LCMS Technology Connects to Your Application

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LCMS Product Specialist

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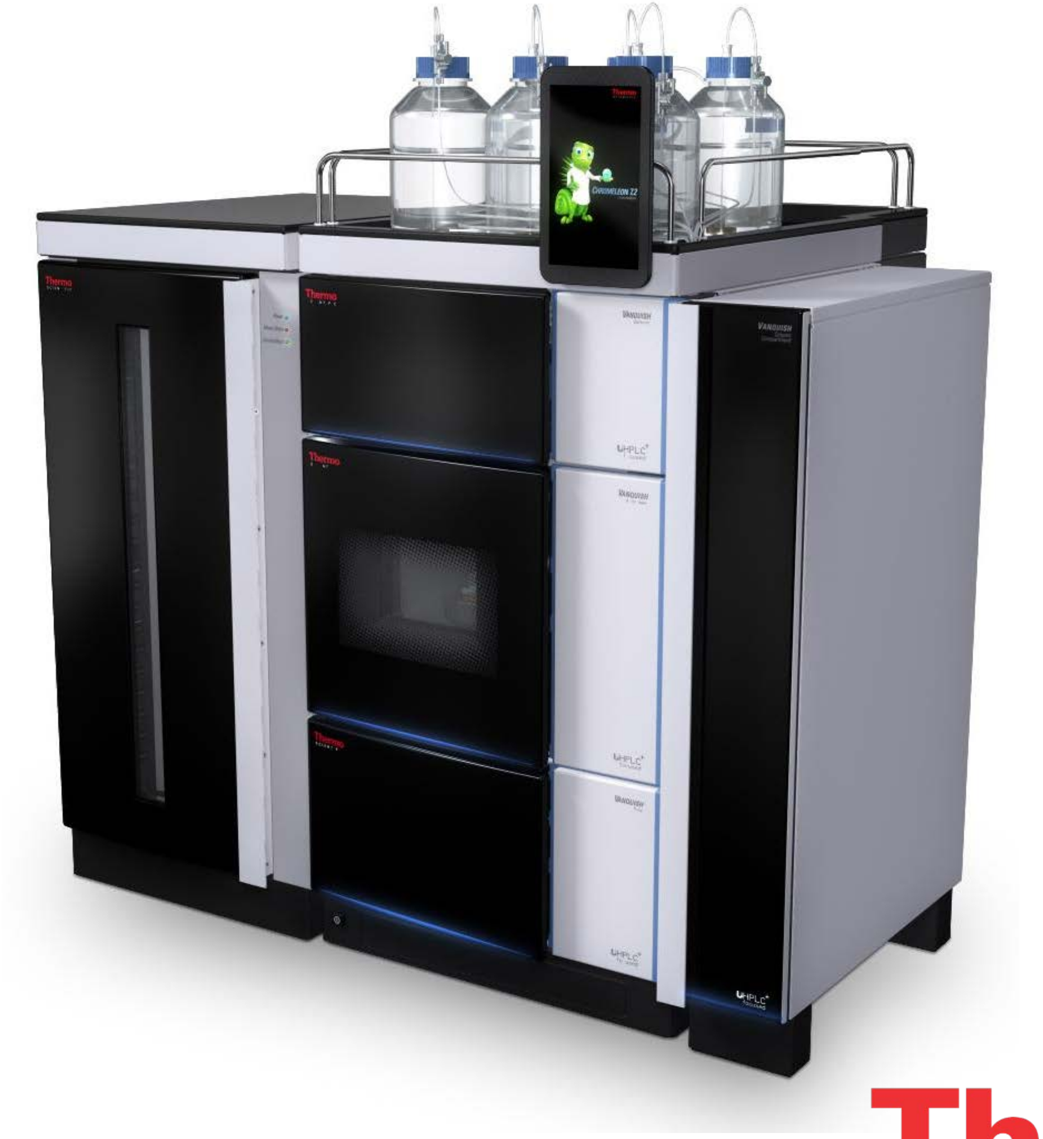
Topics

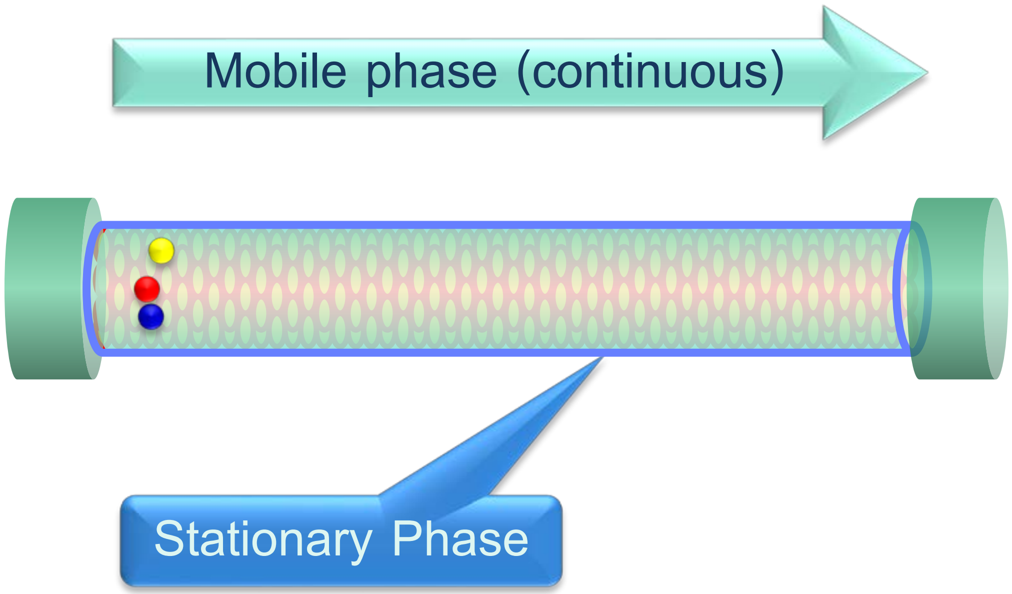
- Technology of Liquid Chromatography
- Type of Mass Spectrometer
- Applications in Food Safety and Pharmaceutical

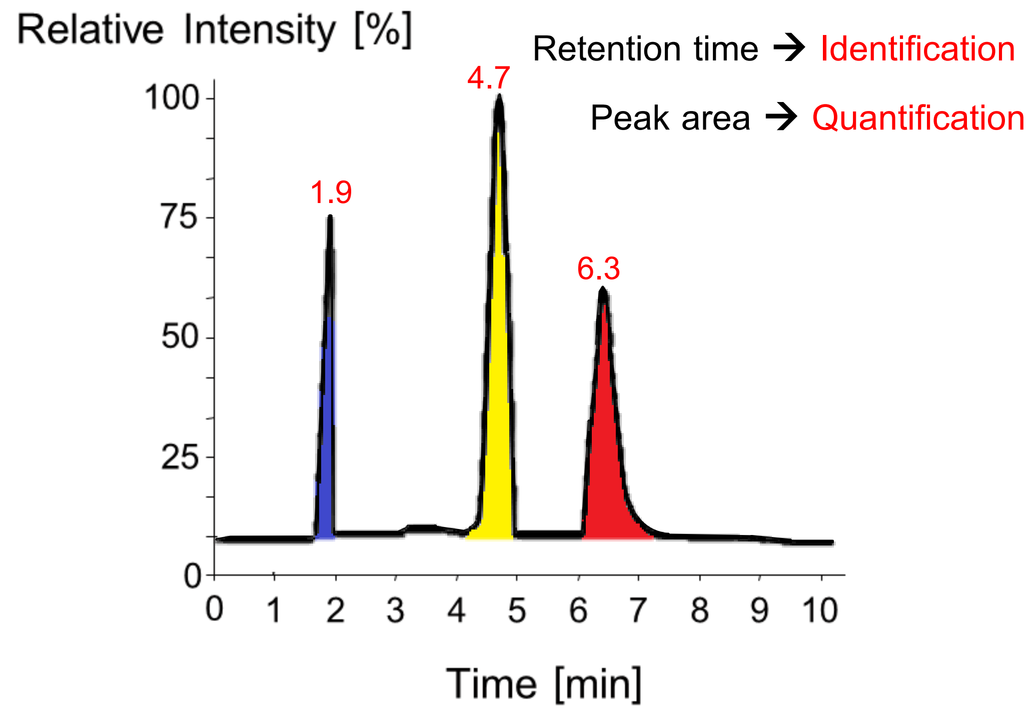
Fundamental of Liquid Chromatography



Thermo SCIENTIFIC

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.

UHPLC System

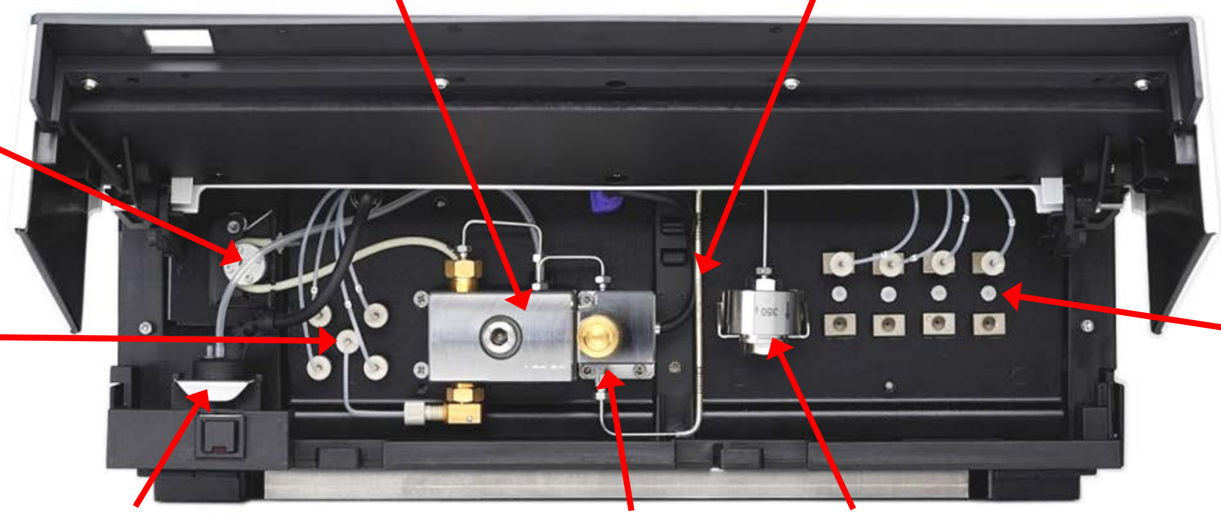
- **Degasser :** Remove air bubble in solvents
- **Pump :** - Mix two or more solvents

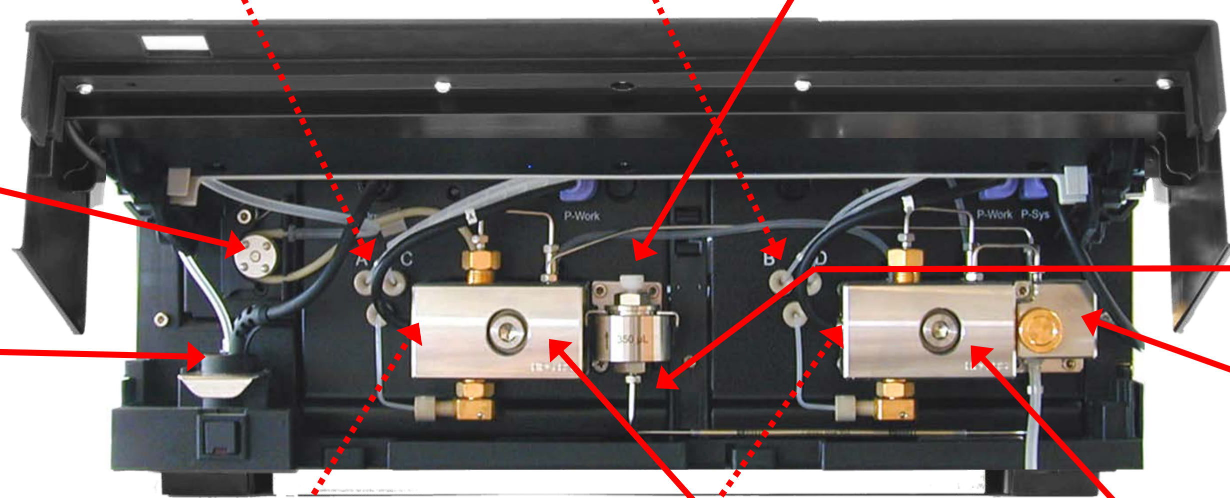
  - Control the flow of mobile phase and analytes
- **Autosampler :** Inject the sample into a running system
- **Column Compartment :** Control a column temperature
- **Detector :** Detect signal from analytes after separation

Technology in UltiMate 3000 for Accurate and Professional Experiments.

ULTIMATE 3000 SERIES PUMPS

Thermo SCIENTIFIC



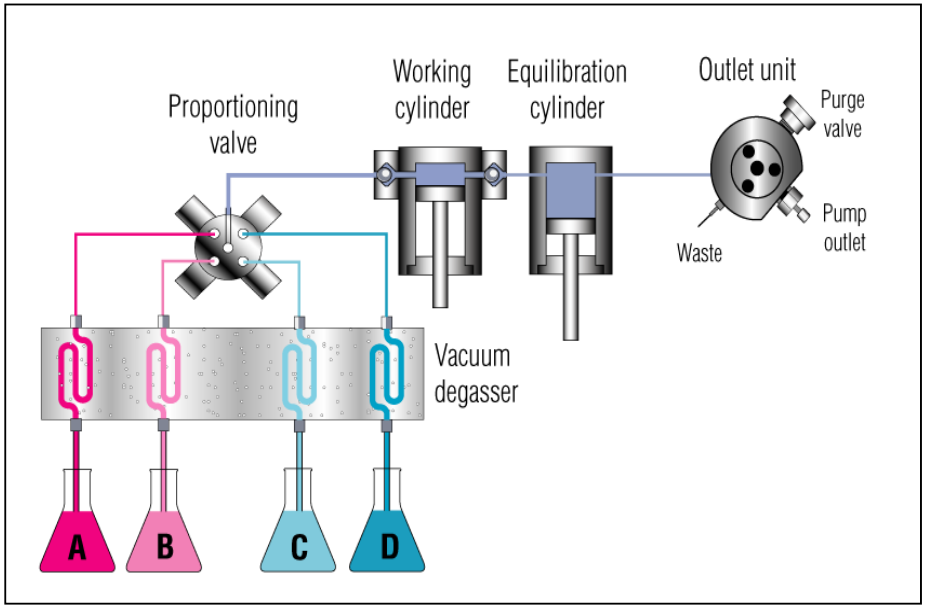


Sci Spec

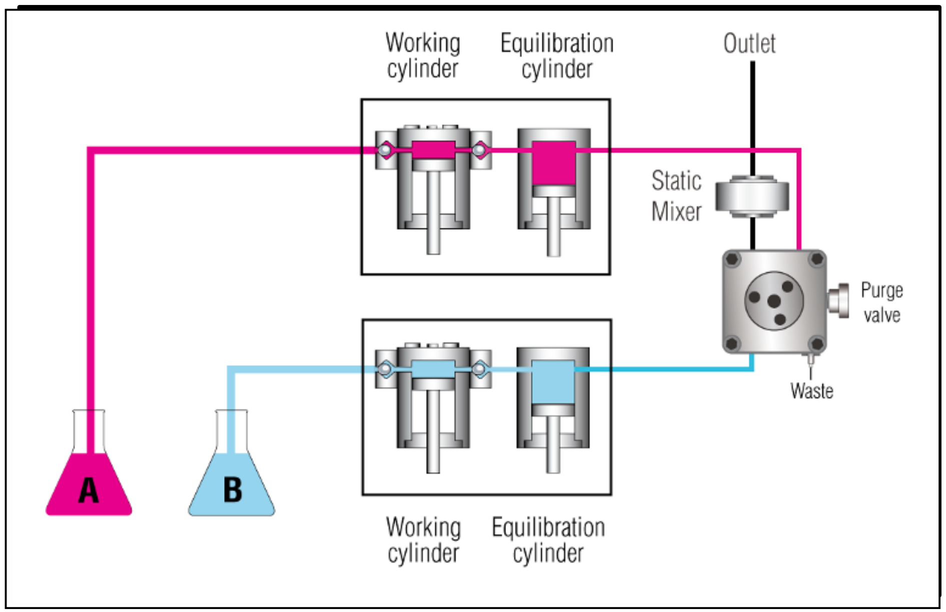
Your Scientific Specialist

General Design

Quaternary Low Pressure Gradient Pump

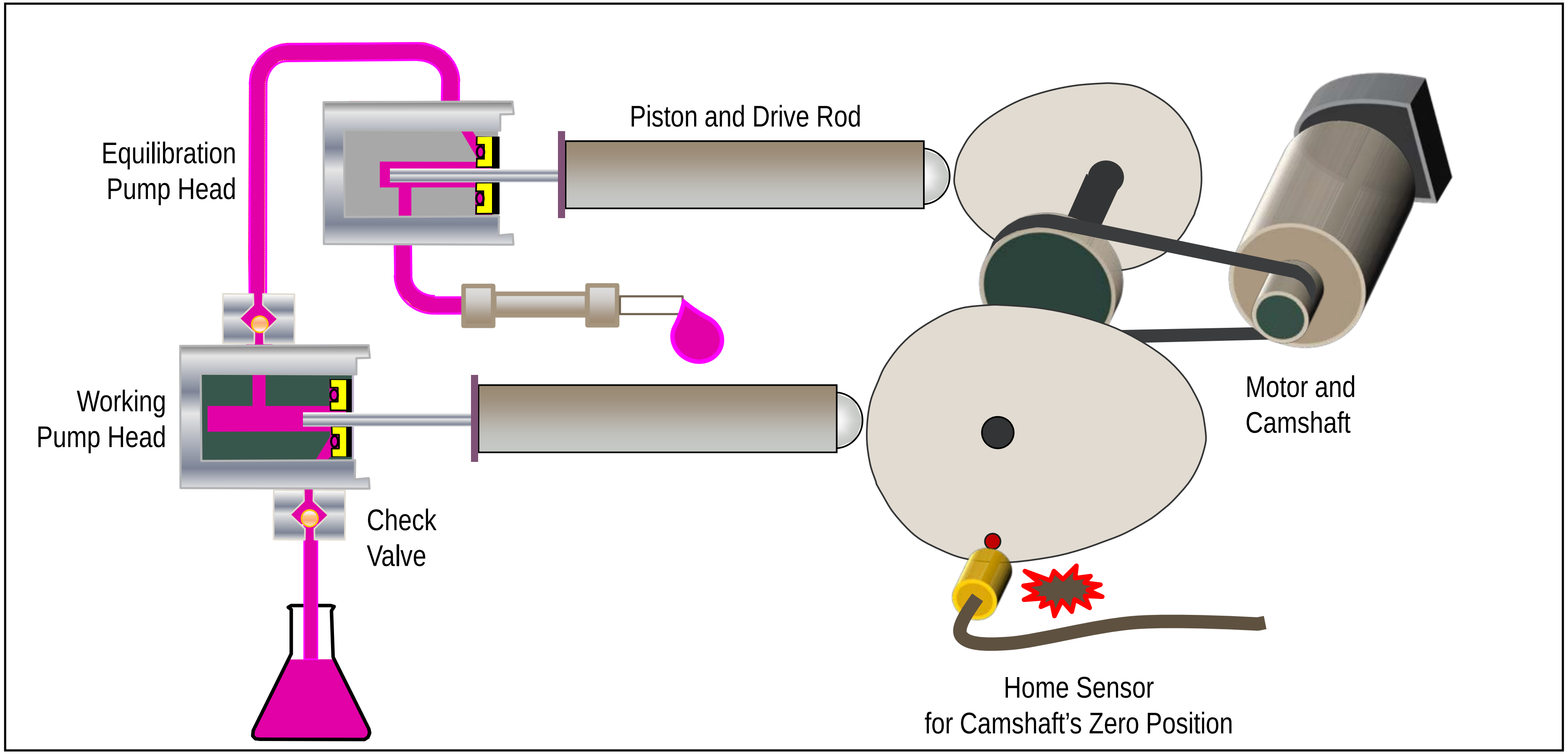


Binary High Pressure Gradient Pump



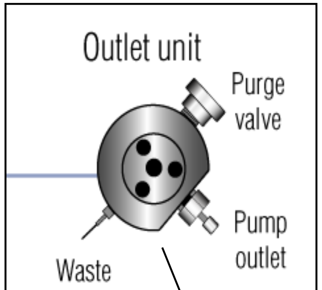
- Main pump parts
  - Working/Equilibration cylinders (for solvent delivery)
  - Degasser
  - Proportioning valve for solvent mixing
  - Dynamic/Static Mixer
  - Outlet unit with purge valve for connecting and removing air

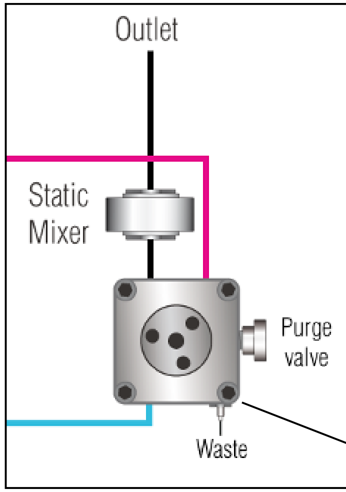
Delivering Solvents



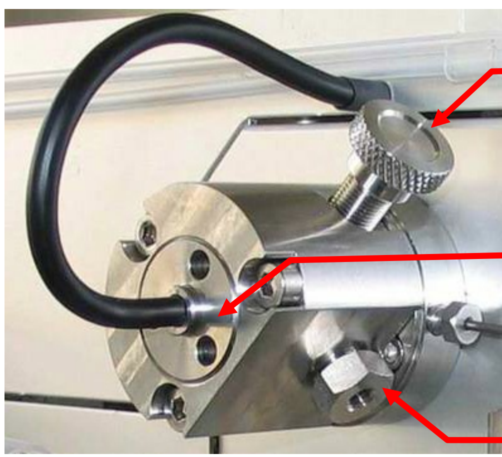
- Two pistons in the pump heads aspirate and displace the solvent
- The pistons are pushed by a camshaft and drive rods
- Camshaft driven by a motor through a gear box (with one or two belts used)
- Sensors for camshaft position and motor speed control

Mixing Solvents – Outlet Unit

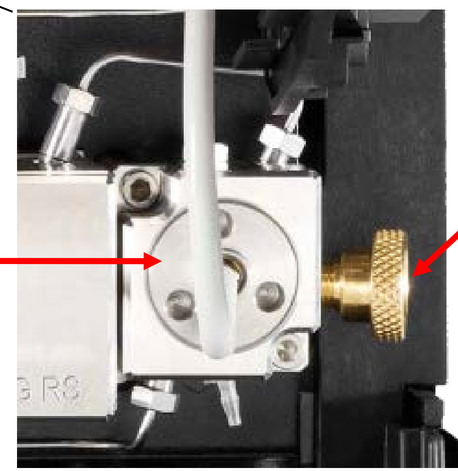




- Outlet Unit
  - Purge valve for priming and removing air
  - Pressure sensor for system pressure
  - ‘Generation 1’ (1G) pumps are equipped with a high pressure filter…
  - ... and with a dynamic mixing chamber (depends on pump model)



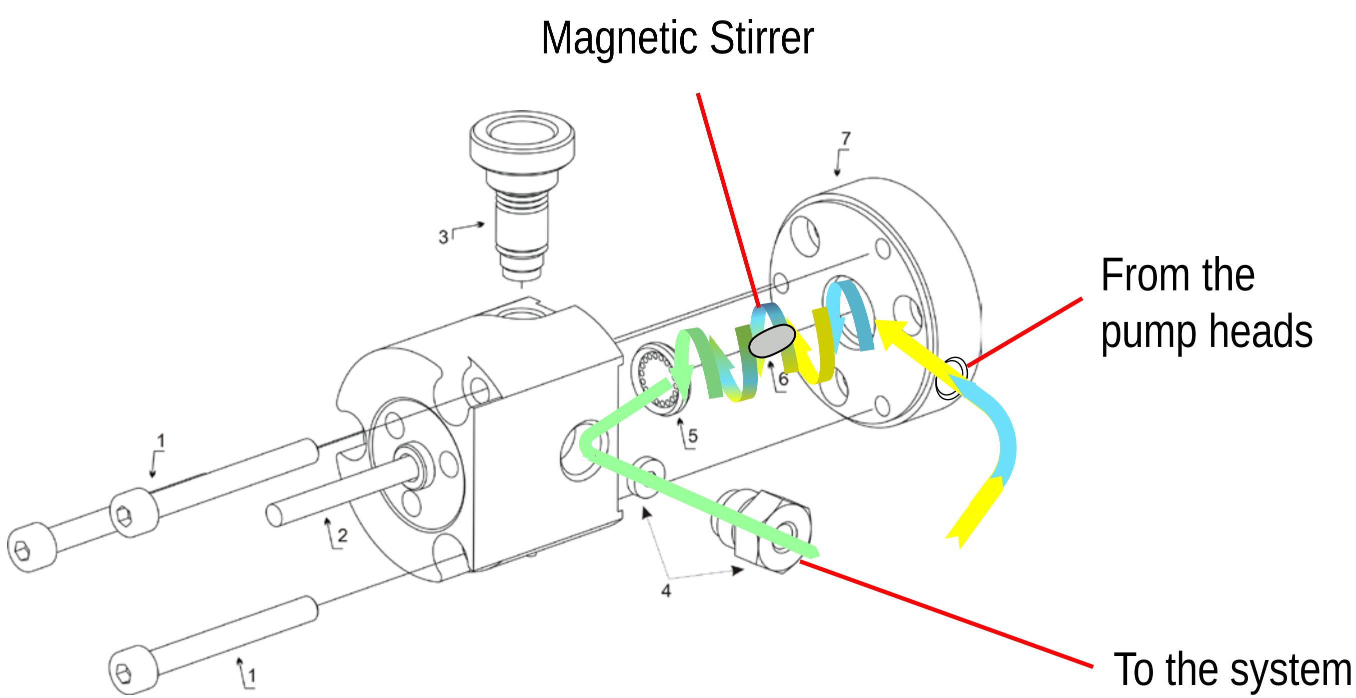
1st Generation (G1)



2nd Generation (2G)

