FIMM
Institute for Molecular Medicine Finland
Nordic EMBL Partnership for Molecular Medicine

Analysis of Polar Metabolites using Mass Spectrometry

TransMed Course: Basics in Clinical Proteomics and Metabolomics. Oct 10-19, 2012

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12/10/12

Outline

- Polar metabolite extraction
- Liquid chromatography
- Mass spectrometry
- Work flow
- Small Test

Automated sample extraction

Polar metabolite extraction

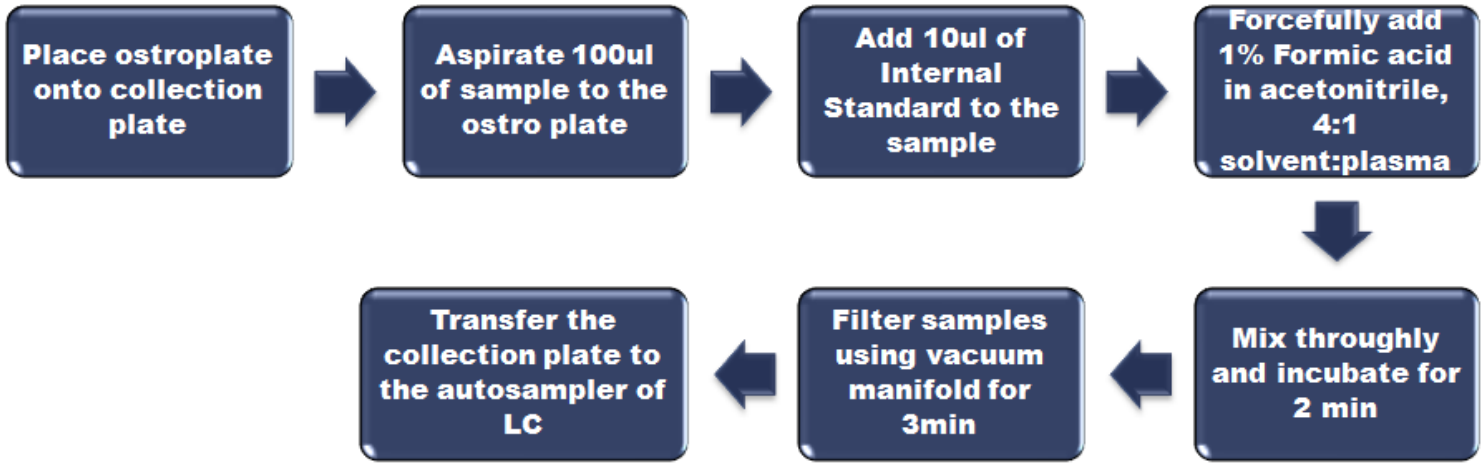


Fig2: : Experimental protocol for metabolite extraction using robot

Why LC/MS/MS?

- **Analysis of labile analytes**
- **Analysis of more polar compounds without derivatization.**
- **Analysis of significantly higher masses**
- **Reduction of lengthy clean-up**

› **LC-MS** (Single quadrupole)

› **LC-MS/MS** (Triple quadrupoles)

› **LC-TOF-MS** (Time-of-flight)

› **Q-TOF-MS** (Quadrupole time-of-flight)

