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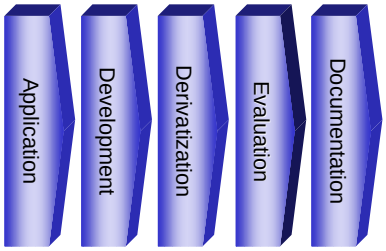
Planar chromatography



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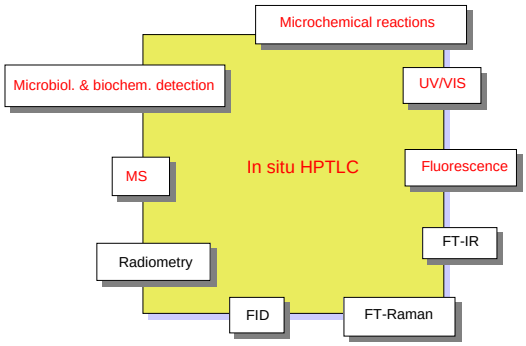
Detection and in situ identification



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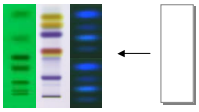
Commonly used in situ detections

Physical
- Optomechanical
- Electronical
- Further techniques

➢ Absorption UV/Vis (‚fluorescence' quenching)
➢ Fluorescence

Microchemical
- Universal reagents
- Group-characterizing reagents
- Pre-chromatogr.
- Post-chromatogr.

Microbiological & biochemical
- Bioautography
- Enzyme-substrat-reaction



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Pre-chromatographic derivatization

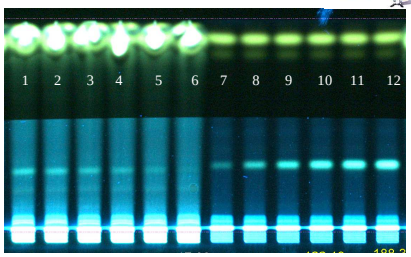

Plate image illuminated at UV 366/>400 nm

D. Müller et al. Poster No.

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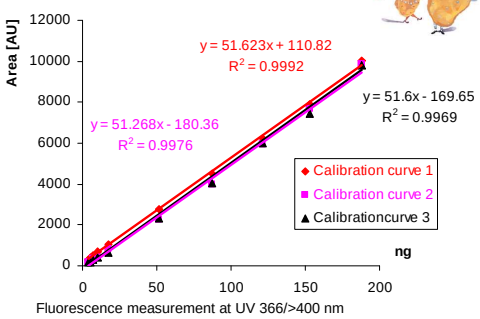
Pre-chromatographic derivatization



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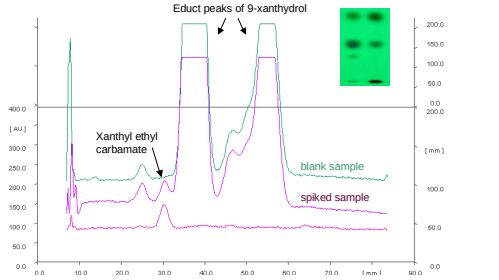
Ethyl carbamate in spirit



G. Morlock, A. Nedele, W. Schwack, Proceedings of EuroFoodChem Hamburg (2005) 513-516

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

Pre-chromatographic derivatization

Advantages

- Enabling the chromatographic separation at all
- Increase in stability of substances to be separated
- Decrease of reactivity of substances with stationary phase, decrease of strong polarities
- Transfer to non-volatile derivatives
- No increase of background by reagents in excess because these can still be removed
- Derivatization in vessel: improved extraction efficacy by changing substance properties

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Pre-chromatographic derivatization

Disadvantages

- Reagent influences other sample and matrix constituents
- Chromatographic properties of different sample constituents can be equalized by formation of great molecule groups
- Varying sample and matrix composition influences quantitative derivatization

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Post-chromatographic derivatization

		Without derivatization
• **Dipping Device**	**RSD 3 %***	⟷ RSD 2 %*
• Spraying → Device	RSD 5 %* → 3 %	
• Evaporation	J_2, Br_2, NH_3, HCl, NO_2	
• Mobile phase	Ninhydrin	
• Incorporation into layer	Amino group	

* see CBS 72

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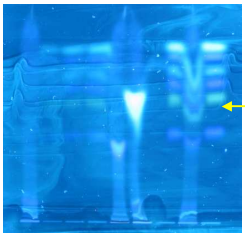
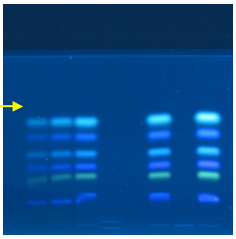
Performance of dipping

Manually



Automated



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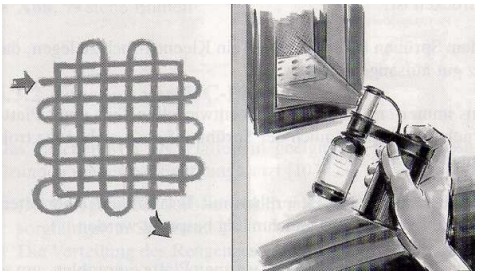
Performance of spraying



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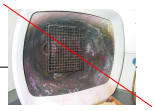
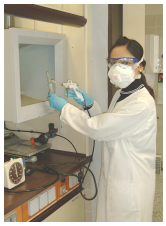
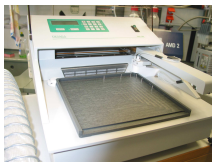
Performance of spraying

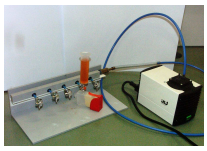


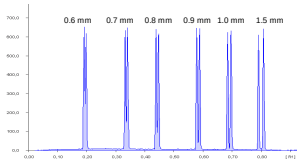




Reagent printer

Bubble jet printer (Canon Pixma iP 3000x)

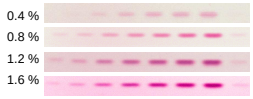


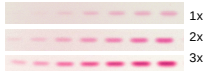


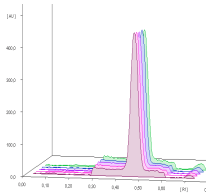
C. Stiefel et al. Poster No.

