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TSQ Operations

Thermo Scientific
Training Institute

TSQ Operations Course



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Course Outline

Day 1

- A. TSQ Quantum – General Presentation
 - 1. TSQ evolution
 - 2. API features and sources
 - 3. LC and LC-MS considerations
 - 4. TSQ Quantum components and principles
- B. TSQ Quantum Tune Master
 - 1. Overview
 - 2. Tune and Calibrate

Day 2

- A. Xcalibur
 - 1. Introduction to Xcalibur (Instrument setup, Sequence setup, Qual Browser)
- B. ESI compound optimization
 - 1. Classification of parameters (manual and automatic optimization)
 - 2. ESI method development
 - 3. Scan modes (Full, SIM, Product, Parent, Neutral Loss, SRM)
 - 4. Data-dependent scan

Course Outline

Day 3

- A. APCI compound optimization
 - 1. Classification of parameters (manual and automatic optimization)
 - 2. APCI method development
 - 3. APCI/APPI set-up
- B. Quantitation considerations - Selected Reaction Monitoring (SRM)
 - 1. Calibration curve - Experimental set-up

Day 4

- 1. Processing setup (Quantitation)
- 2. Quan Browser
- 3. XReport
- 4. Maintenance and troubleshooting
- 5. Technical support, Web-based resources
- 6. Review, Q&A

TSQ – Instrument Evolution

TSQ 15

TSQ 45

TSQ 46

TSQ 70

TSQ 700

TSQ 7000

TSQ Quantum

TSQ Quantum Access



***The World's First High Resolution Triple-Stage
Quadrupole Mass Spectrometer***

TSQ Quantum Series



Quantum Ultra



Quantum Ultra AM



Quantum Ultra EMR



Quantum Discovery MAX



Quantum Access



Quantum with FAIMS



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Chapter 1

MS Basics

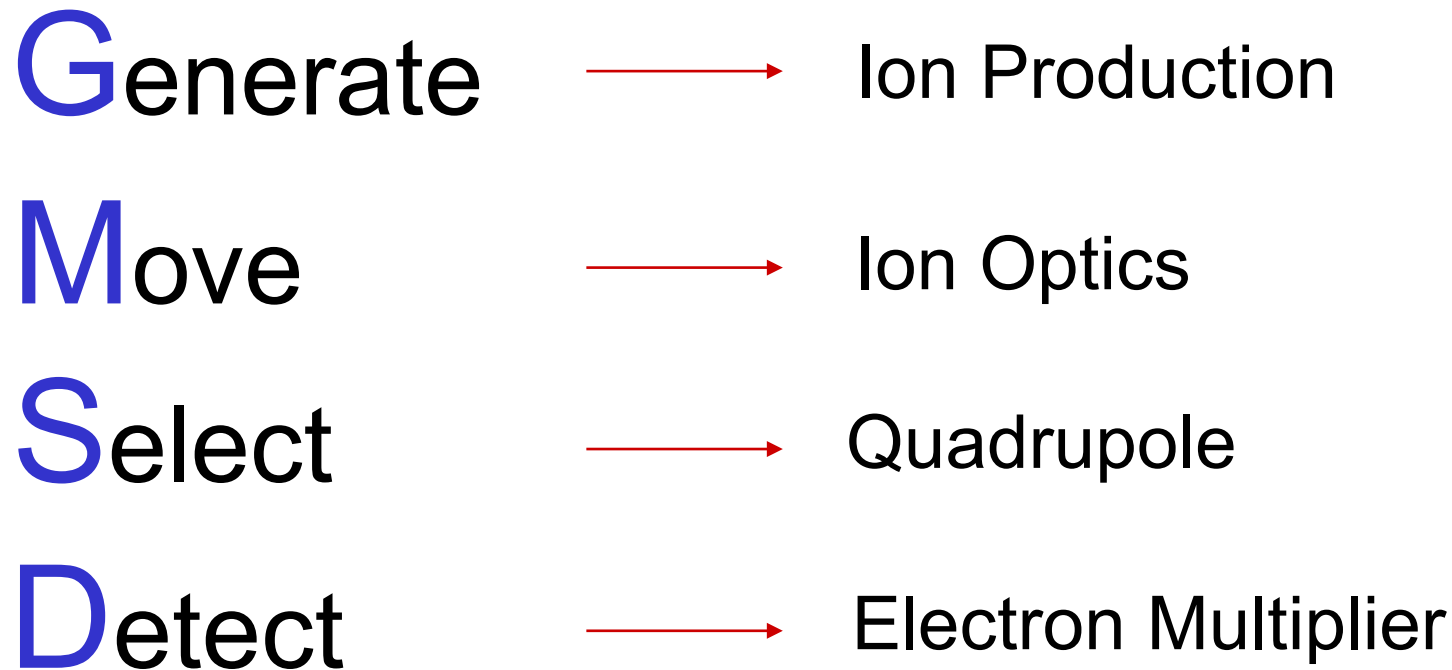


What is Mass Spectrometry?

“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.”

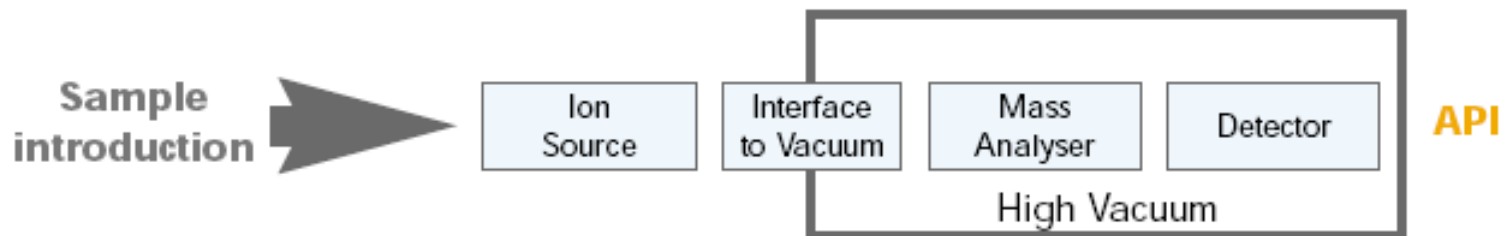
Niessen, W. M. A.; Van der Greef, J., *Liquid Chromatography–Mass Spectrometry: Principles and Applications*, 1992, Marcel Dekker, Inc., New York, p. 29.

Mass Spectrometry “Simplified”



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

API Mass Spectrometry – Block Diagram



Atmospheric Pressure Ionization

Source Types

1. Electrospray (ESI) – Solution phase process.
2. Atmospheric Pressure Chemical Ionization (APCI) – Gas phase process.
3. Atmospheric Pressure Photo-Ionization (APPI) – Gas phase process.

Source Purpose

1. Desolvate sample LC flow for introduction into mass spectrometer.
2. Baffle the first vacuum region of the mass spectrometer from the atmospheric pressure region in the source.
3. Ionize the analyte or allows the transport of ions from solution into the gas phase.
4. Pump away neutrals and opposite charged ions, which would otherwise interfere with the analysis of ions of desired polarity.



Chemistry Considerations

ESI:

Ions formed by solution chemistry

Good for thermally labile analytes

Good for polar / semi-polar analytes

Good for high MW molecules (proteins / peptides)

APCI / APPI:

Ions formed by gas phase chemistry

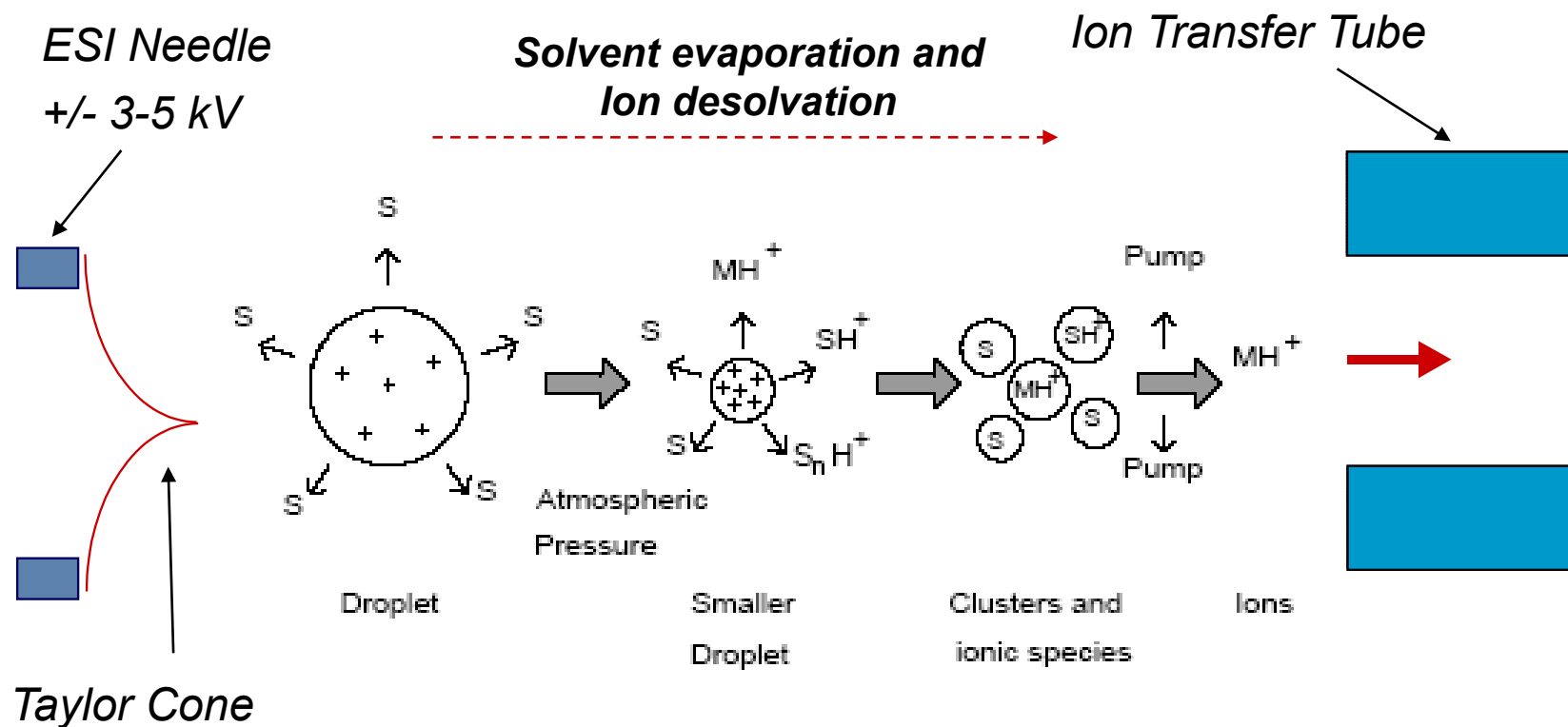
Good for volatile / thermally stable analytes

Good for non-polar / semi-polar analytes

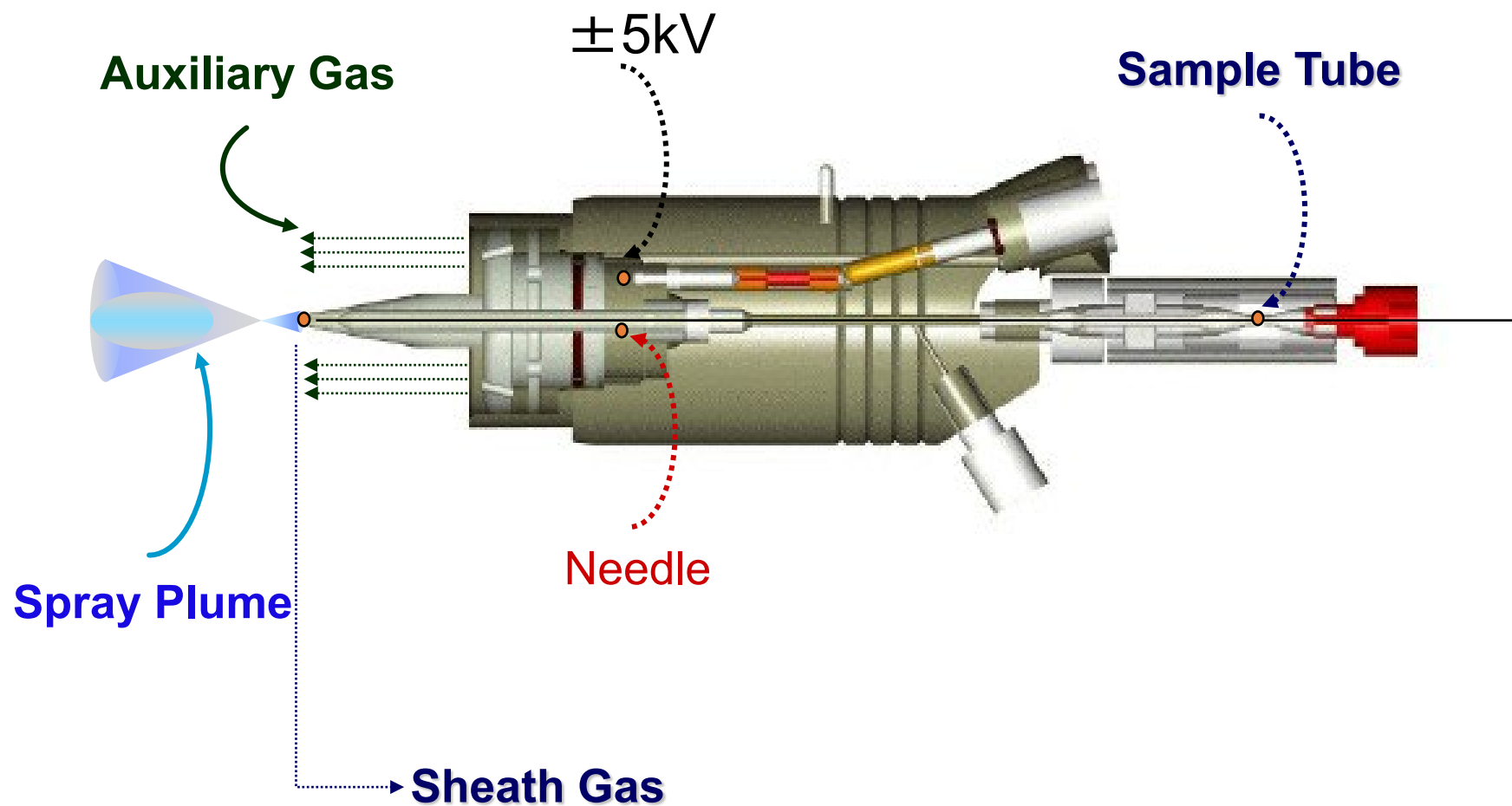
Good for small molecules (i.e. steroids)

Good for ions containing a chromophore (APPI)

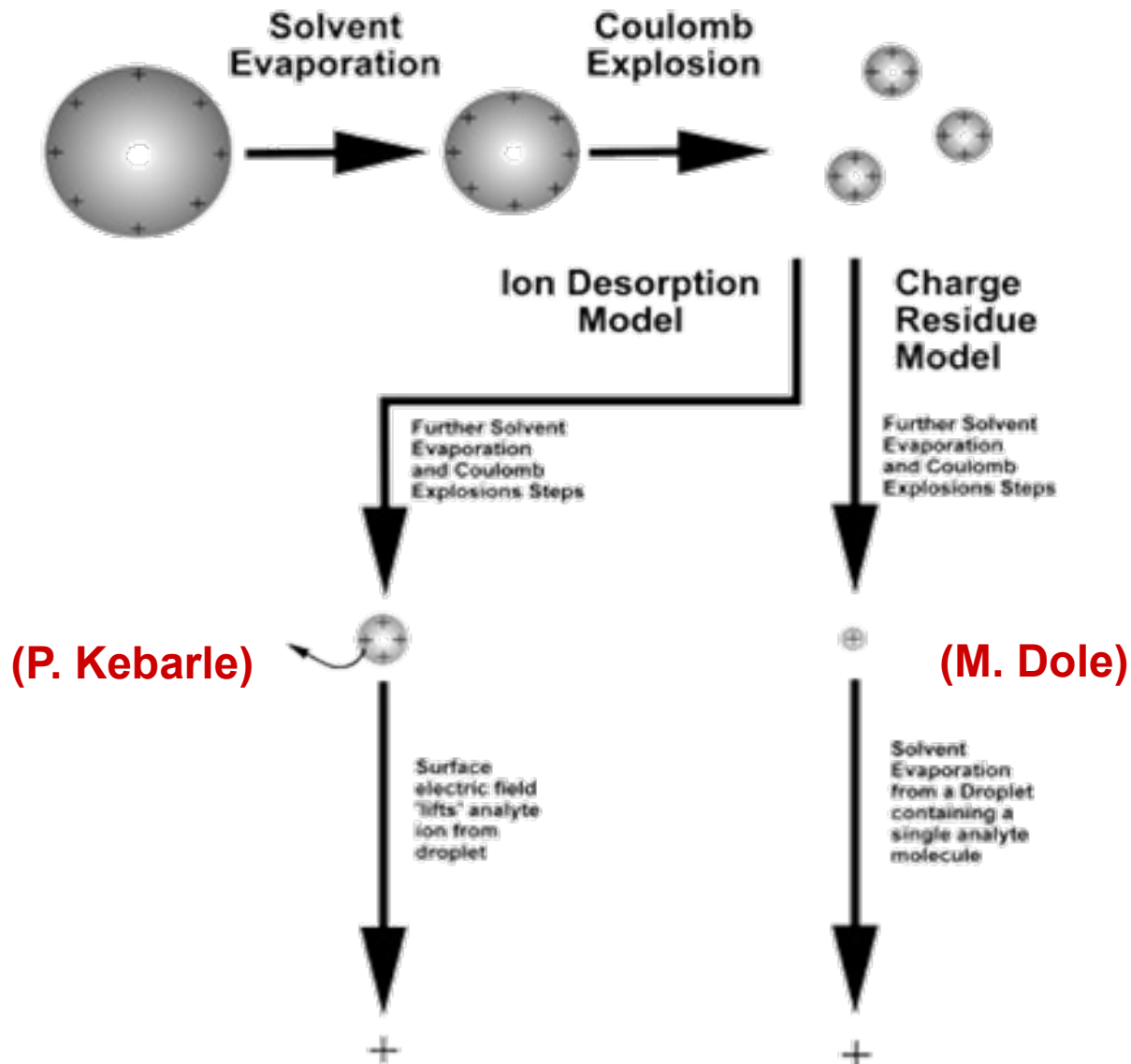
Electrospray - Basic Principle



ESI Nozzle Cross Section



Electrospray – Prevailing Theories



Positive or Negative Ion Mode ?

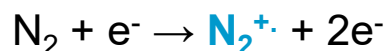
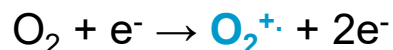
● Basic Functional Groups \longrightarrow $[M+H]^+$
(-NH₂)

● Acidic Functional Groups \longrightarrow $[M-H]^-$
(-COOH, -OH)

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:

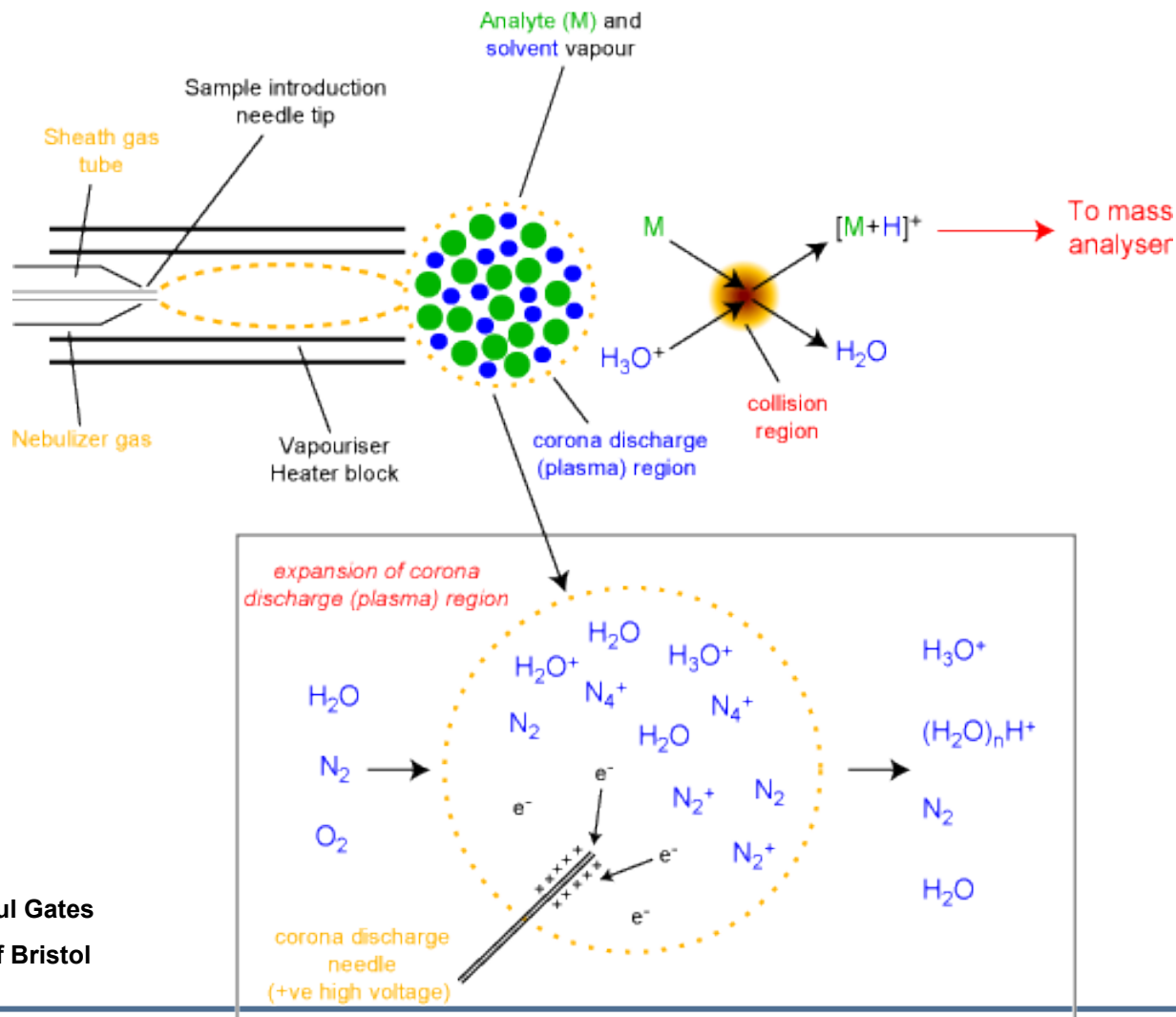


2. Through a complex series of reactions primary ions react with solvent molecules forming reagent ions, H_3O^+ and CH_3OH_2^+

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



Atmospheric Pressure Chemical Ionization (APCI)

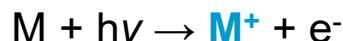


© 2004 Dr. Paul Gates
University of Bristol

Atmospheric Pressure Photo-Ionization (APPI)

- Gas-phase desolvation via APCI mechanism
- Ionization via UV light source
- APPI is a two step process:

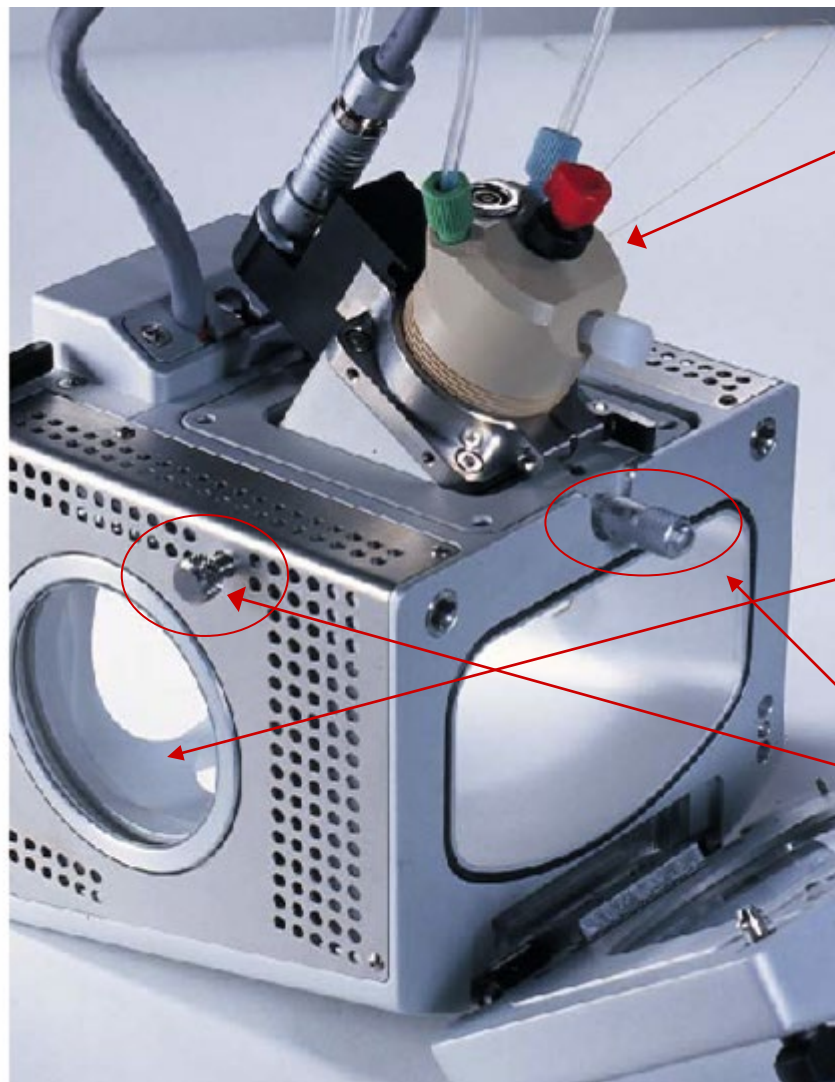
1. Analyte molecules interact with the UV light source (krypton light emits 10.0 eV and 10.6 eV photons). The analyte molecule M is ionized to a molecular ion M^+ if the ionization potential (IP) of the analyte is lower than the photon energy ($h\nu$)



2. In the presence of protic solvents, the analyte ion may extract a hydrogen ion to form a protonated molecule $[M+H]^+$



Ion Max API Source

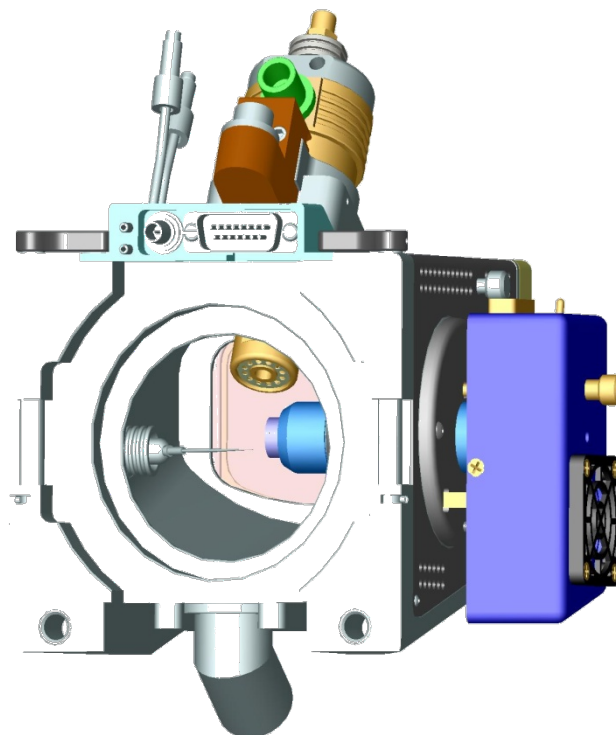
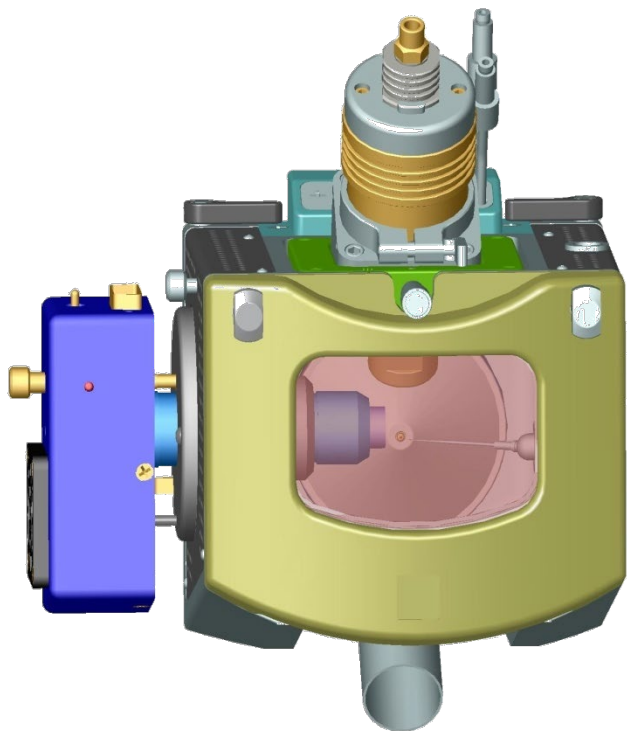


Interchangeable source probe
(ESI probe shown)

Window - Inlet for APPI accessory

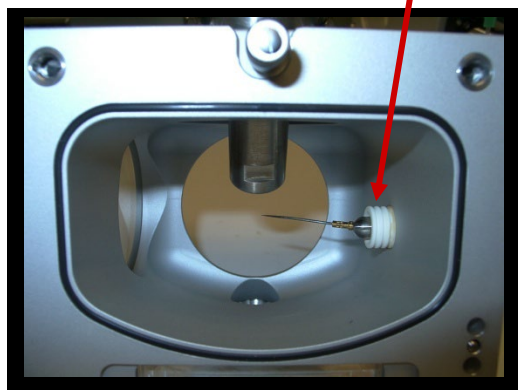
Positional adjusters for source probe

Ion Max Source – APPI Optional Accessory

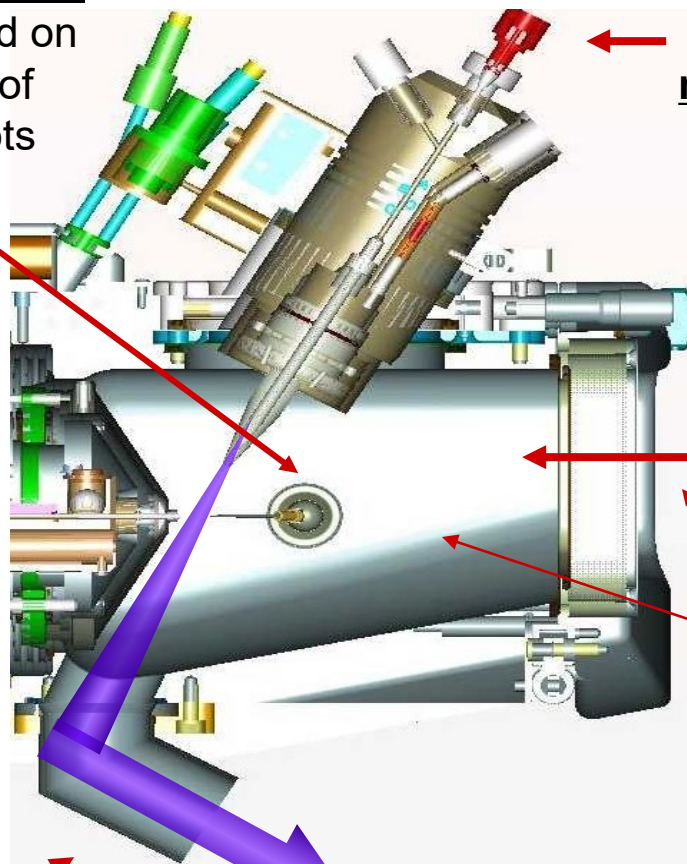


Ion Max Source - Housing Design

Corona discharge pin – **ceramic** and **stainless steel**; located on vertical wall – no pooling of solvent vapor on cold spots



Probe mounted at 60° - liquid drips directly into drain port, **no accumulation of solvent** in housing if nitrogen supply runs out



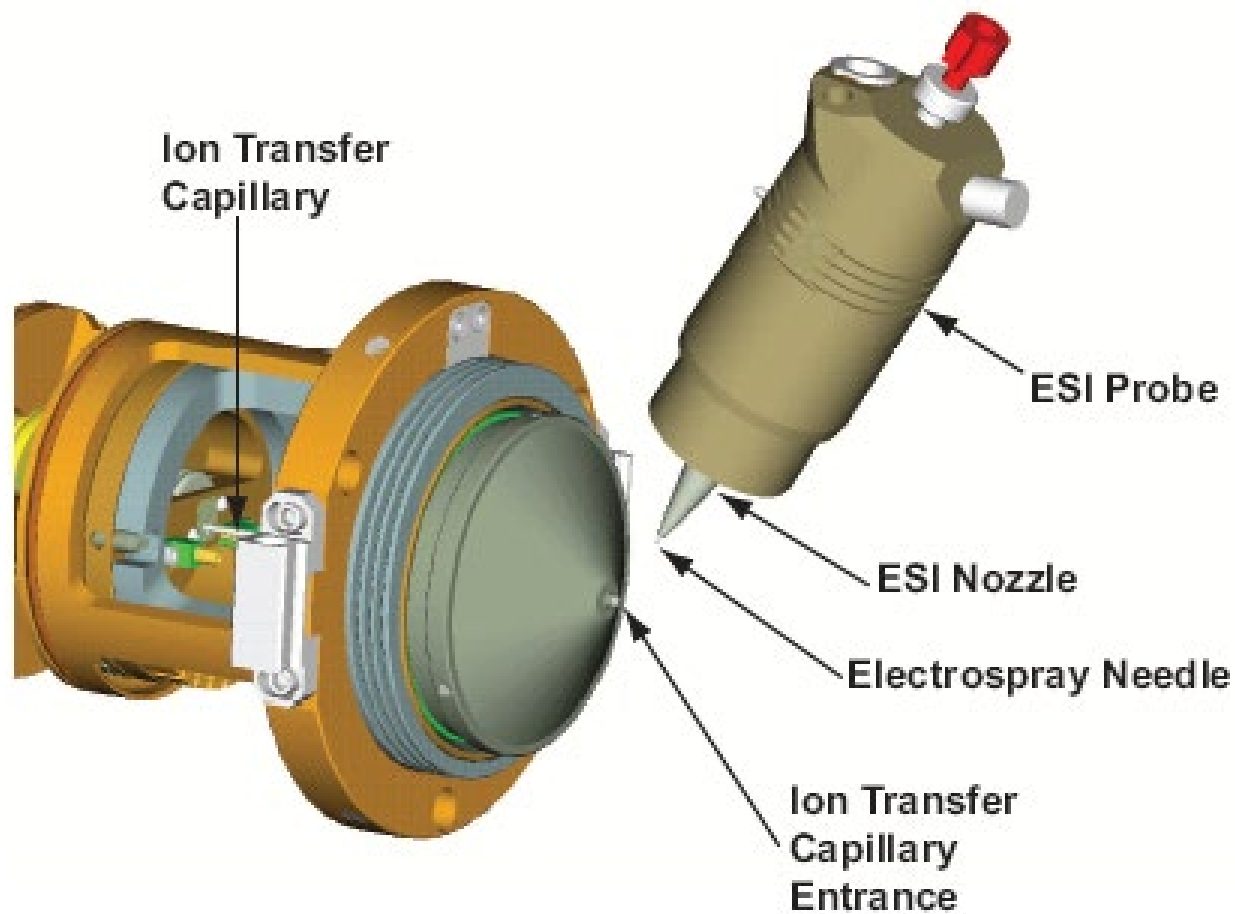
Plastic-free housing

Two view ports for easier visualization

Stainless steel port for improved chamber drainage

Improved drain design, allows streamlined API exhaust removal

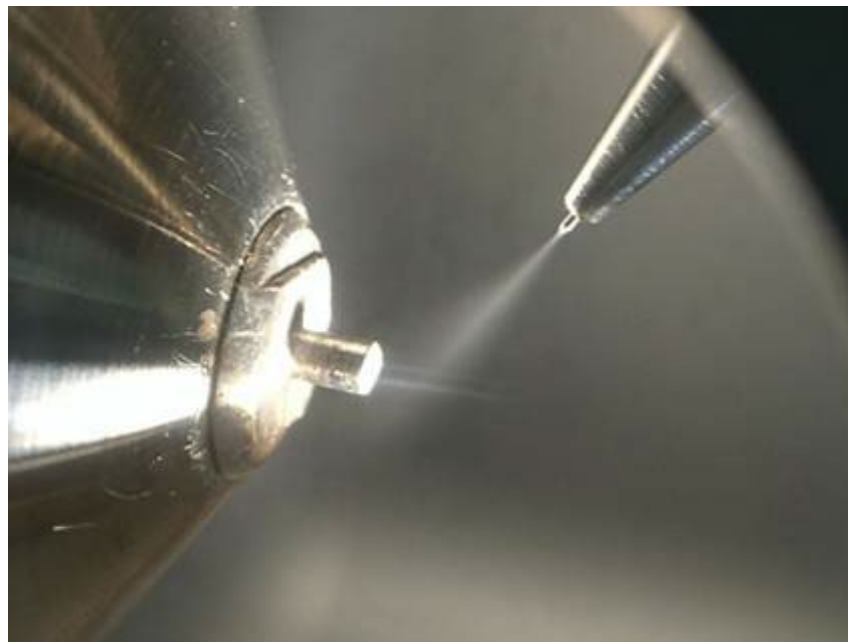
Ion Max Source - ESI



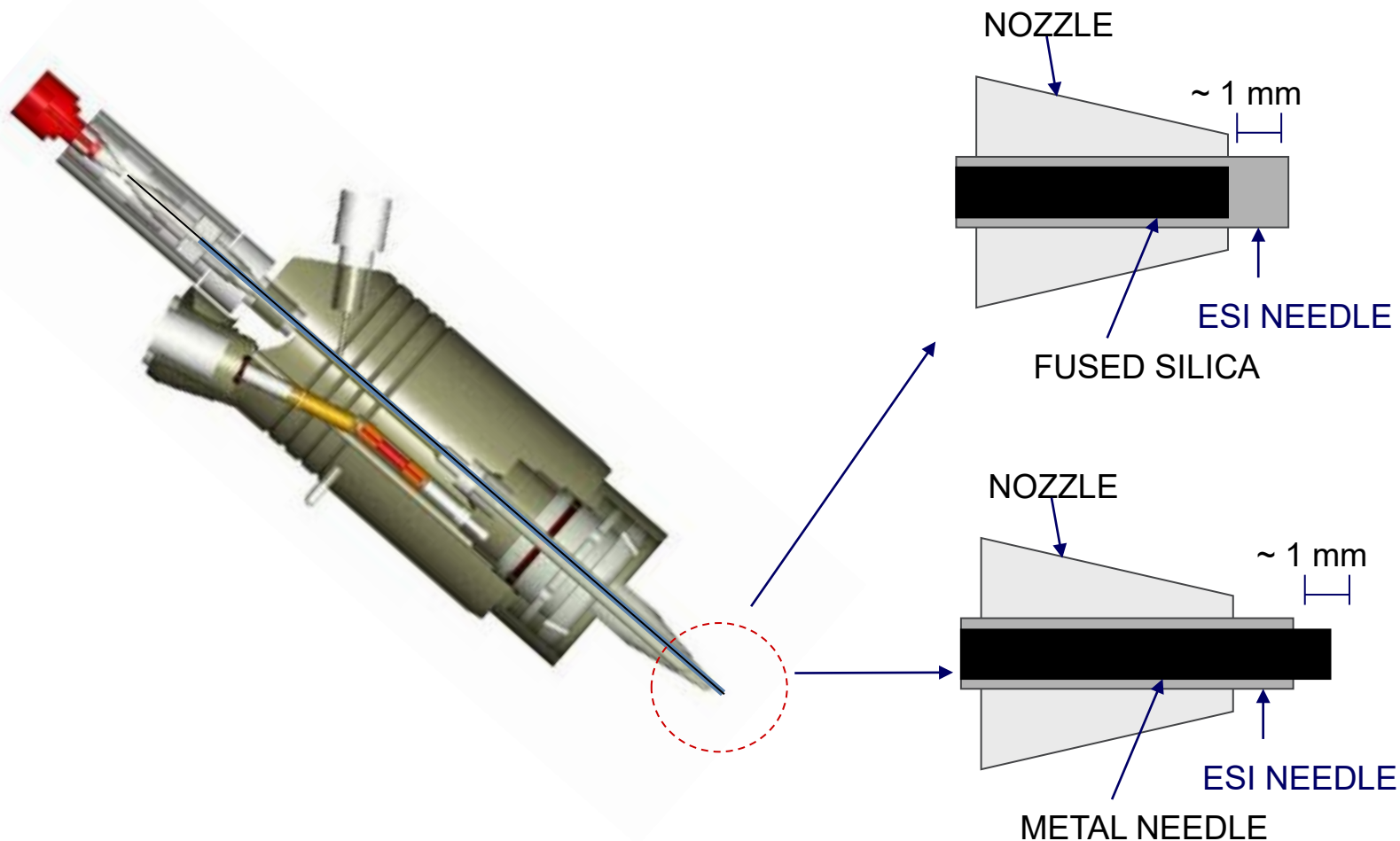
Ion Max Source Design - ESI Probe

ESI probe features:

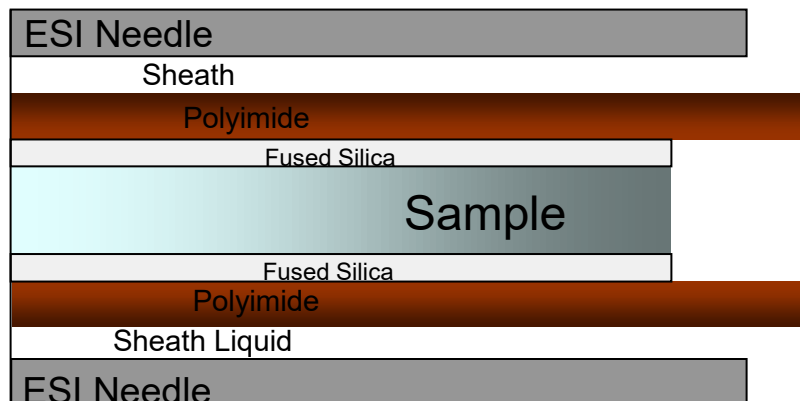
- Fixed spray angle (60 degrees)
- Built-in sheath liquid line (for accurate mass & post-column addition applications)
- X,Y,Z - adjustable for additional spray quality optimization



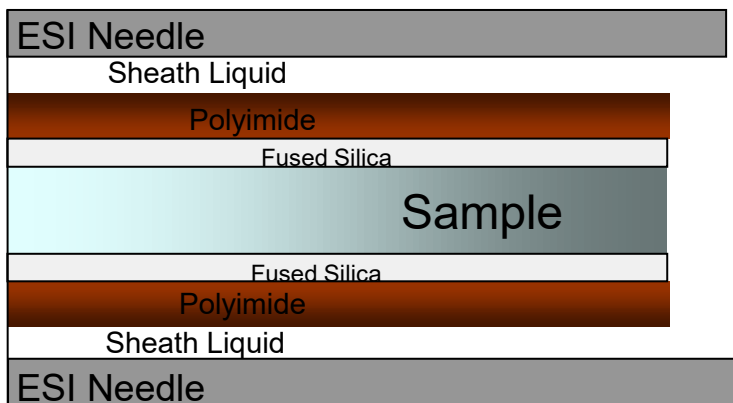
ESI Probe – Sample Tube Choices



Fused Silica Capillary Sample Tube



Elongation of polyimide coating occurs when specific solvents (i.e., acetonitrile) come in contact with the coating.



The sample tube must be cut square to ensure a stable spray.

Best results can be achieved by positioning the sample tube about 1 mm inside the ESI needle.

ESI – Operational Conditions (guidelines)

Liquid Flow Rate (μL/min)	Ion Transfer Tube Temp.* (°C)	Sheath Gas Pressure (arb)	Aux Gas Flow (arb)	Spray Voltage** (V)
5	240	5	0	+2500 (-2500)
200	350	35	5	+3500 (-3000)
1000	400	75	20	+4500 (-3500)

* Optimization of tube lens voltage is recommended following a change in ion transfer tube temperature

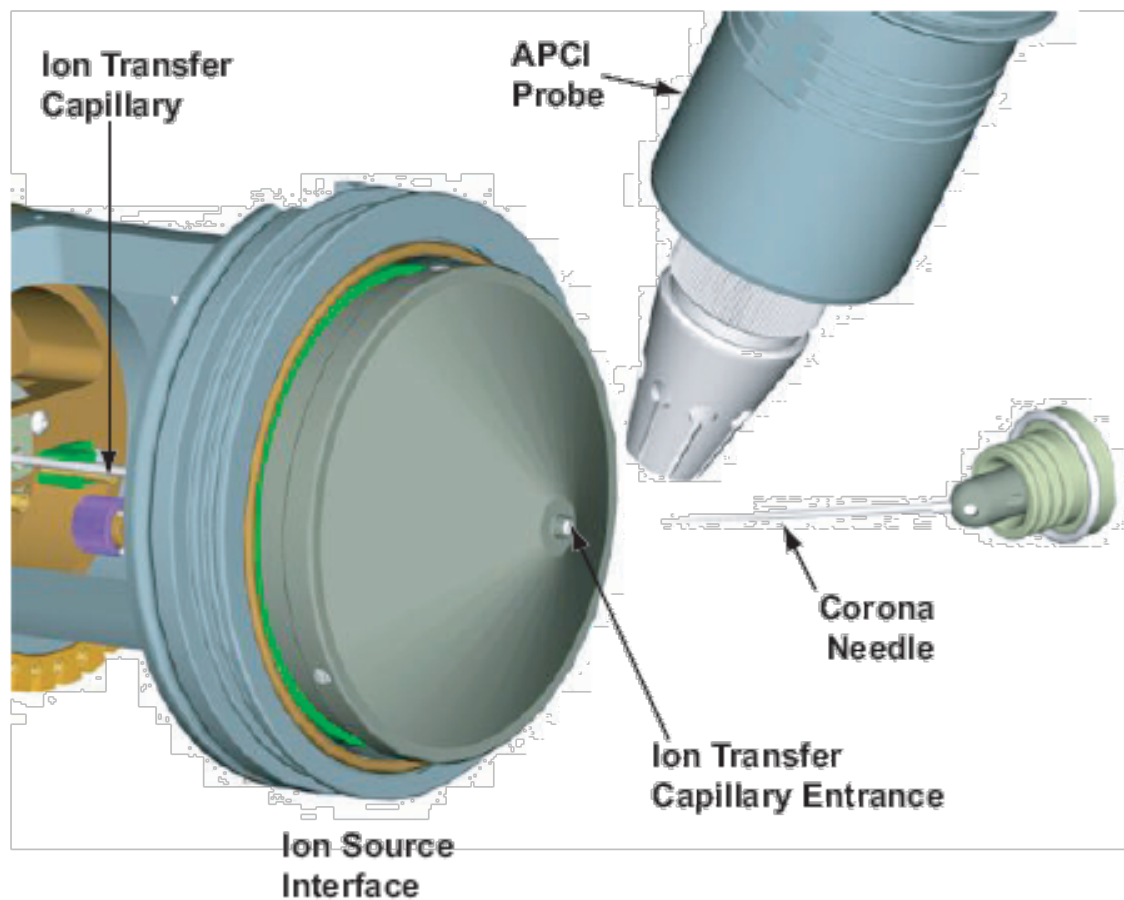
** The spray voltage values may vary for different sample tube materials (i.e., fused silica vs. stainless steel)

Note: Generally, higher flow rates require higher sheath and auxiliary gas flows, as well as a higher ion transfer tube temperature.

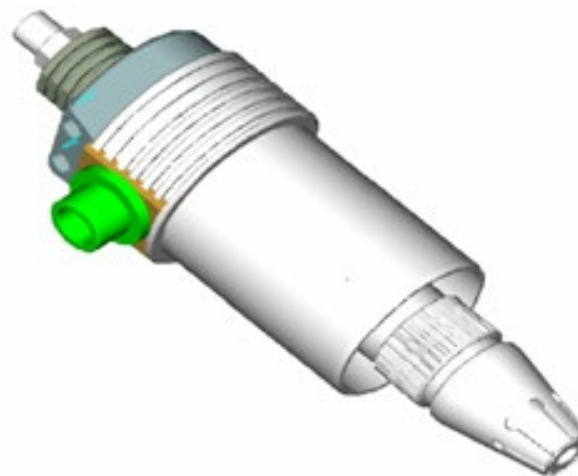
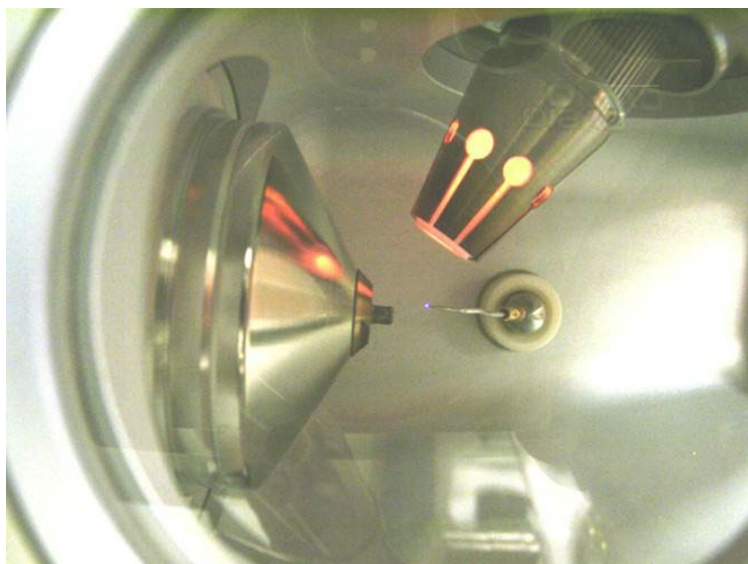
ESI - Microflow Operational Conditions (guidelines)

Stainless Steel Needle Size	Solvent Flow Rate (μL/min)	Capillary Temperature (°C)	Sheath Gas Pressure (Arb. units)	Aux Gas Pressure (Arb. units)	Spray Voltage (kV)
34-gauge	0.5 - 50	150 - 200	0 - 5	0	1.5 - 4.0
32-gauge	3 - 400	200 - 250	5 -15	0 - 5	1.5 - 4.0

Ion Max Source Design - APCI Probe

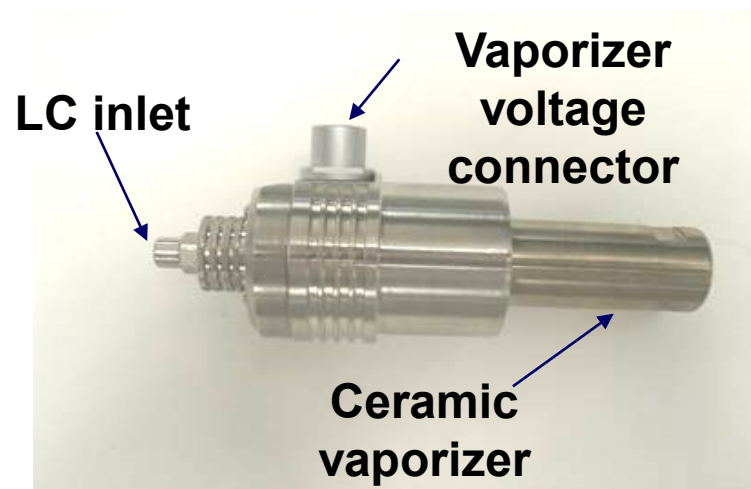


Ion Max Source Design : APCI Probe



APCI probe features:

- Removable sprayer
- Ceramic heater
- Self-cleaning
- External thermocouple
- No plastics in source housing
- Easily changeable nozzle assembly
- X,Y,Z – adjustable



APCI – Operational Conditions (guidelines)

Liquid Flow Rate (μL/min)	Ion Transfer Tube Temp. (°C)*	Sheath Gas Pressure (arb)	Aux Gas Flow (arb)	Vaporizer Temperature (°C)	Corona Discharge Current (μA)
200	250	25	5	350	+4 (-10**)
1000	250	45	5	450	+4 (-10**)

* Optimization of tube lens voltage is recommended following a change in ion transfer tube temperature

** Negative-ion mode

Note: Generally, higher flow rates require higher sheath and auxiliary gas flows, but do not require a higher ion transfer tube temperature.

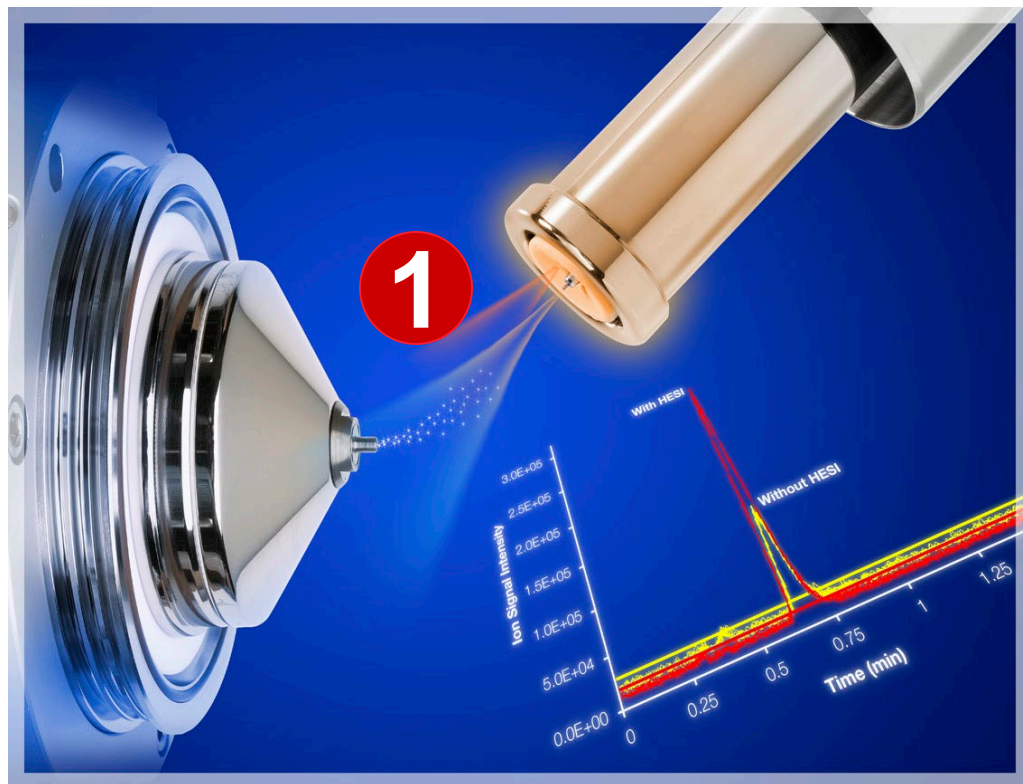
Ion Max Source Design - H-ESI Probe



Dual Desolvation Zone Technology

Zone One— Temperature Control

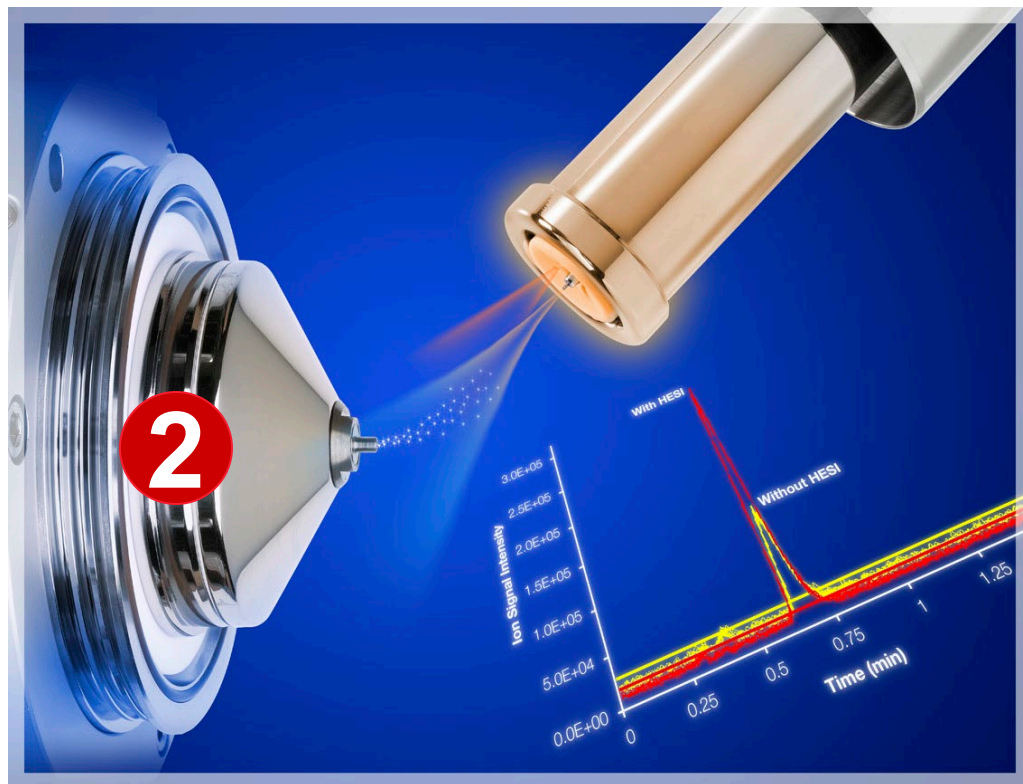
This zone offers full control over the temperature of the auxiliary nitrogen gas. Desolvation is initiated in the source housing by turning on the built-in heat-exchange mechanism which heats the auxiliary gas. This can be done at high LC flow rates or when hydrophilic compounds elute in the high aqueous content of a gradient.



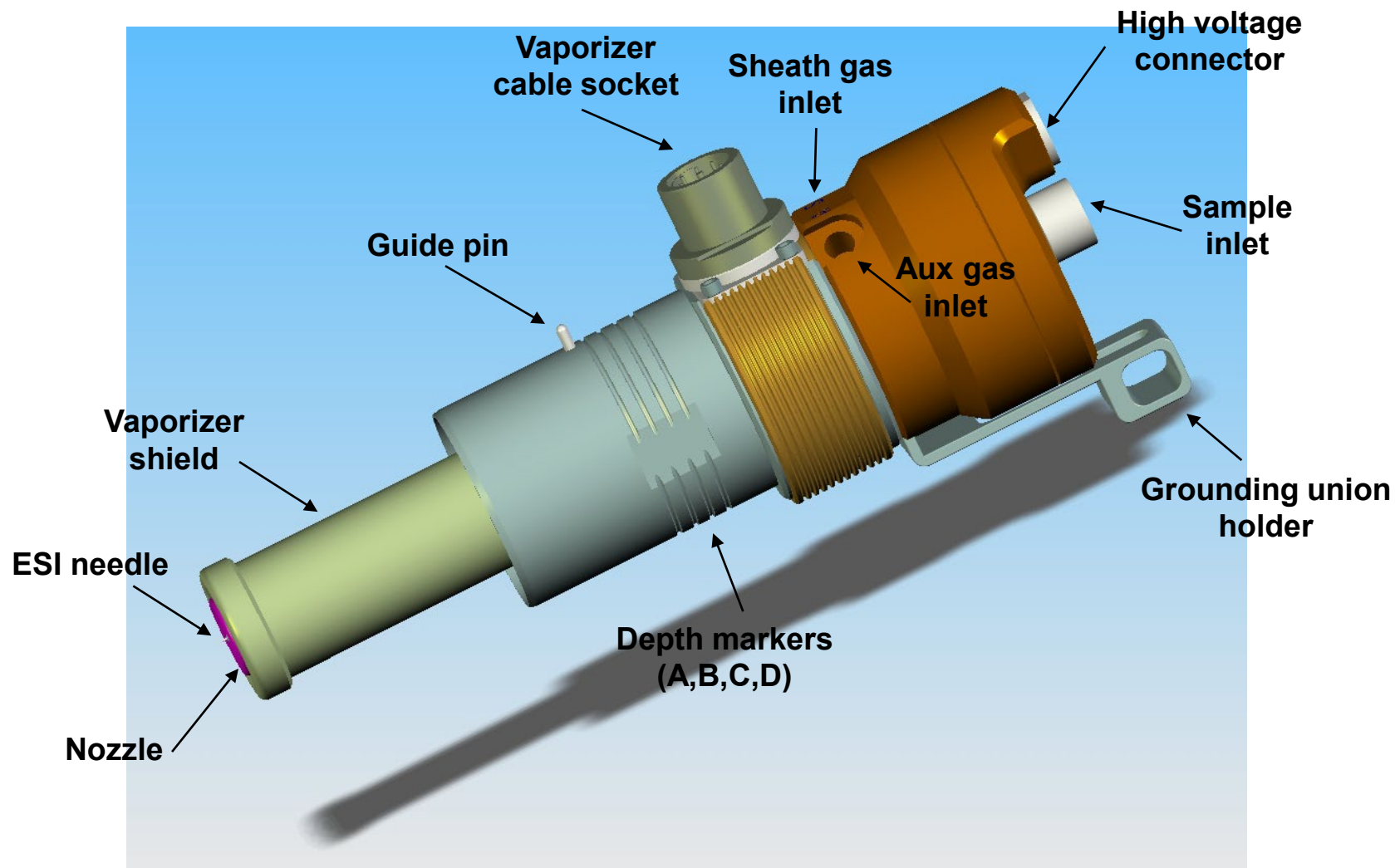
Dual Desolvation Zone Technology

Zone Two— Maximize Ion Efficiency

After being desolvated in zone one, the ions travel into zone two, which maximizes ionization efficiency. This zone allows full control over the temperature of the heated ion transfer tube. The temperature of zone two can be decreased to enhance signal for a wide range of thermally labile compounds.



H-ESI Probe



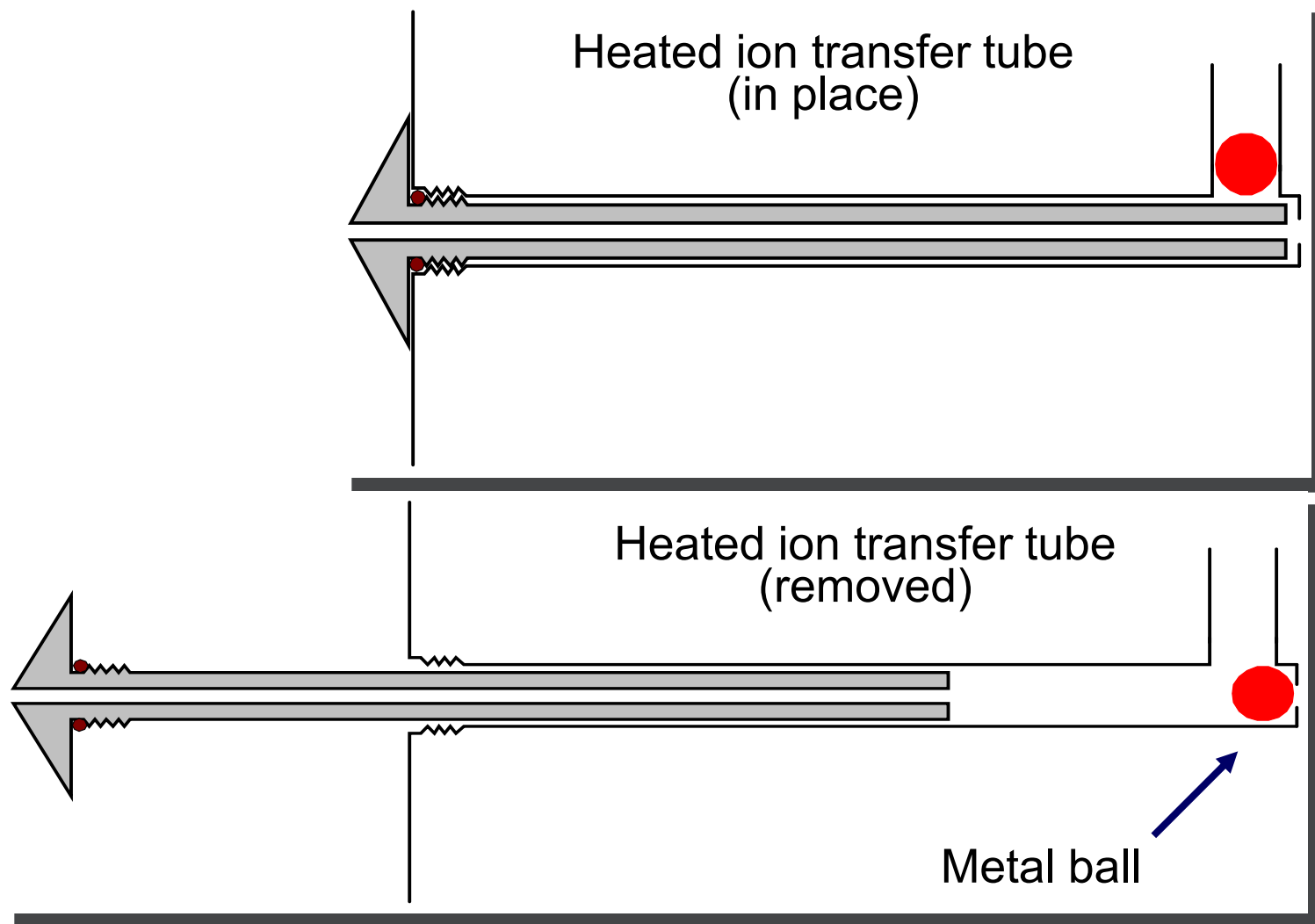
H-ESI - Operational Conditions (guidelines)

Liquid Flow Rate ($\mu\text{L}/\text{min}$)	Ion Transfer Tube Temp. ($^{\circ}\text{C}$)*	Vaporizer Temperature** ($^{\circ}\text{C}$)	Sheath Gas Pressure (arb)	Aux Gas Flow (arb)
5	240	Off - 50	5	0
200	350	250 - 300	35	30
500	380	300 - 400	60	50
1000	400	350 - 450	75	60

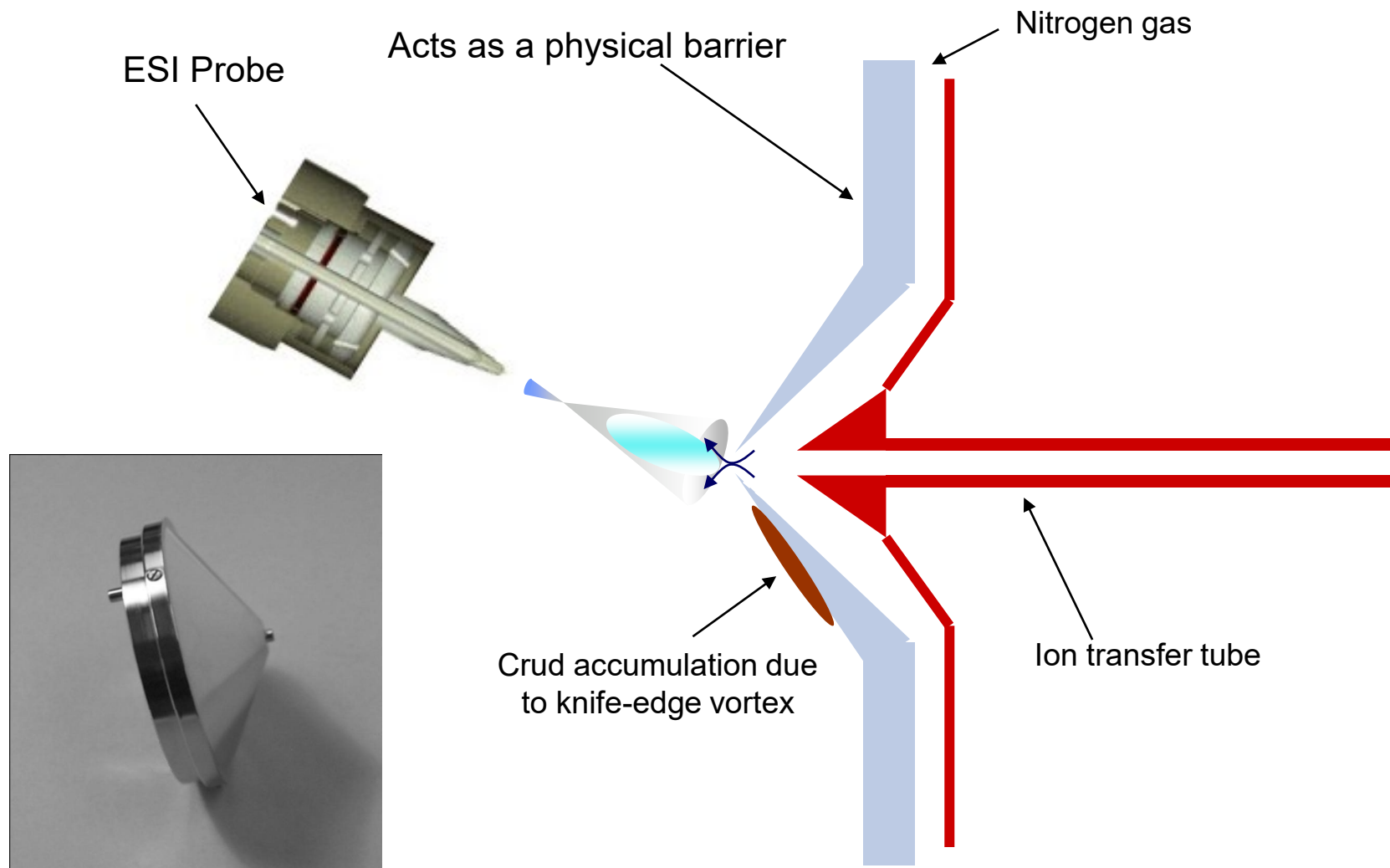
* Optimization of tube lens voltage is recommended following a change in ion transfer tube temperature

** Compound-dependent

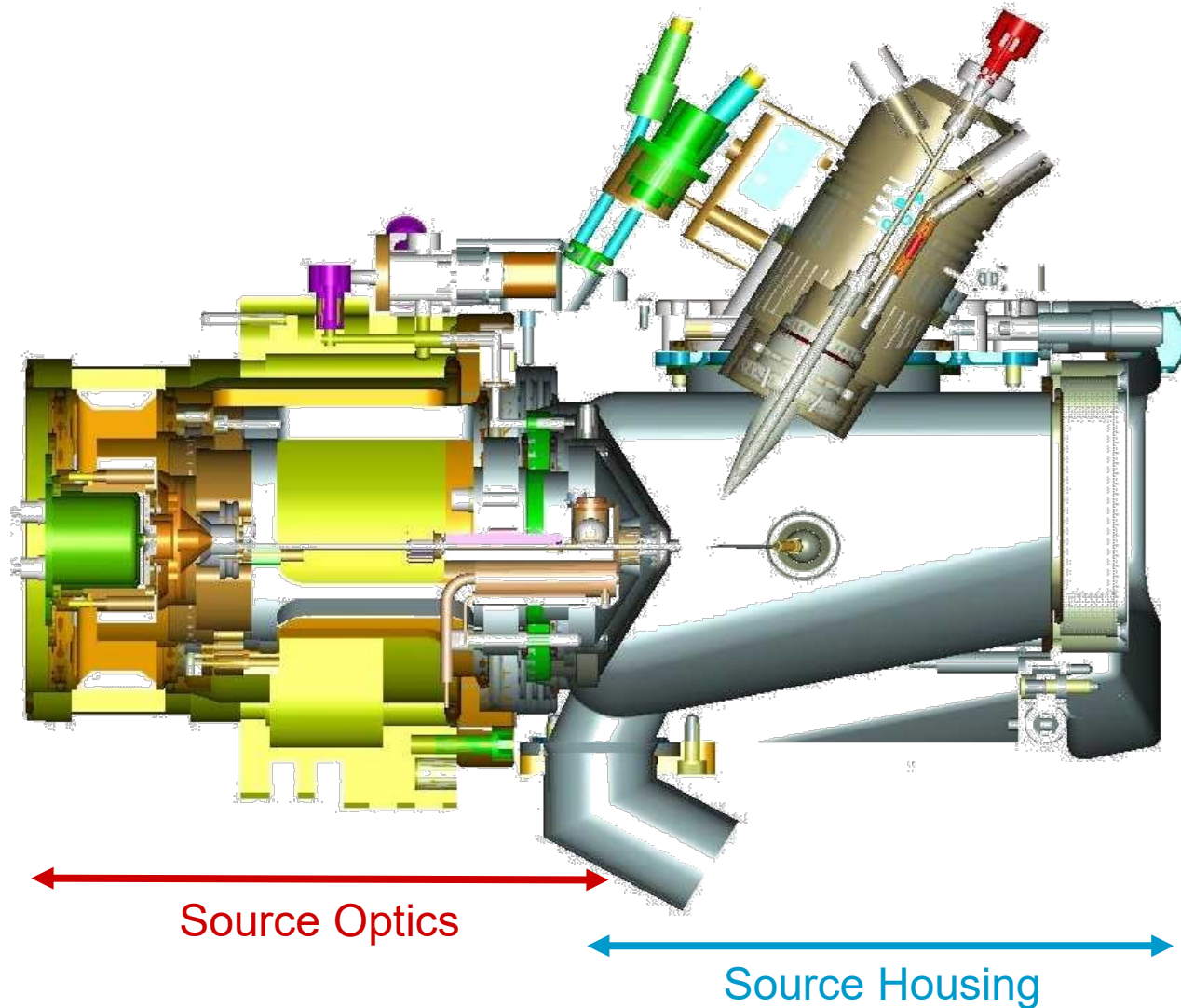
Removable Ion Transfer Tube



The Ion Sweep Gas Function



Ion Max Source - Source Optics and Housing



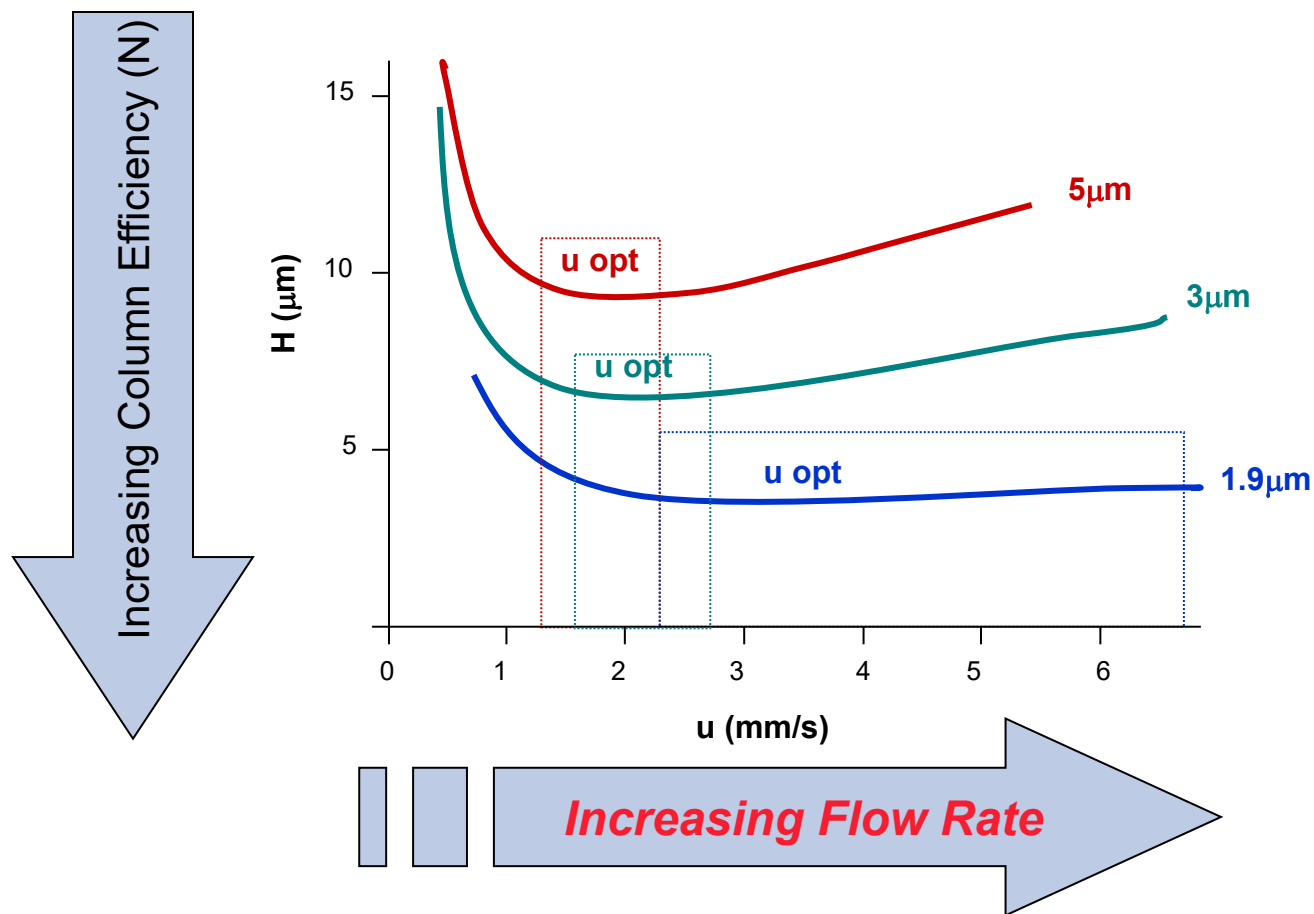


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Chapter 2

Notes on Liquid Chromatography

Optimal Linear Velocities (u_{opt}) Based on Particle Size



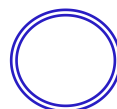
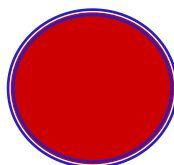
$$H = \frac{N}{L}$$

$$N \propto \frac{1}{d_p}$$



Response Enhancement - Narrow Bore Columns

Col. Diameter, mm	4.6	3.0	2.1	1.0
Flow Rate, $\mu\text{L}/\text{min}$	1000	500	200	50
Theoretical Increase (peak height)	1	2.0	5	20



Once optimal peak shape is achieved, the only way chromatographically to enhance signal is to reduce the columns internal diameter.

