Mass Spectrometry 101

and Beyond

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Introduction and History
LC/MS(/MS)

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What is a mass spectrometer (MS)?

An instrument that measures masses of particles

Who Uses It?

- Forensic Labs
  - City, County, State, FBI
- Hospitals
  - Neonatal Testing
  - Anti-Rejection Therapy
- Universities
- Government Agencies
  - EPA, FDA, USDA, USGS
  - CDC
  - ATF, TSA
  - State Labs, e.g., Agriculture
  - US Army, NASA
- Private Industry
  - Contract Labs

What specifically does an MS measure and display?

A particle's mass to charge ratio (m/z)

- The m in *m/z* is the molecular or atomic mass.
- The z represents the charge(s) carried by the ion.
- A molecule of mass 1000 carrying 2 charges will be observed at 1000/2 or 500 *m/z or m/z 500*.

What unit is used for mass here?

(historically)

Atomic Weight is a relative scale without explicit units.

- 1803 John Dalton **^{1}H = 1.00**
- 1906 Wilhelm Ostwald **H = 1/16 O**
- 1929 Oxygen Isotope is discovered **^{16}O** became **amu** where amu is **a**tomic **m**ass **u**nit
- 1961 **^{12}C** was the relative **u** "**u**nified atomic mass unit"
- 1993 IUPAC tentative approval of the dalton (da)
- 2005 IUPAC endorses the dalton
- 2006 International System of Units (SI) called dalton a non-SI unit.
- 2009 ISO didn't help
- 2010 Oxford University Press style guide – **Dalton**, **Da or kDa**

What must happen for m/z to be measured?

- Sample must be loaded into an MS somehow.
- There is some type of vaporization step.
- The components of the sample must then be ionized.
- The ions must be separated by their m/z ratio.
- The ions then must be detected, often quantitatively.
- The ion signal must be processed into mass spectra.

What are the basic components of a mass spectrometer?

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Sample Introduction and Ionization

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GC MS – Electron Impact or EI

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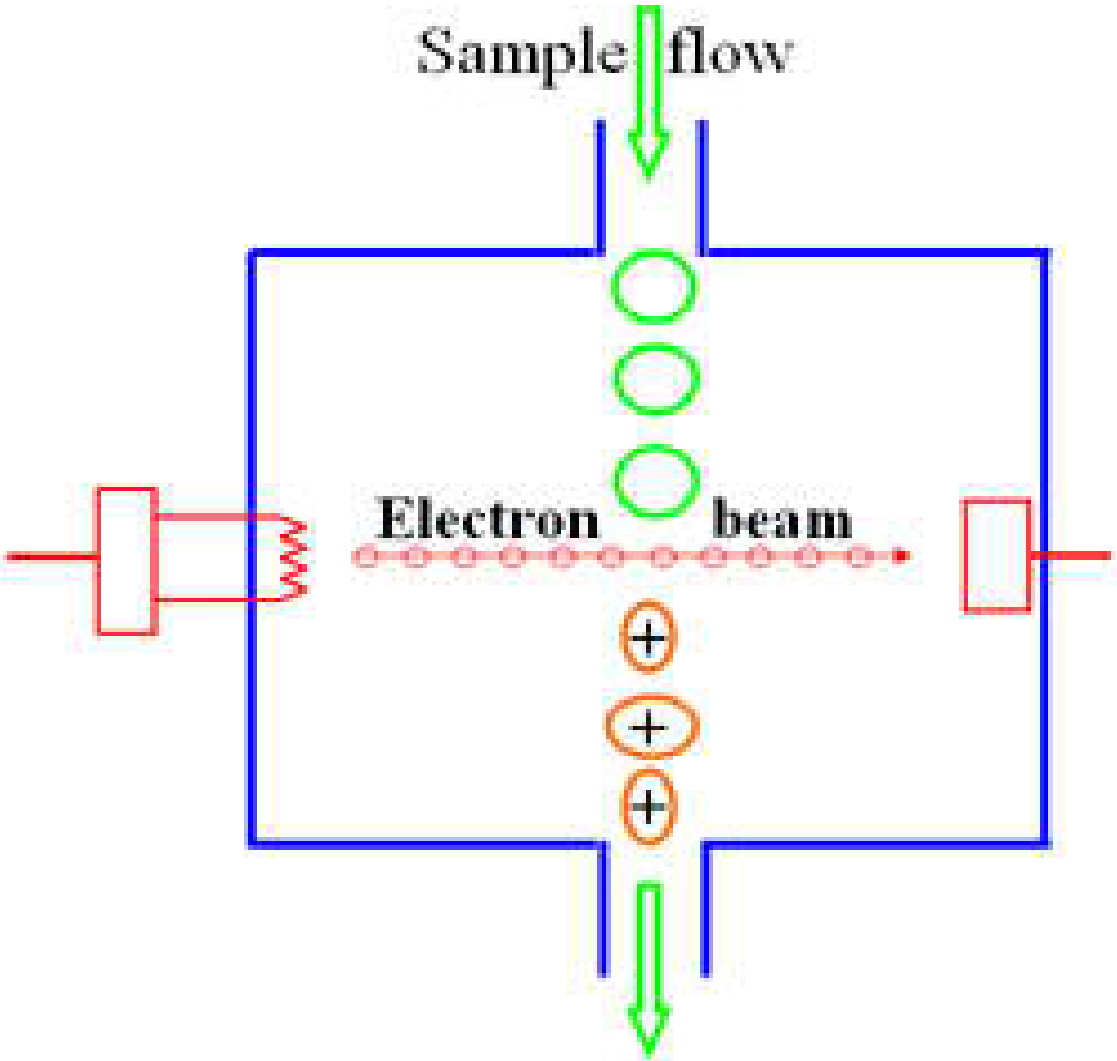
Gas Chromatograph or Solids Probe



Hot Rhenium Filament, 70V

+ Target

Jet Separator

EI Mass Spectrum of Warfarin

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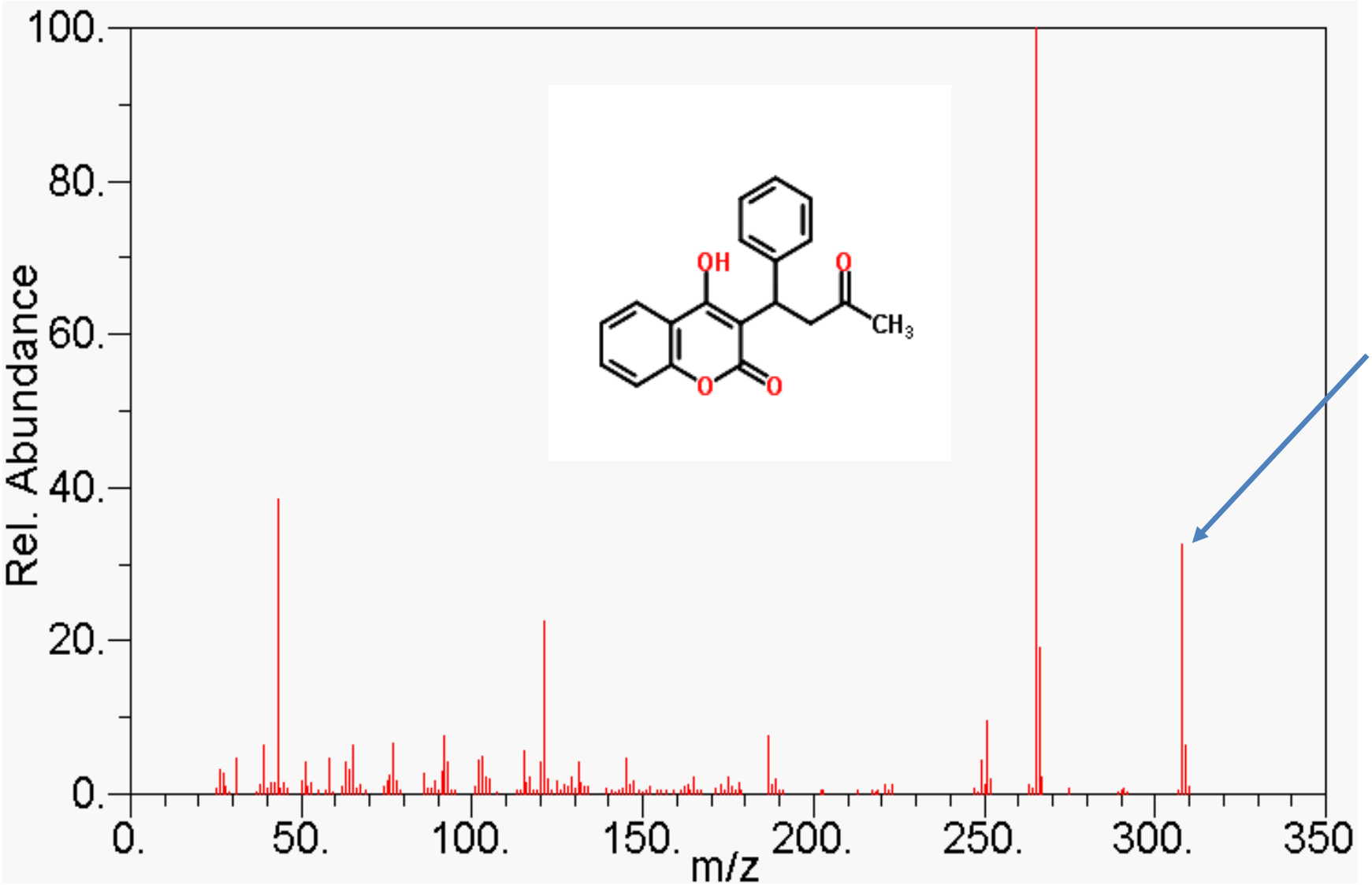

MW = 308

EI Mass Spectrum of Dioctyl Phthalate

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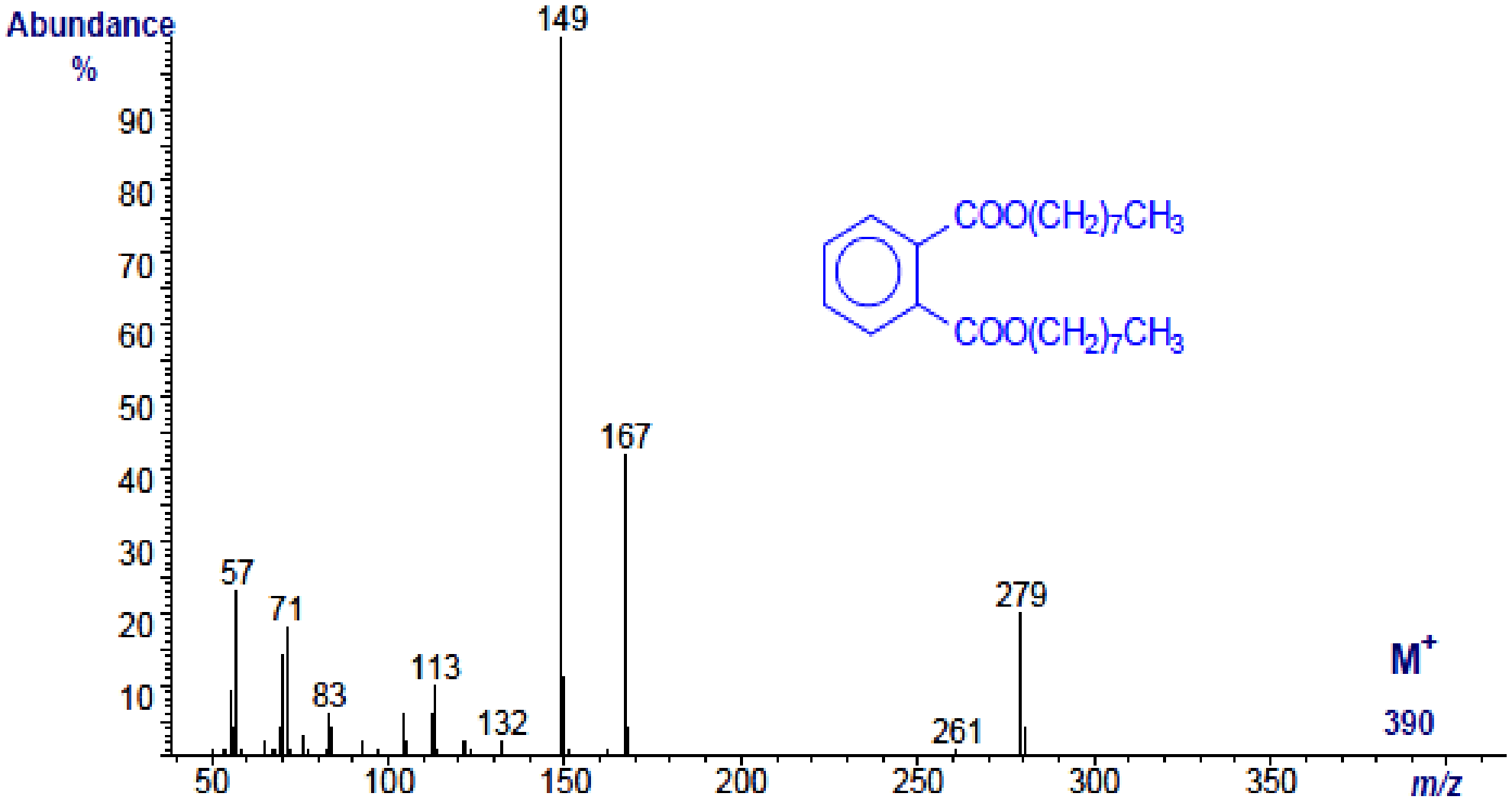

GC MS – Chemical Ionization or CI

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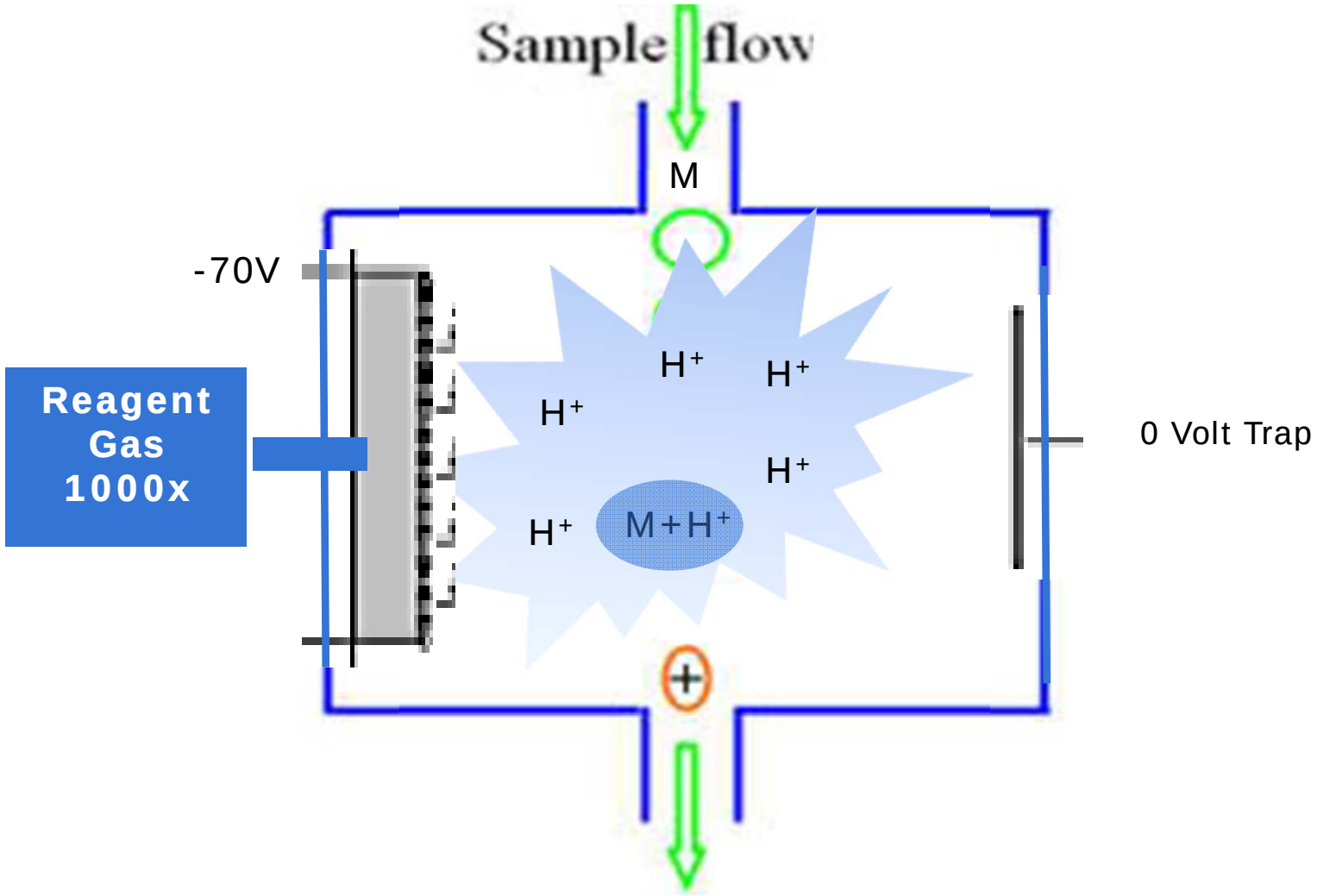
Gas Chromatograph or Solids Probe



Jet Separator

CI Mass Spectrum of Dioctyl Phthalate

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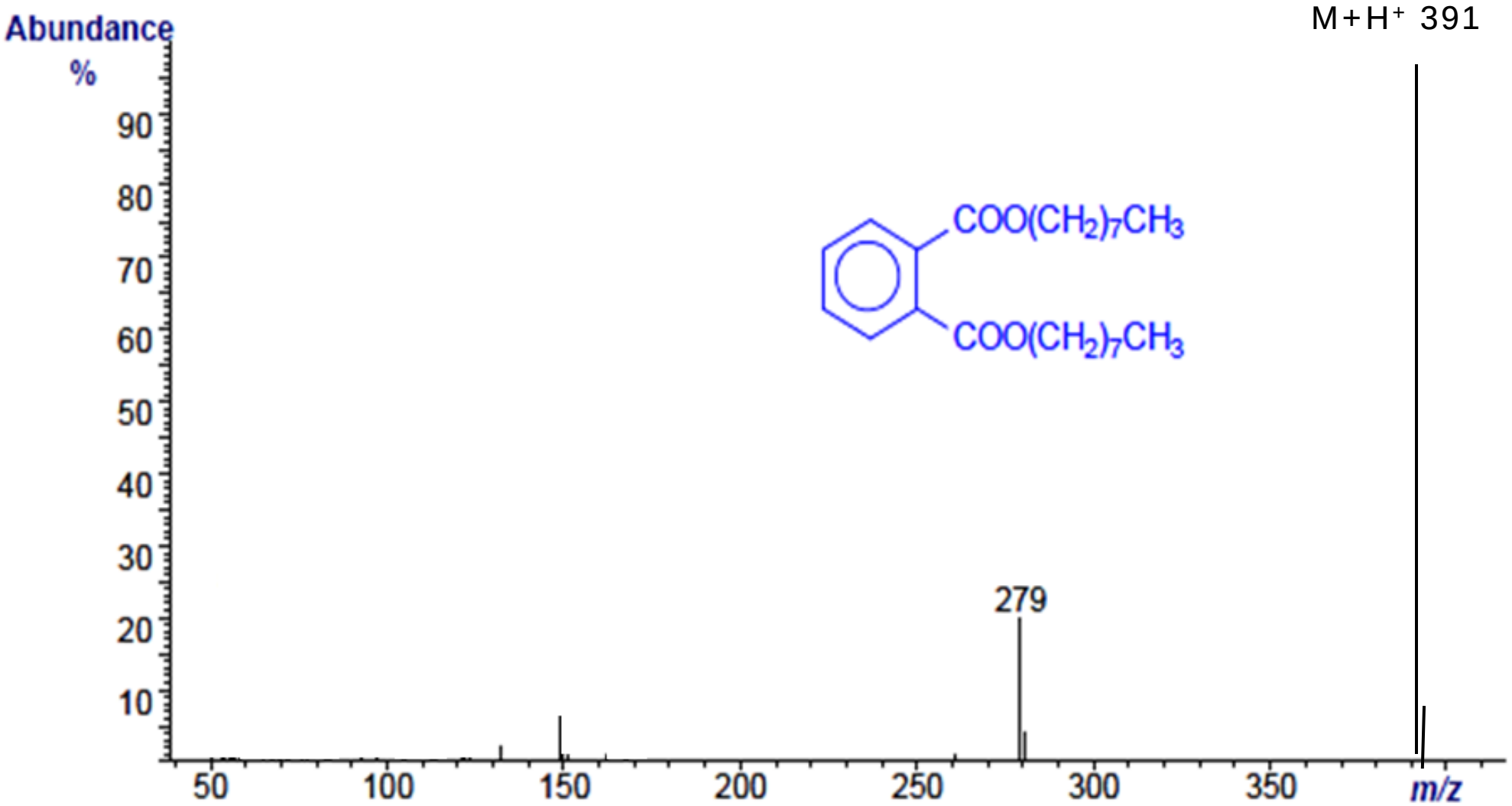
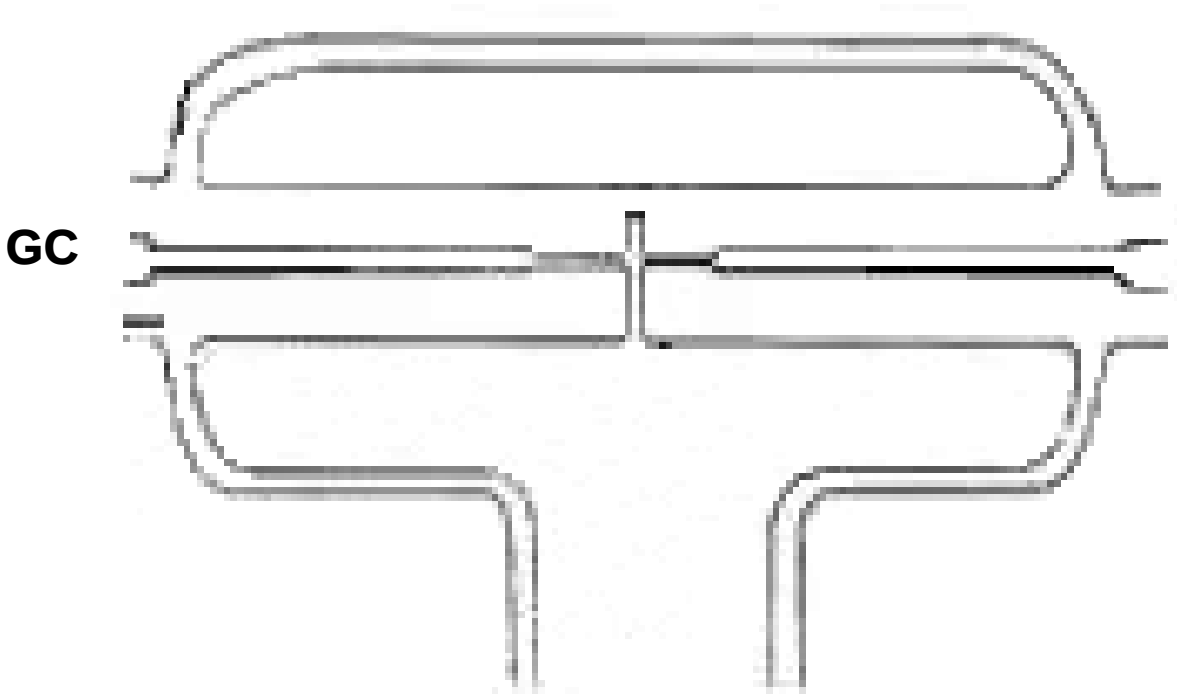
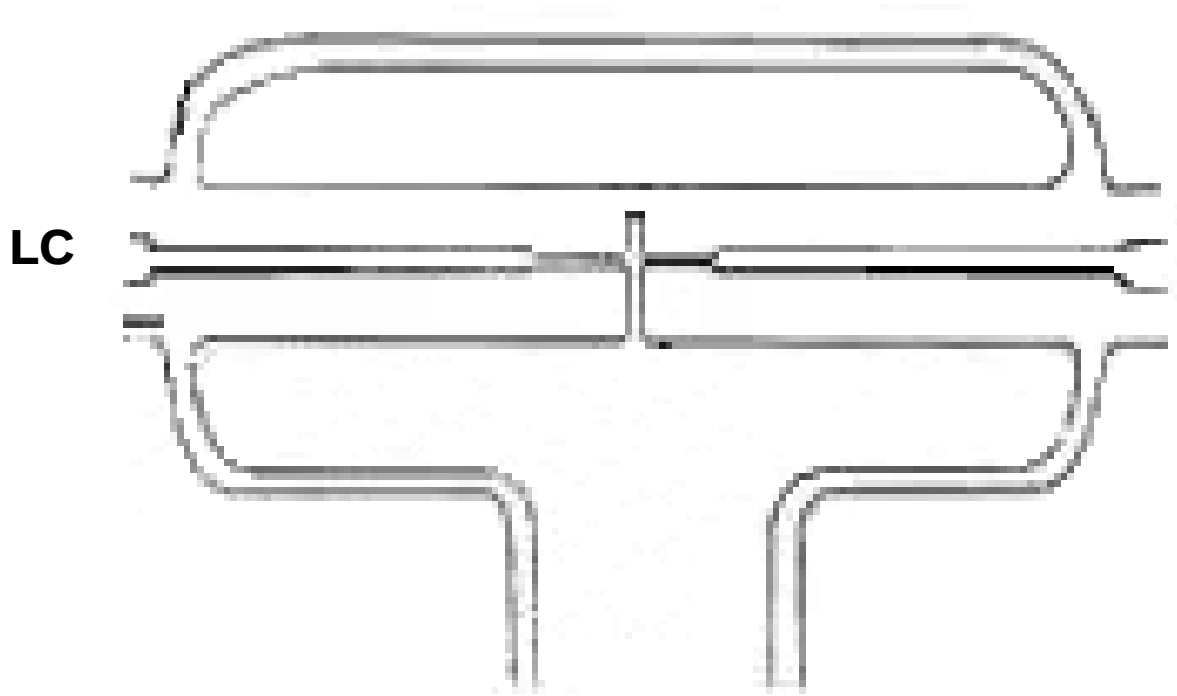


Jet Separator

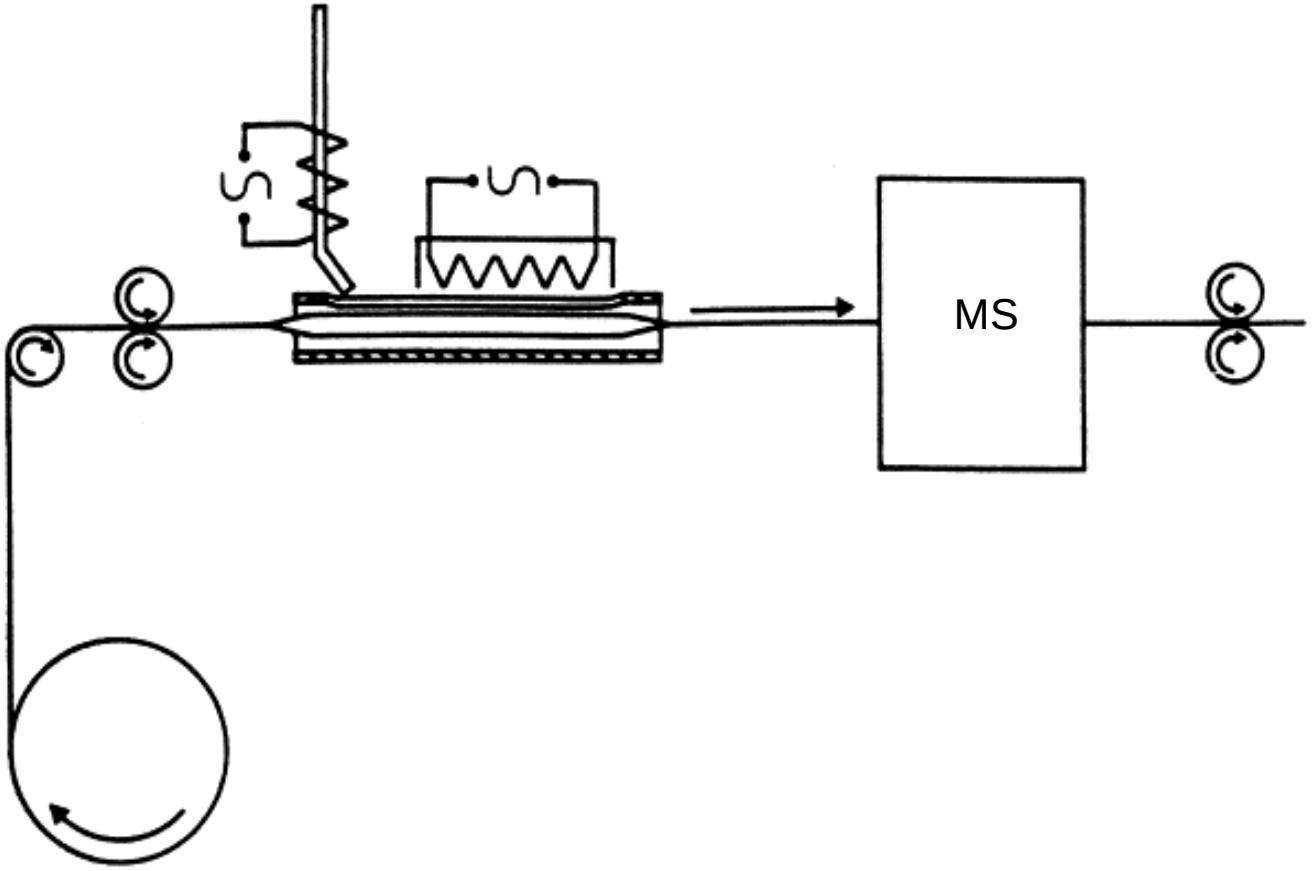


In 1969, LC eluate at 1 µL/min was put into a jet separator.