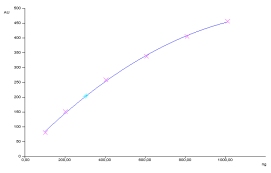
Reagent printer – loading of the plate

Ninhydrin solution for detection of taurine



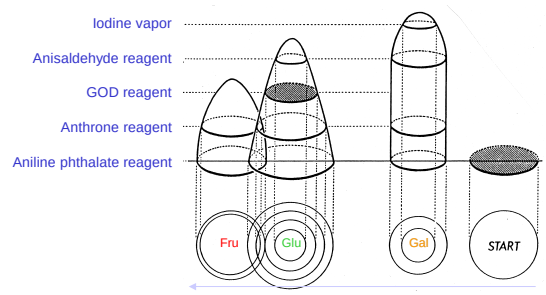






Derivatization and detectability

1 µg sugar each detected by



Jork, H., Funk, W., Fischer, W., Wimmer, H.: Thin-Layer Chromatography, volume 1a and b, VCH Weinheim 1990 and 1994.

Post-chromatographic derivatization

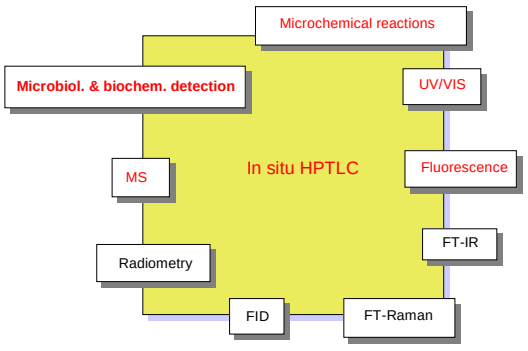
Advantages

- No influence on separation
- Optimal reaction kinetics at different substance concentrations (reaction of substances, not solutions)
- Reaction under identical conditions
- Additional confirmation of results

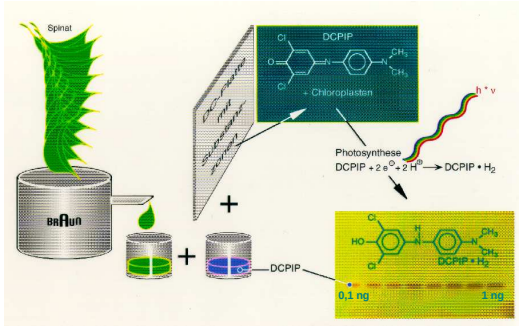
Disadvantages

- Interference by absorption or fluorescence of reagents in excess
- Non-homogeneous background (pH indicator-based reagents)
- Technical difficulties

Detection and in situ identification



Biochemical detection of photosynthesis inhibitors

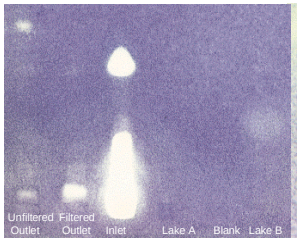


K. Burger, Bayer Industries Inc., Dormagen, Germany

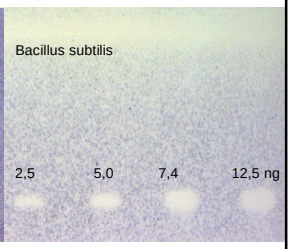
G. Morlock, Institute of Food Chemistry
University of Hohenheim, Stuttgart

Microbiological detection of antibiotics

...in waste water facilities and surface water



...in Supracycline tablets (tetracycline)



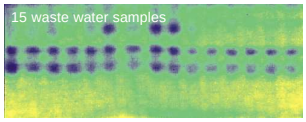
C. Weins, Staatl. Inst. für Gesundheit und Umwelt, Saarbrücken

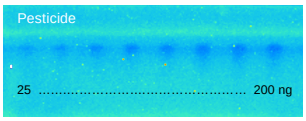
Merck Bioautography Testkit „Chrom Biodip", see CBS 85

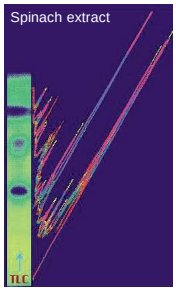
G. Morlock, Institute of Food Chemistry
University of Hohenheim, Stuttgart

Biomonitoring of toxic compounds

Luminographic detection of substances active against luminescent bacterium Vibrio fischeri



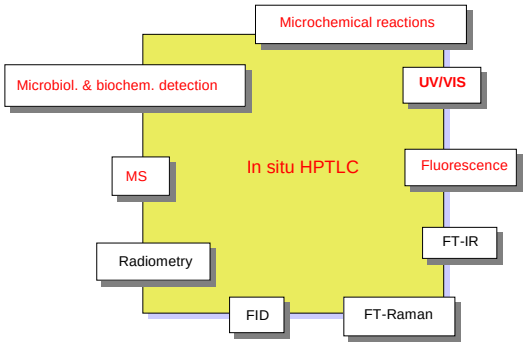




W. Kreiss et al., CAMAG Bibliogr. Service, CBS 88 (2002) 12-13
W. Weber et al., CAMAG Bibliogr. Service, CBS 94 (2005) 2-4

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

Detection and in situ identification



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

Allergenic disperse dyes in textiles

Morlock, G., Bonhoff, A., Reich, E., GIT Spezial Separation 1 (2000) 28-29

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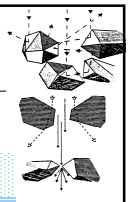
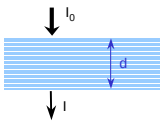
University of Hohenheim, Stuttgart

Why do we obtain a signal?



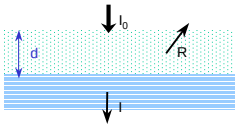
in solution (transmission)



$E = \varepsilon \cdot c \cdot d$

$E \sim c$

on the layer (remission)



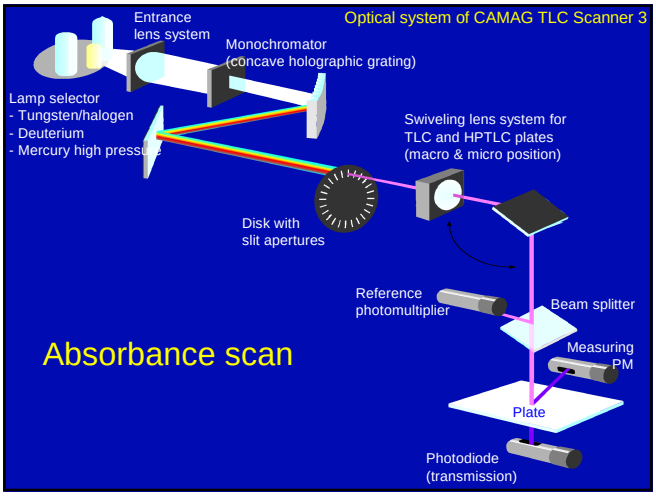
k Absorption coefficient
s Scattering coefficient
R_∞ Absolute remission (d = ∞)

