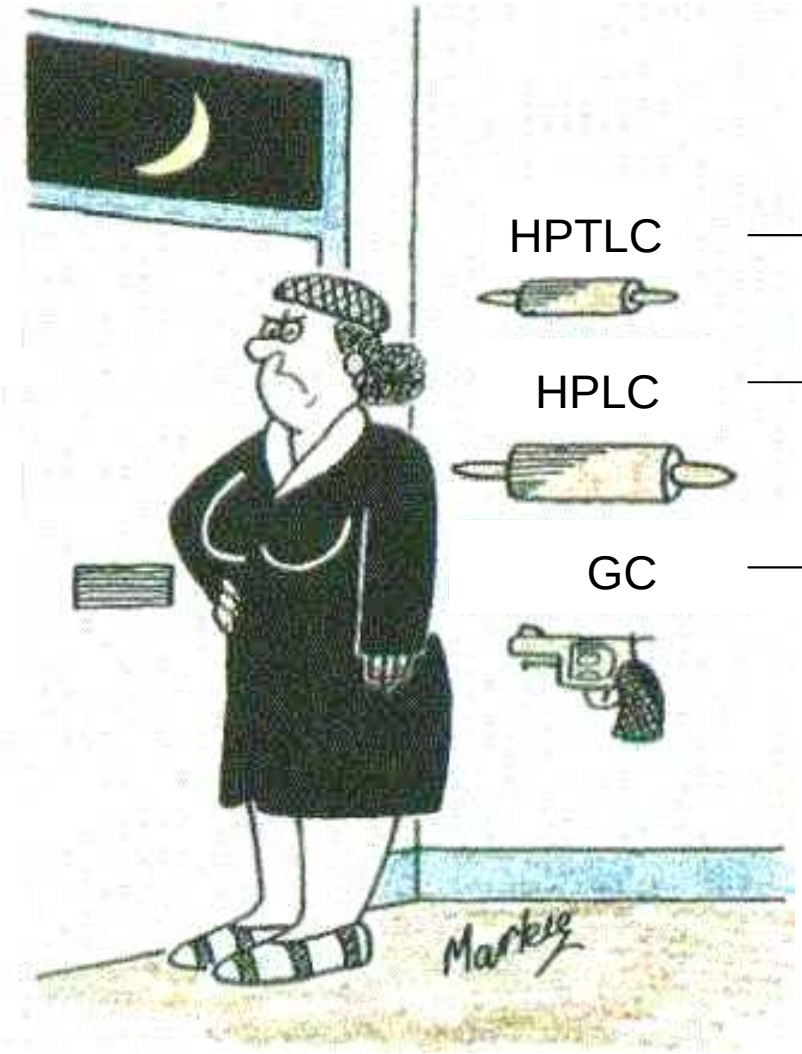

	Plate number *N*
HPTLC →	8.000
HPLC →	30.000
GC →	100.000

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Why HPTLC?

Reaching the water source you have to swim against the mainstream. *Konfuzius*

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Why choosing HPTLC?

1. Gives more information about an unknown
2. Tolerates minimized sample preparation
3. Enables concentration during application up to a factor of 10.000
4. Capable of high throughput (300 runs per day) with minimal costs
5. Runs parallel chromatography under identical environmental conditions
6. Enables selective and simultaneous derivatization (variety of reagents)
7. Enables multiple detection (UV/Vis, FLD, derivatization, MS)
8. Allows toxicity-directed detection (information directed to the effect)
9. Runs highly-targeted, cost-effective HPTLC-MS where separation solvent can be chosen independently from MS
10. Is a very flexible working station

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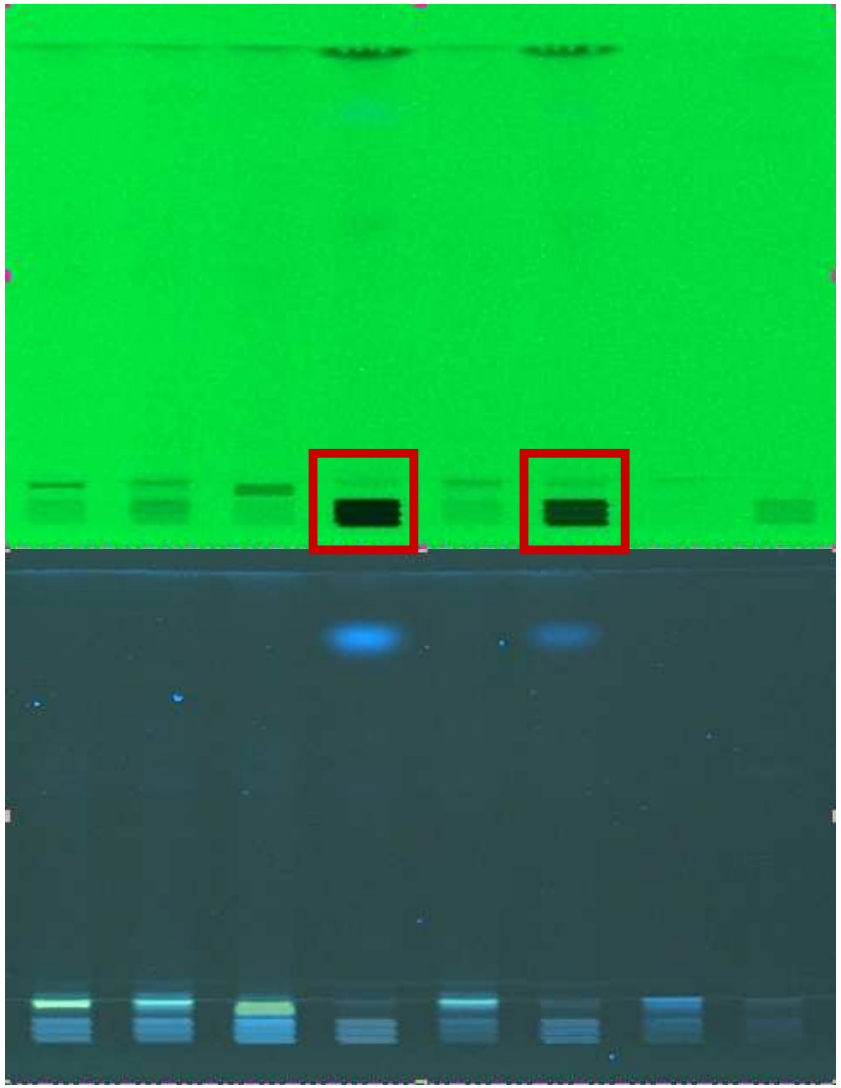
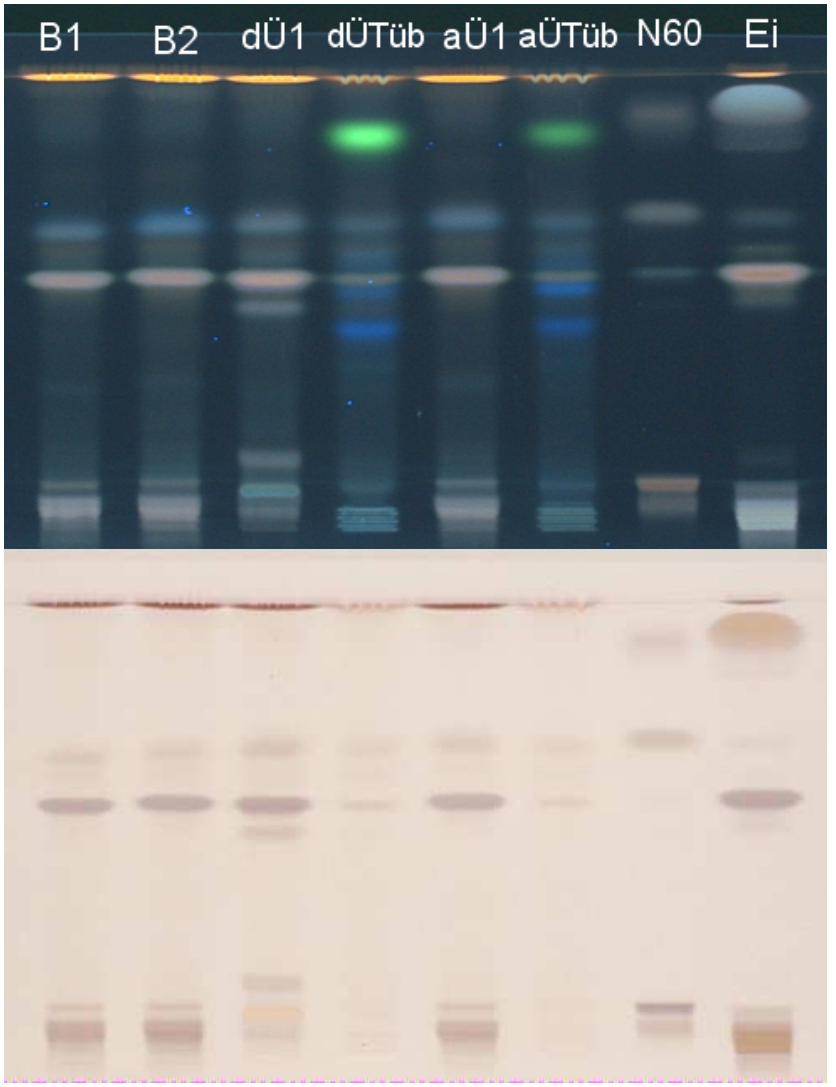
1. Gives more information about an unknown





Project: Find the difference in Lactobacillus fermentum supernatants

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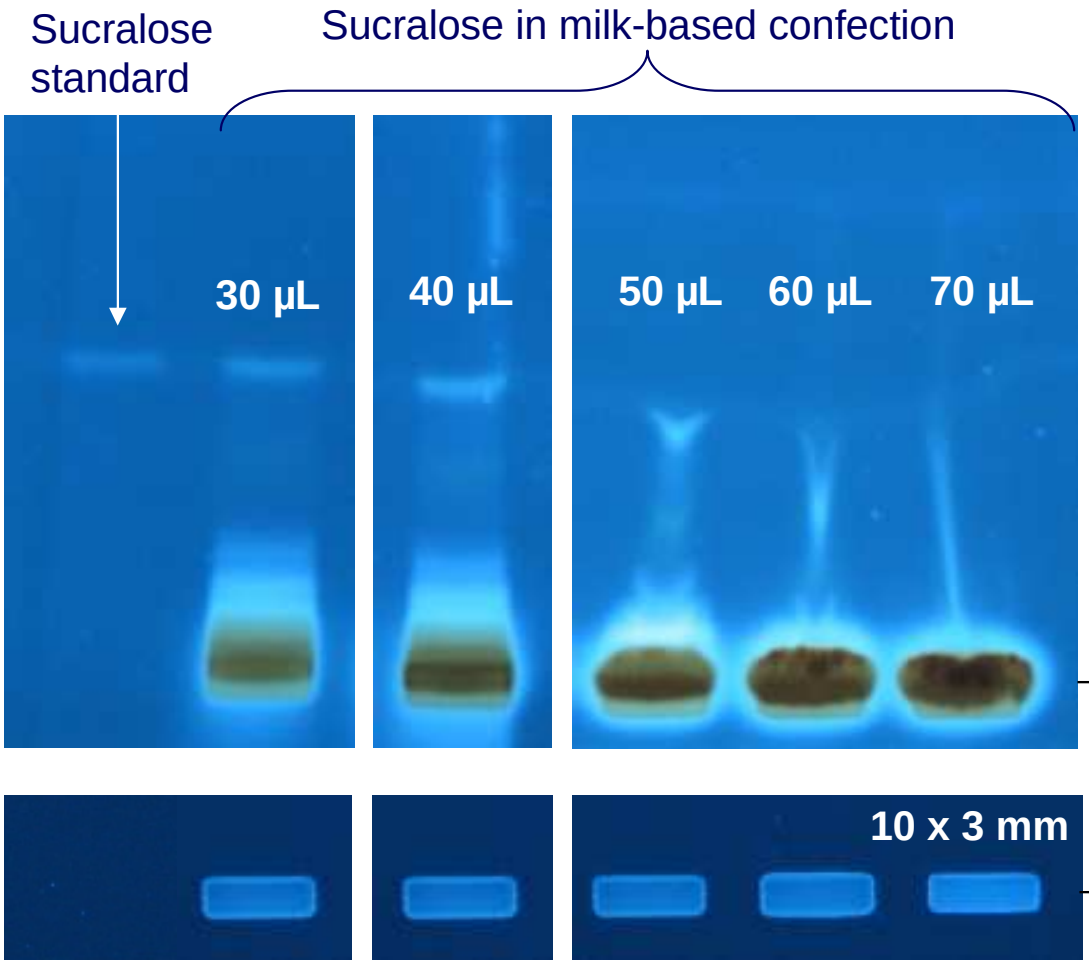
2. Tolerates minimized sample preparation

→ For high matrix-loading choose area application



→ Matrix is left at the start

→ Area application improves LOD by a factor of 10

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Matrix of milk-based confection left at the start

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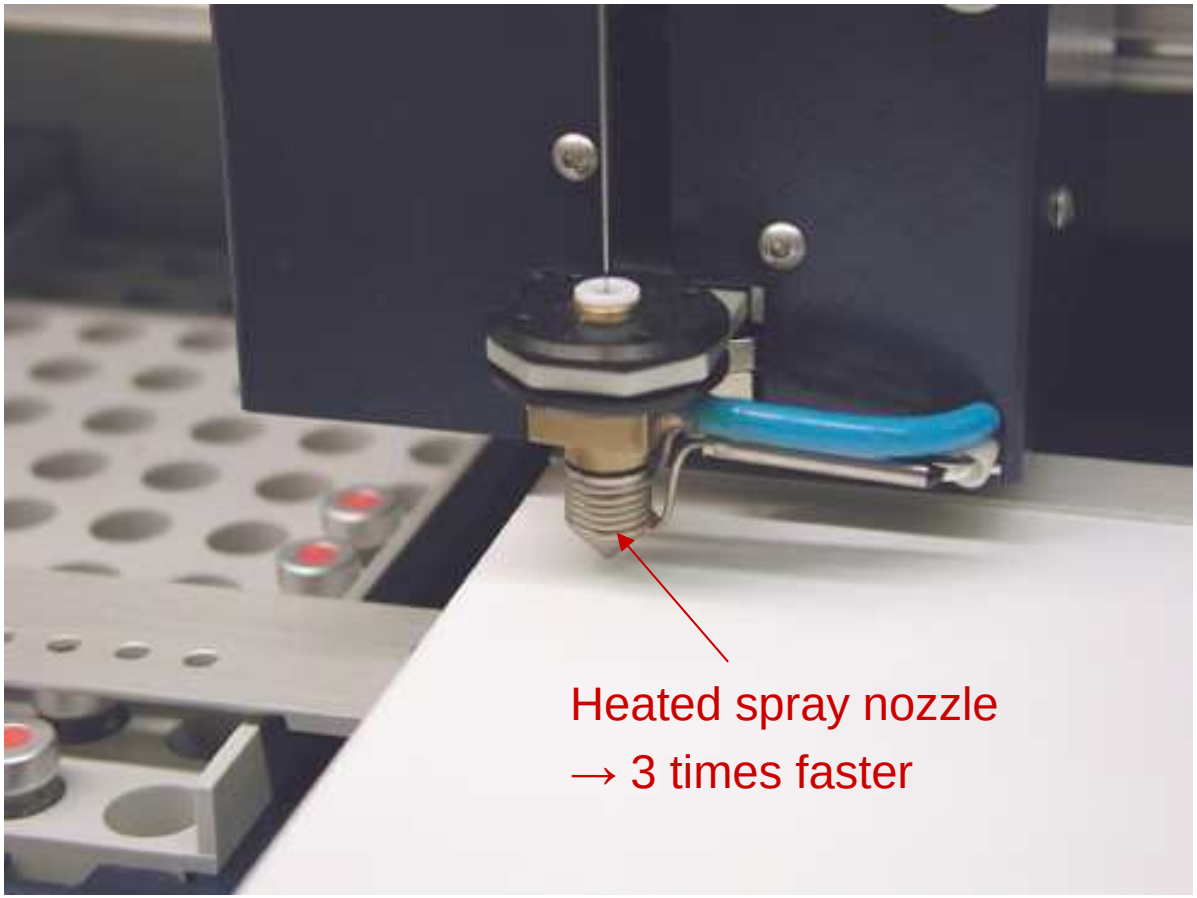
3. Enables concentration during application

→ Dynamic application volumes: 0.1 µL – 1 mL

→ Concentration factor of up to 10.000



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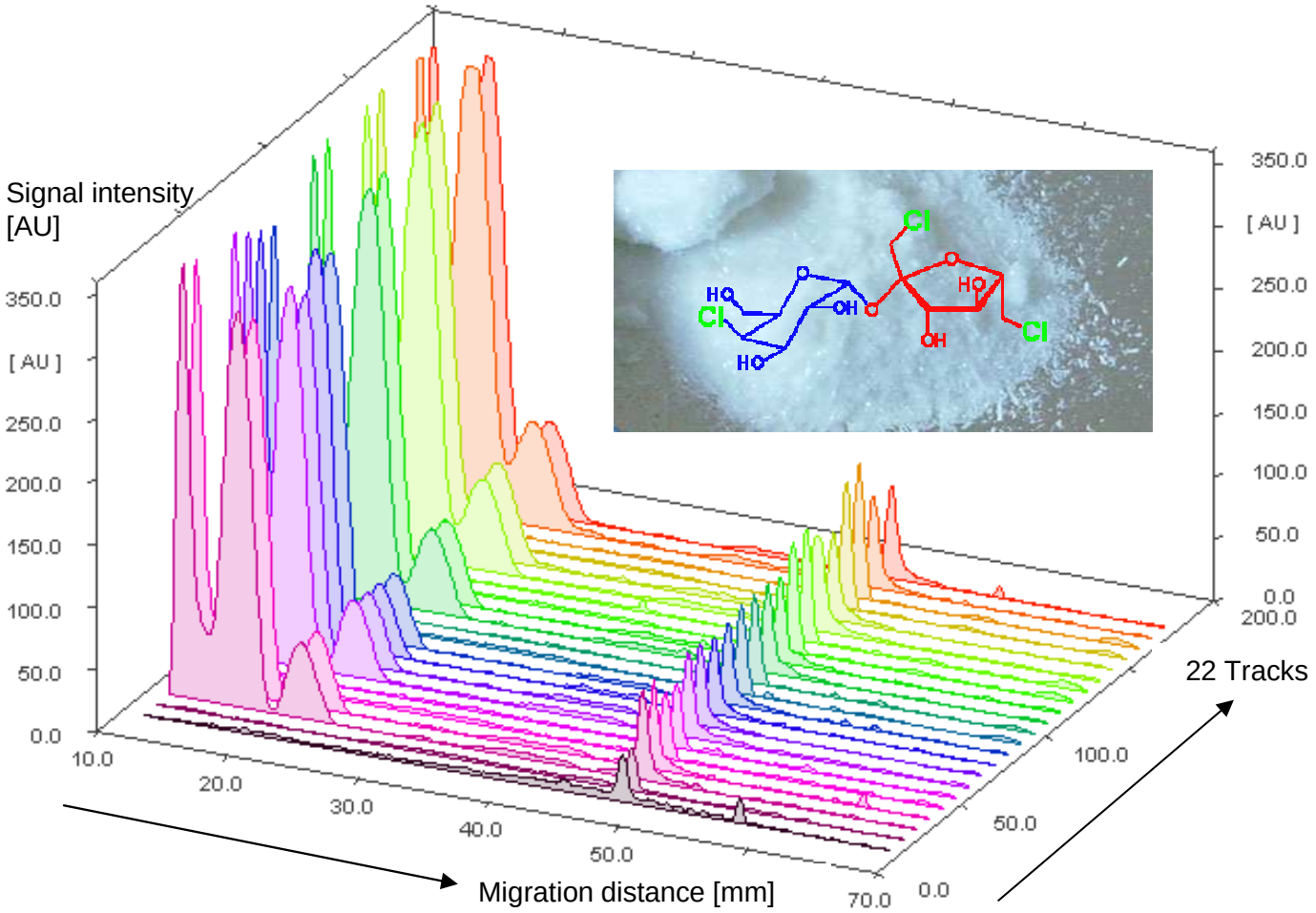
4. Capable of high throughput → parallel…



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

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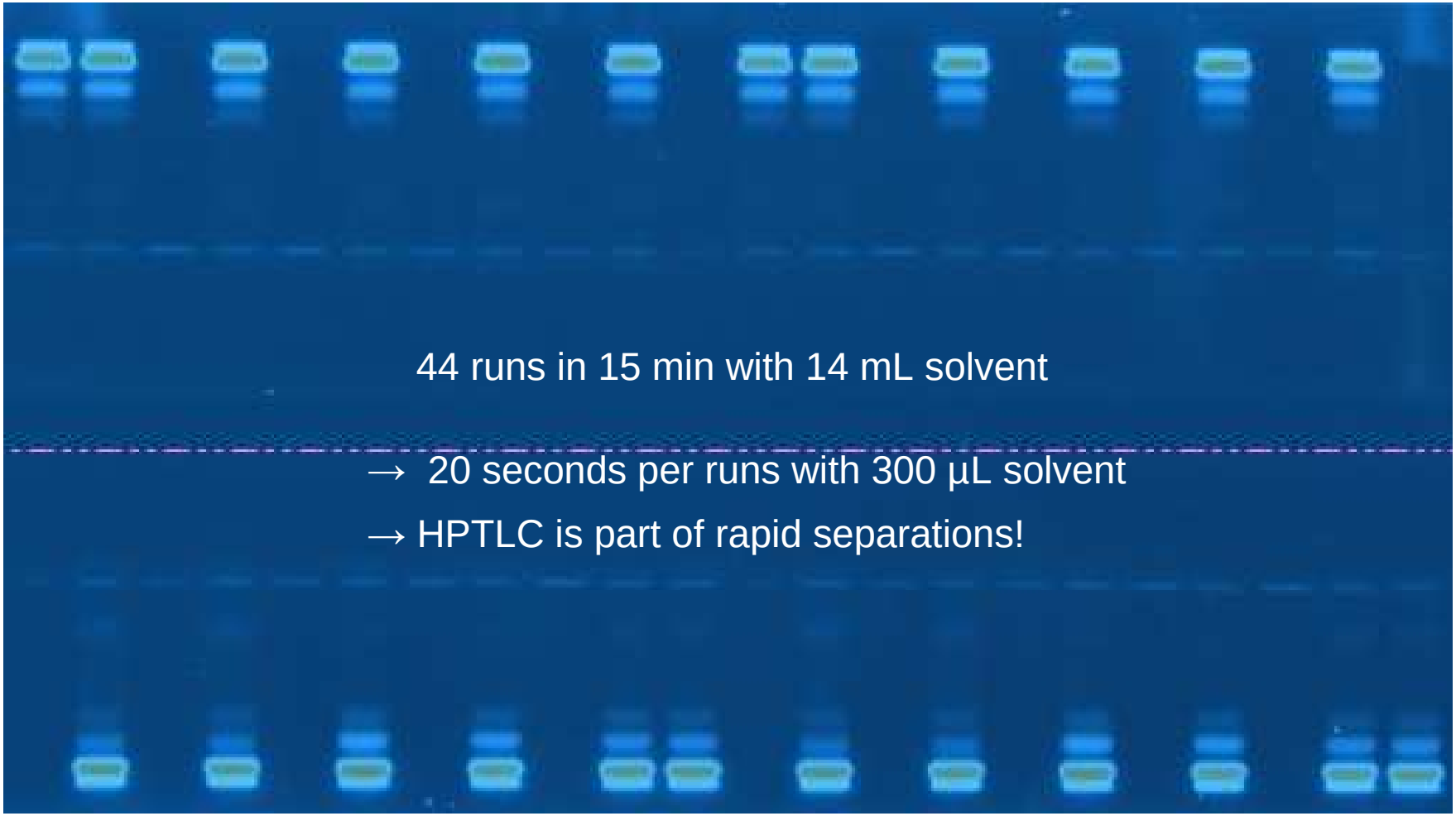
5. ... under identical environmental conditions

A) Sucralose quantification in milk-based confection



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

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Monitoring of products of hydrolysis

Part of the plate image illuminated at 366/>400 nm

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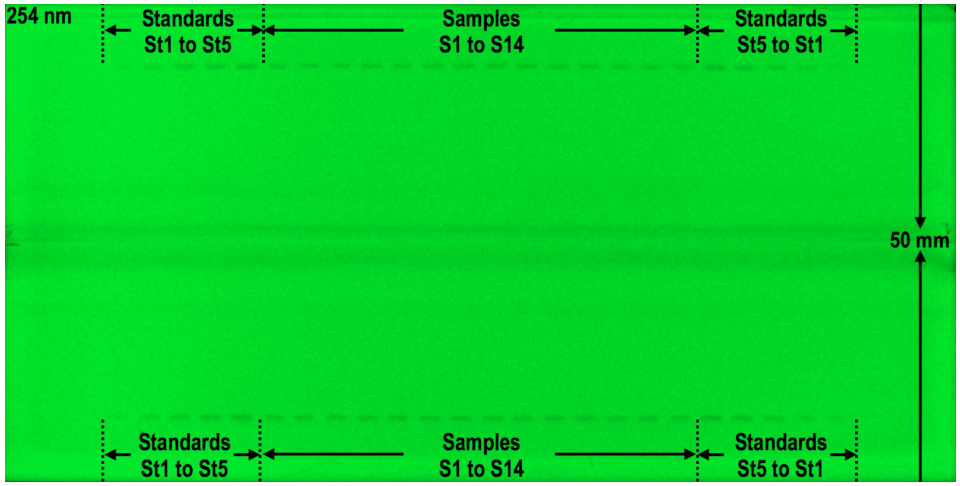
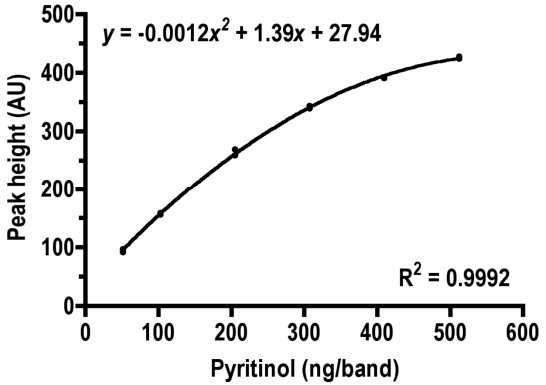
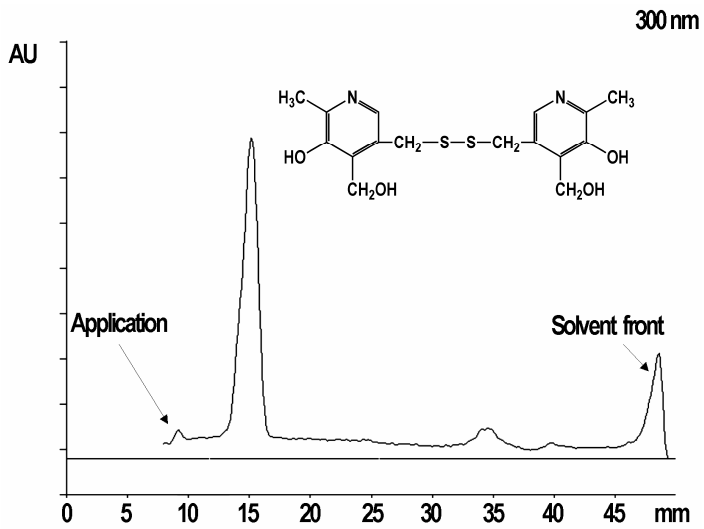
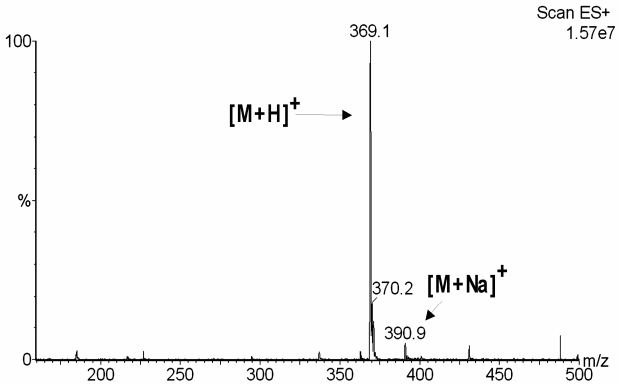
5. ... under identical environmental conditions

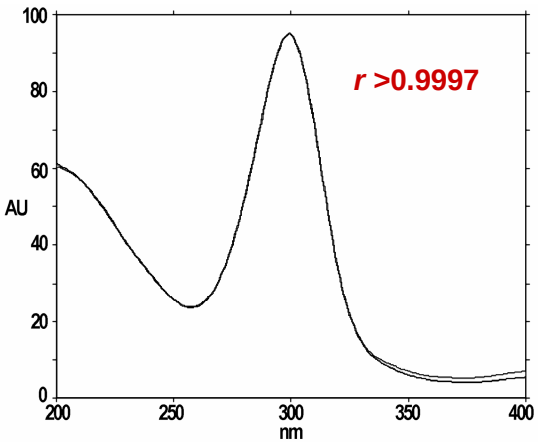
B) Pyridinol quantification in solid formulations











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5. ... under identical environmental conditions

B) Pyridinol quantification in solid formulations

→ Repeatability (n=6) in matrix of RSD = 0.4 %

→ Intermediate precision (n=3) in matrix of RSD = 2.95 %

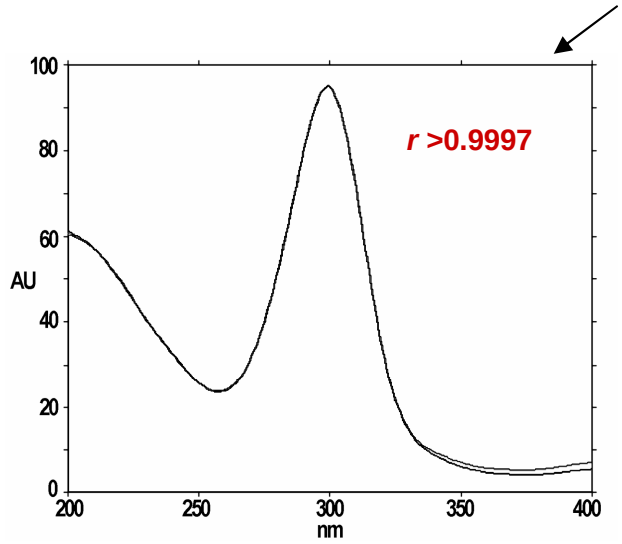
→ Recoveries of spiked samples (three levels) of 98.5 to 101.9% ± 3.6 to 4.7%

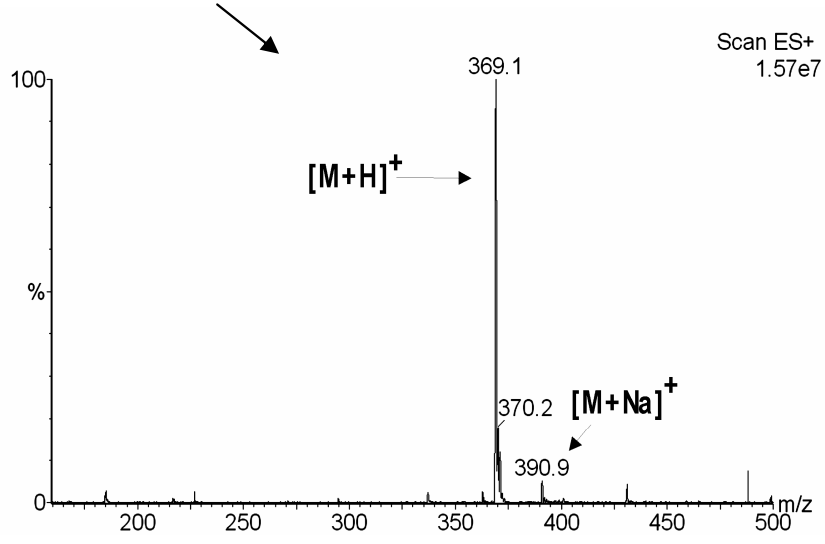
→ LOD/LOQ of 0.6 and 2.0 µg/mL (6 and 20 ng/band)

→ Up to 17 times less mobile phase consumption

→ At least 2 times faster (10 x 10 cm plate, one side)

→ Selectivity proved by spectra purity and MS





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In this case the plate number is highly sufficient!

