Basic theory and introduction of mas spectrometer

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Outline

- Basic MS
- Introduction MS
  - Ion Source: ESI/APCI Ionization
  - Mass Analyzer: QQQ Fundamental
    QTOF Fundamental

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Basic MS

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What is mass spectrometer?

Mass spectrometry (**MS**) is an analytical technique that measures the mass-to-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.

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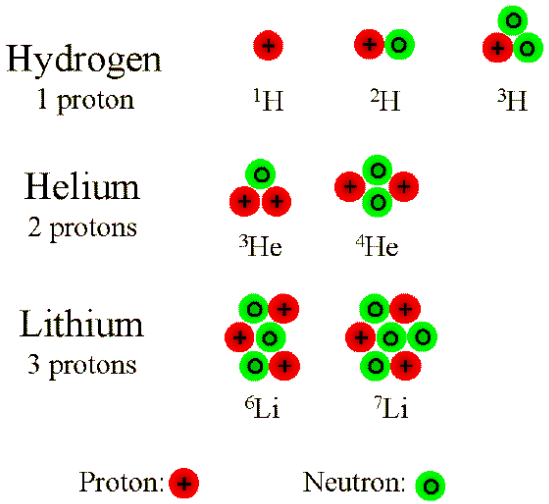
Isotope mass

Most elements are found in nature in form of different so called isotopes. Isotopes of an element have nuclei with the same number of protons (the same atomic number) but different numbers of neutrons.



However, both, protons and neutrons contribute to the weight of an atom, which explains the difference in weight of isotopes.

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Most Elements Have More Than One Isotope

Element	Mass	Natural Abundance
H	**1.0078**	99.985%
	2.0141	0.015
C	12.0000	98.89
	13.0034	**1.11**
N	14.0031	99.64
	15.0001	0.36
O	15.9949	99.76
	16.9991	0.04
	17.9992	0.20
F	18.9984	100
S	31.9721	95.00
	32.9715	0.76
	33.9679	4.22
	35.9671	0.02

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About masses

Integer mass:

Sum of the integer masses of the elements composing the molecule, e.g. $C_6H_{12}O_6$

Integer mass = 6*12+12*1+6*16=180 Da

Monoisotopic mass:

Definition see last slide

Monoisotopic mass = 6*12.000000+12*1.007825+6*15.994915=180.06339 Da

Average mass:

The sum of the average masses of the elements (weighted average of all stable isotopes of an element) composing the molecule

Average mass = 6*12.011+12*1.00794+6*15.994=180.15768 Da

Mass errors – absolute vs. relative

Absolute mass error (Da or mDa)

$$= \textit{Theoretical Mass} - \textit{Measure Mass}$$

$$= 180.06339 - 180.0657$$

$$= -0.00231 \text{ Da}$$

Relative mass error (ppm)

$$= \frac{\boldsymbol{Theoretical\ mass - Measure\ mass}}{\boldsymbol{Theoretical\ mass}} \times \mathbf{1,000,000}$$

$$= \frac{180.06339 - 180.0657}{180.06339} \text{x} 1{,}000{,}000$$

$$= -12.82 \text{ ppm}$$

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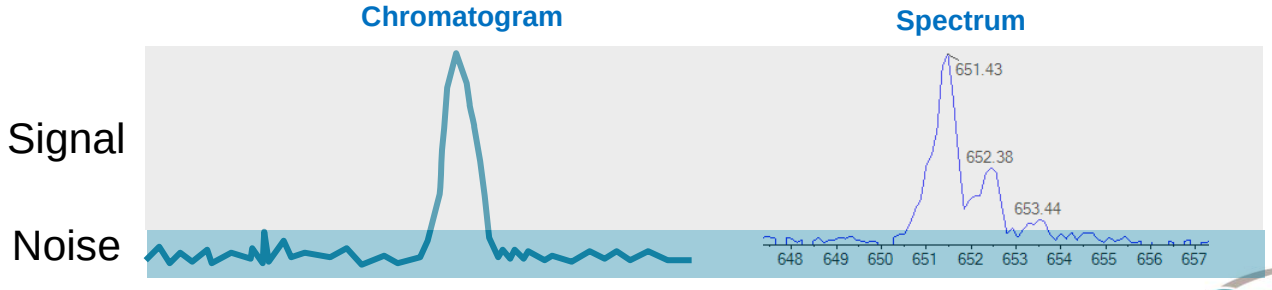
LCMS Specifications terminologies

- What is Signal to noise ratio?

- **Signal** carries information about the analyte that is of interest to us.
- **Noise** is made up of extraneous information that is unwanted because it degrades the accuracy and precision of an analysis
- **Signal-to-Noise Ratio**

Signal-to-noise (S/N) is much more useful figure of merit than noise alone for describing the quality of an analytical method. The magnitude of the noise is defined as the standard deviation s of numerous measurements and signal is given by the mean x of the measurements. S/N is the reciprocal of the relative standard deviation. S/N < 2 or 3 → impossible to detect a signal.



http://faculty.atu.edu/abhuiyan/Course/Chem%204414/Chapter%205.ppt

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LCMS Specifications terminologies

- What is dynamic range?
- Dynamic range is the difference between the smallest and largest usable signal through a transmission or processing chain or storage medium. It is measured as a ratio, or as a base-10 (decibel) or base-(doublings, bits or stops) logarithmic value.

  https://en.wikipedia.org/wiki/Dynamic_range

- Orders of magnitude (log base 10)
  - 1 – 10 = 1 order
  - 1 – 100 = 2 orders
  - 1 – 1,000 = 3 orders
  - 1 – 10,000 = 4 orders
  - 1 – 100,000 = 5 orders
  - 1 – 1,000,000 = 6 orders
- Concentration range of a certain analyte that can be detection on a system
- Minimum detection limit (LOD) would be the lowest value
- Maximum detection limit would be the largest value within linearity limits

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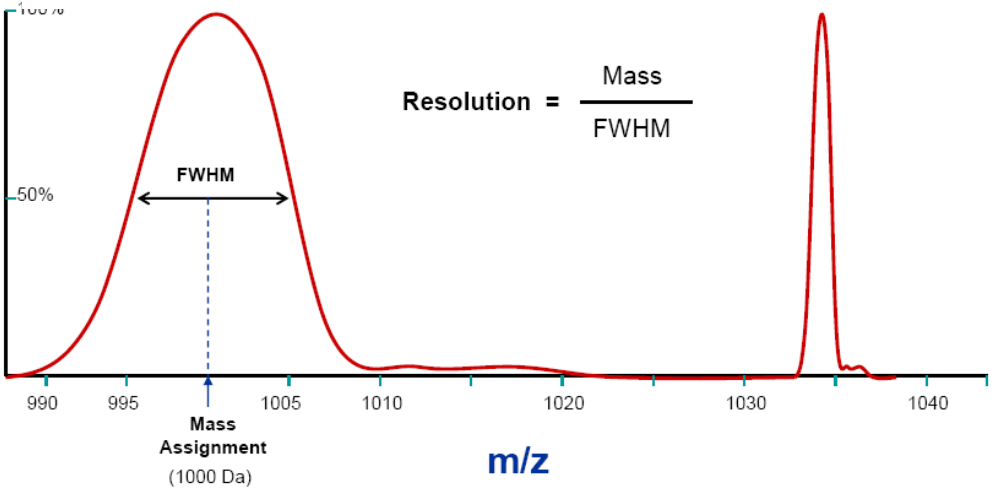
Resolution



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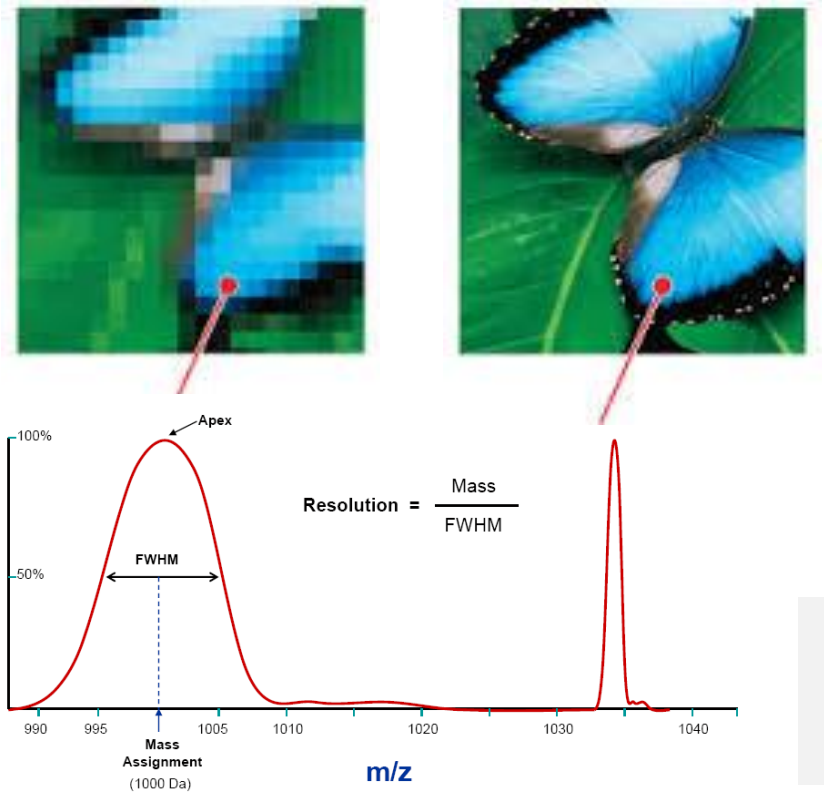
LCMS Specifications terminologies

- Low resolution vs High resolution



TripleQuads and QTRAP are known as nominal mass instruments (low resolution)

TOFs are know as high resolution instruments

System	Resolu-tion	Mass accuracy	Mass error
TQ	1000	0.1	100 PPM
QT	8000	0.01	10 PPM
TT	40000	0.0001	<1 PPM
X500R	40000	0.0001	<1 PPM

$$\text{Parts per Million (PPM)} = \frac{\text{Experimental mass - Theoretical mass}}{\text{Theoretical mass}} \times 10^6$$

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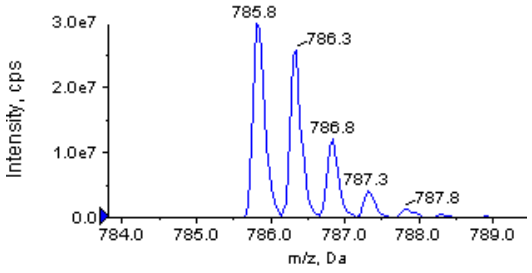
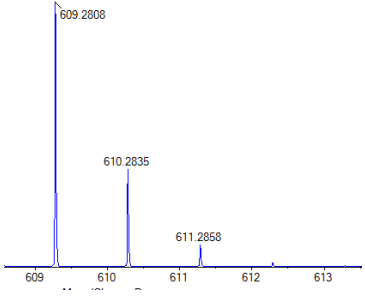
Low resolution MS

QQQ
QTRAP
Ion trap



High resolution MS

QTOF
Orbitrap
FTMS



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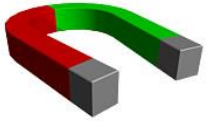
Why do we need ions?

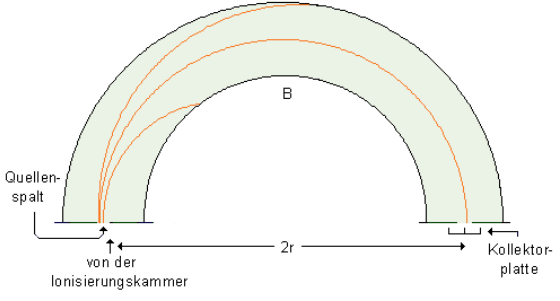
- We want to measure the mass of our analytes. For this we have to separate them according to their masses
- This is typically done in electromagnetic or magnetic fields

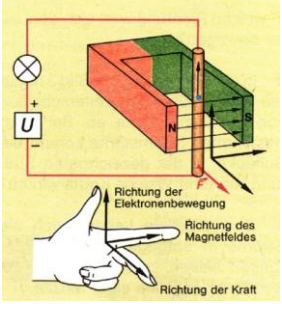


+

→ Well, what should happen???

- A neutral molecule cannot be influenced by such fields
- So we need ions which are effected by these fields!





„Lorentz force"

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Molecules and ions

A **molecule** is defined as a sufficiently stable electrically neutral group of at least two atoms in a definite arrangement held together by strong chemical bonds

An **ion** is an atom or molecule which has lost or gained one or more electrons, making it positively or negatively charged. A negatively charged ion, which has more electrons in its electron shells than it has protons in its nuclei, is known as an **anion** due to its attraction to anodes. Conversely, a positively-charged ion, which has fewer electrons than protons, is known as a **cation** due to its attraction to cathodes.

www.wikipedia.org

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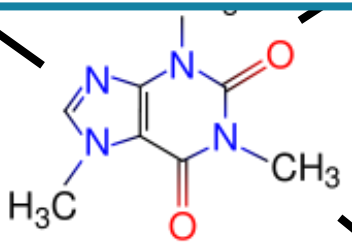
How are ions and molecules related?

Cations

$[C_8H_{11}N_4O_2]^+$

$[C_8H_{10}N_4O_2Na]^+$



$-H^+$

$+CH_3COO^-$

Caffeine

$[C_8H_9N_4O_2]^-$

$[C_{10}H_{13}N_4O_4]^-$

Anions

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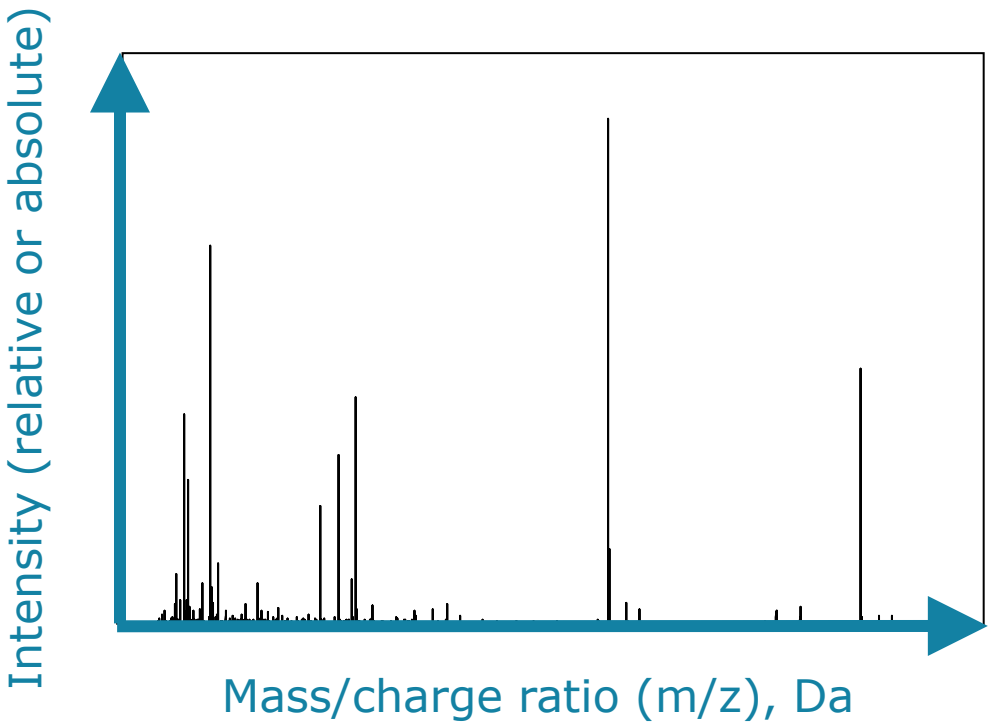
The mass spectrum



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The mass spectrum

The mass spectrum gives us information about

- the mass of a compound
- the charge of a compound
- the isotopic composition of a compound
- the abundance of the compound

It gives no information about the nature of the compound!

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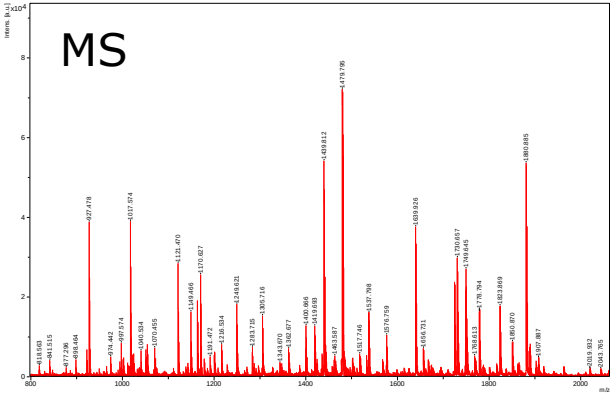
MS versus MS/MS

For an MS spectrum I record the m/z values of all the compounds in my sample

For an MS/MS spectrum, I select one of the signals from the MS spectrum, isolate the signal, fragment it and record all the m/z values of the resulting fragments



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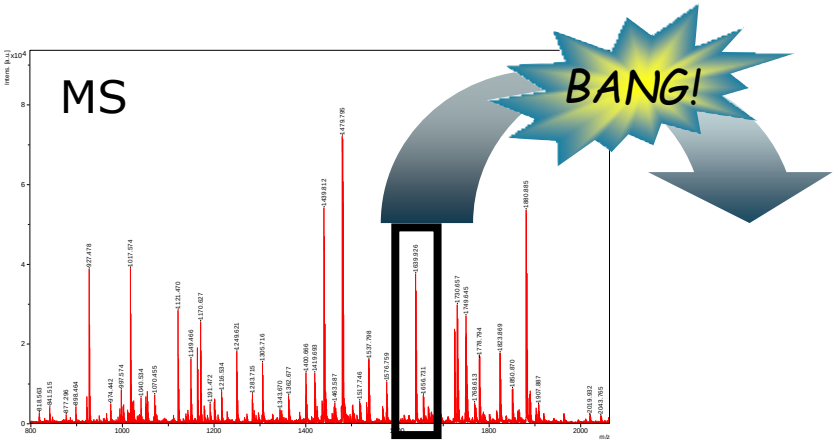

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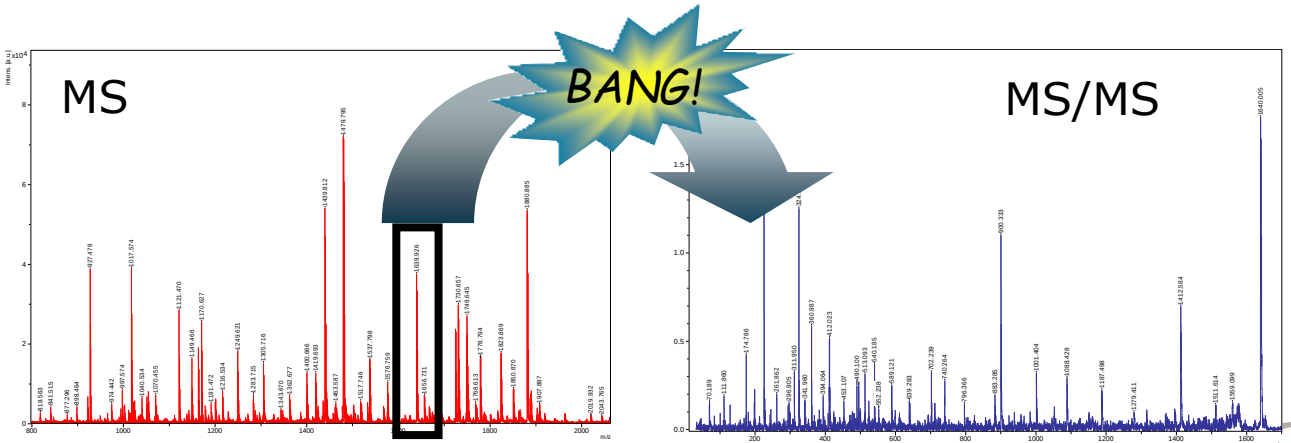

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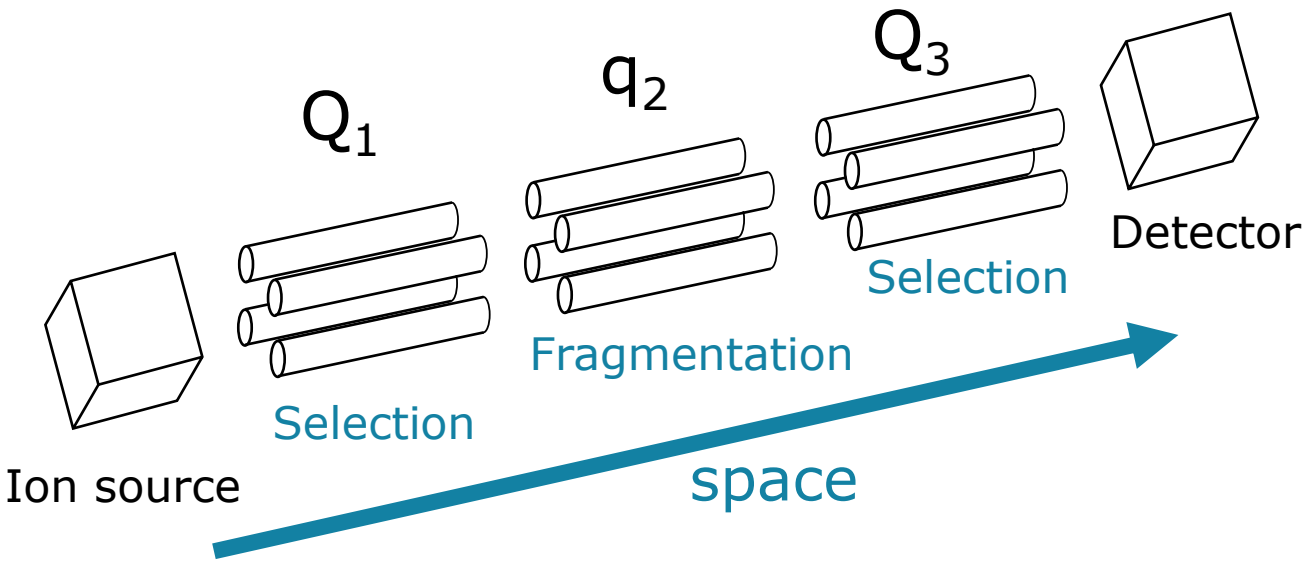
How to do MS/MS

There are two principally different ways to perform MS/MS:

MS/MS in space (selection and fragmentation takes places in different places)
This is typically performed on QqQ, Q(q)TOF, TOF/TOF and QqFT systems

MS/MS in time (selection and fragmentation takes place one after the other but in same location)
This is typically performed on ion trap and FT-ICR systems

MS/MS in space vs in time



General scheme of QqX design. For QTOF replace Q3 by TOF, for Q-FT Q3 by FT-ICR; the collision cell is typically annotated with a small q (but can also be a hexapole (h) or other type (e.g. T-Wave)

