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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."

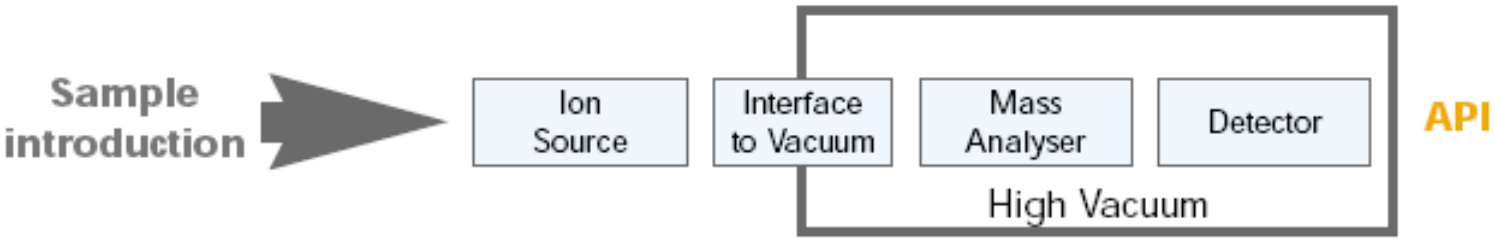
Niessen, W. M. A.; Van der Greef, J., *Liquid Chromatography–Mass Spectrometry: Principles and Applications*, 1992, Marcel Dekker, Inc., New York, p. 29.

Mass Spectrometry “Simplified”

Generate	→	Ion Production
Move	→	Ion Optics
Select	→	Quadrupole
Detect	→	Electron Multiplier

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

API Mass Spectrometry – Block Diagram



Ion Generation (API)

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

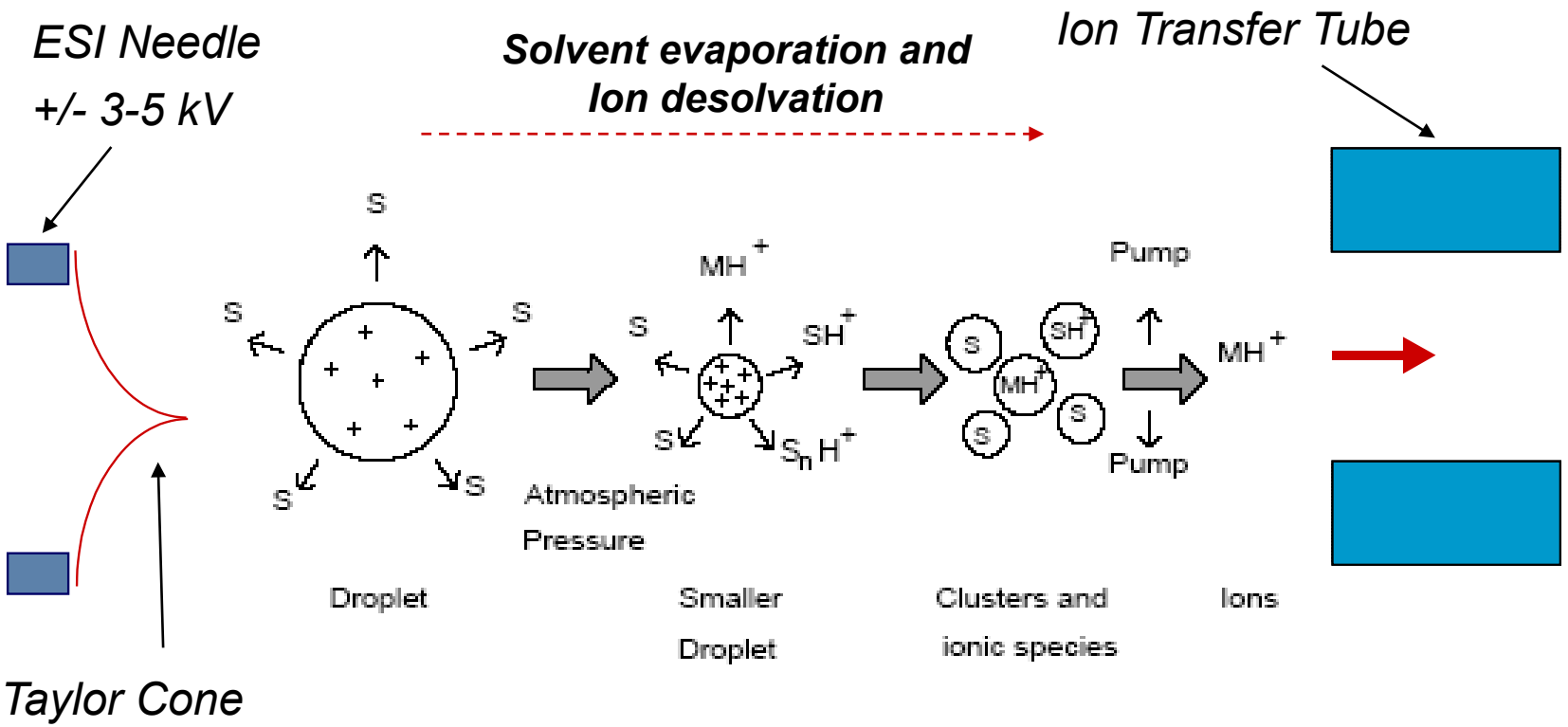
Good for volatile / thermally stable analytes

Good for non-polar / semi-polar analytes

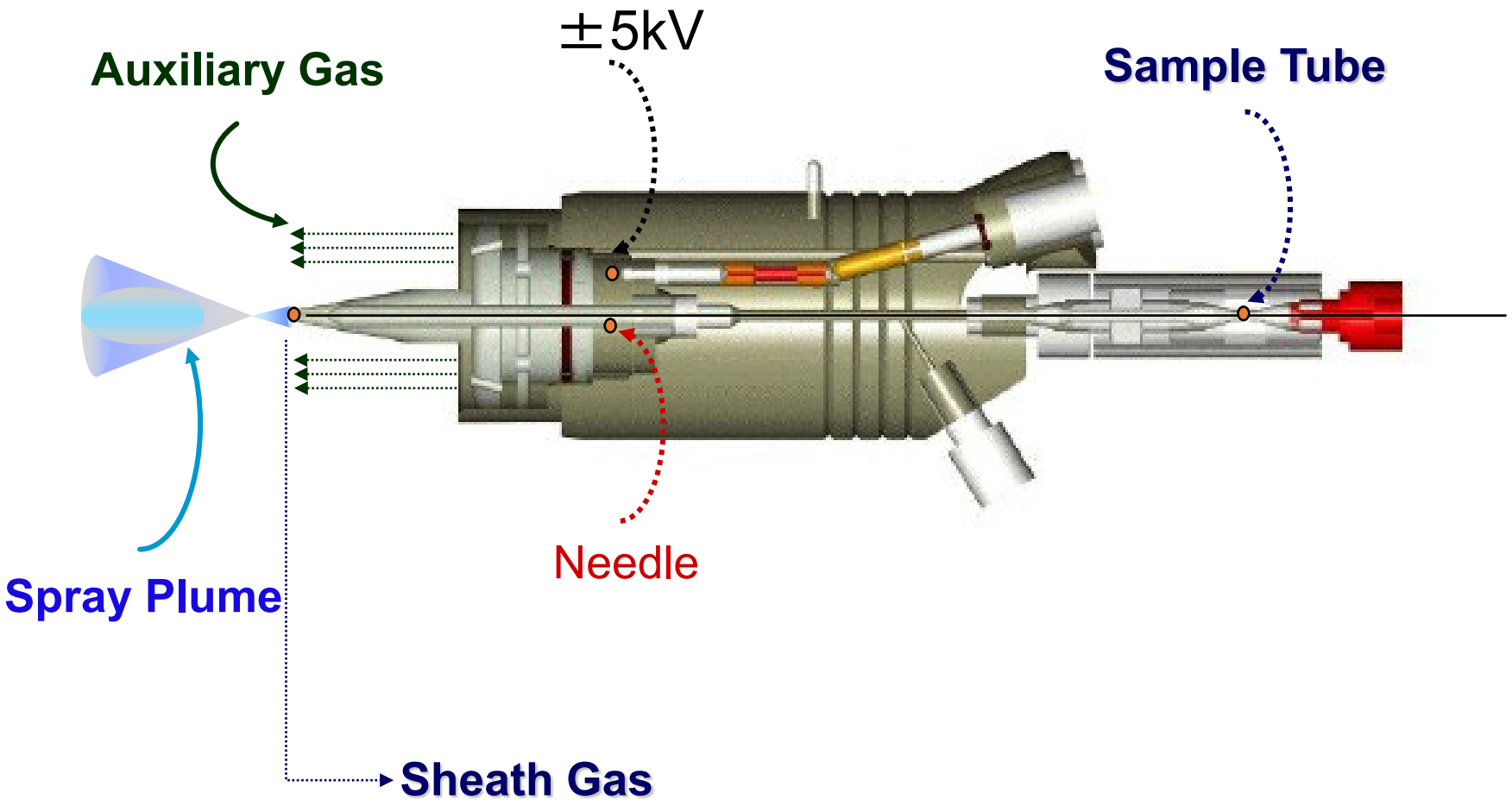
Good for small molecules (i.e. steroids)

Good for ions containing a chromophore (APPI)

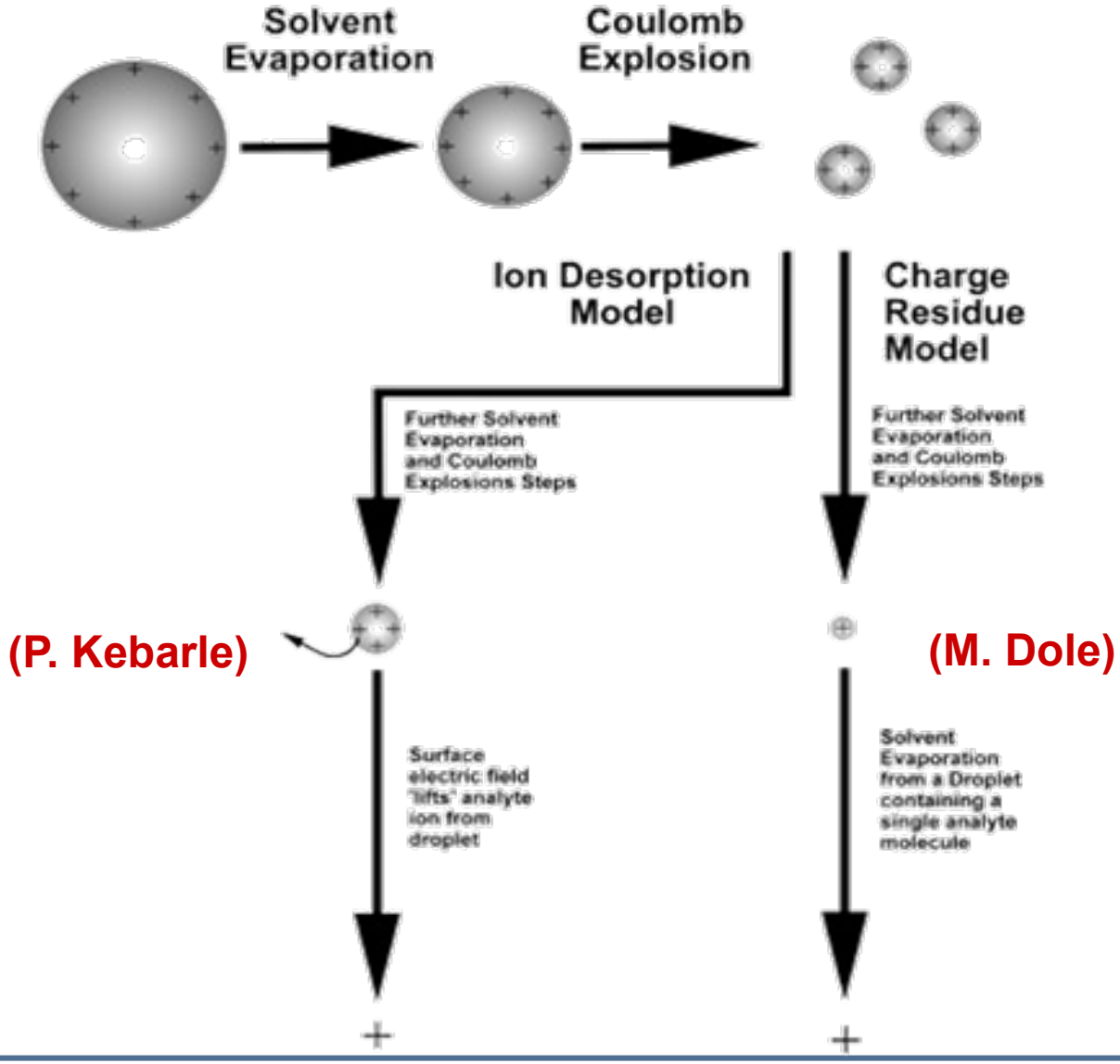
Electrospray - Basic Principle



ESI Nozzle Cross Section



Electrospray – Prevailing Theories



Positive or Negative Ion Mode ?

- Basic Functional Groups → $[M+H]^+$

  ($-NH_2$)

- Acidic Functional Groups → $[M-H]^-$

  (-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:

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

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

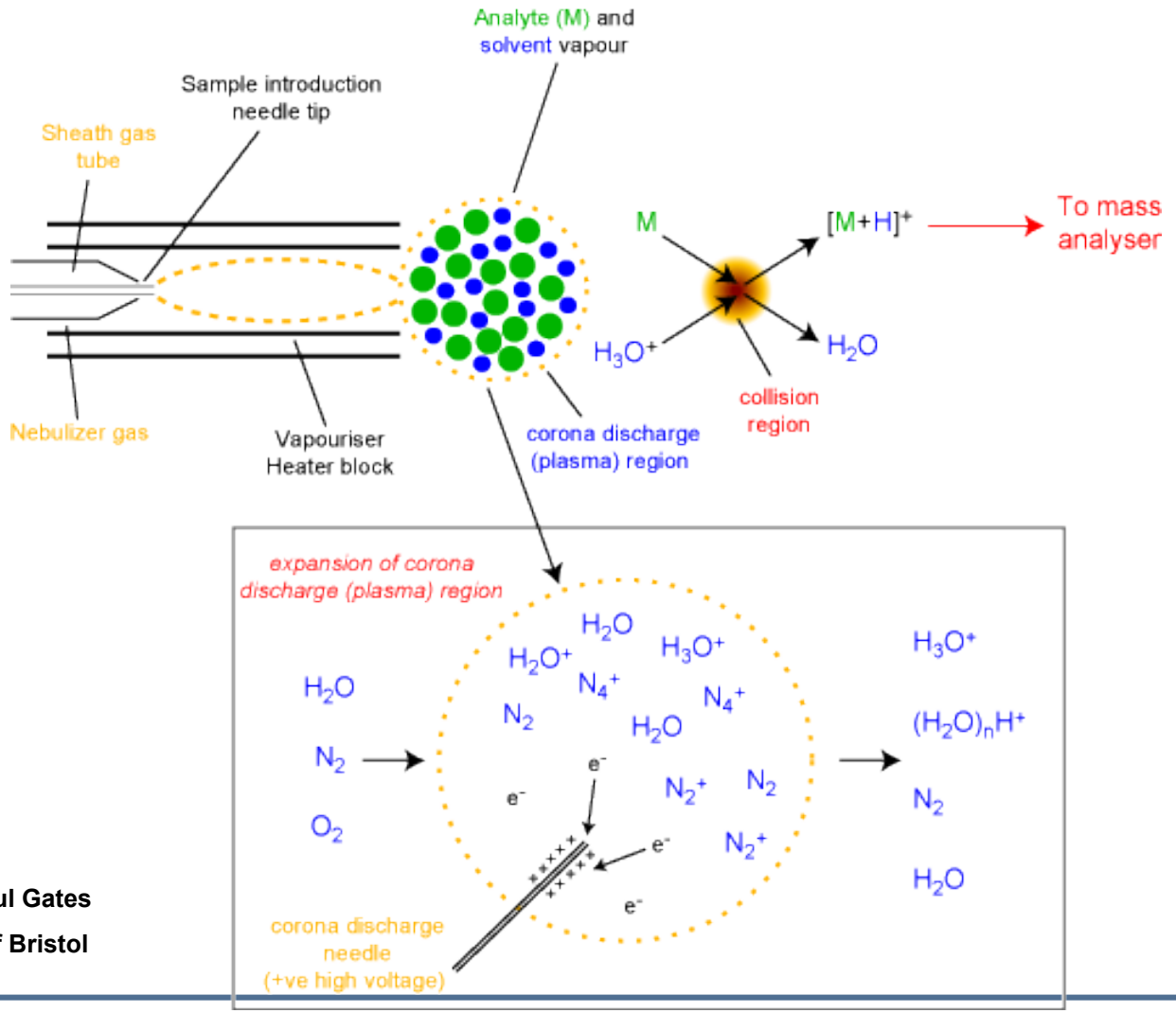
2. Through a complex series of reactions primary ions react with solvent molecules forming reagent ions, $\mathbf{H_3O^+}$ and $\mathbf{CH_3OH_2^+}$

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

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

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

Atmospheric Pressure Chemical Ionization (APCI)



Atmospheric Pressure Photo-Ionization (APPI)

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

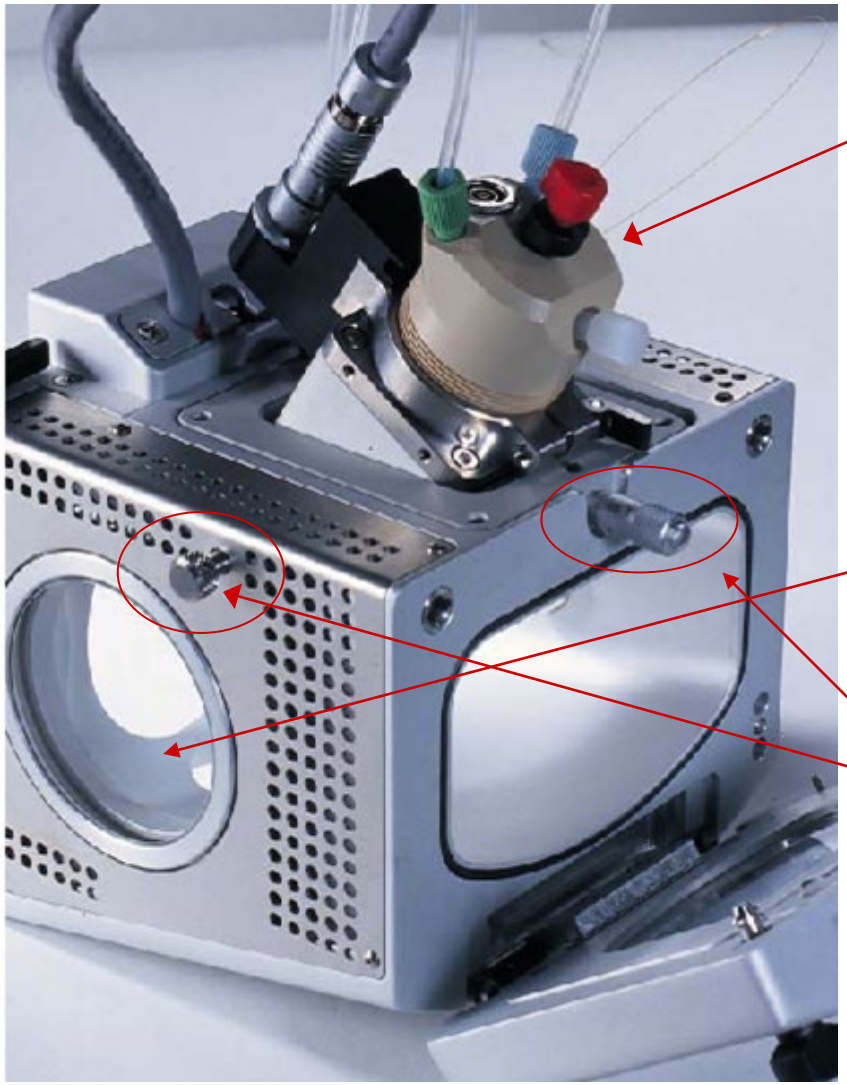
1. Analyte molecules interact with the UV light source (krypton light emits 10.0 eV and 10.6 eV photons). The analyte molecule M is ionized to a molecular ion M^+ if the ionization potential (IP) of the analyte is lower than the photon energy ($h\nu$)

$$M + h\nu \rightarrow \mathbf{M^+} + e^-$$

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

$$M^+ + S \rightarrow \mathbf{[M+H]^+} + [S\text{-}H]$$

Ion Max API Source



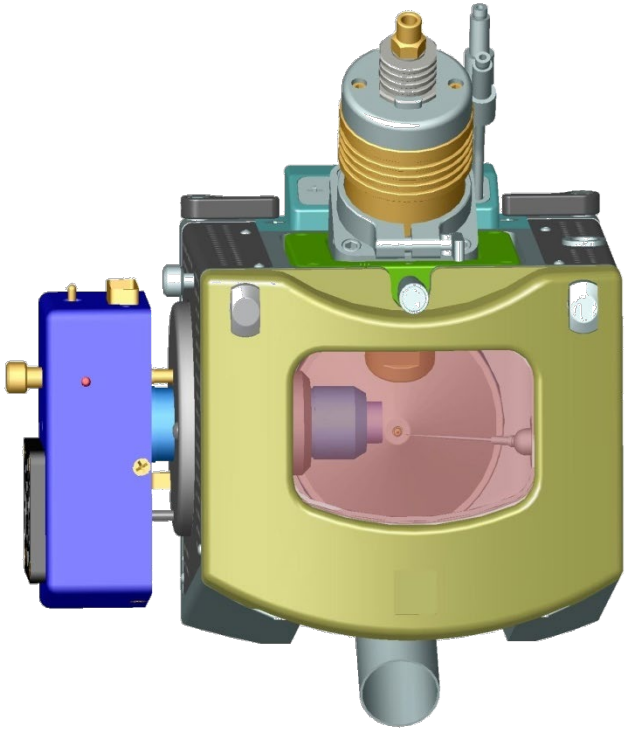
Interchangeable source probe

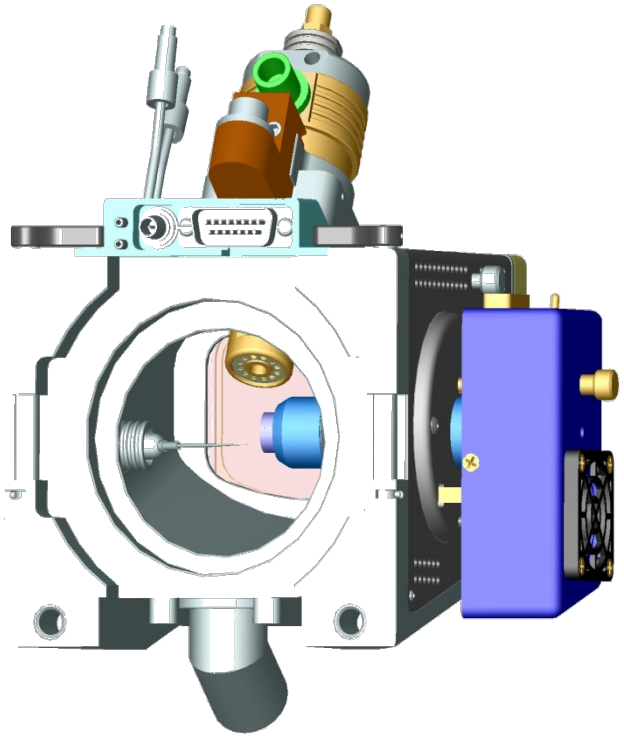
(ESI probe shown)

Window - Inlet for APPI accessory

Positional adjusters for source probe

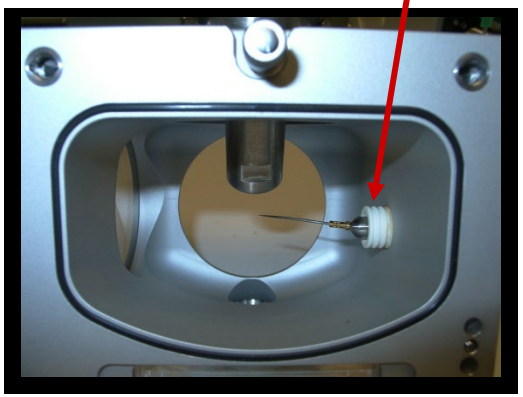
Ion Max Source – APPI Optional Accessory

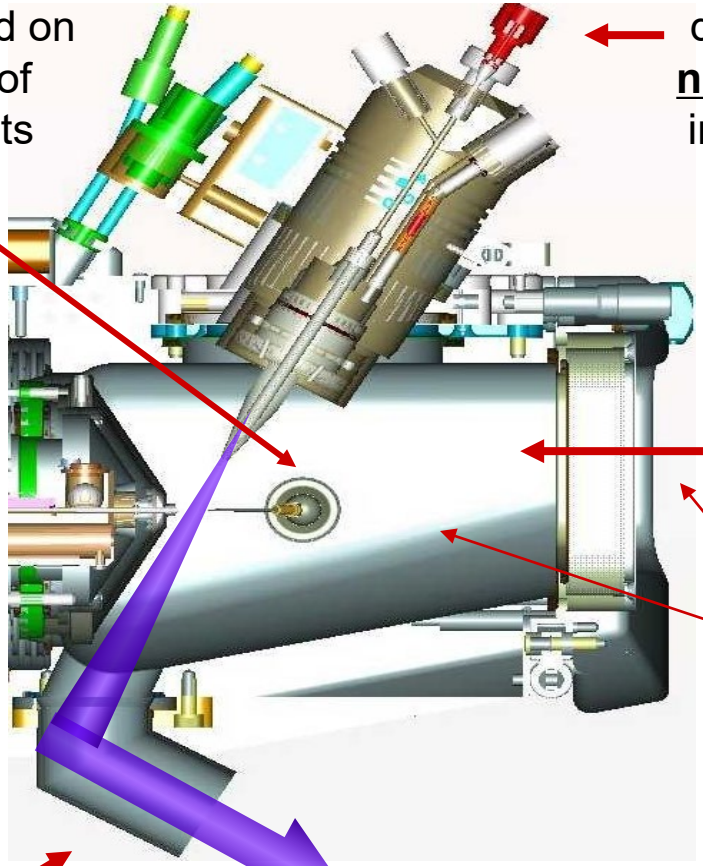




Ion Max Source - Housing Design

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





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

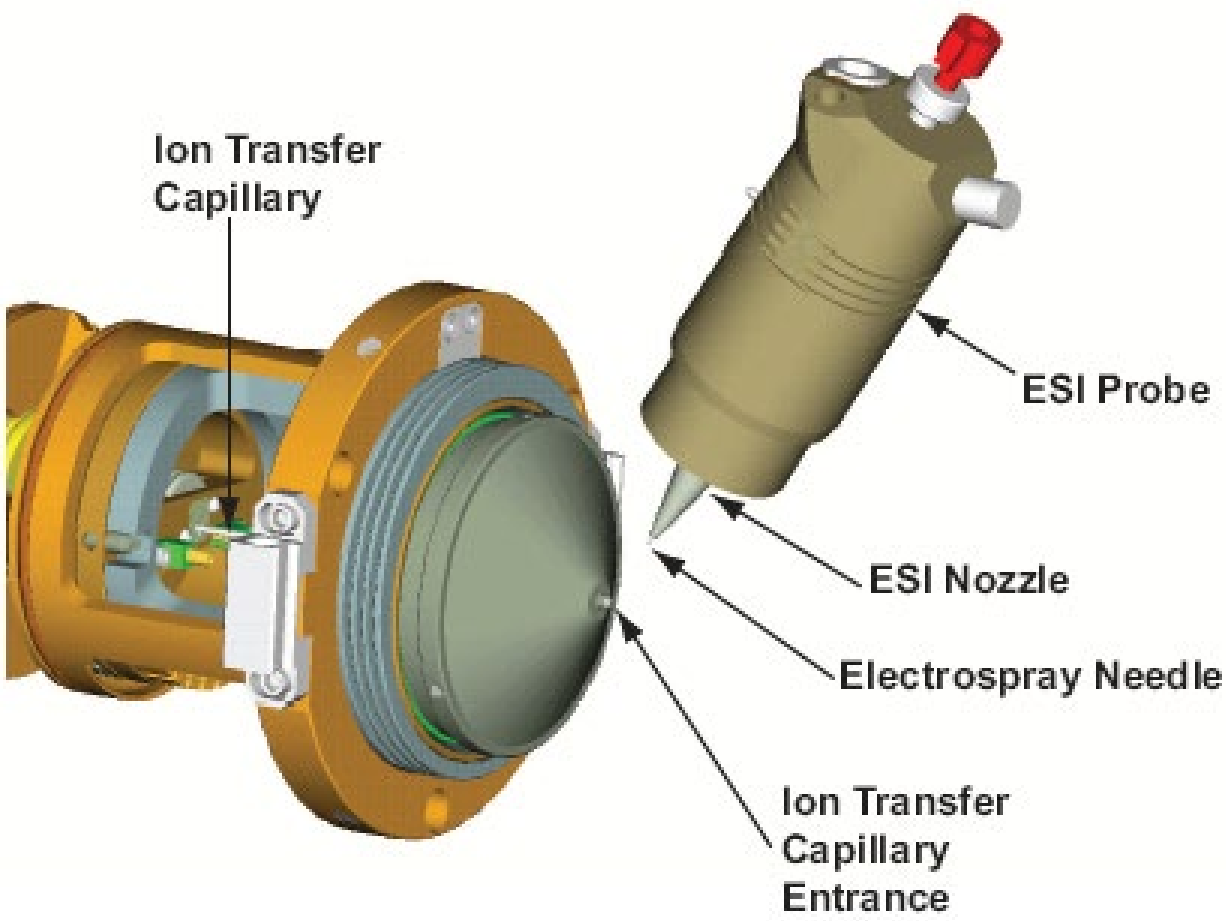
Plastic-free housing

Two view ports for easier visualization

Stainless steel port for improved chamber drainage

Improved drain design, allows streamlined API exhaust removal

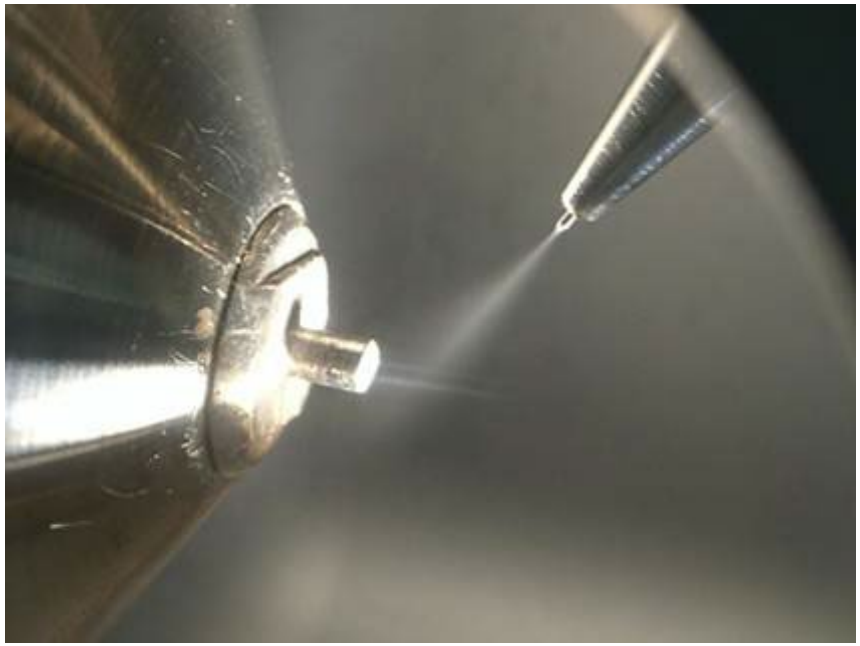
Ion Max Source - ESI



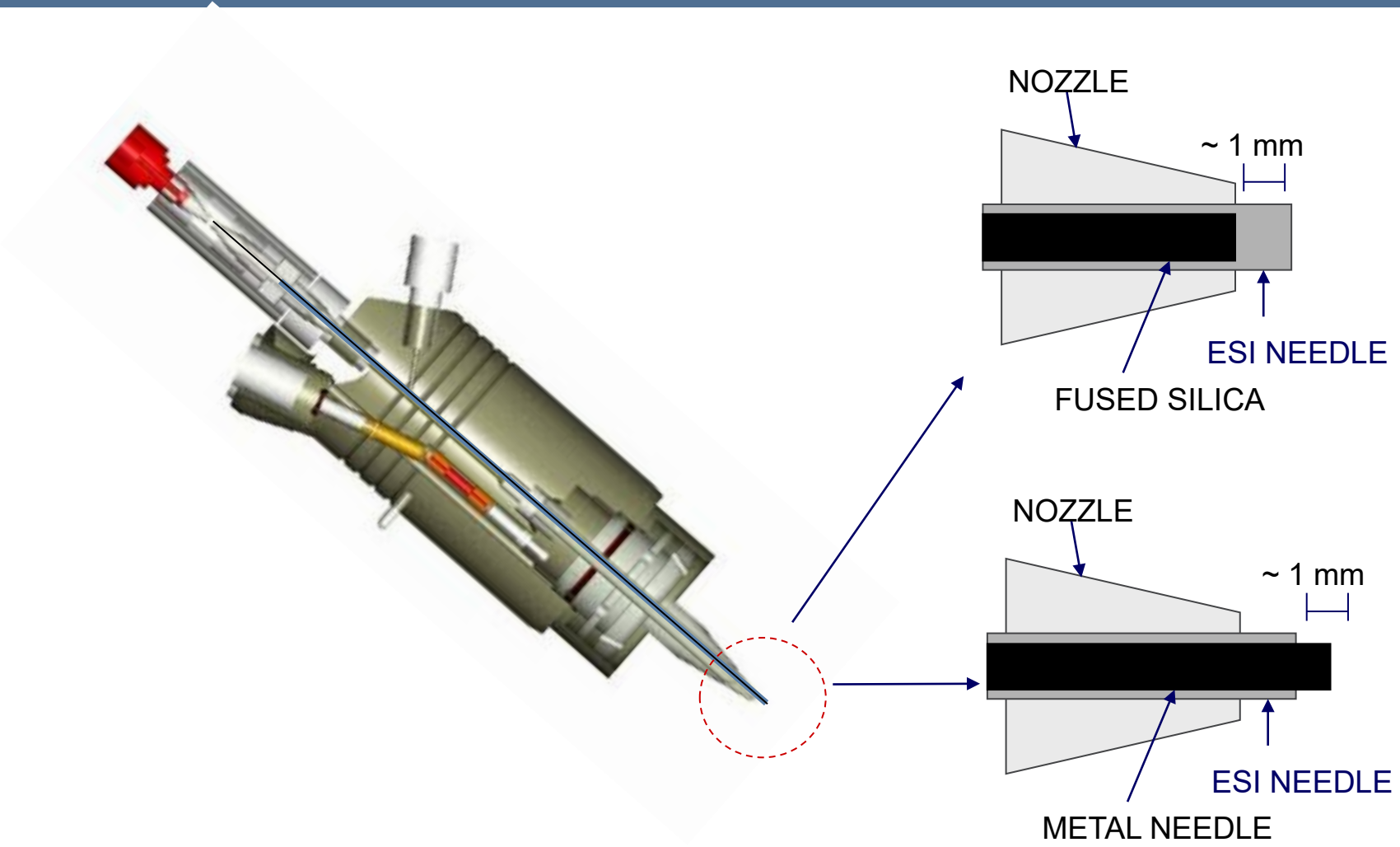
Ion Max Source Design - ESI Probe

ESI probe features:

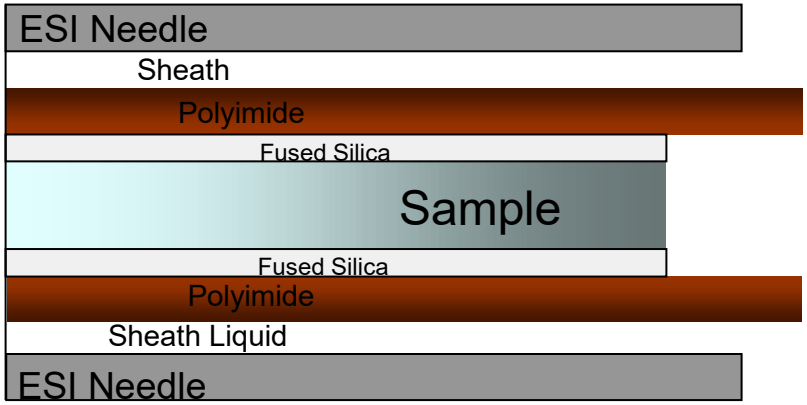
- Fixed spray angle (60 degrees)
- Built-in sheath liquid line (for accurate mass & post-column addition applications)
- X,Y,Z - adjustable for additional spray quality optimization



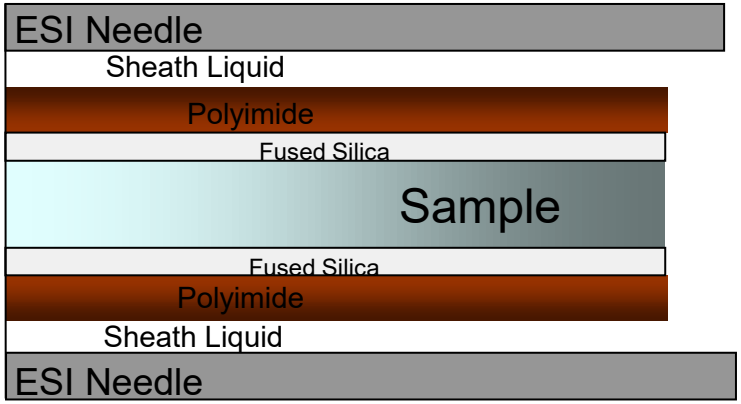
ESI Probe – Sample Tube Choices



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.* (ºC)	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

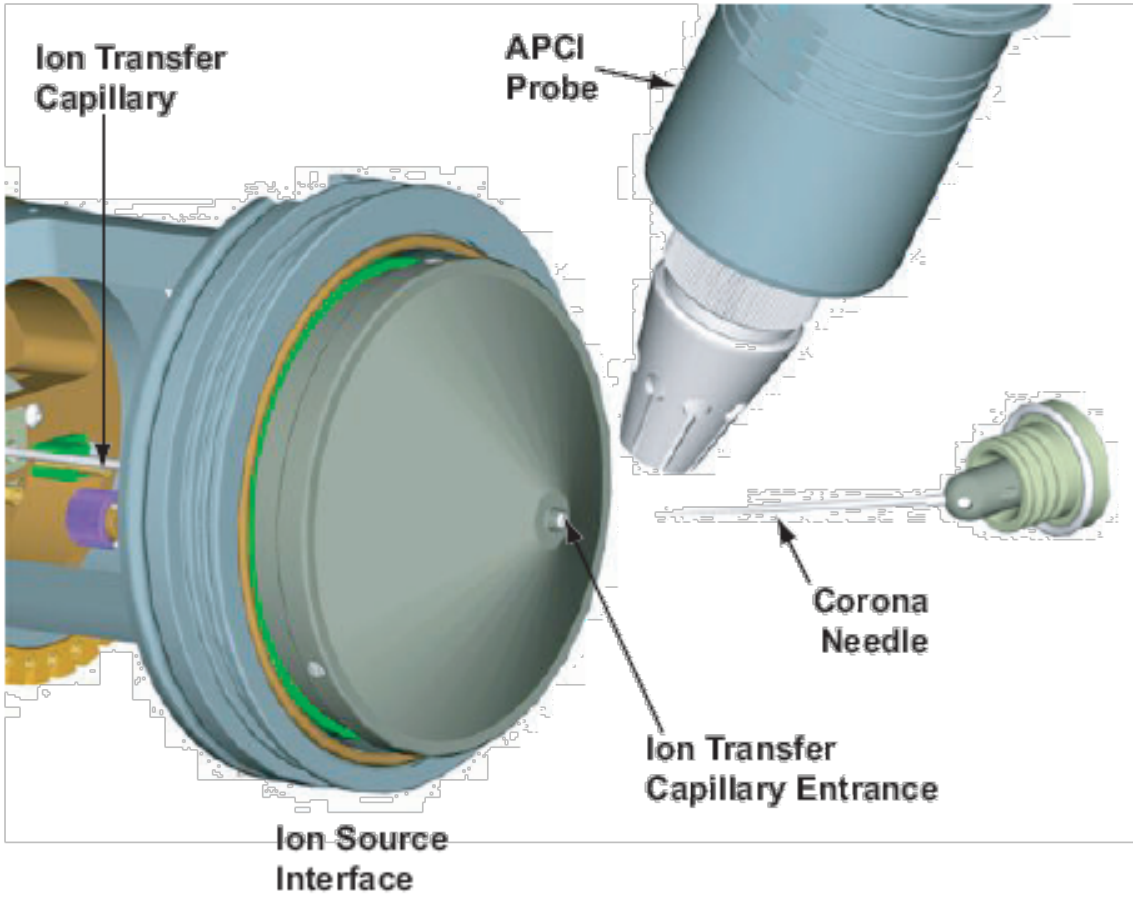
** The spray voltage values may vary for different sample tube materials (i.e., fused silica vs. stainless steel)

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

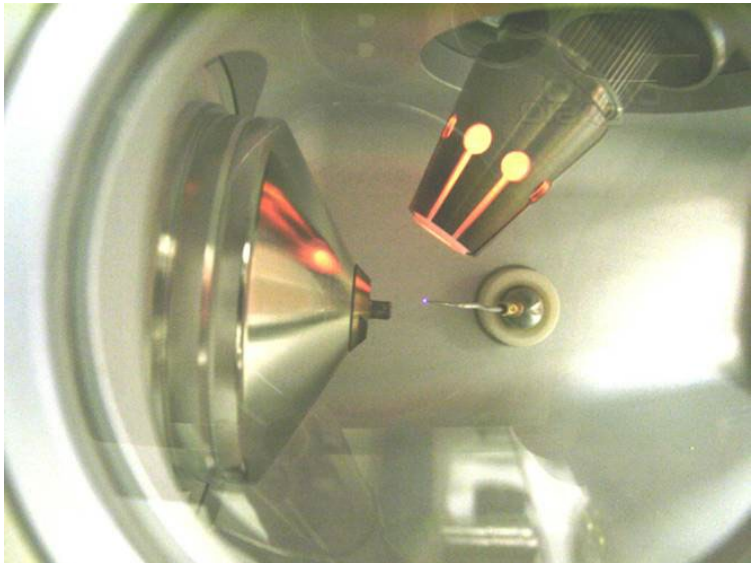
ESI - Microflow Operational Conditions (guidelines)

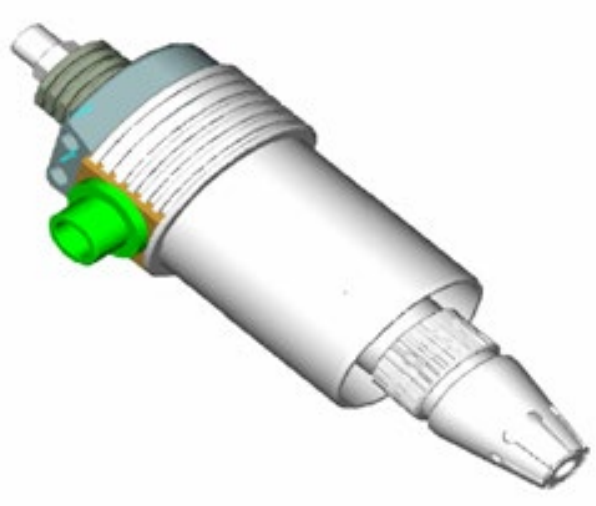
Stainless Steel Needle Size	Solvent Flow Rate (µL/min)	Capillary Temperature (°C)	Sheath Gas Pressure (Arb. units)	Aux Gas Pressure (Arb. units)	Spray Voltage (kV)
34-gauge	0.5 - 50	150 - 200	0 - 5	0	1.5 - 4.0
32-gauge	3 - 400	200 - 250	5 -15	0 - 5	1.5 - 4.0

Ion Max Source Design - APCI Probe



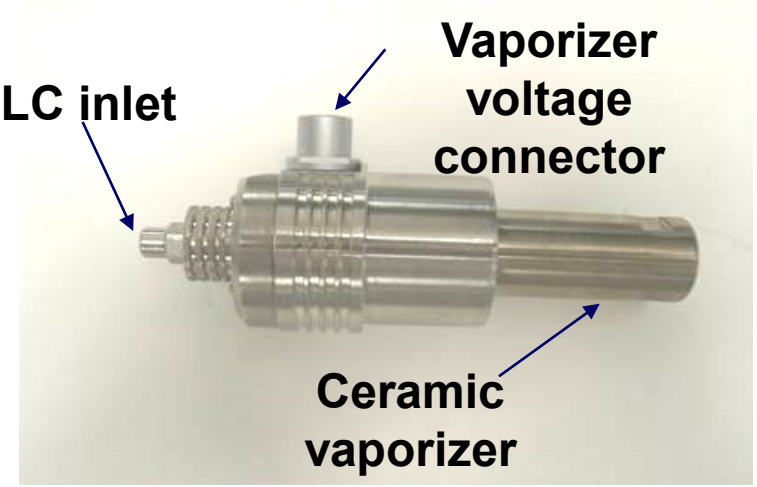
Ion Max Source Design : APCI Probe





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)

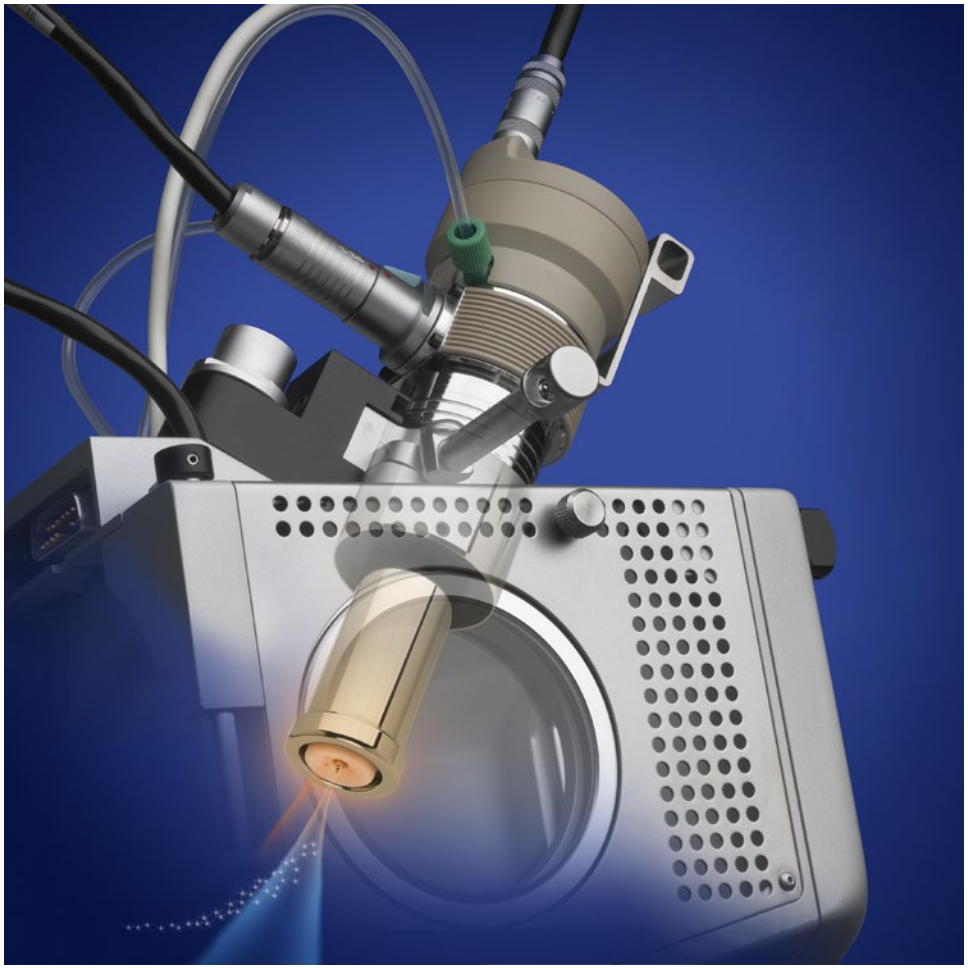
Liquid Flow Rate (μL/min)	Ion Transfer Tube Temp. (ºC)*	Sheath Gas Pressure (arb)	Aux Gas Flow (arb)	Vaporizer Temperature (ºC)	Corona Discharge Current (μA)
200	250	25	5	350	+4 (-10**)
1000	250	45	5	450	+4 (-10**)

* 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.

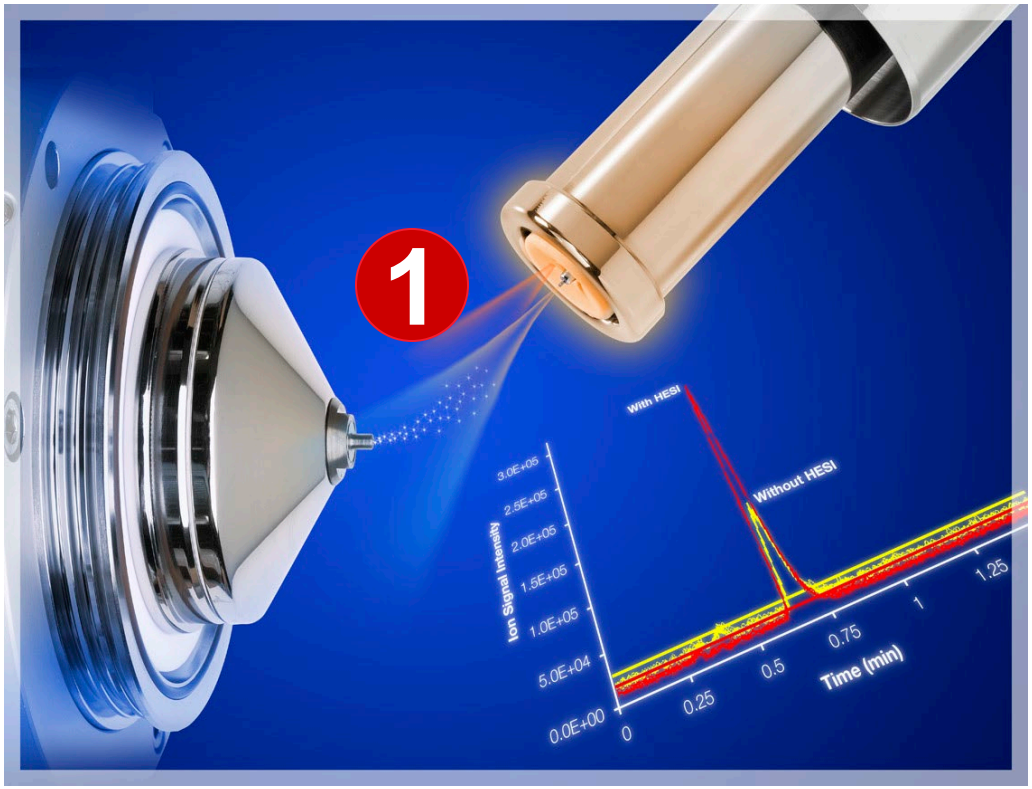
Ion Max Source Design - H-ESI Probe



Dual Desolvation Zone Technology

Zone One–
Temperature Control

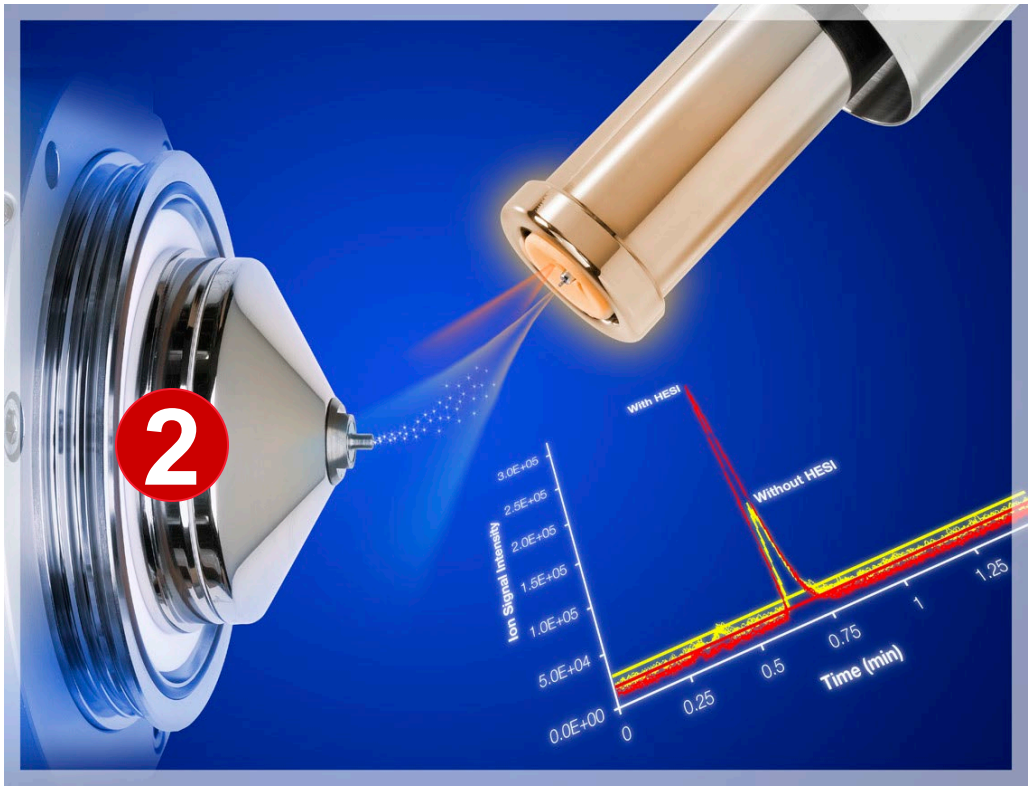
This zone offers full control over the temperature of the auxiliary nitrogen gas. Desolvation is initiated in the source housing by turning on the built-in heat-exchange mechanism which heats the auxiliary gas. This can be done at high LC flow rates or when hydrophilic compounds elute in the high aqueous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



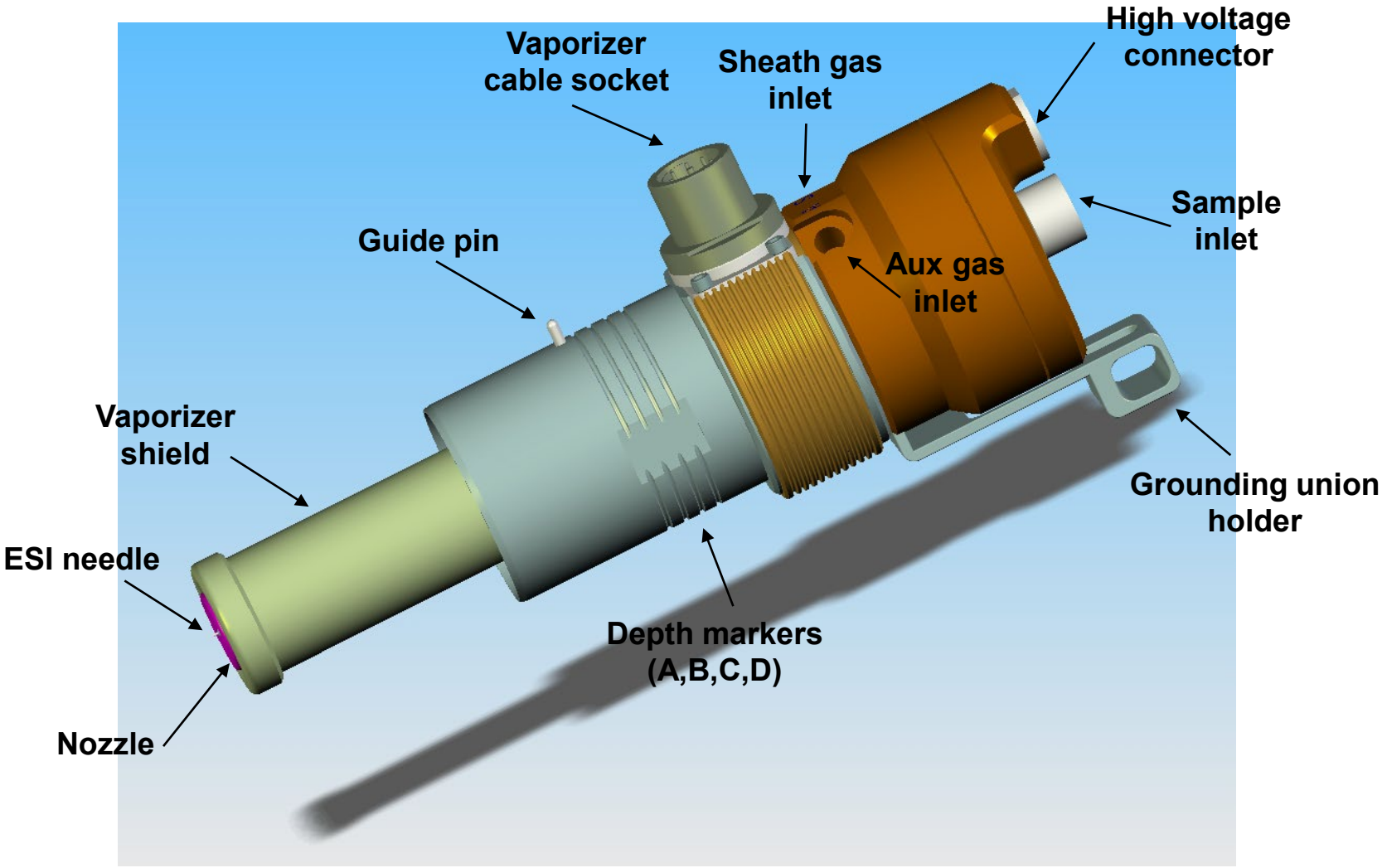
Dual Desolvation Zone Technology

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


High voltage connector
Vaporizer cable socket
Sheath gas inlet
Sample inlet
Guide pin
Aux gas inlet
Vaporizer shield
Grounding union holder
ESI needle
Depth markers (A,B,C,D)
Nozzle

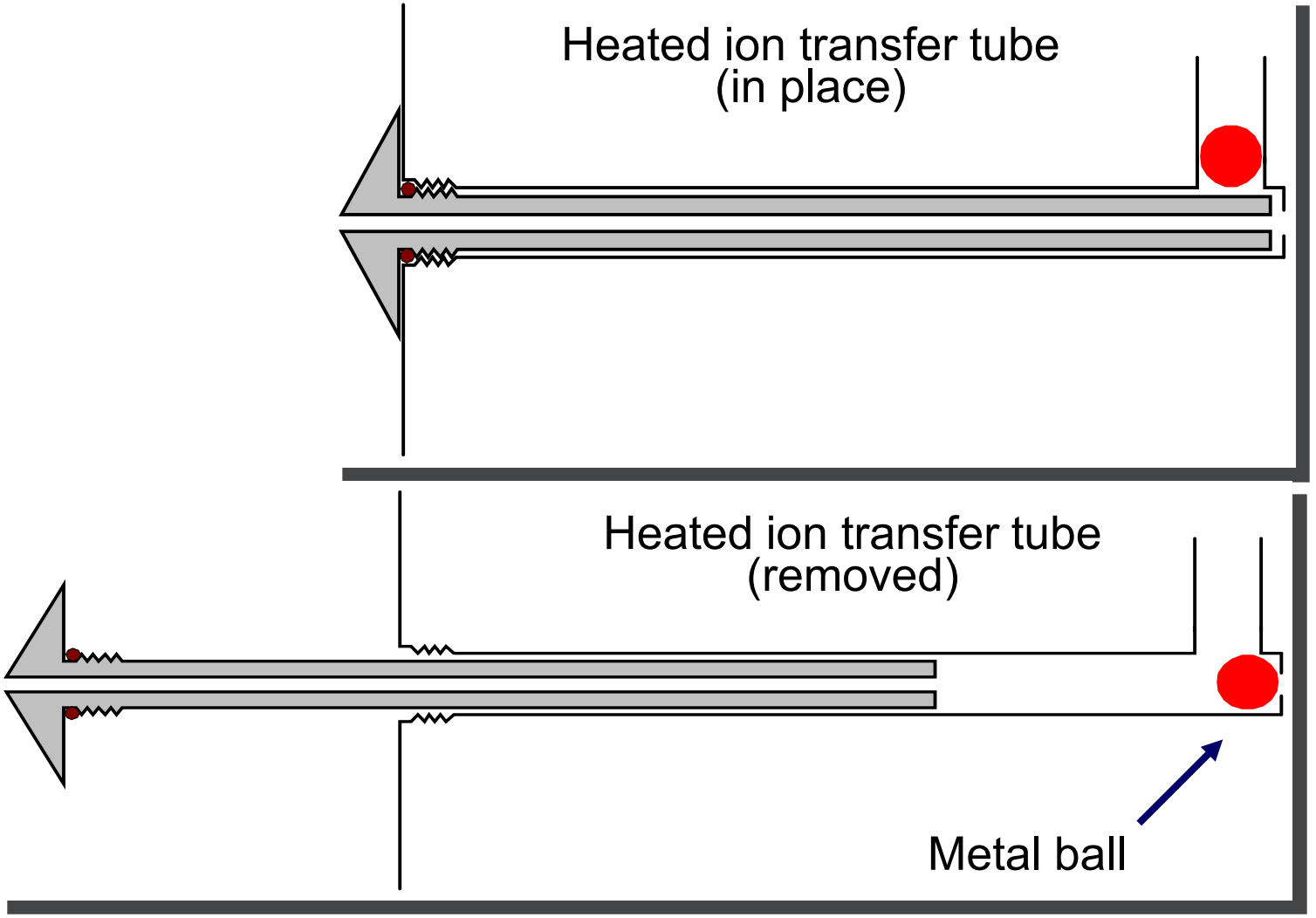
H-ESI - Operational Conditions (guidelines)

Liquid Flow Rate (μL/min)	Ion Transfer Tube Temp. (ºC)*	Vaporizer Temperature** (ºC)	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

