UHPLC-MS Basic Principles and Applications

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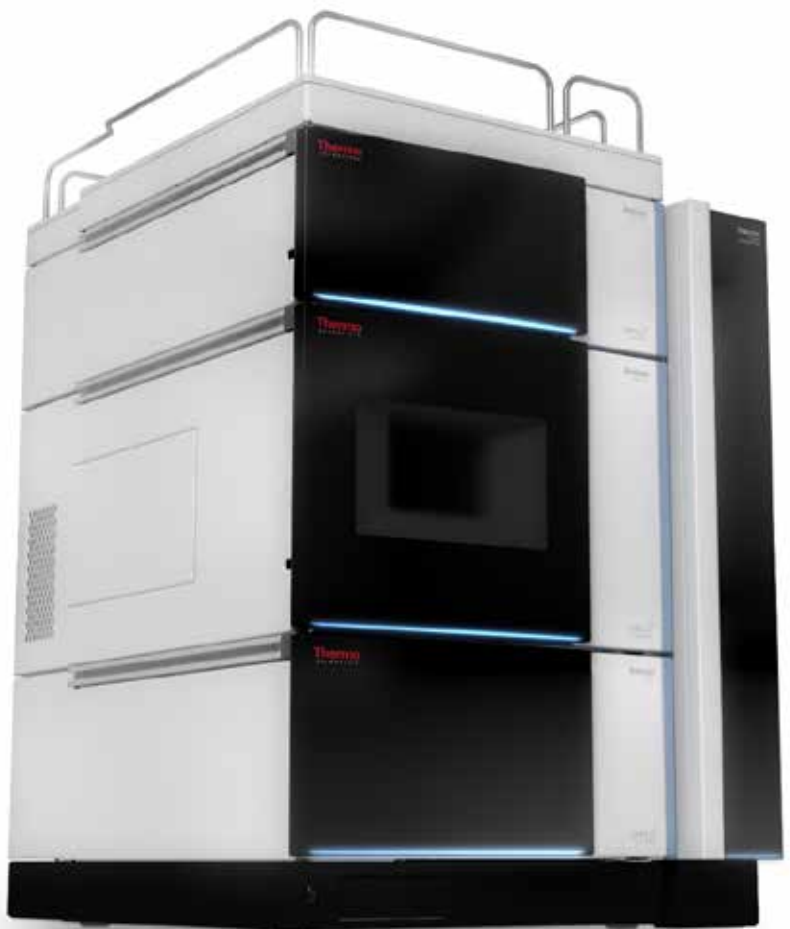
Product Specialist LC/MS

February, 2018

Fundamental of Liquid Chromatography



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

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

Thermo Analytical LC Systems

Vanquish™
Max Pressure 1517 bar

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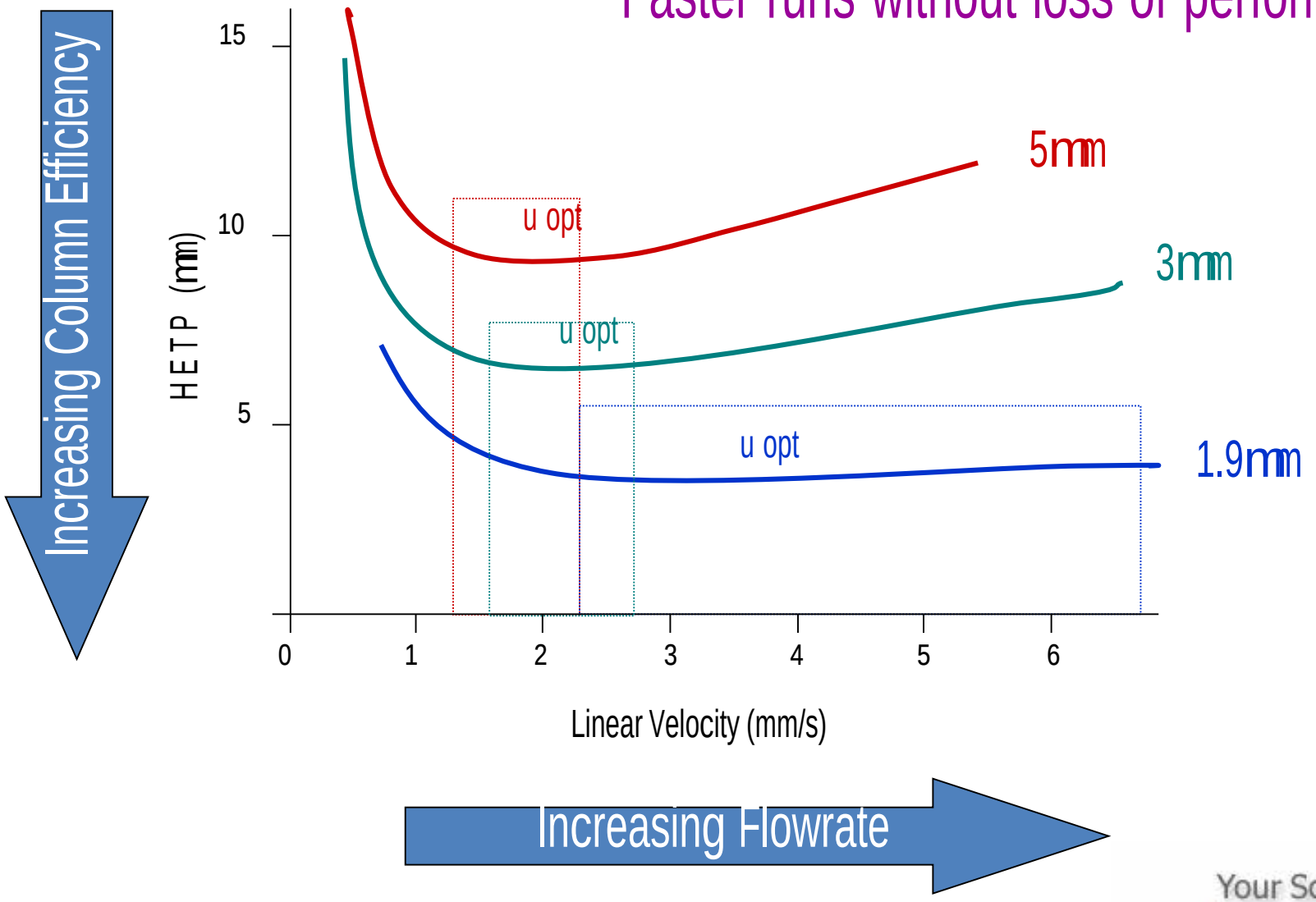
What is the Small Particle Advantage ?

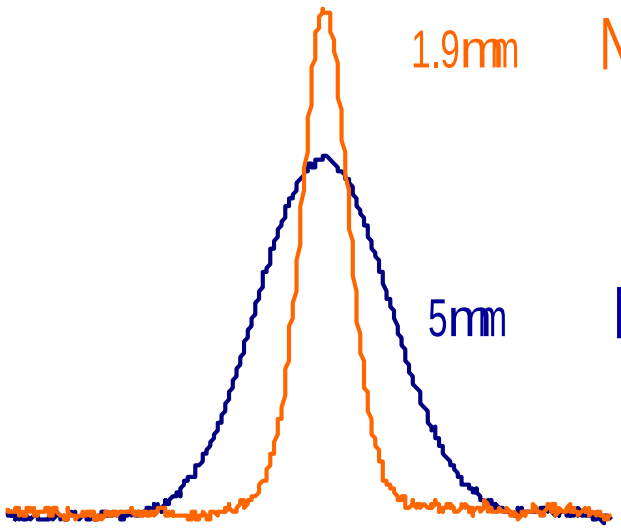
Higher efficiency, independent of flow rate means...

Faster runs without loss of performance



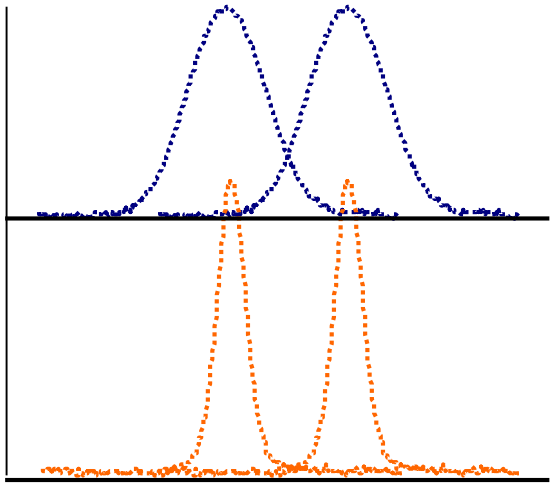
Efficiency is the key!!!

Small Particle Advantage



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

N = 75,000 plates /m



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

Selectivity Efficiency Retention

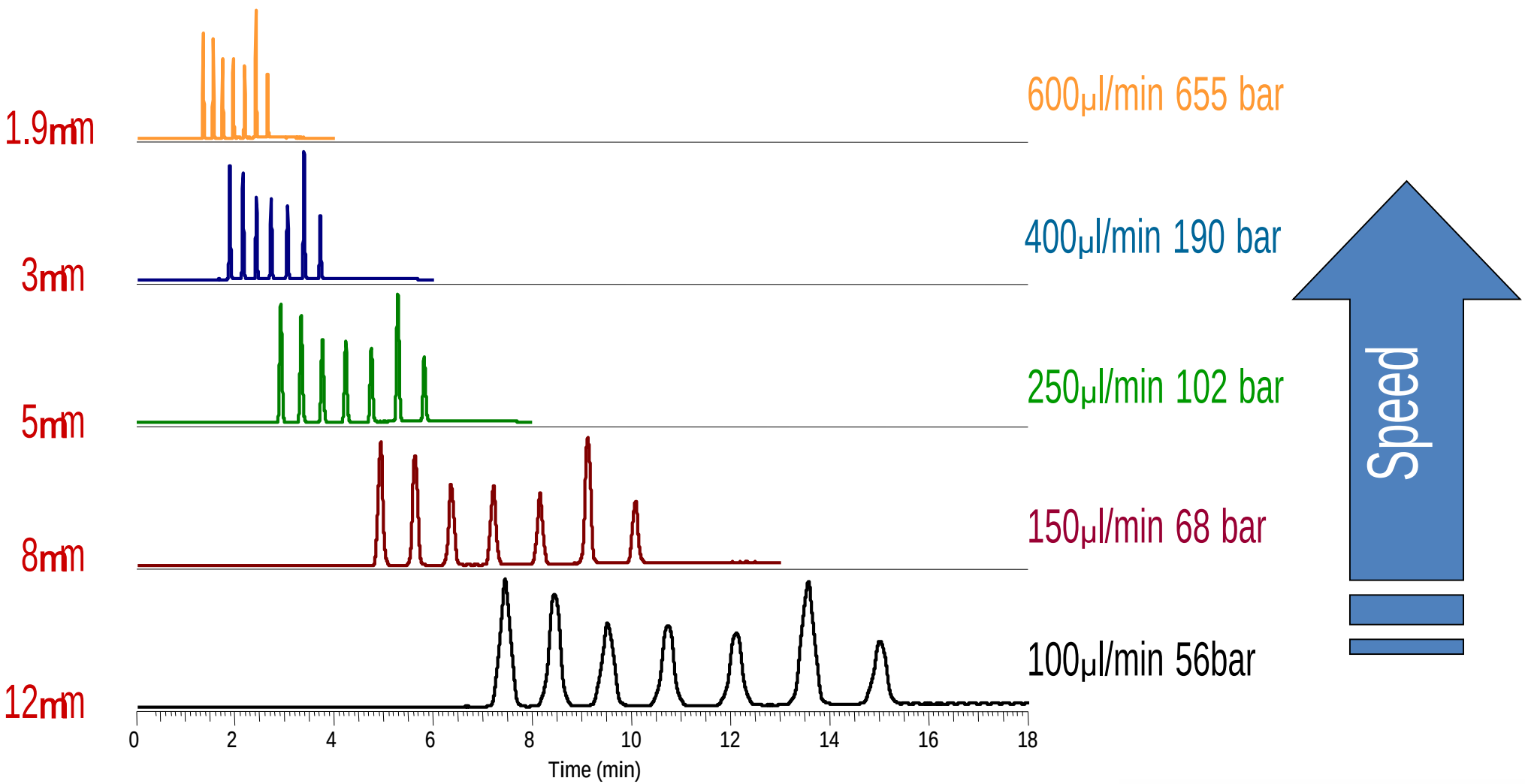
Higher resolution – narrower peaks

Higher sensitivity – taller peaks

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

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

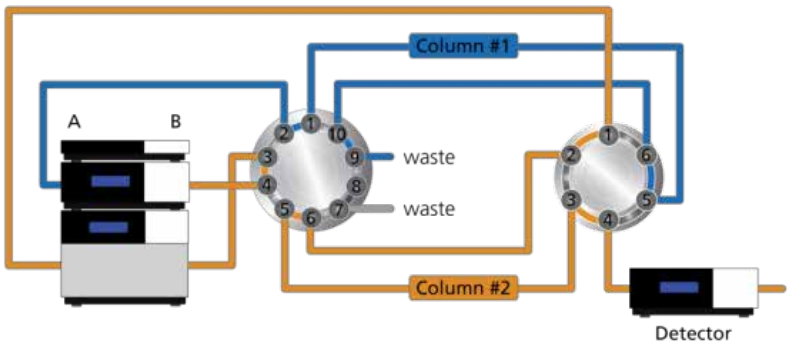
- Standard
- With Valves

Detectors

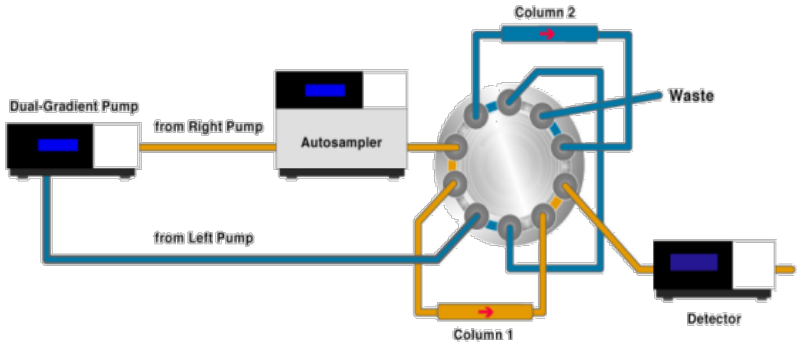
- VWD
- MWD/DAD
- Fluorescence
- Corona
- Coulochem

UHPLC+ Applications

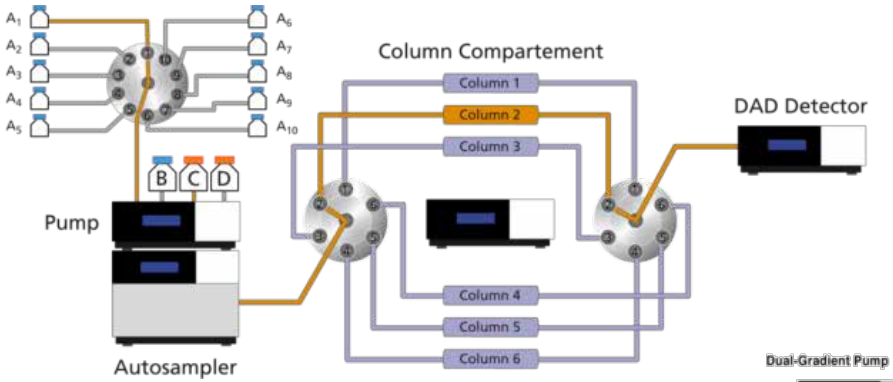
Application Switching



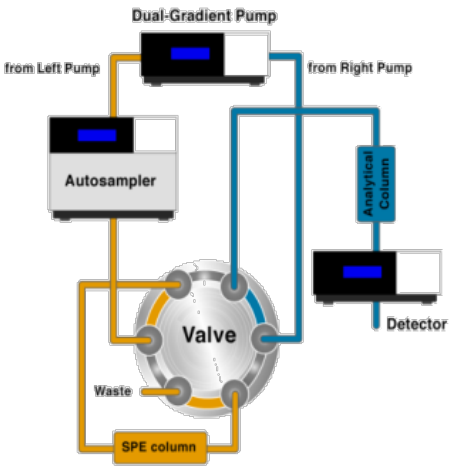
Tandem LC



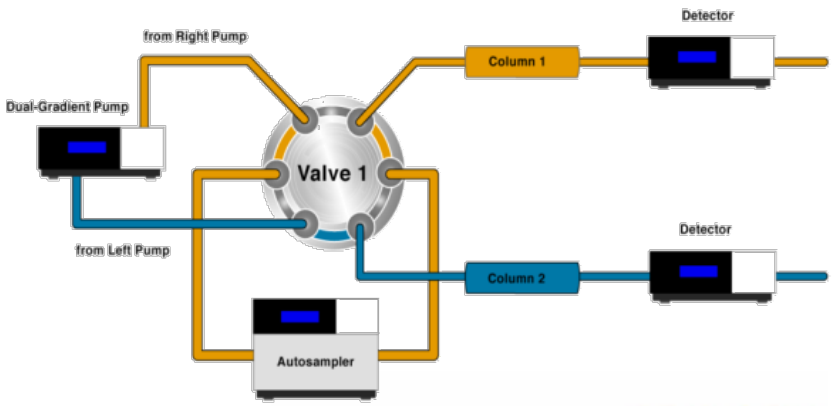
Automated Method Scouting



Online SPE



Parallel LC



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Fundamental of Mass Spectrometry

