LC-FT MS for environmental analysis

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Outline

I. Pollution
II. Chromatography
III. LC-FTMS
IV. Pollutants analysis

POLLUTION

Definition, generalities and environmental impact

What is pollution?

"Pollution means the introduction by man, directly or indirectly, of substances or energy into the environment, resulting in deleterious effects of such a nature as to endanger human health, harm living resources and ecosystems, and impair or interfere with amenities and other legitimate uses of the environment."

OECD 1974

Where does it come from?

Natural: storm, volcanic eruption etc…

Anthropogenic: exhaust gases, industries etc …

Types of pollution

Primary

At the root

Secondary

transformation of a pollutant in natural environment

degradation

?

Polluted environments

Air

Industries, transport, pollen …

Soil

Fertilizer, pesticides, waste…

Water

Fertilizer, industries aqueous waste, pesticides, bacteria…

Incidence

- One-time
  - oil spill, industrial accident …
- Persistent (permanent modification of ecological factor)
  - greenhouse gas, pesticides…

Environmental impact

- Direct or indirect effects on an actor of the environment
- Exposition level
- Ecosystem sensitivity towards the pollutant
- Ecosystem ability to absorb the pollutant

Problematics

Unknown molecules

Traces amount

Big volume

Complex matrix

→Sample preparation

→Instruments with high sensitivity and selectivity

→ Chromatography: separation of compounds

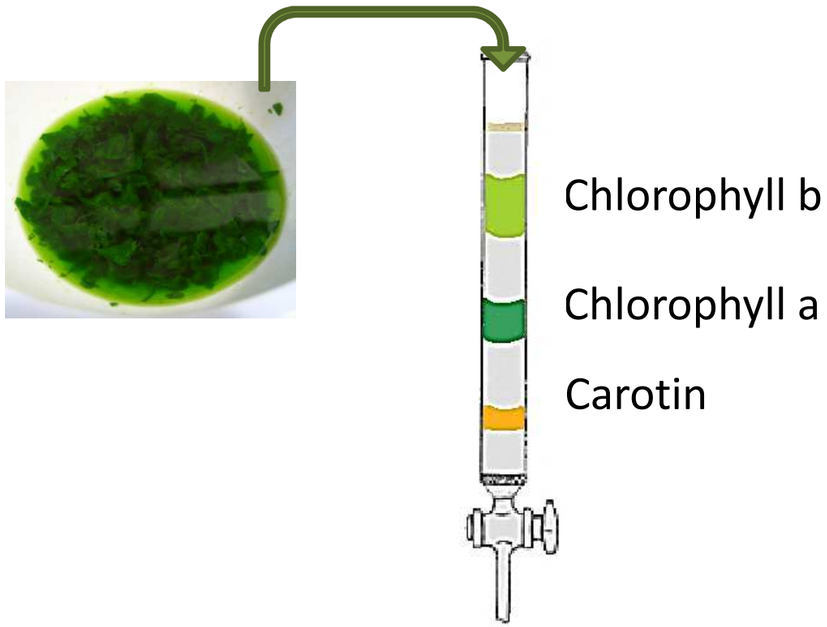
→ Mass spectrometry: sensitive and specific, characterization of compounds

LIQUID CHROMATOGRAPHY

History, separation, resolution

History

1906 : 1st chromatography → plant pigments separation

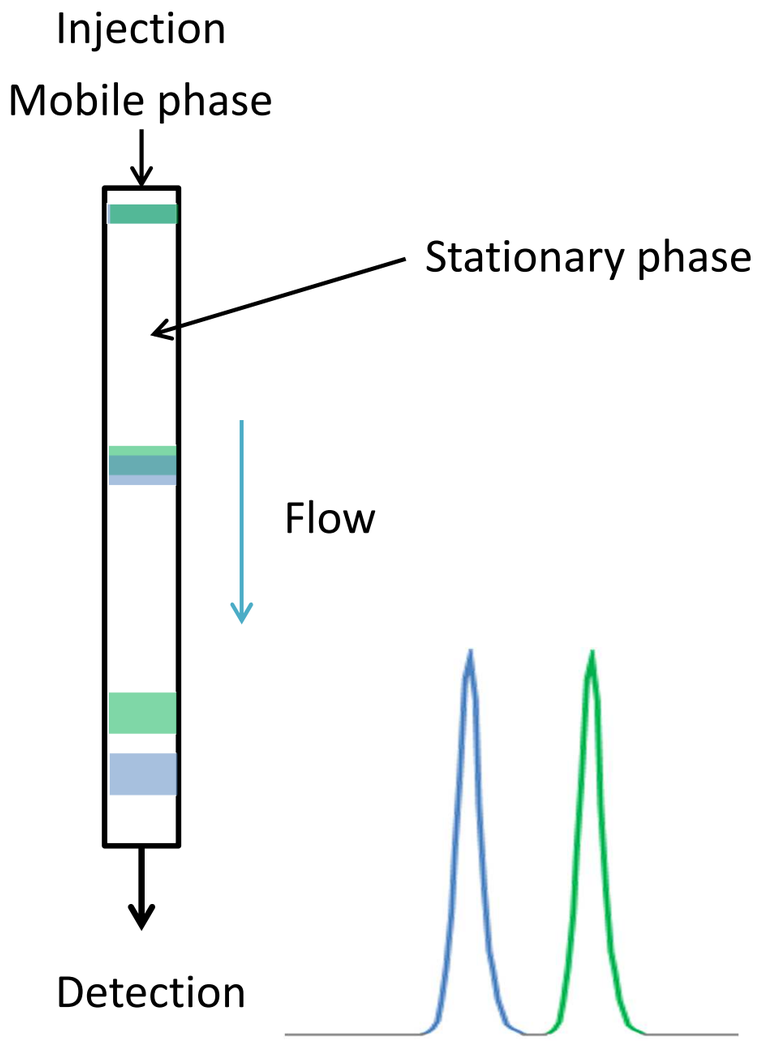


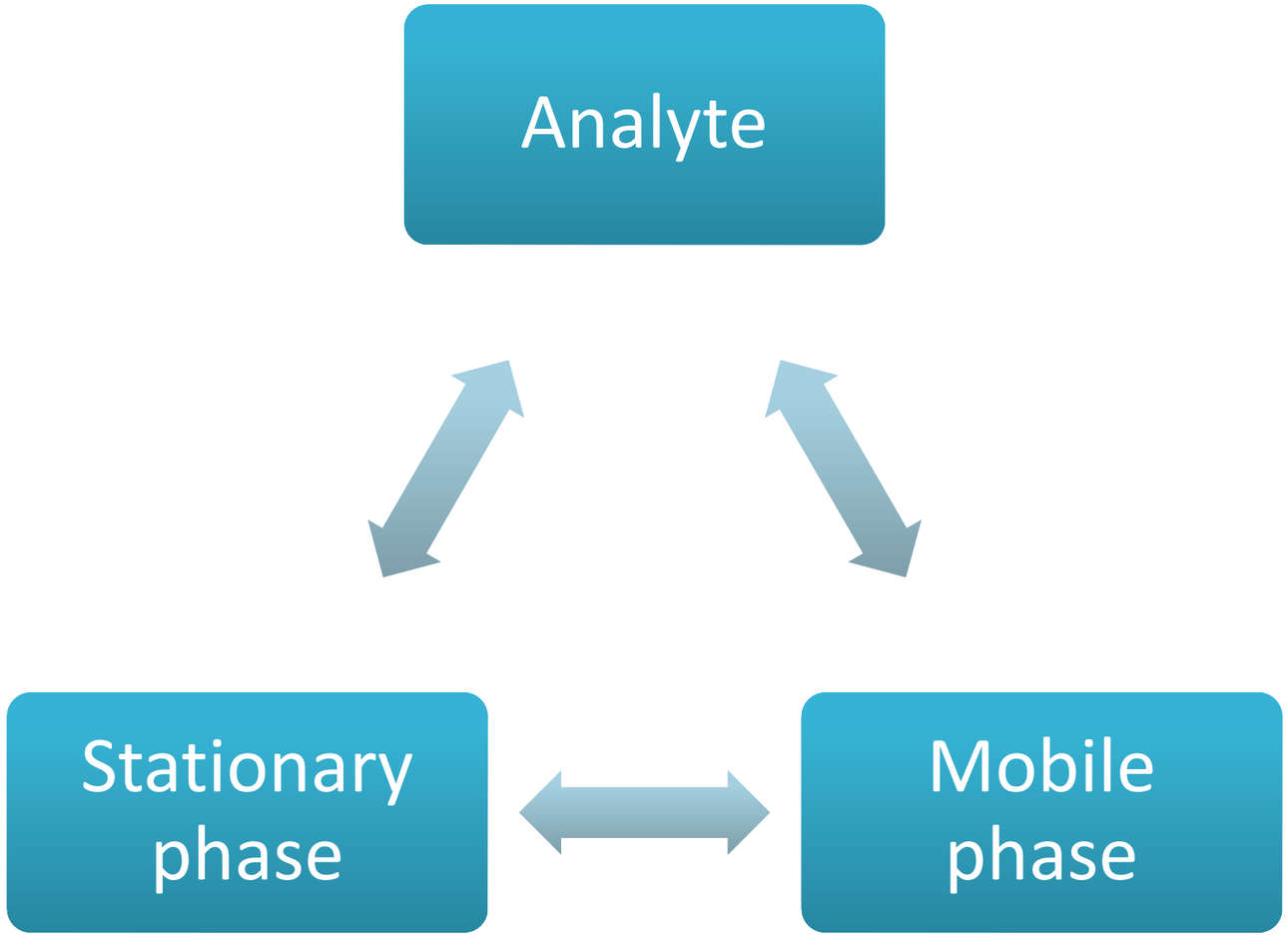
M.S. Tswett, botanist

Chromatography is derived from Greek « *kroma* » (color) and « *graphein* » (to write)

1950 : Gas chromatography
1969 : Liquid chromatography development

Separation


Injection
Mobile phase
Stationary phase
Flow
Detection


Analyte
Stationary phase
Mobile phase

Different chromatography

Gas

Analyte ?
- Volatile
- Thermally-stable

Important parameters ?
- Temperature
- Stationary phase composition

Supercritical fluid

Analyte ?
- Soluble in mobile phase
- Thermally-stable

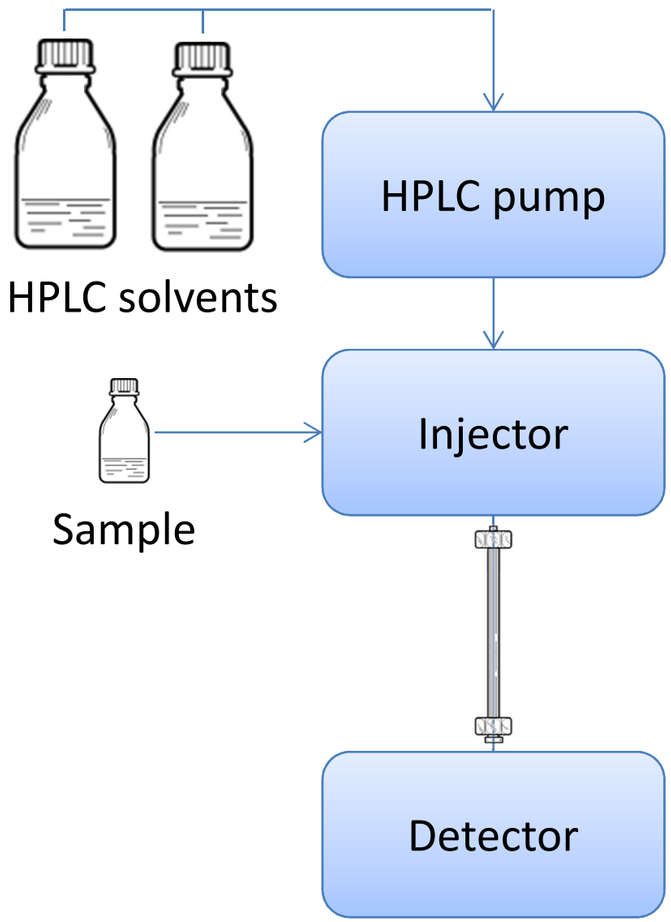
Important parameters ?
- Stationary phase composition
- Mobile phase composition
- Temperature and pressure

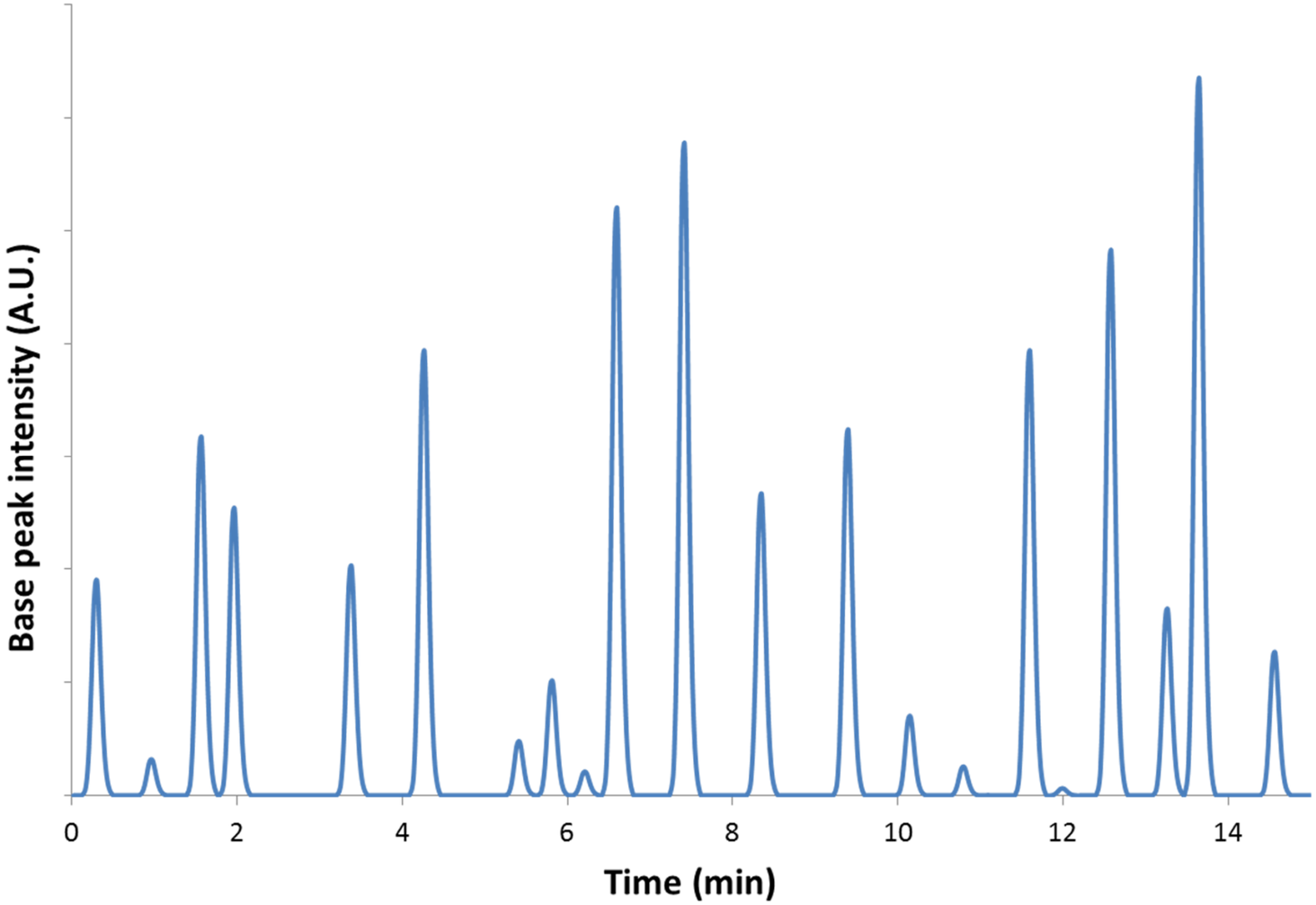
Liquid

Analyte ?
- Soluble in mobile phase

Important parameters ?
- Stationary phase composition
- Mobile phase composition
- Temperature