ESI Probe

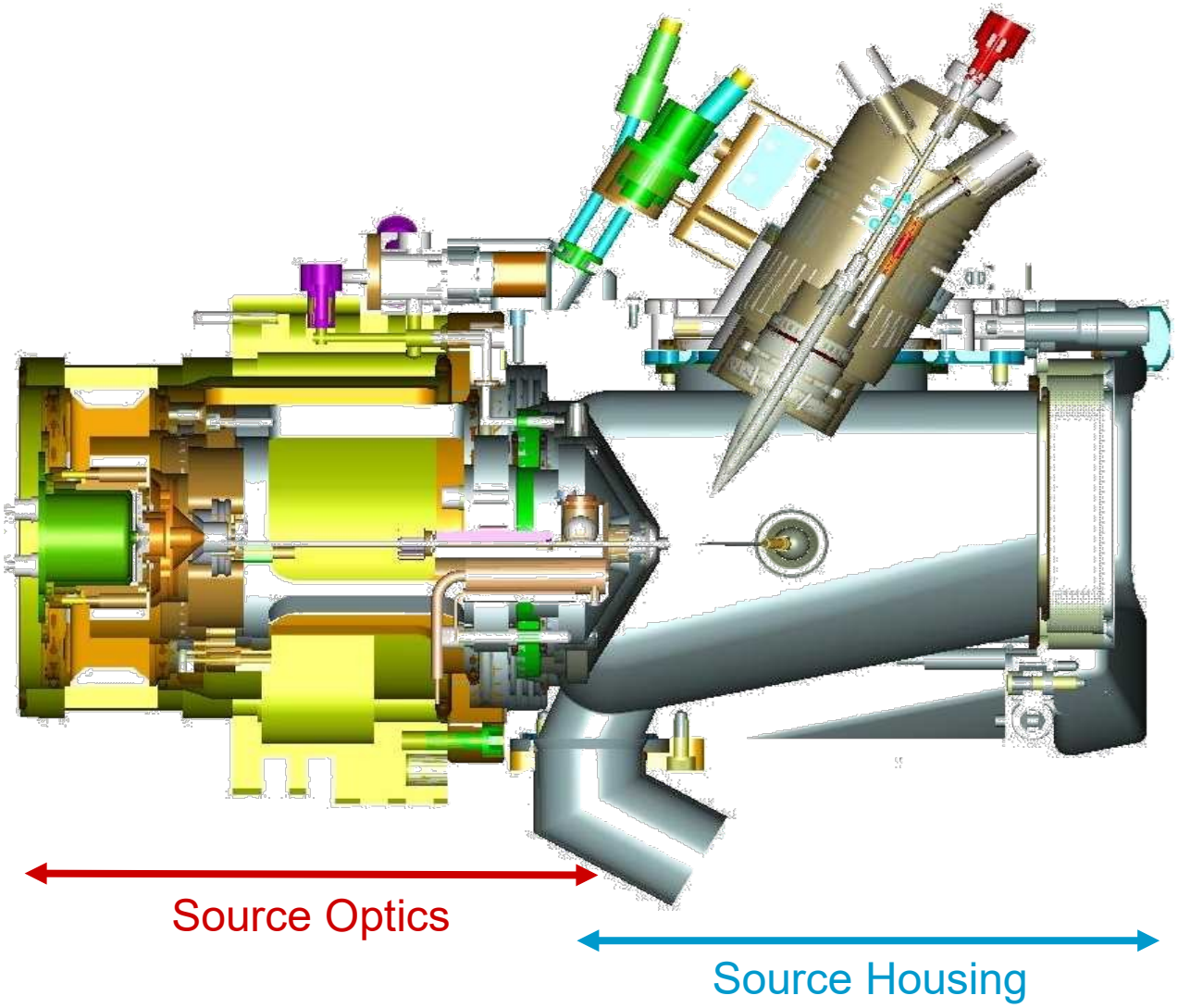
Acts as a physical barrier

Nitrogen gas

Crud accumulation due to knife-edge vortex

Ion transfer tube

Ion Max Source - Source Optics and Housing



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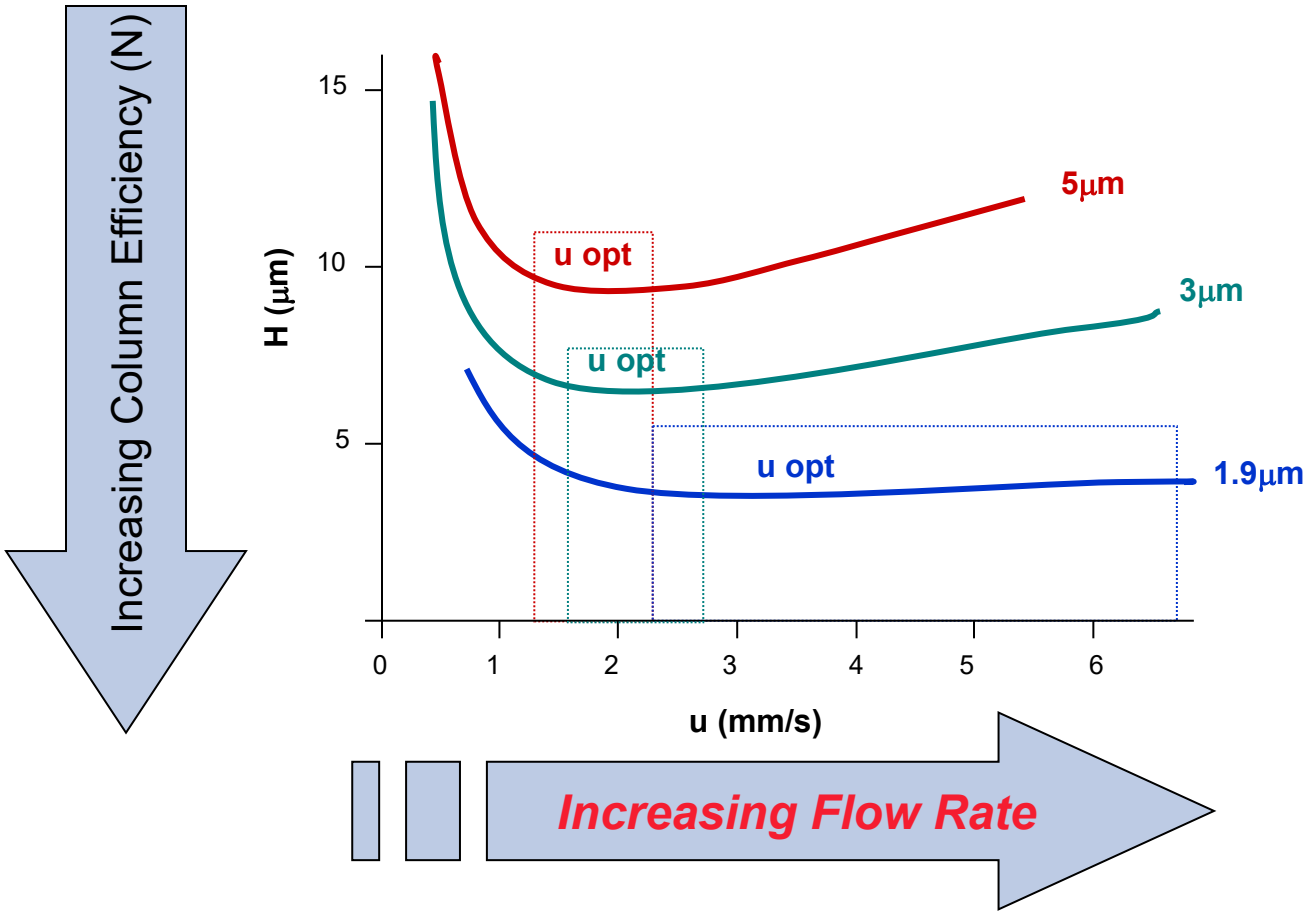
Chapter 2

Notes on Liquid Chromatography

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

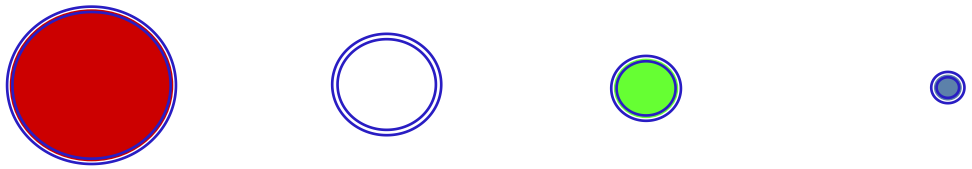


$$H = \frac{N}{L}$$

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

Response Enhancement - Narrow Bore Columns

Col. Diameter, mm	4.6	3.0	2.1	1.0
Flow Rate, µL/min	1000	500	200	50
Theoretical Increase (peak height)	1	2.0	5	20

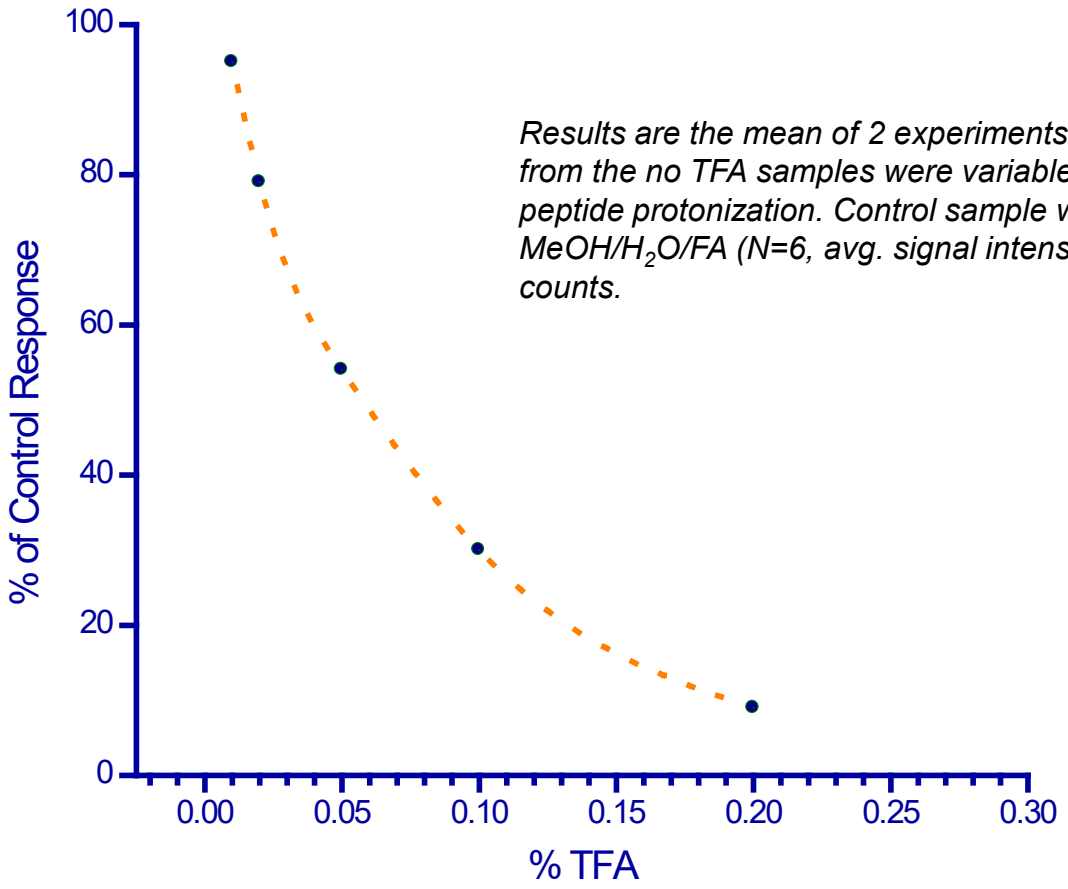


Once optimal peak shape is achieved, the only way chromatographically to enhance signal is to reduce the columns internal diameter.

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.

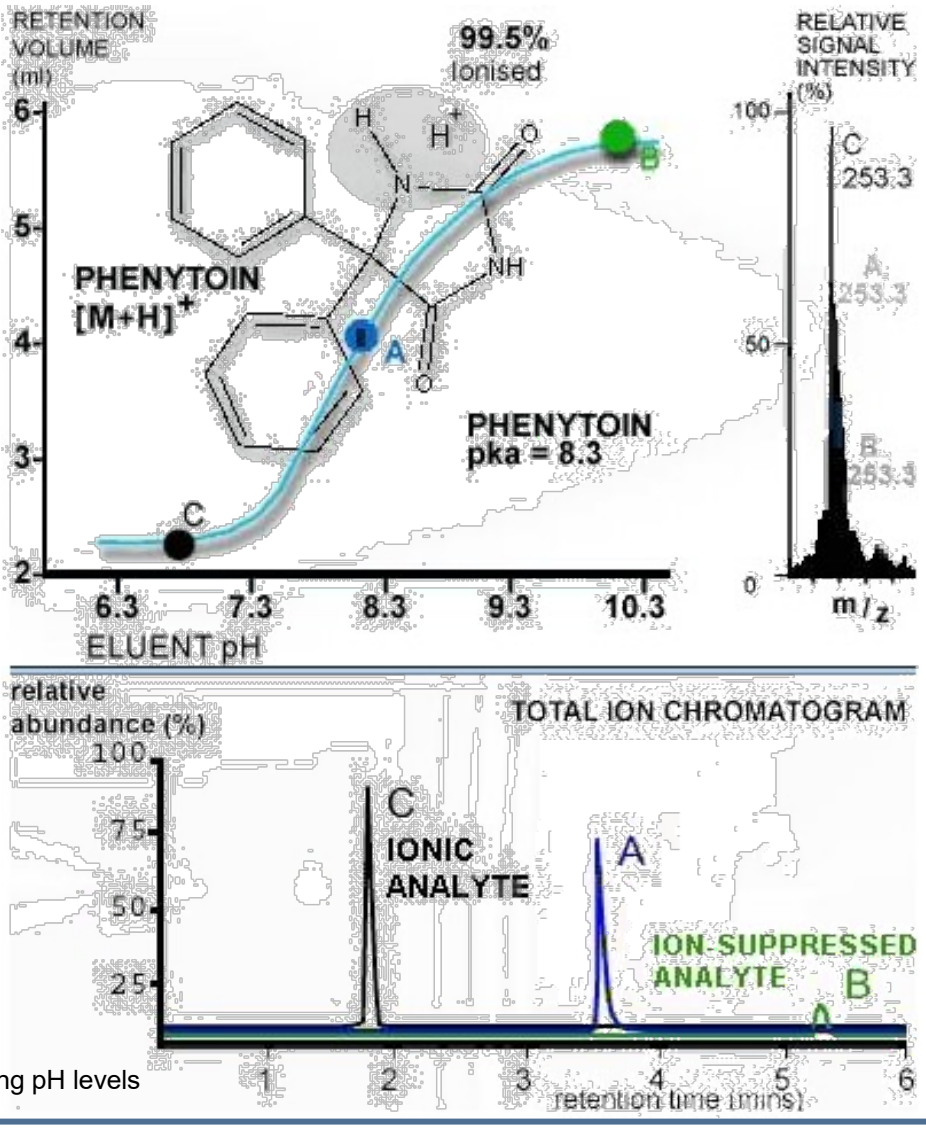
Example - Effect of TFA Levels on MS Signal Intensity



*Results are the mean of 2 experiments. *Note: The results from the no TFA samples were variable due to the lack of peptide protonization. Control sample was 50/50/0.1, MeOH/H_2O/FA (N=6, avg. signal intensity = $1.10x10^6$ counts.*

** 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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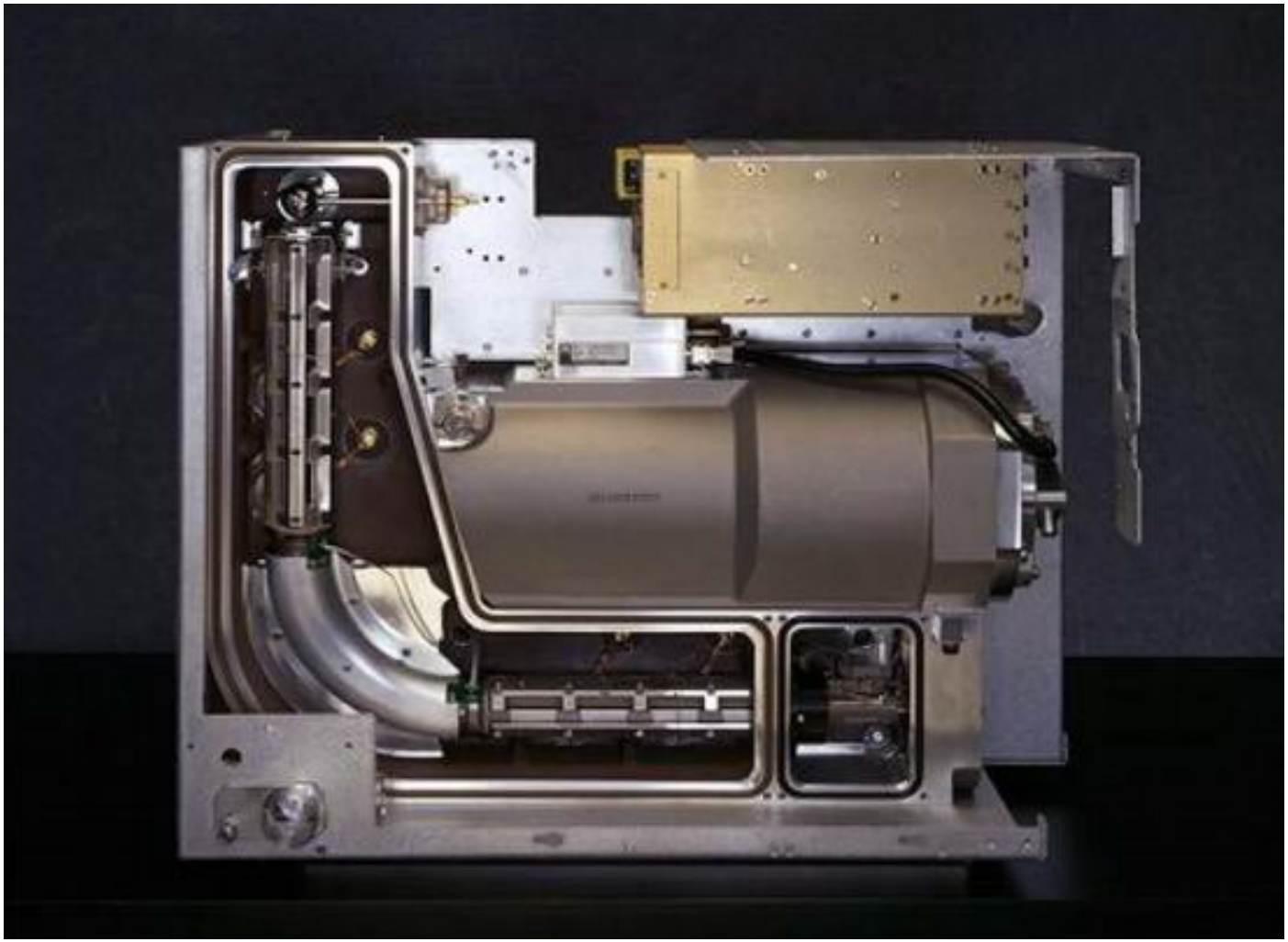
Chapter 3

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:

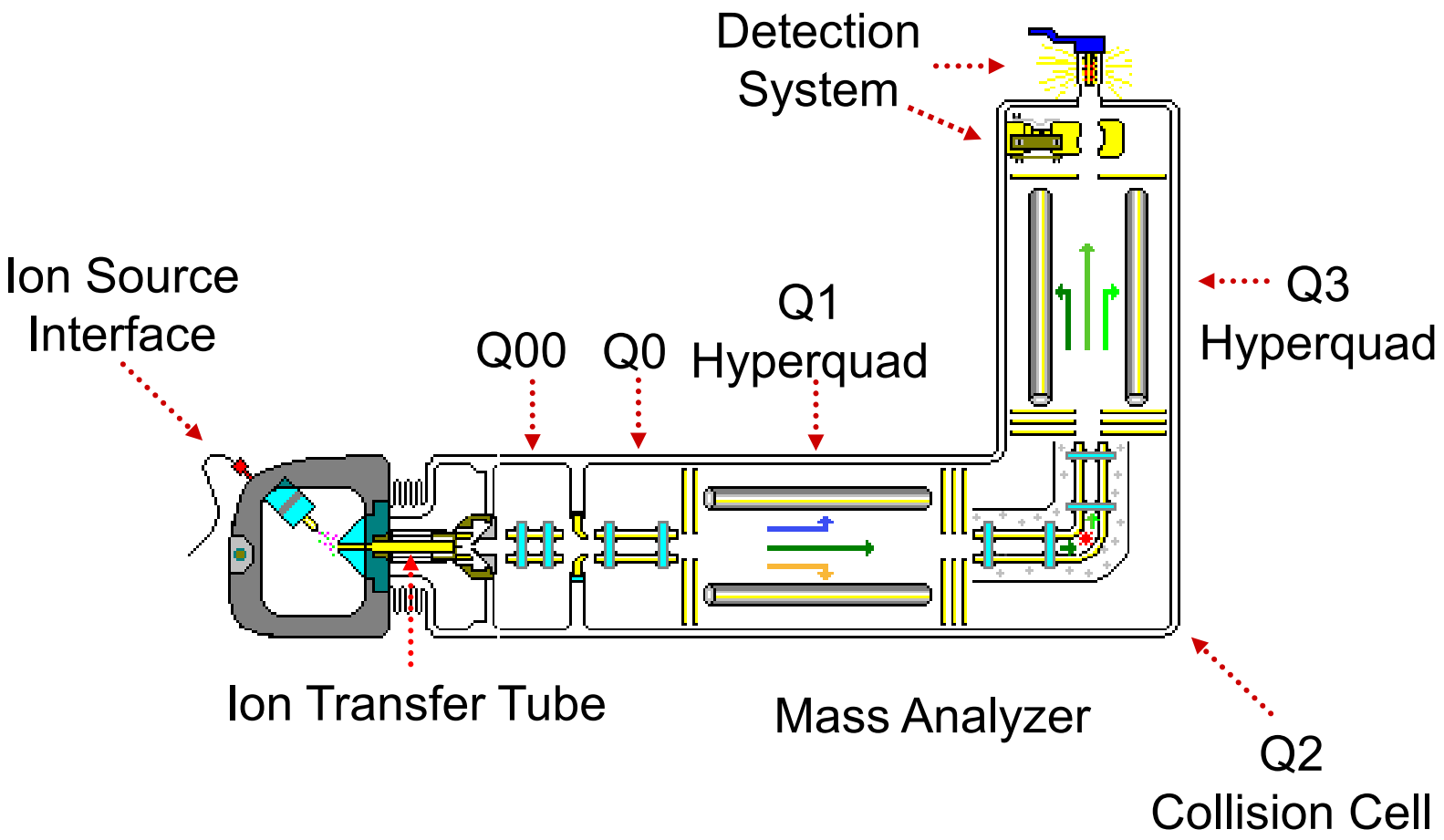
  - The time needed to stabilize the ion source signal
  - The time to switch the conversion dynode voltage (15 kV)

  **** 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.

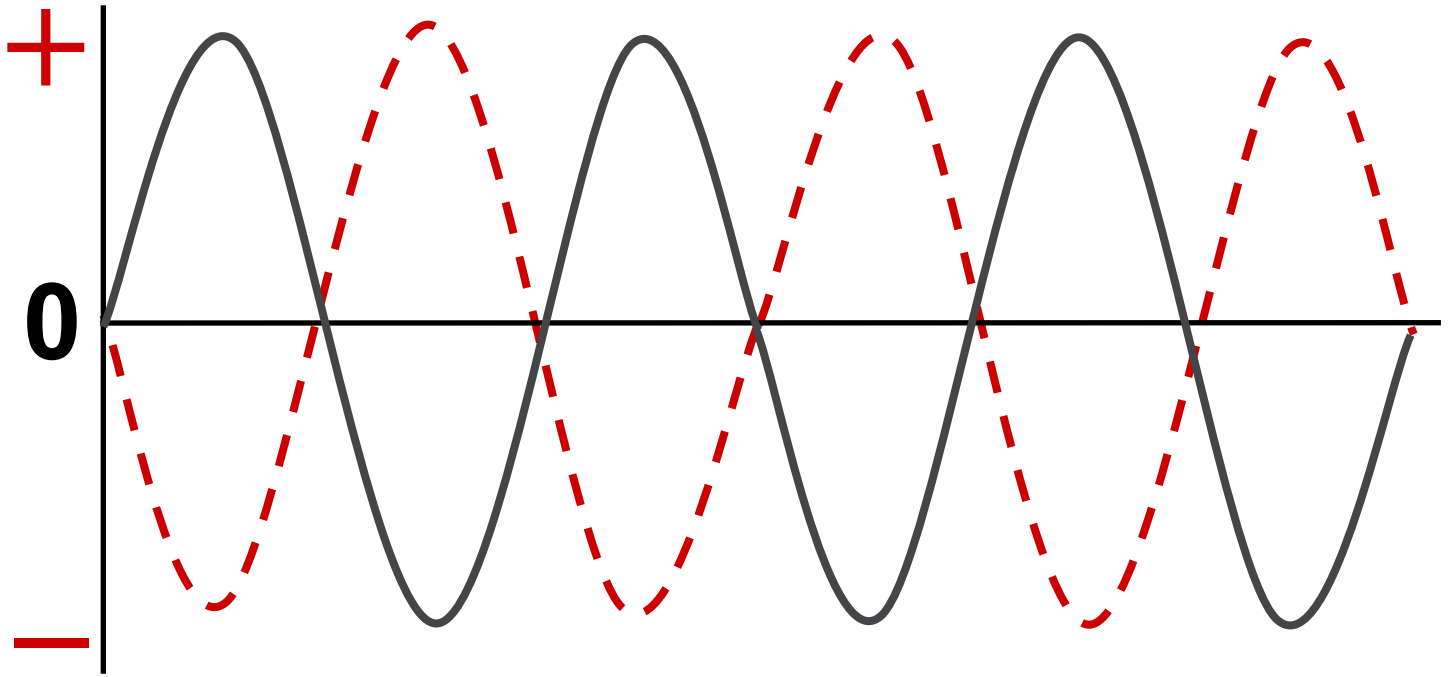
In TSQ Quantum, Q00 and Q0 are quadrupole assemblies of square rods that function solely as ion guides. They focus and transfer the ion beam between the high-pressure region of the ion source and the mass analyzer.

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

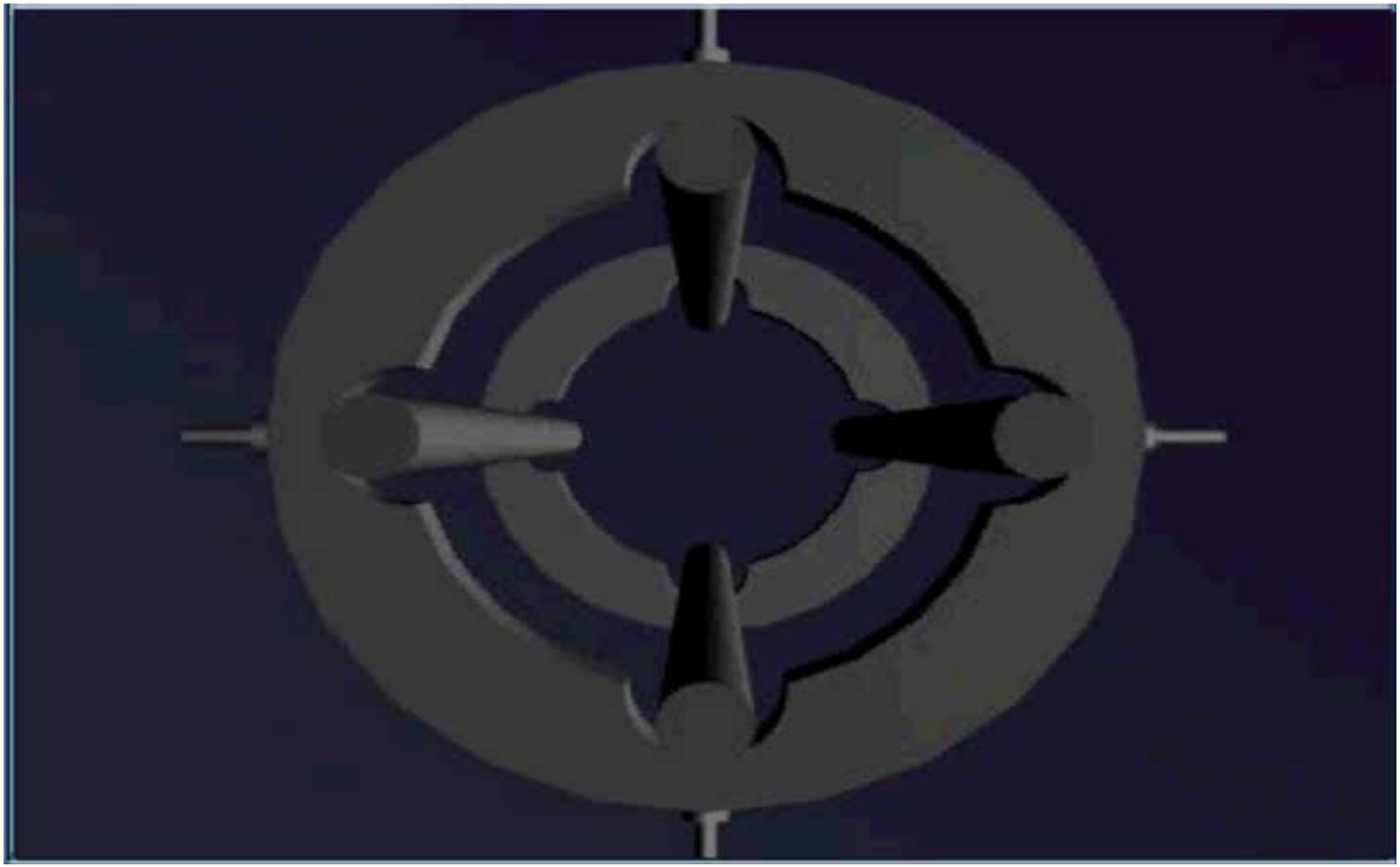
- Reduced size of the inter-quadrupole lens orifices
- Improved vacuum level maintenance at various stages
- Reduced noding in the quadrupole.
- Better collisional dampening in the quadrupole.
- Enhanced transmission of the ion beam.