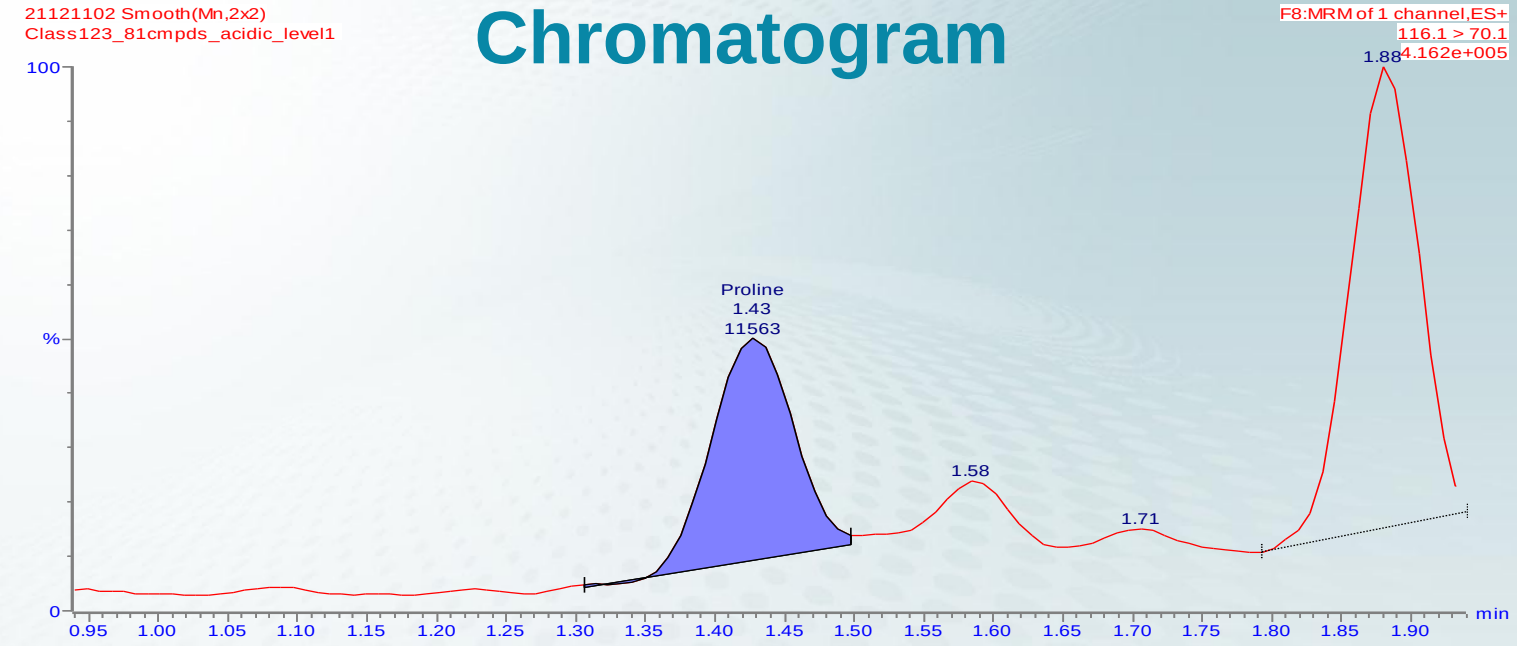
› **LC-Q** (Ion traps, linear ion traps)

› **LC-Q-TRAPS (**Quadrupole linear ion trap)

