UHPLC-MS Basic Principles and Applications

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Product Specialist LC/MS

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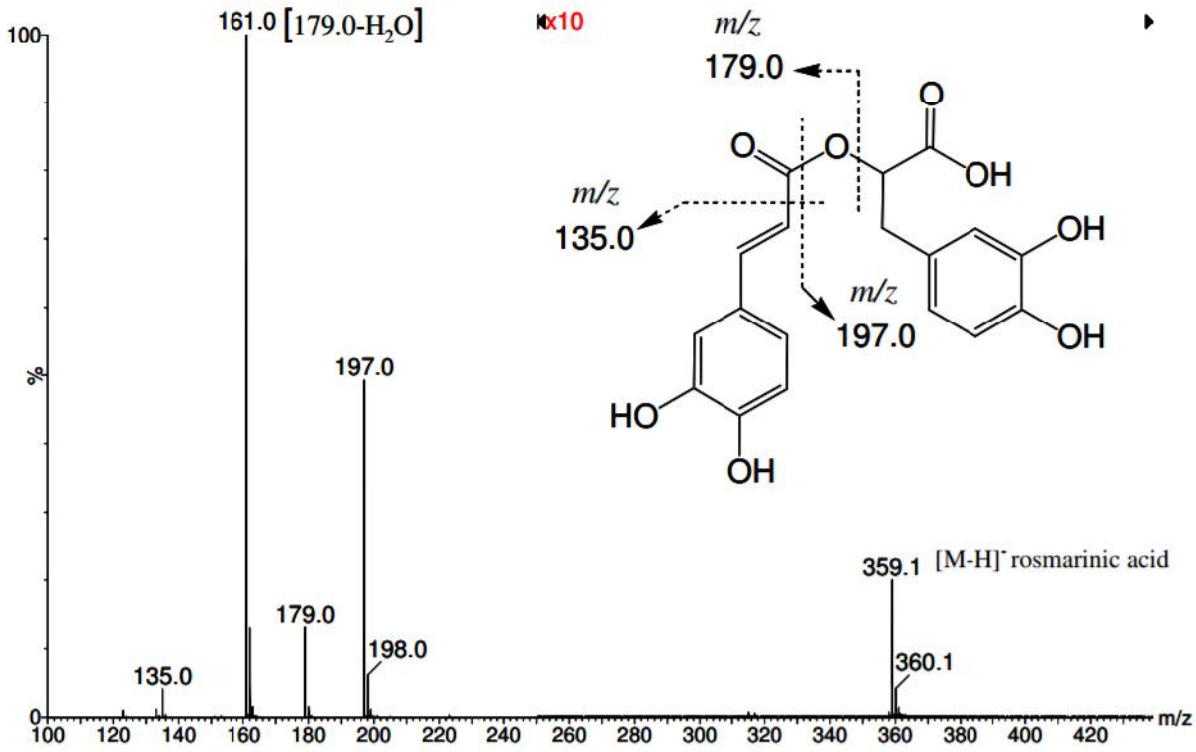
What does a Mass Spectrometer do?

- It measures mass better than any other technique

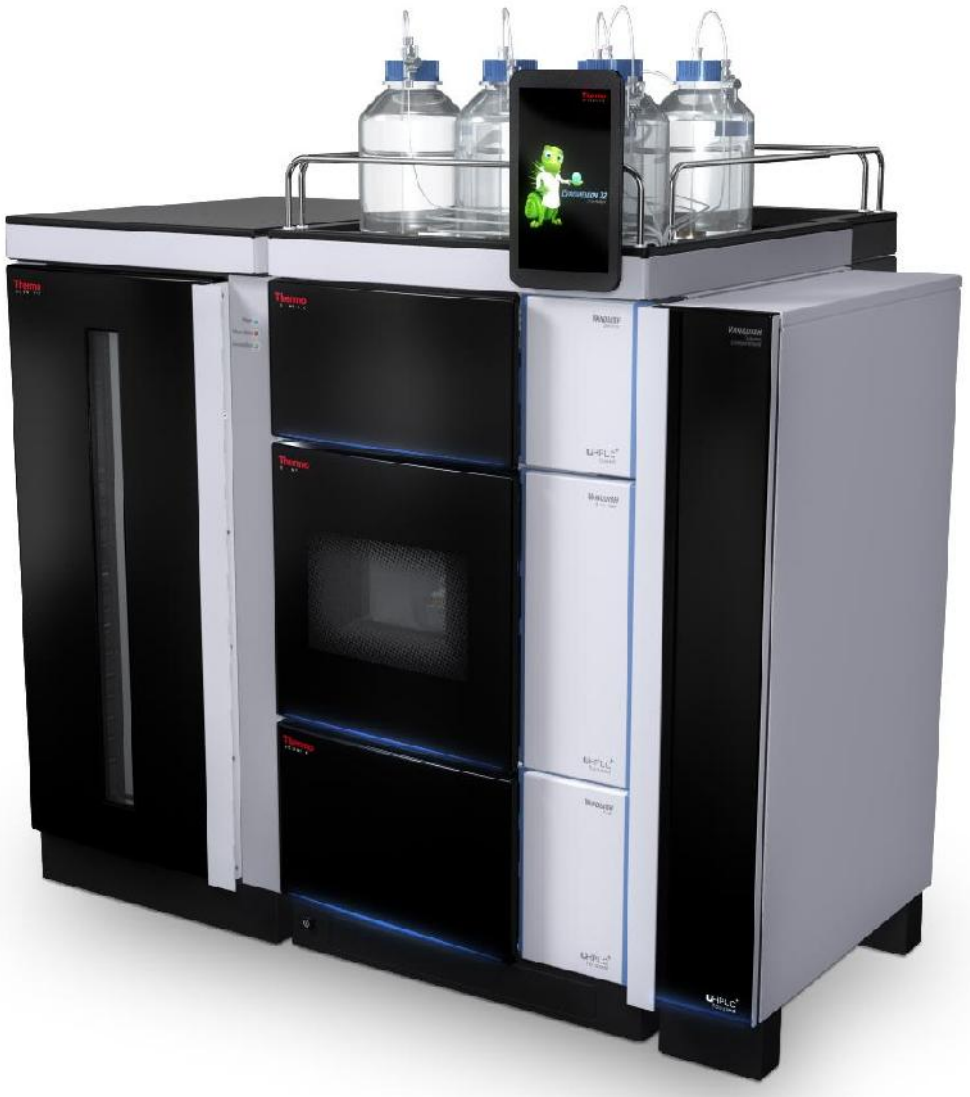
- It can give information about chemical structures.



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

Fundamental of Liquid Chromatography

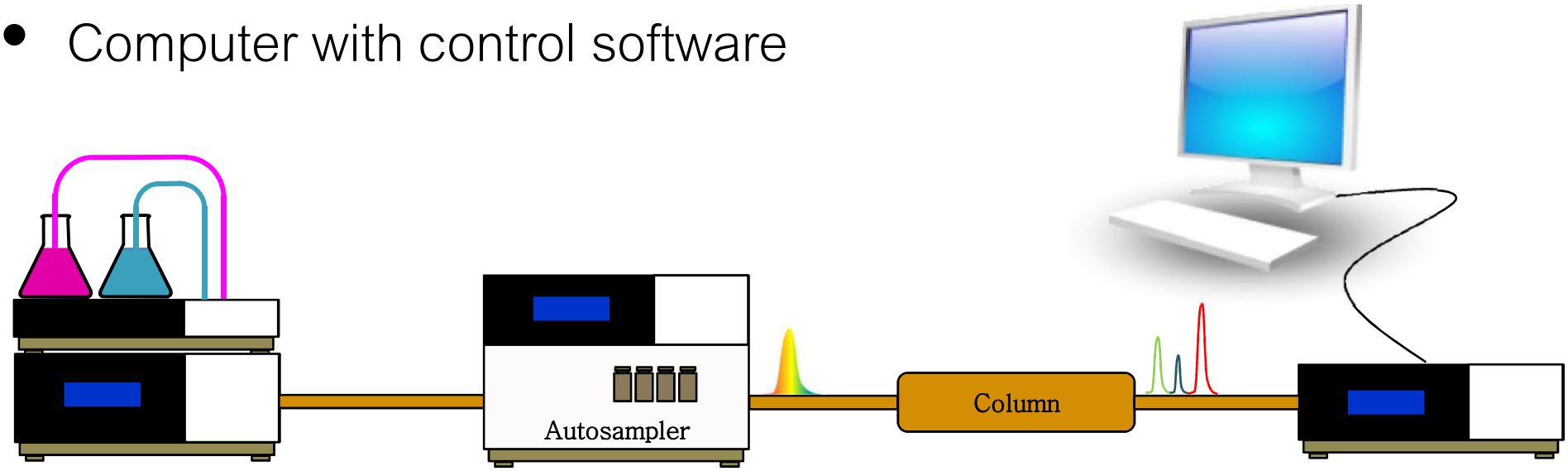


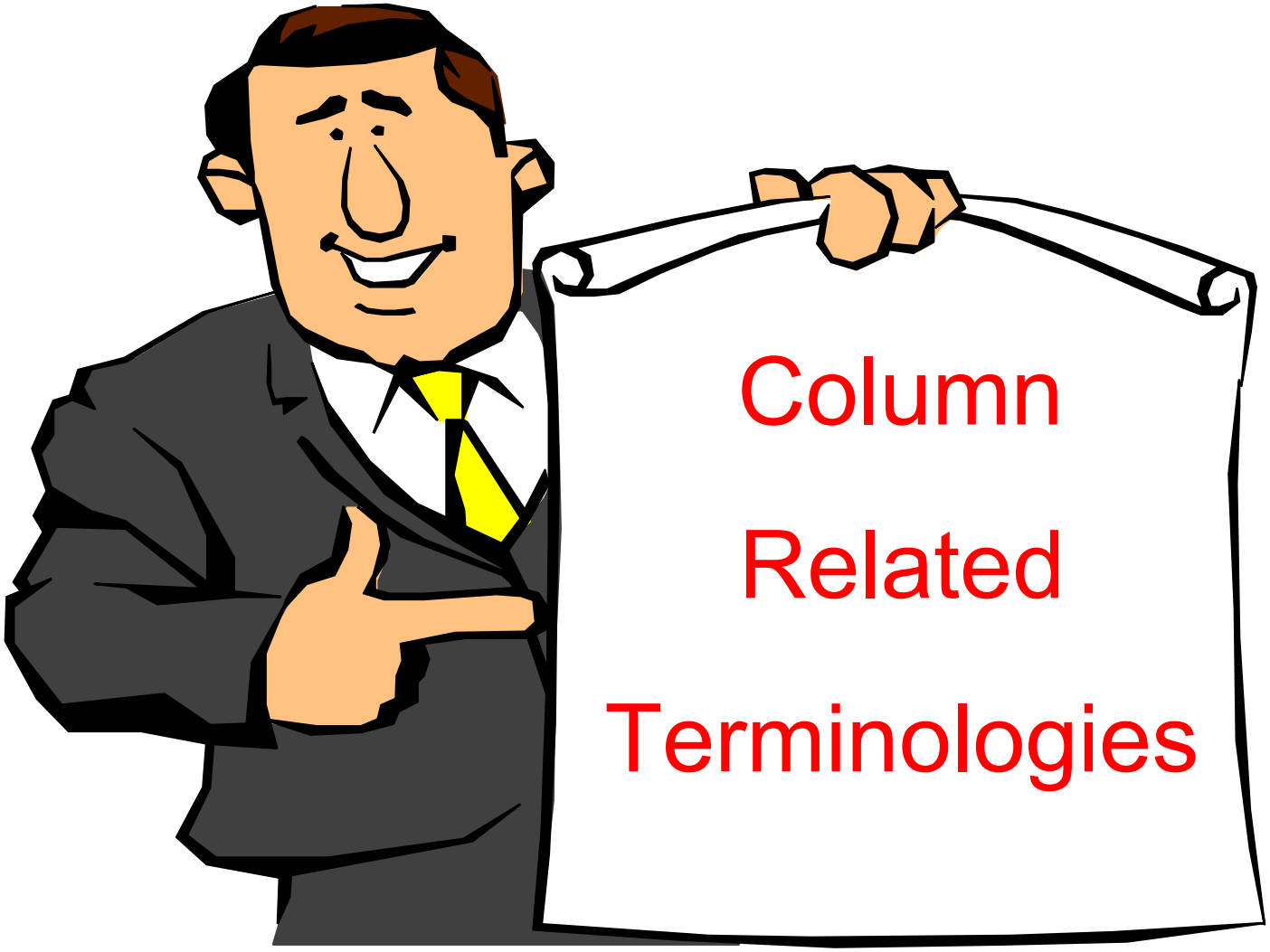
Your Scientific Specialist

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

Introduction – HPLC-System: General Design

- Pump with Degasser
- Autosampler
- Column (installed in a Column Compartment)
- Detector
- Computer with control software




Column
Related
Terminologies

Chromatography Terminology

Sample

The Original Representative Material Which Is To Be Analyzed Also Called The Sample Matrix (Coffee)

Analyte(s)

A Specific Compound(s) Contained In The Sample Which Is(Are) To Be Separated And Analyzed (Caffeine)

Compound

Pure Chemical Component In A Sample, Also Called An Analyte Or Solute

Chromatography Terminology

Stationary Phase

The Chromatographic Packing Material Which Is Held In A Fixed Position Usually Packed Into A Column, Or Coated Onto A Surface. It Performs The Chemical Separation (Also Called The Packing Material, The Chromatographic Material, Or The Adsorbent)

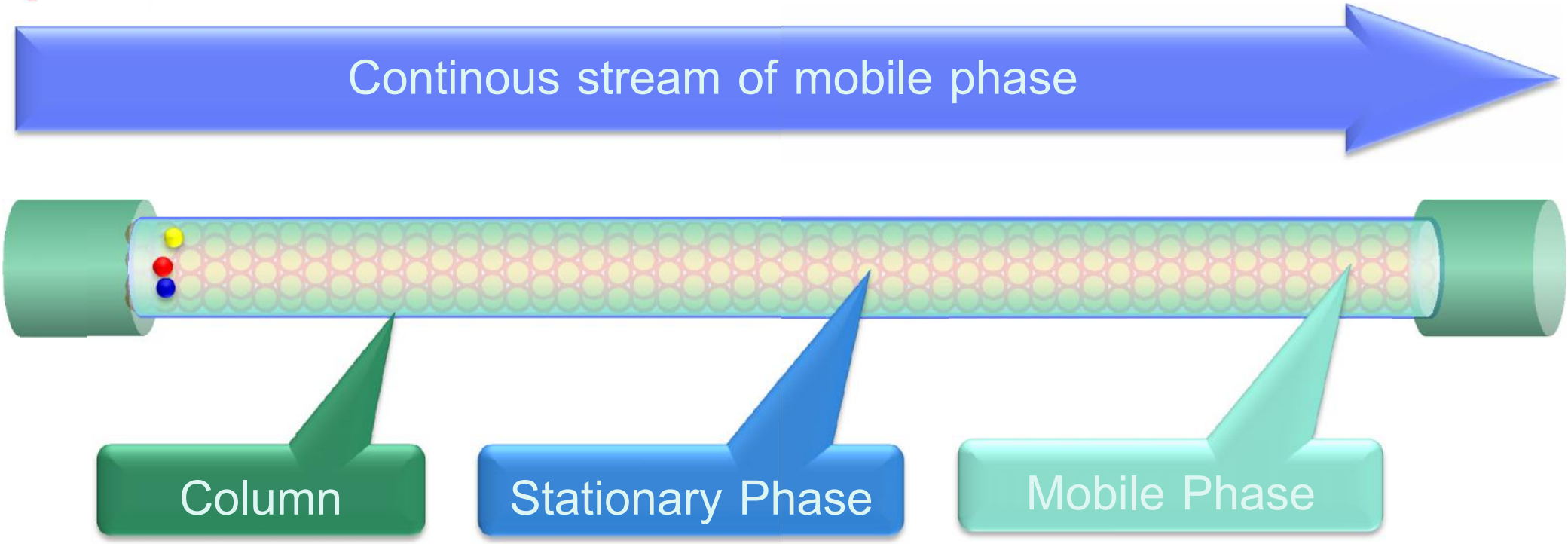
Mobile Phase

Carrier Of The Sample, Moving It Through The Stationary Chromatographic Packing Material. The Mobile Phase Can Be A Liquid (HPLC), Or A Gas (GC)

Remember!

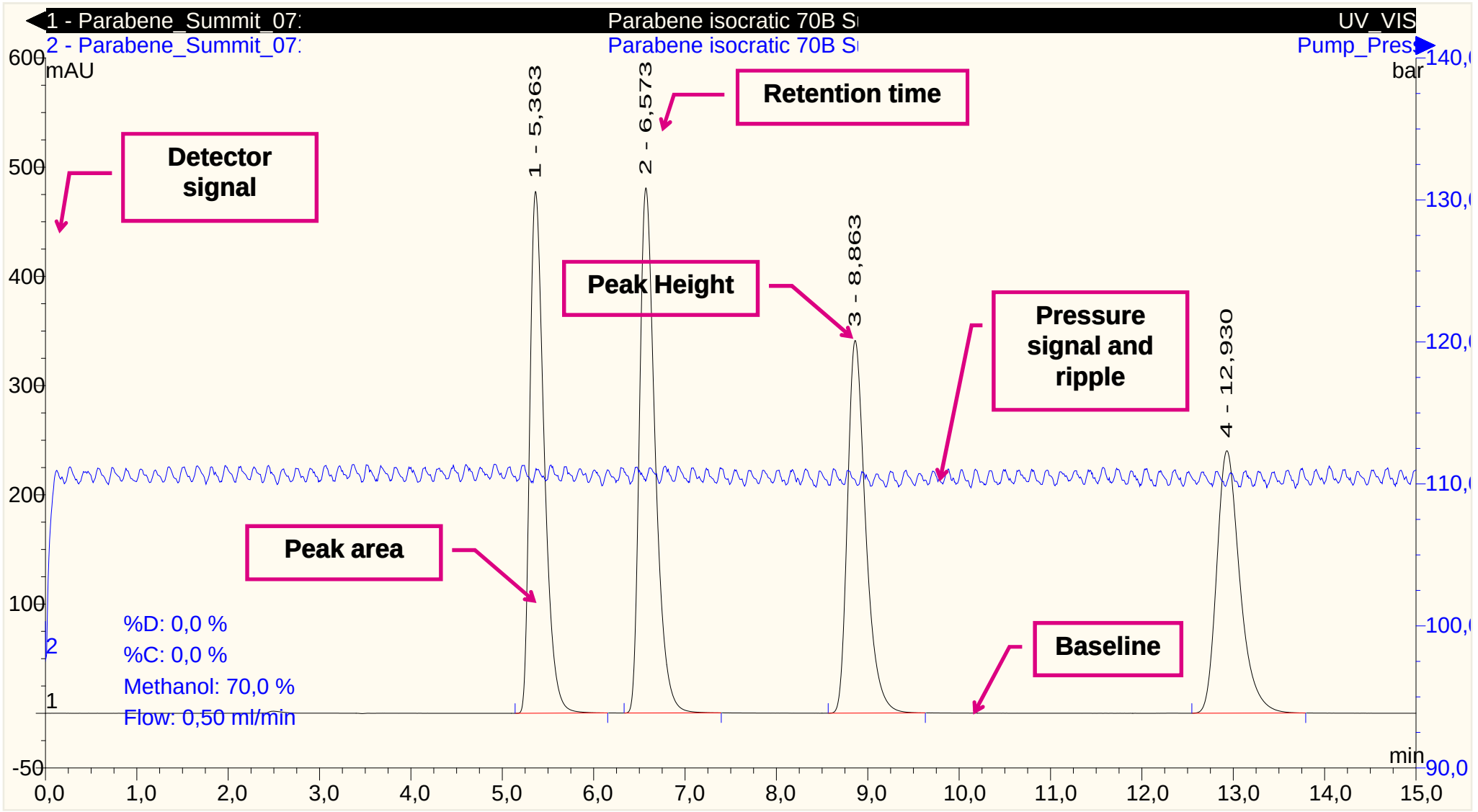
The Stationary Phase and The Mobile Phase will have *OPPOSITE PROPERTIES* to Set-Up Competition For Sample Matrix and Analytes

Introduction – The Chromatographic Process



- The stationary phase retains analytes due to various interactions.
- When different chemical components pass through the column at different rates they become separated in single zones.

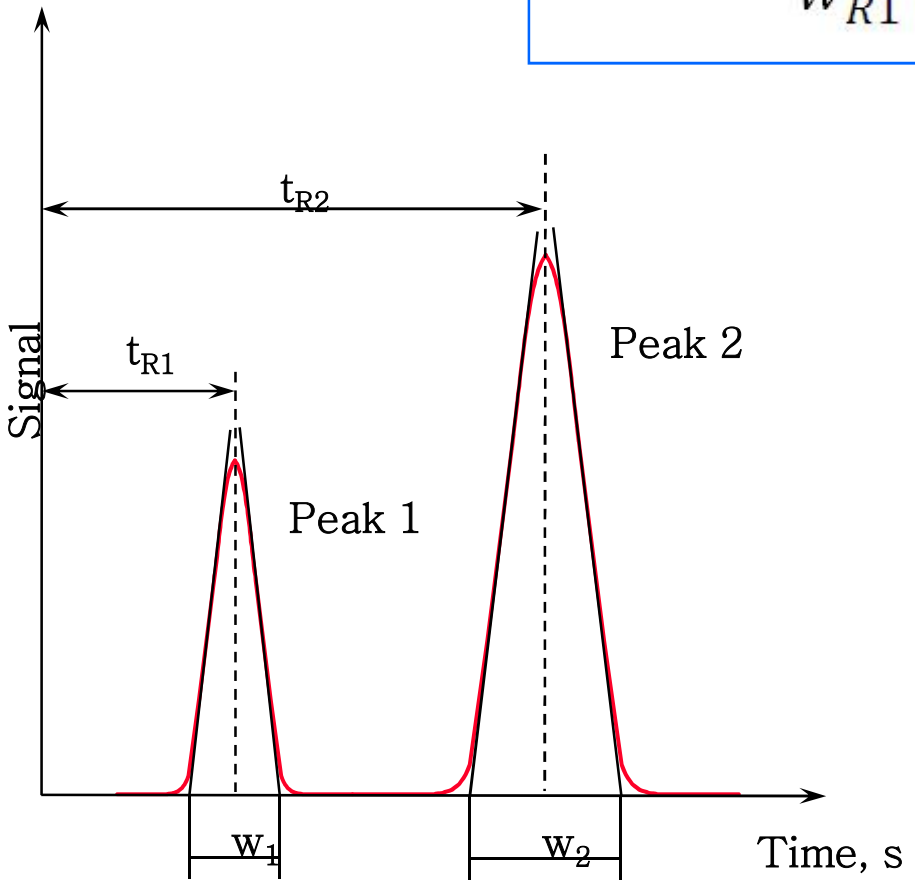
Characteristics of a Chromatogram



Theory of Chromatography

Resolution R of two peaks: Goal of every chromatographic method!

