Liquid and Gas Chromatography (LC/GC) Coupled to a Mass Spectrometry (MS) Based Detector for Metabolite Analyses

Analysing the METABOLOME

1. Metabolite Extraction- last week
2. Metabolite Separation- last week
3. **Metabolite detection (with or without separation)**
4. Data analysis

Common Metabolite Detection Tools

- GC coupled to a Mass Spectrometer (MS)-detector (GC-MS): Naturally volatile or made volatile (any organic-flavors, sugars, lipids, acids)

- NMR – any compound containing hydrogen

- Liquid - Chromatography (LC) + detector

Common detectors-

- UV-PDA-detector
- MS-detector (LC-MS)

Mass Spectrometer

The general operation of a mass spectrometer is:

1. Create gas-phase ions

2. Separate the ions in space or time based on their mass-to-charge ratio (m/z)

3. Measure the quantity of ions of each m/z

Mass Spectrometer

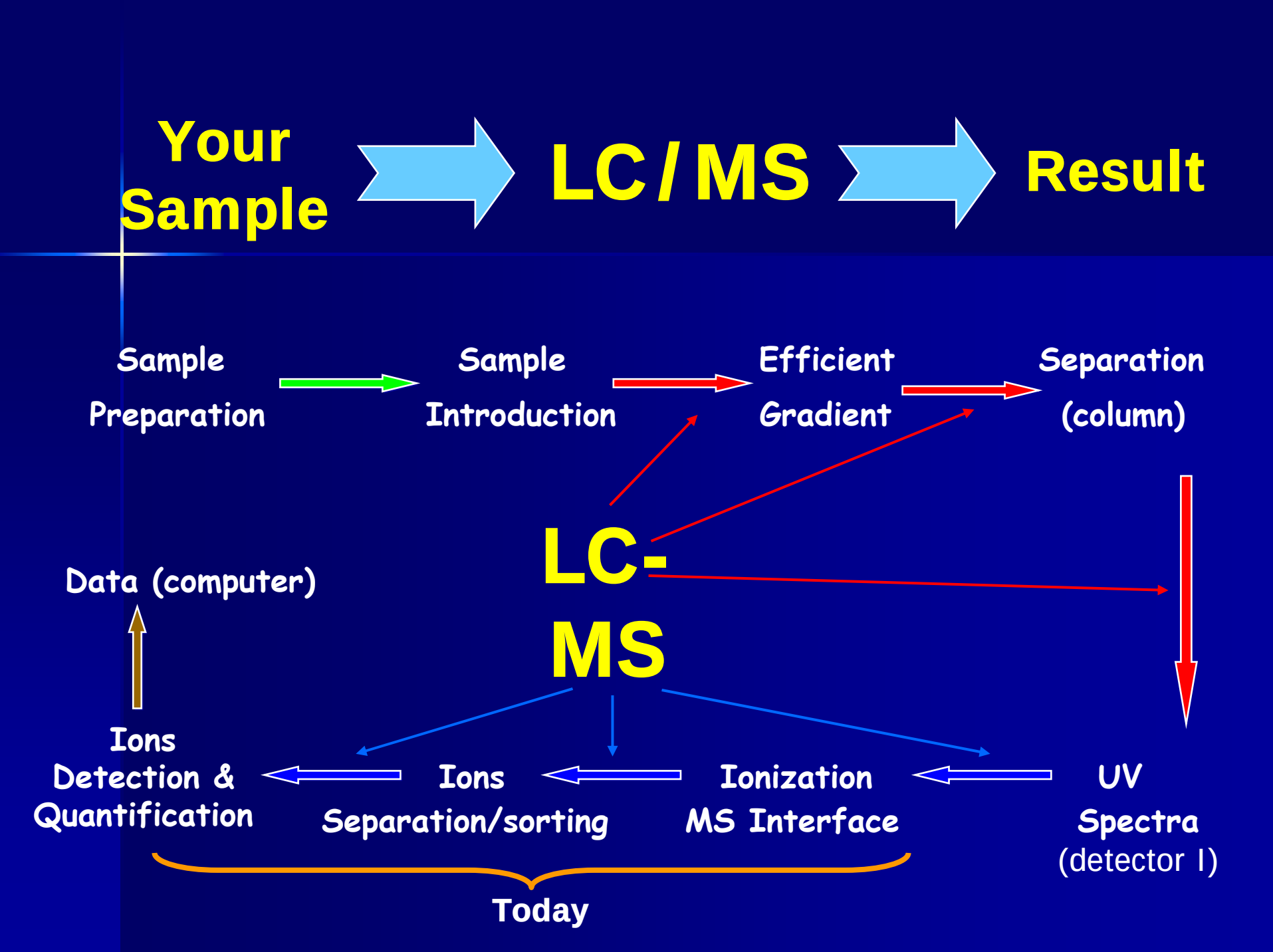
1. Universal detection method
    * compared to UV/VIS (PDA), fluorescence etc.
    * specific compared to NMR
2. More sensitive for most compounds
3. Structural information on metabolite
    * fragmentation pattern
    * accurate mass
4. For both LC and GC

Metabolite Detection

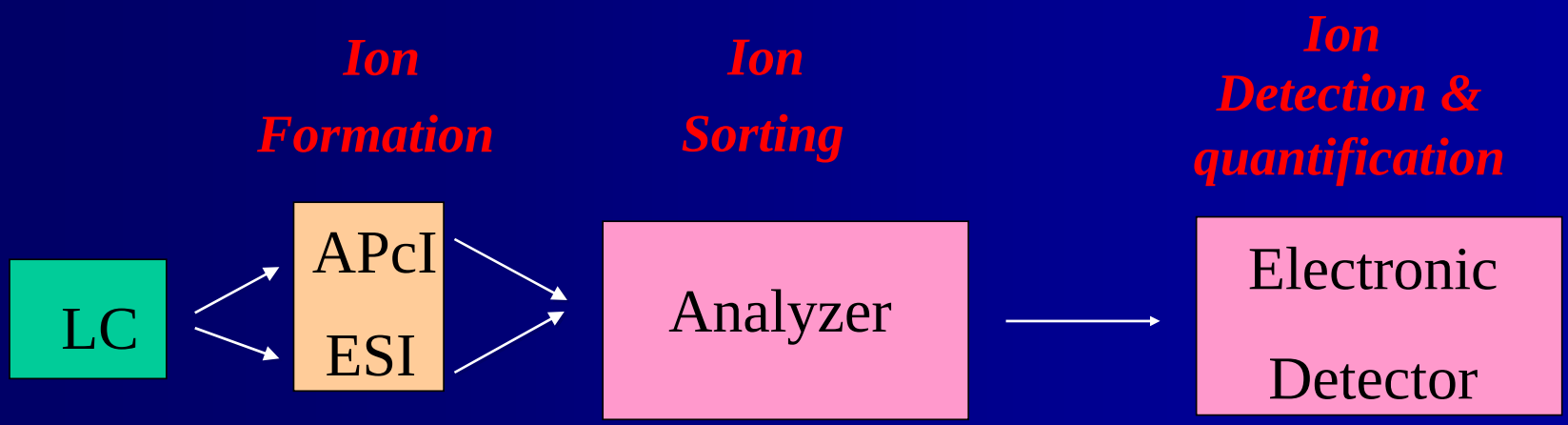
MASS SPECTROMETER (MS) as detector for LC can:

Detect compounds that are not well characterized by other methods (e.g. GC-MS):

- Non volatile
- High molecular weight
- Compounds too sensitive to heat to be analyzed by GC


Your Sample
LC / MS
Result
Sample Preparation
Sample Introduction
Efficient Gradient
Separation (column)
LC-MS
Data (computer)
Ions Detection & Quantification
Ions Separation/sorting
Ionization MS Interface
UV Spectra (detector I)
Today

Common Components in LC-MS



Chemical Separation

Interface:

Atmospheric Pressure Ionization

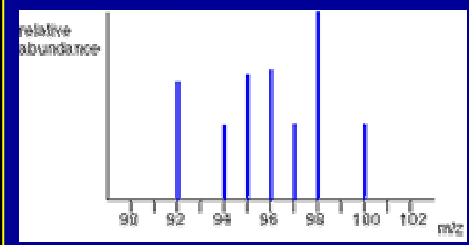
Single Quadropole

Triple Quadrupole

Quadrupole -Time Of Flight

Quadrupole - Ion-Trap

FT-MS



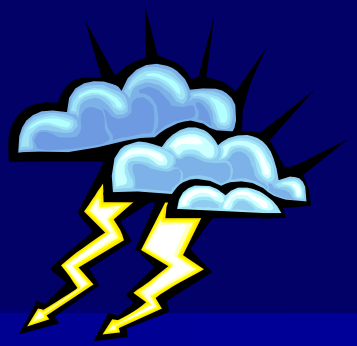
Technology of LC-MS and LC-MS-MS

– Ionization (elimination of solvent and generation of gas-phase ions)

