TUNTWIN

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Workshop/Summer school
Mass Spectrometry
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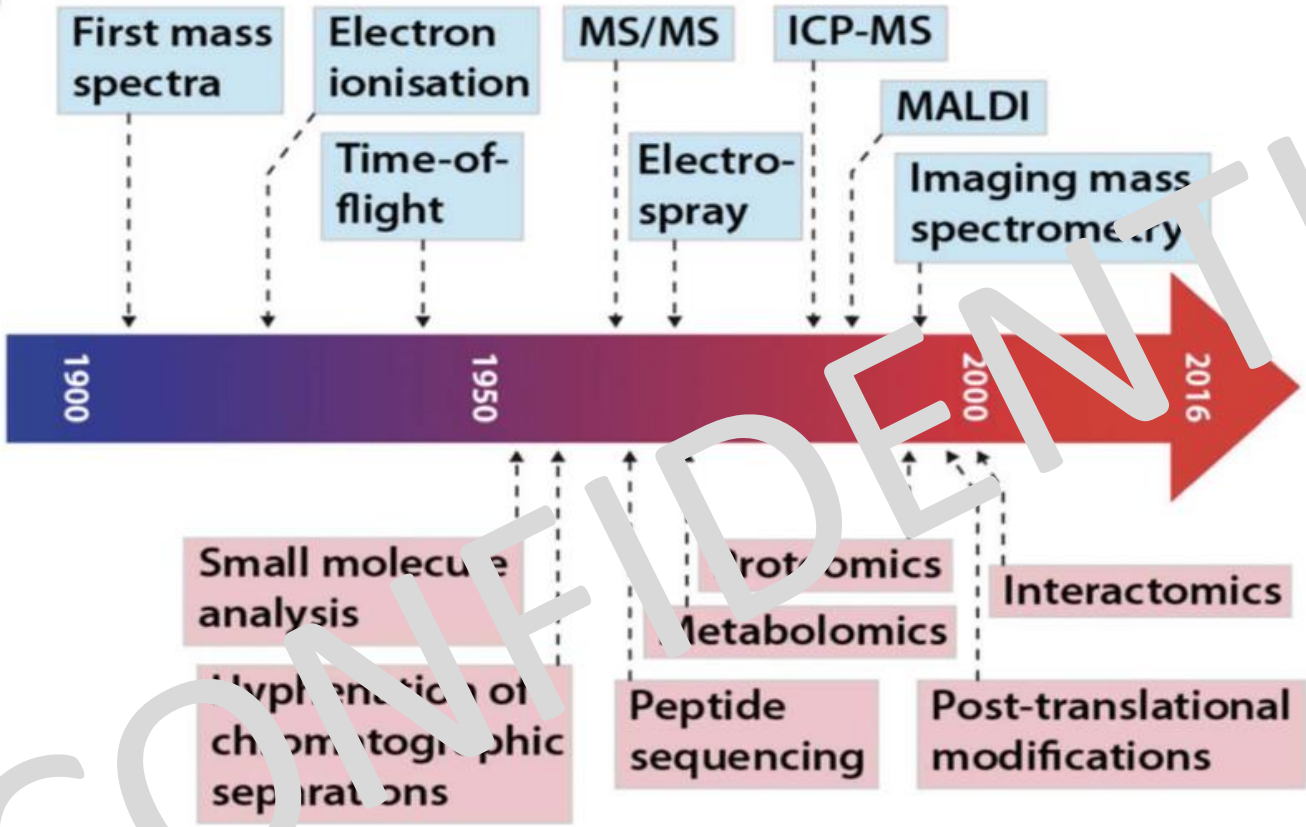
07/03/2023

Funded by the Horizon 2020 Framework Programme of the European Union
under the grant N° *952306*

Summary

Application and evolution of mass spectrometry



A. Doerr, J. Finkelstein, I. Jarchum, C. Goodman and B. Dekker, Nature Milestones: Mass Spectrometry, Nature Publishing Group, 2015.

Application and evolution of mass spectrometry

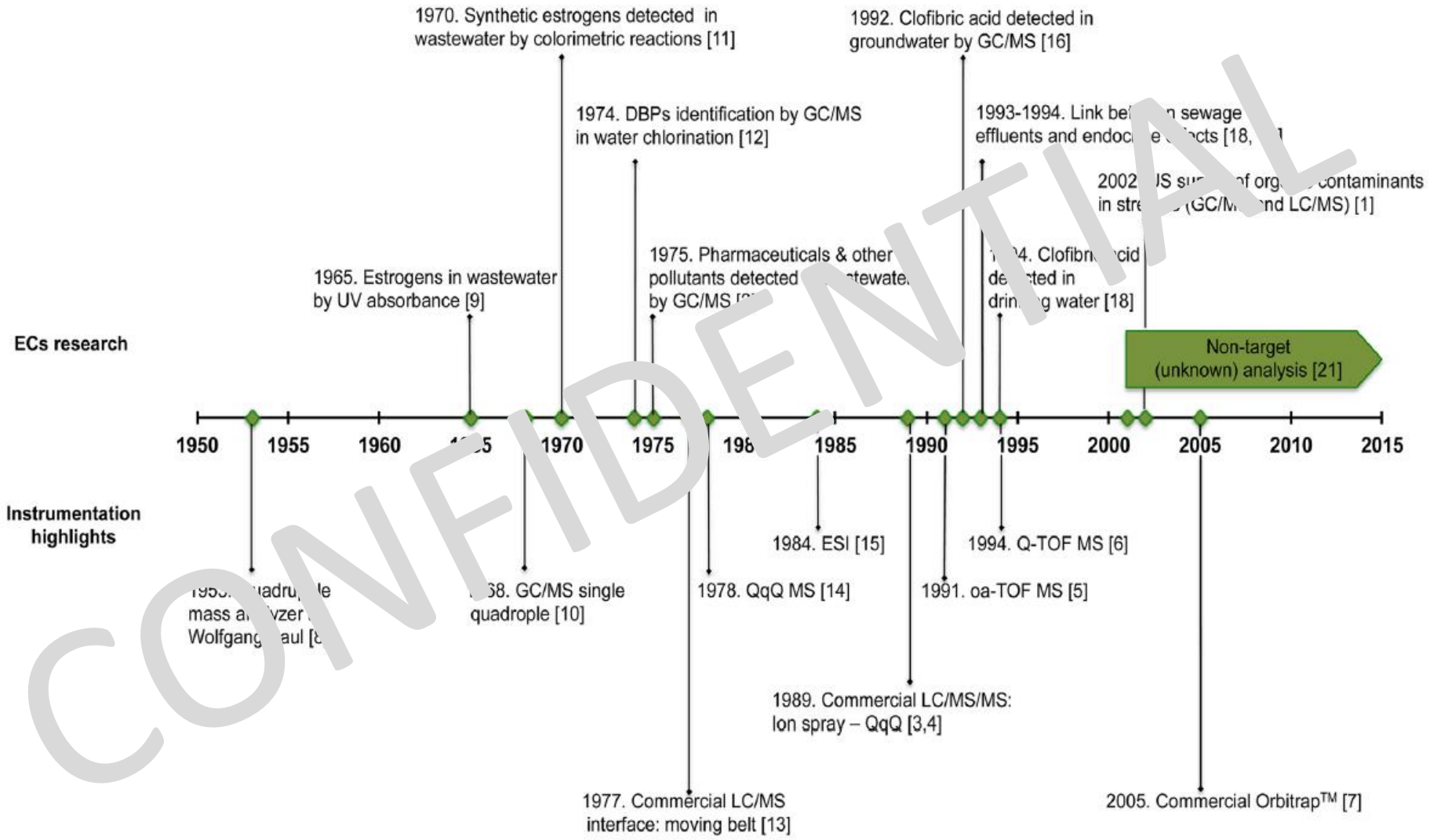


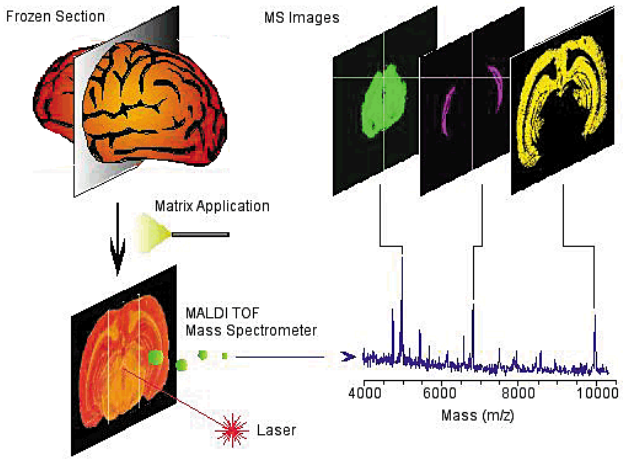
Fig. 1. Timeline highlighting major events in ECs research and related instrumentation development. Numbers in brackets correspond to references. DBPs (disinfection byproducts), ESI (electrospray ionization), MS (mass spectrometry), QqQ (triple quadruple), oa-TOF (orthogonal time of flight), Q-TOF (quadrupole time of flight)[8–20].

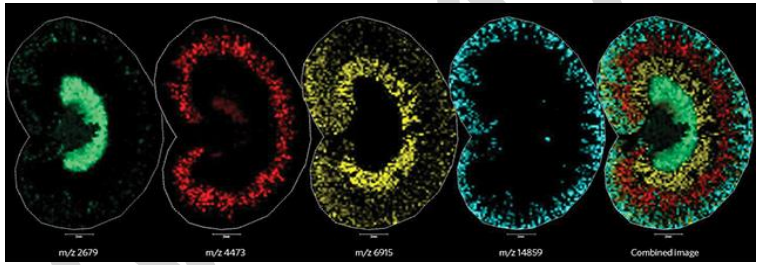
Application and evolution of mass spectrometry

Imaging mass spectrometry : A new technology for The analysis of protein expression in mammalian tissues

M. Stoeckli, R Caprioli et coll.

Nature Medicine, **7**, 493-496 (**2001**)





Multiplex mass-spectrometry image of a section through a rat kidney taken at 150 µm spatial resolution. Each color represents an individual peptide or protein of specific mass-to-charge (m/z) ratio. JUNHAI YANG AND RICHARD CAPRIOLI, MASS SPECTROMETRY RESEARCH CENTER, VANDERBILT UNIVERSITY

Sonde Curiosity

Mission sur Mars (2012)

Mass Spectrometry

- Mass definitions
- Isotope pattern
- Resolution / Accuracy

INTRODUCTION

- ✓ Mass Spectrometry is a discipline dedicated to the study of the **structure** and **reactivity of all types of organic molecules**, from the simplest to the most complex

- ✓ This technique makes it possible to determine **the mass of a molecule** or a combination of molecules. It is also a very effective tool that offers the possibility of **probing the structure of a molecule**, and explaining a break or arrangement of a bond in the gas phase.

- ✓ Among the various analytical methods used in organic chemistry, mass spectrometry has a privileged place today thanks to these characteristics:
  - ➢ Unsurpassed sensitivity and detection limit
  - ➢ Selectivity and rapid quantitative analysis
  - ➢ a wide range of applications





What does a mass spectrometer do?

A mass spectrometer measures the mass of molecules.

To do this, the mass spectrometer must perform the following operations:

1- Volatilise

Separate the molecules from each other: we go from the state of condensed matter to a gaseous state

2- Ionize

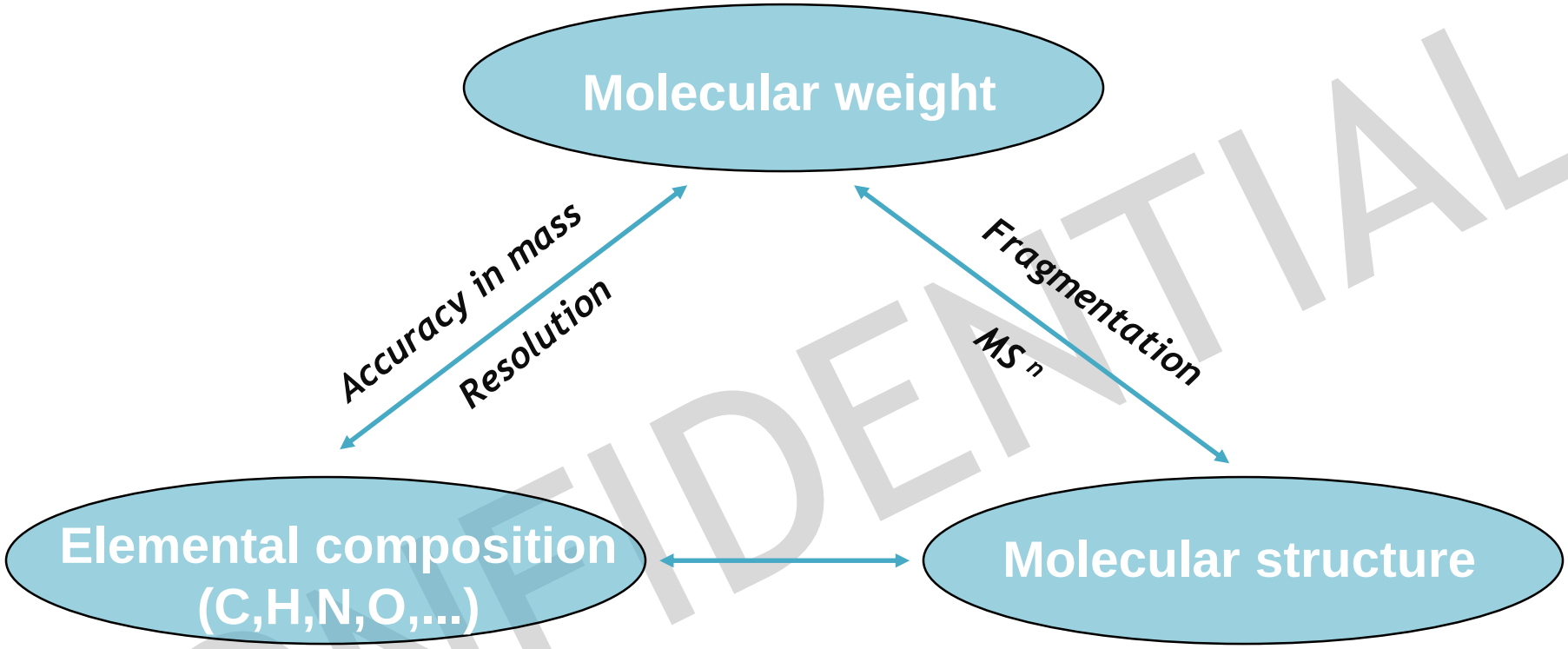
Transforming molecules into ions, as a mass spectrometer works with electric fields

3- Measure m/z ratios

The molecular weight is calculated from the ratio of mass (m)/number of charges (z)

Mass spectrometry = Transformation of molecules in their natural state, into ions in gaseous state, then sorting the ions according to the mass/charge ratio

What we want to obtain by mass spectrometry

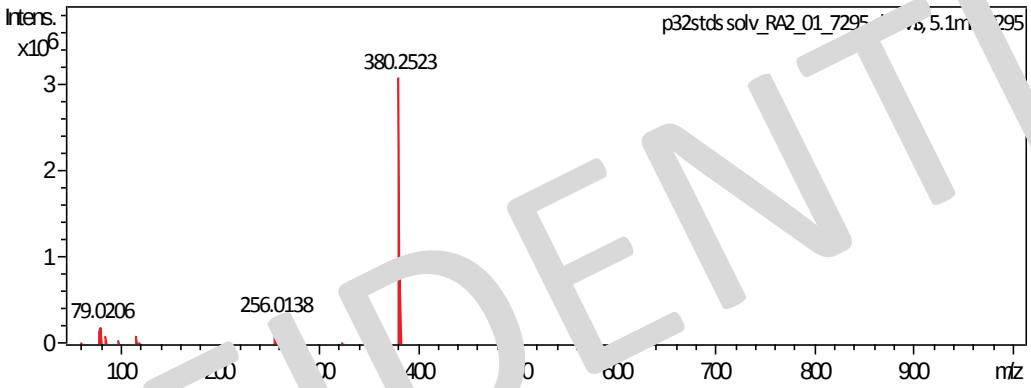


- Nature of the molecules to be analysed
- Ion source to be used ⇔ molecular ion
- Analyzers ⇔ Resolution, MS/MS

Performance of the equipment ⇔Price

What does a mass spectrum show?

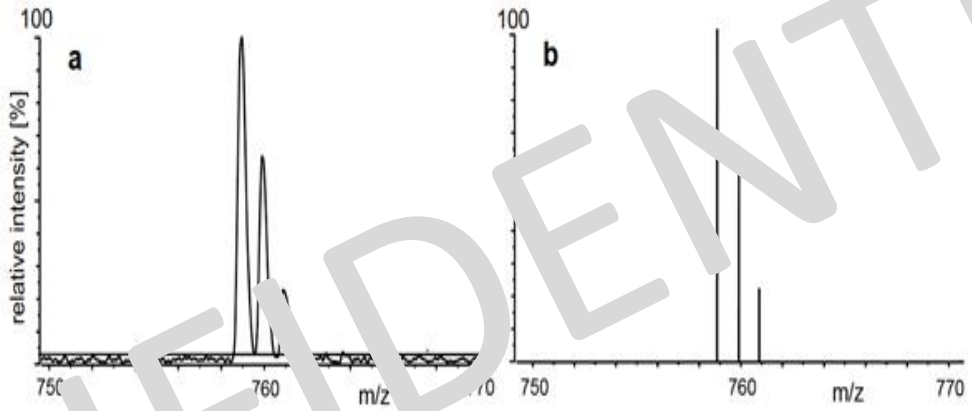
- **The mass spectrum** is the two-dimensional representation **of the signal intensity (y-axis)** versus **m/z (x-axis).**



- **The intensity of a peak** reflects **the abundance of ionic species** of the respective m/z ratio that has been created from the analyte in the ion source.
- **The ratio masse over z (charge) is dimensionless**: it is calculated from the dimensionless mass number of a given ion, and the number of its elementary charges, z.

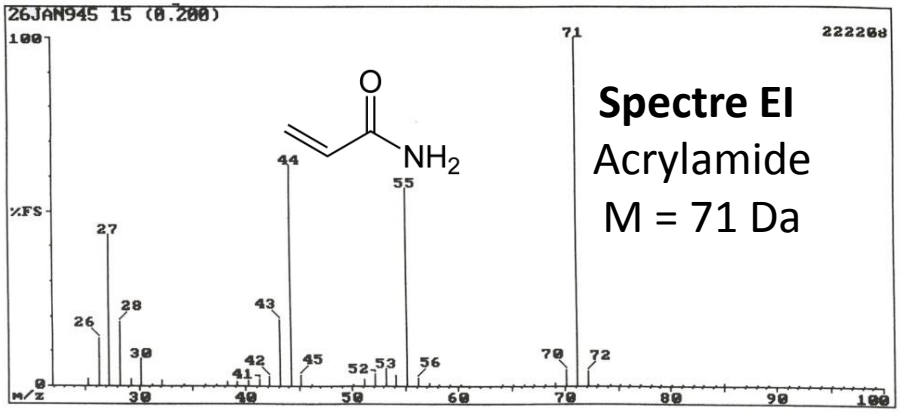
What does a mass spectrum show?

- MS data collected on an instrument are presented as a **profile** (a) or **centroid** (b).



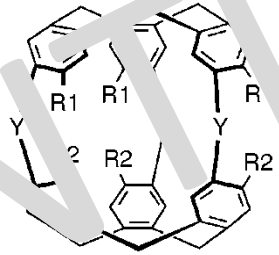
- In profile mode, a peak is represented by a collection of signals over several scans.
- In centroid mode, signals are displayed in discrete m/z with zero widths.

What does a mass spectrum show?

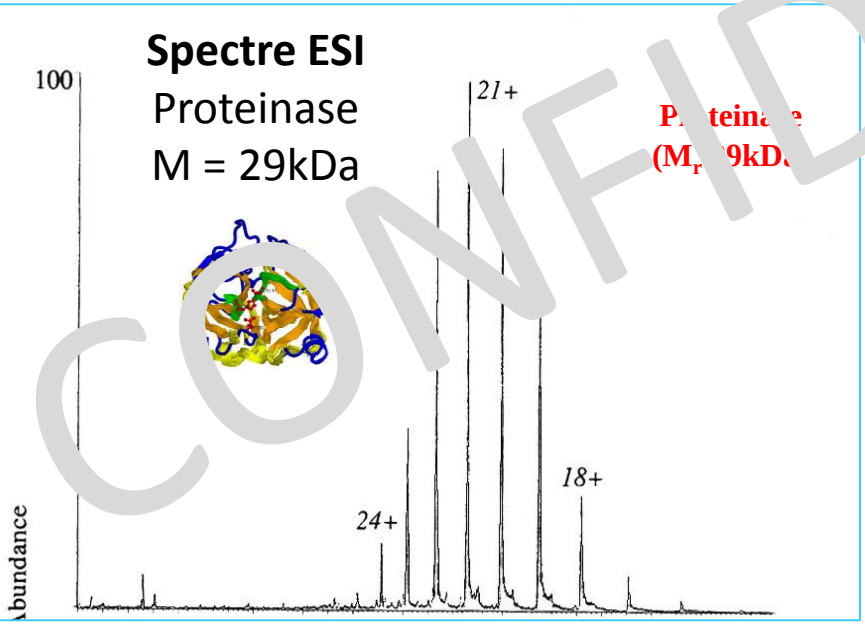


Spectre EI
Acrylamide
M = 71 Da

Spectre MALDI
Cryptophane
M = 1899,3 Da



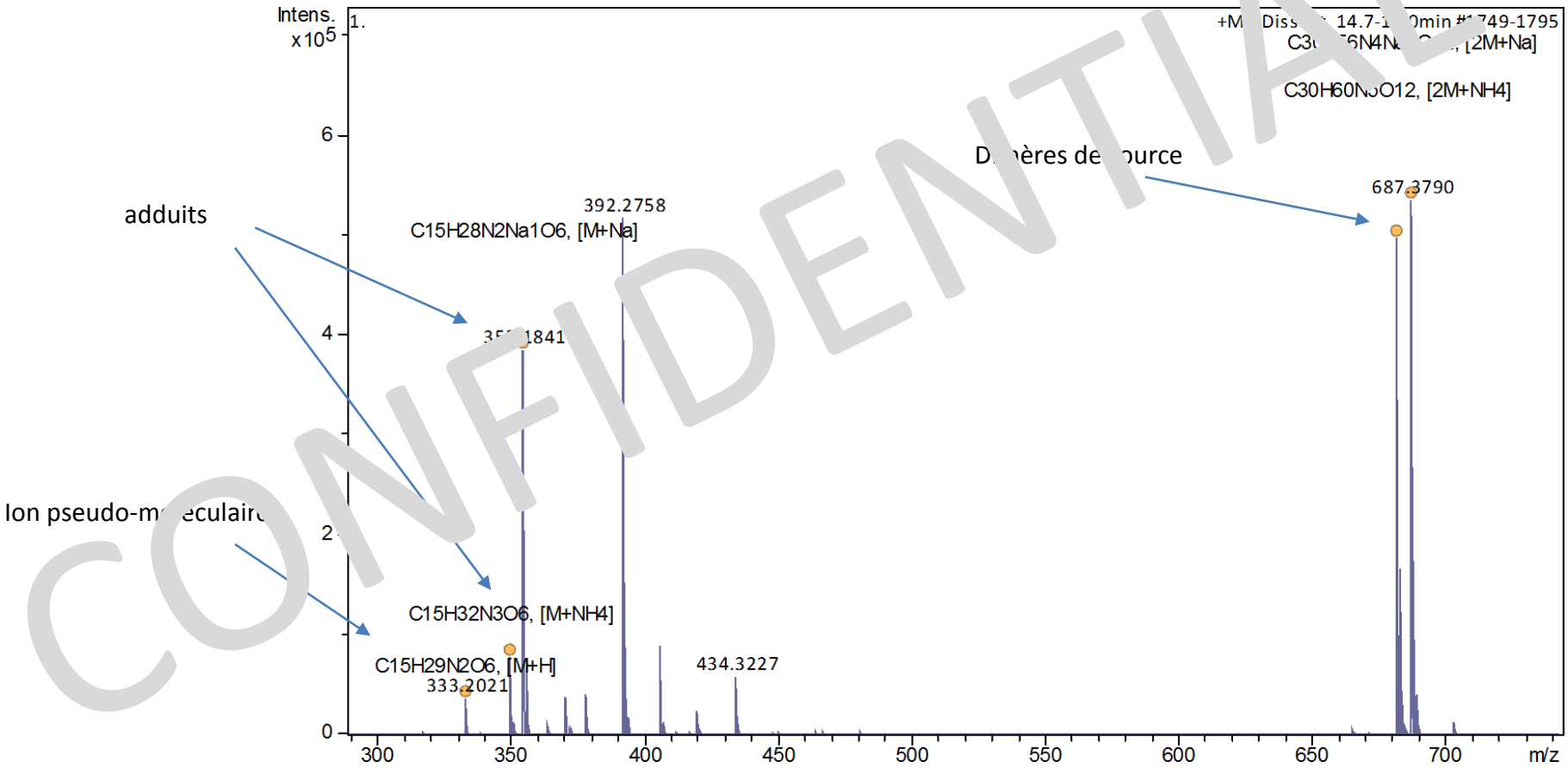
Spectre ESI
Proteinase
M = 29kDa



What does a mass spectrum show?

Molecular ions, pseudo-molecular ions, adducts and fragment ions

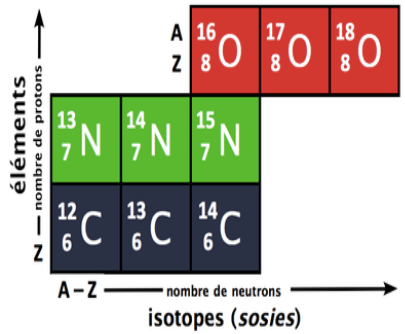
$C_{15}H_{28}N_2O_6$ M = 332,1947



Spectre ESI

MASS DEFINITION

- An element is specified by the number of protons in its nucleus.
- This is equivalent to the **atomic number Z** of the respective element, and thus determines its place in the periodic table of elements.
- **Isotopes** are nucleides that share the **same number of protons** but **have a different number of neutrons**. By extension, a nucleide characterised by its proton number Z and neutron number N (or **mass number A = Z + N**) is often referred to as an isotope, but without distinction as to its spin or energy state



MASS DEFINITION

- **Nominal mass:** calculated using the whole mass of the main isotope of each element (The integral sum of the nucleons in an atom, C = 12, H = 1, O = 16...).
- **Average mass / Atomic mass / Chemical mass:** calculated using the atomic mass of each element which takes into account the relative abundance of natural isotopes (C = 12.01115, H = 1.00797, O = 15.9994...)
- **Monoisotopic mass:** calculated using the exact mass of the main isotope of each element, which takes into account mass defects (C = 12.000000, H = 1.007825, O = 15.994915...)

