UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Overview of advanced instrumental techniques employed in food and feed analysis

Vit Kosek

Food composition

Natural components

Natural toxins

Antinutrition comps.

Primary sensorically active comp.

Antioxidants and other biologically active components

Nutrients

proteins
lipids
saccharides
minerals
vitamins

Fiber

Contaminants

Environmental contaminants

Pesticide / veterinary drug residues

Migrants from plastics

Toxic metals

Processing products

Additives

Biotechnology products

Food and feed analysis

Applications:

- Regulatory
- Food safety
- Quality control
- Research and development

We need the methods to be…

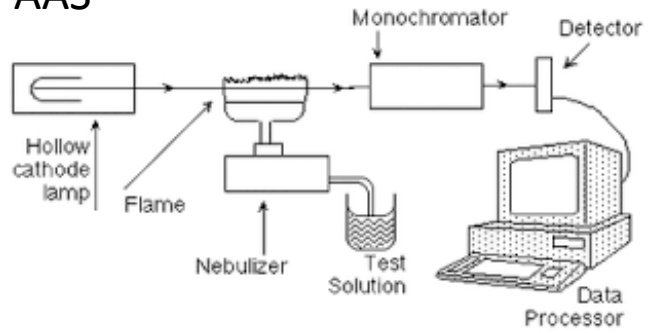
- Precise
- Reproducible
- Accurate
- Simple
- Cheap
- Fast
- Sensitive
- Specific
- Safe
- Destructive/Non-destructive
- Online/Offline
- Official

Techniques must be always fit for purpose!

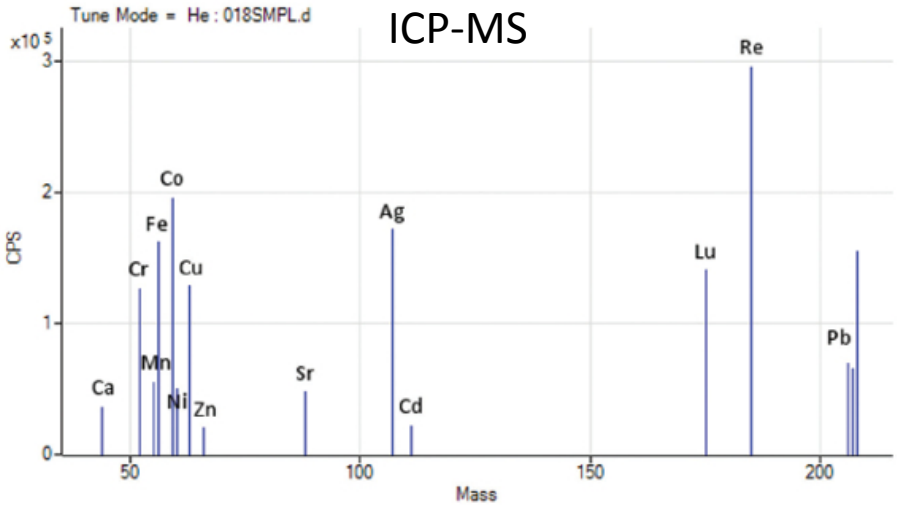
Elemental analysis

- Atomic absorption spectroscopy
- ICP-atomic emission spectroscopy
- ICP- mass spectrometry

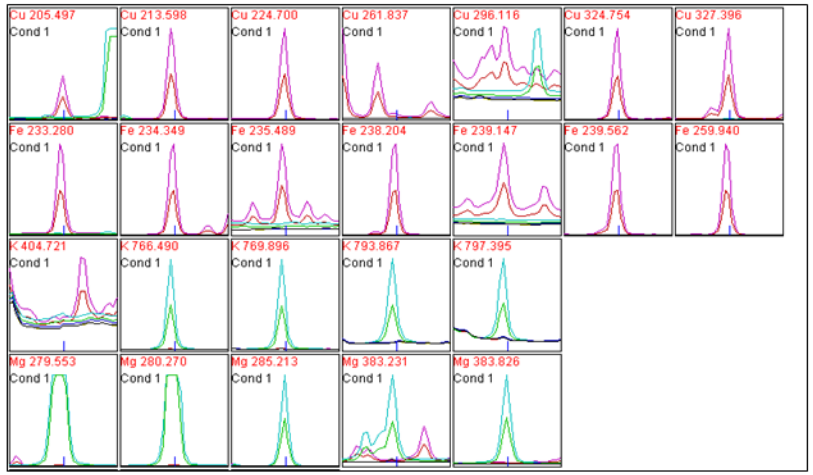
AAS



ICP-MS

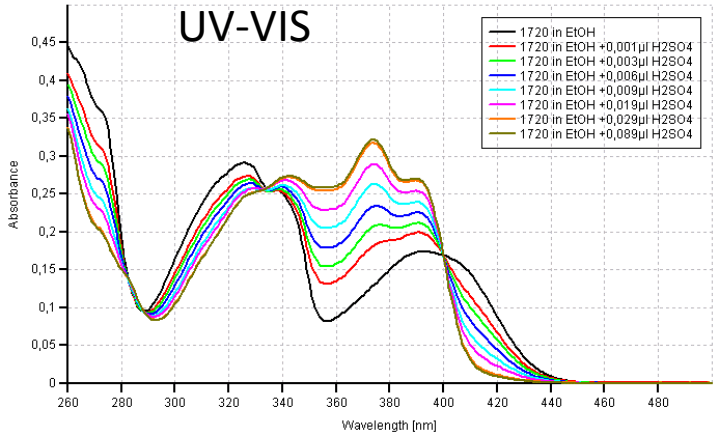


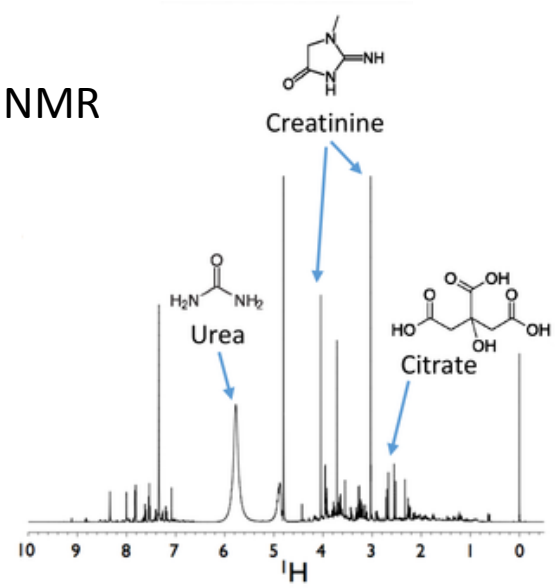
ICP-AES

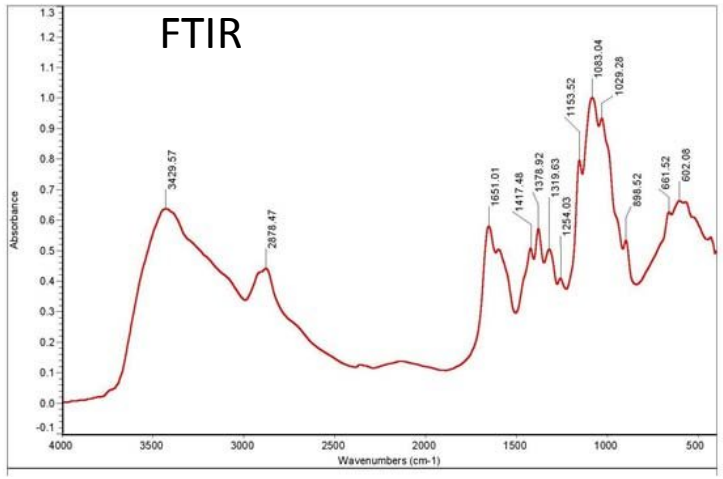


Molecular spectroscopy

- UV-VIS
- Infra-Red
- Nuclear Magnetic Resonance



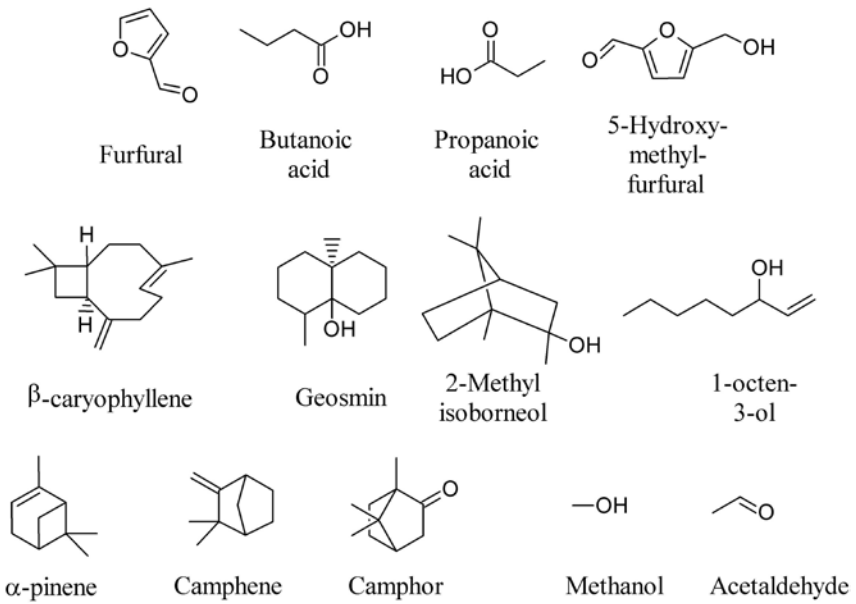




Gas chromatography

- Separation of sample constituents in gas phase
- On the basis of volatility and structure
- Analytes need to be sufficiently volatile and thermally stable
- Analytes usually up to 1000 Da

More on this topic: **Michal Stupák**



Liquid chromatography

- Separation of sample constituents in liquid state
- Wide range of analytes are separable
- No need for temperature stability
- Several mechanisms:
  - Hydrophobic interactions (reverse phase)
  - Polar interactions and hydrogen bonds (normal phase, HILIC)
  - Charge interactions (ion Exchange)

More on this topic: **Vojtěch Hrbek**

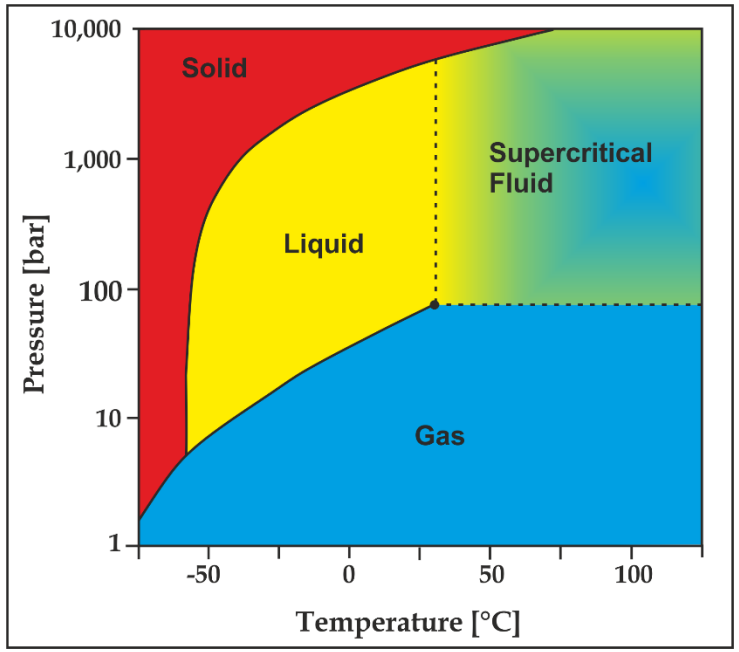
Supercritical fluid chromatography

- Mobile phase **- supercritical CO_2** (Tcrit = 31 °C, Pkrit = 7390 kPa)

- **Fluid with low viscosity and high diffusivity** → high separation efficiency, shortened time of analysis

