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HPTLC hyphenations – potential for structure elucidation

Wolfgang Schwack
University of Hohenheim

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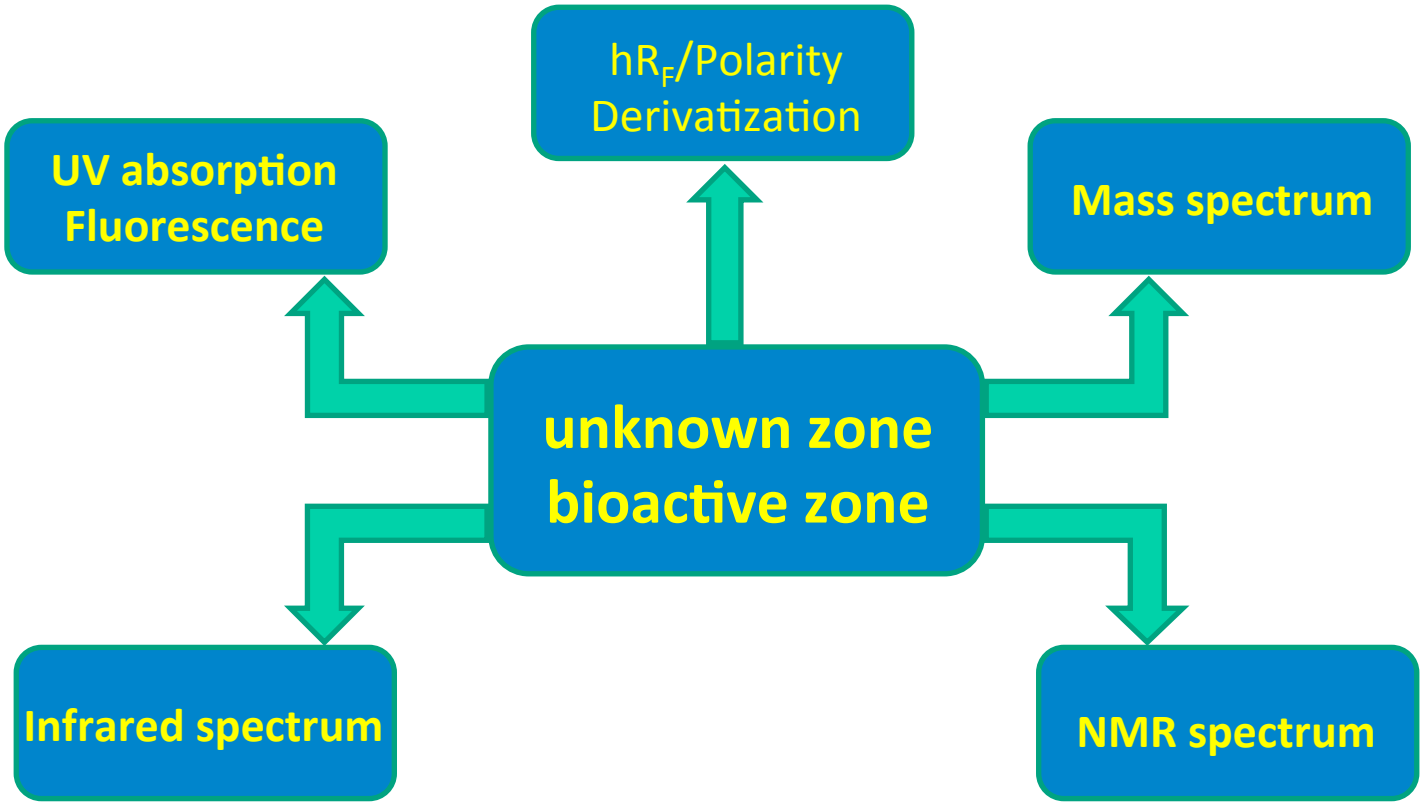
From a detected zone to the chemical structure



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Derivatization/Staining

Iodine: olefins, aromatics

Fluram: amines, amino acids

Ninhydrine: α-amino acids, peptides

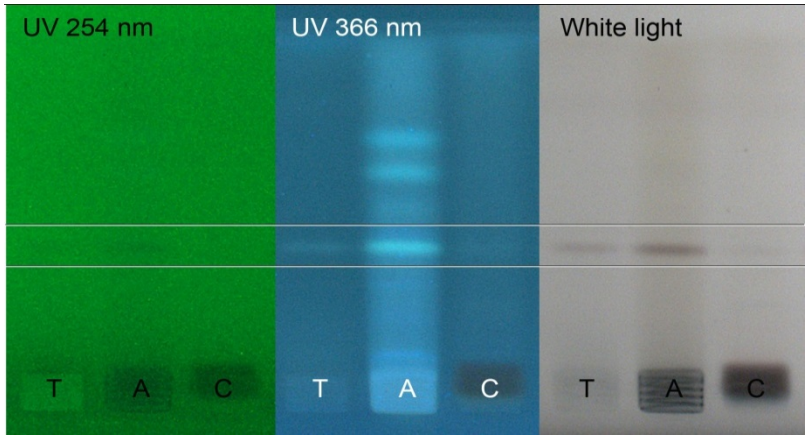
2,4-Dinitrophenylhydrazine: aldehydes, ketones

Primuline: lipids

β-Naphthol: glycosides

p-Aminobenzoic acid: glycosides

...



G. Morlock et al. (2011)

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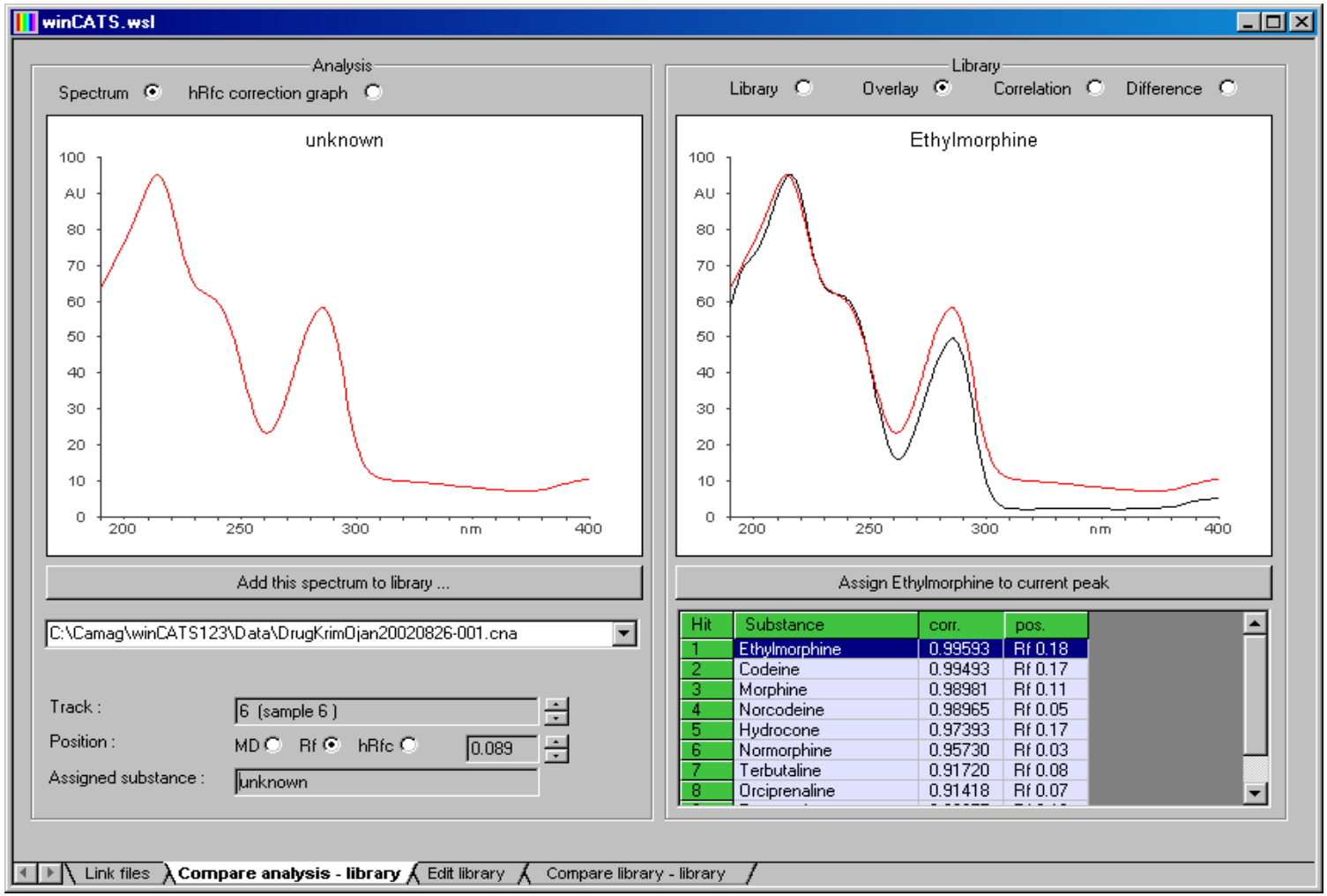
UV/Vis spectrum



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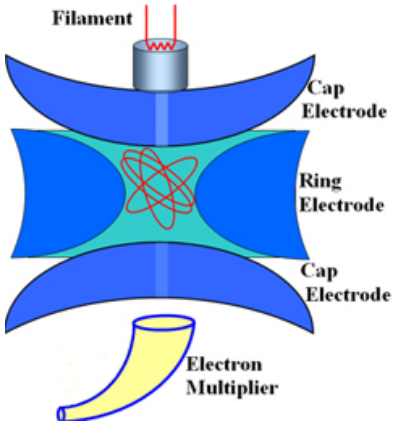
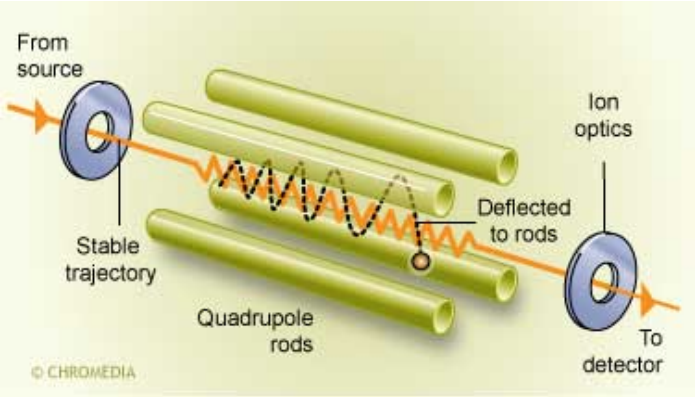
Mass spectrum



Low resolution mass spectrometer (nominal mass)

☞ Some structural information is available:

- Degradation/stability studies (parent is known)
- Organic synthesis or derivatization (parents are known)
- Identification of a bioactive compound in plant X (natural composition of X is well known)



www.chromedia.org

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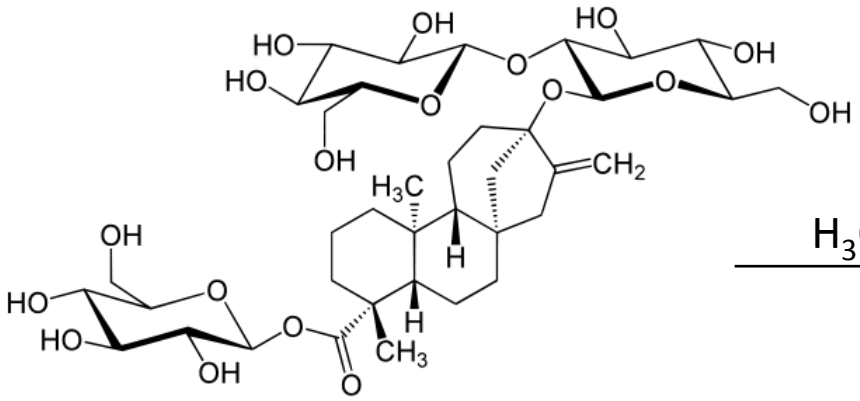
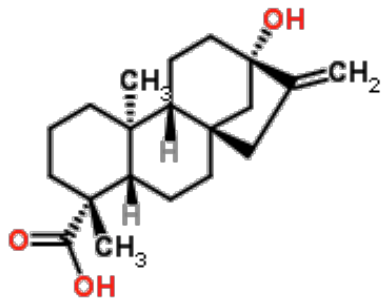
Mass spectrum

Example: *Stevia rebaudiana*



H_3O^+



Stevioside: $[M+H]^+$ = *m/z* 805

☞ Sugar reagents: positive

Steviol: $[M+H]^+$ = *m/z* 319
$[M-H]^-$ = *m/z* 317

☞ Less polar
☞ Sugar reagents: negative
☞ Primuline: positive

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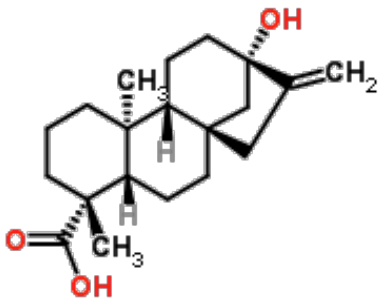
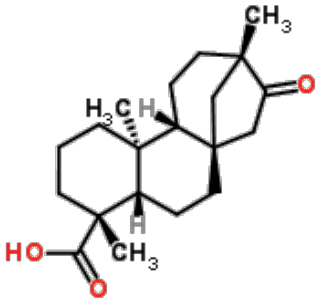
Mass spectrum

However:



Steviol

or



iso-Steviol

☞ IR spectrum, ^{1}H-NMR spectrum ?