A mass spectrometer does not separate ions by element but by isotopic mass

In order to successfully interpret a mass spectrum, it is necessary to know the isotopic masses and their relationship to the atomic weight of the elements, the isotopic abundances and the resulting isotopic patterns.

MASS DEFINITION

Example of calculation of nominal mass :

Ex : H_2O et NH_4^+ (1H, 16O, 14N)

m_{H2O} = 2 x 1 + 1 x 16 = 18 Da

$m_{(NH4)^+}$ = 14 x 1 + 4 x 1 = 18 Da

Not possible to differentiate the 2 by their mass

Example of calculation of monoisotopic mass or exact mass :

Ex : H_2O et NH_4^+ (^{1}H = 1,007825 ^{16}O = 15,994915 ^{14}N = 14,003074)

m_{H2O} = 2 x 1,00783 + 1 x 15,99492 = 18,01058 Da

$m_{(NH4)^+}$ = 14,00307 x 1 + 4 x 1,00783 = 18,03439 Da + $5{,}234^{E-4}$ = 18,03491Da

Mass of charge

if difference of 18,01058 : loss H_2O

if difference of 18,03491 : adduct NH_4^+

MASS DEFINITION

Acrylamide : C_3H_5NO

Nominal mass : 3x12 + 5x1 + 1x14 + 1x16 = 71 u
Exact mass : 3x12,0000 + 5x1,0078 + 1x14,0031 + 1x15,9949 = 71,037 u
Average mass : 3x12,011 + 5x1,0079 + 1x14,006 + 1x15,9994 = 71,1319 u

Hexatriacontane : $C_{36}H_{74}$

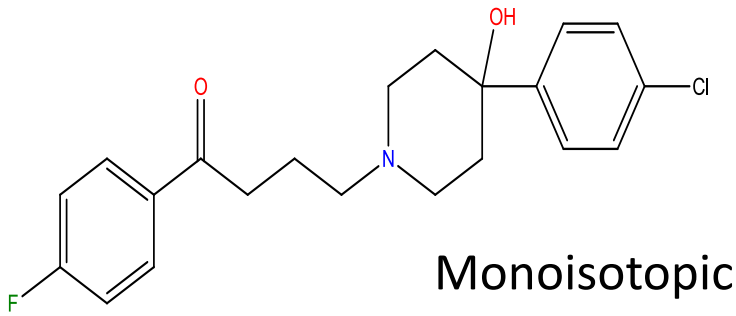
Nominal mass : 506 u
Exact mass : 506,57 u
Average mass : 506,98 u

Glucagon : $C_{153}H_{224}N_{42}O_{50}S$

Nominal mass : 3480 Da
Exact mass : 3481,6 Da
Average mass : 3483,6 Da

- M < 1000 Da : Nominal mass or exact mass
- 1000 < M < 3000 - 4000 Da : exact mass
- M > 4000 Da : Average mass

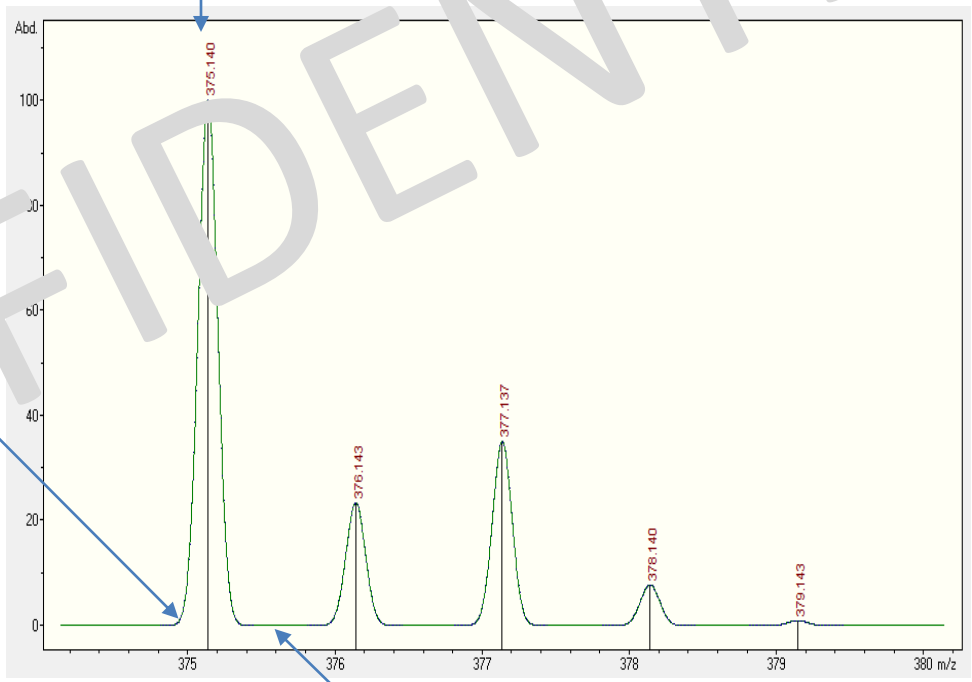
MASS DEFINITION



$C_{21}H_{23}ClFNO_2$

Monoisotopic mass or exact mass

Nominal mass



Average mass

WHAT MASS IS USED ?

Element	Nominal mass	Monoisotopic mass	Average mass
C	12	12,00000	12,01100
H	1	1,007825	1,00794
O	16	15,9941	15,99940
N	14	14,003	14,006
S	32	31,97207	32,06[illegible]

Ex : Average mass ^{12}C = 0,989 x 12 + 0,011 x 13

Isotope	Mass	Natural abundance (%)
^{12}C	12,00000	[illegible]8,9
^{13}C	13,[illegible]0336	[illegible].10
^{14}N	14,0[illegible]07	99,63
^{15}N	15,00[illegible]1	0,37
^{16}O	15,994[illegible]	99,76
[illegible]O	[illegible],9991	0,04
[illegible]	17,9992	0,20
^{1}H	1,00782	99,985
^{2}H	2,01410	0,015

Isotope	Mass	Natural abundance (%)
^{32}S	31,97[illegible]	95,04
^{33}S	32,[illegible]971[illegible]	0,75
^{34}S	33,[illegible]679	4,21
^{35}Cl	34,9[illegible]89	75,77
^{37}Cl	[illegible],9659	24,23
^{54}Fe	53.93960	5,80
^{56}Fe	55.93490	91,72
^{57}Fe	56.93540	2,20
^{58}Fe	57.93330	0,28

Isotope	Mass	Natural abundance (%)
^{19}F	18,9984	100
^{27}Al	26,9815	100
^{31}P	30,9738	100

MASS DEFINITION

Monoisotope, isotopologue and isotopic pattern

- even if the analyte is chemically perfectly pure, it represents a mixture of different isotopic compositions. Therefore a mass spectrum is normally composed of superpositions of the mass spectra of all the isotopic species involved, called an **isotope pattern**
- The polynomial approach is used for the calculation of isotopic distributions of polyisotopic elements or for formulae composed of several non-monoisotopic elements. In general, the isotopic distribution of a molecule can be described by a polynomial product:

$$(a_1 + a_2 + a_3 + \cdots)^m (b_1 + b_2 + b_3 + \cdots)^n (c_1 + c_2 + c_3 + \cdots)^o$$

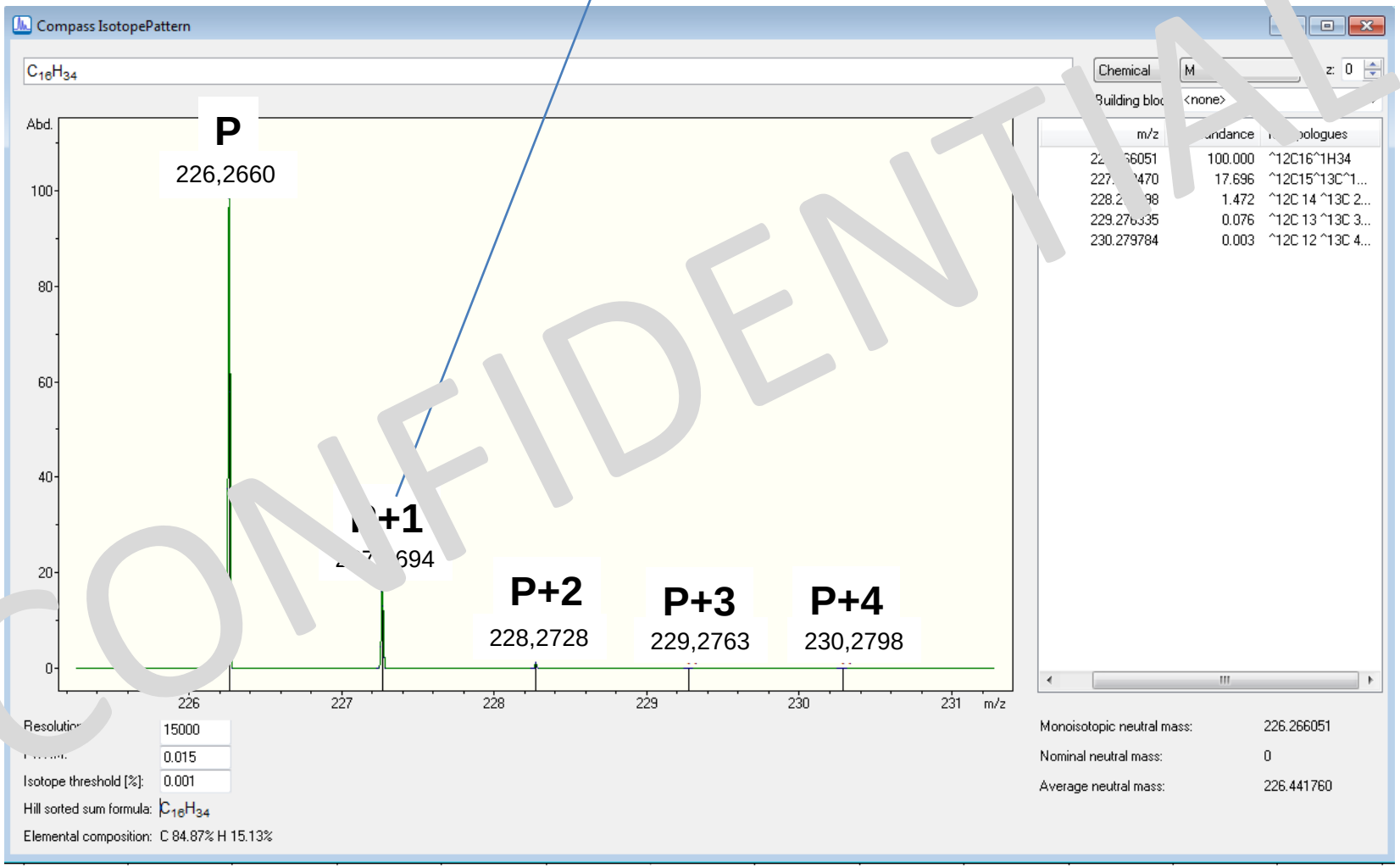
-> a_1, a_2, a_3, represent the individual isotopes of an element
-> b_1, b_2, b_3 represent those of another element and so on until all elements are included
-> m, n, o give the number of atoms of these elements as contained in the empirical formula

Mass spectrometers are usually supplied with software to calculate isotopic distributions. Programs are also available on the internet

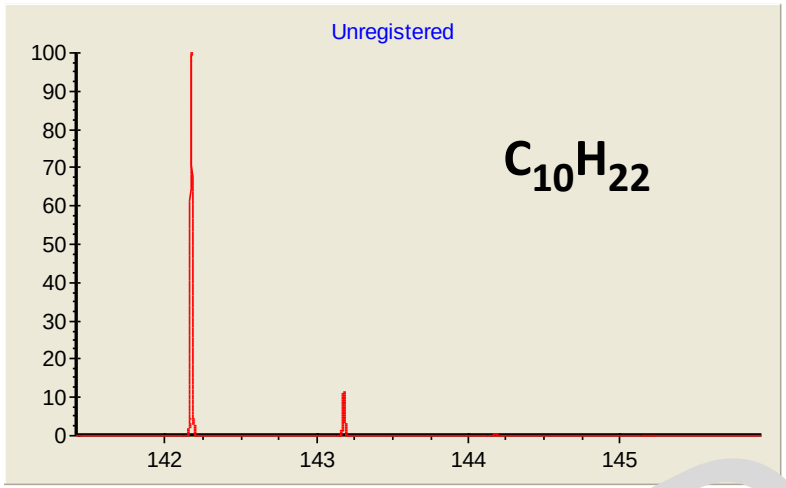
ISOTOPIC PATTERN

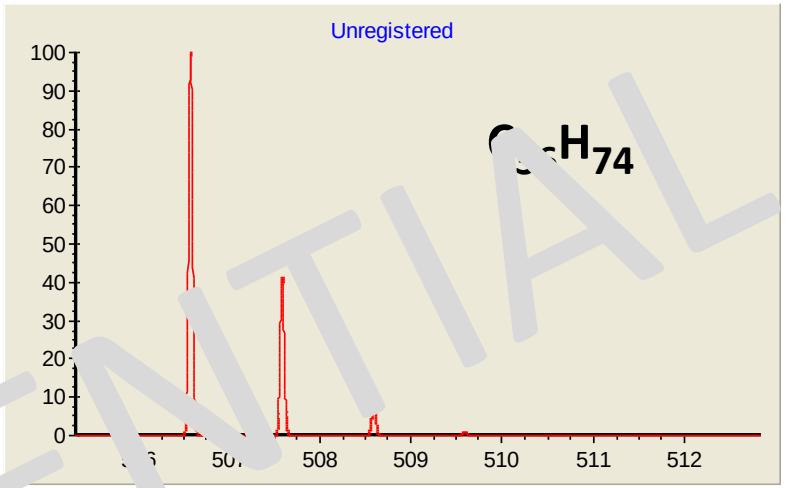
Hexadecane : $C_{16}H_{34}$ 226 u

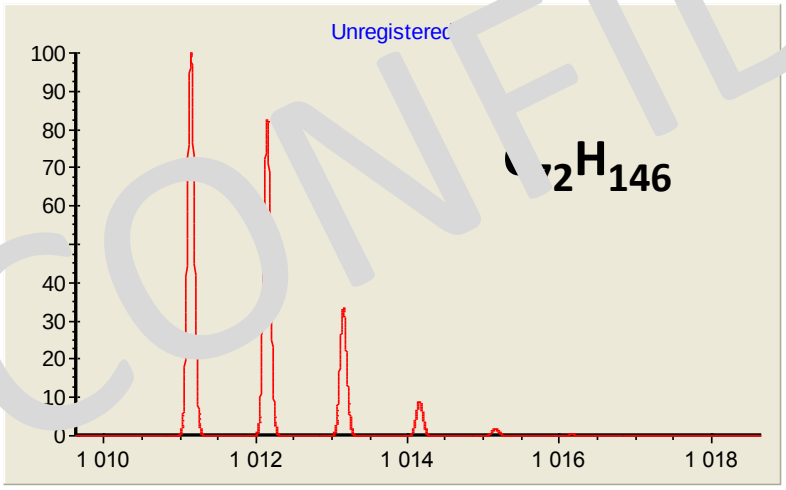
P+1 (^{13}C et ^{2}H) = 16x0,011+34x0,00015 = 0,18

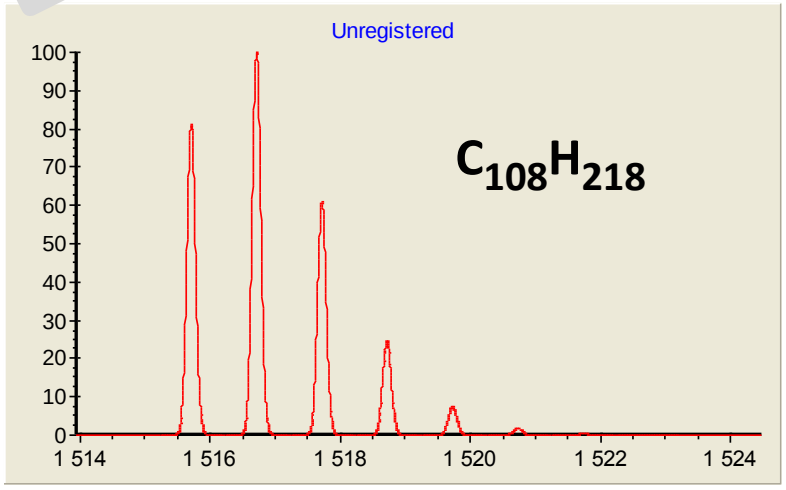


EVOLUTION OF ISOTOPIC PATTERN



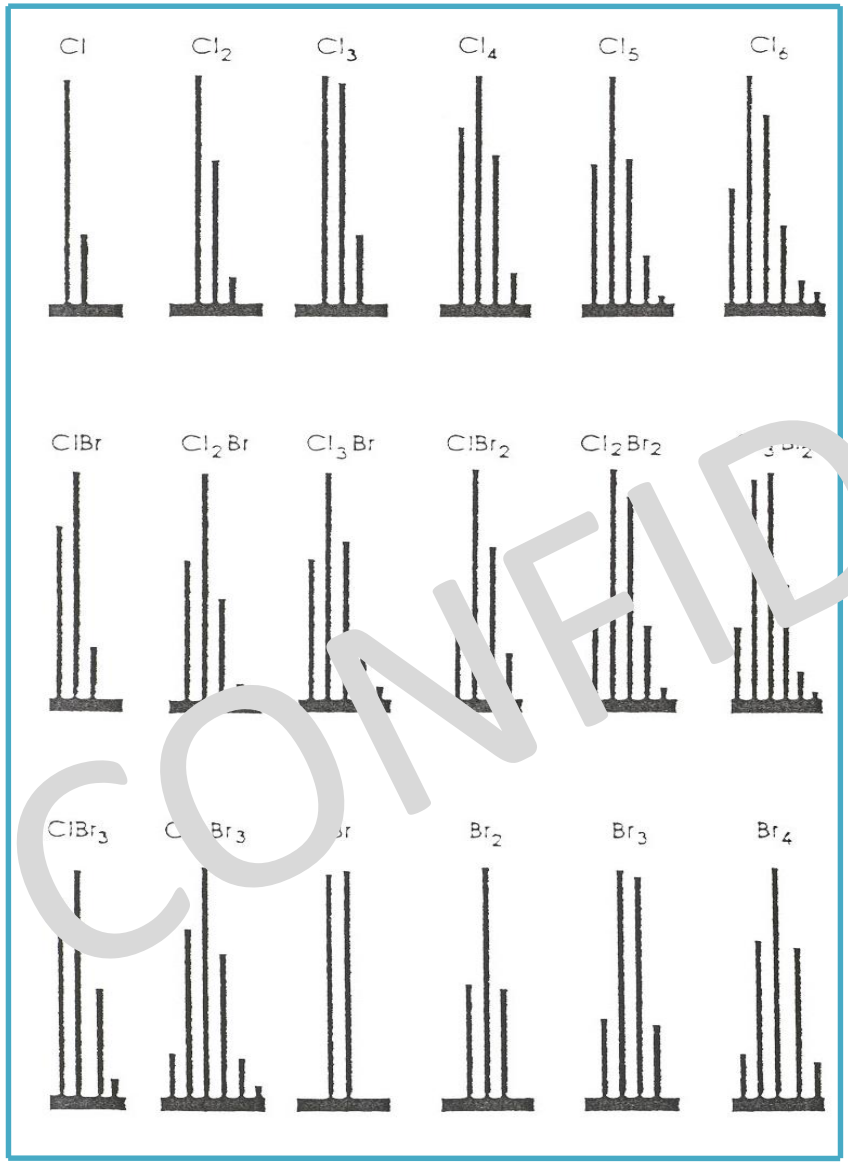






HALOGEN COMPOUNDS

Representation of the isotopic clusters of the combination of 1 to 6 atoms of Cl and/or Br



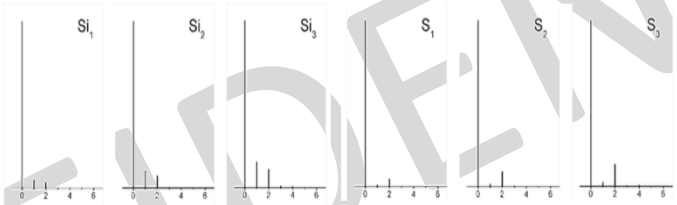
Relative abundance of Cl and Br isotopes

Cl-Br	P	P+2	P+4	P+6	P+8	P+10
Cl	100	32.5				
Cl_2	100	65.0	10.6			
Cl_3	100	97	31.7	3.		
Cl_4	76	100	48.7	10	9	
Cl_5	61.	100	5.0	21.1	3.4	0.2
Cl_6	51.2	100	8 2	35.2	8.5	1.1
lBr	6.6	100	24.4			
Br	61.4	100	45.6	6.6		
Cl_3Br	51.2	100	65.0	17.6	1.7	
$ClBr_2$	43.8	100	69.9	13.7		
Cl_2Br_2	38.3	100	89.7	31.9	3.9	
Cl_3Br_2	31.3	92.0	100	49.9	11.6	1.0
$ClBr_3$	26.1	85.1	100	48.9	8.0	
Cl_2Br_3	20.4	73.3	100	63.8	18.7	2.0
Br	100	98.0				
Br_2	51.0	100	49.0			
Br_3	34.0	100	98.0	32.0		
Br_4	17.4	68.0	100	65.3	16.0	

OTHER PARTICULAR CASES

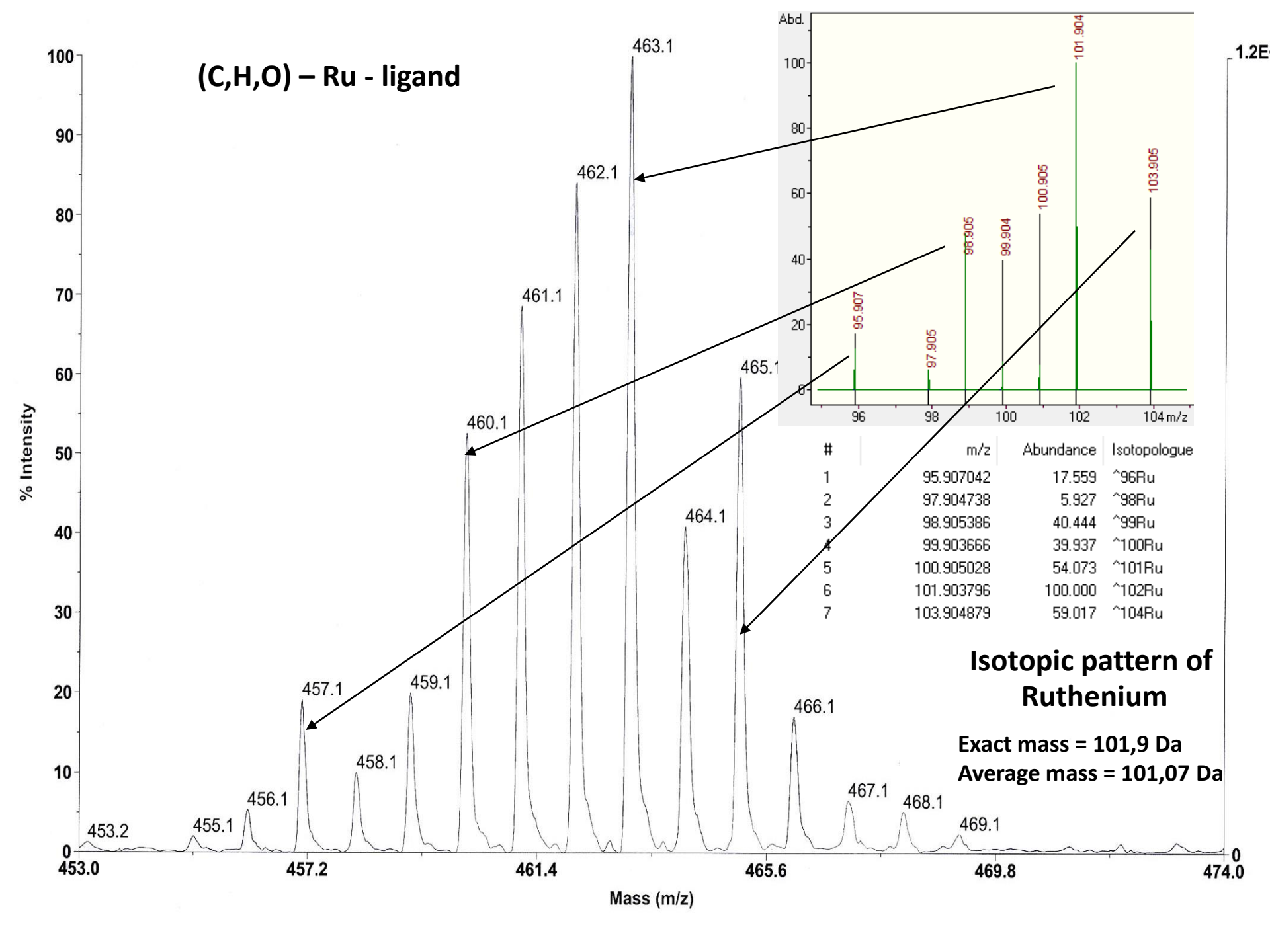
Monoisotope, isotopologue and isotopic pattern

- **Oxygen**, **silicon** and **sulphur** are polyisotopic elements. Oxygen exists as isotopes ^{16}O, ^{17}O and ^{18}O, sulphur as isotopes ^{32}S, ^{33}S and ^{34}S, and silicon as isotopes ^{28}Si, ^{29}Si and ^{30}Si.
- The isotopic profiles of sulphur and silicon are not as characteristic as those of chlorine and bromine, but their contributions are significant enough to infer their presence in a molecule.



- **To check for the presence of S and Si** in a mass spectrum, **the intensity of X+2** must be carefully examined: the intensity of this signal will be too high to be caused by the contribution of $^{13}C2$ alone.