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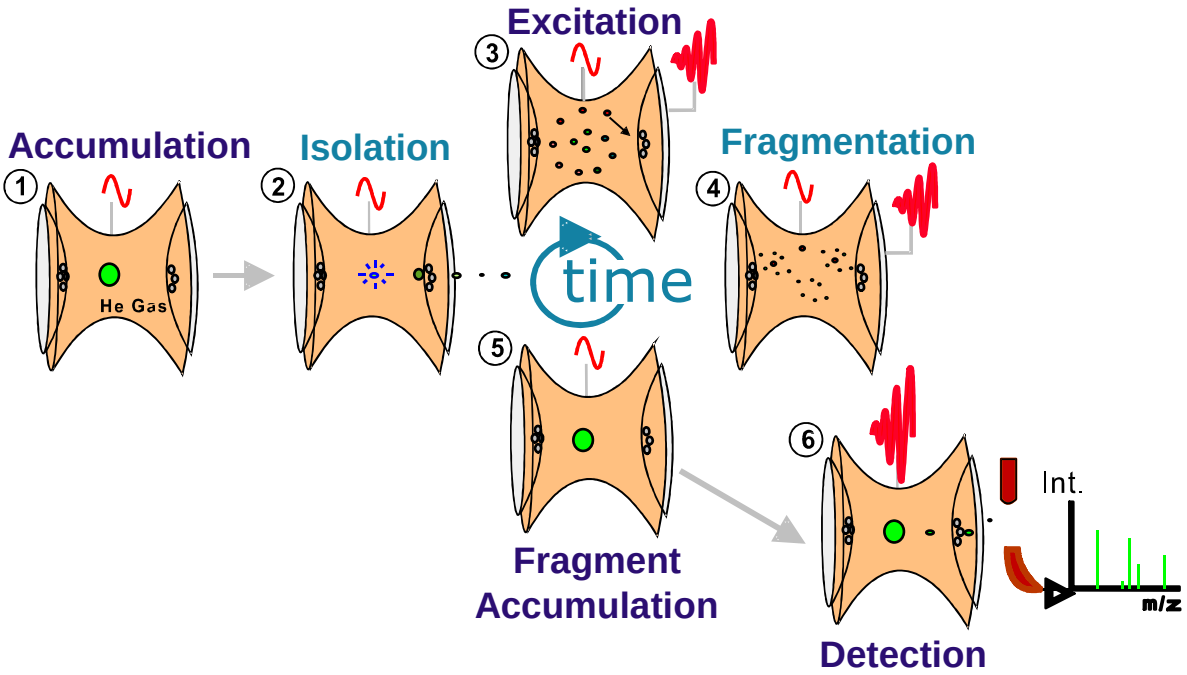
MS/MS in space vs in time



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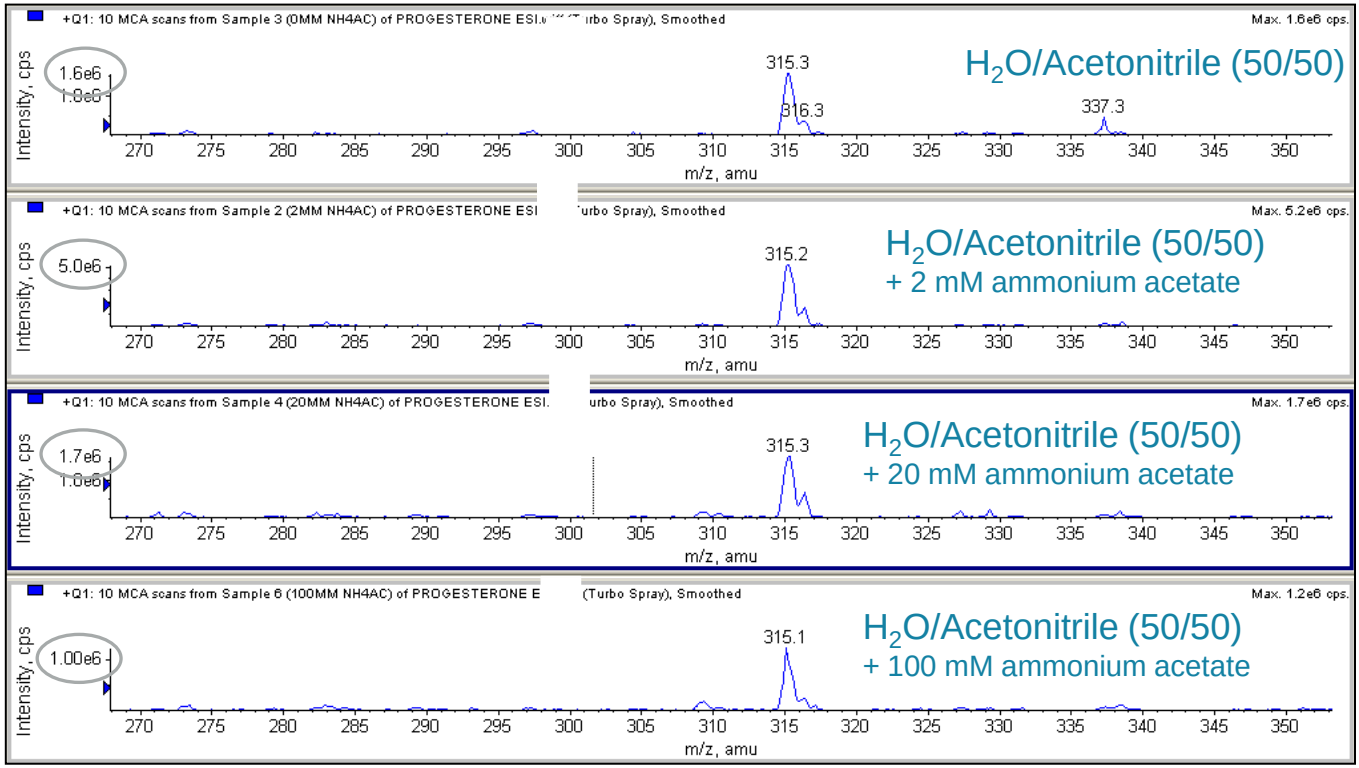
Solvents – What is Recommended

- Mixtures of water with methanol and/or acetonitrile
- Addition of volatile buffers :
  - Ammonium acetate or formate (2-20 mM)
- In positive ionization mode :
  - Adding acetic or formic acid (0.1-1%) to support protonation
- In negative ionization mode :
  - Addition of ammonia (0.1-1%) to support deprotonation

Progesterone (MW 314)

EFFECT OF AMMONIUM ACETATE ON IONIZATION



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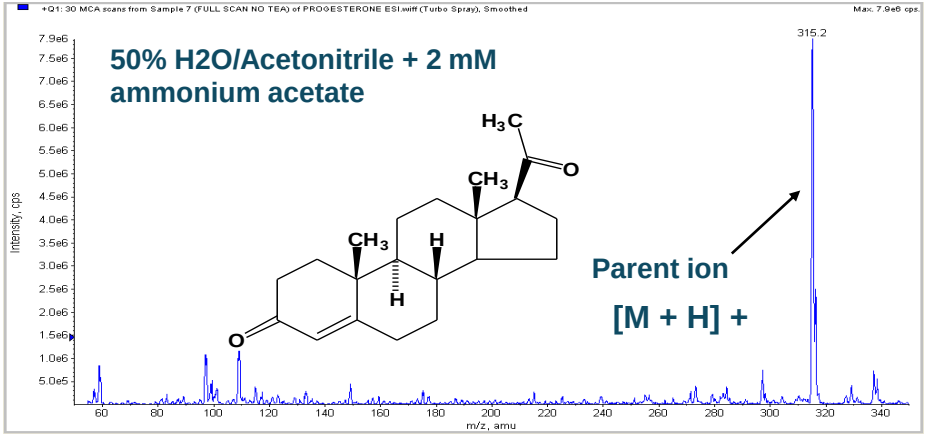
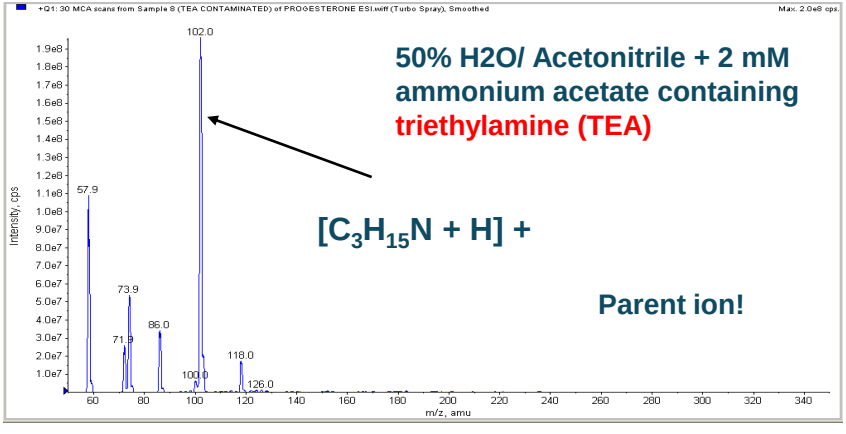
Solvents – What is Not Recommended

- Salts or non-volatile buffers
  - Sodium phosphate, NaCl, etc.
- Inorganic acids
  - Hydrochloric acid, sulfuric acid, etc.
- Other popular modifier
  - Trifluoroacetic acid (TFA)
  - High background due to m/z 113 in negative ion mode
- Triethylamine (TEA, sticky and not volatile !)
  - High background due to m/z 102 in positive ion mode

Effect of a Contaminant, TEA ($C_3H_{15}N$)

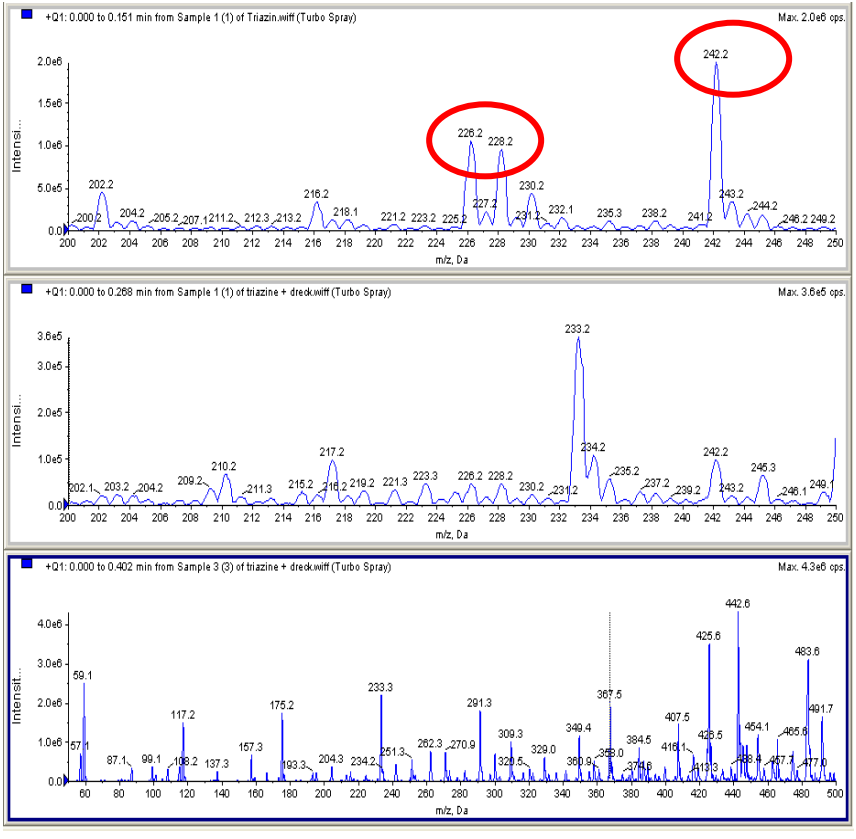
PROGESTERONE INTENSITY IN POSITIVE ESI MODE





Effect of a Contaminated Solvent on Analysis

TRIAZINE MIXTURE @ 20 NG/ML



Scanning area 200 – 250 :
The masses of the Triazine mixture are visible

Scanning area 200 – 250 :
The masses of the Triazine mixture are not visible
Hypothesis : concentration too low

Scanning area 50 – 500 :
The contamination is visible.
It is responsible for the loss of sensitivity

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How to Control the Quality of the Solvents

WHAT CAN YOU DO IF YOU SUSPECT YOUR SOLVENT IS CONTAMINATED?

- Preparation :
  - Change the profile (HW Profile) to “MS and syringe”.
  - Prepare 4 solutions : pure water, organic phase (acetonitrile or methanol) without buffer, water with buffer and organic phase with acid / buffer

- How to check :
  - Fill the syringe with the different solutions (1. water, 2. organic phase, 3. water with buffer, 4. organic with buffer)
  - Run in Manual Tuning a Q1 scan starting from 50 until the highest mass of your method is covered (e.g. 50 – 500 amu)
  - Check the intensity of the masses which are present. Do you see any clusters of masses?

- Note :
  - If you used mixed buffer systems (e.g. formic acid and NH_4 formate) check one buffer after the other to really identify the cause of contamination

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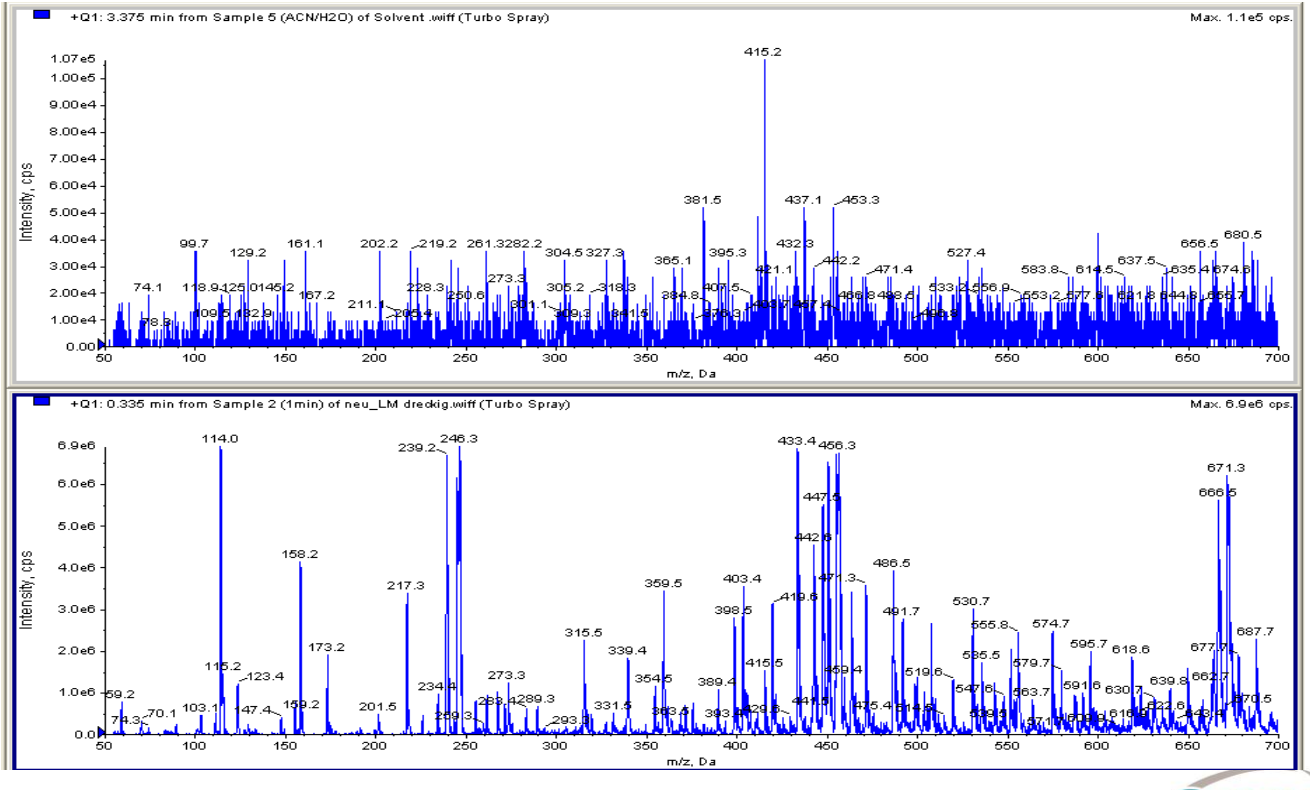
Example of Q1 Scan of Clean and Contaminated Solvent



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High throughput analytical tools

GCMS

LCMS

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In the past……Gas Chromatography

- GC used to be the main instruments in most labs
- For analytes to be analyzed by GC, samples must be in gas phase, or must be volatile enough to become a gas
- If your sample is a liquid that is hard to volatilize or contain high concentration of salt, it is impossible to use GC.
  - Urine, Blood etc….
- Analytes that do not bind well to GC column need to be '**derivatised**' → Lots of sample prep work!
- However, GC separations are very good compared to LC and some compounds need GC for separation e.g. diesel, hydrocarbons

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Other benefits of LCMS

- Shorter run time compared to GC run time (no heating and cooling down)
- No high temperature is involved → suitable for thermal labile compound
- Ionic compounds that are difficult to analysed by GC can now be done by LCMS
- Greater sensitivity can be achieved due to higher injection volume (GC typical inj vol: 1ul; LC typical inj vol: 10ul)
- Easier to maintain e.g. do not need to silanised injector liner or cut GC column

Introduction MS

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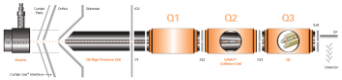
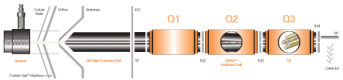
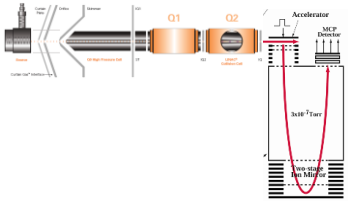
LC-MS/MS for metabolomics







TQ → **QTRAP** → **QTOF**

Targeted Analysis

Untargeted Analysis

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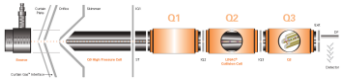
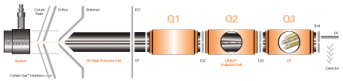
LC-MS/MS for metabolomics



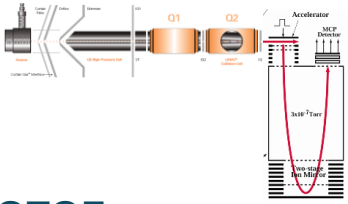
SCIEX Triple Quad™ System

- **Quantitation**
- ID with MRM ratio



QTRAP® system

- Quantitation
- ID with MRM ratio
- **ID with MS/MS library searching**



QTOF

- Quantitation
- ID with accurate mass
- ID with MS/MS library searching
- **ID true unknowns**
- **Retrospective data processing**

Increased confidence in compound ID.

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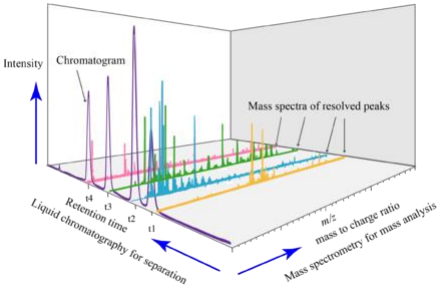
Components of a LC or GC-MS/MS



Separation Techniques

Ion source

API: ESI
APCI
APPI
EI

MALDI

Mass Analyzer

QQQ
QTRAP
Ion trap

Magnetic Sector
QTOF
Orbitrap
FTMS

Detector

- conversion dynode/ electron multiplier
- multichannel plate

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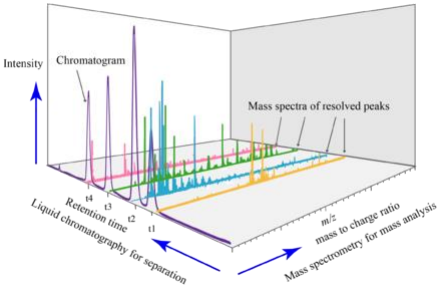
Components of a LC or GC-MS/MS



Separation Techniques

Ion source

API: ESI
APCI
APPI
EI

MALDI

Mass Analyzer

QQQ
QTRAP
Ion trap

Magnetic Sector
QTOF
Orbitrap
FTMS

Detector

- conversion dynode/ electron multiplier
- multichannel plate

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Ion source

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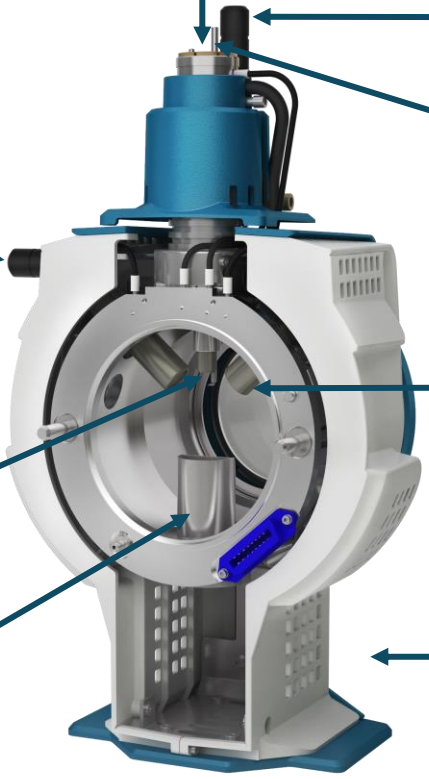
IonDrive™ Turbo V Source



LC input

Vertical adjustment of the probe

Adjustment of the electrode level

Lateral adjustment of the probe

One of the two heating elements IonDrive™ Turbo V source

ESI probe

Support to install the source

Evacuation of the source (exhaust)

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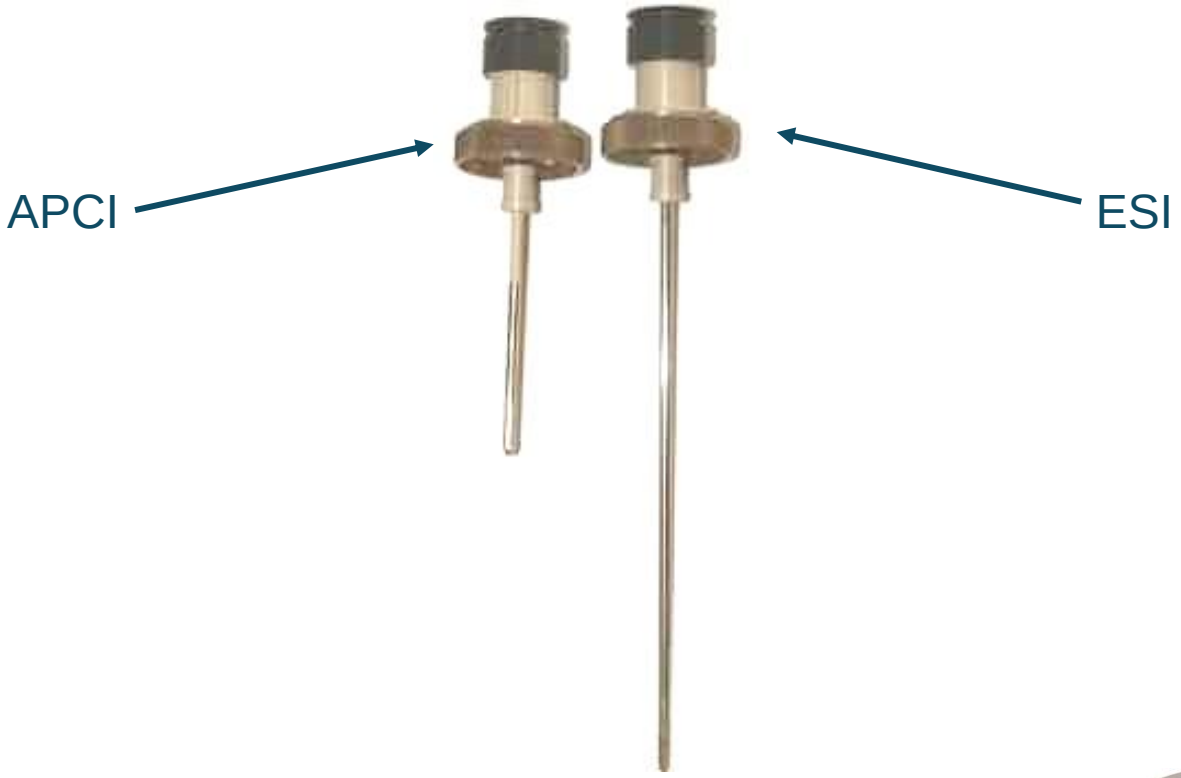
APCI and ESI Probe



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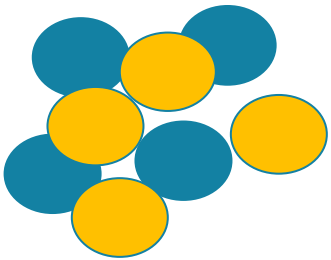
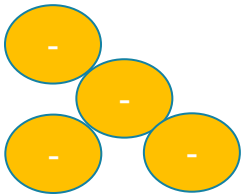
Ionization



- H^+

Ionization

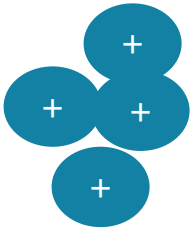
+ H^+



Anion; $[M-H]^-$

Adducts

Acids: $[M+HCOO]^-$, $[M+CH_3COO]^-$



Cation; $[M+H]^+$

Adducts with

Alkali metals, e.g., $[M+Na]^+$, $[M+K]^+$

Ammonia $[M+NH_4]^+$

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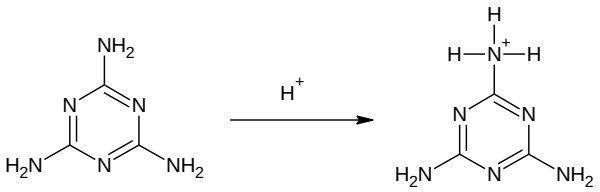
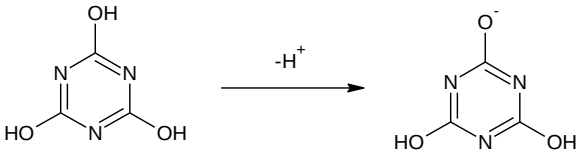
*Positive or Negative Polarity?