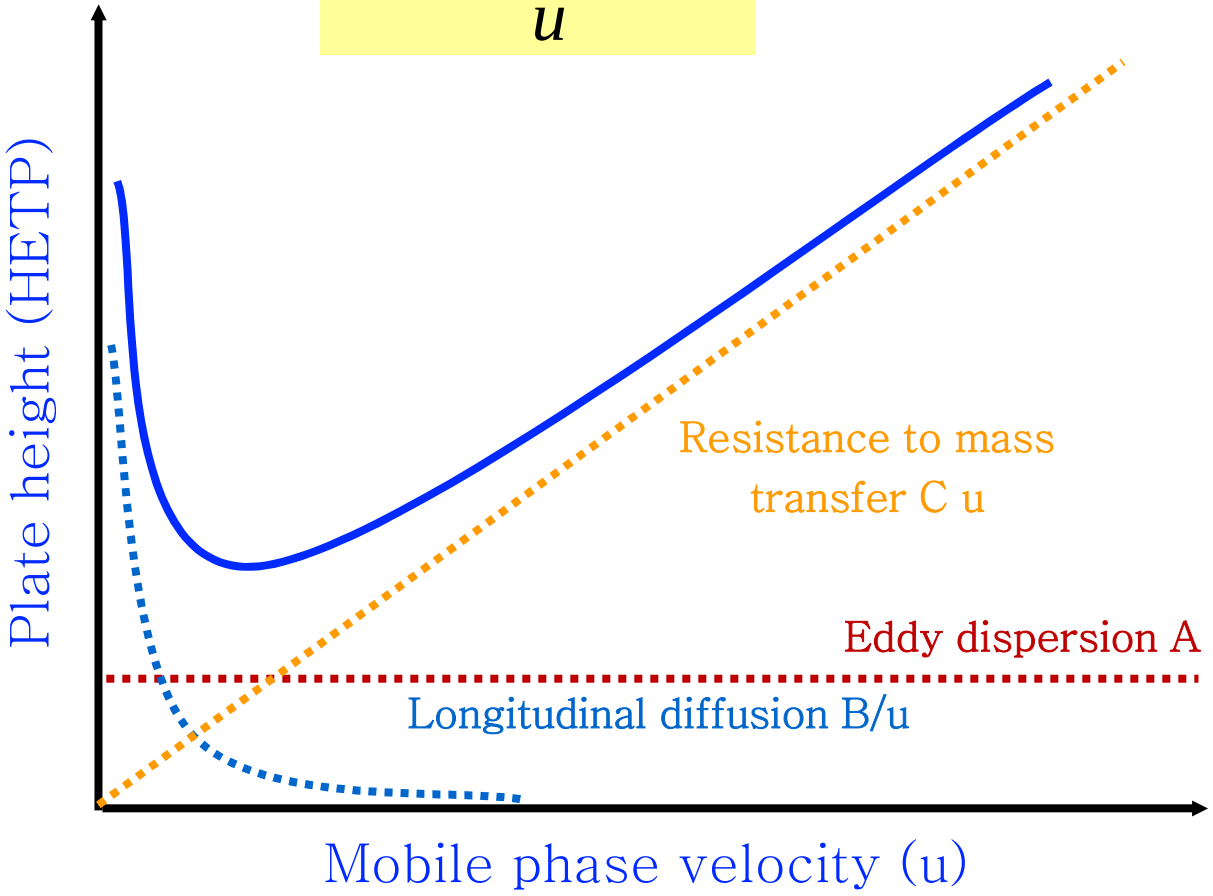
$$R = 2 * \frac{t_{R2} - t_{R1}}{w_{R1} + w_{R2}}$$

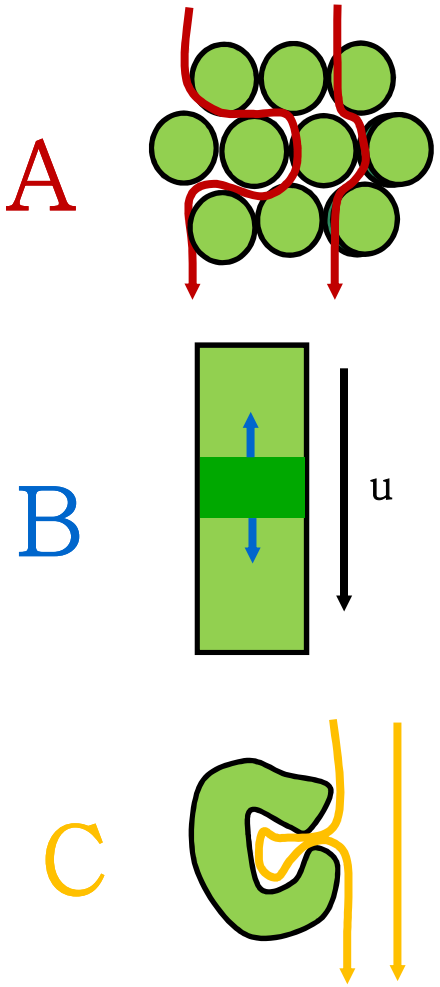


- Distance between the peak centers of two peaks divided by the average base width of the peaks.
- From theory R > 1.50 indicates baseline separation.
- In real life R ≥ 2 is usually the goal (requested in regulated environment).
- Much more resolution than 2 does not improve separation quality but increases analysis time.

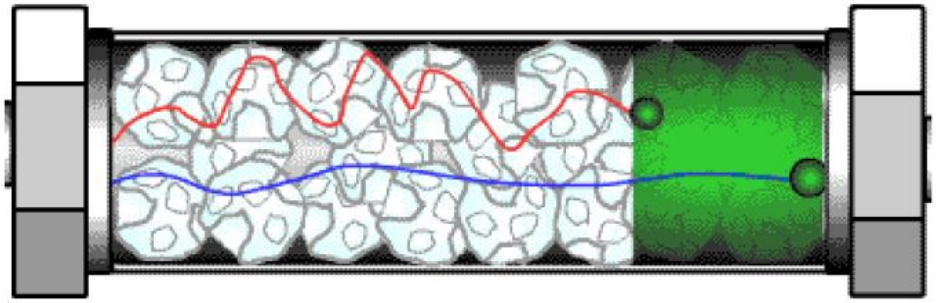
Van Deemter Plot

$$H = A + \frac{B}{u} + C \cdot u$$

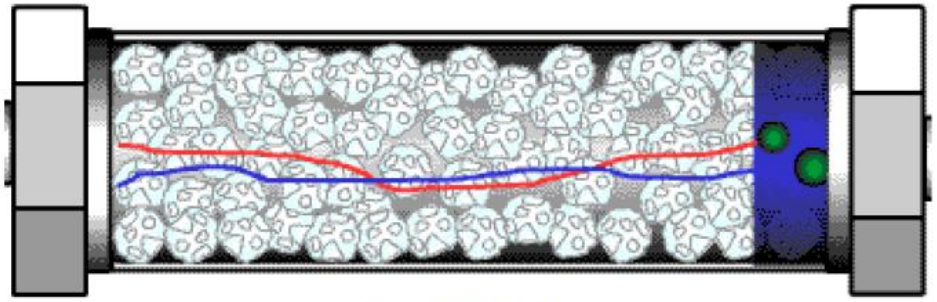




A Term – Eddy Diffusion



Large Particles



Small Particles

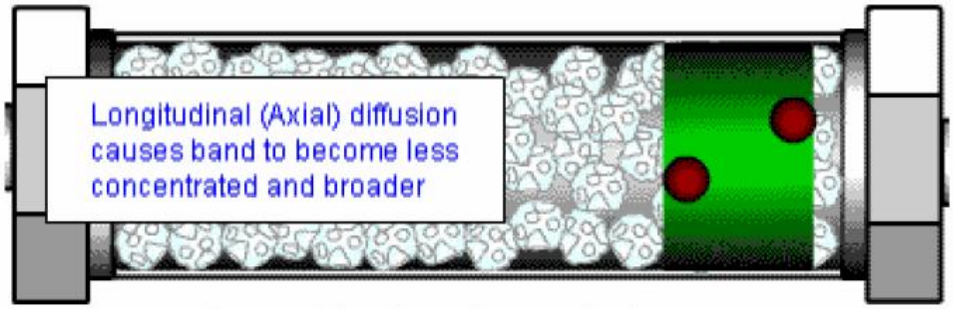
Band broadening due to Eddy Diffusion (A Term) in columns with ***large and small particles*** – effects on chromatographic peak shape (Efficiency (N))

Minimise Eddy Diffusion by:

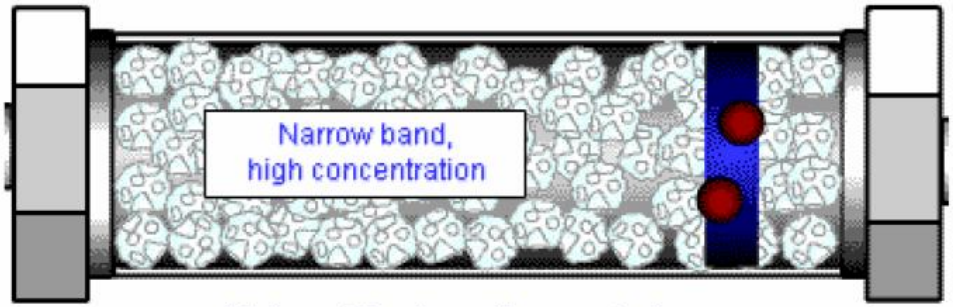
- Selecting well packed columns
- Using smaller stationary phase particles
- Using particles with a narrow size distribution

http://www.chromacademy.com/lms/sco3/Theory_Of_HPLC_Band_Broadening.pdf

B Term – Longitudinal Diffusion



Low mobile phase linear velocity



High mobile phase linear velocity

Band broadening due to Longitudinal Diffusion (B Term) in columns with ***low and high mobile phase linear velocity*** – effects on chromatographic peak shape (Efficiency (N))

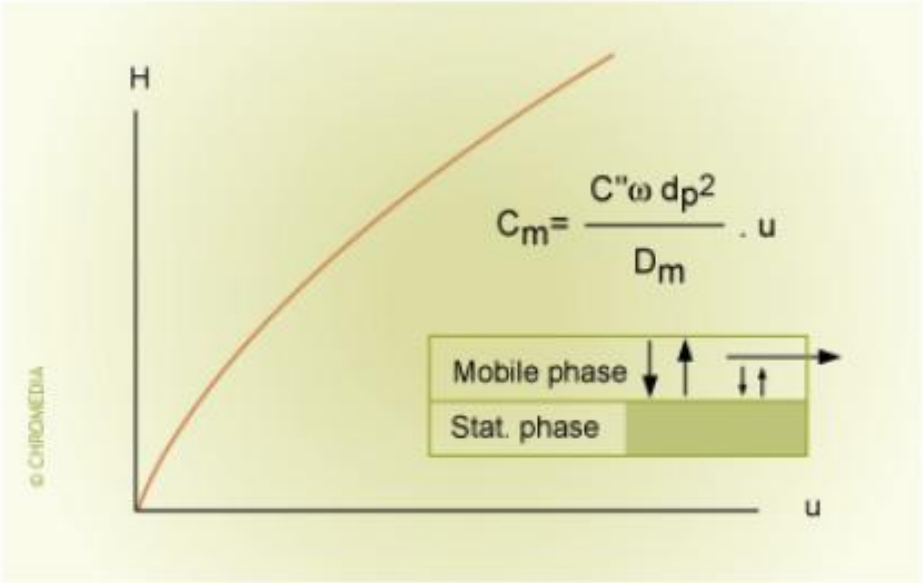
Minimise Longitudinal Diffusion by:

- Using higher mobile phase flow rates
- Keep system tubing short and as a narrow as possible (careful with back-pressure) **(<0.12mm i.d. is ideal)**
- Use correct nuts, ferrules and fittings wherever possible

http://www.chromacademy.com/lms/sco3/Theory_Of_HPLC_Band_Broadening.pdf

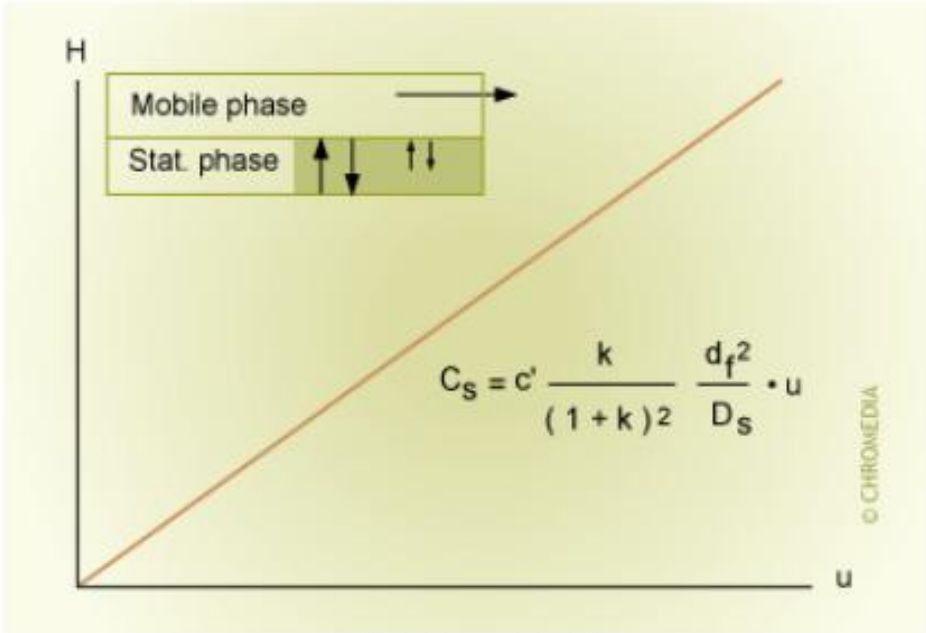
C Term – Mass Transfer

Resistance against mass transfer in mobile phase



C_m – term, describing the contributions to peak broadening in mobile phase

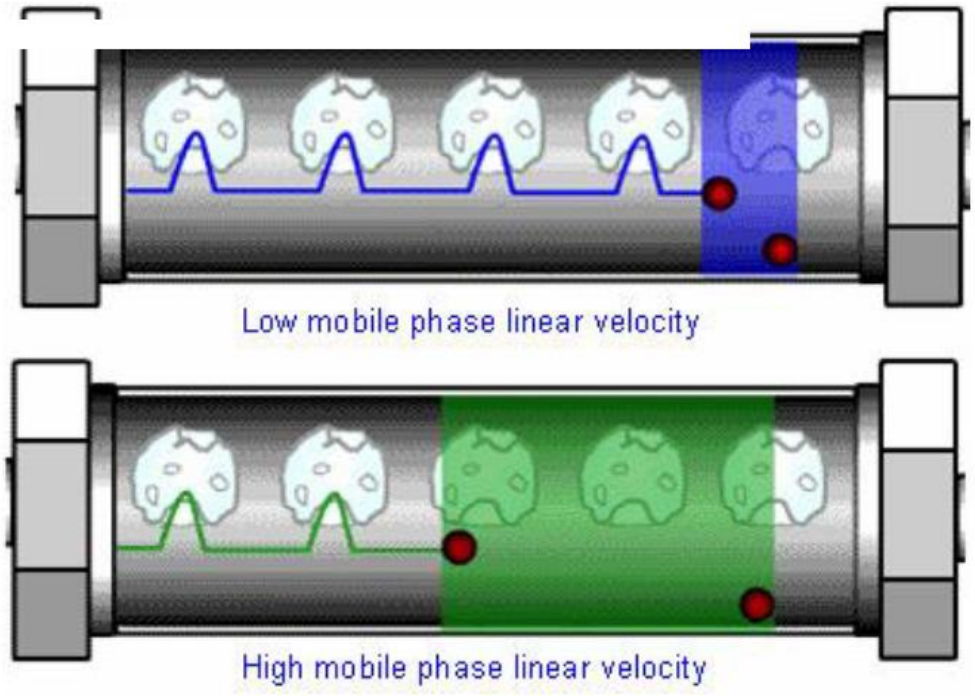
Resistance Against Mass Transfer in Stationary Phase



C_s – term, describing the contributions to peak broadening in stationary phase

https://chem.libretexts.org/Core/Analytical_Chemistry/Chromedia/01Gas_Chromotography_(GC)/Gas_Chromotography%3A_Basic_Theory/13The_Van_Deemter_equation

C Term – Mass Transfer



Band broadening due to Mass Transfer (C Term) in columns with *mobile phase linear velocity and stationary phase particle size* – effects on chromatographic peak shape (Efficiency (N))

Minimise Mass Transfer effects by:

- Using smaller (diameter) stationary phase particles
- Using lower mobile phase flow rates
- Heating the column (at higher temperatures the diffusion processes are speeded up and the differences in elution time from the particle pore are reduced)

http://www.chromacademy.com/lms/sco3/Theory_Of_HPLC_Band_Broadening.pdf

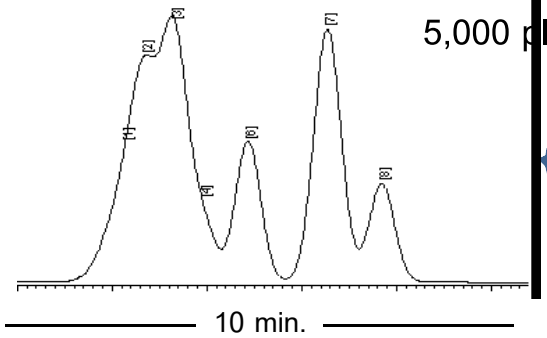
Particle Size Evolution

Late 1960's

40µm pellicular non-porous coated

100-500 psi (7-40 bar)

5,000 plates/meter

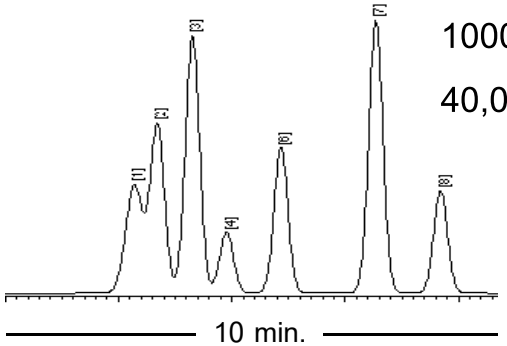


Early 1970's

10µm Irregular micro-porous

1000-2500 psi (70-180 bar)

40,000 plates/meter

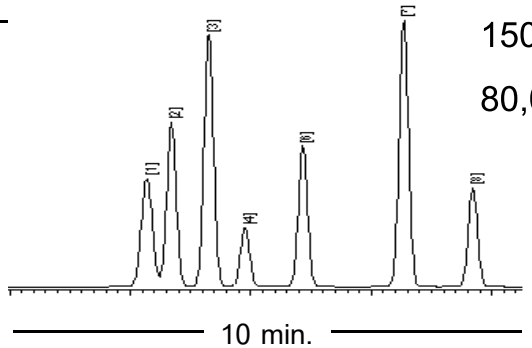


1980's to present day

3.5 - 5µm spherical micro-porous

1500-4000 psi (110-280 bar)

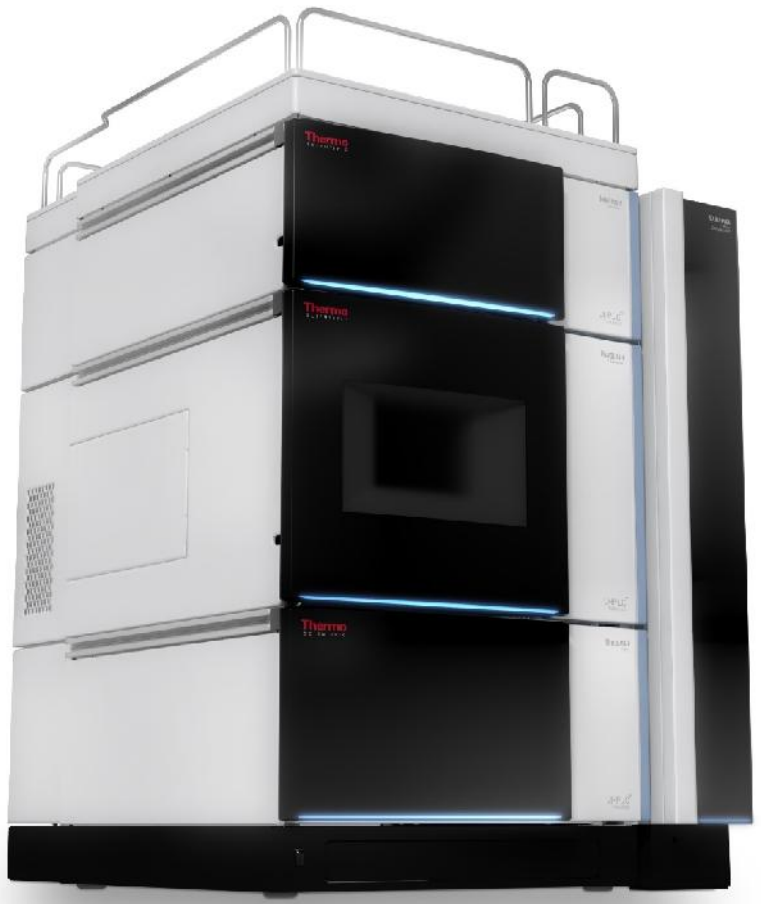
80,000 - 115,000 plates/meter



Thermo Analytical LC Systems

UltimateTH 3000

Max Pressure 1000 bar

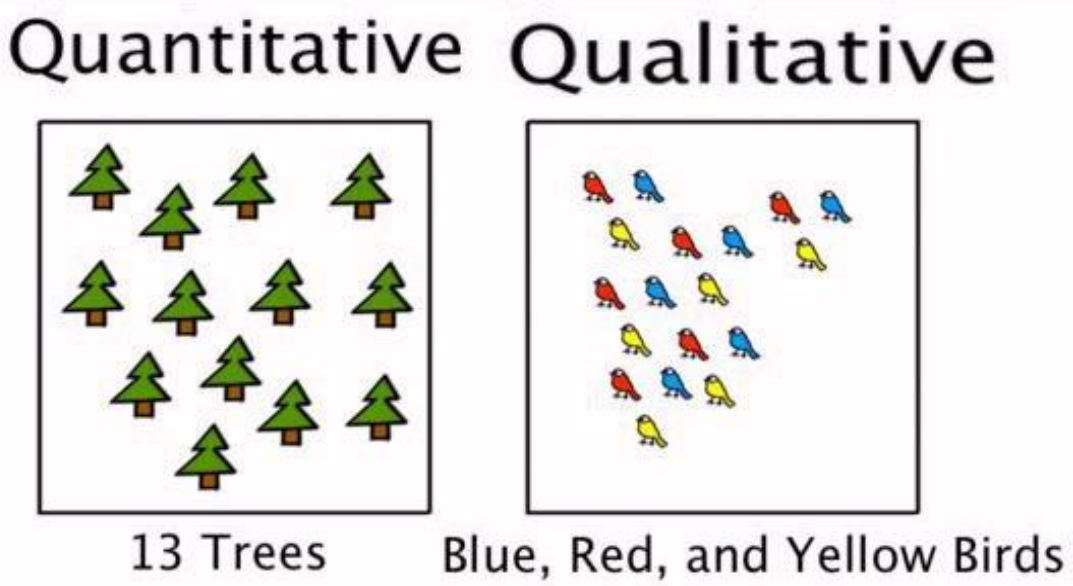


VanquishTH

Max Pressure 1517 bar

System Contribution – What Do We Want?