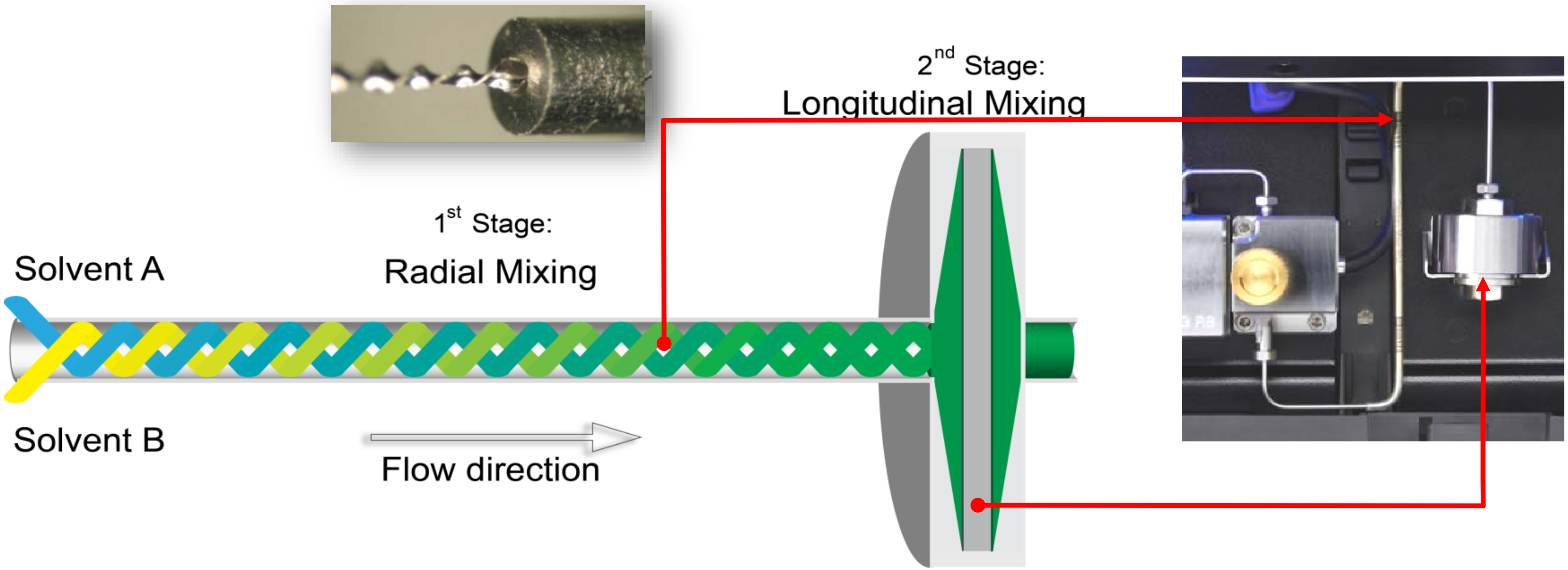
Mixing Solvents – Dynamic Mixer

- 1G pumps equipped with a dynamic mixer
- Magnetic stirrer inside the mixing chamber operated via magnetic force
- Rotation inside mixing chamber volume and ensure homogeneous mix of ‘solvent plugs’



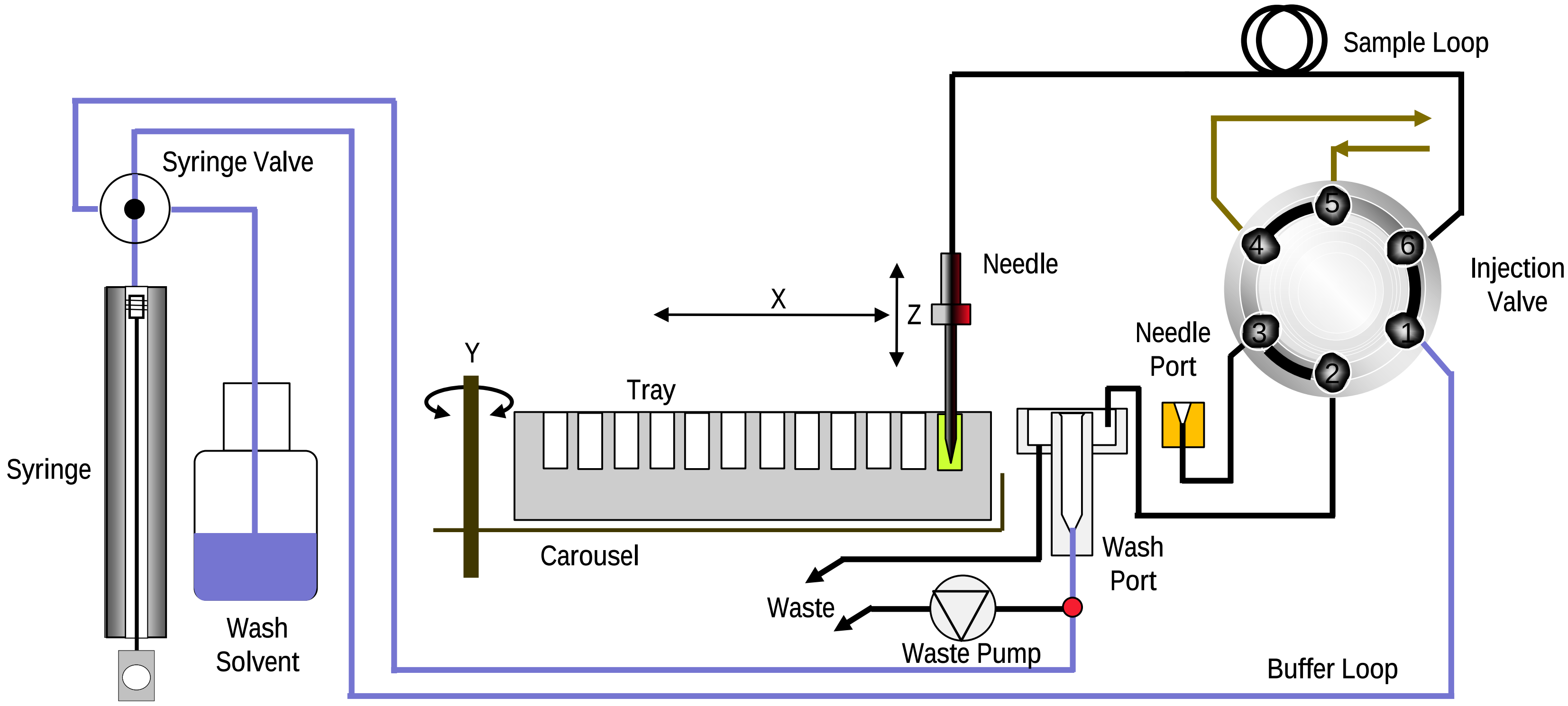
Mixing Solvents – Static Mixer

- 2G pumps are equipped with a static mixing system
- Two-step mixing system:
  - Small volume mixing capillary with helix for radial mixing (25 or 50 µL)
  - Variable static mixer with frit for longitudinal mixing (10 – 1400 µL)

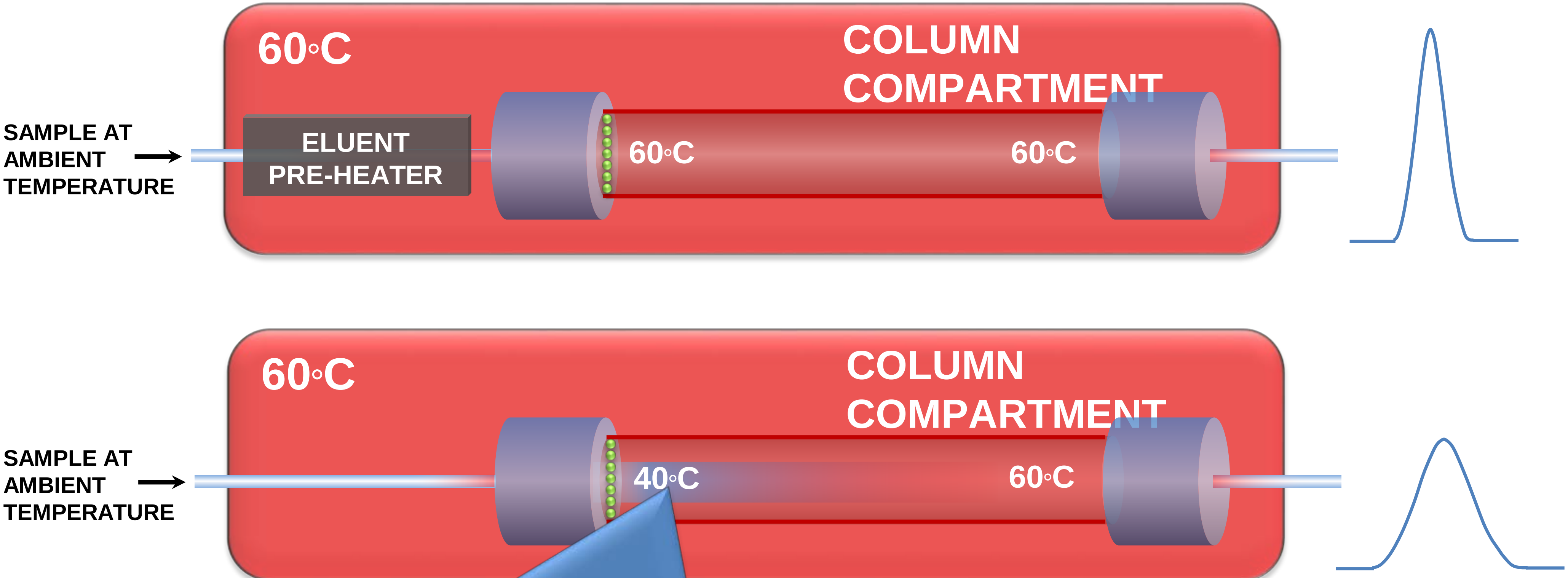


Operating Principle – General Design

- In general, all autosamplers are using the same main parts
  - Needle and sample loop
  - Injection Valve
  - Syringe with syringe valve; Wash port
  - Carousel, trays and needle drive



Loss of Peak Resolution Due to Thermal Mismatch



Mismatch:

- Centre of column below oven temperature
  - Higher viscosity, lower linear velocity in centre
  - Higher retention in centre

Sci Spec

The UltiMate™ 3000 LC Systems

Pumps

Isocratic

Binary

Quaternary

Dual-Gradient

Autosampler

Basic Automated

Standard

Thermostatted

+ Fractionation

Column Compartments

Standard

With Valves

Detectors

VWD

MWD/DAD

Fluorescence

Corona

Coulochem

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

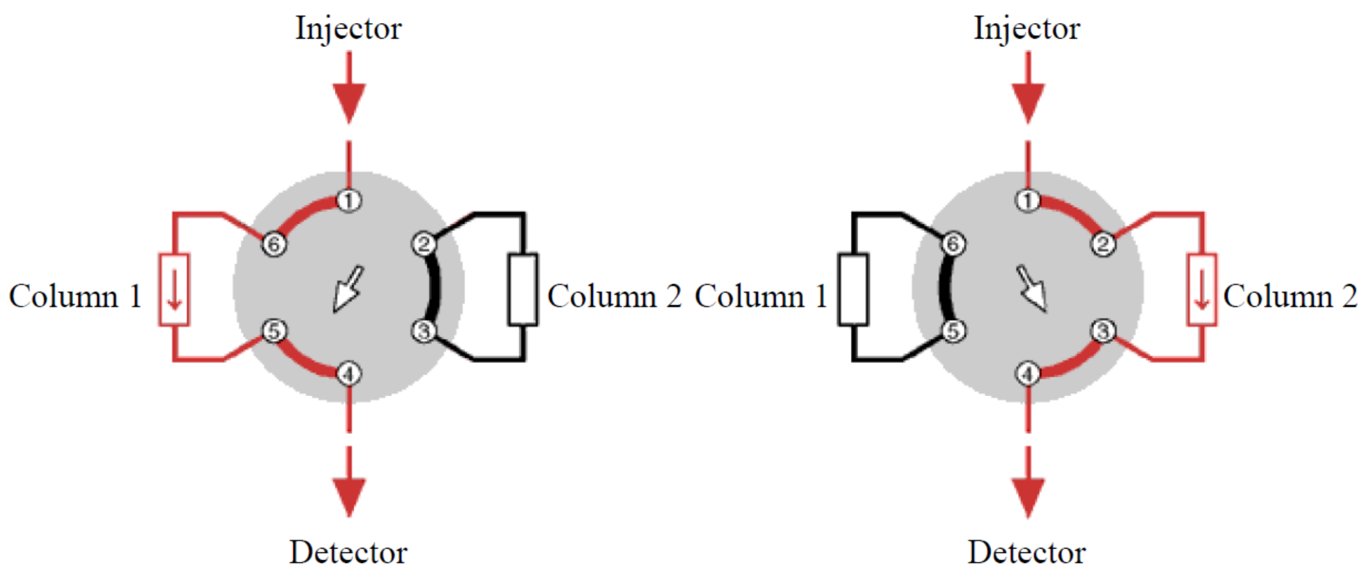
RSLCnano

- 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

UHPLC^{+} Applications

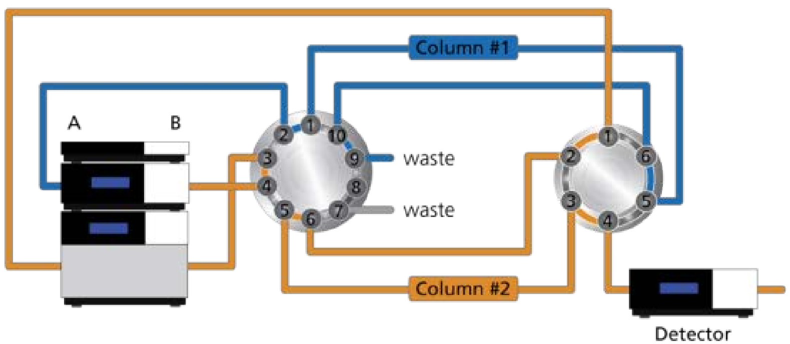
- Built-in column switching valve
- 2-position, 6-port column switching valve



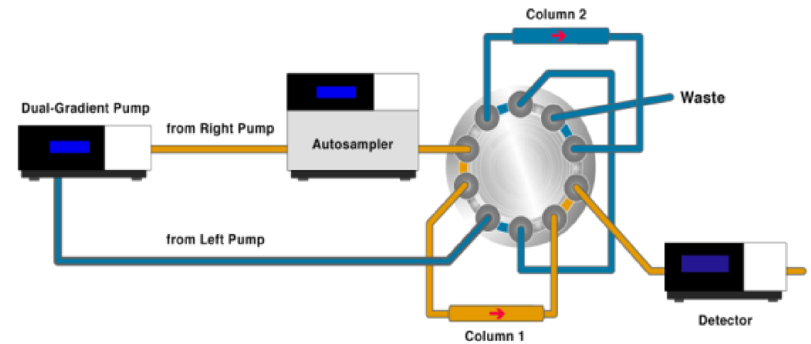
Switching Valve

UHPLC+ Applications

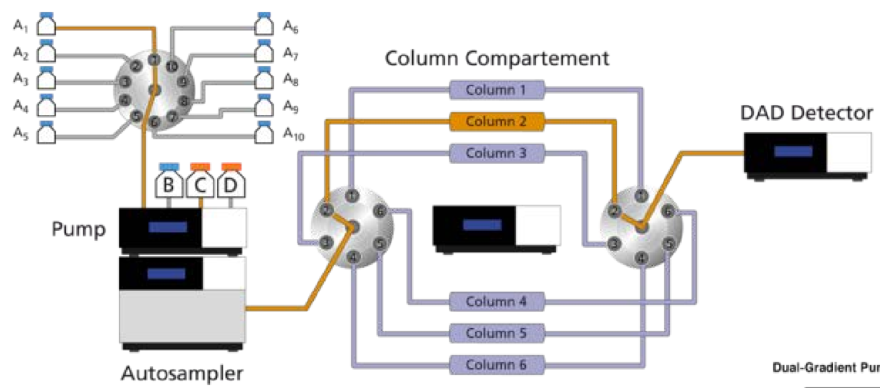
Application Switching



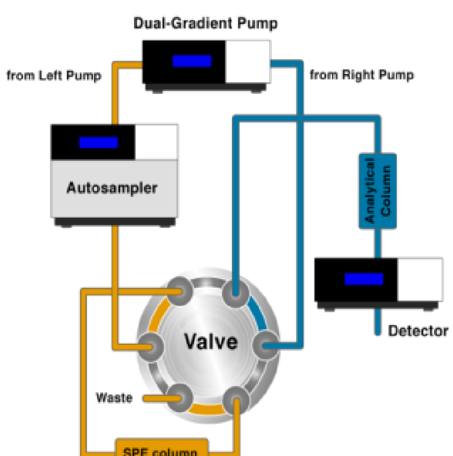
Tandem LC



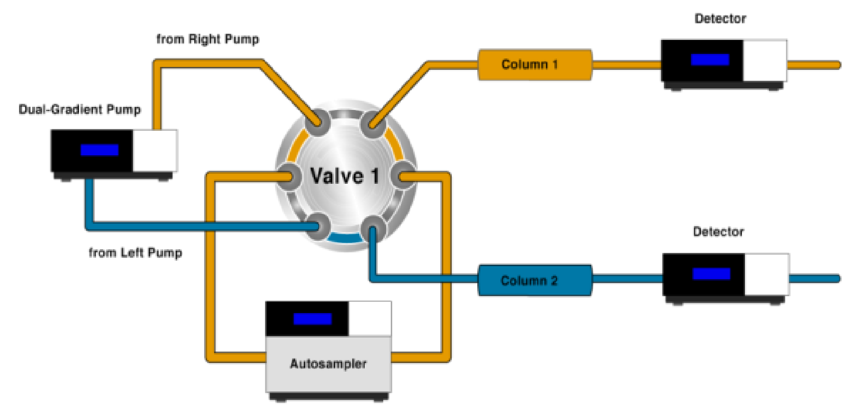
Automated Method Scouting

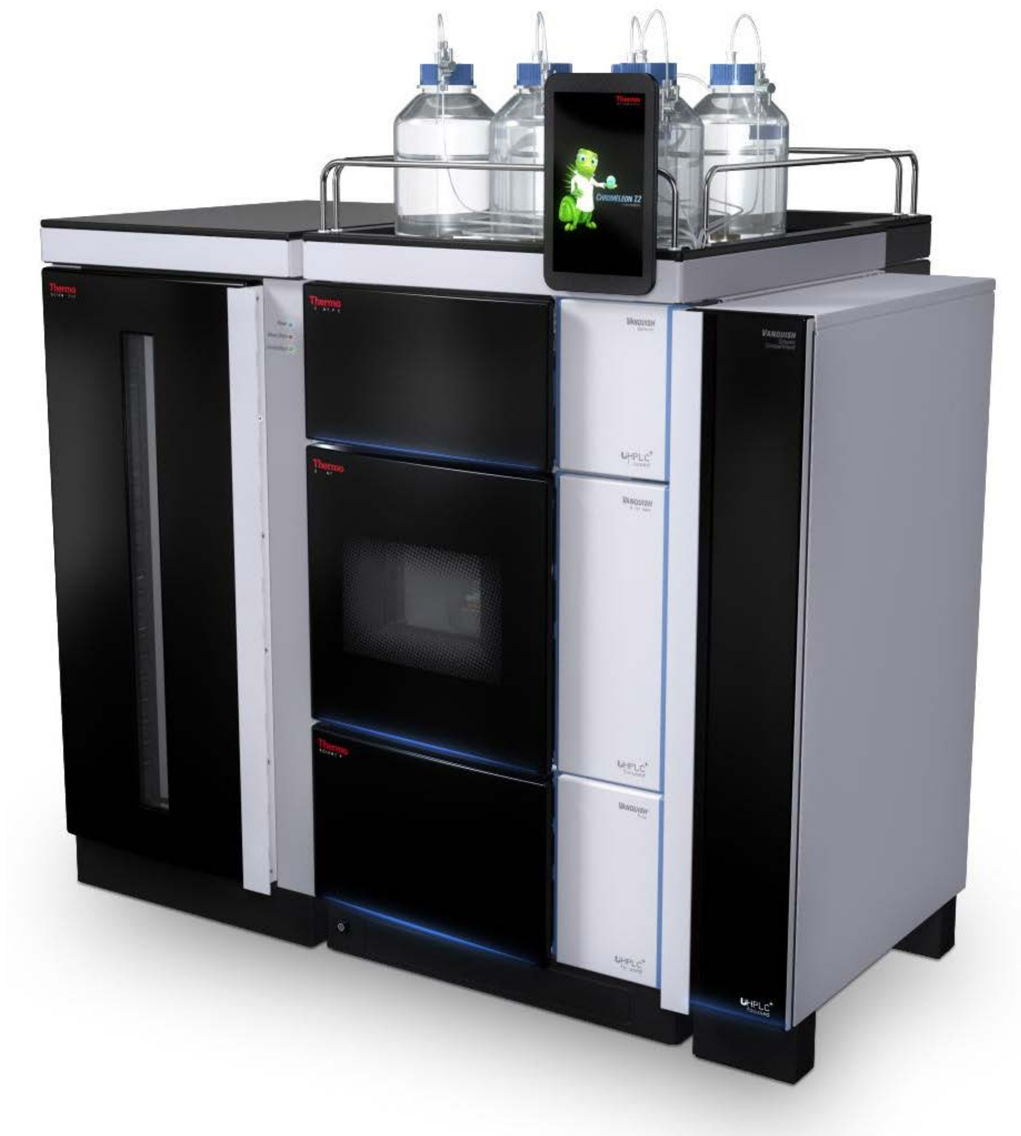


Online SPE



Parallel LC





Vanquish™

Max Pressure 1517 bar

Thermo
SCIENTIFIC

Fundamental of Mass Spectrometry

Sci Spec

Your Scientific Specialist

https://www.thermofisher.com/order/catalog/product/TSQ02-10001?SID=srch-srp-TSQ02-10001

What is Mass Spectrometer?

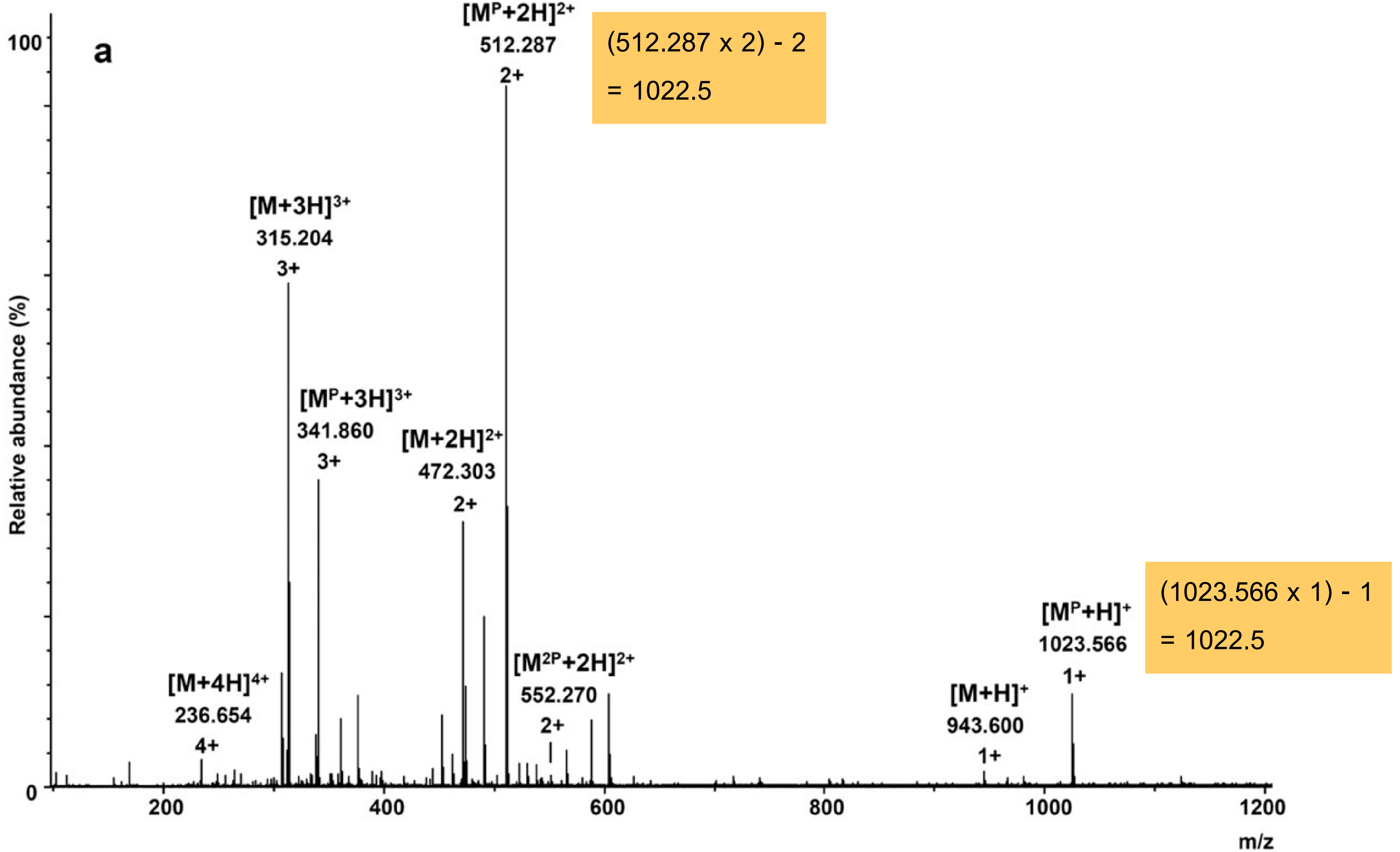
"The basis in mass spectrometry (MS) is the production of ions, that are subsequently separated or filtered according to their **mass-to-charge (m/z) ratio**, and detected. The resulting mass spectrum is a plot of the (relative) abundance of the produced ions as a function of the m/z ratio."