- If the sample has functional groups that readily accept a proton (H+) then positive ion detection is used
  e.g. amines R-NH_2 + H^+ = R-NH_3^+ as in many drugs, proteins or peptides etc



- If the sample has functional groups that readily lose a proton then negative ion detection is used

  e.g. carboxylic acids R-CO_2H = R-CO_2^- and alcohols R-OH = R-O^- as in phenol, saccharides, oligonucleotides etc



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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

Atmospheric Pressure Ionization (API) technique

- **ElectroSpray Ionization (ESI)**
  - Ionization by high voltage (TurboIonSpray® / Turbo V™ Source)

- **Atmospheric Pressure Chemical Ionization (APCI)**
  - Ionization by Corona discharge (Turbo V Source)

- **Atmospheric Pressure Photo Ionization (APPI)**
  - Ionization by UV light (PhotoSpray® Source)

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How to Choose the Correct Ion Source?

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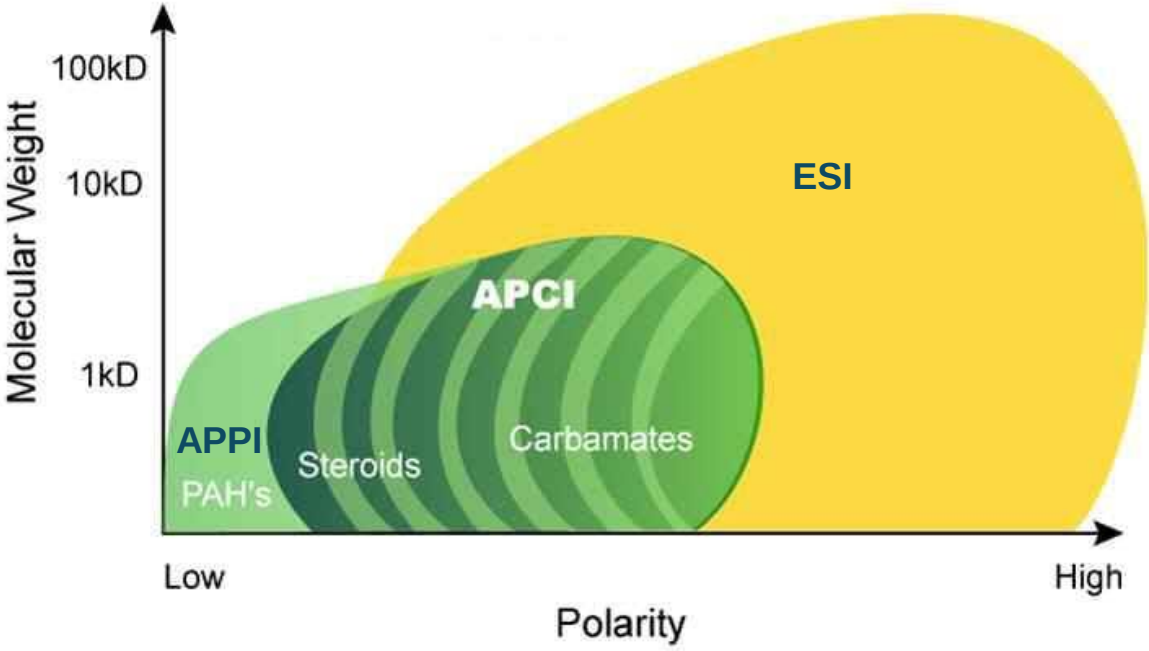
General Source Selection



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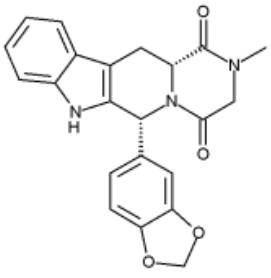
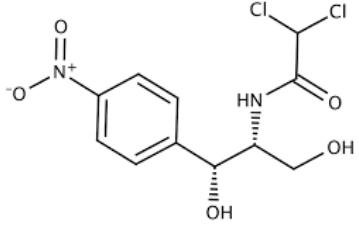
Ionization Modes *vs.* Type of Compounds

- **TurboIon Spray (ESI)** – Tadalafil, Sildenafil, Amphemtamine, Paracetamol, Malachite green, Melamine/Cyanuric acid, Sudan dyes, Acidic/triazines/organophosphates pesticides, Chloramphenicol, Mycotoxin, etc

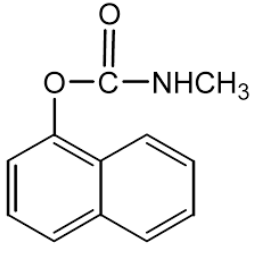
Tadalafil



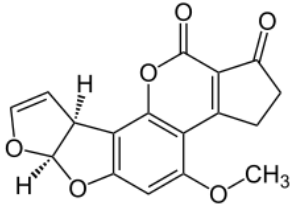
Chloramphenicol



Carbaryl



Alfatoxin B1



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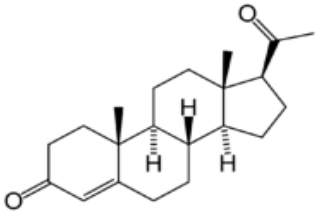
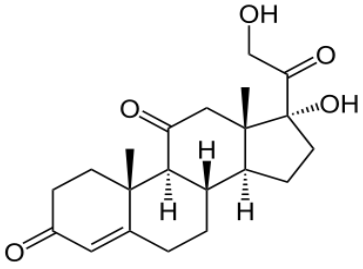
Ionization Modes *vs.* Type of Compounds

- **Heated Nebulizer (APCI)** – Aspartame, Progesterone, Steroids, Vitamin A, Vitamin D2, D3 etc

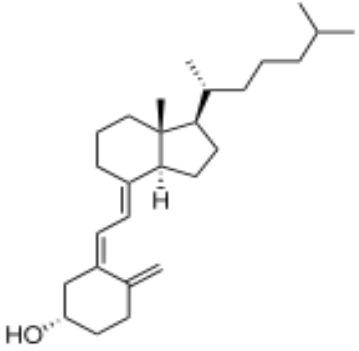
Progesterone



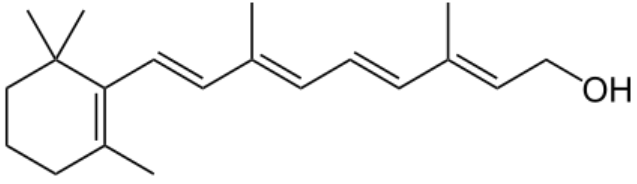
Cortisone



Vitamin D3



Vitamin A



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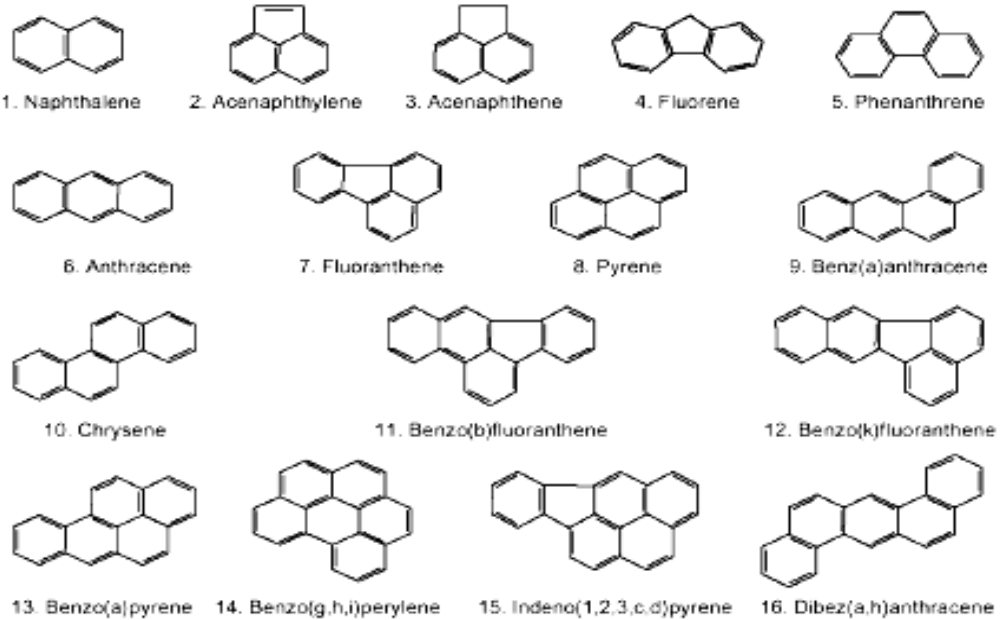
Ionization Modes *vs.* Type of Compounds

- **Photospray (APPI)** – Polyaromatic Hydrocarbons (PAH)

PAHs



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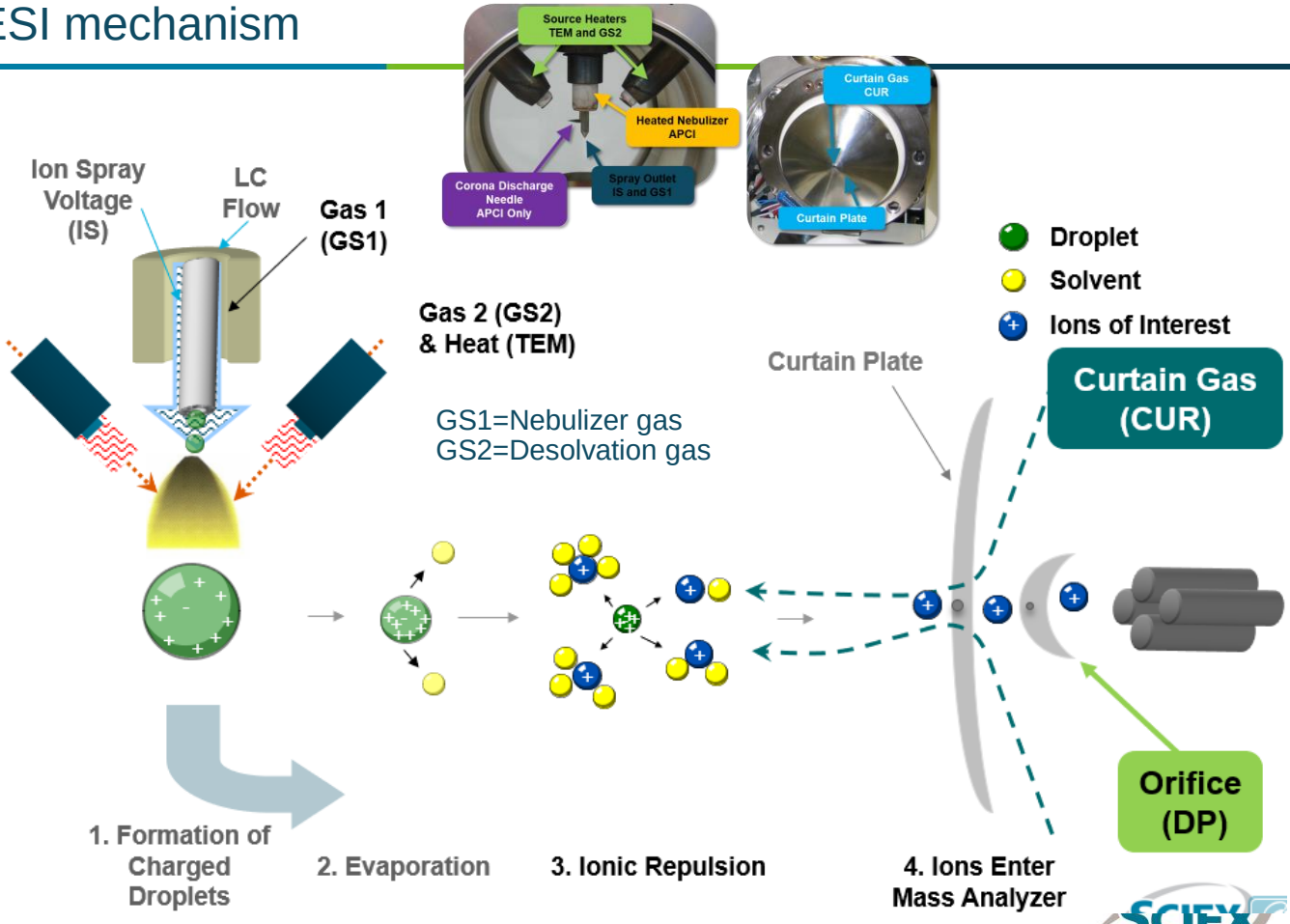
ESI mechanism



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Parameter Optimization for TIS Probe

Parameter	Typical values					Operational Range
LC Flow (µL/min)	5 to 50	51 to 150	151 to 400	401 to 800	Above 800	5 to 3000
Gas 1 – Nebulizer[1] (psi)	15 to 30	30 to 50	40 to 70	50 to 90	60 to 90	15 to 90
Gas 2 – Turbo Gas[1] (psi)	0 to 30	30 to 50	40 to 70	50 to 90	60 to 90	0 to 90
Temperature[2] (ºC)	0 to 150	150 to 250	250 to 500	500 to 650	600 to 750	Up to 750
Curtain Gas (psi)	15 to 20	25	30	35	40	15 to 50
Probe Vertical Position (mm)	5 to 10	3 to 7	0 to 5	0 to 3	0 to 2	0 to 13
Probe Horizontal Position (mm)	4 to 6	4 to 6	4 to 6	4 to 6	3 to 7	3 to 7
Electrode Protusion (mm)	≈ 2	≈ 1	≈ 1	0,5 to 1	0,5	0,5 to 2

1) The maximum setting of the gas depends on the gas source. Usually the sum Gas1 + Gas2 should not exceed 140 psi.

2) The optimal temperature value depends on the compound and the composition of the mobile phase (a significant proportion of water requires a higher temperature)

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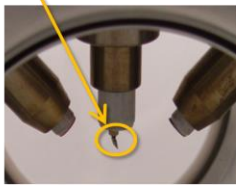
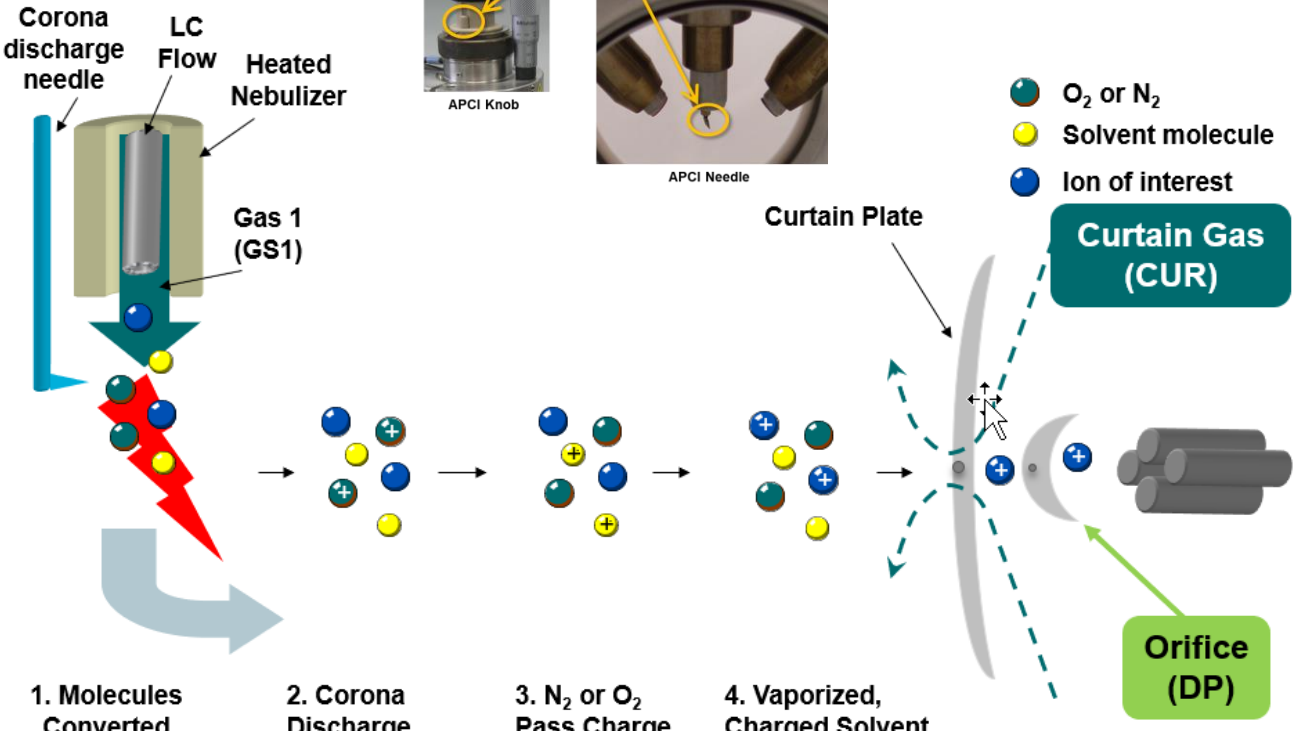
Optimizing ESI Source Conditions

- Aim is a stable spray and high ionization efficiency.
- Generally, high flow rates and a high percentage of water require high nebulizer pressure and dry gas settings.
- As HPLC gradients in reversed phase chromatography start with the highest percentage of water, the parameters determined for the initial conditions will be appropriate for the whole run.
- Check that no “spikes” appear in the chromatogram.
- Monitoring of the TIC in the Control software helps to optimize for maximum signal intensity which corresponds to a maximum transfer of ions into the mass spec.
- The best optimization for LC-MS analysis, e.g. for quantitation is achieved by direct infusion of the target compound and mixing with the respective HPLC eluent using a T-piece.

APCI mechanism





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Atmospheric Pressure Chemical Ionization (APCI)

IONIZATION WITH A CORONA NEEDLE

3 steps ionization process:

1. EI-ionization of gas molecules : $N_2^{\bullet+}$, $O_2^{\bullet+}$
2. Gas molecules ionize solvent molecules : H_3O^+, $CH_3OH_2^+$
3. $CH_3OH_2^+$ and/or H_3O^+ transfer proton to analyte molecule : $[M+H]^+$

Source/Gas Parameters – APCI Probe

Higher flow rates required:

- At least 200 µL/min (up to 2 mL/min)

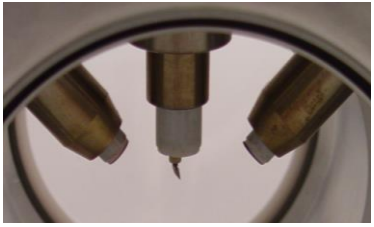
Nebulizer Gas (Gas 1)

- Typical range: 20 psi to 90 psi
- Higher flow rates require higher Gas 1 values

Curtain Gas (CUR)

- Nitrogen
- As high as possible without decreasing the signal
- Depends on flow rate and analyte
- Higher flow rates require higher CUR values
- Typical range: 15 psi to 35 psi

- **Move the Corona Needle**
  - Needle Current (NC)
  - In positive mode about 1 to 5 µA
  - In negative mode about -1 to -5 µA
  - Set the lowest current that produces the highest signal intensity
- Evaporation of sample solution
  - Temperature (TEM) – The optimal spray temperature is affected by the flow rate, the solvent composition, the quantity, the composition, and the type of sample
  - Optimal TEM is the lowest setting that ensures the complete vaporization
  - Default value can be 450^{0}C



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Parameter Optimization for APCI Probe

Parameter	Typical values			Operational range
LC flow (µL/min)	200 to 400	401 to 1000	Above 1000	200 to 3000
Gas 1 – Nebulizer (psi)	40 to 80	50 to 90	60 to 90	0 to 90
Gas 2 – Turbo Gas (psi)	N.A.	N.A.	N.A.	N.A.
Temperature* (ºC)	550 to 650	450 to 650	550 to 750	Up to 750
Curtain Gas (psi)	30	35	40	10 to 50
Probe Vertical position (mm)	0 to 5	0 to 3	0 to 2	0 to 13
Probe Vertical position (mm)	4 to 6	4 to 6	3 to 7	3 to 5

* The optimal temperature value depends on the compound and composition of the mobile phase (A significant proportion of water requires a higher temperature)

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Proper Vacuum is Required for Good Ion Transfer

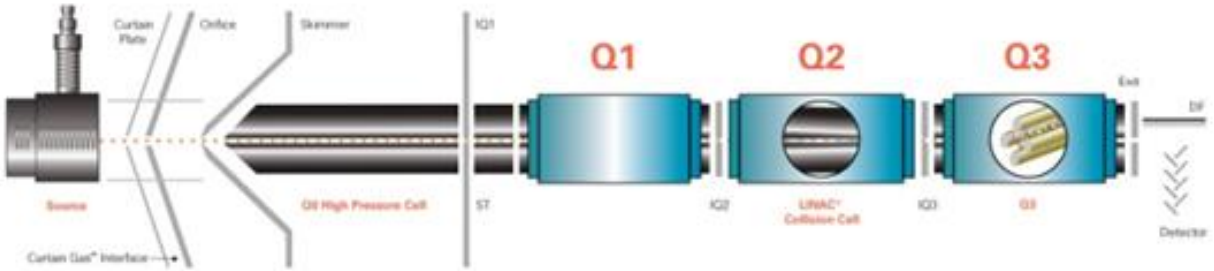

Figure 1. Schematic of Triple Quadrupole Configuration

Roughing Pump

Turbo Pump

Transition to Vacuum

WITHOUT VACUUM - NO MASS SPECTROMETRY !