SciSpec

Your Scientific Specialist

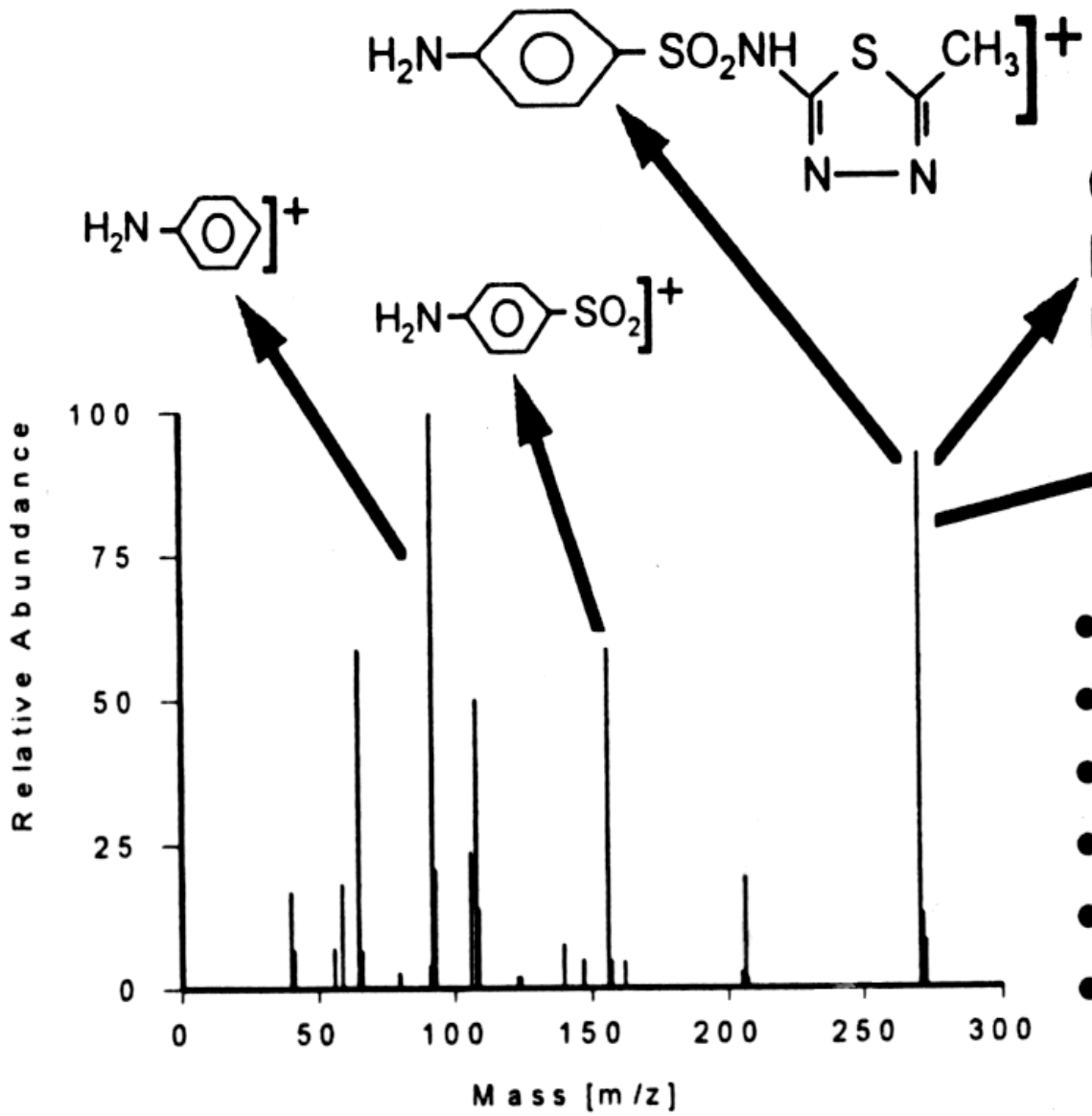
https://www.thermofisher.com/order/catalog/product/TSQ02-10001?SID=srch-srp-TSQ02-10001

What is Mass Spectrometry?

"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, W. M. A.; Van der Greef, J., *Liquid Chromatography–Mass Spectrometry: Principles and Applications*, 1992, Marcel Dekker, Inc., New York, p. 29.

Information Rich Data



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

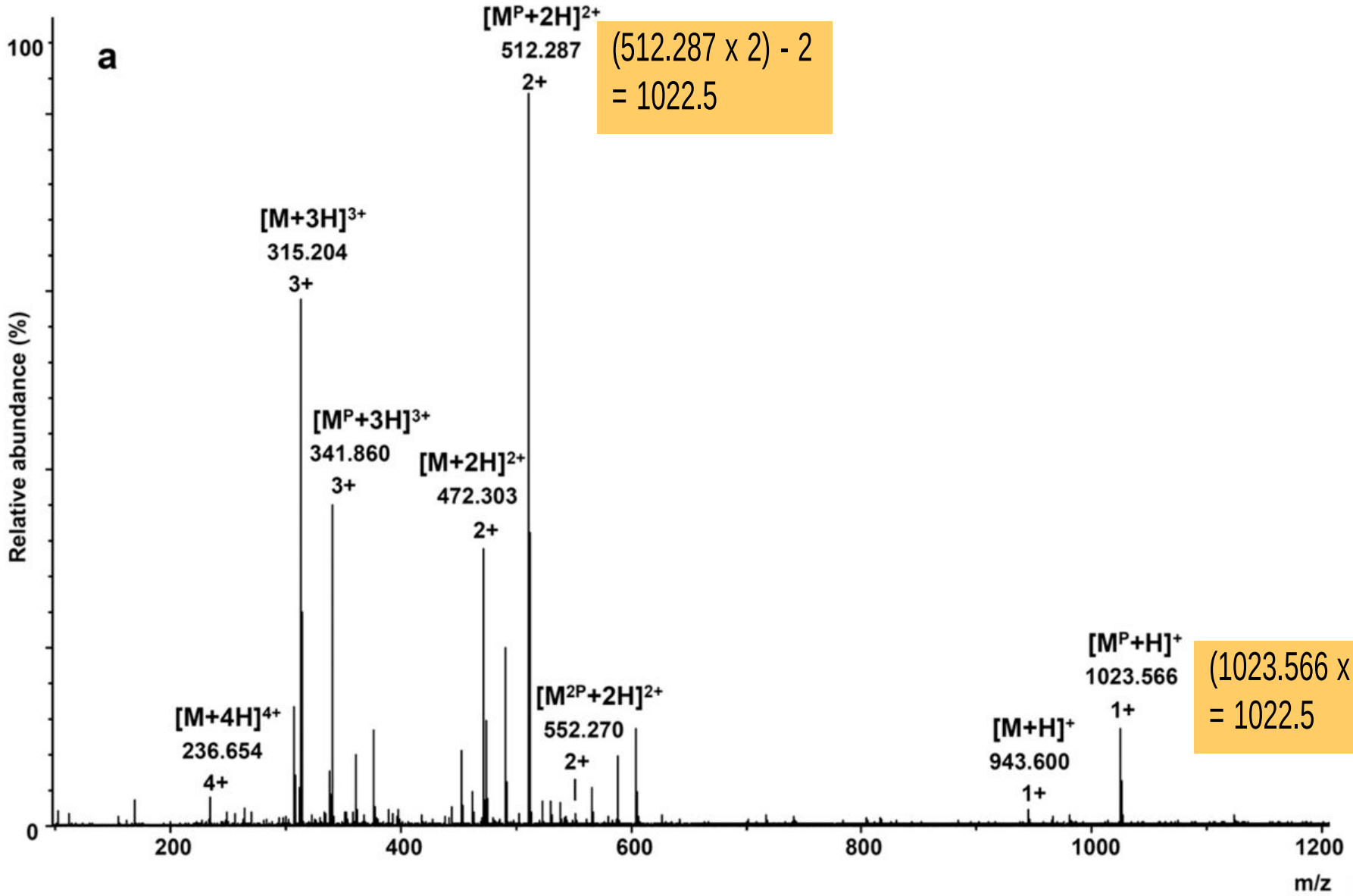
- *Molecular Weight*
- *Elemental Composition*
- *Empirical Formula*
- *Molecular Structure*
- *Unambiguous Identification*
- *Mass Specific Response*

Applications of Mass Spectrometry

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

Mass Spectrum

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



(512.287 x 2) - 2
= 1022.5

(1023.566 x 1) - 1
= 1022.5

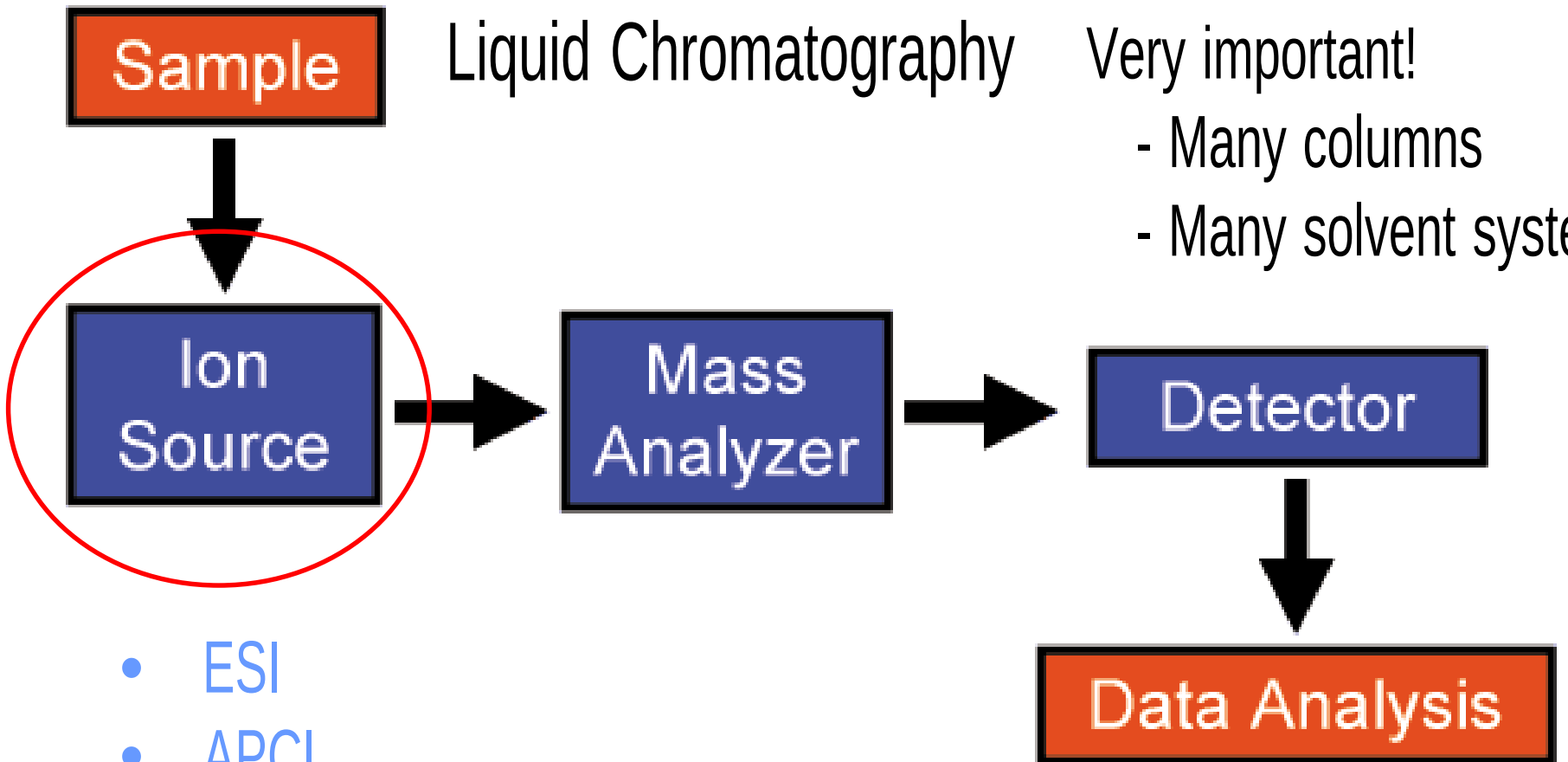
Mass spectrometry Characteristics

- 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 Spectrometry – Block Diagram



ION SOURCE

IONIZATION TECHNIQUES

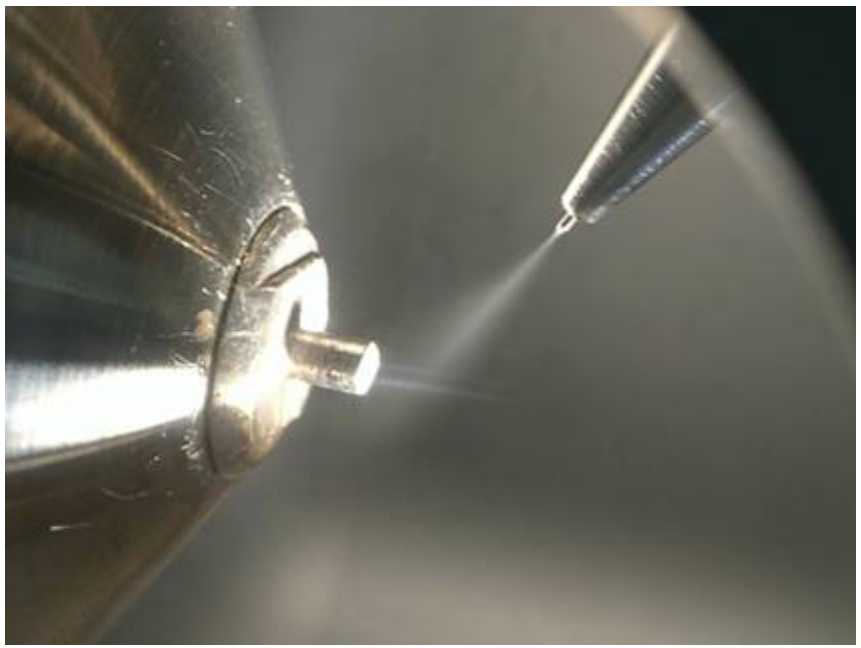
Type of Ionization techniques

- Electron impact (EI)
- Chemical Ionization (CI)
- Atmospheric Pressure Ionization (API)
  - Electrospray Ionization (ESI)
  - Atmospheric Pressure Chemical Ionization (APCI)
  - Atmospheric Pressure Photo-Ionization (APPI)
- Matrix Assisted Laser Desorption/Ionization (MALDI)

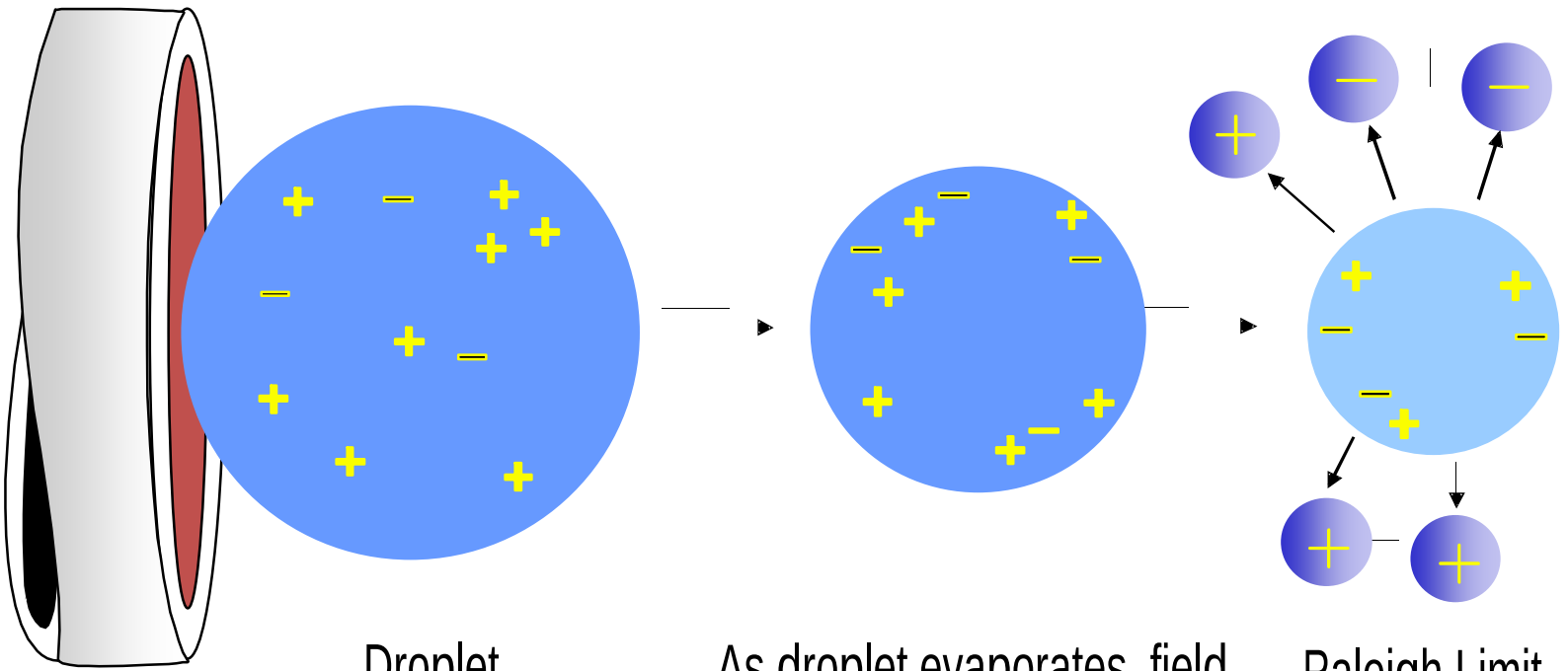
Electrospray Ionization (ESI)

