thermo scientific

SciSpec

Orbitrap™ Technology HRAMS Potentiates Metabolomic Research

Sensitive but Stable High Resolution Mass Spectrometry

by Tosapol Anukunwithaya, PhD

MS Product Specialist

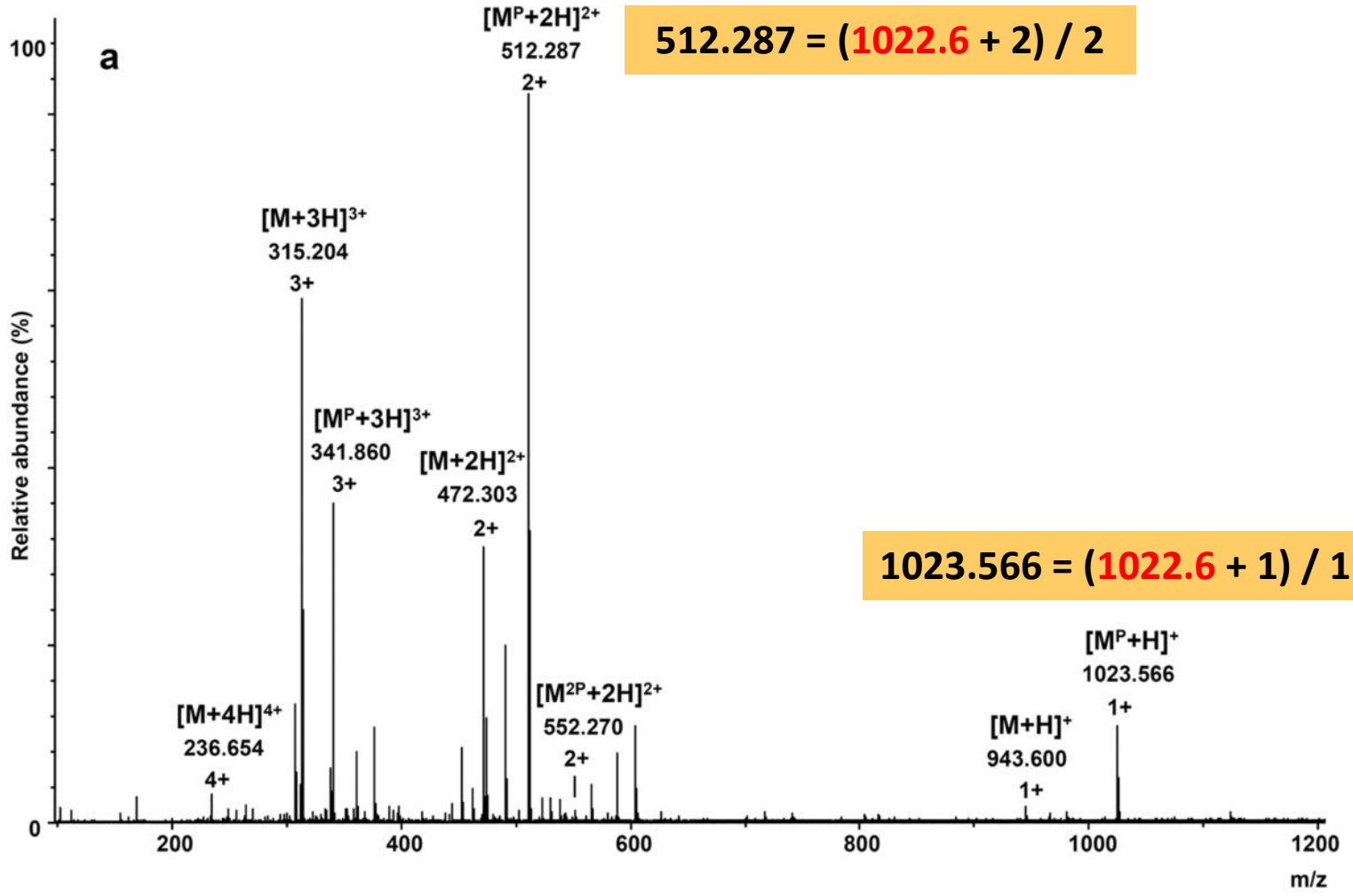
FUNDAMENTAL OF MASS SPECTROMETER

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.

Mass Spectra – *m/z*

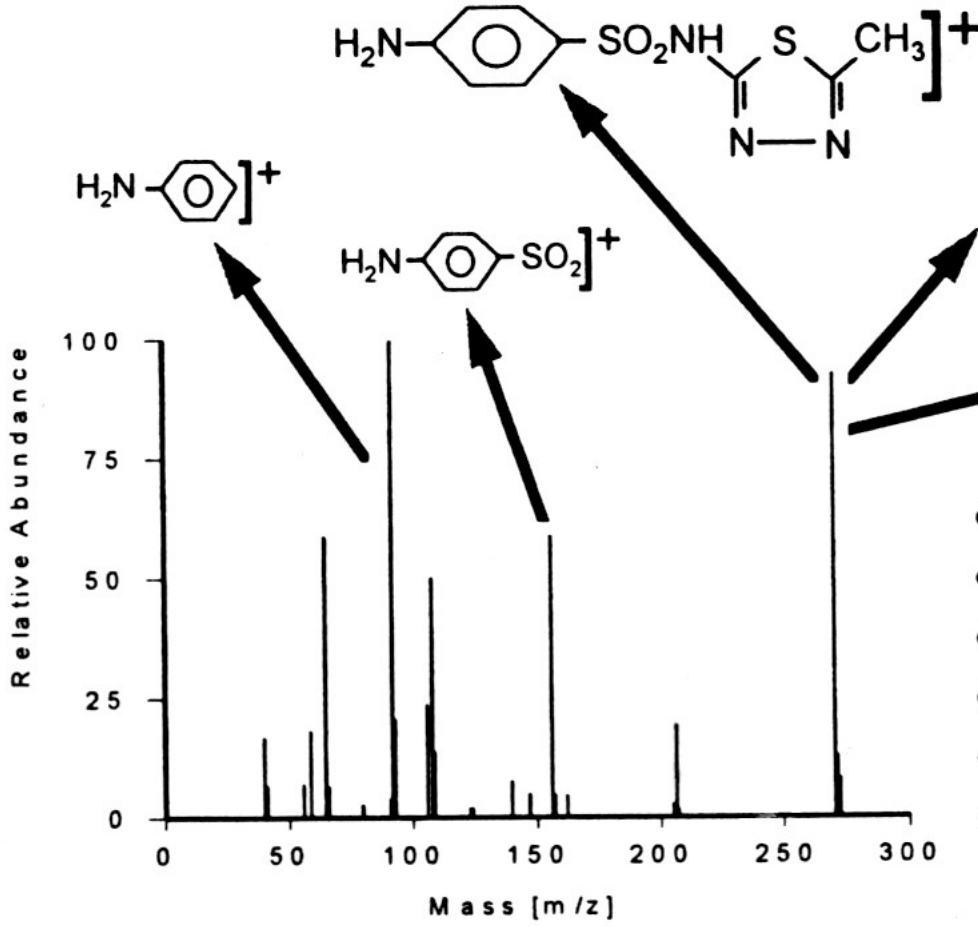
m/z **= (molecular weight + charge) / charge**



512.287 = (1022.6 + 2) / 2

1023.566 = (1022.6 + 1) / 1

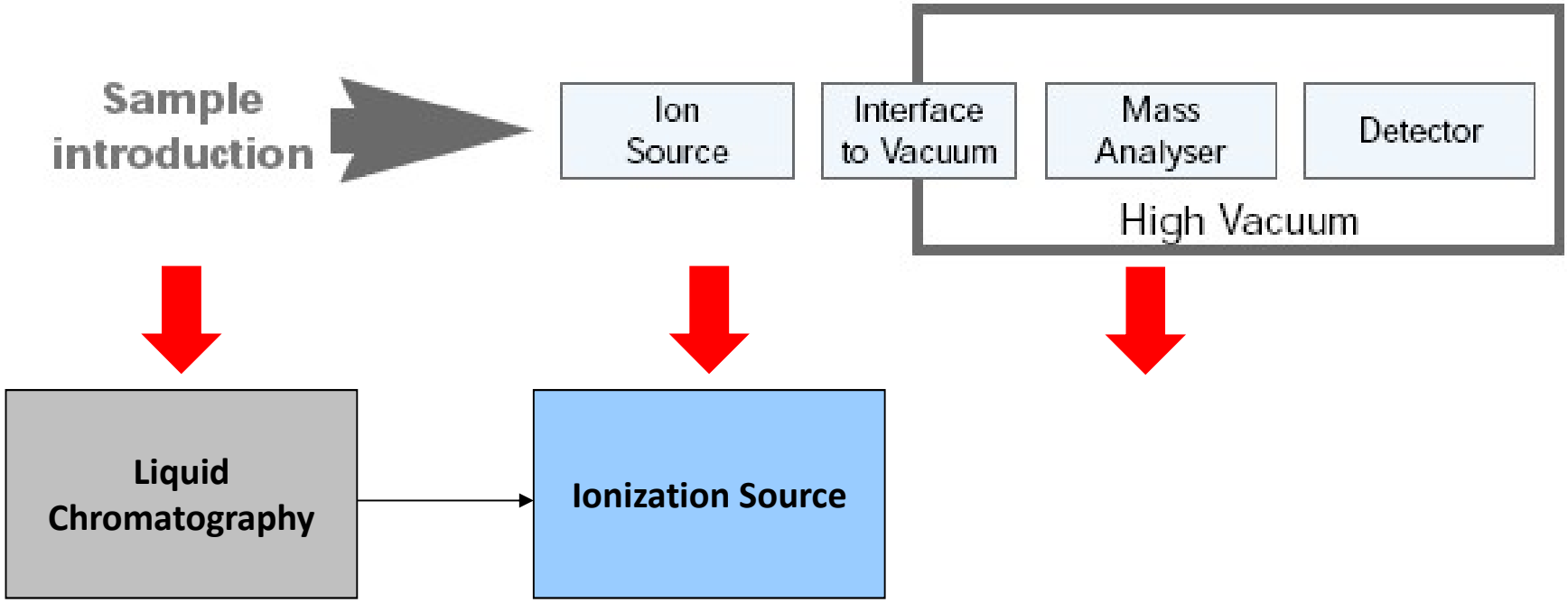
Information Rich Data



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 Spectrometry – Block Diagram



- Columns
- Solvent systems

- ESI
- APCI
- APPI

ION SOURCE

IONIZATION TECHNIQUES

Your Scientific Specialist

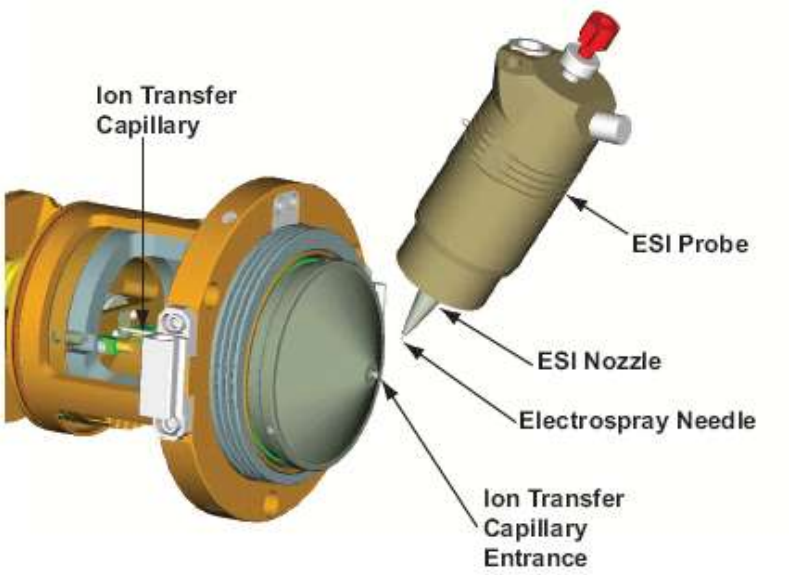
Ionization

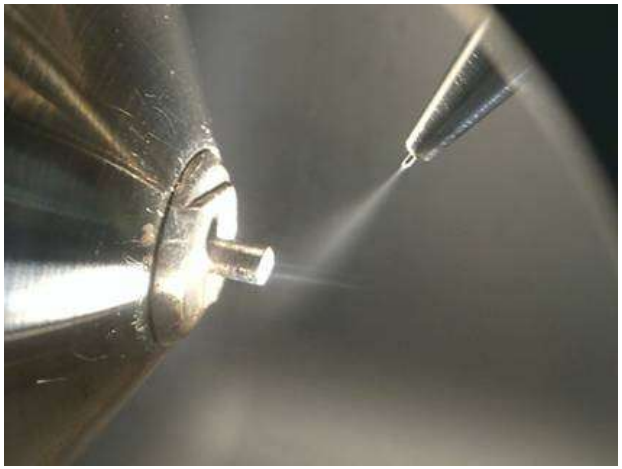
- **Ion sources :** Converts sample molecules (neutral) into charged molecules or molecular ions.

- **Type of ionization techniques**
  - Atmospheric Pressure Ionization (API)
    - Electrospray Ionization (ESI)
    - Atmospheric Pressure Chemical Ionization (APCI)
    - Atmospheric Pressure Photo Ionization (APPI)
  - Matrix Assisted Laser Desorption Ionization (MALDI)

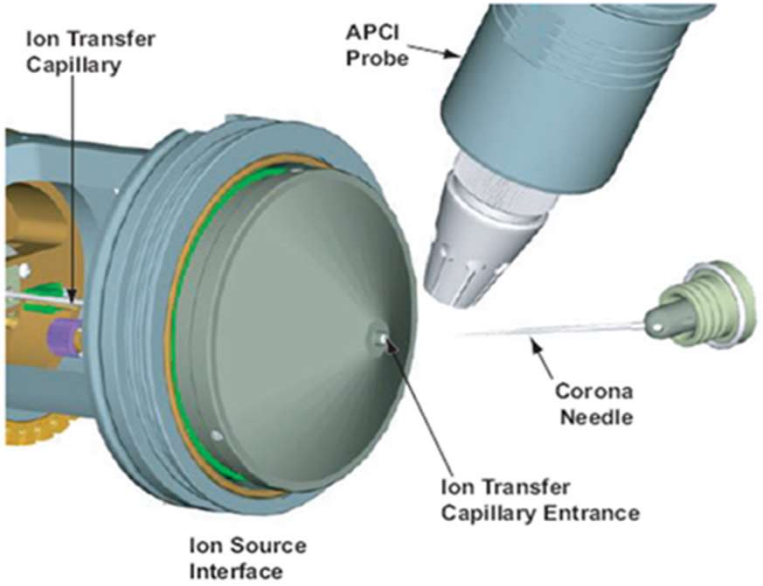
Atmospheric Pressure Ionization (API)

ESI

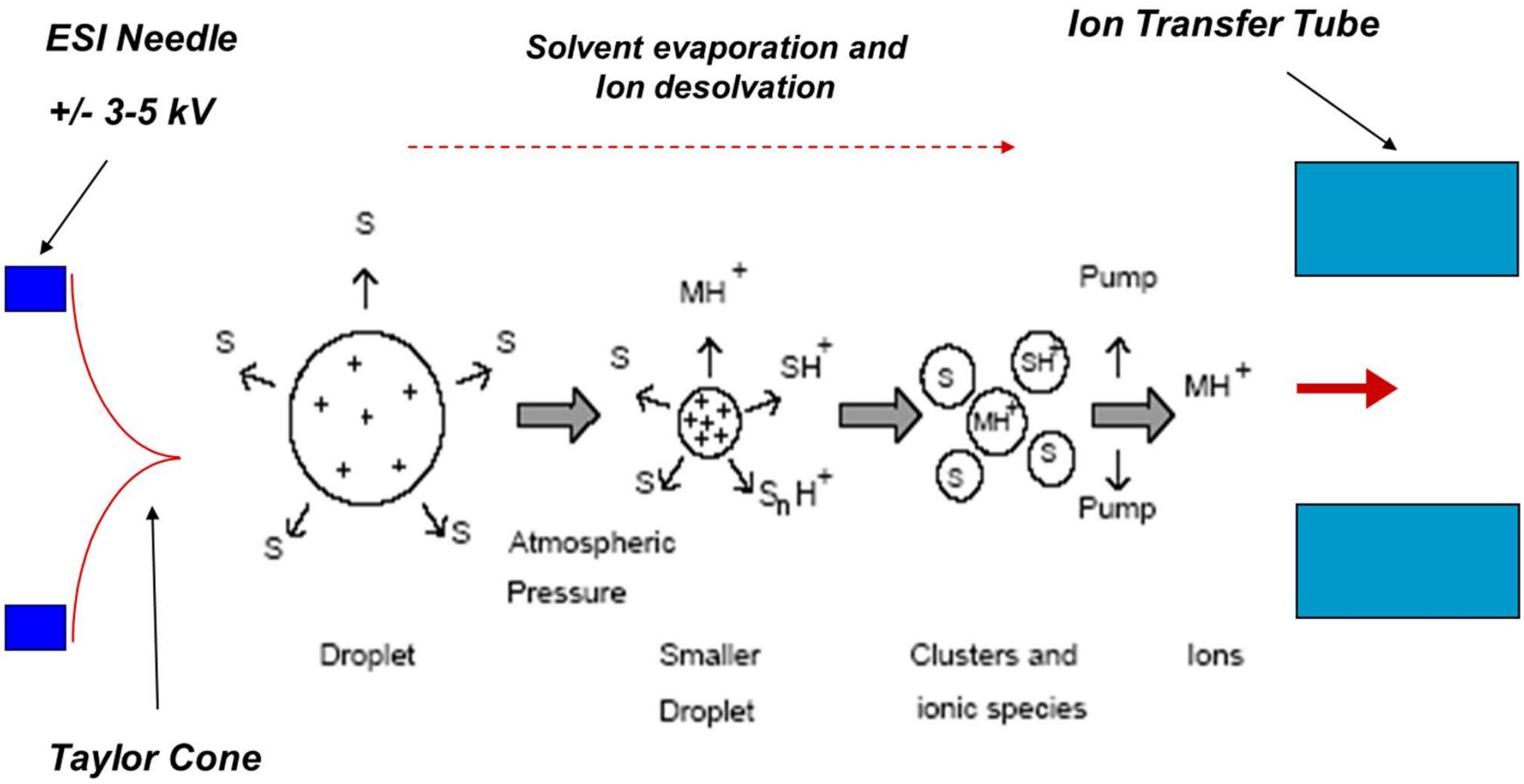




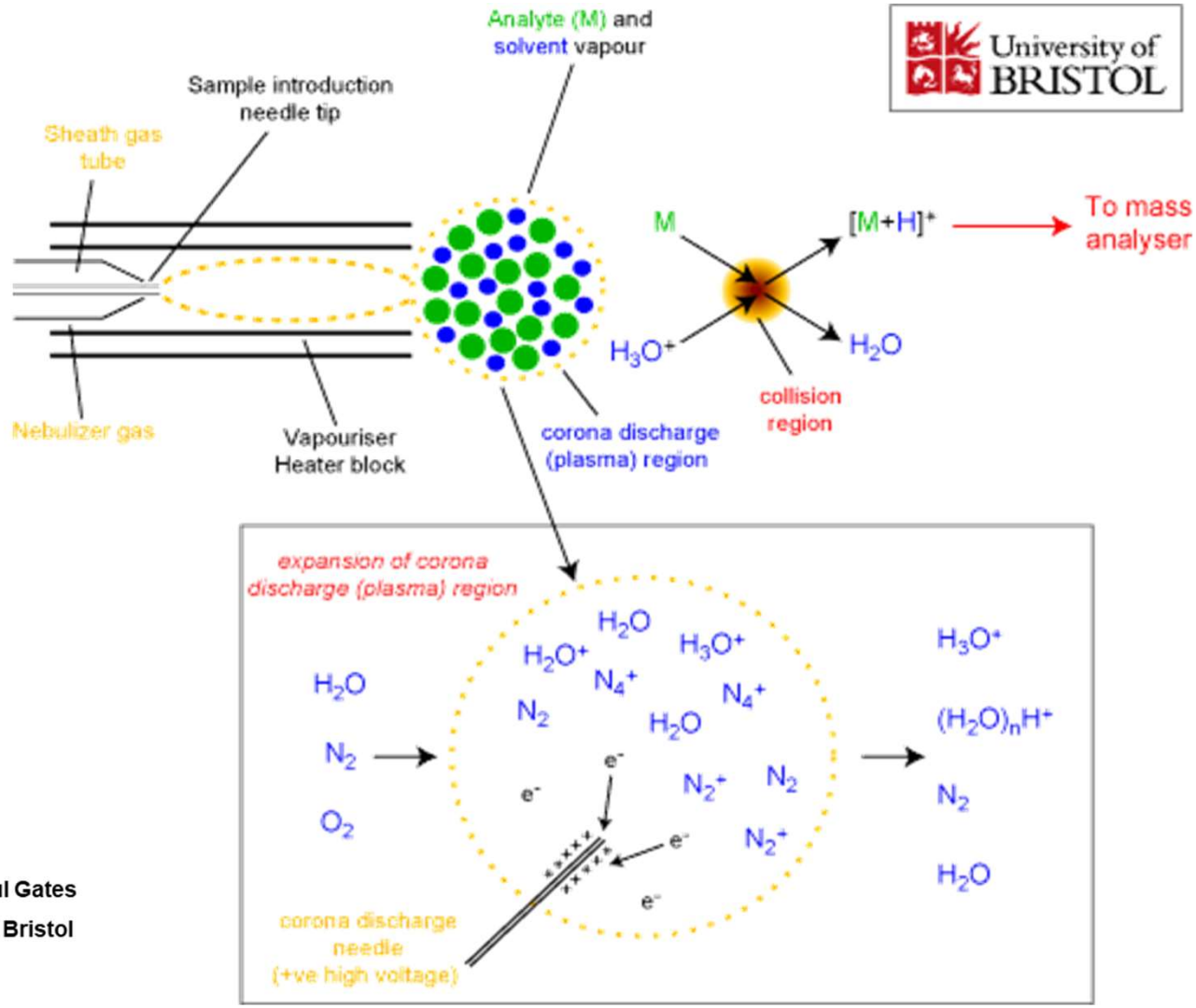
APCI



Electrospray Ionization (ESI)