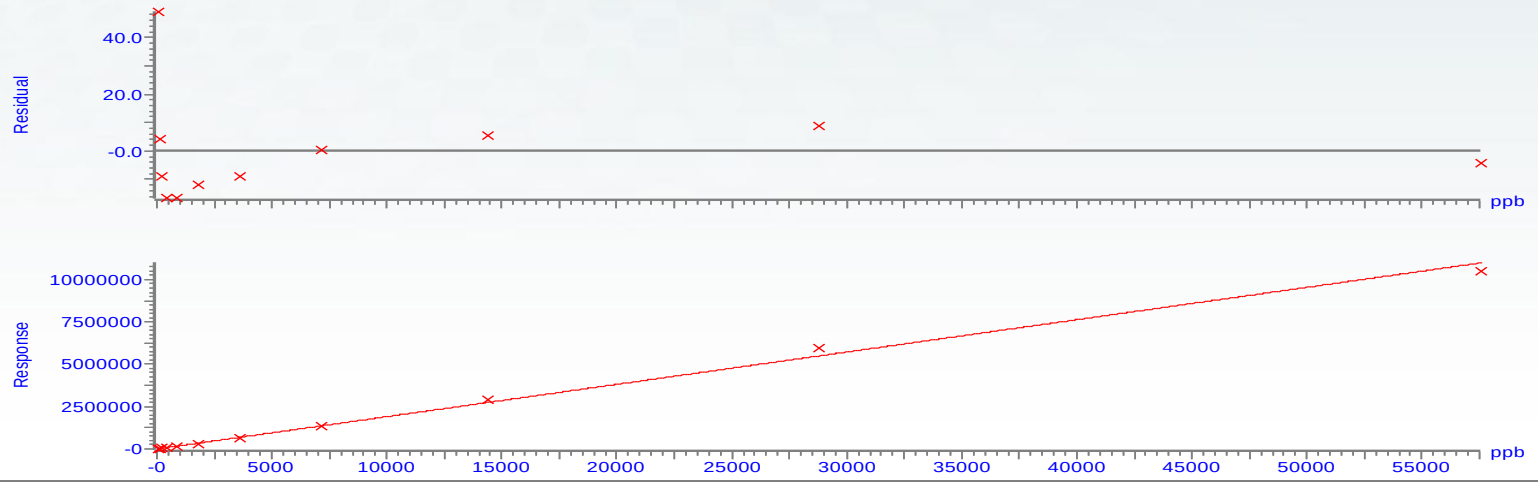
› **MALDI-TOF-MS**

› **FT-MS** (Fourier Transform)

Chromatogram



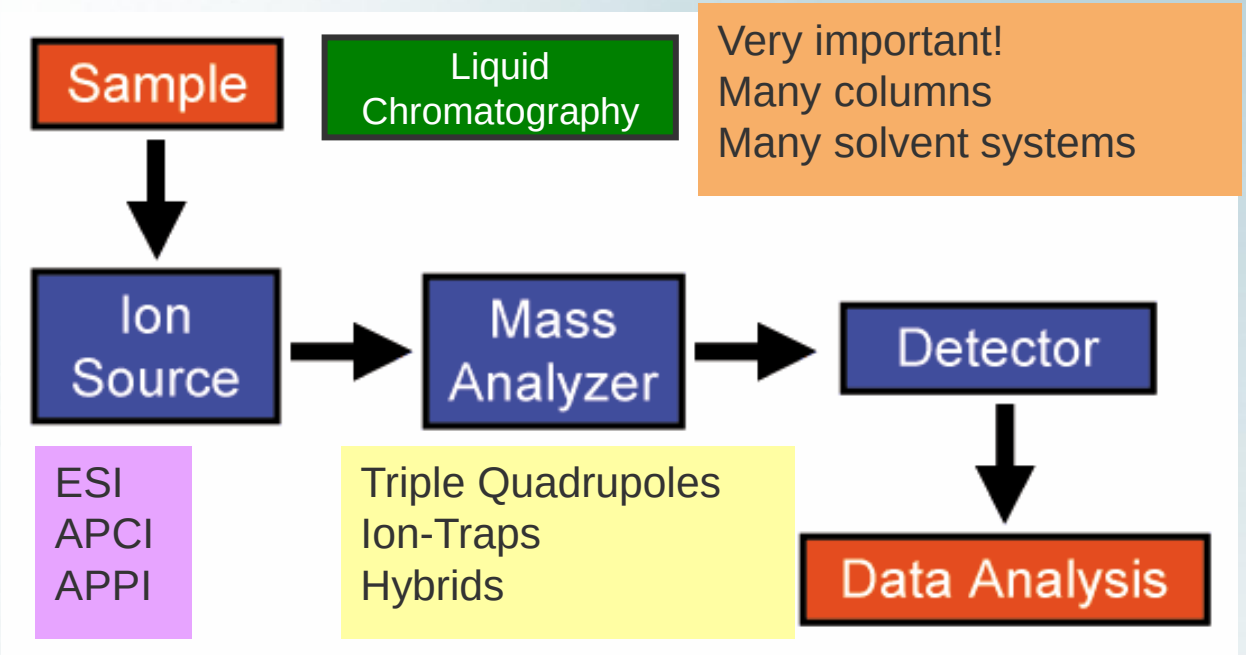
Compound name: Proline
Correlation coefficient: r = 0.997859, r^2 = 0.995722
Calibration curve: 190.945 * x + -4438.66
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



What is Mass Spectrometry (MS)

- MS does not measure the mass of a compound
- Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized compounds to separate them from each other.
- Compounds have distinctive fragmentation patterns that provide structural information to specifically detect each compound very precisely.
- MS – has emerged as an ideal technique for the identification of almost all structurally diverse metabolites.
- MS/MS data provides tremendous structural information for any metabolites

