UHPLC-MS Technology and Applications

Rittichai Charoensapyanan

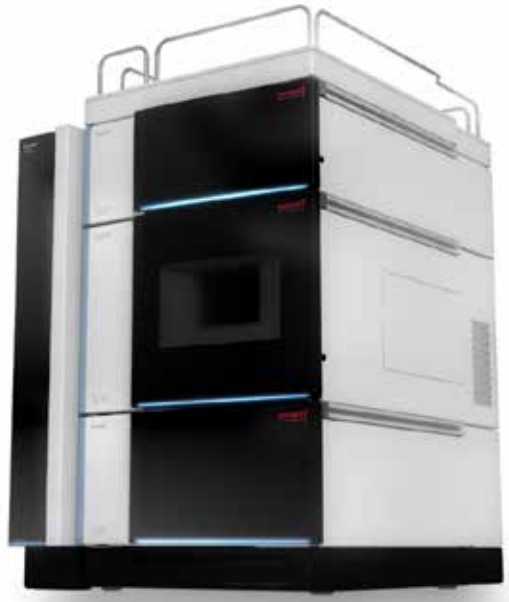
Product Specialist LC/MS

March, 2018

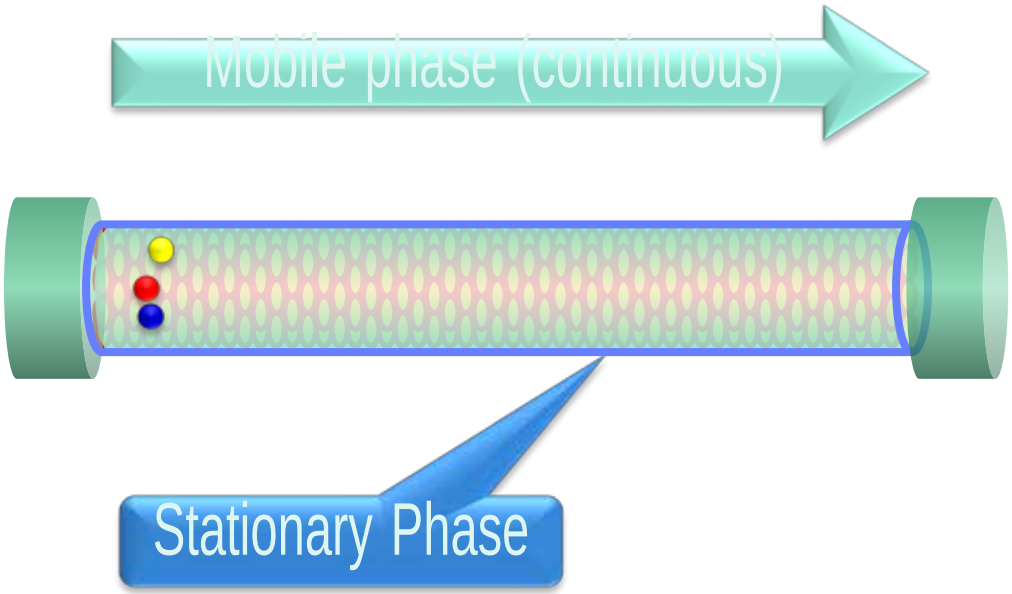
Topics

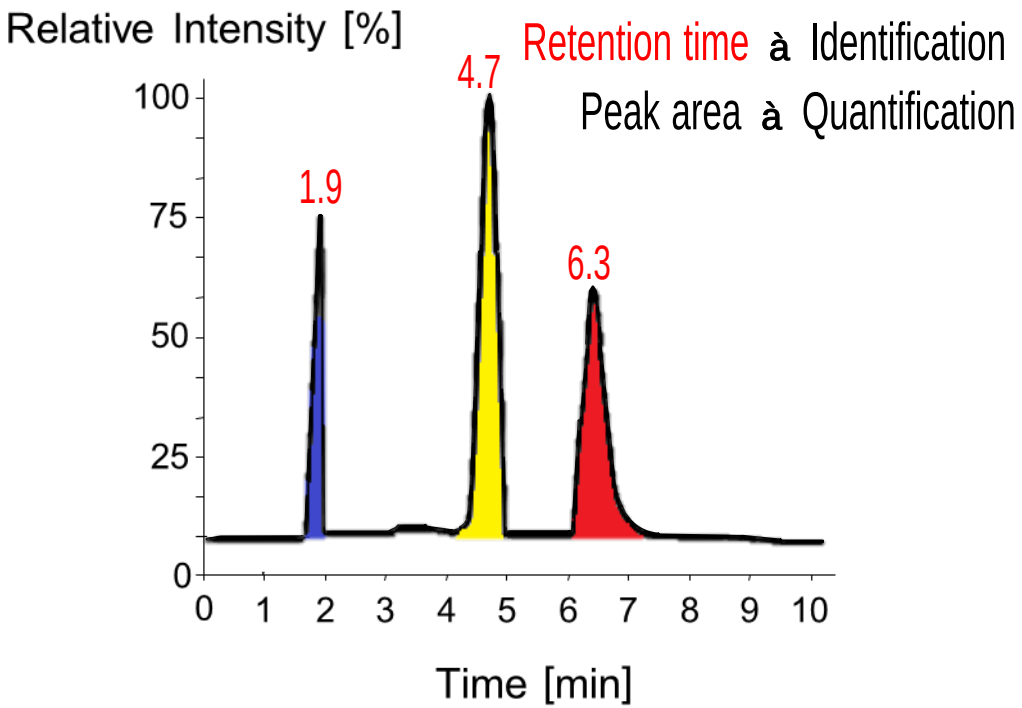
- Fundamental of Liquid Chromatography
- Fundamental of Mass Spectrometer
- Applications in Food Safety, Halal Food and Pharmaceutical

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

- Pump : - Mix two or more solvents
  - Control the flow of mobile phase and analytes
- Degasser : Remove air bubble in solvents
- 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

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 UHPLC

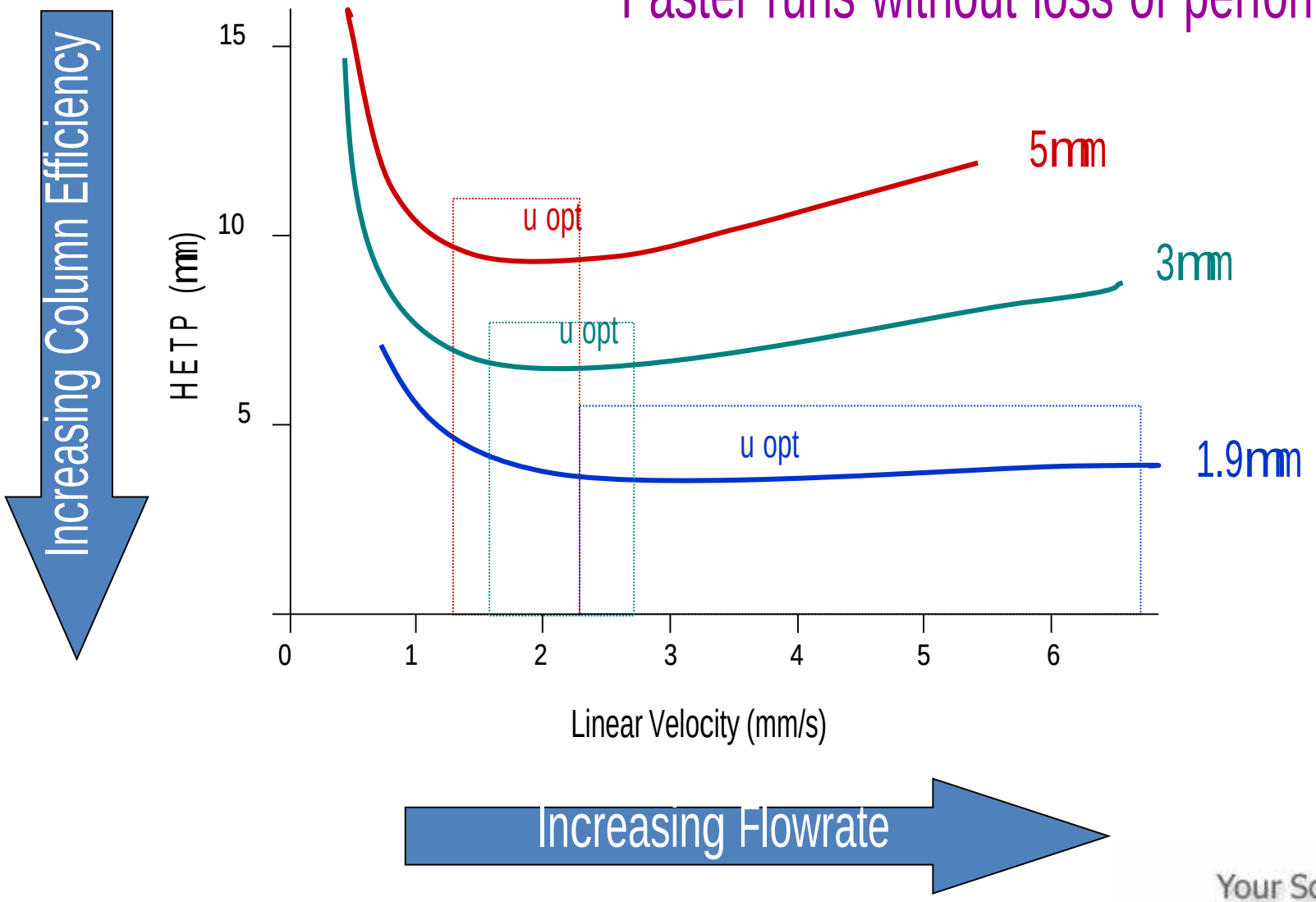
Vanquish™

Max Pressure 1517 bar

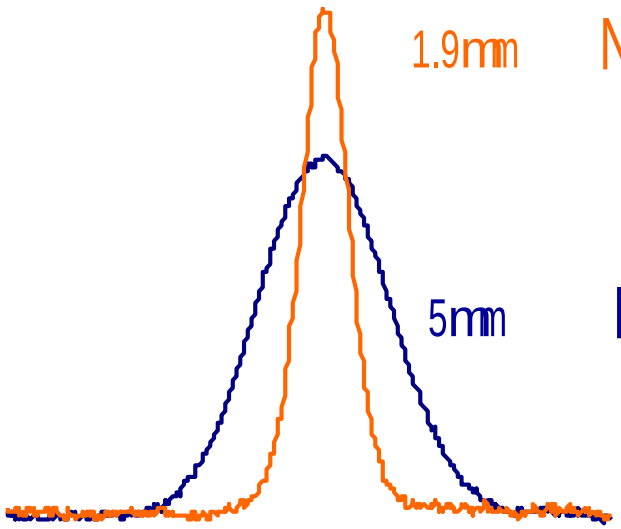
Advantage of Small Particle

Higher efficiency, independent of flow rate means...

Faster runs without loss of performance

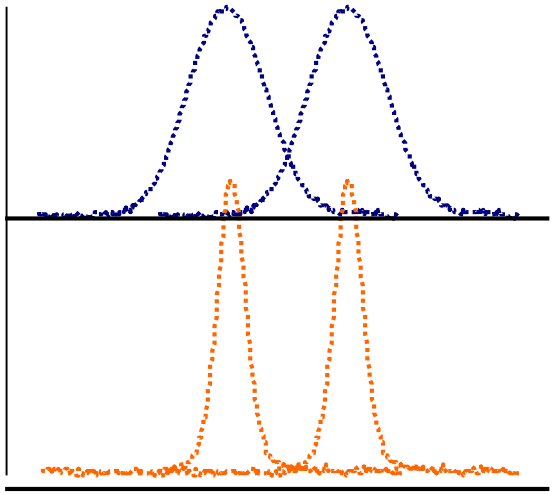


Advantage of Small Particle



N = 142,000 plates/m
(189% higher)

N = 75,000 plates /m



Efficiency is the key!!!

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

Selectivity | Efficiency | Retention

Higher resolution – narrower peaks

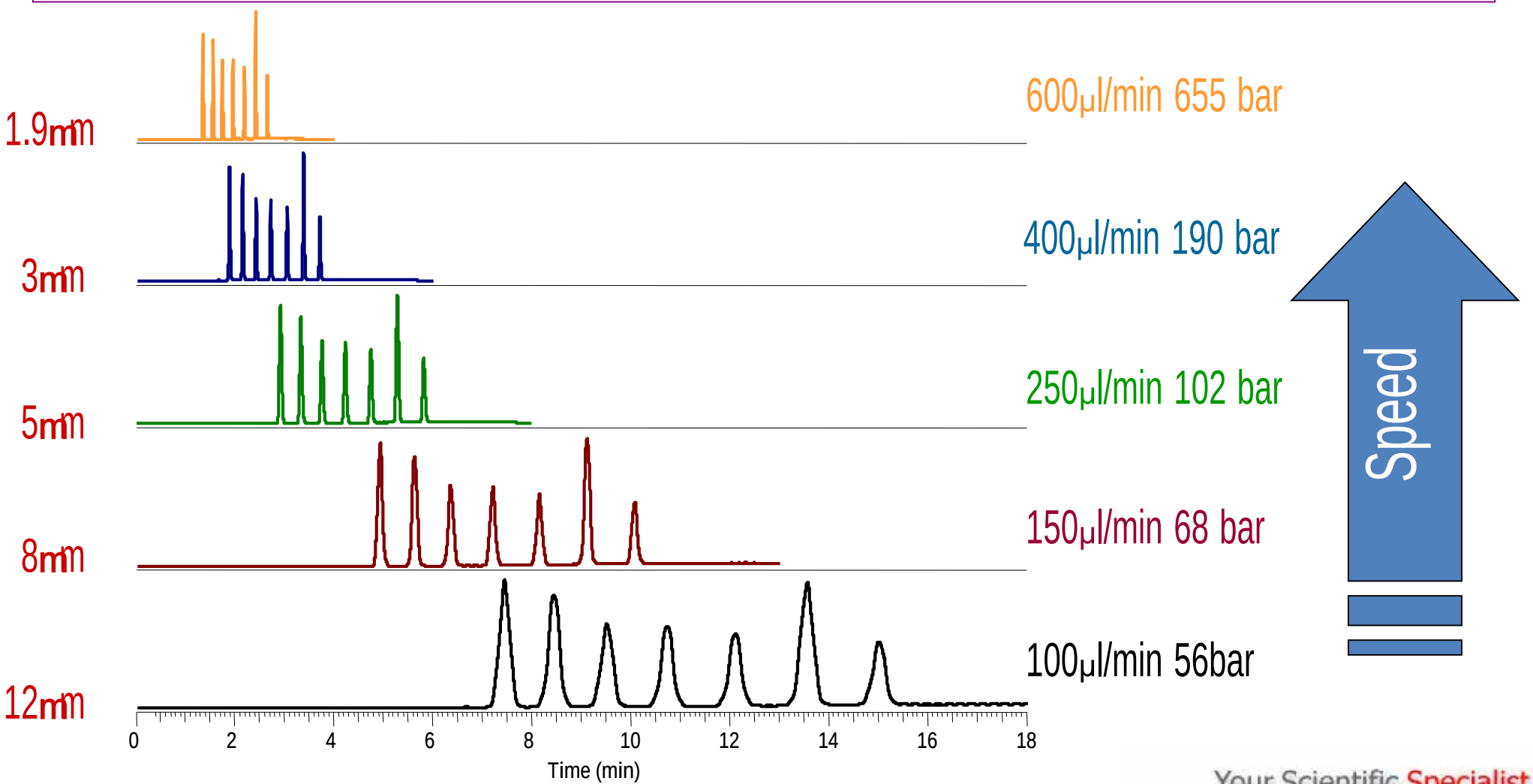
Higher sensitivity – taller peaks

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 mm Hypersil GOLD



The UltiMate™ 3000 LC Systems

Pumps

- Isocratic
- Binary
- Quaternary
- Dual-Gradient

Autosampler

- Basic Automated
- Standard
- Thermostatted
- + Fractionation

Column Compartments

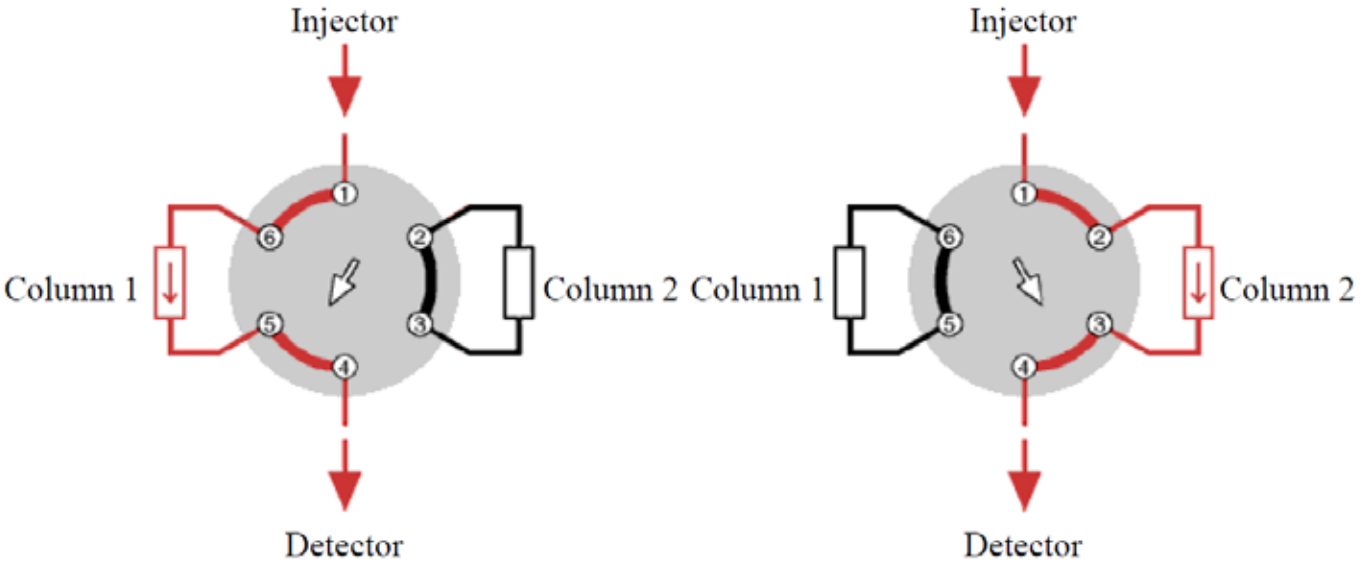
- Standard
- With Valves

Detectors

- VWD
- MWD/DAD
- Fluorescence
- Corona
- Coulochem

UHPLC+ Applications

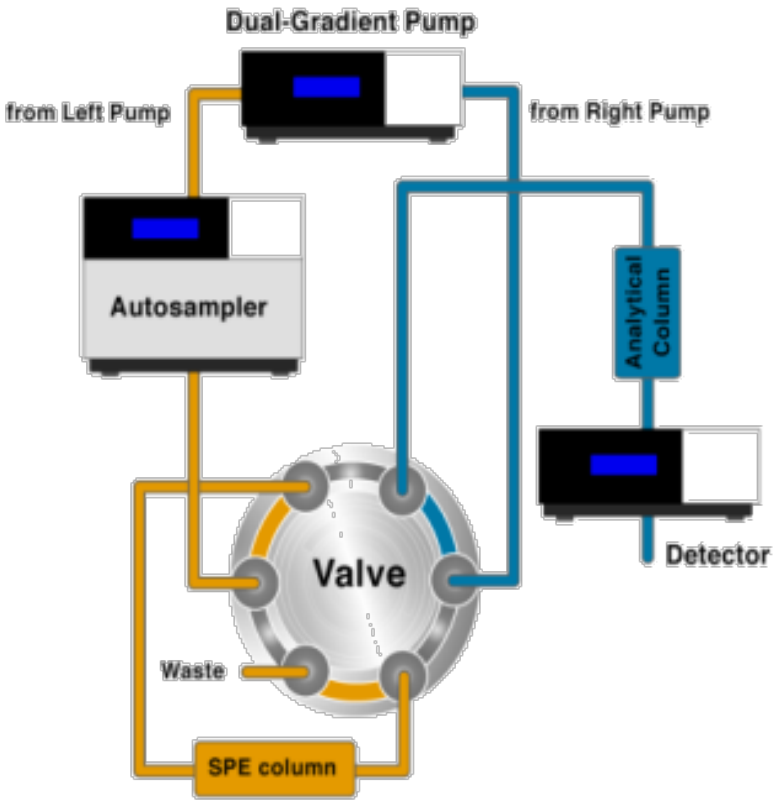
- Built-in column switching valve
- 2-position, 6-port column switching valve