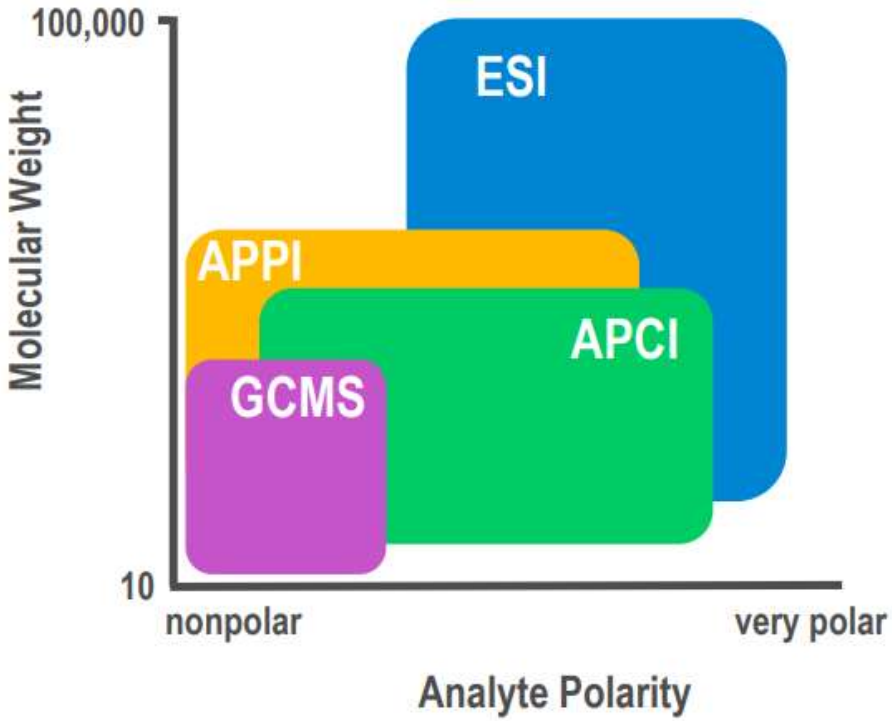


Atmospheric Pressure Chemical Ionization (APCI)



Atmospheric Pressure Ionization (API)

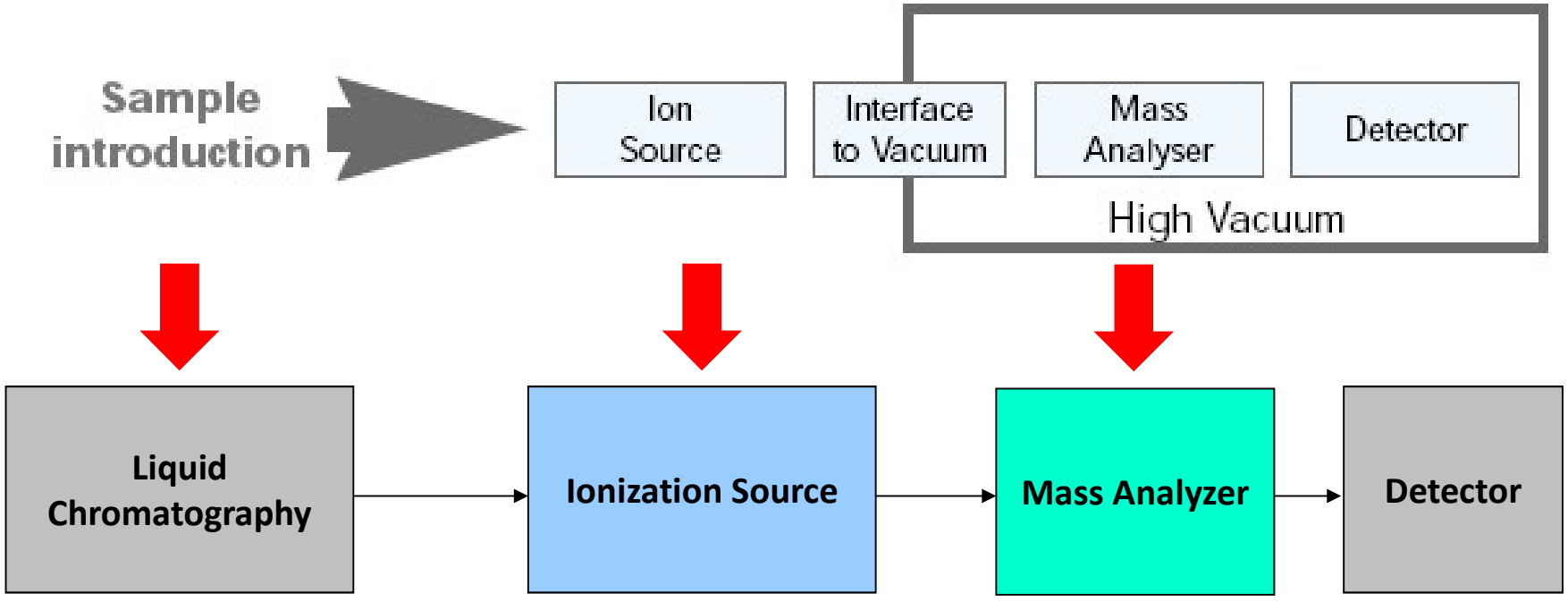
Polarity of analytes determines the ionization source.



ESI	Electrospray ionization
APPI	Atmospheric pressure photo ionization
APCI	Atmospheric pressure chemical ionization
GC/MS	Gas chromatography / Mass spectrometry

MASS ANALYZERS

Mass Spectrometry – Block Diagram

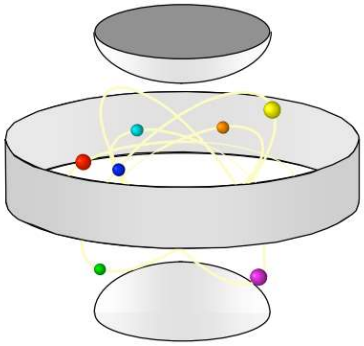


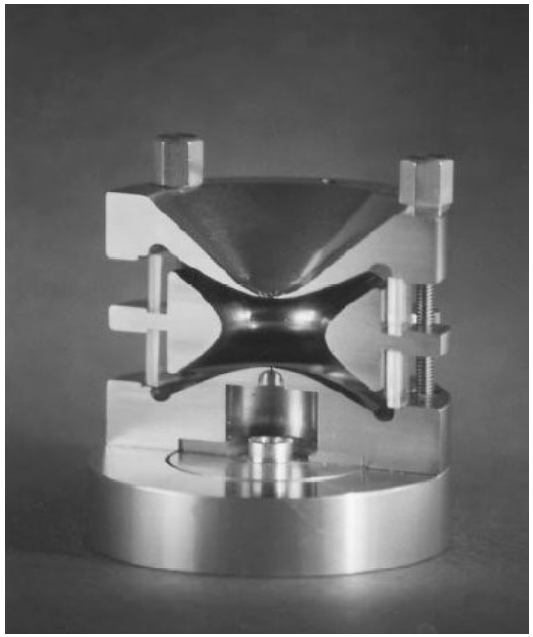
- Ion-Traps
- Triple Quadrapoles
- Time-of-flight
- Orbitrap

Mass Analyzers Separate Ions

- Operate under high vacuum environment (prevent ions from bumping into gas molecules).
- Actually measure mass-to-charge ratio of ions (m/z).
- Key specifications are **resolution**, mass measurement **accuracy**, and **sensitivity**.
- Several kinds exist: for ion traps, quadrupole, time-of-flight and orbitrap are most used.

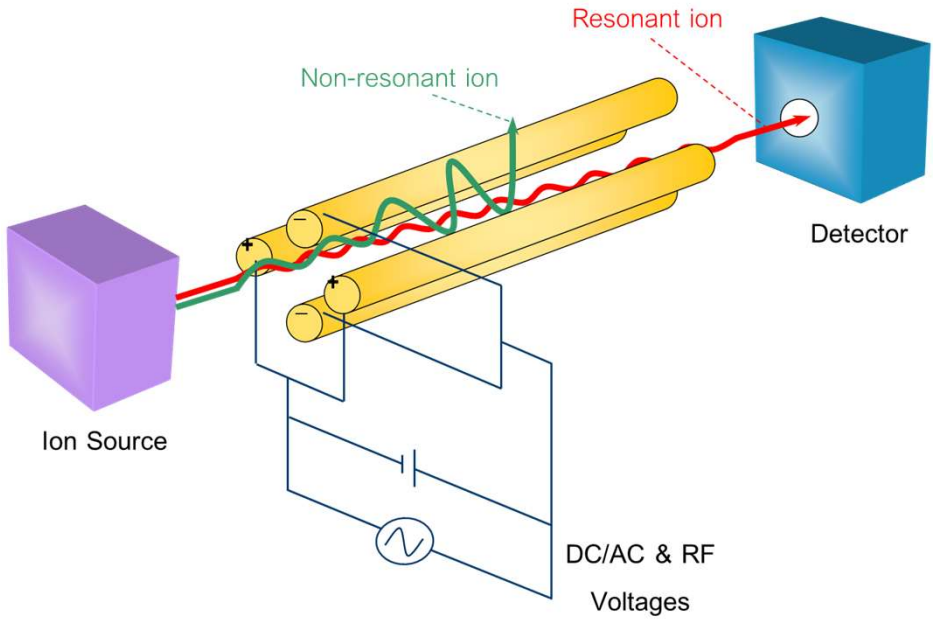
Mass Analyzer : Ion Trap





- Consists of ring electrode and two end caps
- Principle very similar to quadrupole
- Ions stored by RF & DC fields
- Scanning field can eject ions of specific m/z

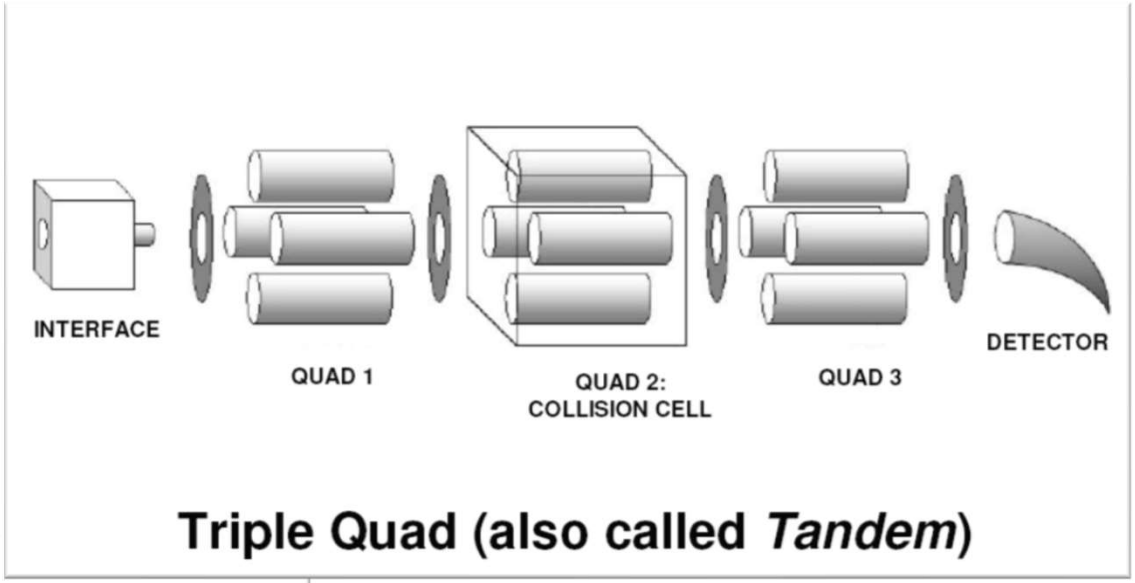
Mass Analyzer : Quadrupole

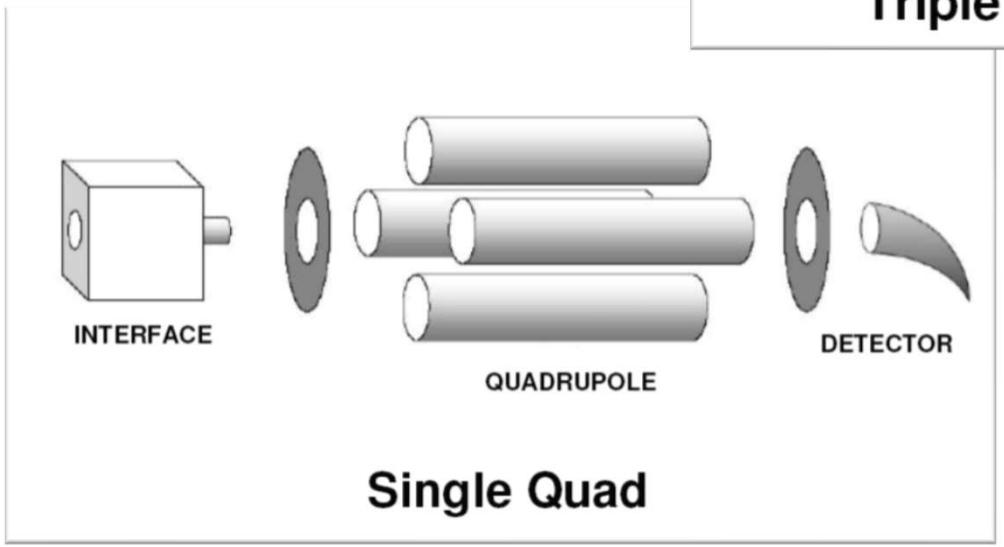


Uses a combination of RF and DC voltages to operate as a mass filter.

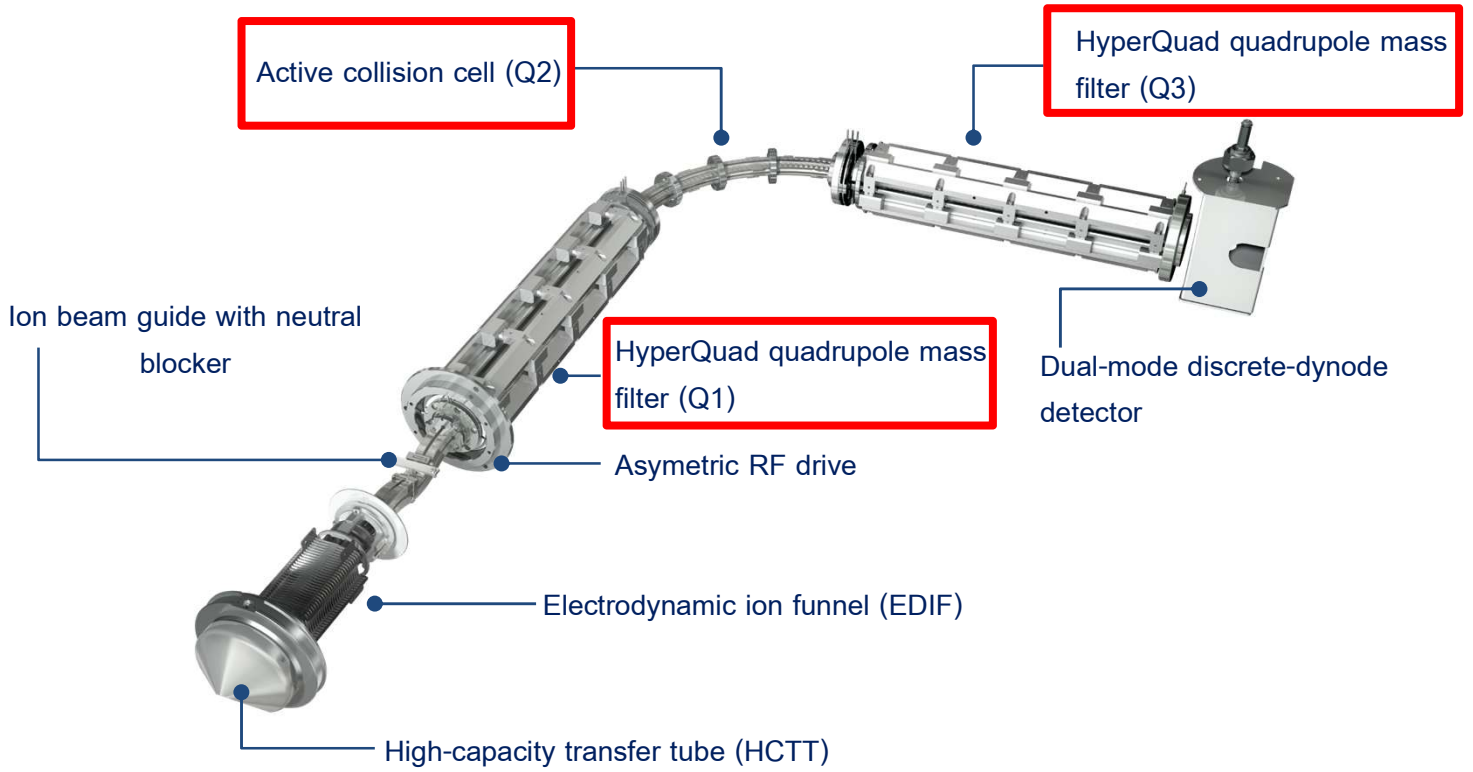
- Has four parallel metal rods.
- Lets one mass pass through at a time.
- Can scan through all masses or sit at one fixed mass.

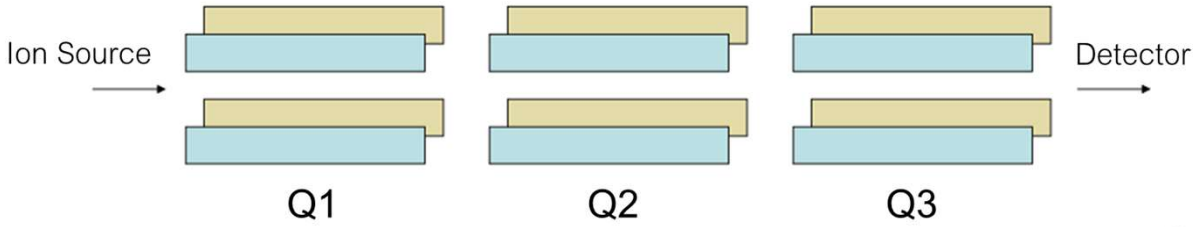
Quadrupole → Triple Quadrupoles (QqQ)





Mass Analyzer : Triple Quadrupoles (QqQ)





Mass Analyzer : Triple Quadrupoles (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

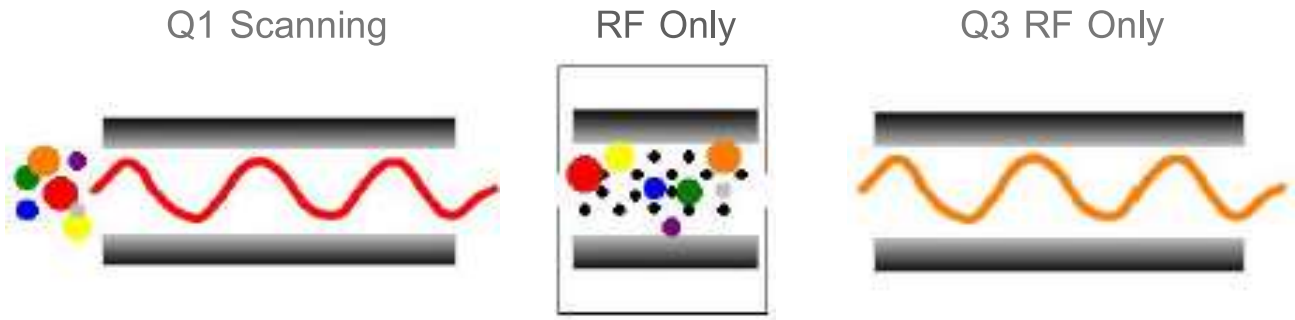
Mass Analyzer : Triple Quadrupoles (QqQ)

Full Scan Mode

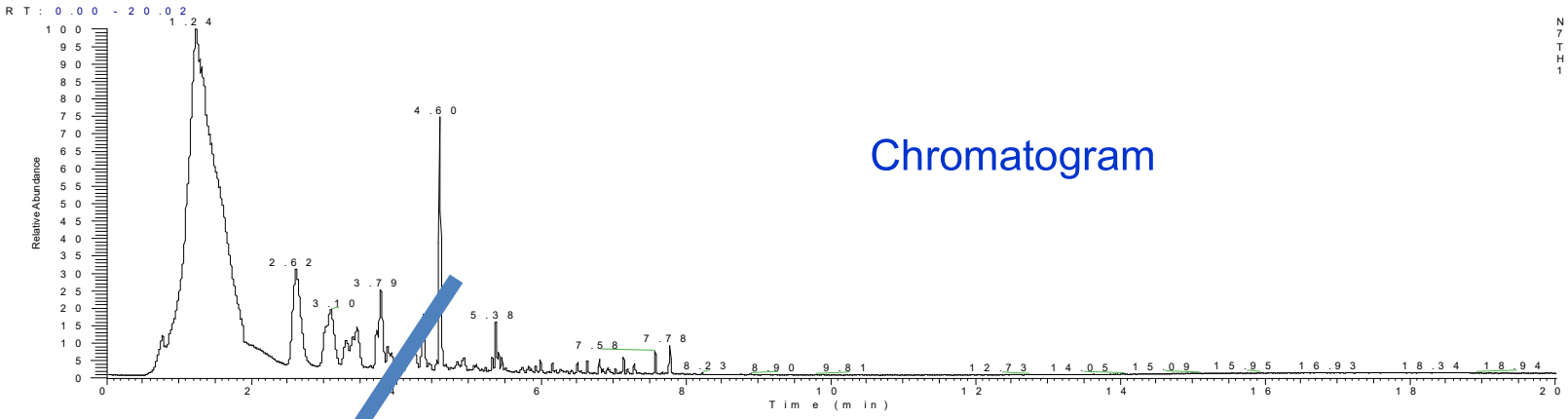
Purpose: Survey scan of a chromatographic peak

Scan Mode	Q1	Q2	Q3	Purpose
Full Scan	Scanning	Pass All	Pass All	MW Info.