This is no longer noticeable when the carbon number becomes too large


(C,H,O) – Ru - ligand
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
453.0
457.2
461.4
465.6
469.8
474.0
453.2
455.1
456.1
457.1
458.1
459.1
460.1
461.1
462.1
463.1
464.1
465.1
466.1
467.1
468.1
469.1
Abd.
100
80
60
40
20
0
96
98
100
102
104 m/z
95.907
97.905
98.905
99.904
100.905
101.904
103.905
| m/z | Abundance | Isotopologue
1 | 95.907042 | 17.559 | ^96Ru
2 | 97.904738 | 5.927 | ^98Ru
3 | 98.905386 | 40.444 | ^99Ru
4 | 99.903666 | 39.937 | ^100Ru
5 | 100.905028 | 54.073 | ^101Ru
6 | 101.903796 | 100.000 | ^102Ru
7 | 103.904879 | 59.017 | ^104Ru
Isotopic pattern of Ruthenium
Exact mass = 101,9 Da
Average mass = 101,07 Da

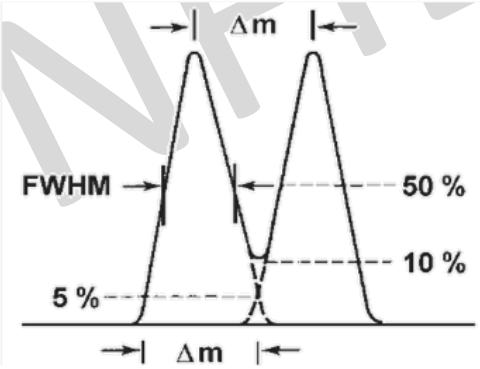
RESOLUTION AND CALCUL OF RESOLUTION

Resolution (R): The ability of the instrument to separate two ions with masses as close as possible

$$R = \frac{M}{\Delta M}$$

Ex : ions at m/z 100 et 100,1 Th
alors R = 1000

For an isolated peak, we take for ΔM the width of the peak at X% of its maximum.
For X=50%, we speak of FWHM (Full Width at Half Maximum) resolution: definition found on instruments

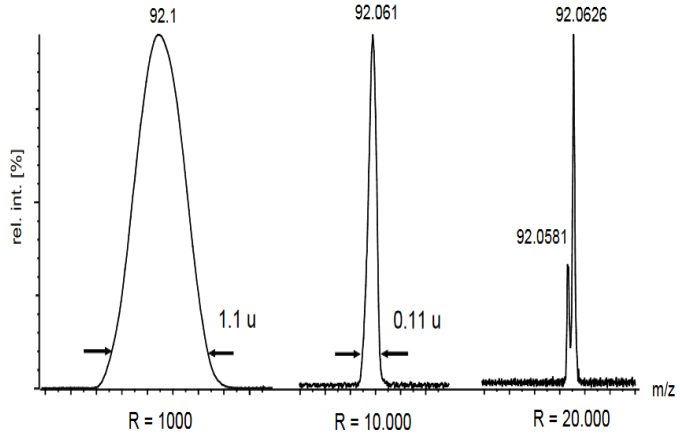


Rs d'un pic

$$Rs = \frac{M \text{ du pic}}{L^{50\%}}$$

RESOLUTION

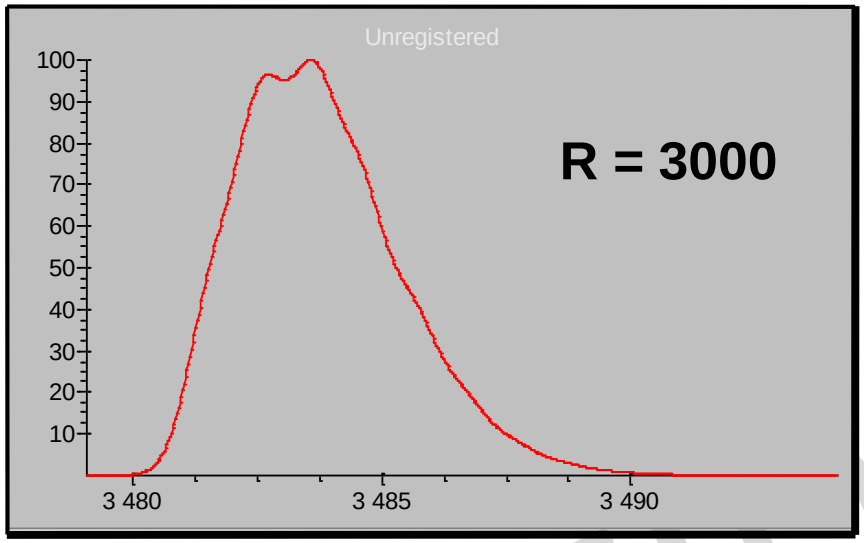
Resolution and mass accuracy



- Increasing the resolution does not affect the relative intensity of the peaks.
- Isotopic profiles are not affected by an increase in resolution up to the threshold of R=100000. Above this, there may be changes due to the separation of different isotopic species of the same nominal mass.
- Accurate mass measurements depend on the resolving power of the apparatus used, as good mass accuracy can only be achieved when the peaks are sufficiently resolved.

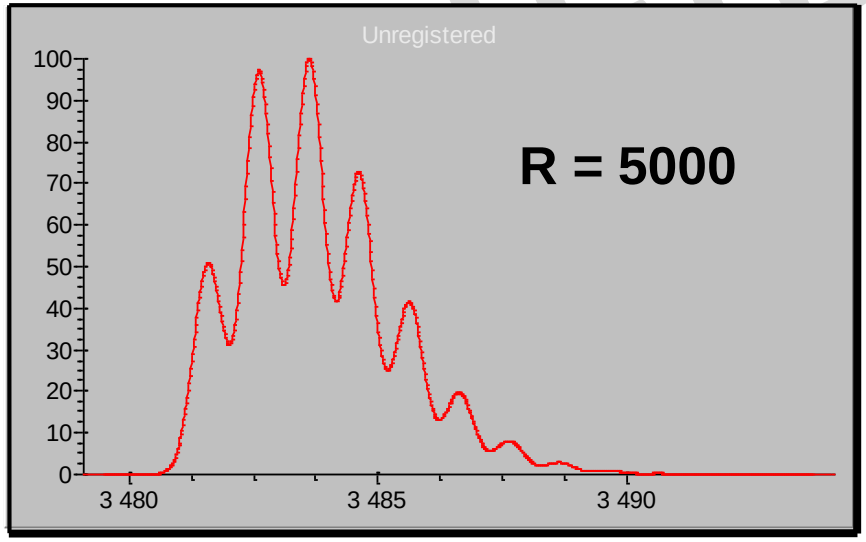
Only high-resolution measurements can be used to determine empirical formulae.

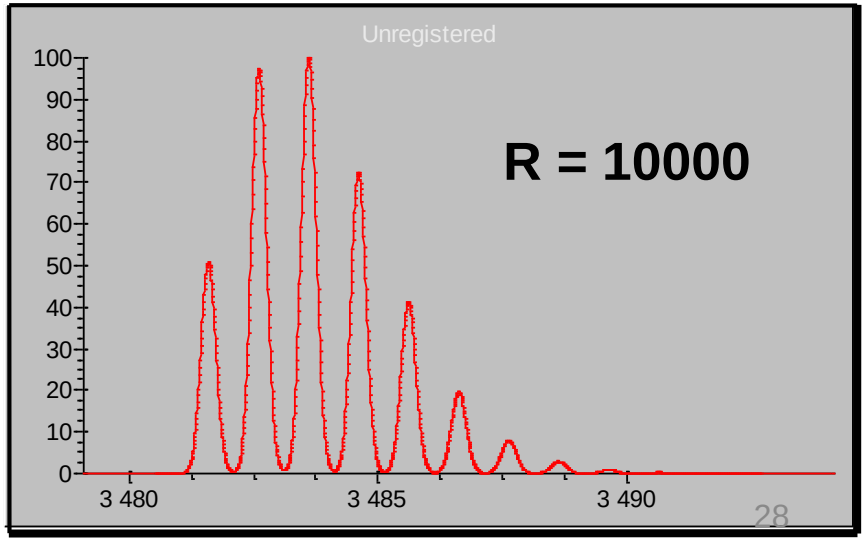
RESOLUTION



Glucagon : $C_{153}H_{224}N_{42}O_{50}S$

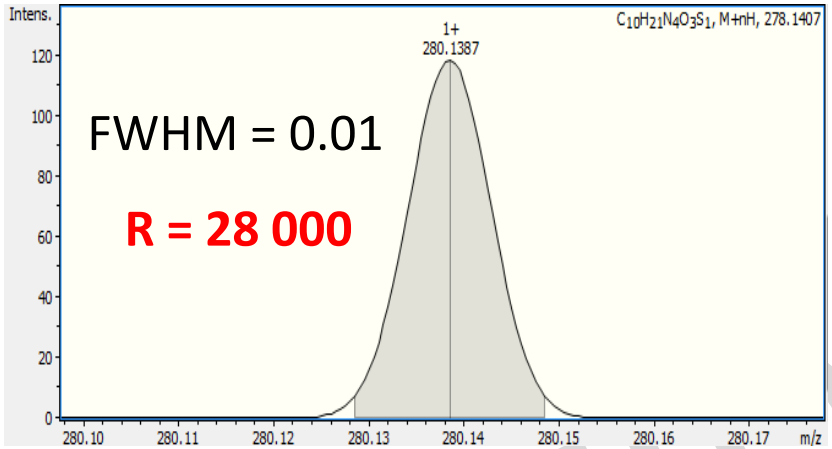
Nominal mass: 3480 Da
Exact mass: 3481,6 Da
Average mass: 3483,6 Da

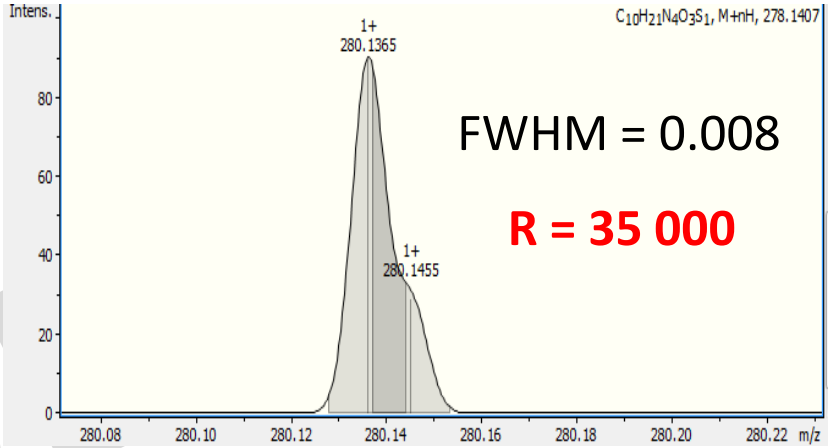


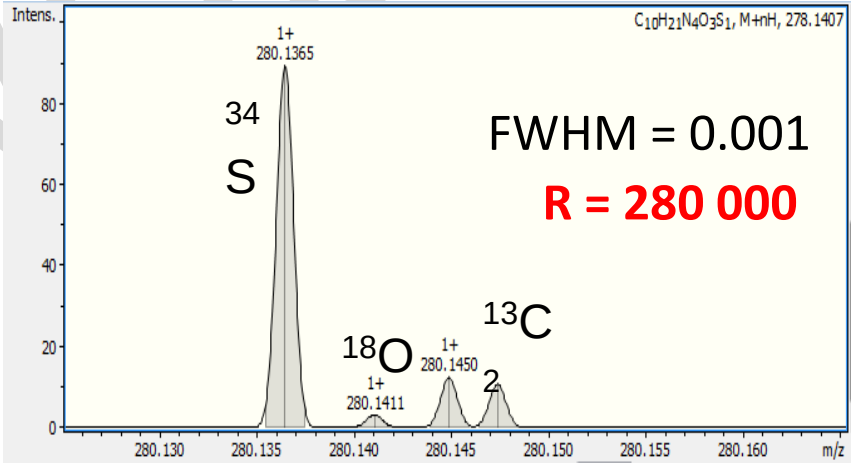


RESOLUTION

Resolution and mass accuracy $C_{10}H_{21}N_4O_3S_1$ Zoom sur P+2







RESOLUTION ET PRECISION

Resolution and mass accuracy

$$Précision\ (ppm) = \frac{|M_{calculée} - M_{expérimentale}|}{M_{calculée}} \times 10^6$$

Instruments must be calibrated to achieve high accuracy in mass

- Lock-mass
- External calibration
- Internal calibration

RESOLUTION

Possible molecules in C, H, O between 298.0 and 298.2 u

Masses	Exact masses	Formula
de 298,0	298,047737	$C_{16}H_{10}O_6$
à	298,062994	$C_{2}H_{10}O$
298,1	298,084123	$C_{17}H_{14}O_5$
	298,0	$C_{21}H_{14}O_2$
de 298,1	298,1 0509	$C_{18}H_{18}O_4$
	298,135	$C_{22}H_{18}O$
à	298,141638	$C_{15}H_{22}O_6$
	298,156894	$C_{19}H_{22}O_3$
298,2	298,178023	$C_{16}H_{26}O_5$
	298,193280	$C_{20}H_{26}O_2$

R=19532

R=5770

RESOLUTION

Resolution and mass accuracy

Why is important ?

- Access to the exact mass
- Access to raw formulas
- Increase confidence in identification
- Improve accuracy of quantification

Measured mass	Tolerance (Da)	Possible Formula	Theoretical mass
32.0	+/- 0.2	O2 CH3OH N2H4 S	31.9898 32.0261 32.0374 31.9721
32.02	+/- 0.02	CH3OH N2H4	32.0261 32.0374
32.0257	+/- 0.002	CH3OH	32.0261

RESOLUTION

Resolution and mass accuracy

Why is important ?

- $[M+H]^+$ = 381.0828 Da (Compound containing only C H N S O)

Tolerance on mass accuracy (ppm)	Possible number of formula
200	265
100	133
30	39
10	14
5	5
3	4

Advantages / Disadvantages

Advantages :

- **Specificity**
- **Sensitivity**
- **Ability to be coupled with separative techniques**
- **Many applications**

Disadvantages :

Mass measurement can only be performed on molecules that have been previously **volatile and ionised molecules**

It is therefore necessary to transform a generally liquid or solid sample into a diluted gas requiring a **high vacuum**

Mass Spectrometer Components

1 bar = 10^5 Pa
1 atm = 1,013 bar
1 torr = 1 mm Hg
= 1,333 mbar

Pump system

Rotary Vane Pumps
Turbo pumps
Diffusion pumps

Introduction of the sample

Ions Source

Mass filter

Detector

Computer

Ball (gaz)
Probe (solid, desorption)
GC
HPLC, UPLC, nanoLC

Target

EI, CI

ESI
APCI
APPI
} **API**

MALDI

Quadrupole
Ion Trap

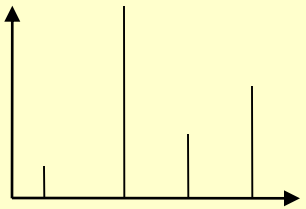
TOF

FTMS
Orbitrap

Electron multipliers

Micro Channel Plate

“Hybrid”



Spectrum

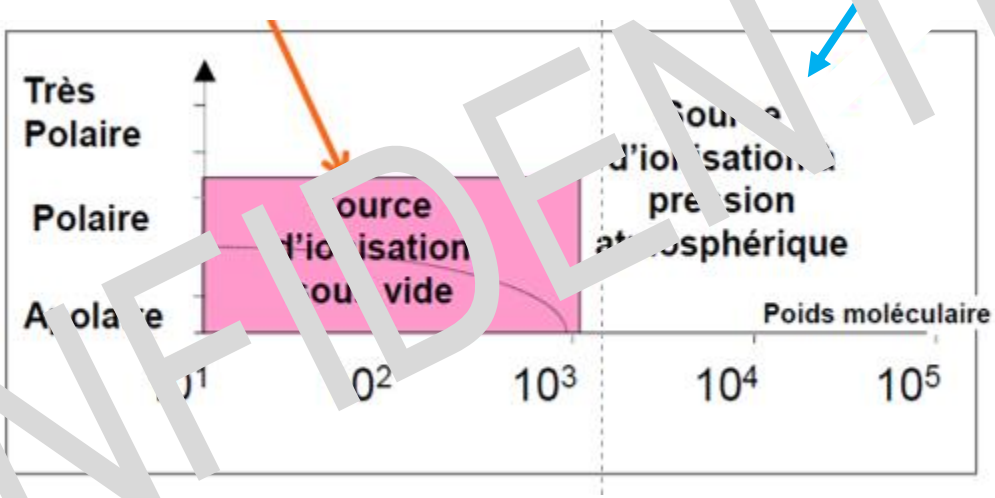
Ions Sources

IONIZATION MODE

2 types of ion sources commonly used

Vacuum ionization source **Atmospheric Pressure ionization source**

Coupling with Gas Chromatography



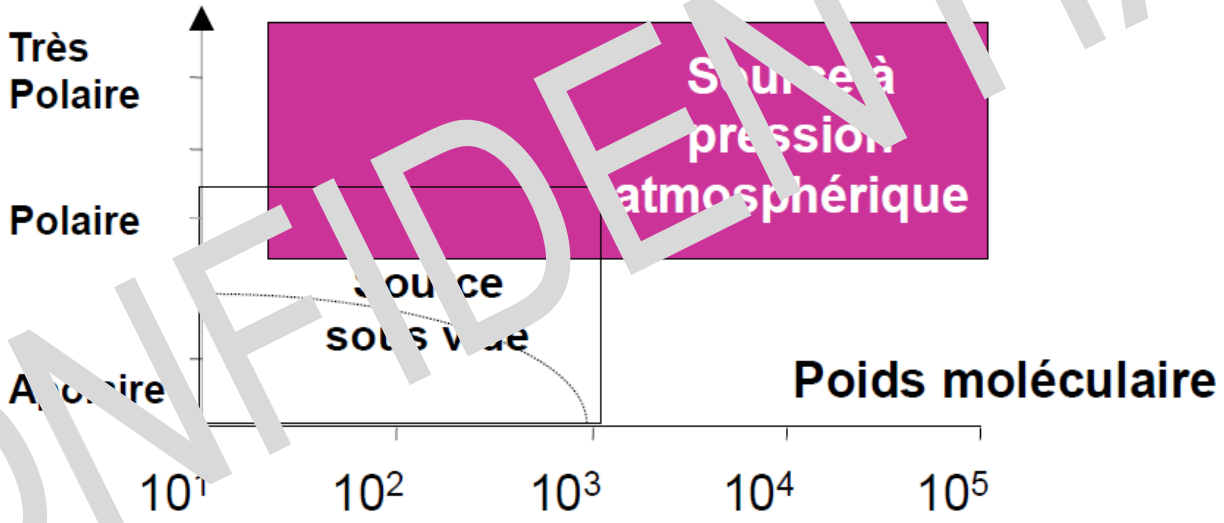
Electronic impact source : strong ionization

Chemical ionization source : soft ionization

IONIZATION MODE

Atmospheric Pressure ionization source

Coupling with **Liquid Chromatography**



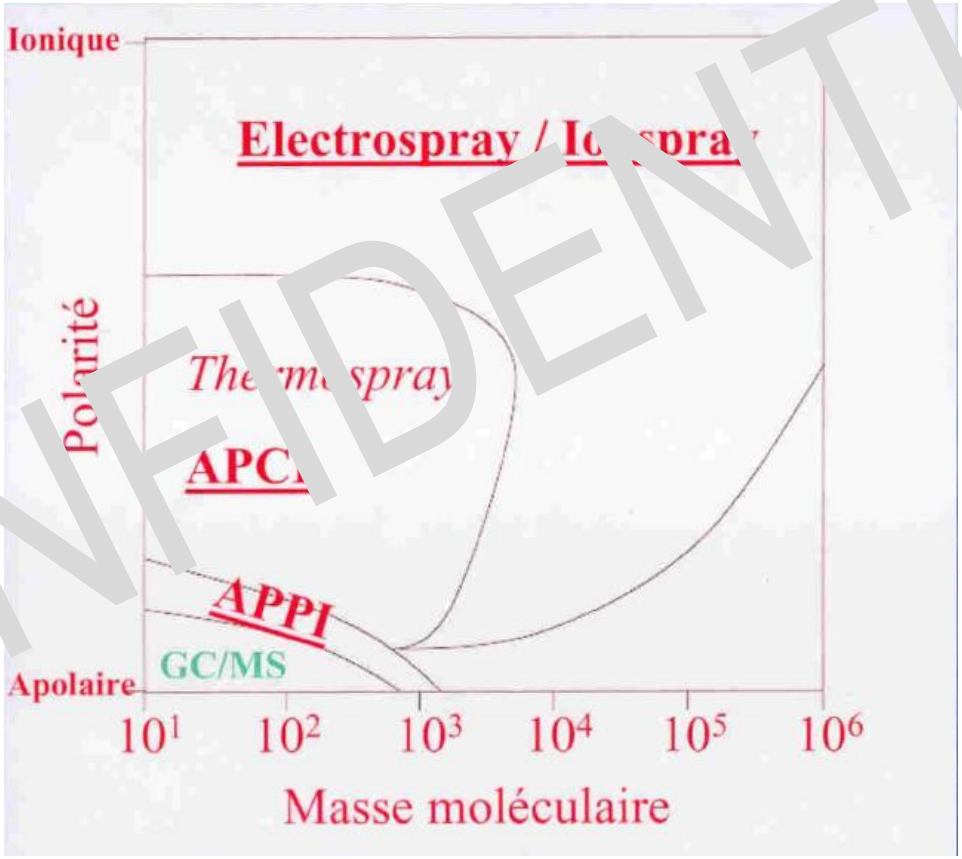
Source ESI : **polar compounds**

Source APCI : non-polar compounds

Source APPI : aromatics compounds

IONIZATION MODE : Atmospheric Pressure Sources

What kind of sources are used?



IONS SOURCES

- **Atmospheric pressure source** : Electrospray, APCI, APPI
- **Desorption Laser source**: MALDI

  - **Soft ionization :**

    - heat sensitive molecules

    - IE and IC not possible

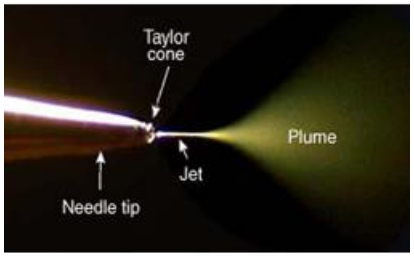
    - low volatile molecules

    - high molecular weight molecules

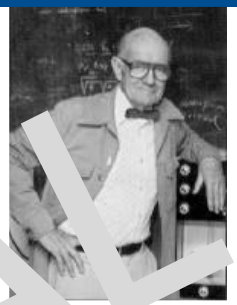
    → **no or little fragmentation**

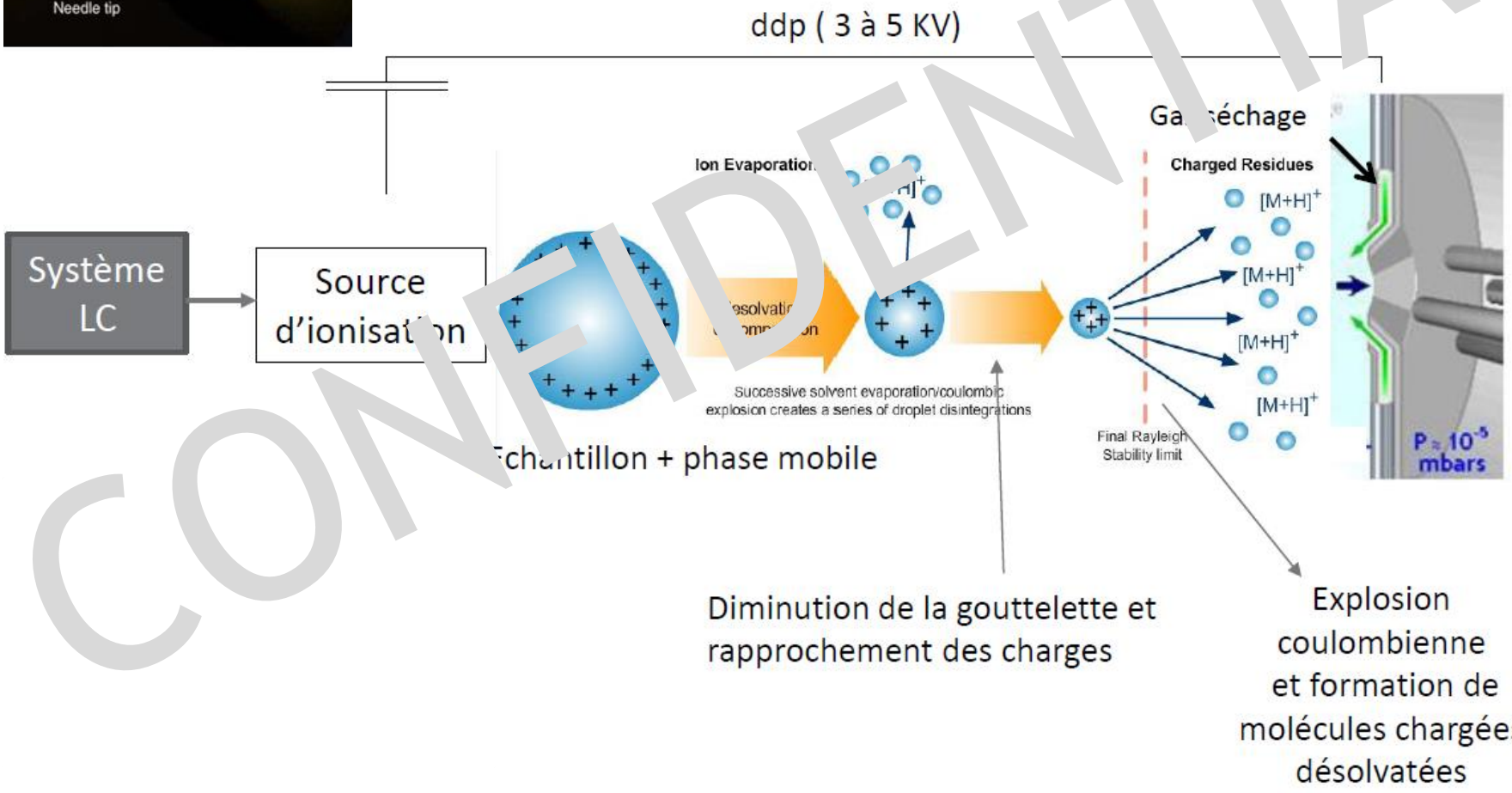
IONIZATION MODE : Atmospheric Pressure Sources

Electrospray ou electronebullization (ESI)



1968 : Malcom Dole
1984 : John Fenn
(Nobel Price Chemistry 2002)





IONIZATION MODE : Atmospheric Pressure Sources

Electrospray or electronebullization (ESI)

- The formation of ions is subject to the laws of solution chemistry
- No or little fragmentation
- Can form both single and multi-charged ions
- Access to very high molecular weight molecules via multi-charged ions [M+nH] n+

$$[M+H]^{+}, [M+2H]^{2+}, [M+3H]^{3+}, \ldots\ldots [M+nH]^{n+}$$

$$\frac{m}{z}$$

$$\left(\frac{M+H}{1}\right)^{+}\left(\frac{M+2H}{2}\right)^{2+}\left(\frac{M+3H}{3}\right)^{3+}\ldots\ldots\left(\frac{M+nH}{n}\right)^{n+}$$

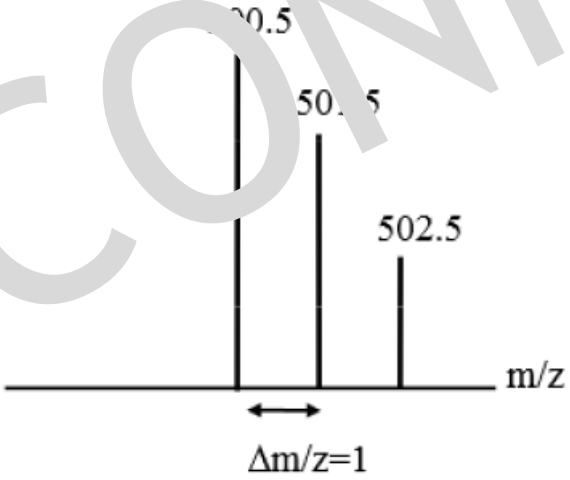
IONIZATION MODE : Atmospheric Pressure Sources

Electrospray or electronebullization (ESI)

Determination of the charge state of a compound under study from the mass spectrum

→ Isotopic pattern are used

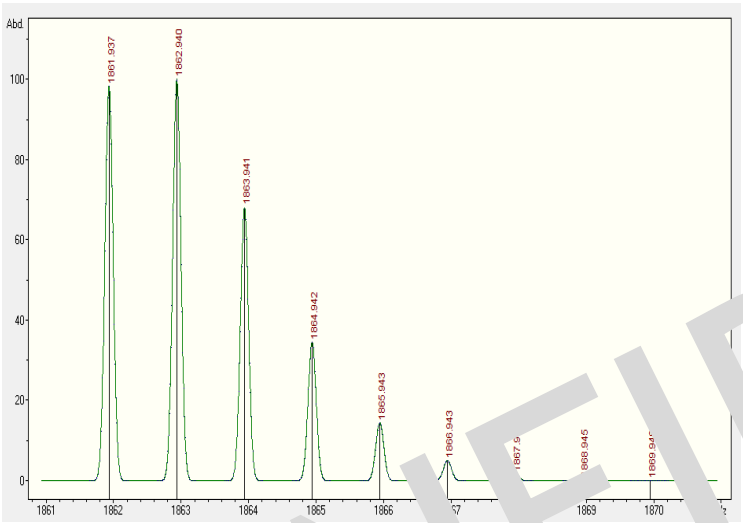
Mass difference brought about by the presence of 1 isotope is 1 Da, so the **m/z ratio varies by 1/z**

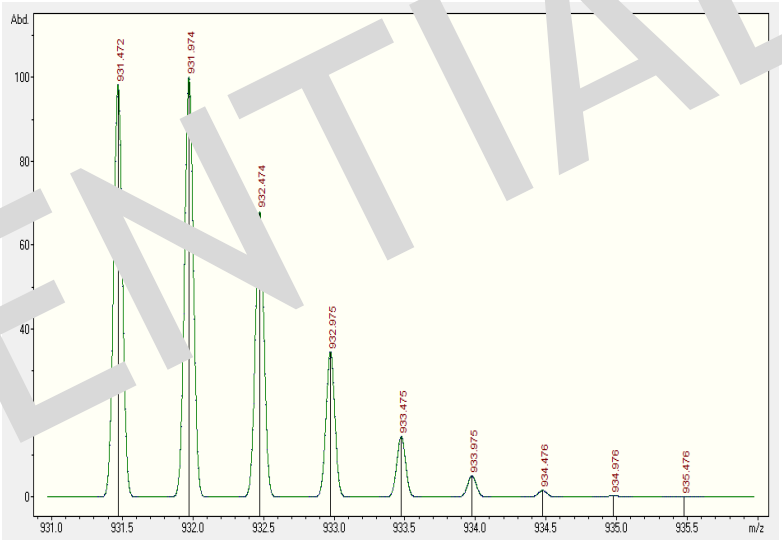


Δm/z=1	z=1
Δm/z=0.5	z=2
Δm/z=0.33	z=3
...	