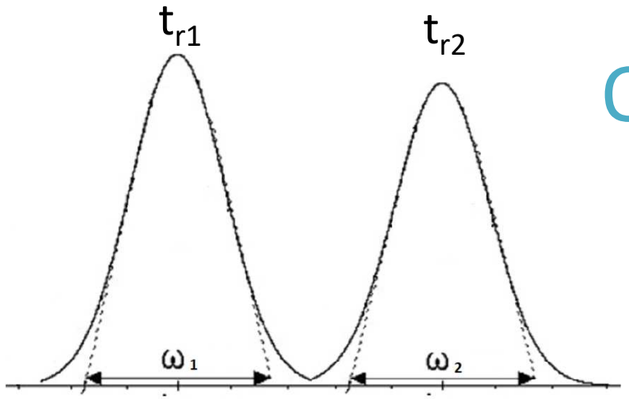
HPLC


HPLC solvents
HPLC pump
Sample
Injector
Detector


Base peak intensity (A.U.)
0
2
4
6
8
10
12
14
Time (min)

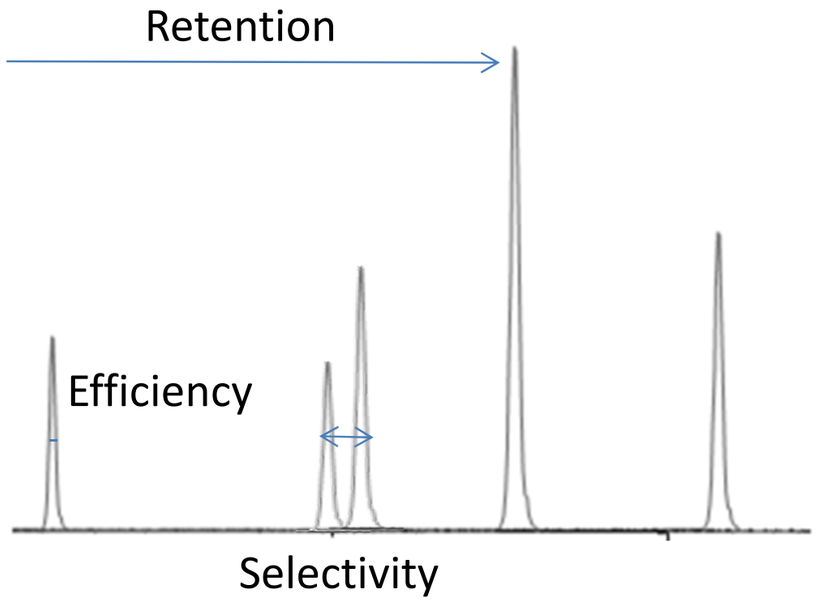
Resolution

$$R_S = \frac{2(t_{r1} - t_{r2})}{\omega_1 + \omega_2}$$



Optimal: 1,4 < Rs < 1,6

$\omega_1 \approx \omega_2$ (Purnell)

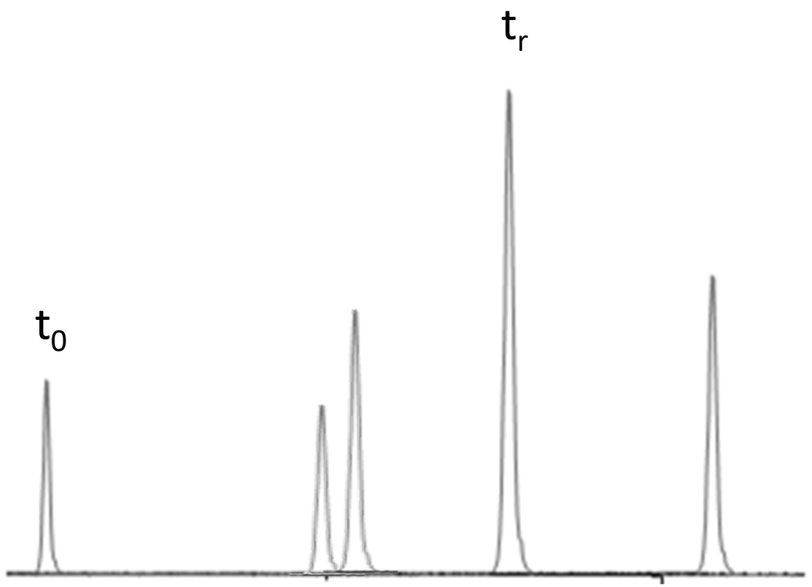


$$R_S = {}^1/_4 \sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{1 + k}$$

Efficiency Selectivity Retention

Retention

$$R_S = {}^1/_4 \sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{1+k}$$



Characterize the compound retention

$$k = \frac{t_r - t_0}{t_0}$$

Parameters affecting retention:

- mobile phase
- stationary phase

Selectivity

$$R_S = {}^1/_4 \sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{1+k}$$

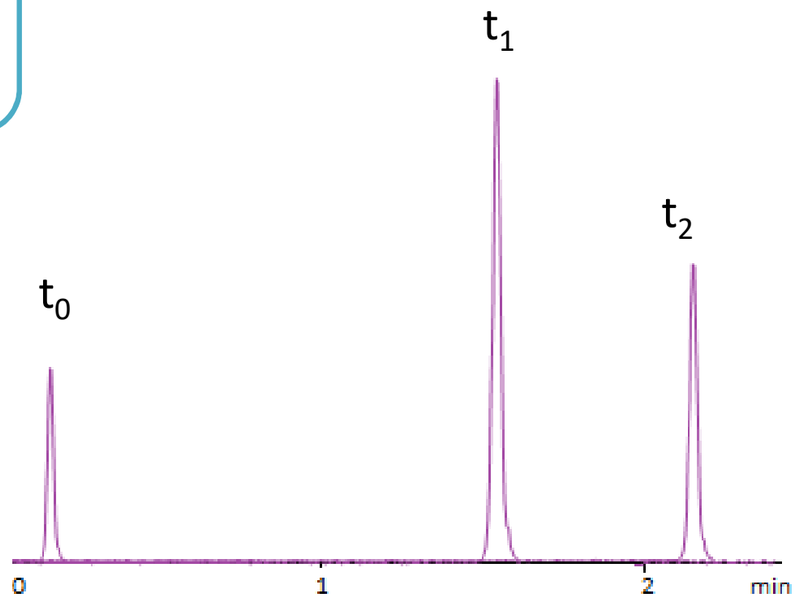
System ability to separate two components

$$\alpha = \frac{t_2 - t_0}{t_1 - t_0} = \frac{k_2}{k_1}$$

α = 1 no separation.

Parameters affecting selectivity:

- Mobile and stationary phase
- Temperature

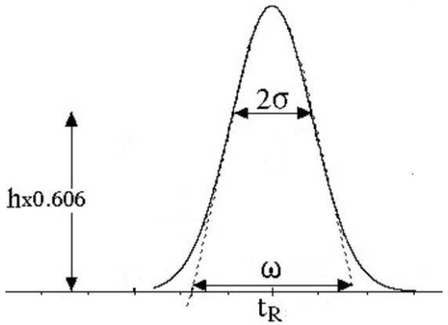


Efficiency

$$R_S = {}^1/_4 \sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{1 + k}$$

N (plate number) reflects the column performance → measure of the peak dispersion on the column

$$N = 16 \left(\frac{t_r}{\omega}\right)^2 = \left(\frac{t_r}{\sigma}\right)^2$$



σ: standard deviation
Normal distribution:
$\omega = 4\sigma$

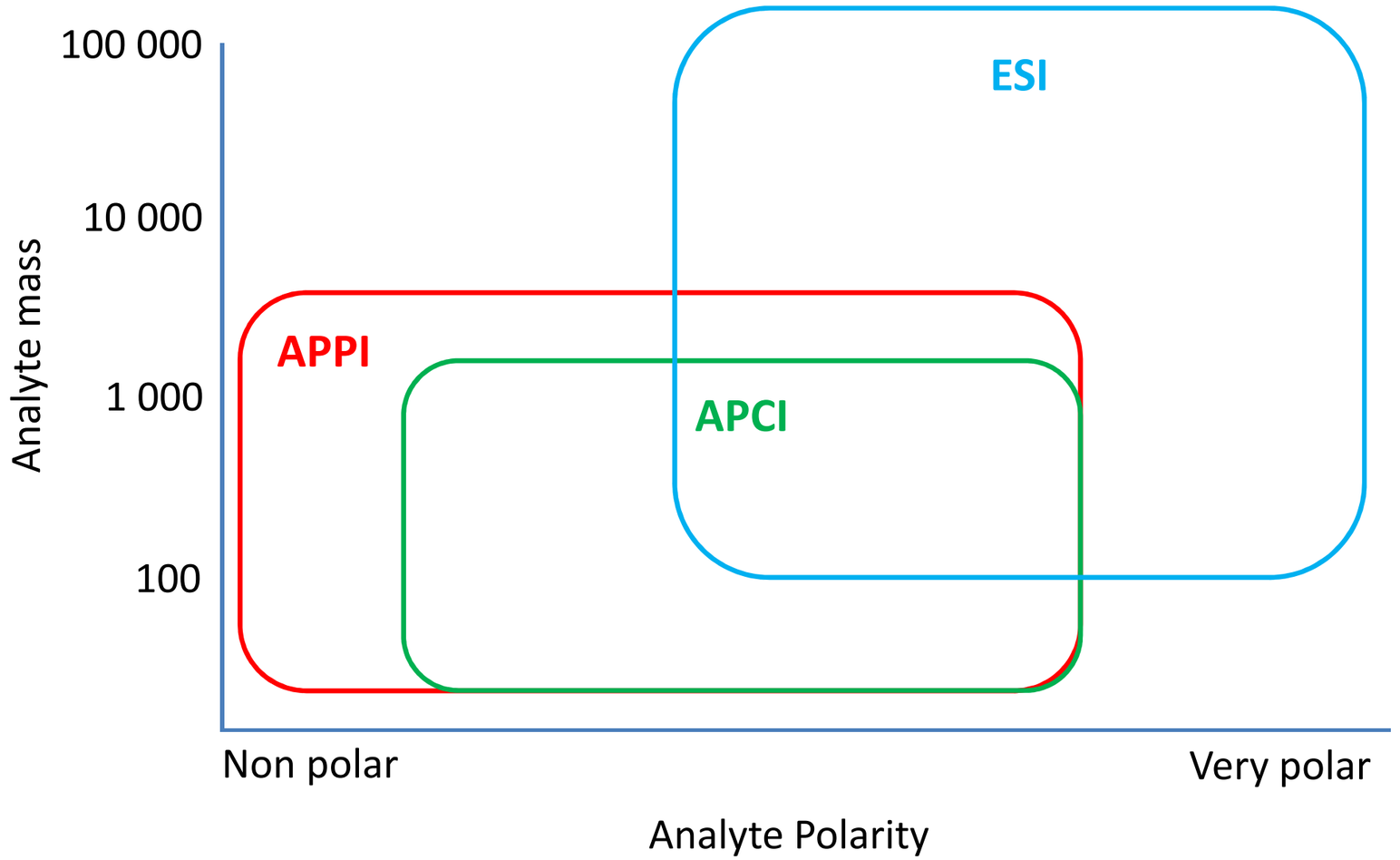
Parameters affecting efficiency:

- Column length
- Mobile and stationary phase (granulometry, interactions etc…)
- Analysis time

LC-FTMS

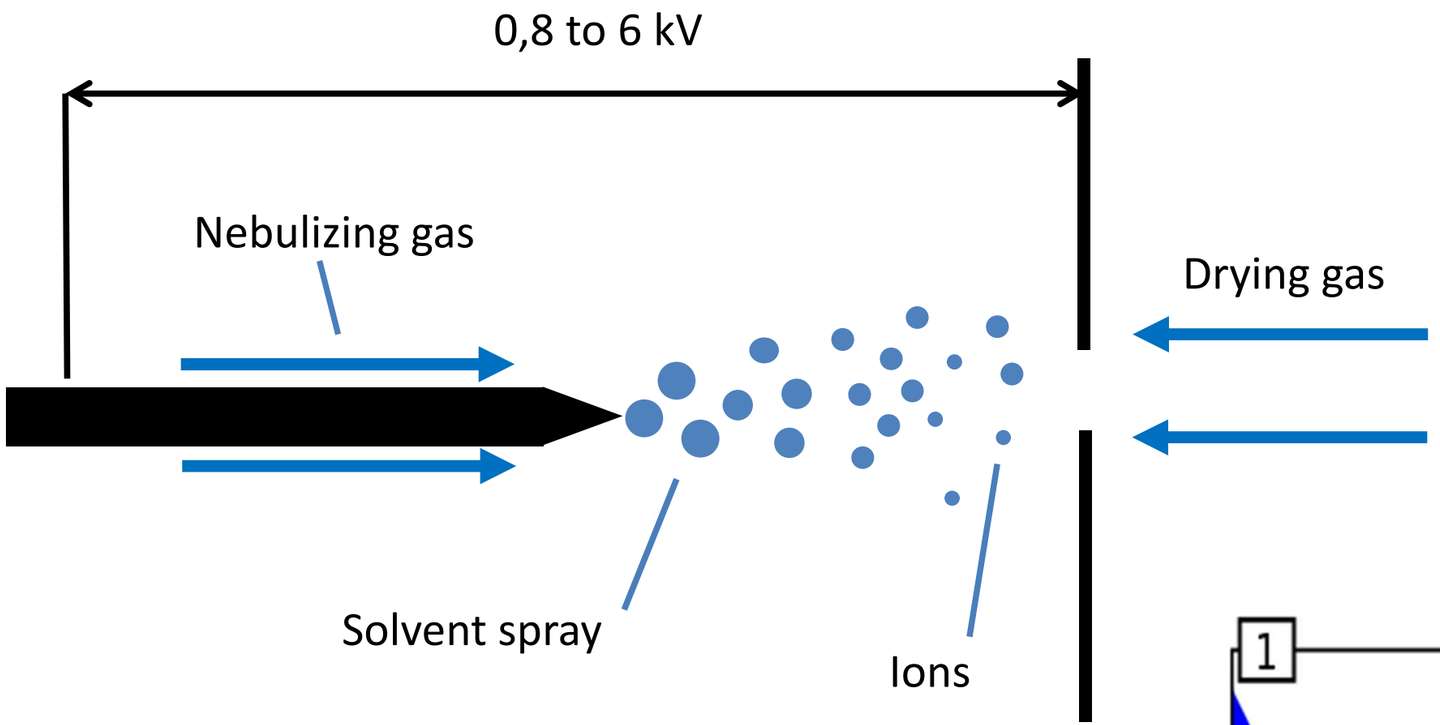
Sources, acquisition frequency, high resolution