Radio Frequency (RF) Voltage

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

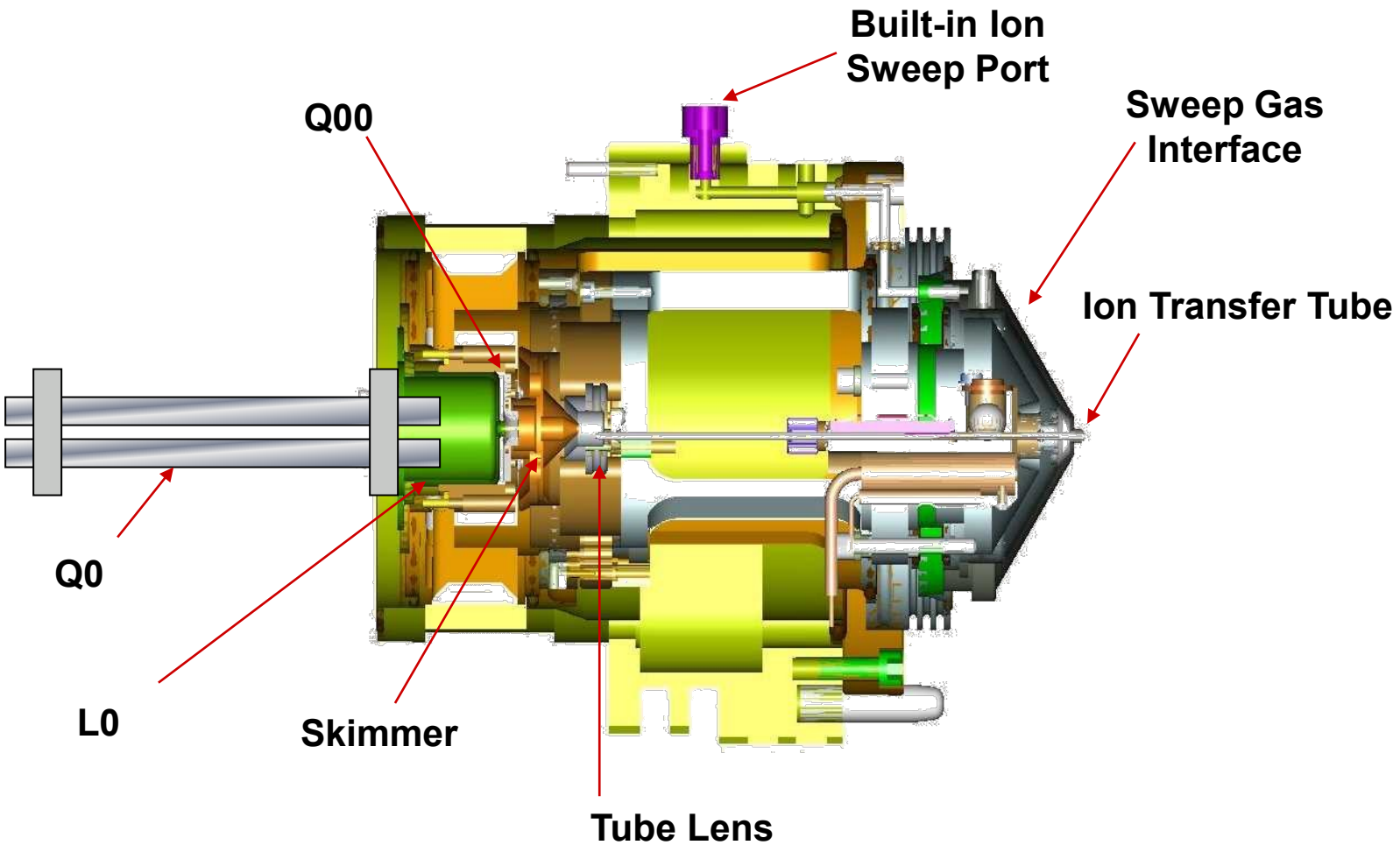


Multipole Transmission (RF-Only)



** 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

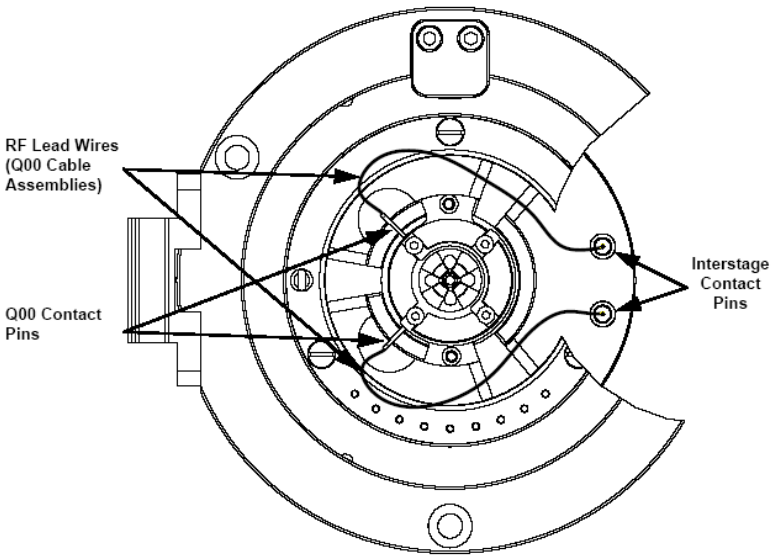


Skimmer

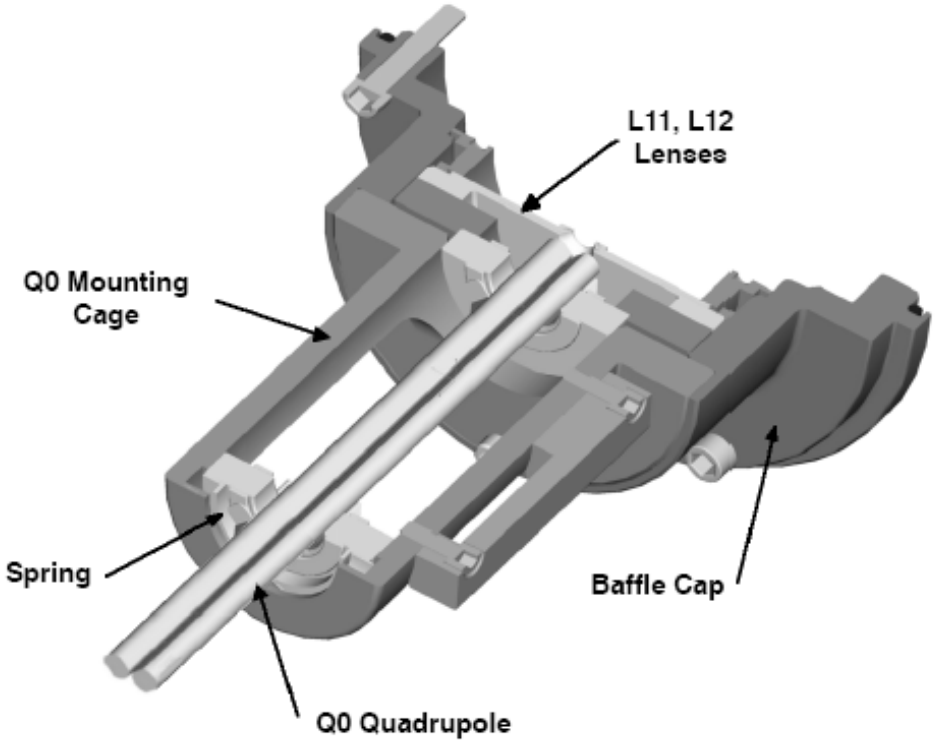
Characteristics:

- 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)

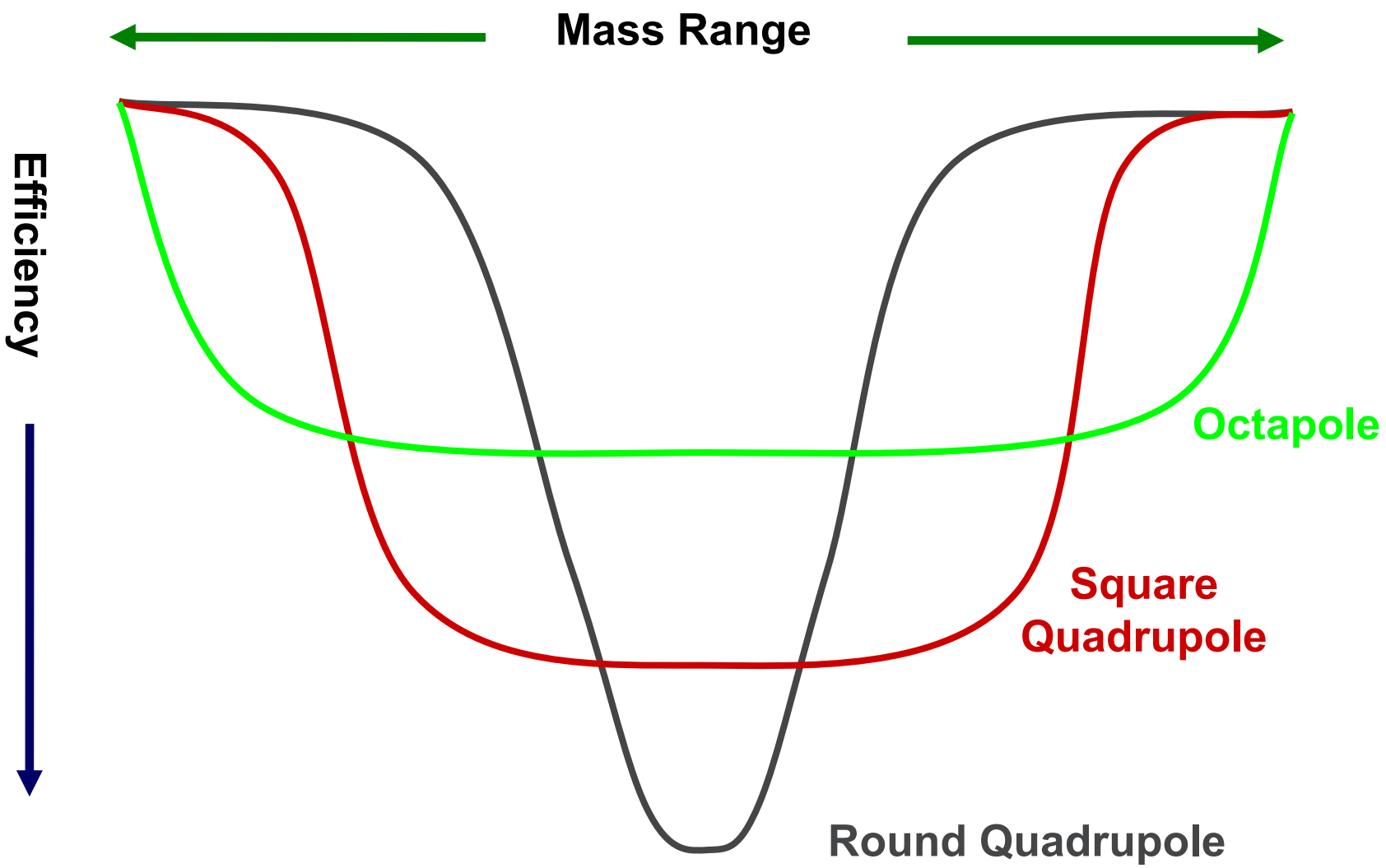


Q00

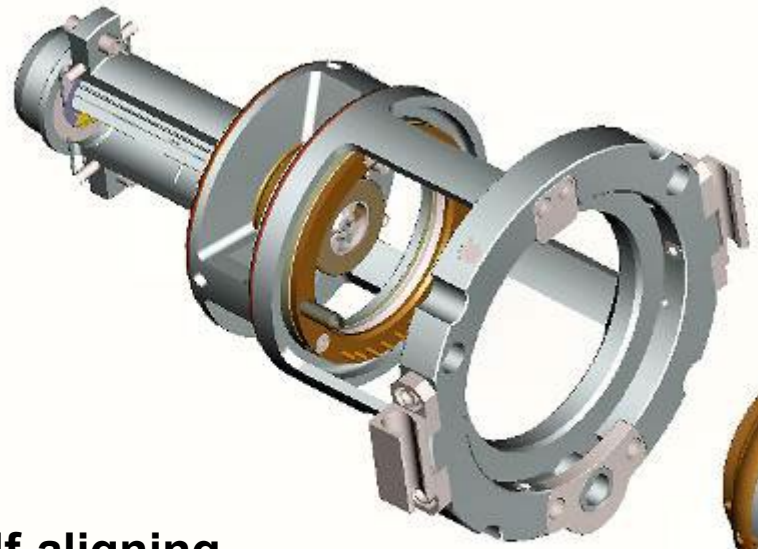


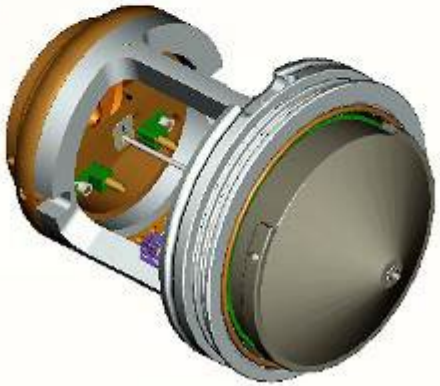
Q0

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

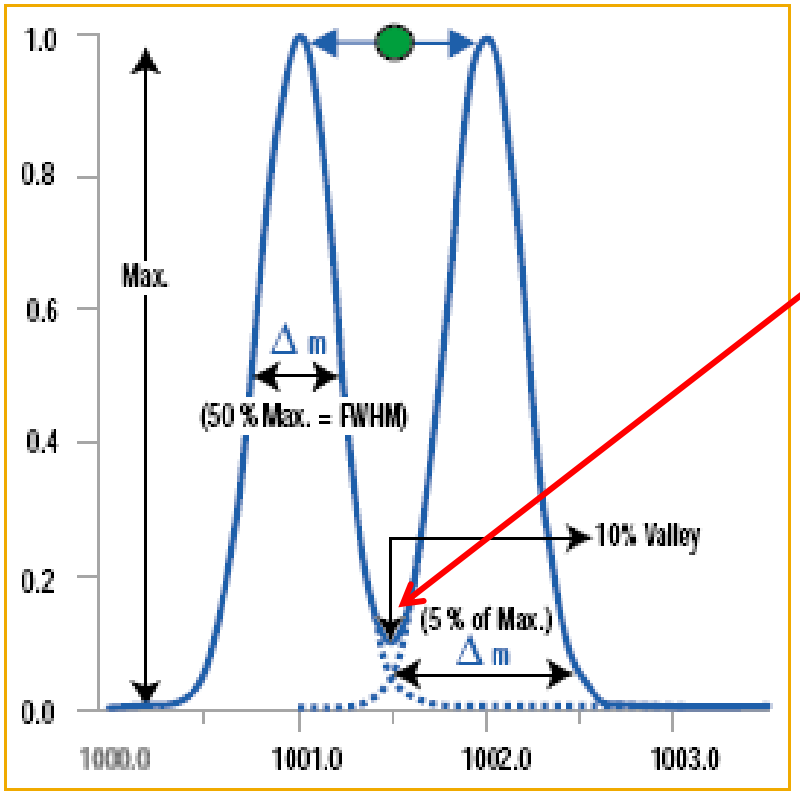
The Patented HyperQuad™ Advantage

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

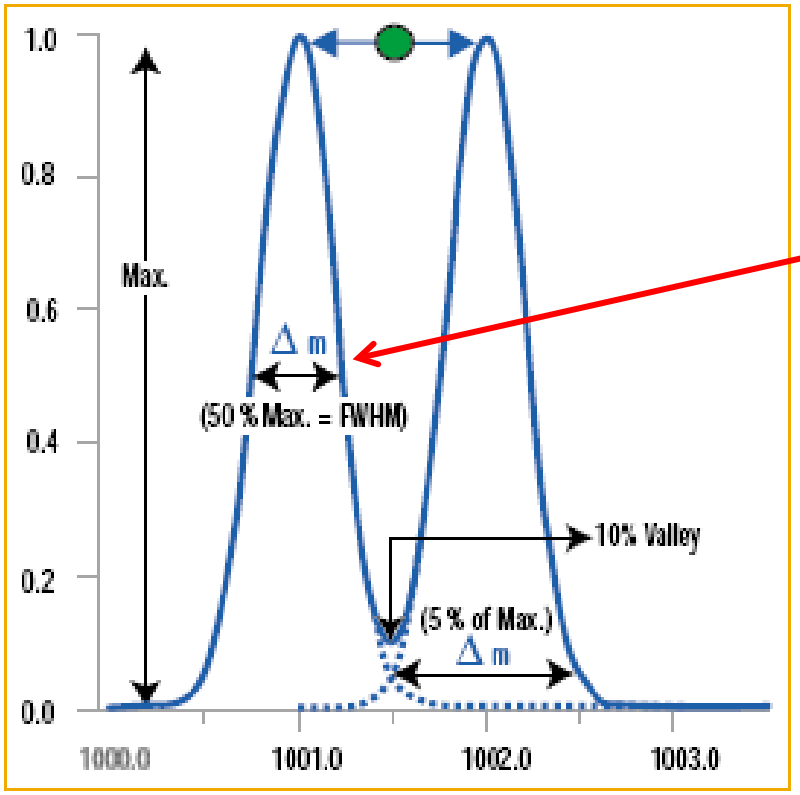
(10% valley definition)

$R_m = m/\Delta m$

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

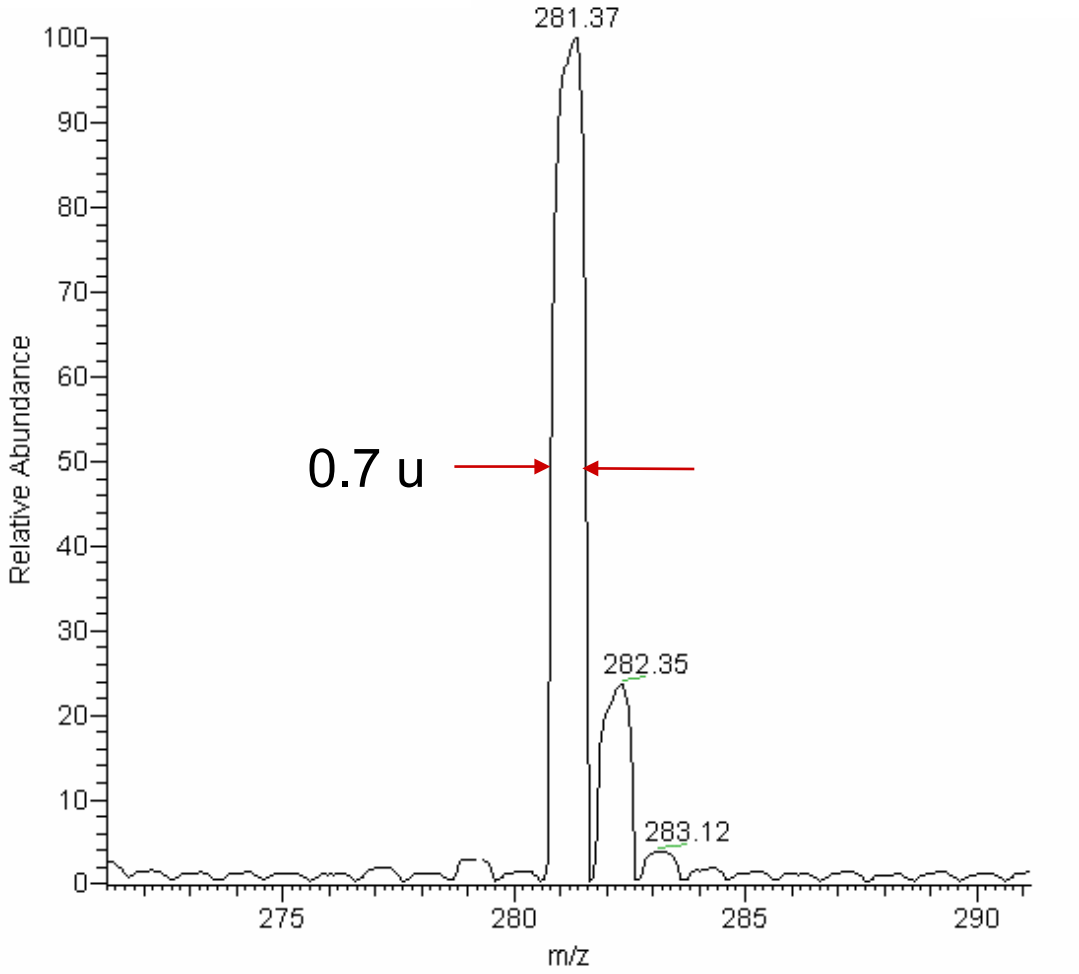
Purpose - to minimize chemical background caused by ions with the same nominal mass but different accurate mass (isobaric compounds) and, therefore, to increase the signal to noise ratio.

Example:

		R= 1000	R= 3000
Compound mass:	281.151 Da	**OVERLAPPING**	**SEPARATION**
Interfering mass:	281.053 Da		

		R= 1000	R=3000	R= 5000
Compound mass1:	372.351 Da	**OVERLAPPING**	**OVERLAPPING**	**SEPARATION**
Compound mass2:	372.421 Da			

Unit Resolution (0.7 FWHM)



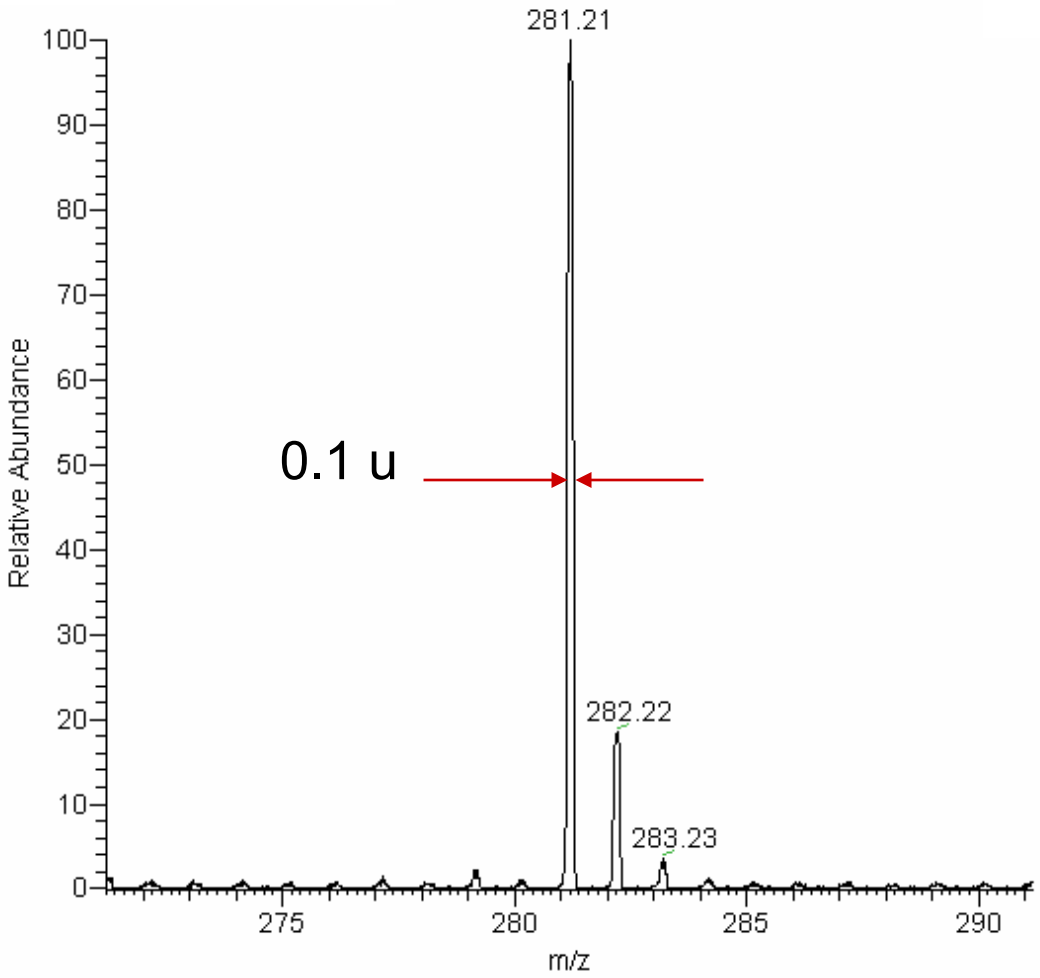
m/z 300/ 0.7 = R 428

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.

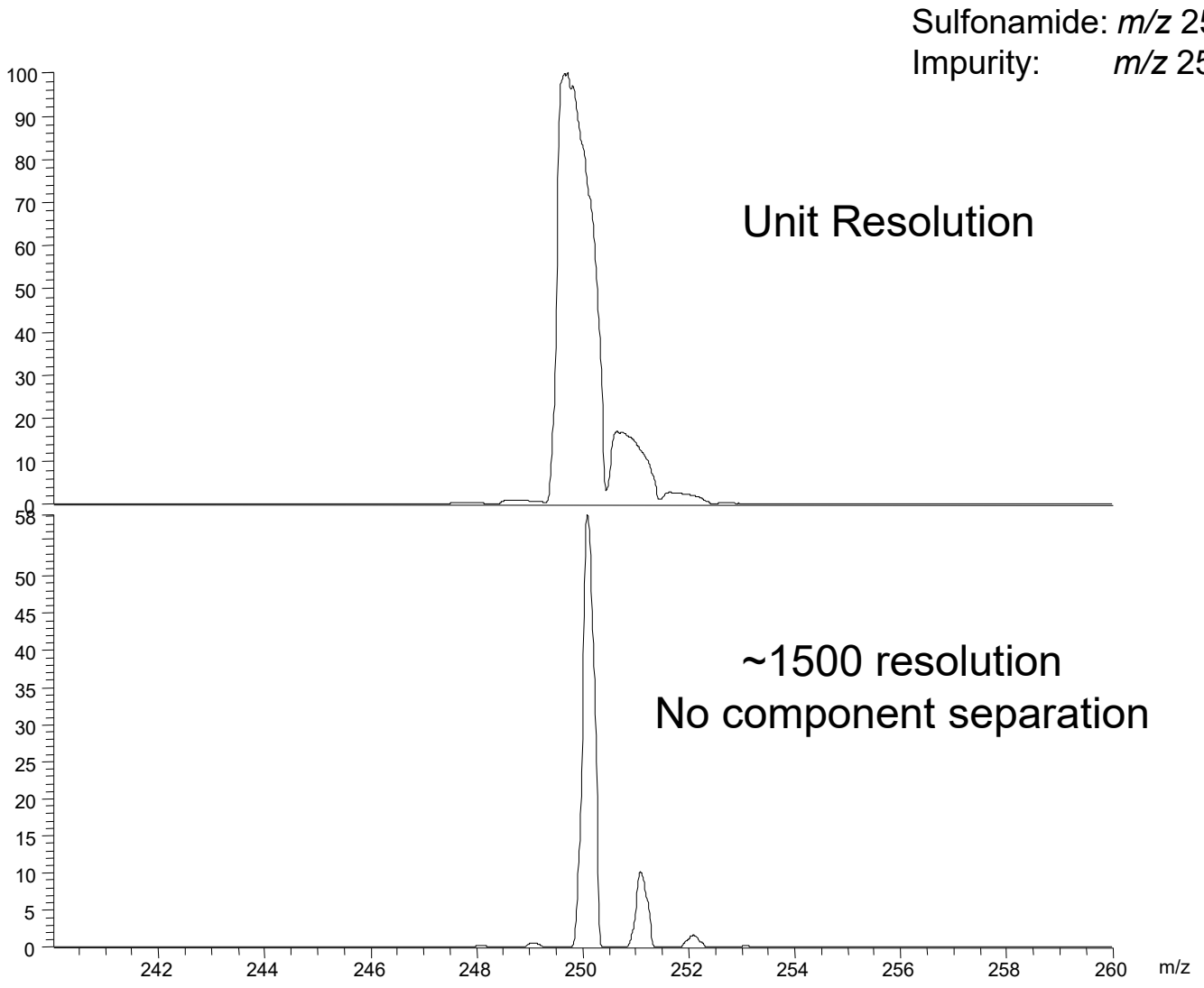
  Sulfonamide: $[M+H]^+$ *m/z* 250.15

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

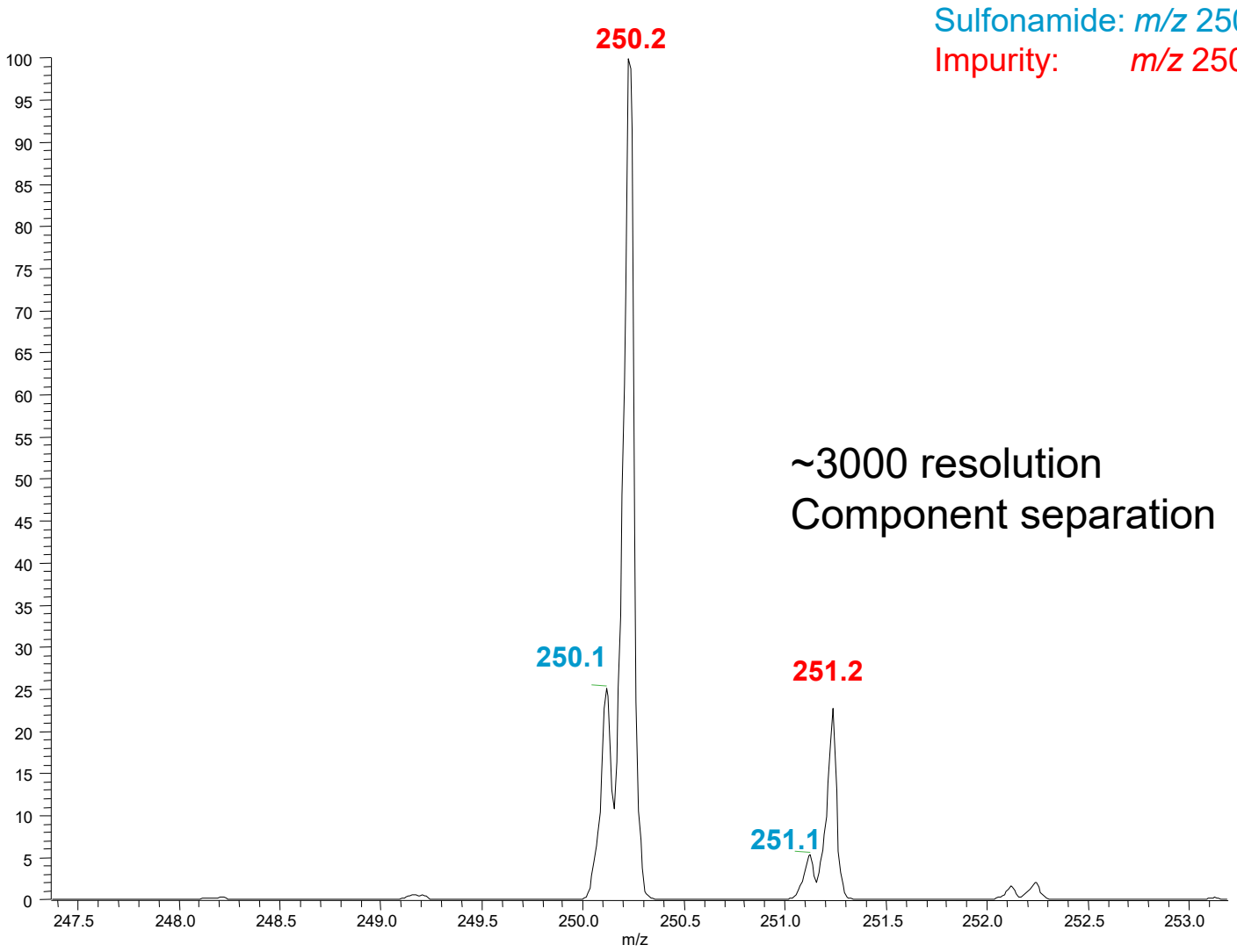
- Chromatographic separation is not an option in the given example

- Precursor ion Hi-Res specificity allows for compound differentiation via the respective product ion MS/MS spectra