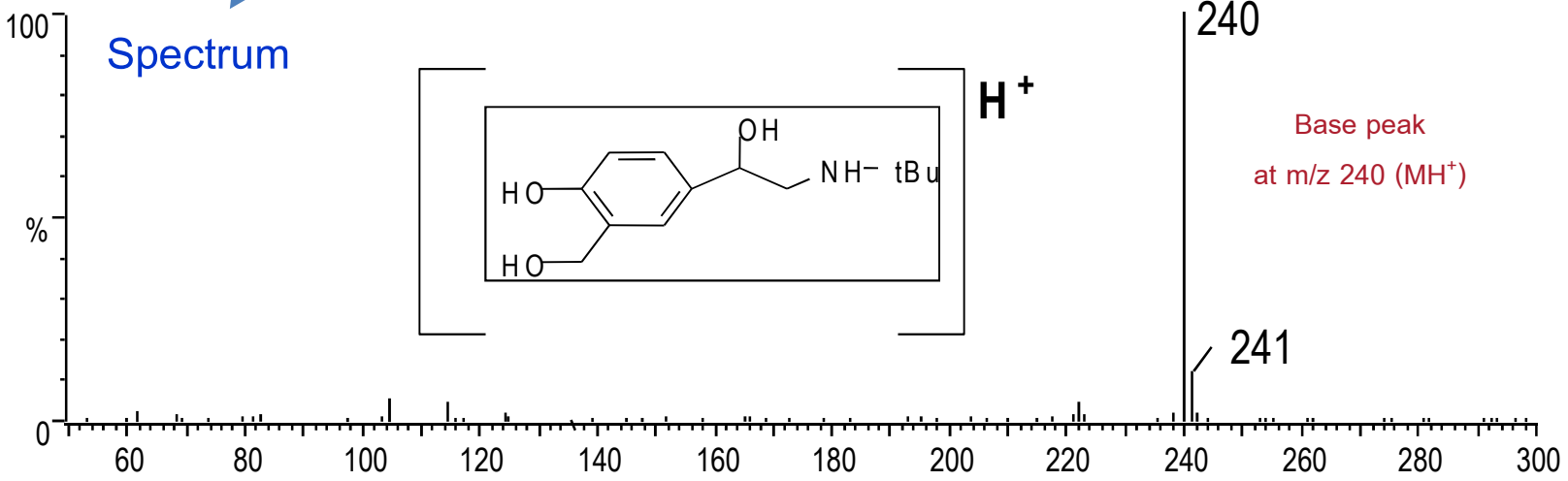
Full scan Q1:



Mass Analyzer : Triple Quadrupoles (QqQ)



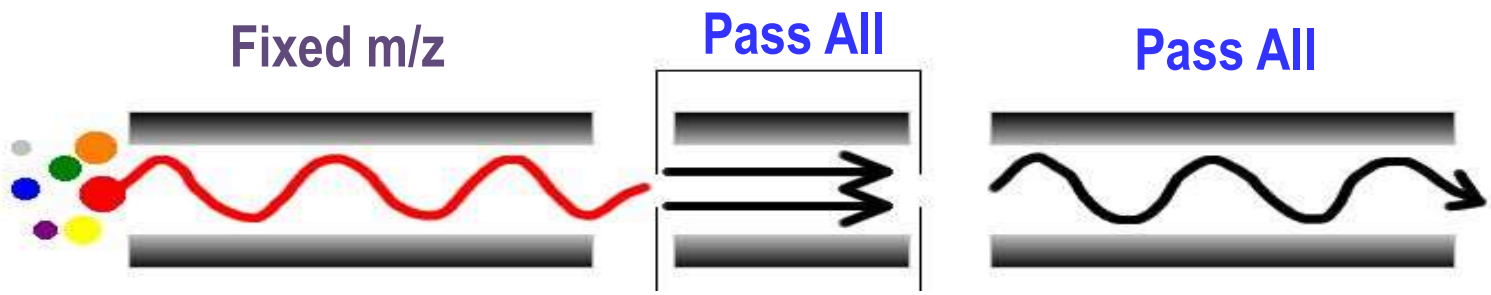
One Click



Mass Analyzer : Triple Quadrupoles (QqQ)

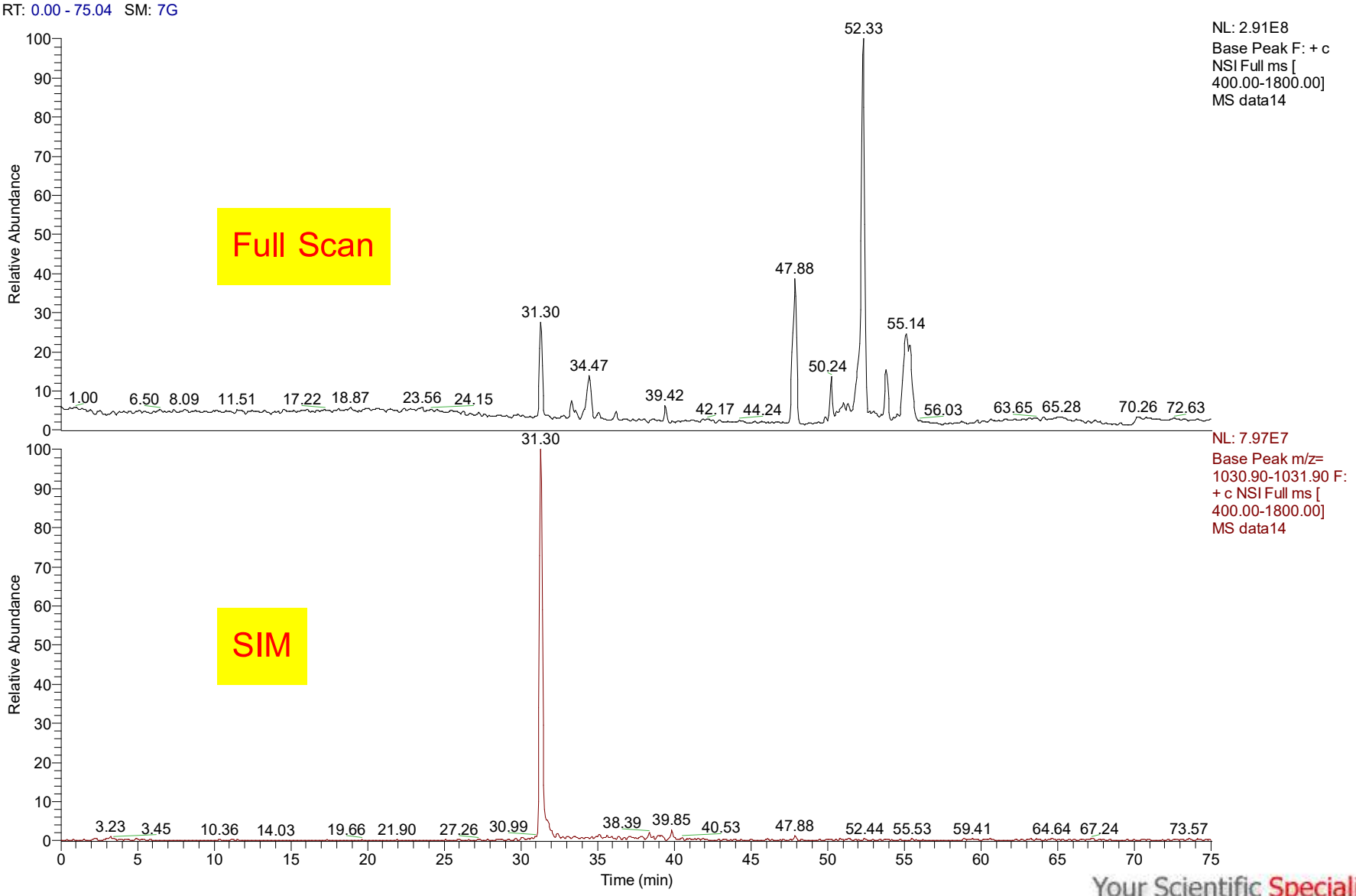
SIM is in essence a full scan acquisition on a relatively narrow mass window (defined as center mass / scan width)

Scan Mode	Q1	Q2	Q3	Purpose
SIM (Selected Ion Monitoring)	Fixed m/z	Pass All	Pass All	Quantitation



- Advantages
  - Targeted analyte monitoring
  - High duty cycle
- Disadvantages
  - Can suffer from interferences
  - Not as sensitive or selective as SRM

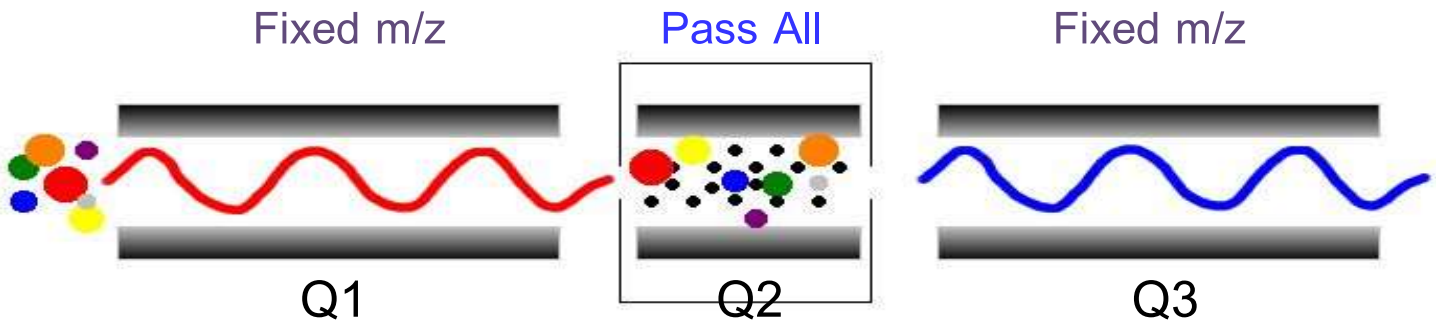
Mass Analyzer : Triple Quadrupoles (QqQ)



Your Scientific Specialist

Mass Analyzer : Triple Quadrupoles (QqQ)

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



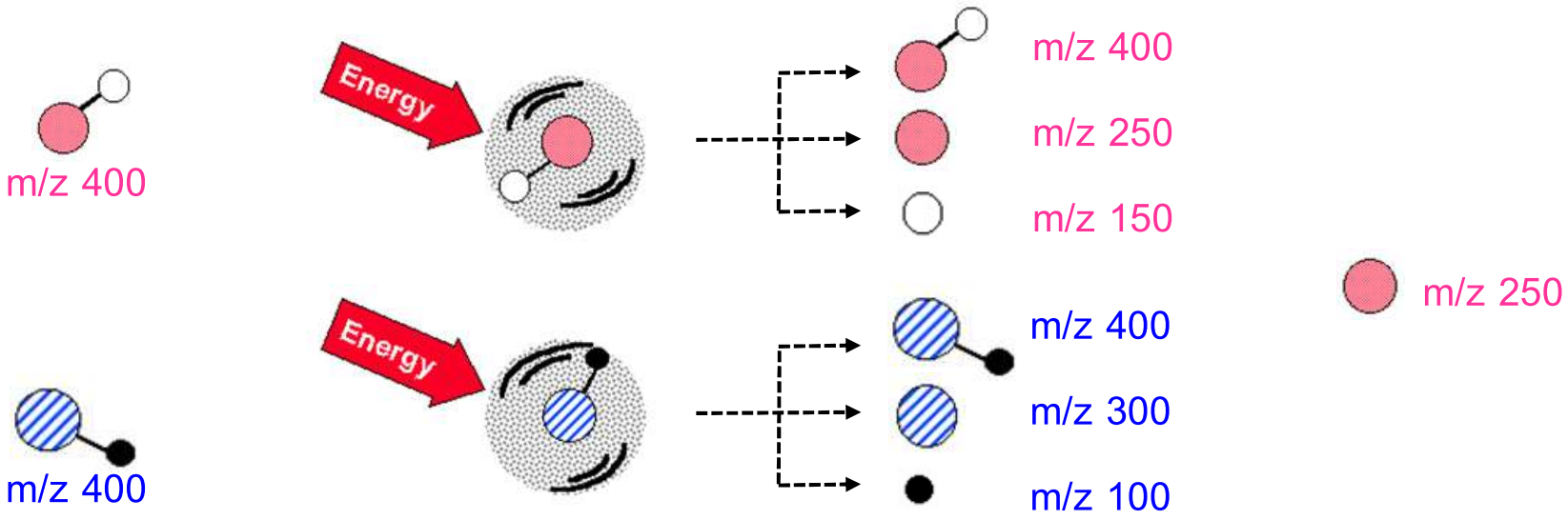
Advantages

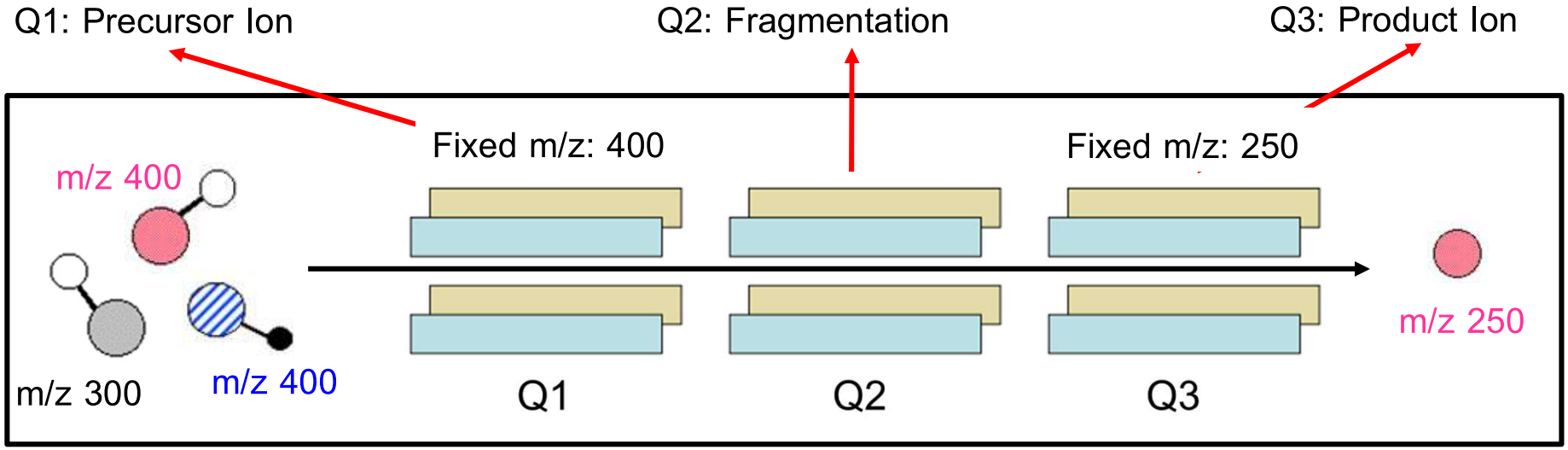
- Targeted analyte monitoring
- High duty cycle
- “Simultaneous” monitoring of multiple transitions

Disadvantages

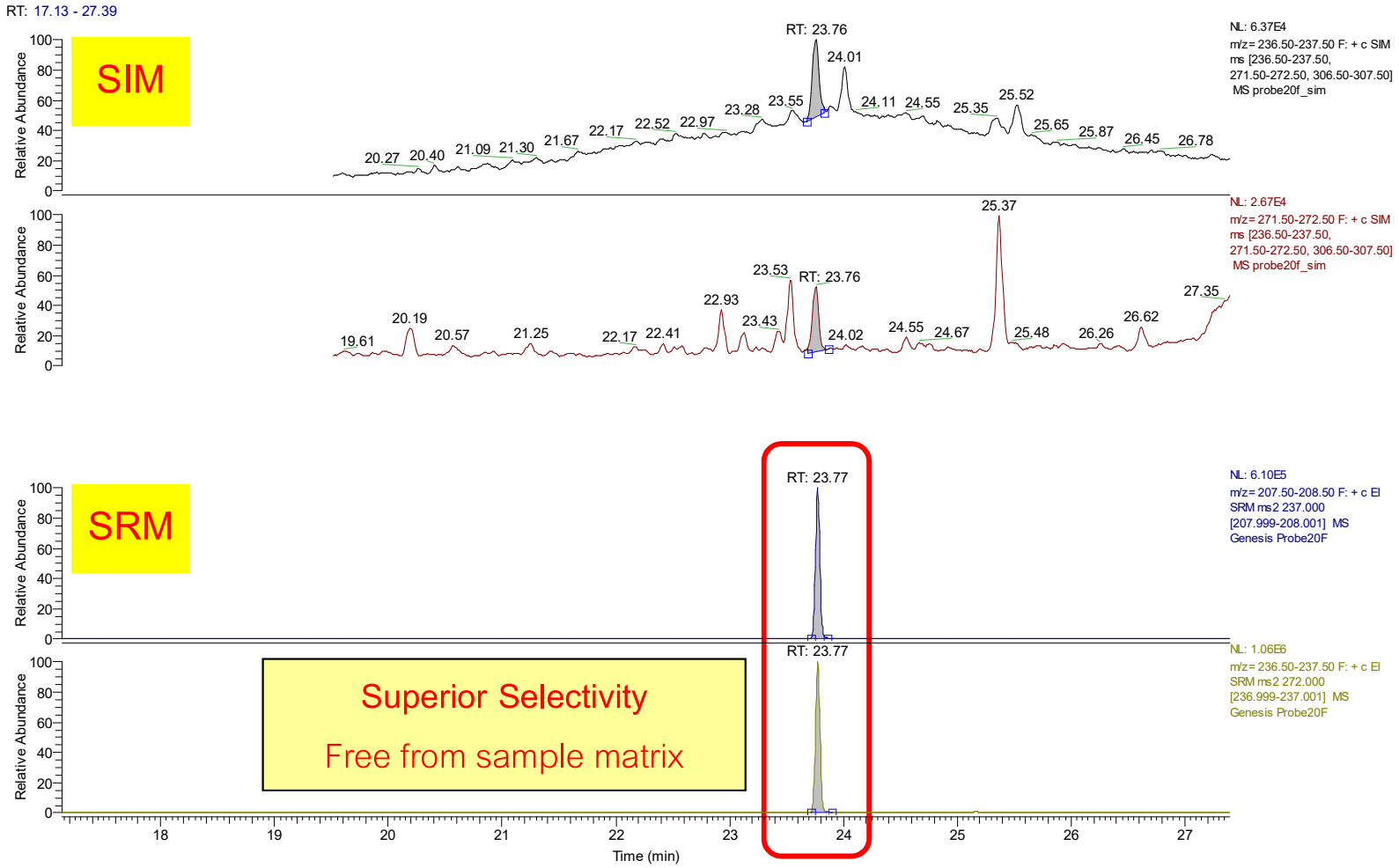
- No structural information

Mass Analyzer : Triple Quadrupoles (QqQ)