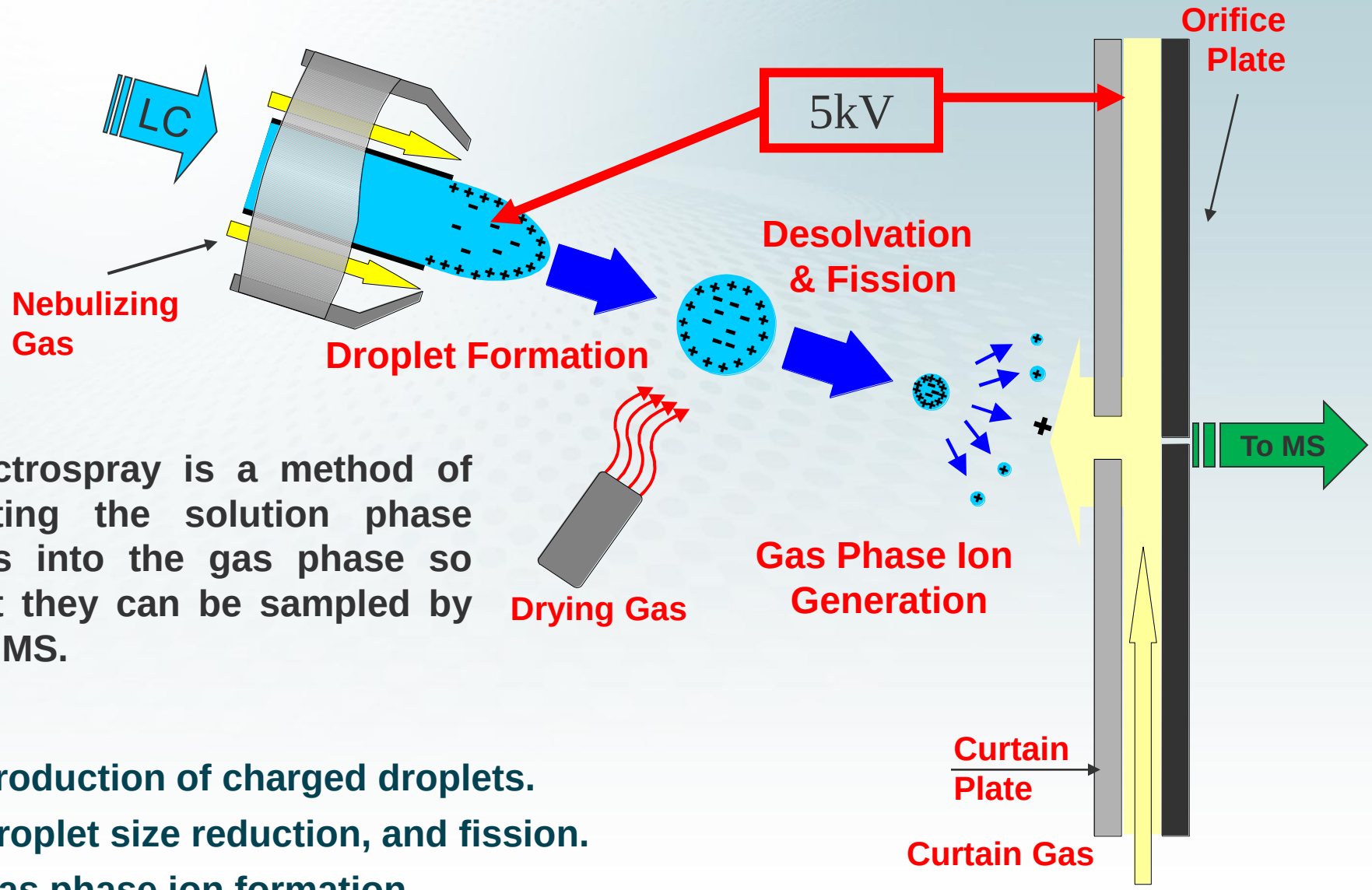
System Configuration



Ionisation Source

- **It depends on the exact application.**
- **Increasing polarity and molecular weight and thermal instability favors electrospray.**
- **Lower polarity and molecular weight favors APCI or APPI**

