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Fundamentals of Single Quadrupole Mass Spectrometry

Waters Corporation

Noud van der Borg

Introduction

- In this presentation we will review the fundamentals of mass spectrometry for current liquid chromatography users

What Is Single Quadrupole Mass Spectrometry and How Is It Used?

Short explanation on other MS techniques and MS-sources

Overview

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- What is mass spectrometry?
  - Who uses it and why?
- How do single quadrupoles work?
- What type of data is collected? What impacts the observed mass-to-charge ratio?

Who Uses MS and Why?

- What industries use MS?
  - Pharmaceutical
    - Reaction Monitoring, Purification, Characterization
    - Drug Metabolism & Pharmacokinetics, Degradation and Bioavailability
  - Food and Environmental
  - Industrial
  - Health Science

- Why?
  - QC
    - Confirmation of analytes
    - Check raw ingredients
  - Synthesis Labs or Reaction Monitoring
    - Confirmation of products
  - Method Development
    - Basic peak tracking
    - Peak purity/co-elutions

Advantages of Adding Mass Spectrometry

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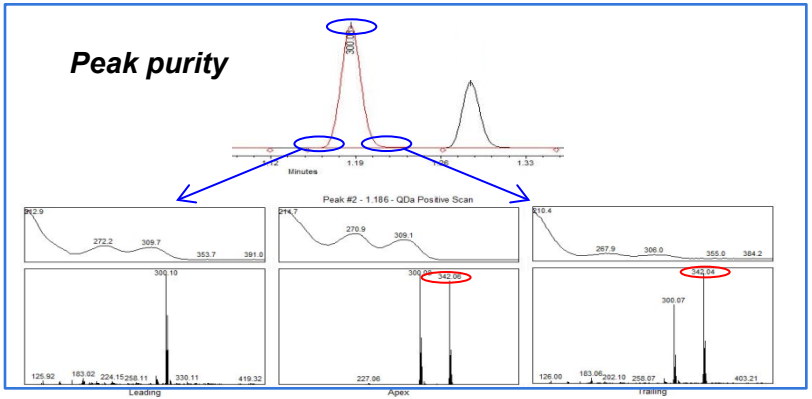

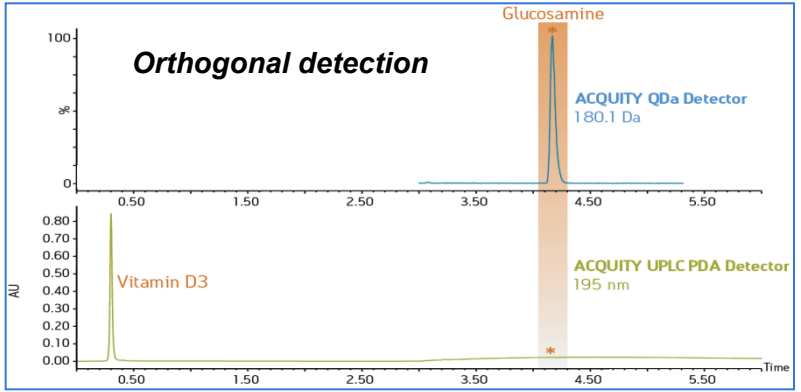

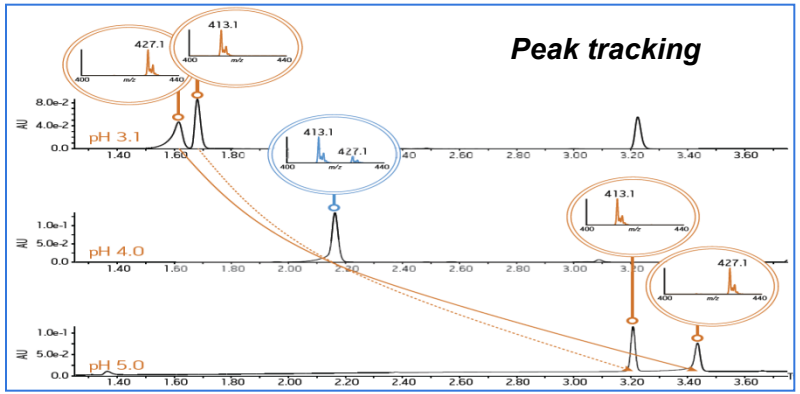

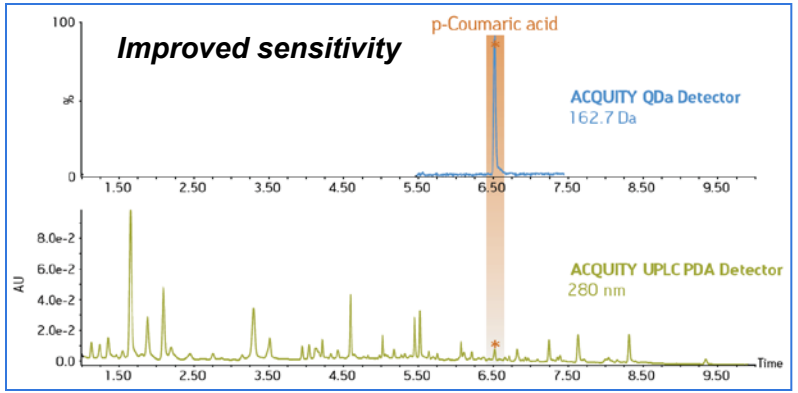

What is Mass Spectrometry?

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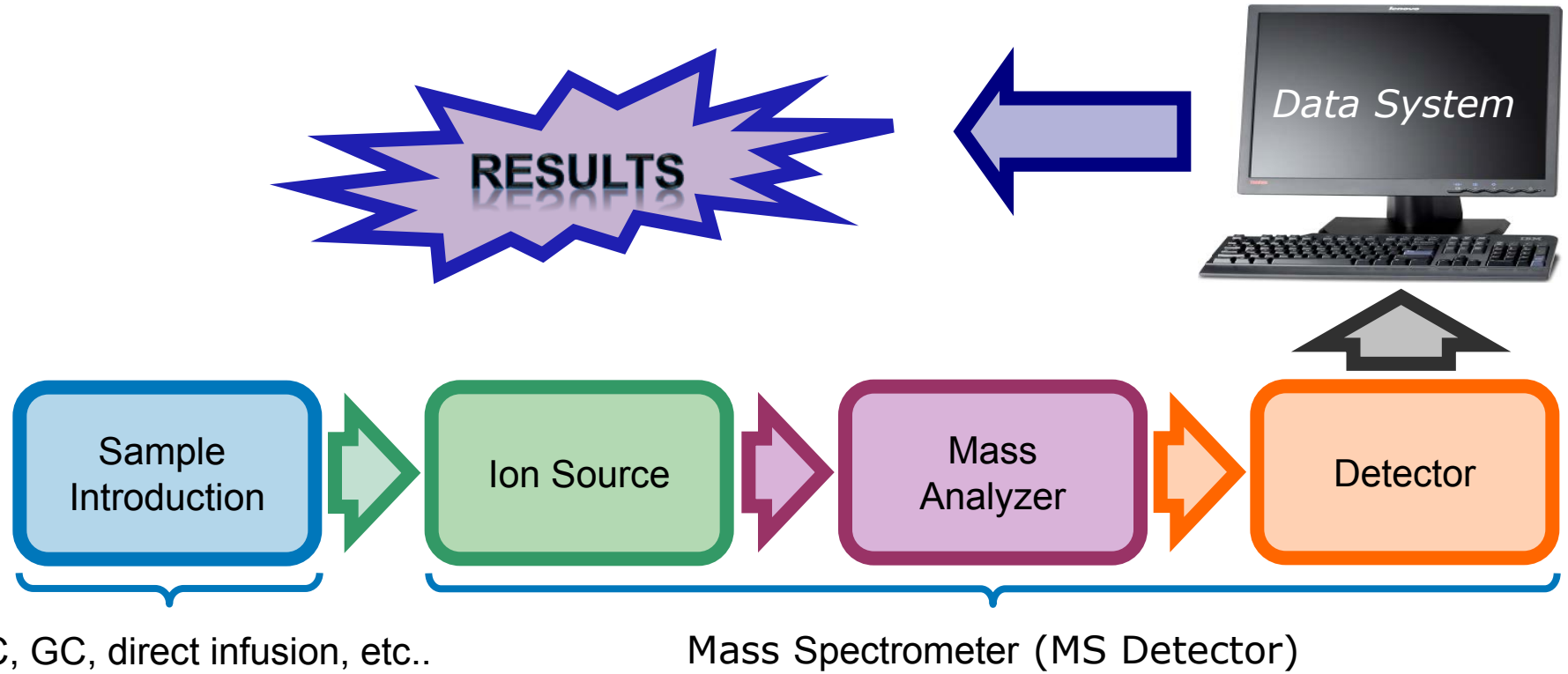
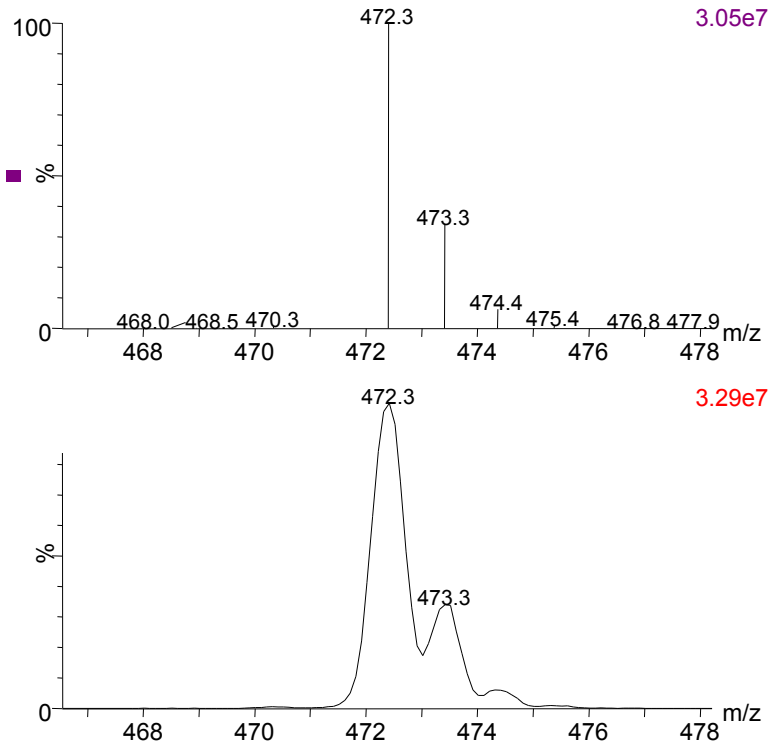


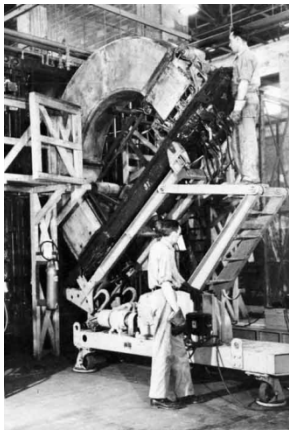
History of MS

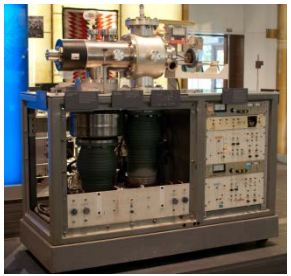


Started in 1886
First only GC-MS systems, ionization of liquid spray was difficult

