Multilple Techniques for simultaneous Quantitative and Qualitative Data Acquisition using a Mass Spectrometer

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What is Mass Spectrometry?

What information does mass spectrometry provide?

- MS Requirements
- General setup of a QQQ
- Ionization Techniques
- Information from a Mass Spectrum
- How ions move in a quadrupole
- QQQ Ion Path
- Quadrupole Scan Modes

Where are mass spectrometers used?

Mass spectrometers are used in industry and academia for both routine and research purposes.

The following list is just a brief summary of the major mass spectrometric applications:

Biotechnology: the analysis of proteins, peptides, oligonucleotides

Pharmaceutical: drug discovery, combinatorial chemistry, pharmacokinetics, drug metabolism

Clinical: neonatal screening, haemoglobin analysis, drug testing

Environmental: PAHs, PCBs, water quality, food contamination

Geological: oil composition

Mass Spectrometry

- Mass spectrometry is an analytical tool used for measuring the **molecular mass** of a sample.
- Powerful technique to characterize & identify unknown compounds
- Mass spectrometers generate a charged species (e.g. molecular ion) and then sort them based on mass-to-charge ratio
- Mass spectrometry is done under high vacuum conditions to increase the efficiency of ion transfers & detection
- Molecules must be charged otherwise there is no method to move them around in the MS
- If the compound is known, the molecular ion will appear at a m/z value corresponding to its molecular weight.
- Structural information can be obtained by MS-MS experiments or by in-source fragmentation driven by high orifice potentials.
- A typical feature of ESI is the good efficiency in producing multiply charged ions.

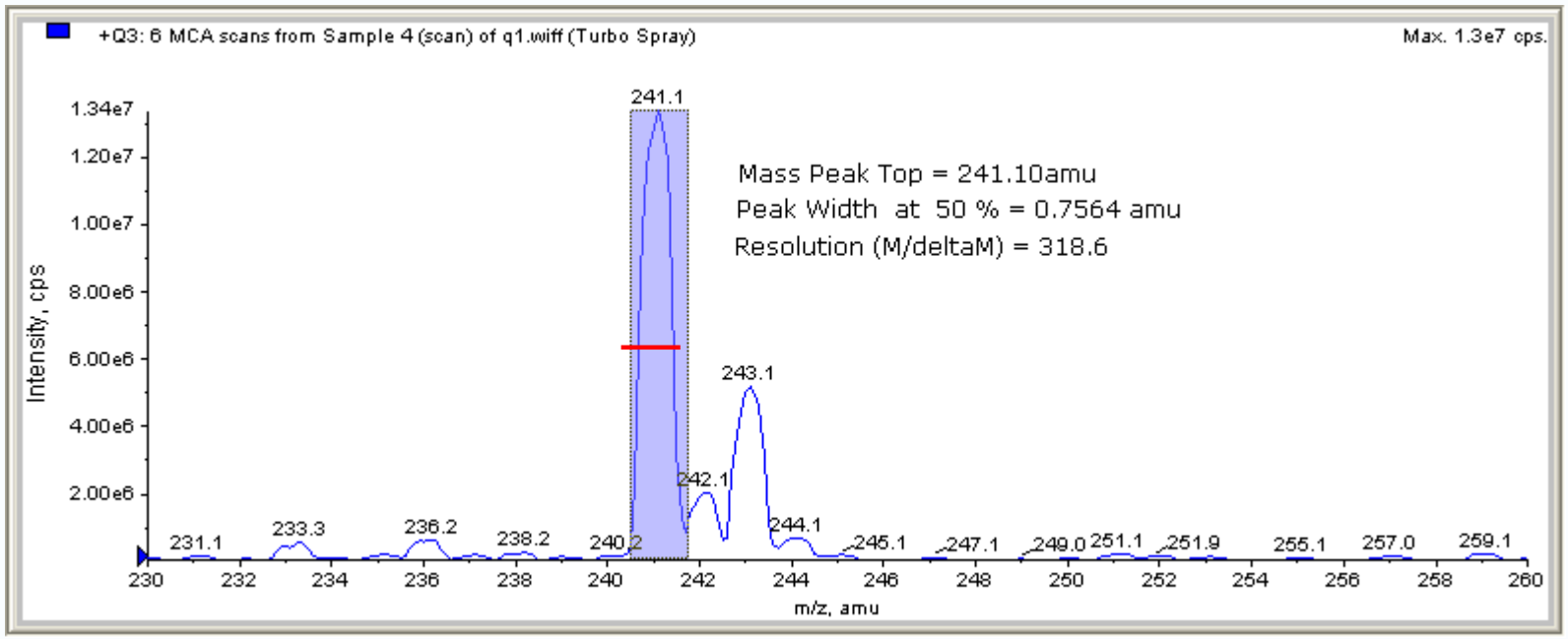
How Many Mass Values Can a Molecule Have?

- Nominal Mass – The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value (i.e. H = 1, C = 12, N = 14, O = 16,…).
- Monoisotopic Mass - The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element (i.e. H = 1.007825, C = 12.00000, N = 14.00307, O = 15.99491, …).
- Average Mass – The mass of an ion or molecule calculated using the average mass of each element weighted for its natural isotopic abundance (i.e. centroid of the distribution).

Information from a Mass Spectrum:

The Molecular Ion

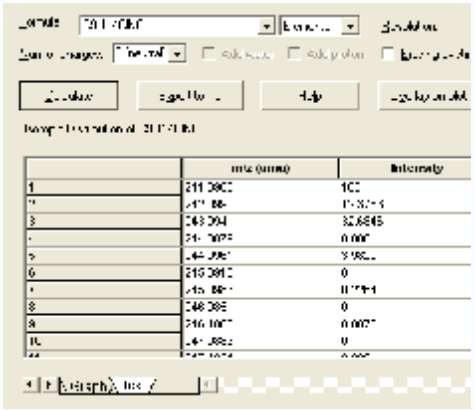
- The molecular ion is formed from the neutral analyte molecule by removing one electron.
- It can be found at a m/z value corresponding to the mass of the analyte.
- If present, hence, provide a direct indication of the analyte molecular weight, the first information we expect from mass spectrometry.
- The molecular ion, and hence the molecular weight, can be expressed by an even or an odd number.

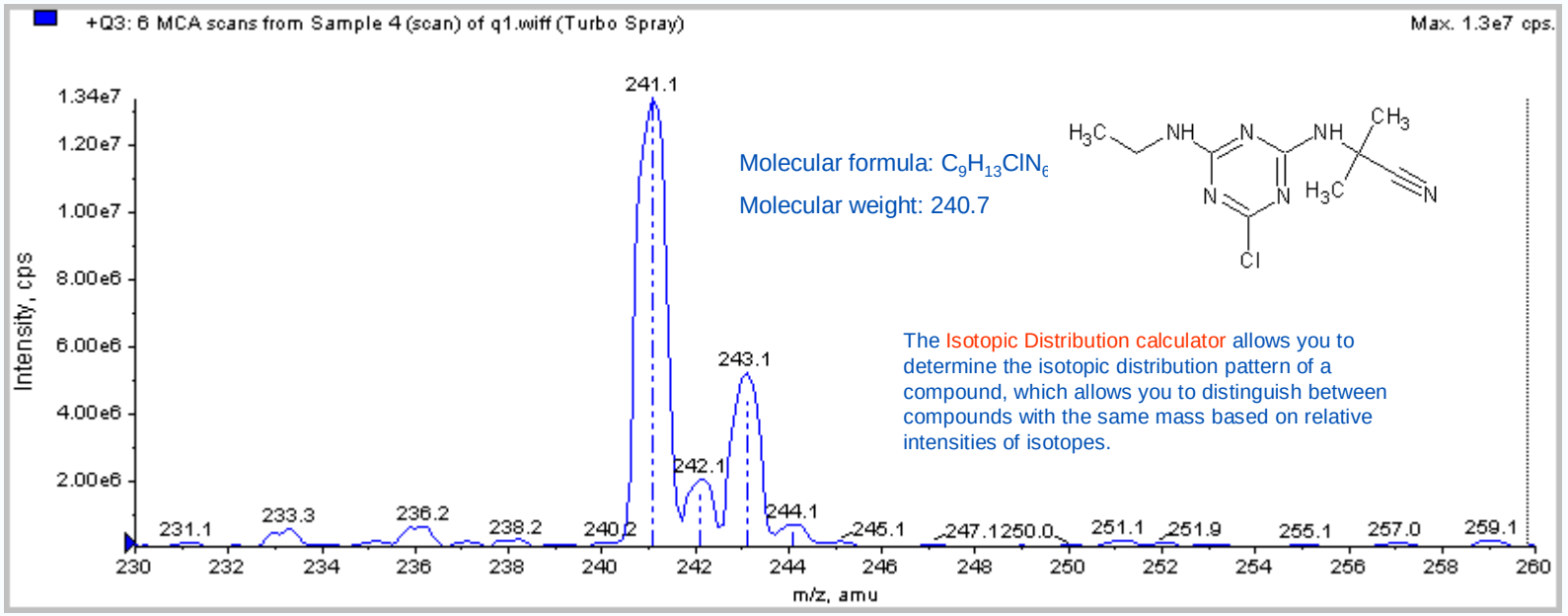


Information from a Mass Spectrum:

The Molecular "Cluster"

- Almost all the elements are present in nature with different isotopic species.
- Isotopes are characterized by the same atomic number, but different atomic mass.
- The aspect of the "isotopic cluster" of the molecular ion can provide further information about the elements present in the molecule.
- Some elements show a clearly evident isotopic composition: Br, Cl (more evident), S e Si (less evident).

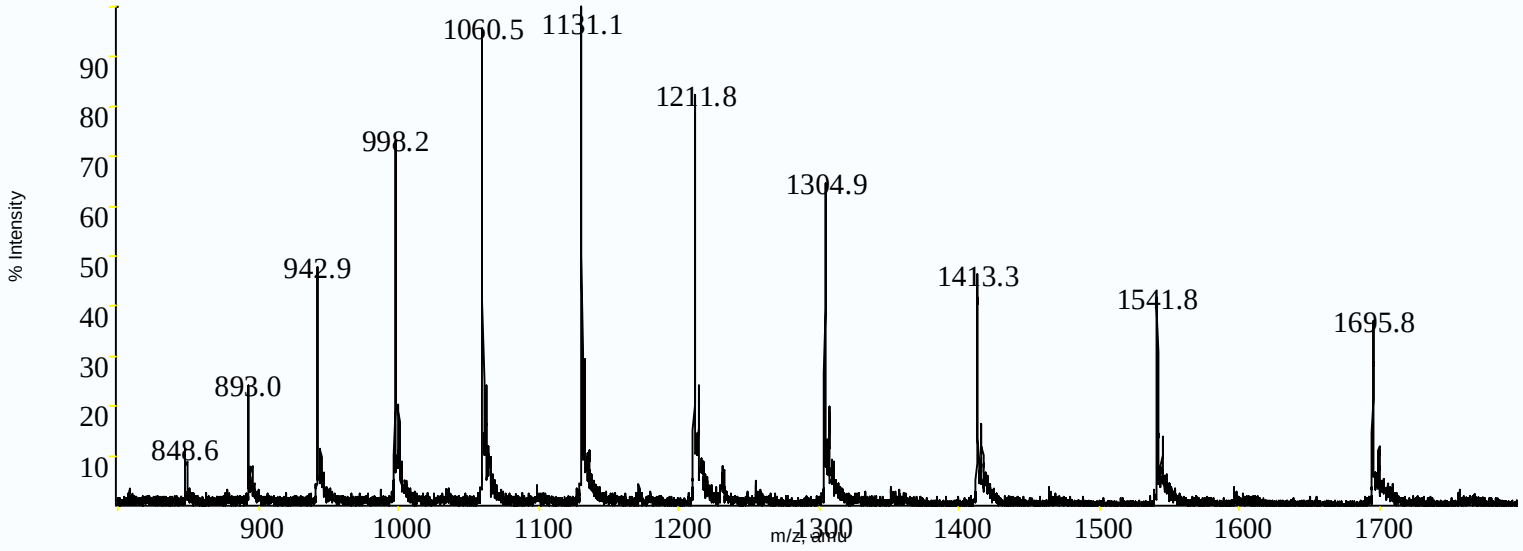




Mass spectrometry can distinguish isotopes: atomic weight of chlorine is 35.5, but the mass spectrum show two distinct ions at m/z 35 and 37 .

Multiply Charged Ions: The m/z ratio

- Mass Spectrometry provides the mass-to-charge ratio (m/z) of the analyte ions.
- ESI is able to put several charges onto the analyte molecule, is this is suitable.
- This can extend the effective range of “low range” analyzers such as quadrupoles.



M = 10000 — Nr. of charges = 10

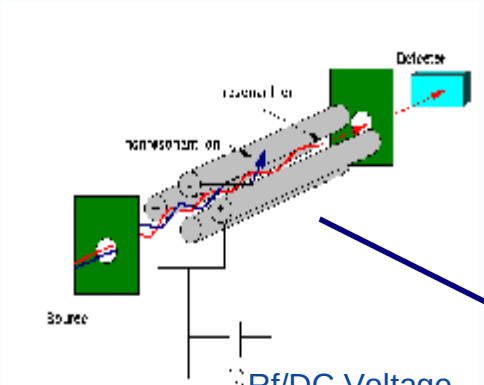
m/z = 1000

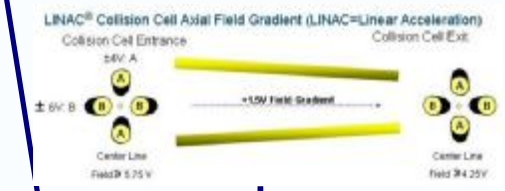
MS Requirements

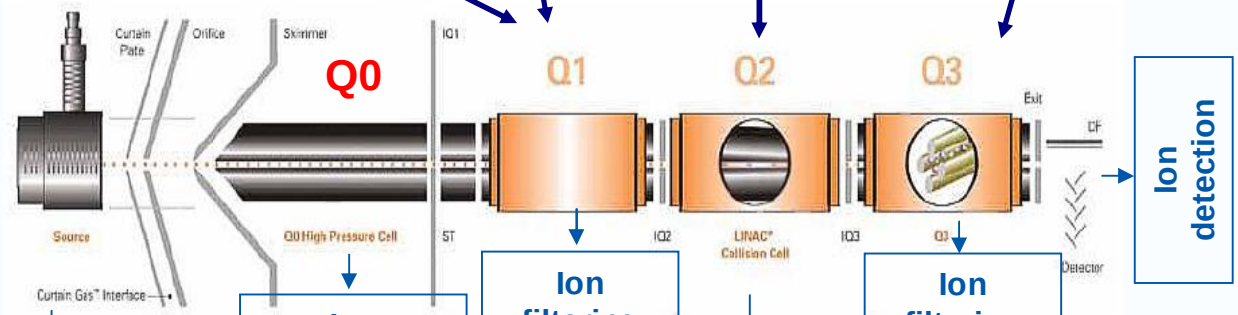
- Sample introduced
- Sample must be ionized
- Ionized sample path is in a vacuum
- Ion sorting
- Ion detection

General setup of a QQQ System Mass Spectrometer

Mass spectrometers can be divided into three fundamental parts, namely the **ionisation source , the analyser , and the detector.**







Ion production

Ion transport

Ion filtering

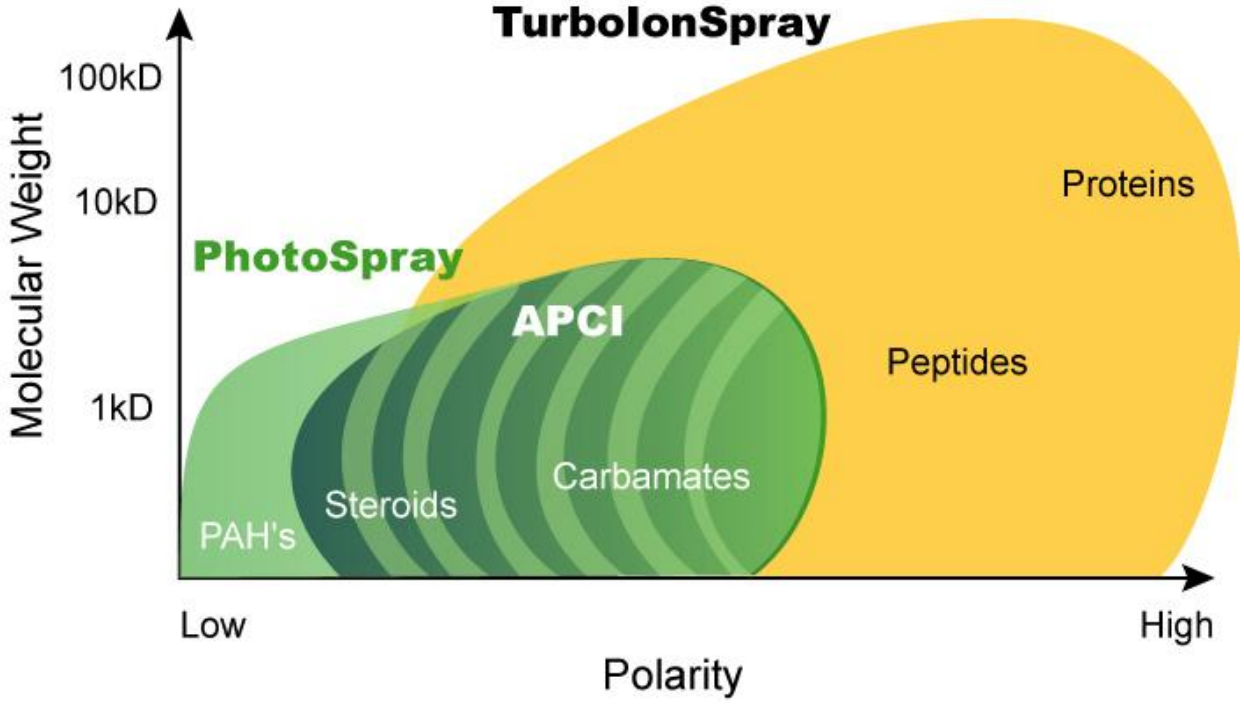
Ion processing

Ion filtering

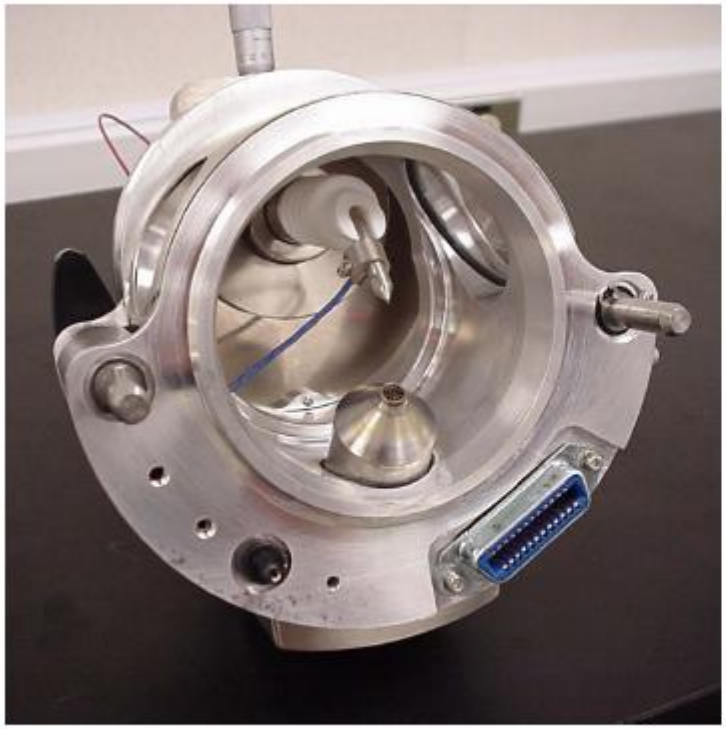
Ion detection

API Polarity/Mw Domains

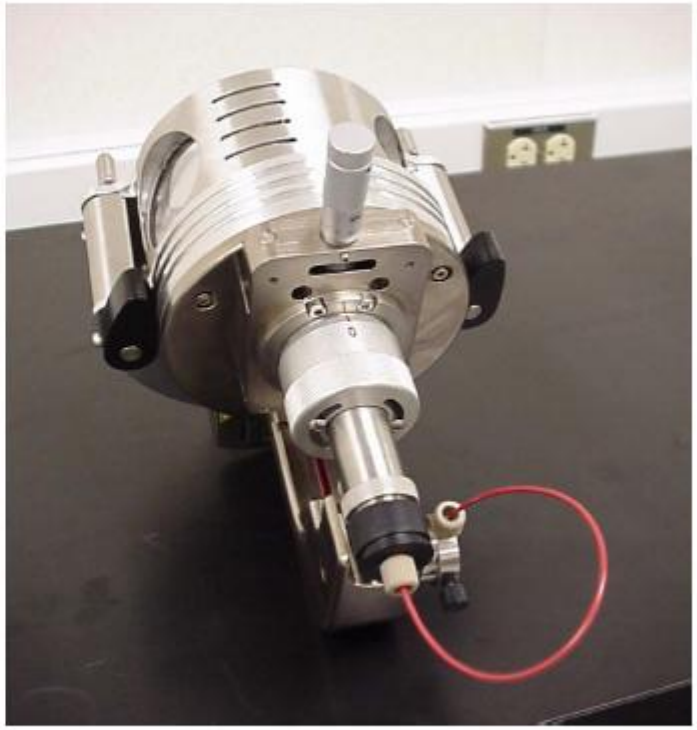
The coupling of Liquid Chromatography (LC) and tandem Mass Spectrometry (MS/MS) is a widely used analytical technique for quantitative and qualitative analysis. Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), or Photo Ionization (APPI) allow the ionization of various semi-volatile, thermally labile, and polar to nonpolar compounds, such as pharmaceuticals, pesticides, personal care products, steroids, explosives, drugs of abuse etc., etc., in trace levels.