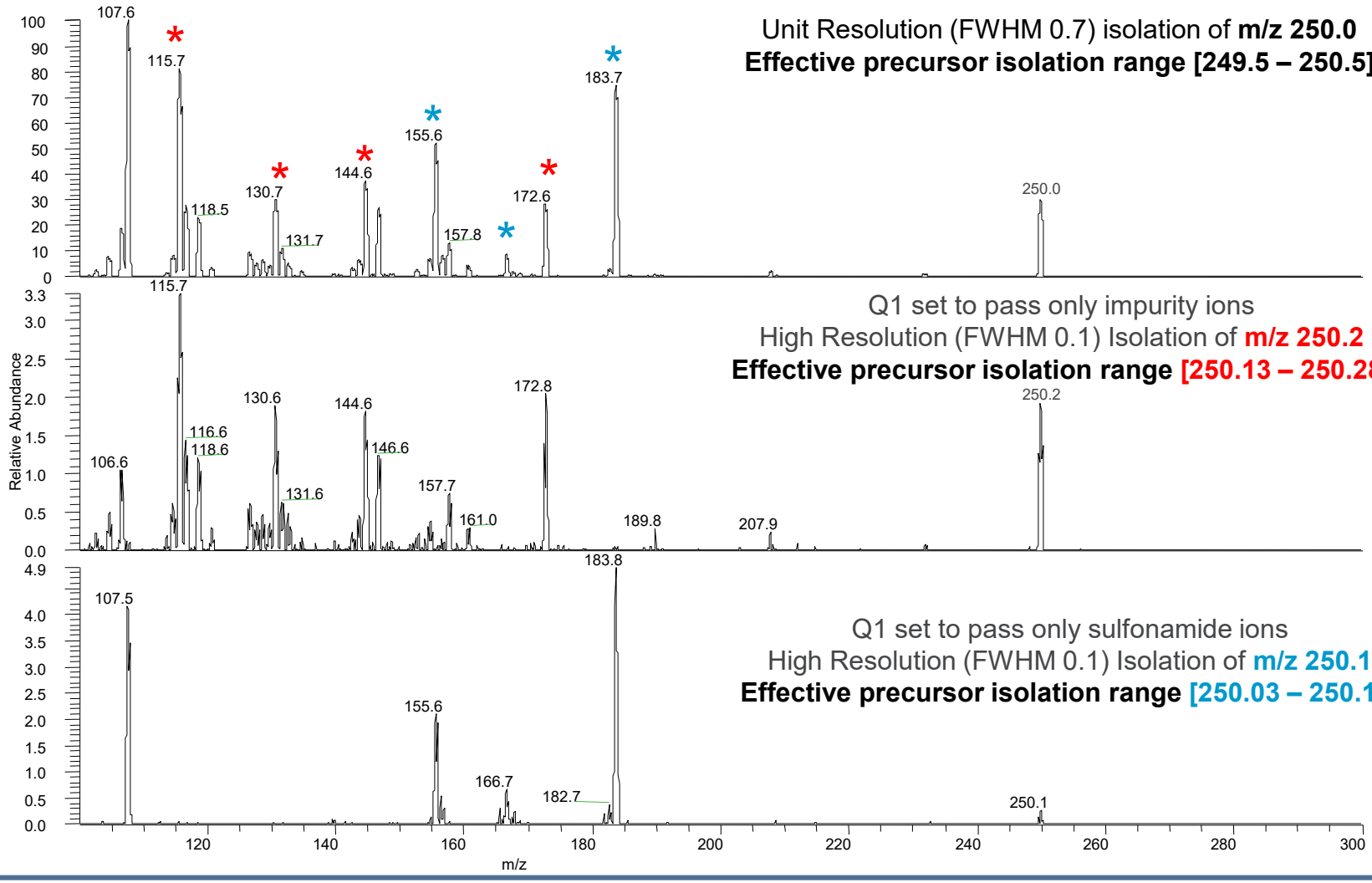
Isobaric Discrimination – Effect of Increasing Resolution



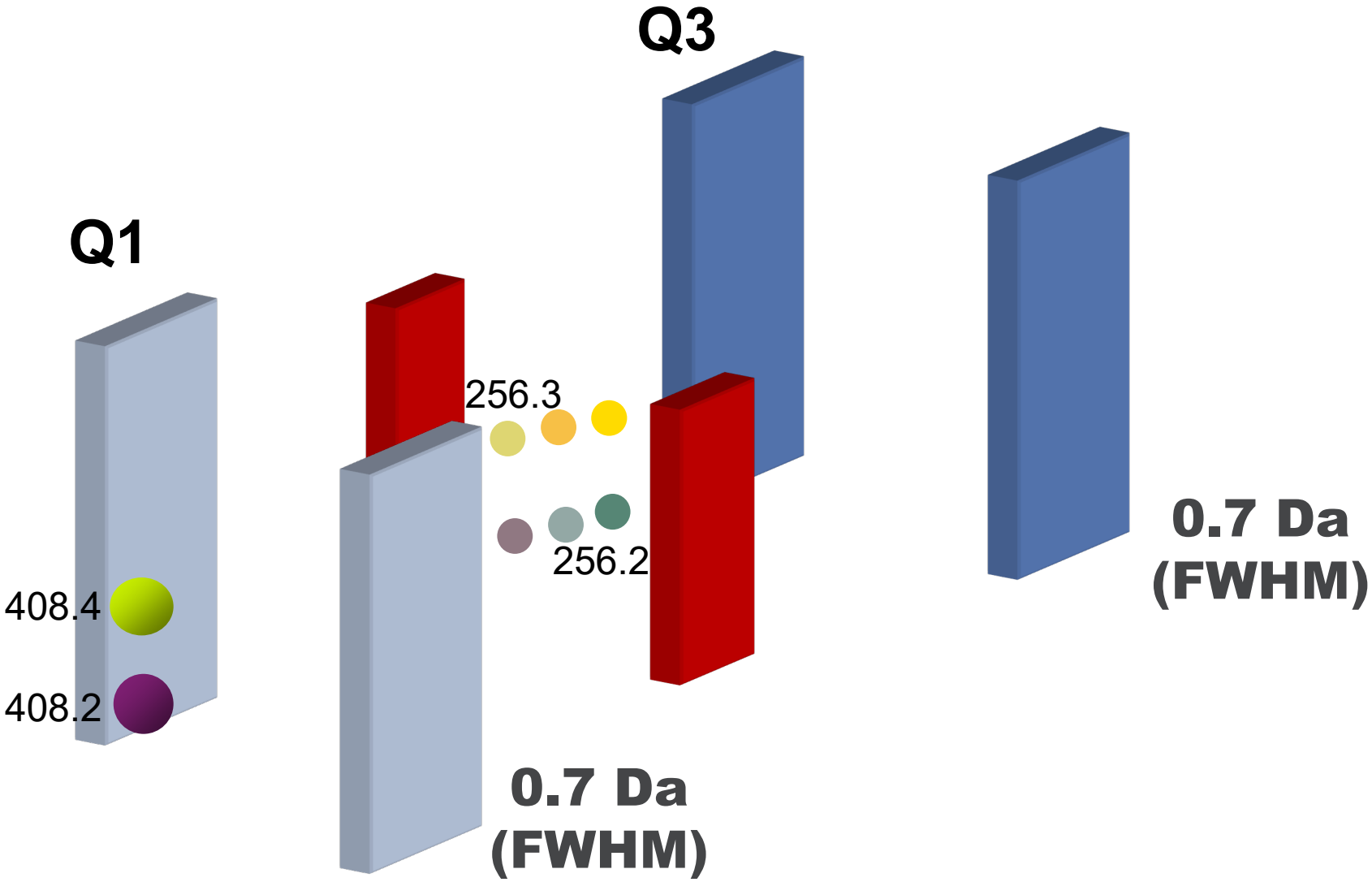
Isobaric Discrimination – Effect of Increasing Resolution



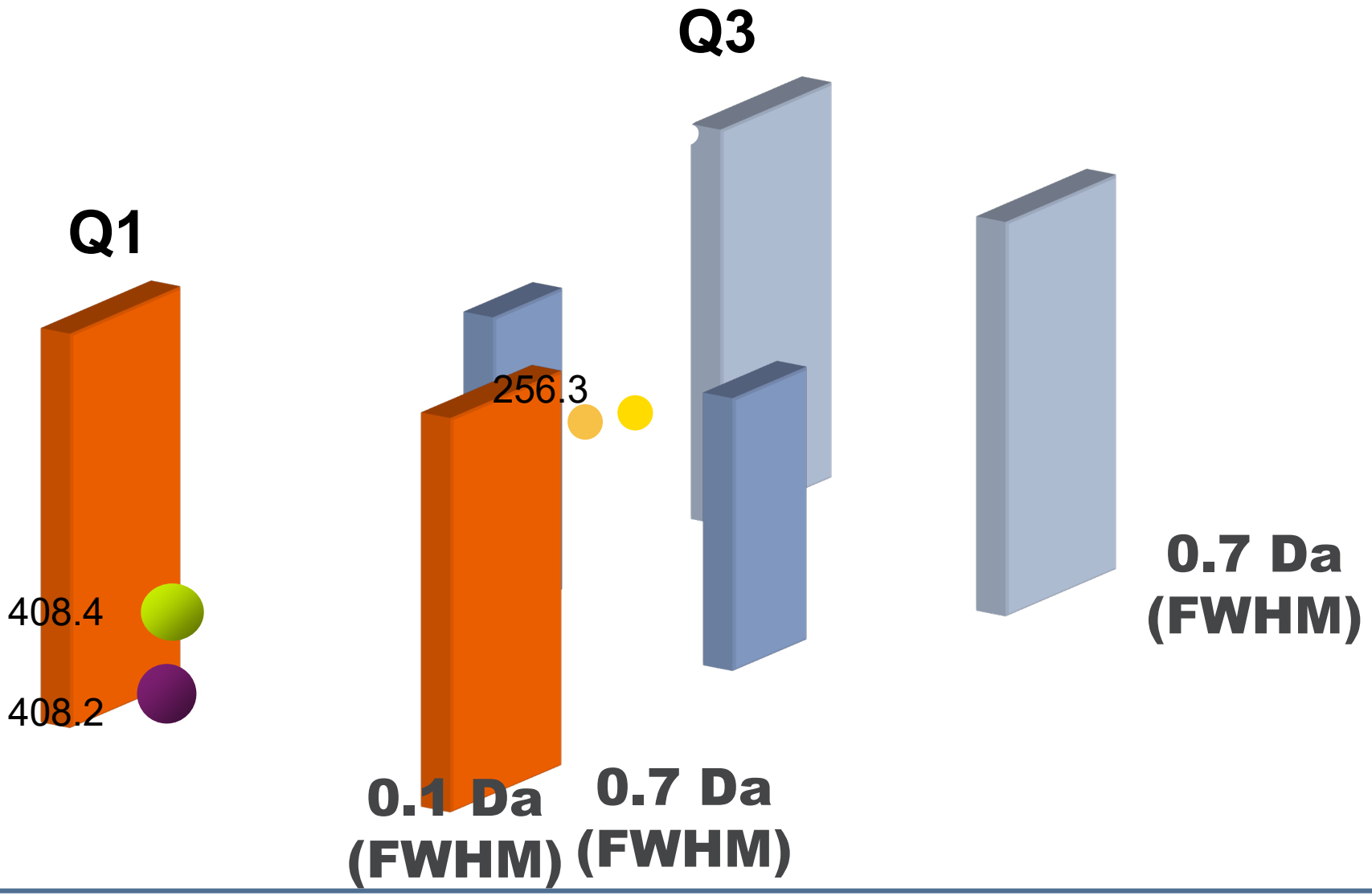
Isobaric Discrimination – Effect of Increasing Resolution



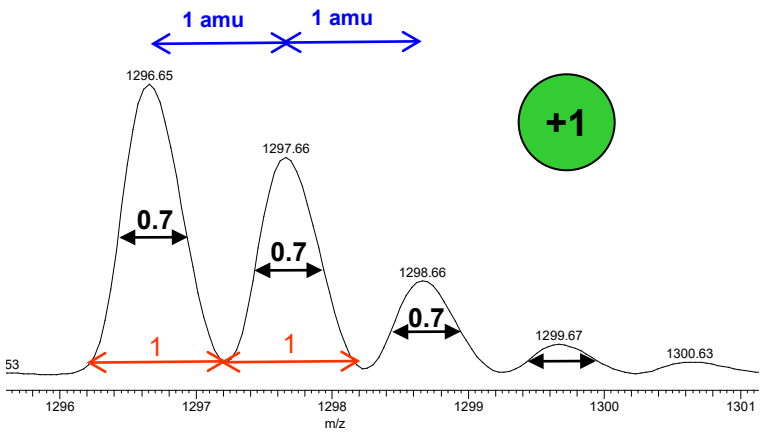
SRM - Selected Reaction Monitoring at Unit Resolution

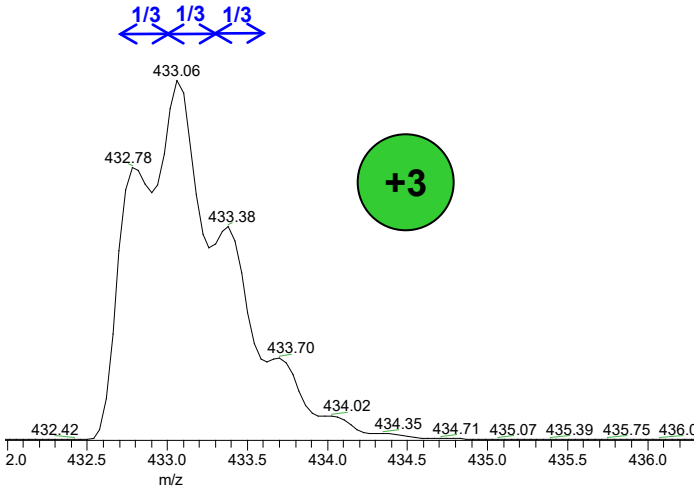


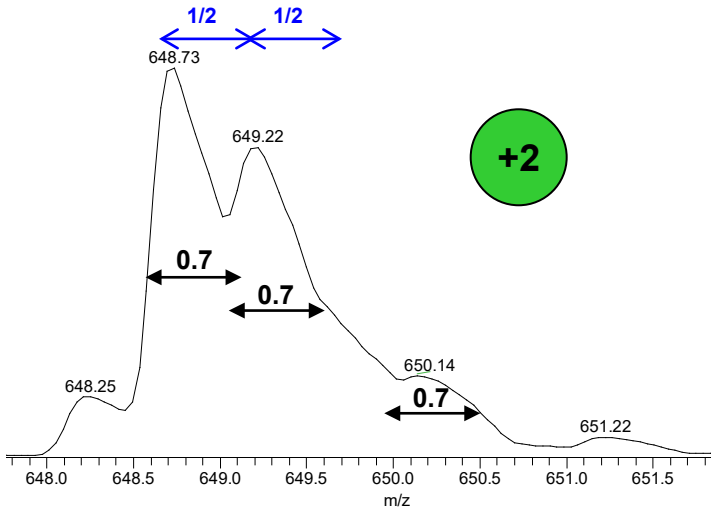
H-SRM - Selected Reaction Monitoring at High Resolution

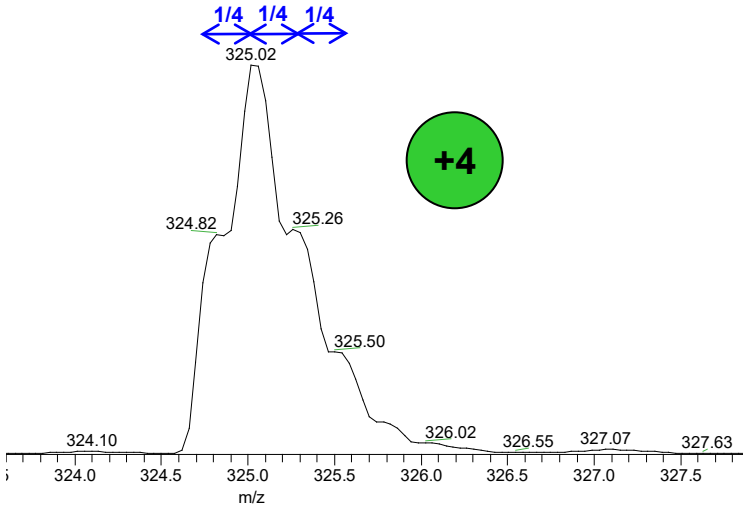


Charge State Determination (FWHM = 0.7)

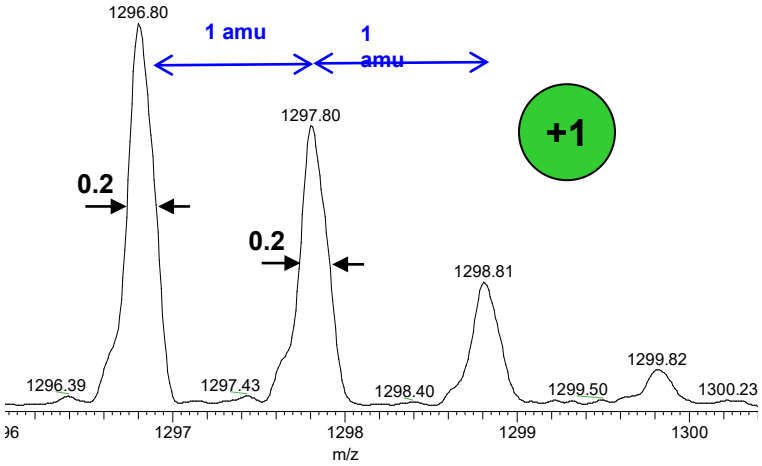


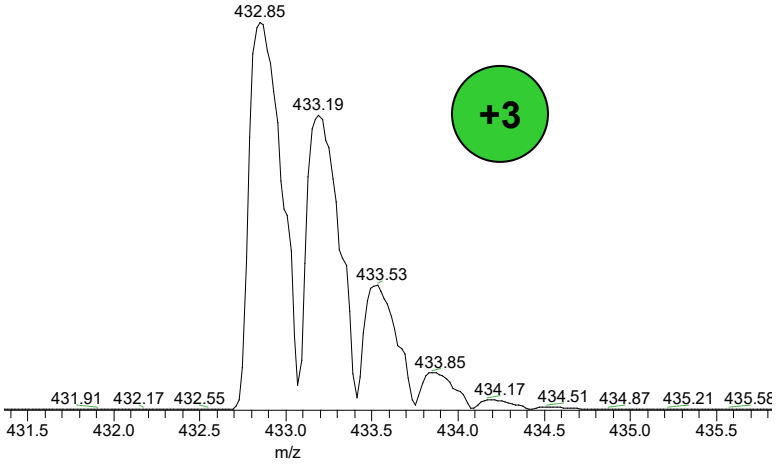


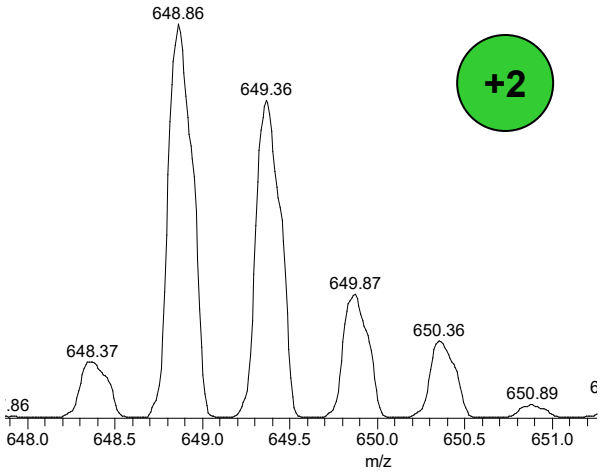


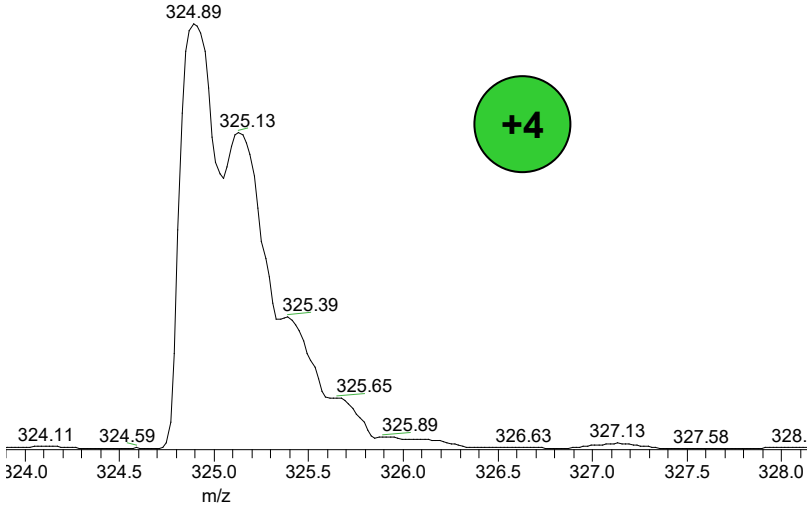


Charge State Determination (FWHM = 0.2)

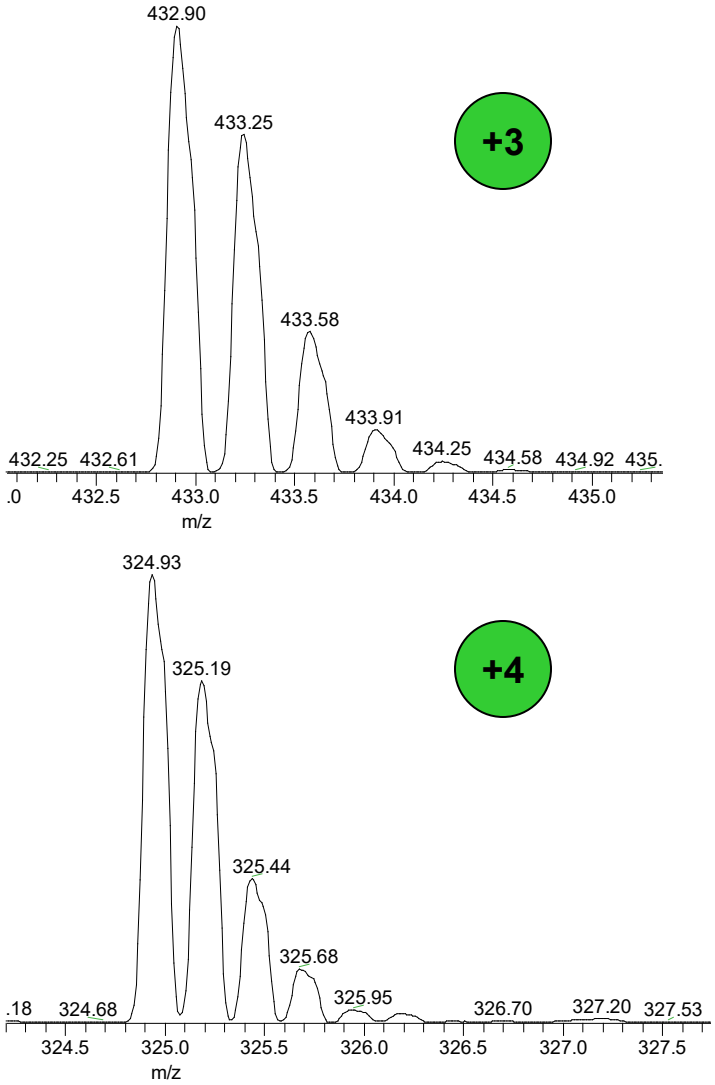








Charge State Determination (FWHM = 0.1)



Quadrupole Ion Selection – Q1 and Q3 as Ion Filters

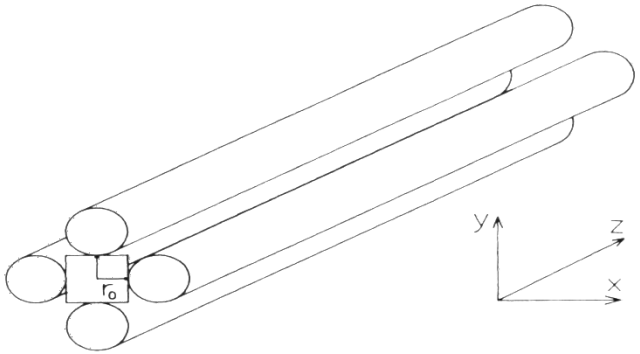


Figure 2.3. Conventional array of circular rods in a quadrupole mass filter.

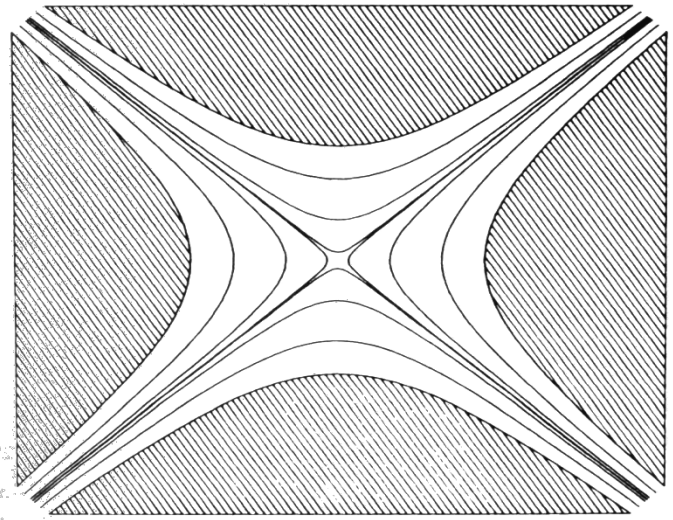
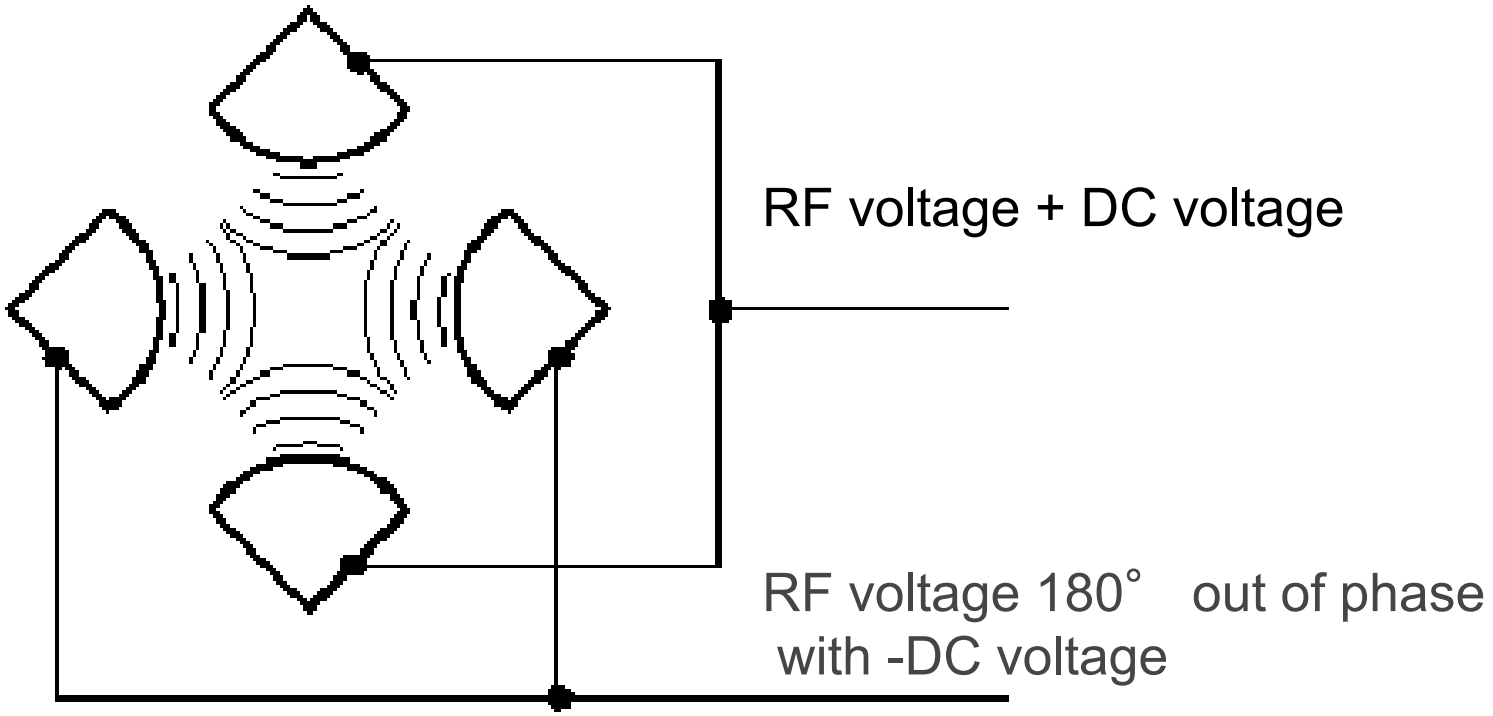


Figure 2.4. Hyperbolic equipotential contours in an ideal two-dimensional quadrupole field.