– Interfaces, from ion formation to the detector - e.g. Z Spray

– Analyzers – Quadrupoles (Q) and Time of Flight (TOF)

LC-MS Interfaces



In MS-

* Measuring the mass of a huge variety of compounds, in a huge variety of matrices

* Need of a range of methods to IONISE all the different compounds

Alternative Ionization Modes

Ionization Methods

- *Fast Atom Bombardment (FAB or SIMS)*
- *Electron Impact (EI)*

"Hard" Ionization

EI ionization can be used with GC

- *Electrospray (ESI)*
- *Atmospheric Pressure Chemical Ionization (APCI)*
- *Laser Desorption (MALDI)*
- *Chemical Ionization*

"Soft" Ionization

ESI, APCI and MALDI can be used with LC

Hard Ionization



Electron Impact (EI)

- Used in GC-MS
- Gas-phase ionization method
- Electron Impact (EI) is the original MS ionization method
- Probably the most widely used of all ionization methods



Hard Ionization

Electron Impact (EI)

- In the EI process, the sample of interest is vaporized into the mass spectrometer ion source, where it is impacted by a beam of electrons with sufficient energy to ionize the molecule

- Ions are pushed by a positive voltage towards the mass analyser

Practical Strengths and Limitations of EI



1. Spectral characteristics

- The ion M^+ is known as the molecular ion
- This species indicate the molecular weight, and thus provides very useful analytical information

The molecular ion will often undergo fragmentation in EI:

$$M1^+ \rightarrow M2^+ + Mn$$

where M 1 is the molecular ion
M 2 is the fragment ion
M n is the neutral fragment

Practical Strengths and Limitations of EI



The fragmentation process is dependent upon many qualities such as:

a) primary metabolite structure
b) electron energy
C) ion source temperature.

- In the best circumstances, fragmentation is advantageous, because one gathers structural information about the molecule under analysis in addition to the molecular weight information

- However, in some cases, all of the molecular ions fragment and no molecular information is gathered

Practical Strengths and Limitations of EI



2. The molecular ion is a radical

- While removing one electron and generating the molecular ion we actually generate a radical

- Radicals are not very stable species and tend to fragment

Therefore EI mass spectra are frequently characterized by intense fragment ions and small molecular ion peaks



Practical Strengths and Limitations of EI

3. Electron energy

- How many ions are made as a fraction of total sample molecules introduced into the instrument (ION YEILD) varies as a function of the electron impact energy

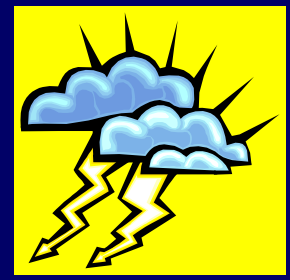
- For most organic molecules, the ion yield is maximum in the region of 70 electron volts (eV) energy

- For this reason, most EI spectra are gathered using 70 eV electron energy

- One can reduce the amount of fragmentation by reducing the energy, and molecular peaks are sometimes observed in low eV spectra

- However, reducing the energy at the same time reduces both the amount of fragmentation and the total number of ions formed.

Practical Strengths and Limitations of EI



- The fragmention in EI under high energy is most reproducible and therfore libraries of spectra produced by different instruments are most usefull

- EI is not always suitable:

• Difficult to get large or in-volatile molecules into the gas phase

• Heating the non-volatile molecules degrades them

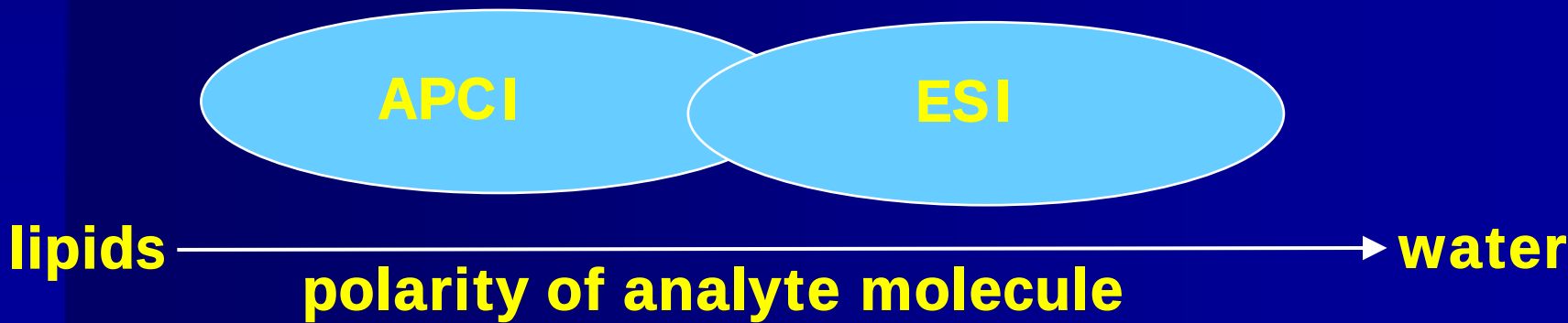


Soft Ionization

- Atmospheric Pressure Ionization (API), in LC-MS

  - Electrospray Ionisation (ESI): polar and semi-polar

  - Atmospheric Pressure Chemical Ionization (APCI): less polar





John Fenn, 2002 , Nobel price in chemistry (for ESI), 4th time for MS pioneers)

Atmospheric Pressure Ionisation (API) Techniques

ESI and APCI differ in…

- **How ions are generated**
  - **ESI - solution phase ionization**
  - **APCI - gas phase ionization**
- **Analyte compatibility**
  - **ESI - polar compounds and large biomolecules**
  - **APCI - less polar, smaller compounds (relative to those ionized by ESI) that have some volatility**
- **Flow rate compatibility**
  - **ESI - 0.001 to 1 mL/min**
  - **APCI - 0.2 to 2 mL/min**

How do the analytes become charged?

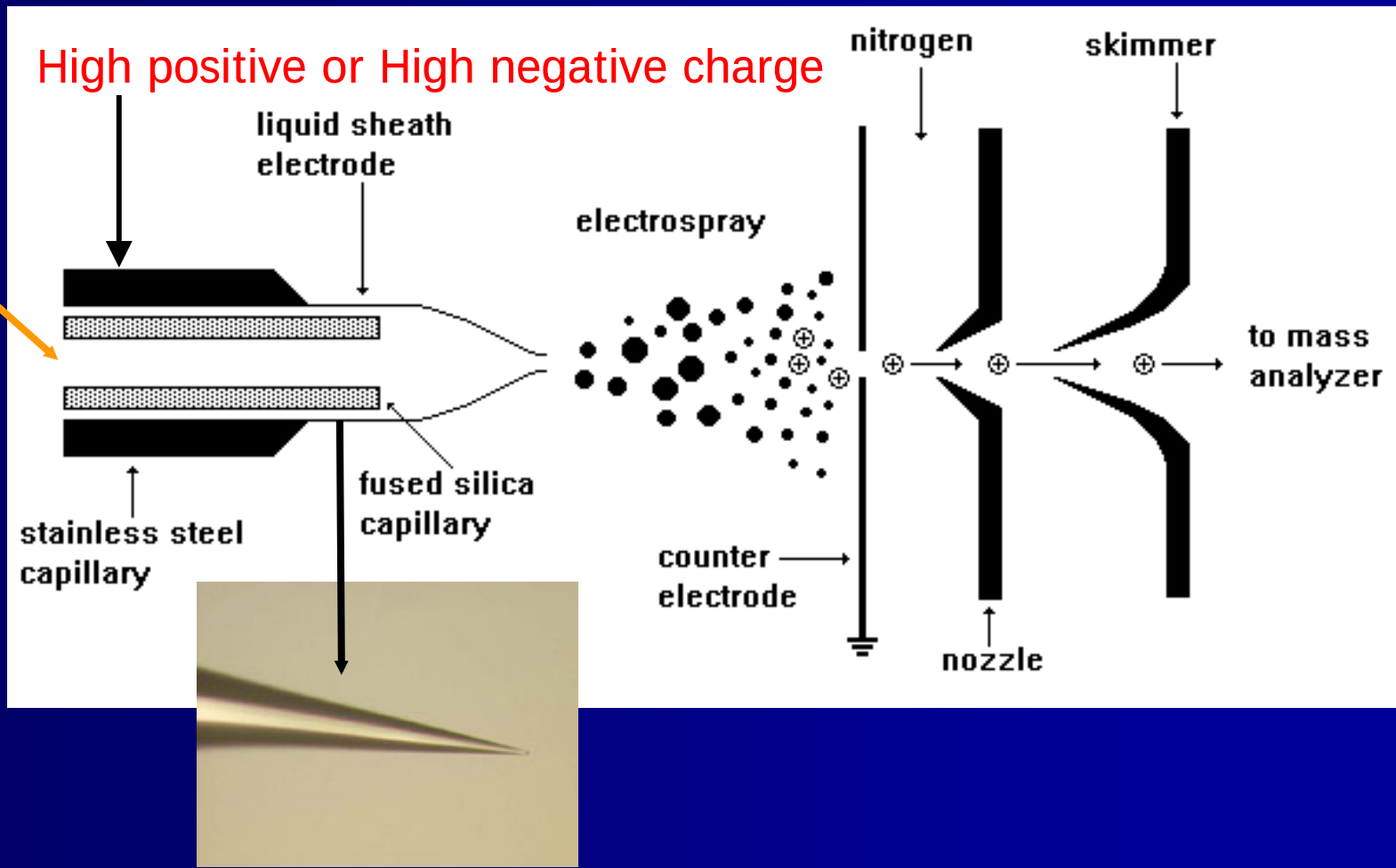
- While in EI, loss of an electron producing a radical molecular ion