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

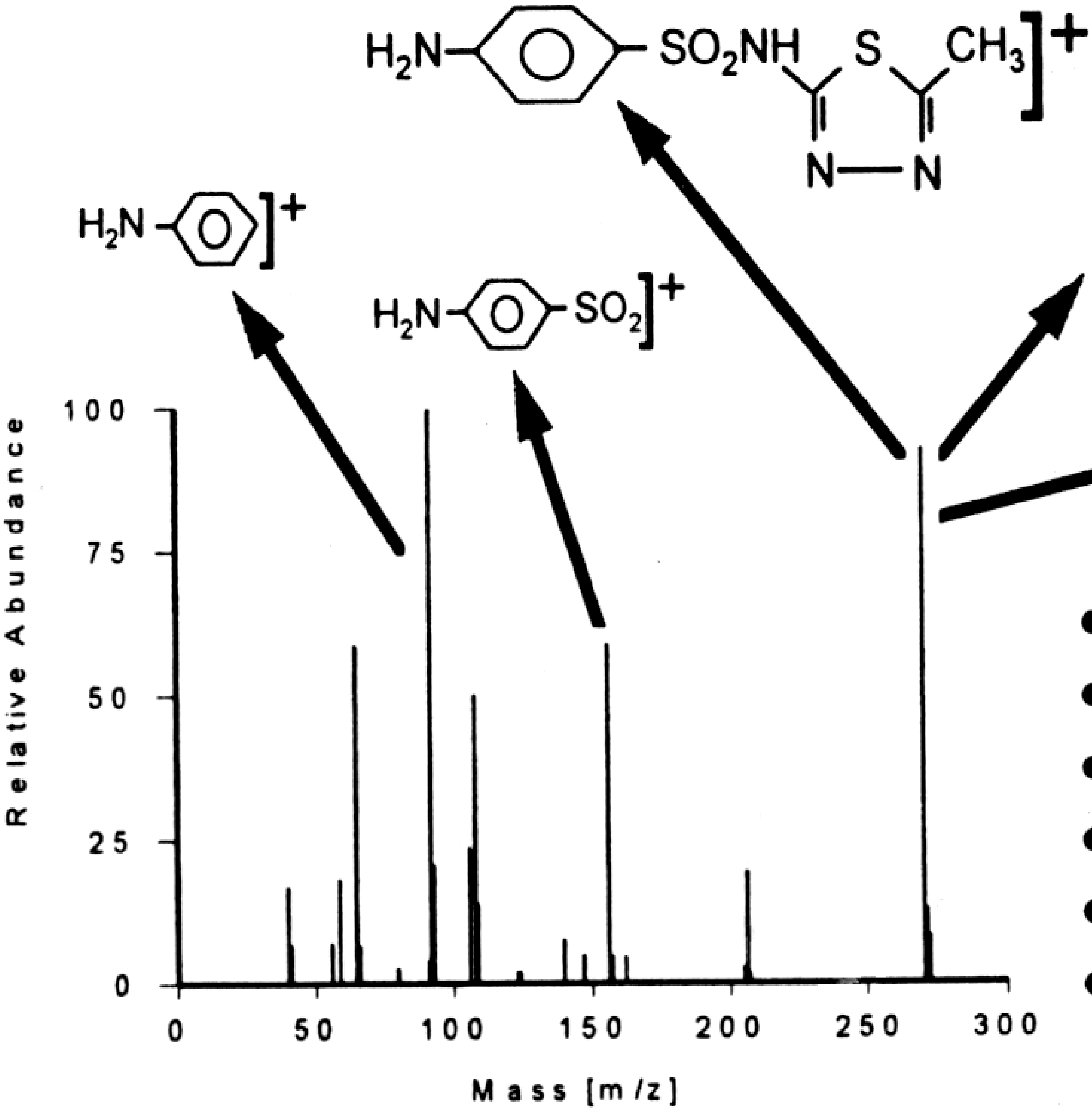
- Operate at very low pressure (10^{-5} to 10^{-7} torr) (Atmosphere = 760 torr)
- Mass spectrometer work with **IONS**
- Measure gas-phase ions
- Determine the mass are separated according to their **mass-to-charge (m/z) ratio**

Mass Spectrum

mass to charge = (molecular weight + charge) / charge



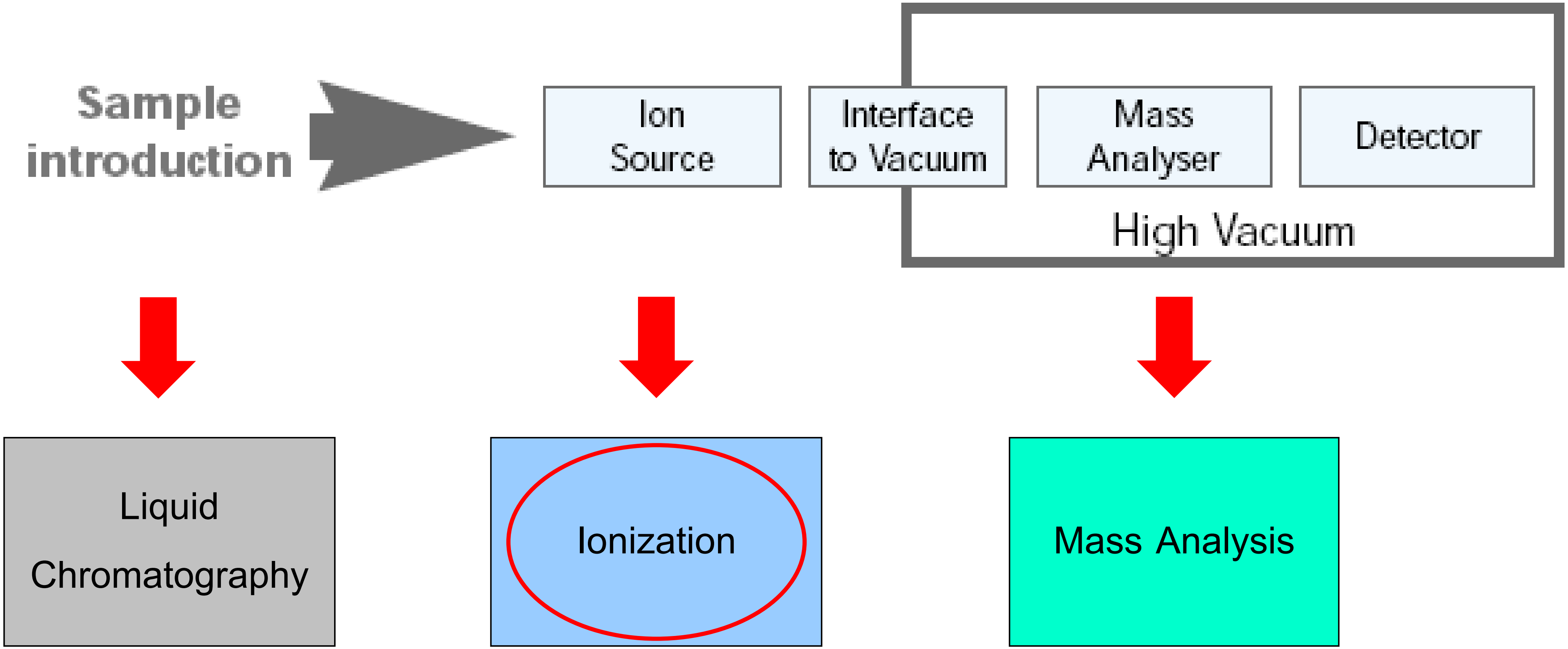
Information Rich Data



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

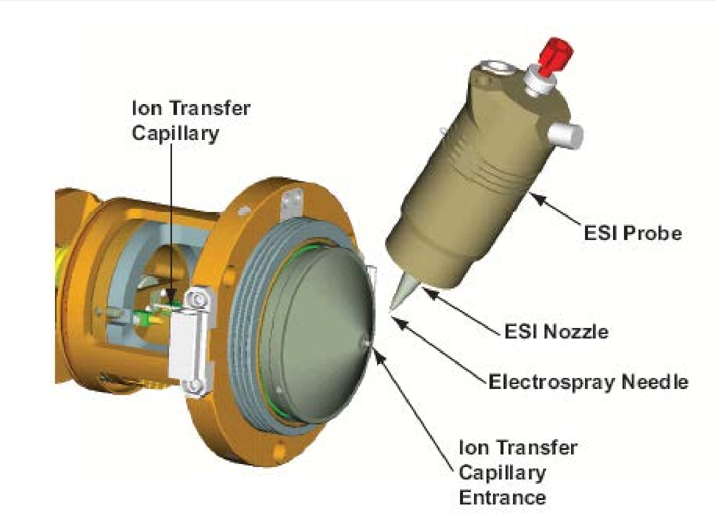


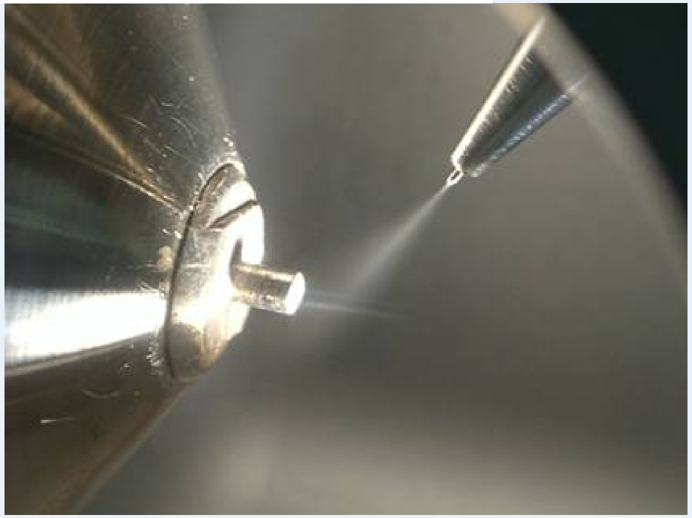
Ionization

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

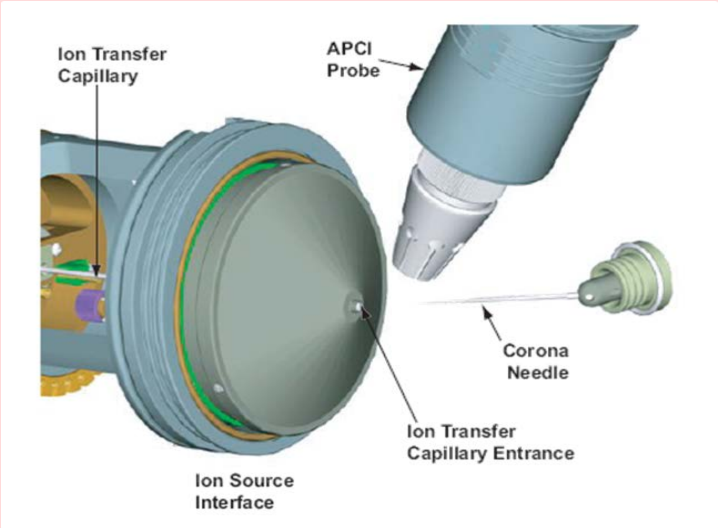
Atmospheric Pressure Ionization (API)

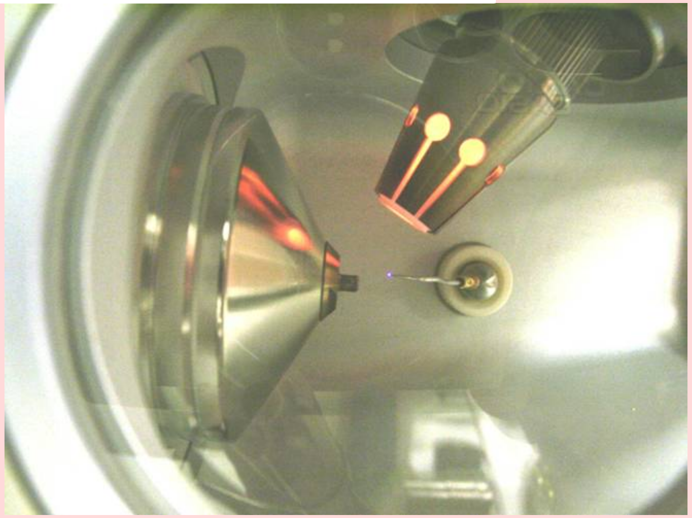
ESI





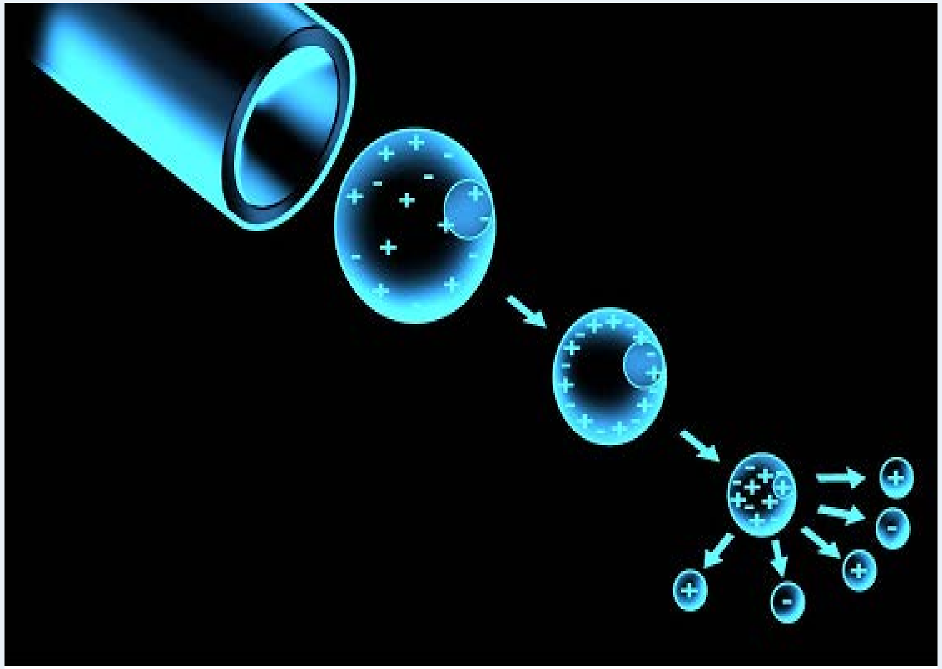
APCI





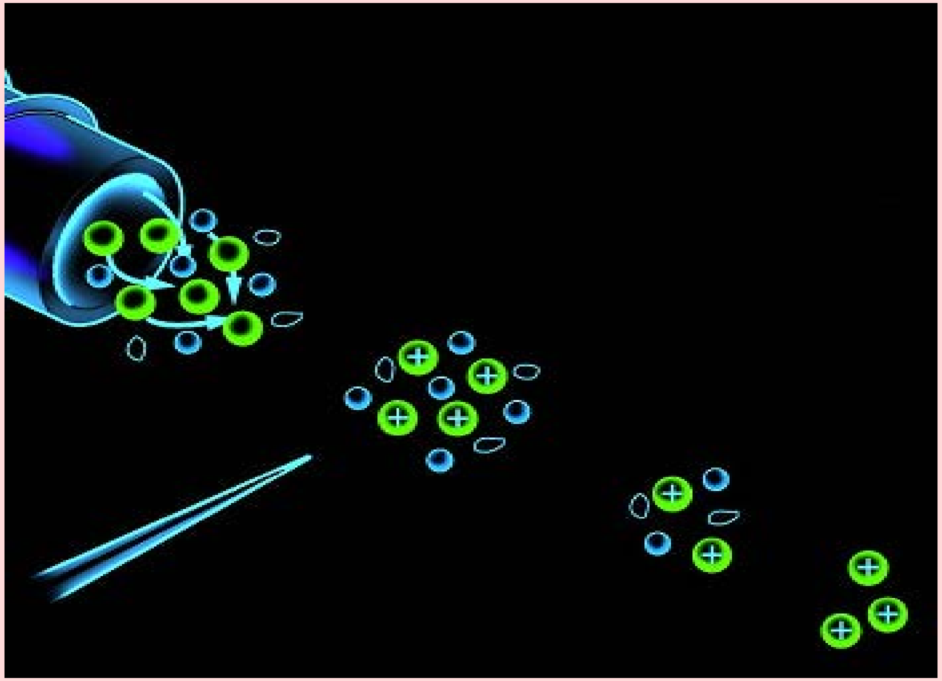
Atmospheric Pressure Ionization (API)

ESI



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

APCI

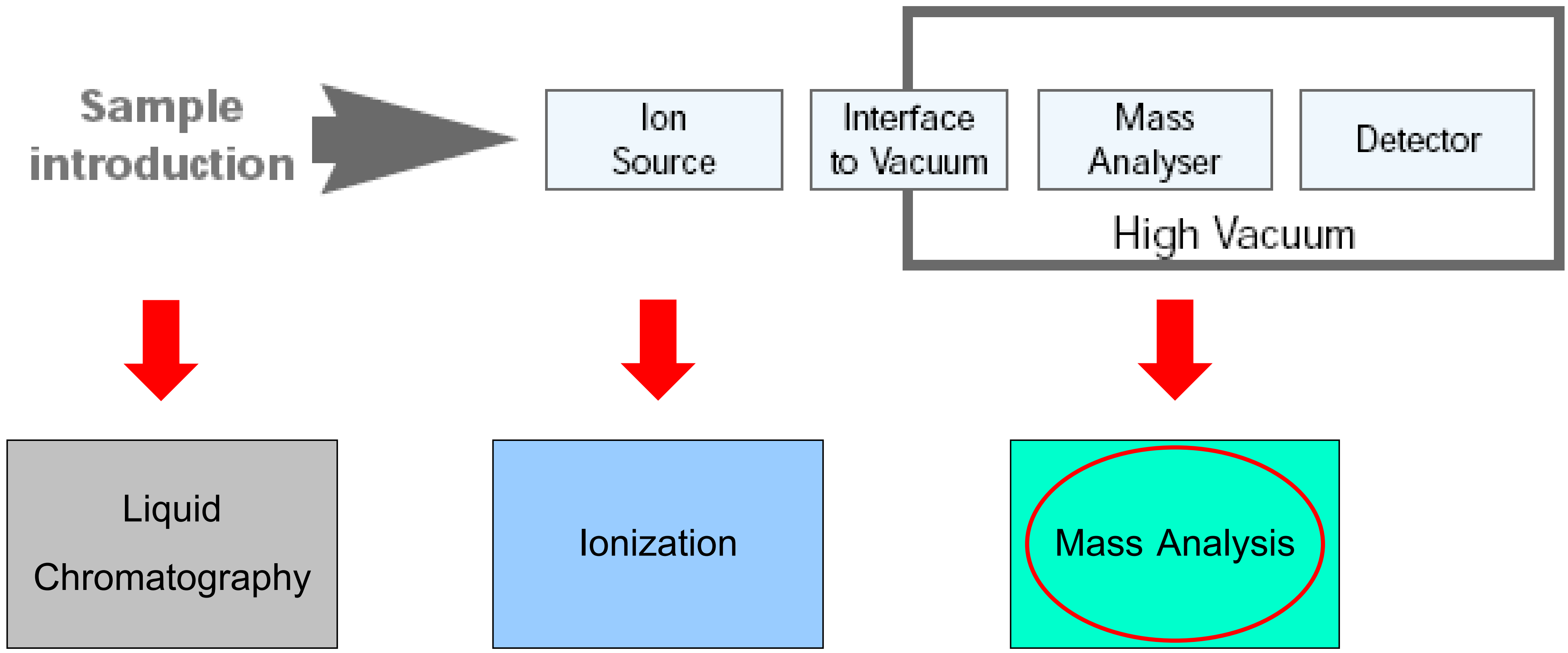


- Ions formed by gas phase chemistry
- Good for volatile / thermally stable
- Good for non-polar analytes
- Good for small molecules (steroids)

Which is Best?

- It depends on the exact application.
- Increasing polarity and molecular weight and thermal instability favors electrospray.
  - Most drugs of abuse are highly polar and are easily analyzed using electrospray.
  - High molecular weight proteins also require electrospray
- Lower polarity and molecular weight favors APCI or APPI.
  - Lower background, but compounds must be more thermally stable.

Mass Spectrometry: Block Diagram



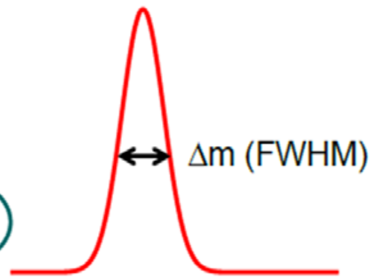
Typical Mass Accuracy and Resolution

Type of MS	Mass accuracy	Resolution	Utility for
Quadrupole	0.1 amu	6,000	Identify
Traps	0.1 amu	8,000	Identify
TOF	0.0001 amu	<20,000 TOF 60,000 Q-TOF	Empirical formula/ composition
Sector	0.0001 amu	10,000	Empirical formula/ composition
Orbitrap	0.0001 amu	1,000,000	Empirical formula/ composition

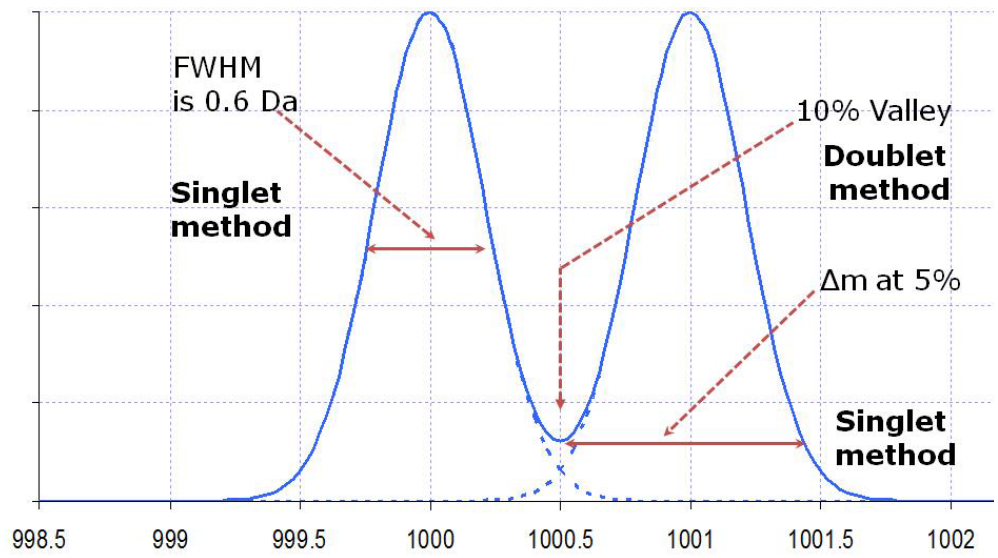
Mass Resolution

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

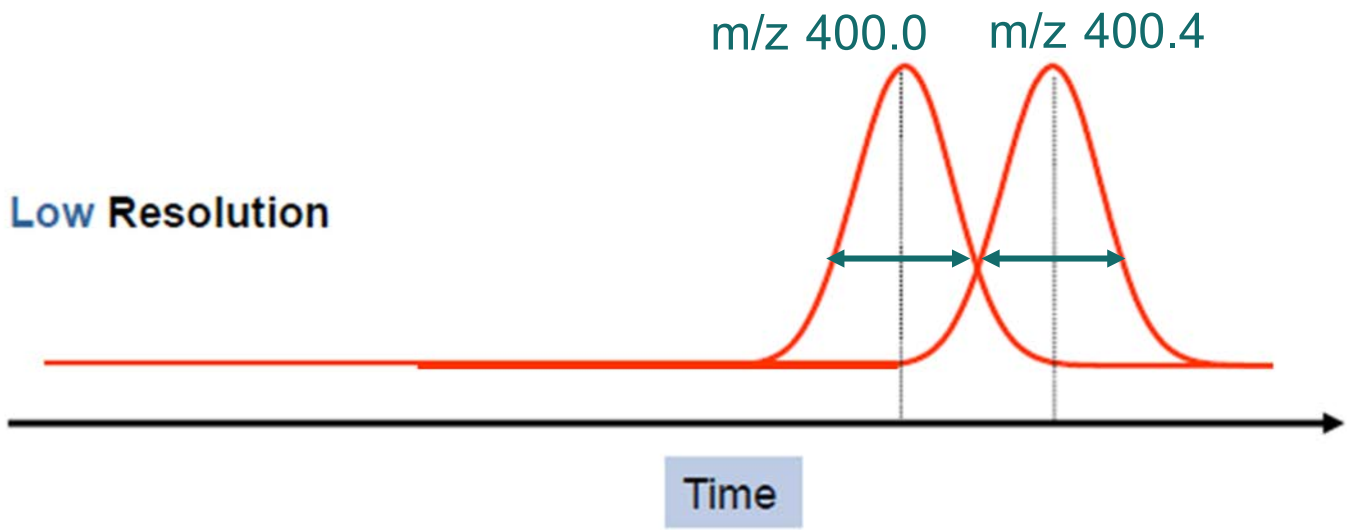
$$R = \frac{m}{\Delta m}$$



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

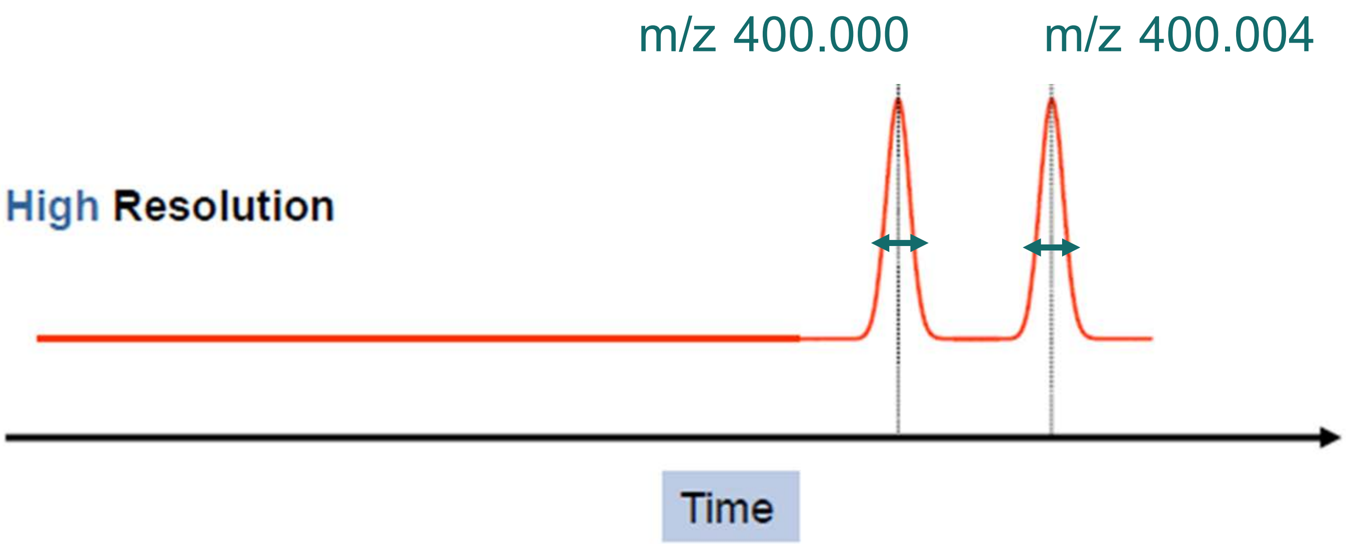


Mass Resolution



- Quadrupole MS

$$R = \frac{400}{0.4} = 1000$$



- **Orbitrap (HRAM) MS**

$$R = \frac{400}{0.004} = 100000$$

Mass Resolution: What is it?