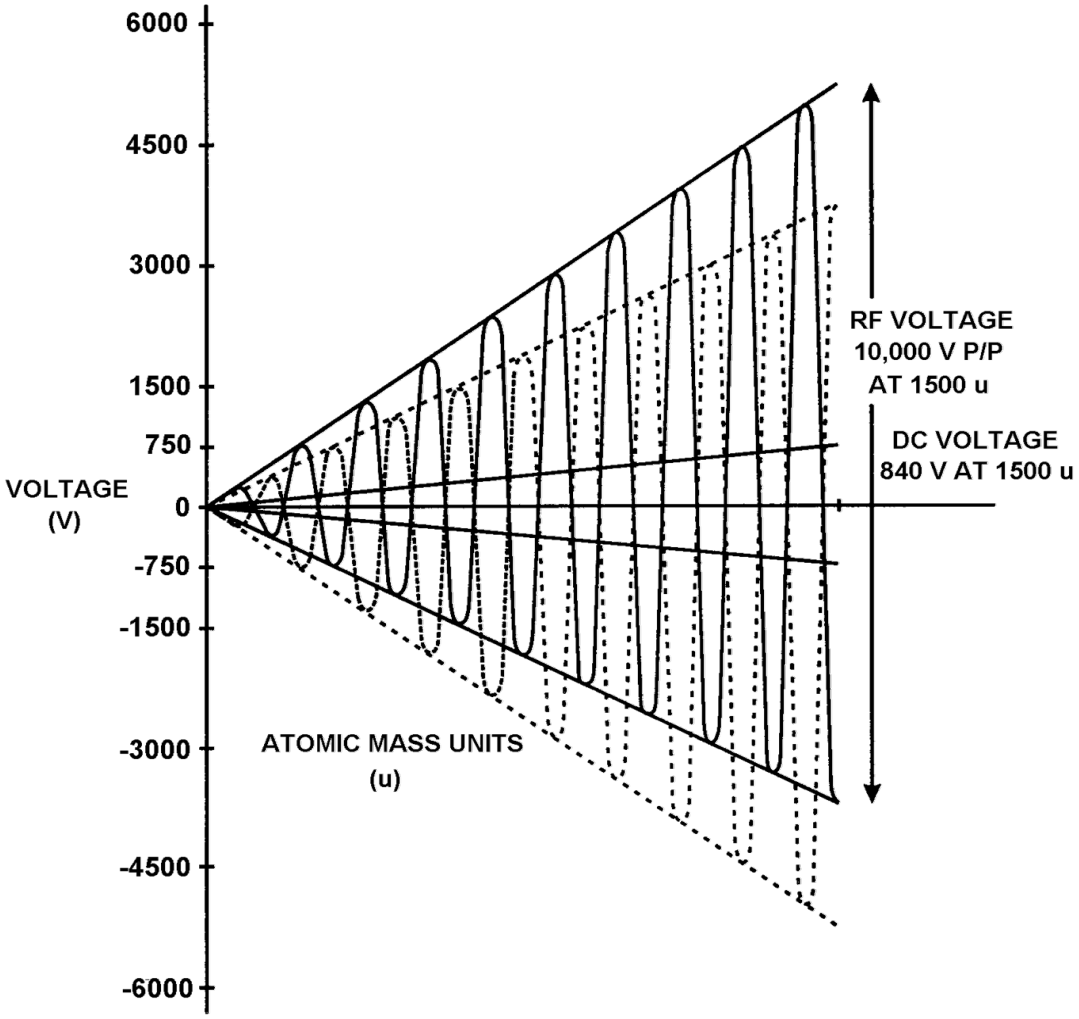
** Raymond E. March, Richard J. Hughes. "Quadrupole Storage Mass Spectrometry." Wiley Interscience, 1989.*

RF and DC Fields Applied to the Quadrupoles

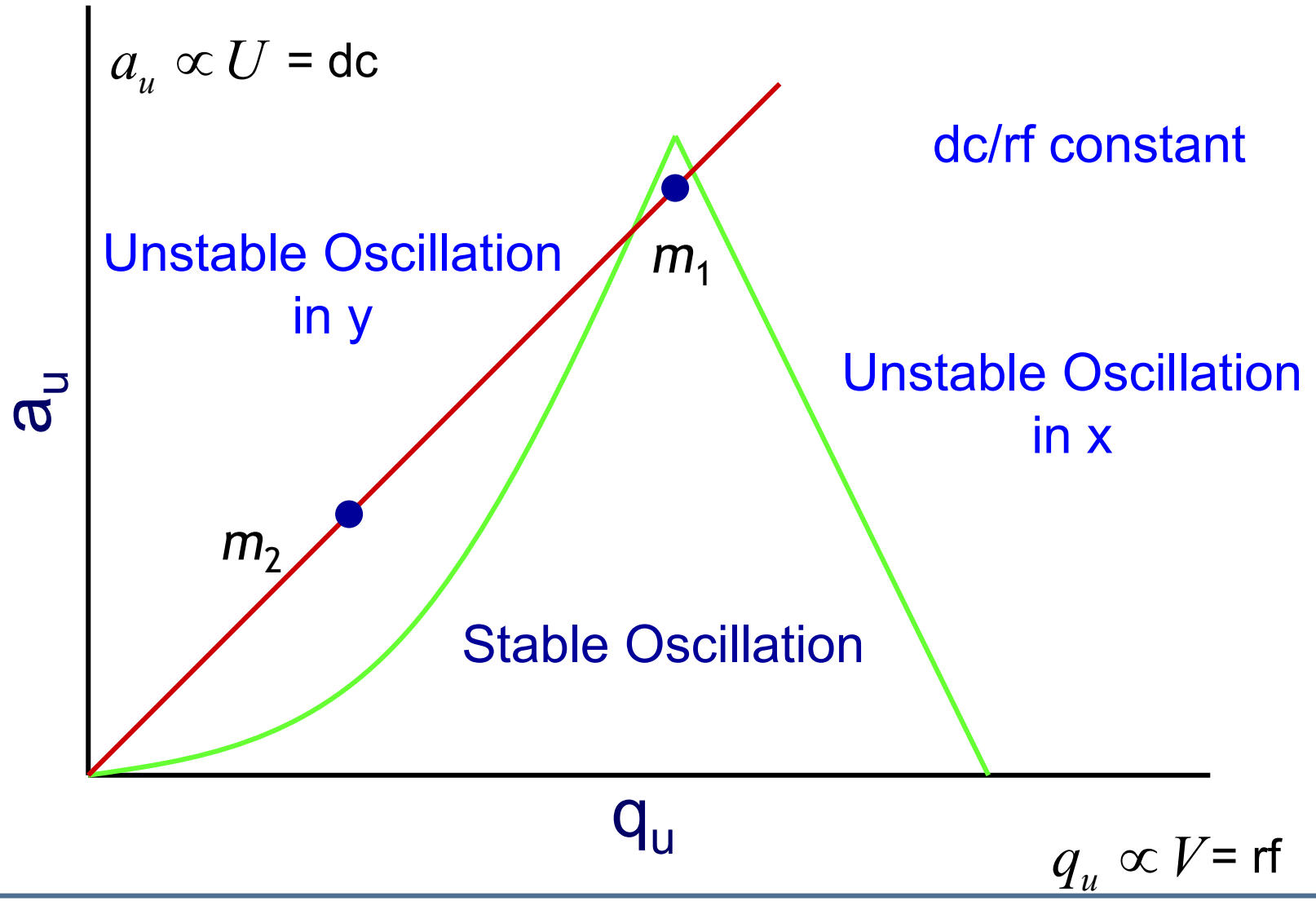
RF = 1.123 MHz and of variable amplitude (0 to 10,000 V peak-to-peak)
DC = 0 to ± 840 V



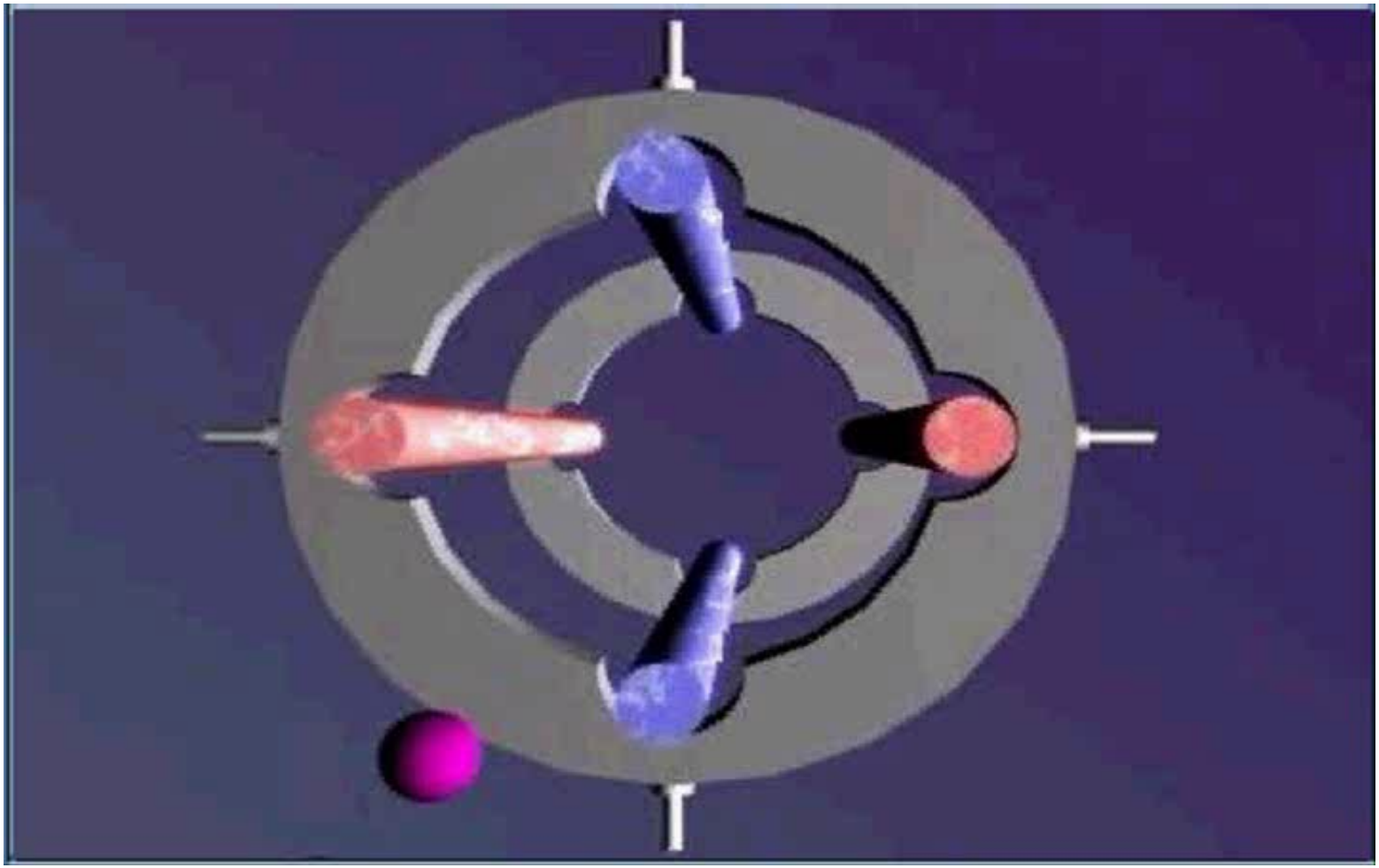
RF and DC Fields Applied to the Quadrupoles



Stability Diagram Transformed to (U,V) Space



RF and DC Fields Applied to the Quadrupoles



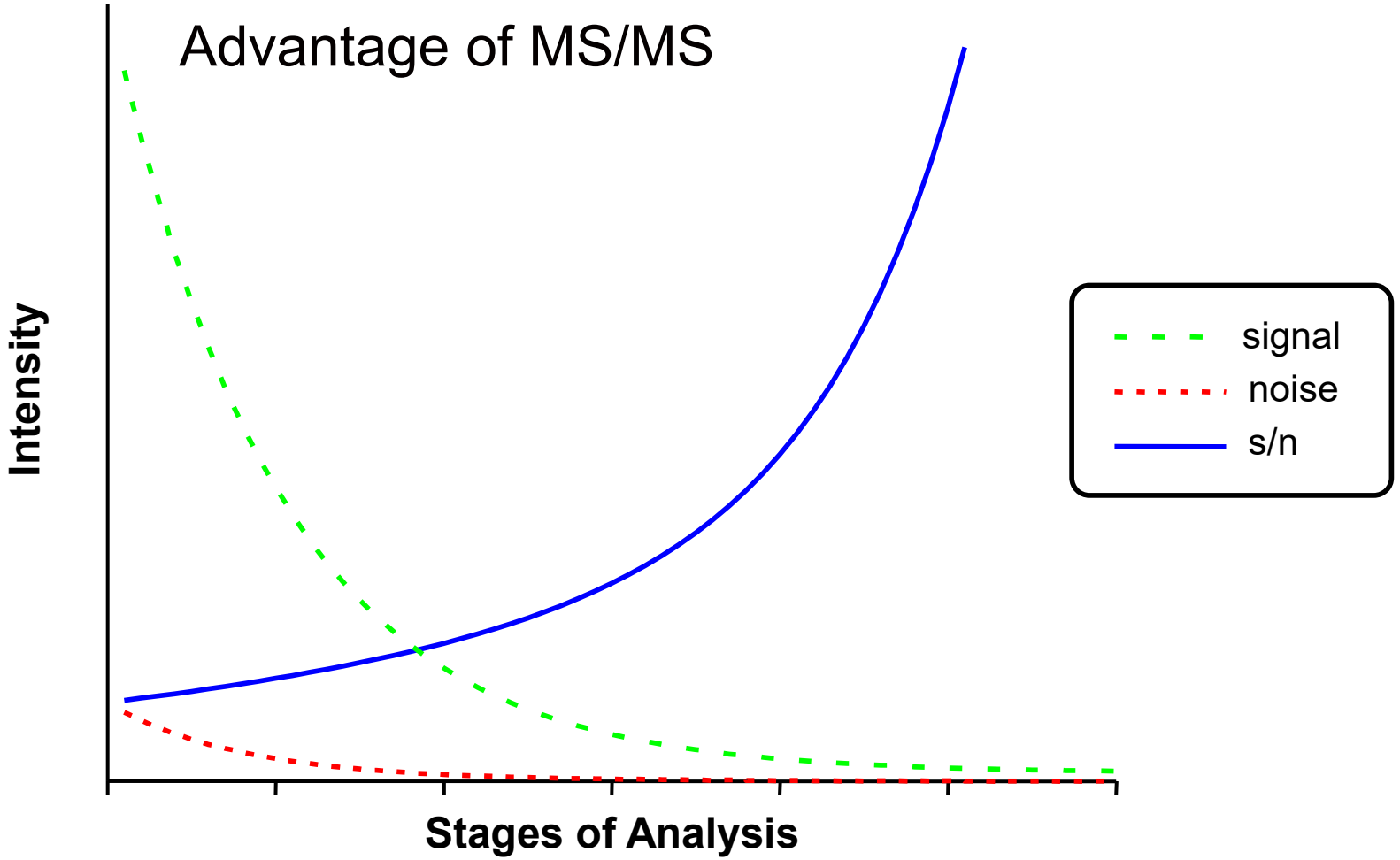
** Crawford Scientific, OPDAC (Online Professional Development in Analytical Chemistry) LC-MS training package. Holm Street, Strathaven, Lanarkshire, ML10 6NB, Scotland, UK*

Summary – Scanning vs. Transmission

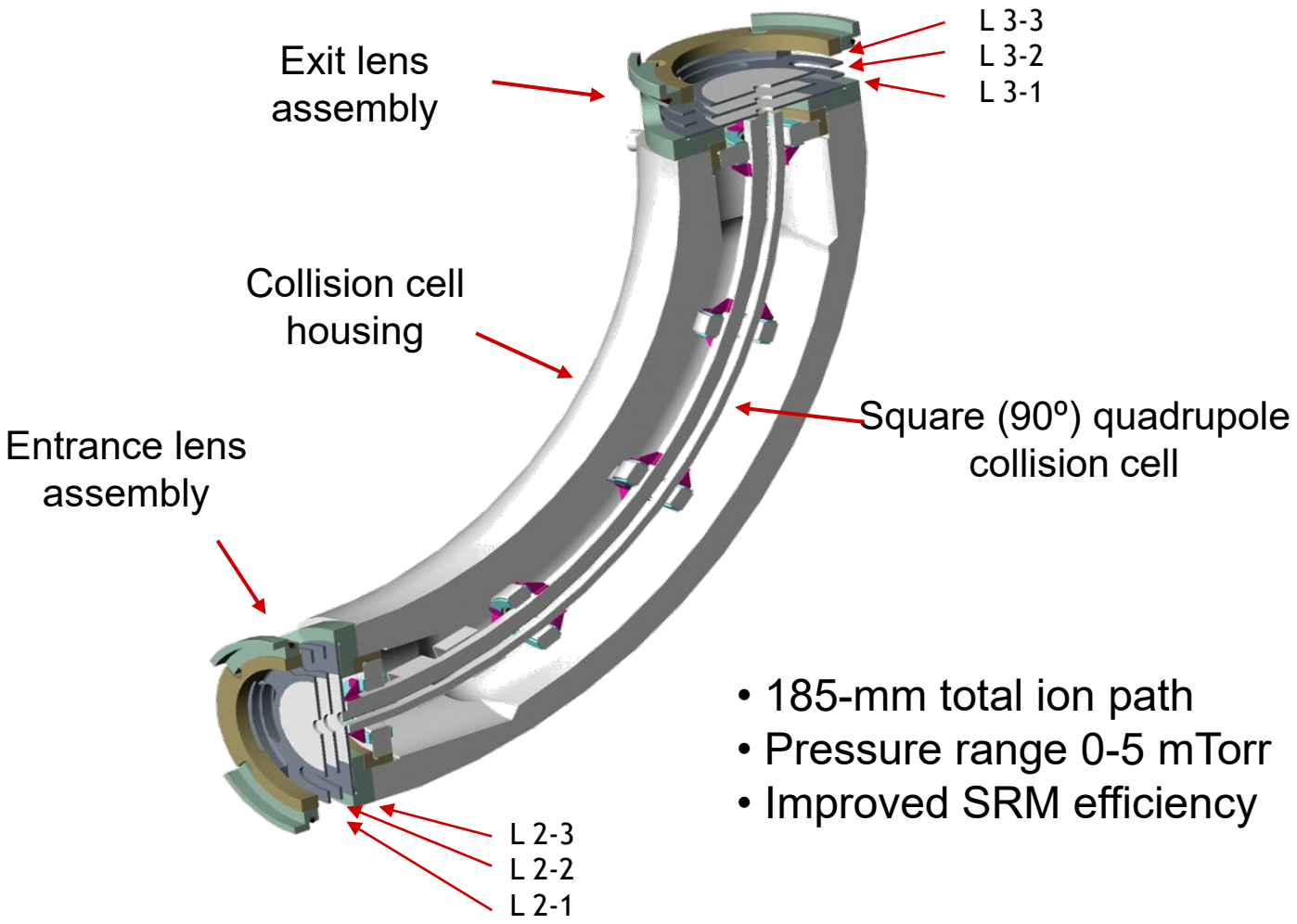
If the RF and DC voltages are ramped upward (i.e.the mass analyzer is scanned upward), discreet ion populations of successively higher *m/z* ratios are allowed to pass through the analyzer, thus having stable trajectories.

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)



- 185-mm total ion path
- Pressure range 0-5 mTorr
- Improved SRM efficiency

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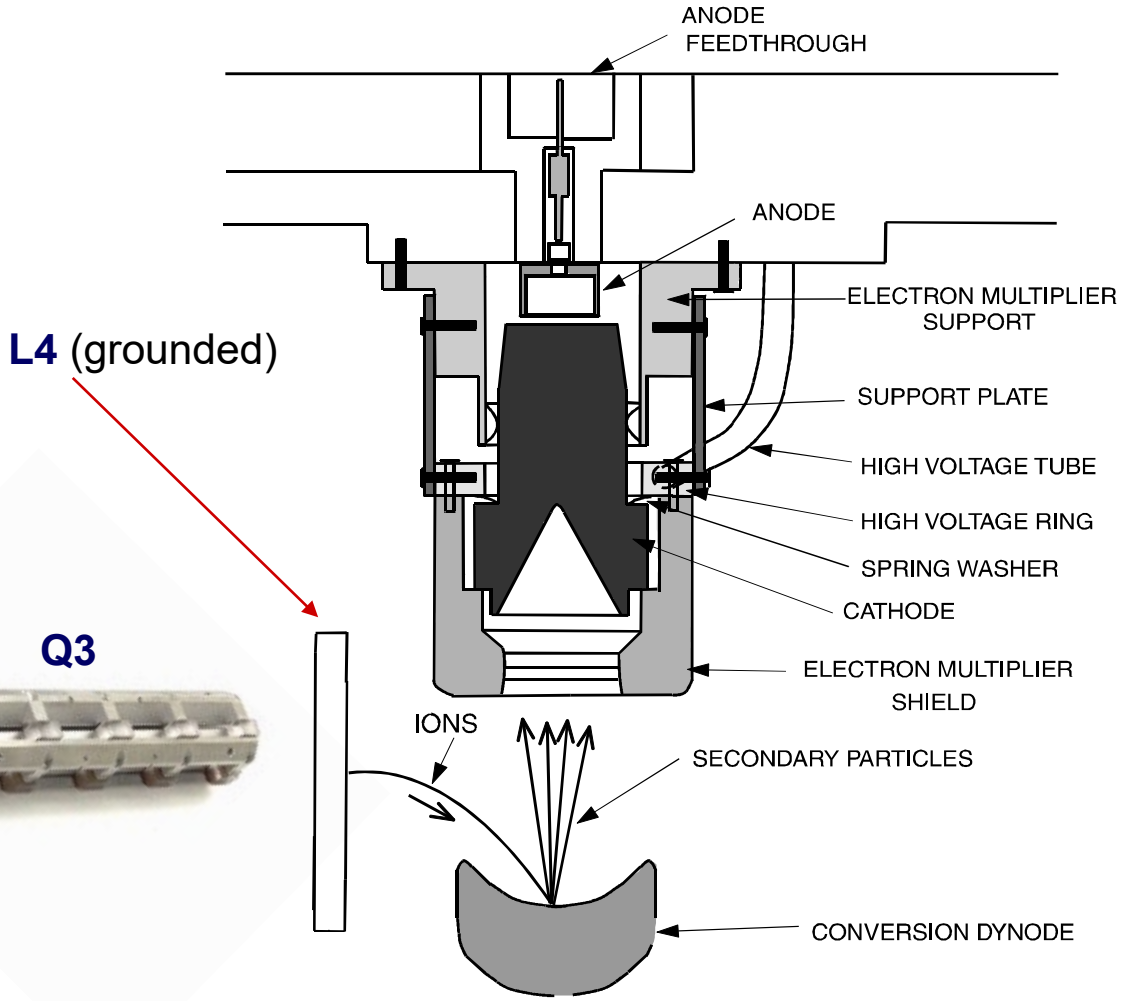
Chapter 5

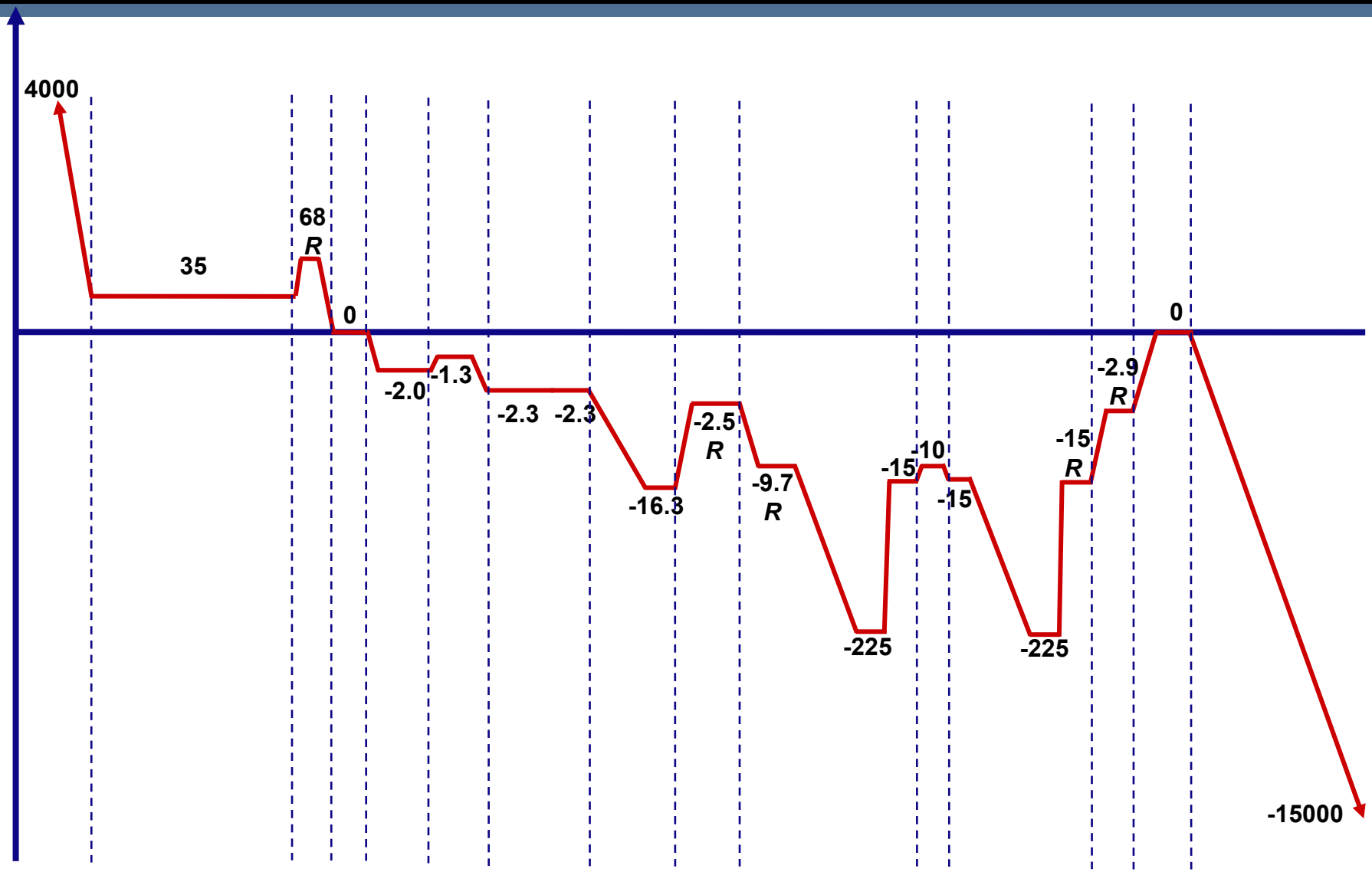
Detection and Vacuum

The Ion Detection System



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

The Ion Optics Potential Energy Diagram



The Vacuum System

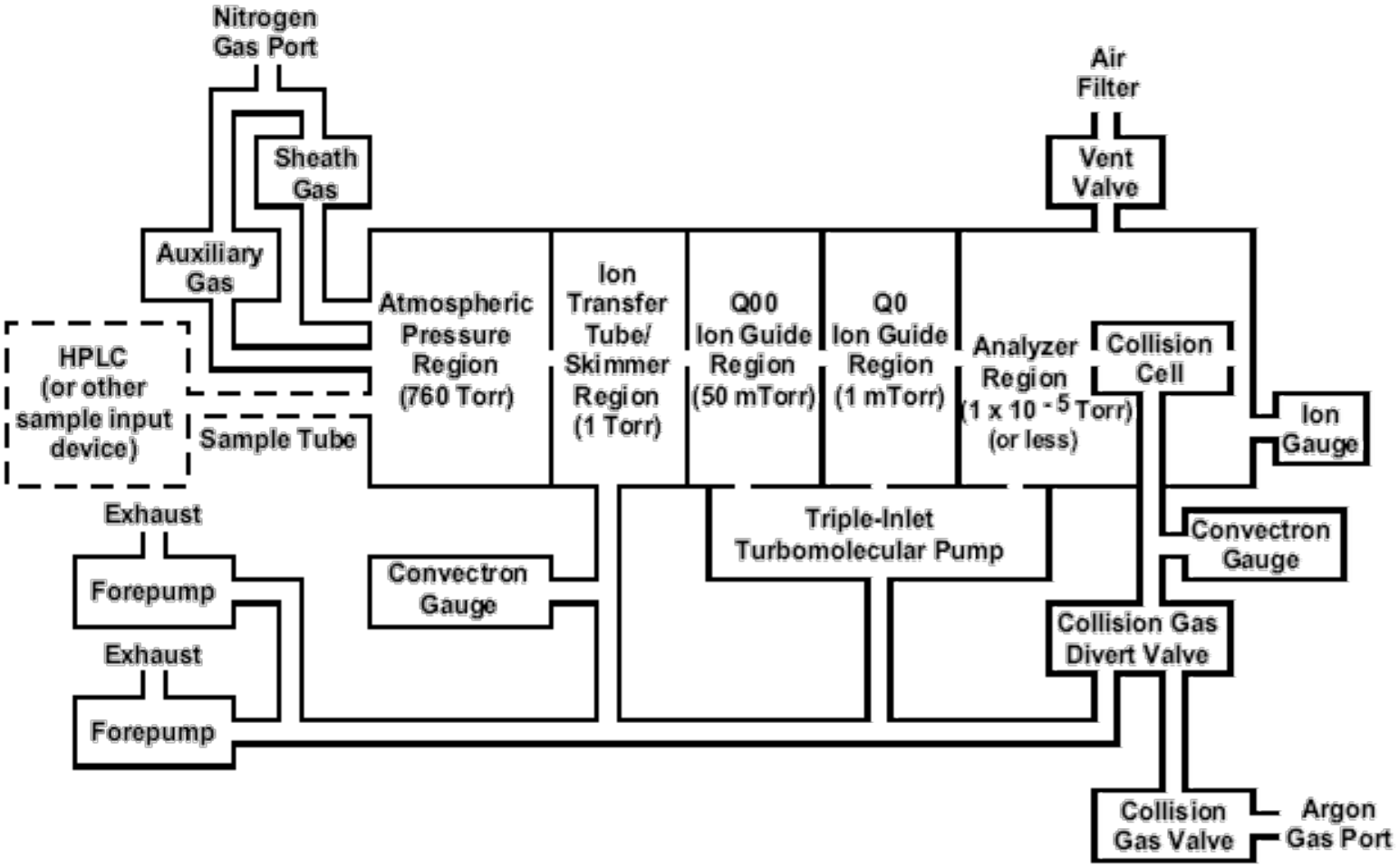
- A mass analyzer must operate under vacuum in order to minimize both ion-molecule and inter-molecular collisions, and allow ions to travel through the elements of the instrument.

- The mean free path of a typical ion (average distance traveled by an ion between collisions) is approximately:

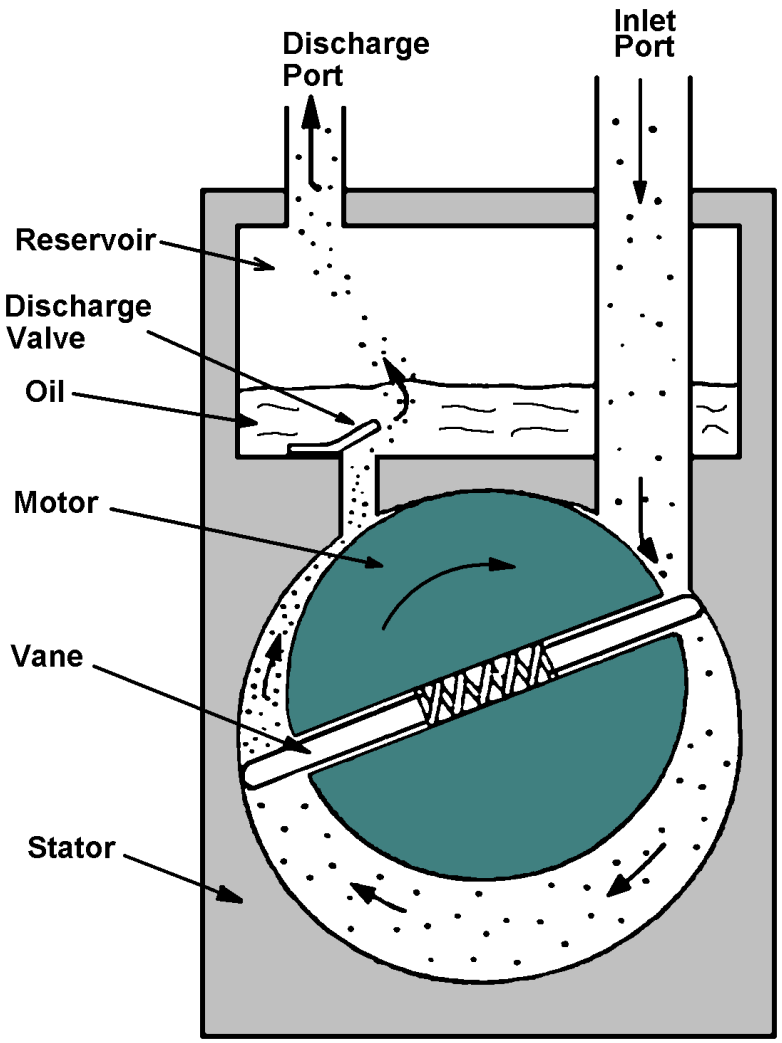
  - 50 nm at atmospheric pressure (760 Torr)
  - 40 mm at 1 mTorr
  - 40 m at 1 μTorr

- 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



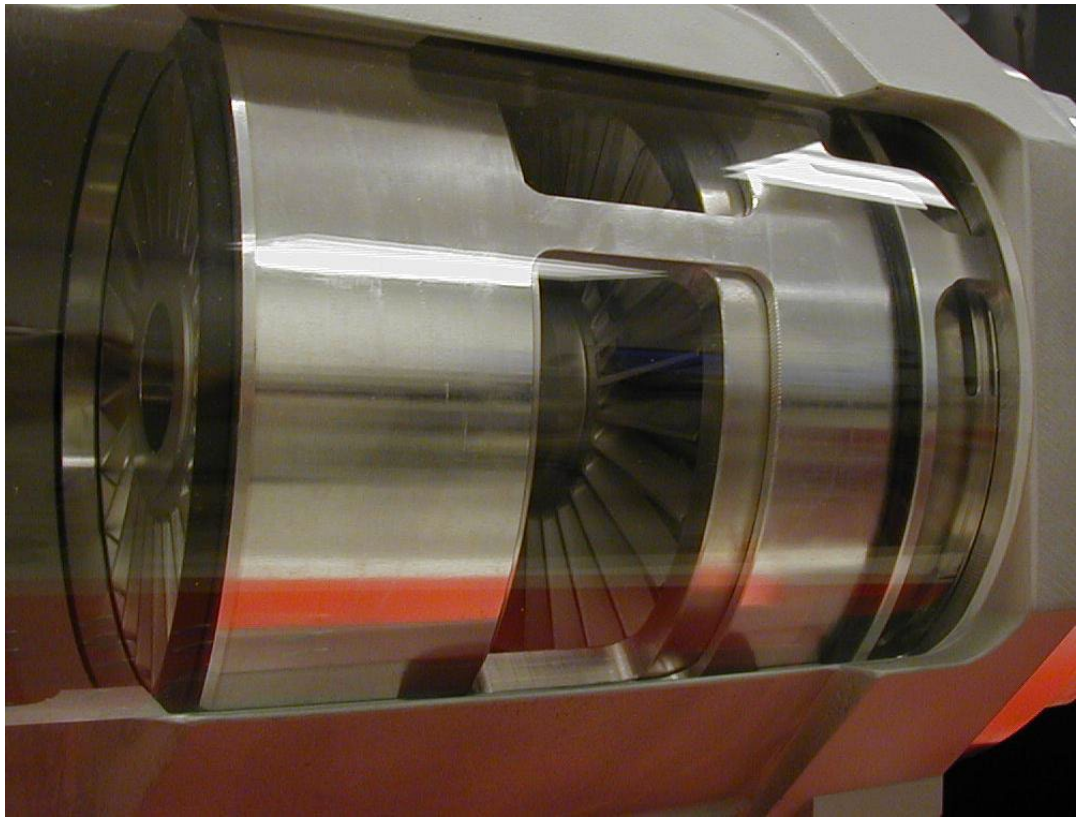
Rough Pump (Forepump)



- 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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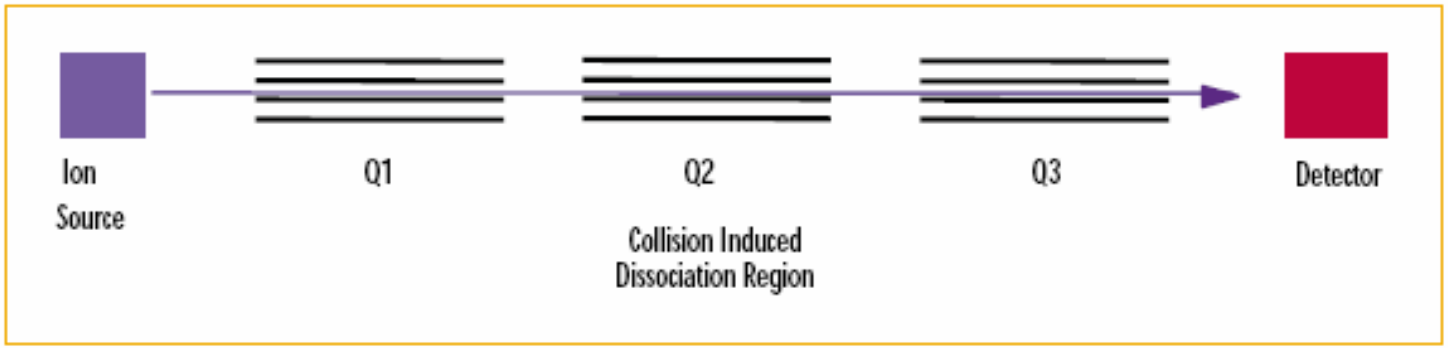
Chapter 6

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.