TurbolonSpray® Source for API 2000™ System



Source with sprayer arm on top and heater on the bottom



Source from the rear, showing adjustment options

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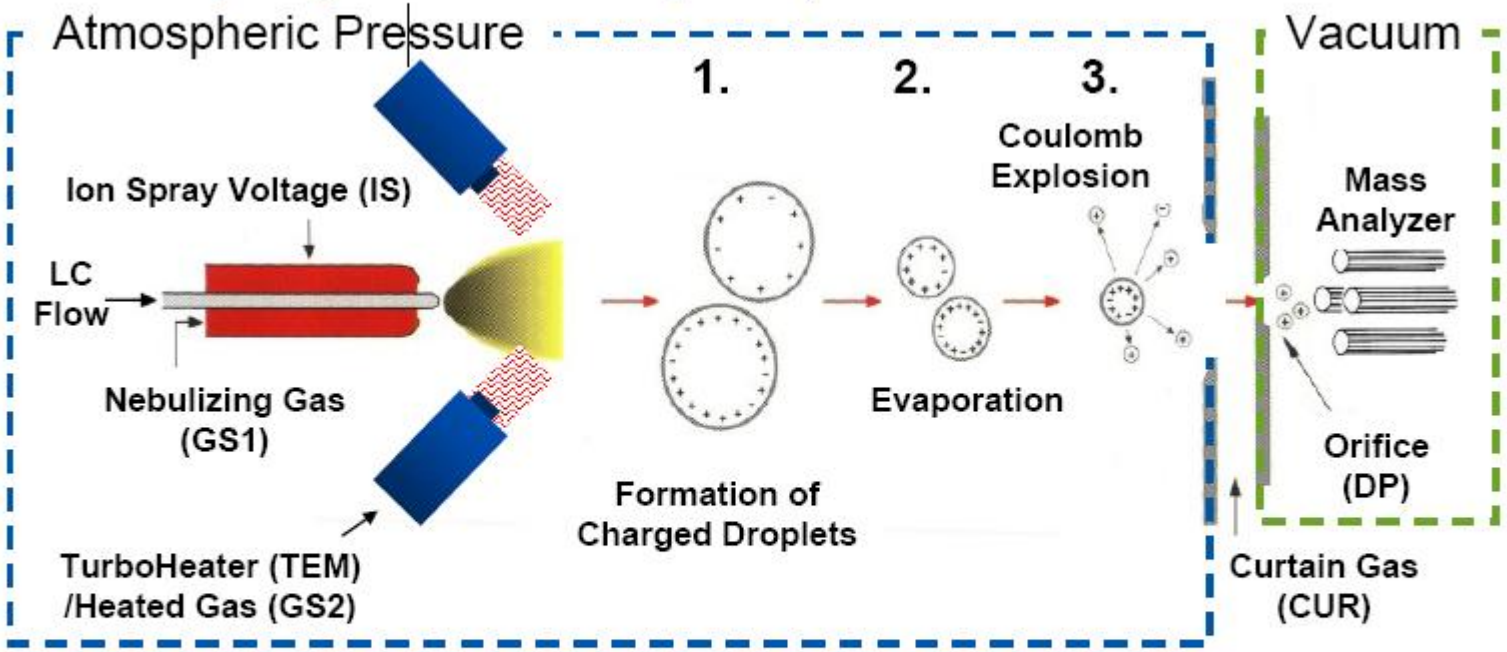
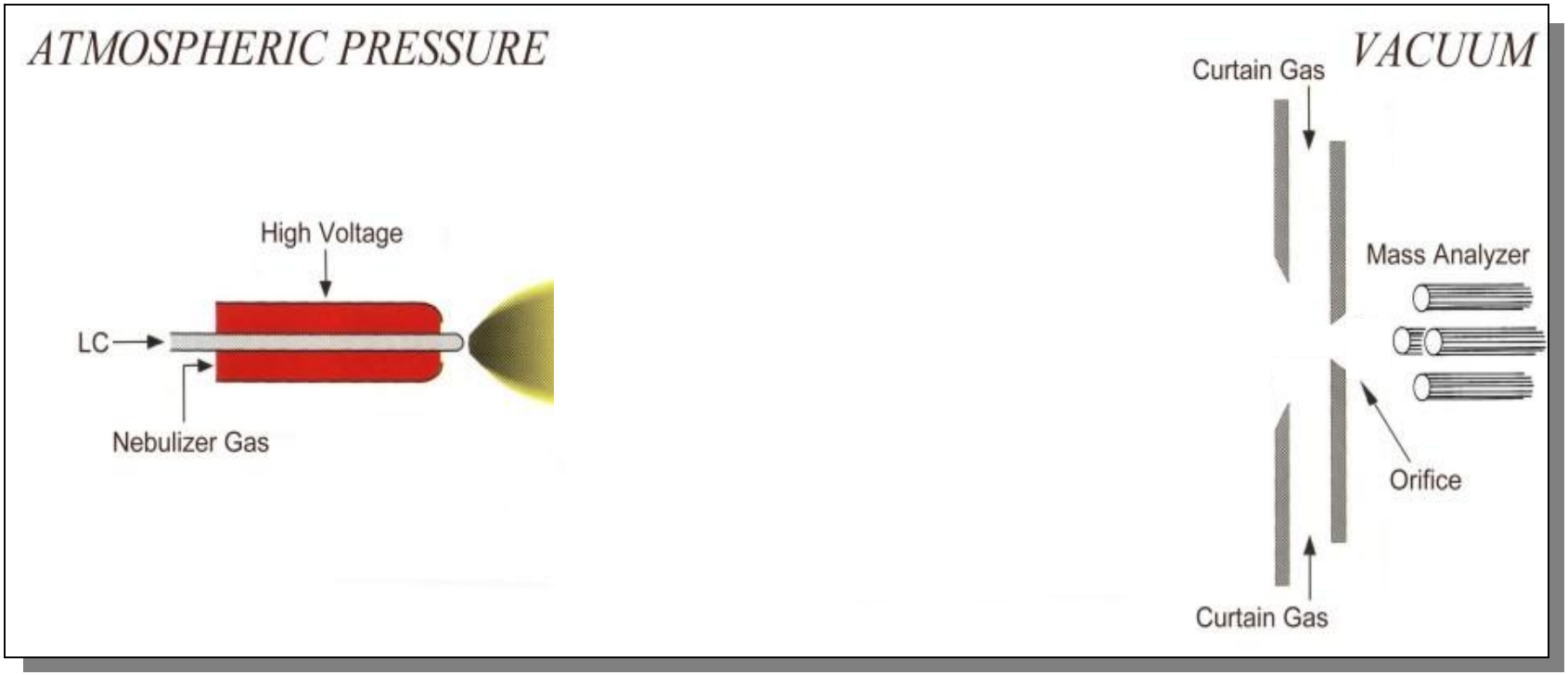
Electrospray Ionization (ESI) - Positive mode shown



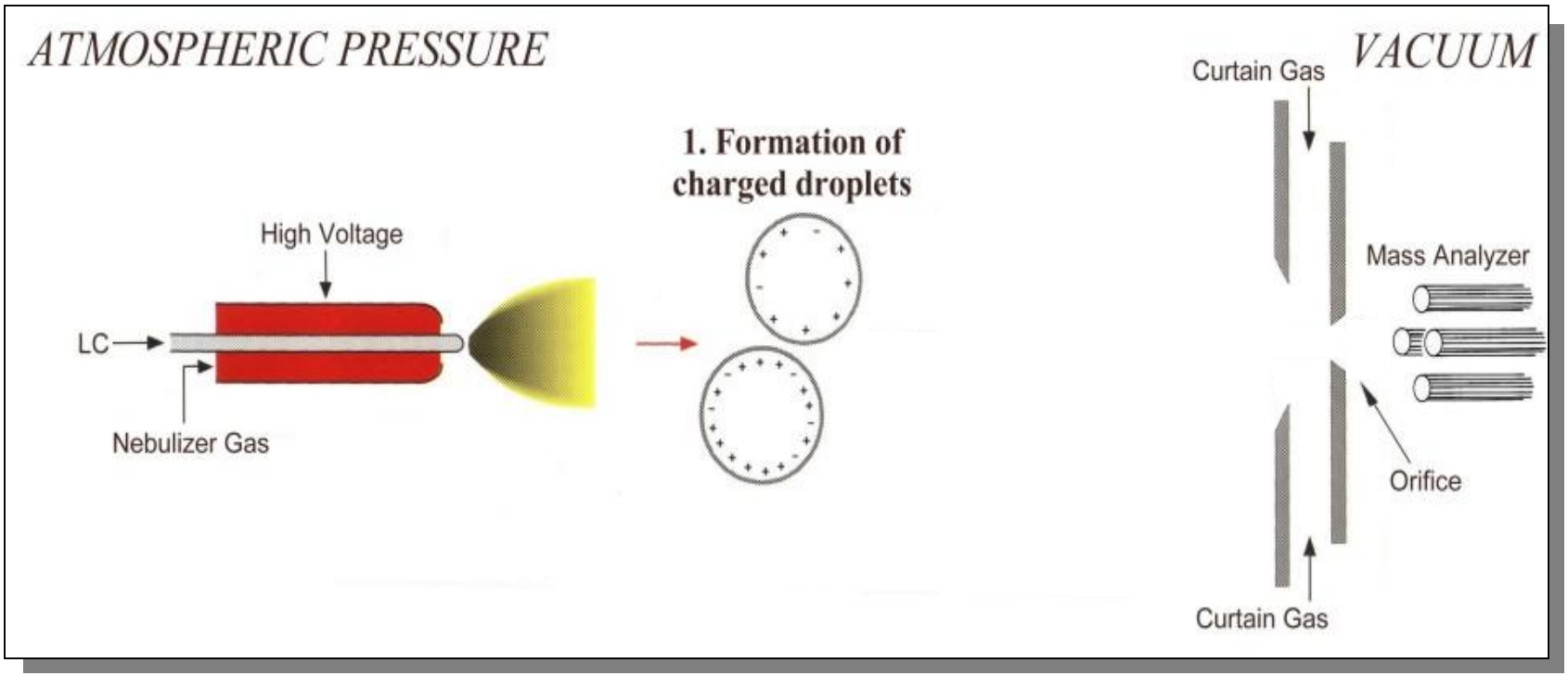
1. Charged droplets are formed
2. The droplets evaporate and the field inside of the droplet increases
3. Ions exit the droplet and are focused into the interface

TurboIonspray™ Principle



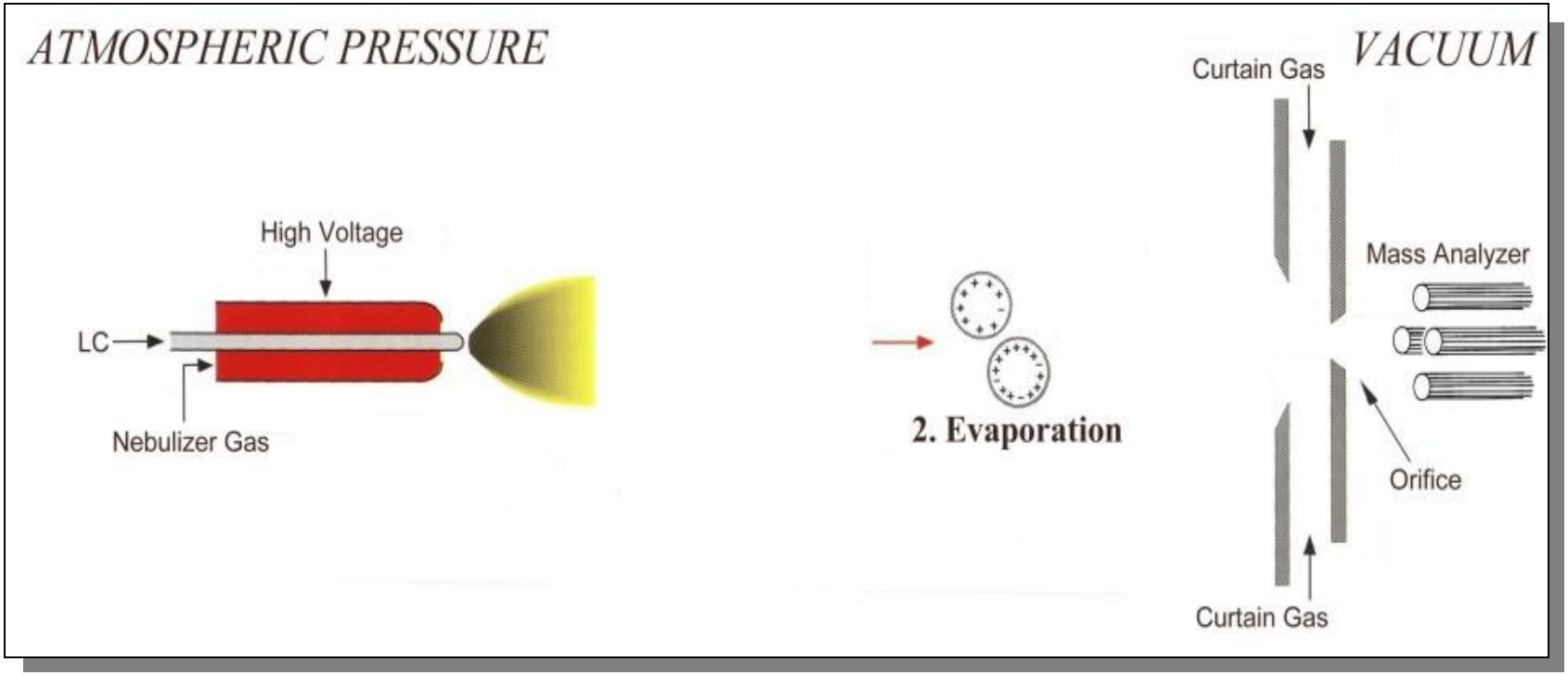
ESI is on one of the softest ionisation technique and ionises polar to ionic substances.

TurboIonspray™ Principle



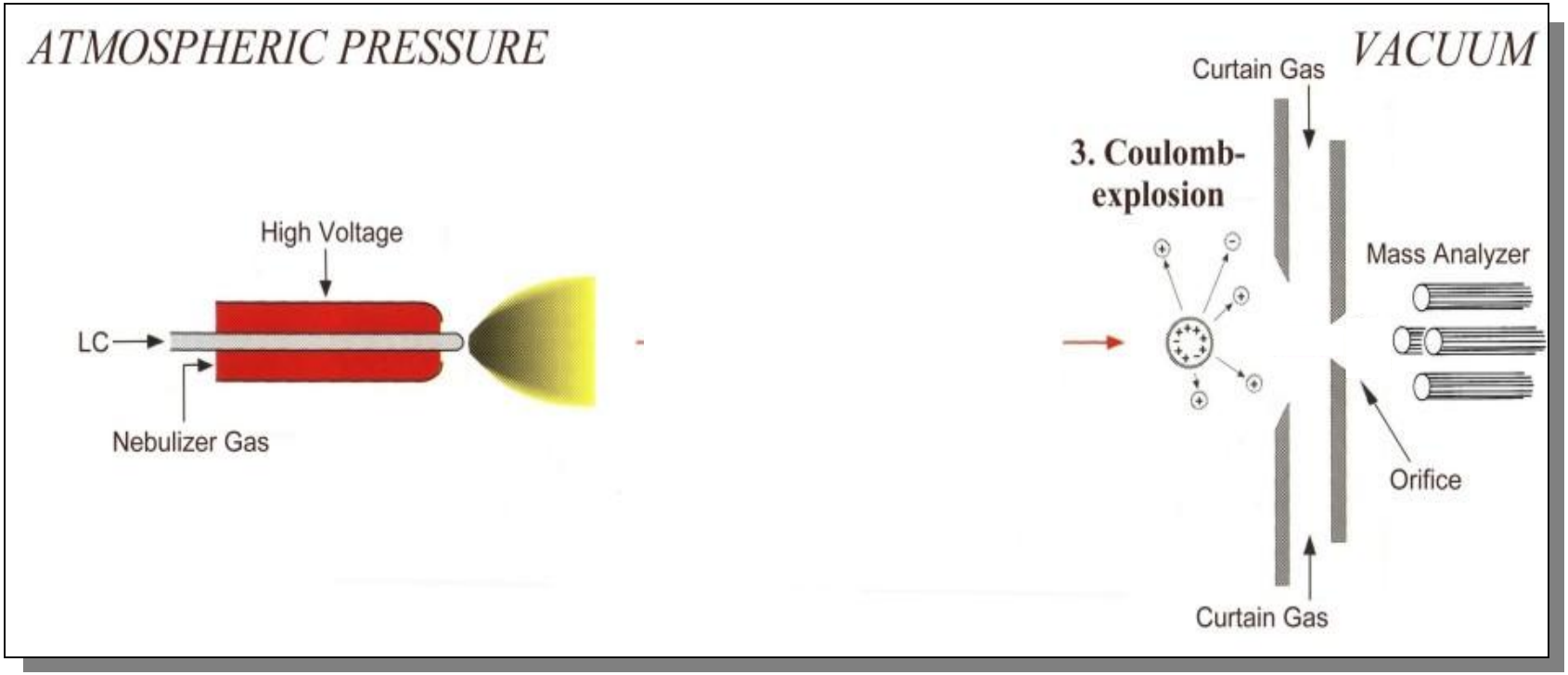
The solvent stream passes down a charged needle so the surface of the flow becomes charged with the same polarity as that of the voltage applied to the needle. This flow then mixes with the a gas stream forming a tailor cone an d charged droplets.