- **Three vacuum steps:**
  - Area OR-QJet® 2 Ion Guide (ca. 1 to 2 torr)
  - Area QO (some Millitorrs)
  - Area Q1 / Q2 / Q3 / CEM (ca. 0.6 x 10^{-5} torr)

- **Bayard Alpert Gauge (BAG) measures the pressure in the Q1/Q2/Q3/CEM area**
  - Pressure increases (up to ca. 3 x10^{-5} torr) if CAD gas is "on"

- **System will not start if:**
  - Nitrogen supply to the CUR gas < 20 psi
  - Pressure > 1x10^{-4} torr (system will also shut down automatically)

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Behind the Source

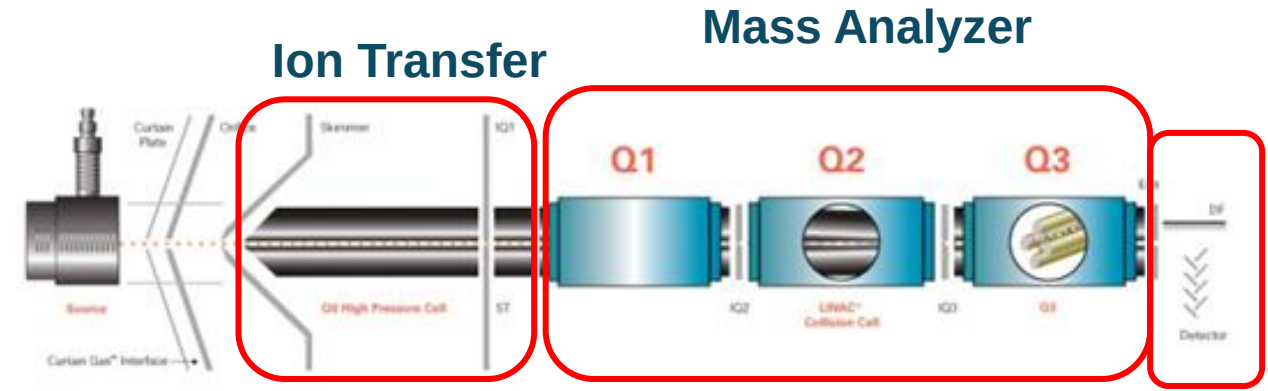

Figure 1. Schematic of Triple Quadrupole Configuration

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Mass Analyzer

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Triple Quadrupole Mass Spectrometer; QQQ

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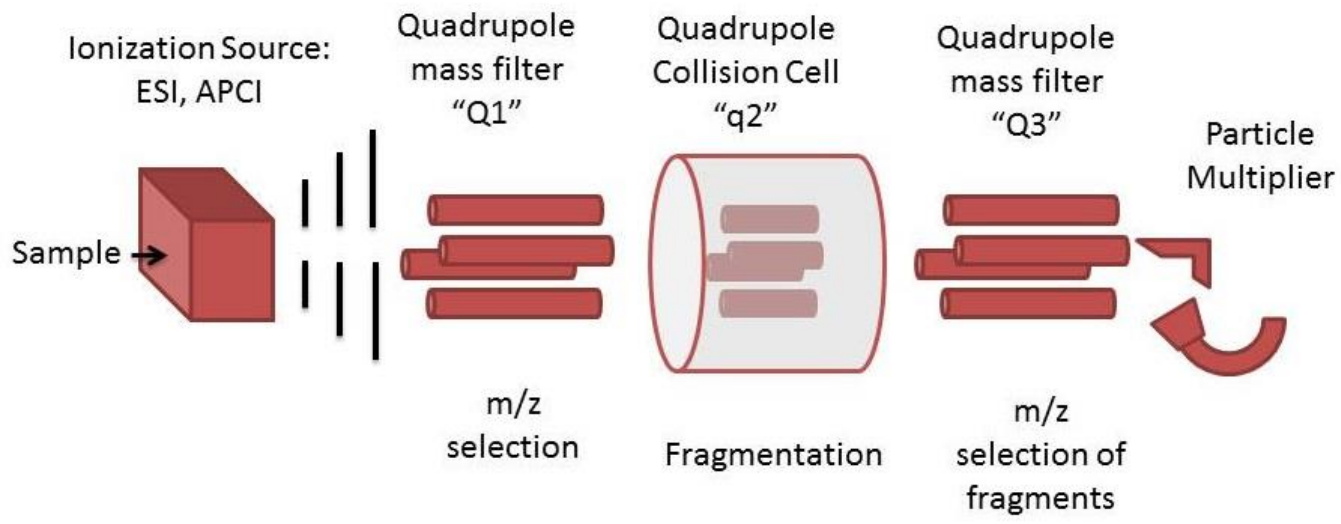
Triple Quadrupole Mass Spectrometer; QQQ



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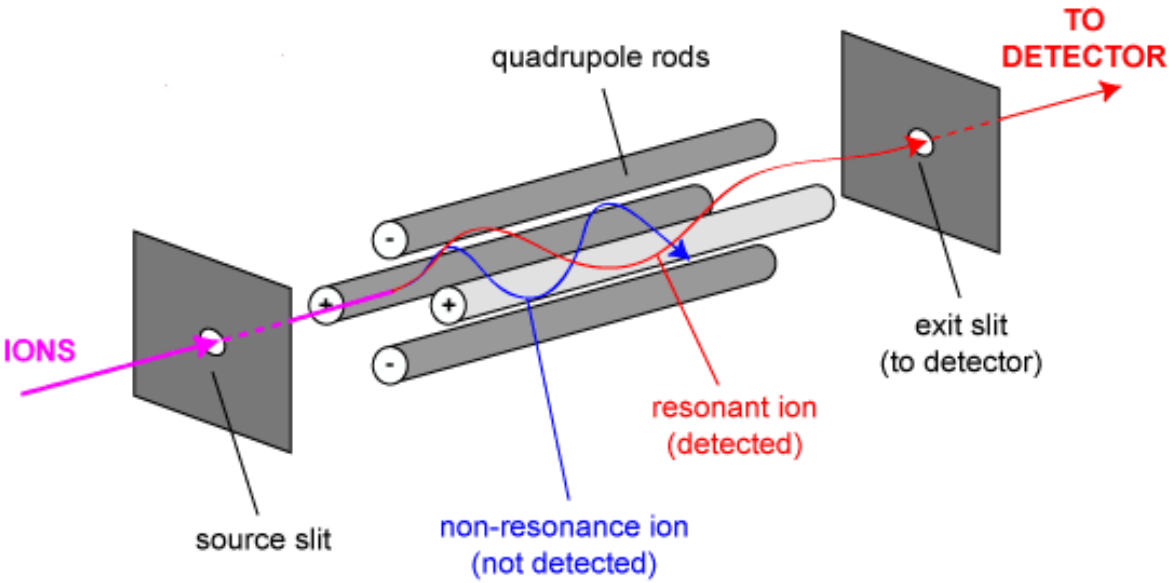
Quadrupole function



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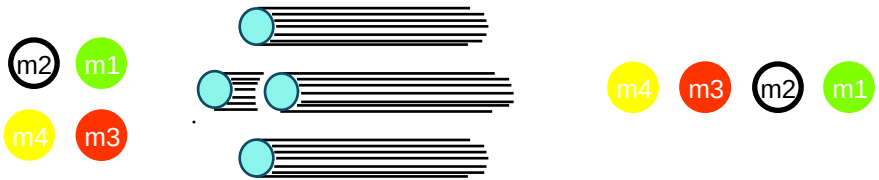
Quadrupoles Mass Filters



mass scanning mode

single mass transmission mode

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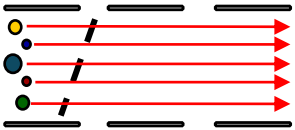
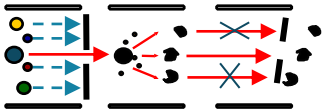
Difference Between Single Quad and Triple Quad

System	Single Quad	Triple Quad
Scan mode	**S**elected **I**on **M**onitoring 	**M**ultiple **R**eaction **M**onitoring 
Quality of Data	- High sensitivity - Prone to high noise level	-Very high sensitivity - Low noise level due to high selectivity

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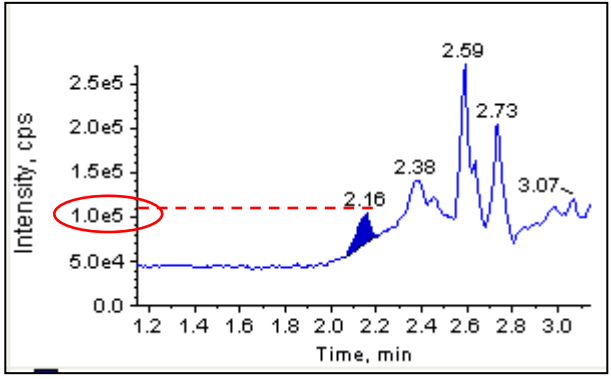
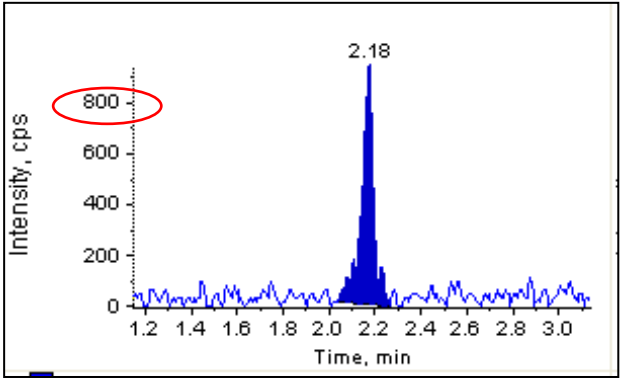
In real sample…

SIM mode



MRM mode



Same compound both at same concentration

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Guidelines for MSMS analysis

- In food safety application: According to European guidelines EC/657/2002
  - Forbidden substance: min. 4 identification points
  - Authorised substance: min. 3 identification points

Technique(s)	Number of ions	Identification Points
GC-MS (EI or CI) or LC-MS	n ions	n
GC-MS/MS or LC-MS/MS	1 precursor + 1 daughters ions	2.5
GC-MS/MS or LC-MS/MS	1 precursor + 2 daughters ions	4
GC-MS/MS or LC-MS/MS	2 precursor, each with 1 daughter	5

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EU Guidelines

IDENTIFICATION OF RESIDUES BY LC/MS/MS ACCORDING TO THE NEW EU GUIDELINES: APPLICATION TO THE TRACE ANALYSIS OF (VETERINARY) DRUGS IN BIOLOGICAL AND ENVIRONMENTAL MATRICES

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Table 1. The relationship between a range of classes of mass fragment and Identification Points earned.

MS technique	Identification Points earned per ion
Low resolutin mass spectrometry (LR)	1.0
LR-MS^n Precursor ion	1.0
LR-MS^n Transition products	1.5
High resolution mass spectrometry (HR)	2.0
HR-MS^n Precursor ion	2.0
HR-MS Transition products	2.5

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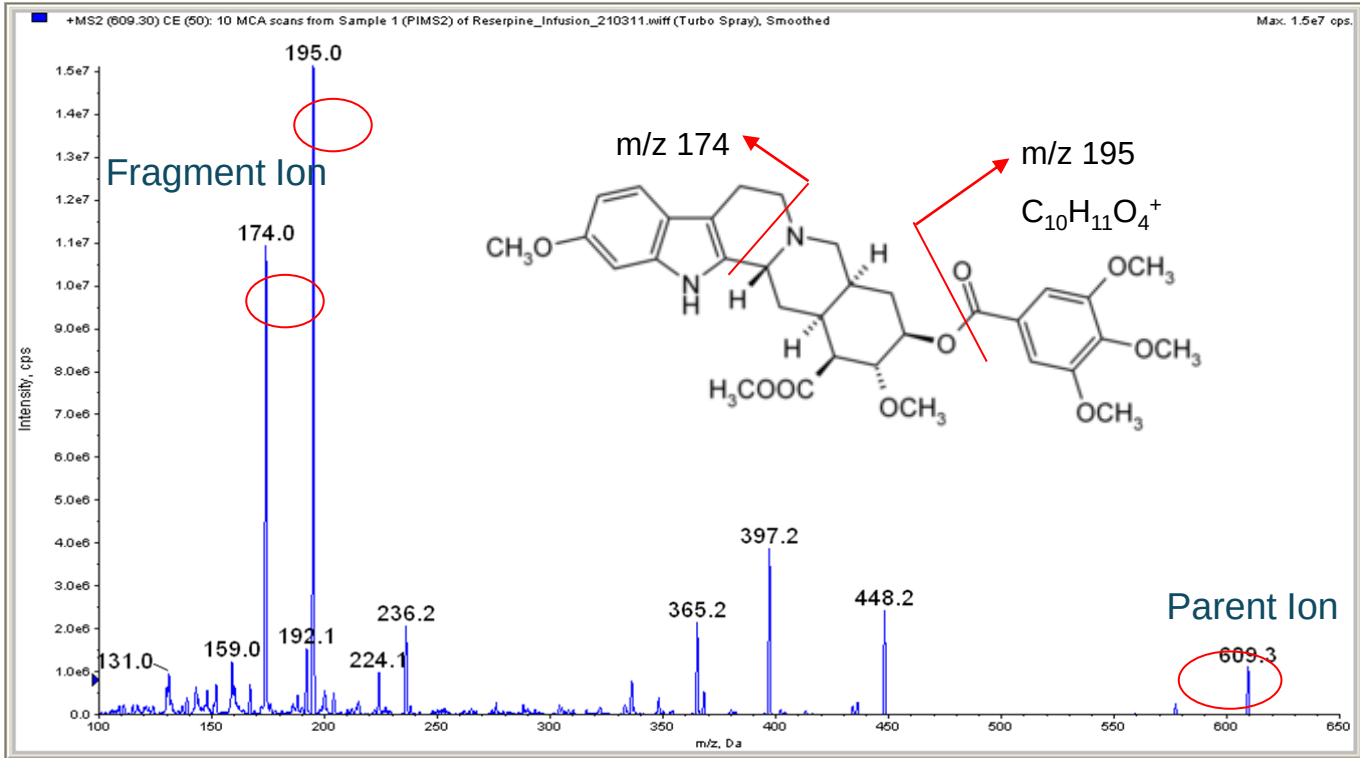
Example: Reserpine MSMS



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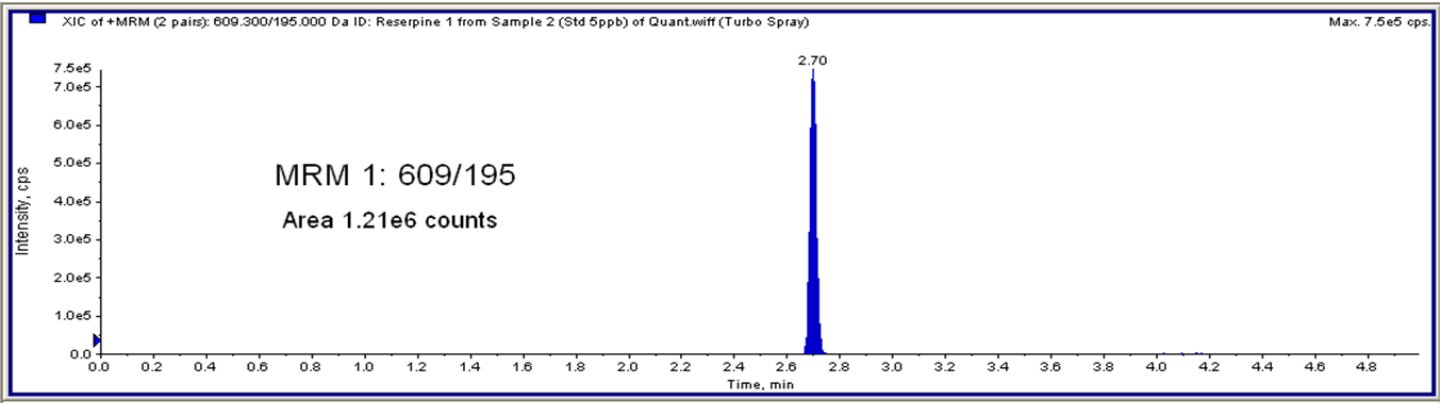
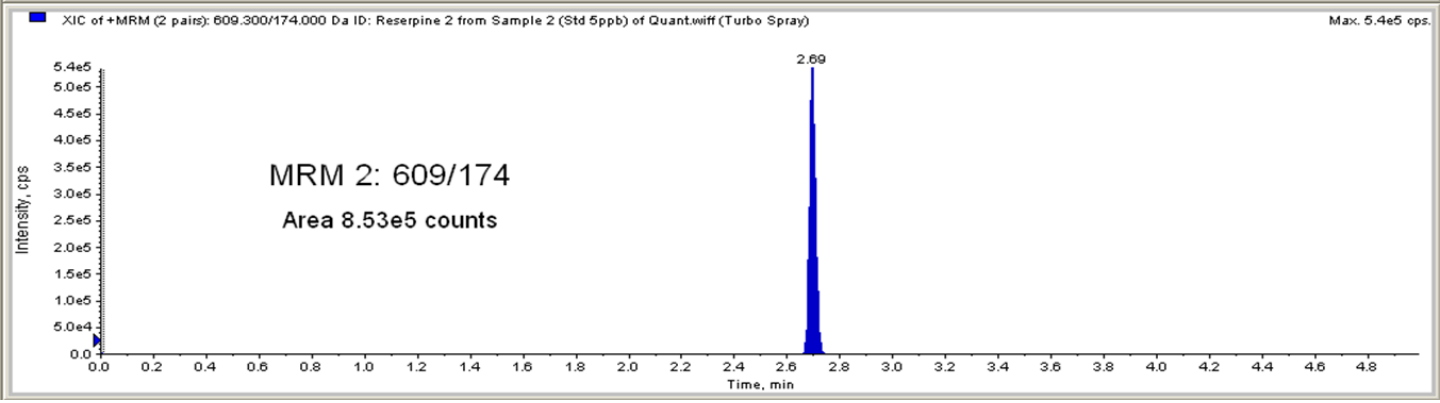
Reserpine
In acquisition method, monitor 2 MRM transitions





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Ion Ratio

$$IonRatio = \frac{A_{MRM\,2}}{A_{MRM\,1}}$$

where

- *MRM 1: Quantitation Trace (i.e Quantifier)*
- *MRM 2: Confirmation Trace (i.e Qualifier)*
- *A: Peak Area*

For Reserpine, ion ratio = 8.53e5 / 1.21e6 = 0.70

Main changes introduced in Document N° SANTE/11312/2021

Table 3. *Identification requirements for different MS techniques.*[2]

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	additionally
Unit mass resolution	Single MS quadrupole, ion trap, TOF	full scan, limited *m/z* range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	

a) preferably including the molecular ion, (de)protonated molecule or adduct ion

b) including at least one fragment ion

c) <1 mDa for *m/z*<200

d) in case noise is absent, a signal should bepresent in at least 5 subsequent scans

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Compound Parameters

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Compound Parameters

- Compound parameters are independent of the Source parameters that ionized the analytes
- Once ions enter the mass spectrometer, the ions are controlled by a series of voltages (compound parameters) across the mass rail

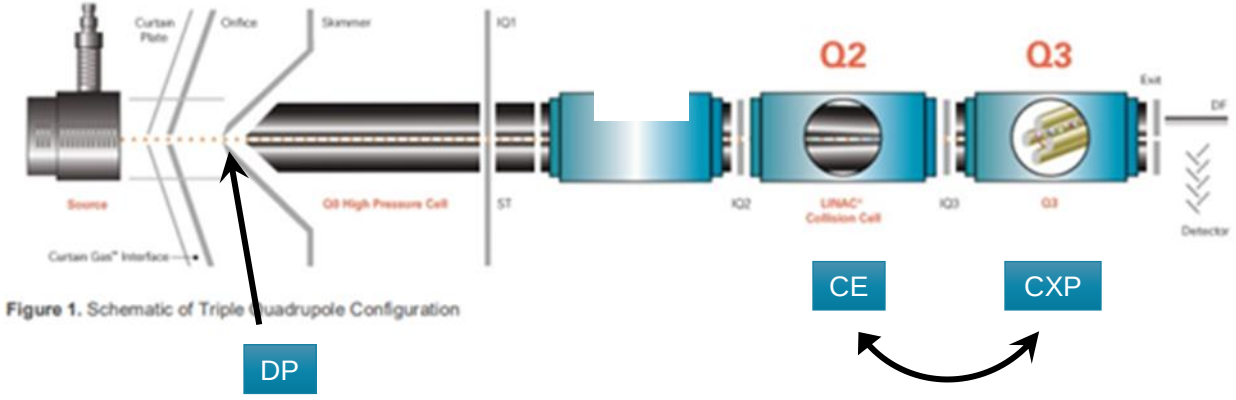

Figure 1. Schematic of Triple Quadrupole Configuration

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Compound Parameters

Voltage	Function
Declustering Potential (DP)	declusters Ions
Collision Energy (CE)	Fragments ions & in Traps helps ions into Trap
Collision Cell Exit Potential (CXP)	Assists ions going into Q3

