LCMS Technology

Connects to Your Application

Dr.Rittichai Charoensapyanan

(LCMS Product Specialist)

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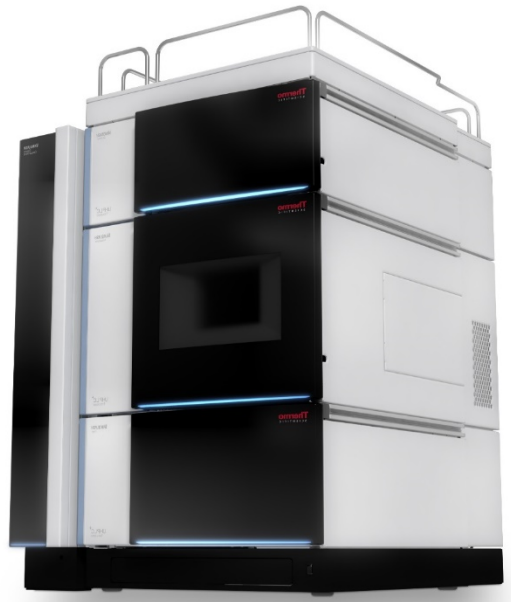
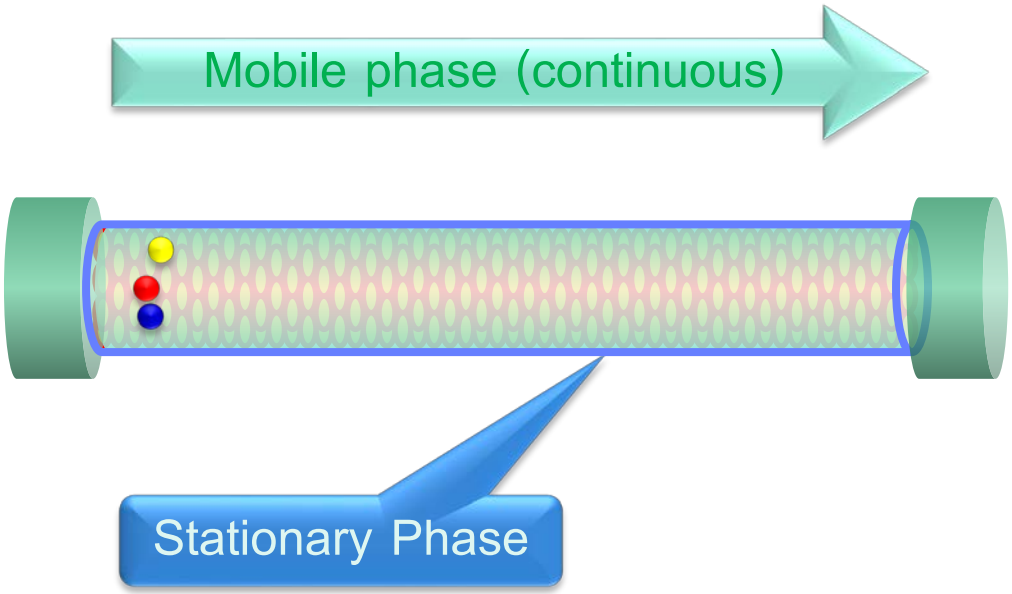
Topics

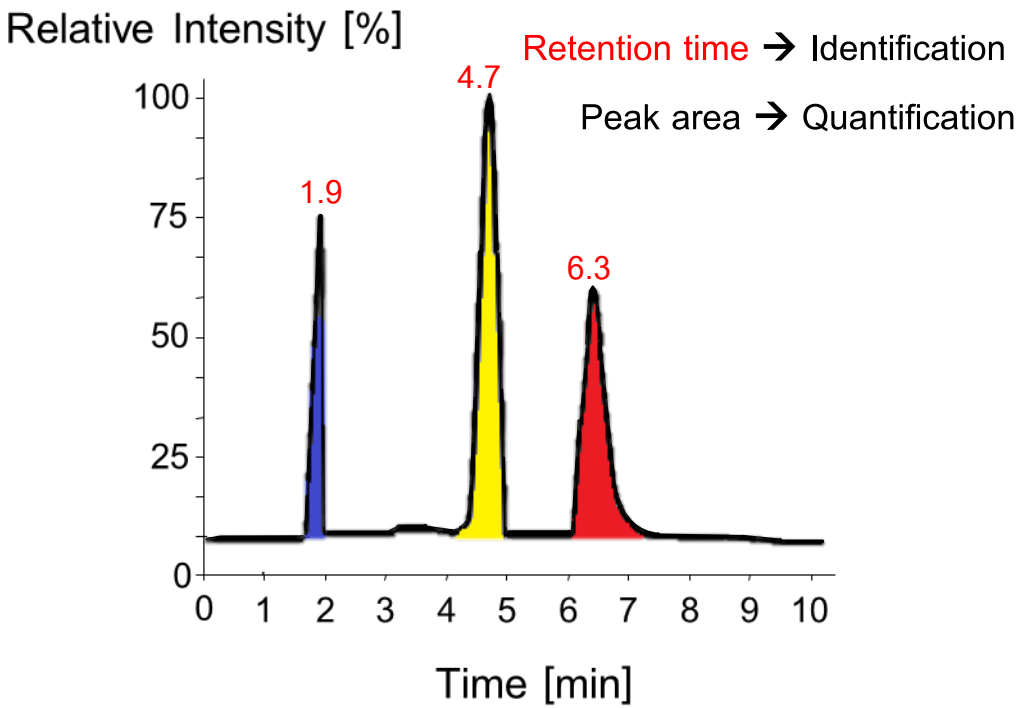
- Fundamental of Liquid Chromatography (LC)
- Fundamental of Mass Spectrometer (MS)
- LCMS Applications

Fundamental of Liquid Chromatography



Liquid Chromatography (LC)





- **Liquid Chromatography (LC)** : Separation technique which liquid is used as mobile phase
- **Separation** : Between two phases (Stationary phase and Mobile phase)
- Compounds are separated from each other based on their difference in affinity for the stationary or mobile phase.

HPLC System

- **Degasser :** Remove air bubble in solvents
- **Pump :** - Mix solvents
  - Control the flow rate of mobile phase and analytes
- **Autosampler :** Inject the sample into a running system
- **Column :** Separate each components
- **Column Compartment :** Control a column temperature
- **Detector :** Detect signal from analytes after separation

DAD (UV, VIS)

Fluorescence

Reflective Index

Mass Spectrometer

HPLC System Range

Basic Automated

- Highly economic & reliable
- 620 bar UHPLC compatible
- Flow rates up to 10 mL/min
- 100 Hz detector range
- Modular flexibility

Standard

- 3rd Generation Modules
- 620 bar UHPLC compatible
- Flow rates up to 10 mL/min
- Oven temp. 5 – 80 °C
- 100 Hz DAD, MWD, VWD, FLD, CAD
- Highest flexibility

x2 Dual LC

- Two systems in one
- 620 bar UHPLC compatible
- Flow rates up to 10 mL/min
- Oven temp. 5 – 80 °C
- Automated Application Switching
- Parallel and Tandem LC
- Online SPE-LC
- Automated method scouting
- Turn key Viper kits for ease of use

RSLC

- Binary and Quaternary UHPLCs
- 1000 bar up to 5 mL/min
- 800 bar up to 8 mL/min
- Oven temp. 5 – 110 °C
- 200 Hz DAD, MWD, VWD, FLD
- Improved sub 2-µm particle column compatibility
- Ultrafast/ultra resolution system

x2 Dual RSLC

- x2 Dual UHPLC System
- Two systems in one
- 1000 bar up to 5 mL/min
- 800 bar up to 8 mL/min
- Oven temp. 5 – 110 °C
- 200 Hz DAD, MWD, VWD, FLD
- Parallel and Tandem LC
- Online SPE-LC
- Automated method scouting
- Offline 2D-UHPLC
- Turn key Viper kits for ease of use

RSLCnano

- UHPLC system for Nano/Cap/Micro
- 20 nL/min – 50 µL/min up to 800 bar
- Continuous direct flow
- New standard in retention time precision
- Snap-in valves
- nanoViper fitting system for easy operation

Basic → Standard → x2 Dual LC → RSLC → x2 Dual RSLC → RSLCnano

The Highest Pressure HPLC

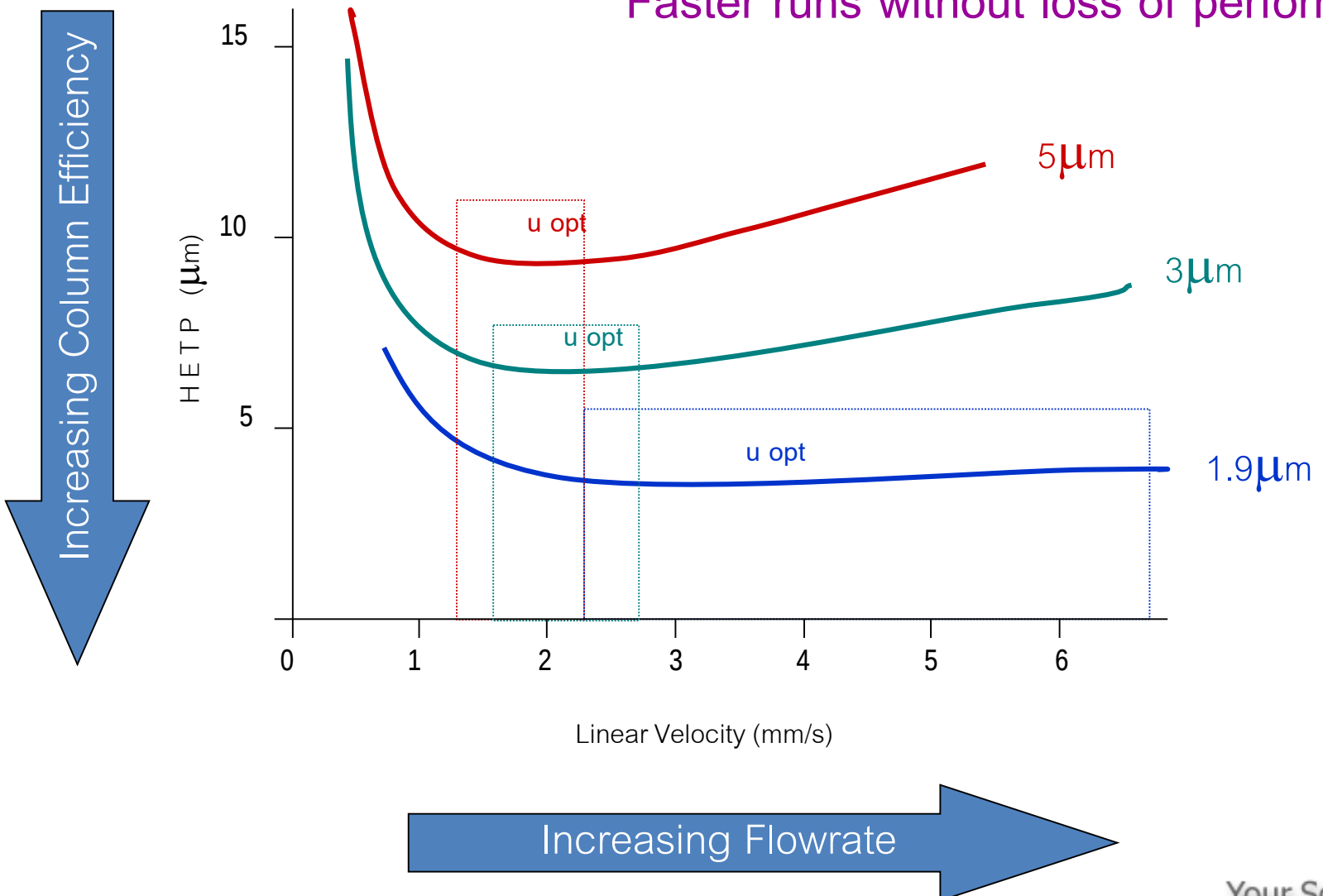
Vanquish™

Max Pressure 1517 bar

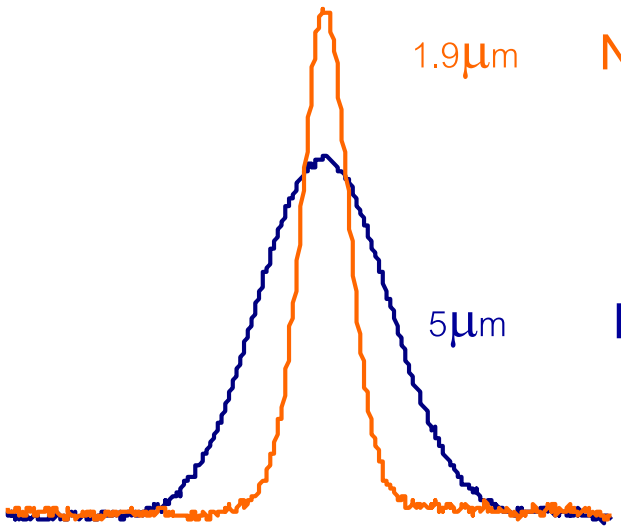
Advantage of Small Particle

Higher efficiency, independent of flow rate means…

Faster runs without loss of performance



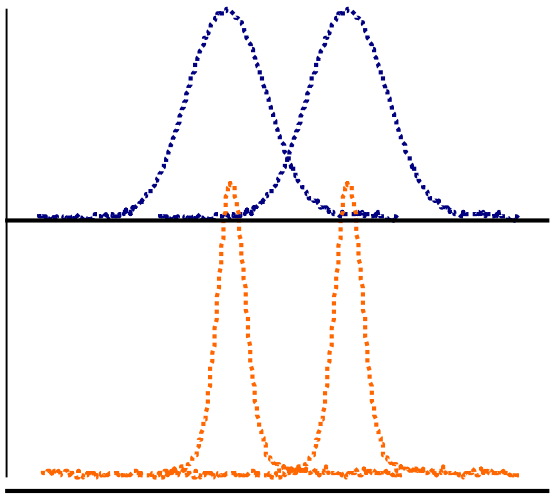
Advantage of Small Particle



1.9µm N = 142,000 plates/m

(189% higher)

5µm N = 75,000 plates /m



$$R_s = \frac{1}{4}\,\frac{(\alpha-1)}{\alpha}\,\sqrt{N}\,\frac{k}{1+k}$$

Selectivity Efficiency Retention

Efficiency is the key!!!

Higher resolution – narrower peaks

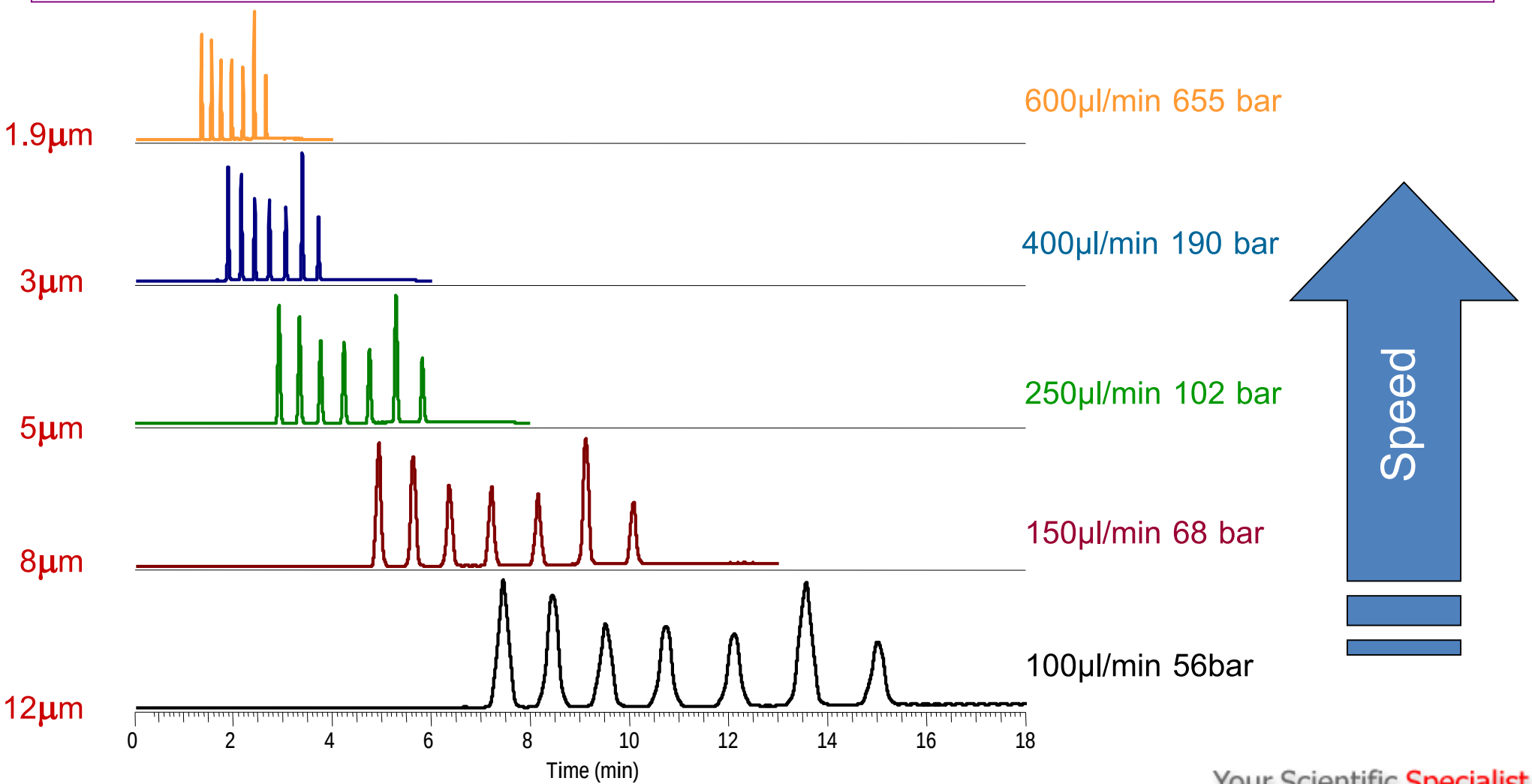
Higher sensitivity – taller peaks

Higher peak capacity (more peaks / unit time) – narrower peaks

Advantage of Small Particle

Increase Speed, Maintain Resolution *200x2.1mm*

Speeding up analysis with 1.9 µm Hypersil GOLD



The UltiMate™ 3000 HPLC Systems

Pump


Isocratic


Binary


Quaternary


Dual-Gradient

Autosampler


Basic Automated


Standard


Thermostatted


+ Fractionation

Column Compartment


Standard


With Valves

Detector


VWD


MWD/DAD

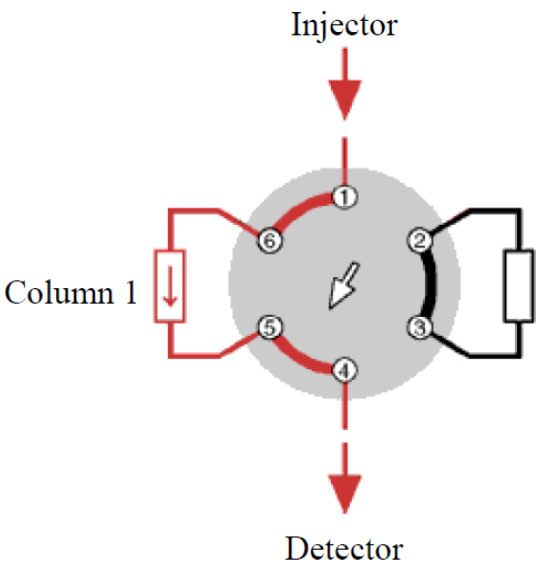

Fluorescence

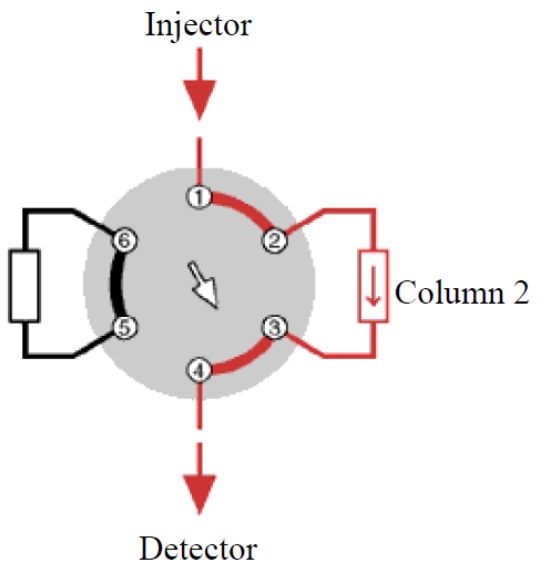
Corona


Coulochem

HPLC Applications

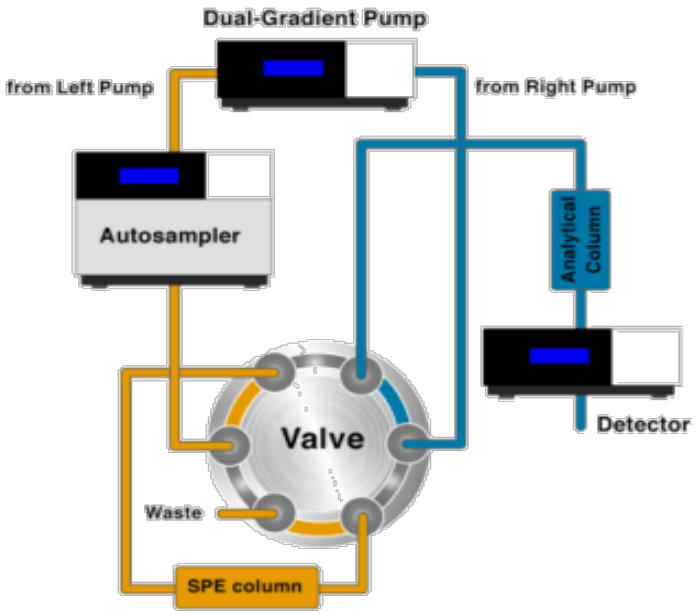
- Built-in column compartment with 2-position, 6-port switching valve



