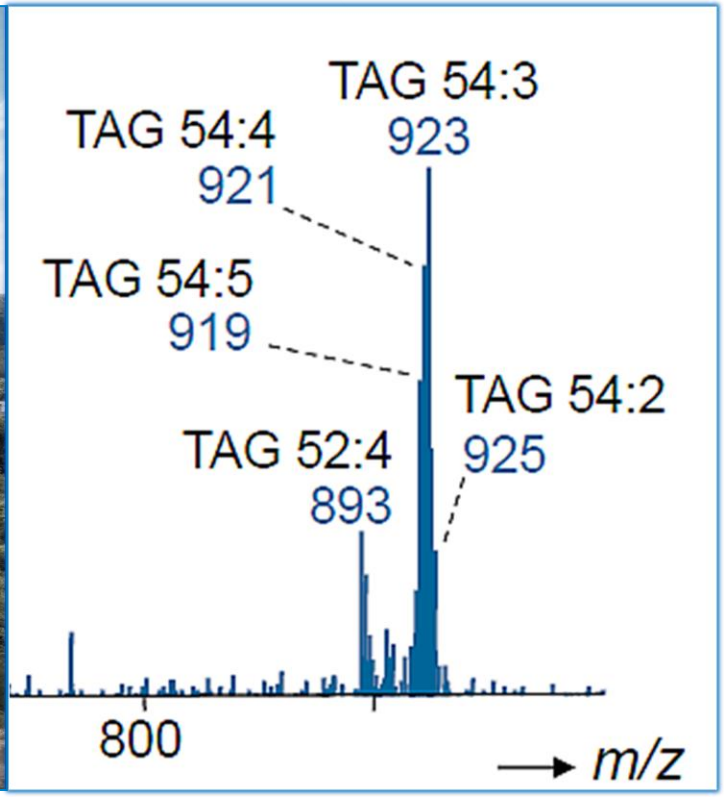
New Ideas. New Materials.

INM



CHEMICAL ANALYTICS AT INM

APPLICATION POTENTIALS OF ATOMIC SPECTROMETRY, CHROMATOGRAPHY AND MASS SPECTROMETRY FOR NEW MATERIALS AND BIOLOGICAL SAMPLES

Dr. Claudia Fink-Straube, 28.06.2019

CHEMICAL ANALYTICS AT INM

1 Introduction
1.1 Qualitative and quantitative analysis, LOD, LOQ
1.2 Source of errors in the analytical chemistry, kind of errors
1.3 Introduction into statistics
1.4 Calibration (external, internal, standard addition)
2 Instrumental equipment of INM
3 Sample preparation
3.1 Introduction
3.2 Digestion techniques
3.3 Micro wave
4 Atomic spectrometry
4.1 Comparison: Atomic absorption and atomic emission
4.2 AAS - Atomic Absorption Spectrometry
4.3 OES - Optical Emission Spectrometry
4.4 ICP-MS – Inductively Coupled Plasma – Mass Spectrometry
4.5 Examples
5 Chromatography
5.1 Chromatographic separation, classification, comparison LC and GC
5.2 Gas chromatography coupled with Mass spectrometry
5.3 Liquid chromatography coupled with Mass spectrometry
5.4 Examples
6 Questions
7 Literature

1.1 QUALITATIVE AND QUANTITATIVE ANALYSES, LOD, LOQ

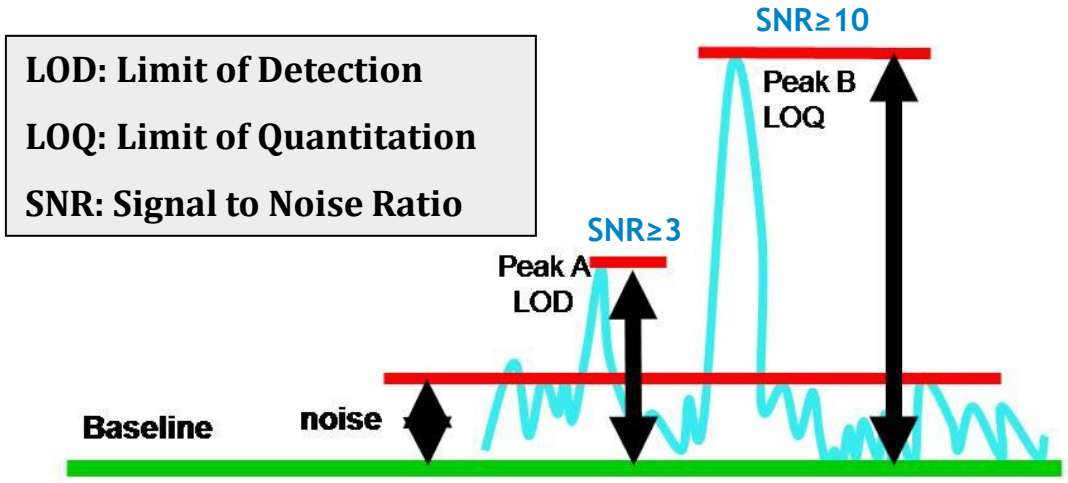
Qualitative: WHAT? What kind of material, which substances?

- identification (evidence, if necessary after separation)
- precondition: enough sample material; **>LOD** → precipitation reaction, flame colouration, GC/MS

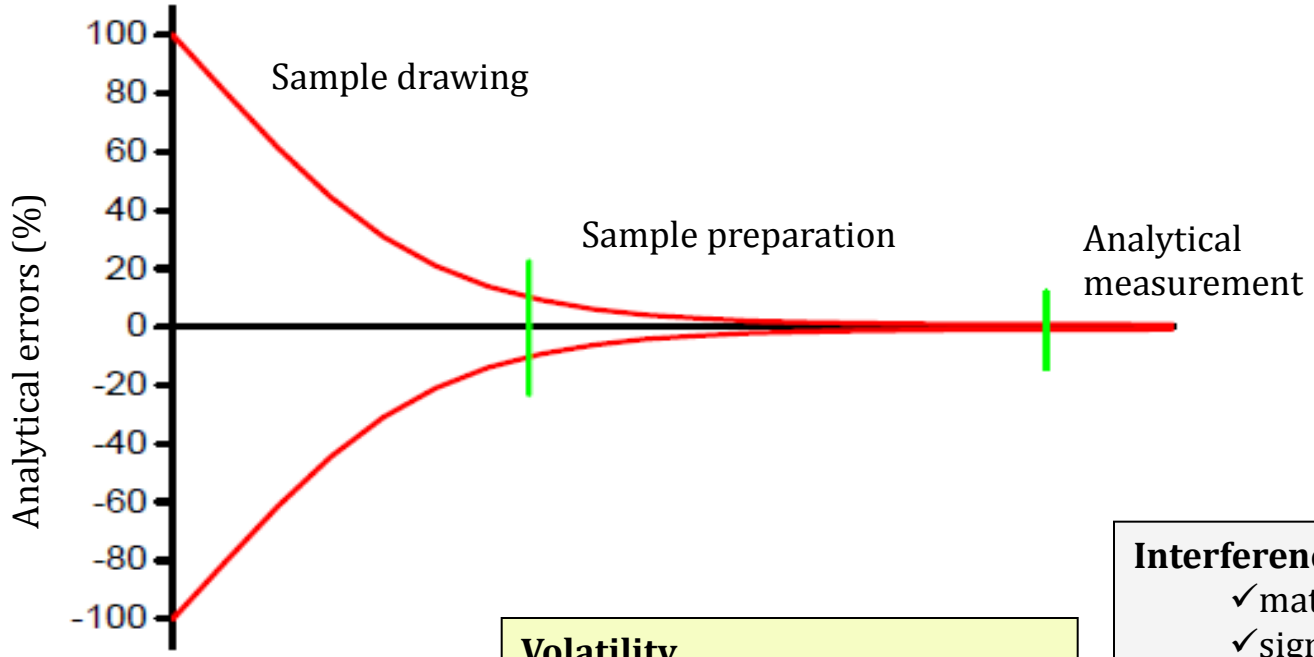
Quantitative: HOW MANY? Amount of analytes

- precondition: qualitatively analysed; **>LOQ** → titration, gravimetry, ICP-OES, AAS, GC

Analytics of structure: **chemical STRUCTURE**, crystal structure → IR-, UV-VIS-spectroscopy, NMR



1.2 SOURCE OF ERRORS, KIND OF ERRORS



Unsuitable sample drawing and storage, contamination
- ✓surface of devices and vessels
- ✓ reagents
- ✓ ambient air

Volatility
- ✓elements (Hg, As, Se..)
- ✓compounds (oxides, halogenids, hydrids etc.)

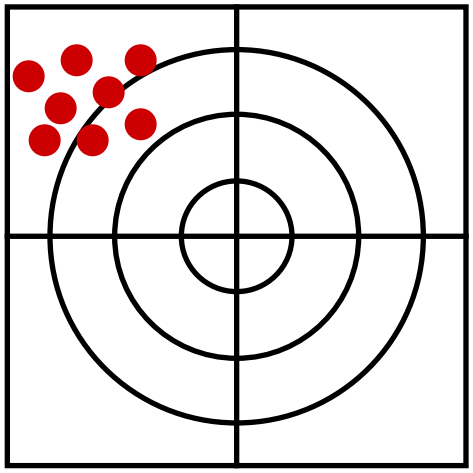
Adsorption / Desorption
- ✓surface of devices and vessels
- ✓filtration/ precipitation

Interferences
- ✓matrix effects
- ✓signal overlap
- ✓background

incorrect calibration and interpretation
- ✓unsuitable standards
- ✓not stable standards
- ✓ blank value
- ✓ calibration error

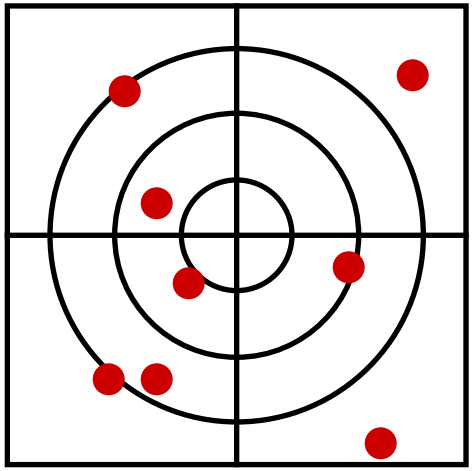
1.2 SOURCE OF ERRORS, KIND OF ERRORS

systematic error
☹ **accuracy**
☺ **precision**



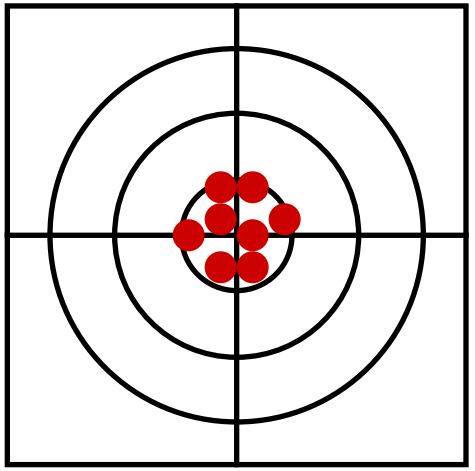
➢ correctable
reference materials,
round robin tests

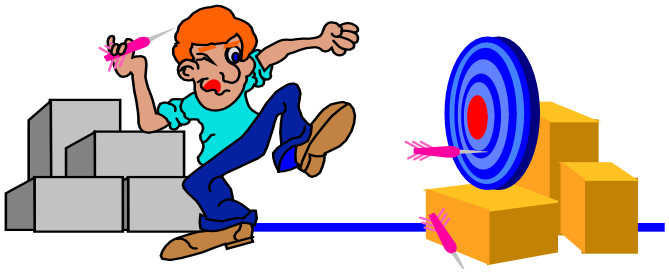
random error
☺ **accuracy**
☹ **precision**



➢ not eliminable
variance of data

accurate result
☺ **accuracy**
☺ **precision**





1.3 INTRODUCTION INTO STATISTICS

TERMS

- Arithmetic mean value

$$\bar{x} = \frac{\sum_i x_i}{n}$$

n = number of measurements

- Distribution

$$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n-1}}$$

mean square error of individual measurement

- Variation coefficient (RSD)

$$CV = \frac{s}{\bar{x}} \cdot 100\%$$

Example: **x ± s (CV)**
19,48 ± 0,31 (1,6%)

- Expectation value

$$\mu = \lim_{n \to \infty} \bar{x}$$

Most probable value of a series of measurements

- Standard deviation

$$\sigma = \lim_{n \to \infty} s$$

Average random deviation of single measuring values of μ

- Variance

$$\sigma^2$$

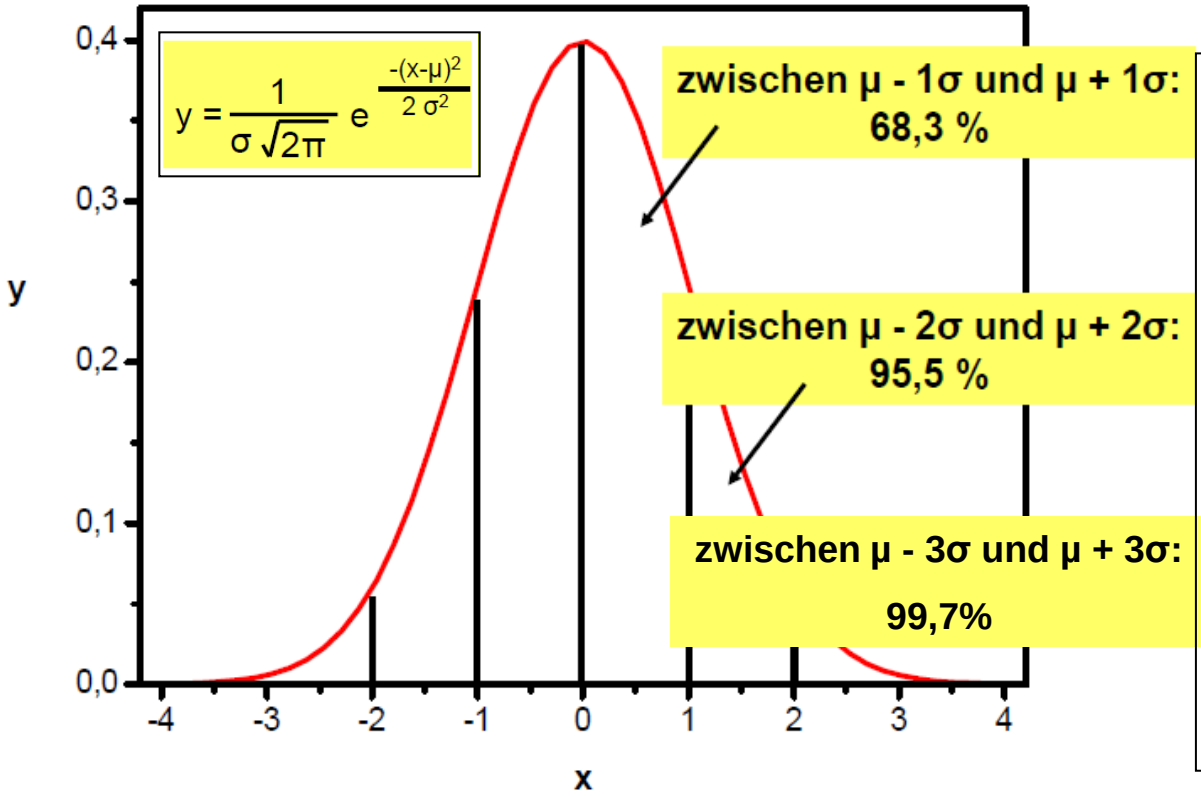
n = finite → s ≠ σ
s → deviation of σ

n = ∞ → s = σ

1.3 INTRODUCTION INTO STATISTICS

STANDARDISED GAUSSIAN DISTRIBUTION

Distribution of all values from arithmetic mean value



Example: **19,48 ± 0,31**

68,3% from **19,17 to 19,79**;
31,7% <19,17 or >19,79

➢ physics, surveying technology

95,5% from **18,86 to 20,10**;
4,5% <18,86 or >20,10

➢ technique, chemical analytics

99,7% from **18,55 to 20,41**;
0,3% <18,55 or >20,41

➢ biology

y → distribution function

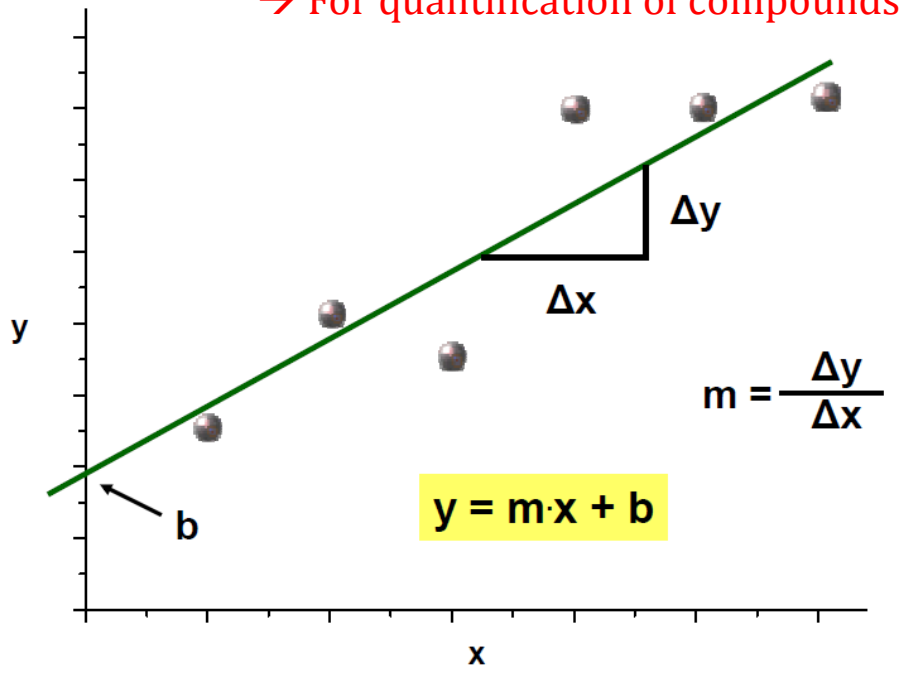
σ → measure of scope of the distribution, standard deviation of x

für n → ∞ x = μ und s = σ

1.4 CALIBRATION

LEAST SQUARES METHOD

→ For quantification of compounds by relative methods



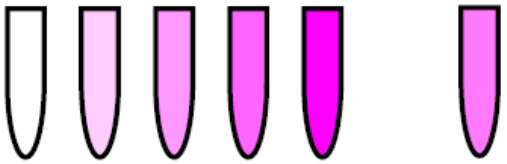
certificated reference materials

b	**blank value (Back ground signal)**
m	**sensitivity (gradient)**
x	**reference value (e.g. concentration)**
y	**measured value (intensity)**

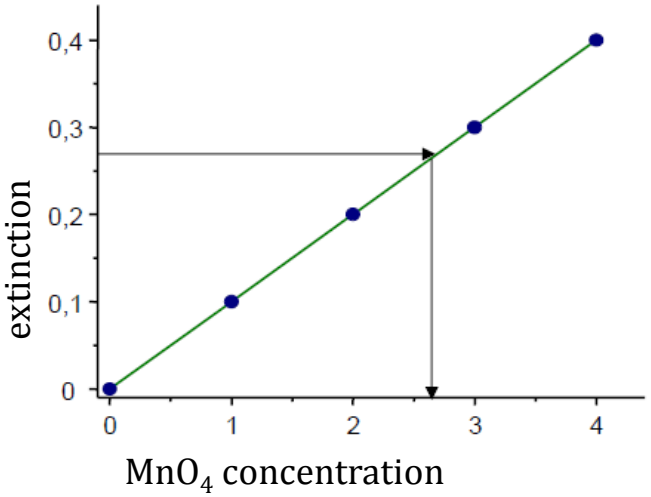
→ Minimum measured value depends on sensitivity

1.4 CALIBRATION

EXTERNAL CALIBRATION



concentr.	0	1	2	3	4	sample
extinction	0	0,1	0,2	0,3	0,4	0,26



Samples similar to standard
little systematic source of errors
high repeatability of all analytic steps

☺ **suitable for routine analysis (many, similar samples)**

☺ **standards re-usable**

☹ **identification of systematic errors are difficult**
☹ **matrix effects are not adjustable**
☹ **not for varying samples**

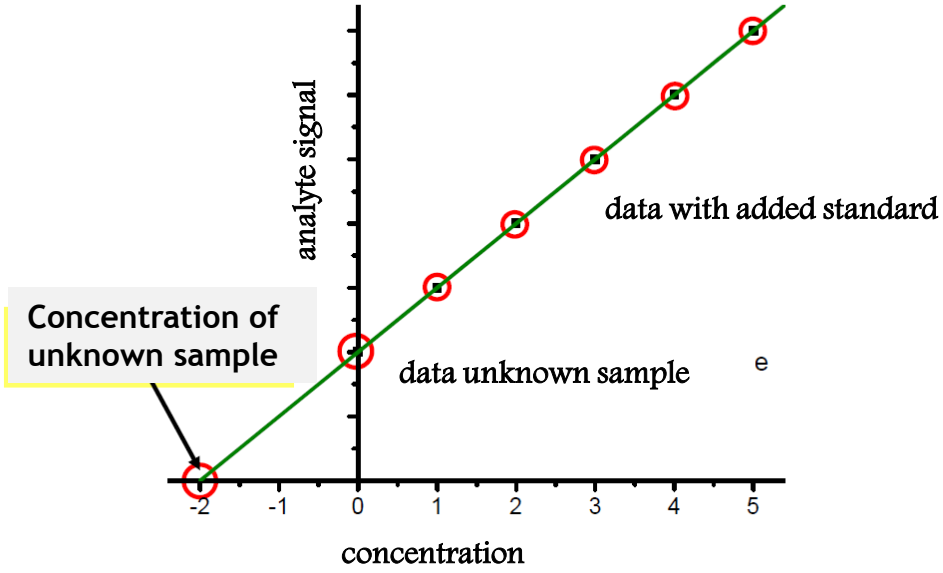
→ **matrix adjustment**

→ **standard addition**

→ **internal standard**

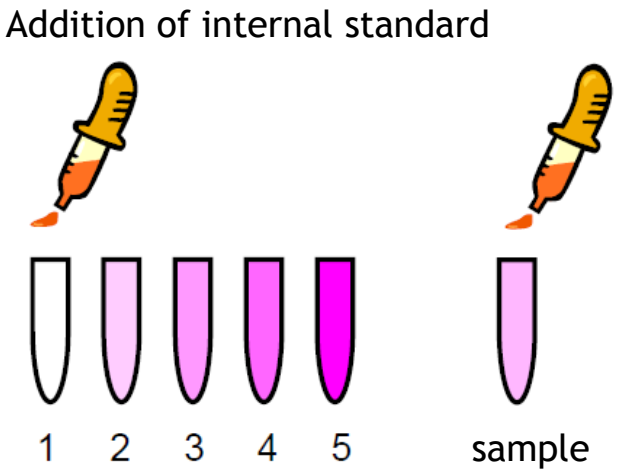
1.4 CALIBRATION

STANDARD ADDITION



→ exact adaption of matrices
→ trace analysis
→ validation of method
→ high effort