Kubelka Munk function

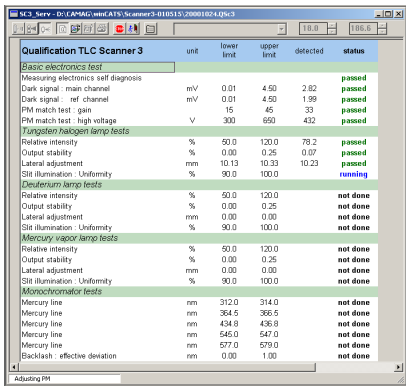
$$F(R_\infty) = \frac{k}{s} c = \frac{(1 - R_\infty)^2}{2 R_\infty}$$

Preconditions: d = ∞
$R_0 = 0$
no direct reflexion
dp < 1 mm

Optical system of CAMAG TLC Scanner 3

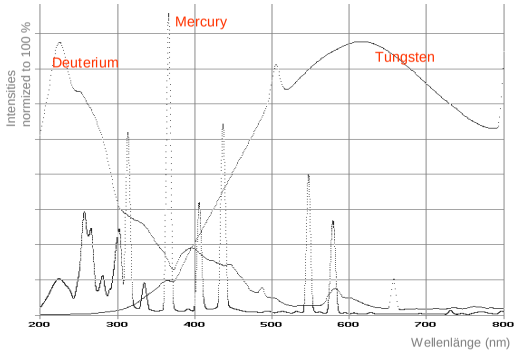


Scanner validation

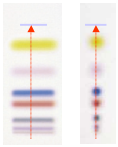


Qualification TLC Scanner 3	unit	lower limit	upper limit	detected	status
Basic electronics test					
Measuring electronics self diagnosis					passed
Dark signal : main channel	mV	0.01	4.50	2.82	passed
Dark signal : ref channel	mV	0.01	4.50	1.99	passed
PM match test : gain		15	45	33	passed
PM match test : high voltage	V	300	650	432	passed
Tungsten halogen lamp tests					
Relative intensity	%	50.0	120.0	78.2	passed
Output stability	%	0.00	0.25	0.07	passed
Lateral adjustment	mm	10.13	10.33	10.23	passed
Slit illumination : Uniformity	%	90.0	100.0		running
Deuterium lamp tests					
Relative intensity	%	50.0	120.0		not done
Output stability	%	0.00	0.25		not done
Lateral adjustment	mm	0.00	0.00		not done
Slit illumination : Uniformity	%	90.0	100.0		not done
Mercury vapor lamp tests					
Relative intensity	%	50.0	120.0		not done
Output stability	%	0.00	0.25		not done
Lateral adjustment	mm	0.00	0.00		not done
Slit illumination : Uniformity	%	90.0	100.0		not done
Monochromator tests					
Mercury line	nm	312.0	314.0		not done
Mercury line	nm	364.5	366.5		not done
Mercury line	nm	434.8	436.8		not done
Mercury line	nm	545.0	547.0		not done
Mercury line	nm	577.0	579.0		not done
Backlash : effective deviation	nm	0.00	1.00		not done

Emission spectra of the lamps



Geometry of the slit scan



Slit length:

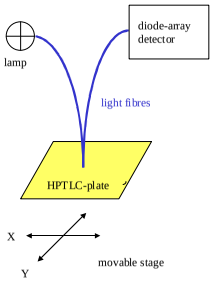
Bands 70 - 80 %

Spots 120 %

Slit width:

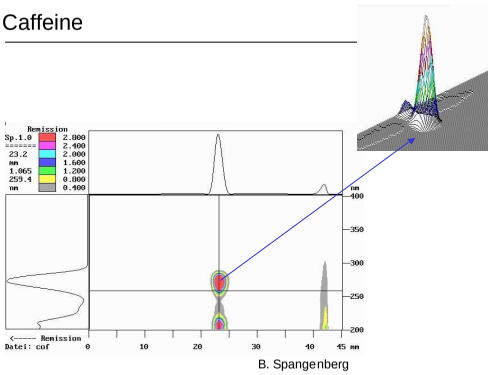
the higher, the better the light intensity, but the worse resolution

DAD scanner (diode-array detector)



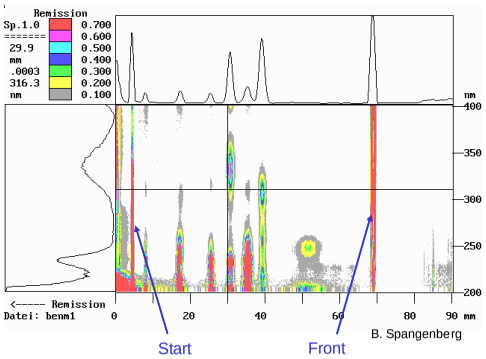
B. Spangenberg, K.-F. Klein *J Chromatogr* 898 (2000) 265-269

Caffeine



B. Spangenberg

Benzodiazepines



B. Spangenberg

Start

Front

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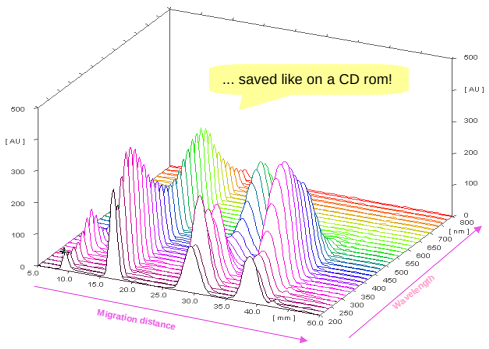
University of Hohenheim, Stuttgart

Multi wavelength scan – an alternative



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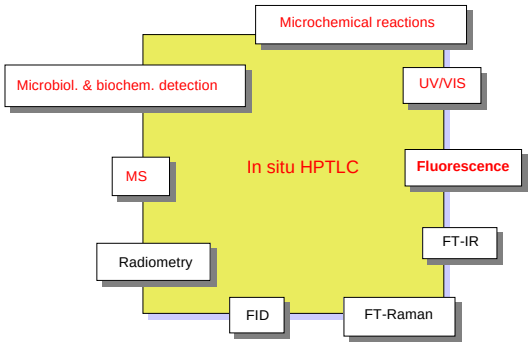
University of Hohenheim, Stuttgart

Detection and in situ identification



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Sucralose in Burfi

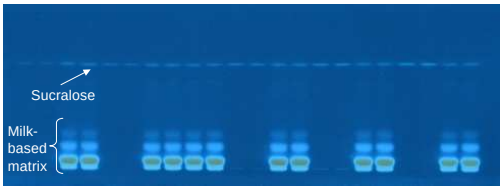

Plate image illuminated at 366/>400 nm

S. Prabha, G. Morlock, CAMAG Bibliogr Service, CBS 94 (2005) 14-15

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

Fluorescent substances are…

- Substances with fixed molecular structure
- Fluorophores

- ✓ Aromatic systems
- ✓ Compounds with conjugated double bonds
- ✓ Carbonyls
- ✓ Condensated heterocycles