TurboIonspray™ Principle

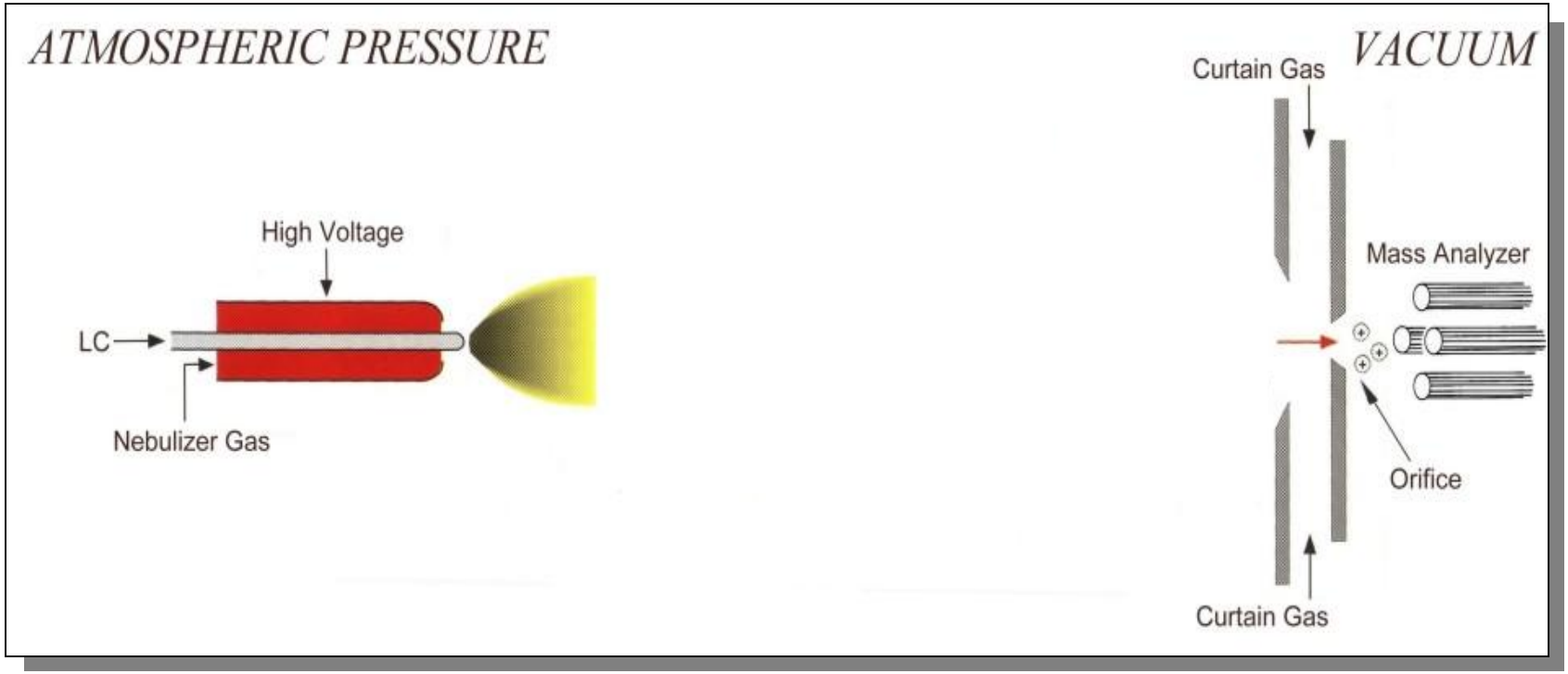


The charged droplets move forwards towards the entrance to the vacuum region due to their initial velocity and the potential difference between the needle and the curtain plate

TurboIonspray™ Principle

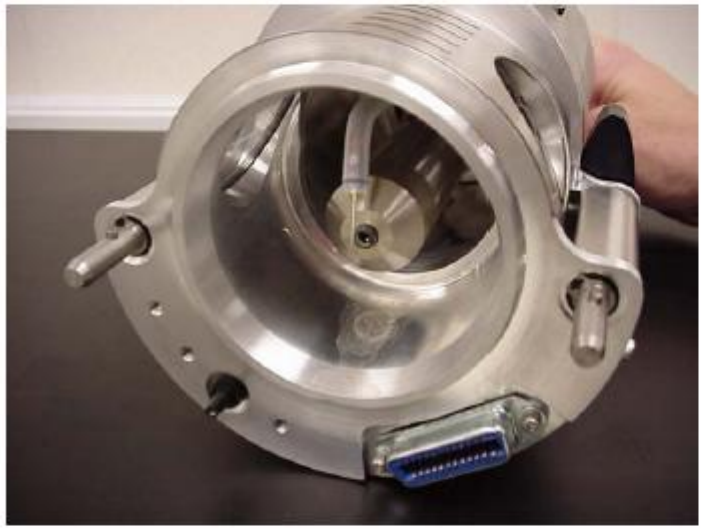


TurboIonspray™ Principle

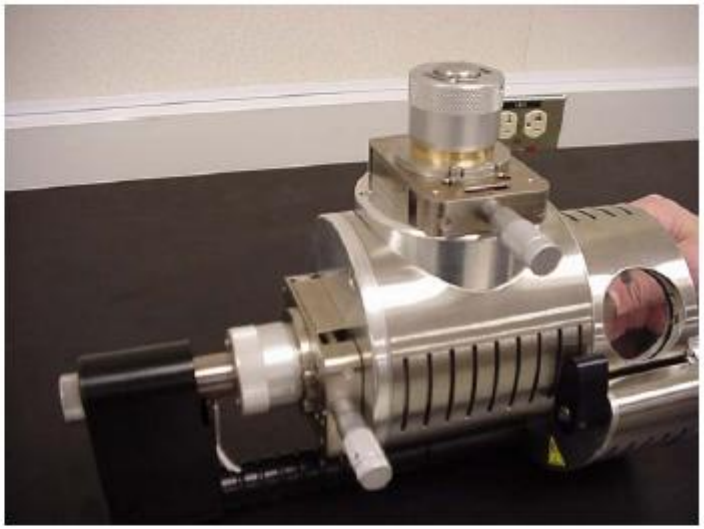


The analyte ions then enter the vacuum region of the system.

APCI Source for API 2000™ System



Inside of Source, showing corona needle offset between orifice and quartz tube



Outside of Source with articulations for the Heated Nebulizer on back and the corona needle on top

APCI Features - Why use APCI?

- APCI can be more sensitive for certain compound classes than ESI-MS
- Matrix effects are often lower in APCI when compared to ESI-MS
- APCI is a high flow inlet (0.2 - 2.0 mL/min.)
- Suitable for non-polar, thermally stable compounds which are usually, MW < 1300 amu
- Probe is heated to facilitate vaporization
- Requires nebulizing and auxiliary gas
- Requires corona discharge needle to produce ionization (APCI)

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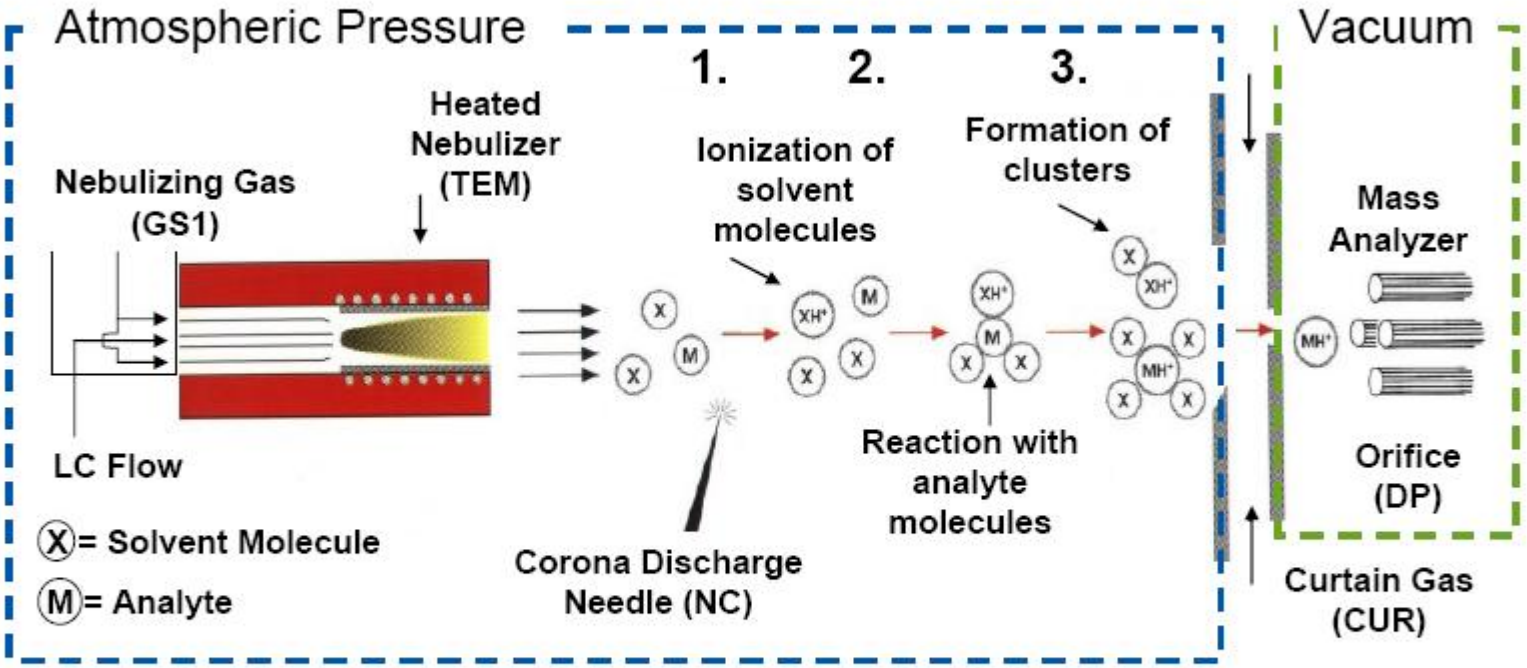
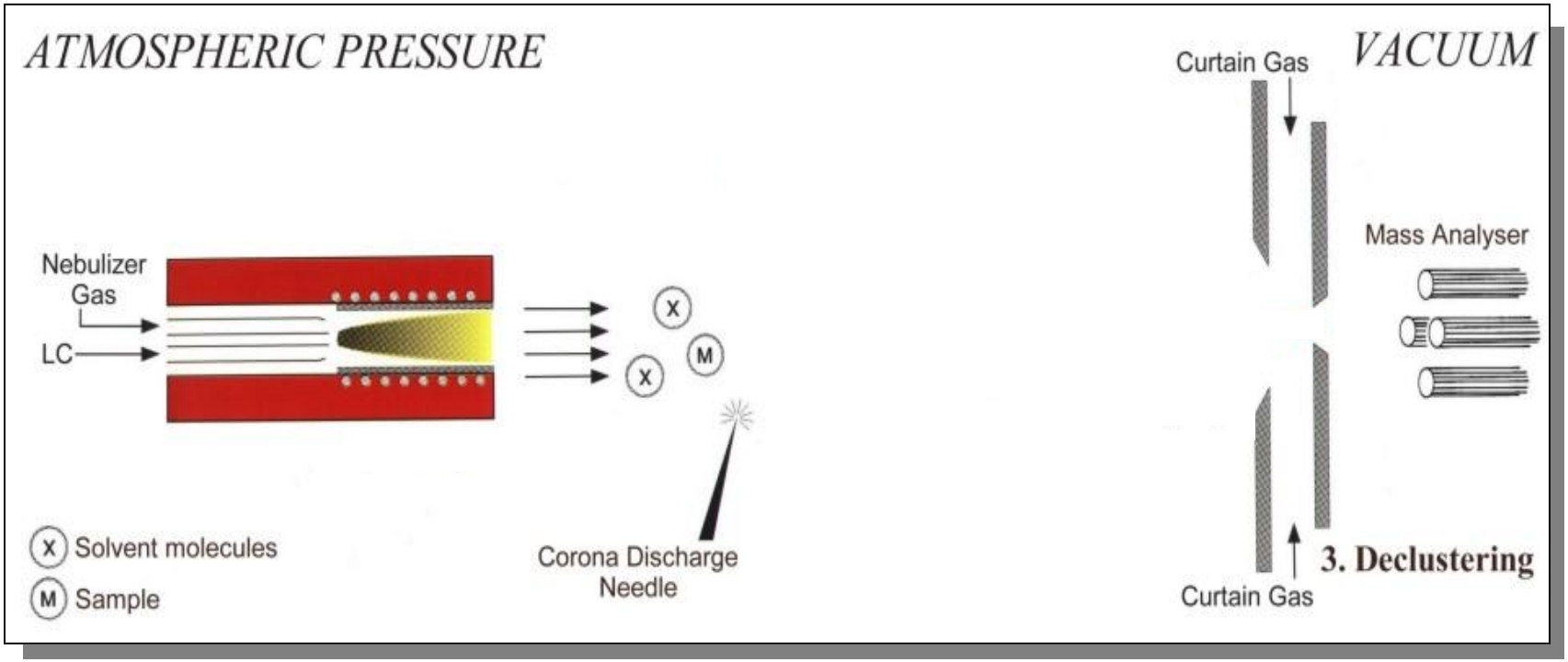
Atmospheric Pressure Chemical Ionization (APCI)



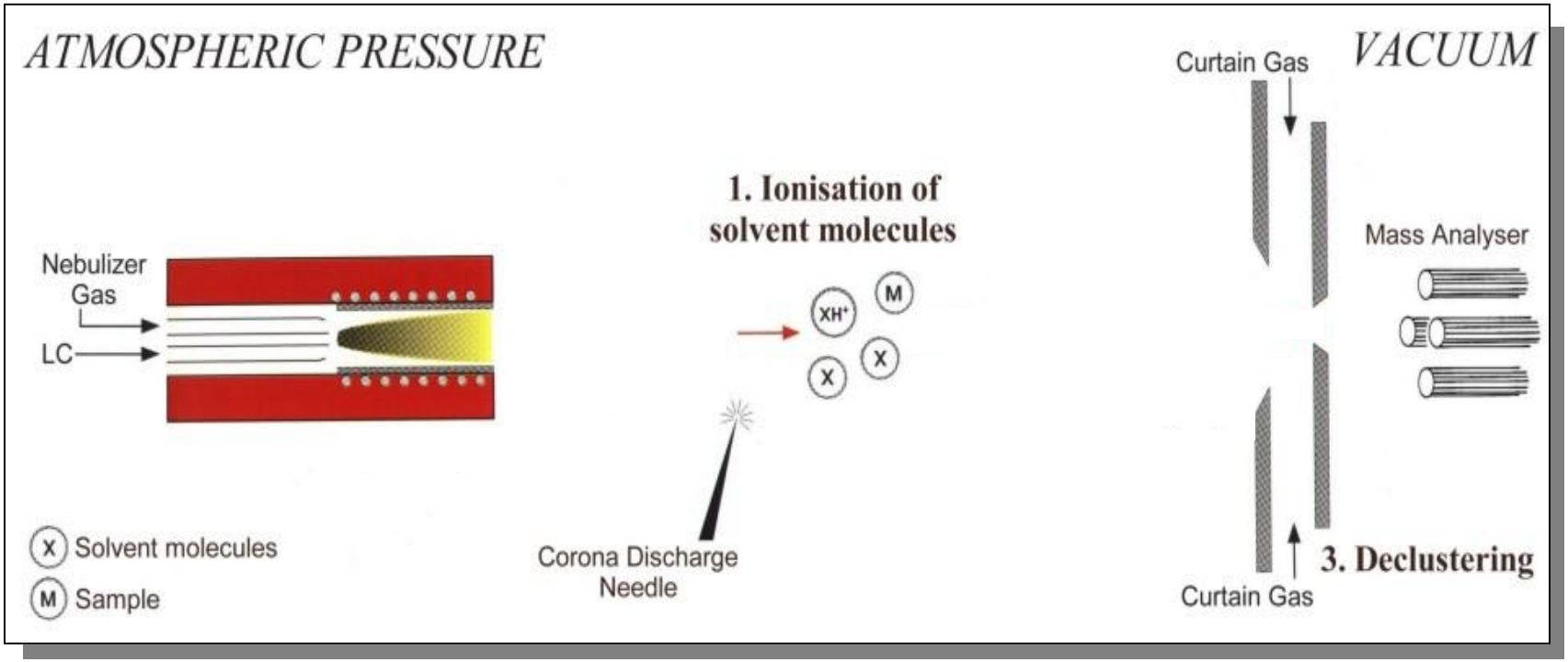
1. Corona discharge needle ionizes N_2 or O_2 in source
2. N_2 or O_2 pass charge to vaporized solvent
3. Vaporized, charged solvent passes charge to analyte

APCI Principle



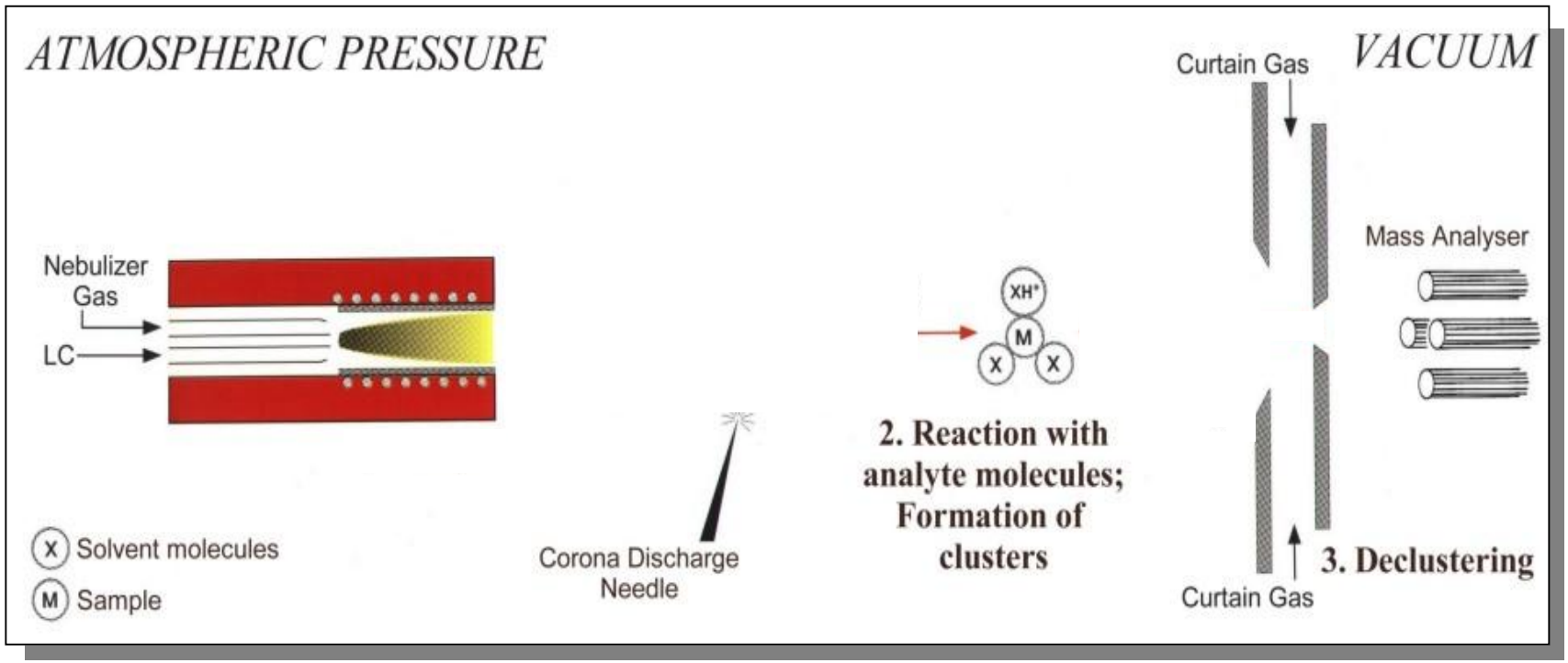
Corona discharge e.g. in positive mode, causes e- removal from atmospheric N2, O2: forms N2+•,O2+•

APCI Principle



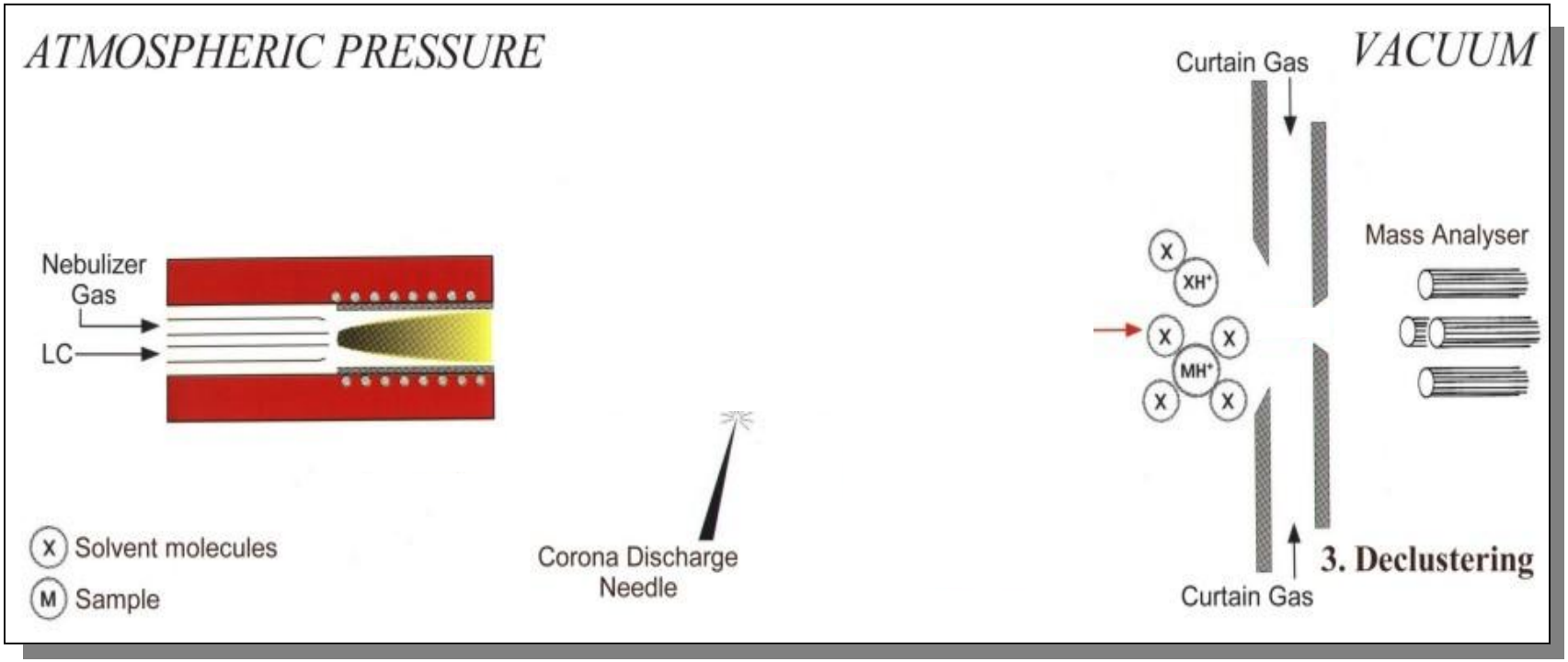
In a complex series of chain reactions the gas ions e.g.N2+•,O2+• react with solvents (H2O, CH3OH) forming reagent ions: e.g., H3O+ , CH3OH2+ the reverse happens in negative mode with an electron being added to the gas molecules.