Sources



- ESI
  - Polar
  - High mass
- APCI
  - Low polarity
  - Small mass
- APPI
  - Non polar
  - Small mass

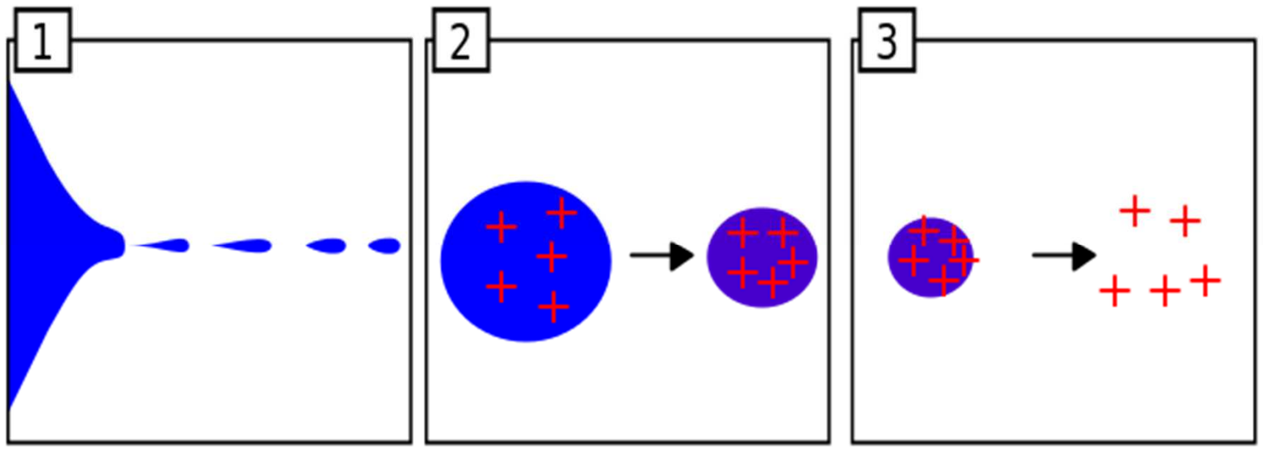
Electrospray ionization (ESI)



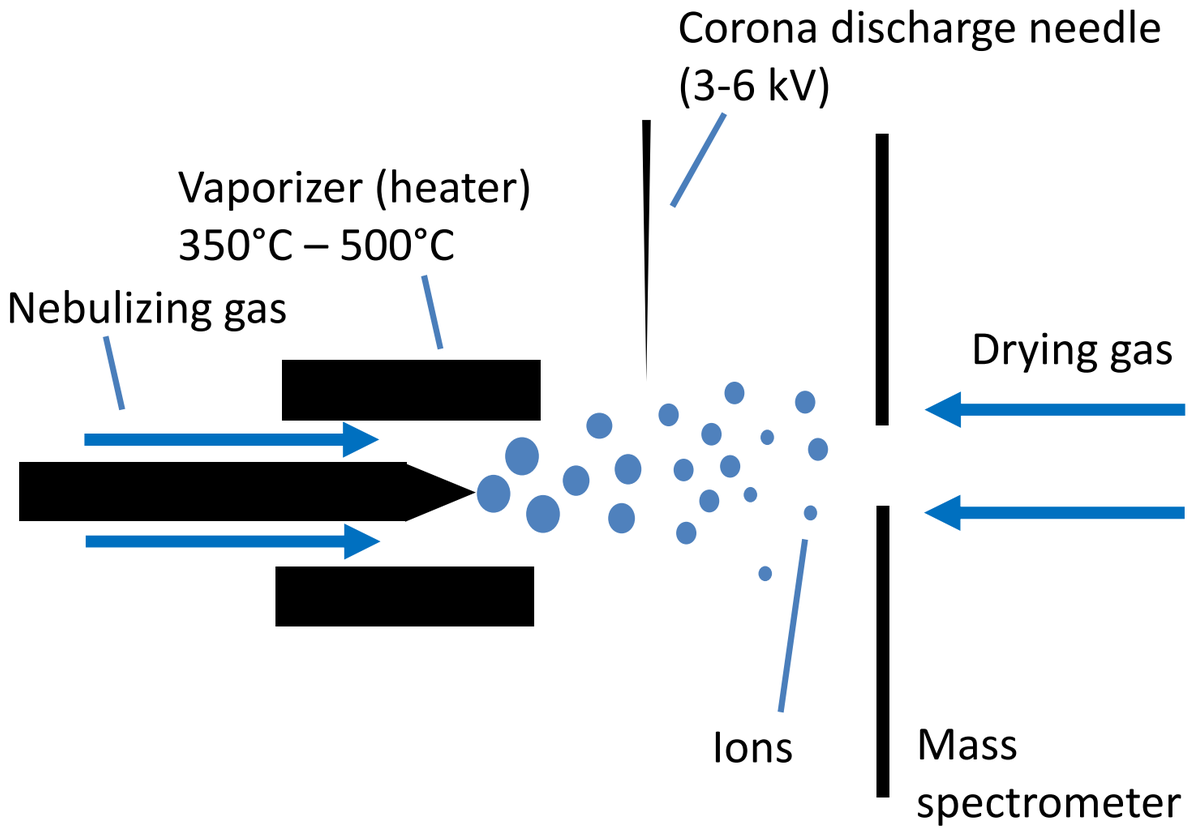
1980 : John Fenn

Wide range of charge states
Soft ionization technique

Salt adducts



Atmospheric pressure chemical ionization (APCI)



Reagent ion formation:

$$N_2 + e^- \rightarrow {N_2}^{+\cdot} + 2e^-$$
$${N_2}^{+\cdot} + 2N_2 \rightarrow {N_2}^{+\cdot} + 2e^-$$
$${N_4}^{+\cdot} + H_2O \rightarrow {H_2O}^{+\cdot} + 2N_2$$
$${H_2O}^{+\cdot} + H_2O \rightarrow H_3O^+ + OH^\cdot$$
$$H_3O^+ + H_2O + N_2 \rightarrow H^+(H_2O)_2 + N_2$$
$$H^+(H_2O)_{n-1} + H_2O + N_2 \rightarrow H^+(H_2O)_n + N_2$$

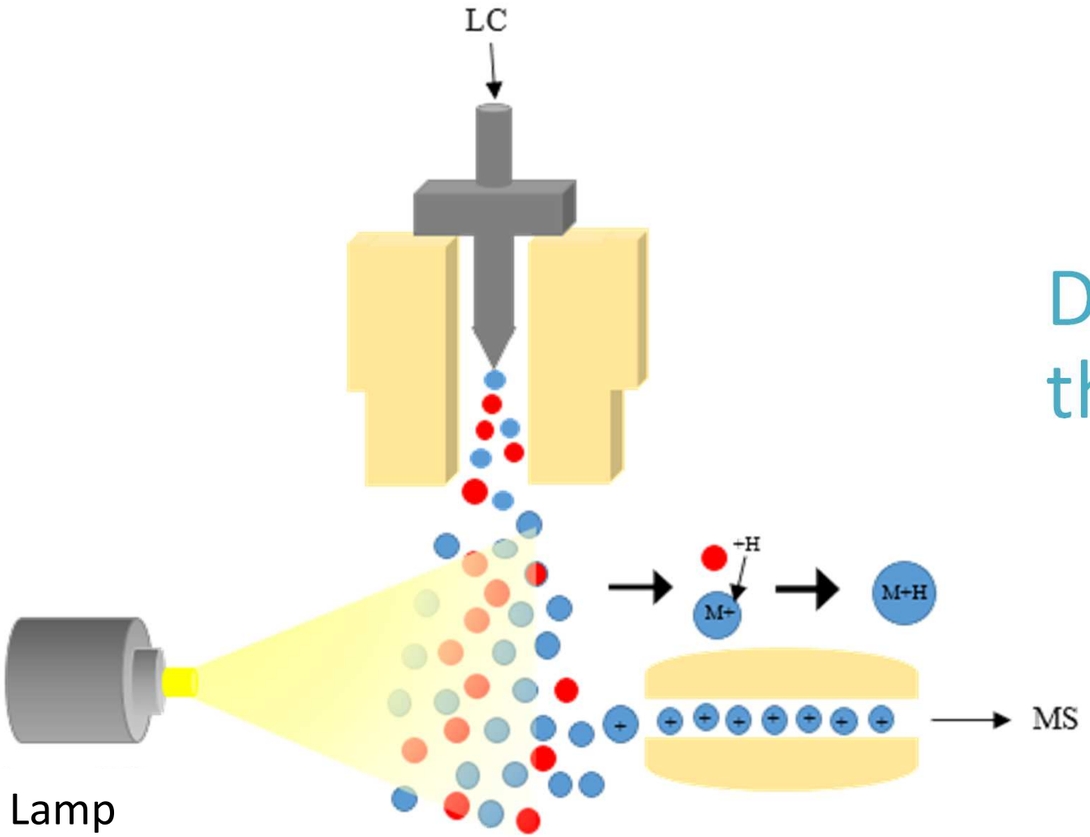
Ionization of product ions:

$$H^+(H_2O)_n + M \rightarrow MH^+(H_2O)_m + (n-m)H_2O$$

Declustering:

$$MH^+(H_2O)_m \rightarrow MH^+ + mH_2O$$

Atmospheric pressure photoionization (APPI)



$$h\nu + M \rightarrow M^{(+\bullet)} + e^-$$

Dopant with lower ionization energy than the sample. (10% to 30% v/v)

$$D + h\nu \rightarrow D^{(+\bullet)} + e^-$$

$$D^{(+\bullet)} + M \rightarrow [M + H]^+ + [D - H]^\bullet$$

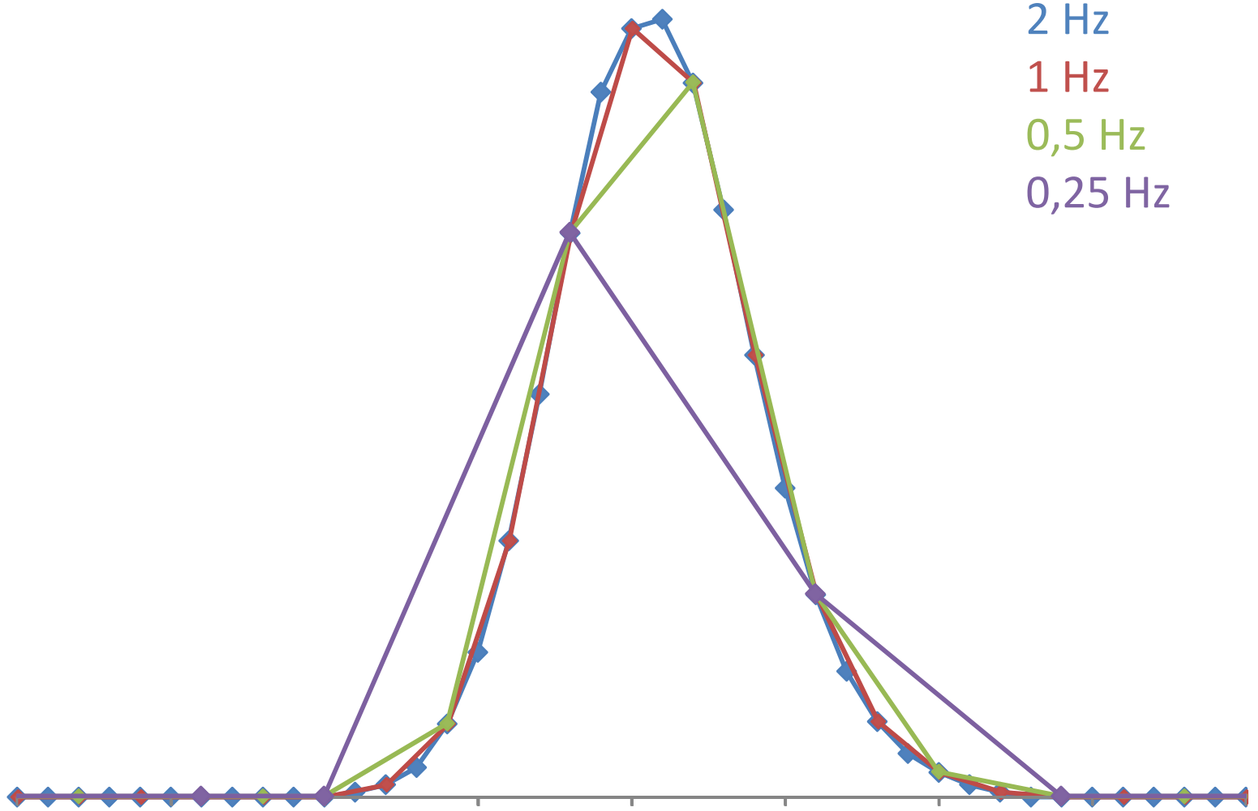
$$D^{(+\bullet)} + M \rightarrow M^{(+\bullet)} + D$$

Comparison ESI / APCI / APPI

	ESI	APCI	APPI
High polarity (drugs, peptides…)	+++	++	++
Medium polarity (steroids, pesticides, lipids…)	+	++	++
Low polarity (PAH…)	--	-	+
High flow rates	+	+++	+
Low flow rates	+++	-	+++
Immunity to ion suppression	--	+	++
Linear dynamic range and quantitation accuracy	+	+++	+++
Reverse phase performance	+++	++	++
Normal phase performance	-	+	+++
Thermally unstable compounds	++	-	+

Acquisition frequency

Higher scan frequency for better chromatographic resolution



Mass resolving power depends on scan rate

Minimum resolution?