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University of Hohenheim, Stuttgart

Fluorescence

Linear correlation between fluorescence intensity and concentration:

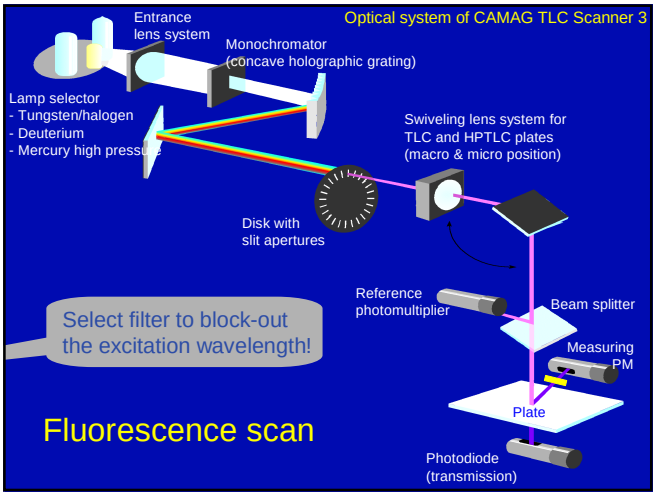
Intensity of light source
Measured fluor. intensity
Concentration

$$I_{fl} = k` * I_0 * e * c * d$$

Substance & instrument factor

Preconditions
- strict monochromatism of the exciting light
- low concentration level

Advantages
- high sensitivity (pg-range)
- high selectivity
- wide linear concentration range



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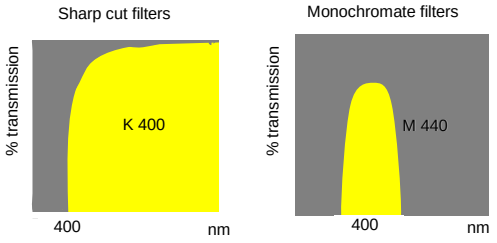
Types of fluorescence filters



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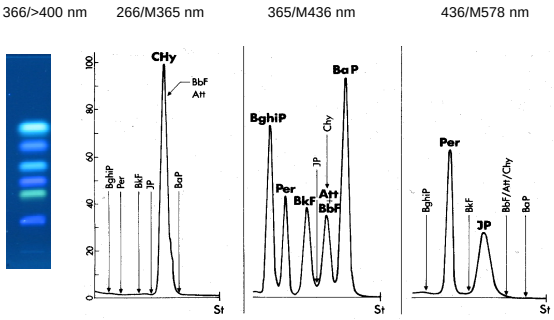
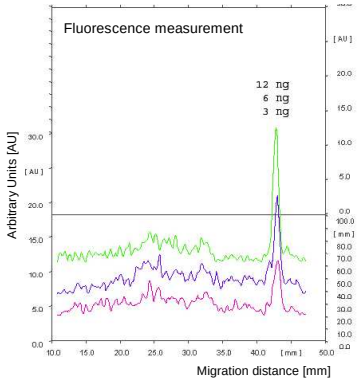
Selectivity by ideal excitation and filter combination



Jork, H., Funk, W., Fischer, W., Wimmer, H.: Thin-Layer Chromatography, volume 1a and b, VCH Weinheim 1990 and 1994.

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

Limit of detection for sucralose



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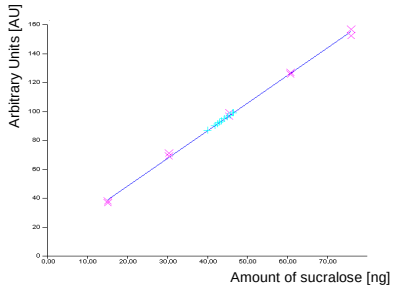
University of Hohenheim, Stuttgart

Calibration of sucralose



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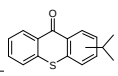
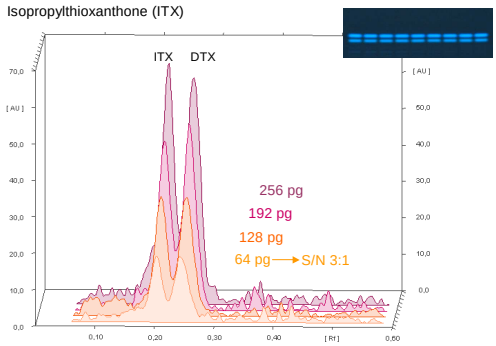
Detectability



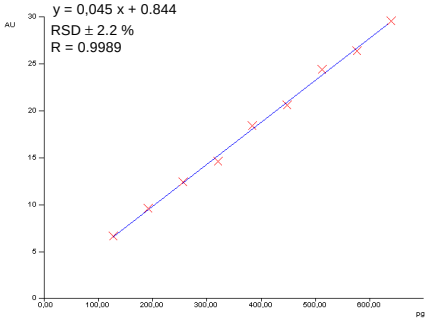
Isopropylthioxanthone (ITX)



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

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

Calibration of ITX from 128 pg to 640 pg



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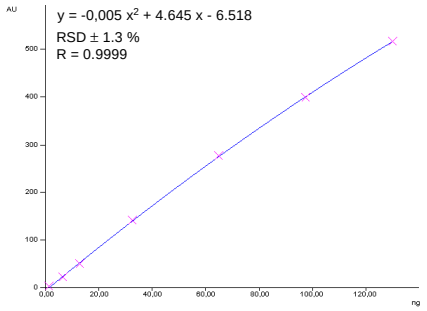
Calibration of ITX from 1.2 to 120 ng



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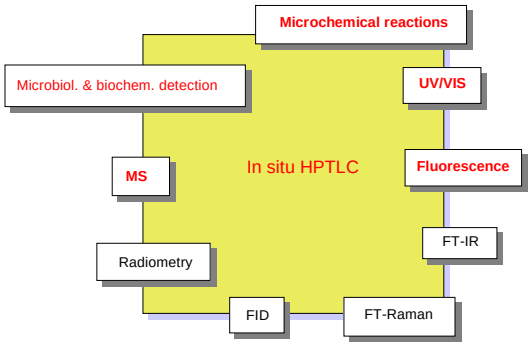
Detection and in situ identification



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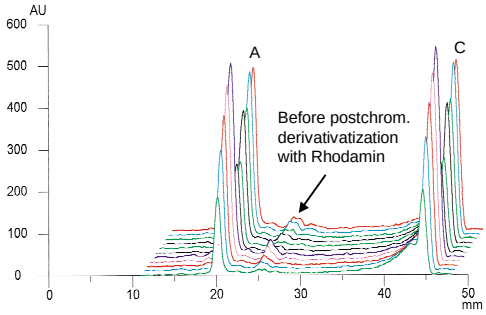
Additives in mineral oil - Multiple detection