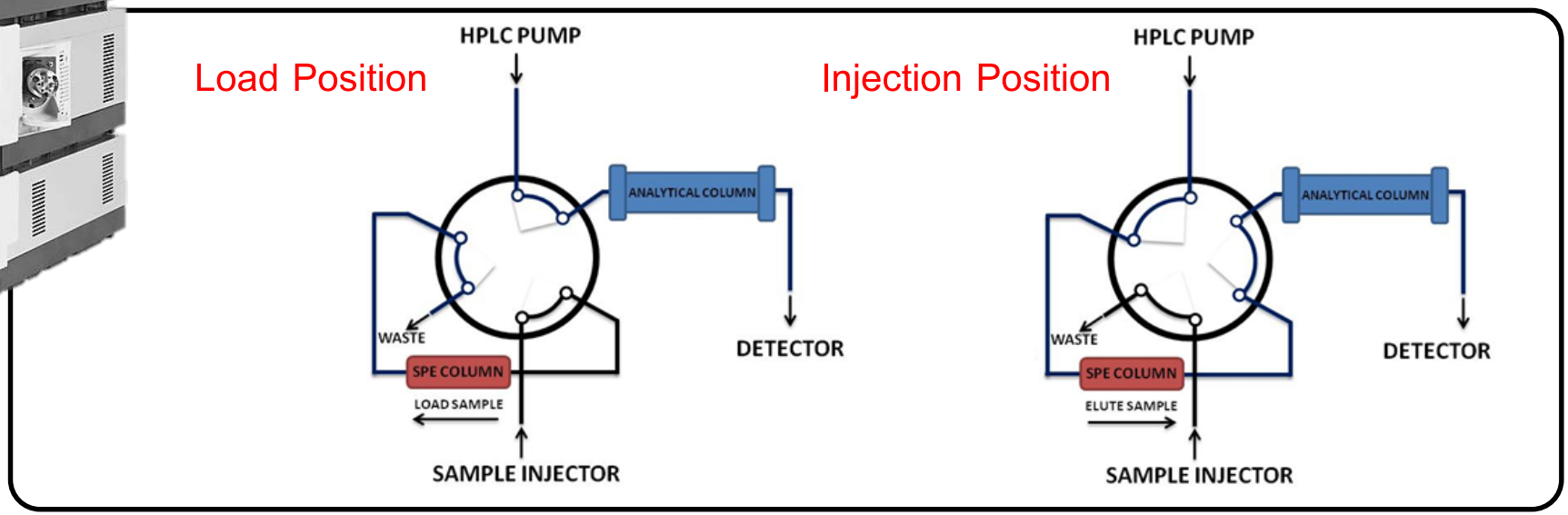


Switching Valve (2-position, 6-port)

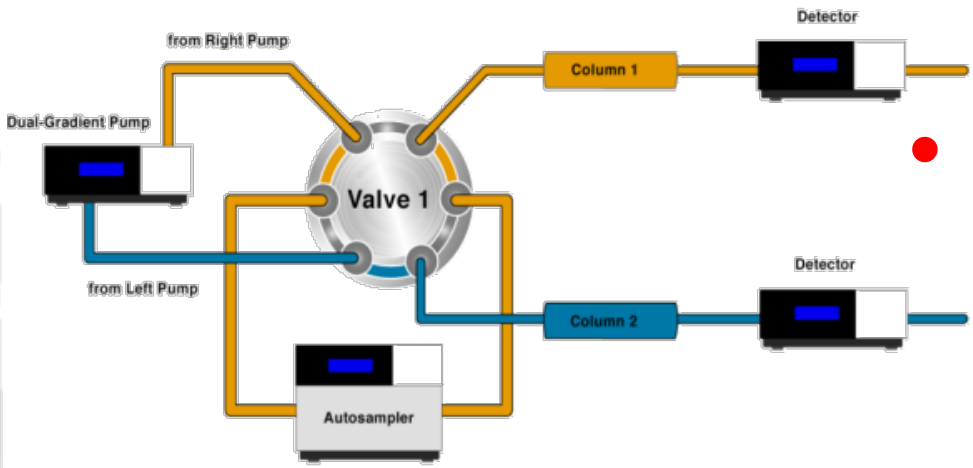
HPLC Applications



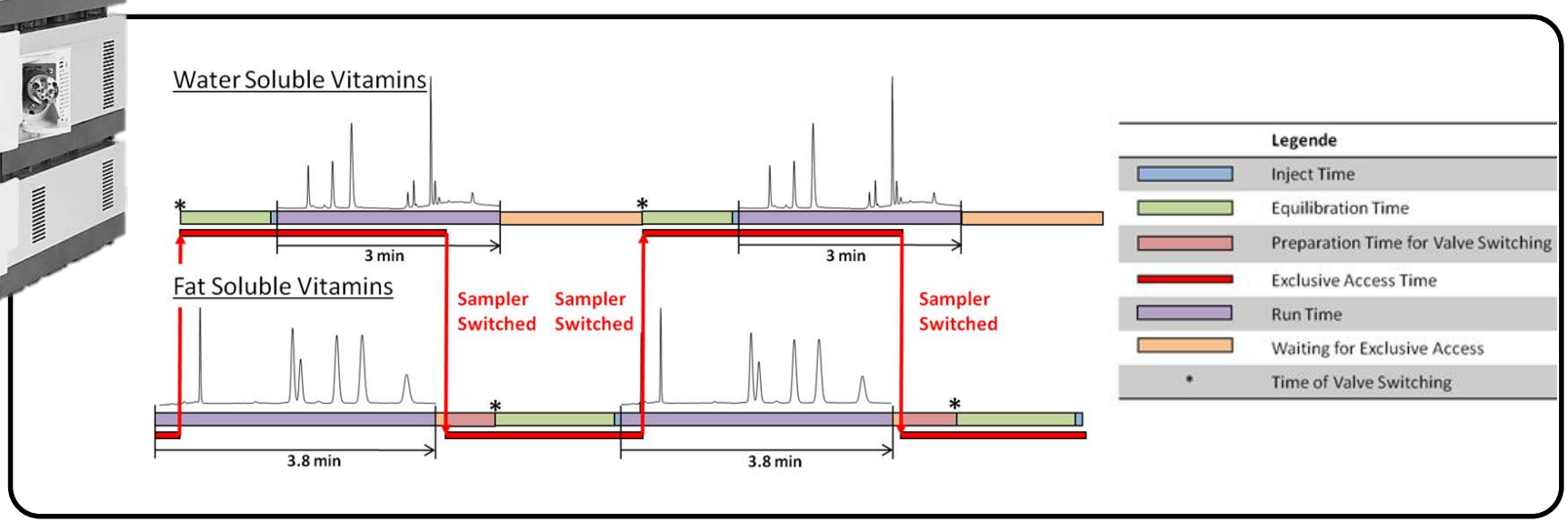
- **Online SPE:** Extraction and separation at the same time !!!



HPLC Applications



- **Parallel LC:** Analysis of two distinct methods !!!



Fundamental of Mass Spectrometer

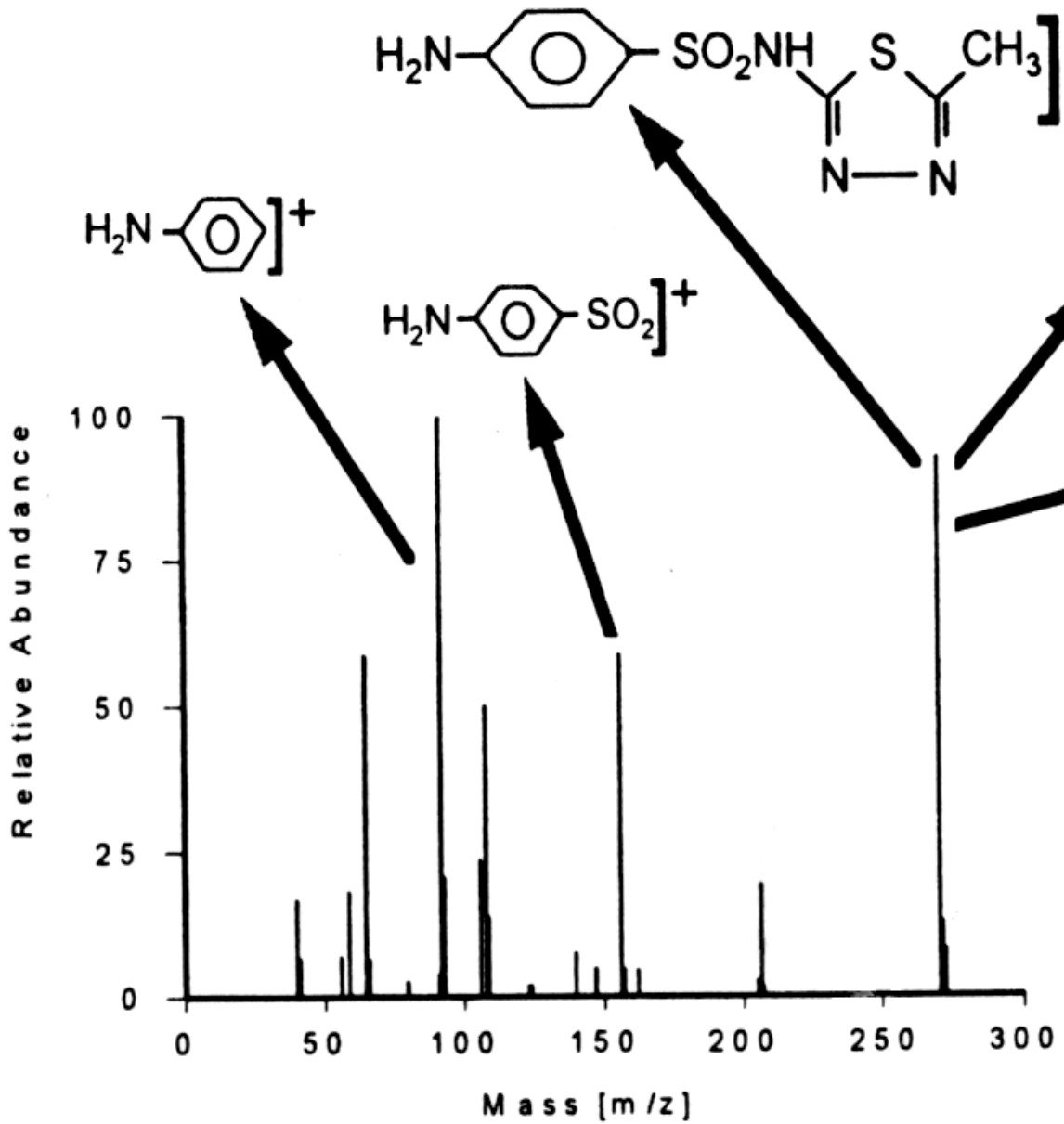
What is Mass Spectrometer?

"The basis in mass spectrometry (MS) is the production of ions, that are subsequently separated or filtered according to their **mass-to-charge (m/z) ratio**, and detected. The resulting mass spectrum is a plot of the (relative) abundance of the produced ions as a function of the m/z ratio."

- Measure gas-phase ions
- Operate at very low pressure (10^{-5} to 10^{-7} torr)
- Mass spectrometer work with **IONS**
- Determine the mass are separated according to their **mass-to-charge (m/z) ratio**

Niessen *et al.*, *LC-MS: Principles and Applications*, 1992, Marcel Dekker, Inc., New York, p. 29.

Information Rich Data

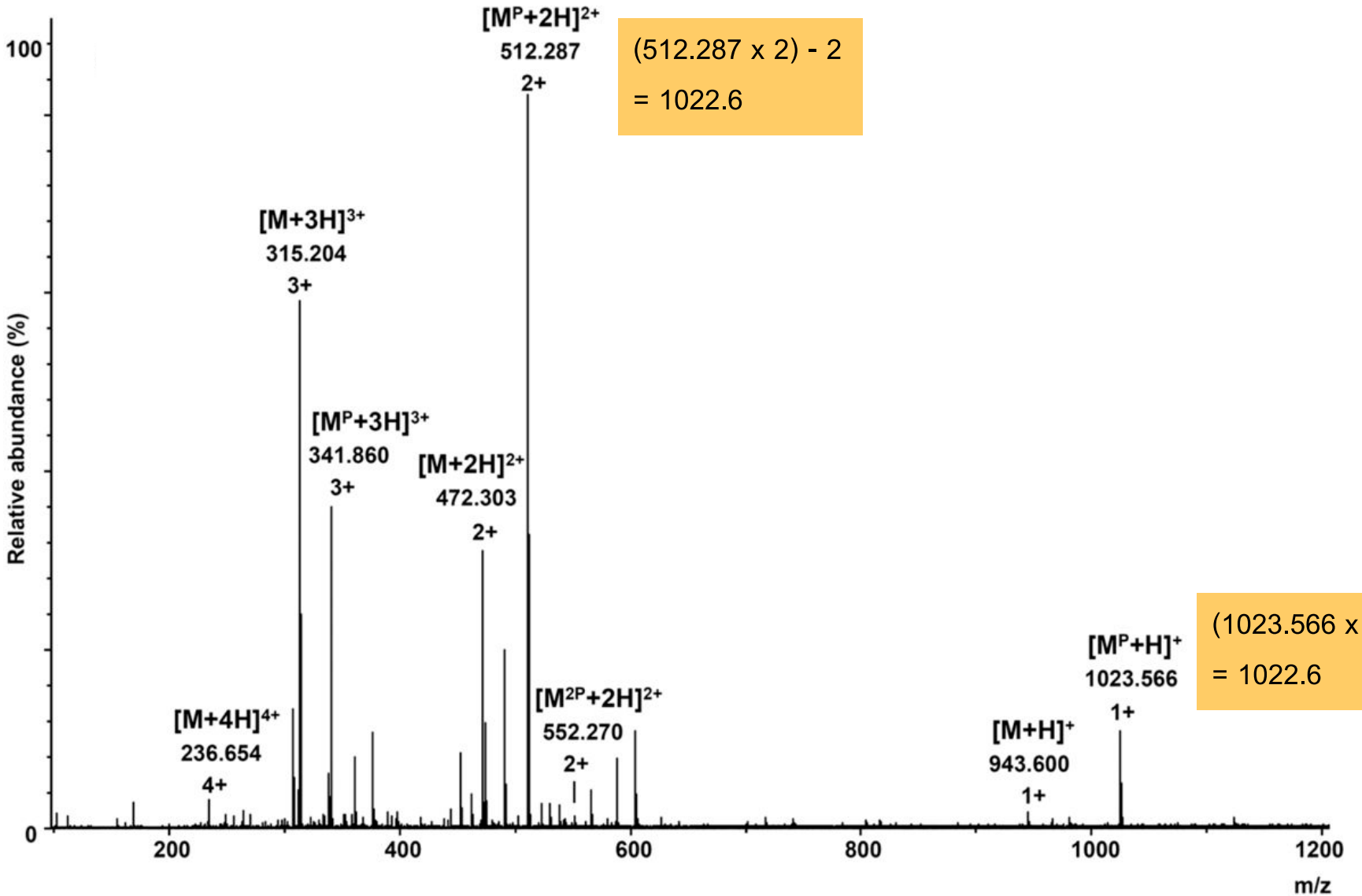


Compound: Sulfamethizole
MW: 270
Empirical Formula:
$C_9H_{10}N_4O_2S_2$

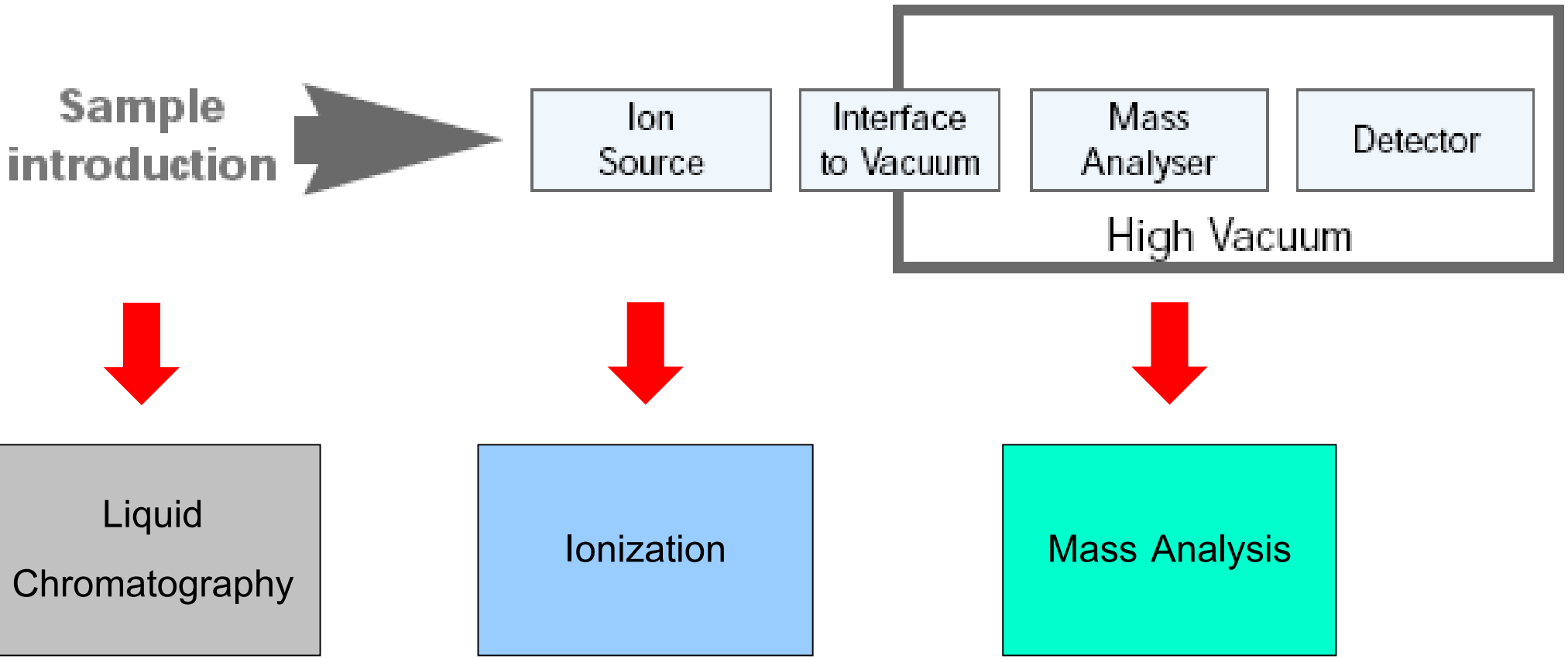
- *Molecular Weight*
- *Elemental Composition*
- *Empirical Formula*
- *Molecular Structure*
- *Unambiguous Identification*
- *Mass Specific Response*

Mass Spectrum

Mass to charge (m/z) = (molecular weight + charge) / charge

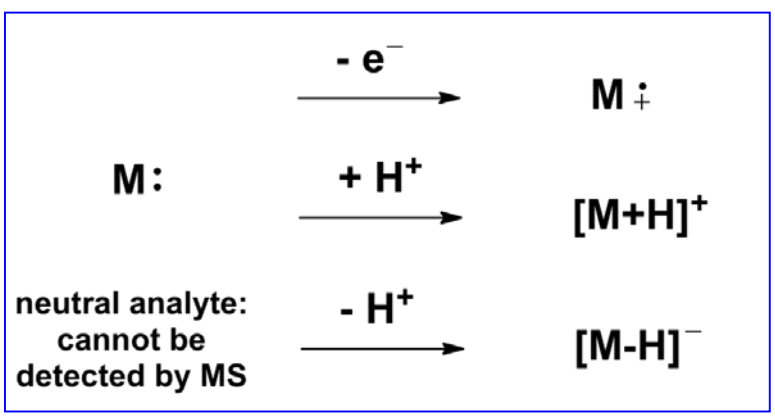


Mass Spectrometry: Block Diagram



Ionization

- **Ion source :** Converts sample molecules (neutral) into charged molecules or molecular ions.
- Type of ionization techniques
  - Matrix Assisted Laser Desorption Ionization (MALDI)
  - Atmospheric Pressure Ionization (API)
    - Electrospray Ionization (ESI)
    - Atmospheric Pressure Chemical Ionization (APCI)

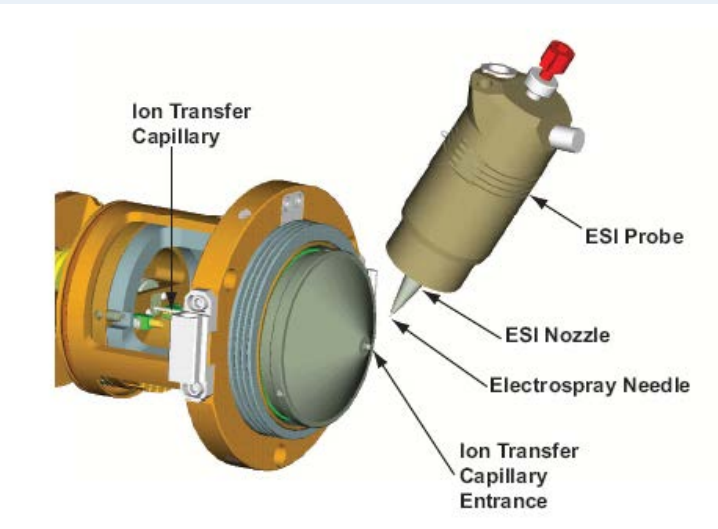


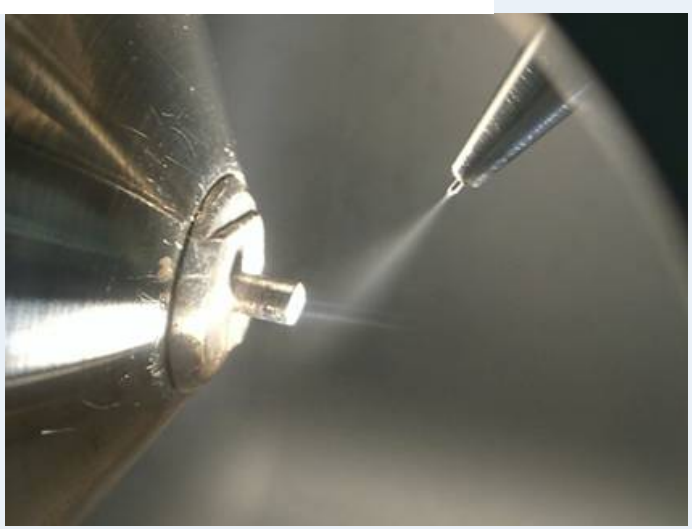
Ion Source

No one ionization technique is applicable to all classes of chemical species !