Jet Separator



In 1981, Moving Belt Interface



Thermospray LC/MS

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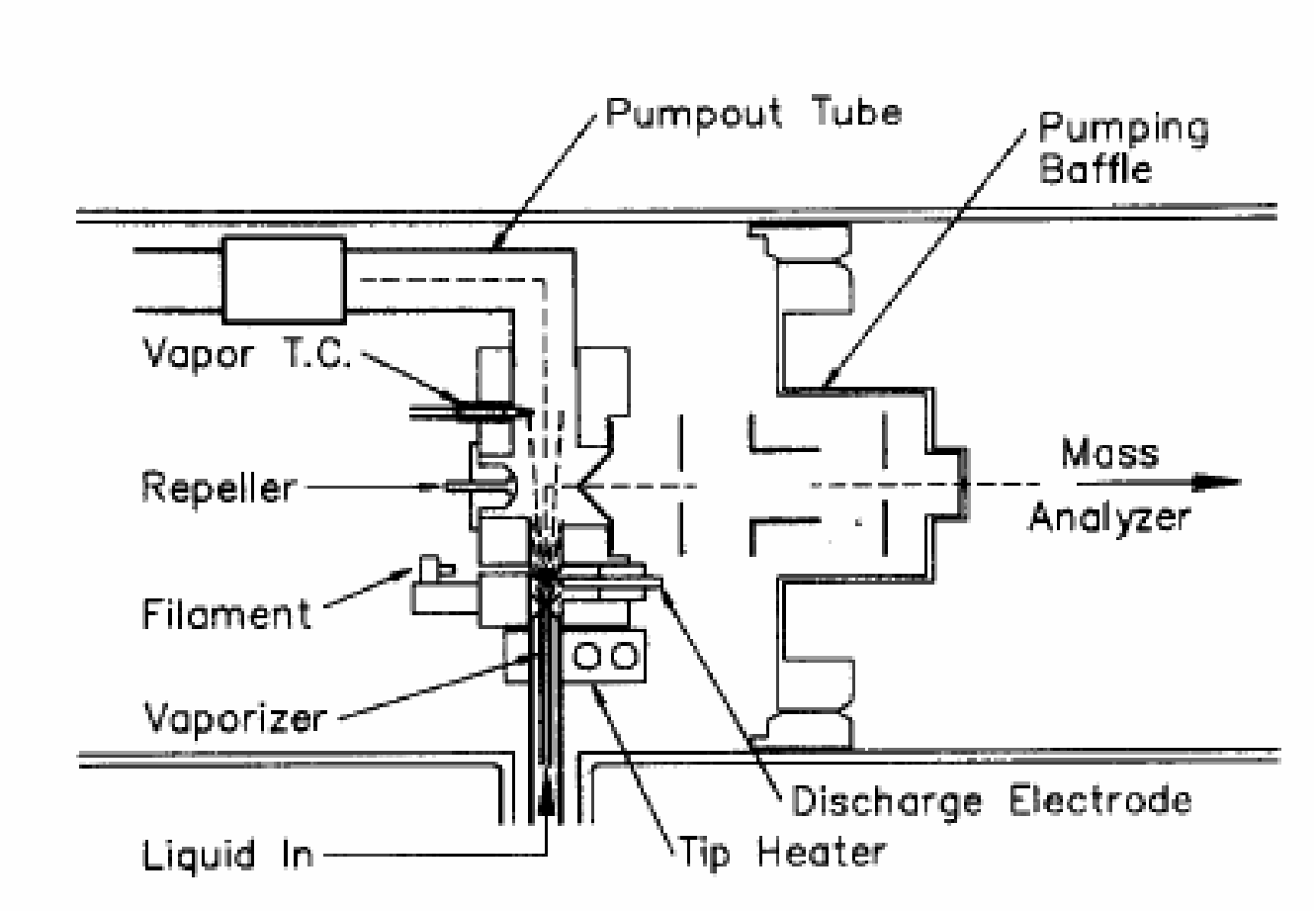
In 1985, Thermospray



ElectroSpray Ionization

ESI

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2002 Nobel Prize

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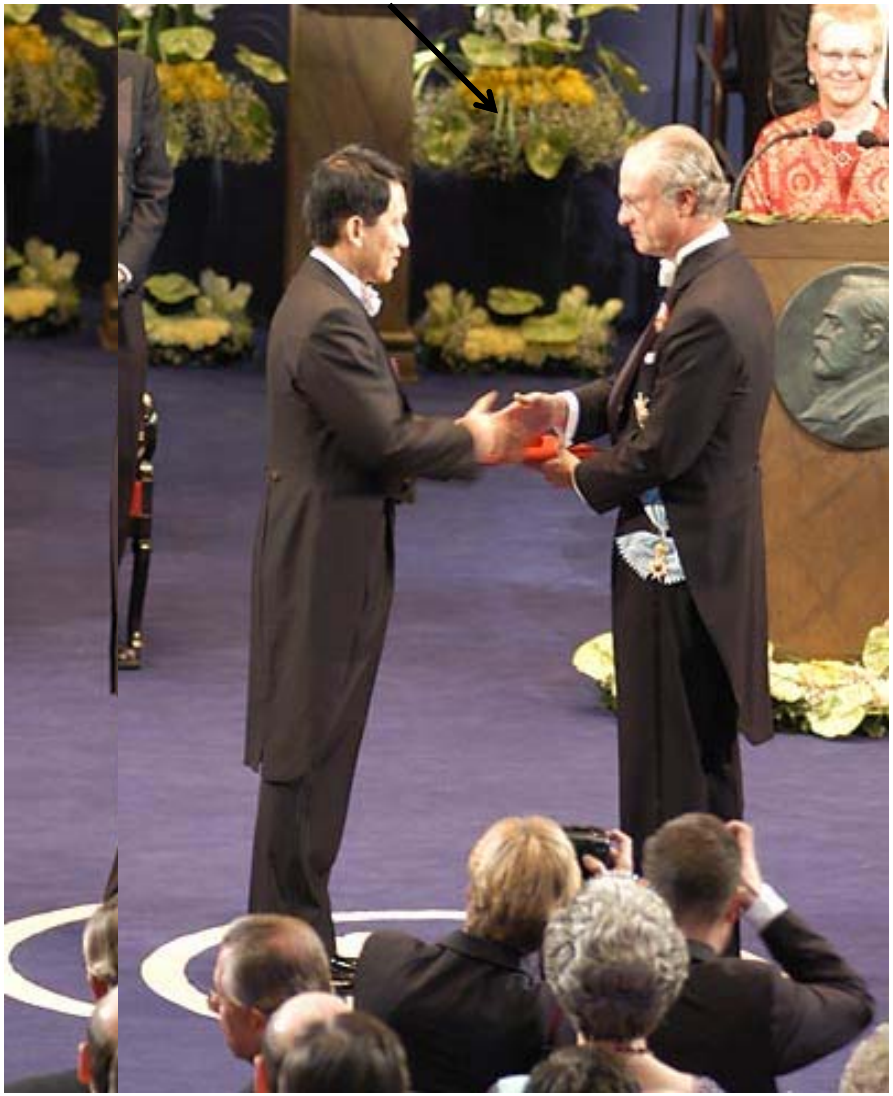
- It was the interface of ESI to MS that allowed widespread use in clinical laboratories

- John Fenn and Koichi Tanaka
  - Nobel Prize in Chemistry 2002
  - "for the development of methods for identification and structure analyses of biological macromolecules“
  - "for their development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules"



Formation of Charged Droplets

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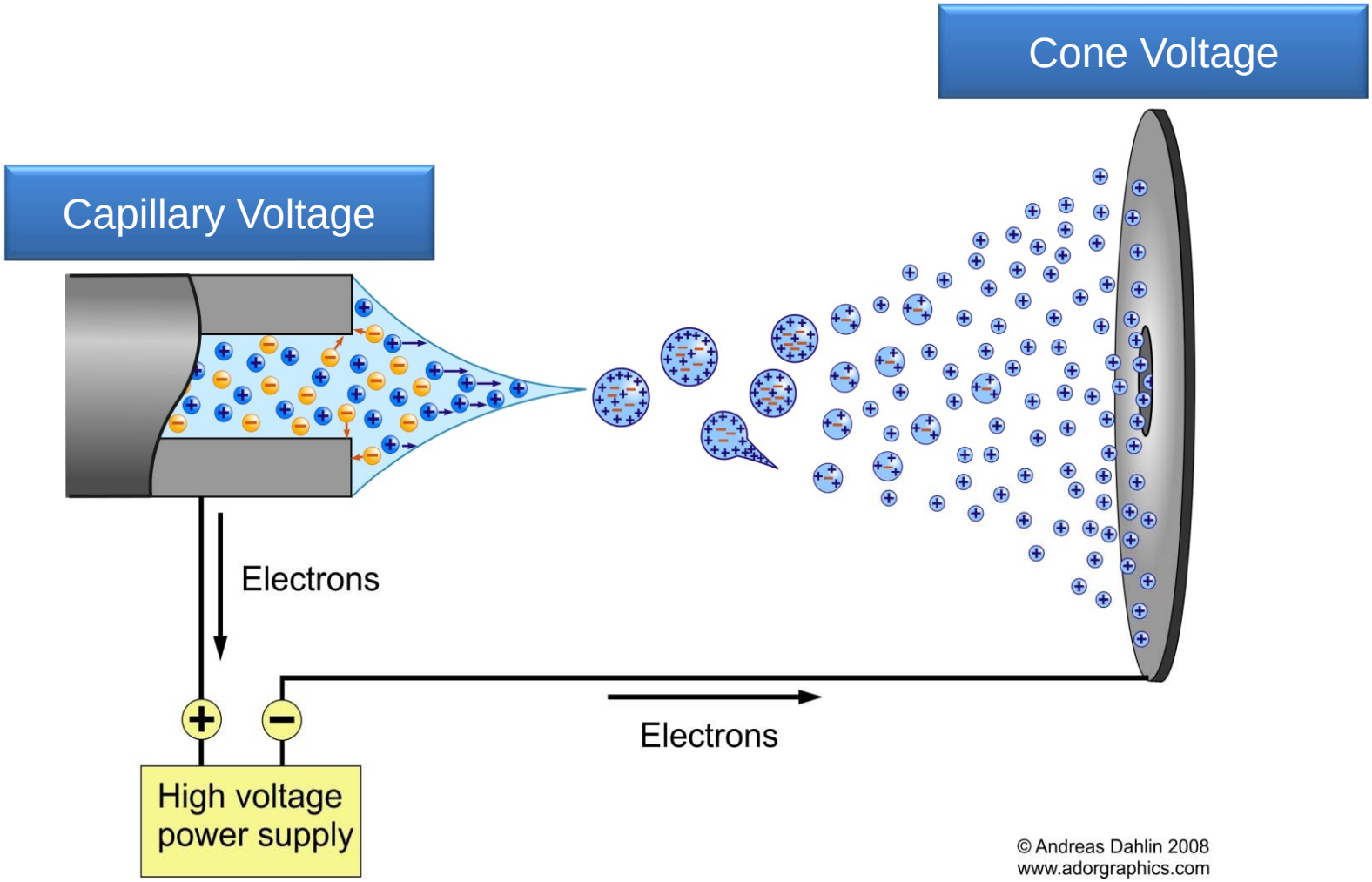

After formation the ions are drawn through an electric field or potential gradient through a drying gas to the counter plate.

Electrospray Ionization
Mechanisms of Ion Formation

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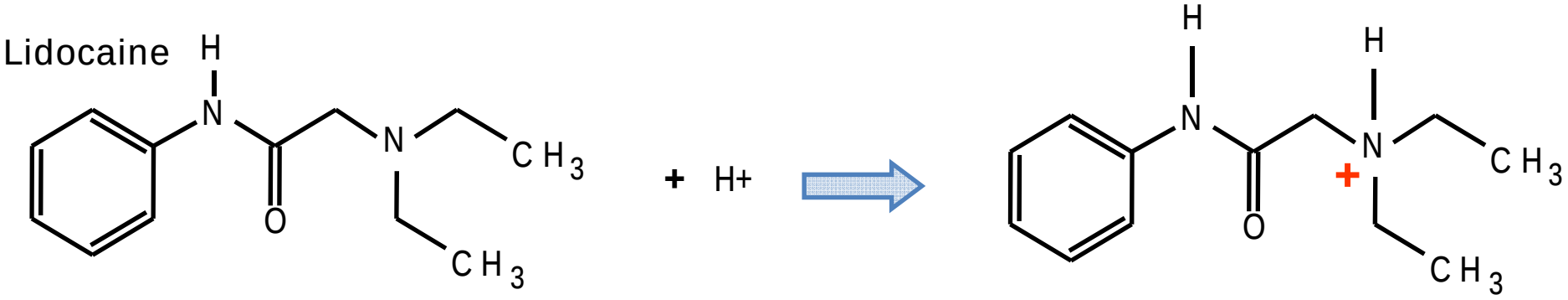
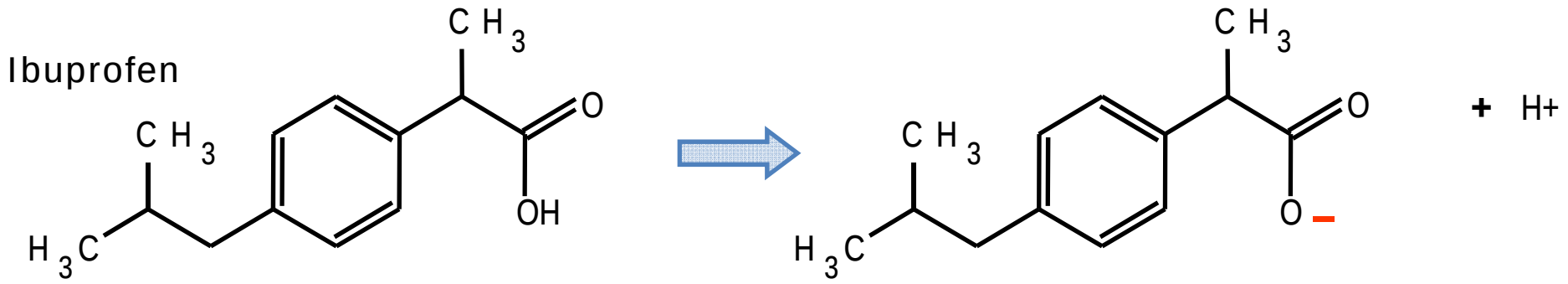
Positive Electrospray Ions



Negative Electrospray Ions



Electrospray Ionization
Z-spray Ion Source

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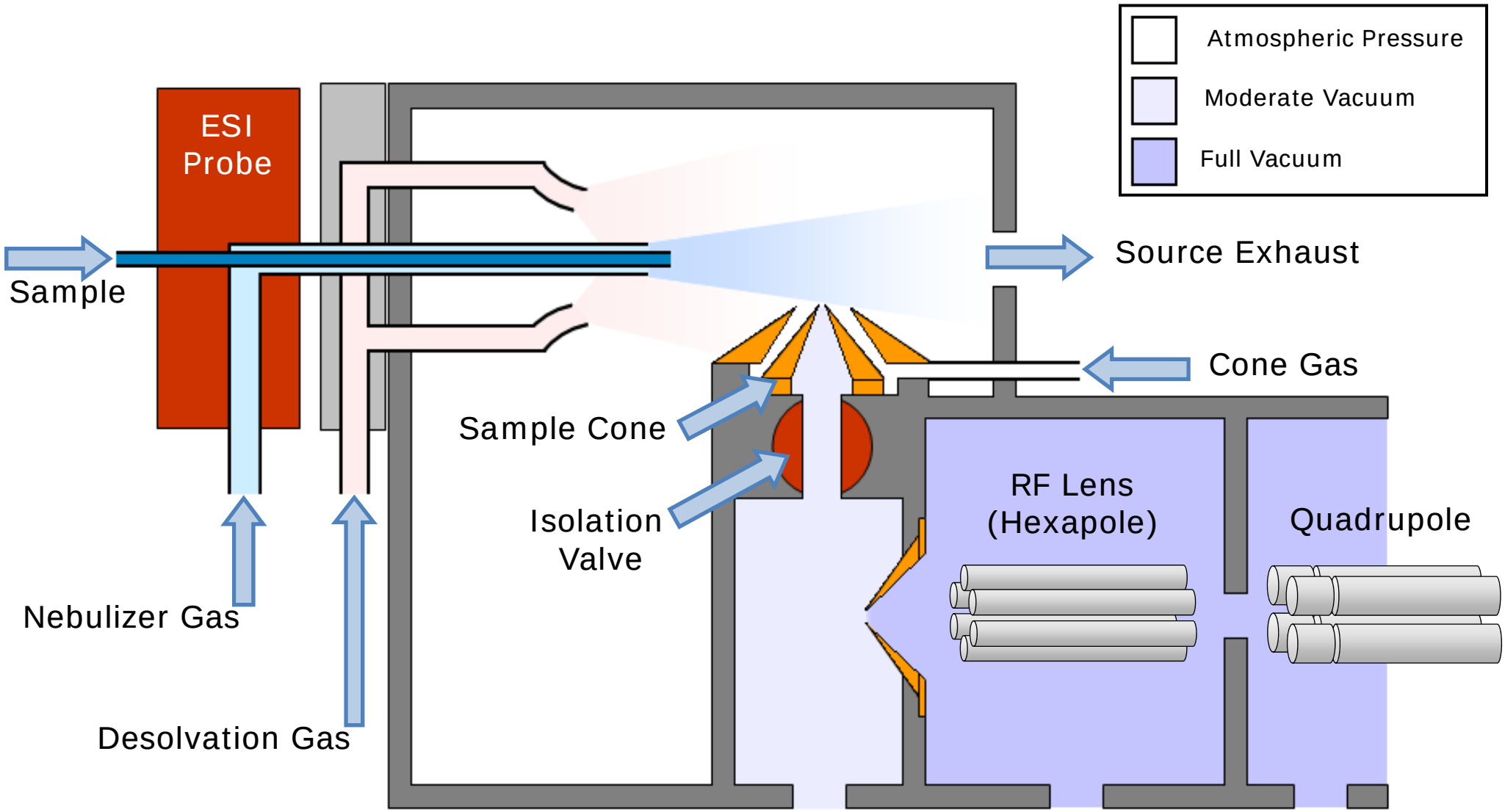

Robustness and Reliability – Z Spray™

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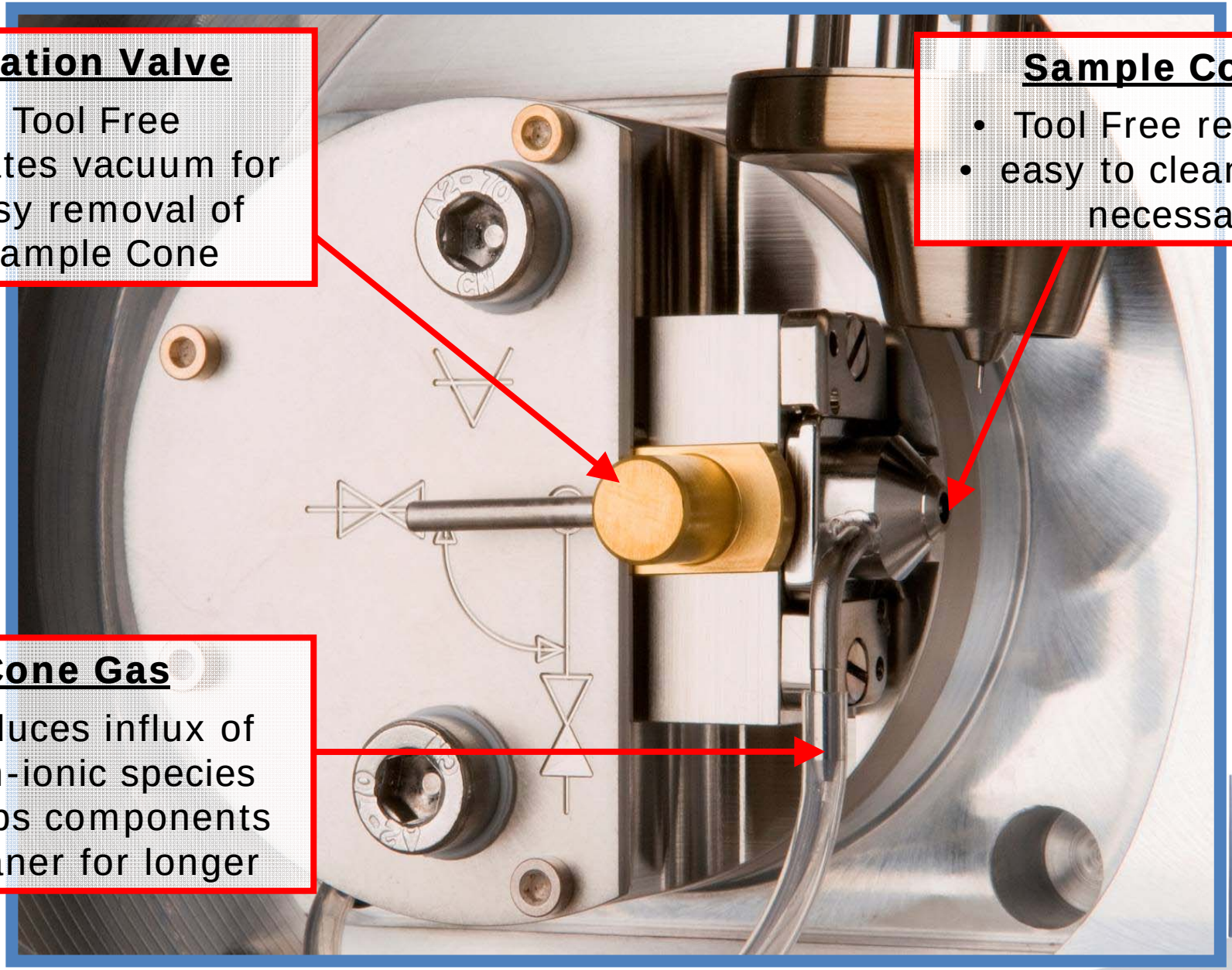
Isolation Valve

- Tool Free
- Isolates vacuum for easy removal of Sample Cone

Sample Cone

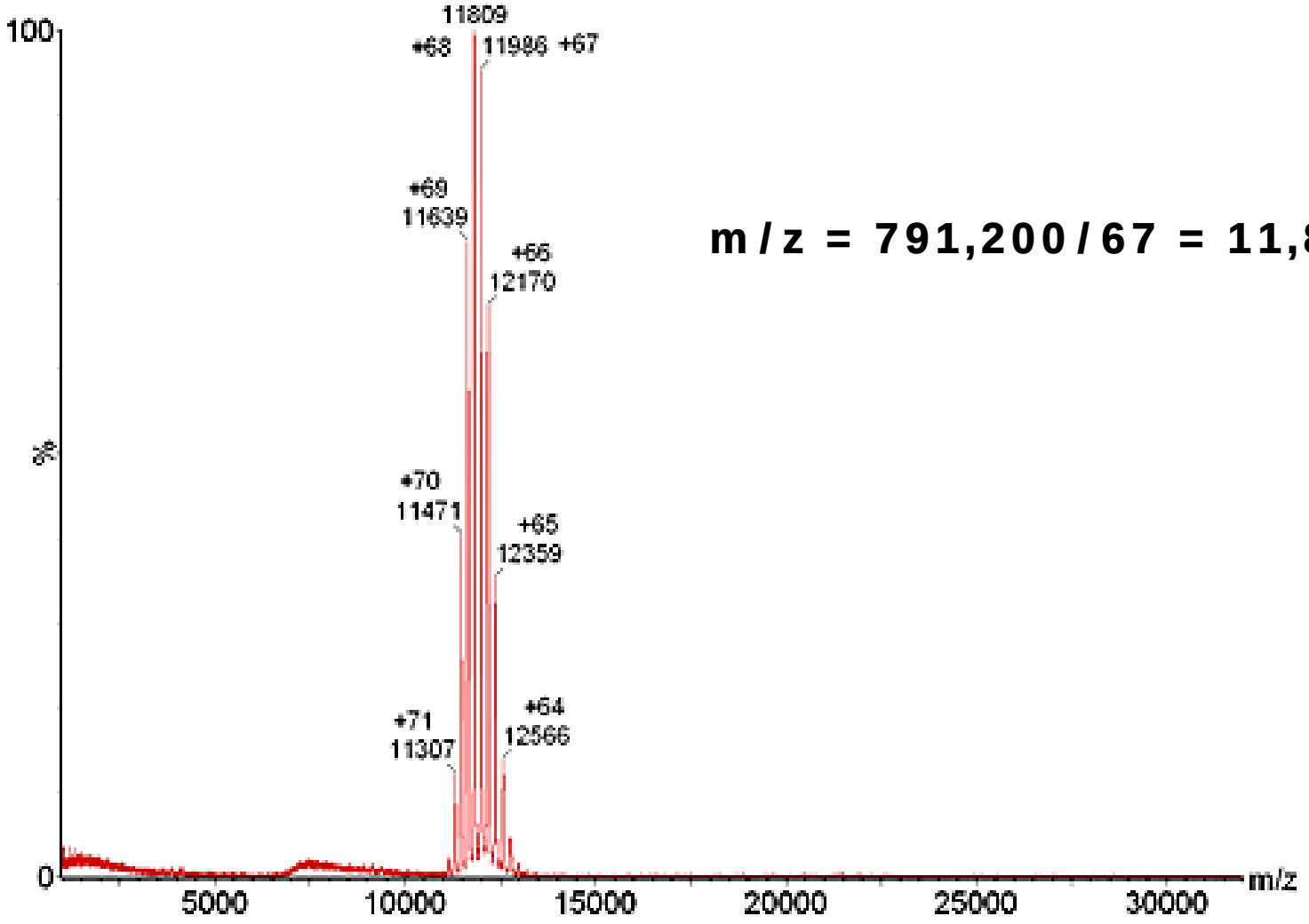
- Tool Free removal
- easy to clean when necessary



Cone Gas

- Reduces influx of non-ionic species
- Keeps components cleaner for longer

GroEL – 800KDa



m/z = 791,200/67 = 11,809

ESI- Summary

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- Electrospray process:
  - Ionization of analyte in solution
  - Droplet fission and desolvation
  - Production of gaseous ions
- “Soft Ionization”
  - Little fragmentation produced by the ESI process
- Adducting and multiply charging is common
- Sensitivity is compound and matrix dependant
- Types of analyte amenable to ESI
  - Must be able to ionize in solution
  - Wide range of masses (<100Da - >30,000Da)
  - Small molecules tend to produce singly charged ions
  - Larger molecules tend to produce multiply charged ions

APCI – Atmospheric Pressure Chemical Ionization

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APCI or APCi

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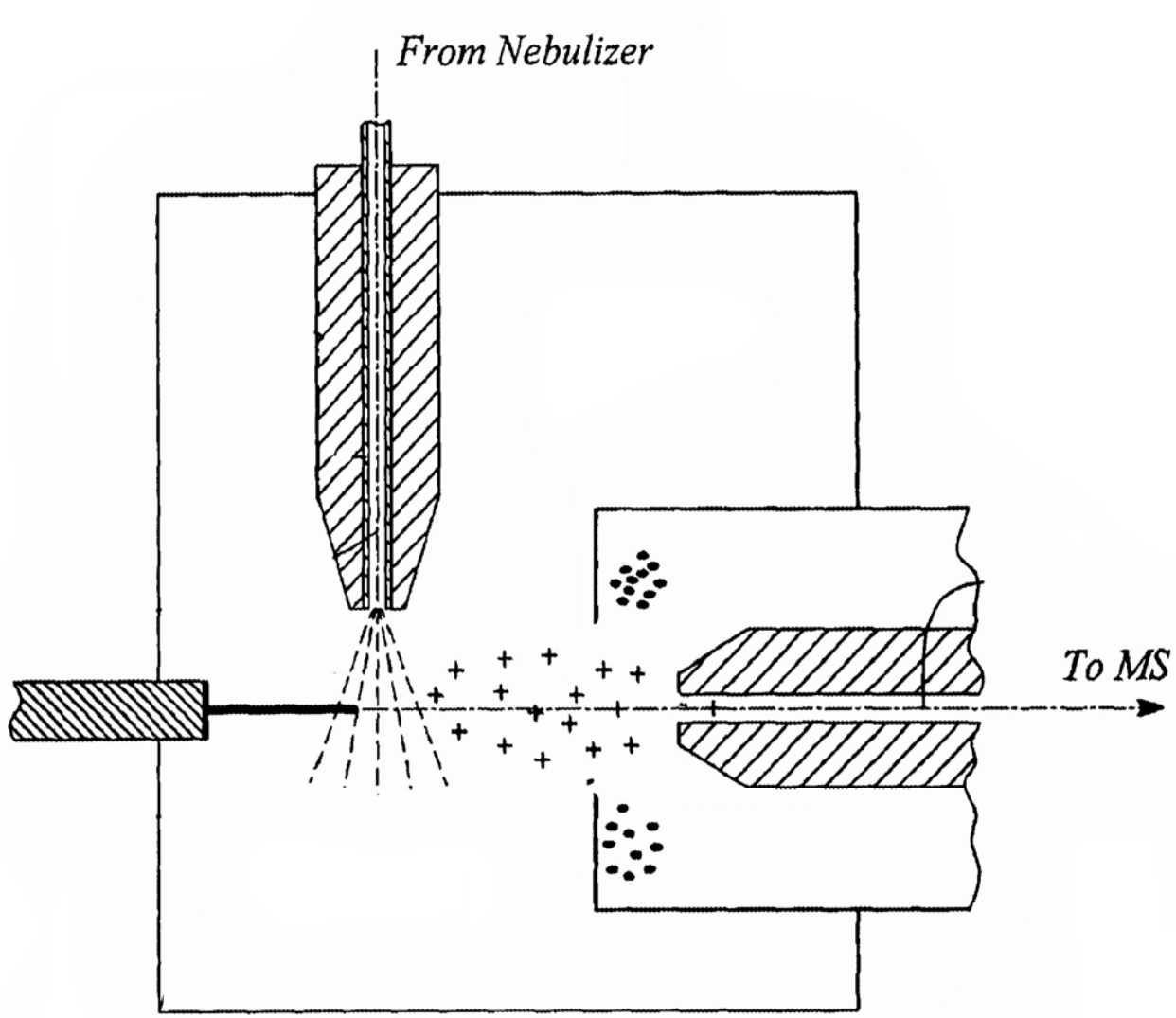
In 2000, Atmospheric Pressure Chemical Ionization (APCI, APCi)



APCI

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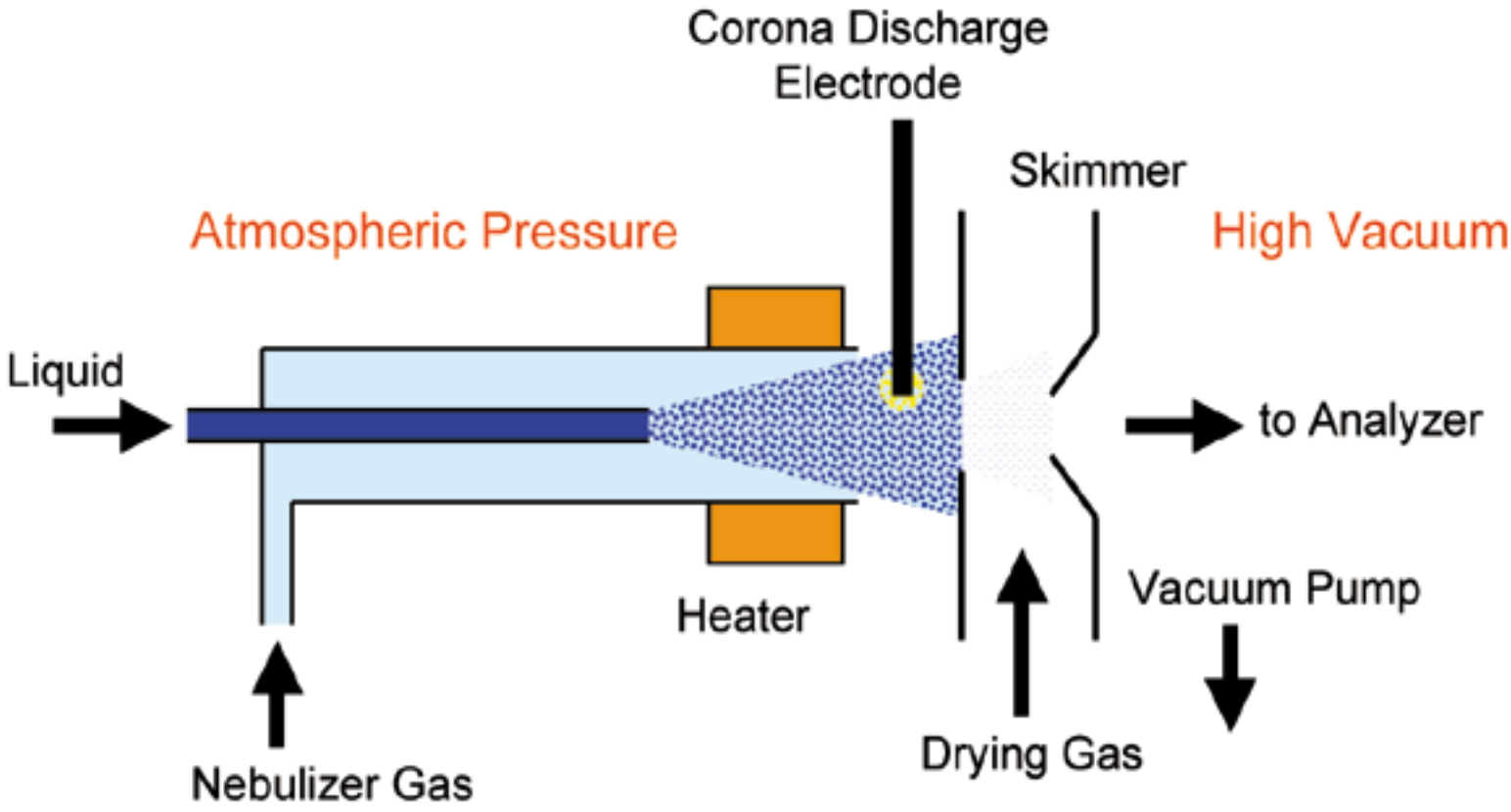