CSID:398979, http://www.chemspider.com/Chemical-Structure.398979.html (accessed 11:00, Jun 9, 2014)

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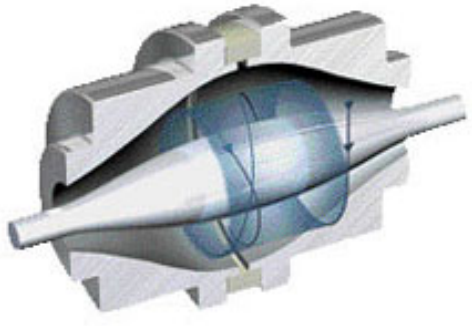
Mass spectrum

High resolution mass spectrometer (exact mass)



☞ No structural information is available:

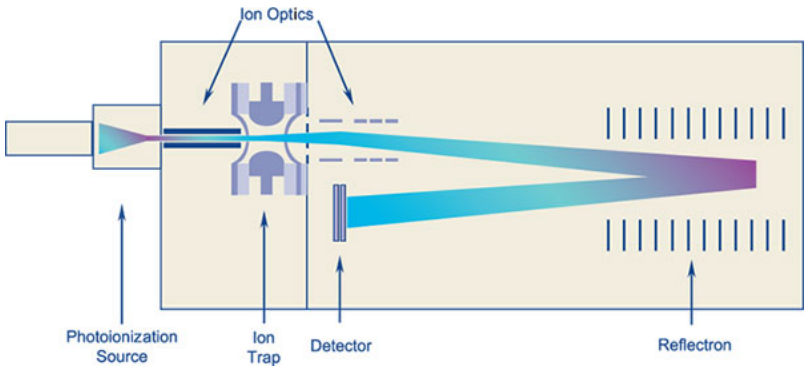
$[M+H]^+ = m/z$ 319.22677

☞ ChemSpider search (www.chemspider.com): compound known?

- Monoisotopic mass ± 0.00032 (1 ppm)

  => 1060 hits (all with $C_{20}H_{30}O_3$)
- Monoisotopic mass ± 0.0032 (10 ppm)

  => 1800 hits



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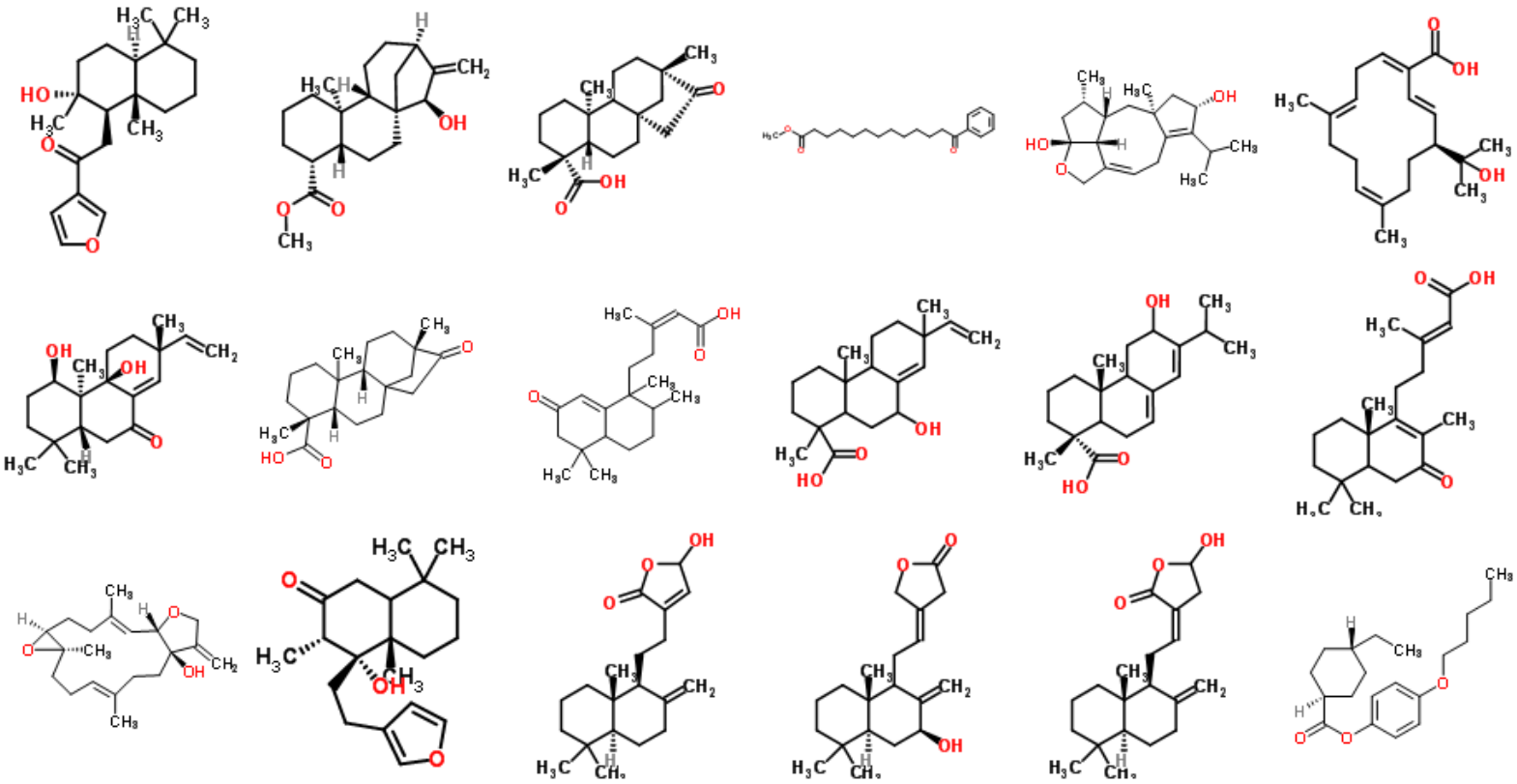
Mass spectrum

Just a selection …



☞ **Additional structural information (filter)?**

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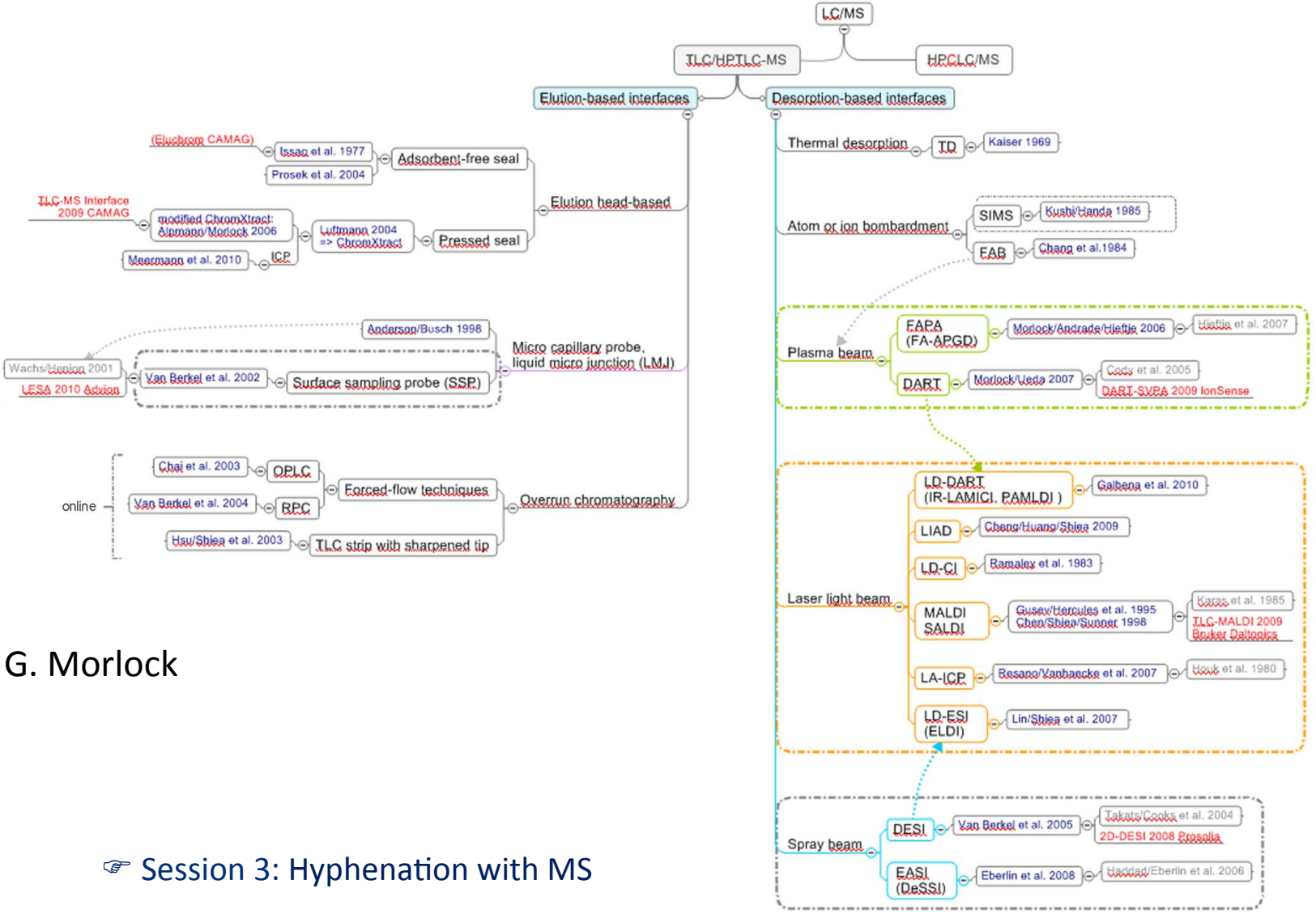
Interfacing HPTLC → MS



G. Morlock

☞ Session 3: Hyphenation with MS

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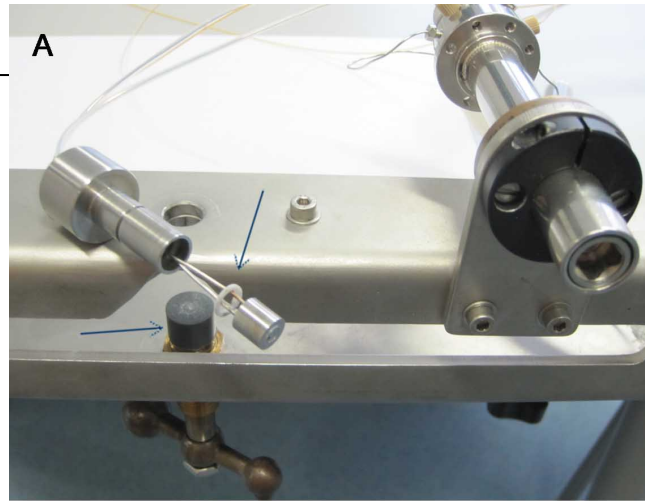
Interfacing HPTLC → MS



1. Elution-based TLC-MS

Pressed seal

- ☞ TLC-MS Interface (CAMAG, 2009)
- ☞ ChromXtract (Luftmann, 2004)
- ☞ ChromXtract modified (Alpmann & Morlock, 2006)

Liquid microjunction (LMJ)

- ☞ In situ microextraction (flowprobe™): Prosolia (2008)
- ☞ Liquid Extraction Surface Analysis (LESA®): Advion (2010)
- ☞ TLC-surface sampling probe (SSP)(Van Berkel et al., 2002)

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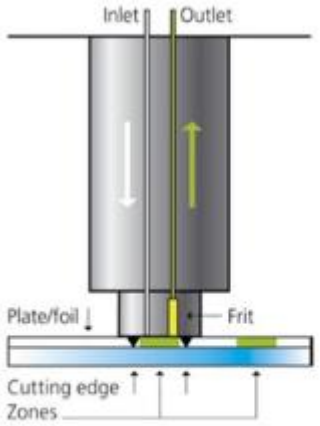
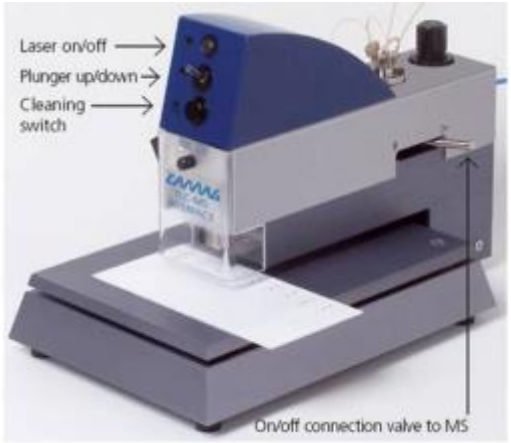
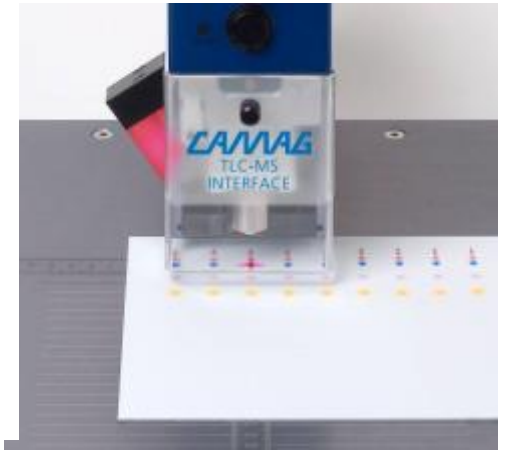
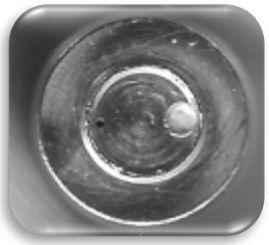
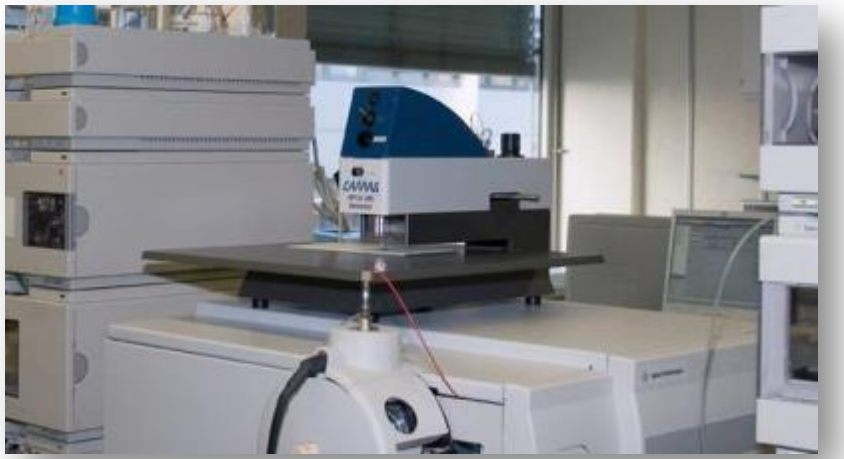
TLC-MS Interface