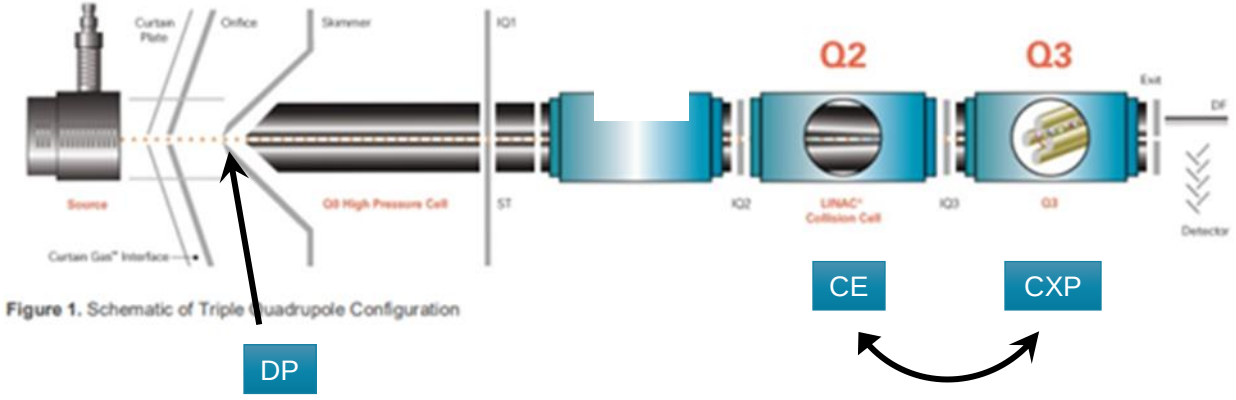

Figure 1. Schematic of Triple Quadrupole Configuration

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Quadrupole Scan Modes

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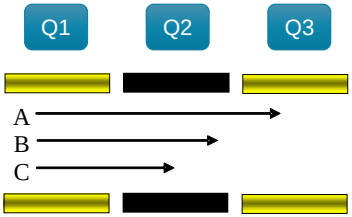
MS and MS/MS Scan Types

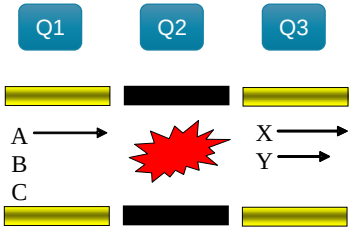


MS Scans:

Q1/Q3 Full Range Scan

Q1/Q3 Multiple Ion Scan

(*aka Single Ion Monitoring*)



MS/MS Scans:

Product Ion Scan

Precursor Ion Scan

Neutral Loss Scan

Multiple Reaction Monitoring (MRM)

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MS scan

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MS mode

- Q1 MS (Q1) scan
- Q1 multiple Ions (Q1 MI) scan
- Q31 MS (Q3) scan
- Q3 multiple Ions (Q3 MI) scan

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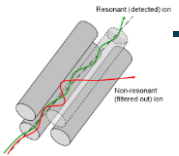
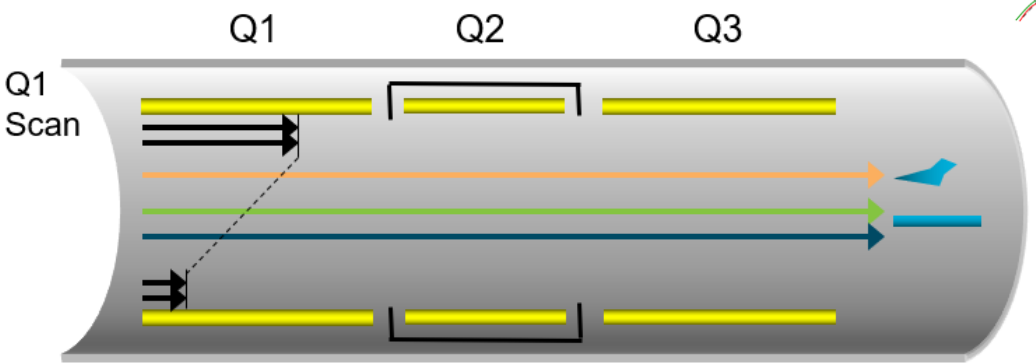
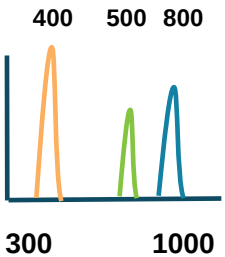
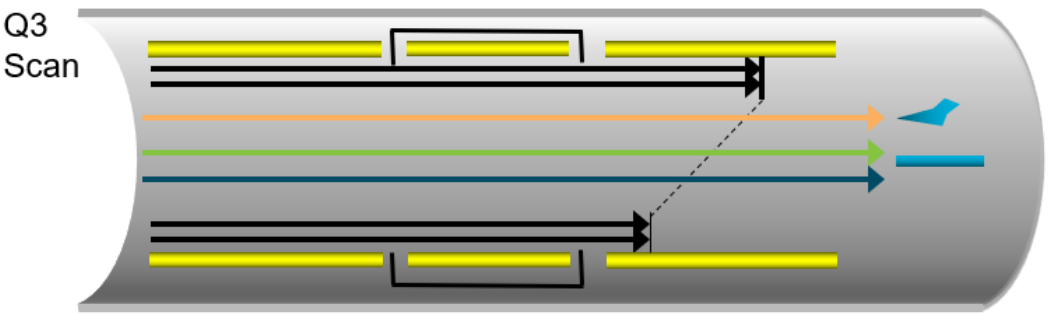
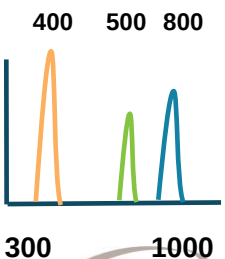
Q1 and Q3 MS Scans – Start/Stop Mode











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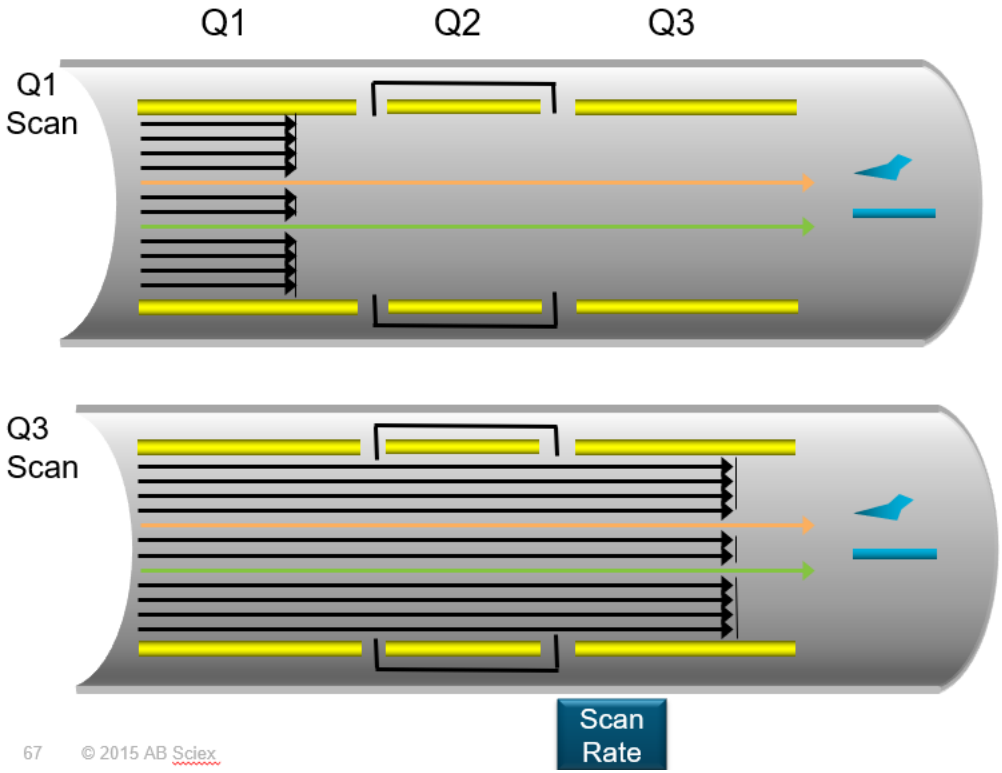
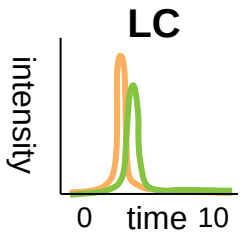
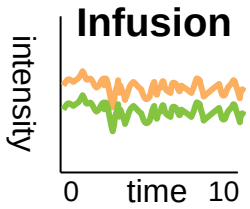
Q1 and Q3 MS Scans – Multiple Ions Mode





or



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MSMS scan

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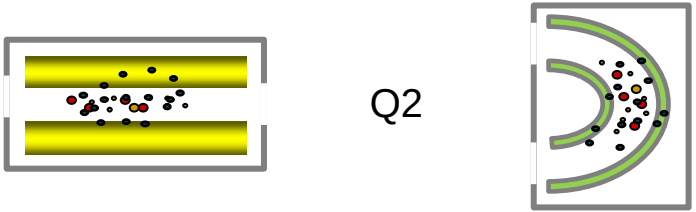
MS/MS: Fragmentation

- Collisional energy, also known as Collisionally Activated Dissociation (CAD), is converted into vibrational energy, and bonds break
  - With CE energy, ions collide with CAD gas (N2)
  - Fragmentation occurs in Q2 (collision cell)
- For singly charged ions, the following formulas apply:

Positive mode: $[Precursor]^+$ → $Product^+$ & Neutral

Negative mode: $[Precursor]^-$ → $Product^-$ & Neutral



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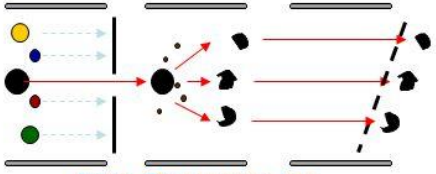
MSMS mode

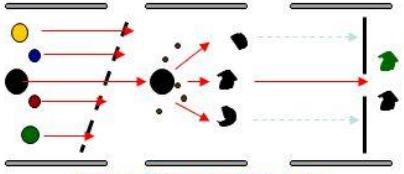
MS/MS: modes of operation

Product Ion Scan



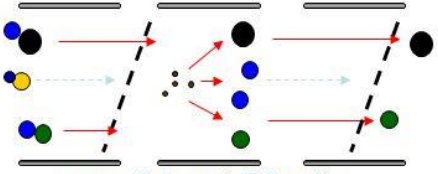
filter Precursor Ion
scan Product Ion

Precursor Ion Scan



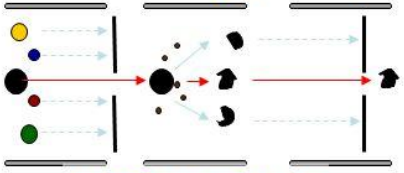
scan Precursor Ion
filter Product Ion

Neutral Loss Scan



scan Q1 and Q3 with
constant mass off-set

Multiple Reaction Monitoring (MRM)



filter Precursor Ion
filter Product Ion

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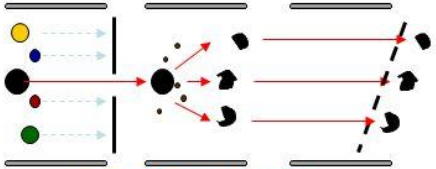
MSMS mode

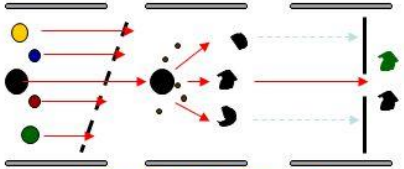
MS/MS: modes of operation

Product Ion Scan



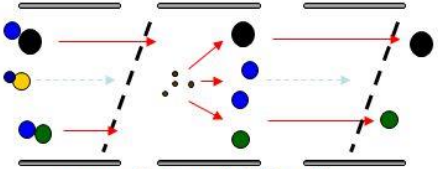
filter Precursor Ion
scan Product Ion

Precursor Ion Scan



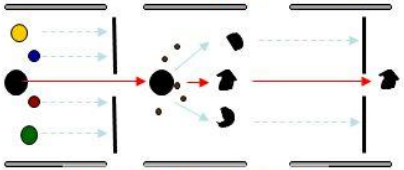
scan Precursor Ion
filter Product Ion

Neutral Loss Scan



scan Q1 and Q3 with
constant mass off-set

Multiple Reaction Monitoring (MRM)



filter Precursor Ion
filter Product Ion

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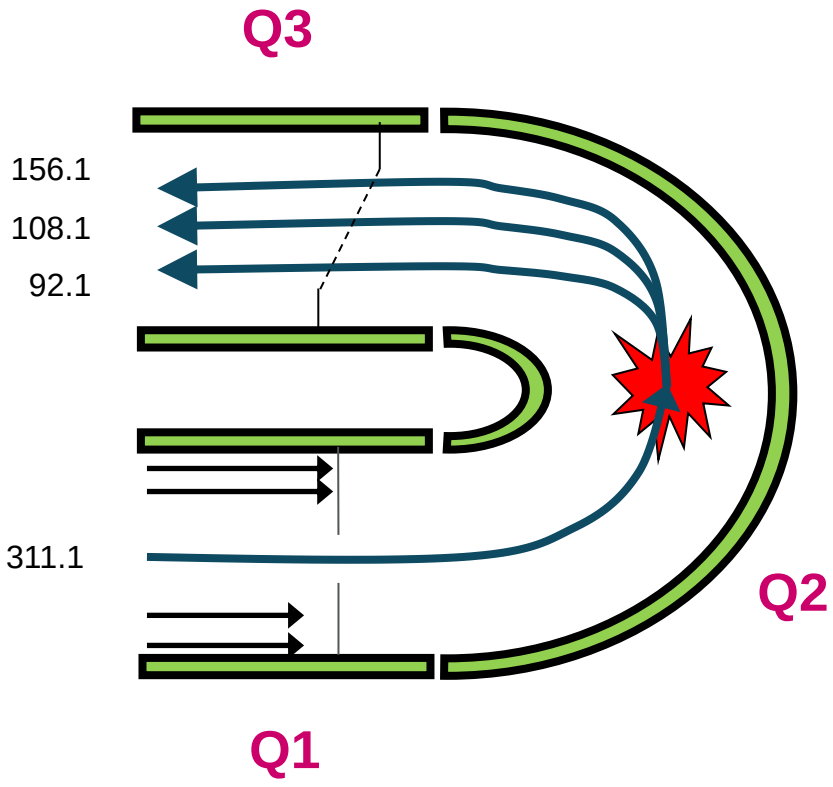
MS/MS – Product Ion Scan



1. Precursor ion selected in Q1
2. Fragmentation of precursor occurs in Q2
3. Product Ions scanned in Q3

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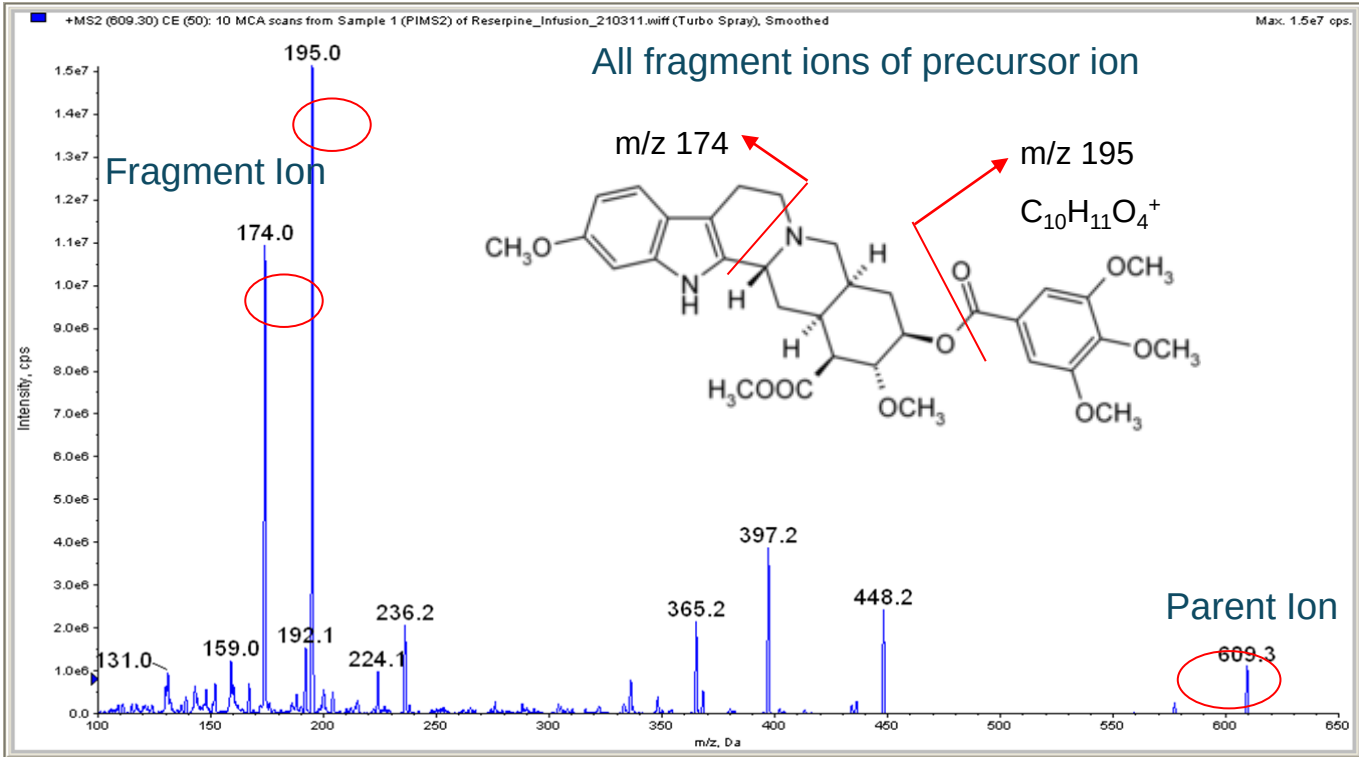
Product Ion Scan



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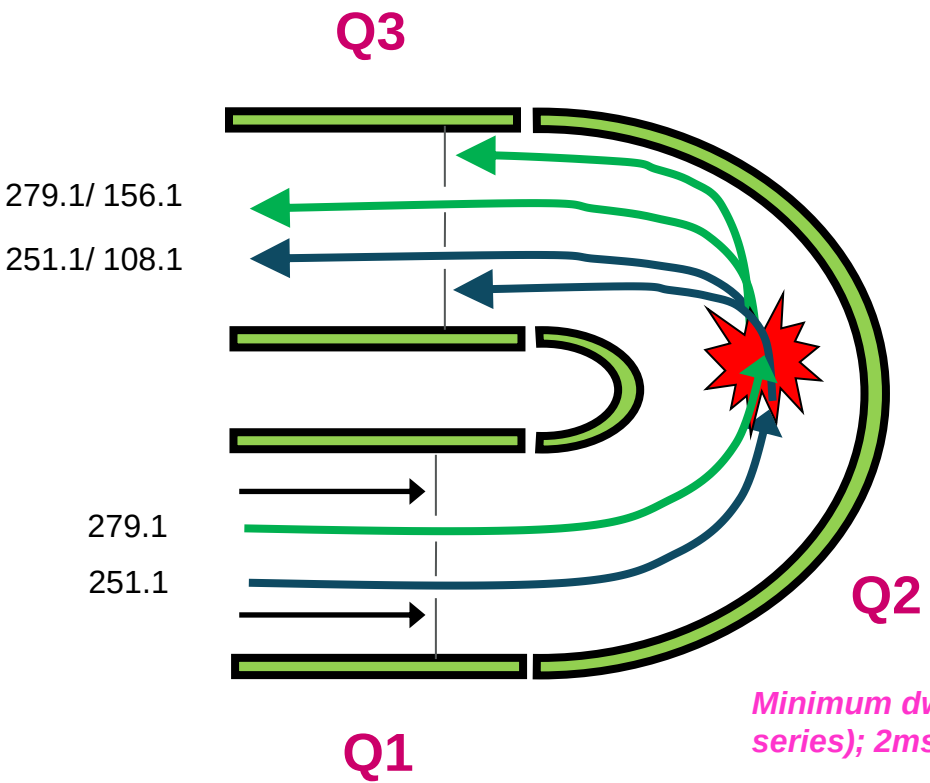
MS/MS – MRM (Multiple Reaction Monitoring)



1. Precursor ions selected in Q1
2. Fragmentation of precursor occurs in Q2
3. Product Ions selected in Q3

Minimum dwell time : 1ms (6500/6500+ series); 2ms (5500/4500 series)

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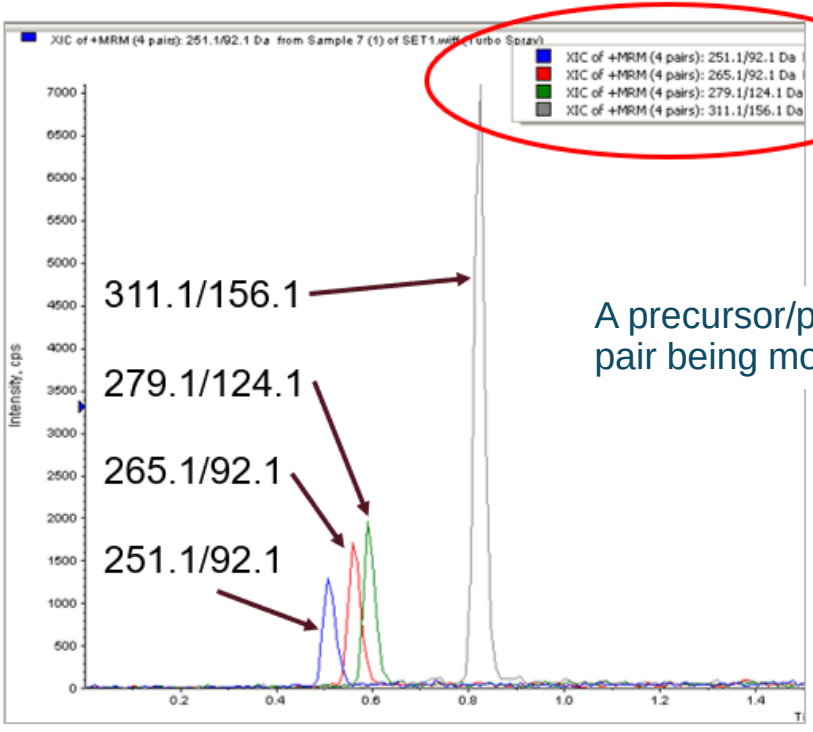
Multiple Reaction Monitoring



A precursor/product ion pair being monitored

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Dwell Time

NEEDED FOR GOOD QUANTITATION

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What is dwell time?