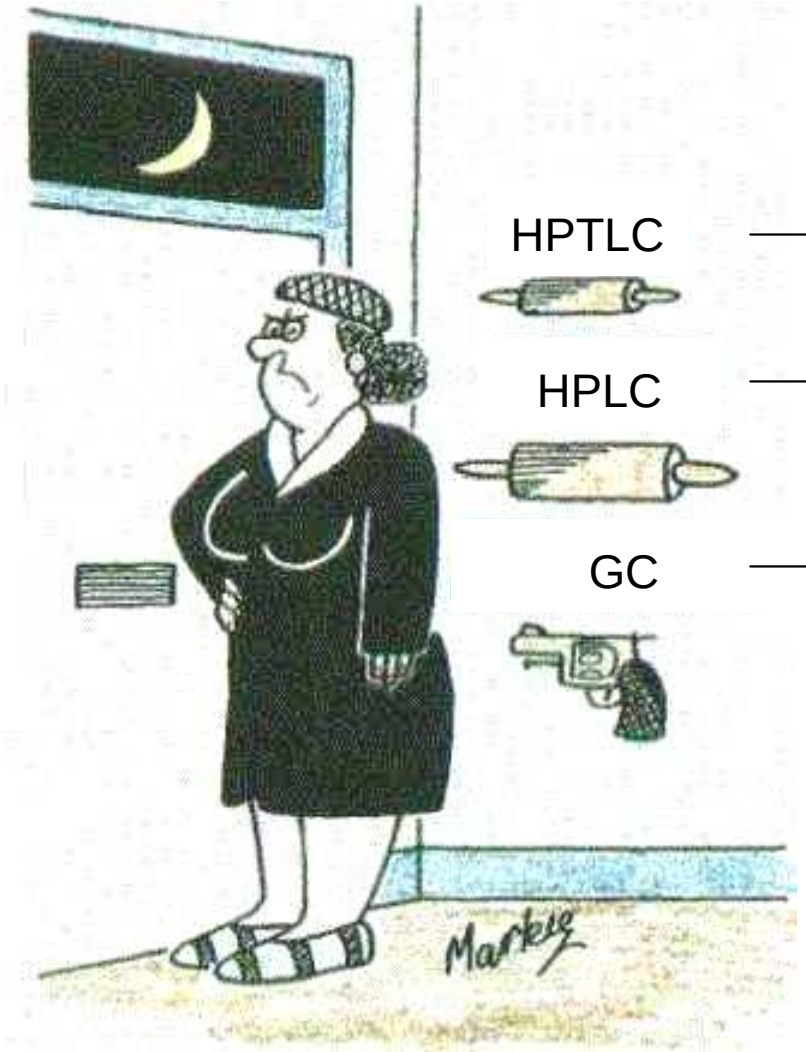

Plate number *N*

HPTLC → 8.000 ✓ *ok*

HPLC → 30.000

GC → 100.000

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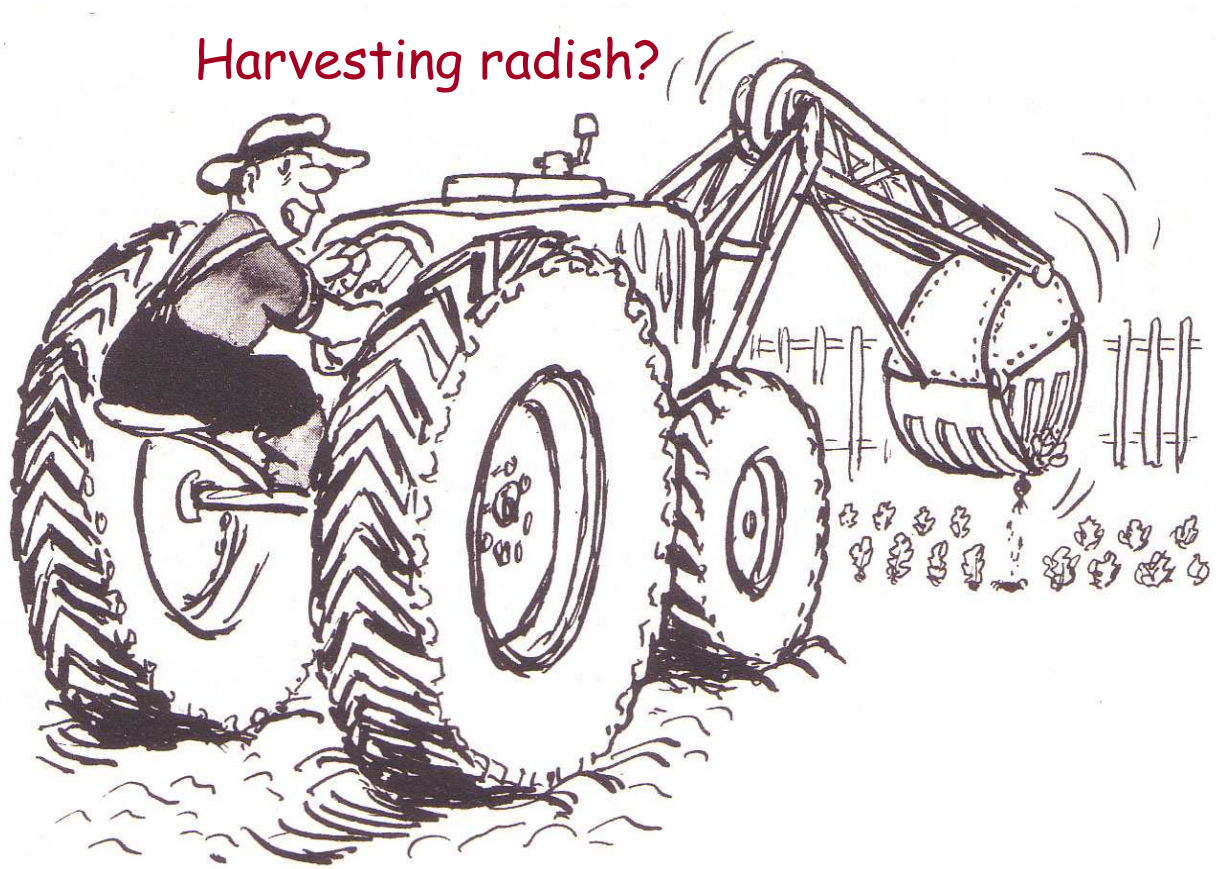
We must ask: Why HPLC?



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We must ask: Why HPLC?

"Personally, I no longer trust the mainstream media."

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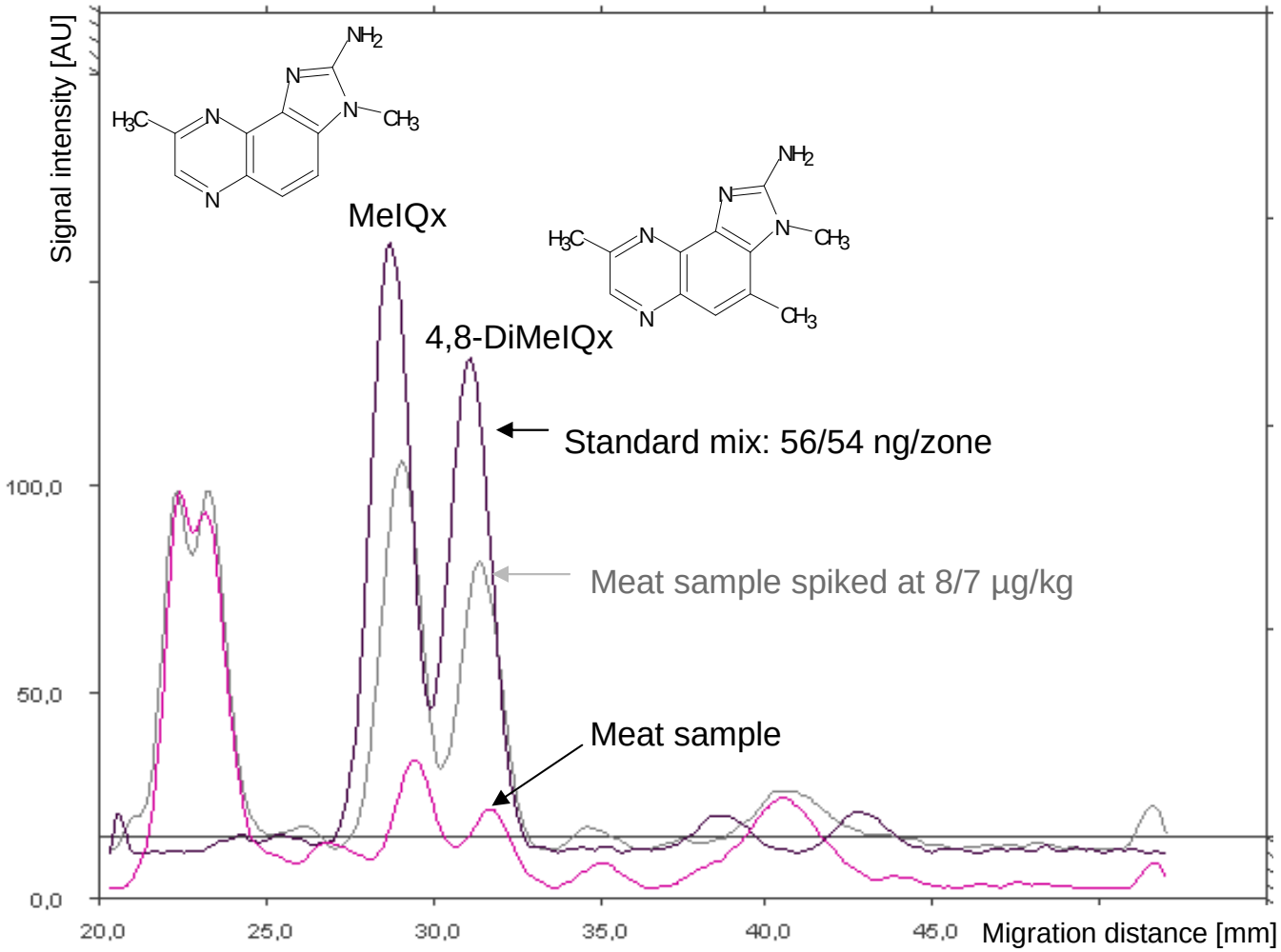
5. High throughput → cost efficiency

Determination of heterocyclic aromatic amines (HAA) in meat



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5. High throughput → cost efficiency

Determination of 5 HAA in meat

Costs	HPLC	HPTLC
Mobil phase (incl. plate precond.)	4,93	0,33
Stationary phase (incl. pre-column)	7,02	4,00
Euro	11,94	4,33
		→ Factor 3 cheaper
Throughput	**HPLC**	**HPTLC**
Application/Injection	1,0	3,0
Chromatography/gradient time	15,6	1,1
Fluorescence intens. & MWL scan	-	0,2
Time [h]	16,6	4,3
		→ Factor 4 faster
Labor	**HPLC**	**HPTLC**
All steps **automated**	online	offline
Stand-by time	→ none	→ 5 min

U. Jautz, M. Gibis, G. Morlock, in preparation

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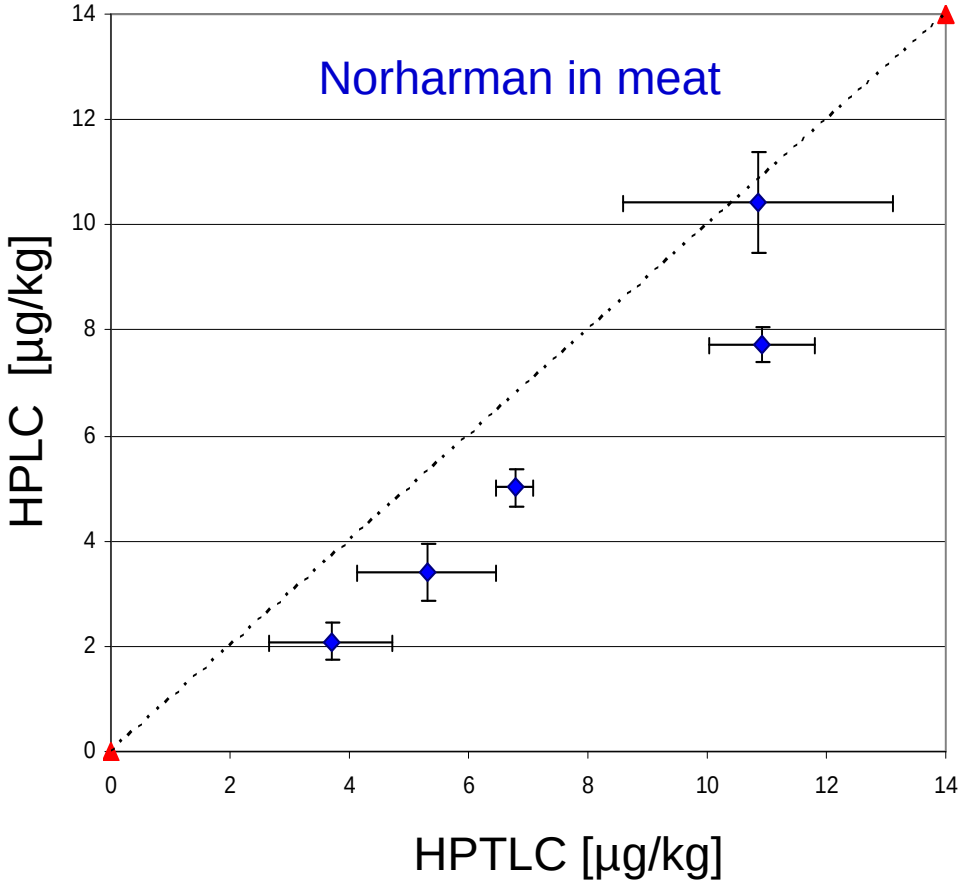
….and comparable results to HPLC



U. Jautz, M. Gibis, G. Morlock, in preparation

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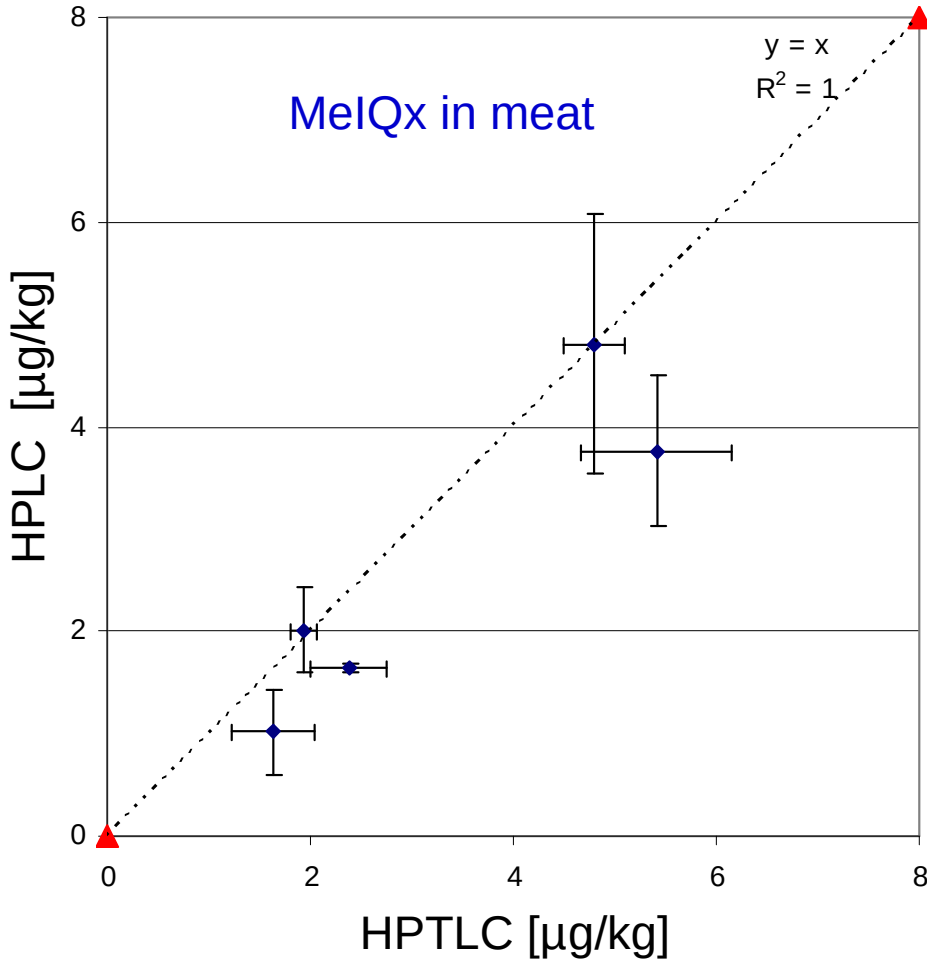
….and comparable results to HPLC



U. Jautz, M. Gibis, G. Morlock, in preparation

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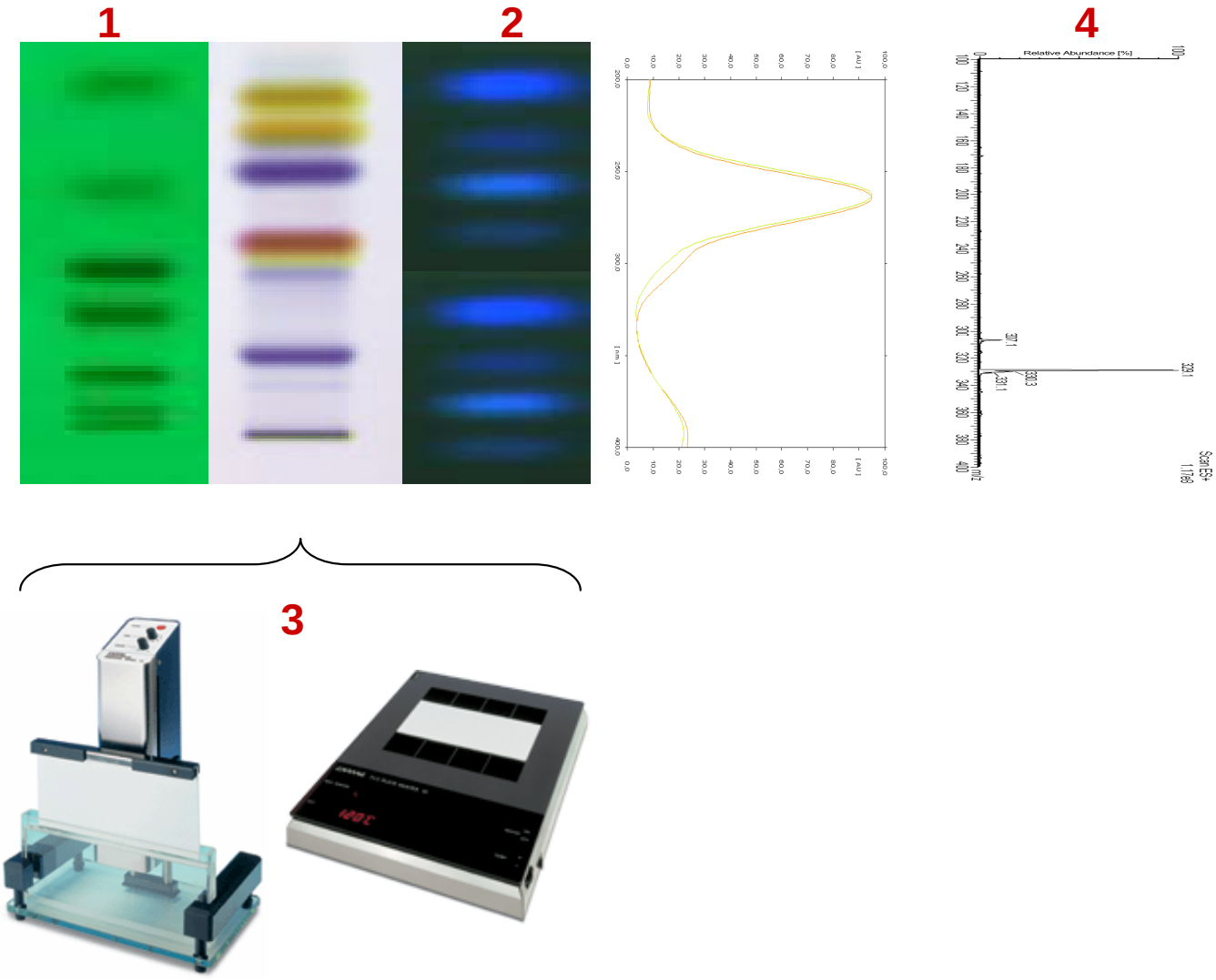
6. Enables selective derivatizations on **one** plate



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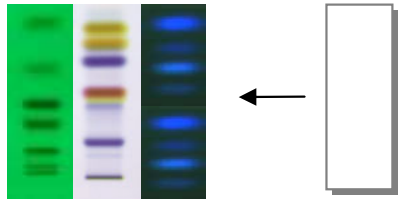
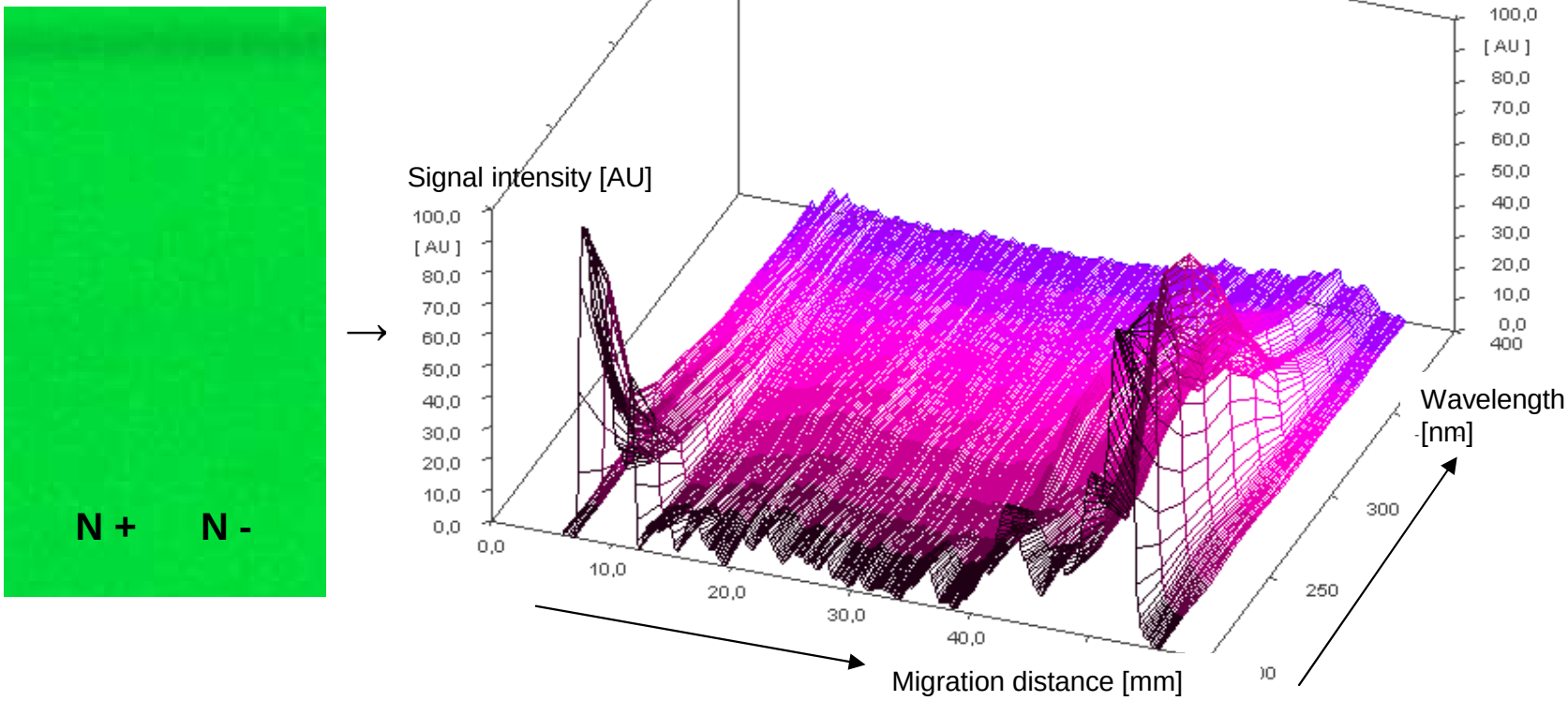
A) Easiness of derivatizations





Project: What substance is in the root exudate of some plants that attract specific N-producing bacteria

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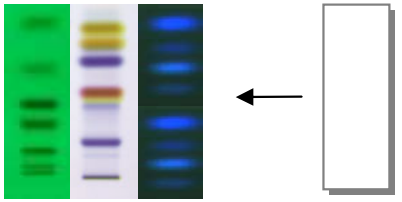
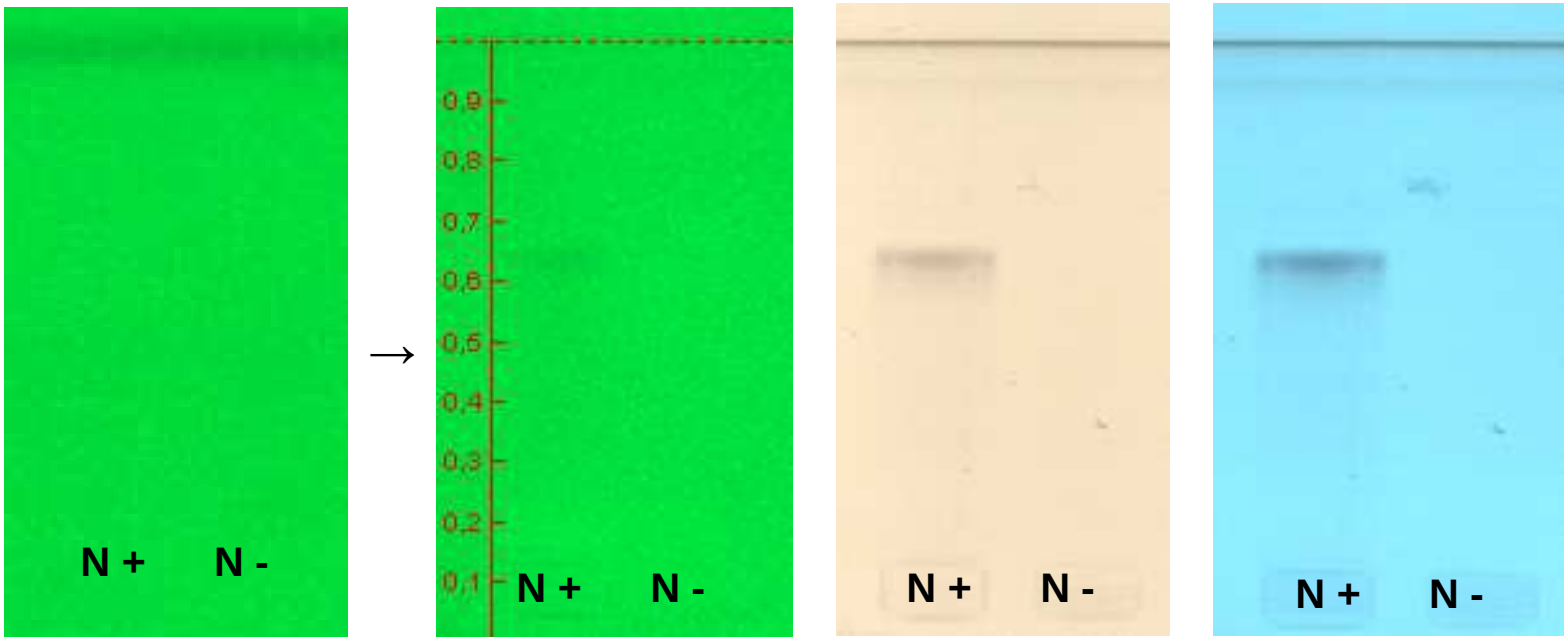
A) Easiness of derivatizations



→ variety of reagents



Project: What substance is in the root exudate of some plants that attract specific N-producing bacteria

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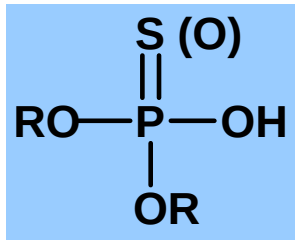
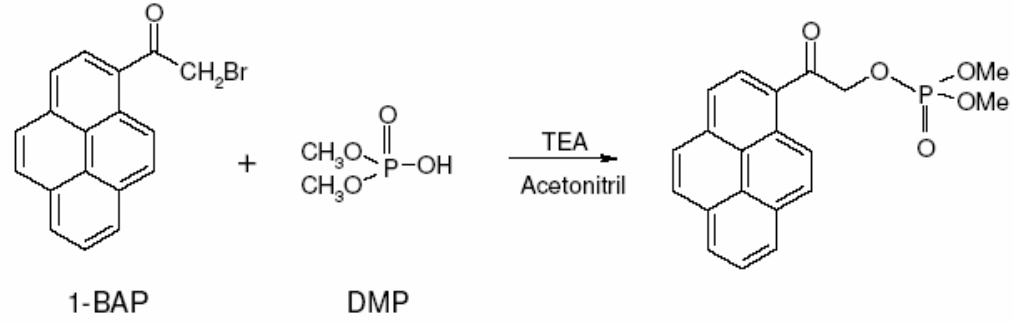
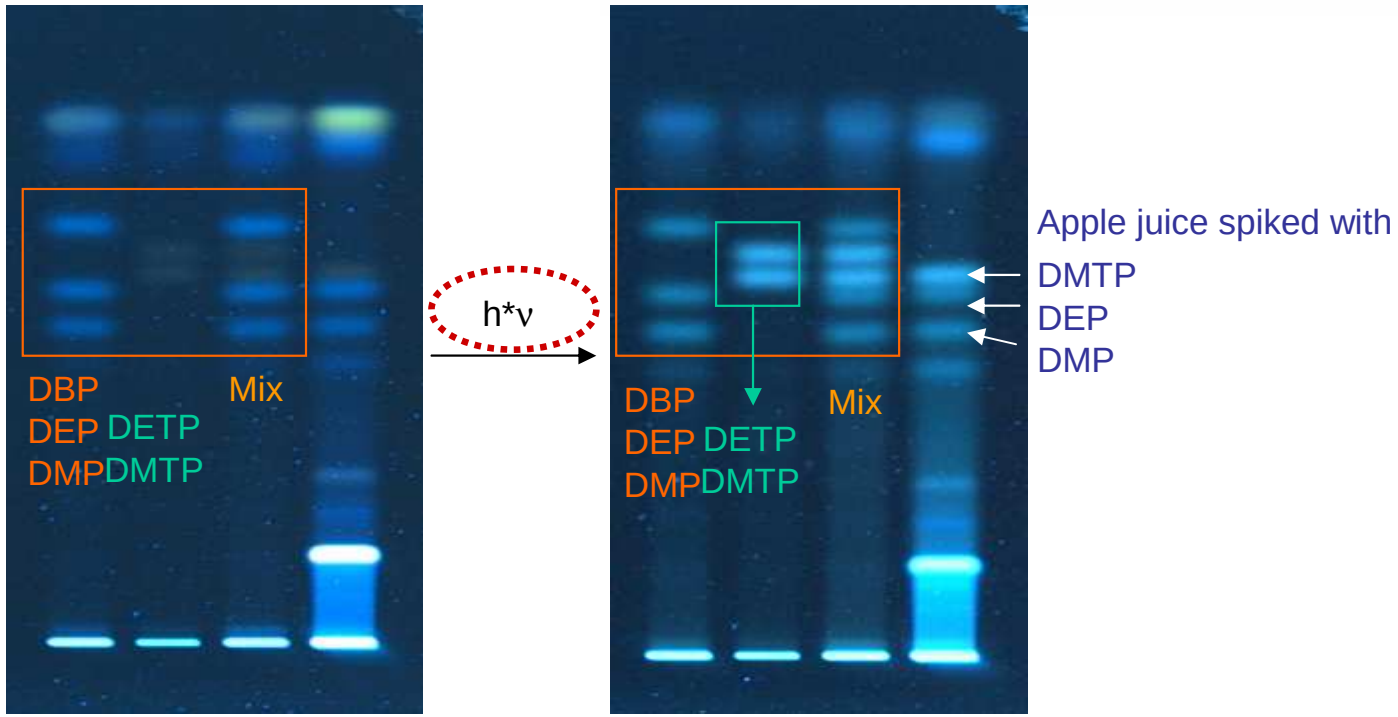
B) Flexibility of derivatizations