INTERNAL STANDARD



→ simultaneous determination of internal standard and analyte

→ calibration line related to internal standard

→ recovery rate; correction of the sample amount

2. INSTRUMENTAL EQUIPMENT OF INM

ATOMIC SPECTROMETRY

Atomic absorption spectrometry (**AAS**)
excitation with flame (**F-AAS**)
or graphite furnace (**GF-AAS**)
HR-CS AAS contrAA 700, Analytik Jena AG
modes: F-AAS, GF-AAS,
option for automated solid sampling

source: Analytik Jena

Optical emission spectrometry (**OES**)

Optical emission with induced coupled plasma (**ICP OES**)

ICP OES Ultima 2, Horiba Jobin Yvon

modes: aqueous and organic samples

high salted samples, halogens

source: Horiba JY

2. INSTRUMENTAL EQUIPMENT OF INM

INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

source: Thermo Fisher SCIENTIFIC

HR-SF-ICP-MS

ELEMENT XR, Thermo Fisher SCIENTIFIC

- Sensitivity down to lower ppt-range
- Isotope Determination
- Single Particle Analysis

2. INSTRUMENTAL EQUIPMENT OF INM

ELEMENTARY ANALYSIS AND SAMPLE PREPARATION

CHNOS analysis

MICRO cube and OXY cube

elementar Analysentechnik GmbH

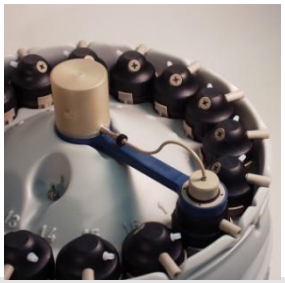
source: elementar

Micro wave for preparation

Multiwave 3000,

Anton Paar GmbH

source: Anton Paar



2. INSTRUMENTAL EQUIPMENT OF INM

CHROMATOGRAPHY

GC/MS (Gas chromatography coupled with mass spectrometry)

GC/MS QP 5050A, GC/MS QP 2010 DI

Autosampler CTC for headspace and direct injection; Shimadzu

GC-FID (flame ionisation detector)

GC-2014, Shimadzu

Direct injection

source: Shimadzu

2. INSTRUMENTAL EQUIPMENT

CHROMATOGRAPHY

LC-ESI-MS (Liquid chromatography-Electrospray Ionization-Mass spectrometry)

HPLC (High Performance Liquid Chromatography)

HPLC including DAD (diode array detector)

RID (refractive index detector)

MSD (mass spectrometer)

LC 1260 Infinity with MSD SL, Agilent

source: Agilent

2. INSTRUMENTAL EQUIPMENT

LC-ESI-QUADRUPOL-TIME-OF-FLIGHT -MASS SPECTROMETRY

source: Agilent

LC-HR-ESI-Q-TOF-MS
6545, Agilent

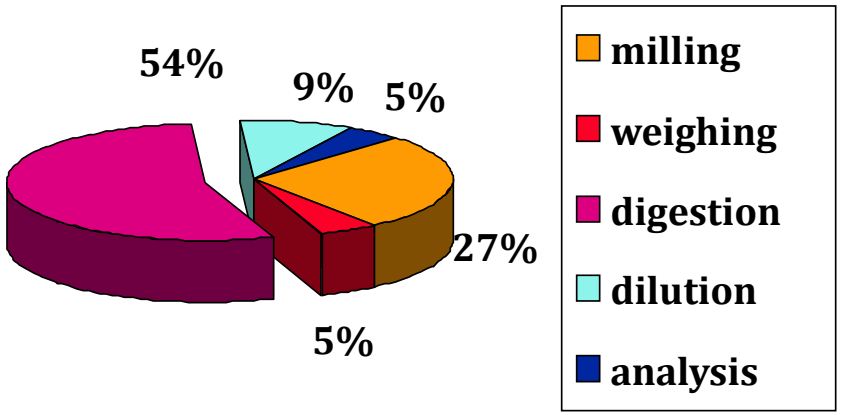
- Improved sensitivity for small molecules and fragile compounds
- Mass accuracy up to 0.8 ppm
- Structure elucidation by auto MS/MS mode

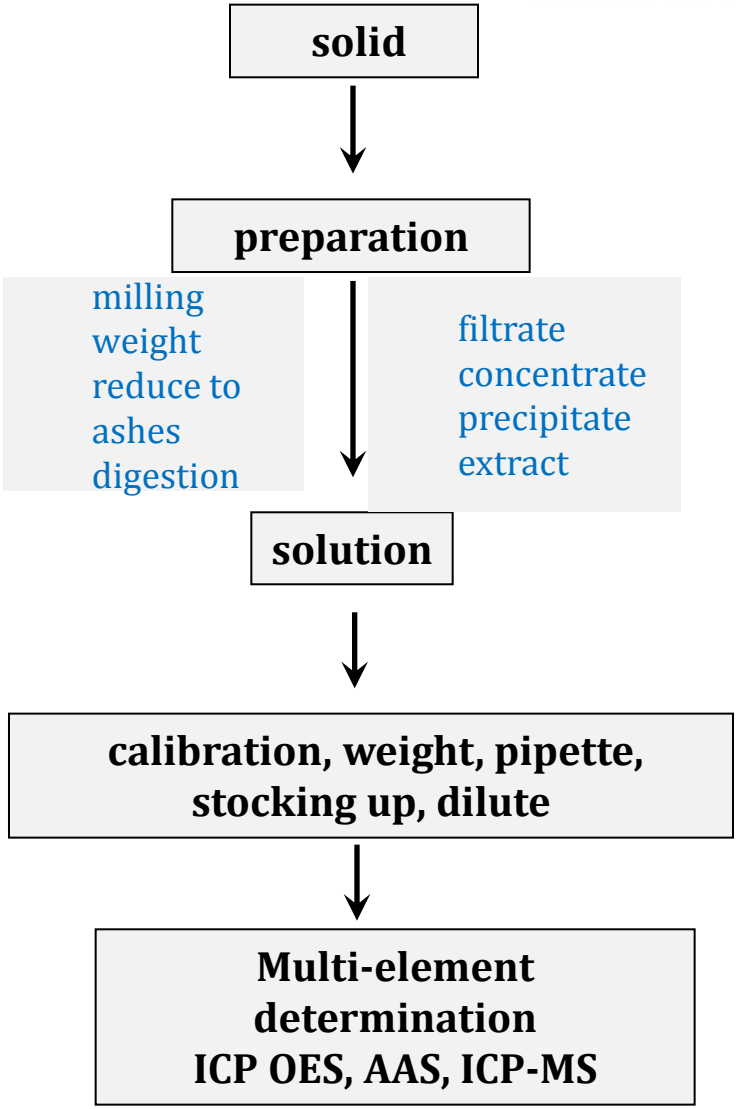
3. SAMPLE PREPARATION

3.1 INTRODUCTION

Wet chemical digestion techniques

- complete solution of analyte
- complete decomposition of matrix
- to avoid loss and contamination
- reduction of handling and process times
- save, reproducible, easy, little manual effort
- economical aspect of sample preparation!





3. SAMPLE PREPARATION

3.2 DIGESTION TECHNIQUE

Conventional methods

Digestion vessels from Berghof

inorganic chemistry

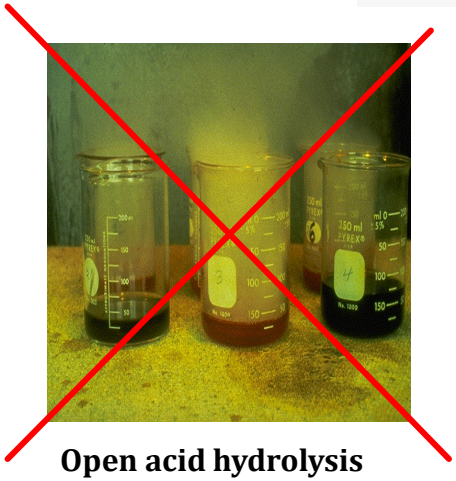
digestion vessel
muffle furnace
hot plate
laboratory sand-bath
Bunsen burner
fusion melt

organic chemistry

Soxhlet extraction
ultrasonic

Digesdahl digestion

Microwave



Open acid hydrolysis

Fusion melt at platinum vessel

Soxhlet extraction

→ As much as necessary, as few as possible !

3. SAMPLE PREPARATION

3.2 DIGESTION TECHNIQUE

Reaction equations

➢ **biologic samples / organic substances**

$(CH_2)_n + 2\ HNO_3 + \Delta\ h \rightarrow CO_2 + 2\ NO + 2H_2O$

➢ **metals**

$6\ H^+ + 3\ Me + 2\ HNO_3 + \Delta\ h \rightarrow 3\ Me^{2+} + 2\ NO + 4H_2O$

➢ **geological samples**

$SiO_2 + 4\ HF + \Delta\ h \rightarrow SiF_4 + 2H_2O$

→ As higher sample weight as greater gas volume and the resulting pressure

Rule of thumb kinetics:

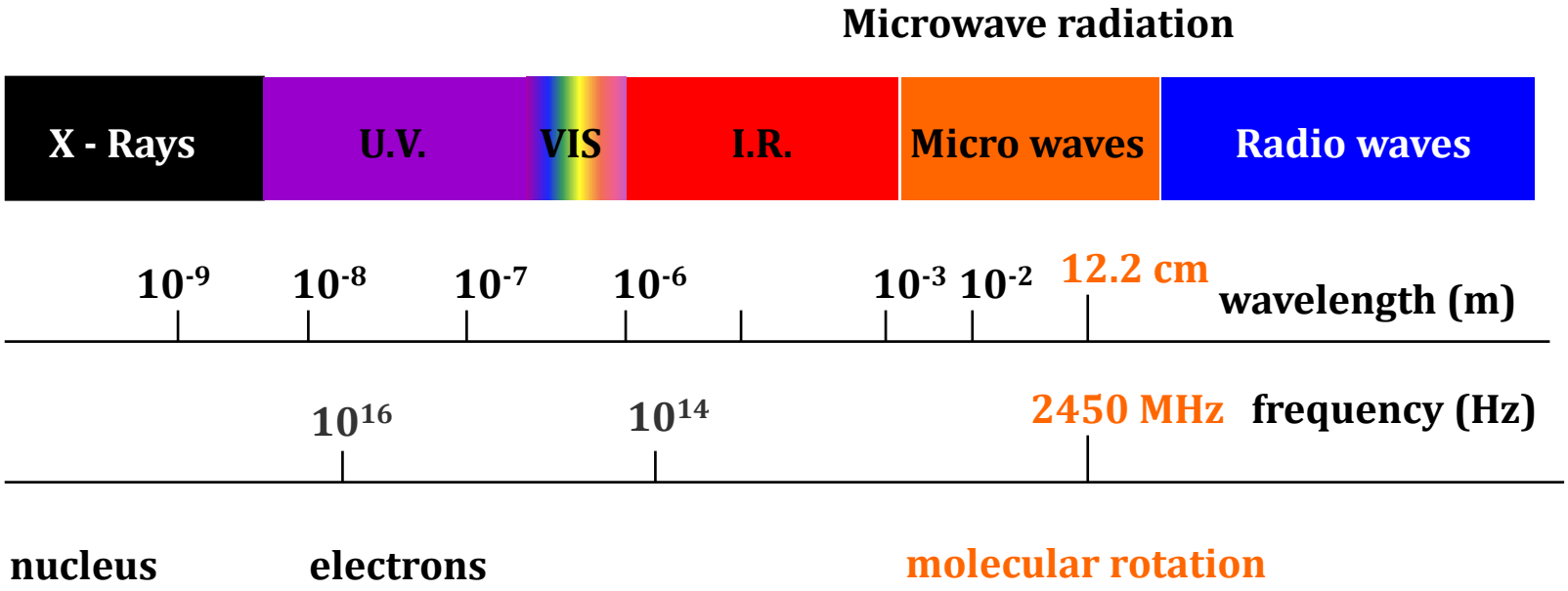
➢ *Increasing of temperature at about 10° C doubled reaction rate*

➢ *Maximum of temperature are limited by boiling points of used acids*

➢ *Reaction in digestion vessels enables higher temperatures*

3. SAMPLE PREPARATION

3.3 MICROWAVE



Examples for

ionisation energy	13 eV
OH bond	5 eV
H_2 bond	2 eV
van der Waals linkage	0,1 eV

$E = h \cdot \nu \rightarrow 10^{-6}$ up to 10^{-3} eV

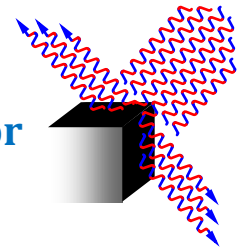
→ No changing of structure!

3. SAMPLE PREPARATION

3.2 MICROWAVE

Interaction material-microwave

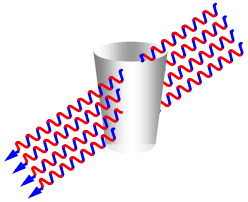
conductor



reflection

no heating

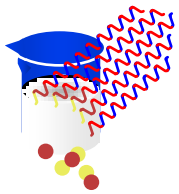
isolator



transparency

no heating

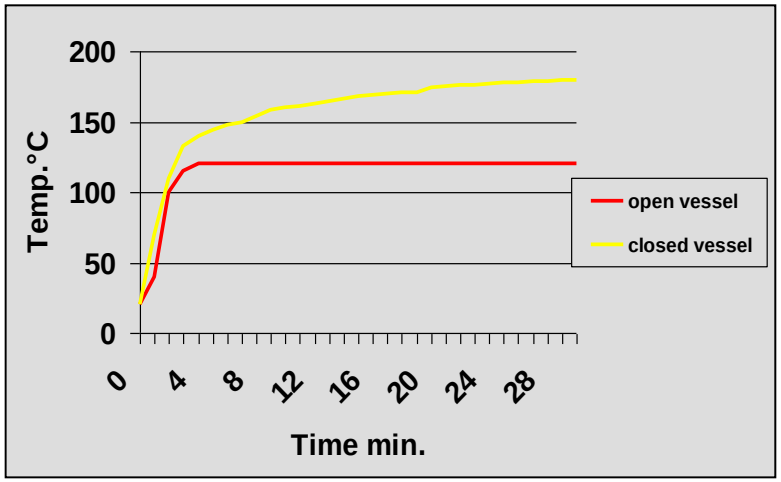
dielectric

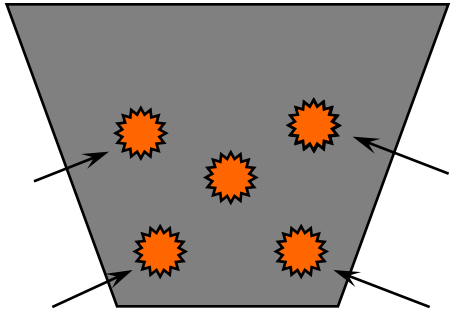


absorption

heating effect

Super-heating effect





absorption of energy from solution

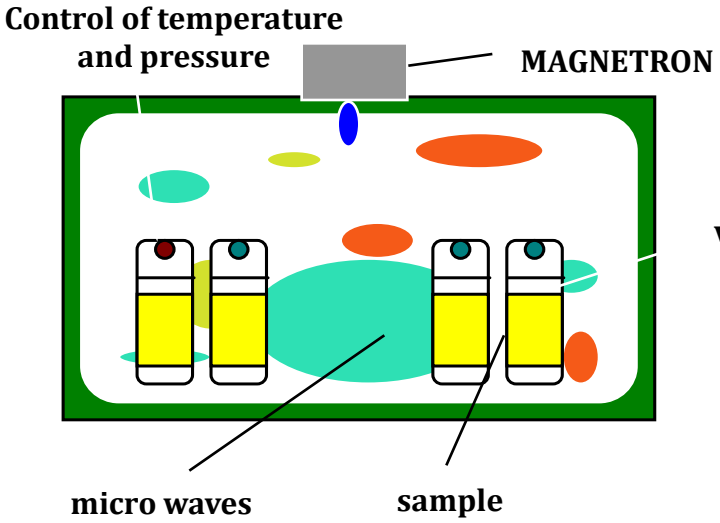
focussed super-heating-effect

conversion of microwave energy into thermal energy

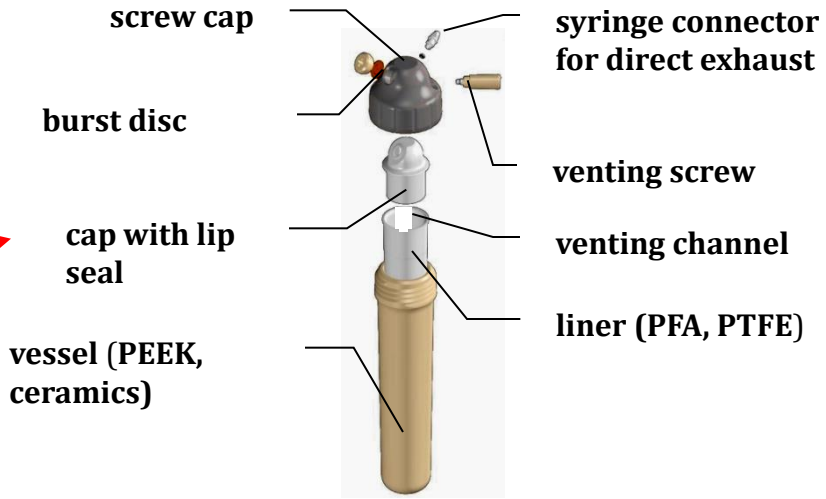
3. SAMPLE PREPARATION

3.3 MICROWAVE

Closed microwave furnace



vessel



Disadvantages

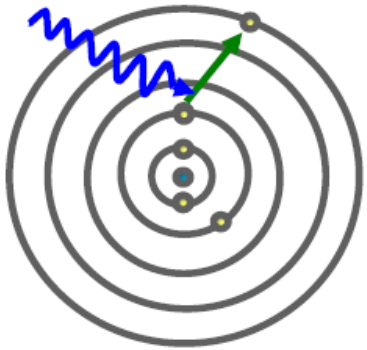
- weighted sample depends on reaction pressure
- problems by pressure spikes
- time-intensive multi-step reactions

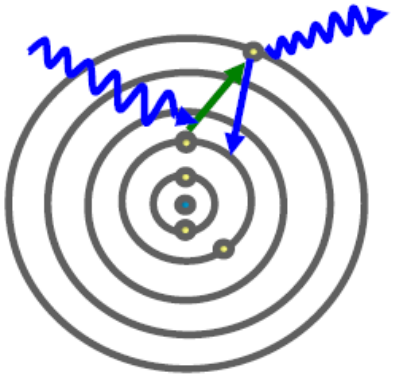
Advantages

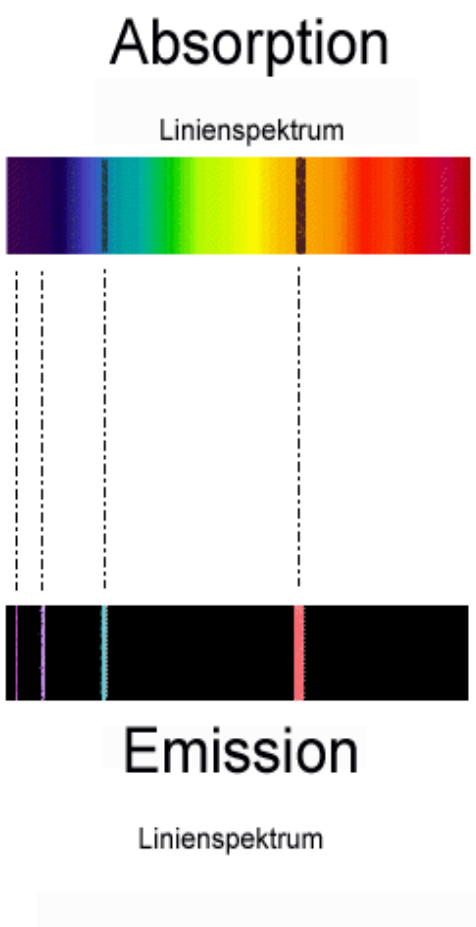
- high temperatures (260°C) and pressures (60-80 bar, max 120 bar) possible
- reduced sample preparation
- contamination risk is minimised
- small consumption of chemicals
- digestion of 8 or more samples side by side
- Use of basic acids and/or acid mixtures
- temperature / pressure control

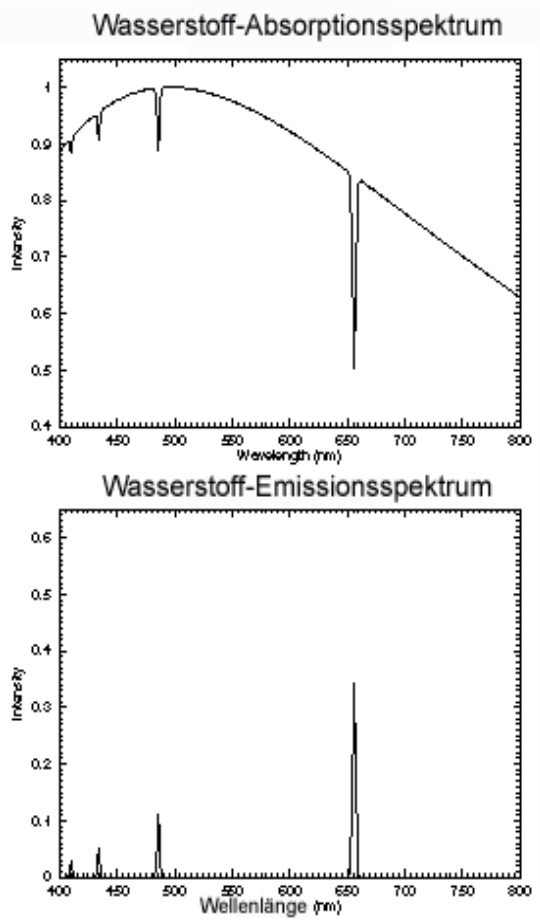
4. ATOMIC SPECTROMETRY

4.1 ATOMIC ABSORPTION, ATOMIC EMISSION





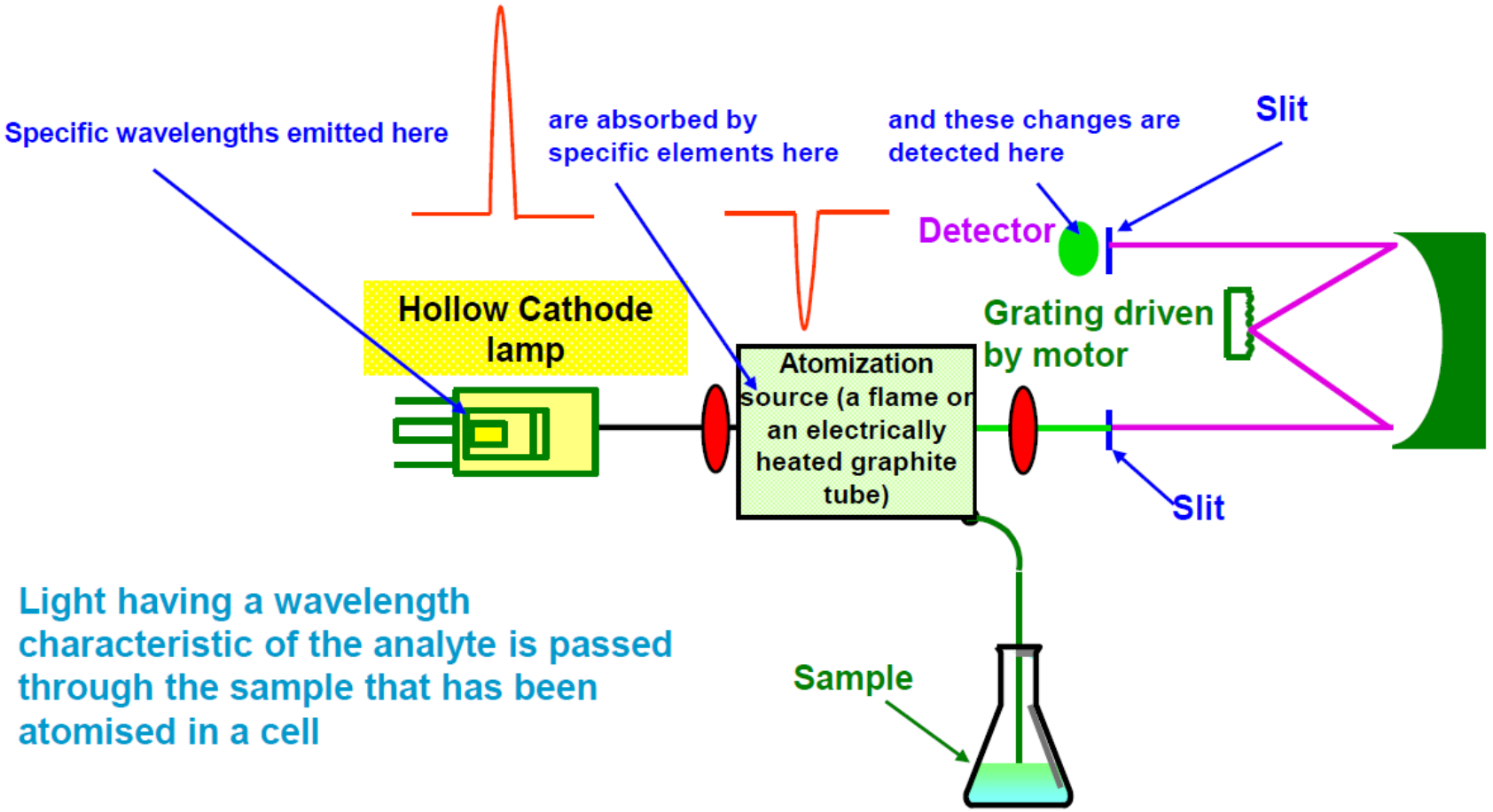




→ Kirchhoff's radiation law: **λ (absorption) = λ (emission)**

4. ATOMIC SPECTROMETRY

4.2 AAS - ATOMIC ABSORPTION SPECTROMETRY



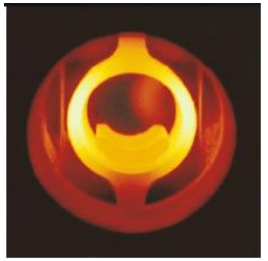
→ **The amount of light absorbed is proportional to concentration**