- Time taken for the ions to be scan out from the mass analyser during MRM or MI mode
- Usually calculate in terms of millisecond (ms)
  - Longer dwell time → less data points will be acquired
  - Shorter dwell time → more data points will be acquired

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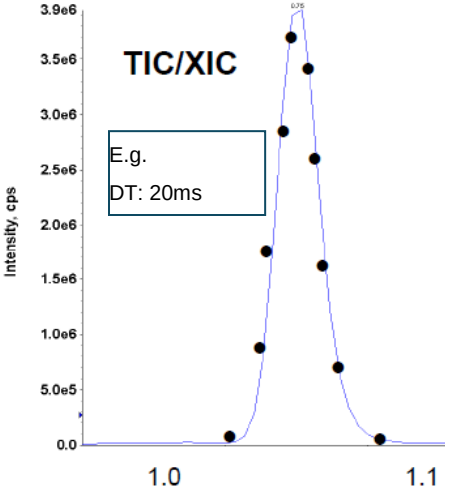
Importance of Dwell Time

Optimum Dwell Time

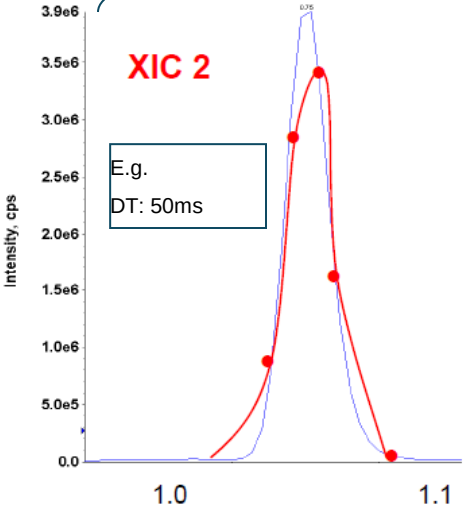


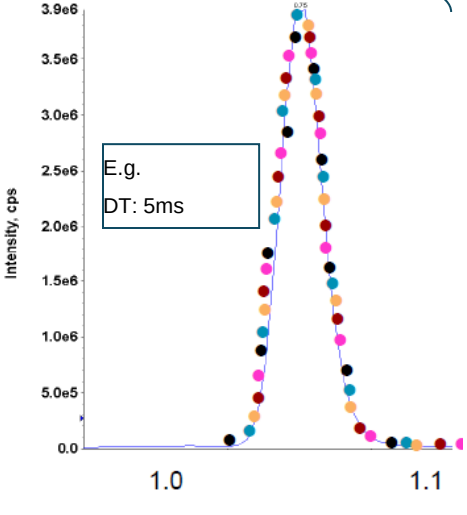
Consequences:

- At least 10-15pt across peak width

- Good repeatability and Quant result

Dwell Time set incorrectly





Consequences:

- Insufficient points or too much points across peak width

- Poor repeatability and Quan result

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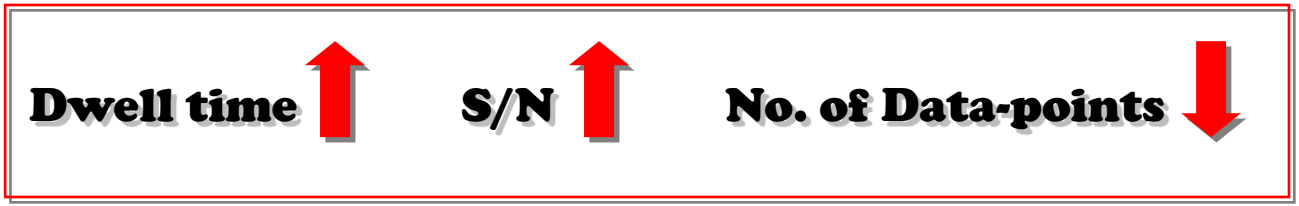
Relationship of Dwell time *vs* S/N and Data-point across the peak

- *As dwell time increases, sensitivity (i.e. S/N) will increase because more product ions are formed.*
- *As dwell time increases, the number of data-points across the peak will decrease because the product ions will take a longer time to reach the detector to produce a signal.*



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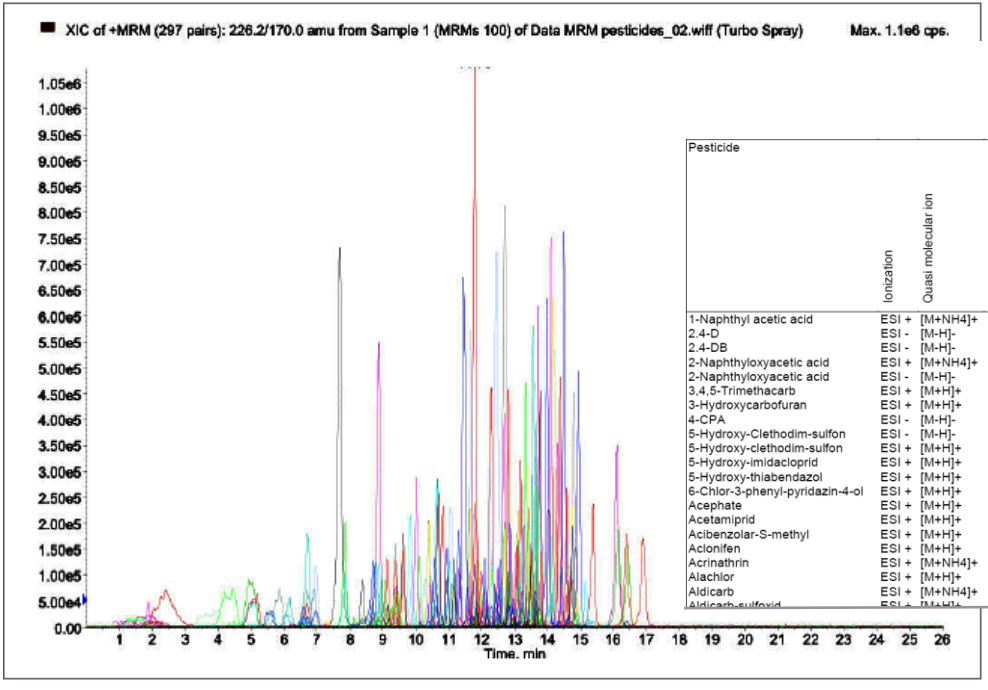
Multi-Component Analysis – 297 Ion Pairs



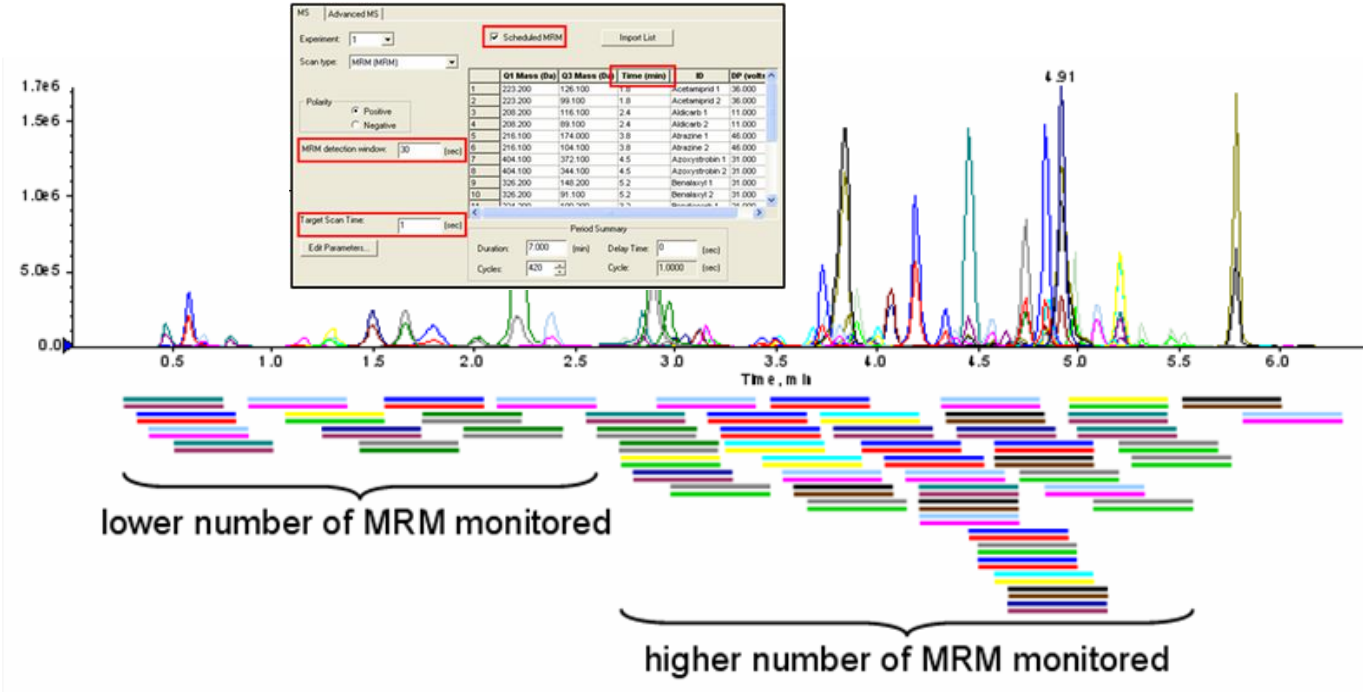
Pesticide	Ionization	Quasi molecular ion	Declustering potential (V)	1st transition Q1 mass (amu)	1st transition Q3 mass (amu)	1st transition Collision energy (V)	2nd transition Q1 mass (amu)	2nd transition Q3 mass (amu)	2nd transition Collision energy (V)	Retention time on endcapped RP phase
1-Naphthyl acetic acid	ESI +	[M+NH4]+	41	204,1	141,1	31	204,1	115,1	63	9,8
2,4-D	ESI -	[M-H]-	-21	219,0	160,9	-14	219,0	124,9	-34	10,8
2,4-DB	ESI -	[M-H]-	-66	247,0	160,8	-12	247,0	124,9	-34	13,4
2-Naphthyloxyacetic acid	ESI +	[M+NH4]+	36	220,1	157,1	19	220,1	127,1	43	10,3
2-Naphthyloxyacetic acid	ESI -	[M-H]-	-71	201,1	143,0	-18	201,1	114,9	-50	10,3
3,4,5-Trimethacarb	ESI +	[M+H]+	61	194,1	137,1	15	194,1	122,0	35	13,4
3-Hydroxycarbofuran	ESI +	[M+H]+	21	238,1	181,1	15	238,1	163,1	19	8,7
4-CPA	ESI -	[M-H]-	-71	185,0	126,8	-18	185,0	140,7	-12	7,4
5-Hydroxy-Clethodim-sulfon	ESI -	[M-H]-	-61	406,1	92,8	-30	406,1	64,1	-80	2,8
5-Hydroxy-clethodim-sulfon	ESI +	[M+H]+	16	408,1	204,0	27	408,1	176,1	35	8,6
5-Hydroxy-imidacloprid	ESI +	[M+H]+	56	272,0	191,1	23	272,0	224,8	23	6,7
5-Hydroxy-thiabendazol	ESI +	[M+H]+	71	218,0	190,9	35	218,0	146,9	43	9,3
6-Chlor-3-phenyl-pyridazin-4-ol	ESI +	[M+H]+	66	207,0	104,0	31	207,0	77,1	43	6,2
Acephate	ESI +	[M+H]+	6	184,1	124,9	25	184,1	142,9	13	1,7
Acetamiprid	ESI +	[M+H]+	36	223,0	126,0	27	223,0	90,1	45	9,1
Acibenzolar-S-methyl	ESI +	[M+H]+	26	210,9	136,1	39	210,9	140,0	31	14,4
Aclonifen	ESI +	[M+H]+	56	265,0	182,1	39	265,0	218,0	33	15,6
Acrinathrin	ESI +	[M+NH4]+	26	559,1	208,1	23	559,1	181,1	43	18,7
Alachlor	ESI +	[M+H]+	31	270,1	238,1	15	270,1	162,2	25	15,1
Aldicarb	ESI +	[M+NH4]+	1	208,1	89,1	21	208,1	116,0	13	10,4
Aldicarb-sulfoxid	ESI +	[M+H]+	36	207,1	89,1	17	207,1	131,9	11	2,3

Figure 2. Standard of 300 pesticides (100 ng/mL) detected in MRM.

For more information go to http://www.bfr.bund.de/cd/5832

Scheduled MRM™ – Aligns MRMs so expected MRMs will be monitored in order

Experimental determination of 75 pesticides



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Ion Detection

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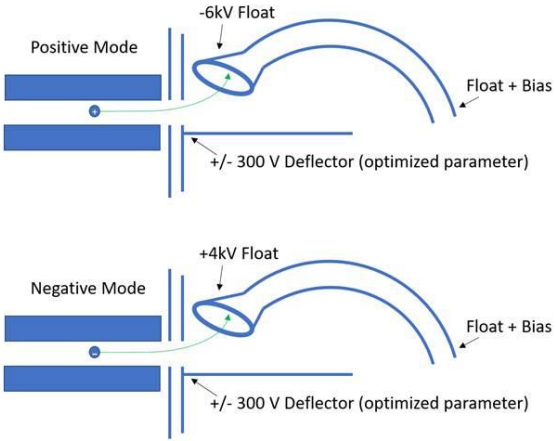
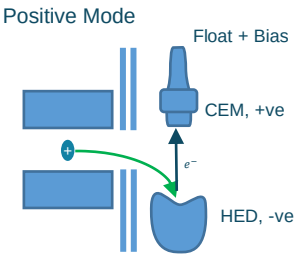
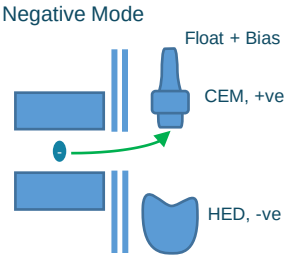
High Energy Dynode (HED) Detector Technology

FOR FAST POLARITY SWITCHING





Positive ions first converted to an electron, then electrons are detected by electron multiplier



Negative ions directly detected by electron multiplier

Improve 1. polarity switching.
2. linear dynamic range.

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High Resolution Accurate Mass Spectrometry; QTOF

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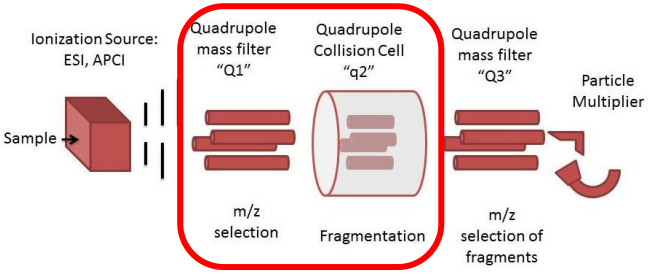
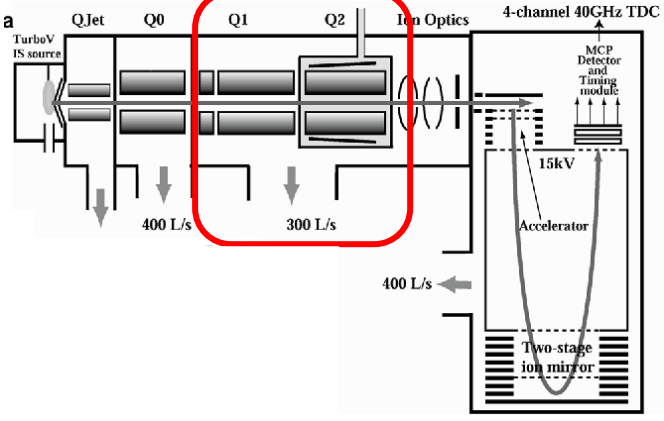
What is different between QQQ vs QTOF?

QQQ



QTOF



Same Q1 and Q2 functionality.

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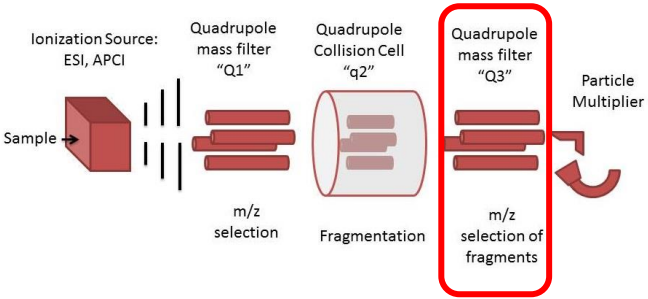
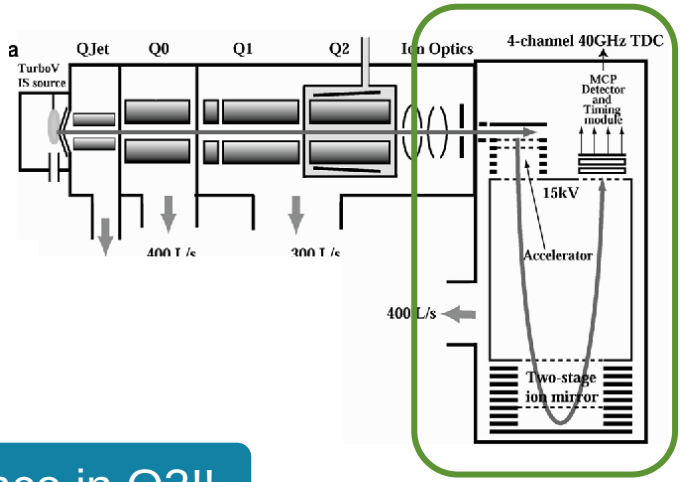
What is different between QQQ vs QTOF?

QQQ



QTOF



Difference in Q3!!

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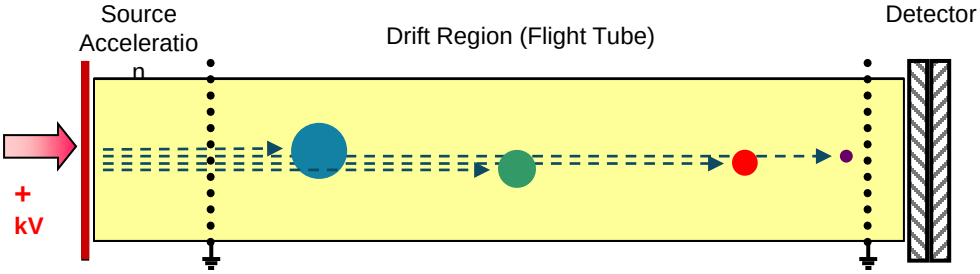
Time of Flight: Linear TOF Analyzer



Principle: All ions are accelerated with the same potential at a fixed initial point and time, and are allowed to drift down the Flight Tube

Ions will separate according to their **mass-to-charge ratios**, with light ions accelerated to a higher velocity that heavy ions.

The lighter ions strike the detector before the heavier ions. The "**time of flight**" (TOF) can be used to calculate the ions' **m/z**.

$$E_K = \frac{1}{2} mv^2$$

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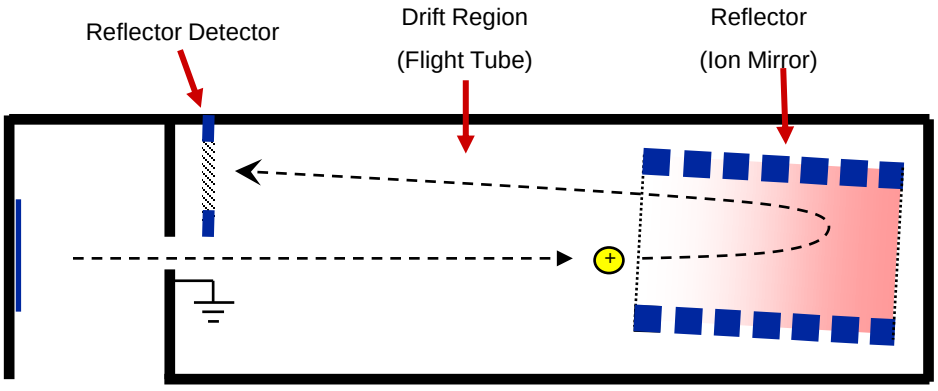
Time of Flight: Reflector TOF Analyzer



The reflector uses an electrostatic field gradient to turn ions around, extending the flight length without increasing the instrument size.

The reflector also compensates any kinetic energy spread of ions having the same mass. The result is **improved resolution**.

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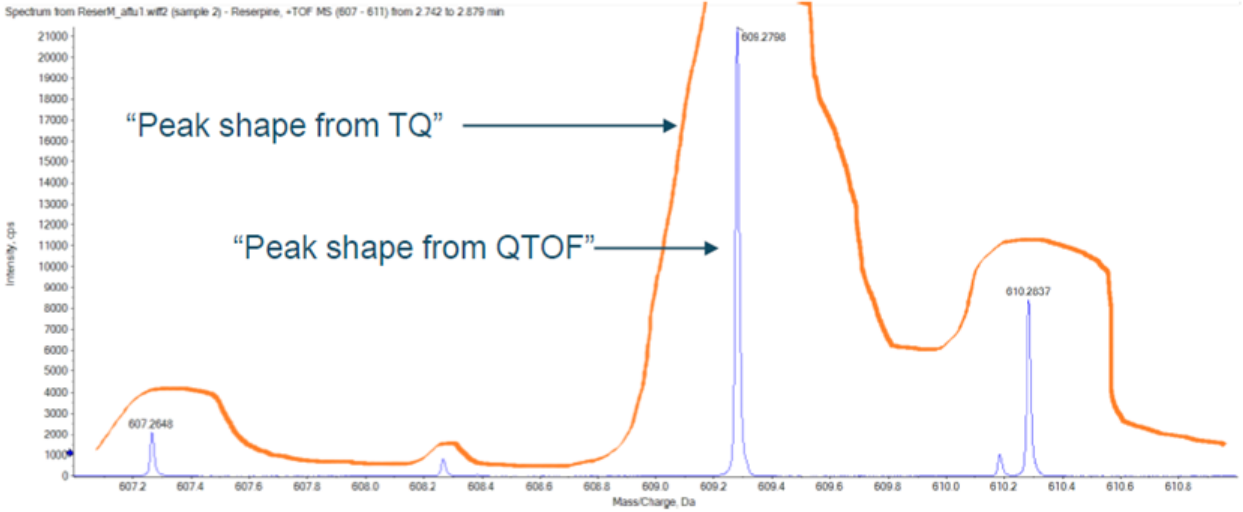
Comparison resolution of QTOF and TQ



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Sensitivity and Resolution

- There is always a trade off between sensitivity and resolution in all quadrupole type mass analyzer
  - *High resolution* = more ion filtering = less ions reaching detector = *less signal*
  - *Low resolution* = less ion filtering = more ions reaching detector = *more signal*

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Unique features of a QTOF vs. triple quadrupole MS

- Applications?
  - High-resolution MRM quantification - high-resolution accurate mass fragments results in greater compound specificity
  - Non-target acquisition with suspect screening - high-resolution accurate mass product scan for compound confirmation
  - Unknown compound ID

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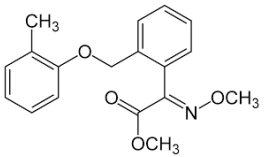
Advantages of high-resolution mass spectrometry

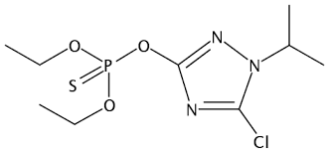
- QTOF can distinguish between compounds of similar mass
  - X500R = 30,000 resolution, 300 Da compound, Δm = 0.01 Da
- QTOF can accurately measure molecular weight to *several decimal places*
  - 300 Da compound, 5 ppm mass error = 0.0015 Da

High-resolution mass spectrometry
can distinguish these pesticides!