APCI

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- APCI process:
  - Flash vaporization of solvent and analyte
  - Gas phase chemical ionization
- More harsh ionization than ESI
  - Fragmentation is more likely than with ESI due to high temperatures and corona discharge involved
- Adducting is common, but multiply charging of compounds is rare
- Sensitivity is compound and matrix dependant
- Types of analyte amenable to APCI
  - Must be volatile to some degree
  - Smaller range of masses than ESI(<1000Da)
- Additives used have less effect on the ionization than in ESI

ESI / APCI

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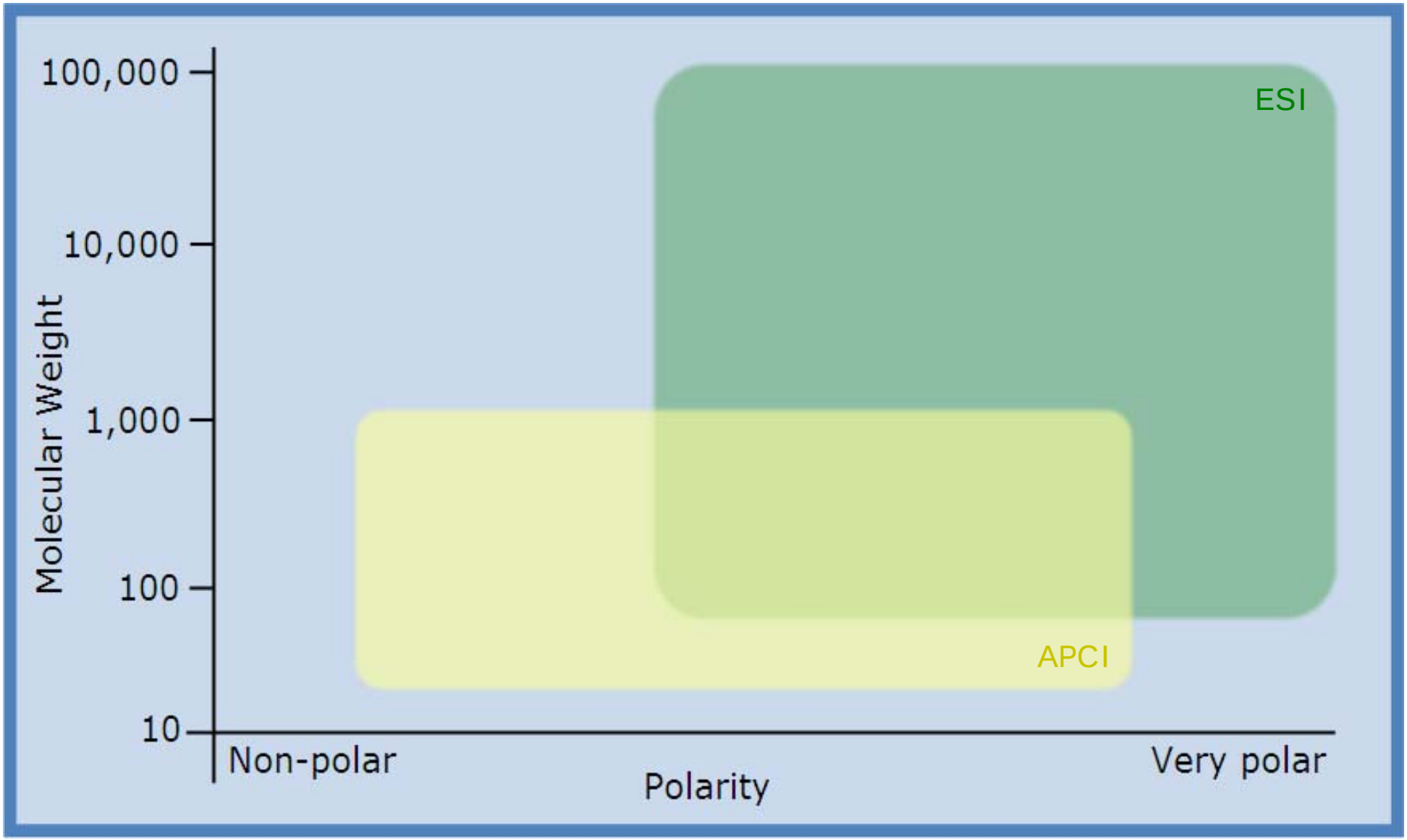
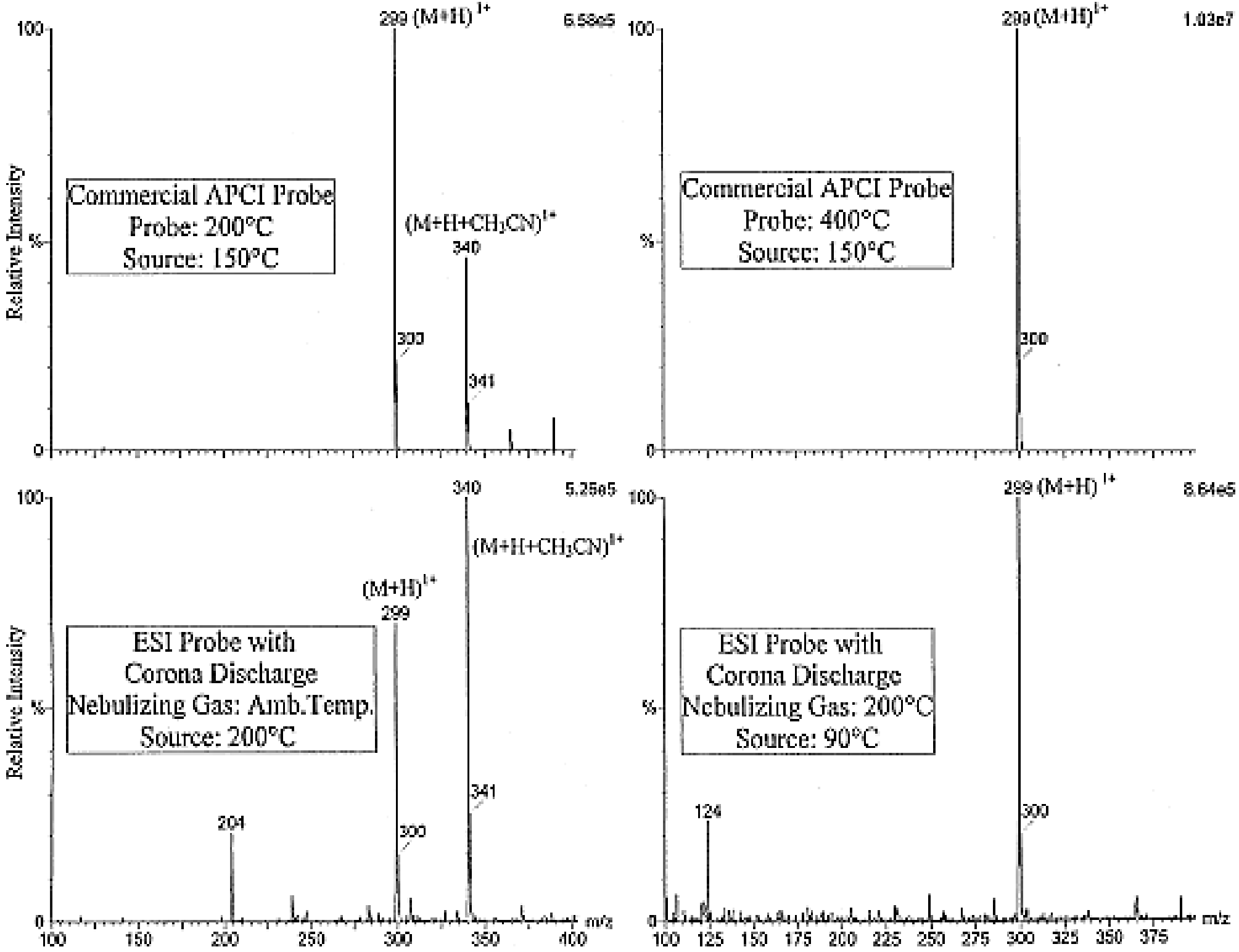


1nmol methyl stearate



Ionization modes: ESI and APCI

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ESI

- Polar compounds
- Multiple-charging occurs
- Proteins/peptides/nucleic acids
- Molecular weight determination
- Appropriate for both volatile and non-volatile solutes
- Good sensitivity
- Especially Na^+ K^+ and NH_4^+ adducts are common

APCI

- Non-polar compounds
- Mostly single charging
- Non-polar small molecules
- Molecular weight determination
- Accommodates LC flow rates up to 2.0 mL/min
- Good sensitivity
- Solvent adducts common

APPI – Atmospheric Pressure Photo Ionization

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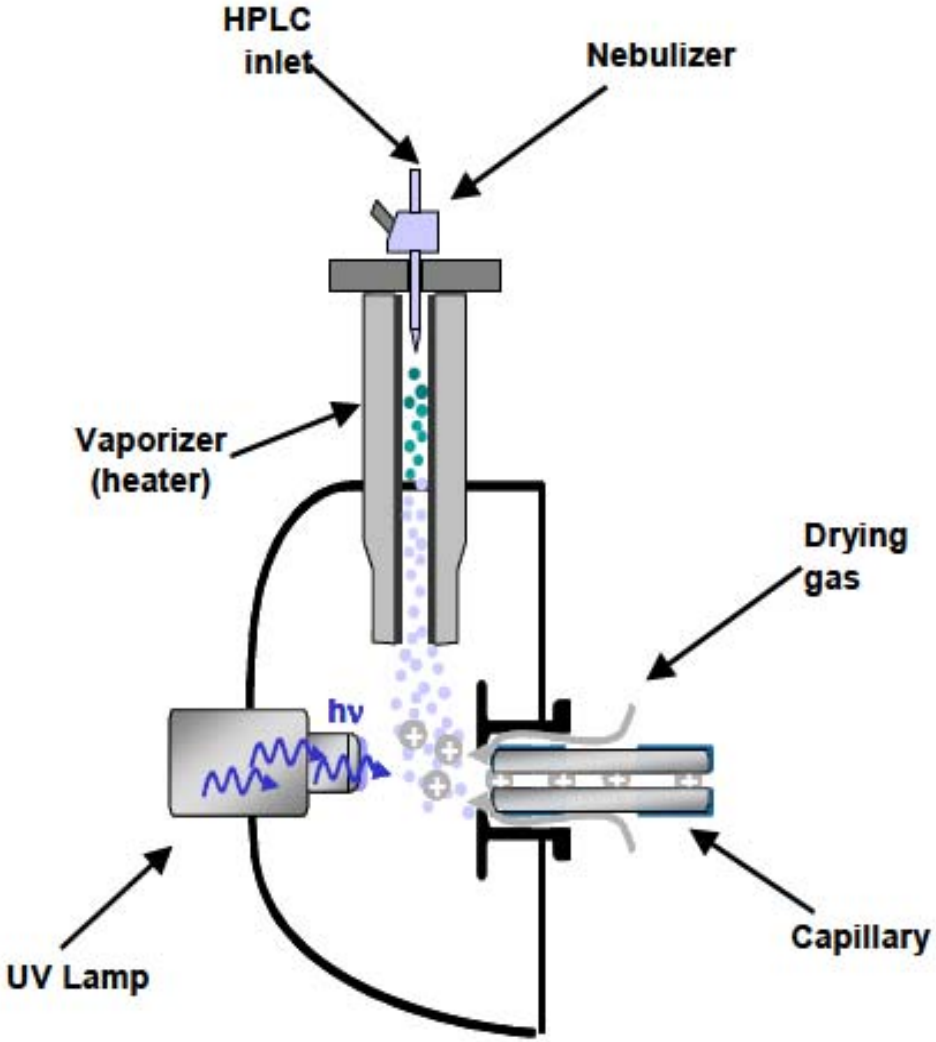
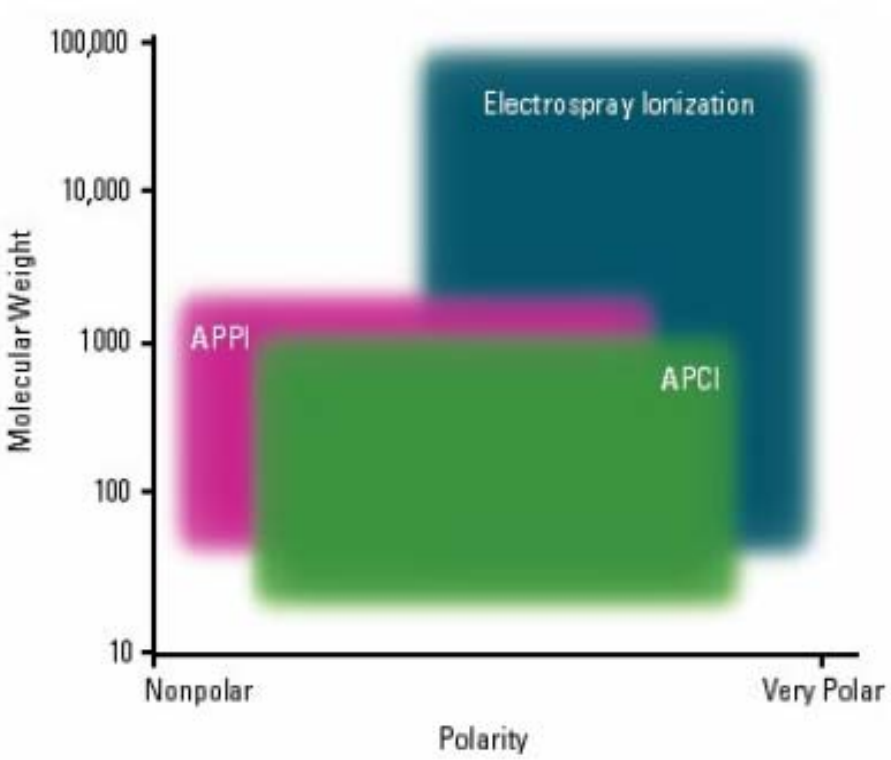
APPI Concept





APPI – Mechanisms of Ion Formation

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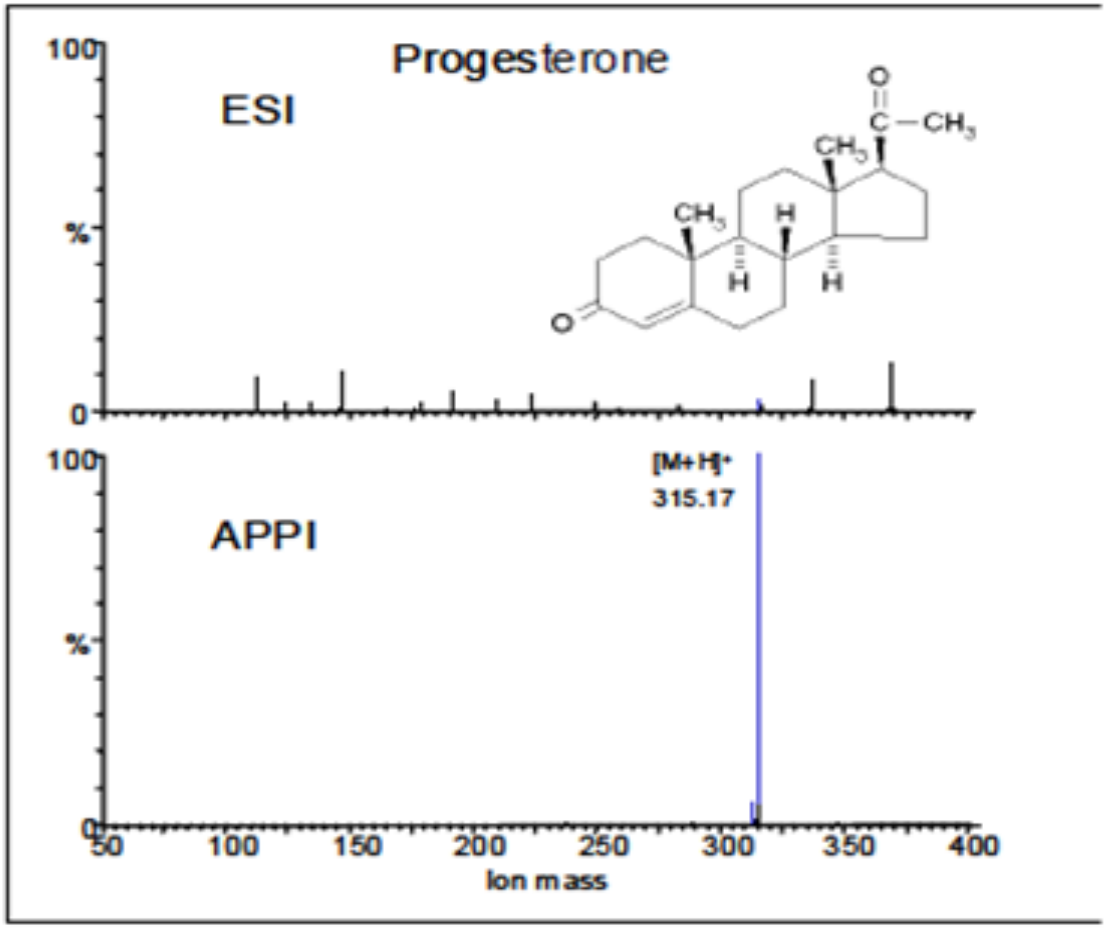
- Direct APPI
  - $M + h\nu \rightarrow M^{+\cdot} + e^-$
    Analyte molecule is ionized to a molecular radical ion if ionization potential is below the energy of the photon
  - $M^+ + Solvent \rightarrow MH^+ + [Solvent\text{-}H]^-$
    In the presence of protic solvents, M^+ may abstract a hydrogen atom to form MH^+
- Dopant APPI
  - $D + h\nu \rightarrow D^{+\cdot} + e^-$
    A photoionizable dopant is delivered in large concentration to yield many D^+ ions.
  - $D^+ + M \rightarrow MH^+ + [D\text{-}H]^-$
    $D^+ + M \rightarrow M^+ + D$
    Dopant ions ionize the analyte by either proton or electron transfer

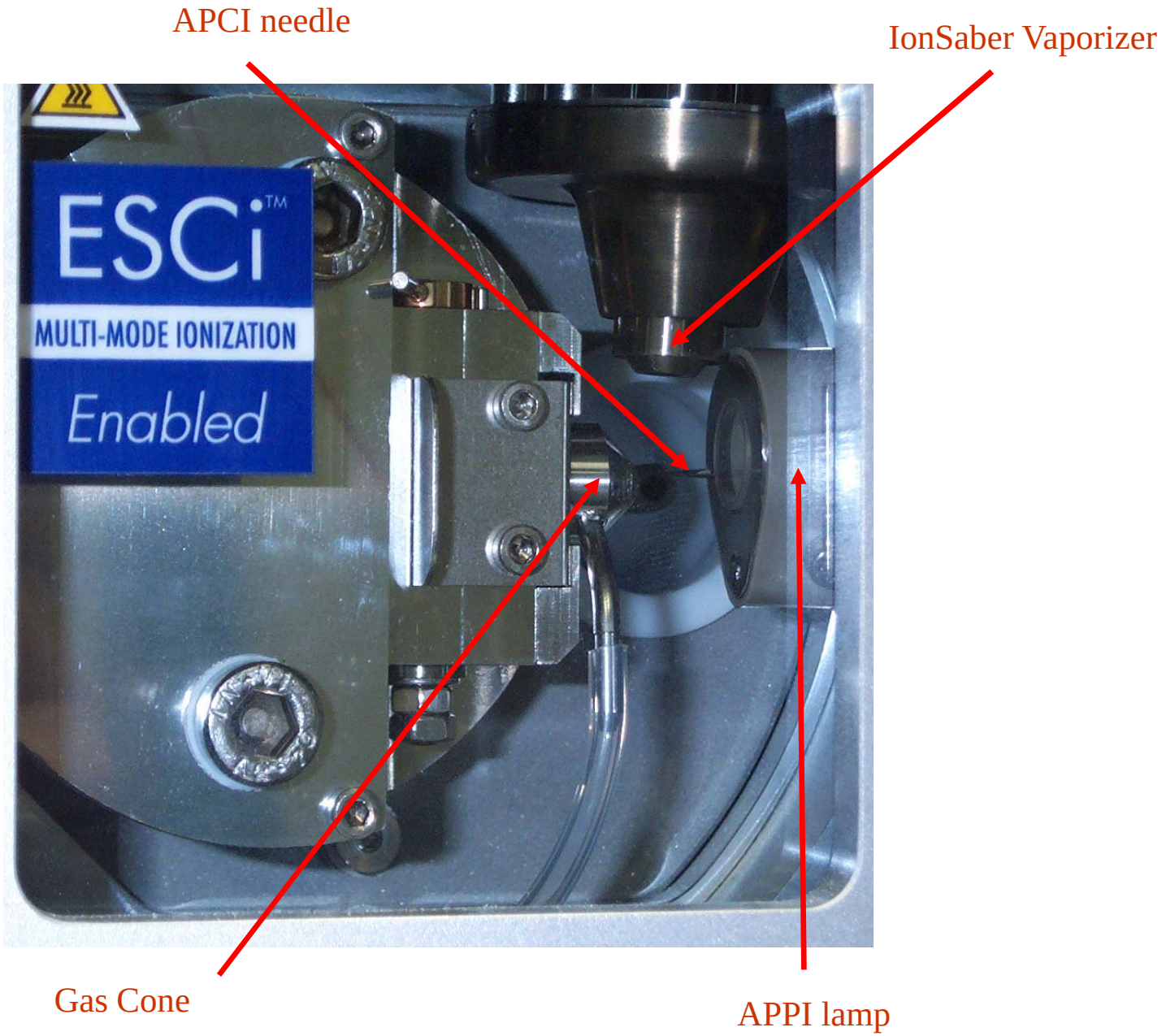
APPI Selectivity



Can be very useful for:

- **PolyNuclear Aromatics**
- **Steroids**
- **Bio-oils / Biodiesel**
- **Plasticizers**
- **Polymers**

APPI is complementary to ESI and APCI by detecting compounds missed by these sources


APCI needle
IonSaber Vaporizer
ESCi™
MULTI-MODE IONIZATION
Enabled
Gas Cone
APPI lamp

APPI - Summary

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- APPI process:
  - Flash vaporization of solvent and analyte
  - Gas phase photoionization (or PI induced CI…)
- Soft ionization technique
  - Fragmentation less common than APPI due to lack of a corona discharge area
- Mainly singly charged parent ions produced
- Sensitivity is compound and matrix dependant
- Dopants often used to enhance sensitivity
- Types of analyte amenable to APPI
  - Must be volatile to some degree
  - Smaller range of masses than ESI(<1000Da)
  - Can ionize some very non-polar compounds not amenable to either ESI or APCI

Ionization Coverage

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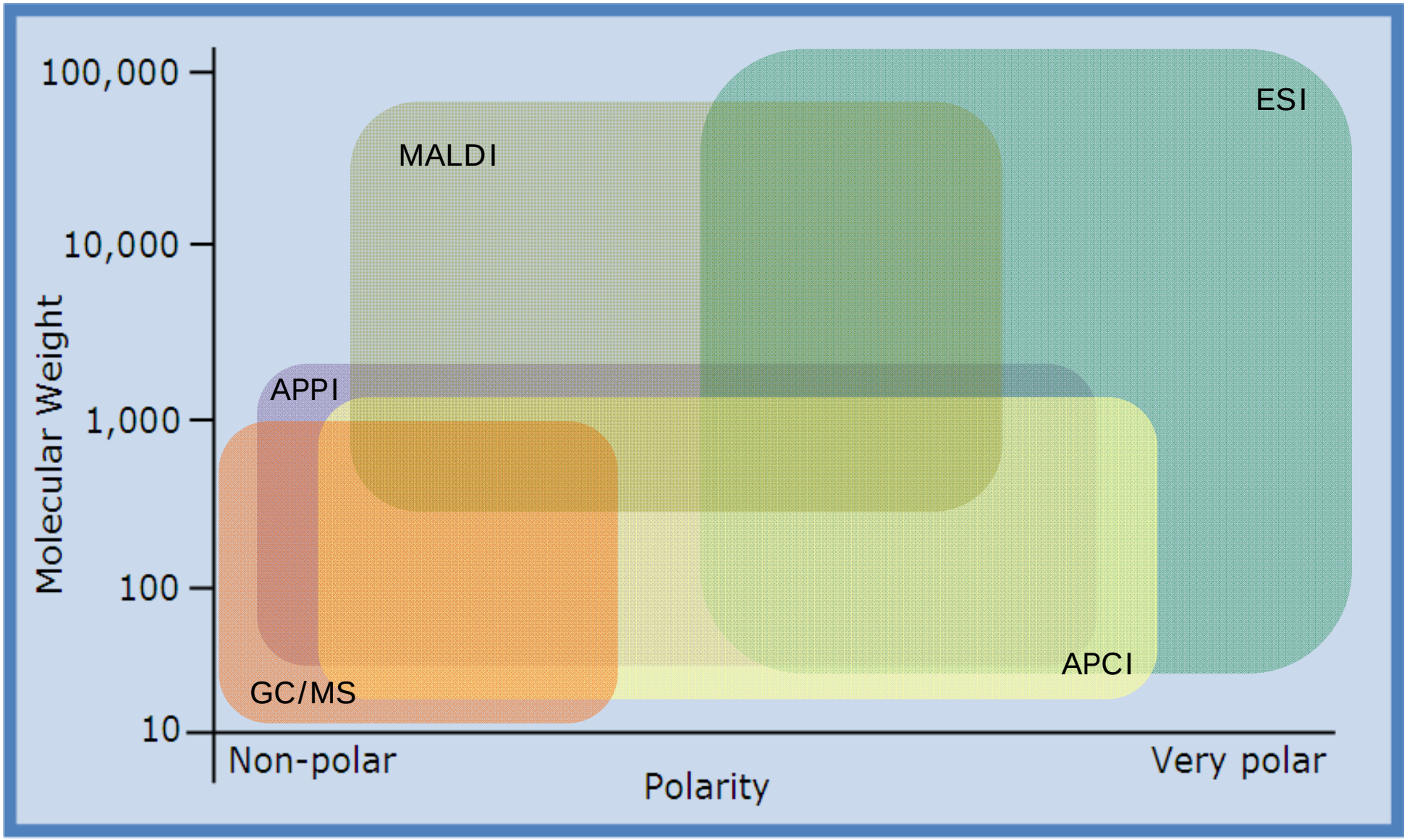

Vacuum

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Roughing or Rotary (vane) Pump (wet)

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- Low vacuum
- Change oil 6 – 12 months
- Vent off-gasses
- Rebuildable
- Somewhat Noisy

Scroll Pump (dry)

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- Low vacuum
- Large footprint
- Oil-less
- Seal Change annually
- Seal Change requires care
- Quiet
- 2-3x Price of Rotary

Turbomolecular (Turbo) Pump

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- High Vacuum
- Oil-less
- Air cooled
- Quiet - usually
- Fairly Expensive
- Potential MS Damages when they rail

Vacuum Regions on Single Quadrupole Instruments

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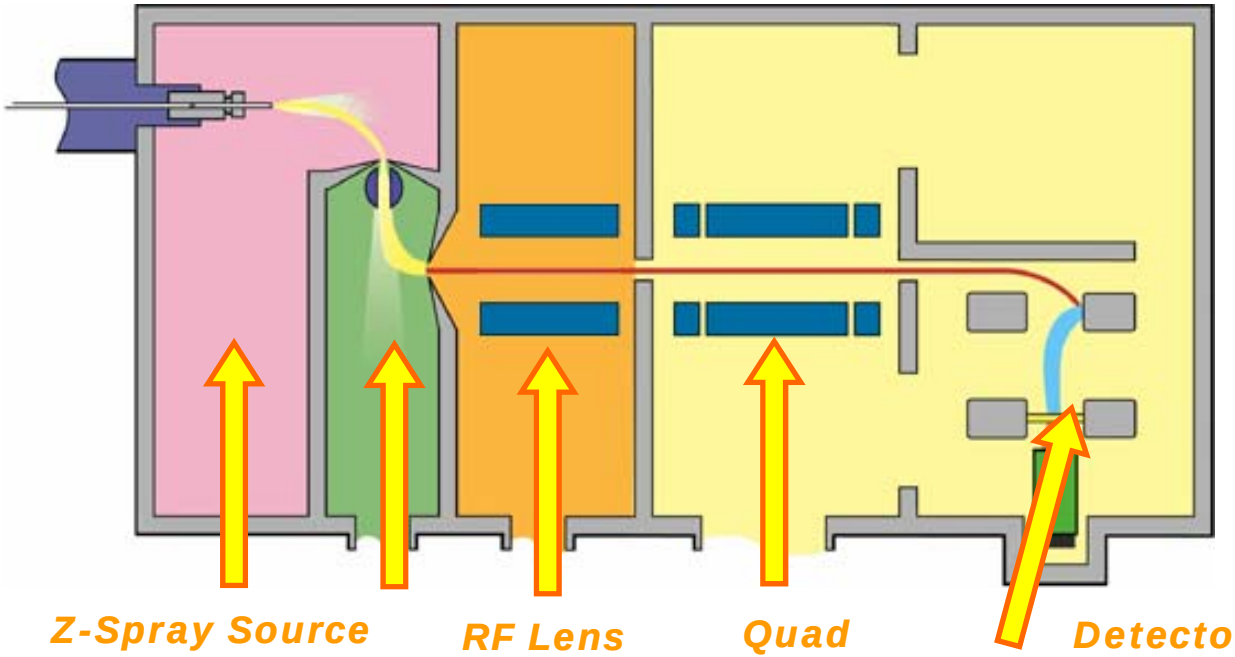

Atmospheric

Roughing

Turbomolecular

Mass Filters

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Mass Analyzers: Quadrupoles vs TOF

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Quadrupoles

- Common identification of unknowns, especially by GC electron impact library
- Quantitation
- Single and Tandem (triple)
- High sensitivity
  - Nominal Mass (only first decimal place significant
- Limited on mass range
- High selectivity

Time-of-Flight (TOF)

- Unknown identifications by empirical formulas
- High resolution
  - Exact or Accurate Mass
- No limit on mass range
- High resolution fragments (after CID)
- Incorporate quadrupoles for precursor selections
- Simultaneous Quantitation with Qualitative now possible