M. Loppacher, R. Rolli, G. Morlock *CBS* 102 (2009)

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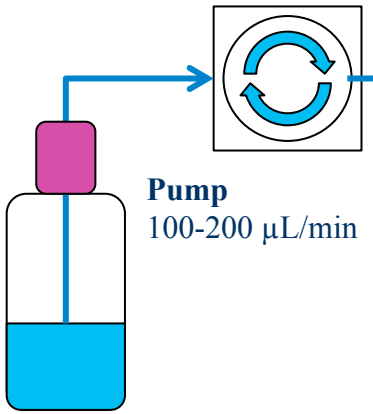
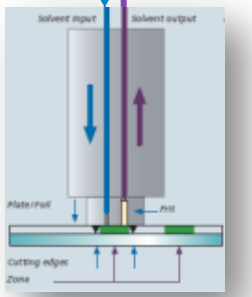
TLC-MS Interface

How it works



Pump
100-200 µL/min

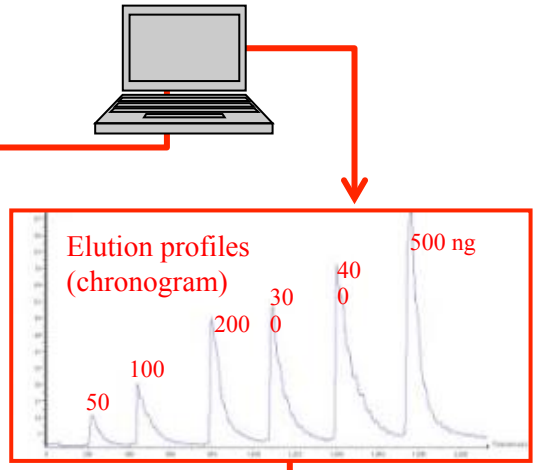
Eluent
Methanol/ammonium formate buffer
(10 mM, pH 4)
19:1 (v/v)

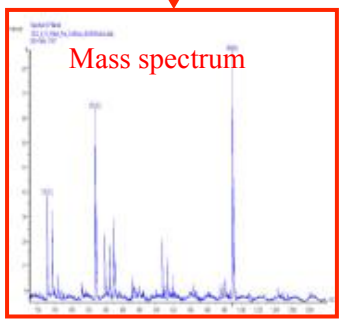


Elution
Zone selection and elution

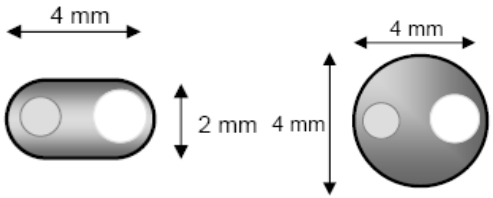
ESI source

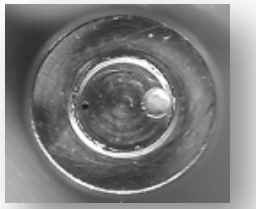
Mass Signals
Online analysis of eluted analytes





Data Evaluation
for structural confirmation, impurity control or search for molecular formula





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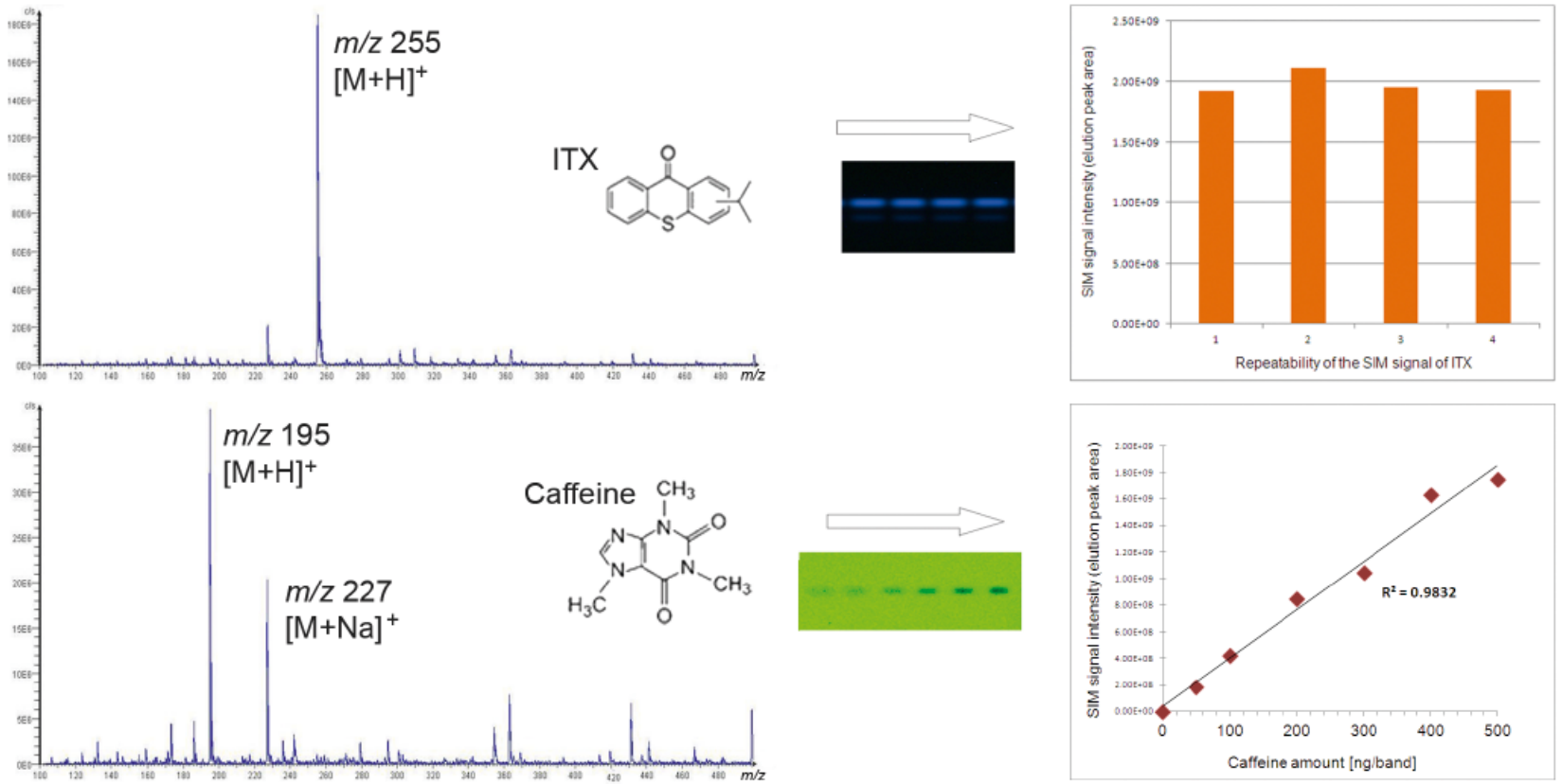
TLC-MS Interface

Performance



Up: Mass spectrum of ITX and its repeatability in the SIM mode (%RSD = 4 %)
Down: Mass spectrum of caffeine and its analytical response in the SIM mode (R^2 = 0.9832)

G. Morlock et al., *CBS* 110 (2013)

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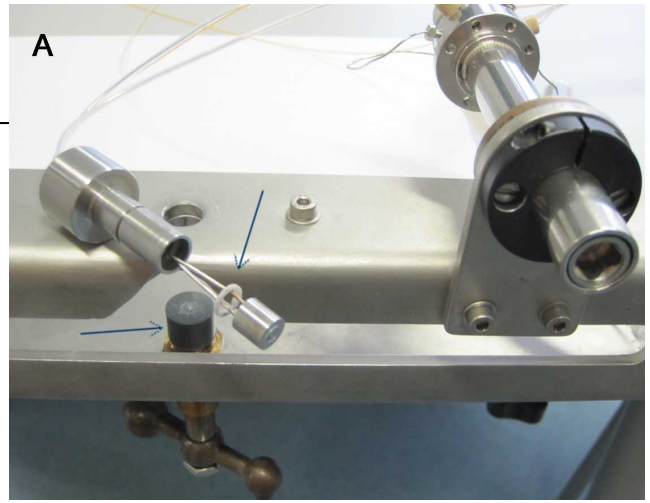
Mass spectrometers/interfaces



1. Elution-based TLC-MS

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- ChromXtract (Luftmann, 2004)
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Liquid microjunction (LMJ)

- In situ microextraction (flowprobe™): Prosolia (2008)
- Liquid Extraction Surface Analysis (LESA®): Advion (2010)
- TLC-surface sampling probe (SSP)(Van Berkel et al., 2002)

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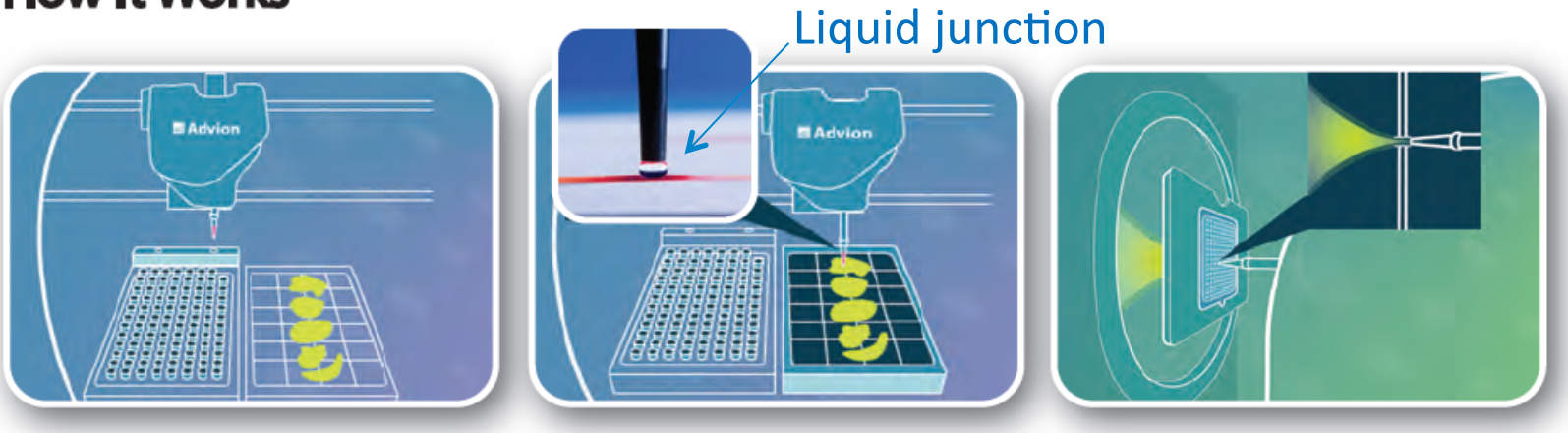
Liquid microjunction

Liquid extraction surface analysis: LESA-TriVersa Nanomate (Advion)

How It Works



The TriVersa NanoMate picks up a pipette tip from the tip rack, then aspirates extraction solvent from the reservoir.

The robot brings the extraction solvent into contact with the surface of the sample. The analyte is extracted from the surface.

The solvent is retracted into the pipette tip and is analyzed by chip-based infusion.

www.advion.com

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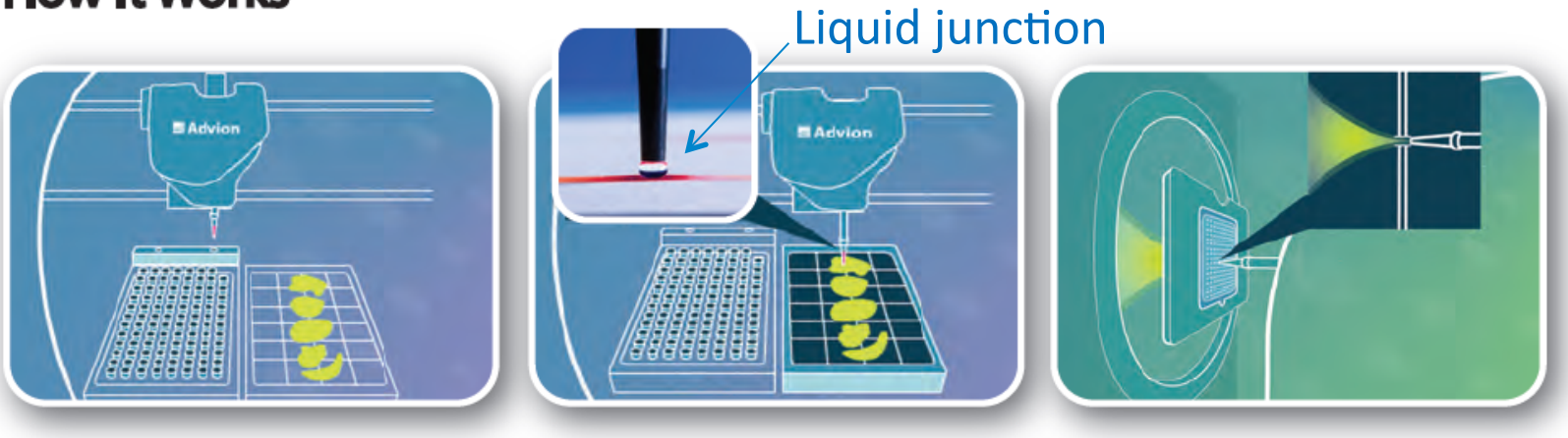
Liquid microjunction

Liquid extraction surface analysis: LESA-TriVersa Nanomate (Advion)

How It Works



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The solvent is retracted into the pipette tip and is analyzed by chip-based infusion.