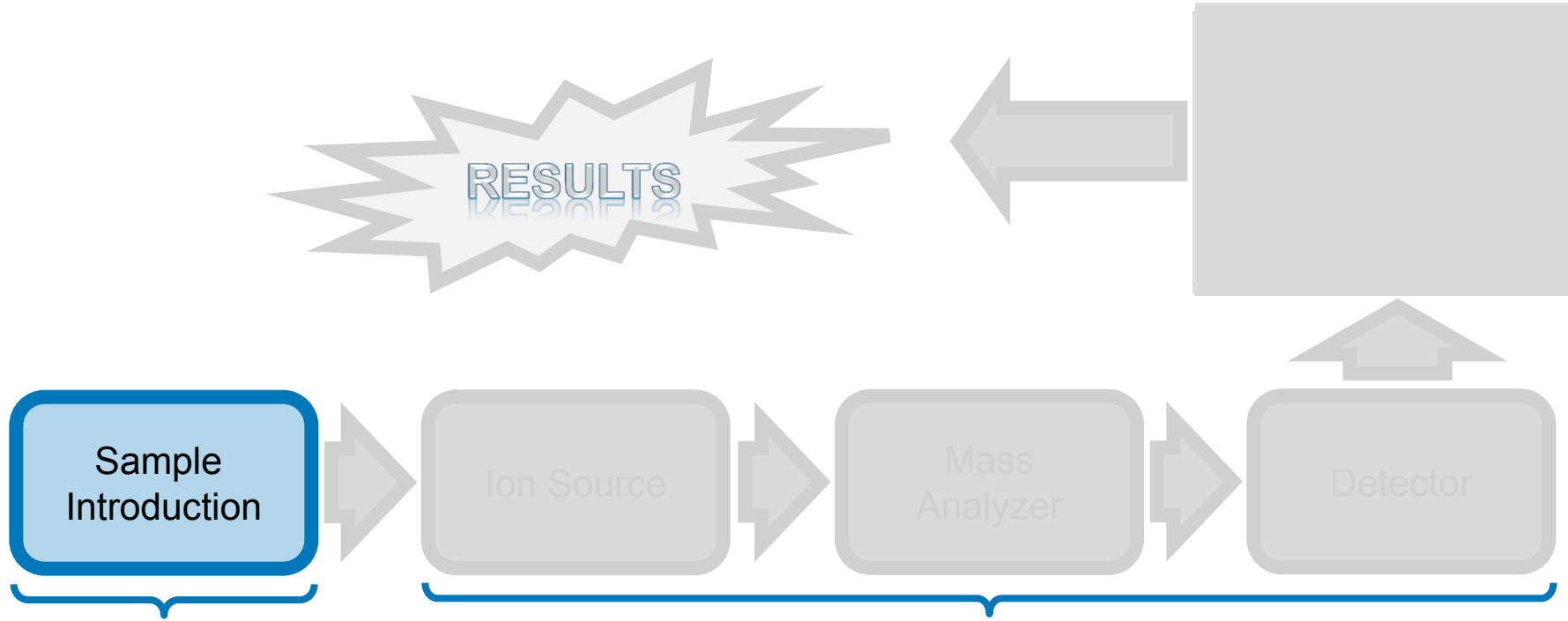
Computers changed MS
TOF was invented before 1940 but start to be useful after 1990 (use of PC's)

PC's could not handle continuum and centroid was developed, now easier to read



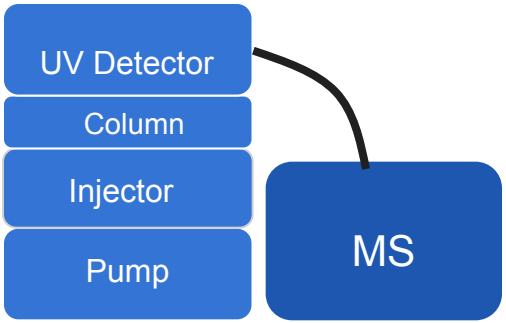


Sample Introduction

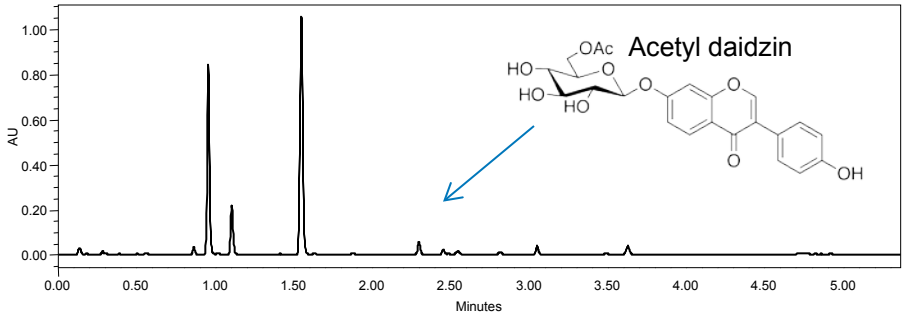


Sample Introduction

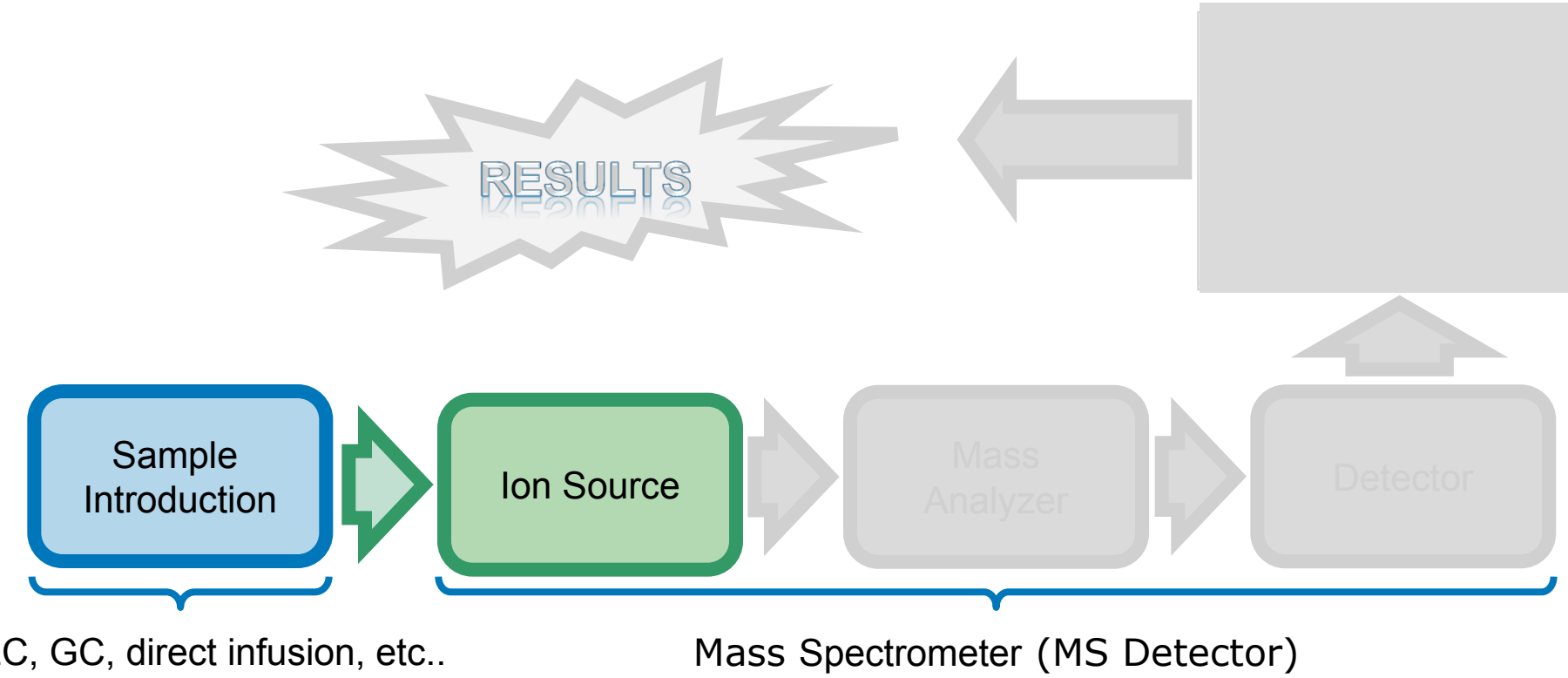
- To analyze a compound by MS detection the sample must be introduced into the system
- For the examples used in this presentation, the MS is coupled to an LC system
- Regardless of the mode of sample introduction, the same data is produced
  - The sample shown contains isoflavones, such as acetyl daidzin, in a dietary supplement