Magnetic Sector Analyzer

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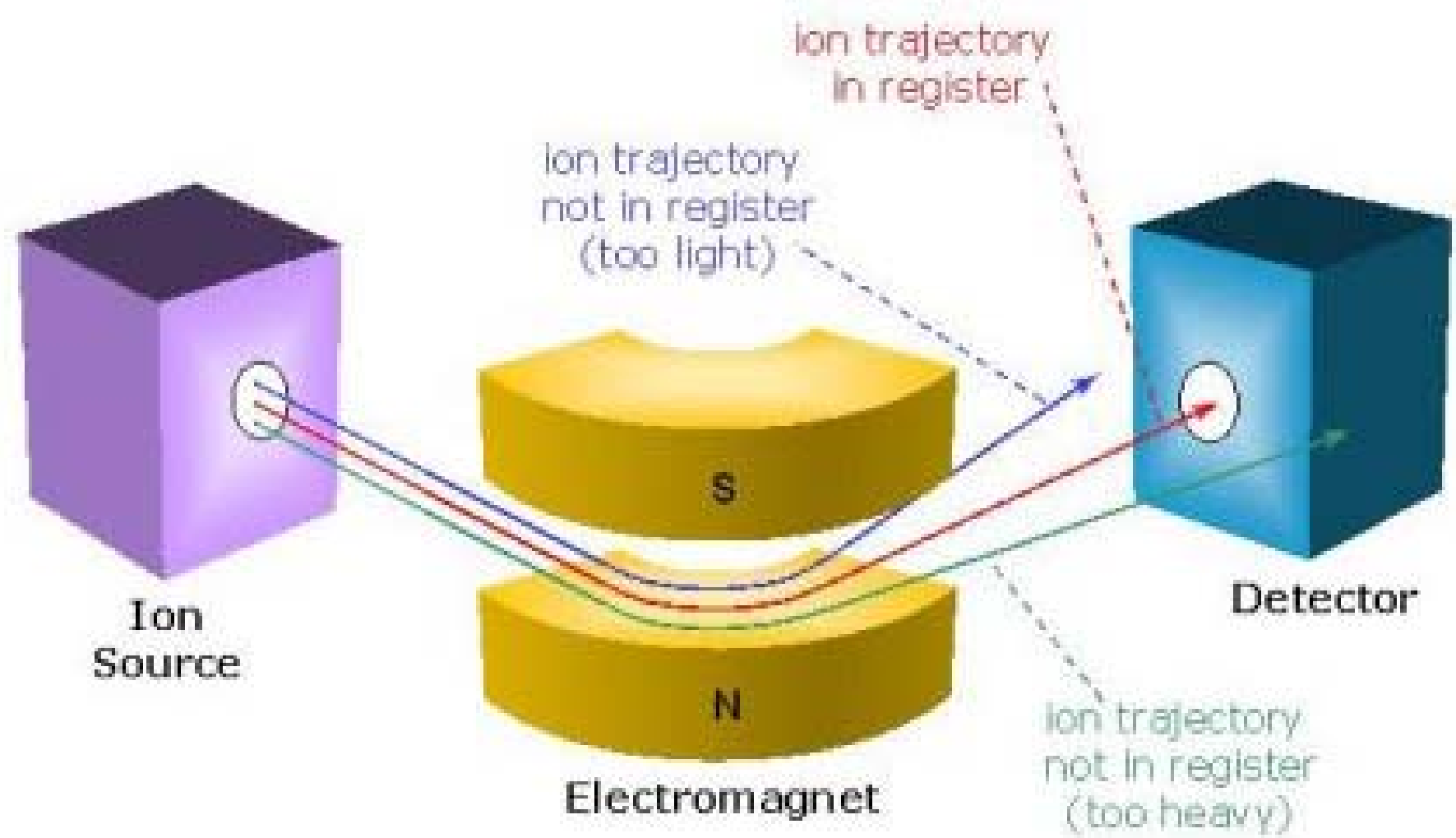
Introduced in 1897 by J. J. Thompson



Ion Trap Mass Analyzer

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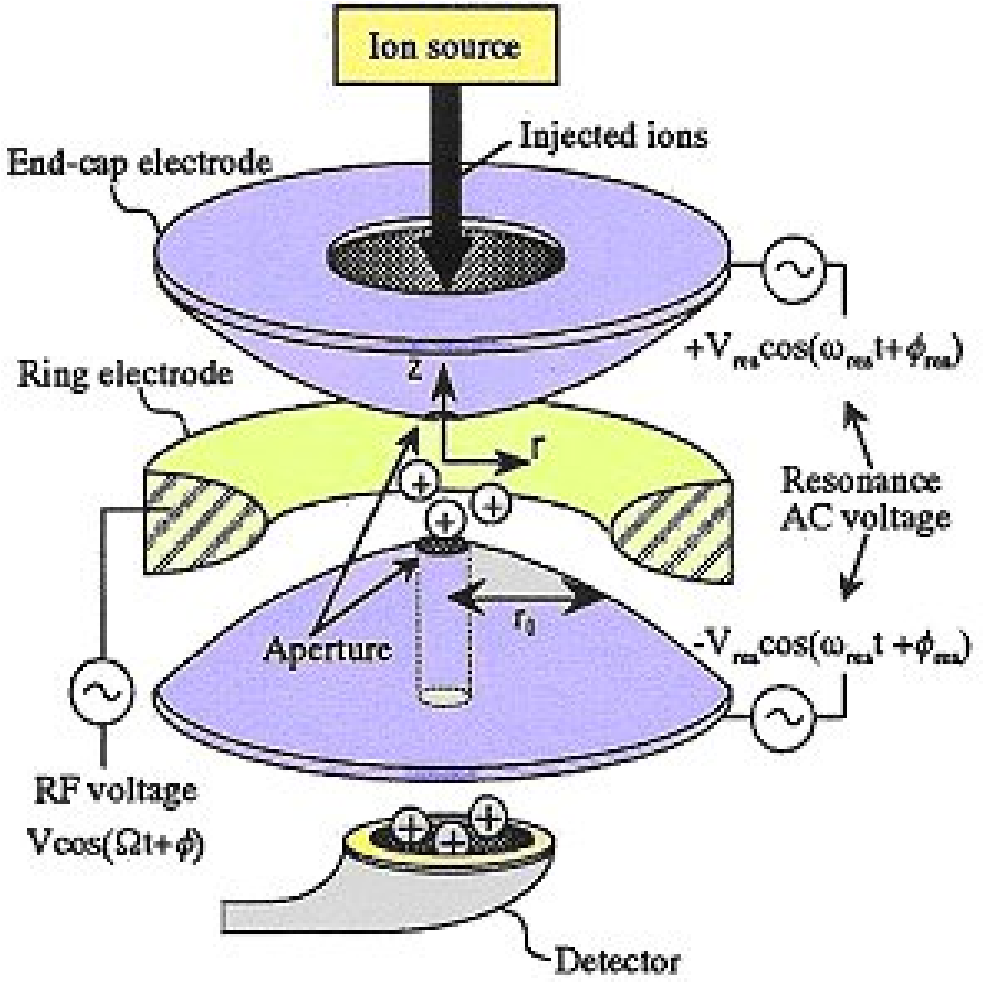
In 1989, Wolfgang Paul received the Nobel Prize.

- Ion traps are ion trapping devices that make use of a 3-D quadrupole field to trap and mass-analyze ions
- Nominal mass resolution
- 1/3 rule limitation
- Challenging quantitation
- Space charge effects



Detectors

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Common Detector Types

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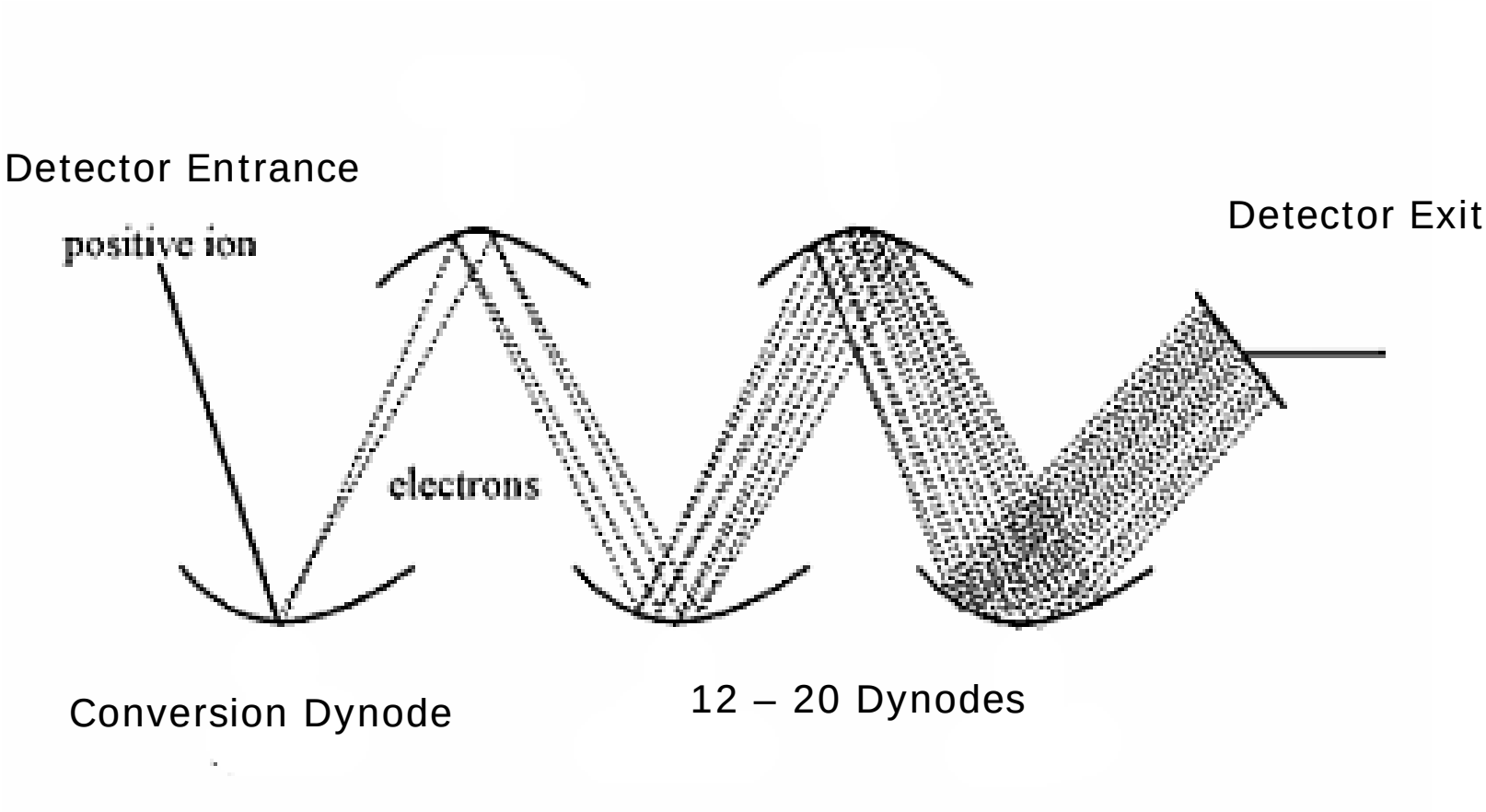
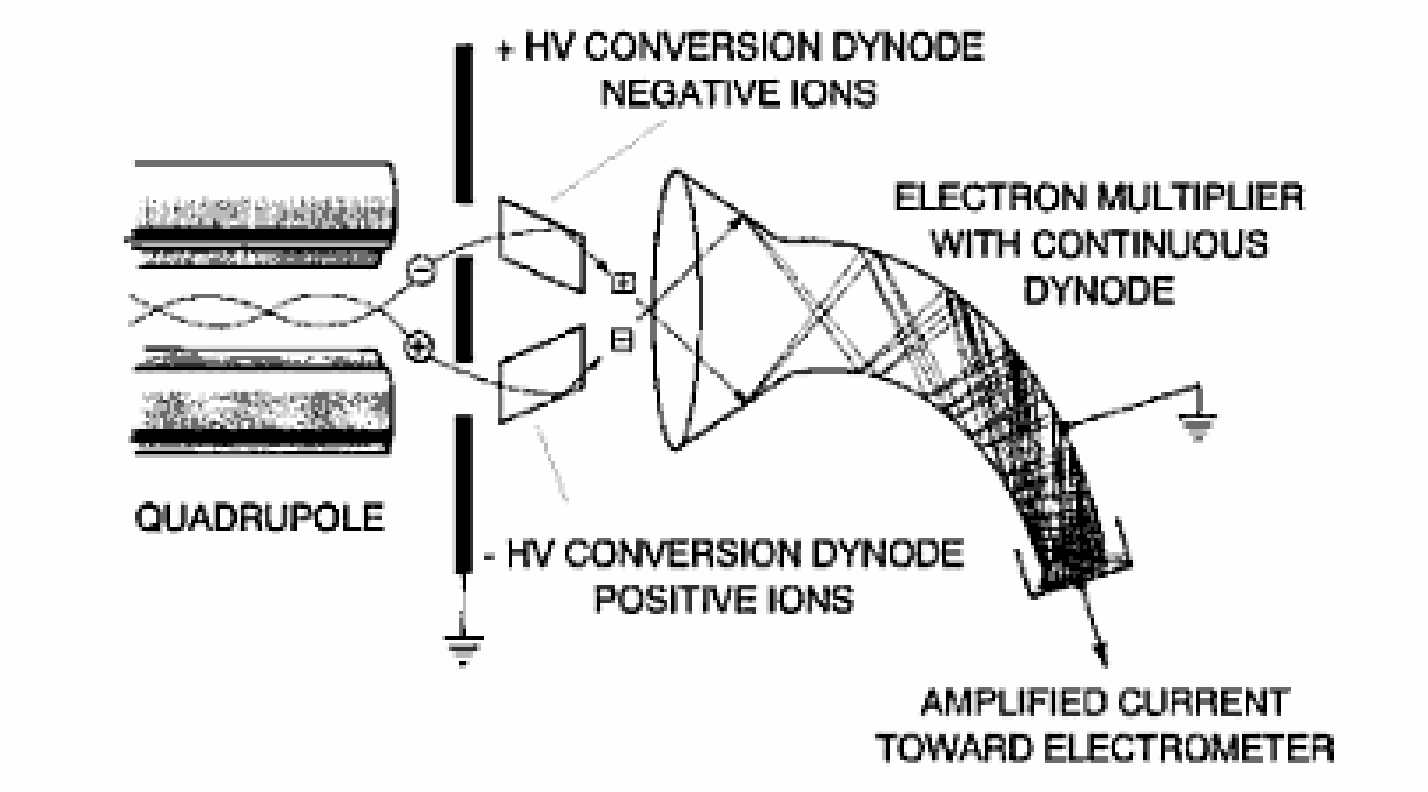
- Electron Multiplier – EM
  - Cascading Dynode
  - Continuous Dynode
- Photomultiplier – PM or PMD
- Microchannel Plate - MCP

Cascading Dynode EM



CONTINUOUS DYNODE EM



Constant Autogain
12 – 18 months lifetime

Photomultiplier Detection (PMD)

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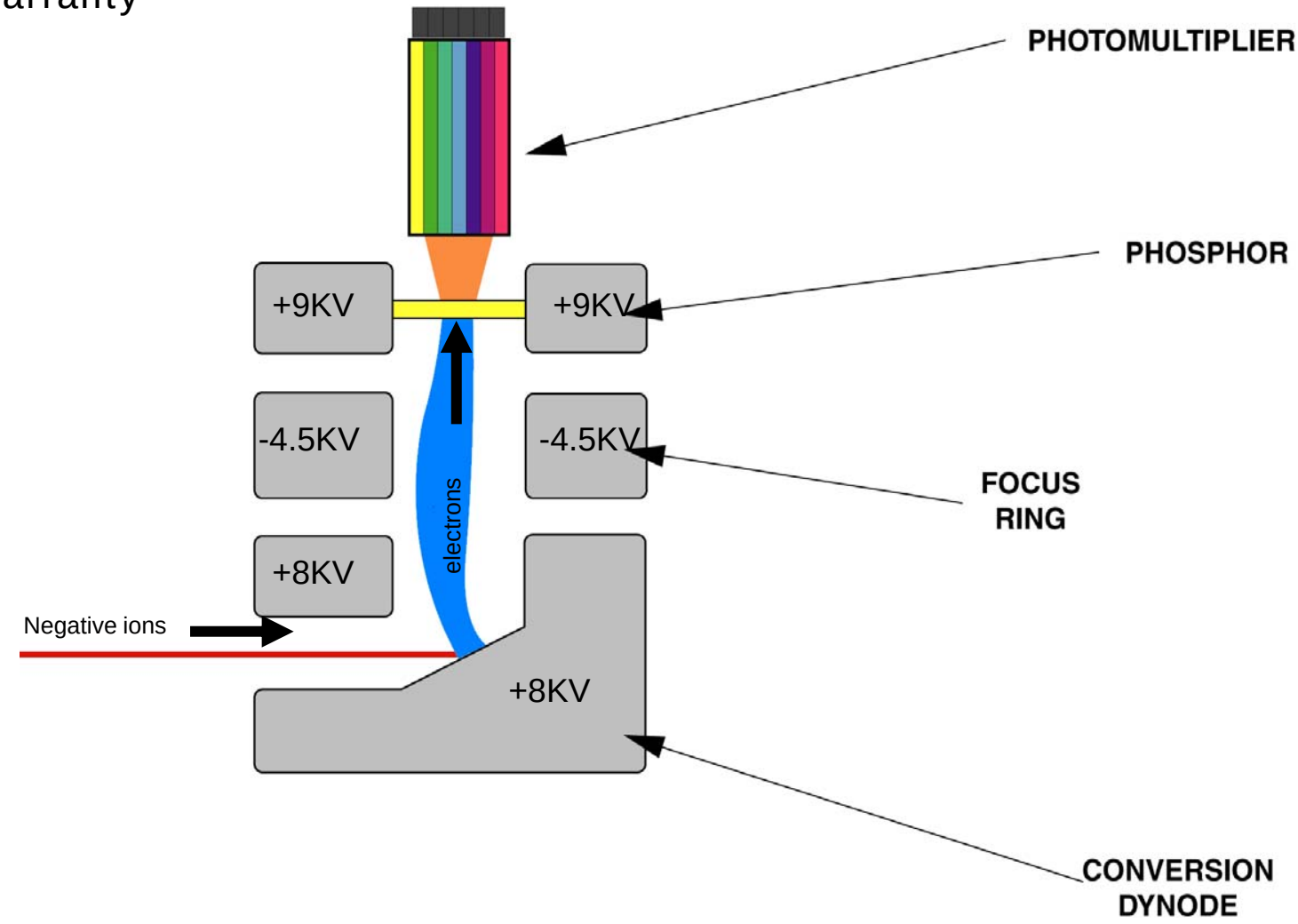
10 Year Warranty



LC/MS (or GC/MS)

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Single Quad Modes of Acquisition

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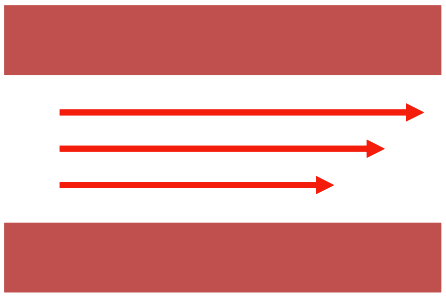
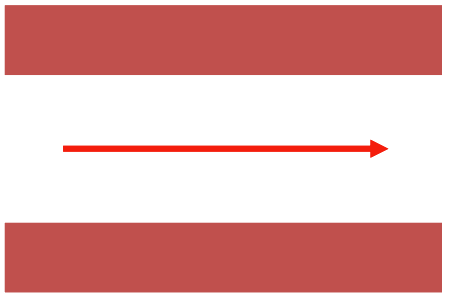
Full Scan (MS mode)



Scanning

LOQ ≈ 500 pg (quantity injected)*

Single Ion Recording Single Ion Monitoring (SIR or SIM Mode)



Static

LOQ ≈ 5 pg (quantity injected)*

*Very compound dependent"

Analyte Injected in Matrix

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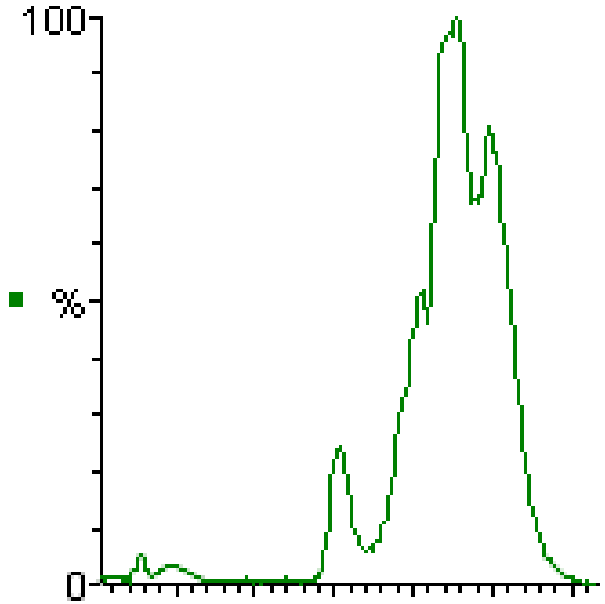
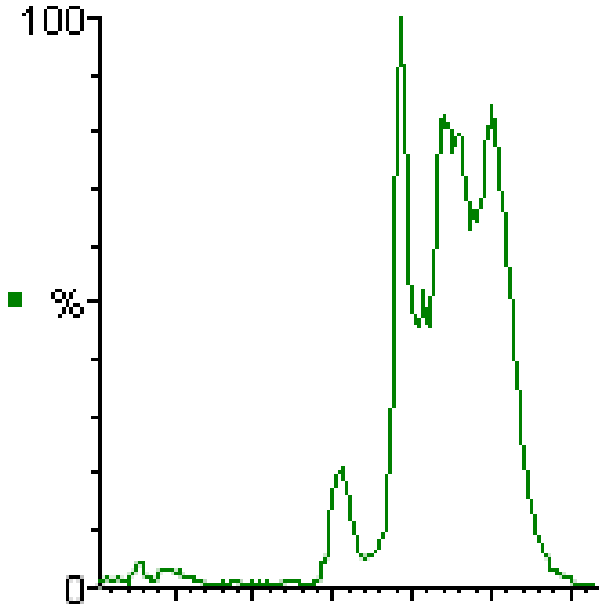
Selected Ion Recording (SIR)

[Low]



[High]



LC/MS/MS

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Differences between Single and Tandem Quadrupole Instruments

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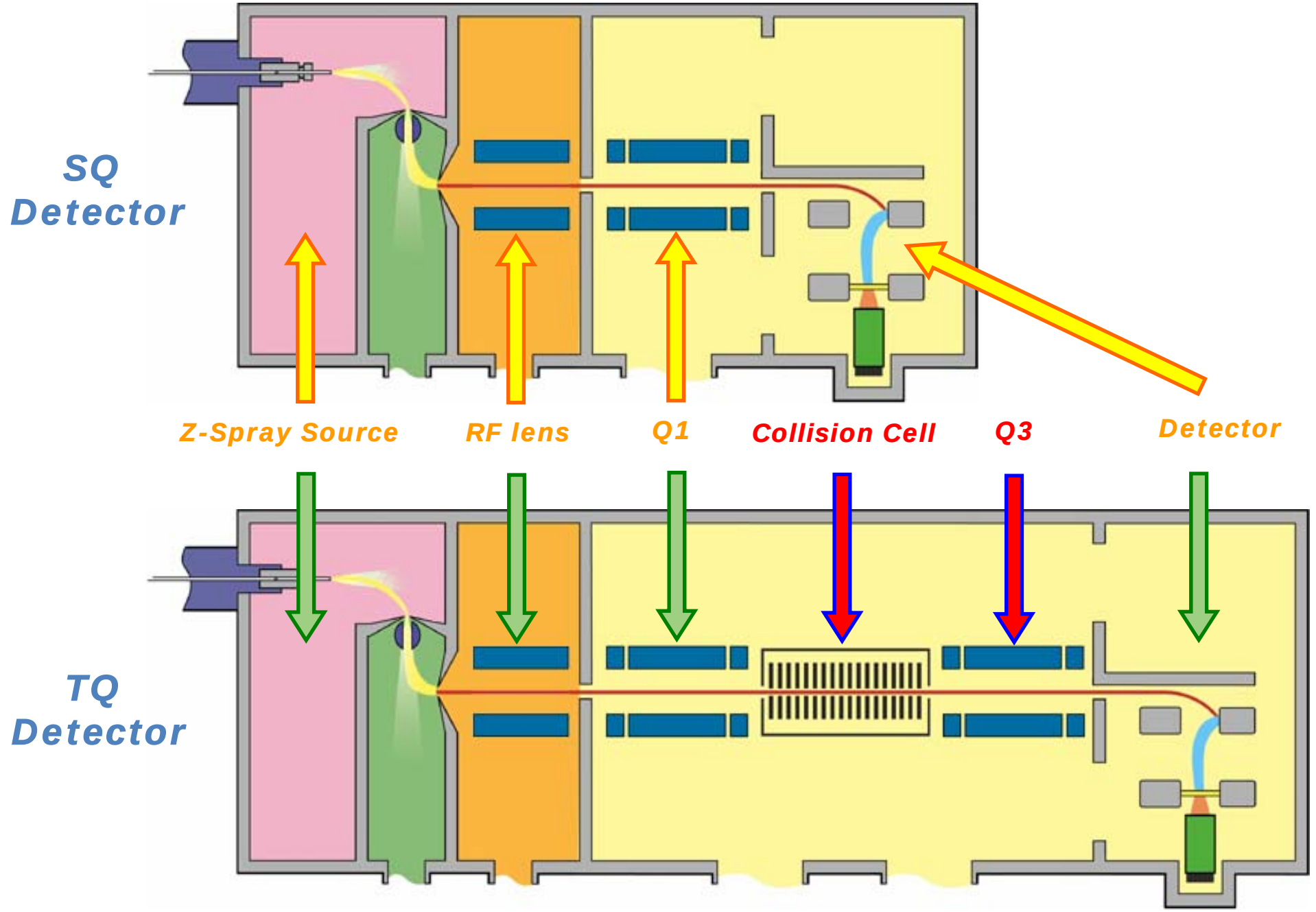
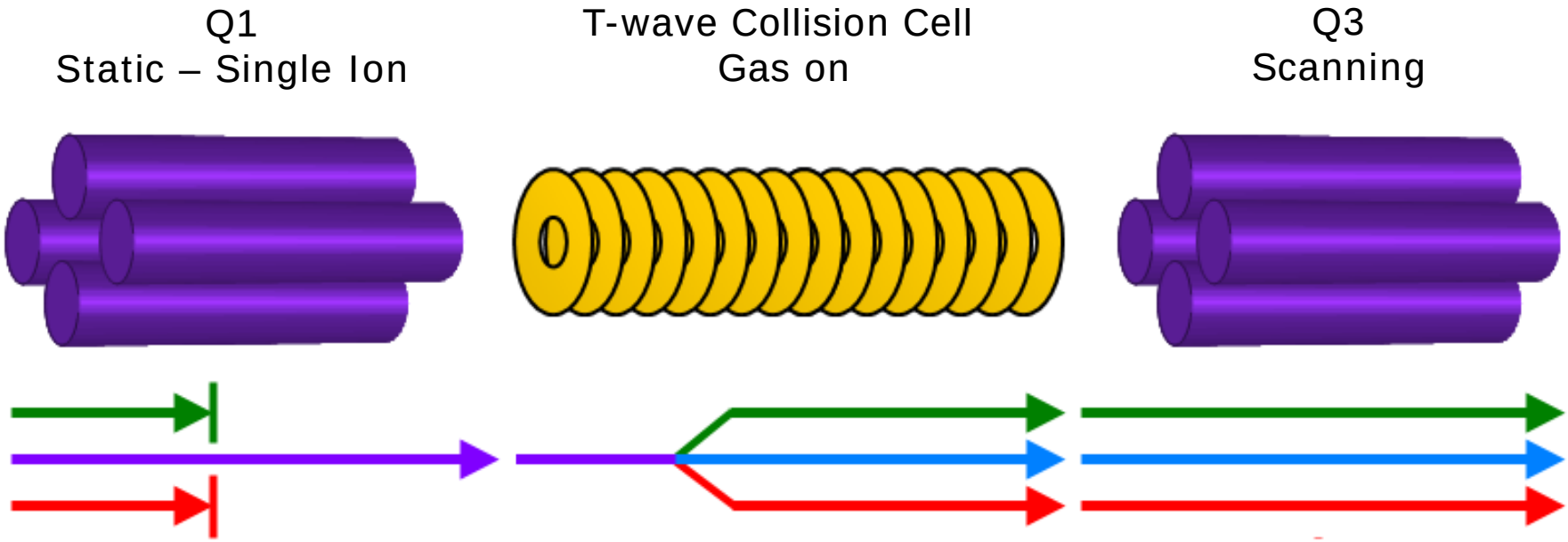


Product (Daughter) Scan
MRM – Multiple Reaction Monitoring



- Selected ions are transmitted through Q1
- Fragmented in the collision cell
- Q3 is then scanned over a defined mass range

- A mass spectrum of the product ions generated by fragmentation is acquired at each time point throughout the acquisition.