- **Quali**tative analysis
  - Resolved analytes
- **Quanti**tative Analyis
  - Reproducible peak areas



We want a reliable method working on a reliable system!

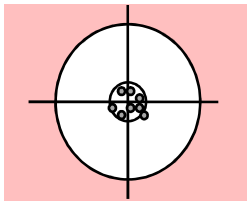
System Contribution – Accuracy and Precision

- Accuracy is the degree of closeness of a measured quantity to its true value

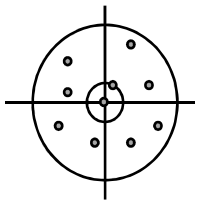
  ⇨ relevance for method transfer

- Precision is the degree of further measurements show the same results (reproducibility) ⇨ deviation of repeated measurements

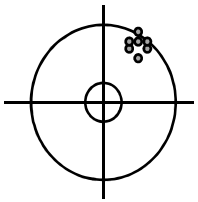
- The target analogy:



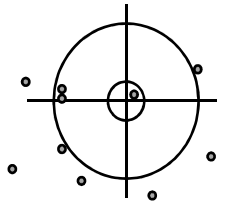
accurate and precise
(ideal result)



accurate, but not precise
(random errors)



precise, but not accurate
(systematic error)

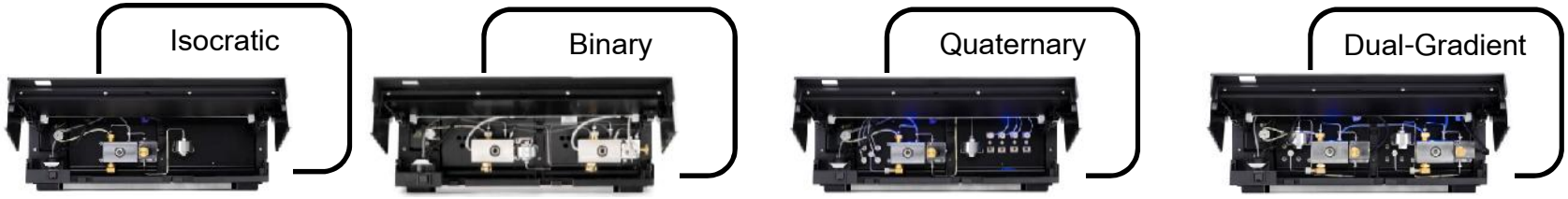


Neither accurate nor precise
(useless)

- A valid method or system is accurate and precise!

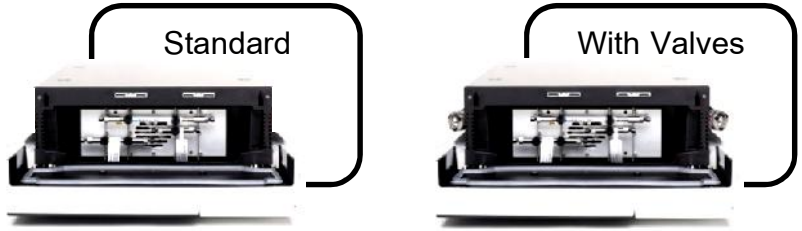
The UltiMate™ 3000 LC Systems

Pumps

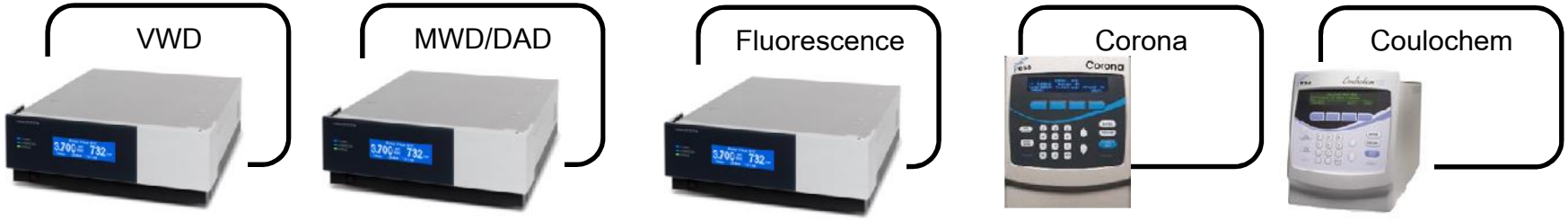


Autosampler

Column Compartments



Detectors



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.

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

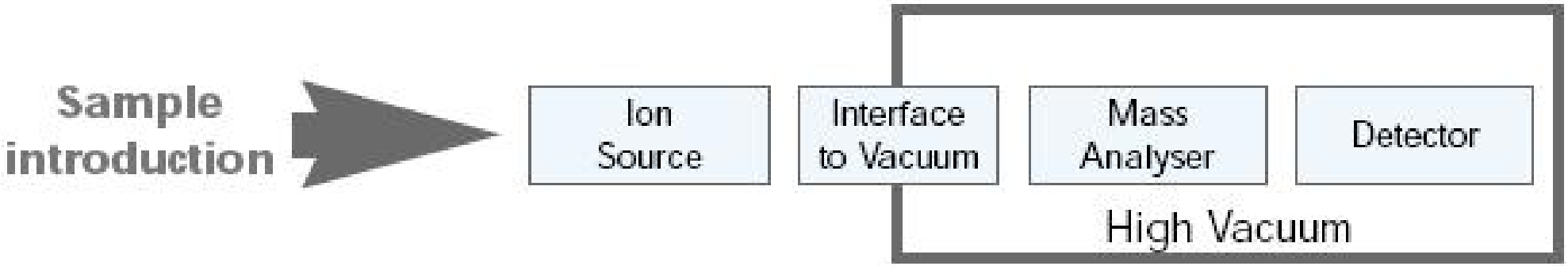
- Determine the mass are separated according to their mass-to-charge (m/z) ratio

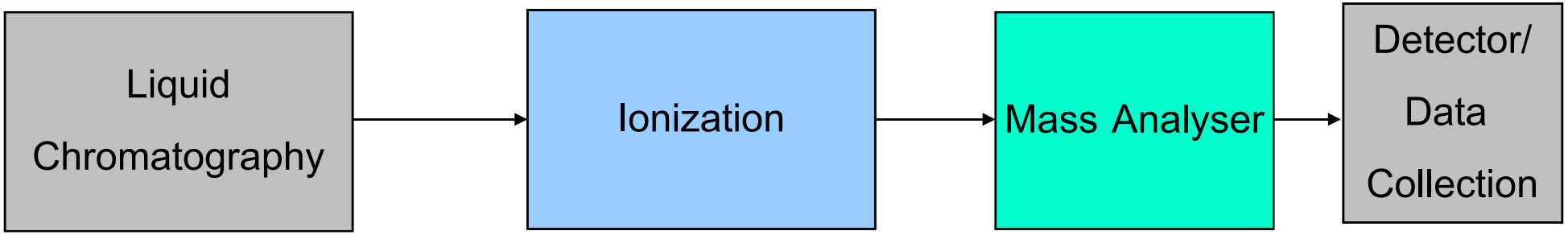
Mass Spectrometry “Simplified”

Generate	→	Ion Production
Move	→	Ion Optics
Select	→	Analyser (Quadrupole, Orbitrap)
Detect	→	Electron Multiplier

The lifetime of an ion from the point of formation to detection is approximately 50 to 100 microseconds

Mass Spectrometry – Block Diagram





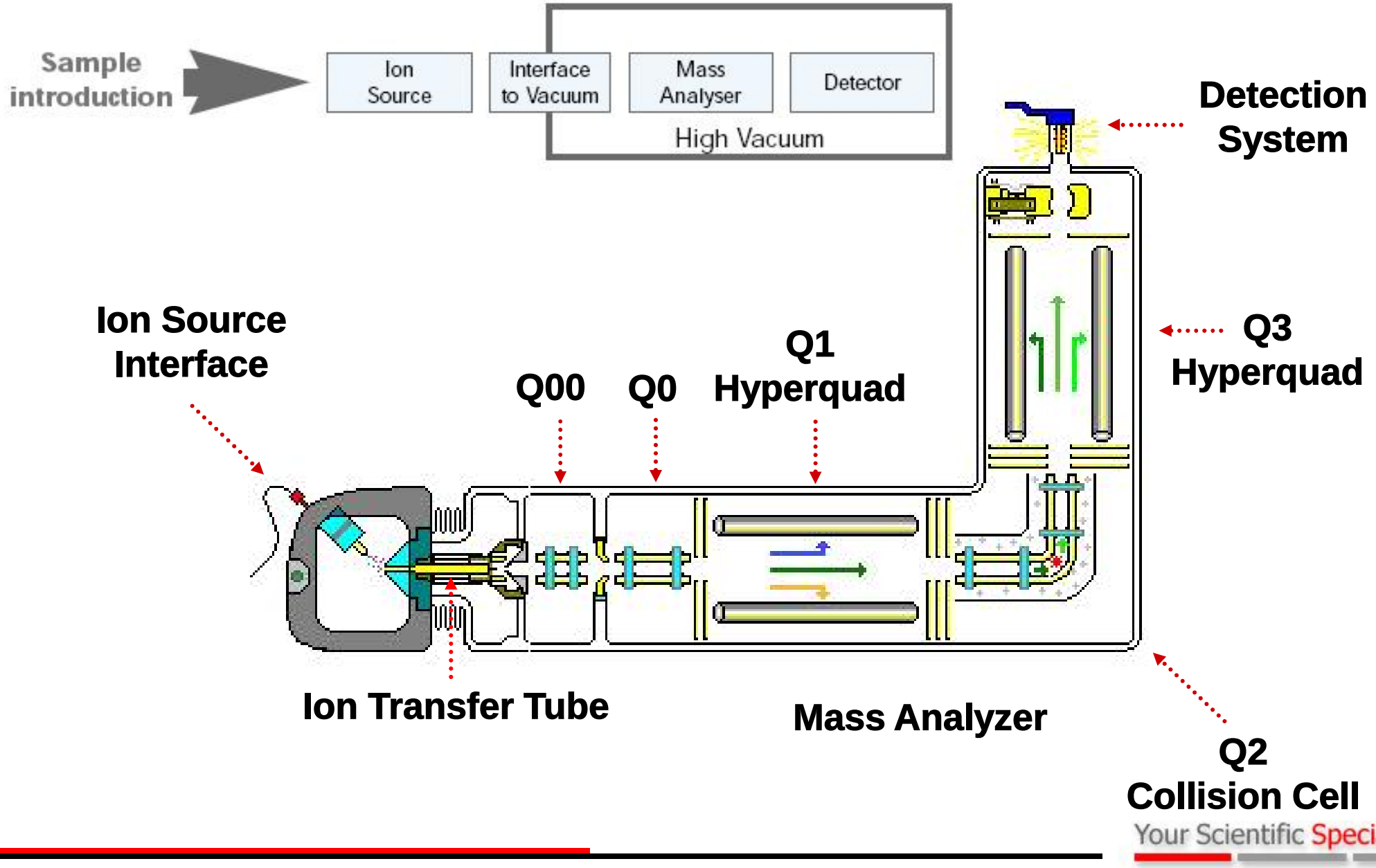
Very important!

- Many columns
- Many solvent systems

- ESI
- APCI
- APPI

- Triple Quadrapoles
- Ion-Traps
- Orbitrap

TSQ Quantum Components



ION SOURCE

IONIZATION TECHNIQUES

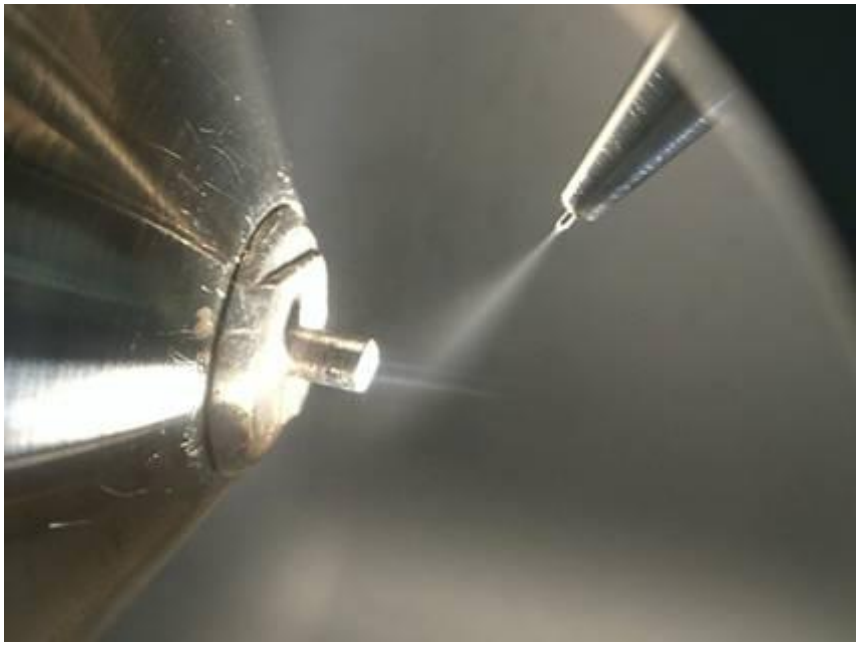
Type of Ionization techniques

- 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)

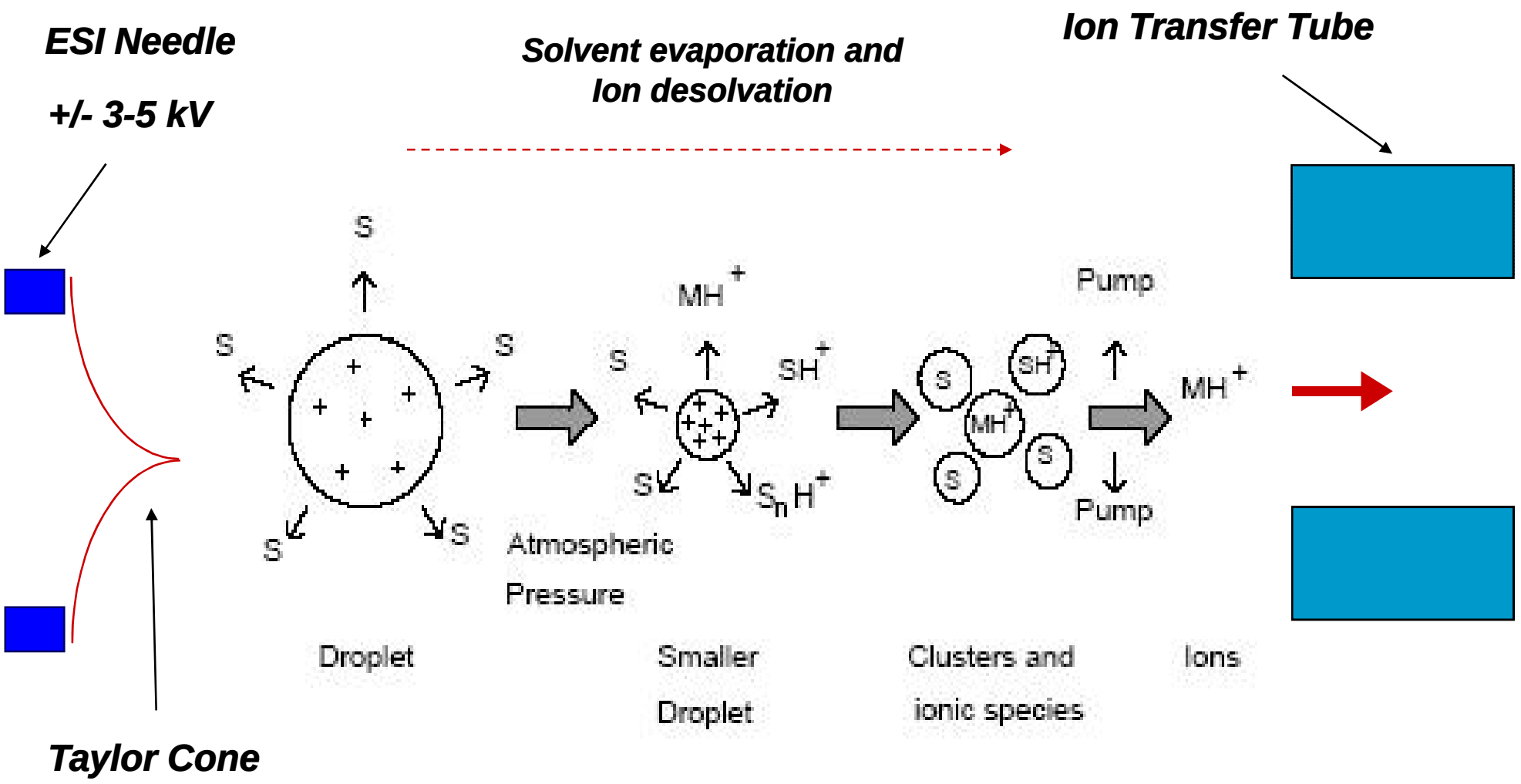
Electrospray Ionization (ESI)

Three Fundamental Processes:

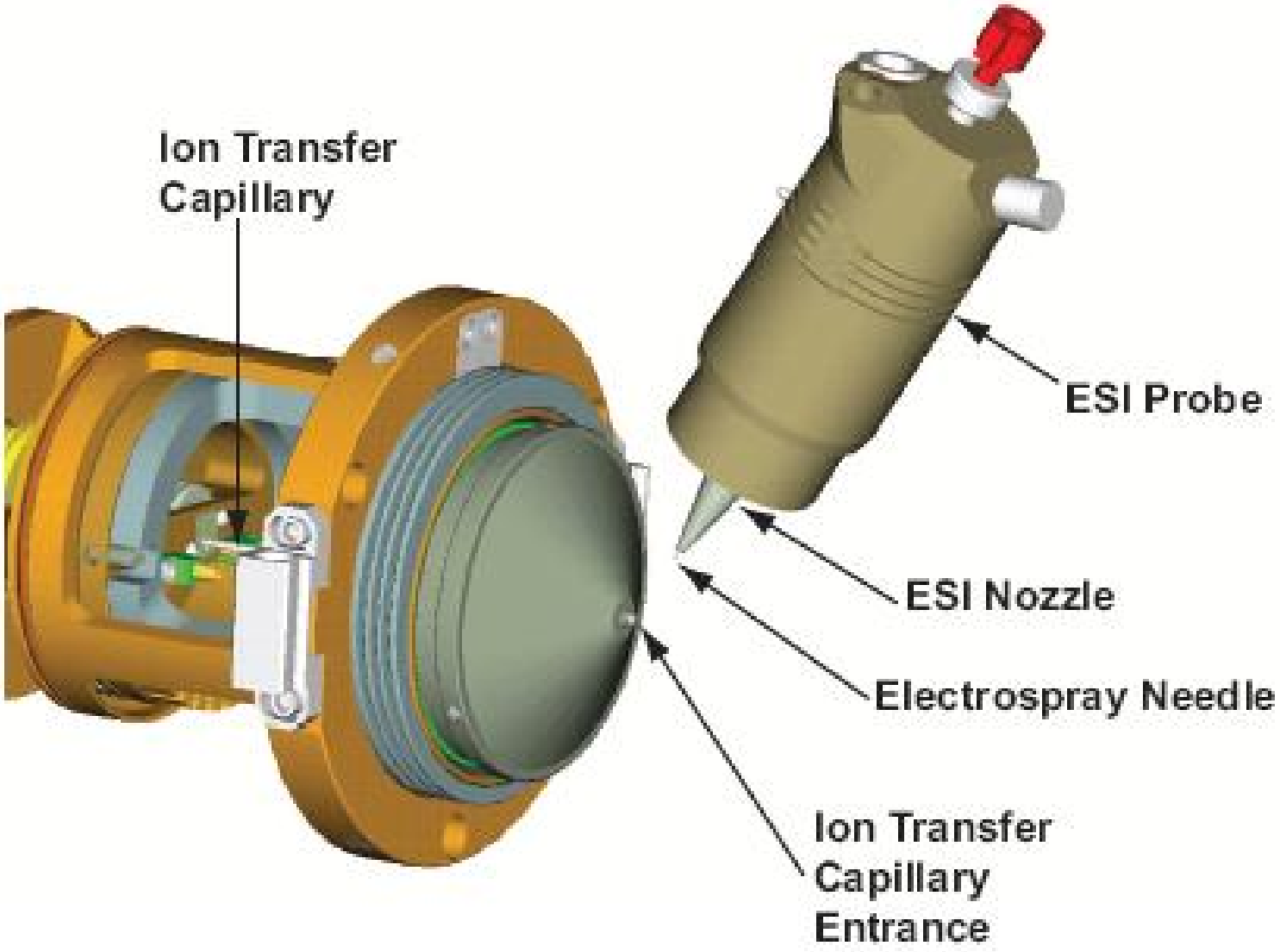
1. Production of **charged** droplets.
2. Droplet **size reduction**, and fission.
3. **Gas phase ion** formation.