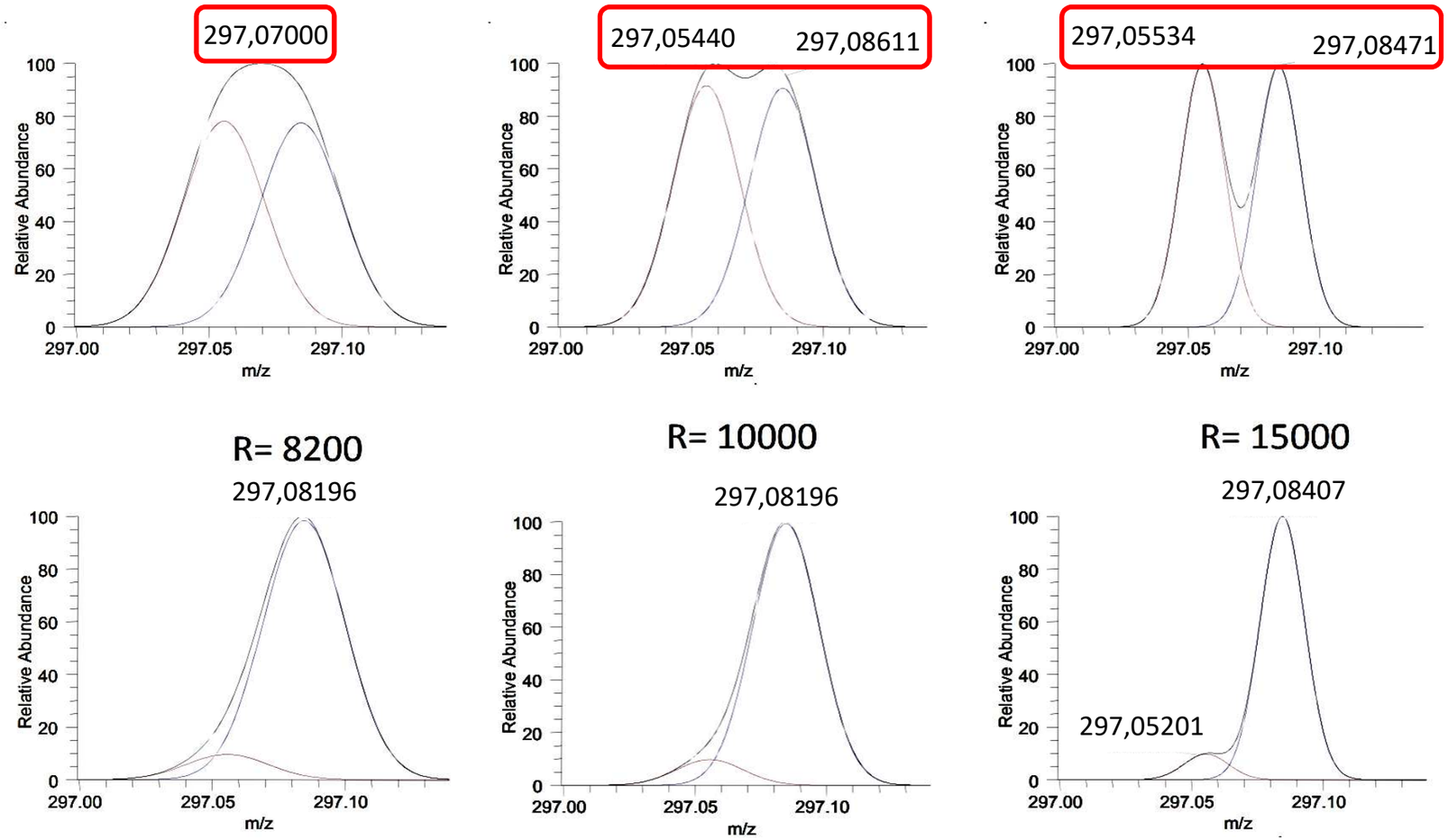
Complex miture spiked with 151 pesticides, veterinary drugs, mycotoxins and plant toxins (10 to 250 ng/g)

- High selectivity for every singly compound in the mixture
- High specificity and sensitivity
- Confirmation of compound identity
- Accurate quantification of analytes found

→ Minimum of resolution?

Mol H. *et al.,* jasms, 2009, 20, 1464-1476

Minimum resolution?



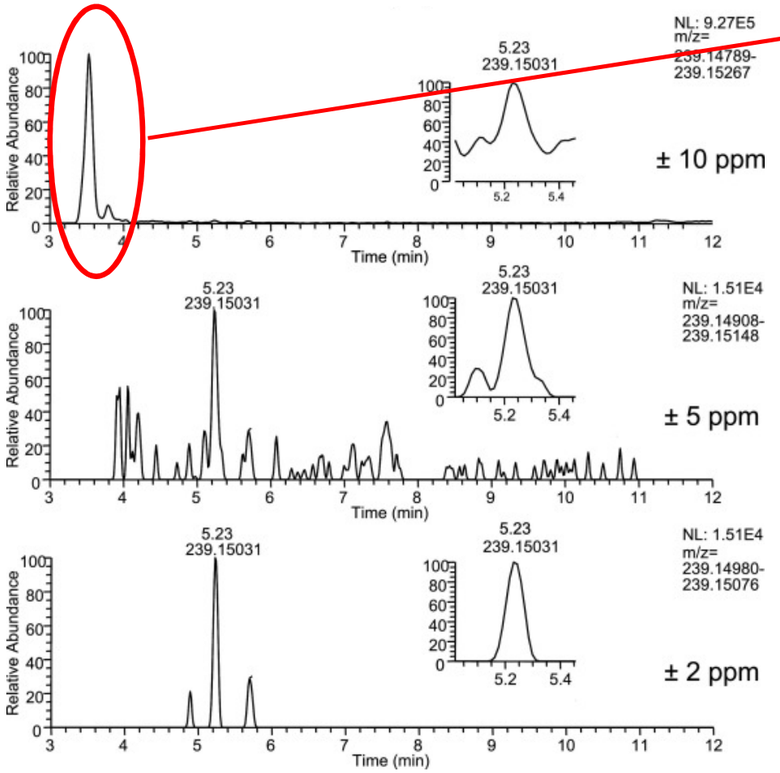
→ Influence of the resolving power on the mass accuracy

LC (RP C_{18}) Orbitrap 10,000 to 100,000 (FWHM @ *m/z* 200)

10 Hz to 1 Hz

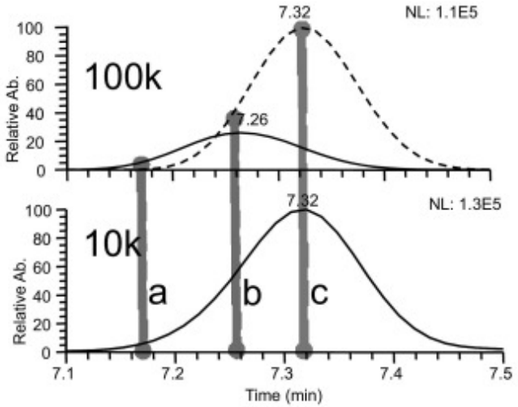
Mol H. *et al.*, jasms, 2009, 20, 1464-1476

Minimum resolution?

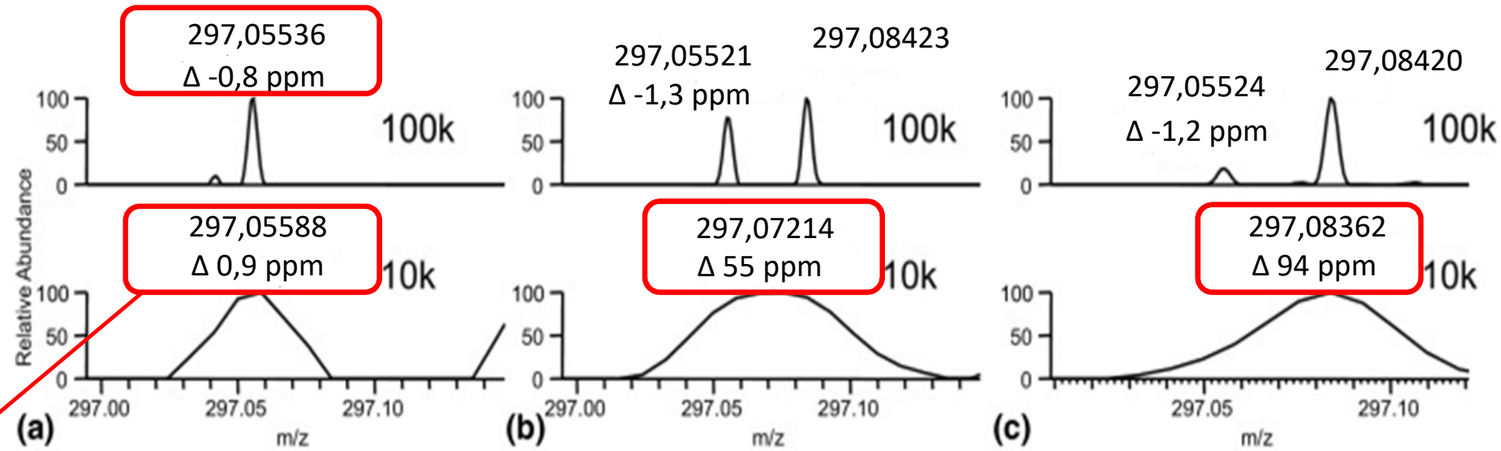


EIC of pirimicarb in horses feed matrix
(RT = 5,23 min)
Resolution @ 100,000

Matrix interference



Mass accuracy



Imazalil (MH^+ 297.05560, $C_{14}H_{14}Cl_2N_2O$, RT = 7,26 min)
Flunixin (MH^+ = 297.08454, $C_{14}H_{11}F_3N_2O_2$, RT = 7,32 min)

Mol H. *et al.*, jasms, 2009, 20, 1464-1476

Minimum resolution?

	% of 151 analytes Mass deviation (ppm)				
Resolution	**< 2**	**2-5**	**5-10**	**10-25**	**>25 / ND**
Honey					
10,000	83	12	5	0	0
25,000	100	0	0	0	0
50,000	100	0	0	0	0
100,000	100	0	0	0	0
Animal feed					
10,000	22	25	16	20	17
25,000	41	17	26	2	14
50,000	93	4	1	0	2
100,000	99	0	0	0	1

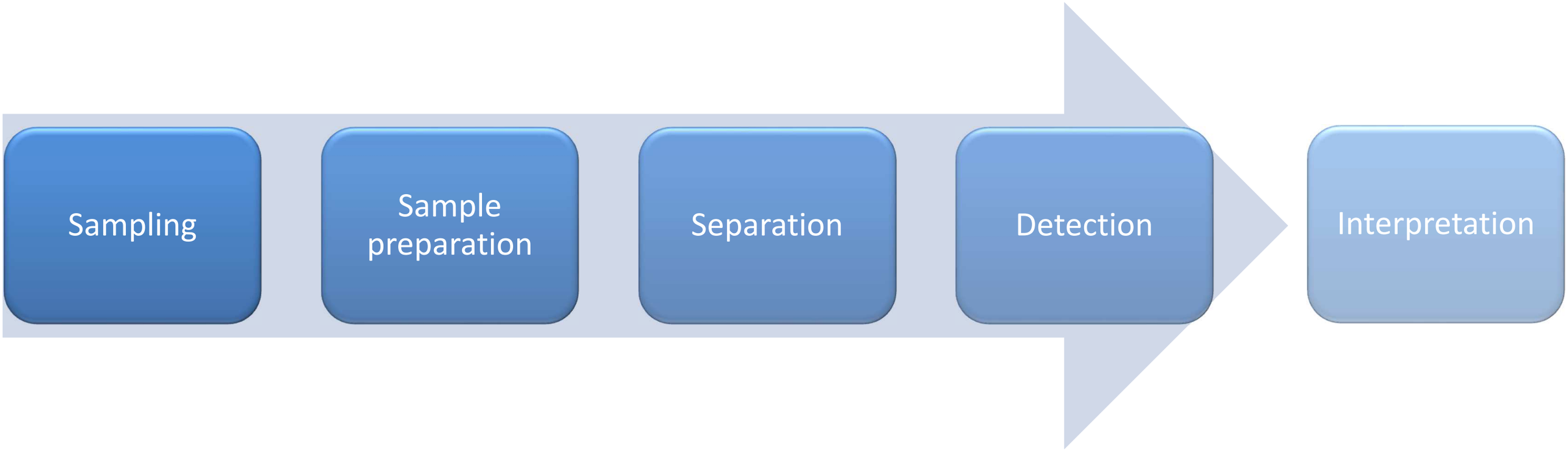
"In **highly complex extracts,** a resolving power of **35,000–50,000 or even 70,000–100,000 is required**."

Mol H. *et al*., jasms, 2009, 20, 1464-1476

POLLUTANTS ANALYSIS

Sampling, preparation, analysis

Analytical process



Sampling

- Soils
  - Excavation
  - Drilling
  - Core sampling…
- Water
  - Water column
  - Rosette sampler
  - Passive sampler…
- Air
  - Bubbling
  - Filters
  - Adsorbants…

Matrix effect

Matrix influence on the analyte

Retention time shift, ion suppression, mass accuracy…

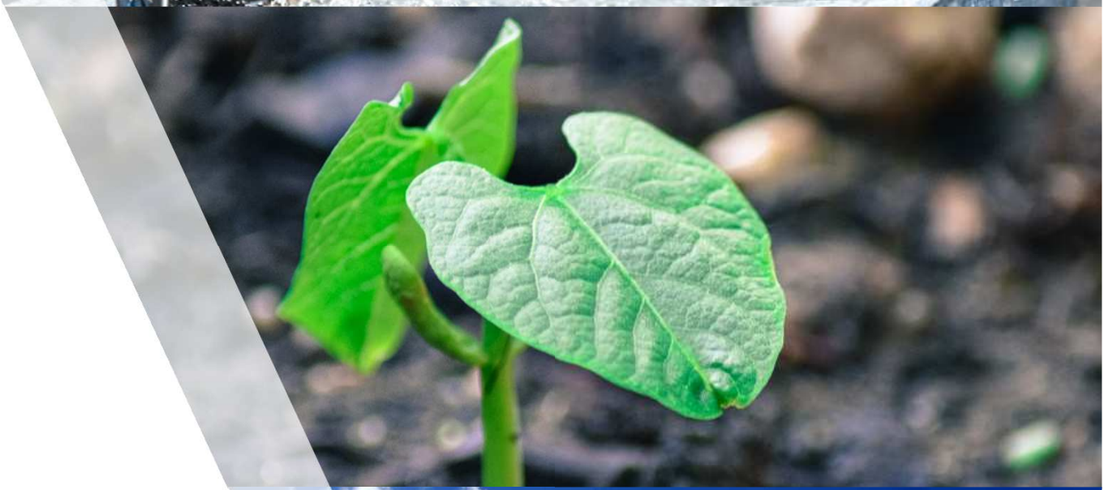
Reduce component of matrix (dilution, sample preparation…)

Improve chromatography (gradient, mobile and stationary phases…)

Change ionisation mode (positif/negatif or ESI/APPI/APCI)