→ Dialkyl phosphates as breakdown products during fruit juice processing







C. Stiefel, W. Schwack, Proceedings of EuroFoodChem 2 (2007) 289-292

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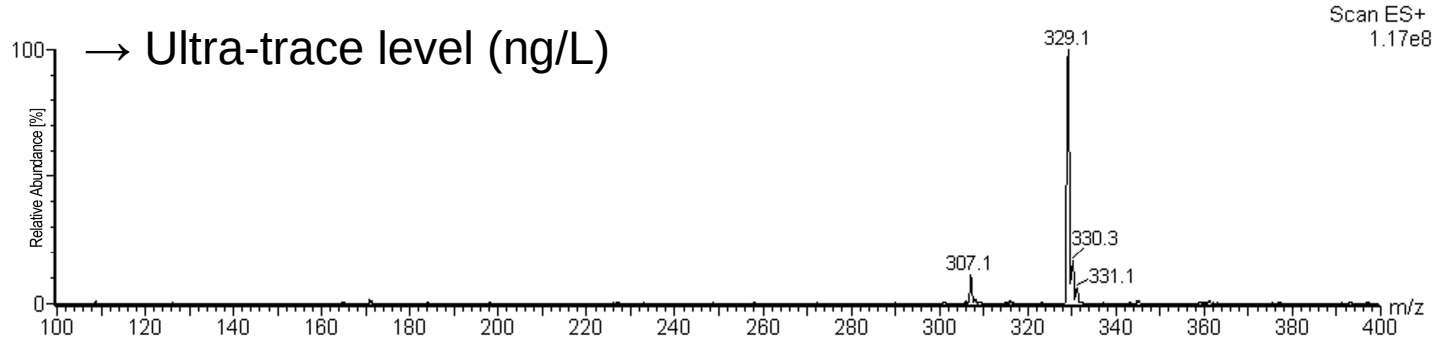
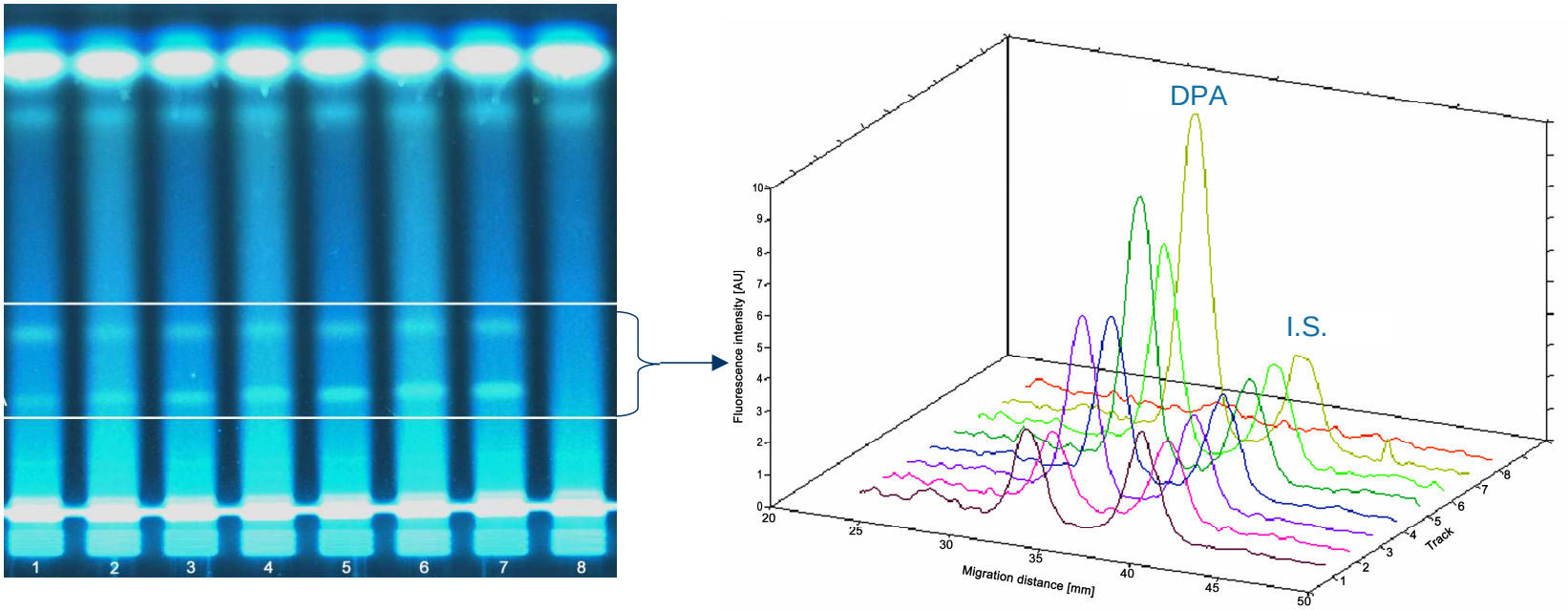
C) Simultaneous derivatization of all tracks

→ Pre-chromatographic derivatization in situ of acrylamid

→ Ultra-trace level (ng/L)





A. Alpmann, G. Morlock, J Sep Sci (2007) in press

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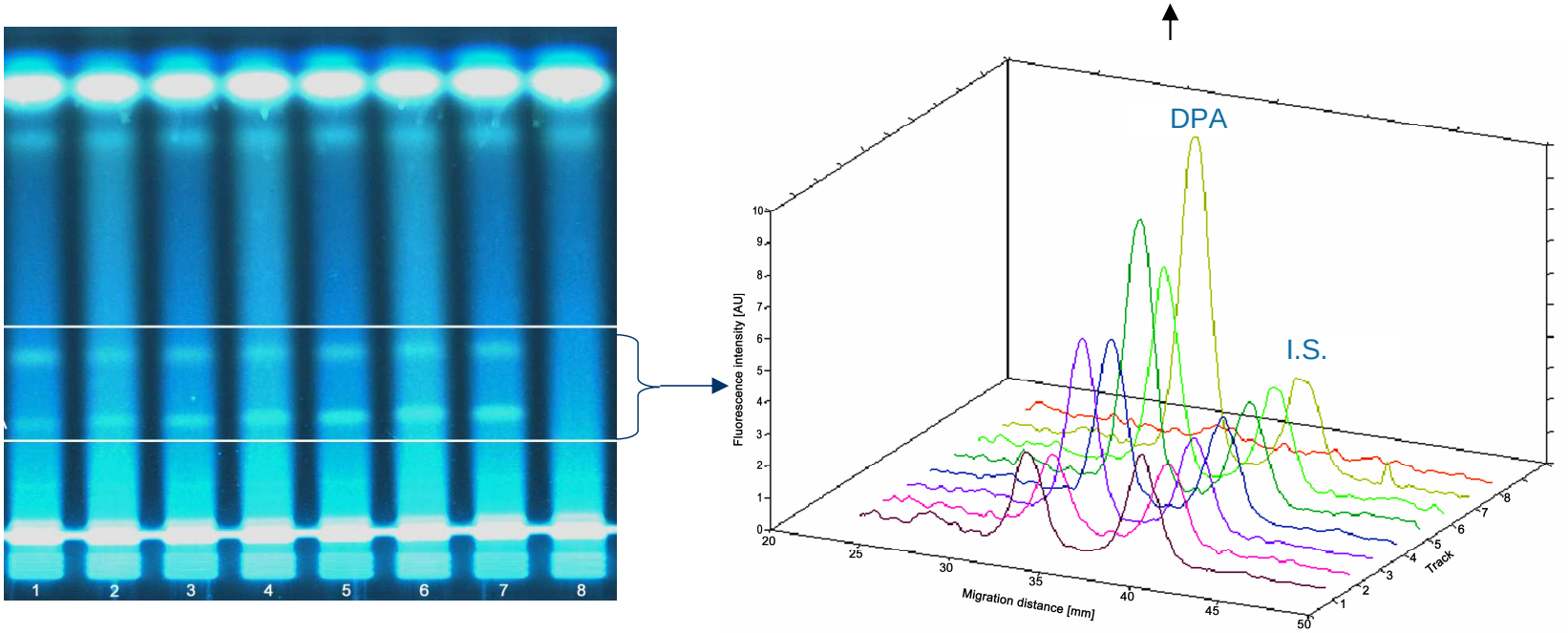
C) Simultaneous derivatization of all tracks

	Ground water spiked with acylamide [µg/L]	HPLC-MS/MS Acylamide [µg/L]	HPTLC/FLD Acylamide [µg/L]
Sample 1	-	< LOQ	< LOQ
Sample 2	0.05	0.07	0.09
Sample 3	0.15	0.18	0.24
Sample 4	0.50	0.59	0.60



A. Alpmann, G. Morlock, J Sep Sci (2007) in press

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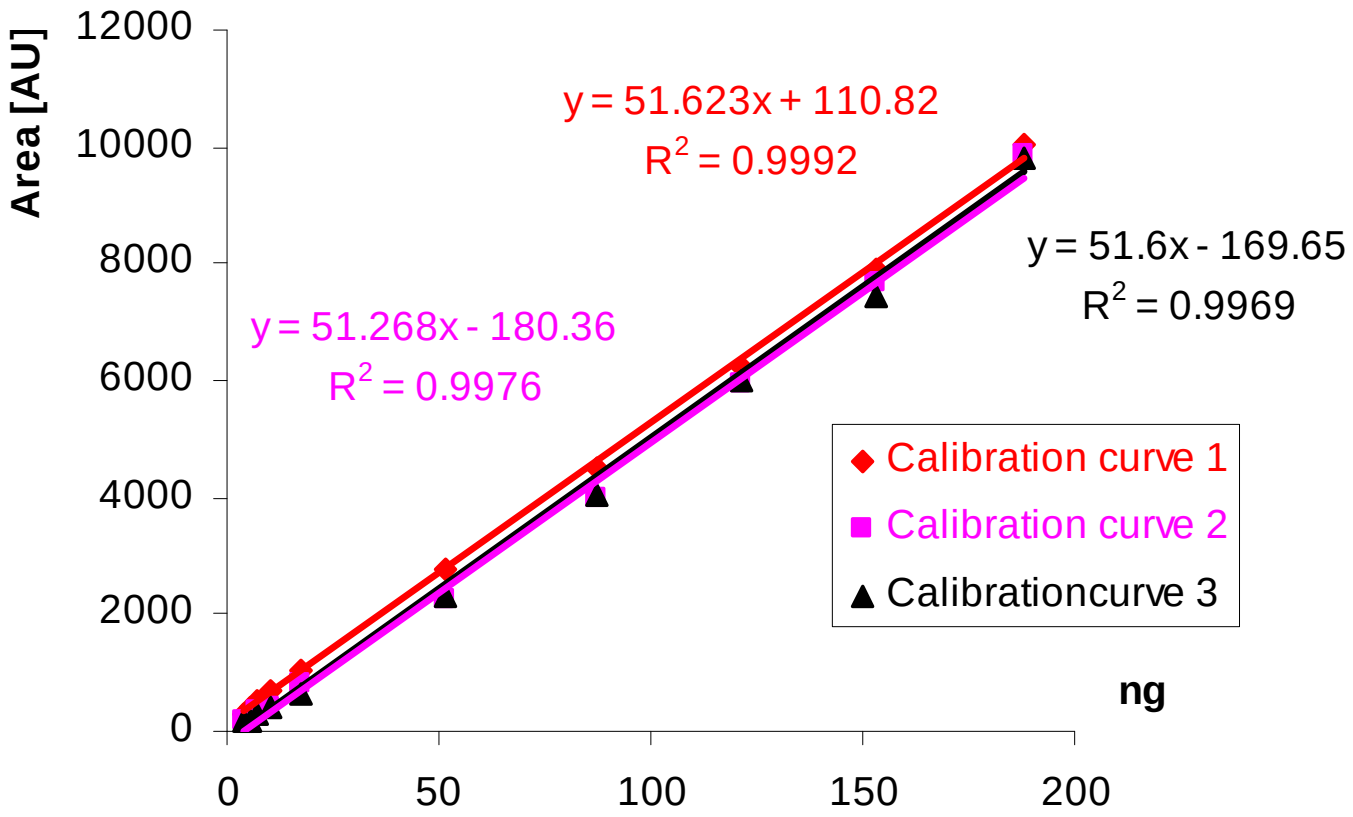
D) Reproducible derivatizations



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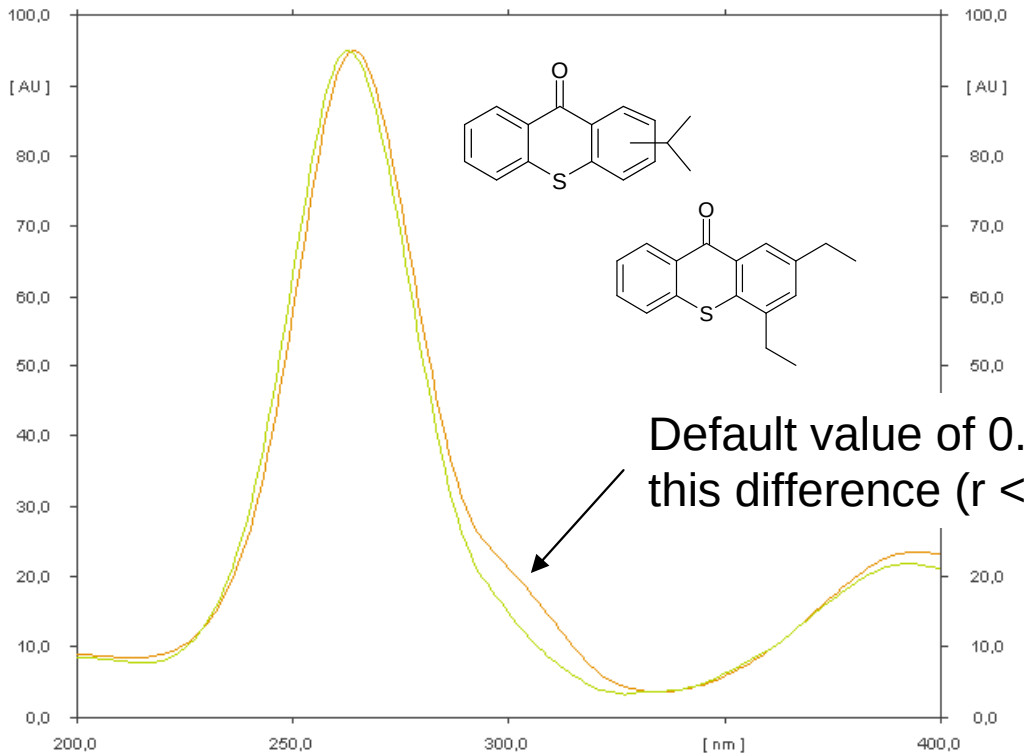
7. Enables multiple detections

→ UV/Vis library search, spectra identity and purity

→ Spectra identity for 3 milk-based samples:

r ≥ 0.99974 for ITX at 5 ng/zone

r ≥ 0.99984 for DTX at 14 ng/zone



G. Morlock, W. Schwack, Anal. Bioanal. Chem 385 (2006) 586-595

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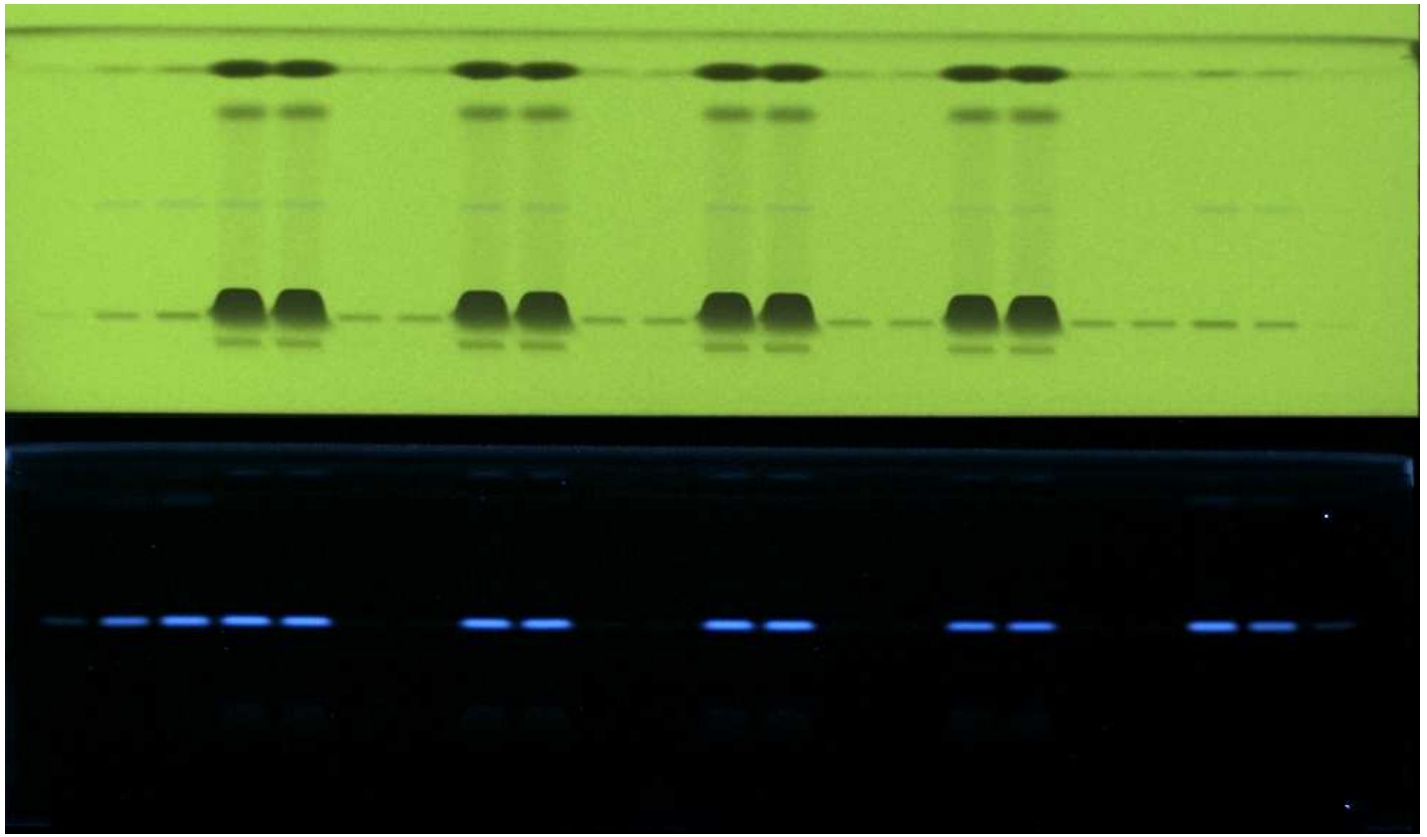
A) MWL scan for UV/FLD

→ Simultaneous determination of caffeine, ergotamine and metamizol



Calibration with $r^2 > 0.999$

Recoveries in pharmaceutical products: 102.8 % ± 2.8 % for ergotamine
106.6 % ± 3.2 % for caffeine
104.7 % ± 2.2 % for metamizol

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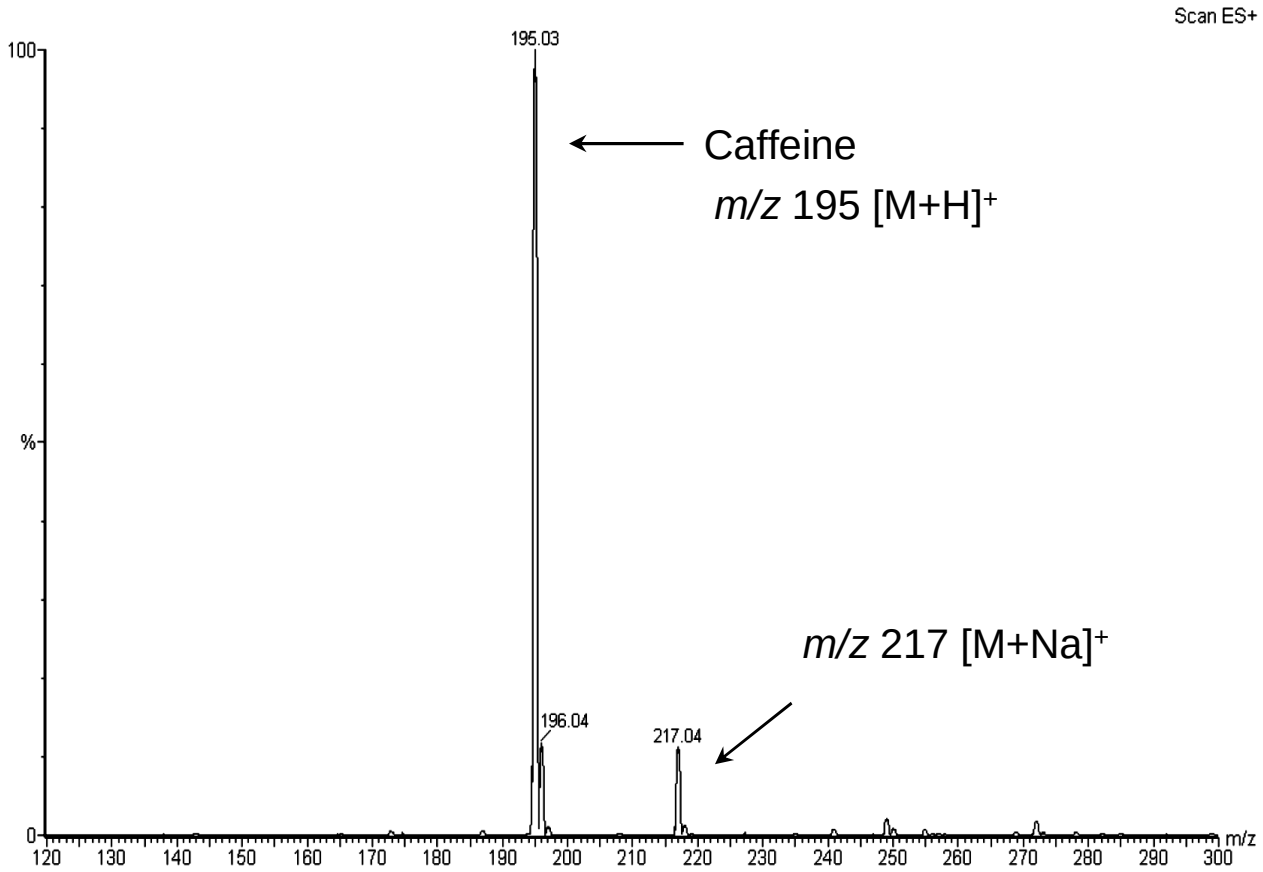
A) Confirmation by MS

→ Simultaneous determination of caffeine, ergotamine and metamizol



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

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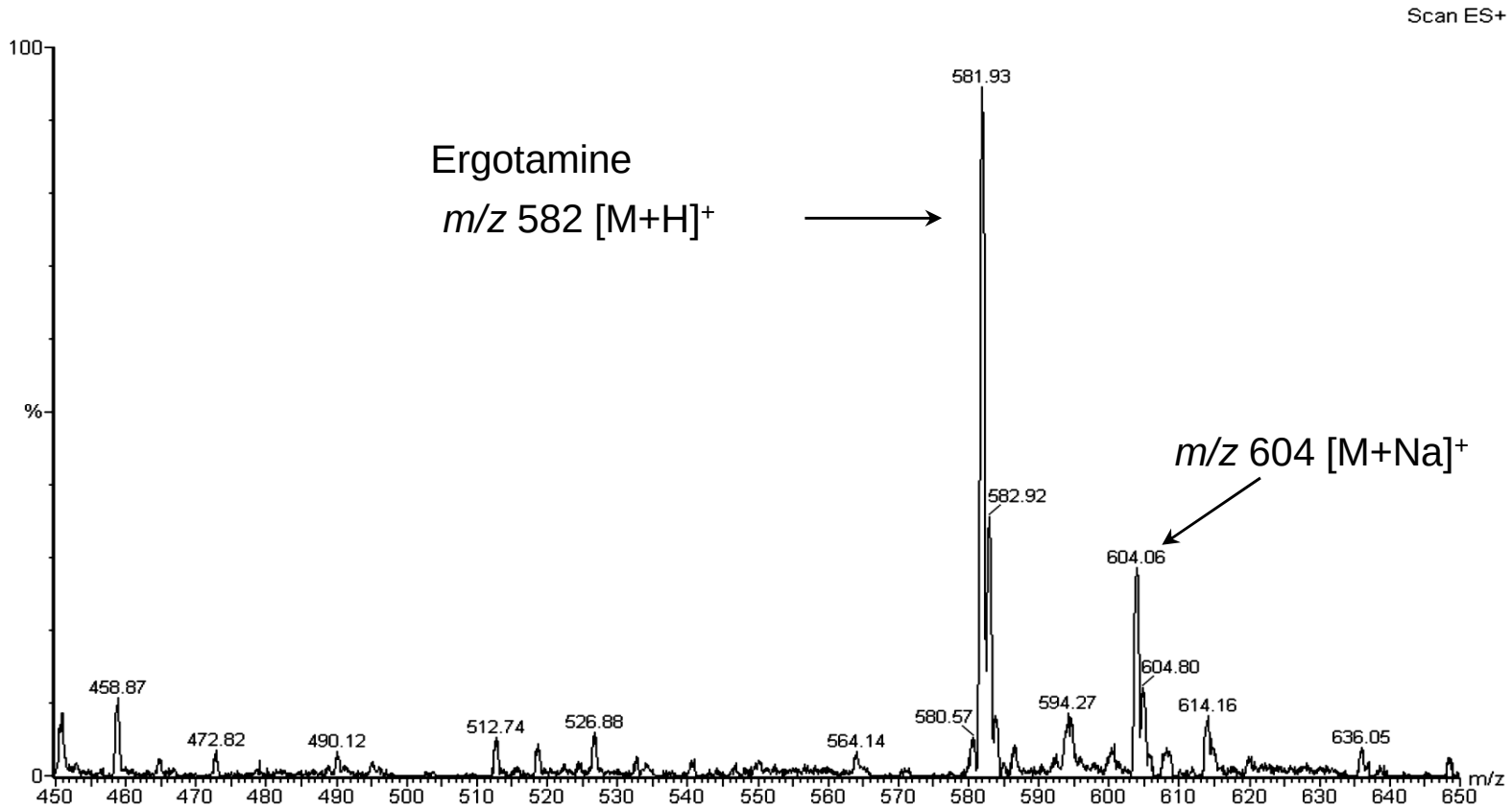
A) Confirmation by MS

→ Simultaneous determination of caffeine, ergotamine and metamizol



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

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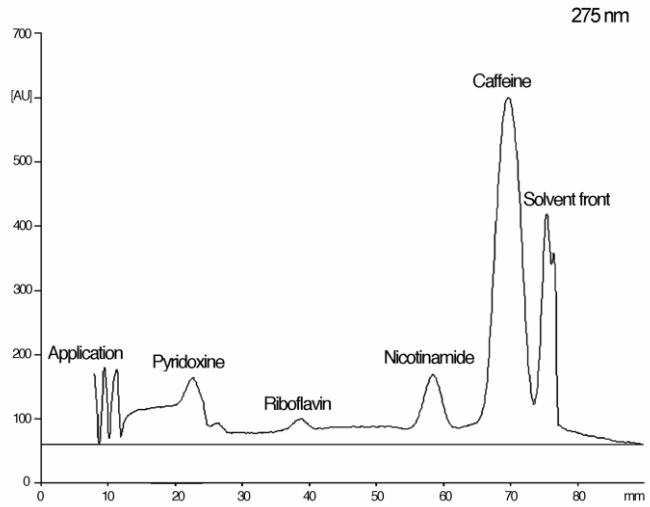
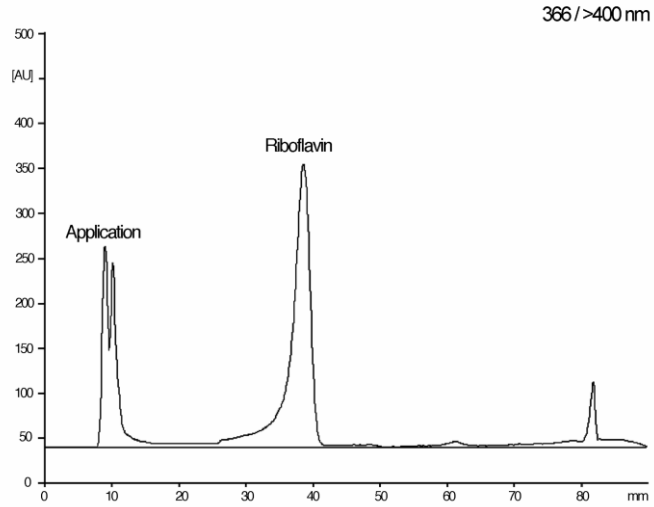
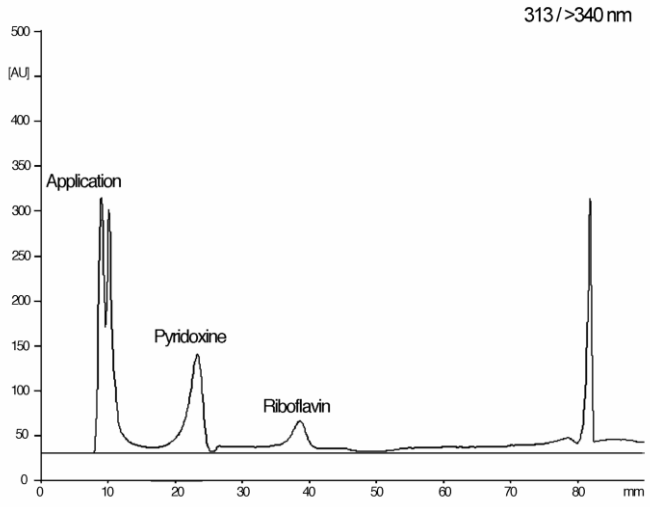
B) MWL scan for UV/FLD → derivatization → Vis

→ Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks







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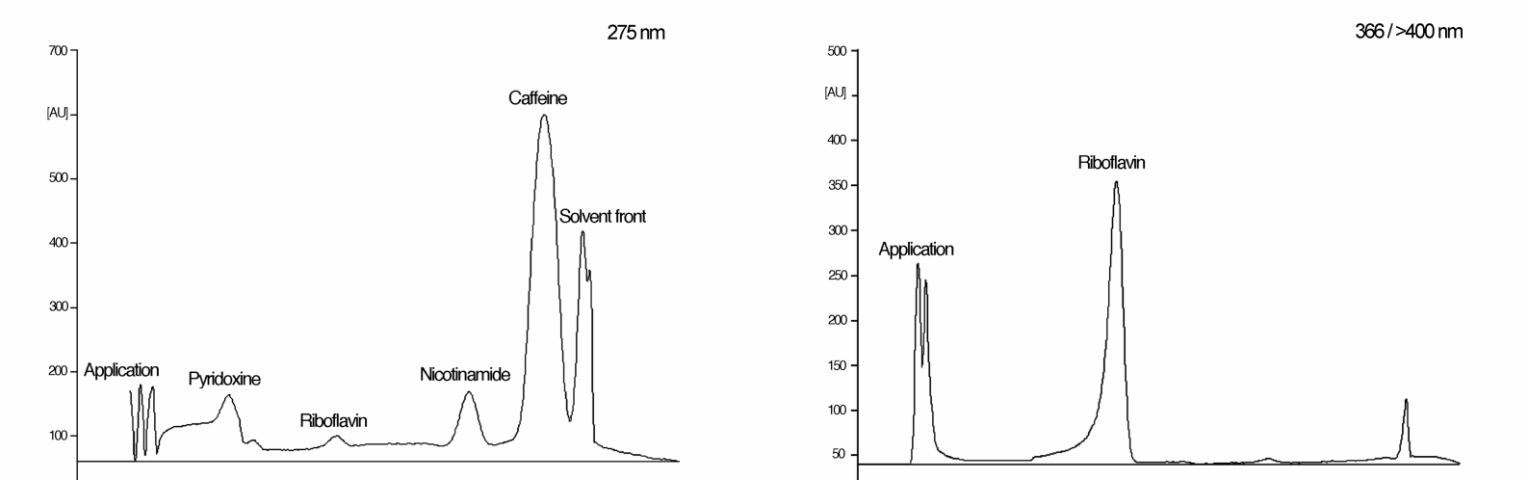
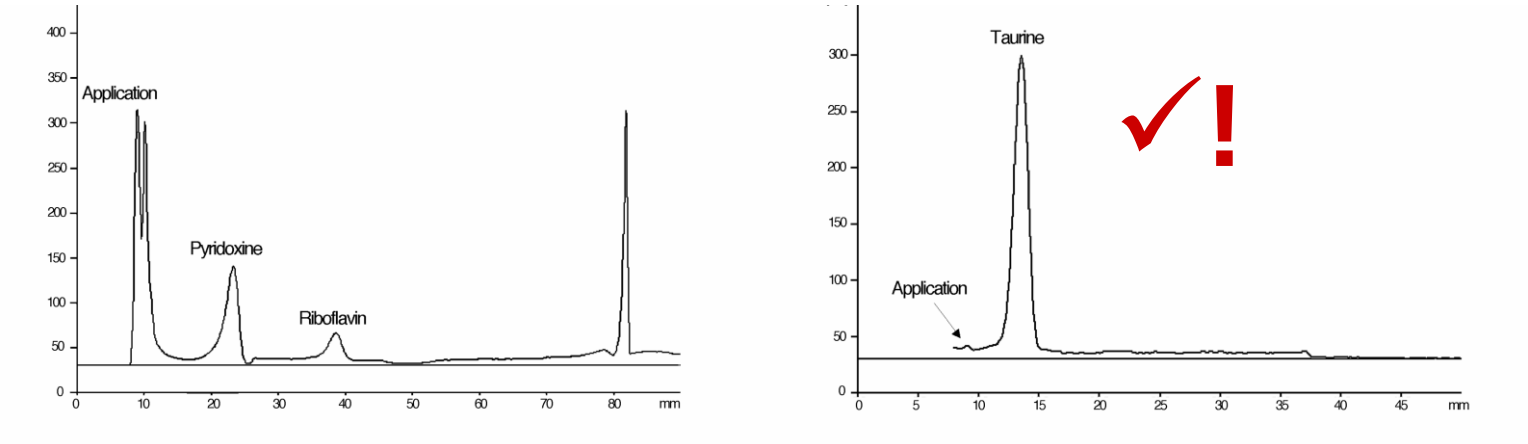
B) MWL scan for UV/FLD → derivatization → Vis

→ Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks



- ✓ Calibration with $r^2 > 0.999$
- ✓ Recoveries in energy drinks (3 levels) between 81 and 106 % with RSD range from 0.5 to 7.4%



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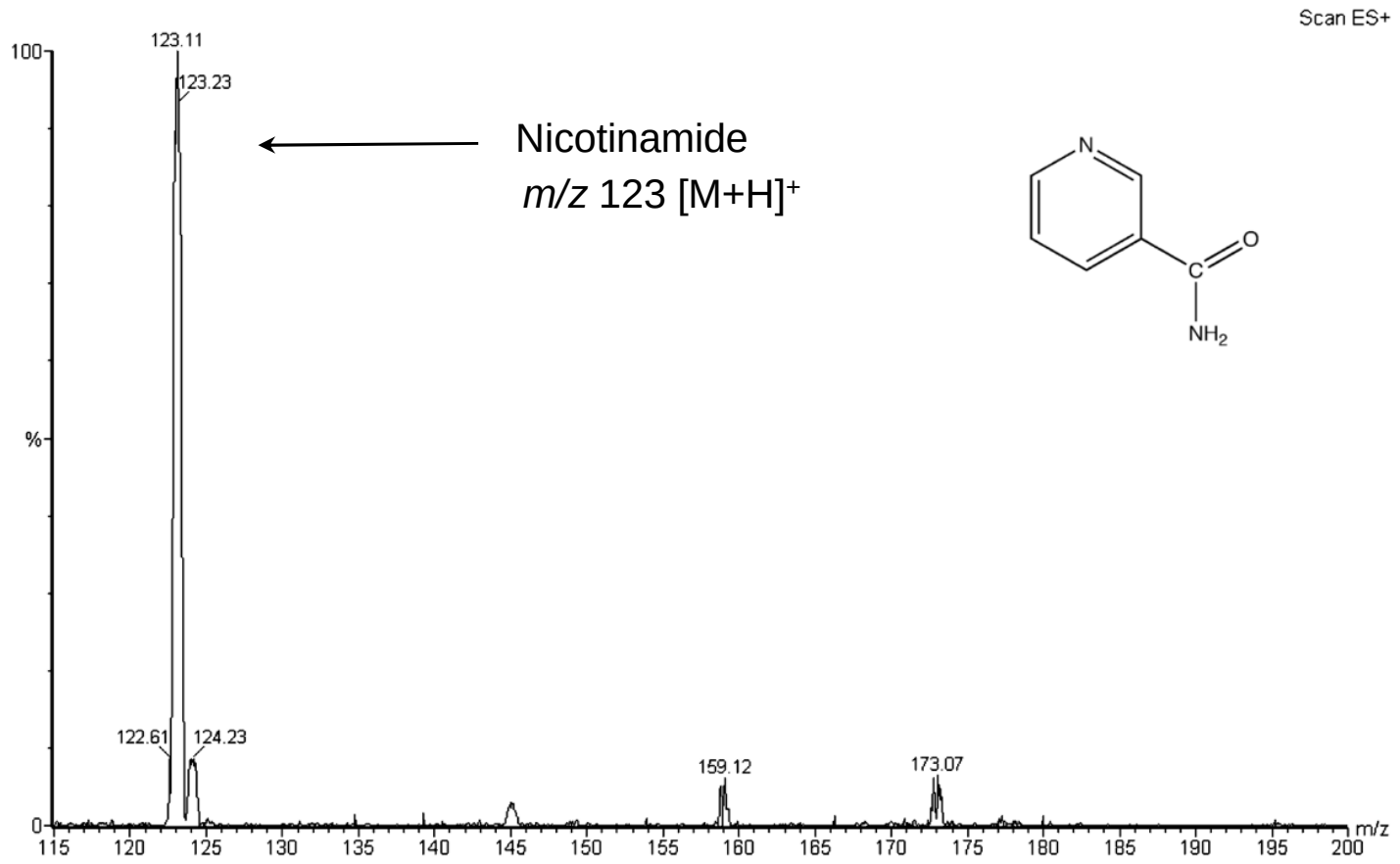
B) Confirmation by MS

→ Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks



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

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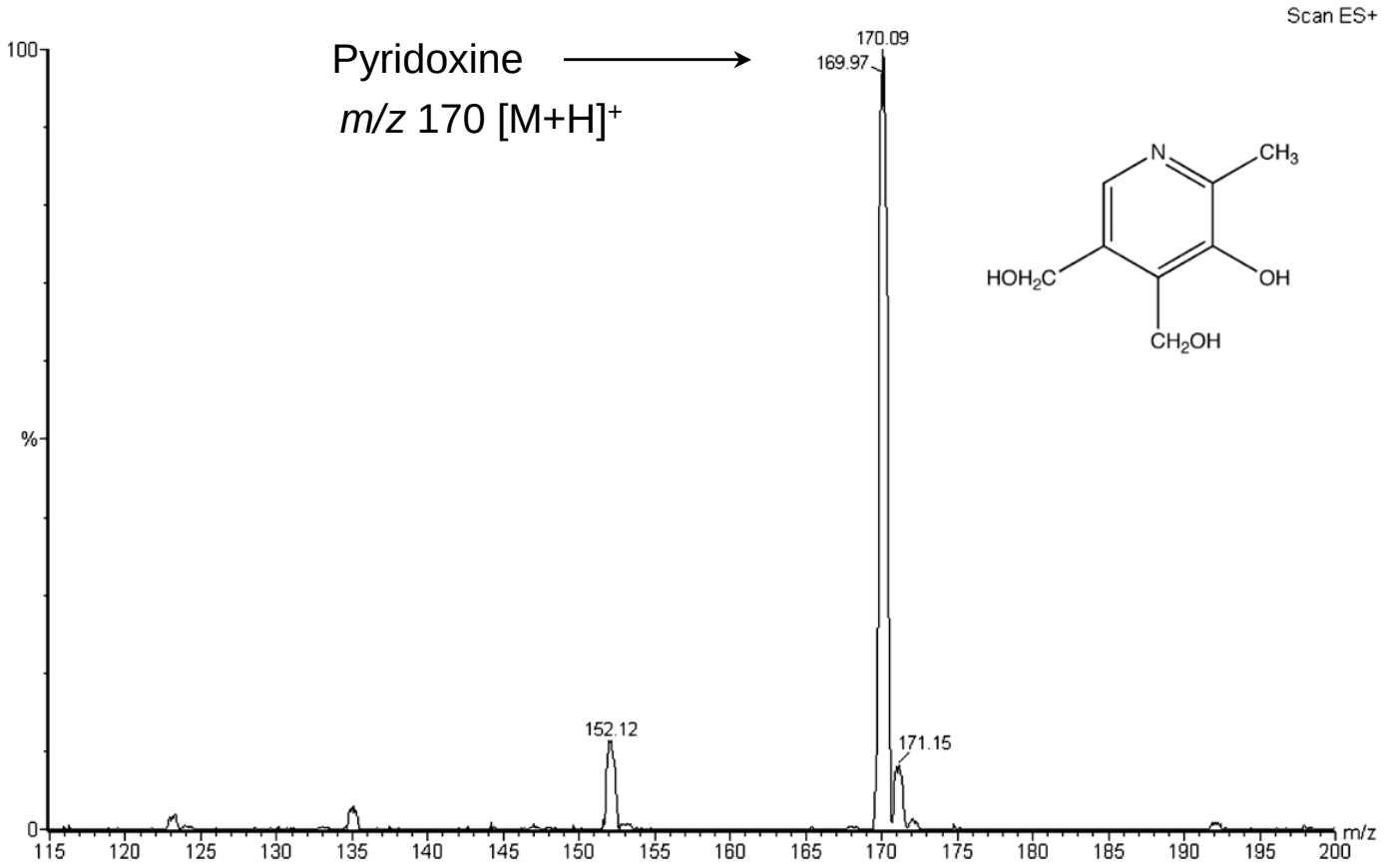
B) Confirmation by MS

→ Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks



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

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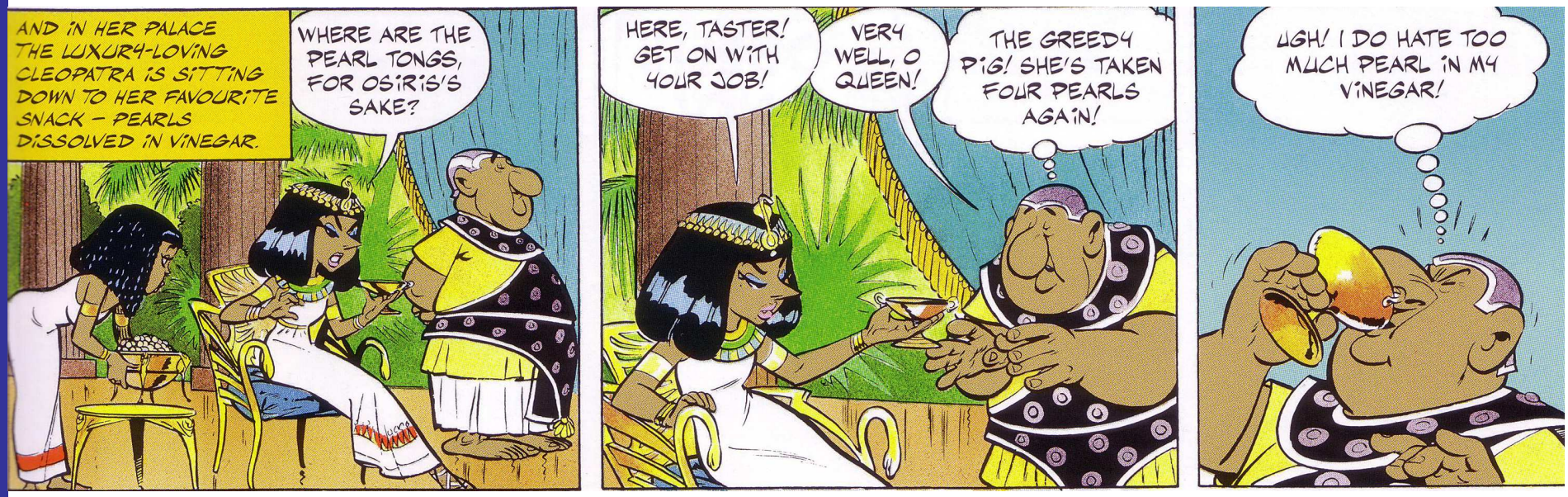
Biomonitoring of toxic compounds



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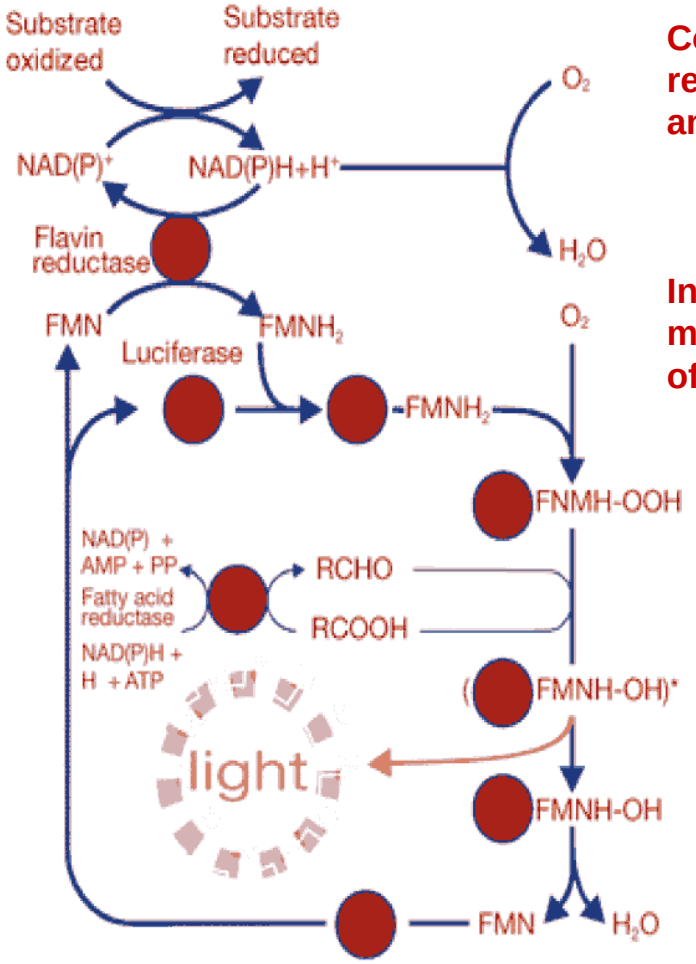
8. Allows toxicity-directed detection

Luminescent bacteria test in cuvette → ISO 11348-3 (1999)

→ detection of toxic compounds as a sum parameter



Coupled to the respiratory chain and ATP pool

Information to metabolic status of the cell

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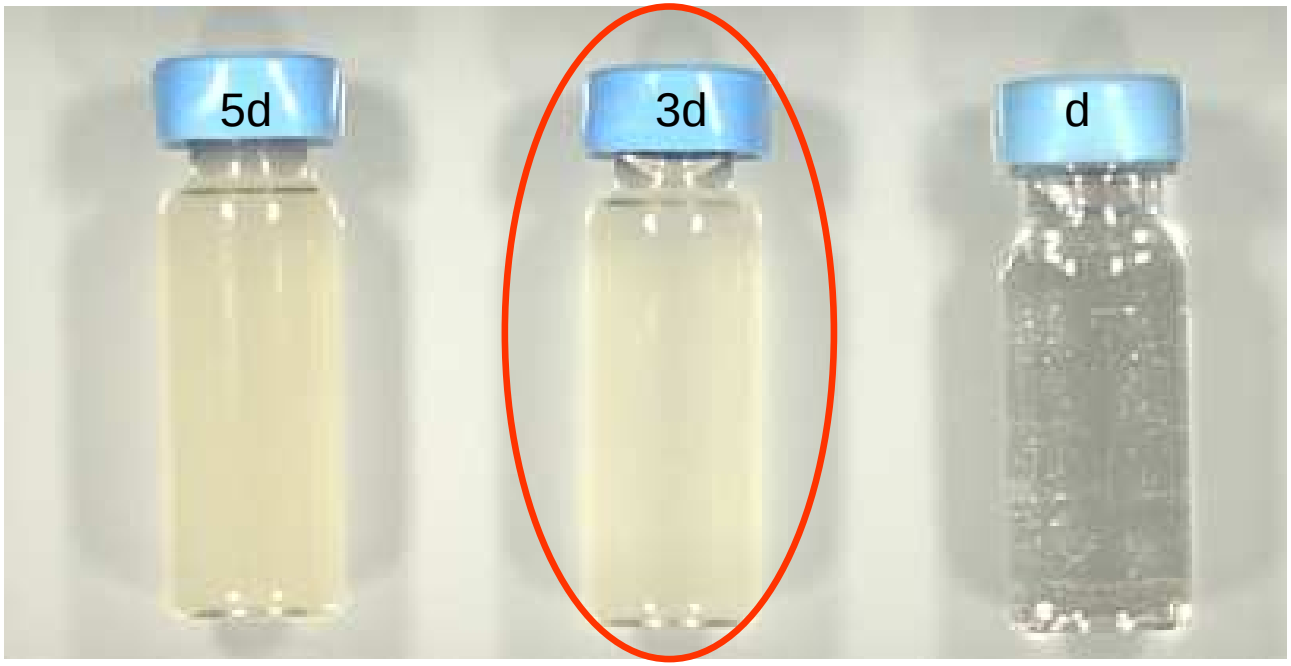
Luminescent bacterium Vibrio Fischeri



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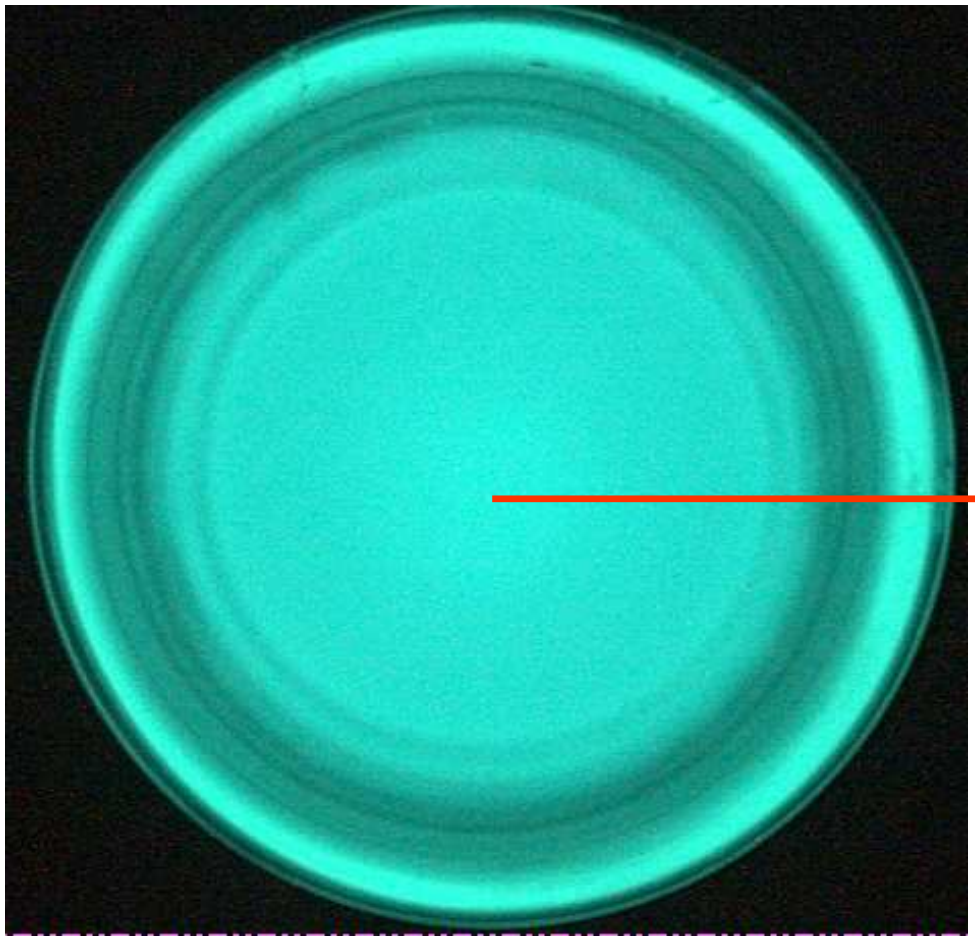
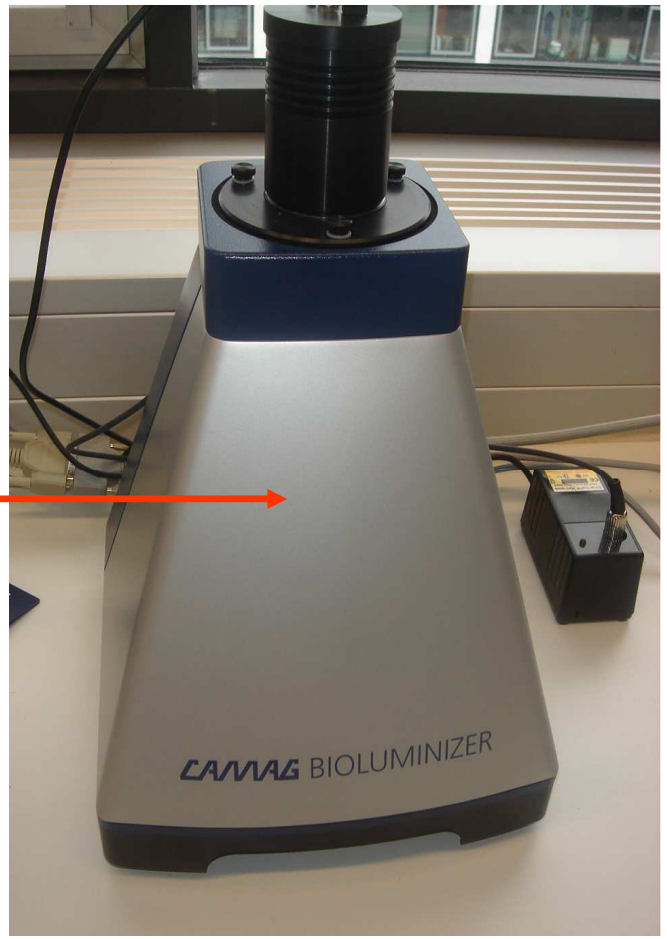
Detection of luminescent bacteria





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Protocol

Luminescent bacteria → **NEW**: combined with HPTLC

Coupling chromatography with a toxicity-directed detection system
→ effect-directed analysis ↔ different approach to target-analysis
→ detection of **single** toxic compounds

EP 0588 139 B1, ChromaDex, www.bioluminex.com/applications

W. Kreiss (Bayer Industries) et al. CBS 88 (2002) 12-13

W. Weber (Federal water supply Langenau) et al. CBS 97 (2006) 2-4

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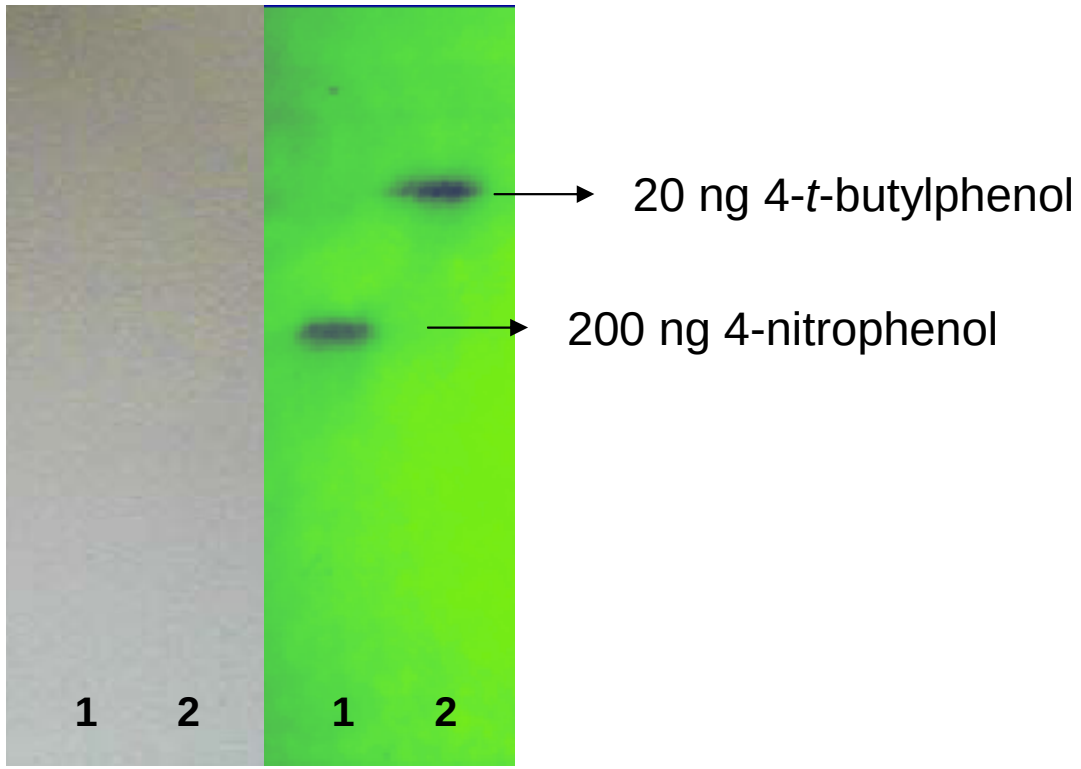
Instead of the sum → the single compound

Example: Phenols (W. Kreiss, Bayer Industries)



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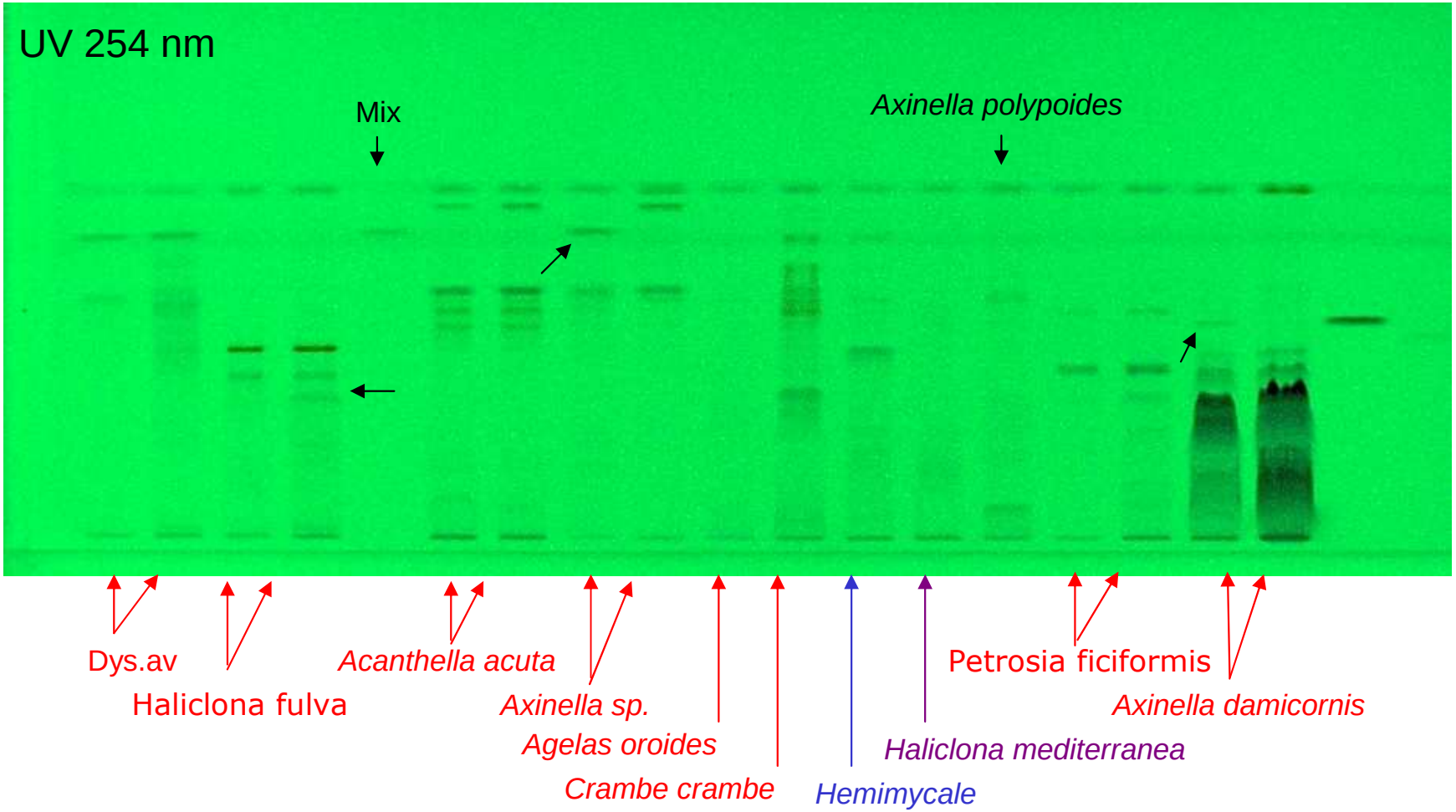
Instead of the sum → the single compound

Project: Screening of marine sponges for toxic compounds



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Instead of the sum → the single compound