Electrospray Ionization (ESI)



ESI - Ion Max Source



Atmospheric Pressure Chemical Ionization (APCI)

- Gas phase ionization via corona discharge
- APCI is a three-step process:

1. High voltage (via corona needle) interacts with both the nitrogen carrier gas and the vaporized HPLC solvent to produce primary ions:

$$O_2 + e^- \rightarrow O_2^{+\cdot} + 2e^-$$

$$N_2 + e^- \rightarrow N_2^{+\cdot} + 2e^-$$

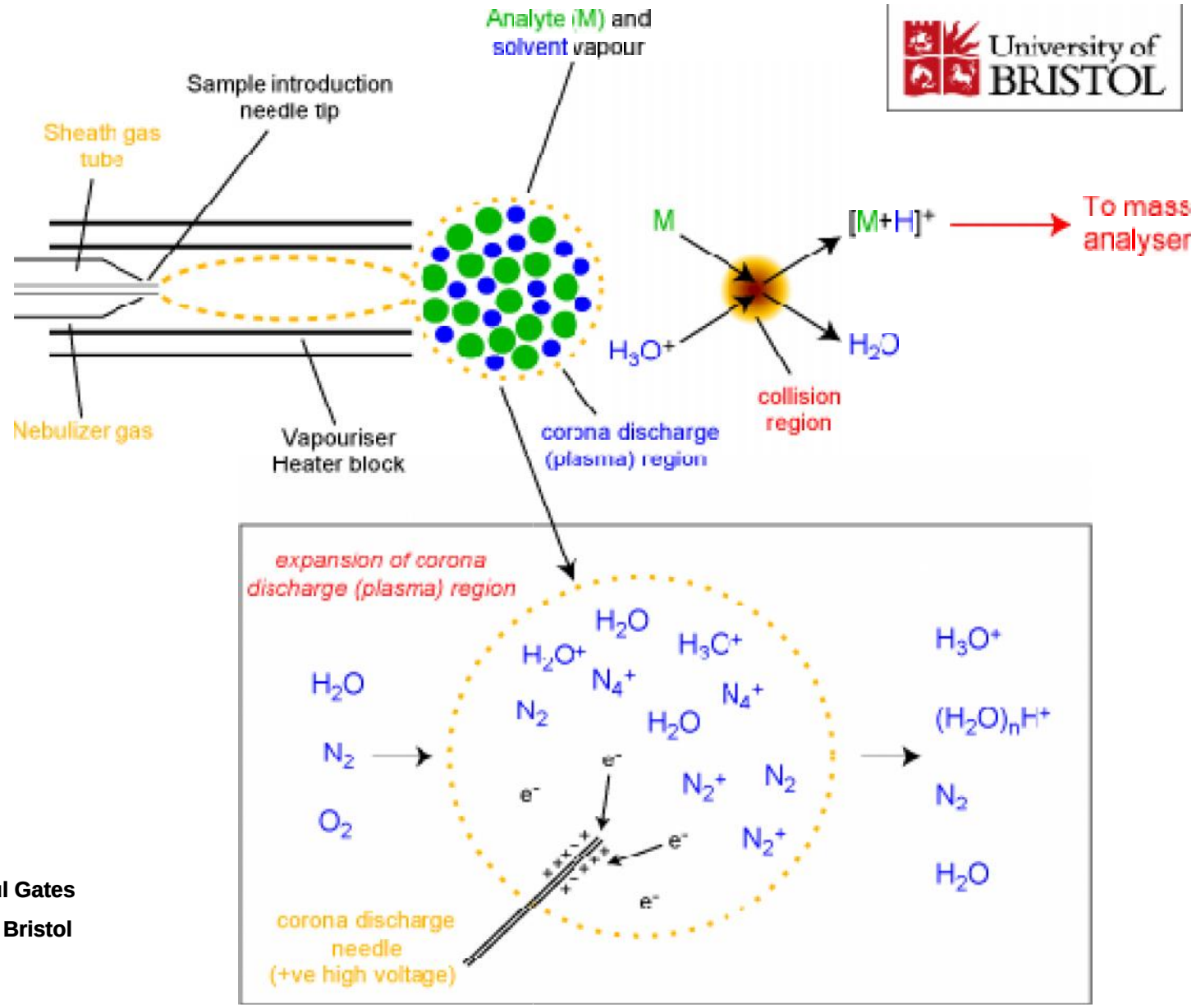
2. Through a complex series of reactions primary ions react with solvent molecules forming reagent ions, H_3O^+ and $CH_3OH_2^+$

3. Reagent ions react with analyte molecules forming $(M+H)^+$ in positive ion mode or $(M-H)^-$ in negative ion mode:

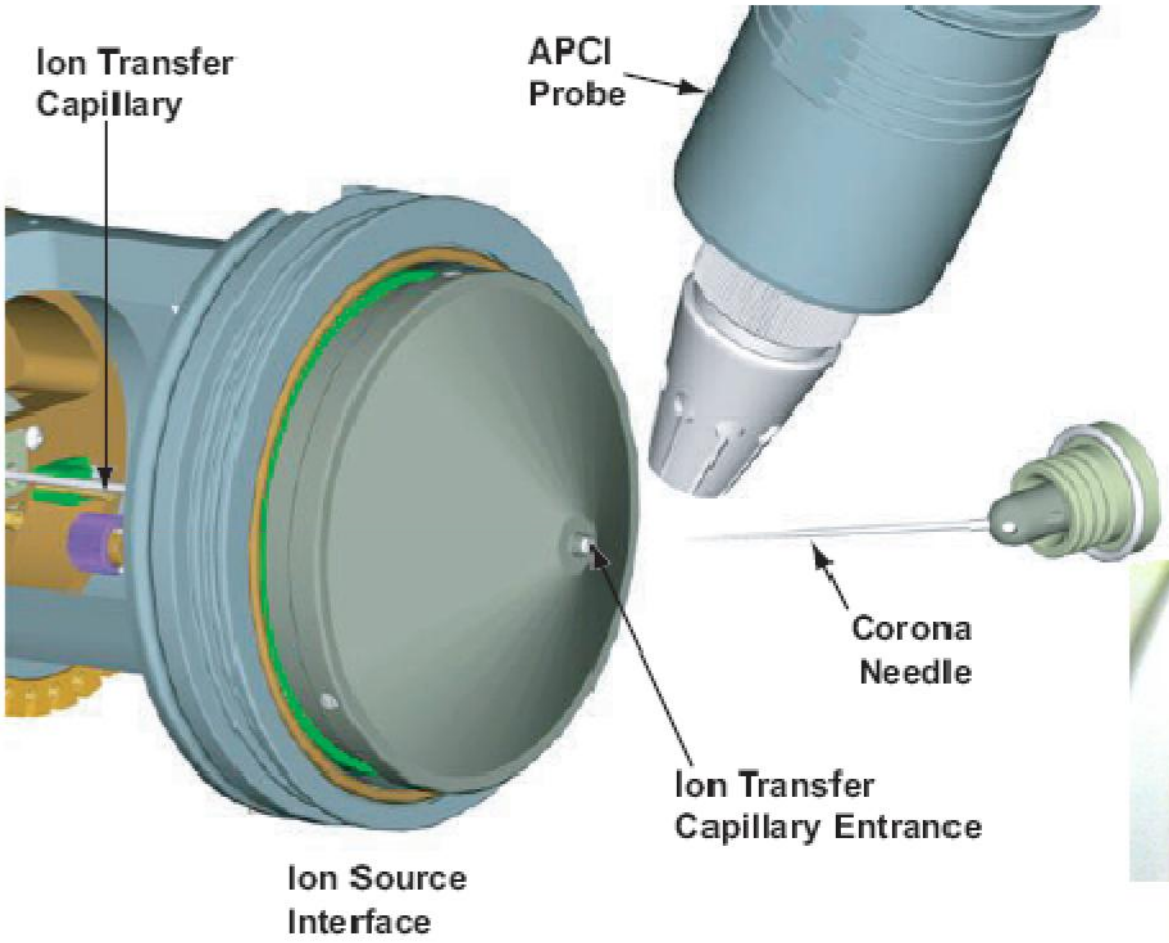
$$H_3O^+ + \text{Analyte} \rightarrow (\text{Analyte} + H)^+ + H_2O$$

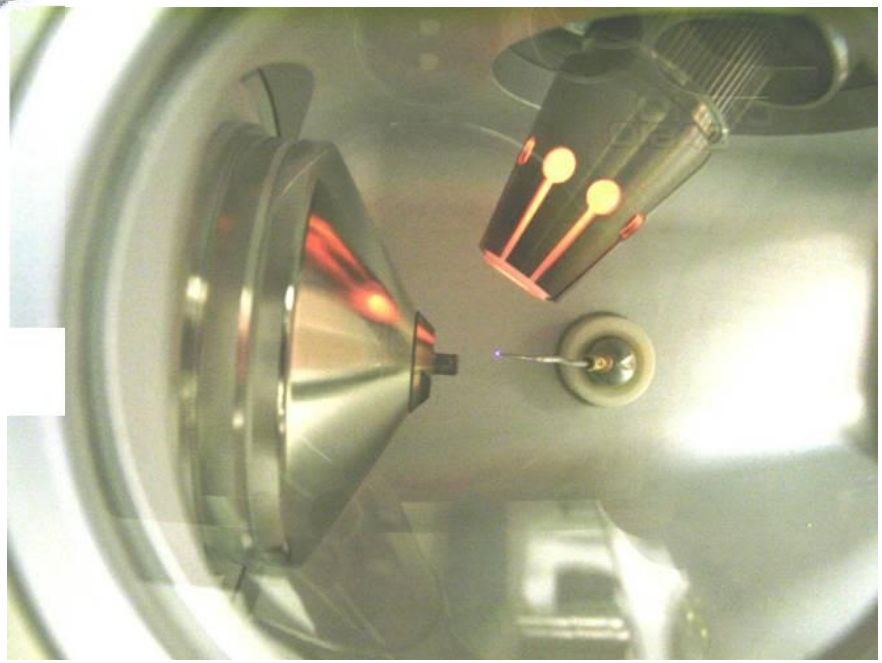
$$OH^- + \text{Analyte} \rightarrow (\text{Analyte} - H)^- + H_2O$$

Atmospheric Pressure Chemical Ionization (APCI)



Ion Max Source Design - APCI Probe

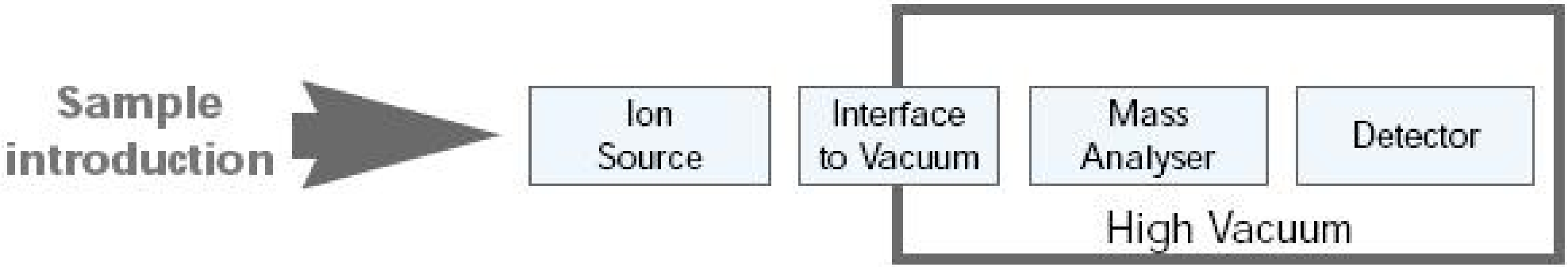


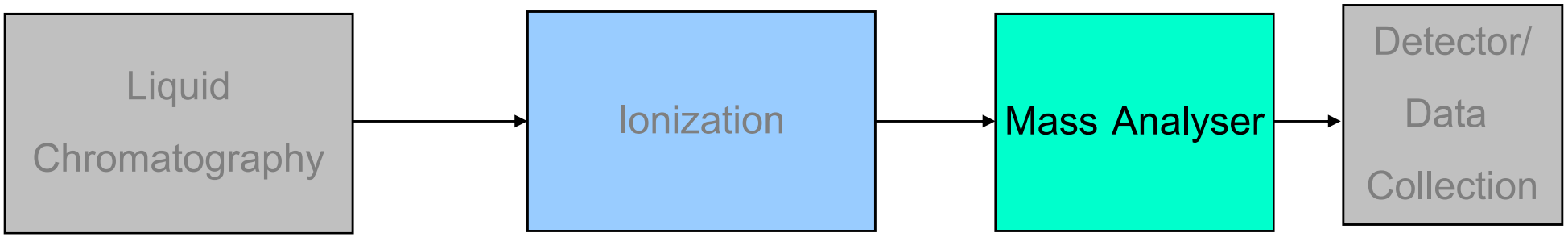


Which is Best?

- 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





- Triple Quadrapoles
- Ion-Traps
- Orbitrap

MASS ANALYSER

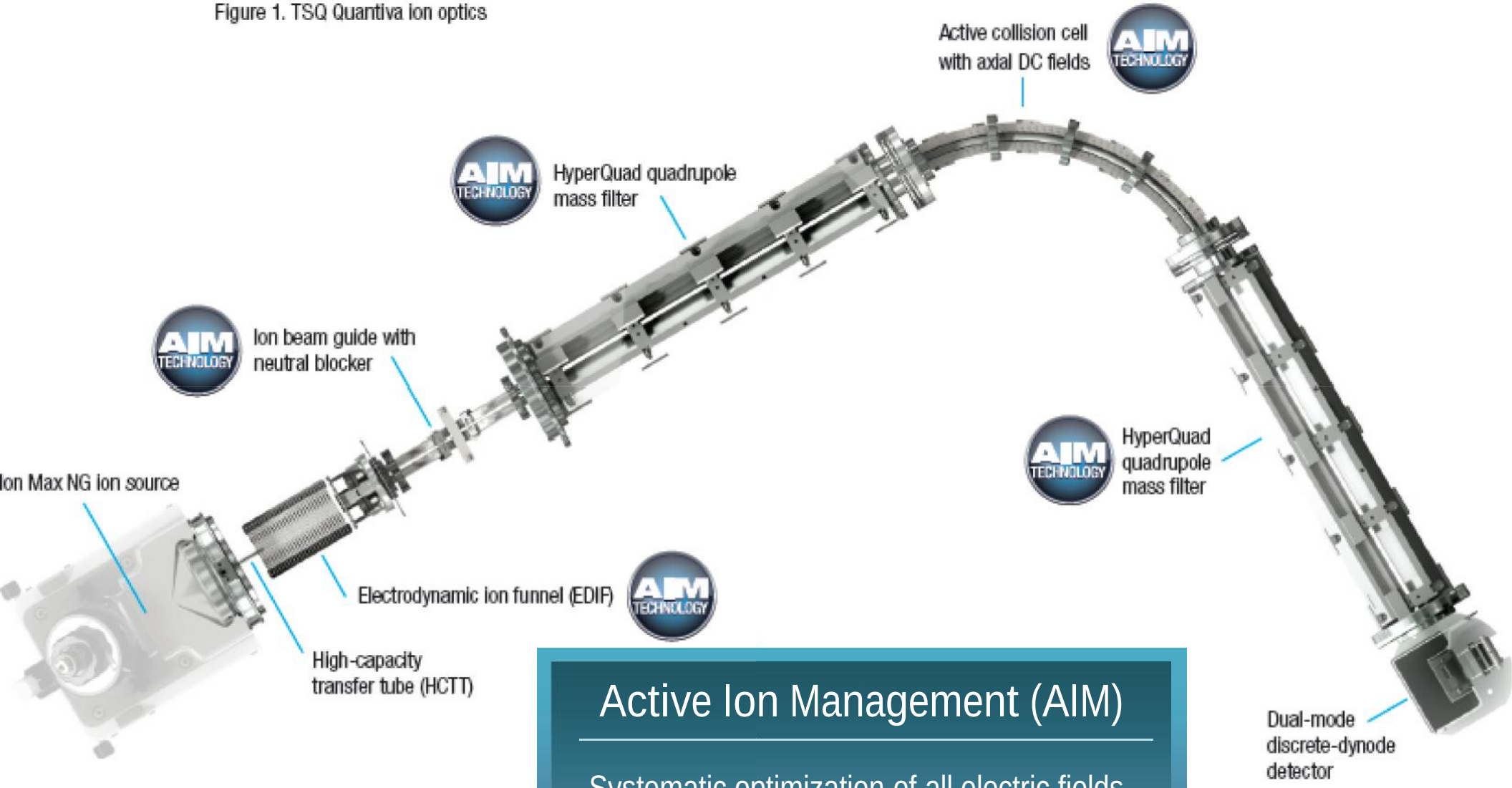
QUADRUPLE

Mass analyzers separate ions

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

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.

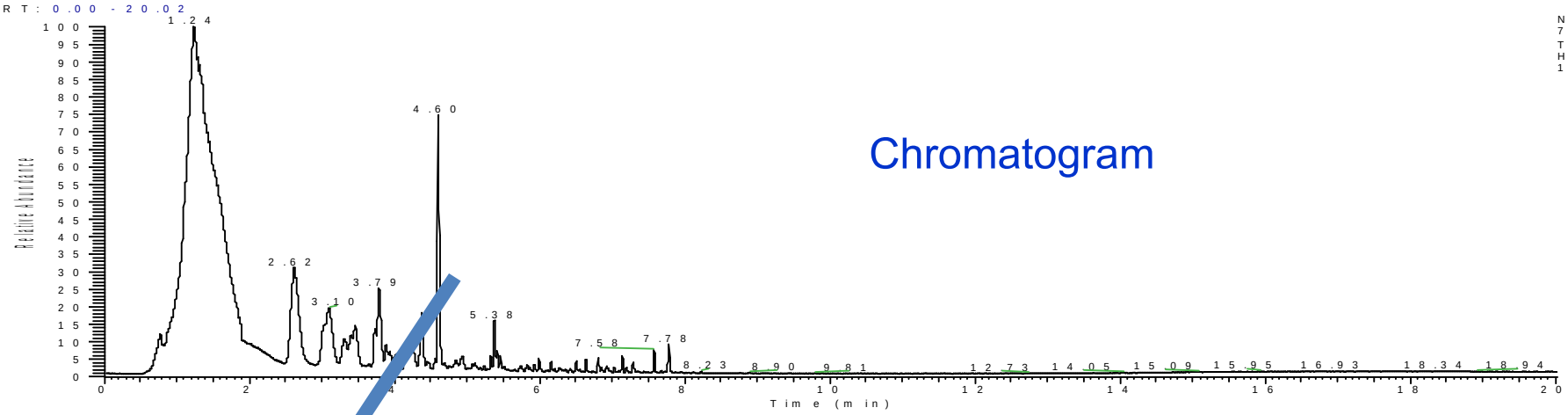
TSQ Quantiva Animation (available on YouTube)

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

Scan Modes

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

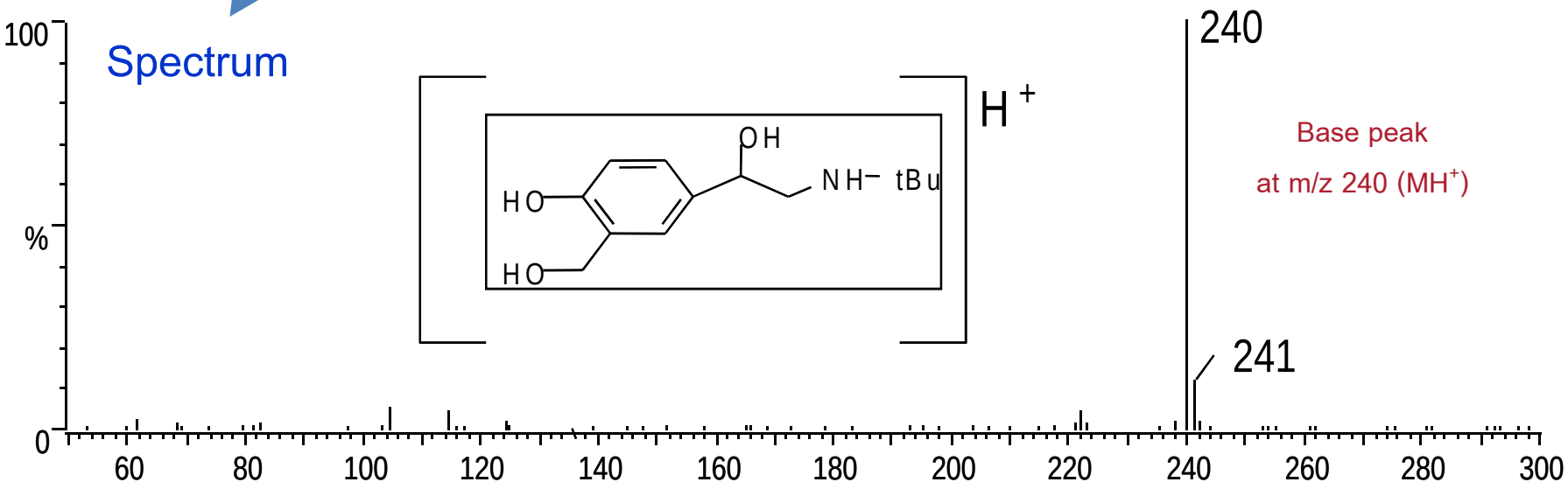
Full Scan Mode



Chromatogram

One Click

Spectrum



Base peak

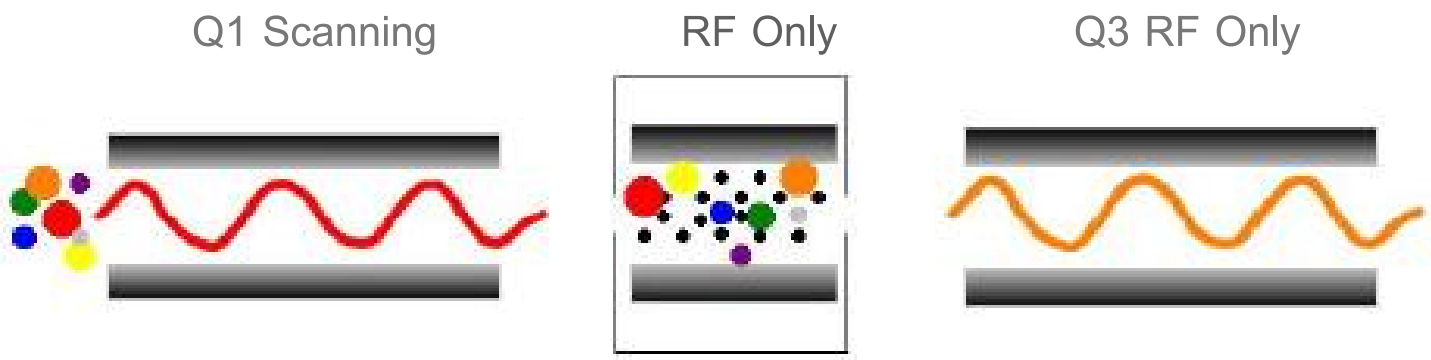
at m/z 240 (MH^+)

Full Scan (Q1 or Q3)

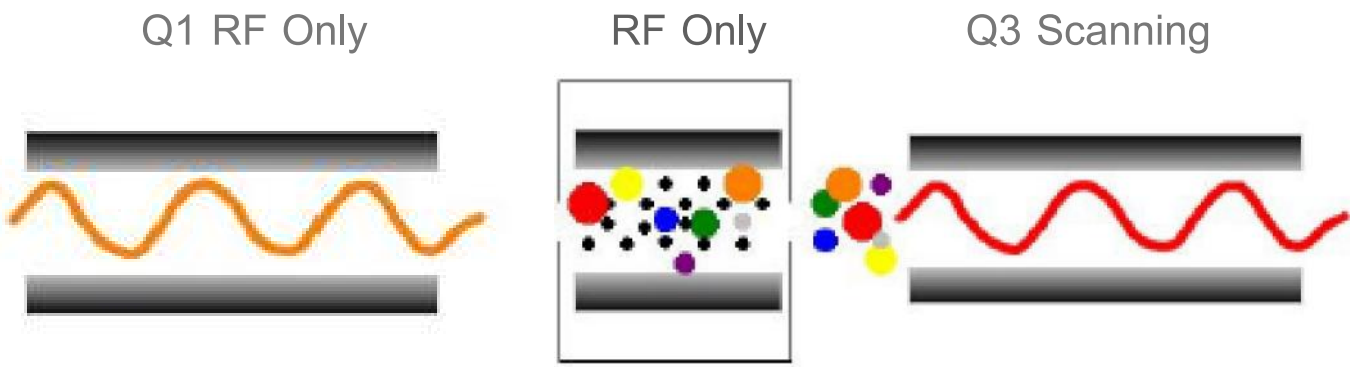
Full Scan Mode

Purpose: Survey scan of a chromatographic peak

Full scan Q1:



Full Scan Q3:

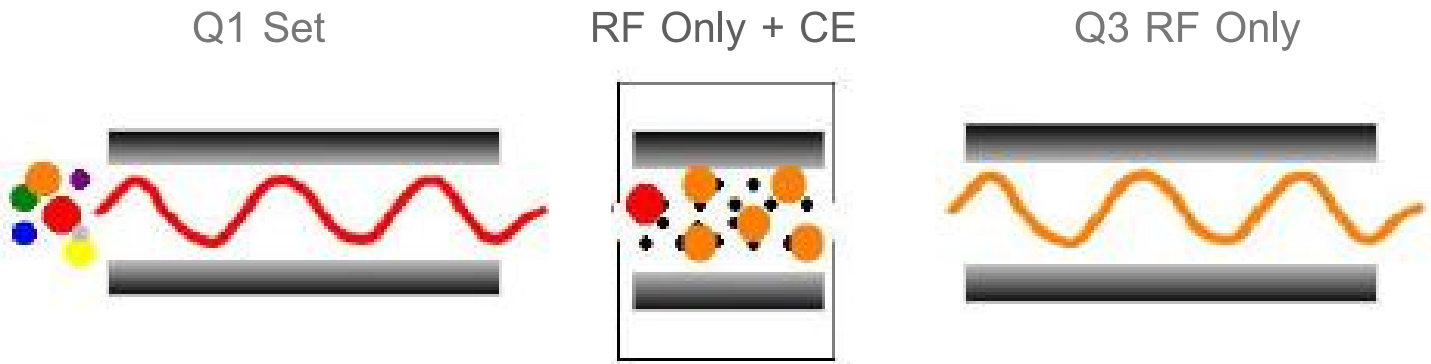


Selected Ion Monitoring – SIM

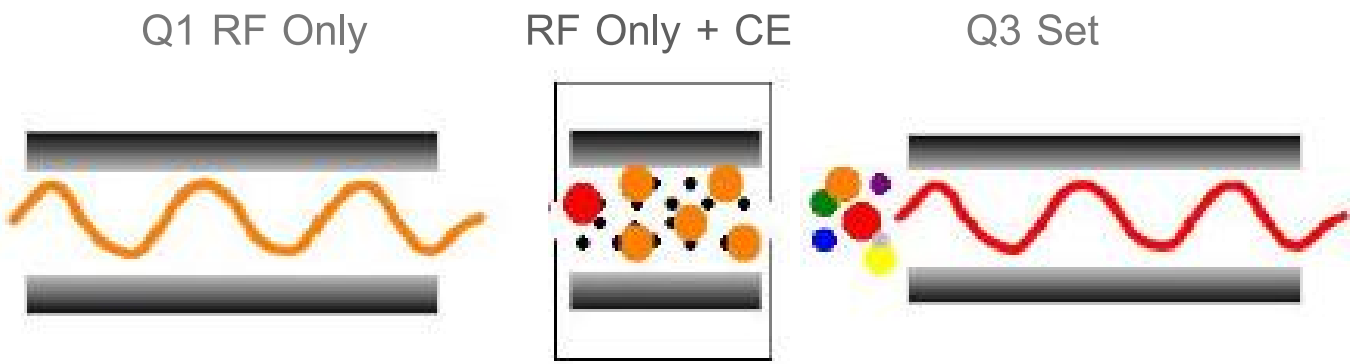
SIM Mode

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

SIM Q1:

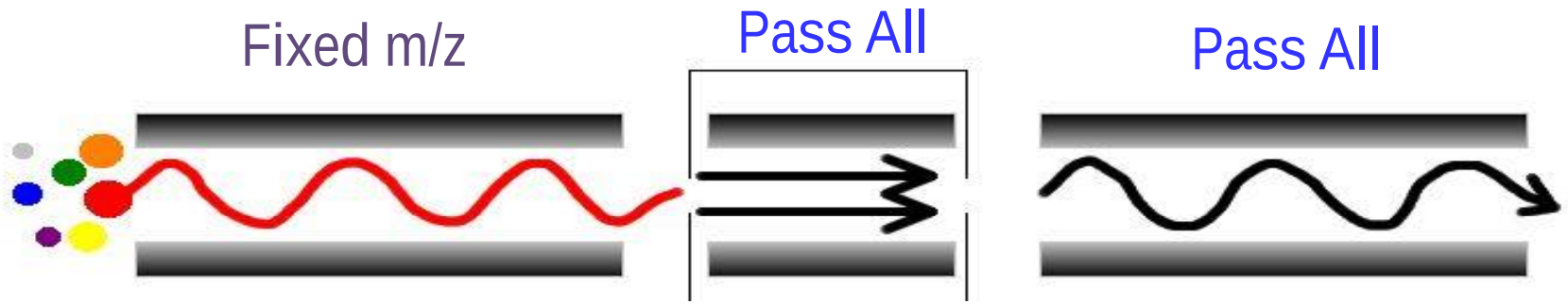


SIM Q3:



Selected Ion Monitoring – SIM

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



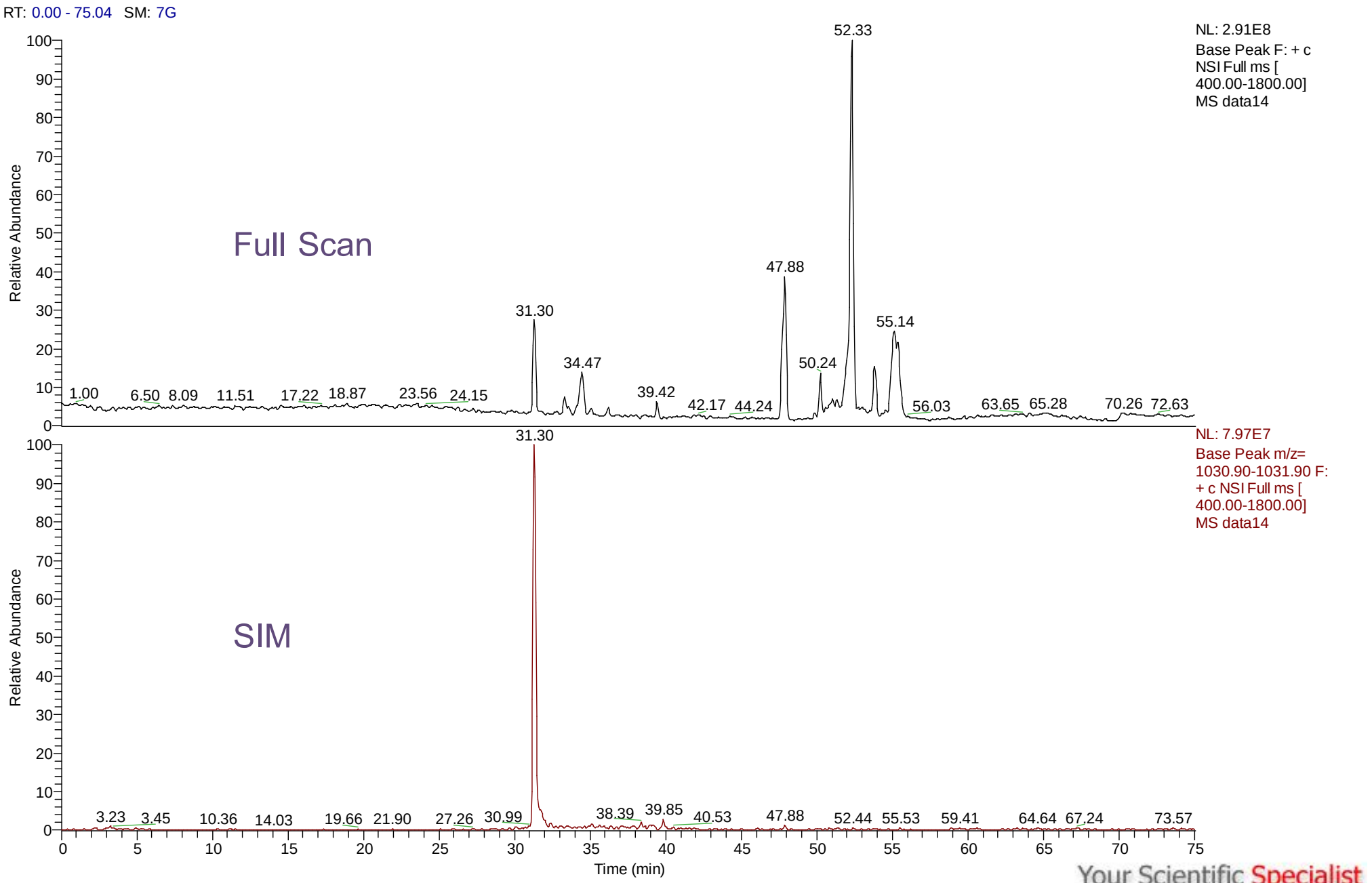
Advantages

- Targeted analyte monitoring
- High duty cycle

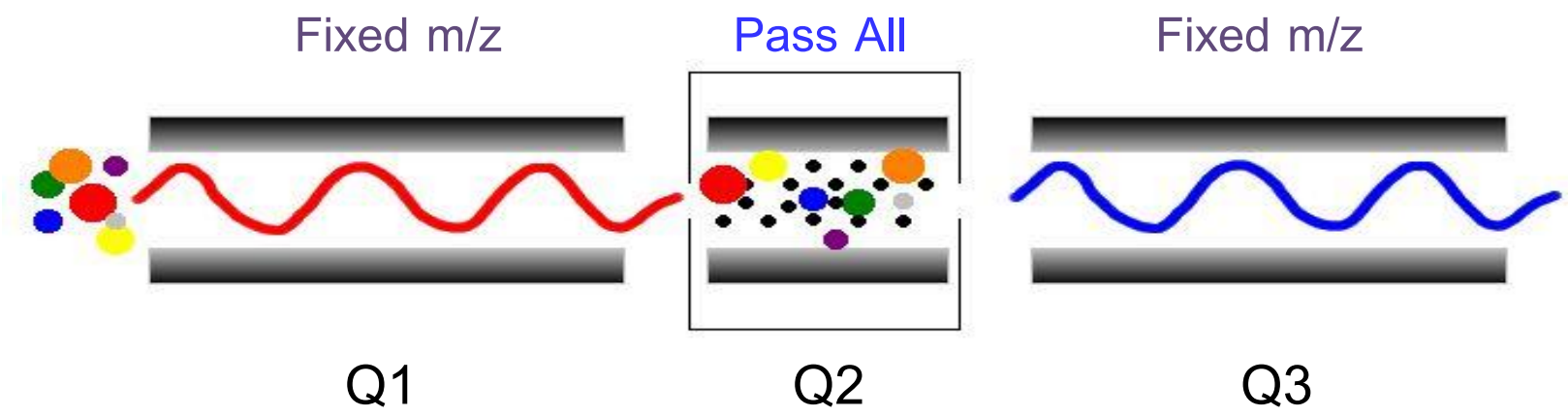
Disadvantages

- Can suffer from interferences
- Not as sensitive or selective as SRM

Full Scan versus SIM



Selected Reaction Monitoring (SRM)



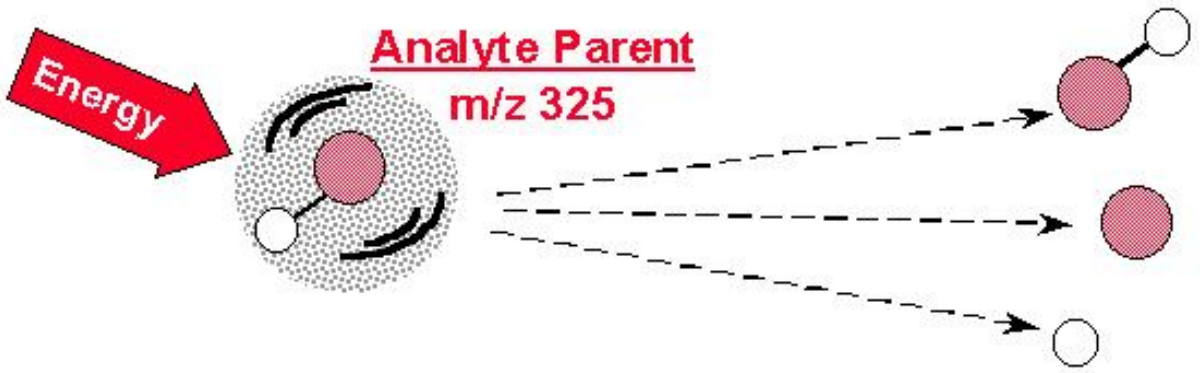
Advantages

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

Disadvantages

- No structural information

The Need for True MS/MS

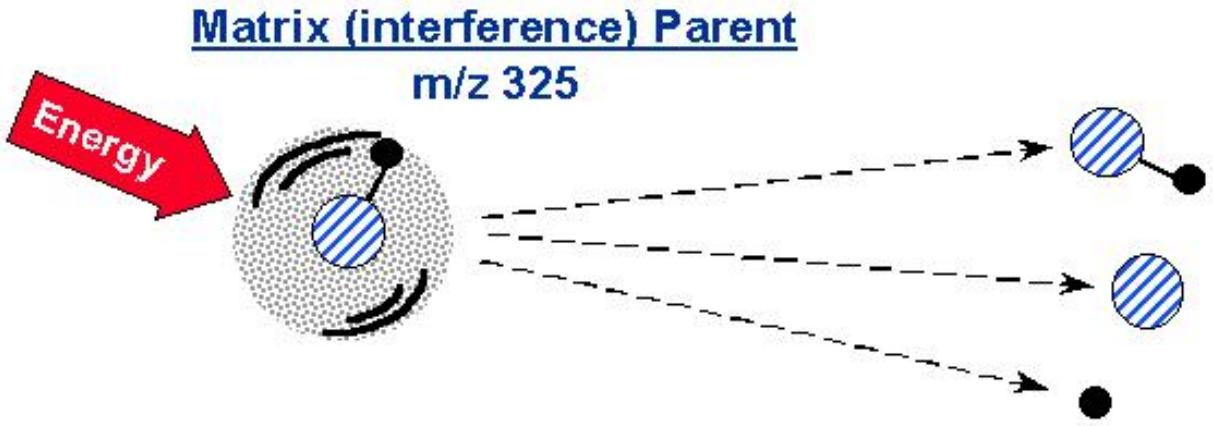


m/z 325 Intact Analyte Parent

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.



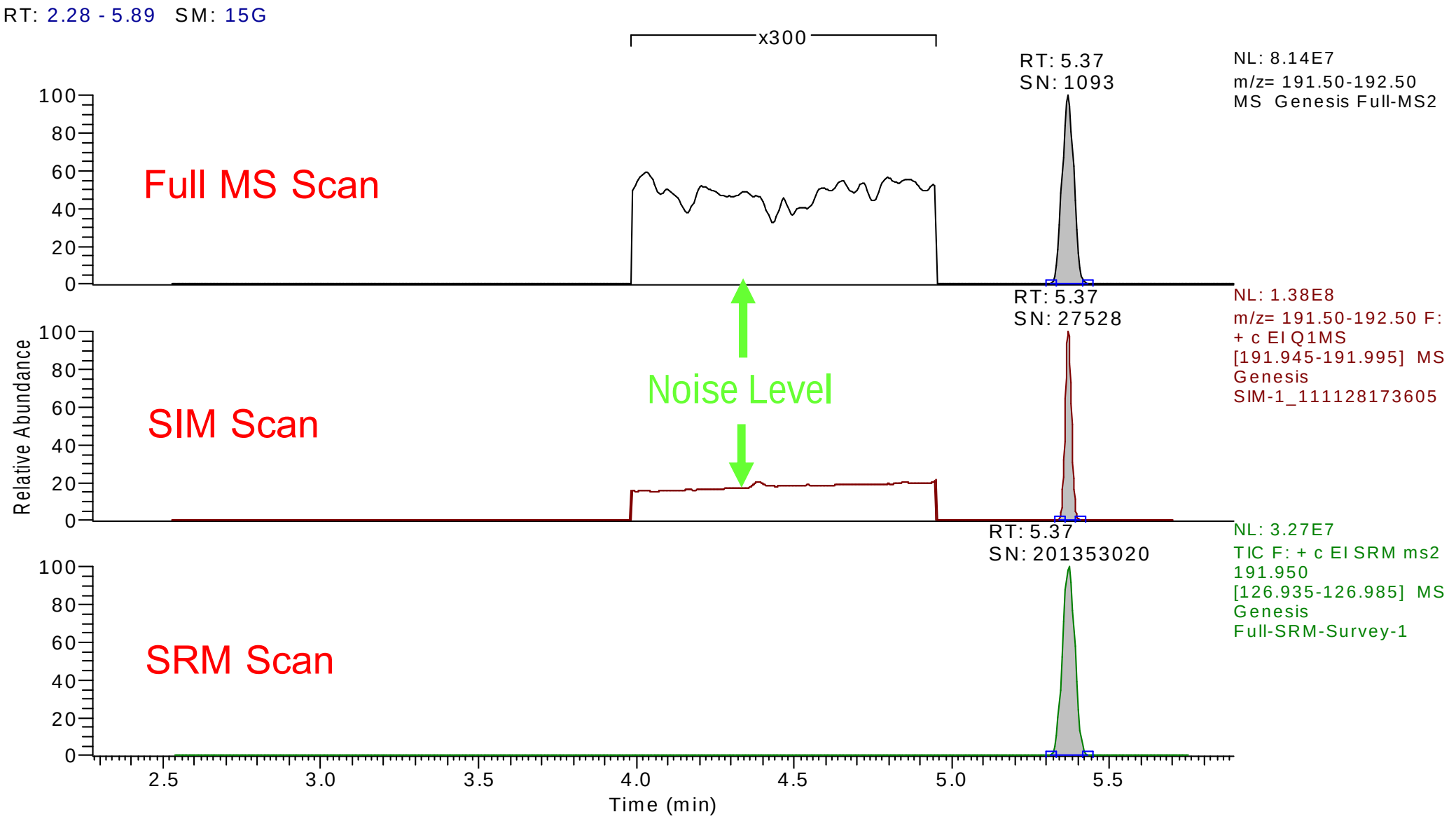
m/z 325 Intact Matrix Parent

m/z 185 Matrix CID Product A

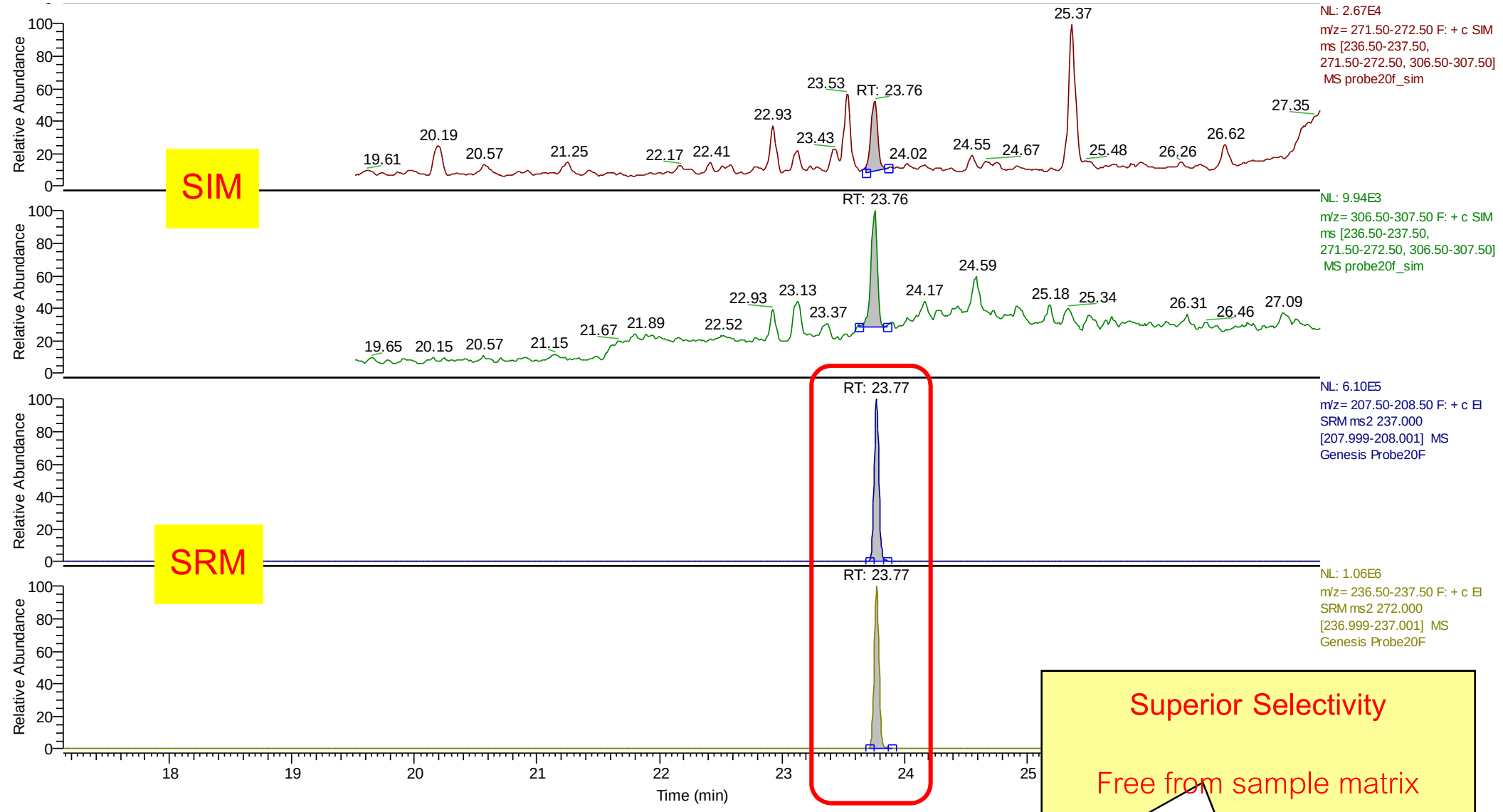
m/z 140 Matrix CID Product B

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

Applications of Triple Quadrupole LC-MS/MS

Confident Quantitation

Any compound, any matrix, any user.

ThermoFisher SCIENTIFIC

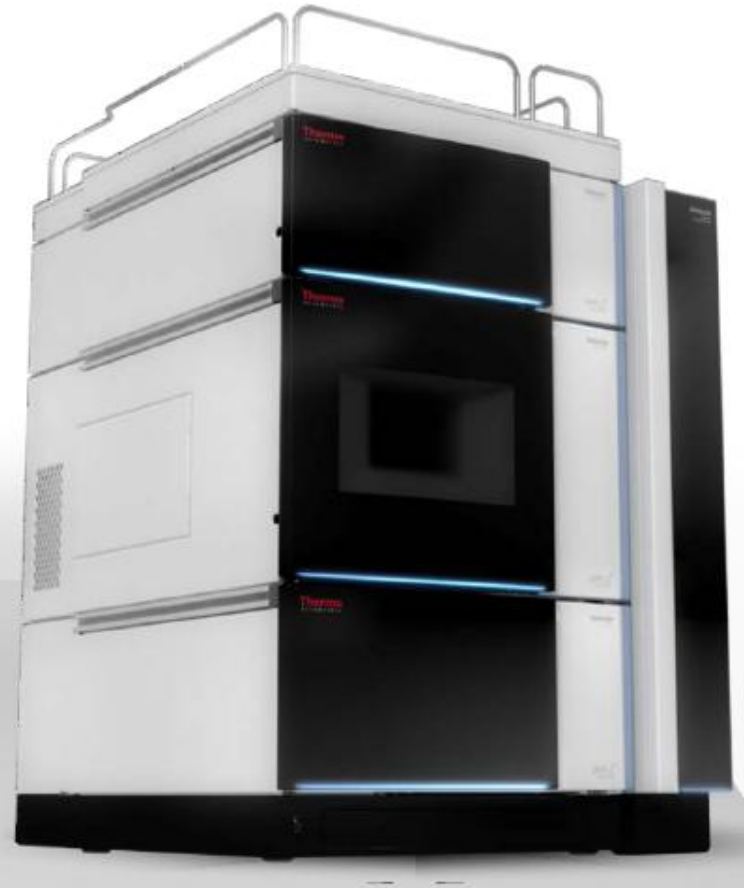
LC-MS/MS Quantitation of ~100 Drugs of Abuse in Urine in Under Two Minutes on the Thermo Scientific™ TSQ Quantis™ MS

The world leader in serving science

Why High-throughput LC-MS/MS for Drugs of Abuse Analyses?