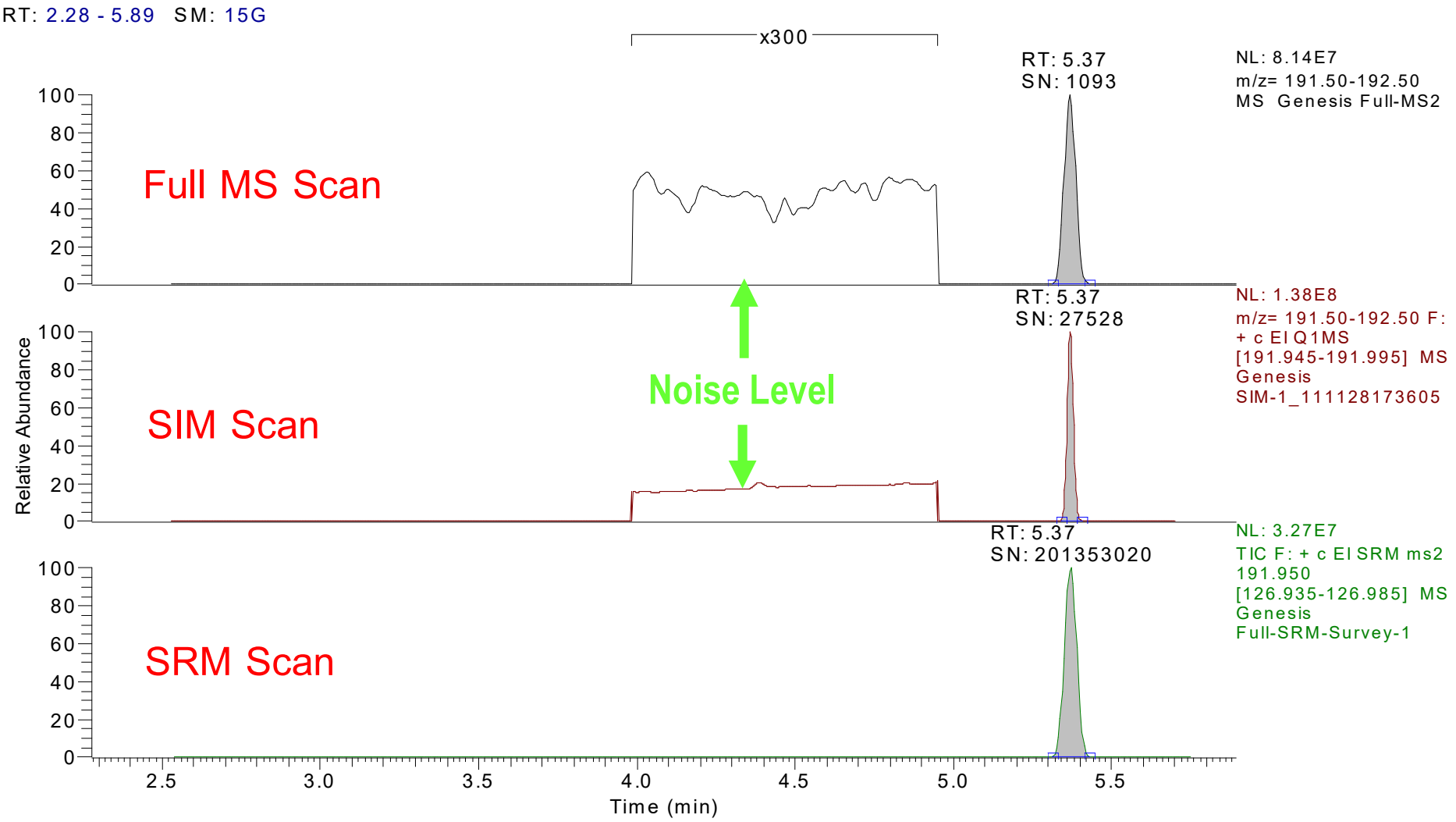




Mass Analyzer : Triple Quadrupoles (QqQ)

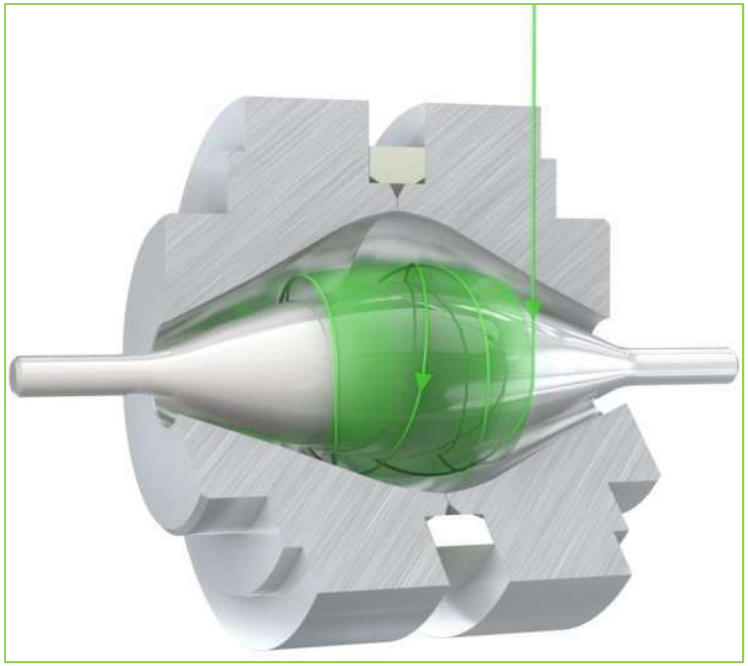


Mass Analyzer : Triple Quadrupoles (QqQ)



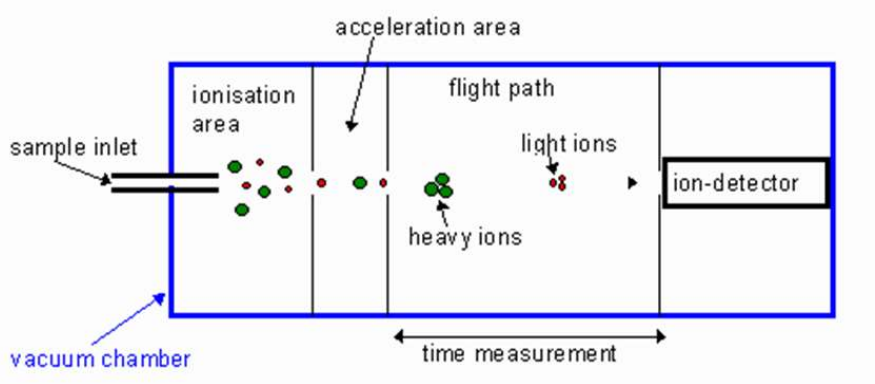
Mass Analyzer : Triple Quadrupoles (QqQ)

http://www.youtube.com/watch?v=LFB14D8pkoc



HIGH RESOLUTION MASS ANALYZERS

Mass Analyzer : Time-of-flight (TOF)

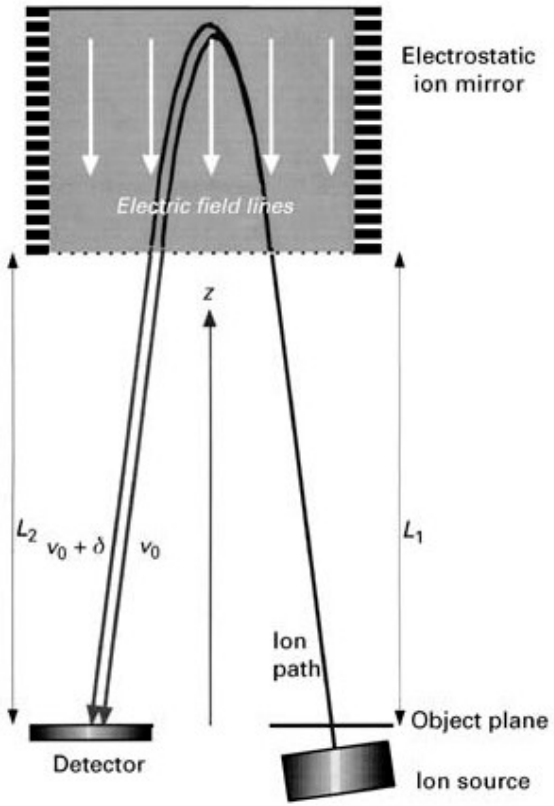


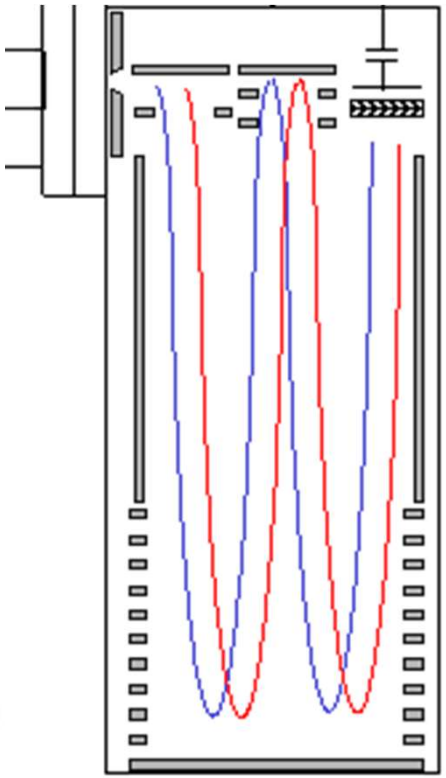
- A package of ions is accelerated by a potential **V** into a field free flight tube.
- The time $\mathbf{t_{flight}}$ needed for ions to reach a detector placed at distance **d** is measure.

- Small ions have higher velocity than large mass ions therefore their time of flight in the tube are different and related to m/z ratio

Mass Analyzer : Time-of-flight (TOF)

- Resolution limited by:
  - **Length of TOF flight tube**
  - Kinetic energy distribution
  - Propagation delay in detector
- Sensitivity limited by:
  - Ion stability
  - Ion transfer efficiency

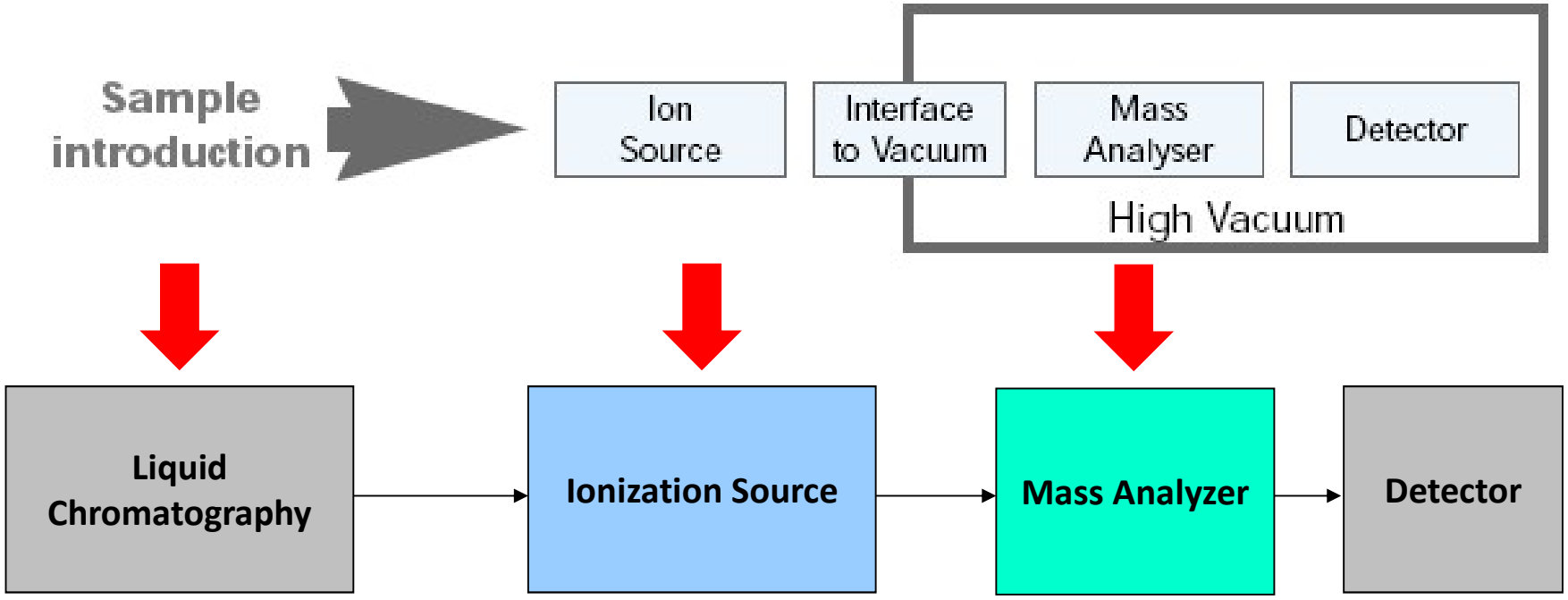




ORBITRAP MASS ANALYZER

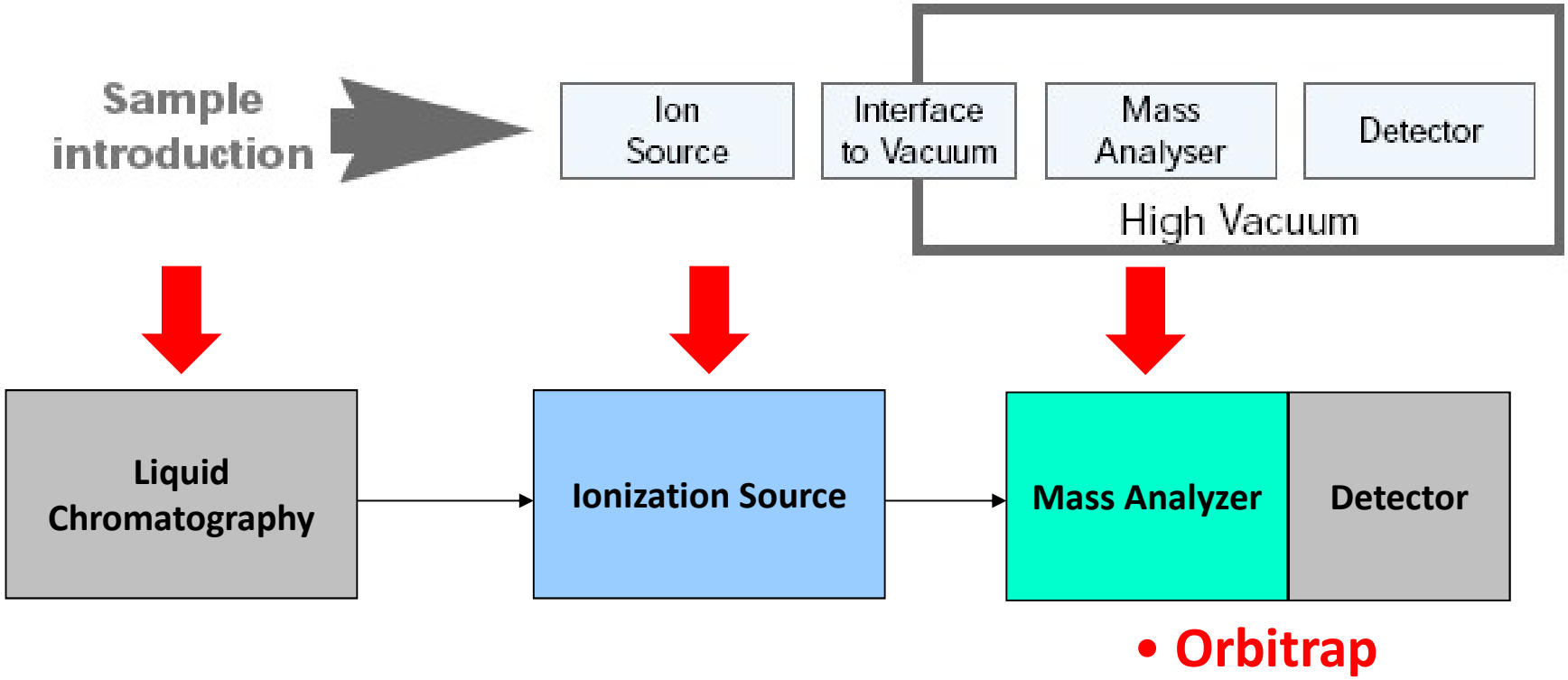
Alexander Makarov

Mass Spectrometry – Block Diagram



- Ion-Traps
- Triple Quadrapoles
- Time-of-flight
- Orbitrap

Mass Spectrometry – Block Diagram



Mass Analyzer : Orbitrap™ Technology

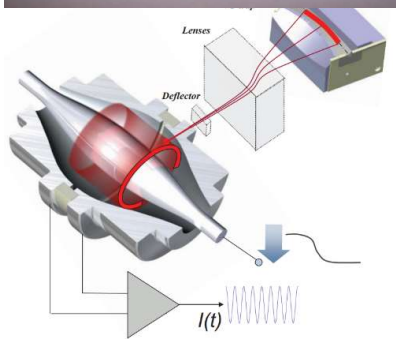
Anal. Chem. **2000,** *72,* 1156−1162

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.



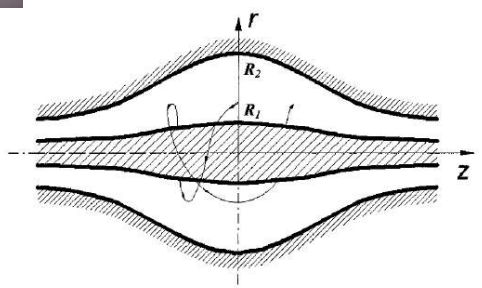
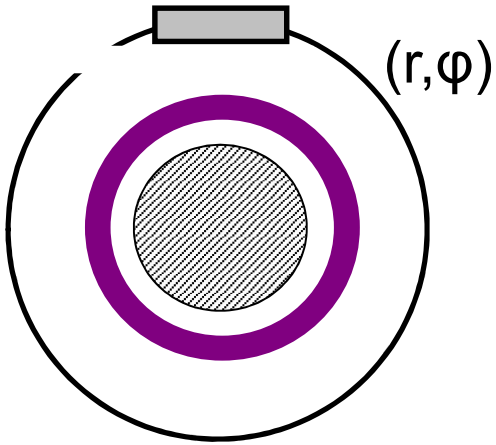
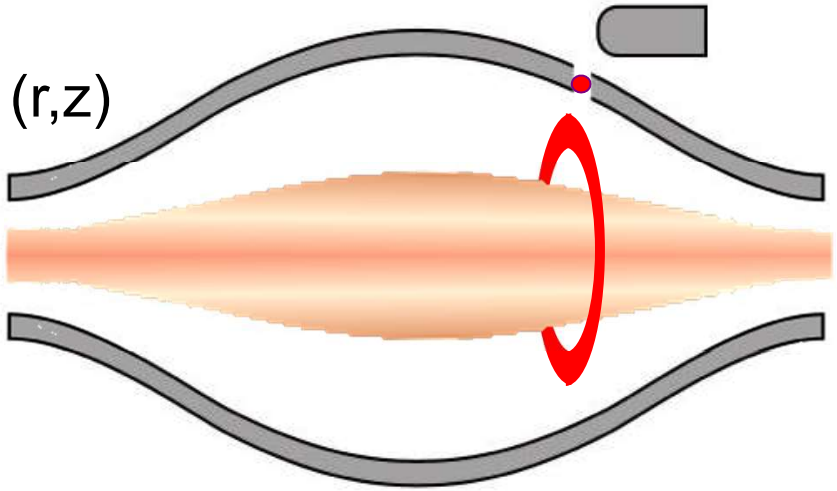


Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

Mass Analyzer : Orbitrap™ Technology

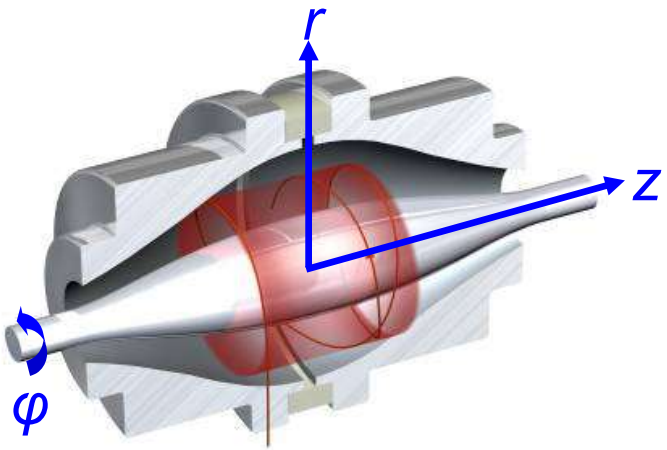
- An ion packet of a selected m/z enters the field.
- Increasing voltage squeezes ions.
- Voltage stabilises and ion trajectories are also stabilized.
- Angular spreading forms a **rotatating ring**.





Mass Analyzer : Orbitrap™ Technology

- Ions trapped in an **electrostatic field.**
- Central electrode kept on high voltage.
- Outer electrode is split and able to pick up an **image current** induced by ion packets moving inside the trap.