- **Polarity of supercritical** CO_2 **~ hexane**

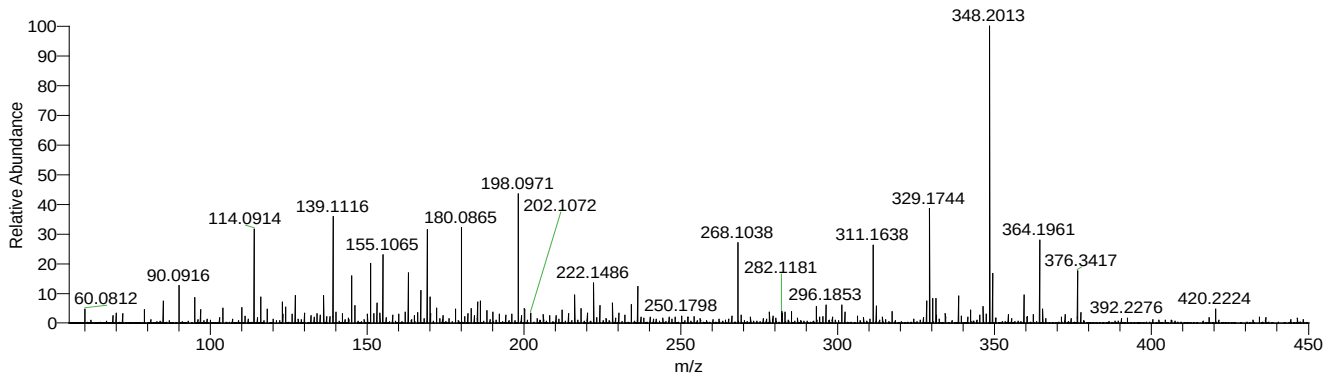
- Amenable for analytes with wide range of polarities



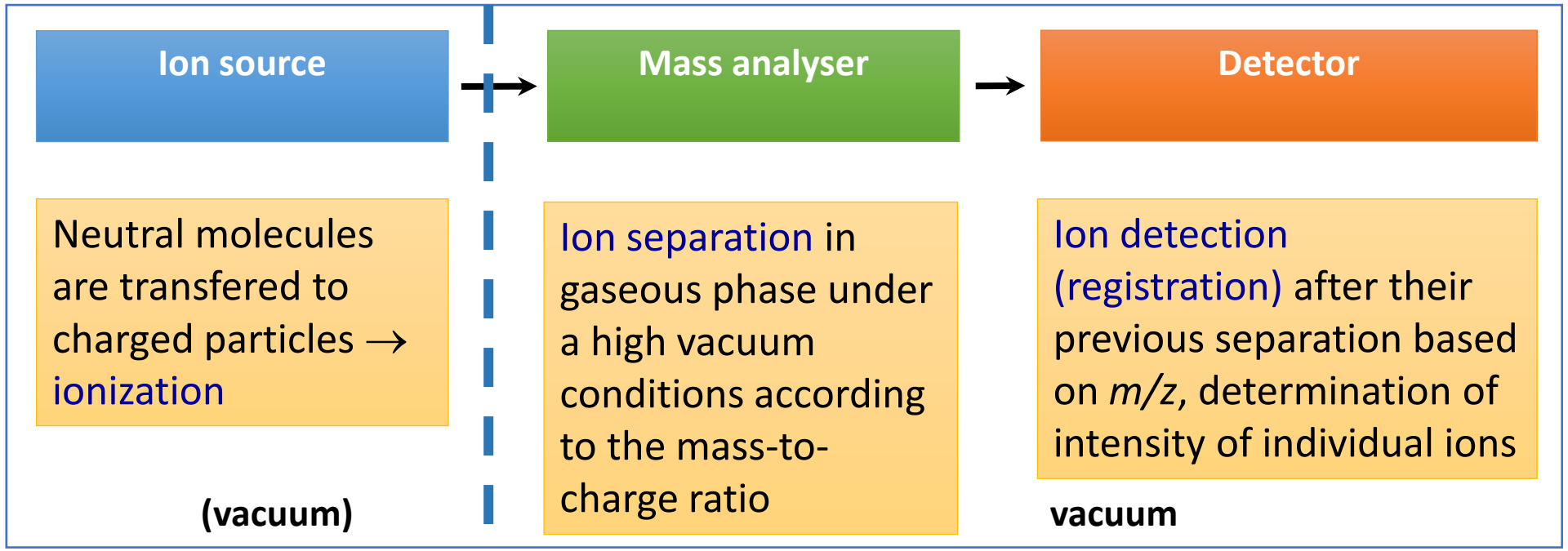
More on this topic with:
Beverly Bělková, Michaela Rektorisová

Mass spectrometry

- Weighing molecules
- Molecules need to be ionised
- Ions can be manipulated with in electric or magnetic field
- Mass spectrum: *m*/*z* X intensity
- Destructive X very sensitive
- Specific



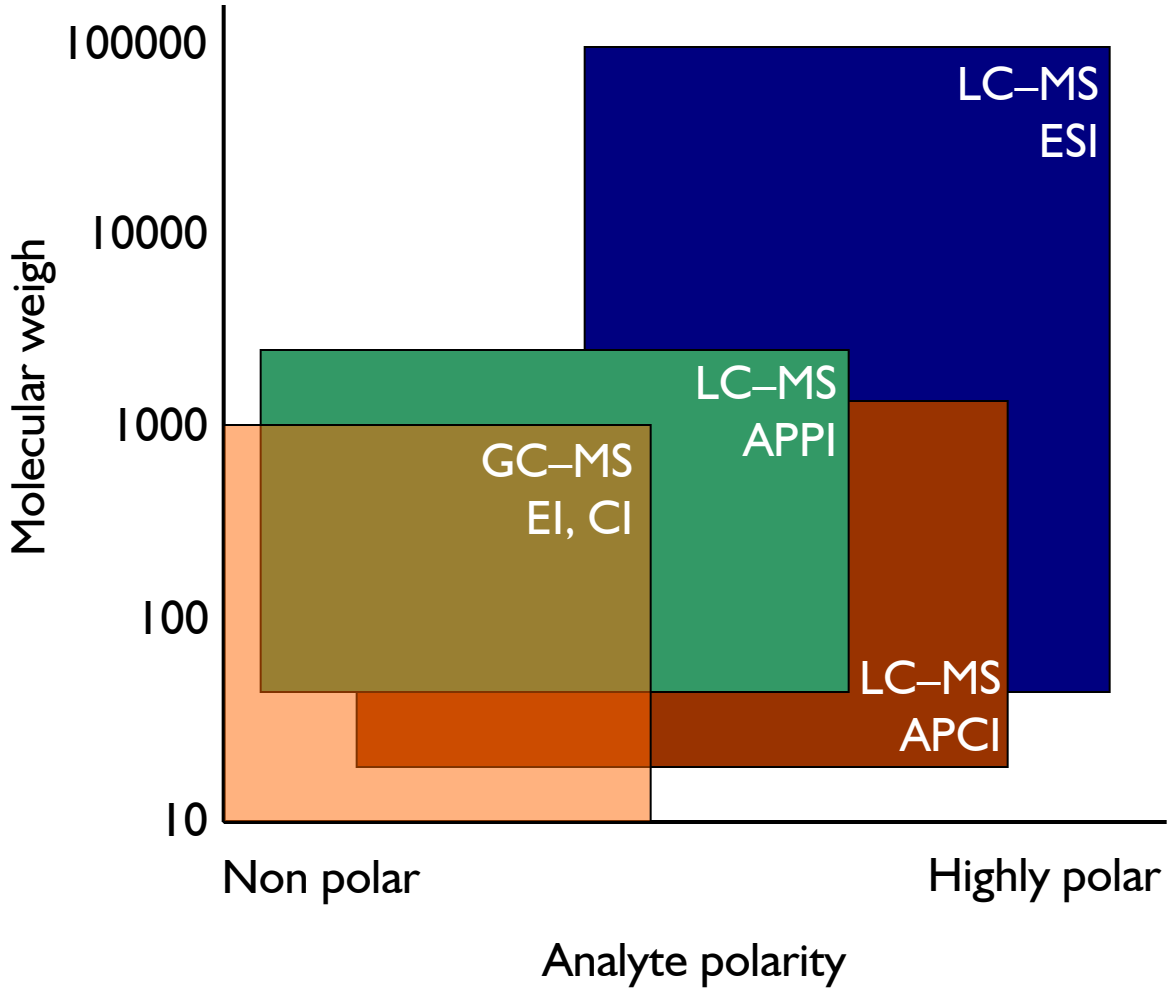
PARTS OF MASS SPECTROMETER



Electron **I**onization
Electrospray
Matrix **A**ssisted **L**aser
Desorption **I**onization

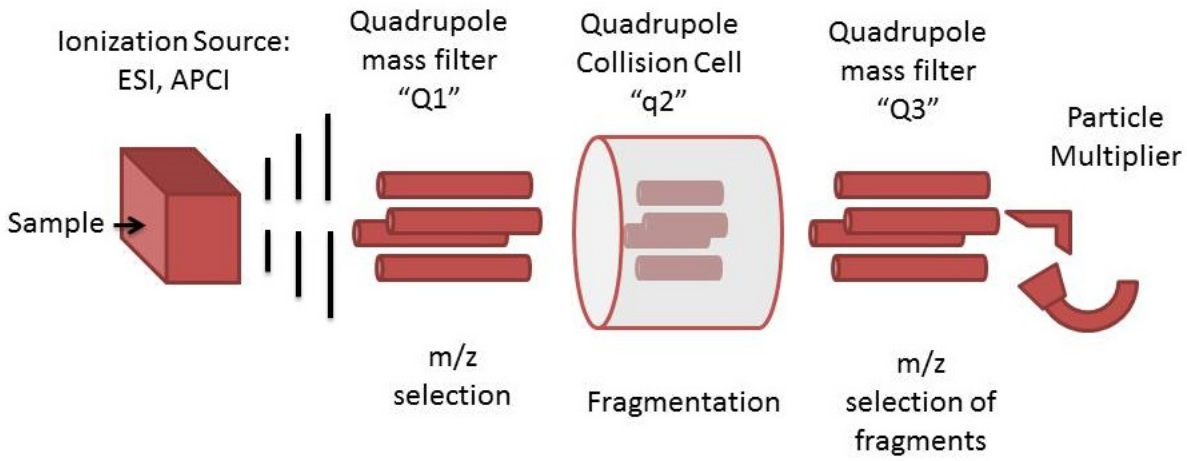
Quadrupole
Ion Trap
Time-of flight
Orbitrap
FT-ICR

Mass spectromectry and separation techniques



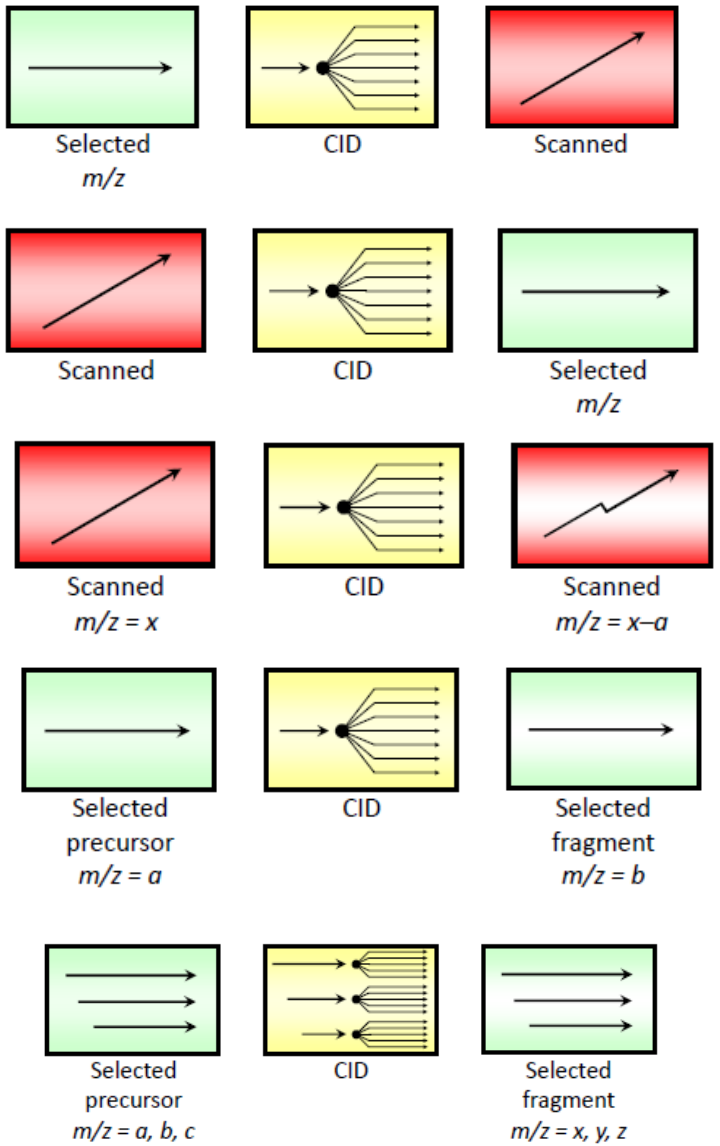
TANDEM MS

- A method comprising at **least two levels of mass analysis** steps: either in connection with a dissociation process or a chemical reaction that causes a change in the ion mass or ion charge
- MS/MS methods involve the **activation of the selected ion** (precursor)
- Activation of ions in space or in time