IONIZATION MODE : Atmospheric Pressure Sources

Charge state and isotopic pattern





Δ(m/z) = 1 Da

Z = 1

Δ(m/z) = 0.5 Da

Z = 2

Δ(m/z) (Da)	z
1	1
0.5	2
0.33	3
0.25	4
0.2	5

IONIZATION MODE : Atmospheric Pressure Sources

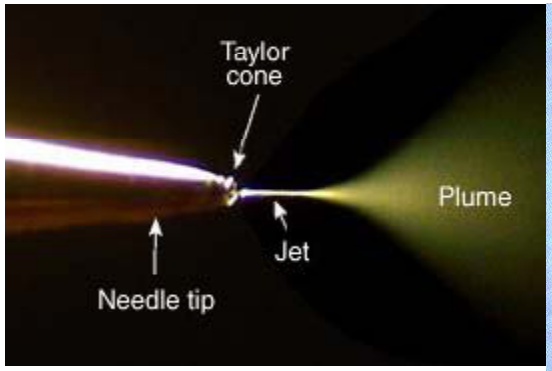
Electrospray or electronebullization (ESI)

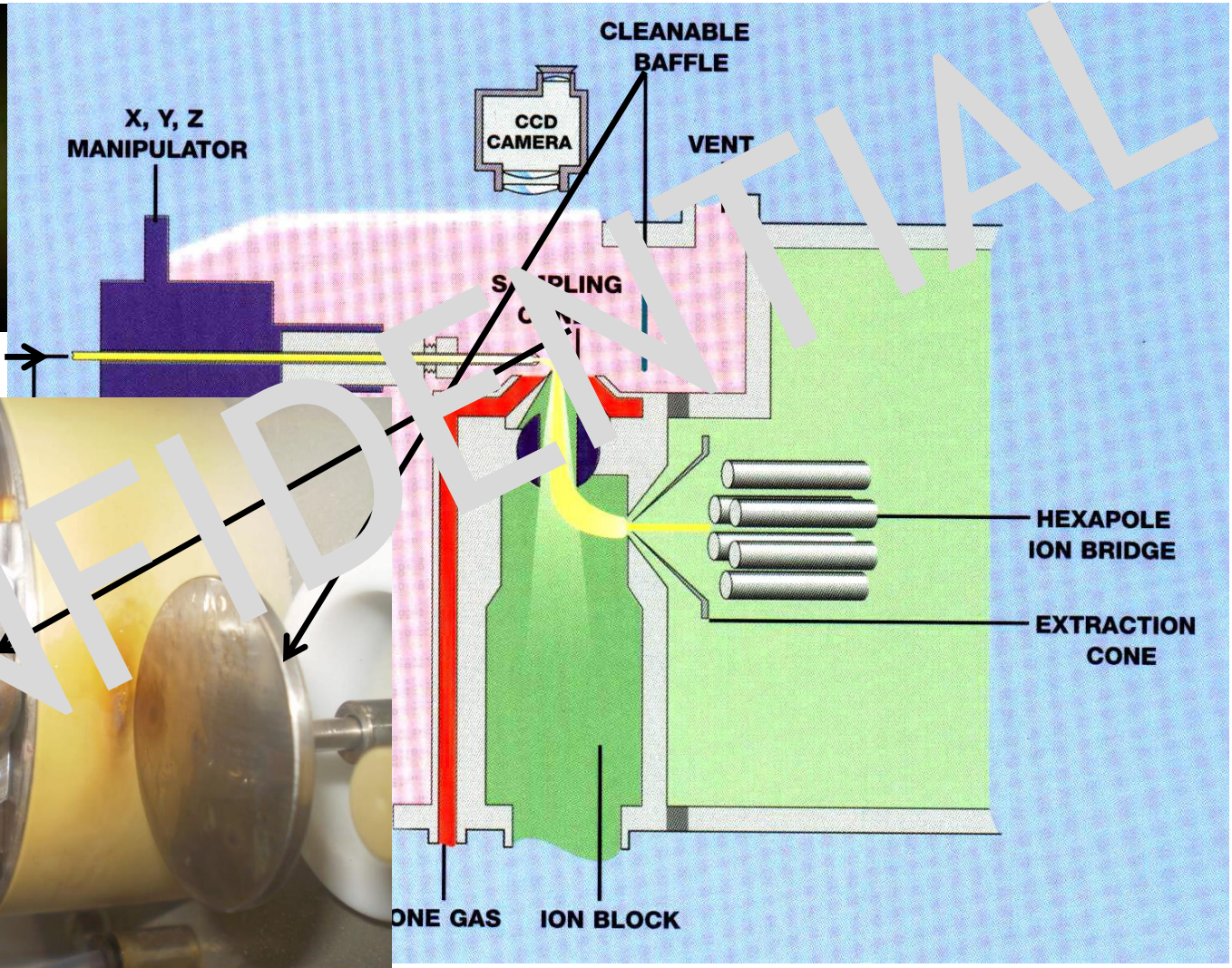
- Masses of 1000 à 10^5 Da (multi-charged ions) :
  - Biopolymers (Proteins, polysaccharides …)
  - Polar polymers
  - Supramolecules

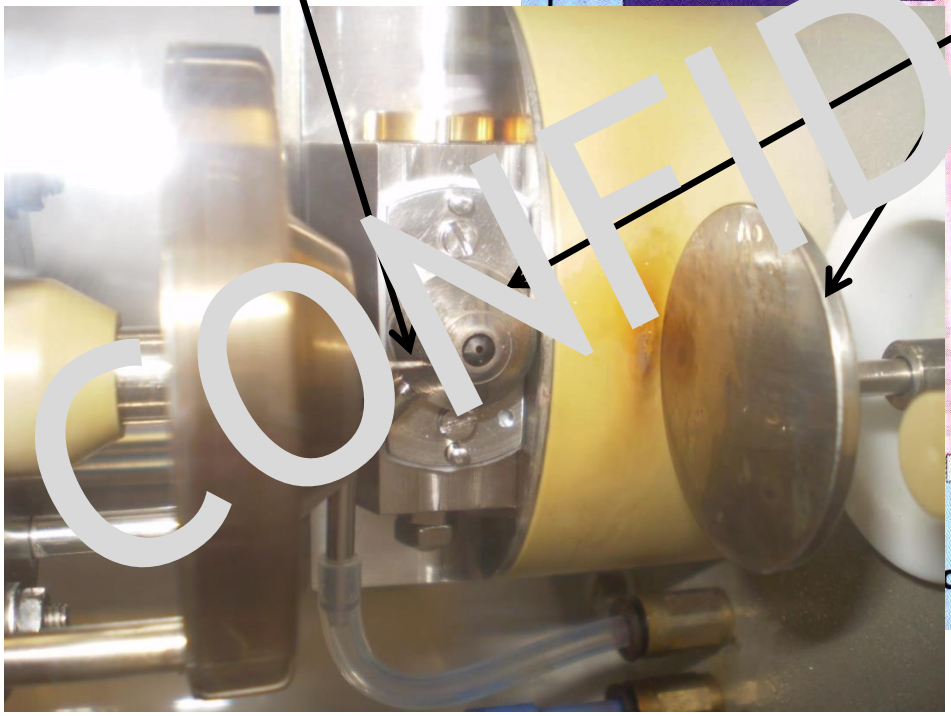
- Masses < 1000 Da :
  Polar, heat-sensitive or non-volatile molecules

- Couplings with chromatography techniques :
  - HPLC, nanoLC, UPLC
  - Capillary electrophoresis (CZE)
  - Maximum flow rate : 200 µl/min (HPLC) à 600 µl/min (UPLC)
    300 nl/min (nanoLC)

ELECTROSPRAY (Source Z-spray (Waters))







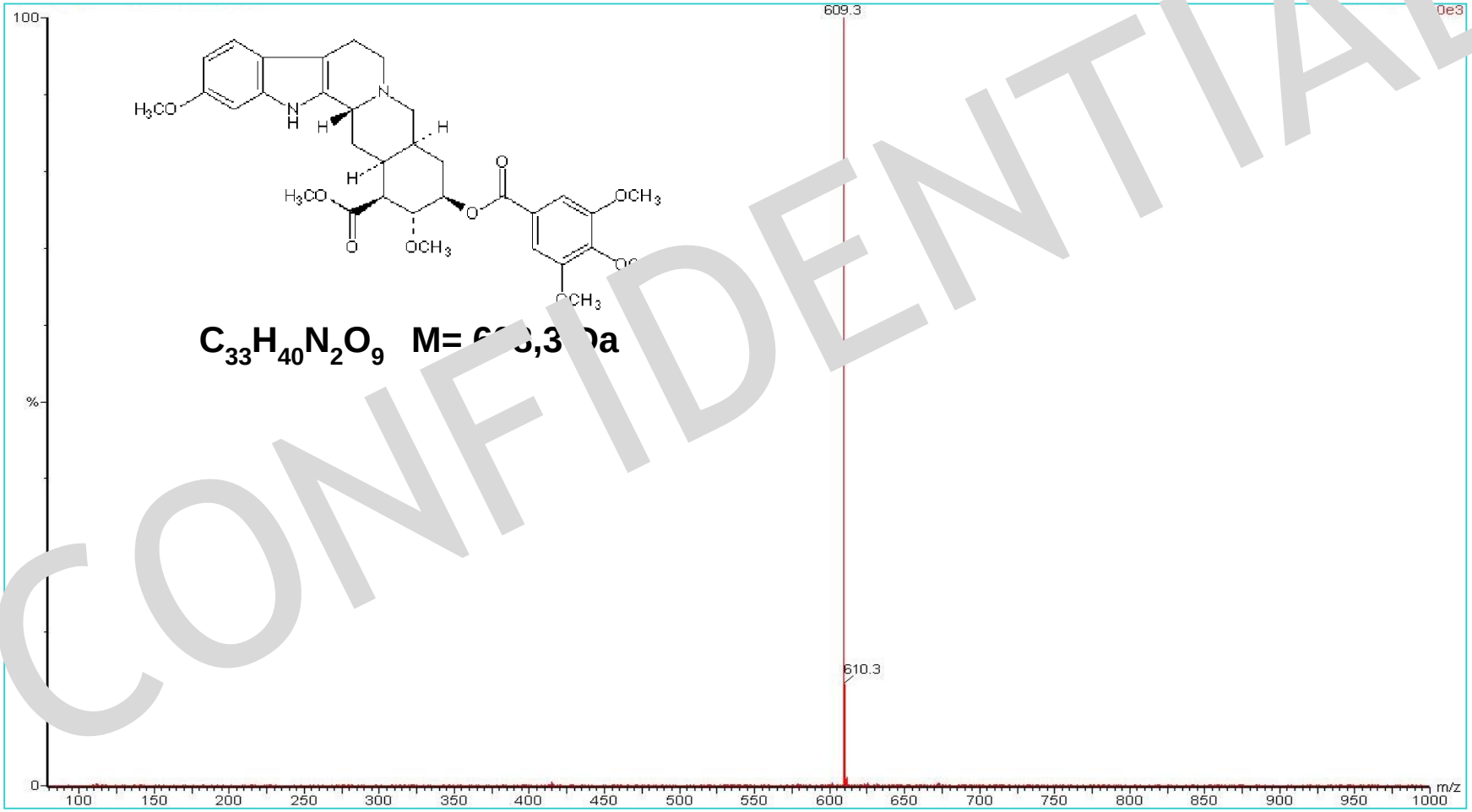
MASS SPECTRUM IN ESI

Types of ions obtained

- In positive mode : protonated molecules [M+H]+ or complexed with alkaline ions [M+Na]+, [M+K]+, [M+NH4]+... as electrospray is applicable to molecules with no ionisable site
- In negative mode : $[M-H]^-$
- Sometimes : $M^{+\cdot}$, $M^{-\cdot}$
- Association Molecule - solvent, formation of non-covalent complexes [Solvent + M + H]+
- Formation of multiply charged species $[M + nH]^{n+}$

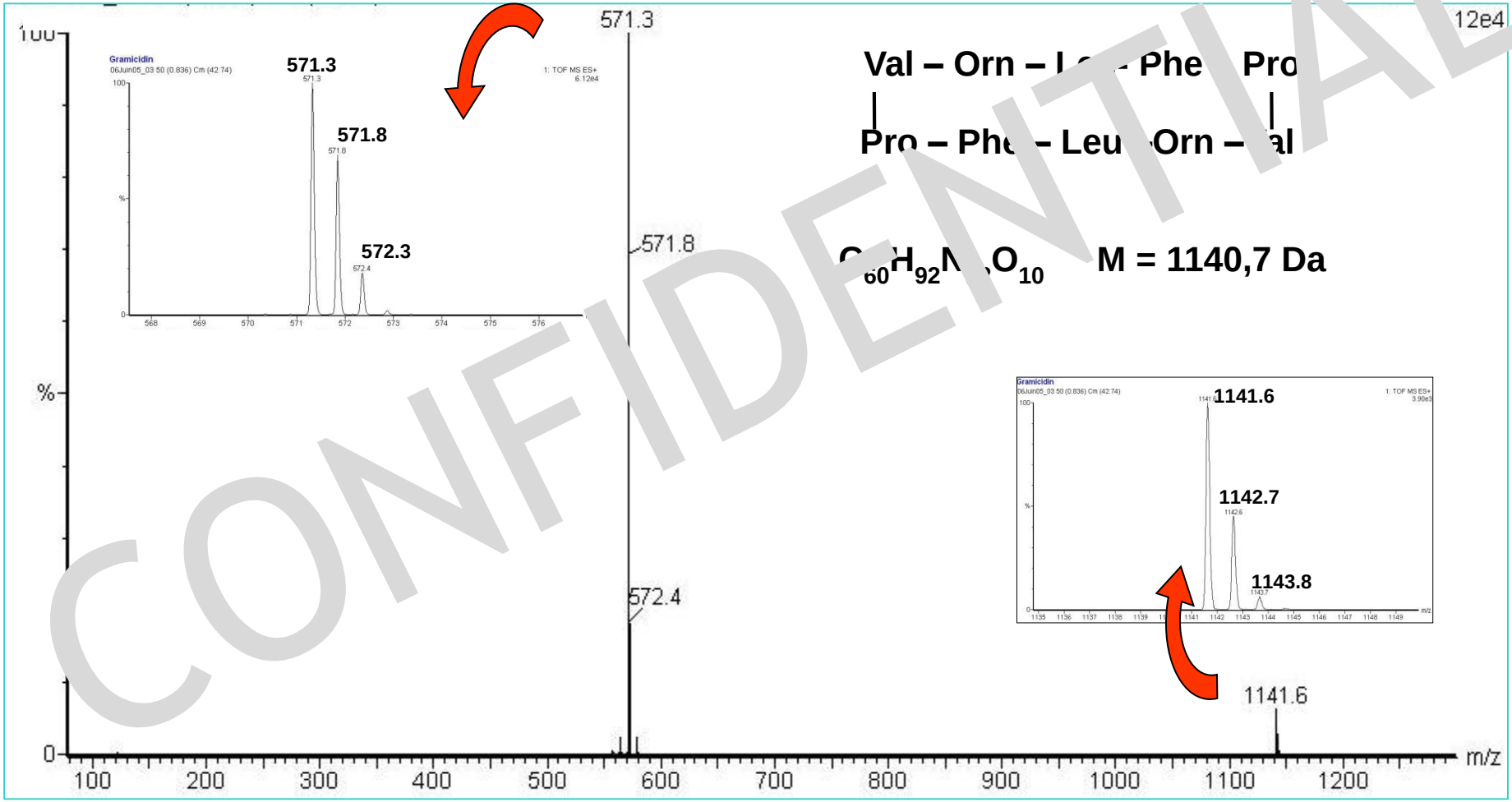
MASS SPECTRUM IN ESI

Spectrum Electrospray of Reserpine



MASS SPECTRUM IN ESI

Spectrum Electrospray of Gramicidine

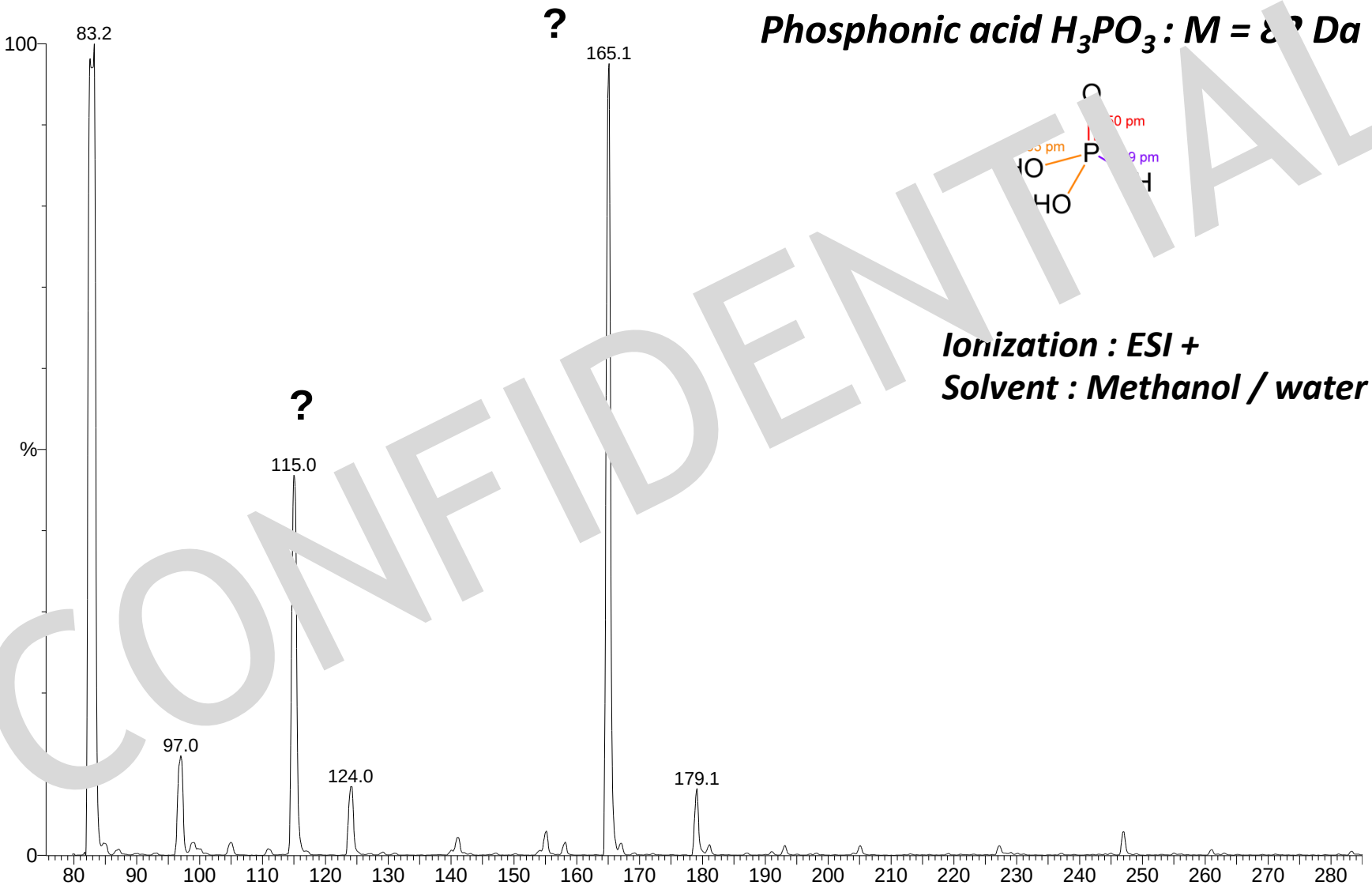


Val – Orn – Leu – Phe – Pro

Pro – Phe – Leu – Orn – Val

$C_{60}H_{92}N_{10}O_{10}$ M = 1140,7 Da

Ionization artefact : Interaction solvent-molecule

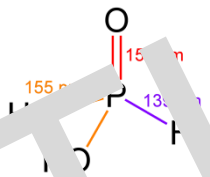


Phosphonic acid H_3PO_3 : M = ?? Da

Ionization : ESI +
Solvent : Methanol / water

Ionization artefact : Interaction solvent-molecule

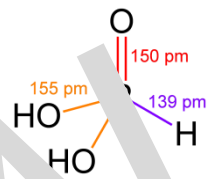
Phosphonic acid H_3PO_3 : M = ? Da



Ionization : ESI +
Solvent : CH_3CN/ water

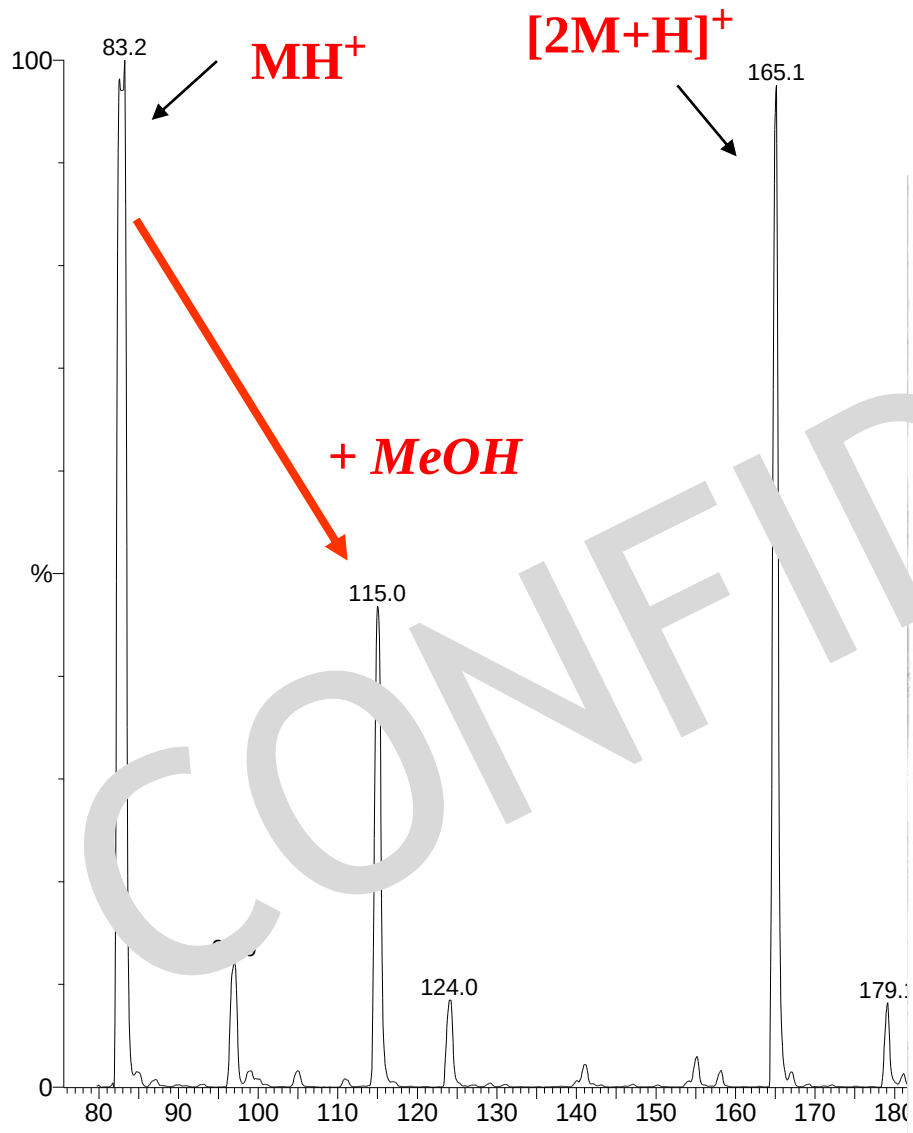
Ionization artefact : Interaction solvent-molecule

Phosphonic acid H_3PO_3 : M = 82 Da



Ionization : ESI +

Solvent : M[illegible]l / w[illegible]



Elemental Compositio Report

Si[illegible]e Mass A[illegible]lys.