Three Fundamental Processes:

1. Production of charged droplets.
2. Droplet size reduction, and fission.
3. Gas phase ion formation.



Ion Evaporation Theory



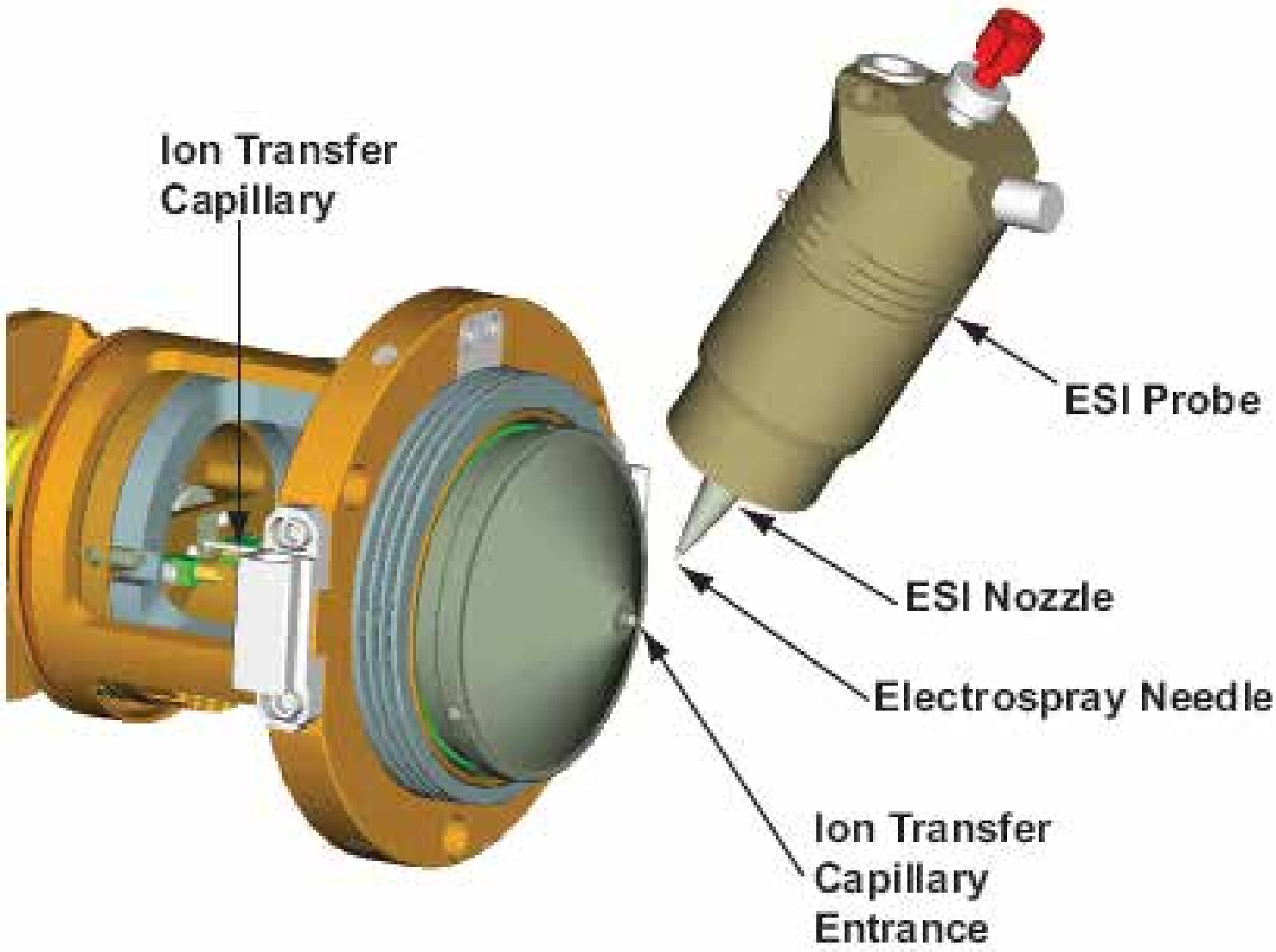
Capillary
+4 kV

Droplet containing ions

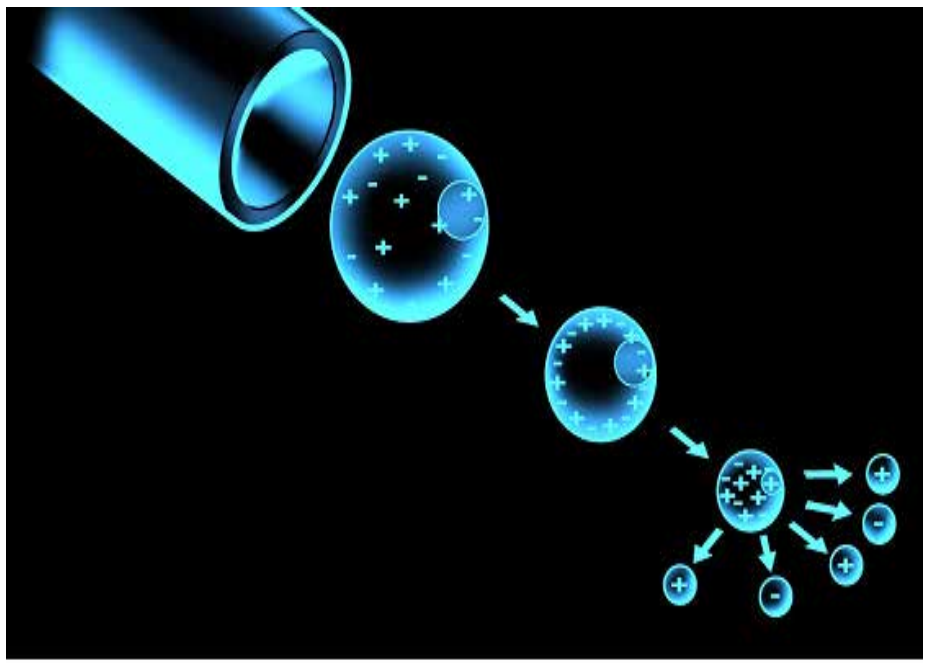
As droplet evaporates, field increases and ions move to surface

Raleigh Limit – Droplet Instability - releases smaller droplets ions

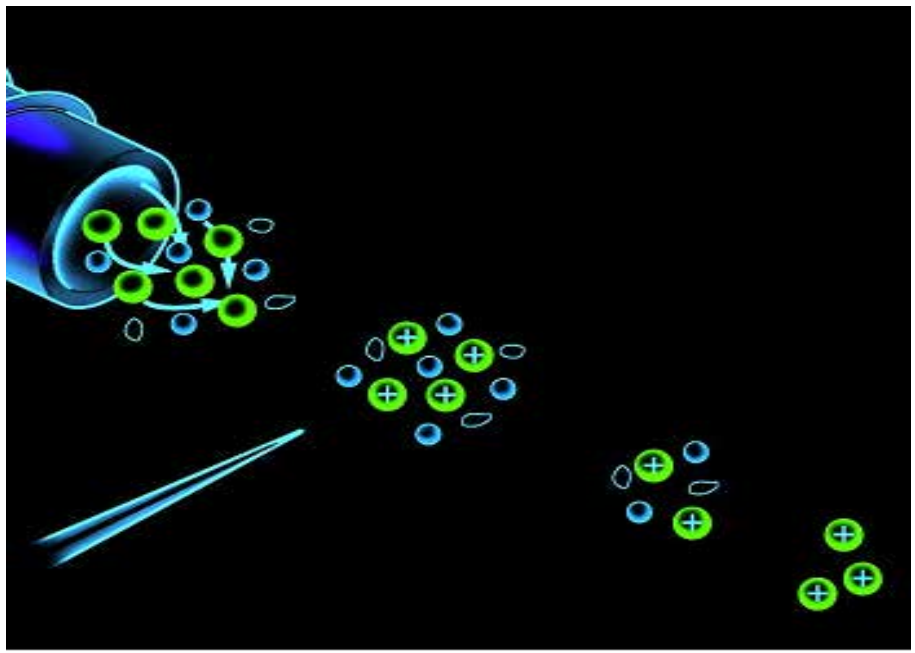
ESI - Ion Max Source



Atmospheric Pressure Ionization



Electrospray Ionization



Atmospheric Pressure
Chemical ionization

Chemistry Considerations ESI or APCI

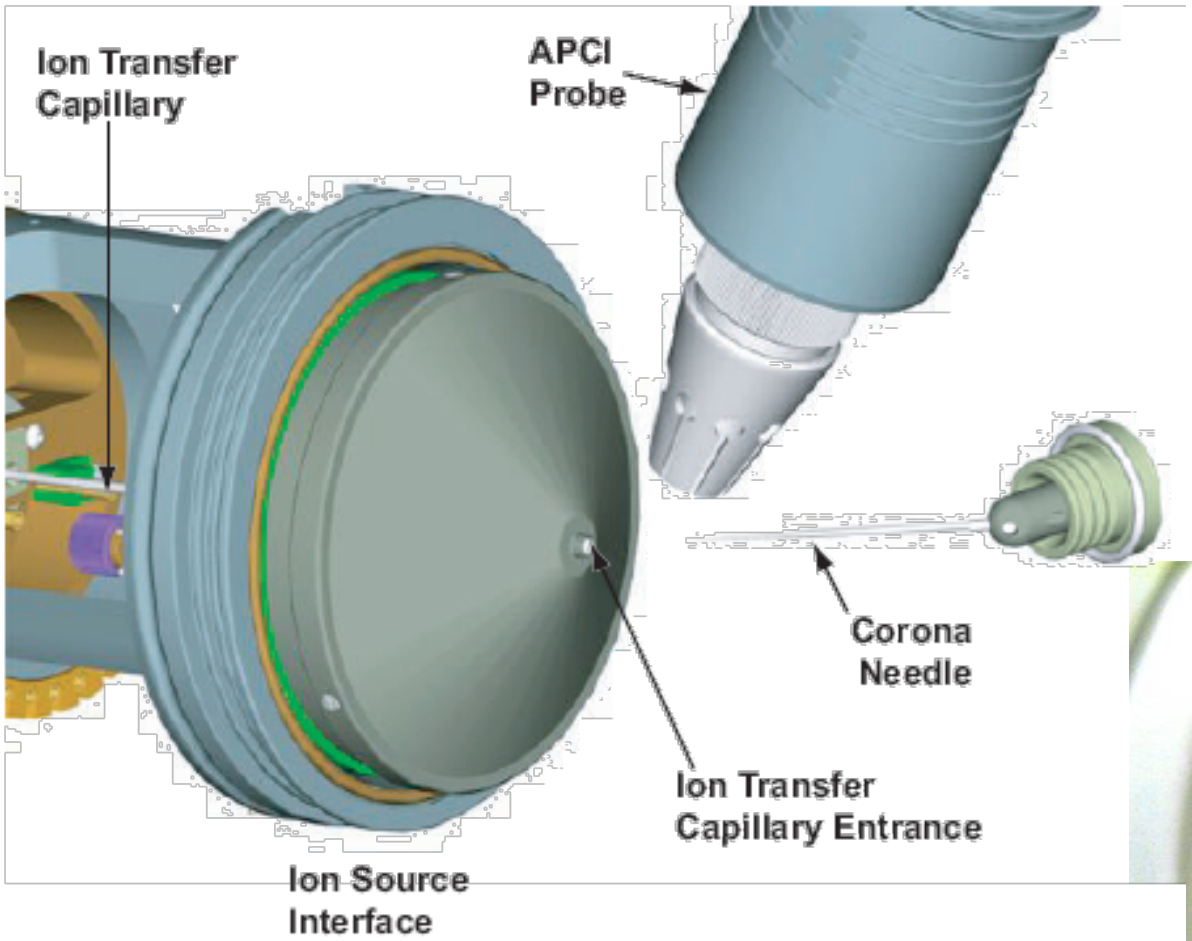
ESI:

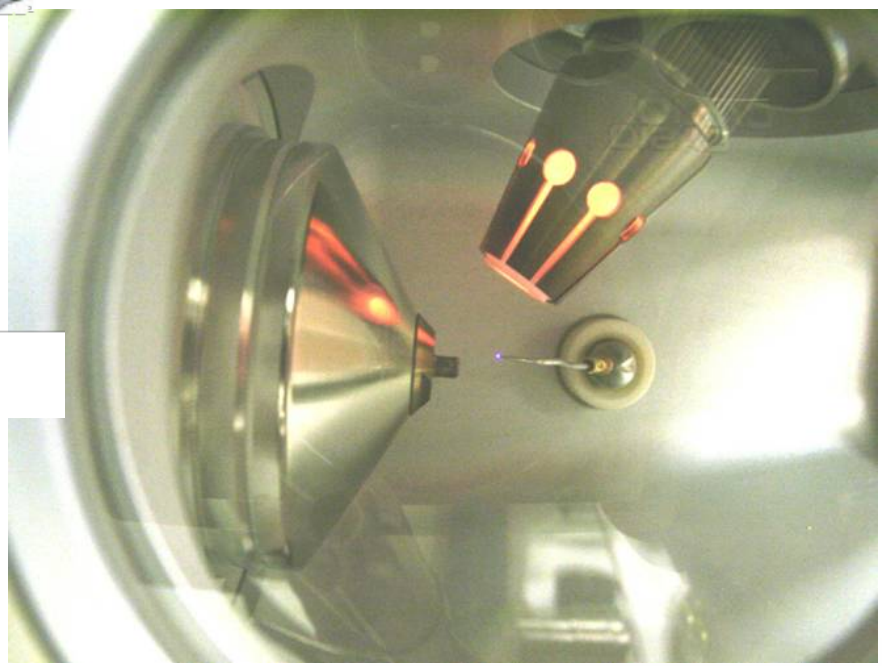
- Ions formed by solution chemistry
- Good for thermally labile analytes
- Good for polar analytes
- Good for large molecules (Proteins / Peptides)

APCI:

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

Ion Max Source Design - APCI Probe

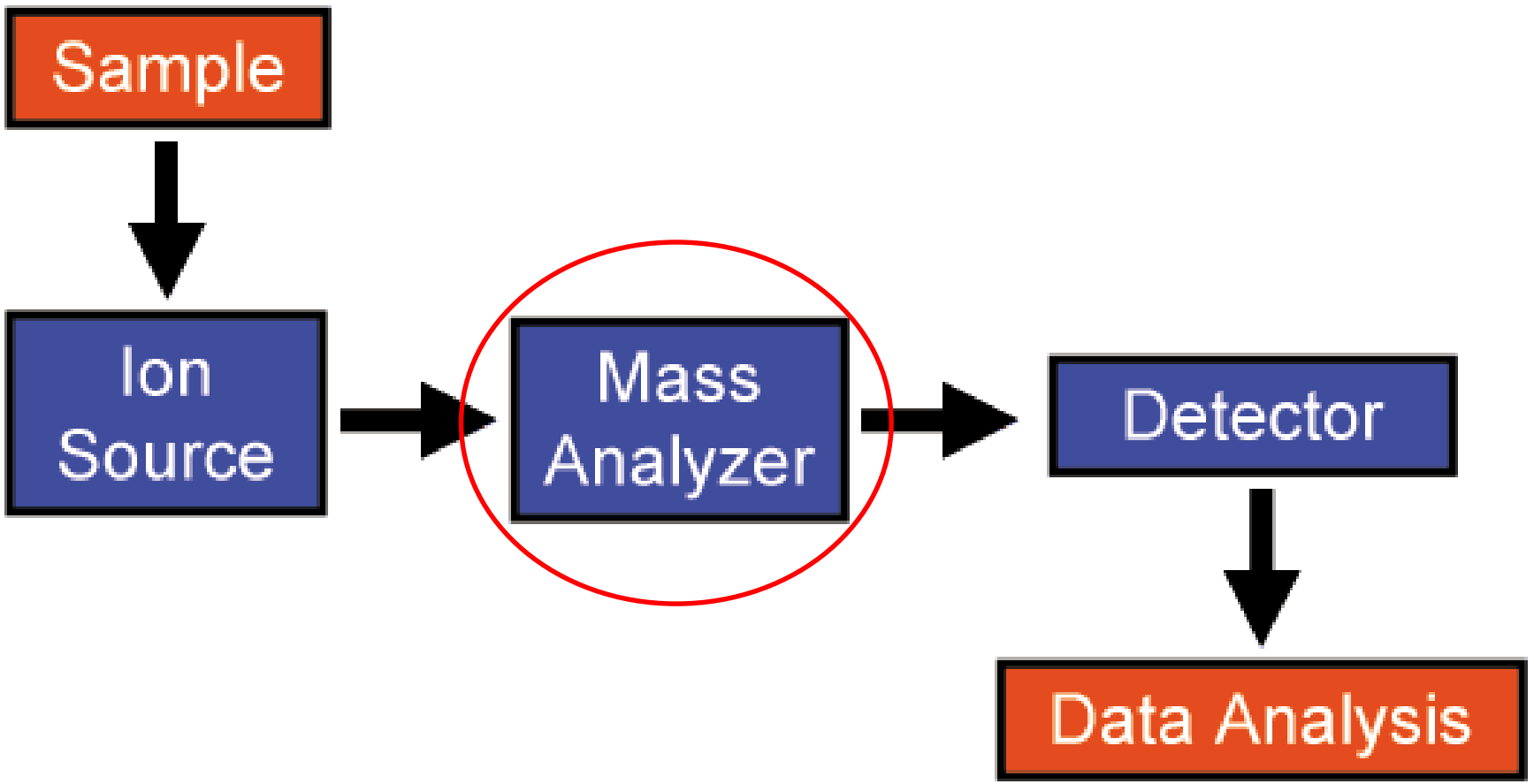




Which is Best?