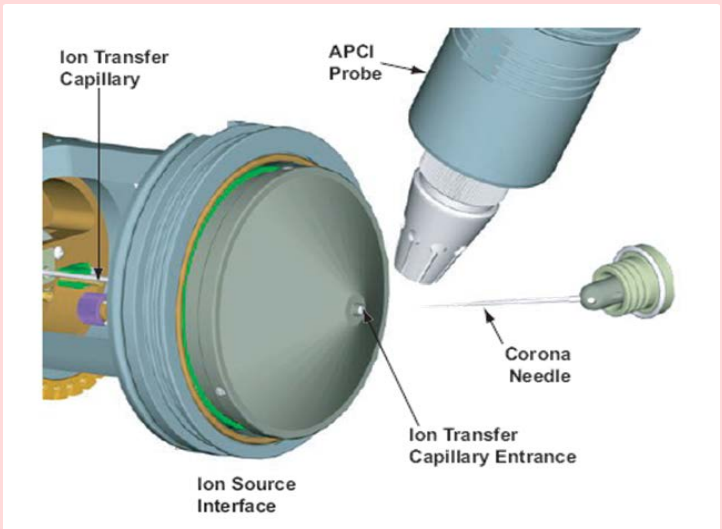
Atmospheric Pressure Ionization (API)

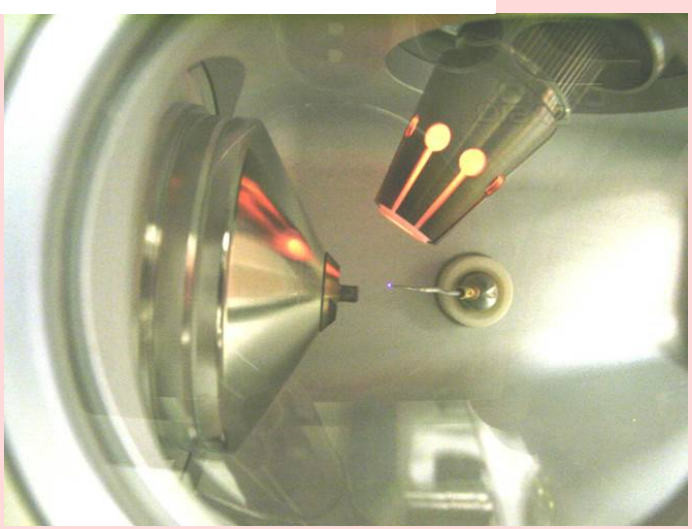
ESI





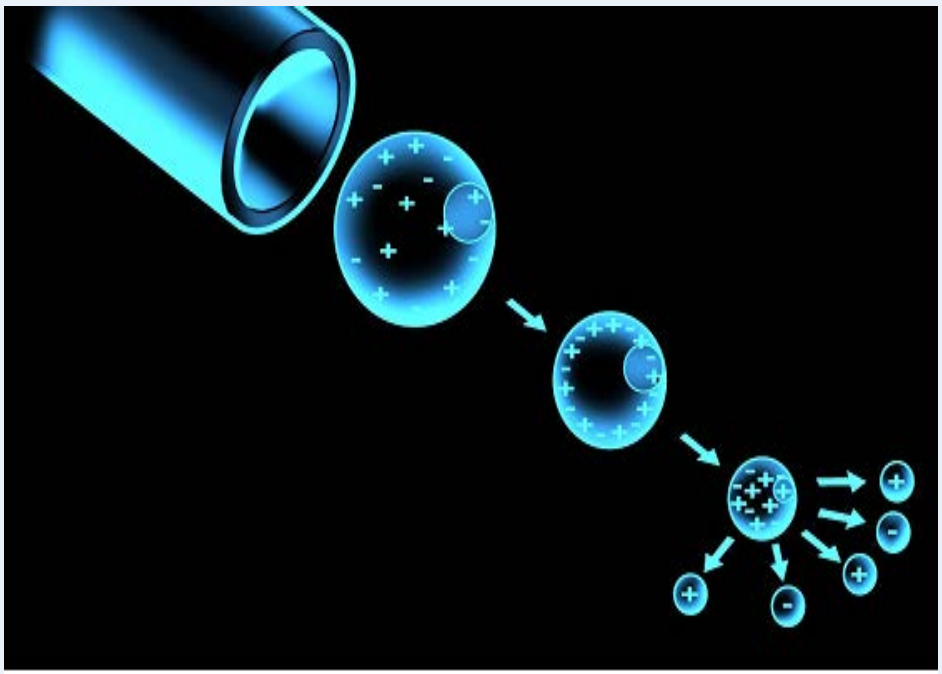
APCI





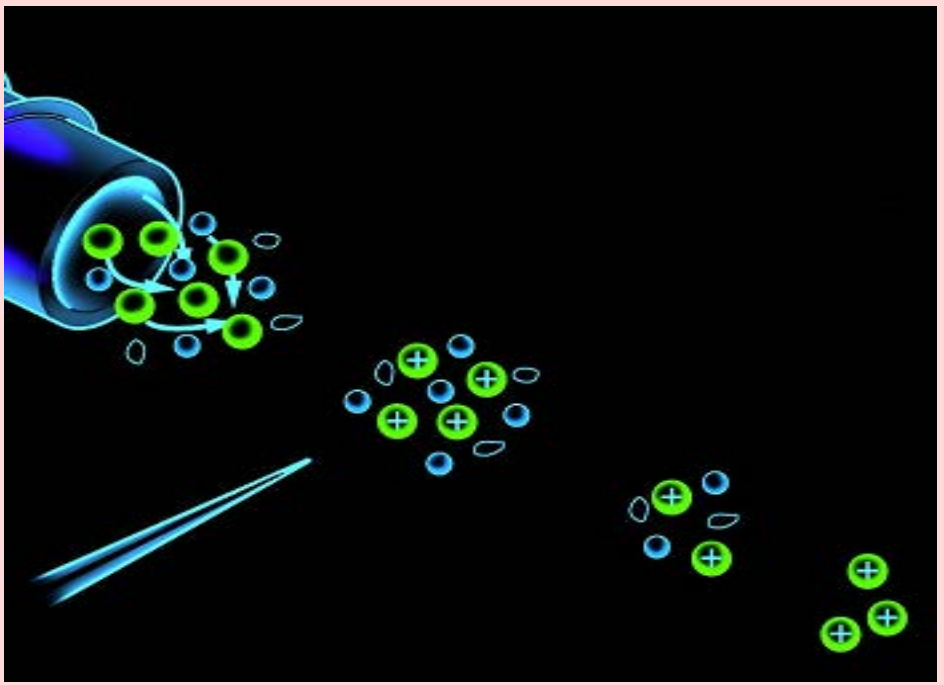
Atmospheric Pressure Ionization (API)

ESI



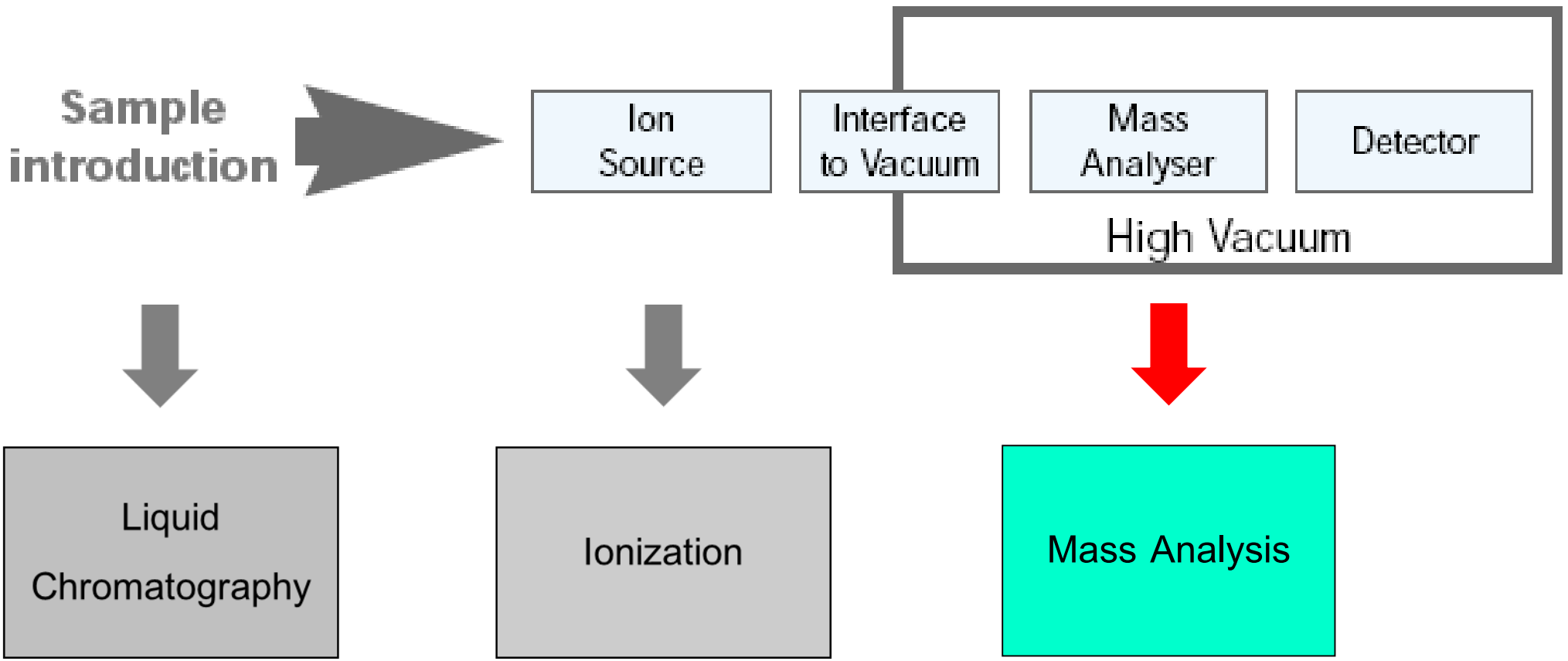
- Ions formed by solution chemistry
- Good for thermally labile analytes
- Good for polar analytes
- Good for large molecules (protein/peptide)

APCI



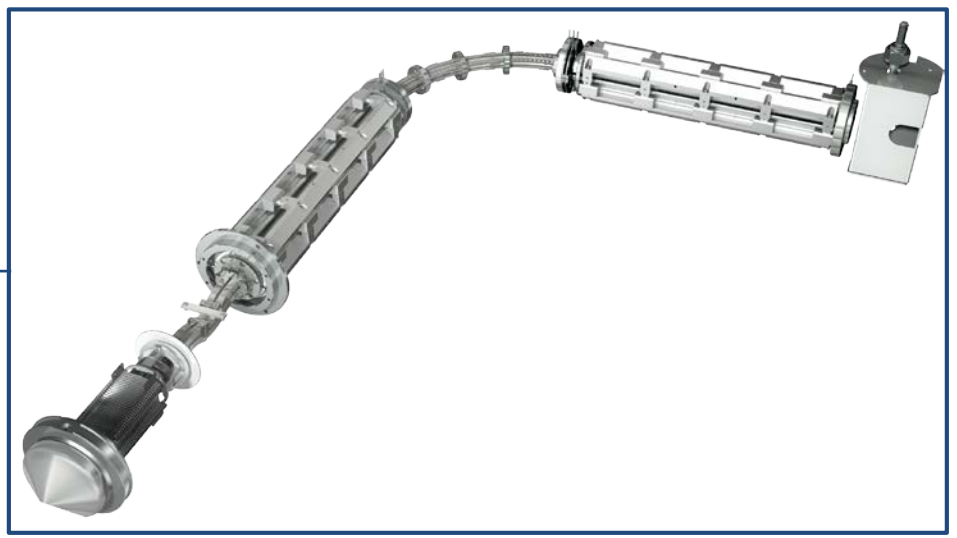
- Ions formed by gas phase chemistry
- Good for volatile / thermally stable
- Good for non-polar analytes
- Good for small molecules (steroids)

Mass Spectrometry: Block Diagram



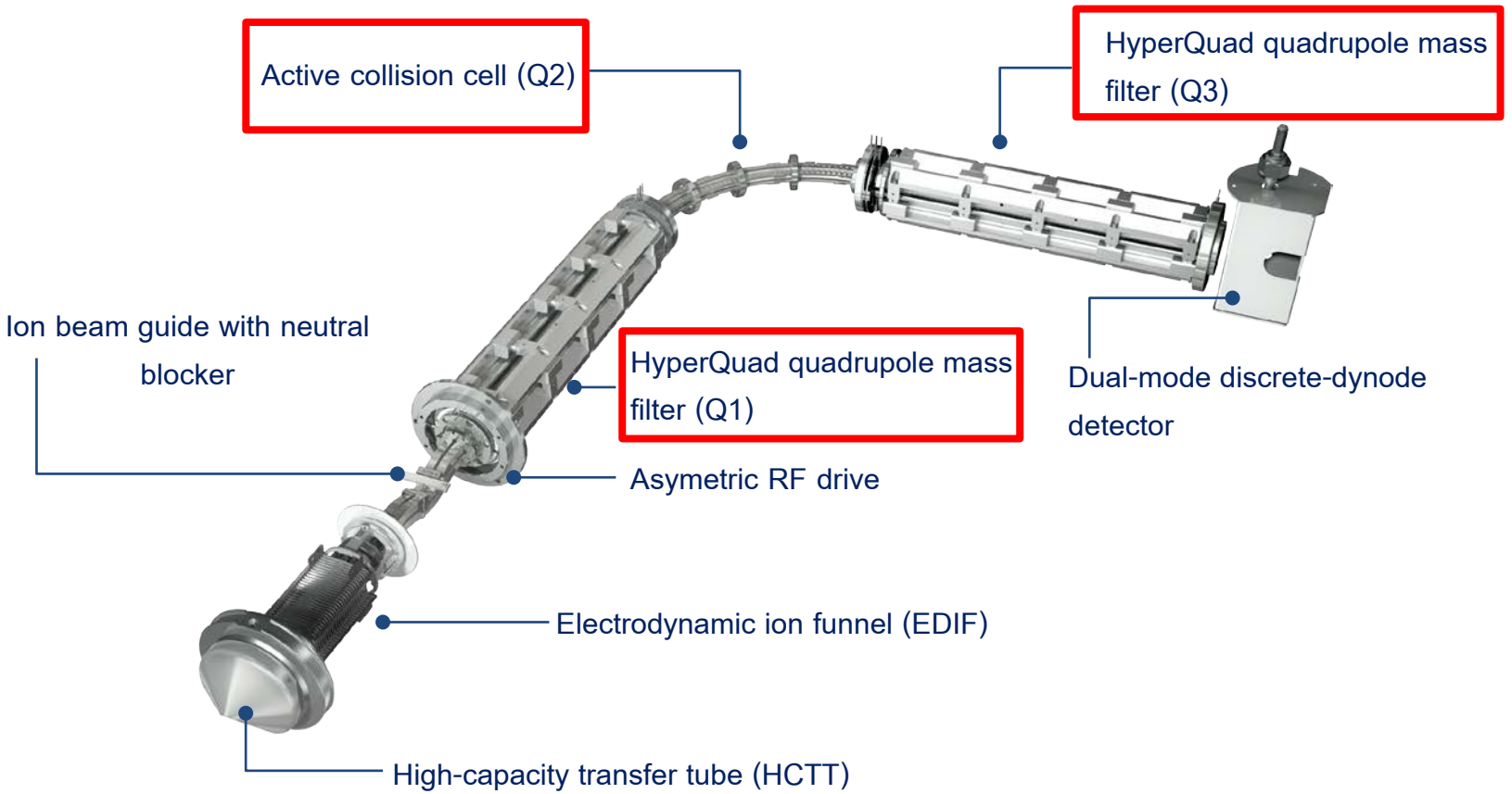
Mass Analyzer

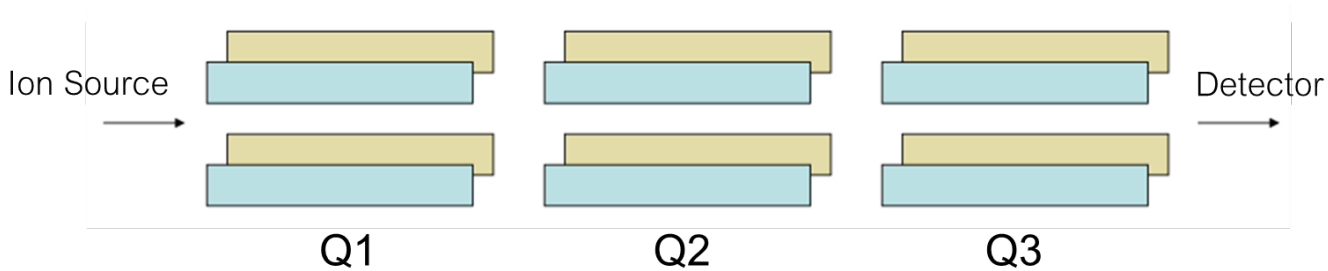
- Triple Quadrupole (QqQ)



- Orbitrap

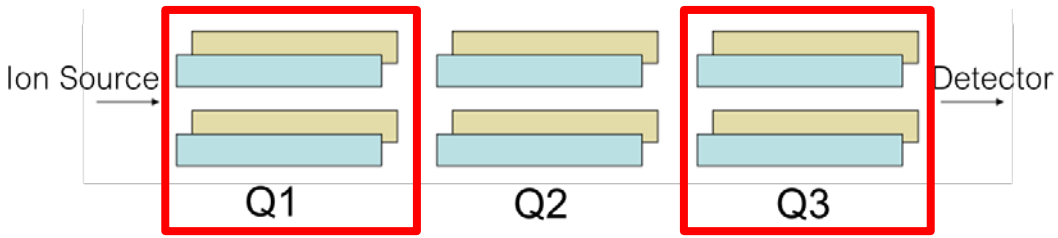
Mass Analyzer: Triple Quadrupoles (QqQ)