Absorption measurement at 220 nm

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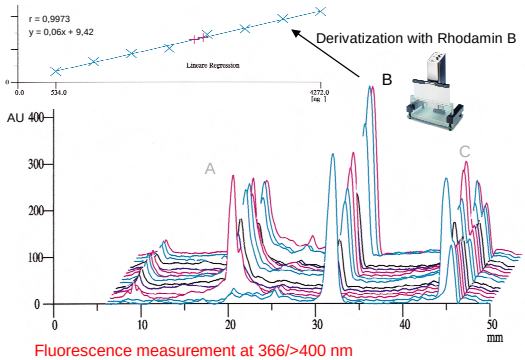
Additives in mineral oil - Multiple detection



Fluorescence measurement at 366/>400 nm

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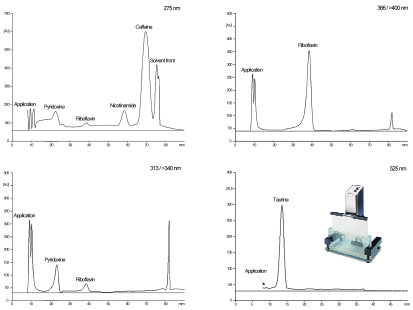
University of Hohenheim, Stuttgart

Energy drinks - Multiple detection

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

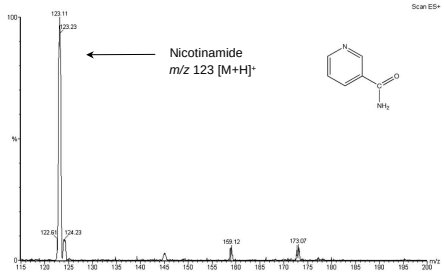


M. Aranda, G. Morlock, J Chromatogr A (2006) DOI 10.1016/j.chroma.2006.07.018

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

Confirmation by HPTLC/ESI-MS

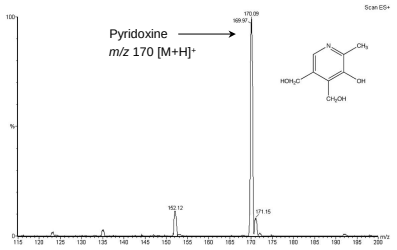
→ Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks



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

Confirmation by HPTLC/ESI-MS

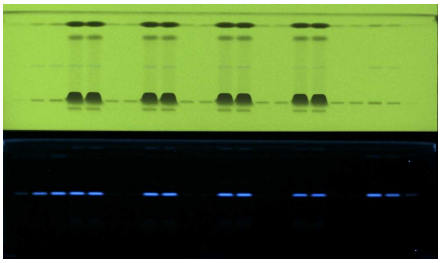
→ Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks



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

Headache tablets - Multiple detection

→ Simultaneous Determination of Caffeine, Ergotamine and Metamizol

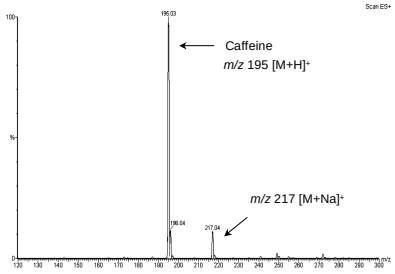


M. Aranda and G. Morlock (2006) J Chromatgr. Sci, in print

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

Confirmation by HPTLC/ESI-MS

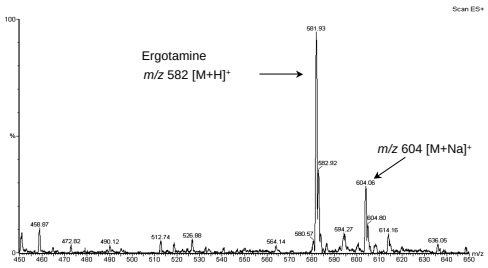
→ Simultaneous Determination of Caffeine, Ergotamine and Metamizol



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

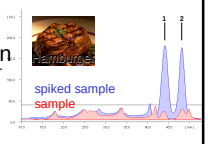
Confirmation by HPTLC/ESI-MS

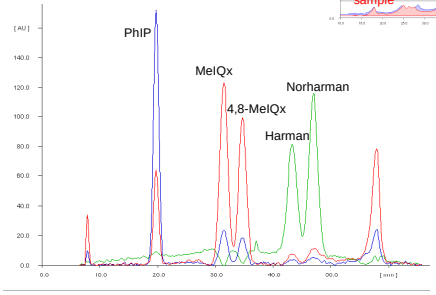
→ Simultaneous Determination of Caffeine, Ergotamine and Metamizol



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

HAAs in meat - Multiple detection



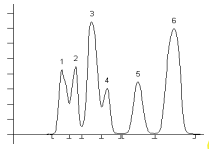


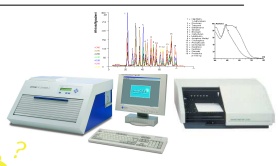
U. Jautz, G. Morlock, Anal Bioanal Chem (2006) in print

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

Electronical evaluation or conventional scan?

Conventional scanner





Scanner UV/Vis/Fluor
Spectra 190-800 nm

Digital System

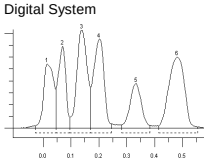
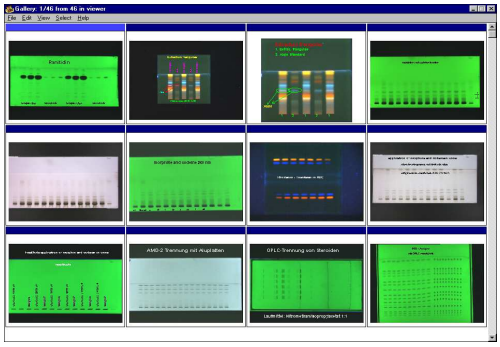
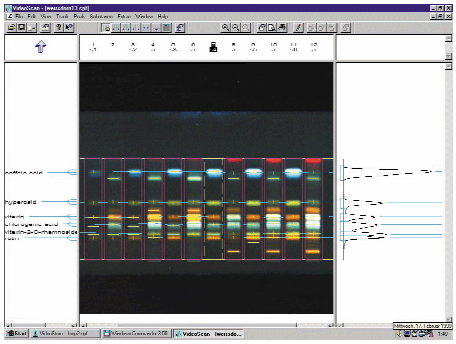