- 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

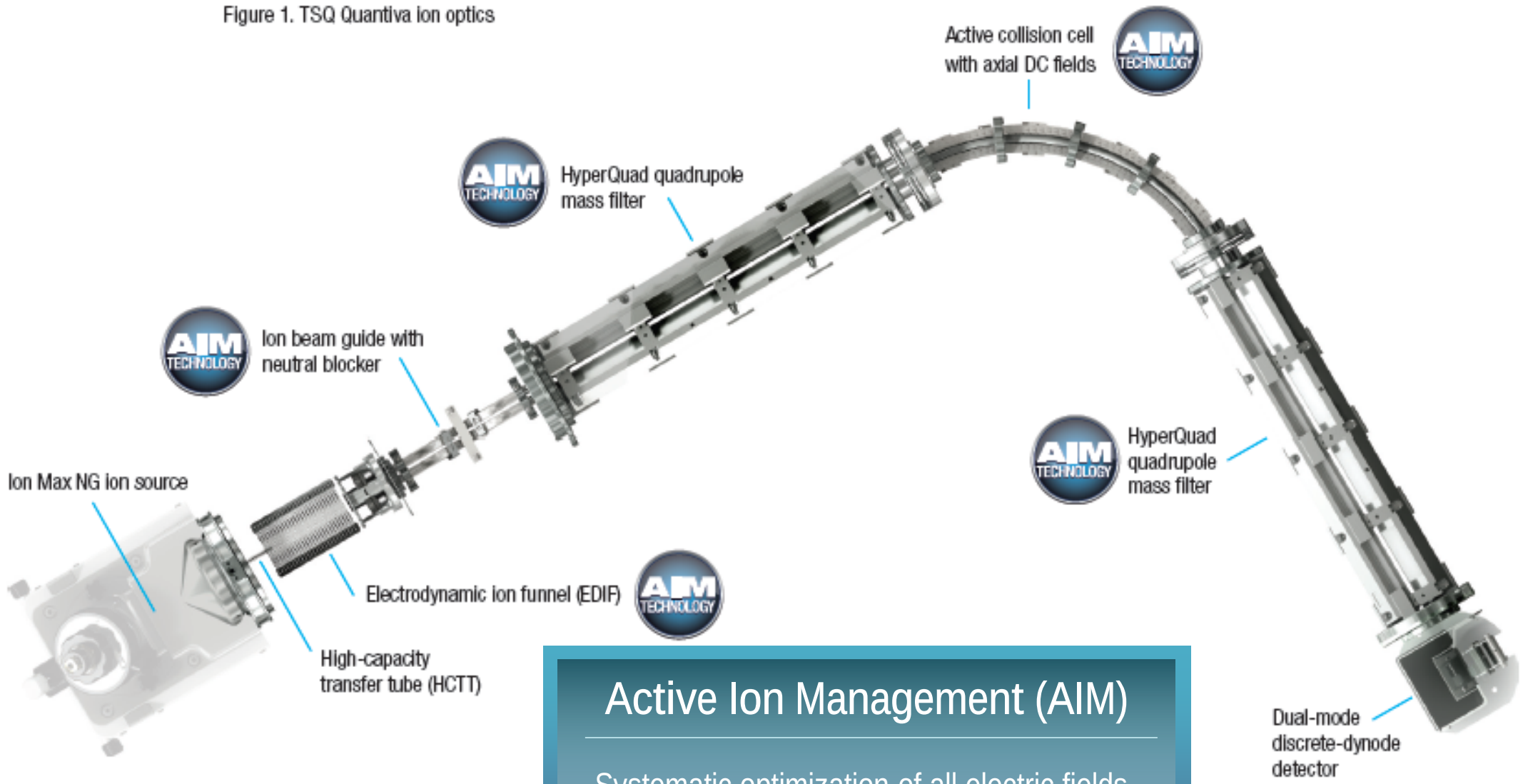
Type of MS	Mass accuracy	Resolution	Utility for
Quadrupole	0.1 amu	6,000	Identify
Traps	0.1 amu	8,000	Identify
TOF	0.0001 amu	<20,000 TOF 60,000 Q-TOF	Empirical formula/ composition
Sector	0.0001 amu	10,000	Empirical formula/ composition
Orbitrap	0.0001 amu	1,000,000	Empirical formula/ composition

MASS ANALYSER

QUADRUPLE

TSQ Quantiva MS—Powered by AIM Technology

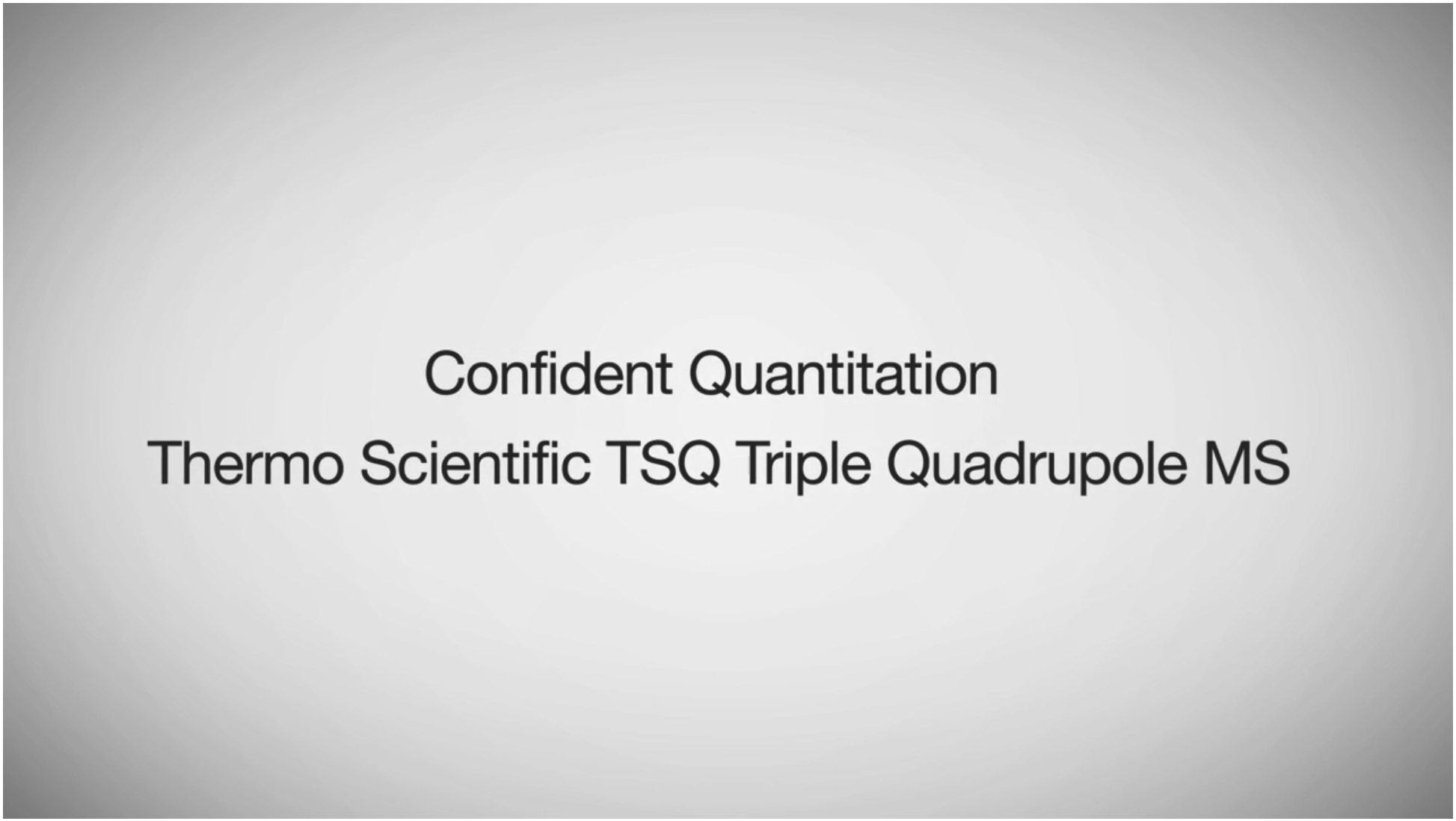
Figure 1. TSQ Quantiva ion optics



Active Ion Management (AIM)

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

TSQ Triple Quadrupole (available on YouTube)

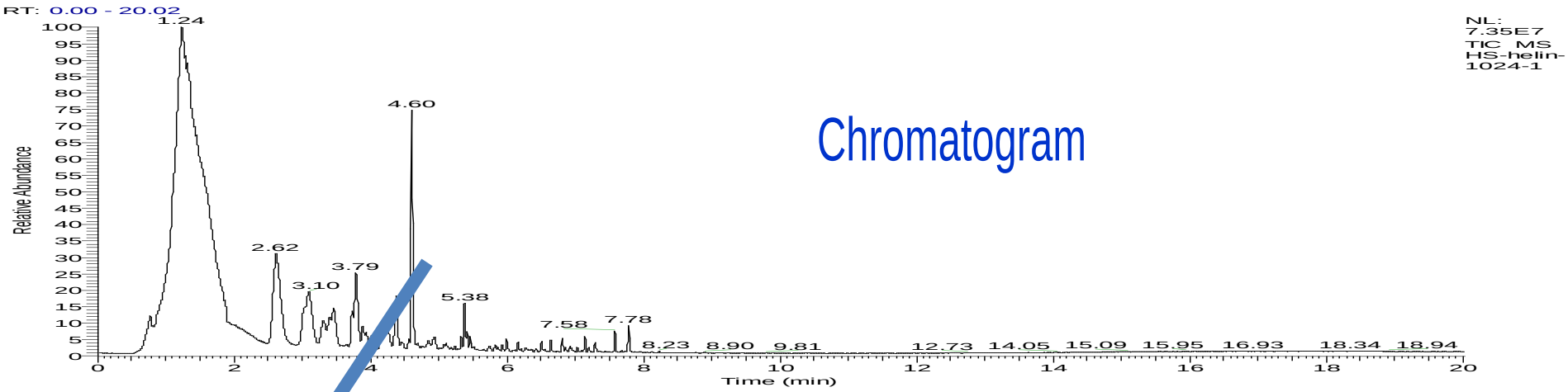


http://www.youtube.com/watch?v=LFB14D8pkoc

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

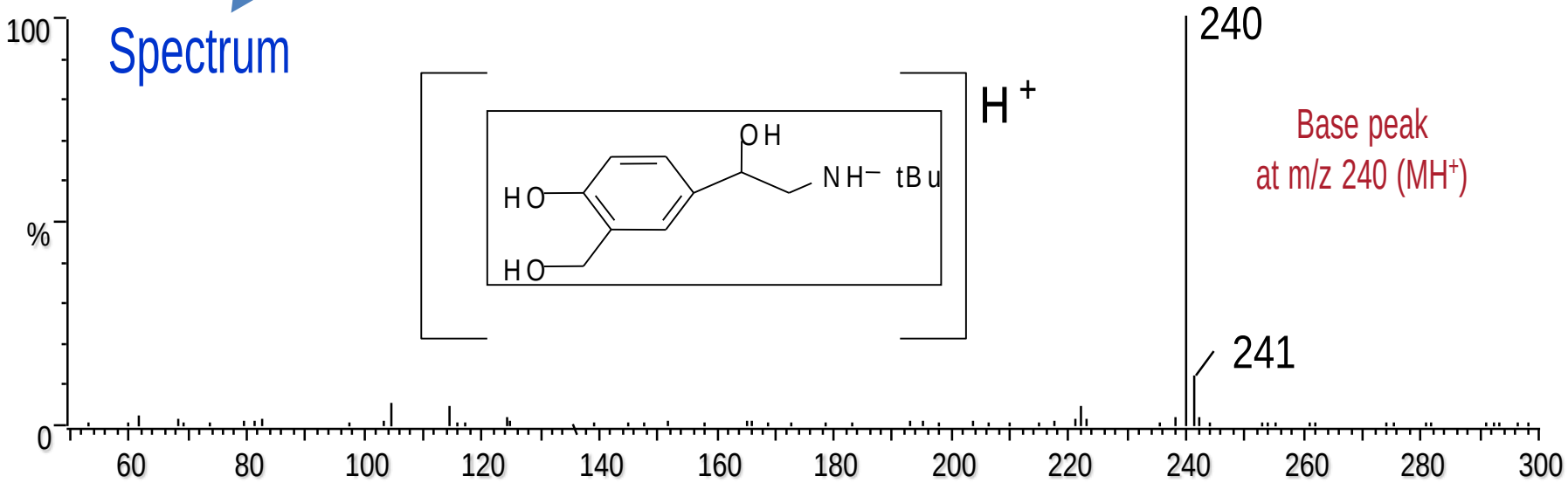
Full Scan Mode



Chromatogram

One Click

Spectrum



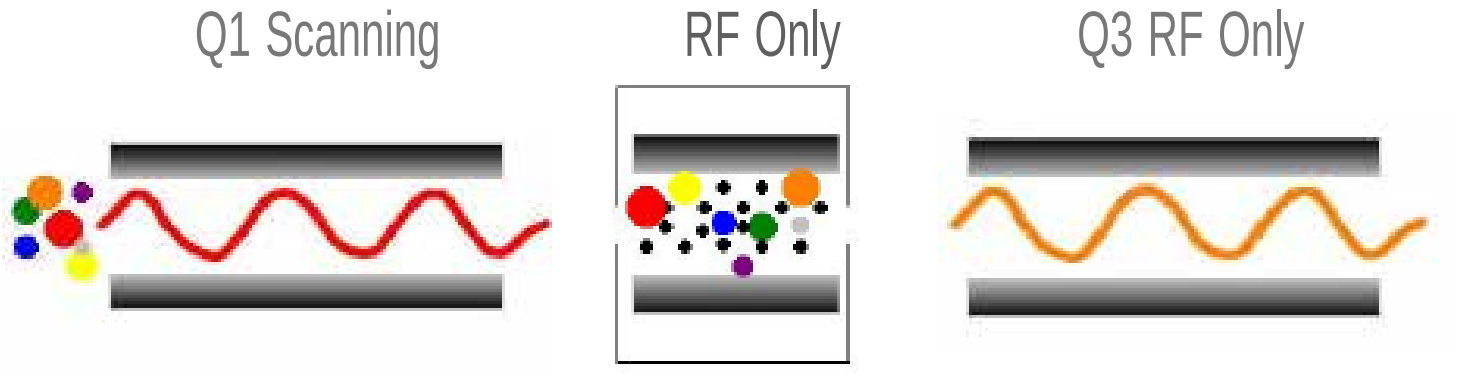
Base peak
at m/z 240 (MH^+)

Full Scan (Q1 or Q3)

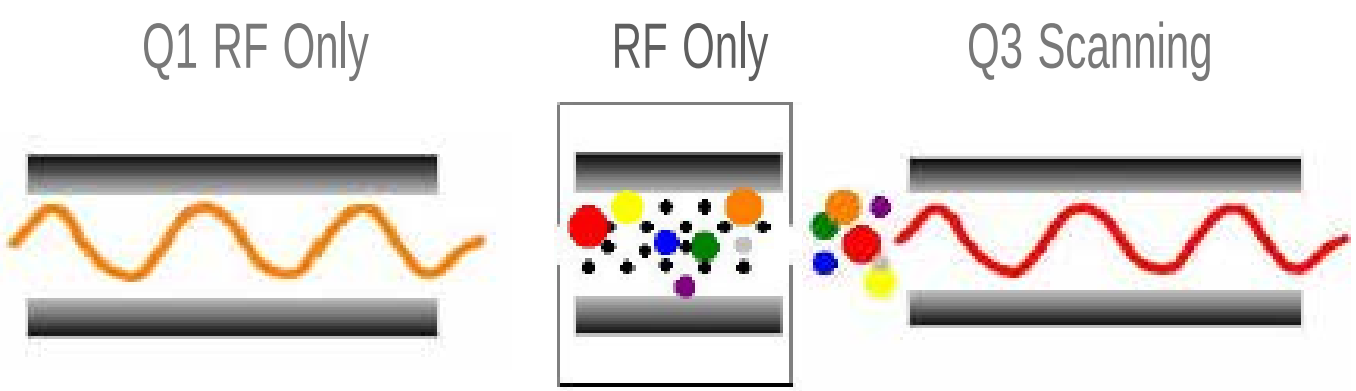
Full Scan Mode

Purpose: Survey scan of a chromatographic peak

Full scan Q1:



Full Scan Q3:

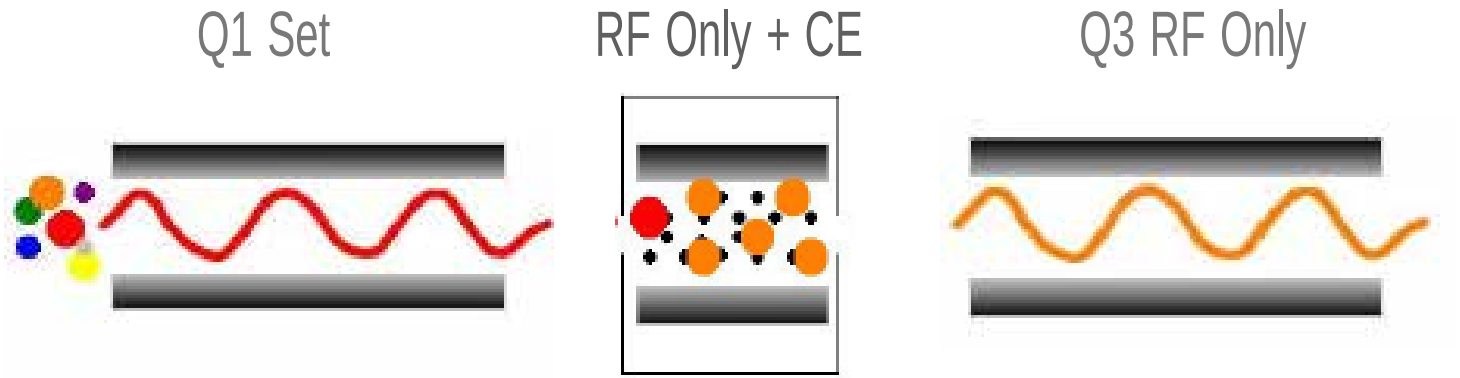


Selected Ion Monitoring – SIM

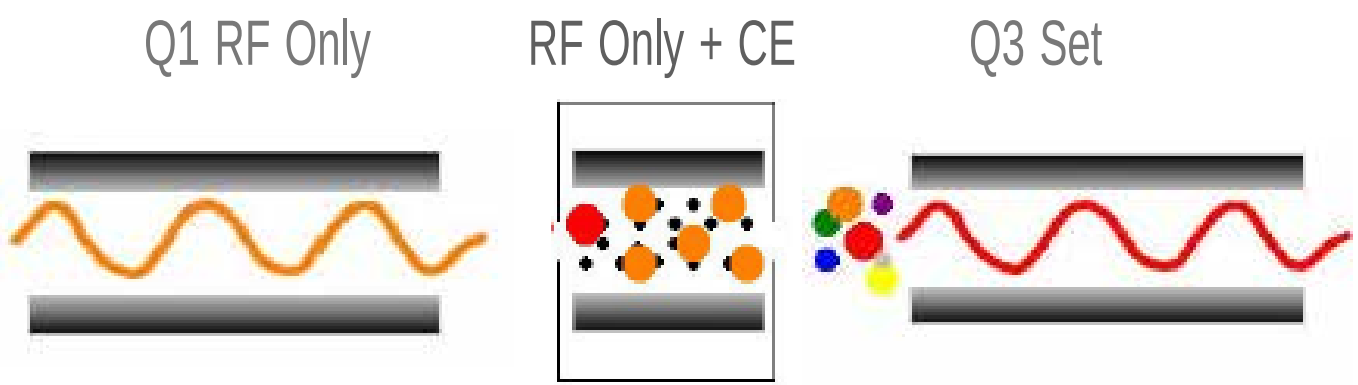
SIM Mode

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

SIM Q1:

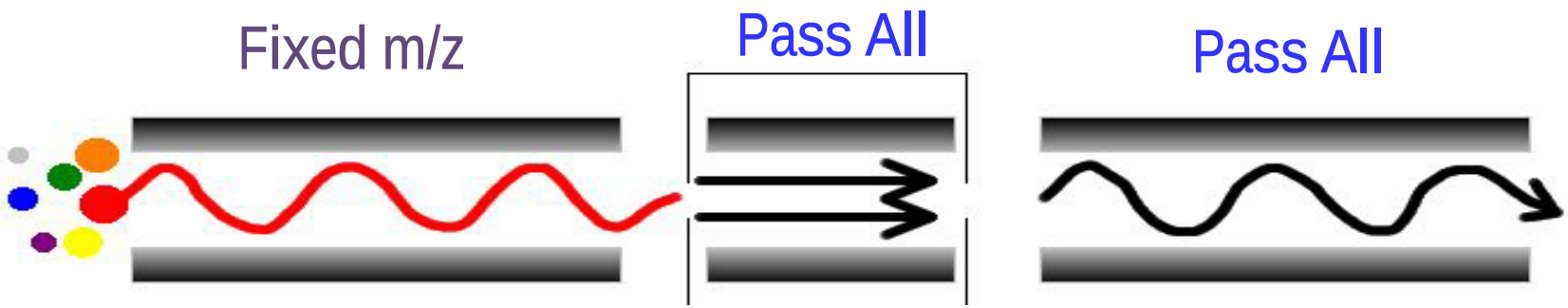


SIM Q3:



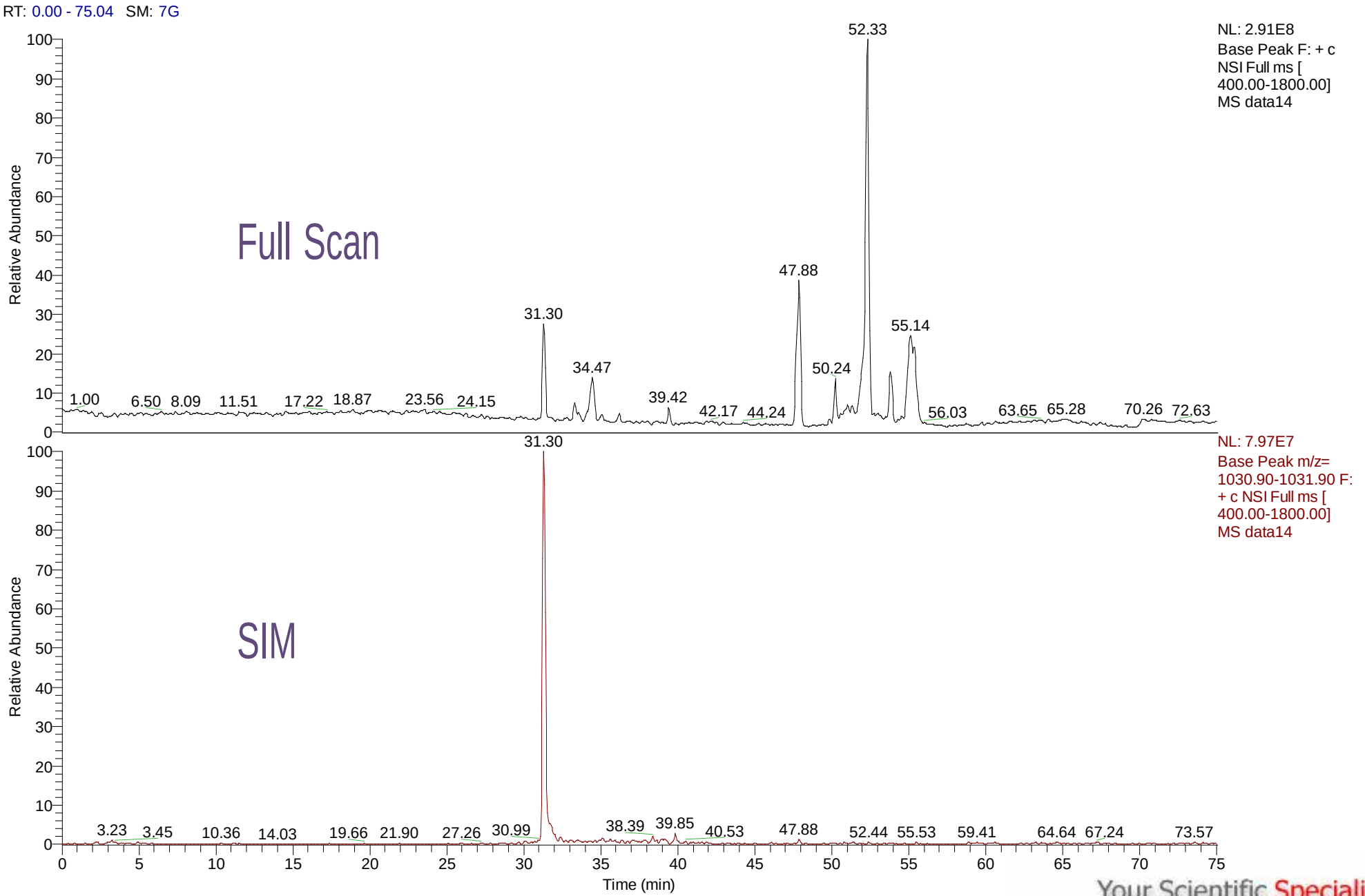
Selected Ion Monitoring – SIM

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

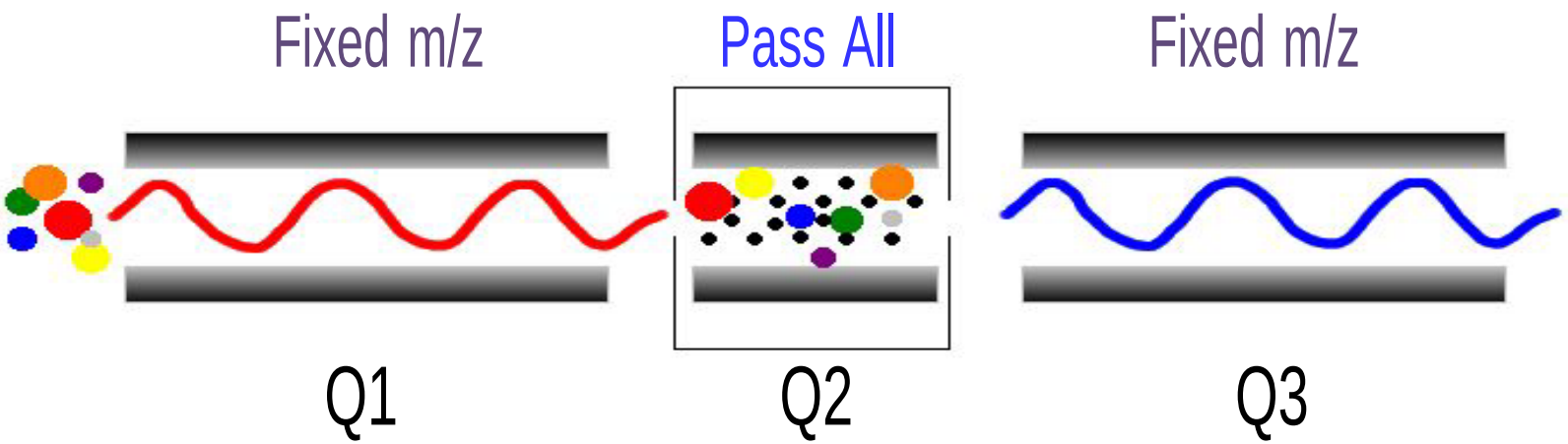


- Advantages
  - Targeted analyte monitoring
  - High duty cycle
- Disadvantages
  - Can suffer from interferences
  - Not as sensitive or selective as SRM

Full Scan versus SIM

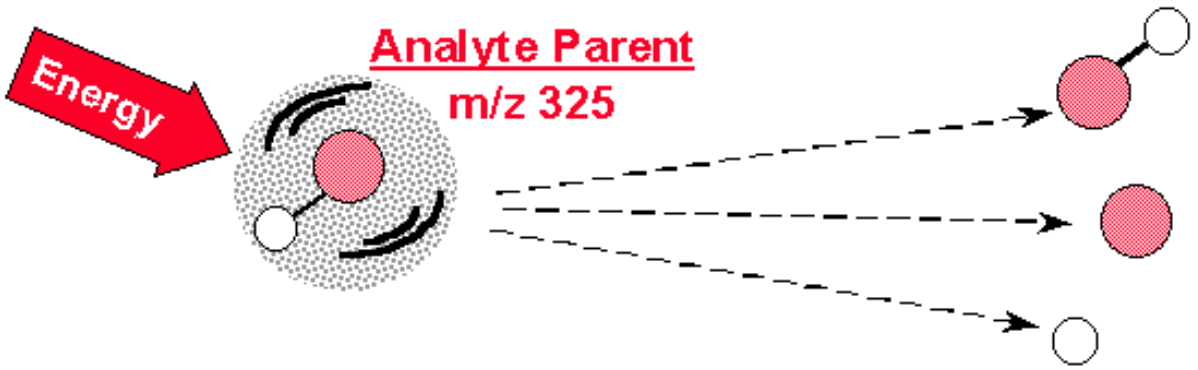


Selected Reaction Monitoring (SRM)



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

The Need for True MS/MS

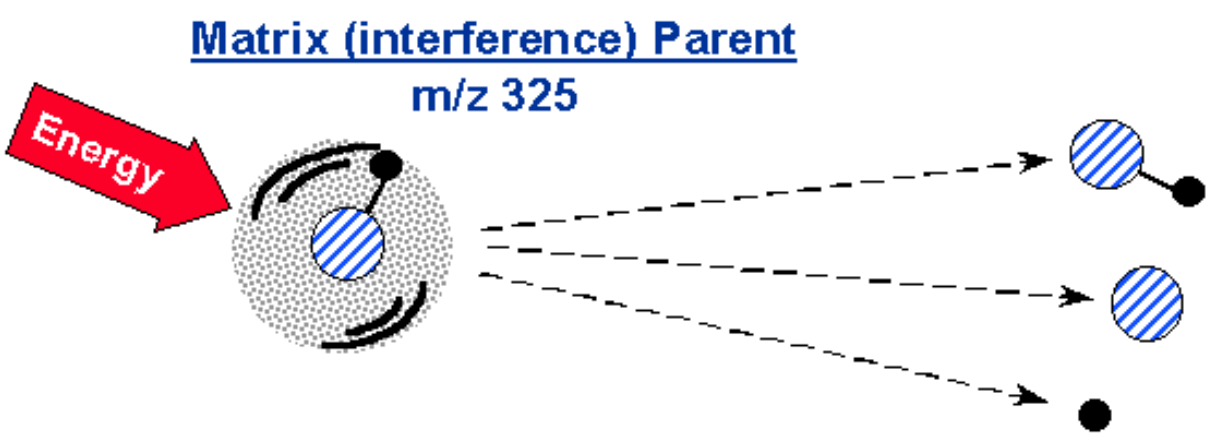


m/z 325 Intact Analyte Parent

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.