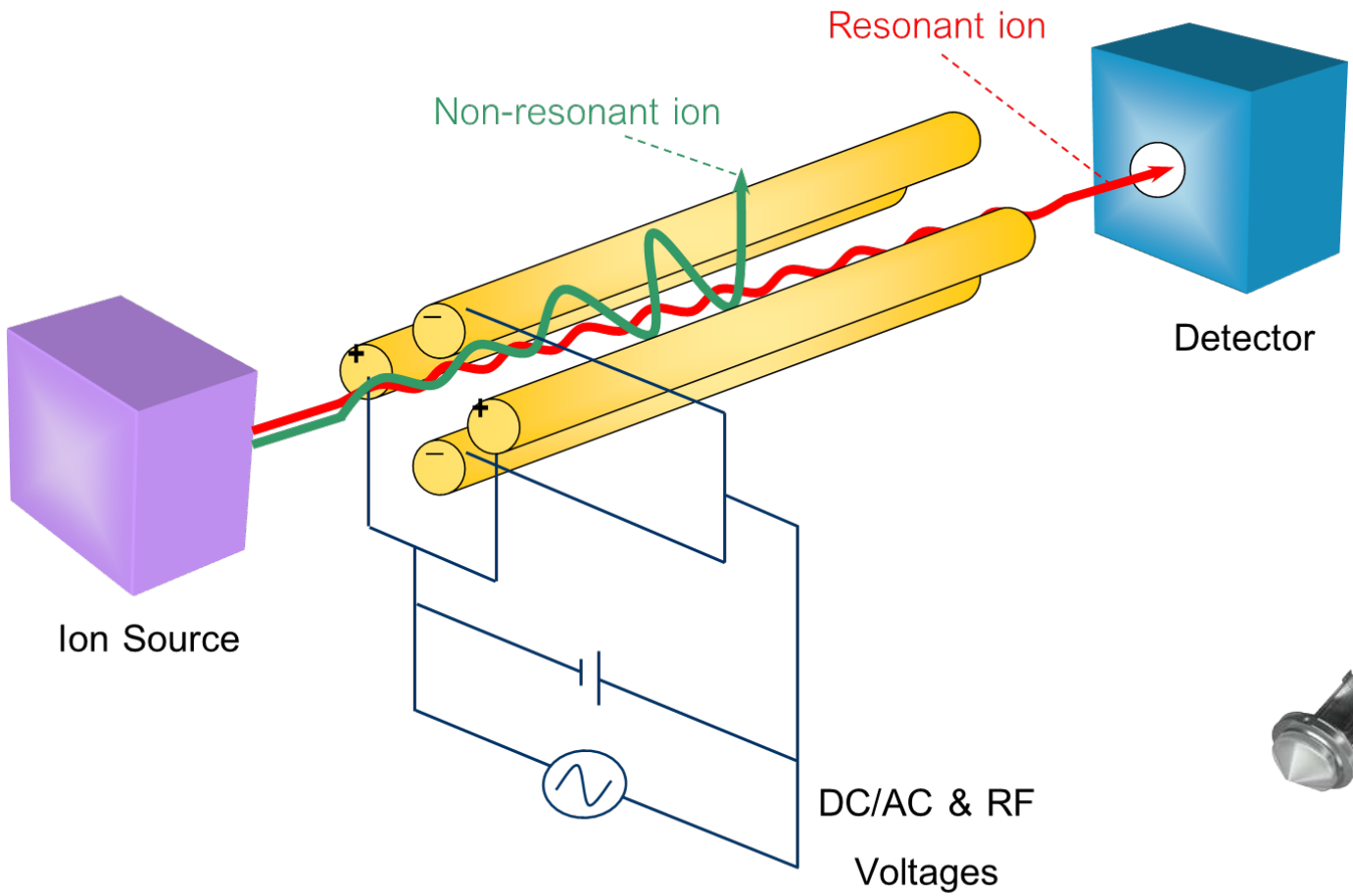


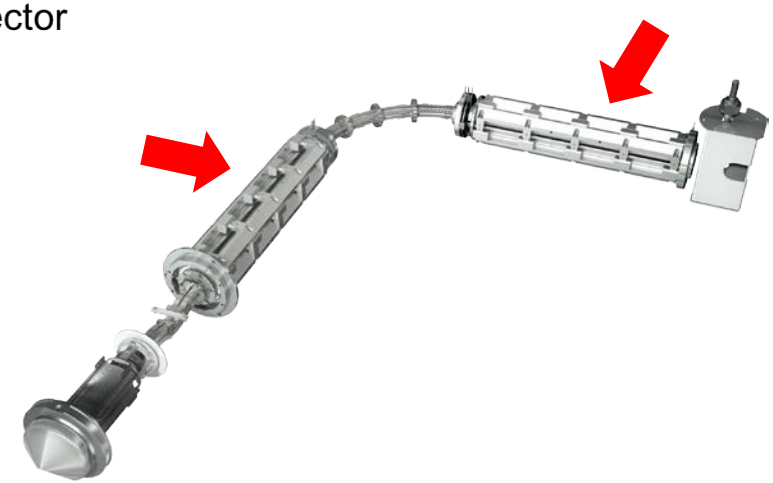


Mass Analyzer: Triple Quadrupoles (QqQ)

- **Q1 and Q3** are "Mass filter" where ions are scanned by varying the DC/AC & RF voltages across the quadrupole set

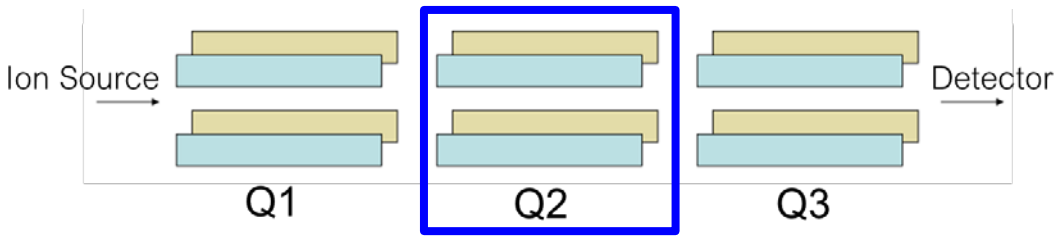


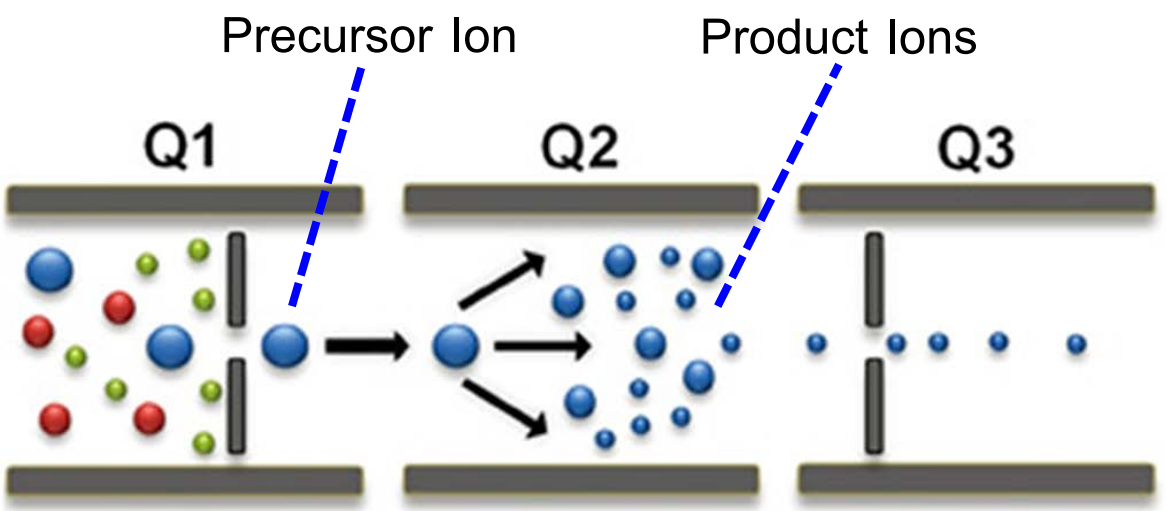




Mass Analyzer: Triple Quadrupoles (QqQ)

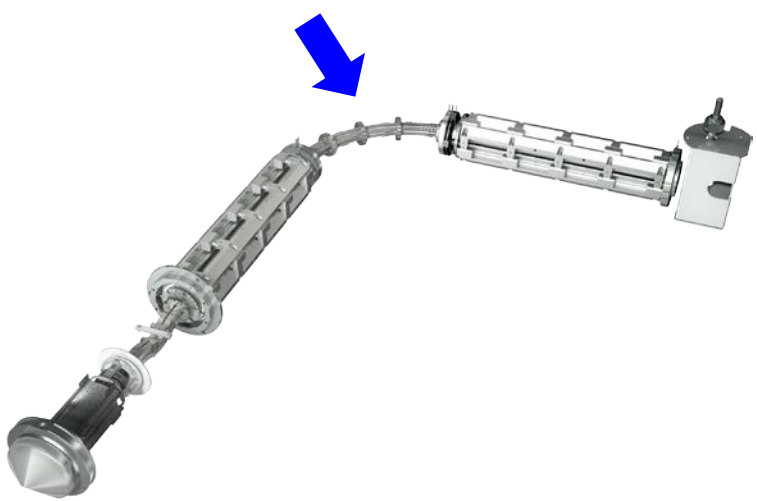
- Q2 is “Collision Cell” where precursor ions are fragmented and pass through Q3 for ion sorting again





Fragmentation

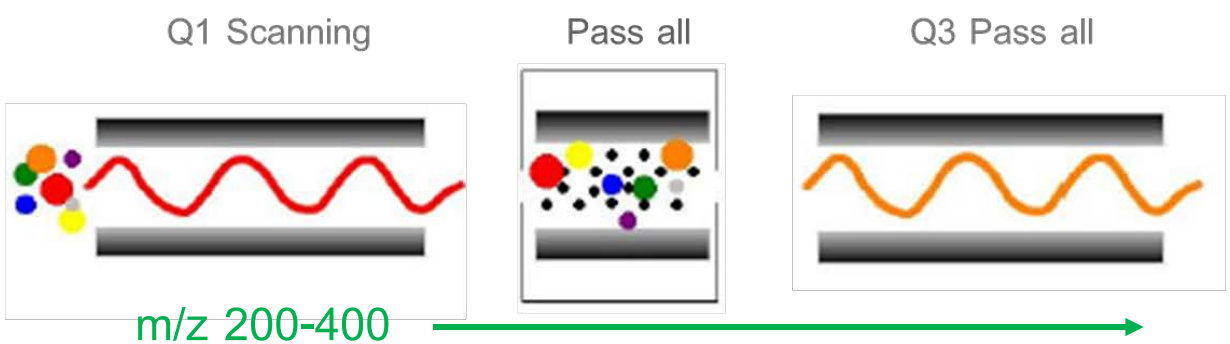
(Collision gas: Ar)



Scan Modes in QqQ

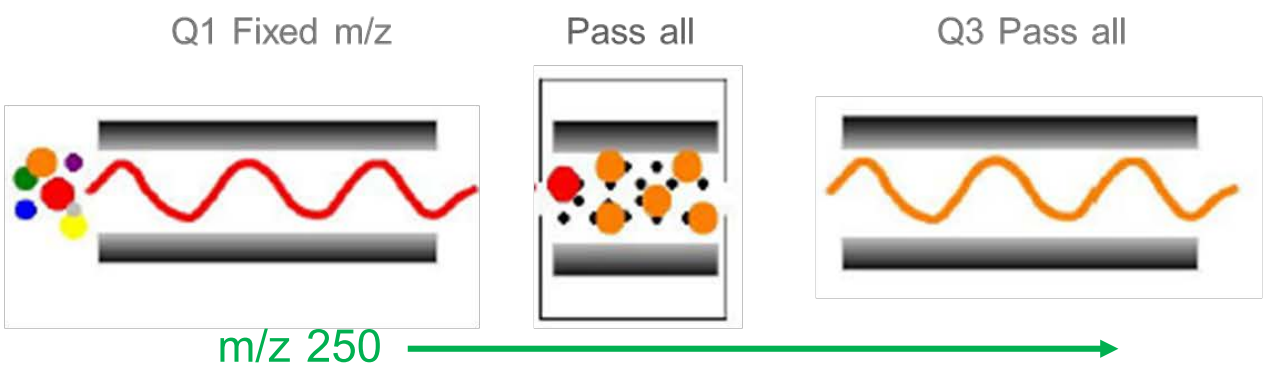
Scan Mode	Q1	Q2	Q3	Purpose
Full Scan	Scanning	Pass All	Pass All	MW Info.
SIM (Selected Ion Monitoring)	Fixed m/z	Pass All	Pass All	Quantitation

Full scan Q1:



Purpose: Survey scan of a chromatographic peak

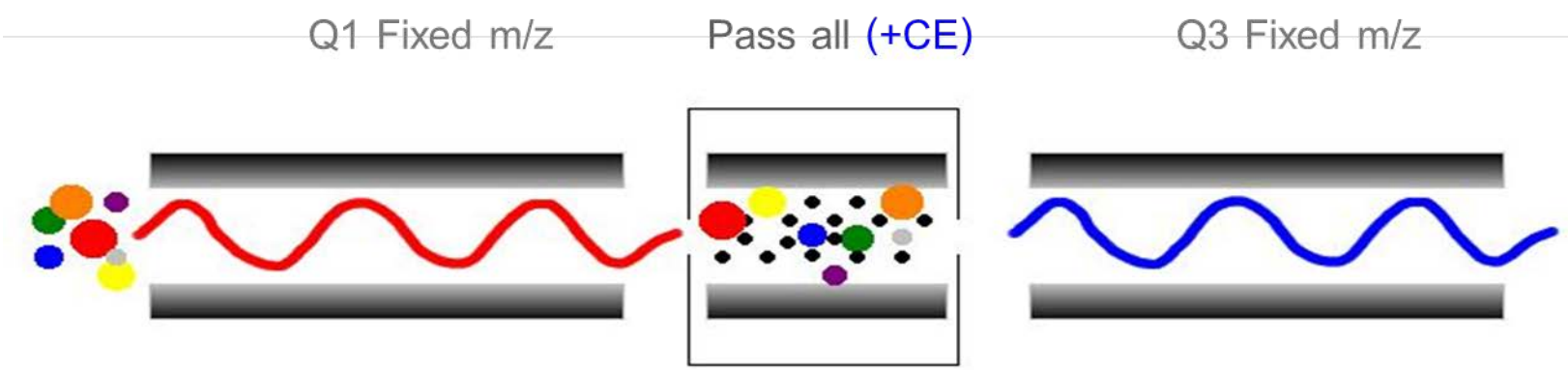
SIM Q1:



Purpose: Quantitation on a specific m/z range of ions

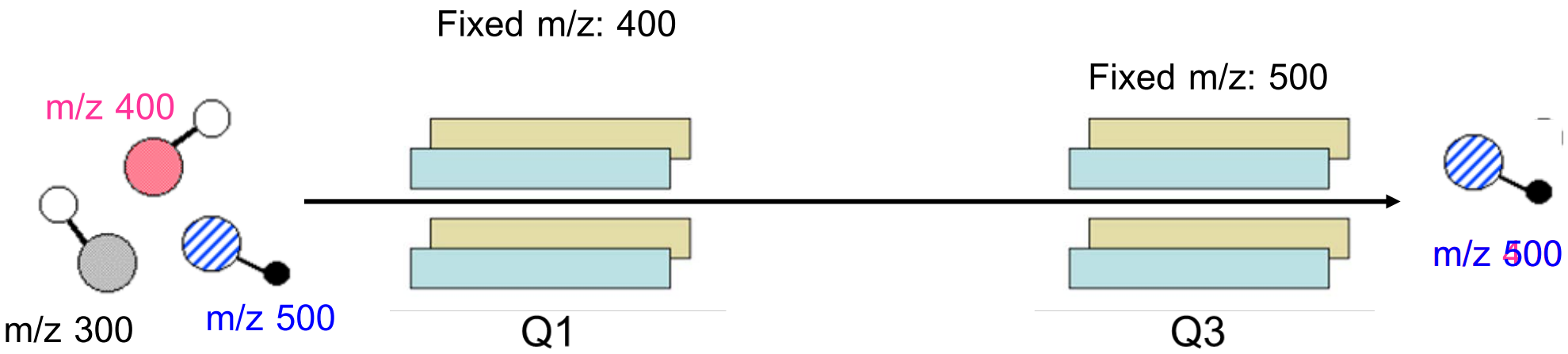
Scan Modes in QqQ

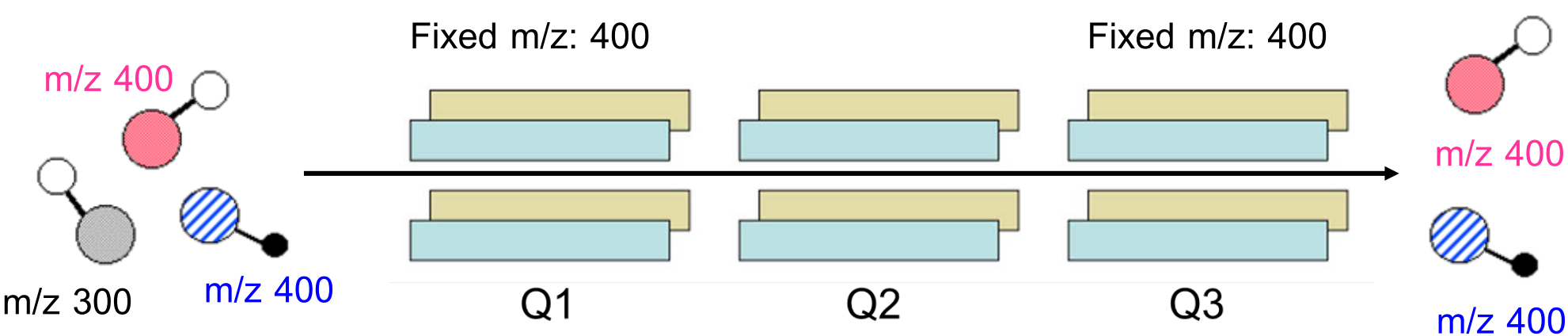
Scan Mode	Q1	Q2	Q3	Purpose
SRM (Selected Reaction Monitoring)	Fixed m/z	Pass All (+CE)	Fixed m/z	Targeted Quantitation