Isoflavones in soy extract

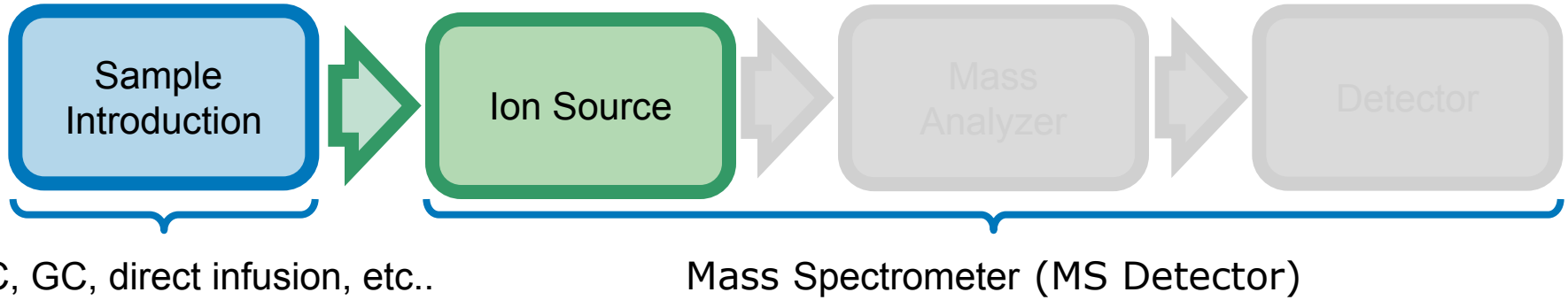


Ion Source

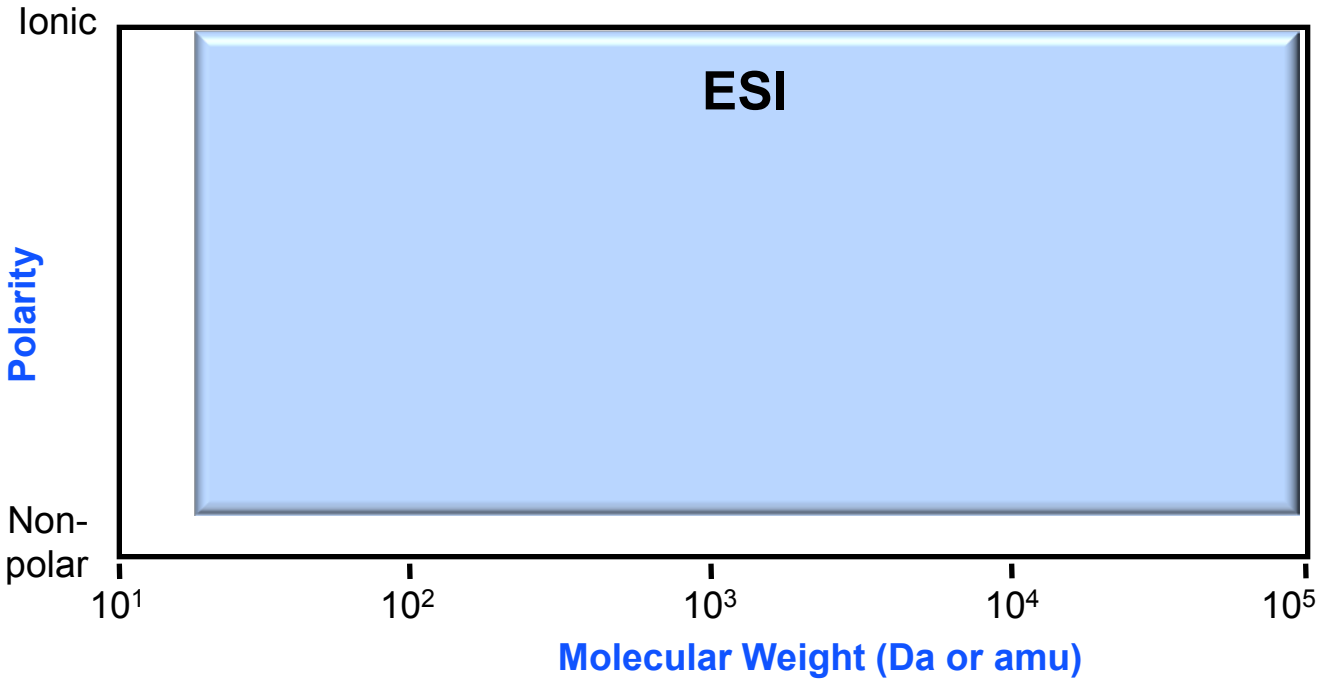


Ion Source

- Some common ionization techniques
  - Electrospray ionization (ESI)
  - Electron ionization (GC,EI)
  - Atmospheric pressure chemical ionization (APCI)
  - Desorption/ionization on silicon (DIOS)
  - Direct analysis in real time (DART)



Electrospray Ionization: Sample Properties



- Electrospray can ionize compounds up to 200,000 – 500,000 Da
- Better for polar or charged compounds (as compared to non-polar analytes)
- Other techniques may have a more limited range

Electrospray Ionization vs. Other Ionization Techniques: Sample Properties

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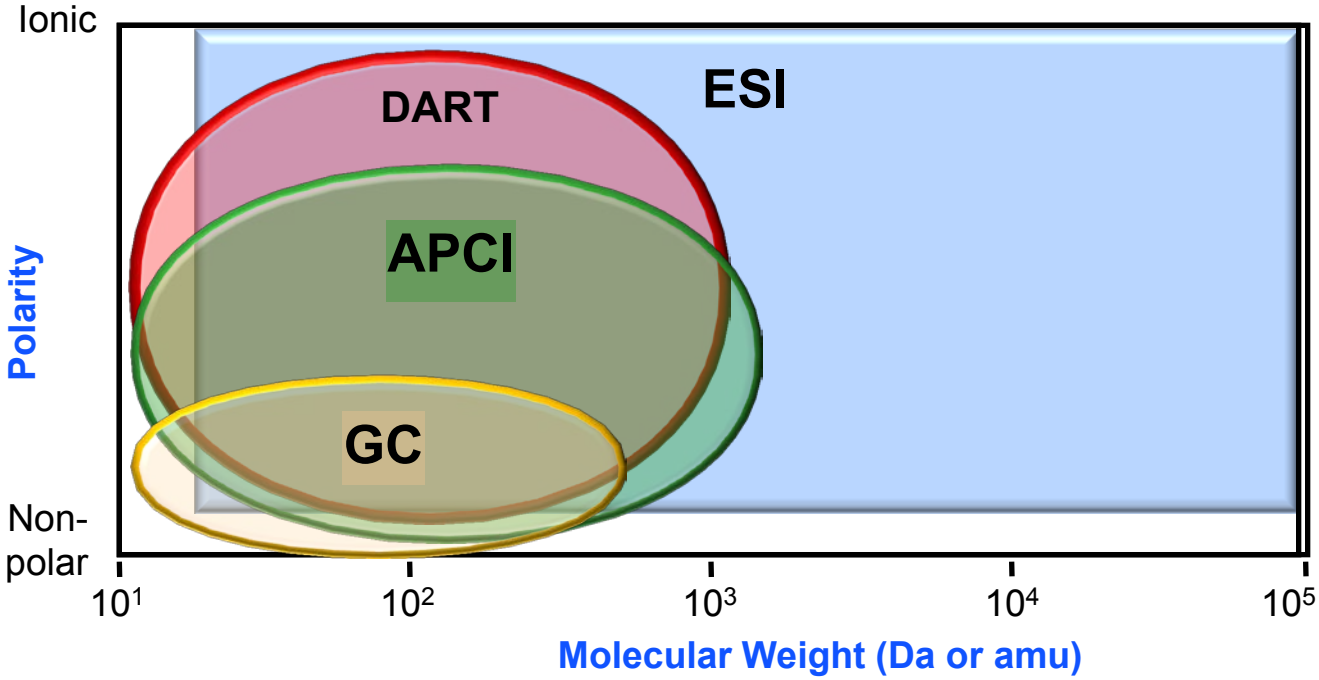
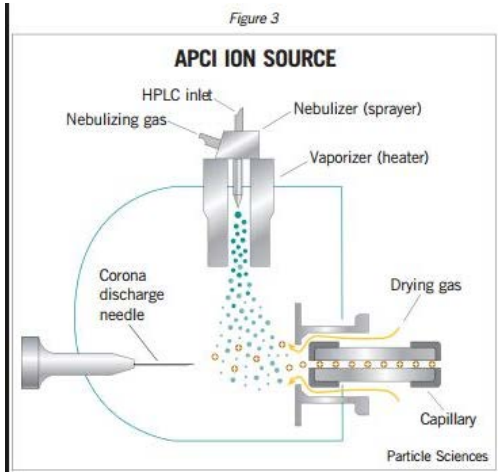
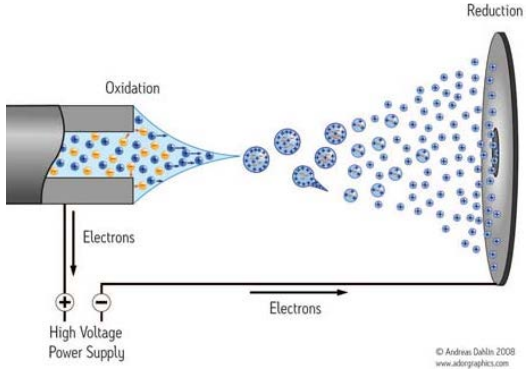


- GC and APCI ionization techniques are optimum for molecules at lower MW range and less polar molecules
- DART ionization technique is applicable for lower MW analytes

Different MS sources





ESI is mostly used

Multimode source
ESI – Electrospray Ionization
APCI – Atmospheric Pressure Chemical Ionization
ESCi® – Dual ESI and APCI

Dual mode source
APPI – Atmospheric Pressure Photo Ionization
APCI – Atmospheric Pressure Chemical Ionization

IonKey Source
with nanoTile Technology.
Plug & Play nanoFlow

nanoFlow™ ESI

Electrospray Ionization
Mechanisms of Ion Formation

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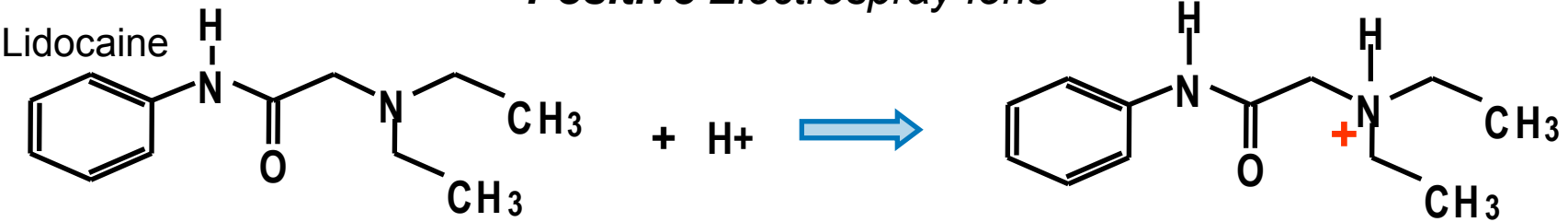
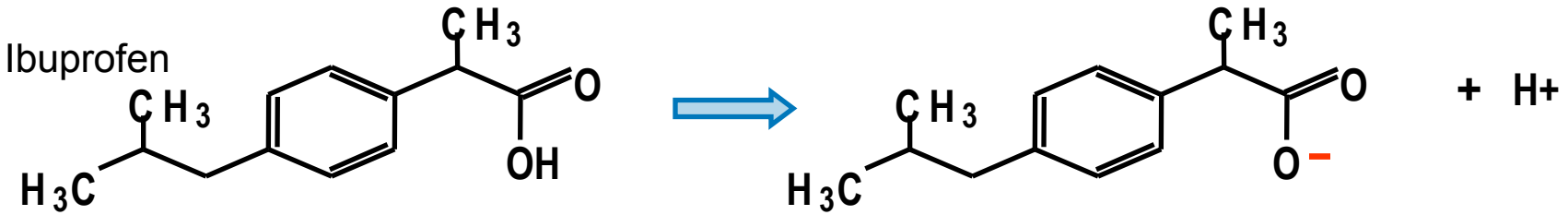
***Positive** Electrospray Ions*