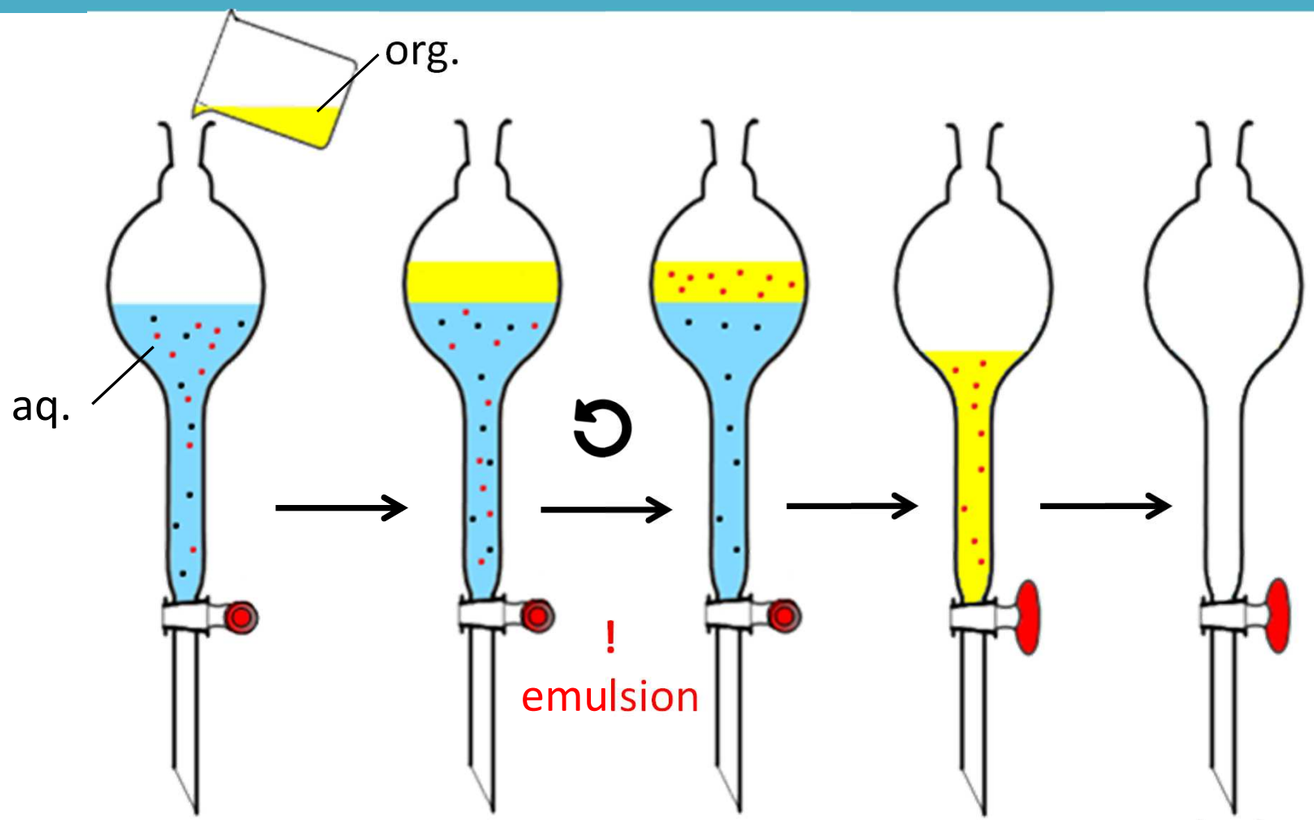
Use of reference matrix and standards

Sample preparation



- Liquid sample
  - Liquid-liquid extraction (LLE)
  - Solid phase extraction (SPE)
  - Dilution…
- Solid sample
  - Ultrasonic assisted extraction (UAE)
  - Microwave assisted extraction (MAE)
  - Liquid-solid extraction (LSE)…

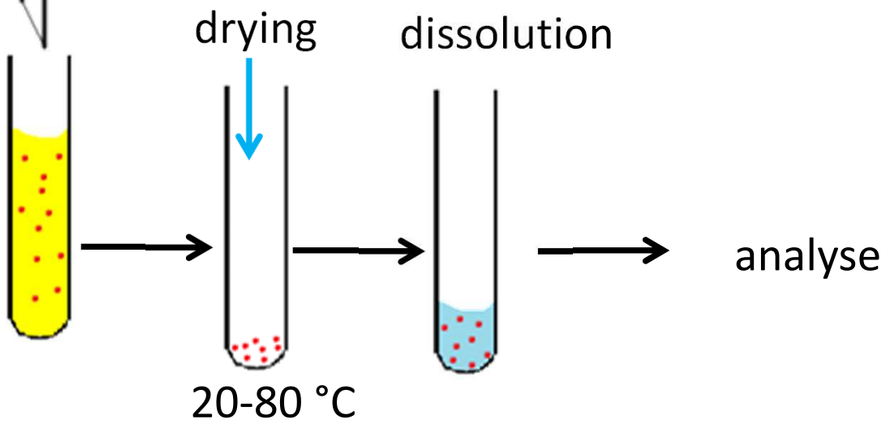
Liquid-liquid extraction (LLE)



Separation of compounds based on relative solubility in immiscible liquids.

Variant:

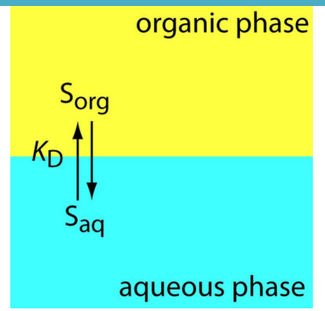
- Dispersive liquid liquid microextraction (DLLME)
- Single drop microextraction (SDME)



Extraction efficiency

K_D : partition coefficient

$$K_D = \frac{[S]_{org}}{[S]_{aq}}$$

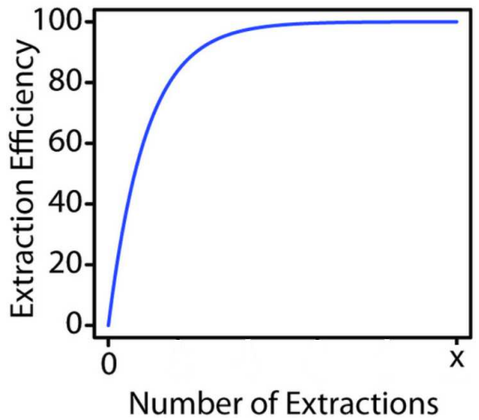


Fraction of solute remaining in aqueous phase after extraction

$$(q_{aq})_1 = \frac{n_{S_{(aq)1}}}{n_{S_{(aq)initiale}}} = \frac{[S]_{aq} * V_{aq}}{[S]_{org} * V_{org} + [S]_{aq} * V_{aq}} = \frac{V_{aq}}{K_D * V_{org} + V_{aq}}$$

Extraction efficiency X for n extractions:

$$X = 1 - (q_{aq})_n = 1 - \left(\frac{V_{aq}}{K_D * V_{org} + V_{aq}}\right)^n$$



Example

$K_D = 5$

$V_{aq} = 50$ mL

$V_{org} = 15$ mL

$n = 1$

X ?

$$X = 1 - \left(\frac{V_{aq}}{K_D * V_{org} + V_{aq}}\right)^n$$

$$X = 1 - \left(\frac{50}{5 * 15 + 50}\right)^1 = 0{,}6$$

$X = 0{,}999$ V_{org}?

$$V_{org} = \frac{V_{aq} - (1 - X) * V_{aq}}{(1 - X) * K_D}$$

$$V_{org} = \frac{50 - (1 - 0{,}999) * 50}{(1 - 0{,}999) * 5} = 9990 \text{ mL}$$

Example

$K_D = 5$

$V_{aq} = 50$ mL

$V_{org} = 15$ mL

$X = 0{,}999$

n ?

$$X = 1 - \left(\frac{V_{aq}}{K_D * V_{org} + V_{aq}}\right)^n$$

$$log(1 - X) = n\, log\left(\frac{V_{aq}}{K_D * V_{org} + V_{aq}}\right)$$

$$n = \frac{log(1 - X)}{log\left(\frac{V_{aq}}{K_D * V_{org} + V_{aq}}\right)} = \frac{log(1 - 0{,}999)}{log\left(\frac{50}{5 * 15 + 50}\right)} = 7{,}53$$

$$n = 8$$

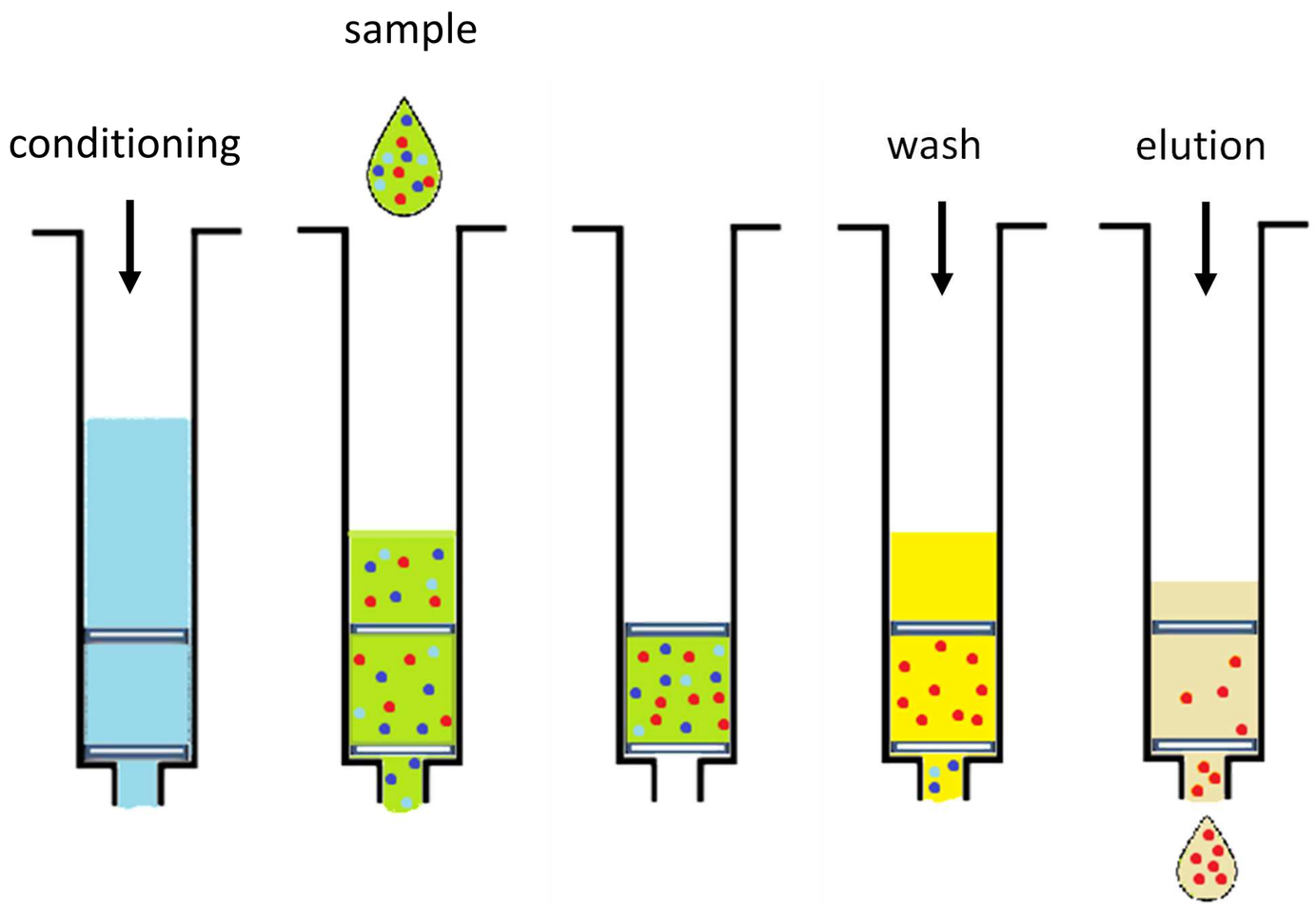
n high, V_{org} small

Strengths and weaknesses

- Wide range of solvents (selectivities and solubilities)
- Easy

- Emulsions
- Time consuming
- Toxicity and volume of solvants
- Number of step

Solid phase extraction (SPE)



Comparable with LC:

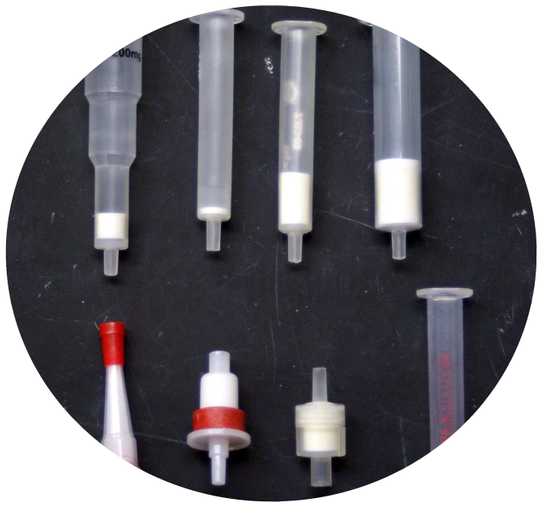
- Stationary phase
  - Reverse phase, normal phase, Ion Exchange…
- Mobile phase

- Variant:
  - Solid-phase microextraction (SPME)
  - Stir bar sorptive extraction (SBSE)…

Analyte Contaminants

Optimisation

- Sample volume
  - Cartridge capacity
- Nature of adsorbant
  - Hydrophilic or hydrophobic
  - Polar or non-polar...
- Sample pre-teatment
  - pH adjustment
  - Filtration
  - Centrifugation…

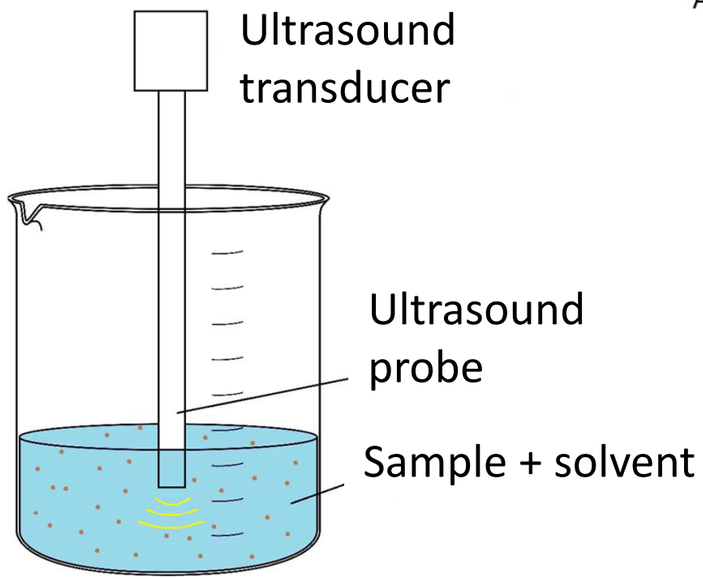


Strengths and weakness

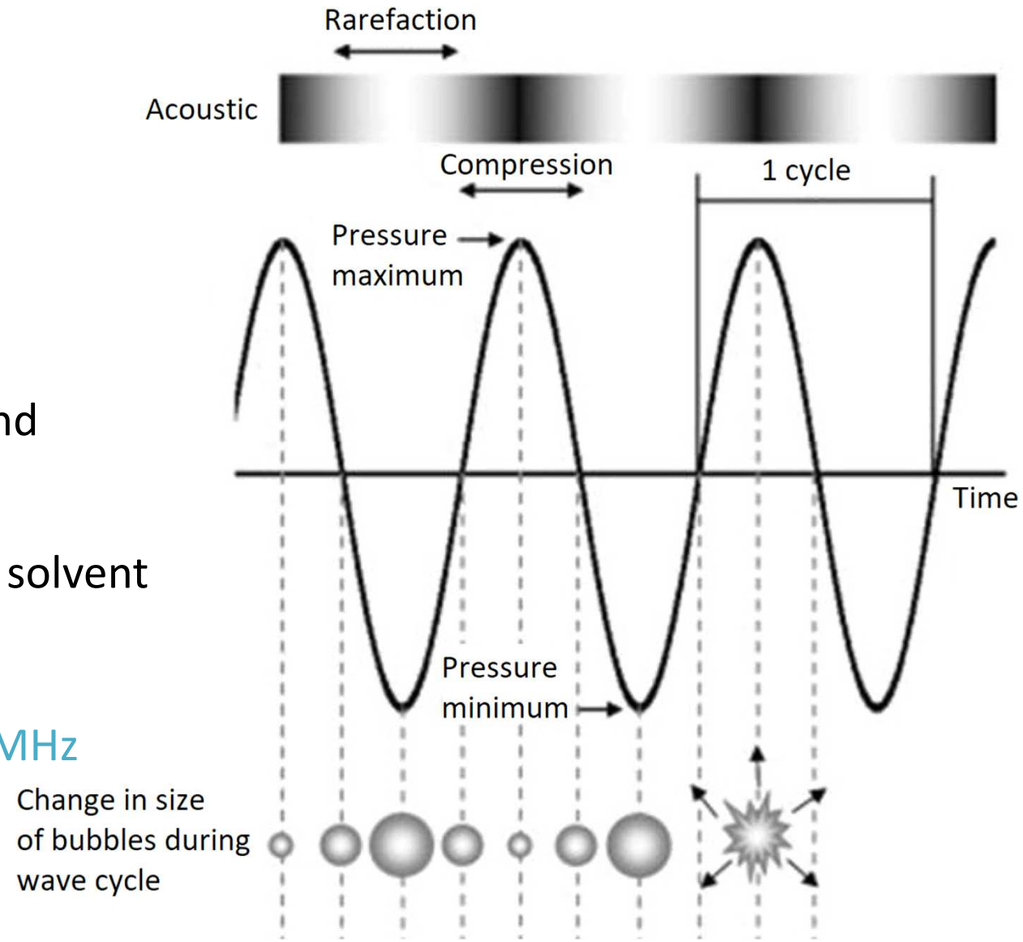
- Time consuming
- Accuracy and precision (flow, volume…)
- Method development