- The most common MRM experiment

Analyte Injected in Matrix

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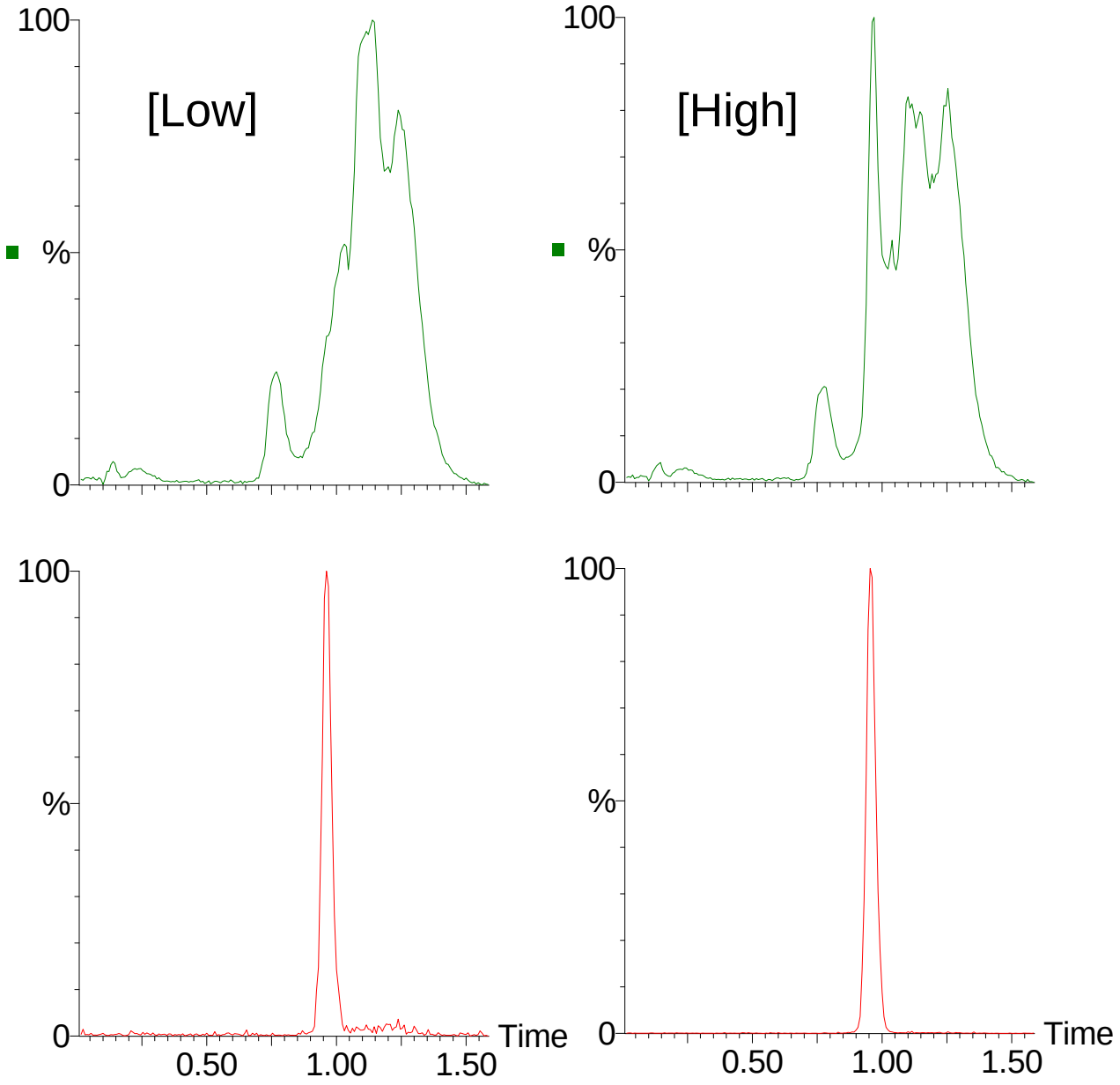
Selected Ion Recording (SIR)

Selected Ion Monitoring (SIM)

Multiple Reaction Monitoring (MRM)



MRM versus SIM

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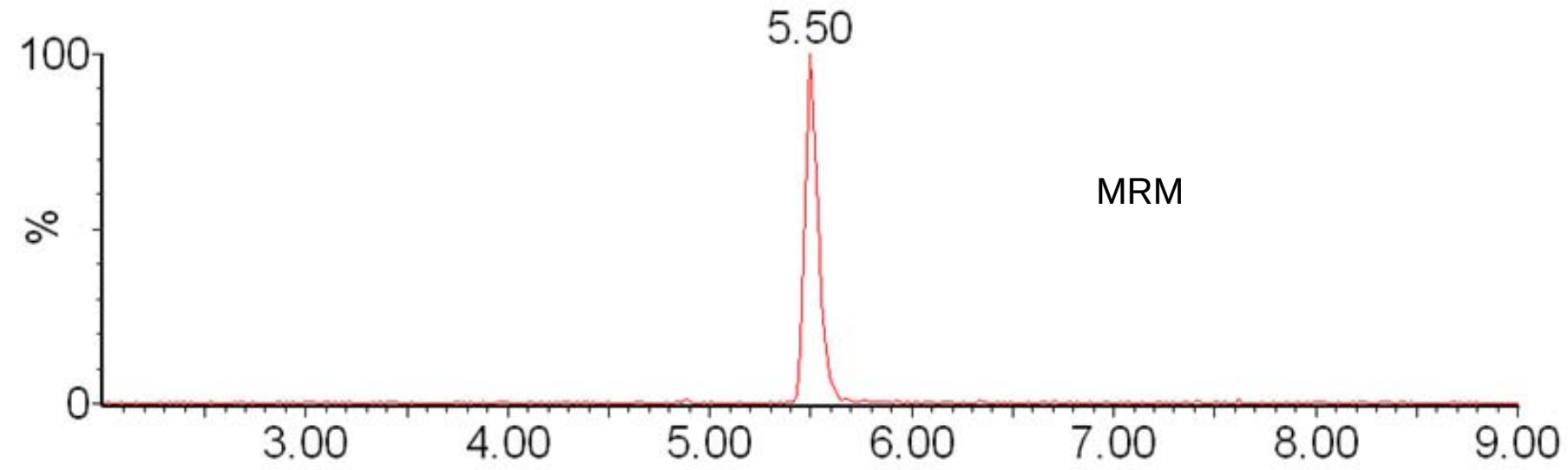
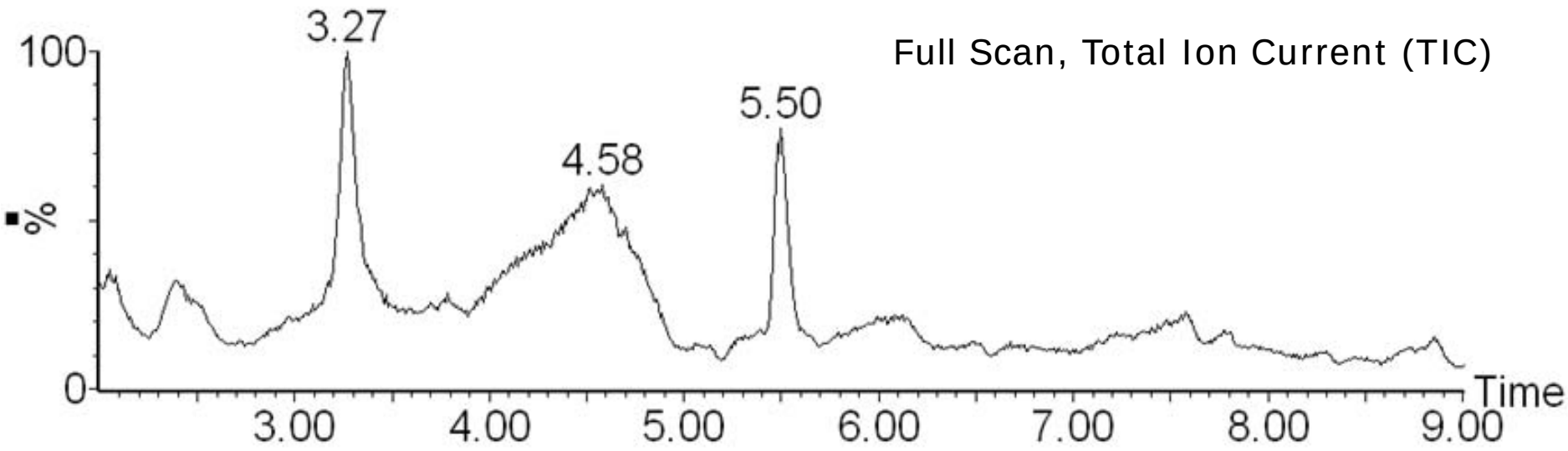


MRM



Full Scan, Total Ion Current (TIC)

ESI+ LC/MS Spectrum for Methamidophos

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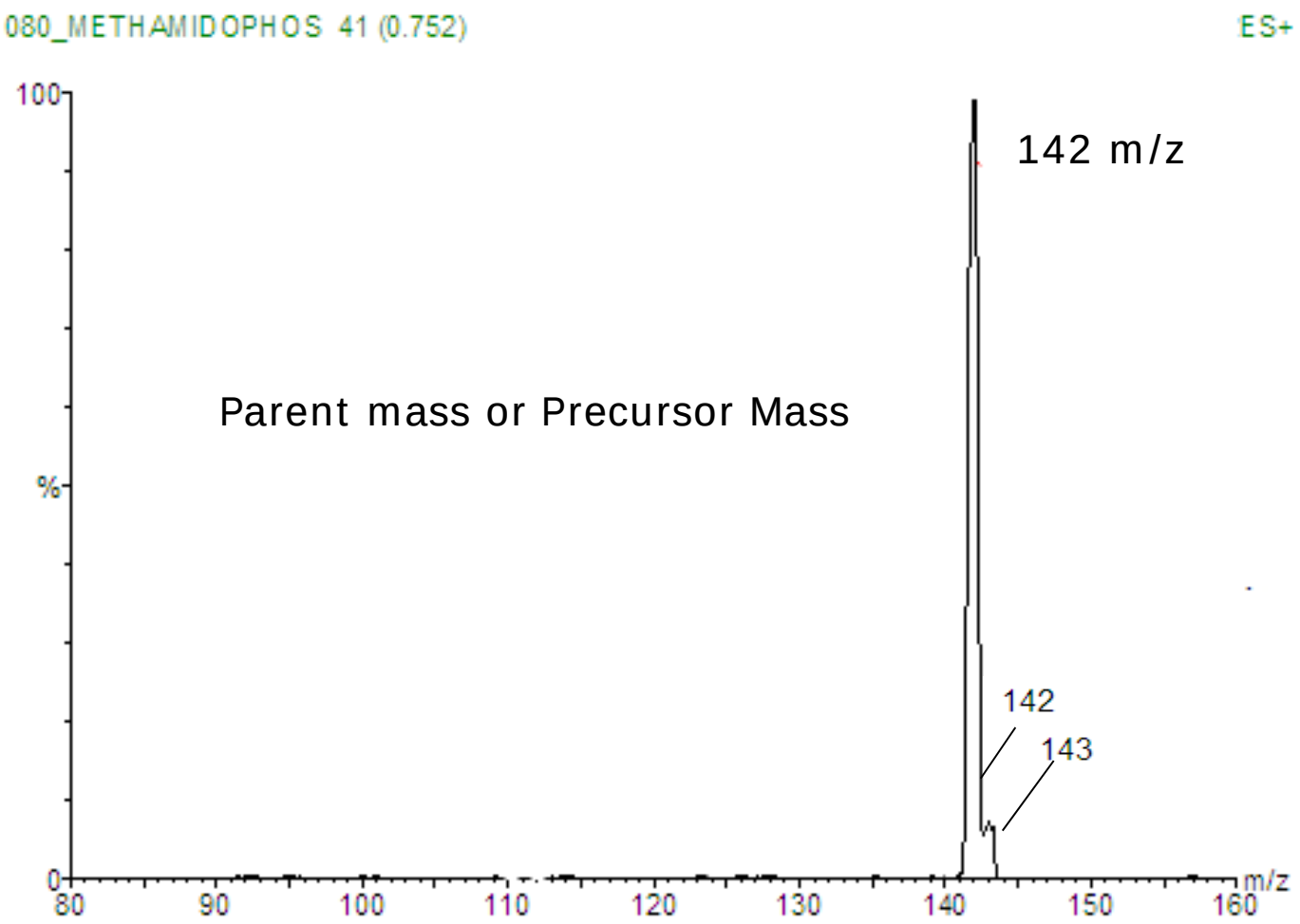

ESI+ LC/MS/MS Spectrum for Methamidophos

Waters
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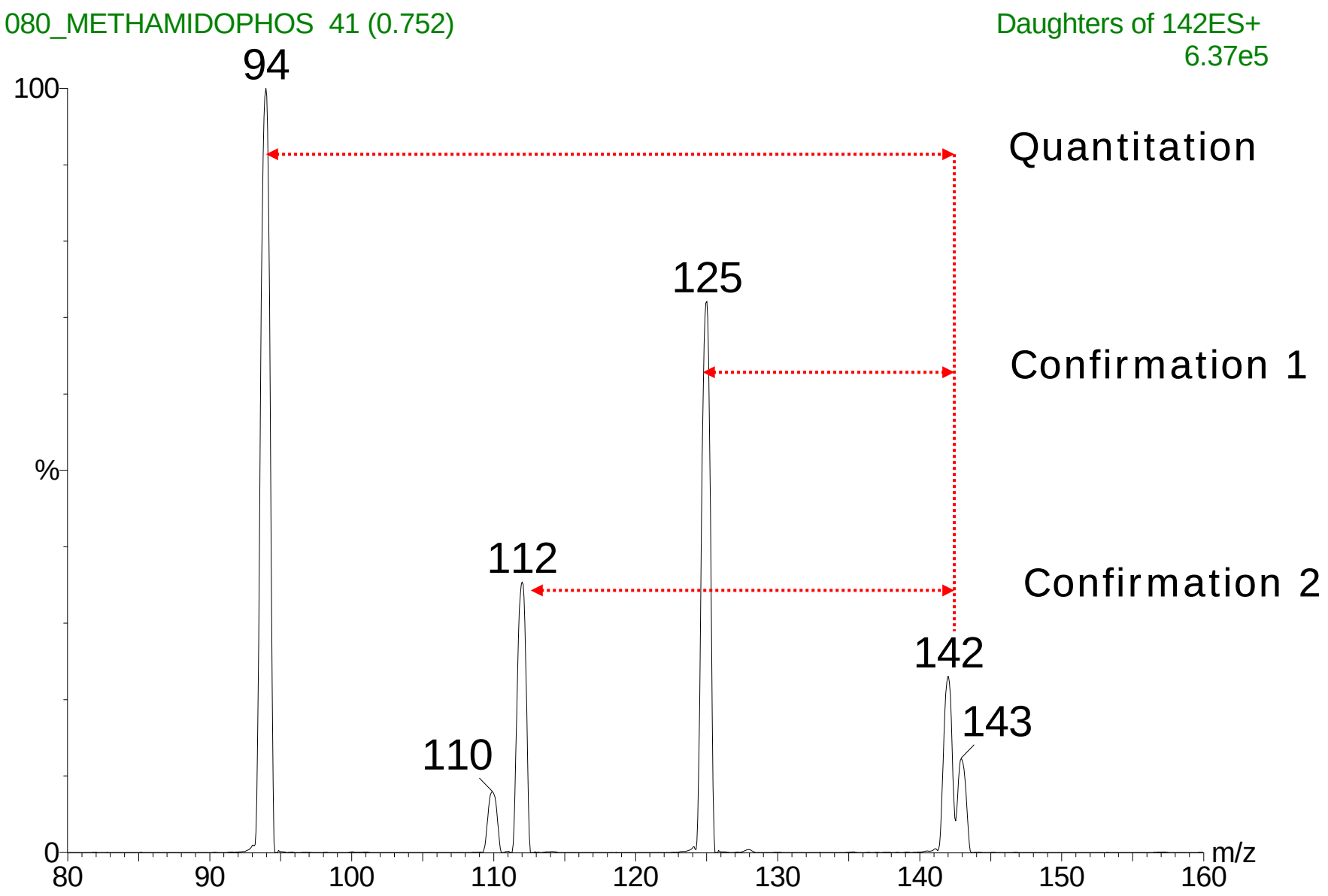
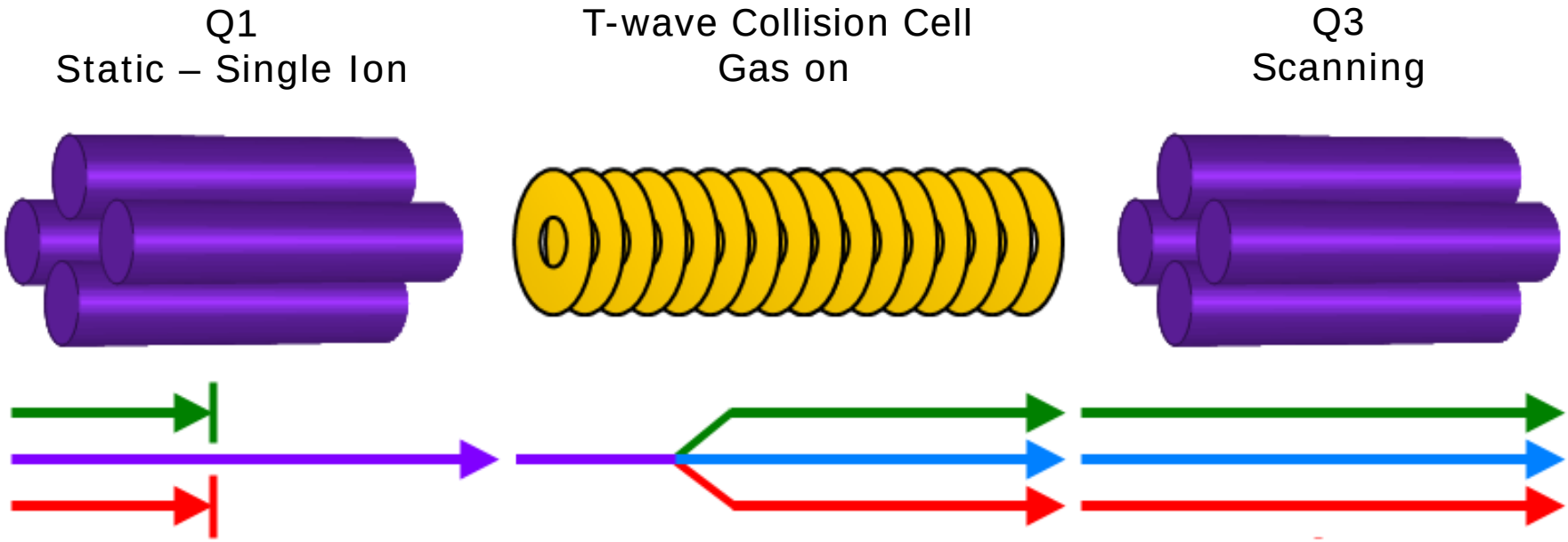


Daughter or Product Masses

Product (Daughter) Scan
MRM – Multiple Reaction Monitoring



- Selected ions are transmitted through Q1
- Fragmented in the collision cell
- Q3 is then scanned over a defined mass range

- A mass spectrum of the product ions generated by fragmentation is acquired at each time point throughout the acquisition.
- This is the most common MRM experiment

Precursor (Parent) Scan

MRM – Multiple Reaction Monitoring

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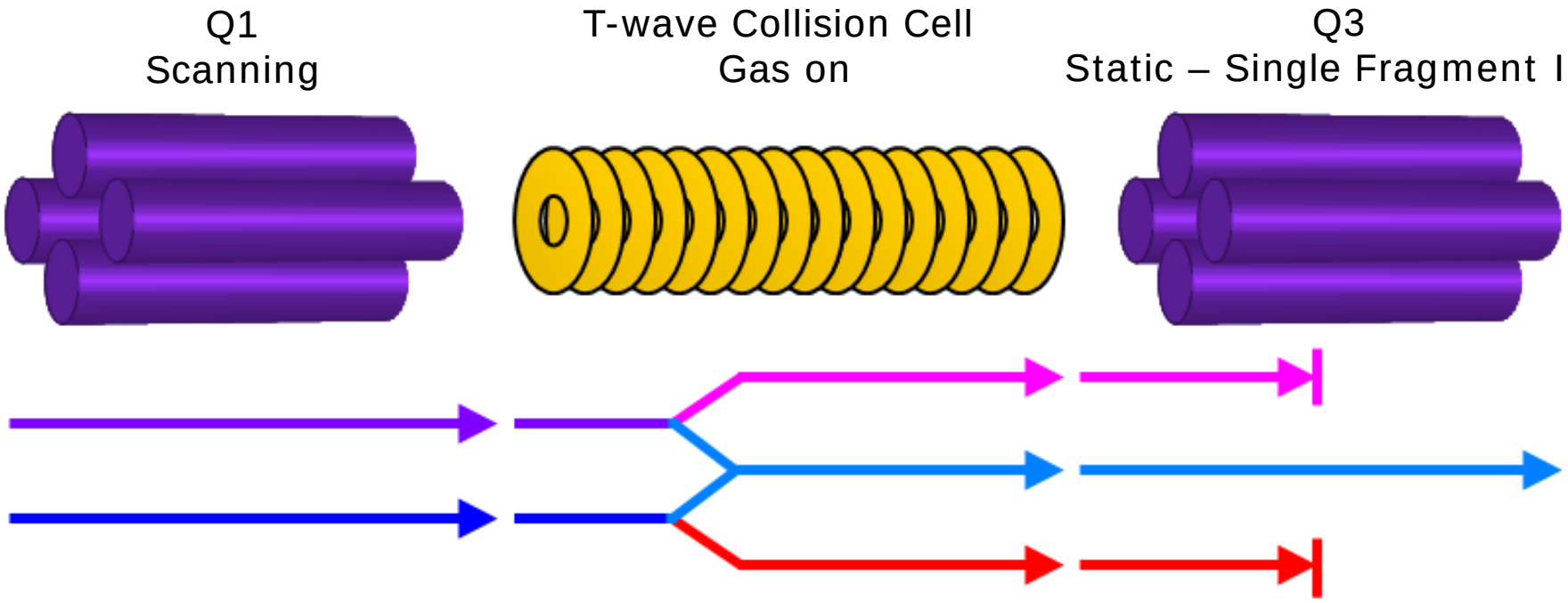
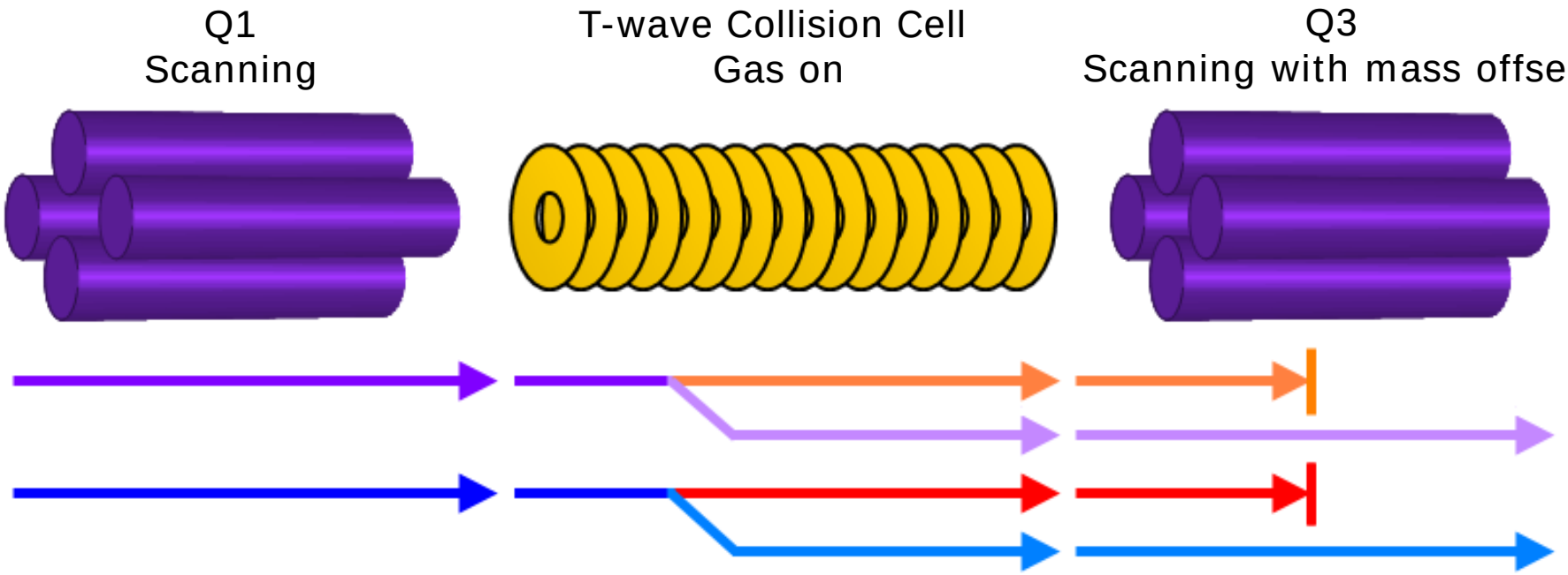


- Q1 is scanned over a specified mass range and
- All ions are sequentially passed through to the collision cell where they are fragmented
- Q3 is set to transmit only the mass of a specific fragment ion and does not scan

- No mass spectra are generated by this MRM experiment
- Monitoring a chemical series e.g., barbituates, sulfonamides

(constant) Neutral Loss



- Q1 is scanned over a specified mass range and
- All ions are fragmented in the collision
- Q3 is scanned in sync with Q1 over the same mass range minus an offset

- A response is only seen if a precursor ion loses a neutral fragment in the collision cell of the same mass to charge ratio as the offset between Q1 and Q3, e.g., 35 m/z (chlorine)

Chromatography is to separate out mixtures by retention time.

Mass spectrometry is to separate out mixtures by mass.

Good analytical chemistry is to use both.

Remember –

Just because a mixture of chemicals have the same elution chromatographically, doesn't mean that they cannot be totally separated by mass.

Screening

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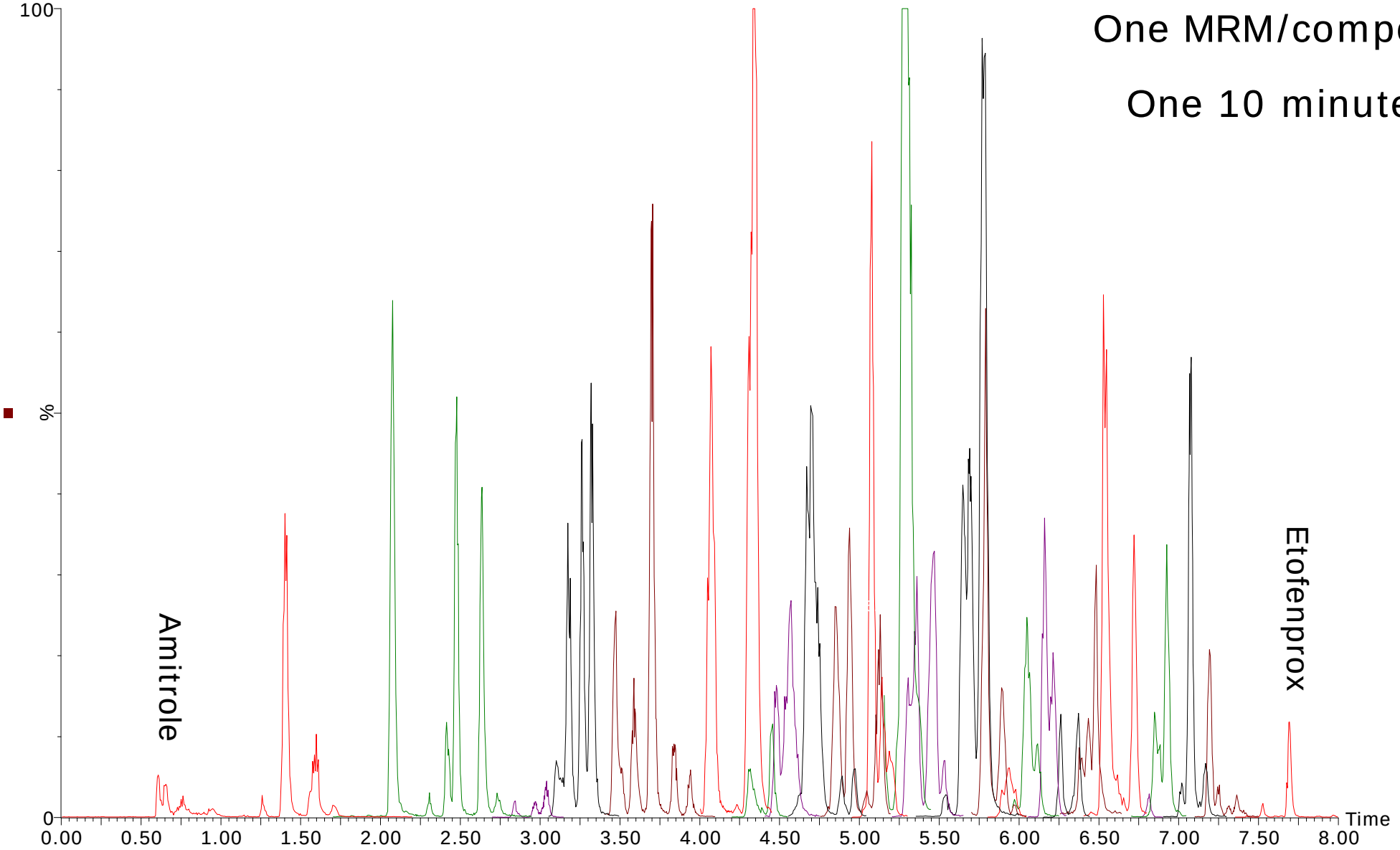
>500 pesticide residues

One MRM/compound

One 10 minute run



Xevo TQ-S
World's Most Sensitive MS

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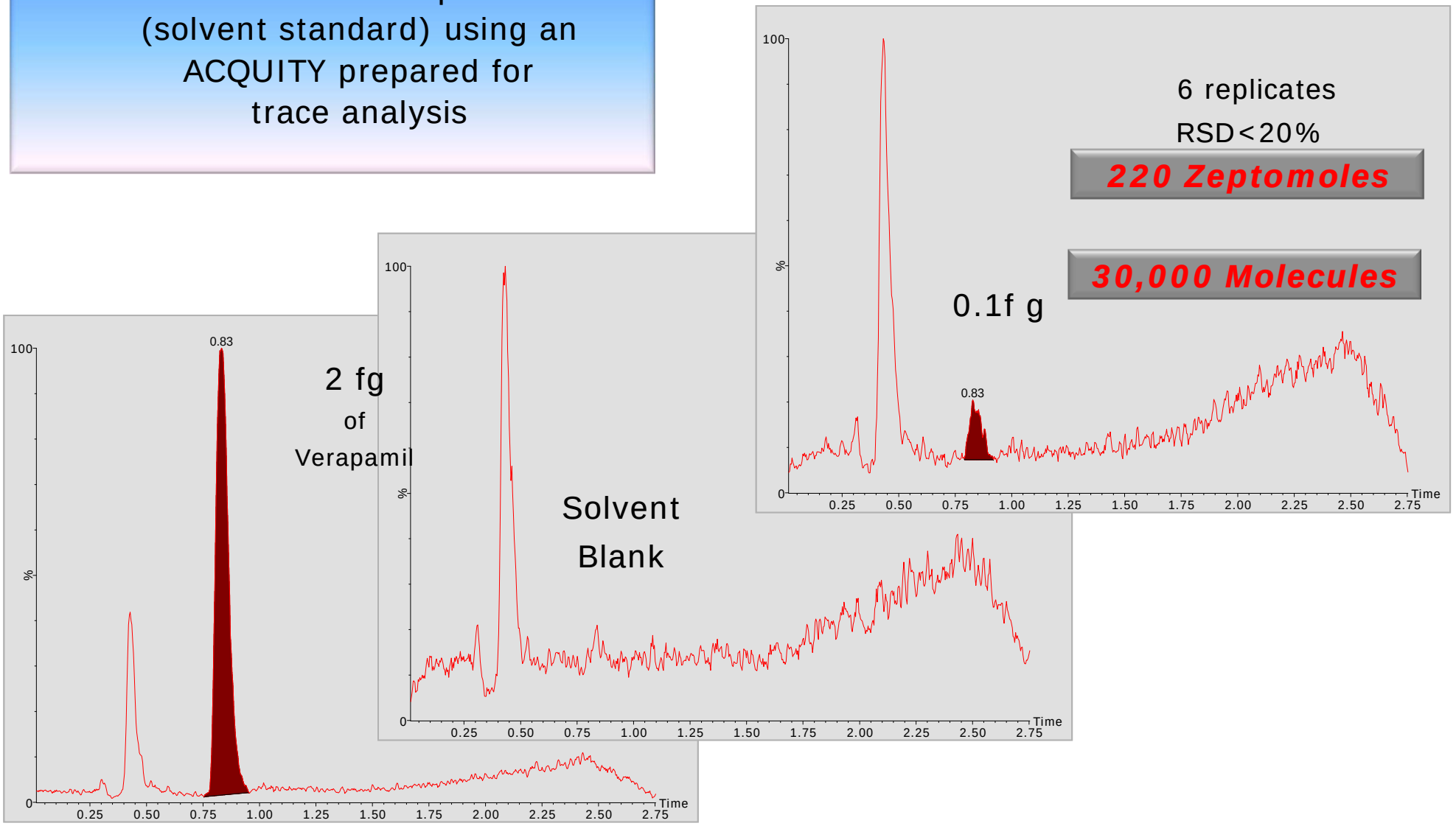
UPLC/MRM of Verapamil (solvent standard) using an ACQUITY prepared for trace analysis



Atmospheric Pressure Solids Analysis Probe

ASAP

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Waters Atmospheric Solids Analysis Probe

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Outer Assembly

Inner Probe

Glass Capillary Tube

Inner Probe Inserted into Outer Assembly

ASAP Introduction

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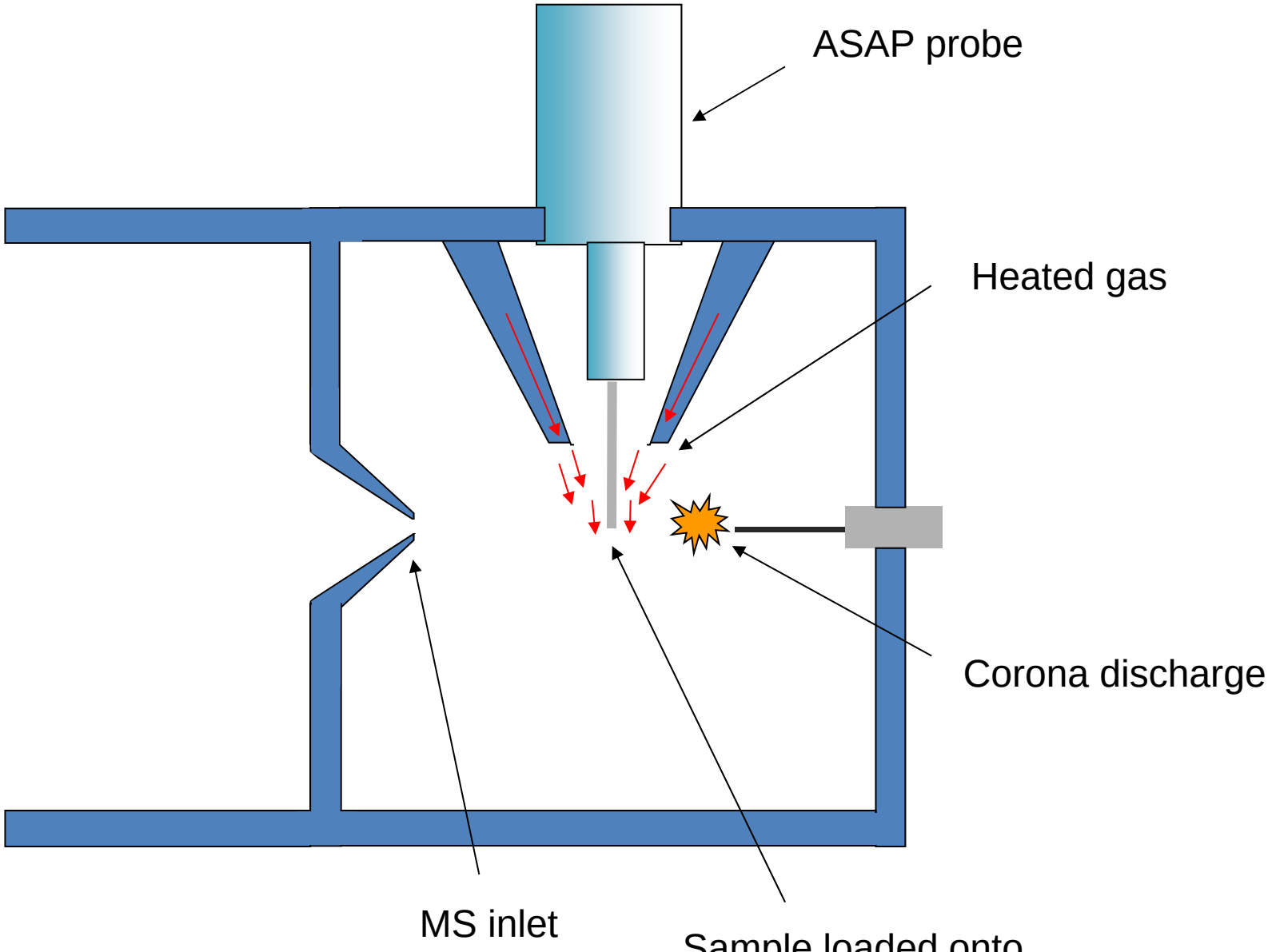
- A solids analysis probe for atmospheric pressure ionization (API) sources
  - Fast
  - No sample preparation
  - No chromatographic separation
  - Simple Construction
  - Low cost analysis
- Suitable for volatile and semi-volatile solid and liquid samples
- Data from non-polar samples normally not seen using API
- Good alternative to GC solids probe (no vacuum lock)
- Enclosed source (safety)