4. ATOMIC SPECTROMETRY

4.2 AAS: CONTRAA 700, ANALYTIK JENA

- liquid (F-AAS, atomisation with flame and GF-AAS, atomisation with graphite furnace) and solid samples (only GF-AAS)
- range: mg/l (F-AAS) to ng/l (GF-AAS)

atomiser:
flame, graphite furnace

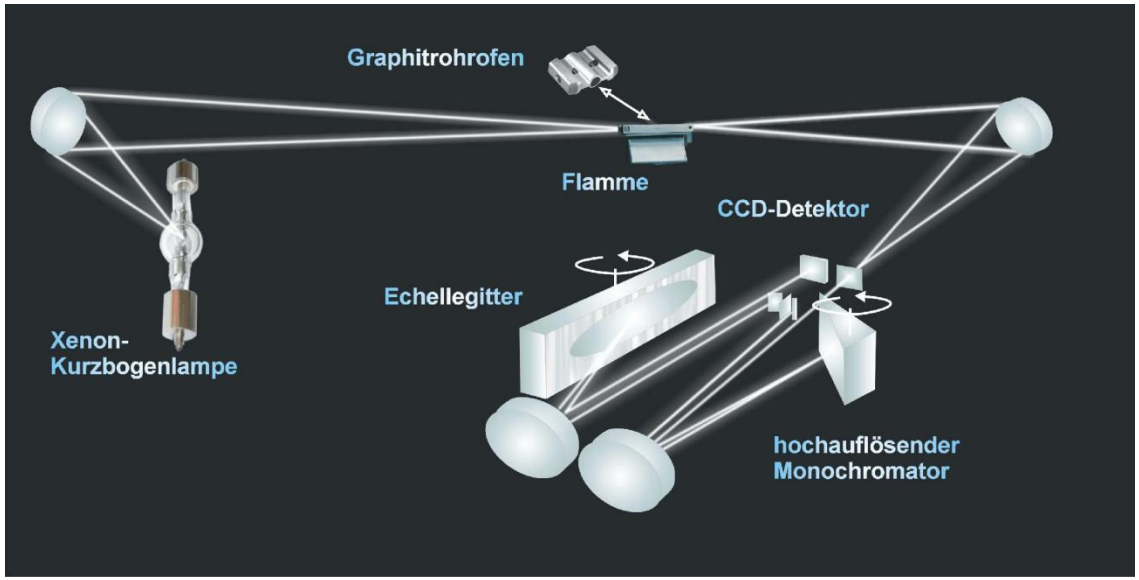


✓ fast and simple change of the modes

✓ Xenon continuum source (185-900 nm)

✓ high resolution Echelle-monochromator

✓ CCD chip detector



source: AnalytikJena

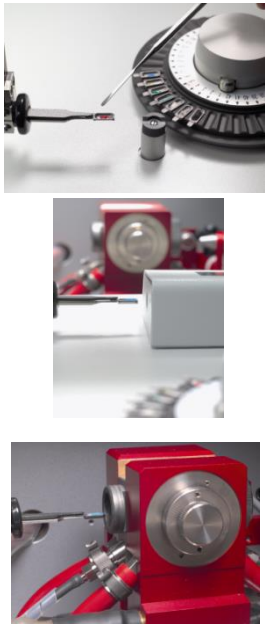
4. ATOMIC SPECTROMETRY

4.2 AAS CONTRAA 700, ANALYTIK JENA

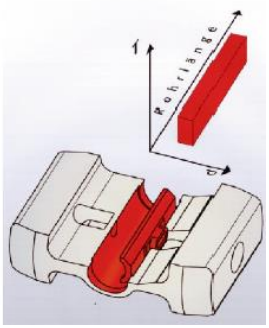
contrAA 700, AnalytikJena

Automated solid-sampling

- Graphite furnace suitable for solid samples
- Simultaneously simple background correction (reference pixel, matrix spectra)
- Range: pg to fg absolutely
- Transversal heated graphite furnace



- ✓ Analysis of original sample
- ✓ No digestion necessary
- ✓ small sample amounts (10-500µg)
- ✓ Decreasing of analytical errors (contamination, blank, dissolution)



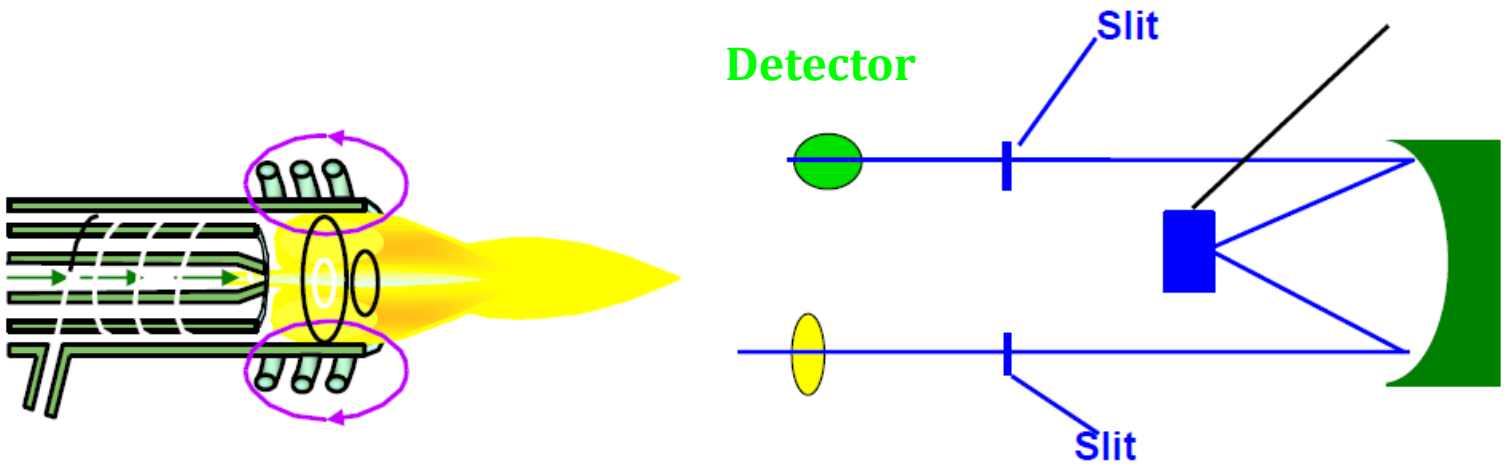
Atomising temperature (°C)		
element	longi-tudinal	trans-versal
Cd	1500	1150
Mn	2400	1600
V	2700	2500

4. ATOMIC SPECTROMETRY

4.3 ICP OES - OPTICAL EMISSION SPECTROMETRY WITH INDUCTIVELY COUPLED PLASMA

Energy from the plasma promotes an electron to a higher energy level (excitation). Electron falls back and emits light at a characteristic wavelength

Grating driven by motor allows users to scan through the wavelengths

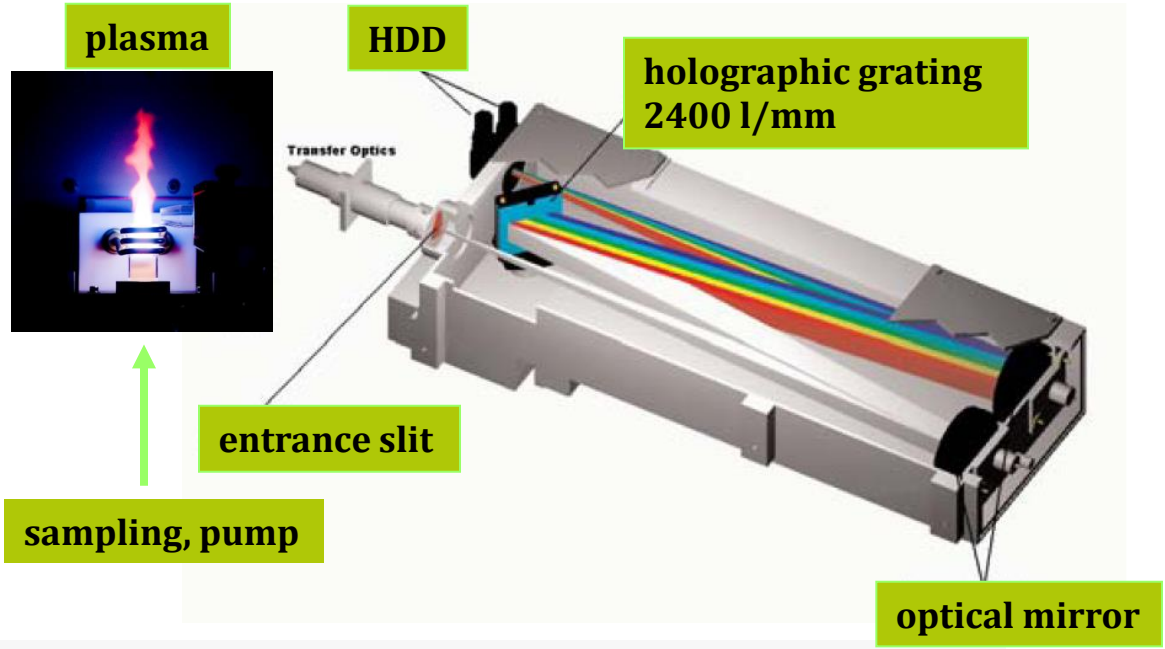


→ Light emission is proportional to concentration

4. ATOMIC SPECTROMETRY

4.3 ICP OES ULTIMA 2, HORIBA

- all elements except C,H,N,O and noble gases
- liquid samples
- organic solutions, high saline solutions
- blank solution necessary
- sub µg/l to mg/l (from sub ppb to ppm)



✓ radial plasma

✓ spectral range: 120-800 nm, resolution 5 pm

✓ simple optical configuration (Czerny Turner)

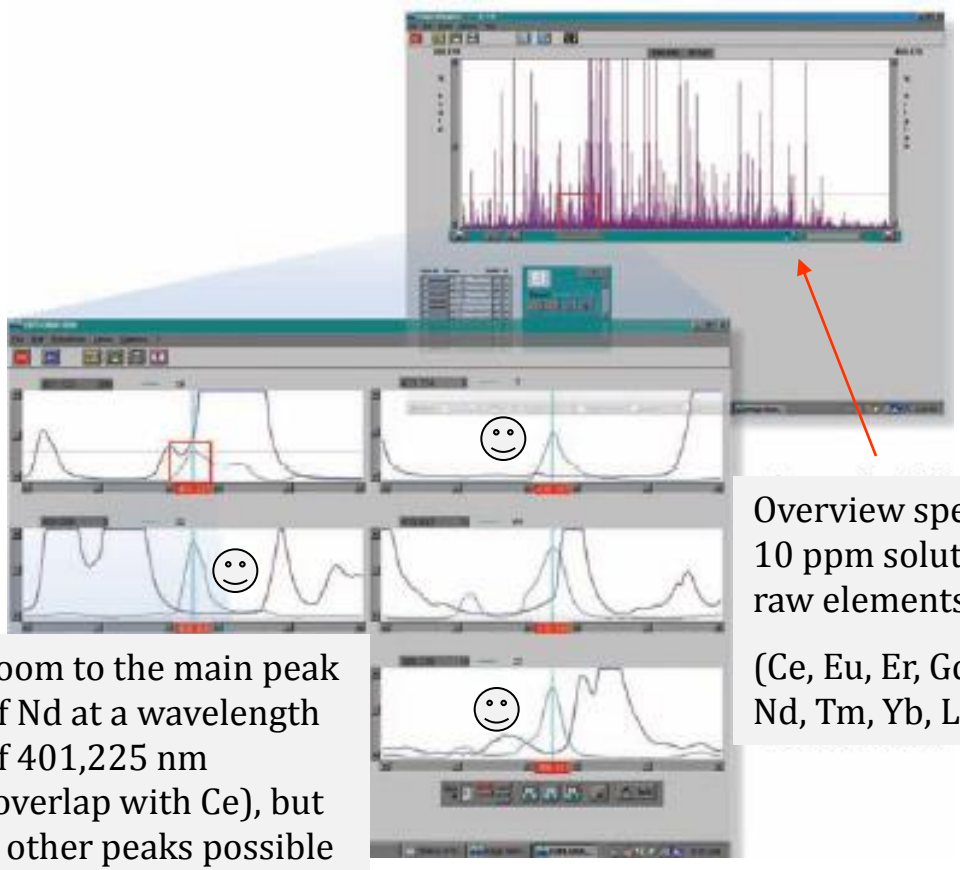
✓ different kind of nebuliser (Meinhard, Miramist, Cross flow, Ringspalt) + -chamber (Scott..)

✓ Win Image

4. ATOMIC SPECTROMETRY

4.3 ICP OES ULTIMA2, HORIBA

ICP OES Win Image-Navigator



zoom to the main peak of Nd at a wavelength of 401,225 nm (overlap with Ce), but 3 other peaks possible (resolution 10 pm)

Overview spectra of 10 ppm solution of raw elements

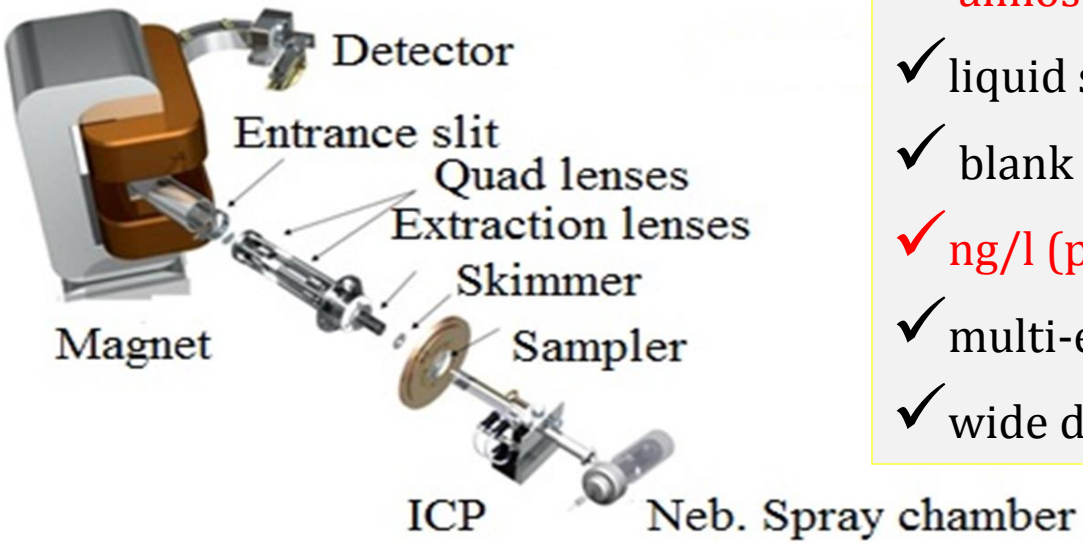
(Ce, Eu, Er, Gd, Ho, Lu, Nd, Tm, Yb, Lu)

Advantages:

- ✓ increasing of capacity
- ✓ half quantitative analysis of almost all elements within a few minutes
- ✓ combination of HDD and Win Image-software
- ✓ combination of high dynamic range (10^9) and fast spectra recording (2-3min, resolution 10 pm)
- ✓ main elements and traces in the same measuring procedure

4. ATOMIC SPECTROMETRY

4.4 HR SF ICP MS



- ✓ almost all elements of periodic table
- ✓ liquid samples
- ✓ blank necessary
- ✓ ng/l (ppt) down to pg/l (ppq) range
- ✓ multi-element analysis
- ✓ wide dynamic range (6-8 decades)

Advantages:

→ isotopic distribution

→ detection of usually hard separated elements showing strong interference with matrix ions

→ Single particle analysis

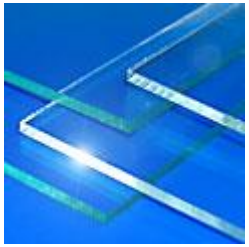
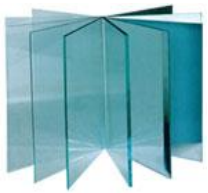
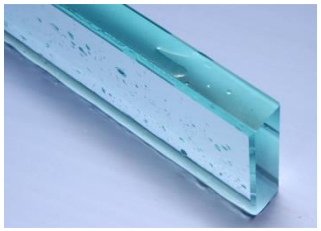
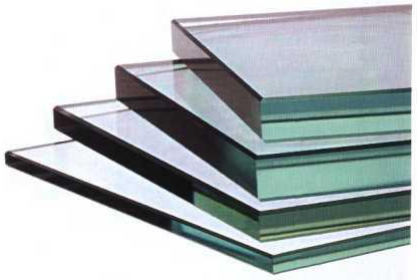
4. ATOMIC SPECTROMETRY

4.5 EXAMPLE ICP OES

Quantitative analysis of float glasses different producers with ICP OES

HF/HNO_3 digestion, Mira Mist nebuliser, Zyklon chamber, power 1000 W, flow 12 l · min^{-1}, pressure 3 bar

wt.-%	SiO_2	Na_2O	K_2O	CaO	MgO	Al_2O_3	Fe_2O_3
INM	69,95 ±0,41	10,15 ±0,07	0,306 ±0,004	12,99 ±0,009	5,76 ±0,07	0,687 ±0,006	**0,147 ±0,003**
Schott	69,59 ±0,39	10,92 ±0,07	0,186 ±0,001	13,28 ±0,05	5,98 ±0,01	0,711 ±0,004	**0,333 ±0,001**
Sekurit	76,22 ±0,77	11,85 ±0,10	0,275 ±0,001	6,544 ±0,006	3,737 ±0,025	1,001 ±0,014	**1,212 ±0,001**
Pilking-ton	76,45 ±1,10	11,91 ±0,10	0,284 ±0,003	7,626 ±0,020	3,349 ±0,033	0,889 ±0,005	**0,992 ±0,012**

4. ATOMIC SPECTROMETRY

4.5 EXAMPLE GF AAS

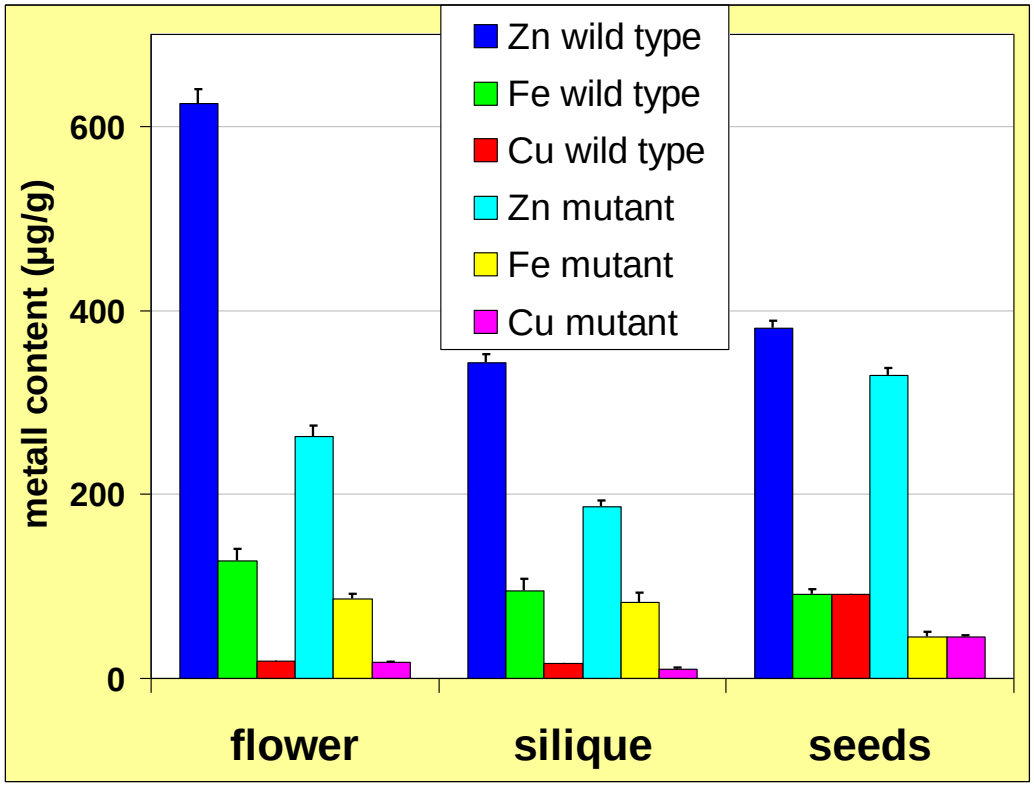
Round-robin test for establishment of direct solid-sampling GF-AAS determination of Pb, Cd and Cu in lichen, herring gull egg and sediment