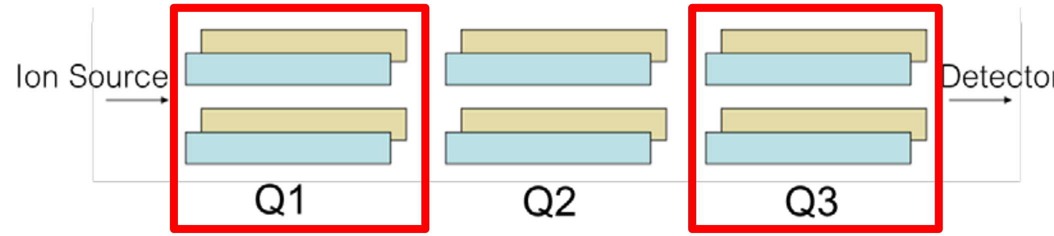
- At minimum the resolution of the mass analyzer should be sufficient to separate two ions differing by one mass unit anywhere in the mass range scanned (unit mass resolution).
- Typical values of resolution for low resolution mass analyzers (e.g. quadrupoles and ion traps) are below **5000**.
- High resolution instruments have a resolution exceeding **15000**.

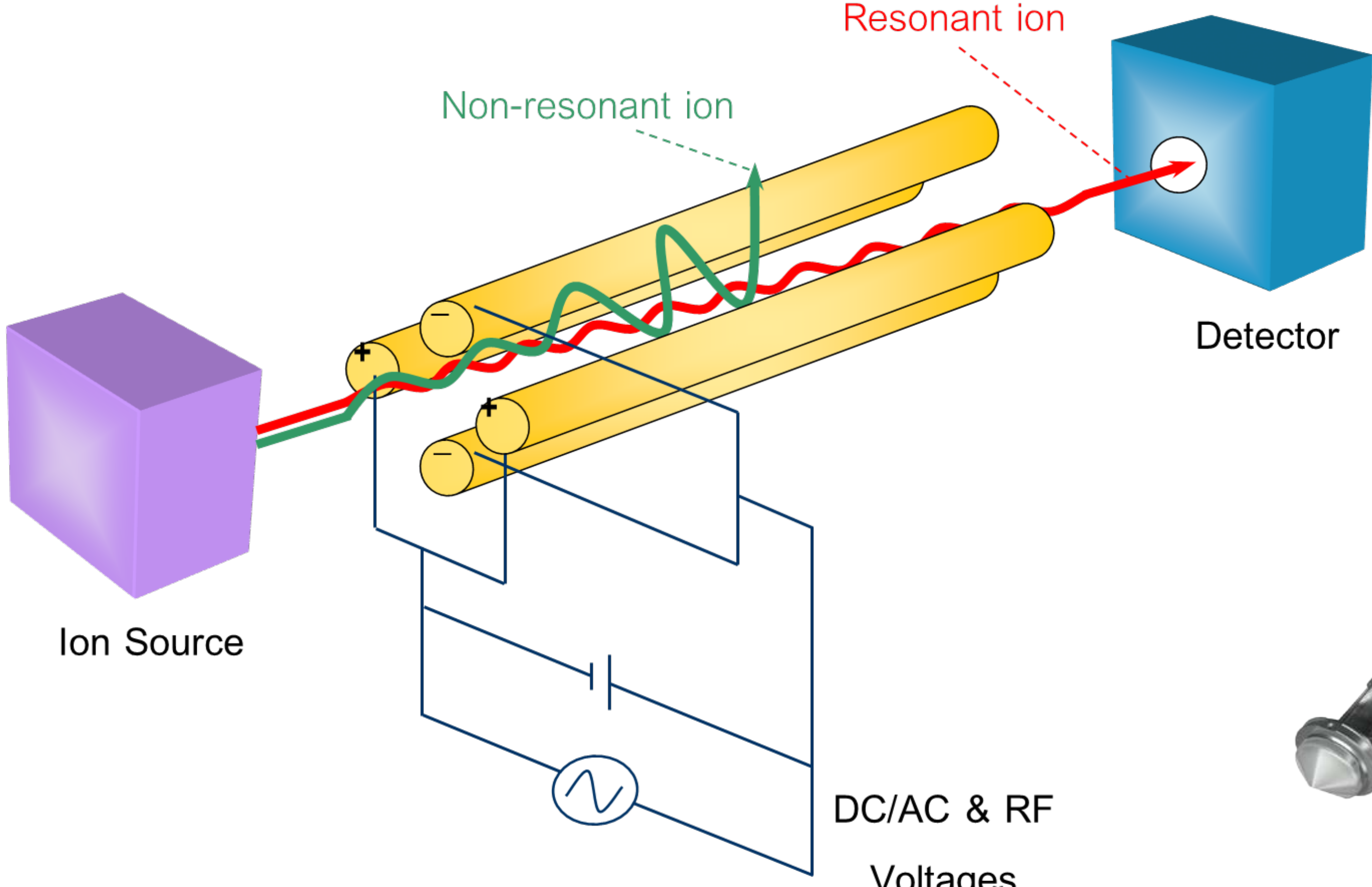
MASS ANALYSER

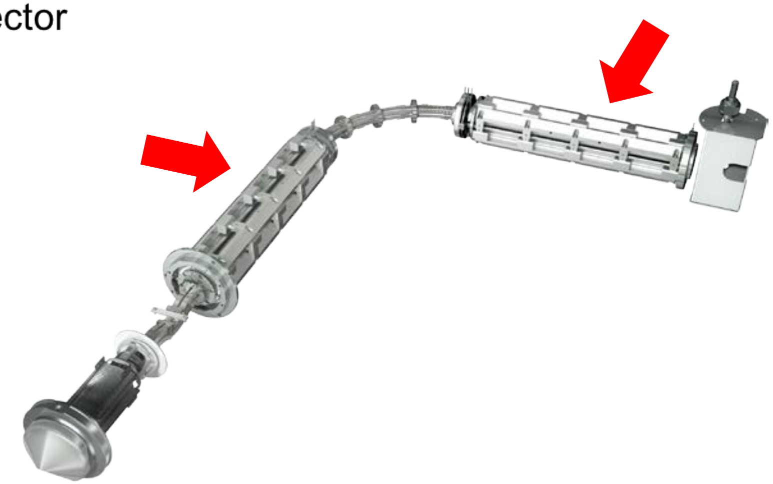
QUADRUPLE

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

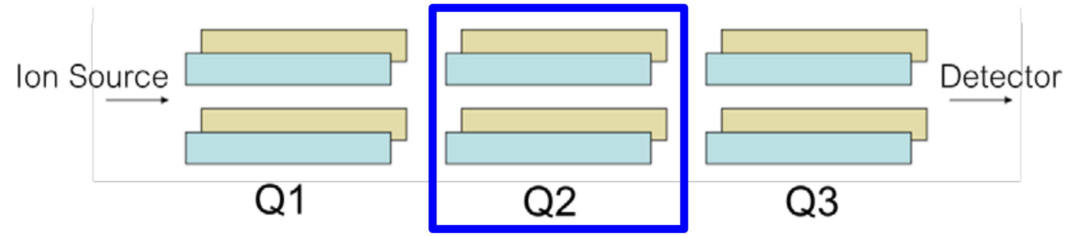


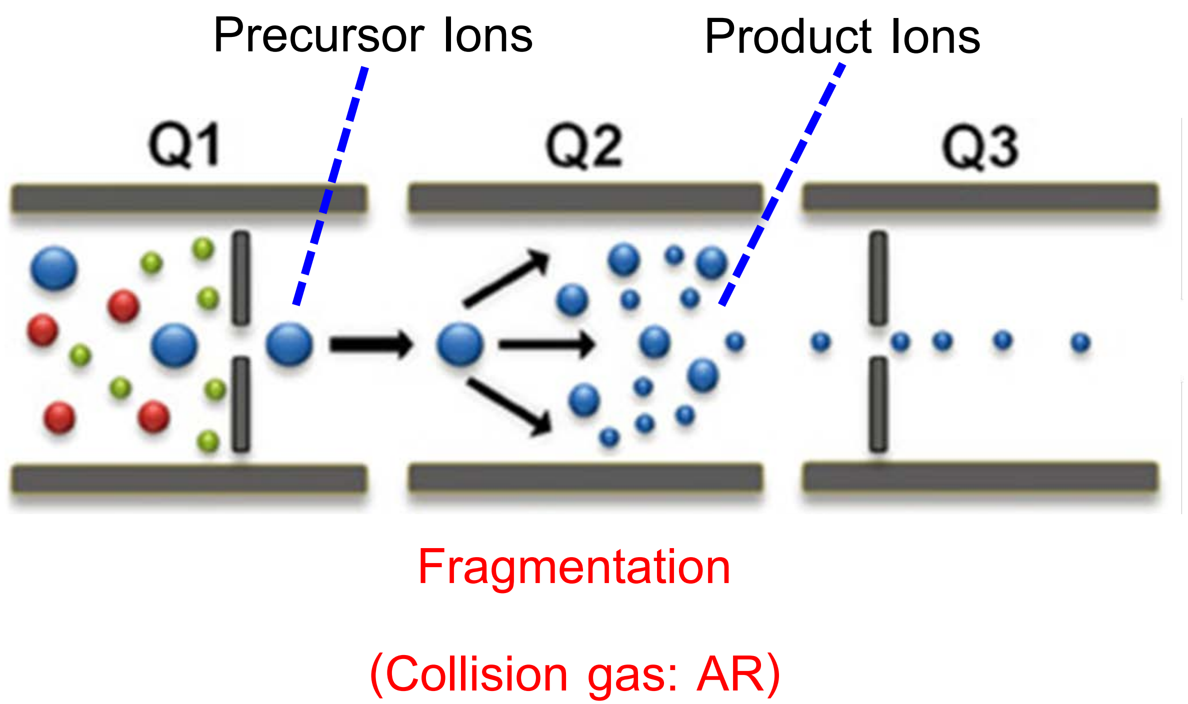


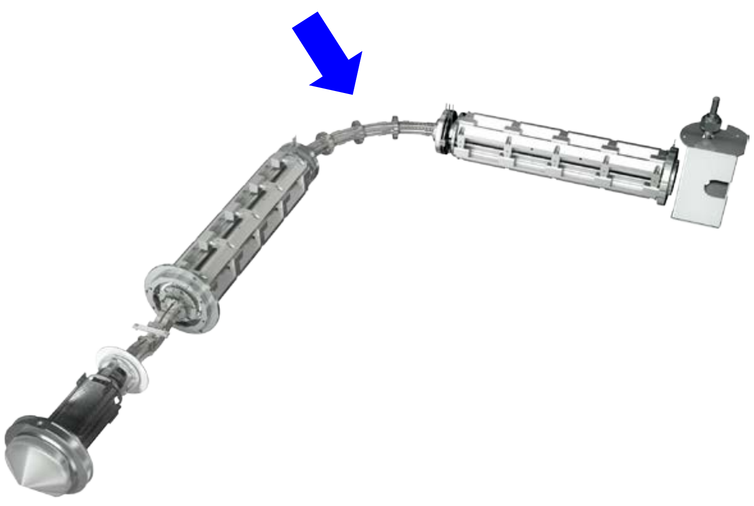


Mass Analyzer: Triple Quadrupoles (QqQ)

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

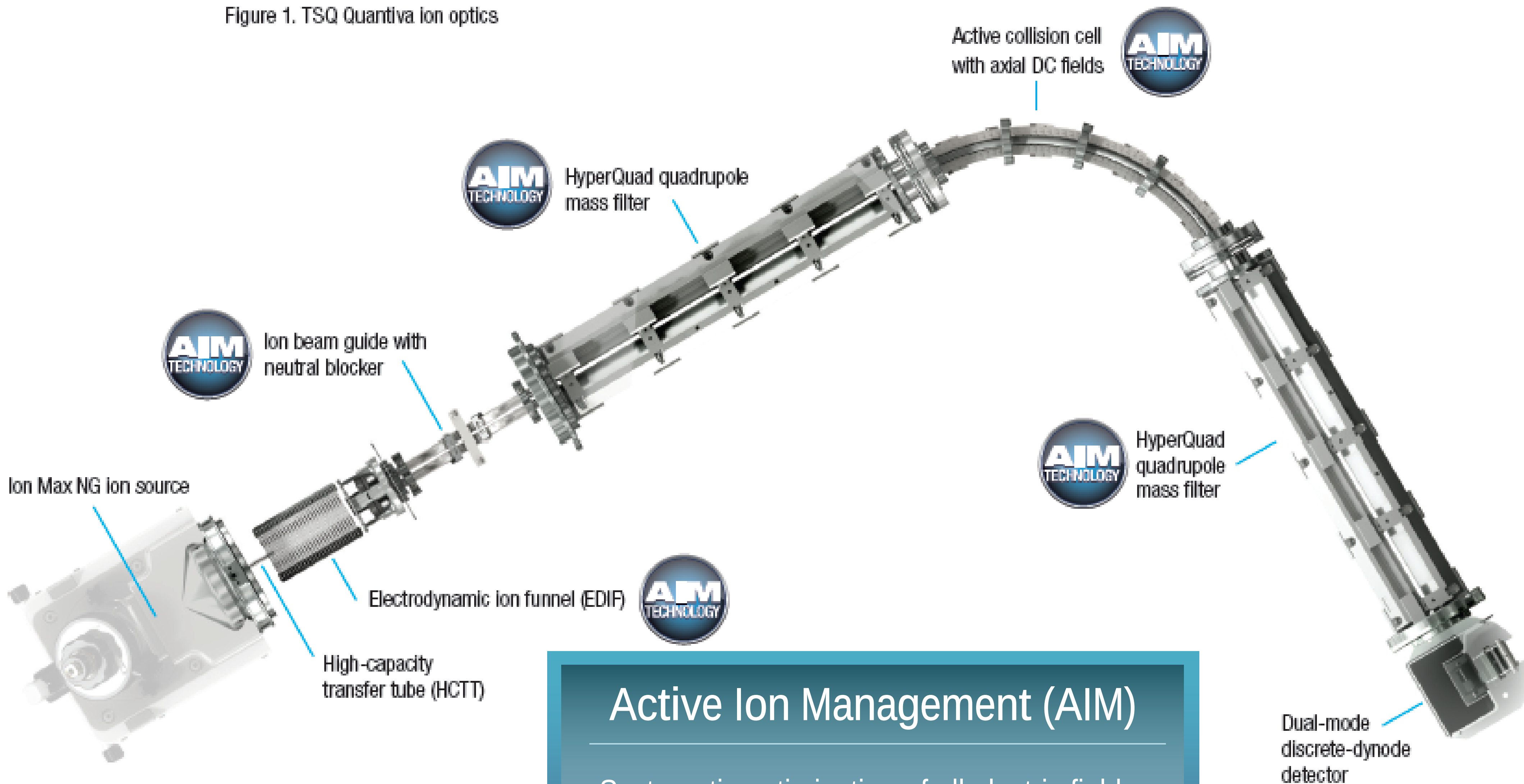






TSQ Quantiva MS—Powered by AIM Technology

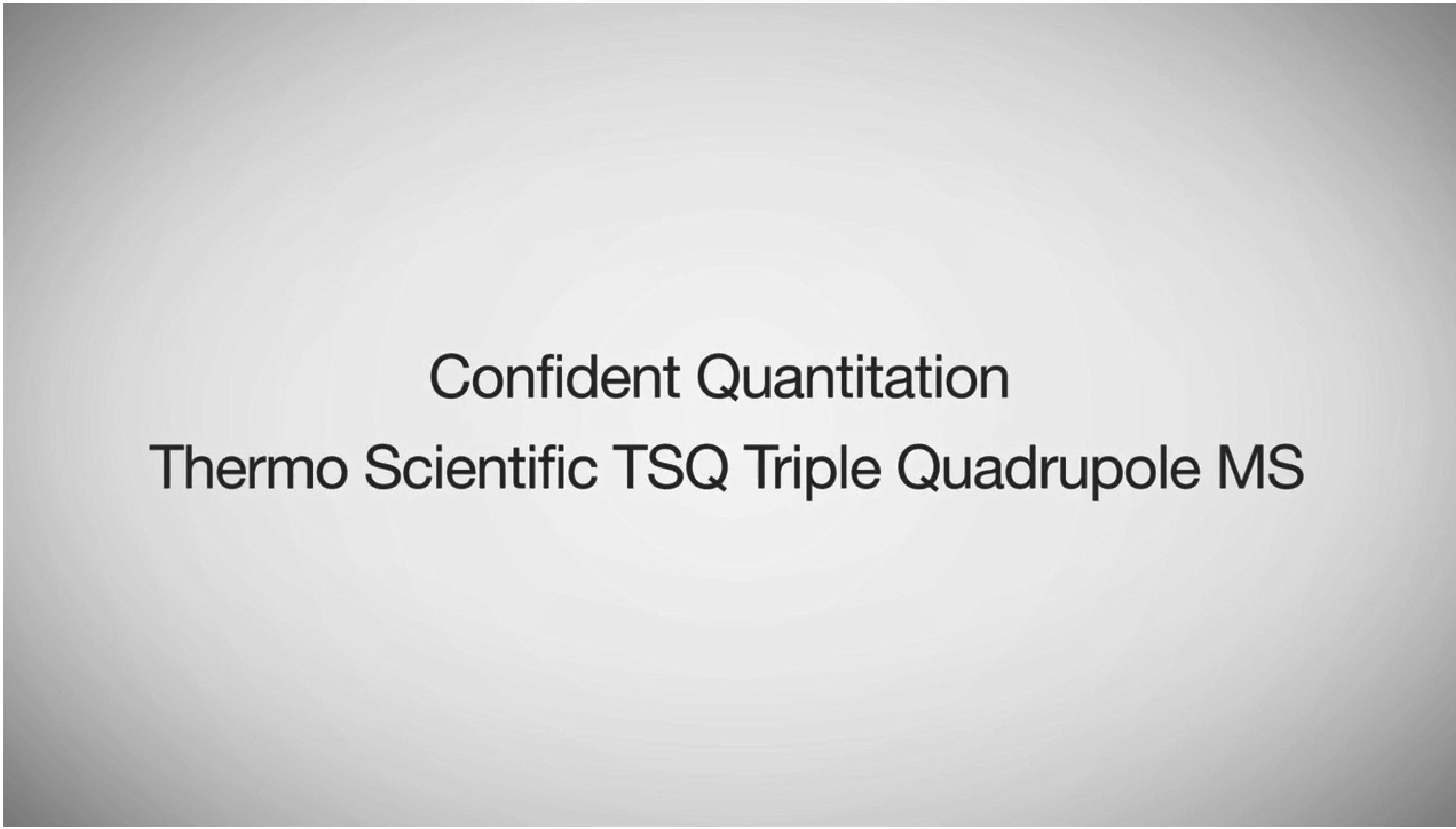
Figure 1. TSQ Quantiva ion optics



Active Ion Management (AIM)

Systematic optimization of all electric fields, in concert, to produce breakthrough performance.

TSQ Triple Quadrupole (available on YouTube)



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

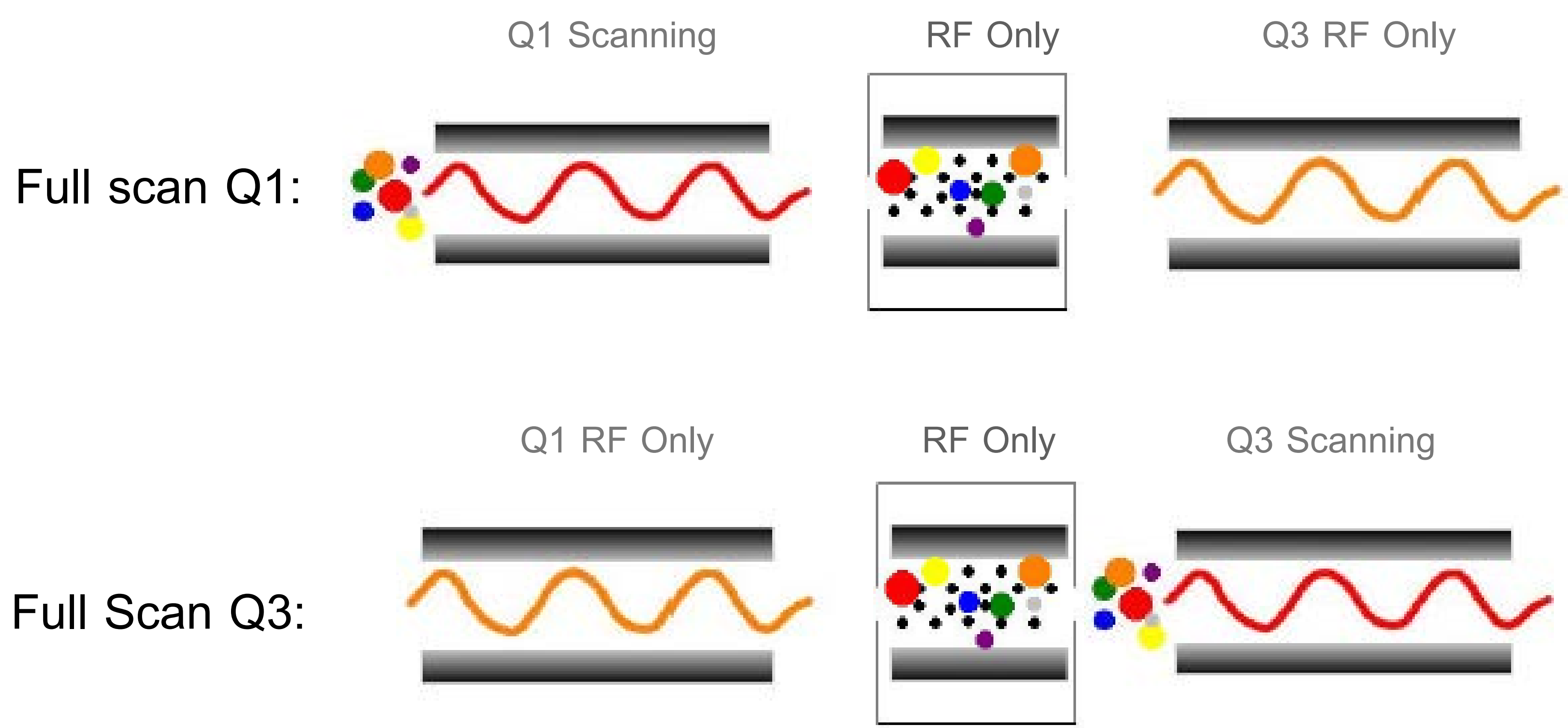
Scan Modes in Quadrupole

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

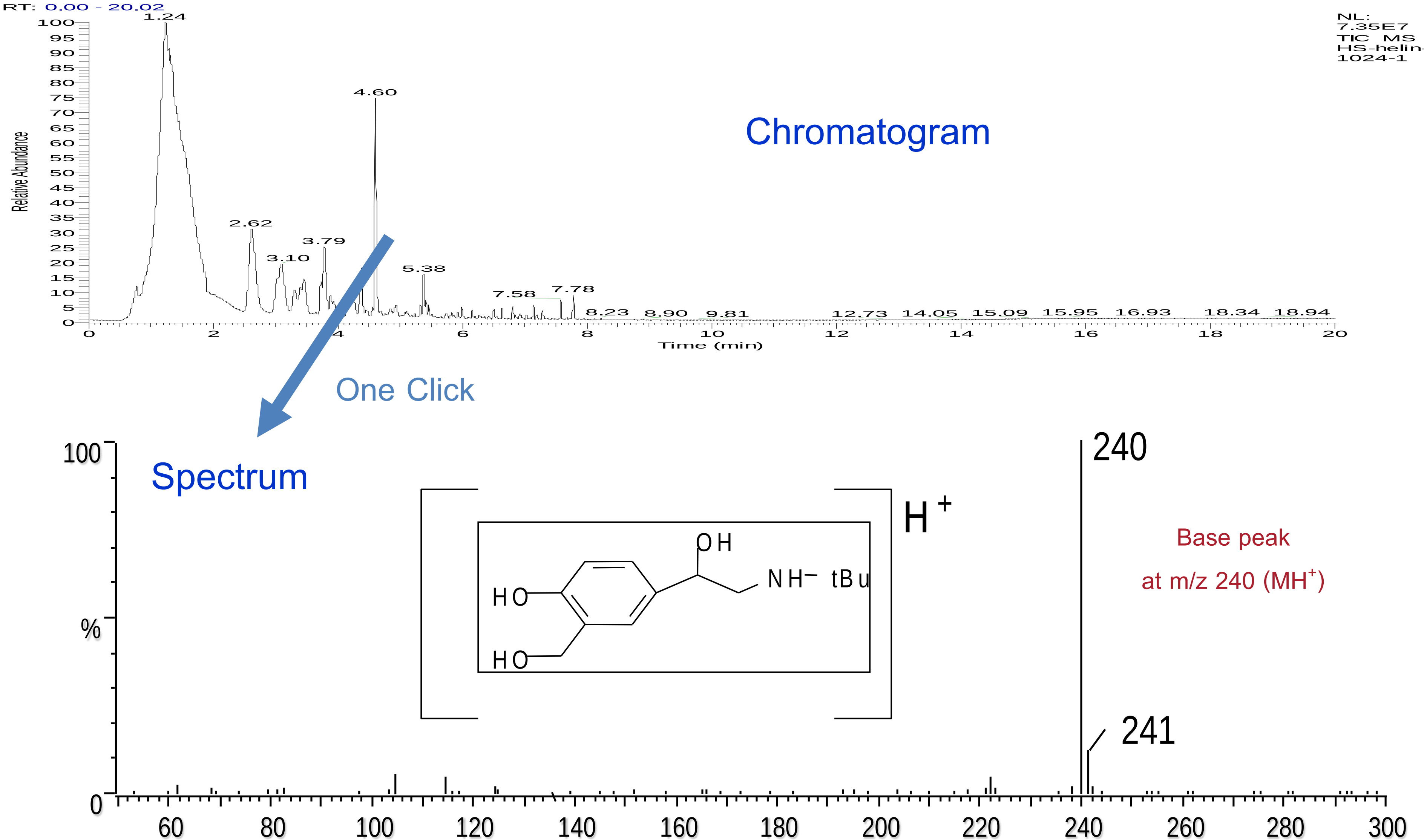
Full Scan (Q1 or Q3)