MODES OF TANDEM MS

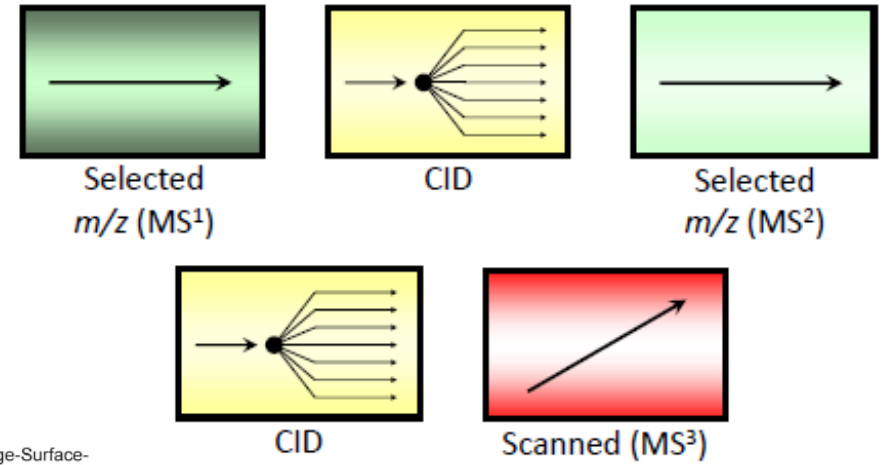
- **Product ion scan**
- **Precursor ion scan**
- **Neutral loss scan**
- **Selected reaction monitoring**
- **Multiple reaction monitoring**