ASAP Overview



Licensed from M&M Mass Spec Consulting

ASAP Probe with Baffle and ESCi Corona Pin

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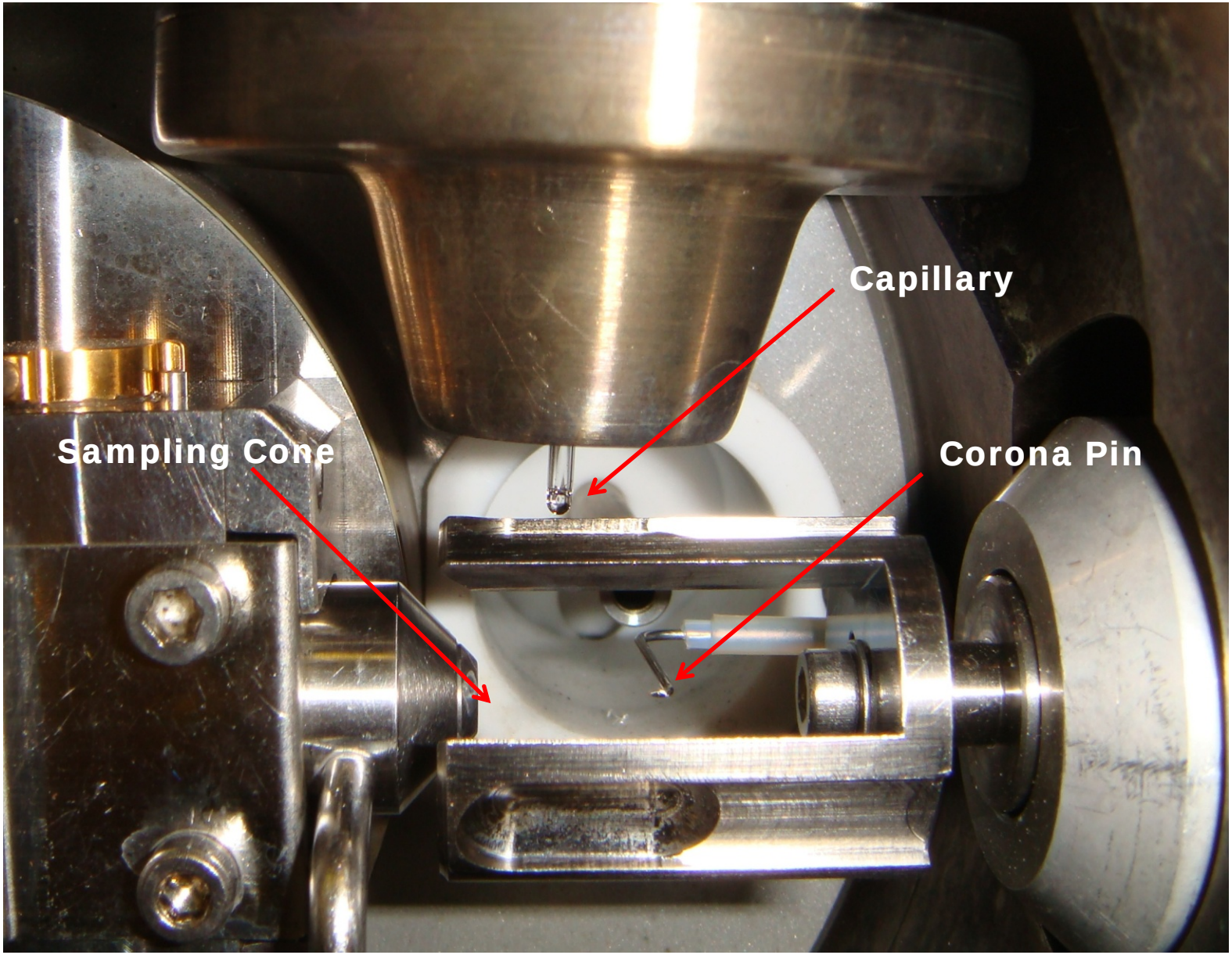

Application Areas

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- **Forensics**
  - Analysis of unknown tablets / counterfeits
  - Analysis of inks
- **Pharmaceuticals**
  - Molecular weight confirmation in drug discovery
  - Formulated products
  - Metabolites in urine
- **Fine Chemicals**
  - Impurity profiling
- **Food Safety**
  - Adulteration of food
- **Environmental**
  - Polynuclear aromatic hydrocarbon contaminants
- **Petrochemical**
  - Analysis of complex samples including polymers and polymer additives (in conjunction with Synapt G2-S HDMS)
- **Synthesis**
- **Raw Material Assays**

Mechanism of Ionization (I)

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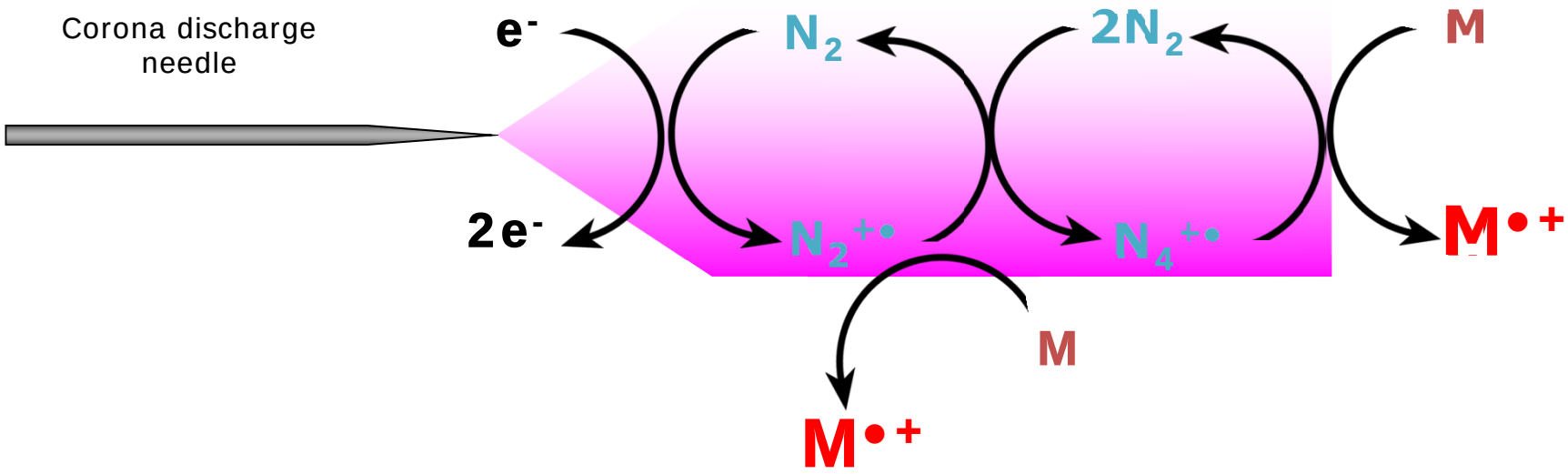
Charge Transfer



- "Dry" source conditions
- Favoured by relatively non-polar compounds

Mechanism of Ionization (II)

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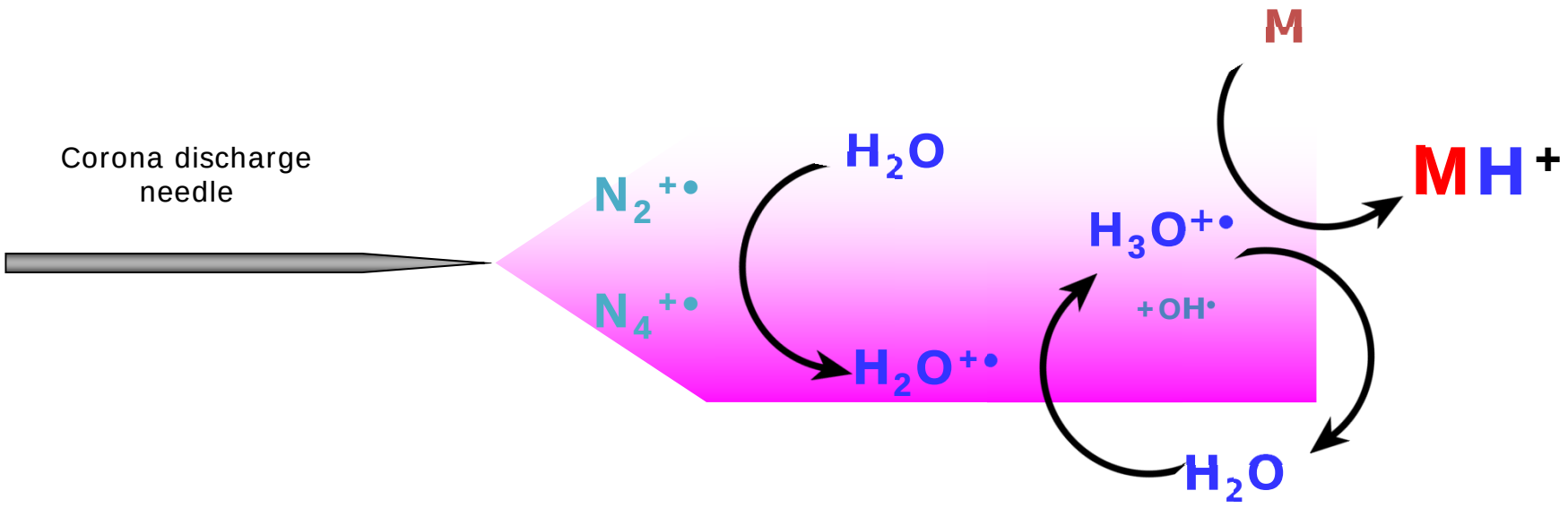
Proton Transfer



- Modified source conditions for example with water or methanol present
- Favoured by relatively polar compounds

Examples of Different ionization Mechanisms
- *Analysis of Steroid Progesterone*

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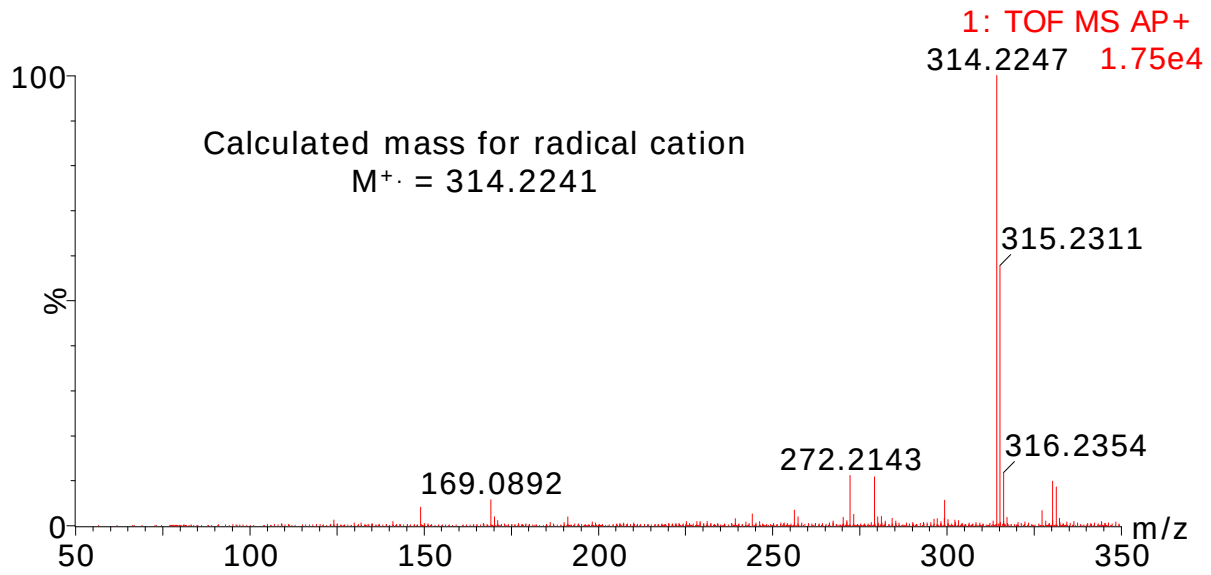
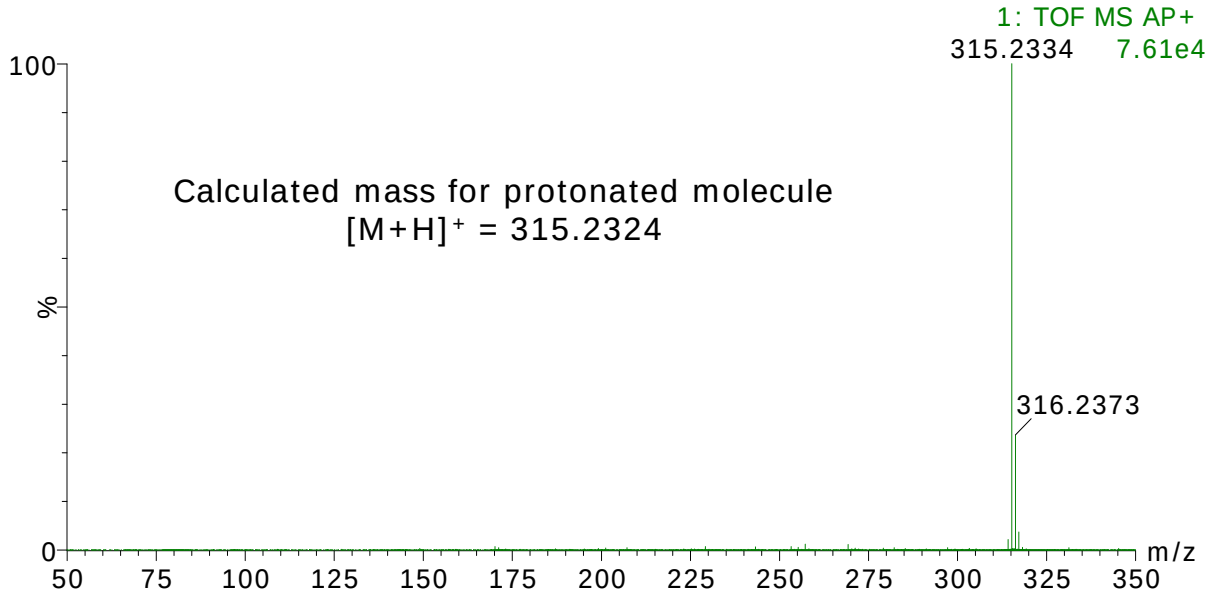
progesterone- no lock ref



Charge transfer
dry source

progesterone



Proton transfer
solvent in source

Metabolites in Urine

...paracetamol and dihydrocodeine

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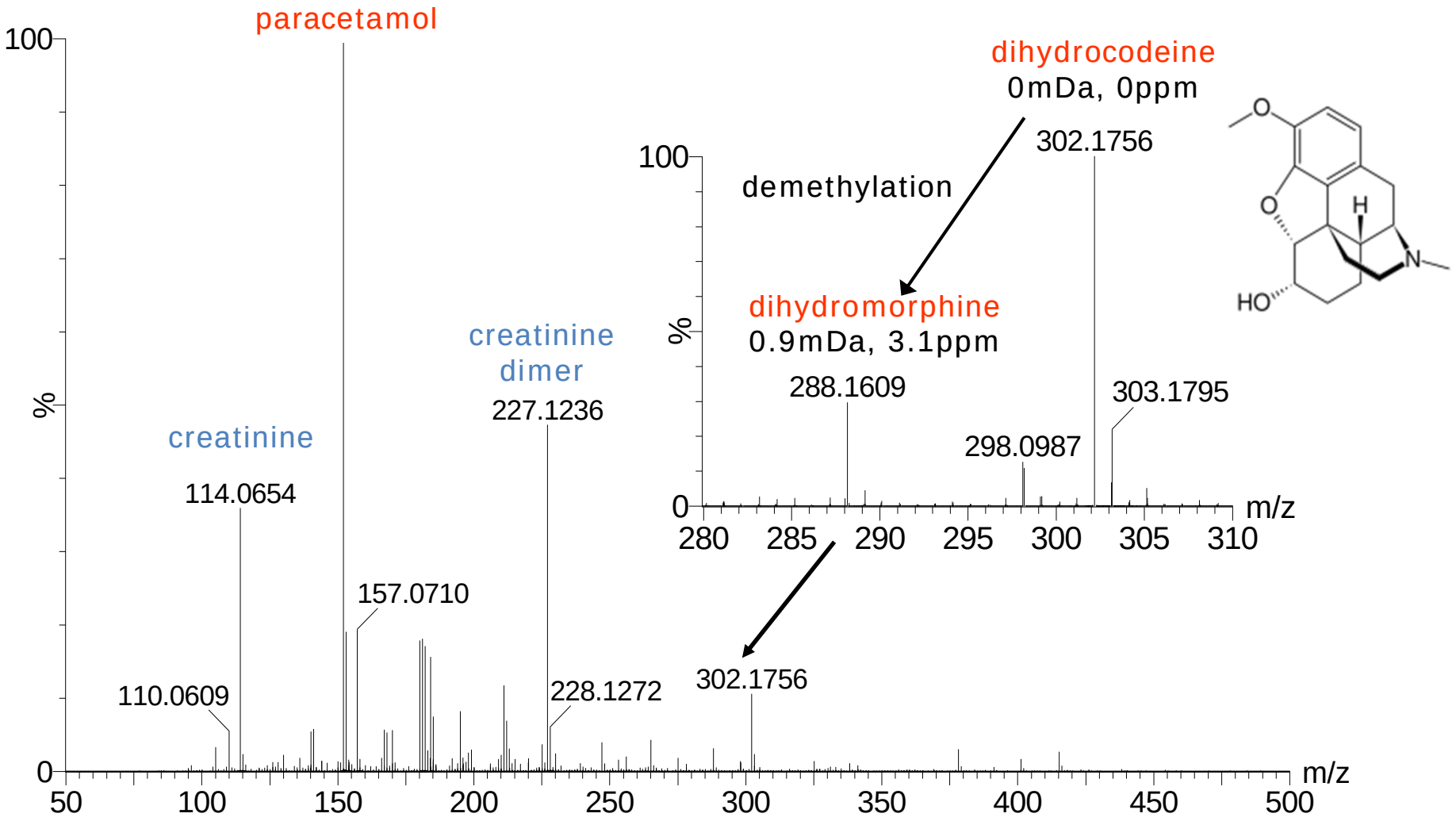
Urine sample from patient after dosing with paracetamol (1000mg) and dihydrocodeine (30mg)



Sample capillary loaded with 1µL neat urine

Identification of inks on paper

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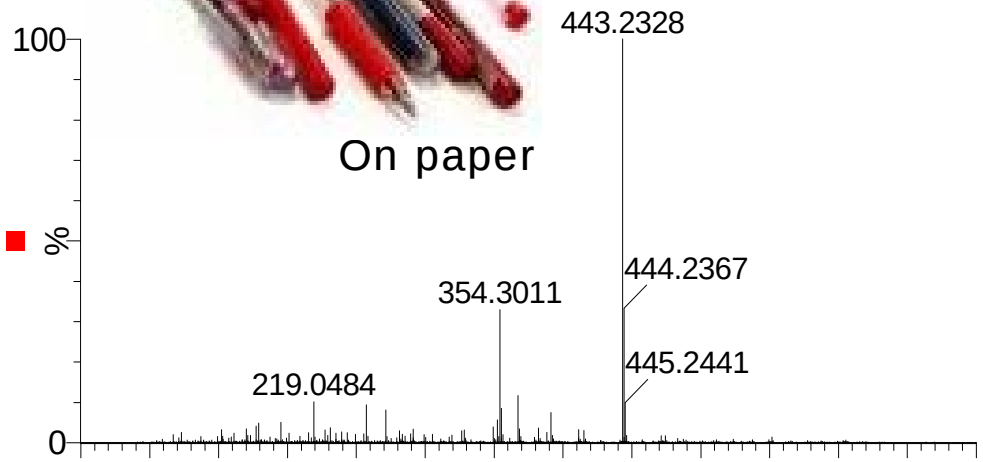
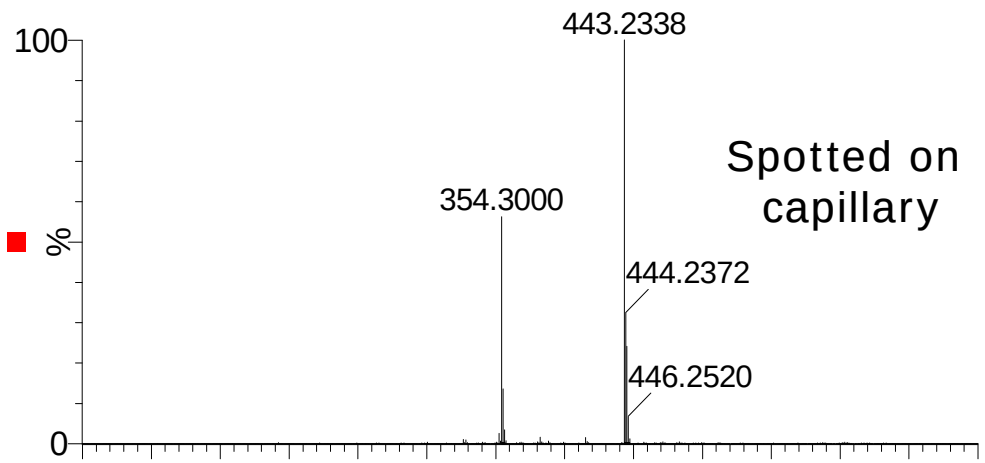
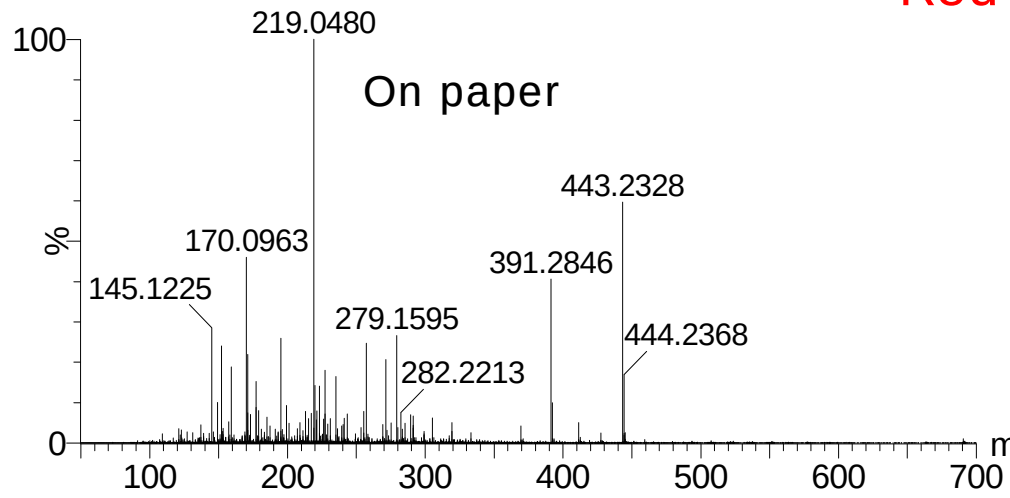
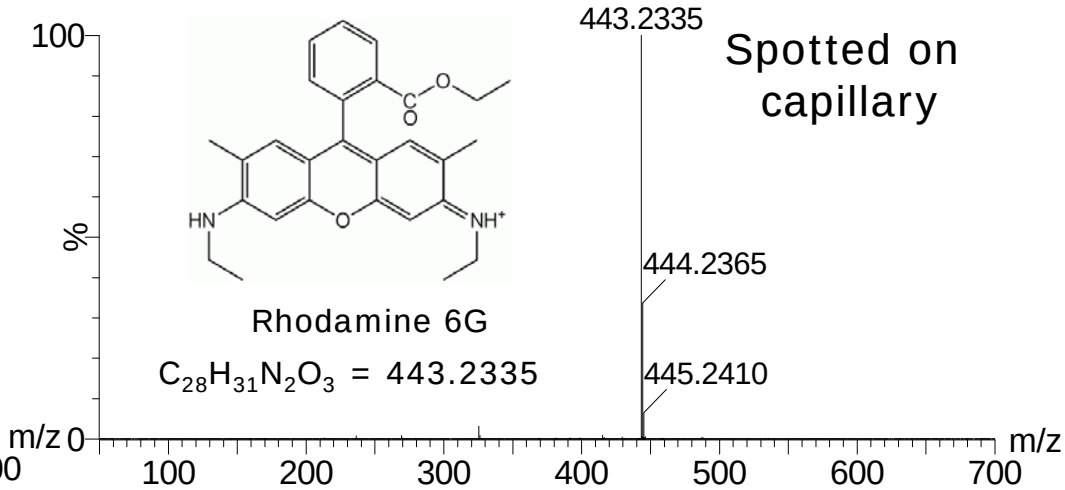
Red ball point ink





Red marker





Rhodamine 6G

$C_{28}H_{31}N_2O_3$ = 443.2335

Glass capillary rubbed across dry ink on surface of paper before introducing into source

Polynuclear aromatic hydrocarbons

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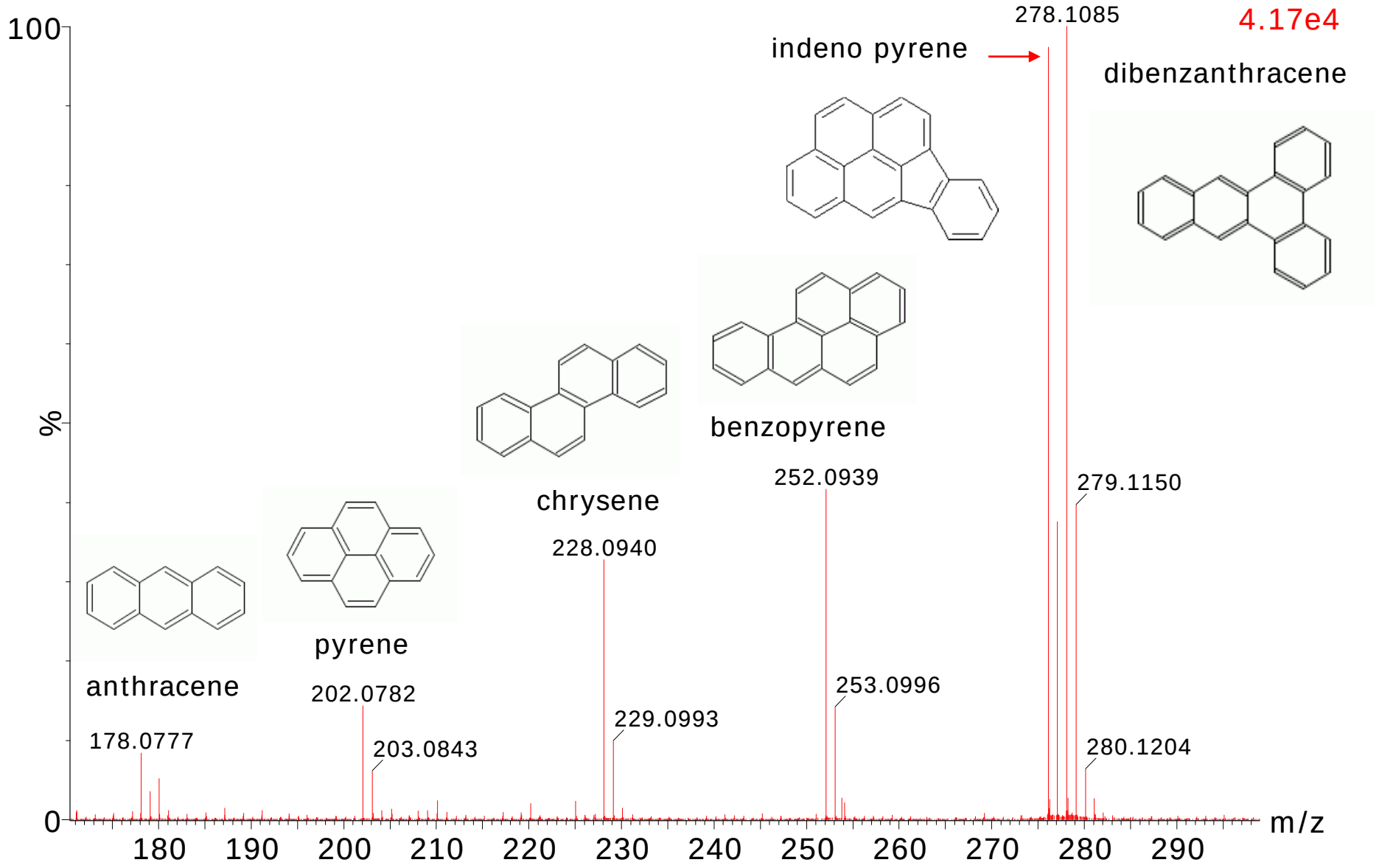