Full Scan Mode

Purpose: Survey scan of a chromatographic peak

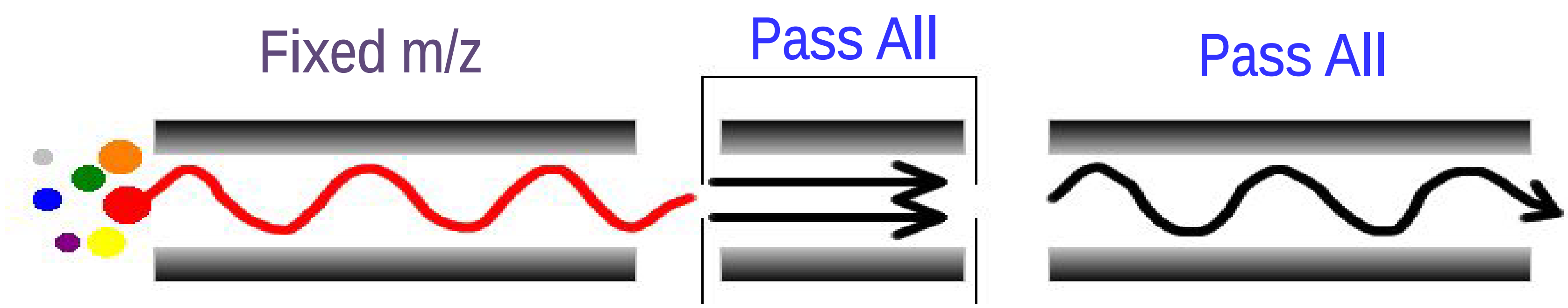


Full Scan Mode



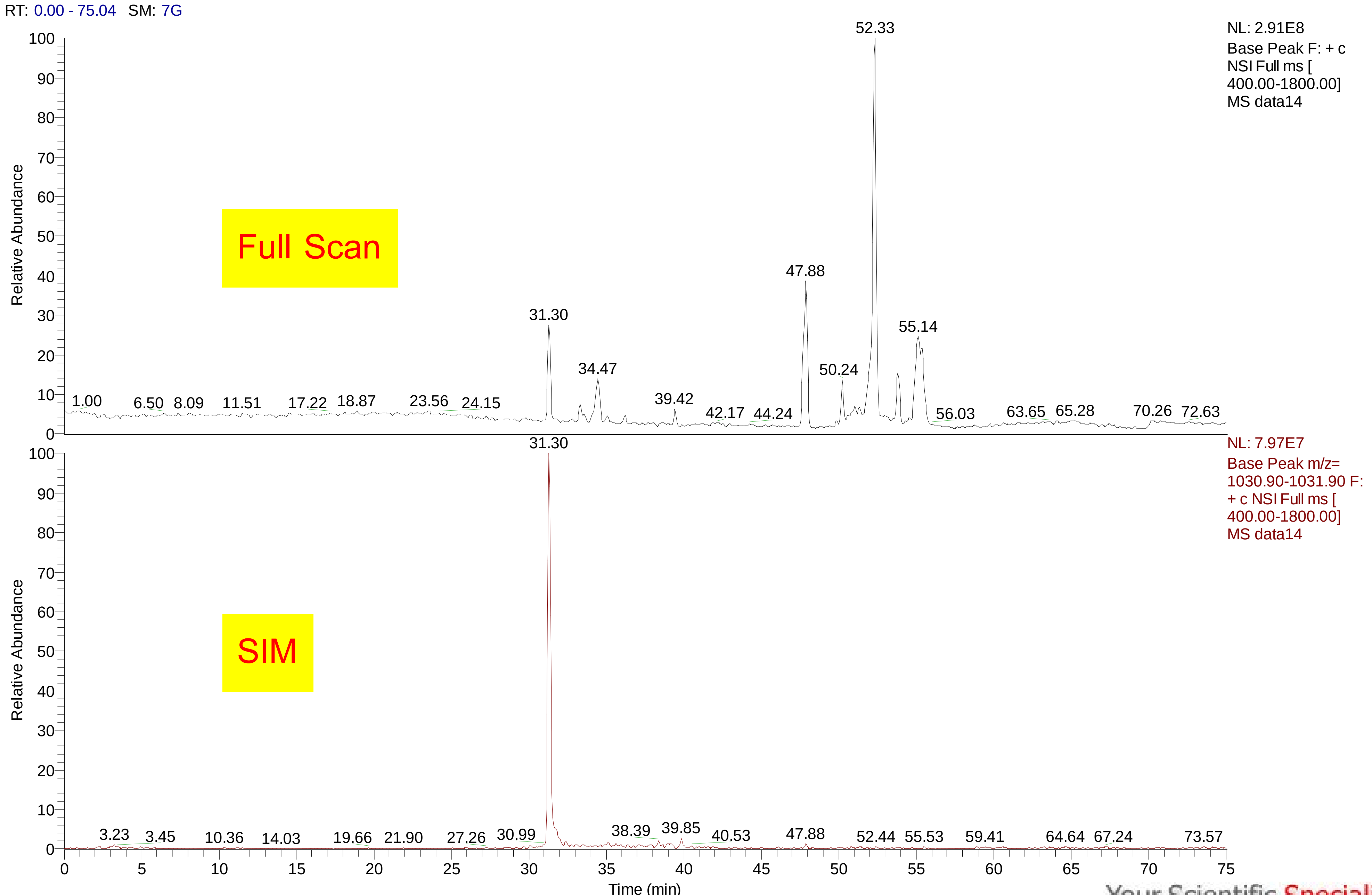
Selected Ion Monitoring – SIM

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



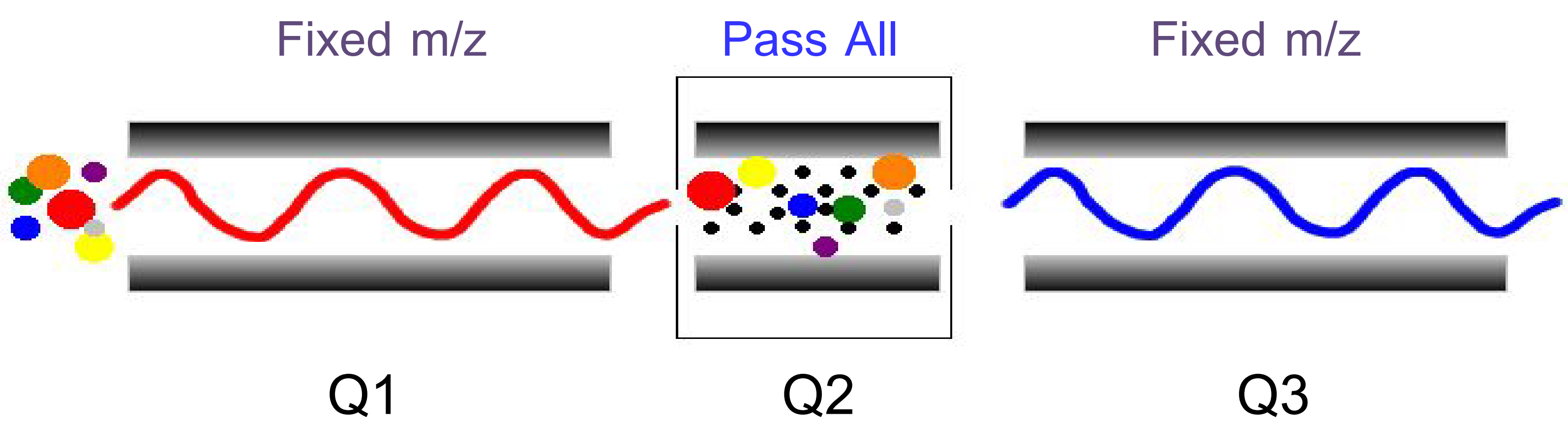
- Advantages
  - Targeted analyte monitoring
  - High duty cycle
- Disadvantages
  - Can suffer from interferences
  - Not as sensitive or selective as SRM

Full Scan VS SIM



Your Scientific Specialist

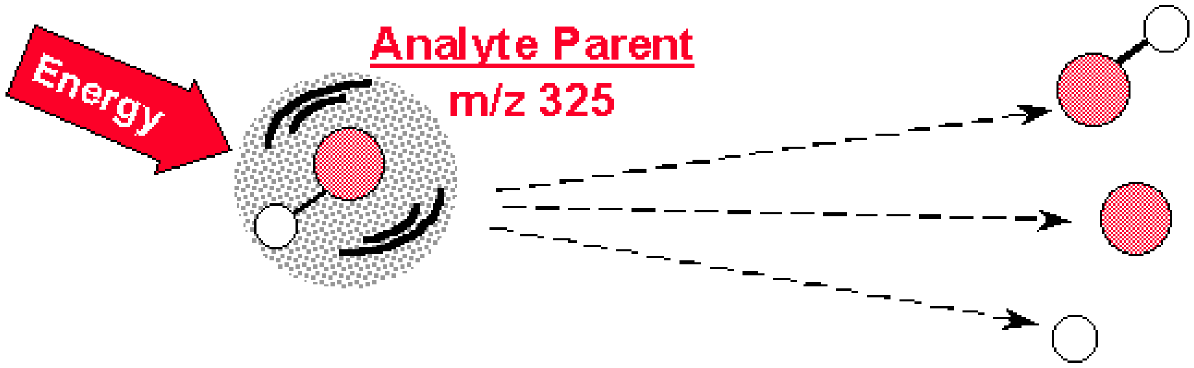
Selected Reaction Monitoring (SRM)



- Advantages
  - Targeted analyte monitoring
  - High duty cycle
  - "Simultaneous" monitoring of multiple transitions
- Disadvantages
  - No structural information

The Need for True MS/MS

Analyte Parent
m/z 325



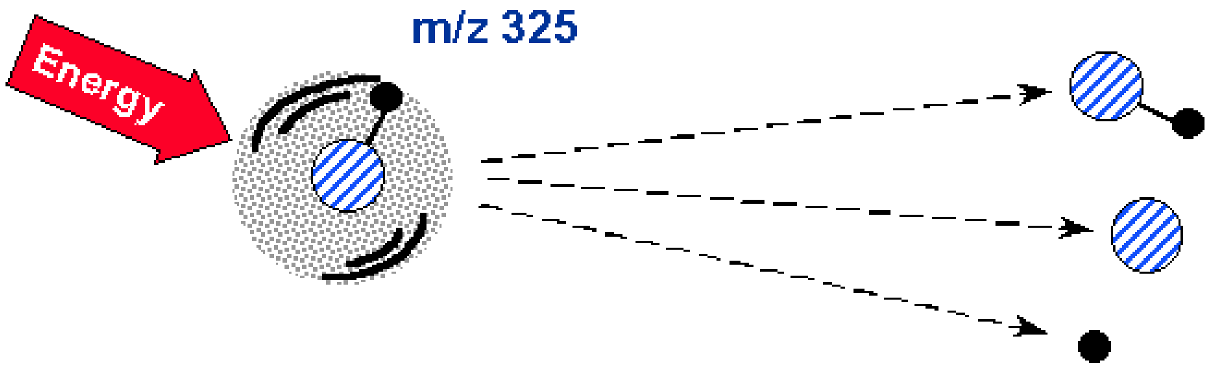
m/z 325 Intact Analyte Parent

m/z 175 Analyte CID Product A

m/z 150 Analyte CID Product B

Different compounds having the same parent m/z have different structures. True MS/MS facilitates Monitoring of a Single Reaction (SRM) for interference free quantitation of analytes in matrix.

Matrix (interference) Parent
m/z 325

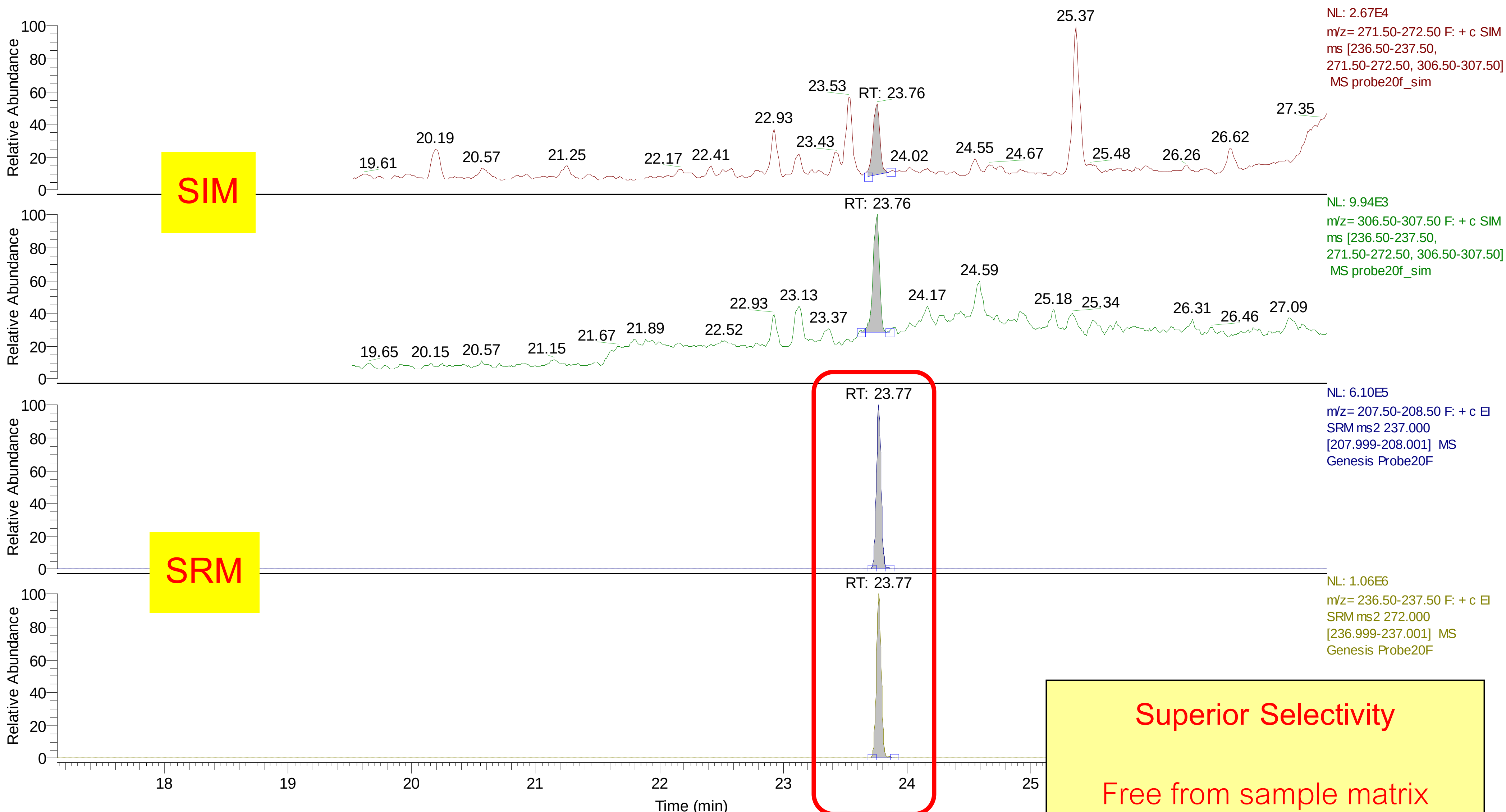


m/z 325 Intact Matrix Parent

m/z 185 Matrix CID Product A

m/z 140 Matrix CID Product B

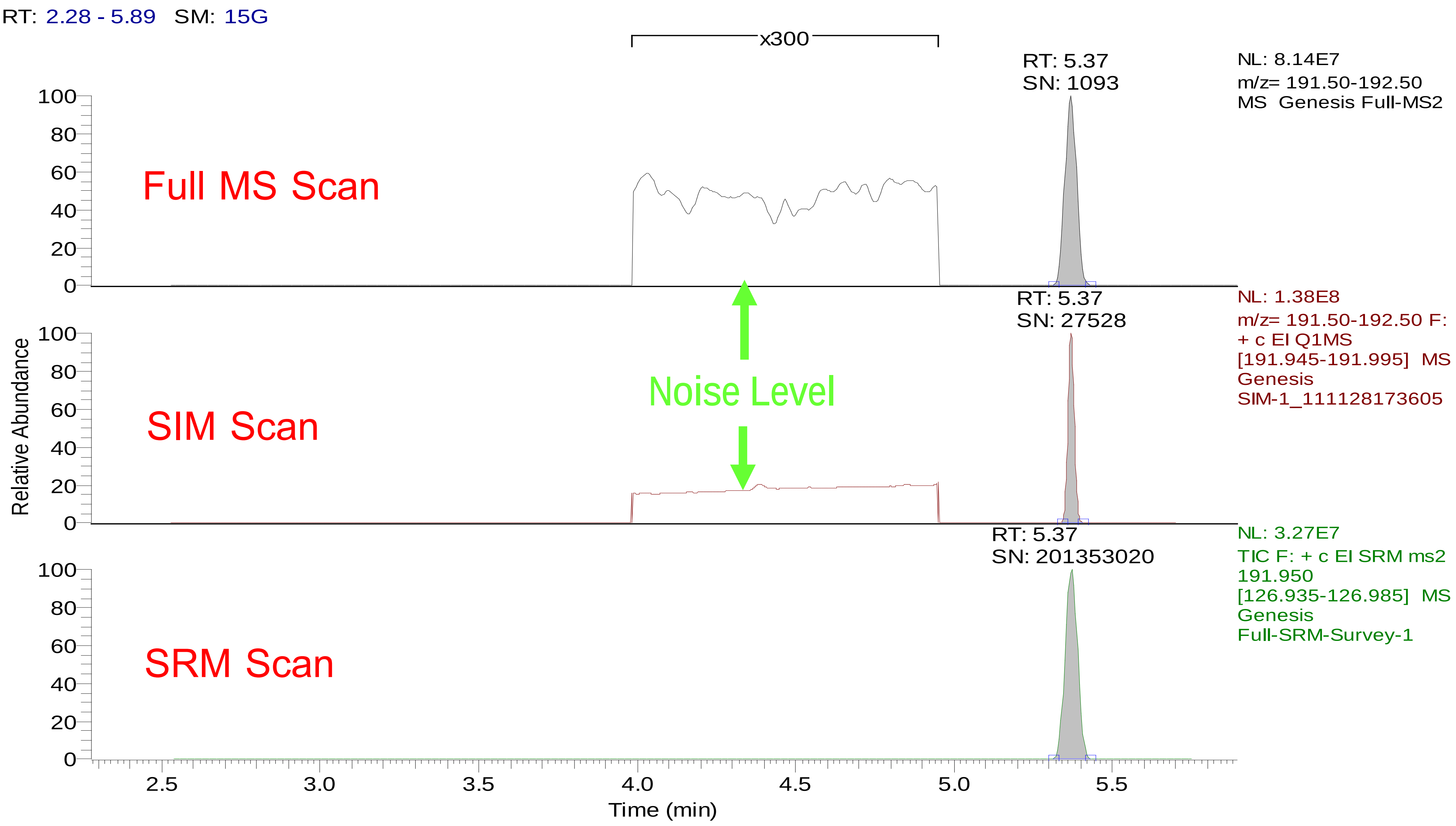
SIM VS SRM



Superior Selectivity

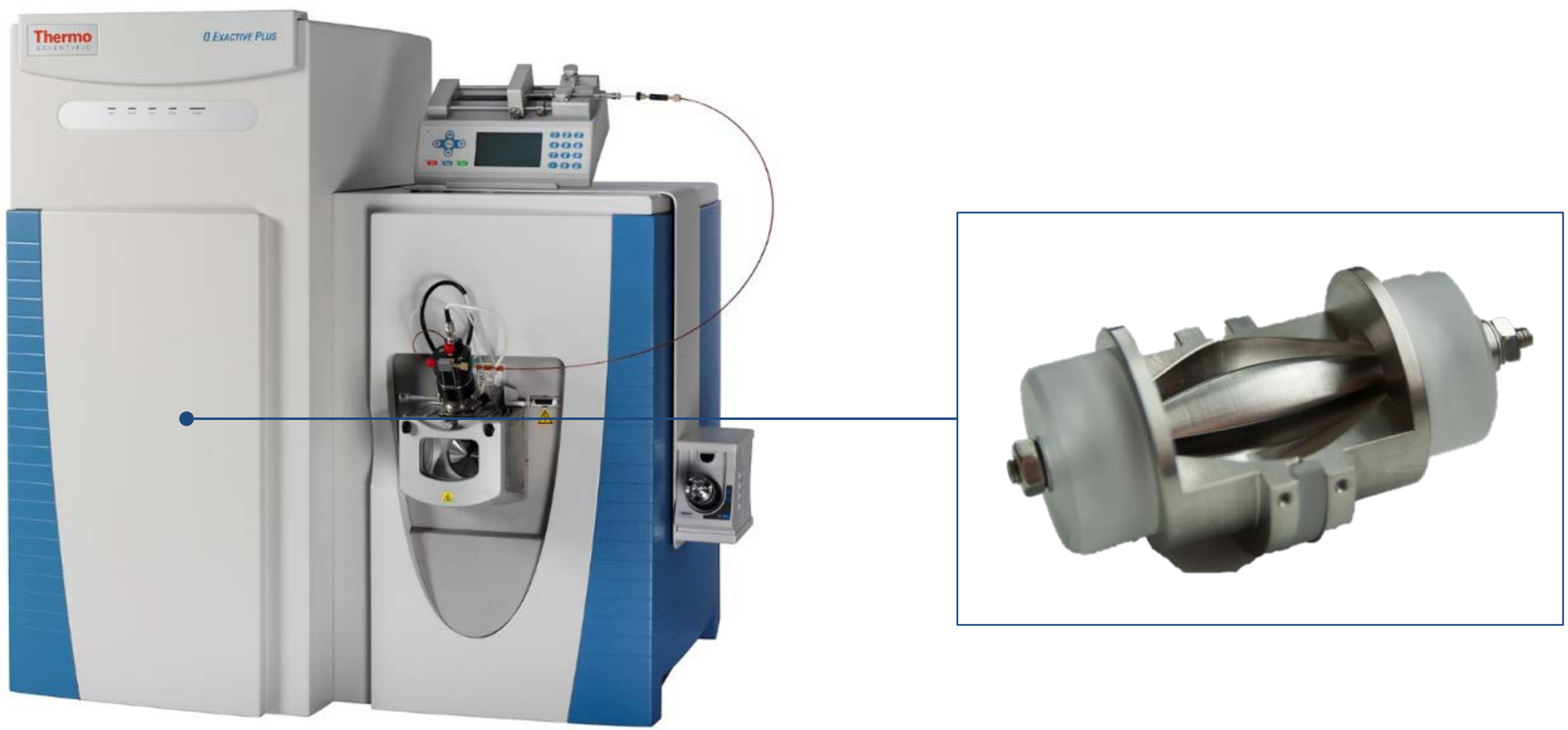
Free from sample matrix

SRM Selectivity in Complex Matrices



Mass Analyzer

- Orbitrap



High Resolution Accurate Mass (HRAM)

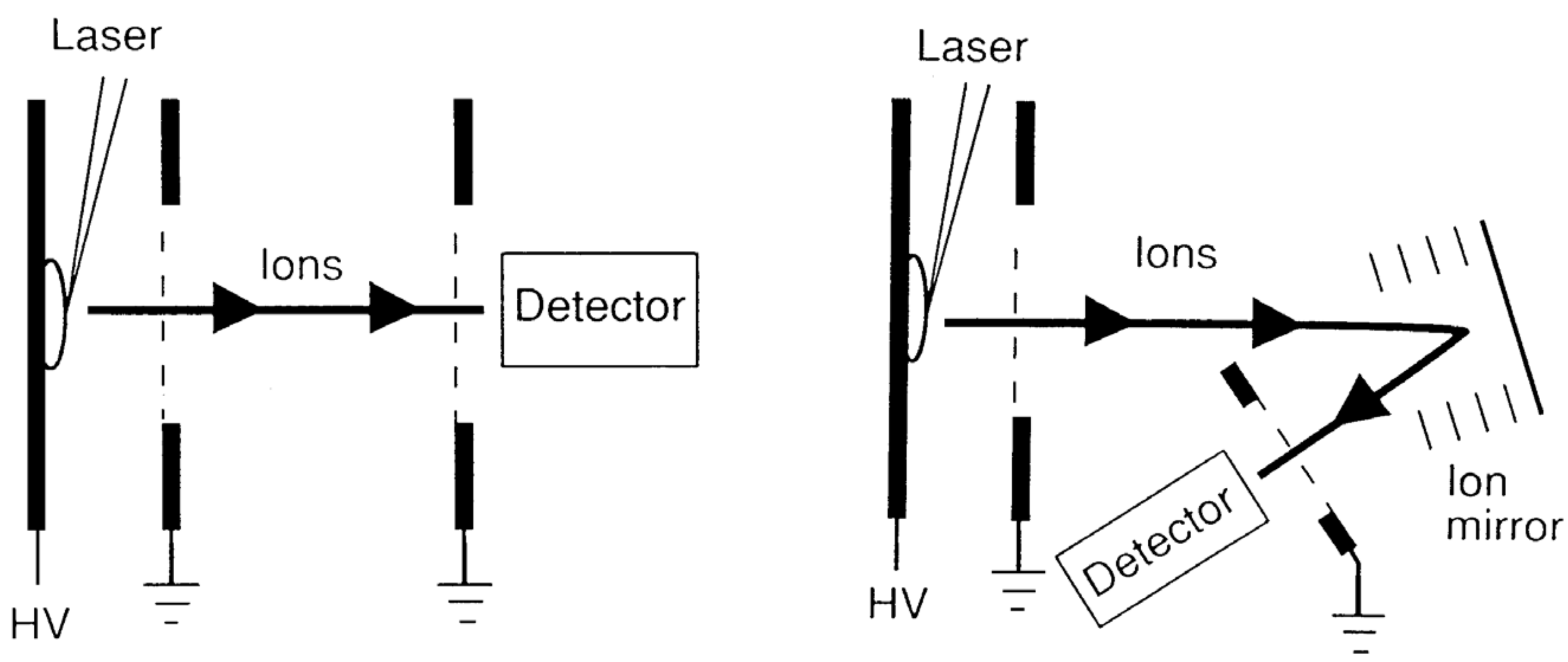
Spectrometer

Time-of-Flight (TOF) Mass Analyzer

$$m/z = \frac{2t^2V}{L^2} \quad \textbf{or} \quad t \propto \sqrt{m/z}$$

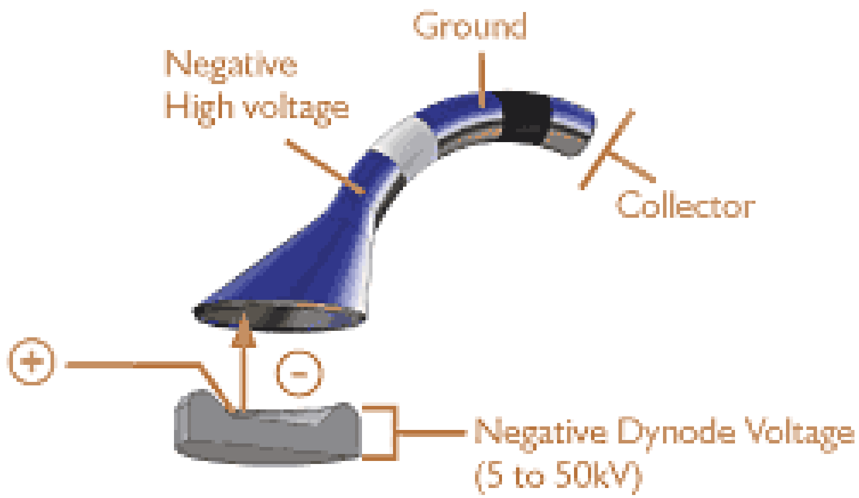
- Ions formed in pulses.
- Measures **time** for ions to reach the detector.