Lidocaine

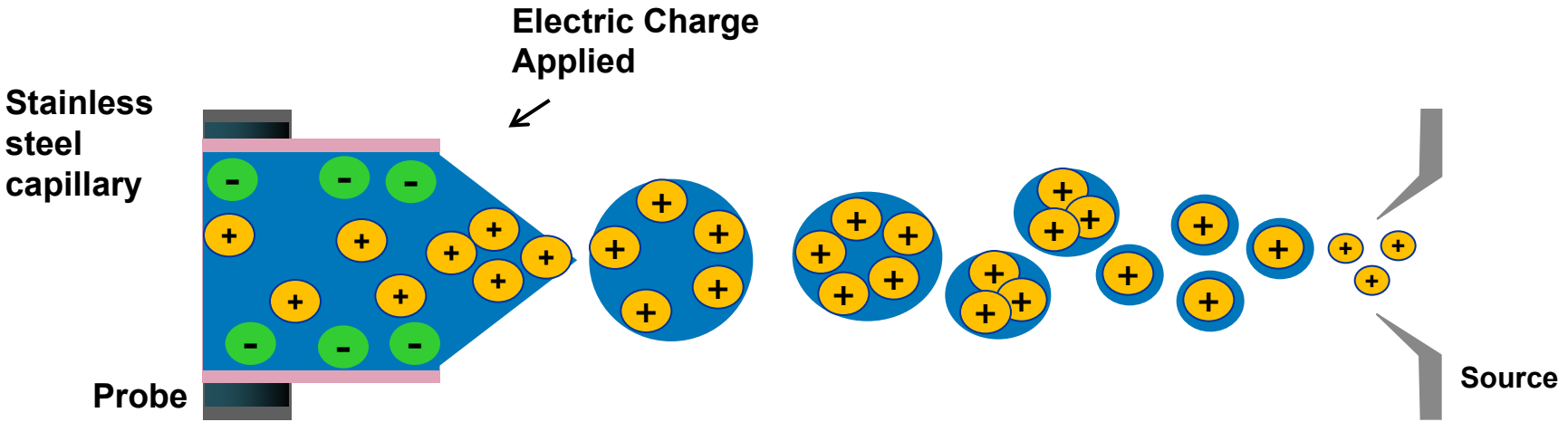


***Negative** Electrospray Ions*

Ibuprofen

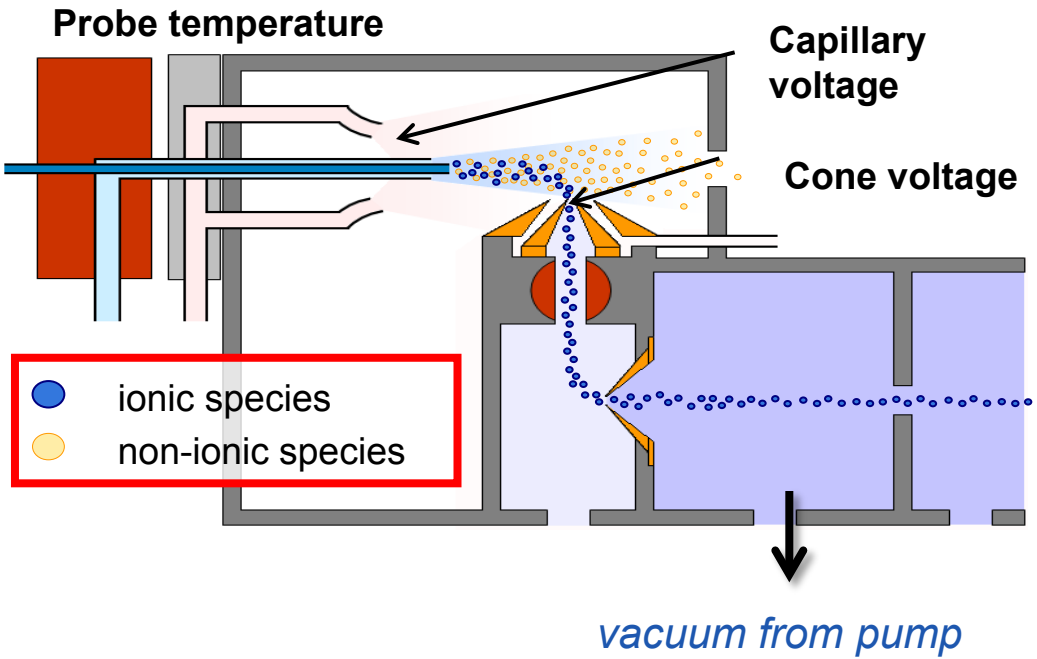


Forming Gas-Phase Ions



- Ionization takes place at atmospheric pressure and has three stages:
  - Formation of charged droplets
  - Solvent evaporation and droplet fission
  - Formation of gas-phase ions
    - Generally produces $(M+H)^+$ or $(M-H)^-$ ions

Introducing Sample into Mass Analyzer



- **Probe temperature**: High temperature applied to the probe
- **Capillary voltage:** Potential used in the capillary of the ESI probe
- **Cone voltage**: Potential applied to the cone located at the entrance of the source
- After ionization, the ions are directed to mass analyzer by vacuum from a pump

Spray, ionization and use of salts …..

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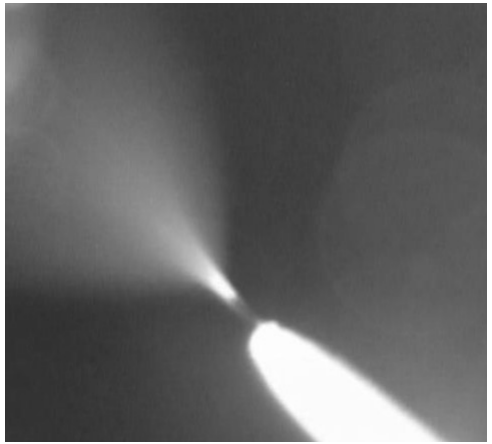
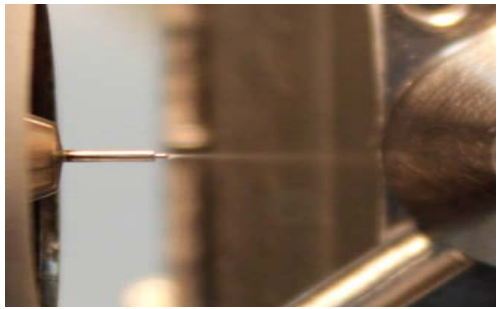
To get a good ionization a spray with small partials is needed.
Creating this, salts will fall out and crystallize





Single Quadrupole Mass Analyzer and Detector

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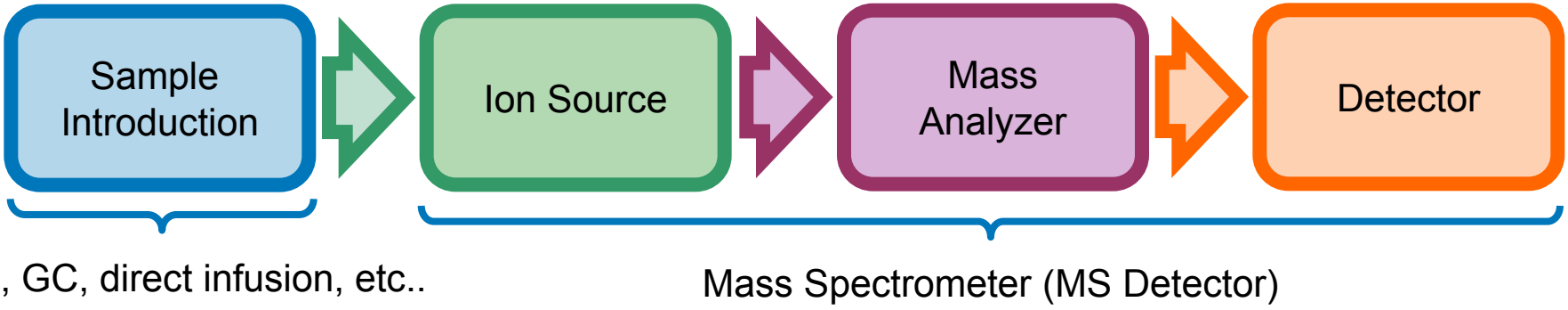
- To detect ions, the ions must be filter or separated
- This occurs in the mass analyzer which includes a pre-filter and the quadrupole
- The measurement of the ions occurs in the detector



Different mass selection options

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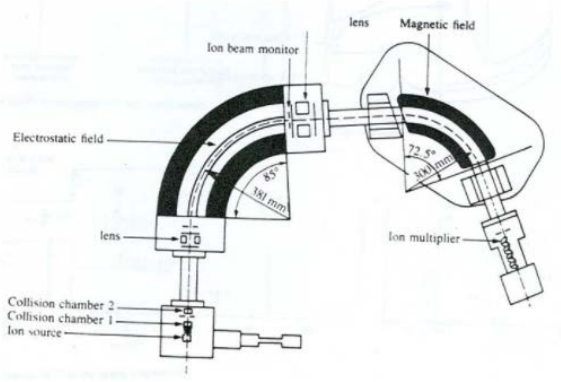
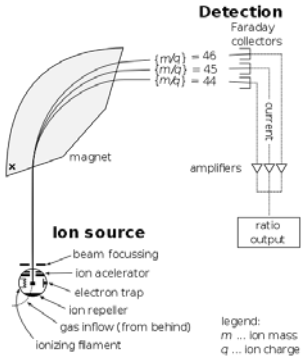
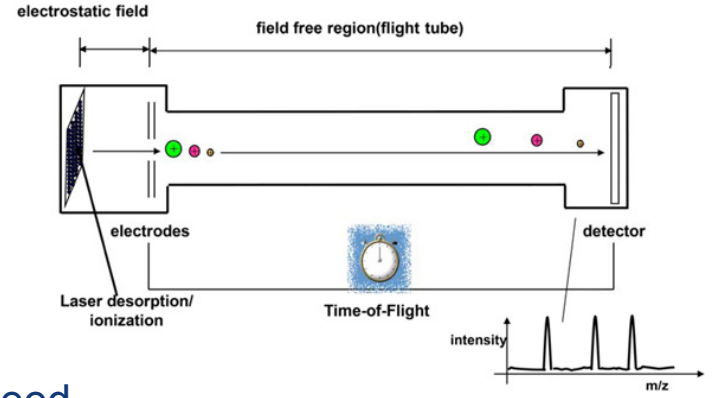
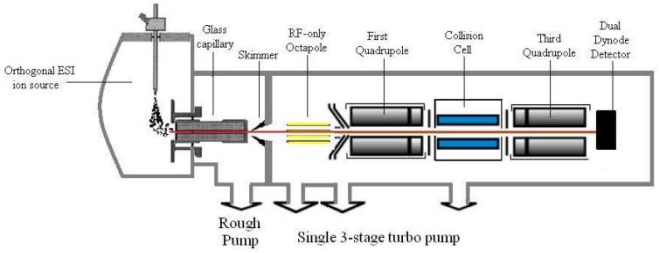