Analysis of Cooking Oils

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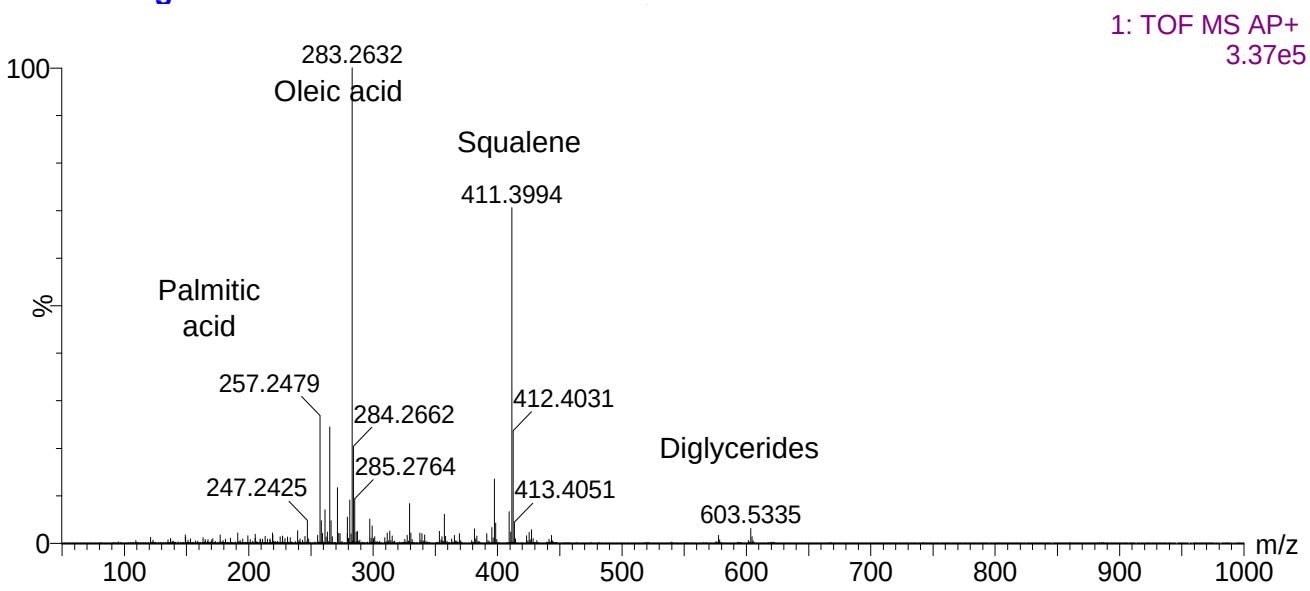
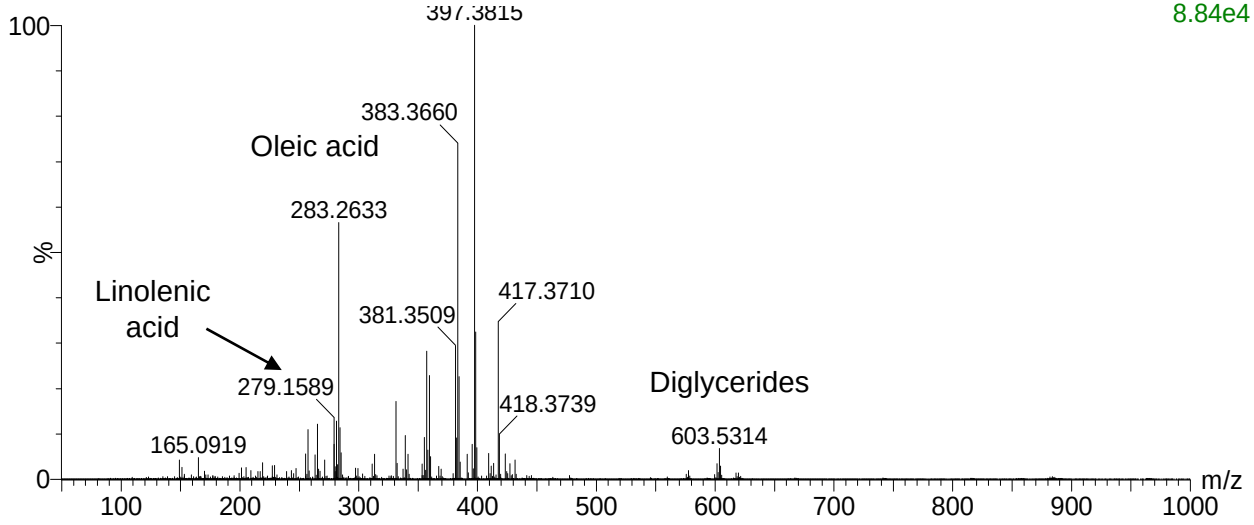
Extra virgin olive oil



Rapeseed oil



Analysis of cooking oils

.... Diglycerides and triglycerides

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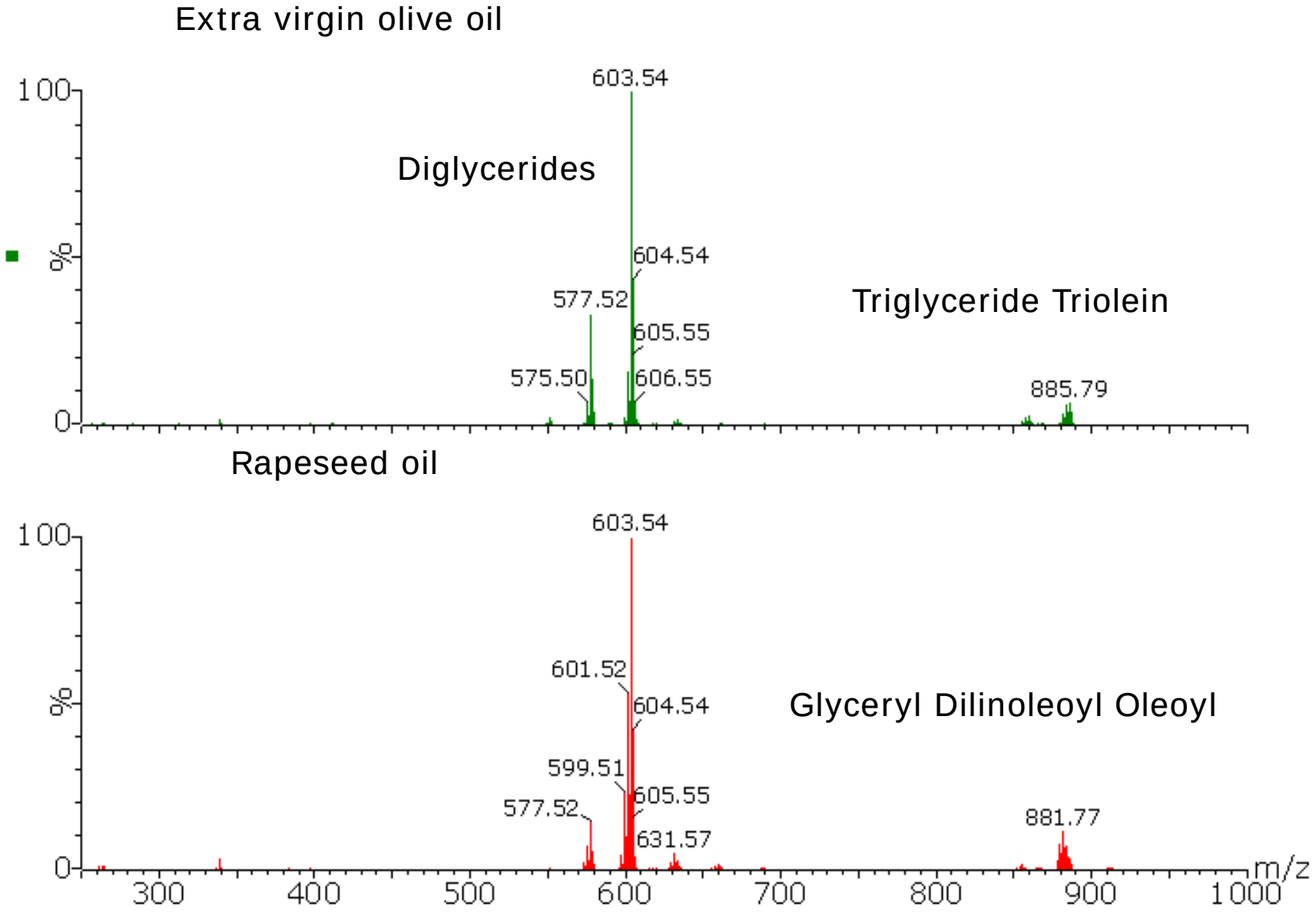

Detection of oleocanthal in extra virgin olive oil

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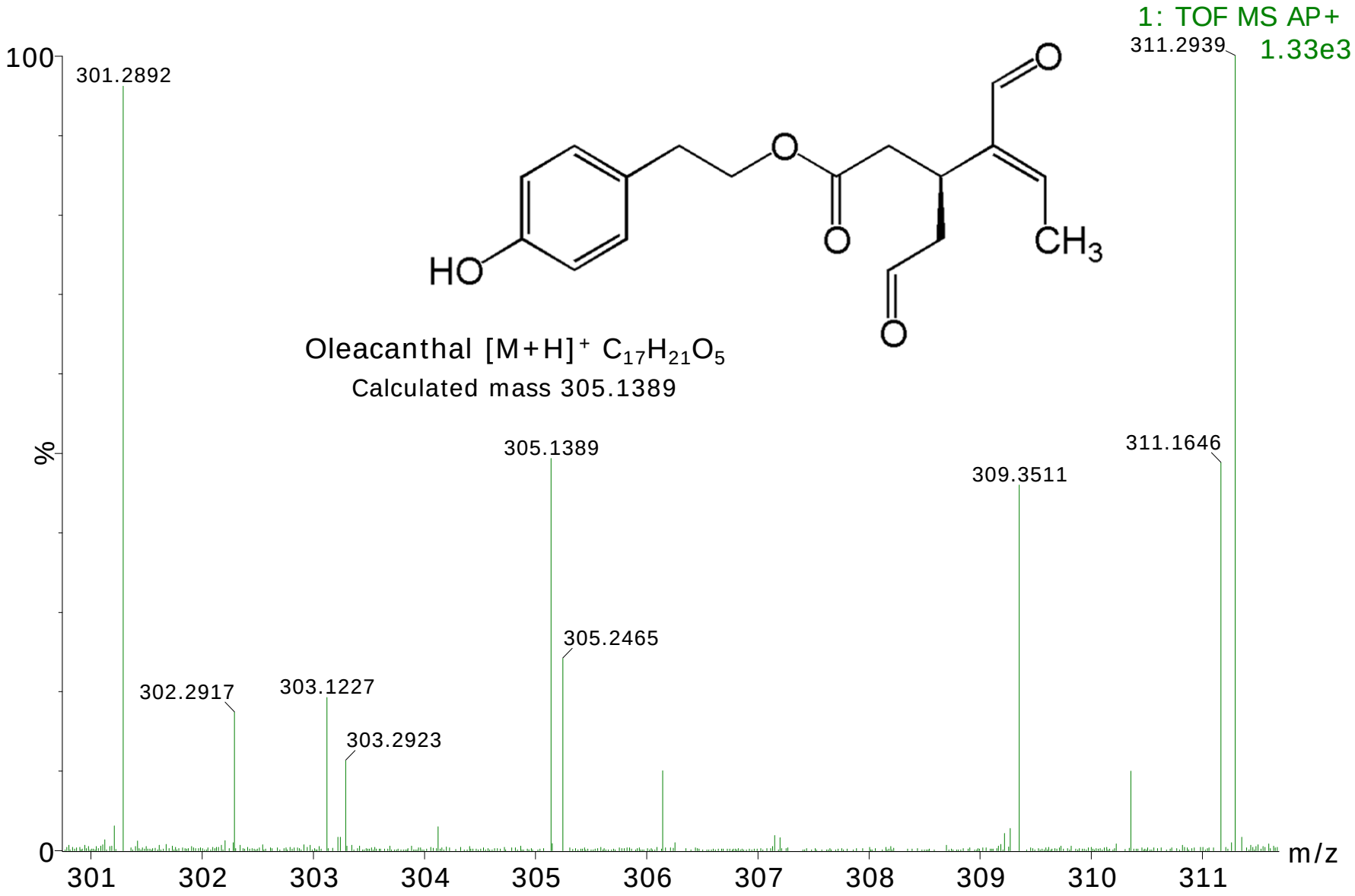

Pesticides in Wheat
Target Strobilurins

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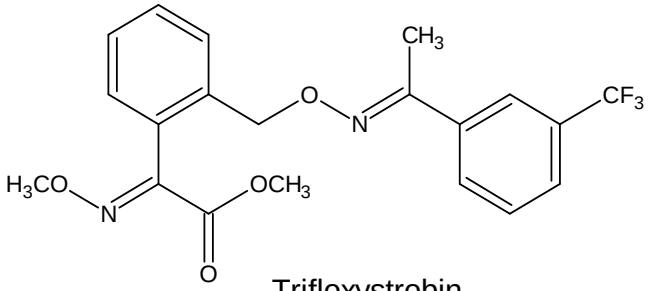
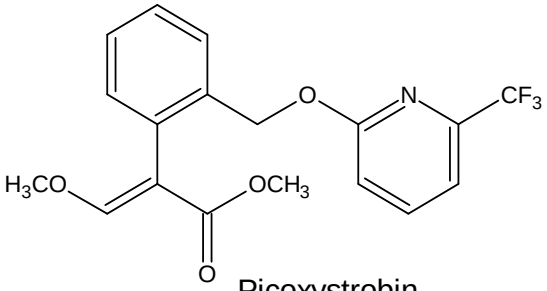
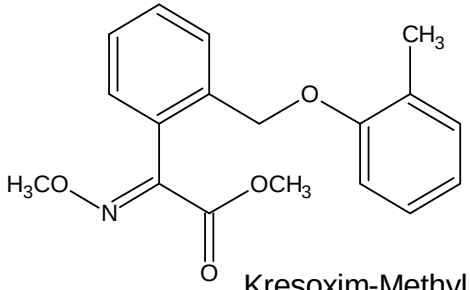


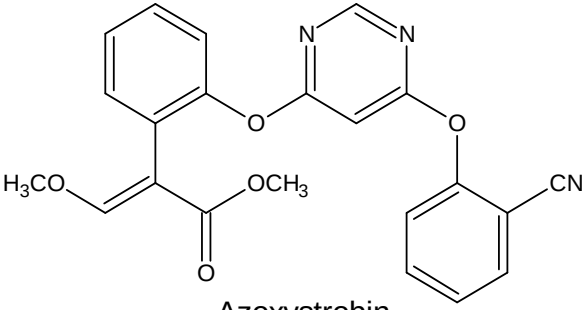
Trifloxystrobin



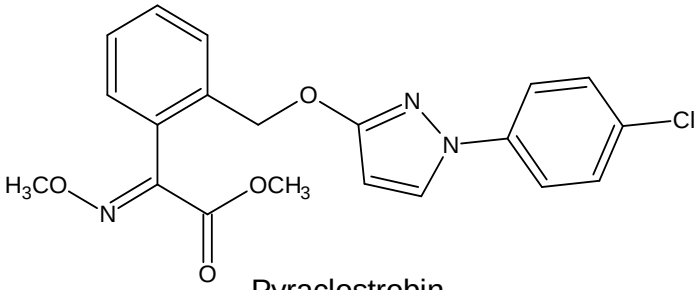
Picoxystrobin



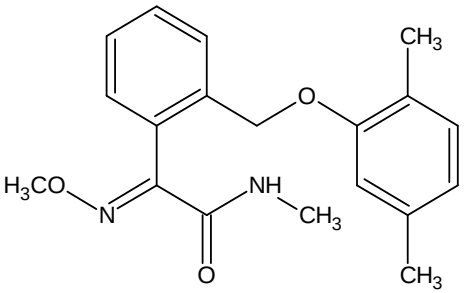
Kresoxim-Methyl



Azoxystrobin



Pyraclostrobin



Dimoxystrobin

fera
The Food and Environment Research Agency

EU MRLs = 0.02 - 0.3mg/kg in wheat

Azoxystrobin Fungicide on Wheat Whole Grain (430µg/kg)

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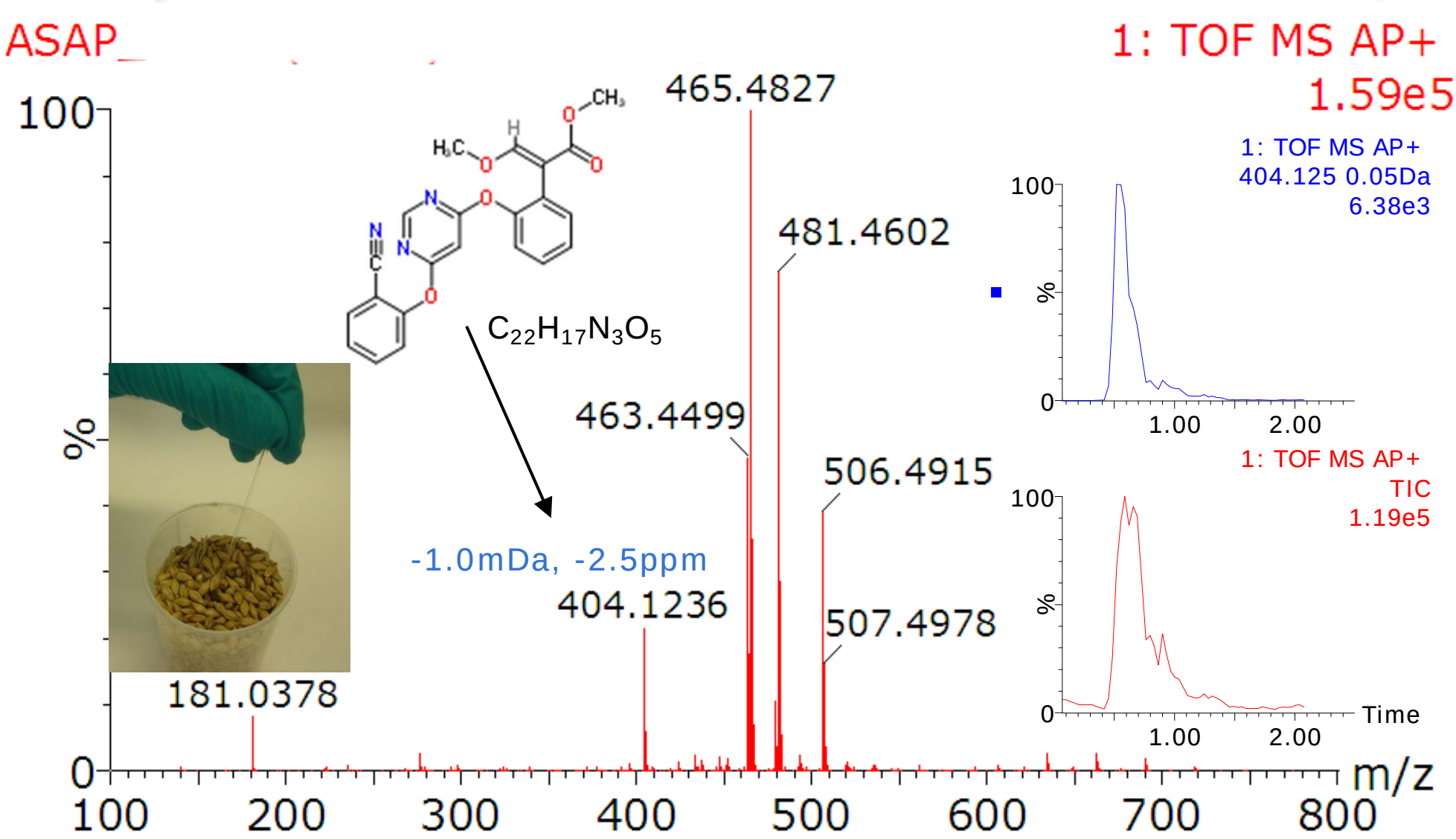

Verapamil 10-10,000ng/mL
....no internal standard

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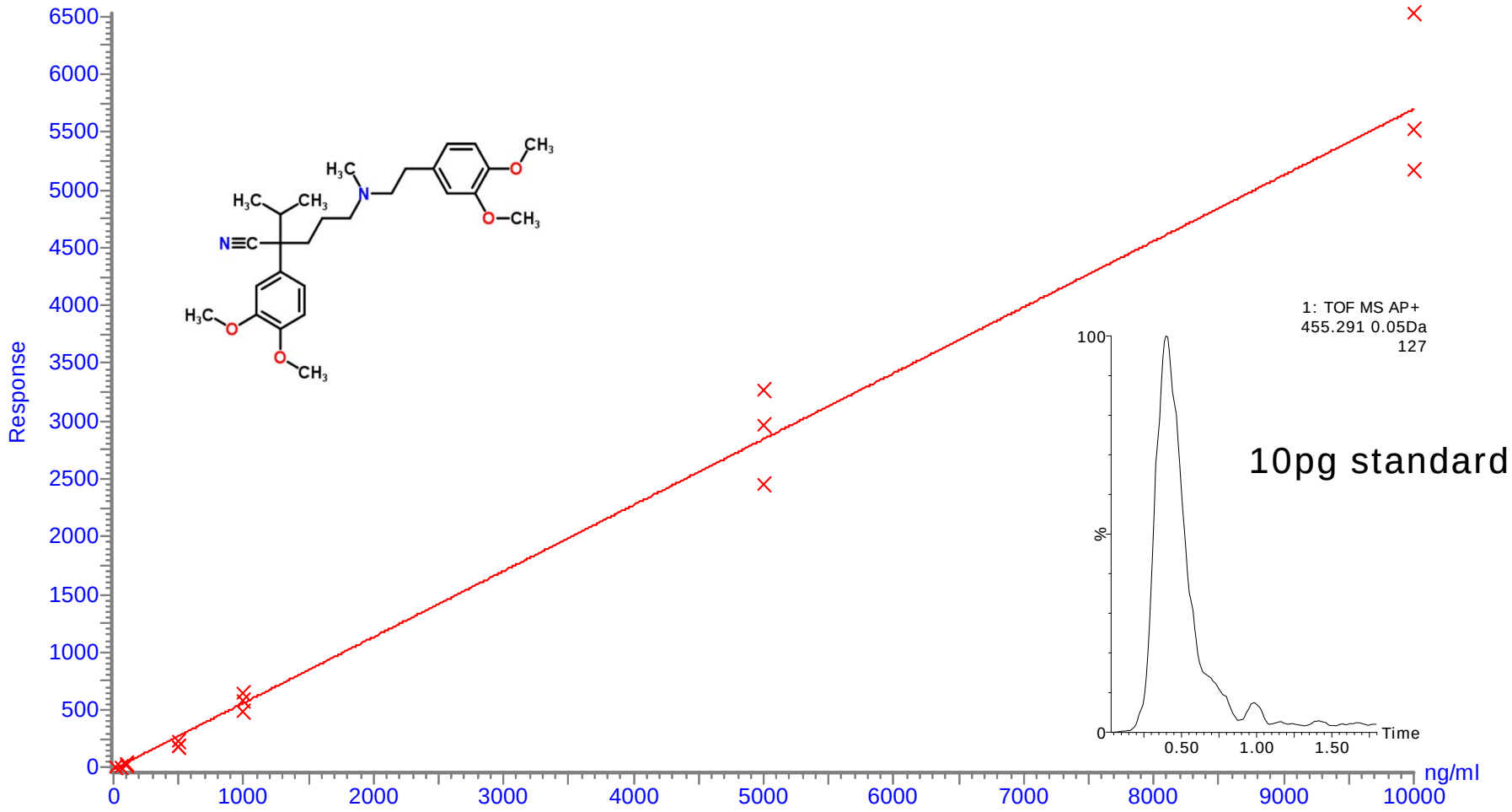
Compound name: Verapamil
Correlation coefficient: r = 0.992187, r^2 = 0.984435
Calibration curve: 0.570819 * x + -10.6168
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Formulated tablets
...scraping from tablet surface

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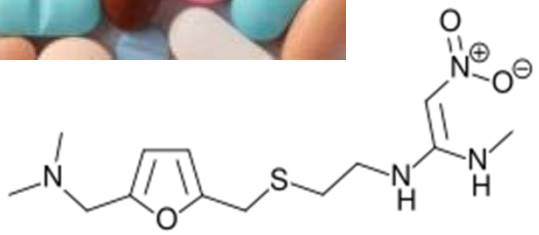

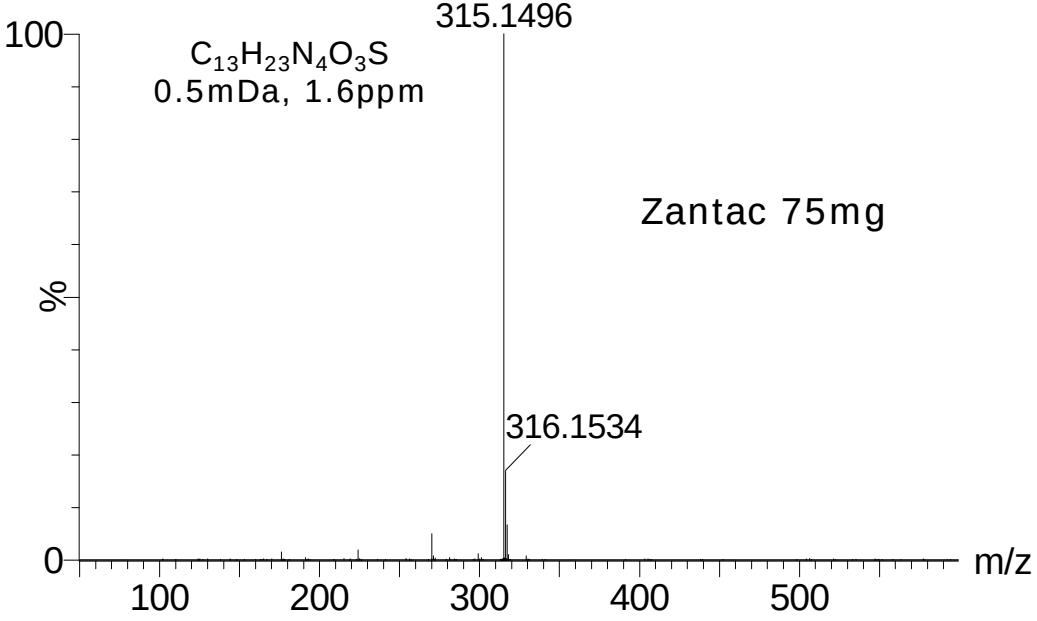

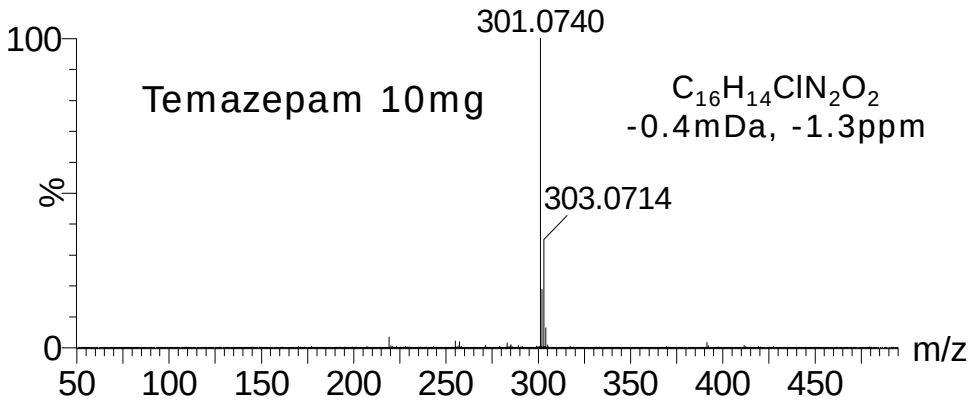

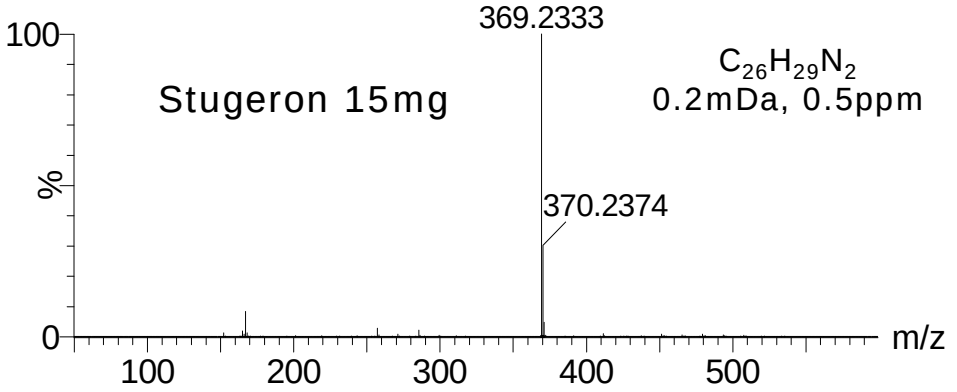

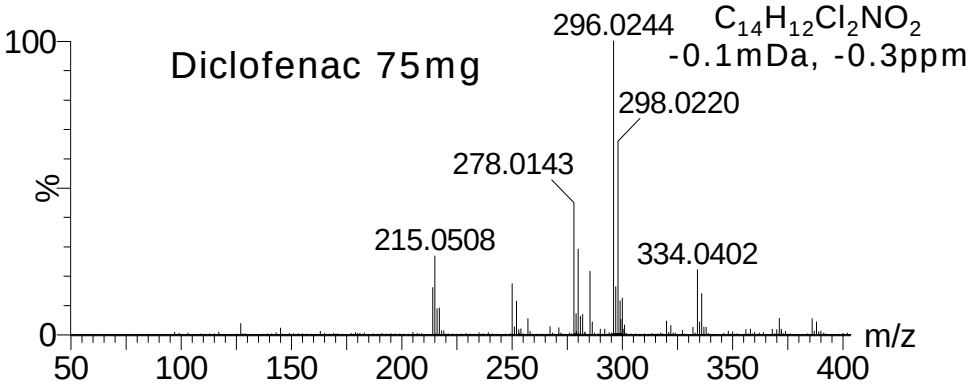

Which are Genuine?
Which are Counterfeit?