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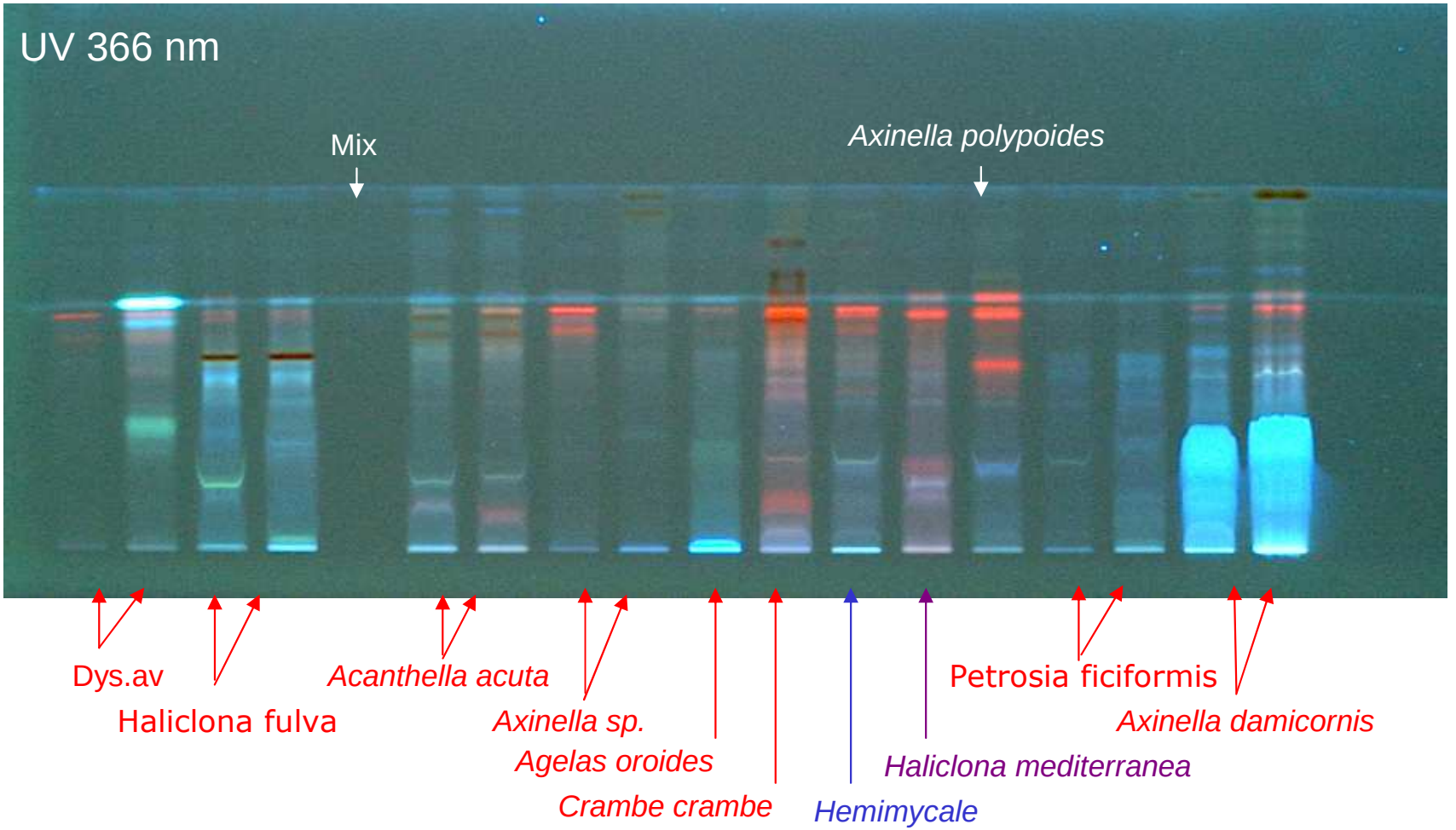

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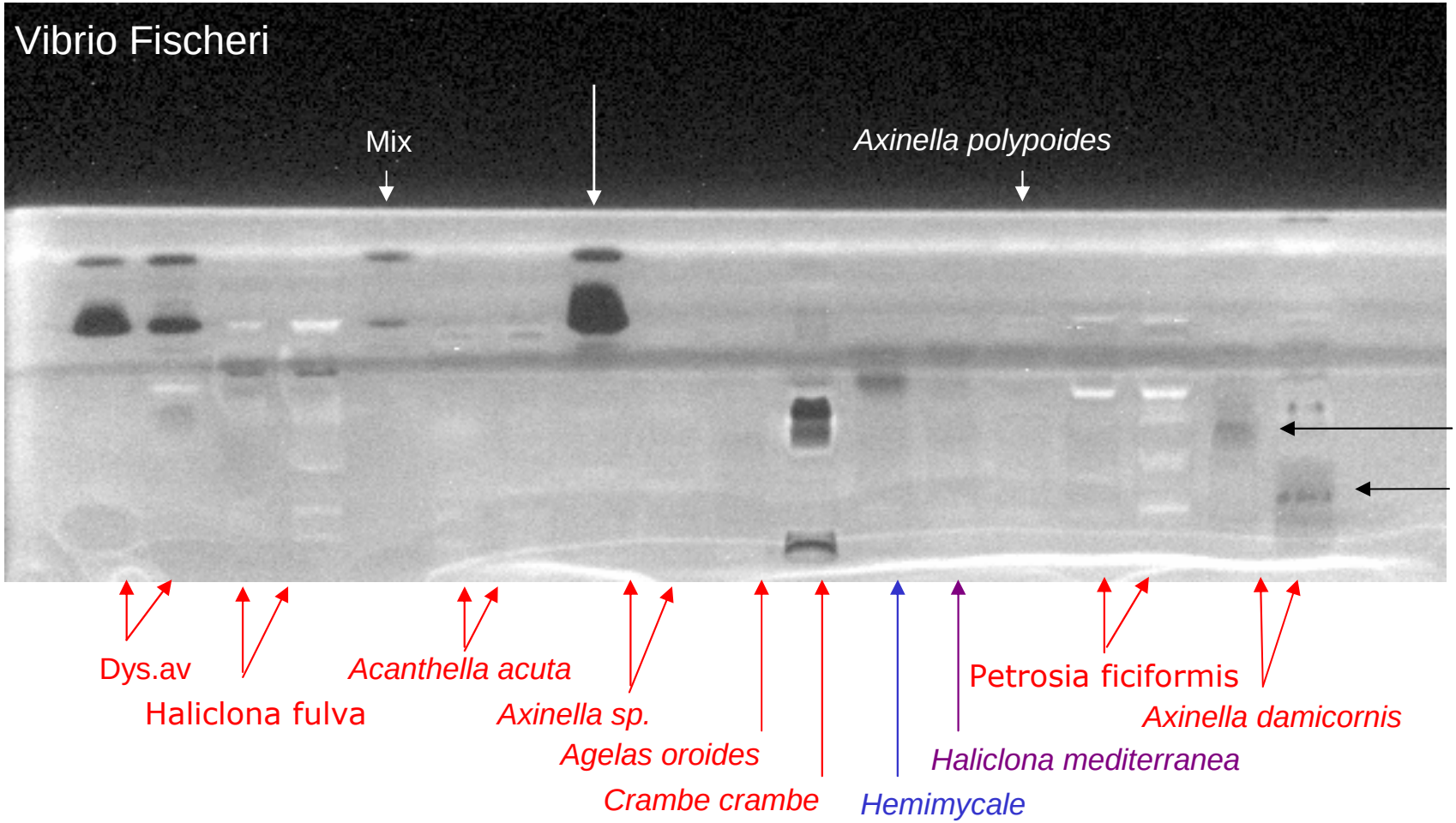
Instead of the sum → the single compound

Project: Screening of marine sponges for toxic compounds

→ avoids laborious isolation of potential toxic compounds each followed, as proof, by the test of bioactivity



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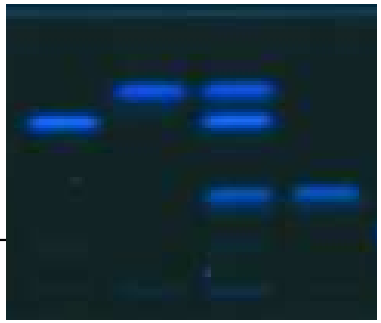
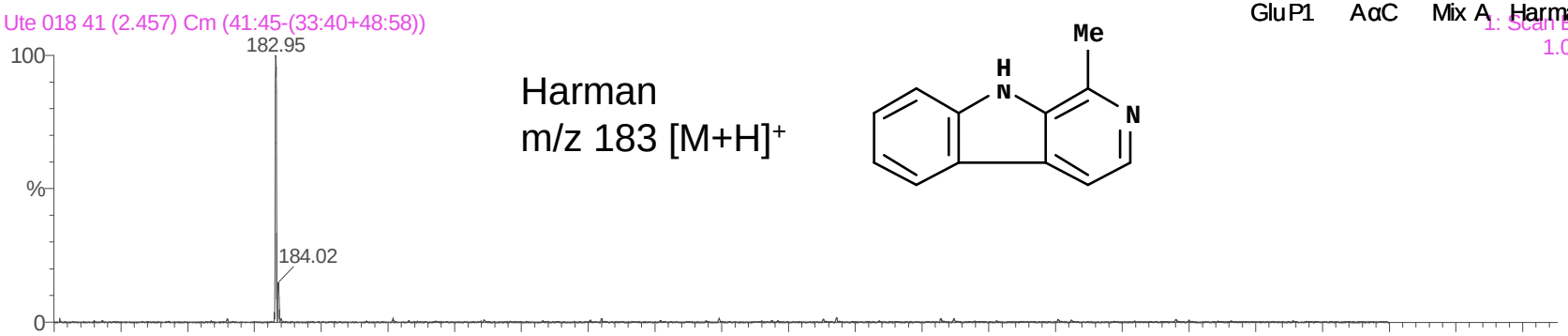
9. Cost-effective coupling with MS



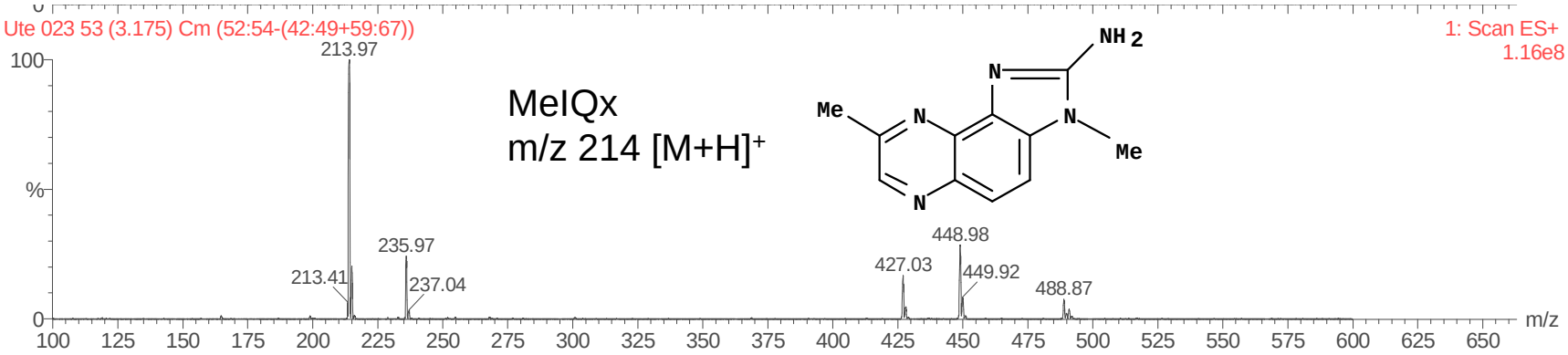


Harman
m/z 183 $[M+H]^+$

→ highly targeted recording

→ reduced costs and storage of data

→ separation solvent independently from mass spectrometry



MeIQx
m/z 214 $[M+H]^+$

U. Jautz, G. Morlock, Anal. Bioanal. Chem. 387 (2007) 1083-1093

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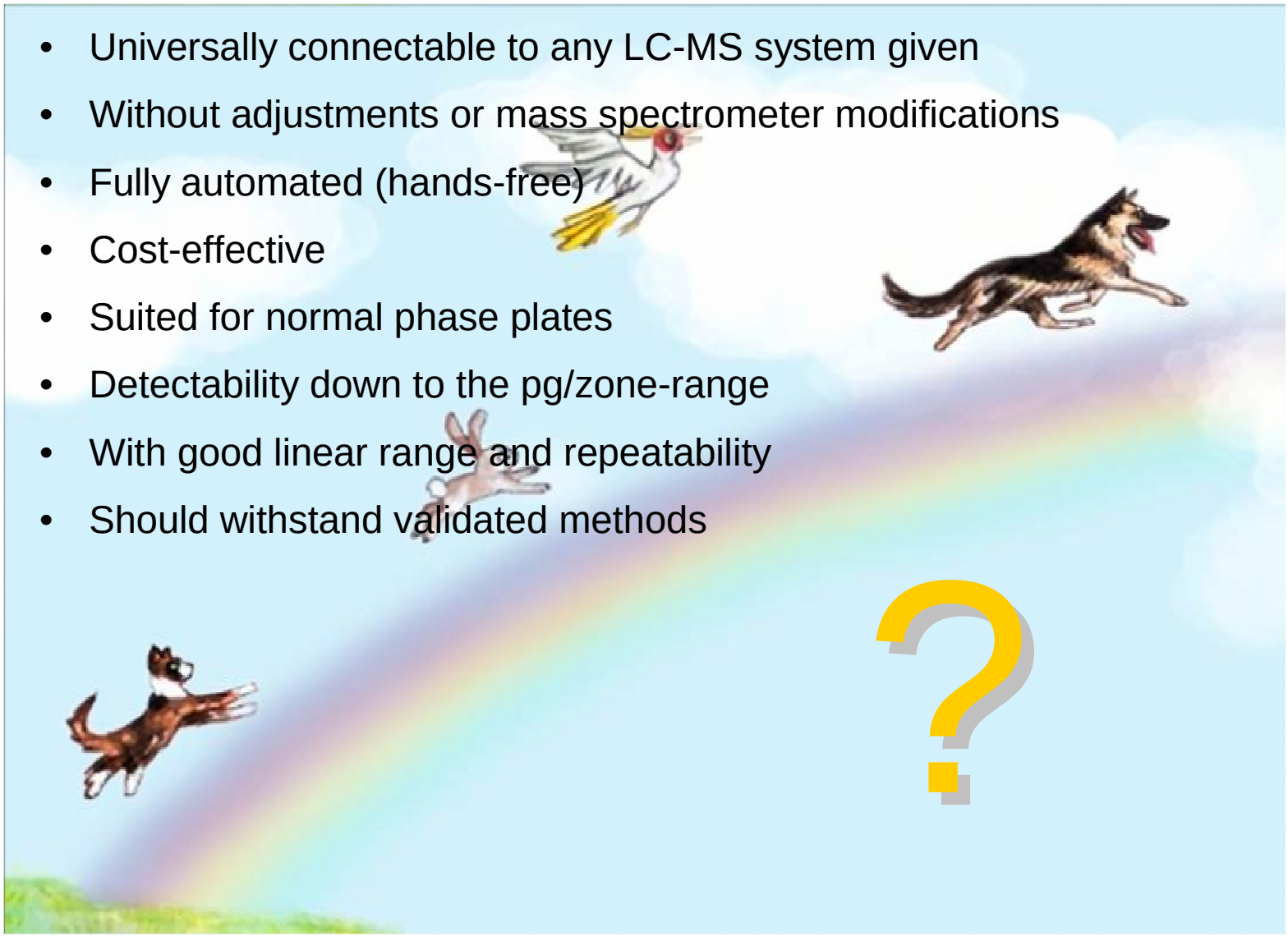
The look of an HPTLC/MS interface

- Universally connectable to any LC-MS system given
- Without adjustments or mass spectrometer modifications
- Fully automated (hands-free)
- Cost-effective
- Suited for normal phase plates
- Detectability down to the pg/zone-range
- With good linear range and repeatability
- Should withstand validated methods



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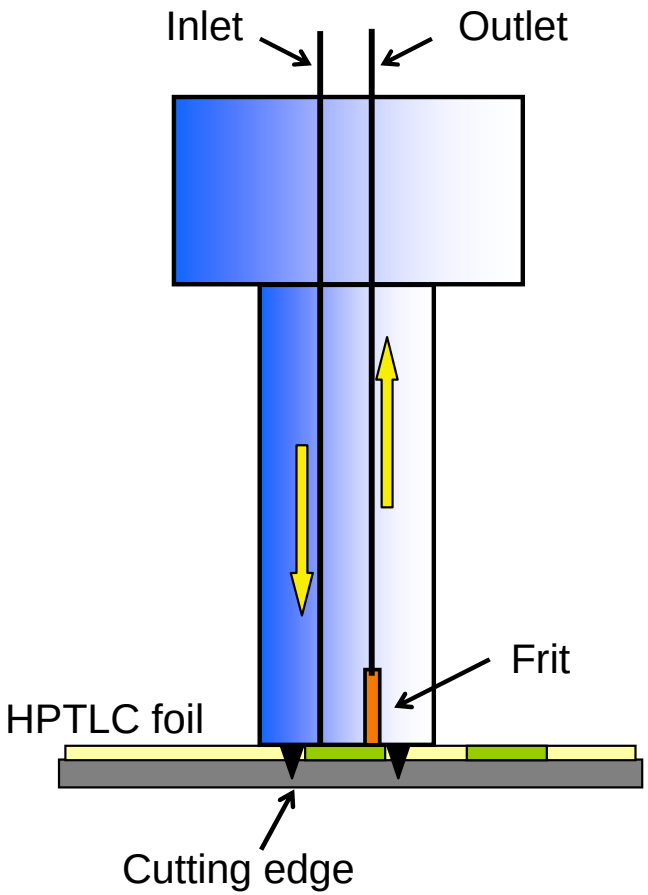
Online extraction



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

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

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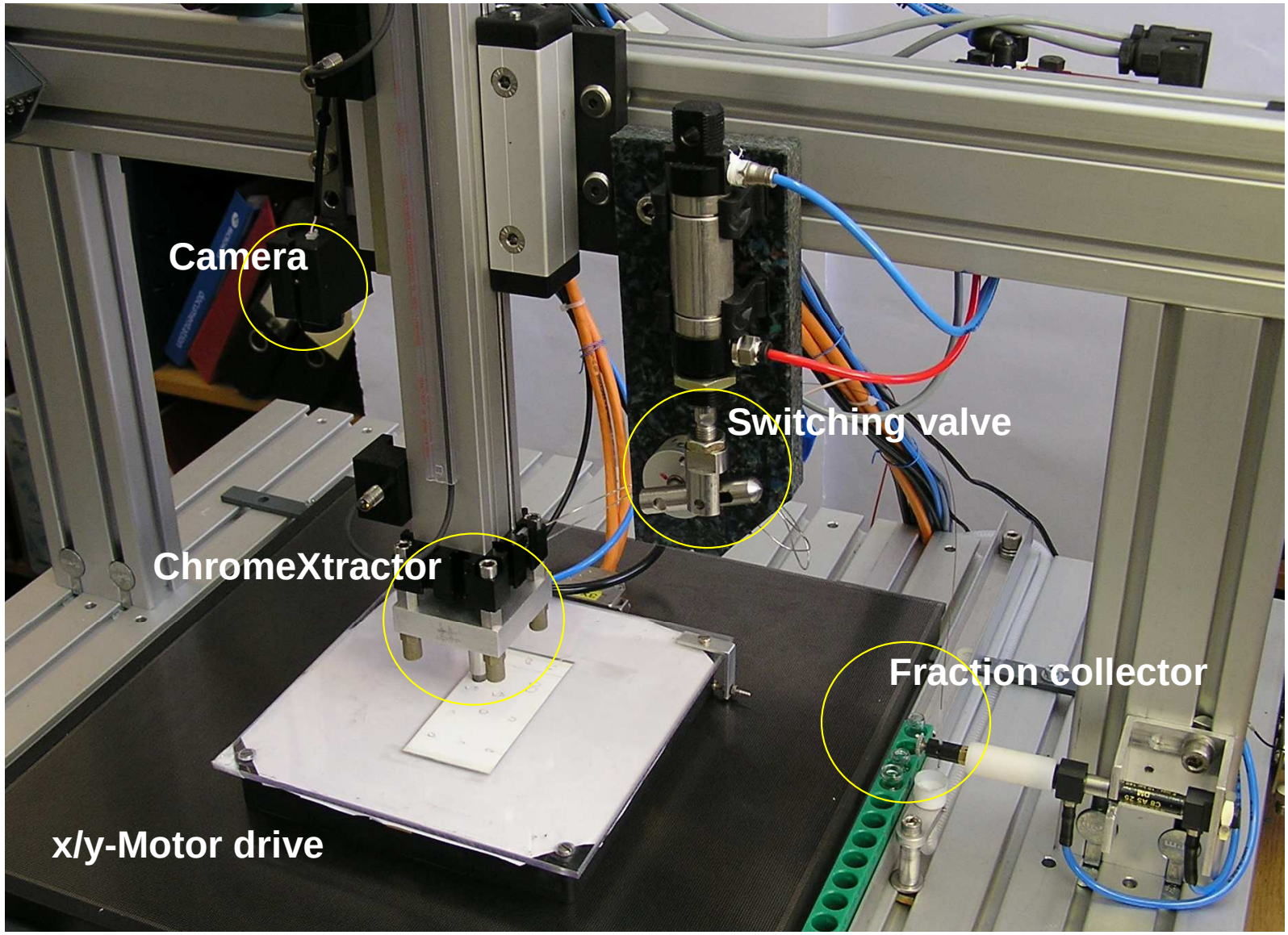
The hands-free interface called ‘R3D3’



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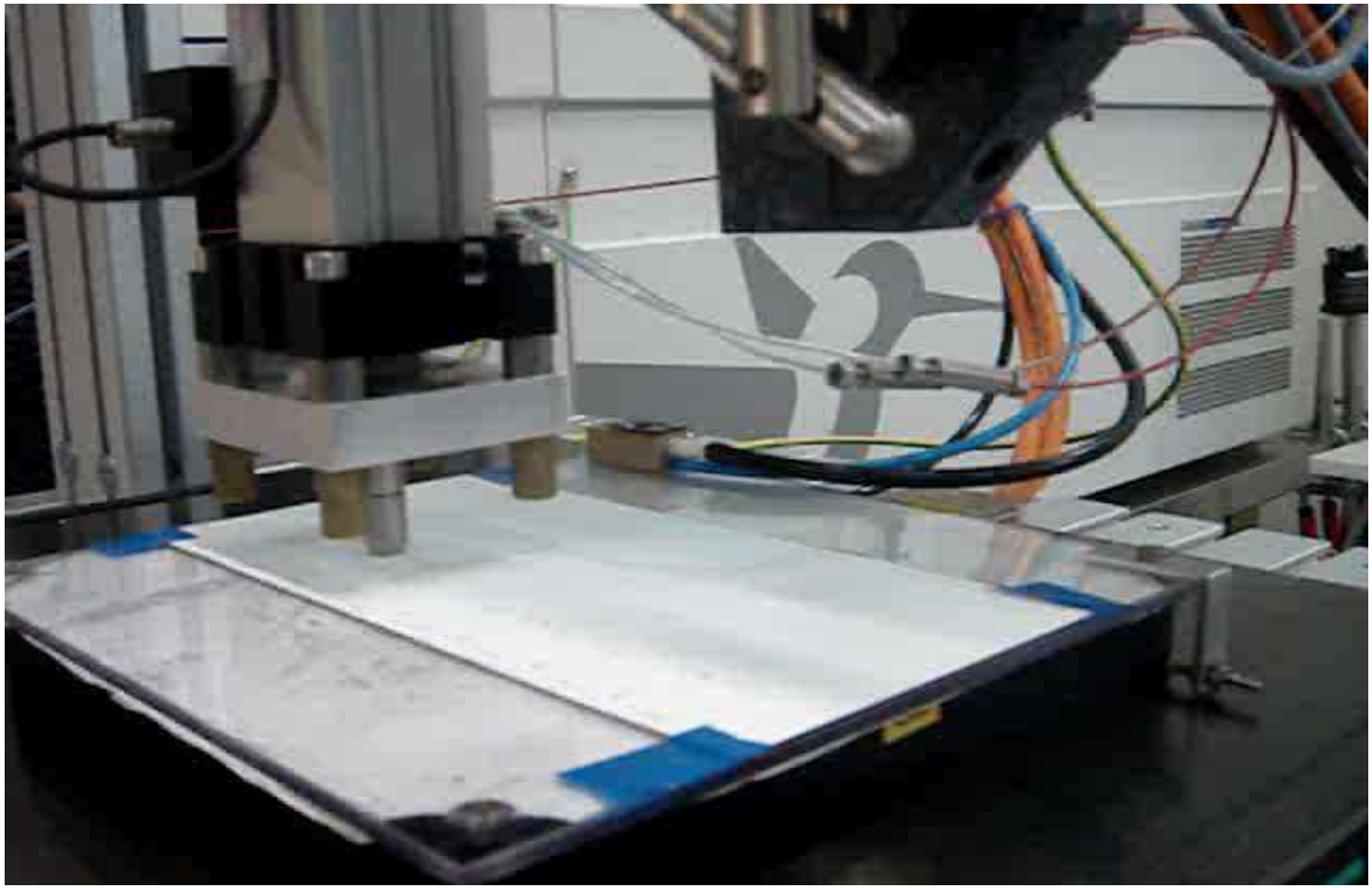
R3D3 working…



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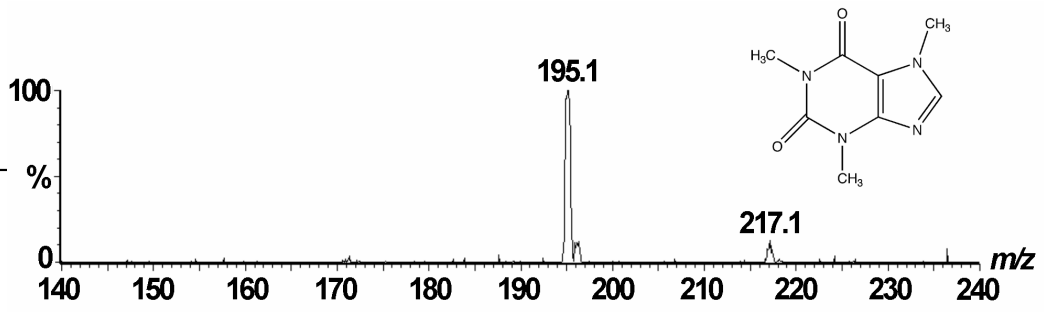
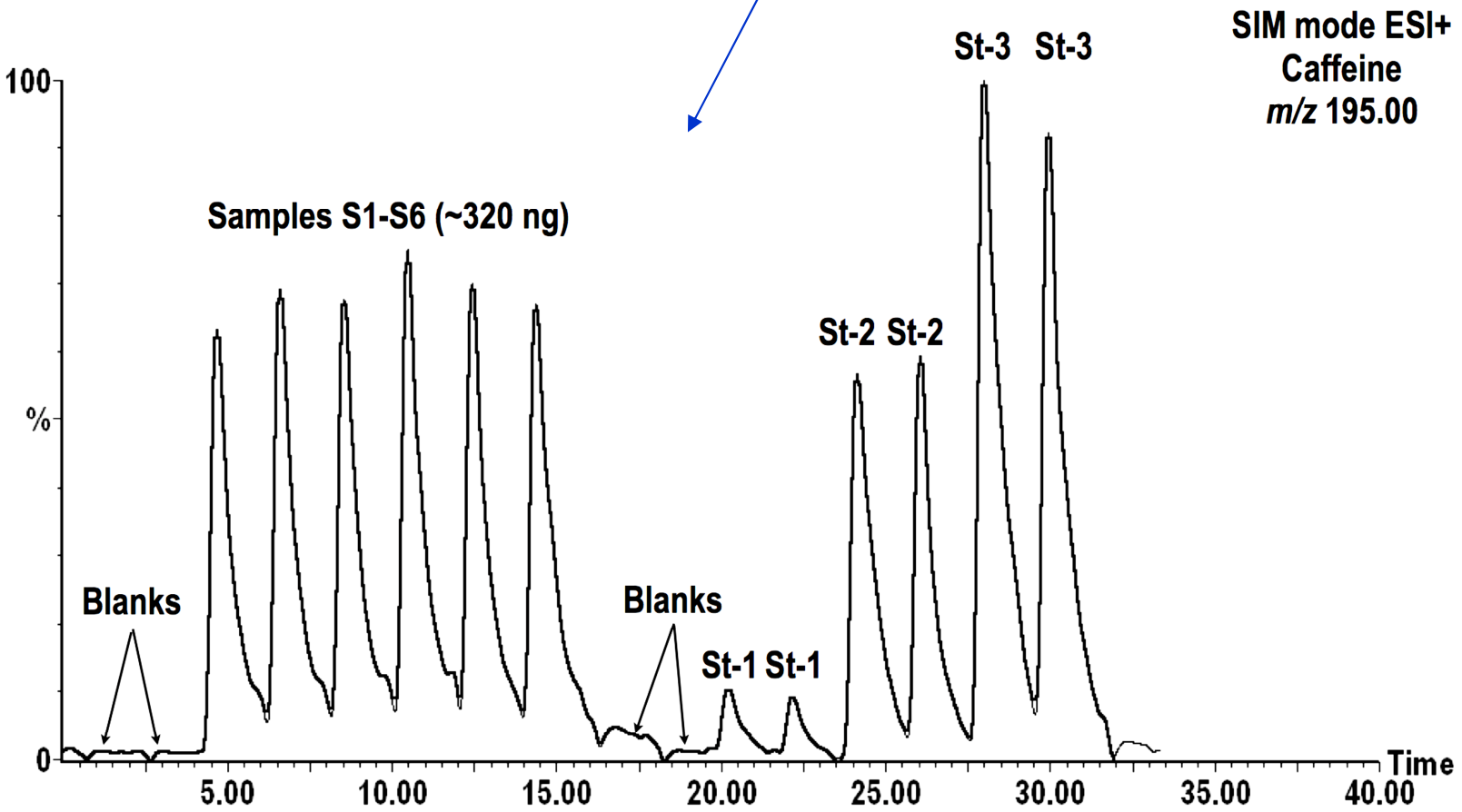
Elution profile





H. Luftmann, M. Aranda, G. Morlock, Rapid Commun Mass Spectrom 21 (2007) in press

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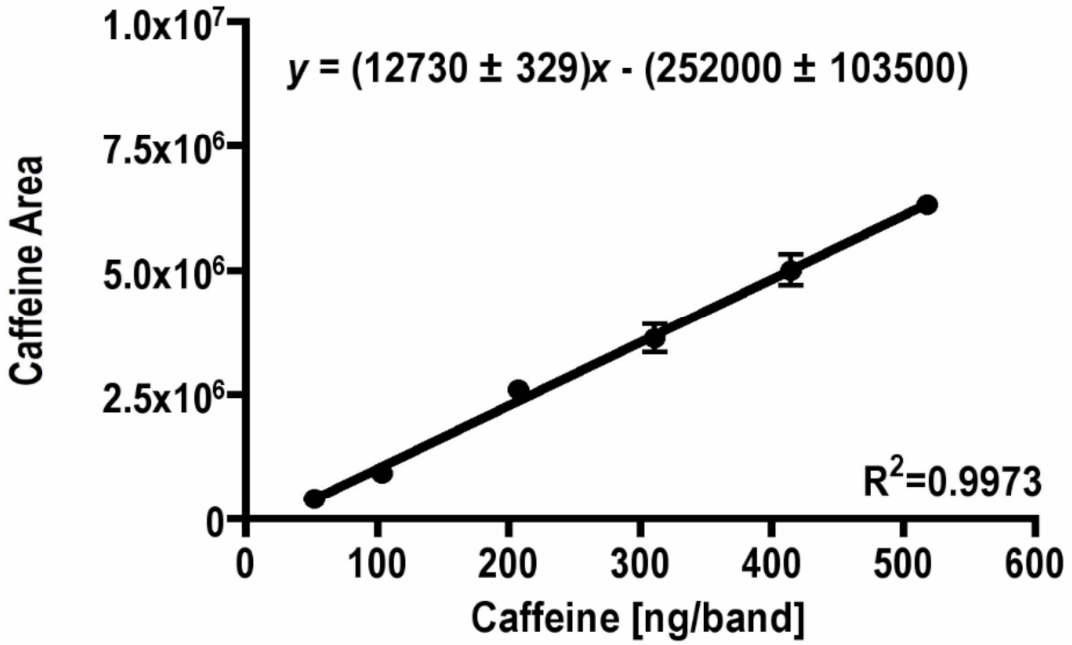
Data of validation without IS