T[illegible]erance = 10.0 PPM / [illegible]E: min = -5.0, max = 100.0

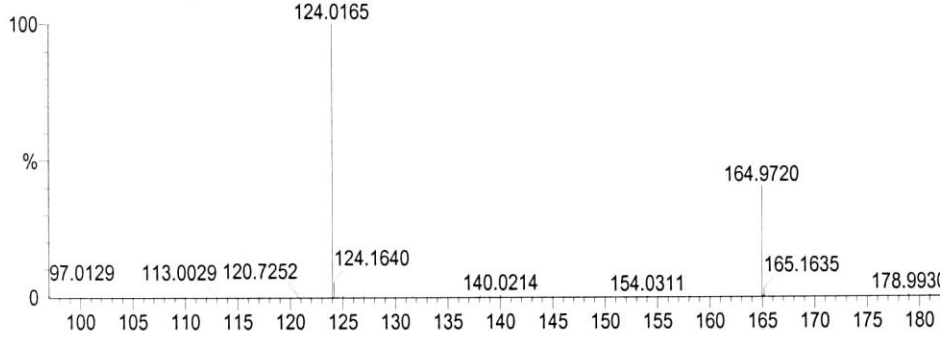
Iso[illegible]luster pa[illegible]meters: Separation = 1.0 Abundance = 1.0%

[illegible]onois[illegible]opic Mass Even Electron Ions

[illegible]form[illegible]e[illegible]luated with 1 results within limits (all results (up to 1000) for each mass)

01751

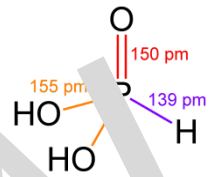
23Mars07_05 28 (0.324) AM (Cen,5, 80.00, Ar,5000.0,294.94,0.80,LS 10); Cm (27:48)



Minimum:				-5.0		
Maximum:		50.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
164.9720	164.9718	0.2	1.5	-1.5	1	H7 O6 P2

Ionization artefact : Interaction solvent-molecule

Phosphonic acid H_3PO_3 : M = 82 Da



Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -5.0 [illegible] = [illegible]0.0

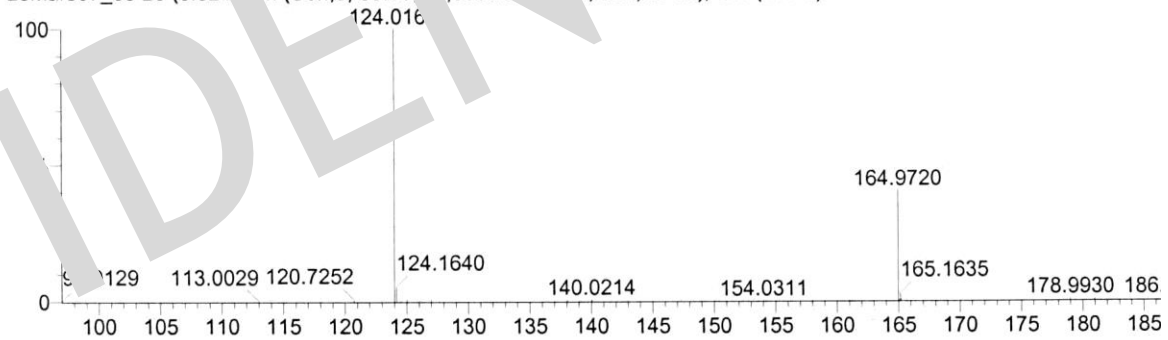
Isotope cluster parameters: Separation [illegible] Abund[illegible]nce = [illegible]

Monoisotopic Mass, Even Electron Ions

74 formula(e) evaluated with 1 [illegible]sults within [illegible]mits (all [illegible]ults (up to [illegible]00) for each mass)

0701751

23Mars07_05 28 (0.324 [illegible]M (Cen,5, 80.00 [illegible],5000. [illegible]4,0.80,LS 10); Cm (27:48)



Minimum:				-5.0		
Maximum:		50.0	10.0	100.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
124.0165	124.0164	0.1	0.9	0.5	1	C2 H7 N O3 P

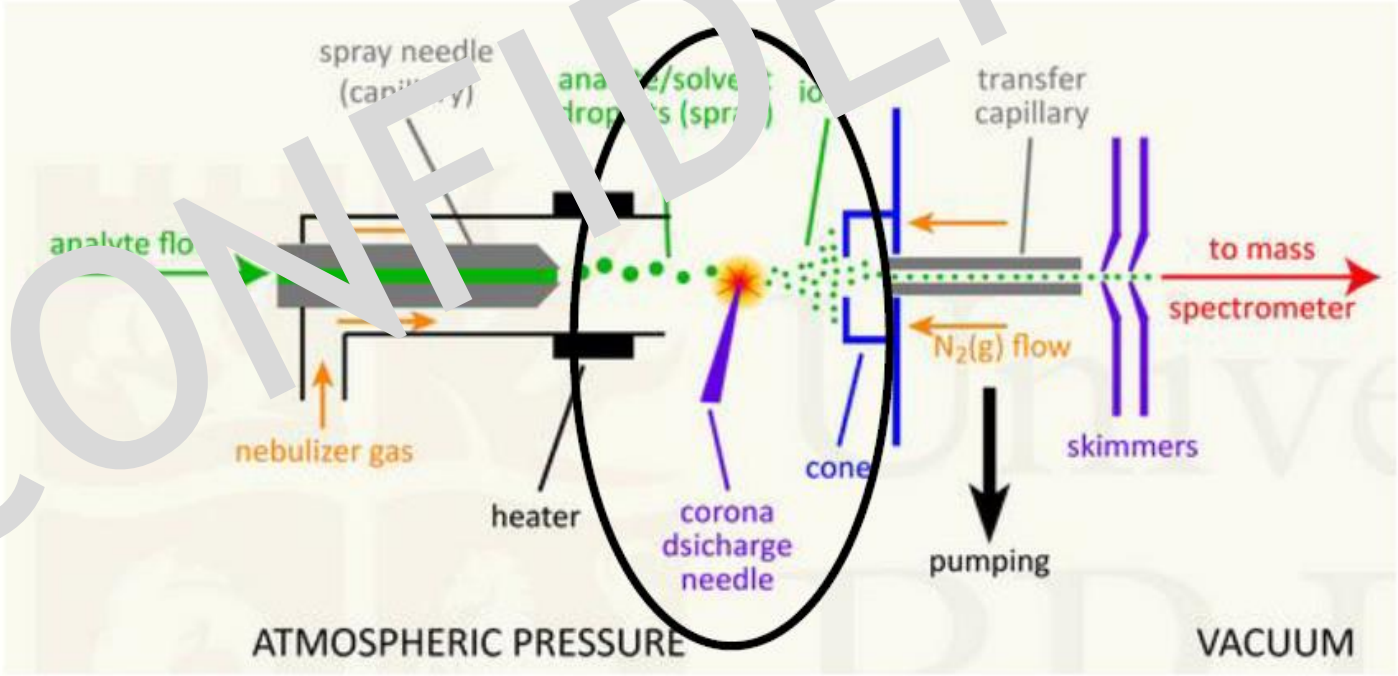
+ CH_3CN ?

[2M +H]

IONIZATION MODE : Atmospheric Pressure Sources

Atmospheric pressure chemical ionisation (APCI)

Neutral or moderately polar, volatile compounds of MW<1000 daltons
Sample solution vaporised at atmospheric pressure under the effect of heat
Gas phase ionisation close to that of chemical ionisation
Little or no fragmentation



IONIZATION MODE : Atmospheric Pressure Sources

Atmospheric pressure chemical ionisation (APCI)

1975 : D.I. Carroli

Principle: Production of electrons by corona discharge

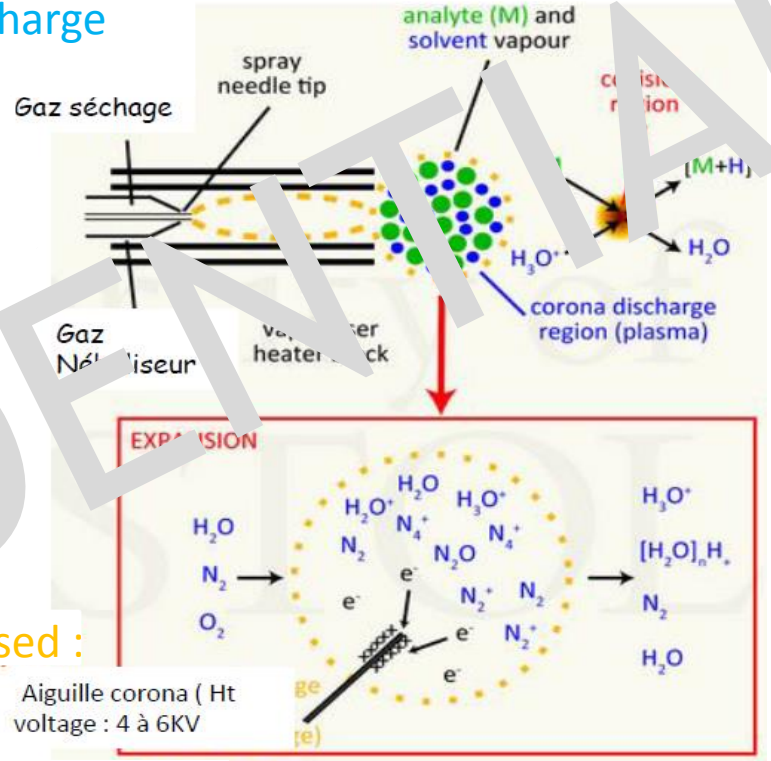
Production of primary ions:

Preferential ionisation of the reactant gas (air or N2)

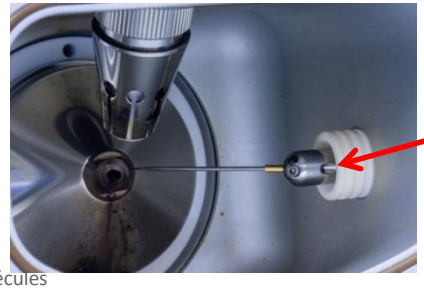
Production of secondary ions:

Reaction primary ion/polar molecule (steam; solvent)

Reaction secondary ions / molecules to be analysed :



Species ionised and mono-charged $[M+H]^+$, $[M]^{+\cdot}$ ou $[M-H]^-$, $[M+OH]^-$



Needle Corona

Atmospheric Pressure Chemical Ionization (APCI)

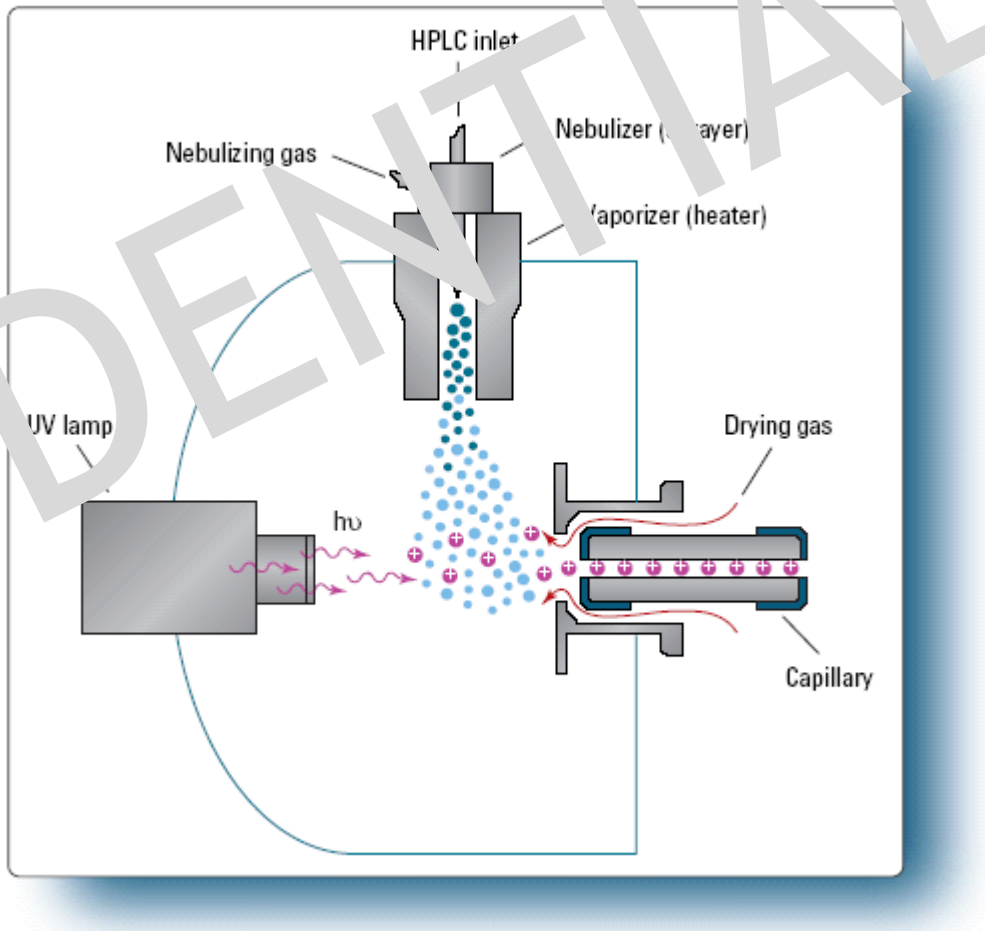
- **Masses < 1000 Da (one charge)** :
  - Low to medium polar, thermally stable molecules

- **Couplings with chromatography techniques:**
  - HPLC, UPLC
  - Capillary electrophoresis (CZE)
  - Maximum flow rate : 0,2 à 2 ml/min

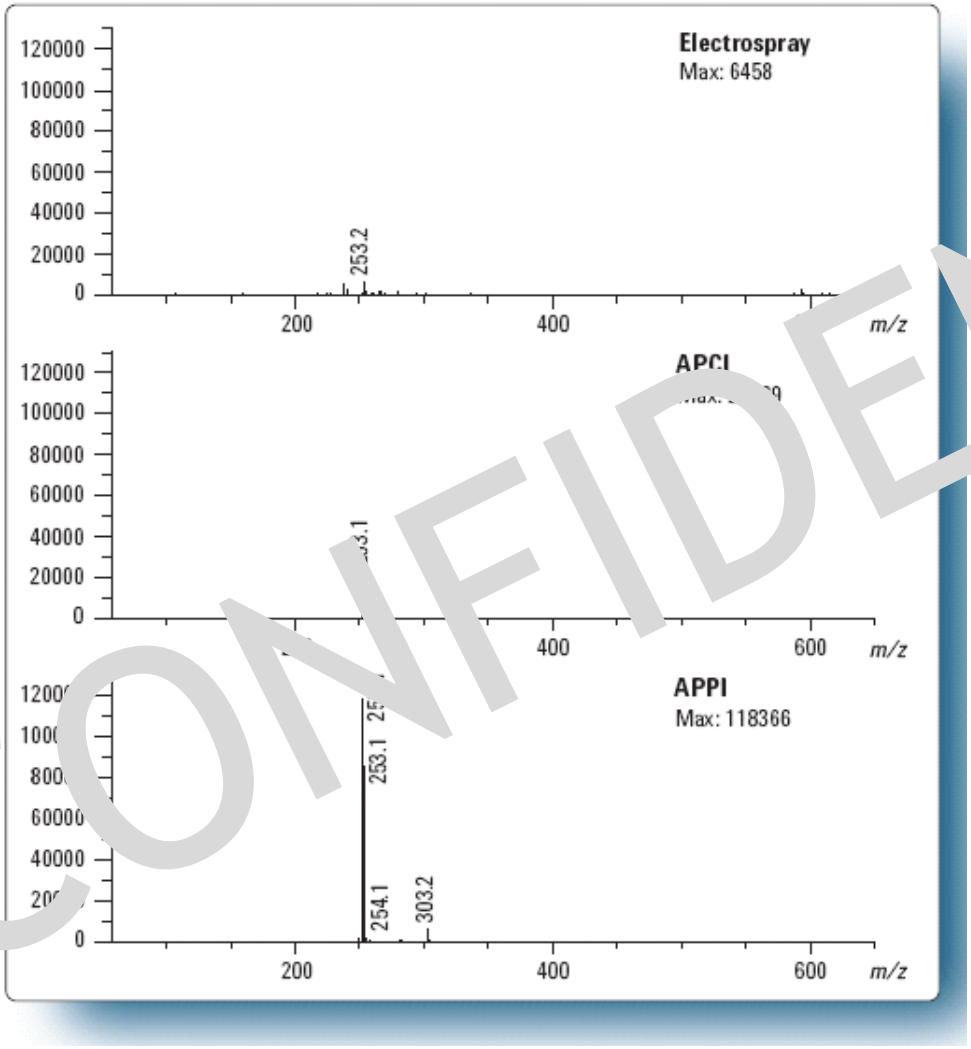
- **Combined source ESCI**

Atmospheric Pressure Photo Ionization (APPI)

Source PhotoMate AGILENT



Atmospheric Pressure Photo Ionization (APPI)



Benzo(a)pyrène - 100 pmol

Positive ions

Matrix Assisted Laser Desorption Ionization (MALDI)

Michael Karas

Franz Hillenkamp

Koichi Tanaka
Prix Nobel de chimie 2002

- Ionisation of very high molecular weight molecules:
  - proteins, synthetic polymers, oligosaccharides...
  - masses up to 300 000 Da (limitation of the ToF analyser)
- Obtaining the pseudomolecular ion MH+, MNa+...
- Extreme sensitivity: picomole, femtomole of deposited sample
  - peptides, proteins: 0.1 to 10 pmol
  - synthetic polymers: 10-4 M
- High salt tolerance (buffers, LC mobile phase)

Principle of MALDI

sample (M)

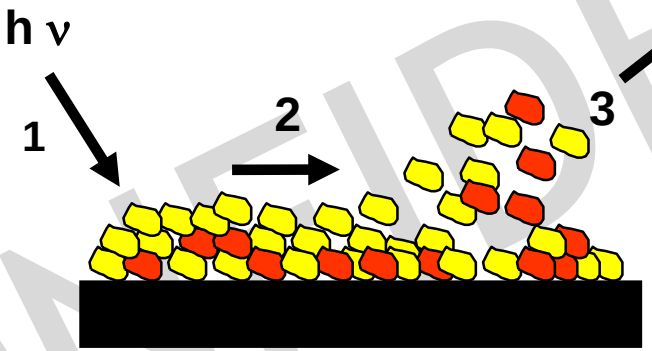
matrix *:*

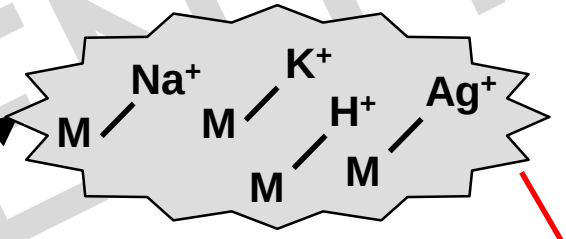
- absorption in UV (aromatic compound)
- donneur de H^+ (alcool, acide)

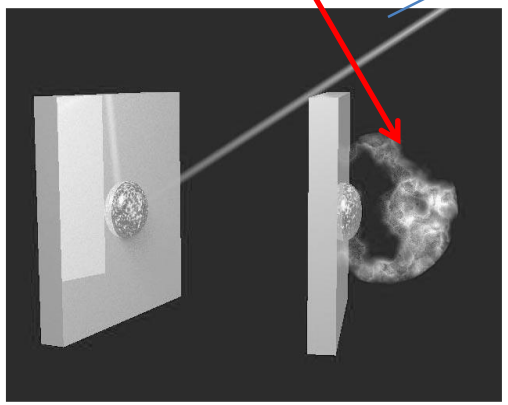
Principle*:*

- Mix of sample and matrix in excess
- Deposit 0.5 to 1 µl of the mix
- Evaporation of the solvent (in air or under vacuum) → **cocristallisation**

Laser N_2 (UV)
337 nm, 3 ns







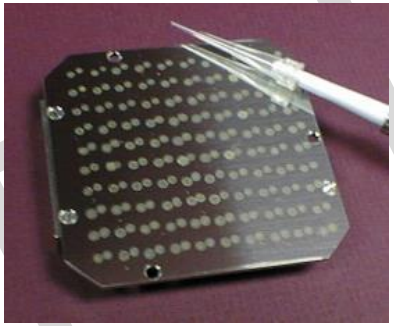
1 - Impact of photons
2 - Energy propagation
3 - Desorption

The matrix facilitates the **desorption and ionisation** of high MM samples while minimising their fragmentation (absorption of the incident laser beam energy)

Preparation of the deposits

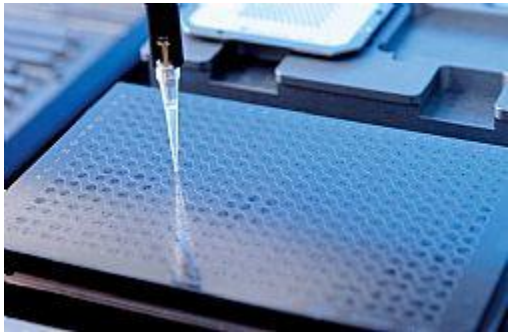
Restrictions:

- Sample must be soluble
- Choice of matrix vs sample
- Compatibility of sample and matrix solvents
- Concentration of matrix and sample
- Ratio matrix / sample (10/1, 100/1...)
- Desalting or addition of salts (Na+, K+, Ag+...)

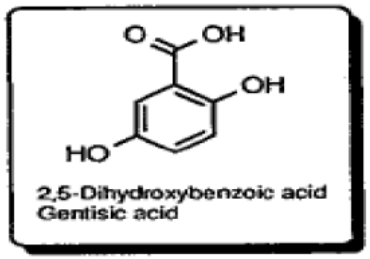


LC-MS :

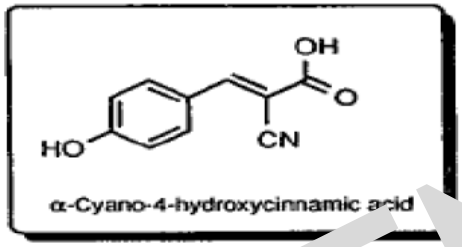
- Offline : deposit with spotter
- Matrix addition



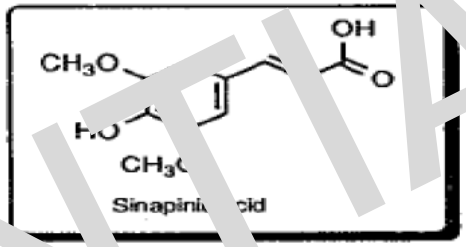
Matrix used in MALDI



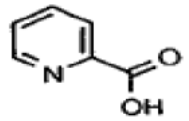
2,5-Dihydroxybenzoic acid
Gentisic acid



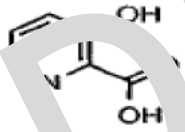
α-Cyano-4-hydroxycinnamic acid



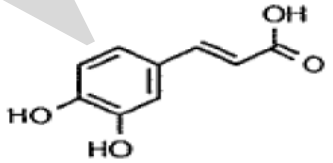
Sinapinic acid



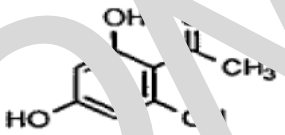
Picolinic acid
Pyridine-2-carboxylic acid



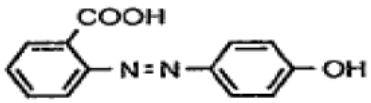
3-Hydroxypicolinic acid
3-Hydroxy-2-pyridinecarboxylic acid



Caffeic acid
3,4-Dihydroxycinnamic acid



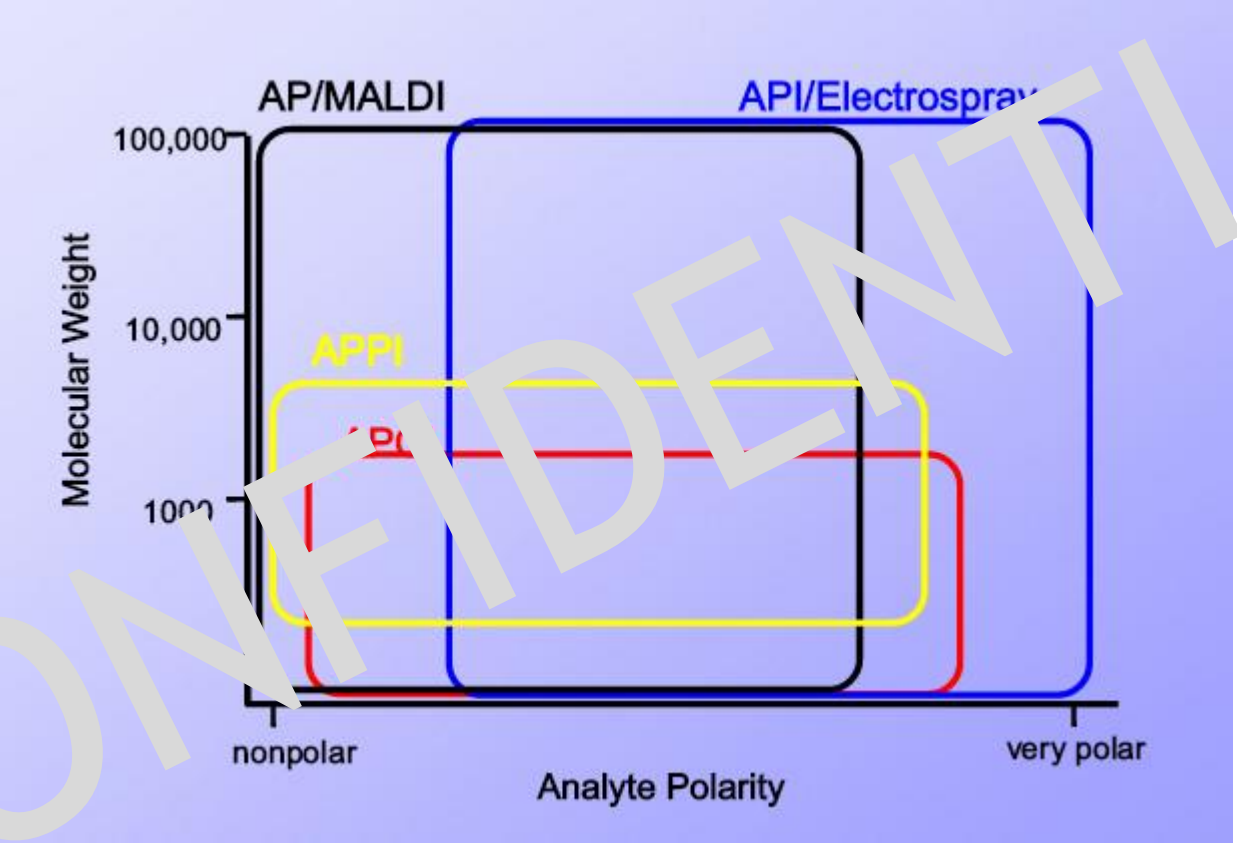
2,4,6-Trihydroxyacetophenone



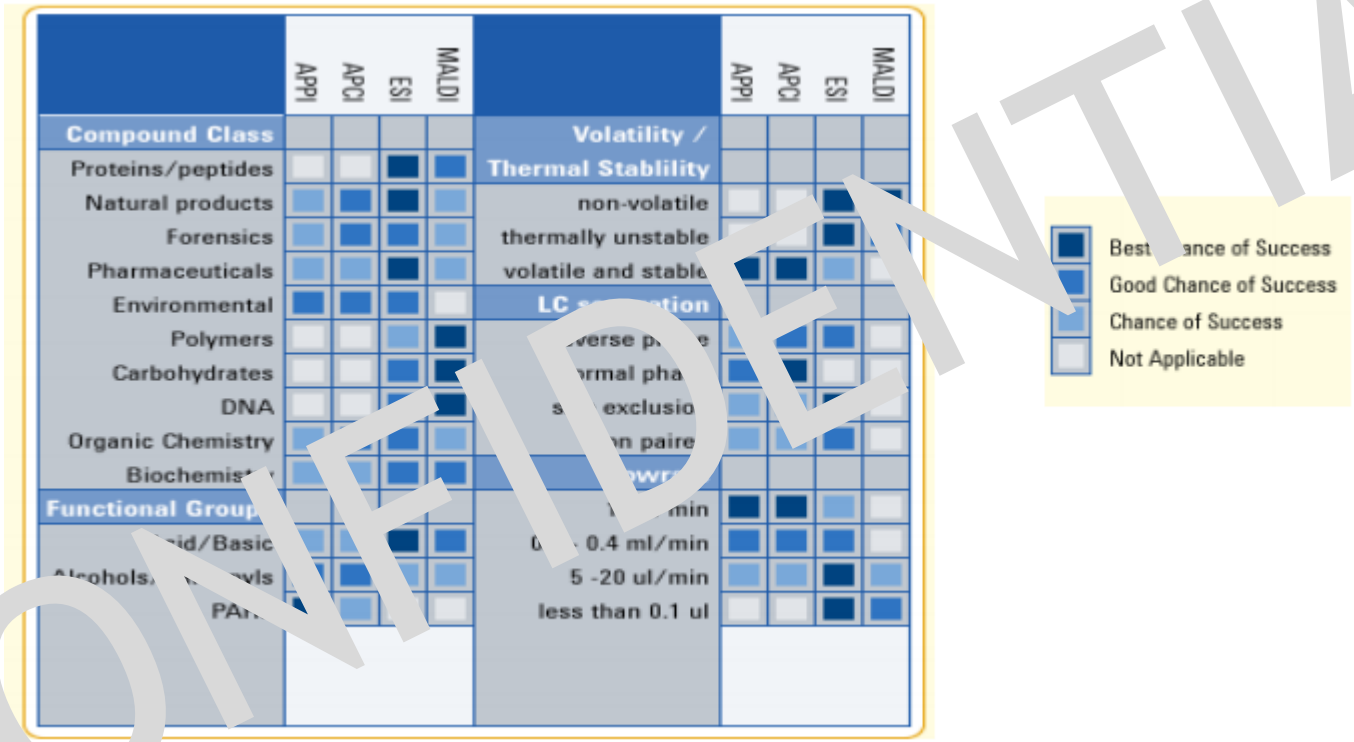
2-(4-hydroxyphenylazo)benzoic acid

they absorb at 337nm and crystallize easily

Differents sources for differents applications



Differents sources for differents applications



Interface Source -> Vacuum

To pass the ions formed at atmospheric pressure into the vacuum chamber of the mass spectrometer analyser, a device called an INTERFACE is required

Obta in ion in the as phase

Transmission and focusing of ions

Ion separation as a function of m/z

Eliminate as many neutrals as possible

Analyzers

- **Spatial separation analyzers** (quadrupole, ToF)
- **Time-separated analyzers** (Trap, Orbitrap)
- **Hybrid analyzers** (QToF, QTrap, QqQ, Q-Orbitrap)

ANALYZERS

Role: To separate ions according to their mass/charge ratio

The ions produced are extracted and pass through the spectrometer without being discharged, or without colliding with other molecules.
They are separated **in space or in time**.