Material	Pb (mg / kg)		Cd (mg / kg)		Cu (mg / kg)	
lichen	12,52 ± 1,03	✓	0,422 ± 0,022	✓	5,556 ± 0,425	✓
herring gull egg	-	✓	-	✓	2,196 ± 0,151	✓
sediment	82,90 ± 6,44	✓	0,534 ± 0,025	✓	64,54 ± 5,56	✓

4. ATOMIC SPECTROMETRY

4.5 EXAMPLE GF AAS

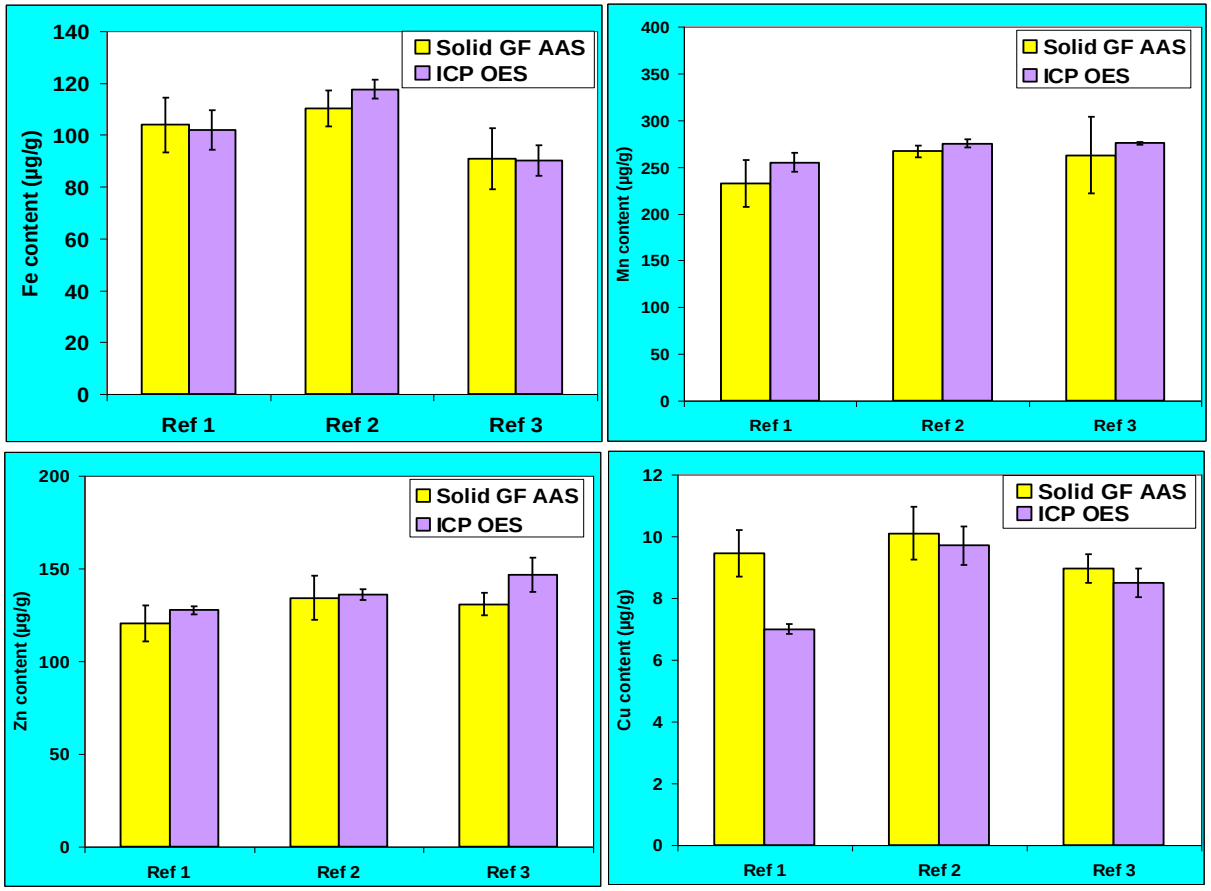
Analysis of metal content in Arabidopsis thaliana
Solid-sampling GF-AAS, Zn, Fe, Cu, N=6
weighted sample: 50-100µg

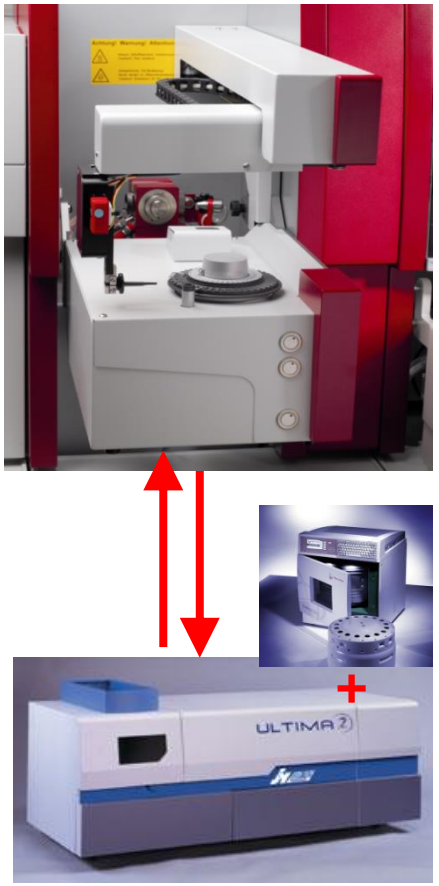


4. ATOMIC SPECTROMETRY

4.5 EXAMPLE GFAAS, ICP OES

Comparison of solid-sampling GF-AAS and ICP OES after Micro wave digestion (50 mg, 4 ml HNO_3, 2 ml H_2O_2, 30min, 1200 W), 3 plants



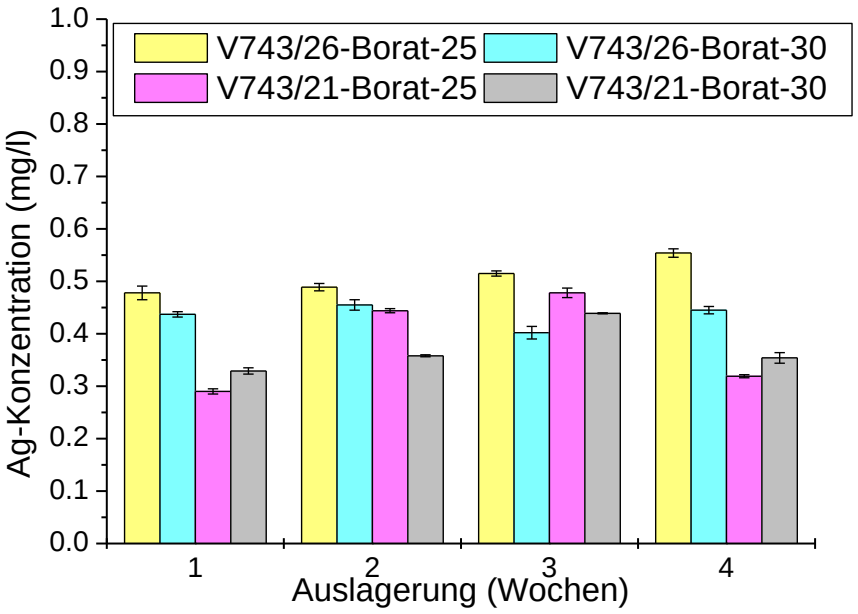


4. ATOMIC SPECTROMETRY

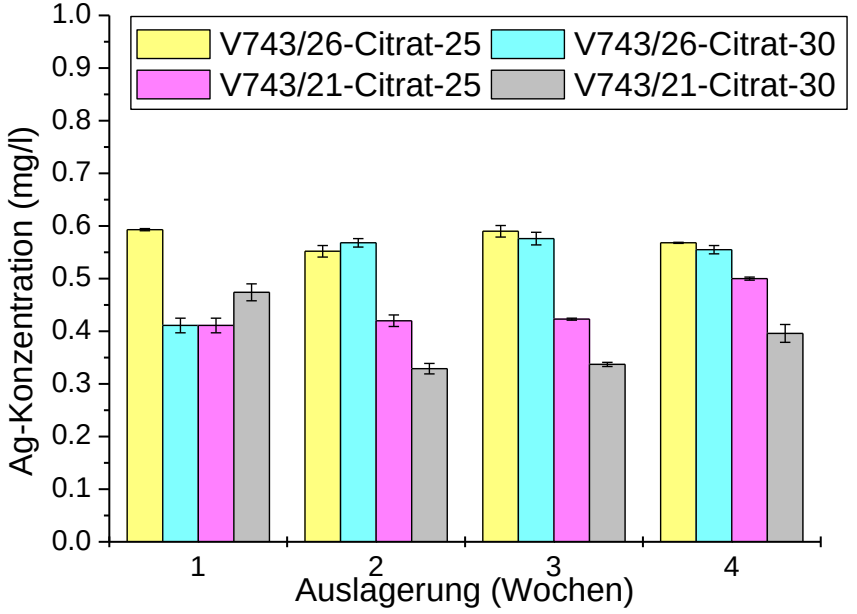
4.5 EXAMPLE ICP OES

Antimicrobial coatings with nano-silver for eye drop flasks
Leaching of coatings in borate- or citrate buffer solutions, determination of Ag content by **ICP OES**

borate buffer



citrate buffer



4. ATOMIC SPECTROMETRY

4.5 EXAMPLE GFAAS, ICP OES

Analysis of silver content in cosmetics and household articles different suppliers

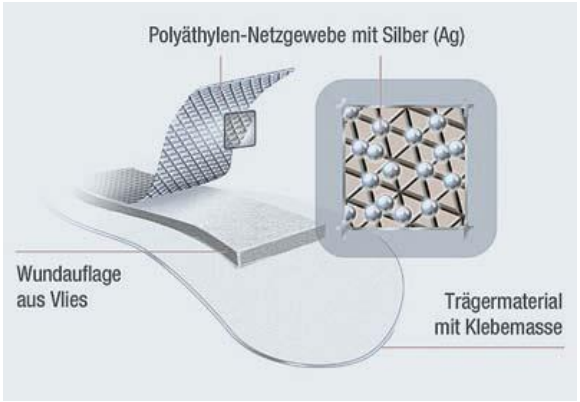
Digestion with HNO_3 und H_2O_2 in micro wave, comparison of **GF AAS** and **ICP OES** measurements

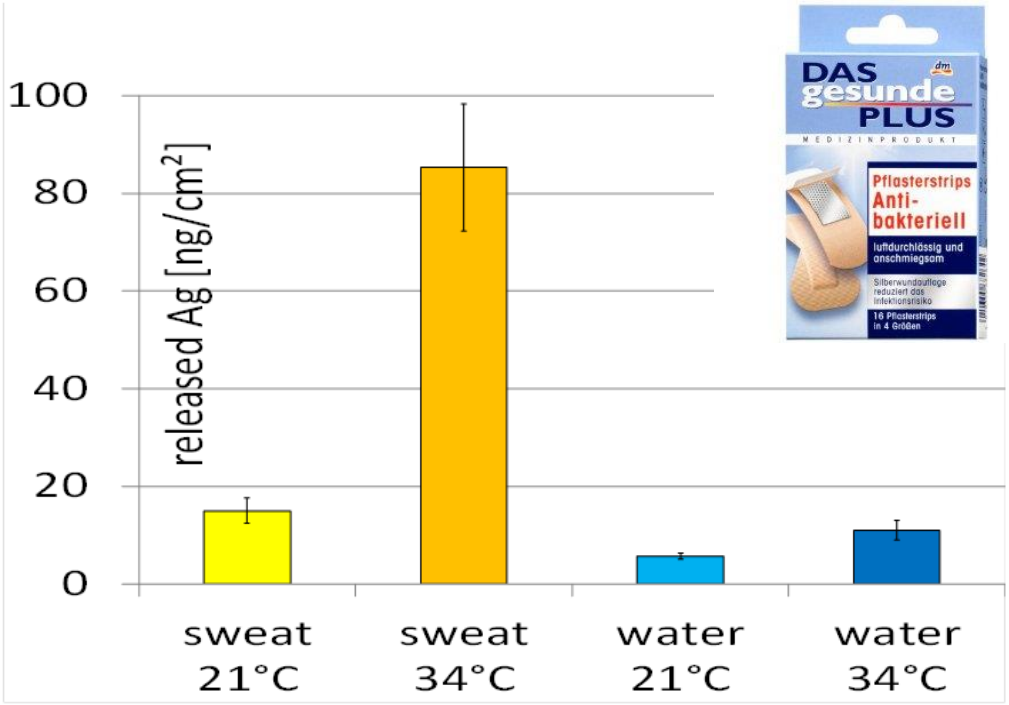
Ag-Konzentration	GFAAS	ICP OES
Nivea men aftershave lotion silver protect	1,937 ± 0,071 mg/100ml	1,95 ± 0,3 mg/100ml
Nivea man aftershave lotion	< LOD	< LOD
Nivea deo for men silver protect	39,61± 0,28 mg/100ml	60,15 ± 0,5 mg/100ml
DM Pflasterstrips antibakteriell	0,994 ± 0,023 µg/cm2	0,923 ± 0,119 µg/cm2
DM Pflasterstrips	0,013 ± 0,004 µg/cm2	0,0133 ± 0,062 µg/cm2
Rossmann Nanosilber Universaltuch	0,518 ± 0,017 µg/cm2	0,517 ± 0,133 µg/cm2
DM Spül- und Wischtuch Nanosilber	0,766 ± 0,012 µg/cm2	0,8568 ± 0,157 µg/cm2
DM Spül- und Wischtuch	0,003 ± 0,0001 µg/cm2	< LOD
Isana men deo roll-on silver	25,91 ± 1,2 µg/100g	< LOD
Isana men deo roll-on	< LOD	< LOD
SOS Microsilbercreme	108,7 ± 0,76 mg/100g	109,39 ± 2,69 mg/100g

4. ATOMIC SPECTROMETRY

4.5 EXAMPLE GFAAS

Silver release of antibacterial band aid with GF-AAS



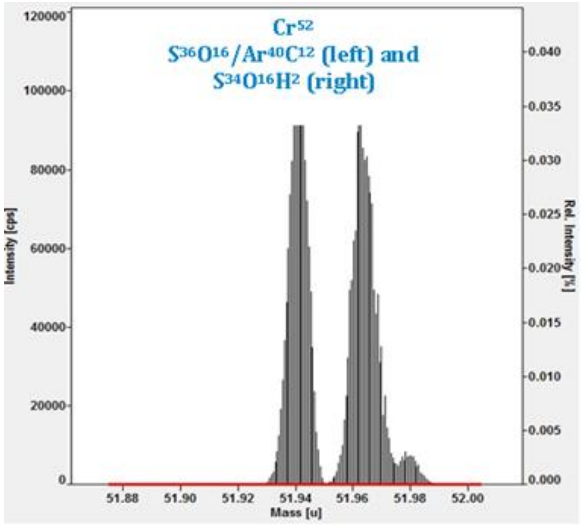


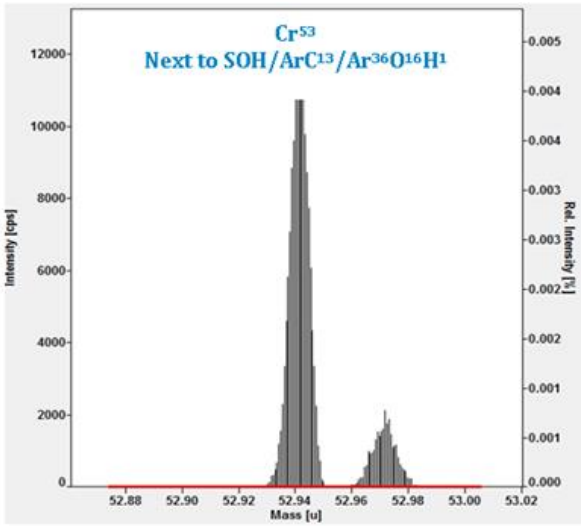
Amount of silver released from band-aid (anti-bacterial, 5 cm^2) into 10 ml artificial sweat or water (24 h incubation).
Total silver content was determined with ICP-OES after microwave assisted acid digestion and amounts to 4.6 µg/cm^2 , this equates to 368 µg/g band-aid.

4. ATOMIC SPECTROMETRY

4.5 EXAMPLE ICP-MS

Cr-Analysis of stainless steel after digestion in sulfuric acid with ICP-MS



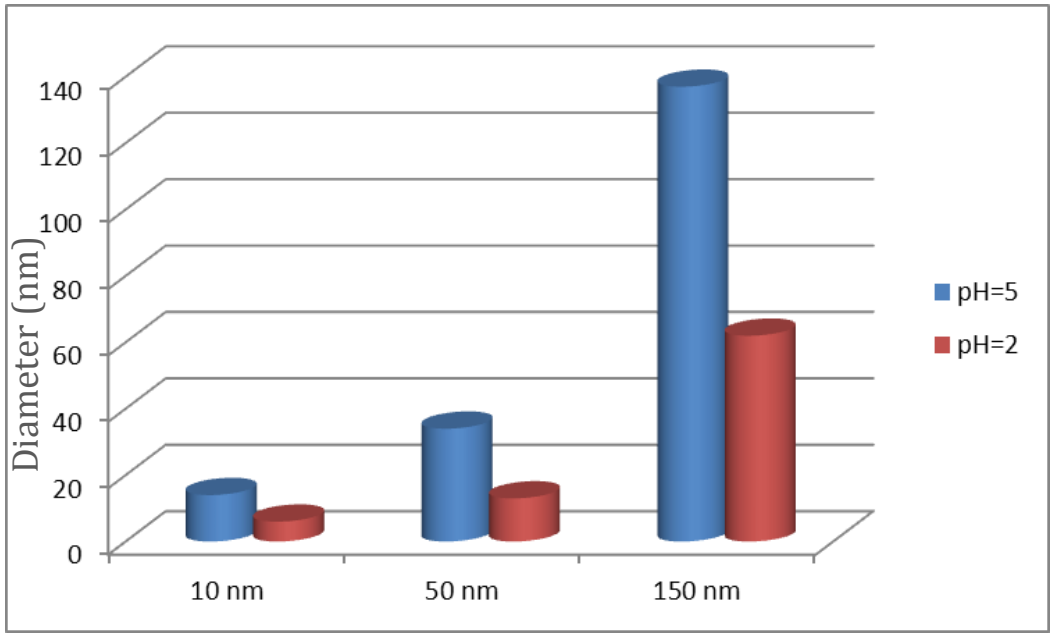


Medium resolution (MR) mass spectra (Resolution 4000) of Cr^{52} (83.8 % isotopic abundance) and Cr^{53} (9.5 %) in 10% H_2SO_4

4. ATOMIC SPECTROMETRY

4.5 EXAMPLE ICP-MS

Dissolution of commercial Au-NPs under acidic conditions analyzed with SP ICP-MS

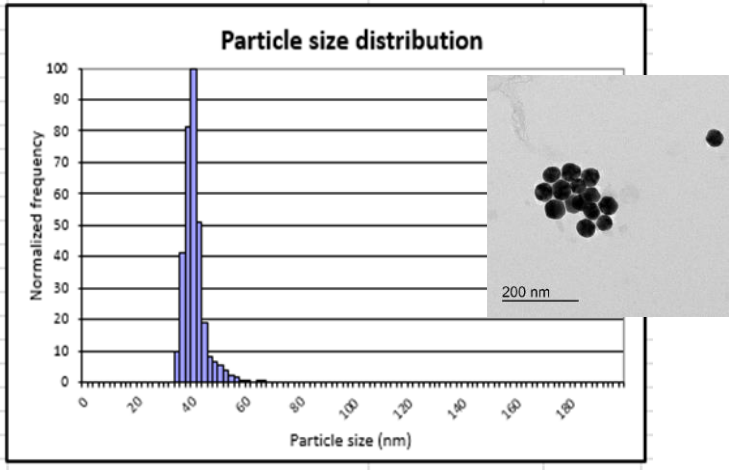


Decrease of diameter of Au-NPs depending on pH values.