→ repeatability in matrix of *RSD* = 5.6 % (*n* = 6)

→ linear response with determination coefficient of R^2 = 0.9973



H. Luftmann, M. Aranda, G. Morlock, Rapid Commun Mass Spectrom 21 (2007) in press

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Analysis of samples containing caffeine

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

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

H. Luftmann, M. Aranda, G. Morlock, Rapid Commun Mass Spectrom 21 (2007) in press

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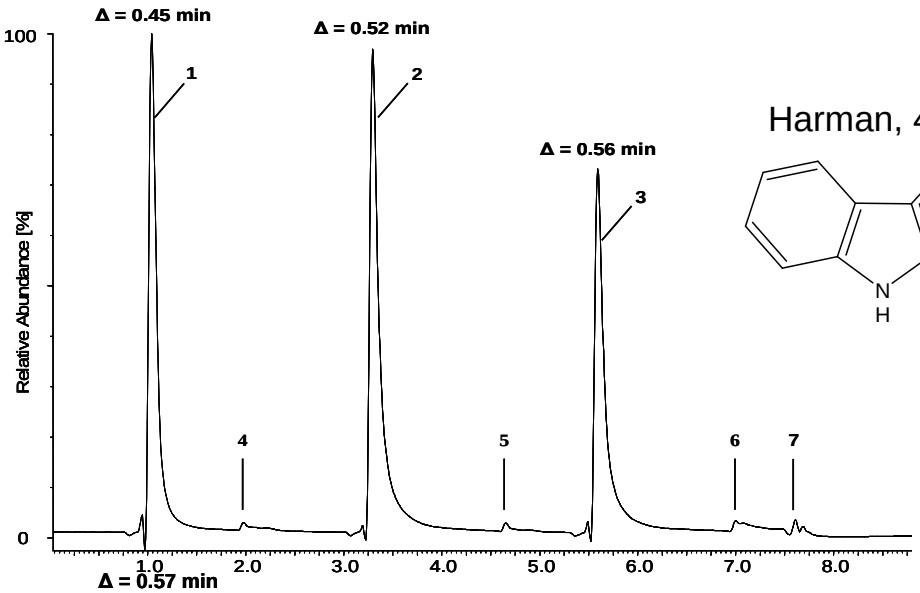
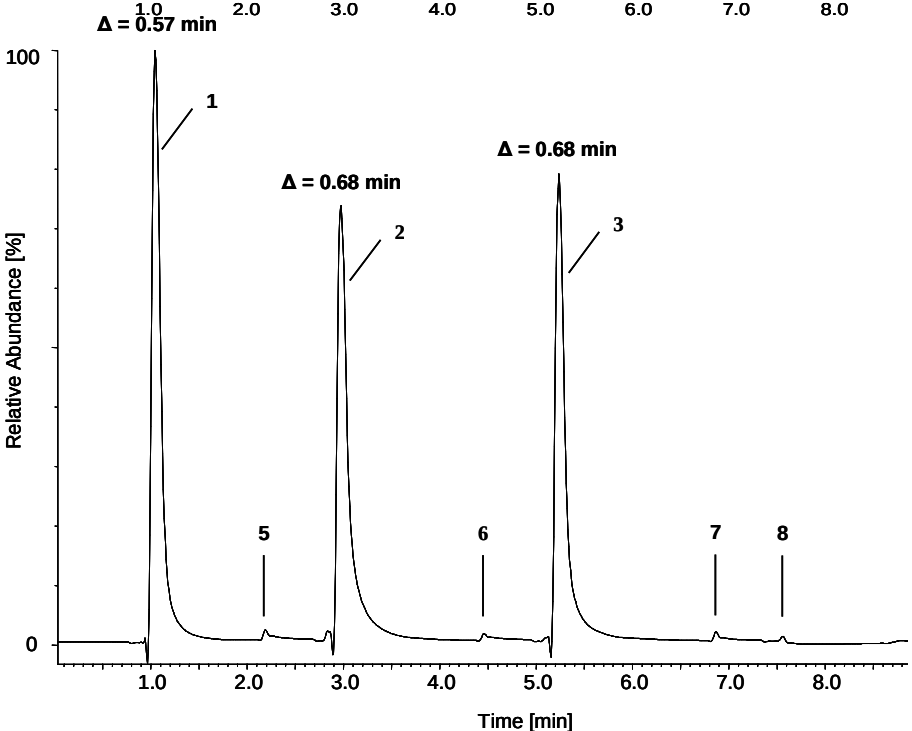
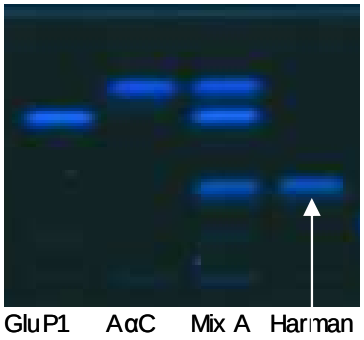
Comparison of different cutting edges

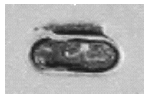


Harman, 4 ng each

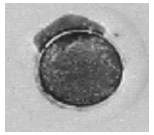


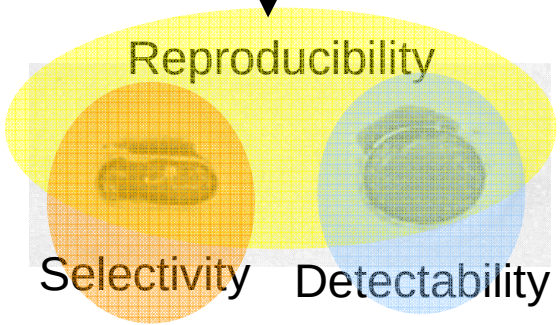


Oval cutting edge



Round cutting edge





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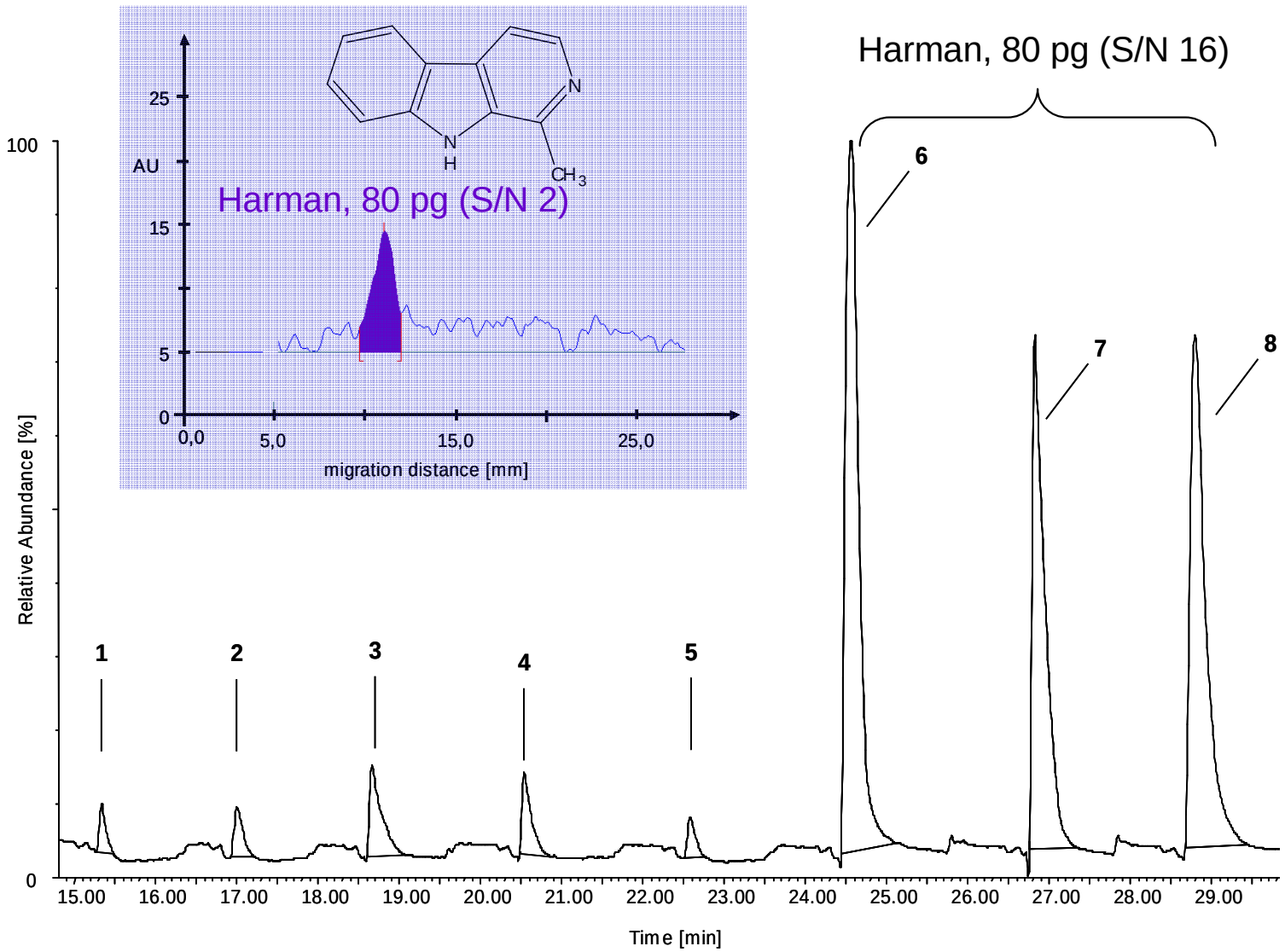
Detectability: FLD versus MSD



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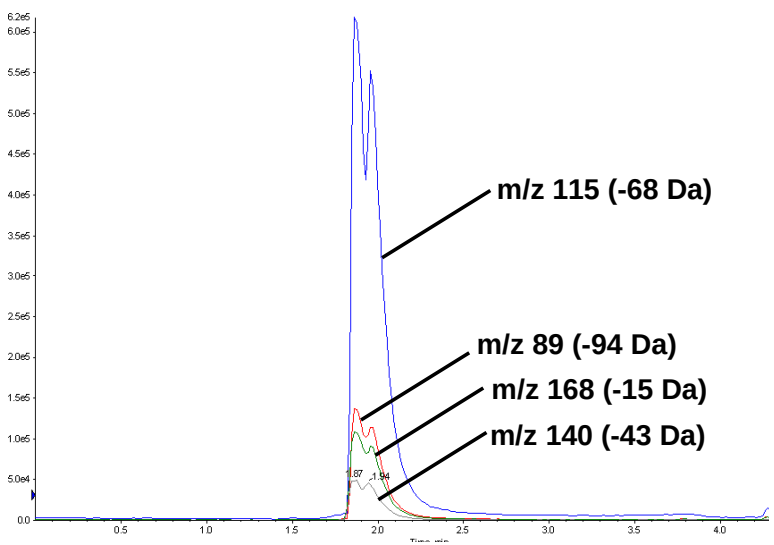
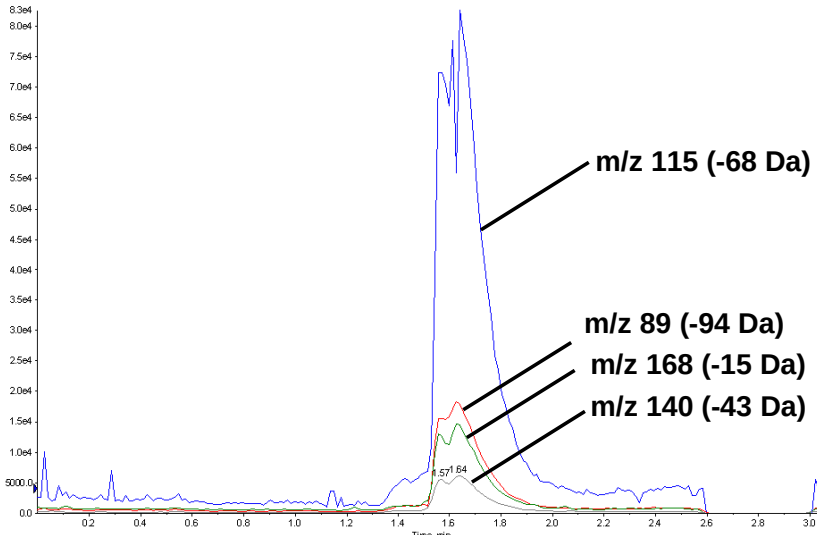
Detectability by HPTLC/ESI-MS-MS

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

→ detectability comparable to HPLC/MS





200 pg/zone Harman → 20 pg/zone Harman

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

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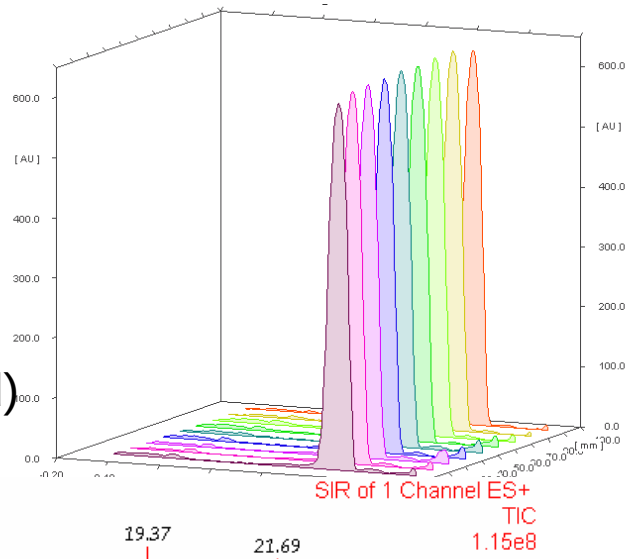
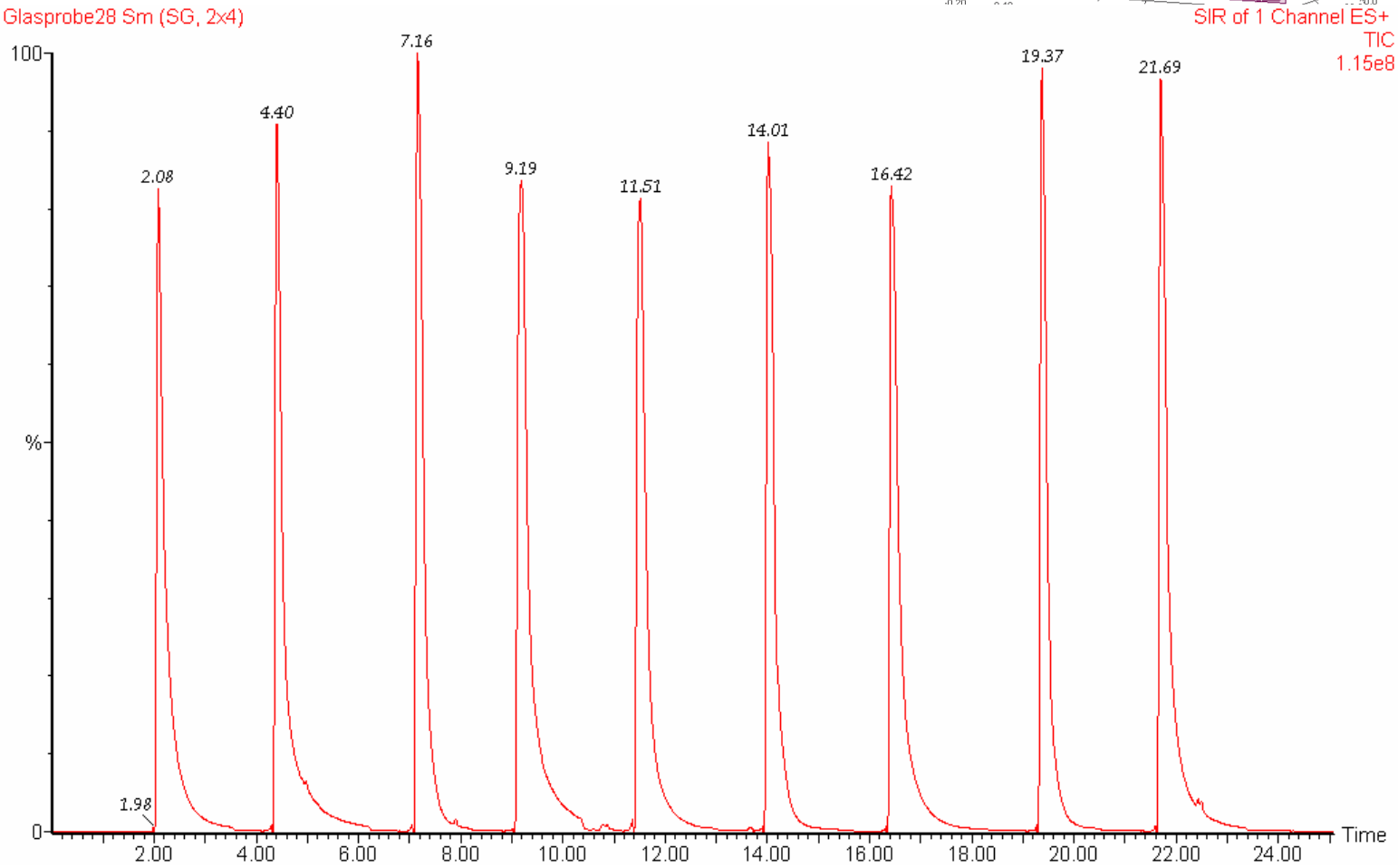
Repeatability of extraction



SIM at *m/z* 329 with RSD 6.6 % (n=9, 1 µg/band)



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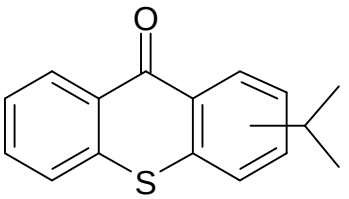
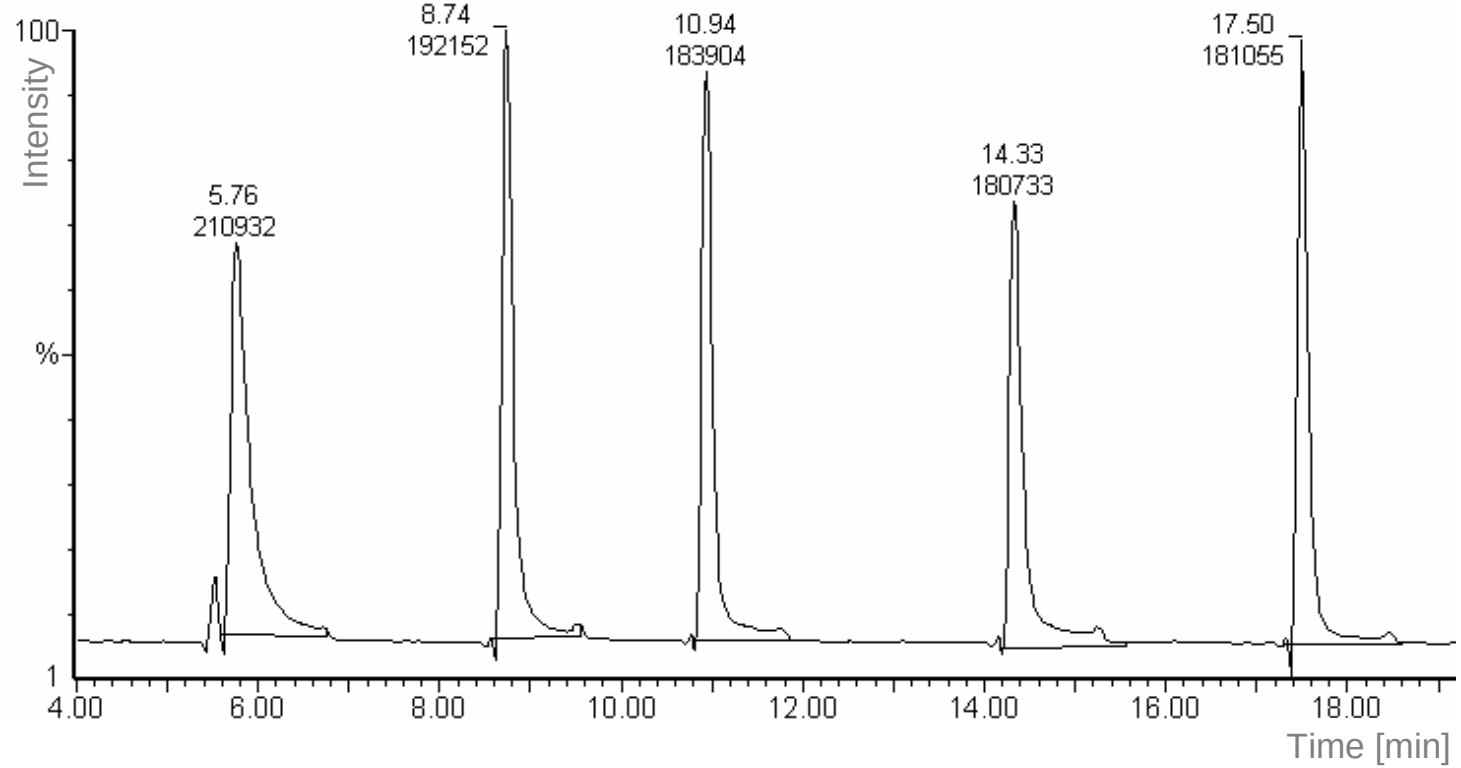
Trace analysis: Food contaminant ITX

Elution profiles of 6 ng ITX each

Repeatability RSD = ± 6.7 % (*n* = 5)



Isopropylthioxanthone



SIM at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$

G. Morlock, W. Schwack, Anal Bioanal Chem 385 (2006) 586-595

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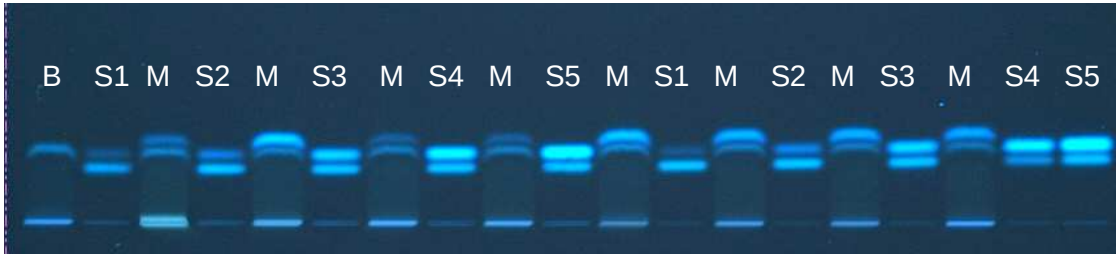
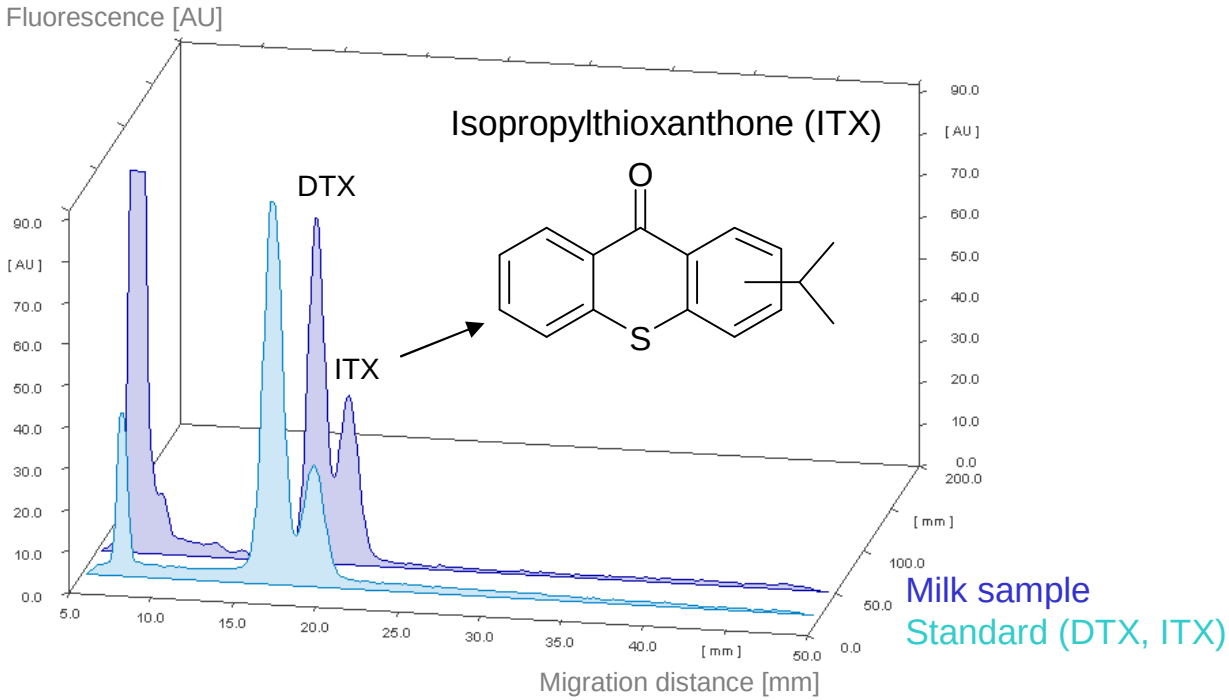
Trace analysis: Food contaminant ITX





G. Morlock, W. Schwack, Anal Bioanal Chem 385 (2006) 586-595

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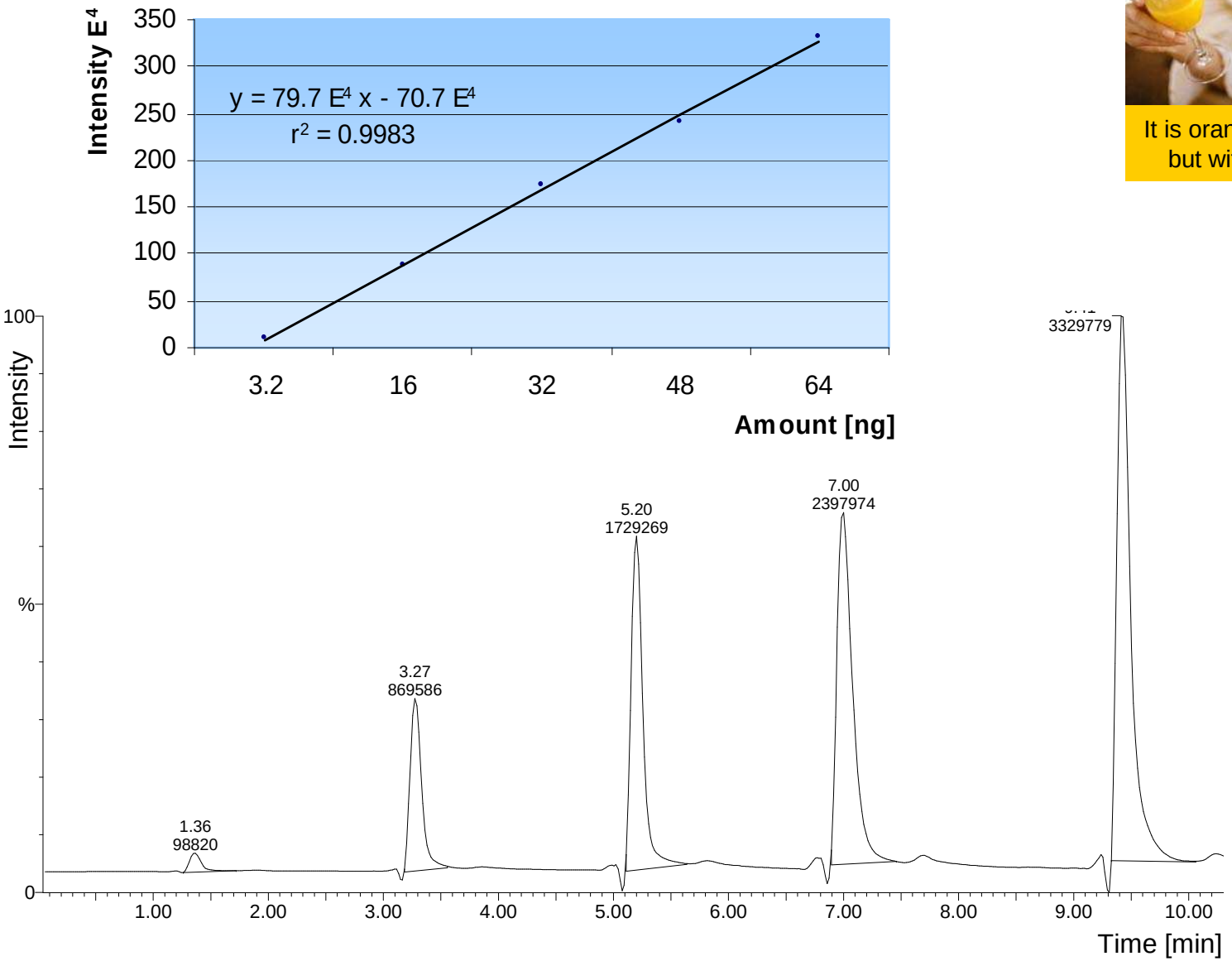
Analytical response

It is orange juice, but with ITX.