→ Need a high vacuum

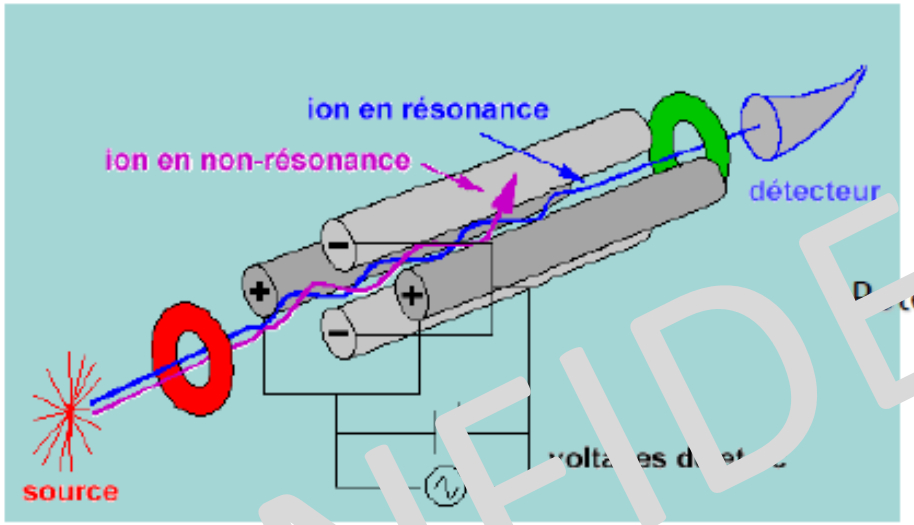
Optimise the lens parameters in the analyzer to allow ions of given m/z to go to the detector

Main qualities of an analyzer :

- Resolution R
- The m/z range it can analyze
- Speed of scan in m/z
- Sensitivity
- Transmission

ANALYSERS: THE QUADRUPOLE ANALYSER

Principle



Potentiels appliqués aux 2 paires d'électrodes :

$+\phi_0 = +(U-V\cos \omega t)$

$-\phi_0 = -(U-V\cos \omega t)$

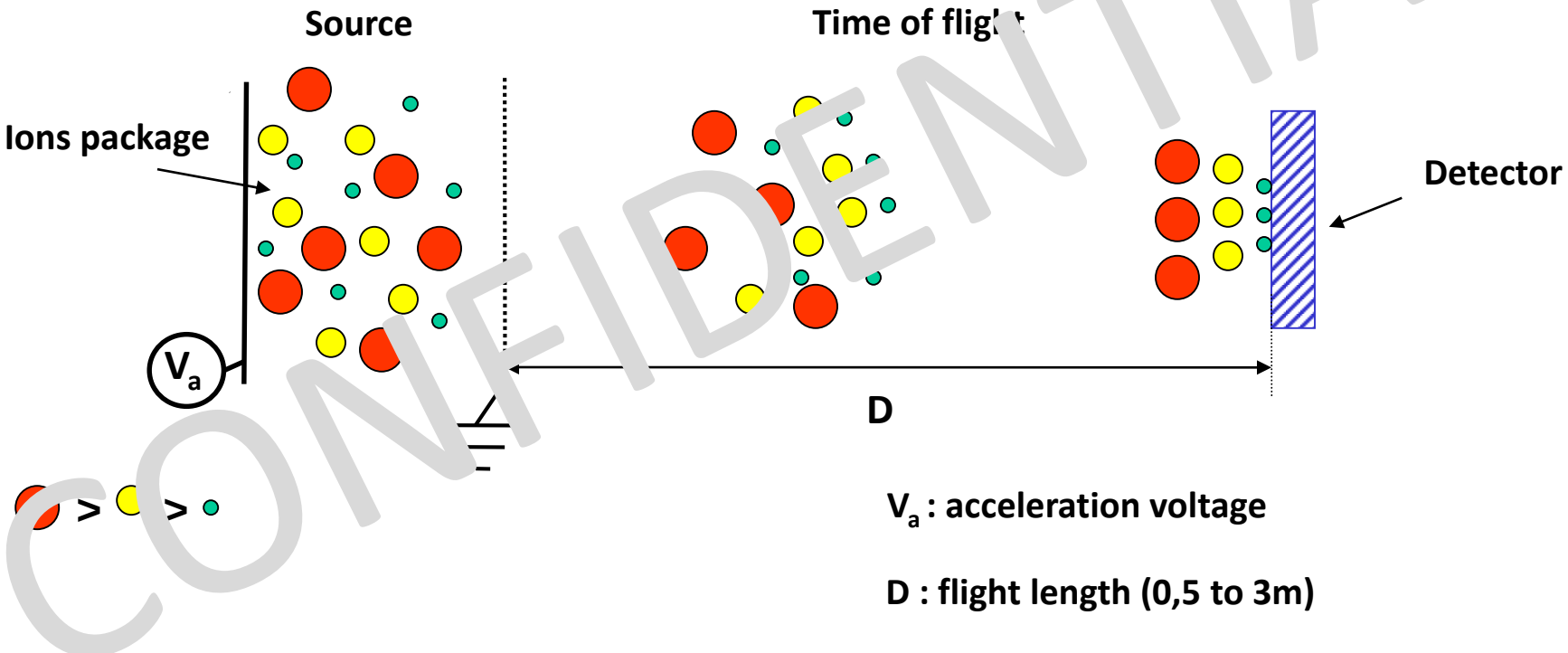
Modes de balayage

Mode scan : acquisition de toutes les masses sur une plage donnée

Mode SIM : sélection de quelques masses d'intérêt (meilleur S/N)

ANALYSERS: TIME OF FLIGHT

- Unlimited mass range
- Speed acquisition and high
- Ion package acquisition(ESI with pusher)
- Higher resolution than quadrupole: R = 5000 – 60 000 (FWHM)



Principle of ToF – Detection in linear mode *(low resolution)*

ANALYSERS: TIME OF FLIGHT

Time of flight (µs)

- Kinetic energy E_c : $E_c = \frac{1}{2}mv^2 = zV_a$

- Time of flight : $t_v = \frac{D}{v}$

. m : *ion mass*
. v and z : *speed and ion charge*.
. $E = zV_a$: *electric field*
. D : flight length

$$\frac{m}{z} = t_v^2 \left(\frac{2V_a}{D^2} \right)$$

At V= 30kV :

m (Da) \ D(m)	0,5	1,0	1,5
1000	6,58	13,1	19,7
5000	14,7	29,4	44,1
10 000	20,8	41,6	62,4
50 000	46,5	93,0	139,0
100 000	65,8	131,5	197,0

ANALYSERS: TIME OF FLIGHT

Improved resolution on a ToF

Why ?

Limiting factors :

- Ion formation time during ionization
- Position of the ions in the source before acceleration
- Initial velocity distribution
- Multiple collisions :
  - between ions and neutral molecules

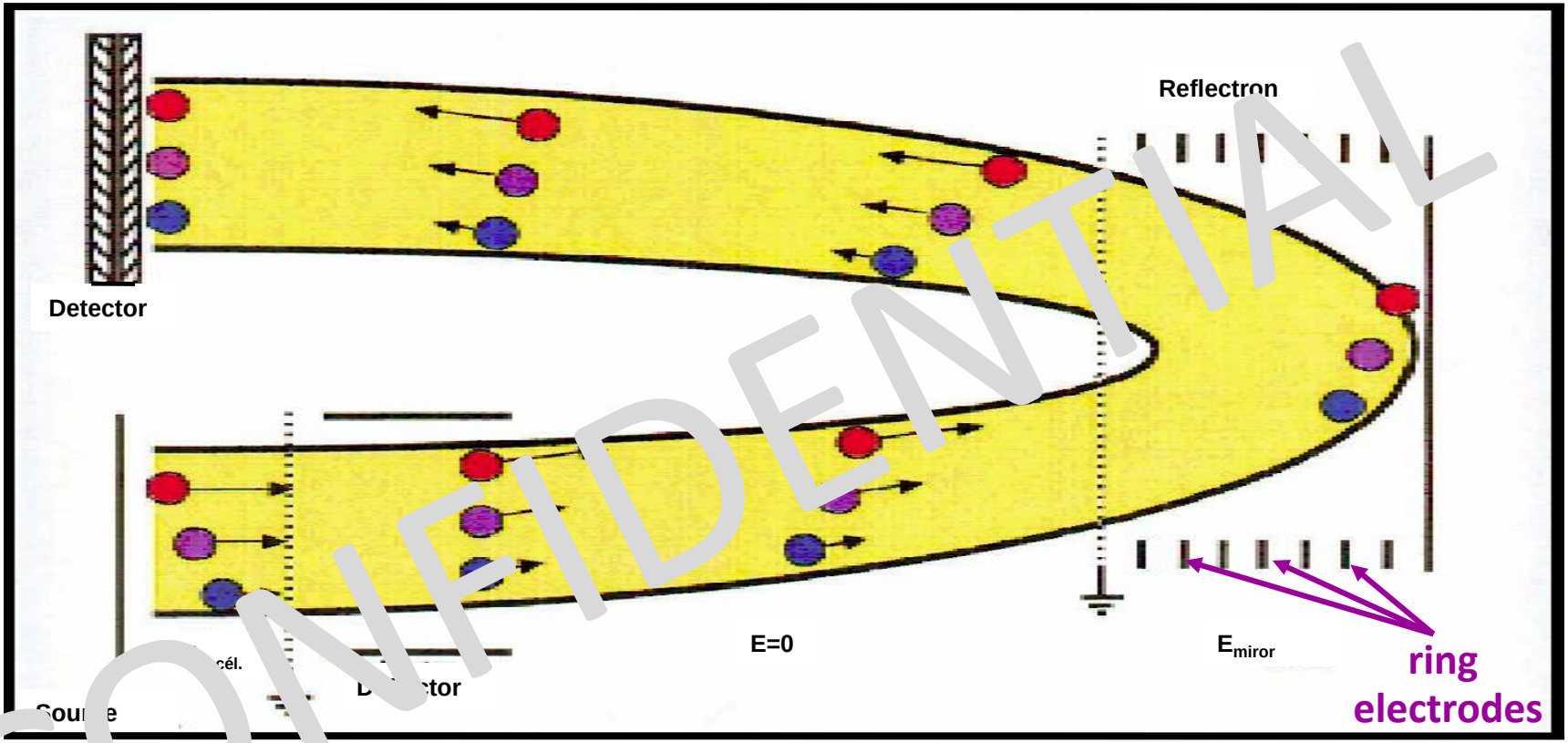
$$R = \frac{M}{\Delta M} = \frac{t}{2\Delta t}$$

- Δt increases so R decreases
- Resolution in linear mode of the order of 500

Reflectron

ANALYSERS: TIME OF FLIGHT

Reflectron: Electrostatic mirror



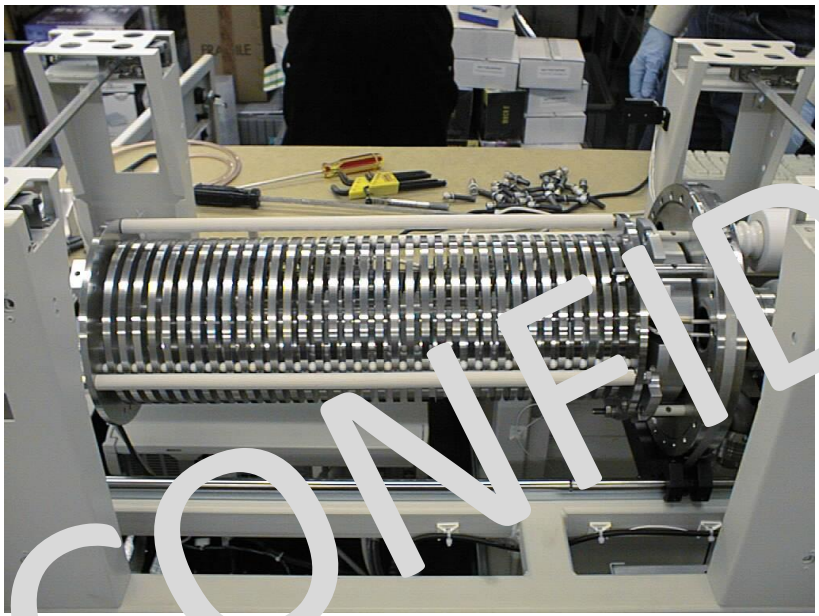
Same m/z

Correction on arrival times at the detector

- **Δt decreases so R increases**
- **Resolution in Reflectron mode ≈ 60 000**

Reflectron

(Voyager DE-STR, AB Sciex)



3 m RP~80 000

2 m RP~50 000

1 m RP~20 000

ANALYSERS: TIME OF FLIGHT

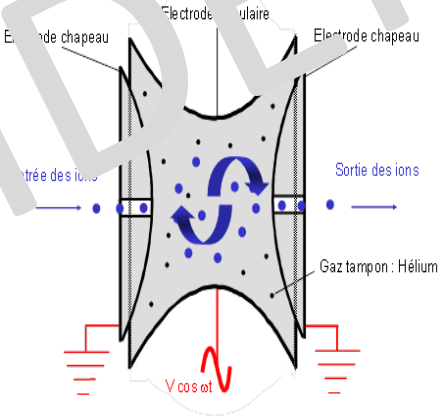
Flight time characteristics

- Simultaneous detection of all ions
- High resolution analyzer
- Excellent transmission
- High acquisition frequency

Determination of exact masses

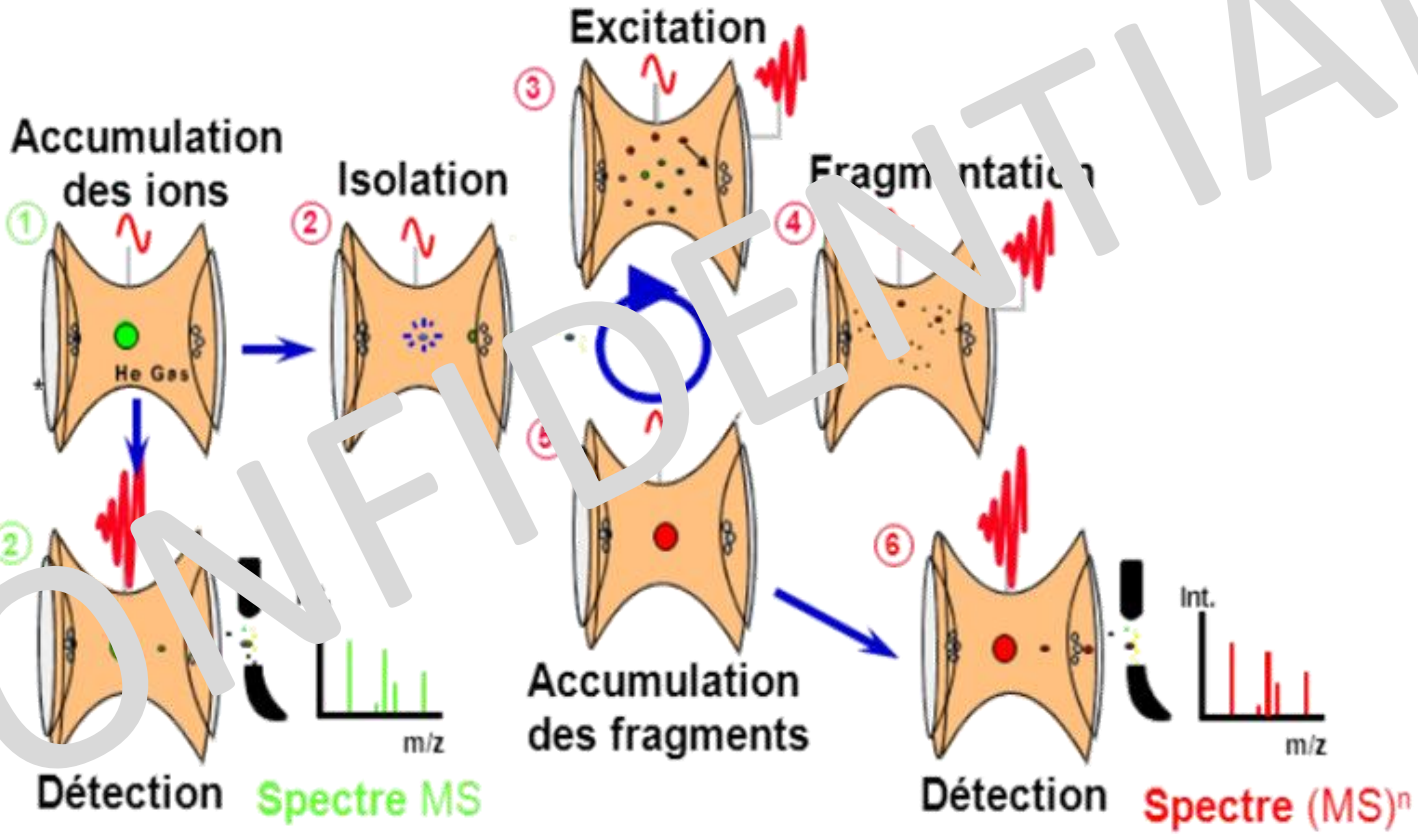
Analyzer Ion Trap

- The **ion trap** is one of the **path stability analyzers**
- The trap consists of **three electrodes with a hyperbolic cross-section** a ring electrode flanked by two cap electrodes (input and output)
- A radio frequency voltage combined or not with a DC voltage U is applied between the central electrode and the two cap electrodes.



- Ion traps have the ability **to accumulate ions and fragment them successively (MSn)**

Analyseur Ion Trap



Ion Mobility Mass Spectrometry

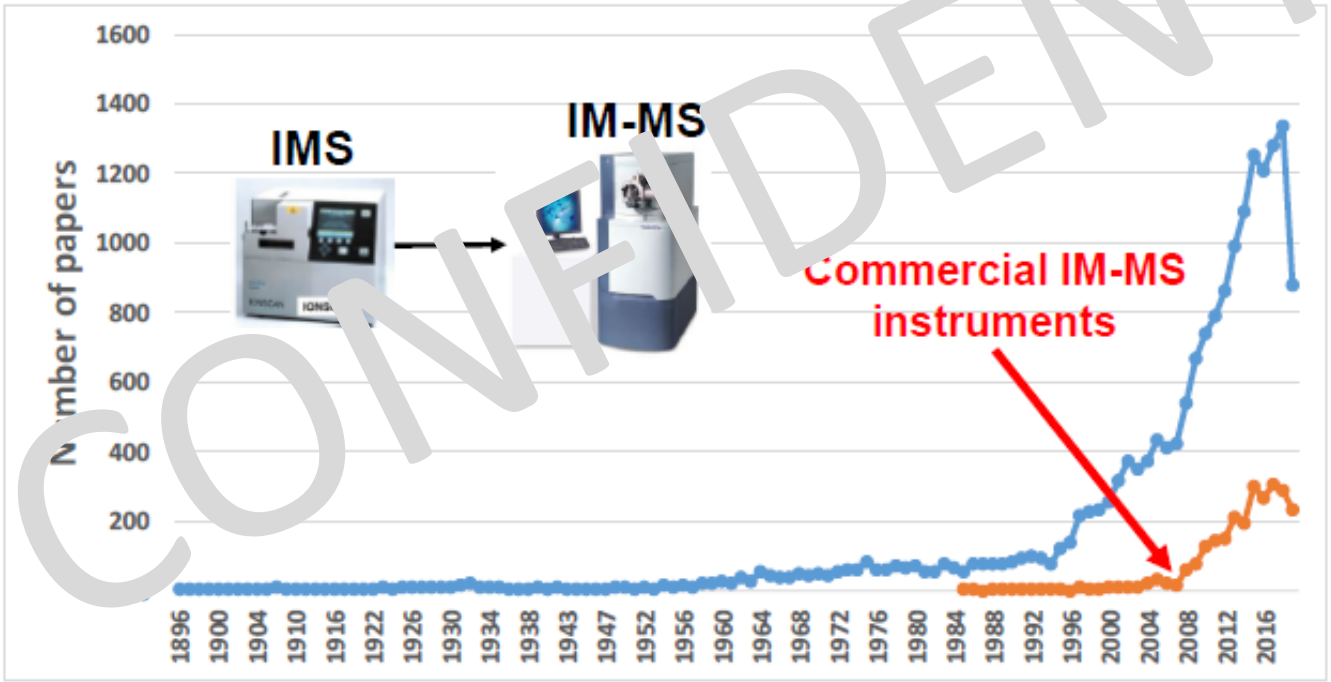
- Detection of dangerous substances (drugs, explosives)

Portable detector
by IMS

SABRE 5000
Smiths Detection®

Detector GC-IMS

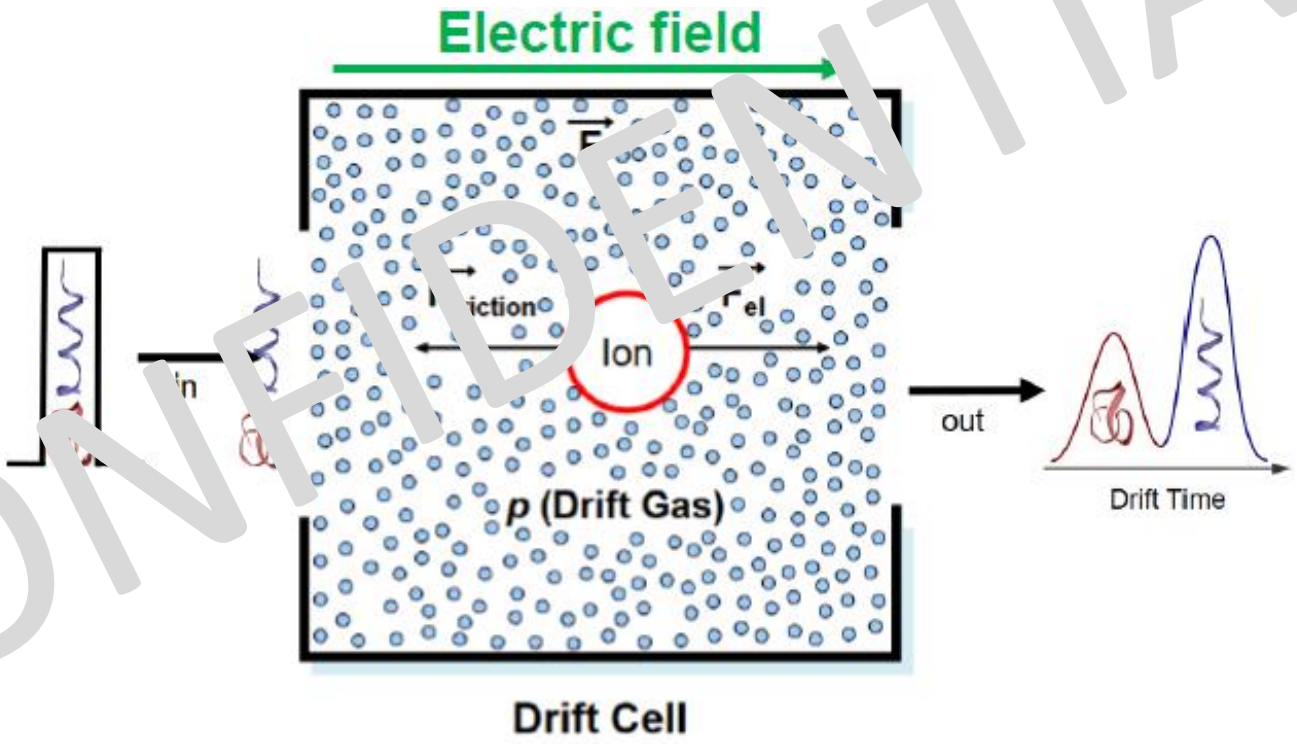
- Coupled with MS: complementary informations