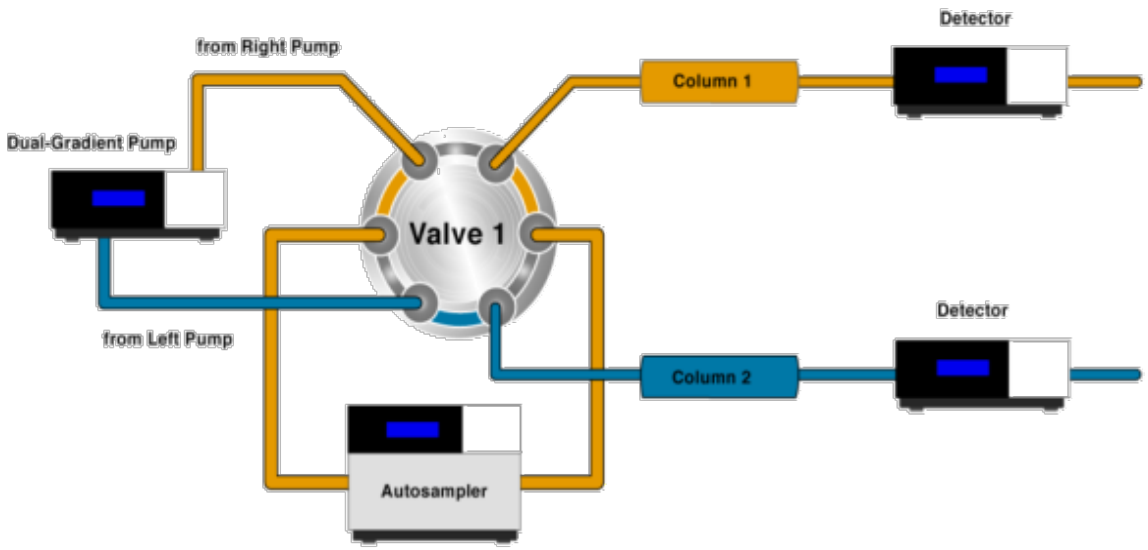
Switching Valve

UHPLC+ Applications

Online SPE



Parallel LC



Fundamental of Mass Spectrometer

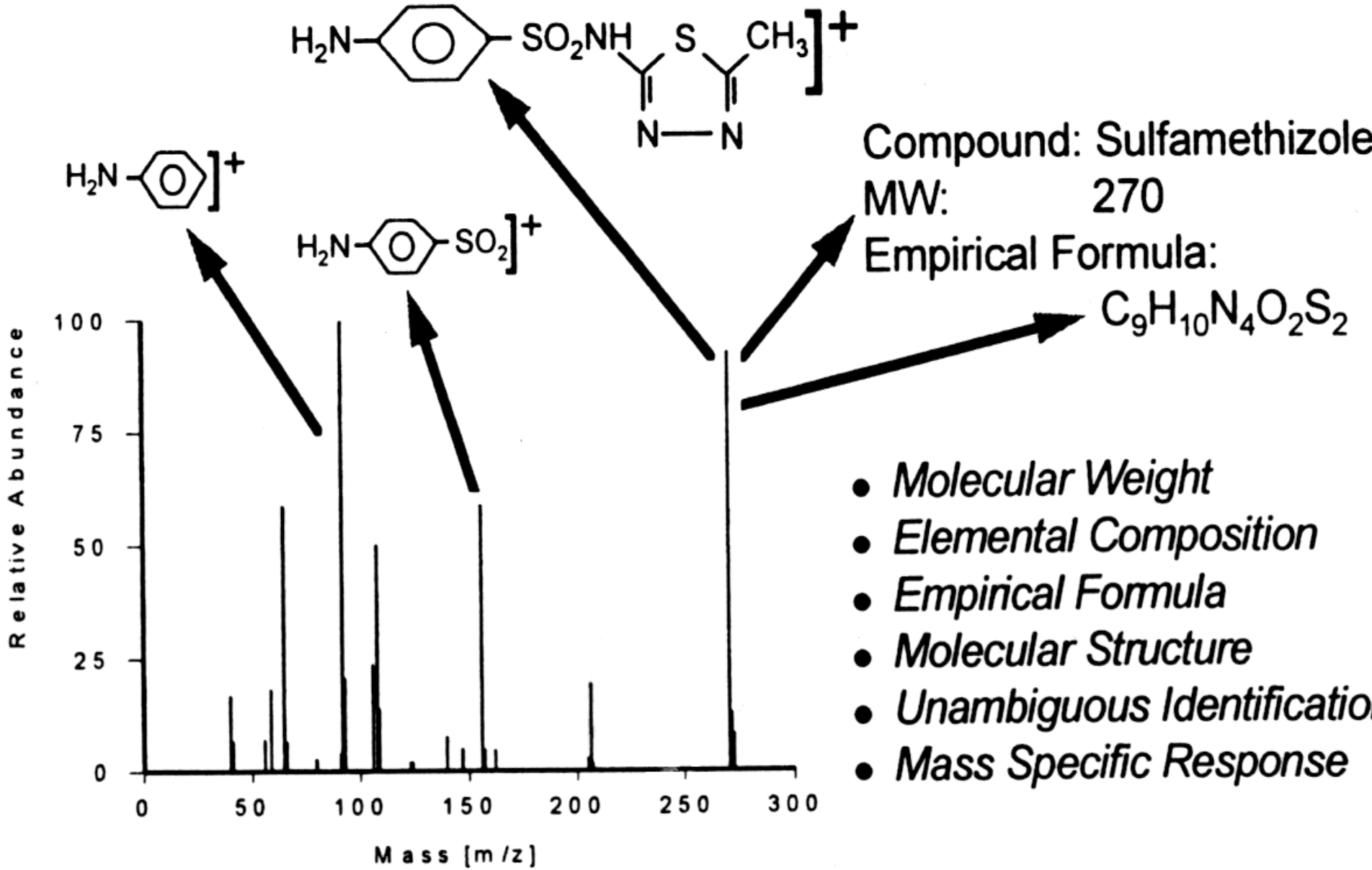
What is Mass Spectrometer?

"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."

- 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

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

Information Rich Data

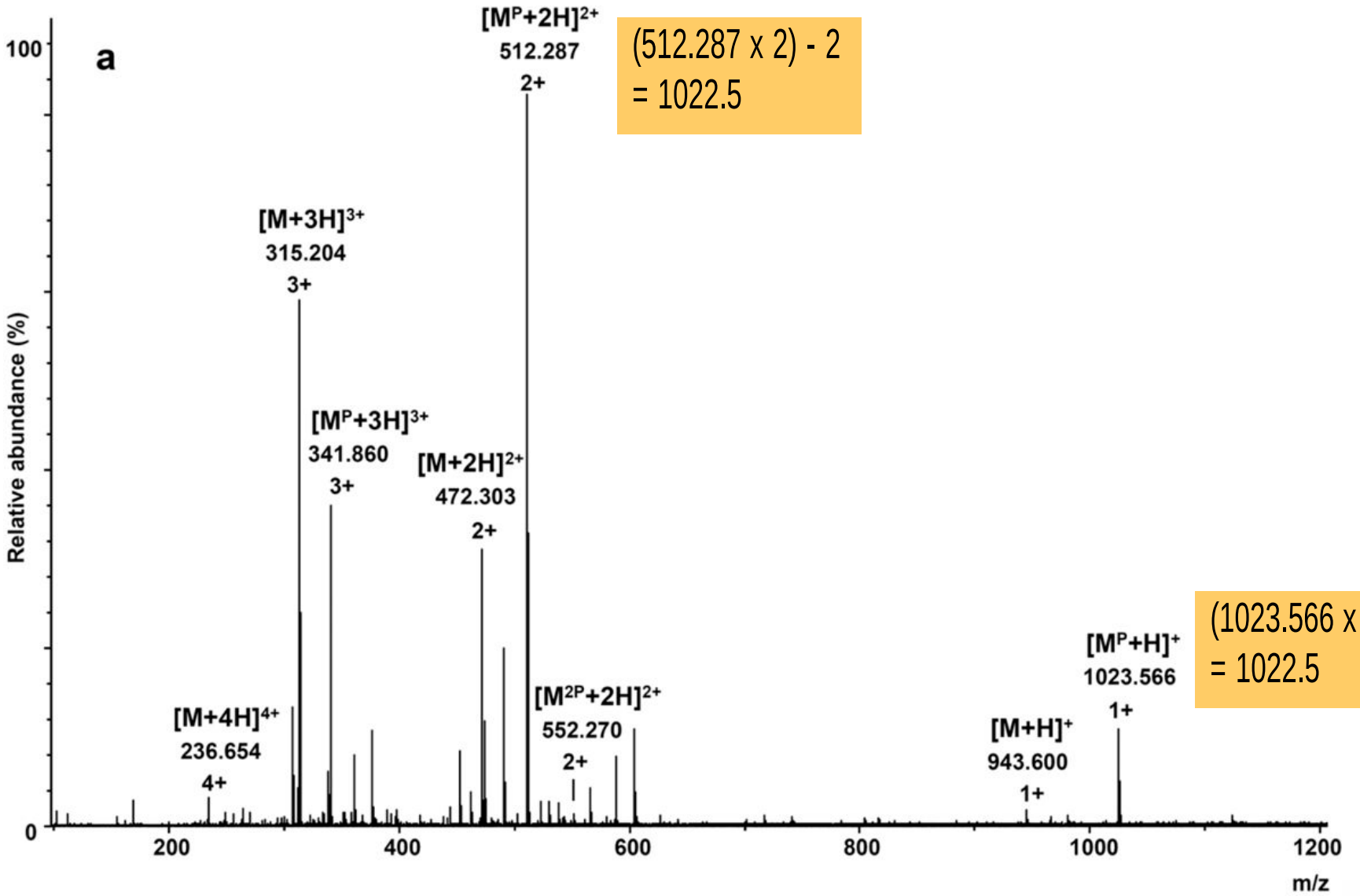


Compound: Sulfamethizole
MW: 270
Empirical Formula:
$C_9H_{10}N_4O_2S_2$

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

Mass Spectrum

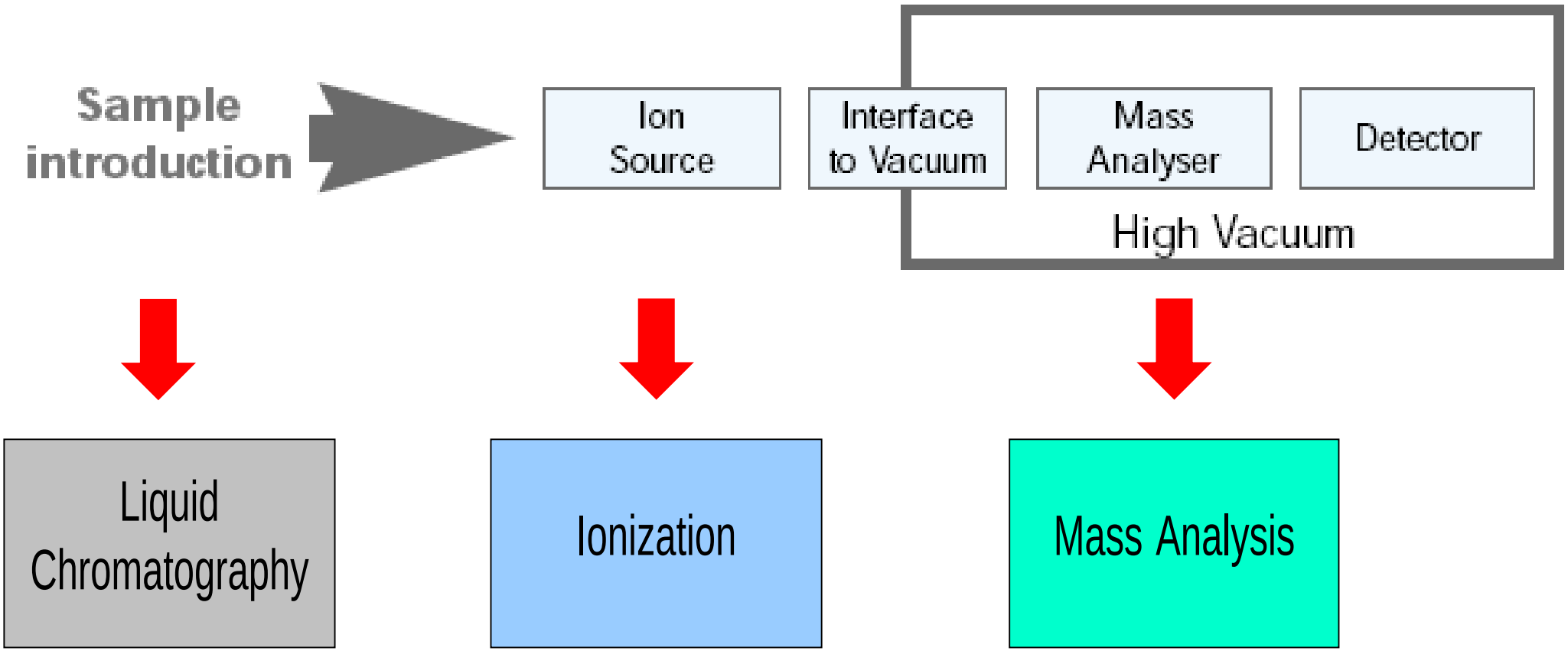
mass to charge = (molecular weight + charge) / charge



(512.287 x 2) - 2 = 1022.5

(1023.566 x 1) - 1 = 1022.5

Mass Spectrometry: Block Diagram



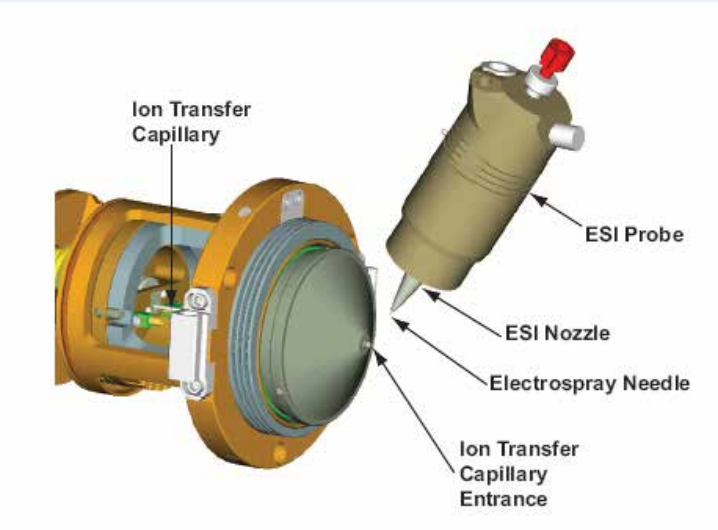
Ionization

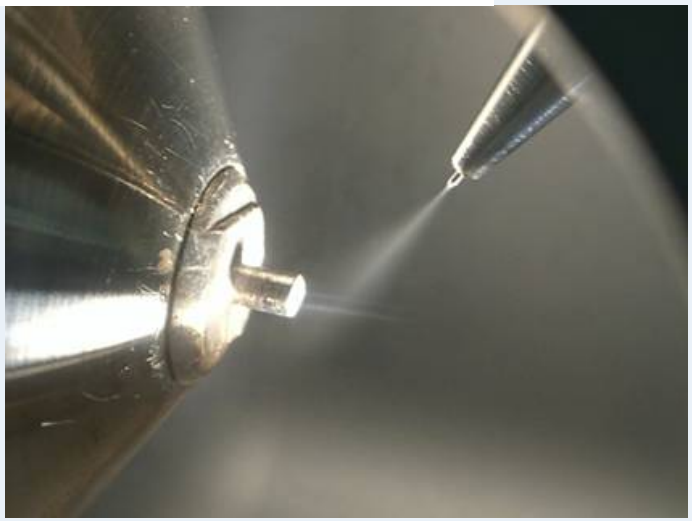
- Ion source : converts sample molecules (neutral) into charged molecules or molecular ions.
- Type of ionization techniques
  - Electron Impact (EI)
  - Chemical Ionization (CI)
  - Matrix Assisted Laser Desorption Ionization (MALDI)
  - Atmospheric Pressure Ionization (API)
    - Electrospray Ionization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