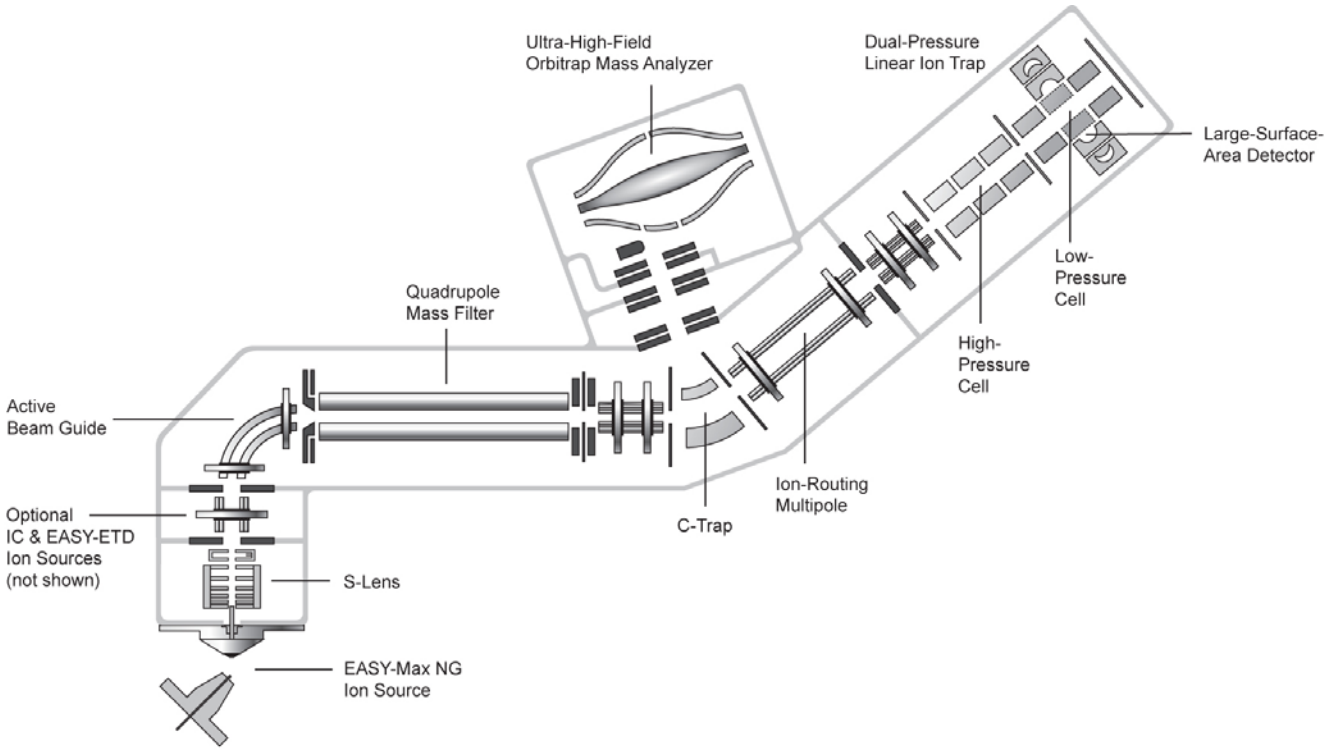


MODES OF TANDEM MS

- **Scan MS^n**

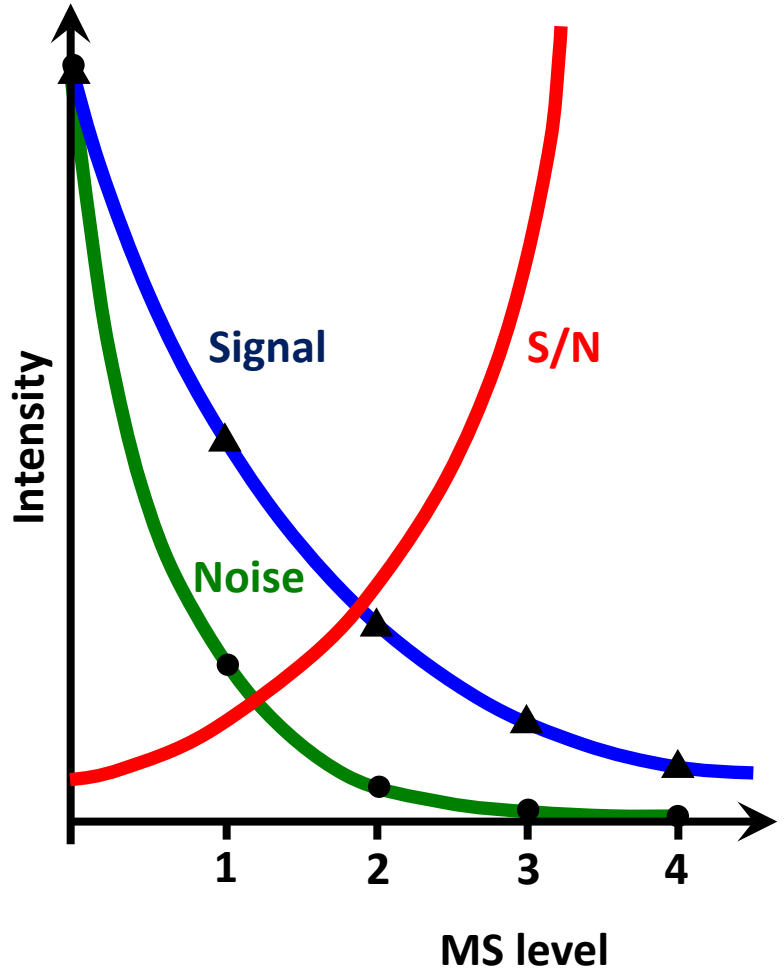
- ***Applicable for ion traps***

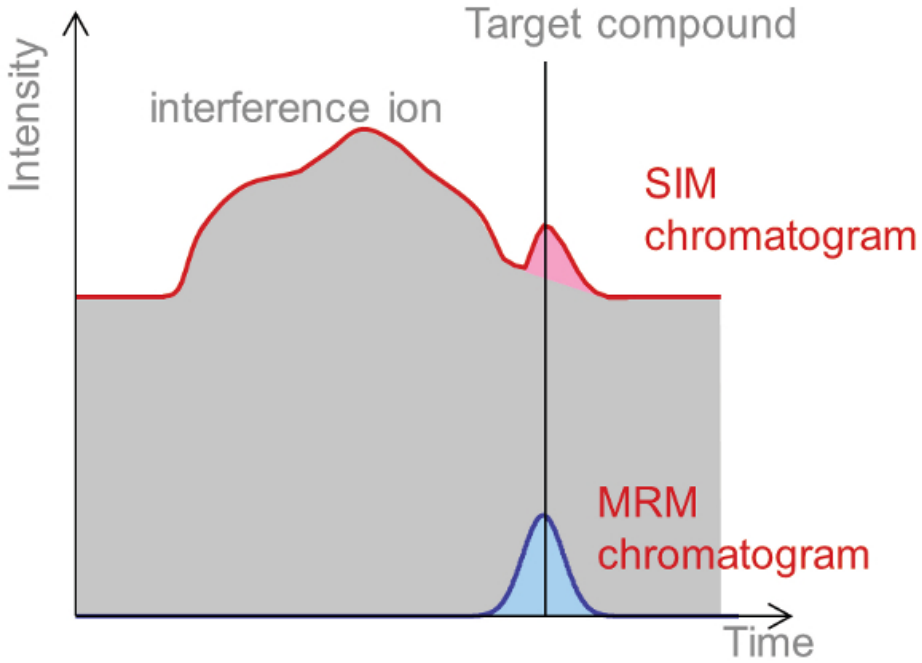




Effect of tandem MS

- **Scan MS^n: selectivity × sensitivity**

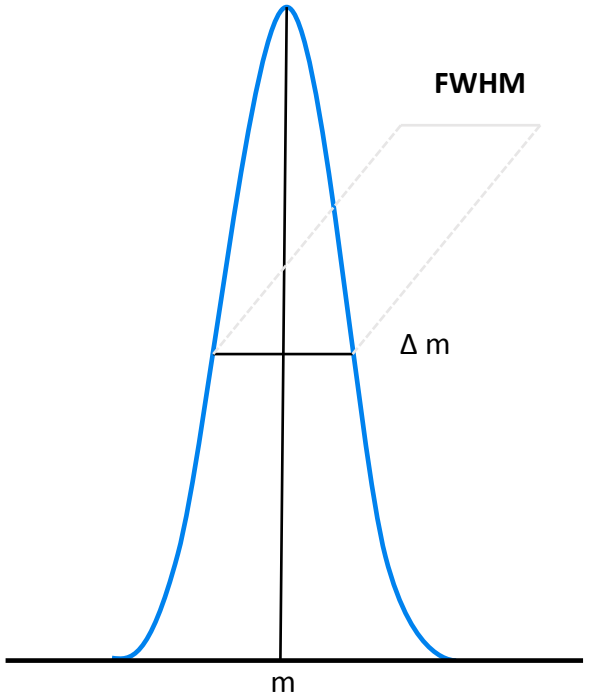




source: R.G. Cooks, K.L. Busch, J. Chem. Educ. 59(11) (1982) 926–933

High resolution mass spectrometry

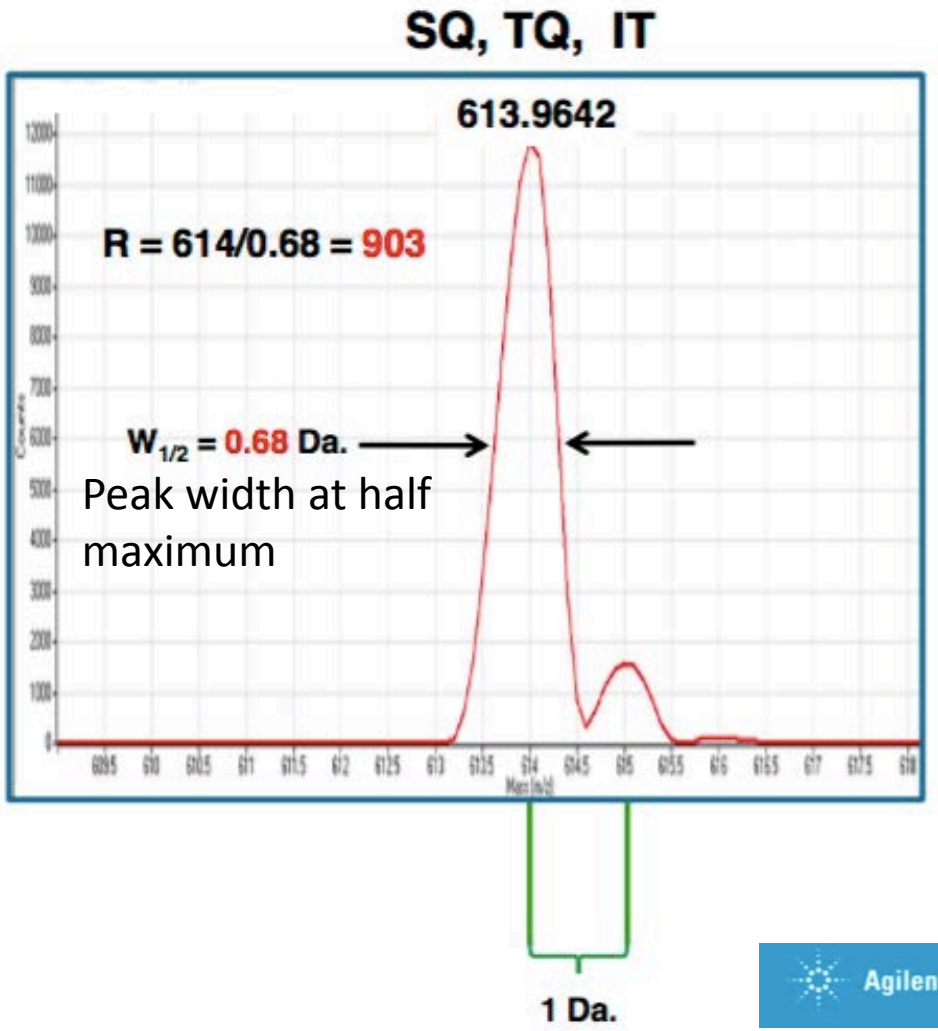
- Mass spectrometer has **high resolving power**
- Definiton according to the width of one peak
- *Full Width at Half Maximum* (FWHM)
  - Mass difference expressed as the peak width of a given mass peak measured (in mass units) at 50% of its height



$$RP = {}^{m}/_{\Delta m}$$

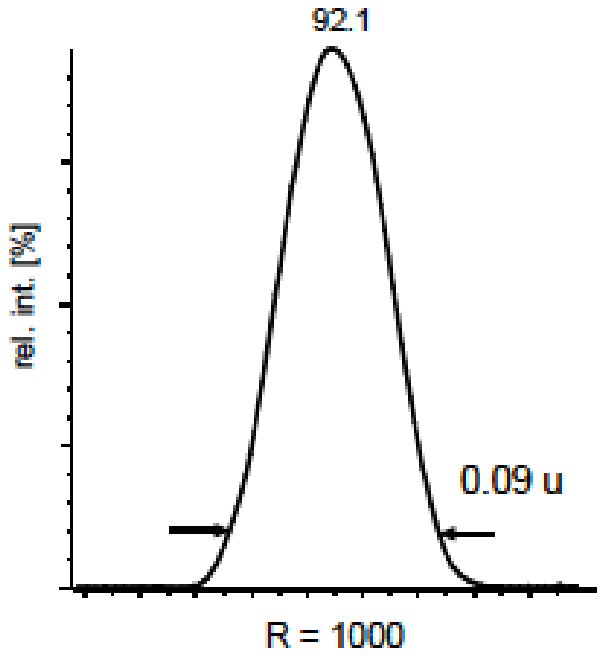
High resolution mass spectrometry

m/z = 613.964203



High resolution mass spectrometry

- A mixture of xylene (*m/z* 92.0581) and toluene (*m/z* 92.0626) at different settings of resolution



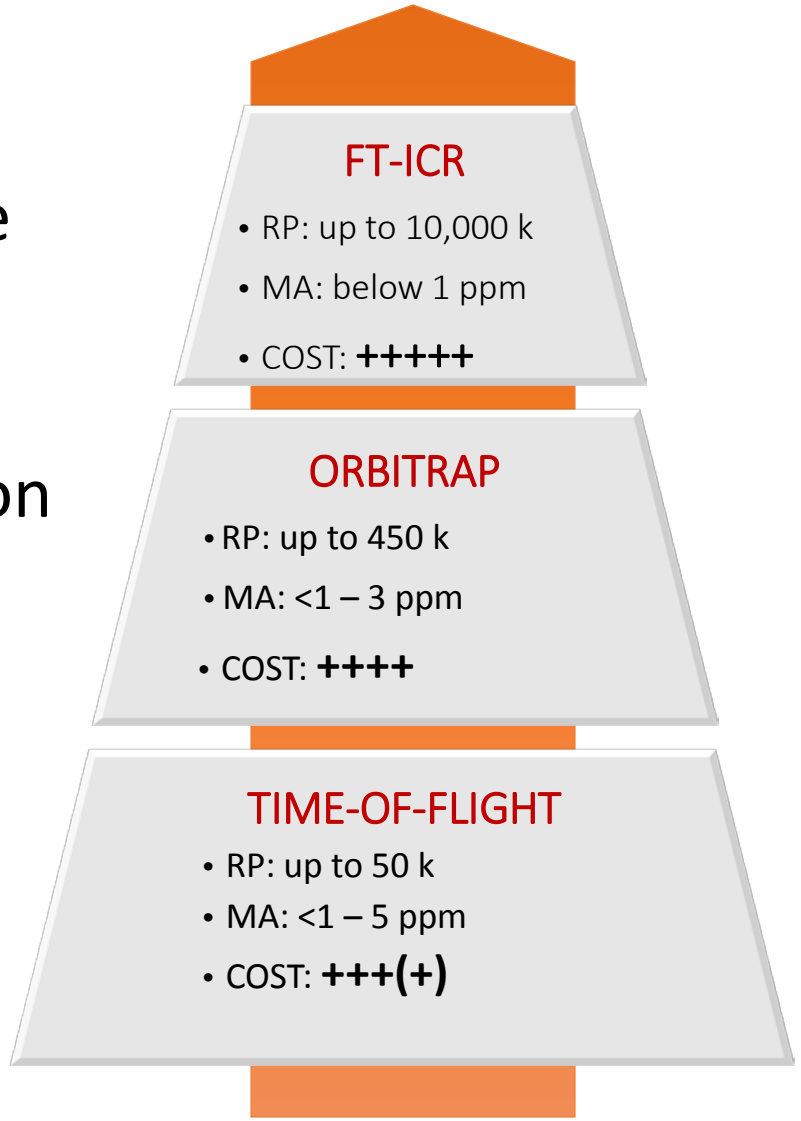
High resolution mass spectrometry

Mass accuracy:

- The deviation between measured mass (accurate mass) and calculated mass (exact mass) of an ion expressed as an error value (mDa, ppm)
- Important for structural interpretation (calculation of elemental composition)

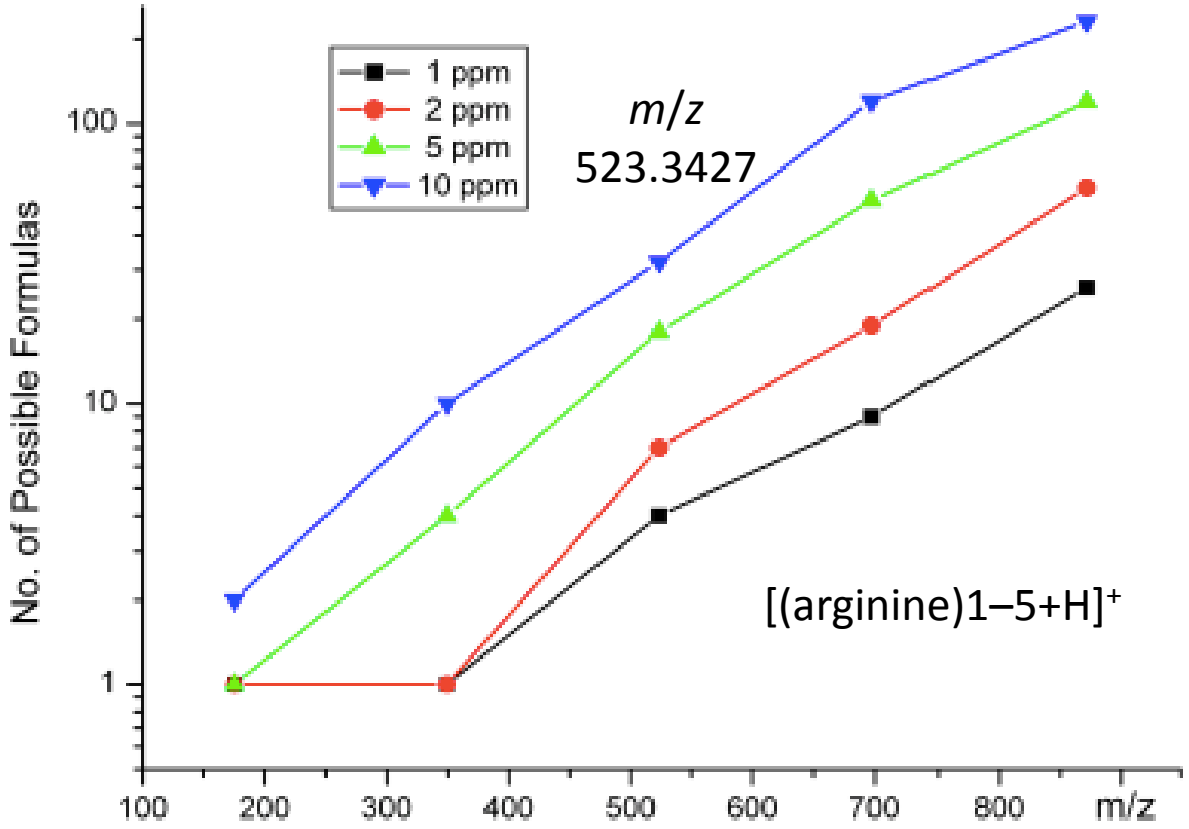
$$\Delta\,(\text{ppm}) = \frac{m_{\text{exp.}} - m_{\text{teor.}}}{m_{\text{teor.}}} \cdot 10^{6}$$

$$\Delta\,(\text{mDa}) = \left(m_{\text{exp.}} - m_{\text{teor.}}\right) \cdot 10^{3}$$