APCI Principle



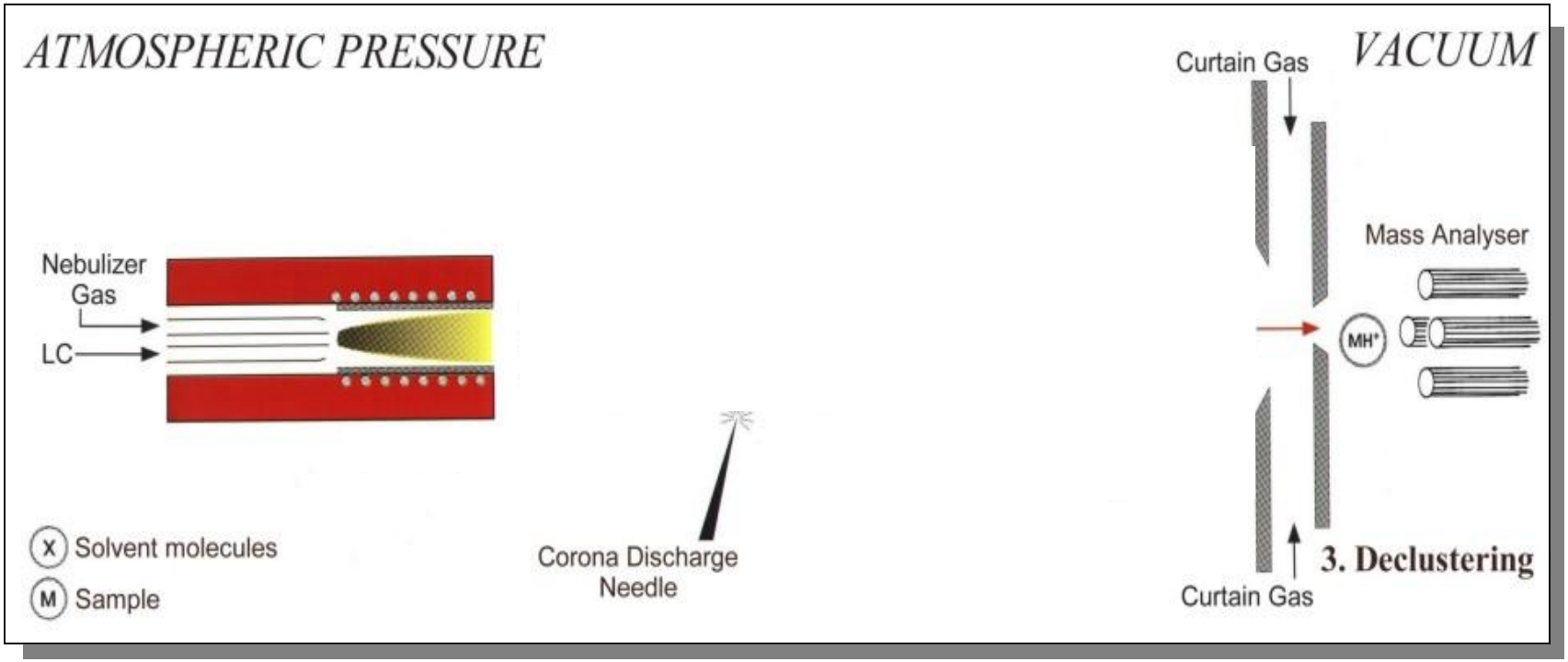
In positive mode the H3O+, CH3OH2+ protonate the analyte forming [M+H]+ . In negative mode the corresponding anion deprotonates the analyte forming [M-H]- .

APCI Principle



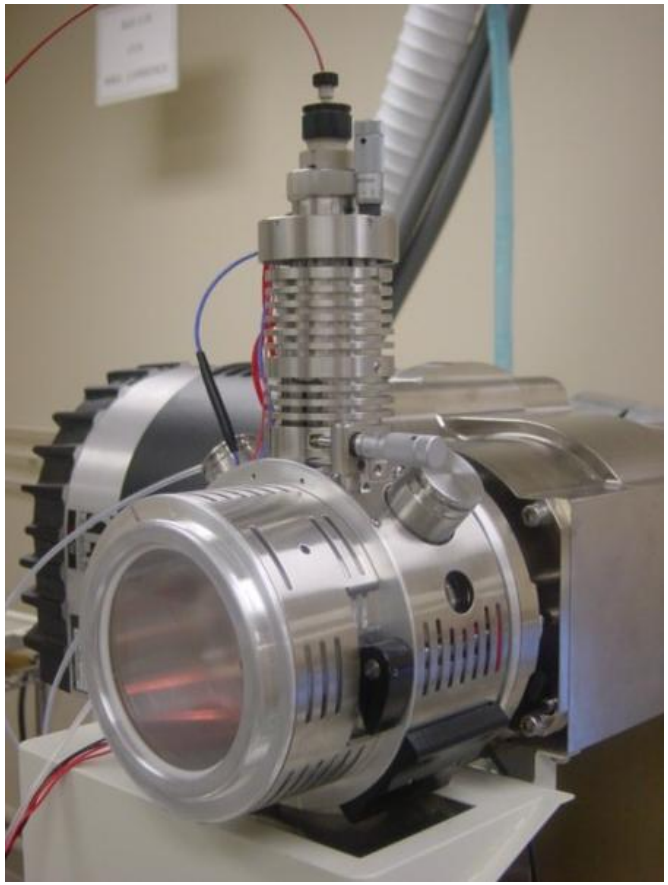
The curtain gas then knocks off the solvent molecules clustered around the analyte ion.

APCI Principle



The ion enters the vacuum region of the LC/MS/MS system.

Turbo V™ Source



TurboV™ Gas Entrainment Ion Source

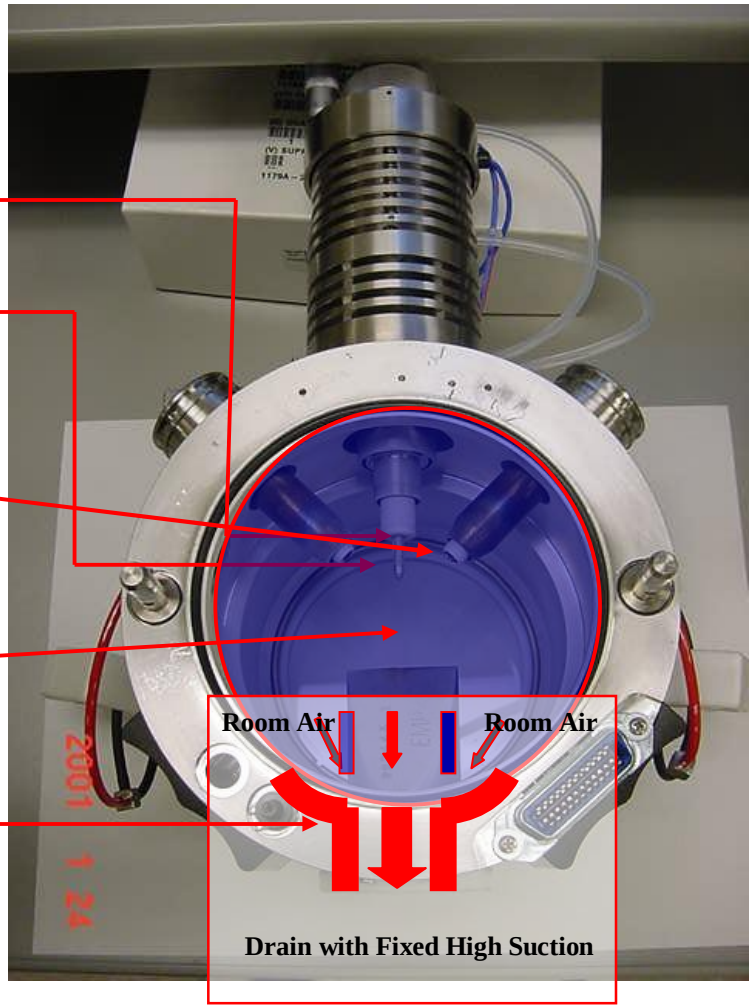
IonSpray voltage

IonSpray electrode tip protrusion
(As API 2000™ System)

Turbo has an extended temperature range
(750°C) and gas range is 0-12 L/min

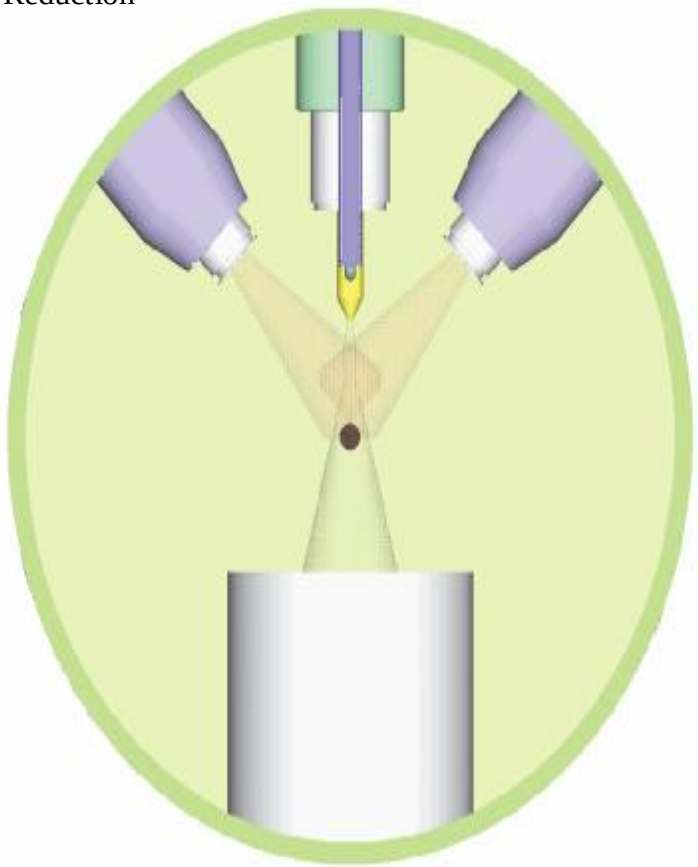
High curtain gas setting
will attenuate signal

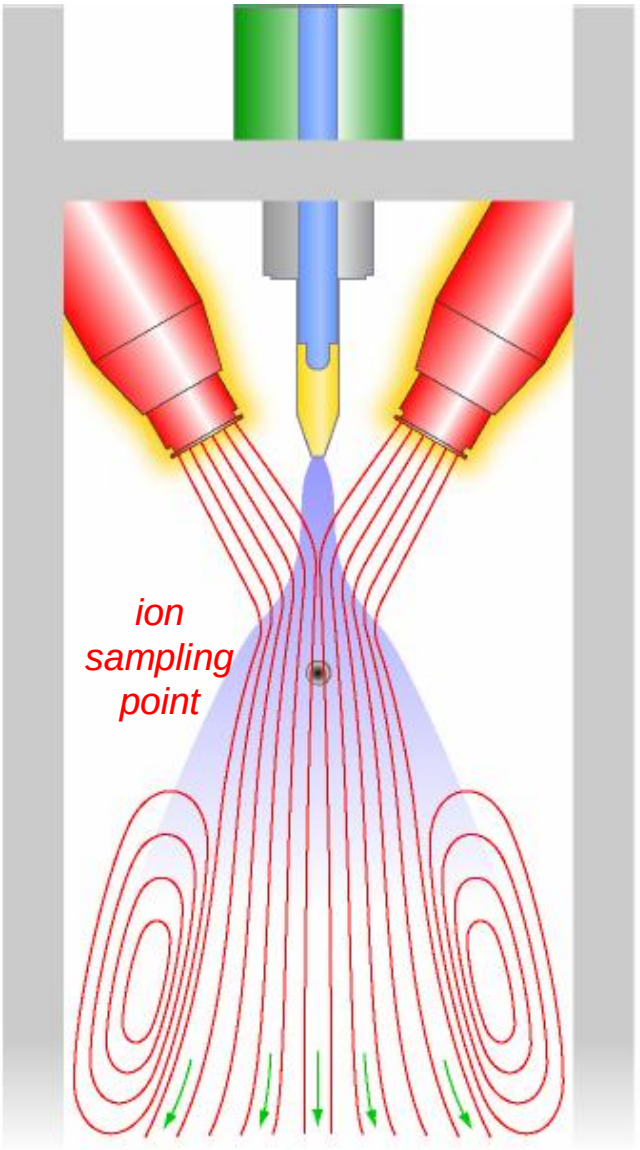
Extended source exhaust
housing



Increased Thermal Efficiency of Heater

Promote Turbulent Mixing
Improved Heat and Mass Transfer
Maximize Desolvation
Background Reduction





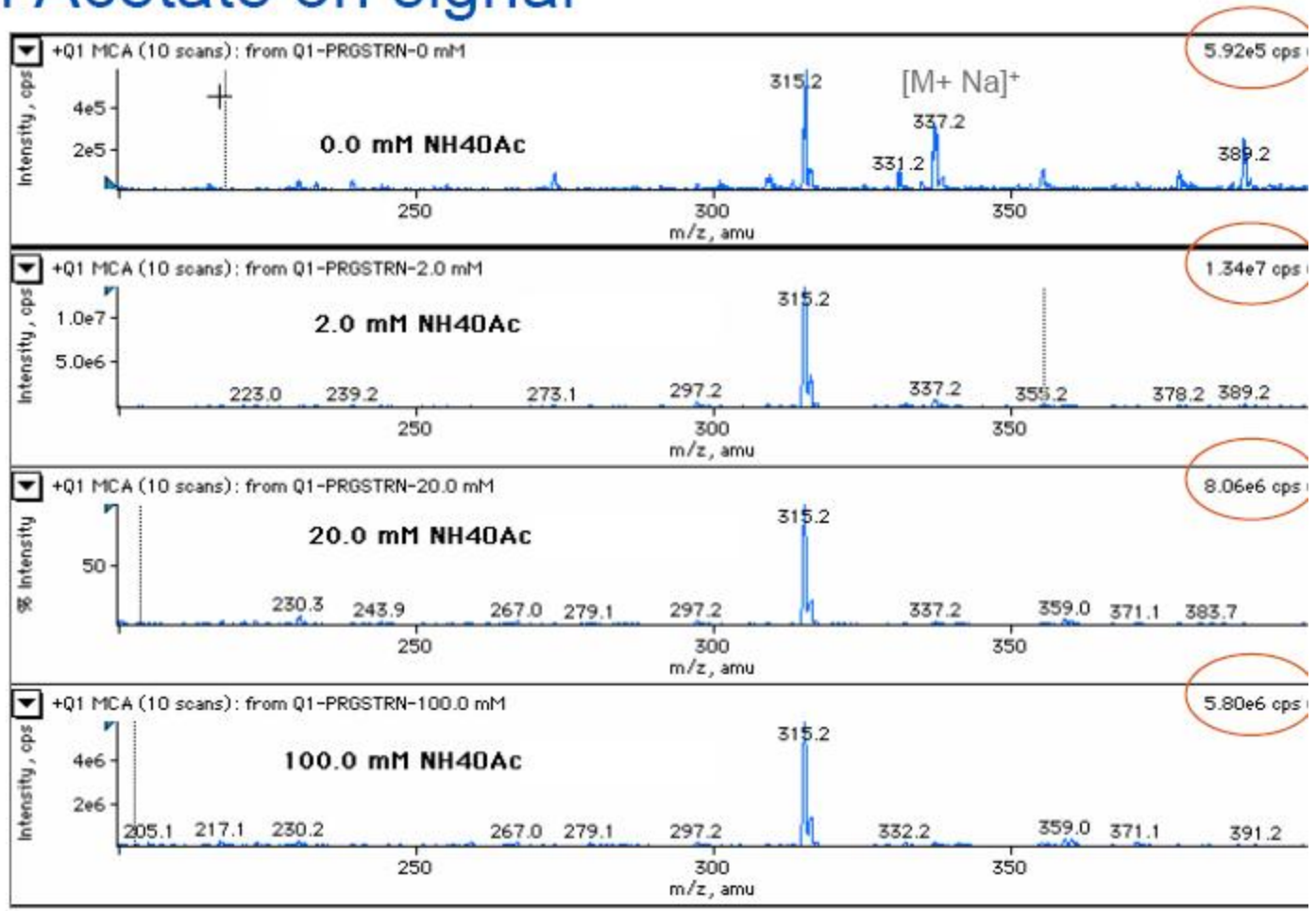
- Common Solvent components for ESI
  - Volatile buffers such as Ammonium Formate or Ammonium Acetate (2 – 10 mM optimum, may cause suppression over 20 mM)
  - Acids
    - 0.1 - 1.0 % Acetic acid
    - 0.1% Formic acid
    - Up to 0.1% TFA for positive ion mode ONLY - may cause suppression

Things to avoid:

- Salts can interfere with ionization and can cluster, complicating spectrum
- Strong bases or quaternary amines such as Triethylamine (TEA) can cause signal suppression in positive ion mode
- Strong acids such as Sulfuric/Sulfonic acids and TFA can cause signal suppression in negative ion mode
- Non-volatile modifiers or buffers such as Phosphate buffers can cause adduct formation and contaminate the source

Progesterone in Positive Ion ESI-MS: Effect of Ammonium Acetate on signal

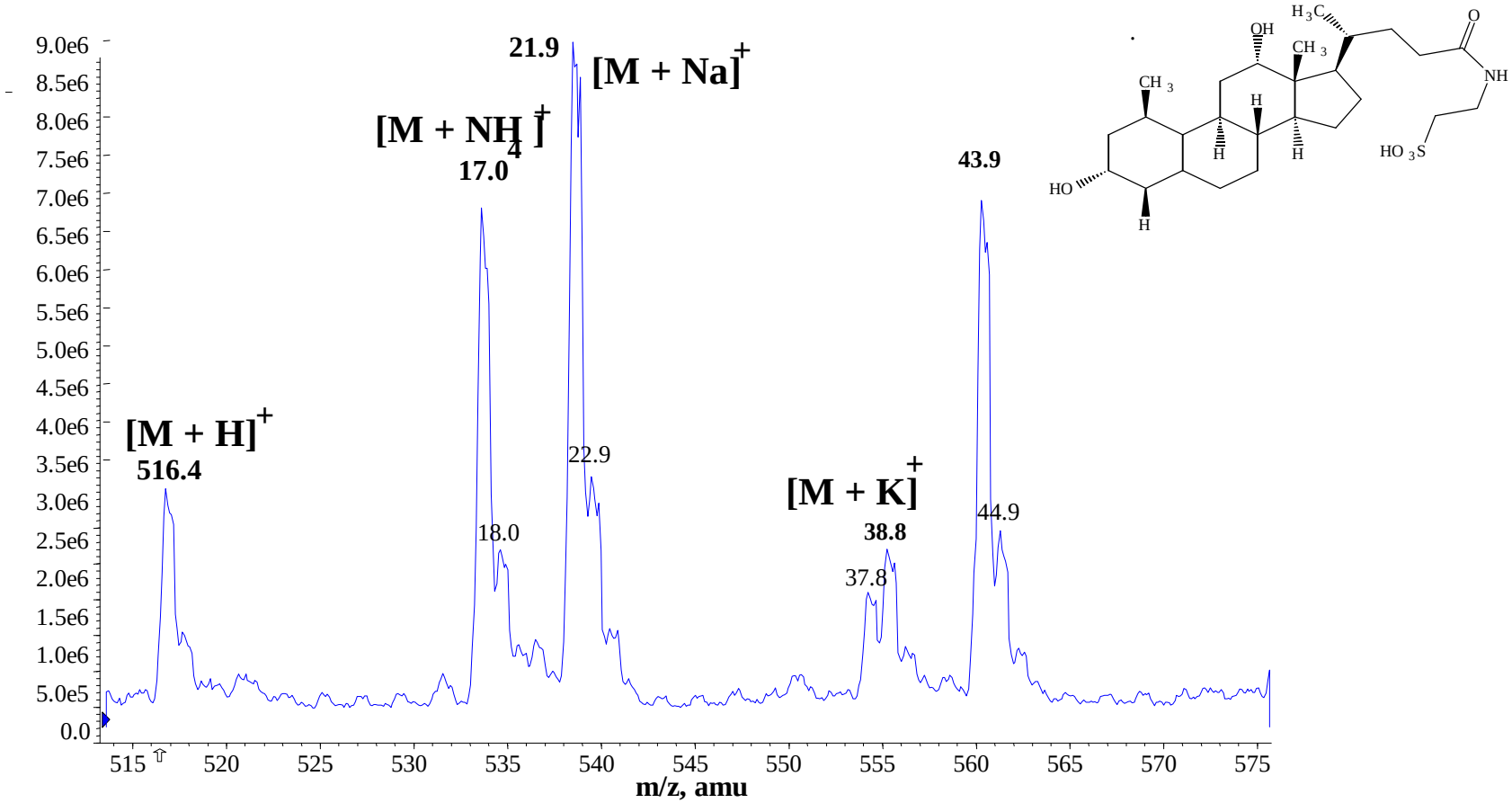
Ammonium Acetate (NH_4OAc) minimizes sodium effect, and with a concentration of only 2mM the signal increased by more than 20 fold.