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synthetic phosphodiesterase type-5 (PDE-5) inhibitors used in the treatment of erectile dysfunction

Direct Analysis of Tablet Samples using ASAP: Sildenafil / Vardenafil / Tadalafil

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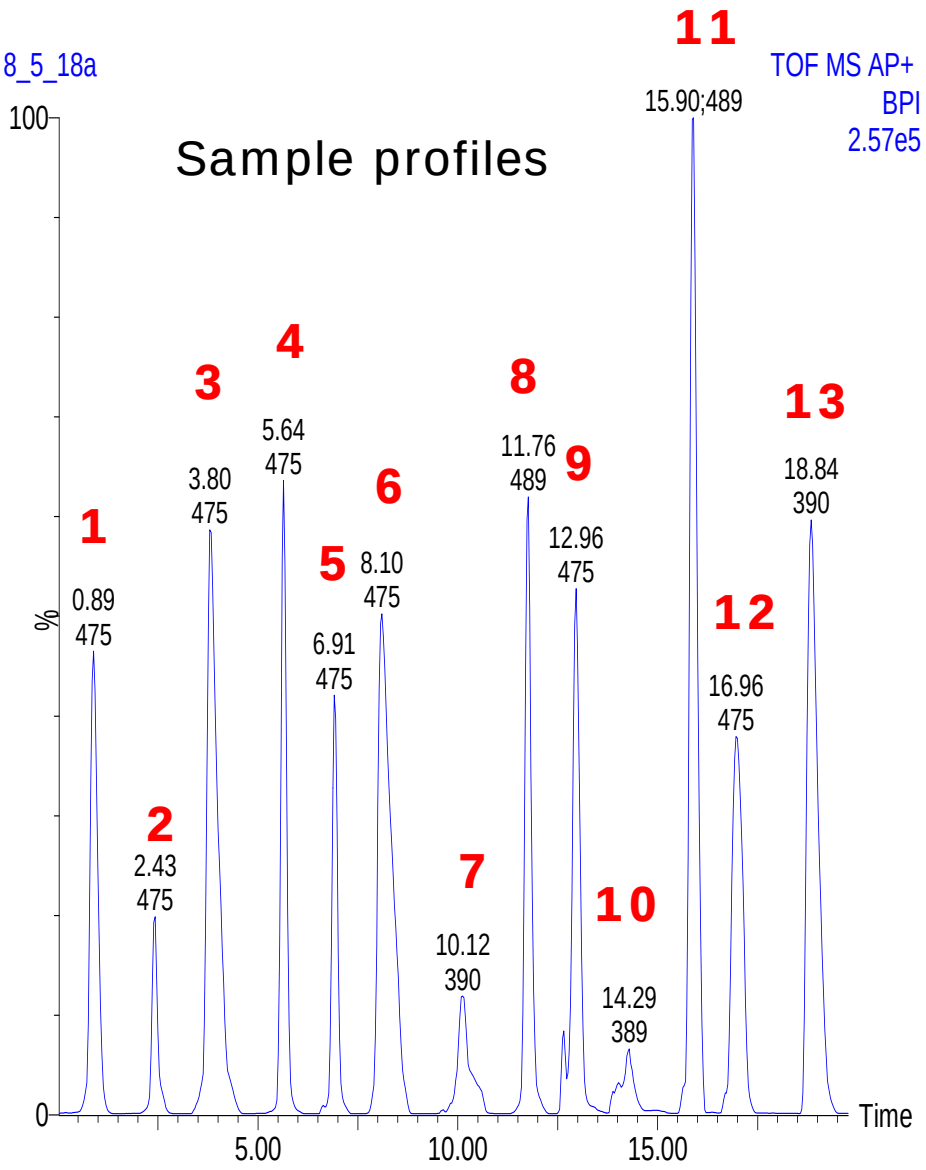
13 Tablet Samples with no sample extraction or chromatographic separation

1) Genuine Brand Sildenafil
2) Internet Pharmacy "Brand" Sildenafil
3) Internet Pharmacy A "Brand" Sildenafil
4) Internet Pharmacy A "Brand" Vardenafil
5) Internet Pharmacy A "Brand" Tadalafil
6) "Generic" Sildenafil
7) Genuine Brand Tadalafil
8) Genuine Brand Vardenafil
9) "Generic" Sildenafil
10) "Generic" Tadalafil
11) "Generic" Vardenafil
12) "Generic" Sildenafil
13) "Generic" Tadalafil



ASAP / TOF MS Data for Genuine Brand Samples and Counterfeit Internet Pharmacy Samples

Waters
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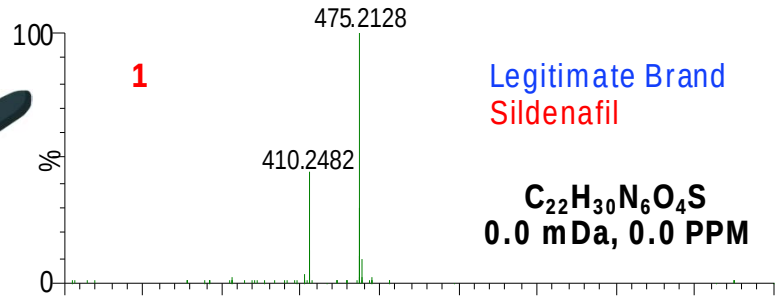
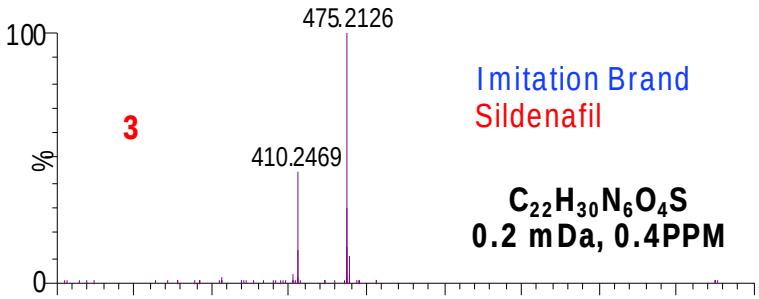


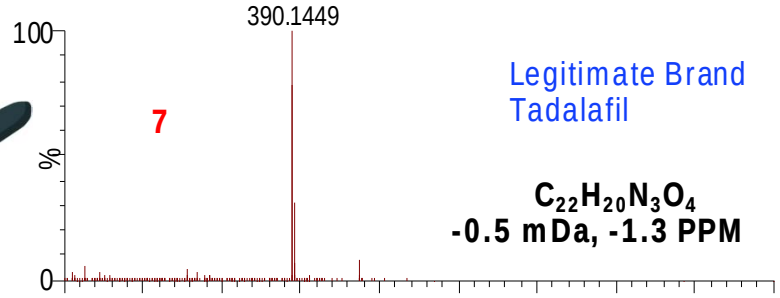
Legitimate Brand
Sildenafil

$C_{22}H_{30}N_6O_4S$
0.0 mDa, 0.0 PPM



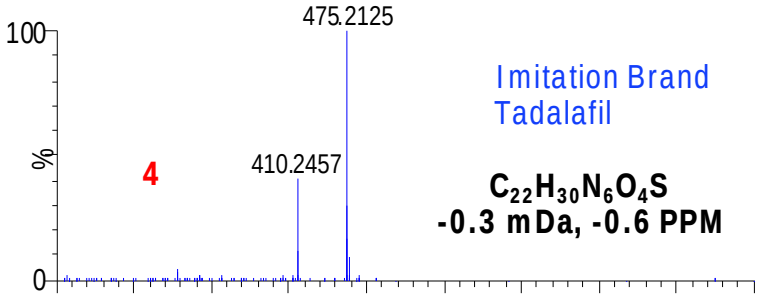
Imitation Brand
Sildenafil

$C_{22}H_{30}N_6O_4S$
0.2 mDa, 0.4PPM



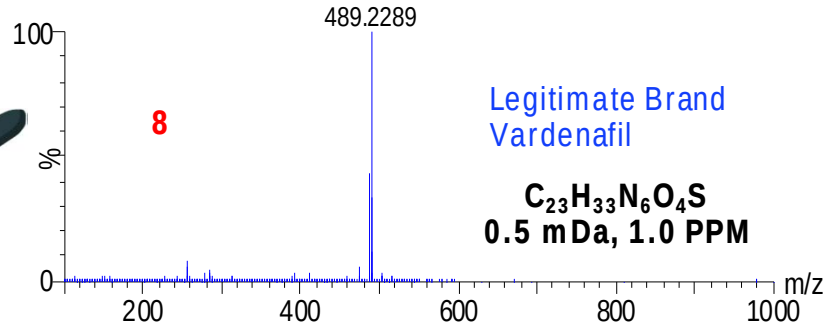
Legitimate Brand
Tadalafil

$C_{22}H_{20}N_3O_4$
-0.5 mDa, -1.3 PPM



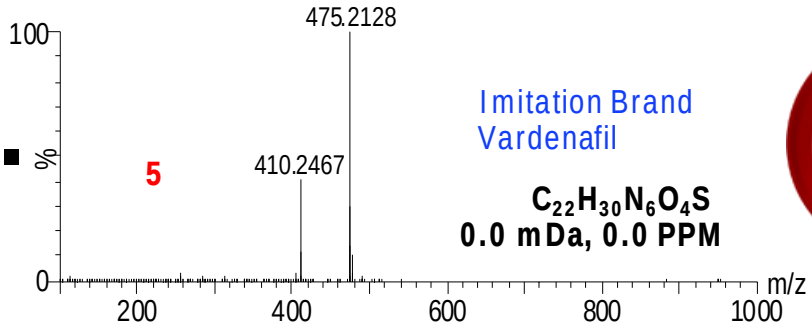
Imitation Brand
Tadalafil

$C_{22}H_{30}N_6O_4S$
-0.3 mDa, -0.6 PPM



Legitimate Brand
Vardenafil

$C_{23}H_{33}N_6O_4S$
0.5 mDa, 1.0 PPM



Imitation Brand
Vardenafil

$C_{22}H_{30}N_6O_4S$
0.0 mDa, 0.0 PPM

Genuine Medicine

Counterfeit Medicine

Applications

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Vitamins by APPI

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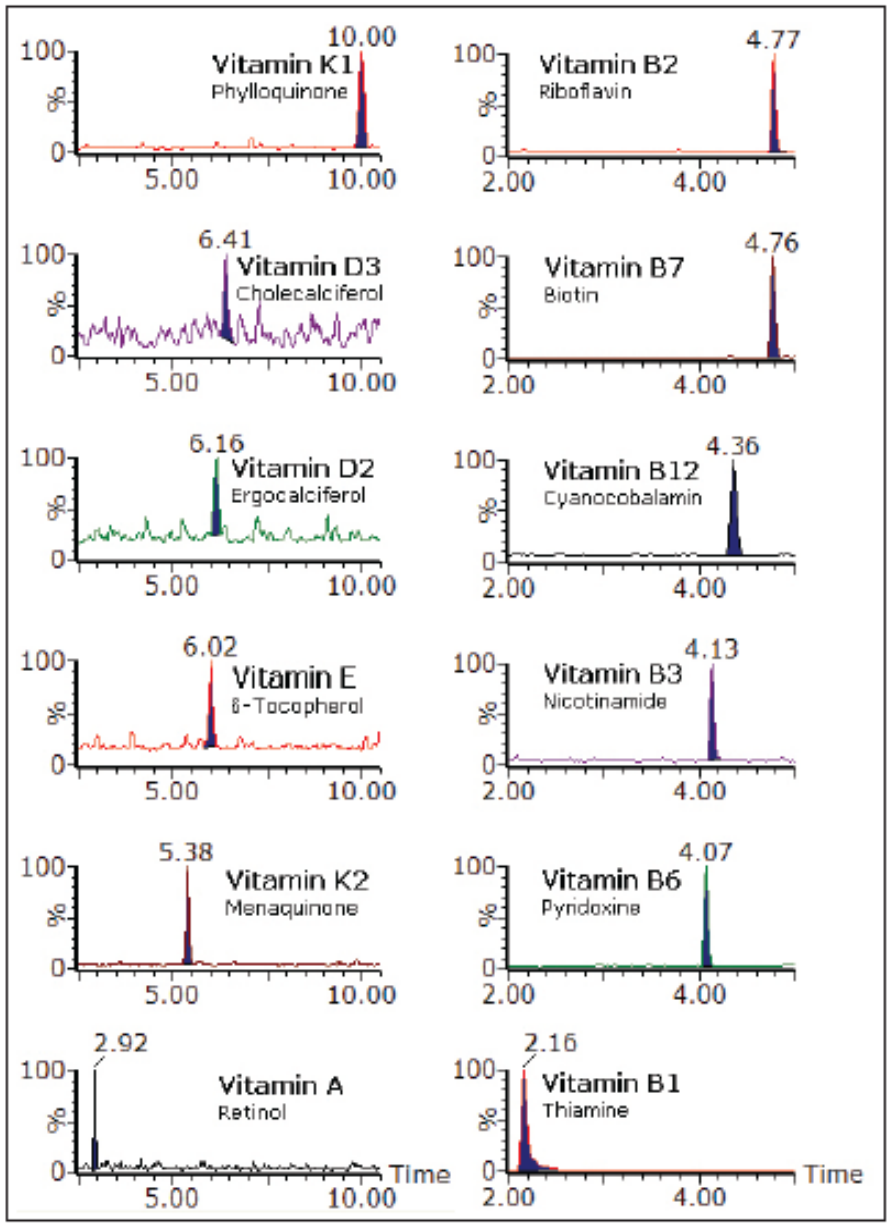
6 Water soluble

6 Fat soluble



Acidic Herbicides in Drinking Water - ppq

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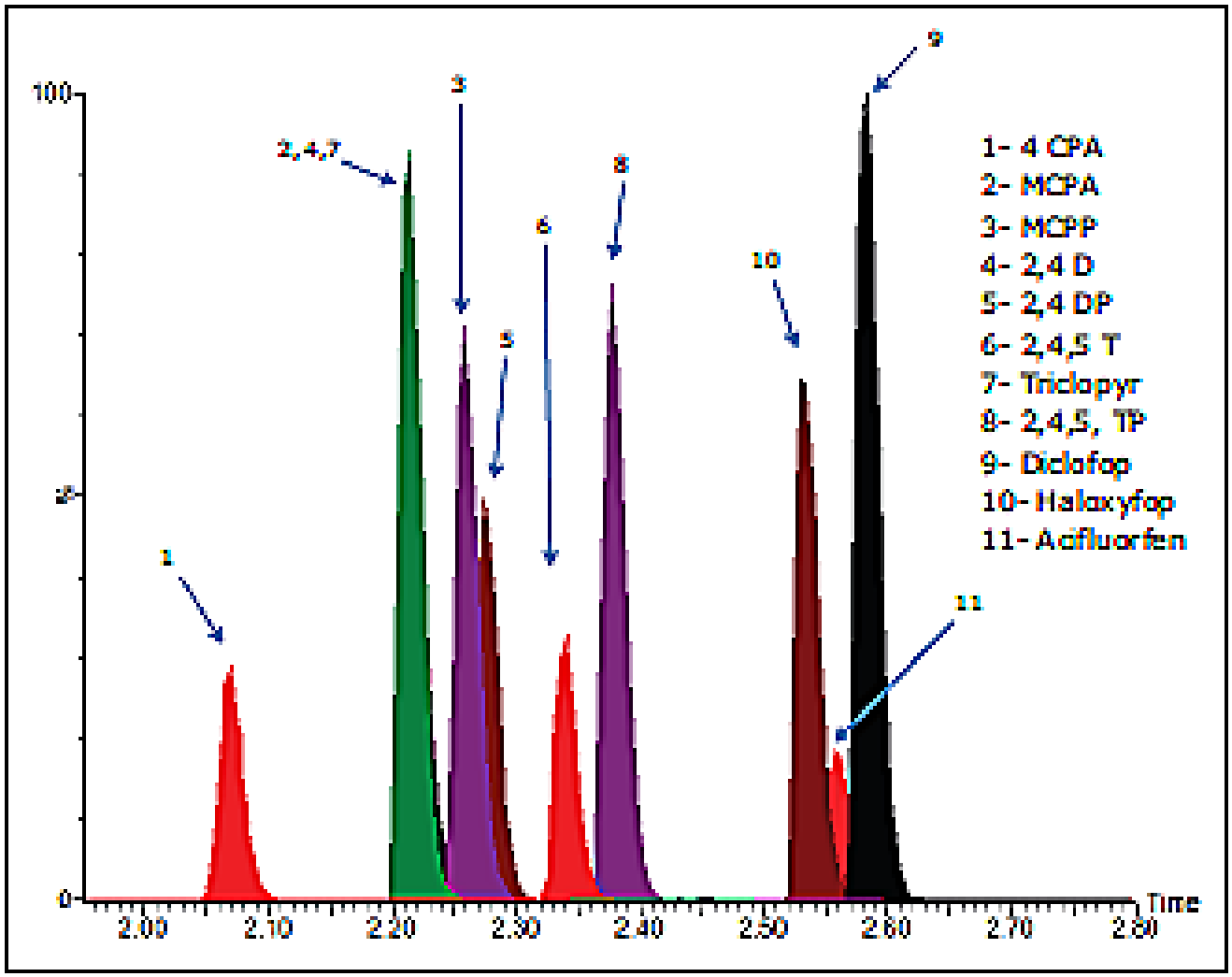

MRM chromatogram of phenoxyacetic acids standards in MilliQ water at 100 ppt.

Lipids by Class

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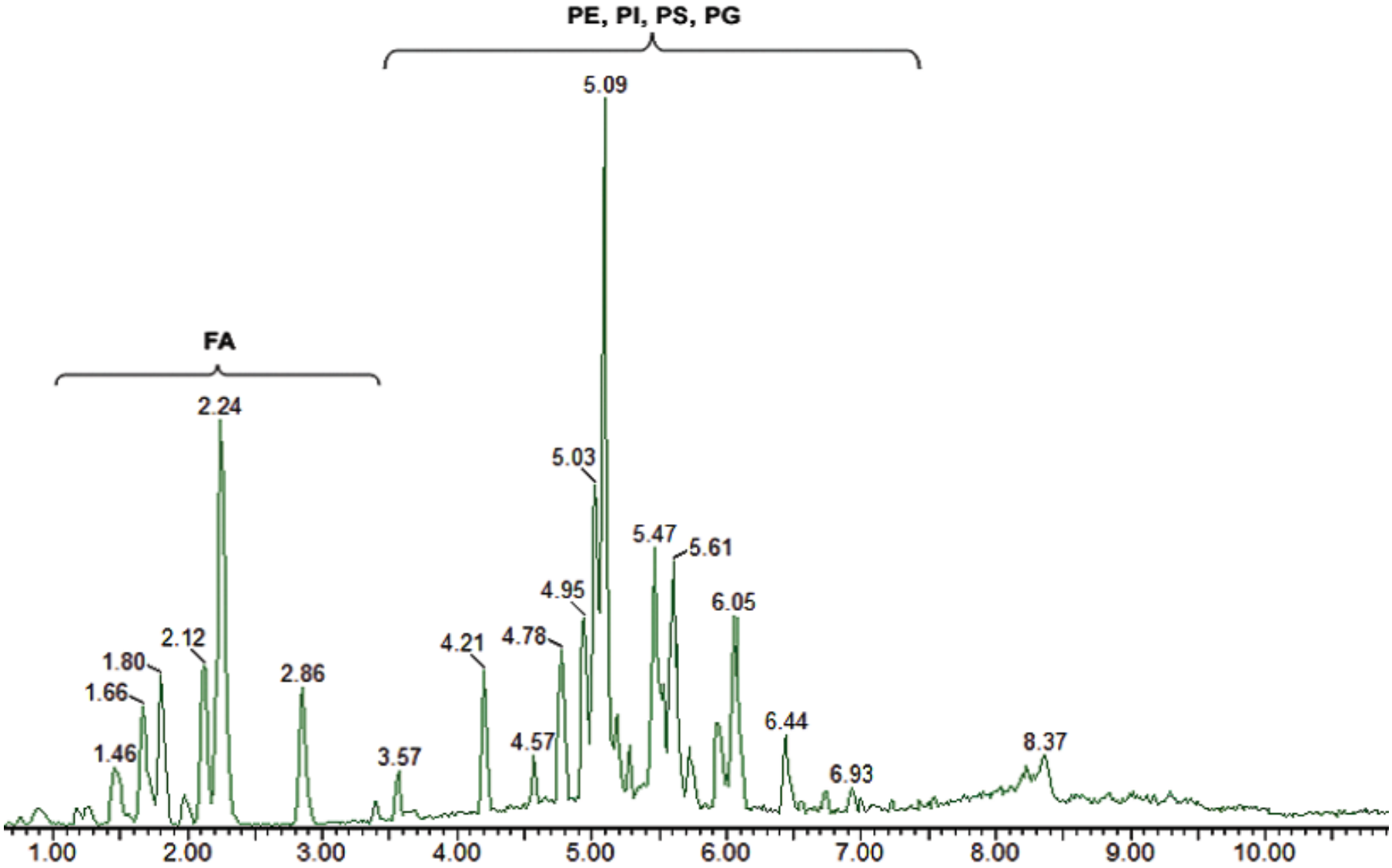

Collection of Whale Blow (snot)

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500 fg of Cortisol and Progesterone

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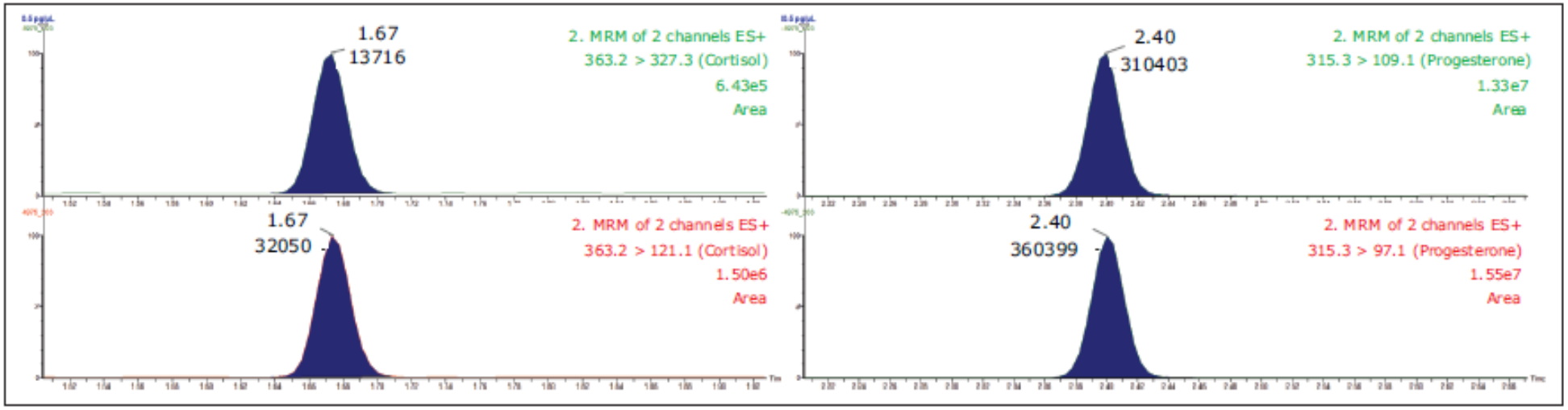
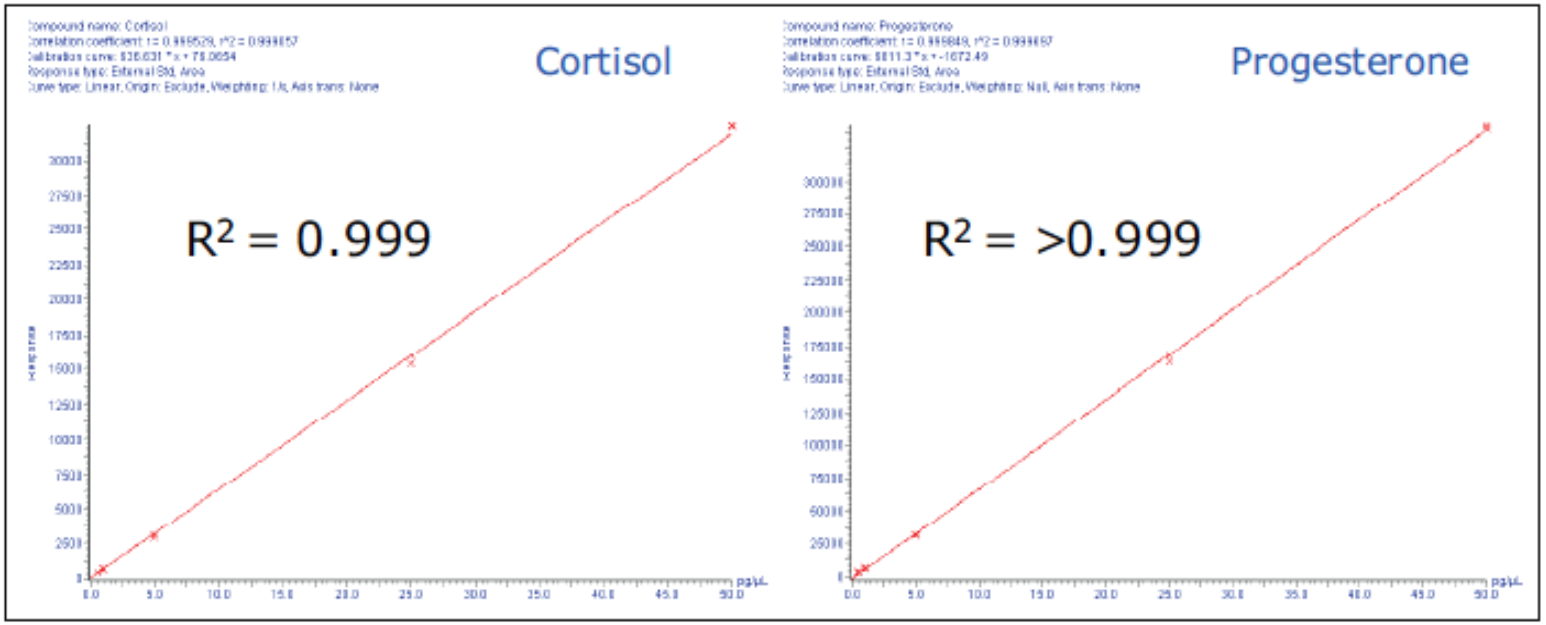


Chro



Analysis of 25 Blow Samples

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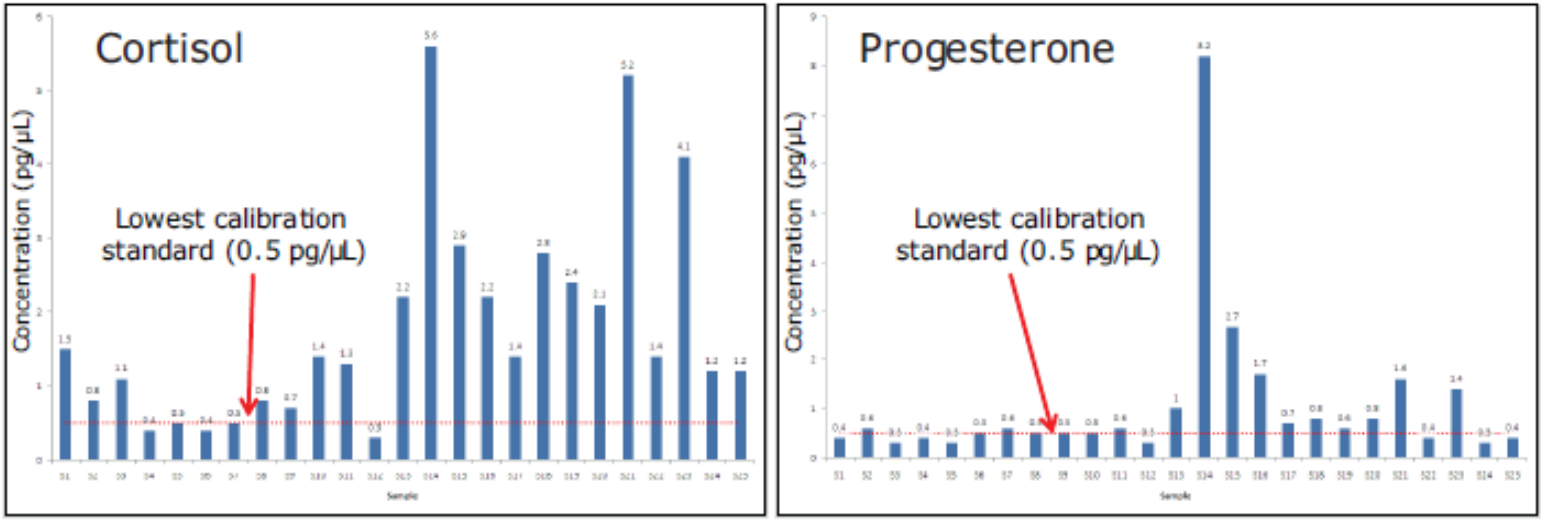
	Cortisol		Progesterone	
Sample code	Area	pg/µL	Area	pg/µL
4975_244	3429	5.4	36408	5.6
4975_245	3348	5.3	35529	5.5
4975_246	3332	5.3	35025	5.4
4975_247	3354	5.3	35474	5.5
4975_248	3290	5.2	35758	5.5
%RSD	1.5	%RSD	1.4	

Table 3. Repeatability data for cortisol and progesterone at 5 pg/uL.



Cortisol and progesterone levels in the whale blow samples.

Exact Mass
Accurate Mass

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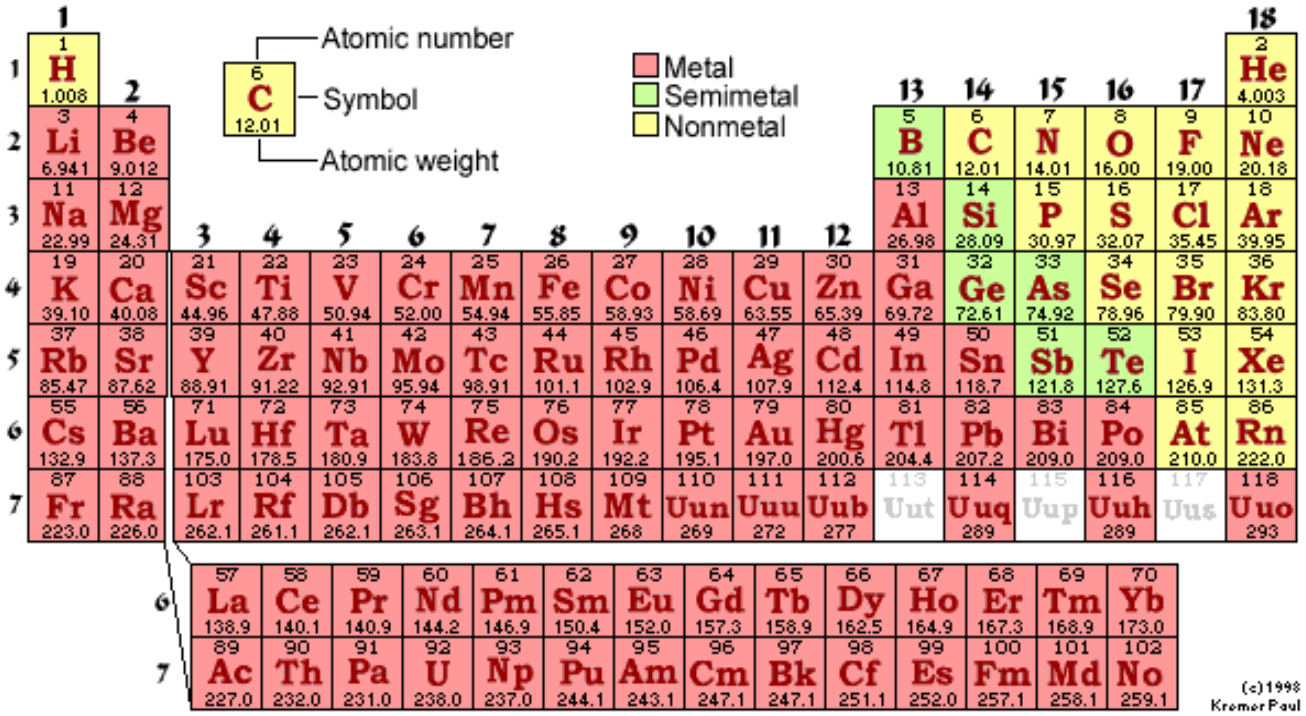
Introduction - Terminology



- Every element found in nature has a unique mass
- Elements are combined to produce compounds with distinct masses and physical properties
- Compounds can be detected by mass spectrometry and thus their masses measured
- If a compound mass can be measured with sufficient accuracy, a unique elemental composition can be inferred – the benefit of exact mass

The Fundamentals of Mass

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- carbon has a mass of 12
- hydrogen has a mass of 1
- oxygen has a mass of 16
- nitrogen has a mass of 14

But these are nominal (integer, whole) units

The Fundamentals of Mass

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- carbon has a mass of 12.0000
- hydrogen has a mass of 1.0078
- oxygen has a mass of 15.9949
- nitrogen has a mass of 14.0031

- It is possible to have combinations of atoms which have the same nominal (or integer) mass but different accurate mass
- If such compounds can be mass measured with sufficient accuracy it is possible to determine elemental composition

Simple Examples

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- CO = 27.9949
- N_2 = 28.0061
- C_2H_4 = 28.0313

- These elemental combinations have the same nominal mass but different exact mass.
- A nominal mass measurement cannot distinguish these.
- If any compounds differ in their elemental compositions by substitution of any of these elements, then the exact mass measurement will show this.

Isotopes

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- Most elements have more than one stable isotope.
  - For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da (^{13}C).
- Why do we care?
  - Mass spectrometers can register isotope peaks if their resolution is high enough.
  - If a instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

Courtesy of Sandler Mass Spectrometry Users' Group

Exact Mass and Isotopic Abundance of Common Elements

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Element		Nominal Nuclide	Exact Mass	Mass Defect	Isotopic Abundance
Hydrogen	H	1	1.0078	0.00783	100.00%
	D	2	2.0141	0.0141	0.02%
Carbon	C^{12}	12	12.0000	0	100.00%
	C^{13}	13	13.0034	0.00336	1.10%
Nitrogen	N^{14}	14	14.0031	0.003074	100.00%
	N^{15}	15	15.0001	0.0001	0.37%
Oxygen	O^{16}	16	15.9949	-0.0051	100.00%
	O^{17}	17	16.9991	-0.0009	0.04%
	O^{18}	18	17.9992	-0.0008	0.20%
Fluorine	F^{19}	19	18.9984	-0.0016	100.00%
Phosphorus	P^{31}	31	30.9738	-0.0262	100.00%

Critical Needs for Accurate Mass

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CONFIDENT COMPOUND ID

DIFFERENT COMPOUND SELECTIVITY

COMPLETE LACK OF ANALYTICAL STANDARDS

MEASUREMENT OF COLLISIONAL CROSS SECTIONS

GAS PHASE CONFORMATIONAL STUDIES

POLYMER STUDIES

LARGE MASS STUDIES

PROTEOMICS

METABOLOMICS

METABONOMICS

LIPIDOMICS

mAb QUALITY CONTROL

Thanks for your attention.

Questions?