LC Additives

➤ Acids (proton donors)

- ❑ Do not use inorganic acids (*will cause source corrosion*)
- ❑ Formic and acetic acid are recommended

➤ Bases (proton acceptors)

- ❑ Do not use alkali metal bases (*will cause source corrosion*)
- ❑ Ammonium hydroxide and ammonia solutions are recommended

➤ Surfactants (improve chromatographic separation)

- ❑ Detergents and other surface active agents may suppress ionization

➤ Trifluoroacetic Acid (TFA) (improves chromatographic separation)

- ❑ May enhance chromatographic resolution, but causes ion suppression in both negative and positive ion mode

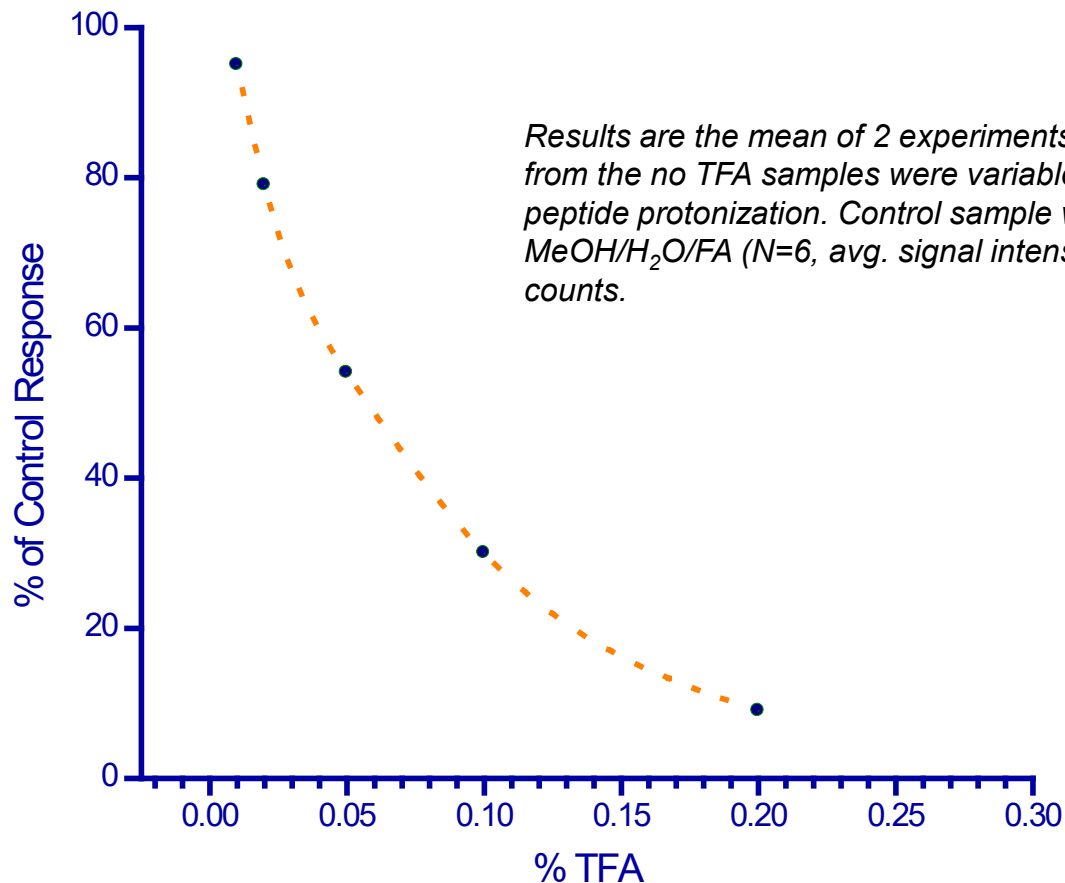
➤ Triethylamine / Trimethylamine

- ❑ May enhance deprotonation in negative ion mode

➤ Buffers

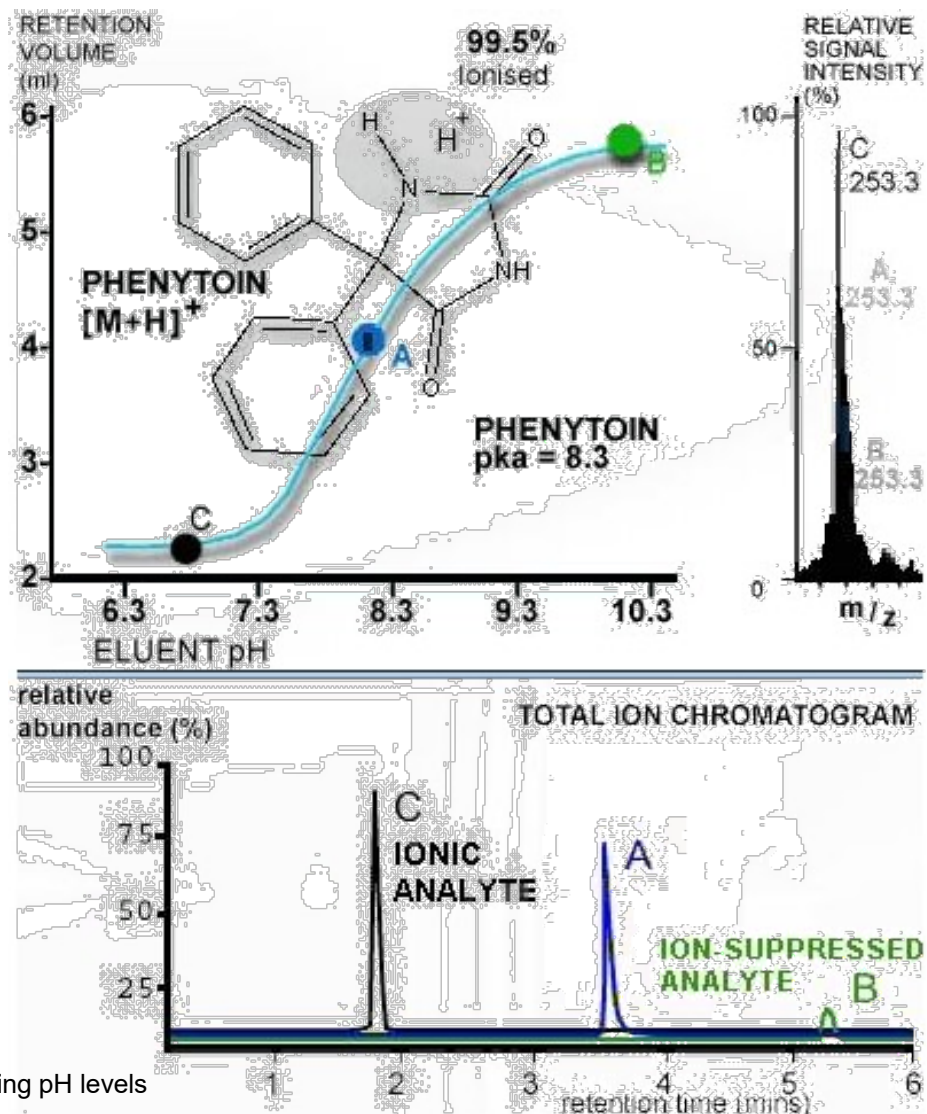
- ❑ Avoid using non-volatile buffers. If used, the sweep cone should be in place. Frequent cleaning of ion transfer tube and Q00 is suggested.

Example - Effect of TFA Levels on MS Signal Intensity



* S. Baldwin, K. Stoney, K. Wheeler, I. Mychreest. "Low pH Solvent Alternatives to TFA Solvents and Their Effect on HPLC/ESI-MS of Peptides", Poster Paper Presented at ASMS '96.

Buffers (pH)



Overlaid chromatograms at the differing pH levels



Buffers

- When using non-volatile buffers, sweep cone should be in place, as a physical barrier; additionally, the use of nitrogen sweep gas will reduce the background contamination.

- If possible, avoid using non-volatile HPLC additives such as:
 - Alkali-metal phosphates
 - Borates
 - Citrates

- When using buffers, more frequent cleaning of the source housing, sweep cone, ion transfer tube, skimmer, and tube lens is necessary.



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Chapter 3

TSQ Quantum Specifics and Optics

Inside the TSQ Quantum



TSQ Quantum - System Specifications

- 1 Triple-port Leybold TW220/150/15S turbomolecular pump
- 2 Rotary forepumps * (Edwards E2M30, 650 L/min)
- All vacuum lines are 1.5 inches in diameter
- 250 mm quadrupoles (Q1 and Q3), 6 mm R_o
- Integrated syringe pump with adjustable delivery rate
- 6-Port divert valve / loop injector
- Collision cell 185 mm, 0 - 5 mTorr pressure range
- Maximum scan range (m/z): 30 – 1500 **
- Maximum scan speed: 2000 Da/sec
- 5000 Resolution FWHM at m/z 500
- Full DS control of every parameter

* TSQ Quantum Discovery is equipped with one E2M30 forepump

** TSQ Quantum Access and Ultra EMR have a maximum scan range (m/z) of 30 - 3000

Instrument Control and Data Acquisition

- The maximum sampling rate is automatically adjusted according to the scan speed and resolution
- Maximum total number of tasks allowed per run is 256 (limited by the experiment control matrix):
 - The individual maxima for these categories are:
 - 64 segments per run
 - 64 scan events per segment
 - 64 SRM transitions per scan event
- Polarity-switching occurs in approximately 0.33 seconds *
 - Rate-limiting items:
 - The time needed to stabilize the ion source signal
 - The time to switch the conversion dynode voltage (15 kV)

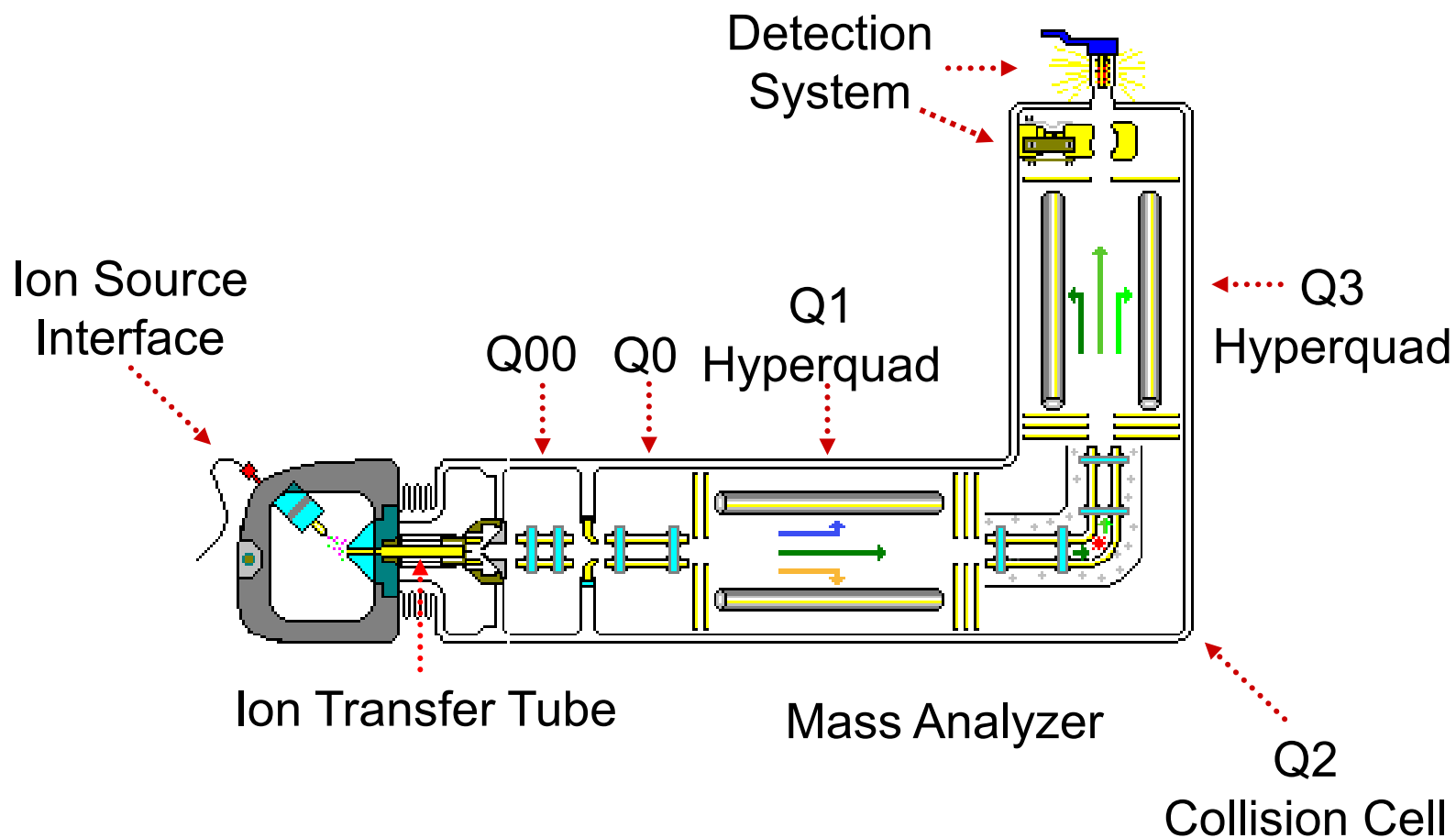
*** *Quantum Access features faster polarity-switching***



TSQ Quantum - Diagnostics

- A full range of diagnostic procedures is available. The procedures concern a wide variety of functions, including power supplies, electrical circuitry, vacuum system, RF tuning, etc.
- The diagnostics workspace is accessible from Quantum Tune View. There is no need to reboot the system to enter / exit the workspace.
- Diagnostic tasks can be selected individually, run as a group, or run in total. All results, including graphs, can be saved for future reference.
- **CAUTION: Running diagnostic tests in an improper succession may lead to circuitry damage or instrument malfunction.**
- Routine application: API spray stability test (recommended as part of auto-tune, calibration, and compound optimization)

TSQ Quantum Components



Ion Guides (a.k.a. “RF-only Devices”)

A multipole rod assembly that is operated with only radio frequency (RF) voltage applied onto the rods. Ideally, in this type of device, virtually all ions have stable trajectories and pass through the assembly.

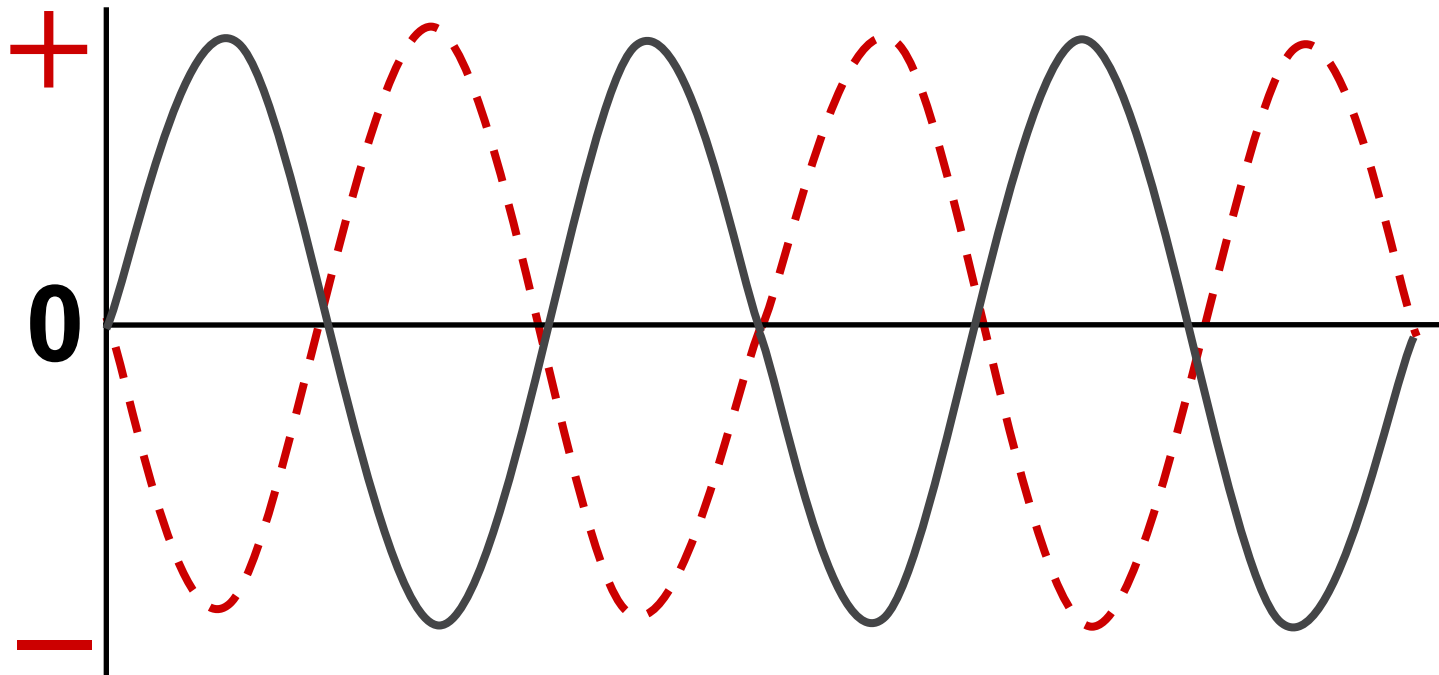
In TSQ Quantum, Q00 and Q0 are quadrupole assemblies of square rods that function solely as ion guides. They focus and transfer the ion beam between the high-pressure region of the ion source and the mass analyzer.

Square-rod quadrupole ion guides offer advantages over multipole ion guides constructed with round rods:

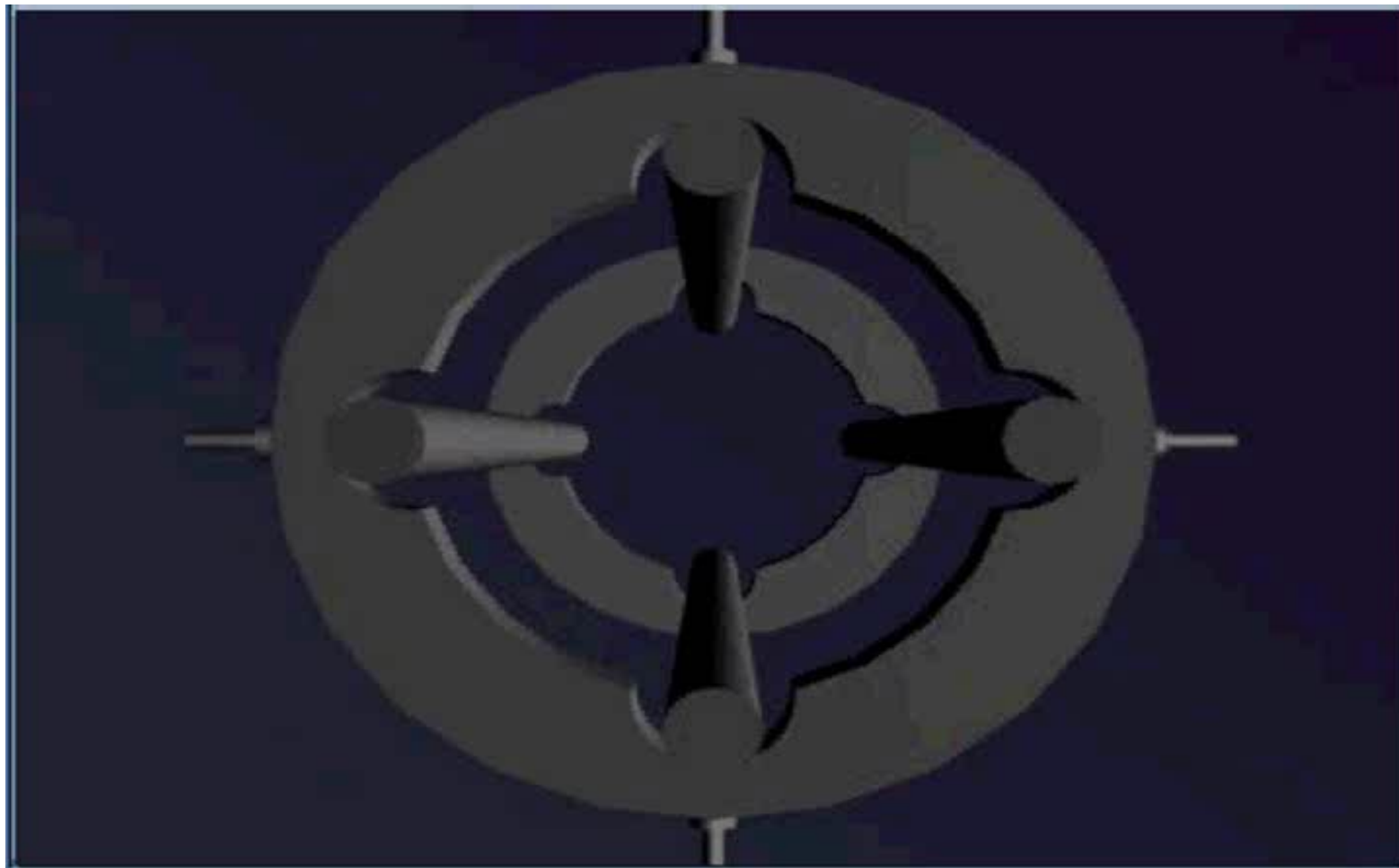
- Reduced size of the inter-quadrupole lens orifices
- Improved vacuum level maintenance at various stages
- Reduced nodding in the quadrupole.
- Better collisional dampening in the quadrupole.
- Enhanced transmission of the ion beam.

Radio Frequency (RF) Voltage

Continuously oscillating voltage of a set amplitude positive and negative relative to a center voltage

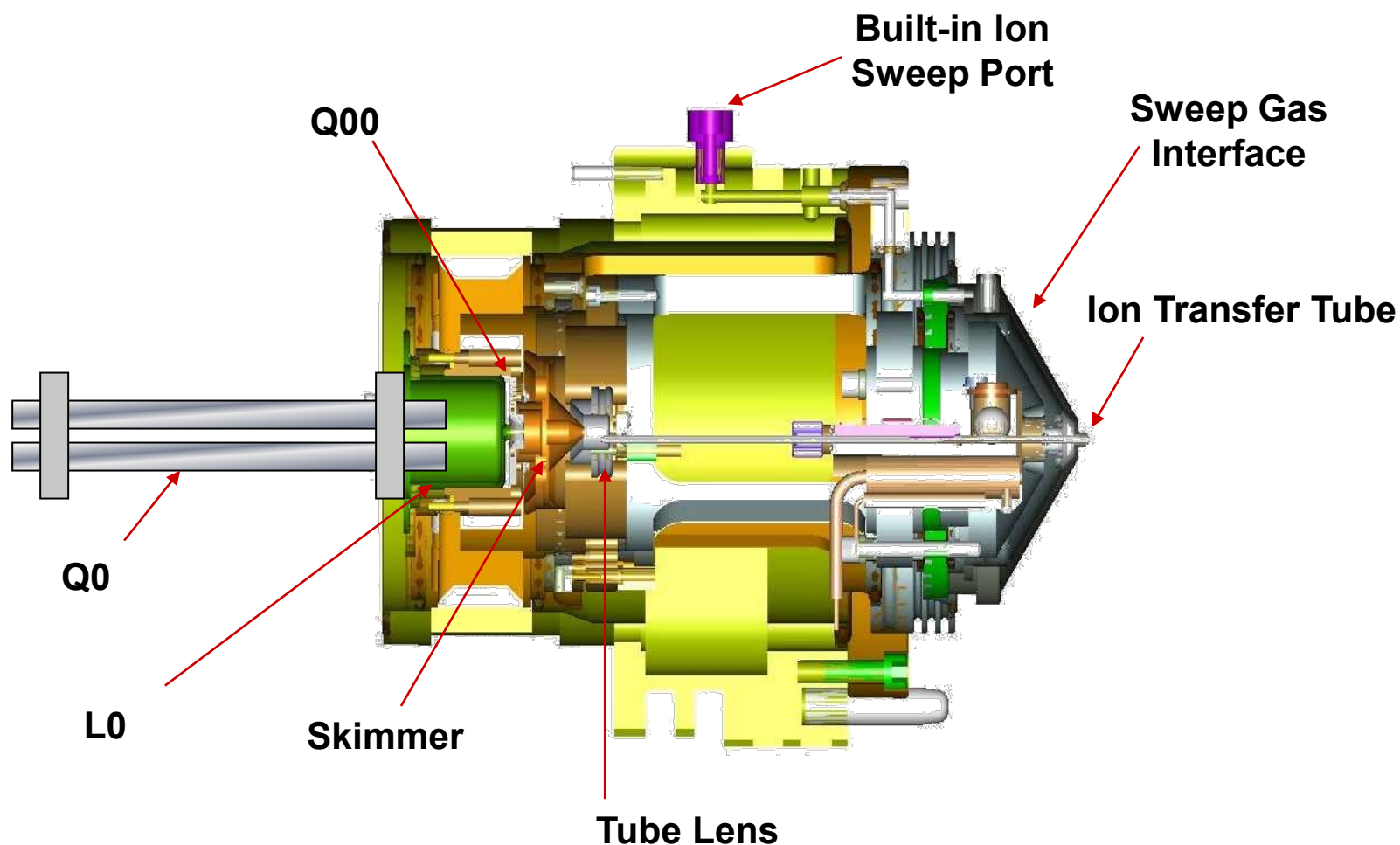


Multipole Transmission (RF-Only)



** Crawford Scientific, OPDAC (Online Professional Development in Analytical Chemistry)
LC-MS training package. Holm Street, Strathaven, Lanarkshire, ML10 6NB, Scotland, UK*

Quantum Ultra - Heater Cage Assembly



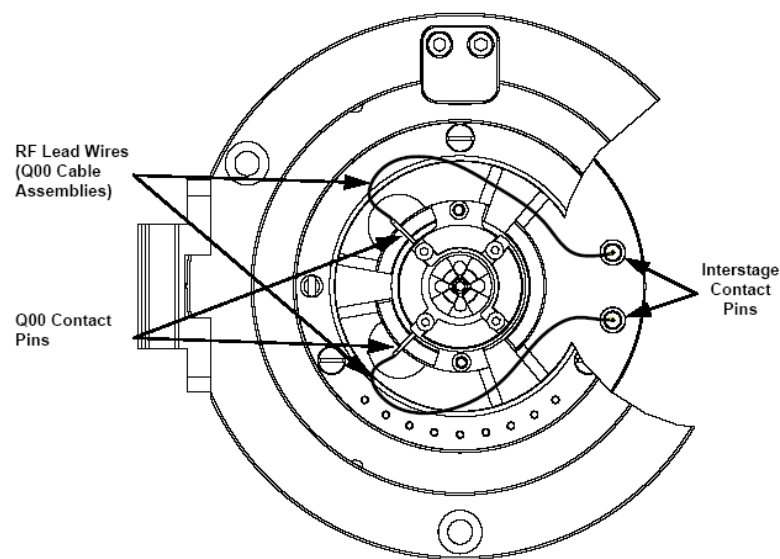
Skimmer

Characteristics:

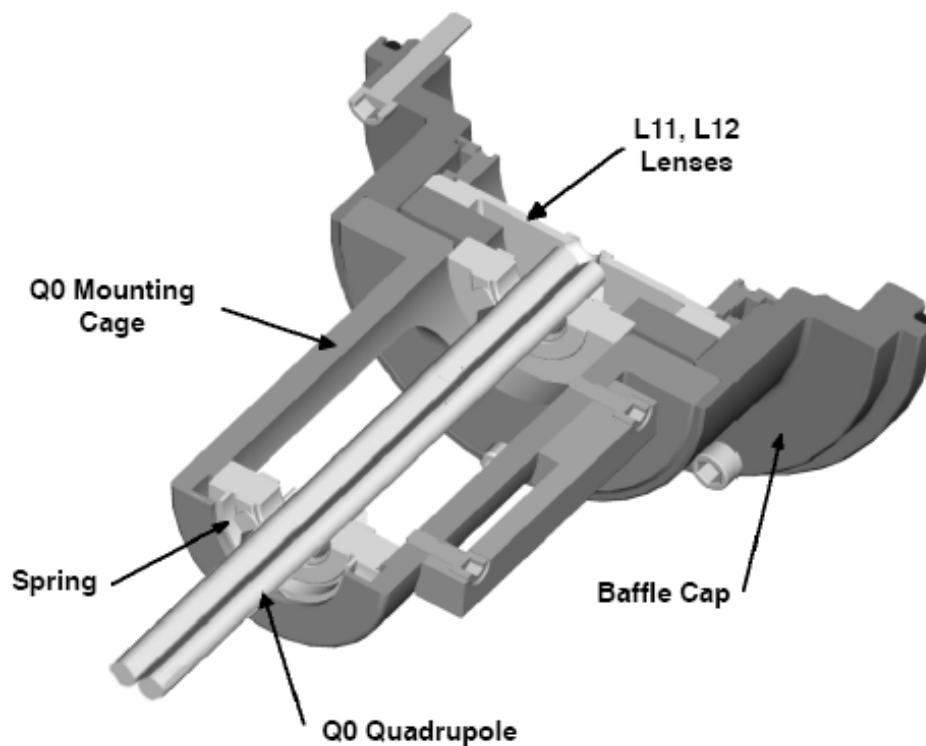
- Bigger diameter for increased sensitivity (Quantum Ultra)
- Made of titanium
- Keeps skimmer at better thermal equilibrium than stainless steel which can act as a heat sink upon cooling



Ion Guide Quadrupoles (Quantum Ultra / Access)



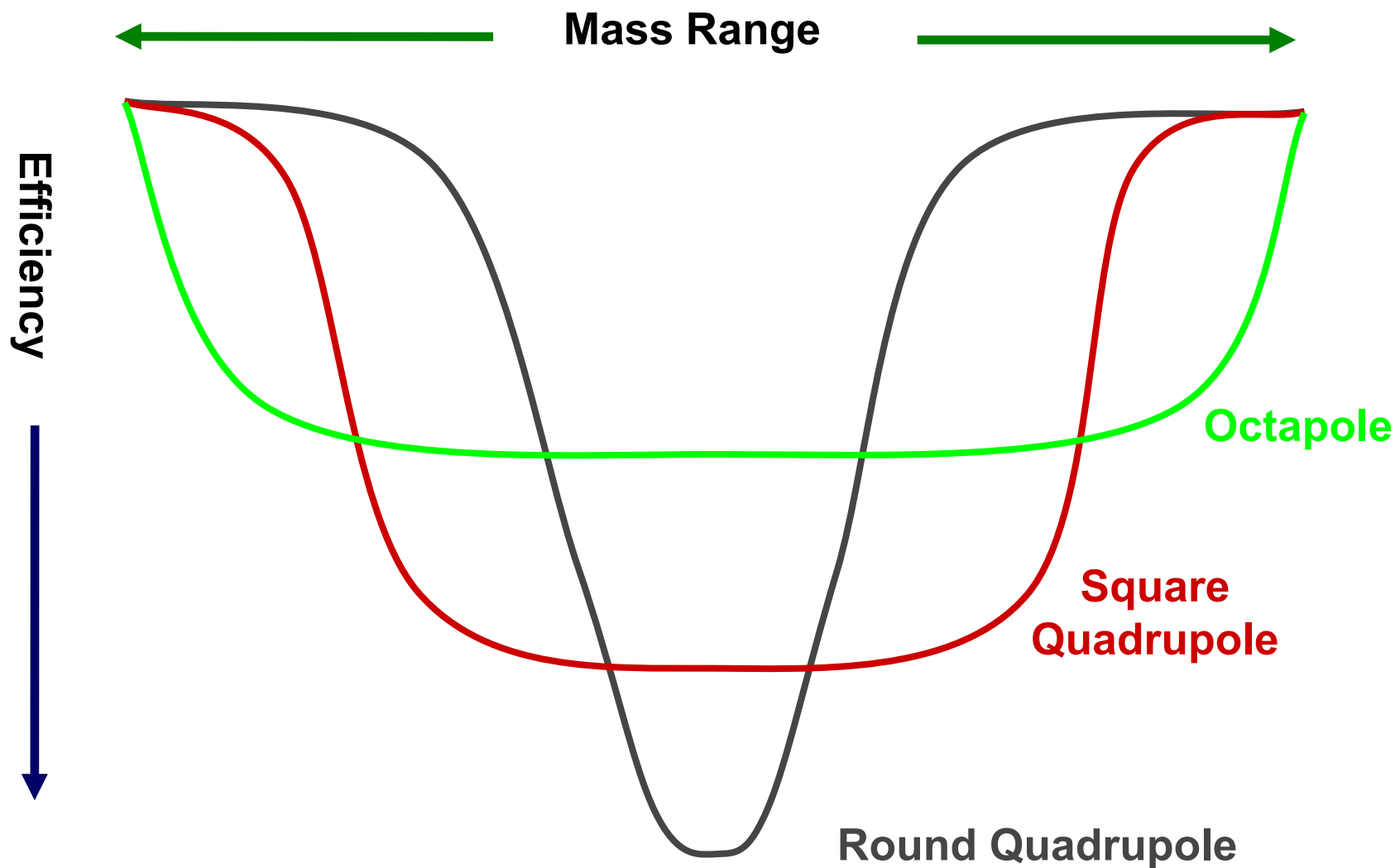
Q00



Q0



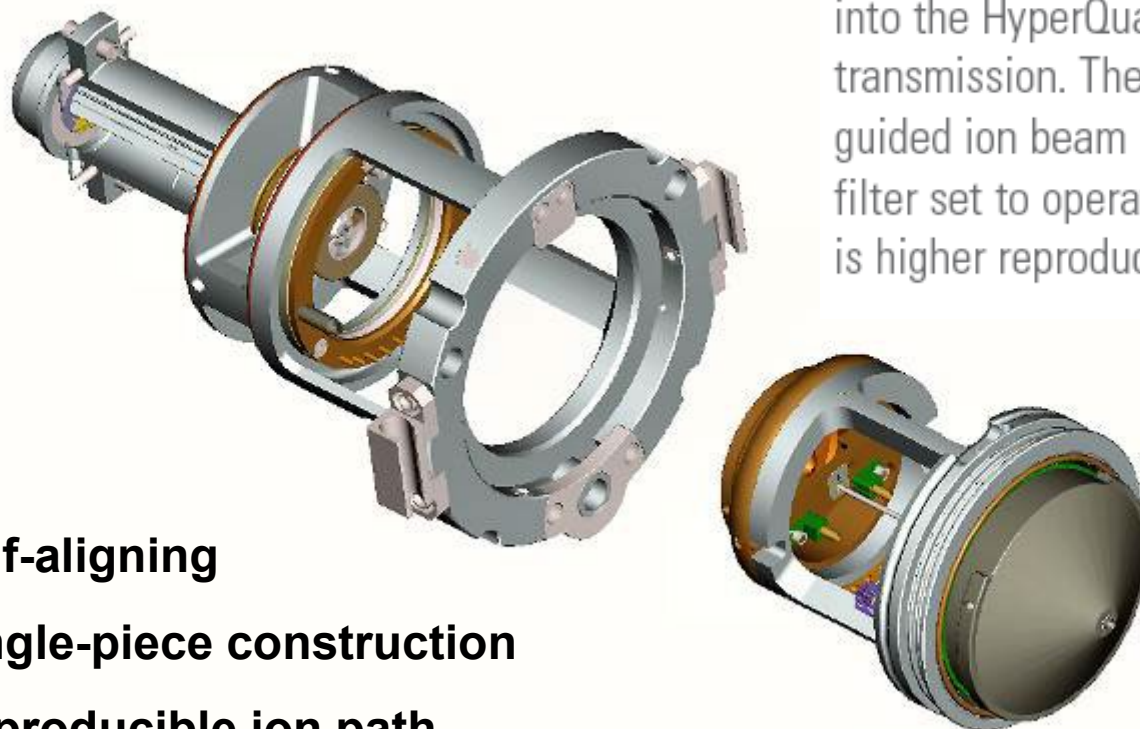
The Choice of Square Quadrupoles as Ion Guides



Ion Vector® Optics

Ion Vector

Ion Vector self-aligning optics ensure efficient transfer of ions from the source into the HyperQuad mass filter for maximum transmission. The result of a precisely guided ion beam into the HyperQuad mass filter set to operate in the H-SRM mode is higher reproducibility and lower LOQs.



- ✓ Self-aligning
- ✓ Single-piece construction
- ✓ Reproducible ion path



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Chapter 4

Resolution and Mass Filtering

HyperQuad™ Technology



The Patented HyperQuad™ Advantage

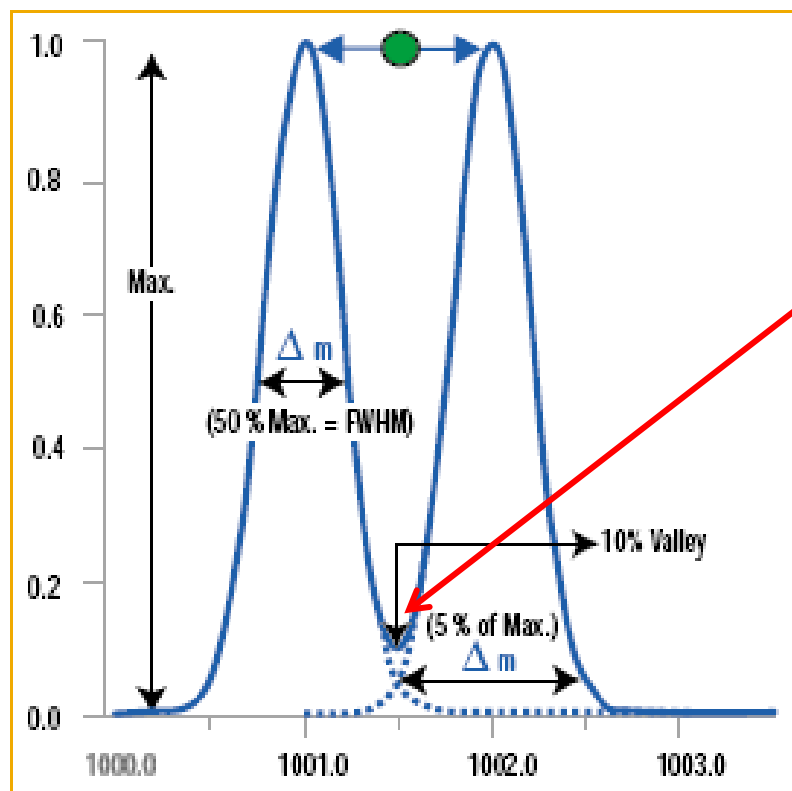
Quadrupole mass filtering fields are defined by:

- Electrode shape
- Field radius
- Frequency
- Voltage
- Length



All TSQ Quantum instruments have true hyperbolic electrodes, large field radius, high frequency and voltage, and 25-cm length

Resolution Basics



Magnetic Sector Instruments

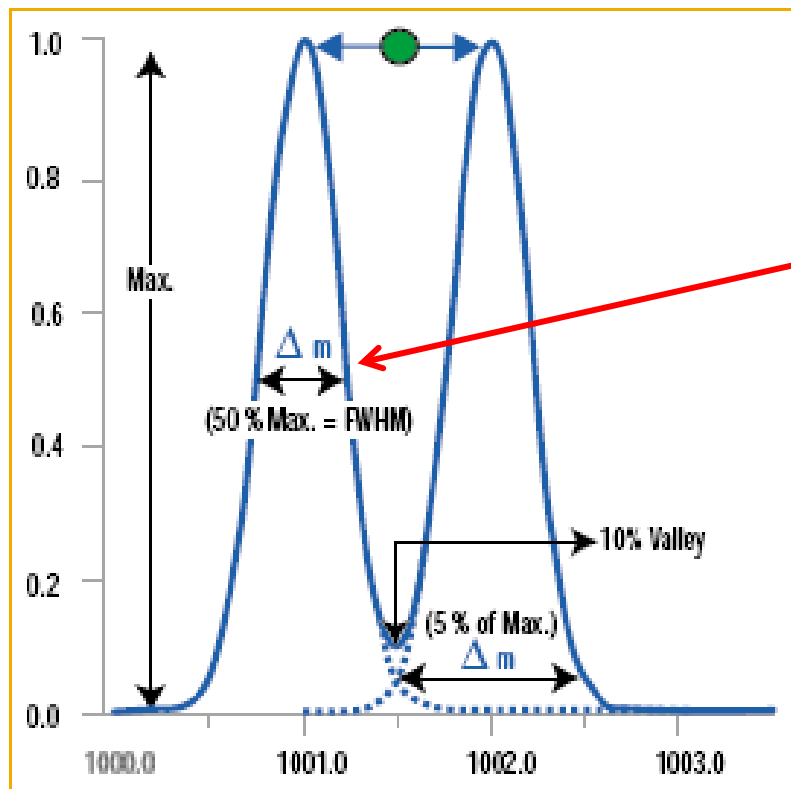
Constant resolution with mass
(10% valley definition)

$$R_m = m/\Delta m$$

m = measured mass (m/z)

Δm = width of a mass peak at a specified height or the difference between two adjacent mass peaks

Resolution Basics



Quadrupoles, Ion traps, TOF's
Constant peak width with mass
(FWHM Definition)

$$R_m = m/\Delta m$$

m = measured mass (m/z)

Δm = width of a mass peak at a specified height or the difference between two adjacent mass peaks

Importance of Resolution

High Resolution Mass Spectrometry

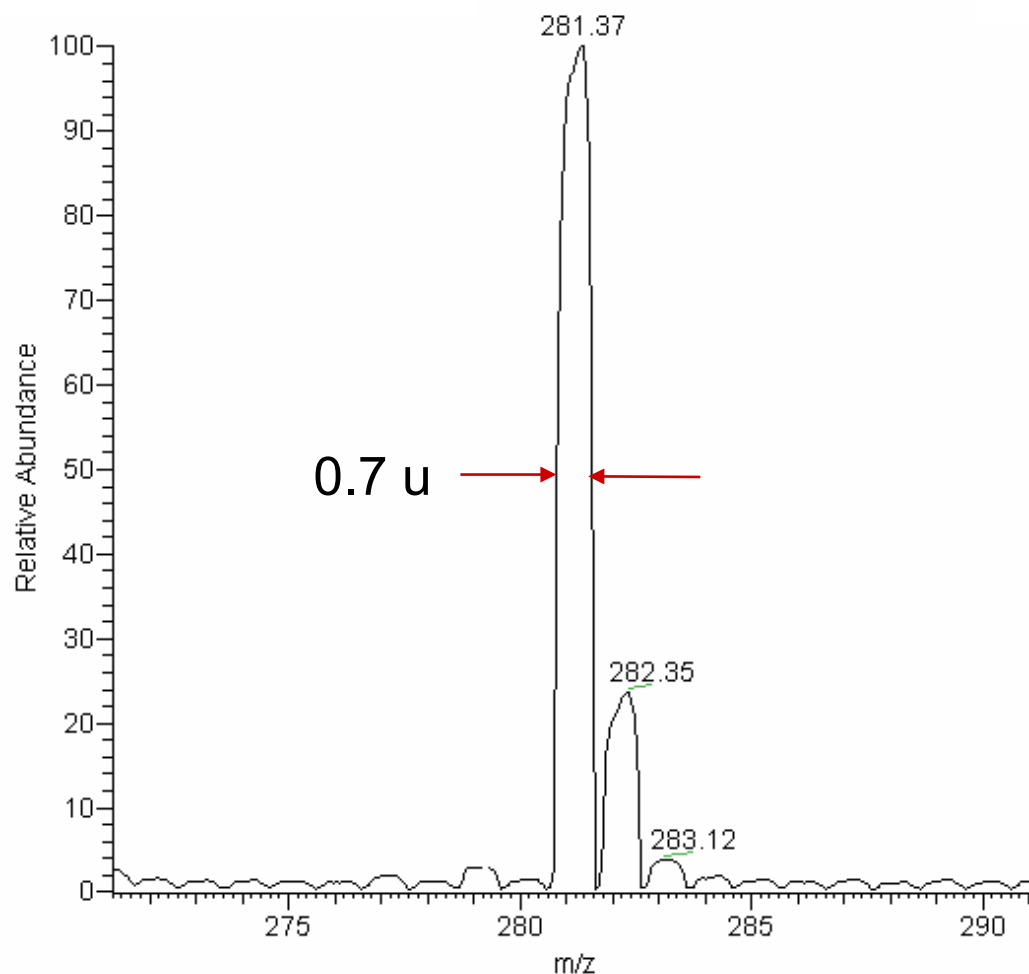
Purpose - to minimize chemical background caused by ions with the same nominal mass but different accurate mass (isobaric compounds) and, therefore, to increase the signal to noise ratio.

Example:

		<u>R= 1000</u>	<u>R= 3000</u>
Compound mass:	281.151 Da	OVERLAPPING	SEPARATION
Interfering mass:	281.053 Da		

		<u>R= 1000</u>	<u>R=3000</u>	<u>R= 5000</u>
Compound mass1:	372.351 Da	OVERLAPPING	OVERLAPPING	SEPARATION
Compound mass2:	372.421 Da			

Unit Resolution (0.7 FWHM)



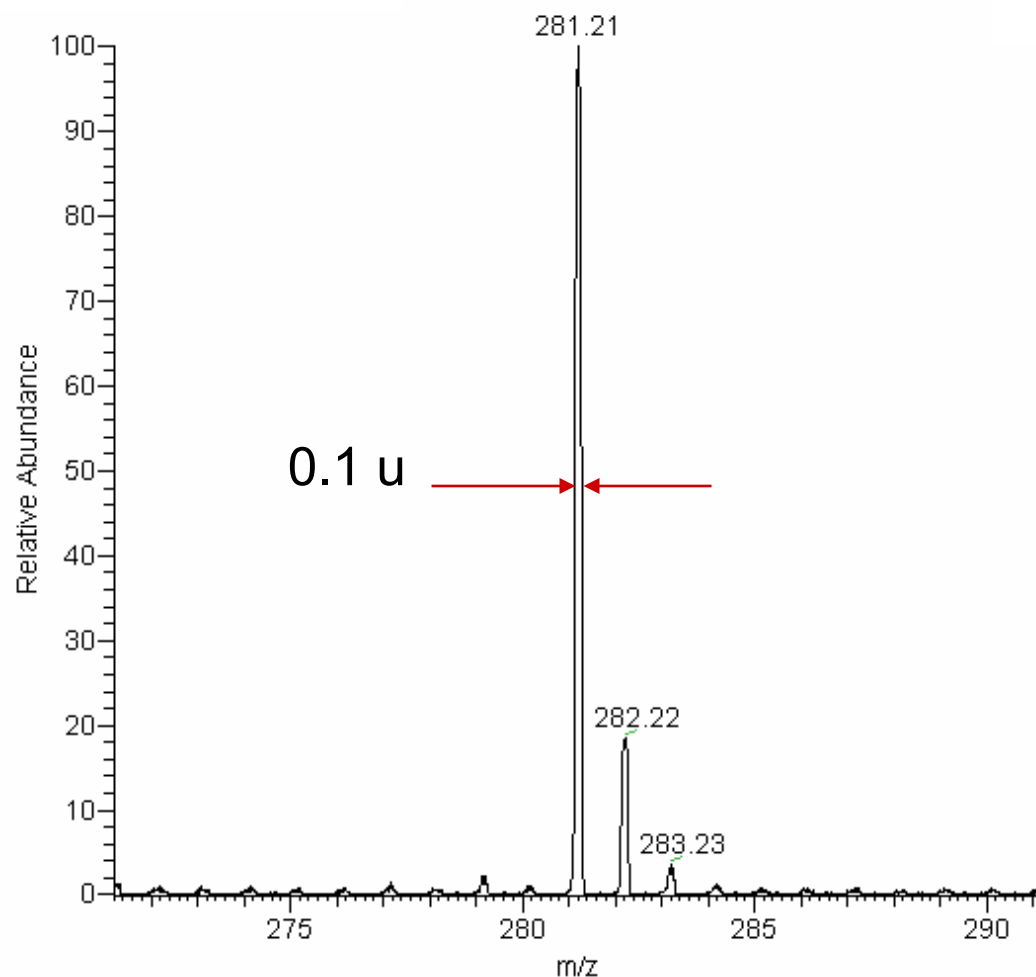
$$m/z\ 300 / 0.7 = R\ 428$$

$$m/z\ 500 / 0.7 = R\ 714$$

$$m/z\ 1000 / 0.7 = R\ 1428$$

Note: 0.7 FWHM is equivalent to 1.0 mass width at base of peak (m/z scale)

High Resolution (0.1 FWHM)



$$m/z \ 300 / 0.1 = R \ 3000$$

$$m/z \ 500 / 0.1 = R \ 5000$$

$$m/z \ 1000 / 0.1 = R \ 10,000$$

Note: 0.1 FWHM is equivalent to 0.15 mass width at base of peak (m/z scale)

The Power of High Resolution Mass Spectrometry

Resolving Target Compounds in the Presence of Interferences

- Example:

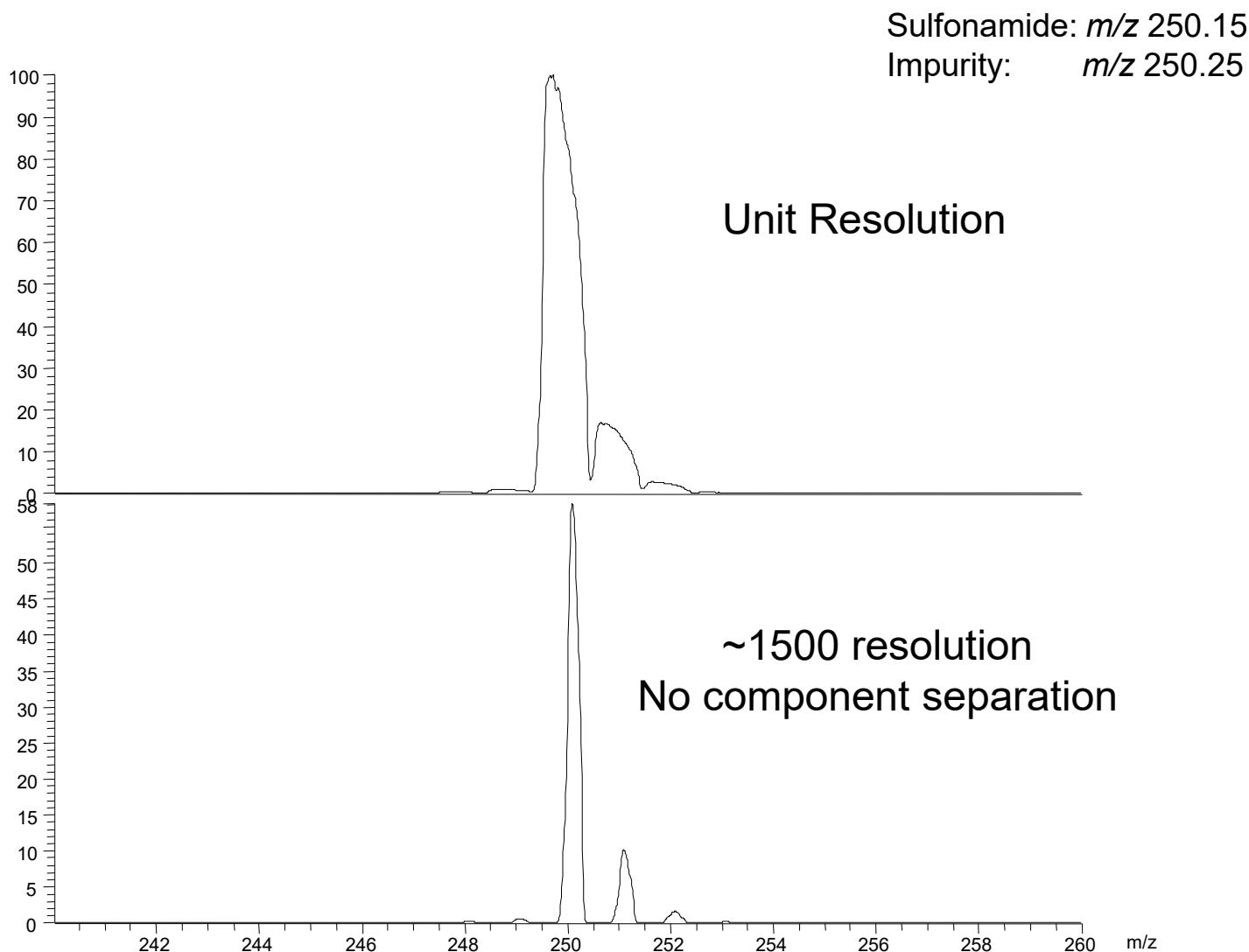
Sulfonamide with an isobaric impurity interference.

Sulfonamide: $[M+H]^+$ m/z 250.15

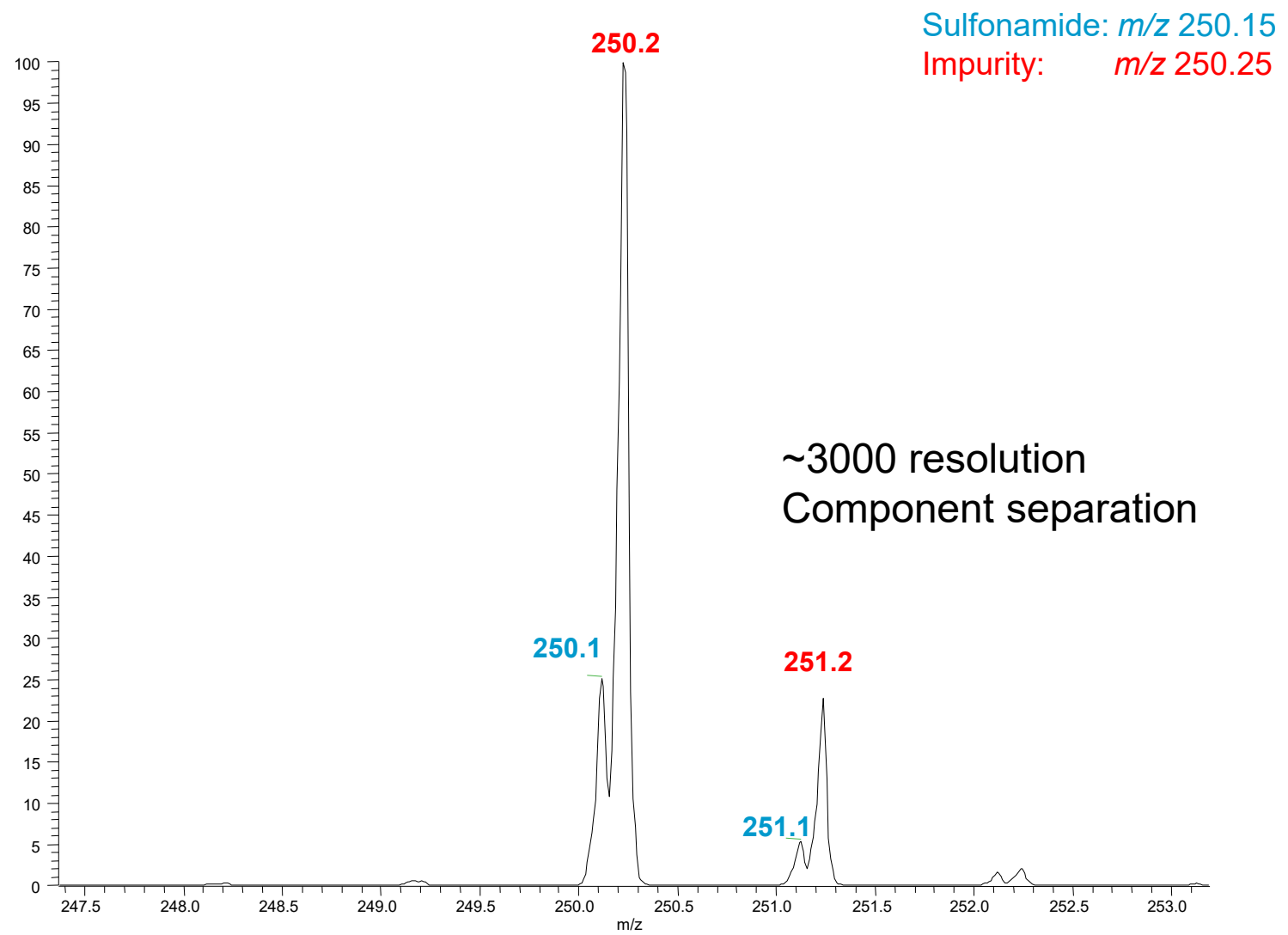
Impurity: $[M+H]^+$ m/z 250.25

- Chromatographic separation is not an option in the given example
- Precursor ion Hi-Res specificity allows for compound differentiation via the respective product ion MS/MS spectra

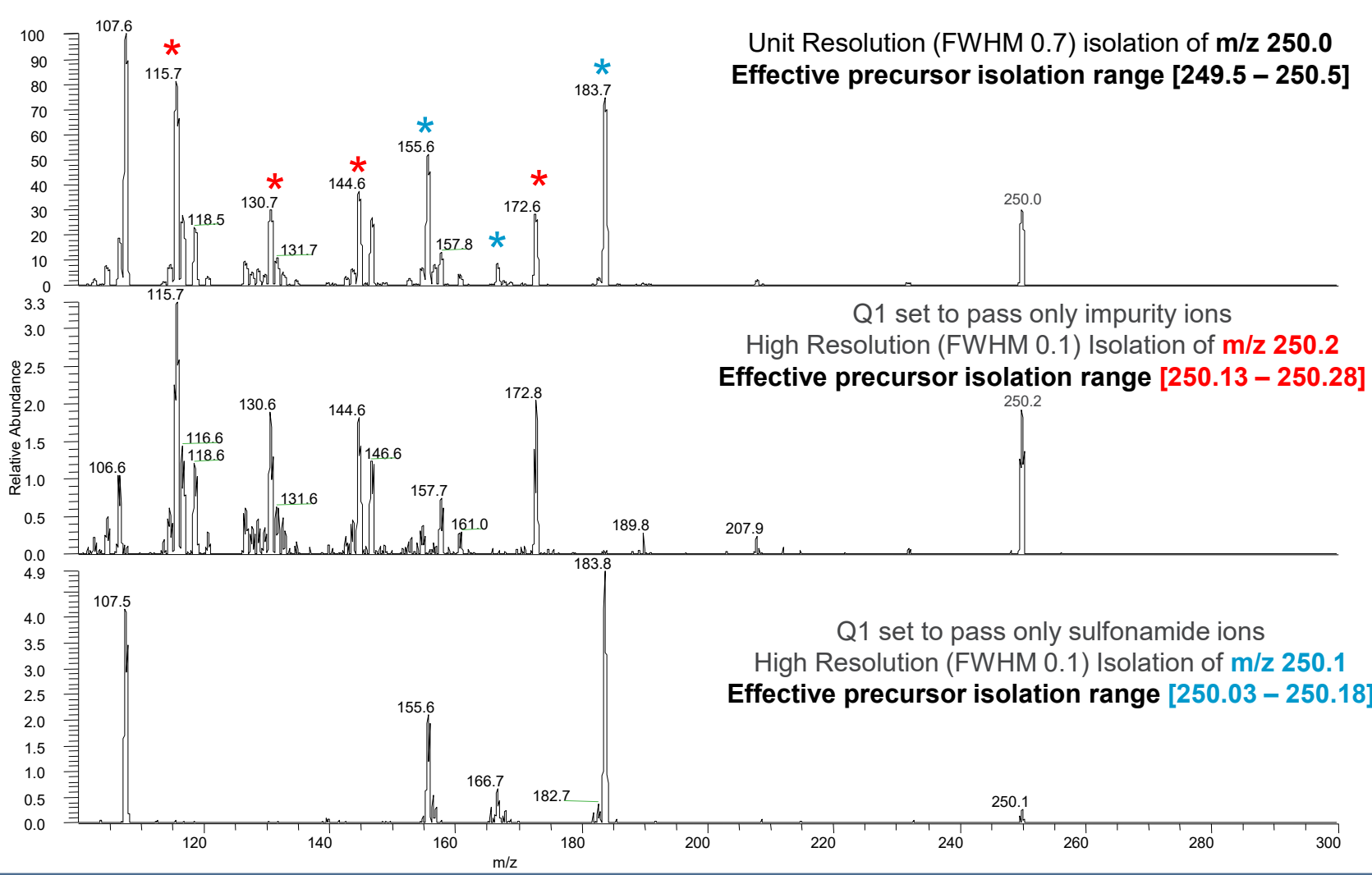
Isobaric Discrimination – Effect of Increasing Resolution



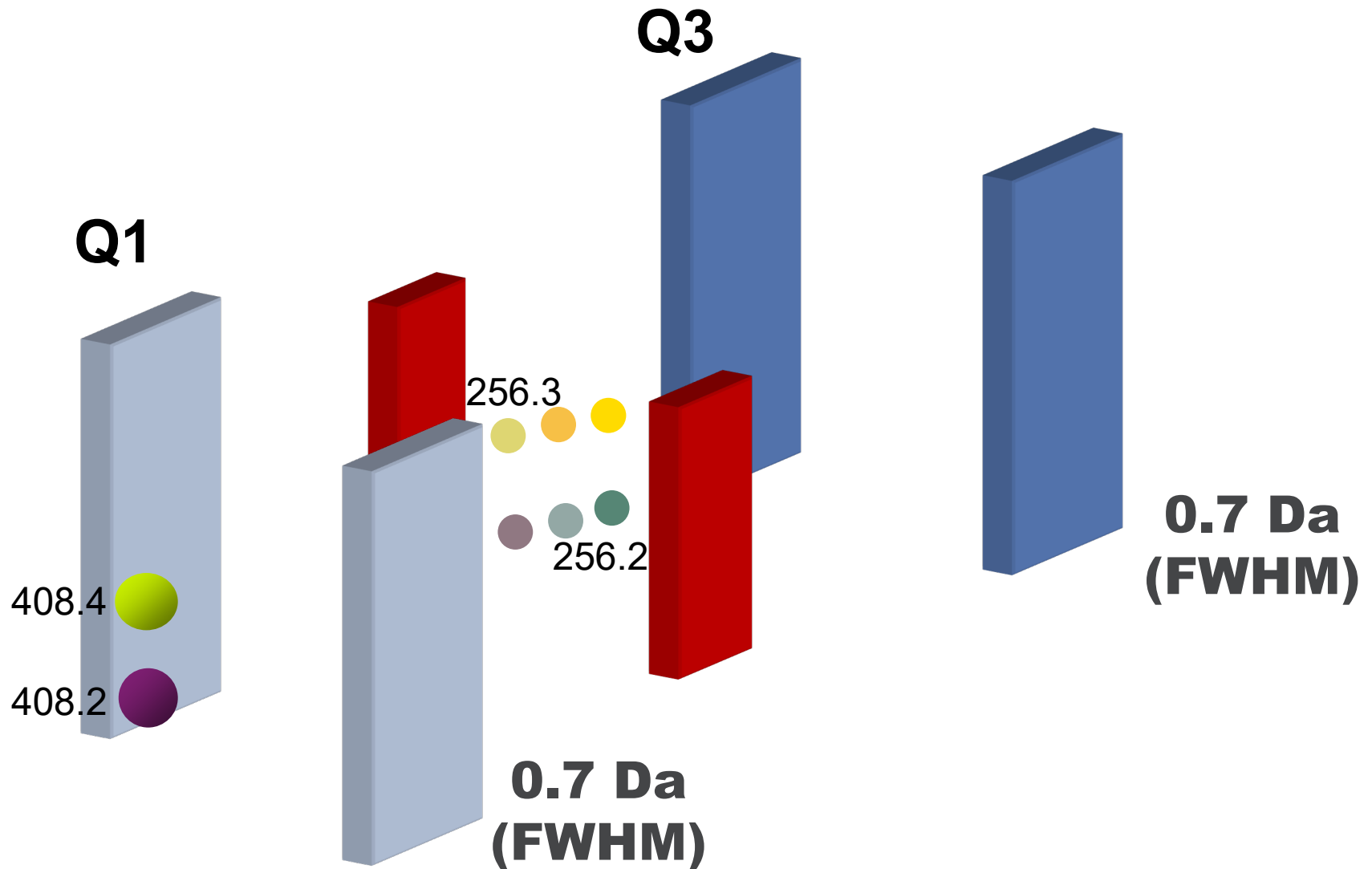
Isobaric Discrimination – Effect of Increasing Resolution



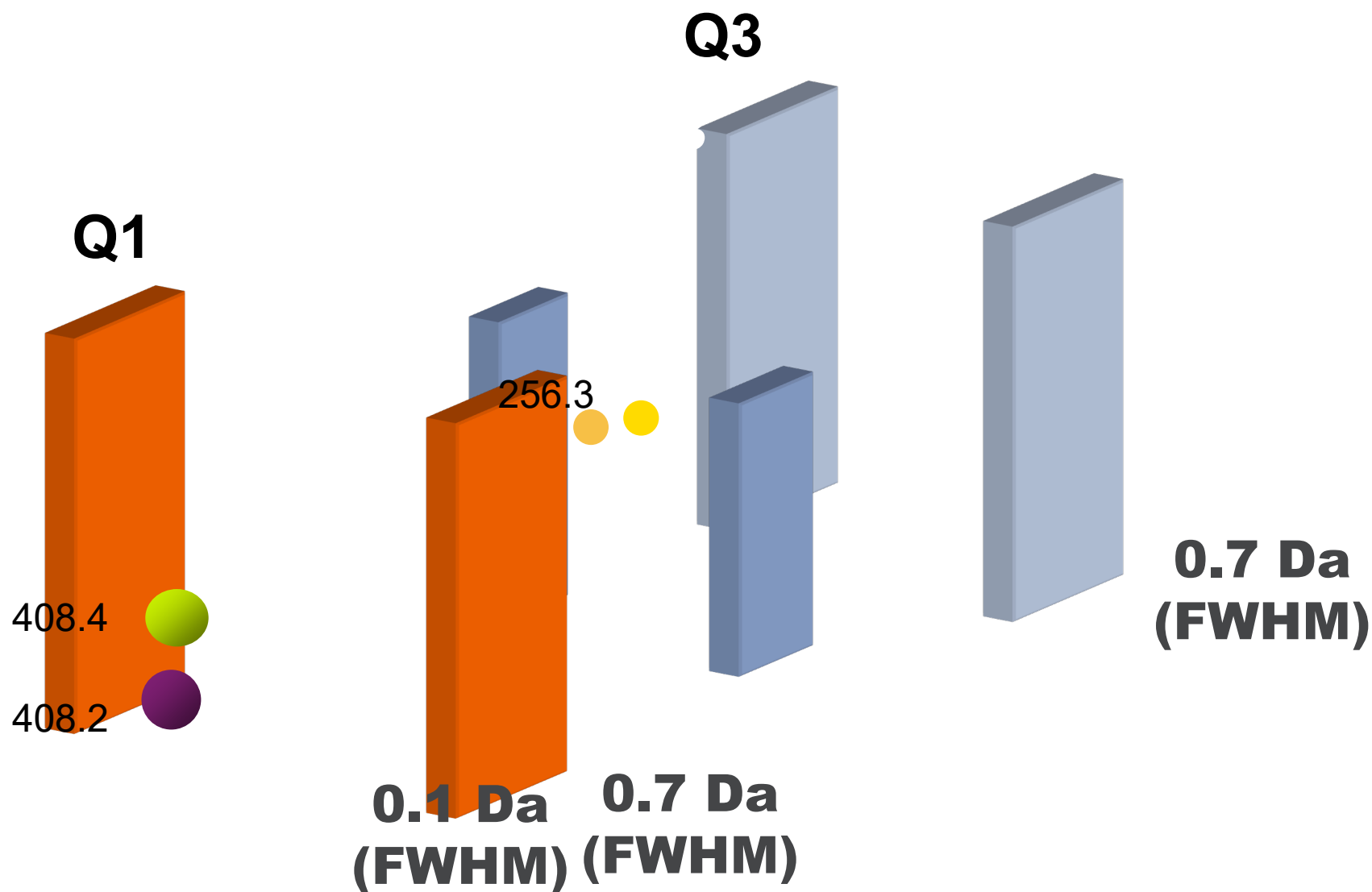
Isobaric Discrimination – Effect of Increasing Resolution