Example: 50 nm, pH 5.5

INSTRUMENT SETTINGS AND CALIBRATION DATA		
Instrument settings:		
ICPMS sample flow	0.2	ml/min
ICPMS dwell time	3	ms
Properties of target particle:		
elemental composition of target particle	Au	
molar mass particle/molar mass analyte	1	
particle density	19.3	g/ml
Calibration data:		
transport efficiency	0.007	
ICPMS response ion standard	61855	cps/(µg/L)
limit for particle detection	319679	cps
number of particles detected	1571	

Results		
Particle concentration (number-based)	1.2E+09	particles/L
Particle concentration (mass-based)	695	ng/L
Particle size	39	nm
Ionic concentration	<1200	ng/L

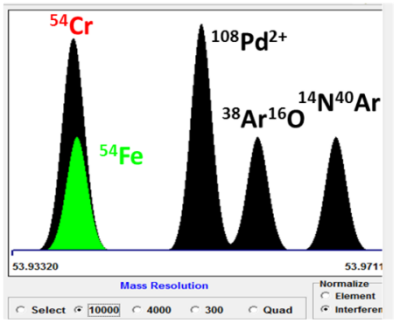


4. ATOMIC SPECTROMETRY

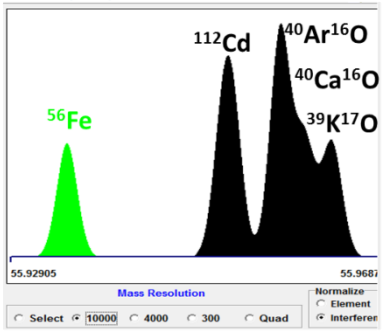
4.5 EXAMPLE ICP-MS

Fe quantification in Mouse oocytes at trace levels, Method validation (HR-SF ICP-MS)

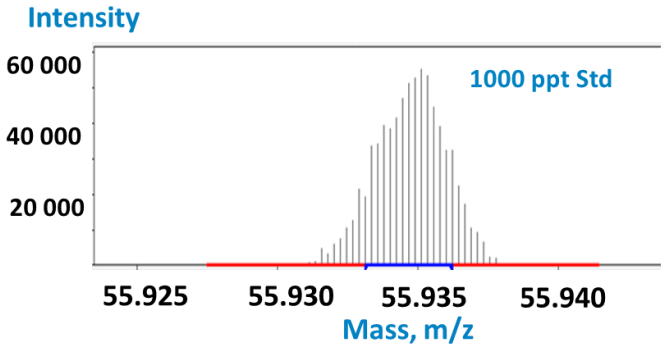
^{54}Fe (HR), Abundance 5.8 %

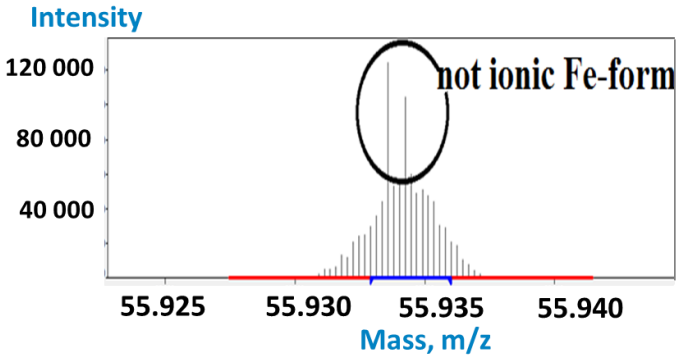


^{56}Fe (HR), Abundance 91.7 %

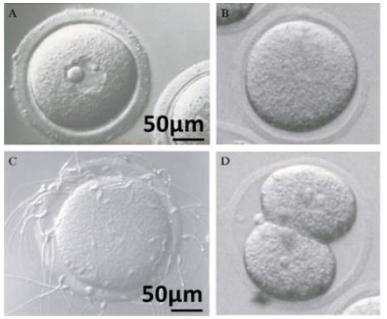


Isotopic interferences in HR mode



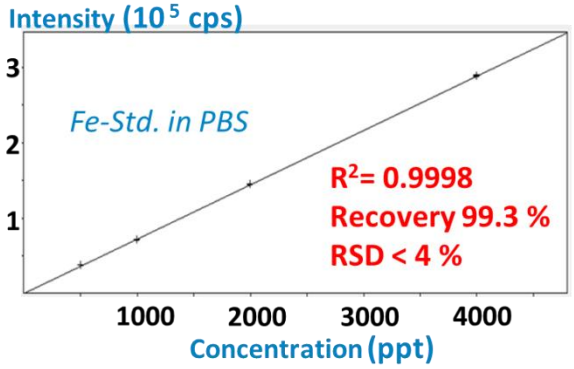


HR-mass spectra obtained for ^{56}Fe: standard solution in PBS-medium (*top*) and intact oocytes (*bottom*)



In vitro fertilisation of mouse oocytes

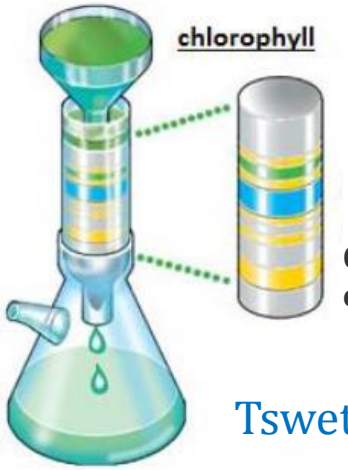
Calibration curve



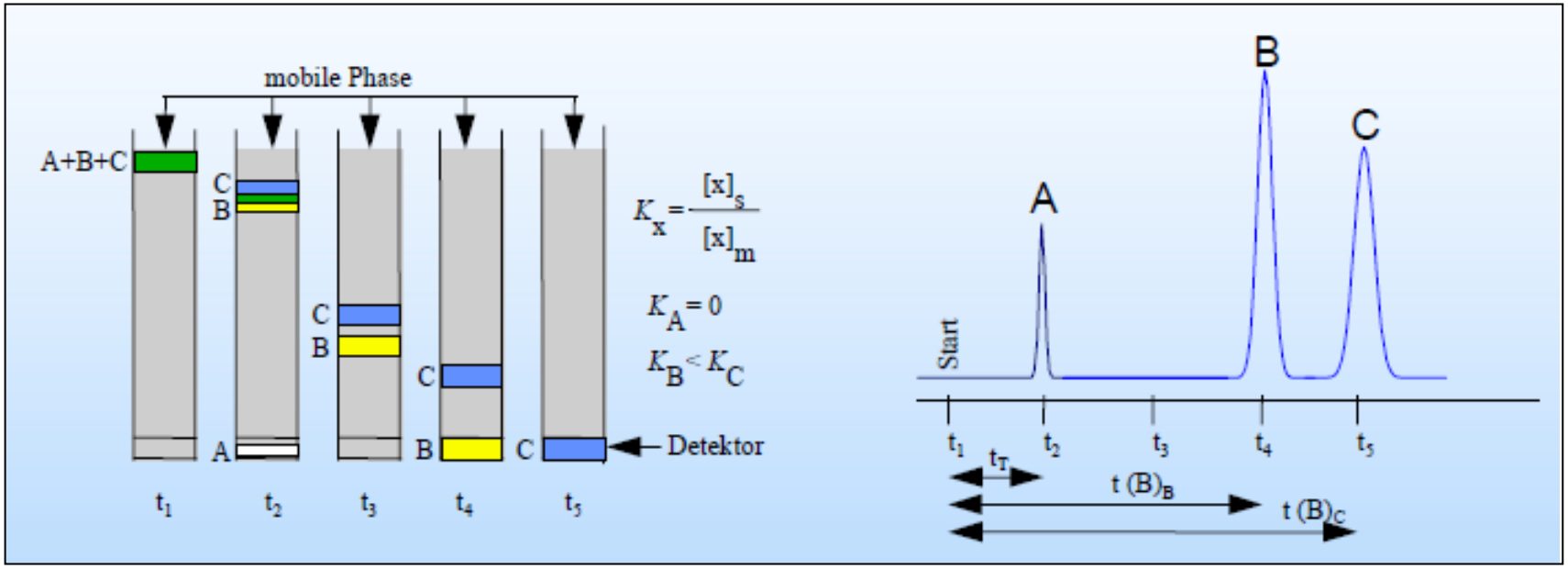
5. CHROMATOGRAPHY

5.1 CHROMATOGRAPHIC SEPARATION

Chromatography: chroma-colour, graphein-write
physico-chemical separation method
Distribution of separated substances between stationary and mobile phase



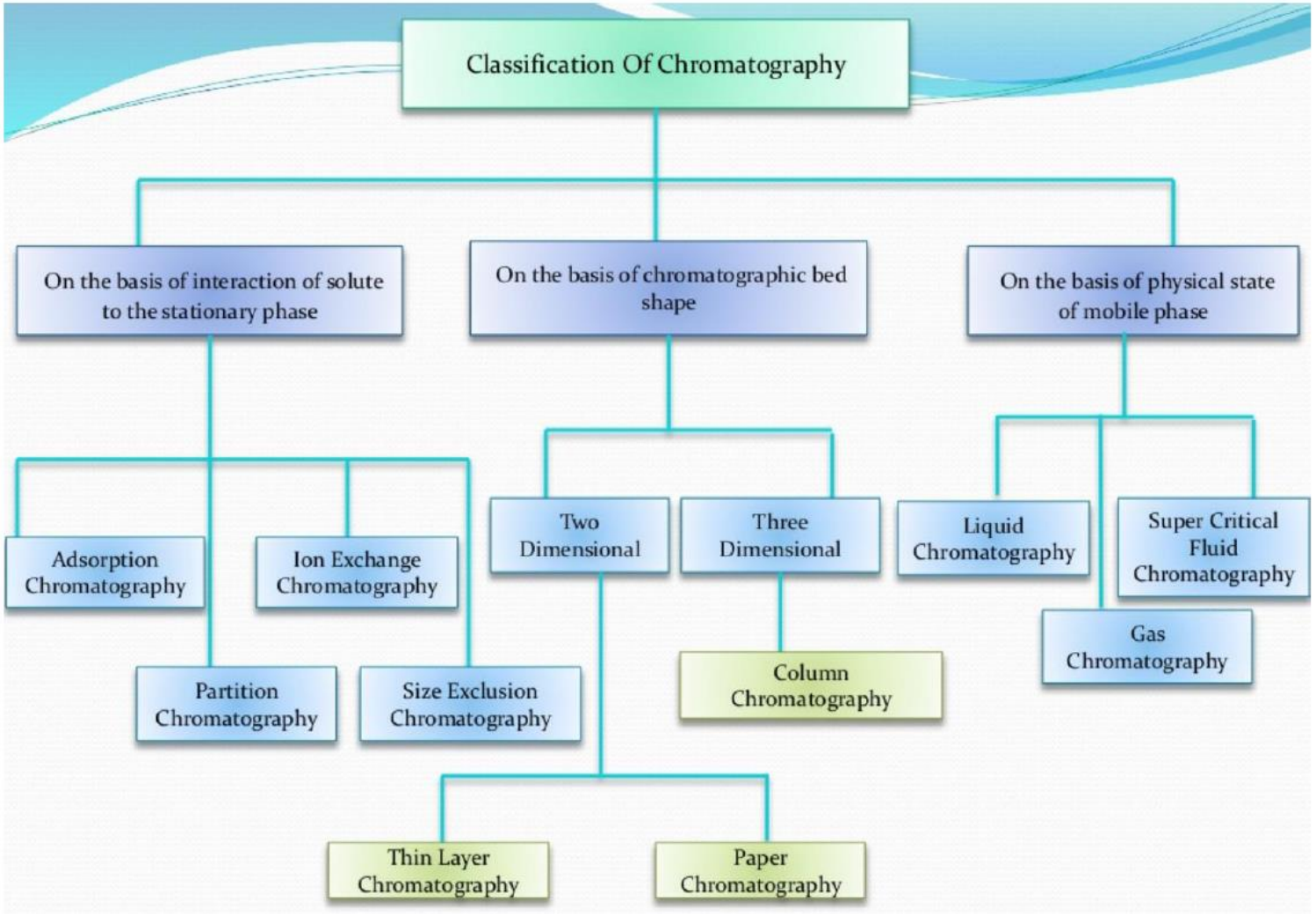
Tswett 1903



→ distribution coefficient K describes relation between concentration of substance x in the mobile and stationary phase at thermodynamic equilibrium

5. CHROMATOGRAPHY

5.1 CLASSIFICATION



5. CHROMAT0GRAPHY

5.1 DIFFERENCES GAS AND LIQUID CHROMATOGRAPHY

- mobile phase gaseous, used as carrier
- sample should be volatile and vaporisable without decomposing
- molar mass < 500 Dalton
- volatile solvent (normally decreasing boiling temperature as sample)
- separation by **boiling temperature and polarity**

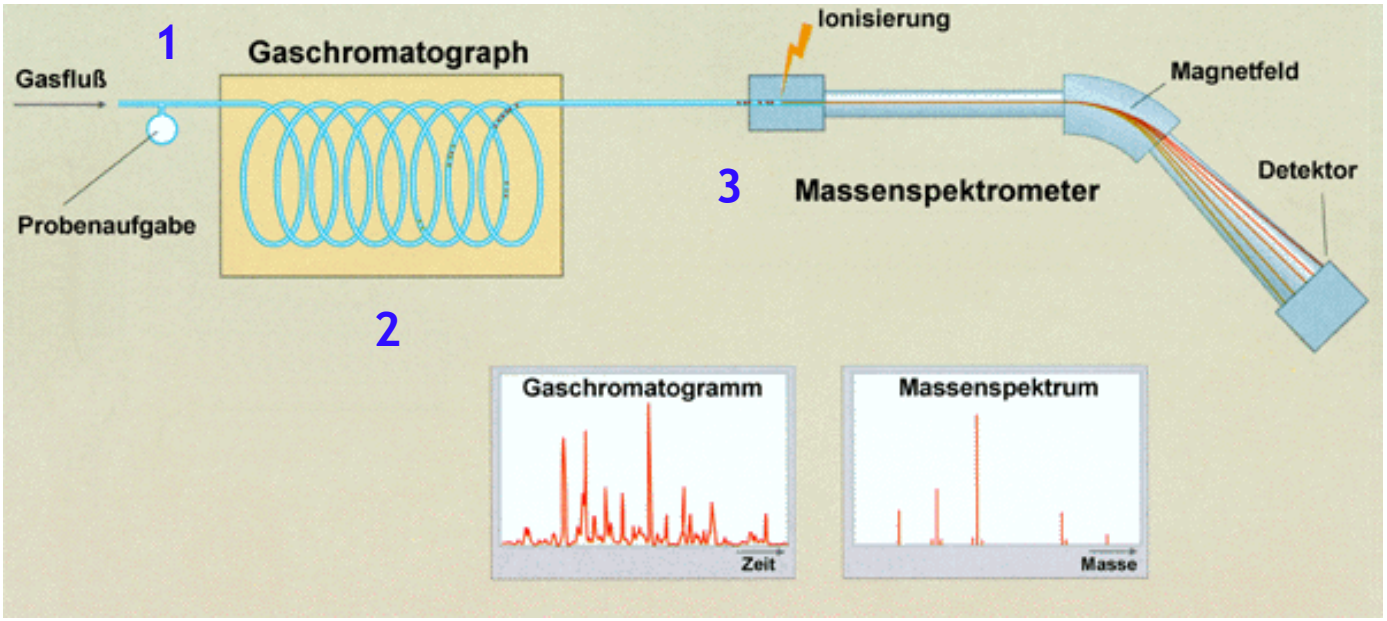
- mobile (liquid) and stationary phases are involved in separation
- no requirement concerning volatility
- must-have: solubility in mobile phase
- no upper limit regarding to molar mass
- analysis at room temperature
- separation by **polarity (Reversed Phase Chromatography) and size (Gel Permeation Chromatography)**

5. CHROMATOGRAPHY

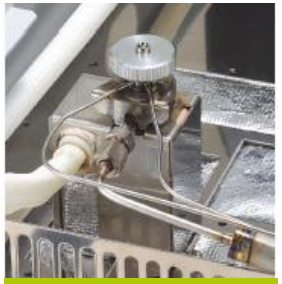
5.2 GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

GC/MS, direct injection

- soluble in organic solvents with preferably low boiling point
- sample volume 0.2 – 5 µl
- range pg (scan) to fg (SIM)
- auto sampler CTC: up to 98 samples, 6 samples for head space and SPME



1



split injector (up to 400 °C)

2

capillary column (30m, 0.25mm ID; 0.25µm film)

3

ion source (70keV)

5. CHROMATOGRAPHY

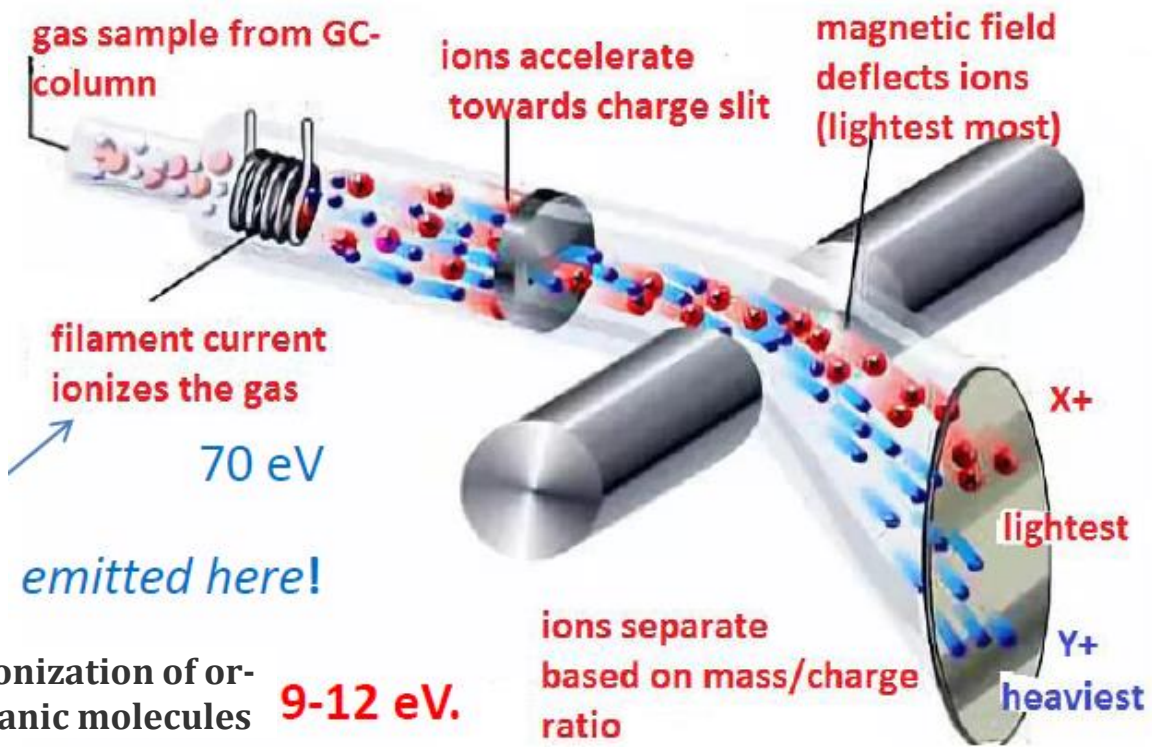
5.2 GC/MS

MS mode: **EI** (Electron Impact Ionization or Electron Ionization)

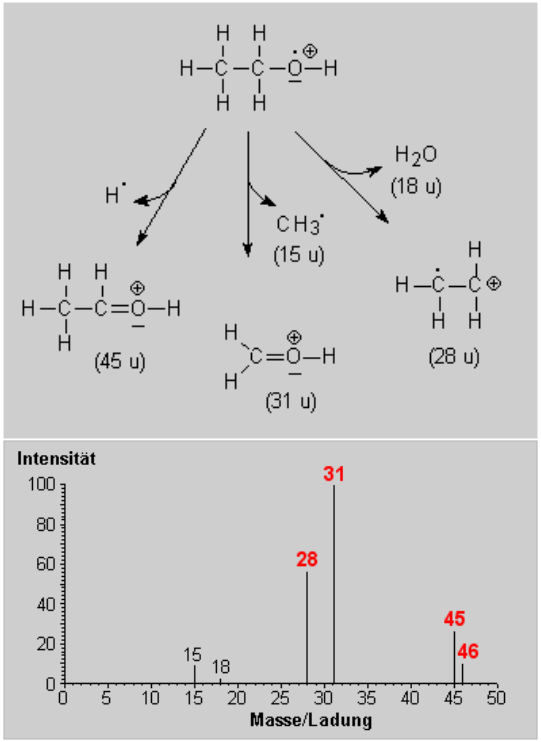
✓ typical „strong“ ionization method (70 eV), fragmenting „fingerprint“

✓comparison with MS data base (Wiley, NIST: more than 300 000 compounds)

✓ mass range 33-900 Dalton

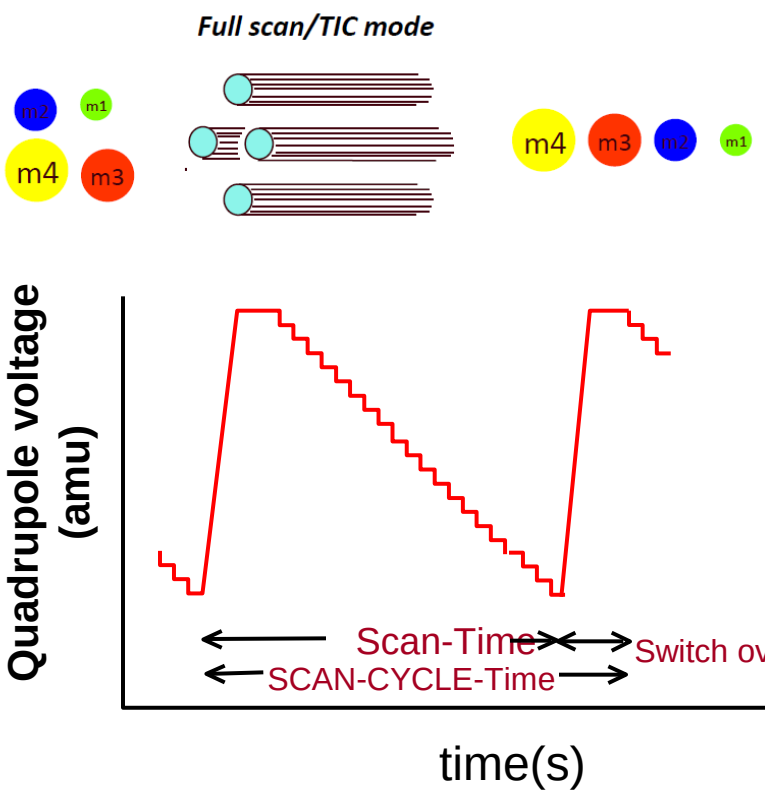


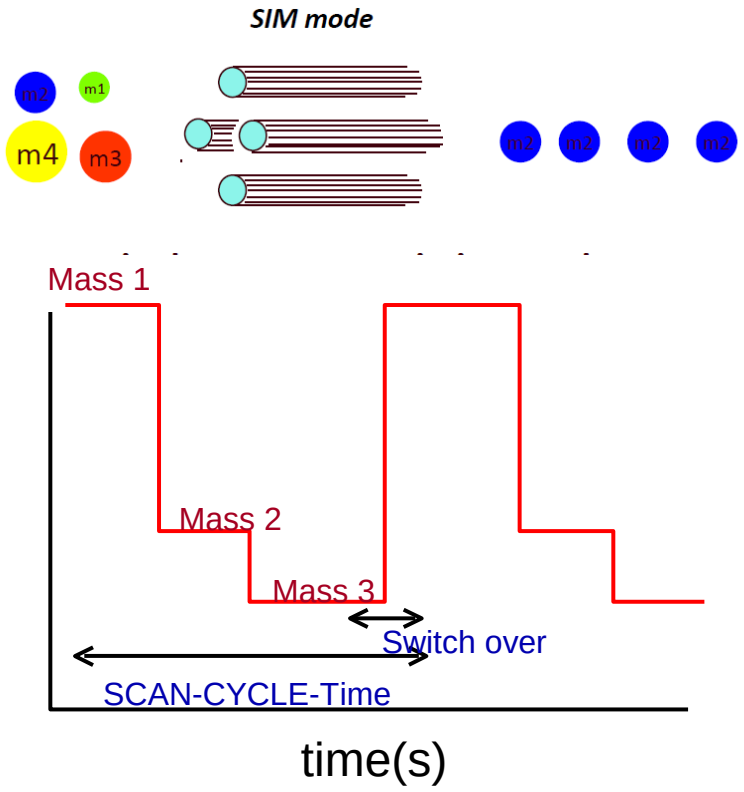
ethanol as example



5. CHROMATOGRAPHY

5.2 GC/MS





✓ unexpected substances

✓ **qualitative analysis**

✓ greatest specificity, selectivity and sensitivity

✓ **quantification**

5. CHROMATOGRAPHY

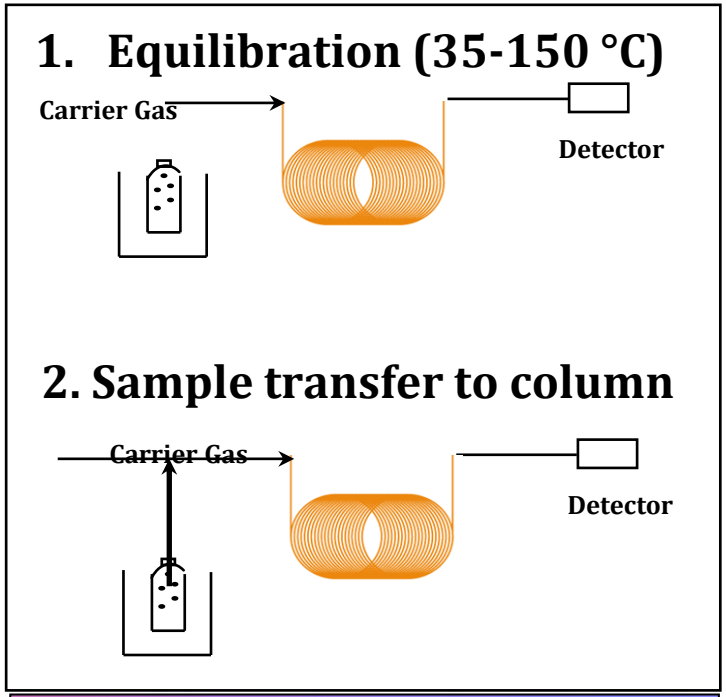
5.2 GC/MS

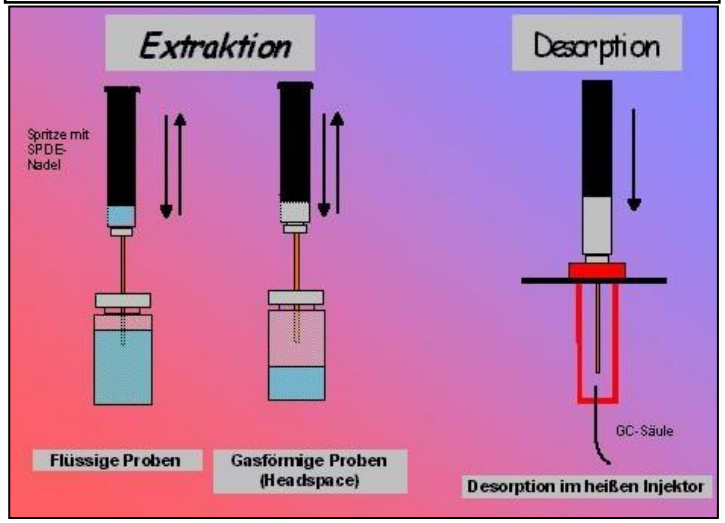
Head space (HS-GC/MS)