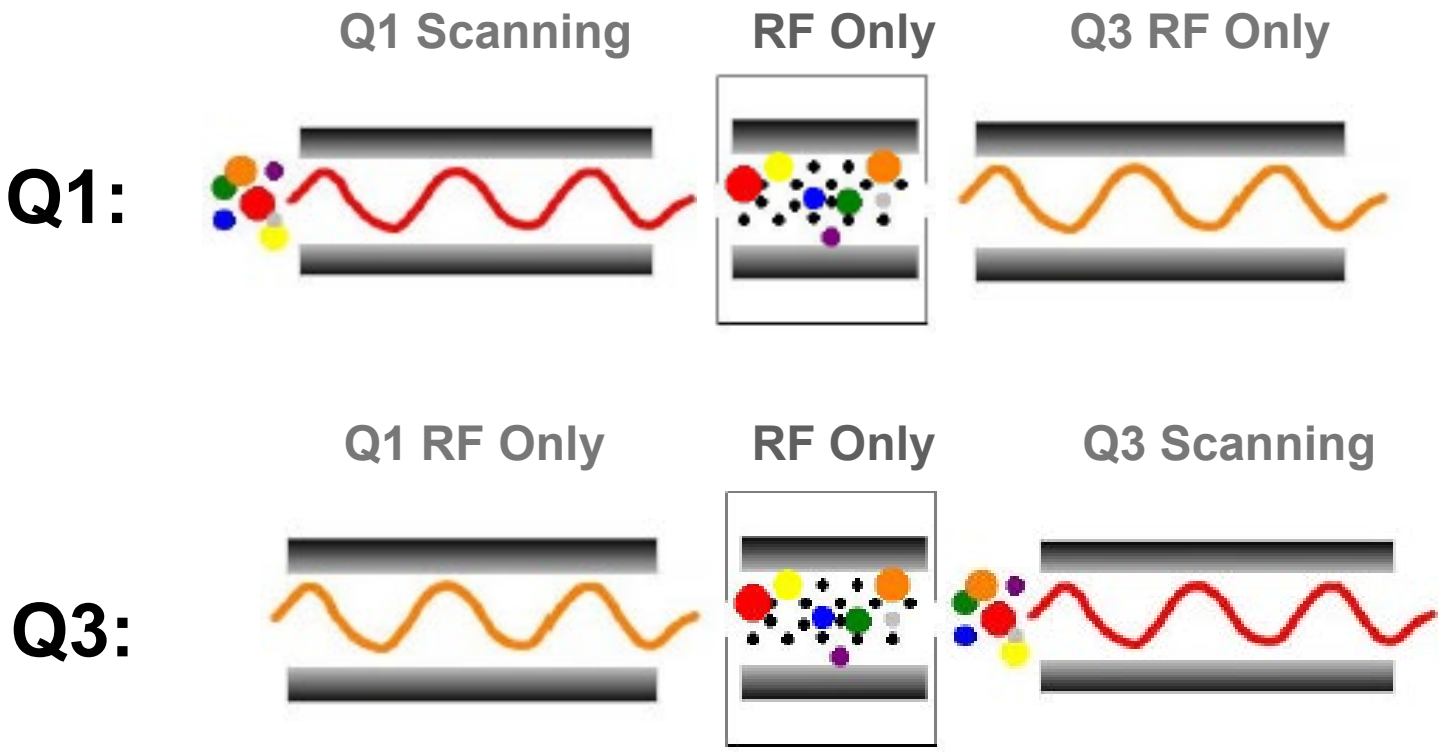
- Precursor ions and product ions are created and analyzed in different physical spaces.

- Ions must be moved from the ion-source to the analyzer (different physical regions) where different functions take place.

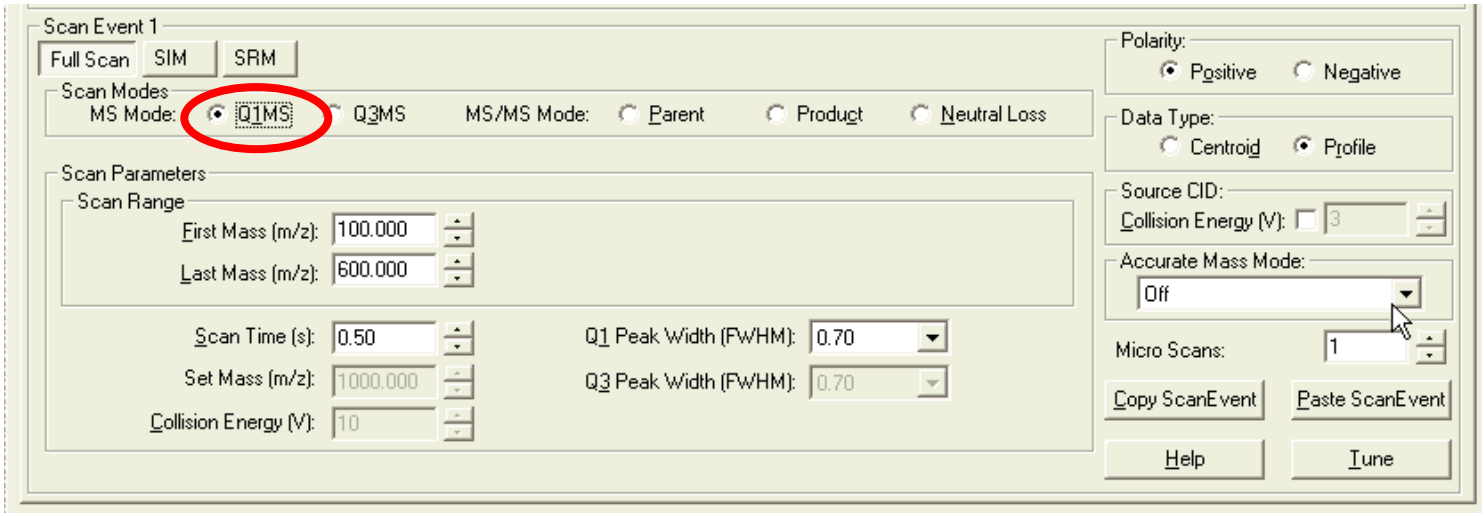
MS Full Scan (Q1 or Q3)

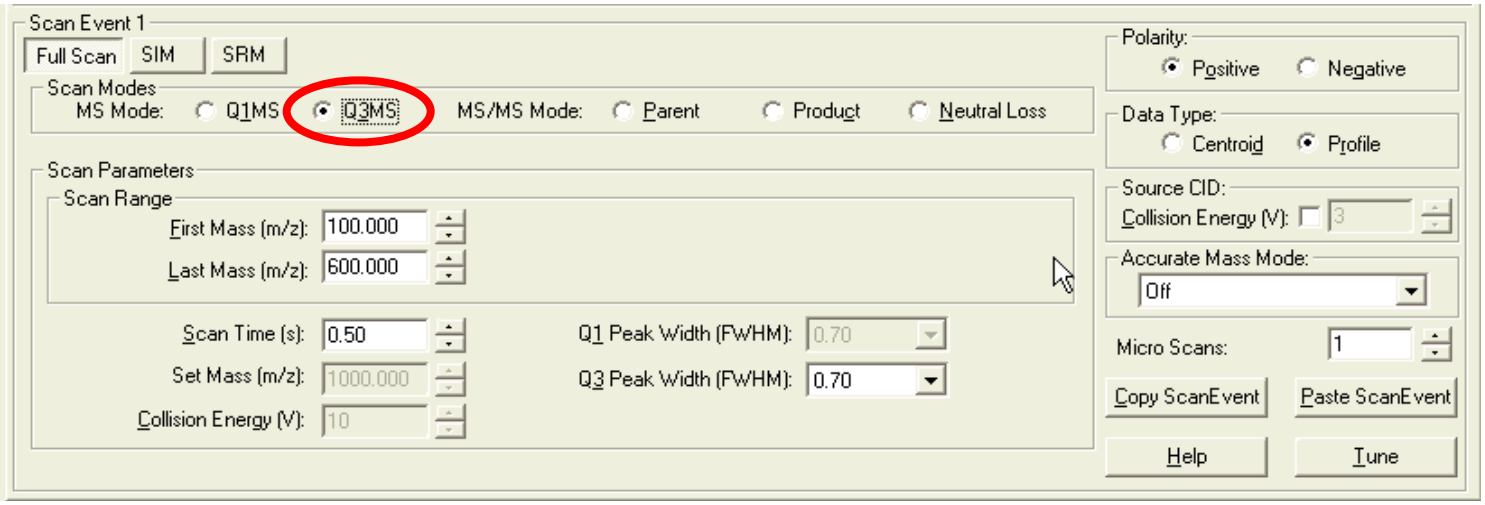
Full Scan Mode

Purpose: Survey scan of a chromatographic peak

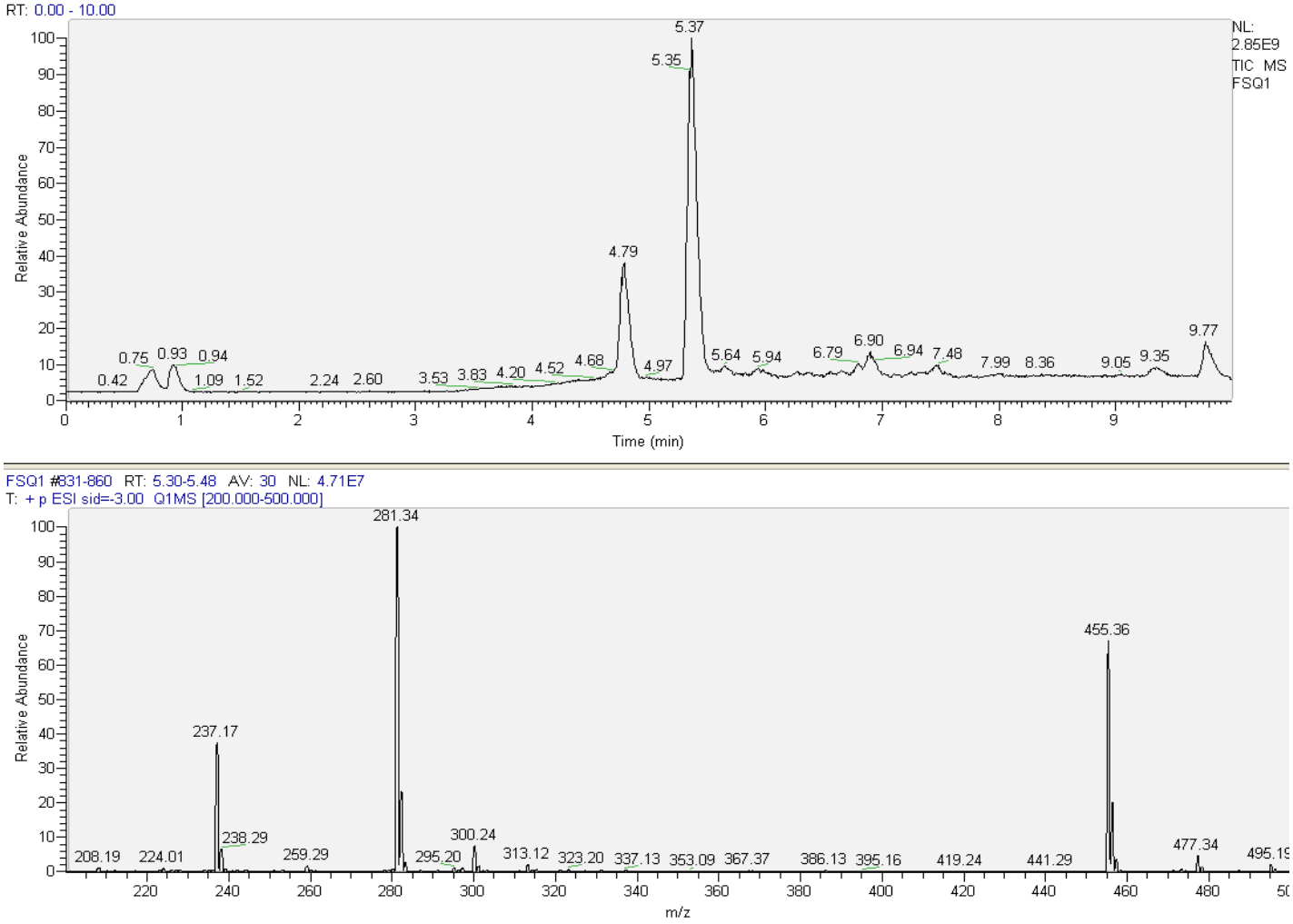


Full Scan (Q1 or Q3)

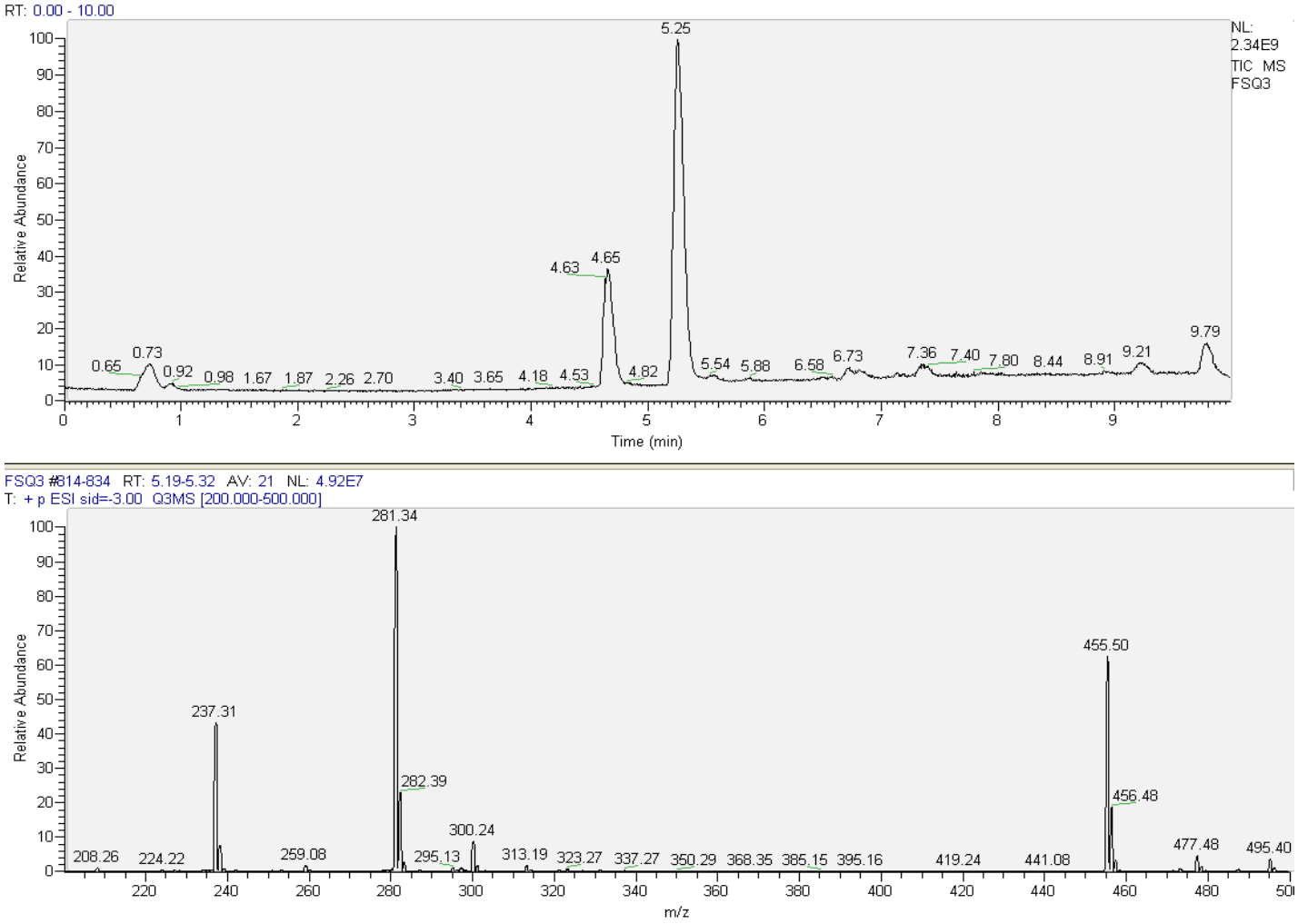




Full Scan Example (Q1)

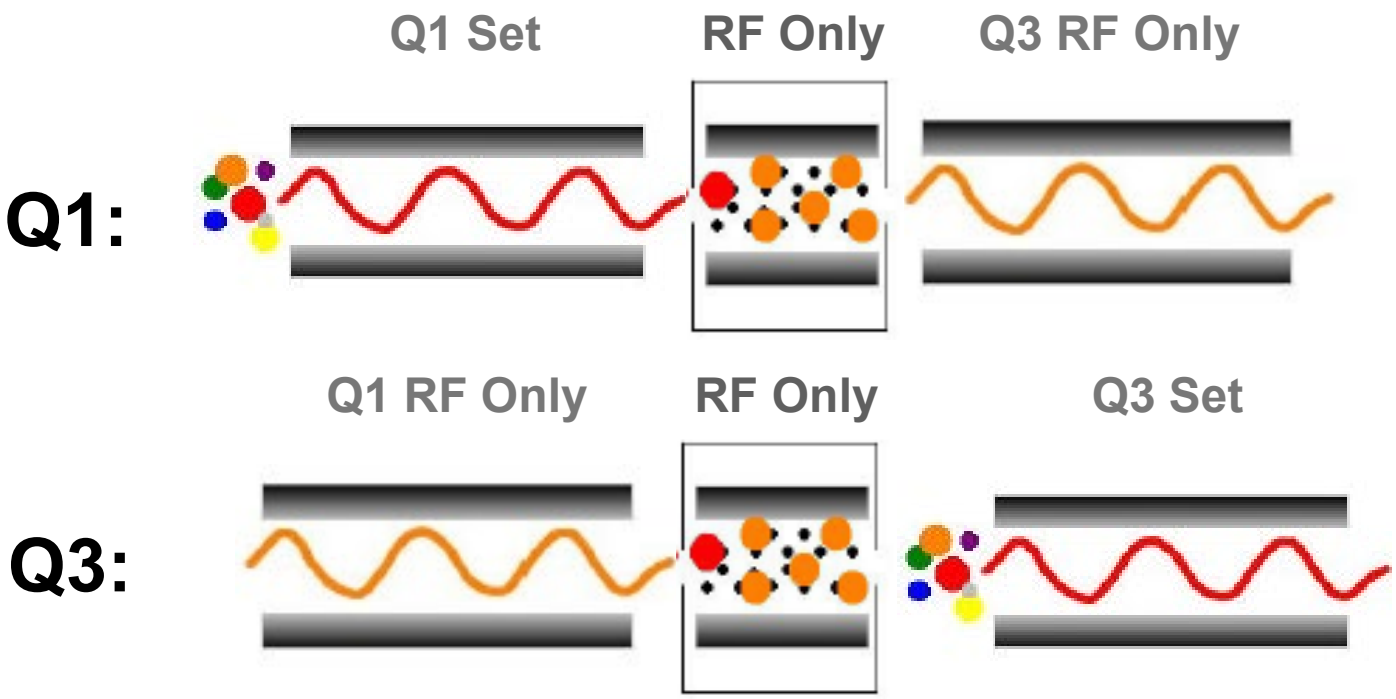


Full Scan Example (Q3)

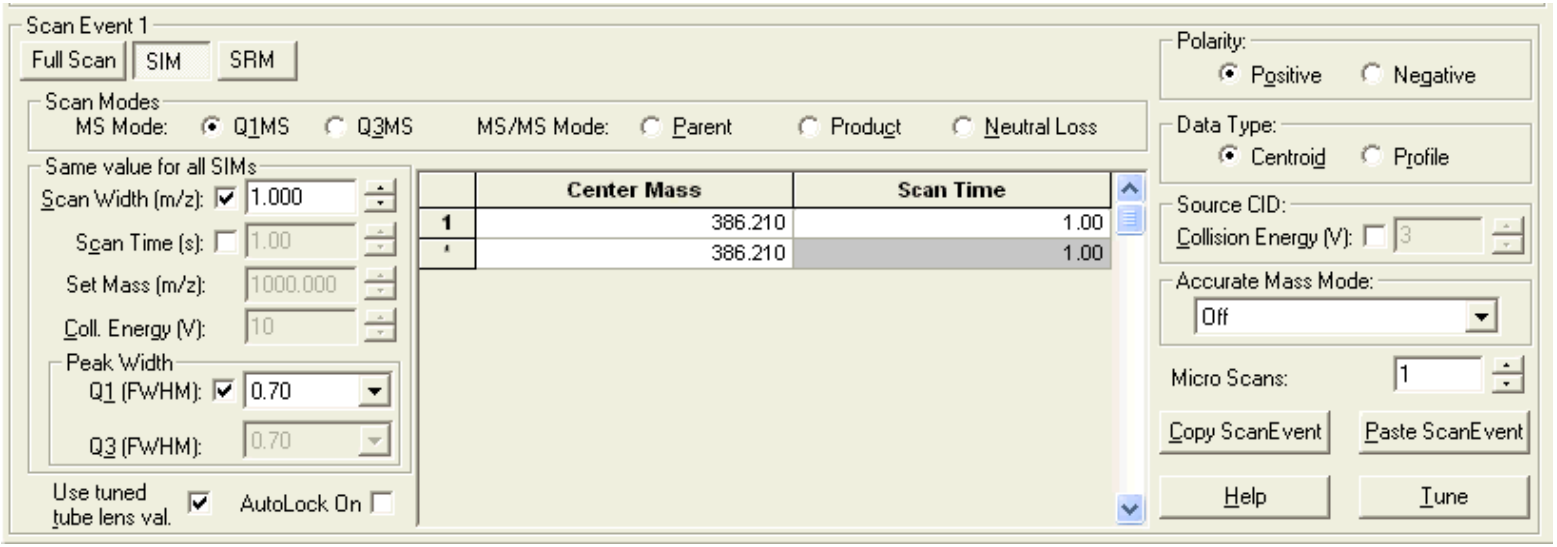


Selected Ion Monitoring - SIM (Q1 or Q3)

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



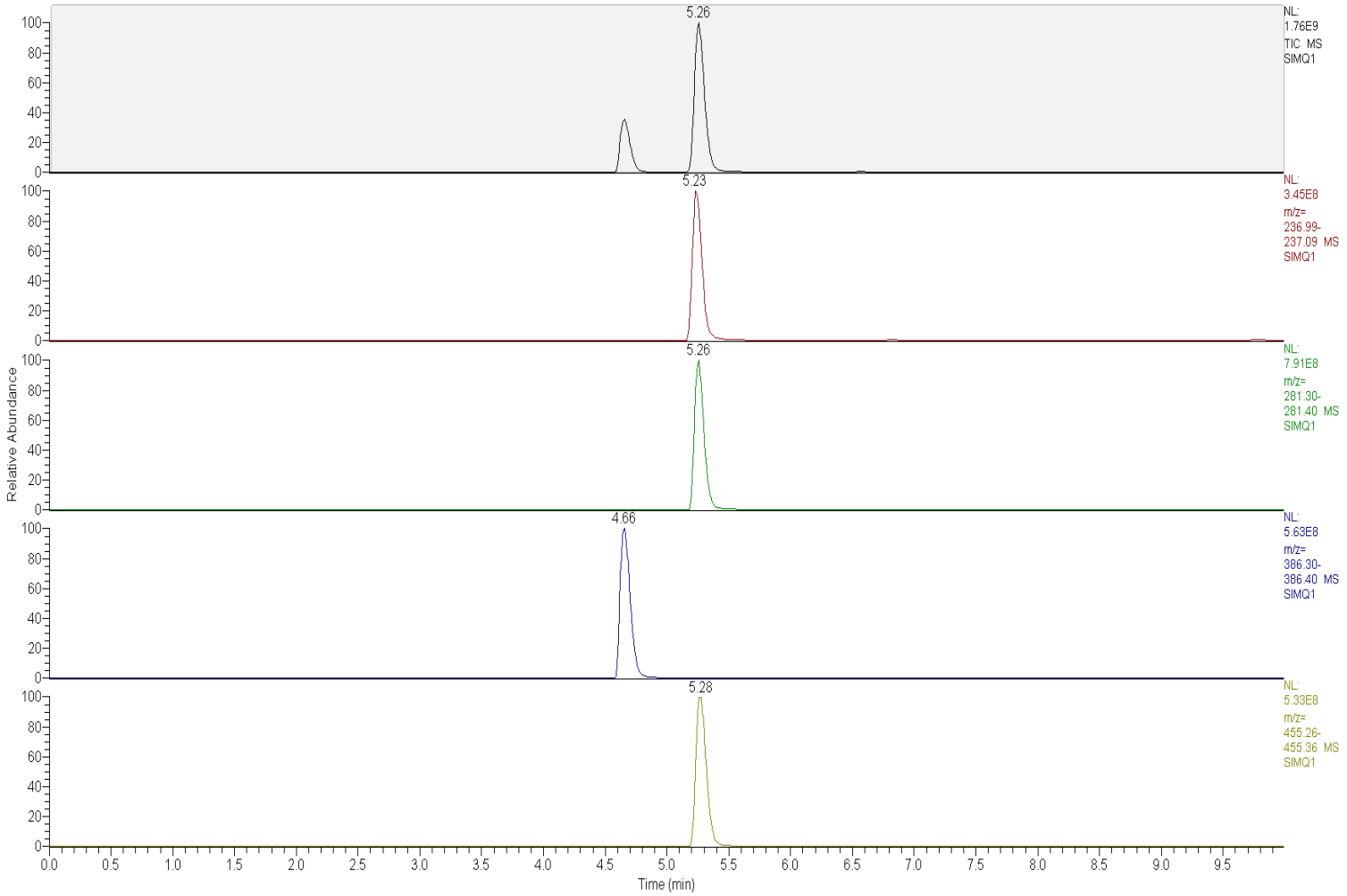
Selected Ion Monitoring - SIM (Q1 or Q3)



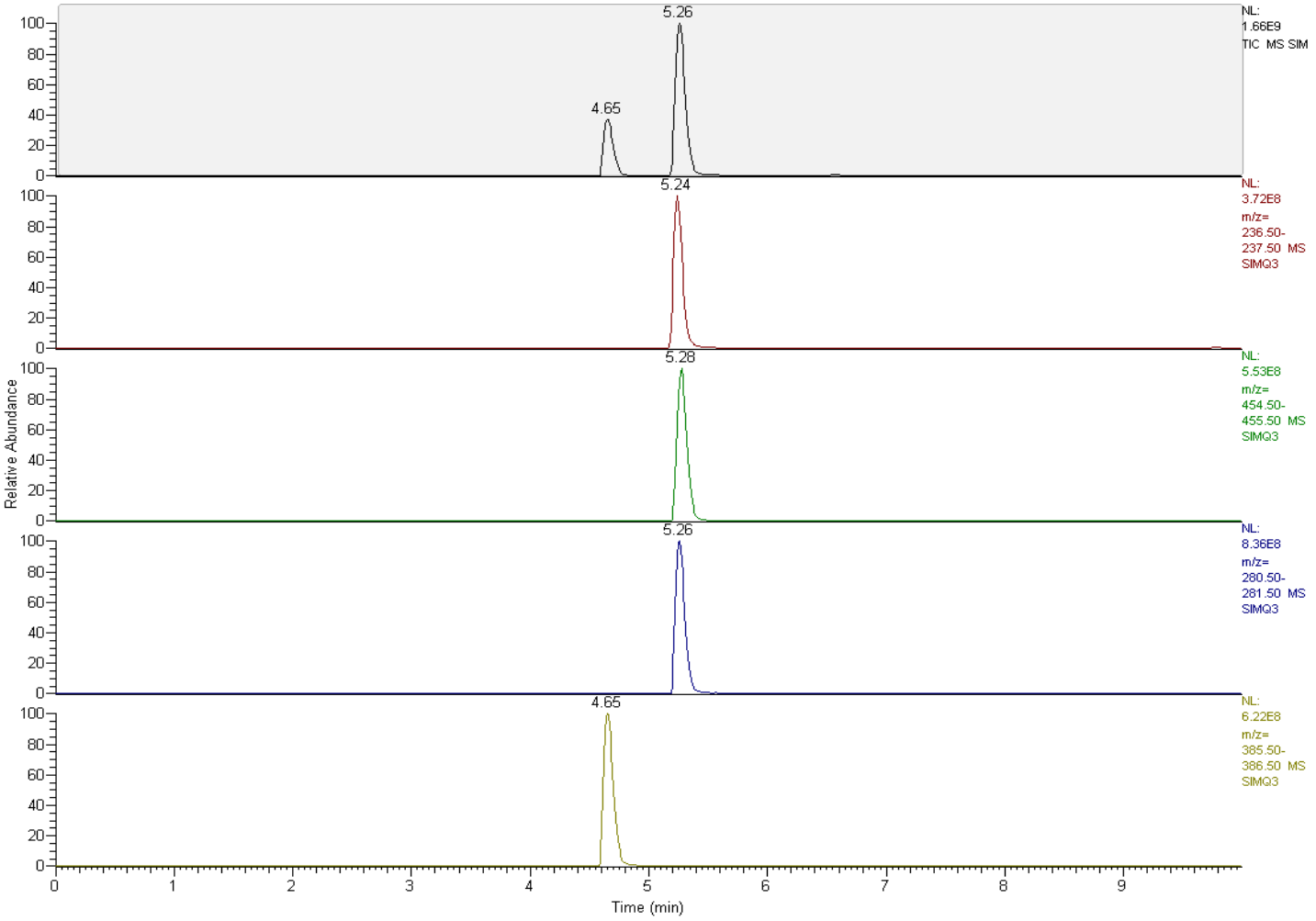
- SIM is in essence a full scan acquisition on a relatively narrow mass window (defined as center mass / scan width)