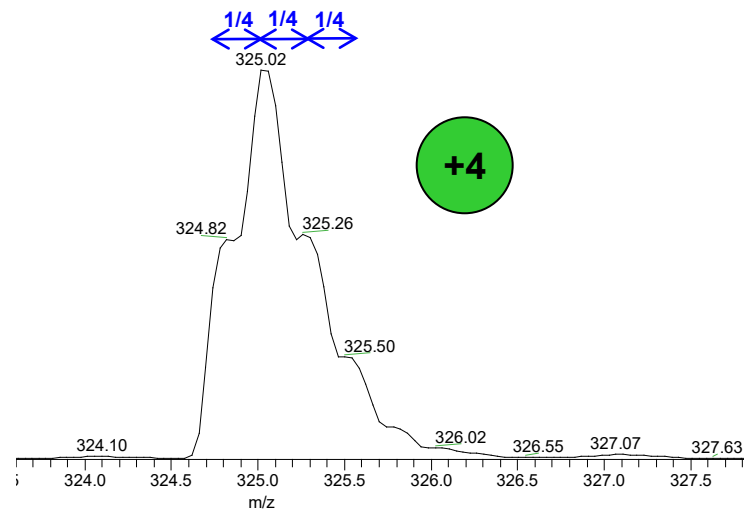
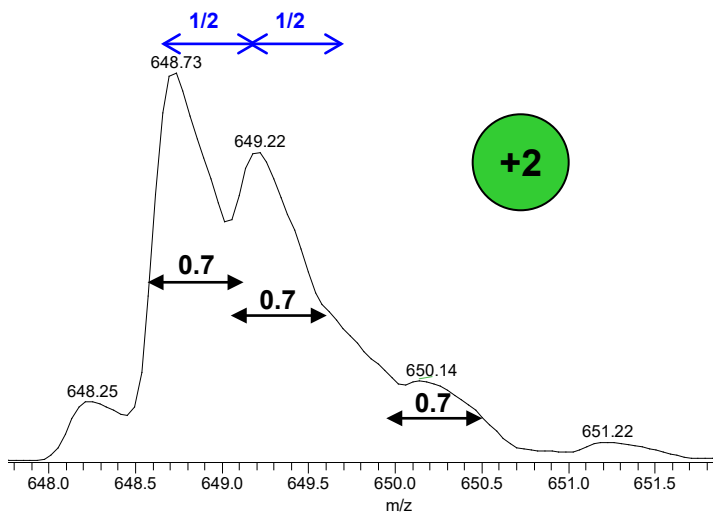
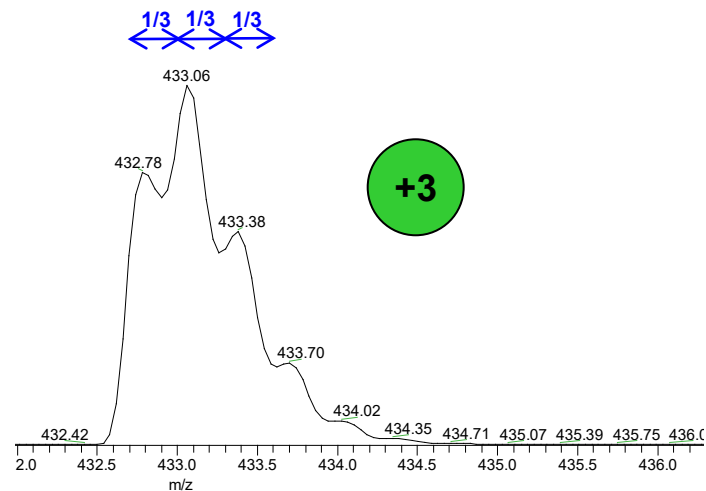
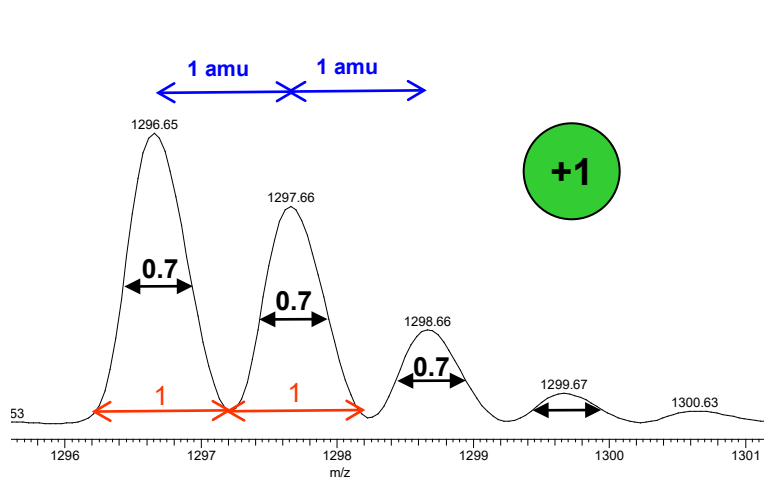
SRM - Selected Reaction Monitoring at Unit Resolution



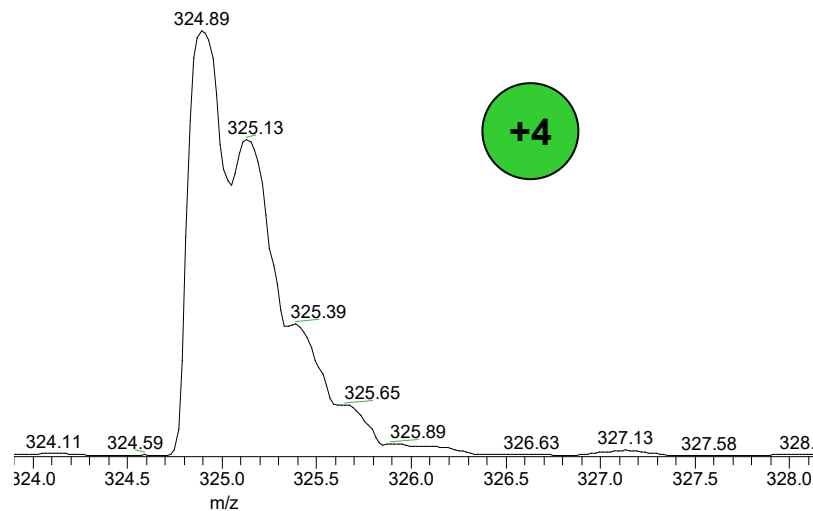
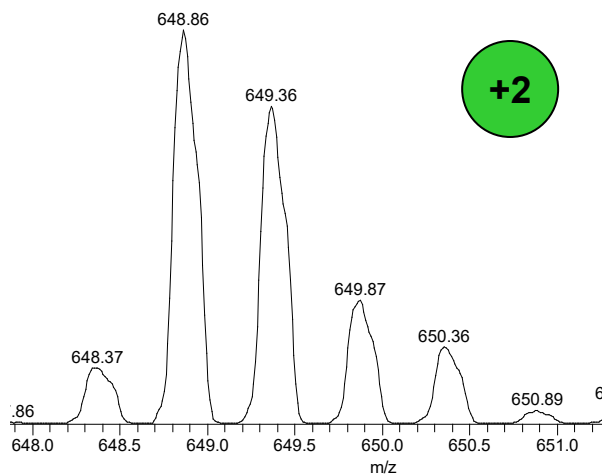
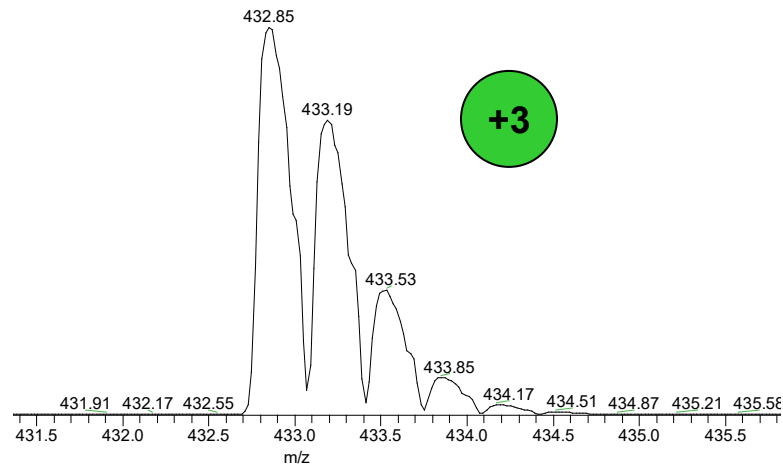
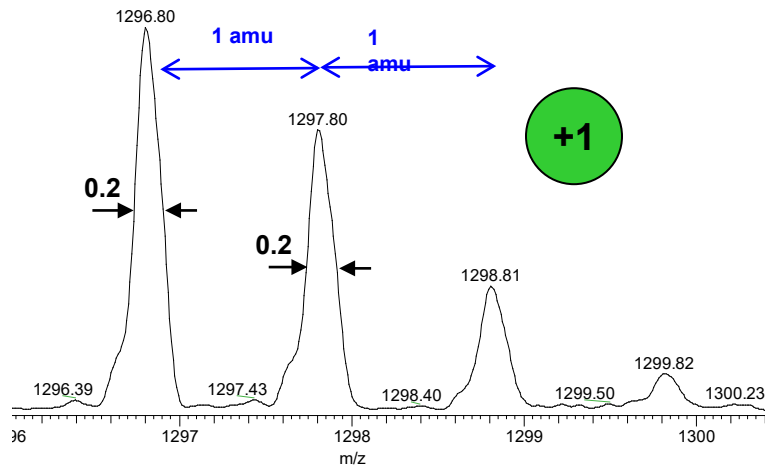
H-SRM - Selected Reaction Monitoring at High Resolution



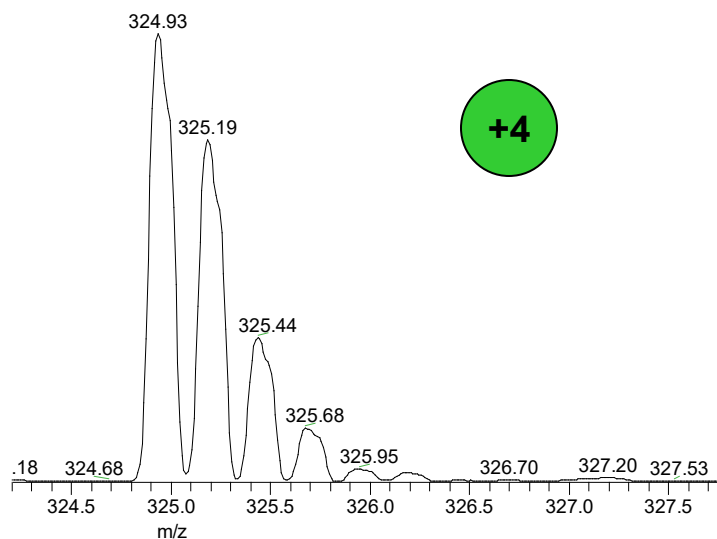
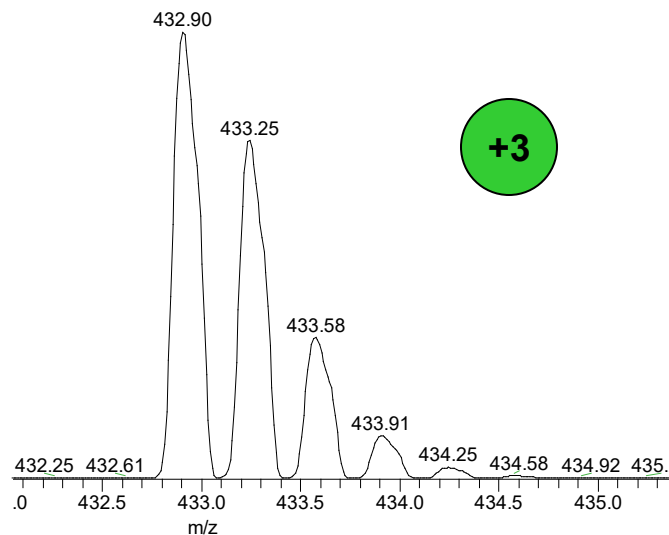
Charge State Determination (FWHM = 0.7)



Charge State Determination (FWHM = 0.2)



Charge State Determination (FWHM = 0.1)



Quadrupole Ion Selection – Q1 and Q3 as Ion Filters

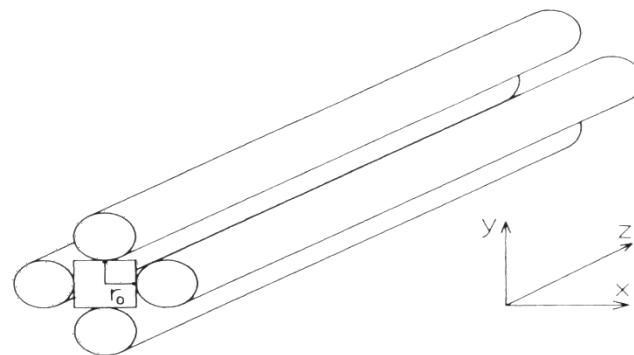


Figure 2.3. Conventional array of circular rods in a quadrupole mass filter.

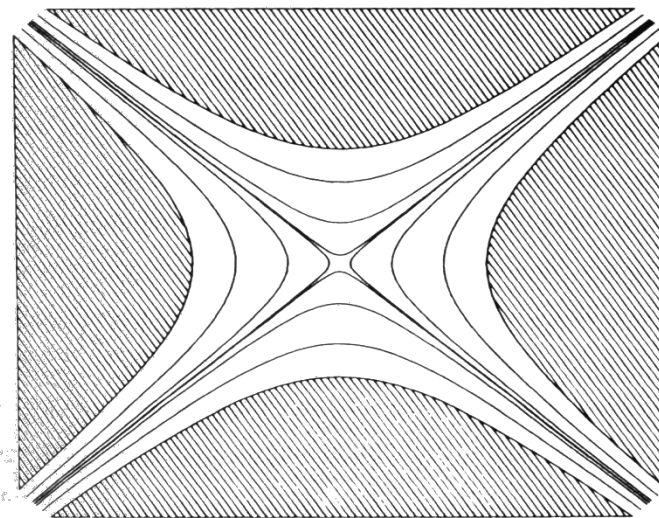
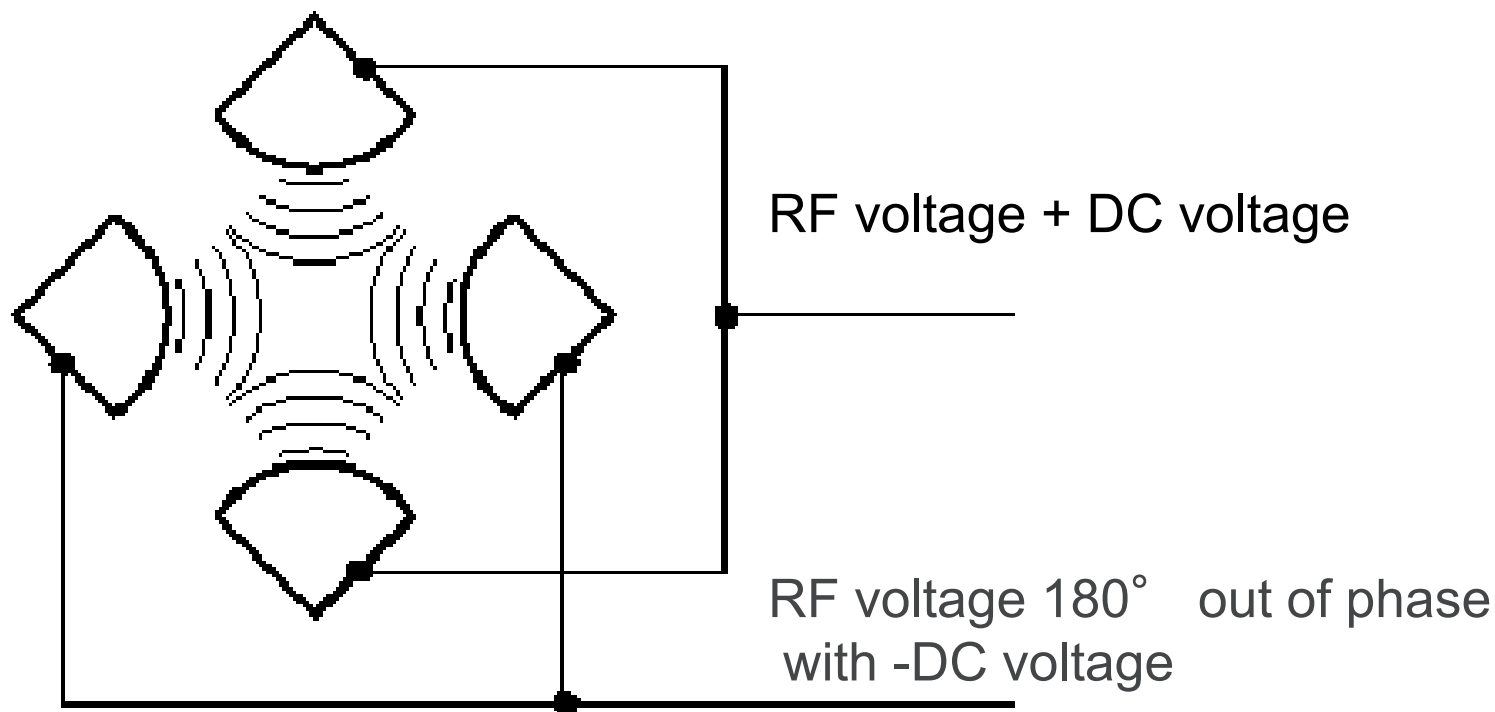


Figure 2.4. Hyperbolic equipotential contours in an ideal two-dimensional quadrupole field.

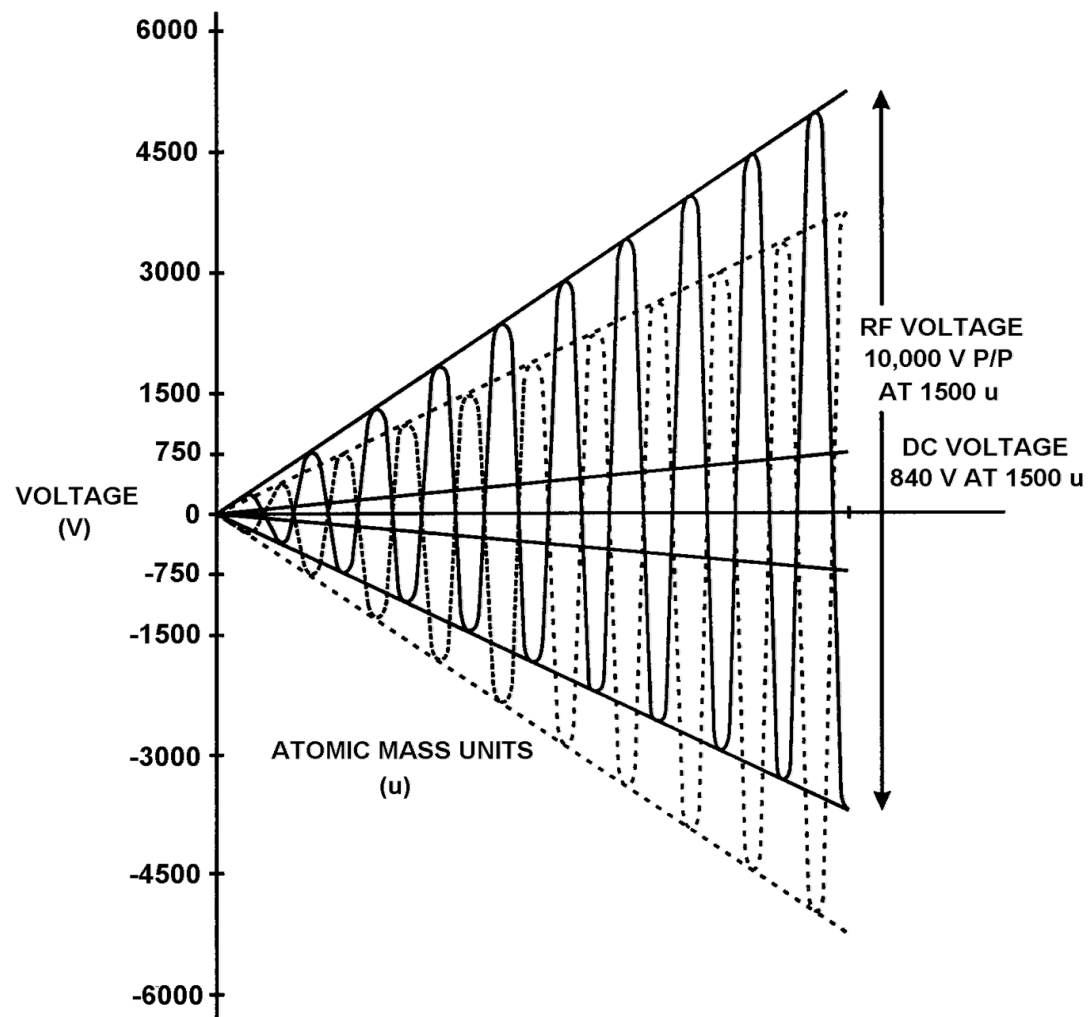
* Raymond E. March, Richard J. Hughes. "Quadrupole Storage Mass Spectrometry." Wiley Interscience, 1989.

RF and DC Fields Applied to the Quadrupoles

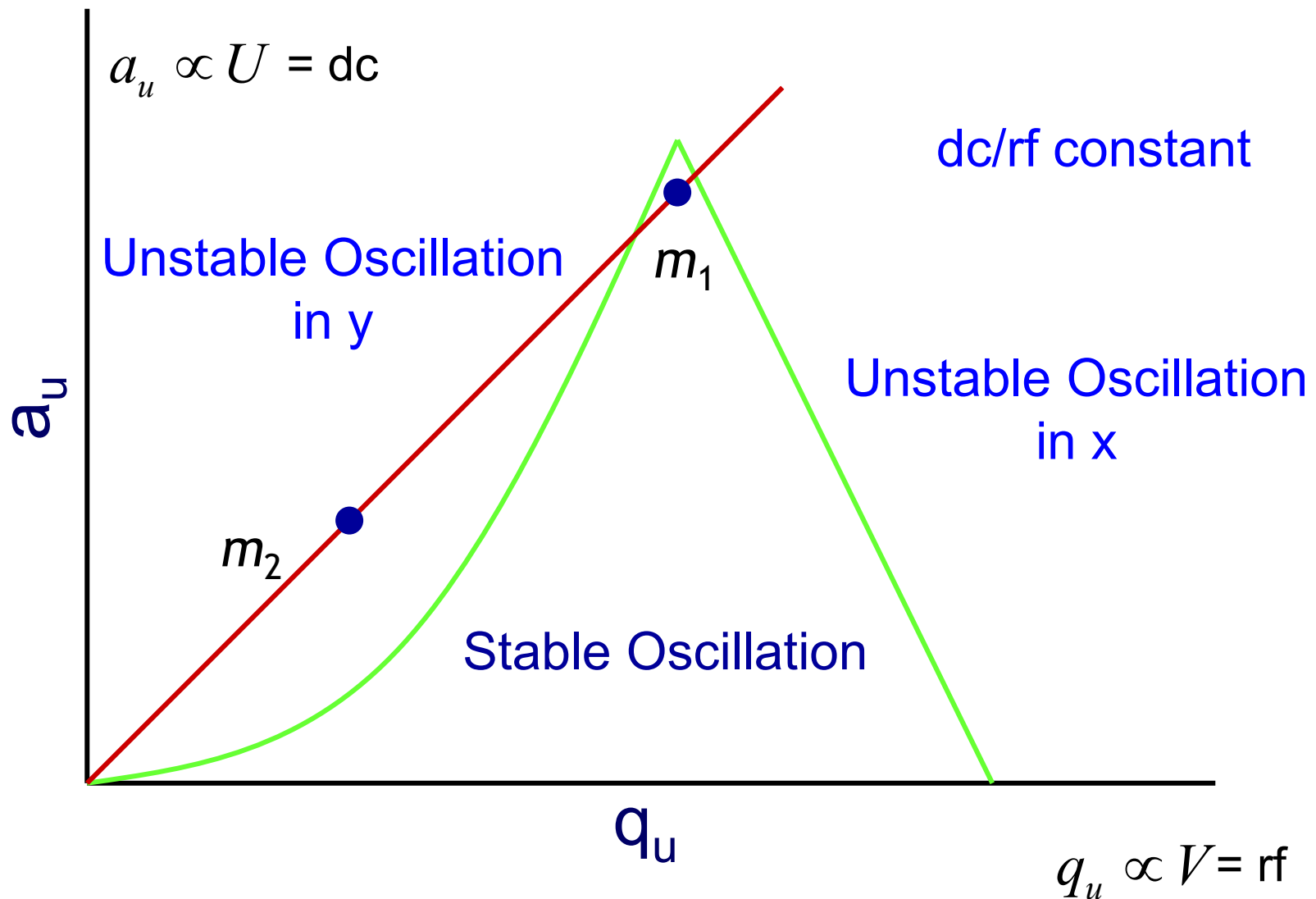
RF = 1.123 MHz and of variable amplitude (0 to 10,000 V peak-to-peak)
DC = 0 to ± 840 V



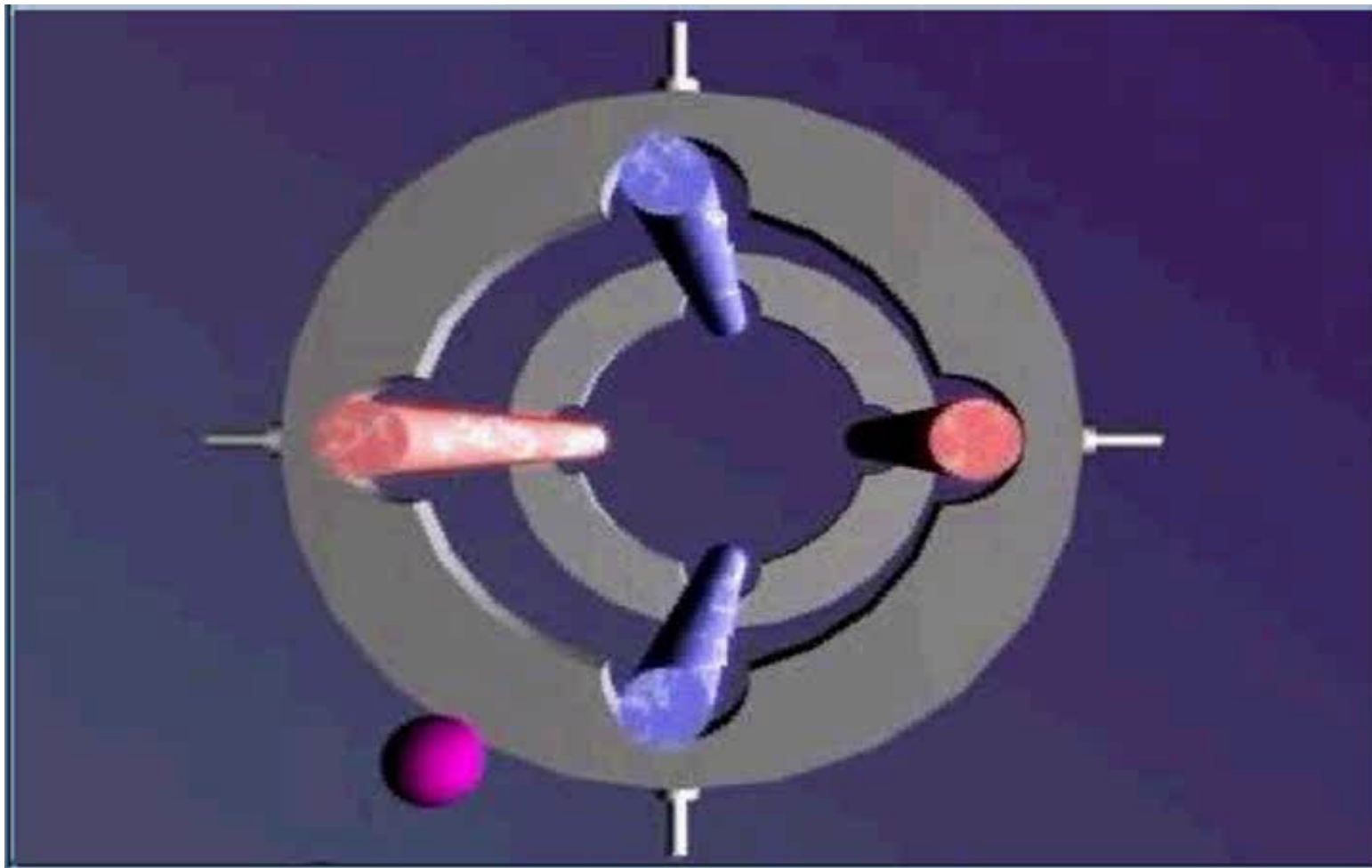
RF and DC Fields Applied to the Quadrupoles



Stability Diagram Transformed to (U,V) Space



RF and DC Fields Applied to the Quadrupoles



** Crawford Scientific, OPDAC (Online Professional Development in Analytical Chemistry)
LC-MS training package. Holm Street, Strathaven, Lanarkshire, ML10 6NB, Scotland, UK*

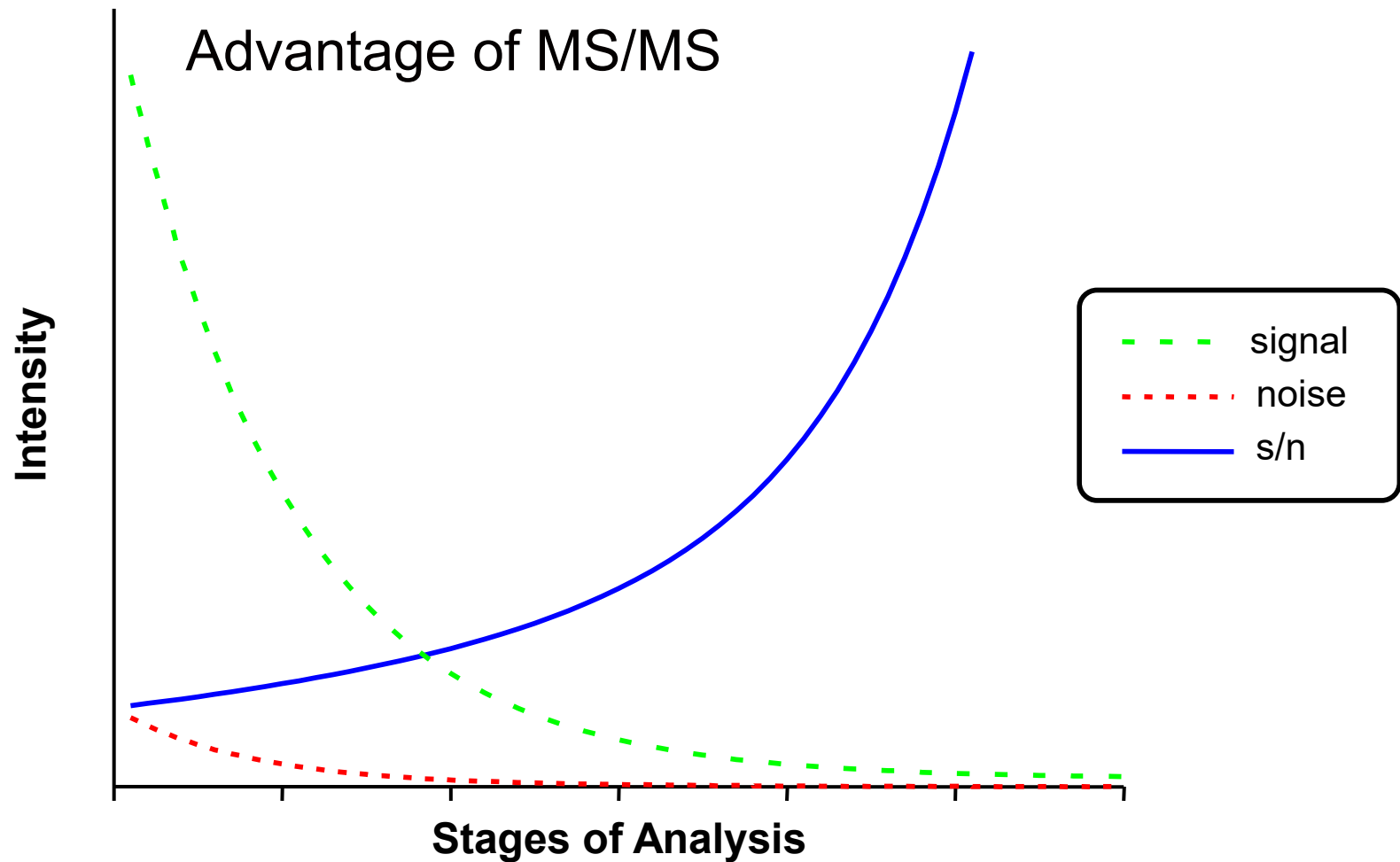
Summary – Scanning vs. Transmission

If the RF and DC voltages are ramped upward (i.e. the mass analyzer is scanned upward), discrete ion populations of successively higher m/z ratios are allowed to pass through the analyzer, thus having stable trajectories.

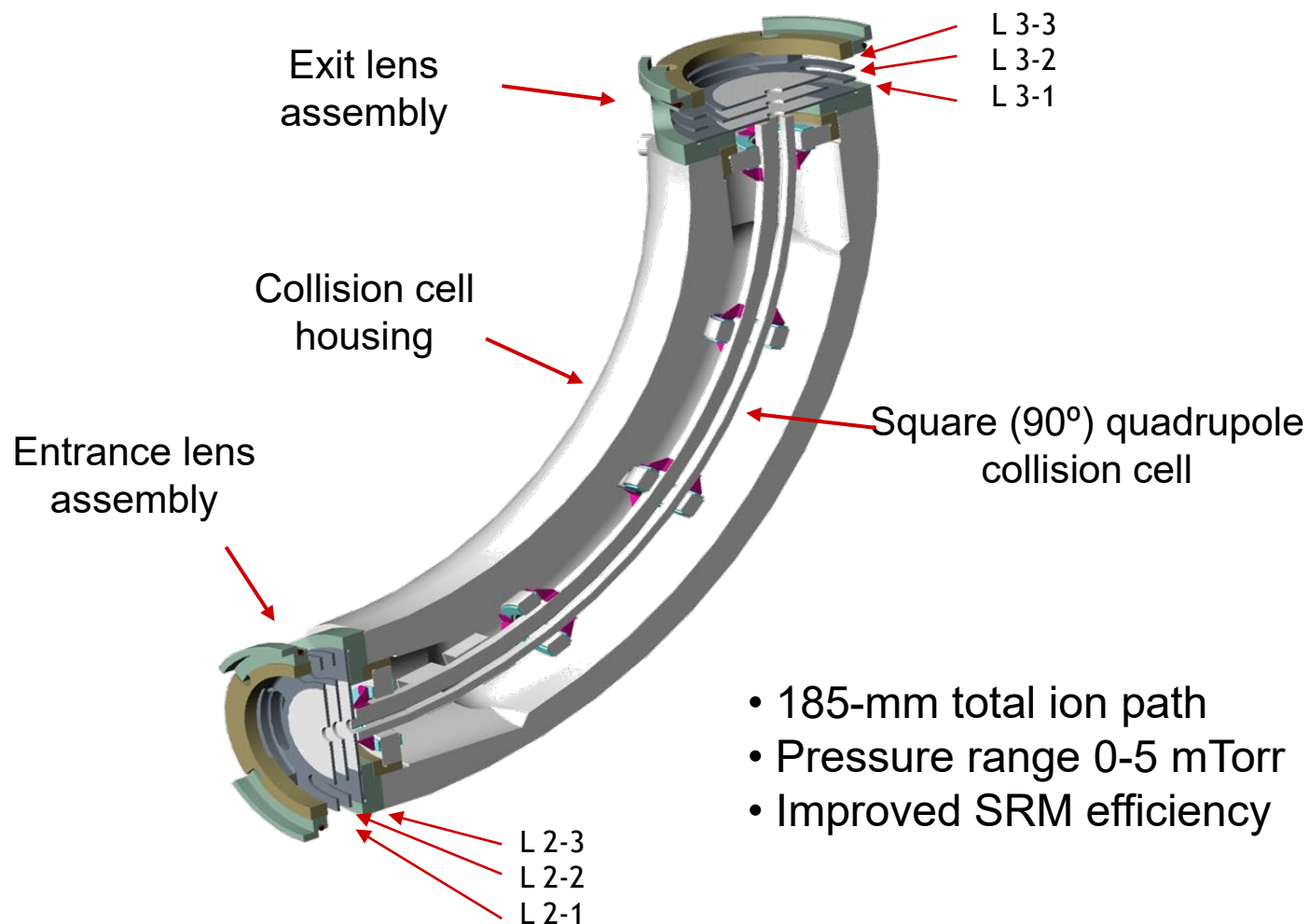
If the RF and DC voltages are held constant, a constant population of ions, defined by a range of m/z values, is transmitted.



Signal-to-Noise Improvement in Multi-Stage Analysis



The Collision Cell (Q2)



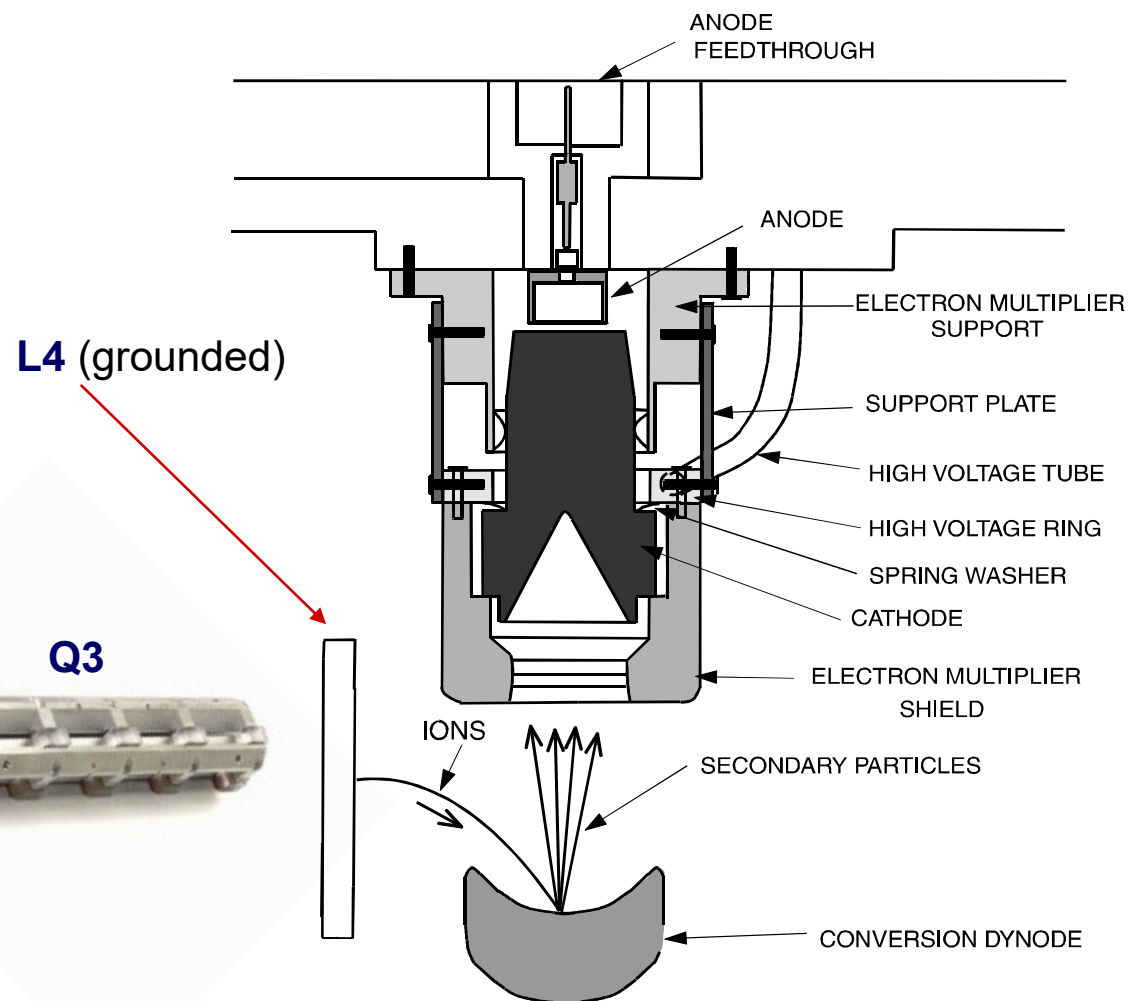


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Chapter 5

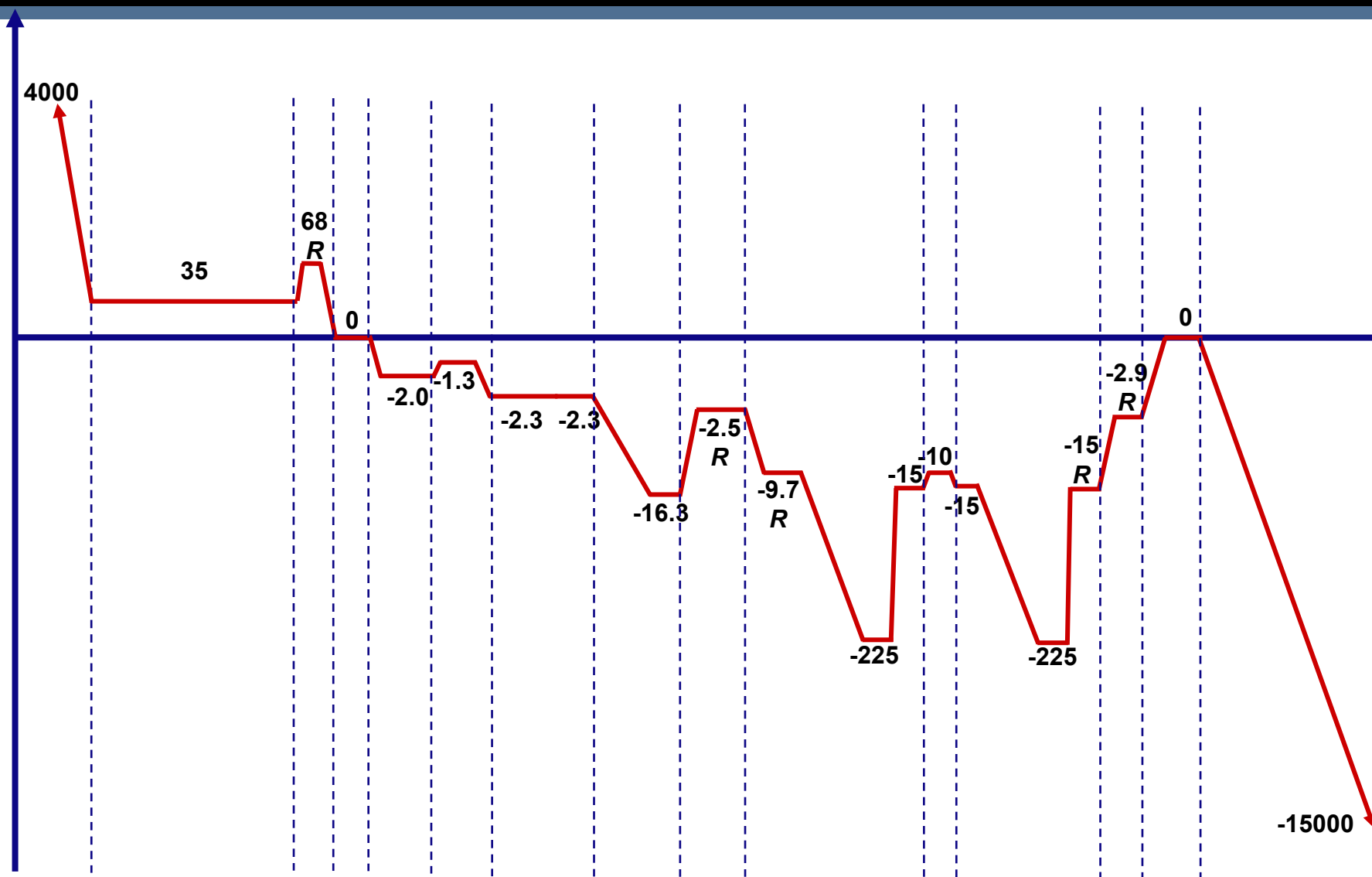
Detection and Vacuum

The Ion Detection System



* Note – The diagram is -90 degrees from proper orientation

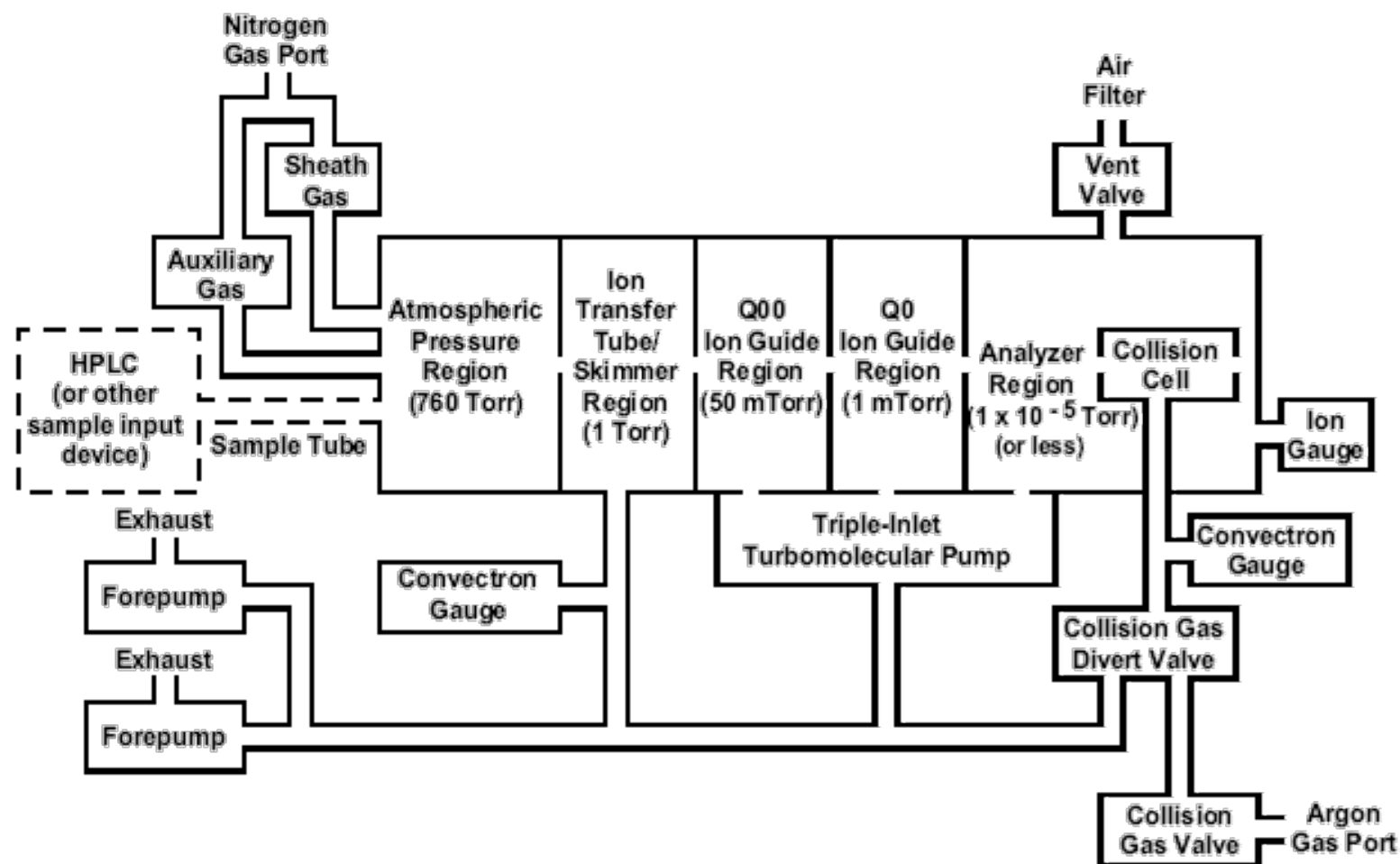
The Ion Optics Potential Energy Diagram



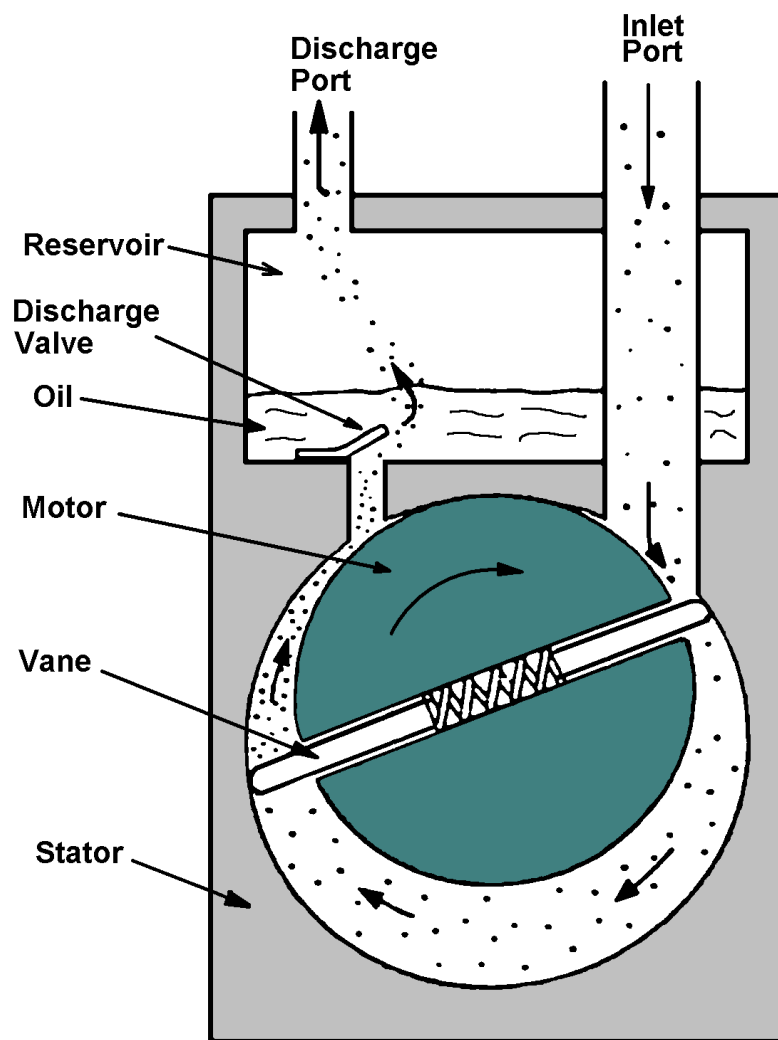
The Vacuum System

- A mass analyzer must operate under vacuum in order to minimize both ion-molecule and inter-molecular collisions, and allow ions to travel through the elements of the instrument.
- The mean free path of a typical ion (average distance traveled by an ion between collisions) is approximately:
 - 50 nm at atmospheric pressure (760 Torr)
 - 40 mm at 1 mTorr
 - 40 m at 1 μ Torr
- Two types of vacuum pumps provide vacuum to the system:
 - Rotary vane pump (a.k.a. forepump or rough pump)
 - Triple-inlet turbomolecular pump

The Functional Diagram of the Vacuum System



Rough Pump (Forepump)



- Provides vacuum (approx. 1 Torr) for the skimmer region
- Provides primary vacuum for the turbomolecular pumps, operates inlet valves, etc.
- Requires low maintenance
- Much of the sample is dissolved in the oil which becomes hazardous waste

Turbomolecular Pump



- Provides working vacuum (1 mTorr to 1 nTorr)
- High speed gas turbine with interspersed rotors (moving blades) and stators (fixed or stationary)
- Rotation forces molecules through the blade system
- Bearing failure is usually catastrophic



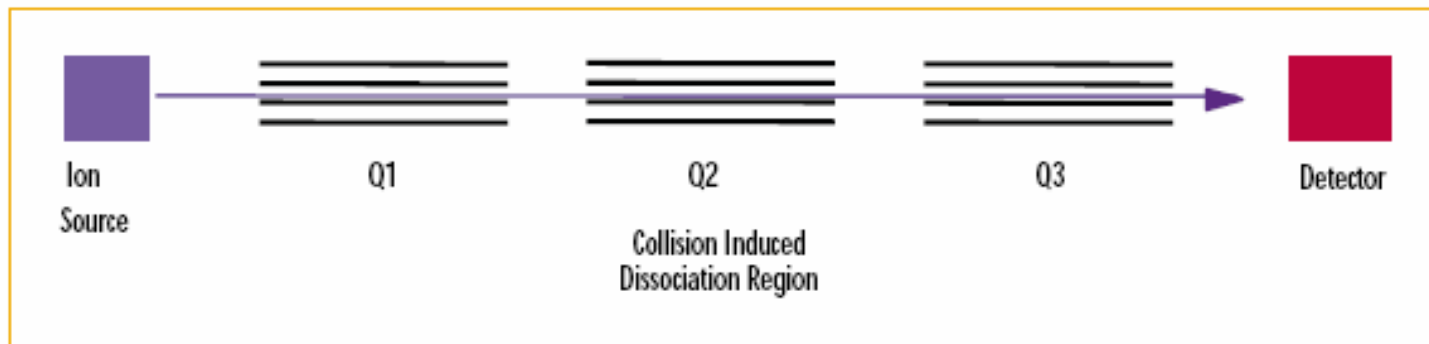
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Chapter 6

Scan Modes

Scan Modes

Triple-Stage Quadrupole (TSQ)

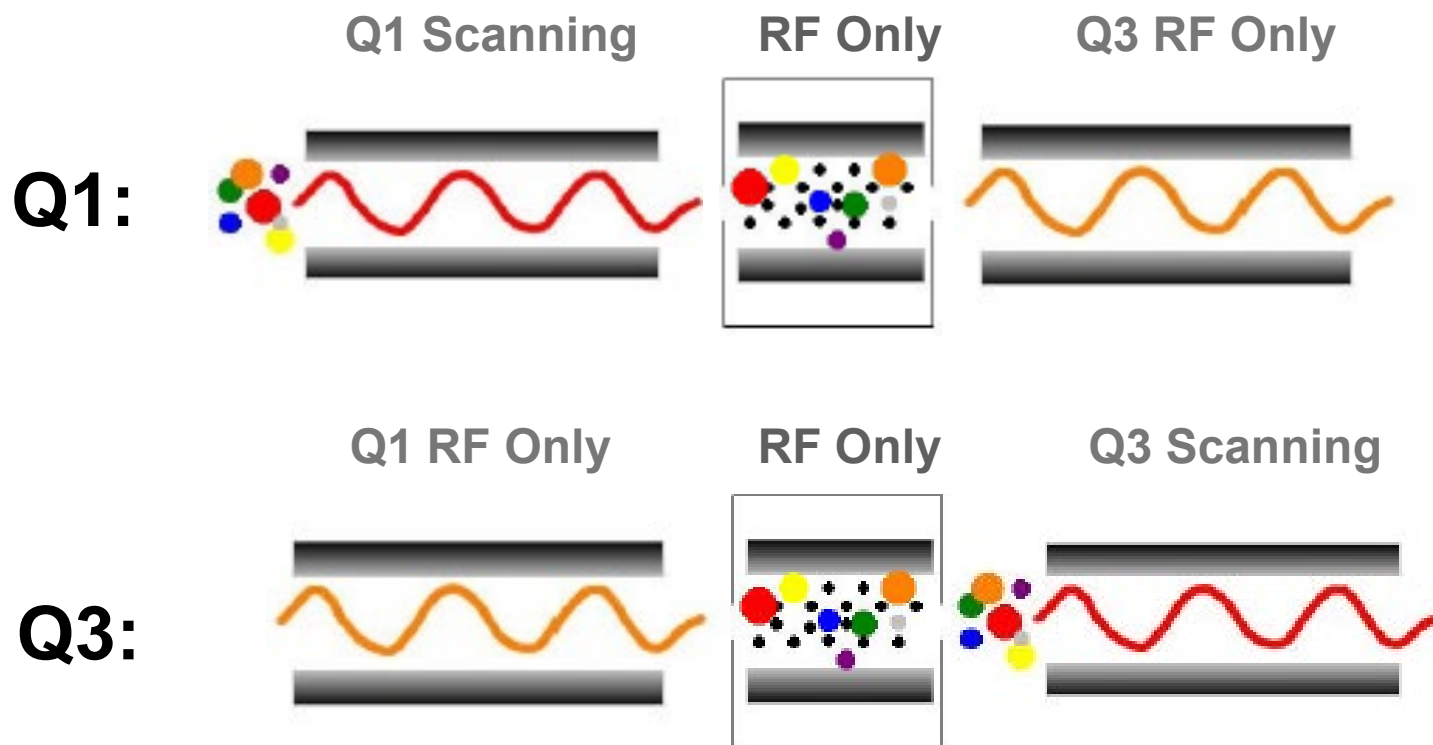


- The analyzer of a “triple quadrupole” instrument consists of two quadrupoles, separated by a collision cell. Such a configuration is often referred as a "tandem in space" arrangement.
- Precursor ions and product ions are created and analyzed in different physical spaces.
- Ions must be moved from the ion-source to the analyzer (different physical regions) where different functions take place.

MS Full Scan (Q1 or Q3)

Full Scan Mode

Purpose: Survey scan of a chromatographic peak



Full Scan (Q1 or Q3)

Scan Event 1

Full Scan SIM SRM

Scan Modes

MS Mode: ☒ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☐ Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 100.000

Last Mass (m/z): 600.000

Scan Time (s): 0.50

Set Mass (m/z): 1000.000

Collision Energy (V): 10

Q1 Peak Width (FWHM): 0.70

Q3 Peak Width (FWHM): 0.70

Polarity: ☒ Positive ☐ Negative

Data Type: ☐ Centroid ☒ Profile

Source CID:

Collision Energy (V): ☐ 3

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Scan Event 1

Full Scan SIM SRM

Scan Modes

MS Mode: ☐ Q1MS ☒ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☐ Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 100.000

Last Mass (m/z): 600.000

Scan Time (s): 0.50

Set Mass (m/z): 1000.000

Collision Energy (V): 10

Q1 Peak Width (FWHM): 0.70

Q3 Peak Width (FWHM): 0.70

Polarity: ☒ Positive ☐ Negative

Data Type: ☐ Centroid ☒ Profile

Source CID:

Collision Energy (V): ☐ 3

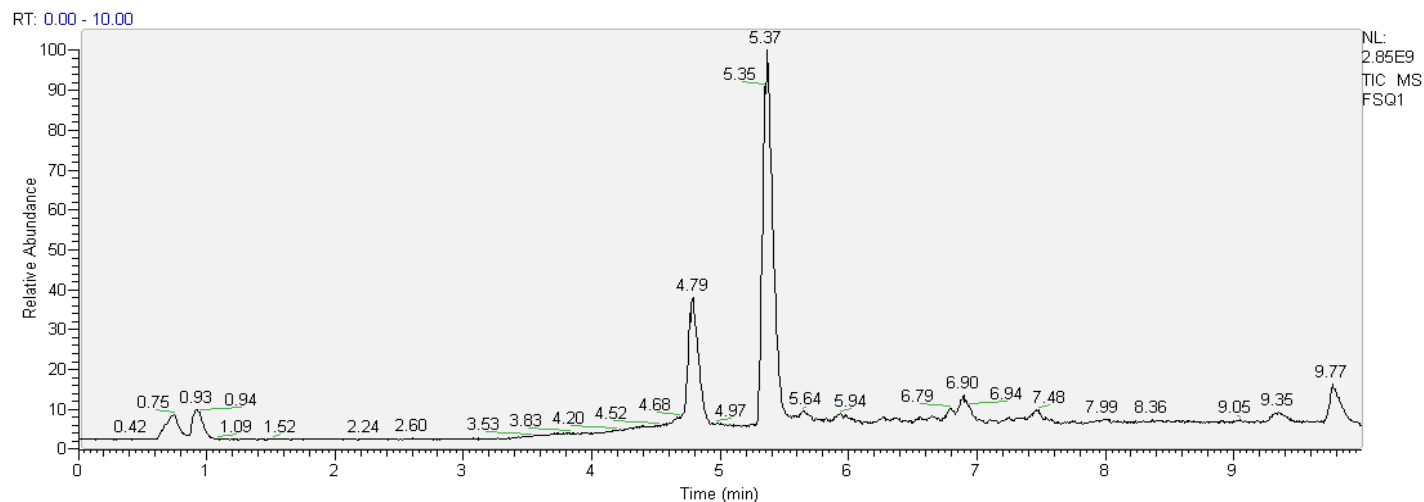
Accurate Mass Mode: Off

Micro Scans: 1

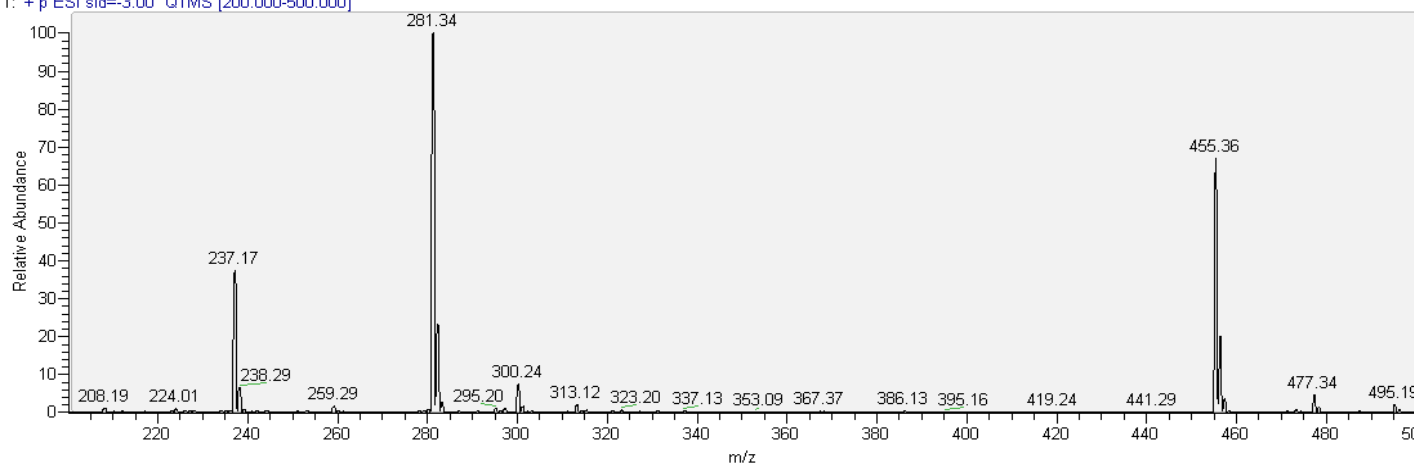
Copy ScanEvent Paste ScanEvent

Help Tune

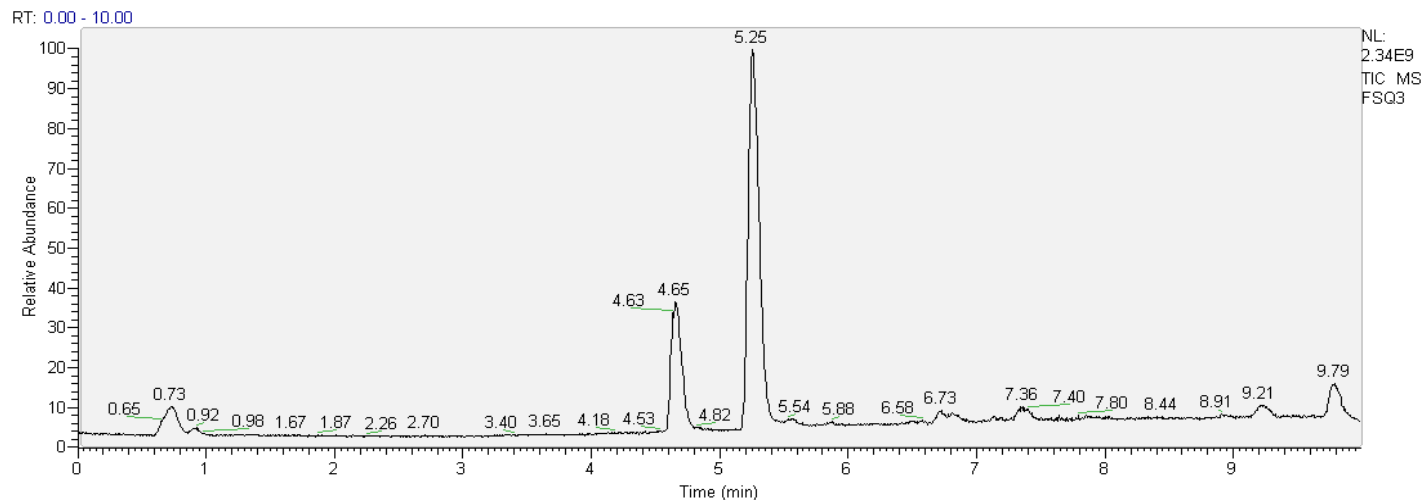
Full Scan Example (Q1)



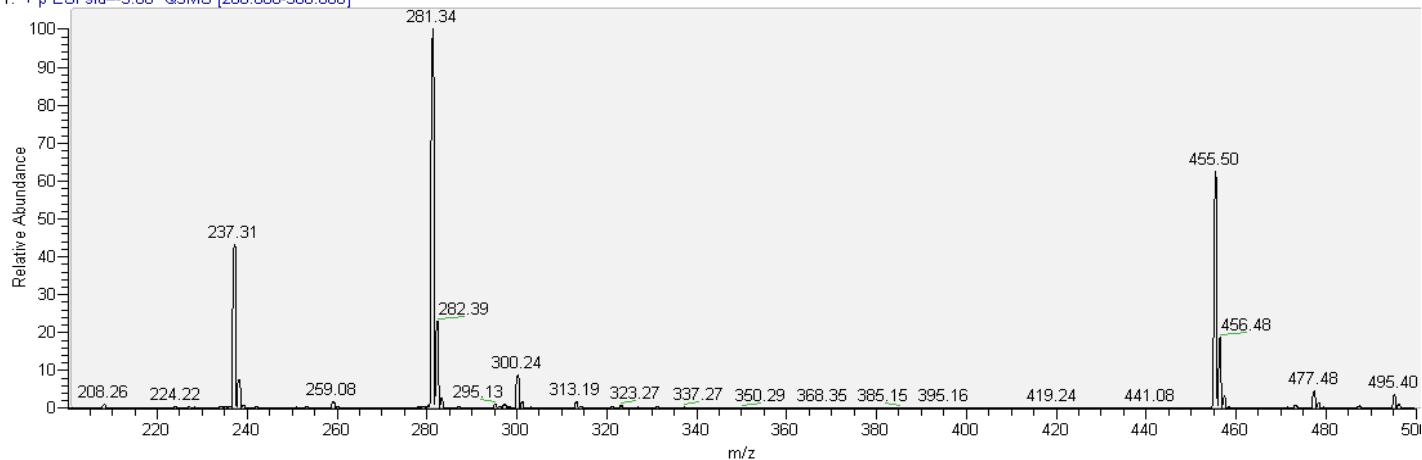
FSQ1 #831-860 RT: 5.30-5.48 AV: 30 NL: 4.71E7
T: +p ESI sid=-3.00 Q1MS [200.000-500.000]



Full Scan Example (Q3)

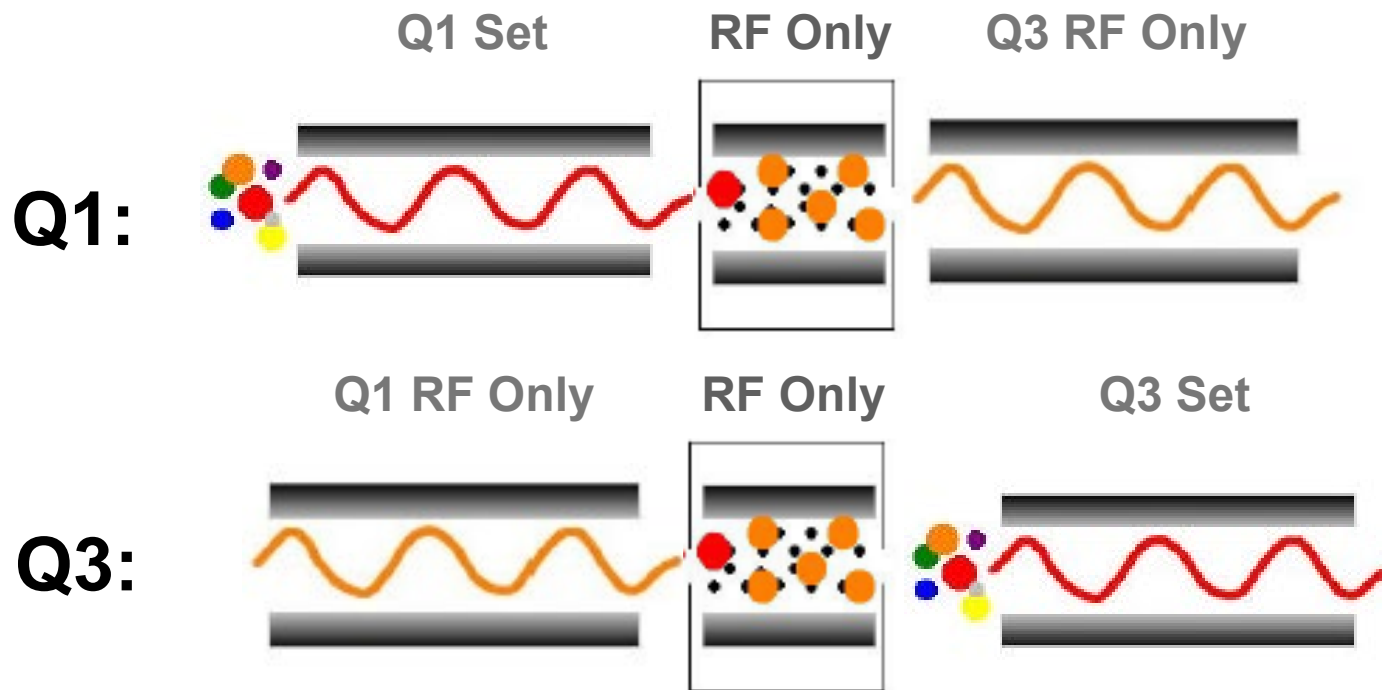


FSQ3 #814-834 RT: 5.19-5.32 AV: 21 NL: 4.92E7
T: + p ESI sid=3.00 Q3MS [200.000-500.000]

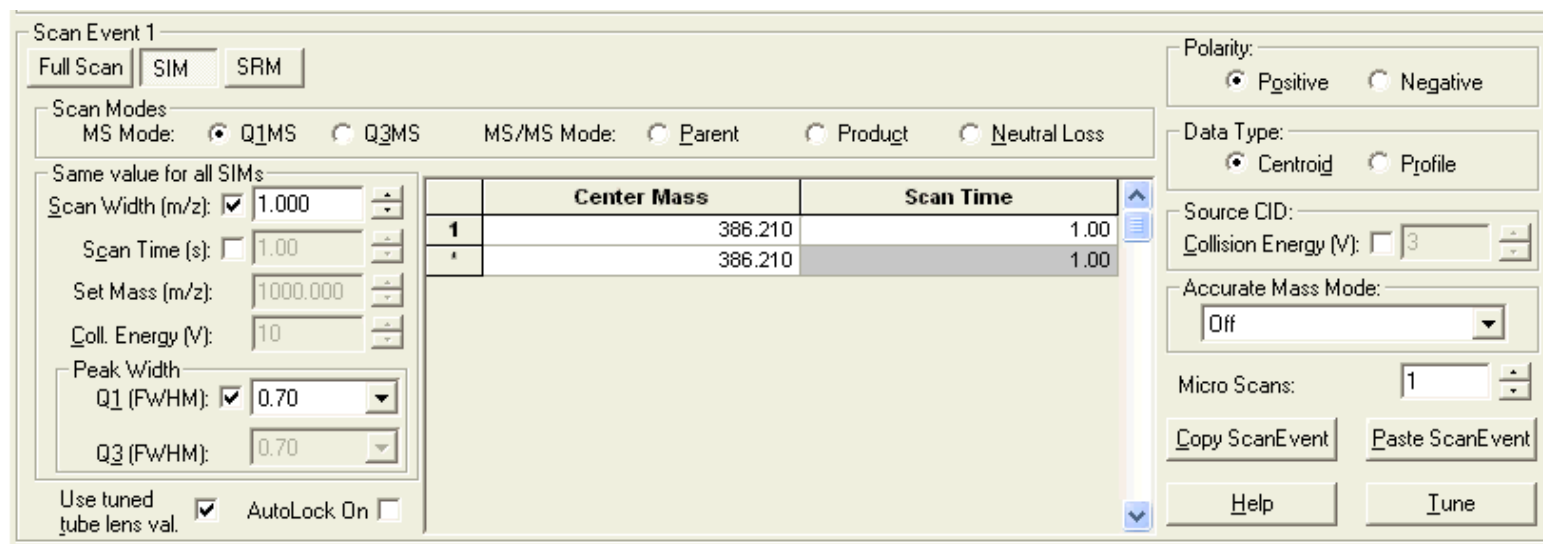


Selected Ion Monitoring - SIM (Q1 or Q3)

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



Selected Ion Monitoring - SIM (Q1 or Q3)



Scan Event 1

Full Scan **SIM** SRM

Scan Modes
MS Mode: ☒ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☐ Neutral Loss