- Analysis of **volatile sample parts from solid or liquid matrices**
- transfer of the gaseous sample parts into GC by gastight syringe after thermal treatment up to 150°C
- CTC Autosampler with 6 vials

SPME (HSPME-GC/MS)

- **Extraction of volatiles** in the HS-vial by adsorption on a polymer-coated fiber
- transfer of the fiber to GC injection port, **desorption**
- fibers: PDMS, Carbowax, Divinylbenzen



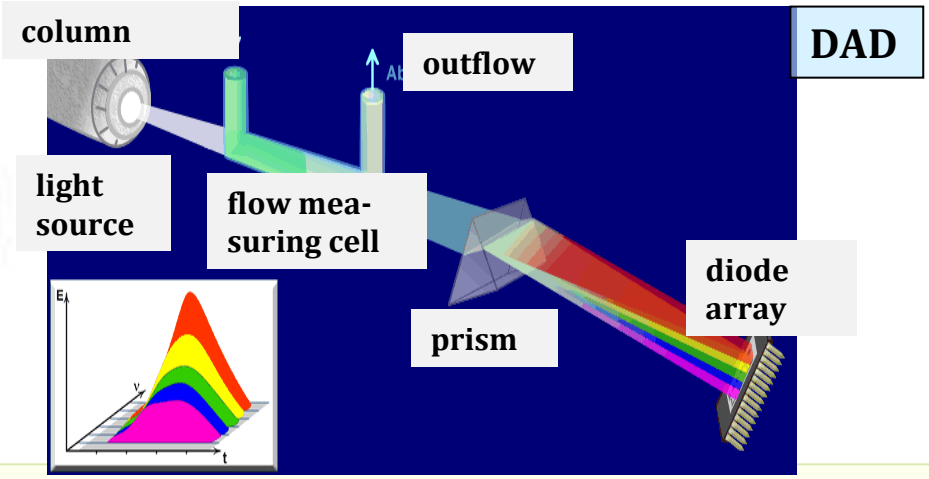


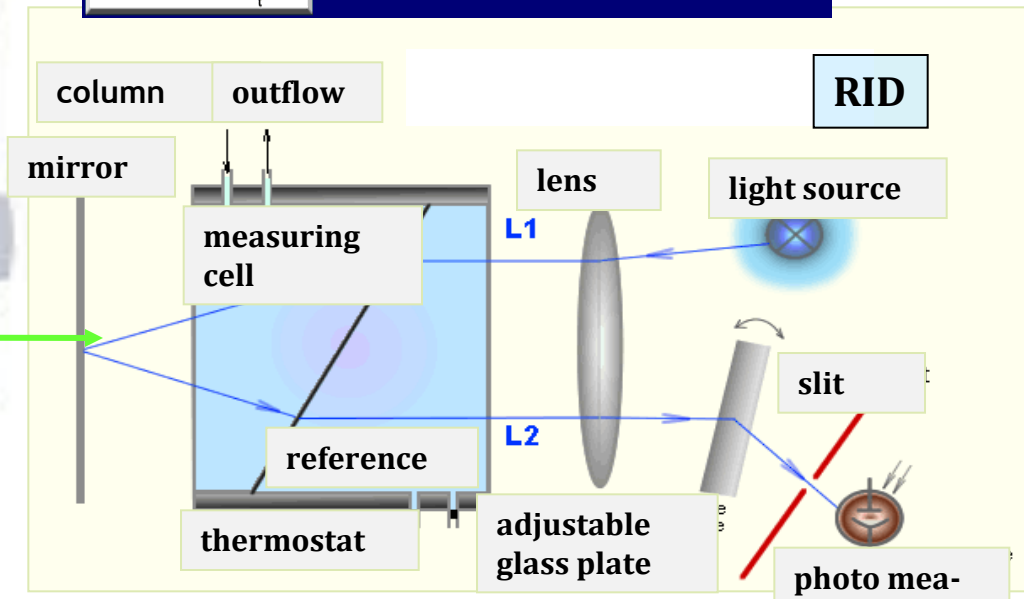
5. CHROMATOGRAPHY

5.3 LIQUID CHROMATOGRAPHY (LC)

1. Eluents (mobile phase)
2. Degasser
3. Quaternary Pump
4. Autosampler
5. Column oven (up to 80 °C)

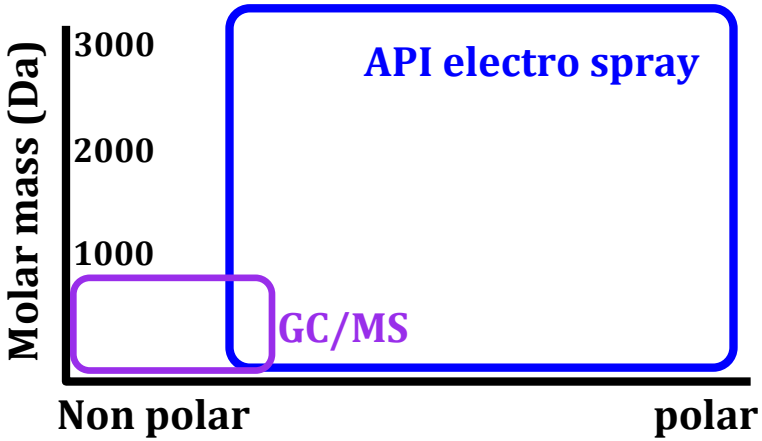
6. Detector
   DAD (100 pg / ml)
   RID (100 ng / ml)





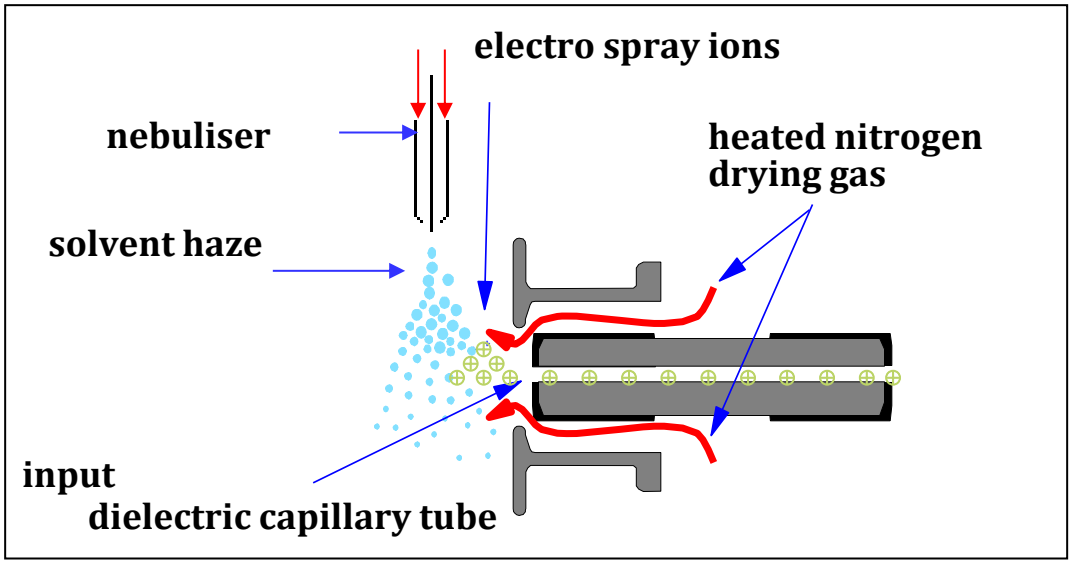
5. CHROMATOGRAPHY

5.3 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY



API-Electro spray (ESI)

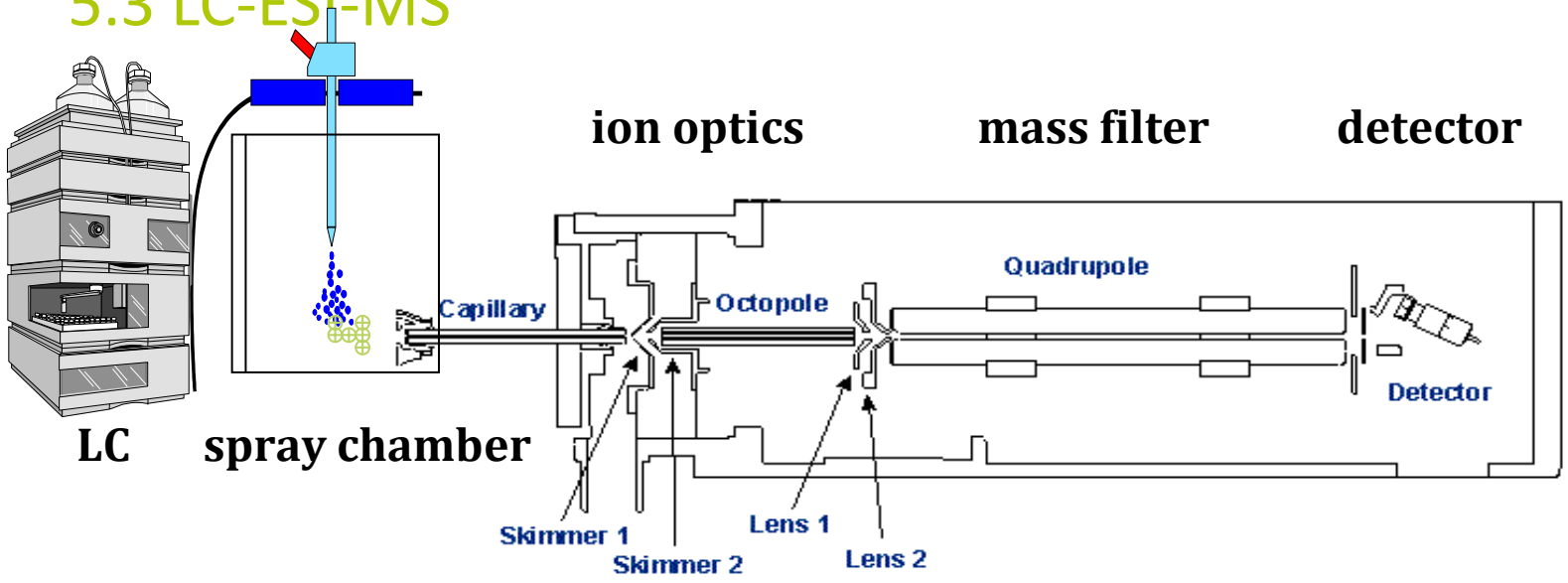
✓ Ionisation with electrical field (max 4.5 kV)
→ production of charged droplets
✓ following production of analyte ions by Ion evaporation
✓ nebuliser pneumatic supported

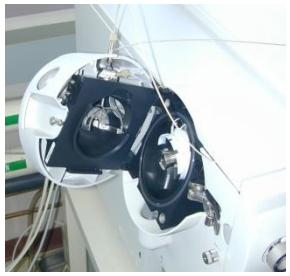


Must-have:
Ions in solution or development of ions (e.g. decreasing of the pH in case of alkaline substances leads to protonation)

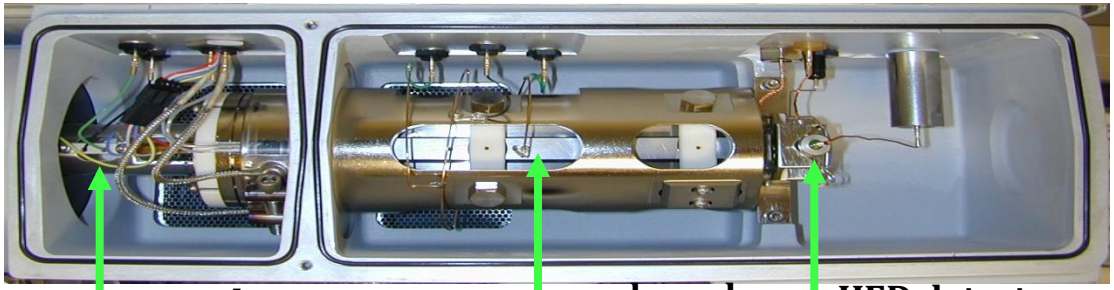
5. CHROMATOGRAPHY

5.3 LC-ESI-MS





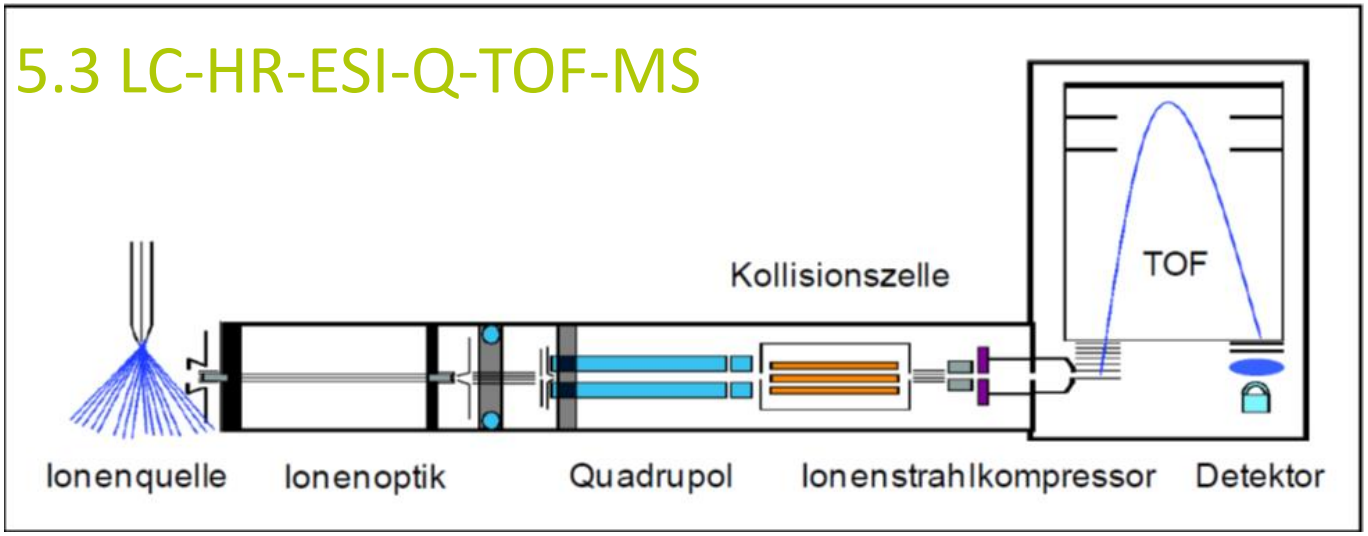
spray chamber



- ✓ after LC separation: drying, ionisation, analysing by mass/ charge and detection
- ✓ **fg up to pg** sensitivity, M/Z range **up to 3000 Dalton**

5. CHROMATOGRAPHY

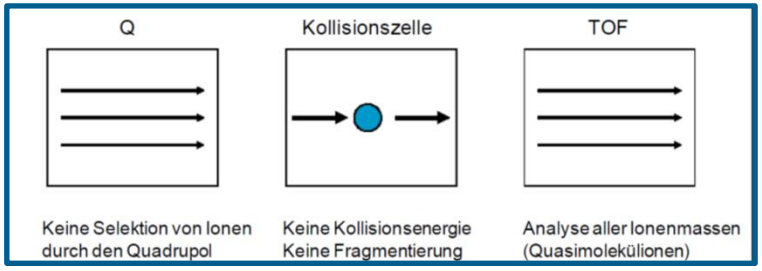
5.3 LC-HR-ESI-Q-TOF-MS

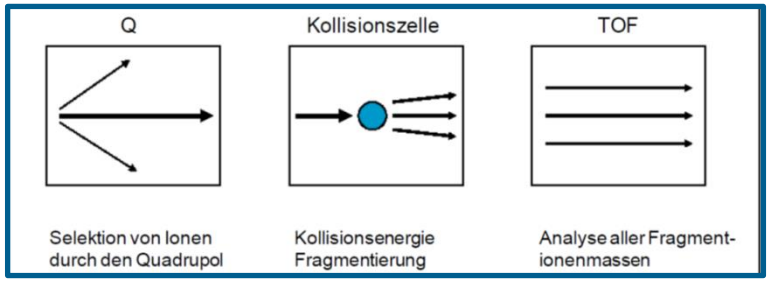


The larger the mass the slower the ion

Typical flight times of ions: 5-1000 µsec

- ✓ after LC separation: drying, ionisation, analysing by mass/ charge and detection
- ✓ sensitivity: **down to pg**, M/Z range: **up to 10000 Da**, accuracy **0.8 ppm**





MS only: maximum sensitivity

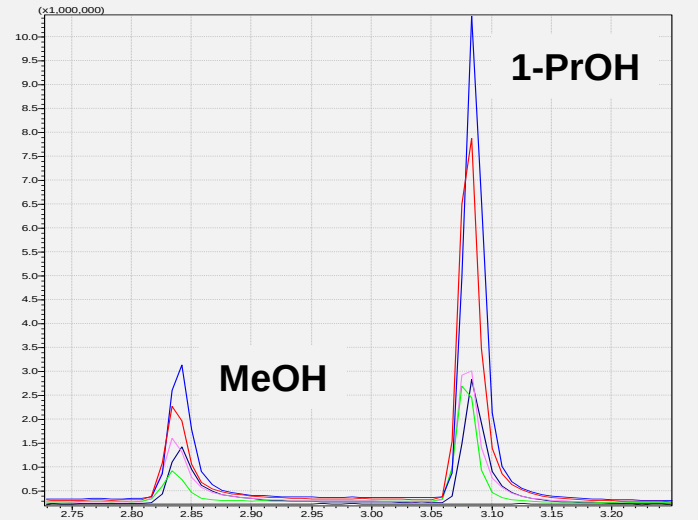
MS/MS: structure elucidation by CID spectra

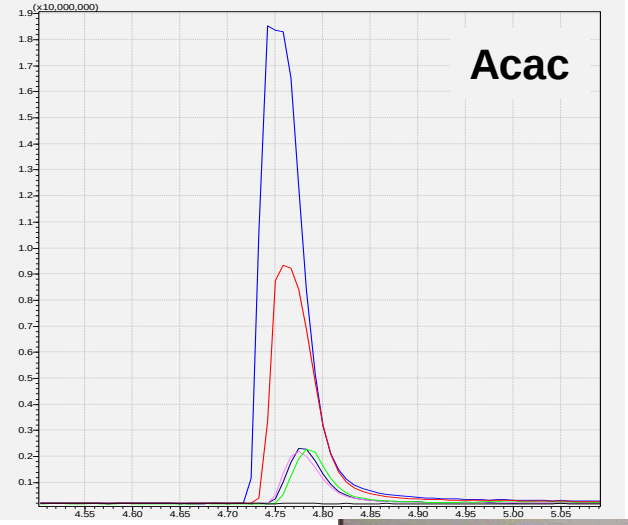
5. CHROMATOGRAPHY

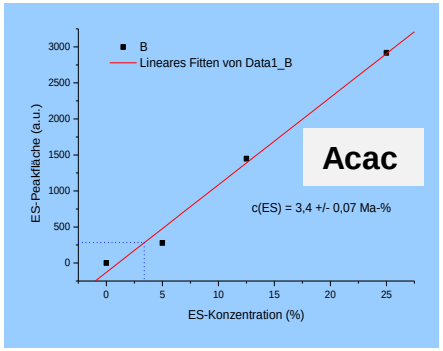
5.4 EXAMPLE HS-GC/MS

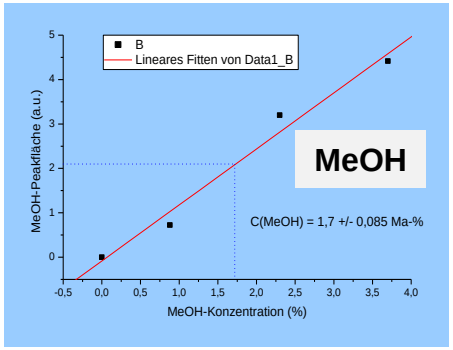
Quantitative determination of volatile sol components

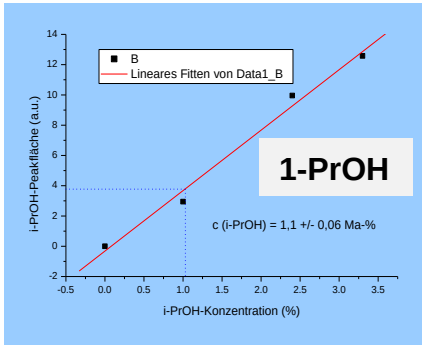
HS GC/MS, 15 min 85 °C and 120 ° C resp., 20 µl volume, 180 ° C, WAXplus