Electrospray: Overview



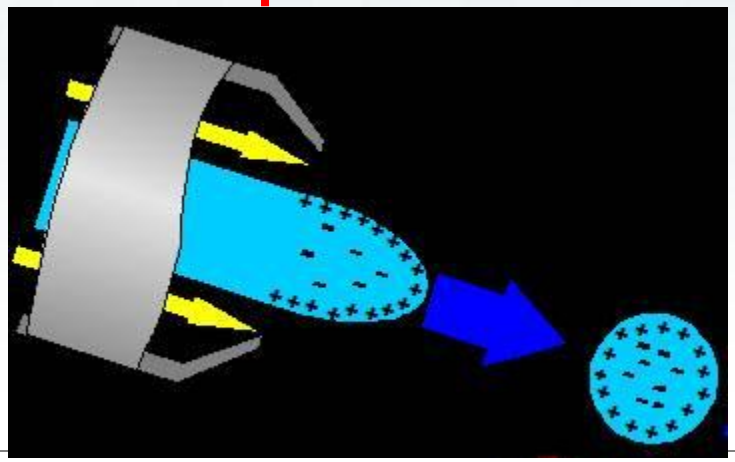
Electrospray is a method of getting the solution phase ions into the gas phase so that they can be sampled by the MS.

1. **Production of charged droplets.**
2. **Droplet size reduction, and fission.**
3. **Gas phase ion formation**

ESI: Production of Charged Droplet

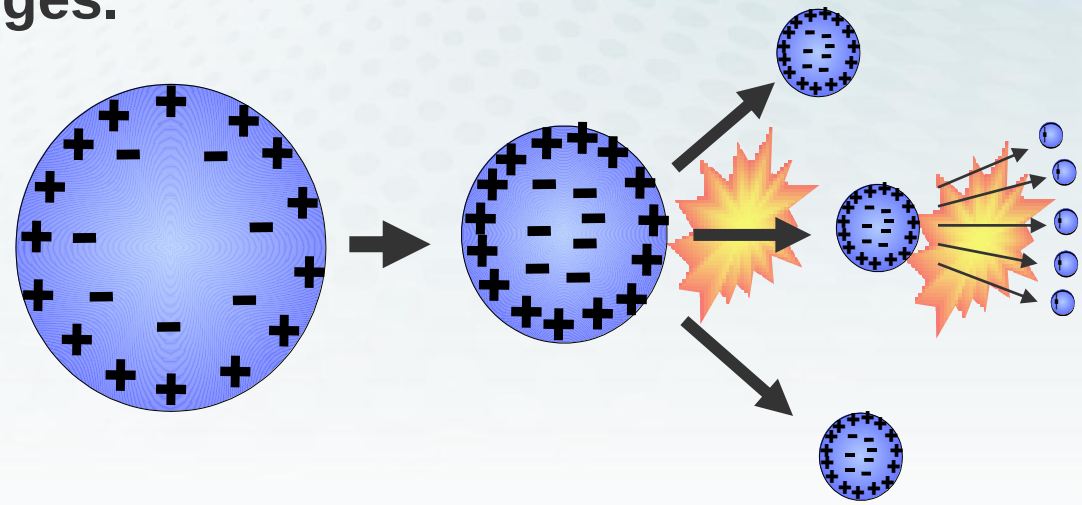
1. A large voltage (up to 6kV) is applied between the end of a capillary carrying the LC mobile phase and the mass spectrometer entrance.
2. Ions (of the same polarity) are drawn out toward the counter electrode (curtain plate) pulling the mobile phase along.
3. When the excess charge at the tip of the capillary overcomes surface tension, a droplet is formed.

Droplet Formation



ESI: Droplet size reduction & fission

- **Droplet size reduction occurs by the continual repetition of two processes:**
  1. **Desolvation (evaporation of neutral solvent and volatile buffers)**
  2. **Droplet fission caused by electric repulsion between like charges.**