- In soft ionisation techniques, analyte molecules are:
protonated $[M + H]^+$
or:
de-protenoated $[M - H]^-$

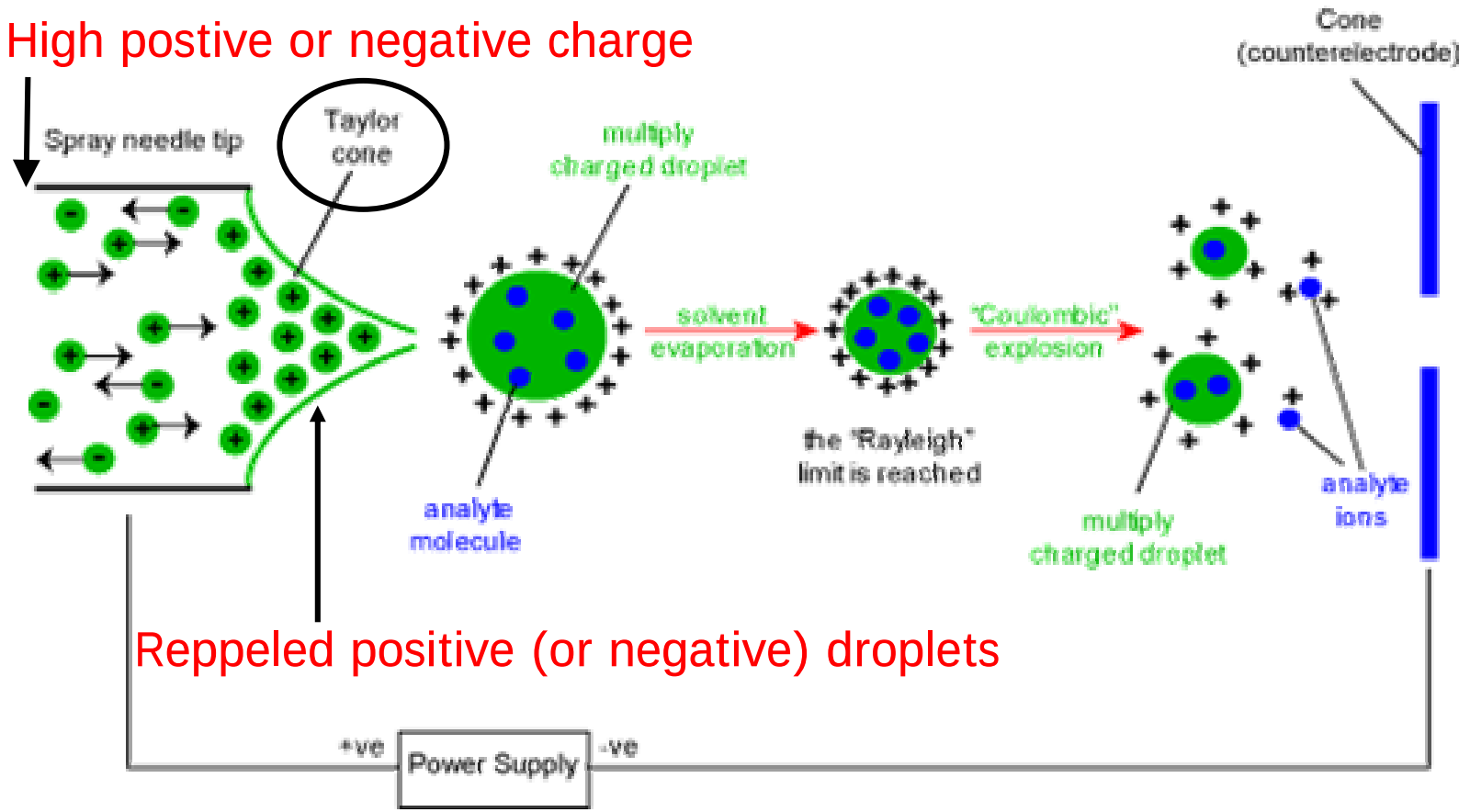
- Could also be sodiated, potassiated etc.. (adducts)

Ion Formation in ESI

Analyte/Eluent from LC or syringe pump



How do the analytes become charged?



Positive or Negative Modes?

The formation of positive or negative ions depends on the sign of the applied electrical field in the cone