☞ RP plates only!

www.advion.com

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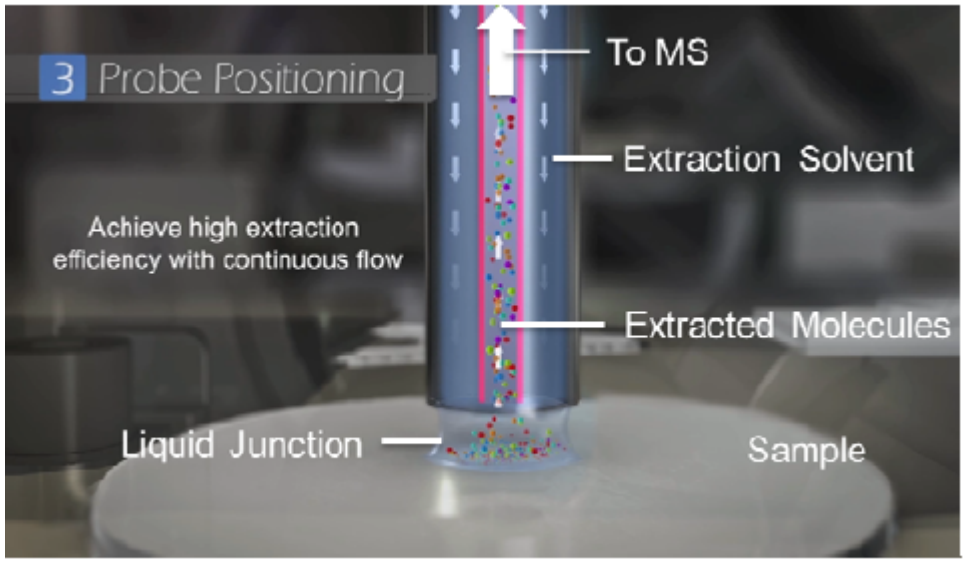
Liquid microjunction

In situ microextraction (flowprobe™): Prosolia

How it works:

The flowprobe uses a two step process: 1) dissolution of the material at the surface by a continuous flow of solvent; and 2) ionization of the analyte(s) via electrospray (ESI) ionization.



www.prosolia.com

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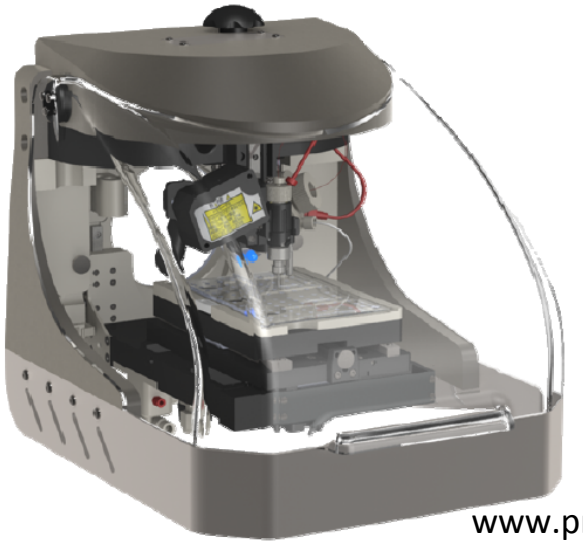
Liquid microjunction

In situ microextraction (flowprobe™): Prosolia



www.prosolia.com

- ☞ RP plates directly (extraction with methanol/water)
- ☞ NP plates sprayed with silicone oil after development [1)]
- ☞ Line scanning option!

1) M. J. Walworth et al., Anal. Chem. (2011)

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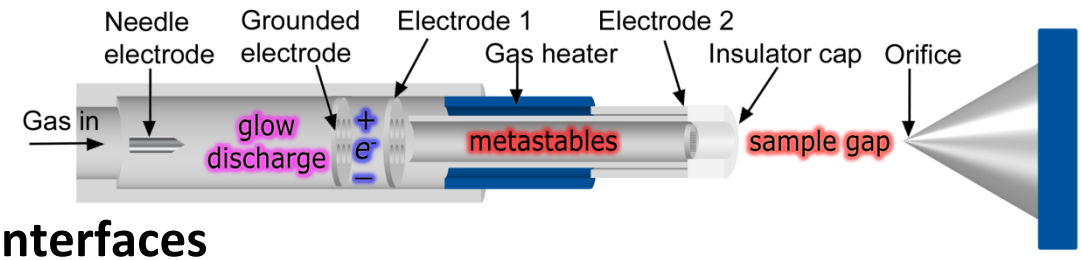
Interfacing HPTLC → MS



2. Desorption-based interfaces

Direct Analysis in Real Time (DART®): Ion Sense (2005)

- ☞ DART-TLC (Morlock & Ueda, 2007)
- ☞ DART SVP 45A – 3+D Scanner (Ion Sense, 2009)

Matrix Assisted Laser Desorption and Ionization (MALDI):

- ☞ TLC-MALDI (Bruker Daltonics , 2009)

Desorption Electrospray Ionization (DESI):

- ☞ TLC-DESI (Van Berkel et al., 2005; Pasilis et al., 2007)
- ☞ Omnispray® ion sources (2D-DESI): Prosolia (2008)

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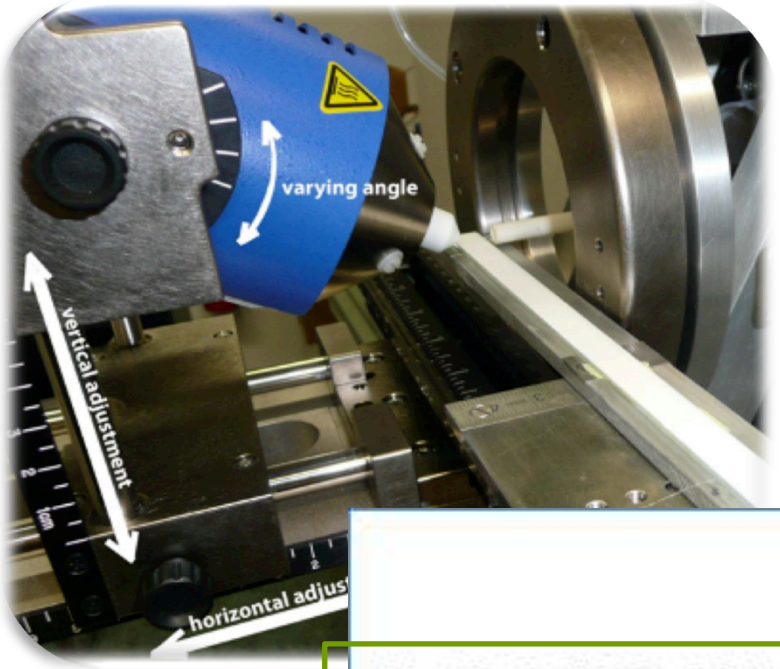
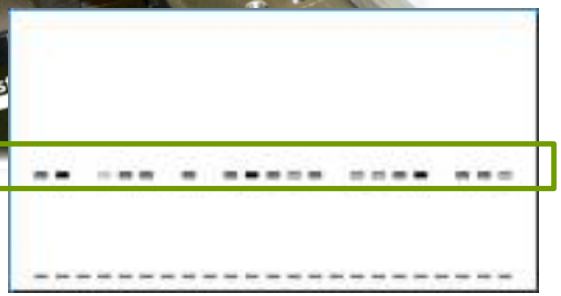
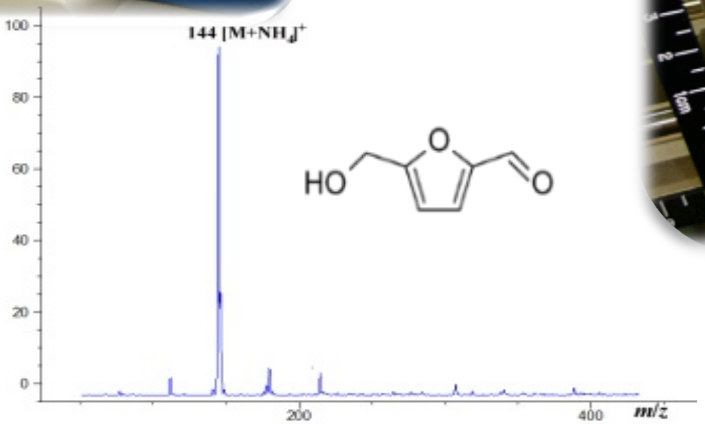
Desorption-based interfaces

DART 100 → DART SVP 45A – 3+D Scanner

2006 ↔ 2011







G. Morlock, Y. Ueda, *J Chromatogr* (2007)
G. Morlock, E. Chernetsova, *Cent Eur J Chem* (2012)

☞ E. Crawford (0-9)

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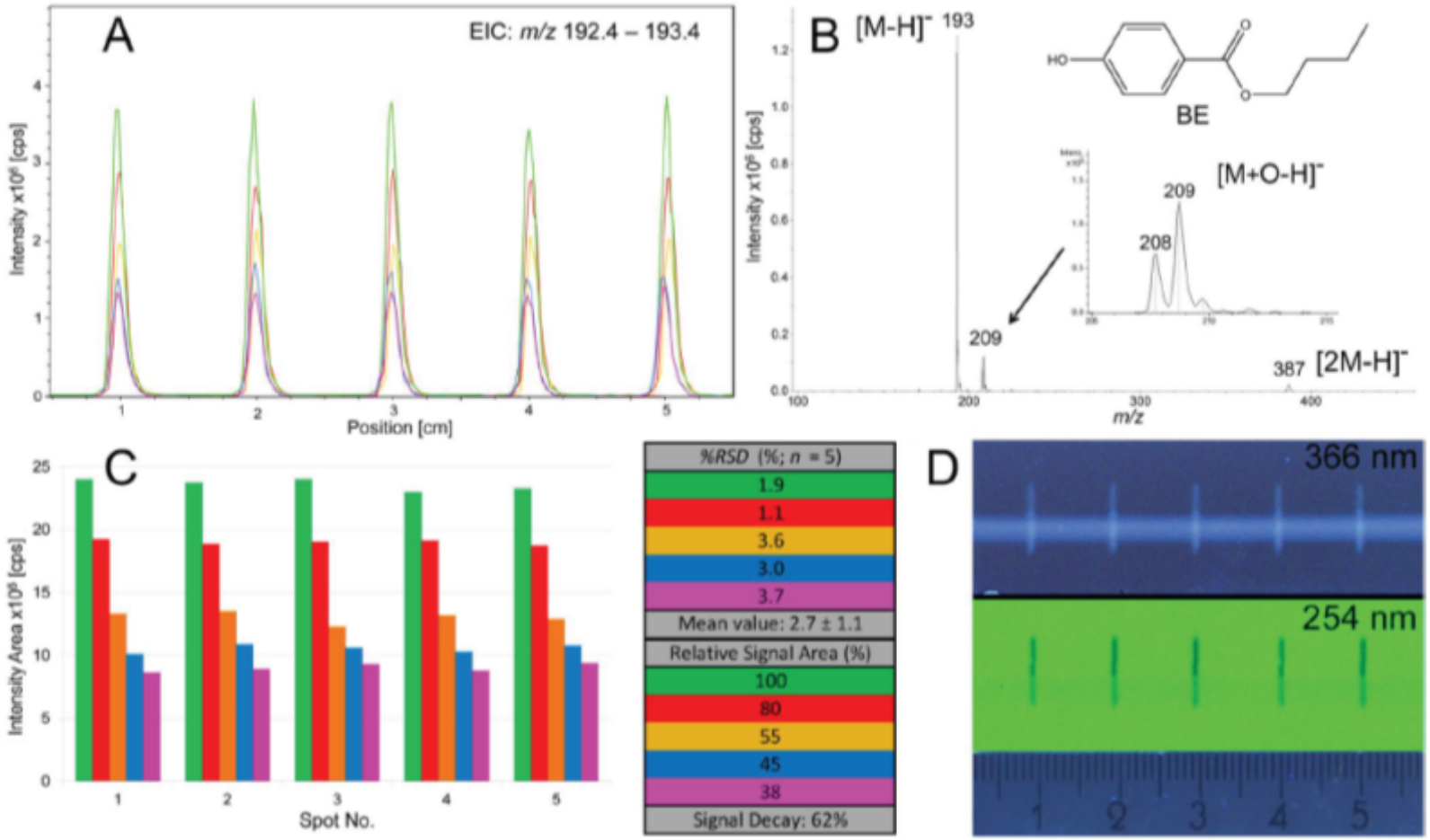
HPTLC-DART SVPA-MS



☞ T. Häbe & G. Morlock (O-15)

Interfacing HPTLC → MS

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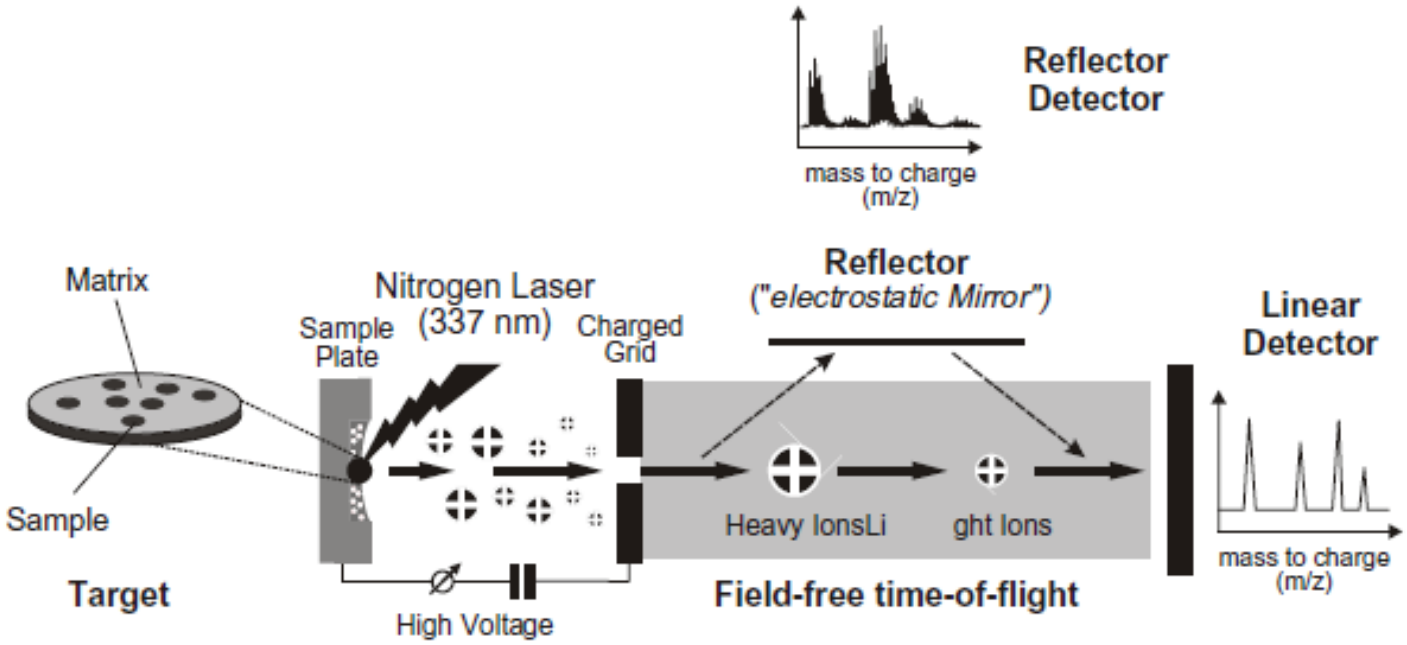
MALDI-TOFMS