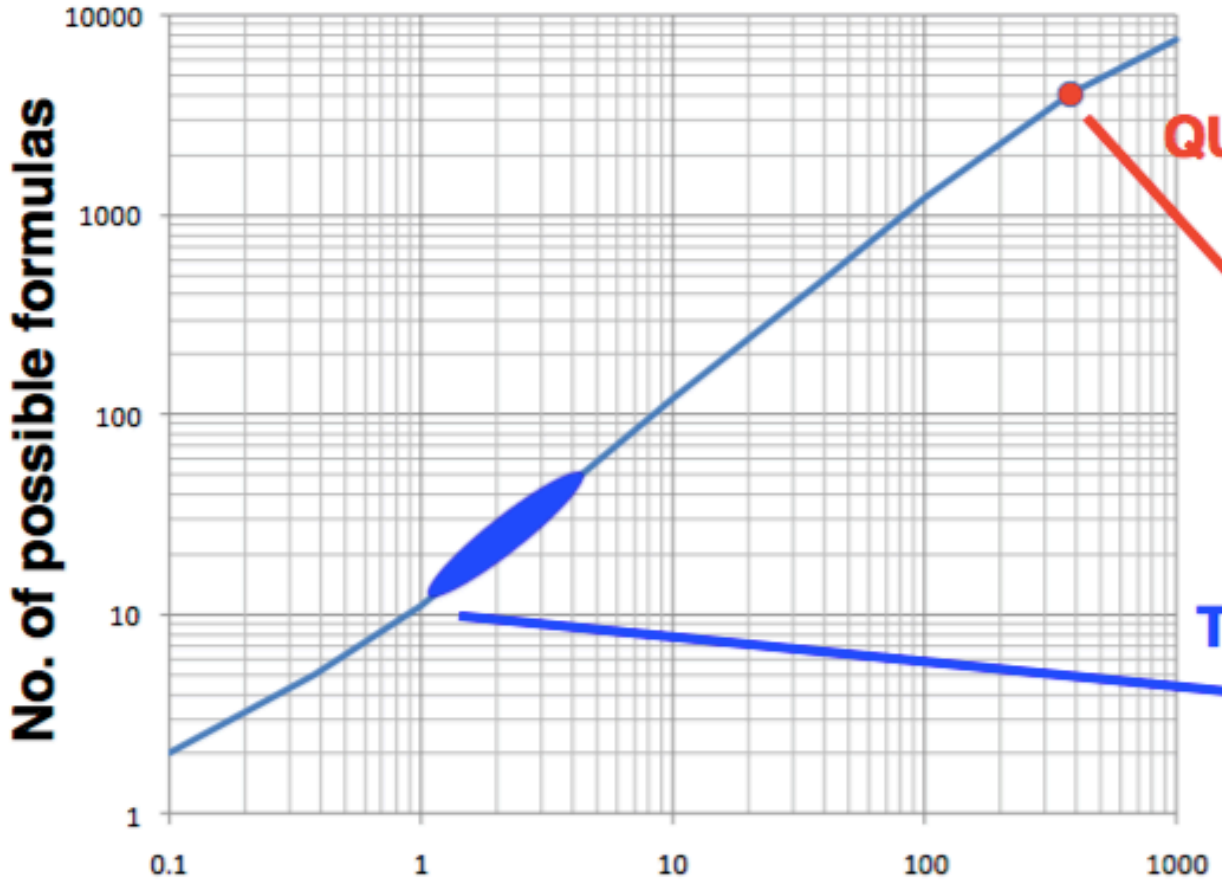
Why do we need accuracy and precision?

Molecular formulas based on a free selection among the elements C, H, N, O as a function of relative mass error vs. *m/z*.



The higher the mass error the larger number of candidates

Possible chemical formulas for m/z = $C_{10}F_8$ = 271.98667



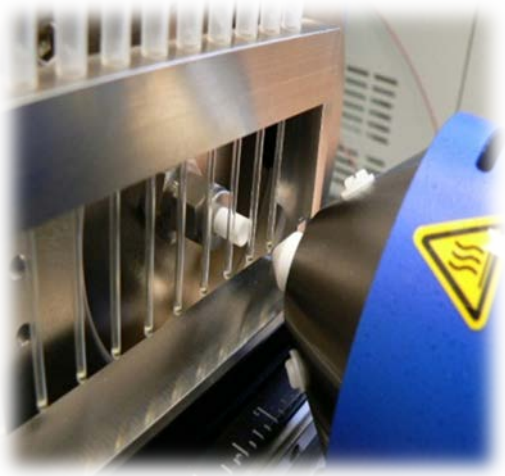
mass uncertainty		# of Possible Formulas
ppm	amu	
1000	0.3	7657
368	0.1	4050
100	0.03	1223
37	0.01	466
10	0.003	120
4	0.001	43
1	0.0003	11
0.4	0.0001	5
0.1	0.00003	2

Formulas made of:
C,H,N,O,F, & Cl

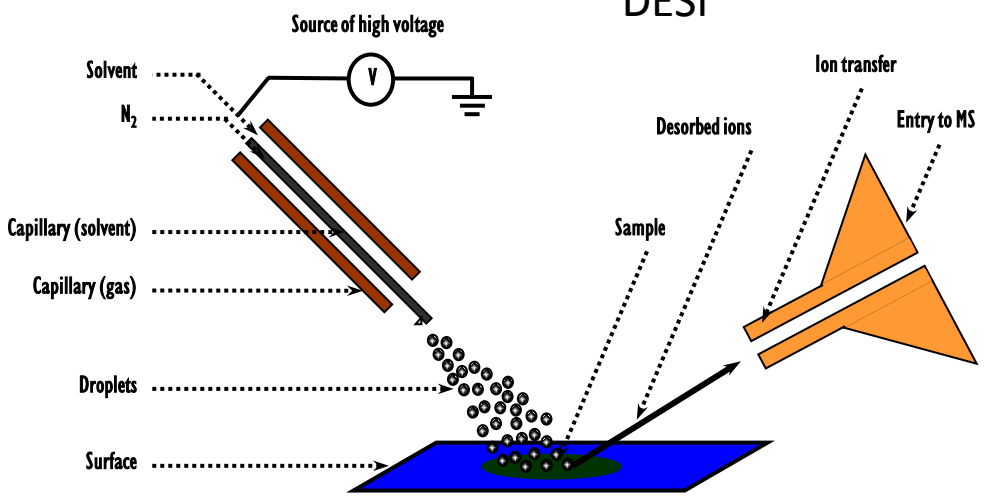
Ambient mass spectrometry

- Sample ionization at atmospheric pressure
- Usually no separation
- Fast response
- MS imaging

DART



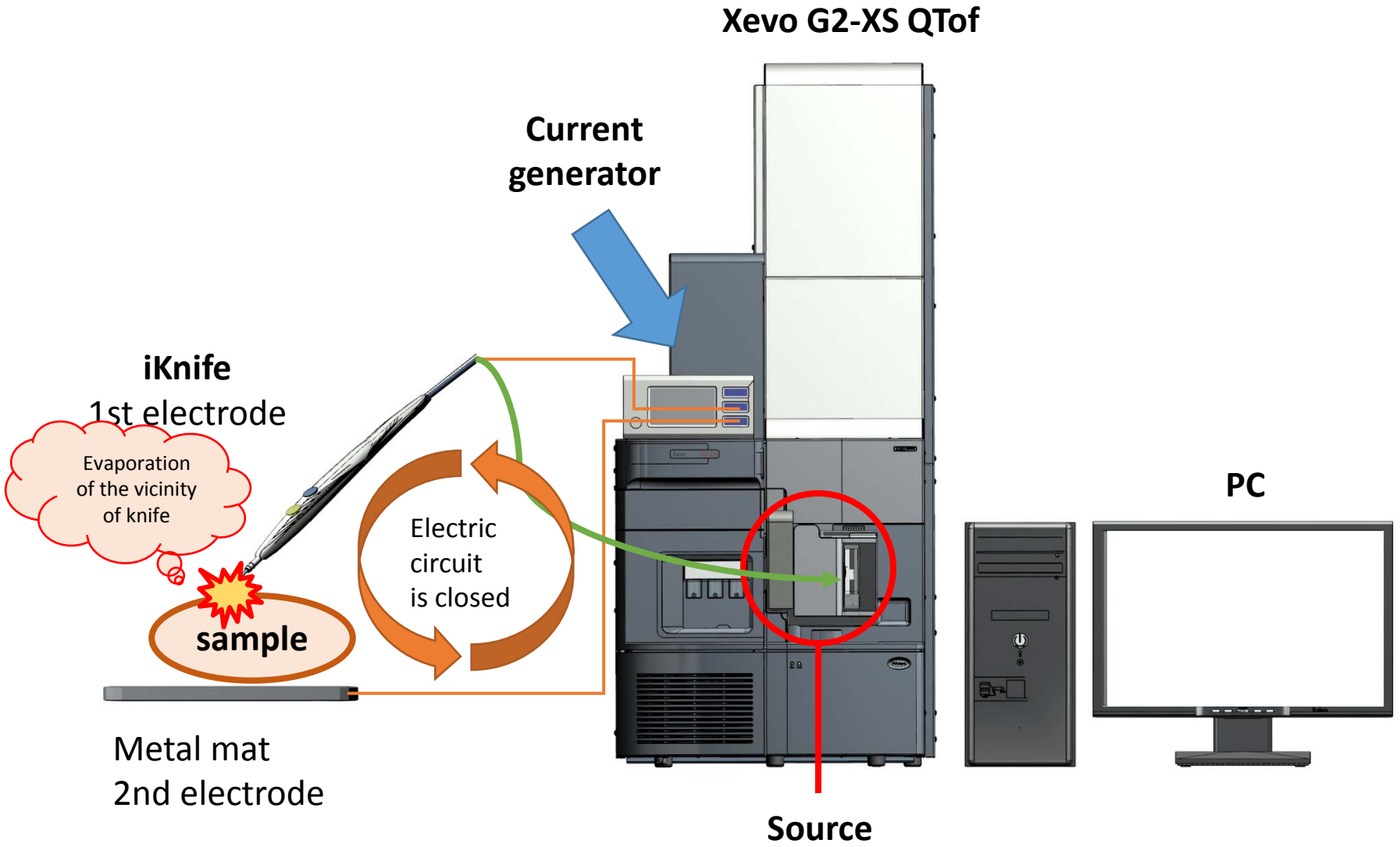
DESI



REIMS

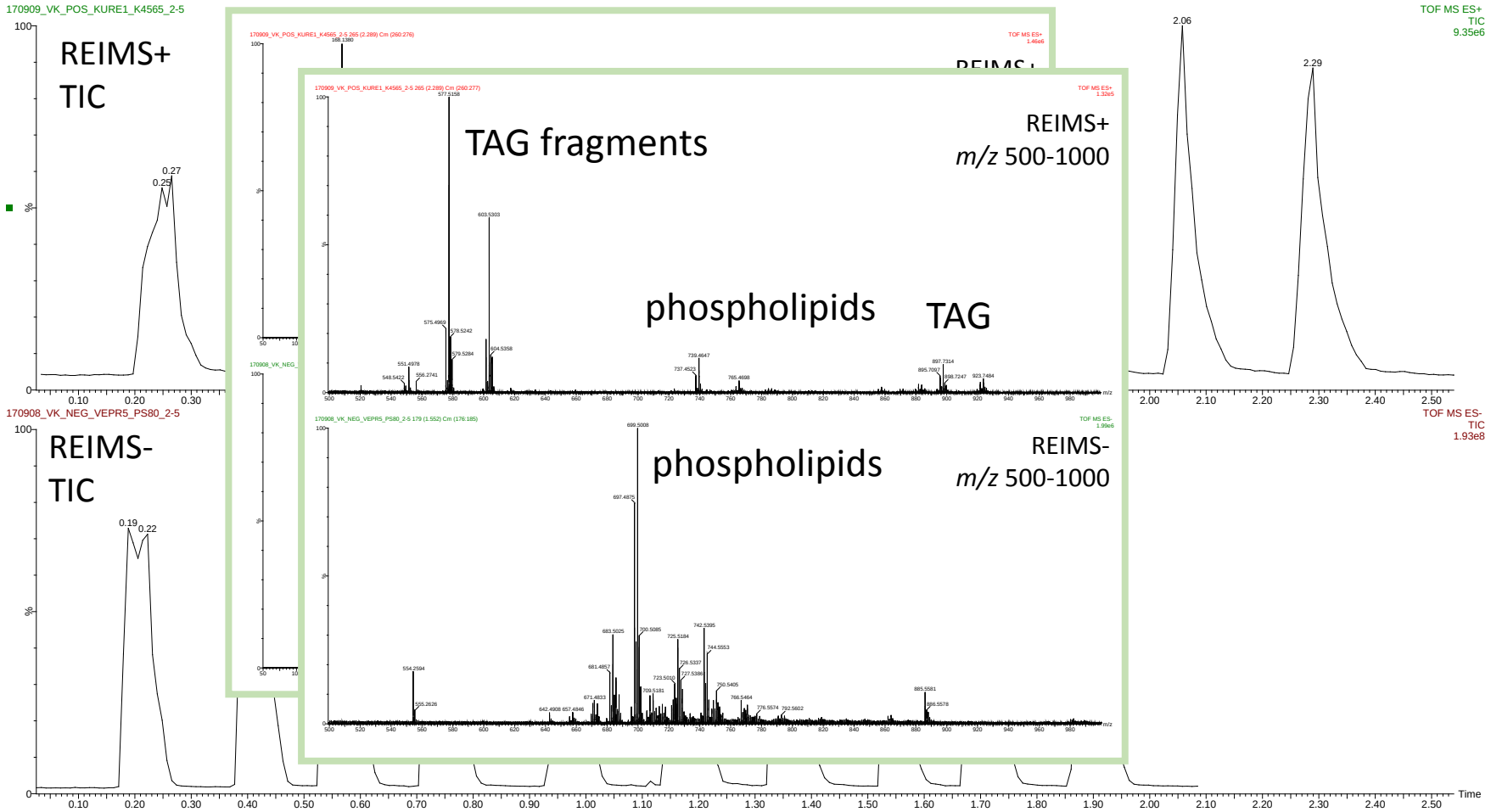
- **R**apid **E**vaporative **I**onization **M**ass **S**pectrometry
- First electrosurgical knife **1926**
- The hyphenation of electrosurgical knife and mass spec in **2010**
- Developed for cancer surgery