Linear and Reflector TOF Analyzers

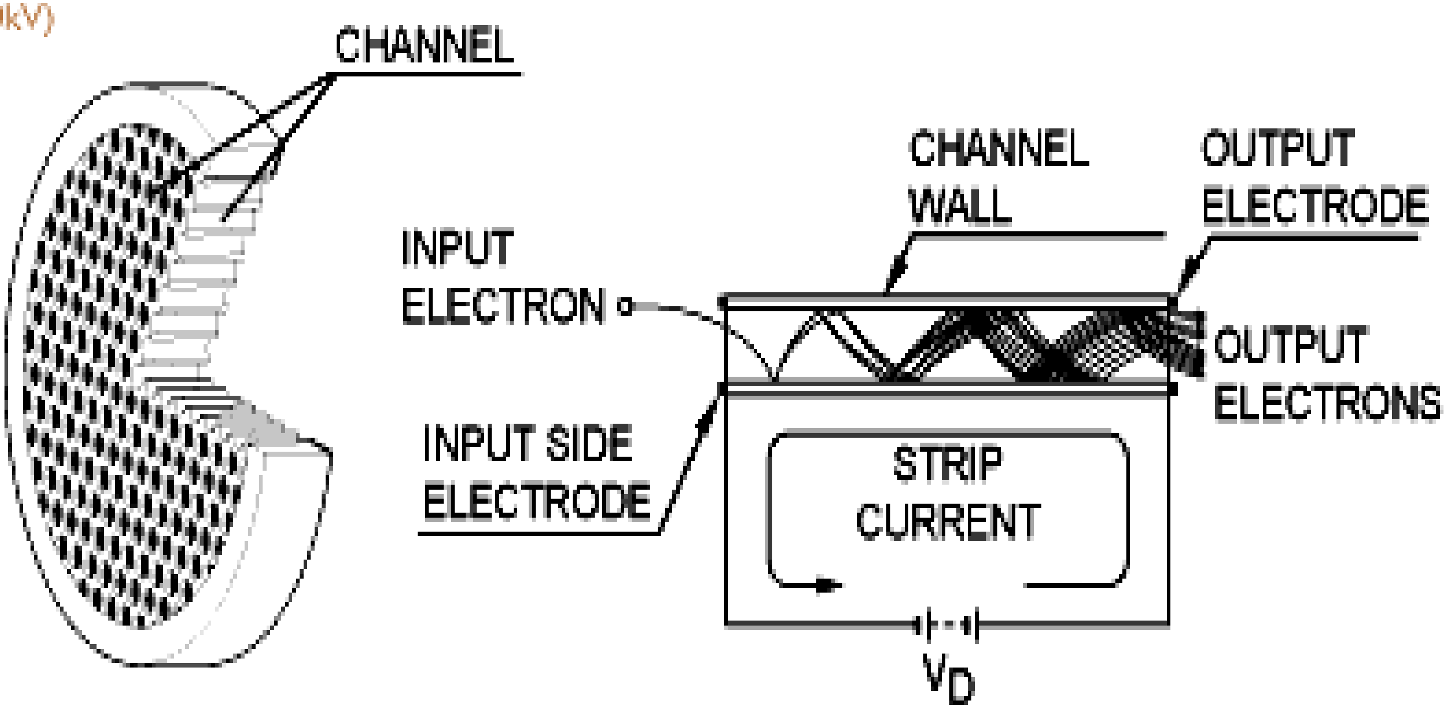


Reflector compensates for initial variation in kinetic energy, improving resolving power and mass accuracy.

Electron Multiplier



Multi-Channel Plate (MCP)



From Detector Technolgy: http://www.detechinc.com/

B. Brehm et al., Meas. Sci. Technol. 6 (1995) 953-958.

TOF Fundamental Limitations

Resolution limited by:

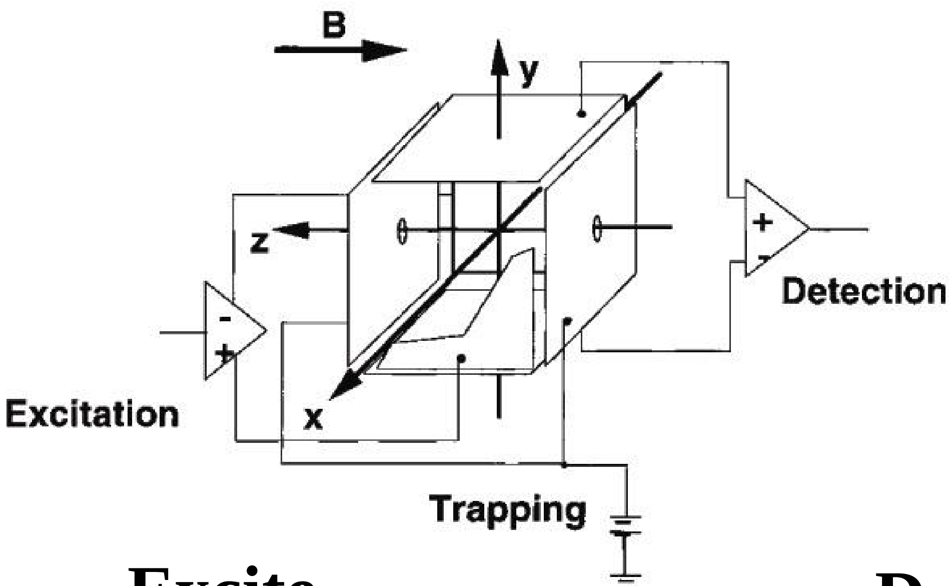
- length of TOF flight tube
- kinetic energy distribution
- propagation delay in detector

Sensitivity limited by:

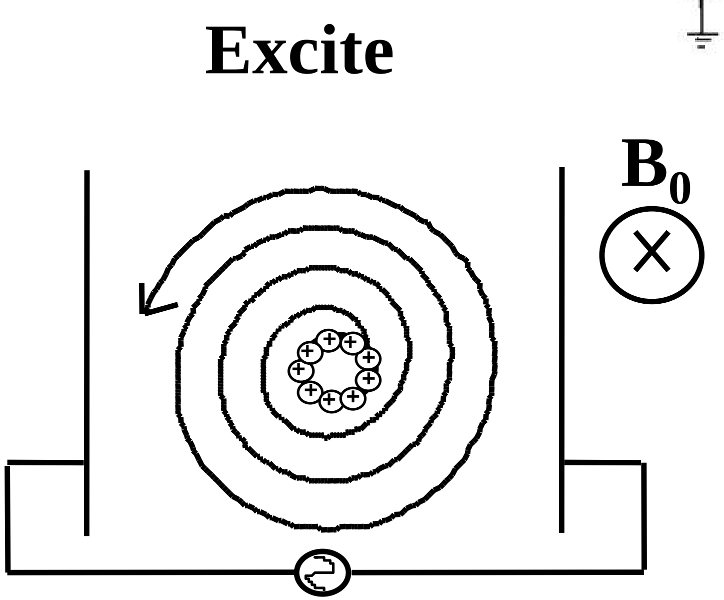
- ion stability
- ion transfer efficiency

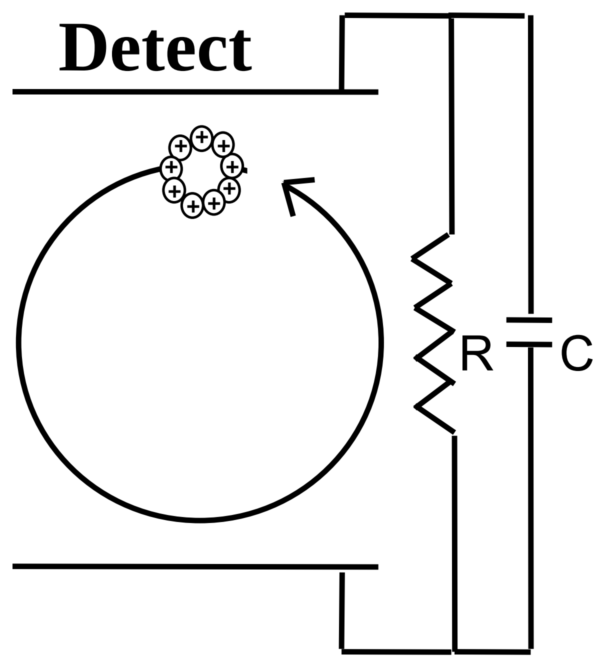
MS/MS is difficult

Fourier Transform Ion Cyclotron Resonance (FT-ICR)



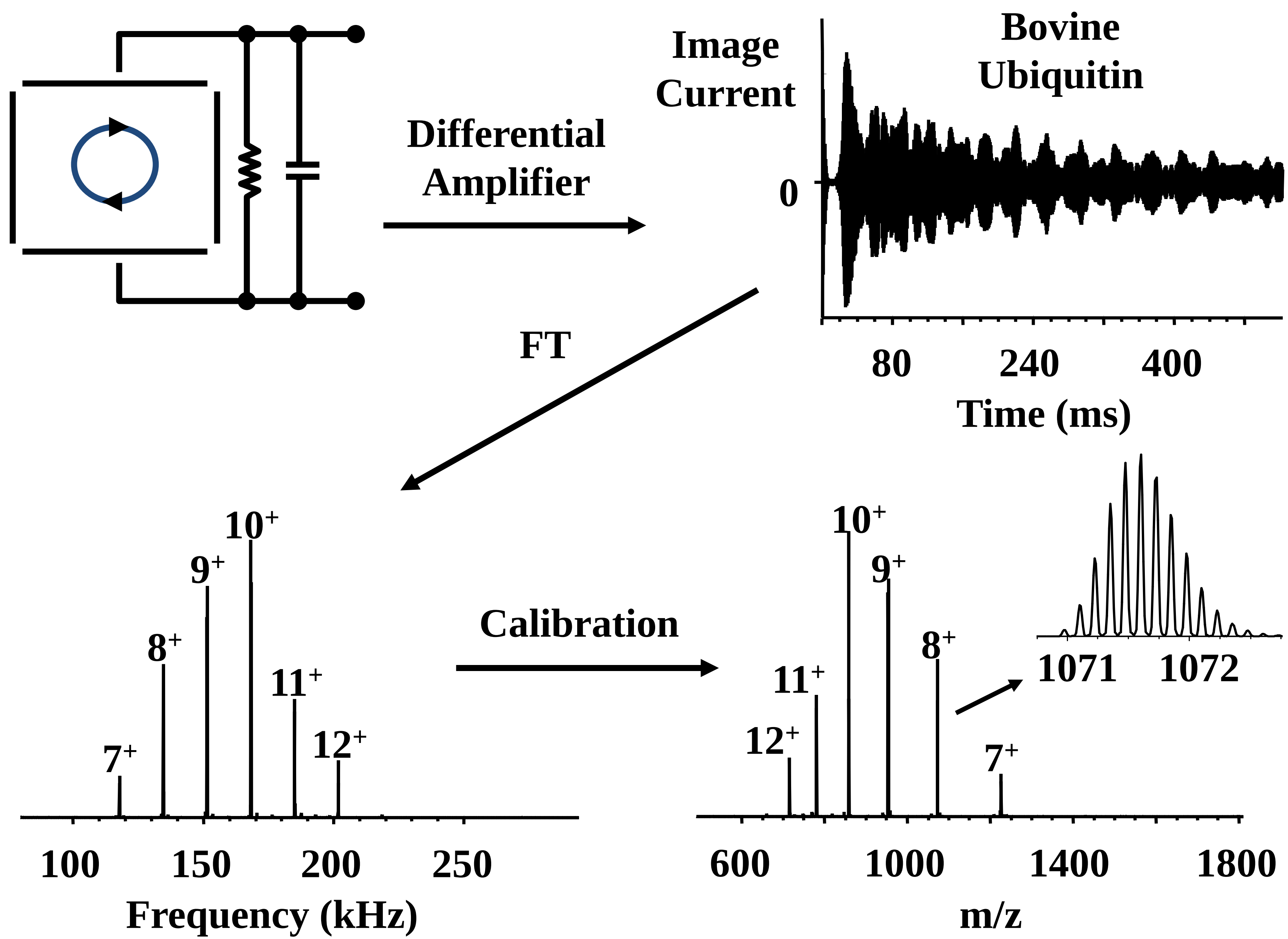
- Ions trapped and measured in ultrahigh vacuum inside a superconducting magnet.





$$\omega \propto \frac{1}{m/z}$$

Fourier Transform Ion Detection



FTICR Fundamental Limitations

- Resolution limited by:
  - Pressure
  - Magnetic field (strength and homogeneity)
  - Electric field (homogeneity)
  - Space charge

- Sensitivity limited by:
  - Preamplifier Noise
  - Magnetic field strength
  - Space charge

- Mass range limited by:
  - Magnetic field
  - Frequency performance of electronics

Mass Analyzer: Orbitrap

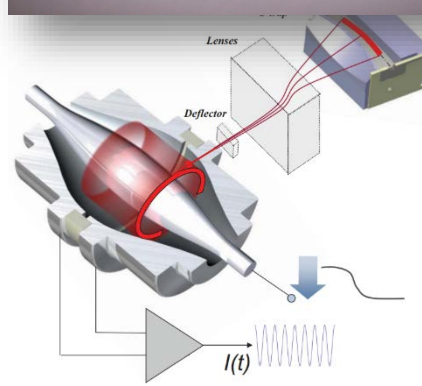
Anal. Chem. **2000,** *72,* 1156−1162

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.



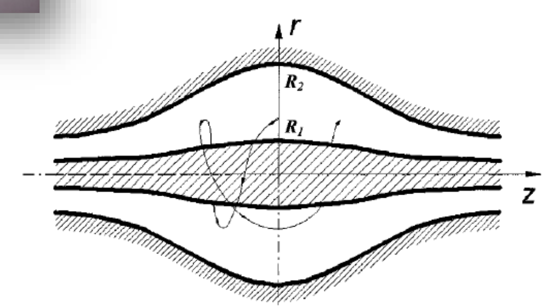
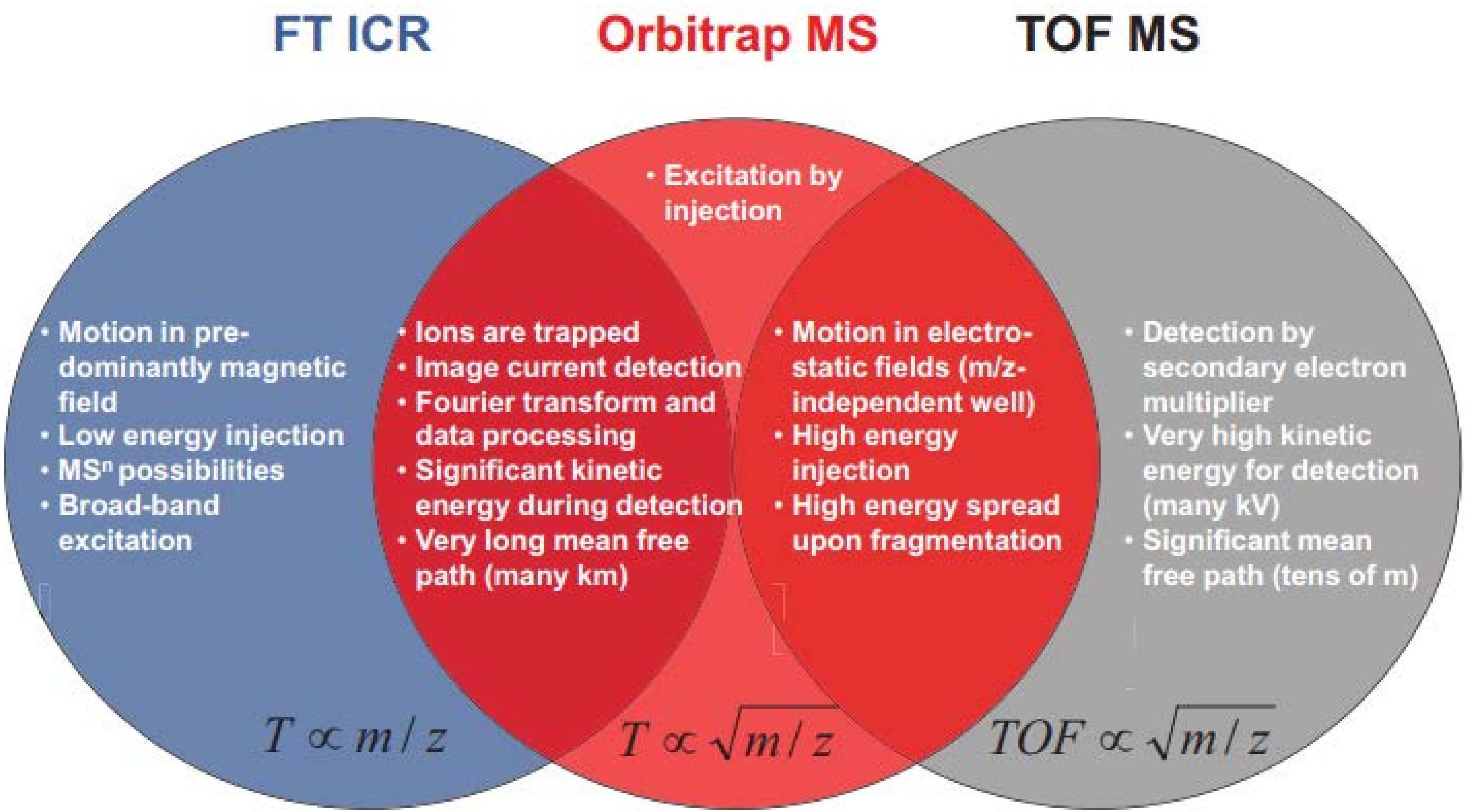
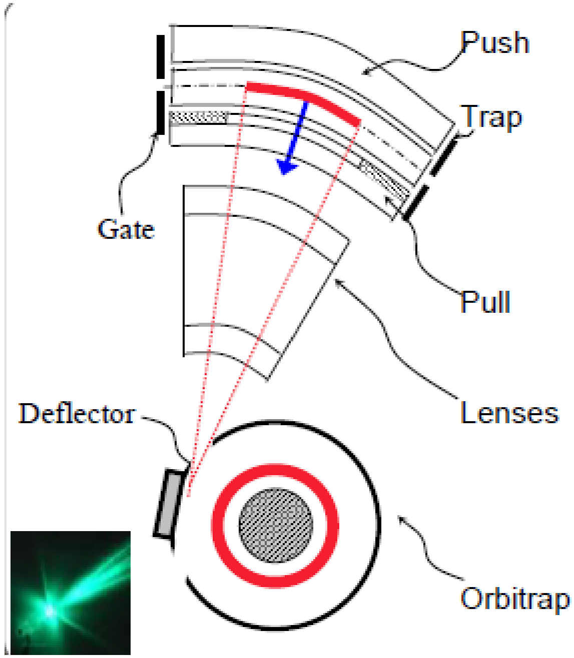


Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

Major accurate-mass analyzers for Life Science



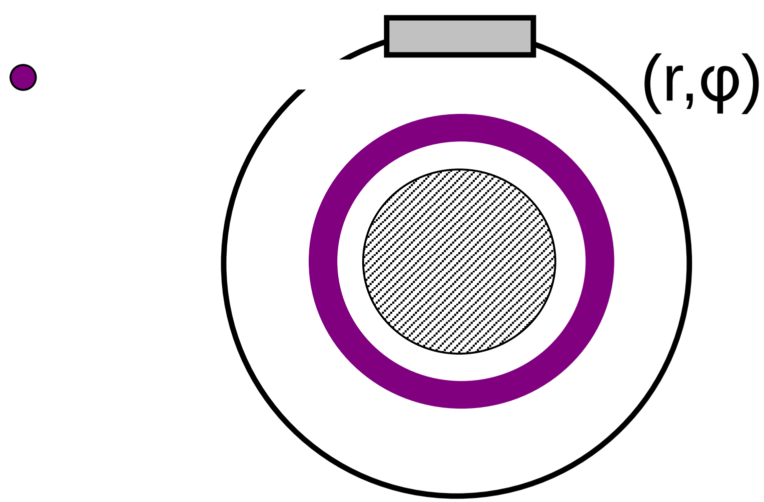
Curved Linear Trap (C-trap): Radial "Fast" Injection

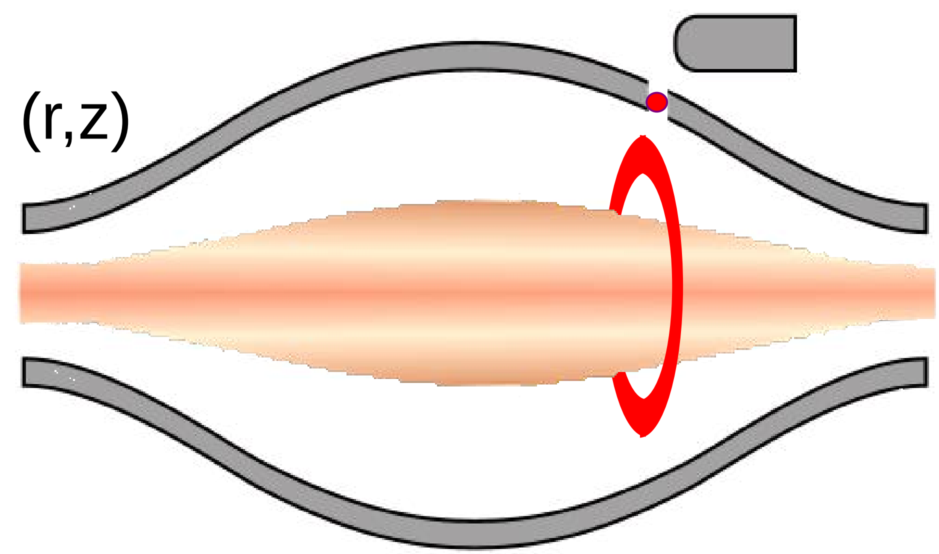


- Ions are stored and cooled in a curves RF-only quadrupole (C-trap)
- RF is ramped down, radial DC is applied
- Ions are ejected along lines converging on the orbitrap entrance
- As ions enter obritrap, they are picked up and squeezed by its electric field

Ion Injection and Formation of Ion Rings

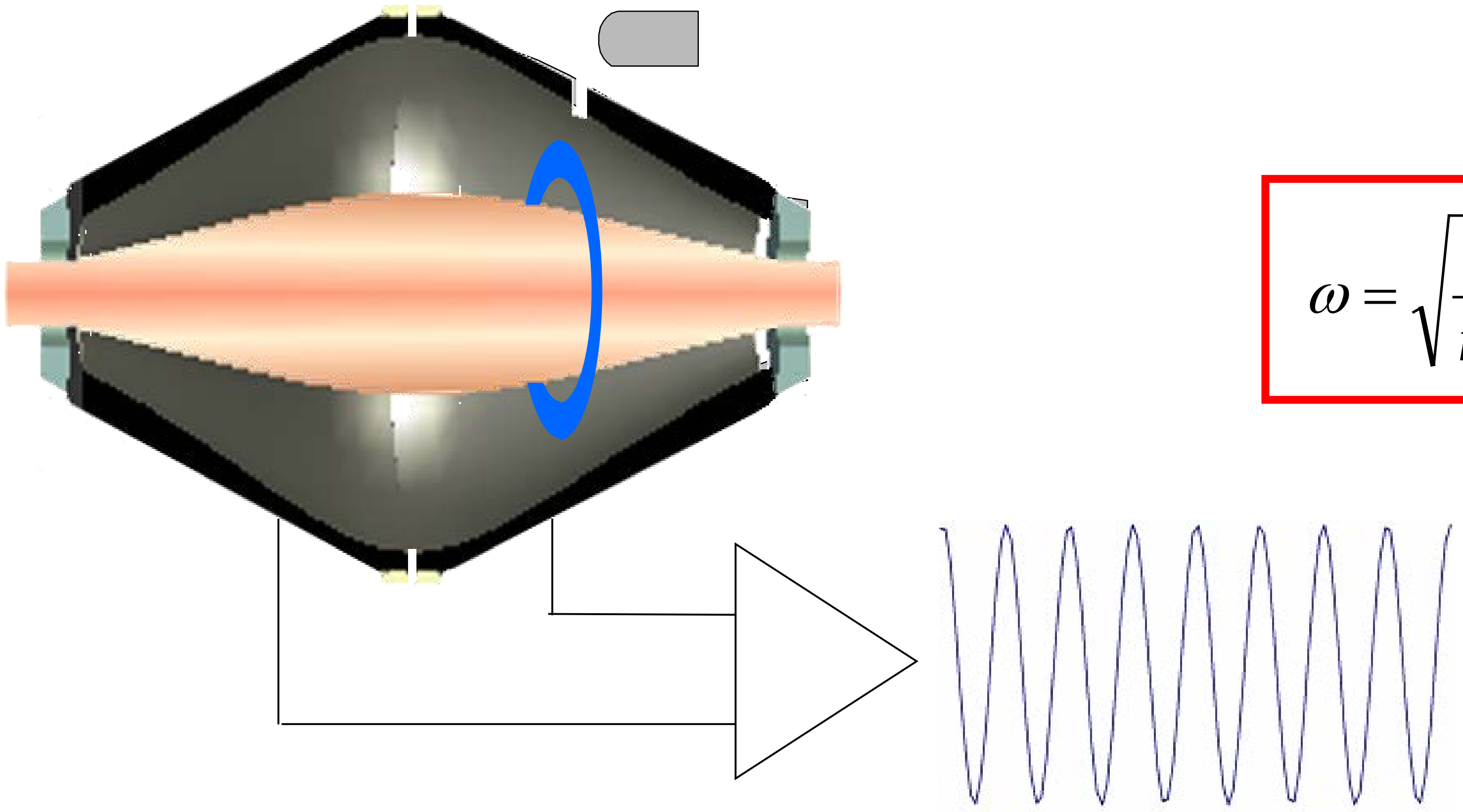
- An ion packet of a selected *m/z* enters the field
- Increasing voltage squeezes ions
- Voltage stabilises and ion trajectories are also stabilized
- Angular spreading forms a ROTATING RING