C.Lapthorn et al., Mass spectrometry rewiews, 32 2013, 43-71

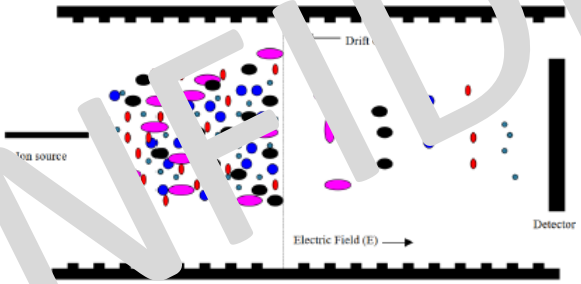
Ion Mobility Mass Spectrometry

Ion Mobility Spectrometry (IMS) is an analytical technique based on the separation of molecular ions according to their mobility in a gas (N_2, He) under the a tion of an electric field

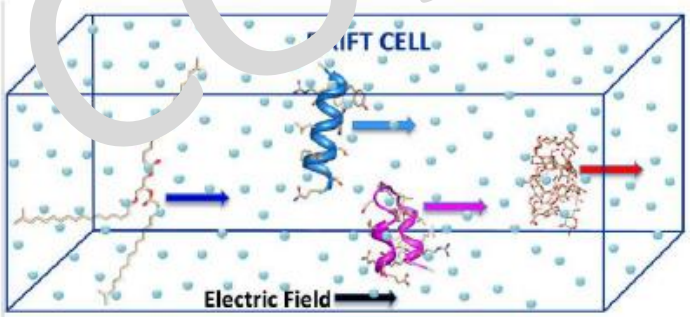


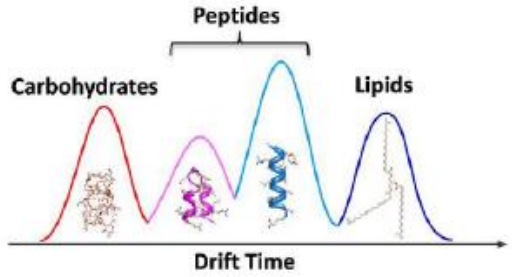
Ion Mobility Mass Spectrometry

- IMS applies to gas phase ions
- Separative technique Different mobility = Different travel speed
  ⇨ Separation of ions in time and space
- Mobility of ions is related to their conformation
  ⇨ Mobility = structural information
- Used alone or in combination with other techniques
  ⇨ Liquid chromatography, gas, mass spectrometry



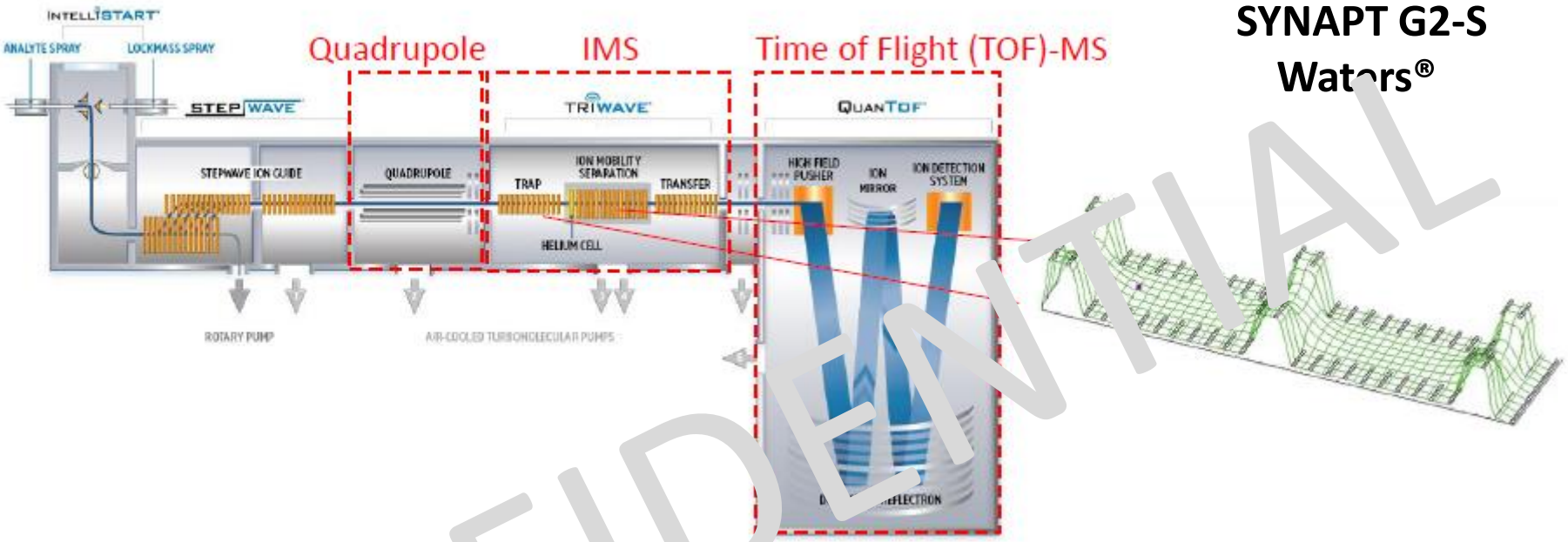
- Size
- Shape
- Charge of the compounds





10ms to 100ms
for one spectrum IMS

TWIMS : Traveling-wave IMS

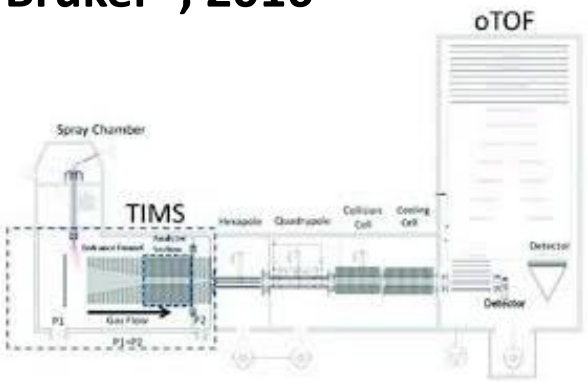


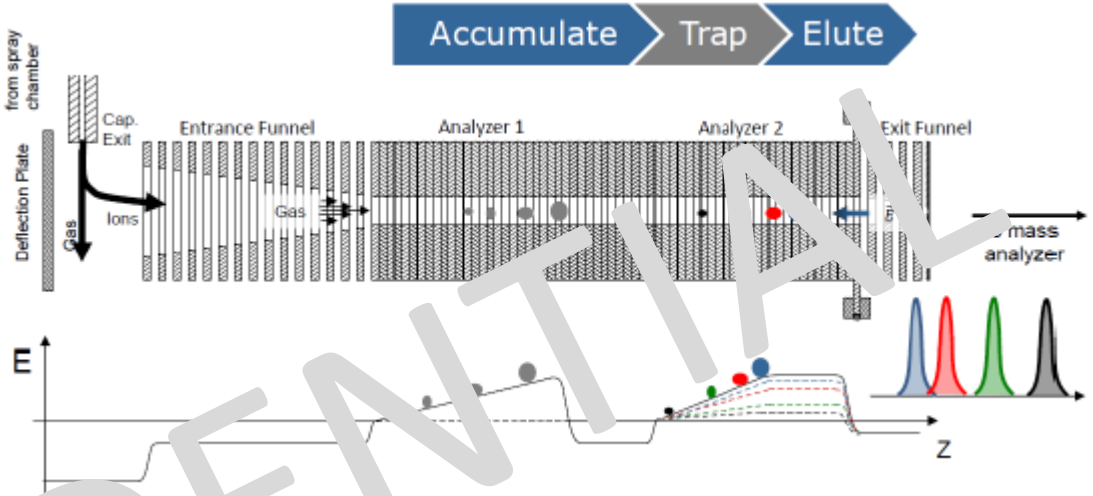
SYNAPT G2-S
Waters®

- Wave of ion's field electric potential pushing ions
- Change in velocity and field strength ➡ Ions separation
- The CCS is determined from the drift time, only after calibration of the ion mobility cell with a mixture of compounds with known CCS

TIMS : Trapped IMS

TIMS-ToF
Bruker®, 2016





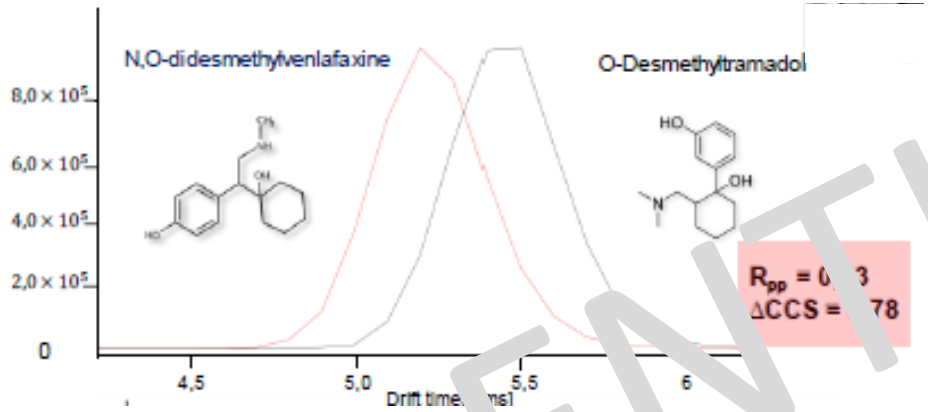
- High flow of N2 to transport ions into the drift cell
- Low electric field applied in the opposite direction which allows separation
- Then the electric field gradually decreases, allowing the release of large ions and then small ones

- The CCS is determined from the drift time, only after calibration of the ion mobility cell with a mixture of compounds with known CCS
- The TIMS cell can achieve a resolution of up to 200

Resolution in IMS: application examples

Isobaric compounds, impossible to separate by HRMS

TWIMS

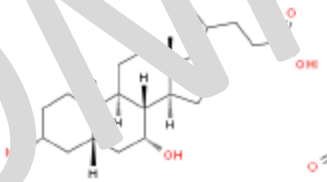


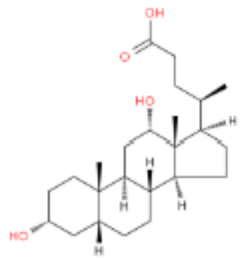
Cyclic- TWIMS, Waters®

Chenodeoxycholic acid

$C_{24}H_{40}O_4$
M=392,2921

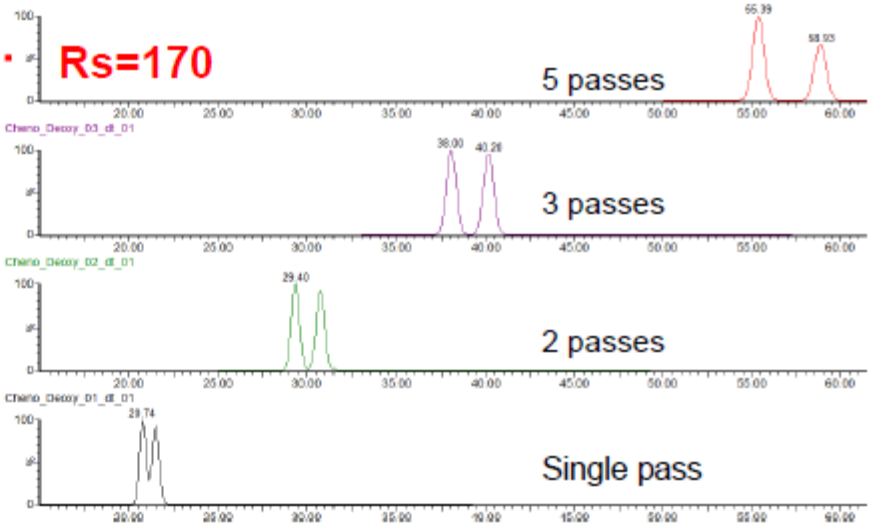
201.51 Å²





$C_{24}H_{40}O_4$
M=392,2921

Deoxycholic acid
200,7 Å²



Tandem mass spectrometry (MS/MS, MS^n)

OBJECTIVES

- **Fragmentation: structural information**
- **Identification and quantification of compounds :**
  - **In trace amounts**
  - **In complex matrices**

↳ **MS/MS selectivity**

- **reaction mechanisms : ions/molecules reaction**
- **Isolating an ion, fragmenting it to :**
  - **Study its specific fragments trace**
  - **Back to the parent ion**

Time-separated analyzers
Hybrid analyzers

$$M^{+\cdot} \longrightarrow F^{+\cdot} + N$$
$$M^{+} \longrightarrow F^{+} + N$$
$$MH^{+} \longrightarrow FH^{+} + N$$

MS to MS/MS

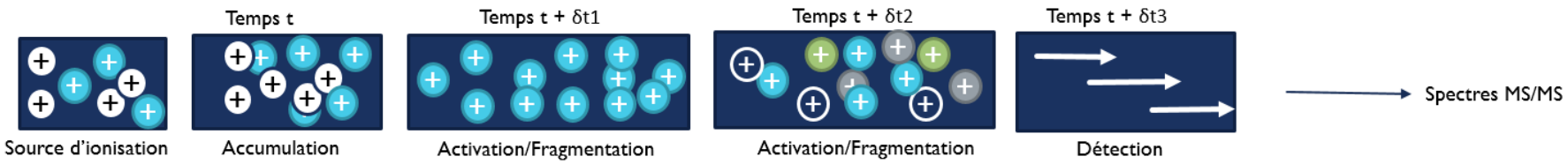
What is it for? How does it work?

- Probing the structure of an analyte
- Increase the confidence of identification
- Improve accuracy of quantification
- Reduce the possibility of raw formulae

Principle: **Activation of fragmentation by collision of an ion with a neutral gas (N2, He, Ar)**

MS/MS in space :

MS/MS in time :



Acquisition mode

- **Full Scan**: Full scan over a specified m/z range
- **SIM (Selected Ion Monitoring):** A mass spectrometry scan mode in which only a limited range of m/z is transmitted/detected by the instrument, as opposed to the full spectrum
- **SRM (Selected Reaction Monitoring):** Acquisition mode consisting of selecting a fragmentation reaction. For this analysis, both the first and second analysers are focused on the selected masses. In this case, the ions selected by the first mass analyser are only detected if they produce a given fragment by a selected reaction.

MS to MS/MS

Acquisition mode

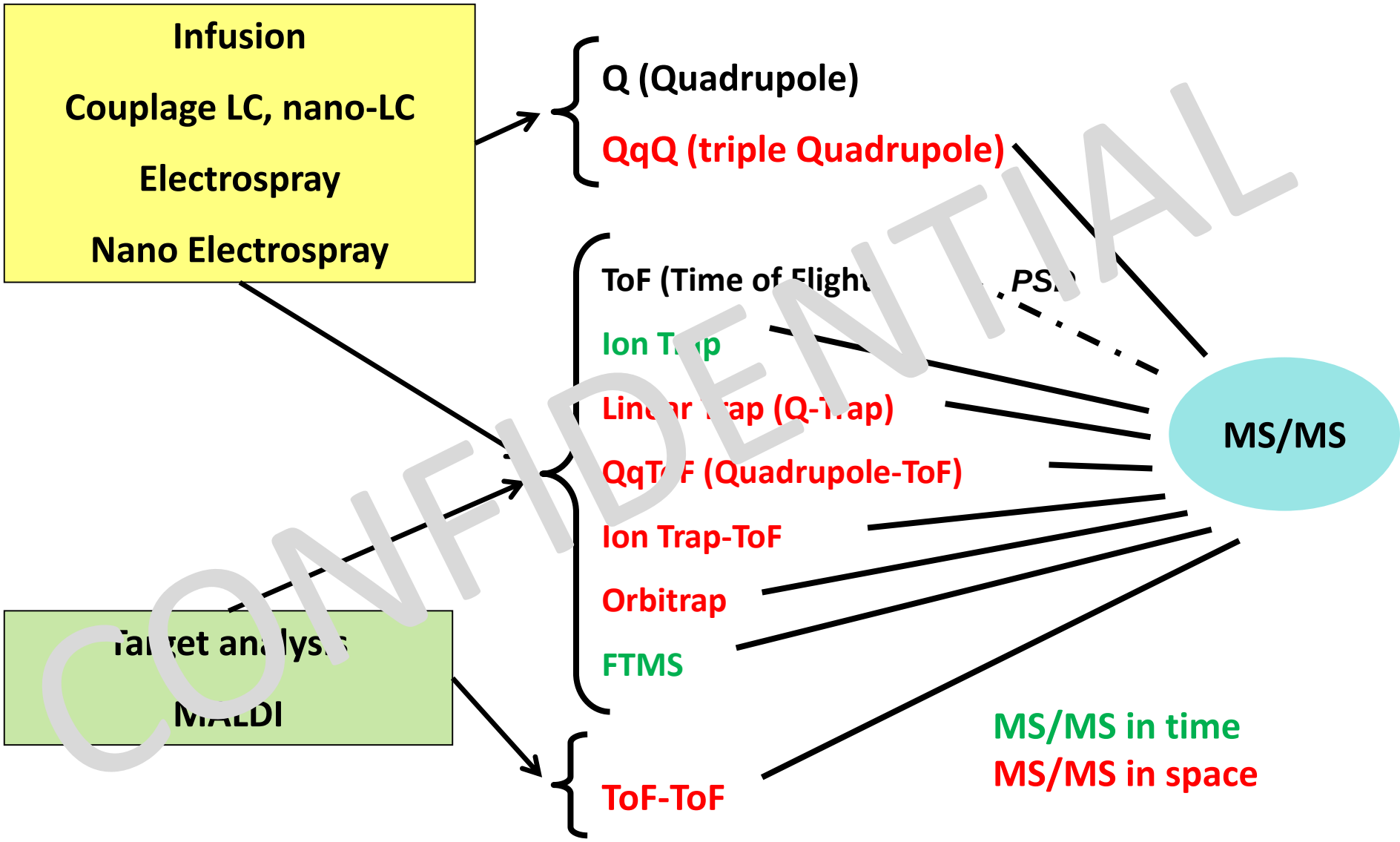
- **DDA (Data-dependent acquisition)**

- A data acquisition mode in which a fixed number of precursor ions whose m/z values have been selected using predetermined rules are subjected to a second fragmentation step
- The selection of the ions is generally based on criteria of intensity, charge state, mass range, isotope pattern, etc.

- **DIA (Data-Independent acquisition):**

- A data acquisition mode in which all ions in a selected m/z range are fragmented and analysed in a second step.
- Fragmentation spectra are acquired either by fragmenting all the ions that enter the mass spectrometer at a given time (called broadband DIA), or by sequentially isolating and fragmenting m/z ranges.

Hybrid Analysers

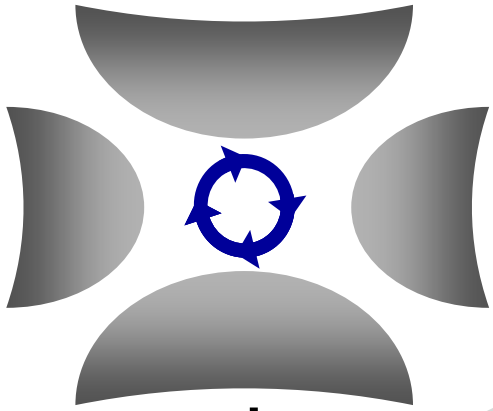


Hybrid Analyser : Q-TRAP™ (AB Sciex)

3D Trap

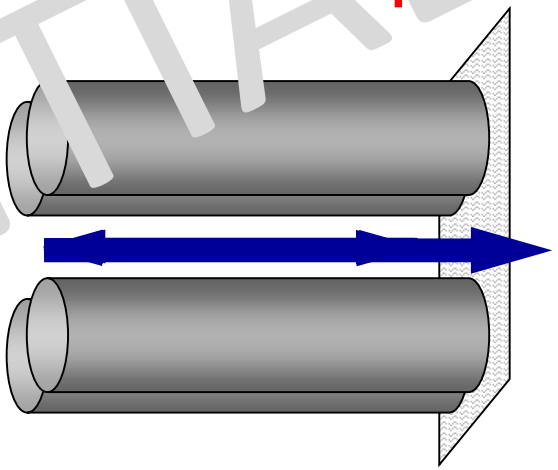
- Sensitivity in Full Scan
- MS^3 (or more)

MS/MS in time



Q Trap

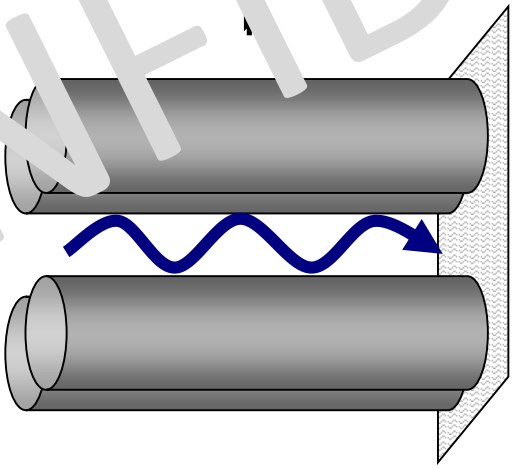
MS/MS
In time and space



- Sensitivity in Full Scan
- Quantification
- MS^3 (or more)
- Sensitivity in MRM
- Neutral loss
- Precursors ions