Kresoxim-methyl
$C_{18}H_{19}NO_4$
Mass = 313.1214 Da



Isazophos
$C_9H_{17}ClN_3O_3PS$
Mass = 313.0417 Da



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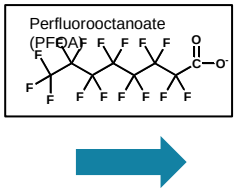
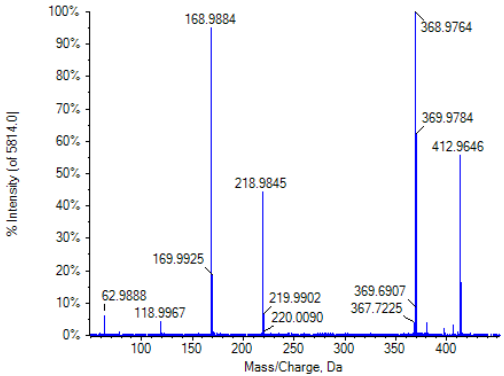
Molecule fragmentation

- Compounds will break apart into characteristic fragments which generally represent pieces of the original (precursor) molecule
- Fragmentation pattern can reveal the chemical structure
- TOF instruments obtain high resolution fragment masses resulting in greater specificity





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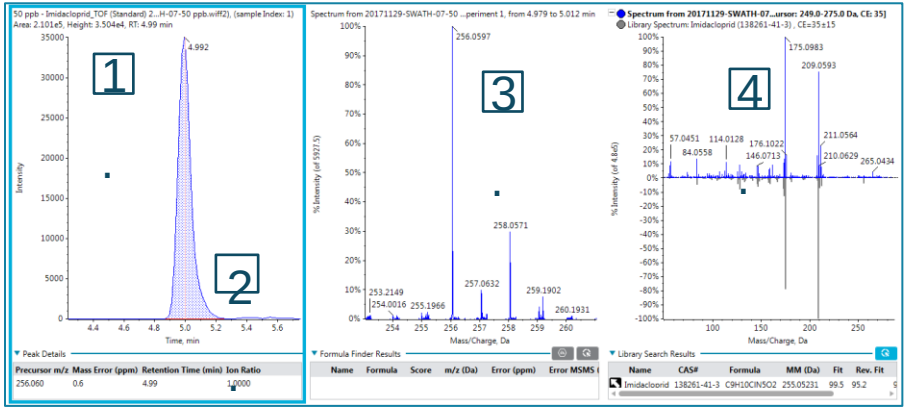
ID unknown compounds and confirm target compound ID

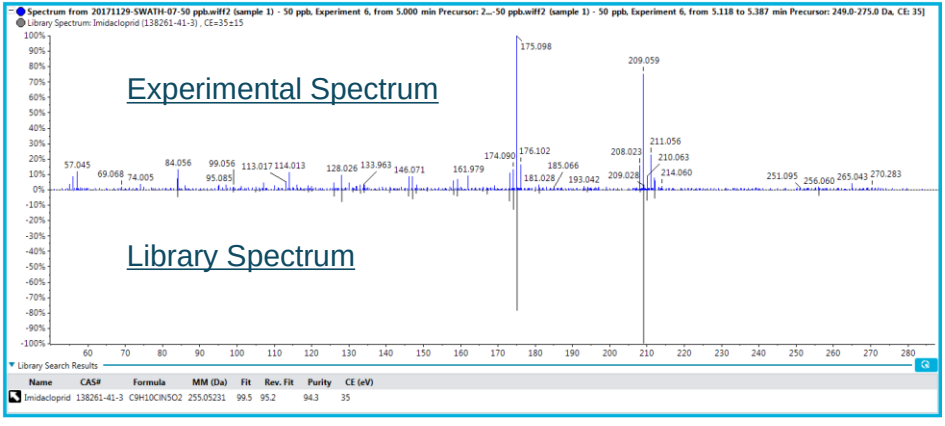
1. Retention time (<2.5%) ✓
2. High resolution accurate mass (<5 ppm) ✓
3. Isotope pattern (>80%) ✓
4. MS/MS fragmentation pattern match with HRMS Library ✓



SCIEX
The Power of Precision

ID unknown compounds and confirm target compound ID

4. Match with high-resolution mass spectrometry library ✓



Library sources:

1. Commercial library (e.g. SCIEX, NIST)
2. User generated

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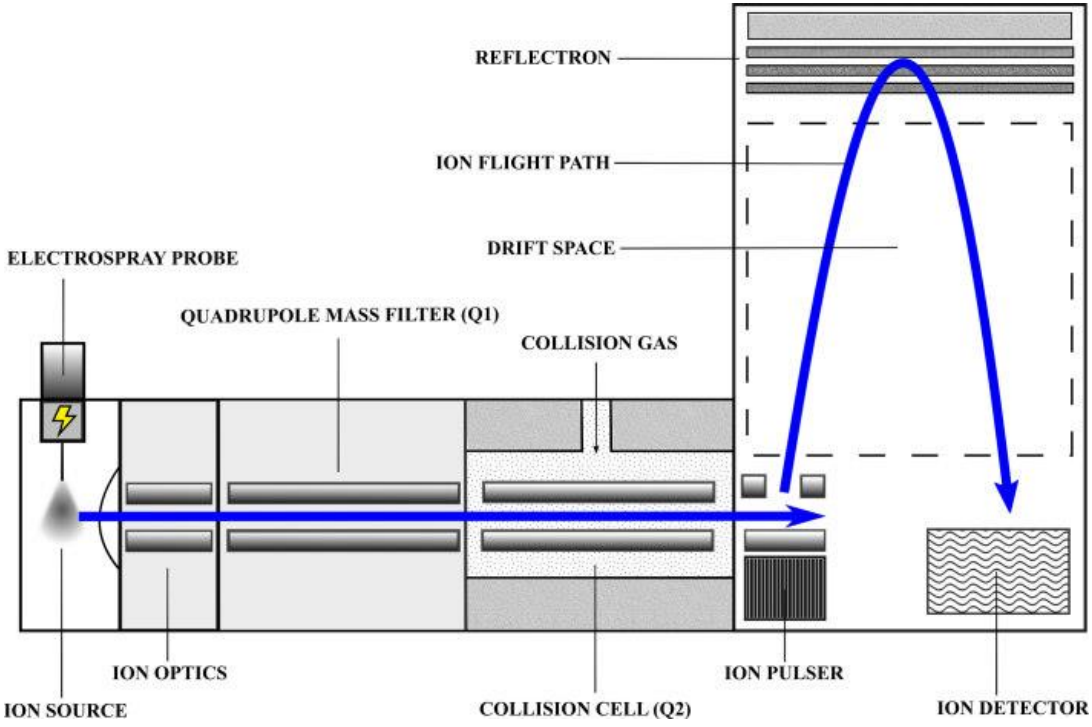
Instrument rail



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Targeted and Untargeted Screening: Acquisition, Processing and Results

Sample Injection → IDA/SWATH® → Pesticide Confirmation → Analyte Quantitation → Cross Sample Comparison

X500R System

Bench Top QTOF

Routine Use

Single Injection Workflow

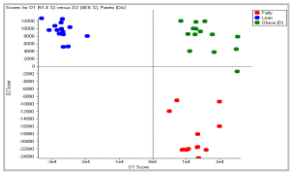
SCIEX OS Software

Pesticide Identification & Quantitation

SCIEX OS Targeted Data Extraction using a Library and Quantitation

MarkerView™ Software

Statistical Analysis



T-tests, PCA analysis, etc.

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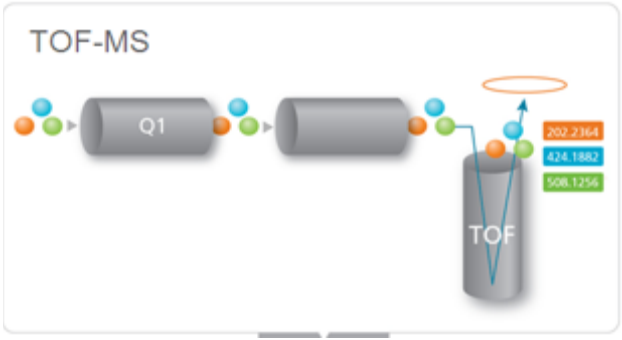
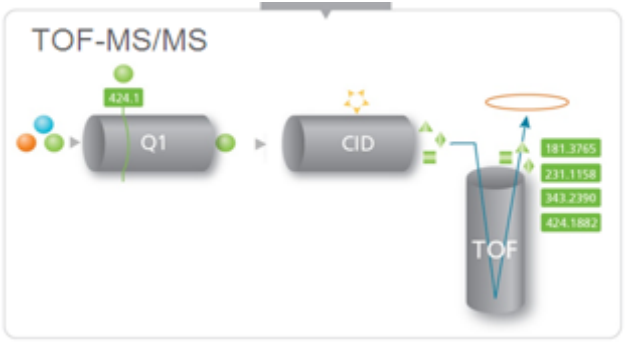
MS and MS/MS Scan Types

MS scan



MSMS scan

- DDA (Data Dependent Acquisition)
- DIA (Data Independent Acquisition)
- MRM^{HR} (Multiple Reaction Monitoring)



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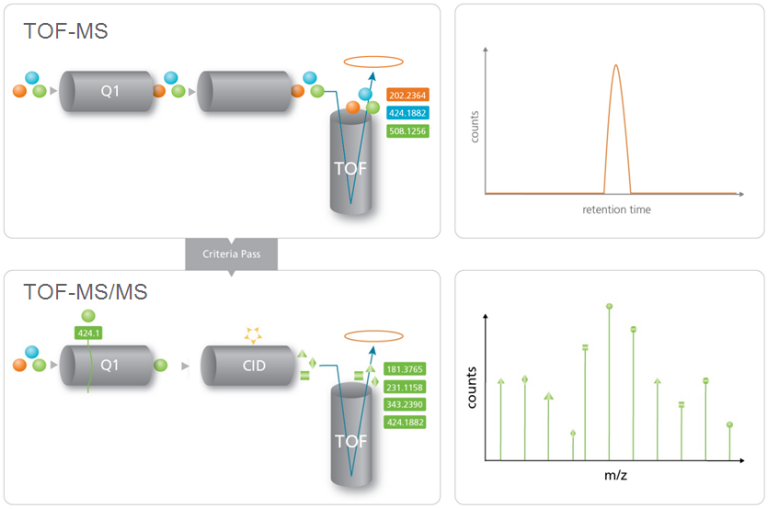
MSMS scan: DDA (Data Dependent Acquisition)

The mass spectrometer selects the most intense peptide ions in a first stage (Q1) of tandem mass spectrometry, and then they are fragmented and analyzed in a second stage of tandem mass spectrometry.

First MS scan

Criteria:
- Number of precursor ion
- Intensity threshold

Second and so on MSMS scan



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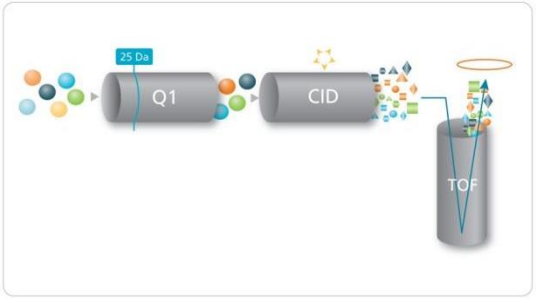
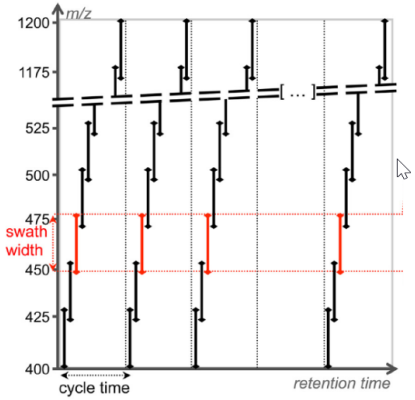
MSMS scan: DIA (Data Independent Acquisition; **SWATH* MS**)

For each cycle, the instrument focuses on a narrow mass window of precursors and acquires MS/MSdata from all precursors detected within that window. This mass window is then stepped across the entire mass range, systematically collecting MS/MS data from every mass and from all detected precursors.





SWATH MS data independent acquisition

*** SWATH** – Sequential Windowed Acquisition of All Theoretical Fragment ions

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Comparison DDA vs DIA

DDA (Data Dependent Acquisition)

- Ions from 1 amu allowed into the collision cell
- MS/MS spectra only from a single parent mass
- Thresholds applied for MS/MS spectral data acquisition
- Very fast, has short cycle times
- Spectra are usually very clean and library searchable
- Can be used for quantitation

DIA (Data Independent Acquisition; SWATH™)

- Ions from several masses allowed into collision cell at one time
- MS/MS Spectra contains fragment ions from all parent ions
- No thresholds applied – always have spectral data
- Can have long cycle times
- Need to carefully cleanup spectra for library searching
- Can be used for quantitation

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DDA/DIA; Targeted and Untargeted Screening

Confirmation Criteria

Targeted screening

Confirmation criteria:

Mass error (<5 ppm) ✔
Retention time (<2.5%) ✔
Library "fit" (>70) ✔

Untargeted screening

Confirmation criteria:

Mass error (<5 ppm) ✔

Library "fit" (>70) ✔

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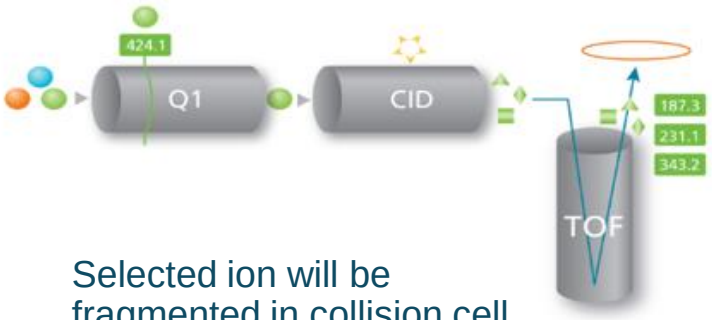
MSMS scan: MRM^{HR} (Multiple Reaction Monitoring)

Also known as Selective Reaction Monitoring – SRM) is a highly specific and sensitive mass spectrometry technique that can selectively quantify compounds within complex mixtures.



Selected ion will be fragmented in collision cell and scan out to TOF analyzer.

Using only Precursor Ion

TOF MSMS

Mass Table — Apply fragment ion mass — Apply TOF start/stop mass — Apply Scan

	Compound ID	Group name	Precursor ion (Da)	TOF start mass (Da)	TOF stop mass (Da)
1	F1.6pp	F1.6	339.00	40.00000	700.00000
2	F1.6pp_13C3	F1.6	342.00	40.00000	700.00000
3	F1.6pp13C6	F1.6	345.00	40.00000	700.00000
4	ATP506	ATP	506.00	40.00000	700.00000
5	ATP507	ATP	507.00	40.00000	700.00000
6	ATP508	ATP	508.00	40.00000	700.00000
7	ATP509	ATP	509.00	40.00000	700.00000
8	ATP510	ATP	510.00	40.00000	700.00000
9	ATP511	ATP	511.00	40.00000	700.00000
10	ATP512	ATP	512.00	40.00000	700.00000
11	ATP513	ATP	513.00	40.00000	700.00000
12	Glutamate146	Glutamate	146.00	40.00000	700.00000
13	Glutamate148	Glutamate	148.00	40.00000	700.00000
14	Citrate191	Citrate	191.00	40.00000	700.00000
15	Citrate192	Citrate	192.00	40.00000	700.00000
16	Citrate193	Citrate	193.00	40.00000	700.00000
17	Citrate194	Citrate	194.00	40.00000	700.00000
18	Citrate195	Citrate	195.00	40.00000	700.00000

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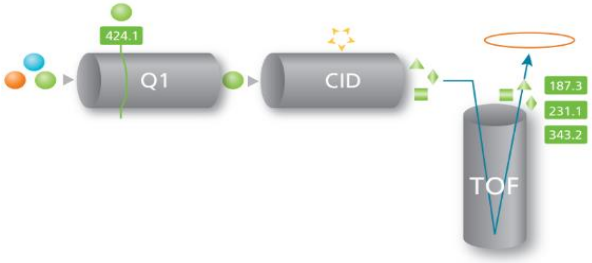
MSMS scan: MRM^{HR} Workflow with Time Scheduling

- Input Q1 and Q3 masses and compound retention time



Using Precursor-Fragment Ion & RT

TOF MSMS

Mass Table ◉ Apply fragment ion mass ○ Apply TOF start/stop mass ☑ Apply Scan Schedule

	Compound ID	Group name	Precursor ion (Da)	Fragment ion (Da)	Accumulation	Retention time (min)
1	F1,6P 1	F1,6P	337.00	96.9693	0.0400	3.70
2	F1,6P 2	F1,6P	337.00	78.9594	0.0400	3.70
3	ATP 1	ATP	506.00	408.0105	0.0400	3.85
4	ATP 2	ATP	506.00	158.9242	0.0400	3.85
5	Glutamate 1	Glutamate	146.00	128.0347	0.0400	2.73
6	Glutamate 2	Glutamate	146.00	102.0559	0.0400	2.73
7	Citrate 1	Citrate	191.00	111.0088	0.0400	3.54
8	Citrate 2	Citrate	191.00	87.0089	0.0400	3.54

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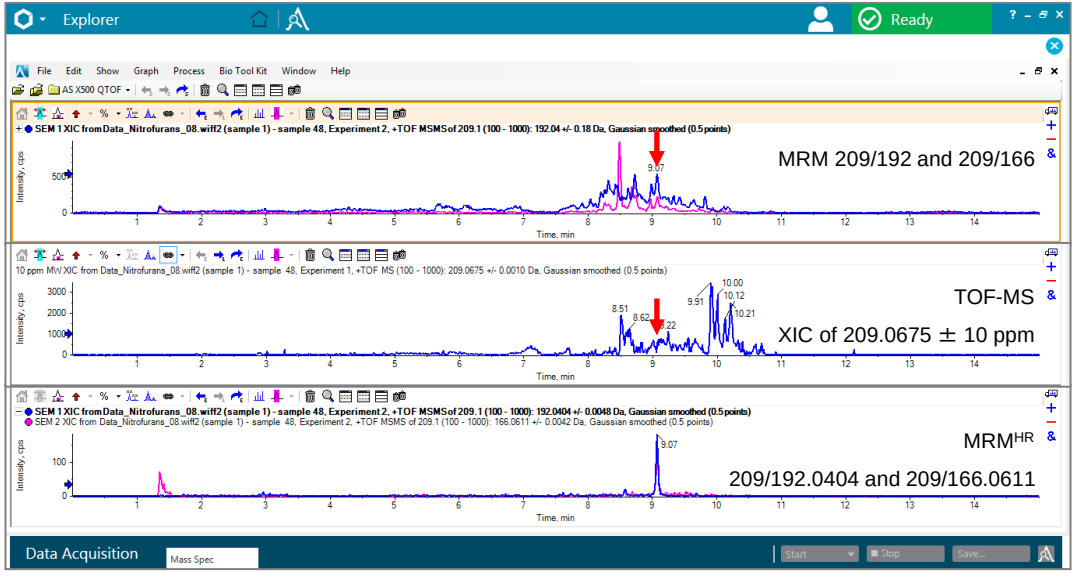
Targeted quantitation – MRM^{HR}

- **Increased selectivity with MRM^{HR}:**
- Feed Sample Tested Positive for NP-Semicarbazide



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LCMSMS applications

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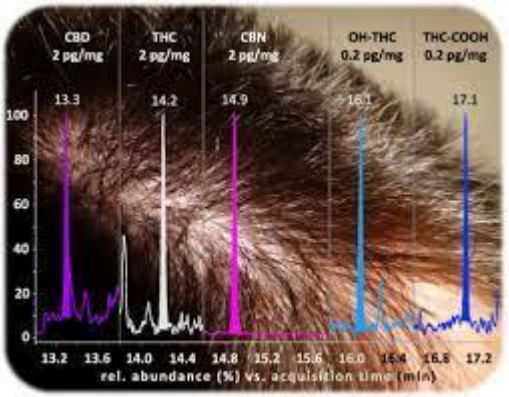
The Power of Precision

Strategy for Easy, Sensitive and Selective Detection of THC Carboxylic Acid Direct from Hair.

The active ingredient in cannabis is THC and its major metabolite is THC carboxylic acid, which is the target analyte for detecting cannabis use. Urine is the typical matrix analyzed for THC use and LC-MS/MS operating in MRM mode is the usual technique of choice. Analysis of urine or plasma for THC carboxylic acid generally detects use only during a time window of up to thirty days.

However, THC carboxylic acid is also stored in hair and, hence, enables monitoring of a person's use of cannabis over an extended period of time.