Ion Source

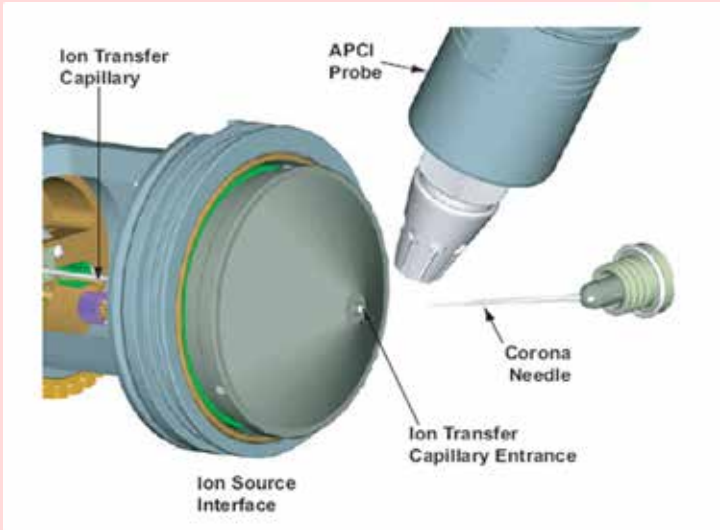
Atmospheric Pressure Ionization (API)

ESI



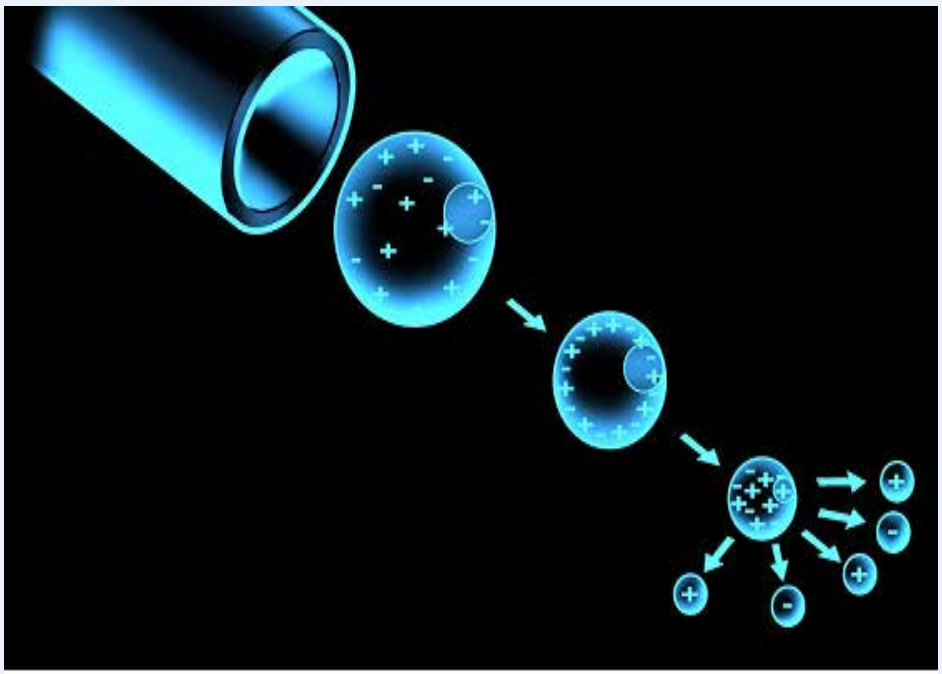


APCI



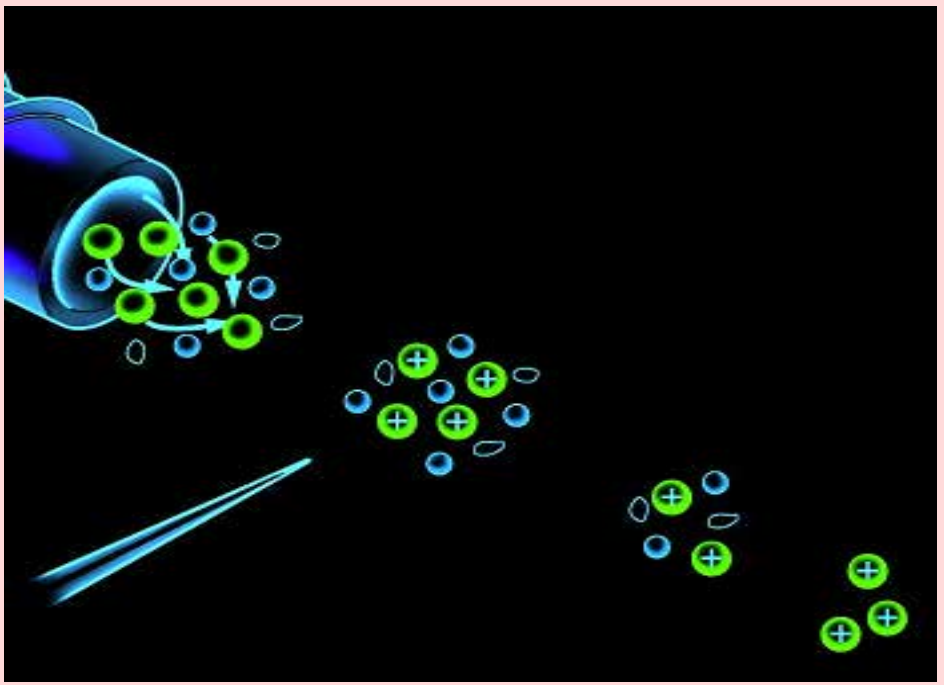
Atmospheric Pressure Ionization (API)

ESI



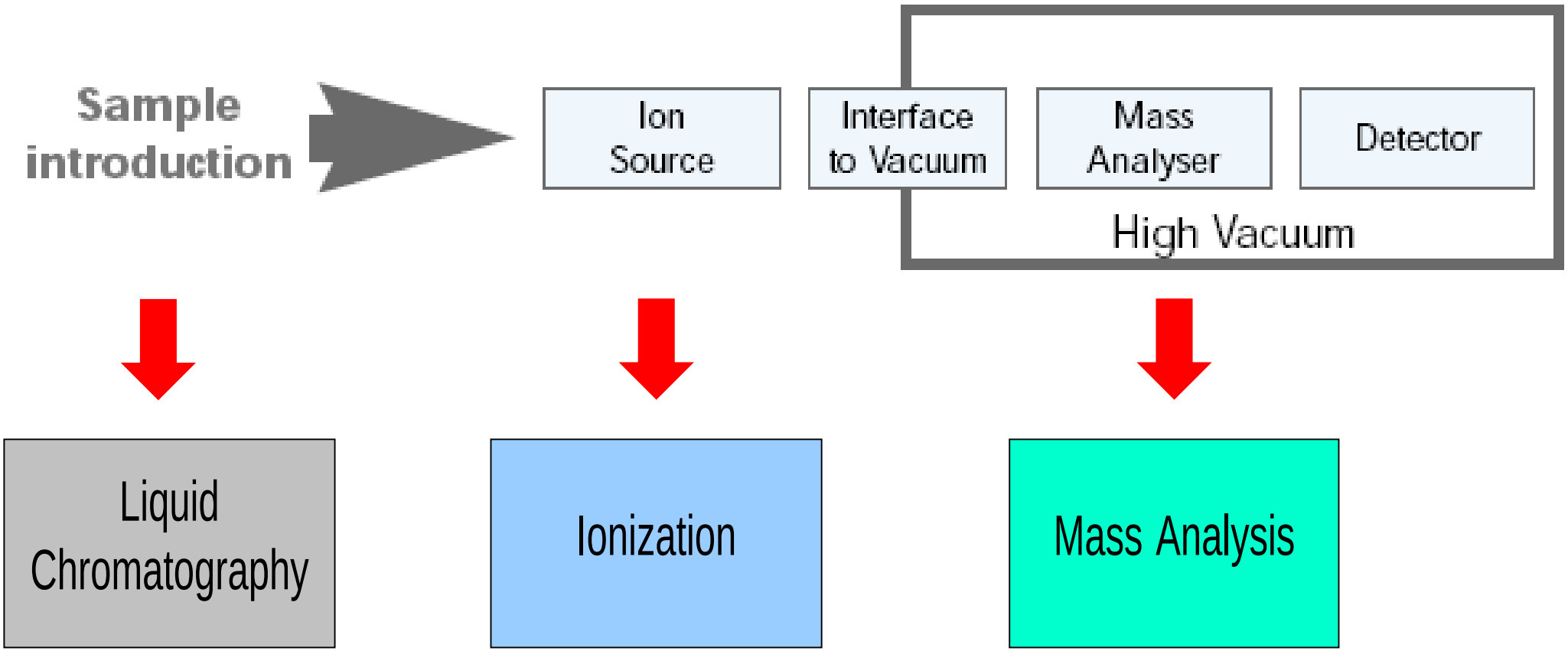
- Ions formed by solution chemistry
- Good for thermally labile analytes
- Good for polar analytes
- Good for large molecules (protein/peptide)

APCI



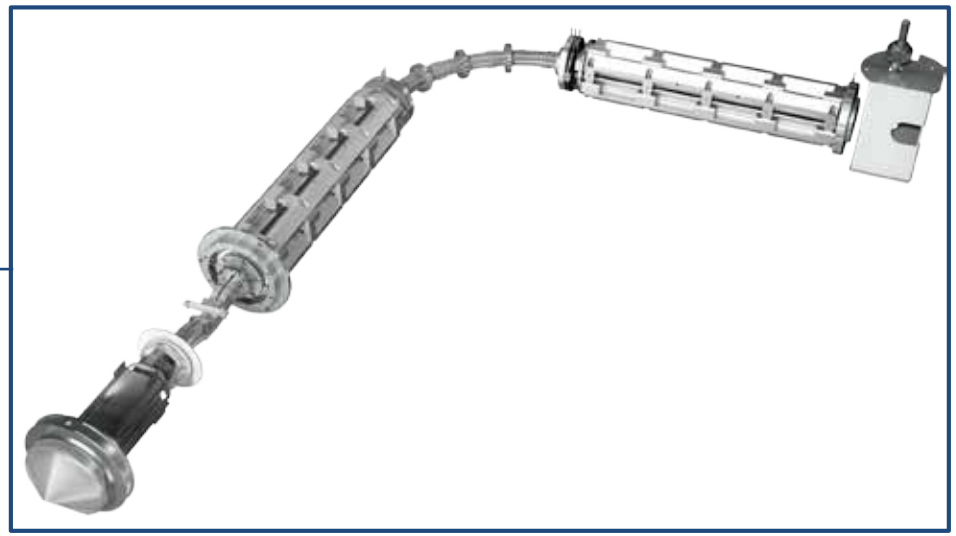
- Ions formed by gas phase chemistry
- Good for volatile / thermally stable
- Good for non-polar analytes
- Good for small molecules (steroids)

Mass Spectrometry: Block Diagram



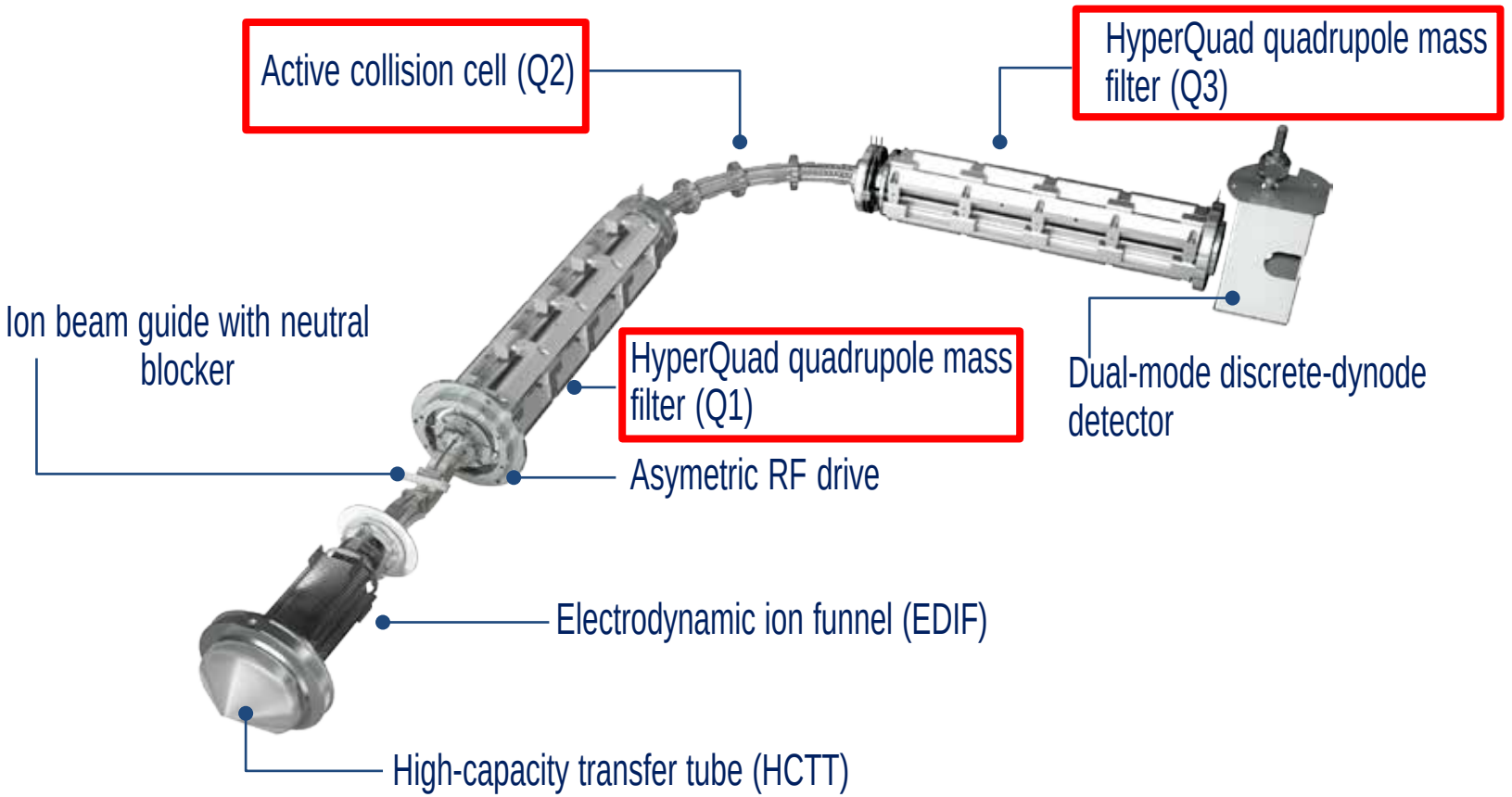
Mass Analyzer

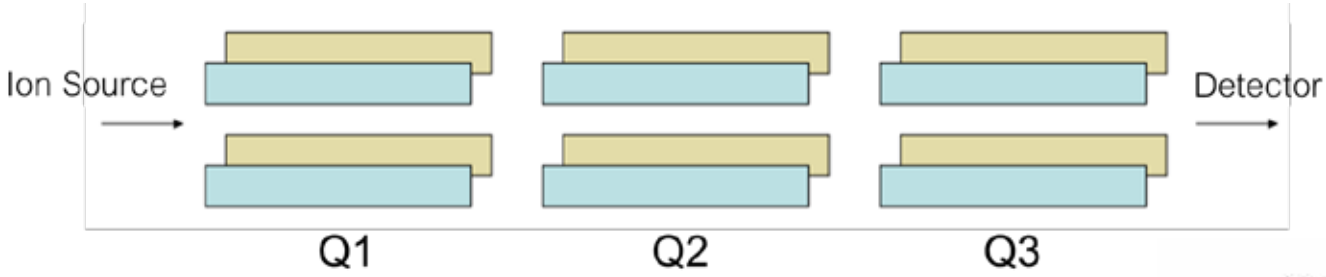
- Triple Quadrupole (QqQ)



- Orbitrap

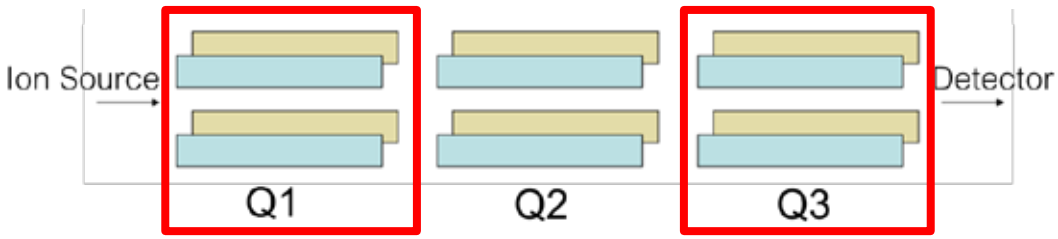
Mass Analyzer: Triple Quadrupoles (QqQ)