MALDI: Matrix Assisted Laser Desorption and Ionization
(Tanaka et al., 1988)



B. Fuchs et al., Progress Lipids Res. (2010)

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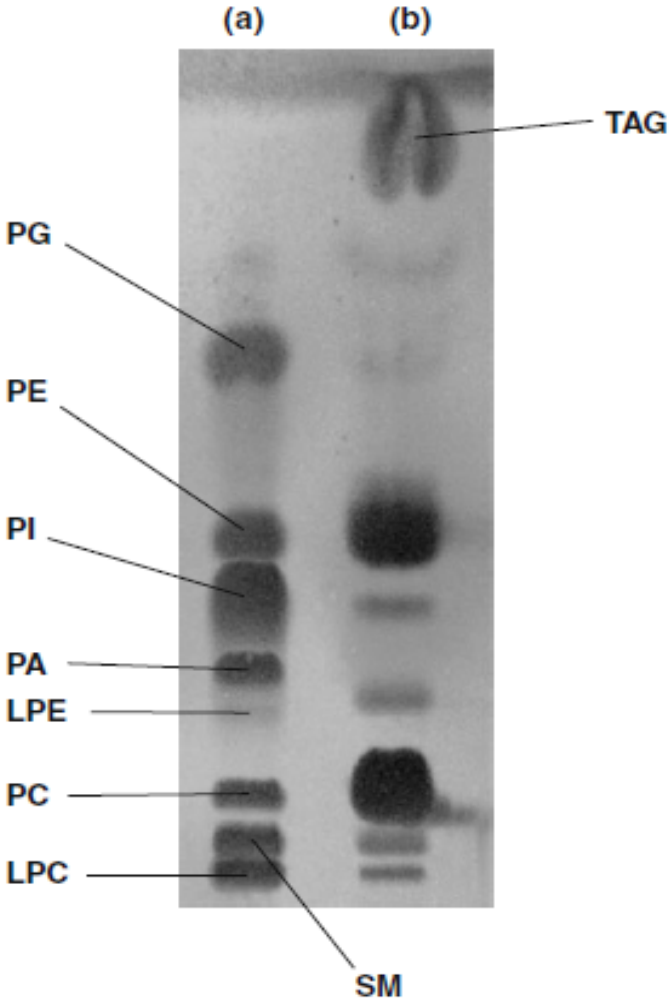
TLC-MALDI-TOFMS

Offline TLC-MALDI-TOFMS:

1. HPTLC and primuline staining
2. Scrape off and extract
3. Mix with matrix
4. MALDI-TOFMS



Phospholipids:
a) standards
b) hen yolk extract

K. Teuber at al., Anal. Bioanal. Chem. (2010)

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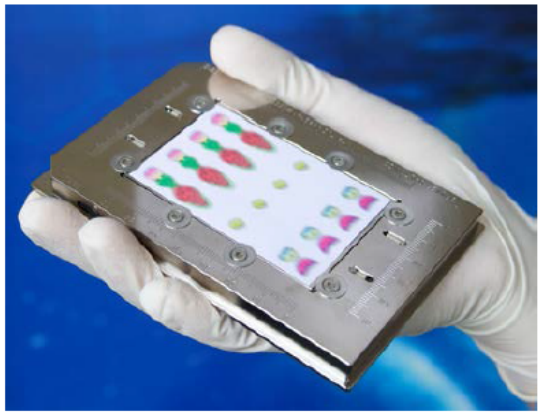
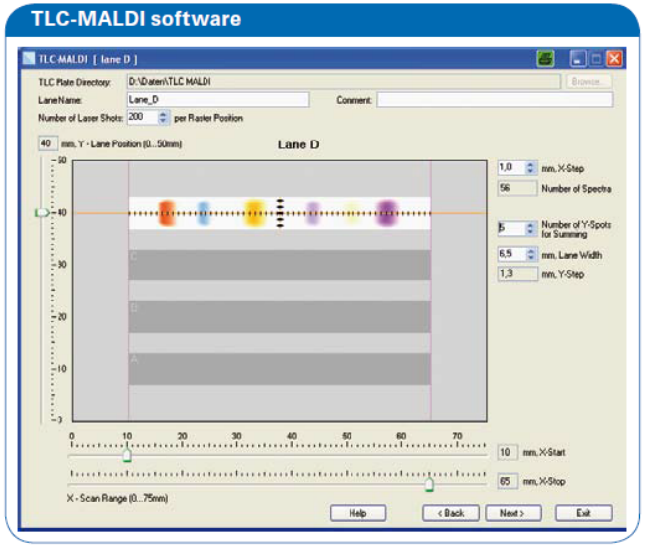
TLC-MALDI-TOFMS

Online TLC-scanning MALDI-TOFMS: Bruker Daltonics (2009)





- Aluminium (electrically conductive) backed plates (7.5 x 5 cm^2)
- Dipping the plate into matrix solution (20 % DHB)
- Scanning up to 4 tracks on a plate
- Scanning time 5 min per 5-cm track
- Imaging time several hours

Bruker Daltonics Application Note MT-94

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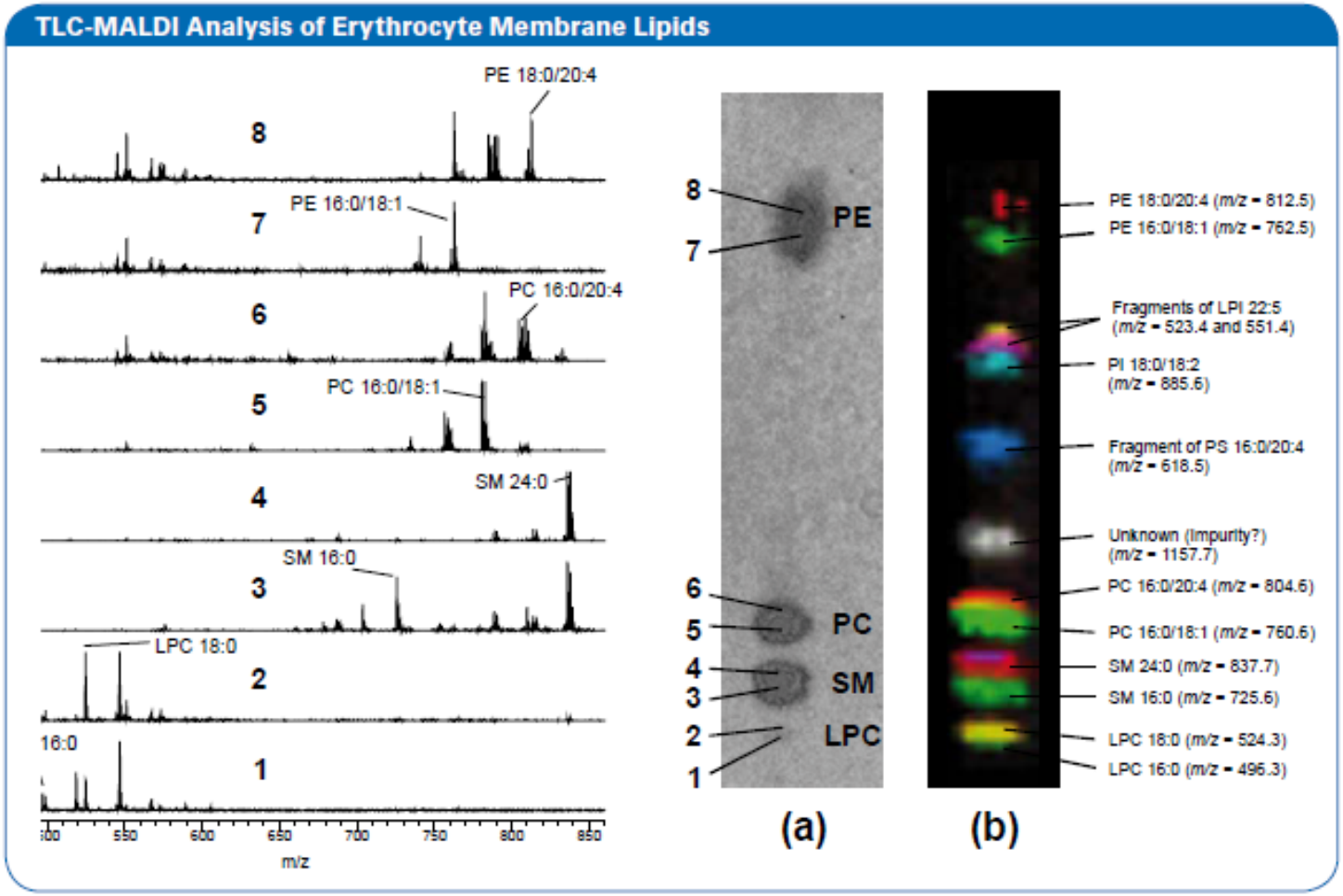
TLC-MALDI-TOFMS



Bruker Daltonics Application Note MT-94

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Interfacing HPTLC → MS

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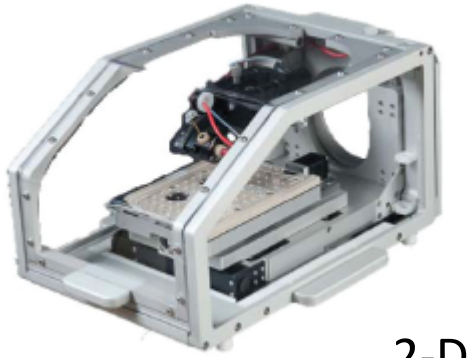
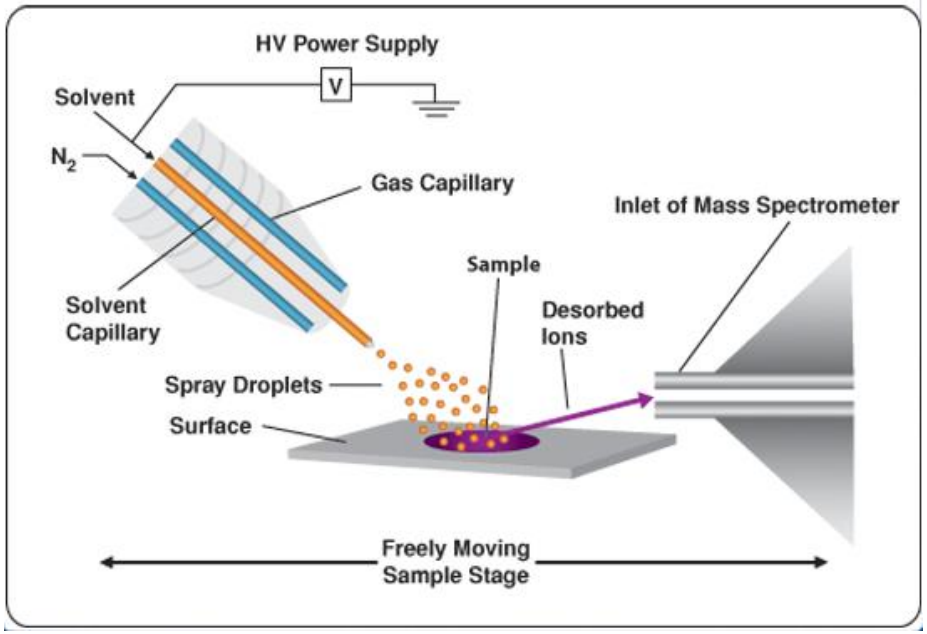
DESI ion sources

Principle of Operation

Desorption Electrospray Ionization (DESI) is carried out by directing high velocity charged droplets produced from a pneumatically-assisted electrospray onto a surface to be analyzed at atmospheric conditions. Ions of chemical species present on the surface are produced through the interaction of the charged droplets and the sample. The resulting mass spectra are similar to ESI mass spectra.



2-D DESI



DESI ion sources

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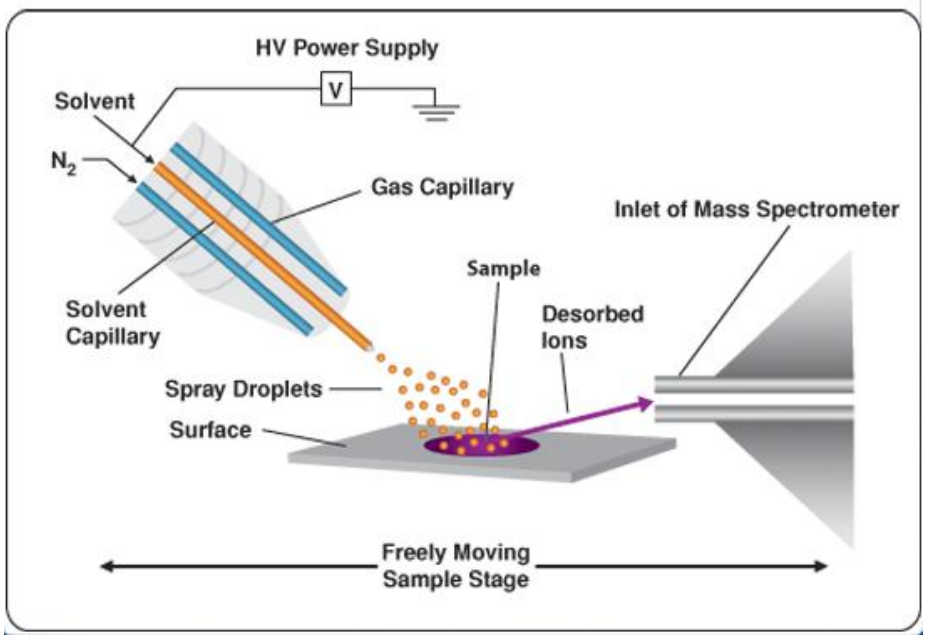
2-D DESI options:

- Scanning
- Imaging
- 2 plates (5 x 5 cm^2)
- NP and RP
- Sensitivity RP >> NP

Omnispray 2-D DESI



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TLC-MS

Generally soft ionization processes (ESI, APCI, DART, MALDI)