REIMS system



REIMS data

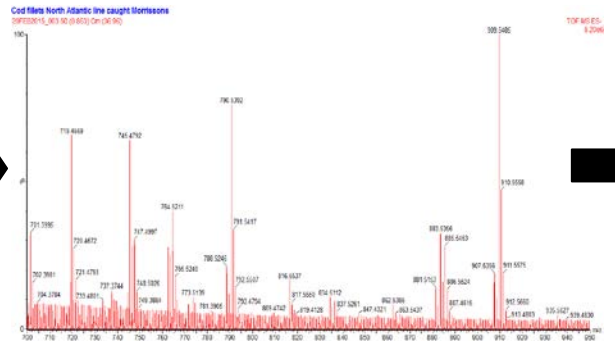
Chronograms



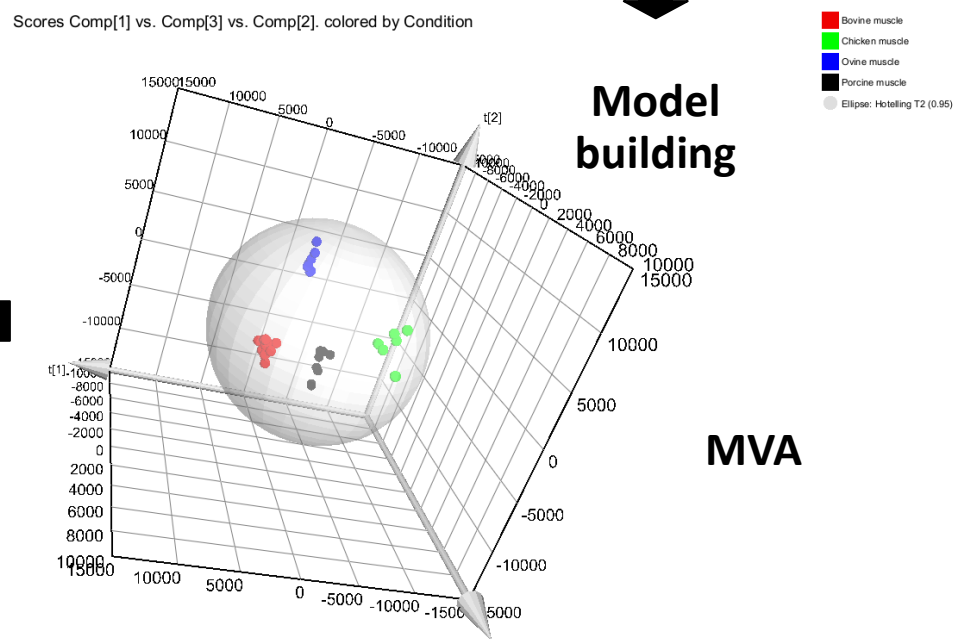
REIMS method workflow

iKnife
+
Mass spec

spectra



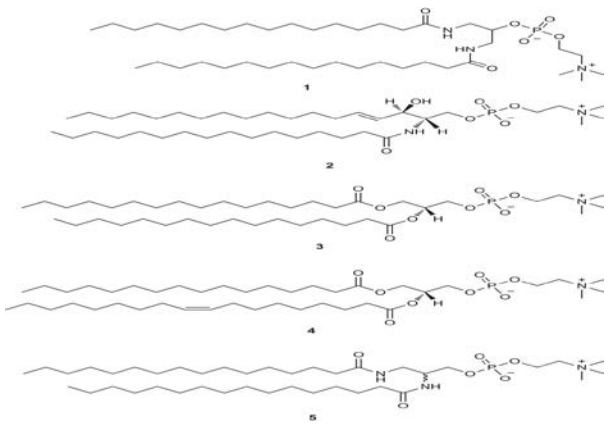
Database creation



Model building

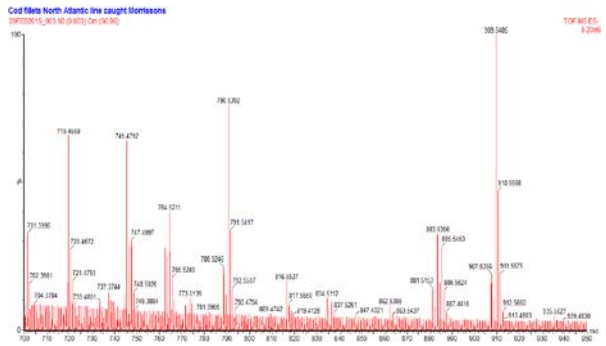
MVA

Tentative identification of important variables

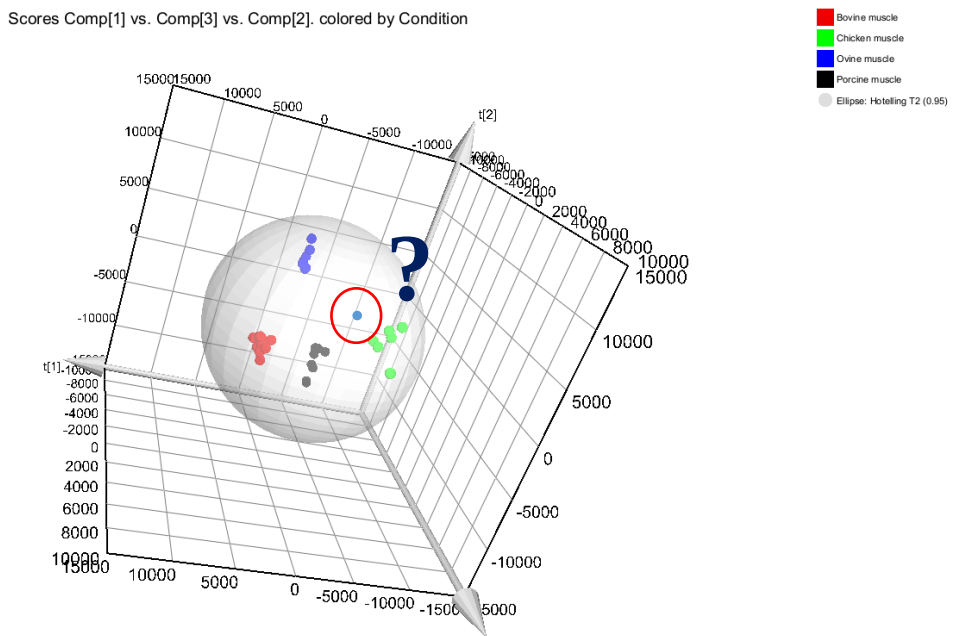


Authentication by REIMS

iKnife
+
Mass spec



Evaluation of the sample



Answer

Chicken muscle

Why REIMS?

Advantages:

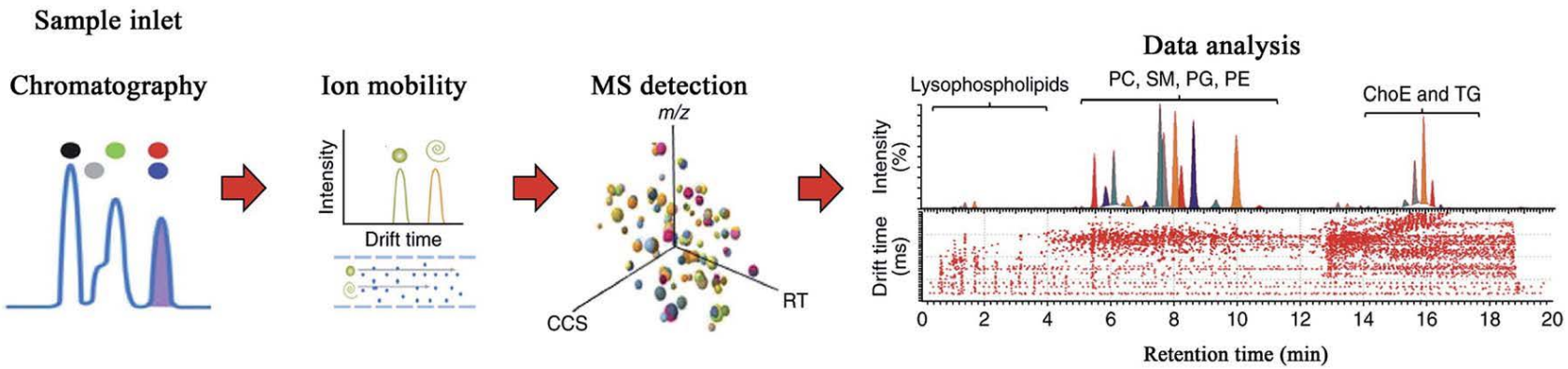
- Quick alternative PCR, MS a LC-MS methods
- Possibility of mobile instruments

Disadvantages:

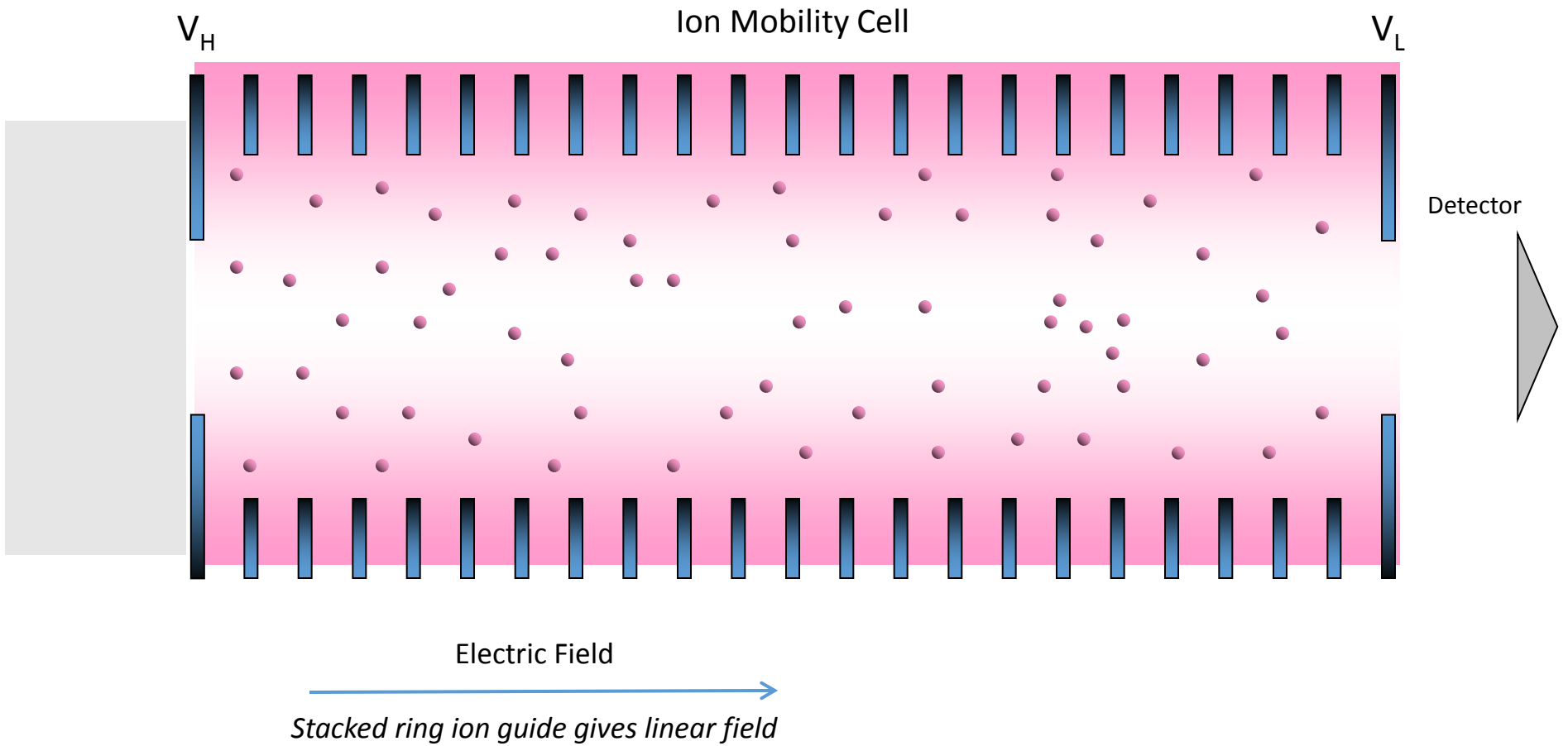
- Low sensitivity
- Limited number of matrices