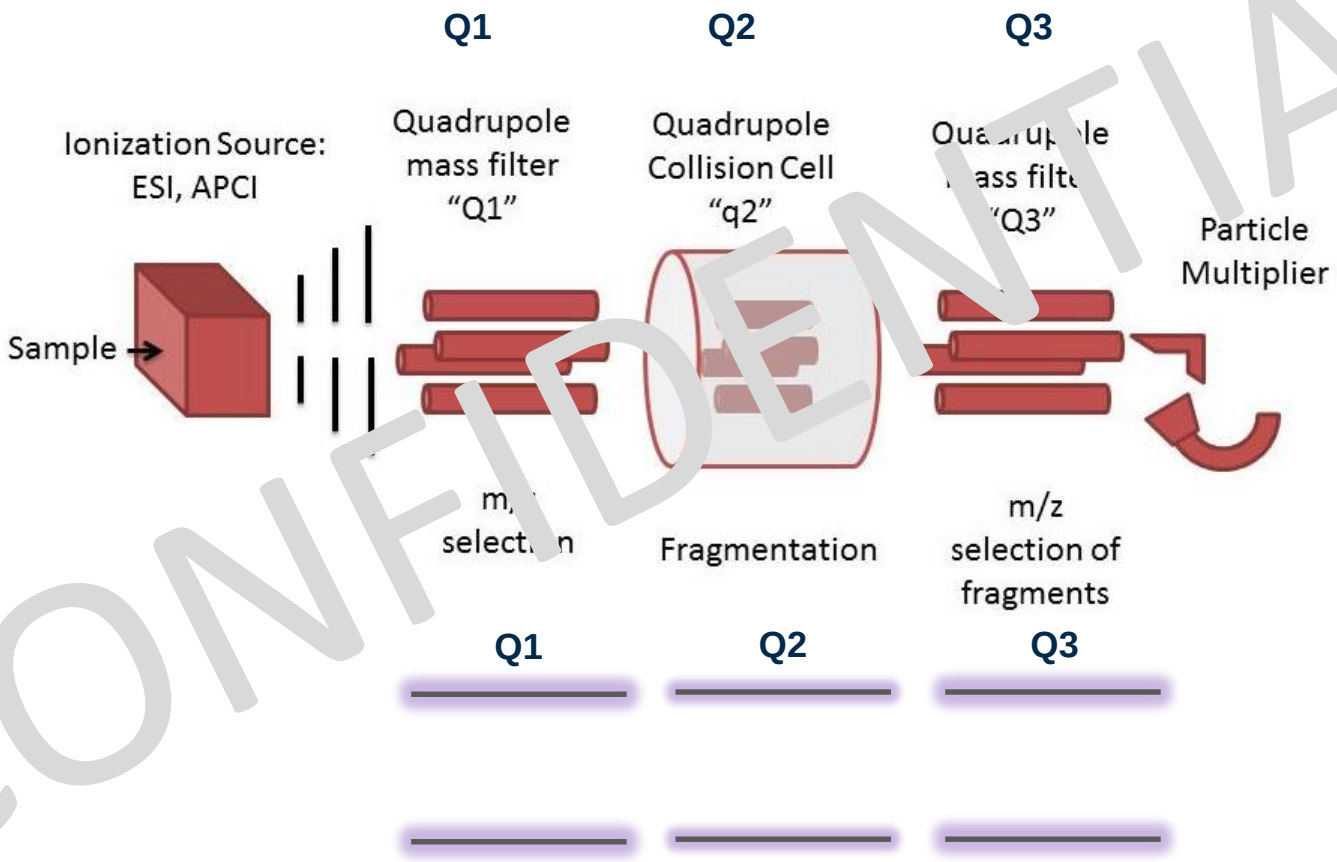
QqQ

- Sensitivity in MRM
- **Quantification**
- Neutral loss
- Precursors ions



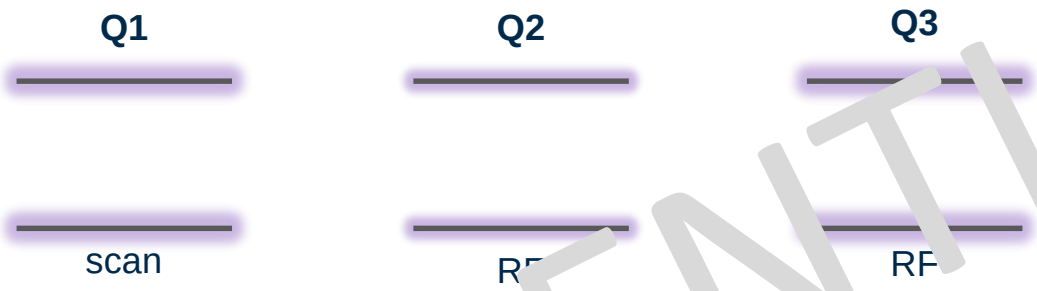
MS/MS in space

Tandem Mass Spectrometry

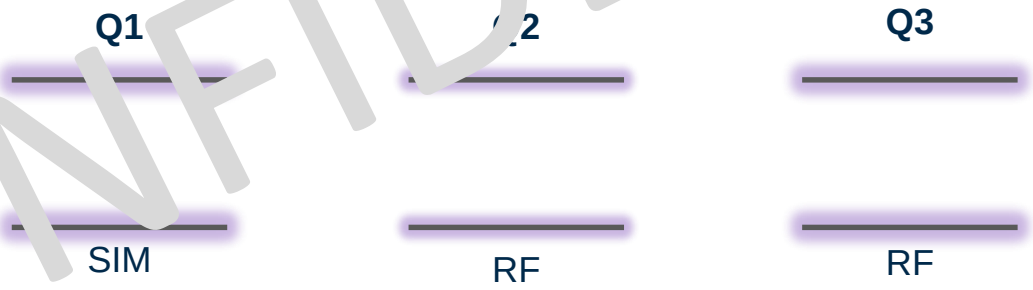


Tandem Mass Spectrometry

Q1 MS mode or Q3 MS mode

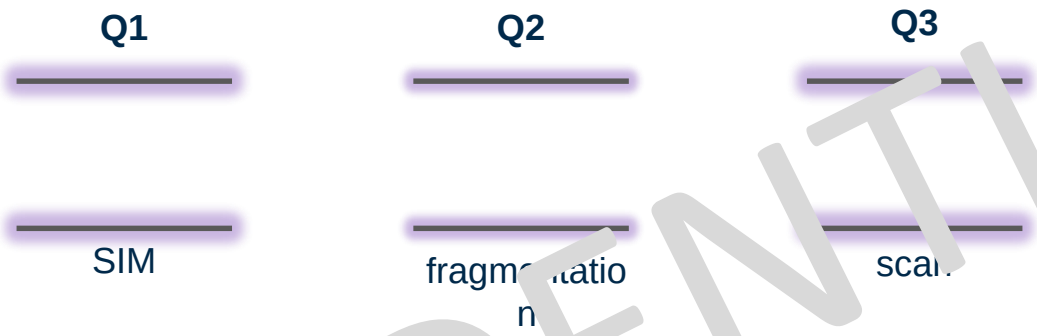


SIM mode : Single Ion Monitoring

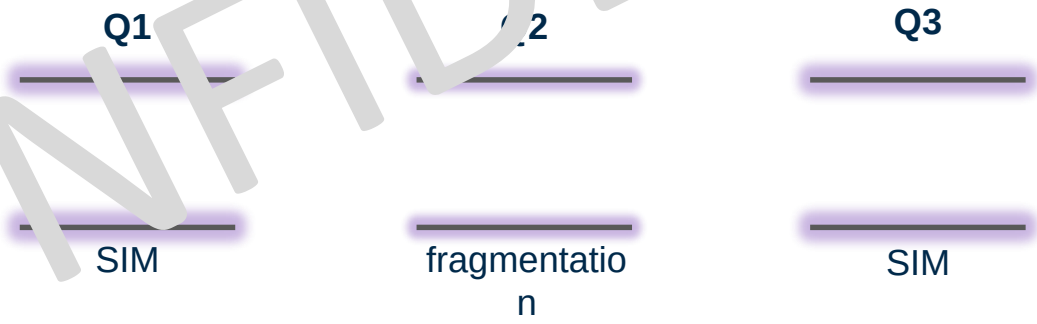


Tandem Mass Spectrometry : QqQ – MS/MS

MS2 mode

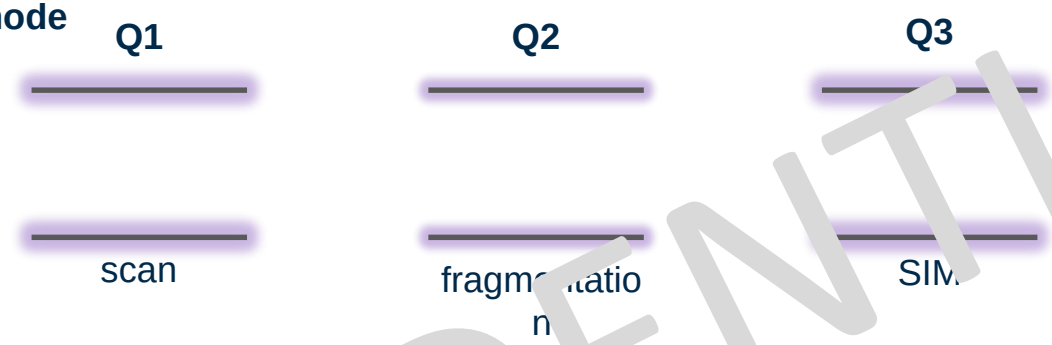


MRM mode (Multiple Reaction Monitoring, or scheduled MRM

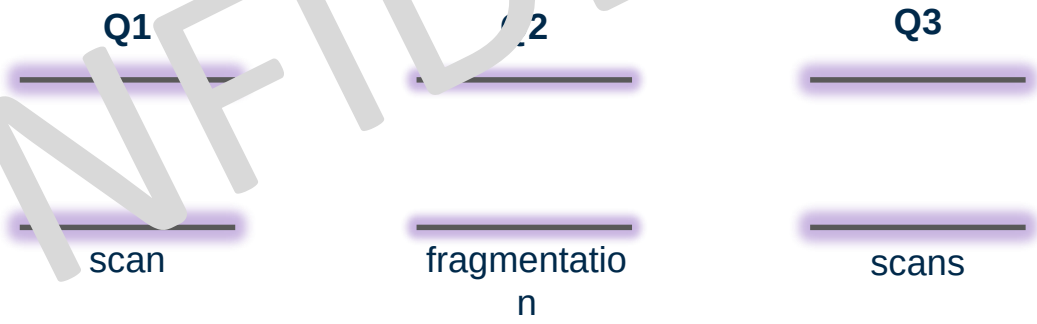


Tandem Mass Spectrometry : QqQ – MS/MS

Precursor Ion Scan mode



Neutral Loss Scan mode



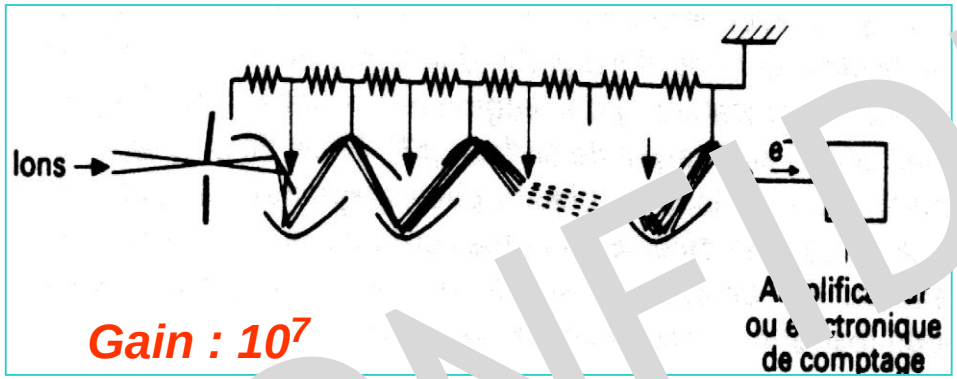
Detectors

DETECTORS

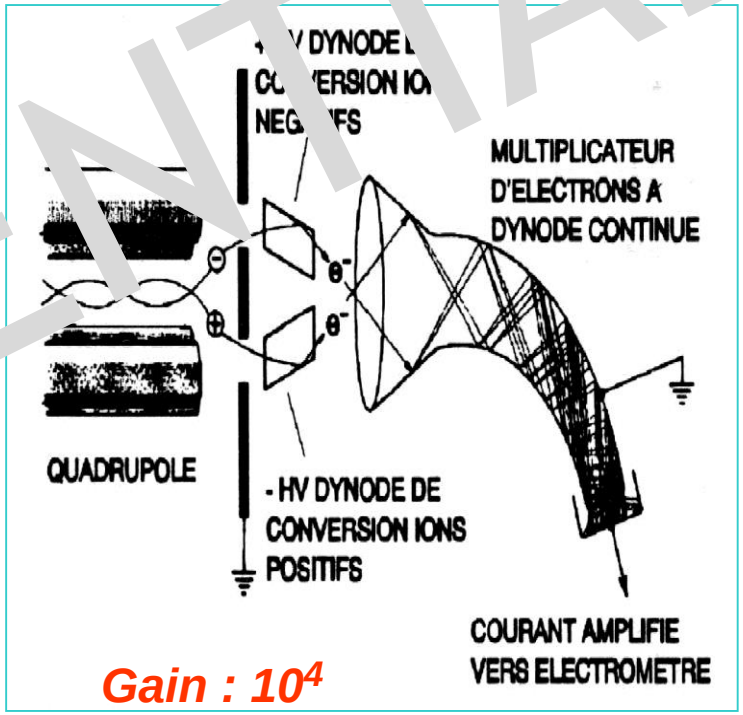
- **Electron multipliers**
- **Micro Channel Plate**
- **Hybrid detectors**

Electron multipliers

They are mainly based on the multiplication of secondary electrons generated by the incident ions.

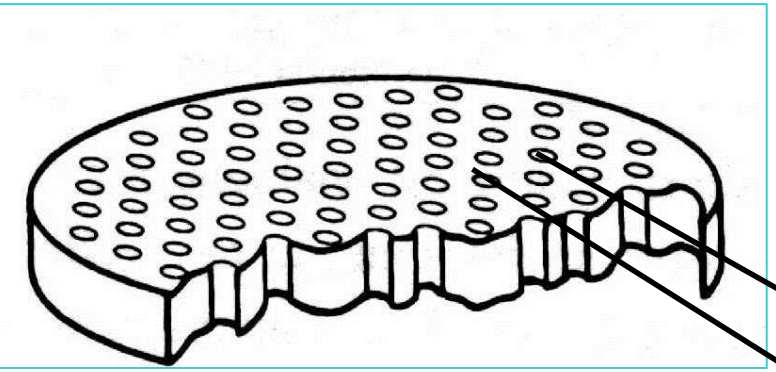


Multiplicateur à dynodes discrètes

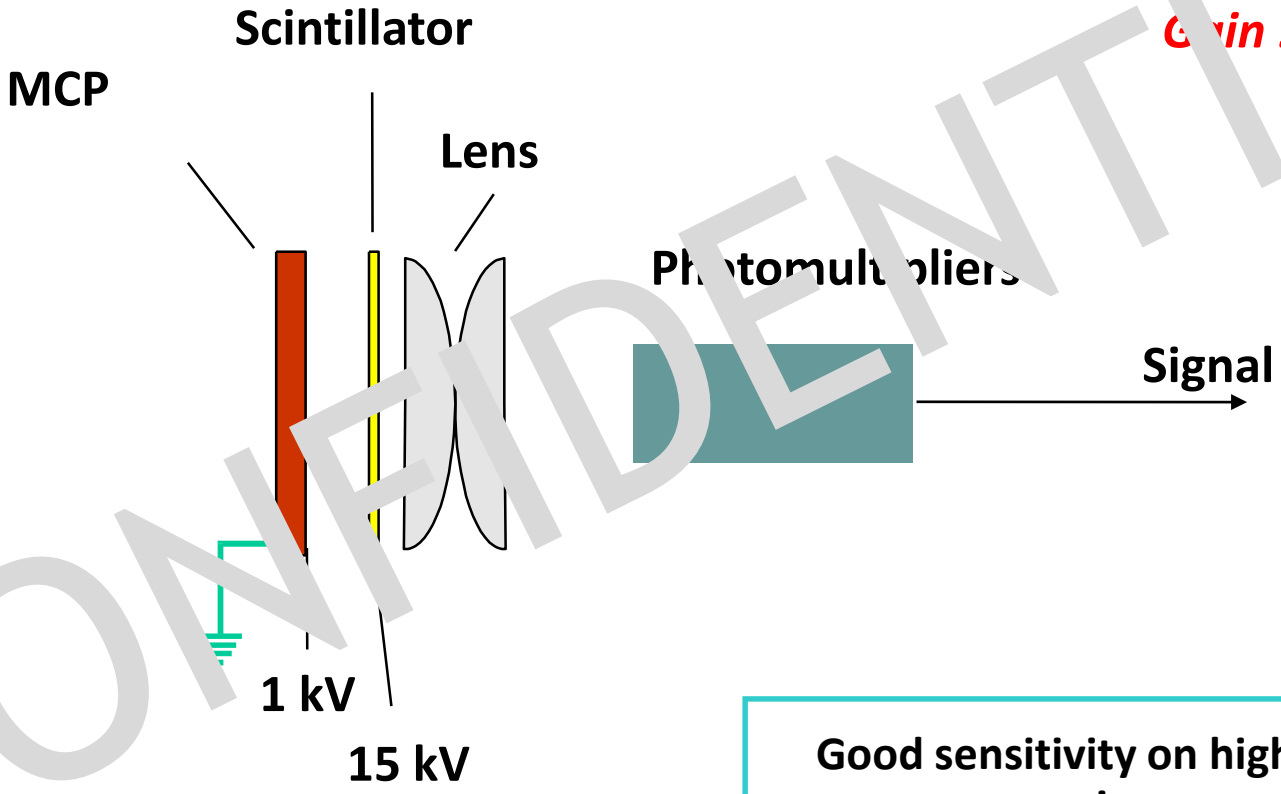


Multiplicateur type Channeltron

Micro Channel Plate (MCP)



Hybrid Detector



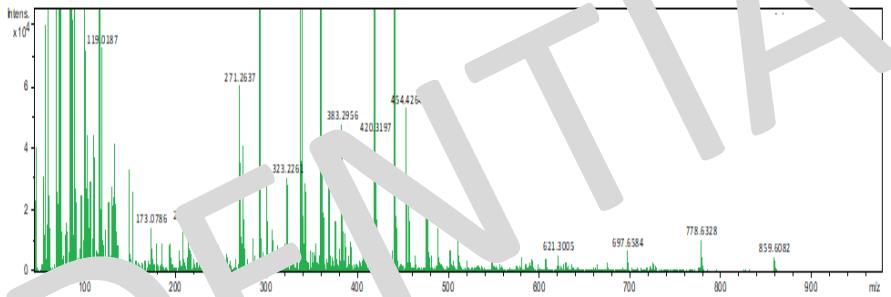
Good sensitivity on higher mass ions

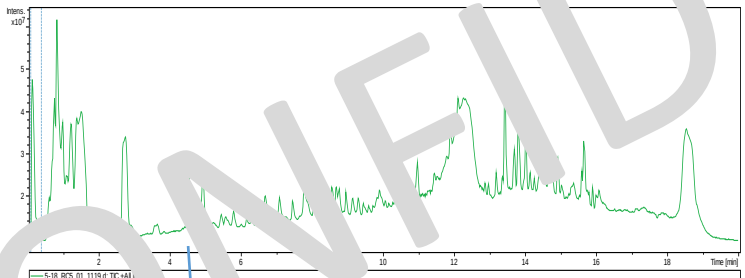
LC-MS

Liquid Chromatography – Mass spectrometry coupling

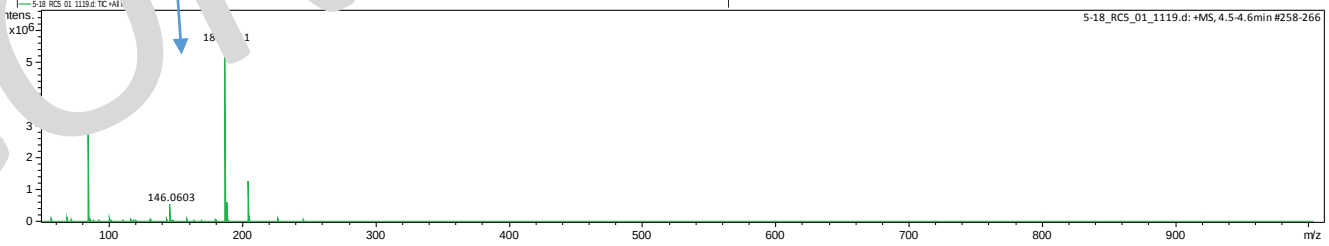
Why Liquid Chromatography ?

Spectrum complexity
Ionization suppression
Loss of information





Separate compounds
Less complex spectrum
Very attenuated ionization suppression



LC-MS/MS Optimisation

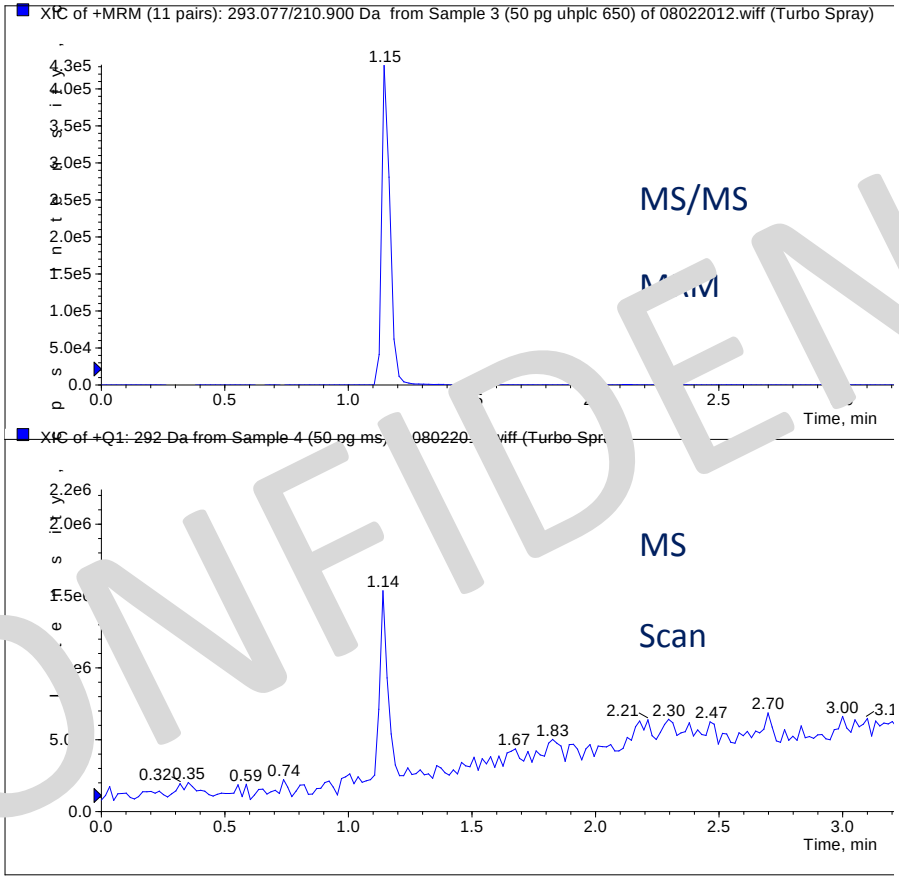
How to increase sensitivity?

Signal

Noise

LC-MS/MS Optimisation

How ?



MS/MS

MRM

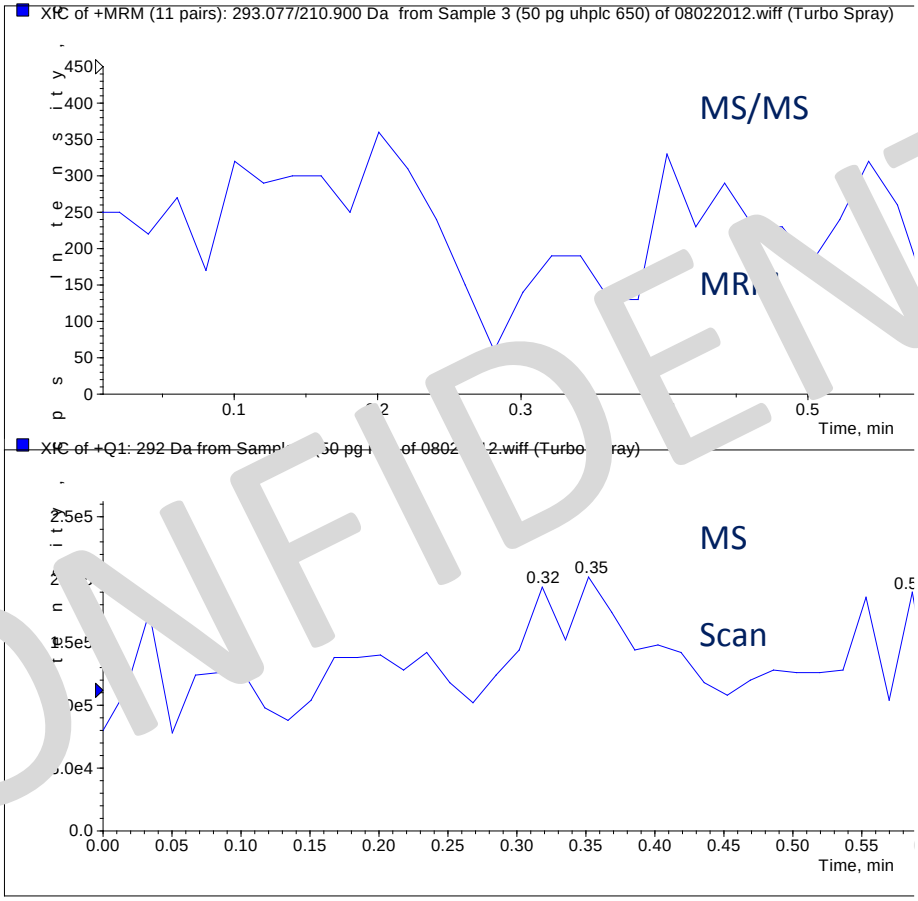
MS

Scan

Reduce Noise

LC-MS/MS Optimisation

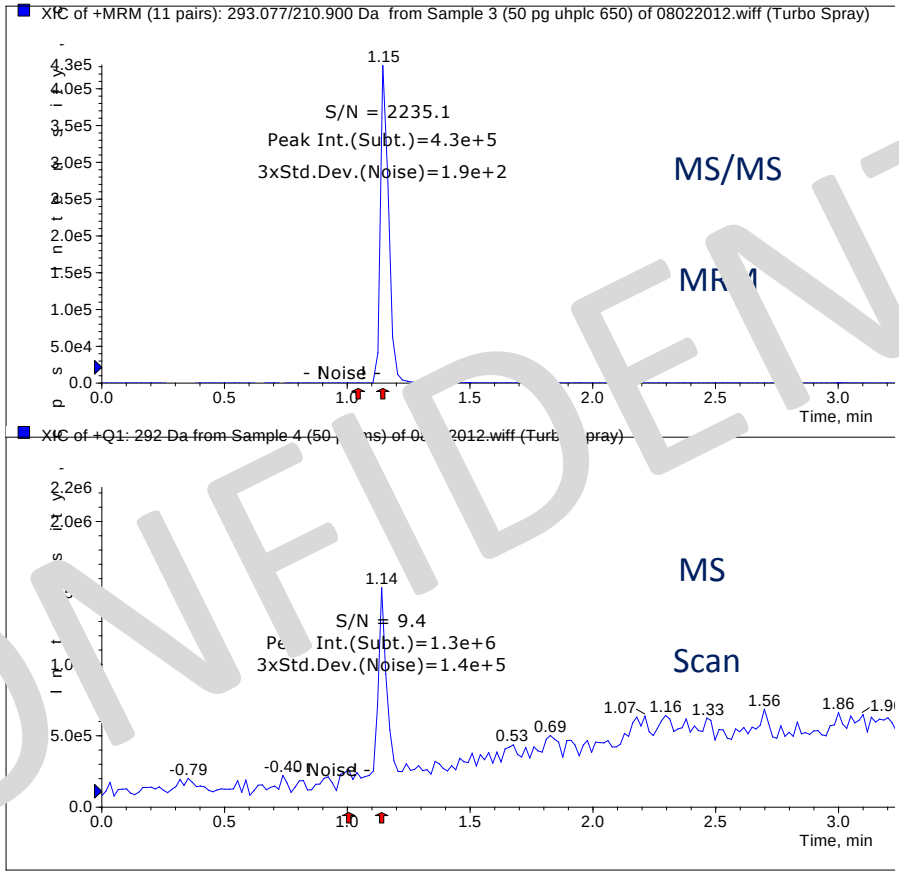
How ?



Reduce Noise

LC-MS/MS Optimisation

How ?



Reduce Noise

LC-MS/MS Optimisation

How ?

1. Detection Parameters

   -> Direct infusion

2. Source Parameters:

   -> FIA (Flow Injection Analysis) or coupled

3. Separation Parameters

   -> Coupled

Standards solution at 100 ng/mL (infusion) or at 10 ng/mL (coupling)

4. Sample preparation

MATRIX

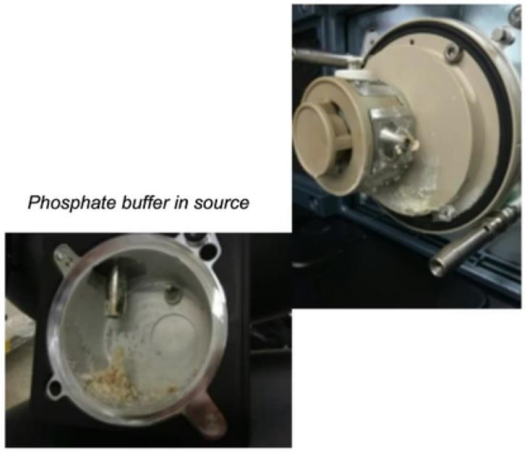
LC-MS/MS Optimisation

volatiles solvents used and additives which increase ionization

- solvents grade LC-MS used
- solvents commonly used in ESI :
  - Water
  - Acetronitrile
  - Methanol
  - Ethanol
  - Isopropanol
- Acid additives :
  - Formic Acid
  - Acetic Acid
  - TFA
- Basic additives :
  - NH_4OH (0,1 – 1%)
  - Ammonium formate
  - Ammonium acetate

LC-MS/MS Optimisation

- Non volatiles salt (phosphate, borate) -> source deposits
- Surfactants/detergents (SDS, nonyl sulfate) -> ionisation suppression
- Inorganic acids (HNO_3, HCl, H_3PO_4, H_2SO_4) -> corrosion



LC-MS/MS Optimisation

Recommended buffers :

- Ammonium formate ou acetate (1-10 mM max)
- 0.1 - 1.0 % acetic acid , 0.1% formic acid
- If TFA used to refine a chromatographic peak, always use with formic or acétic acid with 0.01 - 0.05% TFA
- **Be carreful TFA in negative mode**

sensitivity suppression :

- Salts
- Strong bases and quaternary amines in positive mode (Triethylamine (TEA))
- Sulfuric acid and TFA in negative mode
- Phosphate et ion pairing agents non - volatiles (ex. SDS) : strong ion suppression + complex spectrum

LC-MS/MS Optimisation

buffer	pH range	LC-MS compatible
phosphate (pK_1)	1.1 – 3.1	X
phosphate (pK_2)	6.2 – 8.2	X
phosphate (pK_3)	11.3 – 13.3	X
acetate[1]	3.8 – 5.8	YES
citrate (pK_1)	2.1 – 4.1	X
citrate (pK_2)	3.7 – 5.7	X
citrate (pK_3)	4.4 – 6.4	X
trifluoroacetic acid (0.1%)	2.0	YES
phosphoric acid (0.1%)	2.0	X
formic acid (0.1%)	2.7	YES
ammonium formate	2.7 – 4.7	YES
ammonium bicarbonate	6.6 – 8.6	YES
borate	8.3 -10.3	YES

[1] suitable for LC-MS as ammonium acetate