Image documentation
Digital densitometry

Image documentation

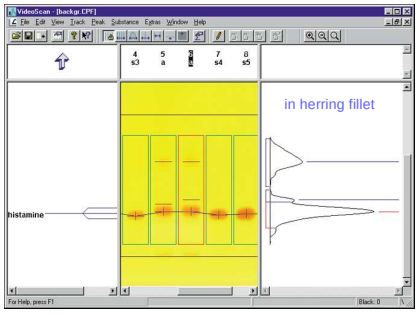


Hawthorn extracts



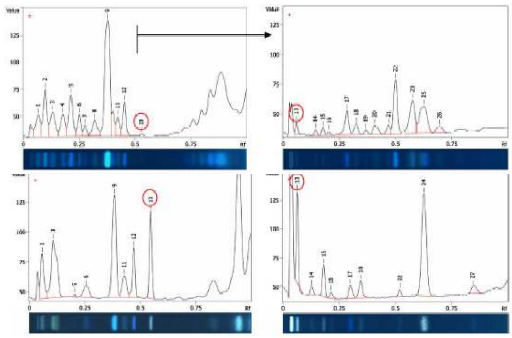
CAMAG Laboratory

Histamine in fish and fish products



M. Stoyke, IVPT, Berlin, see CBS 83

Analysis of herbals



Si-Bao Chen, et al., J Chromatogr A, 1121 (2006) 114–119

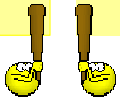
Electronical evaluation or conventional scan?

Digital System

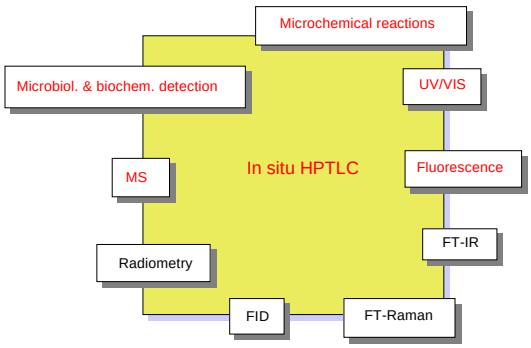
- very fast
- less costs
- image documentation and its quantification
- quantification later
- but...

Scanner

- optimal reproducibility
- the whole UV-range
- spectral selectivity
- spectra recording



Detection and **in situ identification**



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University of Hohenheim, Stuttgart

Spectra recording

$\lambda_{\text{Sample corr.}} = \lambda_{\text{Sample}} - \lambda_{\text{Lamp}} - \lambda_{\text{Background}}$

Difference to spectra in solution

- no solvent
- adsorbed on the layer

Note:

- Compare spectra at similar concentrations!
- RP-NP
- MP (pH)

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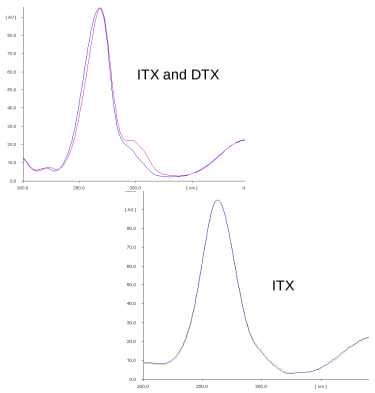
Spectra comparison



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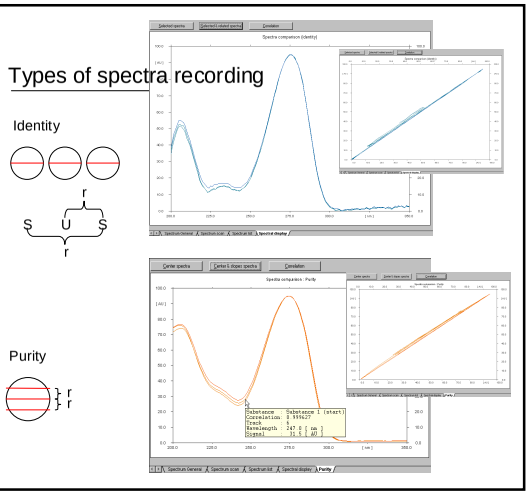
Types of spectra recording



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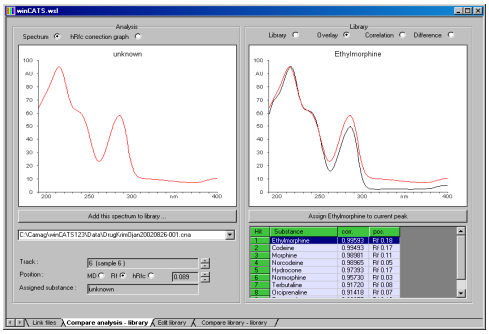
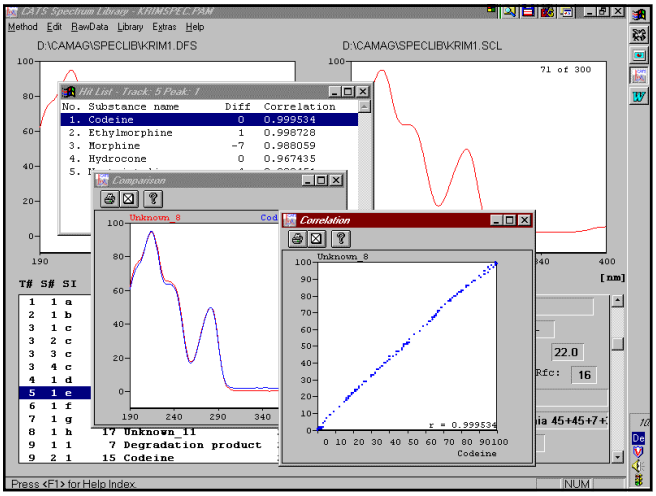
Spectra library





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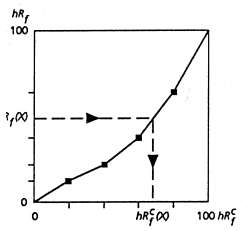
University of Hohenheim, Stuttgart

Calculation of hR_F^c



$$R_f^c(X) = R_f^c(A) + \frac{\Delta^c}{\Delta} [hR_f(X) - hR_f(A)]$$

$\Delta^c = hR_f^c(B) - hR_f^c(A)$ and

$\Delta = hR_f(B) - hR_f(A)$

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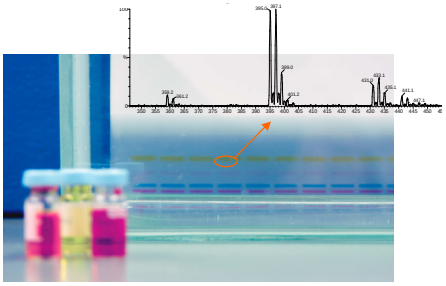
Which mass do I have?