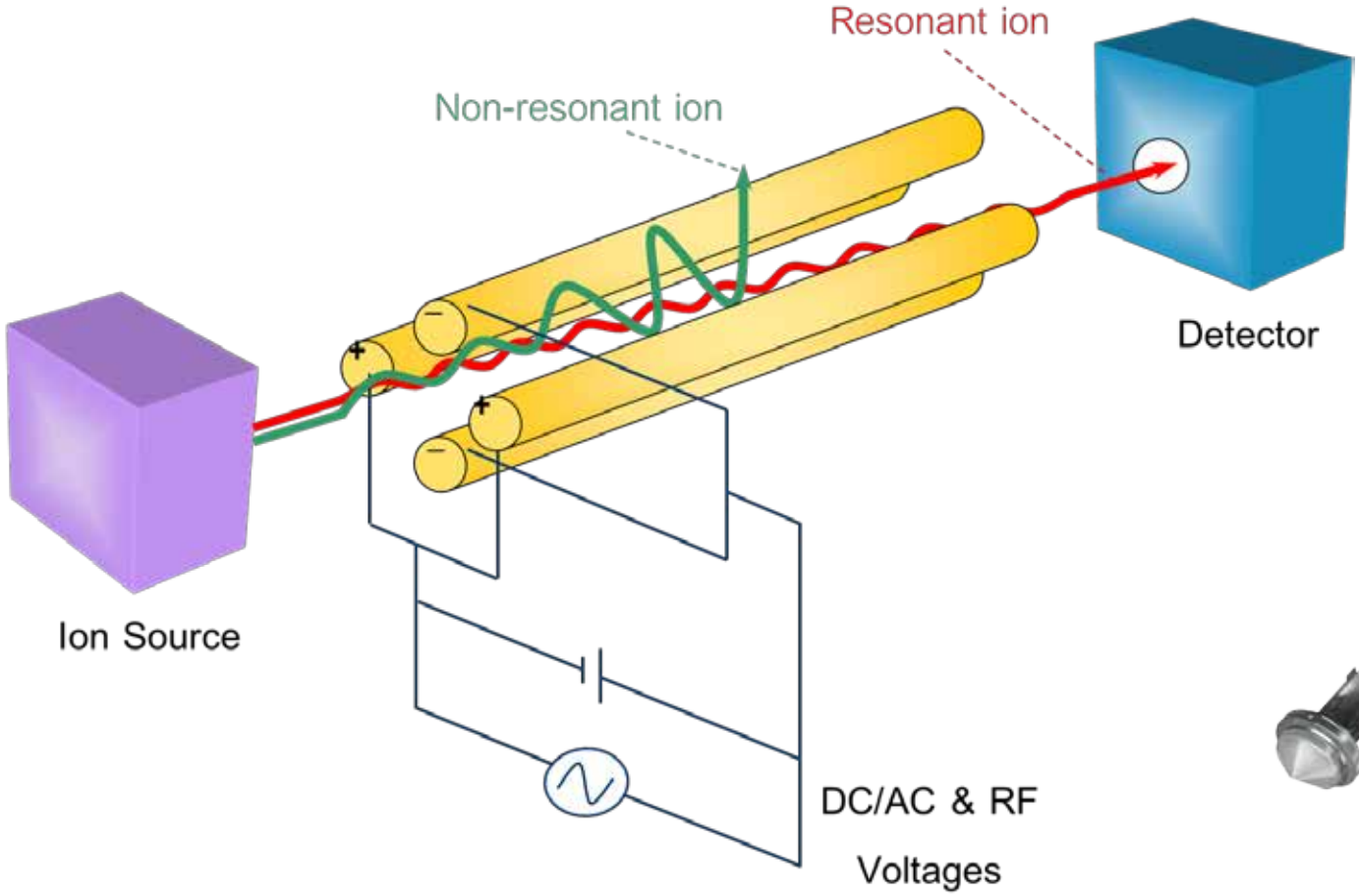


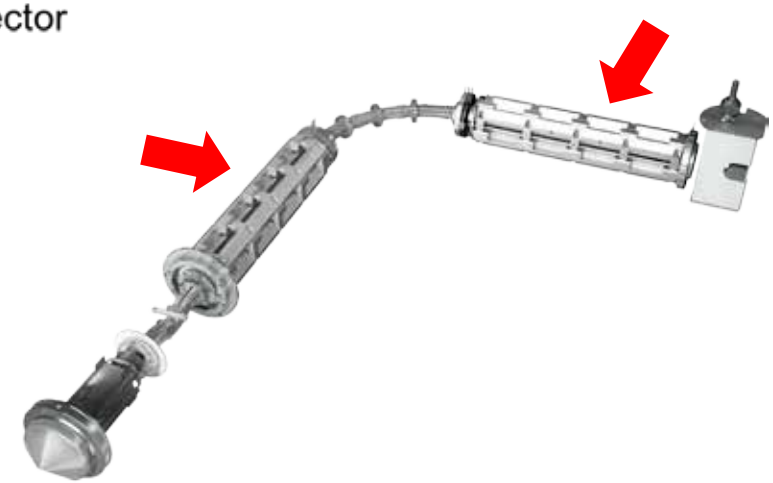


Mass Analyzer: Triple Quadrupoles (QqQ)

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

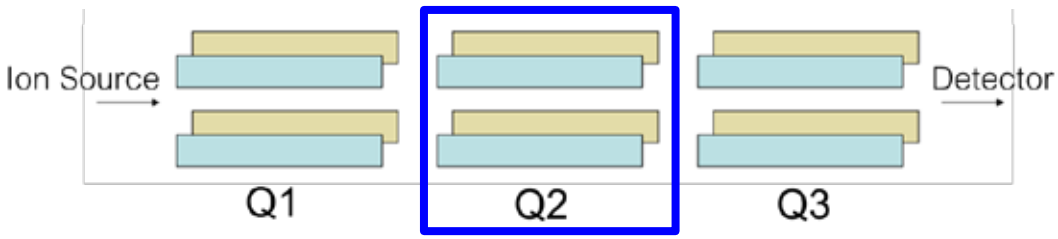


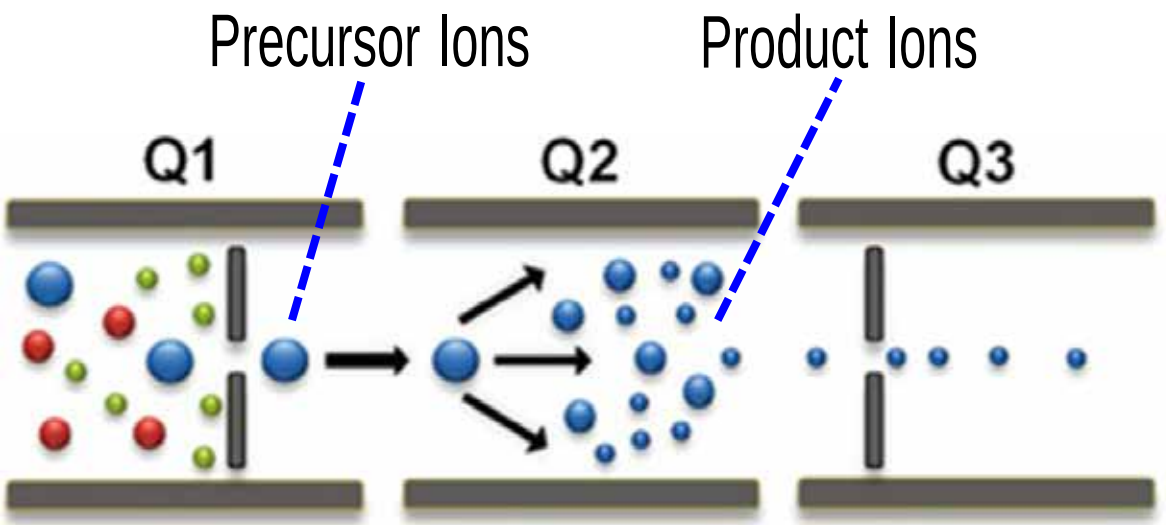




Mass Analyzer: Triple Quadrupoles (QqQ)

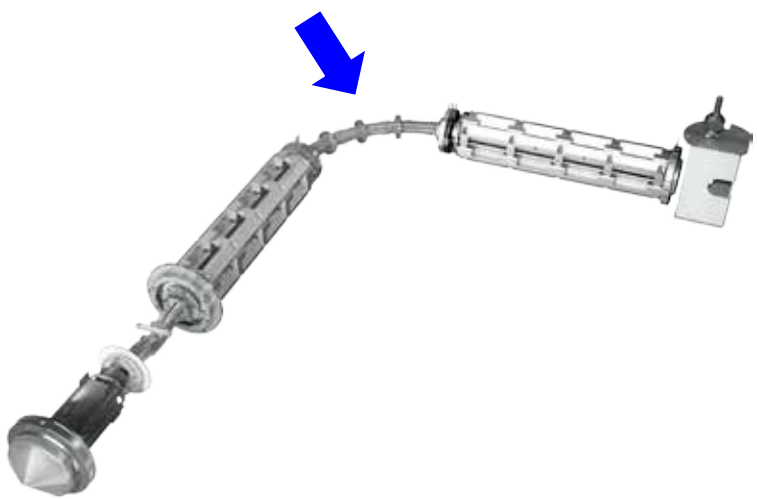
- Q2 is “Collision Cell” where precursor ions are fragmented and pass through Q3 for ion sorting again





Fragmentation

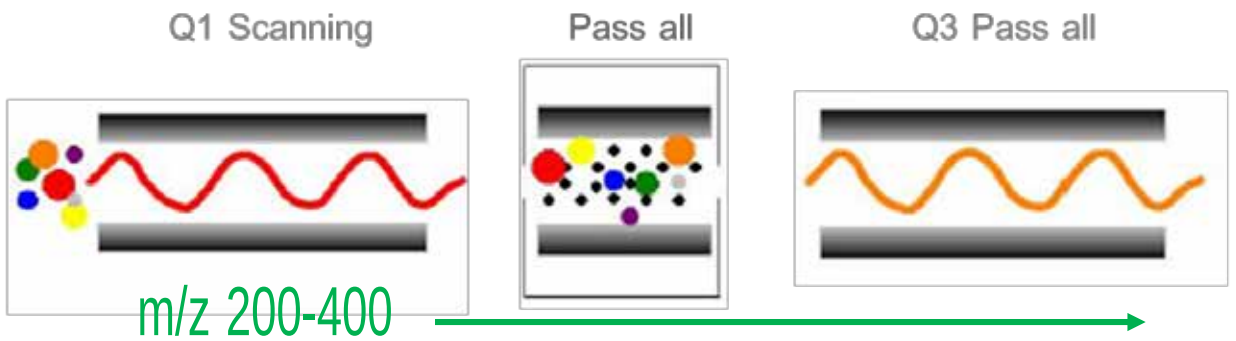
(Collision gas: AR)



Scan Modes in QqQ

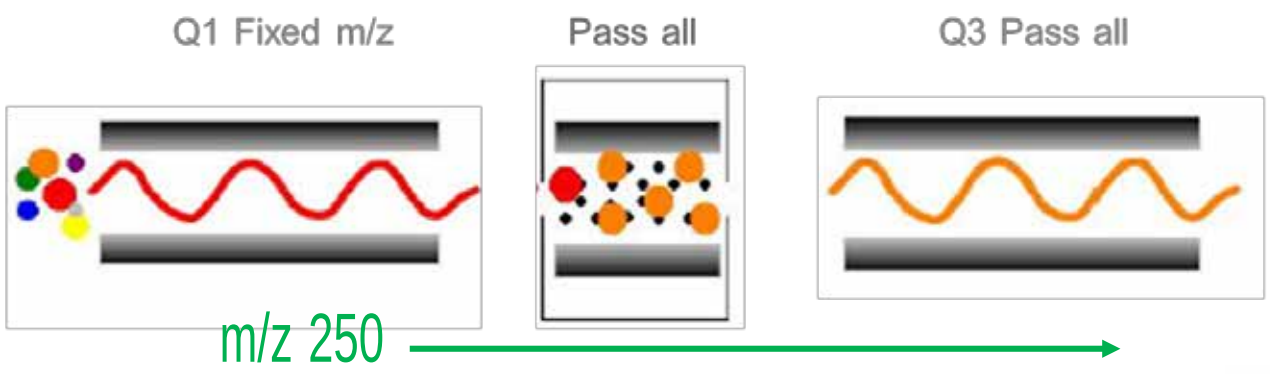
Scan Mode	Q1	Q2	Q3	Purpose
Full Scan	Scanning	Pass All	Pass All	MW Info.
SIM (Selected Ion Monitoring)	Fixed m/z	Pass All	Pass All	Quantitation

Full scan Q1:



Purpose: Survey scan of a chromatographic peak

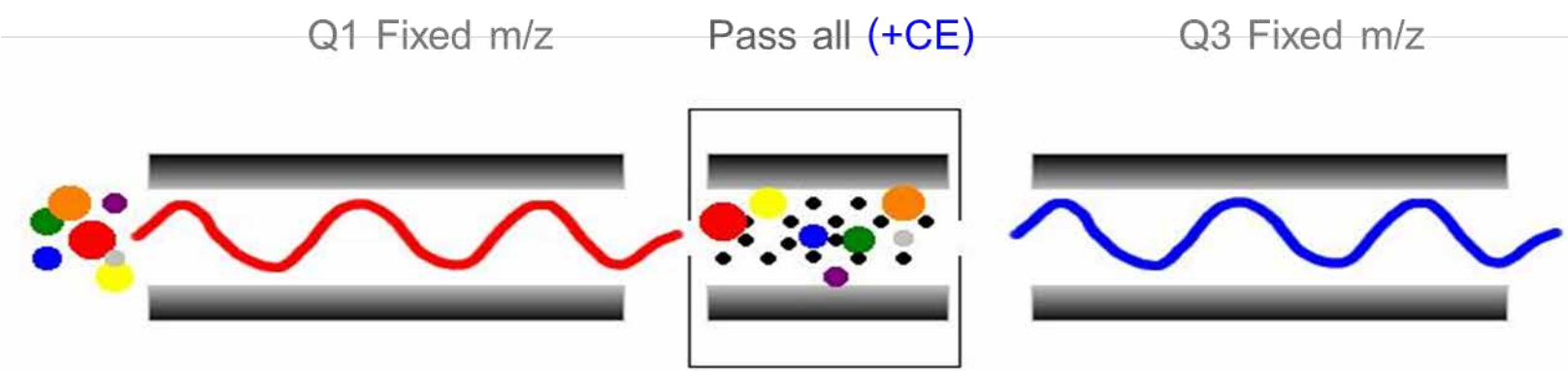
SIM Q1:



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

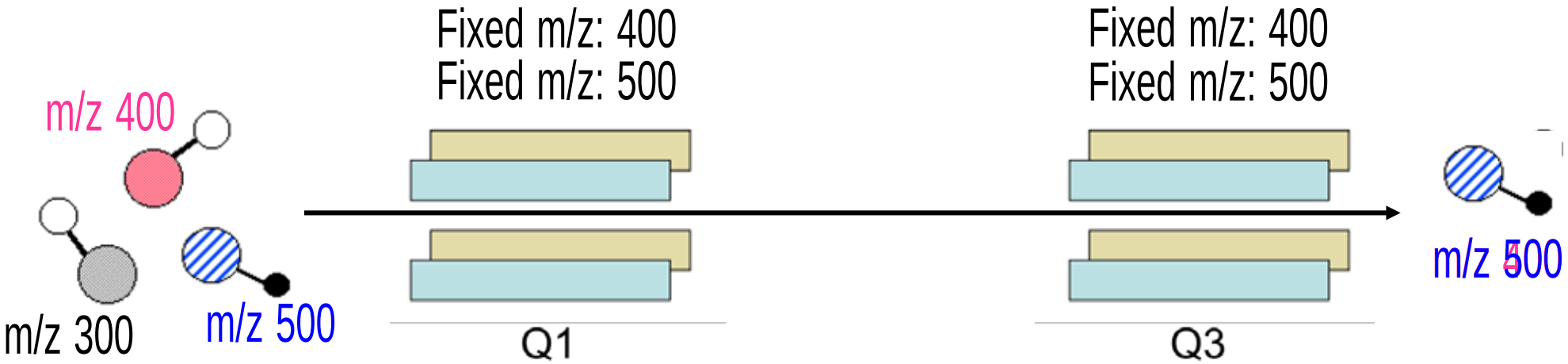
Scan Modes in QqQ

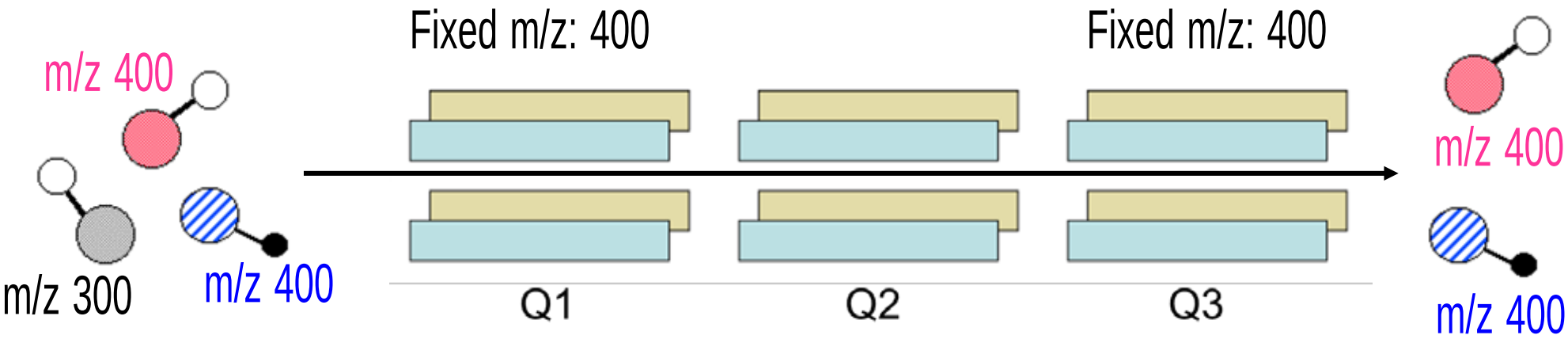
Scan Mode	Q1	Q2	Q3	Purpose
SRM (Selected Reaction Monitoring)	Fixed m/z	Pass All (+CE)	Fixed m/z	Targeted Quantitation