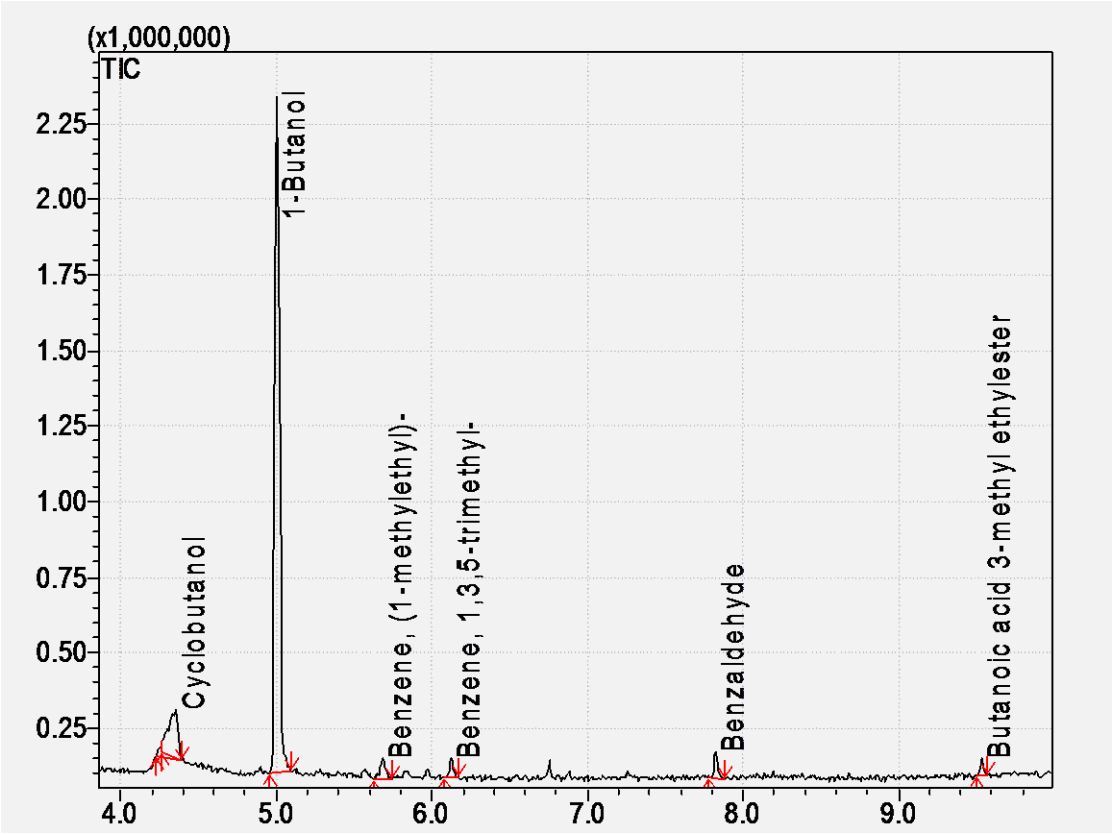
5. CHROMATOGRAPHY

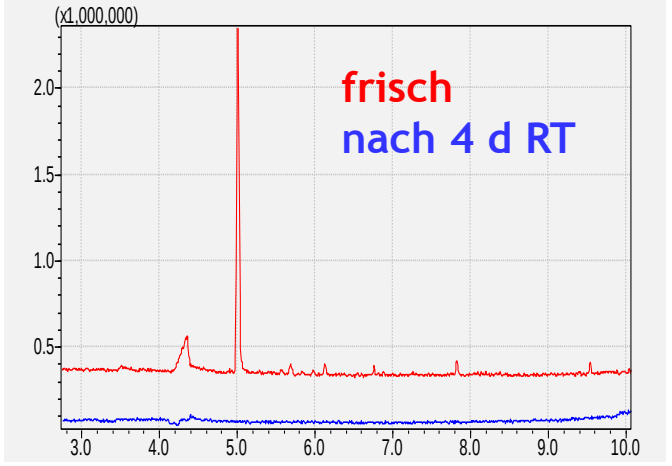
5.4 EXAMPLE HS GC/MS

Qualitative determination of solvents in translucent ink

HS GC/MS

Thermo 15 min 35 °C, 200 ° C, 200 ° C, volume 1 ml, WAXplus

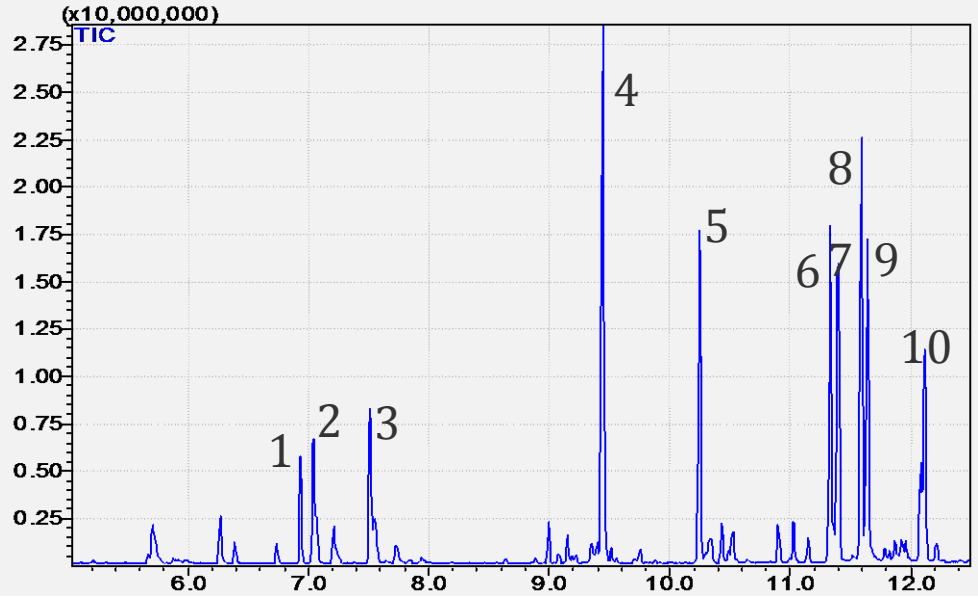




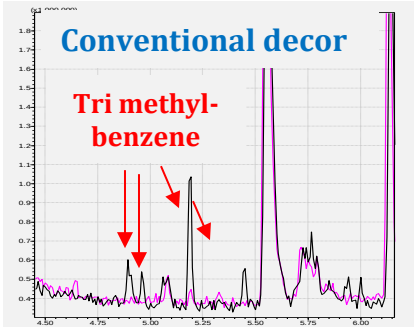
5. CHROMATOGRAPHY

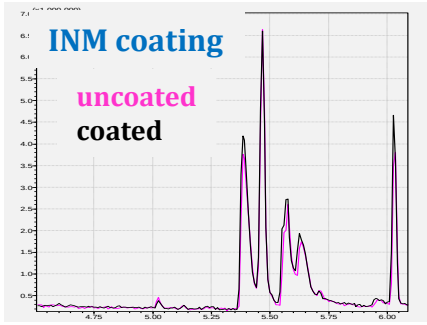
5.4 EXAMPLE GC/MS

Qualitative analysis of Narcise perfume and determination of migration of coating components in Narcise GC/MS, 1:10 in MeOH, 0,2 µl, EC™-1, ms

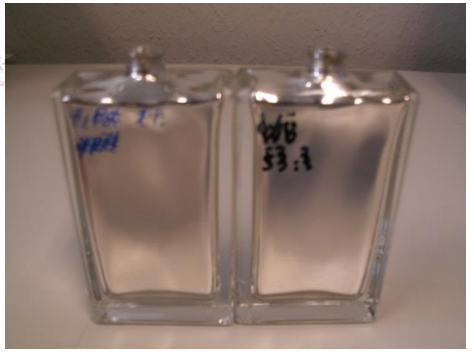


Fragrances	
1	Benzyl Alcohol
2	Phenylethyl Alcohol
3	Linalyl butyrate
4	Lilial
5	Methyl dihydrojasmonate
6	Isocitronellol
7	Isopropyl Myristate
8	Ambrox
9	Isopropyl Myristate
10	Galoxolide





Coated flasks

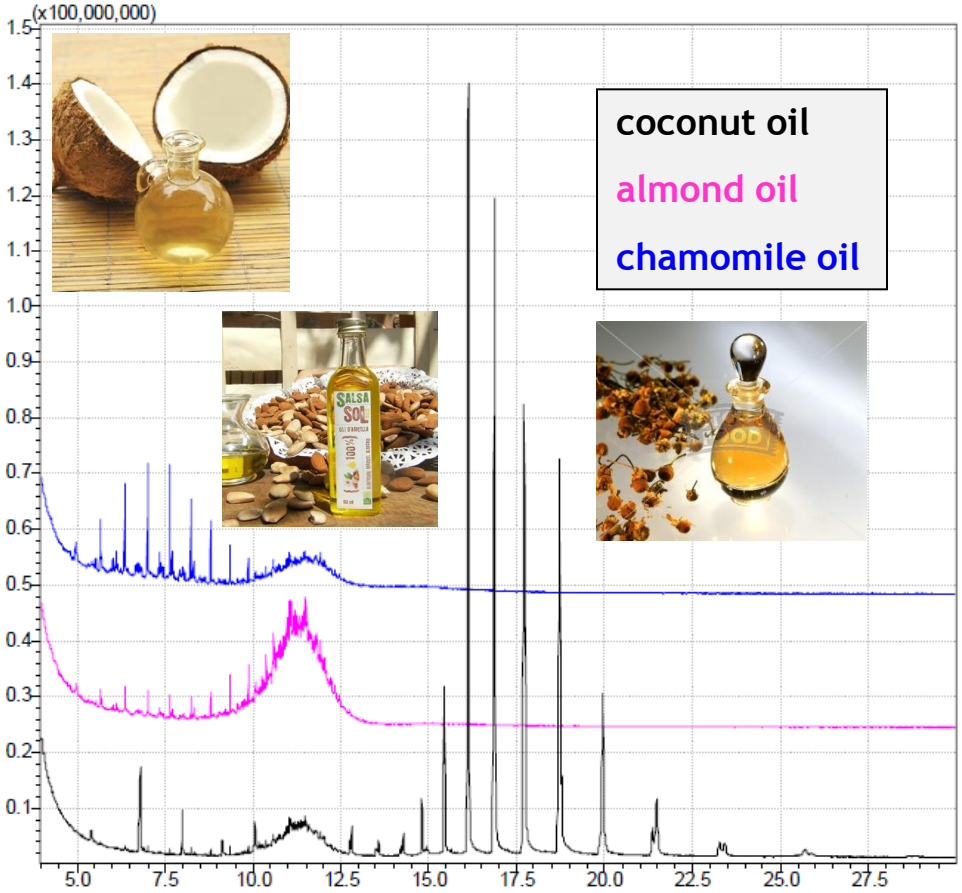


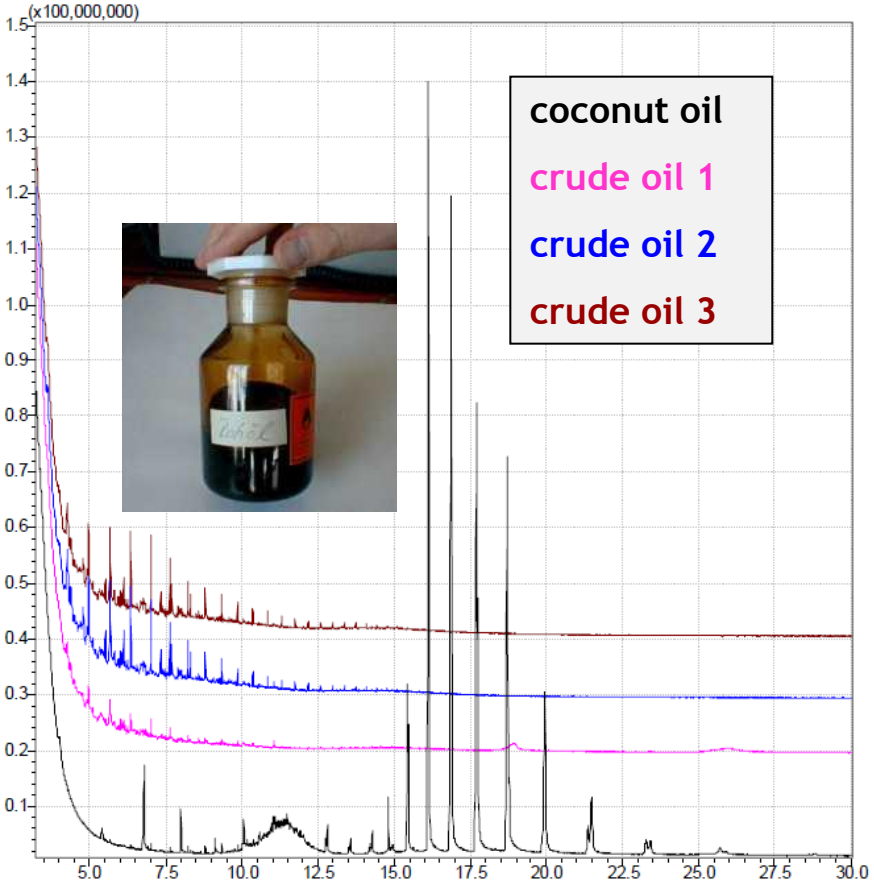
5. CHROMATOGRAPHY

5.4 EXAMPLE GC/MS

Comparison of natural and crude oils

GC/MS, 1:100 in hexane, 1 µl direct, ZB-1 guardian

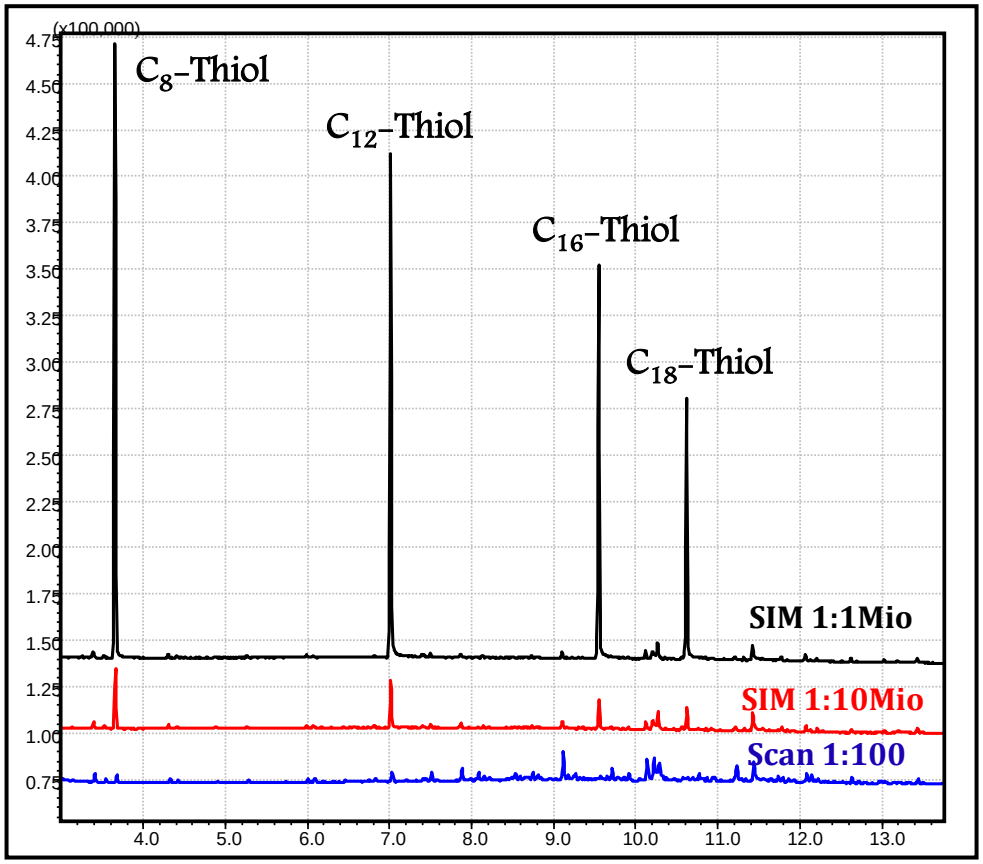




5. CHROMATOGRAPHY

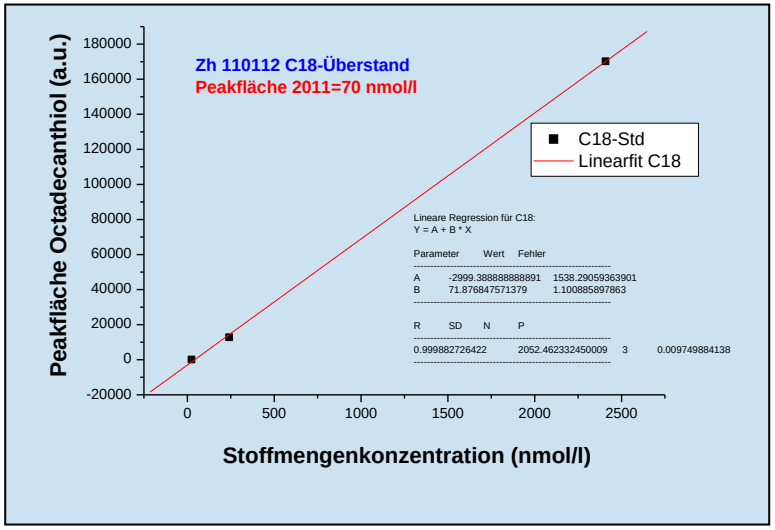
5.4 EXAMPLE GC

Quantitative trace analysis of alkan thiols in non-polar solvents
GC/MS, 5µl direct, ZB-1HT Inferno (2min 100°C, 20K/min 360°C, 1min), MZ 56, 69, 111



LOQ: Scan mode 10 µmol/l
SIM mode 10 nmol/l
(MZ 56, 69 und 111)

real sample: 70 nmol/l C_{18}-thiol

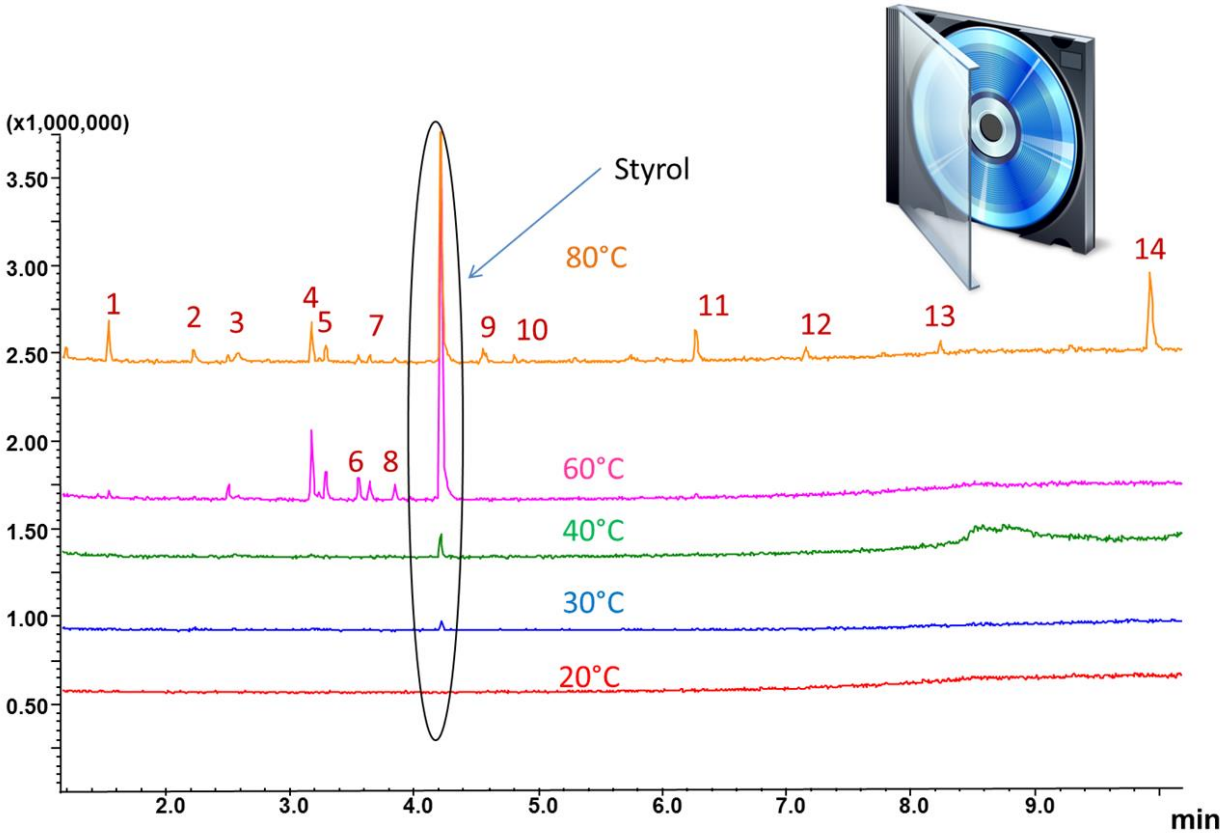


5. CHROMATOGRAPHY

5.4 EXAMPLE HS-GC/MS

Thermal degradation of commercial discs

HS-GC/MS, 5min incubation time, 500 µl direct, WAX plus, (2min 100°C, 20K/min 250°C, 1min)



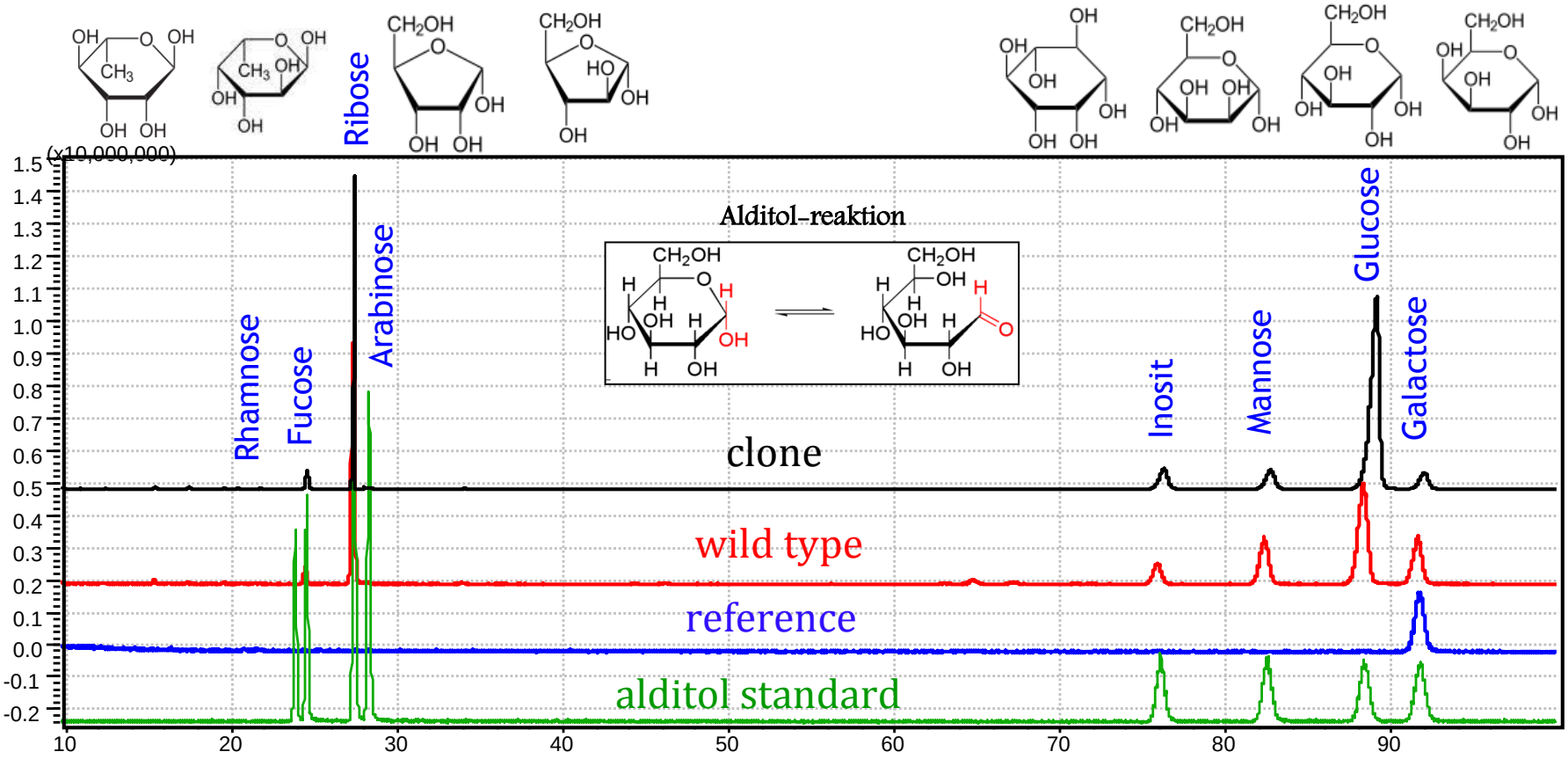
1-tetrahydrofuran
2-acetonitrile
3-viniloxyethanol
4-ethybenzene
5 -o-Xylene
6-isopropylbenzene
7-xylene
8-benzeneacetaldedyde
9-cyclohexanone
10-2-phenylpropene
11-benzyldehyde
12-acetophenone
13-isopropyl laurate
14-1-methoxy-ethylbenzoate