- Automated / On-line
- Large choice of stationary phase, size and extraction solvants
- Preconcentration and extraction

Ultrasound-assisted extraction (UAE)



Frequency from 20 kHz to 10 MHz



Low intensity (< 1 W/cm^2):

non-destructive analysis

High intensity (10 – 1000 W/cm^2):

improve efficiency of sample preparation

Villamiel M. *et al.,* Trends Food Sci. Technol., 2010, 21, 323

Strengths and weakness

- Post-treatment (filtration, concentration…)
- Toxicity of extraction solvent

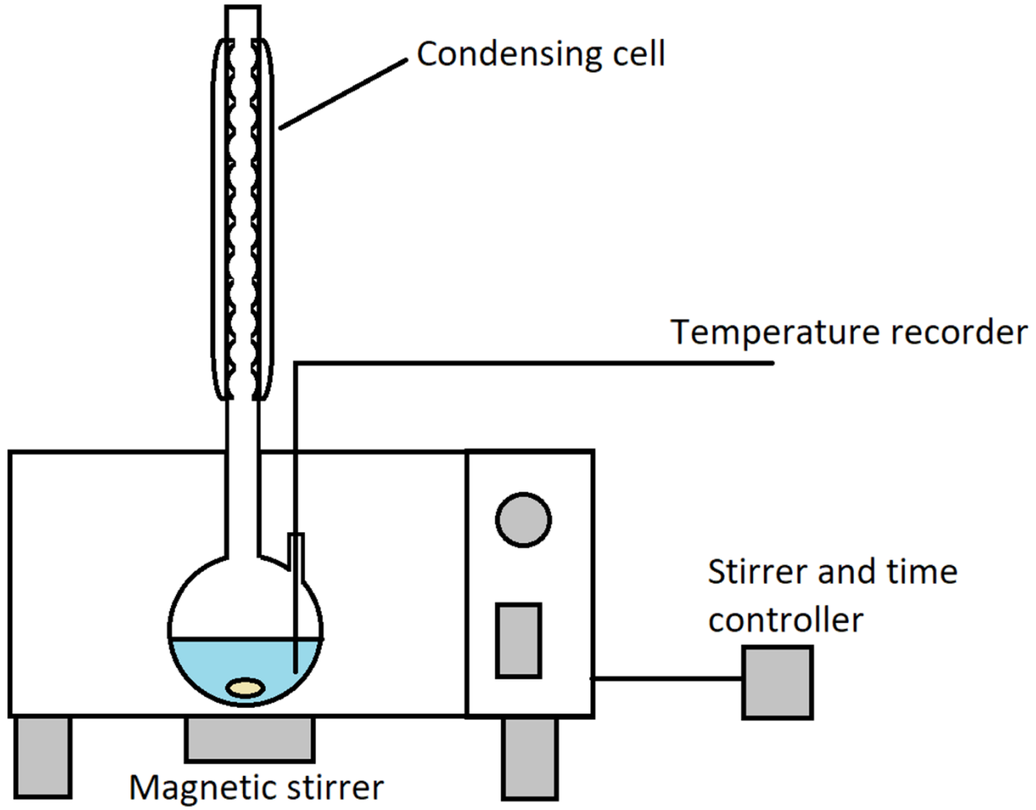
- Fast
- Large scale of application (solids and liquids)
- Unsophisticated instrumentation required

Microwave assisted extraction (MAE)

Microwave radiation interact with dipole of polar and polarizable material (orient in the changing field direction → heated)

Variant:

- Focused microwave-assisted extraction (FMAE)
- Pressurized microwave-assisted extraction (PMAE)
- Dynamic microwave-assisted extraction (DMAE)



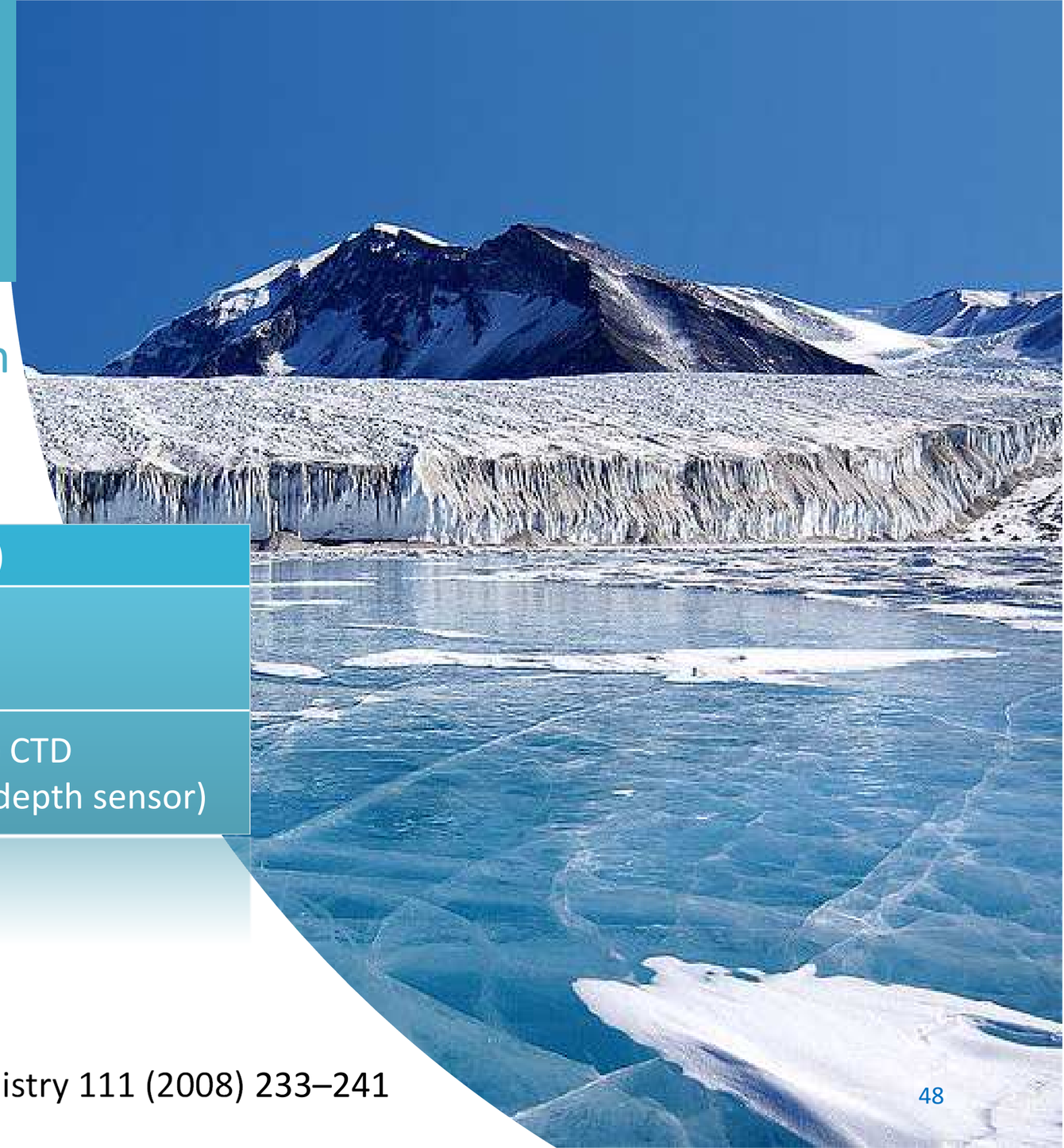
Microwave region frequencies: 300 MHz to 100 GHz. (domestic and scientific ovens operate at 2.45 GHz)

Strengths and weakness

- Expensive instrumentation
- Re-adsorption possible during cooling step
- Post-treatment

- High extraction rate (rapid heating and elevated temperature)
- Fast and easy
- Automatisation possible (on-line)

Characterization of marine dissolved organic by LC-FT-ICR (off-line)



Improvement of DI-FTMS with LC separation

Brine	Drill holes (100 cm of depth)
Edge water	Pumped into clean canister
Under ice water	
Antarctic surface sea water	Rosett sampler connected to CTD (Conductivity, temperature, depth sensor)
Weddell sea deep water	

B.P. Koch *et al.,* Marine Chemistry 111 (2008) 233–241

Characterization of marine dissolved organic by LC-FT-ICR (off-line)

Sample preparation:

160 L of aqueous sample

↓

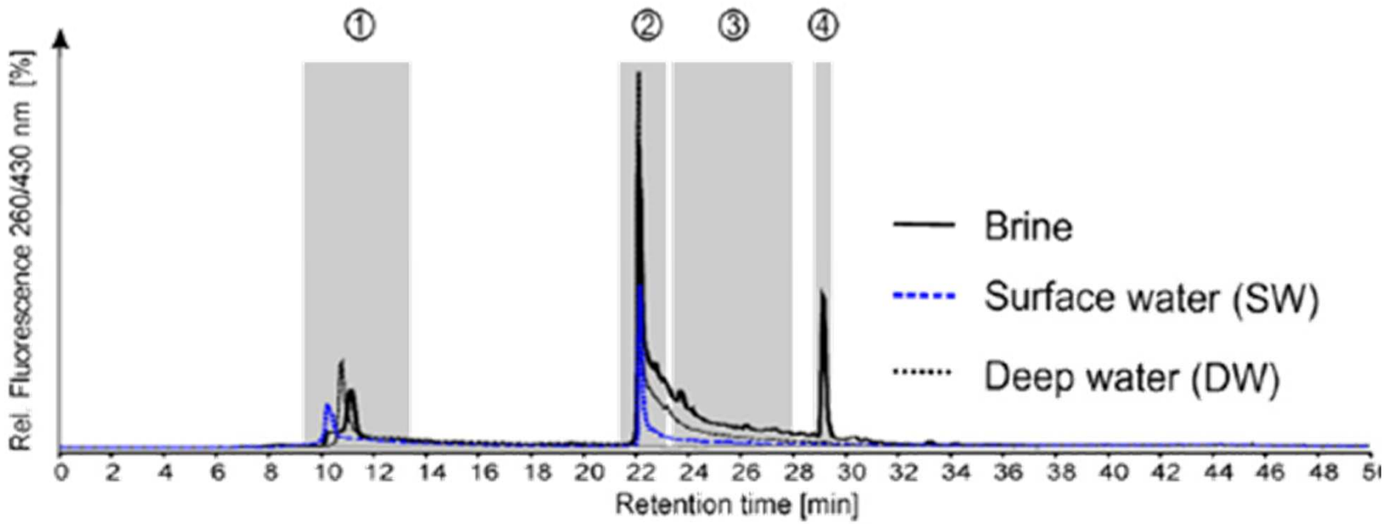
Filtration (0,2 µM) + pH 2 (HCl)

↓

SPE (PPL 5 g, Varian Mega Bond Elut)

Chromatographic separation:

C18 RP Phenomenex, synergi



B.P. Koch *et al.,* Marine Chemistry 111 (2008) 233–241

Characterization of marine dissolved organic by LC-FT-ICR (off-line)

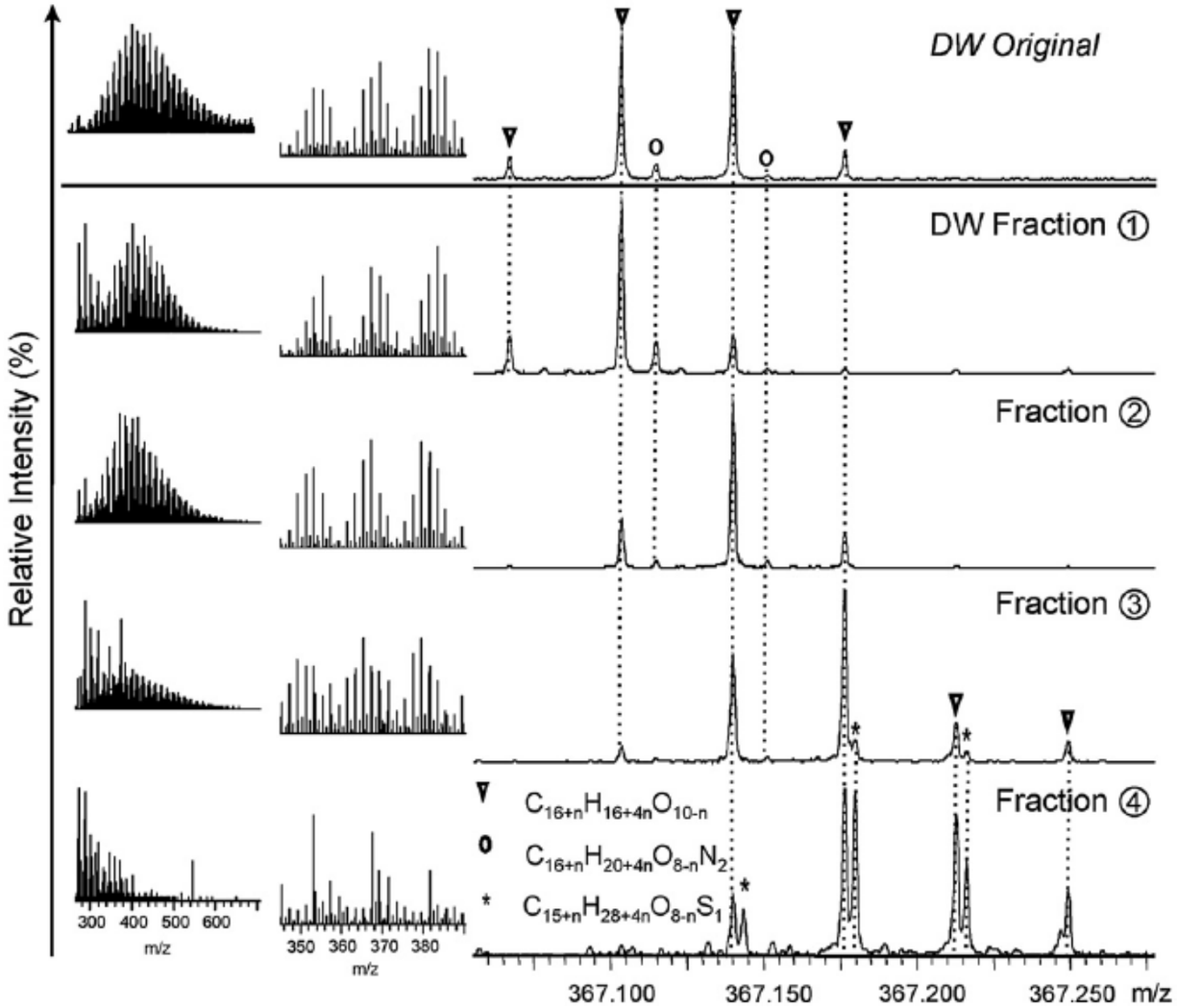
FT-MS analysis:

ApexQe 9,4T (Bruker) ESI neg

3000 pics per fraction → 75% assigned (mass accuracy: 0,5ppm)

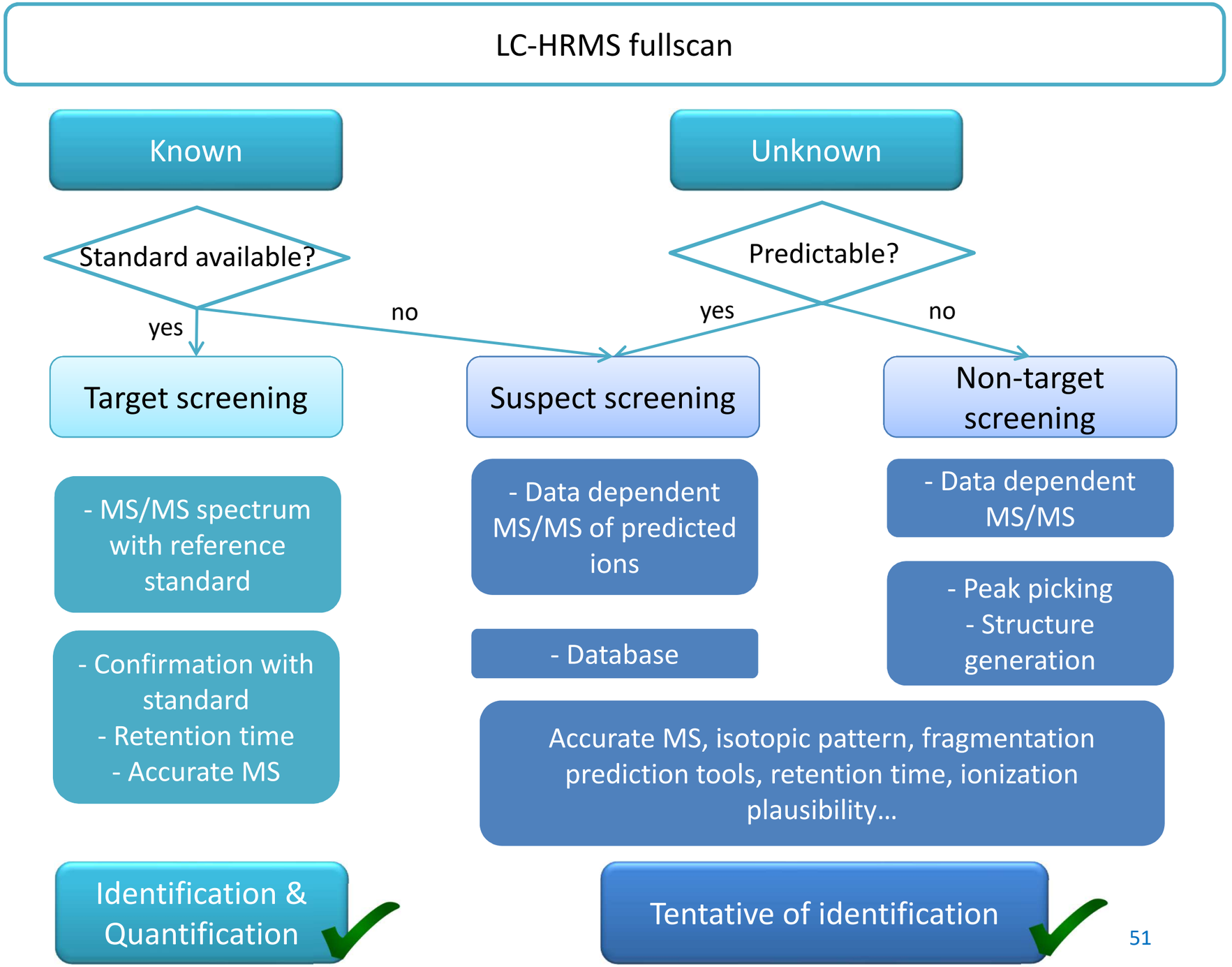
→ 70 to 90 % of unique pic

→ Isomers separation



B.P. Koch *et al.,* Marine Chemistry 111 (2008) 233–241

LC-FTMS analytical strategy



Target screening

Known products and standards available

LC-HRMS (fullscan and MS/MS)

- Increase of specificity and selectivity
- all compounds determinated simultaneously (fullscan mode)

EU decision 2002/657/EC →4 identification points (IP)

MS technique	Identification point earned per ion
Low-resolution MS (LR-MS)	1.0
LR-MS^n precursor ion	1.0
LR-MS^n transition product	1.5
High-resolution MS (HR-MS)	2.0
HR-MS^n precursor ion	2.0
HR-MS^n transition product	2.5

Target screening of 170 pesticides

Fruits and vegetables extracts spiked with 170 selected pesticides (10 µg/kg to 500 µg/kg)

UHPLC-Orbitrap HESI positive:

- R = 17,500; 35,000 and 70,000 (FWHM @ *m/z* 200) → 12; 7 and 3 scans per second
- Thermo scientific accucore AqC_{18}

Orbitrap had similar quantitative performance than QqQ

- Avoiding false positives and negatives
- Allowing a retrospective evaluation

Fernandez-Alba R. *et al.,* J. Chrom A 1360 (2014) 119-127

Target screening of 170 pesticides

"pymetrozine in orange at a 10 g/kg level **was not detected** when analysed **with a 17,500 resolution**. An increase of resolution to 35,000 resulted in a peak containing four points. **A further increase to 70,000 resulted in a peak containing over 40 points**"

"**With a 17,500 resolution it is easy to obtain false negative results** even when ionization was very efficient and a large number of ions reached the detector."

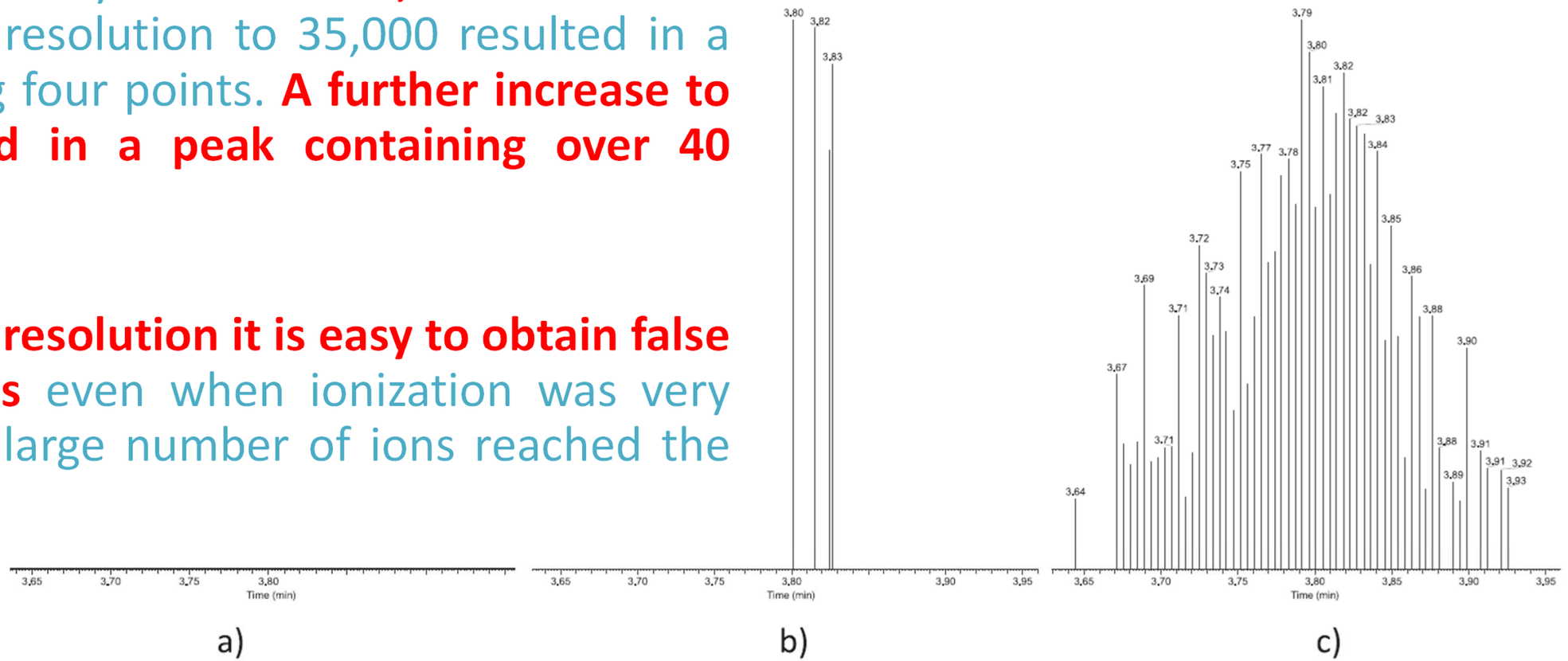


Fig. 4. Increase of number of points per peak at higher resolution. 10 µg/kg of pymetrozine in orange extract, peak obtained with resolution (a) 17,500, (b) 35,000 and (c) 70,000.

Fernandez-Alba R. *et al.,* J. Chrom A 1360 (2014) 119-127

Suspect screening

Known products WITHOUT standards available
or
Suspected unknown pollutants

Use of prediction system for transformation products:

- University of Minnesota Pathway Prediction System (UM-PPS: http://eawag-bbd.ethz.ch/)
- CATABOL (http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity/catabol-301c.aspx)
- PathPred (http://www.genome.jp/tools/pathpred/)
- Meteor (http://www.lhasalimited.org/products/meteor-nexus.htm)

Fragmentation pathway (should be similar to pollutant)


Stockholm, Sweden

Suspect screening of OH-PAH

LC-Orbitrap HESI negative, resolution 120,000 (FWHM):

- Strategy : Targeted and suspects

Sampling of air particulates

- glass fiber filters.
- Extraction: ASE (accelerated solvent extraction system)
- Concentration: solid phase extraction (SPE).

20 hydroxylated polycyclic aromatic hydrocarbons detected:

- 9 using the target screening
- 11 tentatively identified and semi-quantified by suspect screening

Westerholm R. *et al.,* Talanta 165 (2017) 702-708

Non target screening

Unknown pollutants and unpredictable

FullScan HR-MS/MS, mass error < 5ppm

Post-acquisition data-processing	MZmine	http://mzmine.sourceforge.net/
	XCMS	https://xcmsonline.scripps.edu
	EnviMass	http://www.eawag.ch/forschung/uchem/software/enviMass1
Comparison to database	MassBank	http://massbank.ufz.de/MassBank/
	MetLin	http://metlin.scripps.edu/index.php
	m/zCloud	https://www.mzcloud.org/
Fragmentation rules	MOLGEN-MS	http://www.molgen.de/
	ACD/MS Fragmenter	www.acdlabs.com/products/adh/ms/ms_frag
	MassFrontier	http://www.highchem.com/index.php/component/content/article?id=81
	SIRIUS	http://bio.informatik.uni-jena.de/sirius2/

Non target screening of pesticides metabolites

LC-Orbitrap HESI positive, Resolution 50,000 (FWHM)

- Strategy: suspect and non-target screening
- database: 240 pesticides metabolites
- « fragmentation-degradation »: non-target analysis

Sampling of air particulates (Valencia region)

- Quartz fiber filters
- Extraction by MAE
- Concentration, filtration

Identified pesticides and metabolites:

- 34 metabolites identified, 11 confirmed by standards
- 2 elucidated for non target screening