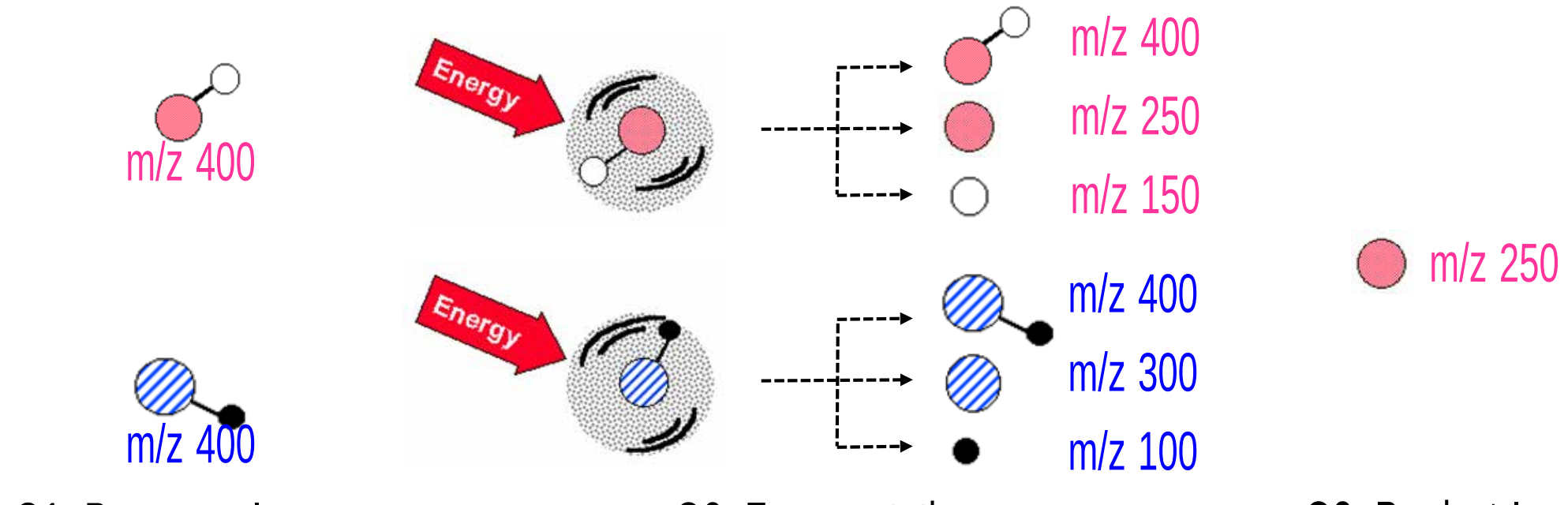
Purpose: Targeted quantitation

Scan Modes in QqQ





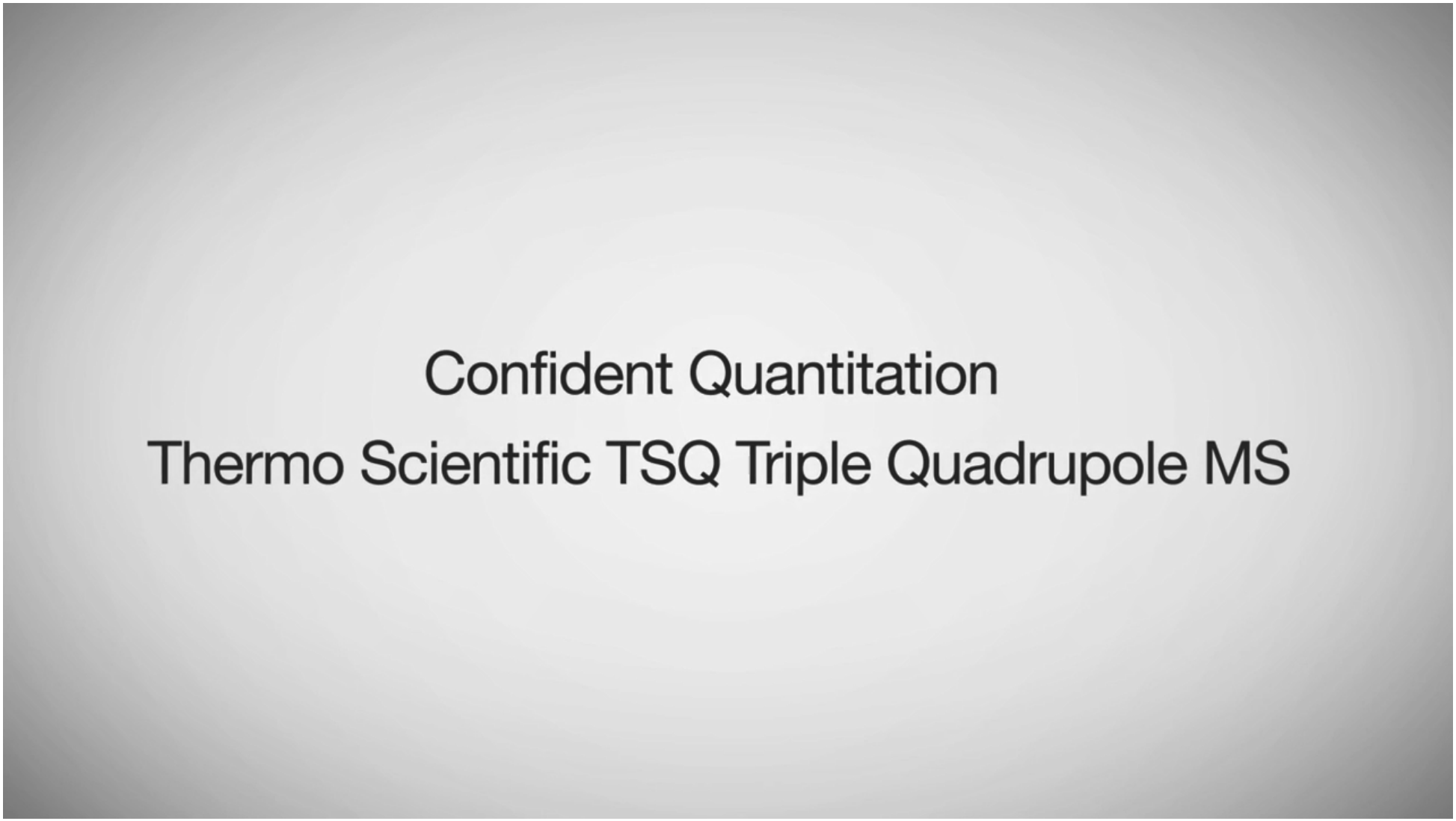
SRM


m/z 400
Energy
m/z 400
m/z 250
m/z 150
m/z 250
m/z 400
Energy
m/z 400
m/z 300
m/z 100
Q1: Precursor Ion
Q2: Fragmentation
Q3: Product Ion
m/z 400
Fixed m/z: 400
Fixed m/z: 250
m/z 250
m/z 300
m/z 400
Q1
Q2
Q3

Scan Modes in QqQ

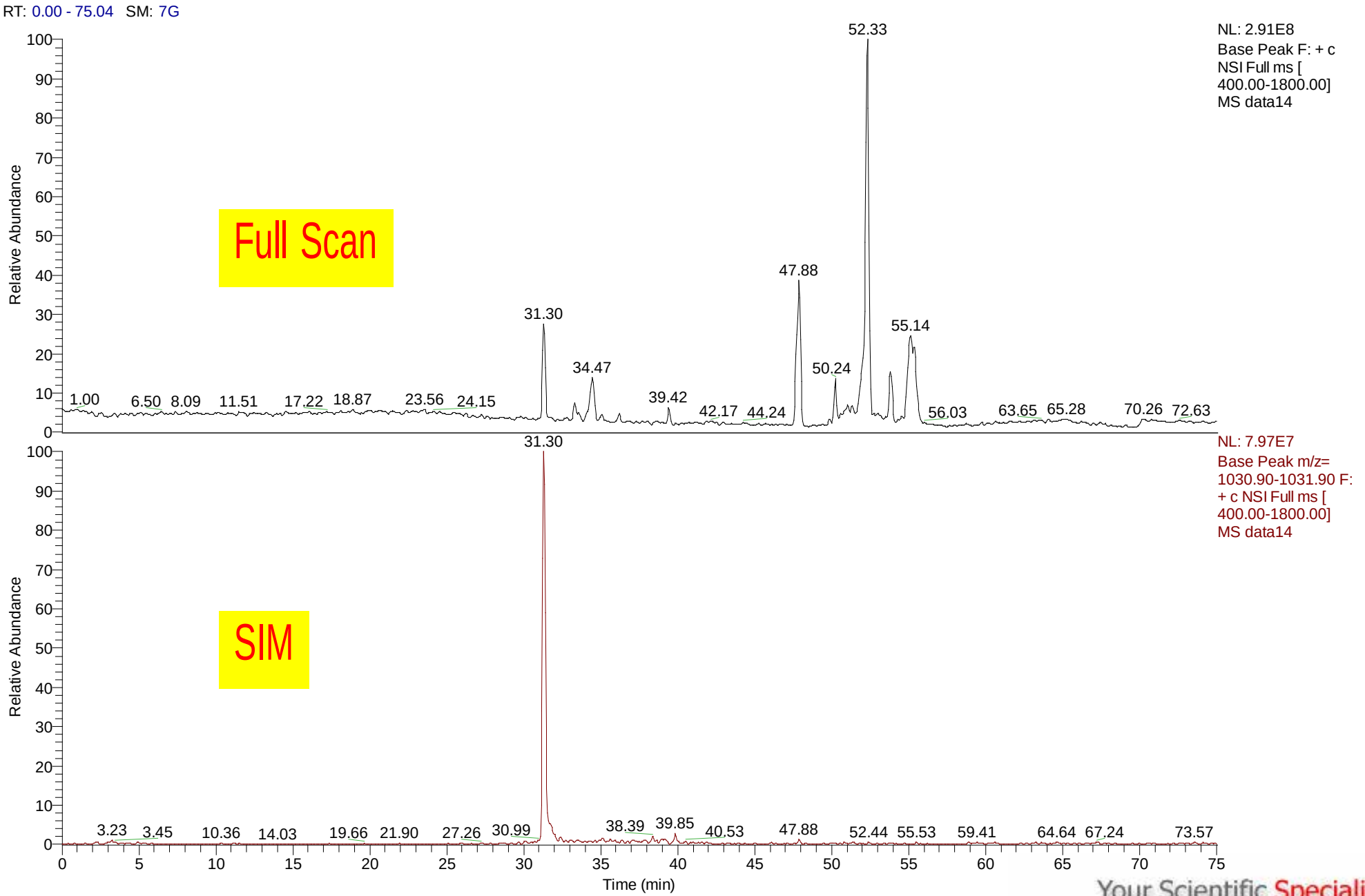
Scan Mode	Q1	Q2	Q3	Purpose
Full Scan	Scanning	Pass All	Pass All	MW Info.
SIM (Selected Ion Monitoring)	Fixed m/z	Pass All	Pass All	Quantitation
SRM (Selected Reaction Monitoring)	Fixed m/z	Pass All (+CE)	Fixed m/z	Targeted Quantitation
Product	Fixed m/z	Pass All (+CE)	Scanning	Structural Info.
Neutral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

TSQ Triple Quadrupole MS



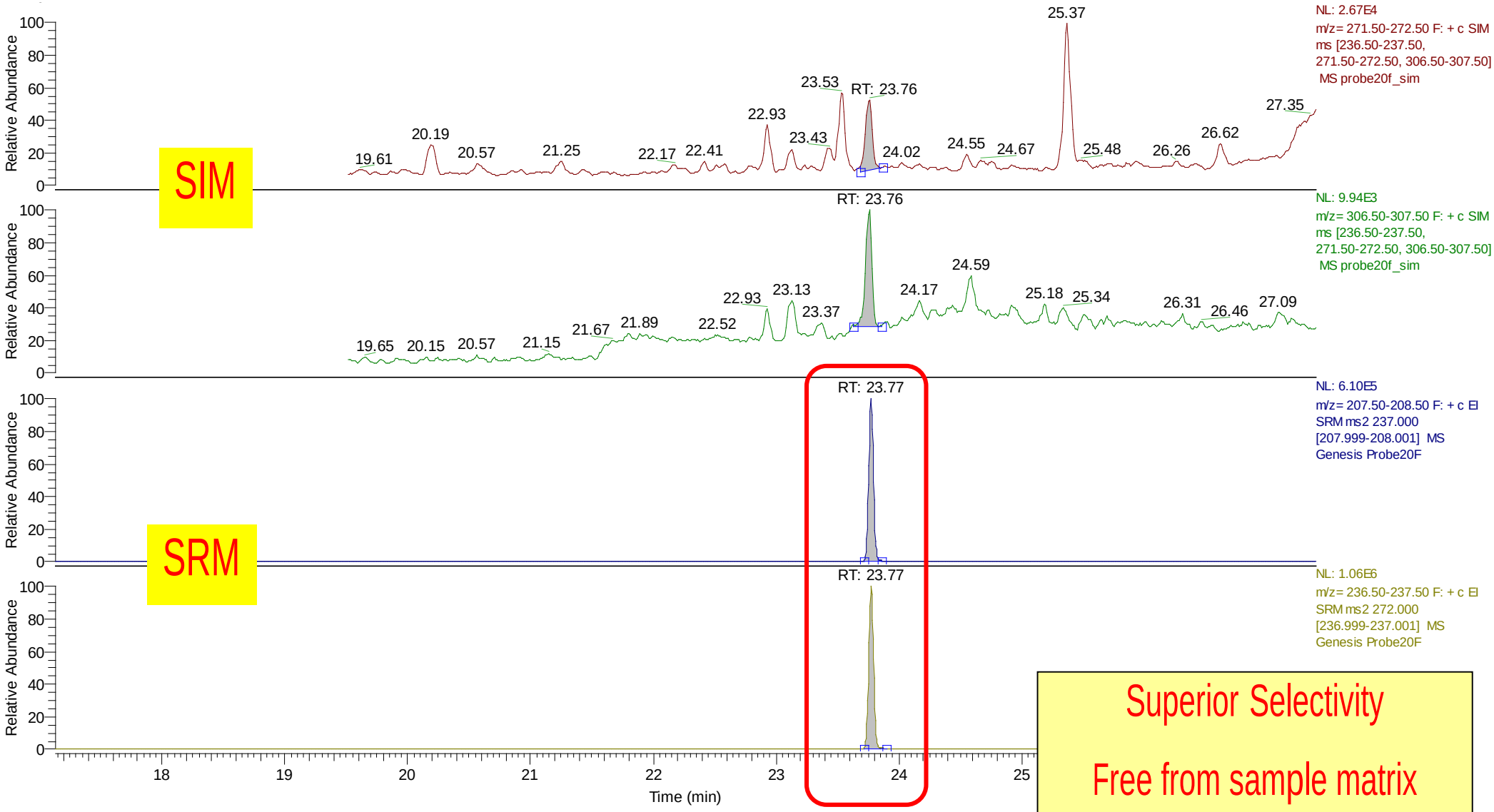
http://www.youtube.com/watch?v=LFB14D8pkoc

Full Scan VS SIM



Your Scientific Specialist

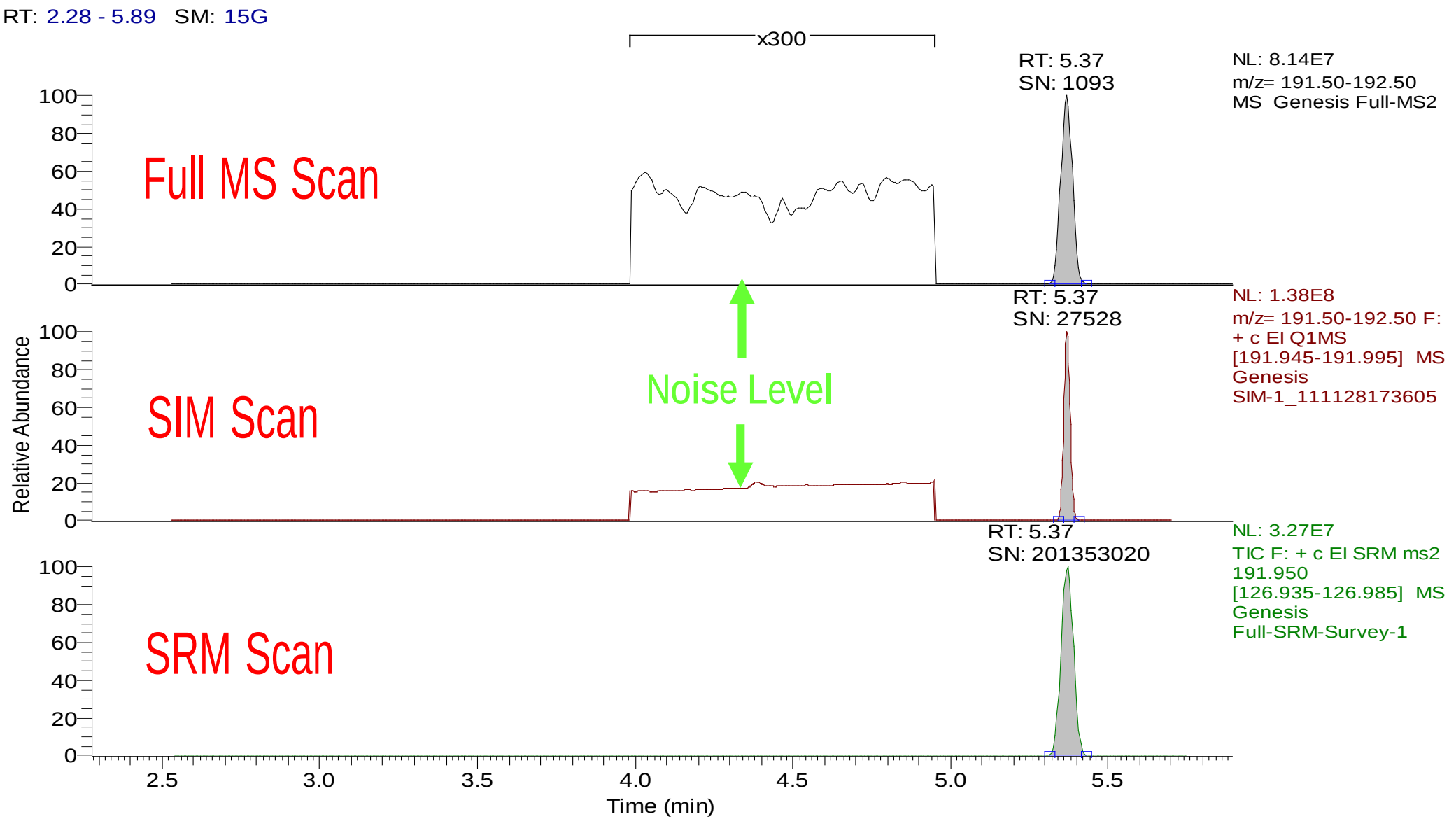
SIM VS SRM



Superior Selectivity

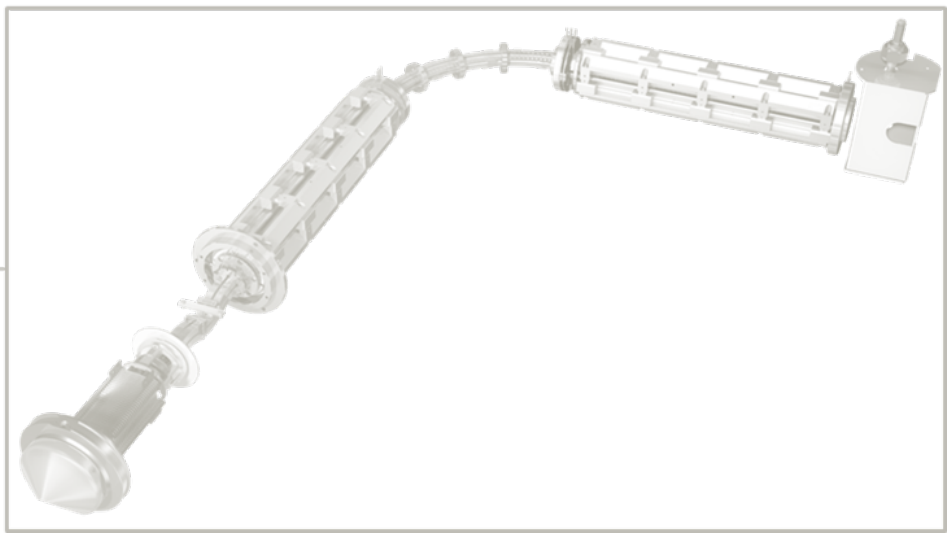
Free from sample matrix

SRM Selectivity in Complex Matrices



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.