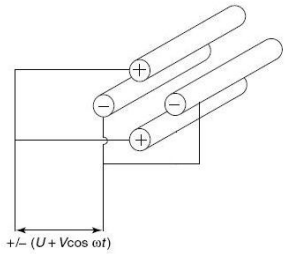


Curve

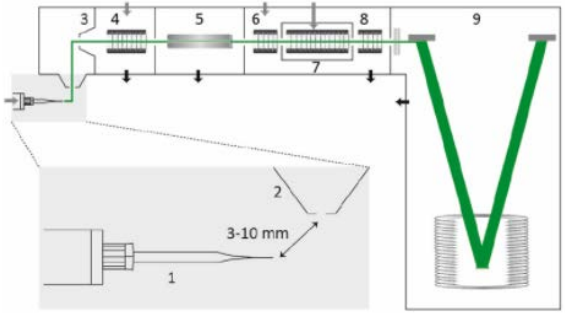


Speed



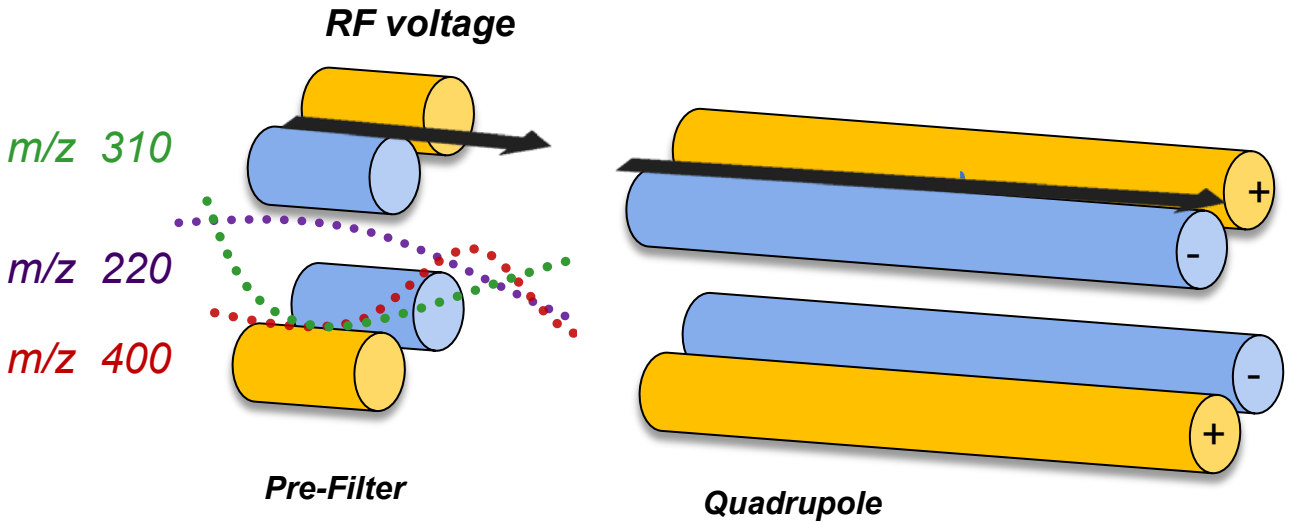


Slalom



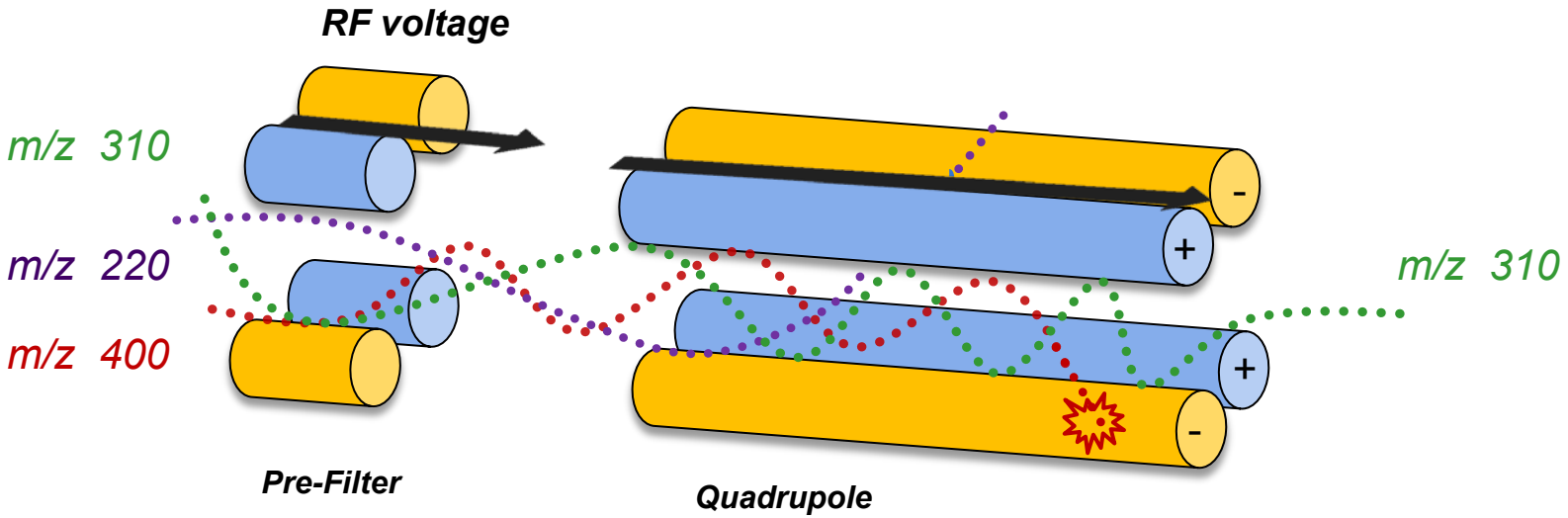
m/z explained ☺

Separation or Filtering of Ions in the Quadrupole



- After the sample is ionized, the sample enters the ion guides and quadrupole
- A combination of RF and DC voltages are applied to the quadrupole to create a fluctuating field
- Opposite rods have same charge applied
- The alternating field guides ions based on *mass-to-charge (m/z) ratio*

Separation or Filtering of Ions in the Quadrupole



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Single Quadrupole Mass Range: Sample Properties

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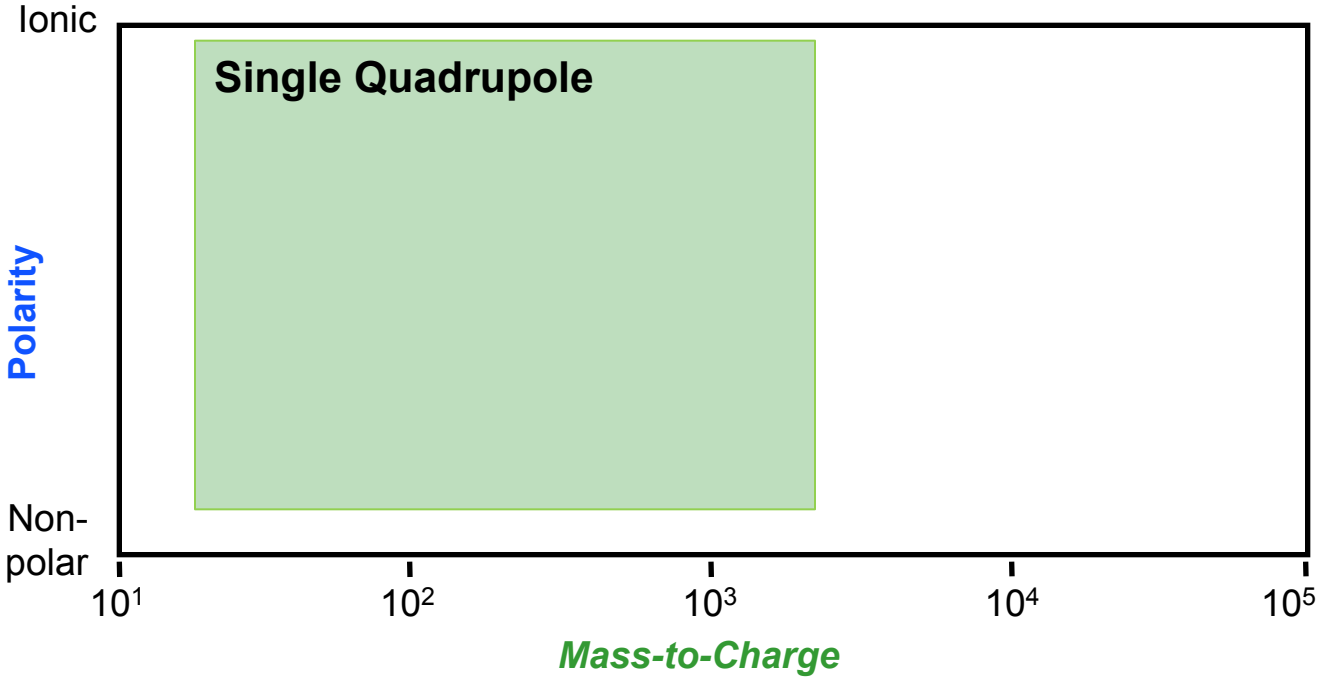

- Single quadrupoles typically have a maximum range of up to 2,000 - 3,000 m/z
- Higher molecular weights analytes with charged states greater than 2 might be observed on a single quadrupole, provided the charged state is less than 2,000 - 3,000 m/z

Single Quadrupole Mass Range: Sample Properties

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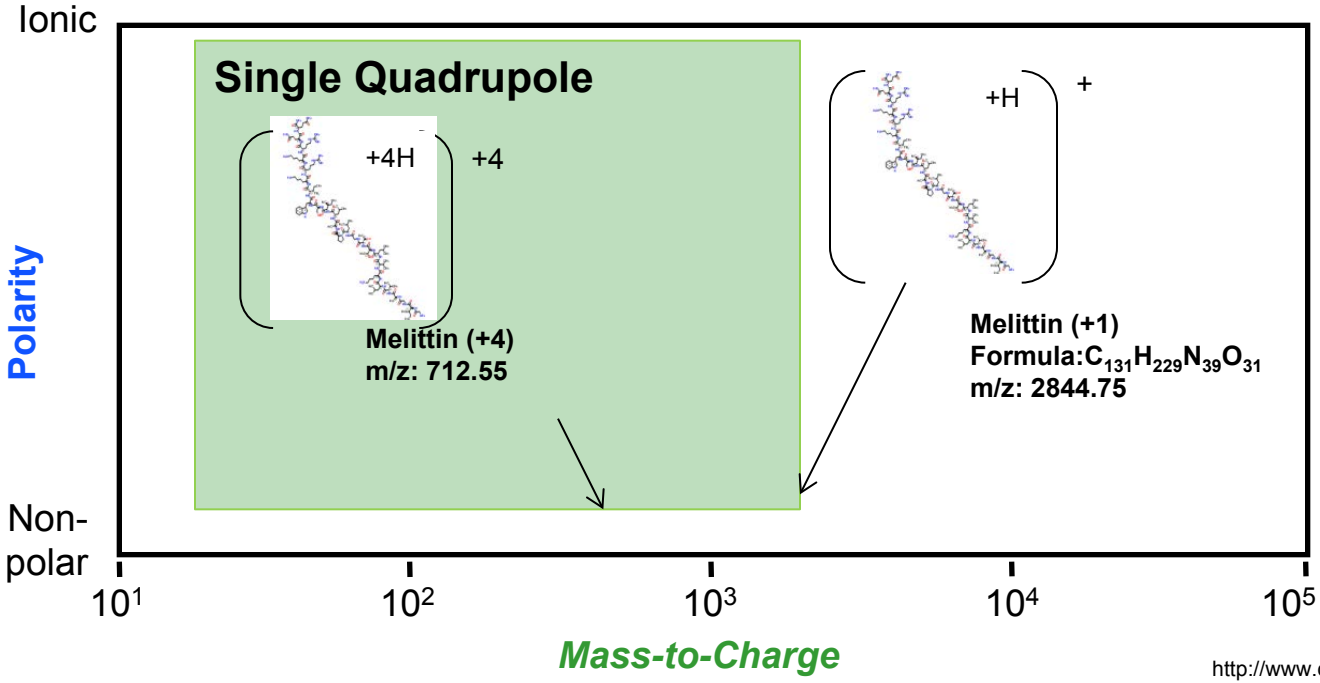

http://www.chemspider.com/Chemical-Structure.17290230.html

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Acquiring Single Quadrupole Mass Spectra

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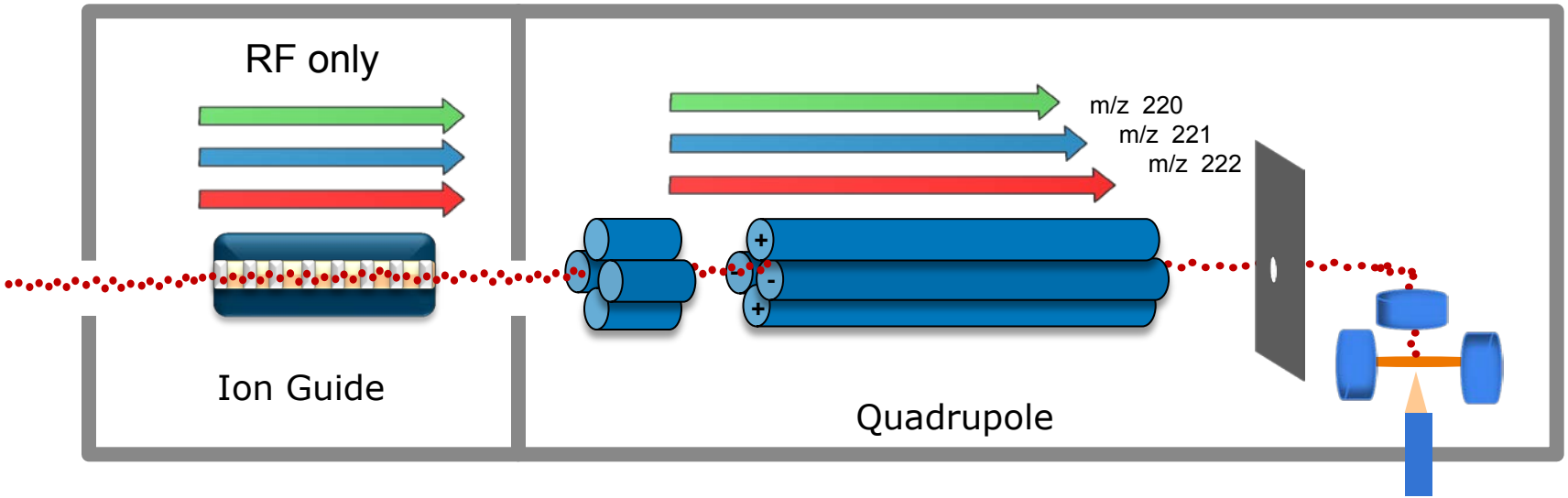
- Mass data can be collected in two modes:
  - Full scan mode
  - Static mode