- Large number of samples
  - Need to reduce analysis times
- Reduce false positives (immunoassays)
  - LC-MS/MS has high selectivity
- Reduce costs
  - Multi-class drugs of abuse require multiple immunoassays
  - Still may require LC-MS/MS confirmation
- Thermo Scientific™ Vanquish™ Horizon UHPLC and TSQ Quantis™ MS can meet these requirements

Thermo Scientific Vanquish Horizon UHPLC



Vanquish Horizon UHPLC Platform:

- Maximize UHPLC separations with 1500 bar (22,000 psi) pump pressure limit
- Unmatched retention time performance via parallel dual piston principle
- Ultra-low Gradient Dead Volume (35 uL) for faster separations
- Viper-based, tool-free fluidic connections
- Biocompatible, iron-free flow path
- Sample pre-compression for better injection reproducibility and longer column lifetimes
- Standard AS capacity: 4 racks (216 vials); expandable Charger module for up to 20 well plates)
- New column thermostatting technology
- Removable doors for easy access

Experimental Design – Liquid Chromatography

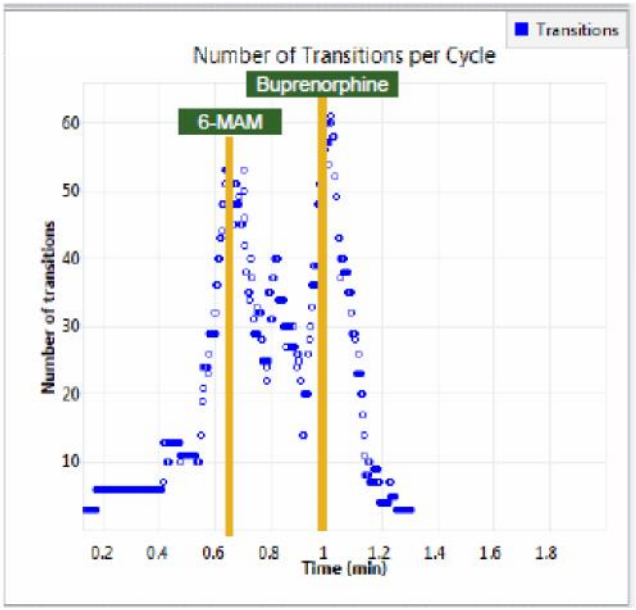
- Thermo Scientific™ Vanquish™ Horizon UHPLC
  - Column: 2.1 x 50 mm, 1.9 um Hypersil Gold AQ
  - Column Temp: 40 C
  - Mobile Phase: [A] H_2O + 0.1% HCOOH; [B] ACN + 0.1% HCOOH
  - Flow Rate: 1.0 mL/min (no split)
  - Gradient: see table
  - Injection Volume: 2 uL

No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	1.000	0.0	5
3	0.400	1.000	22.5	5
4	0.950	1.000	75.2	5
5	1.450	1.000	75.2	5
6	1.470	1.000	0.0	5
7	1.490	1.000	0.0	5
8	1.510	1.200	0.0	5
9	2.150	1.200	0.0	5
10	2.200	1.000	0.0	5

Note: total LC runtime is 2.2 minutes for ~100 drugs of abuse

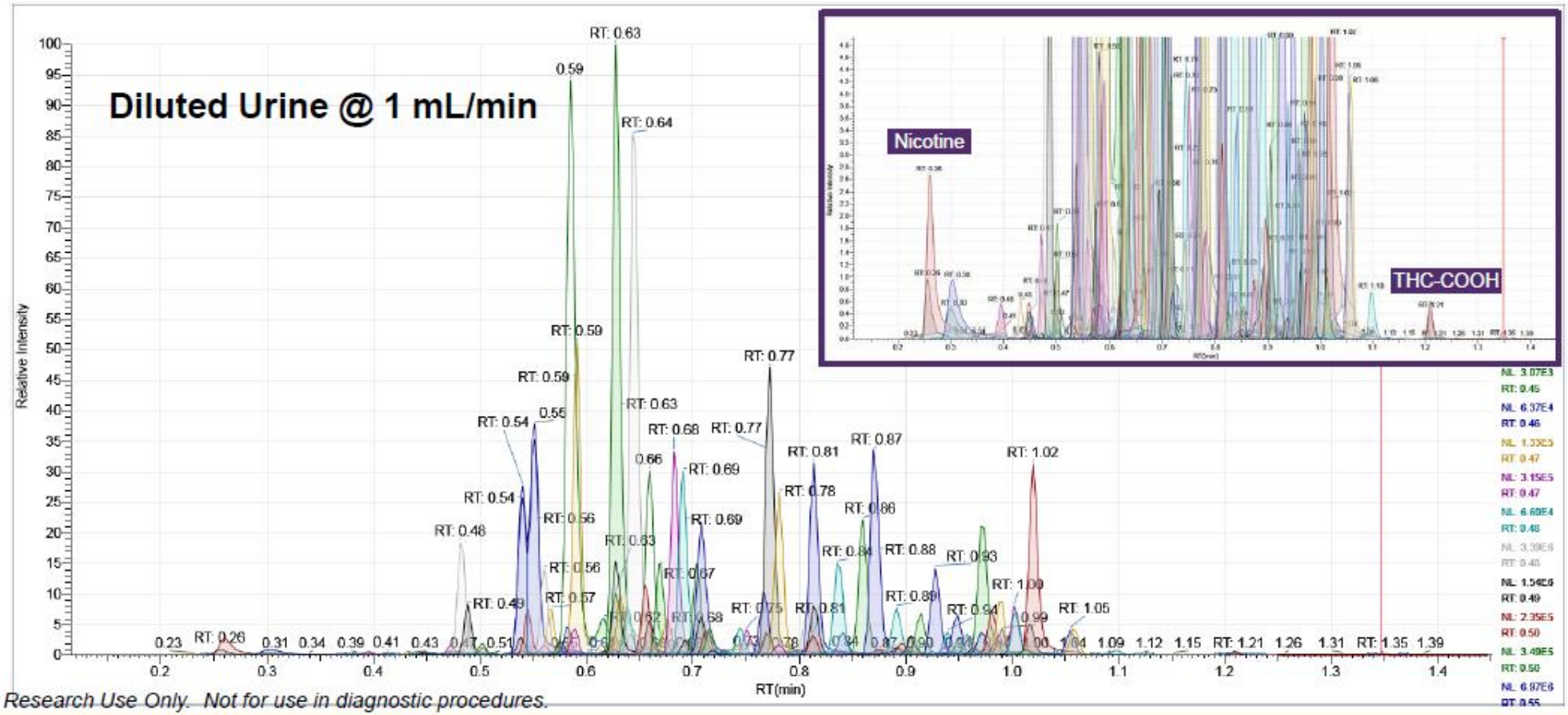
Experimental Design – Mass Spectrometry

- Thermo Scientific™ TSQ Quantis™ MS
  - Ionization Mode: HESI, Positive ion mode
  - MS Acquisition Mode: Selective Reaction Monitoring (SRM) – see # Transitions vs. RT below
  - Cycle time: 0.15 s
  - Quad Isolation (Q1,Q3) = Unit (0.7 Da FWHM)



Note: elution of 6-MAM & Buprenorphine occur at the times of highest # SRM transitions (i.e., during lowest dwell times)

Approximate 100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS

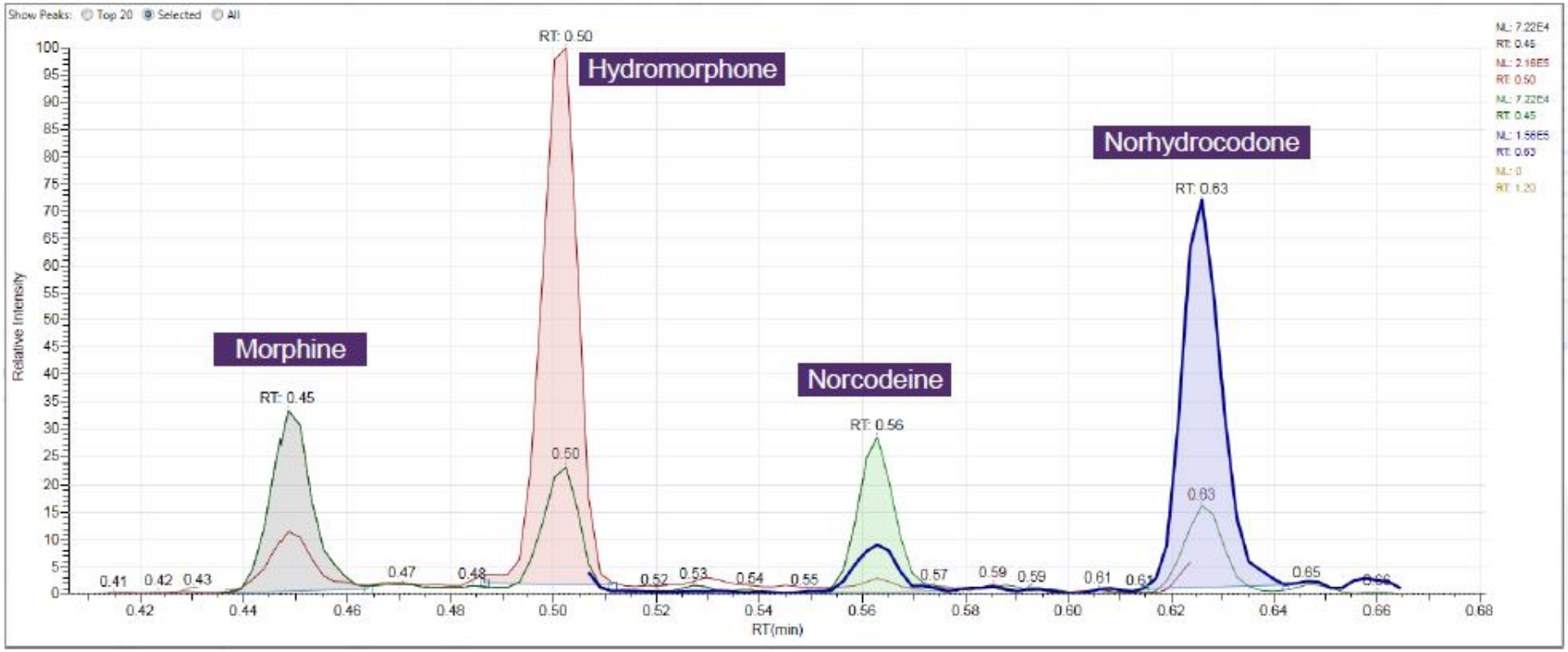


For Research Use Only. Not for use in diagnostic procedures.

SRM chromatograms of ~100 drugs of abuse in under 1.3 minutes [THC-COOH elutes at 1.21 min, inset]

Approximate 100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS

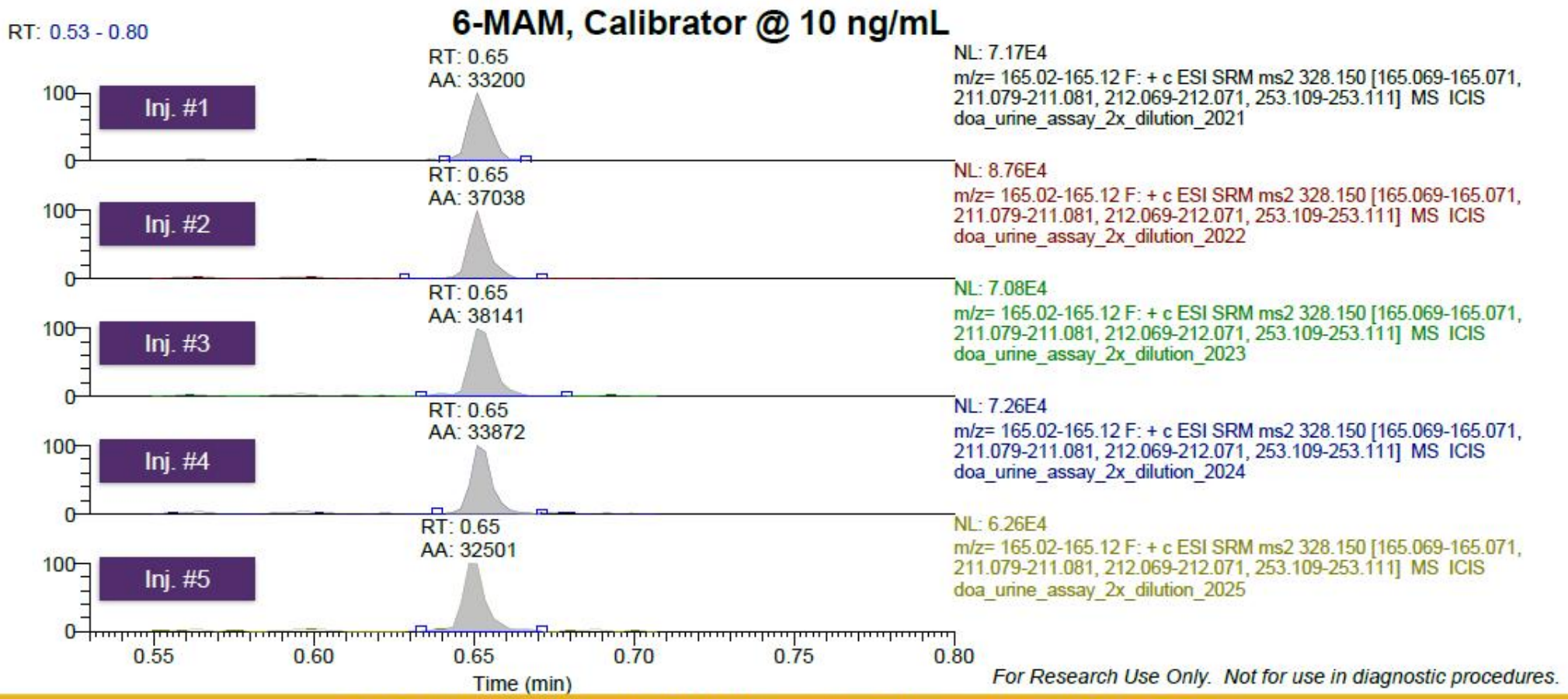
Separation of opiate isomers @ m/z 286



For Research Use Only. Not for use in diagnostic procedures.

Opiate isomers at m/z 286 are well separated in under 12 s [typical LC peak = 1.2 s wide]

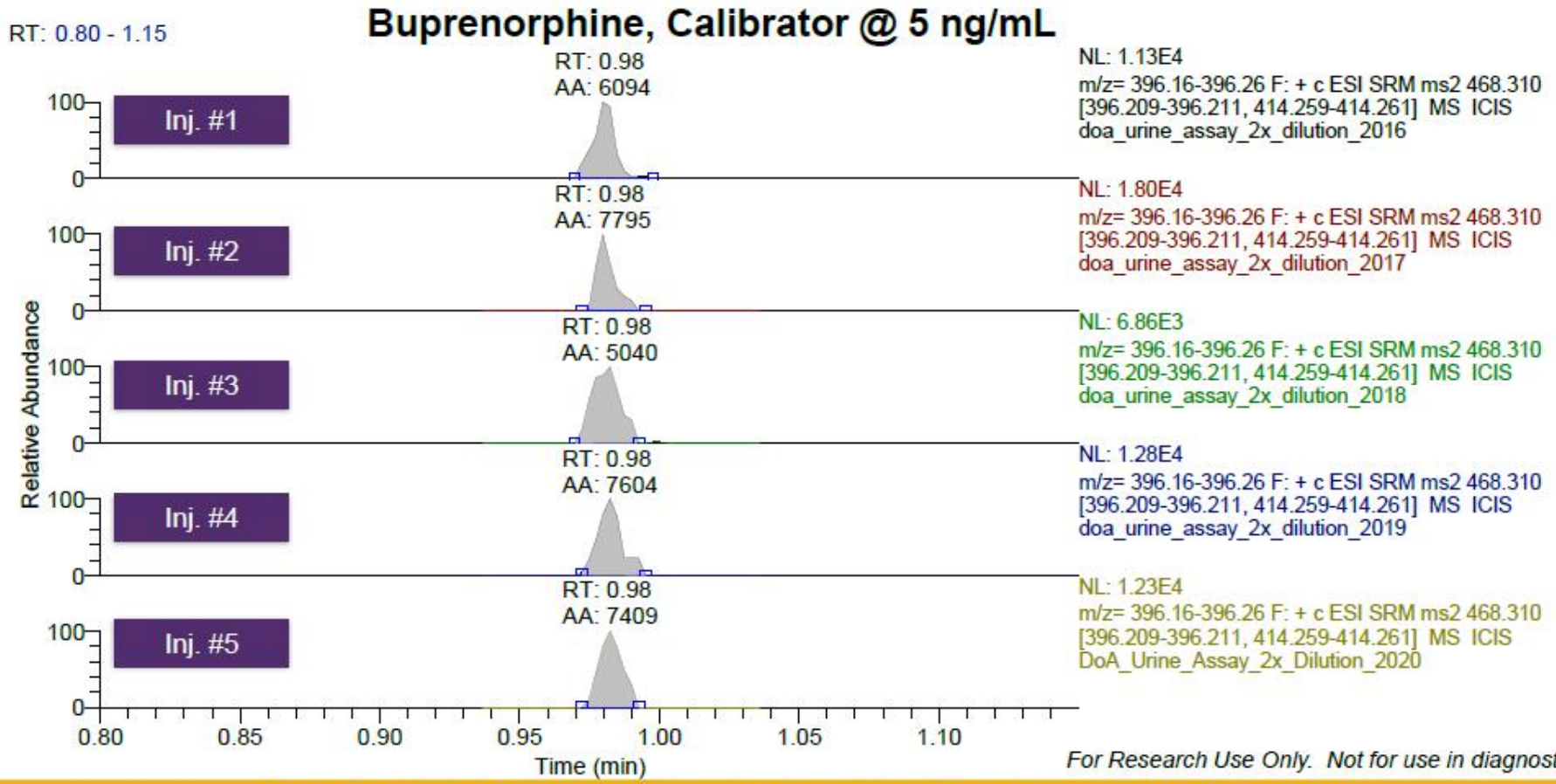
Approximate 100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS



For Research Use Only. Not for use in diagnostic procedures.

- 1:2 dilution of 10 ng/mL 6-MAM in urine, 2 uL injections [%CV = 8.5%];
- Dwell Time = 1.63 ms (50 simultaneous SRM transitions w/ 0.15 s SRM Cycle Time)

Approximate 100 Drugs of Abuse on Thermo Scientific TSQ Quantis MS



For Research Use Only. Not for use in diagnostic procedures.

- 1:2 dilution of 5 ng/mL Buprenorphine in urine, 2 uL injections [%CV = 16.5%];
- Dwell Time = 0.82 ms (60 simultaneous SRM transitions w/ 0.15 s SRM Cycle Time)

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

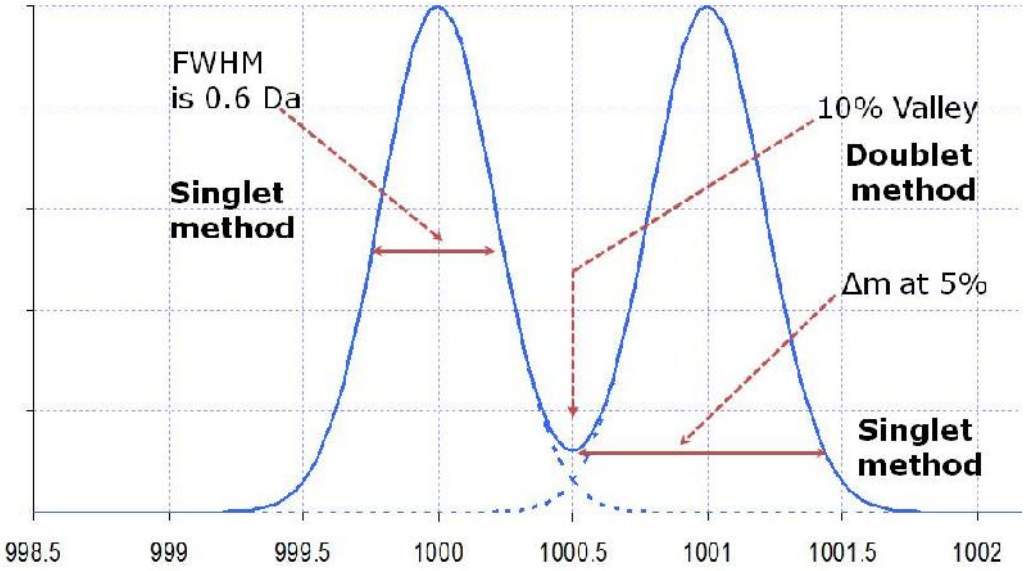
Mass Resolution: What is it?