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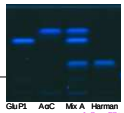
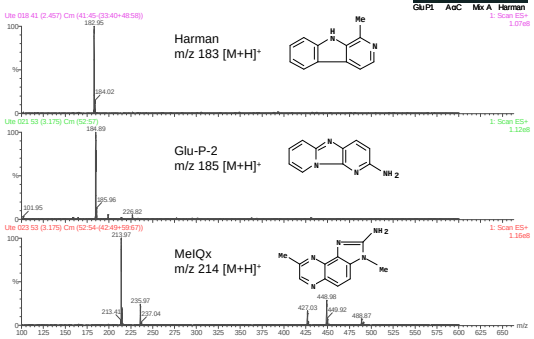
Heterocyclic aromatic amines





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University of Hohenheim, Stuttgart

Various approaches of HPTLC/MS coupling

- **FAB, SIMS, MALDI, LD/CI**
  - Ivleva et al. **2004**
  - Meisen et al. **2004**
  - Dreisewerd et al. **2006**
  - Peng et al. **2005**
- **ESI**
  - Cooks et al. 2004; van Berkel et al. **2005** — DESI
  - Wachs et al. 2001; Kertesz et al. **2005** — Micro-junction
  - Prosek et al. **2004**; Luftmann **2004**; Alpmann et al. **2006** — Extractor
- **DART, APGD**
  - Cody et al. 2005
  - Morlock et al. **2006** in submission
  - Morlock et al. **2006** in preparation

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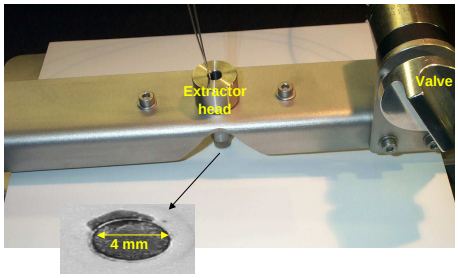
University of Hohenheim, Stuttgart

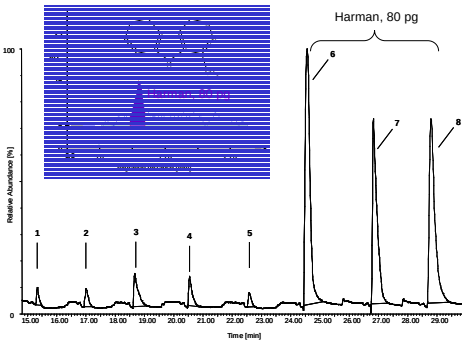
Online Extraction



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

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

Detectability: FLD versus MSD



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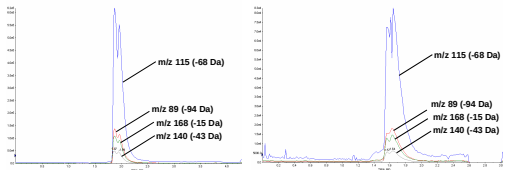
University of Hohenheim, Stuttgart

Detectability by HPTLC/ESI-MS-MS

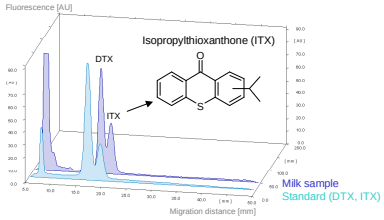
200 pg Harman → 20 pg Harman



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

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

Trace analysis: Food contaminant ITX

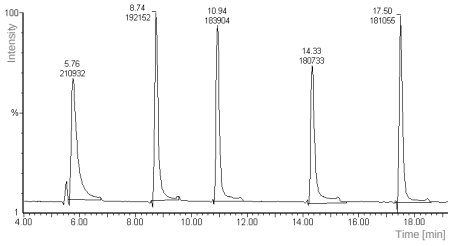


G. Morlock, W. Schwack, Anal Bioanal Chem 385 (2006) 586-595

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

Repeatability

Elution profiles of 6 ng ITX each (SIM at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$)
RSD = ± 6.7 % (*n* = 5)



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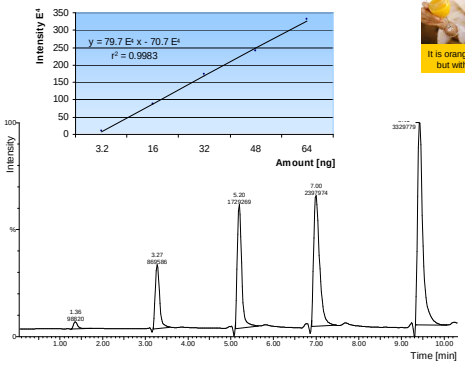
University of Hohenheim, Stuttgart

Analytical response



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

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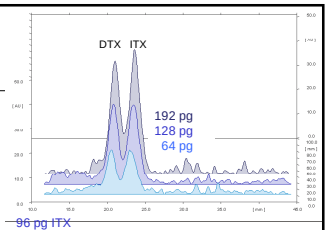
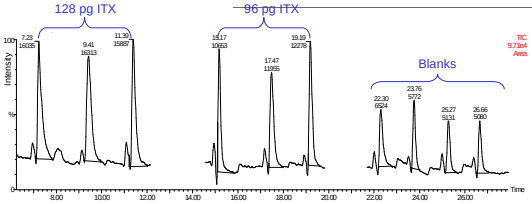
University of Hohenheim, Stuttgart

FLD ↔ MSD

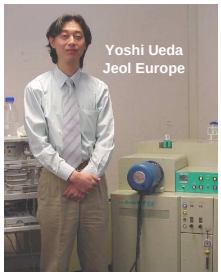




Elution profiles of ITX (SIM at *m/z* 255 $[M+H]^+$ and 277 $[M+Na]^+$)

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

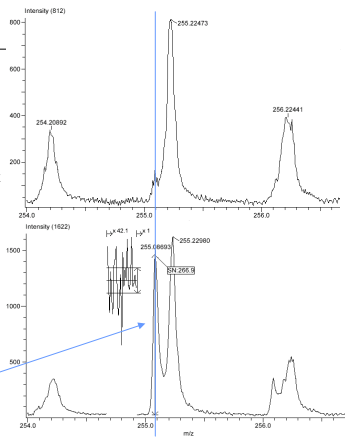
HPTLC/DART-TOF



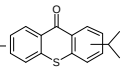
G. Morlock, Y. Ueda: Coupling of (2006) in submission

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

Detectability

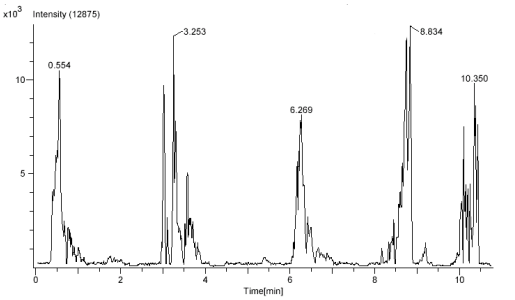


Repeatability



Isopropylthioxanthone (ITX)