- Full scan is analogous to photo diode array (PDA) spectra
  - Mass range unit is mass-to-charge (m/z) (e.g. 50-2000)
  - The data can be viewed in a **Total Ion Chromatogram (TIC)**
    - Individual mass-to-charge (m/z) spectra can be extracted from the TIC

- Static mode is analogous to a single 2D UV channel
  - Static mode produces a **Single Ion Recording (SIR) or Single Ion Mode (SIM)**
  - A single mass-to-charge (m/z) channel is selected

Scanning Mode



- In full scan, the RF and DC voltages are scanning transmitting ions in sequence
- The quadrupole can scan an ion in milliseconds
- More ions are transmitted in scan mode than static but the sensitivity will be lower
- Ideal for screening or scouting

Total Ion Chromatogram (TIC): Comparison to Data in UV Detection

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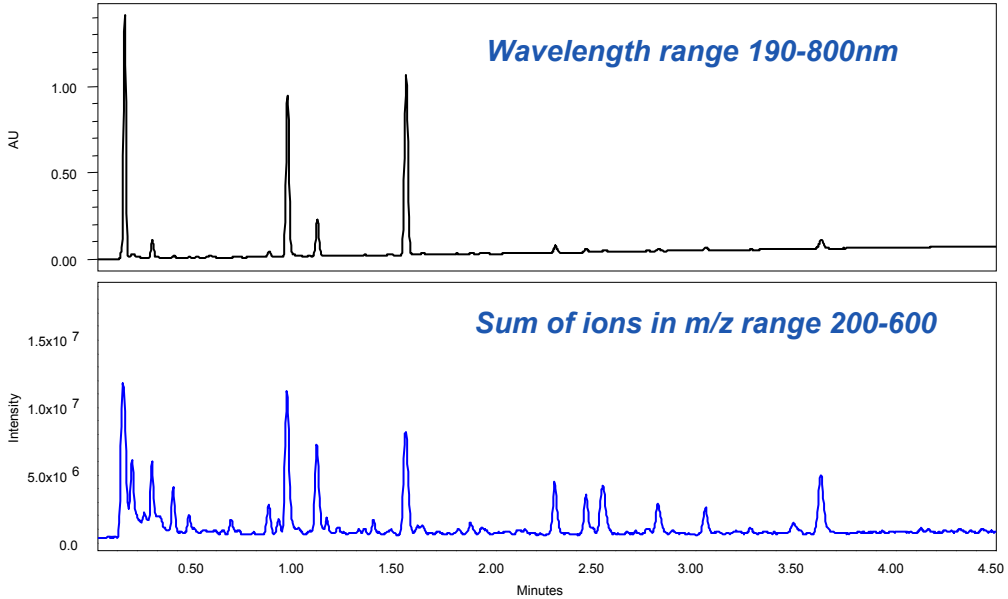
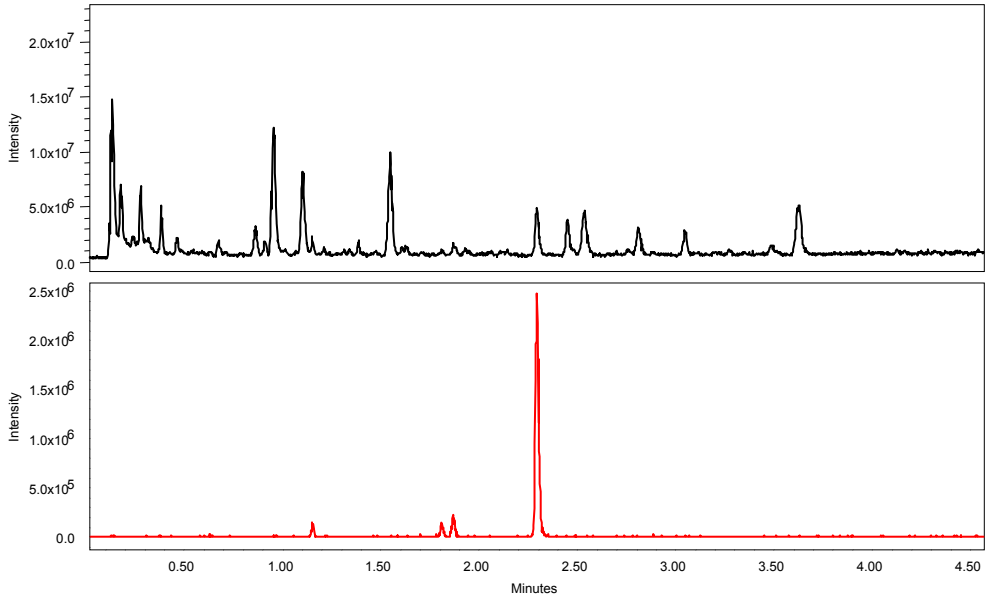
UV 190-800 nm

ESI+ TIC



- Relative response of analytes varies in each technique
- Y-scale equals Counts in MS vs. AU in UV
- Sample: Isoflavones in dietary supplement. Data was collected in ESI+

Extracted Ion Chromatogram (XIC)



- Extracting a specific ion from the chromatogram indicates whether the m/z is present
- In this case, the m/z corresponding to 459.0 is present in multiple peaks
- Sample: Isoflavones in dietary supplement

Comparison of Extracting Single Channel in MS and PhotoDiode Array Detector (PDA)

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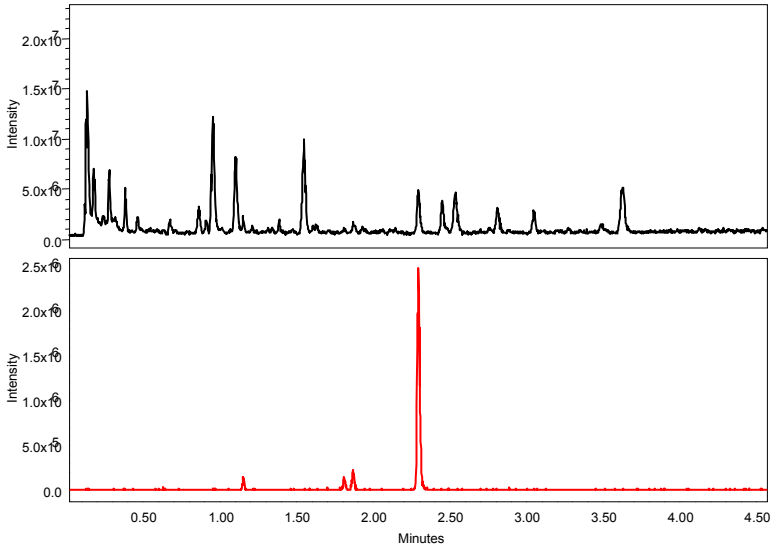
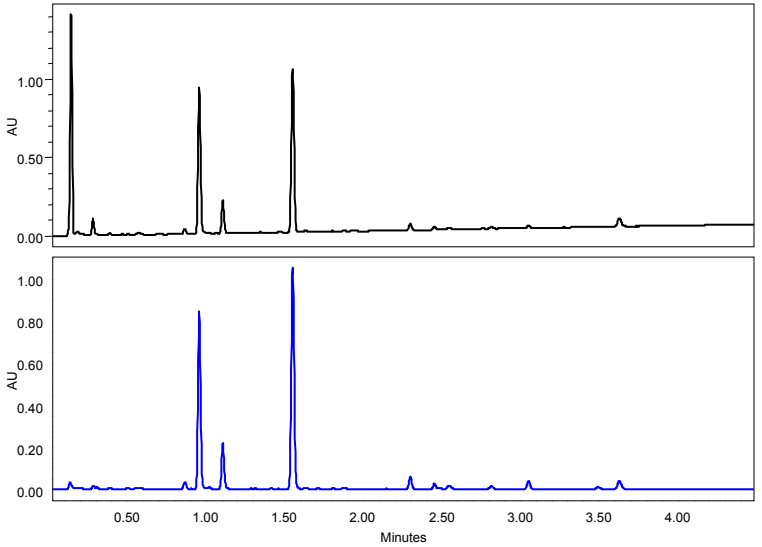
MS