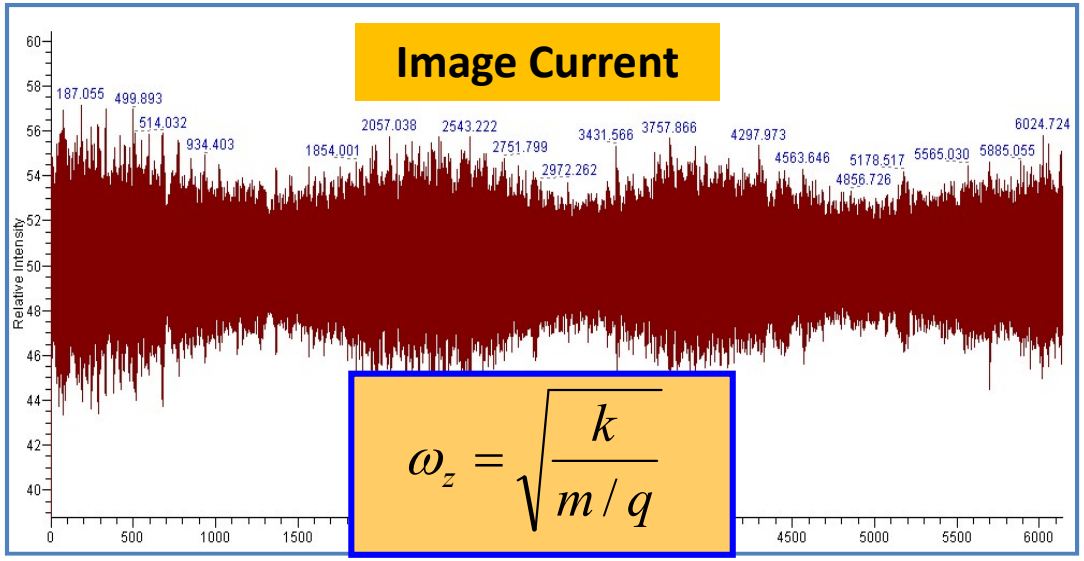
Characteristic frequencies:

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

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

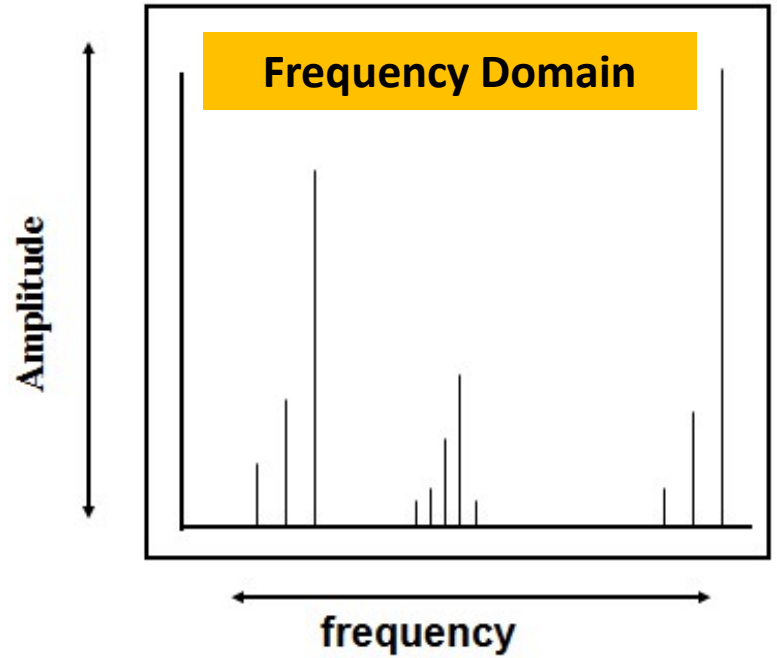
A. Makarov, Anal. Chem 2000, 1156-1162

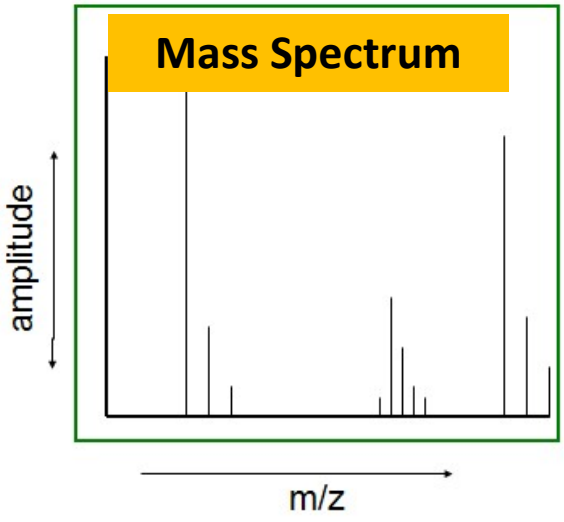
Mass Analyzer : Orbitrap™ Technology



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

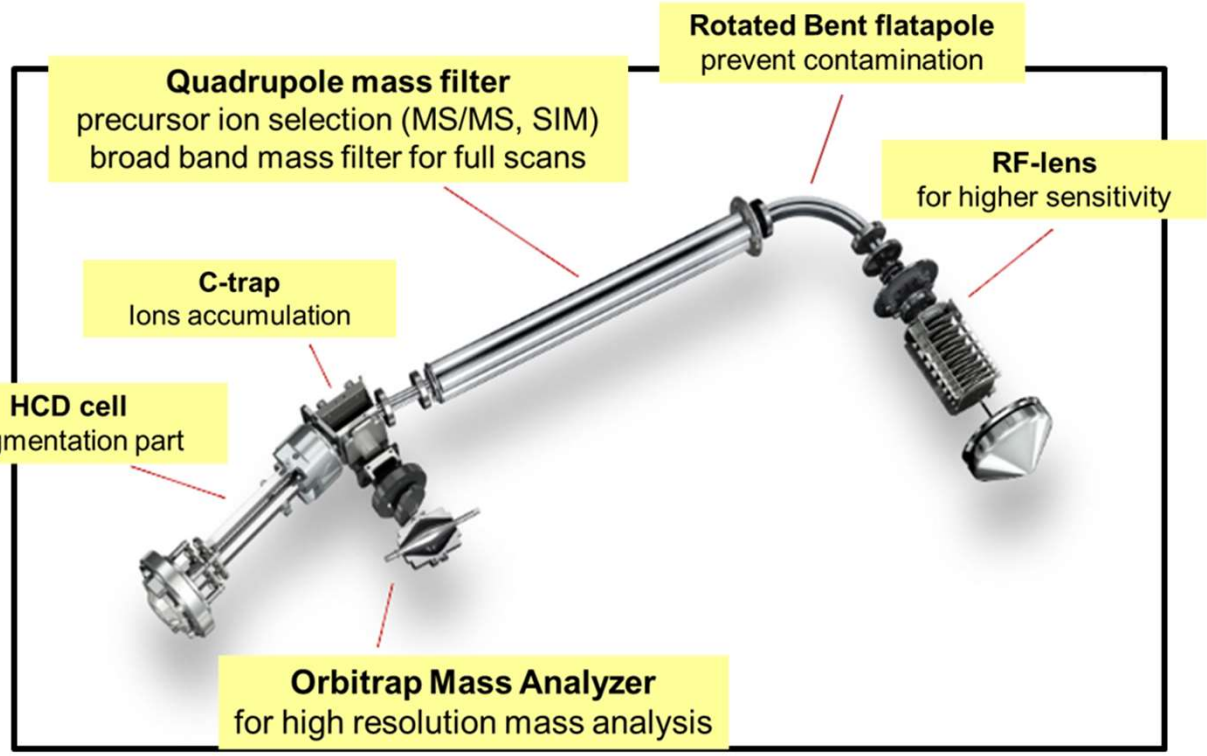
Fourier Transform





Mass Analyzer : Orbitrap™ Technology

Thermo SCIENTIFIC



Mass Analyzer : Orbitrap™ Technology

http://planetorbitrap.com/q-exactive-plus#.WmoCMeRG3IX

thermo scientific

SciSpec

Orbitrap™ Technology HRAMS Potentiates Metabolomic Research

Sensitive but Stable High Resolution Mass Spectrometry

by Tosapol Anukunwithaya, PhD

MS Product Specialist

Orbitrap™ Technology HRAMS

- HRAMS = **H**igh **R**esolution **A**ccurate **M**ass Spectrometry

- **Mass Resolution** = ability of a mass spectrometer to distinguish between ions of nearly equal m/z ratios (isobars).

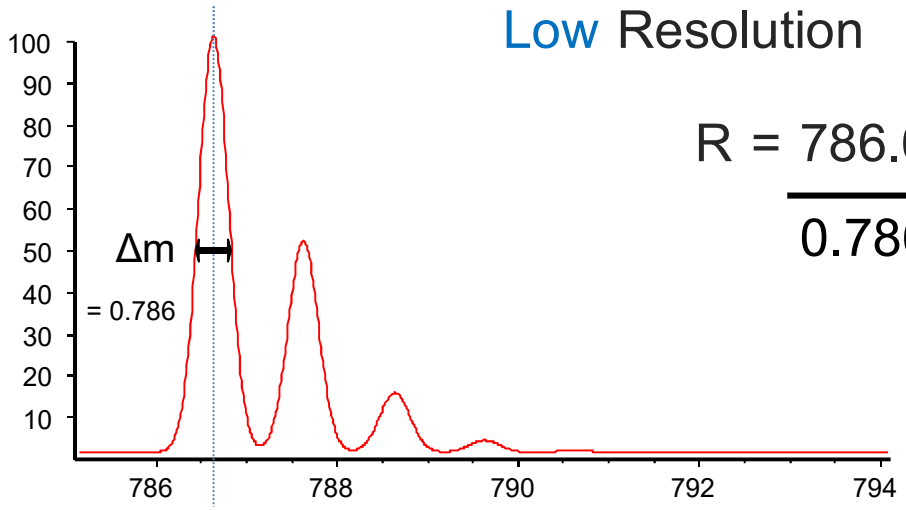
- **Mass Accuracy** = the precision of which the mass is measured by the mass spectrometer.

Mass Resolution

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

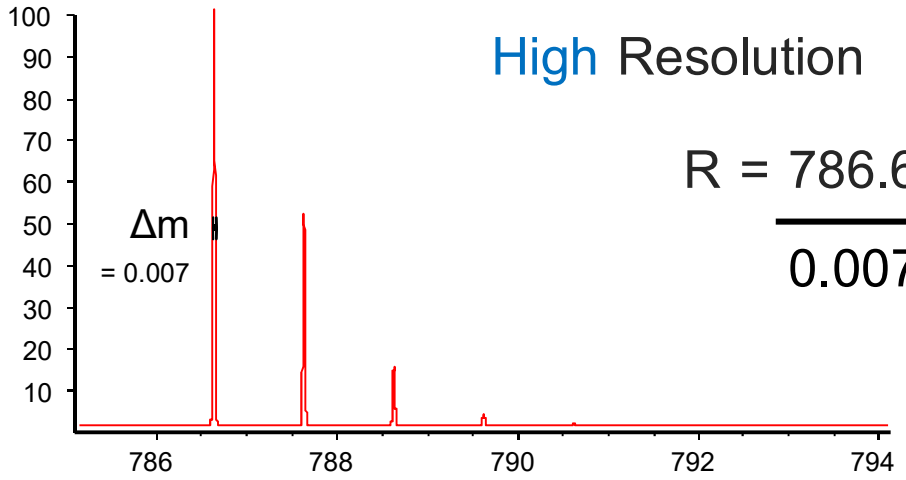
m = measured mass

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



Low Resolution

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



High Resolution

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

Mass Accuracy

- Mass error reporting in **ppm** (relative mass error):

$$\boldsymbol{Mass\ error = \frac{Measured\ -\ Theoretical}{Theoretical} \times 10^6 = ppm}$$

- **Exact Mass** is the mass of an ion with a given empirical formula calculated using the exact mass of the most abundant isotope of each element

Example *M = 249;*

$C_{20}H_9+$	*249.0070*
$C_{19}H_7N+$	*249.0580*
$C_{13}H_{19}N_3O_2+$	*249.1479*

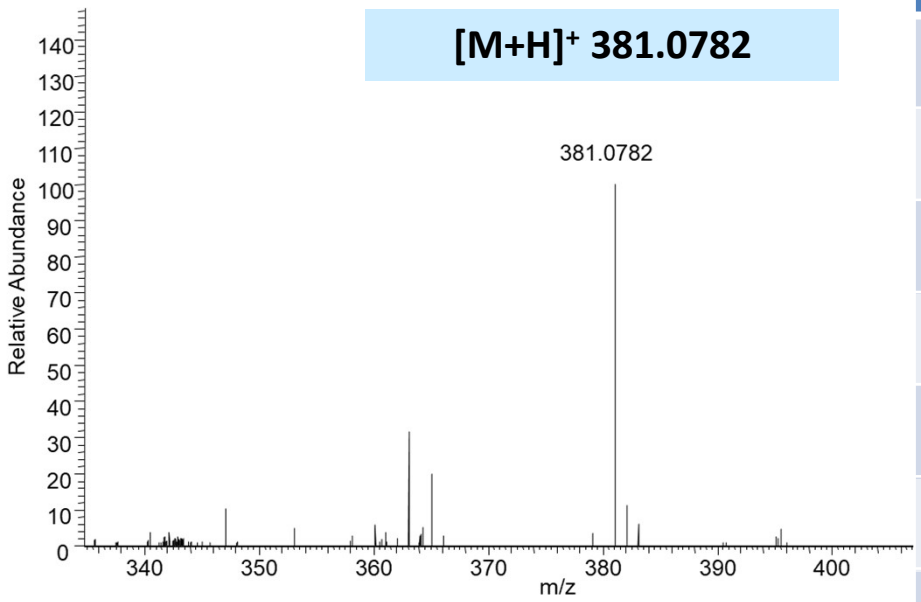
C = 12.0000

H = 1.0078
N = 14.0031

O = 15.9949
S = 31.9721

Mass Resolution and Accuracy

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



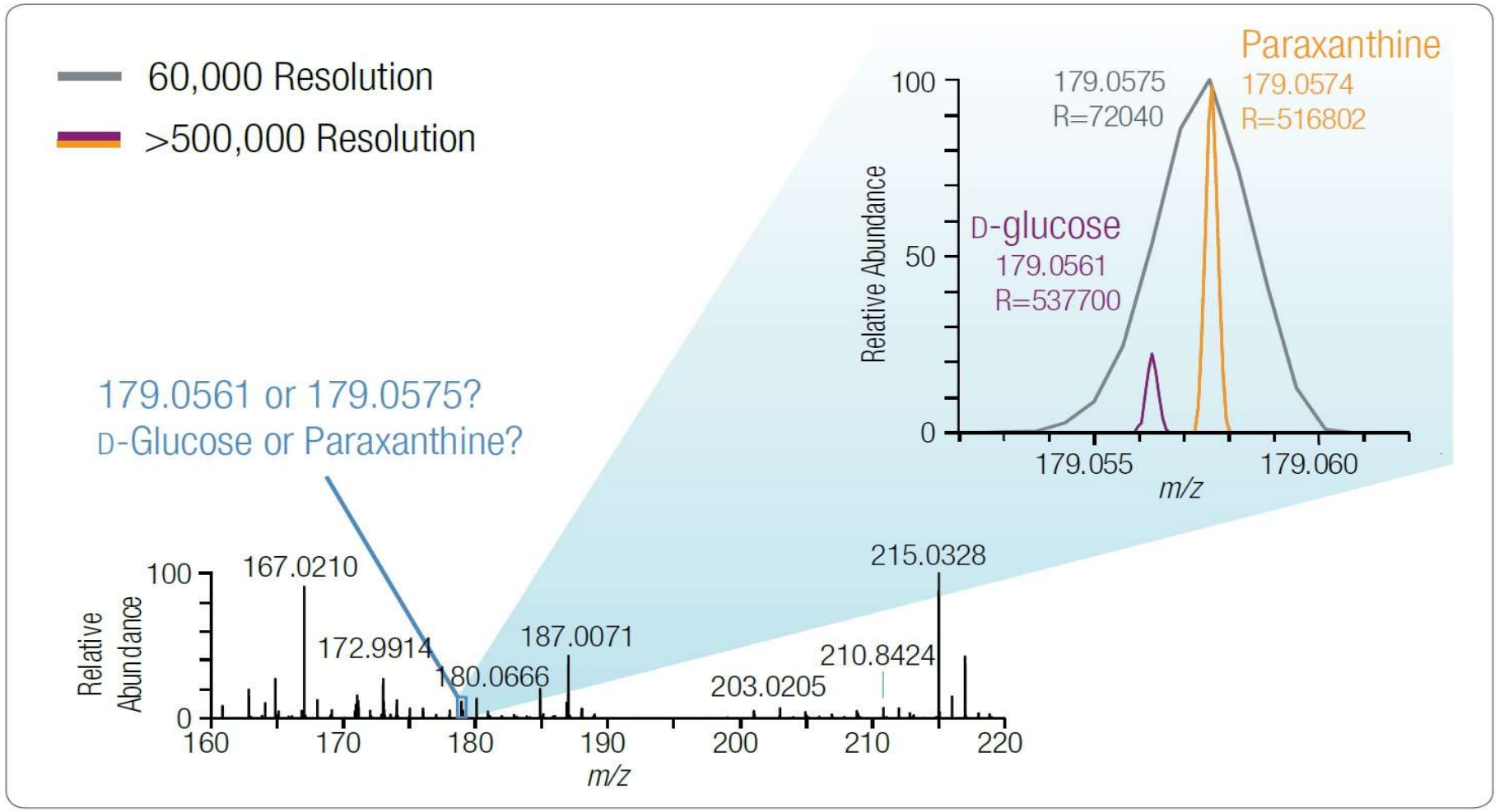
Mass Accuracy	Number of Hits*
± 200 ppm	265
± 100 ppm	133
± 30 ppm	39
± 10 ppm	14
± 5 ppm	5
± 3 ppm	4
± 1 ppm	1

$C_xN_xO_xH_x$

→ $C_{12}H_{22}O_{11}$

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.

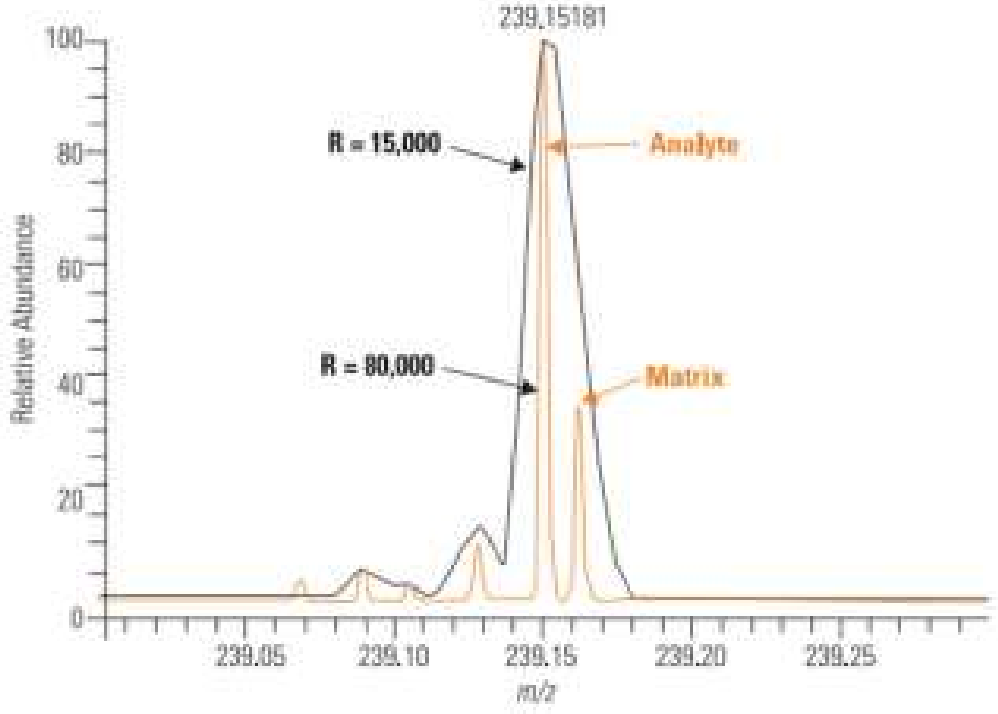
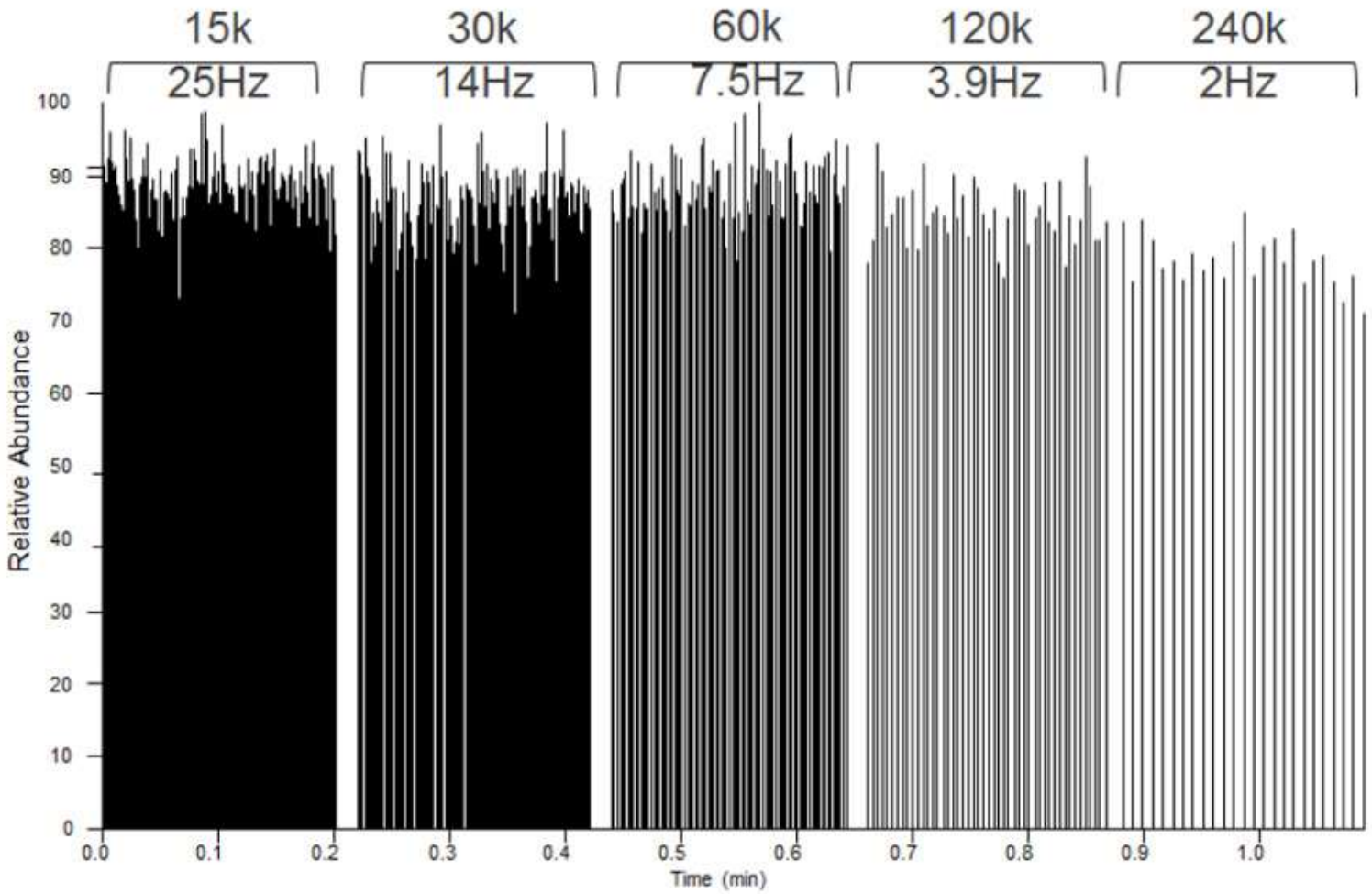


Figure 1: Analysis of the MH+ peak of Primicarb at 15,000 and 80,000 resolution.