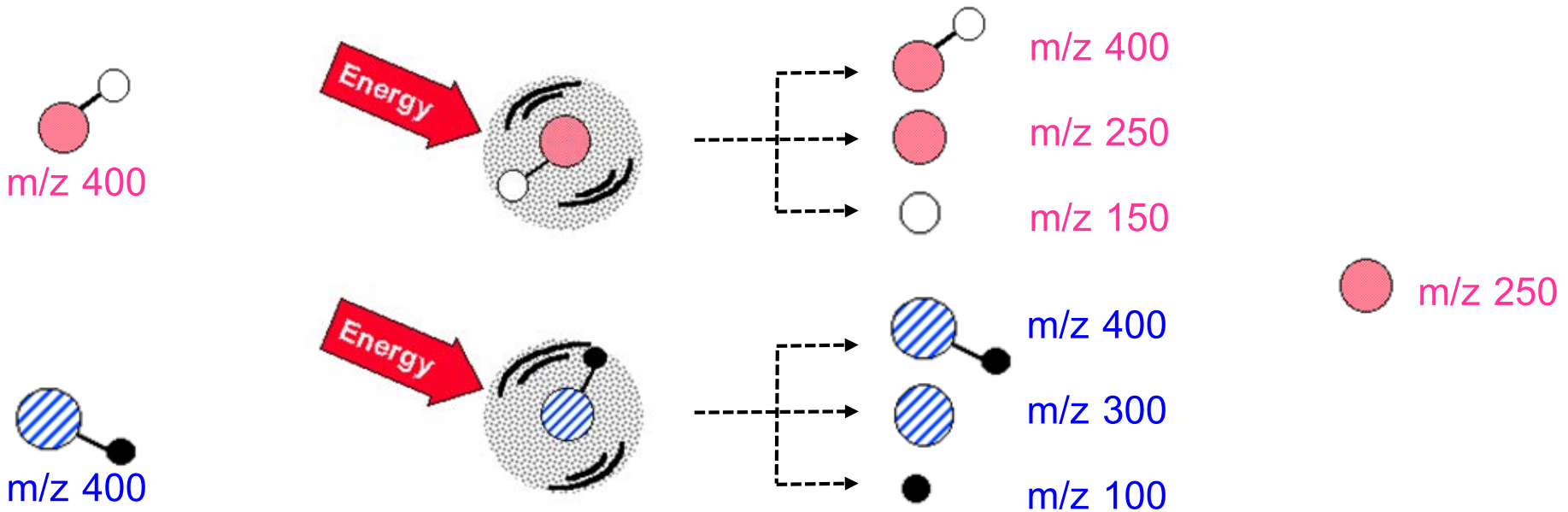
Purpose: Targeted quantitation

Scan Modes in QqQ





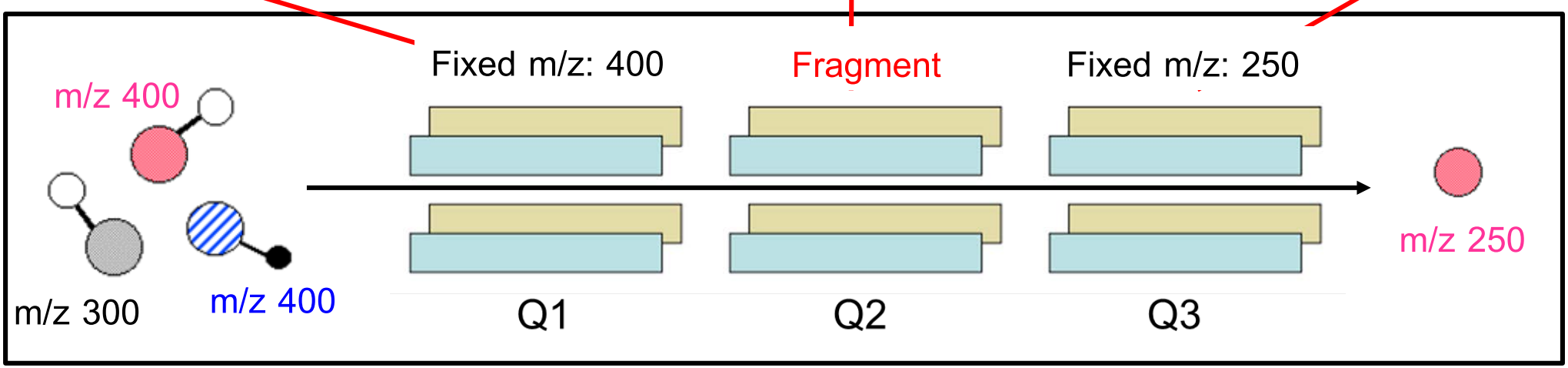
SRM



Q1: Precursor Ion

Q2: Fragmentation

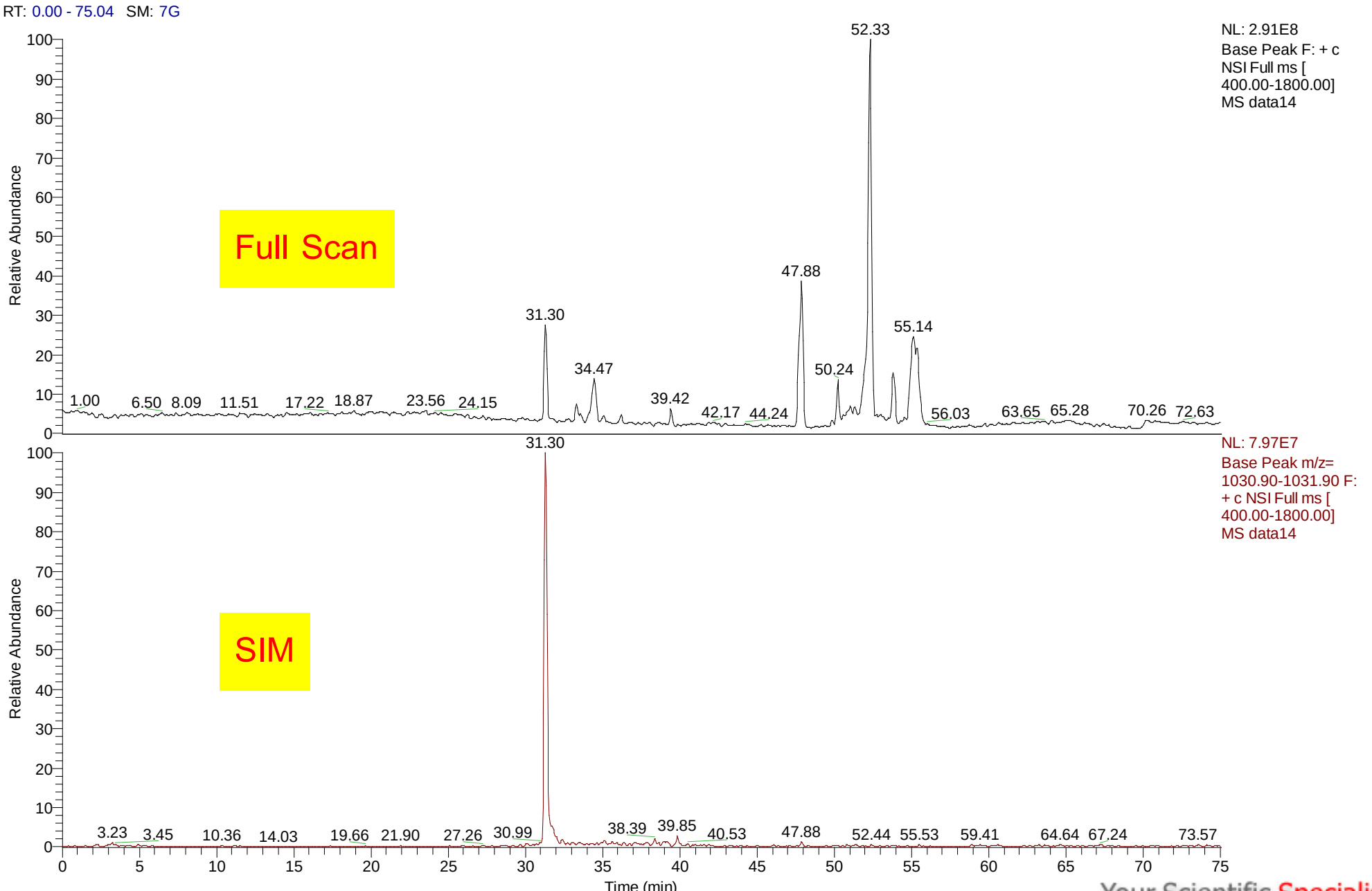
Q3: Product Ion



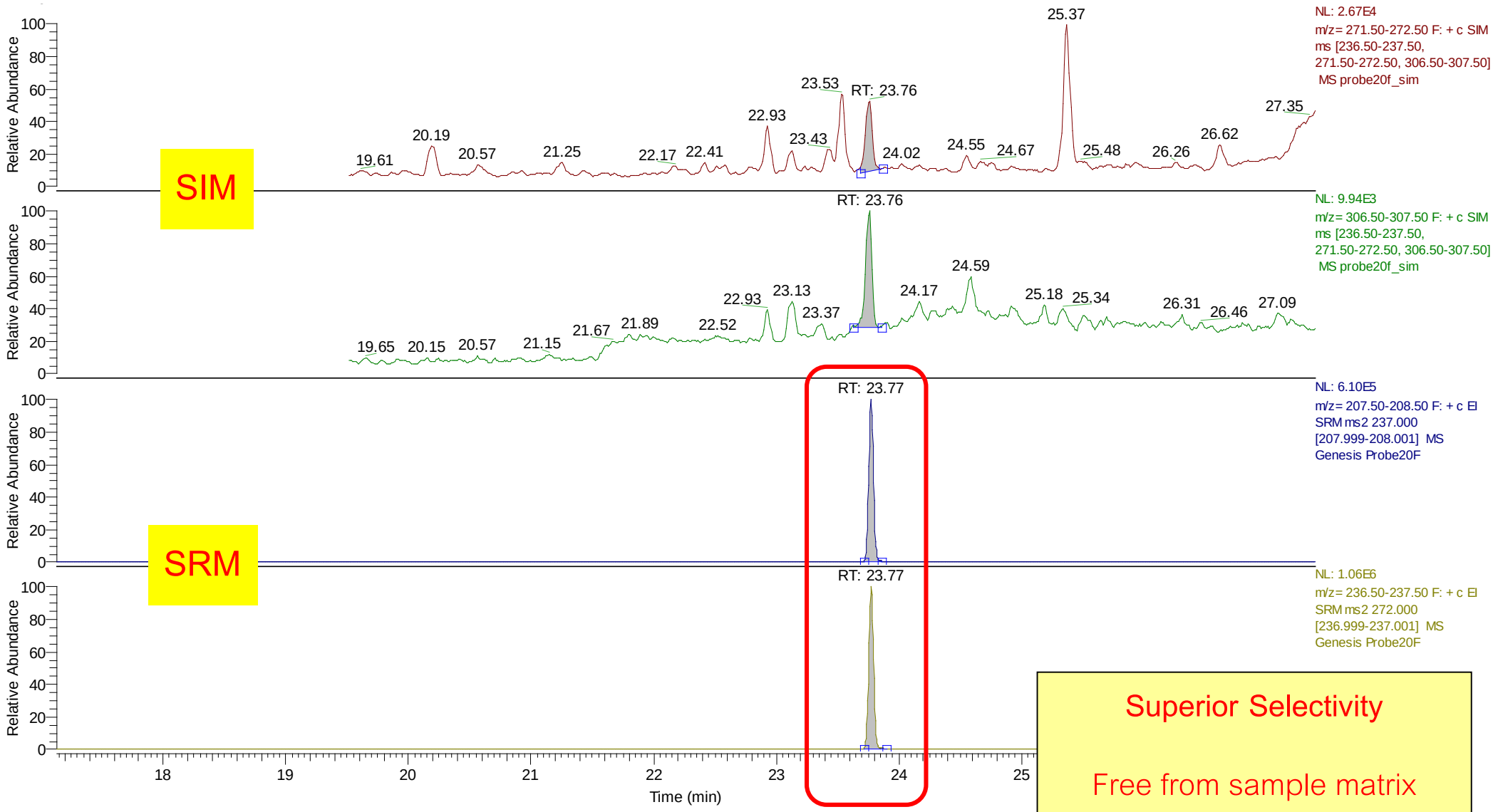
Scan Modes in QqQ

Scan Mode	Q1	Q2	Q3	Purpose
Full Scan	Scanning	Pass All	Pass All	MW Info.
SIM (Selected Ion Monitoring)	Fixed m/z	Pass All	Pass All	Quantitation
SRM (Selected Reaction Monitoring)	Fixed m/z	Pass All (+CE)	Fixed m/z	Targeted Quantitation
Product	Fixed m/z	Pass All (+CE)	Scanning	Structural Info.
Neutral Loss	Scanning	Pass All (+CE)	Scanning	Analyte Screening
Precursor	Scanning	Pass All (+CE)	Fixed m/z	Analyte Screening

Full Scan VS SIM



SIM VS SRM

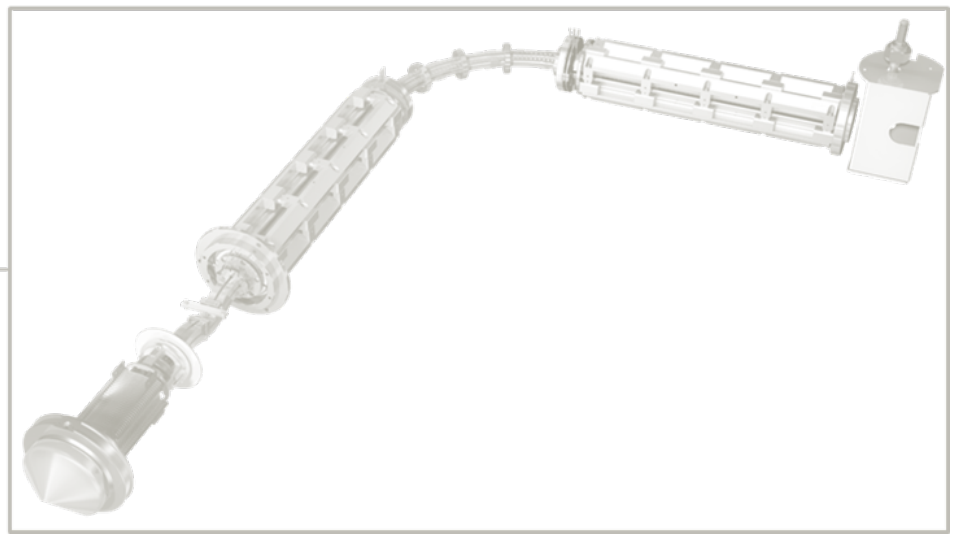


Superior Selectivity

Free from sample matrix

Mass Analyzer

- Triple Quadrupole (QqQ)



- Orbitrap

Mass Analyzer: Orbitrap

Anal. Chem. **2000,** *72,* 1156−1162

Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

Alexander Makarov*

HD Technologies Ltd., Atlas House, Simonsway, Manchester, M22 5PP, U.K.

This work describes a new type of mass analyzer which employs trapping in an electrostatic field. The potential distribution of the field can be represented as a combination of quadrupole and logarithmic potentials. In the absence of any magnetic or rf fields, ion stability is achieved only due to ions orbiting around an axial electrode. Orbiting ions also perform harmonic oscillations along the electrode with frequency proportional to $(m/z)^{-1/2}$. These oscillations are detected using image current detection and are transformed into mass spectra using fast FT, similarly to FT ICR. Practical aspects of the trap design are presented. High-mass resolution up to 150 000 for ions produced by laser ablation has been demonstrated, along with high-energy acceptance and wide mass range.

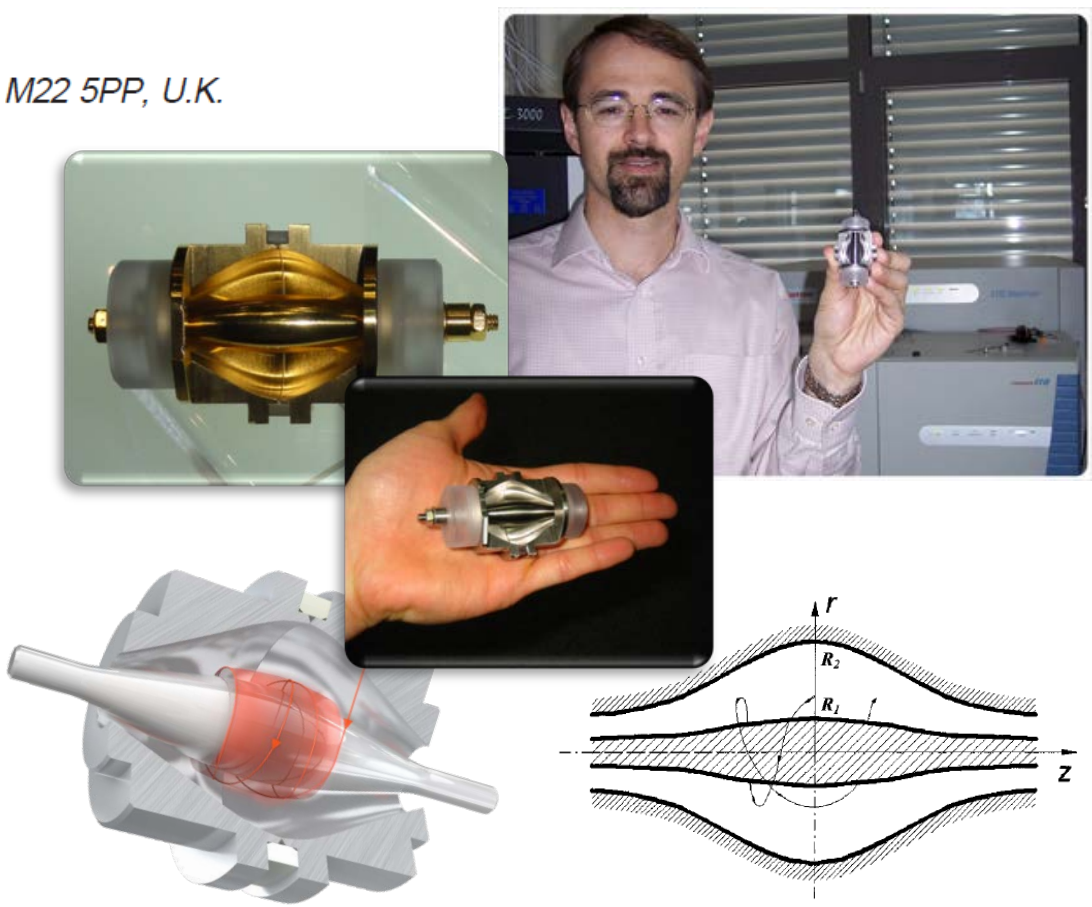
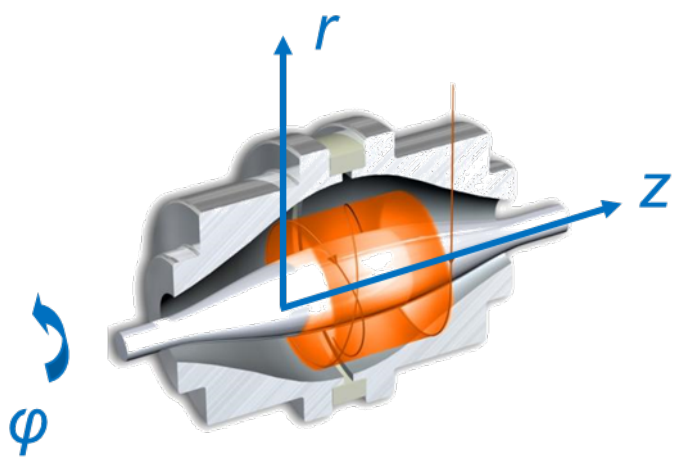


Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

Orbitrap Mass Analyzer: Principle of Operation



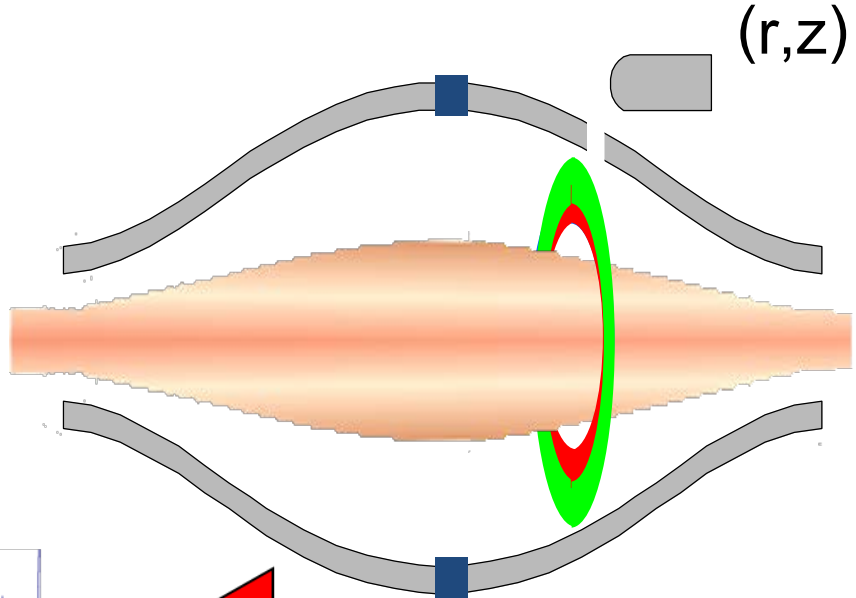
Hyper-logarithmic potential distribution:
"ideal Kingdon trap"

$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

Characteristic frequencies:

- Frequency of rotation ω_φ
- Frequency of radial oscillations ω_r
- Frequency of axial oscillations ω_z

$$\omega_z = \sqrt{\frac{k}{m/q}}$$



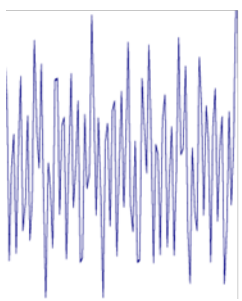
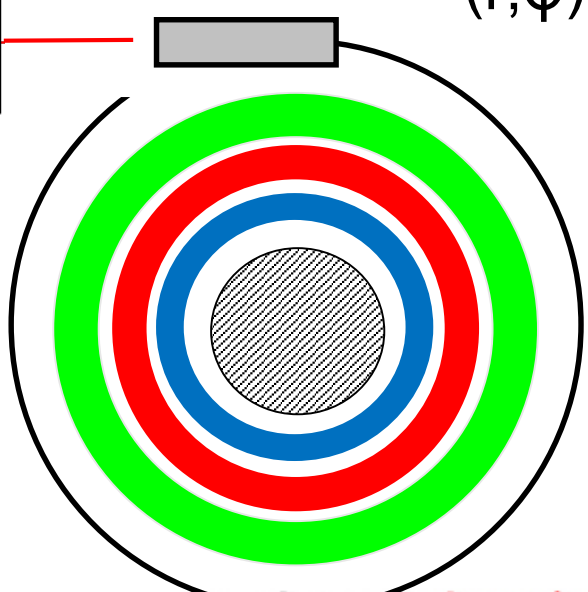
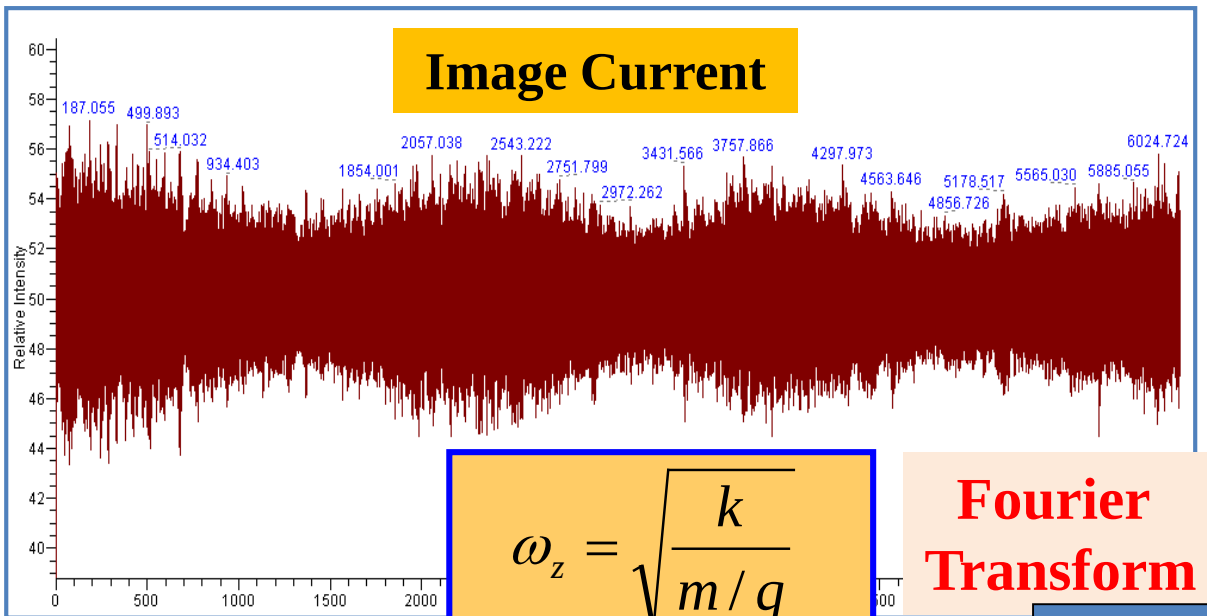