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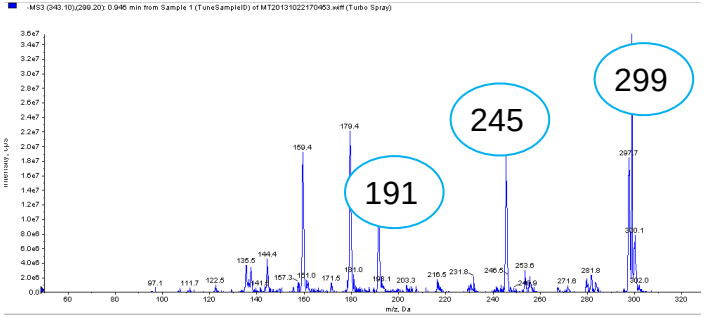
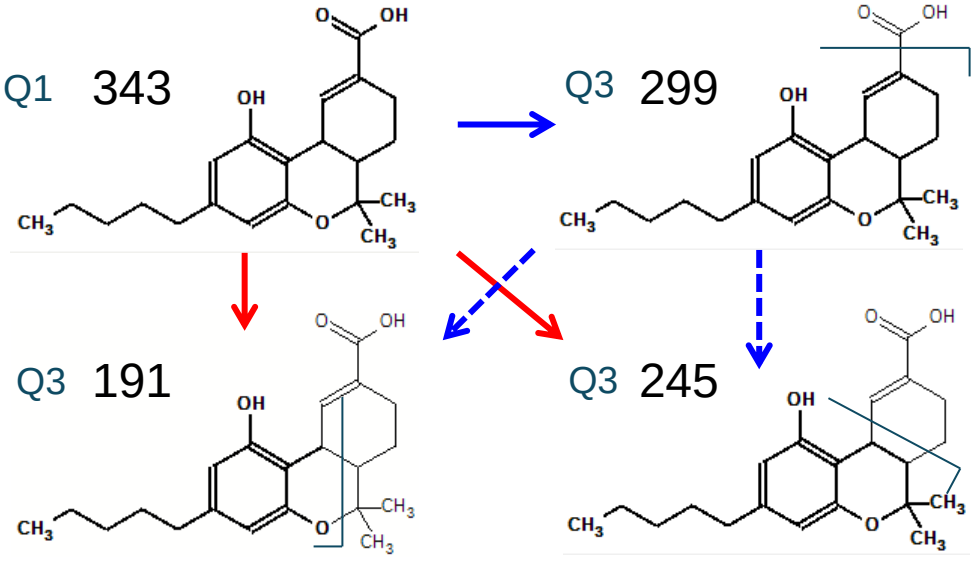
MRM and MRM^3

343 → 299 → MS^3





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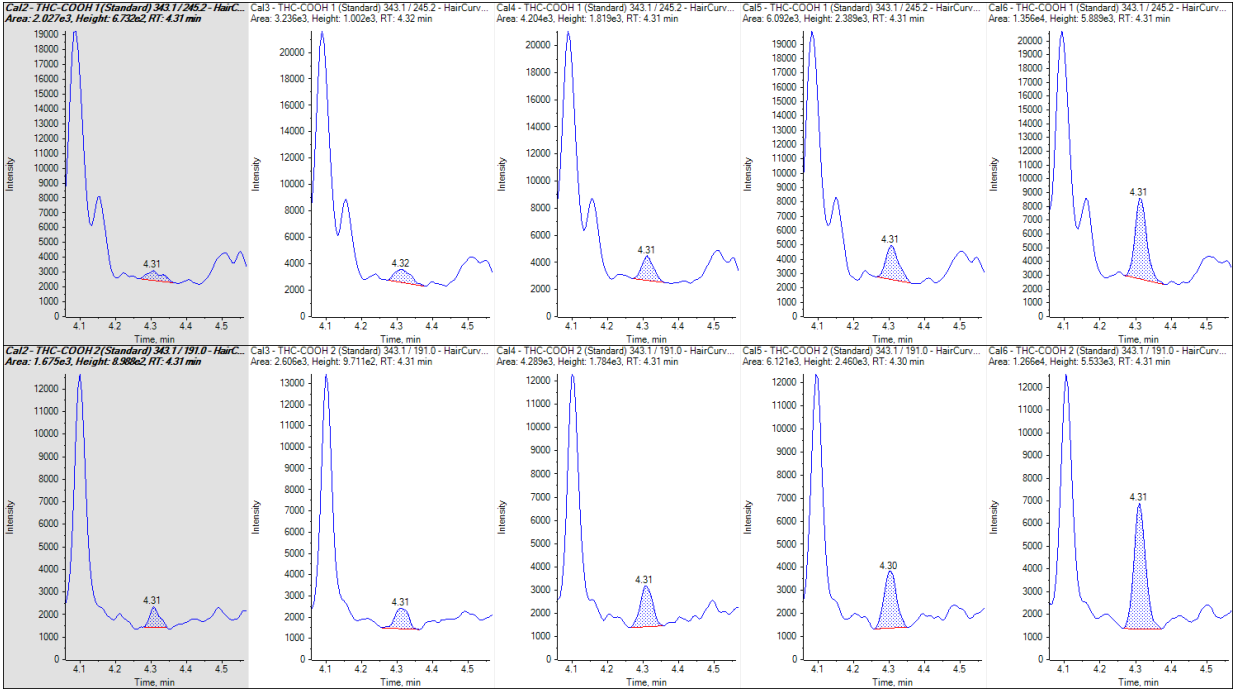
Detection of THC-COOH with MRM

MRM: TOP/343→245/QUANT, BOTTOM/343→191/QUAL

245

191



0.04 pg/mg 0.1 pg/mg 0.2 pg/mg 0.4 pg/mg 1 pg/mg

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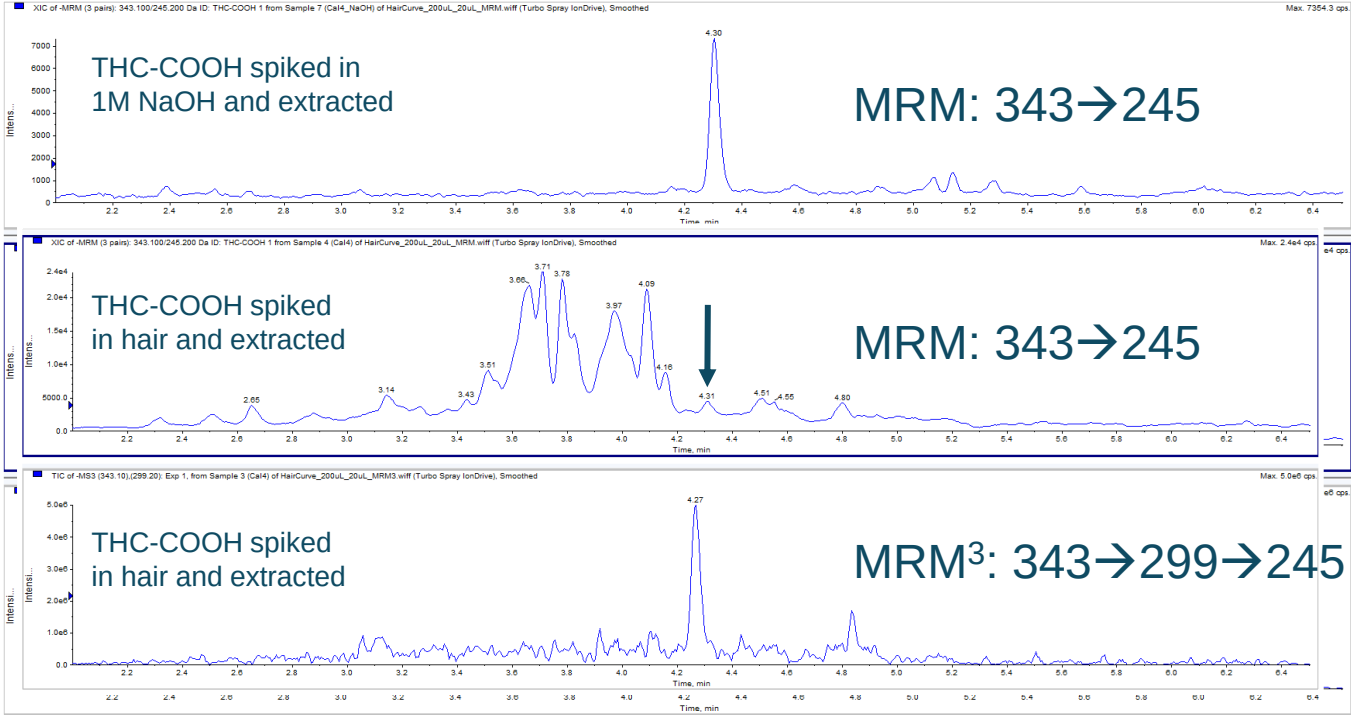
MRM^3 is Necessary

0.2 PG/MG THC-COOH (CUTOFF), 0.4 PG ON-COLUMN



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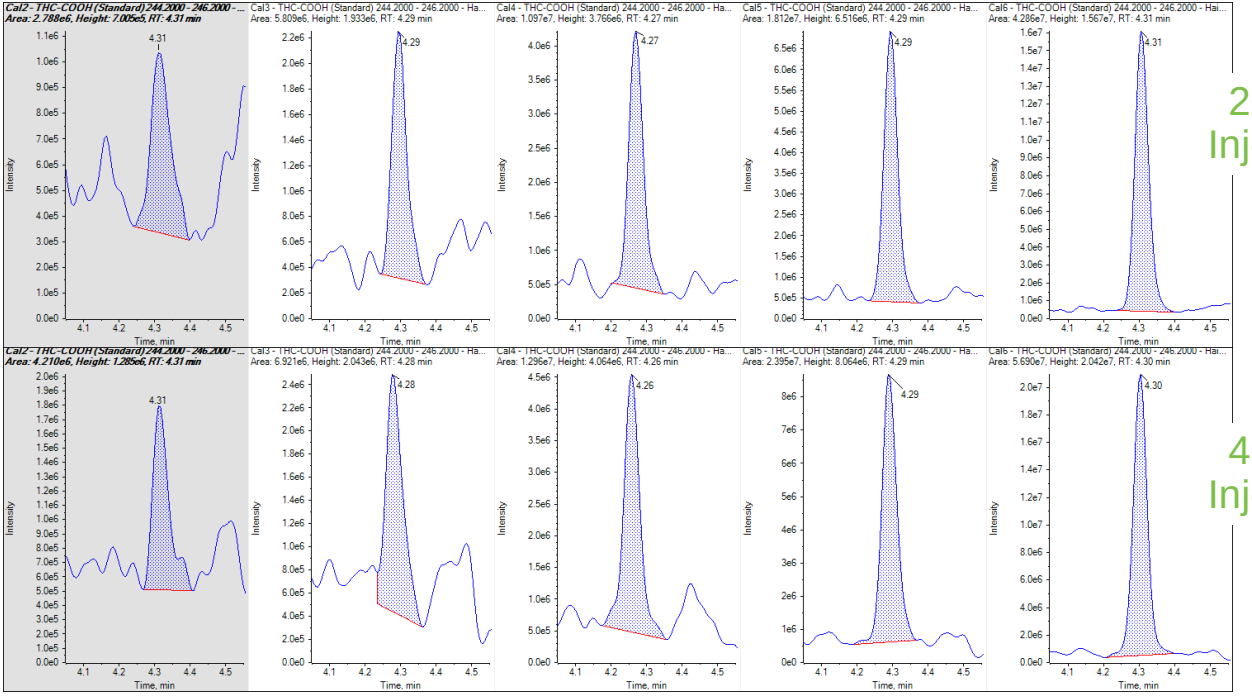
Detection of THC-COOH with MRM^3

343→299→245±1



20 µL Injected

40 µL Injected

0.04 pg/mg 0.1 pg/mg 0.2 pg/mg 0.4 pg/mg 1 pg/mg

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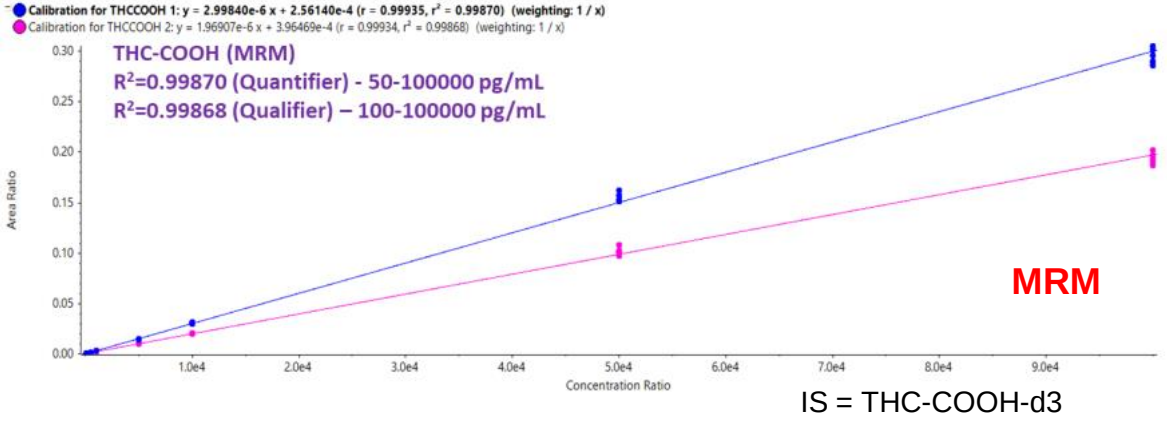
Ultra-sensitive analytical methodology for the quantification of 11-nor-9-carboxy-THC (THC-COOH) in oral fluid

Δ9-tetrahydrocannabinol (THC) has previously been used as the marker of choice to monitor cannabis consumption. 11-nor-9-carboxy-THC (THC-COOH) was proposed as a marker of cannabis intake since it is not detected in oral fluid collected from subjects passively exposed to cannabis



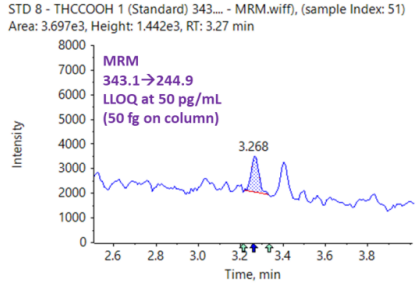
IS = THC-COOH-d3

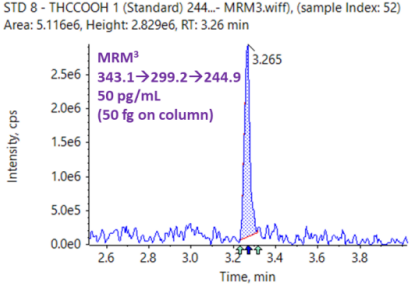
343.1 → 244.9 (Blue line = Transition 1)
191.1 (Pink line = Transition 2)

SCIEX
The Power of Precision

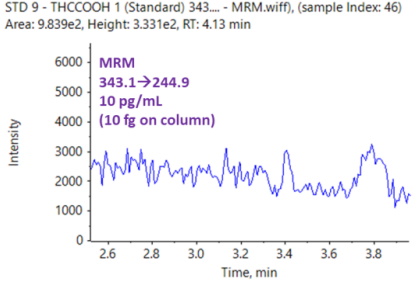
Ultra-sensitive analytical methodology for the quantification of 11-nor-9-carboxy-THC (THC-COOH) in oral fluid

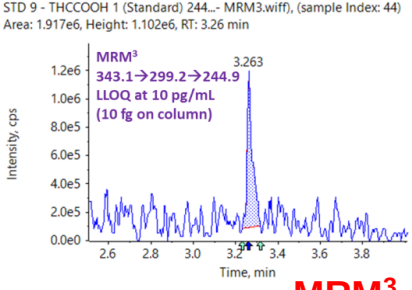
Δ9-tetrahydrocannabinol (THC) has previously been used as the marker of choice to monitor cannabis consumption. 11-nor-9-carboxy-THC (THC-COOH) was proposed as a marker of cannabis intake since it is not detected in oral fluid collected from subjects passively exposed to cannabis





50 pg/mL





10 pg/mL

MRM **MRM³**

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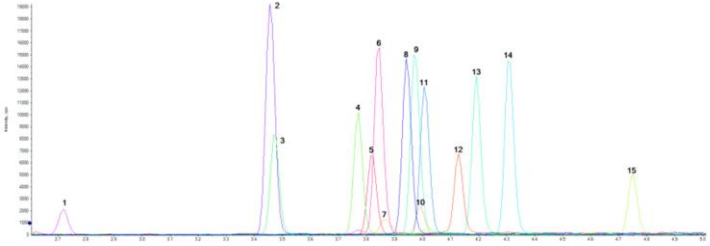
Detection of Fentanyl Analogs and Novel Synthetic Opioids in Hair

The recent outburst of novel synthetic opioids (NSO) into the recreational drug market has been a major contributor to the ongoing opioid crisis. These substances are gaining popularity as substitutes to controlled opioids and are often used as cutting agents or adulterants to heroin and other commonly abused drugs.

Table 1. List of the 15 Target Compounds and 3 Internal Standards Used in This Study.

Fentanyl Analogs	Non-Fentanyl Analogs-Related
Fentanyl	U-47700
Norfentanyl	MT-45
Acetyl Fentanyl	AH-7921
Furanylfetanyl	AH-7921 D6
4-ANPP	
Carfentyl	
Acrylfentanyl	
Ocfentanyl	
α-Methylfentanyl	
4-Fluoro-Butyrfentanyl	
Cyclopropylfentanyl	
Butyrfentanyl	
Fentanyl D5	
Norfentanyl D3	



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Detection of Fentanyl Analogs and Novel Synthetic Opioids in Hair

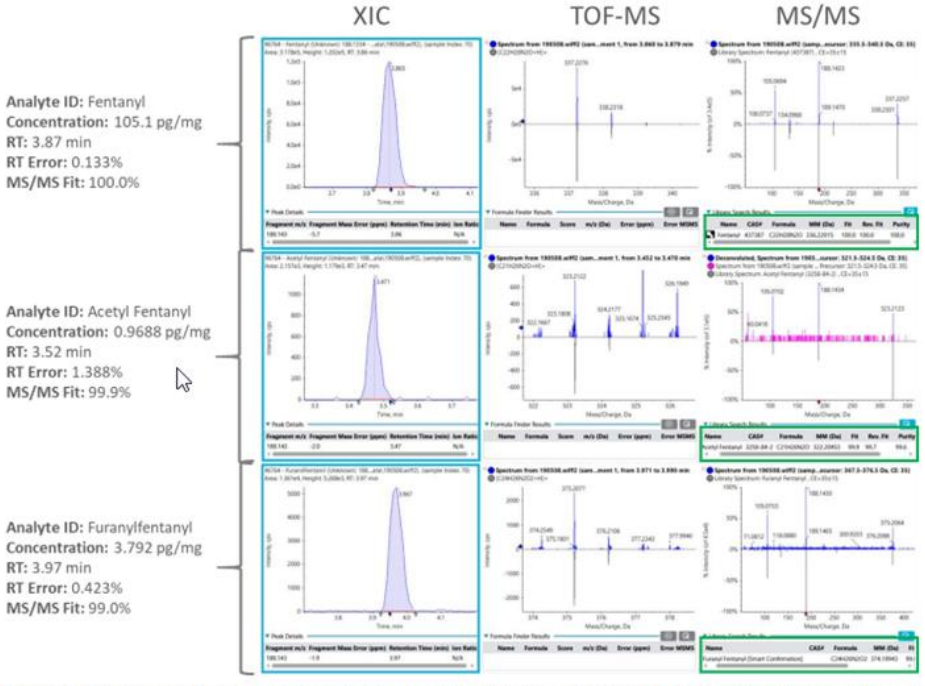

Figure 5: SWATH Acquisition Leads to Accurate Identification and Quantification of NSOs in Real Head Hair Samples. Extracted Ion Chromatograms (XICs), TOF MS and MS/MS spectra showing confident and detailed identification of fentanyl (top), acetyl fentanyl (middle) and furanylfentanyl (bottom) from a real head hair sample. These three NSOs were accurately detected at 105.1 (fentanyl), 0.9688 (acetyl fentanyl) and 3.792 pg/mg (furanylfentanyl) with excellent MS/MS fit value (>99%) and for all three compounds.

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Detection of Fentanyl Analogs and Novel Synthetic Opioids in Hair

Table 2: Validation Study. Average (n=3) Results From the Validation Study Showing Inter-Day and Intra-Day Precision (%CV) and Accuracy (bias%) as Well as the LOD for the 15 Target Analytes Used in This Study.

		Intra-Day Precision (CV %)			Inter-Day Precision (CV %)			Intra-Day Accuracy (Bias %)			Intra-Day Accuracy (Bias %)		
Analyte	LOD (pg/mg)	2 pg/mg	10 pg/mg	100 pg/mg	2 pg/mg	10 pg/mg	100 pg/mg	2 pg/mg	10 pg/mg	100 pg/mg	2 pg/mg	10 pg/mg	100 pg/mg
4-ANPP	0.7	11	11	3	15	17	11	3	-3	12	4	-11	2
4-fluorobutyrfentanyl	0.2	12	9	3	17	16	9	18	-2	5	4	-4	-3
Acetylfentanyl	0.6	25	7	3	24	14	6	6	-14	8	2	-8	-3
Acrilfentanyl	0.6	24	9	5	28	19	16	4	-10	7	5	-8	-0.4
AH-7921	0.6	19	13	6	20	19	20	9	-10	3	3	-8	-2
Butyrfentanyl	0.6	18	7	4	24	15	10	4	-3	5	4	-4	-3
Carfentanyl	0.8	24	8	4	24	12	9	7	-9	2	5	-10	-0.6
Cyclopropylfentanyl	0.7	18	7	3	17	17	11	8	-5	5	5	-9	-0.04
Fentanyl	0.6	24	7	4	19	13	6	6	-12	8	4	-10	-2
Furanilfentanyl	0.6	18	8	3	22	12	7	5	-11	8	2	-6	-3
Methylfentanyl	0.5	19	7	3	21	14	10	5	-9	10	4	-6	-0.9
MT-45	0.7	24	11	5	19	17	8	7	-7	2	3	-7	-3
Norfentanyl	1.2	22	23	12	19	20	18	-0.1	9	-16	19	15	13
Ocfentanyl	0.4	17	8	4	11	16	10	2	-5	13	1	-7	-3
U-47700	0.4	15	14	3	12	16	7	3	-11	9	3	-10	-0.8

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Detection of Fentanyl Analogs and Novel Synthetic Opioids in Hair

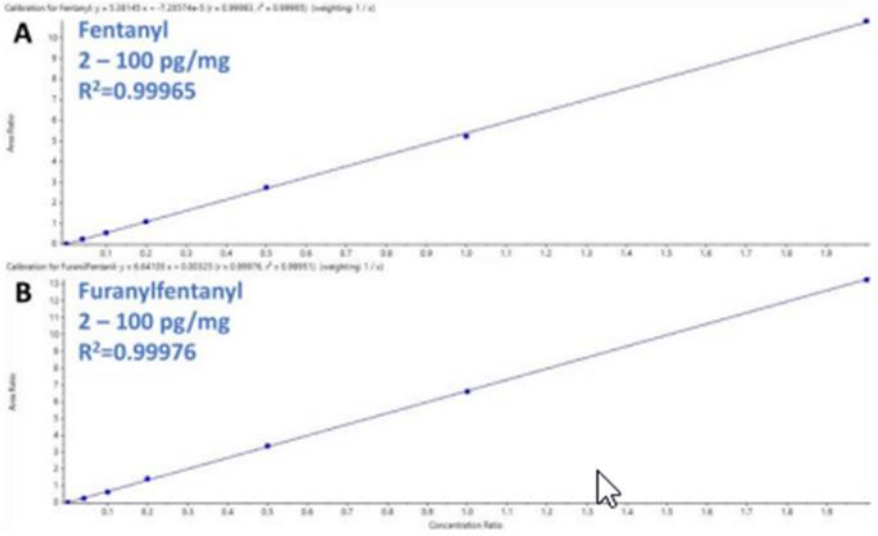

Figure 4. Excellent Linearity and High Dynamic Range for Novel Synthetic Opioids. Calibration curves resulting from the calibration series for fentanyl (A) and furanylfentanyl (B). Excellent linear response and sensitivity were observed with R^2 values of 0.993331 and 0.99844 for fentanyl and furanilfentanil, respectively.

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Thank you!!