Adducts and clusters formed in LC/MS

Cluster/Adduct Ion	Source of cluster	Occurrence (Polarity)	m/z of Cluster Ion
$[M + NH_4]^+$	Ammonia	positive	M + 18
$[M + Na]^+$	Sodium salts	positive	M + 23
$[M + K]^+$	Potassium salts	positive	M + 39
$[M + CH_3CN+H]^+$	Acetonitrile	positive	M + 42
$[M + CH_3OH+H]^+$	Methanol	positive	M + 33
$[M + H_2O+H]^+$	Water	positive	M + 19
$[M + CH_3COO]^-$	Acetic acid	negative	M + 59
$[M + Cl]^-$	Chlorinated solvent	negative	M + 35

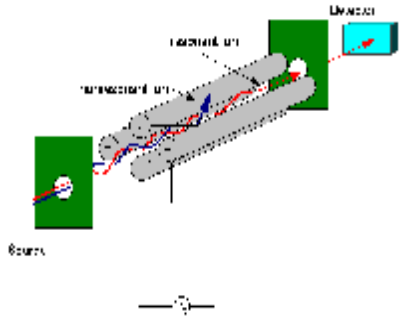
Analysis of Taurocholic Acid by LC-MS



Normally run in negative mode this standard has been run in positive mode to show the ammonium adduct (+ 17 amu) sodium adduct (+ 22 amu) formation di-sodium adduct (+ 44 amu) and potassium adduct (+ 38 amu).

How ions move in a quadrupole

- Forces of repulsion / attraction, combined with kinetic energy of ions (velocity in the quadripole) will impose a oscillating trajectory to ions throughout the quadrupole.

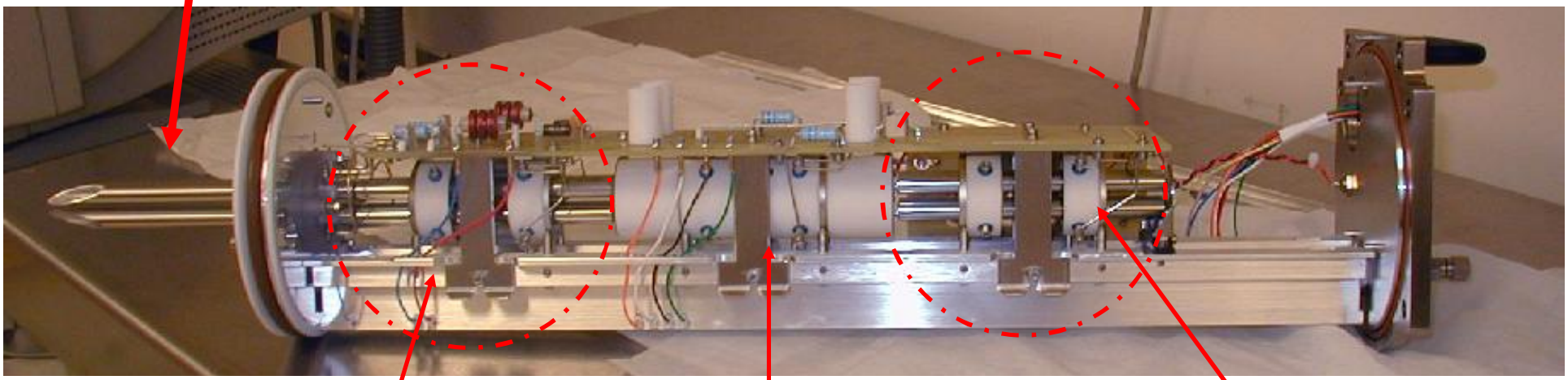


- Two opposite quadrupole rods have a positive, the other two a negative polarity
- The polarities of the two rod pairs are exchanged with high frequency (RF)
- Ions will be trapped circulating between these four rods
- If the potential in front of the quadrupole is higher than at the end, these ions will be pushed through the four quadrupole rods on "corkscrew like" trajectories

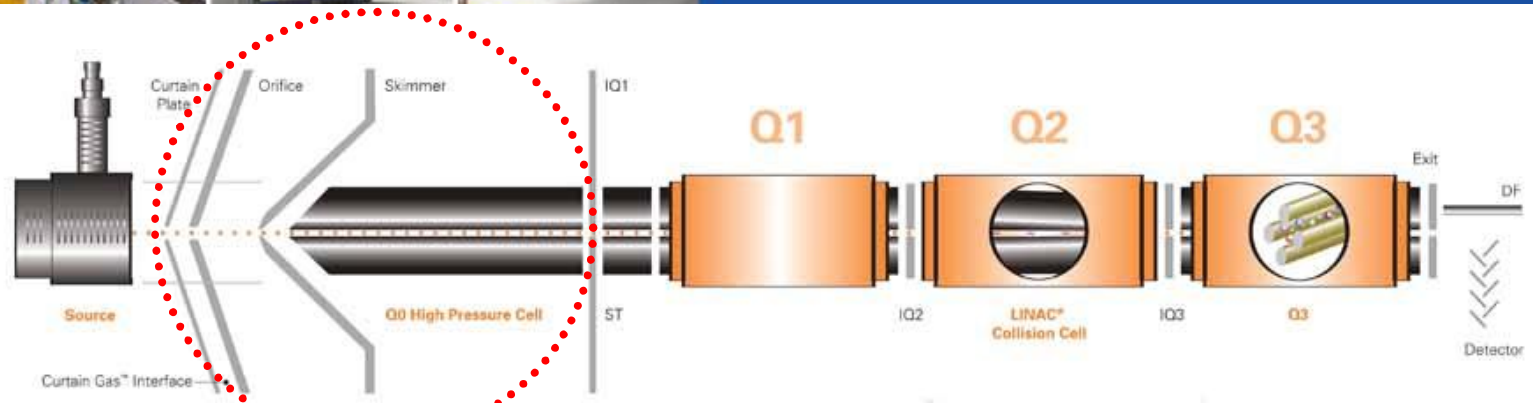
A quadrupole can:

- Transfer ions
- Scan ions (sort according their m/z values)
- Filter ions with specified m/z values

QQQ Ion Path

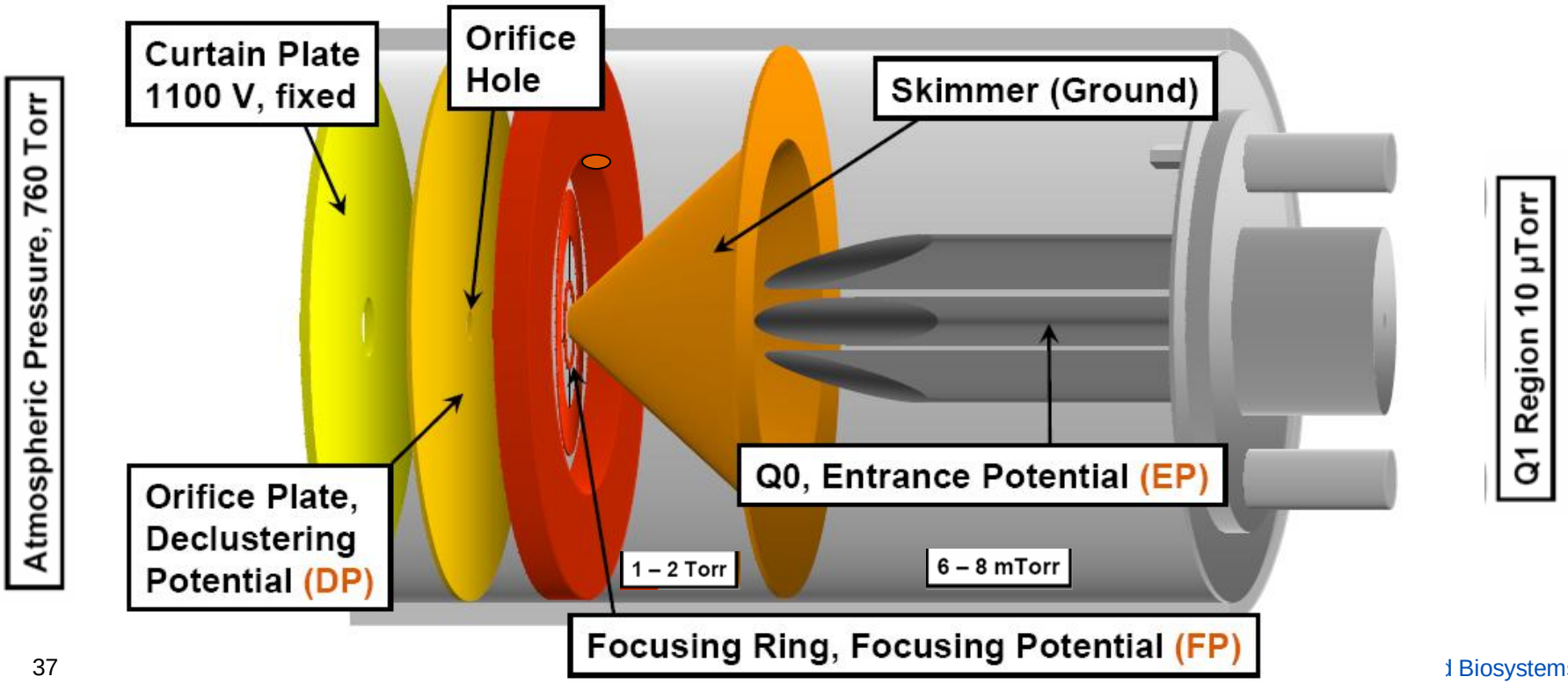


The detector is not visible in this picture



API 2000 – Systems Interface

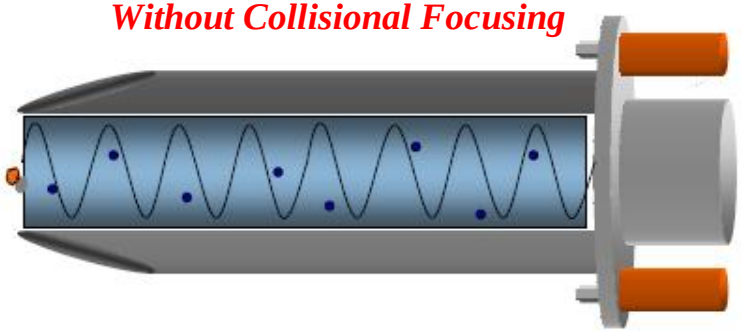
API 2000 – Differentially Pumped Interface and Curtain Gas



Collisional Focusing

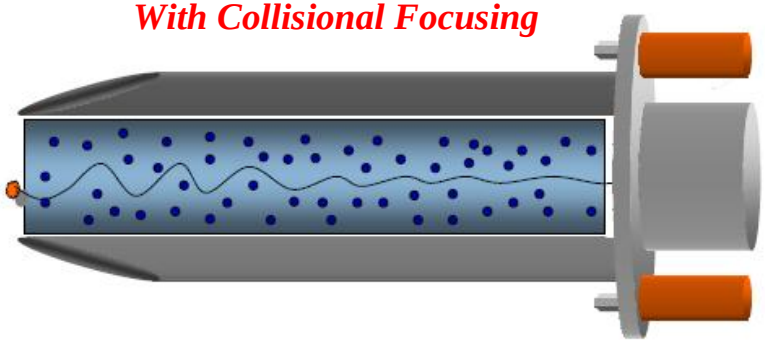
**Collisional focusing* is a patented technique, owned by ABI-Sciex, which improves ion transmission in Q0 and Q2… More later about Q2

Without Collisional Focusing



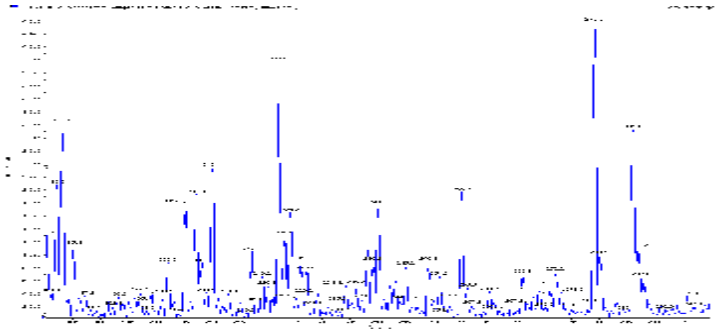
- Lower gas pressure at higher vacuum <8 mTorr
- Not a lot of gas molecules for the ions to collide with
- More "space" for the ions to travel, resulting in a longer time it takes for them to pass through Q0 and enter Q1

With Collisional Focusing



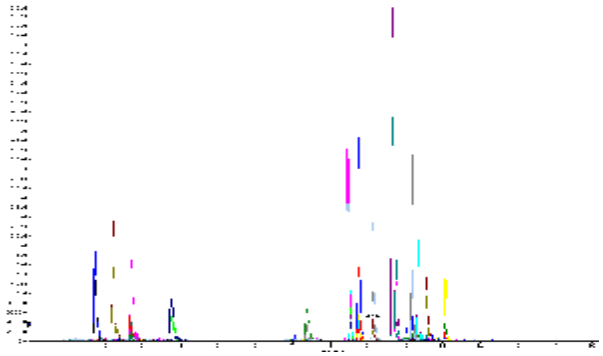
- Higher gas pressure at lower vacuum 8 mTorr
- More gas molecules for the ions to collide with which limits the "space" for the ion path
- Shortens the time it takes for them to pass through Q0 and enter Q1

- A Quadrupole Mass analyzer can operate in two modes:



- *Scanning* Mode

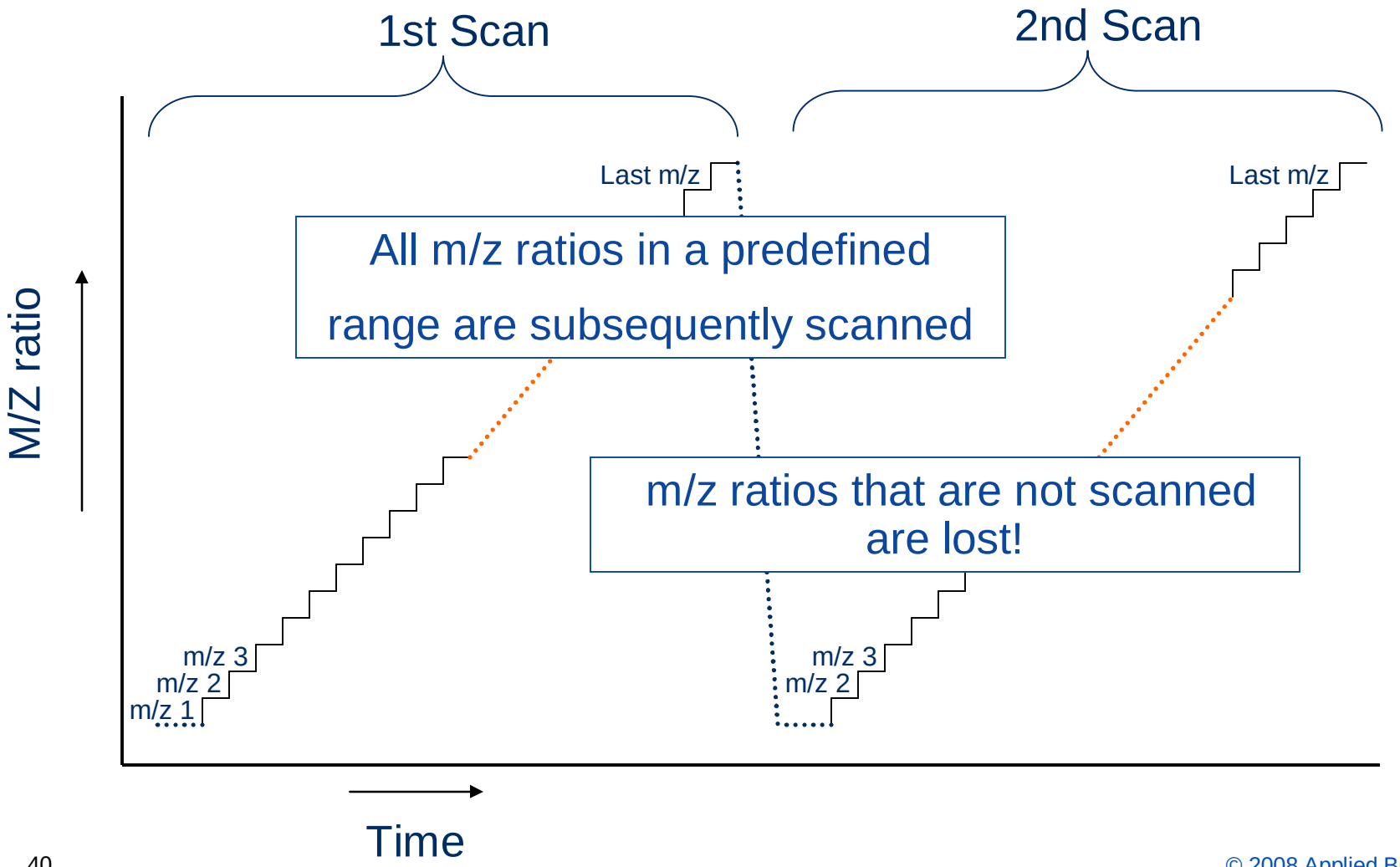
  A pre-defined range of m/z ratios is monitored



- *Selected Ion Monitoring (SIM) Mode*

  The Mass Analyzer monitors only 1 or a few m/z ratios

Scan Mode



Mass Filtering

A combination of a *high frequency Rf* voltage and a *DC* Voltage is applied to the rods

↓

An electrical field is created inside the quadrupole

↓

Only ions of *one* particular *m/z ratio* that enter the quadrupole field follow a *stable* trajectory

↓

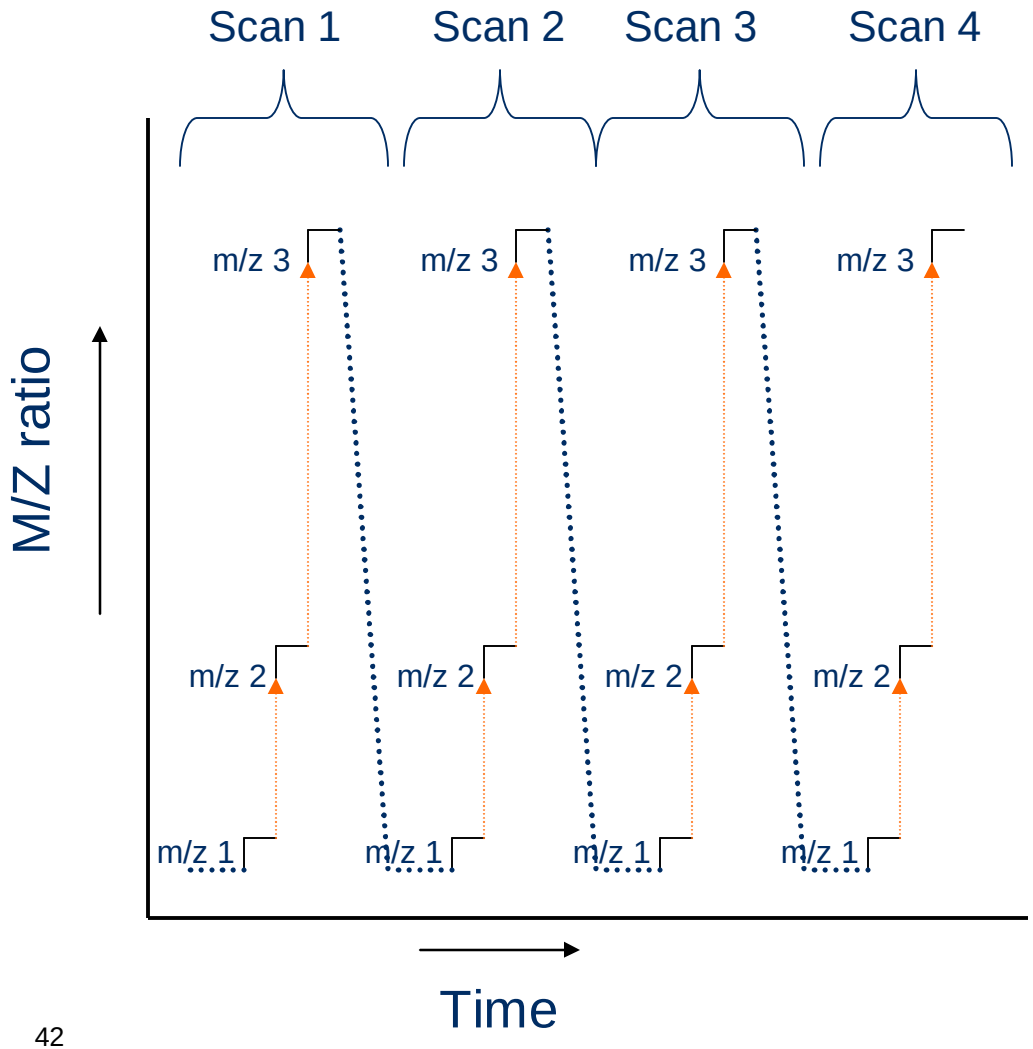
The ions of this m/z ratio can *pass* the quadrupole and *exit* at the end