Protonated/deprotonated molecules
Sodium adducts
Ammonium adducts

Low (in-source) fragmentations: neutral losses (even/odd rule)

$[M+H]^+ - H_2O = [M-17]^+$
$[M+Na]^+ - H_2O = [M+5]^+$
$[M+NH_4]^+ - H_2O = M^+$

Enhanced fragmentations (CID): MS/MS, QTRAP, QTOF
☞ Substance identification, structural information

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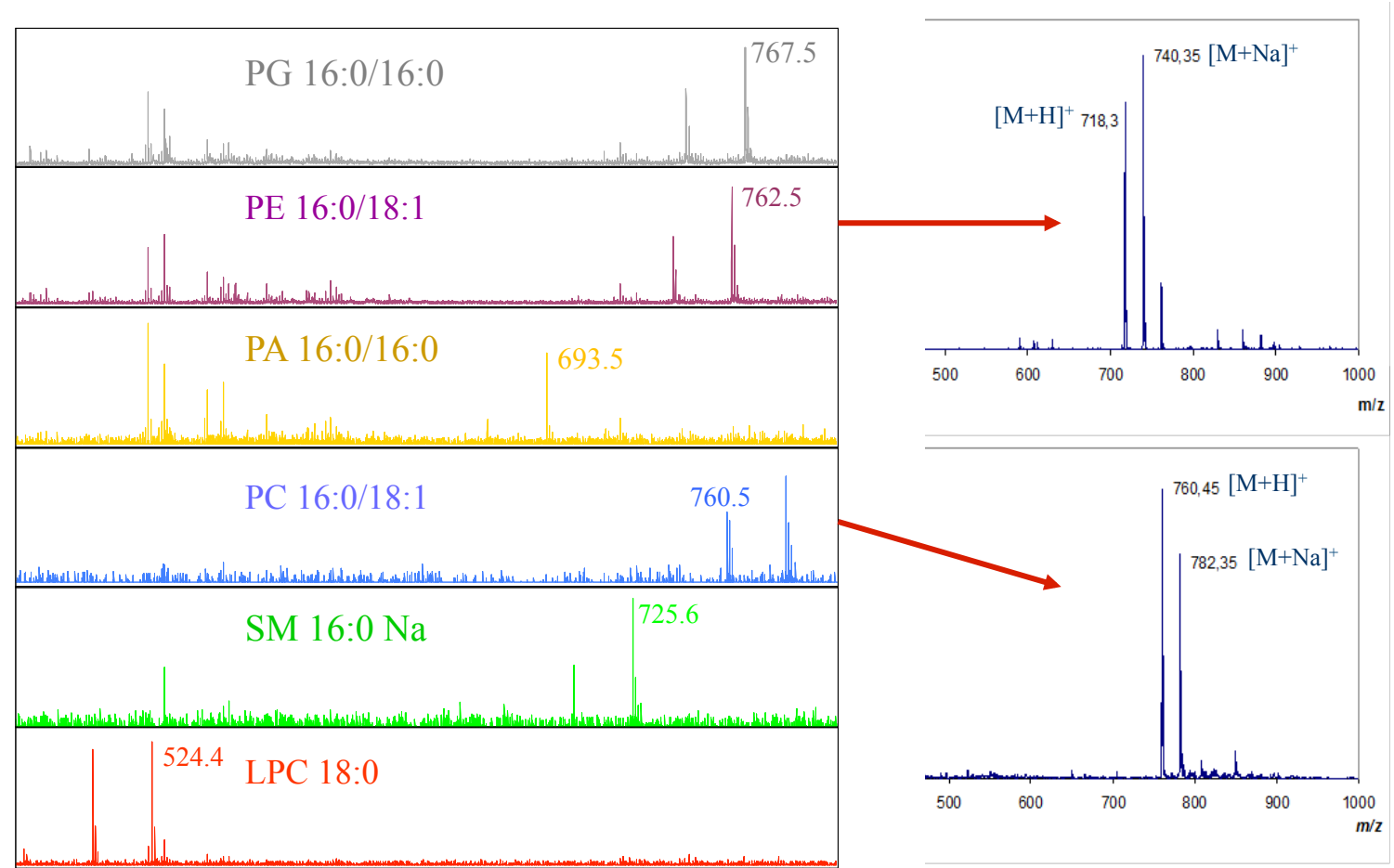
MALDI ↔ ESI



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FTIR spectrum

Mid-infrared ☞ functional groups, carbon skeleton

Diffuse Reflectance Infrared Fourier Transform (DRIFT)

- TLC-DRIFT (scanning device) [1)]

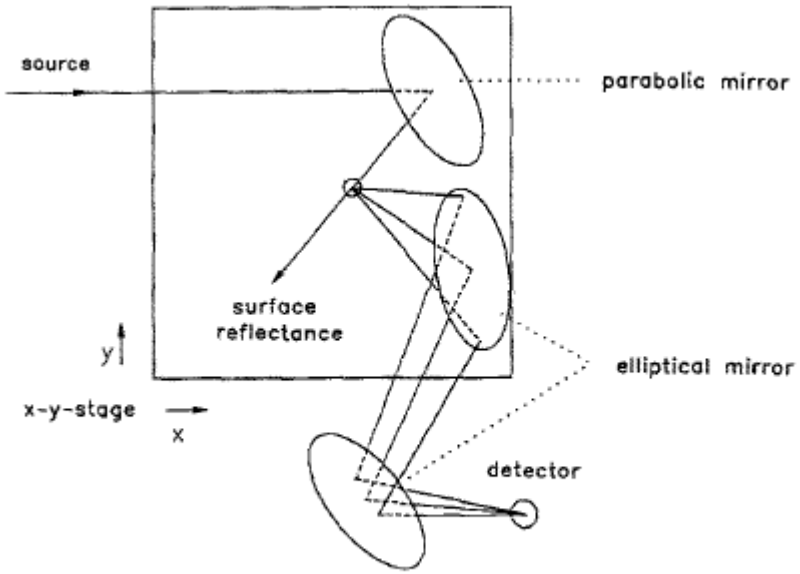
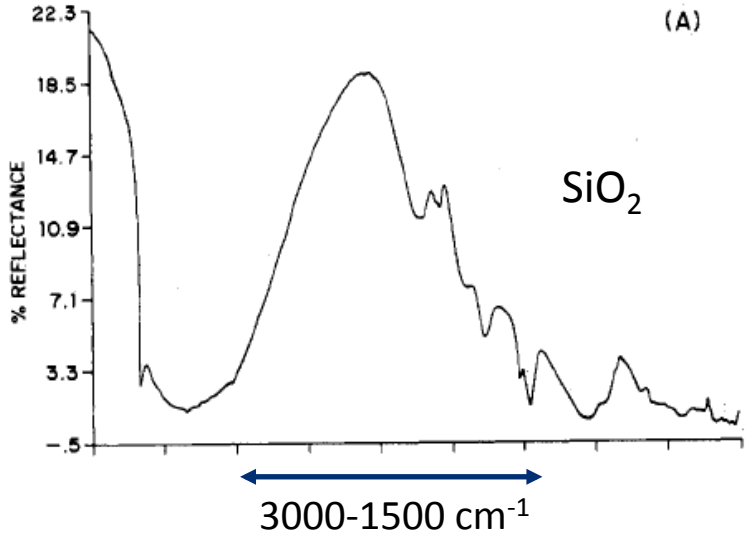


Fig. 1. Scheme of the constructed DRIFT unit for on-line measurements



1) E. Glauninger et al., Fres. J. Anal. Chem. (1990)

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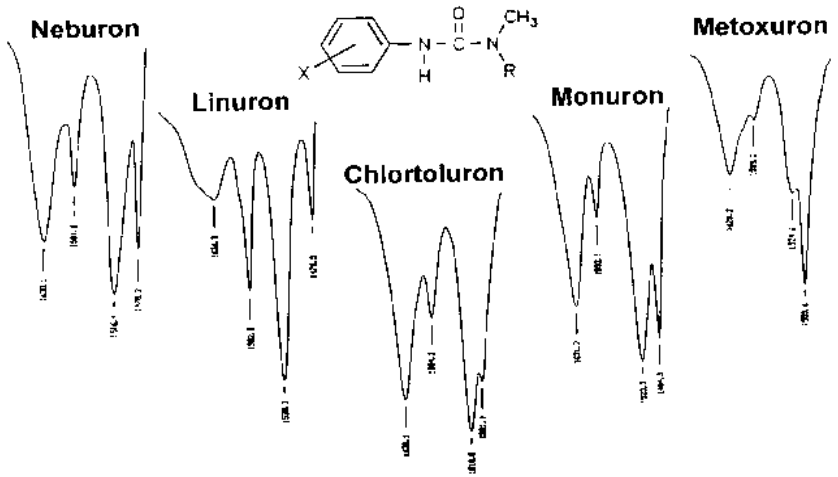
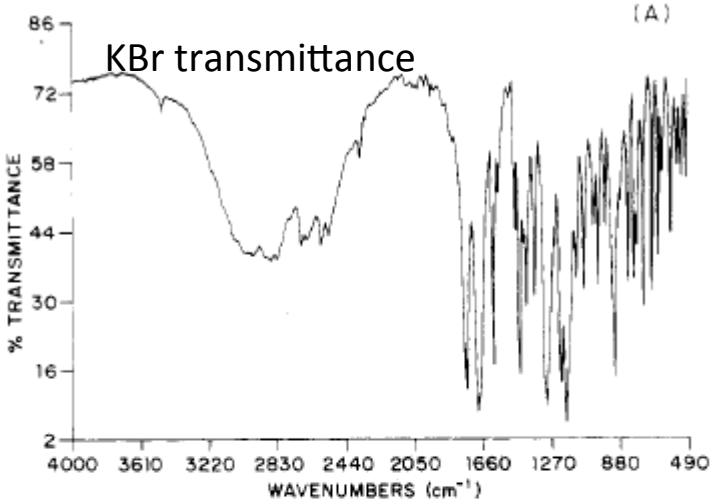
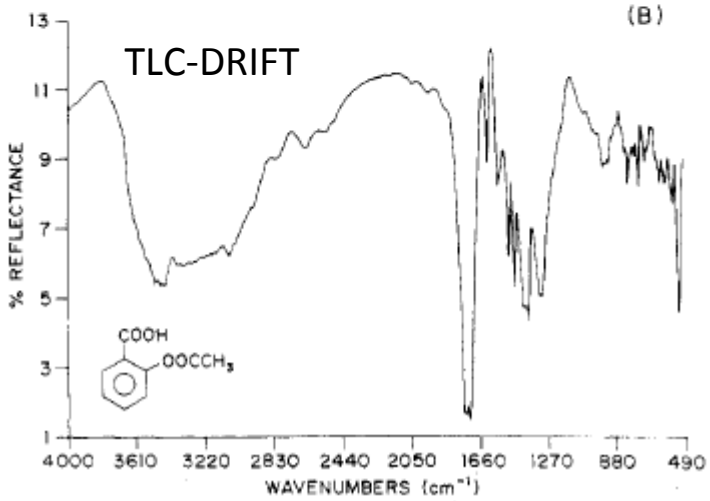
TLC-DRIFT



G. Morlock, PhD thesis (1995)





☞ Reference libraries [1)]

1) E. Glauninger et al., Fres. J. Anal. Chem. (1990)

G. E Zuber et al., Anal. Chem. (1984)

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FTIR spectrum

Diffuse Reflectance Infrared Fourier Transform (DRIFT)

- Actual DRIFT units

  ☞ Small samples (powders, pills, …)

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FTIR spectrum

Elution-based FTIR techniques

☞ Transfer to FTIR-microscopy slides

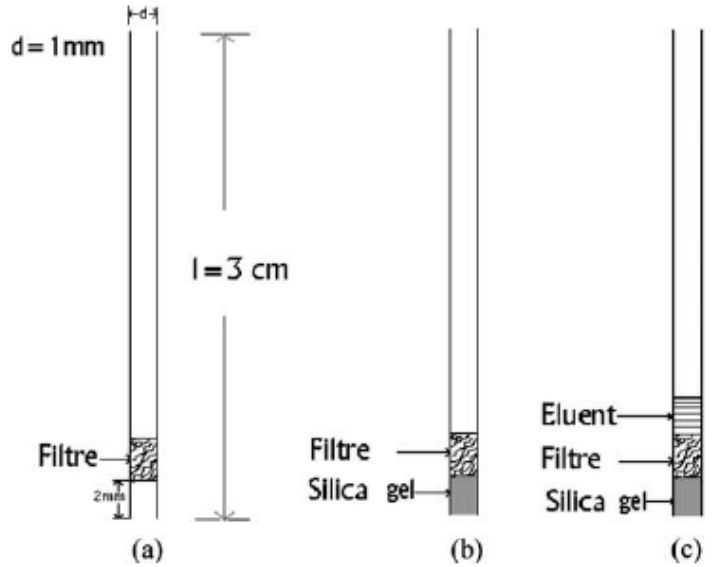

Fig. 2. Capillary transfer technique, showing the steps of transfer technique: (a) with the filter plug; (b) with filter plug and silica gel containing adsorbed compounds; (c) after eluting with the eluent, the upper eluent layer contains separated component.