Elution profiles of ITX (SIM at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$)

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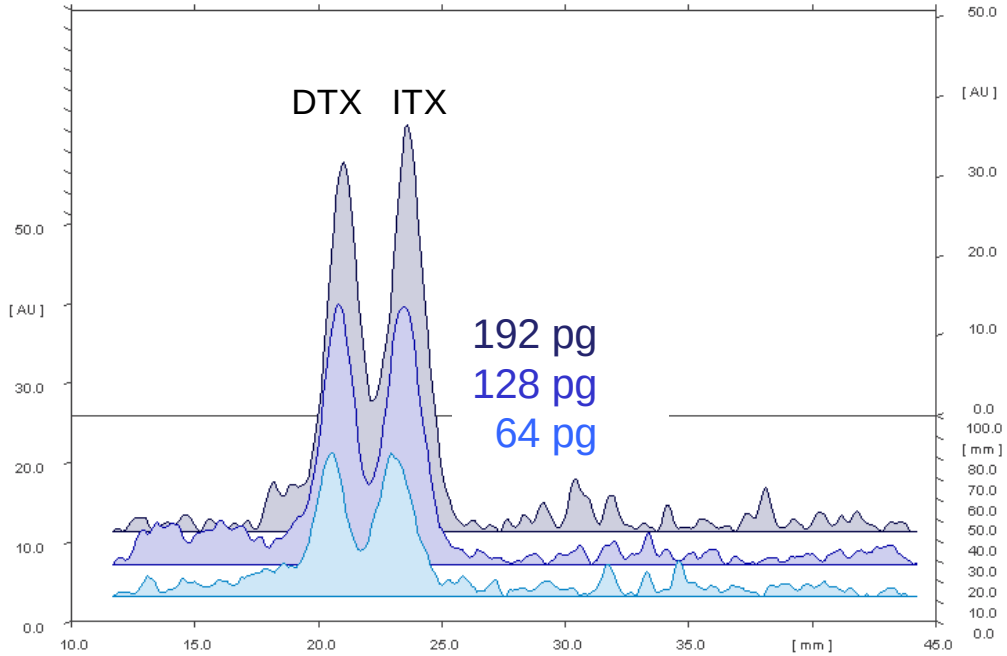
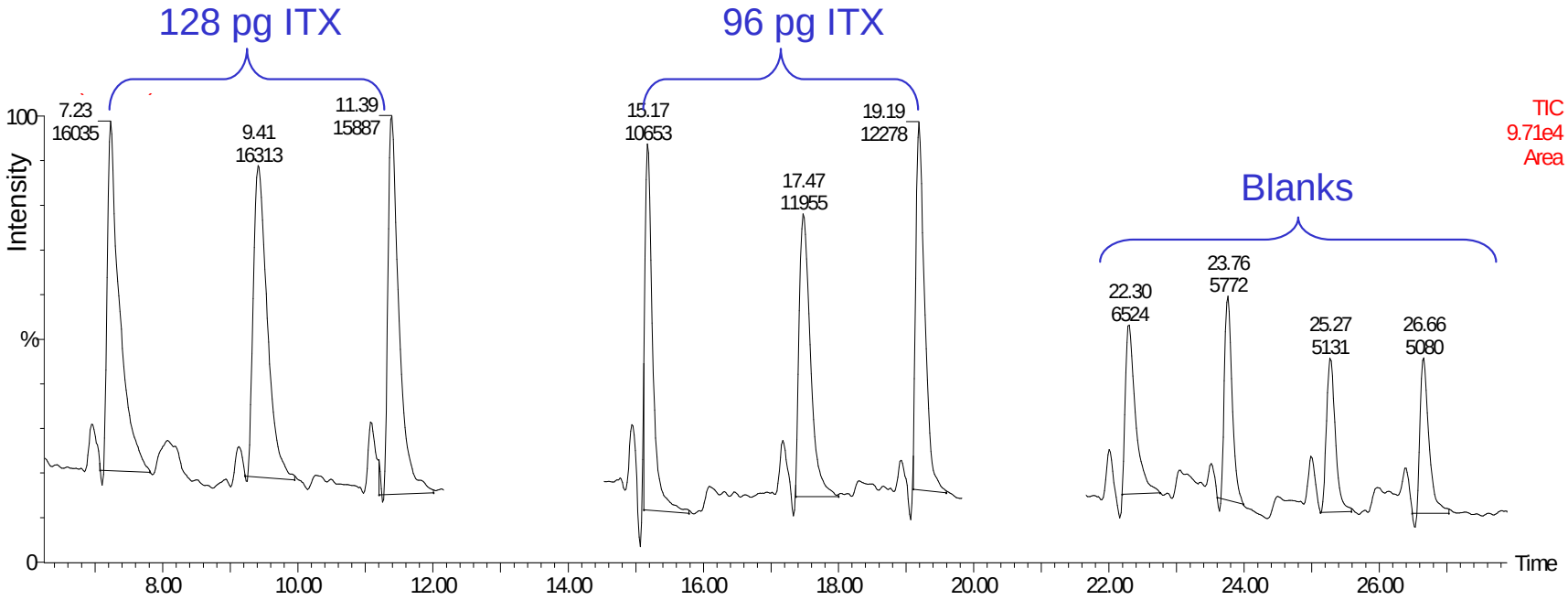
FLD ⟷ MSD





Elution profiles of ITX (SIM at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$)

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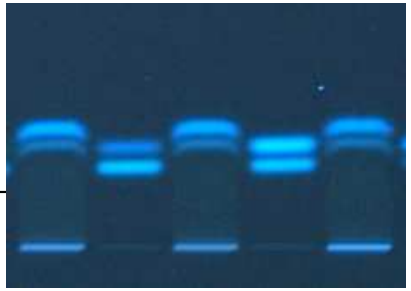
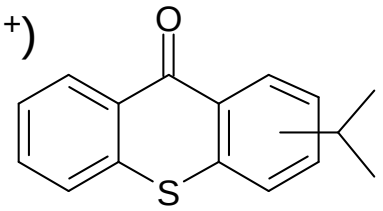
Confirmation by HPTLC/ESI-MS

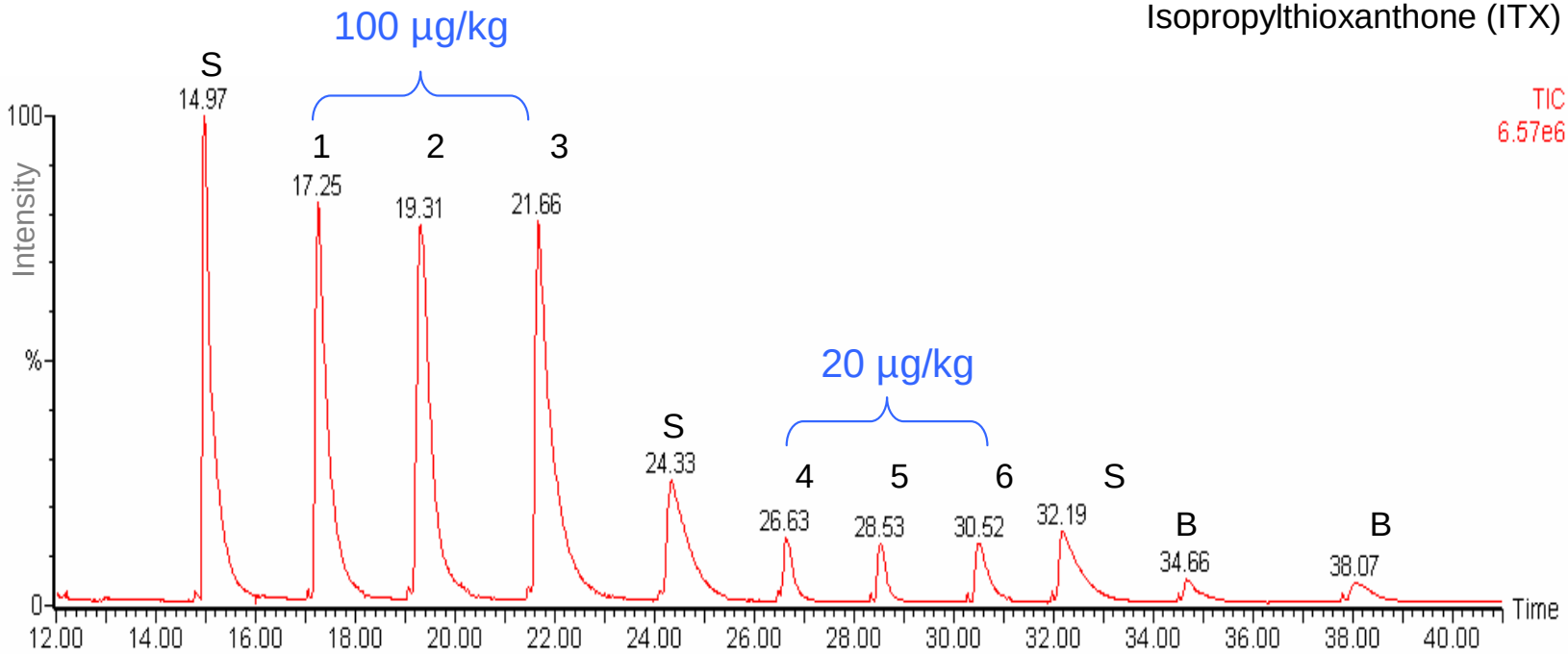


Elution profiles (SIM at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$)

→ Yoghurt samples spiked with ITX



Isopropylthioxanthone (ITX)



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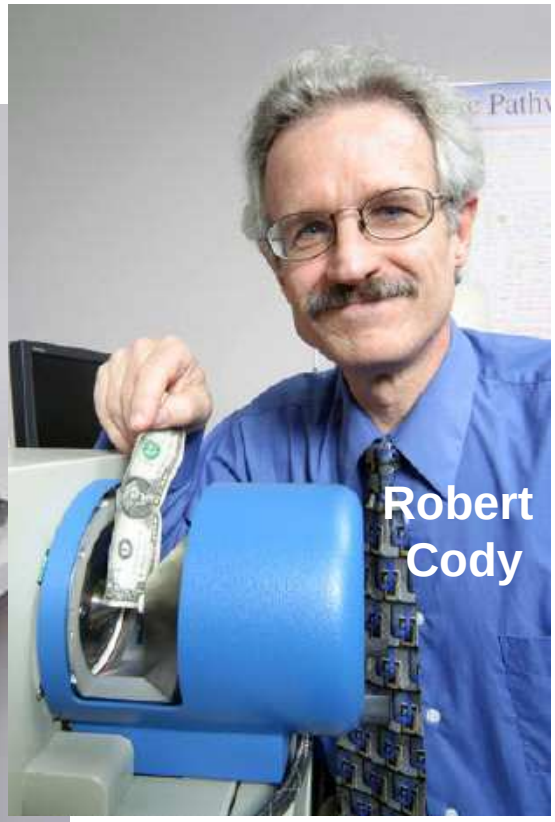
DART - Direct Analysis in Real Time



R. Cody, J. Laramée, H. Dupont Durst Anal Chem 77 (2005) 2297-2302

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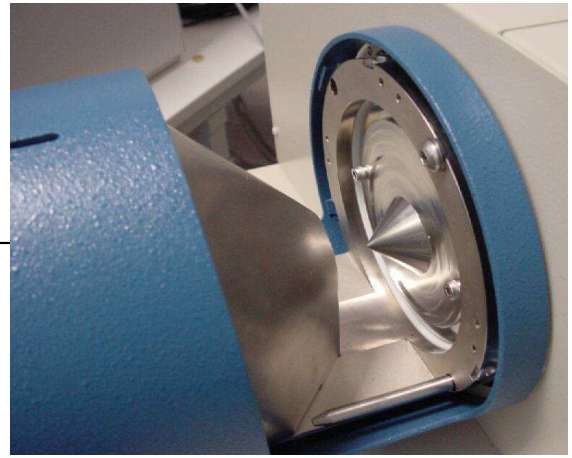
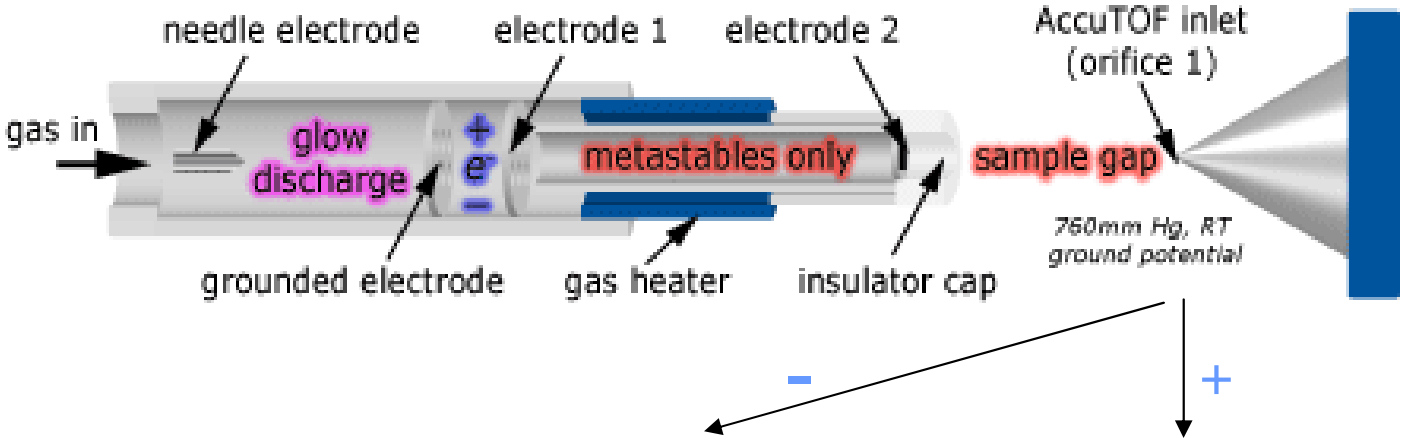
DART mechanisms





$$M^{*} + N \rightarrow N^{+\bullet} + M + e^{-}$$

$$e^{-*} + G \rightarrow e^{-} + G^{*}$$

$$e^{-} + O_2 \rightarrow O_2^{-\bullet}$$

$$O_2^{-\bullet} + S \rightarrow [S\text{-}H]^{-} + OOH^{\bullet}$$

$$O_2^{-\bullet} + S \rightarrow S^{-\bullet} + O_2$$

$$O_2^{-\bullet} + S \rightarrow [S+O_2]^{-\bullet *} + G \rightarrow [S+O_2]^{-\bullet} + G^{*}$$

$$He(2^3S) + H_2O \rightarrow H_2O^{+\bullet} + He(1^1S) + e^{-}$$

$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^{+} + OH^{\bullet}$$

$$H_3O^{+} + nH_2O \rightarrow [(H_2O)_nH]^{+}$$

$$[(H_2O)_nH]^{+} + M \rightarrow MH^{+} + nH_2O$$

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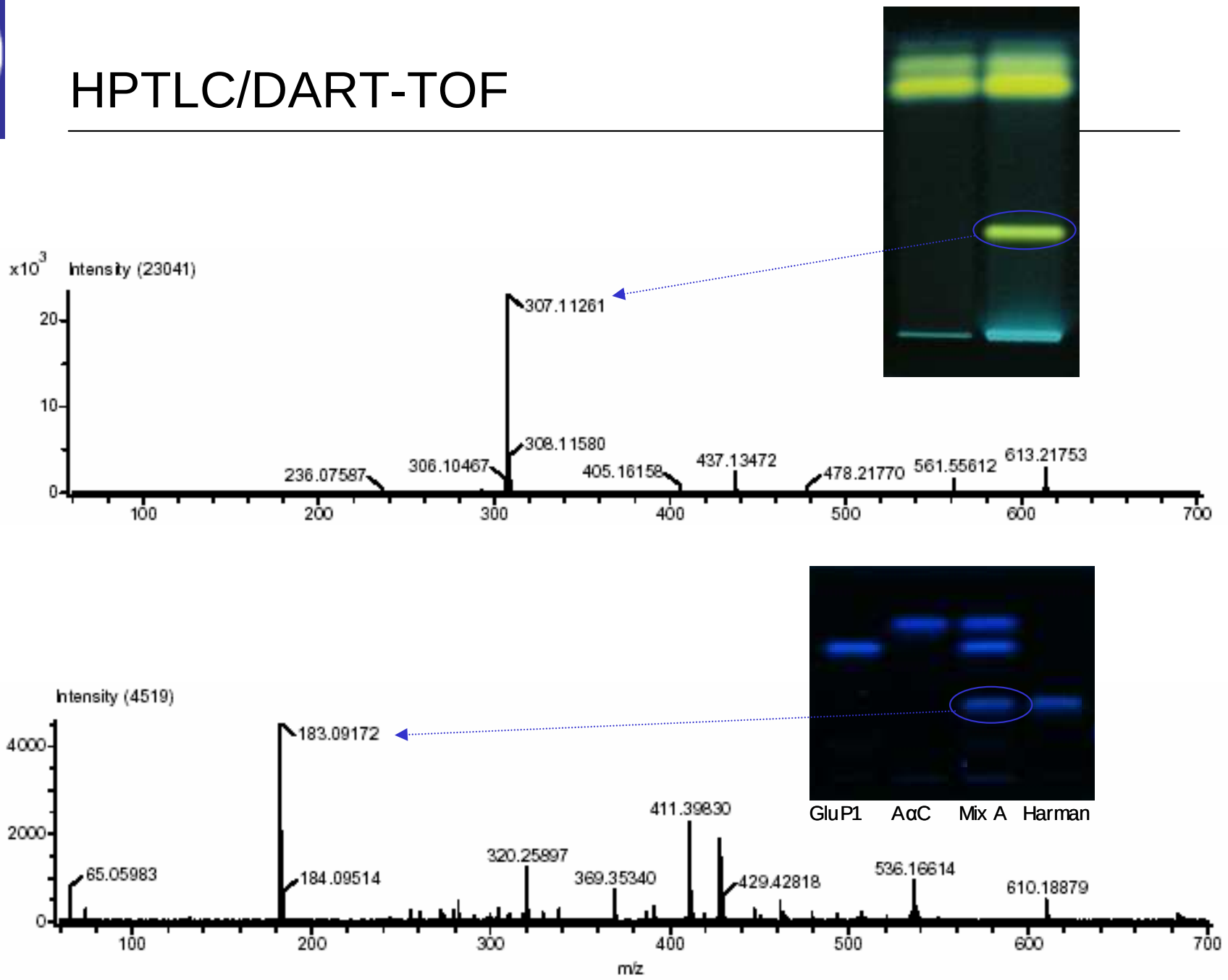
HPTLC/DART-TOF



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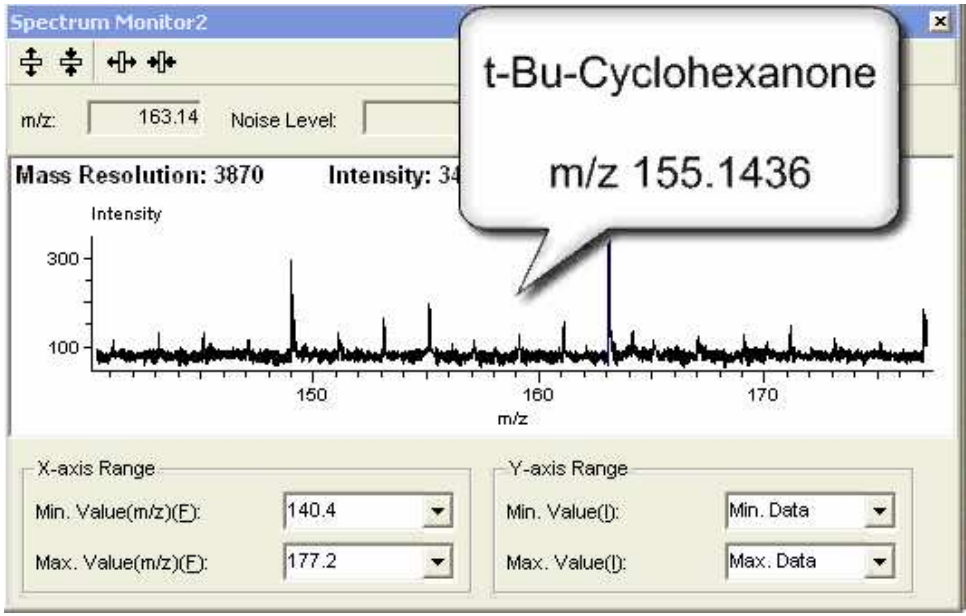
HPTLC/DART coupling



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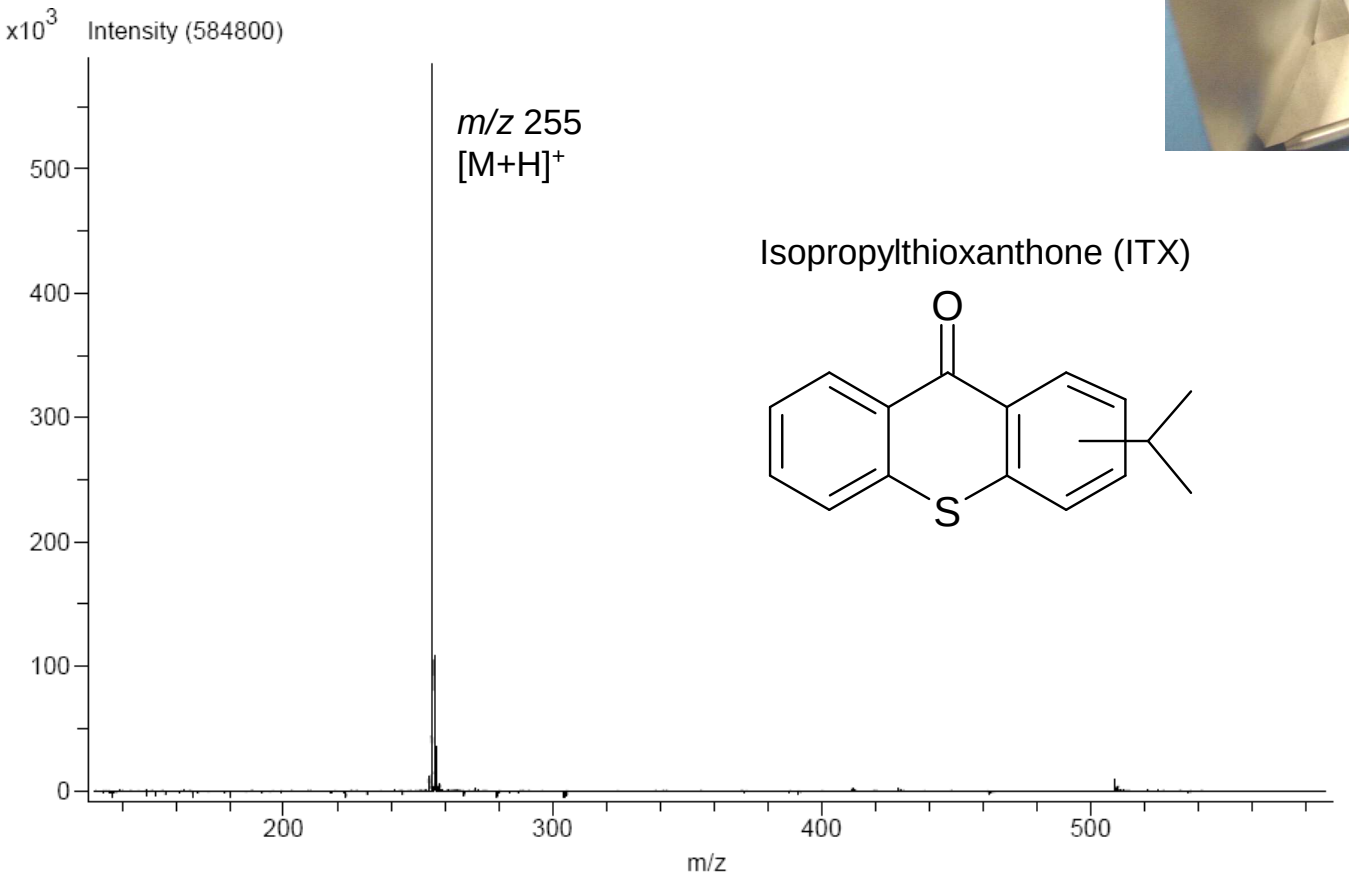
HPTLC/DART-TOF



Isopropylthioxanthone (ITX)

G. Morlock, W. Schwack, Anal Bioanal Chem 385 (2006) 586-595
G. Morlock, W. Schwack, CBS 96 (2006) 11-13

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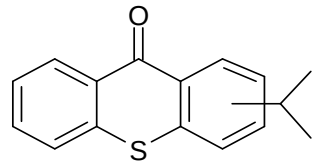
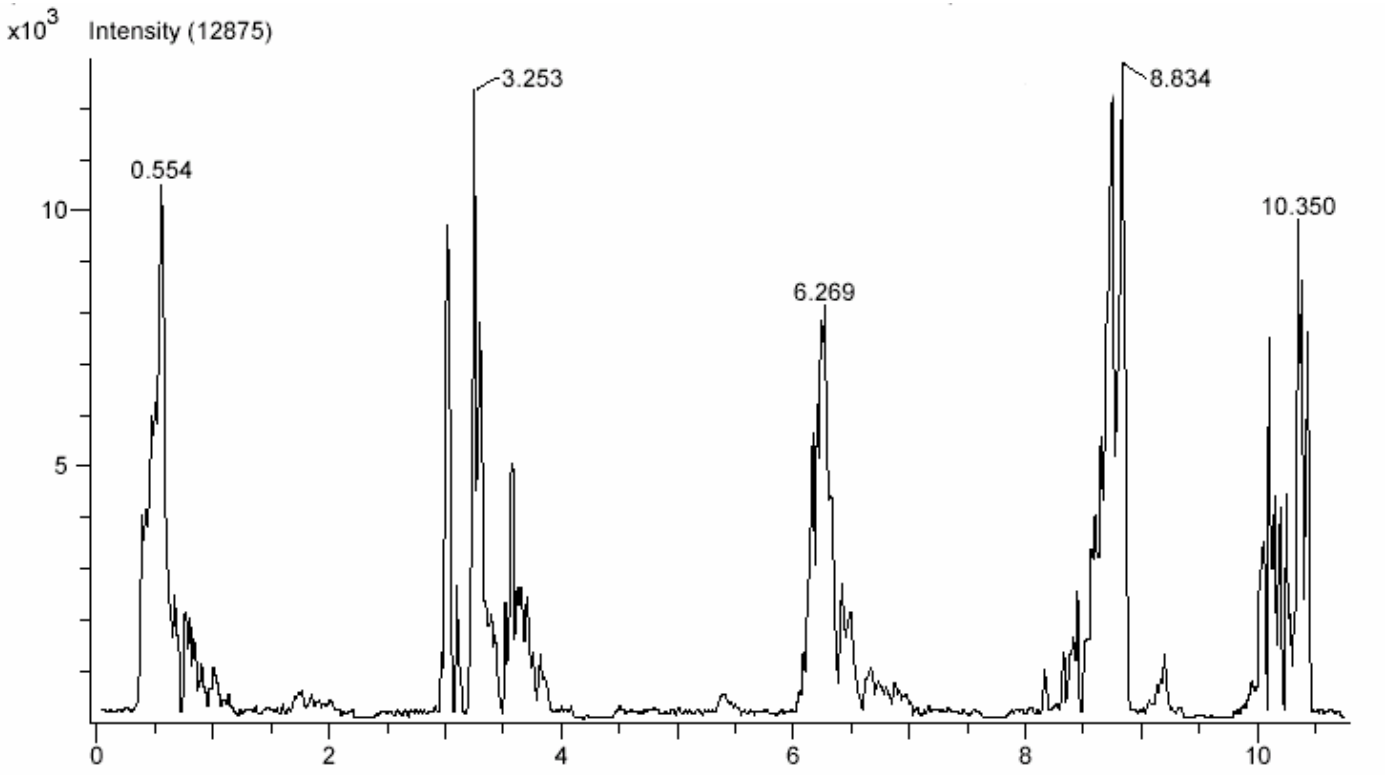
Repeatability



Isopropylthioxanthone (ITX)

5 zones, 32 ng ITX each: RSD = **± 71.1 %**



Mass chromatogram [min] at m/z 255.086 $[M+H]^+$

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

G. Morlock, Y. Ueda, LCGC The Peak June (2007) 7-14

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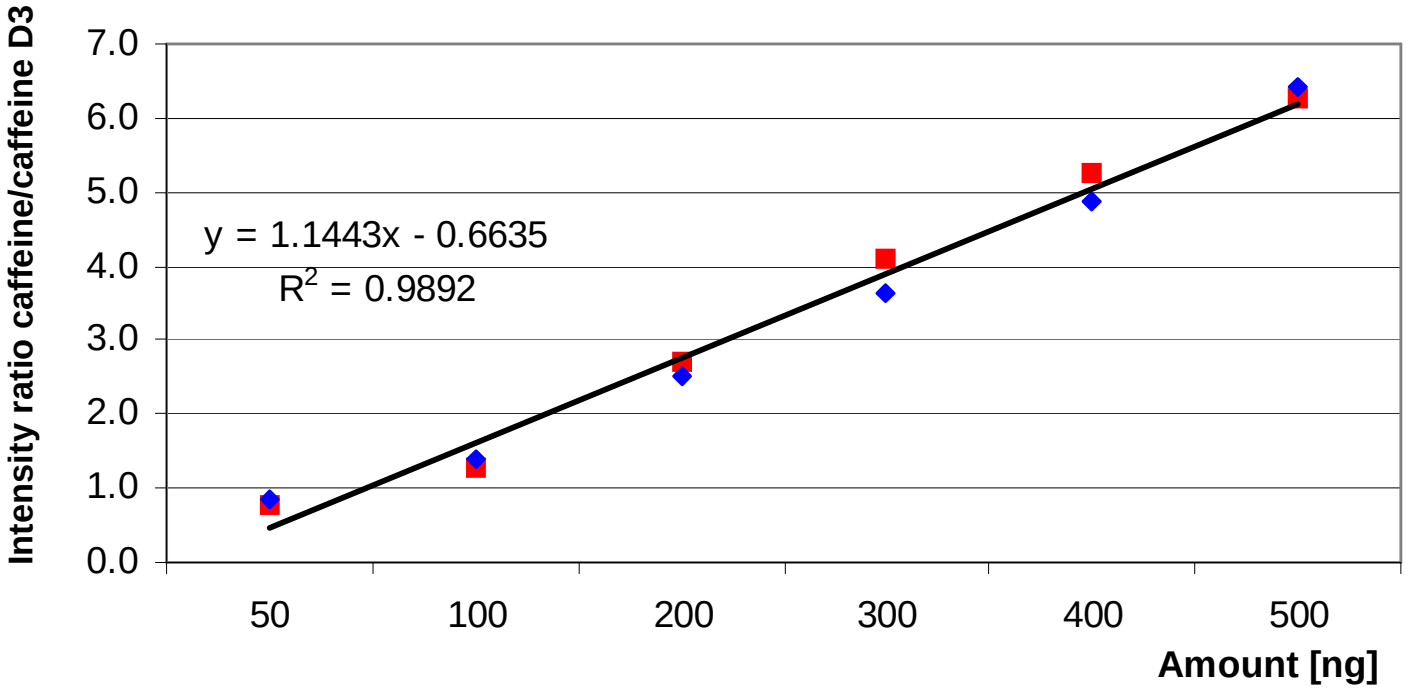
HPTLC/DART-IDA-TOF

- Repeatability RSD **< 5.4 %,** *n* = 6
- Coefficient of determination **R^2 = 0.9892**



Caffeine at *m/z* 195 $[M+H]^+$ corrected by the stable isotope labeled internal standard caffeine D3 at *m/z* 198 [M+H]+

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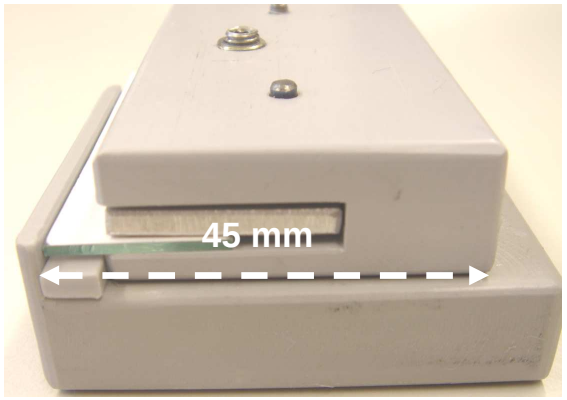
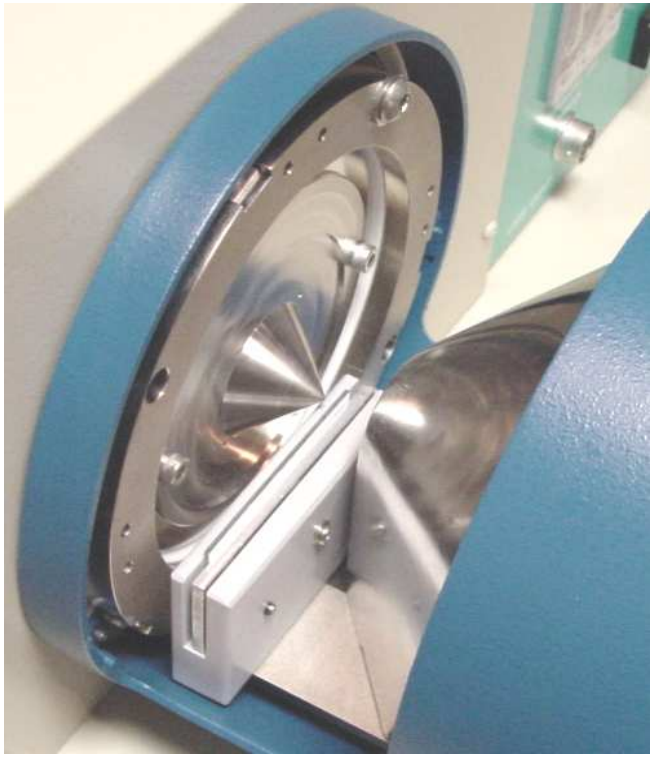
Plate holder