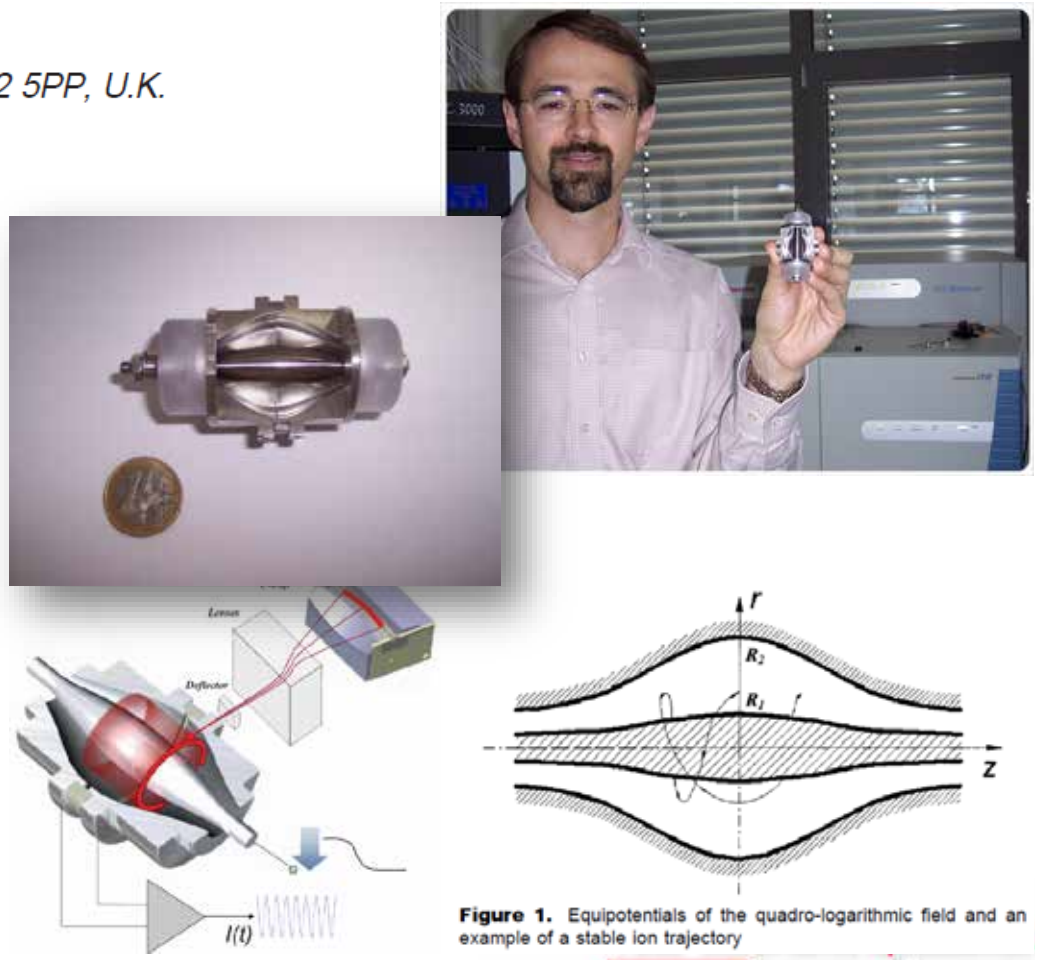
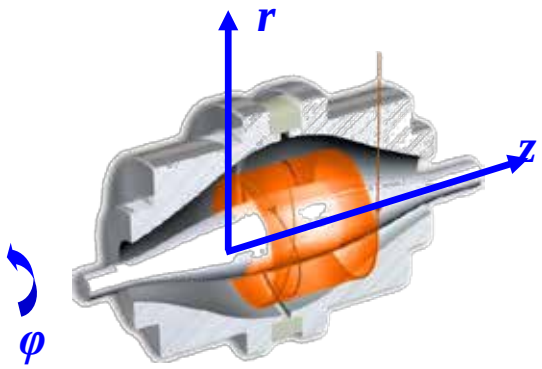


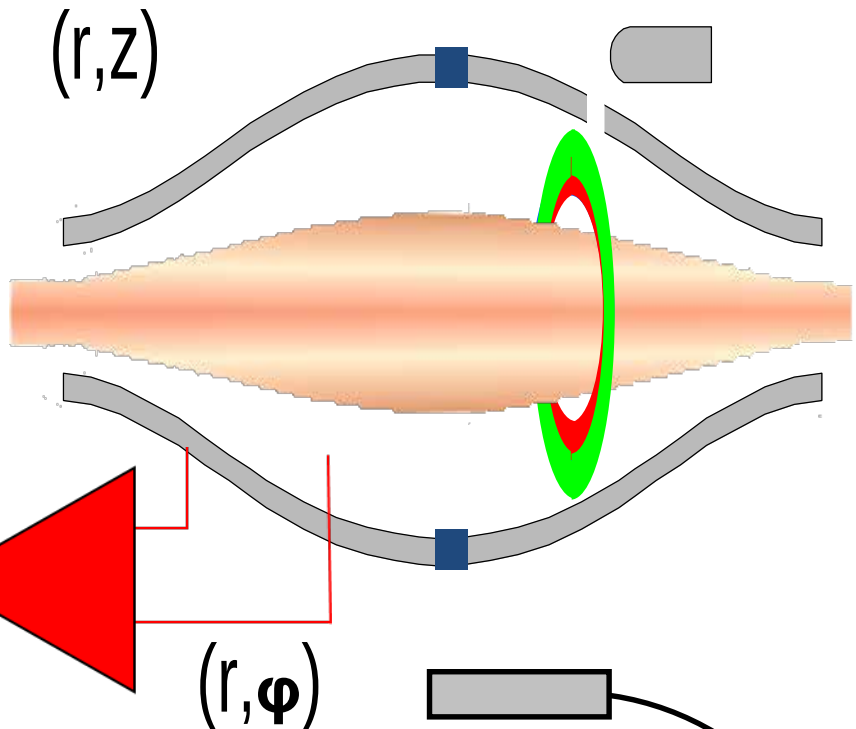
Figure 1. Equipotentials of the quadro-logarithmic field and an example of a stable ion trajectory

Orbitrap Mass Analyzer: Principle of Operation



Hyper-logarithmic potential distribution:
"ideal Kingdon trap"

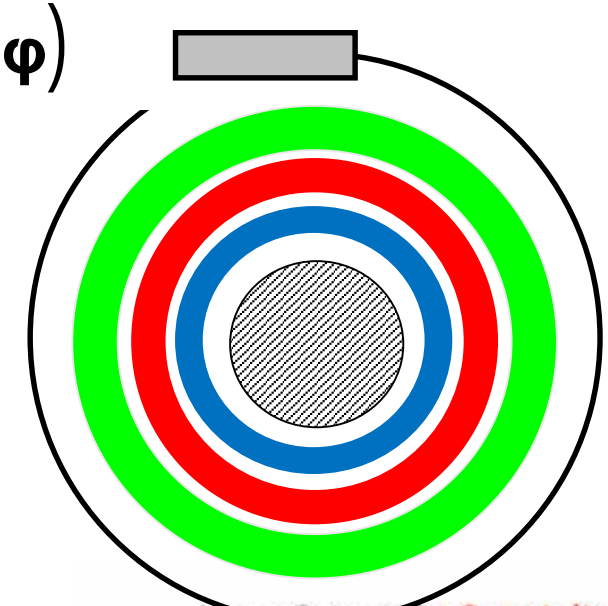
$$U(r,z) = \frac{k}{2}\left\{z^2 - r^2/2 + R_m^2 \ln(r/R_m)\right\}$$



- Characteristic frequencies:
  - Frequency of rotation ω_φ
  - Frequency of radial oscillations ω_r
  - Frequency of axial oscillations ω_z

$$\omega_j = \frac{\omega_z}{\sqrt{2}}\sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \qquad \omega_r = \omega_z\sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

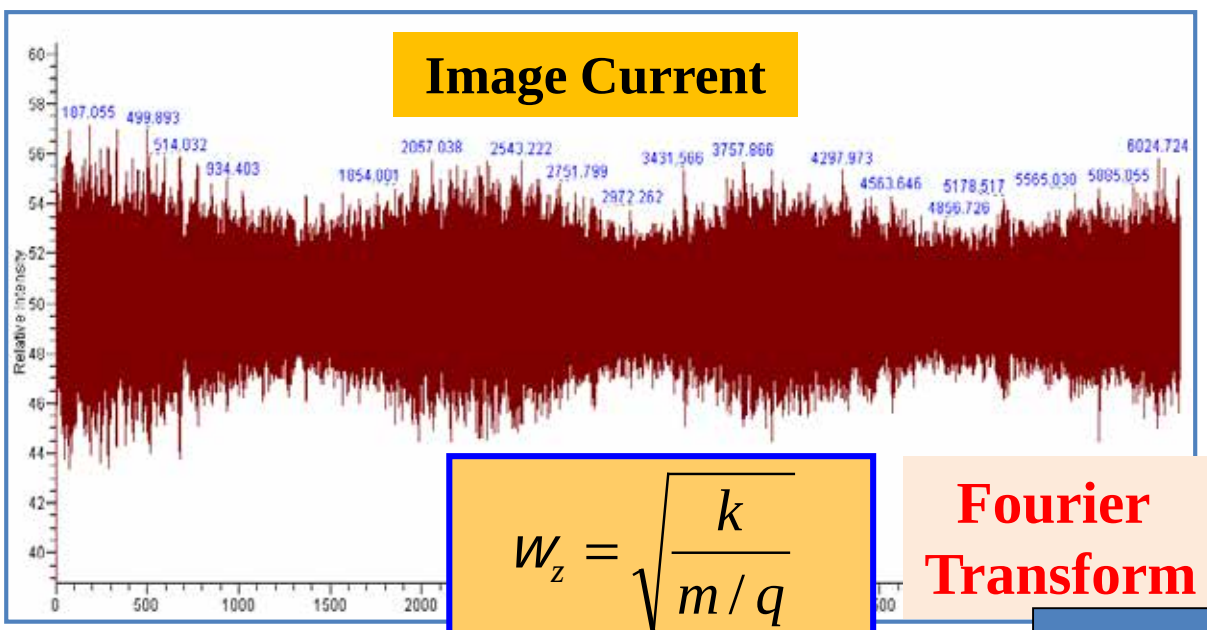
$$\omega_z = \sqrt{\frac{k}{m/q}}$$



Your Scientific Specialist

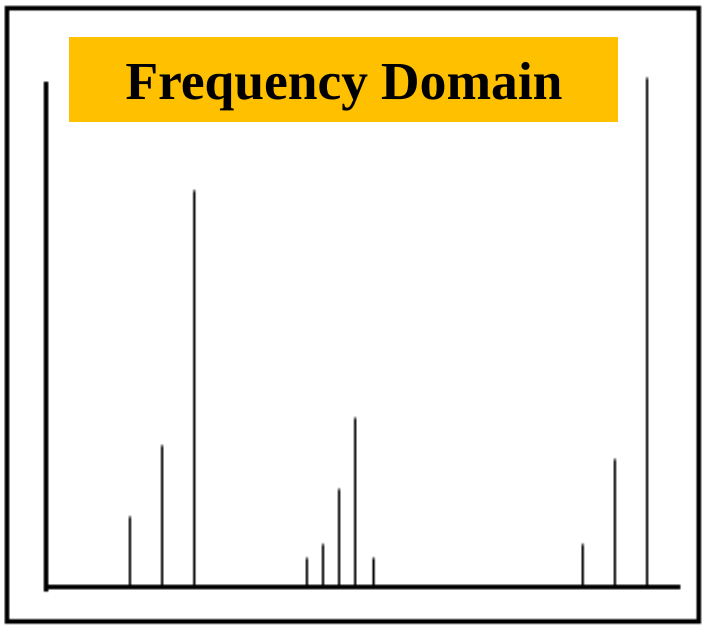
Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

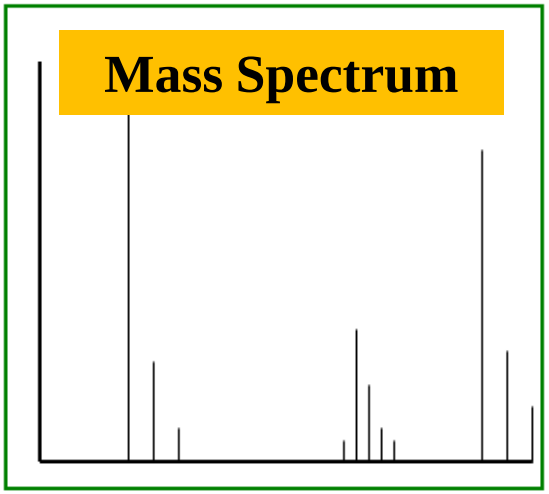
Many Ions Generate a Complex "Transient"