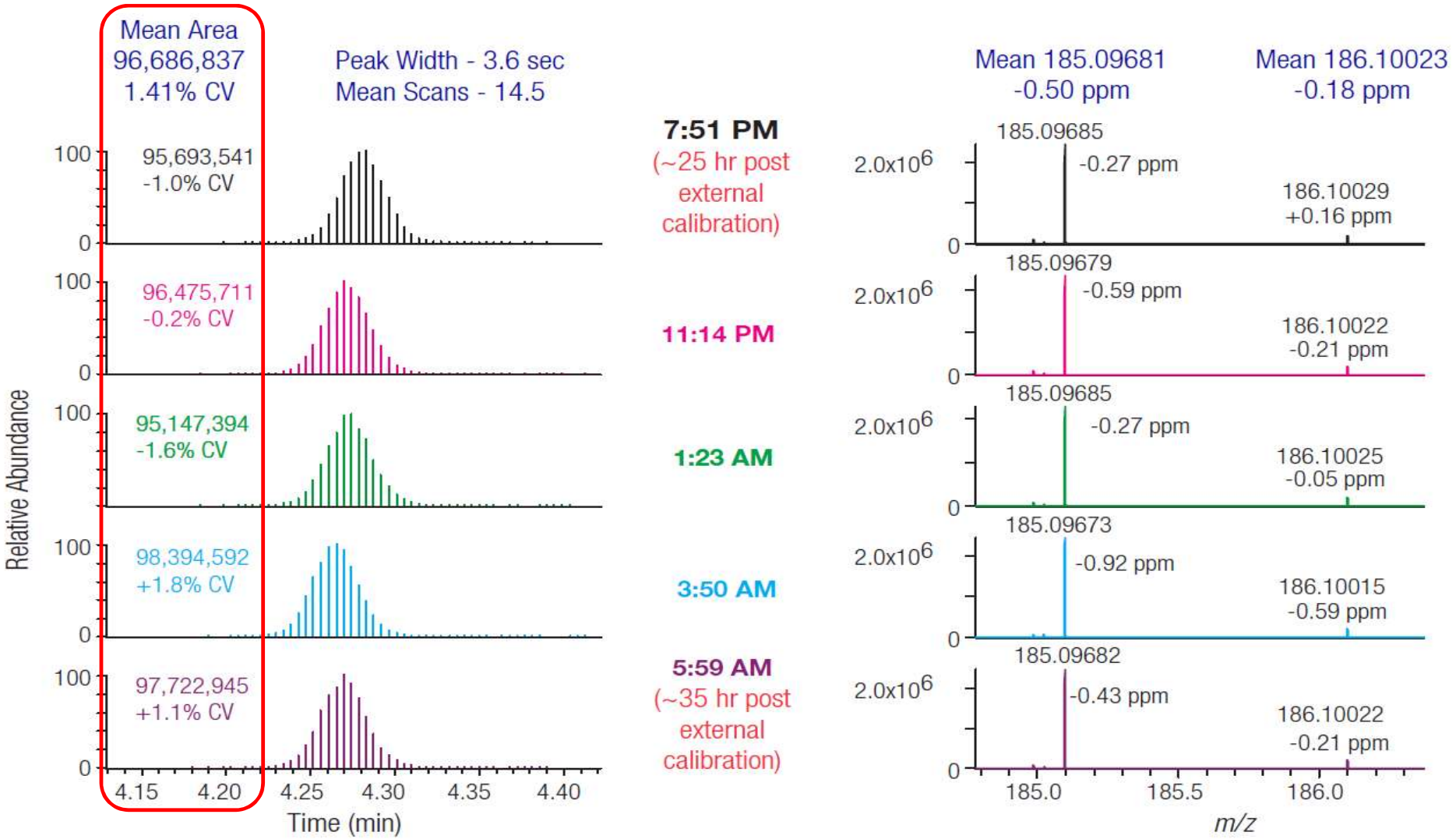
- High resolution is needed for:
  - Separating co-eluting molecules with close masses
  - Mass accuracy
  - Good quantitation results

Sensitivity and Resolution : No Compromise



Signal intensity does not change with resolution with the Orbitrap detector

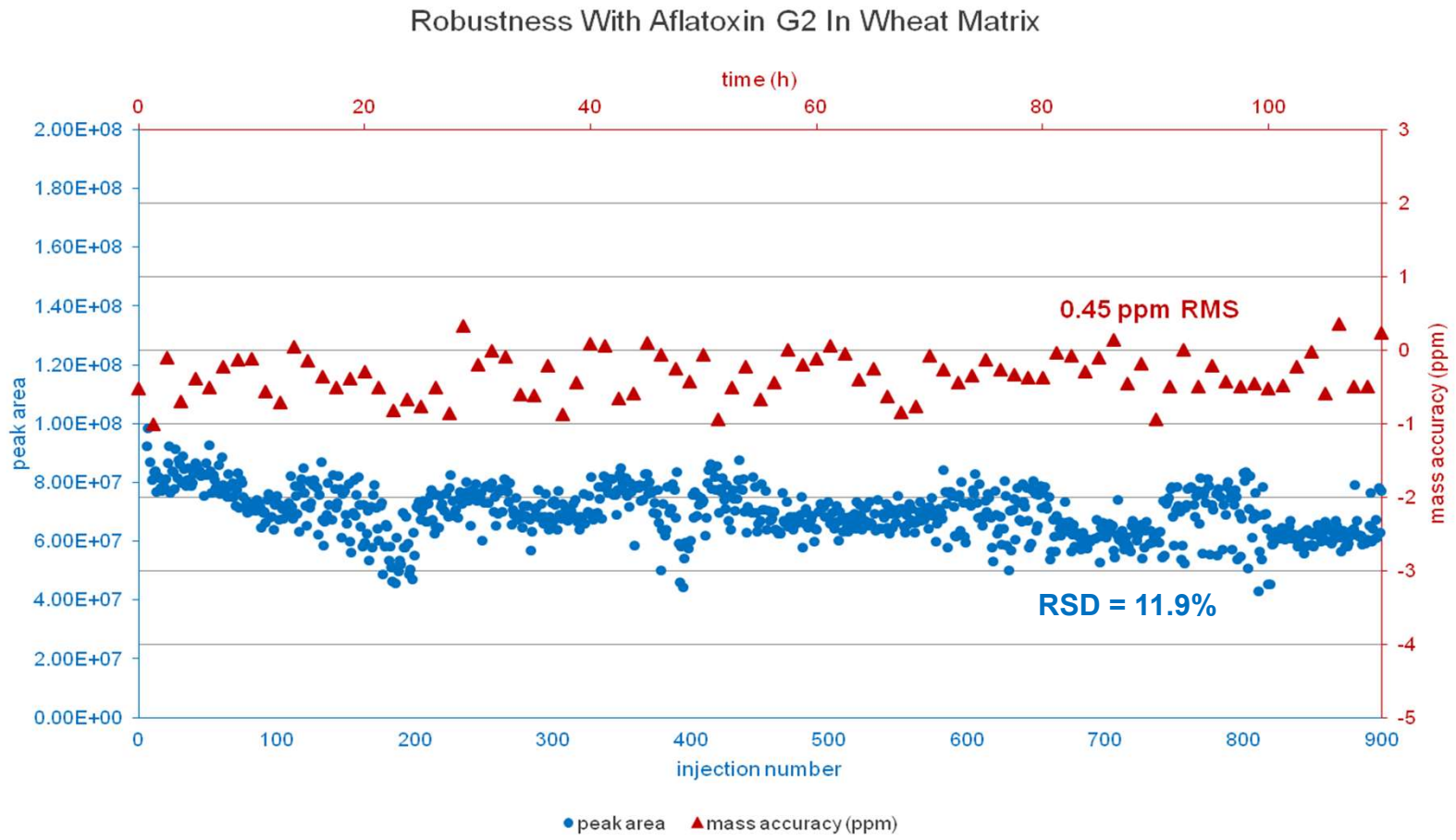
Stability : Robust and Reproducible HRAMS



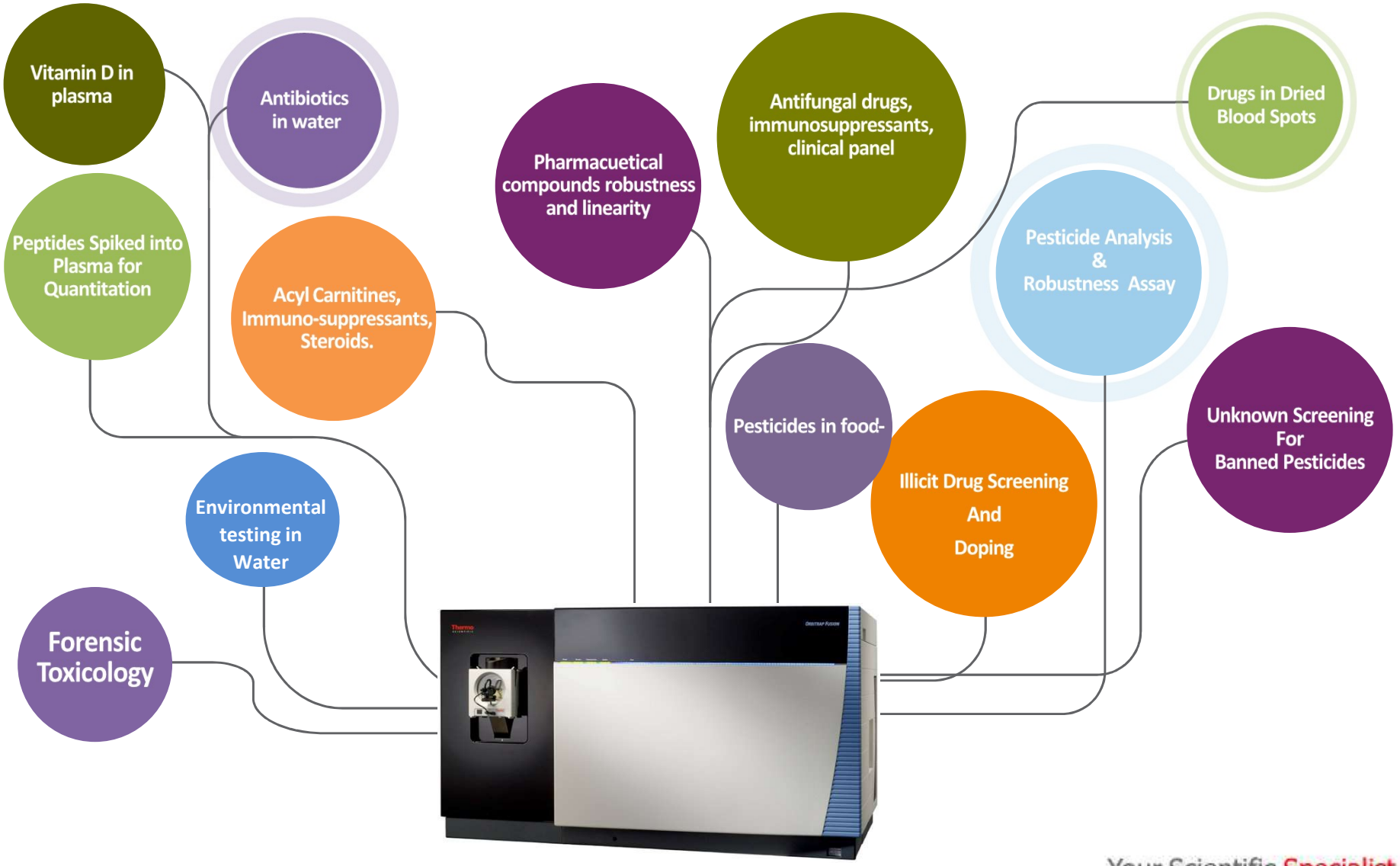
Ultimate 3000 HPG UHPLC coupled to Q Exactive
Full Scan MS: R=70K, d_5-Hippuric acid, theoretical m/z 185.0969

Stability : Robust and Reproducible HRAMS

- Peak intensity (area) variation during 900 injections (blue) with according mass accuracy (peak apex scan, red)



Orbitrap Applications Universe



MS Technology in Pharma Research

Drug Discovery	Drug Formulation	Preclinical Evaluation	
❑ Natural Product • Unknown compound identification • Plant metabolic pathway ❑ Synthetic Compounds • Molecular mass confirmation	❑ Determination of active compound concentration • Solubility test • Stability test	❑ *in vitro* Testing • Permeability test ❑ *in vivo* Testing • Toxicological study • Pharmacokinetic study	❑ Metabolism Study • Target/ Non-target metabolite identification ❑ **Metabolomics**
High Resolution	***High Sensitivity***		***High Resolution***

ORBITRAP™ TECHNOLOGY HRAMS

POTENTIATES METABOLOMIC RESEARCH

SciSpec

Your Scientific Specialist

Metabolomics

- Metabolomics is the scientific study of chemical processes involving metabolites, the **small molecule** intermediates and products of metabolism.

- Specifically, metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their **small-molecule** metabolite profiles.

Metabolomics

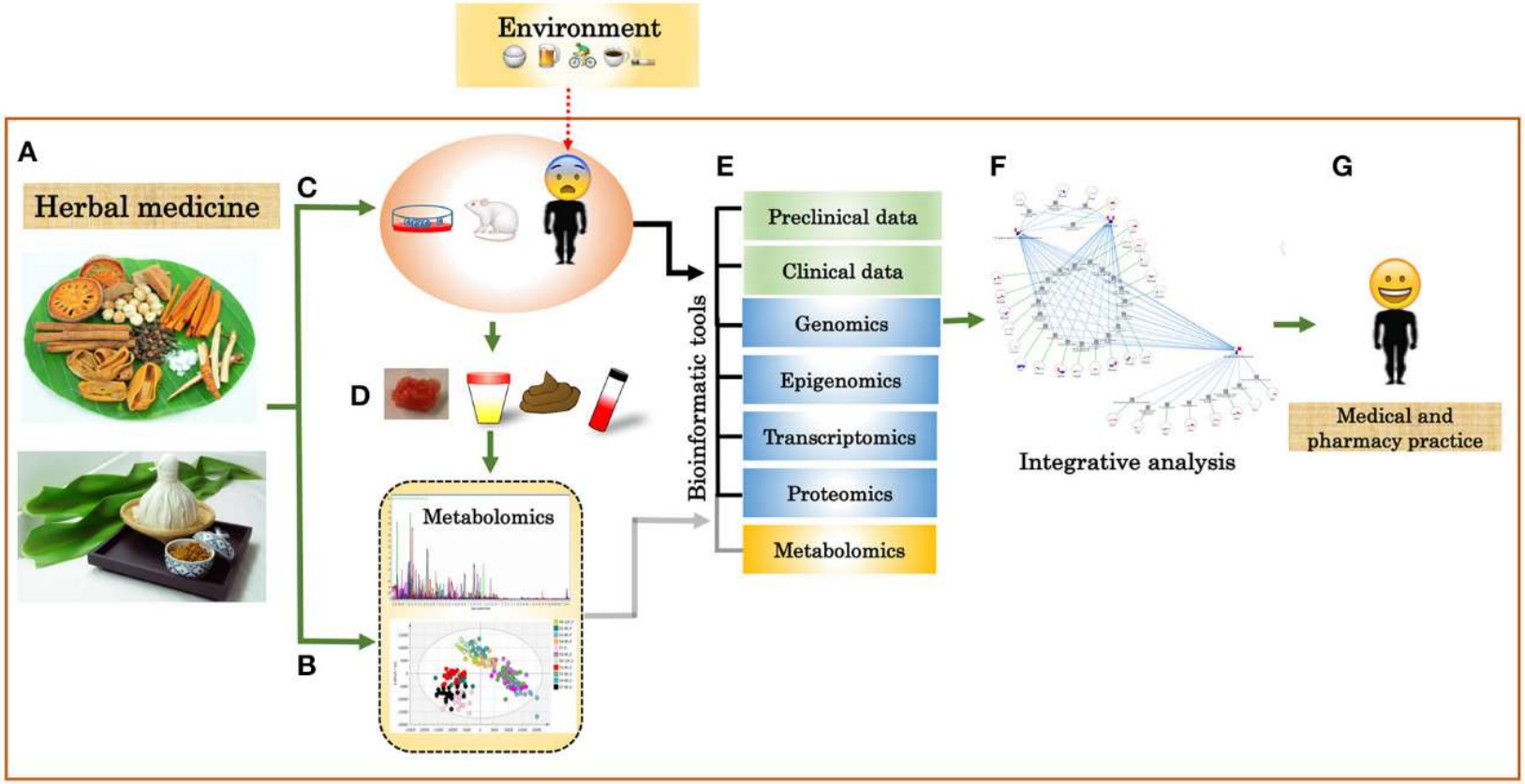
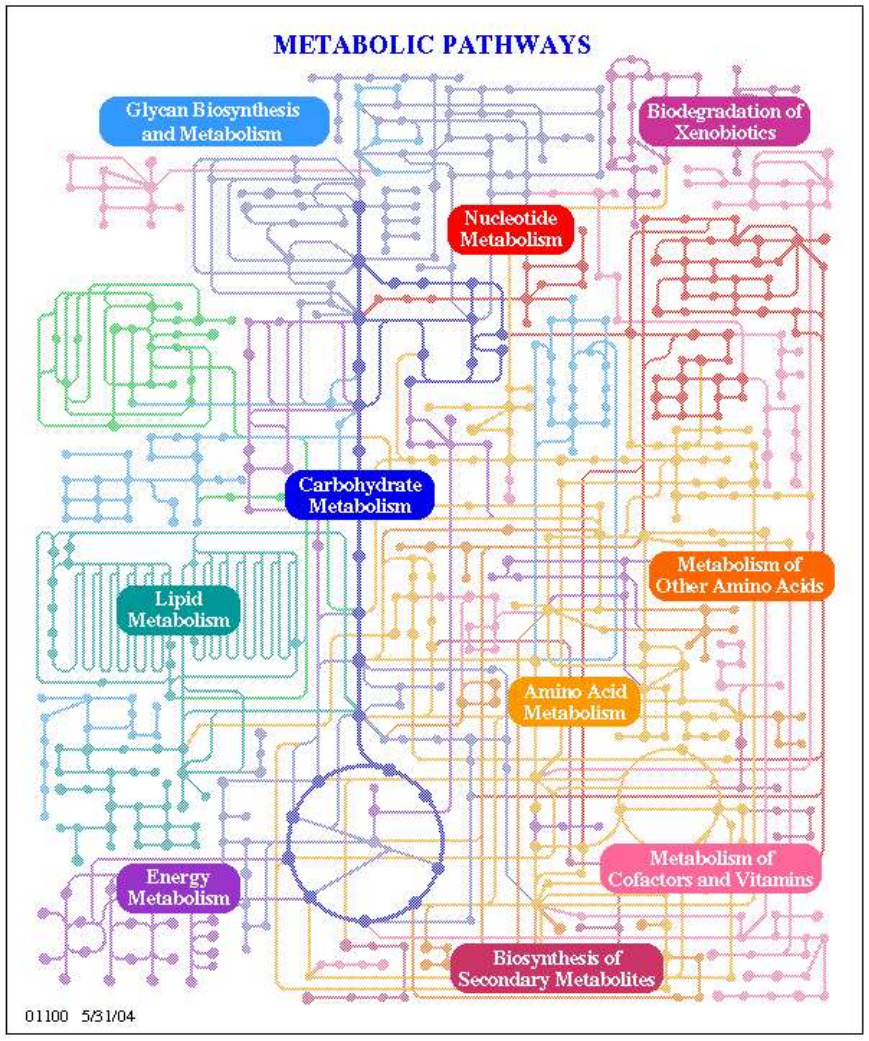


FIGURE 3 | Integration of multi-omics and clinical data into TTM. **(A)** Herbal plants and herbal medicines. **(B)** Chemical characterization of herbal plants, herbal medicines, and biofluids using targeted and untargeted metabolomics. **(C)** Clinical trial of herbal plants and herbal medicines by cell lines, animal, or human models. **(D)** Biofluid for metabolome analysis. **(E)** Integrative analysis of different omics data and clinical data. **(F)** Systems biology network. **(G)** Individualized data.