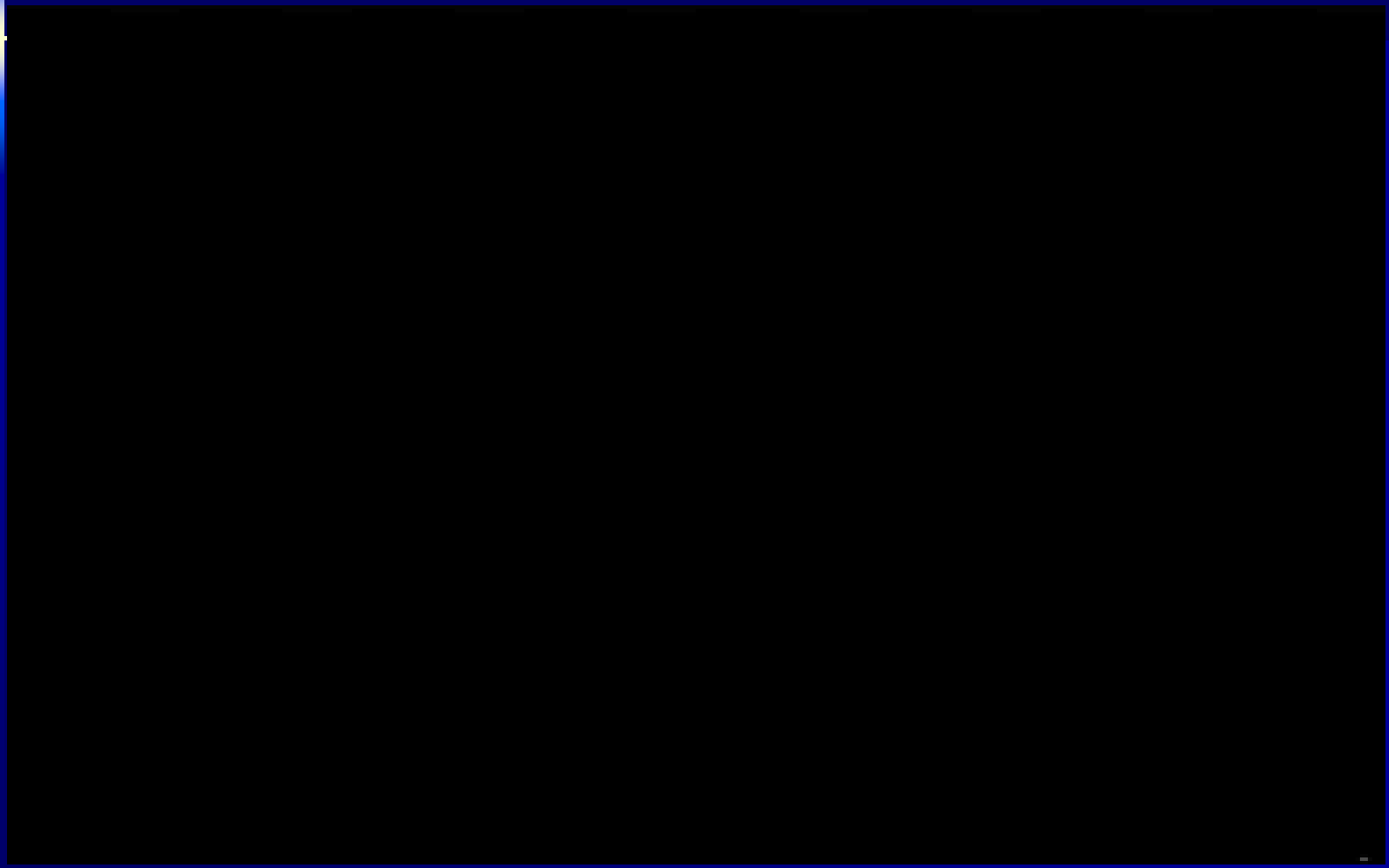
ES+: $(M+H)^+$

Good ionization of basic compounds (get proton)
E.g. with functional groups such as amino, amide, ester, aldehyde / keto

ES-: $(M-H)^-$

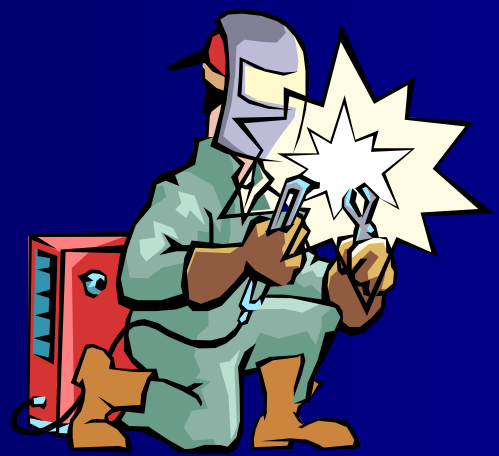
Ionization of Acidic Compounds (give proton) E.g. organic acids, containing OH

Electrospray Theory



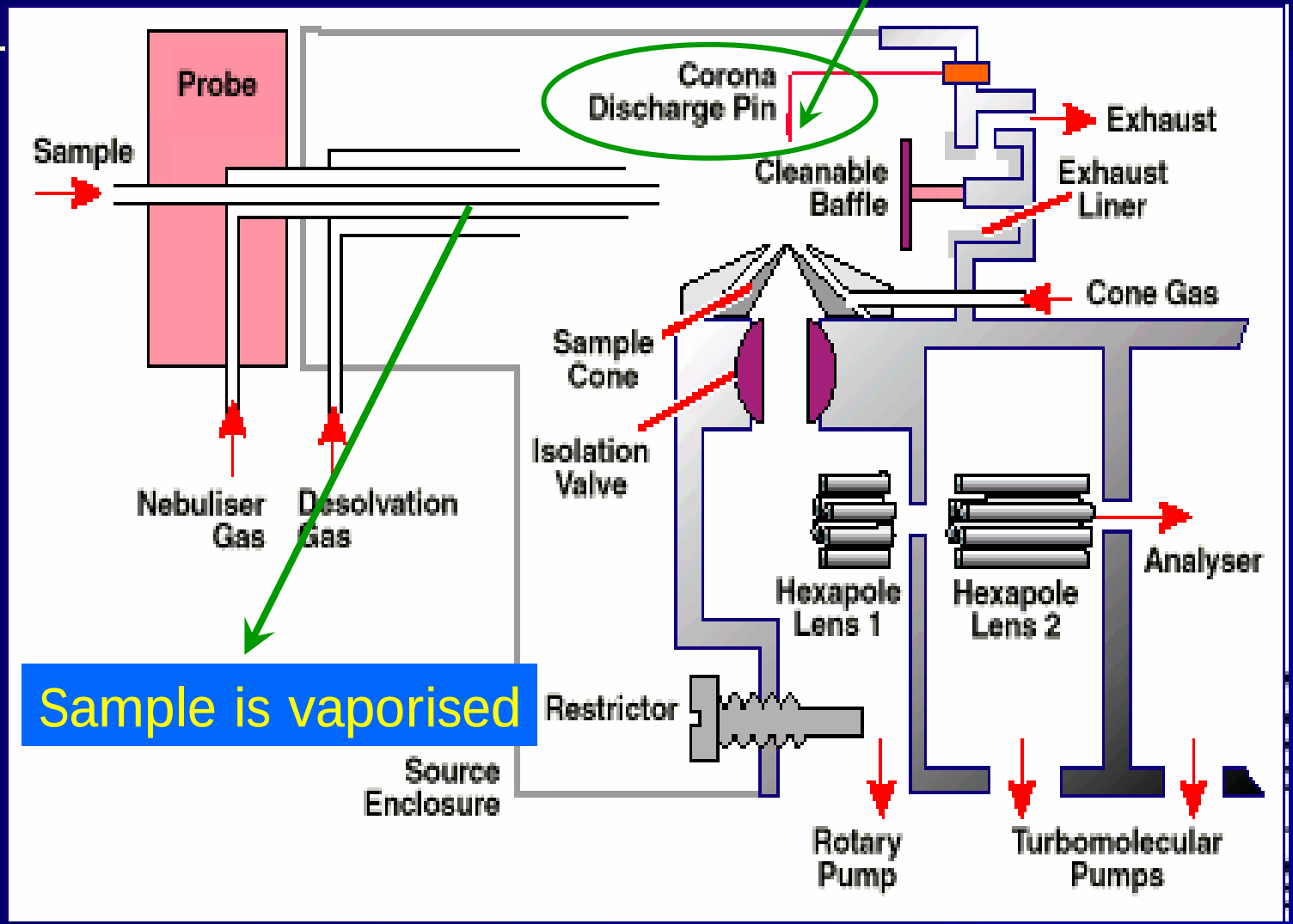
Chemical Atmospheric Pressure Ionization - APCI

Atmospheric Pressure
Ionization Interface



APcI Source

Ionization of solvent
& solvent transfers the charge to analyte



APcI Theory



Atmospheric Pressure Chemical Ionisation (APcI)

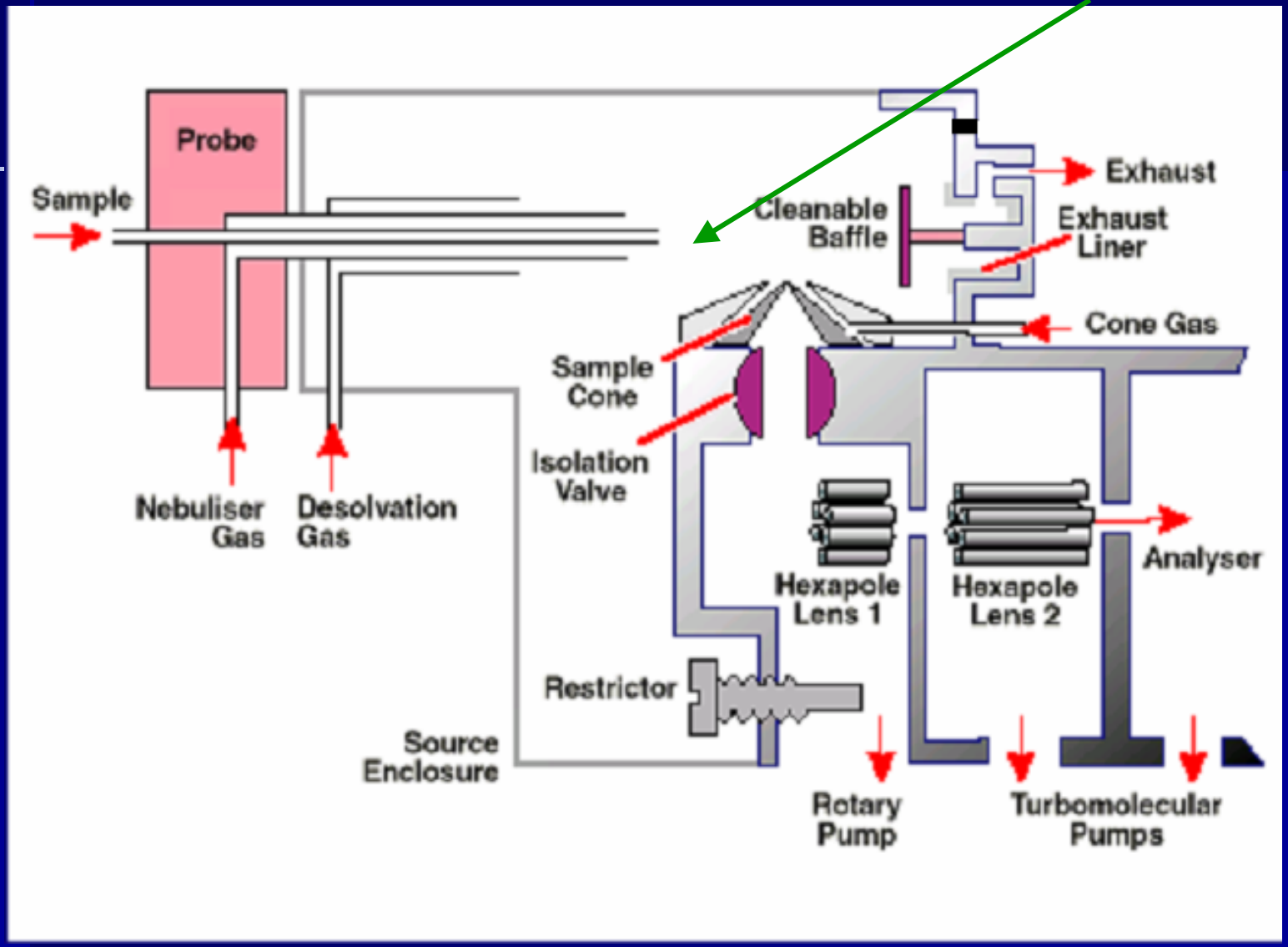
- Low molecular weight (<1000 Da)