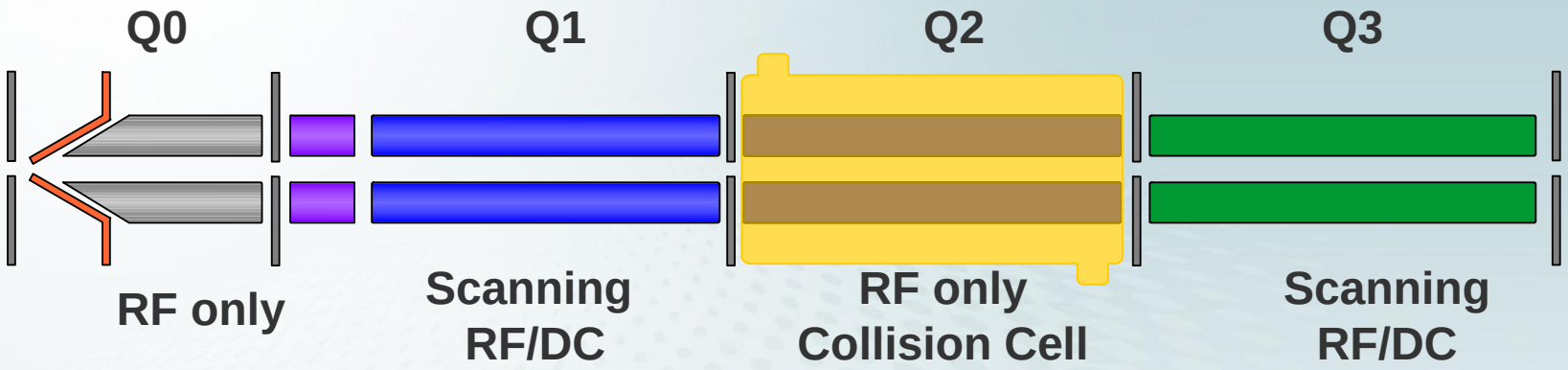


Congratulations!

You have made an ion.

Now what do you do with it?

Triple Quad Configuration

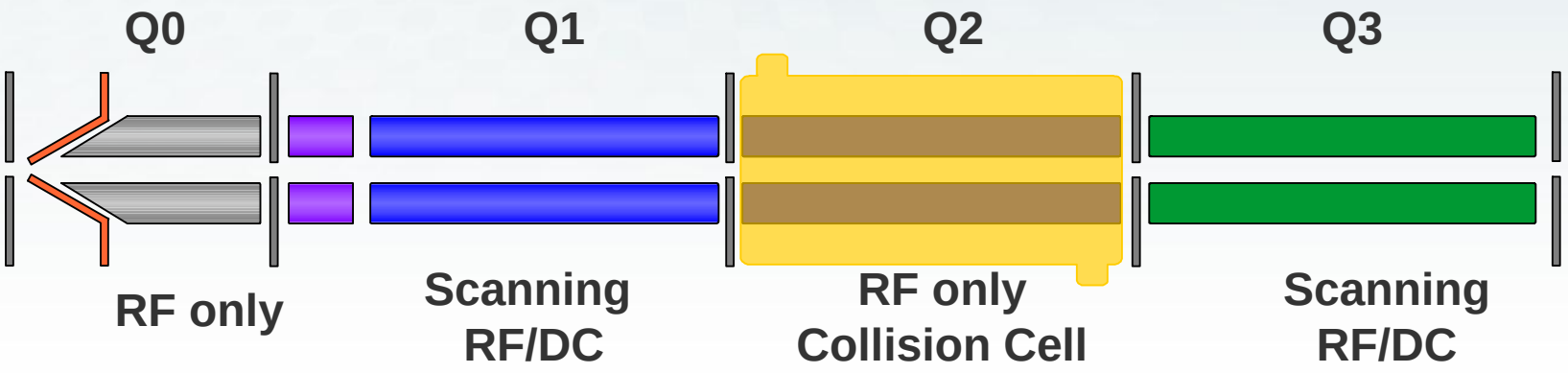


- Q1 and Q3 are standard mass filter quadrupoles.
  - The can scan masses sequentially (e.g. 50 to 500 amu)
  - The can be used to select a single mass.

- Q2 is an RF only quadrupole that is in a gas filled chamber.
  - Q2 is the “collision cell” where mass fragmentation occurs.
  - Q2 does not filter ions. It accepts all ion sent to it by Q1 and passes all ions formed by collision to Q3 to be sorted.