Same value for all SIMs

Scan Width (m/z): ☒ 1.000

Scan Time (s): ☐ 1.00

Set Mass (m/z): 1000.000

Coll. Energy (V): 10

Peak Width
Q1 (FWHM): ☒ 0.70

Q3 (FWHM): 0.70

Use tuned tube lens val. ☒ AutoLock On ☐

	Center Mass	Scan Time
1	386.210	1.00
*	386.210	1.00

Polarity: ☒ Positive ☐ Negative

Data Type: ☒ Centroid ☐ Profile

Source CID:

Collision Energy (V): ☐ 3

Accurate Mass Mode: Off

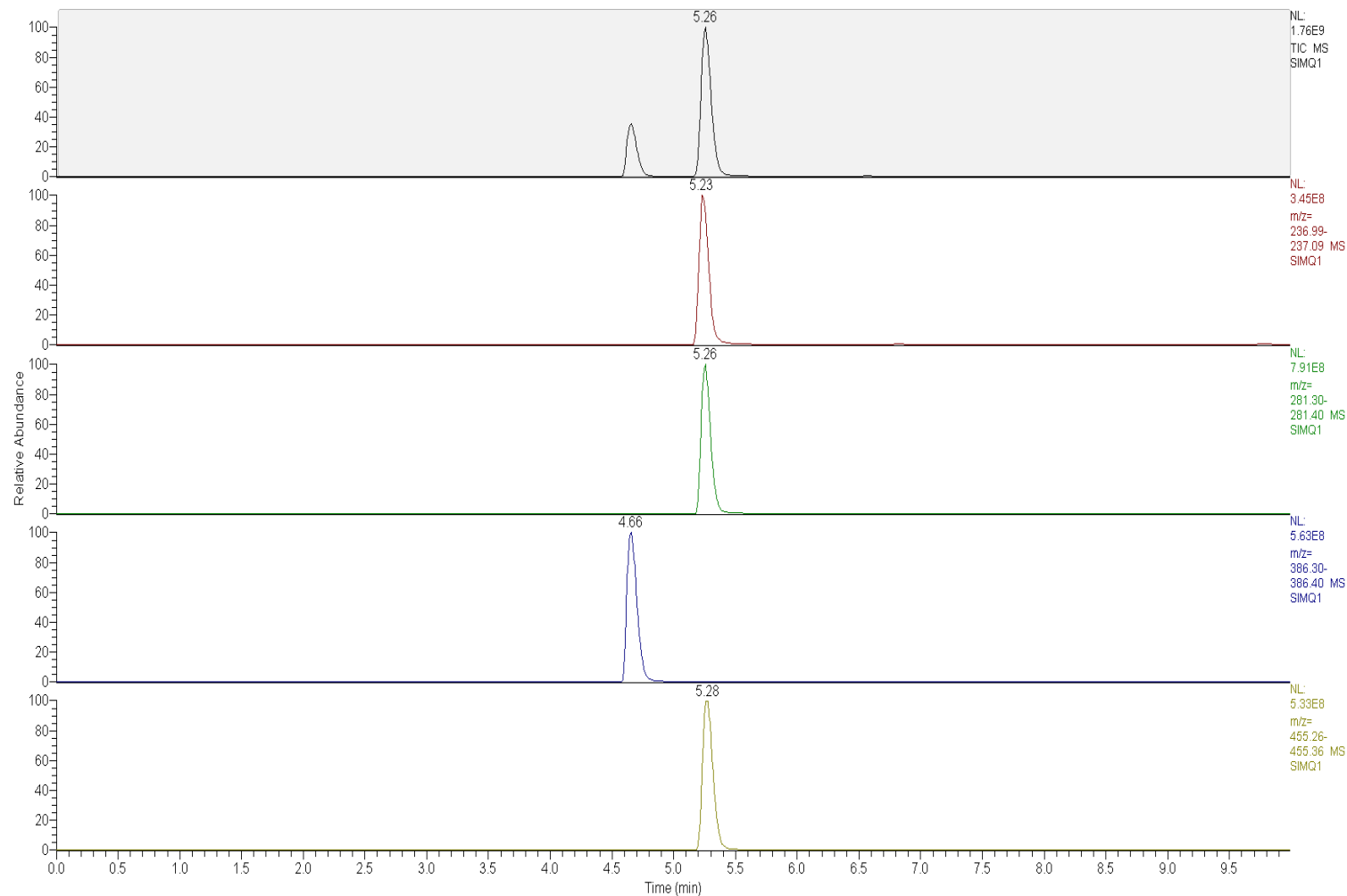
Micro Scans: 1

Copy ScanEvent Paste ScanEvent

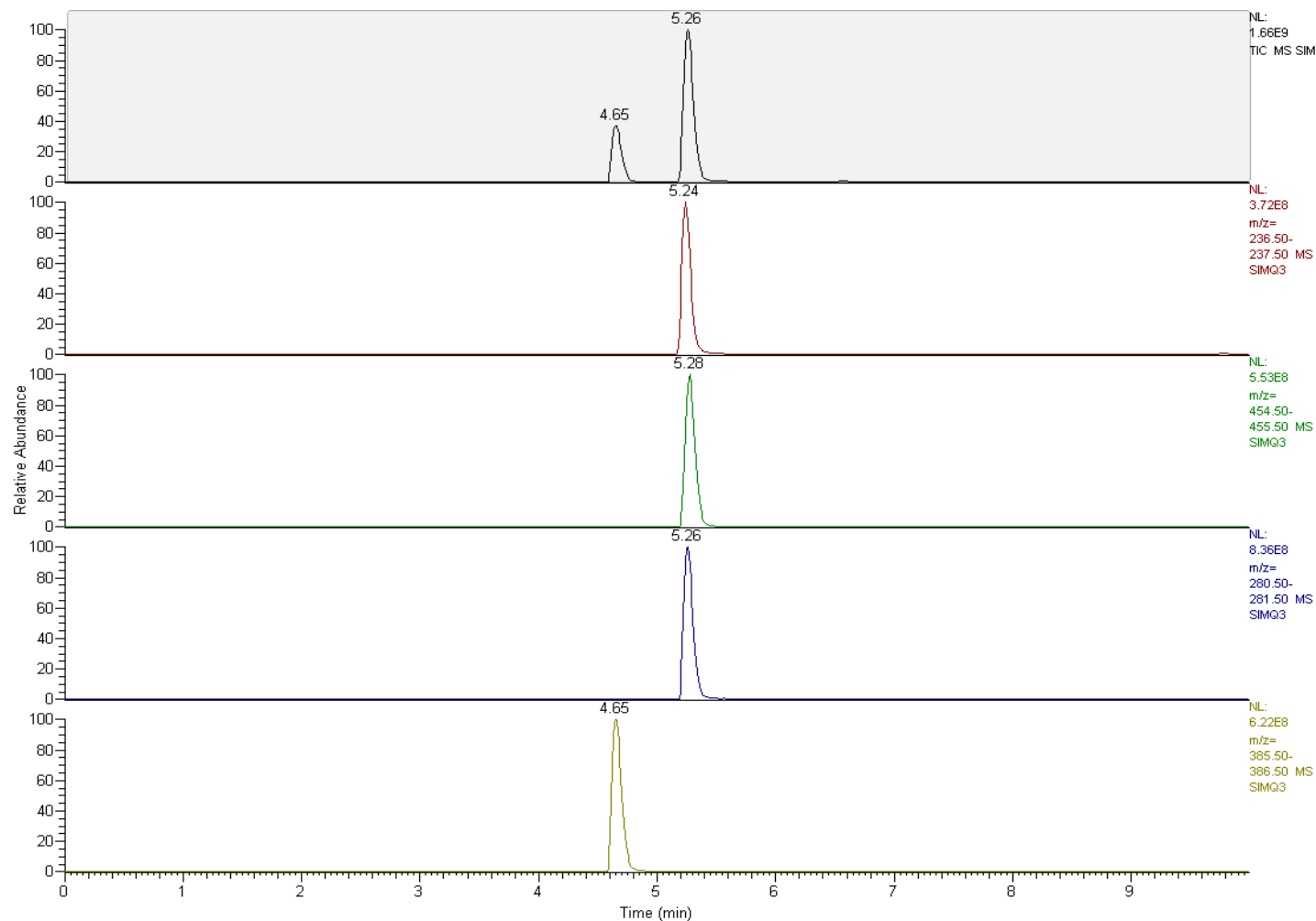
Help Tune

- SIM is in essence a full scan acquisition on a relatively narrow mass window (defined as center mass / scan width)
- The scan window around a set center mass is typically 1 Da (± 0.5 Da)

Selected Ion Monitoring - SIM (Q1)

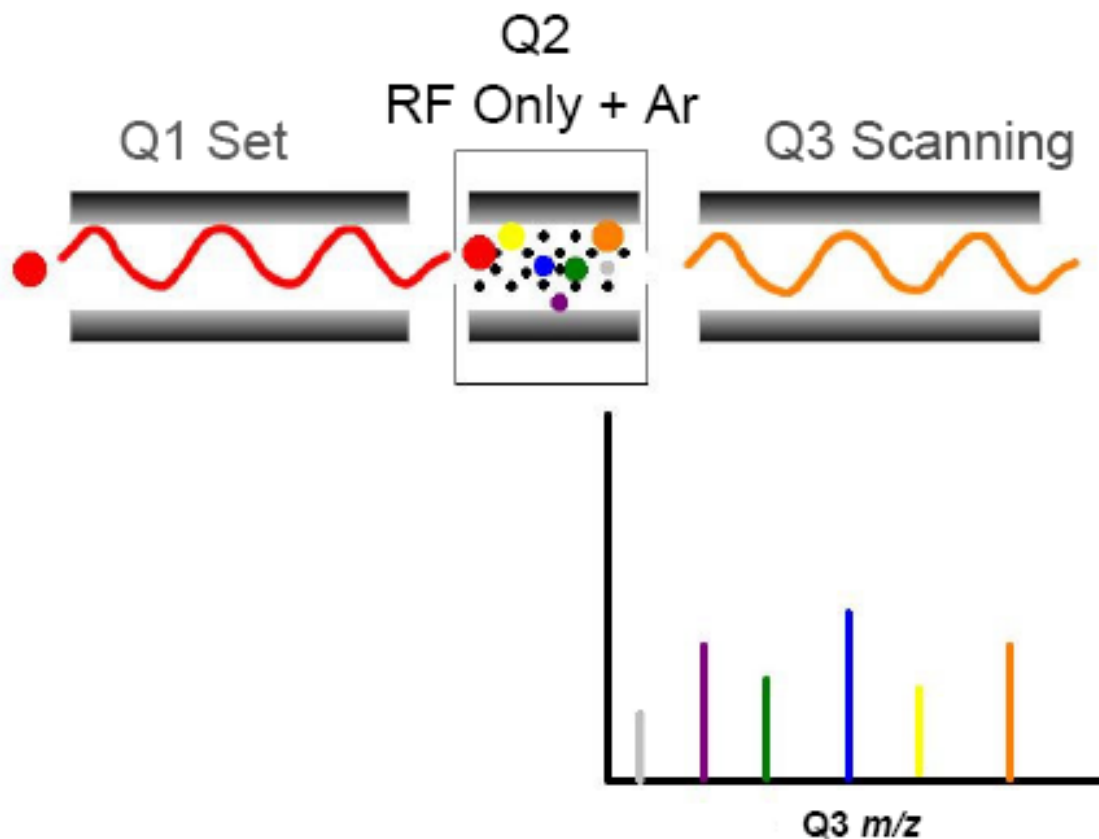


Selected Ion Monitoring - SIM (Q3)



Product Ion Scan (MS/MS)

Purpose: Survey scan of the product ions resulting from controlled fragmentation of a specific population of precursor ions



Product Ion Scan (MS/MS)

Scan Events: 1 Chrom Filter Peak Width (s): ☒ 6 Collision Gas Pressure (mTorr): ☒ 1.2

Current Scan Event: 1 Scan Event 1

Scan Event 1

Full Scan SIM SRM

Scan Modes

MS Mode: ☐ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☒ Product ☐ Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 50.000

Last Mass (m/z): 400.000

Scan Time (s): 1.00

Parent Mass (m/z): 386.210

Collision Energy (V): 42

Q1 Peak Width (FWHM): 0.70

Q3 Peak Width (FWHM): 0.70

Polarity: ☒ Positive ☐ Negative

Data Type: ☐ Centroid ☒ Profile

Source CID:

Collision Energy (V): ☐ 3

Accurate Mass Mode: Off

Micro Scans: 1

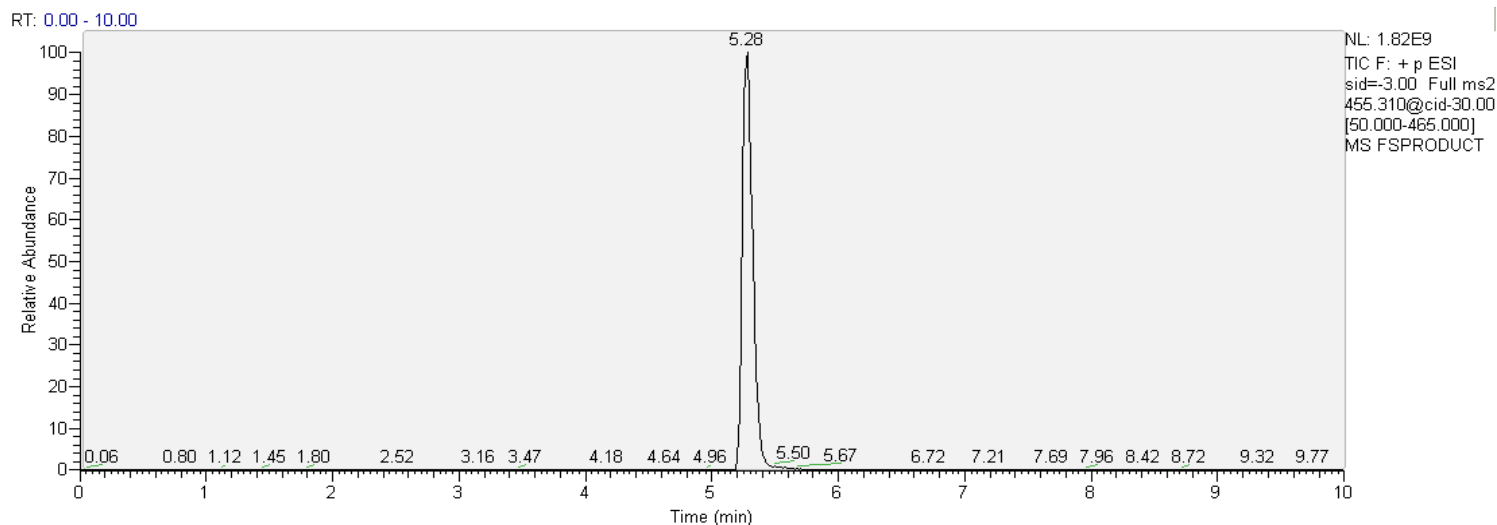
Copy ScanEvent Paste ScanEvent

Help Tune

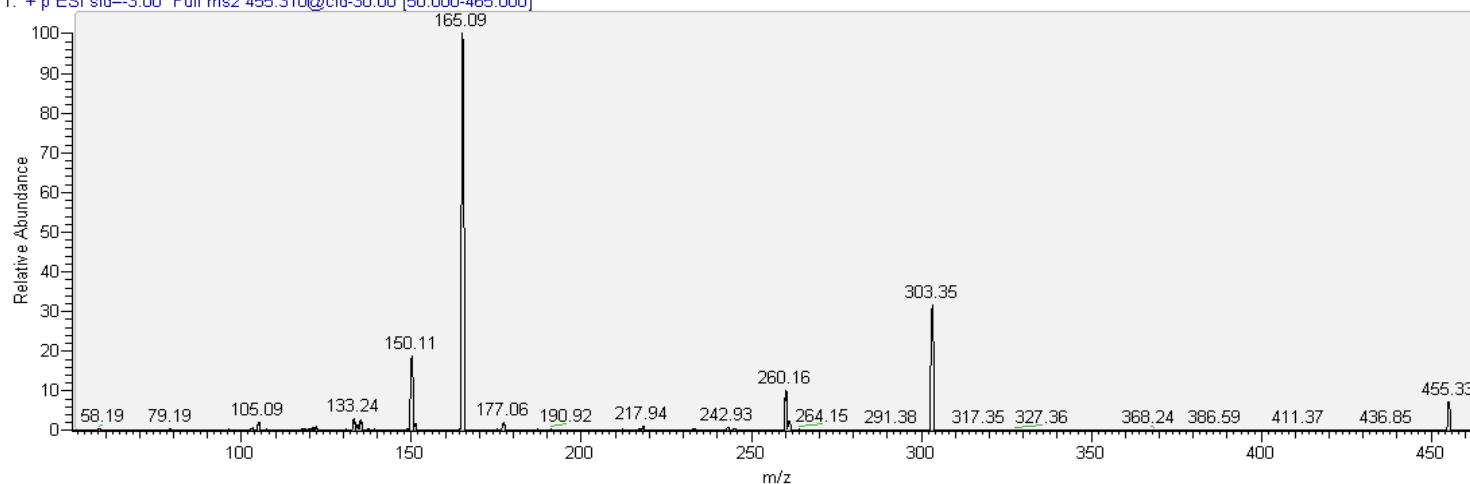
Key experimental parameters:

- Parent ion mass (m/z)
- Peak width (FWHM) of parent mass population
- Collision gas pressure
- Collision energy
- Scan range of product ions of interest

Product Ion Scan (MS/MS)

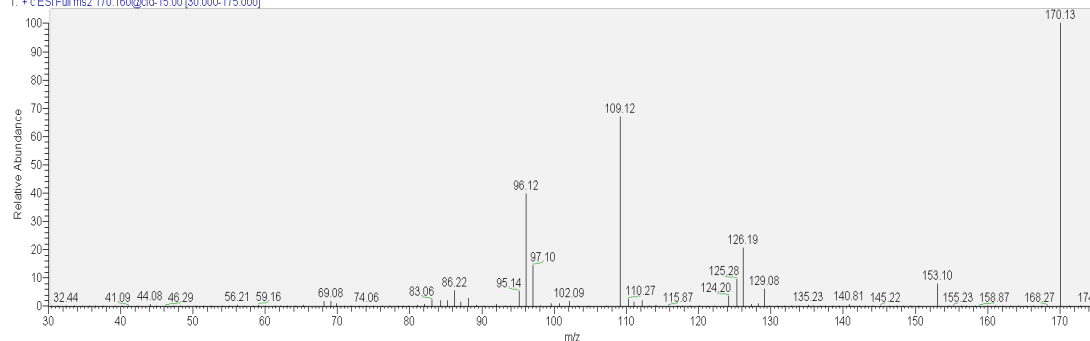


FSPRODUCT #774-794 RT: 5.22-5.34 AV: 10 NL: 5.63E7
T: + p ESI sid=-3.00 Full ms2 455.310@cid-30.00 [50.000-465.000]



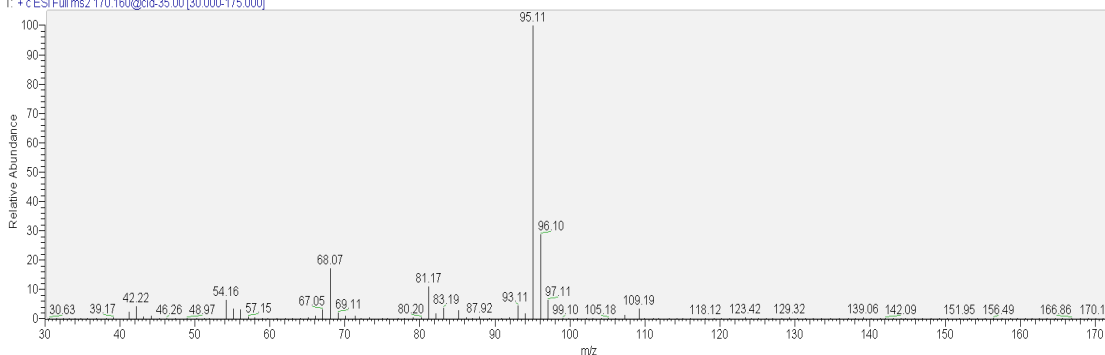
Product Ion Scan – Role of Collision Energy Ramp

ENERGY_RAMP_RE_01#1 RT: 0.00 AV: 1 NL: 6.00E5
T: + c ESI Full ms2 170.160@cid-15.00 [30.000-175.000]



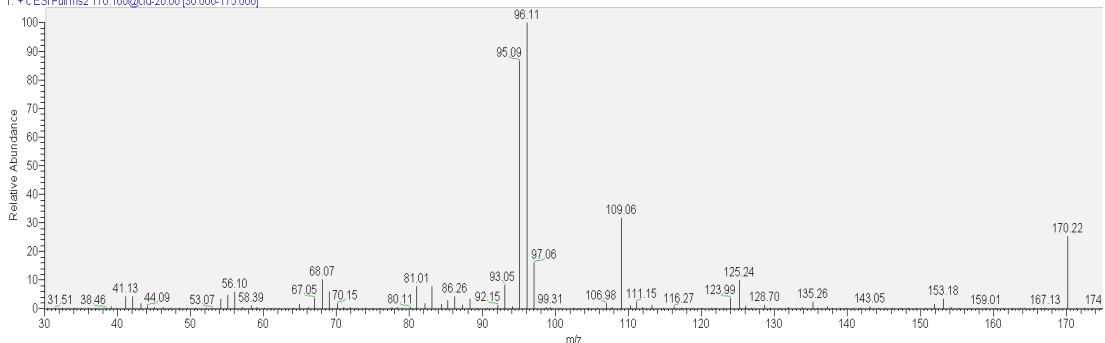
CE = 15 V
ER = 0

ENERGY_RAMP_RE_02#1 RT: 0.00 AV: 1 NL: 3.62E5
T: + c ESI Full ms2 170.160@cid-35.00 [30.000-175.000]



CE = 35 V
ER = 0

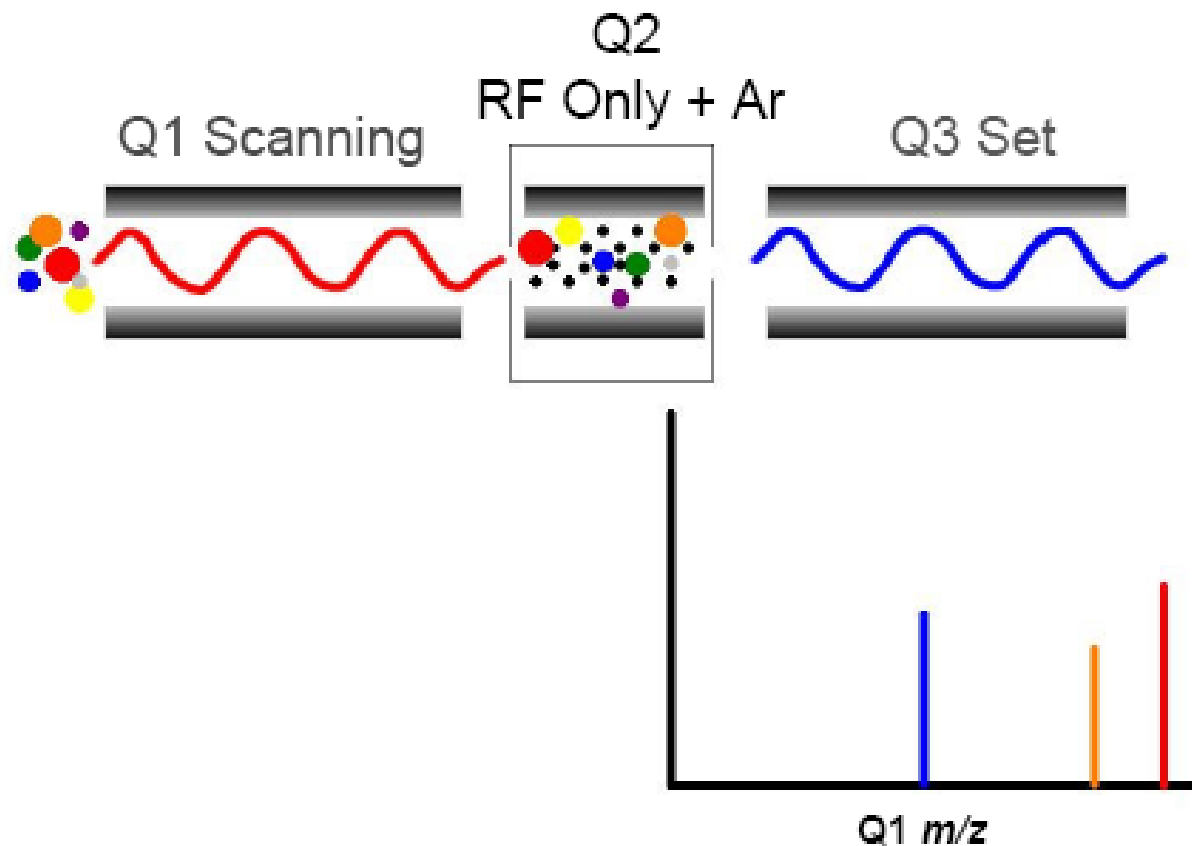
ENERGY_RAMP_RE_04#1 RT: 0.00 AV: 1 NL: 2.12E5
T: + c ESI Full ms2 170.160@cid-20.00 [30.000-175.000]



CE = 20 V
ER = 15 V

TSQ: Precursor Ion Scan (MS/MS)

Purpose: To determine the “origin” of a specific product ion



TSQ: Precursor Ion Scan (MS/MS)

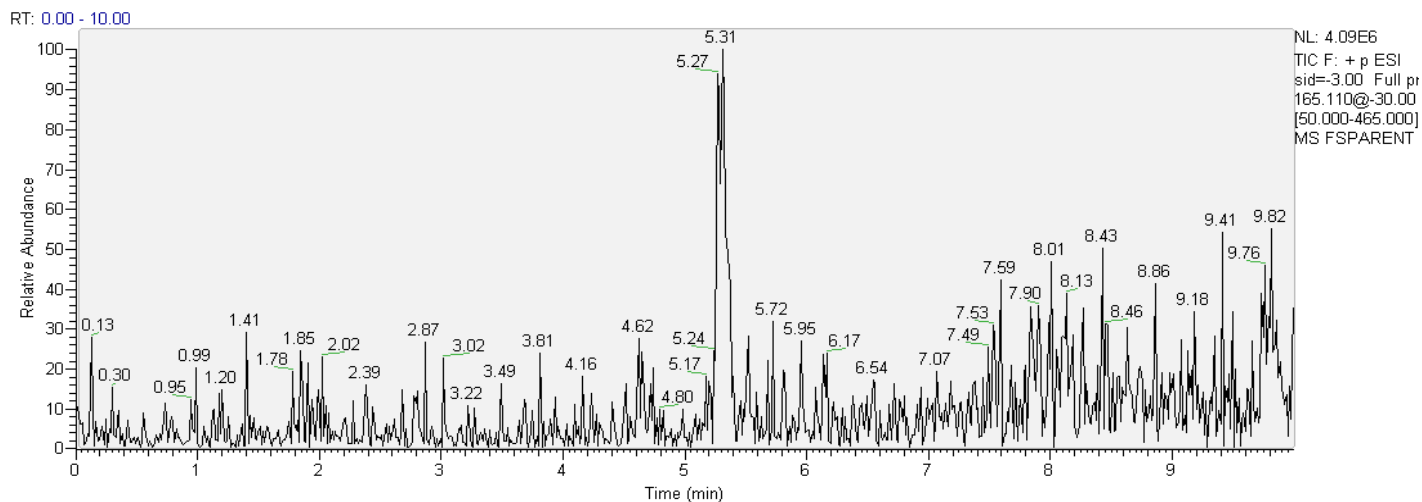
The screenshot shows the configuration window for a Precursor Ion Scan (MS/MS) in the Thermo Scientific TSQ software. The interface is organized into several sections:

- Top Section:** Contains global parameters: Scan Events (1), Chrom Filter Peak Width (s) (6), and Collision Gas Pressure (mTorr) (1.2).
- Current Scan Event:** A dropdown menu showing "Scan Event 1".
- Scan Event 1 Section:**
 - Full Scan:** Includes buttons for SIM and SRM.
 - Scan Modes:** Includes MS Mode (Q1MS, Q3MS) and MS/MS Mode (Parent, Product, Neutral Loss). The "Parent" mode is selected.
 - Scan Parameters:**
 - Scan Range:** First Mass (m/z) 250.000, Last Mass (m/z) 500.000.
 - Scan Time (s):** 1.00.
 - Product Mass (m/z):** 121.970.
 - Collision Energy (V):** 42.
 - Q1 Peak Width (FWHM):** 0.70.
 - Q3 Peak Width (FWHM):** 0.70.
- Right Section:**
 - Polarity:** Radio buttons for Positive (selected) and Negative.
 - Data Type:** Radio buttons for Centroid (selected) and Profile.
 - Source CID:** A dropdown menu.
 - Collision Energy (V):** 3.
 - Accurate Mass Mode:** A dropdown menu set to "Off".
 - Micro Scans:** 1.
 - Buttons:** Copy ScanEvent, Paste ScanEvent, Help, and Tune.

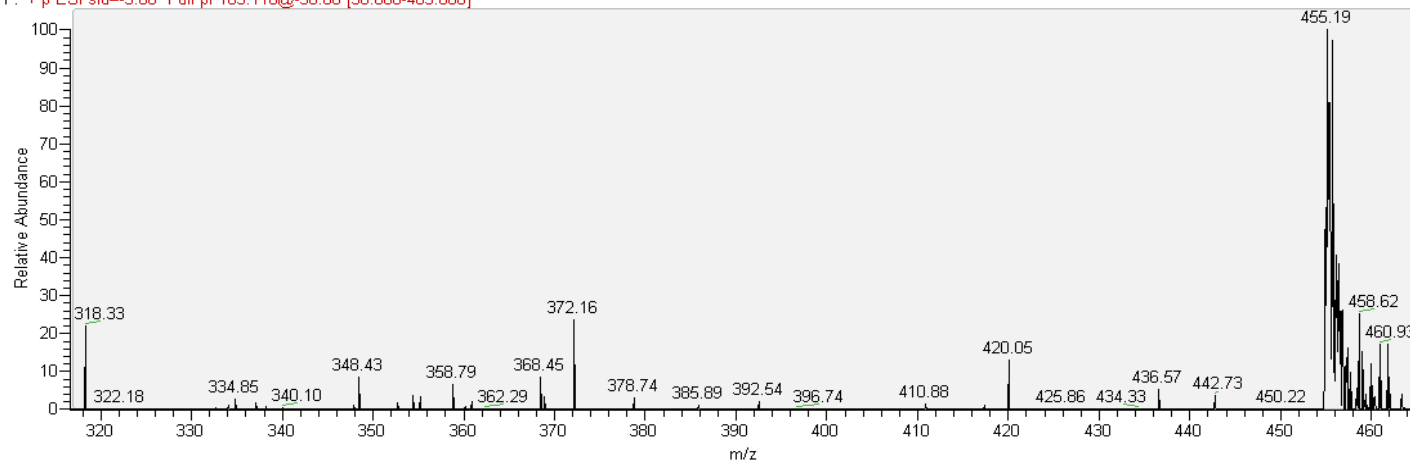
Key experimental parameters:

- Product ion mass (m/z)
- Collision gas pressure
- Collision energy
- Scan range of precursor ions of interest

TSQ: Precursor Ion Scan (MS/MS)

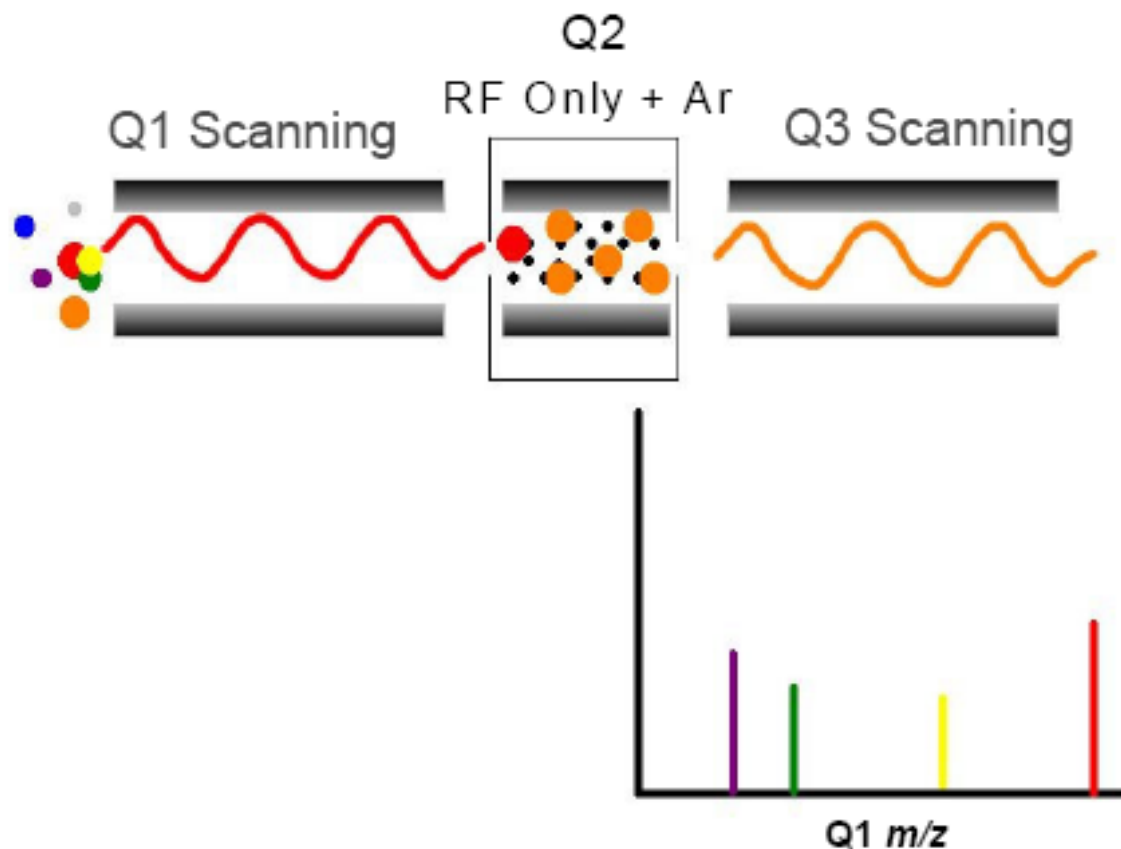


FSPARENT #778-795 RT: 5.24-5.35 AV: 9 NL: 1.74E5
F: + p ESI sid=-3.00 Full pr 165.110@-30.00 [50.000-465.000]



TSQ: Neutral Loss Scan (MS/MS)

Purpose: Compound class identification, when the class-identifying product ion does not retain the charge, following CID (i.e. phosphorylated compounds)



TSQ: Neutral Loss Scan (MS/MS)

Scan Events: 3 Chrom Filter Peak Width (s): ☒ 6 Collision Gas Pressure (mTorr): ☒ 1.2

Current Scan Event: 1 Scan Event 1 Scan Event 2 Scan Event 3

Scan Event 1

Full Scan SIM SRM

Scan Modes

MS Mode: ☐ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☒ Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 225.000 Last Mass (m/z): 500.000

Scan Time (s): 0.30 Q1 Peak Width (FWHM): 0.70

Neutral Loss Mass (m/z): 195.000 Q3 Peak Width (FWHM): 0.70

Collision Energy (V): 22

Polarity: ☒ Positive ☐ Negative

Data Type: ☒ Centroid ☐ Profile

Source CID: Collision Energy (V): 3

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Key experimental parameters:

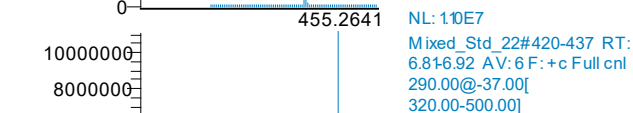
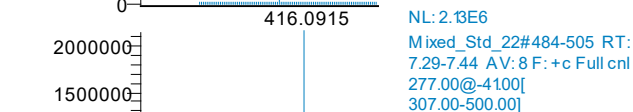
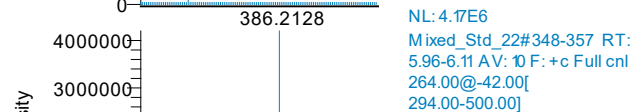
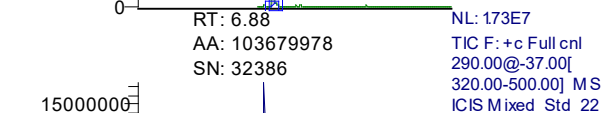
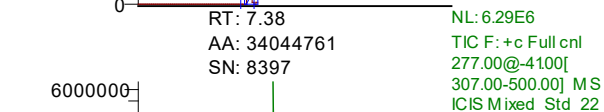
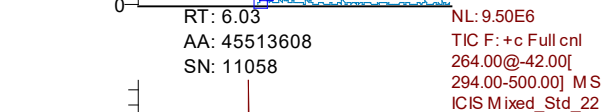
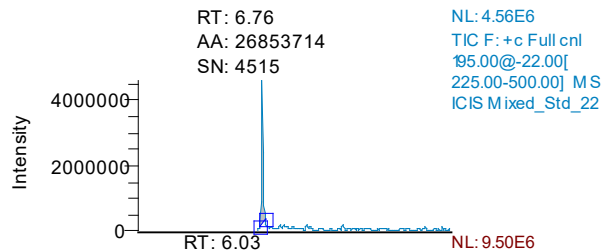
- Neutral loss mass
- Collision gas pressure
- Collision energy
- Scan range of precursor ions of interest

TSQ: Neutral Loss Scan (MS/MS)

E:\Training\Mixed_Std_22

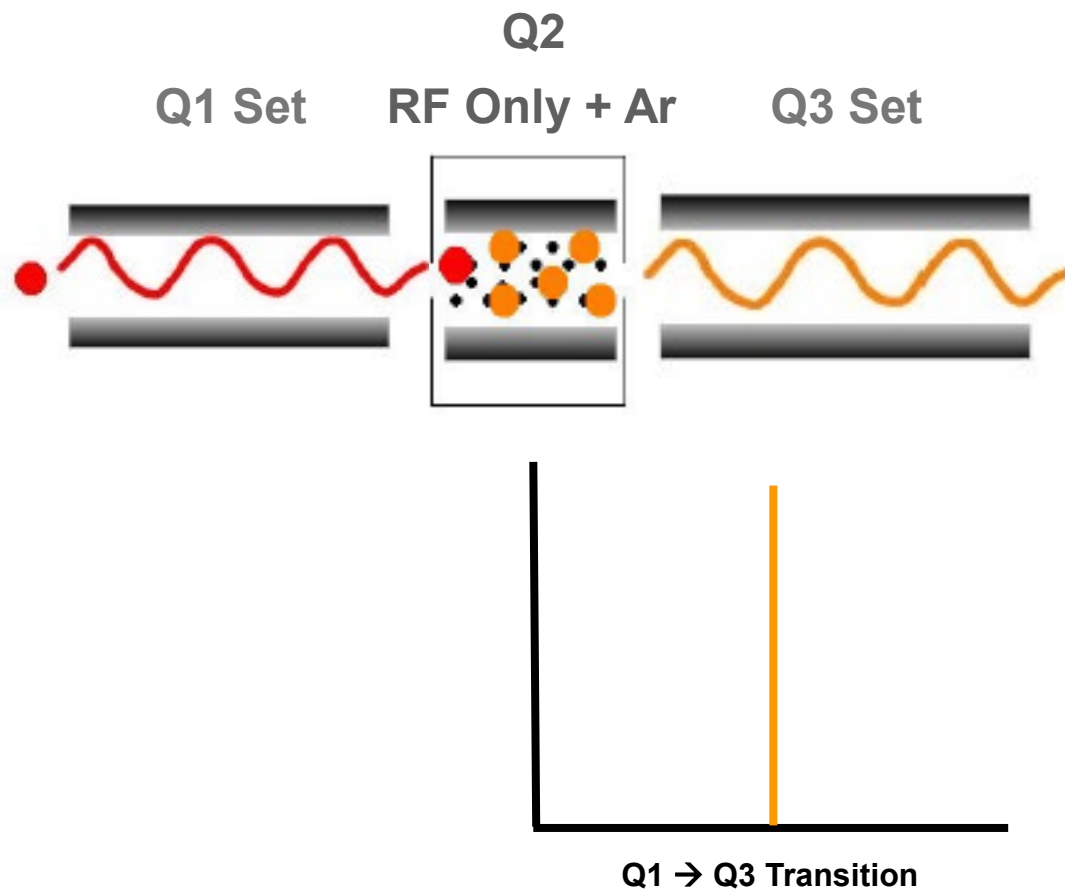
7/10/2004 2:56:47 PM

RT: 0.00 - 16.99 SM: 7G



SRM (Selected Reaction Monitoring)

Purpose: Quantitation on a single product ion population



SRM (Selected Reaction Monitoring)

Scan Event 1

Full Scan SIM SRM

Same value for all SRMs

Scan Width (m/z): ☒ 1.000

Scan Time (s): ☐ 1.00

Coll. Energy (V): ☐ 10

Peak Width

Q1 (FWHM): ☒ 0.70

Q3 (FWHM): ☒ 0.70

Use Tuned Tube Lens Value: ☒

	Parent Mass	Product Mass	Scan Time	Collision E
1	386.210	122.180	1.00	42
*	386.210	122.180	1.00	42

Polarity: ☒ Positive ☐ Negative

Data Type: ☒ Centroid ☐ Profile

Source CID:

Collision Energy (V): ☐ 3

Accurate Mass Mode: Off

Micro Scans: 1

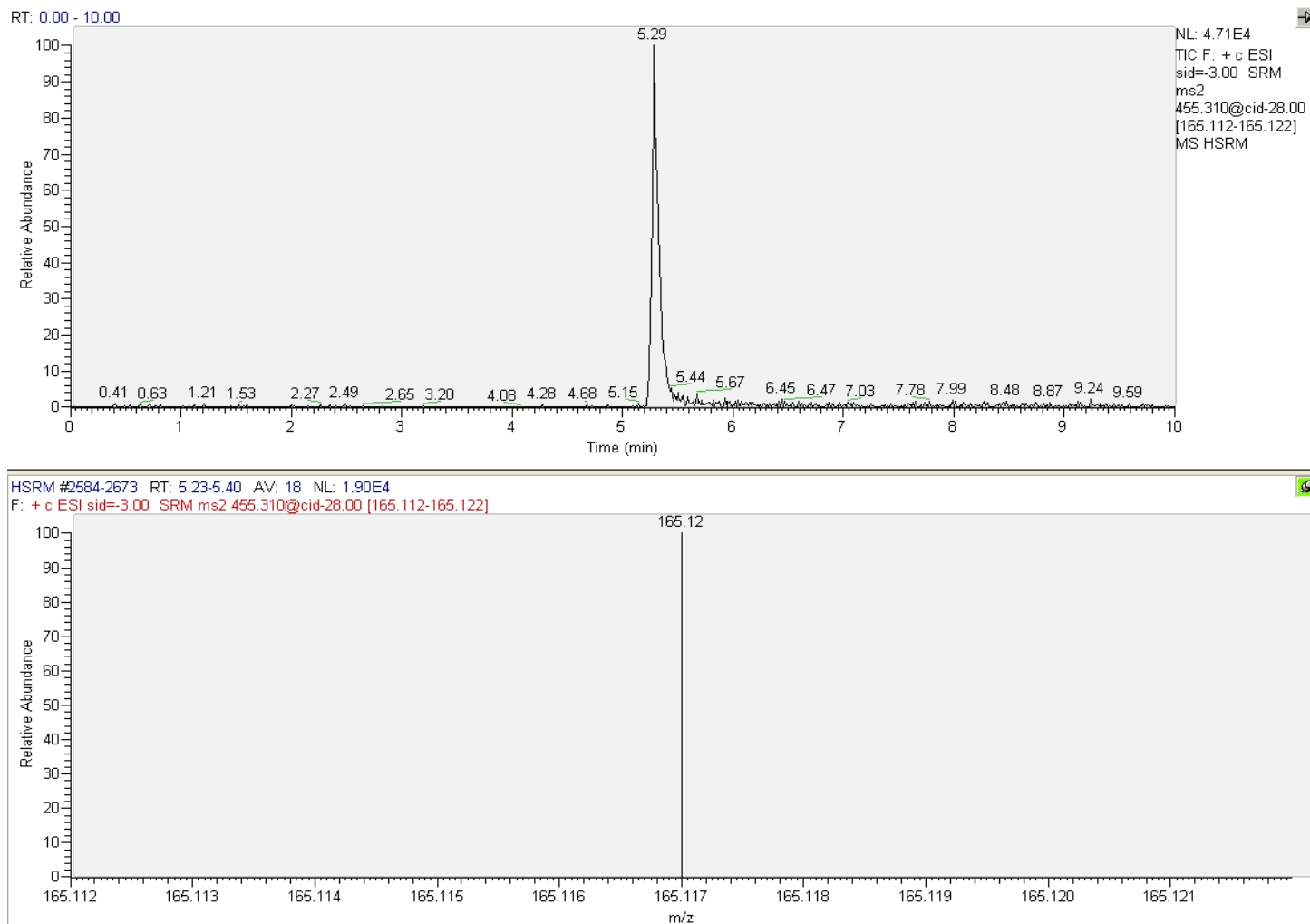
Copy ScanEvent Paste ScanEvent

Help Tune

Key experimental parameters:

- Precursor ion mass (m/z)
- Product ion mass (m/z)
- Scan time
- Collision energy
- Collision gas pressure
- Peak width (FWHM) of precursor ion

SRM (Selected Reaction Monitoring)

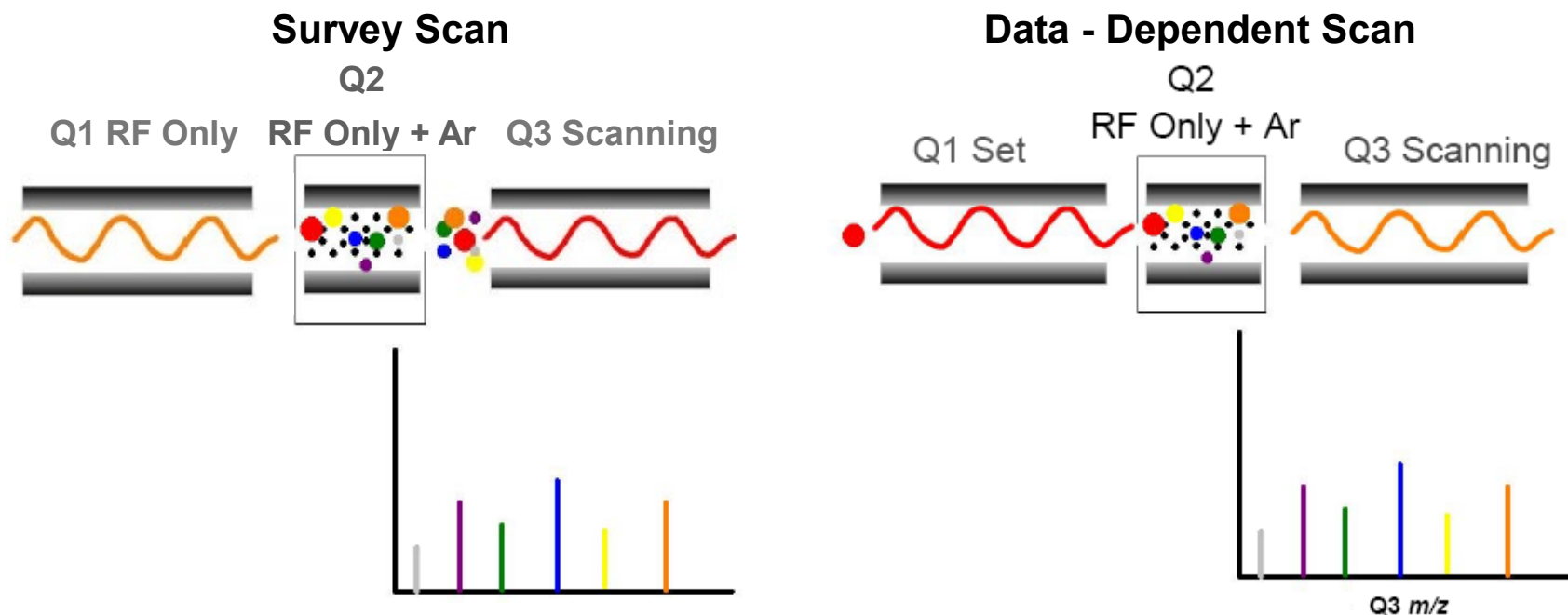


Data-Dependent Scan (Q3)

Purpose: Simultaneous acquisition of full-scan, as well as product ion information, for qualitative purposes

Survey Scan: **Full Scan Q3 Mode**

Data-Dependent Scan: **Product Ion Mode**



Data-Dependent Scan (Q3)

Survey Scan

Scan Events: 2 Chrom Filter Peak Width (s): 10 Collision Gas Pressure (mTorr): 0.8

Current Scan Event: 1

Scan Event 1

Full Scan SIM SRM

Scan Modes

MS Mode: Q1MS Q3MS MS/MS Mode: Parent Product Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 100.000 Last Mass (m/z): 600.000

Scan Time (s): 0.30 Q1 Peak Width (FWHM): 0.70

Set Mass (m/z): 1000.000 Q3 Peak Width (FWHM): 0.70

Collision Energy (V): 10

Polarity: Positive Negative

Data Type: Centroid Profile

Source CID: Collision Energy (V): 3

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Data-Dependent Scan

Scan Events: 2 Chrom Filter Peak Width (s): 10 Collision Gas Pressure (mTorr): 0.8

Current Scan Event: 2

Scan Event 1 Scan Event 2

Scan Event 2

Full Scan SIM SRM Dependent Scan AutoSIM

Scan Selection

Mass determined from scan event: 1 From Scan From Parent List

Nth Most Intense Ion: 1 If no acceptable parent found convert to most intense from scan

Signal Threshold (10⁴ counts): 2.0 Weighting Factor: 0.0

Scan Parameters

Scan Time (s): 0.70 Collision Energy (V): 30 Q1 Peak Width (FWHM): 0.70

Charge State: 1 CE grad(V per m/z): 0.1000 Q3 Peak Width (FWHM): 0.70

Source Delta(m/z): 1.000 DD Delta(m/z): 1.000

Advanced Data Dependent Settings And Activation

Dynamic Exclusion Isotope Ratio Specify mass lists in sequence row (Global Setting)

Advanced Settings...

Copy ScanEvent Paste ScanEvent

Help Tune

Data-Dependent Scan (Q3)

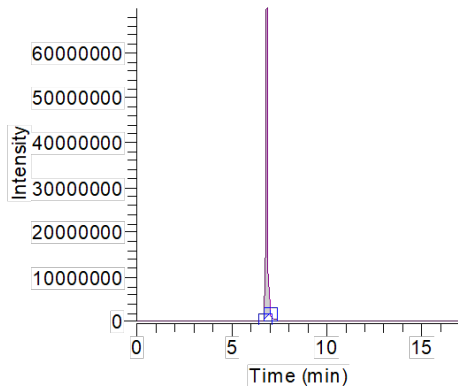
E:\Training\Mixed_Std_14

7/8/2004 11:59:27 AM

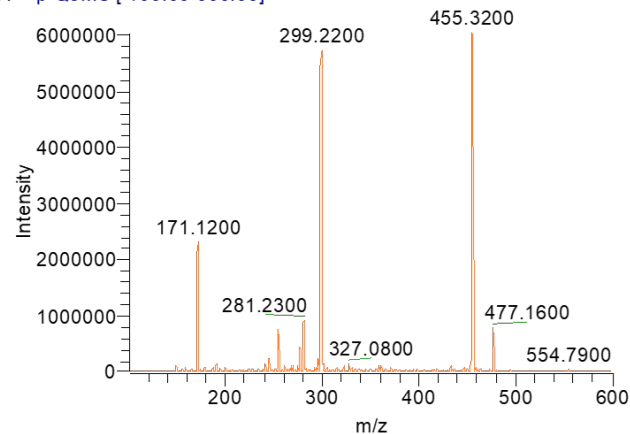
RT: 0.00 - 16.99 SM: 5B

RT: 6.85
AA: 533612692
SN: 2629_{RMS}

NL: 6.99E7
m/z=
454.9000-
455.1000 F: + p
Q3MS [
100.00-600.00]
MS ICIS
Mixed_Std_14



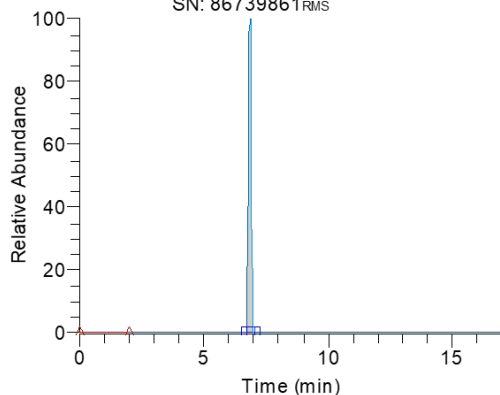
Mixed_Std_14 #542-585 RT: 6.58-7.10 AV: 22 SB: 151
T: + p Q3MS [100.00-600.00]



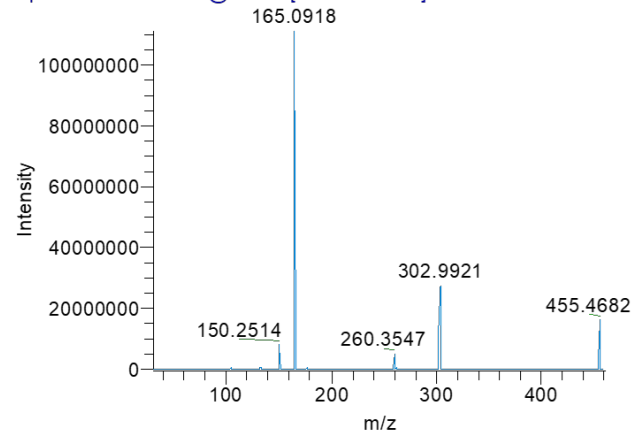
RT: 0.00 - 16.99 SM: 5B

RT: 6.86
AA: 660361798
SN: 86739861_{RMS}

NL:
8.67E7
m/z=
454.4749-
455.7680 F: + p
d Full ms2 MS
ICIS
Mixed_Std_14



Mixed_Std_14 #553-575 RT: 6.81-6.94 AV: 6 NL: 1.11E8
T: + p d Full ms2 455.23@-30.00 [30.00-460.23]





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Chapter 7

***Quantum Tune Page
with Tuning and Calibration***



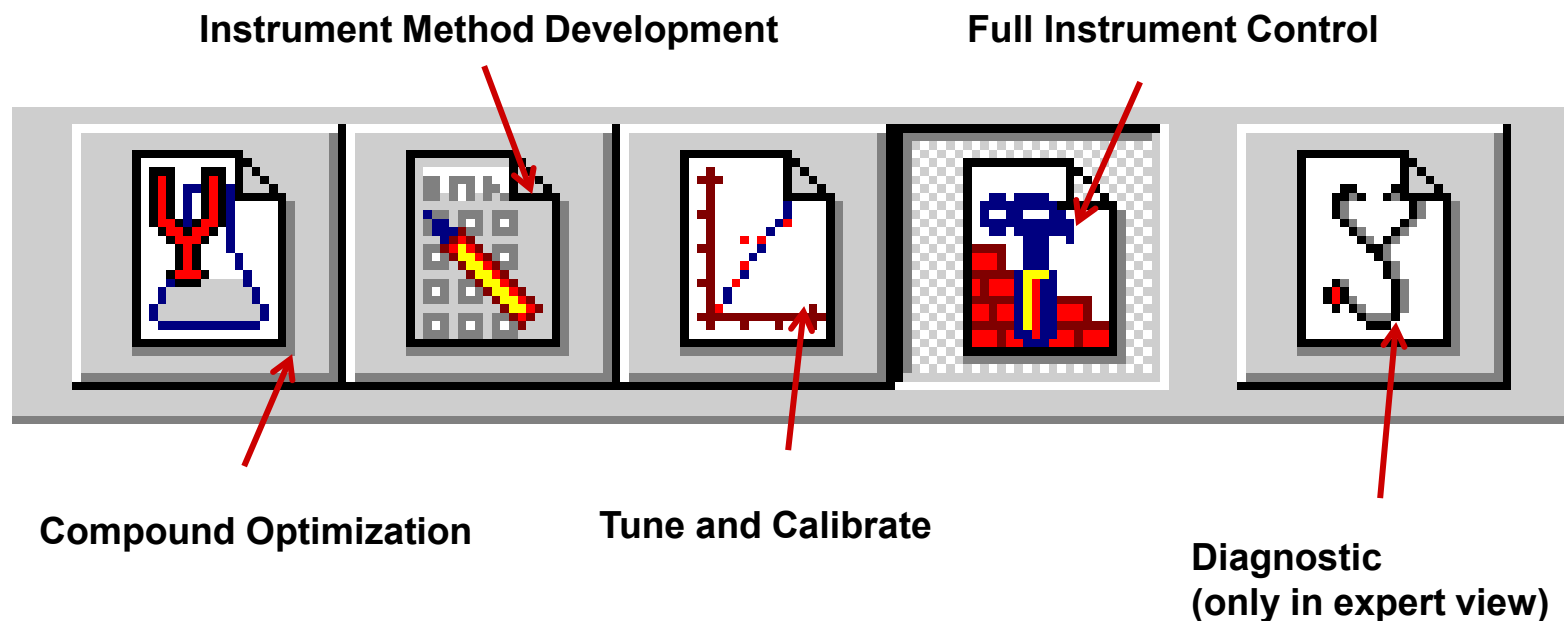
Work Space based for functional tasks:

- » **Tune and Calibrate**
- » **Instrument Method Development**
- » **Compound Optimization**
- » **Diagnostics**

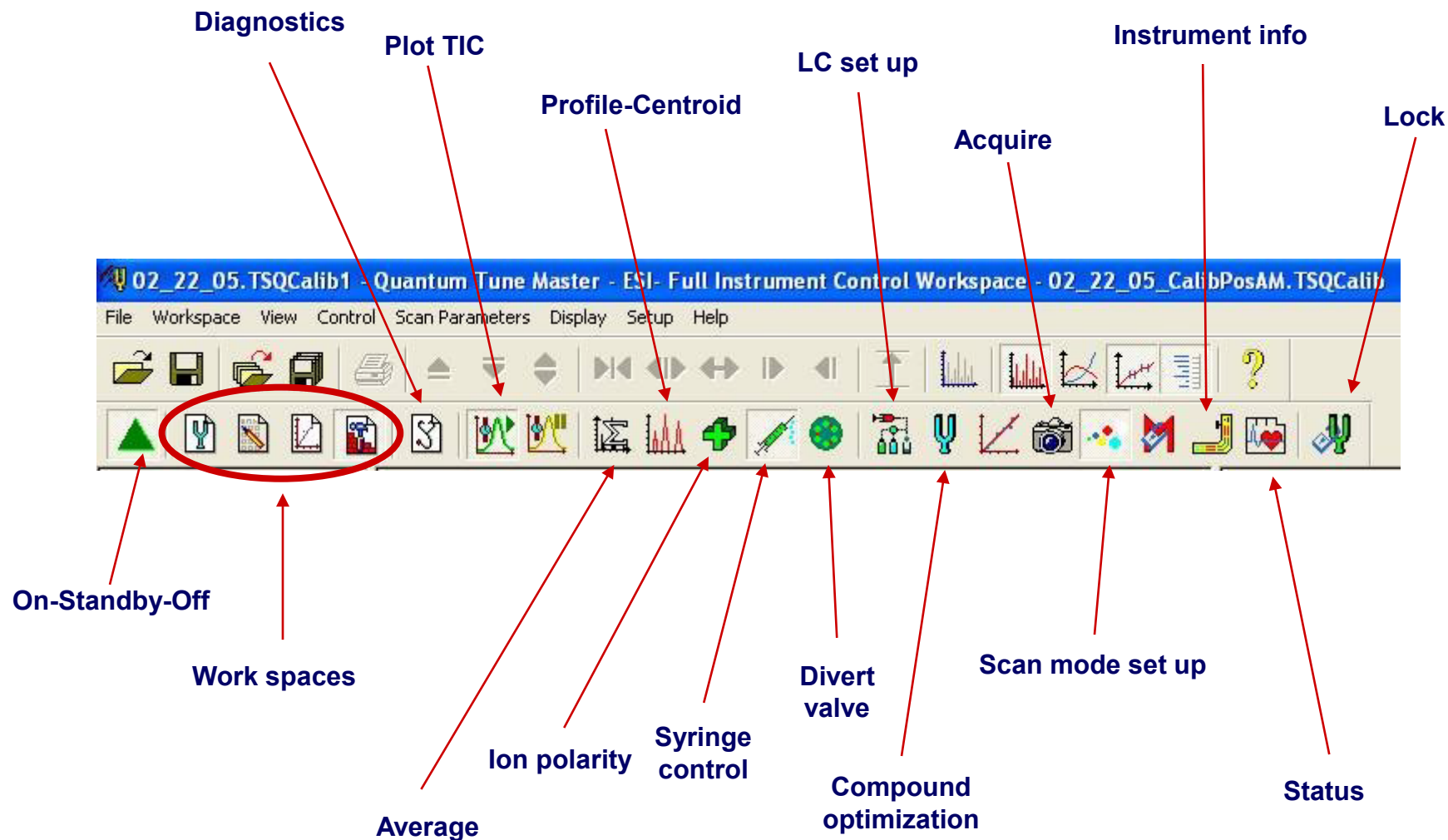
Key Features

- User-defined tune and calibration masses (optional)
- Full instrument development environment, MS and LC
- Automated optimization of MS/MS conditions
- Transferable methods from Tune Window to experimental editor in Xcalibur Instrument Setup

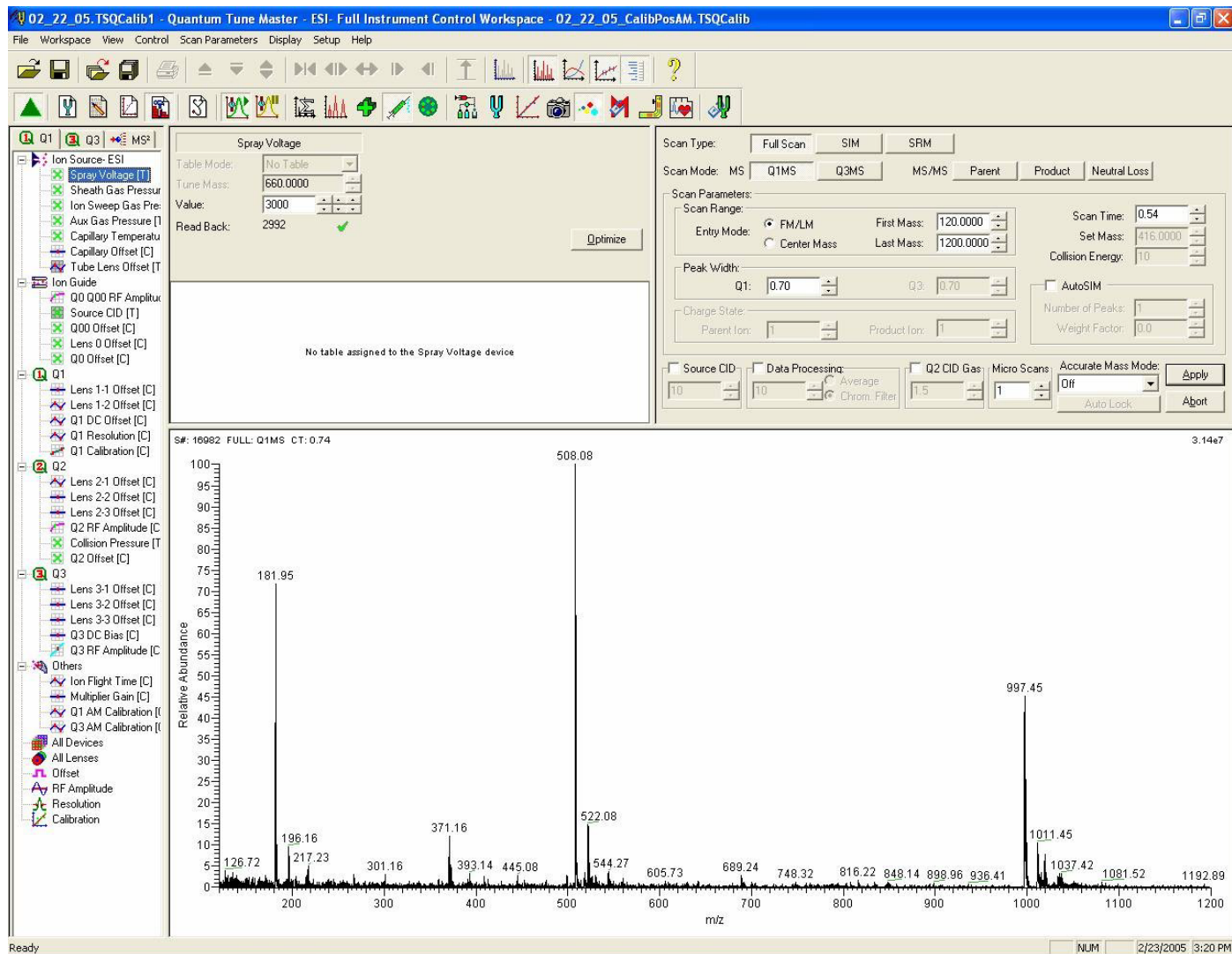
Instrument Work Spaces



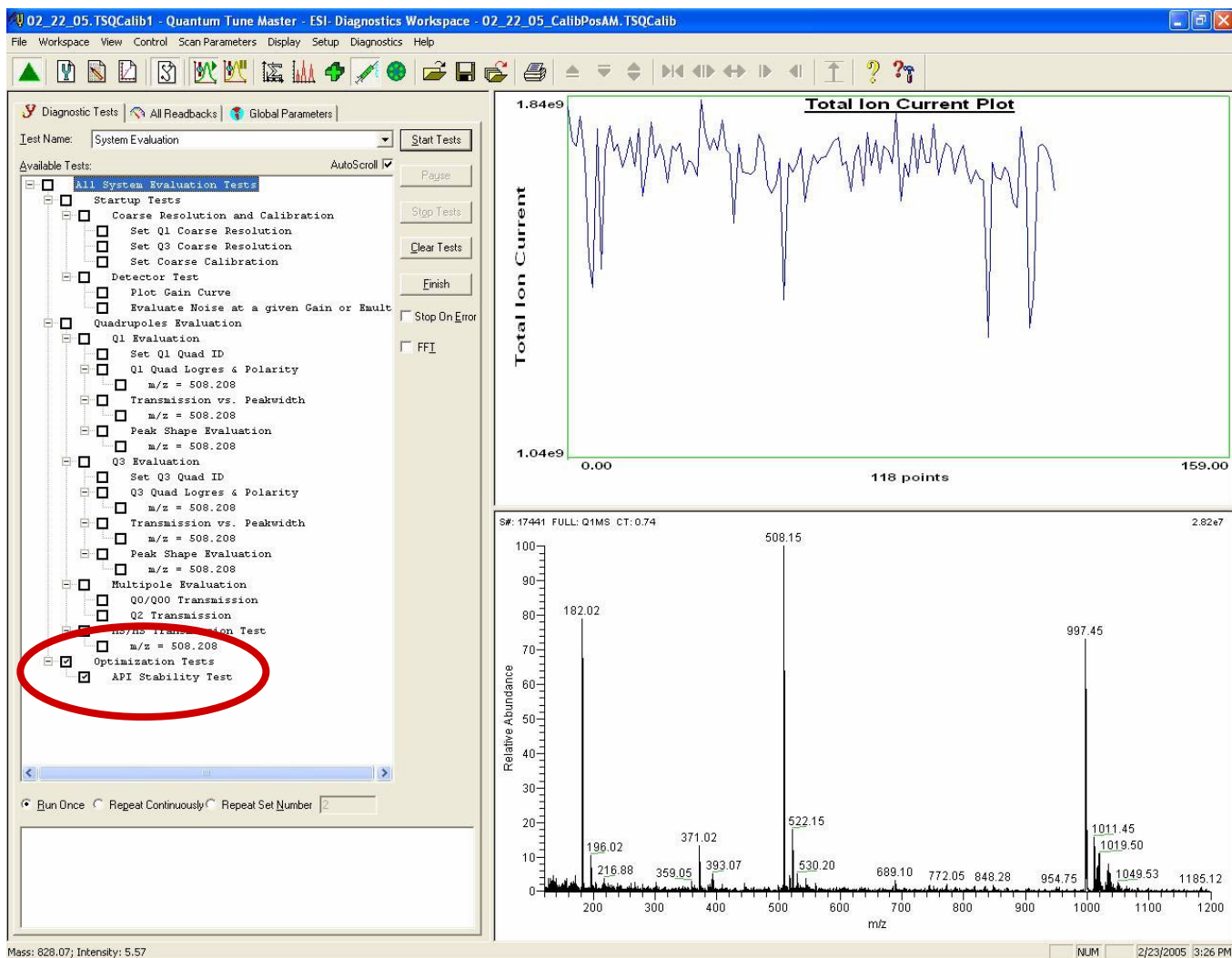
All Instrument Control Tool Bar



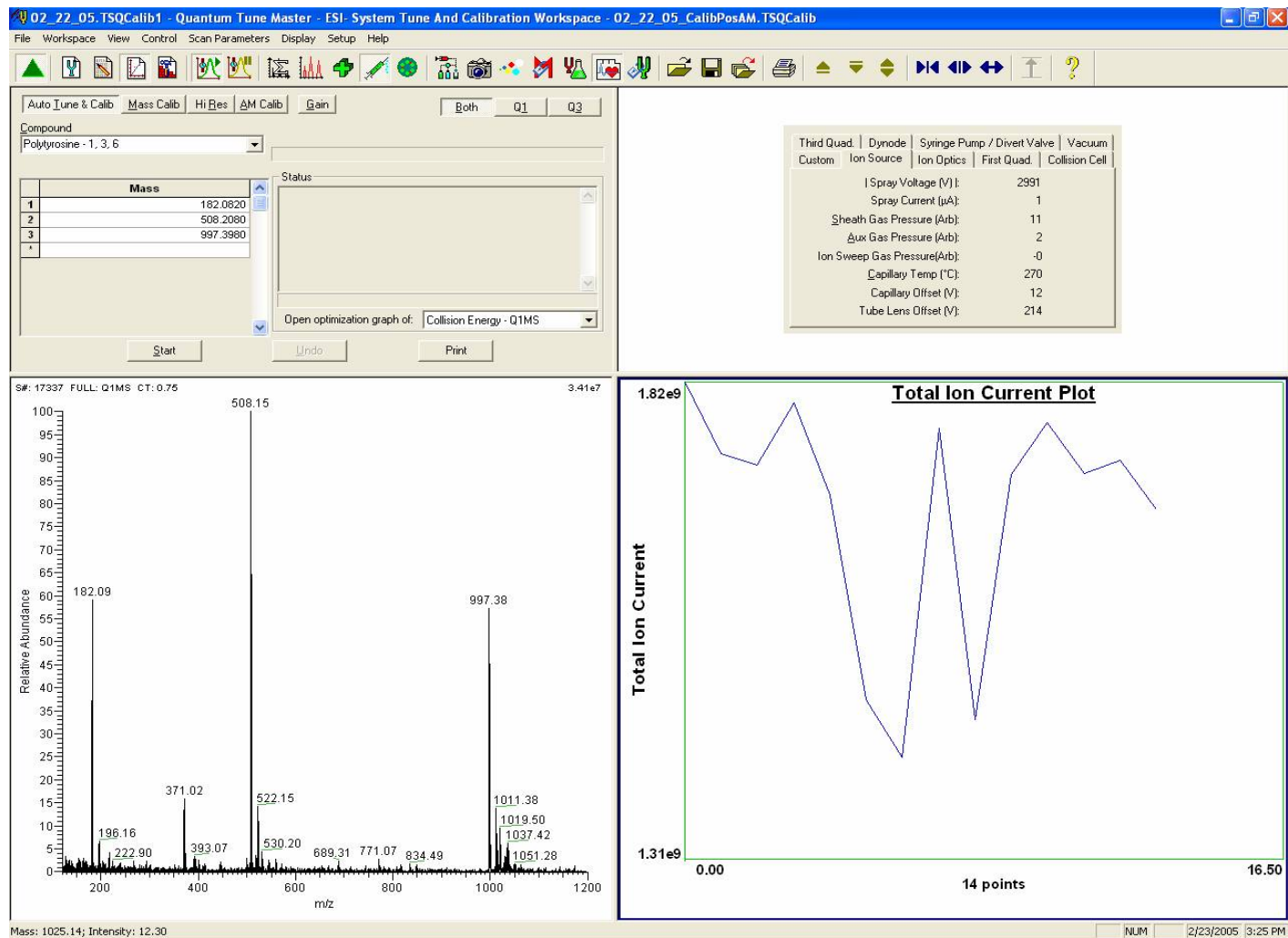
Full Instrument Control-Expert View



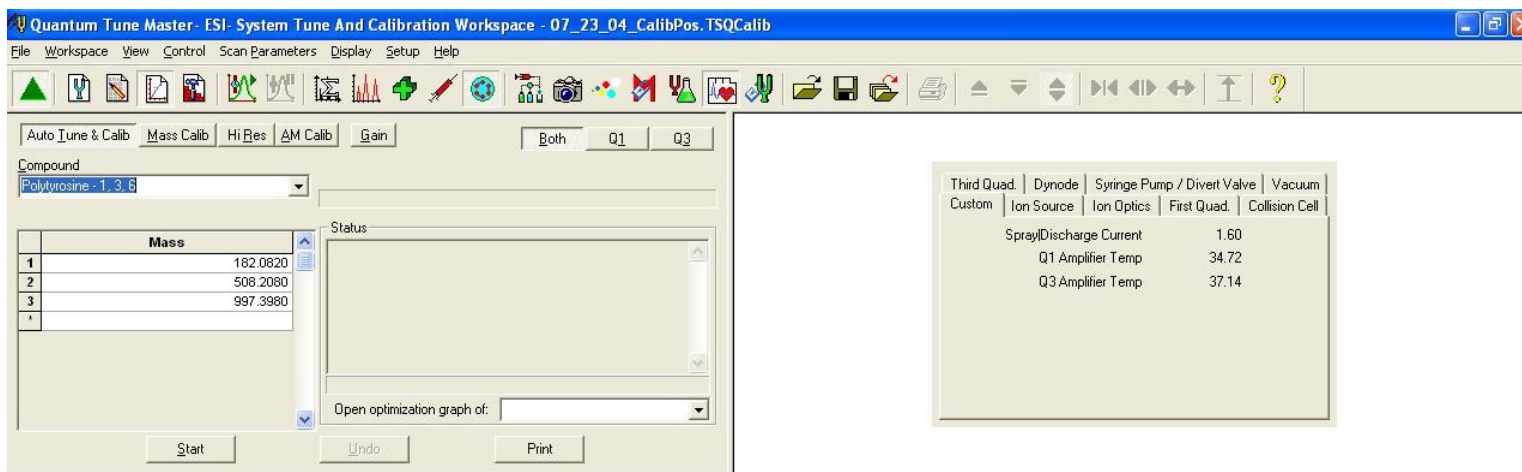
Diagnostics



Auto Tune and Calibrate



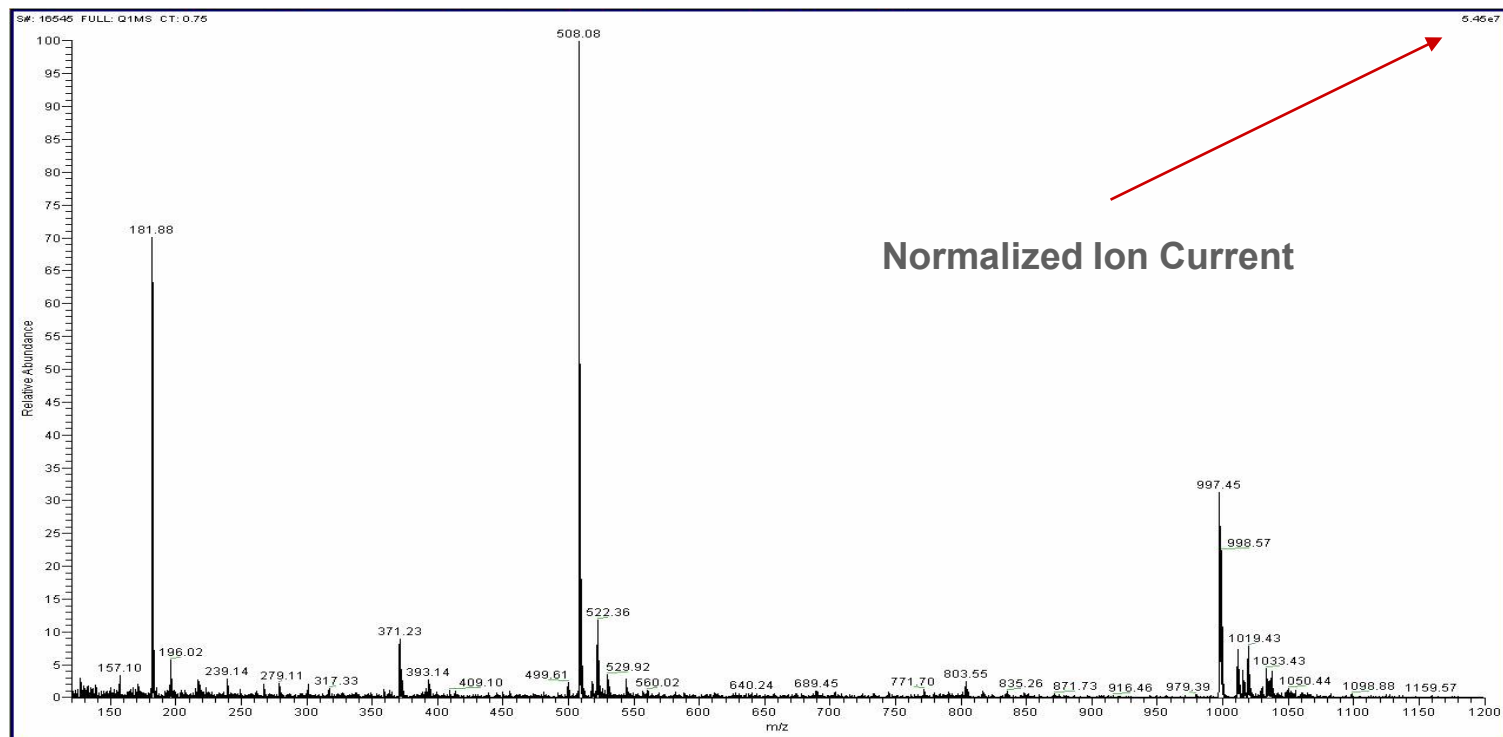
Tune and Calibrate



Pull-down calibration mass list:

- User-customizable
- Masses of pre-set compounds are protected

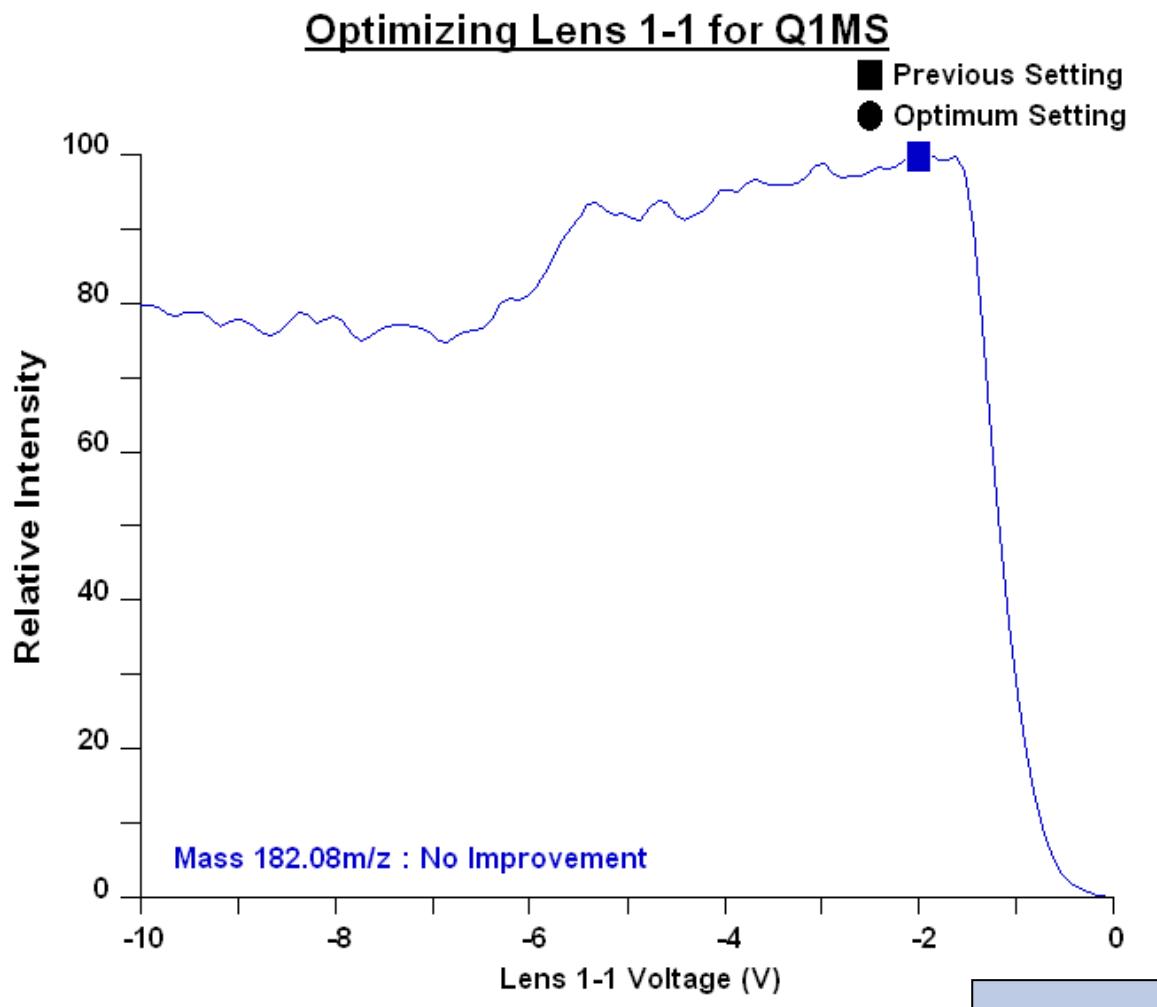
1, 3, 6 - Polytyrosine



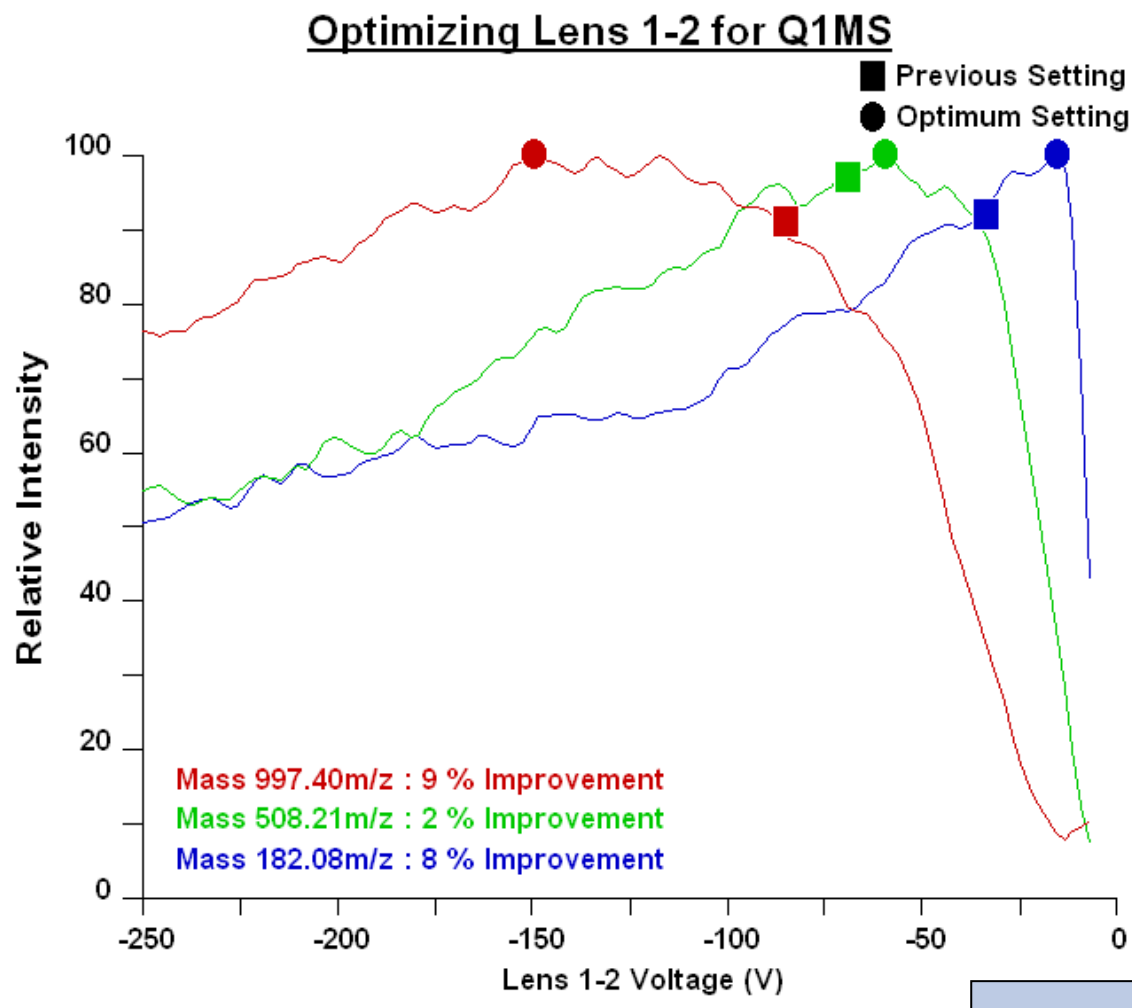
Prerequisites for successful auto-tune and calibration:

- Ions of interest must be present in the full-scan view
- Satisfactory spray stability (via diagnostics)
- The intensity of base peak should be around 1E7 (or 1E6 in negative ion mode)

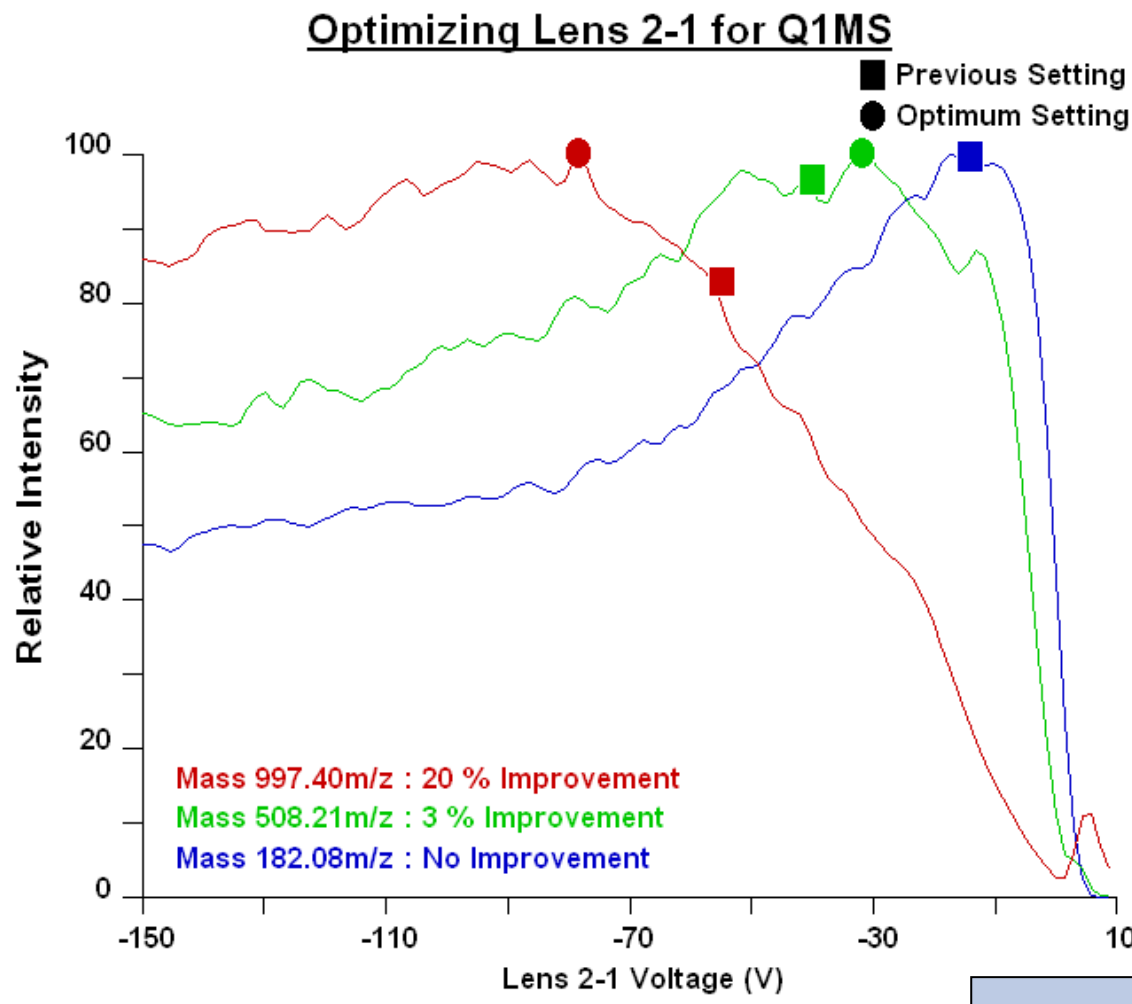
Auto Tune and Calibration



Auto Tune and Calibration

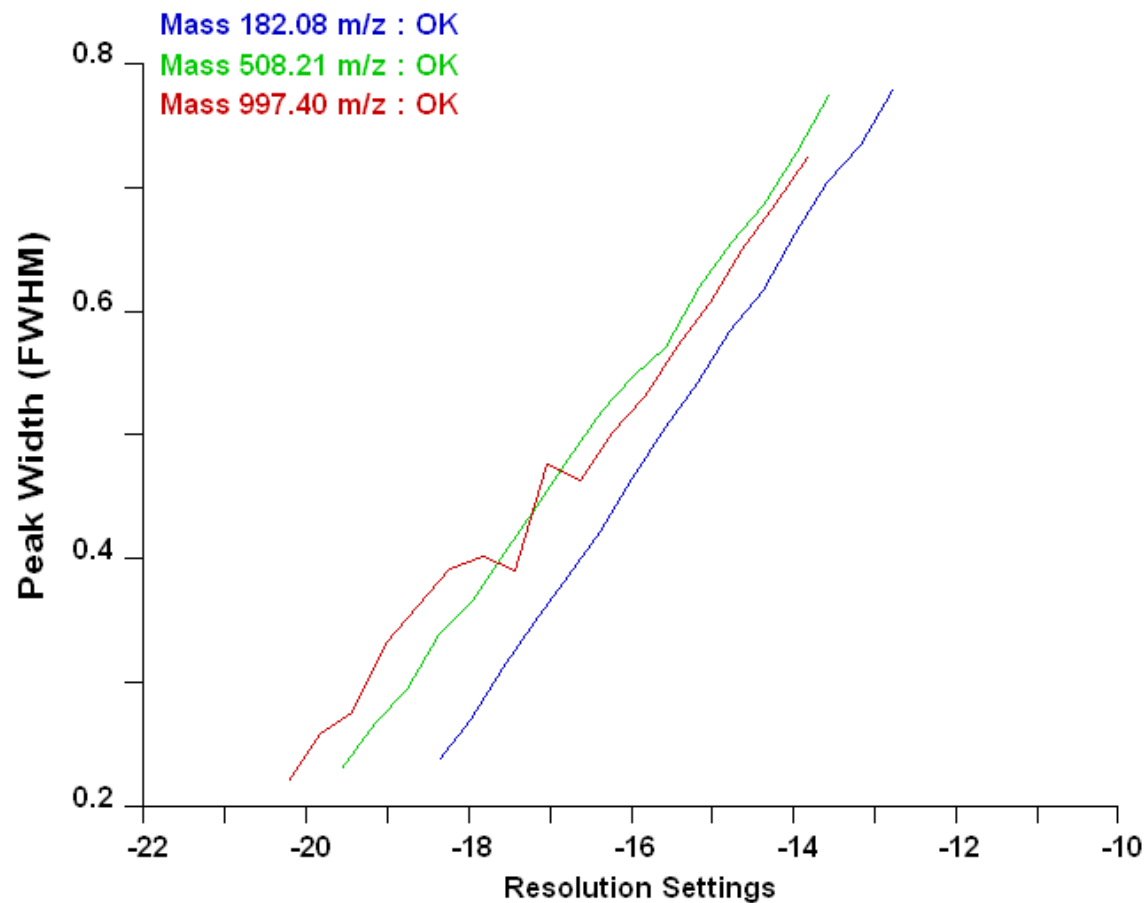


Auto Tune and Calibration



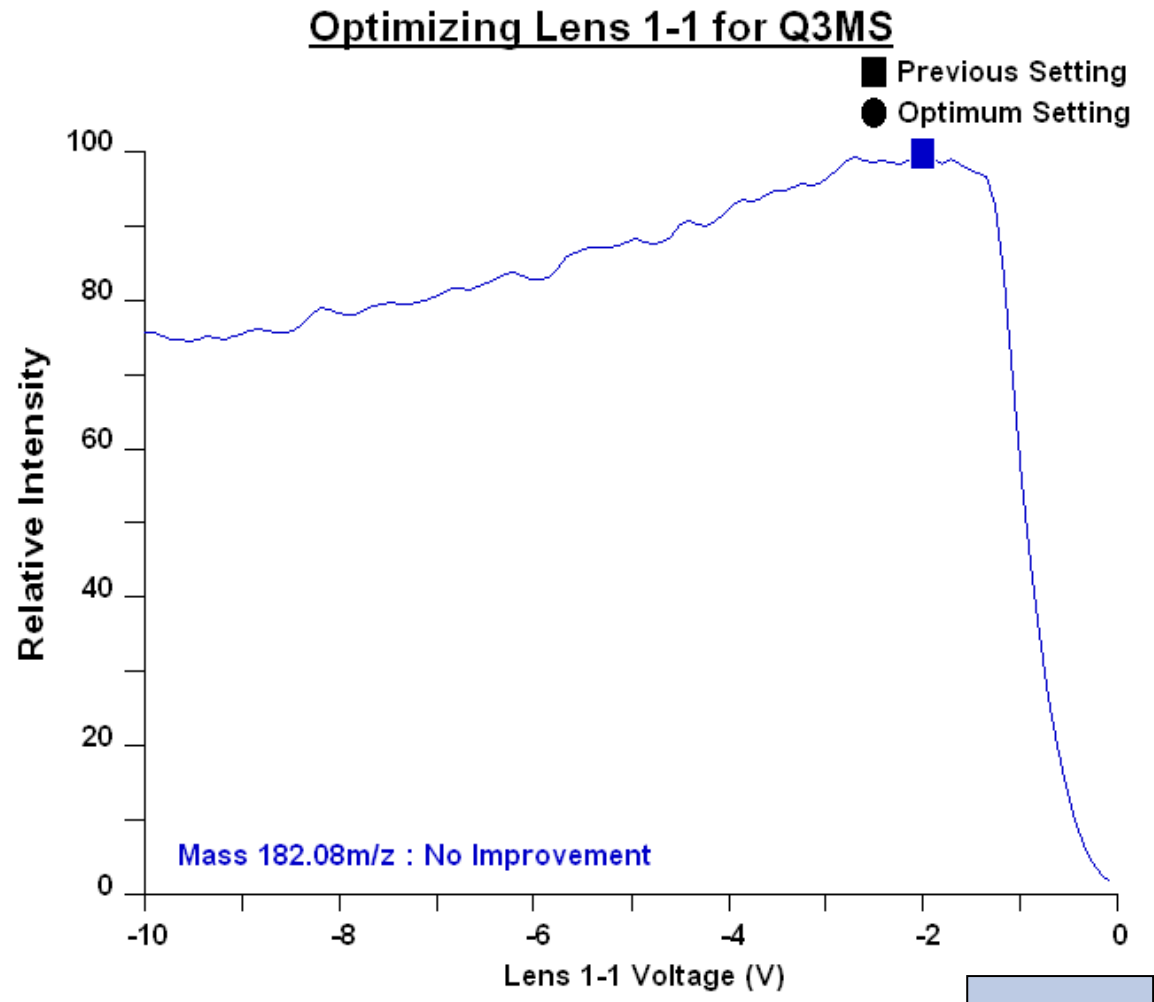
Auto Tune and Calibration

Peak Width vs. Resolution for Q1

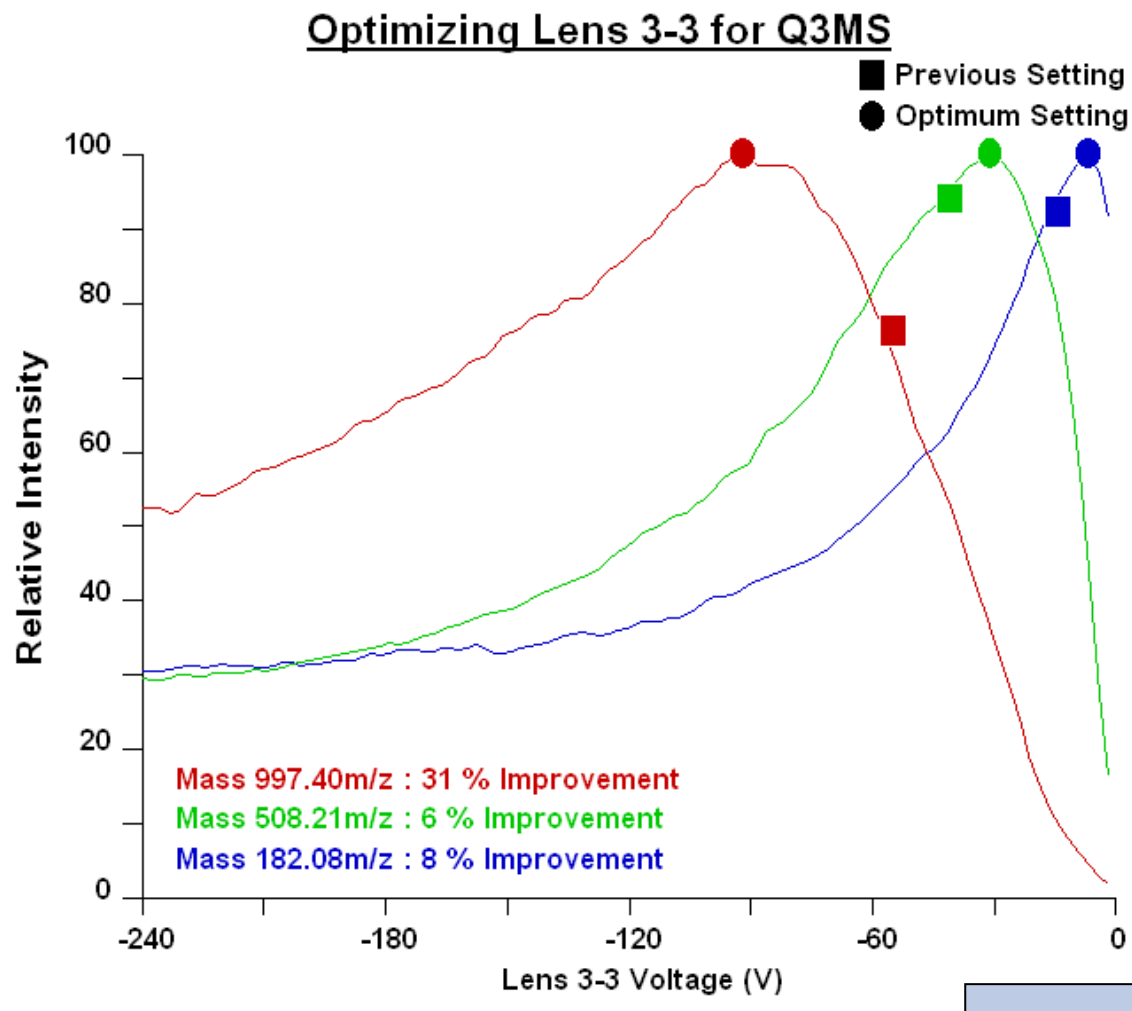


Auto Tune and Calibration

The process is repeated for Q3, one mass at a time, one parameter at a time

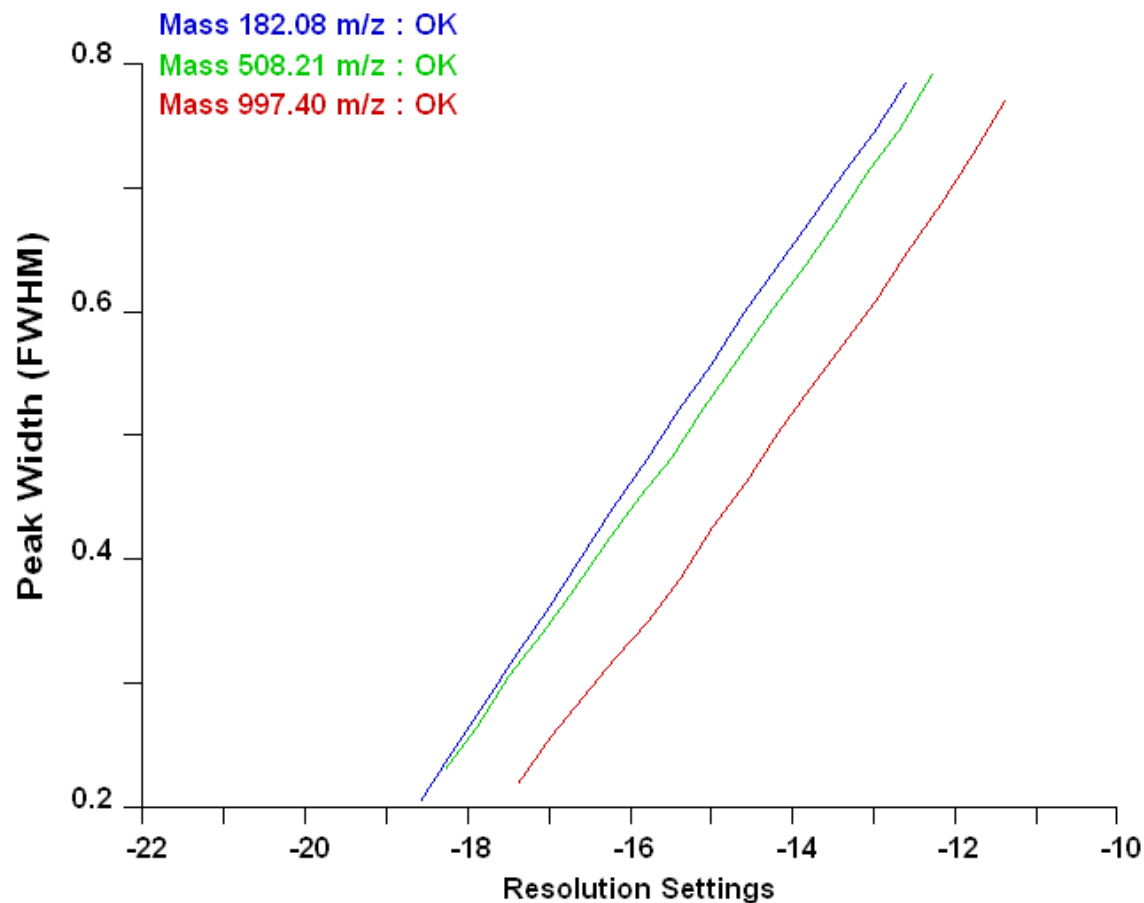


Auto Tune and Calibration



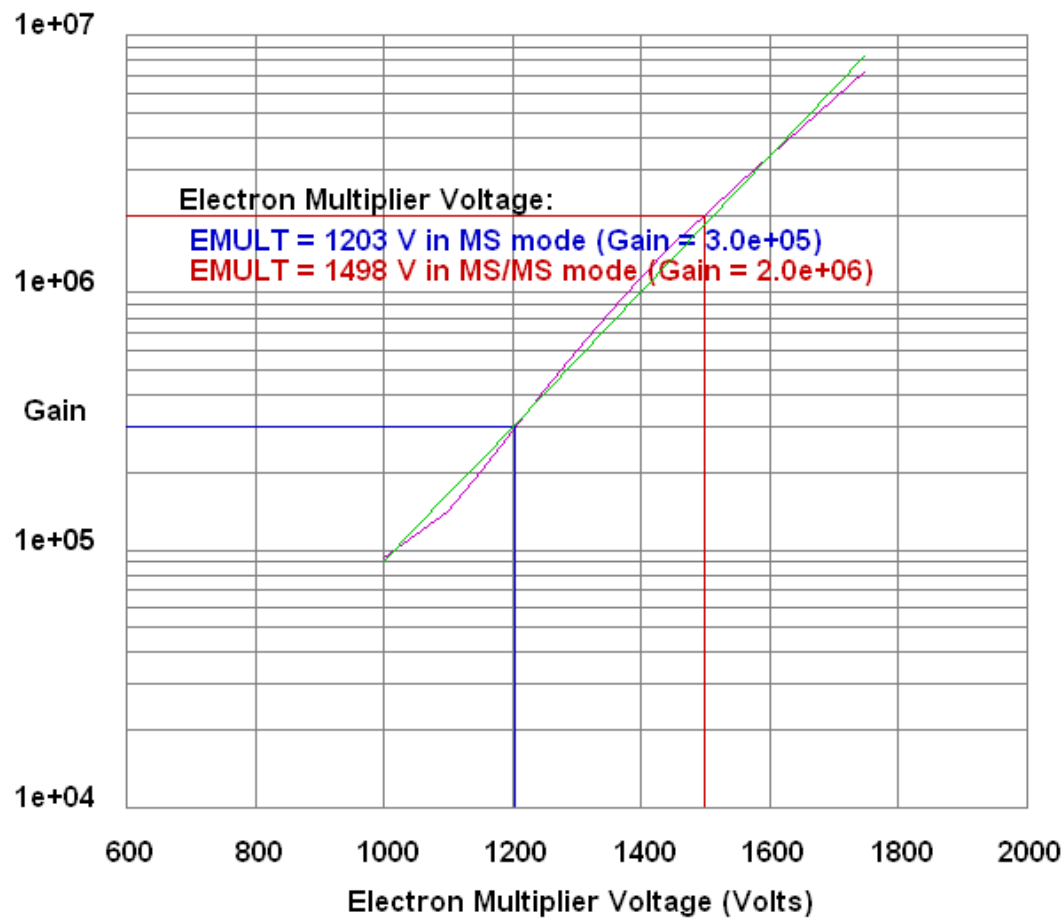
Auto Tune and Calibration

Peak Width vs. Resolution for Q3

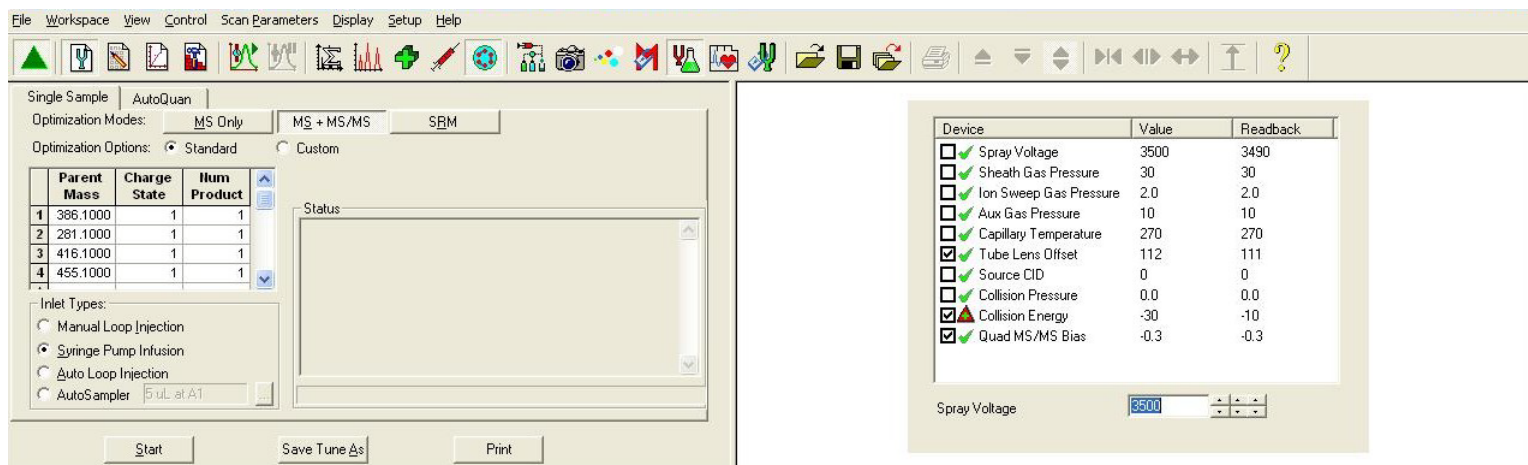


Gain Curve

Gain Curve @ m/z 997.4 (Positive Ion)



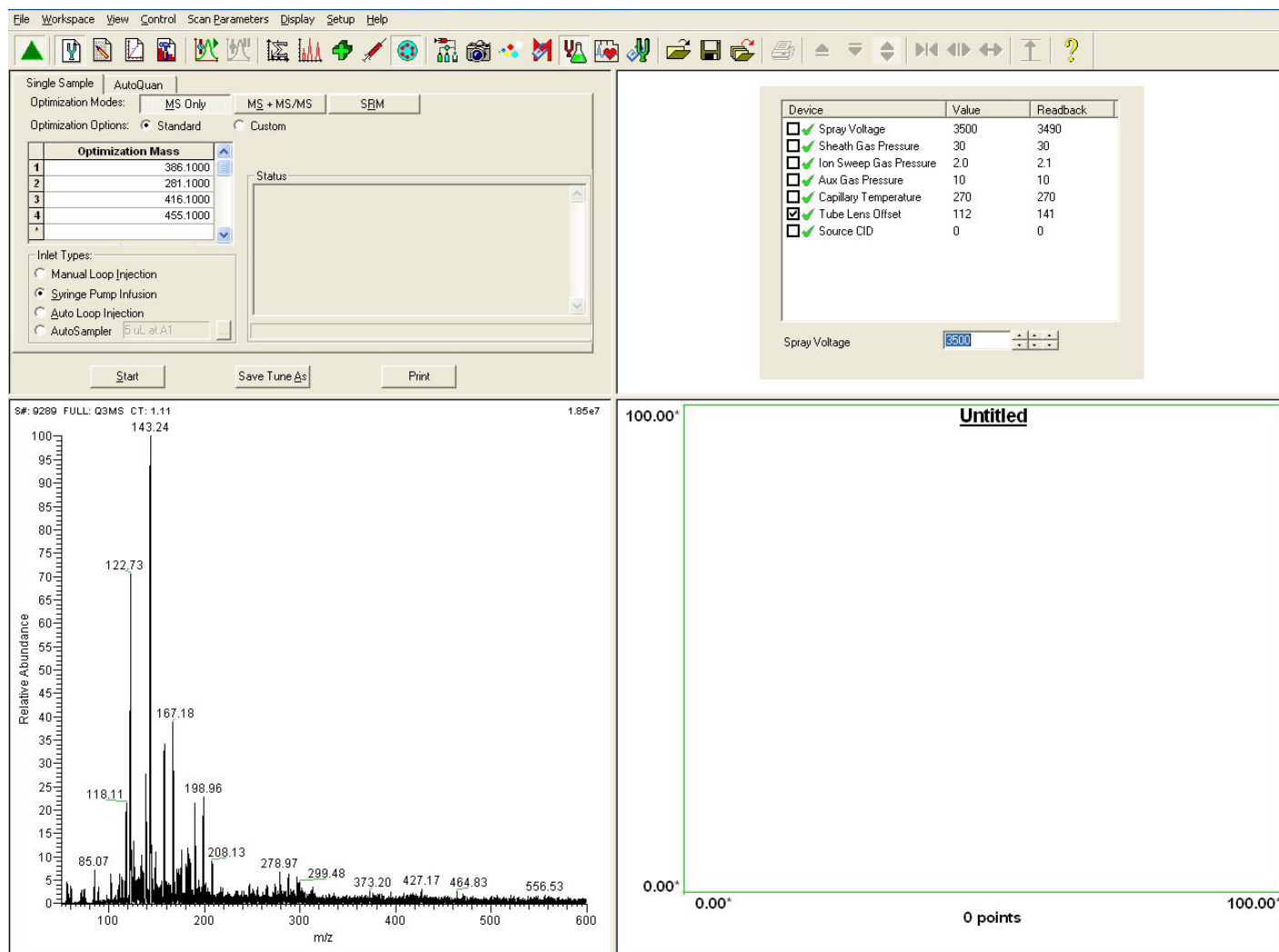
Automatic Compound Optimization



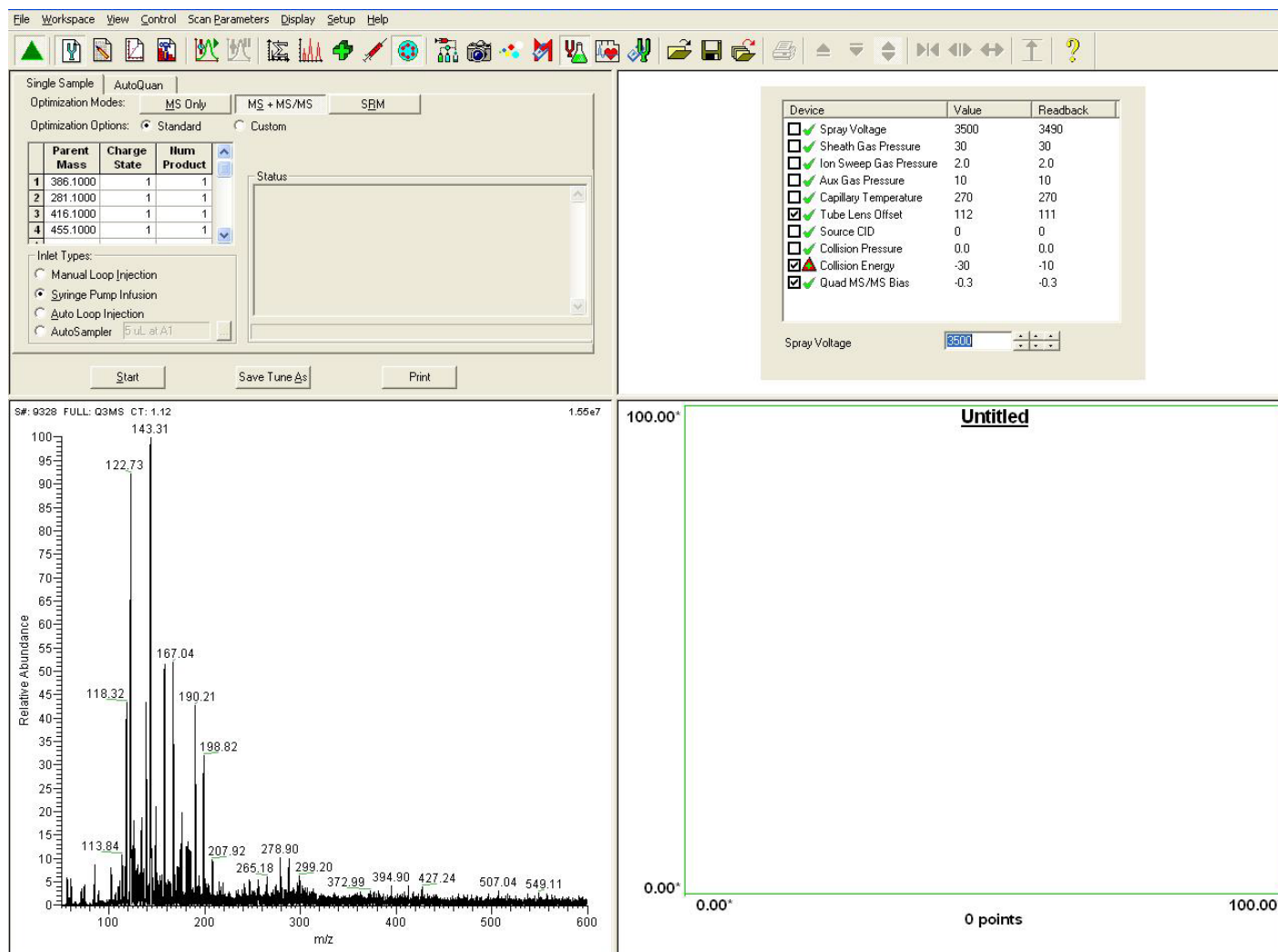
Optimization (a.k.a. “Compound tuning”) options:

- Tuning of all selected parameters for specified elements
- Auto optimization of collision energy for the specified number of product ions

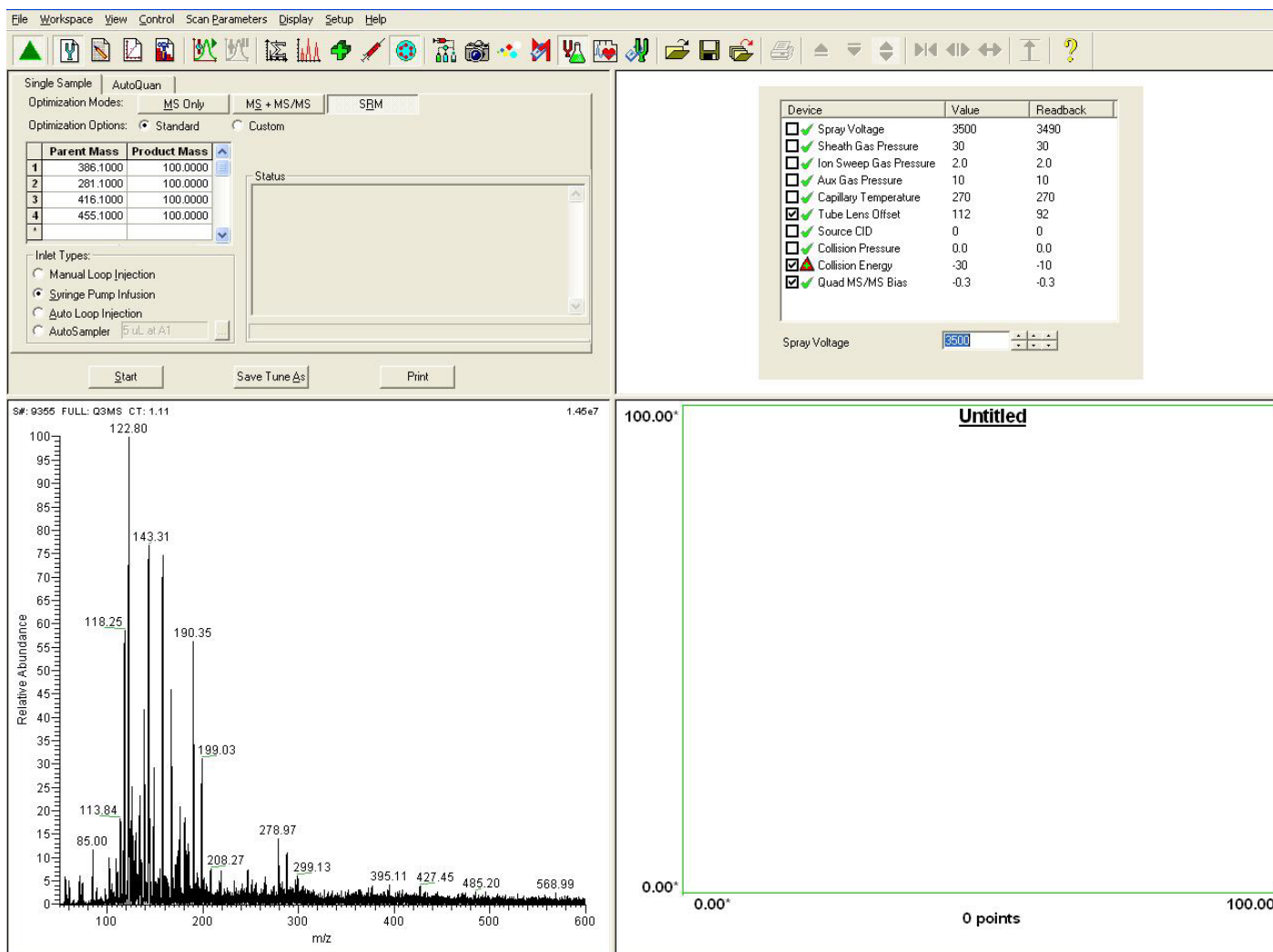
Automatic Compound Optimization - MS Only



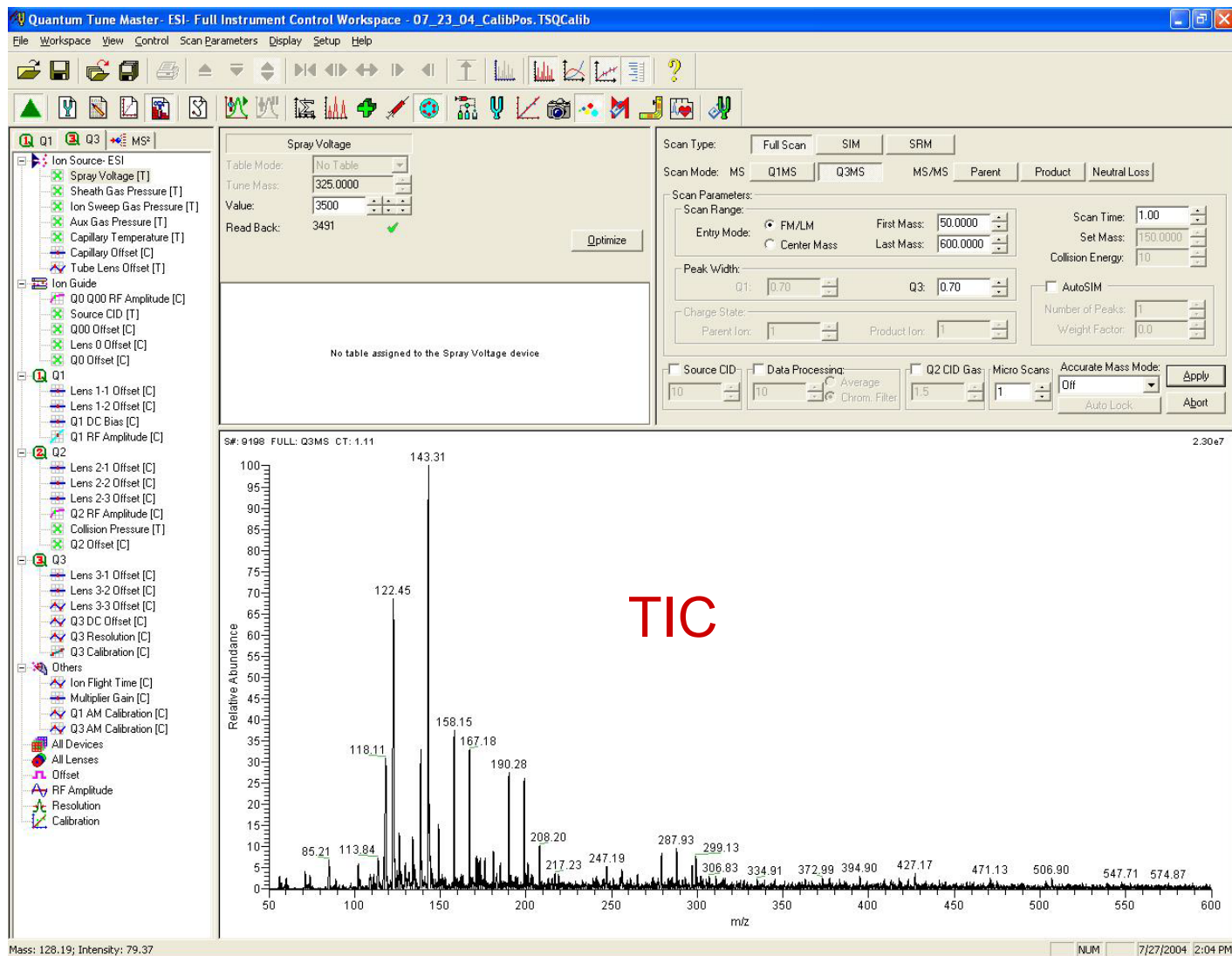
Automatic Compound Optimization - MS+MS/MS



Automatic Compound Optimization - SRM



Compound Optimization – Manual Procedure



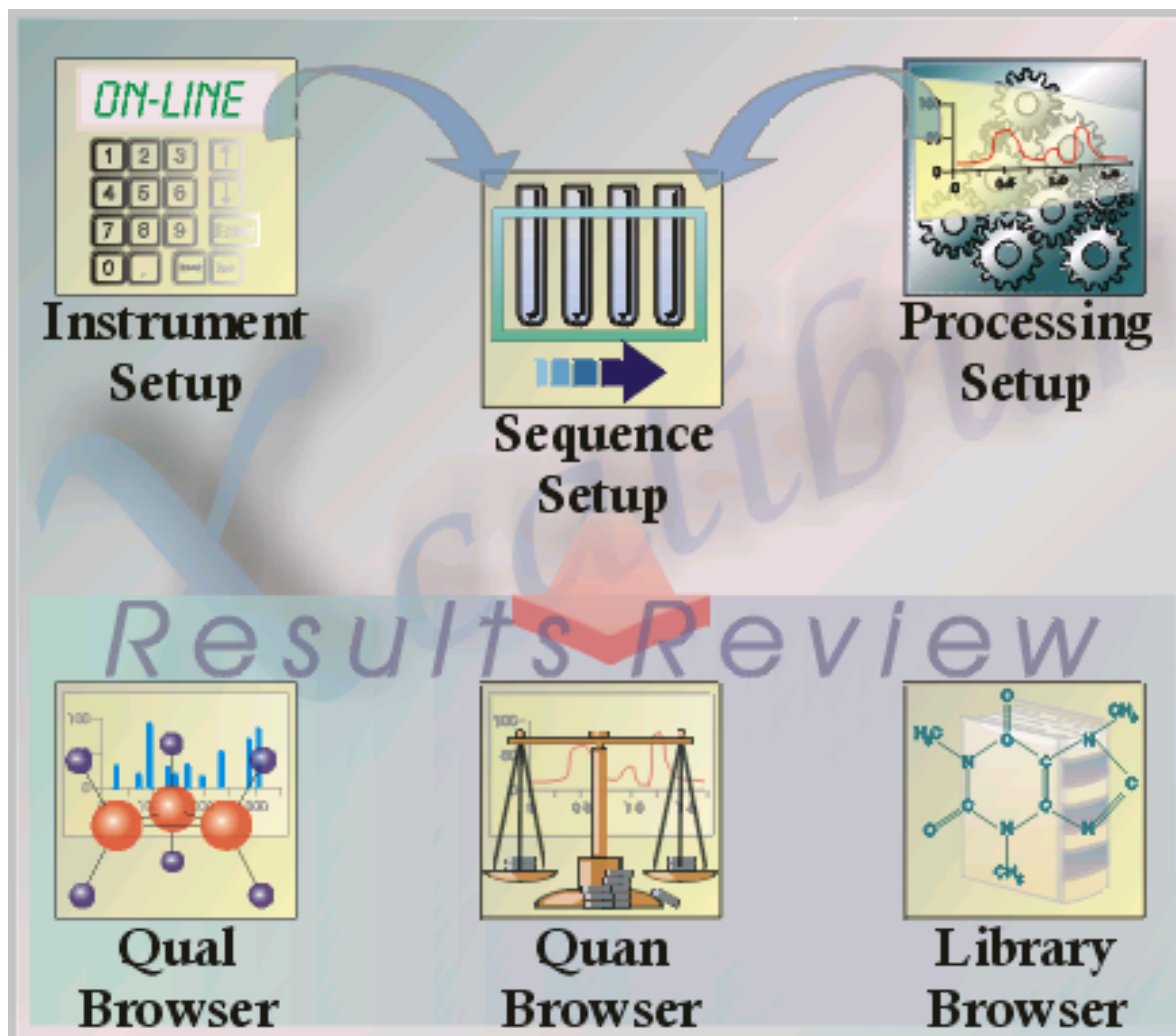


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Chapter 8

Xcalibur Instrument Configuration Method Setup

Xcalibur[®] 2.0



Thermo Software Standard

- TSQ Quantum Access / Discovery / Discovery MAX / Ultra / Ultra AM / EMR / Classic
- LCQ^{Fleet} / LCQ^{Advantage} / LCQ^{Advantage MAX} / LCQ^{Deca XP Plus} / LCQ^{Duo} / LCQ^{Deca} / LCQ^{Classic}
- LTQ / LTQ-FTMS / LTQ Orbitrap / LTQ Orbitrap Discovery / LTQ Orbitrap XL
- Tempus / PolarisQ (Polaris, GCQ)
- TraceDSQ
- TraceMS (Voyager, MD800)
- aQa (Navigator) / MSQ / MSQ+





Supported LC Peripherals

- Surveyor (*LC/MS/MS Plus pumps, AS/ASLite/AS Plus/AS Plus Lite, PDA/PDA Plus, UVvis 2000*)
- TSP (*P2000/P4000, AS1000/AS3000, UV2000/UV6000*)
- CTC Analytics (*PAL Autosampler*)
- Waters (*2690, 2695, 2795, 2487 UV*)
- HP/Agilent (*LC 1050/1090/1100, AS 1100, DAD 1100, VWD 1100*)
- Shimadzu (*LC-10Avp series*)
- Flux Instruments AG (*Rheos 2000/dual, IC8*)
- Dionex/LC Packings (*Ultimate*)
- Other Analog Devices

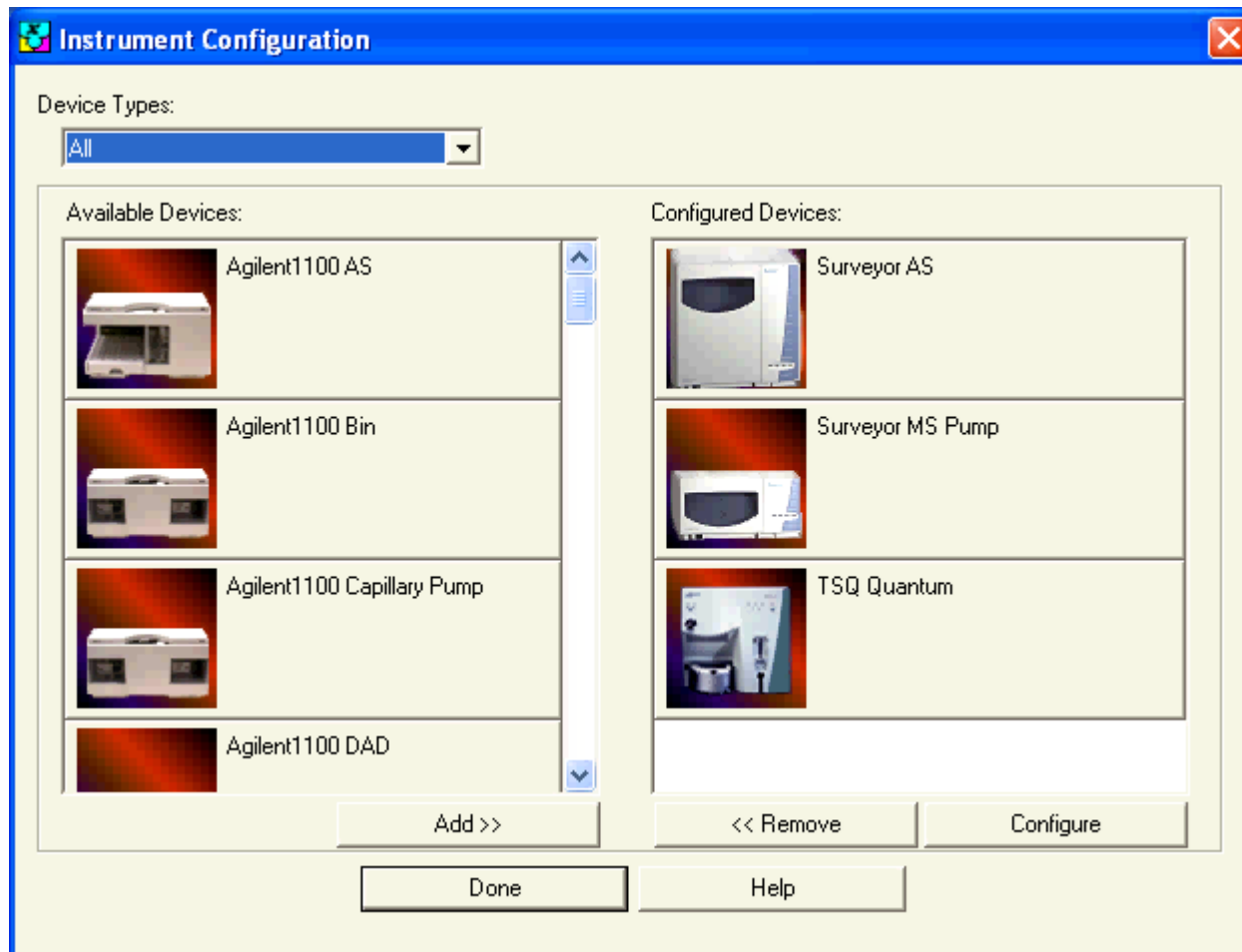
Xcalibur® File Types

.raw	Acquired data files
.sld	Sequence setup files
.pmd	Processing setup method
.meth	Instrument setup method
.rst	Result files from quantitation
.lyt	Qual browser layout
.lqn	LCquan files
.xqn	Quan browser files
.xrt	XReport files

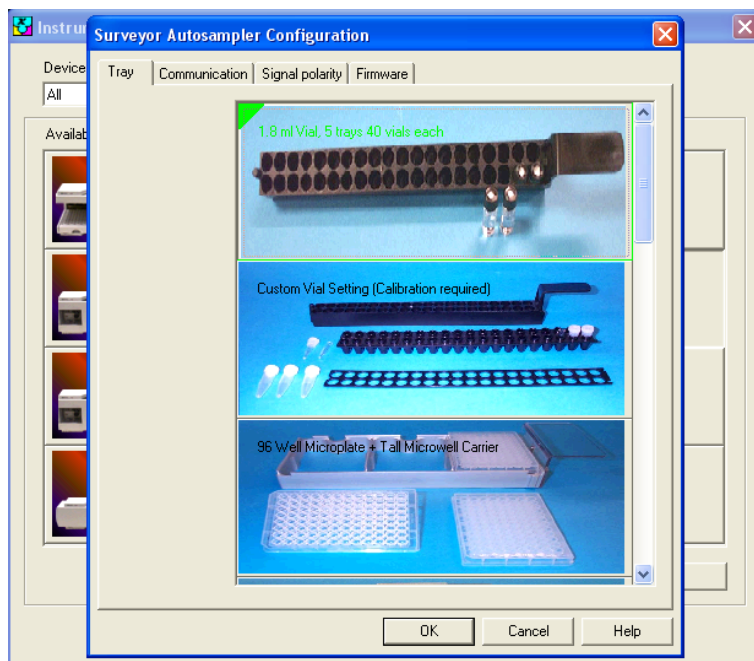
Instrument Configuration, Setup, and Control



Instrument Configuration

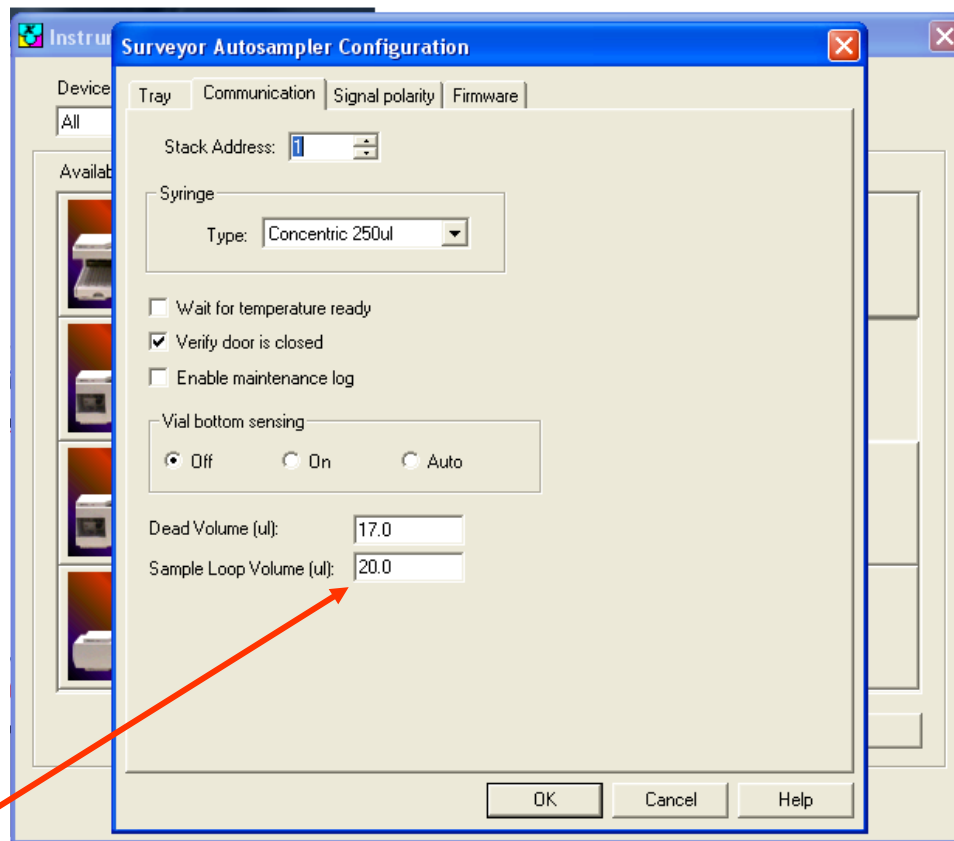


Autosampler Configuration



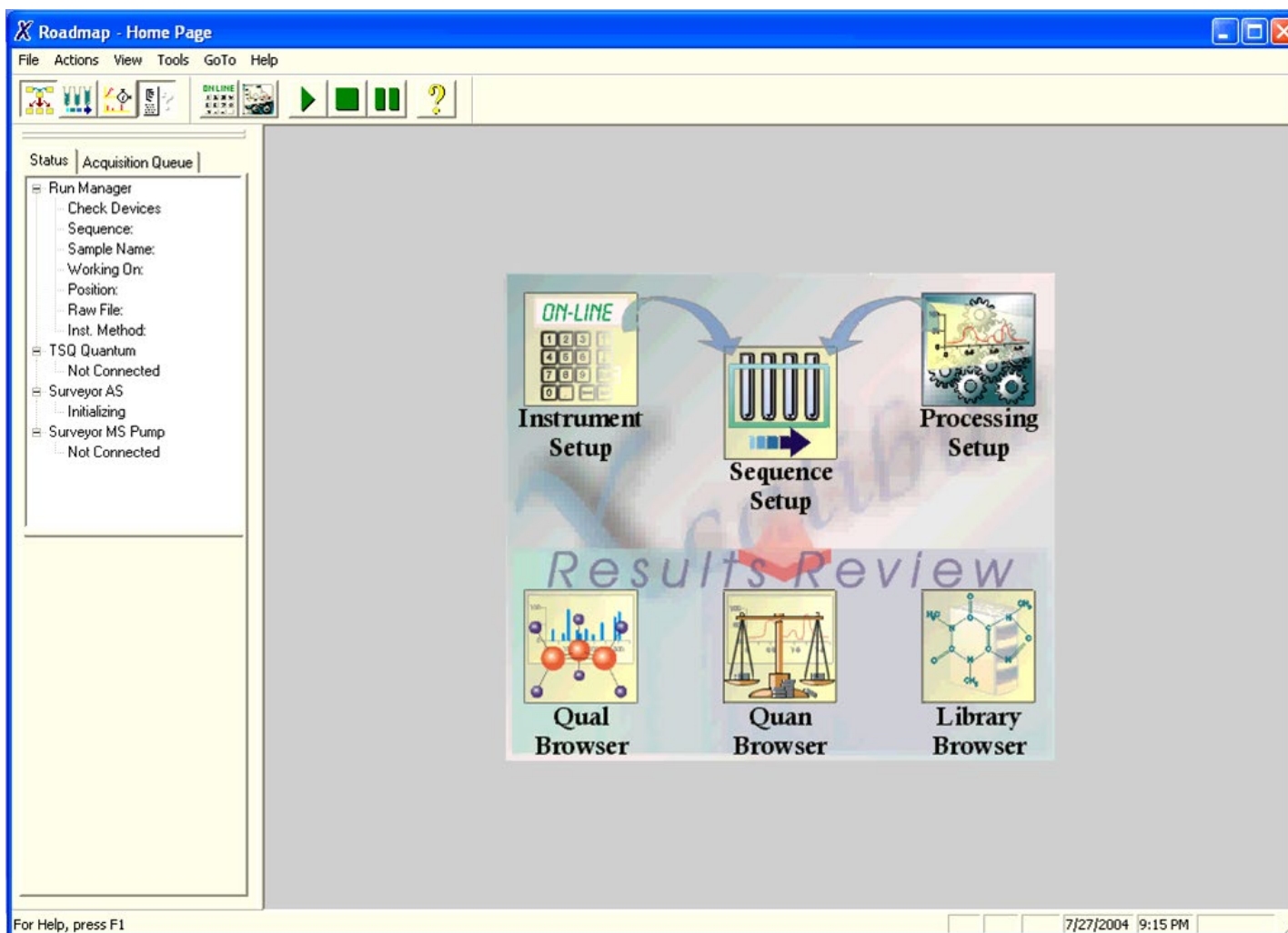
Autosampler configuration tab: Tray

Autosampler configuration tab: Communication

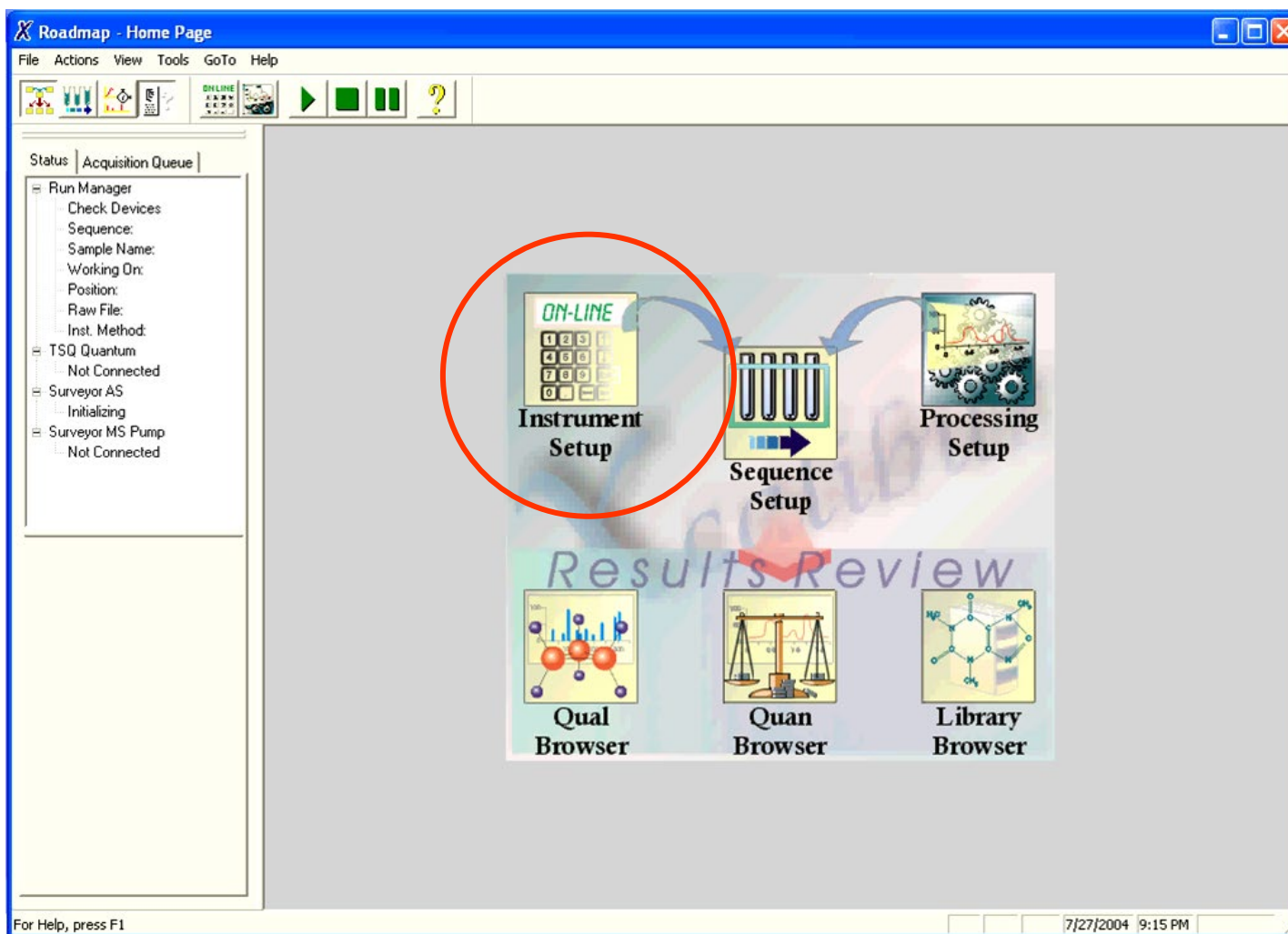


Sample Loop Volume must match loop installed for reproducible injections!!!