↓

Ions of *other m/z ratio* follow an *unstable* trajectory, hit the rods and are *lost*

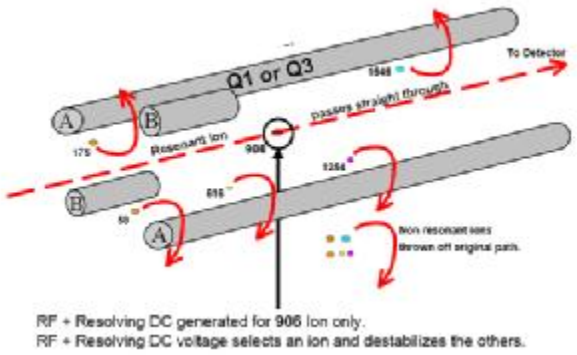
Mass Filtering

SIM Mode

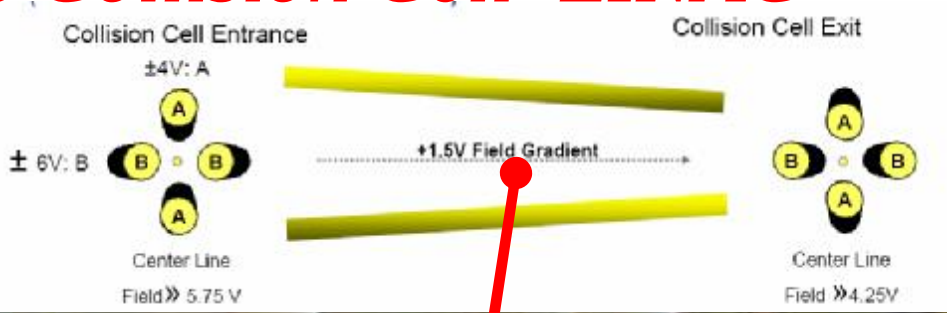


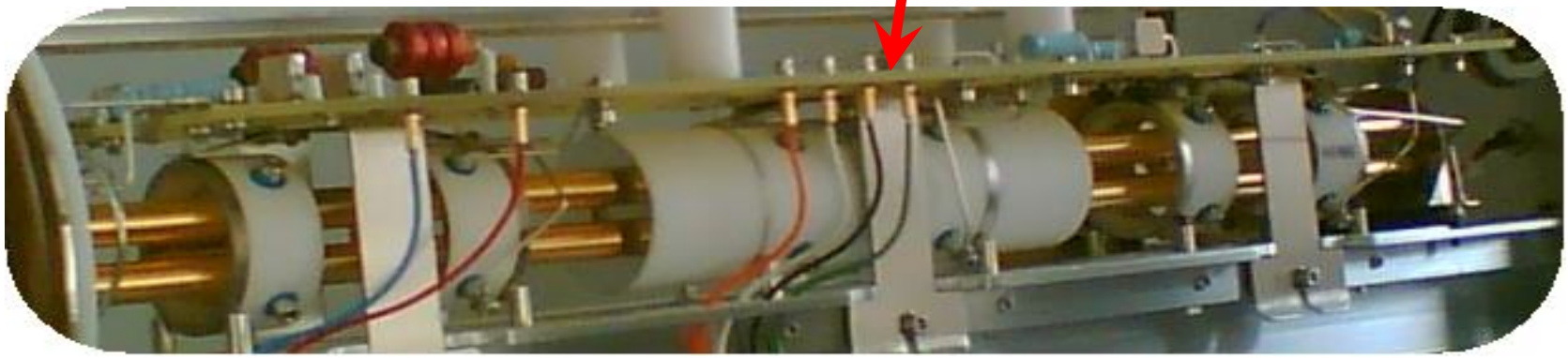
Only one or a few m/z ratios are subsequently scanned

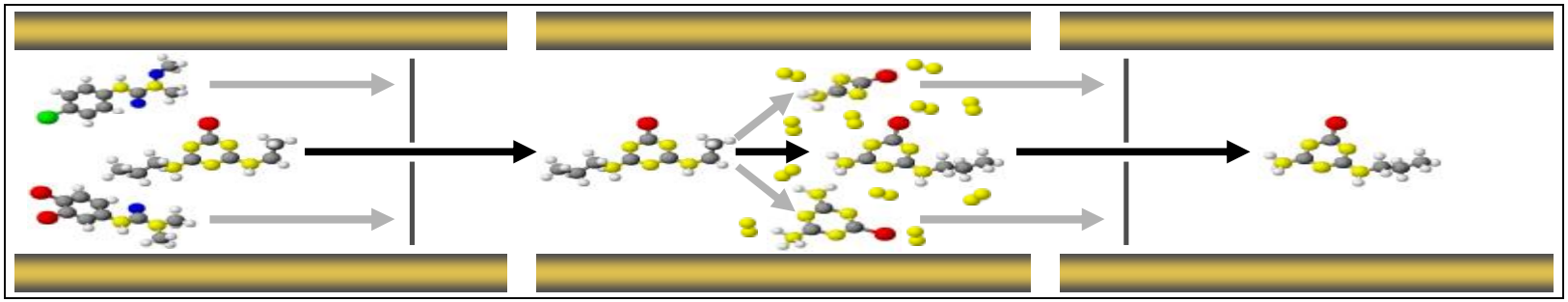
m/z ratios that are not scanned are lost!



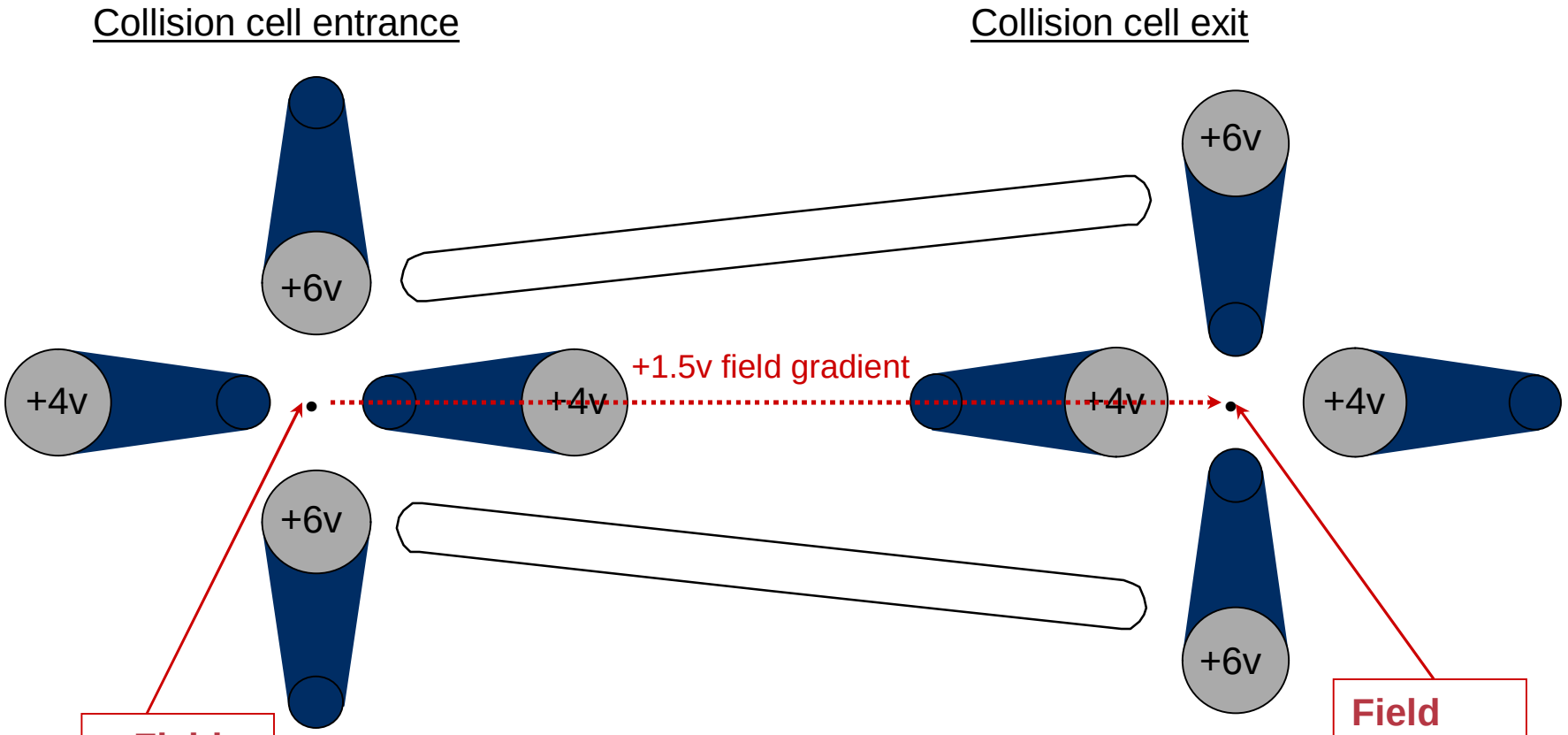
The Collision Cell "LINAC ®"





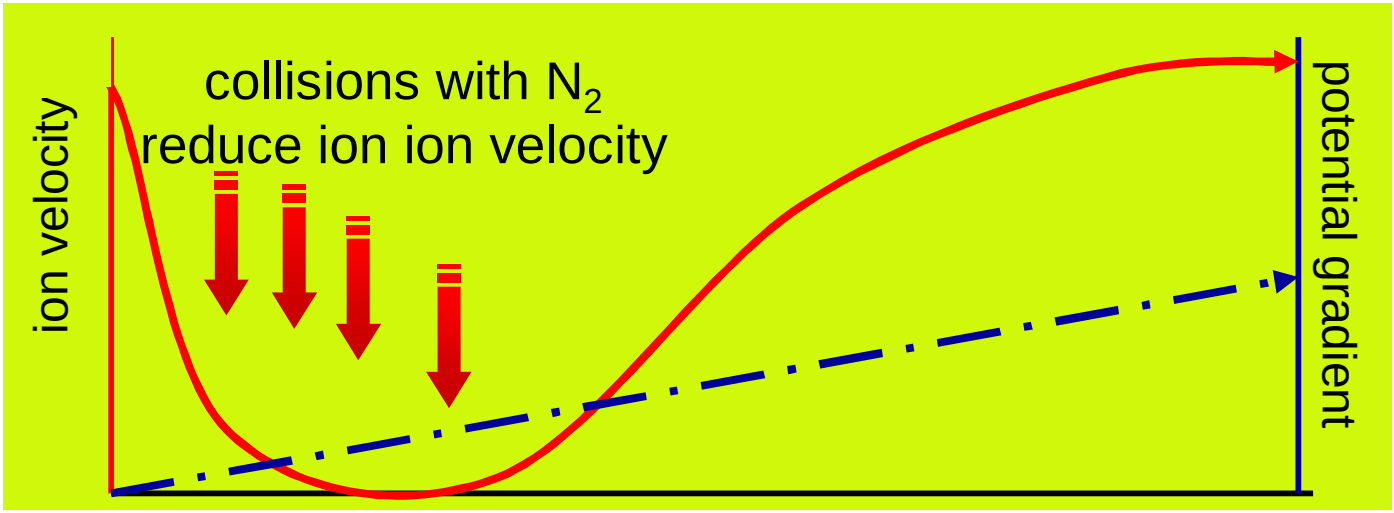


Q_2 = collision cell = LINAC™(LIN*ear* AC*celerator*)



- Because of the Field gradient, ions pass through the collision cell much faster then standard collision cells
- Q2 LINAC® Collision Cell eliminates cross-talk and allows faster MS/MS scanning without sensitivity losses
- Q2 rods are tilted and separate DC potentials are applied to each pair of rods to create an axial electric field

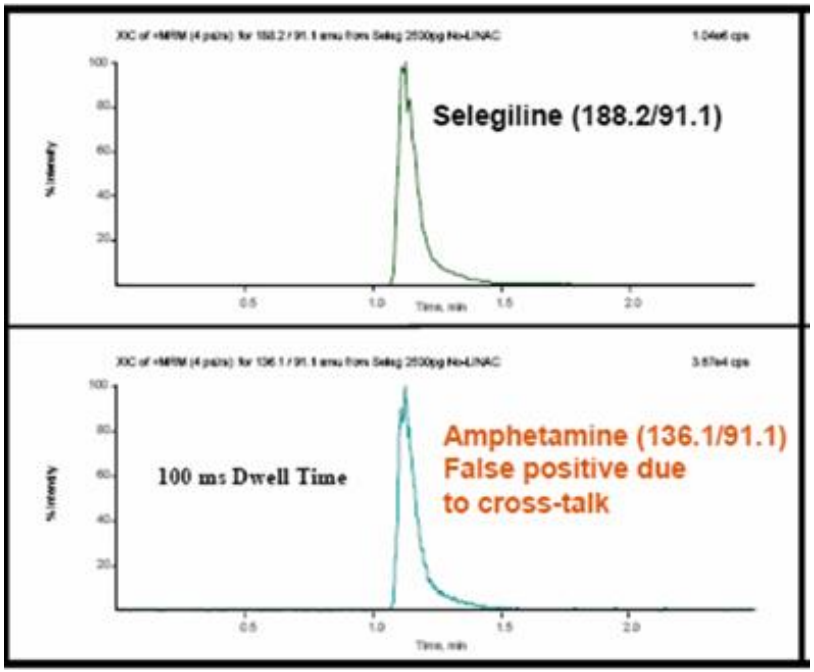
The Collision Cell (Linear Accelerator, LINAC)



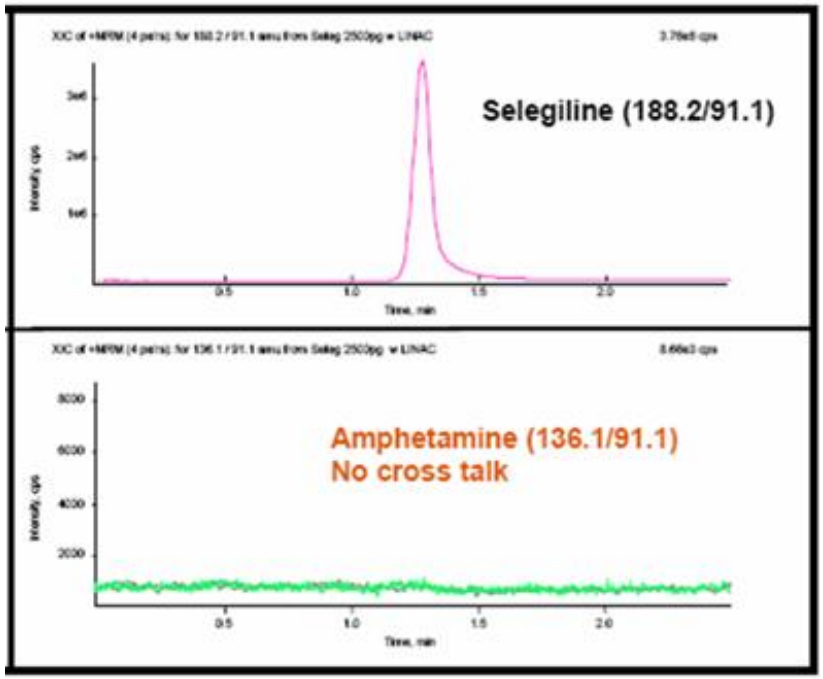
- Q2 Linac eliminates cross-talk allows faster MS/MS scanning without sensitivity loss

- Q2 Linac eliminates cross-talk allows faster MS/MS scanning without sensitivity loss

Without LINAC® Collision Cell

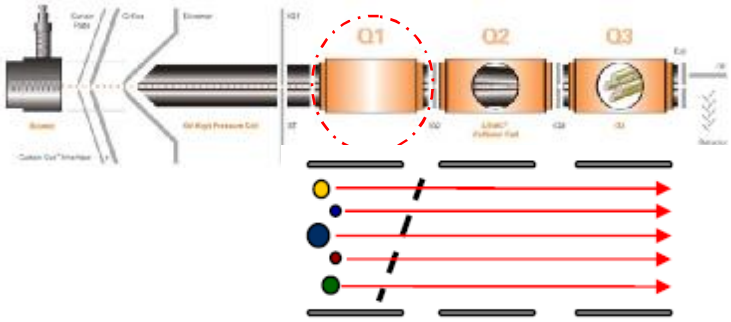


With LINAC® Collision Cell

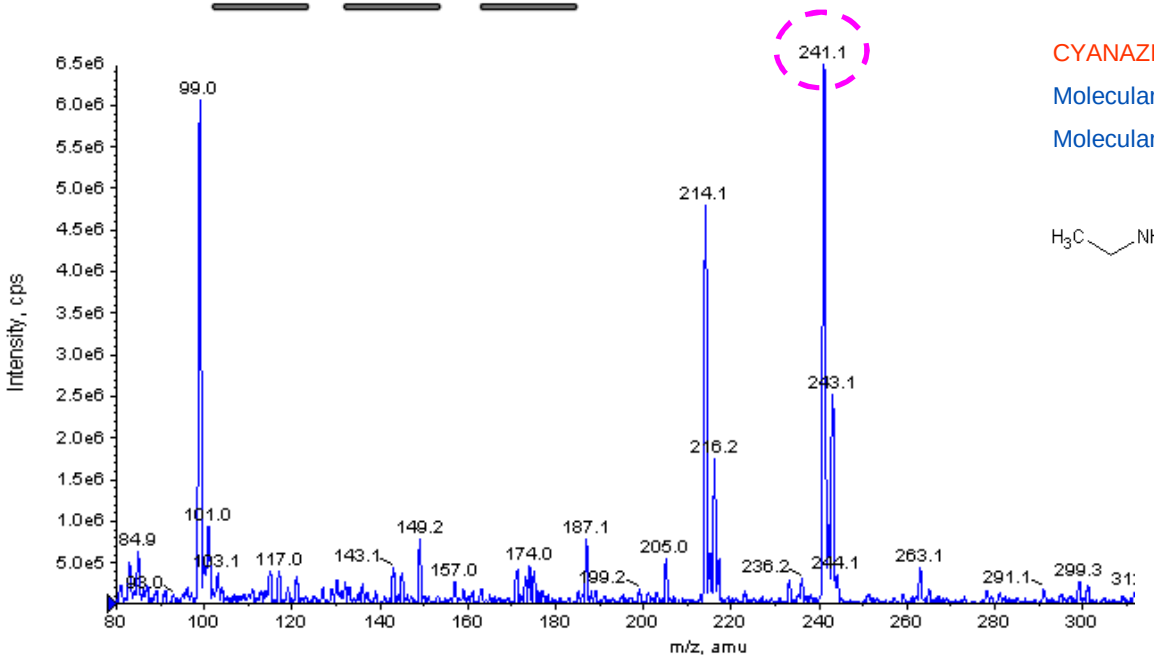


Q1 scan (or Q3 Scan): full scan (Start – Stop)

Ø Only one specific m/z passes through the rods at any one time. When the detector receives a signal (ions), we can extrapolate back to which voltage is on the rods, and thus know the m/z of the ions.