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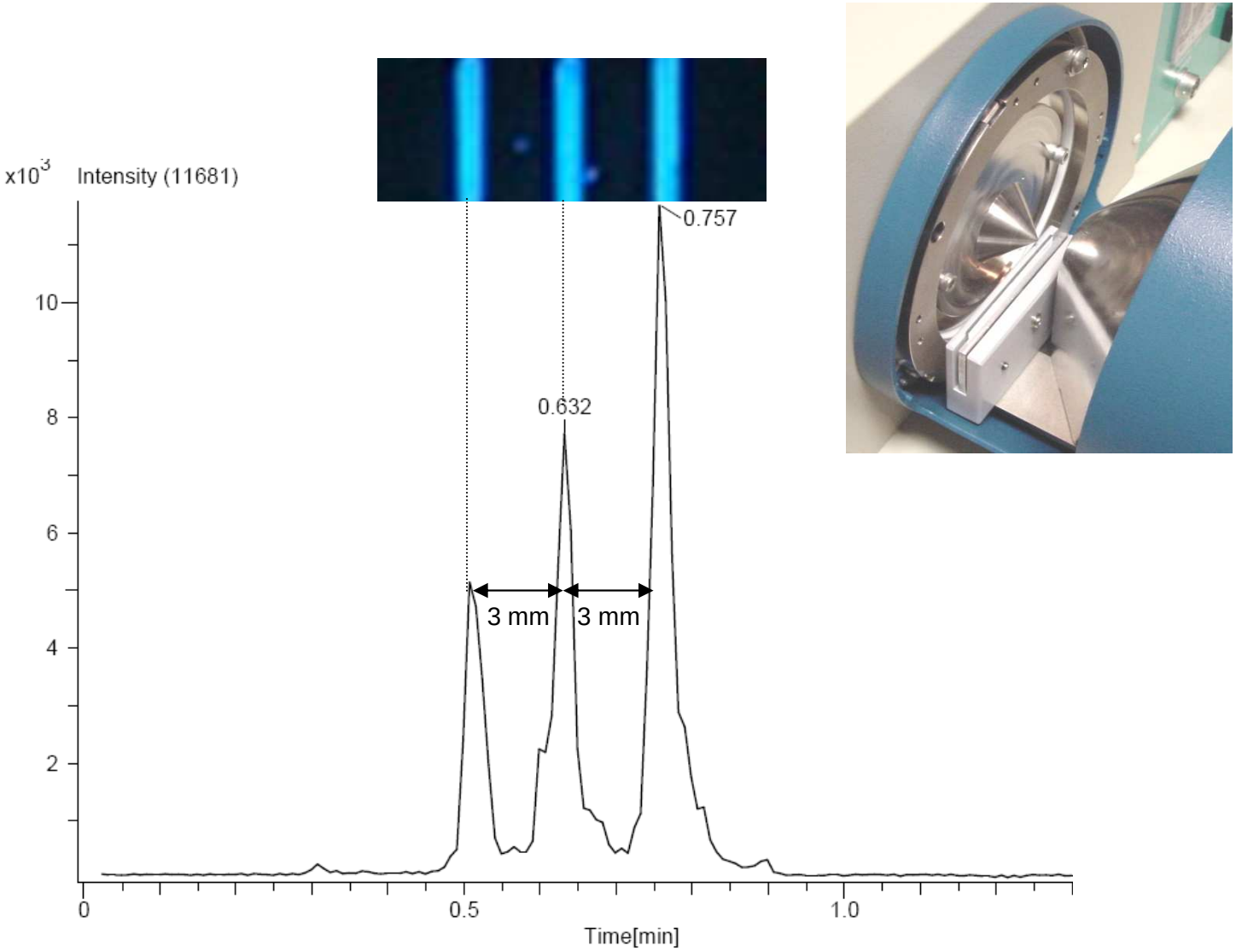
Spatial resolution of DART



Speed: ~2 mm/10s

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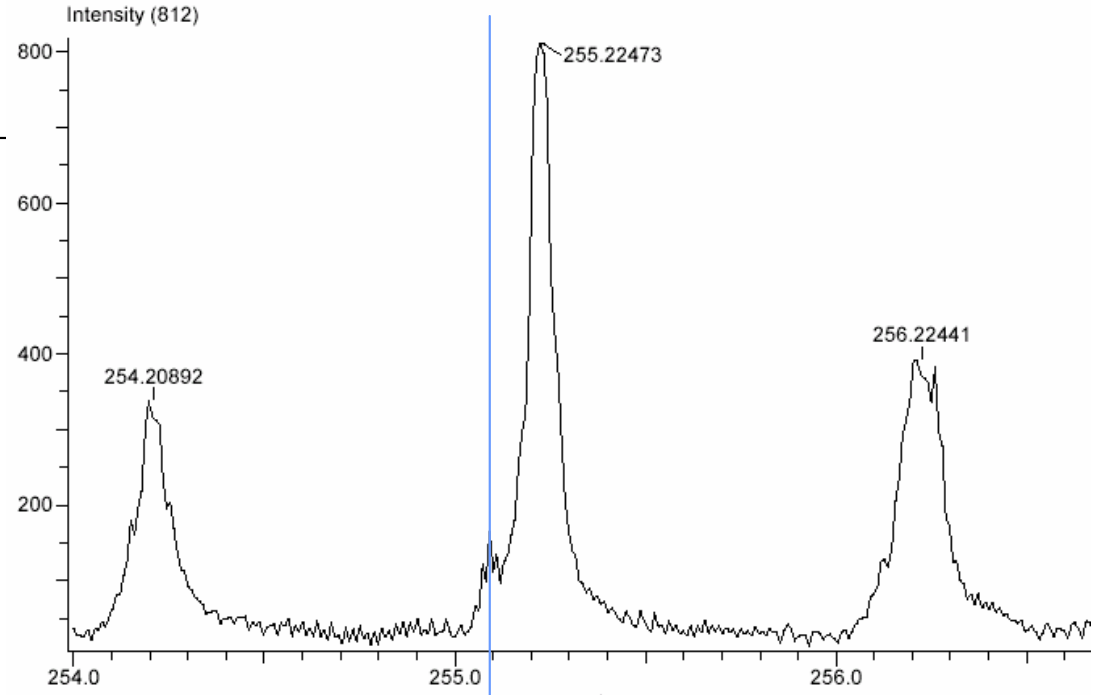
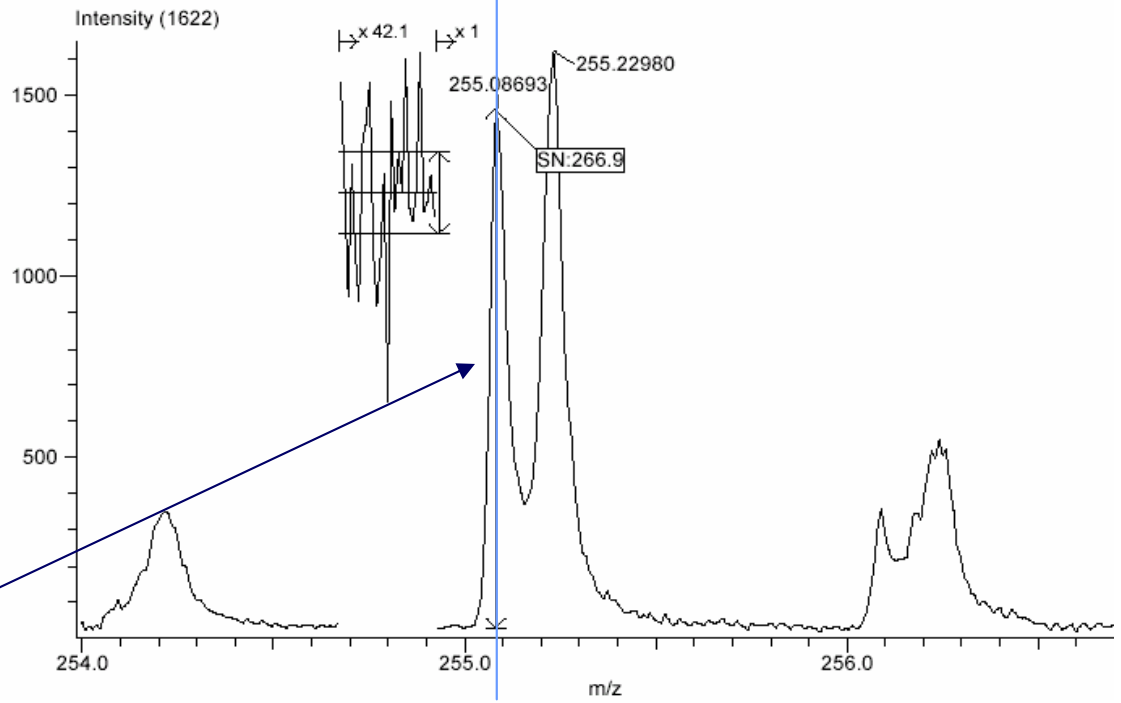
Detectability

Blank
m/z 255.08693



3.2 ng ITX zone
m/z 255.08693
S/N 267



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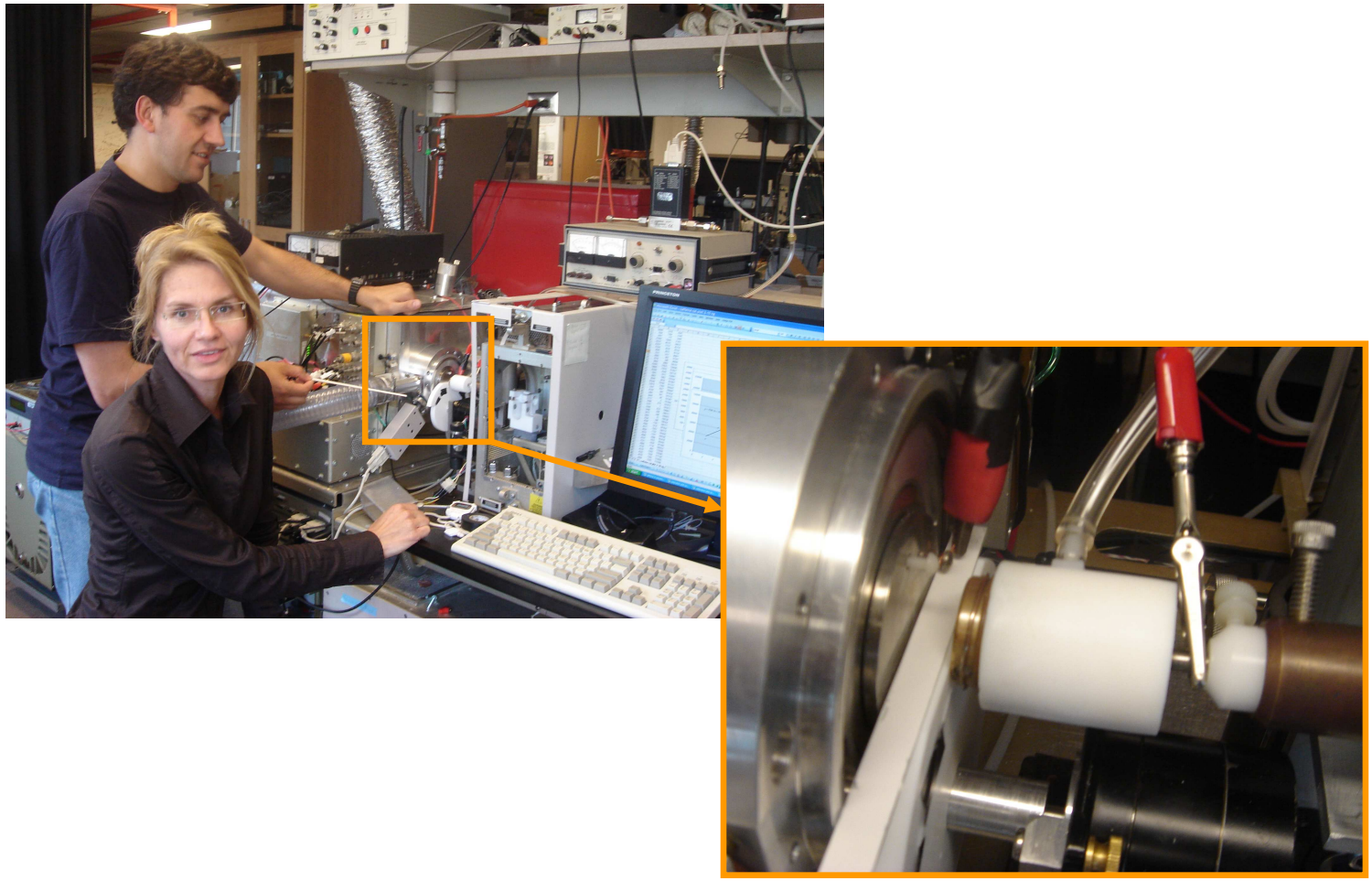
HPTLC/APGD-TOF coupling



G. Morlock, F. Andrade, G. Hieftje, in preparation

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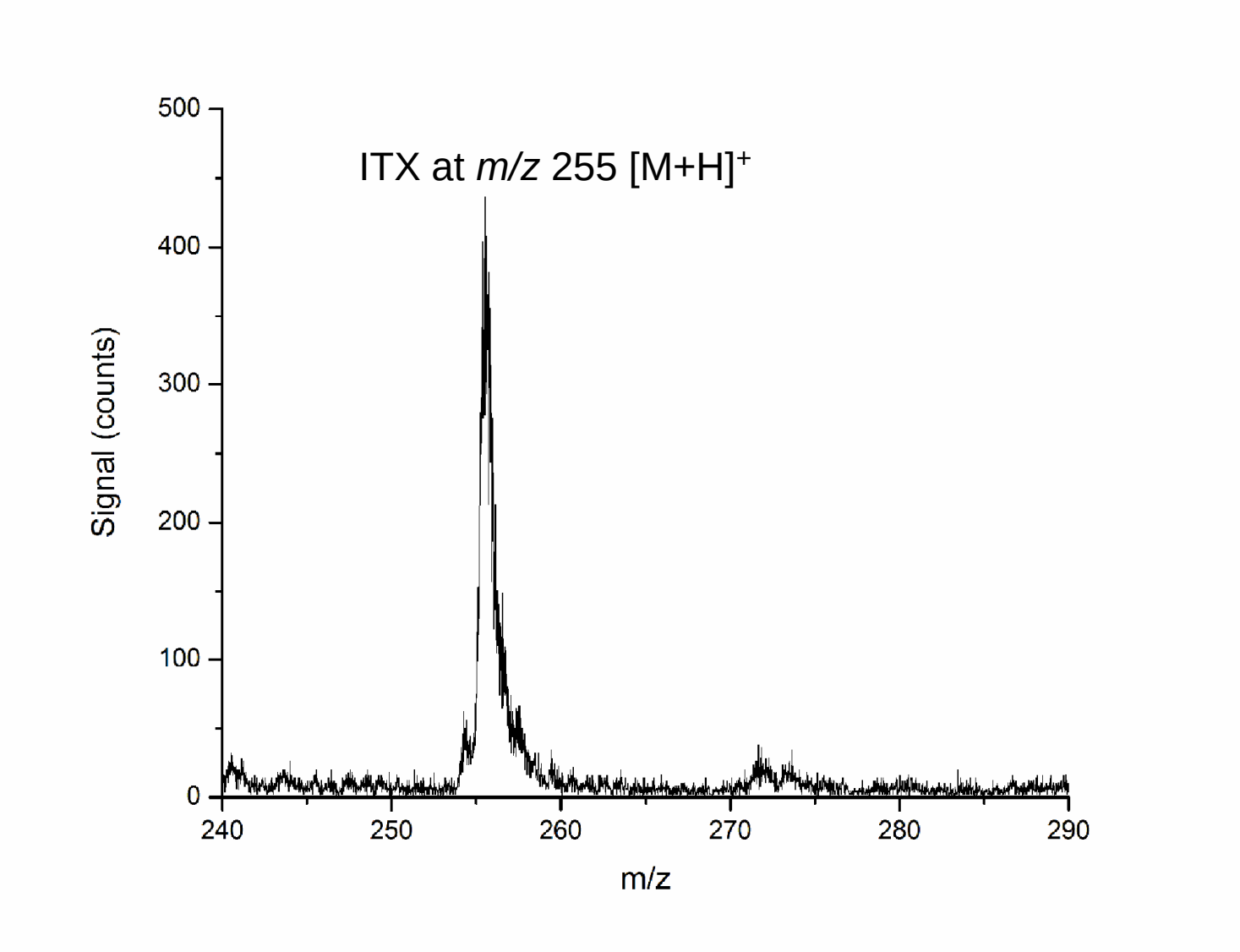
HPTLC/APGD-TOF



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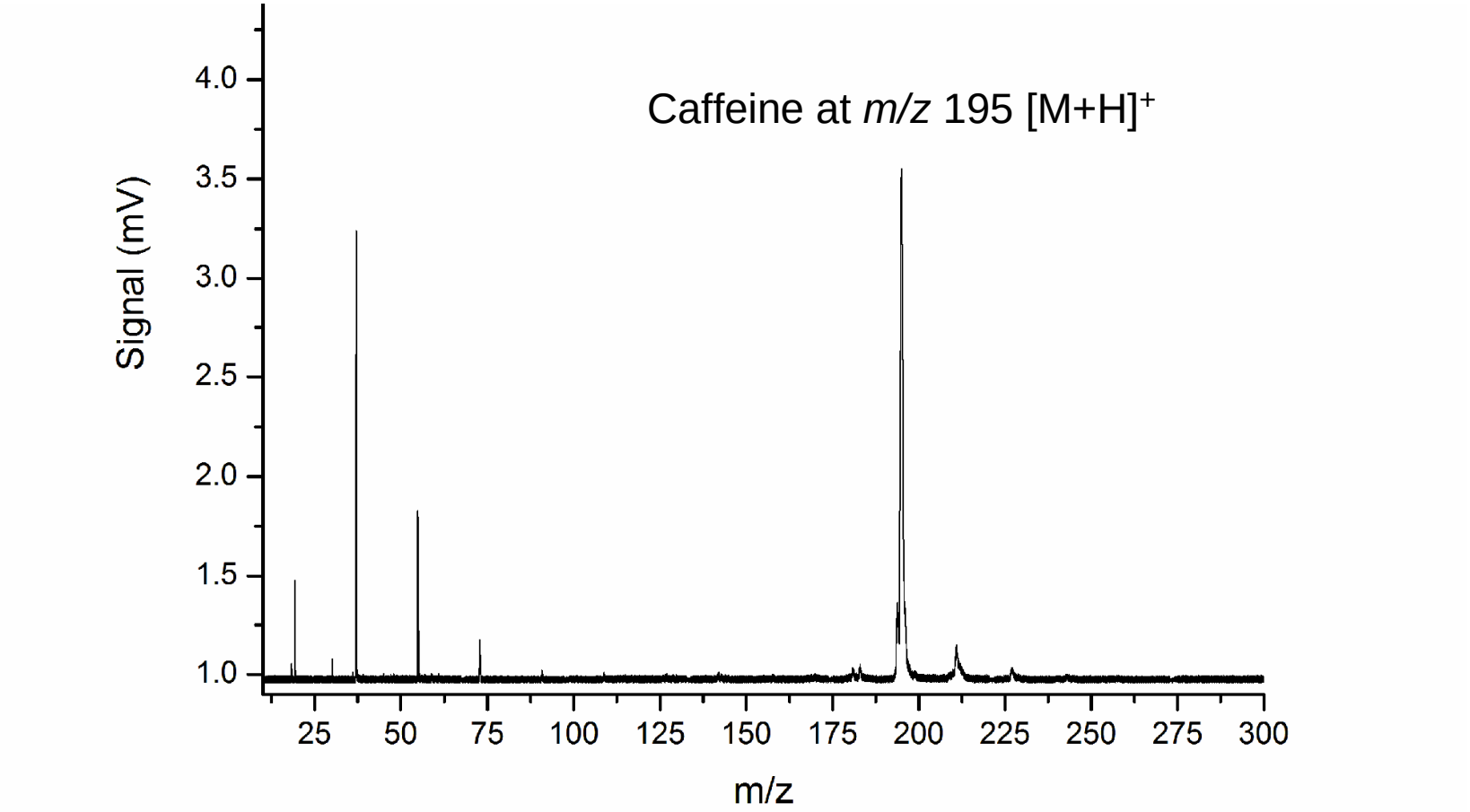
HPTLC/APGD-TOF



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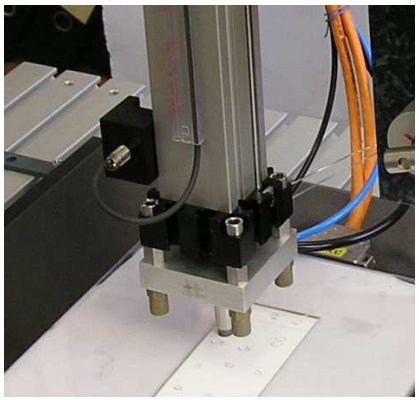
Comparison of interfaces

DART & APGD

- → dry desorption technique ⟷ DESI
- → no plate preparation etc. ⟷ SALDI, MALDI
- → eased handling (ambient conditions)
- → simple spectra ⟷ MALDI
- → quantitativ *with* internal standard → scanfunction

ESI via R3D3

- ✓ universally connectable to any LC-MS system given
- ✓ without adjustments or mass spectrometer modifications
- ✓ fully automated (hands-free)
- ✓ whole plate (no cut)
- ✓ all layers and carriers
- ✓ cost-effective
- ✓ detectability in the pg/zone-range
- ✓ with good linear range and repeatability
- ✓ withstand validated methods



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10. Flexible working station

At one HPTLC working place → 4 persons work on 4 different projects
→ 300 runs per day (staggered system)

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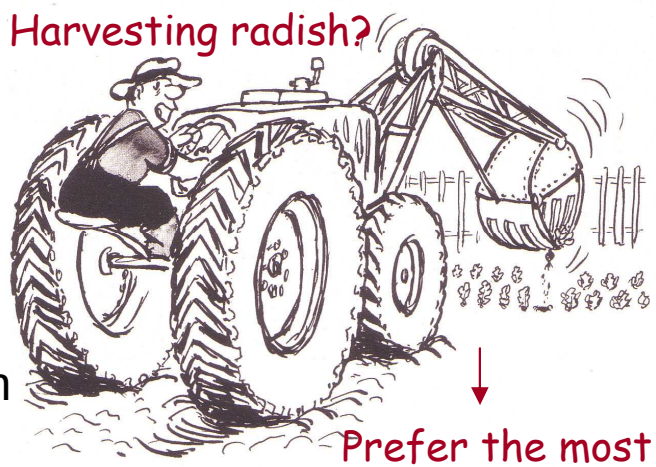
Why choosing HPTLC?



1. Gives more information about an unknown
2. Tolerates minimized sample preparation
3. Enables concentration during application up to a factor of 10.000
4. Capable of high throughput (300 runs per day) with minimal costs
5. Runs parallel chromatography under identical environmental conditions
6. Enables selective and simultaneous derivatization (variety of reagents)
7. Enables multiple detection (UV/Vis, FLD, derivatization, MS)
8. Allows toxicity-directed detection (information directed to the effect)
9. Runs highly-targeted, cost-effective HPTLC-MS where separation solvent can be chosen independently from MS
10. Usage as flexible working station

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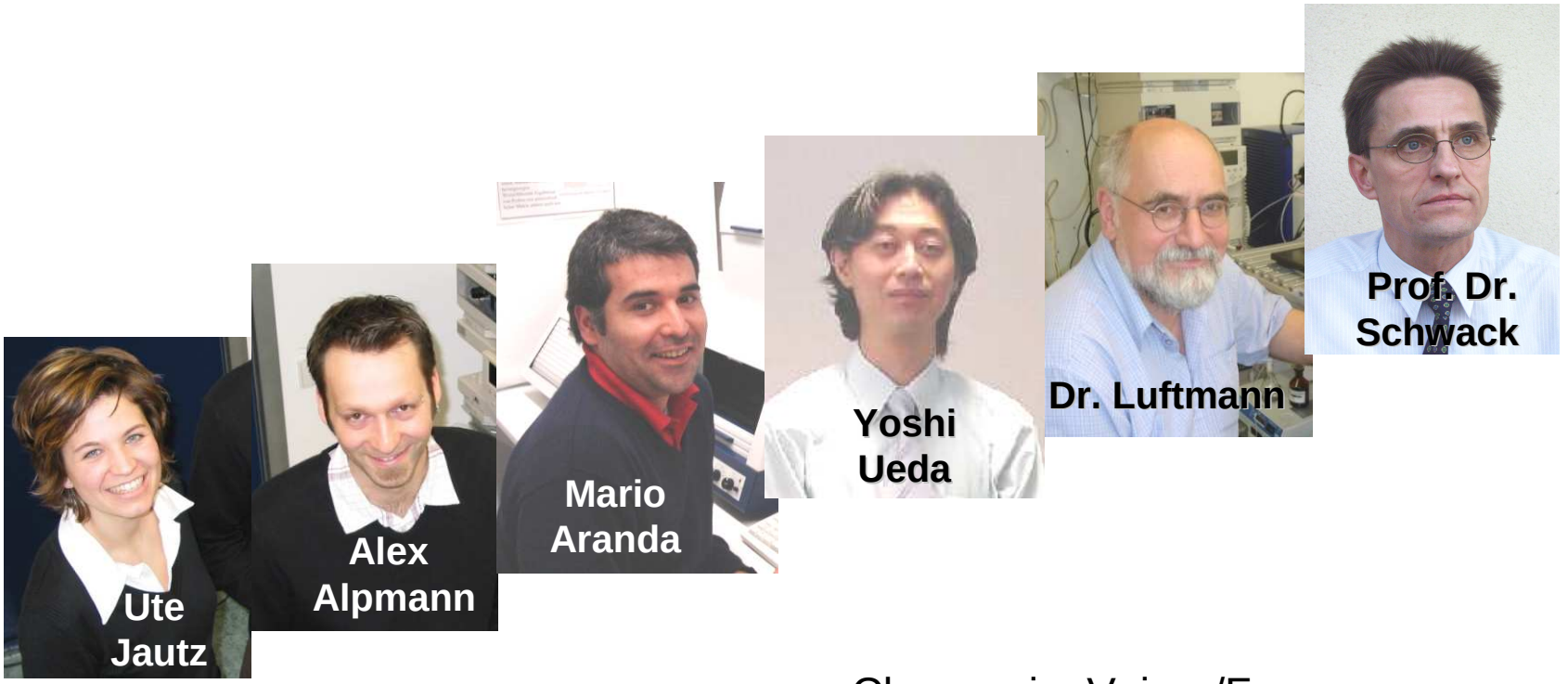
Special thanks go to …



Chromacim Voiron/F,

CAMAG, Muttenz/CH

Merck, Darmstadt/D

Jeol (Europe), Paris/F

ChromAn, Holzhausen/D

Landesstiftung BW (Projekt Nr. P-LS-E2/25)

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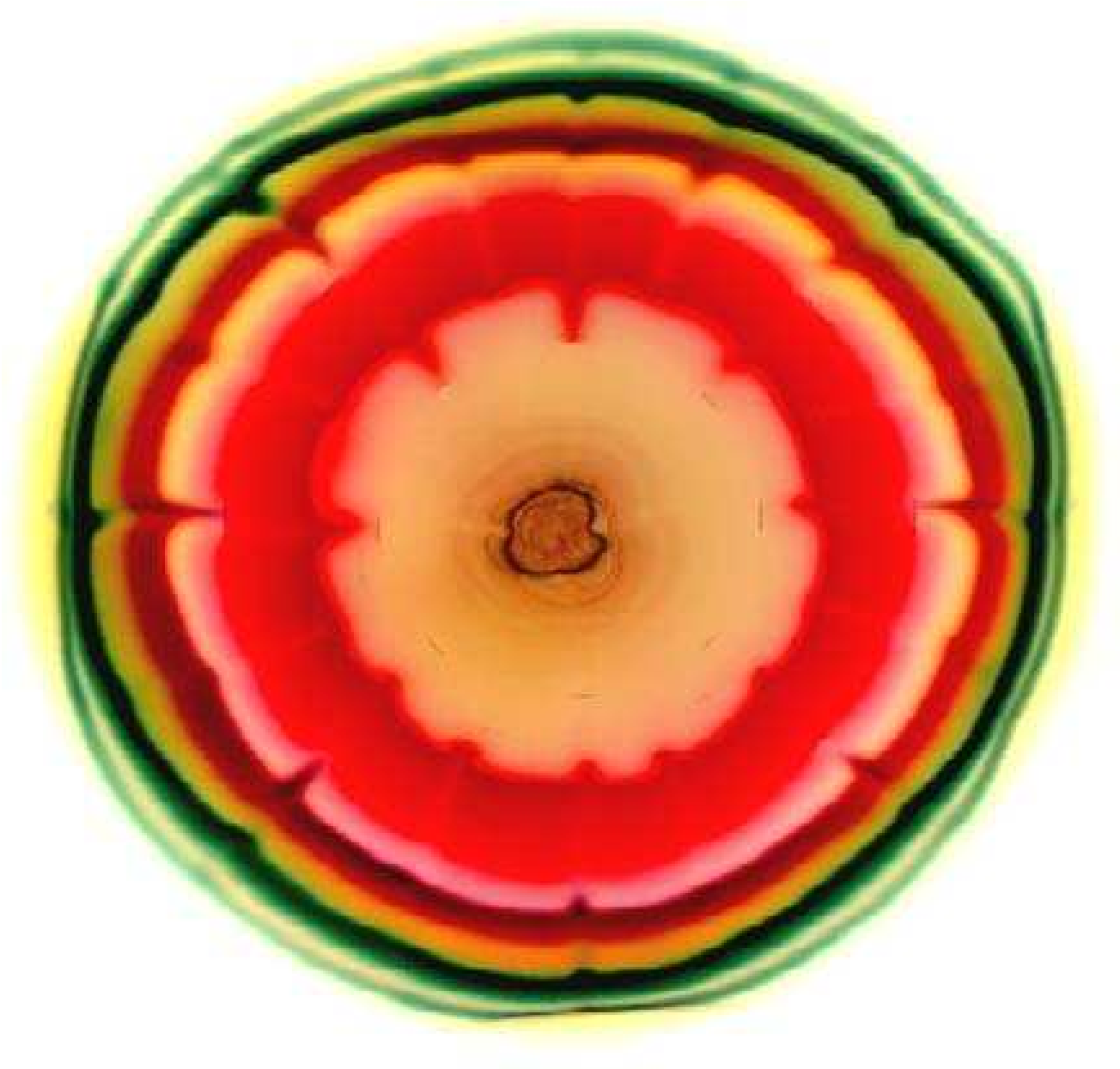

CHROMart by Drs. Karla und Herbert Halpaap