Ion mobility MS

- Additional separation dimension
- Standalone MS or hyphenated with LC
- Several types of ion mobility

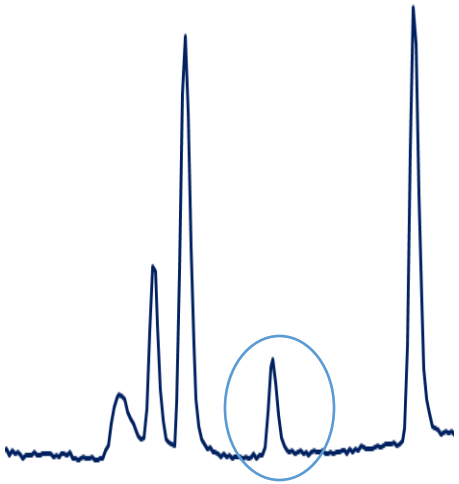


Basic operation of ion mobility

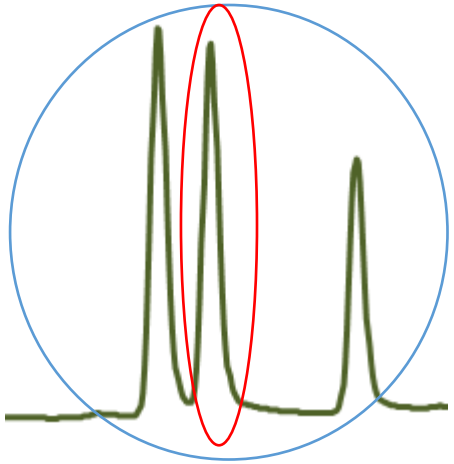


Source: Agilent Technologies

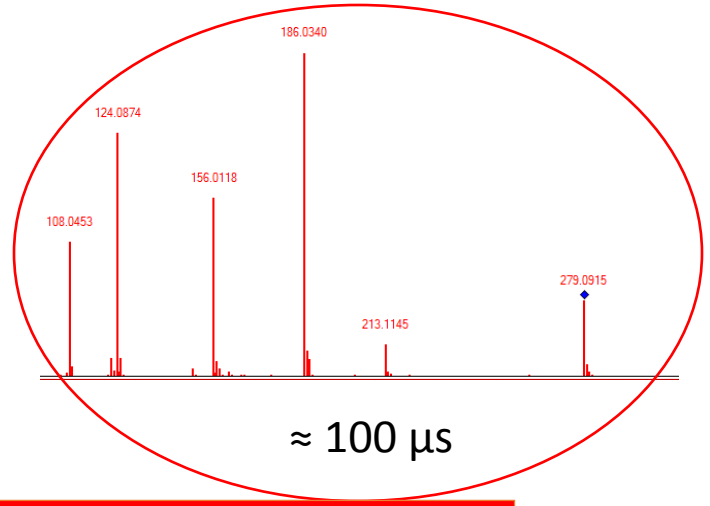
Adding Ion Mobility Spectrometry in LC/MS



≈seconds



≈ 60 ms



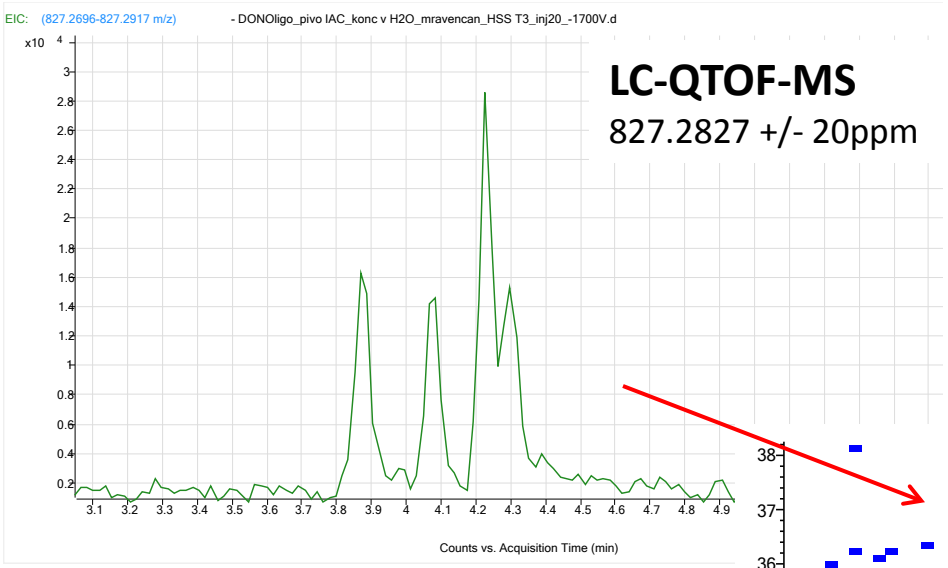
≈ 100 µs

IMS fits between LC and TOF MS on the separation time scale!

Source: Agilent Technologies

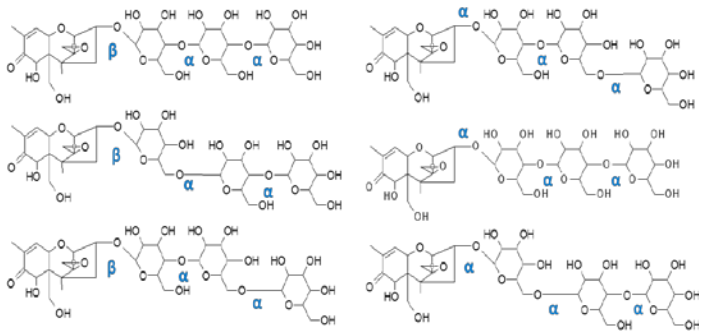
IM separation of masked mycotoxins

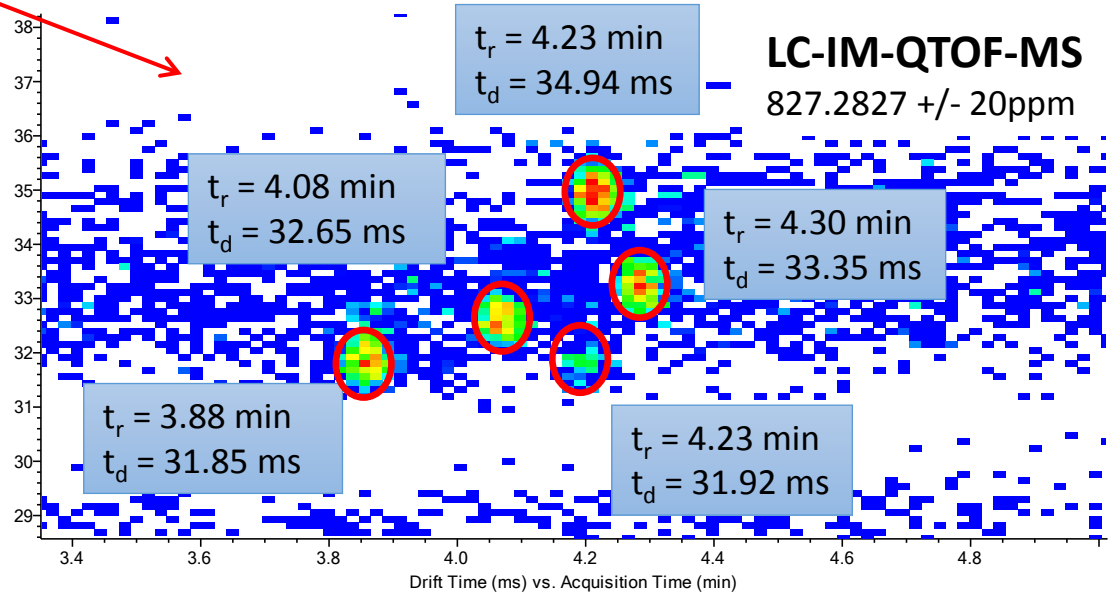
DON-3-triGlc [M+HCOO]$^-$



LC-QTOF-MS
827.2827 +/- 20ppm

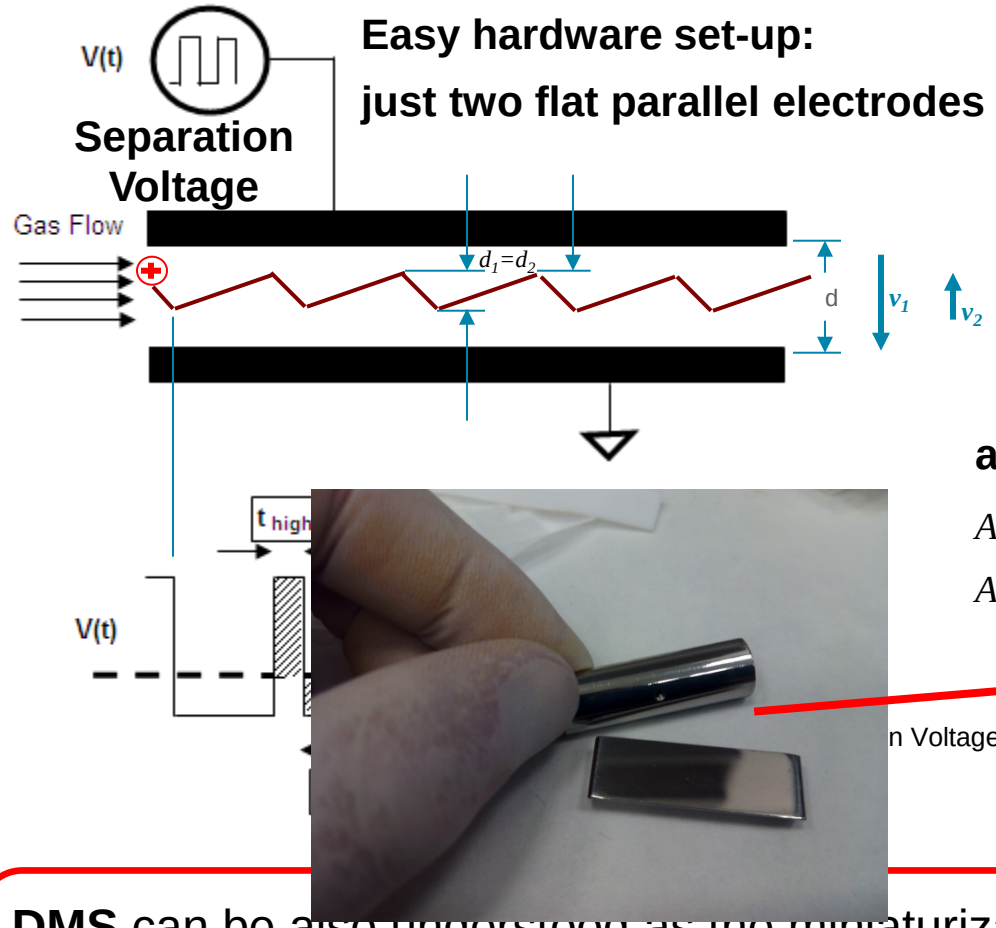
Five isomers found in IM!