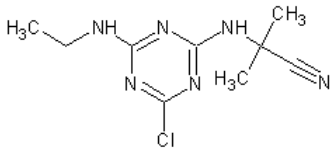
Ø Which ions are present?
Ø Q1 usually acts as the single MS analyzer.
Ø Used primarily for identity of precursor ions.
Ø Q3 operates in RF-only mode



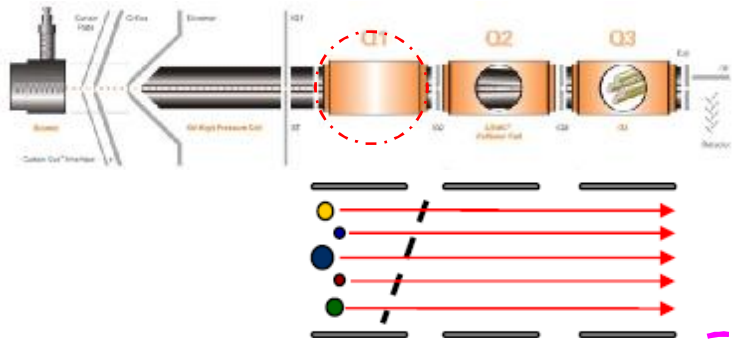
CYANAZINE

Molecular formula: $C_9H_{13}ClN_6$

Molecular weight: 240.7



Q1 scan (or Q3 Scan): full scan (Start – Stop)



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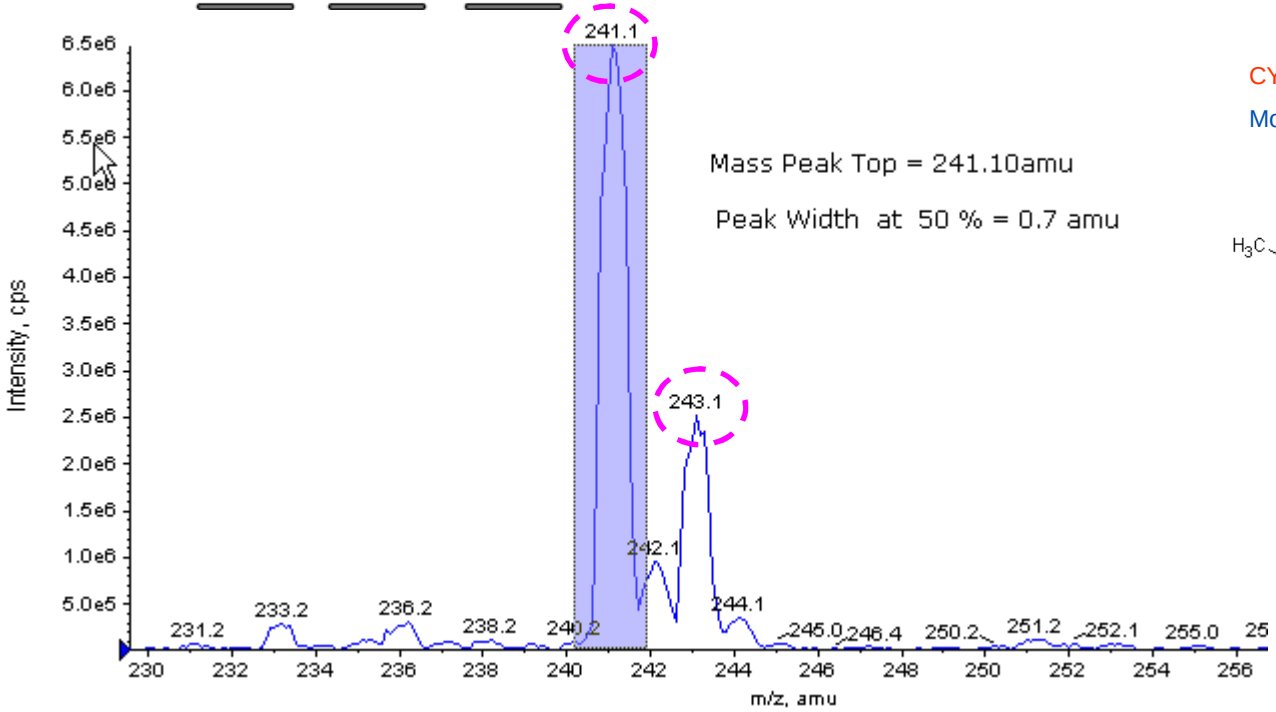

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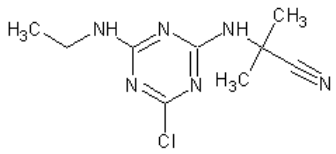
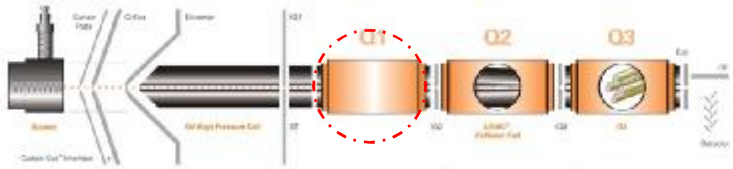
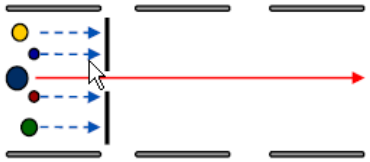
Molecular formula: $C_9H_{13}ClN_6$

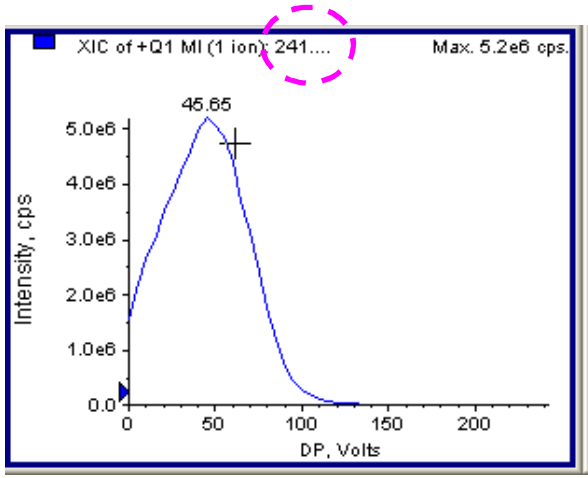


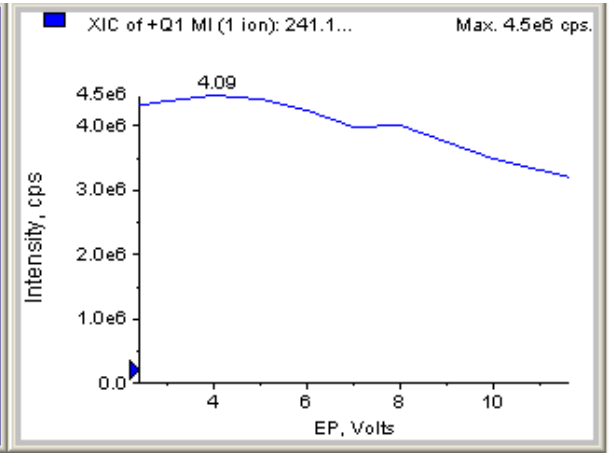
Q1 Multiple Ion (Single Ion Monitoring)





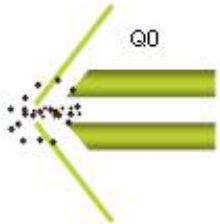
- Used to optimize analyzer for specific ions
- Used for quantitative analyse
- Used to “optimize” DP, EP, precursor ion
- Maximize signal in preparation for MS/MS



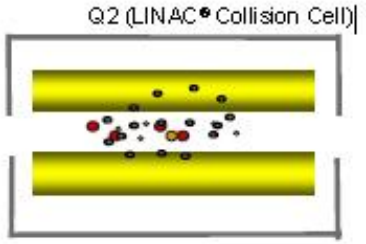


Tandem mass spectrometry: *Fragmentation*

- With singly charged ions, the following formulas apply:
  - Positive mode: $[Precursor]^+ \rightarrow Product^+ + Neutral$
  - Negative mode: $[Precursor]^- \rightarrow Product^- + Neutral$
- Collisional energy is converted into vibrational energy, and bonds break
- Two modes of fragmentation on Sciex Instruments:
  - Collision-Induced Dissociation (CID) or in-source fragmentation
    - With DP energy, Ions collide with N_2 from curtain gas
    - fragmentation occurs between orifice and skimmer

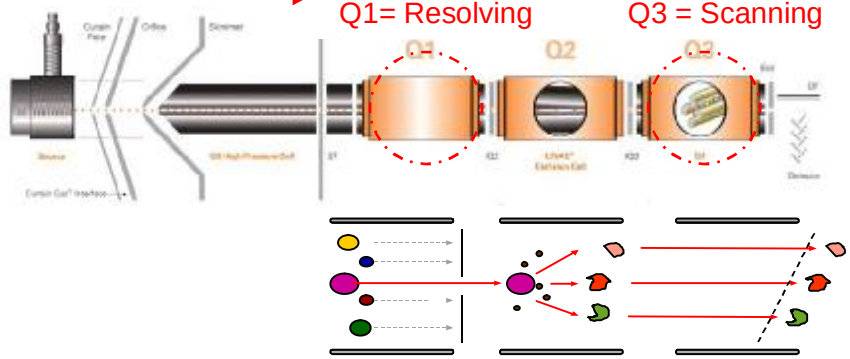


  - Collisionally Activated Dissociation (CAD)
    - With CE energy, ions collide with N_2 from CAD gas
    - fragmentation occurs in Q2 collision cell



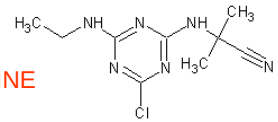
Product Ion Scan (MS²) - Fragmentation

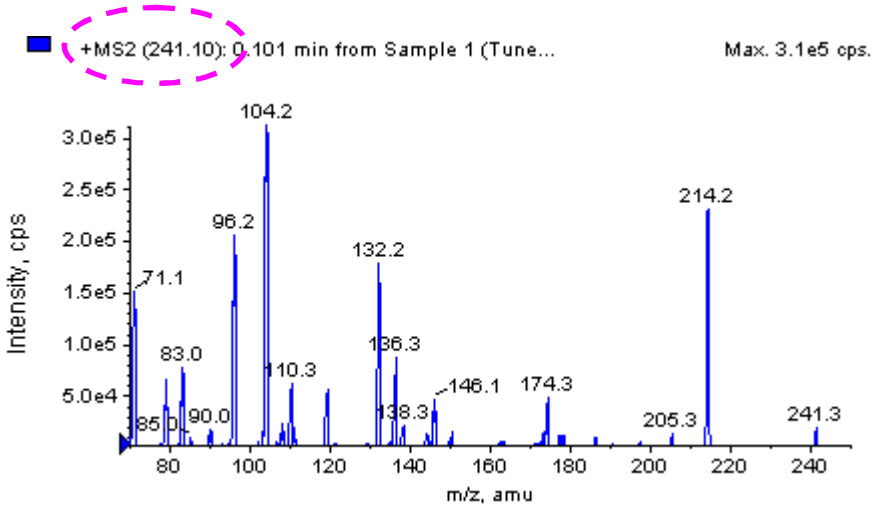
Continuous Ion Flow

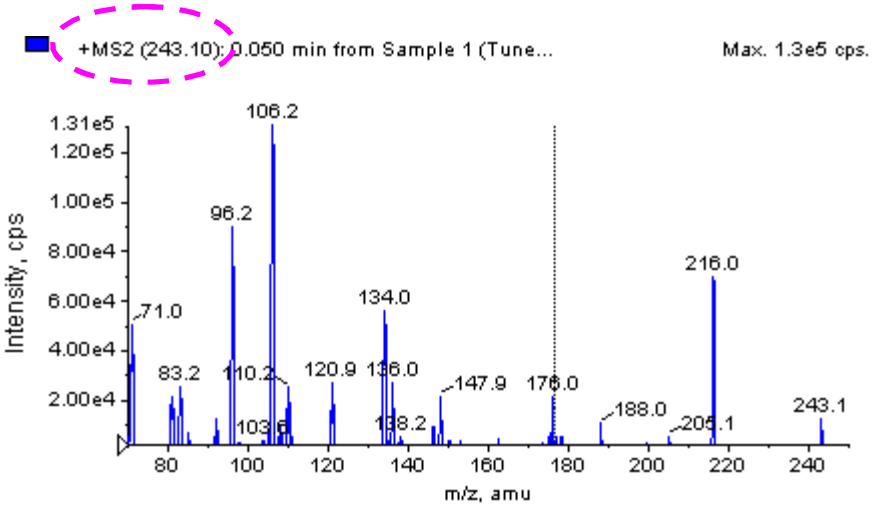


- Used for structural information and identification of product ions
- After identification, the precursor ion is sent into the collision cell and fragmented by nitrogen
- Q1 is fixed, filters precursor ions of interest
- Q3 sweeps a given mass range

CYANAZINE

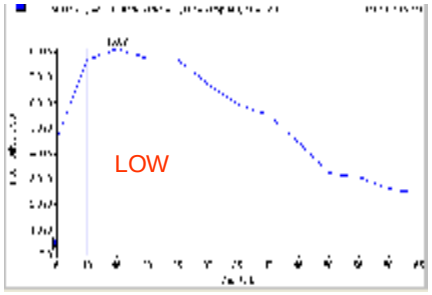


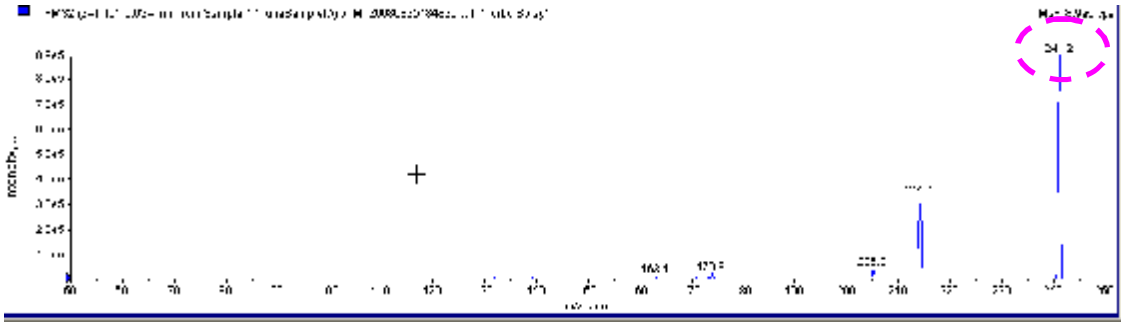


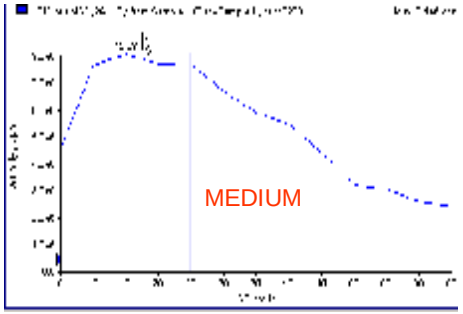


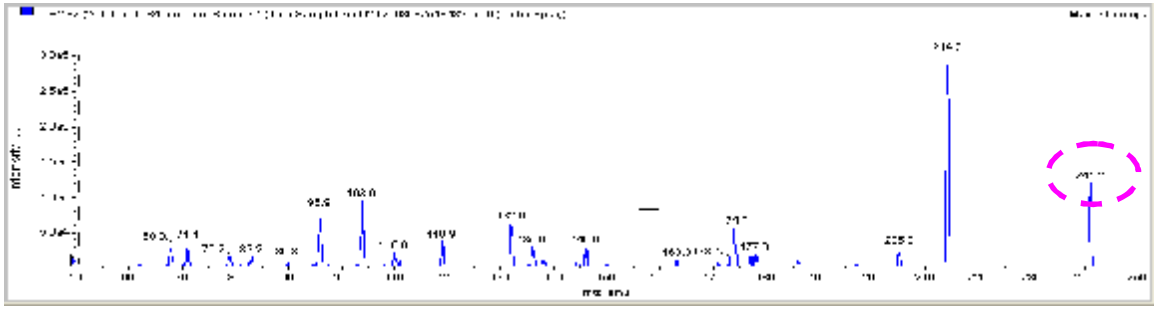
Mass spectrometry can distinguish isotopes: atomic weight of chlorine is 35.5, but the mass spectrum show two distinct ions at m/z 35 and 37 .

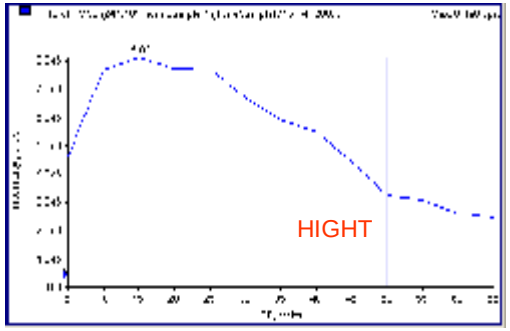
collision energy / fragmentation

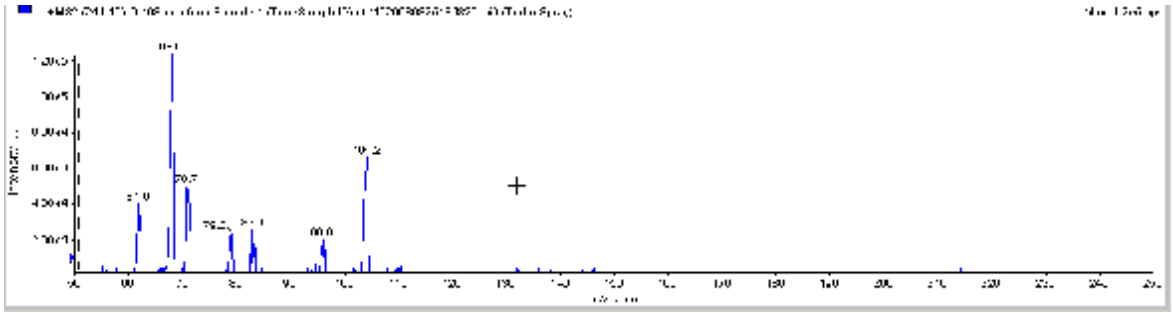




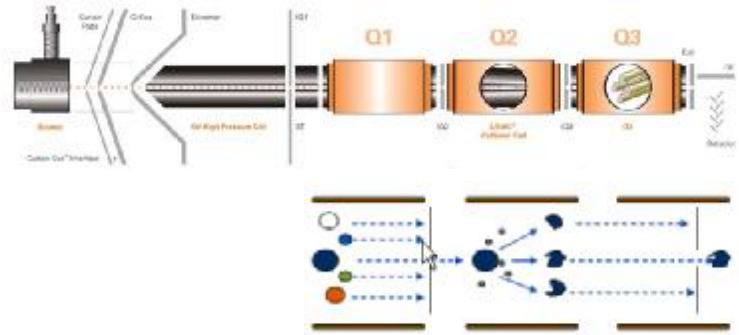








MRM (Multiple reaction Monitoring)

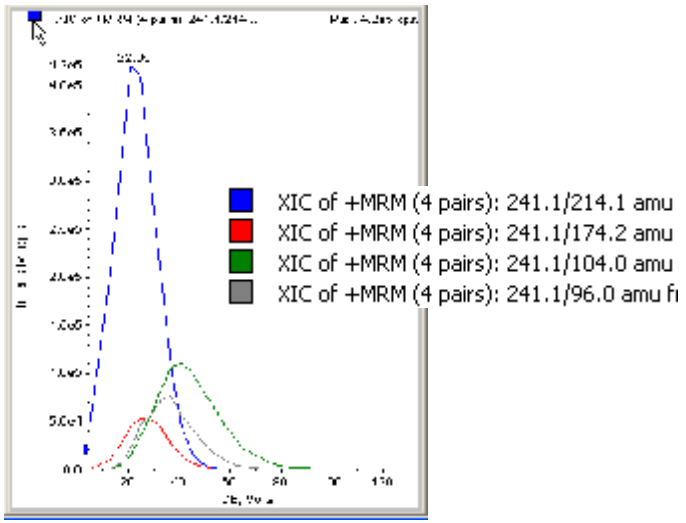


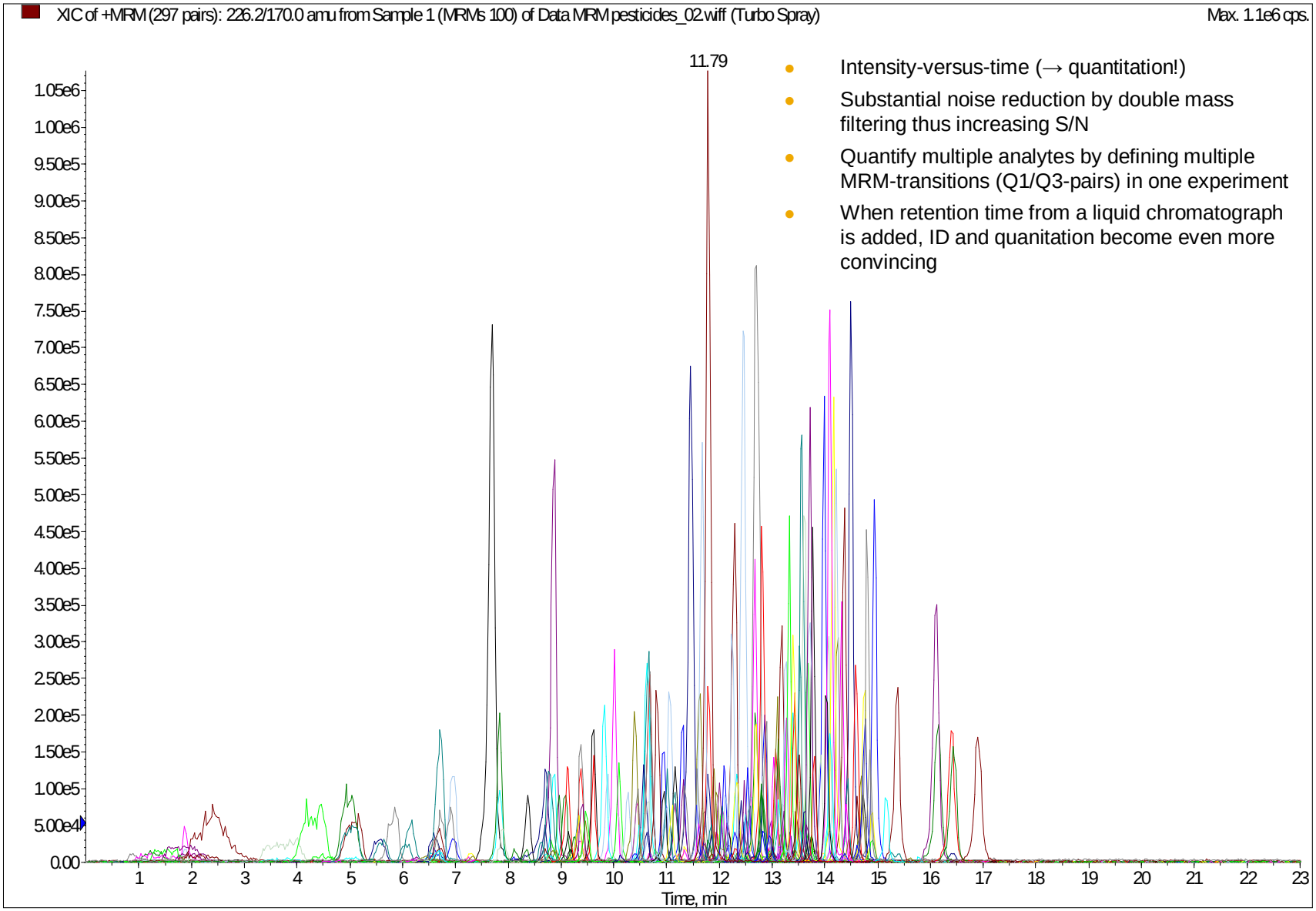
1. Q1 filters the analyte mass (= precursor ion)
2. Q2 fragments this mass
3. Q3 filters the mass of one of its fragments (= product ion) = Multiple Reaction Monitoring (MRM)

Many precursor to product ion pairs can be monitored.
MRM analysis is the best way to maximize signal/noise ratio of compounds.
MRM used primarily for quantitation studies.