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

Fourier Transform



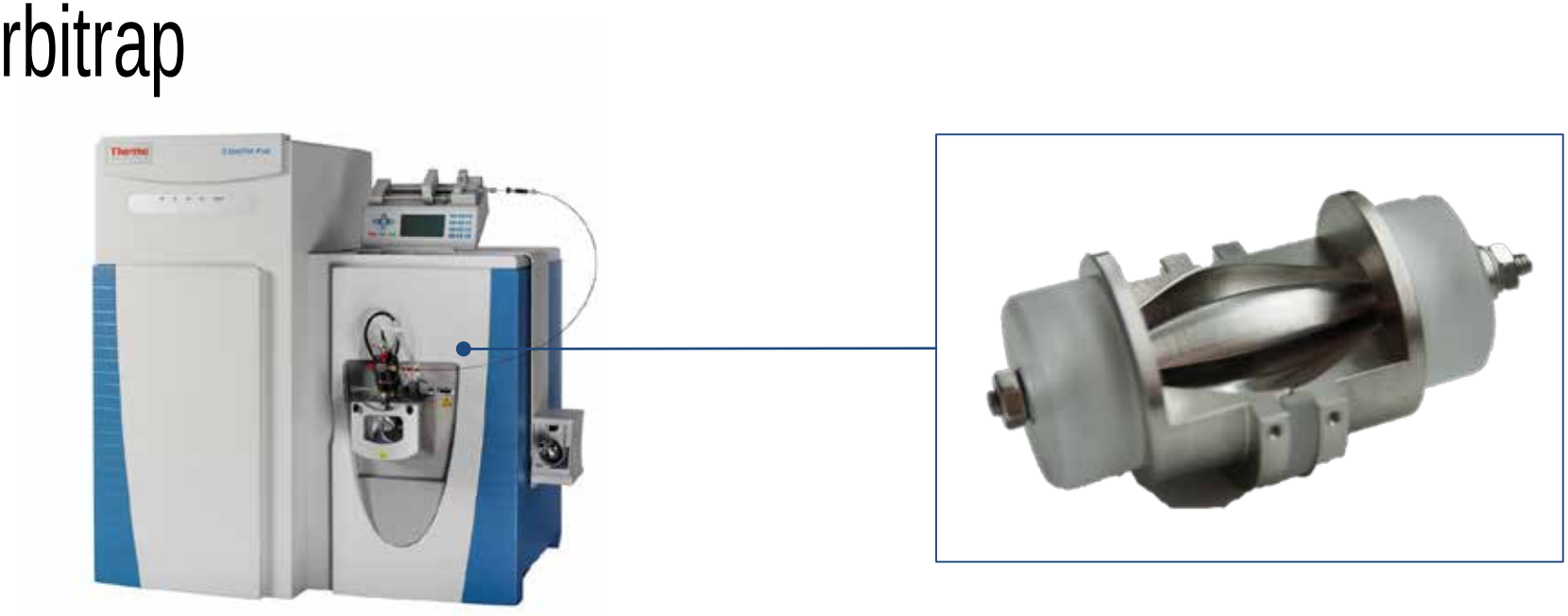


Orbitrap MS

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

Mass Analyzer

- Orbitrap

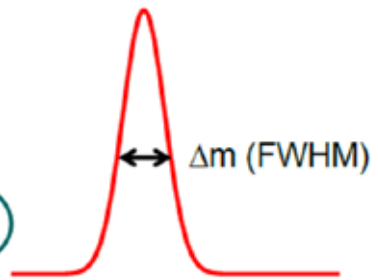


High Resolution Accurate Mass (HRAM) Spectrometer

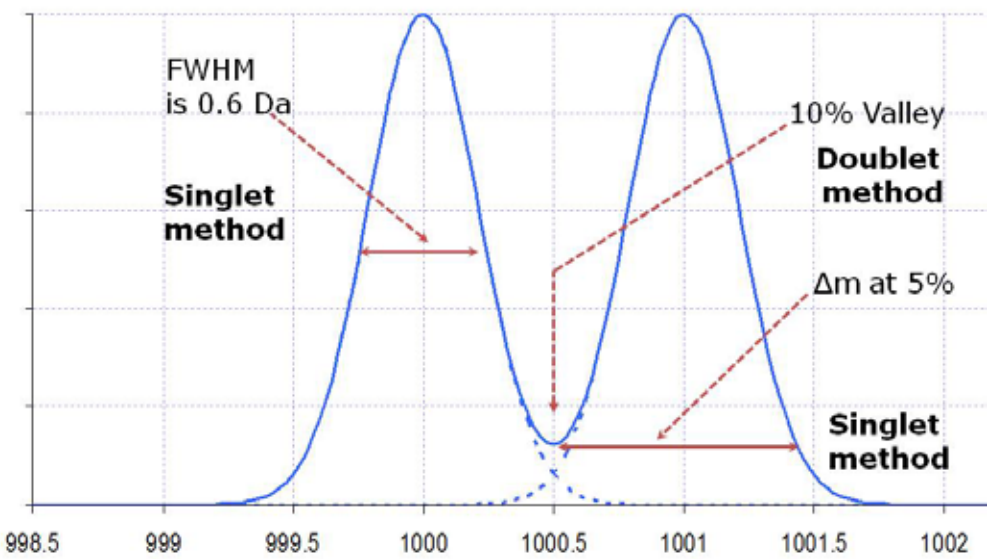
Mass Resolution

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

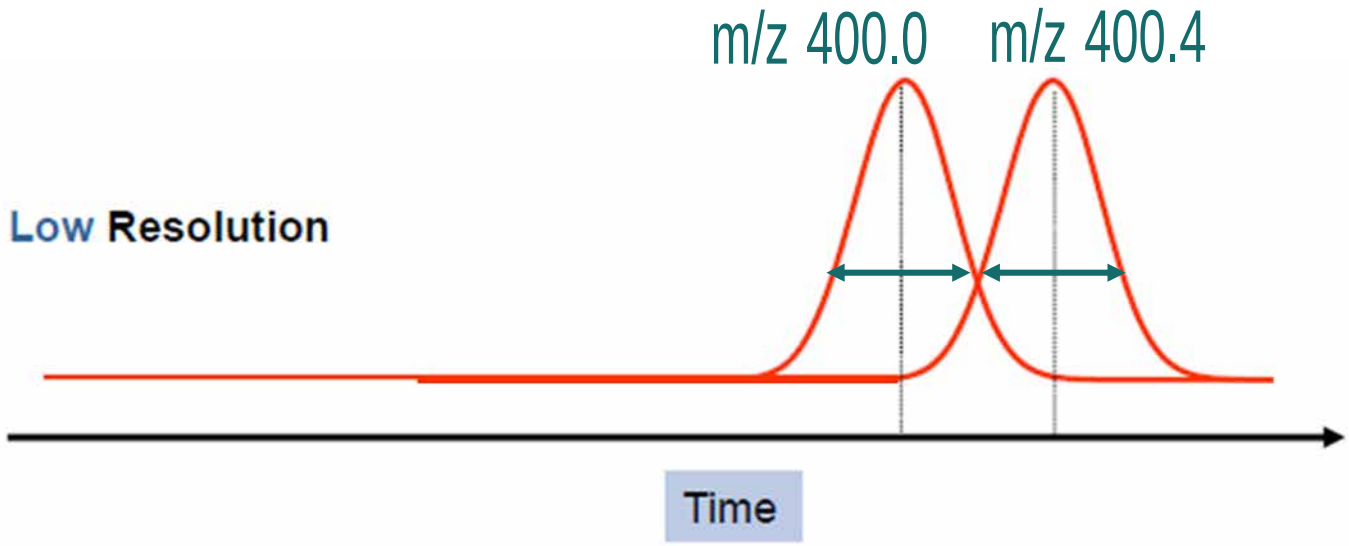
$$R = \frac{m}{\Delta m}$$



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

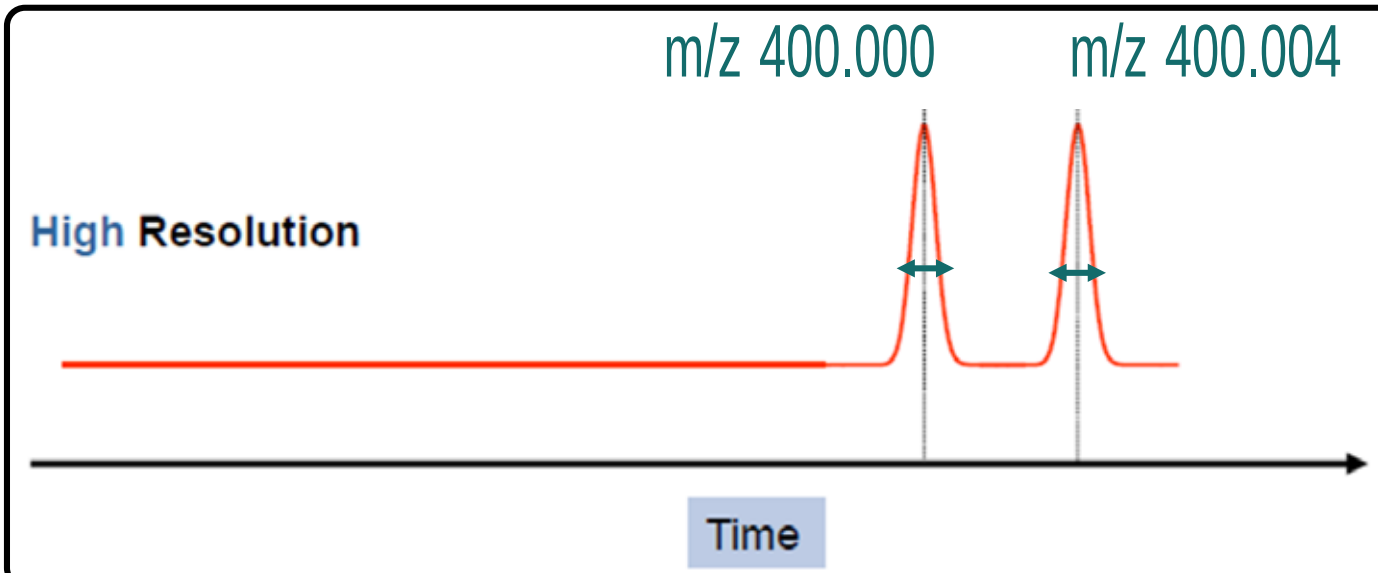


Mass Resolution



- Quadrupole MS

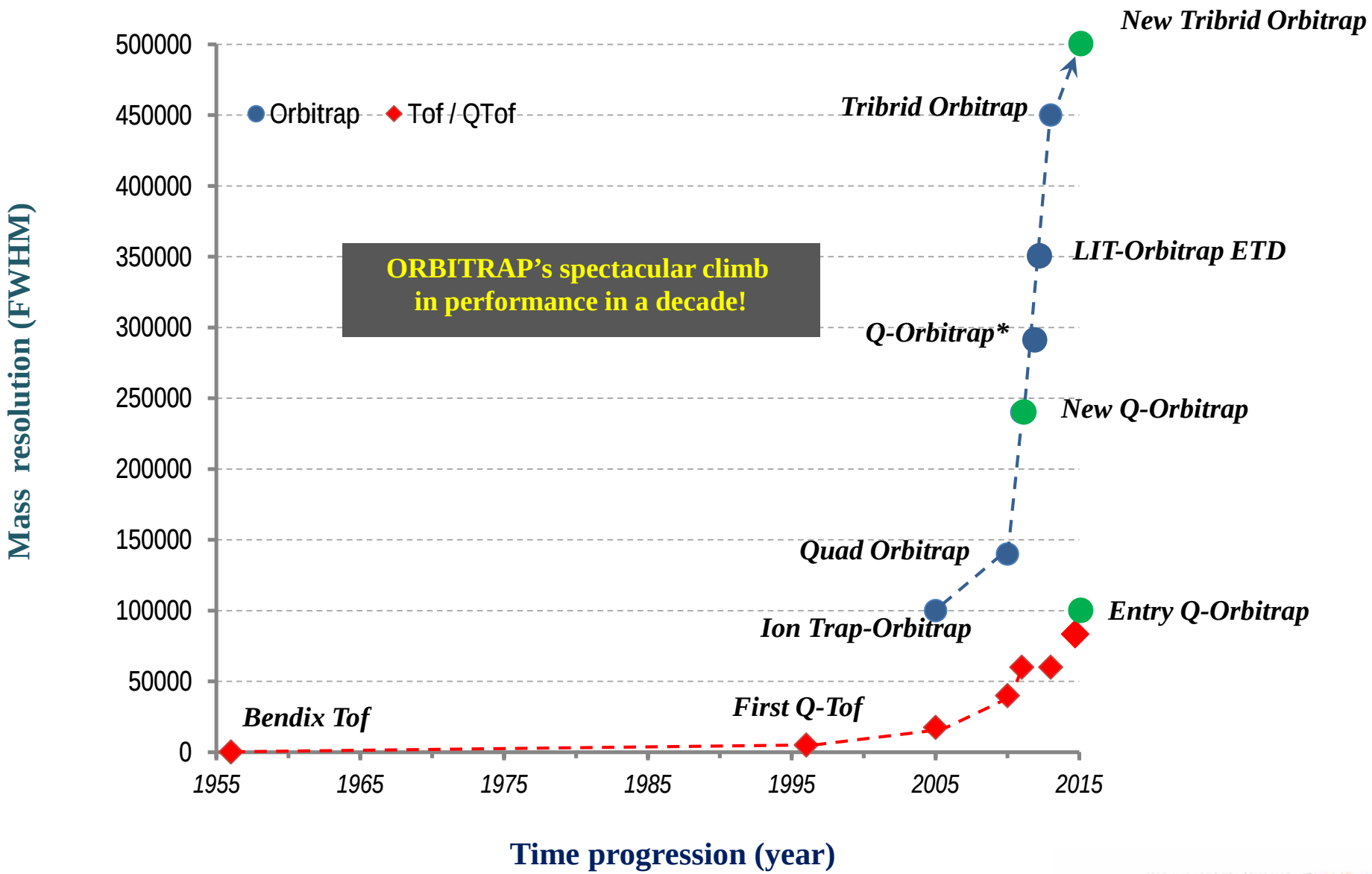
$$R = \frac{400}{0.4} = 1000$$



- **Orbitrap (HRAM) MS**

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

Commercial High Resolution MS Technology Race



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
S = 31.9721

H = 1.0078
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

$$= \frac{500.1 - 500.0}{500} \cdot 10^6 = 200 ppm$$

- Orbitrap MS

$$= \frac{500.10314 - 500.10214}{500.10314} \cdot 10^6 = 2 ppm$$

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

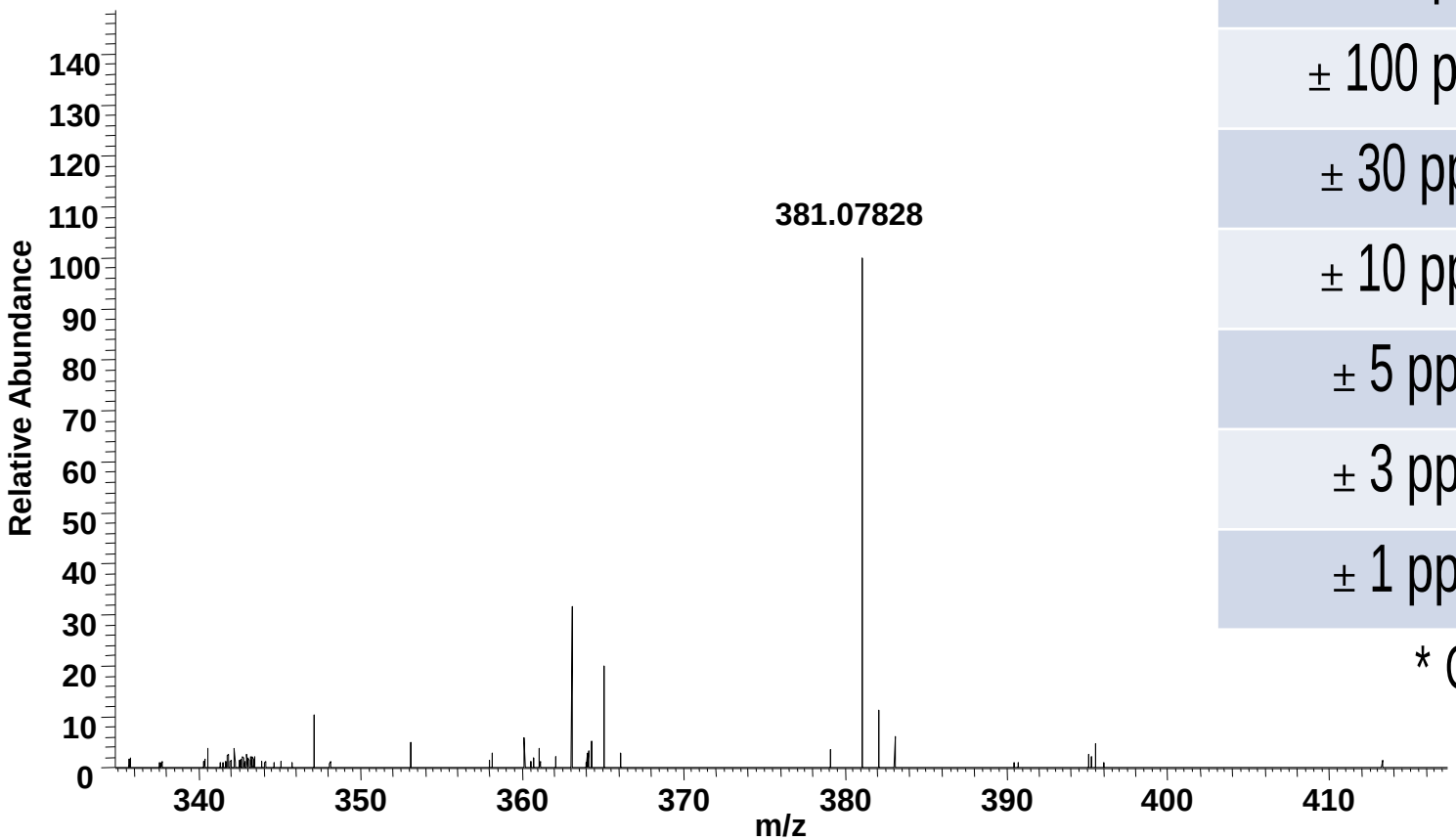
Mass Accuracy

- 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

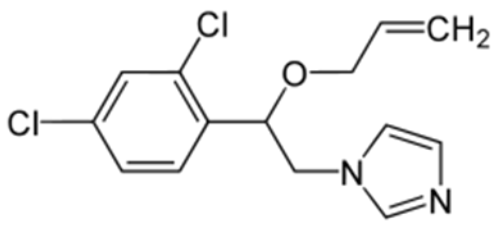
O = 15.9949
S = 31.9721

H = 1.0078
N = 14.0031

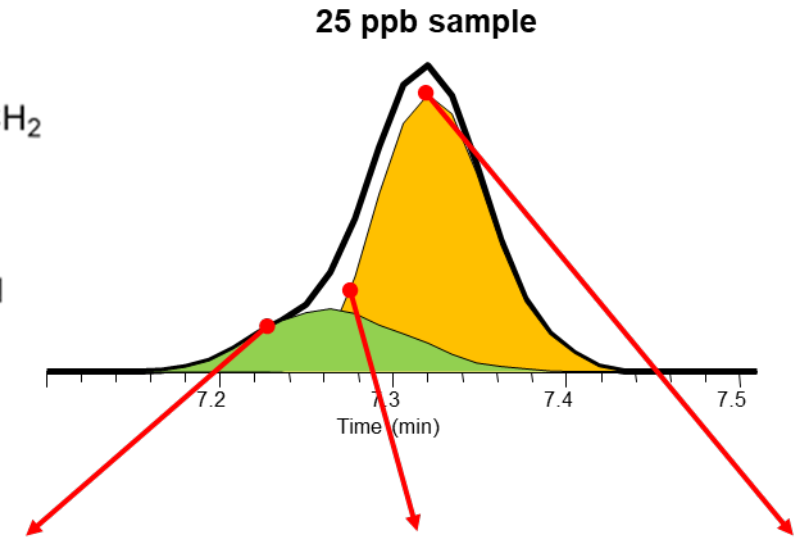
- Main advantage: the possibility to determine the elemental composition of individual molecular or fragment ions, a powerful tool for the structural elucidation or confirmation.

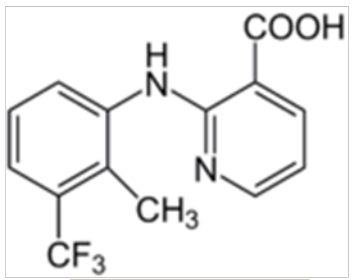
Mass Resolution and Accuracy

- Isobaric compounds separation

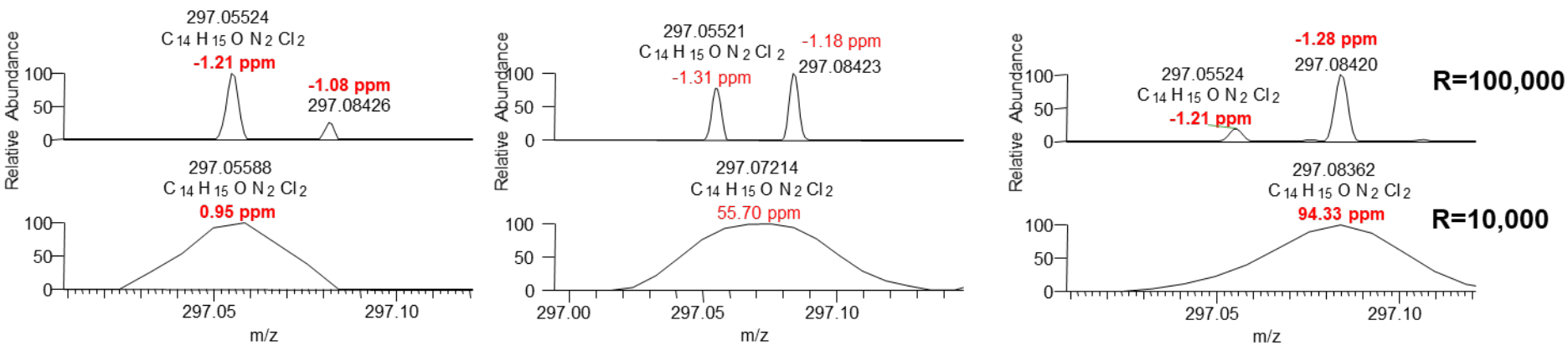


Imazalil
$C_{14}H_{15}Cl_2N_2O$
297.05558





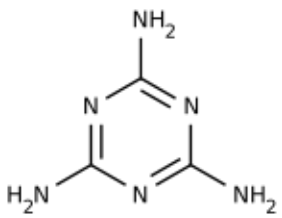
Flunixine
$C_{14}H_{12}F_3N_2O_2$
297.08458