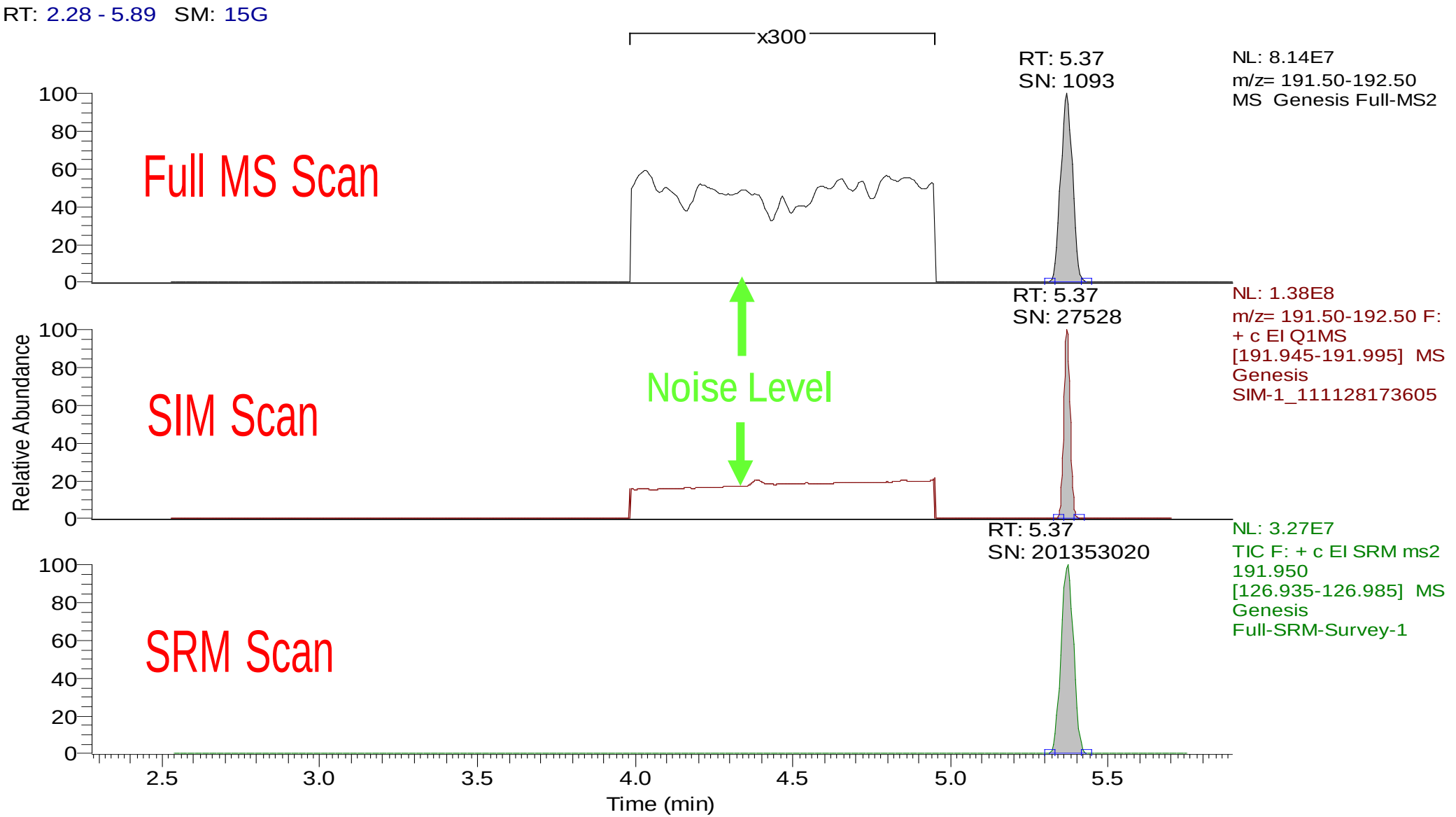
m/z 325 Intact Matrix Parent

m/z 185 Matrix CID Product A

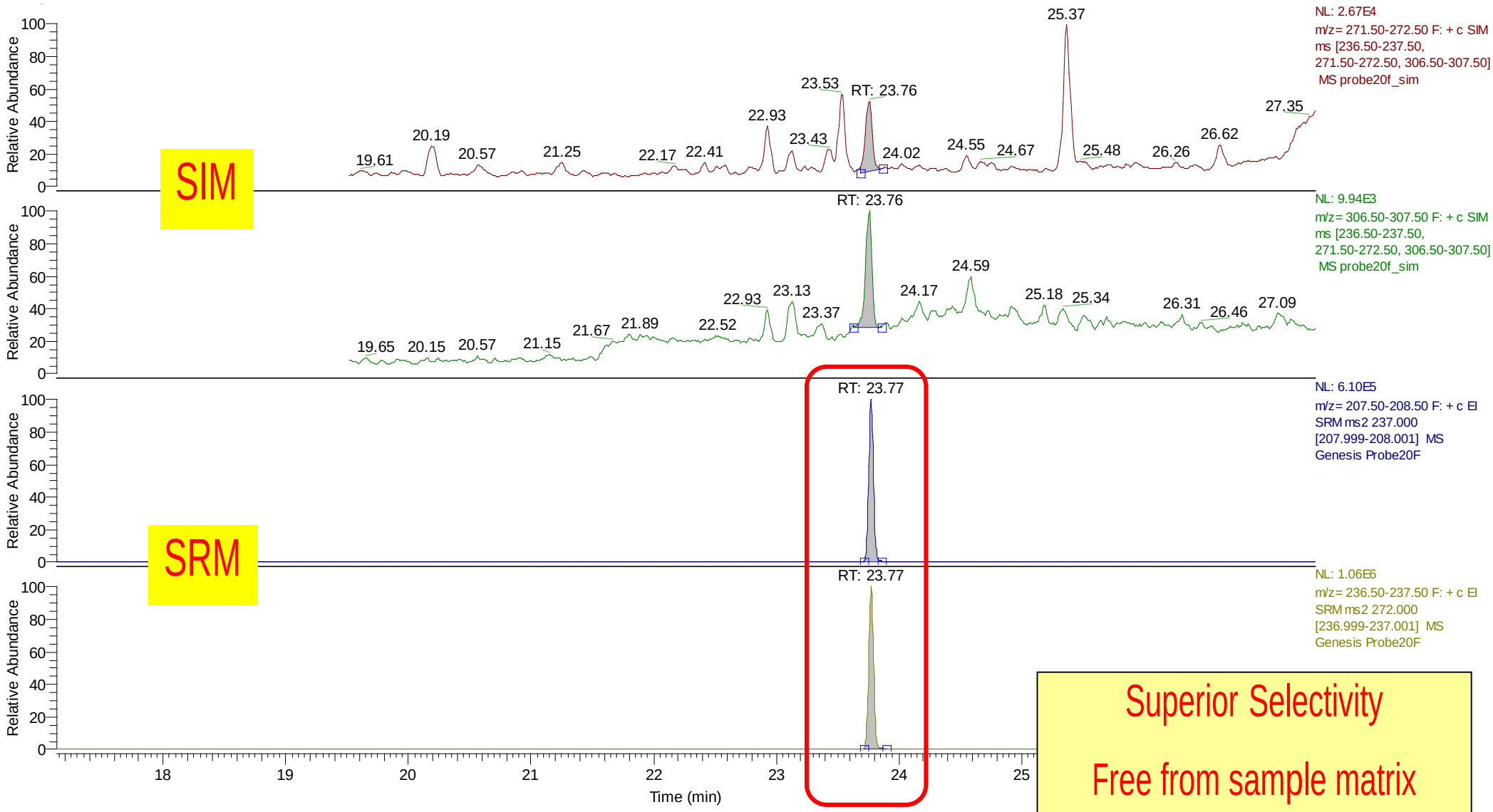
m/z 140 Matrix CID Product B

LC/MS Cross Roads -- Revision 7.5 -- 8/27/98 -- Richard Klein

SRM Selectivity in Complex Matrices



Comparison of SIM and SRM



Superior Selectivity

Free from sample matrix

HIGH RESOLUTION MASS ANALYSER

- ORBITRAP

Mass Analyzers Parameters

- Nominal Mass

The mass of an ion with a given empirical formula calculated using the integer mass numbers of the most abundant isotope of each element

Ex : M=249 $C_{20}H_9^+$ or $C_{19}H_7N^+$ or $C_{13}H_{19}N_3O_2^+$

- 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

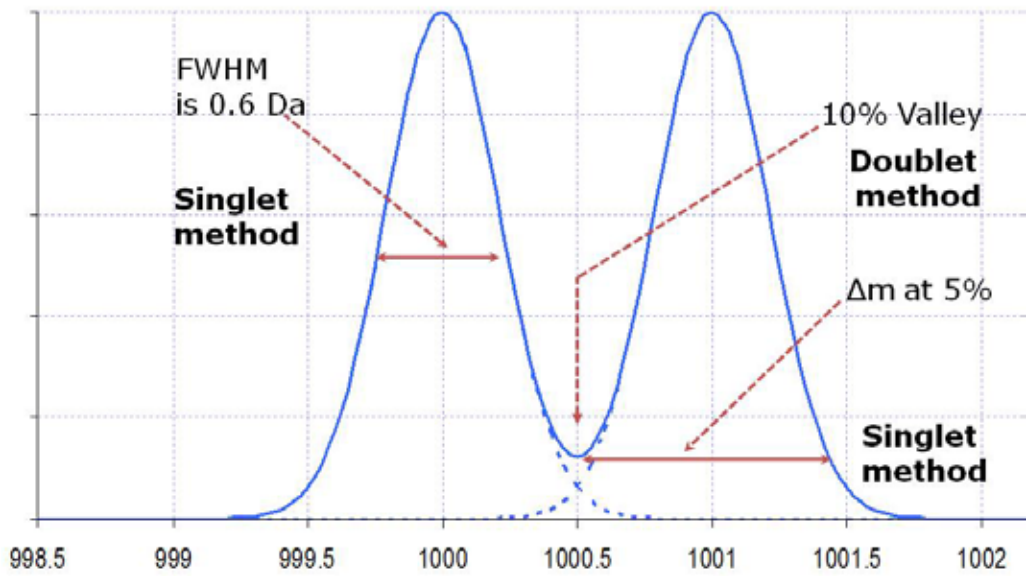
MASS RESOLUTION

Mass Resolution: What is it?

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

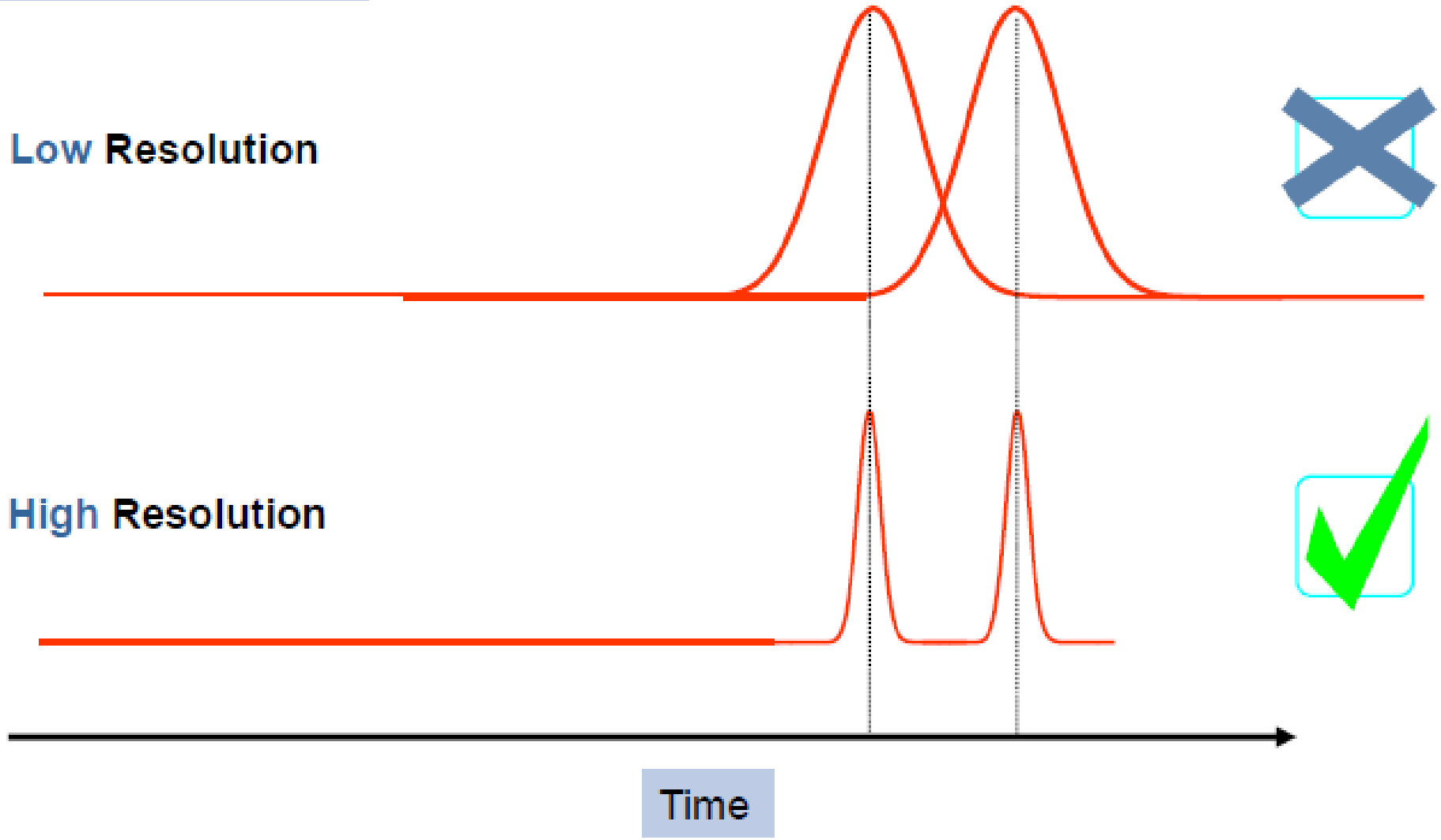
$$R = \frac{m}{\Delta m}$$

- m - measured mass
- Δm - peak width measured at 50% peak intensity (Full Width Half Maximum)
  - or the mass difference between two adjacent peaks of equal intensity, in this case pw @ 10% valley definition is used.



Resolution & Peak Width

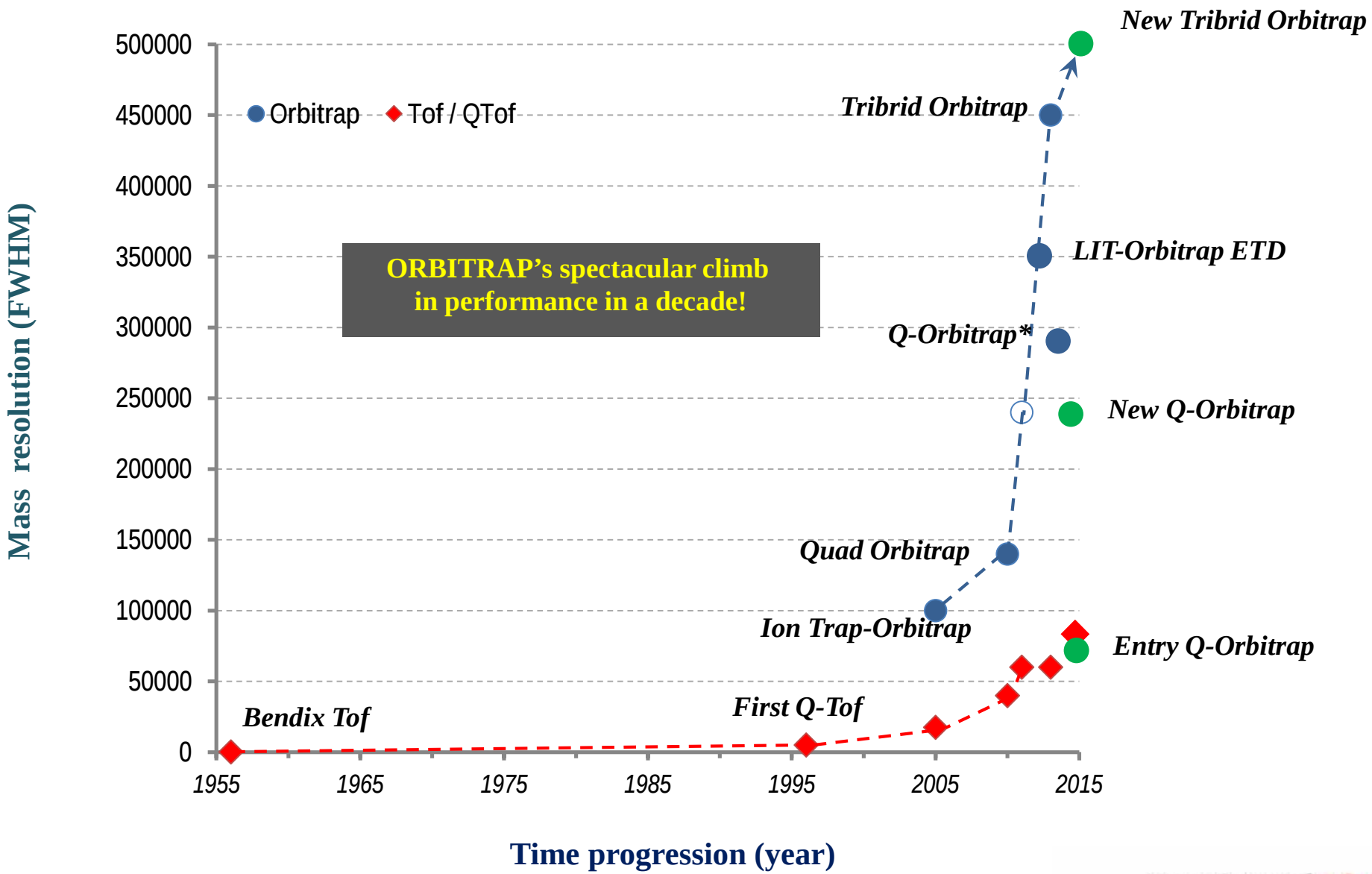
Chromatography



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.