Image Current



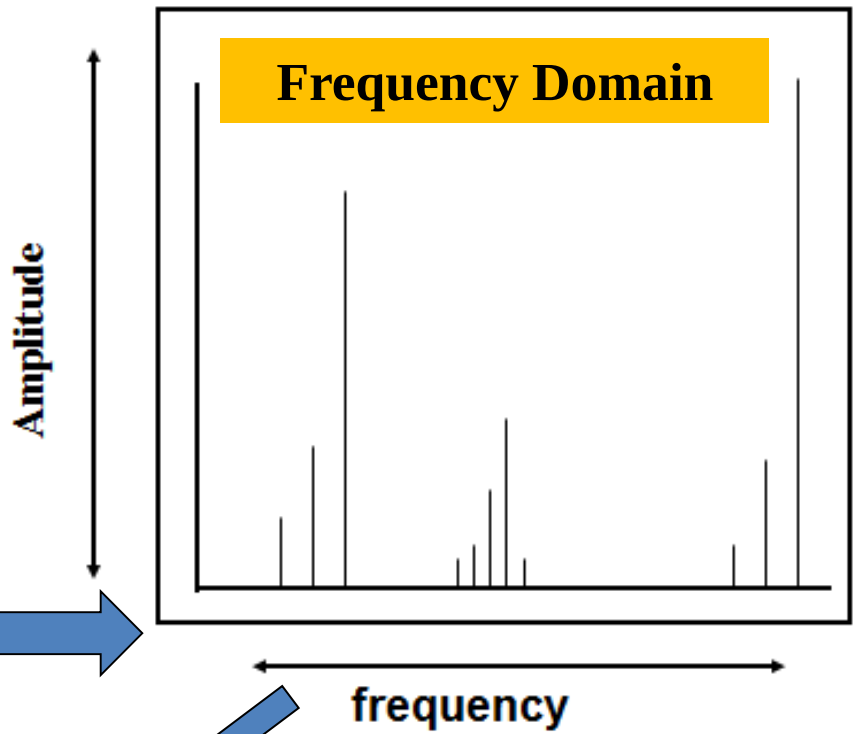
Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

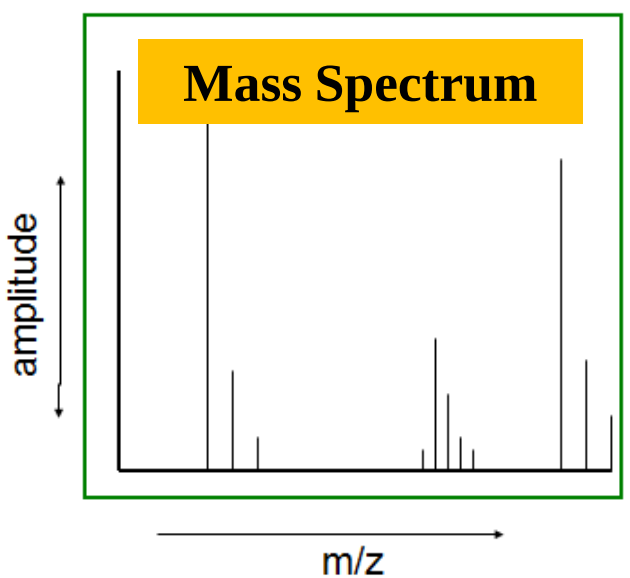
Many Ions Generate a Complex "Transient"



$$\omega_z = \sqrt{\frac{k}{m/q}}$$

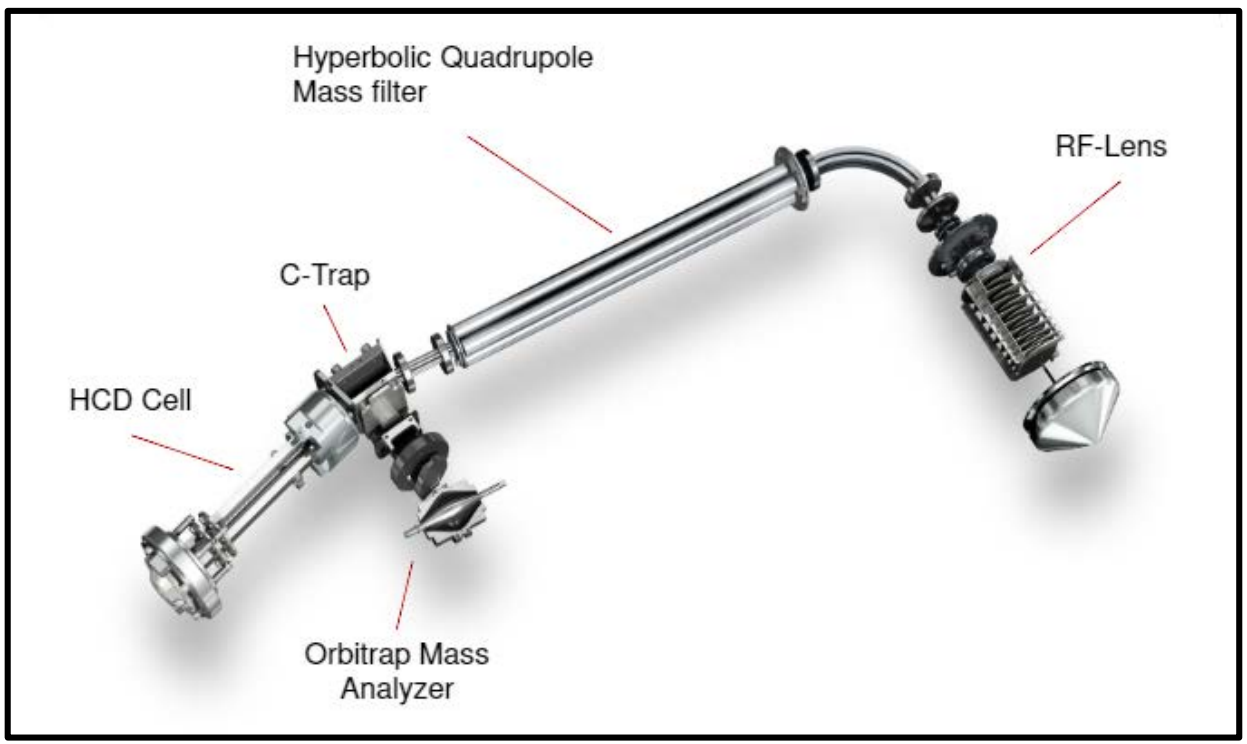
Fourier Transform





Q Exactive™

Thermo SCIENTIFIC



Mass Analyzer

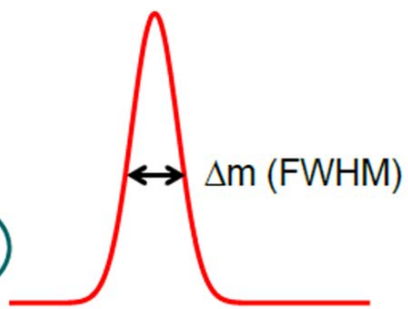
- Orbitrap

High Resolution Accurate Mass (HRAM)

Mass Resolution

- Ability of a mass spectrometer to distinguish between ions of nearly equal m/z ratios (isobars).

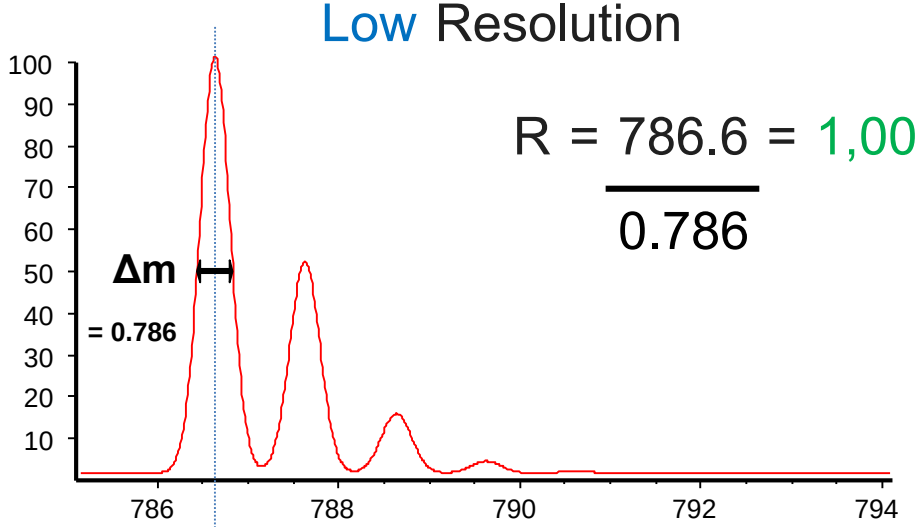
$$R = \frac{m}{\Delta m}$$



m - measured mass

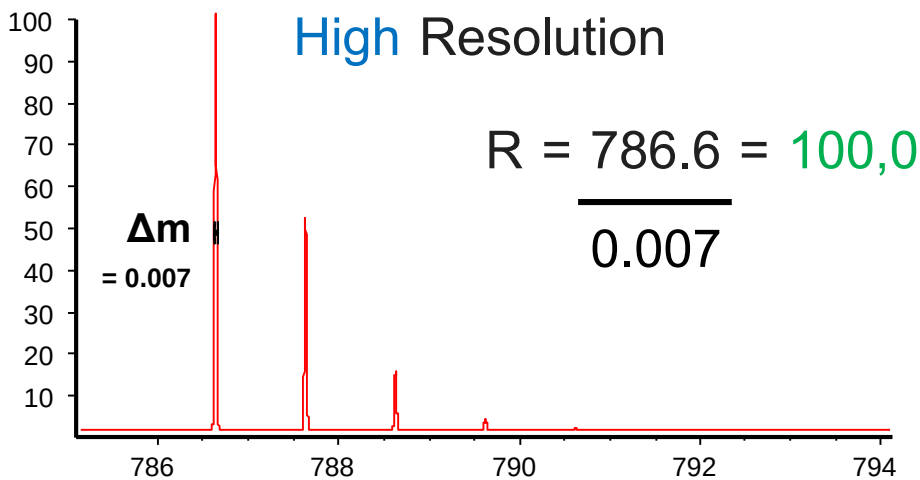
Δm - peak width measured at 50% peak intensity (Full Width Half Maximum)

Low Resolution



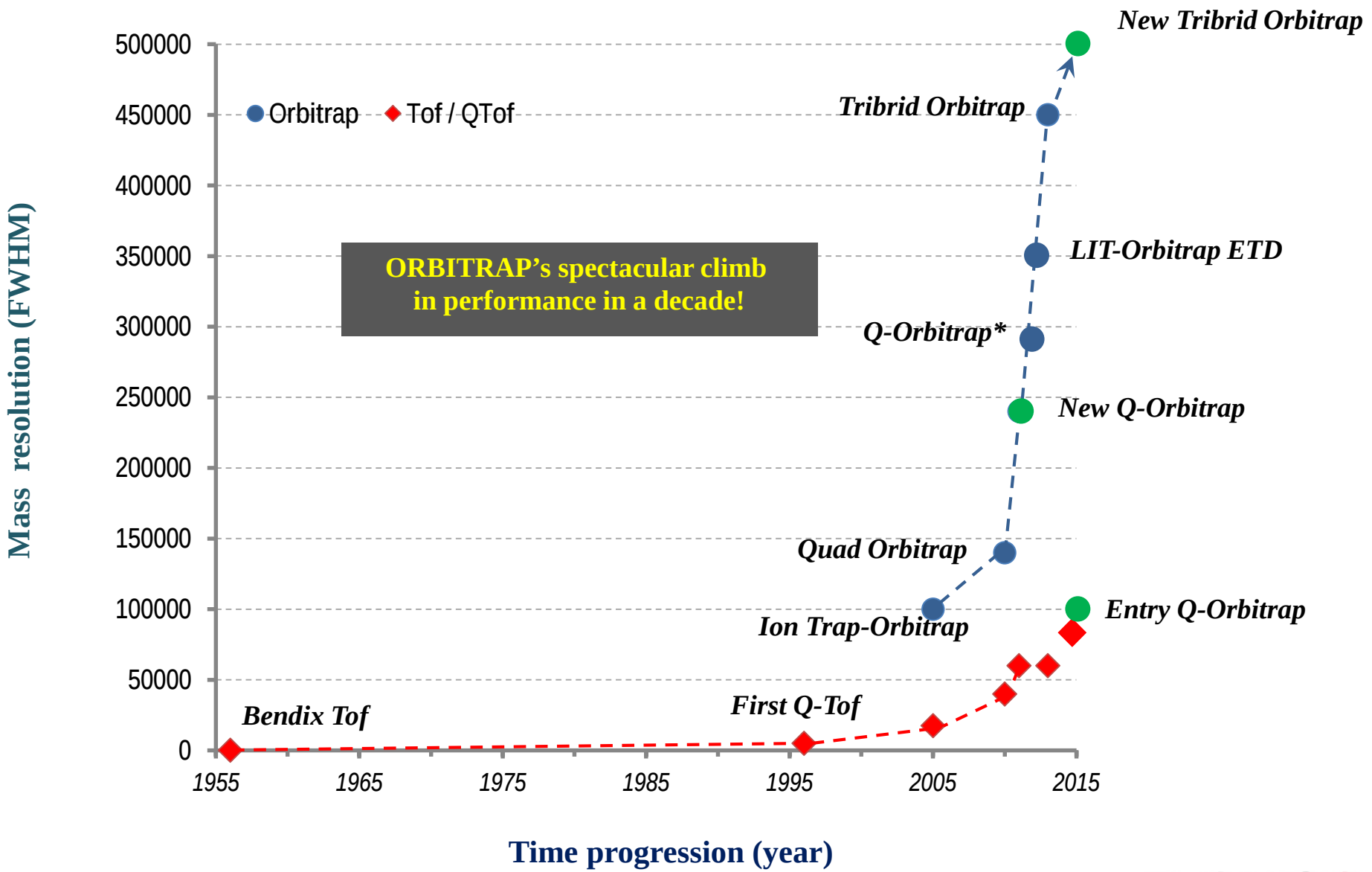
$$R = \frac{786.6}{0.786} = 1{,}000$$

High Resolution



$$R = \frac{786.6}{0.007} = 100{,}000$$

Commercial High Resolution MS Technology Race



Mass Accuracy

- The precision of which the mass is measured by the mass spectrometer.
- Typical way of reporting mass error in **ppm** (relative measure) or **mDa** (absolute measure)

$$\text{Mass error} = \left(\frac{\text{Measured} - \text{Exact Mass}}{\text{Exact Mass}} \right) \times 10^6$$

C = 12.0000

O = 15.9949

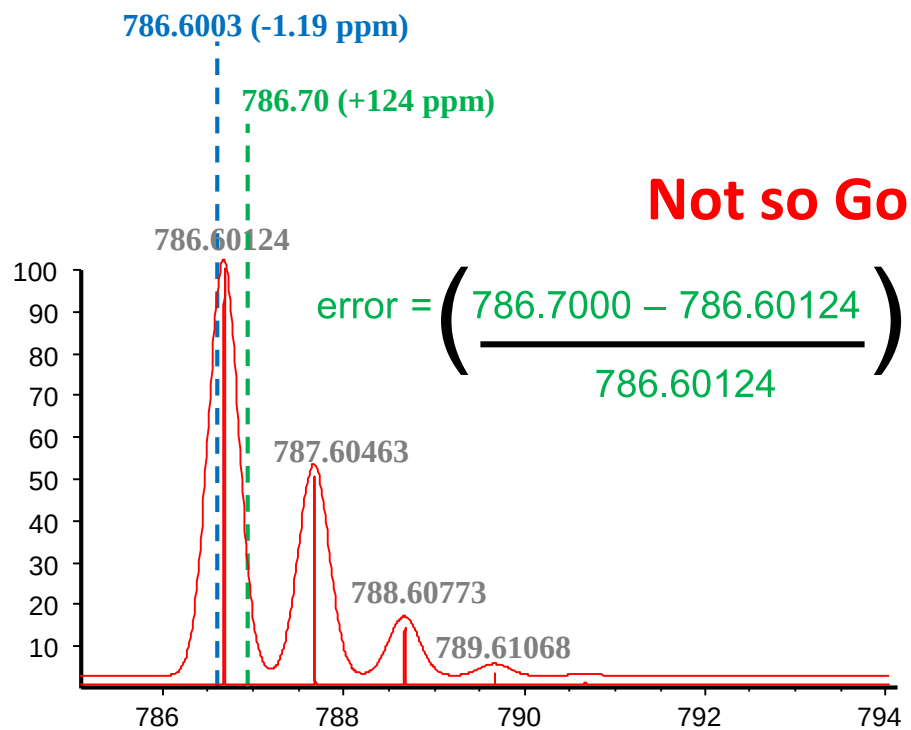
S = 31.9721

H = 1.0078

N = 14.0031

Good

$$\text{error} = \left(\frac{786.6003 - 786.60124}{786.60124} \right) \times 10^6$$



Not so Good

$$\text{error} = \left(\frac{786.7000 - 786.60124}{786.60124} \right) \times 10^6$$

Mass Accuracy

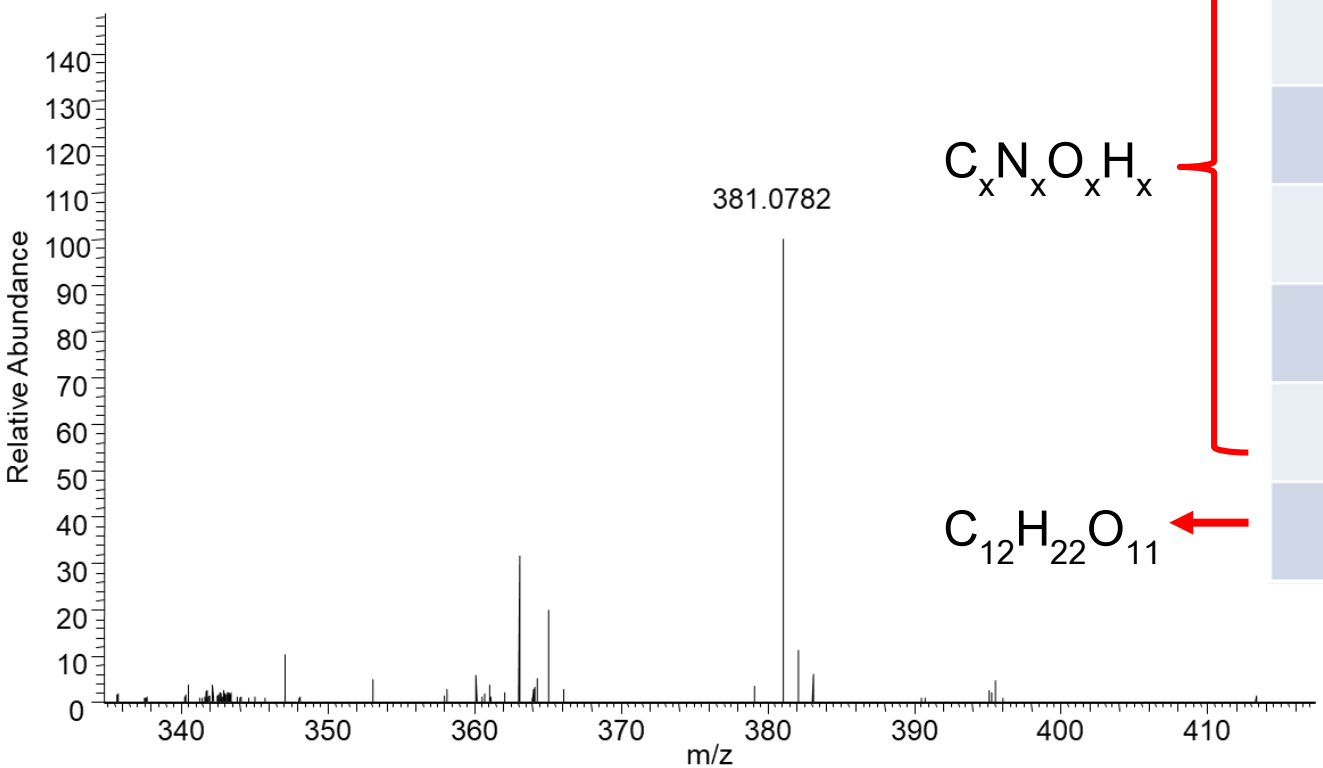
- Typical mass accuracy capability for various MS types