Homepage (Roadmap) – Status View



Instrument Setup



Instrument Setup – Surveyor MS Pump Plus

Untitled - Instrument Setup

File Surveyor MS Pump Plus Help

MS Pump

MS Pump

Comment:

Solvent A:

Solvent B:

Solvent C:

Solvent D:

Start settings: Surveyor AS injection logic

Method finalizing: First line conditions

Pumping efficiency (%): 100

Min pressure (bar): 0.0

Max pressure (bar): 400.0

Pressure stability (bar): 10.0

☐ Home before run

Pressure units: bar

Pumping efficiency (%) takes into account solvent compressibility (for common LC solvents this can be left at 100)

Min/Max pressure will stop the sequence if the pump pressure read-back goes below or above the specified values

Pressure stability dictates how stable the backpressure must be before an injection takes place

Instrument Setup – Surveyor MS Pump Plus

Untitled - Instrument Setup

File Surveyor MS Pump Plus Help

Agilent1100 AS

TSQ Quantum

Surveyor MS Pump Plus

Surveyor AS

Pump General Gradient Program

MS Pump

	Time	A%	B%	C%	D%	μl/min	P2
0	0.00	100.0	0.0	0.0	0.0	10.0	
1		100.0	0.0	0.0	0.0	10.0	

Solvent colors:

A B

C D

Type of view:

Solvent Gradient

Build the gradient profile here

Note: Flow is in $\mu\text{L}/\text{min}$ and there will always be an extra line that does not contribute to the pumps gradient logic

Instrument Setup – Surveyor Autosampler

Surveyor AS Method | Sample Preparation | Reservoir Content | Timed Events

Injection volume (ul): 20.0

Needle height from bottom (mm): 2.0

Syringe speed (ul/s): 8.0

Flush volume (ul): 500

Flush/Wash source: bottle

Wash volume (ul): 500

Flush speed (ul/s): 150.00

Post-injection valve switch time (min): 0.0

Loop loading speed (ul/s): 8.00

Injection Mode

- ☐ Partial loop
- ☒ Full loop
- ☐ No waste

Tray Temperature Control

☐ Enable tray temperature control

Temperature (°C): 0.0

Column Oven Control

☒ Enable column oven control

Temperature (°C): 40.0

Help

Instrument Setup – Surveyor Autosampler

Surveyor AS Method **Sample Preparation** Reservoir Content | Timed Events |

Prep Operations

- Deposit liquid in sample
- Deposit liquid in reservoir
- Draw from reservoir
- Draw from sample
- Flush to waste
- Mix at sample
- Mix at reservoir
- Transfer from reservoir to reservoir
- Transfer from reservoir to sample
- Transfer from sample to reservoir
- Transfer from sample to sample
- Wait time
- Wash needle

[Add To Task List >>](#)

Sample Location:

☒ Absolute location:

☐ Relative location:

Volume (ul):

Syringe speed (ul/s):

Needle height (mm):

Method

Sample Preparation

- ☒ Flush to waste
- ☒ Wash needle

[Remove Task](#) [Clear All Tasks](#)

File name: [Import](#)

[Help](#)

Instrument Setup – Surveyor Autosampler

Surveyor AS Method | Sample Preparation | **Reservoir Content** | Timed Events

Reservoir 1:

Reservoir 2:

Reservoir 3:

Reservoir 4:

Wash Bottle:

Instrument Setup – Surveyor Autosampler

Surveyor AS Method | Sample Preparation | Reservoir Content | **Timed Events**

	Time(min)	TF1	TF2	TF3	TF4
1	0.0	Off	Off	Off	Off
*	0.0	Off	Off	Off	Off

[Help](#)

Instrument Setup - Mass Spectrometer

Full Scan

Scan Editor | Syringe Pump | Divert Valve | Accurate Mass | Method Summary

Run Settings

MS Acquire Time (min): 17.00 Segments: 1 Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1

Segment 1 Settings

Segment Time (min): 17.00 Tune Method: C:\Xcalibur\Patrick\Methods\Training Methods\7_07_04_Full_Scan.TSQ Tune

Scan Events: 1 Chrom Filter Peak Width (s): 10 Collision Gas Pressure (mTorr): 0.8

Current Scan Event: 1 Scan Event 1

Scan Event 1

Full Scan | SIM | SRM

Scan Modes

MS Mode: **Q1MS** Q3MS MS/MS Mode: Parent Product Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 100.000 Last Mass (m/z): 600.000

Scan Time (s): 0.50 Q1 Peak Width (FWHM): 0.70

Set Mass (m/z): 1000.000 Q3 Peak Width (FWHM): 0.70

Collision Energy (V): 10

Polarity: ☒ Positive ☐ Negative

Data Type: ☐ Centroid ☒ Profile

Source CID: Collision Energy (V): 3

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Instrument Setup - Mass Spectrometer - SIM

Scan Editor | Syringe Pump | Divert Valve | Accurate Mass | Method Summary

Run Settings

MS Acquire Time (min): 17.00 Segments: 2 Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1 Segment 2

0 1 2 3 4 5 6 7 Retention Time (min) 0 11 12 13 14 15 16 17

Segment 1 Settings

Segment Time (min): 6.50 Tune Method: C:\Xcalibur\Patrick\Methods\Training Methods\7_07_04_Full_Scan.TSQ Tune

Scan Events: 1 Chrom Filter Peak Width (s): ☒ 6 Collision Gas Pressure (mTorr): ☐ 0.8

Current Scan Event: 1

Scan Event 1

Full Scan | **SIM** | SRM

Scan Mode

MS Mode: ☒ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☐ Neutral Loss

Same value for all SIMs

Scan Width (m/z): ☒ 1.000

Scan Time (s): ☐ 1.00

Set Mass (m/z): 1000.000

Coll. Energy (V): 10

Peak Width

Q1 (FwHM): ☒ 0.70

Q3 (FwHM): 0.70

Use tuned tube lens val. ☒ AutoLock On ☐

	Center Mass	Scan Time
1	386.210	1.00
*	386.210	1.00

Polarity: ☒ Positive ☐ Negative

Data Type: ☒ Centroid ☐ Profile

Source CID:

Collision Energy (V): ☐ 3

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Instrument Setup - Mass Spectrometer - SRM

Scan Editor | Syringe Pump | Divert Valve | Accurate Mass | Method Summary

Run Settings
MS Acquire Time (min): 10.00 Segments: 3 Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

0 1 2 3 4 5 6 7 8 9 10
Retention Time (min)

Segment 1 2 Segment 3

Segment 1 Settings
Segment Time (min): 3.80 Tune Method: C:\Xcalibur\Patrick\Methods\Training Methods\7_07_04_Full_Scan.TSQ Tune
Scan Events: 1 Chrom Filter Peak Width (s): 6 Collision Gas Pressure (mTorr): 0.8
Current Scan Event: 1 Scan Event 1

Scan Event 1
Full Scan | SIM | **SRM**

Same value for all SRMs
Scan Width (m/z): 1.000
Scan Time (s): 1.00
Coll. Energy (V): 10
Peak Width
Q1 (FWHM): 0.70
Q3 (FWHM): 0.70
Use Tuned Tube Lens Value: ☒

	Parent Mass	Product Mass	Scan Time	Collision E
1	386.210	122.180	1.00	42
*	386.210	122.180	1.00	42

Polarity: ☒ Positive ☐ Negative
Data Type: ☒ Centroid ☐ Profile
Source CID:
Collision Energy (V): 3
Accurate Mass Mode: Off
Micro Scans: 1
Copy ScanEvent Paste ScanEvent
Help Tune

Instrument Setup – MS - Data-Dependent Scan

Scan Editor | Syringe Pump | Divert Valve | Method Summary

Calibration Correction Method ☐

Run Settings
MS Acquire Time (min): 10.00 Segments: 1 Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1

Segment 1 Settings
Segment Time (min): 10.00 Tune Method: C:\Xcalibur\Methods\ex.TSQ Tune
Scan Events: 2 Chrom Filter Peak Width (s): 10 Collision Gas Pressure (mTorr): 1.0
Current Scan Event: 2 Scan Event 1 Scan Event 2

Scan Event 2
Full Scan | SIM | SRM | **Dependent Scan** | AutoSIM

Scan Selection
Mass determined from scan event: 1 From Scan ☒ From Parent List ☐
Nth Most Intense Ion: 1 ☐ If no acceptable parent found convert to most intense from scan
Signal Threshold (10⁴ counts): 10.0 Weighting Factor: 0.0

Scan Parameters
Scan Time (s): 1.000 Collision Energy (V): 10 Q1 Peak Width (FWHM): 0.70
Charge State: 1 CE grad(V per m/z): 0.0000 Q3 Peak Width (FWHM): 0.70
Source Delta(m/z): 1.000 DD Delta(m/z): 1.000 Energy Ramp (V): 0

Advanced Data Dependent Settings And Activation
☐ Dynamic Exclusion ☐ Isotope Ratio ☐ Specify mass lists in sequence row (Global Setting) Advanced Settings...

Polarity: ☒ Positive ☐ Negative
Data Type: ☒ Centroid ☐ Profile
Skimmer Offset: ☐ Skimmer Offset (V): 10
Micro Scans: 1
Copy ScanEvent Paste ScanEvent
Help Tune

Instrument Setup - Mass Spectrometer

Scan Editor **Syringe Pump** | Divert Valve | Accurate Mass | Method Summary

Syringe Pump Settings

☐ Use Syringe Pump

Syringe Type

☐ Hamilton Volume (μL): 500

☒ Urimetrics Syringe ID (mm): 3.260

☐ Other

Flow Rate (μL/min): 5.00

☐ Stop Syringe Pump at End of Run

Syringe Pump Settings for Segments

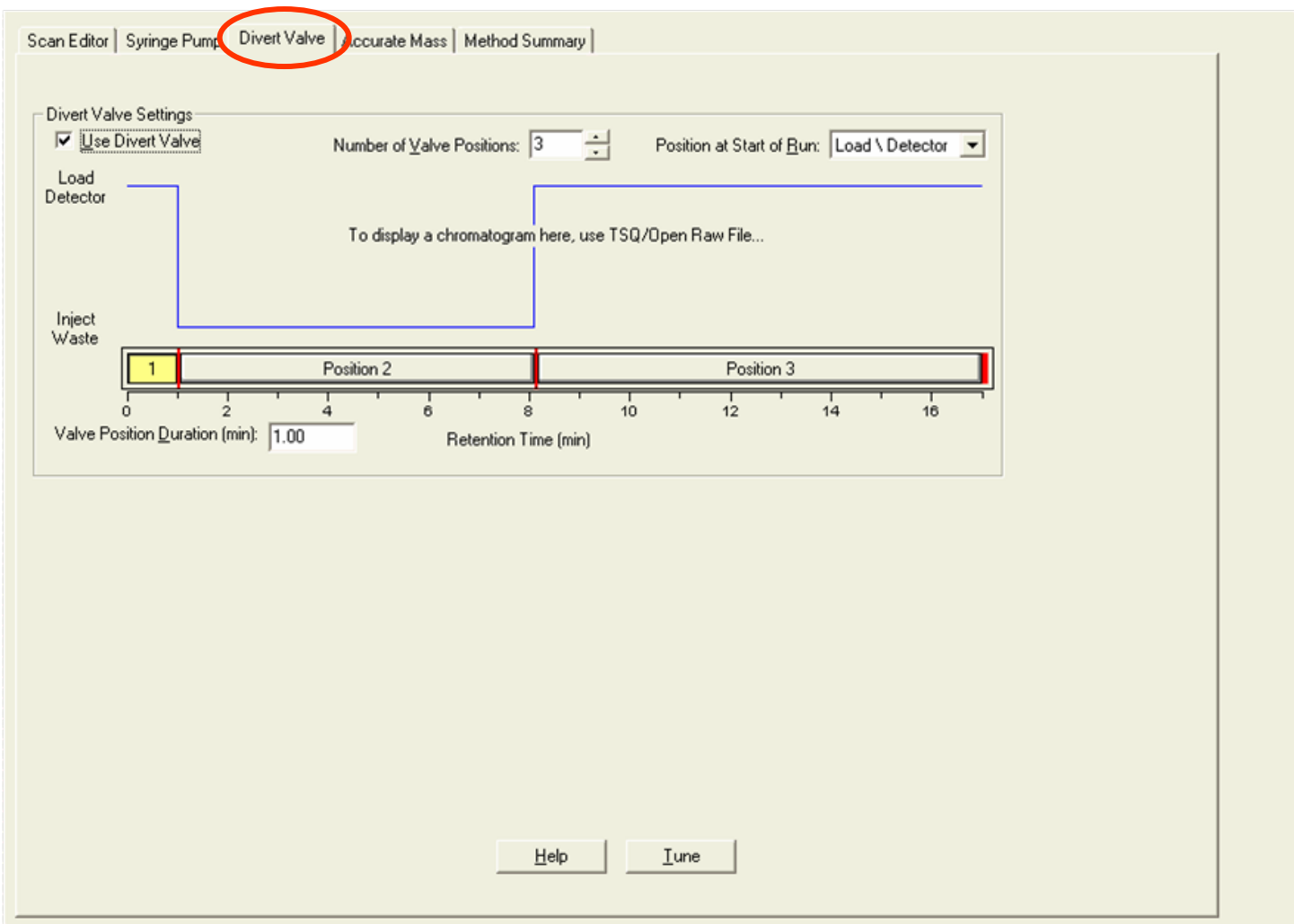
Segment 1

☐ On ☐ Off

Check All Uncheck All

Help Tune

Instrument Setup - Mass Spectrometer



Instrument Setup - Mass Spectrometer

Scan Editor | Syringe Pump | Divert Valve | Accurate Mass | **Method Summary**

Creator: patrick.jeanville Last modified: 7/27/2004 by patrick.jeanville

MS Run Time (min): 17.00

TSQ MS Method Settings:

Segment	1
Duration (min)	17.00
Scan Events	2

Segment 1:

Tune Method C:\Xcalibur\Patrick\Methods\Training Methods\7_07_04_Full_Scan.TSQTune

Chrom filter: Not used

Q2 Gas Pressure: 0.8

Syringe Pump: Off

Data Dependent Parent Mass List: (none)

Data Dependent Reject Mass List: (none)

Scan Events:

1: + p Full Q3MS, Accurate Mass Off, Micro Scans 1,
Scan Time 0.30, Q3 PW 0.70, [100.000-600.000]

2: + p Data Dep. Most intense ion from scan 1, Min. Signal Required 20000.0,
Weighting Factor 0.0, Accurate Mass Off, Scan Time 0.70, Collision Energy 30,
Collision Energy Gradient 0.1000, Default Charge State 1, Source Delta 1.000,
Data Dependent Delta 1.000, Q1 PW 0.70, Q3 PW 0.70, Dynamic Exclusion not enabled,
Isotopic Ratios not enabled

Global Data Dependent Settings:

No override of Data Dependent Parent and Reject masses and AutoSIM Target and
reject masses allowed via user columns in sequence

Dynamic Exclusion not in use

Syringe pump not in use

Divert Valve: in use during run

Divert Time (min)	Valve State
=====	=====

Help Tune



The world leader in serving science

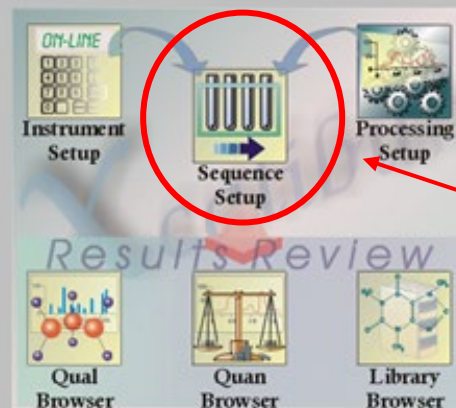
Chapter 9

Setting Up and Running Sequences

Xcalibur Home Page Sequence Setup

To open
Sequence
Setup, can
click View >
Sequence
Setup View

- ✓ Roadmap View
- Sequence Setup View
- Real Time Plot View
- ✓ Info View
- ✓ View Toolbar
- ✓ Roadmap Toolbar
- Sequence Editor Toolbar
- Plot Toolbar
- ✓ Show Large Toolbar
- Customize Toolbars...



Can also click on
Sequence Setup button

Creating a Sequence

If you have a small number of samples to run, it is easiest to create the sequence from the Sequence Setup Home Page

	File Name	Path	Inst Meth	Position	Inj Vol
1	steroids	C:\Vcalibur\data	C:\Vcalibur\TRAINING 01_2007\ESI_SRM_Jan_07	CSik1-01:1	10.000
*					0.000

**Minimum Information Required to Run the Sequence:
File Name, Path, Inst Meth, Position, Inj Vol**

Creating a Sequence

[Open] - Sequence Setup - Home Page

File Edit Change Actions View GoTo Help

Hotkey F2 puts the cursor in the boxes and makes the fields editable. Hitting F2 twice opens up a text box for editing

	File Name	Path	Inst Meth	Position	Inj Vol
1	steroids	C:\Vcalibur\data	C:\Vcalibur\TRAINING 01_2007\ESI_SPM_Jan_07	CSik1-01:1	10.000
					0.000

Browse
Open File
Paste Cells...
Insert Row...

To open the Inst Meth from the sequence, right-click and select Open File

Run Manager

- Ready To Download
- Sequence:
- Sample Name:
- Working On:
- Position:
- Raw File:
- Inst. Method:

TSQ Quantum

- Ready to Download

Surveyor MS Pump Plus

- Ready to Download

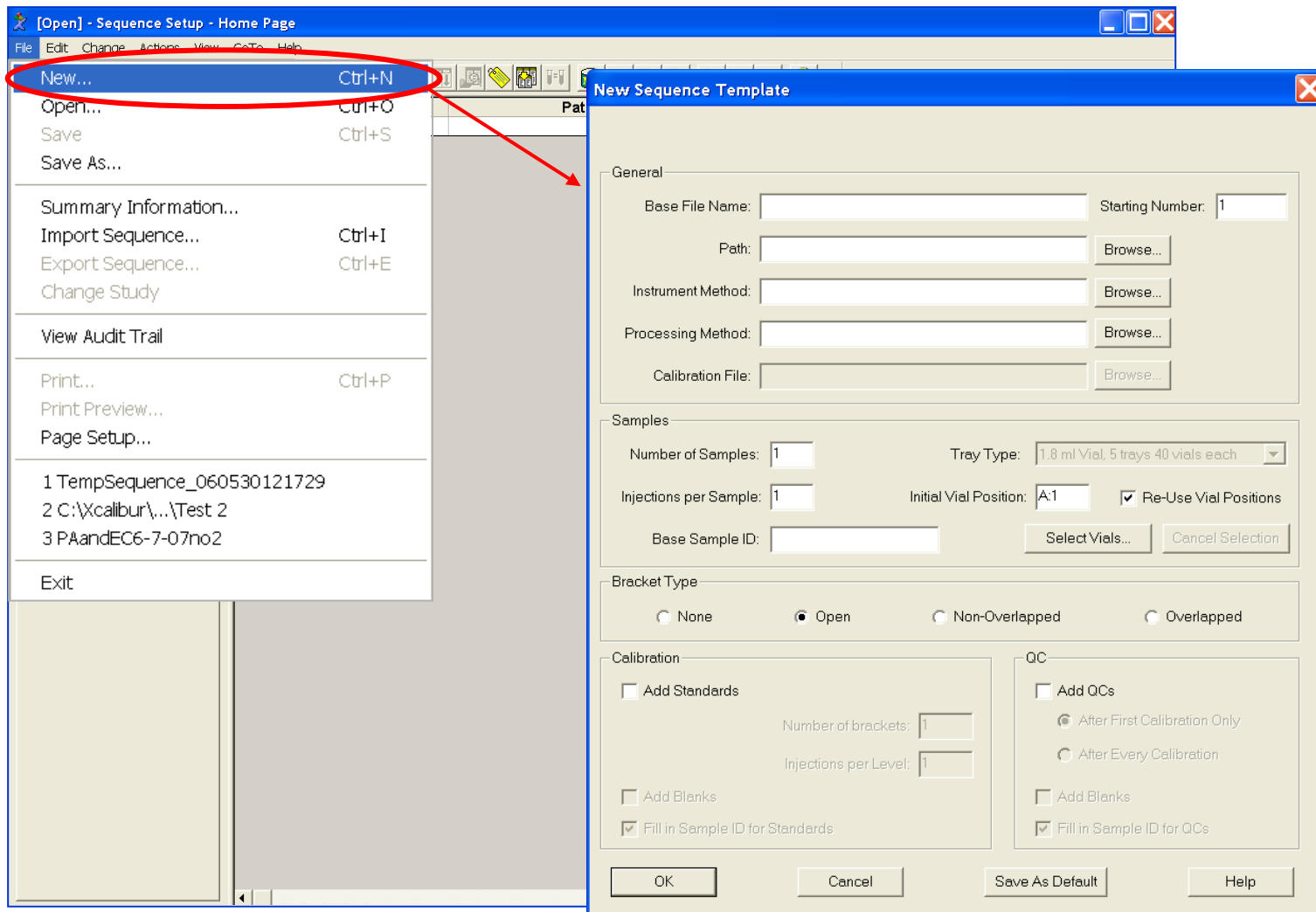
Thermo Pal

- Ready to Download

Creating a Sequence Using the New Sequence Template

If you have a larger number of samples to run, it is easier to use the New Sequence Template to create the sequence

1. Click
New



New Sequence Template

1. Choose a Base File Name, Path, & Instrument Method

2. Enter the number of unknown samples

3. Select the Initial Vial Position

4. If you already have a Processing Method, specify it (above) and you can Add Standards, Blanks and QCs. The sequence will be populated with these rows as established in the processing method.

The screenshot shows the 'New Sequence Template' dialog box with the following fields and options:

- General:**
 - Base File Name: Steroids
 - Path: C:\XCALIBUR\DATA\
 - Instrument Method: C:\Xcalibur\methods\Test
 - Processing Method: (empty)
 - Calibration File: (empty)
 - Starting Number: 1
- Samples:**
 - Number of Samples: 1
 - Tray Type: 1.8 ml Vial, 5 trays 40 vials each
 - Injections per Sample: 1
 - Initial Vial Position: A:1
 - Re-Use Vial Positions: ☒
 - Base Sample ID: (empty)
 - Select Vials... (button)
 - Cancel Selection (button)
- Bracket Type:**
 - None (radio button)
 - Open (radio button, selected)
 - Non-Overlapped (radio button)
 - Overlapped (radio button)
- Calibration:**
 - Add Standards: ☐
 - Number of brackets: 1
 - Injections per Level: 1
 - Add Blanks: ☐
 - Fill in Sample ID for Standards: ☒
- QC:**
 - Add QCs: ☐
 - After First Calibration Only: ☒
 - After Every Calibration: ☐
 - Add Blanks: ☐
 - Fill in Sample ID for QCs: ☒

Buttons at the bottom: OK, Cancel, Save As Default, Help.

Open Bracket

Standard 1

Standard 2

Standard 3

Unknown 1

Standard 4

Standard 5

Standard 6

Standard 7

Standard 8

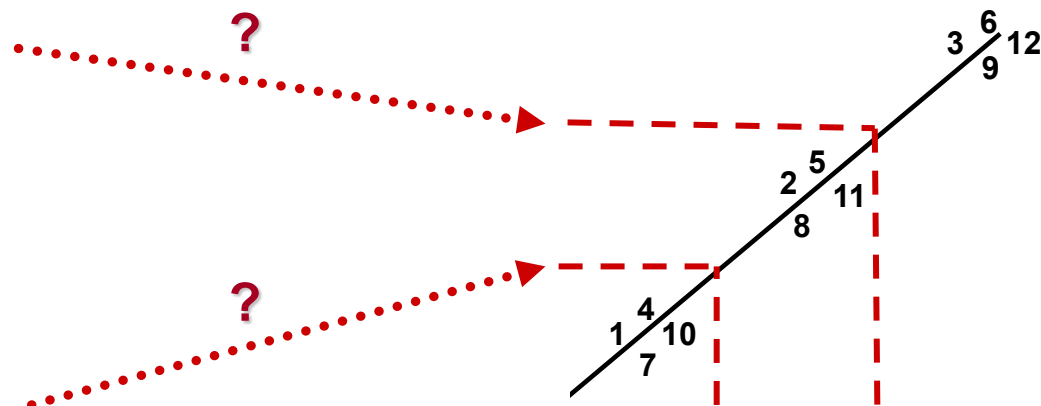
Standard 9

Unknown 2

Standard 10

Standard 11

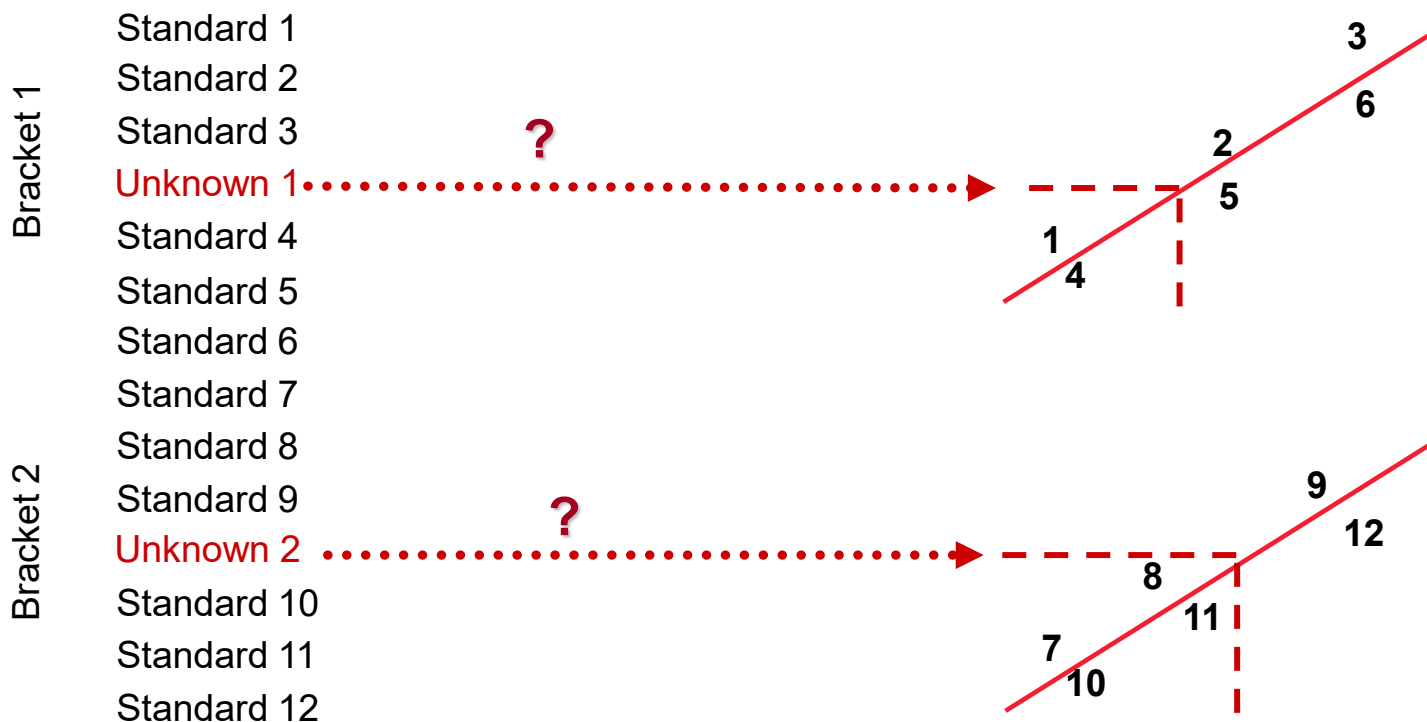
Standard 12



One calibration curve for all unknowns (**1** and **2**)

All standards are equally involved in interpolation

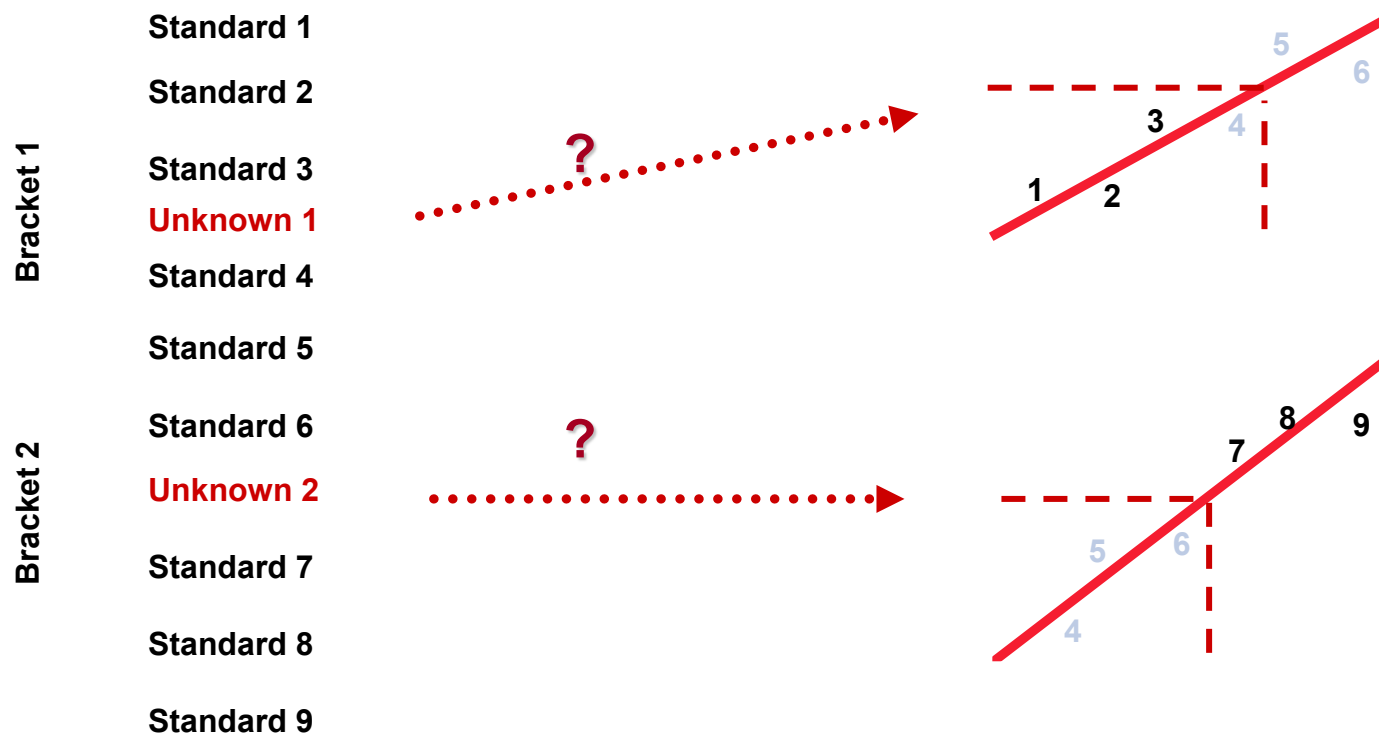
Non-Overlapping Bracket



Separate calibration curves for each unknown (**1** or **2**) (or group of unknowns)

Separate sets of standards are used, for each concentration range (i.e., low, high)

Overlapping Bracket

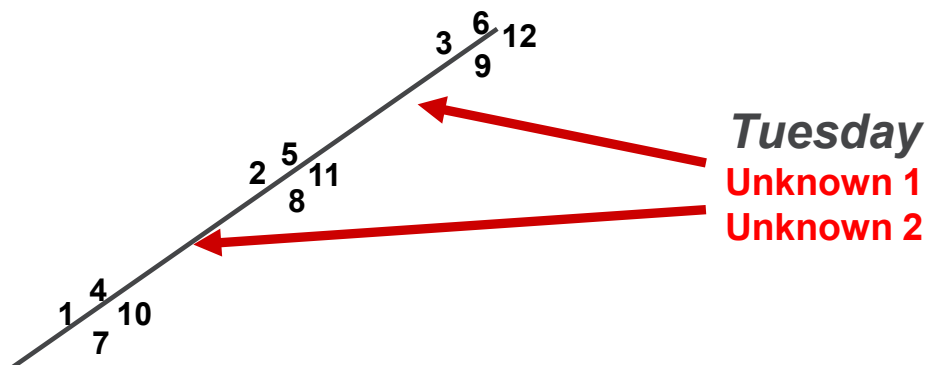


Separate calibration curves for each unknown (**1** or **2**) (or group of unknowns)

Separate sets of standards are generally used, for each concentration range (i.e., low, high), but they contain a common subset of standards (4, 5, 6)

Monday

Standard 1
Standard 2
Standard 3
Standard 4
Standard 5
Standard 6
Standard 7
Standard 8
Standard 9
Standard 10
Standard 11
Standard 12



Two types of designated standards: **Standard Clear** (kept from a previous experimental batch)

Standard Update (newly acquired and substituted in the series of standards)

New Sequence Template

[Open] - Sequence Setup - Home Page

File Edit Change Actions View GoTo Help

Run Manager

- Ready To Download
- Sequence:
- Sample Name:
- Working Dir:
- Position:
- Raw File:
- Inst. Method:

TSQ Quantum

- Ready to Download

Surveyor MS Pump Plus

- Ready to Download

Thermo Pal

- Ready to Download

	Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol	Level
1	Unknown	Steroids01	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
2	Unknown	Steroids02	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
3	Unknown	Steroids03	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
4	Unknown	Steroids04	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
5	Unknown	Steroids05	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
6	Unknown	Steroids06	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
7	Unknown	Steroids07	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
8	Unknown	Steroids08	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
9	Unknown	Steroids09	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
10	Unknown	Steroids10	Tray01:1	C:\Vcalibur\data\	C:\Vcalibur\TRAINI		Tray01:1	10.000	
*	Unknown							0.000	

Once, you click OK on the New Sequence Template, the File Name is automatically incremented starting with the Base File Name you specified

New Sequence Template

If you want to type a new File Name:

2. Select Edit
and click Fill
Down

1. Type File name

The screenshot shows the 'Sequence Setup - Home Page' window. The 'Edit' menu is open, and the 'Fill Down...' option is highlighted with a red oval. A red arrow points from the '1. Type File name' box to the 'Steroids_New_01' entry in the 'File Name' column of the table.

	File Name	Path	Inst Meth	Position	Inj Vol
1	Steroids_New_01	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.1	10.0
2	Steroids02	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.2	10.0
3	Steroids03	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.3	10.0
4	Steroids04	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.4	10.0
5	Steroids05	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.5	10.0
6	Steroids06	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.6	10.0
7	Steroids07	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.7	10.0
8	Steroids08	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.8	10.0
9	Steroids09	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.9	10.0
10	Steroids10	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.10	10.0
*					0.1

Changing the Sequence Column Arrangement

1. Select Change and click Column Arrangement

User Labels...
Tray Name...
Column Arrangement...
Transfer Row Info...

2. Select which columns to add from the available columns

Column Arrangement

Available Columns

Client
Comment
Company
Dil Factor
ISTD Corr Amt
Laboratory
Level
Phone
Proc Meth
Sample ID
Sample Type
Sample Vol

3. Click Add

Add

Remove

Move Up

Move Down

Displayed Columns

File Name
Path
Inst Meth
Position
Inj Vol

4. Can also change the order by clicking Move Up or Down

OK

Cancel

Help

Changing the User Labels

1. Select Change and click User Labels

User Labels...

Tray Name...

Column Arrangement...

Transfer Row Info...

2. Modify labels and the new labels will be incorporated as column headings in the sequence

User Labels

Heading 1 Study

Heading 2 Client

Heading 3 Laboratory

Heading 4 Company

Heading 5 Phone

Default Headings

OK

Cancel

Help

Changing the Tray Name

1. Select Change and click Tray Name

2. Select tray to use

Tray Selection

Select Tray Type with which to validate vial position in the sequence.

96 Well Microplate + Tall Microwell Carrier

OK Cancel Help

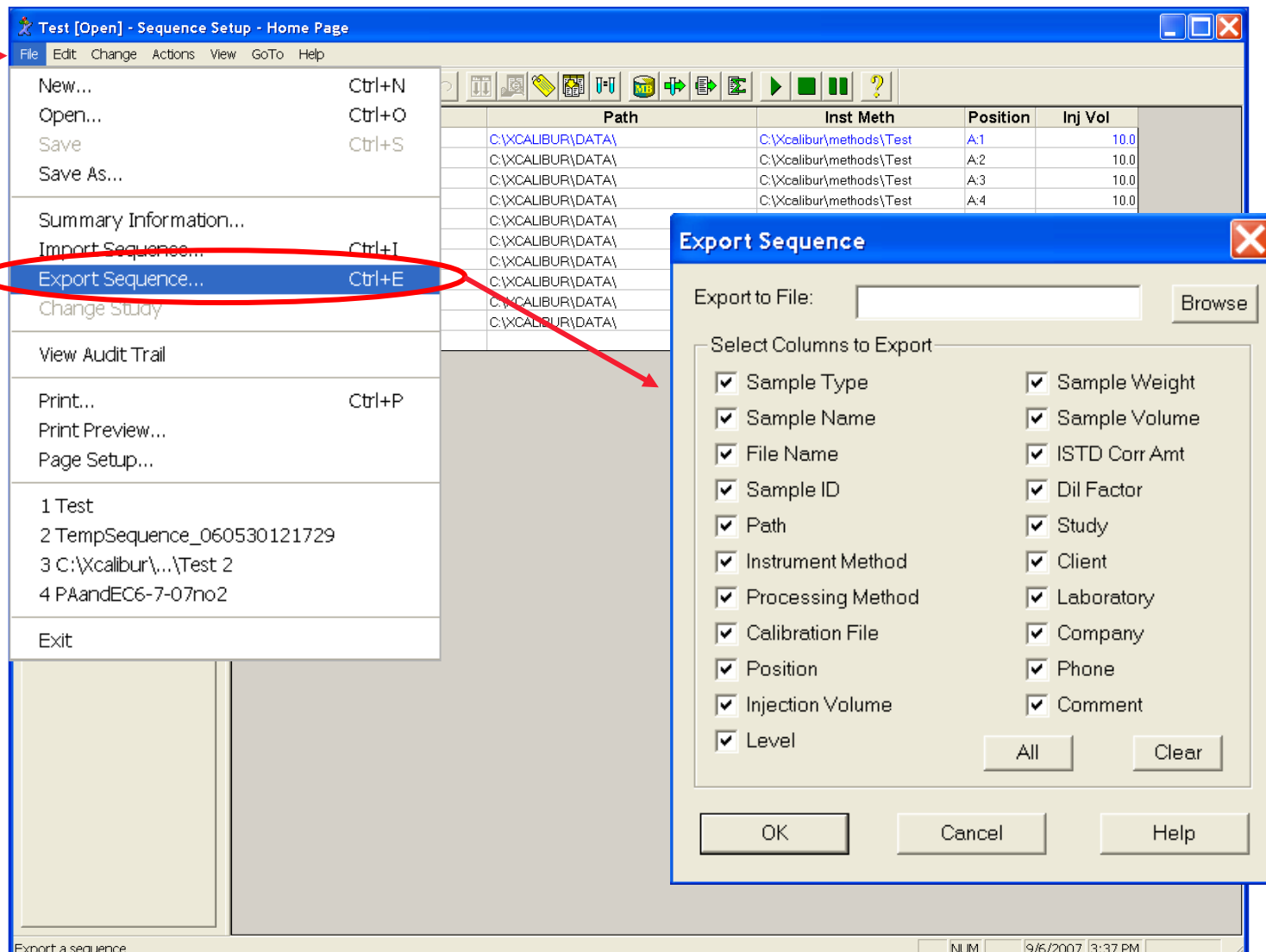
The Tray Types displayed in the list are all of those that are available for the currently configured autosampler

The screenshot shows the 'Sequence Setup - Home Page' window. A context menu is open over the 'Tray Name' column header, with 'Tray Name...' selected. A 'Tray Selection' dialog box is open, showing a list of tray types with '96 Well Microplate + Tall Microwell Carrier' selected. The dialog box has 'OK', 'Cancel', and 'Help' buttons. A red box highlights the tray types available for the configured autosampler.

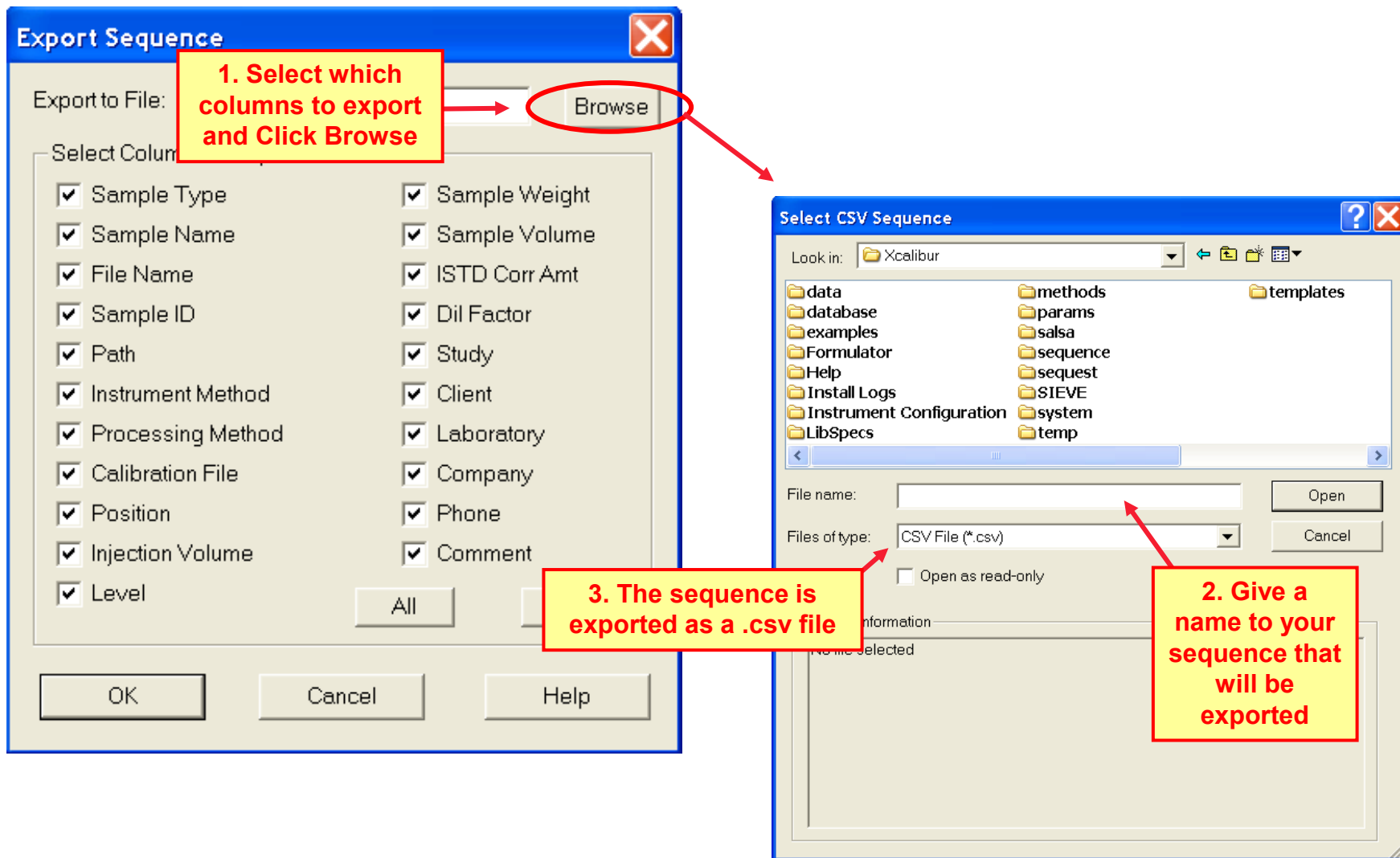
	Path	Inst Meth	Position	Inj Vol	
	calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:1	10.000	
	calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:2	10.000	
	calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:3	10.000	
	calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:4	10.000	
	calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:5	10.000	
6	Steroids06	C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:6	10.000
7	Steroids07	C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:7	10.000
8	Steroids08	C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:8	10.000
9	Steroids09	C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:9	10.000
10	Steroids10	C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:10	10.000
*					0.000

Exporting a Sequence to Excel

1. Select File and click Export Sequence



Exporting a Sequence to Excel



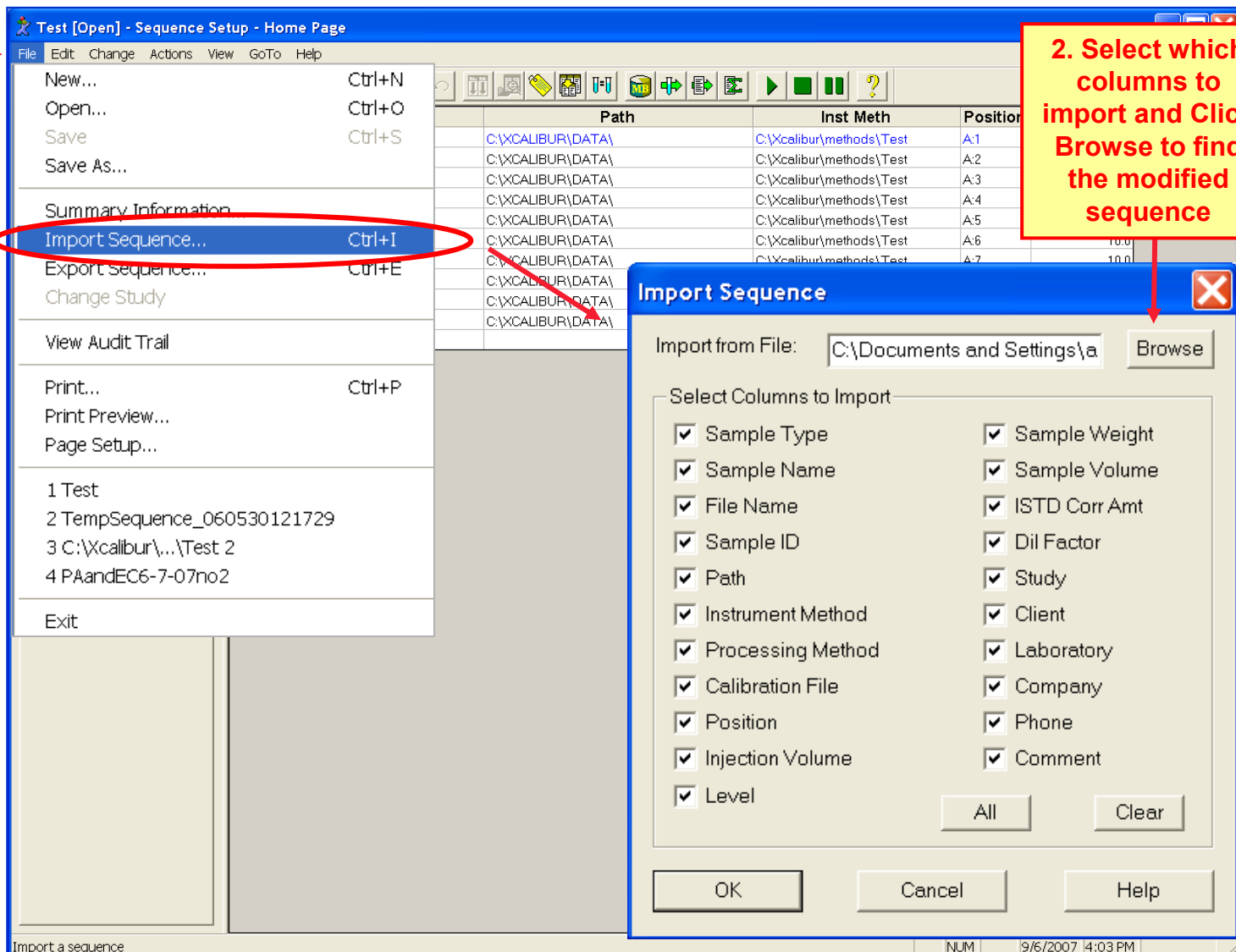
Example of an Exported Sequence

**To be able to import the sequence back into Xcalibur, the first row must contain the text *Bracket Type=n* where n=1-4. Each number represents a particular bracket type as follows:
1= Overlapped, 2= None, 3= Non-overlapped, 4= Open**

	A	B	C	D	E	F	G	H	I	J	K	
1	Bracket Type=4											
2	File Name	Path	Instrument Method	Position	Inj Vol	Sample Type	Sample ID	Process Method	Calibration File	Level	Sample Wt	Sam
3	Steroids_New_01	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:1	10	Unknown	A:1				0	
4	Steroids02	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:2	10	Unknown	A:2				0	
5	Steroids03	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:3	10	Unknown	A:3				0	
6	Steroids04	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:4	10	Unknown	A:4				0	
7	Steroids05	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:5	10	Unknown	A:5				0	
8	Steroids06	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:6	10	Unknown	A:6				0	
9	Steroids07	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:7	10	Unknown	A:7				0	
10	Steroids08	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:8	10	Unknown	A:8				0	
11	Steroids09	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:9	10	Unknown	A:9				0	
12	Steroids10	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:10	10	Unknown	A:10				0	
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												
26												

Importing a Sequence from Excel

1. Select File and click Import Sequence



2. Select which columns to import and Click Browse to find the modified sequence

Running the Sequence

1. Can either Run One Sample or Run Sequence

Check Disk Space...

Run This Sample...
Run Sequence...

Batch Reprocess...

Open File

Start Analysis
Stop Analysis
Pause Analysis

Devices On
Devices Standby
Devices Off
Automatic Devices On

Reinstate Warnings

Path	Inst Meth	Position	Inj Vol
C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:1	10.000
C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:2	10.000
C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:3	10.000
C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:4	10.000
C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:5	10.000
C:\calbur\data\	C:\calbur\TRAINING 01_2007\ESI_SRM_Jan_07	Tray01:6	10.000

Run Sequence

Acquisition Options

Instrument: Surveyor AS
Start Instrument: Yes

Surveyor MS Pump
TSQ Quantum

☒ Start When Ready Change Instruments...

Instrument Method

Start Up: Browse...
Shut Down: Browse...

Programs

Pre Acquisition: Browse...
Post Acquisition: Browse...

Run Synchronously

☒ Pre Acquisition ☒ Post Acquisition

After Sequence Set System:

☒ On ☐ Standby ☐ Off

User: patrick.jeanville

Run Rows: 1-7

☐ Priority Sequence

Processing Actions

☐ Quan
☐ Qual
☐ Reports
☐ Programs
☐ Create Quan Summary

OK Cancel Help

Running the Sequence

Run Sequence

Acquisition Options

Instrument	Start Instrument
Surveyor AS	Yes
Surveyor MS Pump	
TSQ Quantum	

☒ Start When Ready Change Instruments...

Instrument Method

Start Up Browse...

Shut Down Browse...

Programs

Pre Acquisition Browse...

Post Acquisition Browse...

Run Synchronously

☒ Pre Acquisition ☒ Post Acquisition

After Sequence Set System:

☒ On ☐ Standby ☐ Off

OK Cancel Help

User: patrick.jeanville

Run Rows: 1-7

☐ Priority Sequence

Processing Actions

☐ Quan

☐ Qual

☐ Reports

☐ Programs

☐ Create Quan Summary

If not checked, the sequence will not go into the queue until you click Actions > Start Analysis

Displays all instruments that have been configured using Instrument Configuration

Can specify Instrument Method to run before or after the sequence

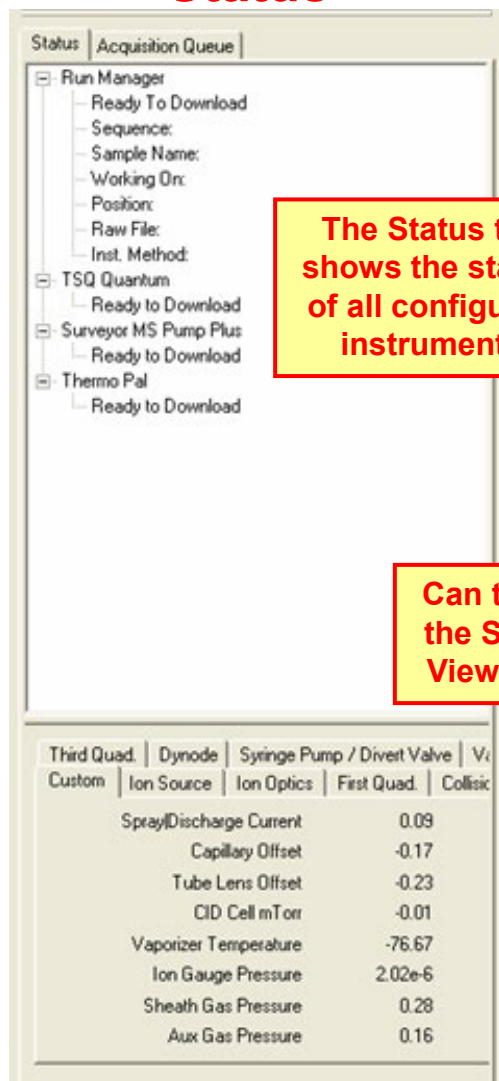
Make sure these are the rows to run

Select if you want to run sequence ASAP

Allows you to process samples automatically

The Info View

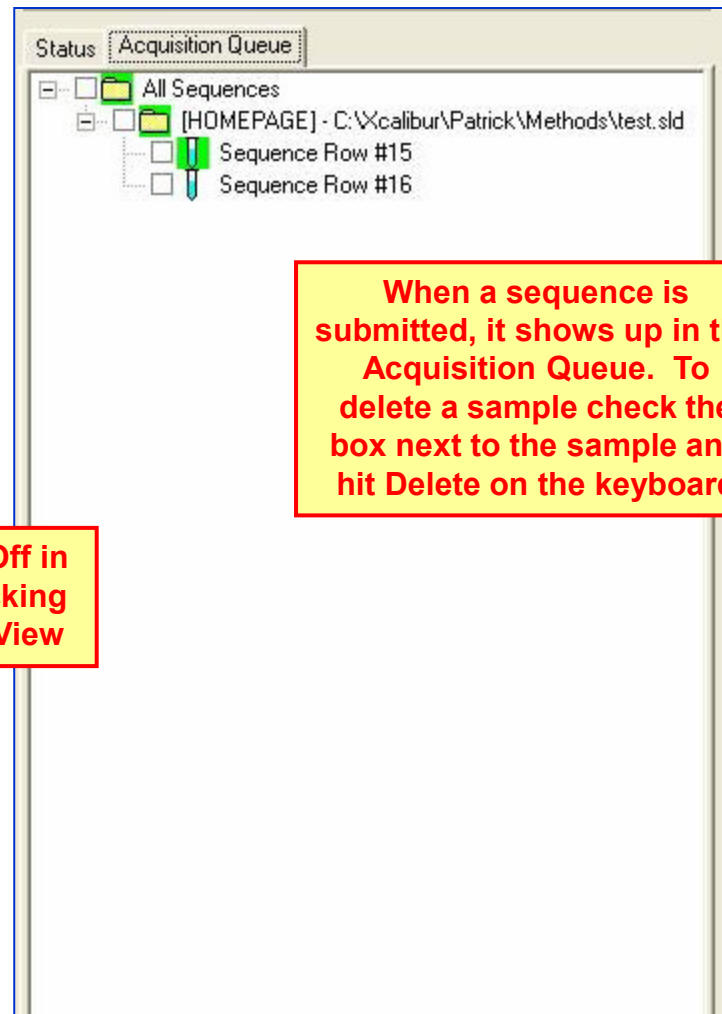
Status



The Status tab shows the status of all configured instruments

Can turn the Info View On/Off in the Sequence Setup by clicking View and unchecking Info View

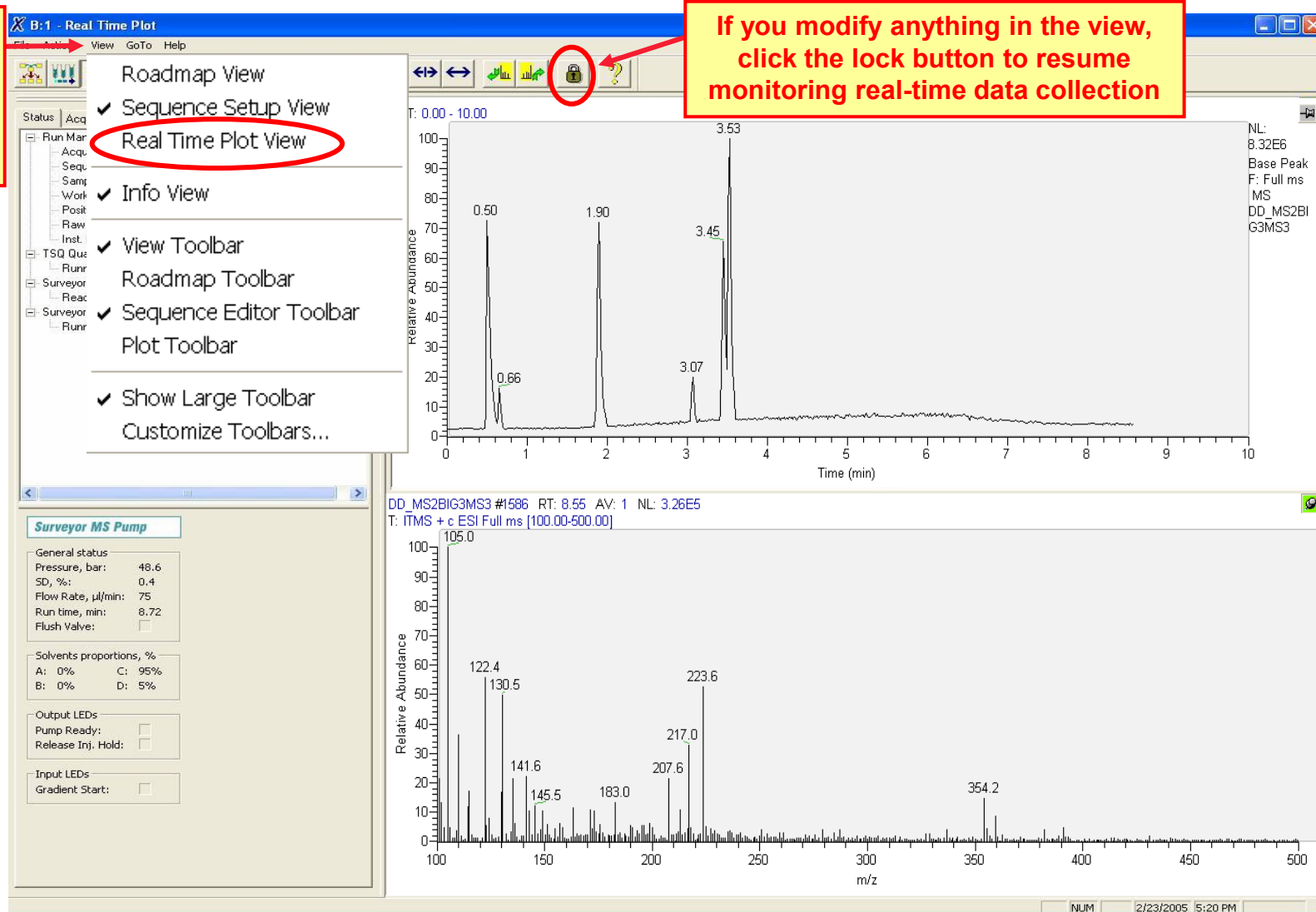
Acquisition Queue-Sequence Progress



When a sequence is submitted, it shows up in the Acquisition Queue. To delete a sample check the box next to the sample and hit Delete on the keyboard

Real Time Plot View

1. Select View and click Real Time Plot View





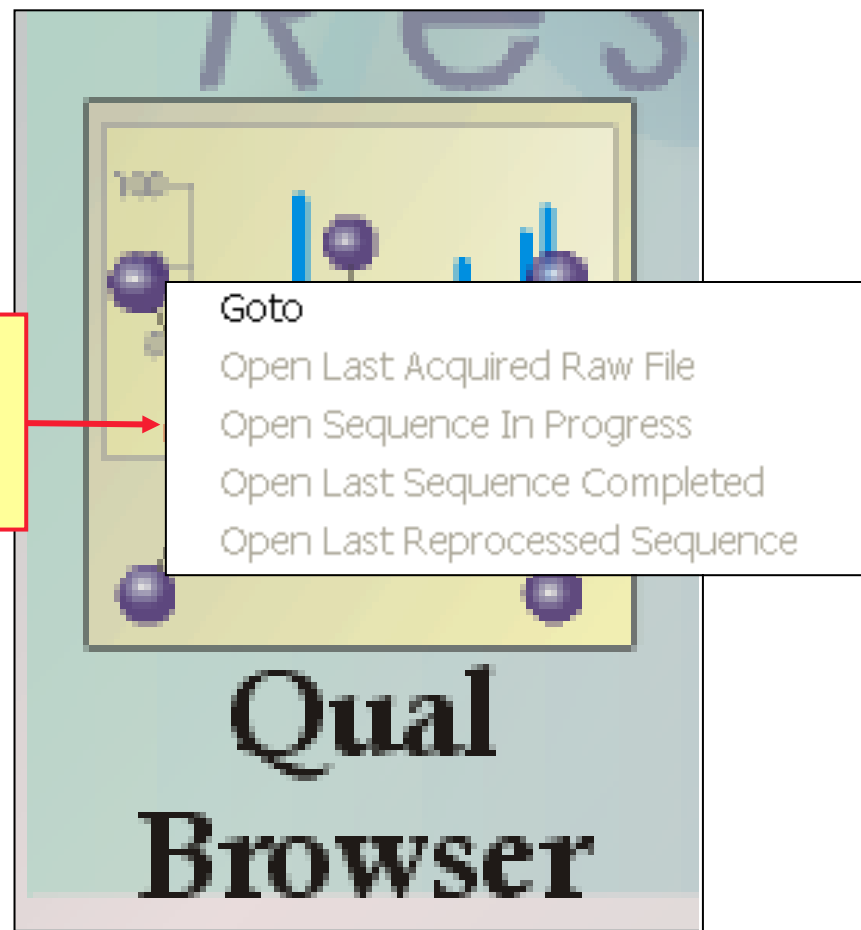
The world leader in serving science

Chapter 10

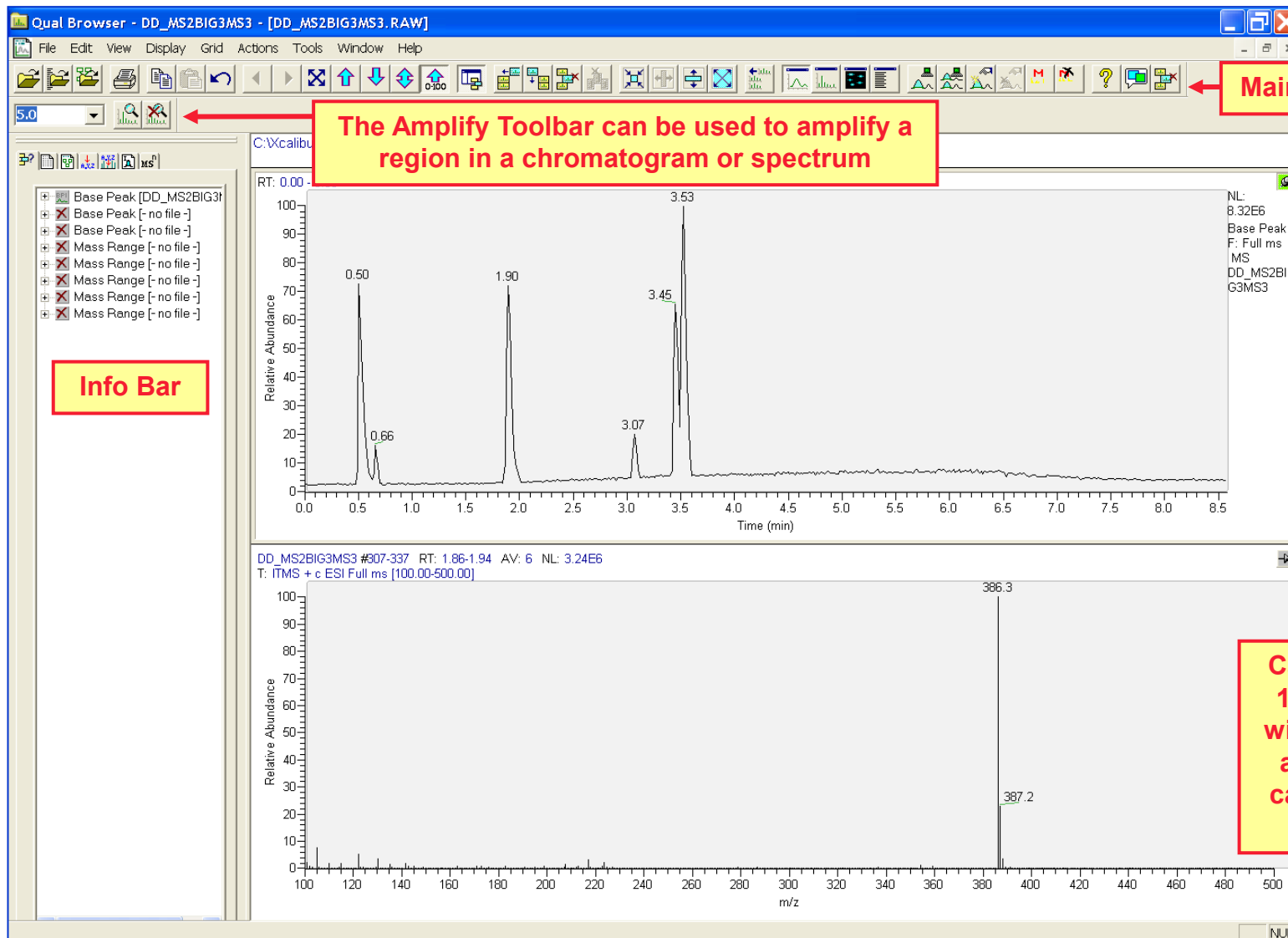
Qual Browser

Opening Qual Browser

To open Qual Browser, you can right-click on the Qual Browser button on the Xcalibur Homepage to have options to open various raw files or sequences

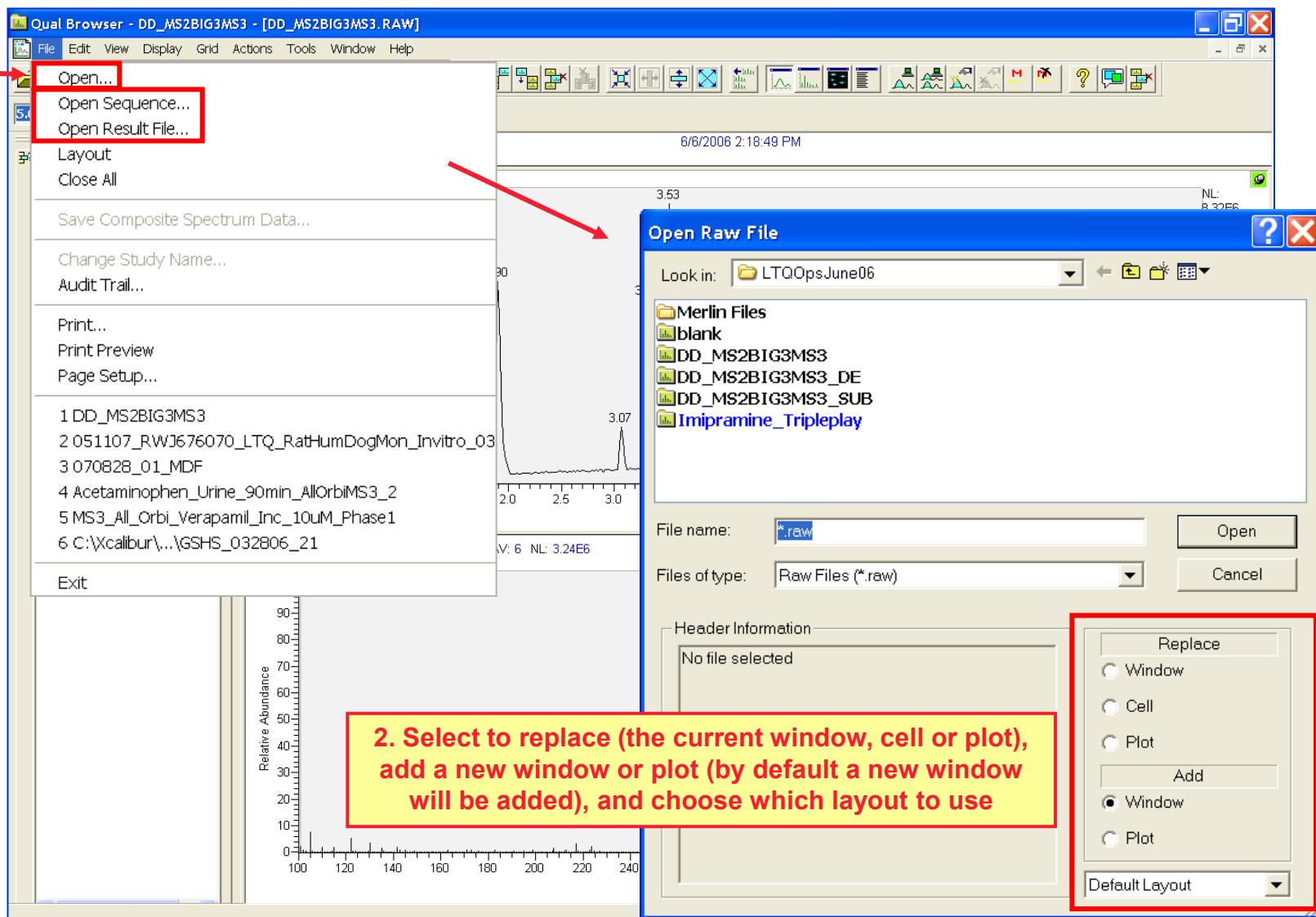


Qual Browser Main View



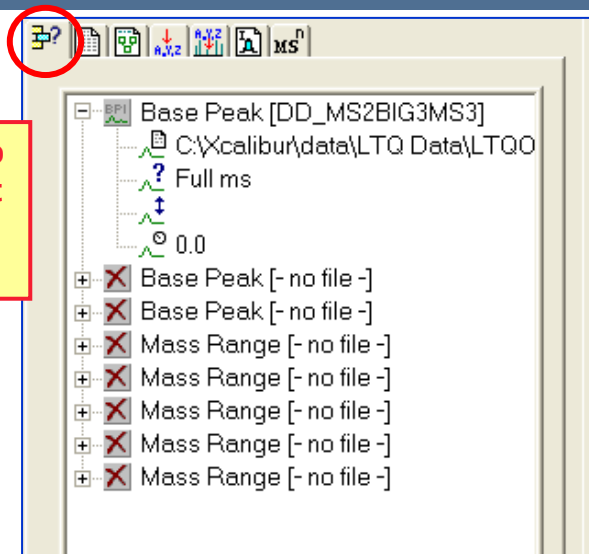
Opening Data in Qual Browser

1. Click File and select Open (can also open sequences or result files)

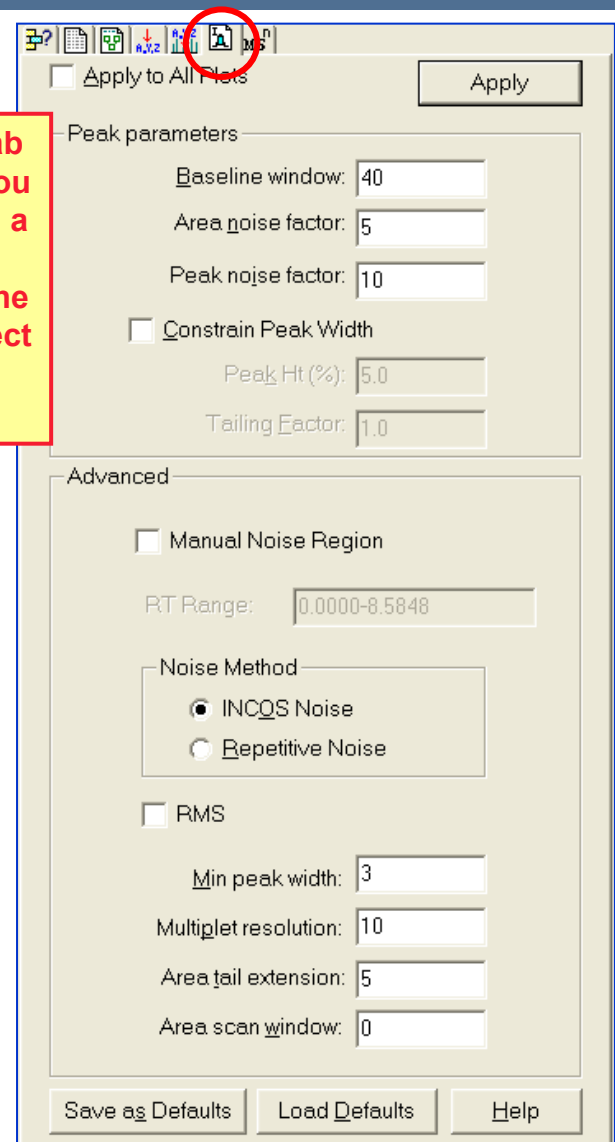


The Info Bar

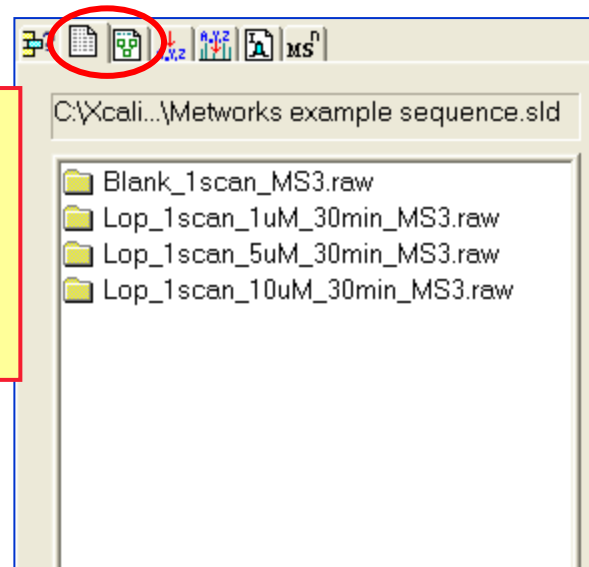
The Cell Info tab gives info about each plot within a cell



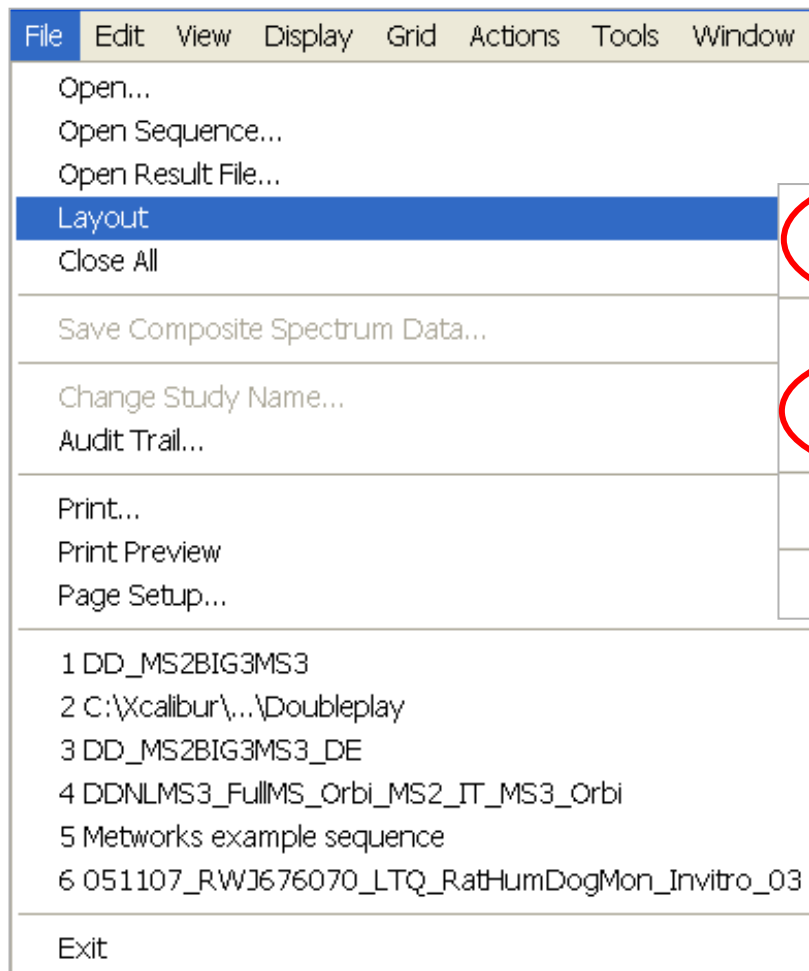
The Integration tab shows up when you integrate peaks in a chromatogram. You can change the parameters to affect how peaks are integrated.