Applications in Food Safety and Halal Food

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

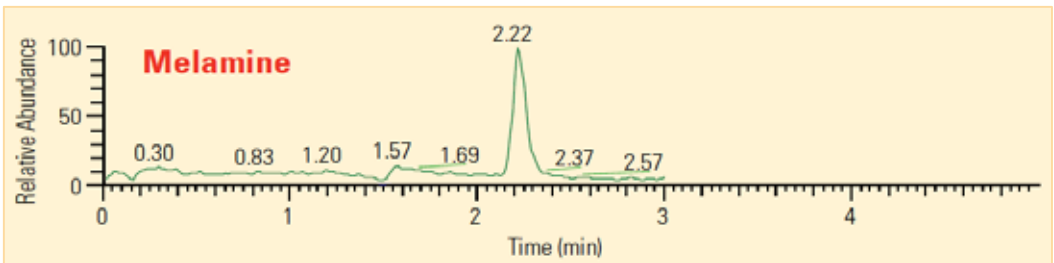
Modes: Targeted SRM

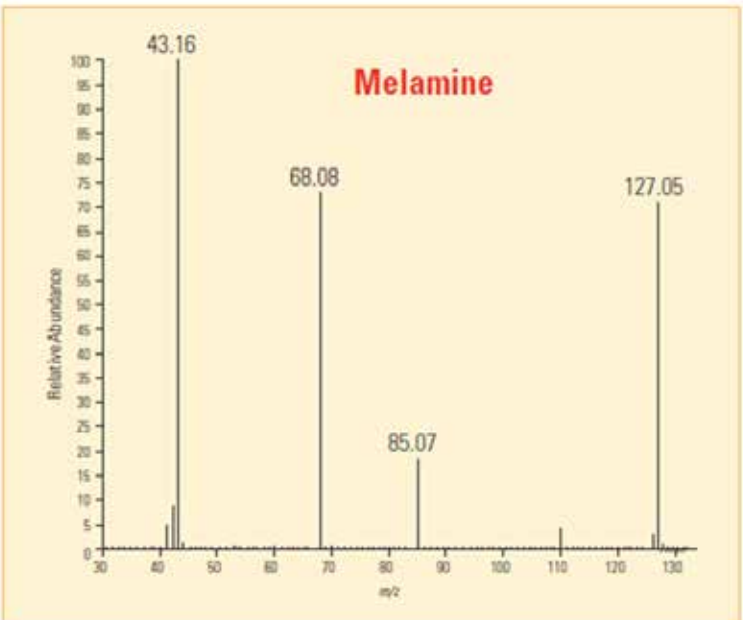
Your Scientific Specialist

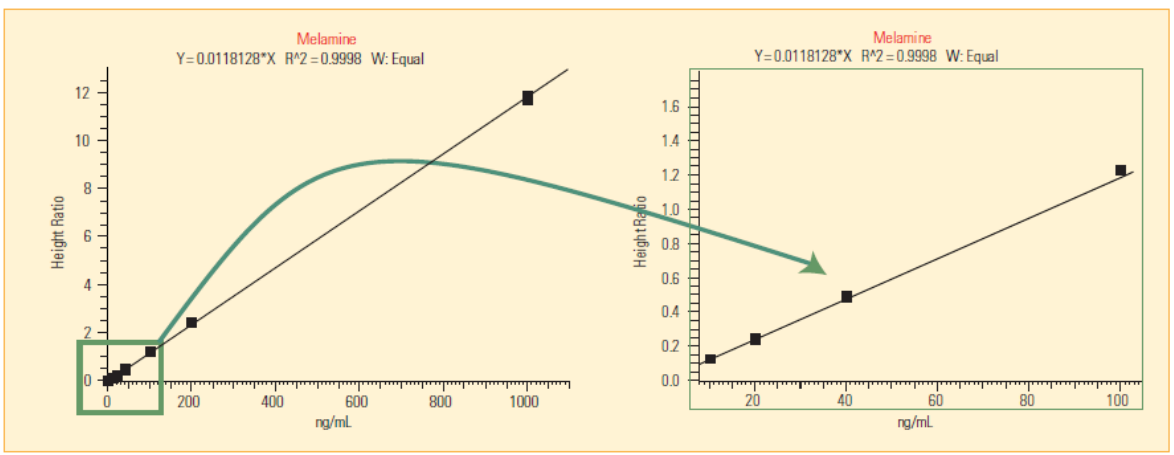
Varelis et al. *Thermo AN62732*. 2008

Application in Food Safety

Identification and Quantitation of Melamine in Milk







- Limit of Detection (LOD): <1 ppb

Your Scientific Specialist

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)

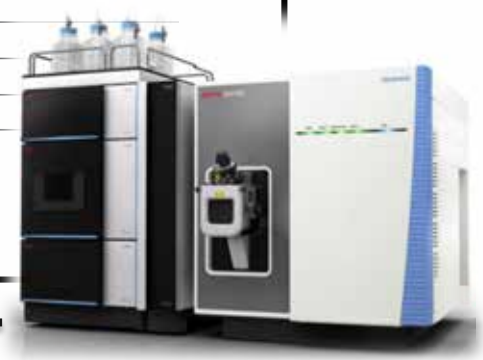
1. Weigh 10 g sample in 50 mL extraction tube.
2. Add 10 mL acetonitrile (20 mL water + 10 mL acetonitrile for wheat flour).
3. Shake for 10 min and centrifuge at 5000 rpm for 5 min.
4. Transfer supernatant into LC vial and place it in the autosampler.

Homogenization → Sample Weighing → Extraction (QuEChERS) → LC-MS/MS

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

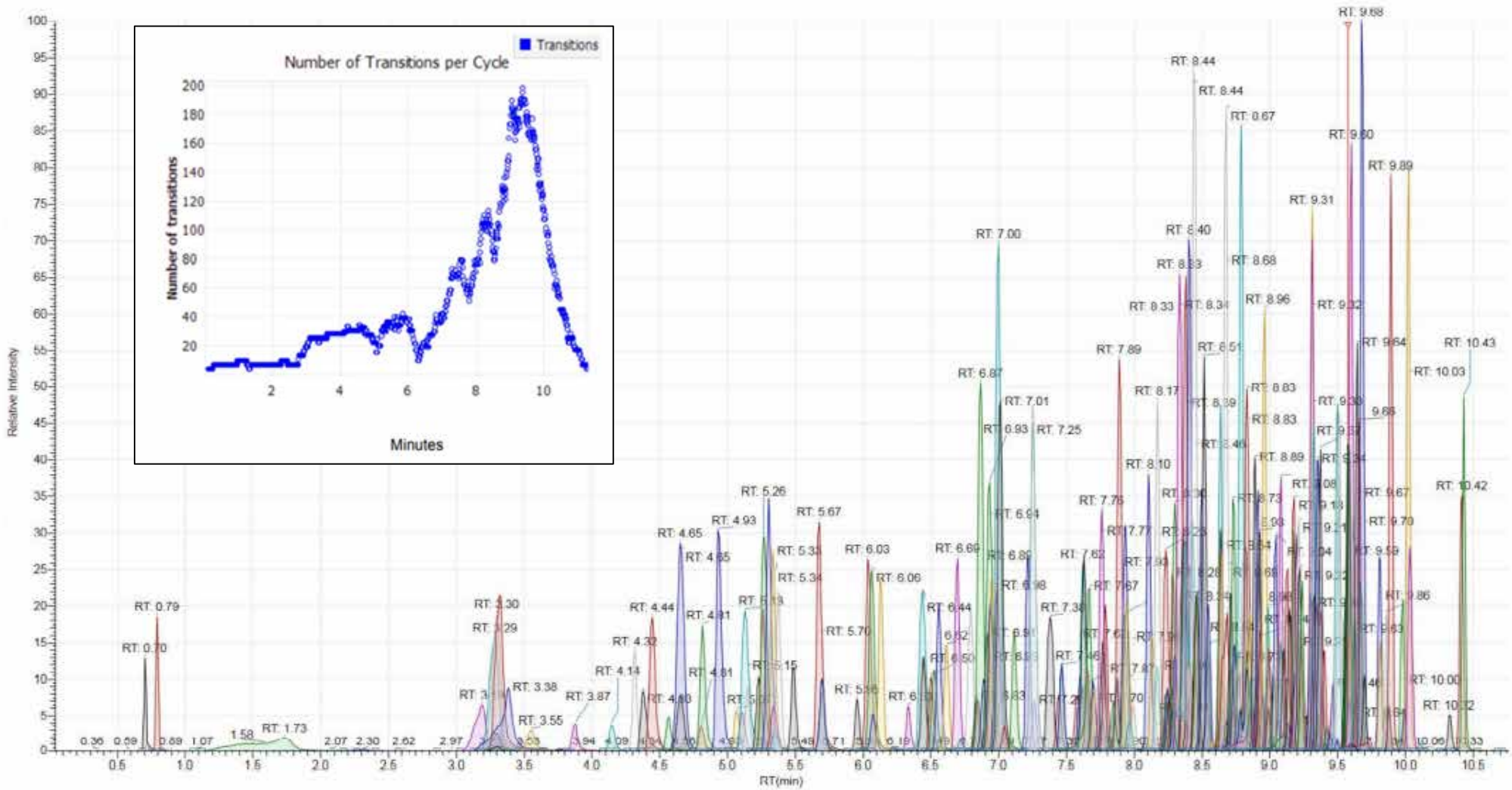


Bousava et al. *Thermo AN64971*. 2017

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

Protein Extraction

Tryptic Digestion

Analysis

Meat Sample | Protein Extracts | Digested Peptides | 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

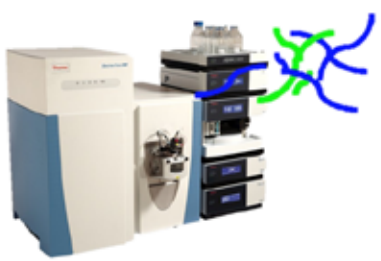
Orduna et al. *Thermo AN64677*. 2016

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

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 mM NH_4FA + 0.1% FA; B) MeOH + 10 mM NH_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

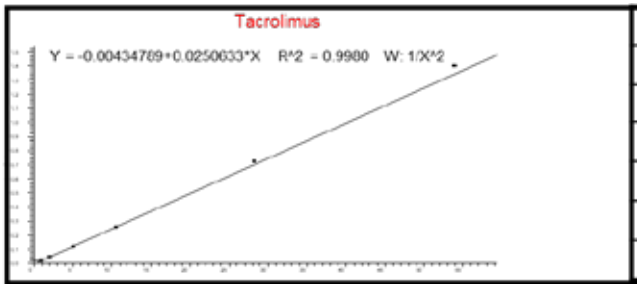
QC sample	Tacrolimus (ng/mL)	Sirolimus (mg/mL)	Everolimus (ng/mL)	Cyclosporin A (ng/mL)
QC1	2.97	3.06	2.93	31.0
QC2	13.66	12.74	13.58	134.0
QC3	33.06	30.66	32.40	386.8

Gao et al. *Thermo AN64504*. 2016

Application in Pharmaceutical

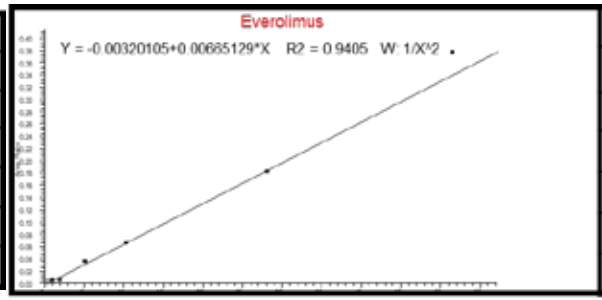
Quantitative Analysis of Immunosuppressant Drugs

Tacrolimus



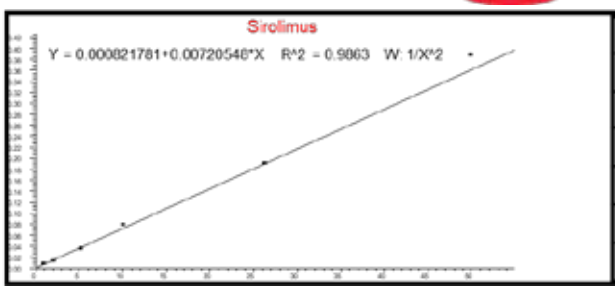
Standard	% Difference
0.97 ng/mL	1.04
2.07 ng/mL	0.34
5.11 ng/mL	-6.41
10.57 ng/mL	-1.62
28.22 ng/mL	2.77
53.92 ng/mL	3.88

Everolimus



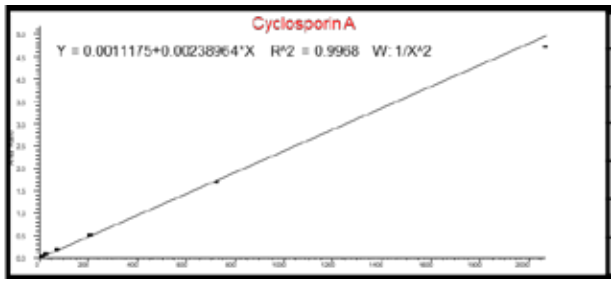
Standard	% Difference
1.02	17.85
1.95	-39.05
5.13	12.53
10.36	-0.81
28.17	-1.06
51.57	10.62

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	8.61
73.0	1.65
208.8	0.93
725.1	-3.24
2067.2	-4.69

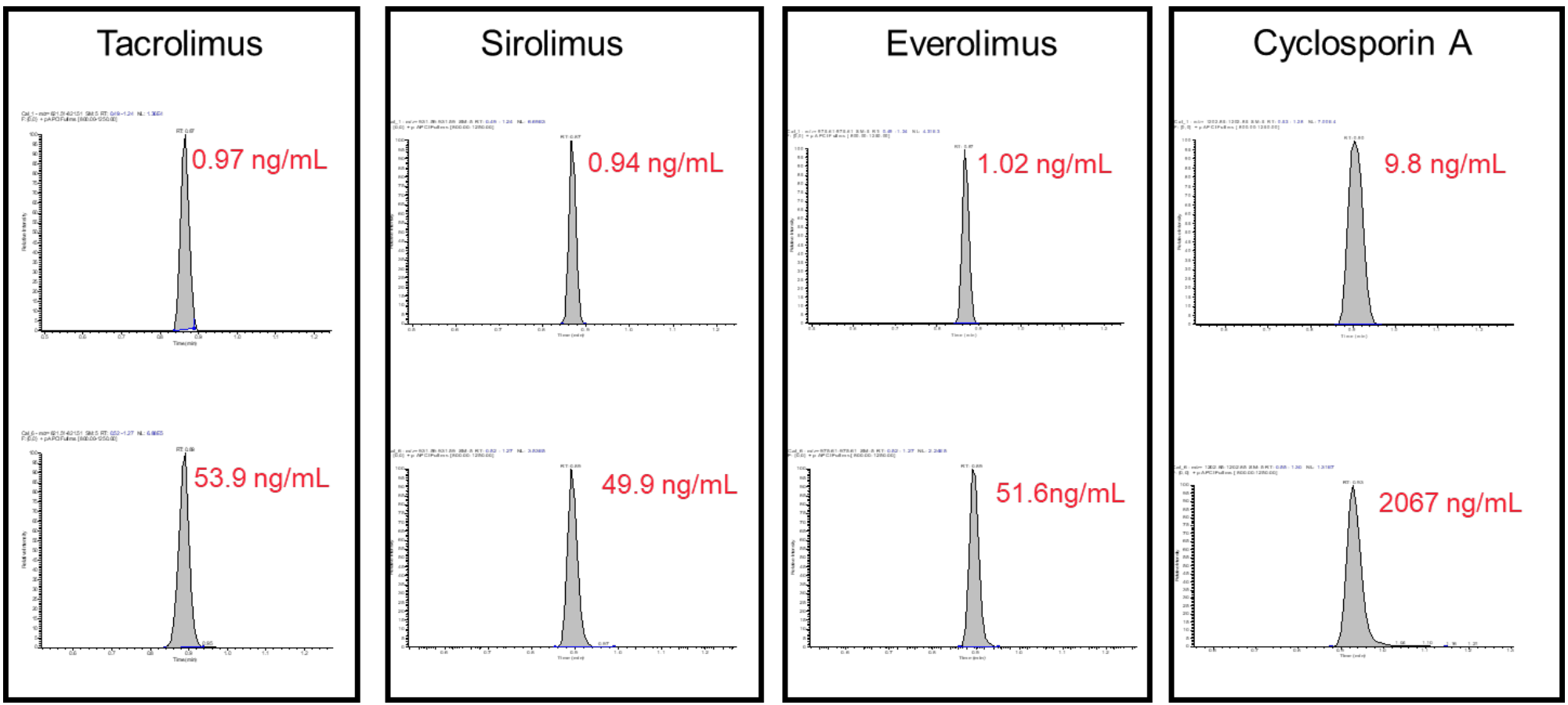
Excellent Linearity and Accuracy

Your Scientific Specialist

Gao et al. *Thermo AN64504*. 2016

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**

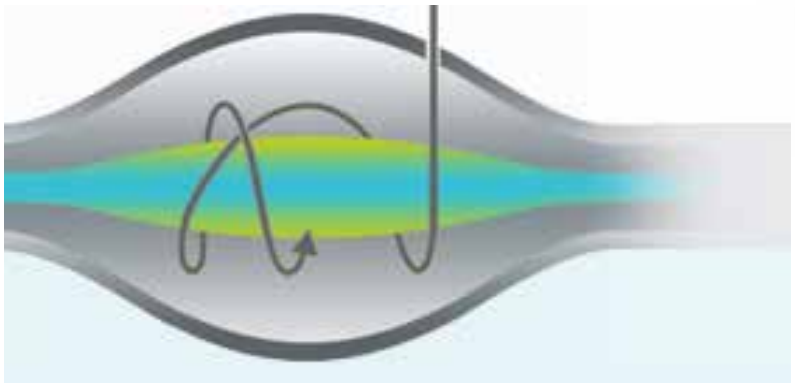
Cyclosporin A

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

UHPLC, MS Technology and Applications



http://planetorbitrap.com/

ThermoFisher SCIENTIFIC

Proteomics Workshop @ CU

Proteomics Workshop

Complete Proteomics Workflow

Chulalongkorn University
Center of Excellence in Systems Biology

21st – 25th May, 2018

Venue

3rd Floor Aor Por Ror Building
Faculty of Medicine
Chulalongkorn University

Real Hands-on Laboratory Experience !!!

Contents

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

Registration

On-line registration at: www.scispec.co.th

Registration Fee*	Early Bird (before 31st Mar)	Regular (1st Apr - 18th May)
2-day (Lecture Only)	2,500 ฿	3,000 ฿
5-day (Lecture & Lab)	7,000 ฿	8,000 ฿

*50% discount to students

For more information please contact:
Rittichai Charoensapyanan (095-539-1652)
E-mail: rittichai@scispec.co.th

Proteomics Workshop

Complete Proteomics Workflow

Chulalongkorn University
Center of Excellence in Systems Biology

21-25 May 2018

@CU

www.scispec.co.th

21st – 25th May, 2018
Registration: www.scispec.co.th

Thank You for Your Attention

Questions?

Follow Us

@scispec or

SCISPEC

www.scispec.co.th