Detection of Ions

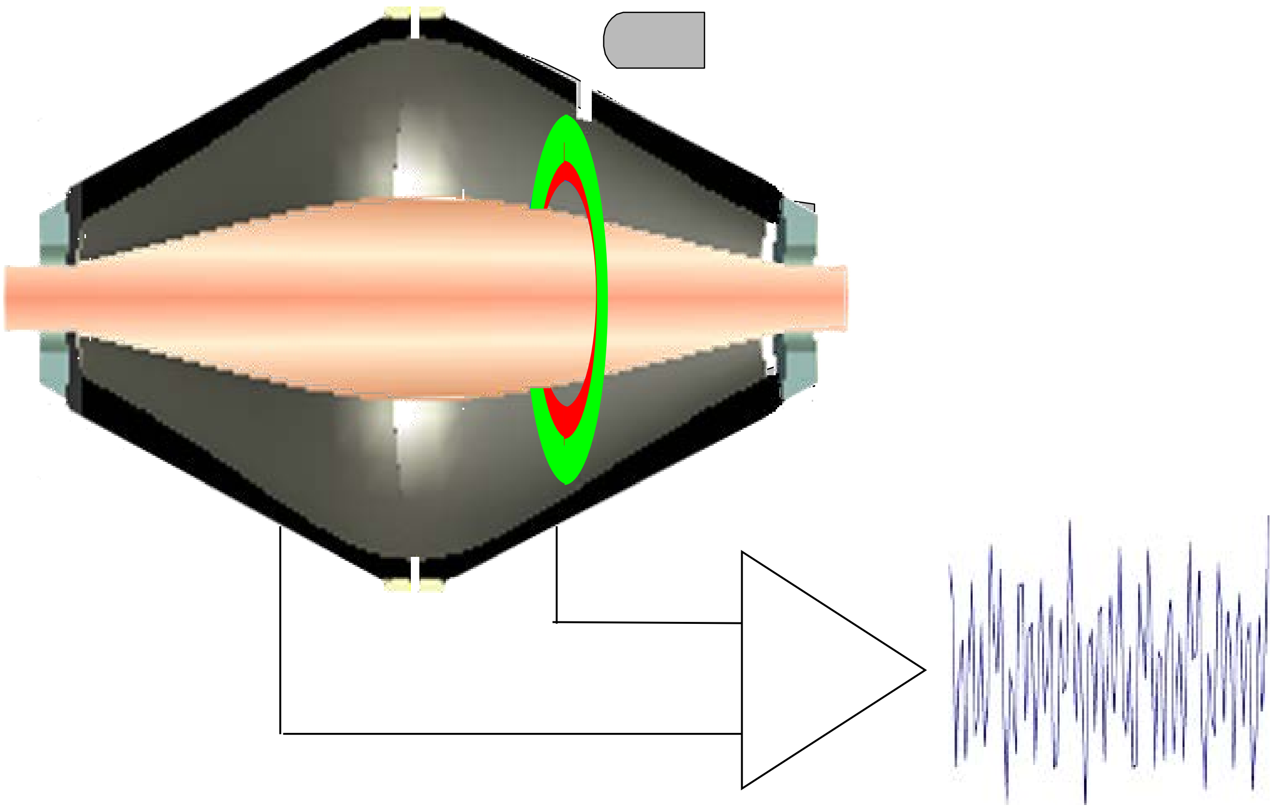
- Ion packets enter the analyzer slightly off axis
- The field inside the trap effects an oscillation of the ion packets/rings
- The moving ion rings induce an image current on outer electrodes
- The frequency of harmonic oscillations is proportional to ions' *m/z*



$$\omega = \sqrt{\frac{k}{m/z}}$$

Fourier Transform

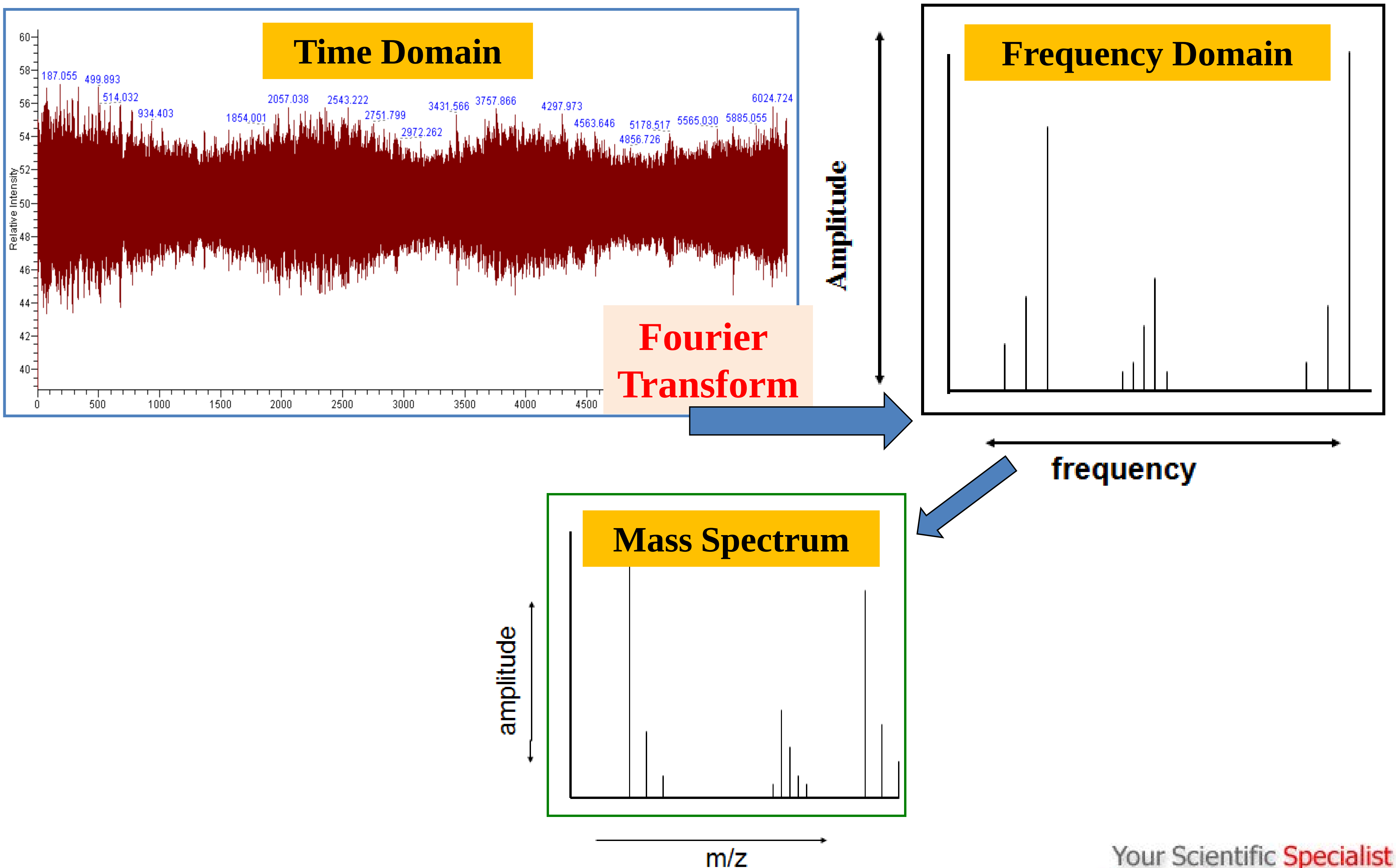
- Mathematical operation transforms frequency signal into a time domain spectrum
- Orbitrap is a Fourier transform-based mass analyzer



Baron Joseph Fourier

Scigelova et al. Mol. Cellular Proteomics 2011, 10: M111.0009431

Many Ions Generate a Complex "Transient"



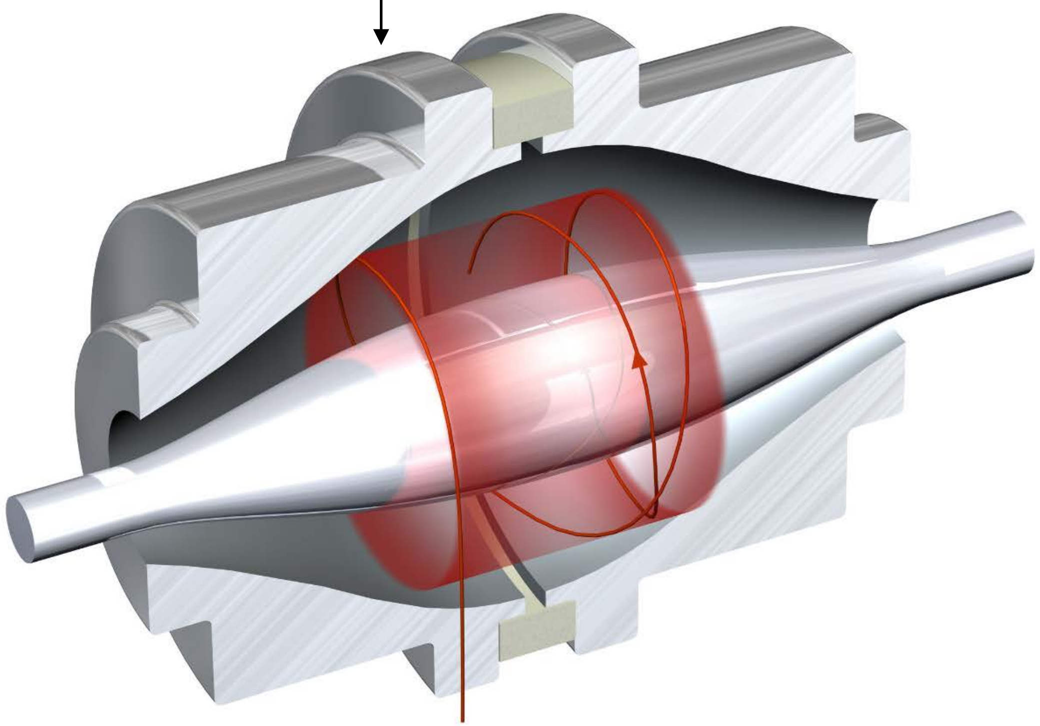
Orbitrap

TOF

- Simultaneous excitation

FTICR

- Confined ion trajectory
- Image current detection
- Fourier transform data conversion



Unique to Orbitrap

- 3D electric field trapping
- No need for magnet
- Easy access
- Final detection device

Alexander Makarov, Anal. Chem. 2000, 72, 1156-1162

Orbitrap MS

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

Mass Accuracy

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

- Typical way of reporting mass error in **ppm (relative mass error)**:

$$mass\ error = \left(\frac{exact\ mass - measured\ mass}{exact\ mass}\right) * 10^6$$

C = 12.0000	
O = 15.9949	H = 1.0078
S = 31.9721	N = 14.0031

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

Ex : M=249	$C_{20}H_9$+	249.0070
	$C_{19}H_7N$+	249.0580
	$C_{13}H_{19}N_3O_2$+	249.1479

Mass Accuracy

$$mass\ error = \left(\frac{exact\ mass - measured\ mass}{exact\ mass}\right) * 10^6$$

- Quadrupole MS $= \frac{500.1 - 500.0}{500} \cdot 10^6 = 200ppm$
- Orbitrap MS $= \frac{500.10314 - 500.10214}{500.10314} \cdot 10^6 = 2ppm$

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

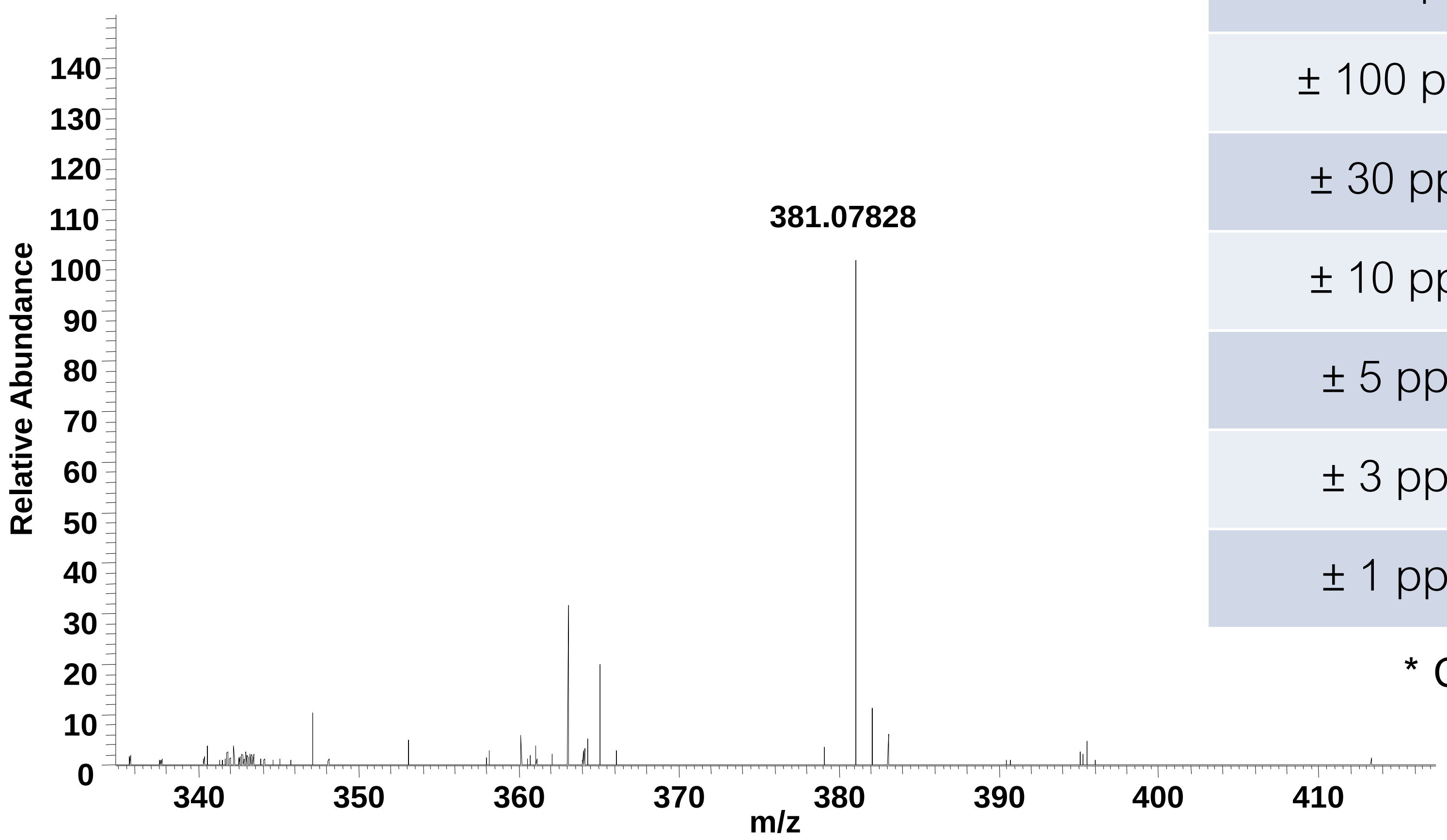
Mass Accuracy

- Increases confidence in identification

$[M+H]^+$ 381.07828

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

* 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

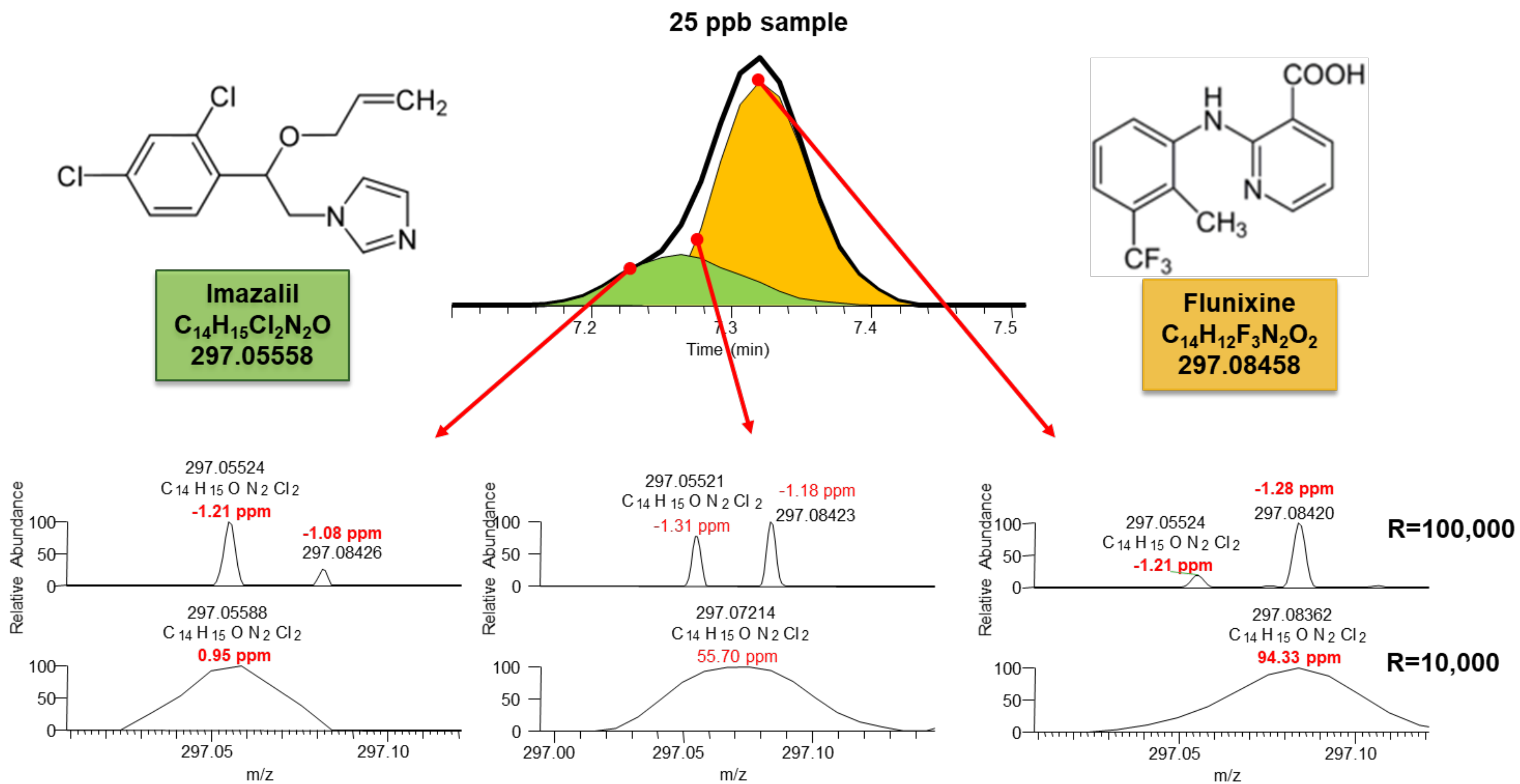
O = 15.9949
S = 31.9721

H = 1.0078
N = 14.0031

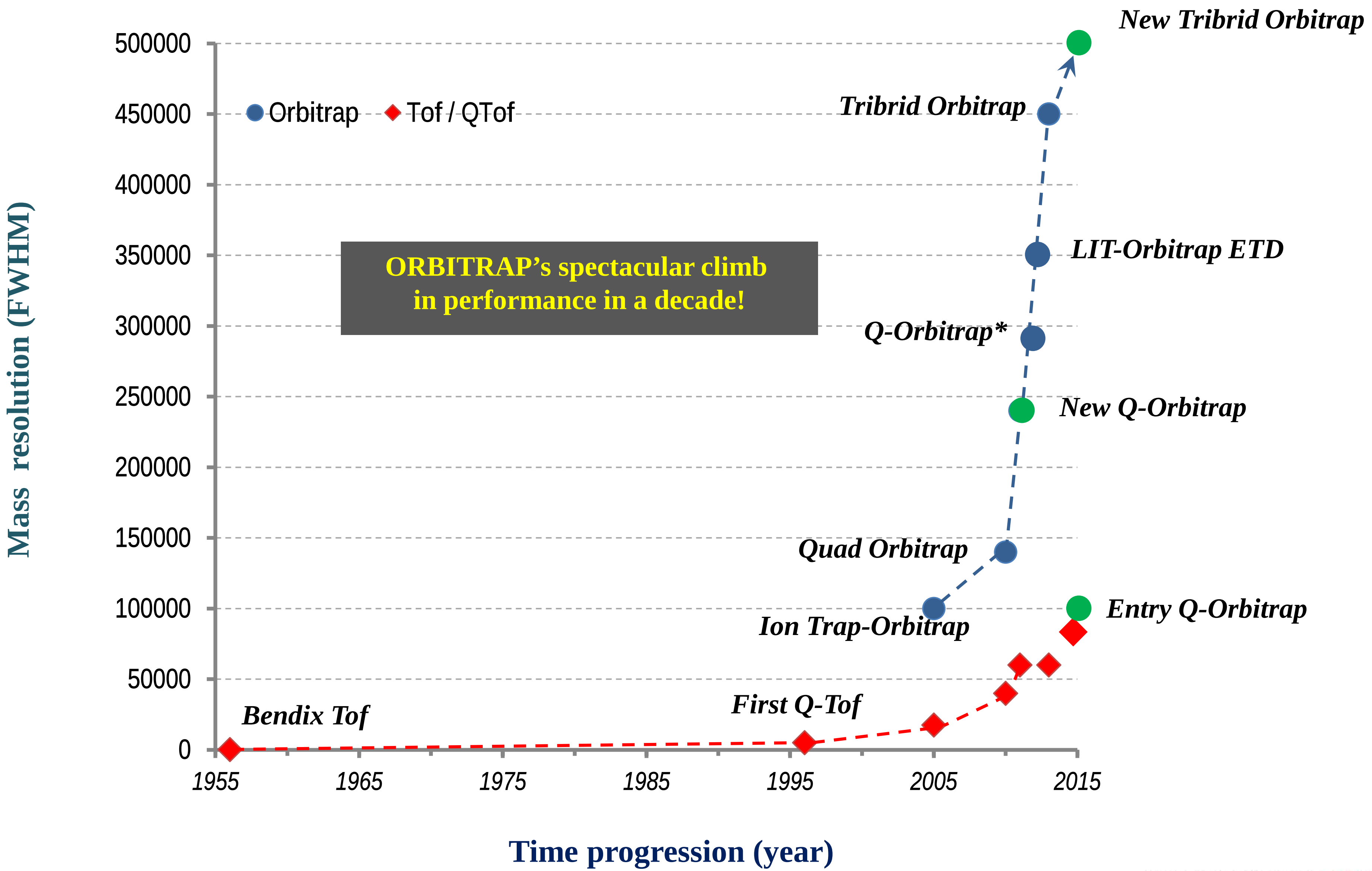
- **Main advantage:** the possibility to determine the elemental composition of individual molecular or fragment ions, a powerful tool for the structural elucidation or confirmation.

Mass Resolution and Accuracy

- Isobaric compounds separation



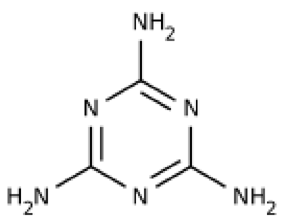
Commercial High Resolution MS Technology Race



LC-MS/MS for Applications in Food Safety

Application in Food Safety

Identification and Quantitation of Melamine in Milk



Melamine

SRM Transitions

(Q1) 127 -> 68 (Q3)

(Q1) 127 -> 85 (Q3)

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

LC: Accela™ System

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

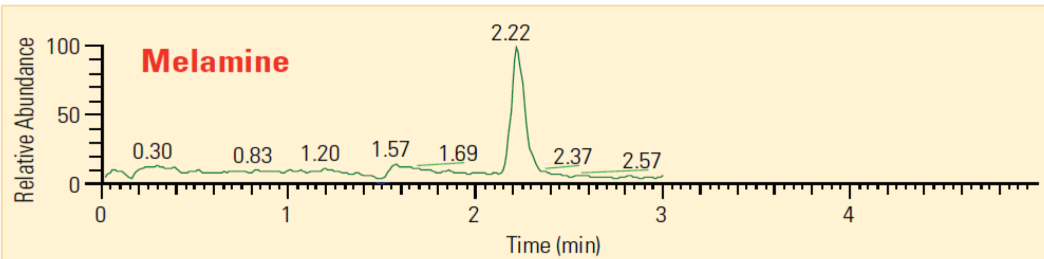
Ionization: Positive ESI

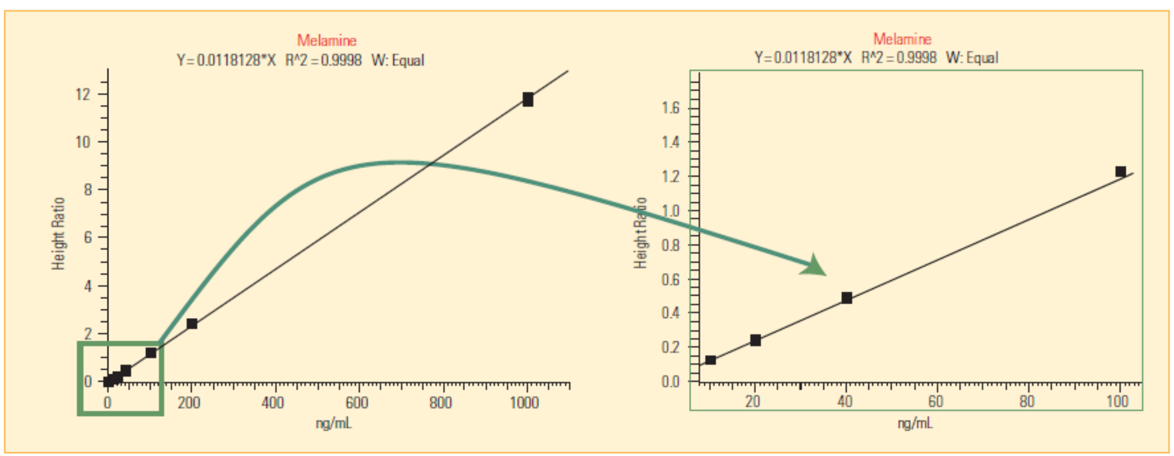
Modes: Targeted SRM

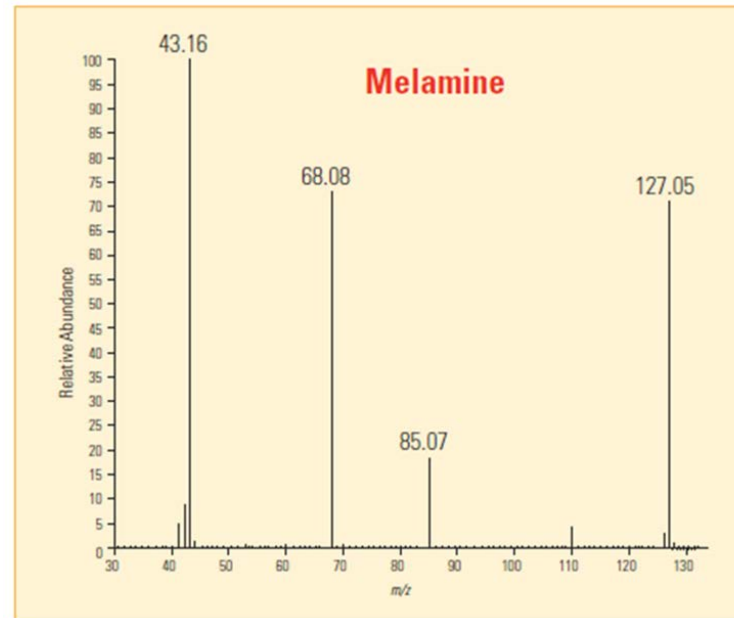
Varelis et al. *Thermo AN62732*. 2008

Application in Food Safety

Identification and Quantitation of Melamine in Milk







- Limit of Detection (LOD): <1 ppb

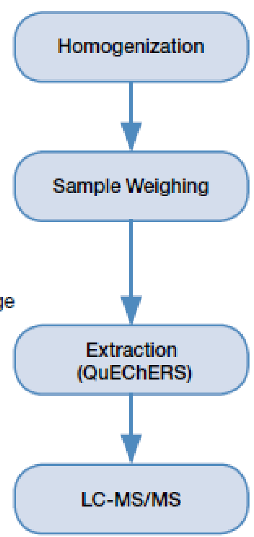
Varelis et al. *Thermo AN62732*. 2008

Application in Food Safety

Rapid and Robust Identification of Pesticides in Leek

Extraction (QuEChERS) → LC-MS/MS (timed-SRM)

1. Weigh 10 g sample in 50 mL extraction tube.
2. Add 10 mL acetonitrile (20 mL water + 10 mL acetonitrile for wheat flour).
3. Shake for 10 min and centrifuge at 5000 rpm for 5 min.
4. Transfer supernatant into LC vial and place it in the autosampler.