- Singly charged species

ESI vs APcI

Technique	Flow Rate (ml/min)	MW Range	Species Produced
ESI	0.001 – 0.3	<200,000 Da	$(M+H)^+$ $(M-H)^-$ $(M+nH)^{n+}$
APcI	0.2 – 2.0	<1000 Da	$(M+H)^+$ $(M-H)^-$

Z *SPRAY*™ Source



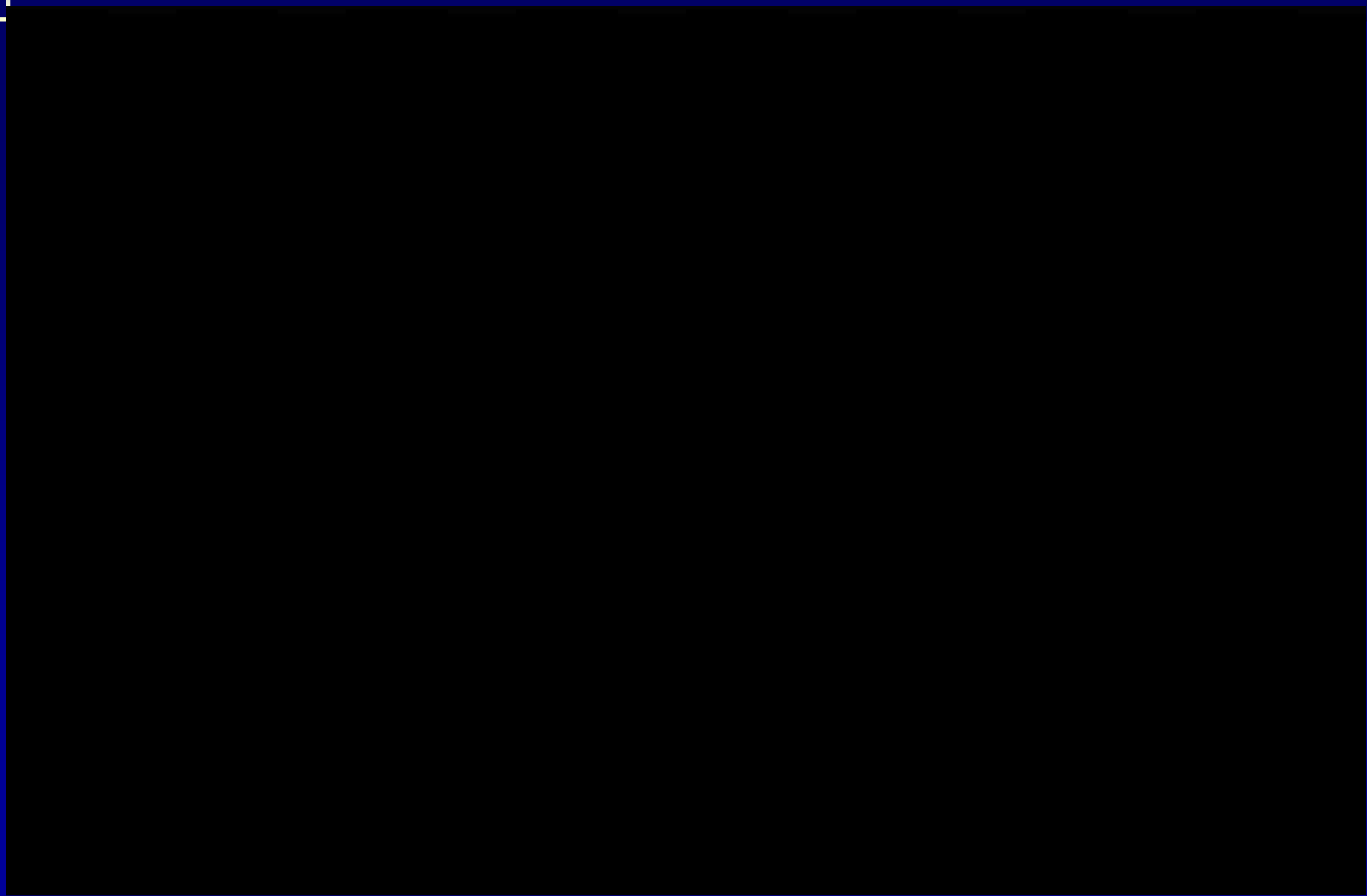
Technology of LC-MS and LC-MS-MS

– Ionization (elimination of solvent and generation of gas-phase ions)

– Interfaces, from ion formation to detector - e.g. Z Spray

– Analyzers – Quadrupoles (Q) and Time of Flight (TOF)

Z-Spray Interface



Technology of LC-MS and LC-MS-MS

– Ionization (elimination of solvent and generation of gas-phase ions)

– Interfaces, from ion formation to detector - e.g. Z Spray

– Analyzers, ion sorting – Quadrupoles (Q) and Time of Flight (TOF)

Components in LC-MS

Ion Formation

Ion Sorting

LC → APcI / ESI → Analyzer → Electronic Detector

Chemical Separation

Interface:

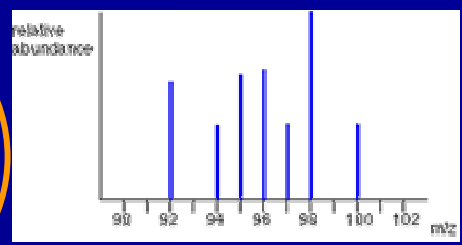
Atmospheric Pressure Ionization

Triple Quadrupole

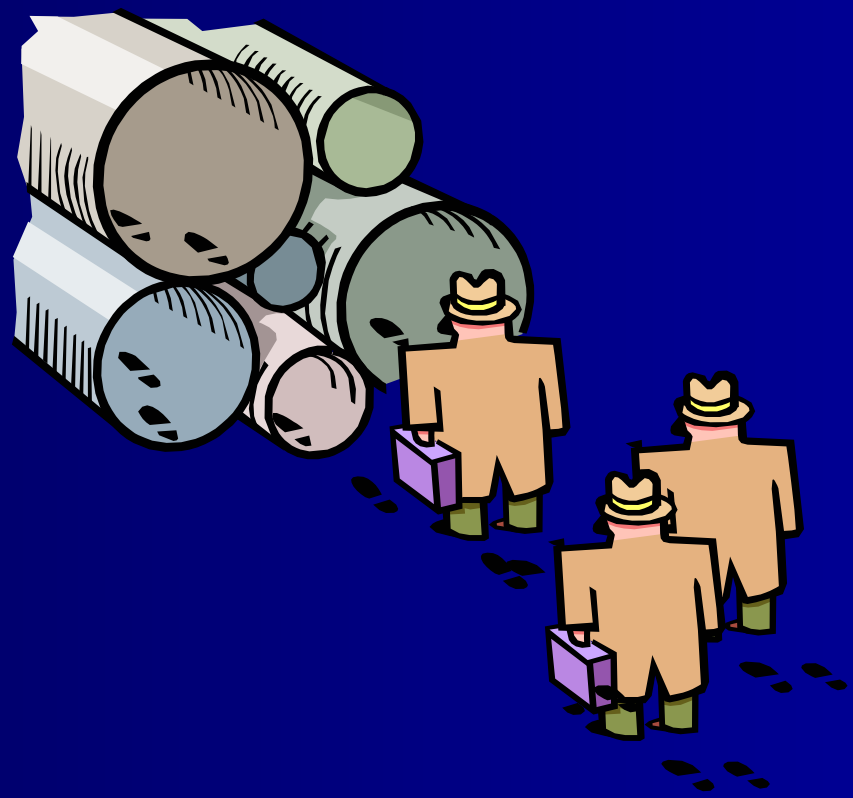
Quadrupole -Time Of Flight

Quadrupole - Ion-Trap

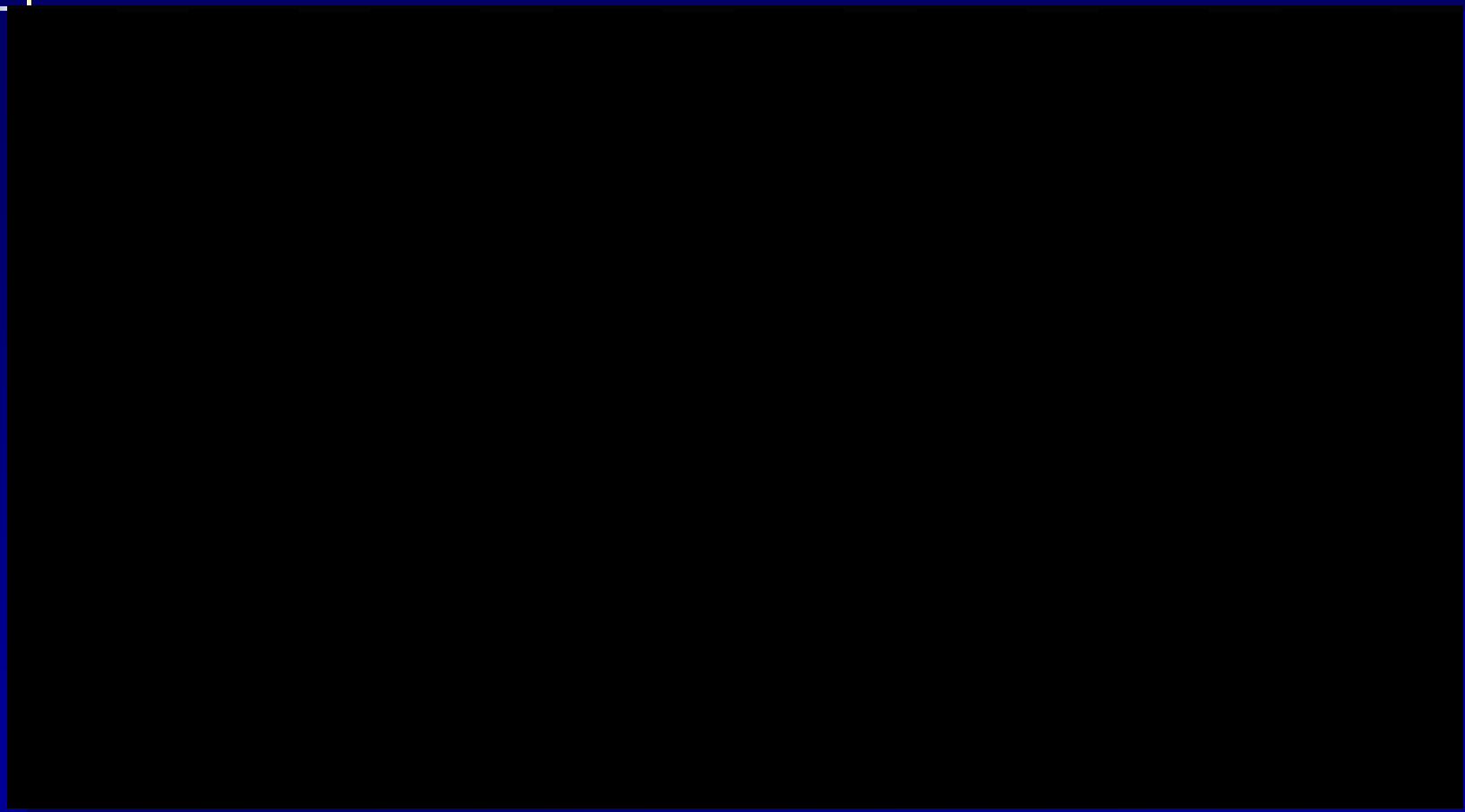
FT-MS



Quadrupole and Tandem Quadrupole



Quadrupole Theory



Resolution & Accuracy of a Mass-spectrometer

Resolution of an MS Detector

Resolution, (or Resolving Power) of a mass sectrometer:

A measure of its ability to separate adjacent ions

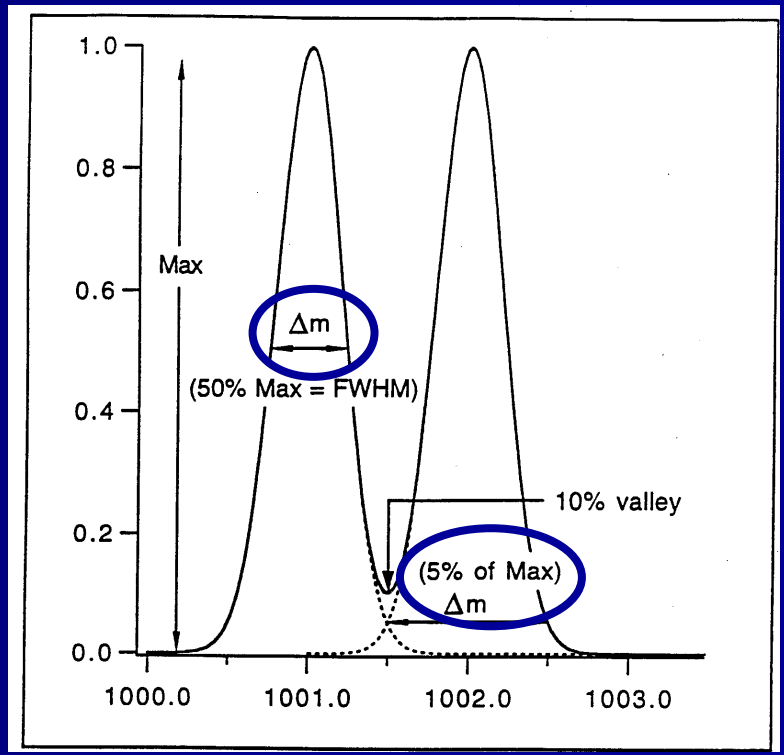
At higher resolution, small differences may be detected