If you open a sequence or result files, they appear in the second and third tabs of the Info Bar, respectively



Qual Browser Layouts

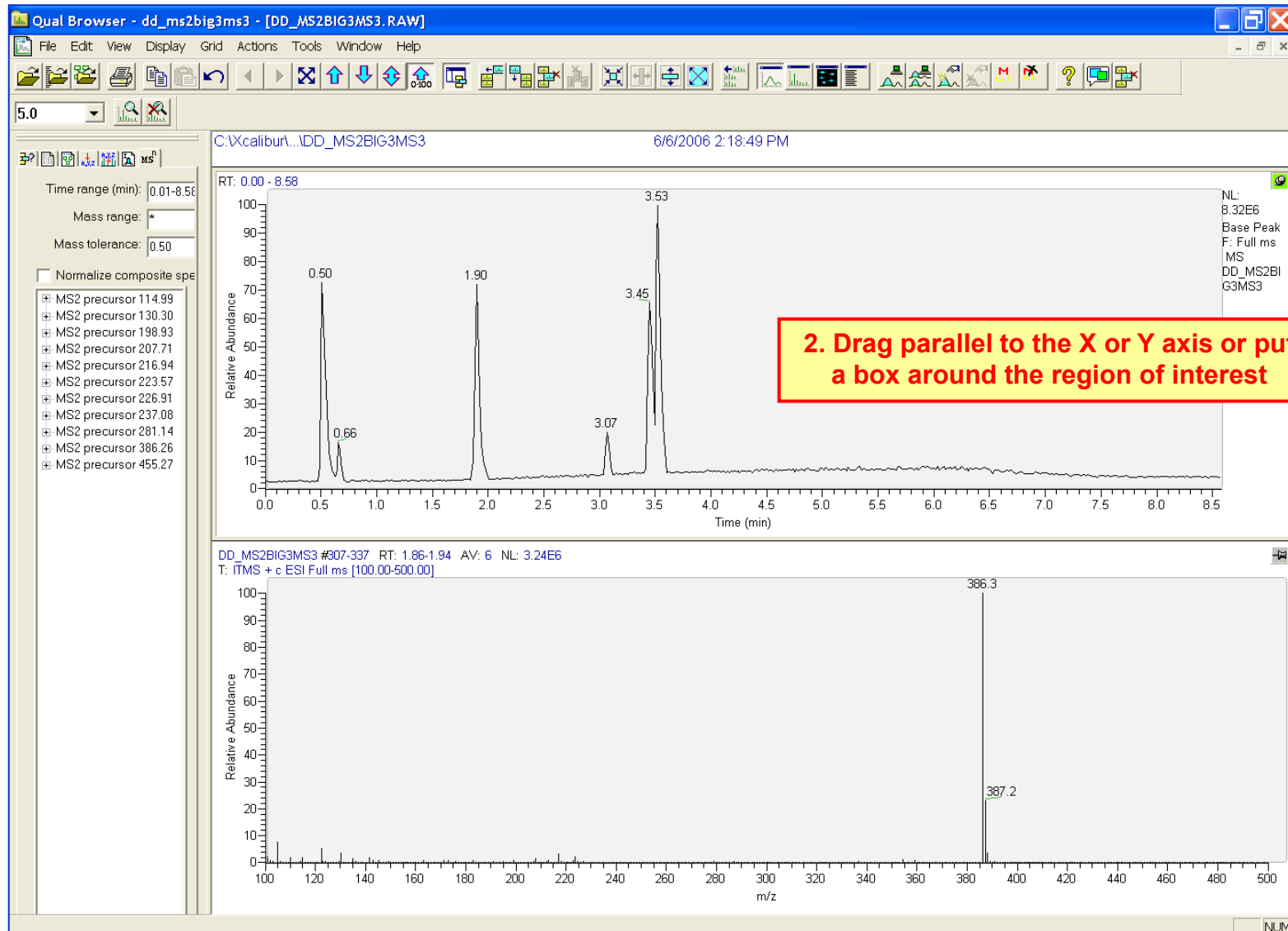


1. Set the cells, plots, integration, etc. to your specifications

3. Apply the layout to subsequent samples

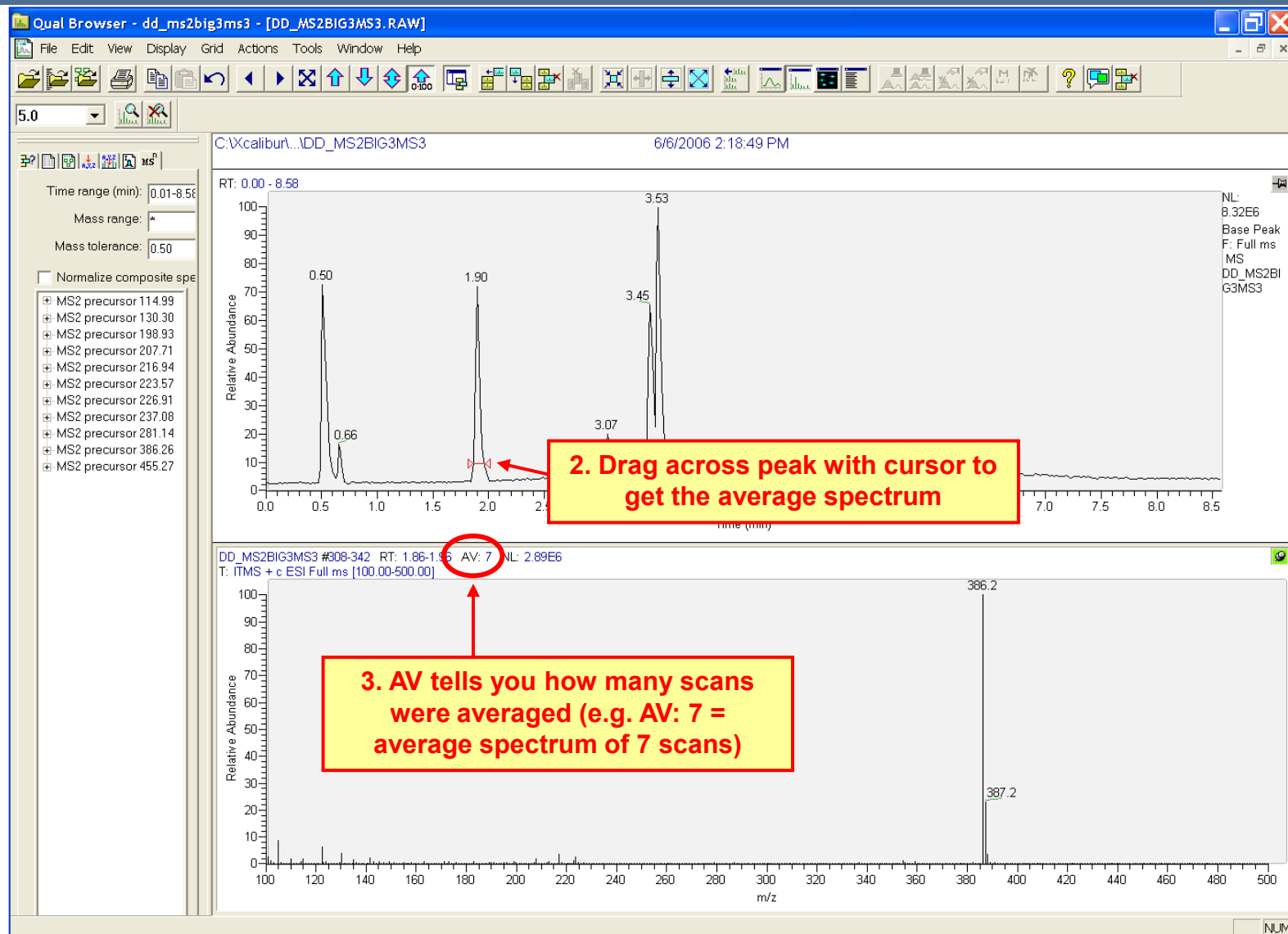
2. Save the layout or save the layout as the default layout

To Zoom In...

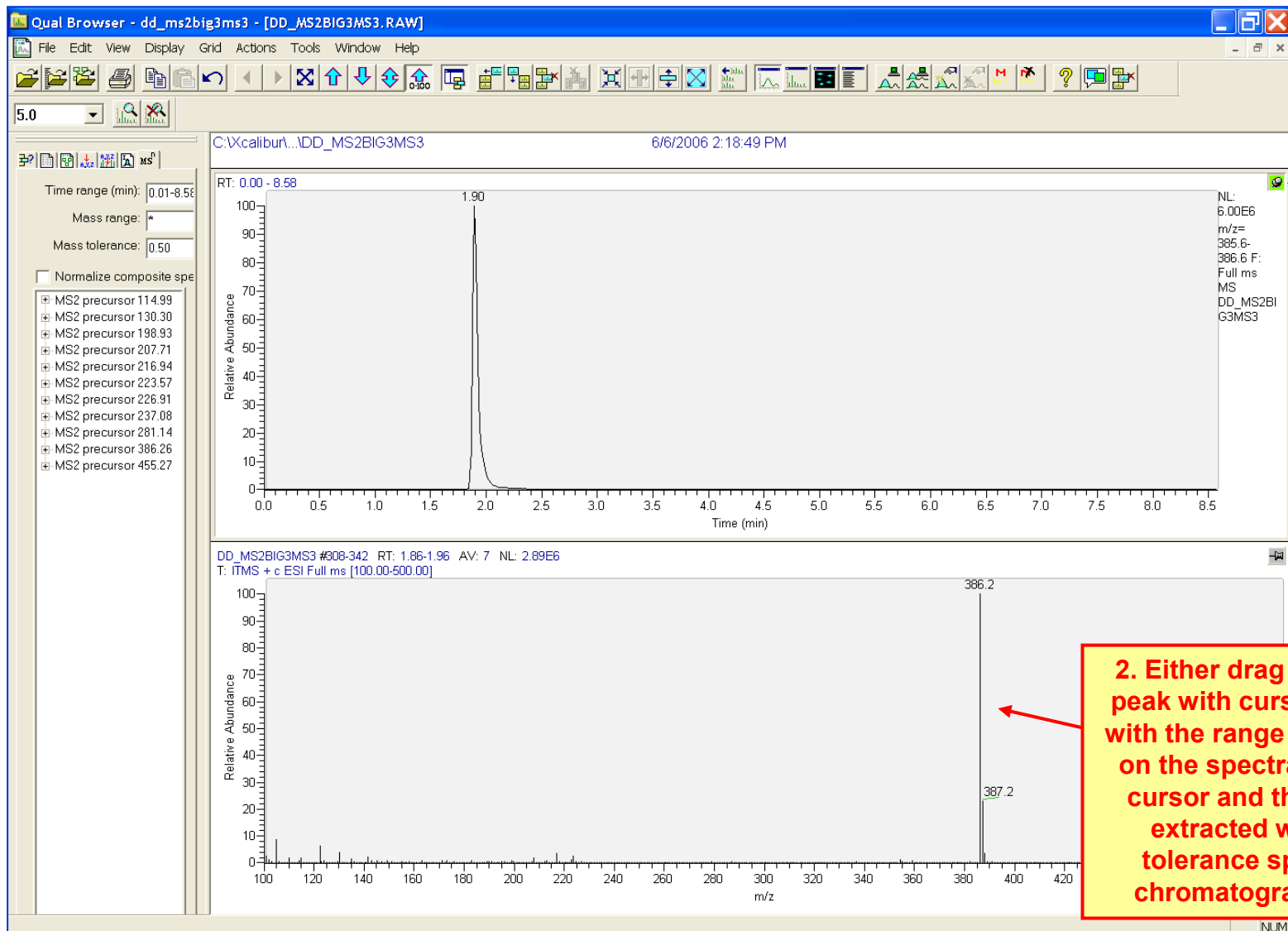


1. Pin cell

Getting an Average Spectrum of a Peak in the Chromatogram



Extracting an Ion from the Chromatogram



1. Pin chromatogram

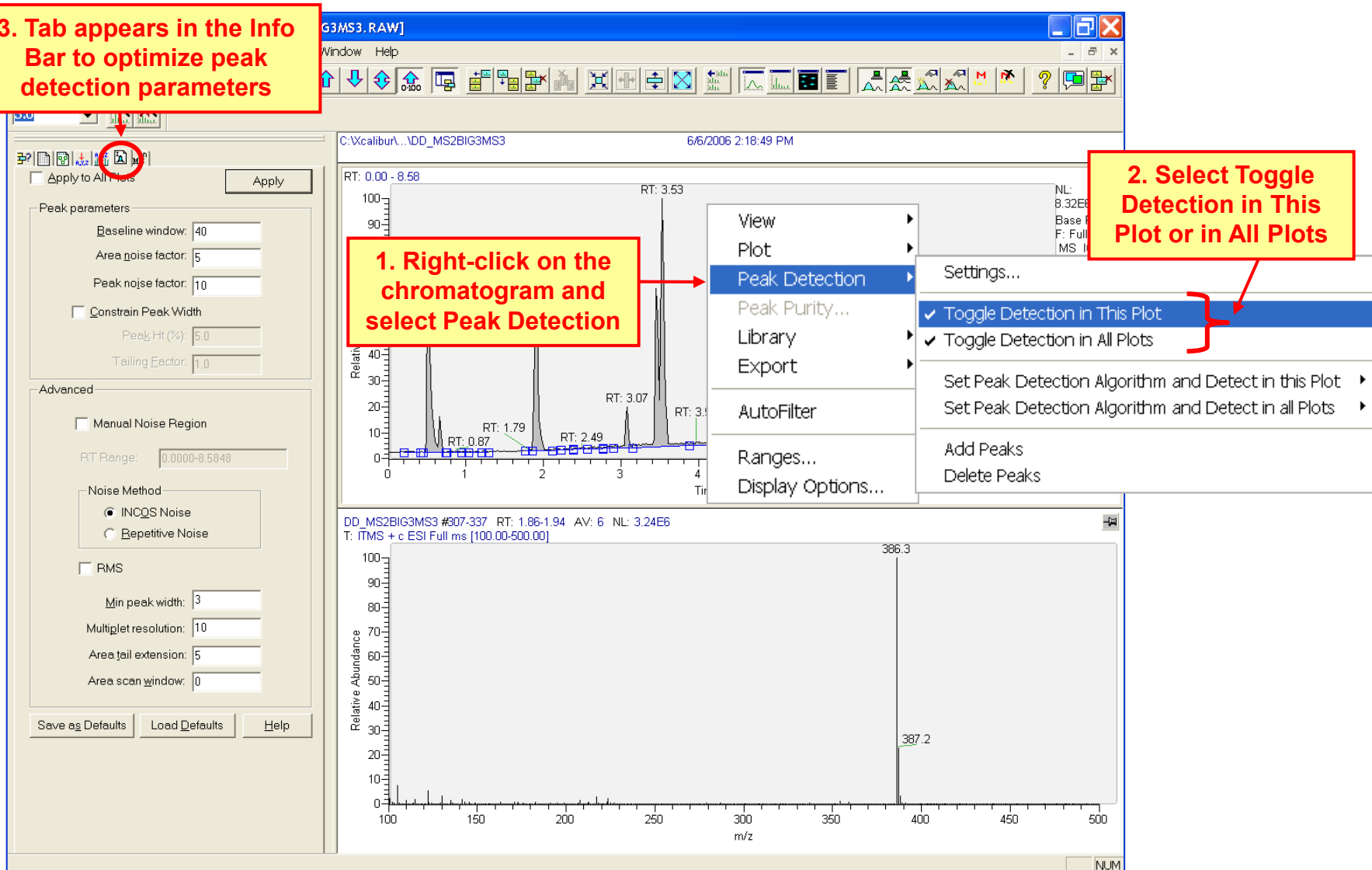
2. Either drag across spectral peak with cursor to get an EIC with the range dragged or click on the spectral peak with the cursor and the mass will be extracted with the mass tolerance specified in the chromatogram ranges box

Chromatogram Right-Click Menu – Peak Detection

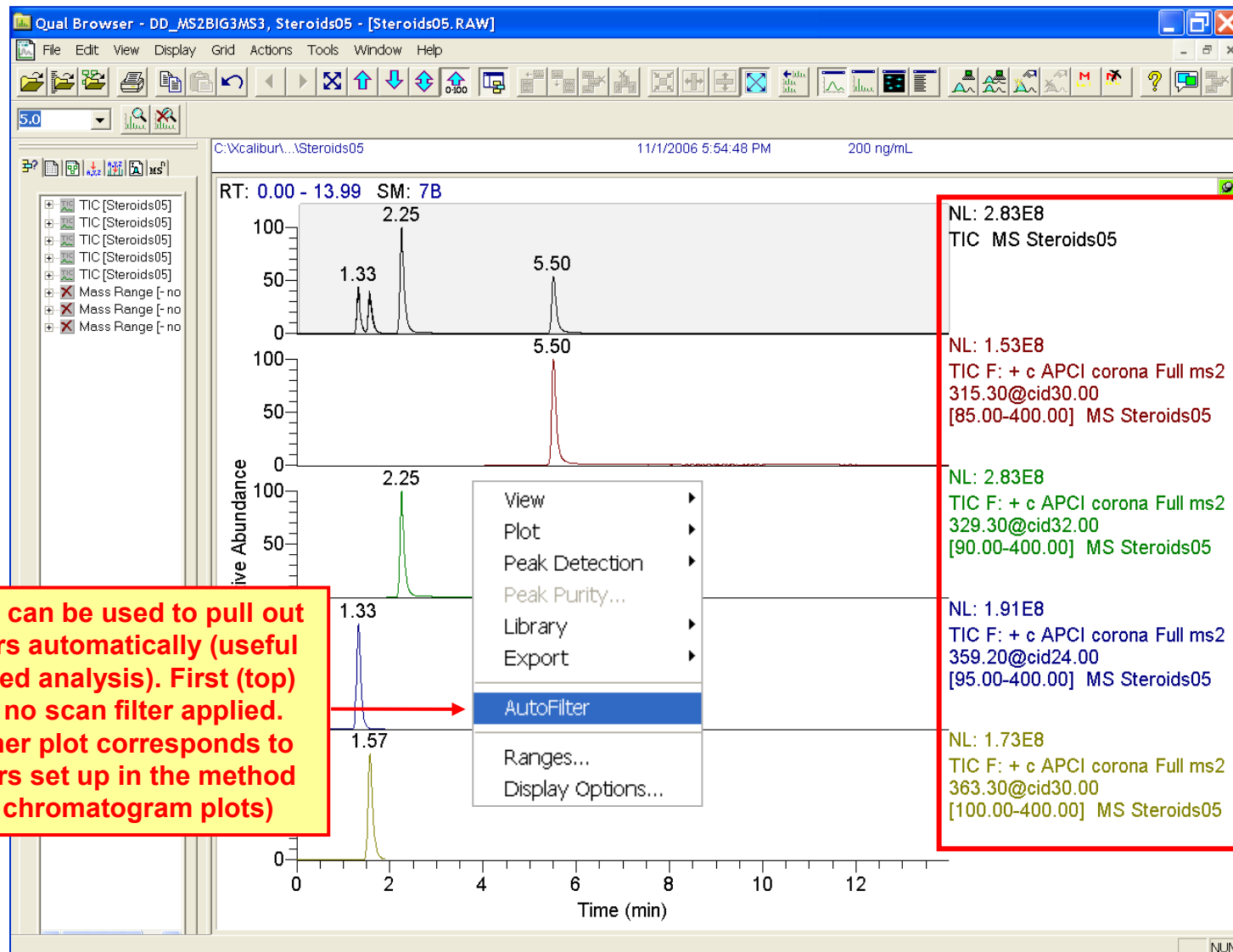
3. Tab appears in the Info Bar to optimize peak detection parameters

1. Right-click on the chromatogram and select Peak Detection

2. Select Toggle Detection in This Plot or in All Plots



Chromatogram Right-Click Menu – AutoFilter



Chromatogram Right-Click Menu – Chromatogram Ranges

1. Right-click on the chromatogram and select Ranges...

The screenshot shows the Qual Browser interface with a chromatogram plot. A right-click menu is open over the plot, and the 'Ranges...' option is selected. The 'Chromatogram Ranges' dialog box is displayed, showing the 'Automatic processing' tab. The 'Range' section has a 'Time range (minutes)' field. The 'Type' section has a table with columns: Type, Range, Scan filter, Delay (...), Scale, and Raw file. The 'Plot properties' section has fields for Raw file, Scan filter, Plot type, Range(s), Detector, Peak algorithm, Delay (min), and Fix scale to.

Type	Range	Scan filter	Delay (...)	Scale	Raw file
<input checked="" type="checkbox"/> Base Peak	-	Full ms	0.00	-	C:\xcalibur\data\LTQ Data...
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-

Plot properties

Raw file: c:\xcalibur\data\ltq_data\ltqopsjune06\dd_ms2big3ms3.raw
Scan filter: Full ms
Plot type: Base Peak
Range(s):
Detector: MS
Peak algorithm: ICIS
Delay (min): 0.00
Fix scale to: 1000000.00

OK Cancel Help

Chromatogram Right-Click Menu – Chromatogram Ranges

Chromatogram Ranges

Ranges | Automatic processing

Range

Time range (minutes): ☐ Fixed scale

Type	Range	Scan filter	Delay (...)	Scale	Raw file
<input checked="" type="checkbox"/> Base Peak	-	Full ms	0.00	-	C:\xcalibur\data\LTQ Data...
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-

Plot properties

Raw file: ...

Scan filter:

Plot type:

Range(s):

Detector:

Peak algorithm:

Delay (min):

Fix scale to:

OK Cancel Help

Check to
add plots
(8 max)

Click to change
the raw file name

Can change
the
Detector,
Peak
detection
algorithm,
and Delay
time here

Chromatogram Ranges – Scan Filter

The screenshot shows the 'Chromatogram Ranges' dialog box. The 'Ranges' tab is active, and the 'Automatic processing' sub-tab is selected. The 'Range' section has a 'Time range (minutes):' field with an asterisk and a 'Fixed scale' checkbox. Below this is a table with columns: Type, Range, Scan filter, Delay (...), Scale, and Raw file. The first row is checked and shows 'Base Peak' with a blank range, 'Full ms' scan filter, 0.00 delay, and a raw file path. Below the table is a 'Plot properties' section with fields for 'Raw file', 'Scan filter' (set to 'Full ms'), 'Plot type', and 'Range(s)'. The 'Range(s)' field contains a list of scan ranges. A red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. Another red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A tenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A eleventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twelfth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirteenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fourteenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifteenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixteenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventeenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighteenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A nineteenth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twentieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A twenty-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirtieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A thirty-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fortieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A forty-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fiftieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A fifty-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixtieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A sixty-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A seventy-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eightieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. An eighty-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninetieth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-first red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-second red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-third red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-fourth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-fifth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-sixth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-seventh red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-eighth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A ninety-ninth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text. A hundredth red arrow points from the 'Scan filter' dropdown to the 'Full ms' text.

Can type any general scan filter here (e.g. Full ms, Full ms2, Full ms3, etc. pulls out all MS, MS², MS³ scans, respectively). The layout can then be saved as default so that if the scan ranges are changed, there is no need to modify the scan filter. If you leave the Scan filter blank, it will show all scans that were acquired (whether MS or MSⁿ)

Can also click down arrow and select any of these more specific scan filters

Chromatogram Ranges – Plot Types

Chromatogram Ranges

Ranges | Automatic processing

Range

Time range (minutes): * ☐ Fixed scale

Type	Range	Scan filter	Delay (...)	Scale	Raw file
<input checked="" type="checkbox"/> Base Peak	-	Full ms	0.00	-	C:\Xcalibur\data\LTQ Data...
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-

Plot properties

Raw file: c:\xcalibur\data\ltq data\ltqopsjune06\dd_ms2big3ms3.raw ... Detector: MS

Scan filter: Full ms Peak algorithm: ICIS

Plot type: Base Peak

Range(s):

Mass Range

TIC

Base Peak

Neutral Fragment

Delay (min): 0.00

OK Cancel Help

1. Click to change the Plot type

TIC – plots the sum of all ions for each scan.
Base Peak – plots the most intense ion for each scan.
Full ms data normally looks better as a Base Peak chromatogram since much of the noise gets filtered out.

Chromatogram Ranges – Extracted Ion Chromatogram

There are different ways to extract an ion in your chromatogram using the Chromatogram Ranges box:

The screenshot shows the 'Chromatogram Ranges' dialog box with the 'Automatic processing' tab selected. The 'Range' section has a 'Time range (minutes):' field with an asterisk and a 'Fixed scale' checkbox. Below is a table with columns: Type, Range, Scan filter, Delay (...), Scale, and Raw file. The first row is checked and shows 'Base Peak', '-', 'Full ms', '0.00', '-', and 'C:\Xcalibur\data\LTQ Data...'. Below the table is the 'Plot properties' section with fields for 'Raw file', 'Scan filter', 'Plot type', and 'Range(s)'. The 'Scan filter' is set to 'Full ms'. The 'Plot type' dropdown is open, showing options: 'Base Peak', 'Mass Range', 'TIC', 'Base Peak', and 'Neutral Fragment'. The 'Range(s)' field is empty. Three red callout boxes provide instructions: 1. Change the Scan filter to Full ms or delete the Scan filter to see all scans (pointing to the Scan filter field). 2. Can either choose Mass Range (TIC) or Base Peak (pointing to the Plot type dropdown). 3. Type mass or mass range in the Range(s) box. If one mass is typed, the range will be defined by the mass tolerance set in the Automatic processing tab (pointing to the Range(s) field).

Chromatogram Ranges

Ranges | Automatic processing

Range

Time range (minutes): * Fixed scale

Type	Range	Scan filter	Delay (...)	Scale	Raw file
<input checked="" type="checkbox"/> Base Peak	-	Full ms	0.00	-	C:\Xcalibur\data\LTQ Data...
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-
<input type="checkbox"/>	-	-	-	-	-

1. Change the Scan filter to Full ms or delete the Scan filter to see all scans

Plot properties

Raw file: c:\xcalibur\data\ltq data\ltqops\june06\dd_ms2big3ms3.raw

Scan filter: Full ms

Plot type: Base Peak

Range(s):

2. Can either choose Mass Range (TIC) or Base Peak

3. Type mass or mass range in the Range(s) box. If one mass is typed, the range will be defined by the mass tolerance set in the Automatic processing tab

Chromatogram Ranges – Automatic Processing tab

1. Can enable smoothing for the chromatogram plot

Smoothing

☒ Enable

Type: Boxcar Points: 7

Baseline subtraction

☐ Enable

Polynomial order: 2

Below curve (%): 10

Tolerance: 0.01

☐ Flatten edges

☒ Overlay graph of fitted polynomial

Mass tolerance

☐ Use user defined

Mass tolerance: 500.0

Units: ☒ mmu ☐ ppm

Mass precision

Decimals: 2

Include peaks

☐ Reference and exception peaks

Smoothing points must be an odd number

OK Cancel Help

Chromatogram Right-Click Menu - Display Options

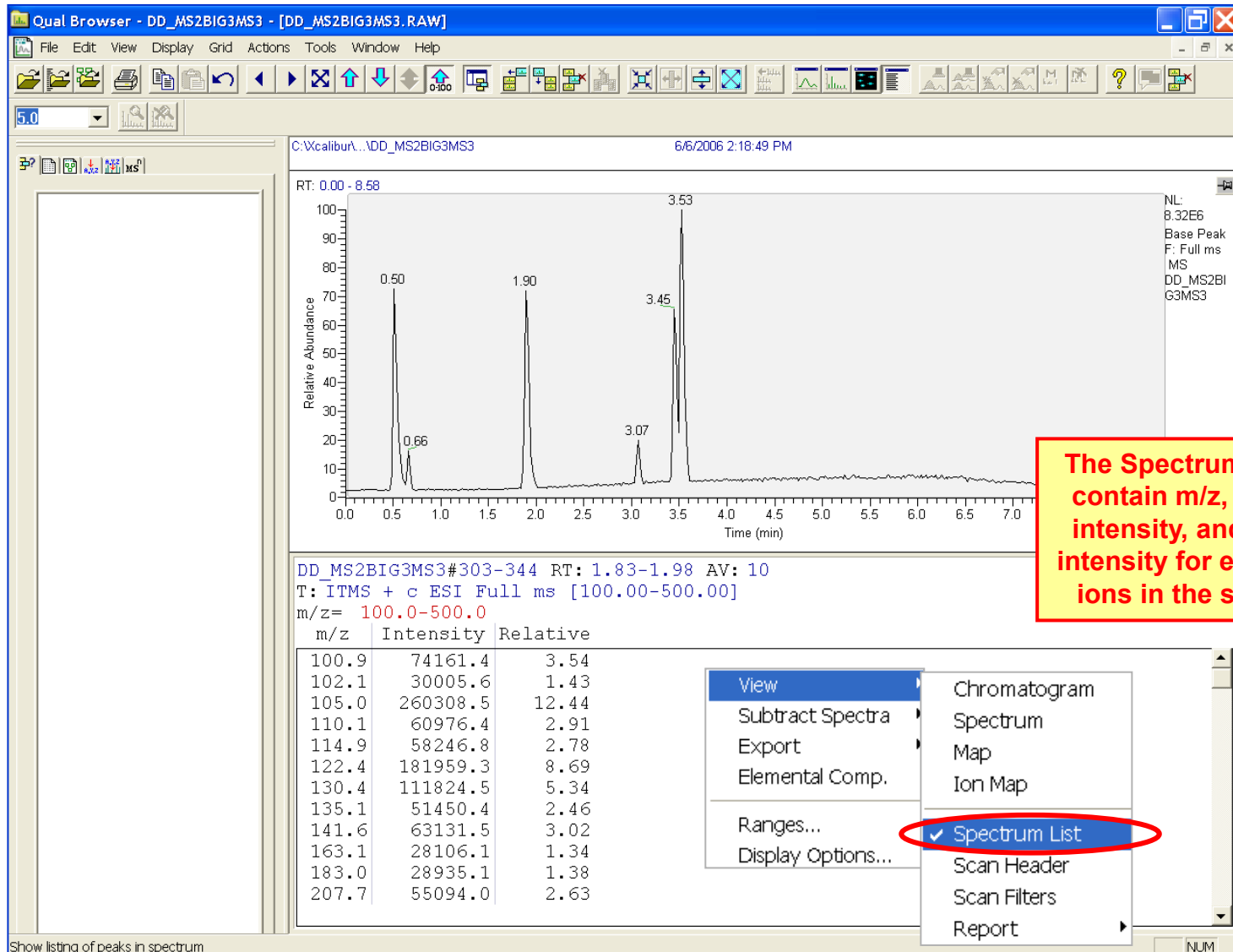
The screenshot shows the Qual Browser interface with a chromatogram plot. A right-click context menu is open over the plot, and the 'Display Options...' option is highlighted. A red box with an arrow points to this option, containing the text: '1. Right-click on the chromatogram and select Display Options...'. The 'Display Options' dialog box is also open, with the 'Style' tab selected. A red box with an arrow points to the 'Style' tab, containing the text: 'The Display Options box allows you to modify the appearance of the chromatogram view (Style, Color, Labels, Axis & Normalization)'. Inside the dialog, the 'Plotting' section has 'Point To Point' selected, and the 'Arrangement' section has 'Stack (2D)' selected. A red box with an arrow points to the '3D' plot area, containing the text: 'Xcalibur displays the results of the current settings in the graphic on the right side'. The '3D' plot area shows a chromatogram with peaks labeled at retention times 0.50, 3.45, and 3.53. The 'Style' tab also includes 'Elevation' and 'Skew' sliders, and a 'Draw Backdrop' checkbox. The 'Normalization' tab is also visible. The background chromatogram shows a peak at 3.53 minutes. The Qual Browser window title is 'Qual Browser - DD_MS2BIG3MS3 - [DD_MS2BIG3MS3.RAW]'. The file path is 'C:\Xcalibur\...DD_MS2BIG3MS3'. The date and time are '6/6/2006 2:18:49 PM'. The plot shows 'Relative Abundance' vs 'Time (min)'. The 'Display Options' dialog box has 'OK', 'Cancel', and 'Help' buttons. The 'Style' tab has 'Plotting' and 'Arrangement' sections. The 'Plotting' section has 'Point To Point' and 'Stick' options. The 'Arrangement' section has 'Stack (2D)' and 'Overlay (3D)' options. The '3D' plot area has 'Elevation' and 'Skew' sliders, and a 'Draw Backdrop' checkbox. The 'Normalization' tab is also visible. The background chromatogram shows a peak at 3.53 minutes. The Qual Browser window title is 'Qual Browser - DD_MS2BIG3MS3 - [DD_MS2BIG3MS3.RAW]'. The file path is 'C:\Xcalibur\...DD_MS2BIG3MS3'. The date and time are '6/6/2006 2:18:49 PM'. The plot shows 'Relative Abundance' vs 'Time (min)'. The 'Display Options' dialog box has 'OK', 'Cancel', and 'Help' buttons.

1. Right-click on the chromatogram and select Display Options...

The Display Options box allows you to modify the appearance of the chromatogram view (Style, Color, Labels, Axis & Normalization)

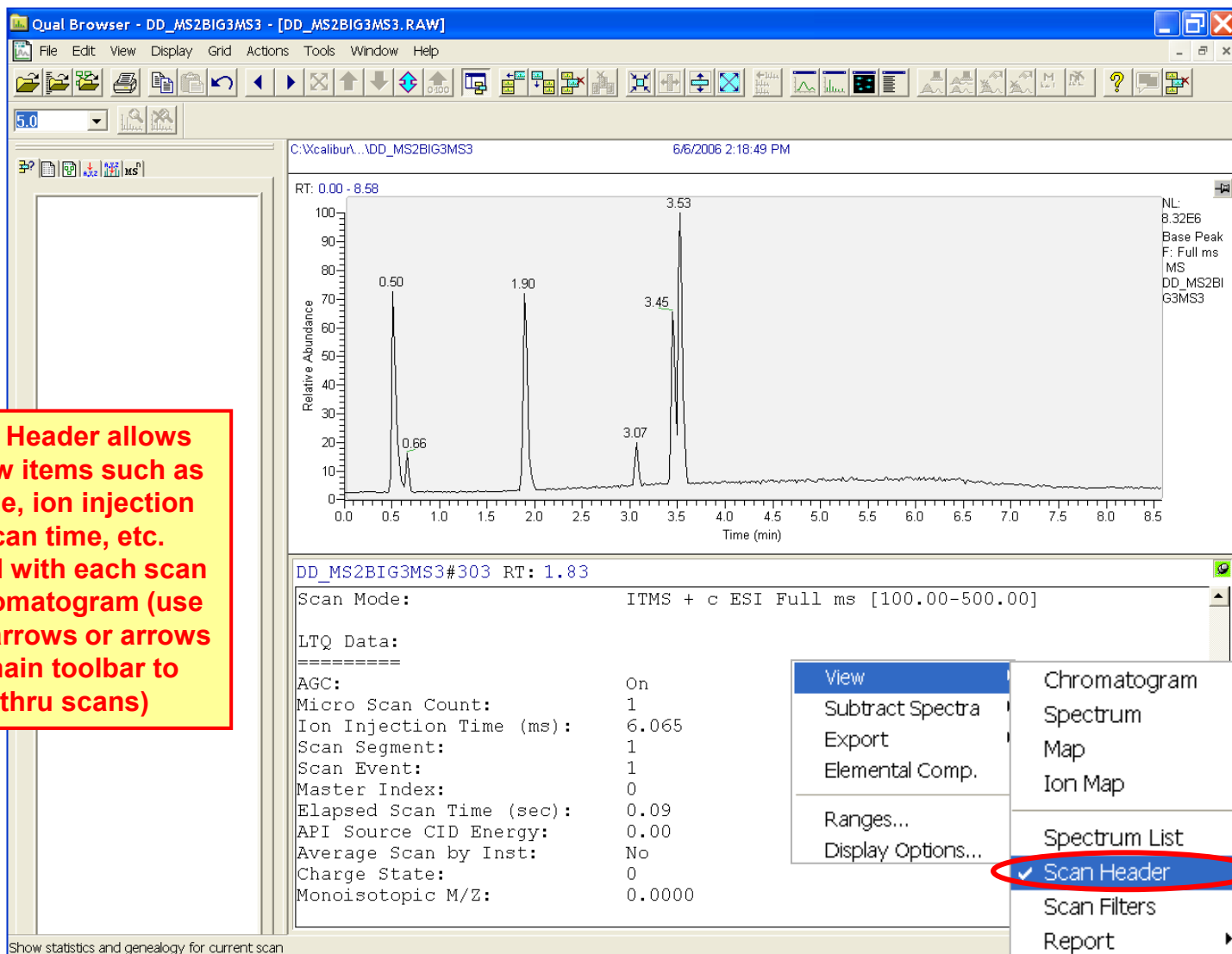
Xcalibur displays the results of the current settings in the graphic on the right side

Spectrum Right-Click Menu – Spectrum List



The Spectrum List can contain m/z, absolute intensity, and relative intensity for each of the ions in the spectrum

Spectrum Right-Click Menu – Scan Header



The Scan Header allows you to view items such as scan mode, ion injection time, scan time, etc. associated with each scan in the chromatogram (use keyboard arrows or arrows on the main toolbar to scroll thru scans)

Spectrum Right-Click Menu – Tune and Instrument Methods

Tune and Instrument Methods are stored in the raw file. When selecting the Instrument Method, the MS method shows up on the first page. Scroll with the keyboard arrows or arrows on the main toolbar to get the pump and autosampler methods.

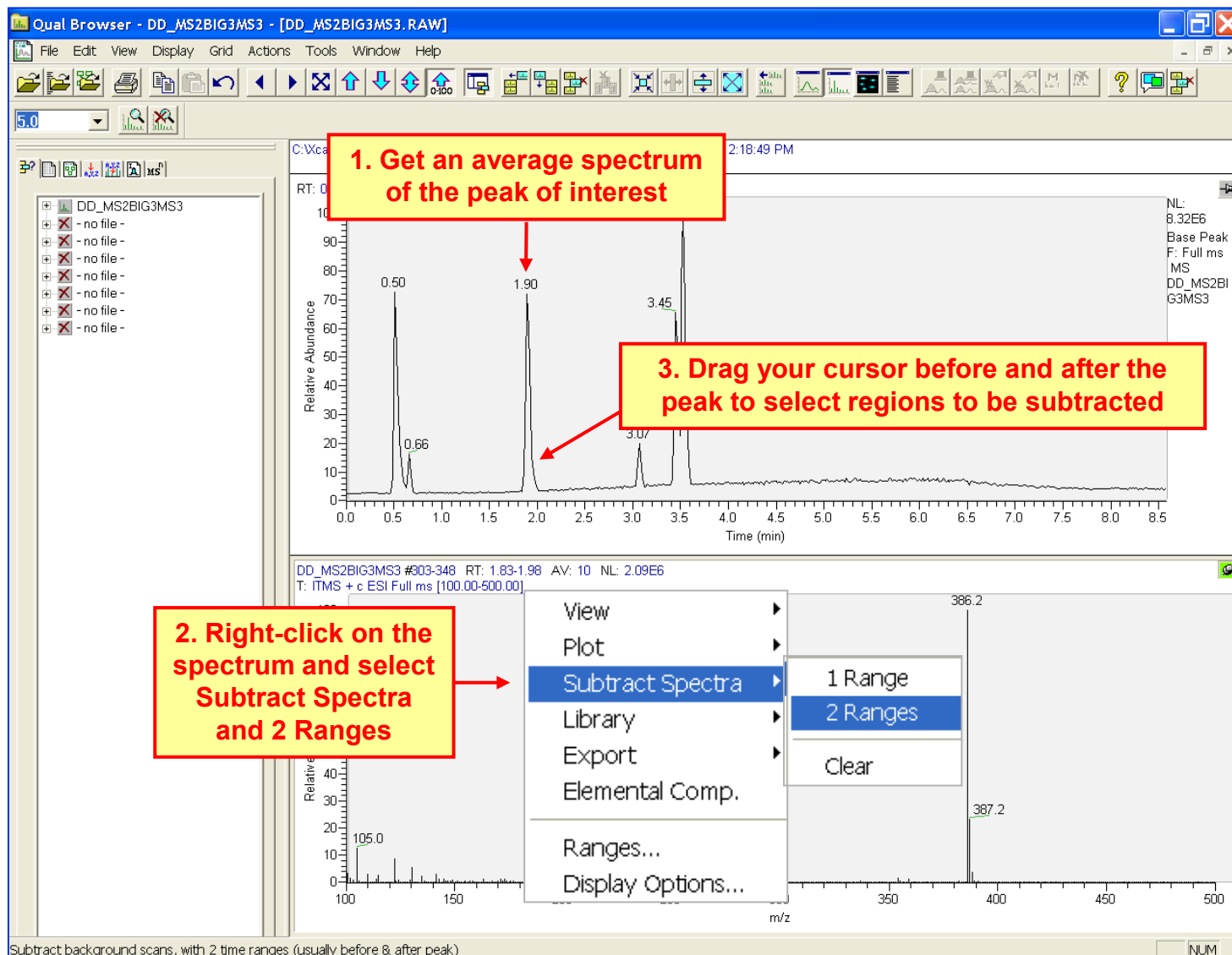
The screenshot displays the Qual Browser interface for file DD_MS2BIG3MS3. The top panel shows a chromatogram with relative abundance on the y-axis (0 to 100) and time in minutes on the x-axis (0.0 to 8.5). Several peaks are labeled with their retention times: 0.50, 0.66, 1.90, 3.07, 3.45, and 3.53. The right side of the chromatogram panel contains metadata: NL: 8.32E6, Base Peak, F: Full ms, MS, DD_MS2BIG3MS3.

The bottom panel shows the 'Segment 1 Information' for the selected method, DD_MS2BIG3MS3, LTQ MS. The 'Tune Method' is listed as 'hydrocortisone'. Below this, 'Scan Event Details' are listed for five scans, all using ITMS with various mass ranges and ion selection criteria. A 'Data Dependent Settings' section is also present.

A right-click context menu is open over the 'View' button. The menu options are: Chromatogram, Spectrum, Map, Ion Map, Spectrum List, Scan Header, Scan Filters, Report, Tune Method, Instrument Method (highlighted with a red circle and a checkmark), Sample Information, Status Log, and Error Log.

At the bottom of the window, a status bar reads: 'Display the instrument method used to collect this raw file'.

Spectrum Right-Click Menu – Spectral Subtraction



Spectrum Right-Click Menu - Spectrum Ranges

The screenshot shows the Qual Browser interface with a mass spectrum plot. The plot displays relative abundance versus m/z, with peaks labeled at 0.50, 0.66, and 387.2. A right-click menu is open over the spectrum, and the 'Ranges...' option is highlighted. A red arrow points from a text box to this option.

1. Right-click on the spectrum and select Ranges...

The 'Spectrum Ranges' dialog box is open, showing the 'Automatic Processing' tab. The 'Range' section has 'Mass range' set to 100.0-500.0, 'Average' checked, and 'Fix scale' set to 1000000.0. The 'Plot properties' section shows 'Detector' as MS, 'Time' as 1.83-1.99, 'Filter Type' as Scan, 'Filter' as Full ms, and 'Raw file' as c:\xcalibur\data\ltq data\ltqops\june06\dd_ms2big3ms3.r. The 'Background Subtraction' section has 'Time range 1' and 'Time range 2' both set to 0.01, and 'Simulation' unchecked.

Time	Filter	Raw File	Subtract 1	Subtract 2
<input checked="" type="checkbox"/> 1.83-1.99	Full ms	C:\xcalibur\data\LTQ Data...	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-
<input type="checkbox"/>	-	-	-	-

OK Cancel Help

Spectrum Right-Click Menu - Spectrum Ranges

Spectrum Ranges

Ranges | Automatic Processing

Range

Mass range: ☒ Average ☐ Fix scale:

Time	Filter	Raw File	Subtract 1	Subtract 2
<input checked="" type="checkbox"/> 1.83-1.99	Full ms	C:\Xcalibur\data\LTQ Data...	-	-
<input type="checkbox"/> -	-	-	-	-
<input type="checkbox"/> -	-	-	-	-
<input type="checkbox"/> -	-	-	-	-
<input type="checkbox"/> -	-	-	-	-
<input type="checkbox"/> -	-	-	-	-
<input type="checkbox"/> -	-	-	-	-

Plot properties

Detector: Time:

Filter Type: ☒ Scan ☐ Process

Filter:

Raw file: ...

Background Subtraction

☐ Time range 1:

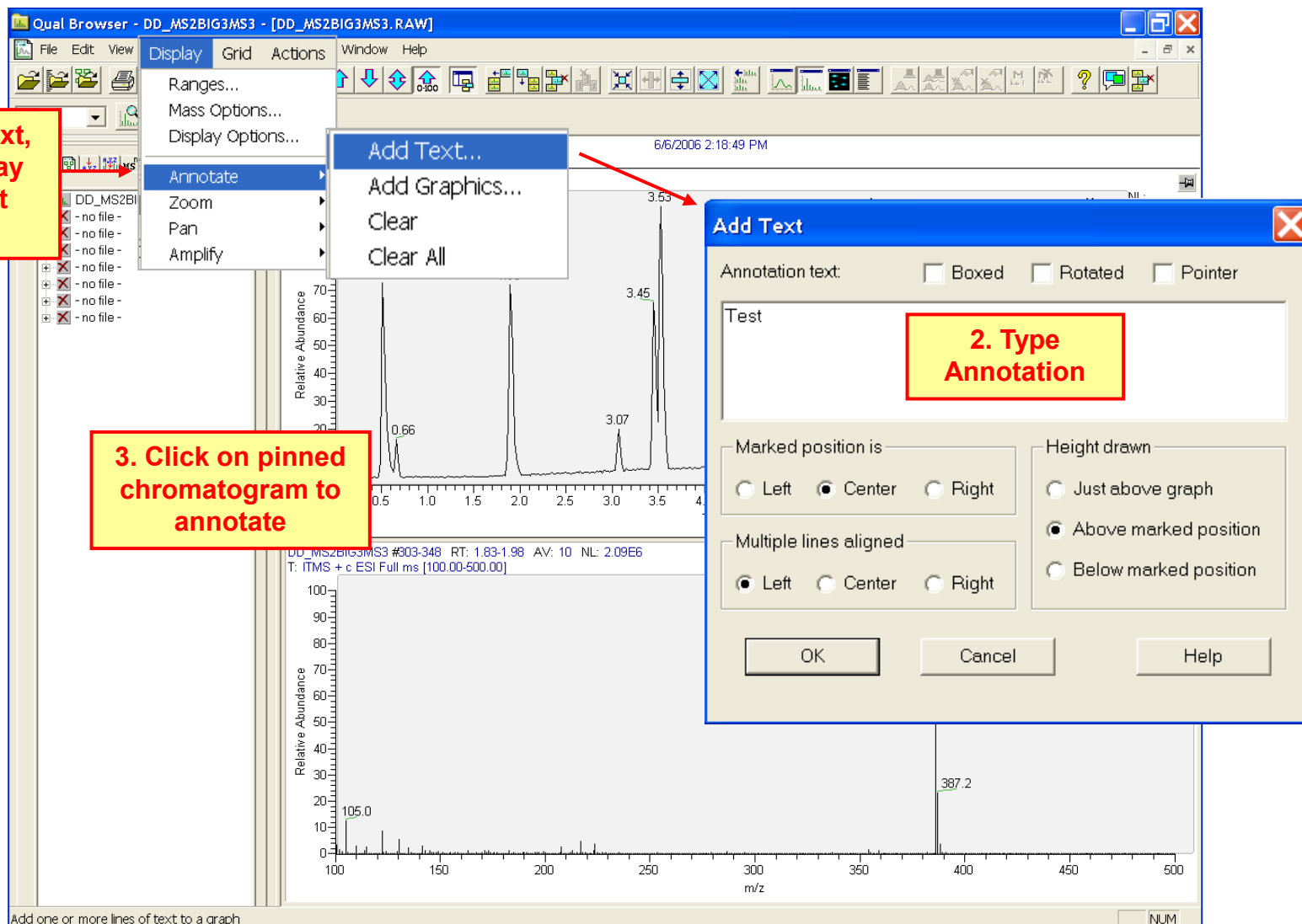
☐ Time range 2:

☐ Simulation

OK Cancel Help

The Spectrum Ranges box is similar to the Chromatogram Ranges box. Can also enable Background Subtraction for the spectrum here.

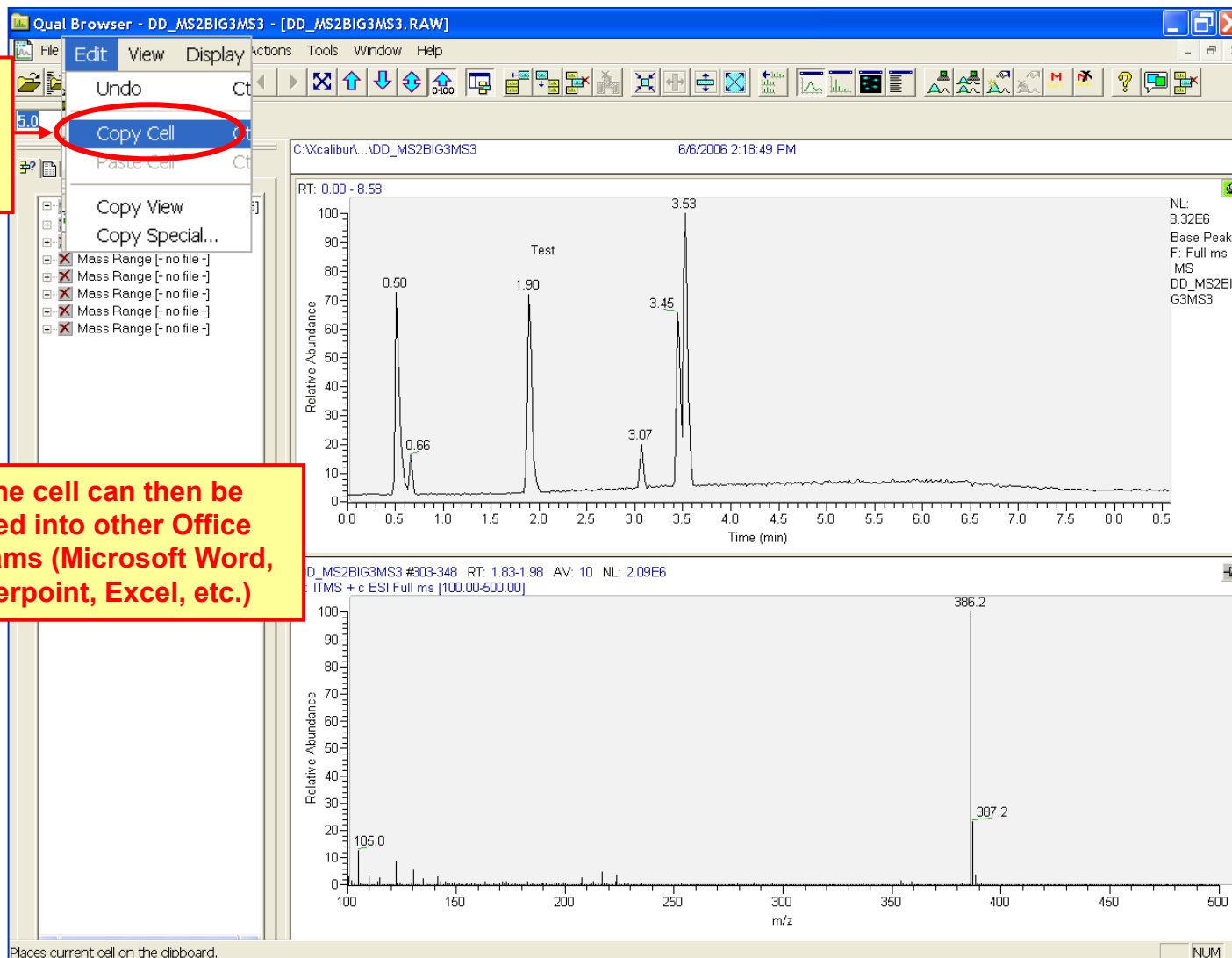
Presentation



Chromatogram Capture

1. Go to Edit
> Copy Cell
to copy the
pinned cell

2. The cell can then be
pasted into other Office
programs (Microsoft Word,
Powerpoint, Excel, etc.)





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Chapter 11

Quantitative Processing

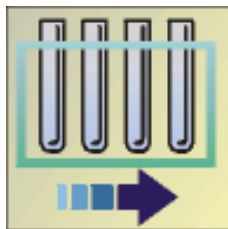
Quantitative Processing

1. Processing Setup



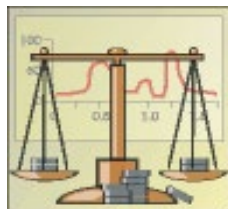
Input known compounds for identification
Set up peak detection/integration parameters
Choose calibration/QC type, levels, weighting
Select advanced chromatographic processing

2. Sample Processing/Reprocessing



Input new sequence setup parameters
Identify calibration file and bracketing type
Process/Reprocess data

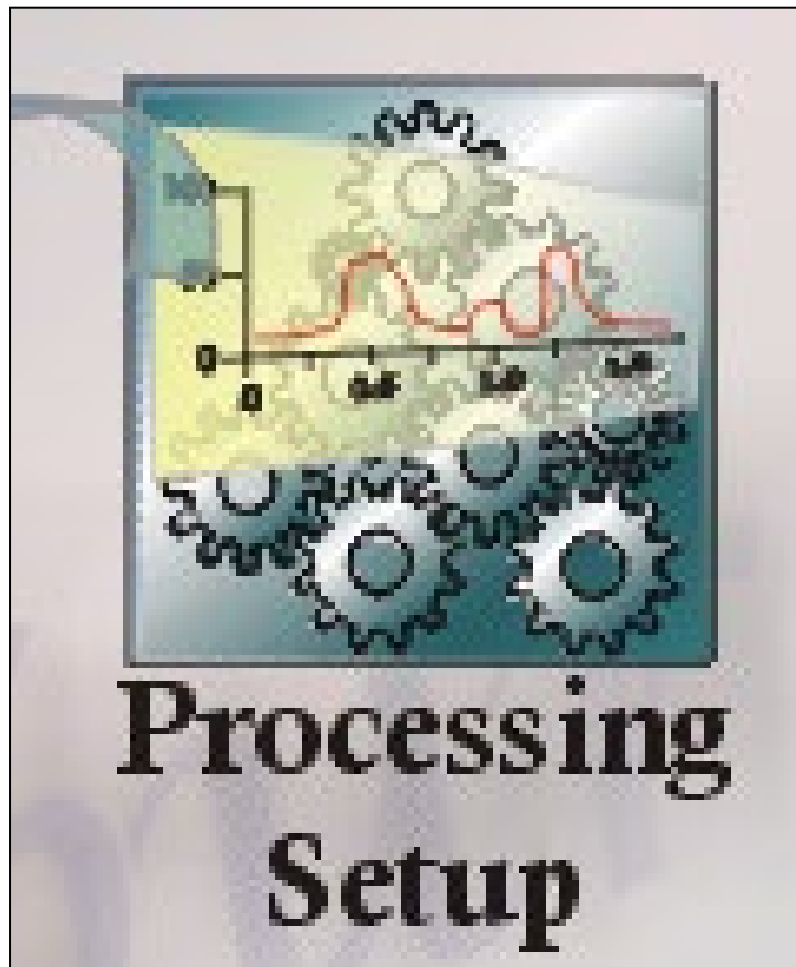
3. Quan Browser



View quantitative results
Evaluate standard curve, QCs, and flags
Recalculate peaks with different parameters
Analyze detailed quantitation information

Quan Processing Setup

Click Processing Setup
button on the Xcalibur
Homepage to begin
setting up the quantitative
processing method



Quantitation Options

1. Click Options

2. Click Chromatography By...

3. Select LC

4. Select Calibration Options...

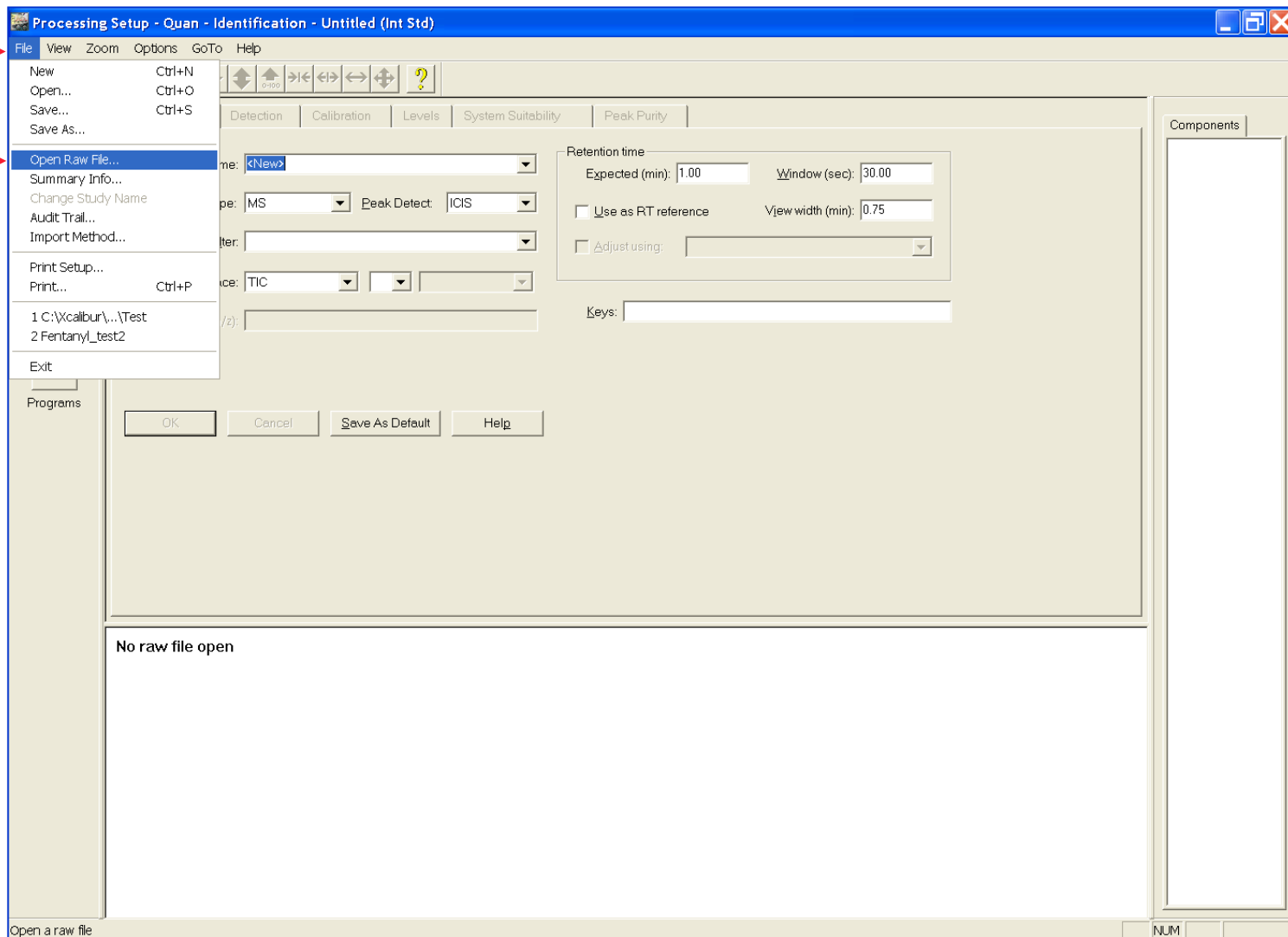
5. Choose whether to use an Internal or External standard

The image shows a software interface for quantitation. The main window is titled 'Processing Setup - Quan - Identification - Test (Int Std)'. It has a menu bar with 'File', 'View', 'Zoom', 'Options', 'GoTo', and 'Help'. Below the menu bar is a toolbar with various icons. A red circle highlights the 'Options' menu item. A red arrow points from the 'Options' menu to the 'Chromatography By...' option. Another red arrow points from the 'Chromatography By...' option to the 'LC' radio button in the 'Chromatography Options' dialog box. A third red arrow points from the 'Calibration Options...' option in the 'Options' menu to the 'Calibration Options' dialog box. A fourth red arrow points from the 'Internal standard' radio button in the 'Calibration Options' dialog box to a yellow box containing the text '5. Choose whether to use an Internal or External standard'. The 'Chromatography Options' dialog box has a title bar with a close button (X). It contains a section titled 'Chromatography by' with two radio buttons: 'GC' and 'LC'. The 'LC' radio button is selected. Below the radio buttons are four buttons: 'OK', 'Cancel', 'Save As Default', and 'Help'. The 'Calibration Options' dialog box also has a title bar with a close button (X). It contains a section titled 'Calibration by' with two radio buttons: 'Internal standard' and 'External standard'. The 'Internal standard' radio button is selected. A red bracket groups the two radio buttons.

Open a Raw File to Set Up the Processing Method

1. Click File

2. Click Open Raw File...



Quan Processing – Identification Tab

Processing Setup - Quan - Identification - Untitled (Int Std)

File View Zoom Options GoTo Help

1. Select <New> and type name of component

2. Select Detector type and Peak Detection algorithm

3. Select scan filter and trace type

4. Click OK to update chromatogram

Identification

Name: Steroid_X

Detector type: MS Peak Detect: ICIS

Filter: ITMS + c APCI corona Full ms2 315.30@cid35.00

Trace: TIC

Retention time Expected

Use as

Adjust using

Mass (m/z):

Keys:

OK Cancel Save As Default Help

Components

Steroid_X

Chromatogram changes to reflect scan filter and trace type selected

SteroidQuantitation-5-30-0606 5/30/2006 1:22:45 PM

RT: 0.00 - 9.99 SM: 1G

100

80

20

0

0 1 2 3 4 5 6 7 8 9

Time (min)

3.22 4.34 4.75 5.59 5.99 7.24 8.04 8.83 9.57

NL: 6.61E5

TIC F: ITMS + c APCI corona Full ms2 315.30@cid35.00 [85.00-330.00] MS SteroidQuantitation-5-30-0606

SteroidQuantitation-5-30-0606 #467 RT: 2.91 AV: 1 NL: -

F: ITMS + c APCI corona Full ms2 315.30@cid35.00 [85...

100

80

60

40

20

0

100 150 200 250 300

m/z

96.94240 171.14845 215.14893 279.25766 297.20245

NUM NOT SAVED

Quan Processing – Identification Tab

The screenshot shows the 'Processing Setup - Quan - Identification - Untitled (Int Std)' window. The 'Identification' tab is selected. A red circle highlights the 'Identification' tab. A red arrow points to the 'Expected (min):' field in the 'Retention time' section, with a callout box stating '1. Populate Expected RT (min)'. Another red arrow points to the 'Window (sec):' field, with a callout box stating 'Window (sec) = allowable RT window for component elution'. A third red arrow points to the 'Use as RT reference' checkbox, with a callout box stating 'If using an internal standard, you can select to use it as a RT reference'. A fourth red arrow points to the 'OK' button, with a callout box stating '2. Click OK to update chromatogram'. The window displays two plots: a Total Ion Chromatogram (TIC) on the left and a mass spectrum on the right. The TIC plot shows a single sharp peak at 8.04 minutes. The mass spectrum plot shows several peaks, with the base peak at m/z 297.20245. The status bar at the bottom indicates 'Ready' and 'NOT SAVED'.

Processing Setup - Quan - Identification - Untitled (Int Std)

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Retention time

Expected (min): 1.00 Window (sec): 30.00

☐ Use as RT reference View width (min): 0.75

☐ Adjust using: [dropdown]

Keys: [text box]

Trace: TIC [dropdown] [dropdown] [dropdown]

Mass (m/z): [text box]

OK Cancel Save As Default Help

SteroidQuantitation-5-30-0606 5/30/2006 1:22:45 PM

RT: 0.00 - 9.99 SM: 1G

Relative Abundance

Time (min)

8.04

3.22 4.34 4.75 5.59 5.99 7.24 8.83 9.57

NL: 6.61E5
TIC F: ITMS + c APCI
corona Full ms2
315.30@cid35.00
[85.00-330.00] MS
SteroidQuantitation-5-30-0606

SteroidQuantitation-5-30-0606 #467 RT: 2.91 AV: 1 NL: [text box]

F: ITMS + c APCI corona Full ms2 315.30@cid35.00 [85.00-330.00] MS

Relative Abundance

m/z

96.94240 171.14845 215.14893 279.25766 297.20245

Ready NUM NOT SAVED

***Note:** If using an internal standard, the ISTD component should be set up first since all target components will refer to the ISTD. For all other components, you can select 'Adjust using' and choose the ISTD name.