Injection volume	1 µL
Column temperature	25 °C
Flow rate	300 µL/min
Analytical column	Accucore aQ column 100 × 2.1 mm, 2.6 µm
Run time	15 minutes
Tray temperature	5 °C
Needle-cleaning solvent	10% Methanol in water
Sample loop	25 µL
Mobile phases	A: 98% water with 2% methanol, 5 mM ammonium formate, and 0.1% formic acid B: 98% methanol with 2% water, 5 mM ammonium formate, and 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0	0.300	100	0
0.5	0.300	100	0
7	0.300	30	70
9	0.300	0	100
12	0.300	0	100
12.1	0.300	100	0
15	0.300	100	0

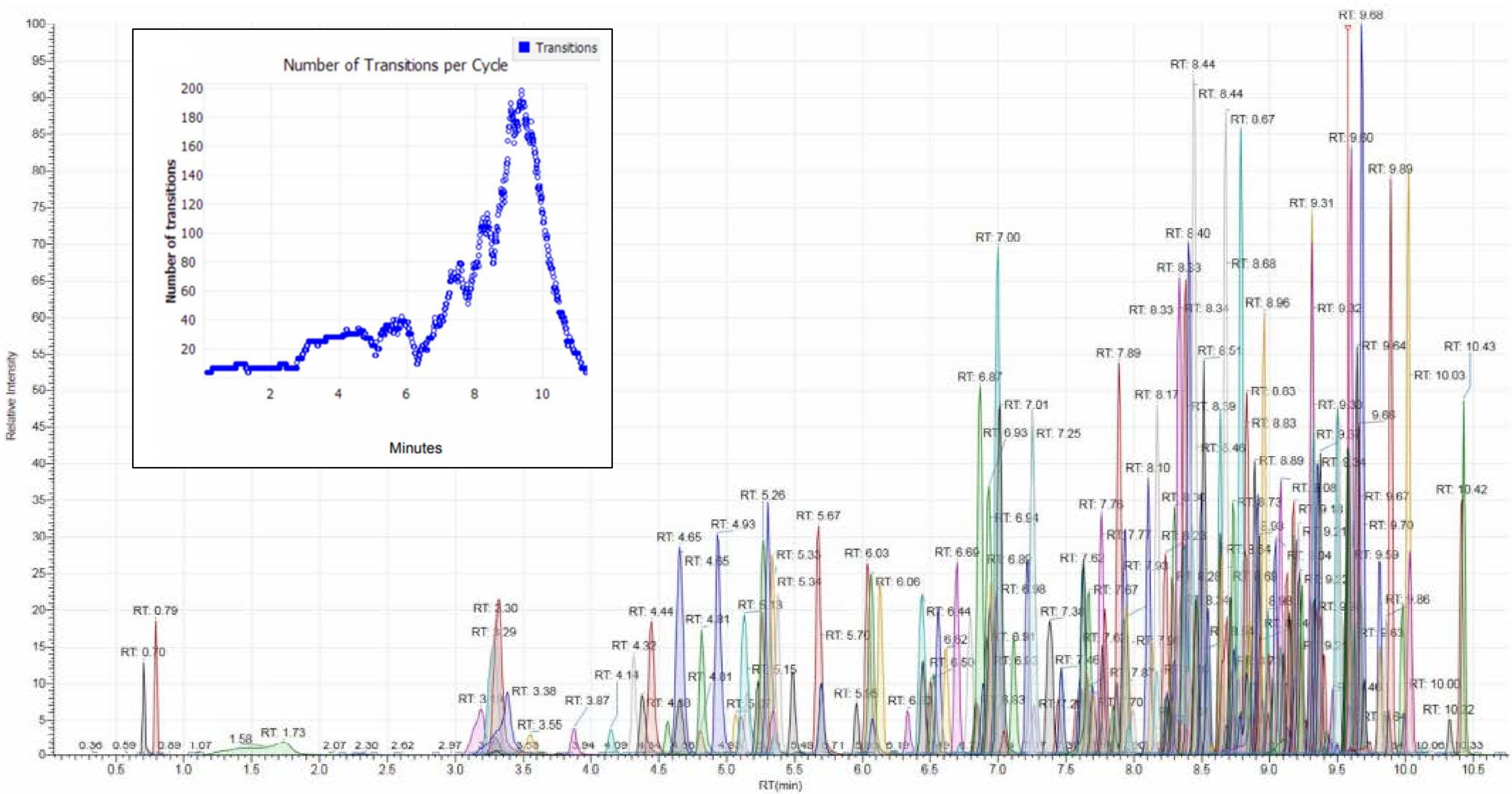
Ionization mode	Heated Electrospray (HESI)
Scan type	timed-SRM
Polarity	Positive/Negative switching
Spray Voltage for Positive mode	3700 V
Spray Voltage for Negative mode	2500 V
Sheath gas pressure	30 arbitrary units (Arb)
Aux gas pressure	6 Arb
Sweep gas pressure	1 Arb
Ion transfer tube temperature	325 °C
Vaporizer temperature	350 °C
CID gas pressure	2 mTorr
Cycle time	0.5 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7

Bousava et al. *Thermo AN64971*. 2017

Application in Food Safety

Rapid and Robust Identification of Pesticides in Leek

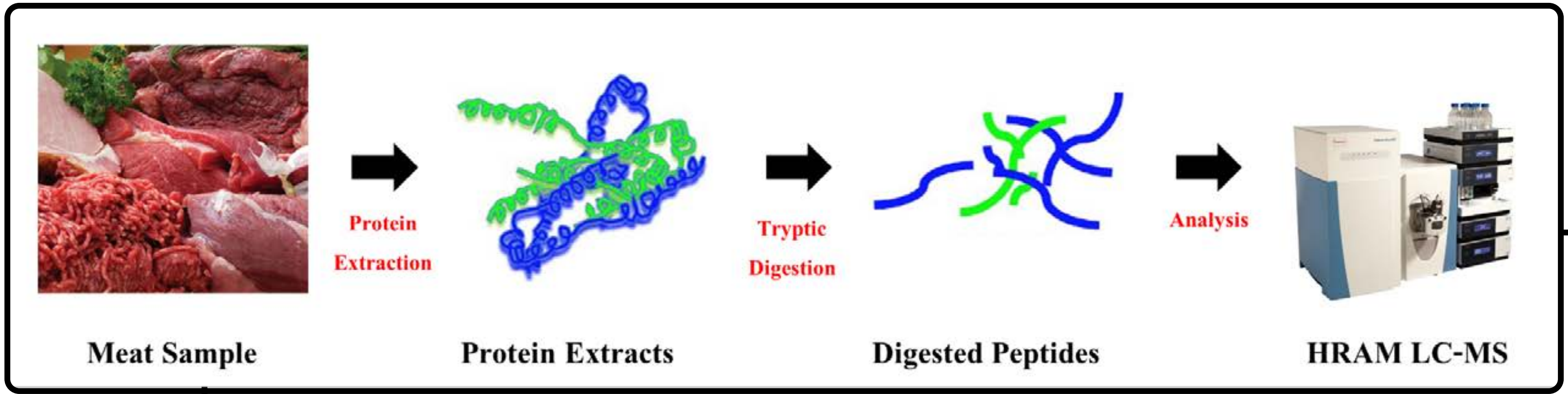
- LC-MS/MS chromatogram of more than 250 pesticides in leek extract at 100 μg/kg



Bousava et al. *Thermo AN64971*. 2017

Application in Halal Food

Determination of Meat Authenticity



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

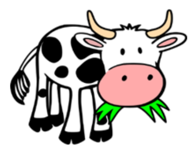
MS Conditions	
MS:	Thermo Scientific Q Exactive benchtop quadrupole-Orbitrap mass spectrometer
Scan Type:	Full scan MS
Resolving Power:	140,000 (FWHM)
AGC:	3.0×10^{6}
Maximum IT:	200 ms
Scan Range:	*m/z* 500–2000
njection Volume:	2 µL
Spray Voltage:	4 kV
Capillary Temperature:	300 °C
Sheath Gas Flow 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

LC-MS/MS for Applications in Pharmaceutical

Application in Pharmaceutical

Quantitative Analysis of Immunosuppressant Drugs

Sample Preparation

50 uL blood + 150 uL of protein precipitation solution containing internal standards
↓
Vortex
↓
Shake for 30 min
↓
Centrifuge 10 min @ 13200 RPM
↓
Supernatant
↓
50 µL inject

→ LC & HRAM MS

Full scan @ 50,000

LC: Accela™ System

Column: C18 column

Column Temperature: 80°C

Injection Volume: 50 µL

Mobile Phase: A) Water + 10 mMNH_4FA + 0.1% FA; B) MeOH + 10 mMNH_4FA + 0.1% FA; C) CAN/IPA/Acetone 45:45:10 v/v/v

Flow Rate: 800 µL/min   Run Time: 2 min

MS: Q Exactive

Ionization: APCI

Modes: Full scan MS at 50,000 Resolution

Calibration Standards

Calibrator	Tacrolimus (ng/mL)	Sirolimus (ng/mL)	Everolimus (ng/mL)	Cyclosporin A (ng/mL)
Cal 1	0.97	0.94	1.02	9.8
Cal 2	2.07	2.10	1.95	26.4
Cal 3	5.11	5.21	5.13	73.0
Cal 4	10.57	10.02	10.36	208.8
Cal 5	28.22	26.28	28.17	725.1
Cal 6	53.92	49.91	51.57	2067.2

QC Samples – Expected Concentration

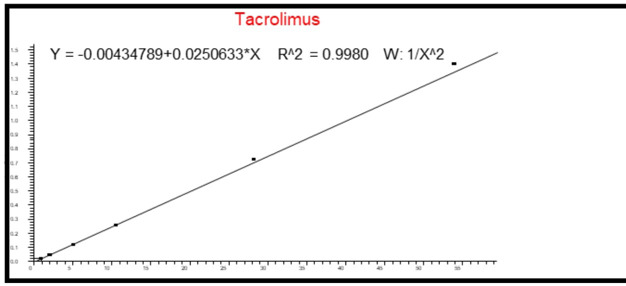
QC sample	Tacrolimus (ng/mL)	Sirolimus (mg/mL)	Everolimus (ng/mL)	Cyclosporin A (ng/mL)
QC1	2.97	3.06	2.93	31.0
QC2	13.66	12.74	13.58	134.0
QC3	33.06	30.66	32.40	386.8

Gao et al. *Thermo AN64504*. 2016

Application in Pharmaceutical

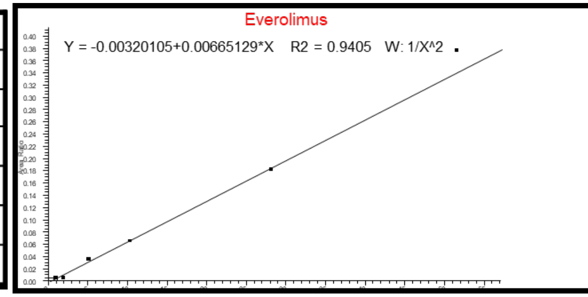
Quantitative Analysis of Immunosuppressant Drugs

Tacrolimus



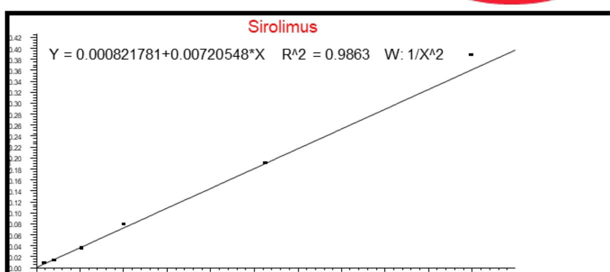
Standard	% Difference
0.97 ng/mL	1.04
2.07 ng/mL	0.34
5.11 ng/mL	-6.41
10.57 ng/mL	-1.62
28.22 ng/mL	2.77
53.92 ng/mL	3.88

Everolimus



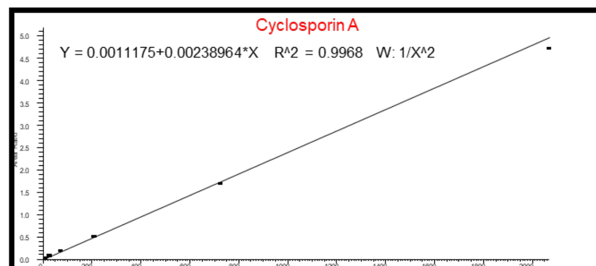
Standard	% Difference
1.02	17.85
1.95	-39.05
5.13	12.53
10.36	-0.81
28.17	-1.06
51.57	10.62

Sirolimus



Standard	% Difference
0.94	4.83
2.1	-12.57
5.21	3.31
10.02	1.88
26.28	-1.61
49.91	4.16

Cyclosporin A



Standard	% Difference
9.8	-3.47
26.4	8.81
73.0	1.65
208.8	0.93
725.1	-3.24
2067.2	-4.69

Excellent Linearity and Accuracy

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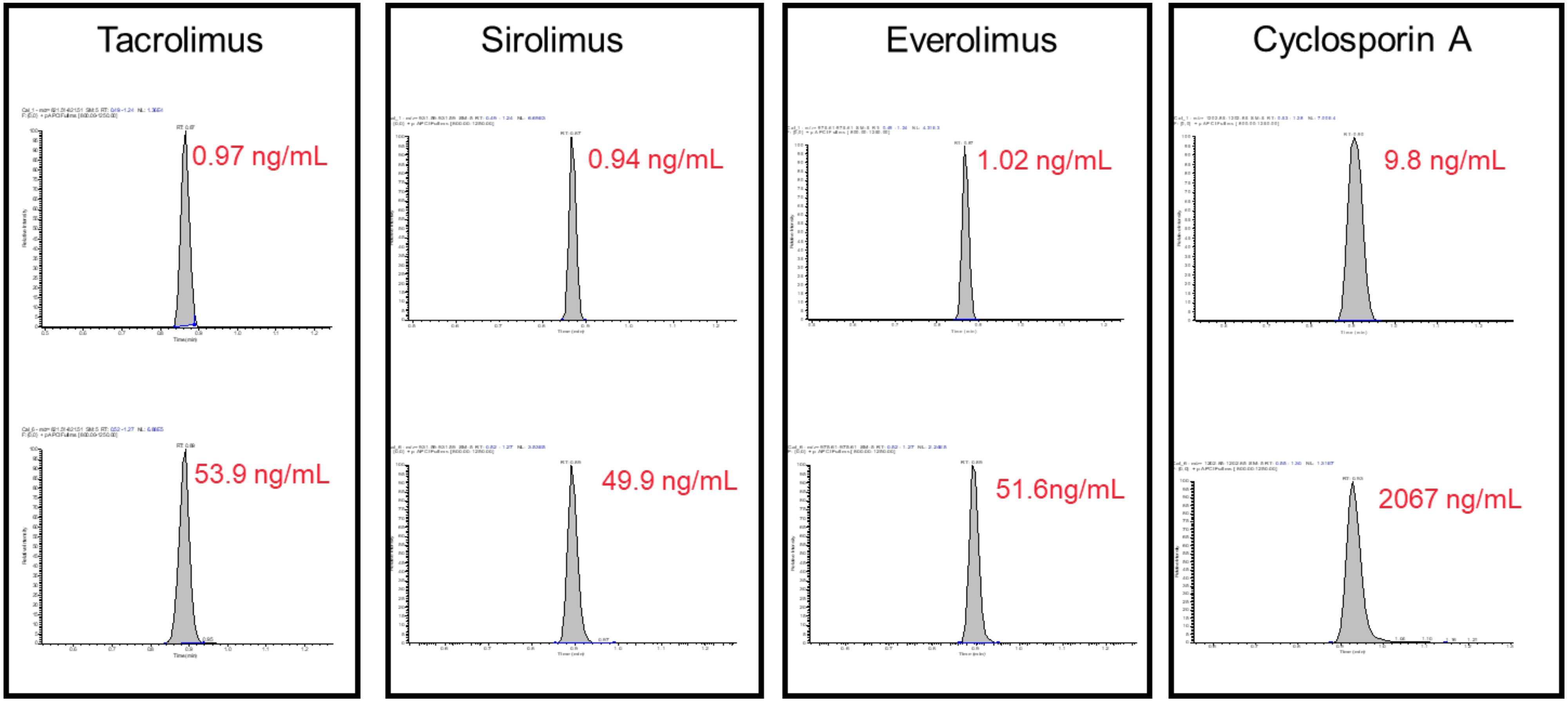
Gao et al. *Thermo AN64504*. 2016

Sci Spec

Application in Pharmaceutical

Quantitative Analysis of Immunosuppressant Drugs



Excellent Specificity and Peak Shape

Gao et al. *Thermo AN64504*. 2016

Application in Pharmaceutical

Quantitative Analysis of Immunosuppressant Drugs

Tacrolimus

No	QC1 (2.97 ng/mL)	QC2 (13.66 ng/mL)	QC3 (33.06 ng/mL)
Replicate 1	3.43	15.03	35.07
Replicate 2	3.21	18.89	35.77
Replicate 3	2.81	14.68	35.94
Replicate 4	3.18	14.15	34.06
Replicate 5	3.02	12.93	34.3
Mean	**3.13**	**14.13**	**35**
SD	**0.23**	**0.81**	**0.84**
%RSD	**7.34**	**5.71**	**2.39**
%Accuracy	**105**	**103**	**106**

Everolimus

No	QC1 (2.93 ng/mL)	QC2 (13.58 ng/mL)	QC3 (32.40 ng/mL)
Replicate 1	2.21	14.79	31.24
Replicate 2	3.05	12.53	40.91
Replicate 3	2.69	16.29	36.66
Replicate 4	2.67	12.02	35.77
Replicate 5	2.06	11.59	34.14
Mean	**2.54**	**13.4**	**35.74**
SD	**0.4**	**2**	**3.55**
%RSD	**15.8**	**15**	**9.93**
%Accuracy	**86.6**	**99**	**110**

Sirolimus

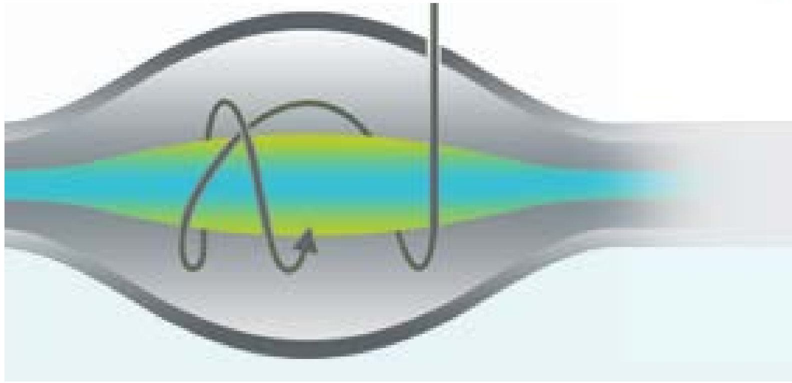
No	QC1 (3.06 ng/mL)	QC2 (12.74 ng/mL)	QC3 (30.66 ng/mL)
Replicate 1	3.3	13.75	29.59
Replicate 2	3.04	14.47	32.46
Replicate 3	3.03	11.74	31.89
Replicate 4	2.63	13.47	32.24
Replicate 5	2.92	9.56	35.3
Mean	**2.98**	**12.6**	**32.3**
SD	**0.24**	**1.97**	**2.03**
%RSD	**8.2**	**15.6**	**6.3**
%Accuracy	**97.5**	**98.9**	**105**

Cyclosporin A

No	QC1 (31.0 ng/mL)	QC2 (134.0 ng/mL)	QC3 (386.8 ng/mL)
Replicate 1	28.49	125.7	377.2
Replicate 2	27.71	128.8	372.5
Replicate 3	28.4	132.4	360.6
Replicate 4	29.88	131.6	383.7
Replicate 5	29.54	122.1	396.6
Mean	**28.8**	**128.1**	**378.1**
SD	**0.08**	**4.3**	**13.3**
%RSD	**3.08**	**3.35**	**3.5**
%Accuracy	**92.9**	**95.6**	**97.7**

Excellent Accuracy and Precision

Gao et al. *Thermo AN64504*. 2016



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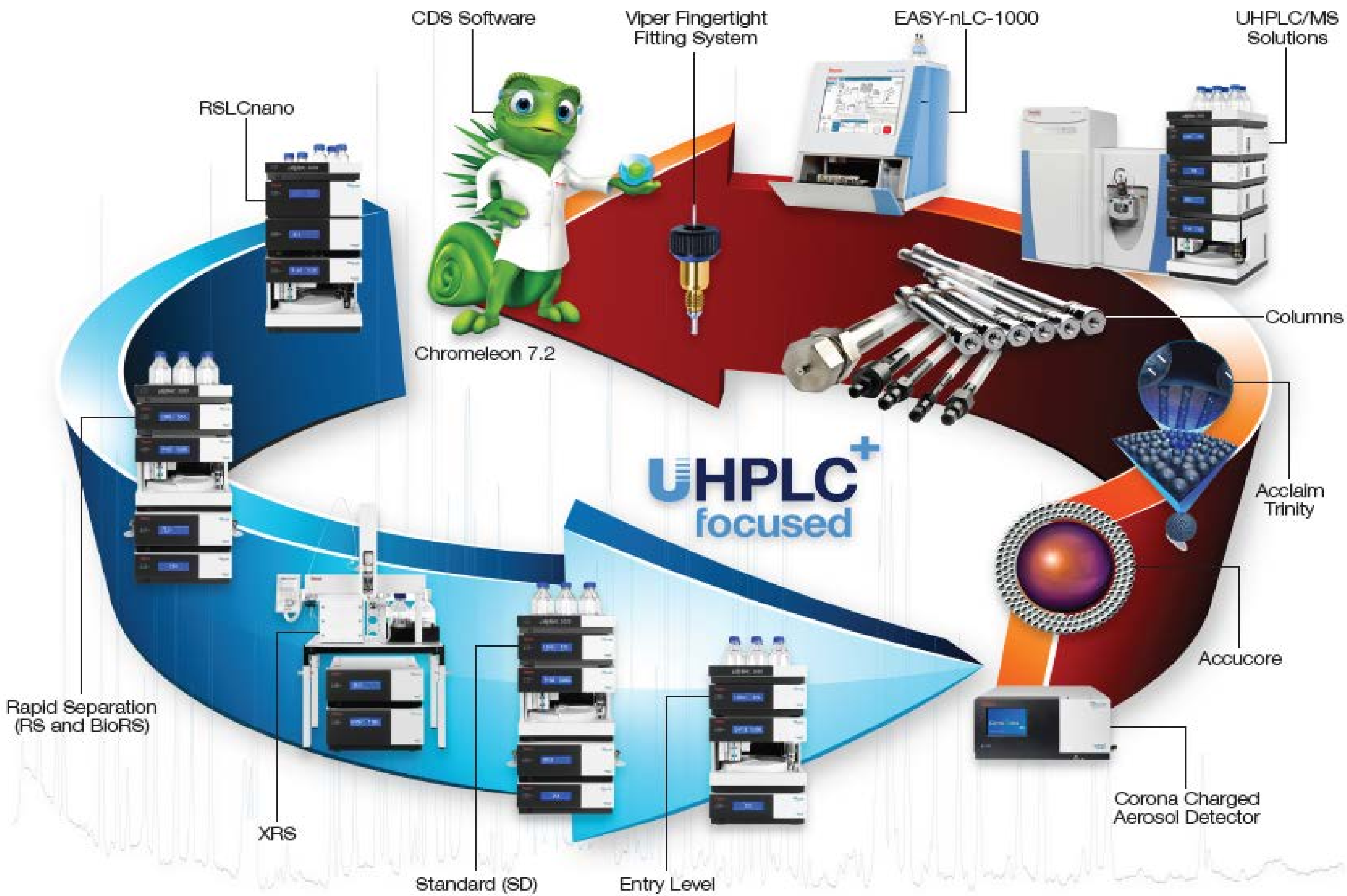

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Sample Preparation

- Tryptic peptide preparation by In-gel and In-solution digestion
- Labeling method for quantitative proteomics (Dimethyl and TMT labeling)
- Phosphopeptide enrichment

LC-MS/MS

- Liquid Chromatography Mass Spectrometry based proteomics using Q-Exactive Plus

Data Analysis

- Protein identification, quantification and statistical analysis by Proteome Discoverer, MaxQuant and Perseus

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