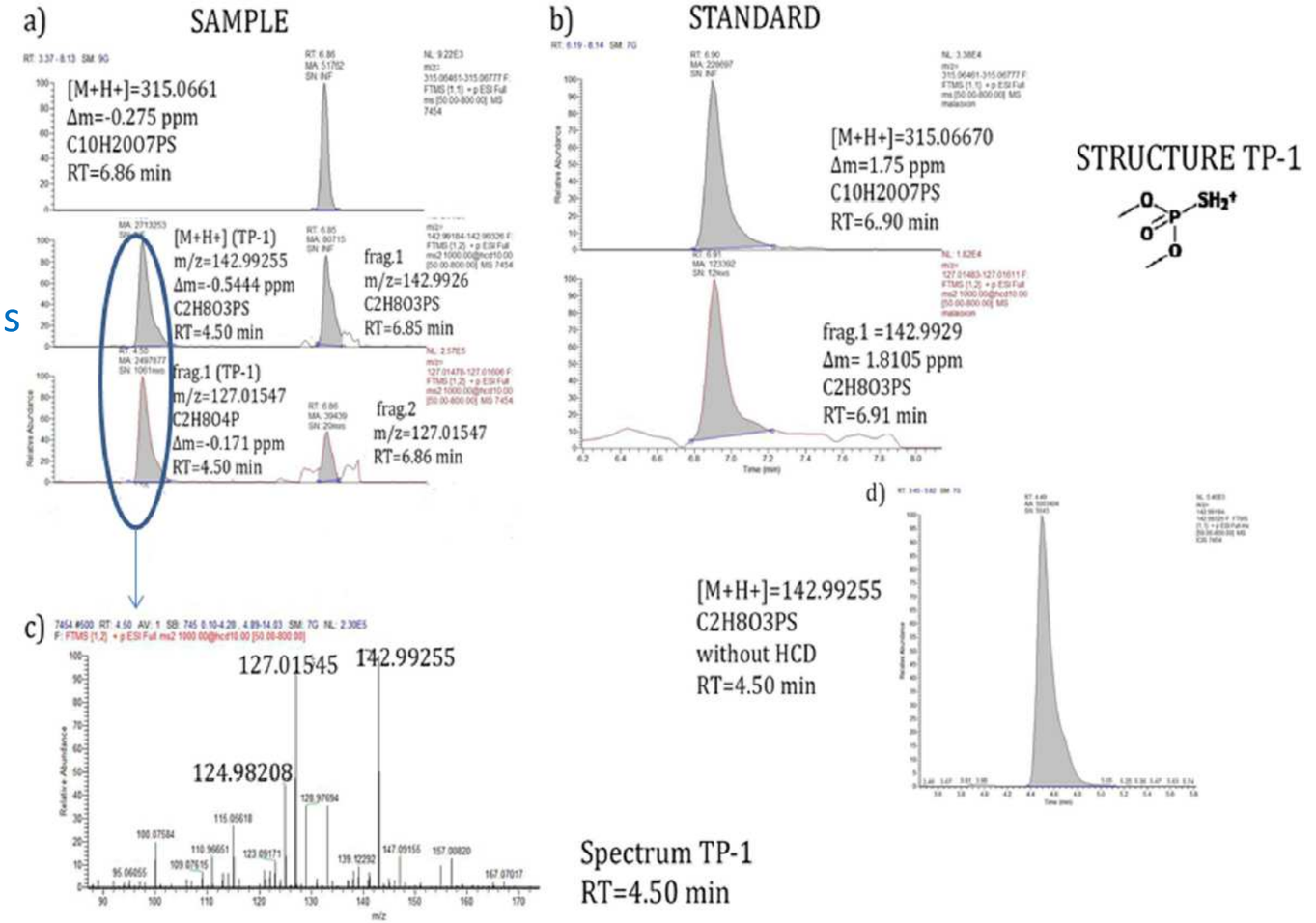
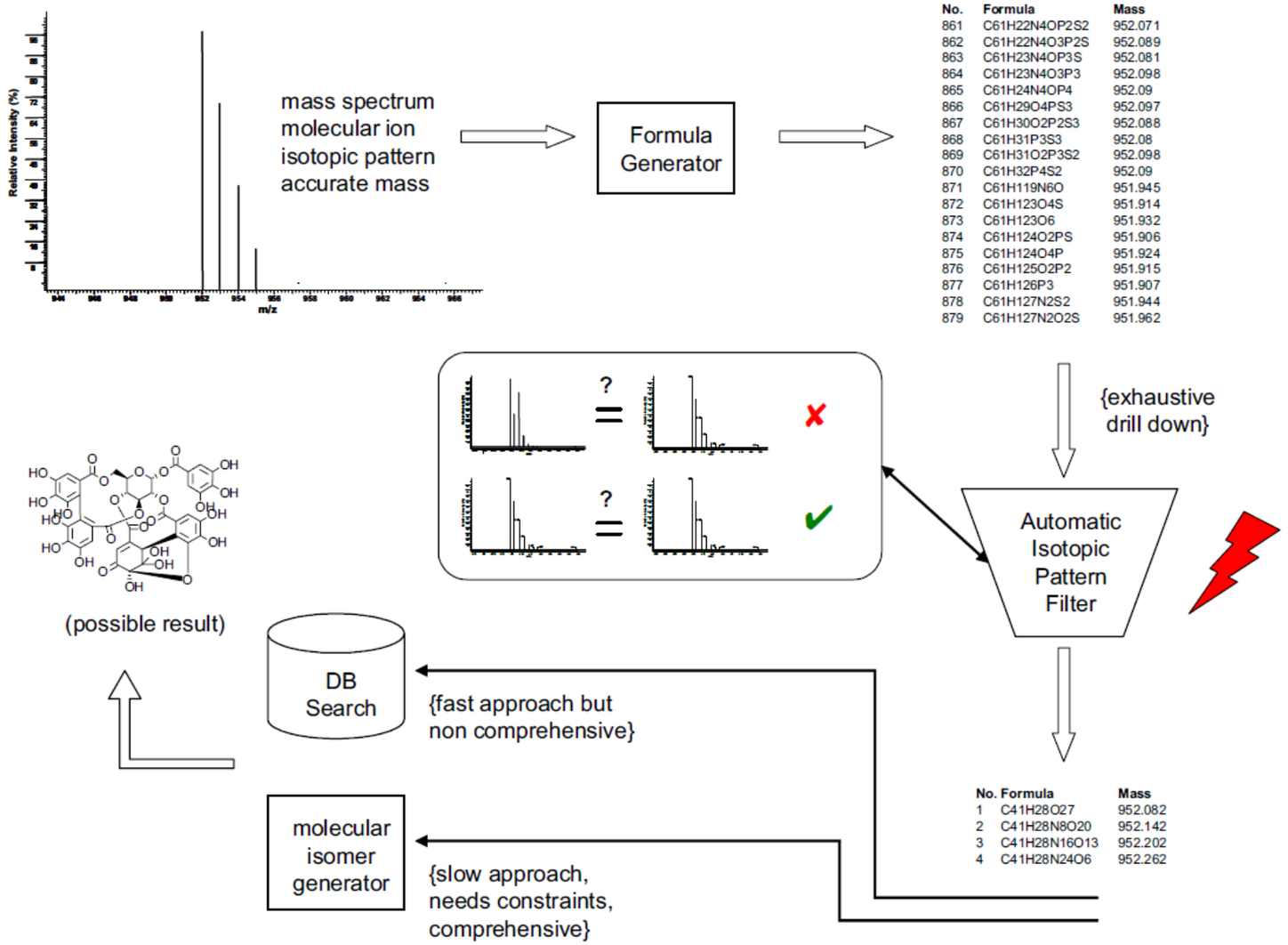


Fig. 4. (a) Extracted ion chromatograms (XIC) of malaoxon (m/z=315.0661), its fragments (m/z 142.9926; m/z 127.01547); and of a transformation product (TP-1) of malathion/malaoxon (m/z 142.9926) obtained from air sample; (b) XIC of malaoxon standard and a characteristic ion fragment; (c) Accurate mass spectrum at 4.50 min, which corresponds to malathion/malaoxon transformation product-1 (TP-1); d) XIC of PT-1 (m/z=142.99255) without HCD.

A. López *et al.*, Talanta 150 (2016) 27–36

Mass accuracy and isotopic abundance accuracy



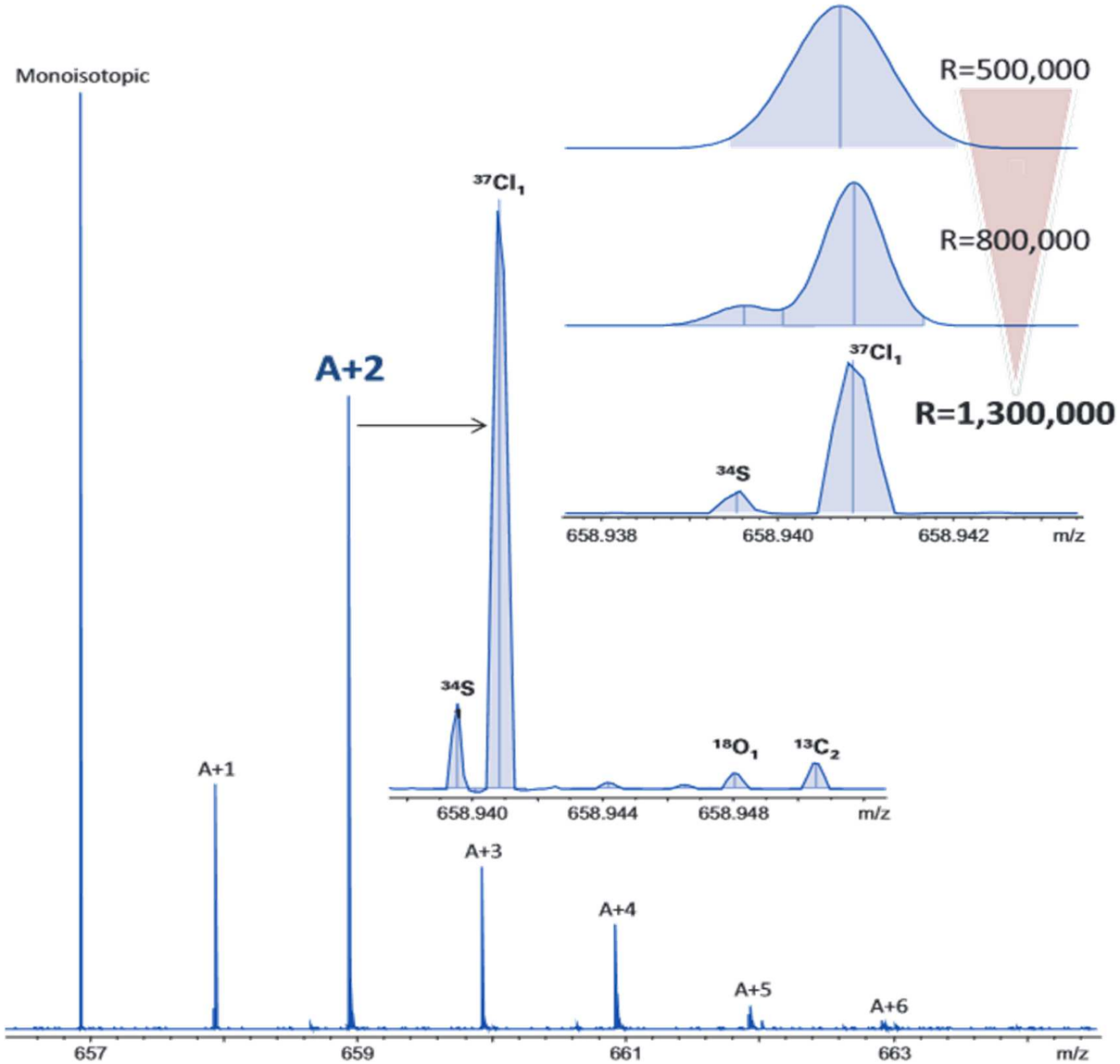
Kind T. and Fiehn O., BMC Bioinformatics (2006) 7:234

Mass accuracy and isotopic abundance accuracy

Without isotope abundance information						2% isotopic abundance accuracy	5% isotopic abundance accuracy
Molecular mass (Da)	**10 ppm**	**5 ppm**	**3 ppm**	**1 ppm**	**0,1 ppm**	**3 ppm**	**5 ppm**
150	2	1	1	1	1	1	1
200	3	2	2	1	1	1	1
300	24	11	7	2	1	1	6
400	78	37	33	7	1	2	13
500	266	115	64	21	2	3	33
600	505	257	155	50	5	4	36

Kind T. and Fiehn O., BMC Bioinformatics (2006) 7:234

Ultra high resolution



Isotopic Fine Structure 09-2016 (1847374), Bruker

Conclusions

DI-FTMS

Sample fingerprint

Fast (< 5 min)

Less solvent

Isomers separation

Ion suppression

Dynamic range

Pollutant in low concentration

Conclusions

LC-FTMS

Isomers separation
Increase of dynamic range
Separation of mixture
Decrease of matrix effect on FT-MS
Better selectivity

Increase analysis time (10 to 50 min)
Matrix effect on retention time
Optimisation compounds dependent
Solvent

Conclusions

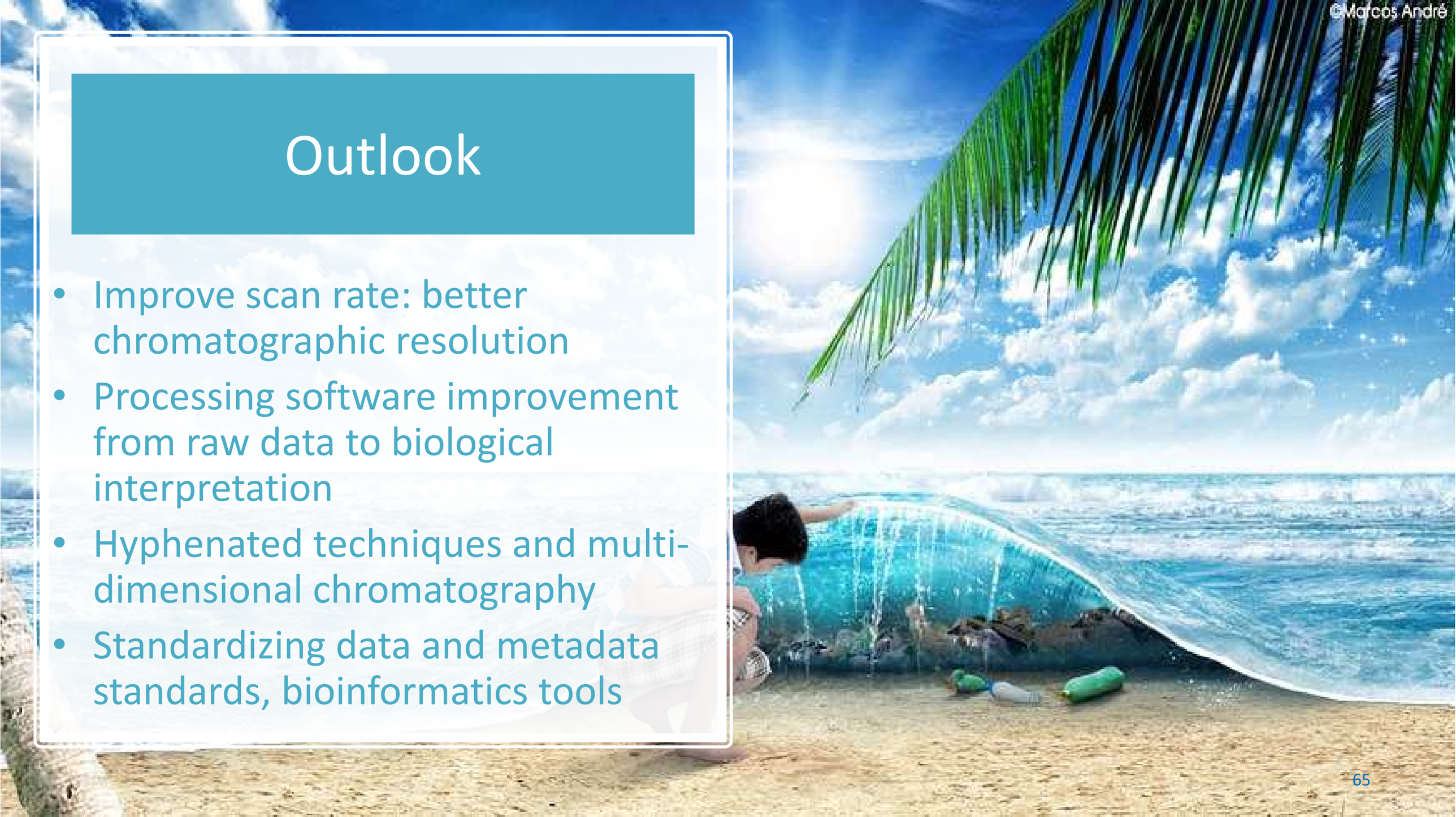
LC-FTMS vs LC-QqQ
Avoiding falses negatives and falses positives
Retrospective analysis

Resolution 500,000 to 10,000,000
Mass accuracy < 0,1 ppm
Femtogram-level sensitivity
Fast scanning rate at 15 Hz

Development of new data processing algorithms, software and databases

Outlook

- Improve scan rate: better chromatographic resolution
- Processing software improvement from raw data to biological interpretation
- Hyphenated techniques and multi-dimensional chromatography
- Standardizing data and metadata standards, bioinformatics tools




Thank you!