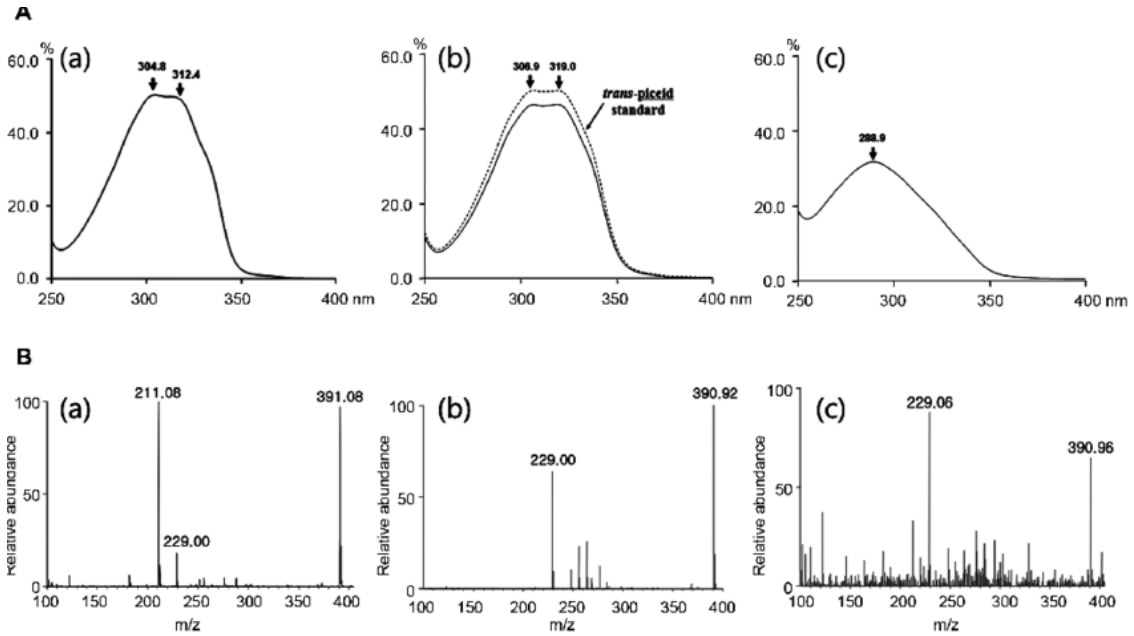


PDA



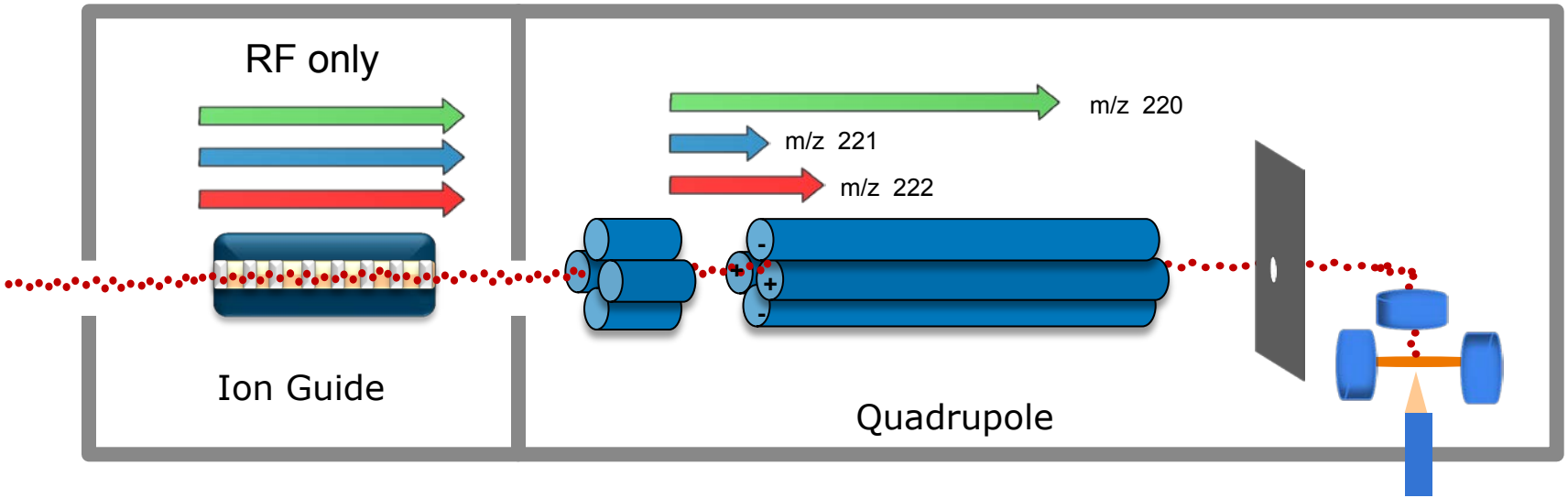
- Exacting a single mass-to-charge channel from a MS is analogous to extracting a single wavelength from a photo diode array detector
- Each may show different responses than the full TIC or full scan in PDA
- Full scan in PDA or Max Plot is maximum spectral absorbance measured at each time point

Difference UV and MS spectra



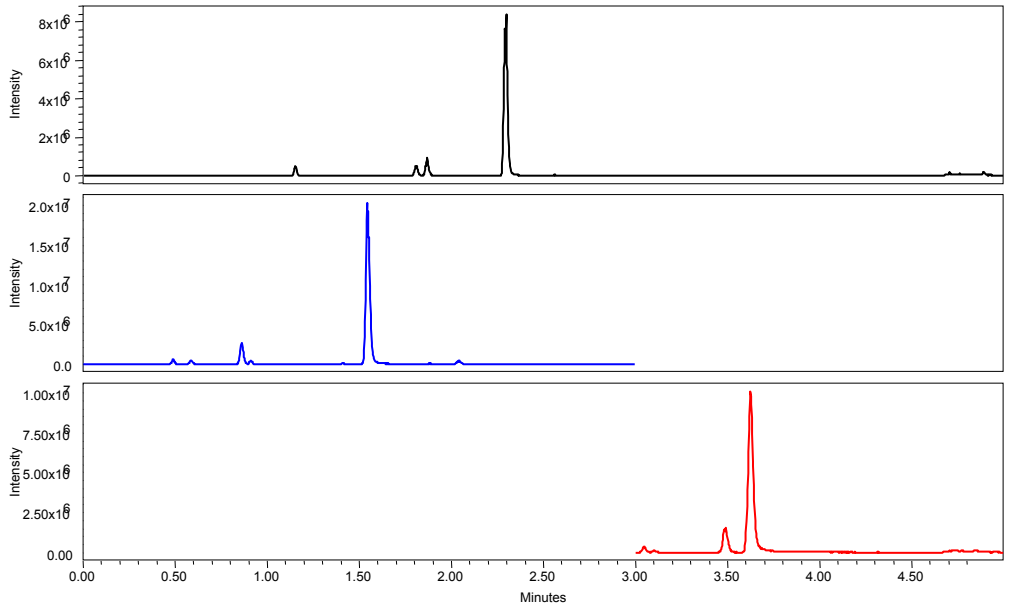
If these 3 peaks would not be chromatographically separated you could not do any quantification on the Spectral difference, With MS you could measure a) easily and b) and c) most likely. The more MS resolution the better change you can detect or measure the compounds.

Static Mode:
Single Ion Recording or Single Ion Mode



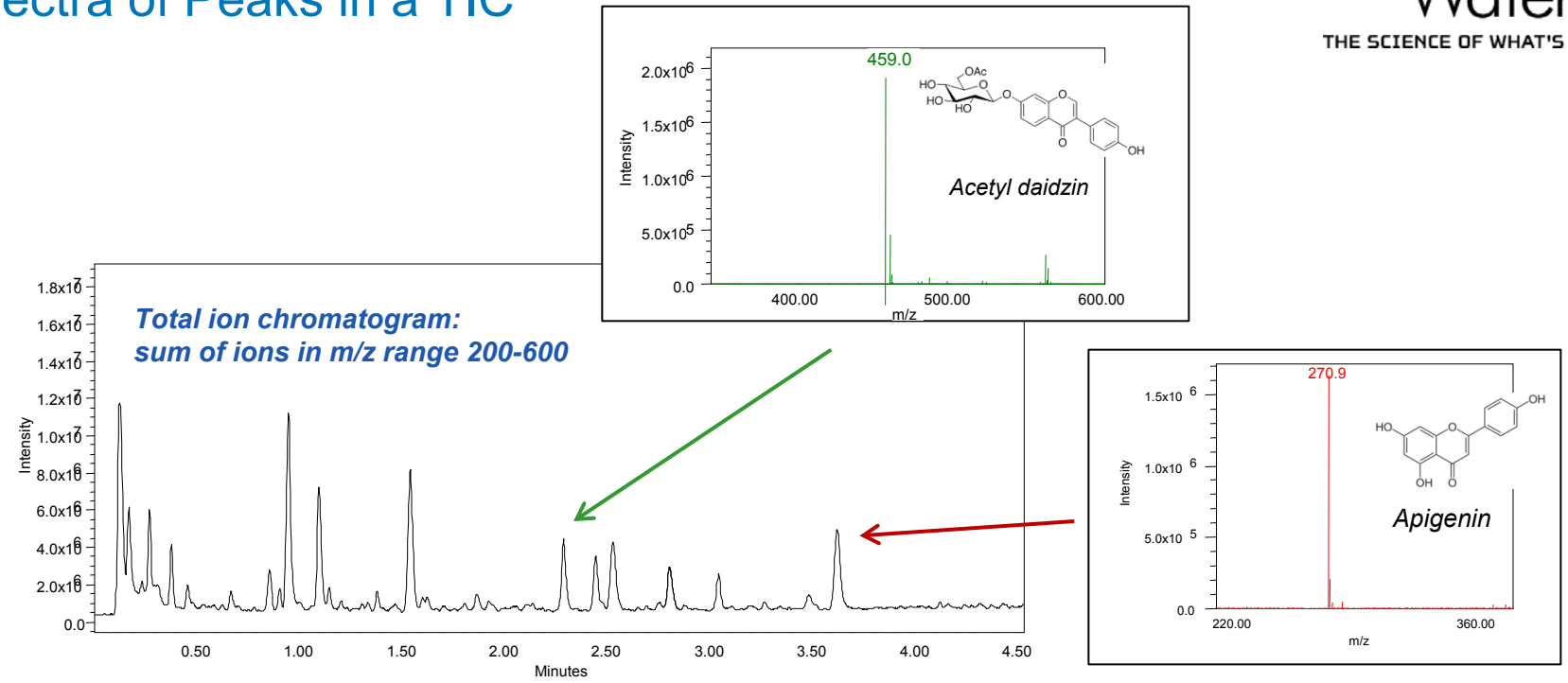
- In this example, the quadrupole settings are fixed so only a single mass-to-charge (*m/z*) ratio is transmitted
- Highest sensitivity because entire dwell time is spent on single *m/z*
- *Most common for quantitative analysis*

Single Ion Recording (SIR) Channels



- In static mode a single ion recording channel is collected
- Each single ion recording channel represents a specific mass-to-charge ratio
- Multiple single channels can be collected in a single run
- Single channels can be collected over the entire analysis or for a specific amount of time

Spectra of Peaks in a TIC



- Each peak in the TIC has a spectrum associated with it
- The spectrum includes the observed mass-to-charge ratios present for that peak

What Impacts Results?

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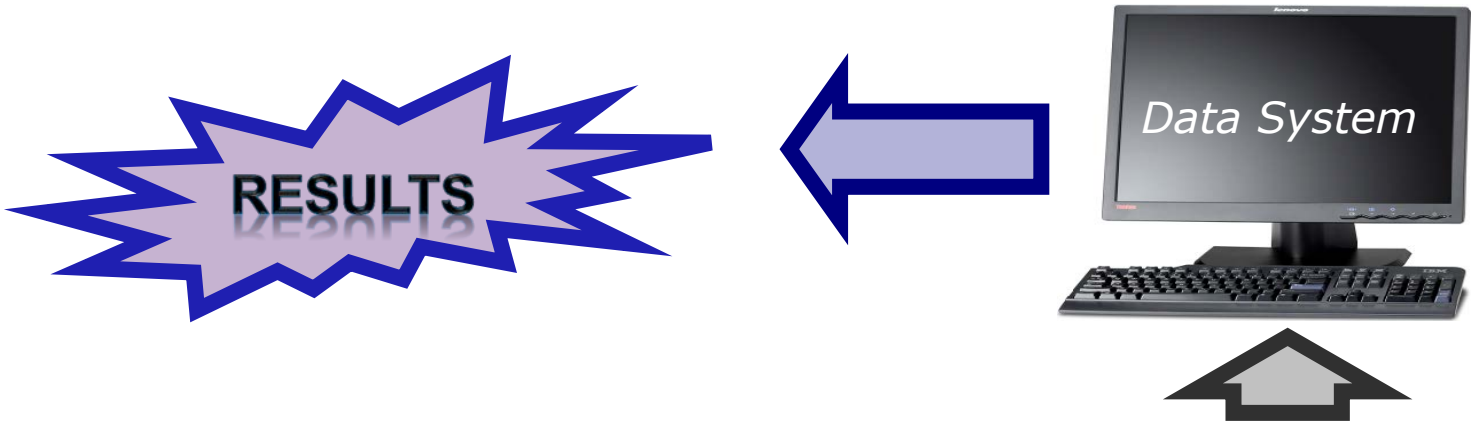

Sample Introduction → Ion Source → Mass Analyzer → Detector

LC, GC, direct infusion, etc.