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

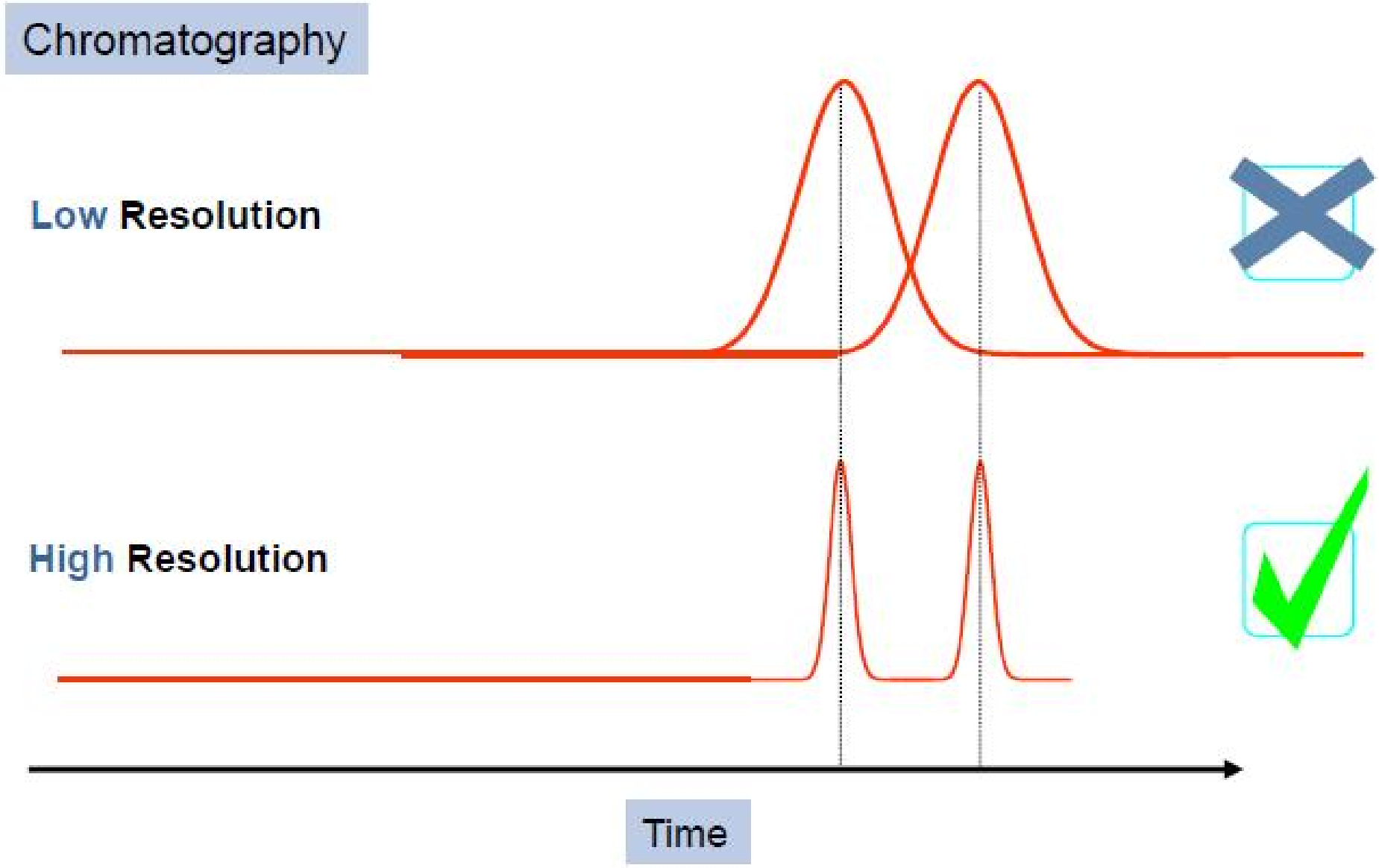
$$R = \frac{m}{\Delta m}$$

- 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



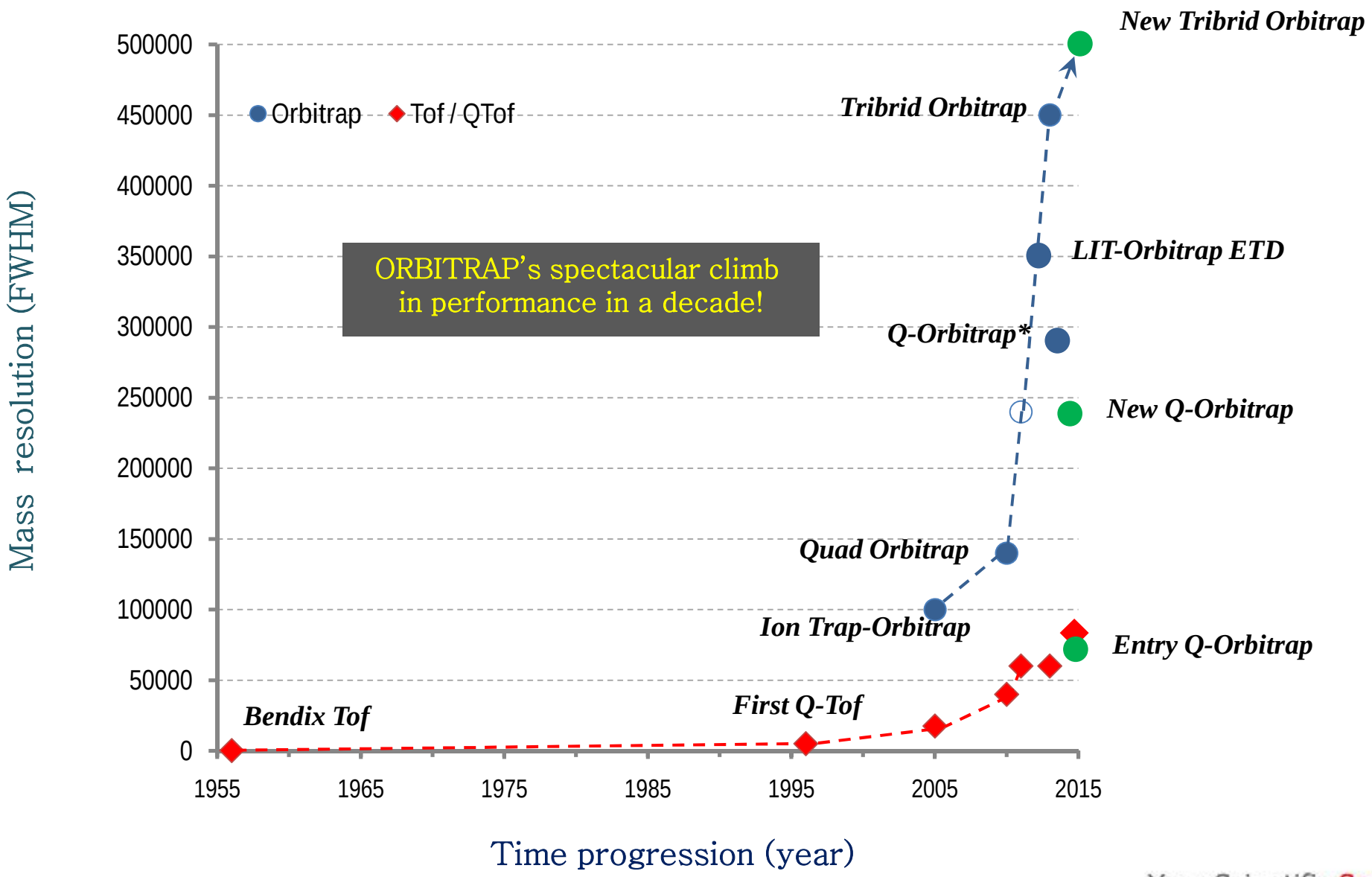
Mass Resolution: What is it?

- 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 Resolution: What is it?

Mass spectrometer	Resolving Power (FWHM)
FT-ICR-MS	1,000,000
Orbitrap	500,000
HR-ToF	60,000
Magnetic Sectors	10,000
Quadrupole / IonTrap in UltraZoom mode	5,000
Quadrupole / IonTrap	1,000

Commercial High Resolution MS Technology Race



MASS ACCURACY

Mass Accuracy: What is it?

- **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)**:

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

- Absolute mass error can be used (**mDa**).

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

Mass Accuracy

- Accurate mass measurements take advantage of the fact that the combination of elements contained in a molecule have a very specific, non-nominal molecular weight:

  - Carbon has a mass of 12.0000
  - Hydrogen has a mass of 1.0078
  - Oxygen has a mass of 15.9949
  - Nitrogen has a mass of 14.0031

Element	Isotope Nominal Mass	Relative Abundance (%)	Average Mass	Exact mass
H	1	100	1.008	**1.0078**
	2	0.016		**2.041**
C	12	100	12.011	**12**
	13	1.08		**13.0034**
N	14	100	14.007	**14.0031**
	15	0.38		**15.0001**
O	16	100	15.999	**15.9949**
	17	0.24		**16.9991**
F	19	100	18.998	**18.9984**
P	31	100	30.974	**30.9738**
S	32	100	32.06	**31.9721**
	33	0.78		**32.9715**
	34	4.4		**33.9679**
Cl	35	100	35.453	**34.9689**
	37	32.5		**36.9659**
Br	79	100	79.904	**78.9183**
	81	98		**80.9163**
I	127	100	126.905	**126.9045**

Mass accuracy depends on resolution

Higher resolution allows for better mass accuracy

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
Triple Quad	3 - 5 ppm
Linear IonTrap	50-200 ppm (10 ppm in Ultra-Zoom)

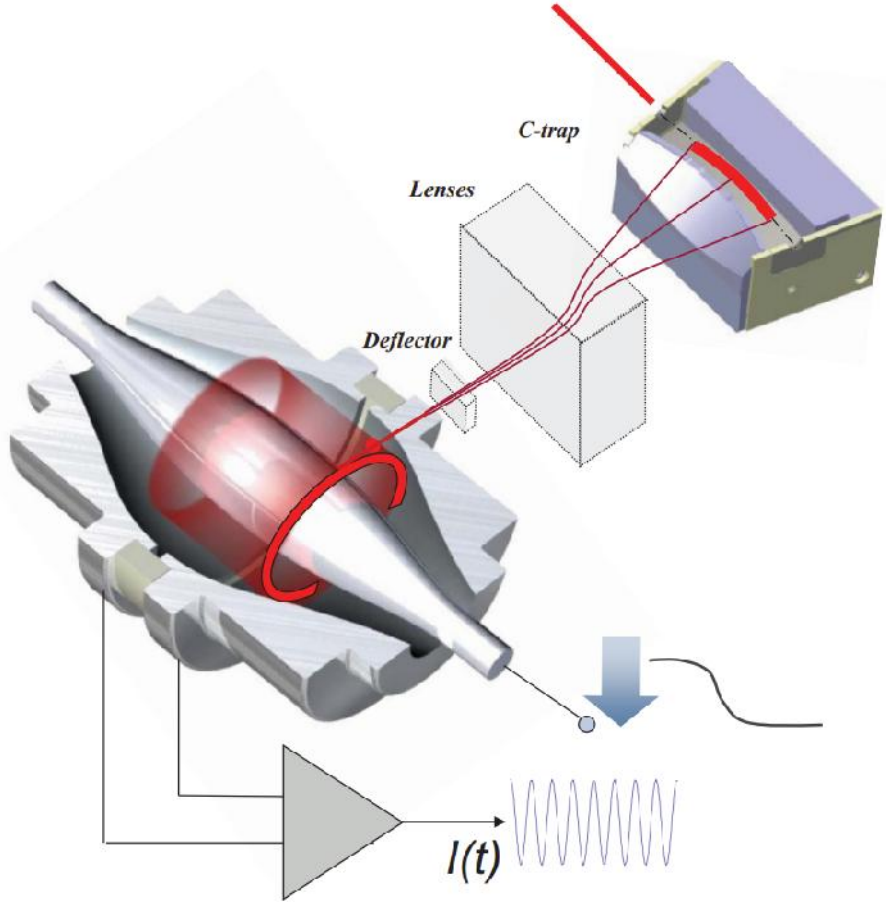
Source: Metabolomics Fiehn's lab

Anal. Chem. **2000,** *72,* 1156−1162

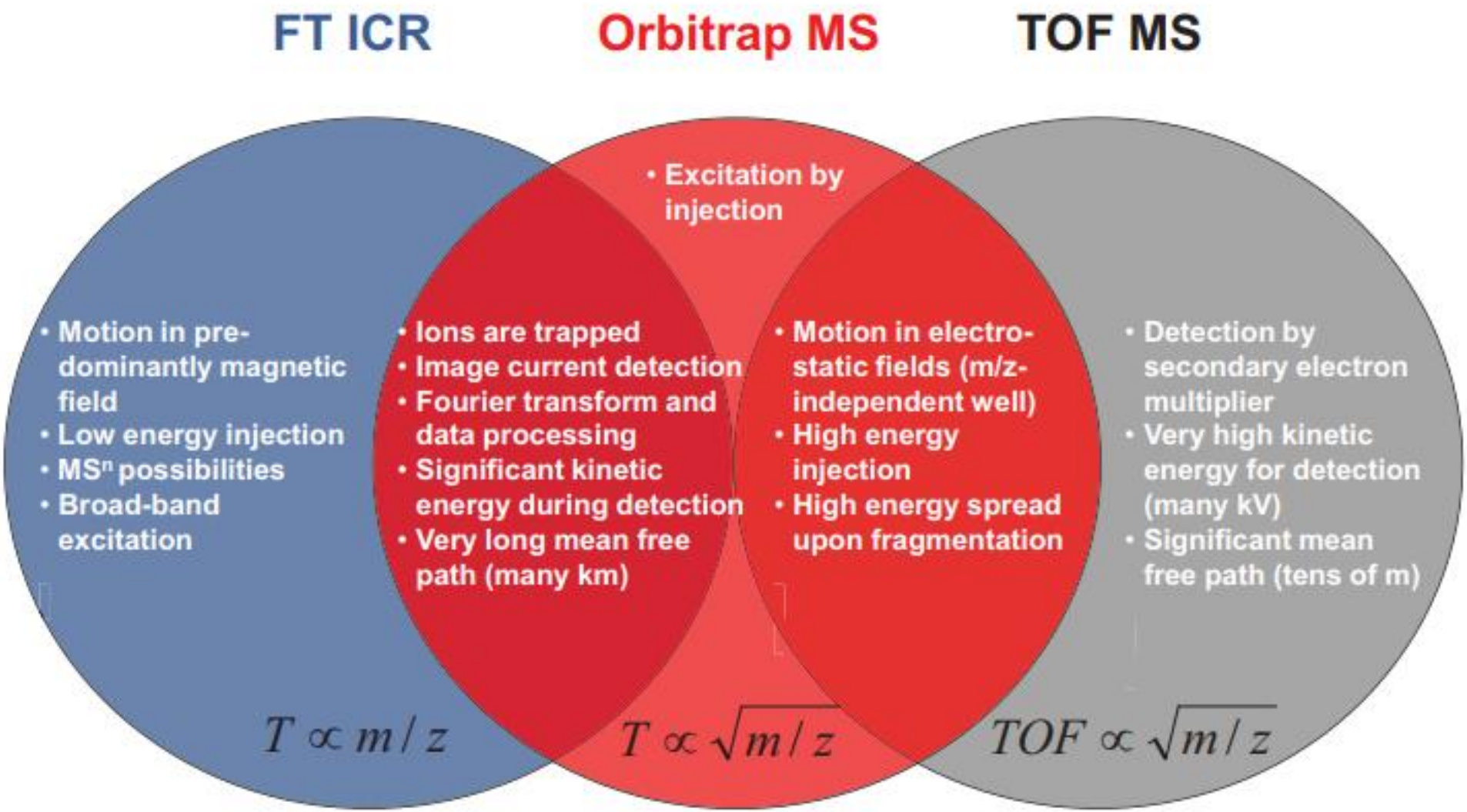
Electrostatic Axially Harmonic Orbital Trapping: A High-Performance Technique of Mass Analysis

Alexander Makarov*

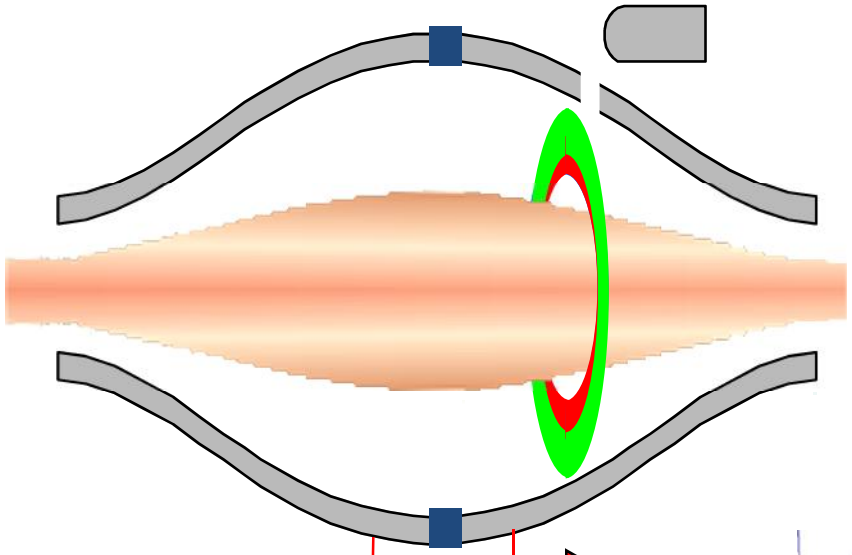
HD Technologies Ltd., Atlas House, Simonsway, Manchester, M22 5PP, U.K.

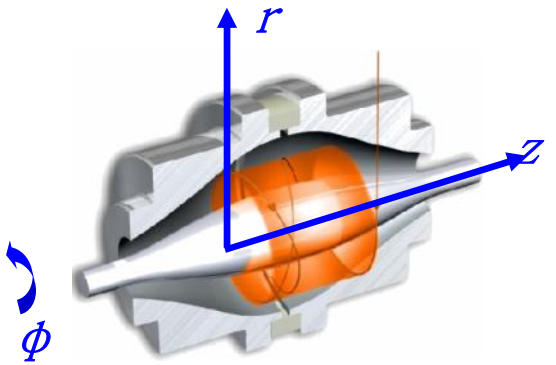


Major accurate-mass analyzers for Life Science



Orbitrap Mass Analyzer: Principle of Operation





Hyper-logarithmic potential distribution:
"ideal Kingdon trap"

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

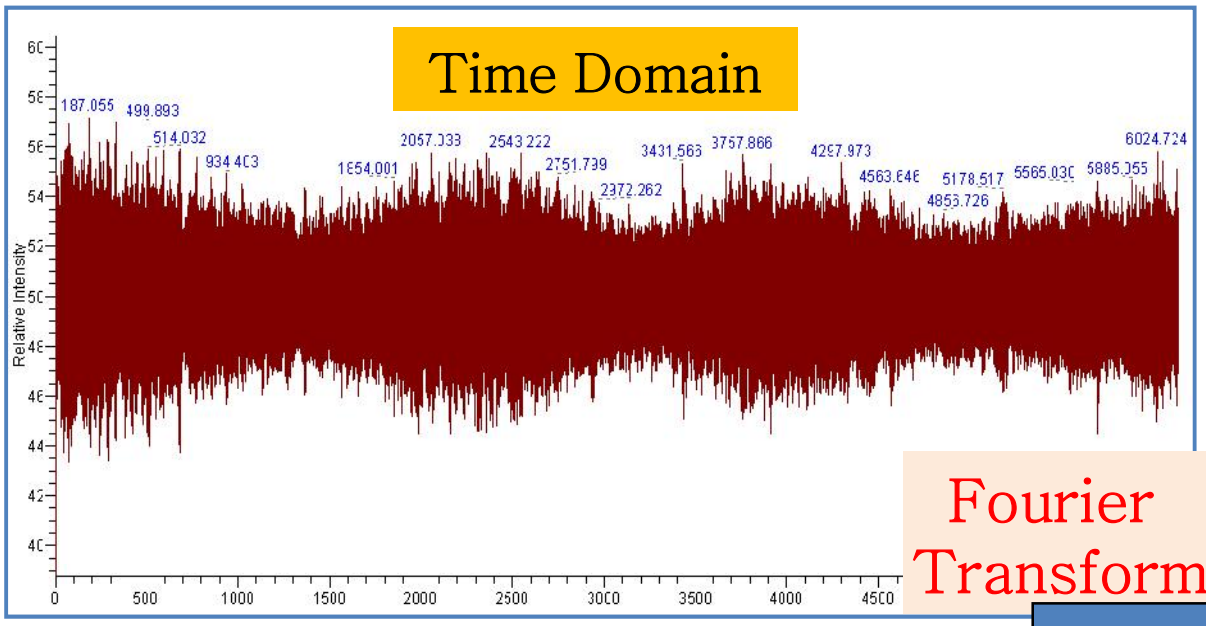
- Characteristic frequencies:
  - Frequency of rotation ω_φ
  - Frequency of radial oscillations ω_r
  - Frequency of axial oscillations ω_z

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

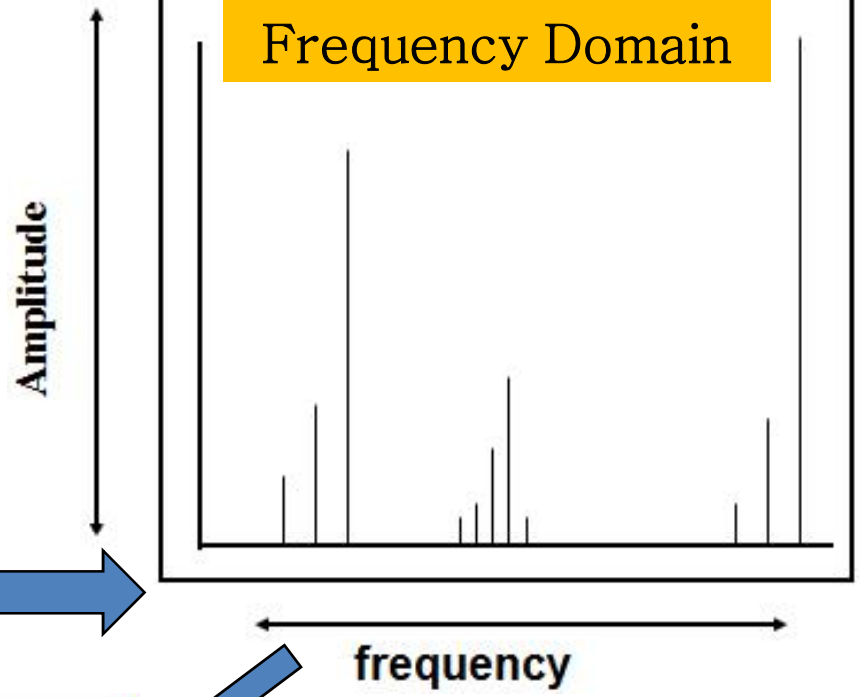
$$\mu_{..} = \frac{\mu_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \qquad \mu_r = \mu_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

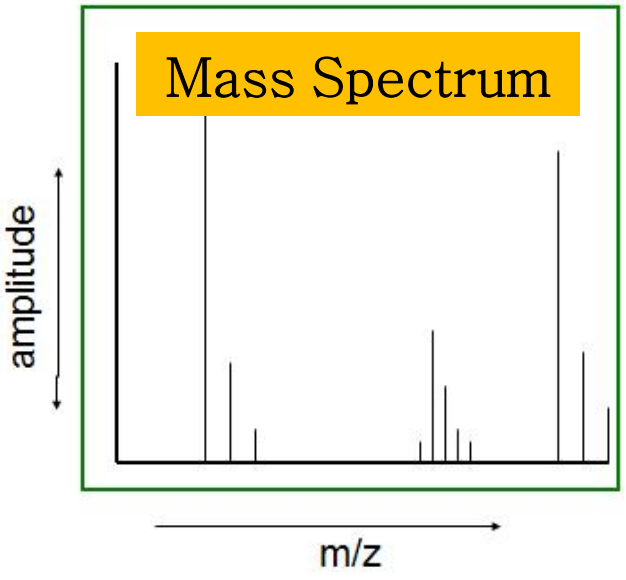
Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

Many Ions Generate a Complex "Transient"



Fourier Transform





ThermoFisher
SCIENTIFIC

Importance of High Mass Resolution & Mass Accuracy – Unknown Compounds Identification