LC-IM-QTOF-MS
827.2827 +/- 20ppm

Differential Mobility Spectrometry



Easy hardware set-up:

just two flat parallel electrodes

The ion traveling from a starting point will
distance from
.

as

A_h high

A_l low

n Voltage)

if

$$v_1 = K \cdot E_1$$

$$v_2 = K \cdot E_2$$

DMS can be also understood as the miniaturiza

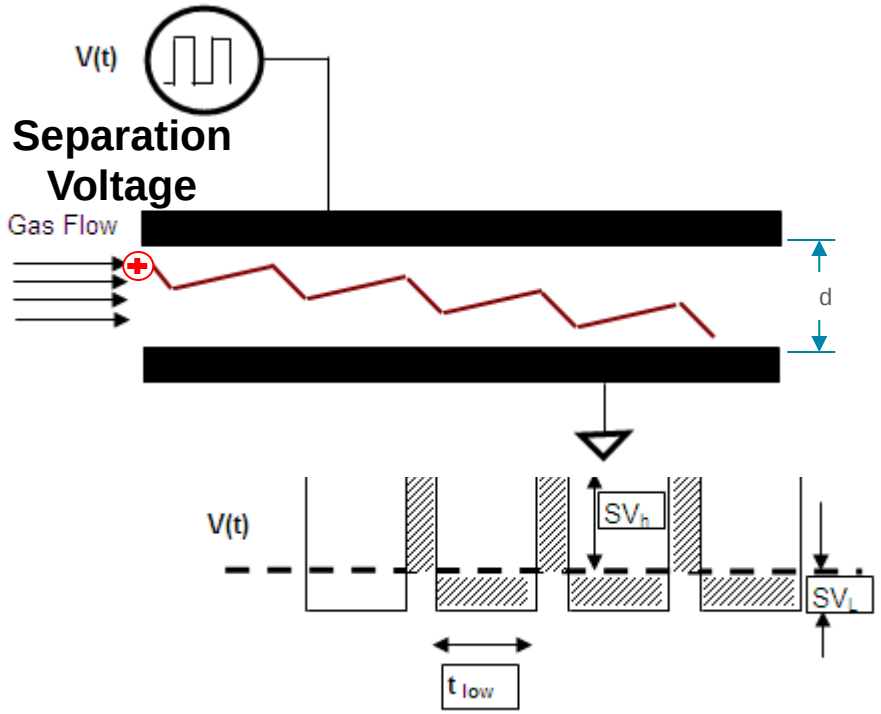
DMS takes advantage of the differences in the mobility of ions in high and low electric fields (**Separation voltage**)

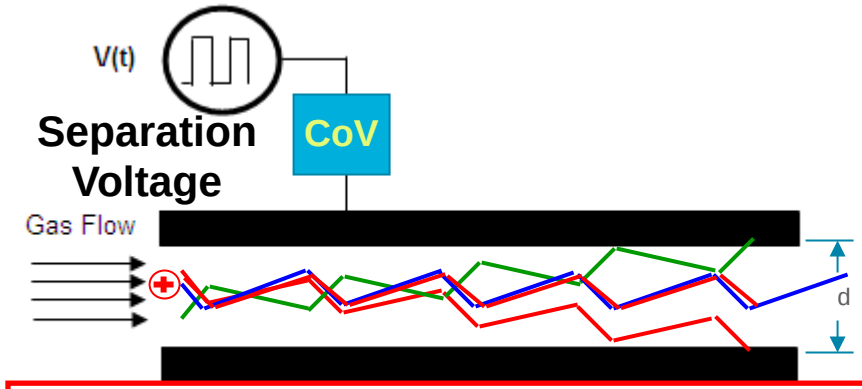
Differential Mobility Spectrometry

However, if the waveform is applied by high SV, the mobility of the ion during application of the peak voltage deviate from its low-field value (dependance of K from E). In this instance, during the higher voltage portion of the waveform, the ion travels at a velocity different than it would absent; this change in mobility:

$$v = K_{(E)} \cdot E$$

The ion traveling from a starting point will therefore *not return to exactly this same distance* from the electrode after one cycle and, thus, drift towards one of the electrodes.



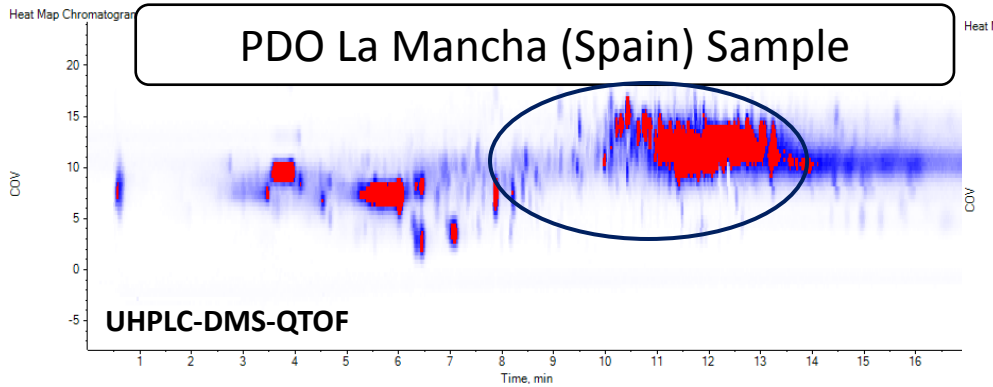


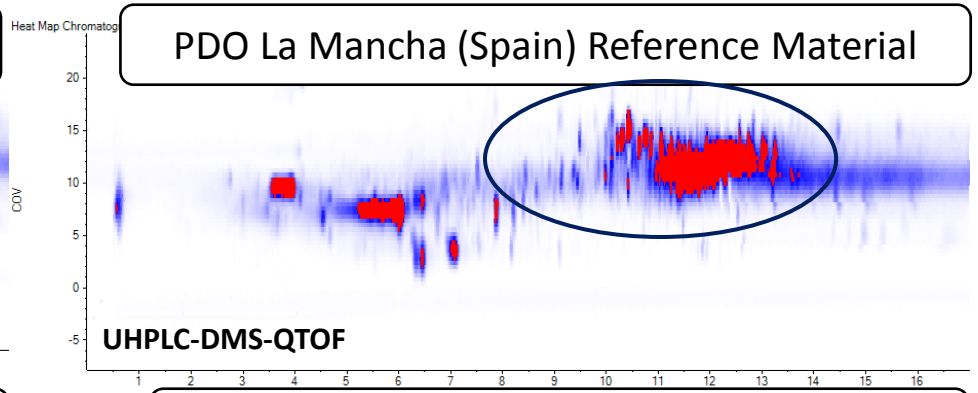
Compensation voltage (CoV):

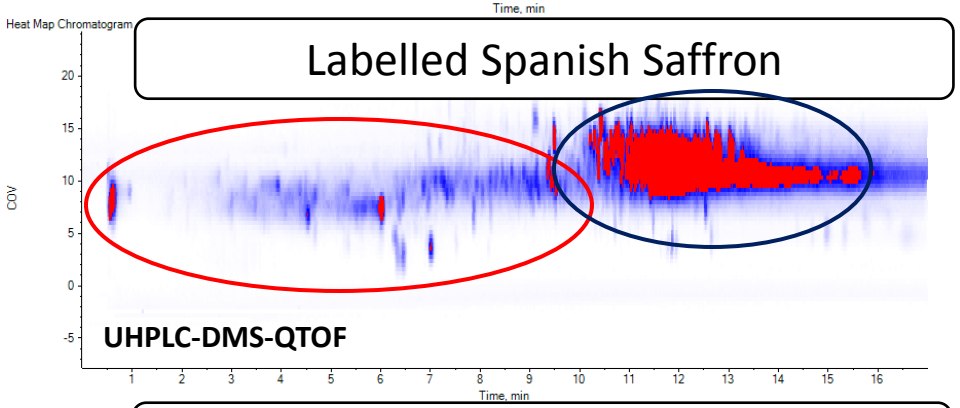
Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

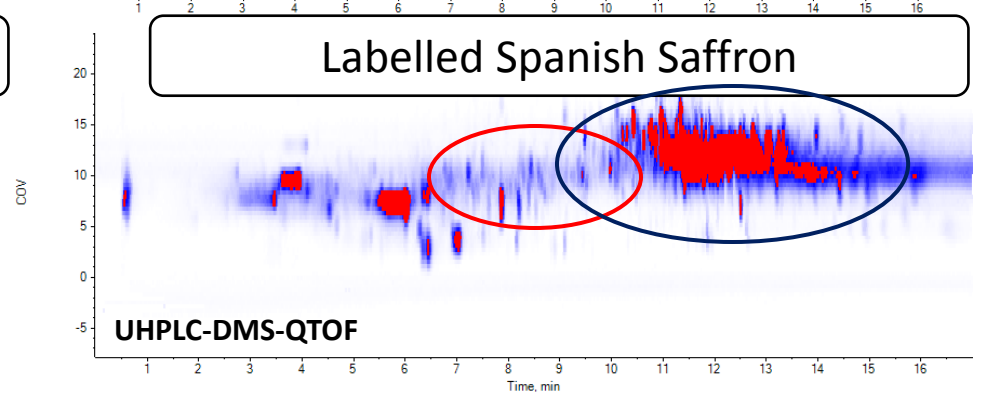
Heat Map Chromatograms

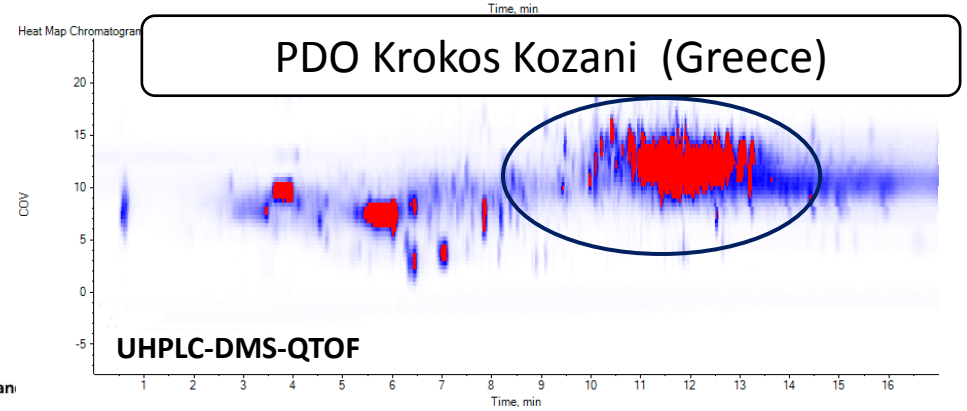
m/z 100 to 1200
CoV -8 to 24
Run time 17 min

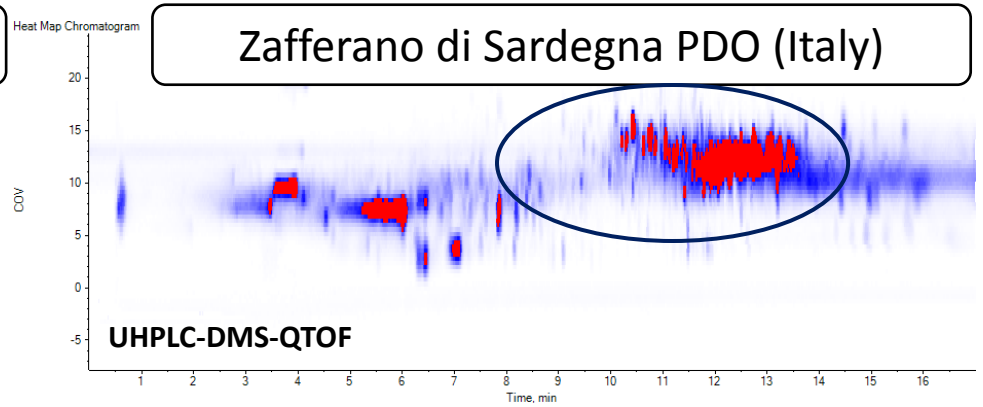












Conclusions

- Wide array of techniques exist
- Domination of separation techniques and mass spectrometry
- Manufacturesrs are routinely assisting in development of methods
- **More exciting instrumental techniques to come!**