Mass Spectrometer (MS Detector)

Ionized Molecules and Adducts

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Positively charged adducts

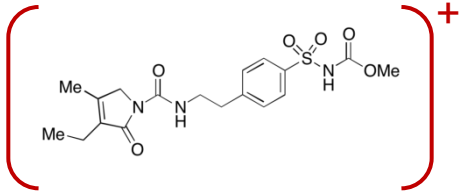
Adduct Ion Formed	m/z of ion
M+H	M + 1.00
$M+NH_4$	M + 18.03
M+Na	M + 22.99
$M+CH_3OH+H$	M + 33.03
M+K	M + 38.96
M+ACN+H	M + 42.03
M+2Na-H	M + 44.97
M+IsoProp+H	M + 61.06
M+ACN+Na	M + 64.02

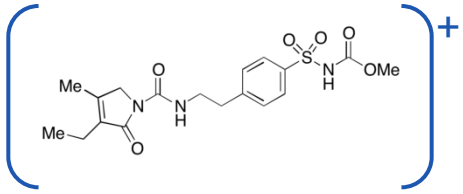
Negatively charged adducts

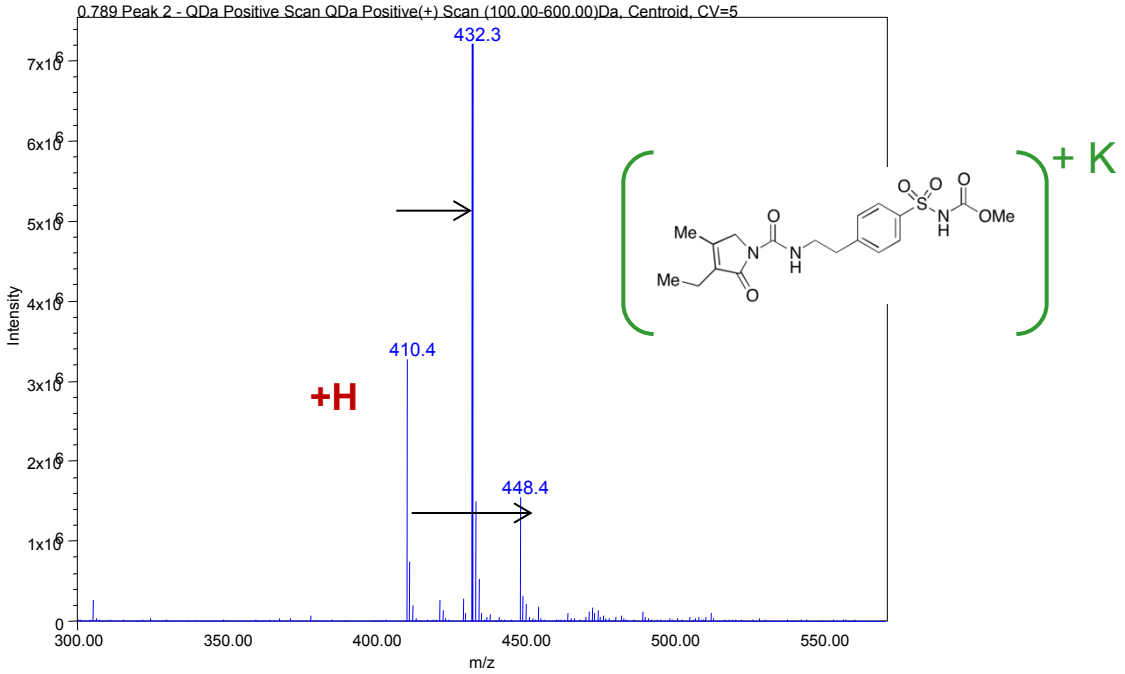
Adduct Ion Formed	m/z of ion
M-H	M - 1.00
M+Na-2H	M + 20.97
M+Cl	M + 34.97
M+K-2H	M + 36.95
M+FA-H	M + 45.00
M+Br	M + 78.92
M+TFA-H	M + 112.99

- REF http://fiehnlab.ucdavis.edu/staff/kind/Metabolomics/MS-Adduct-Calculator

Adducts Observed in MS







- Some compounds are more likely to form adducts other than H+
- Common adducts observed in positive mode include Na and K adducts

Glimepiride related compound C
Formula: $C_{18}H_{23}N_3O_6S$
MW:409.46

Multiple Charging in Electrospray Ionization

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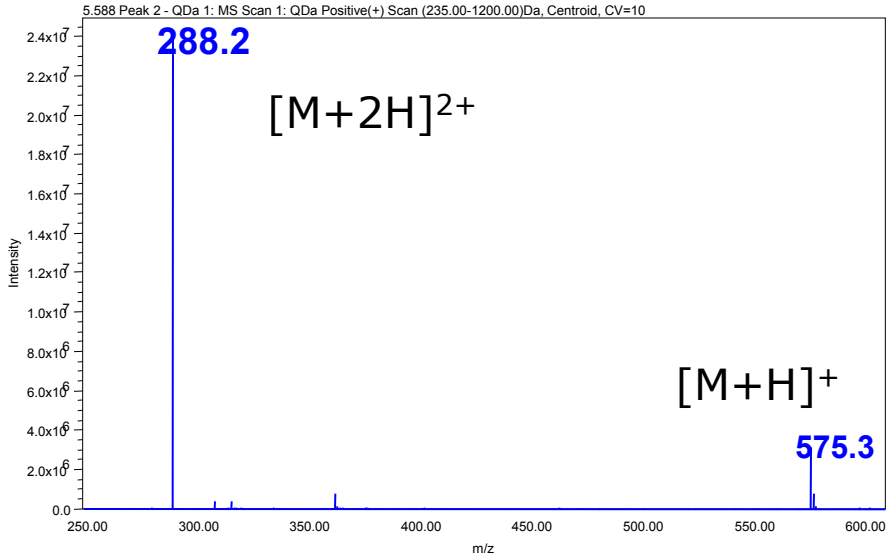
- Mass spectrometers separate ions on the basis of mass-to-charge ratio (*m/z*)
  - Singly charged ion: $(M + H)^+$ *m/z* = (M+ H)/1
  - Doubly charged ion: $(M+ 2H)^{2+}$ *m/z* = (M+ H)/2
  - n charged ion: $(M+ nH)^{n+}$ *m/z* = (M+ nH)/n

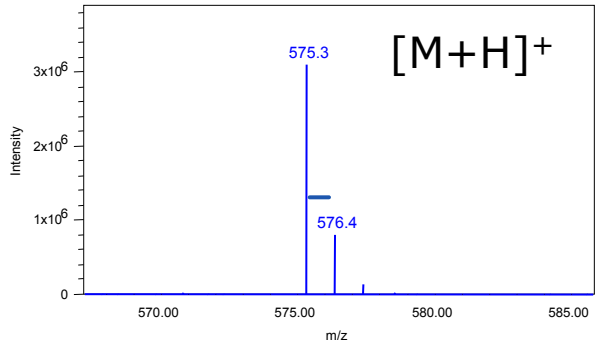
- Isotope peaks of an ion with n charges are separated by 1/n m/z
  - e.g. isotope peaks of a doubly charged ion would be separated by 0.5 m/z

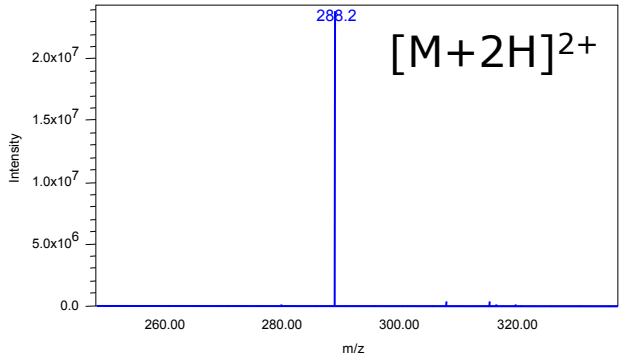
- Multiple charging of an analyte molecule may happen when more than one location on a molecule can accept a charge

- Multiple charge states are more likely observed with analytes of higher mass (peptides, proteins, etc.)

Distinguishing Between Multiply Charged Peaks







- NIST MAb peptide
  - Peptide^{+} – 574.3
  - Single charged: (574.3+1)/1=575.3
  - Doubly charged: (574.3+2)/2 = 288.2

Impact of Instrument Resolution on Charge State

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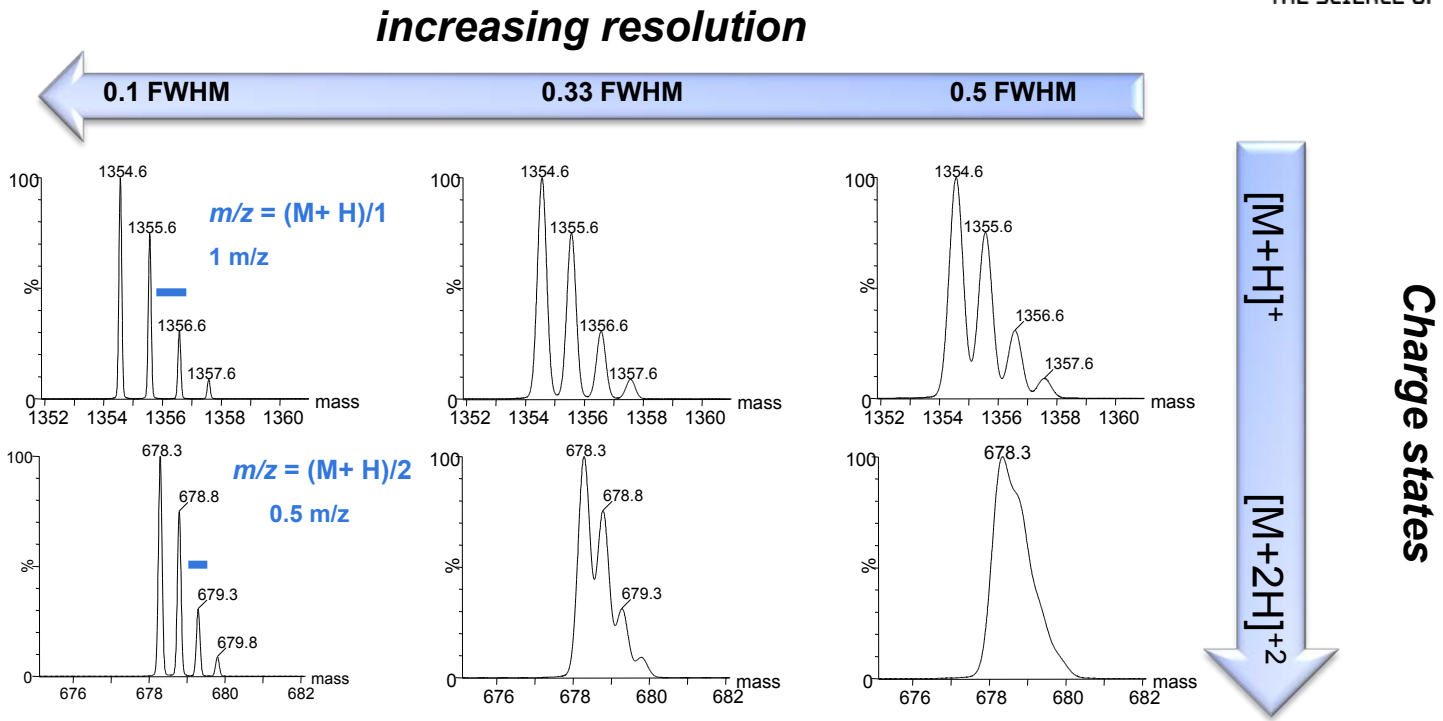

- Simulation of Vitamin B^{12} isotope models for singly and doubly charged isotopes
- Singly charged species separated by 1 m/z, doubly charged species isotopes separated by 0.5 m/z

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Questions??