W. He et al., Vibrational Spectroscopy 30 (2002)
W. He et al., Spectrochimica Acta A 61 (2005)

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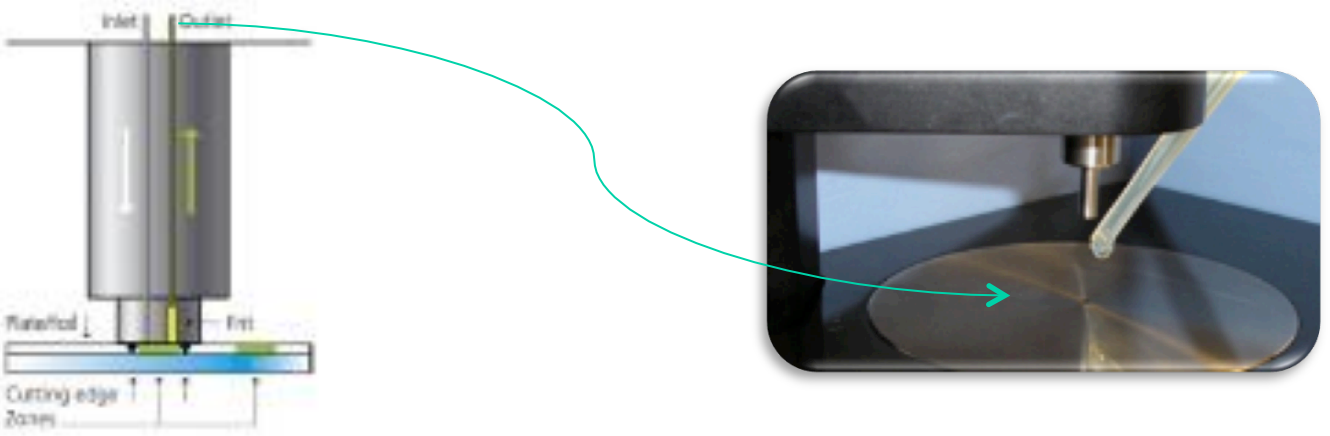
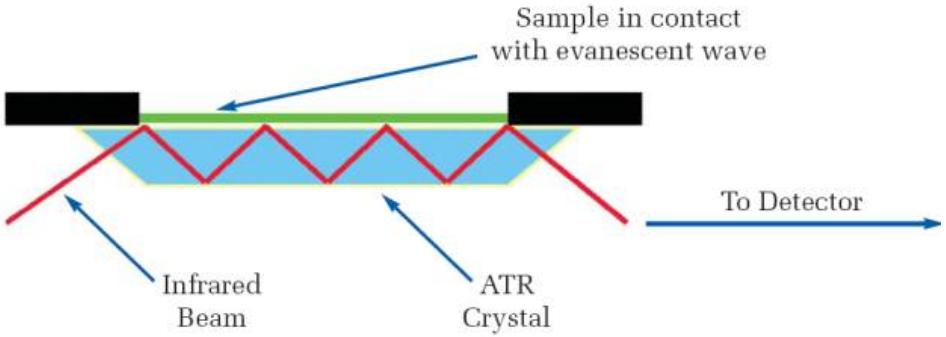
FTIR spectrum

Elution-based FTIR techniques

☞ Transfer to an ATR unit (attenuated total reflection)





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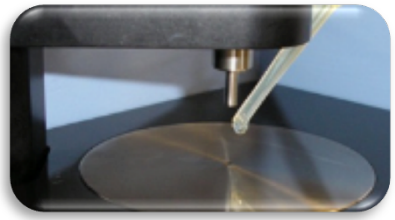
FTIR spectrum

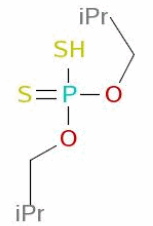
Attenuated Total Reflection (ATR)

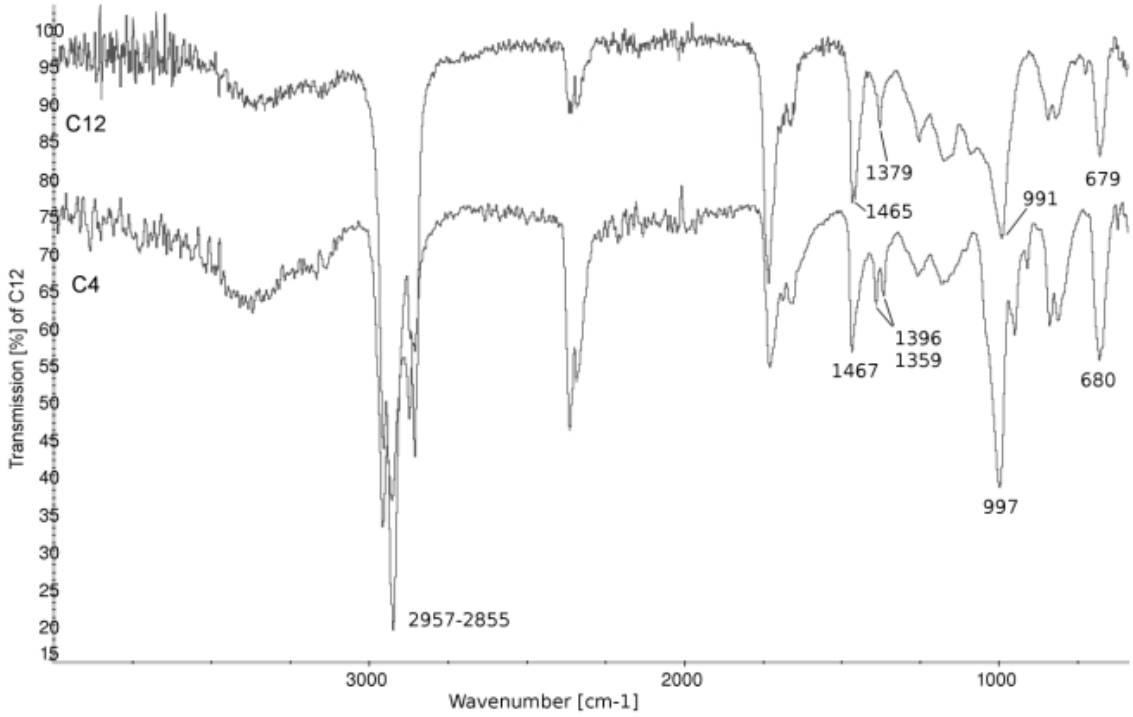
☞ Additives in mineral oils



Zinc-bis(O,O'-didodecyl)-dithiophosphate

Zinc-bis(O,O'-diisobutyl)-dithiophosphate





E. Dytkiewitz, G. Morlock, J. AOAC Int. (2008)

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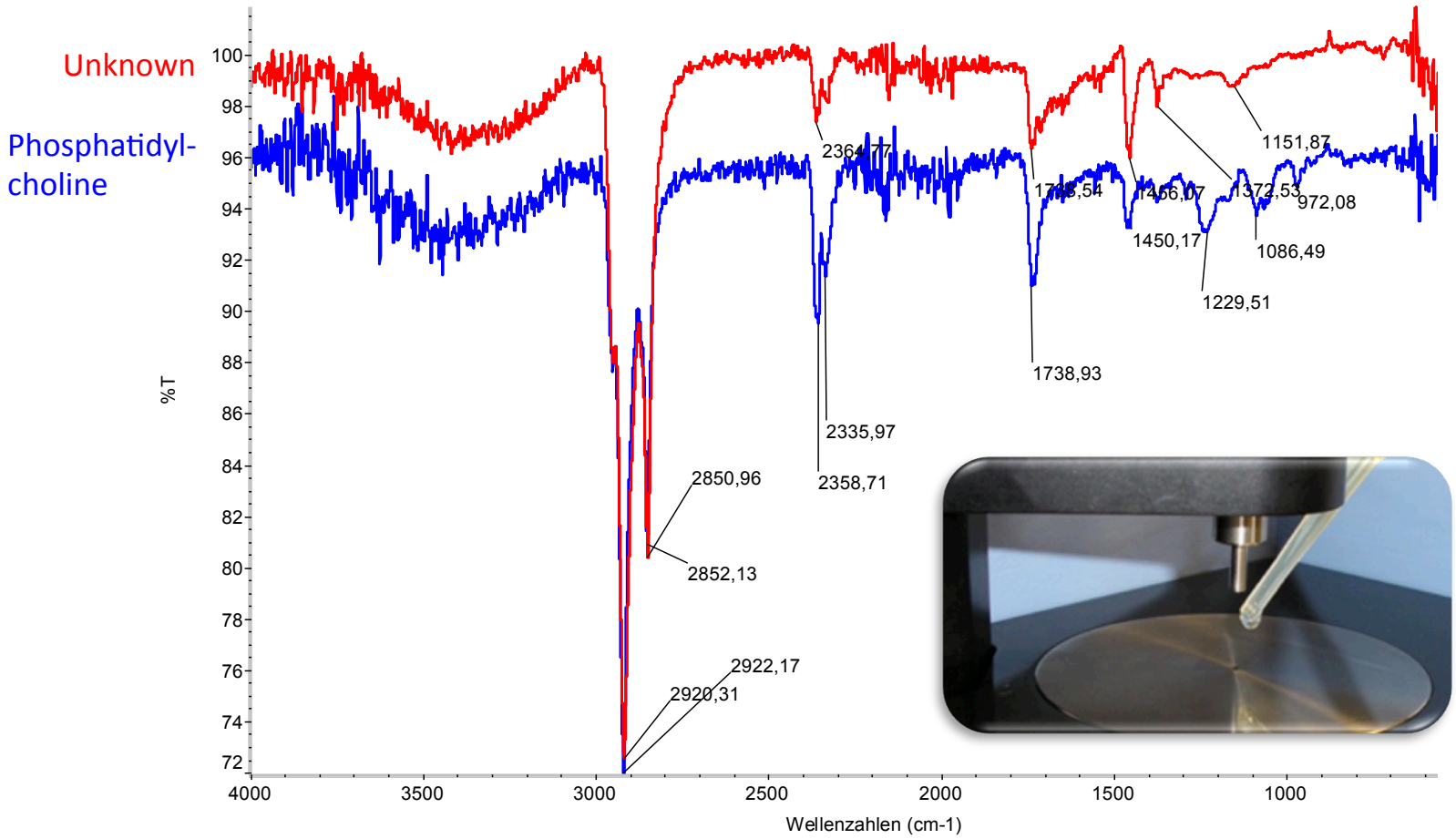
FTIR spectrum

Bioactive components of *Lactobacillus fermentum*



G. Morlock et al. (in prep.)

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FTIR spectrum

Additional structural information from IR: carboxylic acid

☞ ChemSpider search (www.chemspider.com)

$[M+H]^+ = 319.22677$

- Monoisotopic mass ± 0.00032 (1 ppm)

  => 1060 hits (all with $C_{20}H_{30}O_3$)
- Including name fragment (substring): „acid“

  => 185 hits

☞ **Additional structural information: NMR ?**

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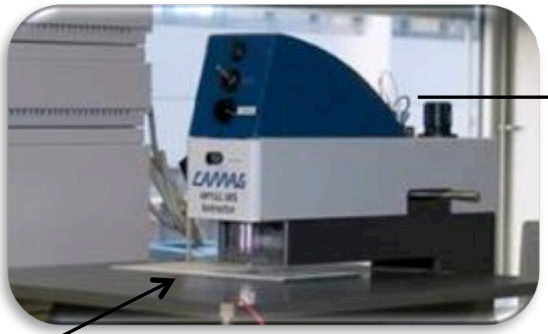
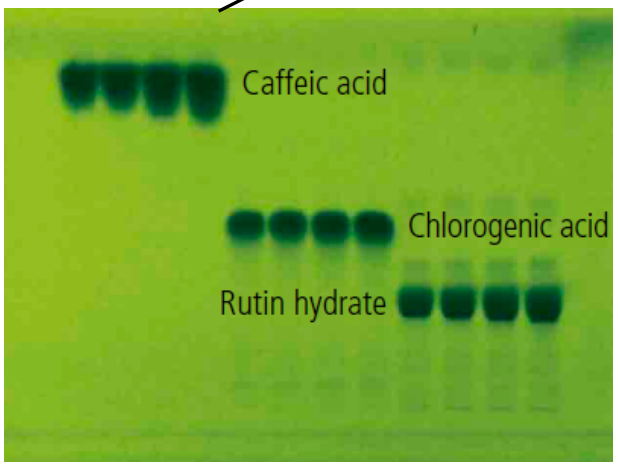
^{1}H-NMR spectrum

^{1}H-NMR: proton spin systems





A. Gössi, U. Scherer, G. Schlotterbeck, Chimia (2012)
CBS 110 (2013)

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^{1}H-NMR spectrum

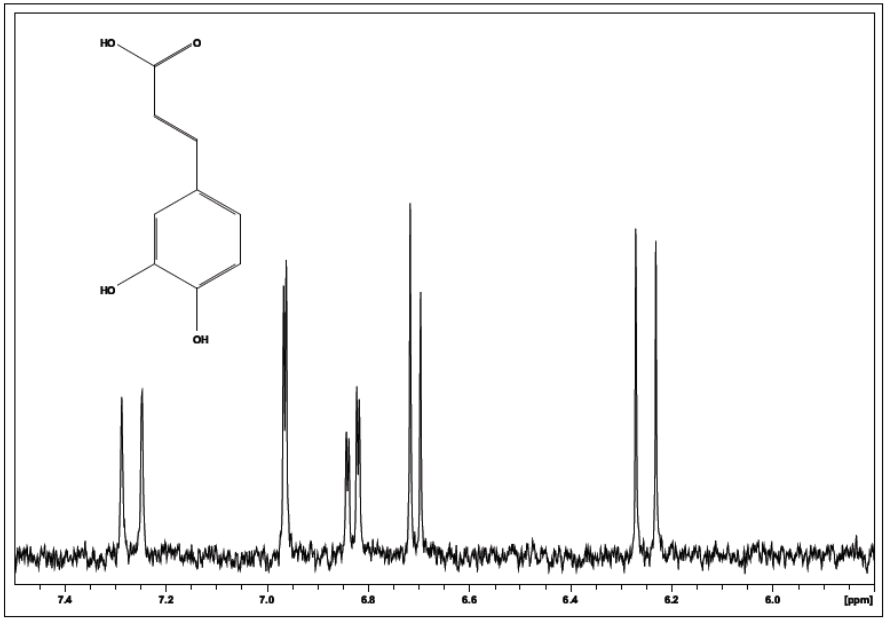

Fig. 1. Aromatic region of ^{1}H NMR Spectrum of 15.6 µg caffeic acid extracted from a TLC plate after development and measured at 400 MHz.