Collision Cell

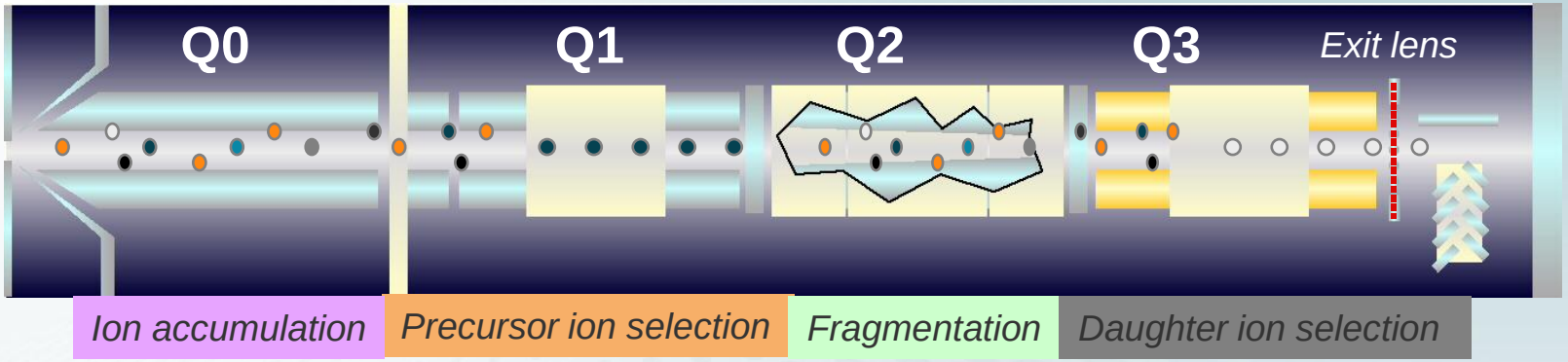
- LINAC (linear accelerator) Collision Cell
  - Filled with N_2 gas at roughly $3x10^{-5}$ torr.
  - Drives ions out, reducing “cross-talk”
- The analyte molecules undergo collision activated disassociation by energetic collision with the N_2 molecules.
- The N_2 also acts to “cool” fragments, facilitating transport to the detector.



Triple Quads…

- In scanning mode 99% ions lost between the rods.
  - Poorer full scan sensitivity
- In SIM mode 100% of selected ion reaches detector.
  - Makes them highly sensitive and great for quantitation!
- Mass resolution typically limited to “unit” (+/- 0.2 amu)
- Fragmentation is controlled by the energy ions have when they enter the collision cell.
  - Higher energy >> greater fragmentation.

Multiple Reaction Monitoring (MRM)



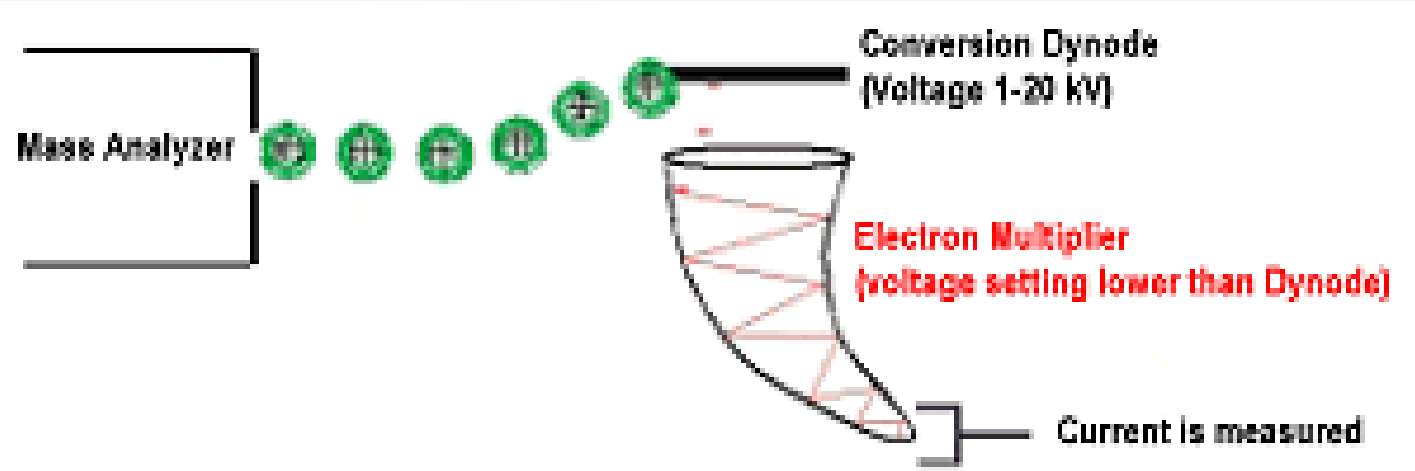
- Q1 Selects an $[M+H]^+$
- Q2 fragments the selected ion.
- Q3 monitors only one daughter ion
- Only the daughter ion reaches the detector.
- Sensitivity of MRM is a function of how much of the daughter ion is produced.
- The parent ion fragmentation to daughter ion is commonly referred to as a “transition”

Detectors

- Electron multipliers,
- Dynolyte photomultiplier,
- Microchannel plates.