5. CHROMATOGRAPHY

5.4 EXAMPLE GC/MS

Quantitative analysis of alditol acetate derivates

GC: 1:100 in ethyl acetate, 2 µl direct, ZB-1701(30m, 0,25mm, 0,25µm), 200°C isotherm **MS:** SIM, 230 kV, M/Z 115, 6-35 min

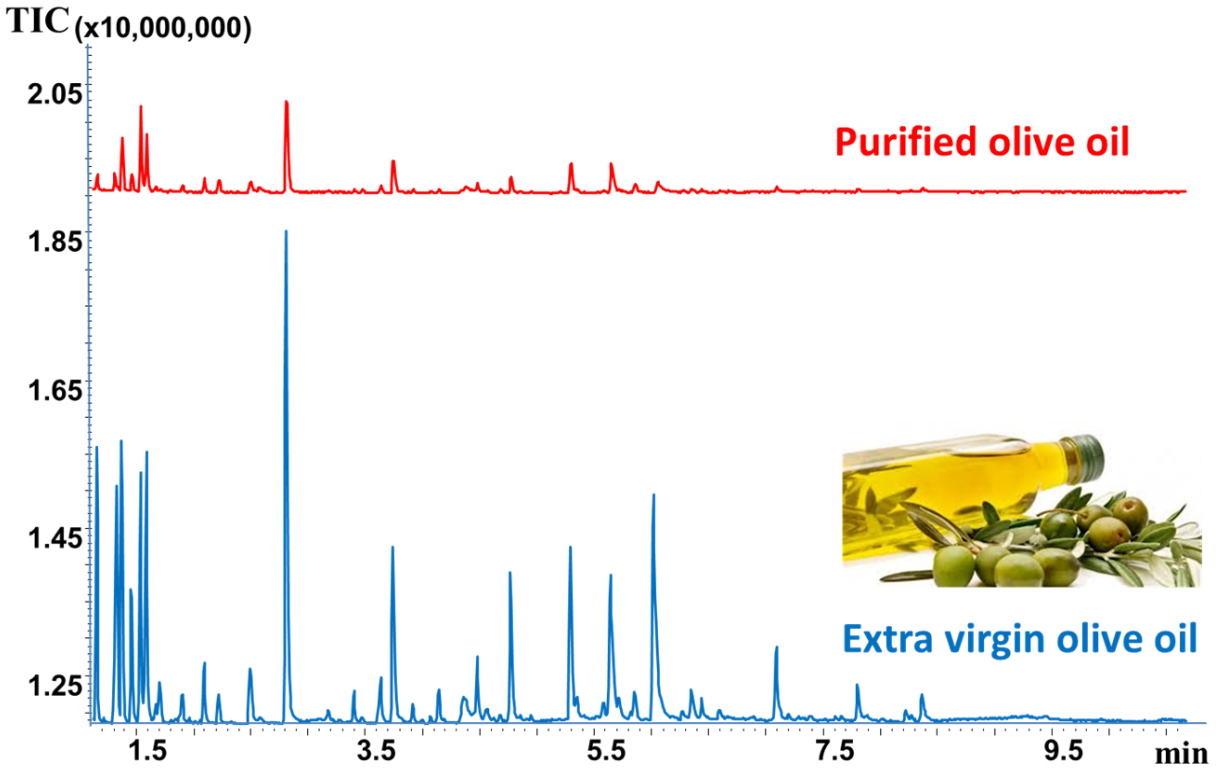


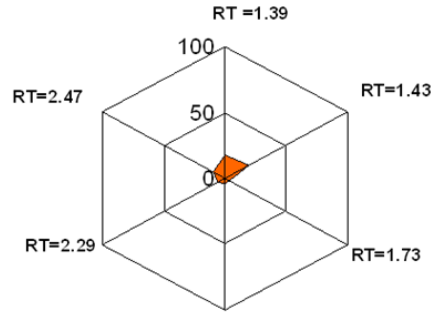
5. CHROMATOGRAPHY

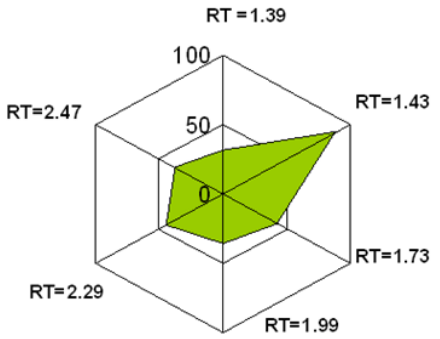
5.4 EXAMPLE GC/MS

Screening of plant oils by HS-GC/MS

Total ion chromatograms of extra virgin and purified olive oils in comparison after 5 min in HS vial held at 30 °C, column ZB-WAX plus (left) and visual prints (right)





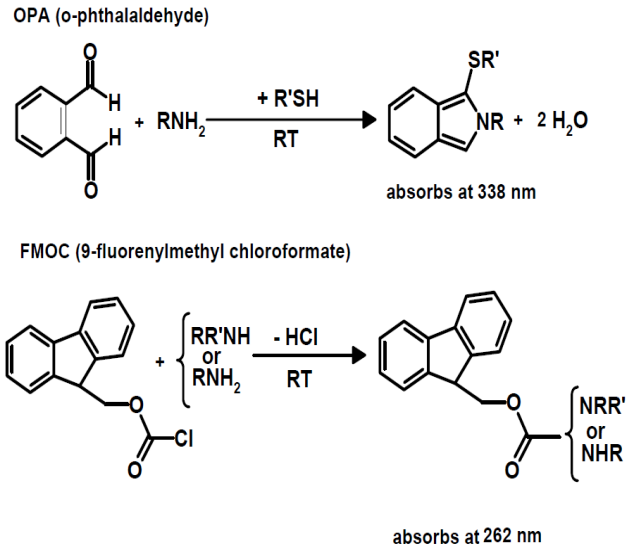


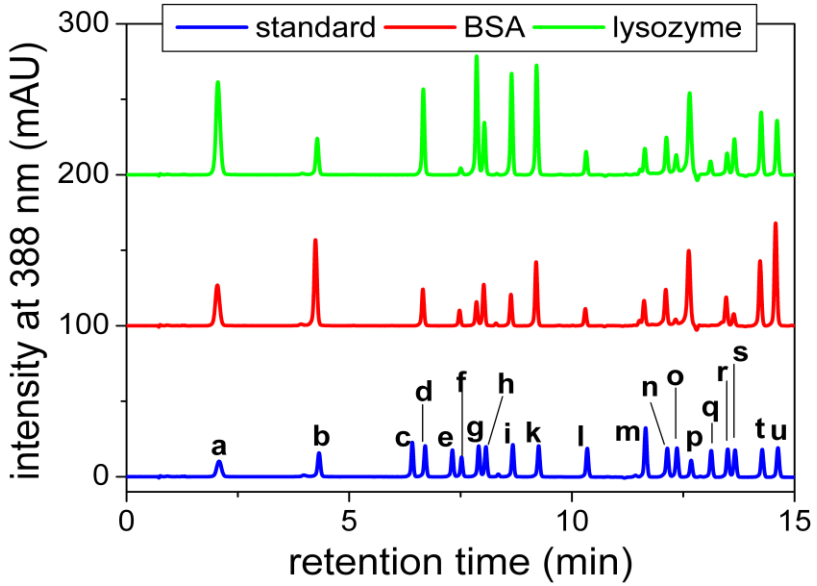
5. CHROMATOGRAPHY

5.4 EXAMPLE LC

Quantitative HPLC analysis of amino acids in proteins

mobile phase: ACN-MeOH-KH_2PO_4 buffer, 1ml·min^{-1} , stationary phase: Zorbax Eclipse AAA (4,6 mm x 150 mm, 5 µm), 0,5 µl volume, online-derivatization with OPA and FMOC, DAD 338nm+262nm





no	abbr
1	ASP
2	GLU
3	ASN
4	SER
5	GLN
6	HIS
7	GLY
8	THR
9	ARG
10	ALA
11	TYR
12	CY2
13	VAL
14	MET
15	NVA
16	TRP
17	PHE
18	ILE
19	LEU
20	LYS

✓**high resolution: 10 pmol/µl**

✓**rapid: 26 min for primary + secondary amino acids**

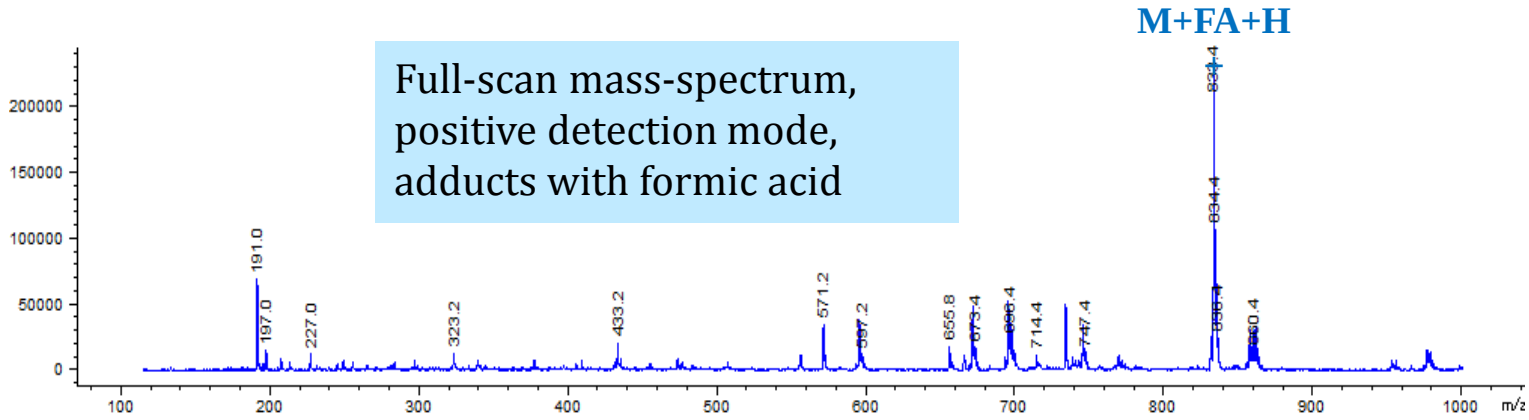
✓**reproducible: N=6, $RSD_{ret.time}$<0,3%; $RSD_{peak\ area}$<2%**

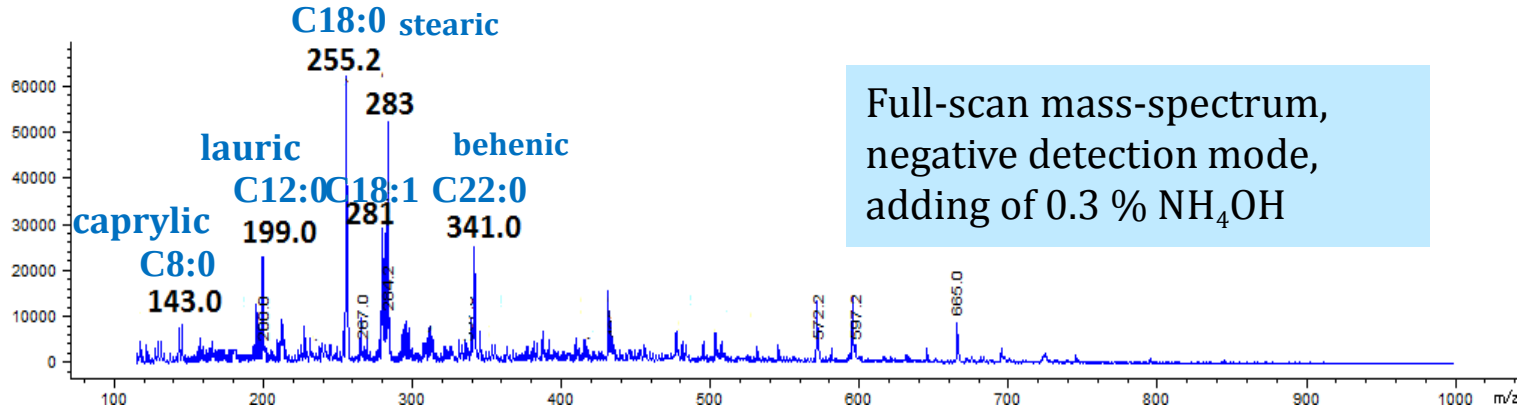
5. CHROMATOGRAPHY

5.4 EXAMPLE LC/MS

LC-ESI-MS of soy lecithine (phosphatidylcholine, phospholipid)

mobile phase: MeOH-H2O, 0.3 ml·min^{-1},stat. phase: capillary, 5 µl



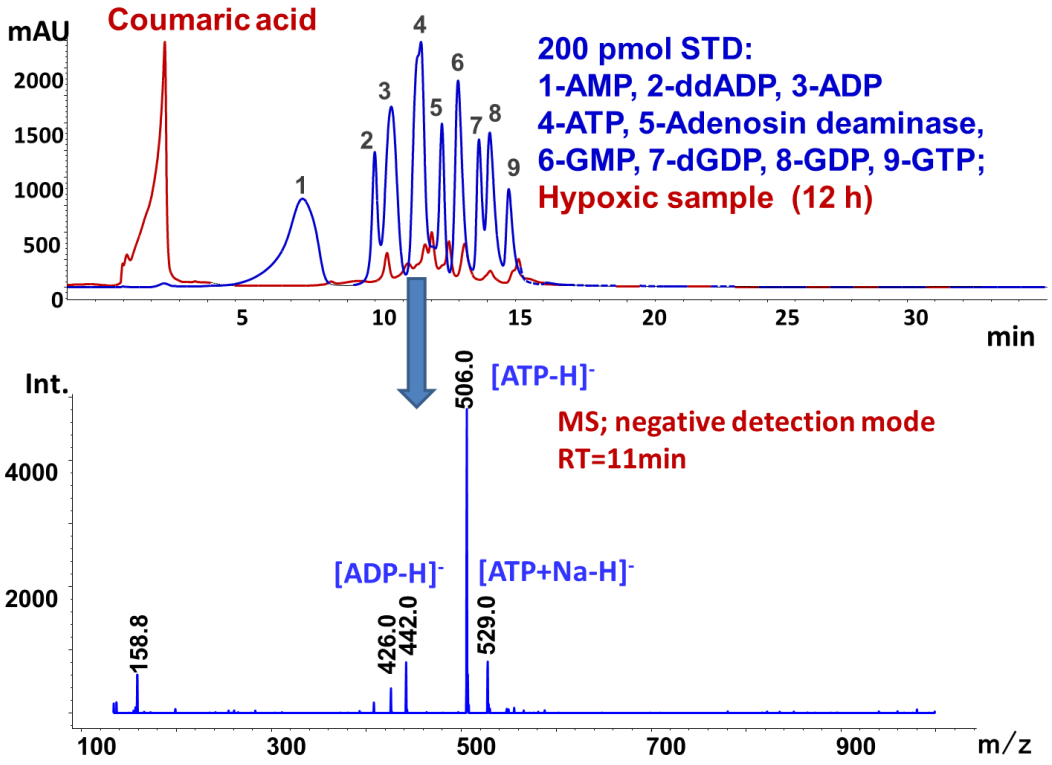


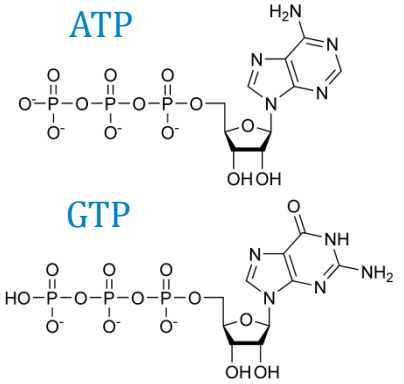
5. CHROMATOGRAPHY

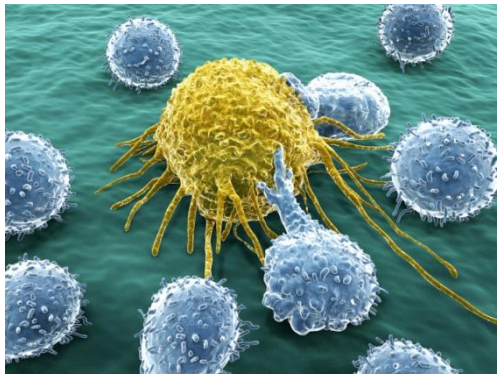
5.4 EXAMPLE LC/MS

Quantification of nucleotides in human cells with LC-ESI-MS

LC: stationary phase: Ascentis C 18 (150 x 4.6mm, 3.5 µm), 27°C
mobile phase: gradient of 10mM ammonium acetate, pH=10 and 100 % ACN, 350µl/min
MS: negative mode, full scan 100-950 Da, 3500 V, dry gas flow 8 l/min, dry gas 350°C



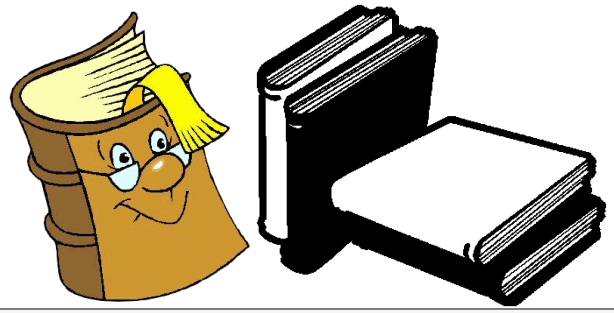




6. QUESTIONS

- Define the terms of LOD and LOQ
- Which kinds of errors are in analytical chemistry? Examples
- Different calibration modes, examples
- Reaction equations for digestion of samples, Rule of thumb kinetics
- Scheme of electromagnetic radiation spectrum, allocation of wavelengths and frequencies
- Advantages and disadvantages of microwave digestion
- Difference between the terms absorption (spectra) and emission (spectra)
- Optical path of AAS and OES analytical instrument
- Measuring ranges, sensitivities of AAS, OES, MS in comparison
- Differences between Gas- and Liquid-Chromatography
- Different detectors in chromatography
- Assign of application examples (Atomic spectrometry, Chromatography, Mass spectrometry)

7. LITERATURE



Veronika R. Meyer: **Praxis der Hochleistungsflüssigchromatographie,** 9. Auflage, WILEY-VCH (2004)

Stavros Kromidas: **Der HPLC-Experte, Möglichkeiten und Grenzen der modernen HPLC**, Wiley-VCH (2014)

Peter J. Baugh: **Gaschromatographie**, Friedr.Vieweg & Sohn (1997)

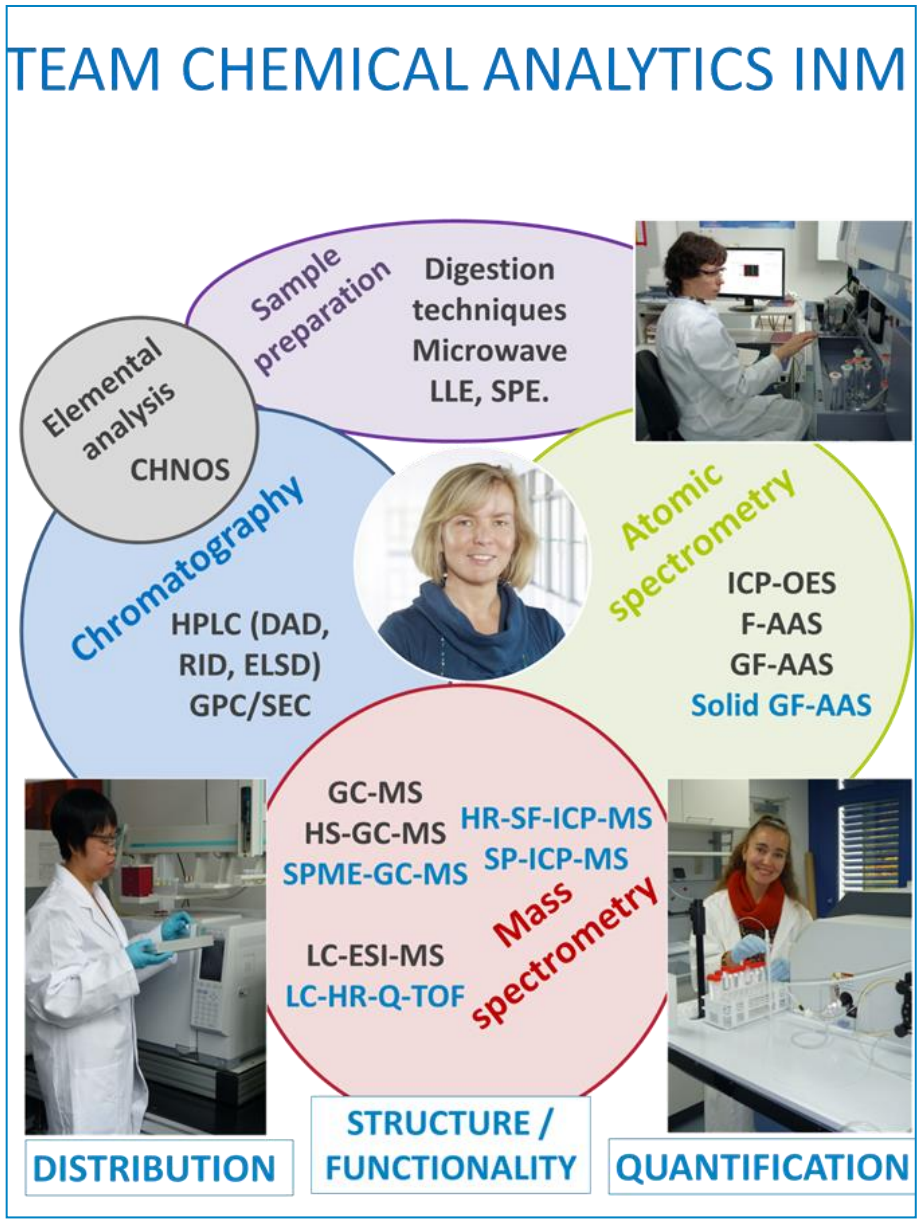
Hans-Joachim Hübschmann: **Handbook of GC/MS**, 2. Auflage, WILEY-VCH (2009)

B. Welz: **Atomabsorptionsspektrometrie**, 4. Auflage, WILEY-VCH (1997)

Joachim Nölte: **ICP Emissionsspektrometrie für Praktiker**, WILEY-VCH (2002)

→ 05.07.19 Nanosafety (Annette Kraegeloh)

INM



THANK YOU VERY MUCH FOR YOUR ATTENTION

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