Quan Processing – Detection Tab

1. Click on Detection tab

2. Modify parameters to achieve satisfactory peak integration

3. Click OK for changes to update on chromatogram

Processing Setup - Quan - Detection - Untitled (Int Std)

File View Zoom Options GoTo Help

Detection Calibration Levels System Suitability Peak Purity

ICIS Peak Integration

Smoothing points: 7

Baseline window: 150

Area noise factor: 5

Peak noise factor: 10

☐ Constrain peak width

Peak height (%): 5.0

Tailing factor: 1.0

ICIS Peak Detection

☒ Highest peak

OK Cancel Save As Default Advanced... Flags... Help

SteroidQuantitation-5-30-0606 5/30/2006 1:22:45 PM

RT: 7.05 - 9.05 SM: 7G

Relative Abundance

RT: 8.05

NL: 6.45E5

TIC F: ITMS + c APCI corona Full ms2

315.30@cid35.00

[85.00-330.00] MS ICIS

SteroidQuantitation-5-30-0606

Relative Abundance

RT: 8.05 AV: 1 NL: 6.45E5

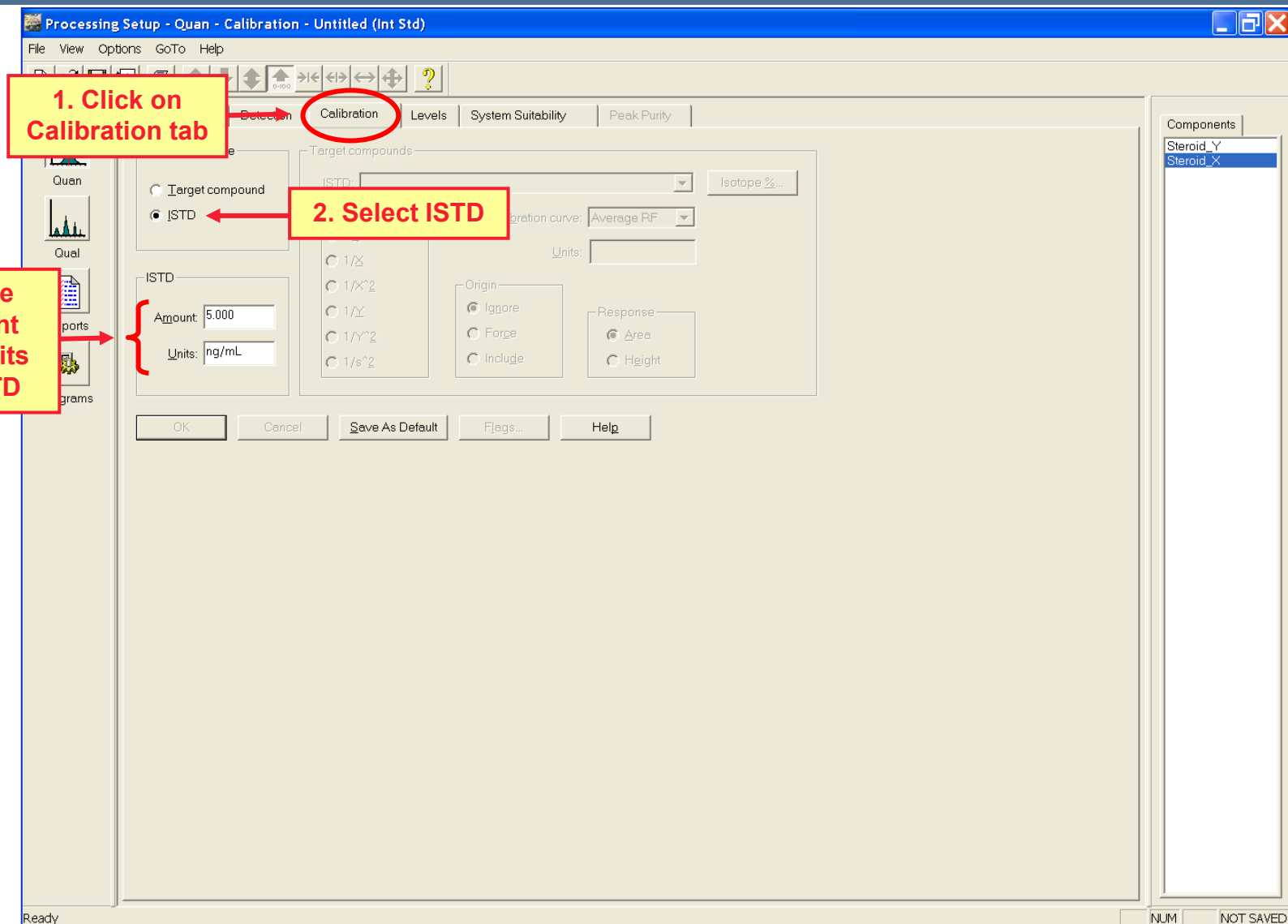
F: ITMS + c APCI corona Full ms2 315.30@cid35.00 [85.00-330.00]

96.94200 108.94489 173.11150 215.23209 279.26367 297.23602

NUM NOT SAVED

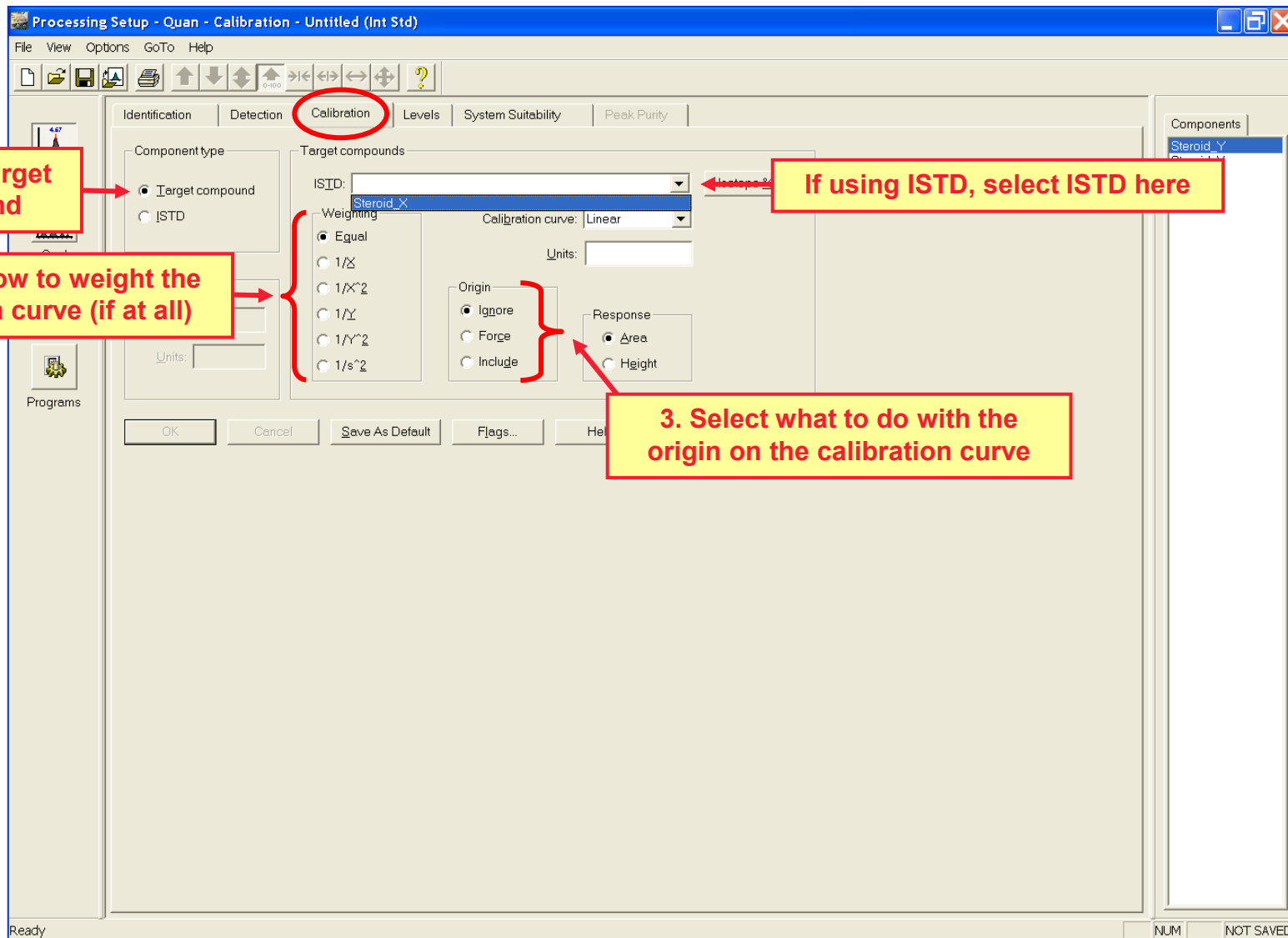
Quan Processing - Calibration Tab

Internal Standard Setup



Quan Processing - Calibration Tab

Target Compound Setup



Quan Processing – Levels Tab

Processing Setup - Quan - Levels - Untitled (Int Std)

File View Options GoTo Help

1. Click on Levels tab

2. Enter name for each calibration level

	Cal Level	Amount
1	2 ng/mL	2.000
2	10 ng/mL	10.000
3	50 ng/mL	50.000
4	200 ng/mL	200.000
5	1000 ng/mL	1000.000
*		0.000

3. Enter amount for each calibration level

QC Level Amount % Test

1	QC1	5.000	20.00
*		0.010	0.00

4. Enter name, amount and % Test for each QC level

OK Cancel Save As Default Help

Ready

NUM NOT SAVED

Copying Levels to All Target Compounds

Processing Setup - Quan - Levels - Manual (Int Std)

File View Options GoTo Help

Identification Detection Calibration **Levels** System Suitability Peak Purity

Units:

	Cal Level	Amount
1	2 ng/mL	2.000
2	10 ng/mL	10.000
3	50 ng/mL	50.000
4	200 ng/mL	200.000
5	1000 ng/mL	1000.000
*		

	QC Level	Amount	% Test
1	QC1	5.000	20.00
*			

Delete Rows
Insert Row
Copy Levels to All Target Components

Delete Rows
Insert Row
Copy Levels to All Target Components

OK

Components
Steroid_Y
Steroid_X

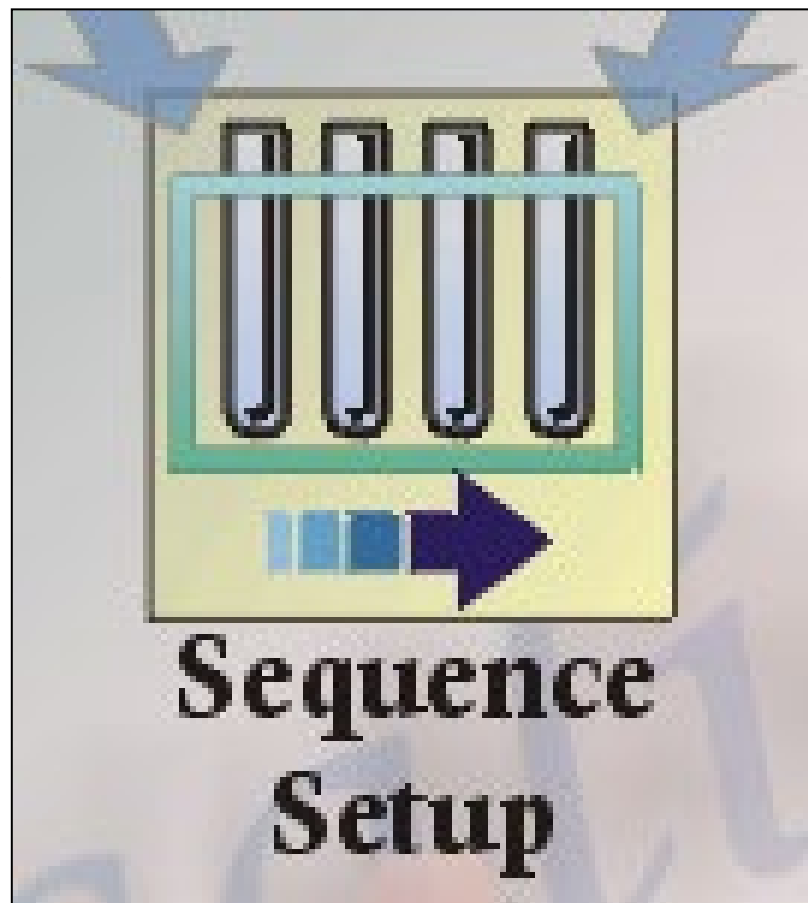
Ready

NUM

The information in the Levels tab only needs to be entered for one target compound. To copy the levels to the other compounds or QCs, right-click and select 'Copy Levels to All Target Components'.

Quan Processing/Reprocessing

Click Sequence Setup button to open the sequence and add information before processing/reprocessing



Open the Sequence and Add Extra Columns

1. Click Change and select Column Arrangement

TempSequence_060530121729 [Open] - Sequence Setup - Home Page

File Edit Change Actions View GoTo Help

User Labels...
Tray Name...
Column Arrangement...
Transfer Row Info...

Sample ID Path Inst Meth Proc Meth Position Inj Vol Level

1	5-30-0601	1A1	C:\Xcalibur\Data\Orbitrap Data\SteroidQuant	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A1	20.0	
2	5-30-0602	1A2	C:\Xcalibur\Data\Orbitrap Data\SteroidQuant	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A2	20.0	Cal1
3	Std Bracket	SteroidQuantitation-5-30-0603	1A3	C:\Xcalibur\data\Ort	1A3	20.0	Cal2	
4	Std Bracket	SteroidQuantitation-5-30-0604	1A4	C:\Xcalibur\data\Ort	1A4	20.0	Cal3	
5	Std Bracket	SteroidQuantitation-5-30-0605	1A5	C:\Xcalibur\data\Ort	1A5	20.0	Cal4	
6	Std Bracket	SteroidQuantitation-5-30-0606	1A6	C:\Xcalibur\data\Ort	1A6	20.0	Cal5	
7	Blank	SteroidQuantitation-5-30-0607	1A1	C:\Xcalibur\data\Ort	1A1	20.0		
8	QC	SteroidQuantitation-5-30-0608	1A8	C:\Xcalibur\data\Ort	1A8	20.0	Low	
9	QC	SteroidQuantitation-5-30-0609	1B1	C:\Xcalibur\data\Ort	1B1	20.0	Mid	
10	QC	SteroidQuantitation-5-30-0610	1B2	C:\Xcalibur\data\Ort	1B2	20.0	High	
11	B							
12	U							
*							0.1	

Column Arrangement

Available Columns

- Dil Factor
- ISTD Corr Amt
- Laboratory
- Level**
- Proc Meth**
- Sample Type**
- Sample Vol
- Sample Wt
- SampleName
- Study

Displayed Columns

- File Name
- Path
- Inst Meth
- Position
- Inj Vol

Add Remove Move Up Move Down


OK Cancel Help

2. Add Level, Proc Meth and Sample Type columns into the sequence

Enter Information into the Sequence

TempSequence_060530121729 [Open] - Sequence Setup - Home Page

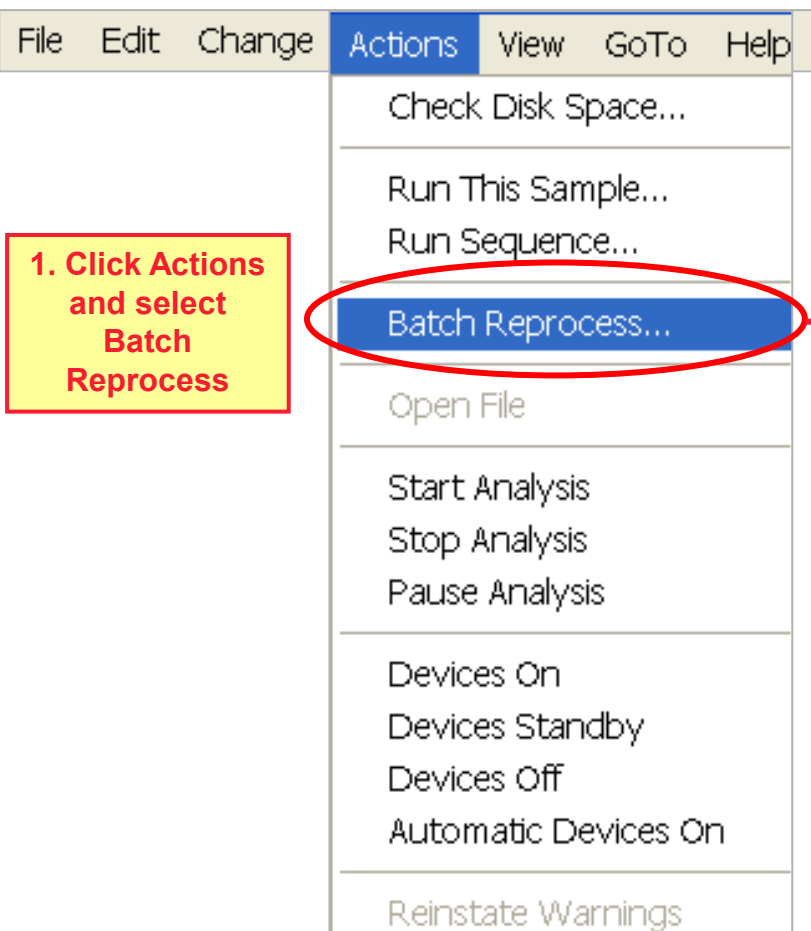
File Edit Change Actions View GoTo Help



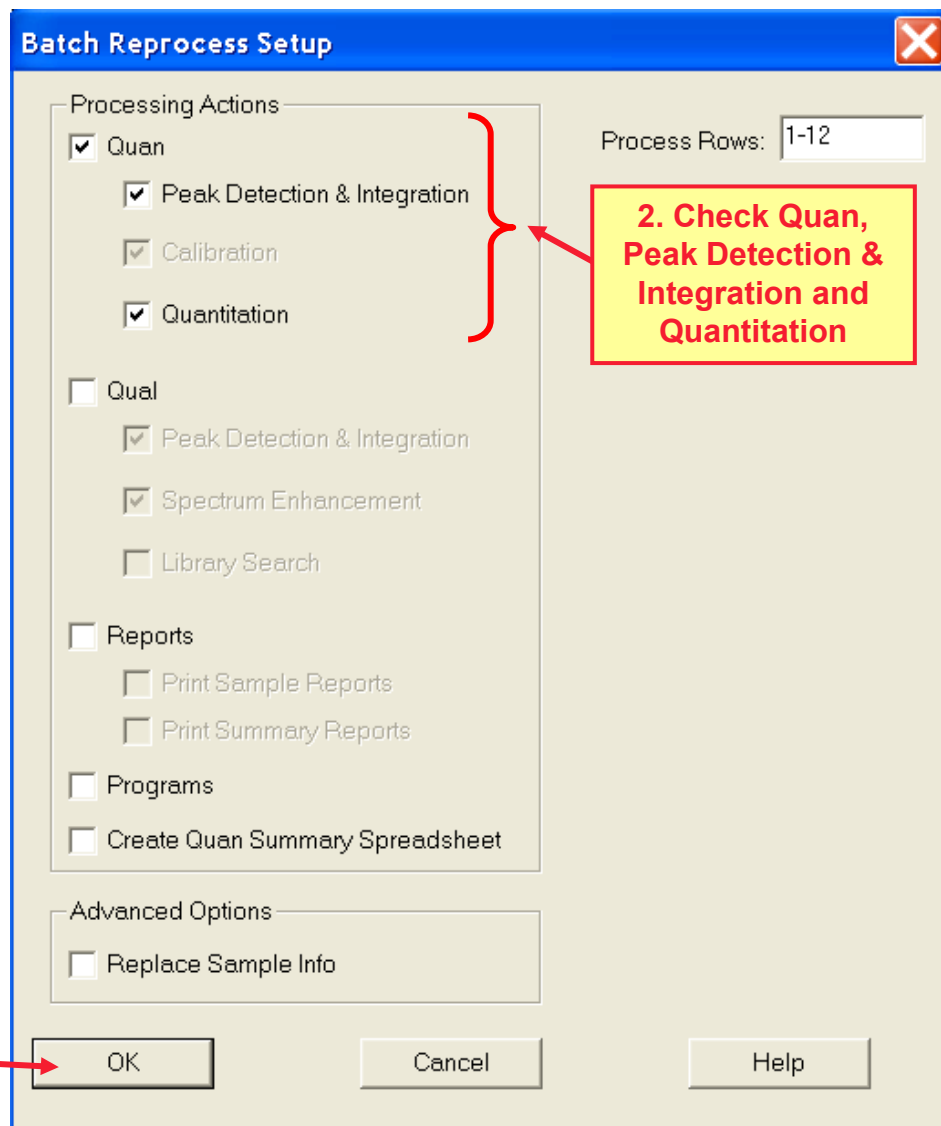
	File Name	Path	Inst Meth	Position	Inj Vol	Proc Meth	Sample Type	Level
1	SteroidQuantitation-5-30-0601	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0601	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1A1	20.0	C:\Xcalibur\data\Ork\Blank		
2	SteroidQuantitation-5-30-0602	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0602	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1A2	20.0	C:\Xcalibur\data\Ork\Std Bracket	Cal1	
3	SteroidQuantitation-5-30-0603	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0603	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1A3	20.0	C:\Xcalibur\data\Ork\Std Bracket	Cal2	
4	SteroidQuantitation-5-30-0604	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0604	C:\Xcalibur\methods\Amber\SteroidQuan_IT		20.0	C:\Xcalibur\data\Ork\Std Bracket	Cal3	
5	SteroidQuantitation-5-30-0605	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0605	C:\Xcalibur\methods\Amber\SteroidQuan_IT		20.0	C:\Xcalibur\data\Ork\Std Bracket	Cal4	
6	SteroidQuantitation-5-30-0606	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0606	C:\Xcalibur\methods\Amber\SteroidQuan_IT		20.0	C:\Xcalibur\data\Ork\Std Bracket	Cal5	
7	SteroidQuantitation-5-30-0607	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0607	C:\Xcalibur\methods\Amber\SteroidQuan_IT		20.0	C:\Xcalibur\data\Ork\Blank		
8	SteroidQuantitation-5-30-0608	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0608	C:\Xcalibur\methods\Amber\SteroidQuan_IT		20.0	C:\Xcalibur\data\Ork\QC	Low	
9	SteroidQuantitation-5-30-0609	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0609	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1B1	20.0	C:\Xcalibur\data\Ork\QC	Mid	
10	SteroidQuantitation-5-30-0610	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0610	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1B2	20.0	C:\Xcalibur\data\Ork\QC	High	
11	SteroidQuantitation-5-30-0611	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0611	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1A1	20.0	C:\Xcalibur\data\Ork\Blank		
12	SteroidQuantitation-5-30-0612	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantitation-5-30-0612	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1B4	20.0	C:\Xcalibur\data\Ork\Unknown		
*					0.1			

Populate the Proc Meth, Sample Type and Level columns in the sequence

Batch Reprocessing Quantitative Data



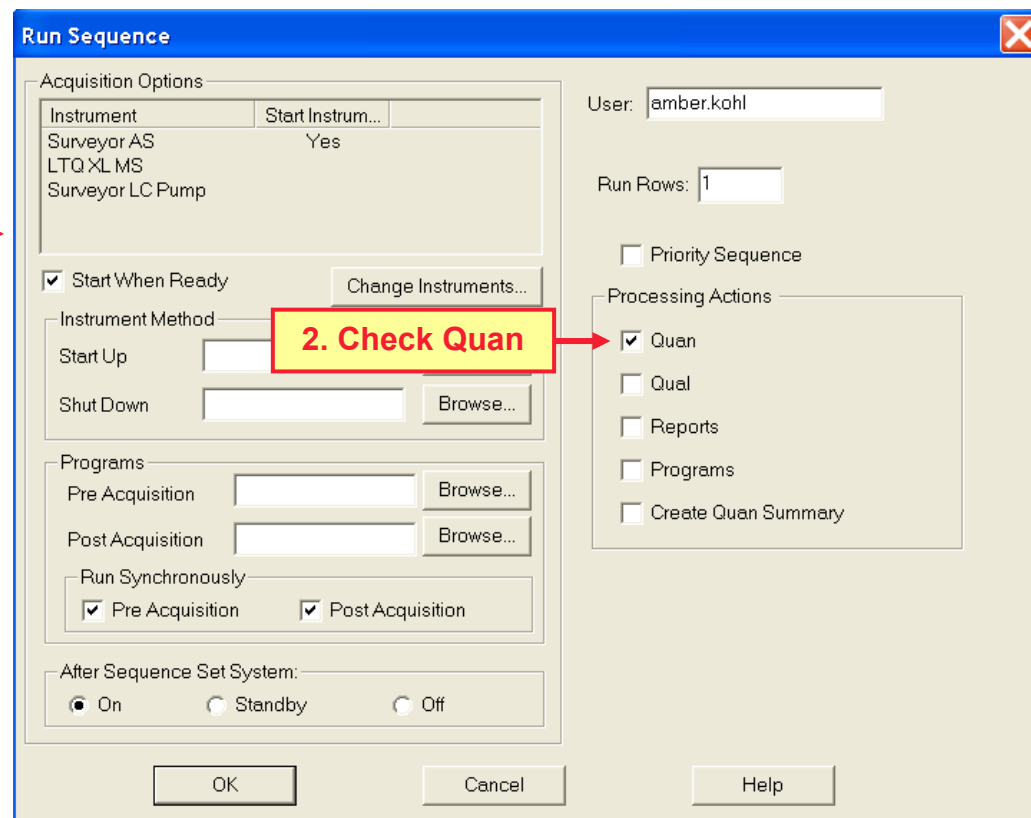
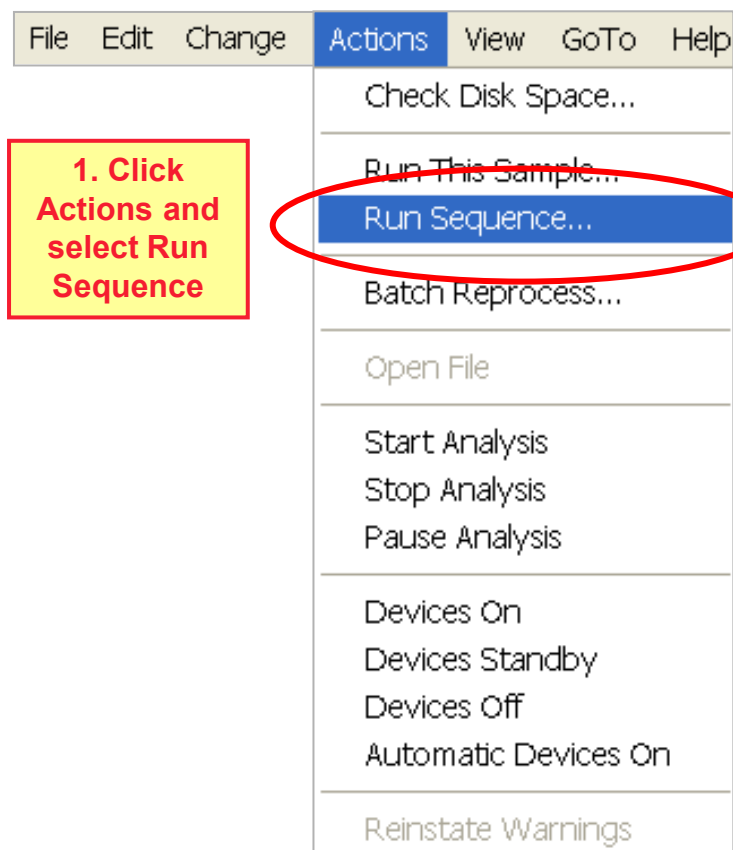
1. Click Actions and select Batch Reprocess



2. Check Quan, Peak Detection & Integration and Quantitation

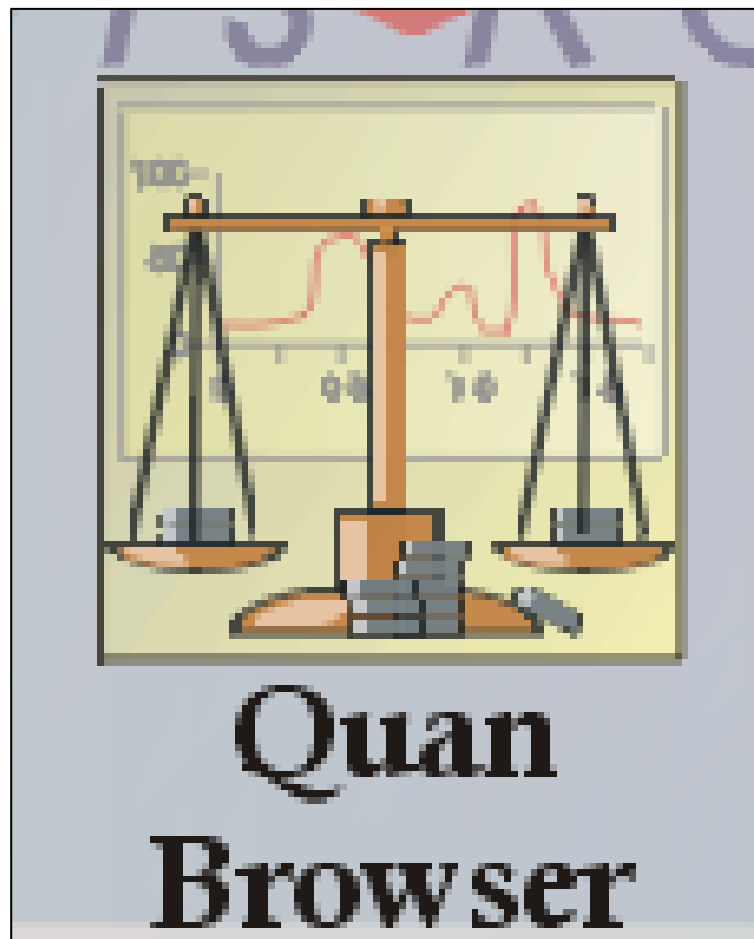
3. Click OK to process

Enabling Quantitative Processing During Acquisition

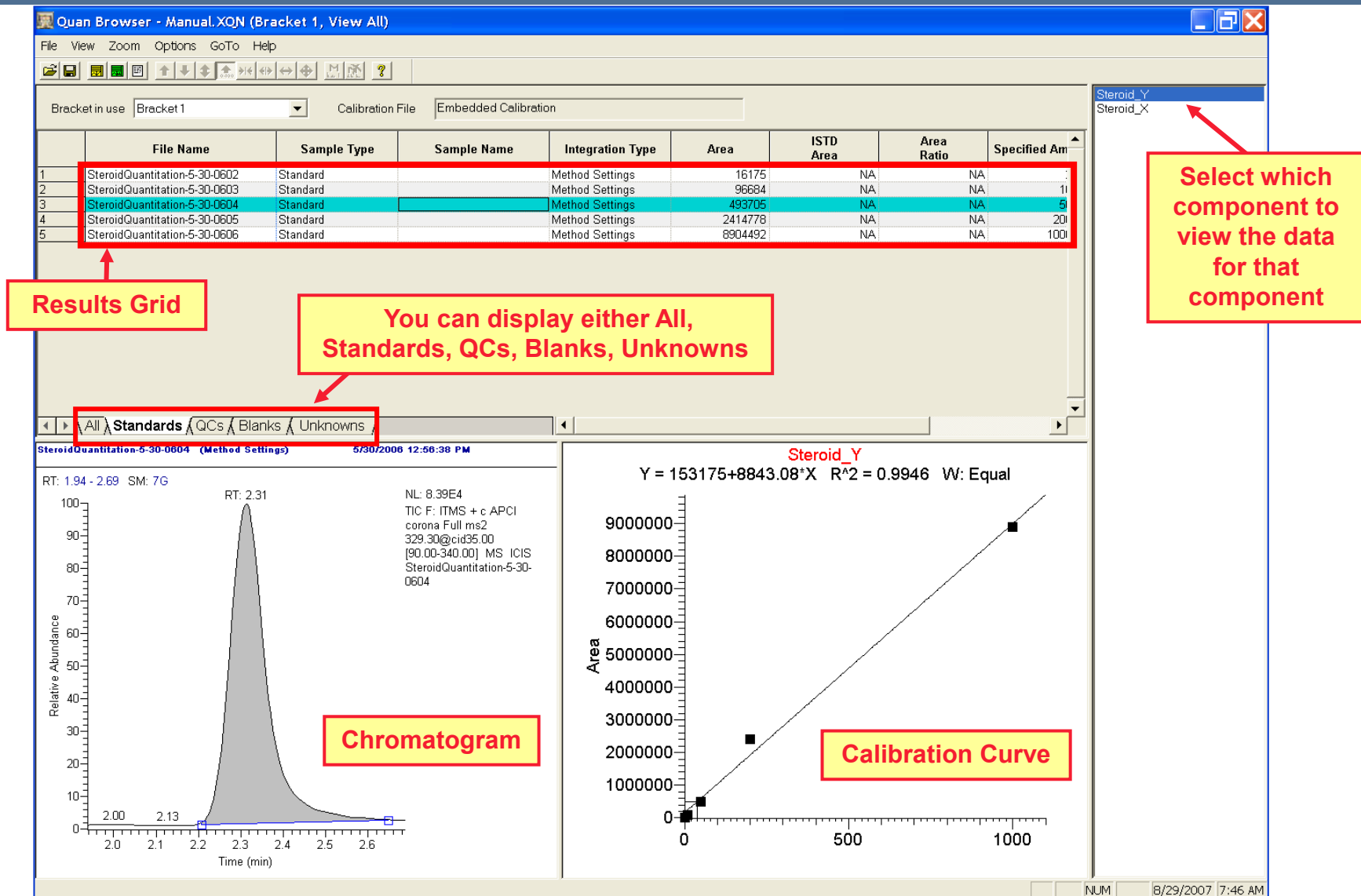


Quan Browser

To view the
processed data,
click on the Quan
Browser button on
the Xcalibur
Homepage



Quan Browser Main View



Changing the Results Grid Display

The screenshot displays the Quan Browser interface. The main window shows a table of results with columns: File Name, Sample Type, Sample Name, and Integration Type. A context menu is open over the table, with options: Columns..., Delete Selected Samples, Add Sample..., Copy Row, Set Sorting Order..., and Send to Qual Browser. Two dialog boxes are also shown: 'Result List Column Hiding' and 'Quantitation Results Sorting Order'. A yellow callout box points to the 'Columns...' option in the context menu, and another yellow callout box points to the 'Set Sorting Order...' option. A third yellow callout box points to the 'Result List Column Hiding' dialog box, and a fourth yellow callout box points to the 'Quantitation Results Sorting Order' dialog box. The bottom of the screen shows a chromatogram plot of Relative Abundance vs. Time (min) with a peak at RT: 2.31. The status bar at the bottom right shows the date and time: 8/29/2007 7:46 AM.

1. To change what is displayed in the Results Grid, right-click on the Results Grid

Can change the columns that are displayed in the Results Grid

Can change the sorting order of the columns

	File Name	Sample Type	Sample Name	Integration Type
1	SteroidQuantitation-5-30-0602	Standard		Method Settings
2	SteroidQuantitation-5-30-0603	Standard		Method Settings
3	SteroidQuantitation-5-30-0604	Standard		Method Settings
4	SteroidQuantitation-5-30-0605	Standard		Method Settings
5	SteroidQuantitation-5-30-0606	Standard		Method Settings

RT: 1.94 - 2.69 SM: 7G

RT: 2.31

Area

Relative Abundance

Time (min)

NL: 8.39E4
TIC F: ITMS + c APCI
corona Full ms2
329.30@cid35.00
[90.00-340.00] MS ICIS
SteroidQuantitation-5-30-0604

Result List Column Hiding

Selected Columns

- ☒ File Name
- ☒ Sample Type
- ☒ Sample Name
- ☒ Integration Type
- ☒ Area/Height
- ☒ ISTD Area/Height
- ☒ Area/Height Ratio
- ☒ Specified Amount
- ☒ Calculated Amount
- ☒ Percent Difference
- ☐ Percent RSD
- ☐ Peak Status
- ☒ Levels
- ☐ Units
- ☒ Retention Time
- ☐ Sample ID
- ☒ Exclude

OK Cancel Help

Quantitation Results Sorting Order

Sorting

First Order: Acquisition Date

☐ Sort in descending order

Second Order: File Name

☐ Sort in descending order

Third Order: Level Name

☐ Sort in descending order

OK Cancel Save As Default Help

Changing Peak Detection/Integration Parameters

4. Integration Type changes to User Integration

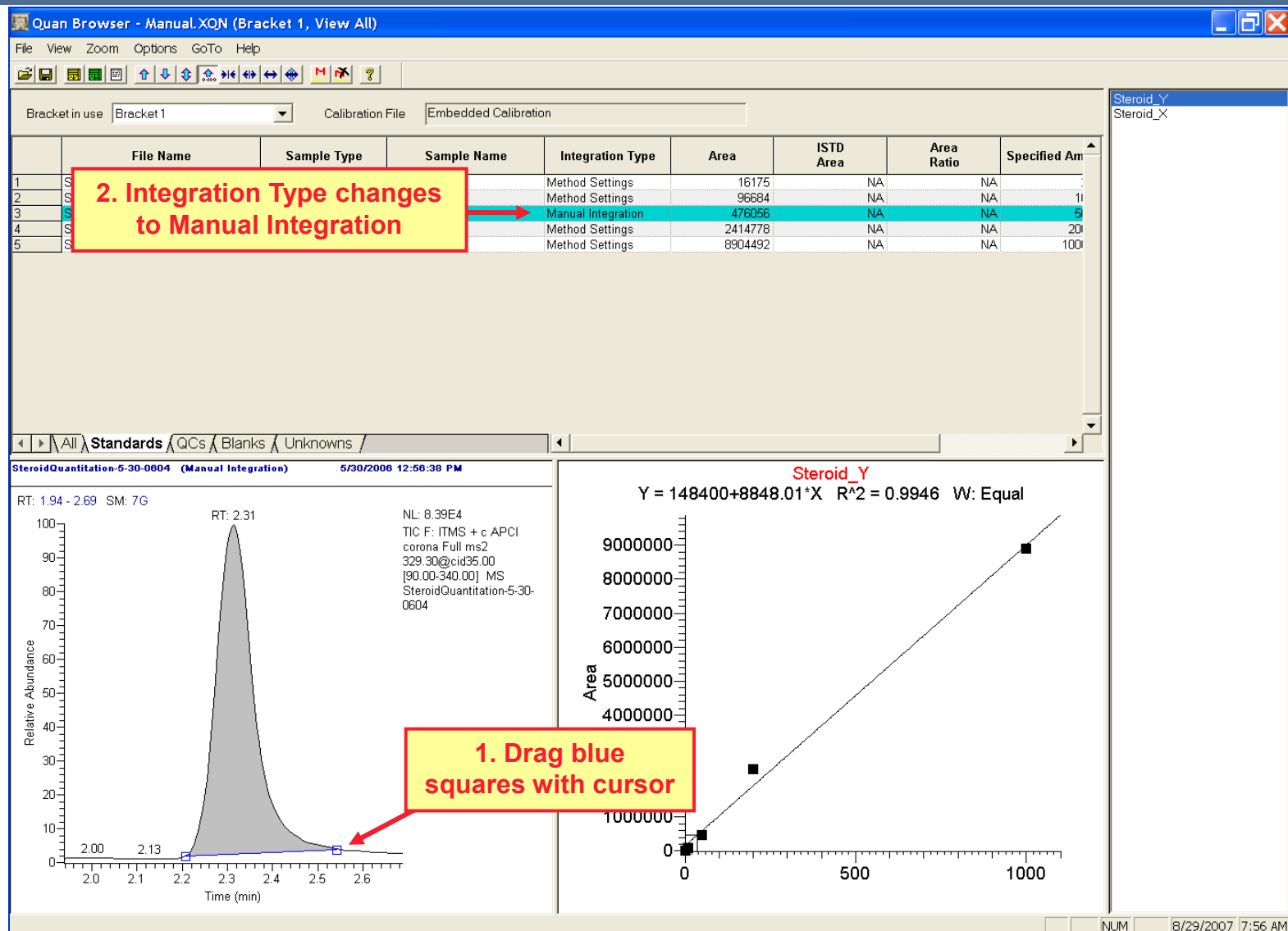
2. Change settings (most used settings are in Identification and Integration tabs)

1. To change how the peaks are detected and integrated, right-click on the chromatogram

User Peak Detection Settings...

3. Click to Apply new settings to the selected plot or to Apply to All Plots

Changing Peak Integration Parameters Manually



Changing Back to Original Integration (Method Settings)

Quan Browser - Manual.XQN (Bracket 1, View All)

File View Zoom Options GoTo Help

Bracket in use: Bracket 1 Calibration File: Embedded Calibration

	File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specified Am
1				Method Settings	16175	NA	NA	
2				Method Settings	96684	NA	NA	11
3				Method Settings	493705	NA	NA	5
4				Method Settings	2414778	NA	NA	20
5				Method Settings	8904492	NA	NA	100

2. Integration Type changes back to Method Settings

1. If you want to change the integration settings back to when the data was first opened, click Method Settings

Method Settings
User Settings
Manual Integration
Show Peak Info...
User Peak Detection Settings...
Display Options...
Manually Add Peak
Set Peak to Not Found Status
Update Expected Retention Time
Reset Scaling

Relative Abundance

Time (min)

2.00 2.13

500 1000

Steroid_Y

175+8843.08*X R^2 = 0.9946 W: Equal

NUM 8/29/2007 8:51 PM

Changing Calibration Parameters

Quan Browser - Manual.XQN (Bracket 1, View All)

File View Zoom Options GoTo Help

Bracket in use: Bracket1 Calibration File: Embedded Calibration

	File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specifi
1	SteroidQuantitation-5-30-0602	Standard		Method Settings	16175	NA	NA	NA
2	SteroidQuantitation-5-30-0603	Standard		Method Settings	96684	NA	NA	NA
3	SteroidQuantitation-5-30-0604	Standard		Method Settings	493705	NA	NA	NA
4	SteroidQuantitation-5-30-0605	Standard		Method Settings	2414778	NA	NA	NA
5	SteroidQuantitation-5-30-0606	Standard		Method Settings	8904492	NA	NA	NA

RT: 1.94 - 2.69 SM: 7G

Relative Abundance

Time (min)

1. To change the calibration parameters, right-click on the calibration curve

Calibration Settings...

Save Calibration File

Exclusion List...

Show Spectrum Plot

Reset Scaling

Copy Graph

Area

Y = 153175+8843.08*X R^2 = 0.9946 W: Equal

Calibration Settings

Type Curve Levels Isotope% Flags

Calibration Curve

Linear

Origin

☒ Ignore

☐ Force

☐ Include

Response

☒ Area

☐ Height

Weighting

☒ Equal

☐ 1/X

☐ 1/X^2

☐ 1/Y

☐ 1/Y^2

☐ 1/s^2

Units:

OK Cancel Apply Help

Calibration Settings

Type Curve Levels Isotope% Flags

	Cal Level	Amount
1	2 ng/mL	2.000
2	10 ng/mL	10.000
3	50 ng/mL	50.000
4	200 ng/mL	200.000
5	1000 ng/mL	1000.000

	QC Level	Amount	% Test
1	QC1	5.000	20.00
*		0.010	0.00

Units:

OK Cancel Apply Help

Ways to Exclude a Calibration Curve Point

Quan Browser - Manual.XQN (Bracket 1, View All)

File View Zoom Options GoTo Help

Bracket in use: Bracket 1 Calibration File: Embedded Calibration

	File Name	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	% Diff	Level	RT	Exclude
1	SteroidQuantitation-5-30-0602	NA	NA	2.000	-15.492	-874.62	2 ng/mL	2.29	<input type="checkbox"/>
2	SteroidQuantitation-5-30-0603	NA	NA	10.000	-6.388	-163.88	10 ng/mL	2.29	<input type="checkbox"/>
3	SteroidQuantitation-5-30-0604	NA	NA	50.000	38.508	-22.98	50 ng/mL	2.31	<input checked="" type="checkbox"/>
4	SteroidQuantitation-5-30-0605	NA	NA	200.000	255.748	27.87	200 ng/mL	2.27	<input type="checkbox"/>
5	SteroidQuantitation-5-30-0606	NA	NA	1000.000	989.624	-1.04	1000 ng/mL	2.29	<input type="checkbox"/>

To exclude a calibration curve point, you can check to exclude in the Results Grid

RT: 1.94 - 2.69 SM: 7G

RT: 2.31 NL: 8.39F4

Relative Abundance

Time (min)

To exclude a calibration curve point, right-click on the calibration curve

Exclude

Calibration Settings...

Exclusion List...

Show Spectrum Plot

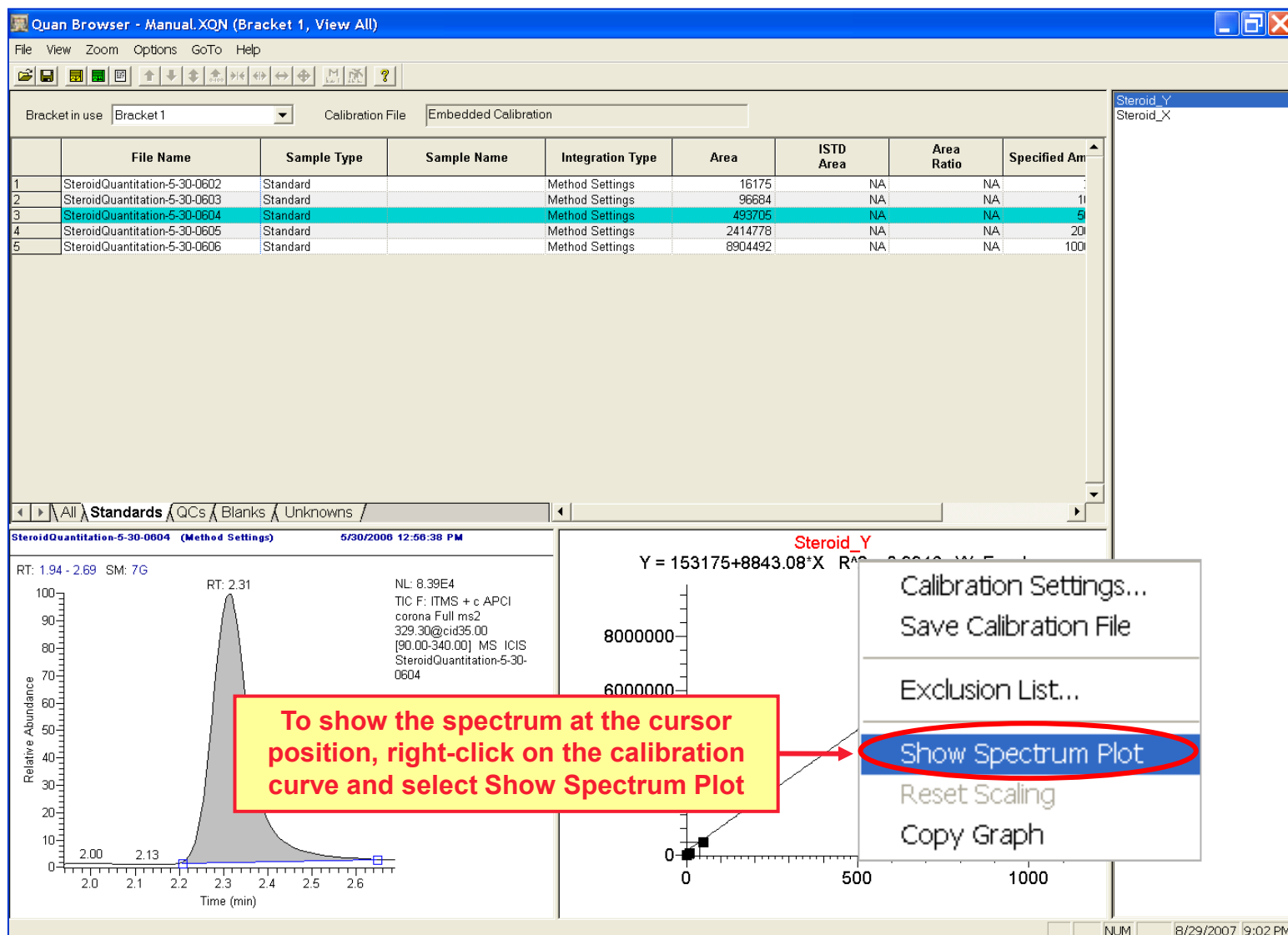
Reset Scaling

Copy Graph

Use Exclude to exclude one point at a time or the Exclusion List to exclude more than one point at a time

NUM 8/30/2007 7:53 PM

Showing the Spectrum Plot Instead of the Calibration Curve



Exporting Data to Excel and Printing Reports

Quan Browser - Manual.XQN (Bracket 1, View All)

File View Zoom Options GoTo Help

Open... Ctrl+O
Save Ctrl+S
Save As...
Save All

Export Method...
Export data to Excel
Summary Information...
Change Dataset Name...
Audit Trail...

Print Setup...
Print

1 Manual
2 TempSequence_060530121729
3 FT_List_061018172746
4 Steroid Quantitation Sequence

Exit

Calibration File Embedded Calibration

ISTD Area	Area	Specified Amount	Calculated Amount		Exclude
15.492				2.29	<input type="checkbox"/>
-6.388				2.29	<input type="checkbox"/>
38.508				2.31	<input type="checkbox"/>
255.748				2.27	<input type="checkbox"/>
989.624				2.29	<input type="checkbox"/>
NA	NA	1000.000			

Click to export data to Excel

Export Short Excel report
Export Long Excel report

Reports Dialog...

Print All Enabled Reports
Print Enabled Sample Reports
Print Enabled Summary Reports

Steroid_Y
Steroid_X

RT: 1.94 - 2.69 SM: 7G
NL: 8.39E4
TIC F: ITMS + c APCI corona Full ms2 329.30@cid35.00 [90.00-340.00] MS ICIS SteroidQuantitation-5-30-0604

RT: 2.31

Relative Abundance

Time (min)

SteroidQuantitation-5-30-0604 #255 RT: 2.31 AV: 1 NL: 1.23E4
F: ITMS + c APCI corona Full ms2 329.30@cid35.00 [90.00-340.00]

Relative Abundance

m/z

NUM 8/30/2007 8:06 PM

Printing Reports

Reports

Sample Reports - 0 selected samples

	Enabled	Std	QCs	Unks	Other	Save As	Report Template Name
	Yes	Yes	Yes	Yes	Yes	None	C:\Xcalibur\templates\QuanPeakResults_ESTD.xrt
		Yes	Yes	Yes	Yes	None	

1. Click to enable reports

2. Select report template to use

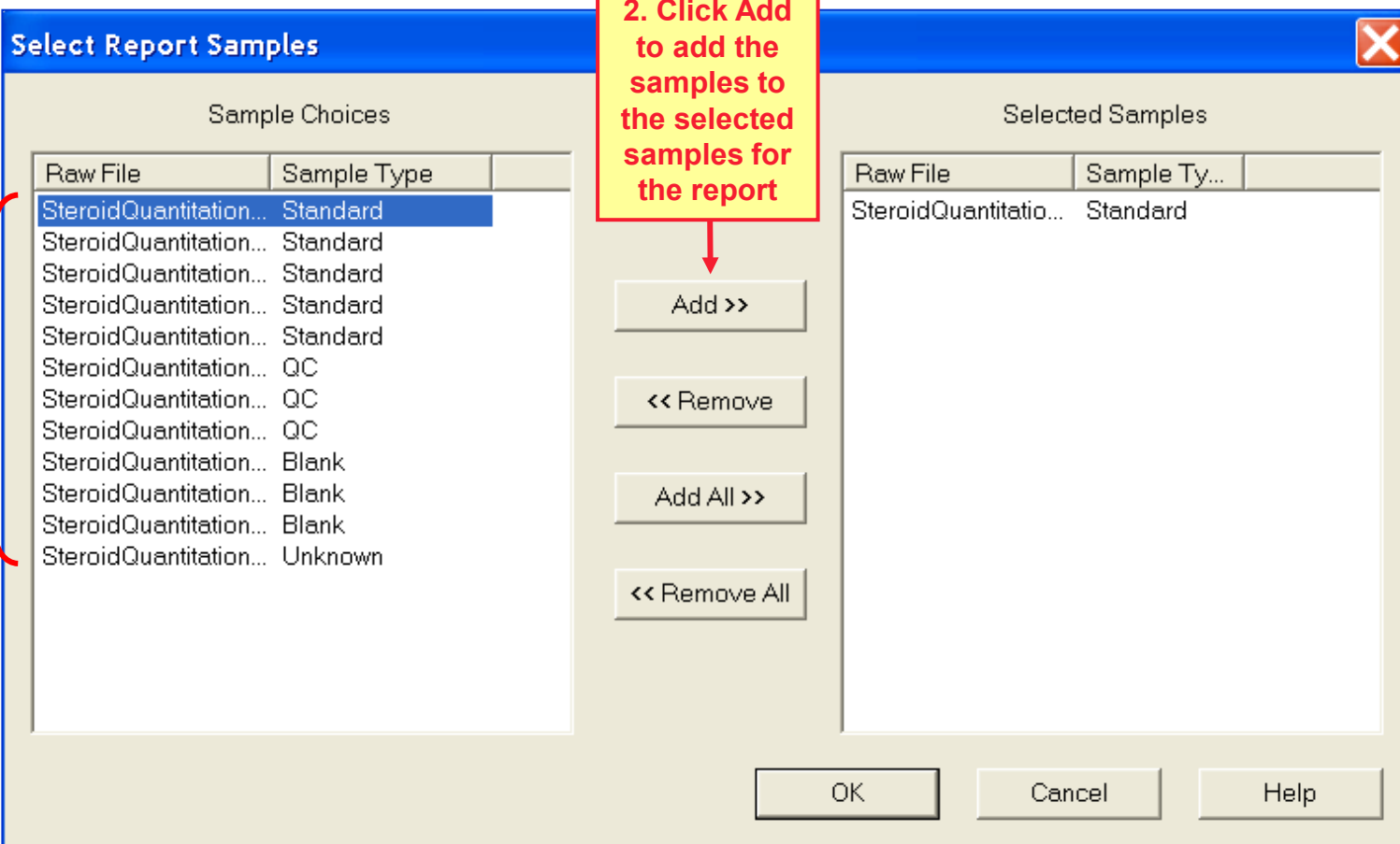
Summary Reports

	Enabled	Save As	Report Template Name
*		None	

3. Click to select samples

☒ Include Sample Reports ☒ Include Summary Reports

Selecting Samples to Include in the Report



ThermoFisher
SCIENTIFIC

The world leader in serving science

XReport 1.0



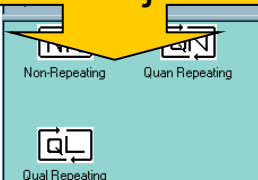
XReport 1.0 :

The Reporting Application for Xcalibur 2.0

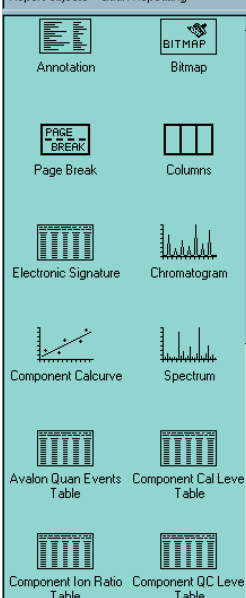
- **Simple to create your report templates!**
- **Report as DOC, TXT ,HTML, RTF,**
- **Configurable properties (i.e. size, decimal places, chromatogram summaries, etc.) of objects and sections**

Drag and Drop Interface: Quan Peak Results Canned Template

Available Sections and Objects



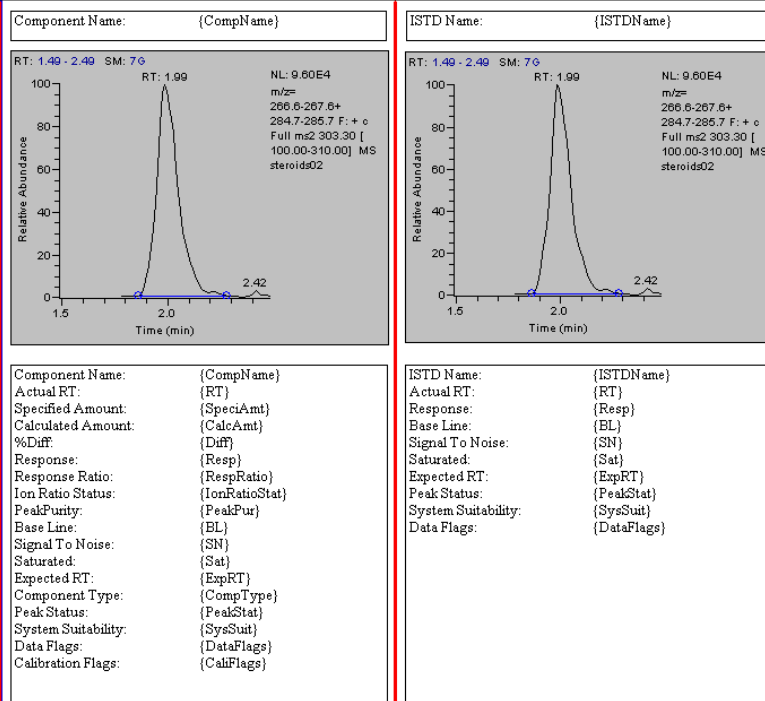
Report objects - Quan Repeating



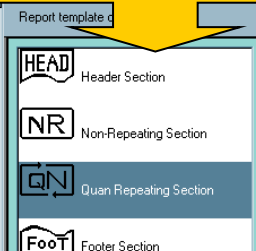
Report Template

Component Name[A]	Component Name[B]	Component Name[C]	Area[D]	Peak Status[E]

Section End



Report Outline



Steps to XReport Reporting

1. XReport



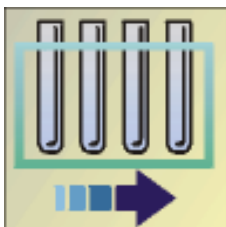
- Open XReport 1.0
- Drag and Drop required items into appropriate fields
- Specify Data Sources to view example report
- Save Report Template

2. Processing Setup



- Open Processing Setup
- Click on the Reports Icon
- Enable Reports and Select the Report Template
- Save the Processing Setup

3. Reprocess Selected Files



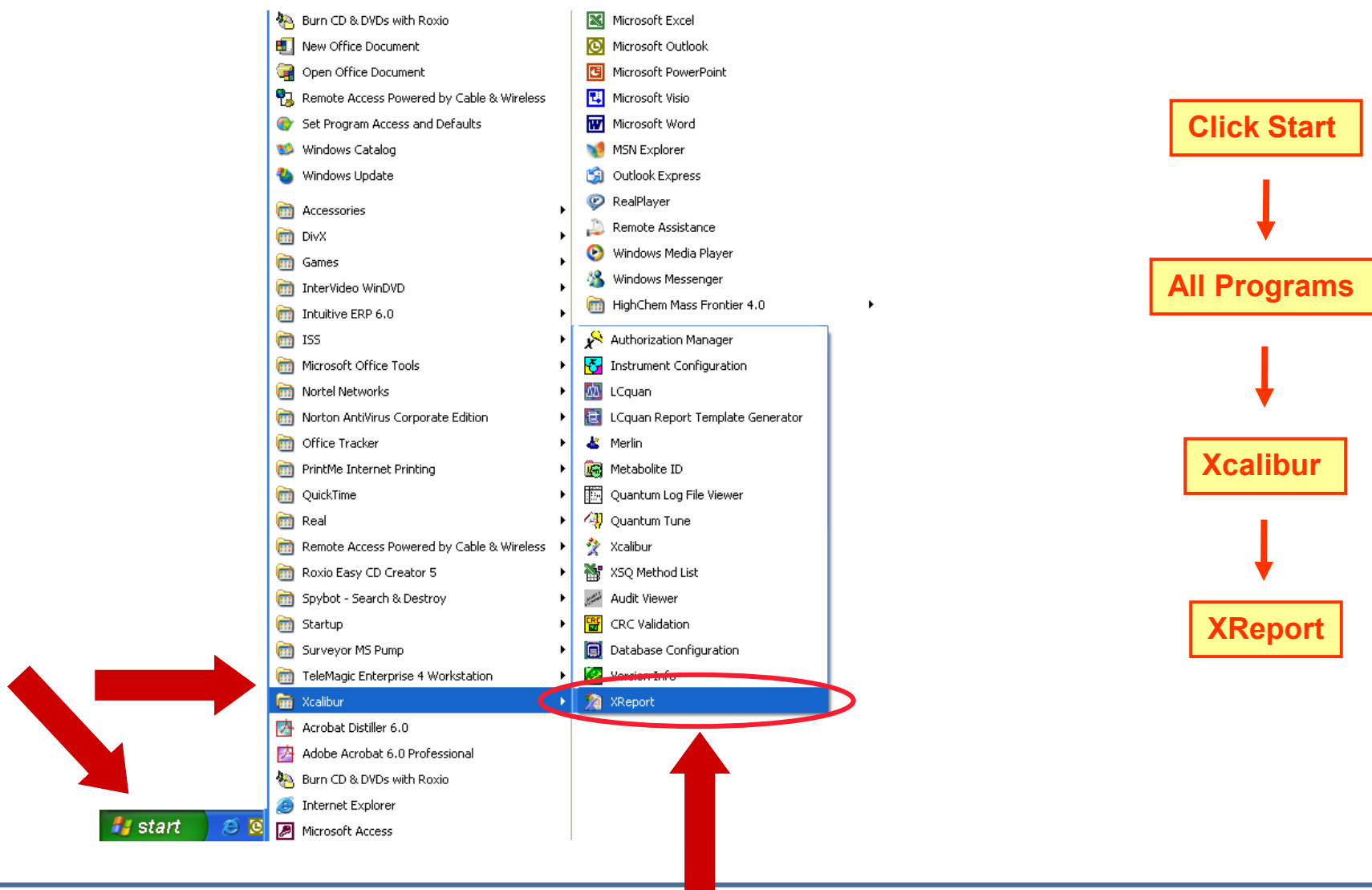
- Go to Home Page - Sequence Setup
- Open/Make a Sequence
- Click Actions : Batch Reprocess...
- Check Reports and Print Reports Boxes

Before you Start



- » Decide what objects you want on the report and how they should be laid out.

Open XReport



Specify Data Sources...

1. Click Report and select Data Sources

Data Sources...

Simulate Report

Resolve Report

Print Report

2. Specify Data Sources

Data Sources

Calibration File

Processing Method File

Raw Data File

Result File

Sequence List File

OK

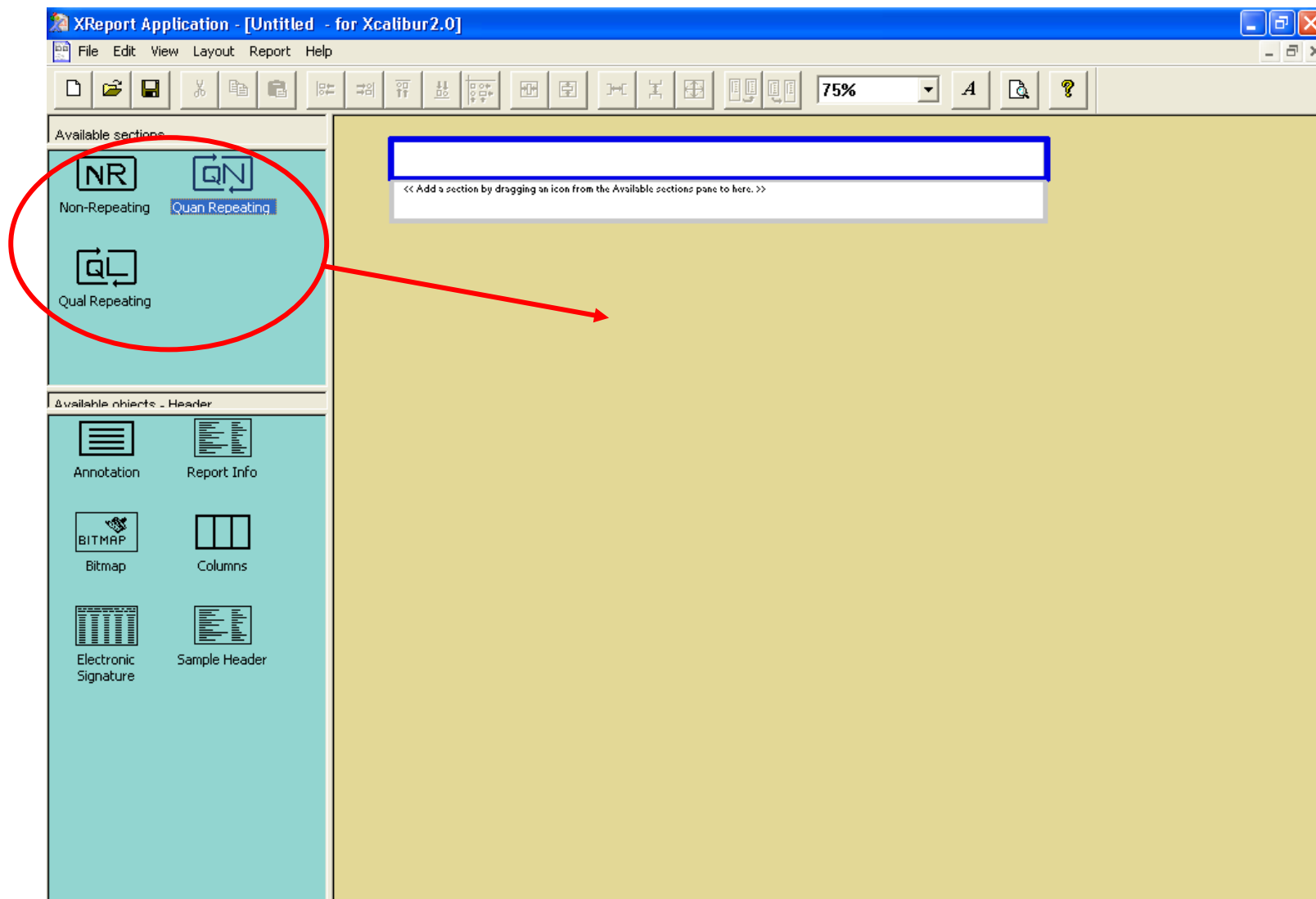
Cancel

Apply

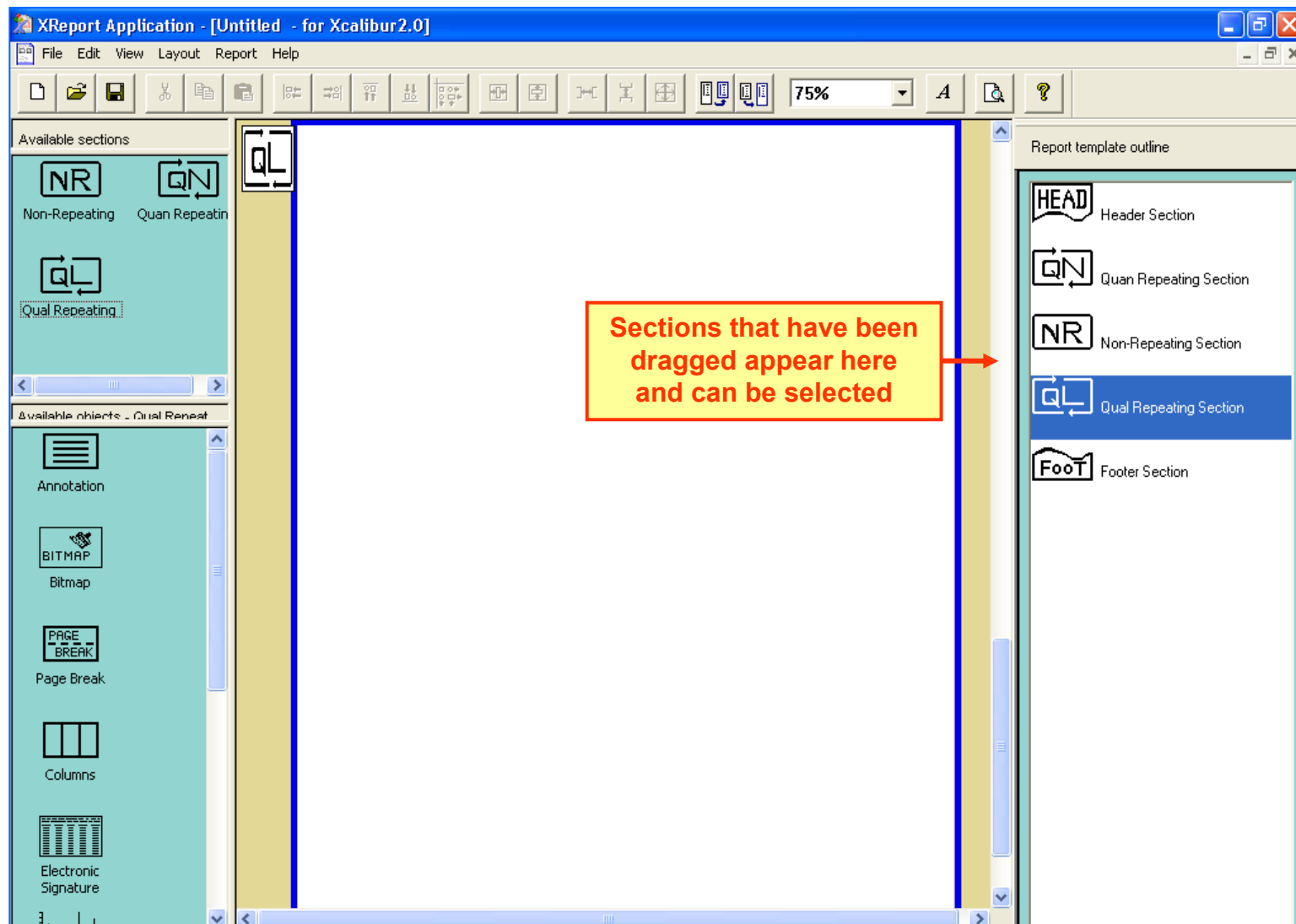
Help

Default...

Drag and Drop Sections...



Drag and Drop Sections...



Drag and Drop Individual Objects...

XReport Application - [Untitled - for Xcalibur2.0]

File Edit View Layout Report Help

Available sections:

- NR Non-Repeating
- QN Quan Repeating
- QL Qual Repeating

Available objects - Quan Repeating:

- Annotation
- BITMAP Bitmap
- PAGE BREAK Page Break
- Column
- Electronic Signature
- Chromatogram
- Component Calcurve
- Spectrum
- Avalon Quan Events Table
- Component Cal Level Table

Report template outline:

- HEAD Header Section
- QN Quan Repeating Section
- NR Non-Repeating Section
- QL Qual Repeating Section
- FOOT Footer Section

Chromatogram: RT: 1.49 - 2.49 SM: 7 G RT: 1.99

Area: 1000000

Spectrum: 281.4, 356.3, 220

Sample Table (Quan Results)

Sample ID	Data File Name	Area
[A]	[B]	[C]

Formatting Objects...

The screenshot shows the XReport Application window. The main area displays a chromatogram with a peak at 1.99 minutes and a mass spectrum with peaks at 220.0 and 281.4 m/z. A right-click context menu is open over the chromatogram, with the 'Properties...' option highlighted in red. The menu options are: Cut (Ctrl+X), Copy (Ctrl+C), Paste (Ctrl+V), Delete (Del), Edit Object (Ctrl+E), Properties... (Alt+Enter), and Delete Section. The sidebar on the left shows available sections (NR, QN, QL) and available objects (Annotation, Bitmap, Page Break, Columns, Electronic Signature, Chromatogram, Component Calcurve, Spectrum, Avalon Quan Events Table, Component Cal Level Table). The right sidebar shows the report template outline with sections: HEAD (Header Section), QN (Quan Repeating Section), NR (Non-Repeating Section), QL (Qual Repeating Section), and FOOT (Footer Section).

Sample ID	Data File Name	Area
[A]	[B]	[C]

- Any inserted object can be formatted to some degree
- Right Click
- Select Properties
- Follow the Instructions

Viewing the Report...

XReport Application - [Untitled - for Xcalibur2.0]

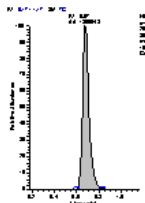
File Edit View Layout Report Help

75% A

Click to view the report

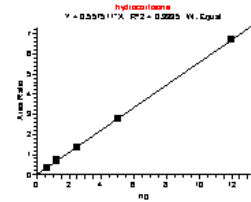
XReport Application - [Untitled - for Xcalibur2.0:2]

Print... Save... First Page Prev Page Page 1 of 5 Next Page Last Page Two Page Zoom In Zoom Out Close Help

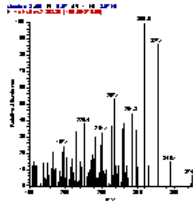



Peak Data:

Peak	Retention Time (min)	Area	Height
1	1.5	189294.27	1.5



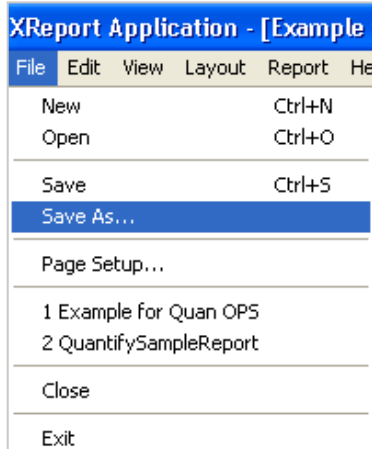
Calibration Curve:

$$Y = 0.000117X \quad R^2 = 0.9999 \quad W. Equal$$



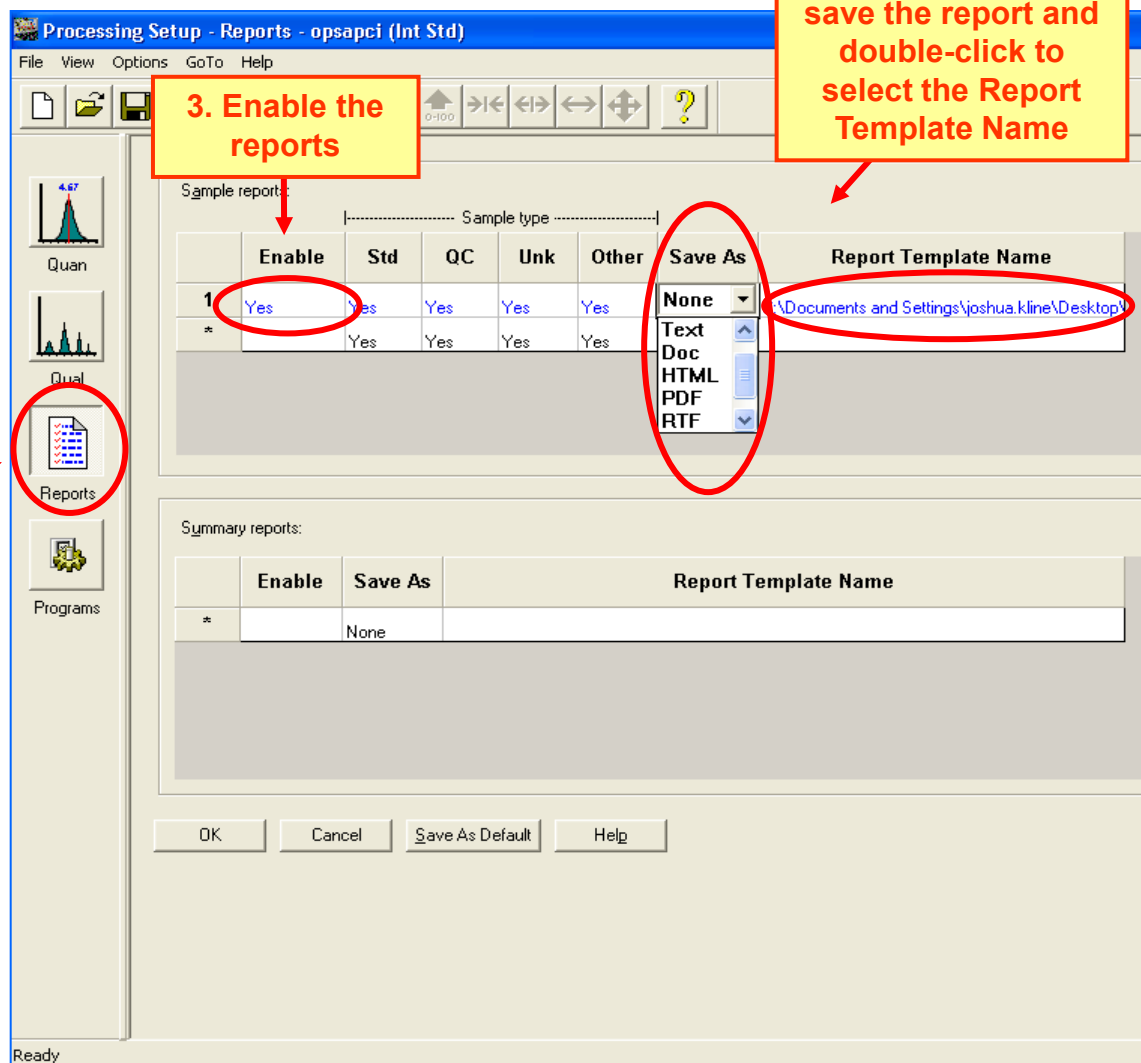
Sample ID	Data File Name	Area
Sample02	sample02	189294.27
Sample03	sample03	95450.72
Sample04	sample04	55134.75
Sample05	sample05	245328.14
Sample06	sample06	1842830.39
Sample09	sample09	501011.50
Sample10	sample10	5455747.72
Sample11	sample11	278701.72
Sample12	sample12	489259.80
Sample13	sample13	1859442.71
Sample14	sample14	896332.77
Sample15	sample15	515053.04
Sample16	sample16	4429085.34

Save, Insert, Use...

1. Click to save the report



2. In Processing Setup, click Reports



Save, Insert, Use...

Run Sequence

Acquisition Options

Instrument: Surveyor AS
Start Instrum...: Yes

LTQ XL MS
Surveyor LC Pump

☒ Start When Ready

Change Instruments...

Instrument Method: [] Browse

Start Up: []

Shut Down: []

Programs

Pre Acquisition: []

Post Acquisition: [] Browse...

Run Synchronously

☒ Pre Acquisition ☒ Post Acquisition

After Sequence Set System:

☒ On ☐ Standby ☐ Off

User: []

Run Rows: 1

☐ Priority Sequence

Processing Actions

☒ Quan

☐ Qual

☒ Reports

☐ Programs

☐ Create Quan Summary

OK Cancel Help

1. Click to enable reports when you run the sequence

Batch Reprocess Setup

Processing Actions

☒ Quan

☒ Peak Detection & Integration

☒ Calibration

☒ Quantitation

☐ Qual

☒ Peak Detection & Integration

☒ Spectrum Enhancement

☐ Library Search

☒ Reports

☒ Print Sample Reports

☐ Print Summary Reports

☐ Programs

☐ Create Quan Summary Spreadsheet

Advanced Options

☐ Replace Sample Info

Process Rows: 1

OK Cancel Help

2. Click to enable reports after the sequence has run