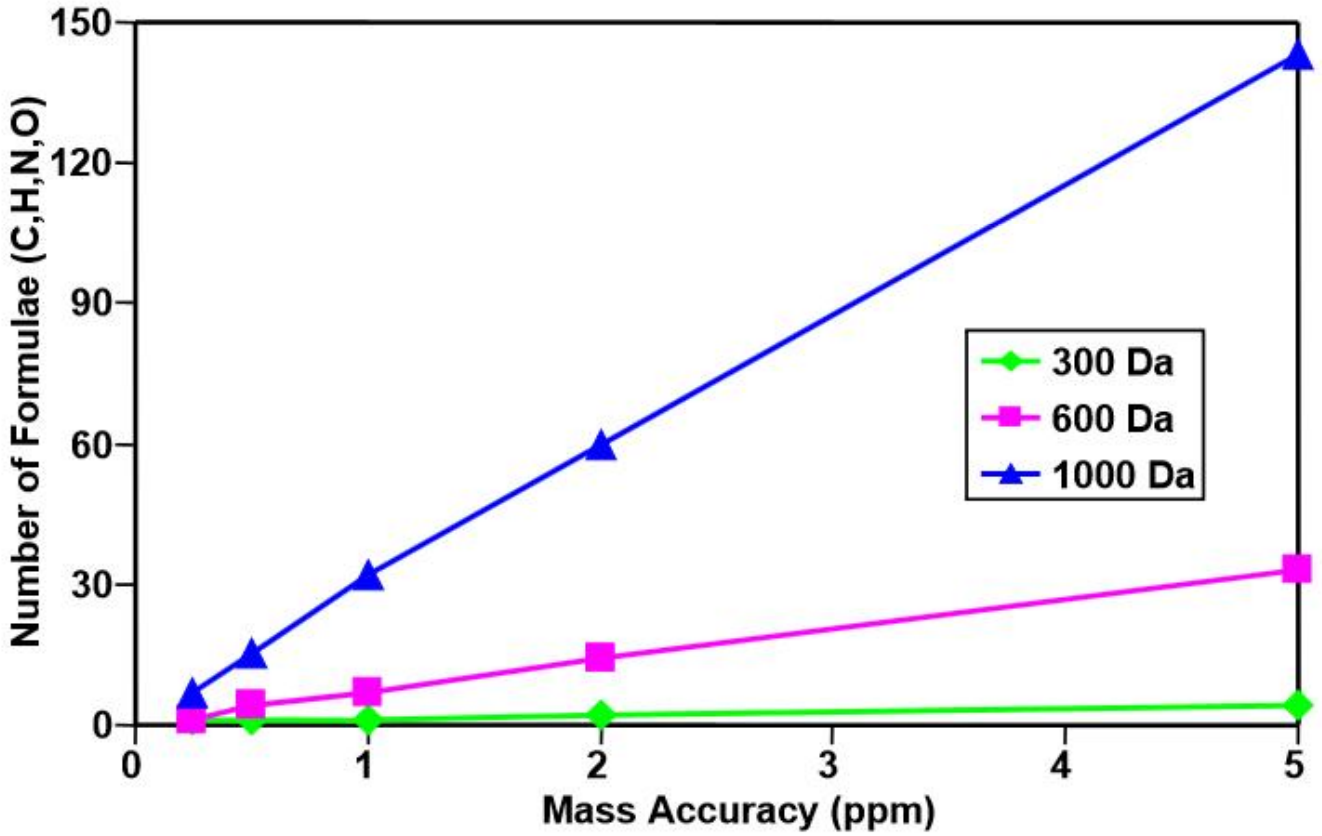
The world leader in serving science

Mass Accuracy: I.D. of unknown compounds

- Accurate mass measurement is the experimentally determined mass measured to an appropriate degree of accuracy and precision (*Gross, J. Am. Soc. Mass Spectrom.,1994*)
- Accurate mass measurements narrow down the list of possible formulae for a particular molecular weight
- Mass spectrum and analyst complete the picture:
  - Isotope distributions indicate/eliminate elements (e.g. Cl, Br, Cu)
  - User-supplied info eliminates others (e.g. no F, Co)
  - Suggested formula has to make chemical sense: ($C_6H_{28}O_2$ is not reasonable nor is $Cl_3H_2Co_4$)

Mass accuracy for chemical formulae ID

- The effect of mass accuracy and molecular weight on the number of potential chemical formulae.



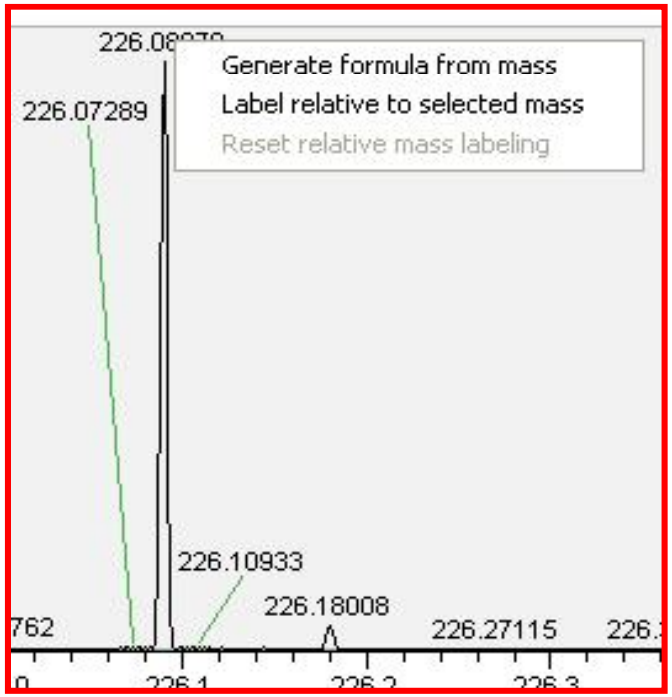
Generate Formula from Monoisotopic Mass

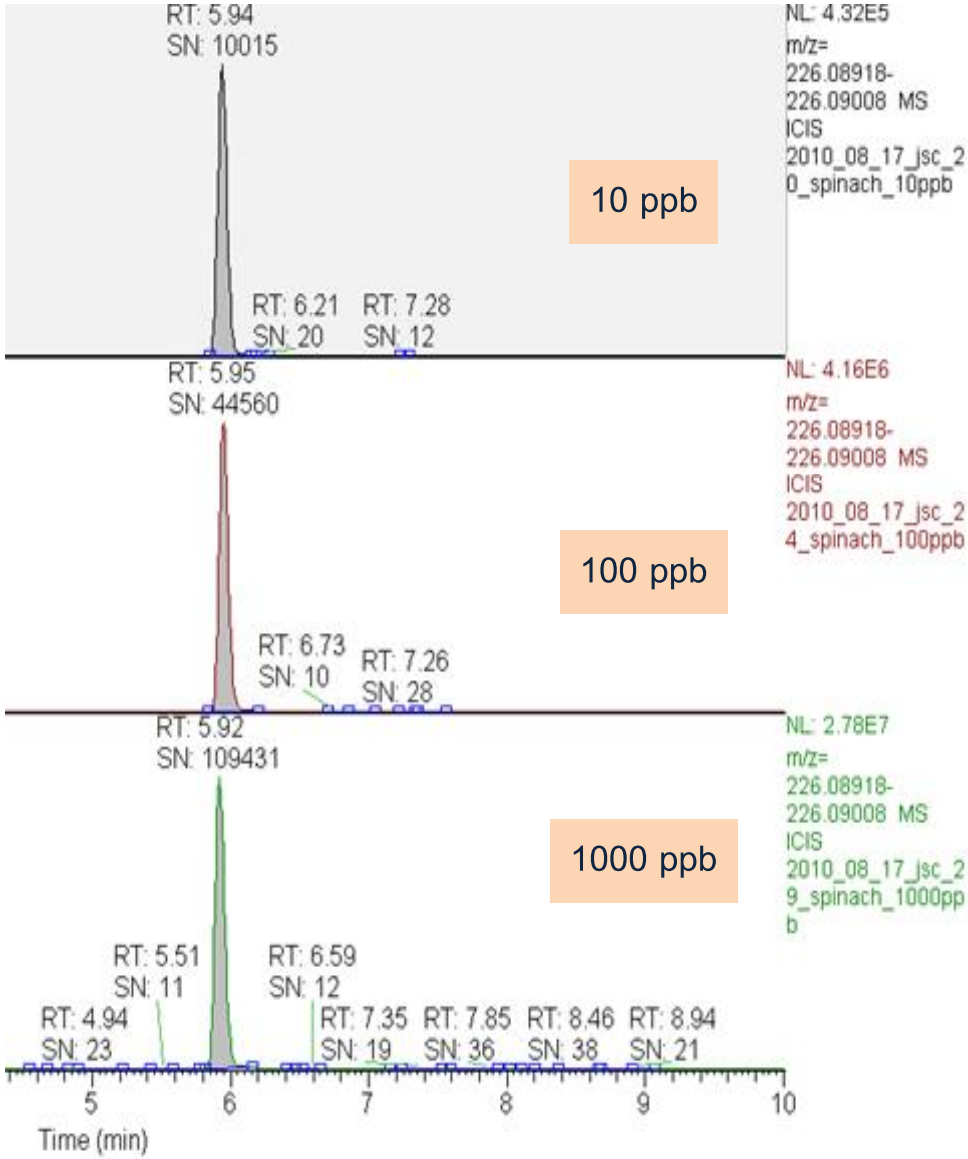
0.549 ppm Mass Accuracy

Right click on mass

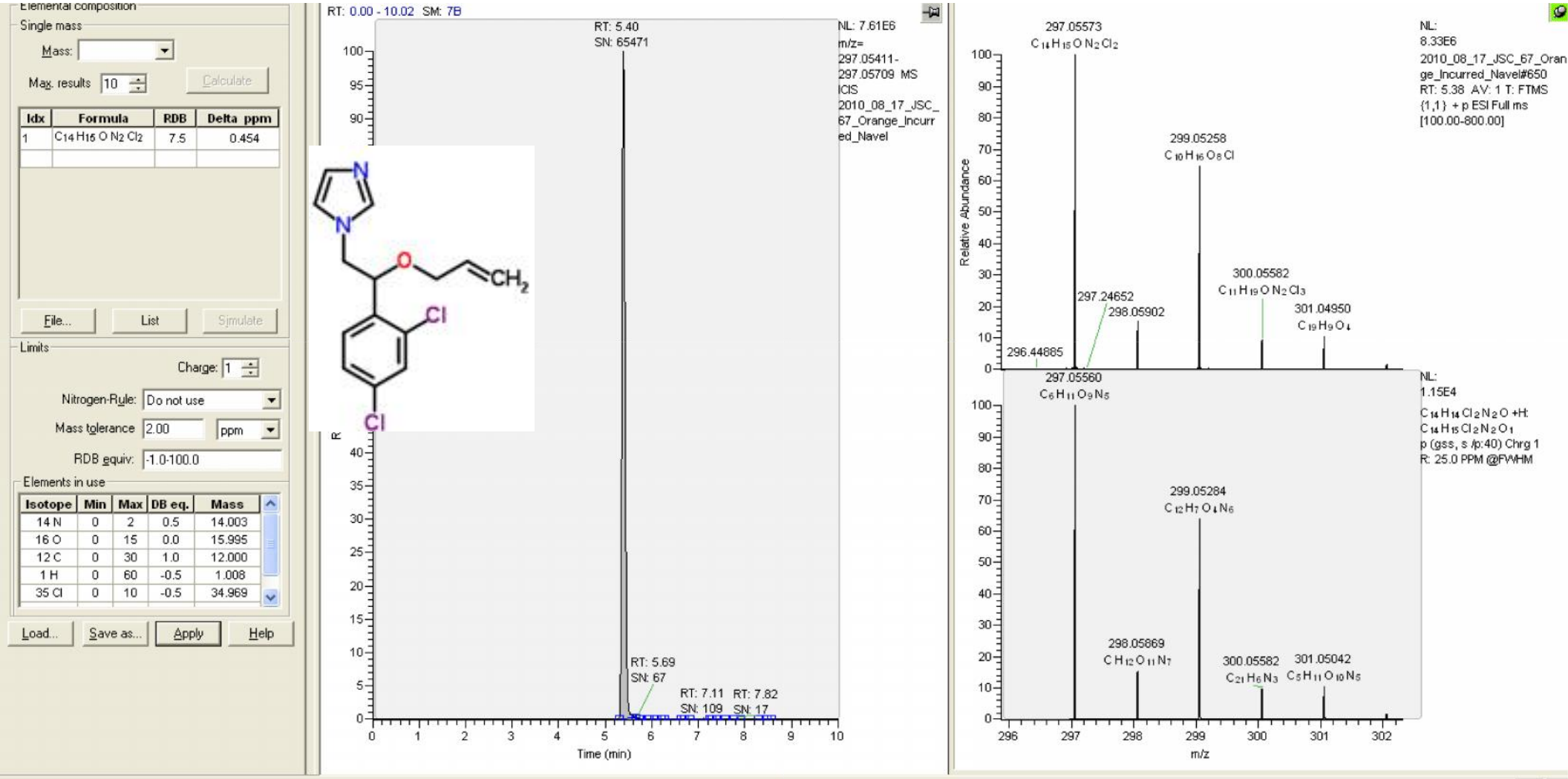
Then select

Generate formula form mass



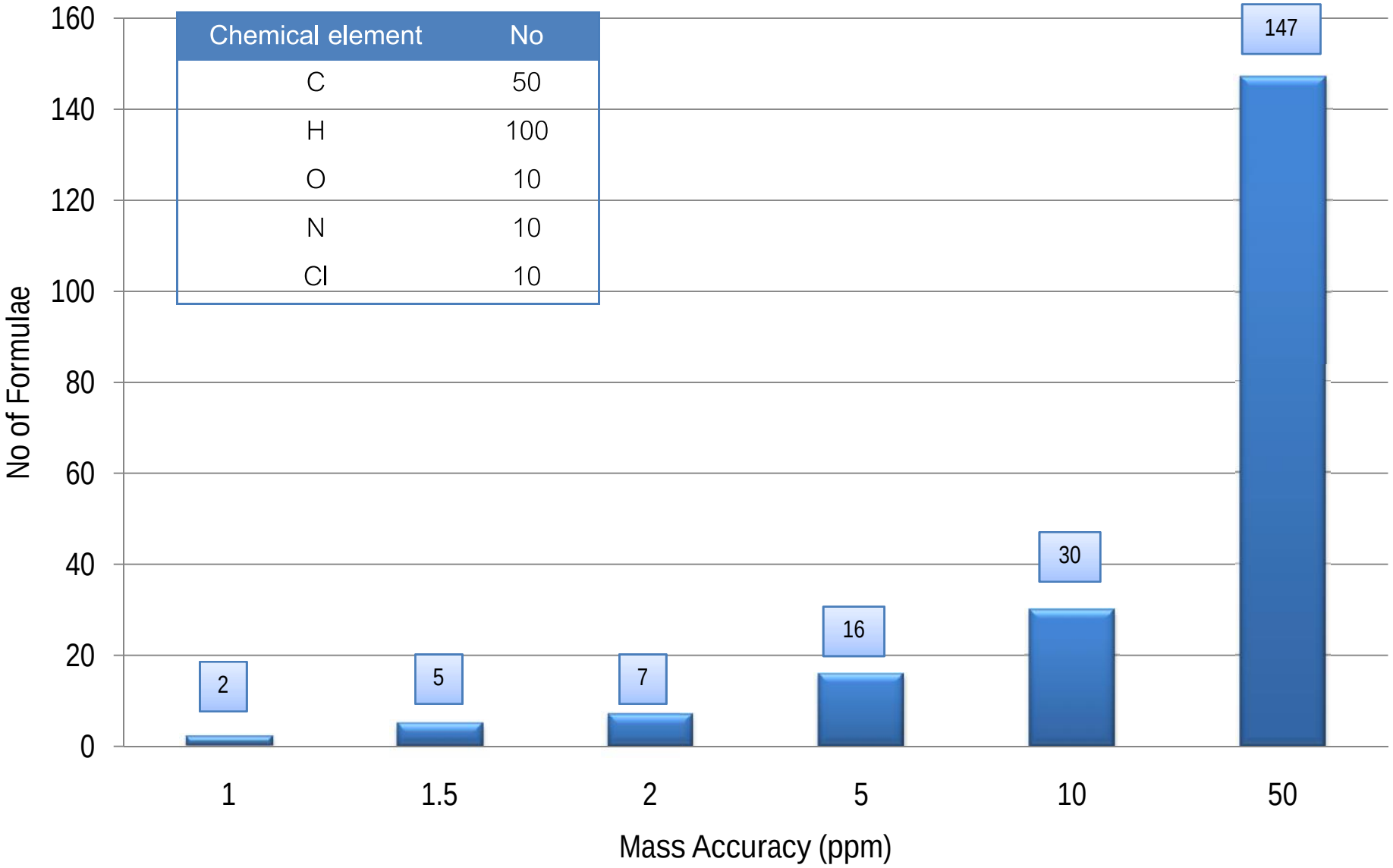


Elemental Composition Generates by Monoisotopic Mass

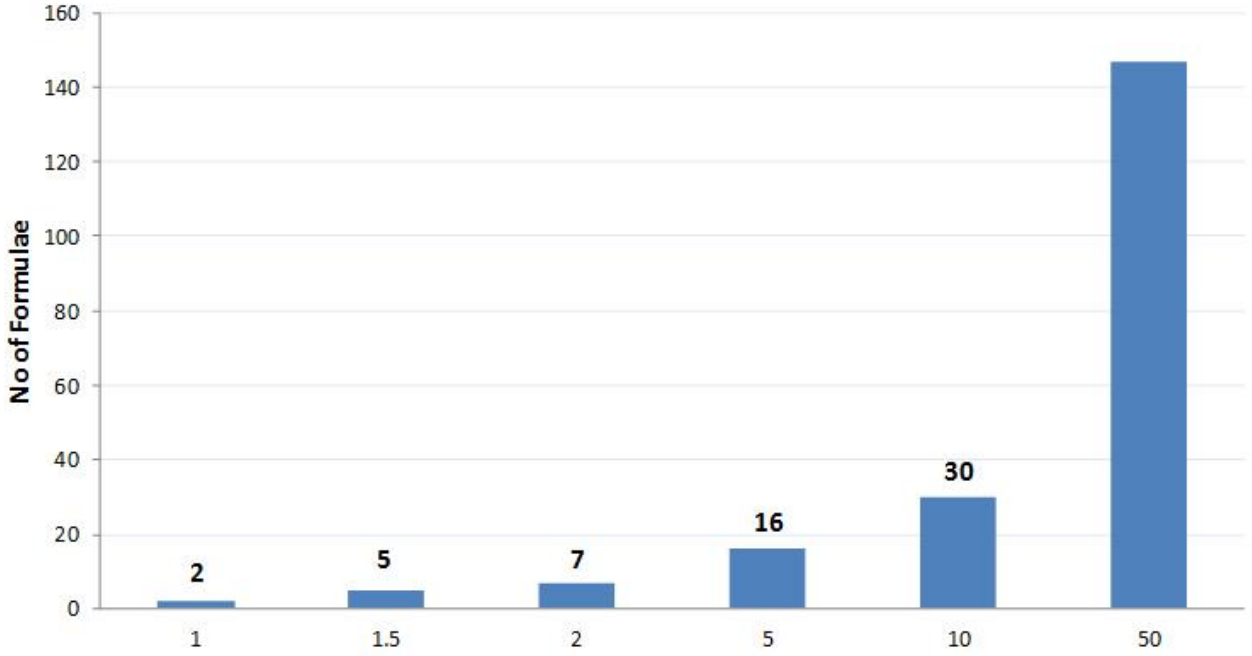


Elemental Composition Statistics

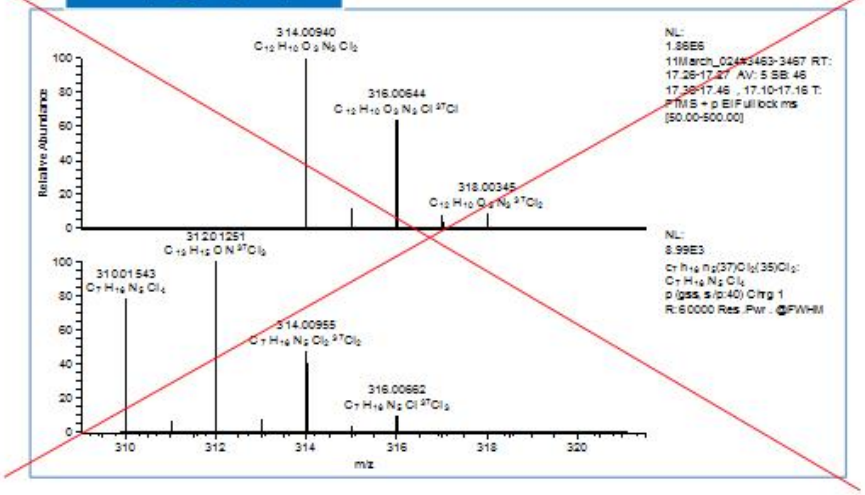
Chemical element	No
C	50
H	100
O	10
N	10
Cl	10



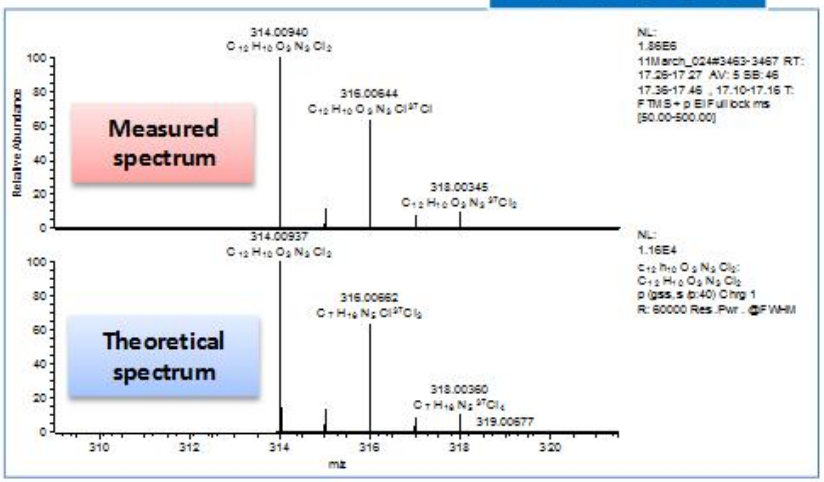
Compound ID confirmation through Isotopic Pattern Match



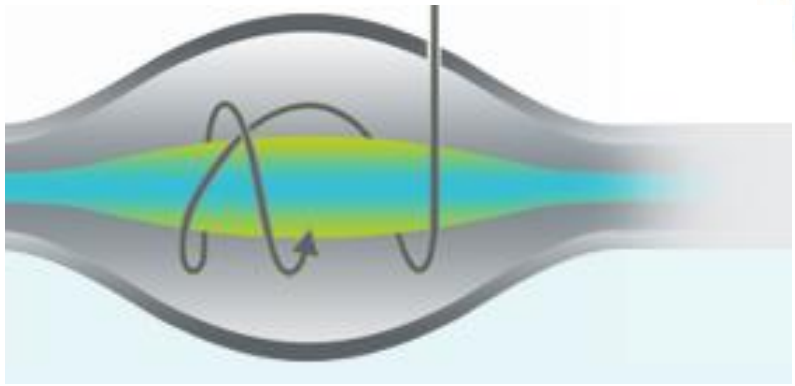
$C_7H_{16}N_5Cl_4$



$C_{12}H_{10}N_5O_3Cl_2$



UHPLC with Q Exactive Mass Spectrometer



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