Determining Resolution

Single Ion method

Full Width at Half Maximum
(50% Max, FWHM)
or at 5% of the peak height

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



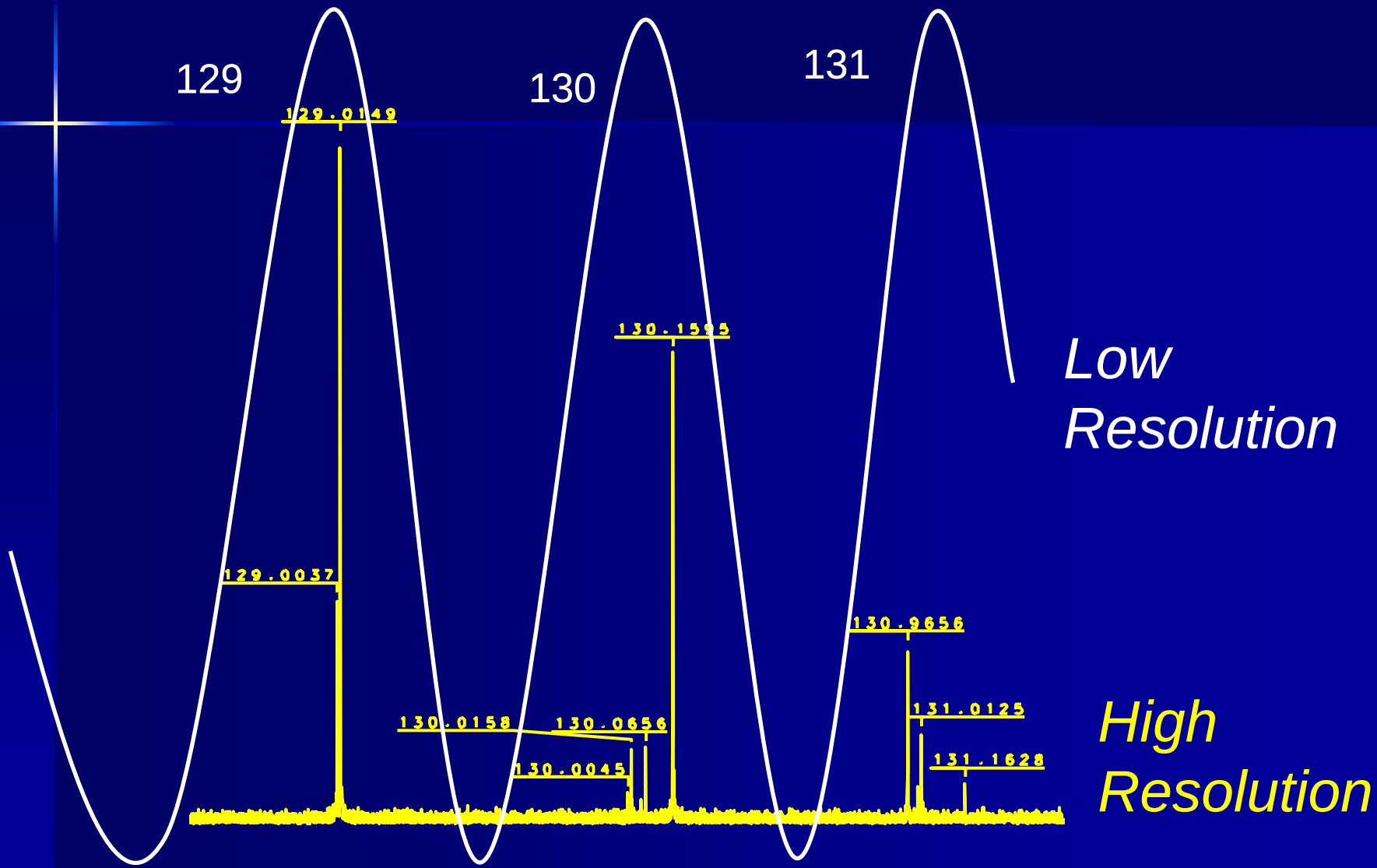
Mass Analyzers

- *Ion Cyclotron (FT-ICR-MS)*
- *Time of Flight (TOF)*
- *Magnetic Sector*
- *Quadrupole Ion Trap*
- *Quadrupole*

"High Resolution" Instruments

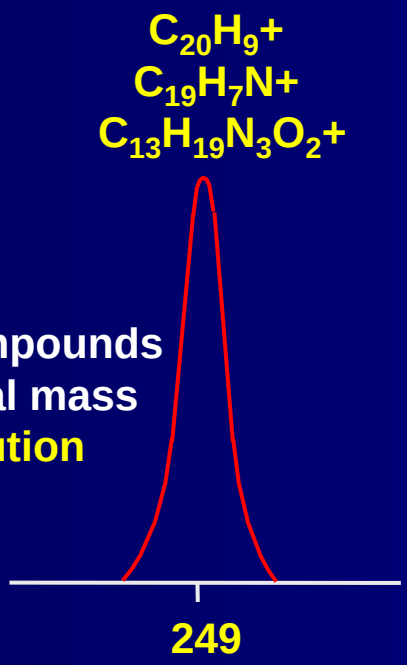
"Low Resolution" Instruments

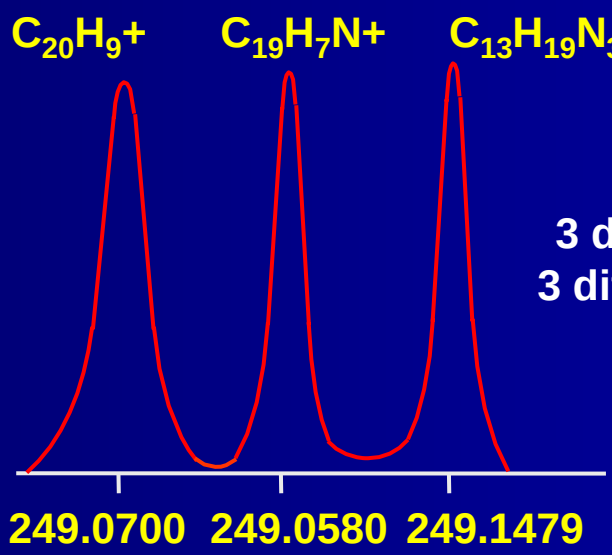
High Resolution vs. Low Resolution



Resolution

3 different compounds
Same nominal mass
Low resolution





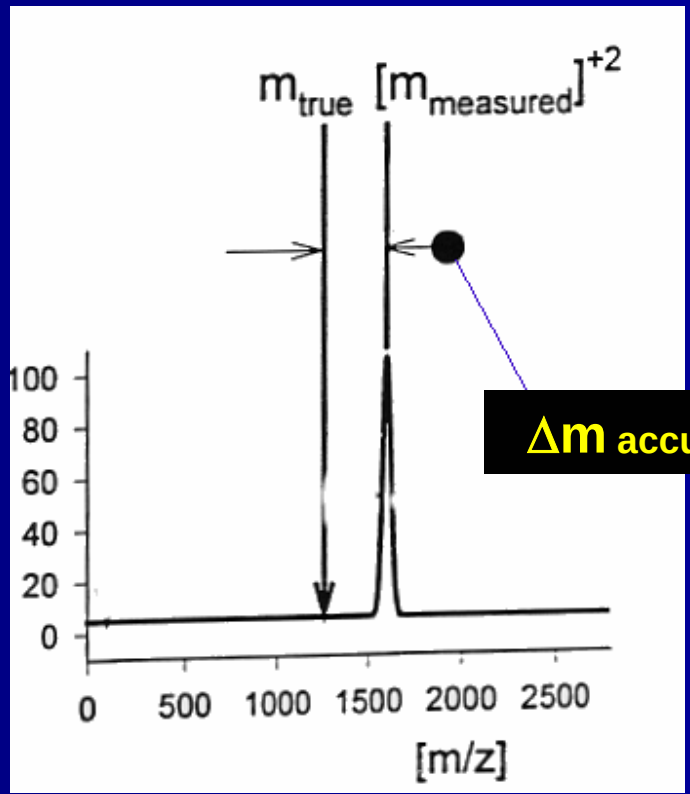
3 different compounds
3 different exact masses
High resolution

Mass Accuracy

Ability of a mass analyzer to assign the mass of an ion close to its true value (exact mass)

$\Delta m_{accuracy} = m_{true} - m_{measured}$

$\underline{\text{In ppm}} = 10^6 * \Delta m_{accuracy} / m_{measured}$

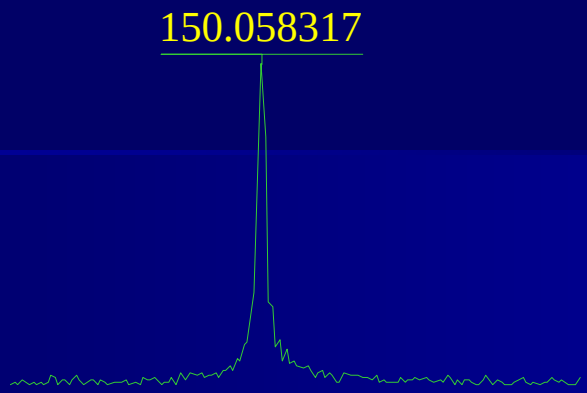


Mass Accuracy

High mass accuracy (exact mass measurement) is usually associated to high resolution analyzers