5 zones, 32 ng ITX each: CV = ± 71.1 % (18.3 %)



Mass chromatogram at m/z 255 $[M+H]^+$

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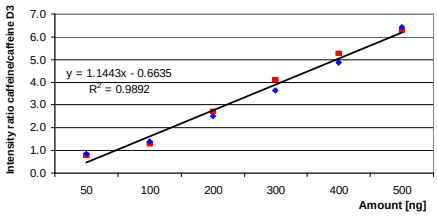
HPTLC/DART-IDA-TOF

- Caffeine at *m/z* 195 [M+H]+ in the range of 50 – 500 ng/zone corrected by the stable isotope labeled internal standard caffeine D3 at *m/z* 198 [M+H]+



- Repeatability RSD < ±5.4 %, *n* = 6

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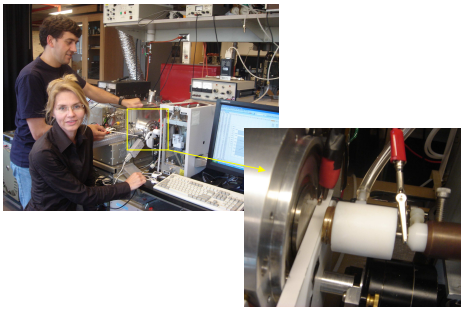
HPTLC/APGD coupling



G. Morlock, F. Andrade, G. Hieftje: Coupling of planar chromatography with atmospheric pressure glow discharge mass spectrometry (2006) in preparation

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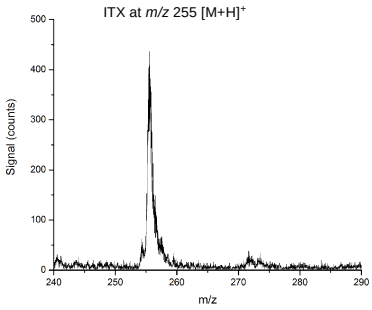
University of Hohenheim, Stuttgart

HPTLC/APGD coupling



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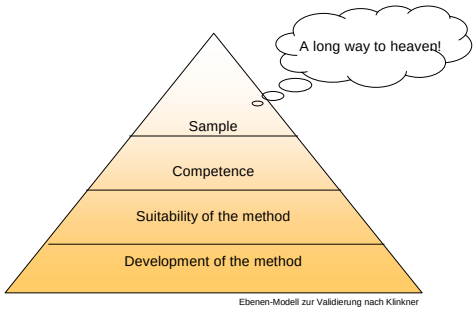
Method validation



Ebenen-Modell zur Validierung nach Klinkner

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Definition

= official qualification of an analytical method showing that it fulfills the intended purpose

Prerequisites

- characterized, homogeneous sample
- exact formal analytical method
- characterized reference substances (stability tested, of known purity and origin)
- validated instruments (IQ, OQ, PQ)
- statements concerning tolerated values & deviations, limit values, purpose of the procedure

Instrumental qualification

SOP	Standard Operating Procedure
IQ	Installation Qualification
OQ	Operational Qualification
PQ	Performance Qualification
MQ	Maintanance Qualification
	Logbuch
	21CFR-part 11

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Validation parameters

1. Specificity
   Differentiate specifically the impurities, degradation products, byproducts etc. from the analyte
   ➢ R_F value, chromatogram & spectra comparison, blank sample, standard addition, ruggedness tests

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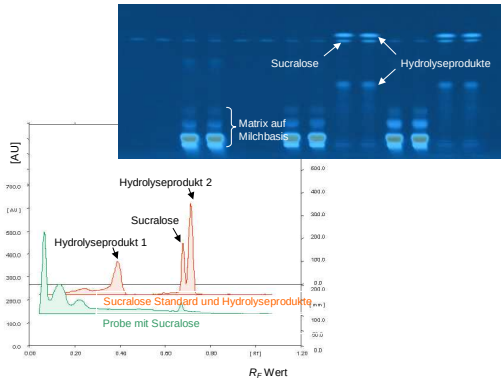
Specificity



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Validation parameters

1. Specificity
   Differentiate specifically the impurities, degradation products, byproducts etc. from the analyte
   ➢ R_F value, chromatogram & spectra comparison, blank sample, standard addition, ruggedness tests

2. Selectivity
   Differentiate selectively the impurities, degradation products, byproducts etc. from the analyte
   ➢ R_F value, resolution, peak asymmetry, spectra purity

3. "Linearity" ⇒ analysis function, analytical response
   Functional correlation between measured value and concentration
   ➢ RSD, determination coefficient, linearity test acc. to Mandel, at least 5 levels (better 10 levels), equidistant

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Validation parameters

4. Sensitivity
   Change of concentration per change of signal, reliability of the results
   ➢ Slope of the function

5. Working range
   Calibration range in which quantitative statements are allowed
   ➢ Starting at the limit of quantification, variances-F-test

6. Limit of quantification (LOQ)
   Concentration, at which a substance can be quantitatively evaluated and is statistically significant different from zero
   = 2-3 fold detection limit (LOD)
   ICH, Eurachem, ISO
   Modes: Calibration curve, blank value, S/N

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Validation parameters

7. Trueness True result of an analysis
   - ➢Systematic errors... recovery rate, recovery function with and without matrix (difference in slope and intercept with the y-axis, variances-F-test)

8. Precision
   Deviation of the results
   - ➢Random errors.... RSD, coefficient of variation, confidence interval
     - repeatability (same day/person/instruments)
     - intermediate precision (different days/persons/instruments)
     - reproducibility

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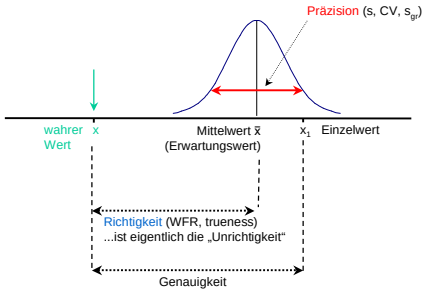
And what is accuracy?

= systematic & random errors



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Validation parameters

9. Ruggedness (stress test)
   Usage of the method under varying conditions,
   stability test of the whole procedure
   - ➢RSD, coefficient of variation, confidence interval, resolution, R_F value, selectivity, sensitivity

10. Control charts (mean value, recovery)
    Check by control standard over a long period,
    confirms trueness and reliability of a procedure
    Warning limit (2s) and control limit (3s)