Type	Mass Accuracy
FT-ICR-MS	0.1 - 1 ppm
Orbitrap	0.5 - 1 ppm
Magnetic Sector	1 - 2 ppm
TOF-MS	3 - 5 ppm
Q-TOF	3 - 5 ppm

Source: Metabolomics Fiehn's lab

Mass Accuracy

- Increases confidence in identification



$[M+H]^+$ 381.0782

Mass Accuracy	Number of hits*
± 200 ppm	265
± 100 ppm	133
± 30 ppm	39
± 10 ppm	14
± 5 ppm	5
± 3 ppm	4
± 1 ppm	1

$C_xN_xO_xH_x$

$C_{12}H_{22}O_{11}$

* Compounds containing CNOH

Mass Resolution and Accuracy

Measured Mass	Mass Error (Da)	Possible Formula	Exact Mass
32.0	± 0.2	O_2	31.9898
		CH_3OH	32.0261
		N_2H_4	32.0374
		S	31.9721
32.02	± 0.02	CH_3OH	32.0261
		N_2H_4	32.0374
32.0257	± 0.002	CH_3OH	32.0261

C = 12.0000

O = 15.9949

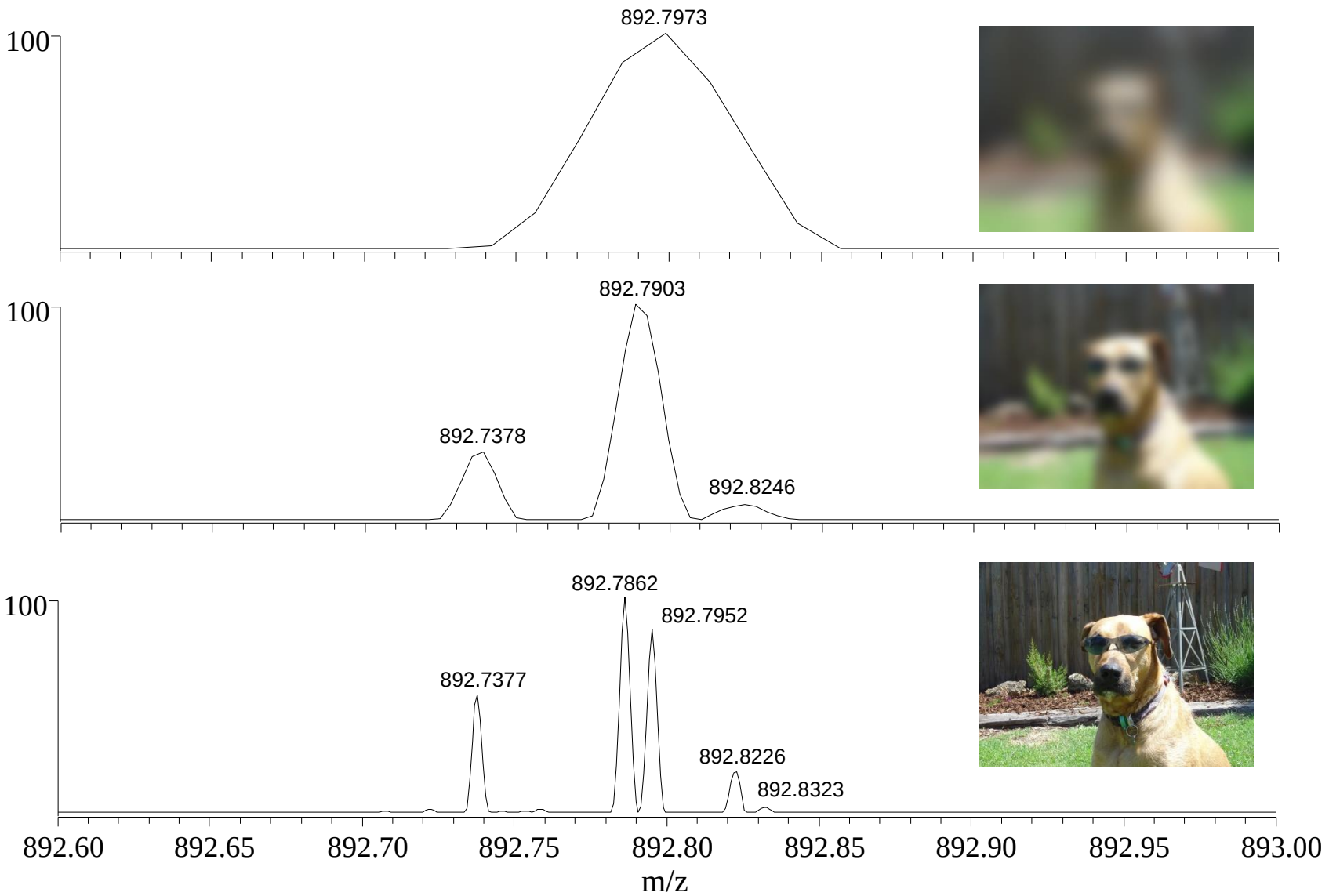
S = 31.9721

H = 1.0078

N = 14.0031

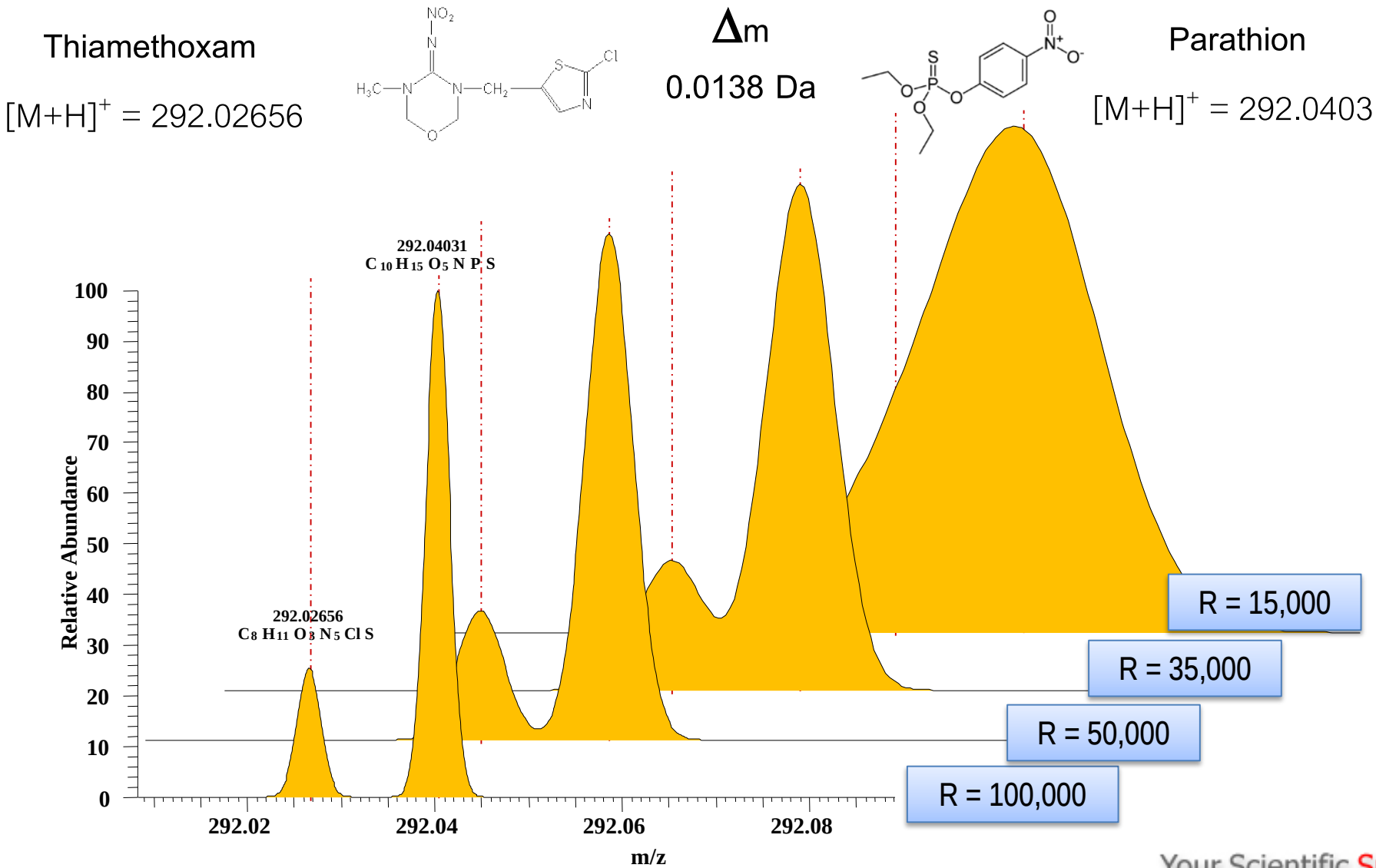
- **Main advantage:** the possibility to determine the elemental composition of individual molecular or fragment ions, a powerful tool for the structural elucidation or confirmation.

Mass Resolution and Accuracy



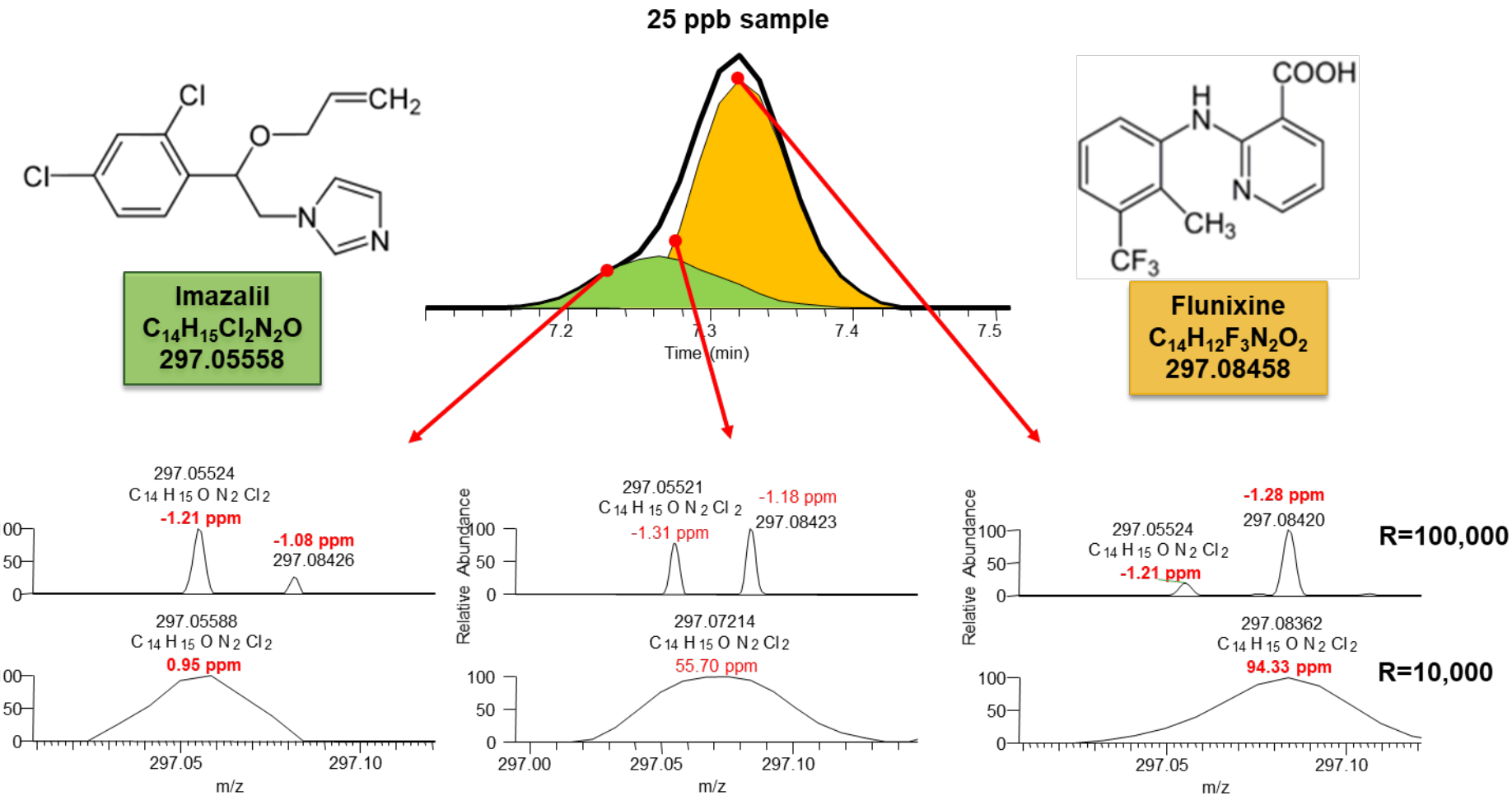
Mass Resolution and Accuracy

- Isobaric compounds separation



Mass Resolution and Accuracy

- Isobaric compounds separation



Mass Resolution and Accuracy

- Removing interferences

High resolution is very important for samples with complex matrix (e.g. biological, food), since they will contain a significant number of background ions

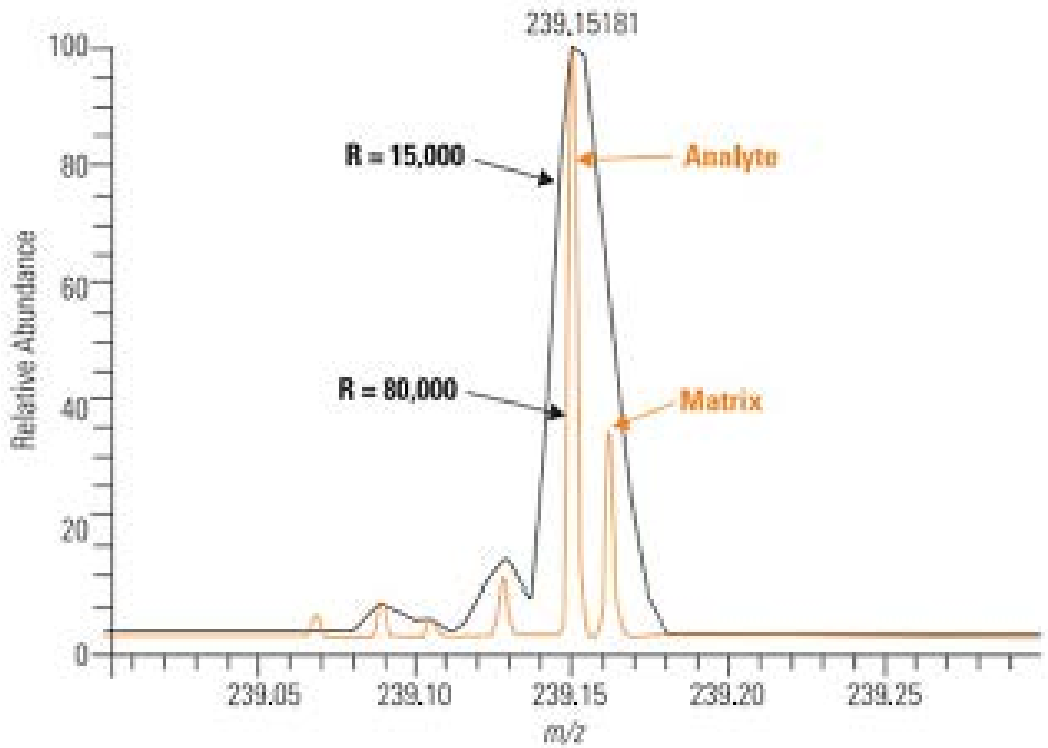


Figure 1: Analysis of the MH+ peak of Pirimicarb at 15,000 and 80,000 resolution.

LCMS Applications

LCMS Applications

- Biomolecule characterization
  - Proteomics
  - Oligonucleotides
- Environmental analysis
  - Pesticides on foods
  - Soil and groundwater contamination
- Forensic and clinical analysis
- Toxicology
- Pharmaceutical analysis
  - Bioavailability studies
  - Drug metabolism studies, pharmacokinetics
  - Characterization of potential drugs
  - Drug degradation product analysis
  - Screening of drug candidates
  - Identifying drug targets
- Etc.

Application in Food

Identification and Quantitation of Melamine in Infant Milk

Melamine

SRM Transitions

(Q1) 127 -> 68 (Q3)

(Q1) 127 -> 85 (Q3)

Sample Prep (SPE) → LC-MS/MS (Targeted SRM)

Instrument conditions

LC: Accela™

Column: BioBasic AX (Ion Exchange)

Column Temperature: 30°C

Injection Volume: 1 µL

Mobile Phase: A) 85% ACN + 10% IPA + 5% Ammonium acetate; B) 90% water and 10%ACN

Flow Rate: 400 µL/min Run Time: 5 min

MS: TSQ Quantum Ultra™

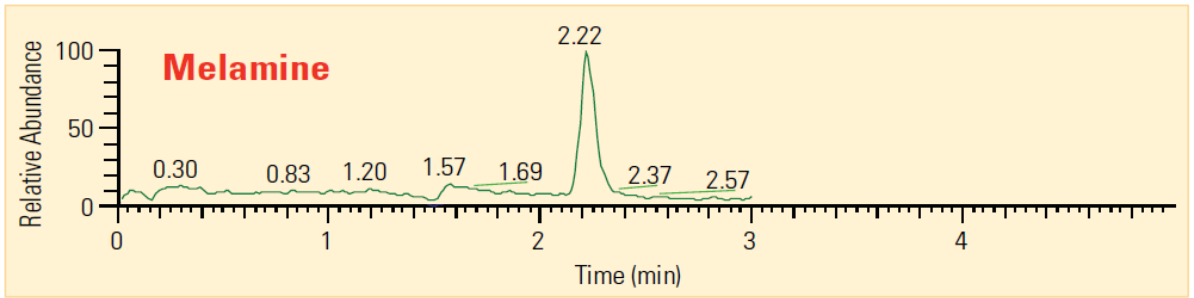
Ionization: Positive ESI

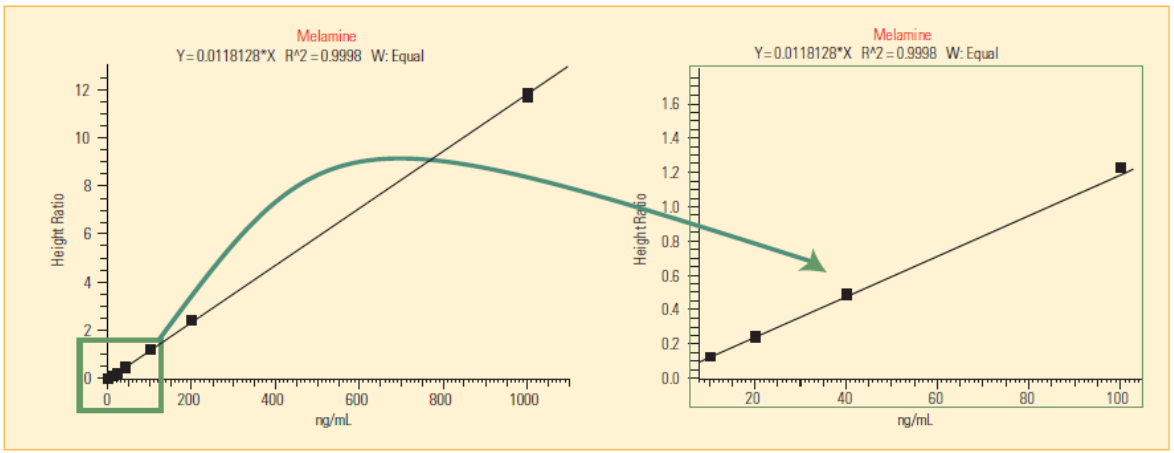
Modes: Targeted SRM

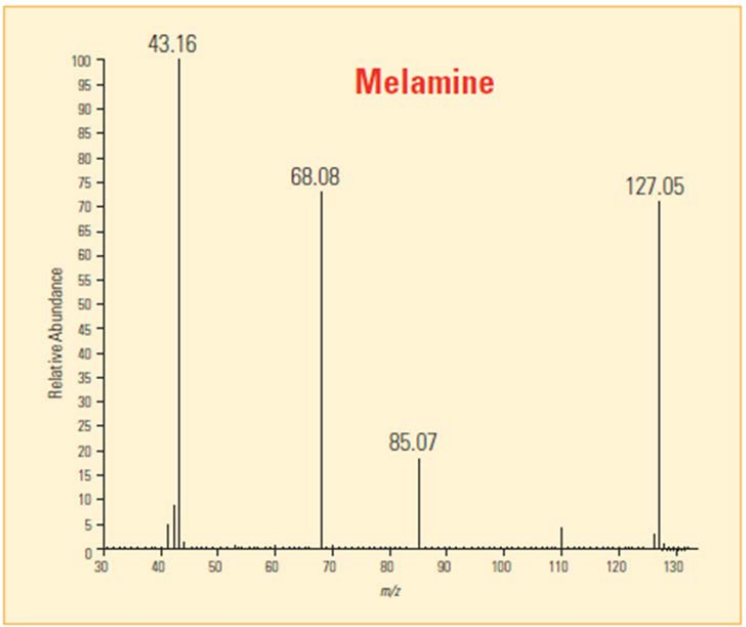
Varelis *et al*. *Thermo AN62732*. 2008

Application in Food

Identification and Quantitation of Melamine in Infant Milk







- Limit of Detection (LOD): <10 ppb (below the FDA requirement at 1 ppm)

Varelis *et al.* *Thermo AN62732*. 2008

Application in Halal Food

Determination of Meat Authenticity

Meat Sample

Protein Extraction

Protein Extracts

Tryptic Digestion

Digested Peptides

Analysis

HRAM LC-MS

LC & HRAM MS Conditions

HPLC Conditions	
System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column:	Thermo Scientific™ BioBasic™ C8 (5 µm, 100 × 1 mm)
Mobile Phases:	(A) water + 0.1% formic acid (B) acetonitrile + 0.1% formic acid
Inj. Volume:	2 µL
Flow Rate:	75 µL/min

MS Conditions	
MS:	Thermo Scientific Q Exactive benchtop quadrupole-Orbitrap mass spectrometer
Scan Type:	Full scan MS
Resolving Power:	140,000 (FWHM)
AGC:	3.0×10^6
Maximum IT:	200 ms
Scan Range:	*m/z* 500–2000
njection Volume:	2 µL
Spray Voltage:	4 kV
Capillary Temperature:	300 °C
Sheath Gas Flow Rate:	10 Arb
Auxilliary Gas Flow Rate:	5 Arb
Product Ion Spectra Obtained with:	
Resolving Power:	17,500 (FWHM)
Collision Energy:	25
AGC:	1.0×10^{-6}
Maximum IT:	100 ms
Isolation Window:	1.5 Da

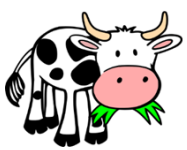
Orduna et al. *Thermo AN64677*. 2016

Application in Halal Food

Determination of Meat Authenticity

Peptide Detection

by HRAM LC-MS

Type of Meat	Peptide Marker Sequence	Precursor Ion Mass (z=2)	Product Ion m/z (z=1)
	HPSDFGADAQAAMSK	766.8	1298.5681 1395.6209
	HPGDFGADAQGAMTK	751.8	1268.5576 1365.6103
	HPGDFGADAQGAMSK	744.8	1254.5419 1351.5957
	HPSDFGADAQGAMSK	759.8	1285.5525 1381.6053

Orduna et al. *Thermo AN64677*. 2016

Application in Pharmaceutical Products

Discovery and Characterization of Natural Components

Samples ground in liquid nitrogen.