	Q1 Mass (a	Q3 Mass (a	Time (msec
1	241.100	214.100	100.0
2	241.100	174.200	100.0
3	241.100	104.000	100.0
4	241.100	96.000	100.0
5			

HIGHLY SPECIFIC
HIGHLY REPRODUCIBLE

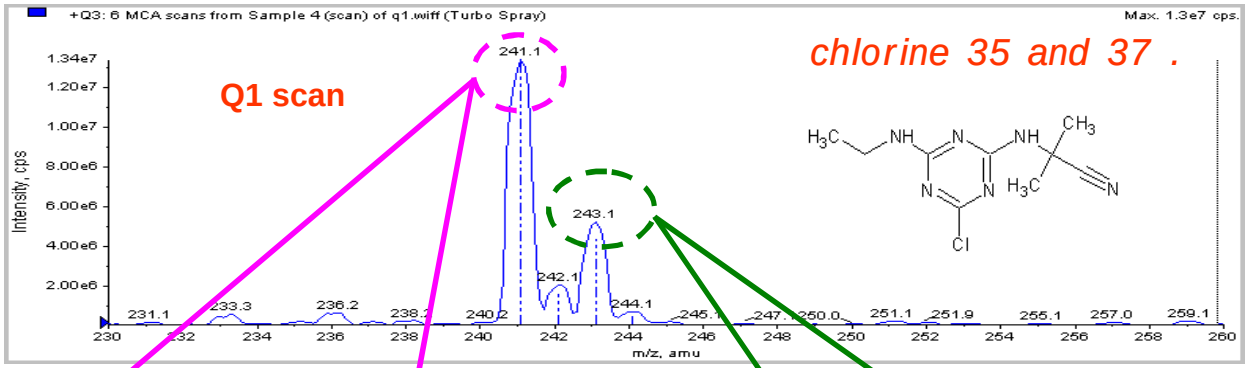


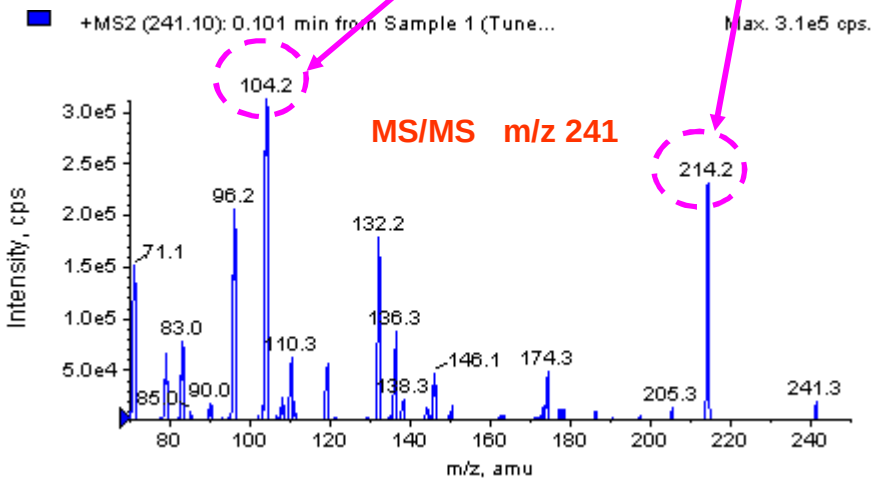


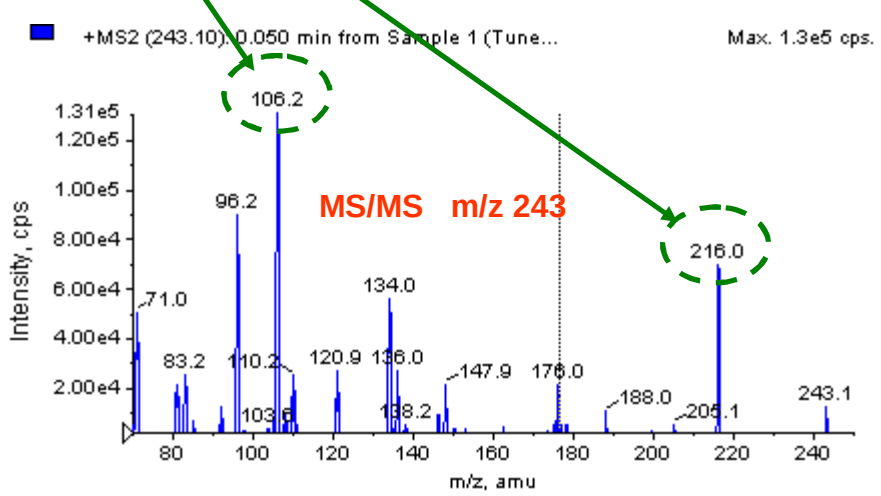
- Intensity-versus-time (→ quantitation!)
- Substantial noise reduction by double mass filtering thus increasing S/N
- Quantify multiple analytes by defining multiple MRM-transitions (Q1/Q3-pairs) in one experiment
- When retention time from a liquid chromatograph is added, ID and quanitation become even more convincing

Additional MSMS scans

Precursor Ion Scan - Neutral Loss Scan





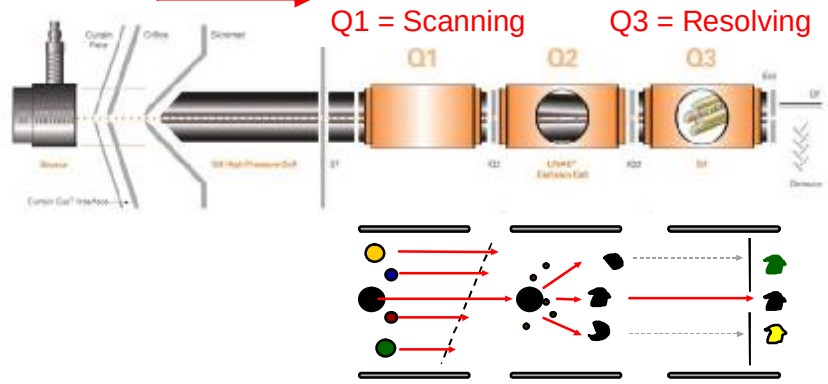


Precursor Ion Scan

Additional MSMS scans

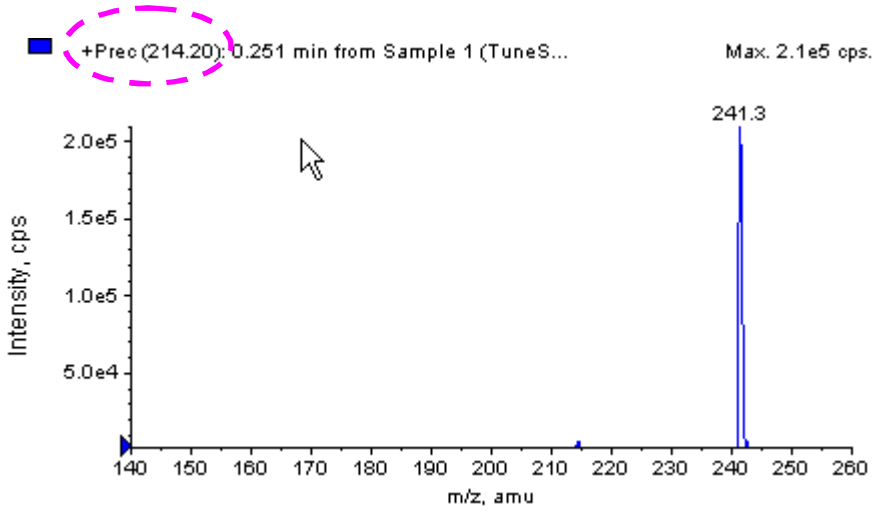
Continuous Ion Flow

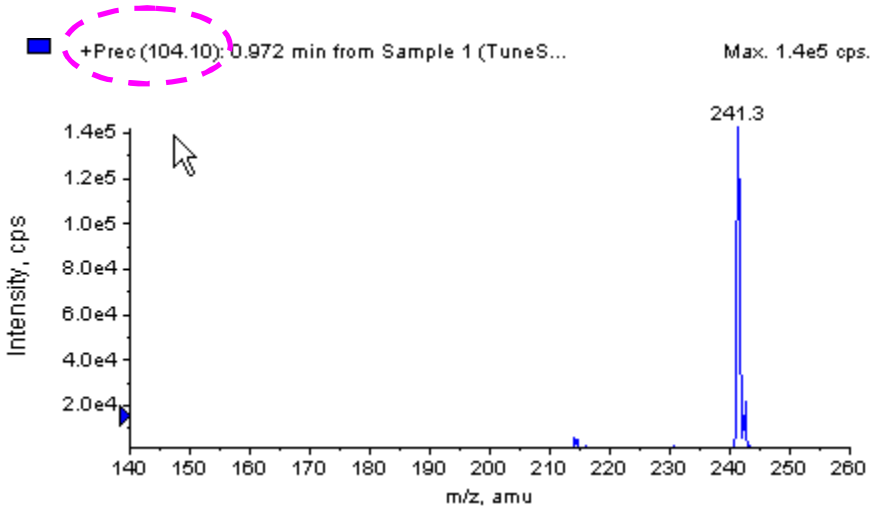
Q1 is scanning while Q3 is filtering the mass of a charged fragment



- Research of ions sharing the same fragment
- Q3 is fixed
- Q1 sweeps a given mass range
- Used to determine the "origin" of particular product ion(s) created in the collision cell
- Frequently used for drug metabolite identification (common product ion observed in the metabolites)

Q1 mass: 241.1 → **Q3 mass: 214.2**, **Q3 mass: 104.1**



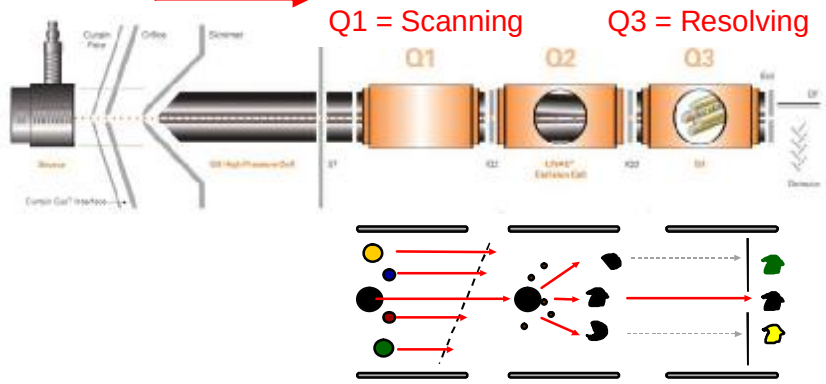


Additional MSMS scans

Precursor Ion Scan

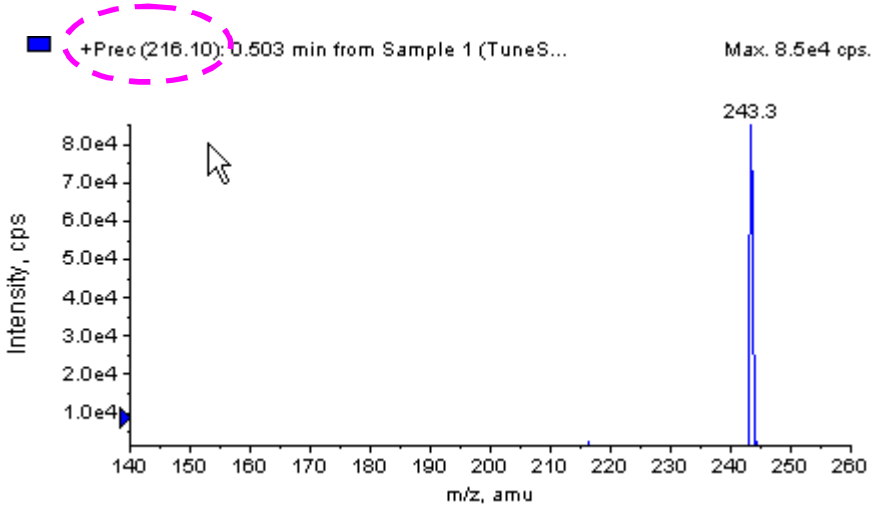
Continuous Ion Flow

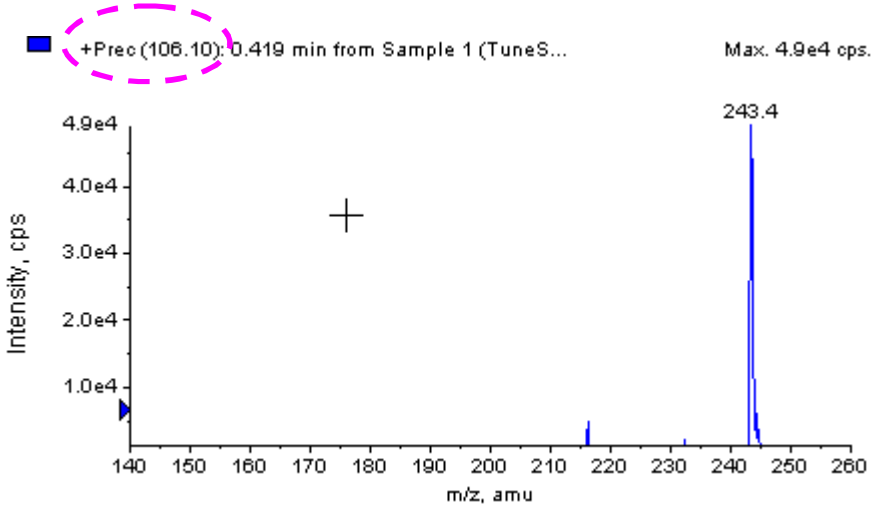
Q1 is scanning while Q3 is filtering the mass of a charged fragment



- Research of ions sharing the same fragment
- Q3 is fixed
- Q1 sweeps a given mass range
- Used to determine the "origin" of particular product ion(s) created in the collision cell
- Frequently used for drug metabolite identification (common product ion observed in the metabolites)

Q1 mass: 243 → **Q3 mass: 216.1**, **Q3 mass: 106.1**

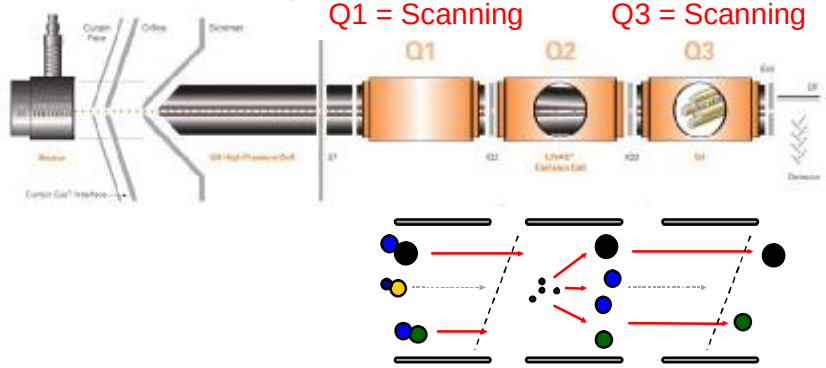




Additional MSMS scans

Neutral Loss Scan

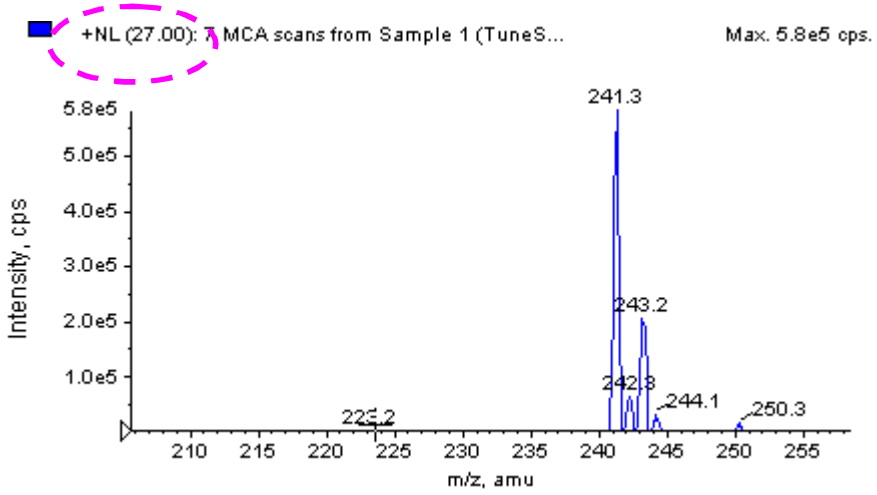
Continuous Ion Flow

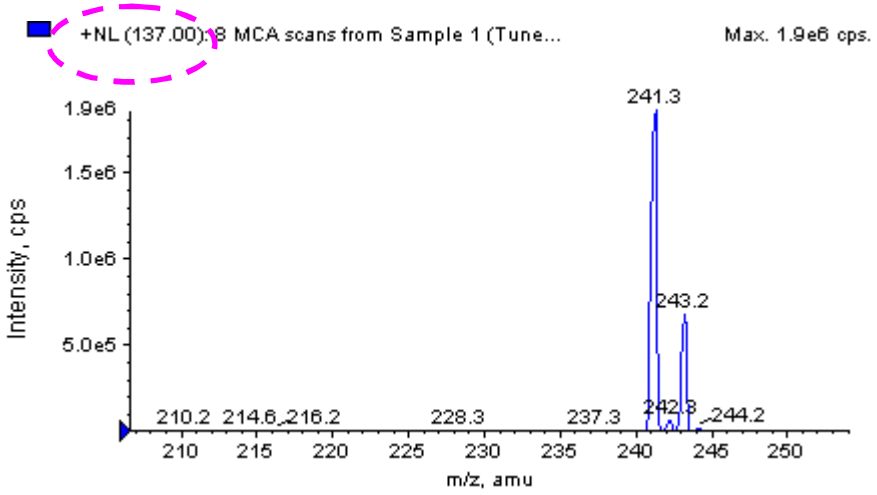


- Q1 & Q3 both scan a given mass range but with a constant difference between the ranges scanned
- Spectrum indicates which ions lose a neutral species equal to Q1 - Q3 difference
- Complement to Precursor Ion Scan
- Neutral "gain" indicates a multiply charged precursor ion was fragmented

Q1 mass: 241.1 → Loss of: 27 → **Q3 mass: 214.2**

Q1 mass: 241.1 → Loss of: 137 → **Q3 mass: 104.1**





Applications

- Food Analysis (pesticides, mycotoxins, acrylamide, antibiotics, nitrofuranes, artificial colors, etc.)
- Environmental Analysis (drinking water, eco-systems, etc.)
- Development of pesticides, fungicides, etc.
- Forensics
  - Toxicity, alcohol control, drug abuse (cocain, etc.)
- Doping (EPO, testosterone, etc.)
- Clinical Analysis (neonatal screening, immunsuppresiva)
- .....

Thank you for listening