Electron multiplier

- A conversion dynode is used to convert either negative or positive ions into electrons. These electrons are amplified by a cascade effect in a horn shape device, to produce a current. This device, also called channeltron, is widely used in quadrupole and ion trap instruments.



MS provides info about

- The elemental composition of samples of matter
- The structures of organic, inorganic and biological molecules
- The qualitative and quantitative composition of complex mixtures
- Isotopic ratios of atoms and samples
- Structure and composition of solid surfaces

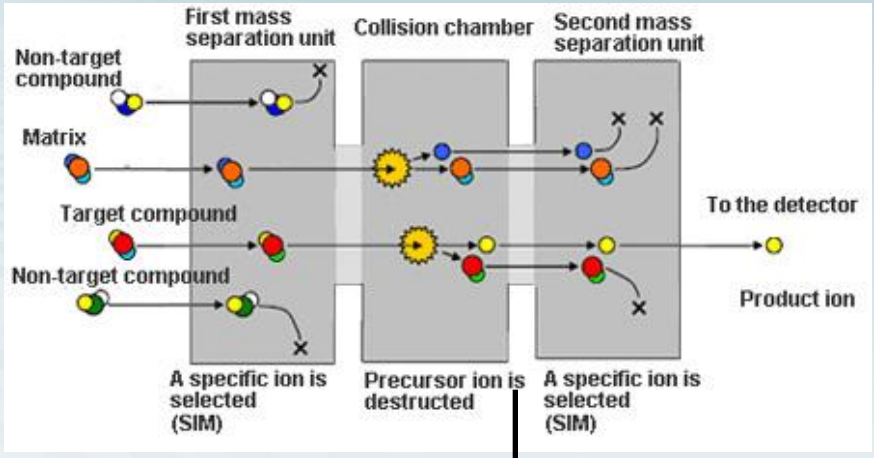
Analysis of the extract

Extracted Metabolites
+ ISTDs

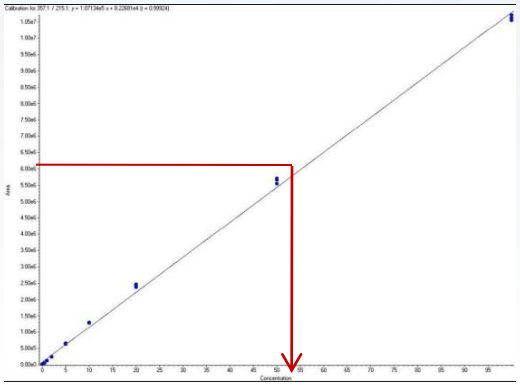
UPLC/3Q

RPLC/HILIC

MRM



Calibration curves

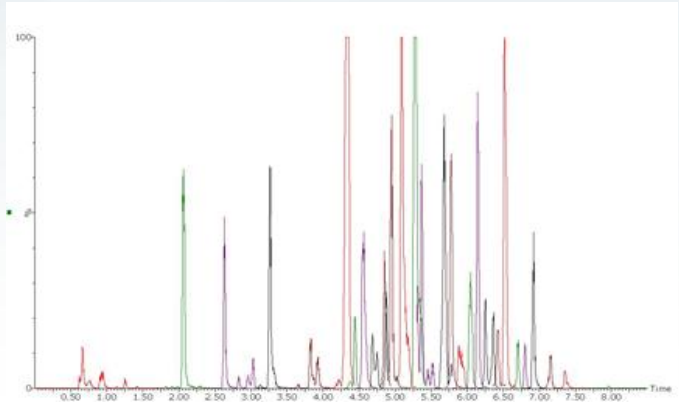


Extracted sample
A_E/A_I

Concentration

Quantification

Daughter ions chromatogram



Retention time