Unknown compound determination
Exact mass helps to define its atomic composition

Mass Accuracy Determining Empirical Formula, Structural Elucidation



Methionine

$C_5H_{12}NO_2S$

0.06 ppm

#	12C	1H	16O	14N	31P	32S	23Na	39K	mass	error
1	5	12	2	1	0	1	0	0	150.0583257	5.826e-08
2	1	15	2	2	1	1	0	0	150.0586364	2.128e-06
3	3	15	0	2	0	1	0	1	150.0587525	2.902e-06
4	9	11	0	0	1	0	0	0	150.0592883	6.473e-06
5	3	15	0	1	2	0	1	0	150.0571936	7.486e-06
6	5	14	0	1	2	0	0	0	150.0595989	8.543e-06
7	4	16	1	0	1	0	0	1	150.0570349	8.544e-06
8	3	10	1	4	0	1	0	0	150.0569831	8.889e-06
9	2	16	3	0	2	0	0	0	150.0569188	9.318e-06

Mass Accuracy- FTMS

- Instrument Calibration -

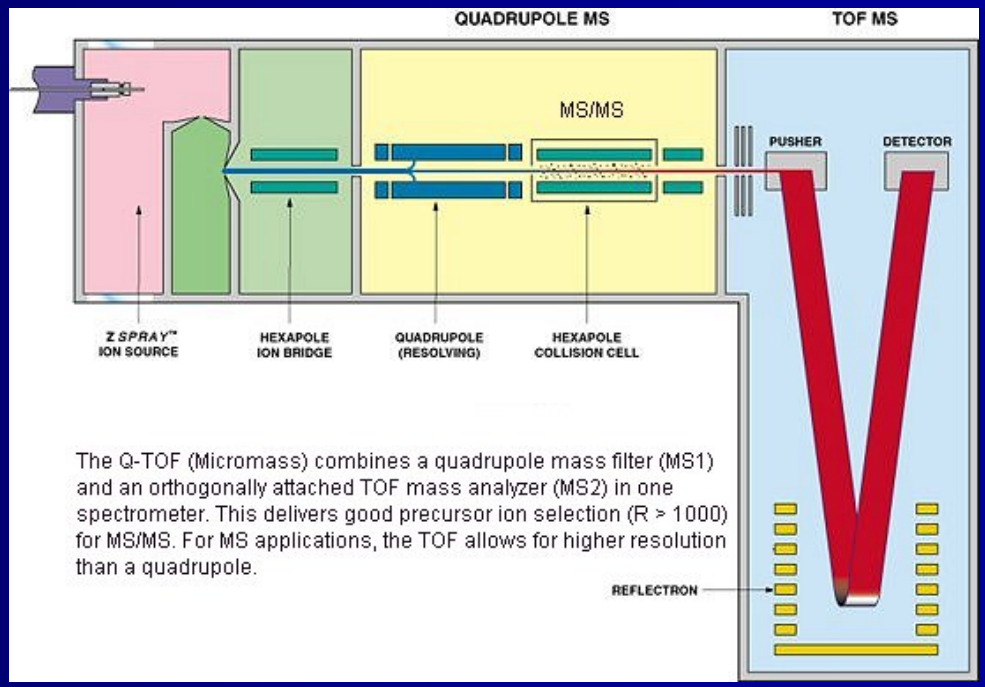
Calibrant	Exact Mass	Experimental Mass	Difference (ppm)
Alanine	90.0550	90.054998	0.0175
Lysine	147.1128	147.112783	0.1173
Glutamate	148.0604	148.060420	0.1353
Methionine	150.0583	150.058317	0.1109
Tyrosine	182.0812	182.081184	0.0866
Tryptophan	205.0972	205.097192	0.0380

Mass Analyzers

	Resolving Power	Mass Accuracy
•Ion Cyclotron (FT-ICR-MS)	200,000	<1ppm
•Time of Flight (TOF)	20,000	3-10ppm
•Magnetic Sector	60,000	2-5ppm
•Quadrupole Ion Trap	1,000	n/a
•Quadrupole	1,000	n/a

Benefits of Time-Of-Flight MS

- high mass resolution (up to 10 or 5 ppm)
- exact mass

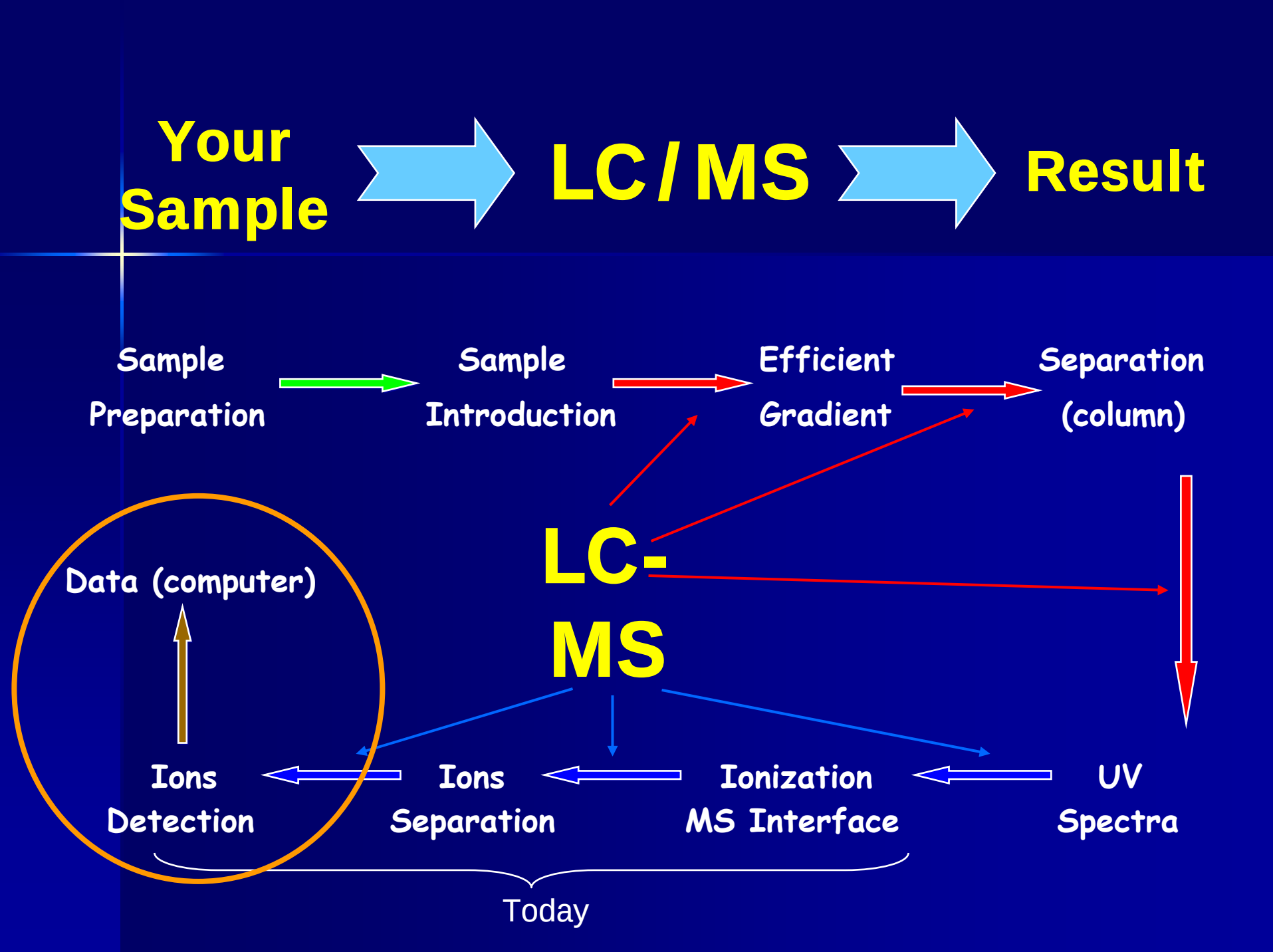


Scan Speed (or rate)

- The rate at which we can acquire a mass spectrum, (mass units/sec).
- Will affect the amount of information (qualitative and quantitative) that can reasonably be attained with a given mass analyzer

Mass Analyzers

Analyzer	Range amu	Accuracy amu	Resolution	Speed amu/s
Quad	< 4000	0.1	1000-2000	4000
Ion Trap	< 20 000	0.1	1000-2000	4000
TOF	1 000 000	0.0001	500-10 000	1 000 000


Your Sample
LC / MS
Result
Sample Preparation
Sample Introduction
Efficient Gradient
Separation (column)
LC-MS
Data (computer)
Ions Detection
Ions Separation
Ionization MS Interface
UV Spectra
Today

Visit to Instruments used In-house

UPLC-Q-Tof-MS



GC-MS



Next Week

Data after ion detection in LC/GC-MS