Powder samples extracted with suitable strong solvents.

Extracts cleaned by centrifugation, filtration, fractionation, or appropriate methods.

Samples concentrated by evaporation/lyophilization and reconstituted in (U)HPLC-suitable solvent.

Instrument conditions

LC: Ultimate 3000™

Column: Hypersil Gold C18

Column Temperature: 35°C

Injection Volume: 1 µL

Mobile Phase: ACN + 0.1% FA (linear gradient)

Flow Rate: 300 µL/min Run Time: 50 min

MS: Q Exactive™

Ionization: Positive and Negative ESI

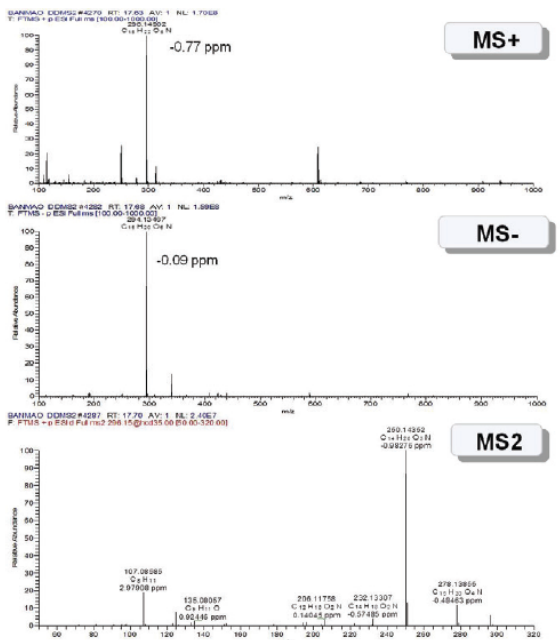
Modes: dd-MS^2

Li *et al*. *Thermo AN64427*. 2015

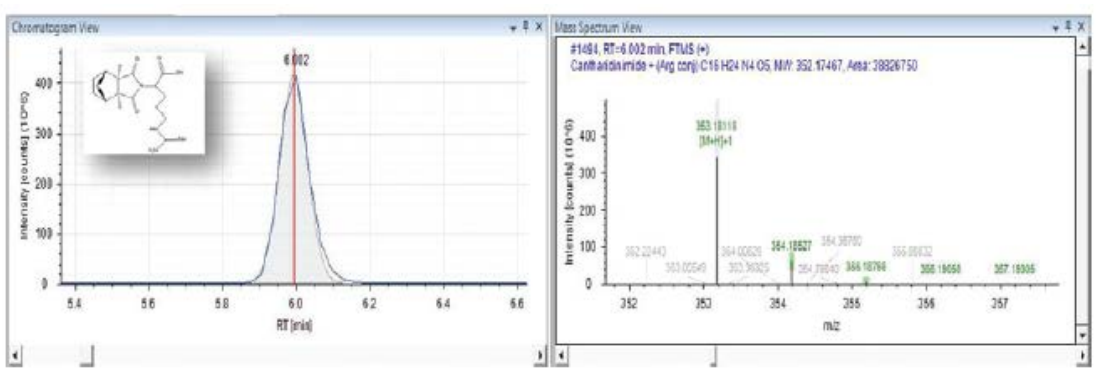
Application in Pharmaceutical Products

Discovery and Characterization of Natural Components

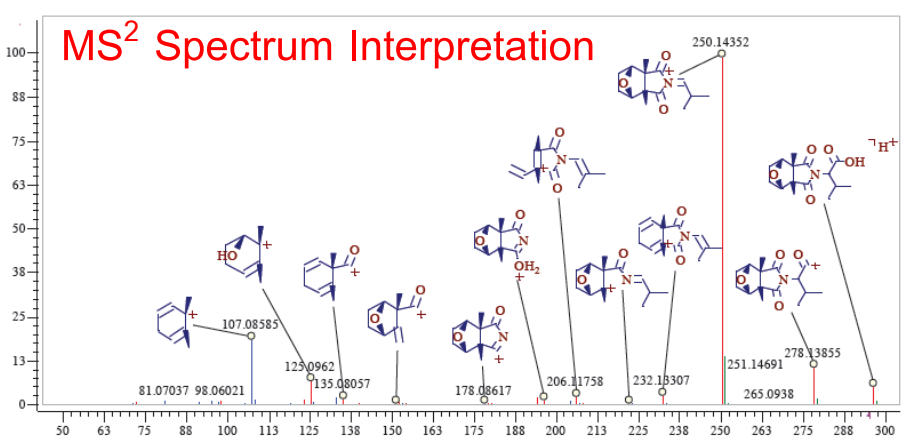
MS and MS^2 Spectrum



Extracted Ion Chromatogram and Isotopic Pattern



MS^2 Spectrum Interpretation



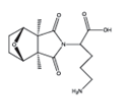
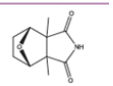
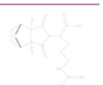
Li *et al.* *Thermo AN64427*. 2015

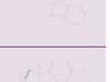
Application in Pharmaceutical Products

Discovery and Characterization of Natural Components

- 21 Cantharidins were detected in the blister beetle extract.

- 16 new Cantharidins were discovered and identified with mass accuracy <1 ppm by fragment ion search (FISh) function, using cantharidinimide as the parent structure.

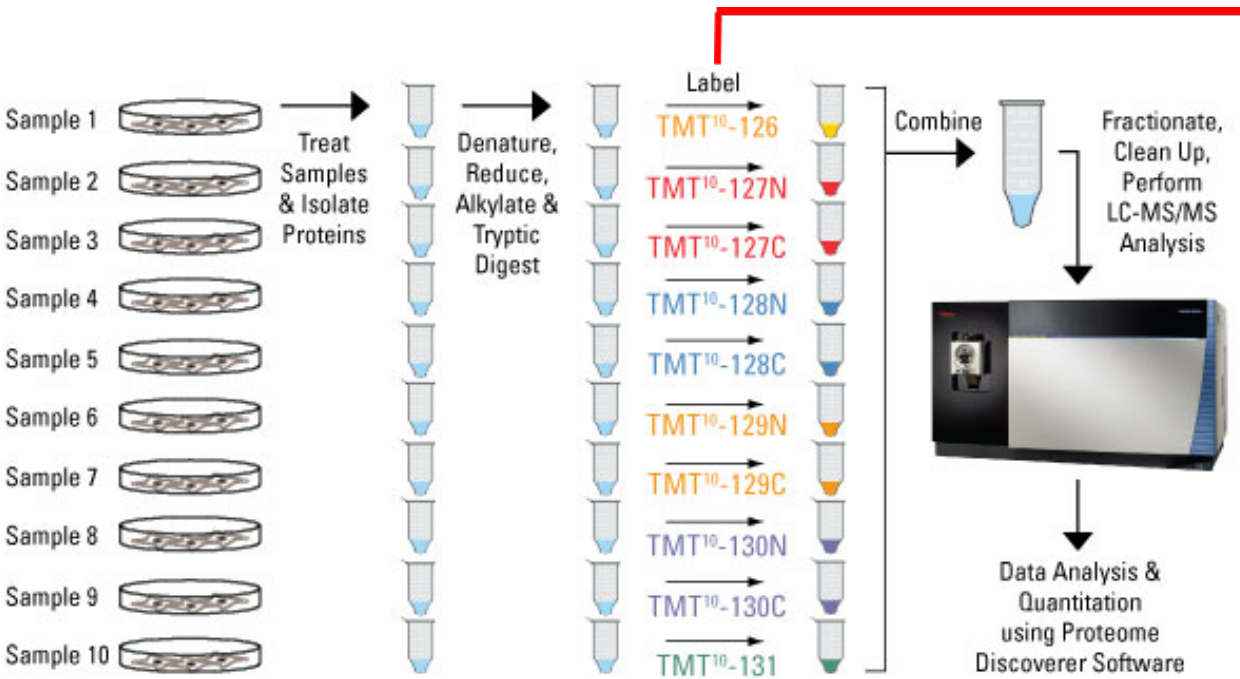
No.	RT (min)	Formula	Theoretical Mass	Measured Mass	Precision (ppm)	Structure
1	4.5	C15H22O5N2	311.16015	311.15994	-0.67	
2	4.56	C10H13O3N	196.09682	196.0968	-0.1	
3	5.4	C16H24O5N2	325.1758	325.17551	-0.89	
4	6	C16H24O5N4	353.18195	353.18179	-0.45	
5	7.21	C13H17NO6	284.11286	284.11282	-0.15	
6	7.43	C14H18N2O6	311.12376	311.12366	-0.33	
7	7.78	C14H17NO7	312.10778	312.1077	-0.25	
8	7.81	C15H20N2O6	325.13941	325.13937	-0.13	
9	8.62	C12H15NO5	254.1023	254.10219	-0.43	
10	9.18	C14H19NO6	298.12851	298.12845	-0.21	

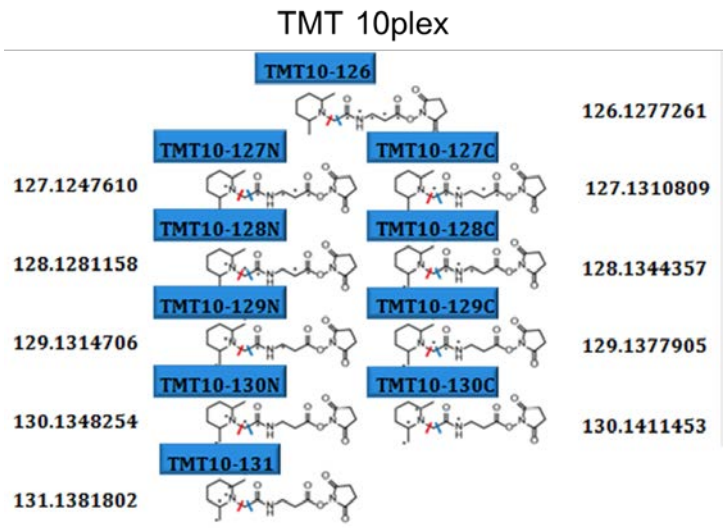
No.	RT (min)	Formula	Theoretical Mass	Measured Mass	Precision (ppm)	Structure
11	9.24	C15H19NO7	326.12343	326.12347	0.13	
12	9.67	C13H17NO5	268.11795	268.11792	-0.11	
13	10.03	C10H12O4	197.08084	197.08084	0.03	
14	14.05	C22H35O6N3	438.25986	438.25983	-0.07	
15	17.23	C15H21NO5S	328.12132	328.12128	-0.12	
16	17.63	C15H21NO5	296.14925	296.14902	-0.77	
17	18.39	C21H30O8N2	439.20749	439.20743	-0.14	
18	19.94	C21H22N2O5	383.16015	383.16003	-0.31	
19	20.66	C16H23NO5	310.1649	310.16467	-0.74	
20	21.34	C16H23NO5	310.1649	310.16479	-0.35	
21	26.2	C22H34O6N2	423.24896	423.24863	-0.78	

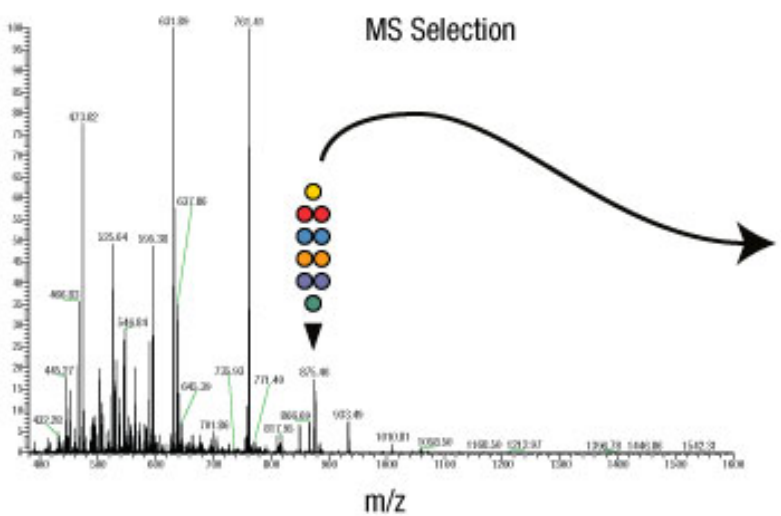
Li *et al.* Thermo AN64427. 2015

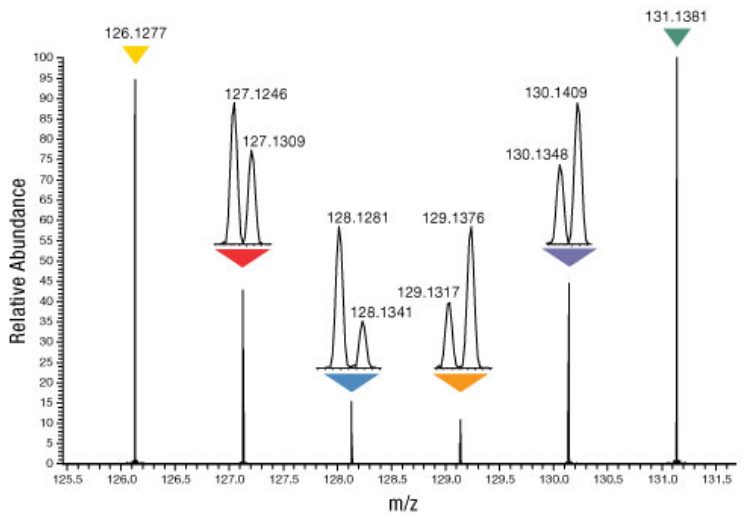
Application in Proteomics

- Peptide Labeling (multiple studies with a single experiment)

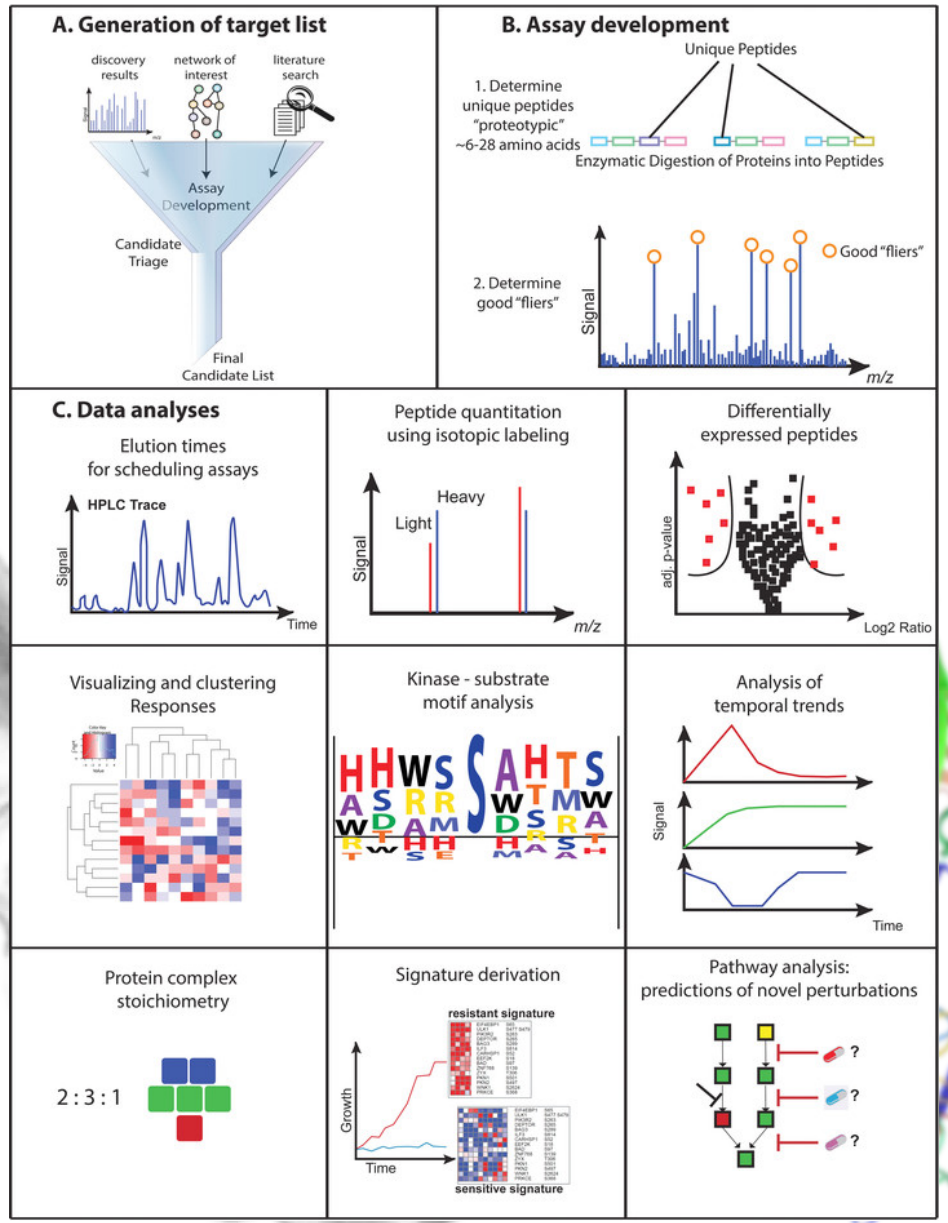






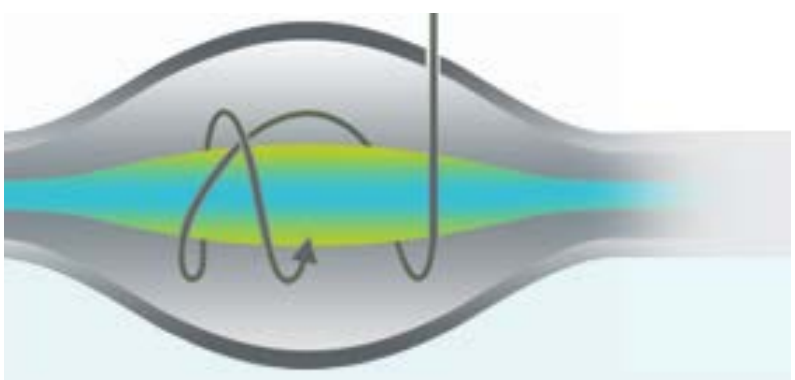


Application in Proteomics



- Protein Identification
- Expression Level of Protein
- Protein Modification
- Protein Localization
- Peptide Mapping
- Pathways
- Etc.

LCMS Technology and Applications



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