Khoomrung et al. (2017)

Challenges of Metabolomics

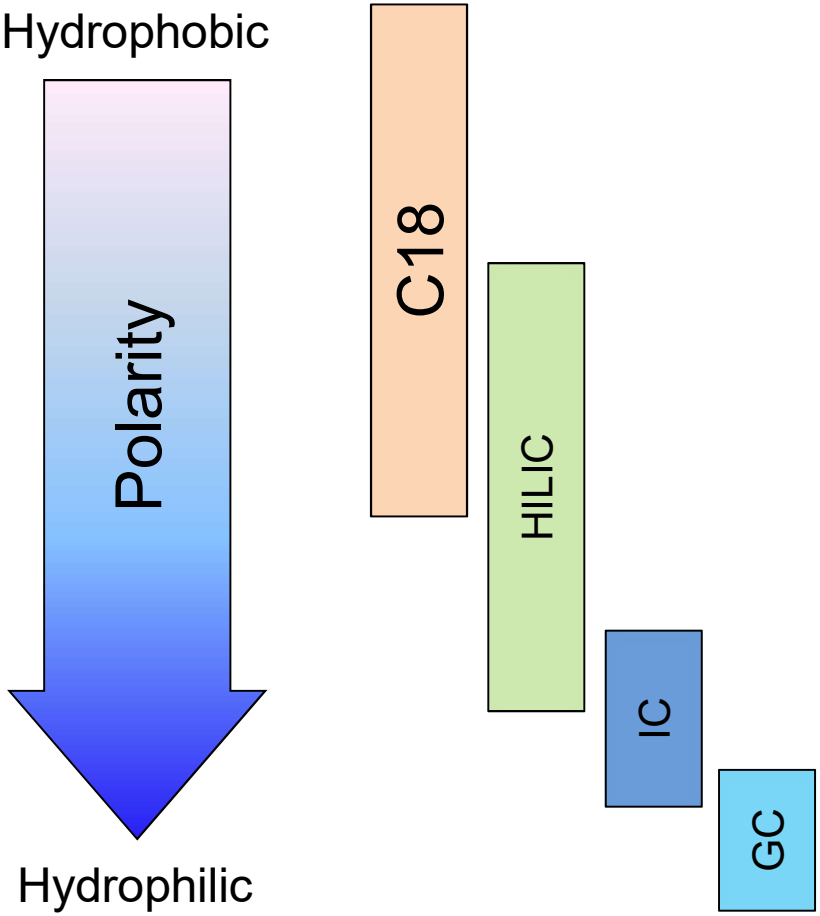


All the images shown in this frame are downloaded or parsed from KEGG

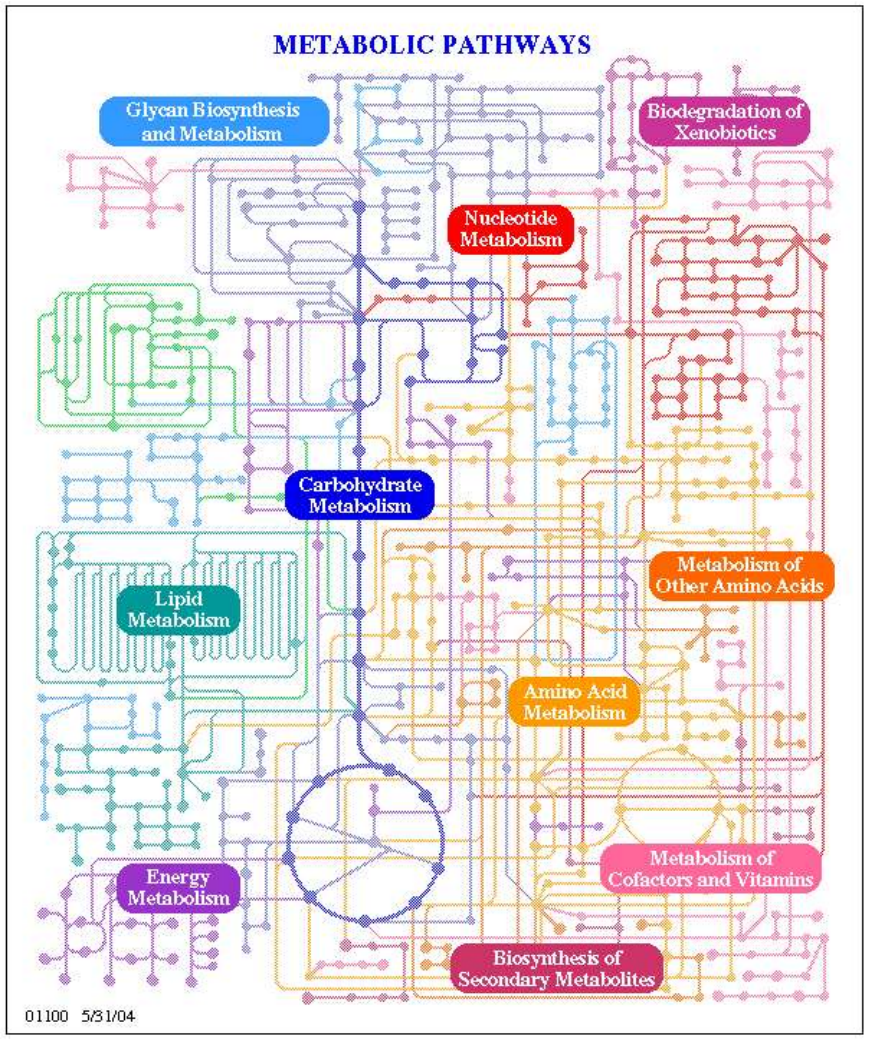
- **Diversity in structures and physical chemical properties**
  - **Require multiple technologies to capture a metabolome**
- Many isomeric and isobaric species
  - Require high resolving power for correct ID
- Very low to very high concentrations
  - Require High sensitivity and wide dynamic range
- No single database to identify all unknown metabolites
  - Require extensive library or fragment ion prediction based on compound structure

Diverse Physical Properties among Metabolites

Metabolites	MS ionization
triglycerides	+
cholesterol esters	+
diacylglycerols	+
sphingomyelines	+
phosphatidylcholines	+
phosphatidylethanolamines	+
other phospholipids	+& -
fatty acids	-
eicosanoids & metabolites	-
bile acids	-
bilirubin	+ or -
amino acids, amines	+
organic acids	-
sugars	-
Other polar:	-
e.g. purines & pyrimidines	



Challenges of Metabolomics



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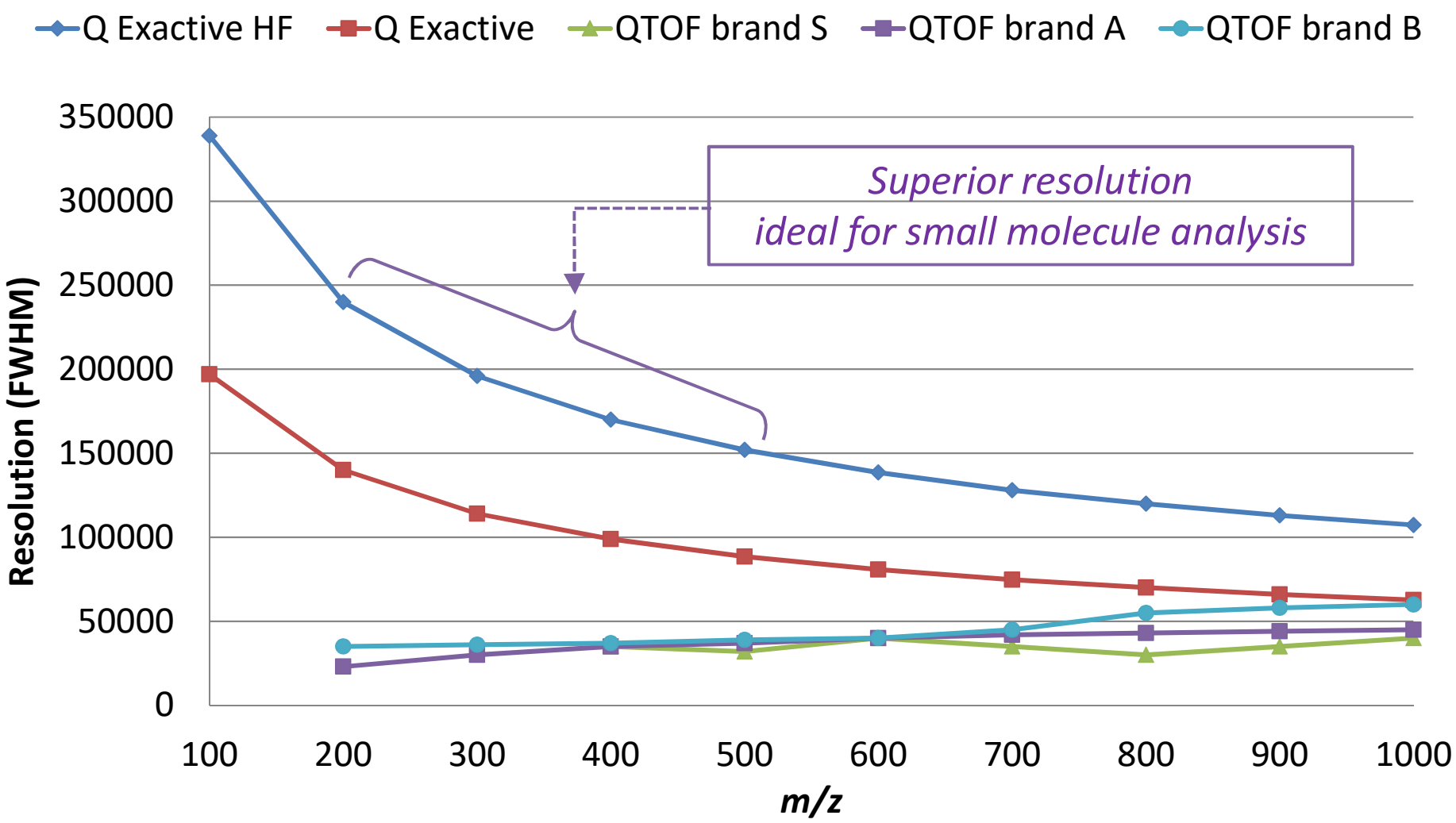
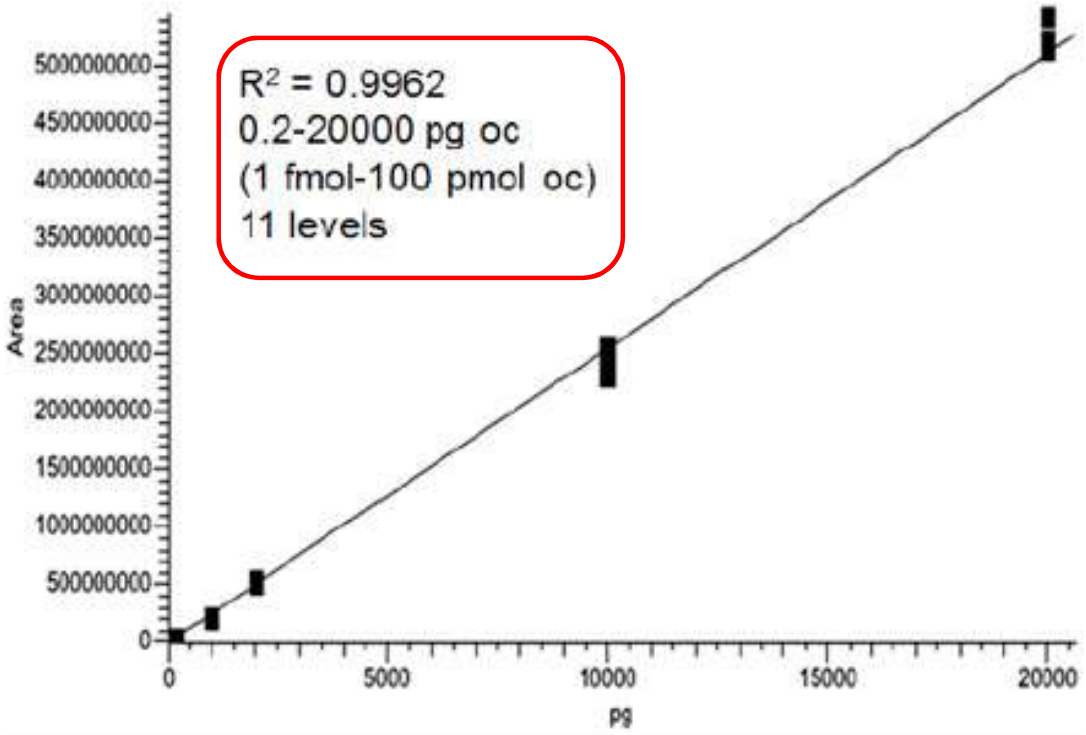
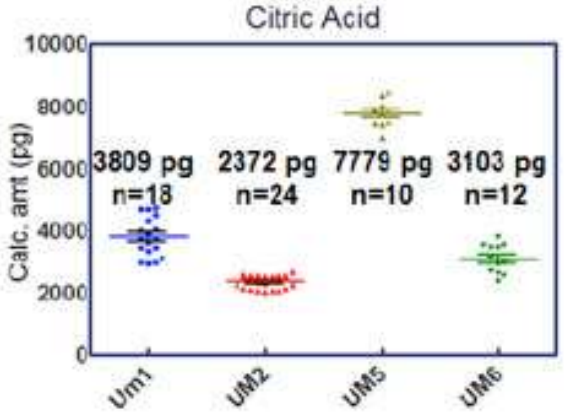
Resolution: QE HF, QE vs. State-of-Art QTOFs



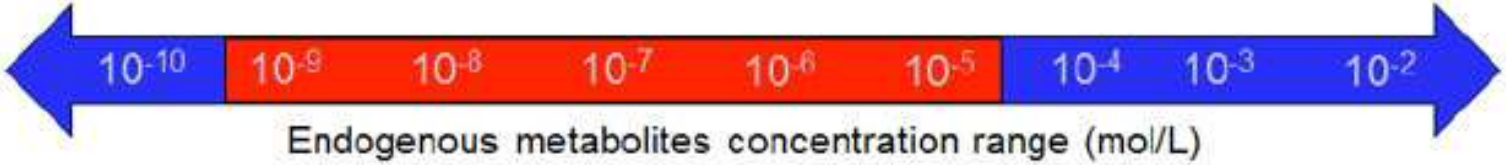
Sensitivity : 5 Orders of Dynamic Range

Quantitation of Citric Acid using orbitrap analyzer achieved at low fmole sensitivity using full scan MS



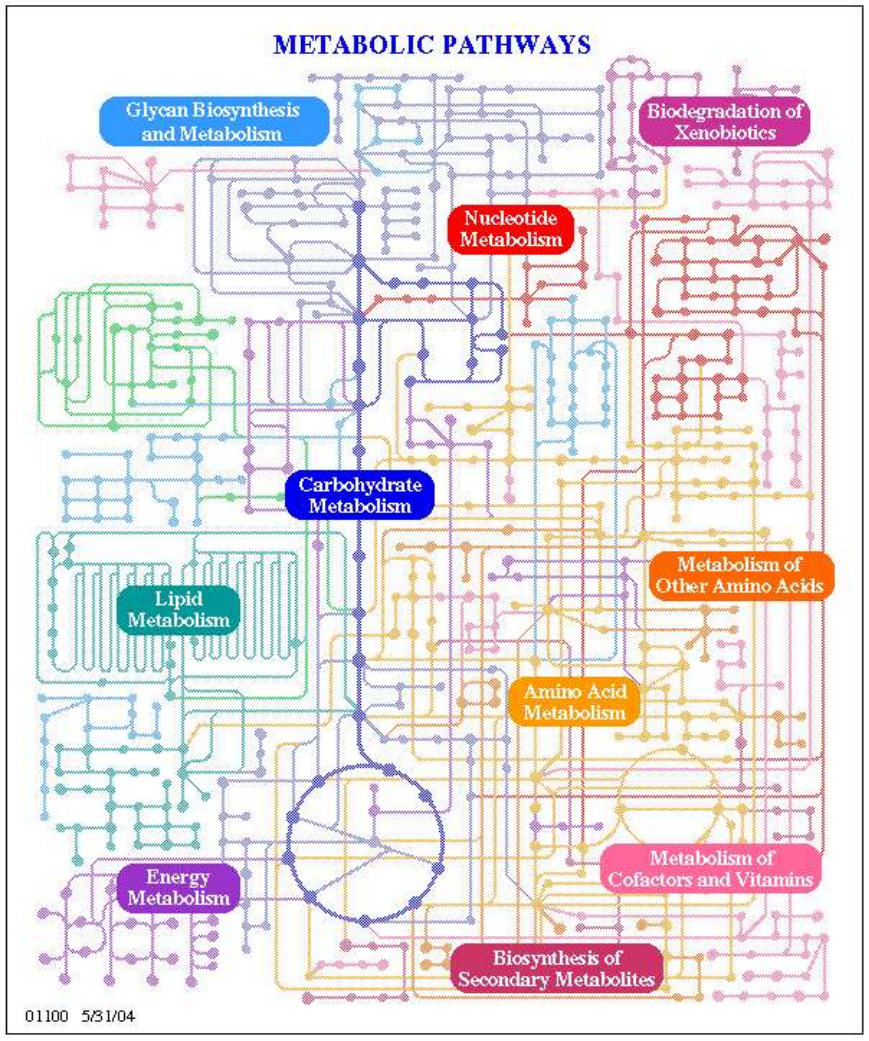


Endogenous Citric Acid amount / plate (~3M cells/plate)
UM1 cells, very invasive
UM2 cells, less invasive
UM5 cells, highly invasive
UM6 cells, less invasive



Endogenous metabolites concentration range (mol/L)

Challenges of Metabolomics



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Untargeted Metabolomics Workflow

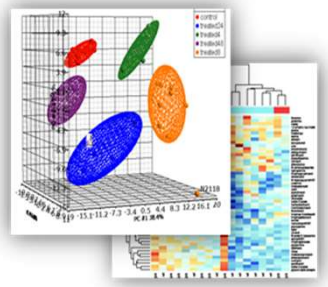
Collect Plasma, Urine, Feces, Tissues, *etc.*