600 µL d_4-methanol (400 MHz)

^{1}H NMR	Measurements in solution			After elution from the plate		
Substance	Linearity r^2	LOD (µg)	LOQ (µg)	Quantity (µg/band)	Recovery (%)	*%RSD* (n=3)
Rutin	0.9976	2.3	6.9	20.3	101.8 ± 4.0	3.9
Caffeic acid	0.9978	2.5	7.3	17.1	103.4 ± 1.0	1.5
Chlorogenic acid	0.9991	3.3	10.1	19.6	100.5 ± 3.1	3.1

A. Gössi, U. Scherer, G. Schlotterbeck, Chimia (2012); CBS 110 (2013)

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NMR spectrum

^{1}H-NMR/^{13}C-NMR including 2-D correlation spectra

☞ More substance than some µg is needed

Due to the highest yield and purity, zones 1 and 6 were isolated for NMR spectroscopy and determination of their biological activity. For this purpose fractions A5 and A6 were applied on HPTLC plates as 16 cm lines with concentrations of 5.0 µg/mm. From 20 HPTLC plates 1.58 and 1.75 mg of the substances 1 and 6 were obtained, respectively. The structures were characterised by one- and two-dimensional ^{1}H- and ^{13}C-NMR spectroscopy and mass spectrometry. The accurate molecular masses of the two oily substances were determined in an UHPLC-QTOF MS system. Flow injection analysis was performed with 0.3 µL sample solution and a flow rate of the mobile phase of 0.4 mL/min in an isocratic mode. The mobile

H.-R. Adhamai et al., Phytochem. Anal. (2013)

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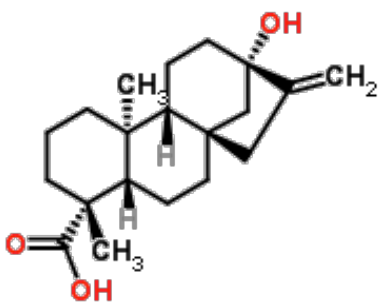
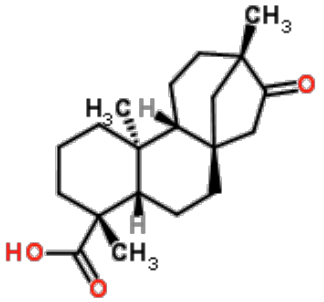
^{1}H-NMR spectrum



Steviol

or



iso-Steviol

δ (ppm) =

0.9 (s, 3 H, -CH_3)
1.0 (s, 3 H, -CH_3)
4.9 (s, 2 H, =CH_2)

CSID:398979, http://www.chemspider.com/Chemical-Structure.398979.html (accessed 11:00, Jun 9, 2014)

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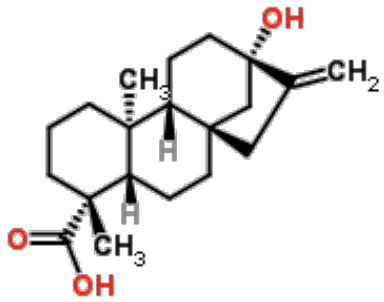
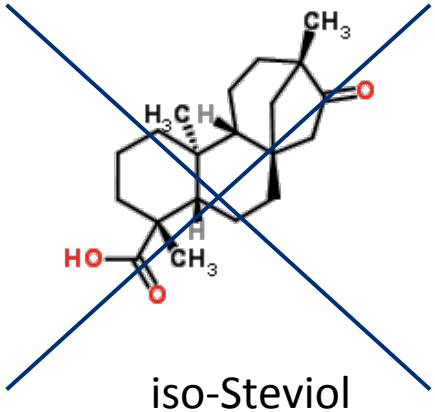
^{1}H-NMR spectrum



Steviol

or



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1.0 (s, 3 H, -CH_3)
4.9 (s, 2 H, =CH_2)

CSID:398979, http://www.chemspider.com/Chemical-Structure.398979.html (accessed 11:00, Jun 9, 2014)

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Conclusions

- HPTLC-MS - the most sensitive interface?
- Scanning MS devices ☞ selective detectors
- From a detected zone to the chemical structure?
  - ☞ High-resolution MS alone does not simply provide the structure of an unknown compound.
  - ☞ Strong support by IR and NMR spectra is required.
- TLC-MS interface
  - ☞ mass spectra, ATR-FTIR spectra, ^{1}H-NMR-spectra
  - ☞ analytical scale (MS: ng/zone, FTIR/^{1}H-NMR: µg/zone)

- Good luck for your study from the detected zone to the chemical structure!

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Many thanks to Gerda Morlock for providing literature and materials for my presentation and for helpful discussions.

Many thanks to you for your kind attention!

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Liquid microjunction

In situ microextraction (flowprobe™): Prosolia

www.prosolia.com

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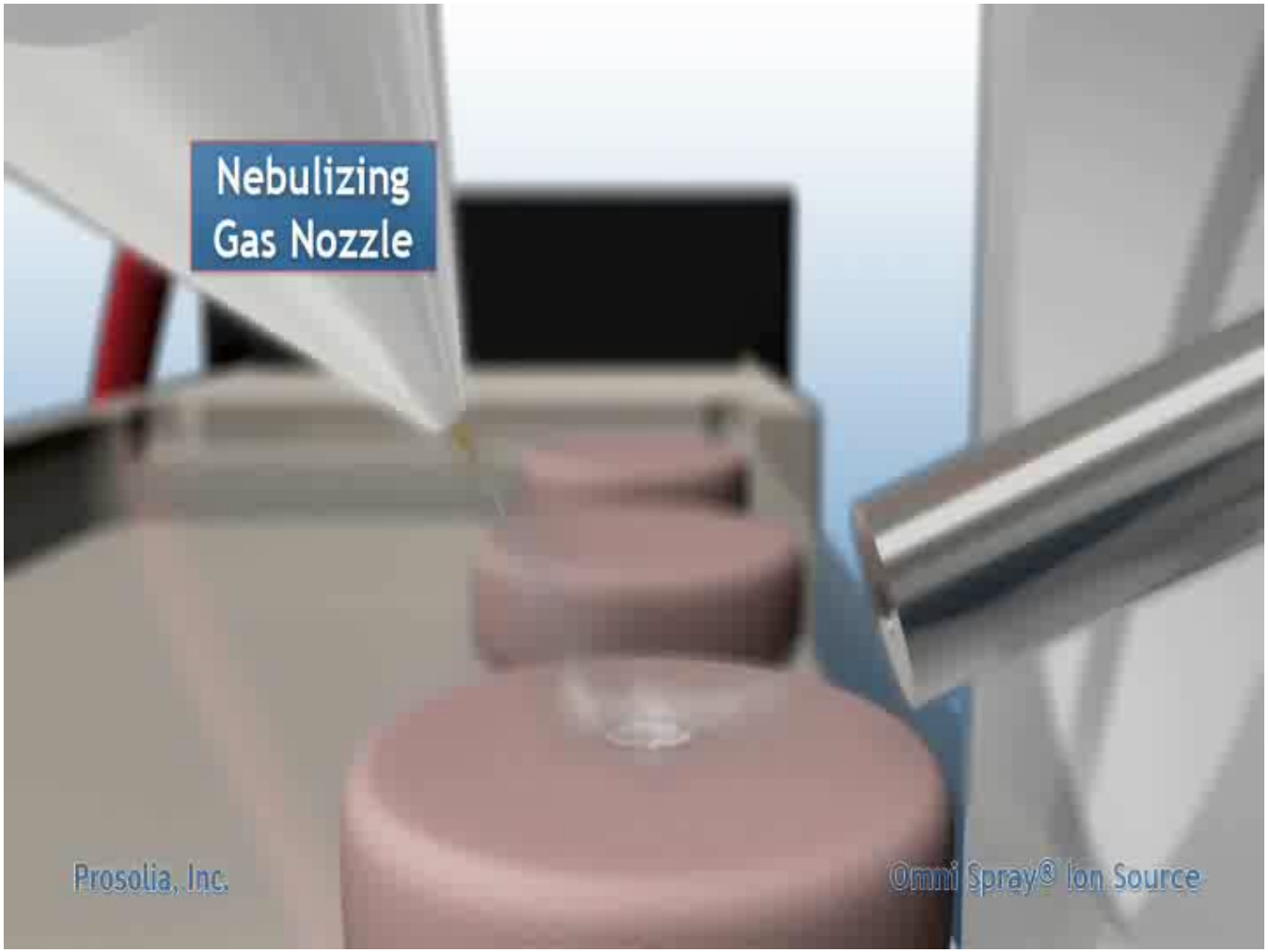
DESI ion sources



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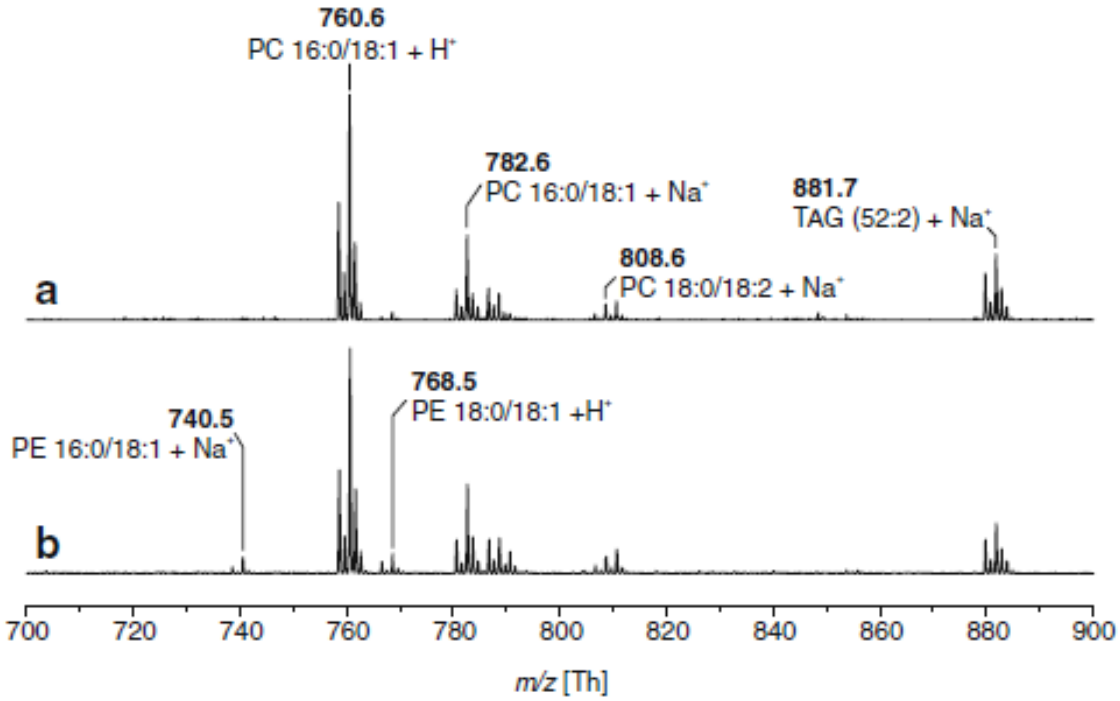
TLC-MALDI-TOFMS

Offline TLC-MALDI-TOFMS:

1. TLC and primuline staining
2. Scrape off and extract
3. Mix with matrix
4. MALDI-TOFMS

Hen yolk extract
a) directly
b) after HPTLC



Teuber at al., Anal. Bioanal. Chem. (2010)

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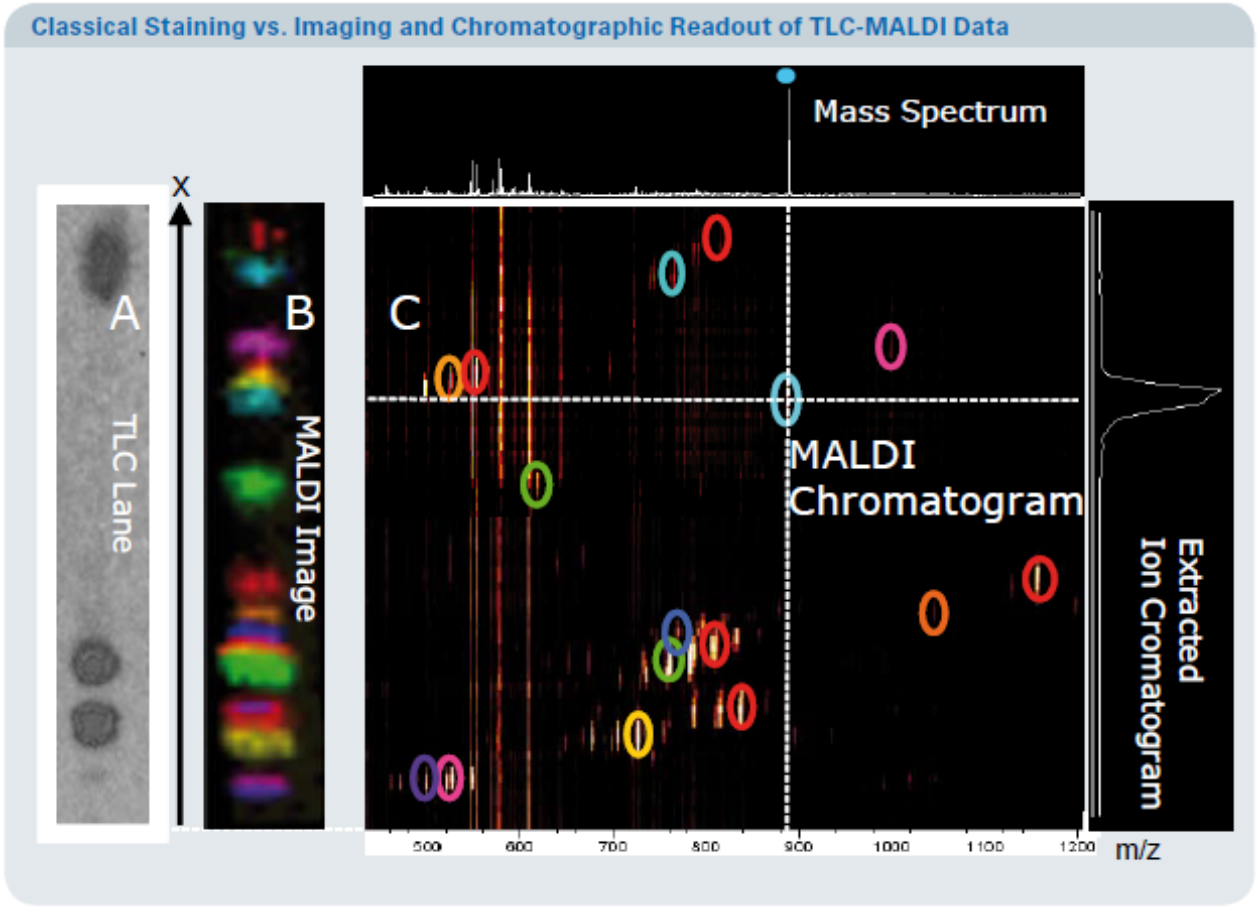
TLC-MALDI-TOFMS

TLC-scanning MALDI-TOFMS: Bruker Daltonics (2009)



Bruker Daltonics Application Note MT-94