Commercial High Resolution MS Technology Race

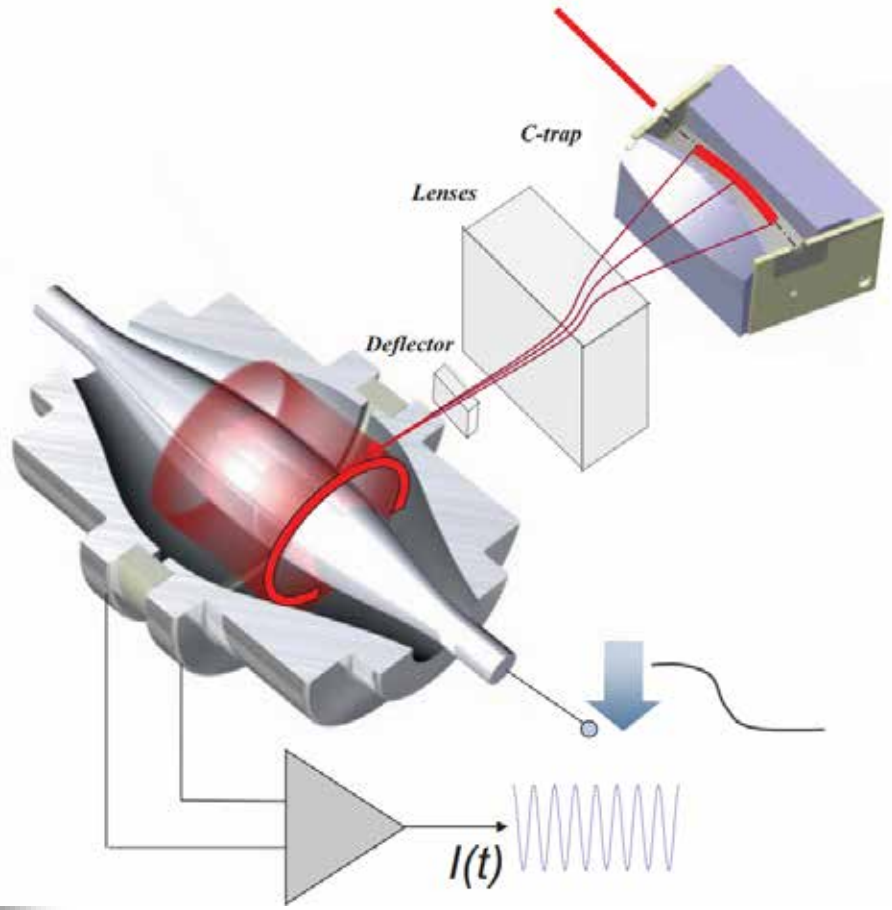


Anal. Chem. **2000,** *72,* 1156−1162

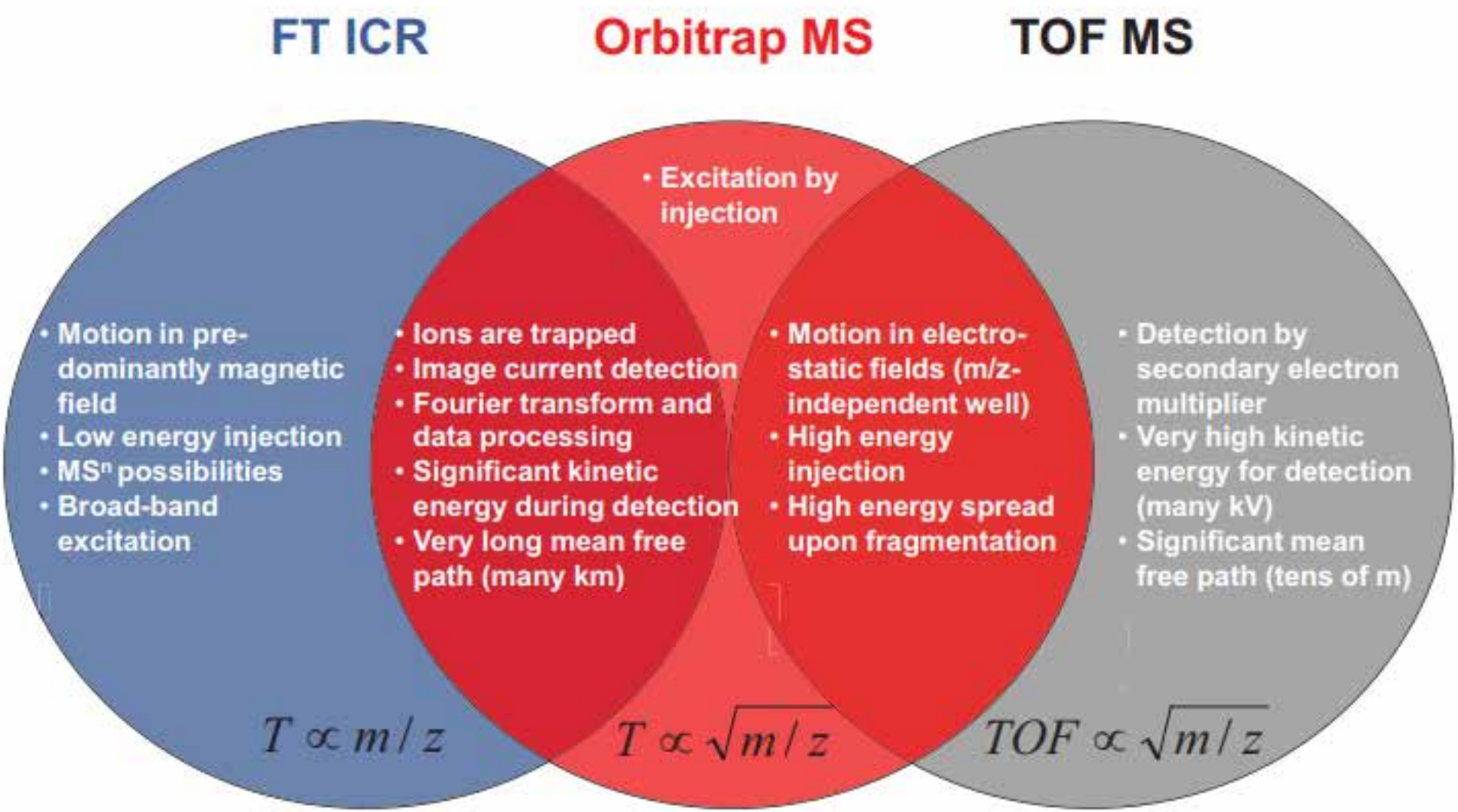
Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

Alexander Makarov*

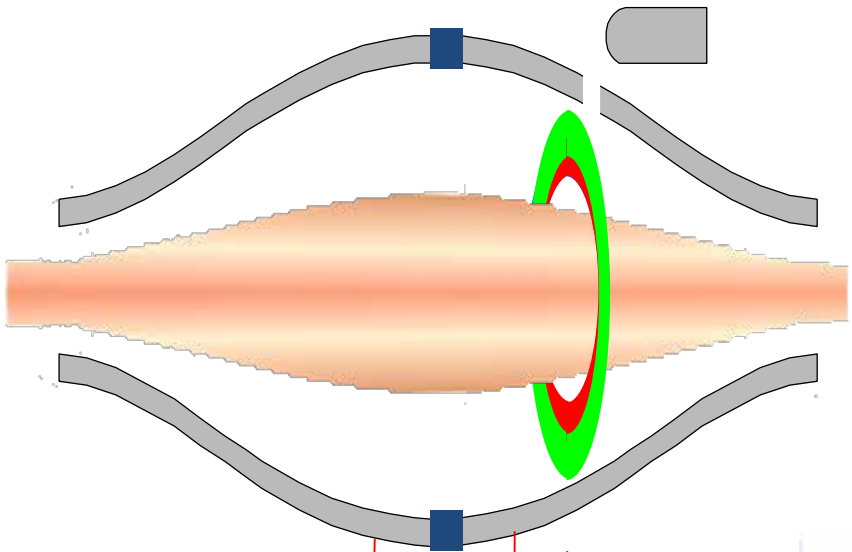
HD Technologies Ltd., Atlas House, Simonsway, Manchester, M22 5PP, U.K.

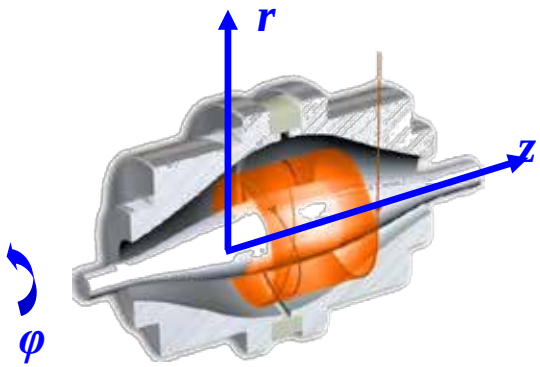


Major accurate-mass analyzers for Life Science



Orbitrap Mass Analyzer: Principle of Operation





Hyper-logarithmic potential distribution:
"ideal Kingdon trap"

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

§ Characteristic frequencies:

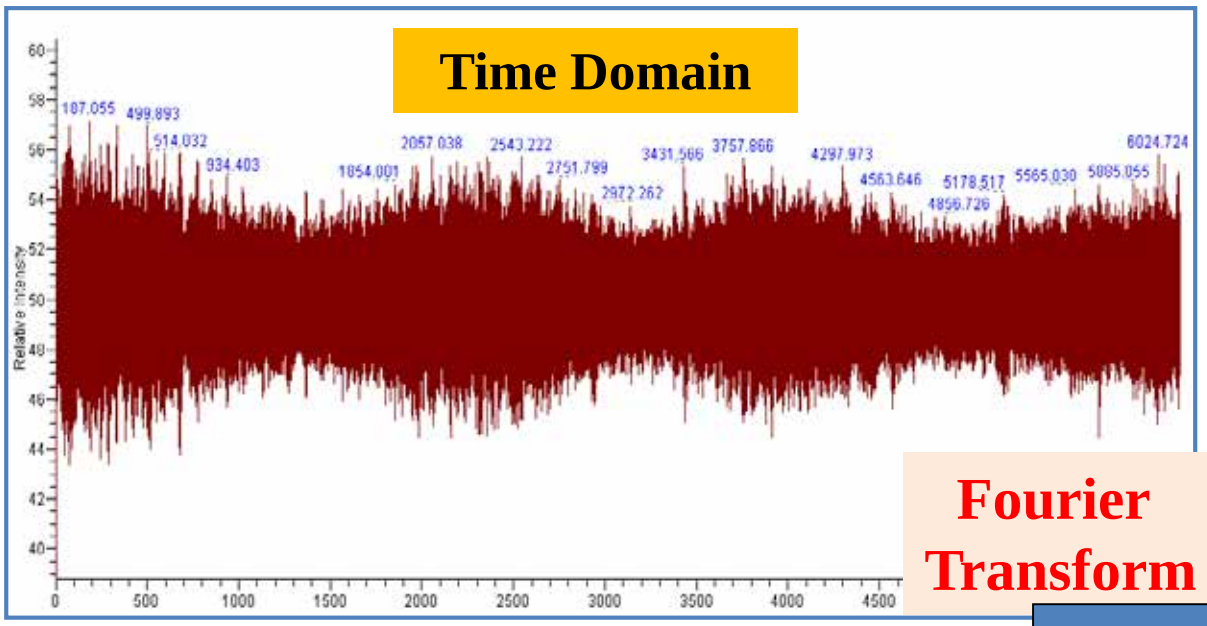
- Frequency of rotation ω_φ
- Frequency of radial oscillations ω_r
- Frequency of axial oscillations ω_z

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

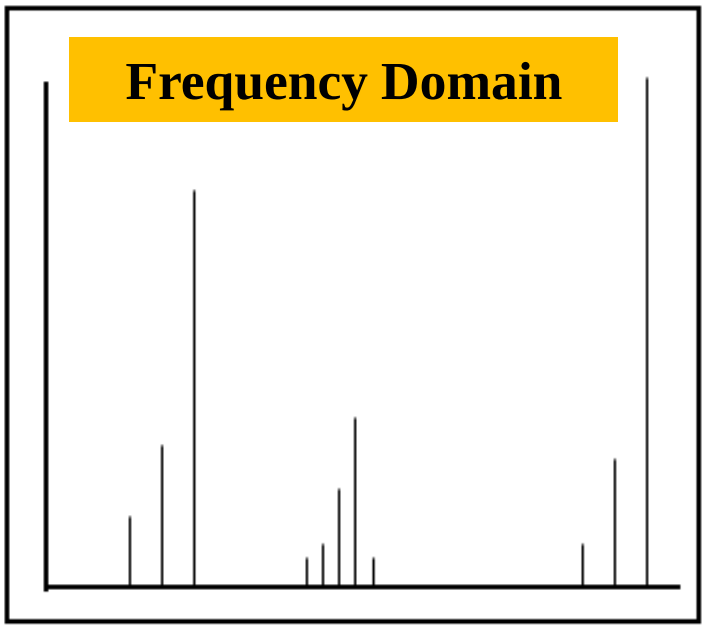
$$\omega_j = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \qquad \omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

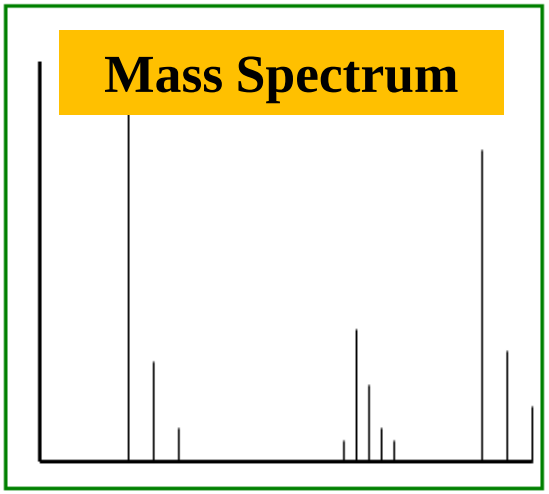
Makarov A. ***Anal. Chem.*** **2000,** ***72,*** **1156-1162.**

Many Ions Generate a Complex "Transient"

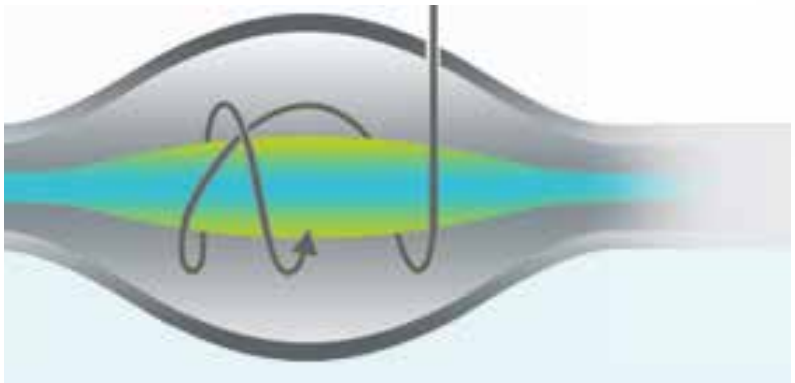


Fourier Transform





UHPLC with Q Exactive Mass Spectrometer



http://planetorbitrap.com/

ThermoFisher SCIENTIFIC

Applications of Triple Quadrupole LC-MS/MS

TSQ Quantis Sensitivity Study: Pesticides

Quantitation of more than 250 Pesticides below MRLs in Leek

LC: Vanquish Flex Binary System

Column: Accucore aQ (2.1 × 100 mm, 2.6 µm)

Column Temperature: 25°C

Injection Volume: 1 µL

Mobile Phase: A) 98% water with 2% methanol; B) 98% methanol with 2% water— Both containing 0.1% formic acid and 5 mM ammonium formate

Flow Rate: 300 µL/min

Run Time: 15 min

Thermo Scientific Vanquish Flex UHPLC

- Maximize UHPLC separation with 1034 bar (15,000 psi) pump pressure limit
- Viper-based, tool-free fluidic connections
- Biocompatible, Iron-free flow path
- Sample pre-compression for better injection reproducibility and longer column lifetimes
- Standard Autosamper capacity: 4 racks (216 vials)
- New column thermostatting technology
- Removable doors for easy access