Sample Preparation

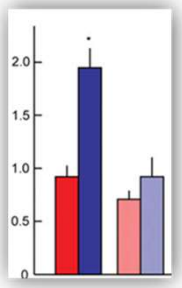
Collect High Resolution LC/MS Profiles

Data Processing

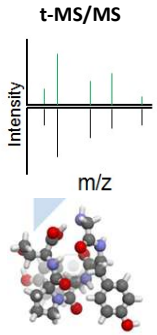
Lists of Compounds



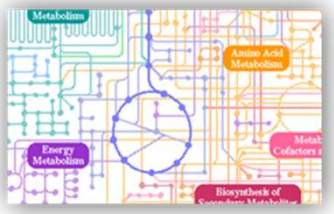
Statistical Analysis



Trends



Metabolites



Pathways

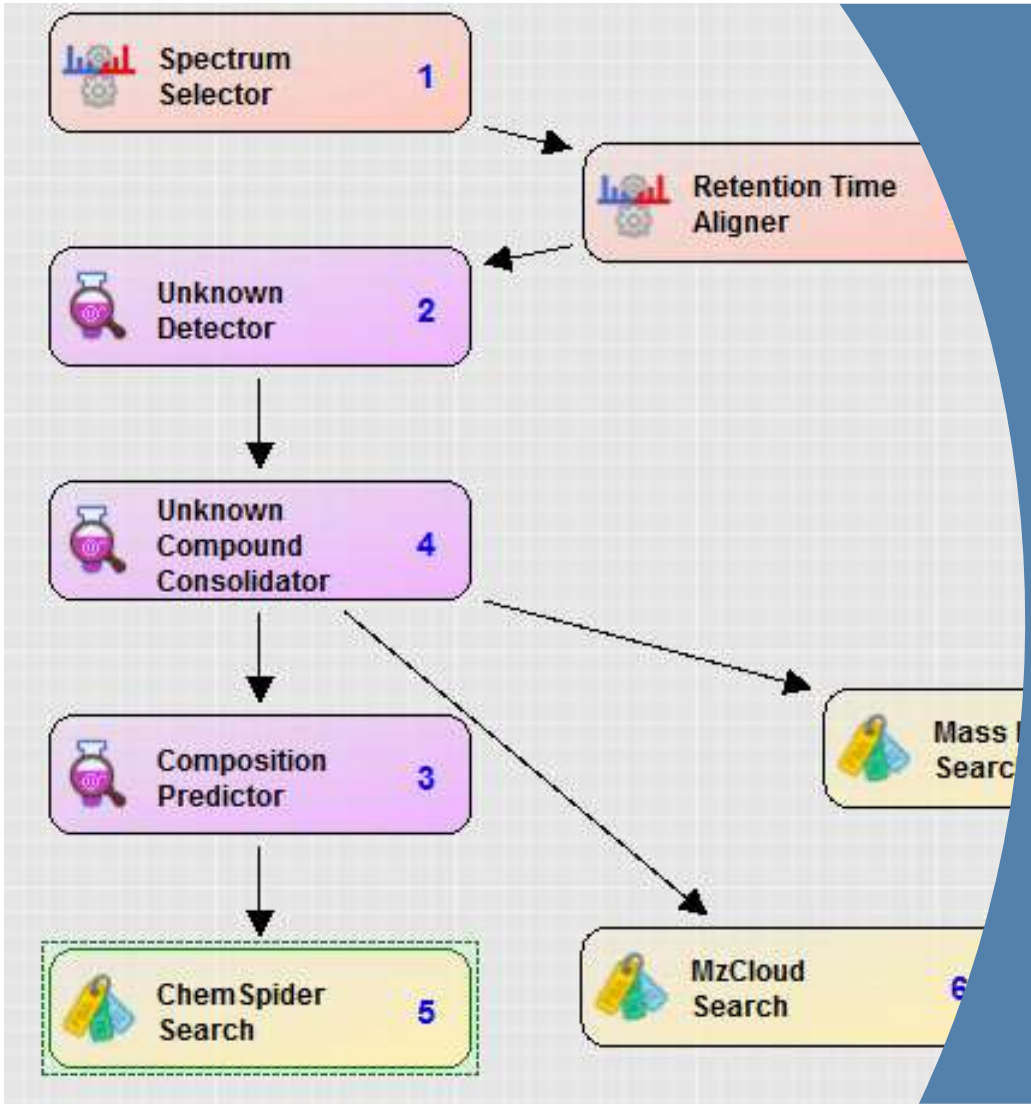
Metabolic Pathway

Your Scientific Specialist

Enhance your Research with Compound Discoverer

https://goo.gl/YvAS2j

Compound Discoverer : Retrospective for Unknowns



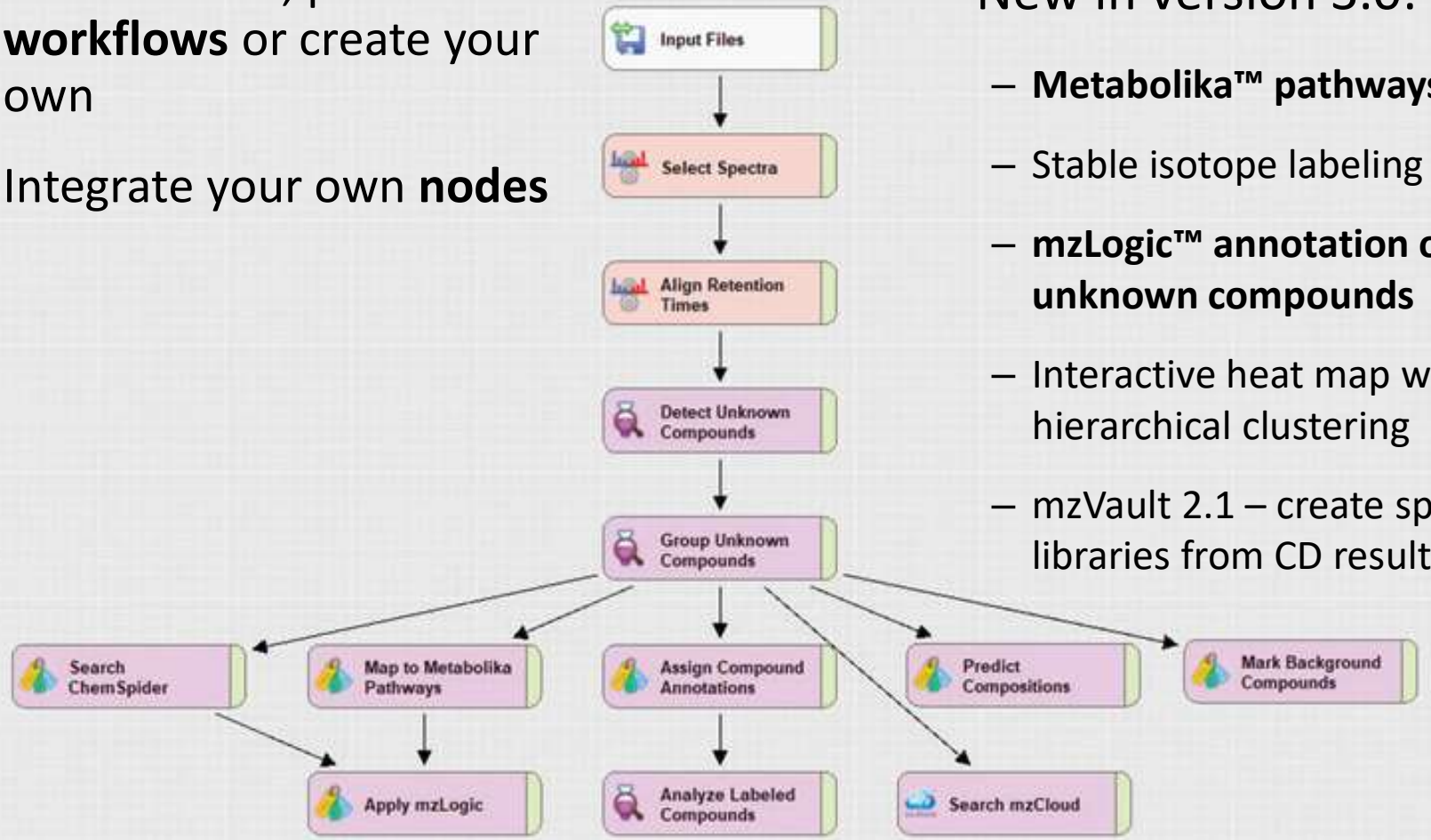
Compound Discoverer

Complete small molecule structure identification in a **Next Generation** platform.

- Unknown Analysis
- Identification
- Statistics

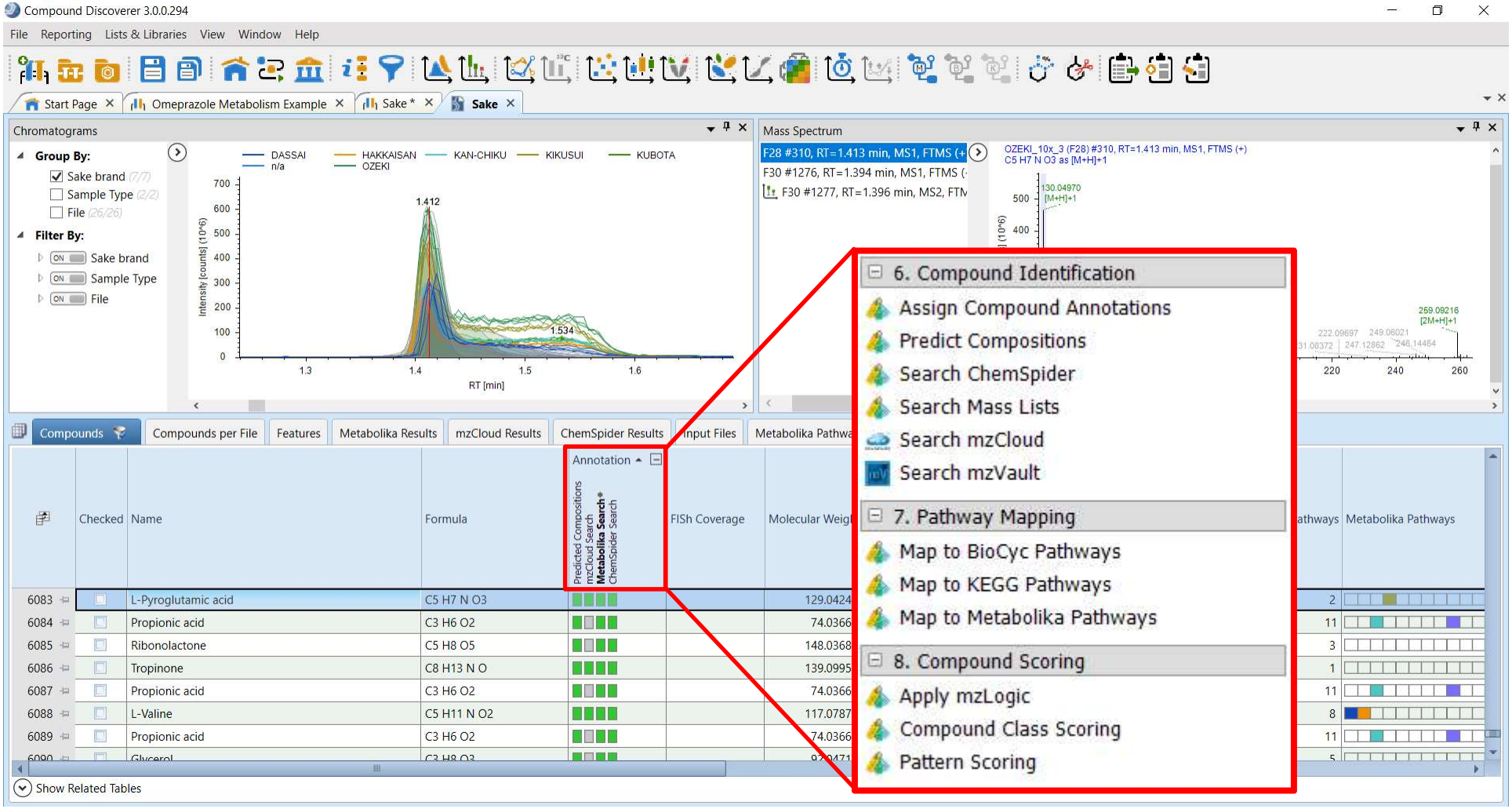
Compound Discoverer : Flexible Workflows

- Use common, predefined **workflows** or create your own
- Integrate your own **nodes**



- New in version 3.0:
  - **Metabolika™ pathways**
  - Stable isotope labeling
  - **mzLogic™ annotation of unknown compounds**
  - Interactive heat map with hierarchical clustering
  - mzVault 2.1 – create spectral libraries from CD results

Compound Discoverer : Identifying Unknowns



Compound Discoverer : Database Hub

KEGG Natural toxins

[Brite menu | Download htext | Download json]

Natural toxins

One-click mode

- **Fungal toxins**
  - Mushroom toxins
  - Mycotoxins
    - Aflatoxins
      - C06800 Aflatoxin B1
      - C16753 Aflatoxin B2
      - C16754 Aflatoxin G2
      - C16755 Aflatoxin G1
      - C16756 Aflatoxin M1
    - Trichothecenes
      - C09662 Diacetoxyscirpenol
      - C19952 HT-2 Toxin
      - C09738 T-2 Toxin
      - C19583 Fusarenone X
      - C06080 Nivalenol
      - C09747 Vomitoxin
      - C20017 Satratoxin H
    - Fumonisins
      - C19241 Fumonisin B1
      - C19242 Fumonisin B2
  - Ergot alkaloids
  - Indole diterpene alkaloids
  - Others

mzCLOUD Advanced Mass Spectral Database

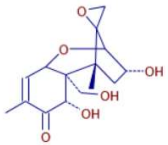
Server location : US

search for compounds... Search

Home About Features App Database Partners Contact

Deoxynivalenol

enlarge



Systematic / IUPAC Name: (3β,7α)-3,7,15-Trihydroxy-12,13-epoxytrichothec-9-en-8-one

ID: Reference6191

Other Names: 4-Deoxynivalenol;
4-Desoxynivalenol;
Dehydronivalenol;
Desoxynivalenol;
Rd Toxin ; more

Formula: $C_{15}H_{20}O_6$

Class: Natural Toxins

Vomitoxin, also known as **deoxynivalenol** (**DON**), is a type B trichothecene, an epoxy-sesquiterpenoid. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and corn, and less often in rice, sorghum, and triticale.

ChemSpider Search and share chemistry

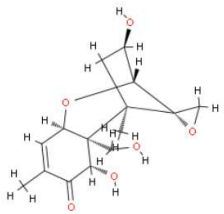
Search ChemSpider

Simple Structure Advanced History

Found 1 result

Search term: **Deoxynivalenol** (Found by approved synonym)

COMMENT ON THIS RECORD



3D

Deoxynivalenol

Molecular Formula	$C_{15}H_{20}O_6$
Average mass	296.316 Da
Monoisotopic mass	296.125977 Da
ChemSpider ID	36584

- 7 of 7 defined stereocentres

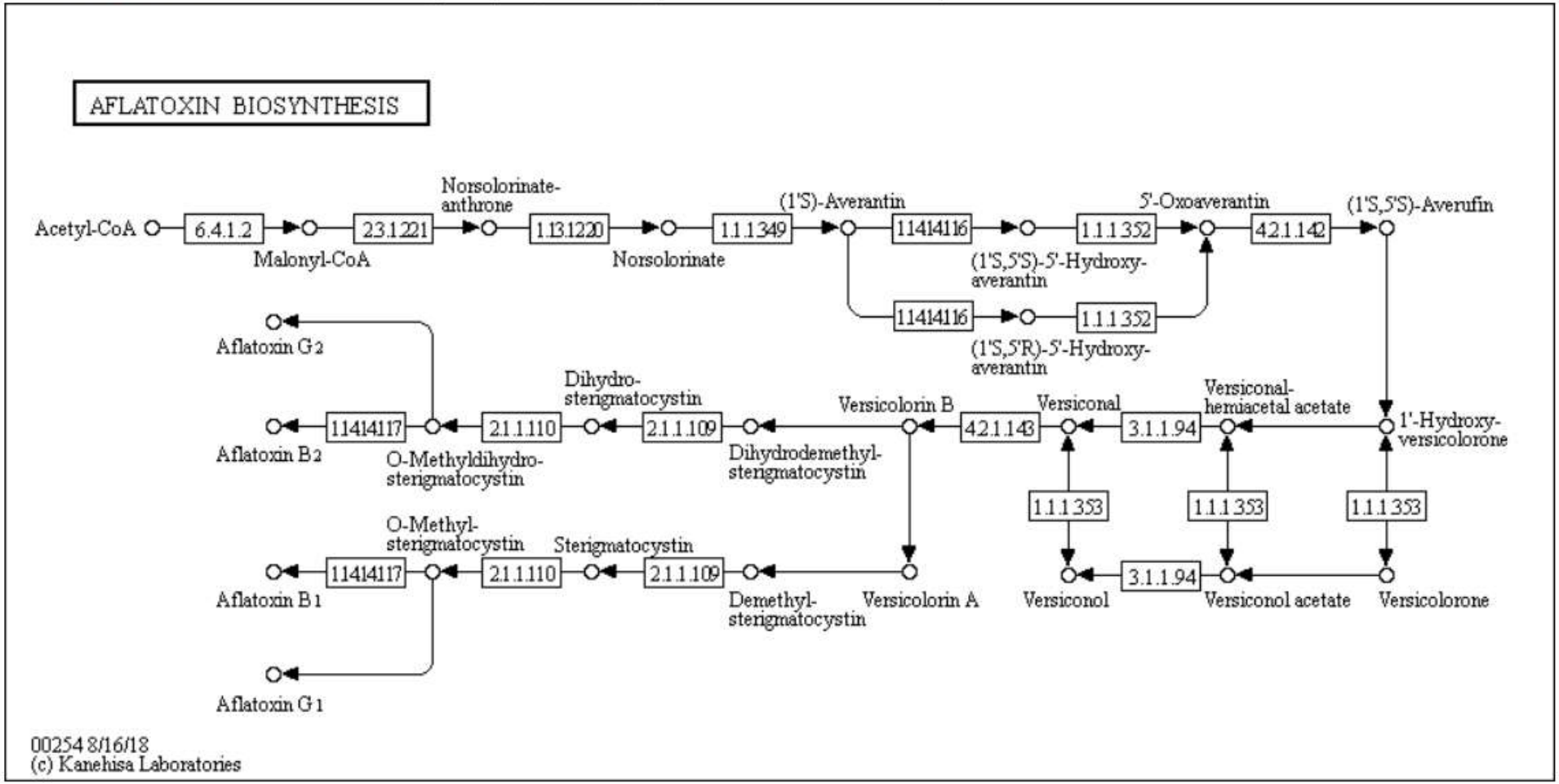
Your Scientific Specialist

Compound Discoverer : Metabolic Pathway

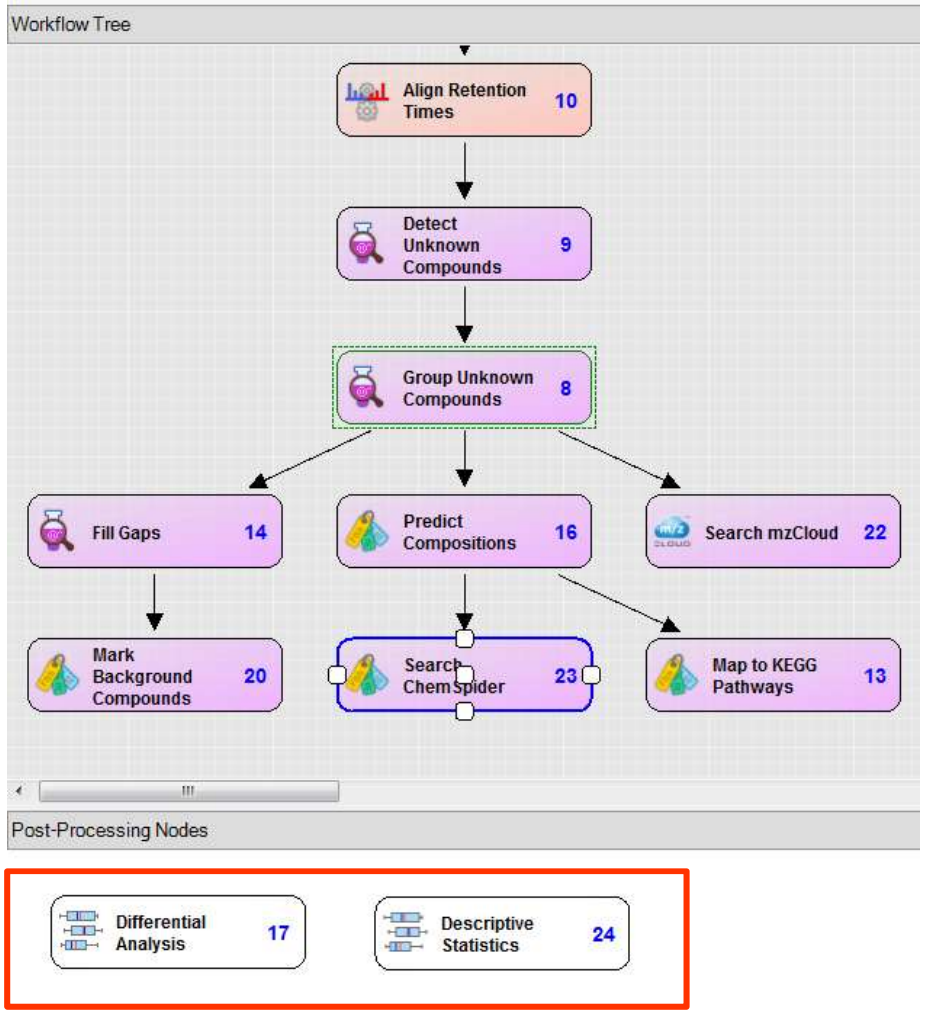
KEGG **Aflatoxin biosynthesis - Reference pathway**

[Pathway menu | Organism menu | Pathway entry | User data mapping]

Reference pathway ▾ Go 100% ▾



Compound Discoverer : Statistics



- Differential Analysis : t-test, ANOVA /w post-hoc tests, ratios & fold changes
- p-value adjustment for FDR
- Principal Component Analysis (PCA)
- Normalization
- Descriptive Statistics

Summary

- Introduction of mass spectrometry
- Key Features of Orbitrap™ Technology HRAMS
  - High Resolution and Accurate
  - Sensitive but Stable
- Compound Discoverer
  - Powerful workflow to identify non-target compounds for metabolomic research

Reference Sites for Orbitrap Analyzer (1)

No.	Company	Department	Model	Application
1	Faculty of Medicine, Siriraj Hospital	Medical Proteomics Unit	LTQ XL Orbitrap	Education
2	Chulabhorn Research Institute (CRI)	Biotechnology Laboratory	Orbitrap ELITE	Research
3	Charoen Pokphand Foods (CPF)	Quality Control Department	Q Exactive	R & D
4	Department of Livestock Development, Ministry of Agriculture and Cooperatives	Bureau of Quality Control of Livestock Product	Q Exactive	Food and Agriculture
5	Faculty of Medicine, Siriraj Hospital	Department of Pharmacology	Q Exactive	Education
6	Faculty of Medicine, Chulalongkorn University	Center of Excellence in Systems Biology	Q Exactive Plus	Research
7	National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA	Biomolecular Analysis and Application Laboratory (SBMA)	Orbitrap Fusion	Research

Reference Sites for Orbitrap Analyzer (2)

No.	Company	Department	Model	Application
8	Synchrotron Light Research Institute (SLRI)	Applied Research for Industry Division	Q Exactive Plus	Research Service
9	Chulabhorn Graduate Institute (CGI)	Chemical Biology	Q Exactive Focus	Research
10	Kokoku Innovative Technology	Center of Rubber Innovation	Q Exactive Focus	Research
11	National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA	Plant Physiology and Biochemistry Laboratory	Q Exactive Focus	Research
12	Institute of Forensic Medicine (IFM), Police Hospital	Department of Toxicology	Q Exactive Focus	Forensic Toxicology
13	National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA	National Omics Center	Q Exactive HF-X	Research

Thank You for Your Attention

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