TSQ Quantis Parameter

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

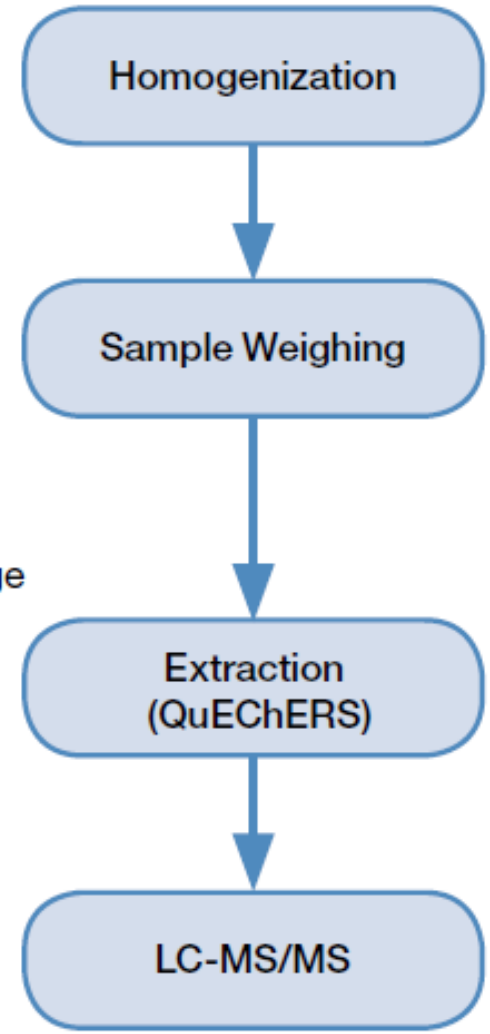
Sample Preparation

1. Weigh 10 g sample in 50 mL extraction tube.

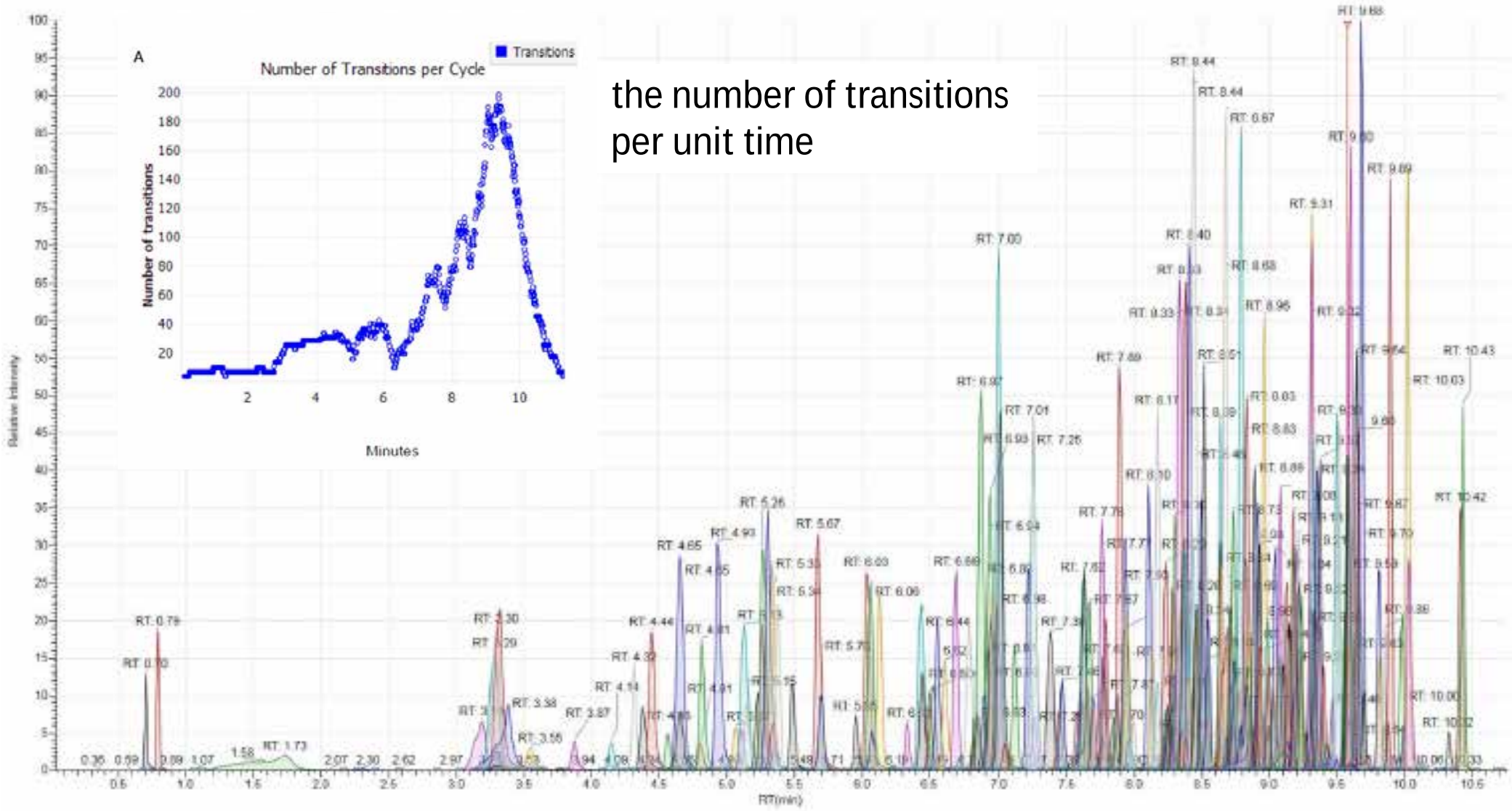
2. Add 10 mL acetonitrile (20 mL water + 10 mL acetonitrile for wheat flour).

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.

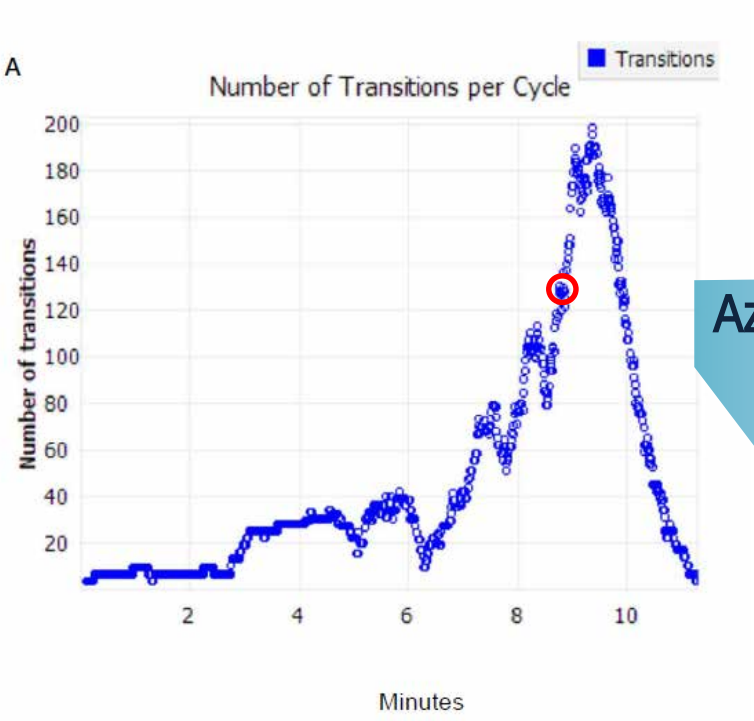


Chromatogram of more than 250 pesticides

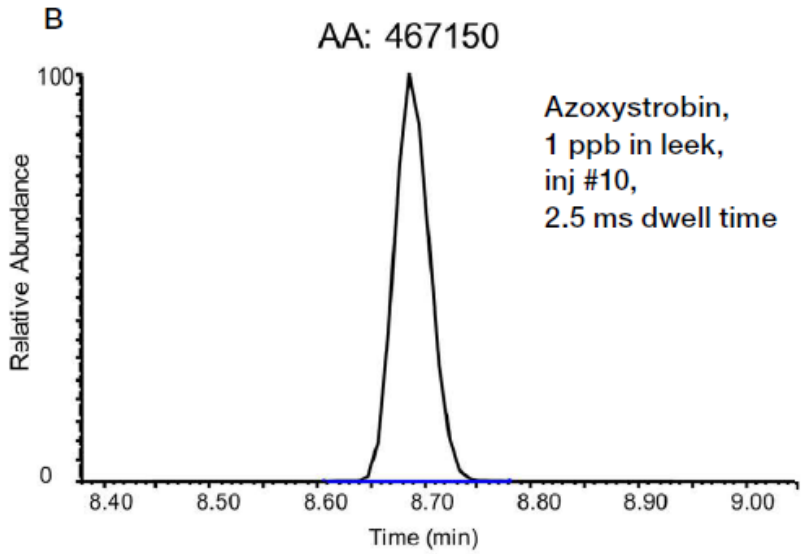


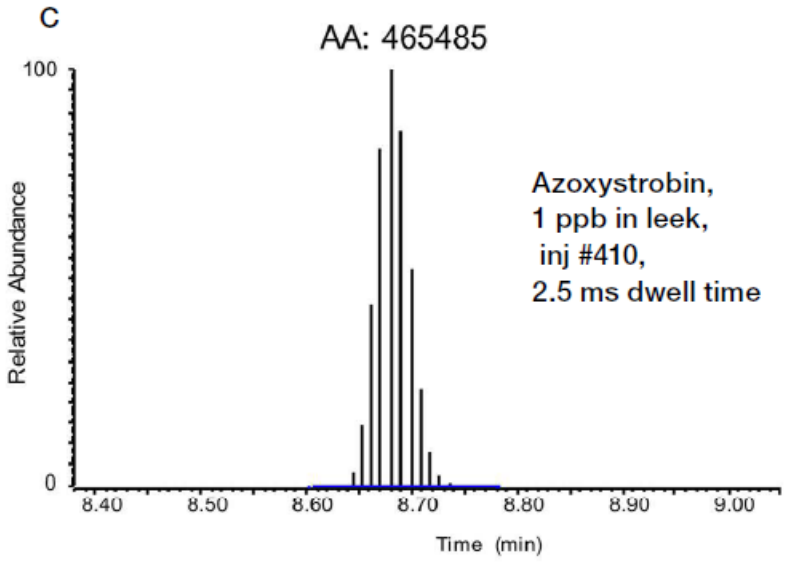
LC-MS/MS chromatogram of more than 250 pesticides in leek extract at 100 µg/kg.

Azoxystrobin elutes at 8.69 min



Azoxystrobin

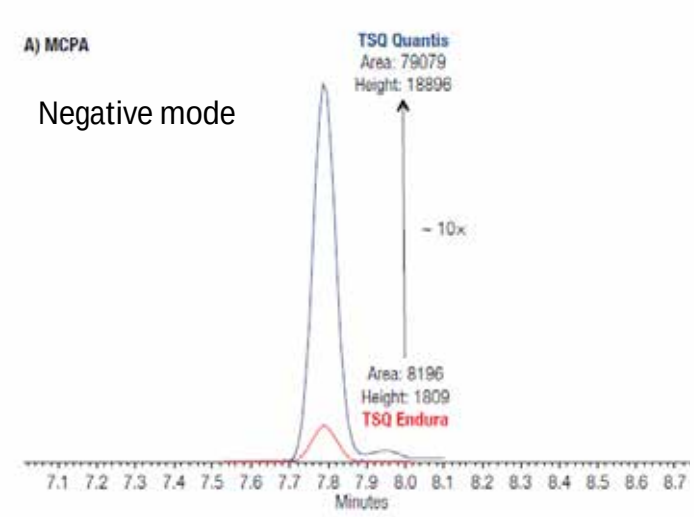


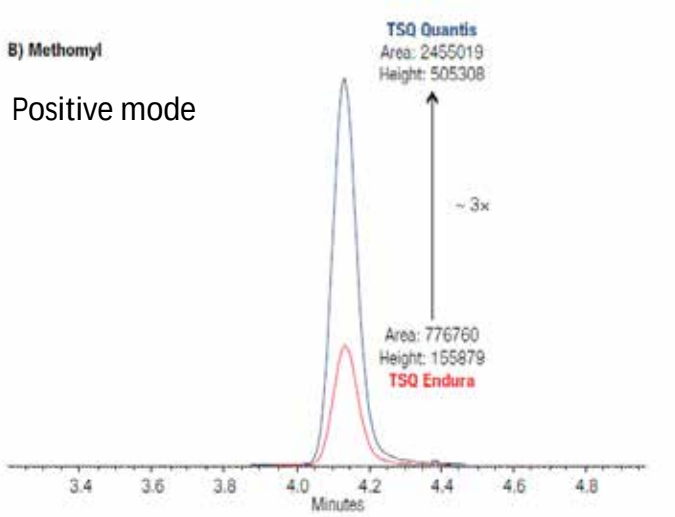


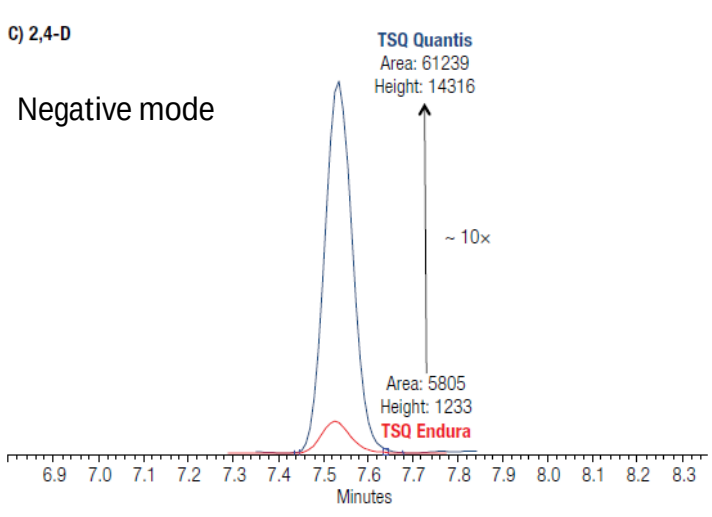
Panels A the number of transitions per unit time

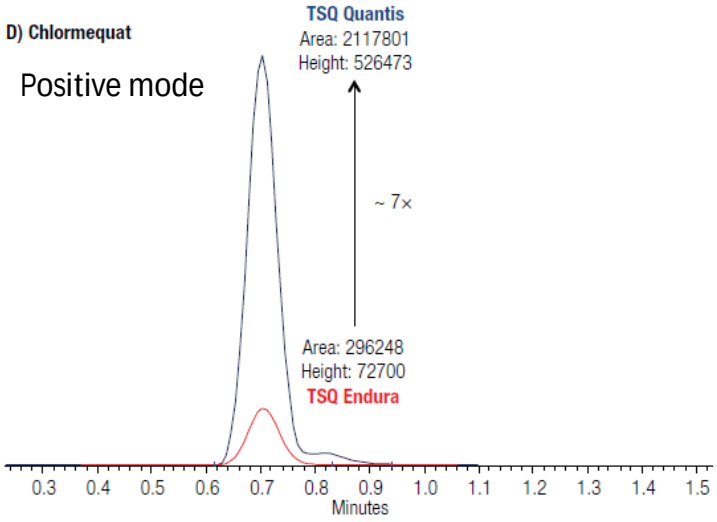
Panels B and C show the reproducibility of azoxystrobin comparing the 10th injection to 410th injection

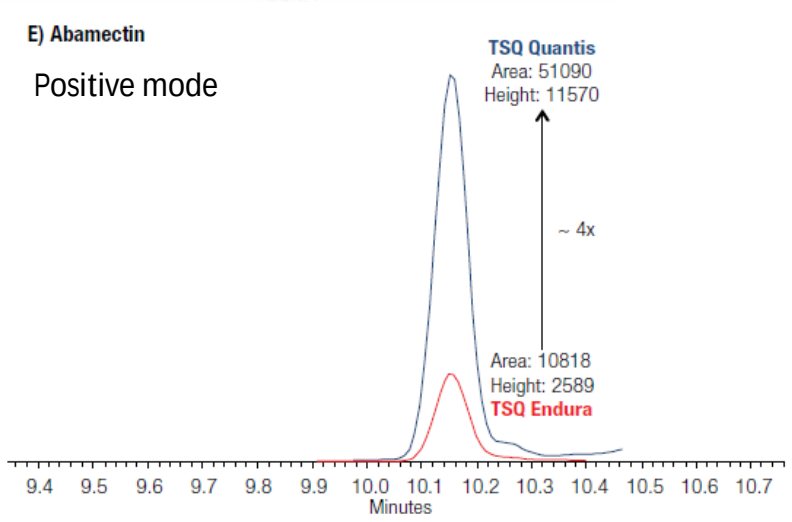
TSQ Quantis MS *VS* TSQ Endura MS





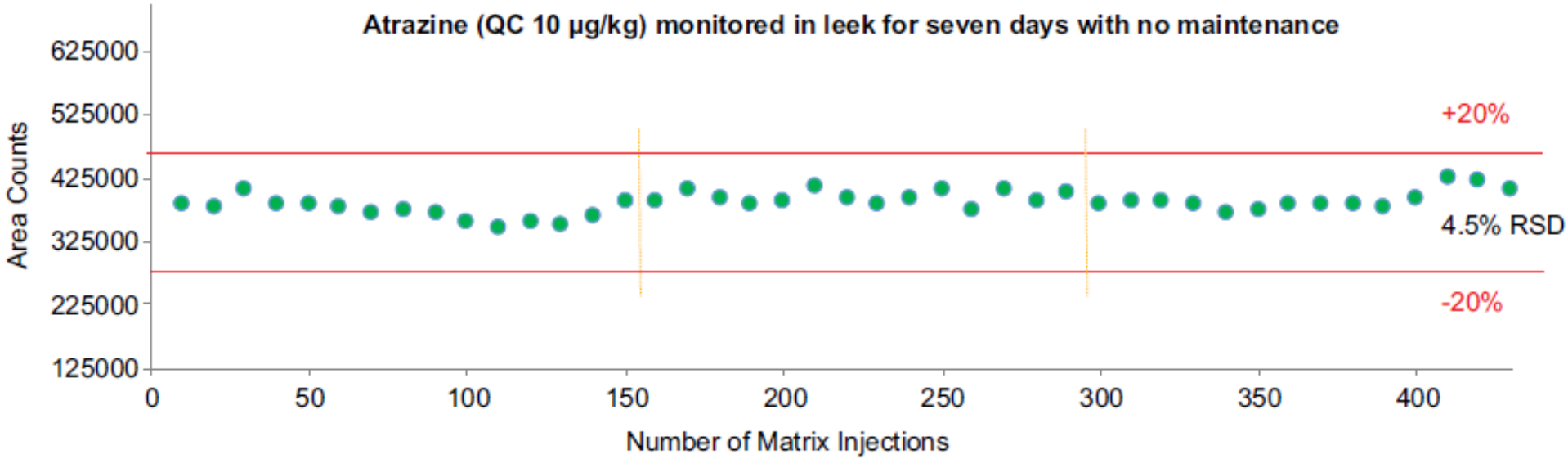






Differences in performance are shown in peak area and peak height.

Reliable Performance when Starting from Standby mode



- Red lines represent —20% of Atrazine response at 10 µg/kg.
- Yellow lines show the exact moment the system was placed in standby mode for 12 h (no maintenance was performed).
- The data shows that the response was within the expected —20% range for at least 400 injections of 10 ppb QC in leek.

Conclusion

- rapid and robust quantitation of more than 250 pesticides in leek at or below their respective MRLs.
- selectivity and sensitivity enabled analysis of only 1 µL sample
- without need for dispersive SPE sample cleanup or sample dilution.

Thank You for Your Attention

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