- The scan window around a set center mass is typically 1 Da (±0.5 Da)

Selected Ion Monitoring - SIM (Q1)

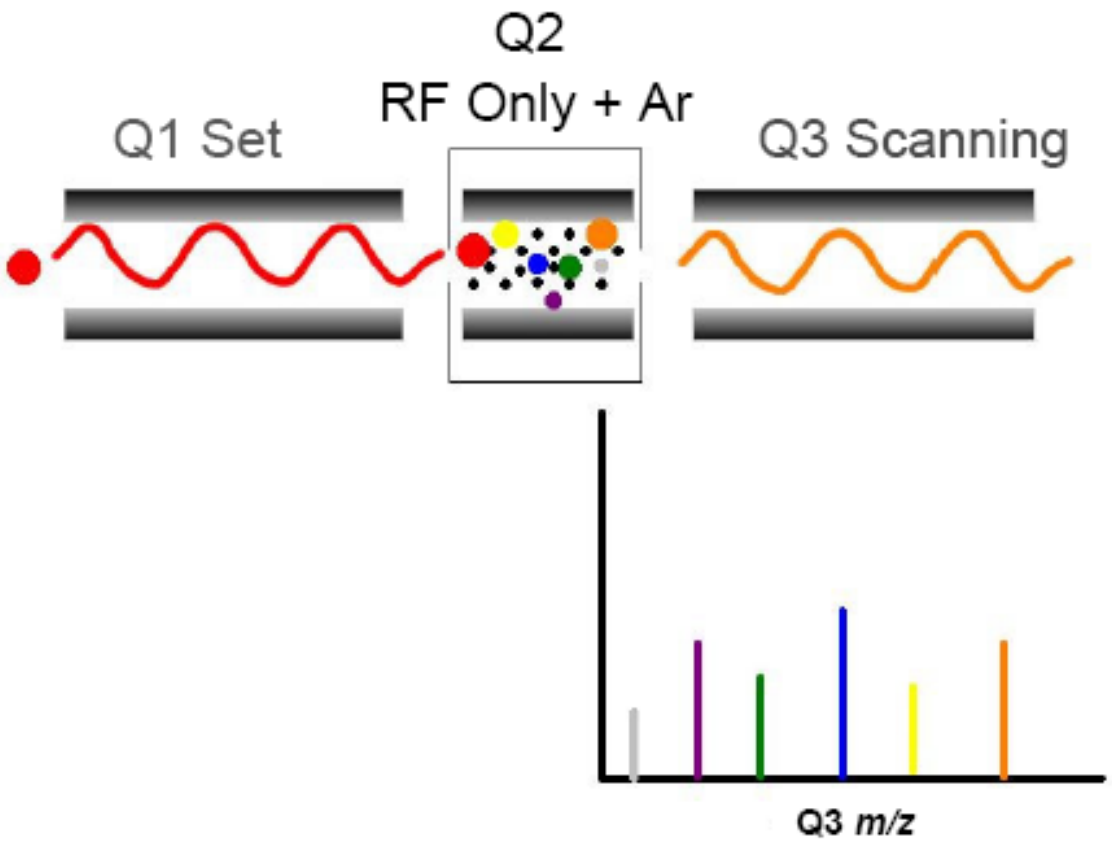


Selected Ion Monitoring - SIM (Q3)

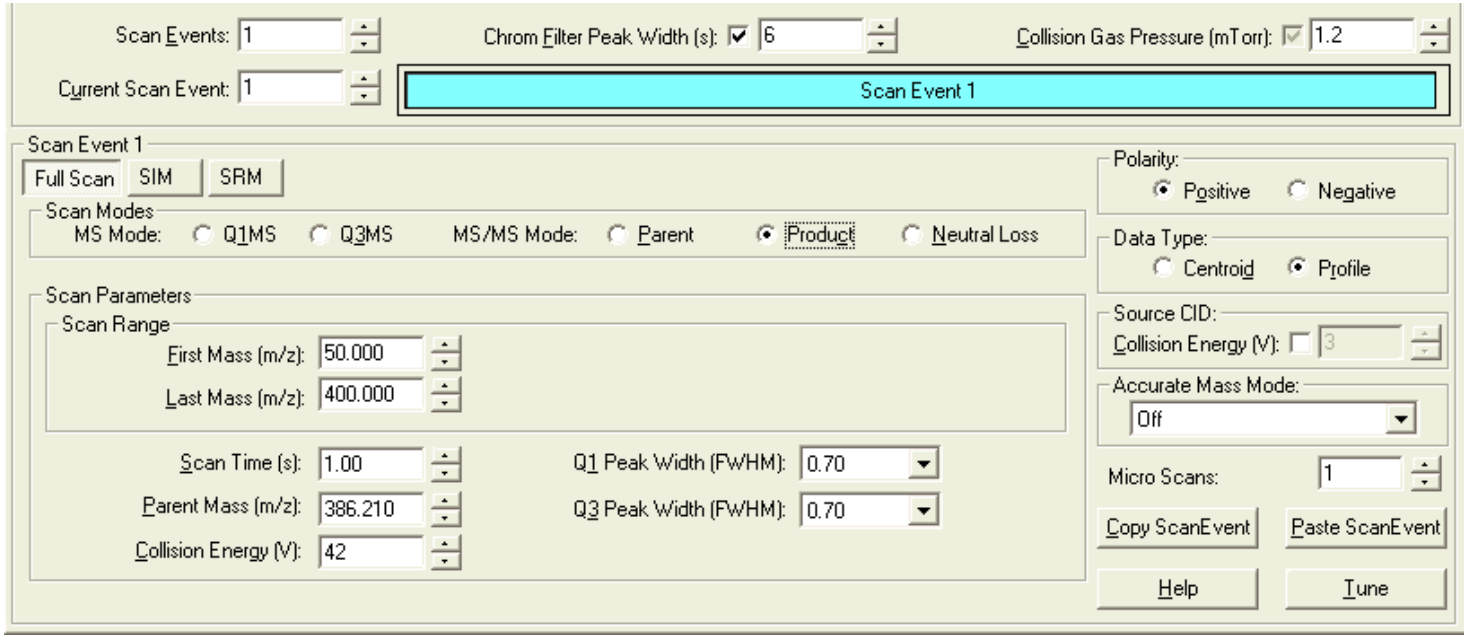


Product Ion Scan (MS/MS)

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



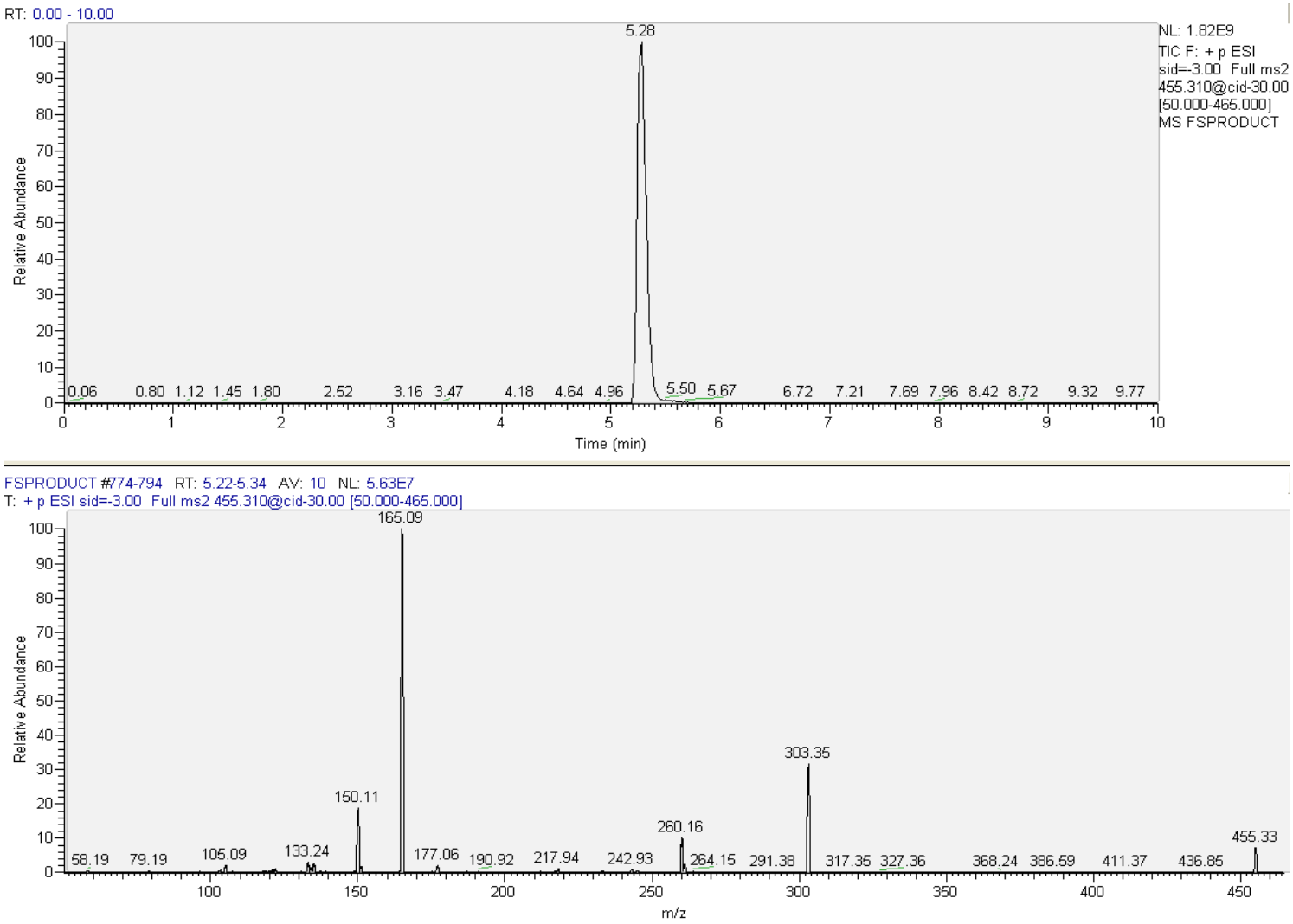
Product Ion Scan (MS/MS)



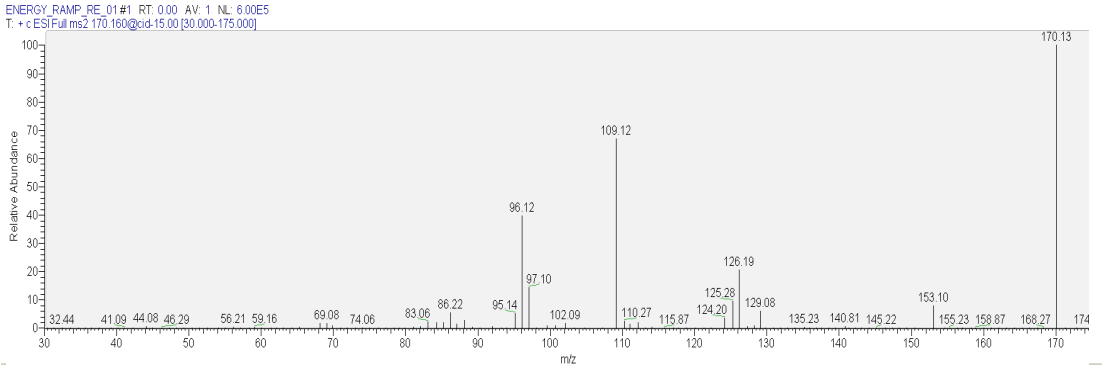
Key experimental parameters:

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

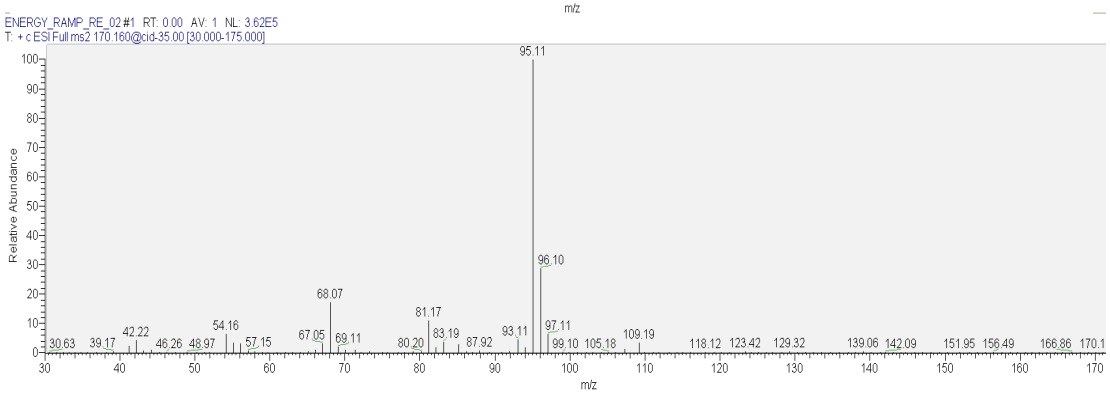
Product Ion Scan (MS/MS)



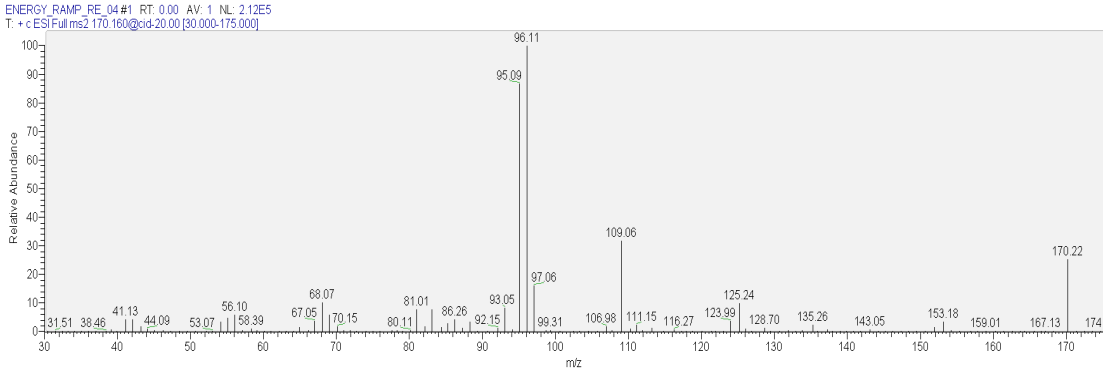
Product Ion Scan – Role of Collision Energy Ramp



CE = 15 V
ER = 0



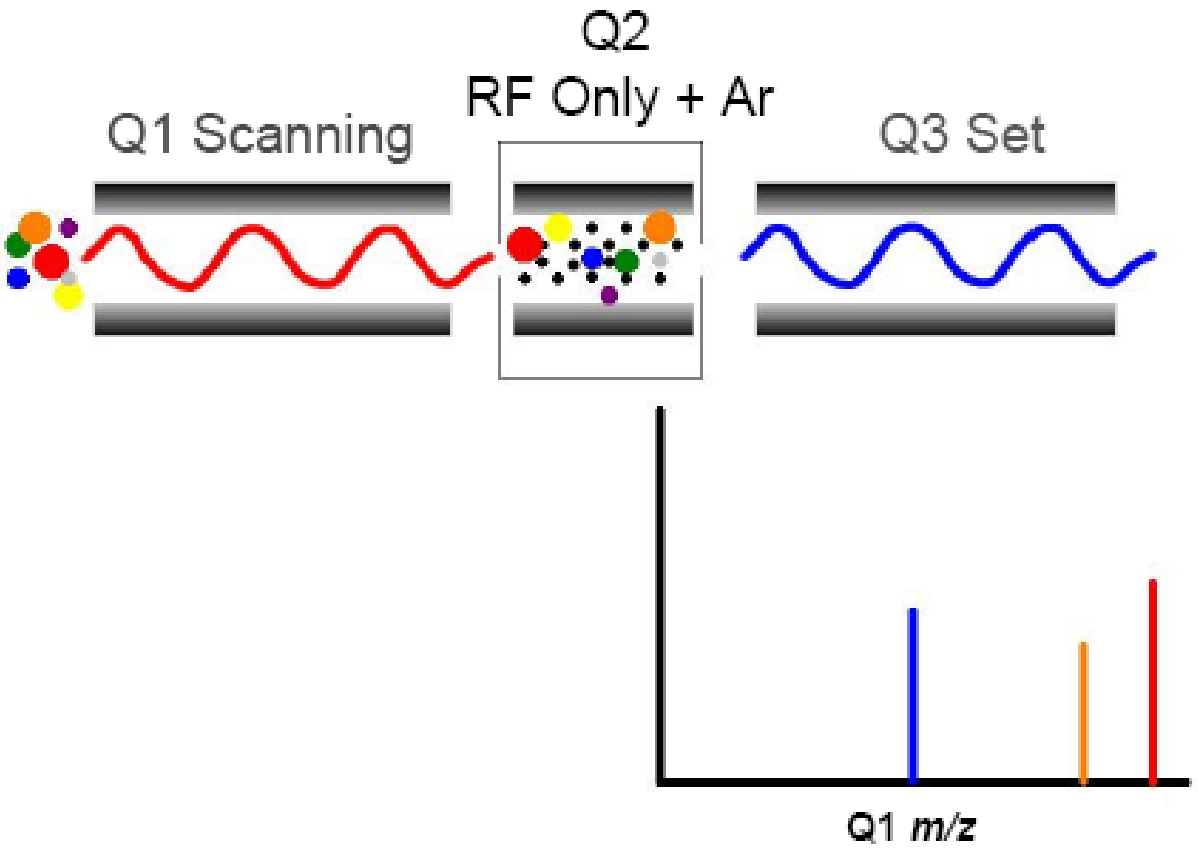
CE = 35 V
ER = 0



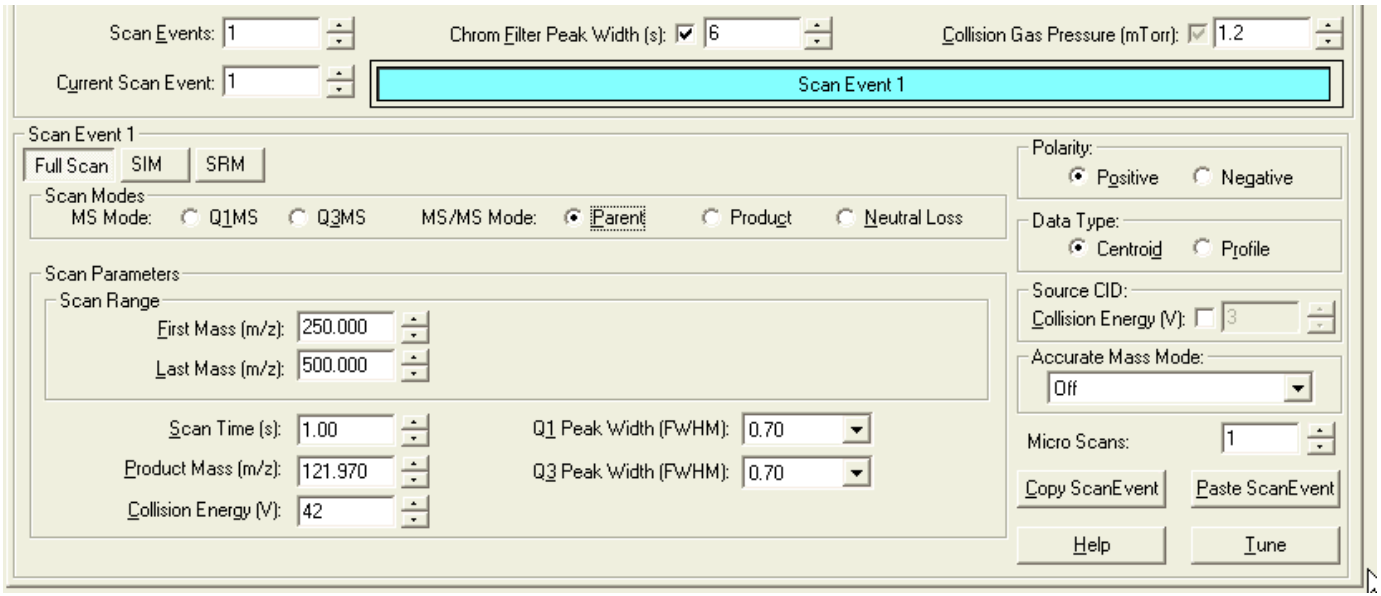
CE = 20 V
ER = 15 V

TSQ: Precursor Ion Scan (MS/MS)

Purpose: To determine the "origin" of a specific product ion



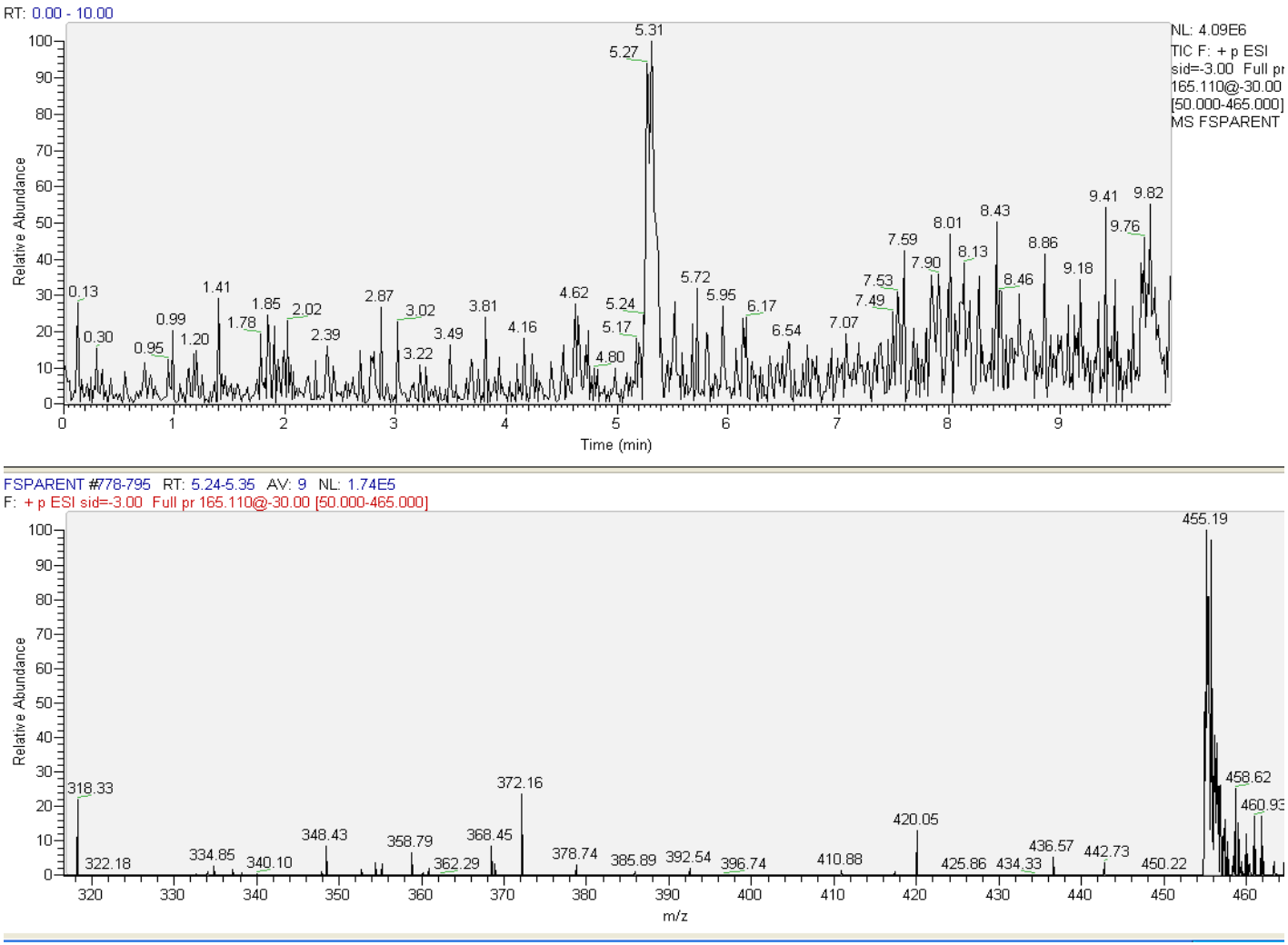
TSQ: Precursor Ion Scan (MS/MS)



Key experimental parameters:

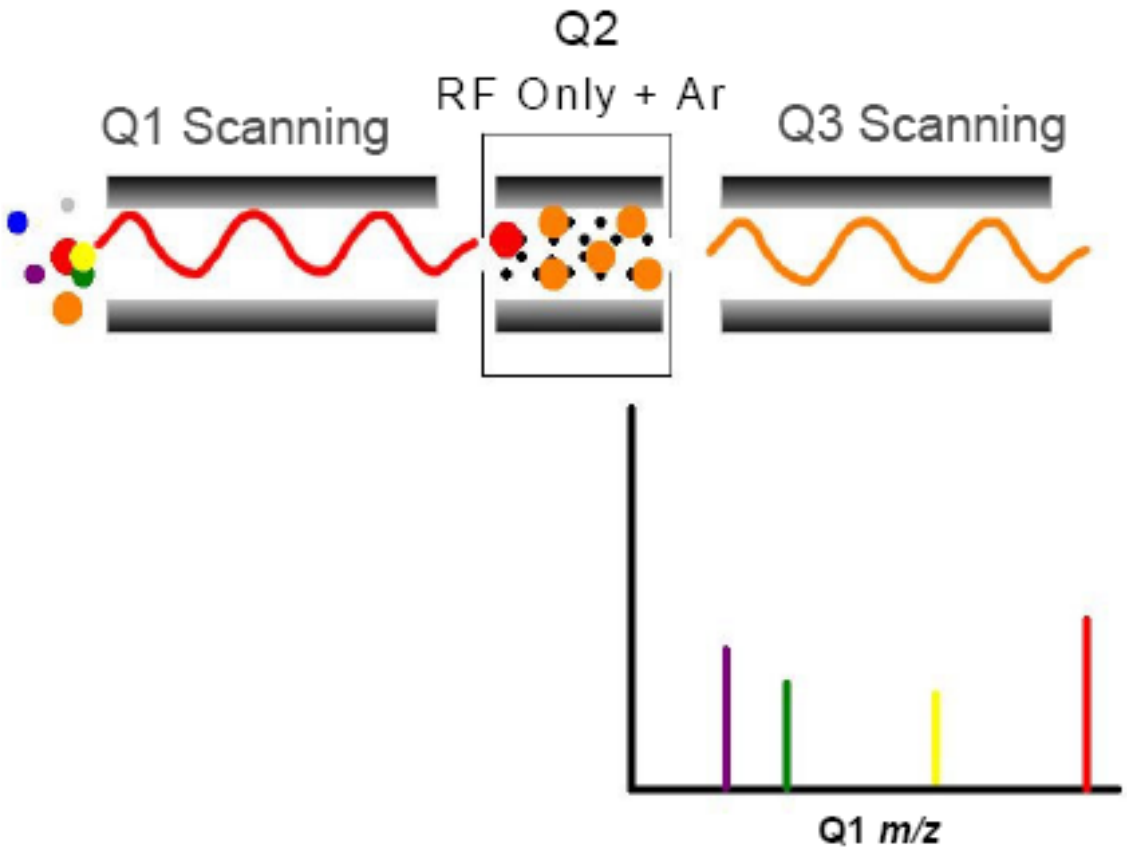
- Product ion mass (*m/z*)
- Collision gas pressure
- Collision energy
- Scan range of precursor ions of interest

TSQ: Precursor Ion Scan (MS/MS)

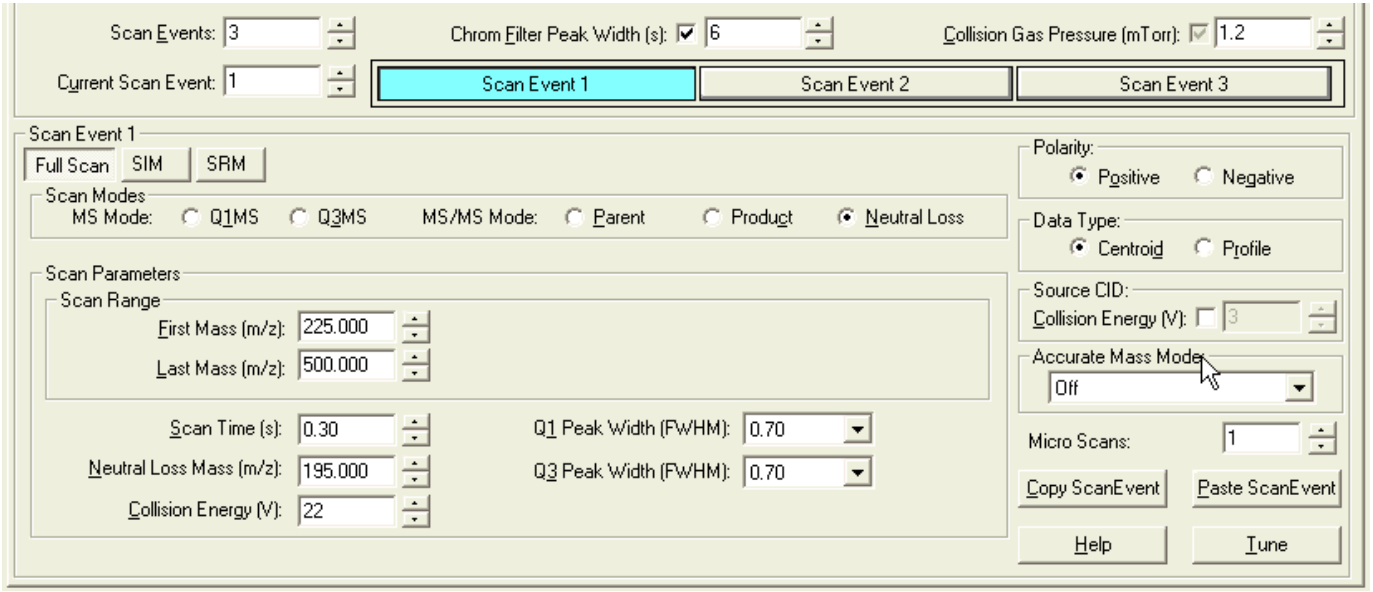


TSQ: Neutral Loss Scan (MS/MS)

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



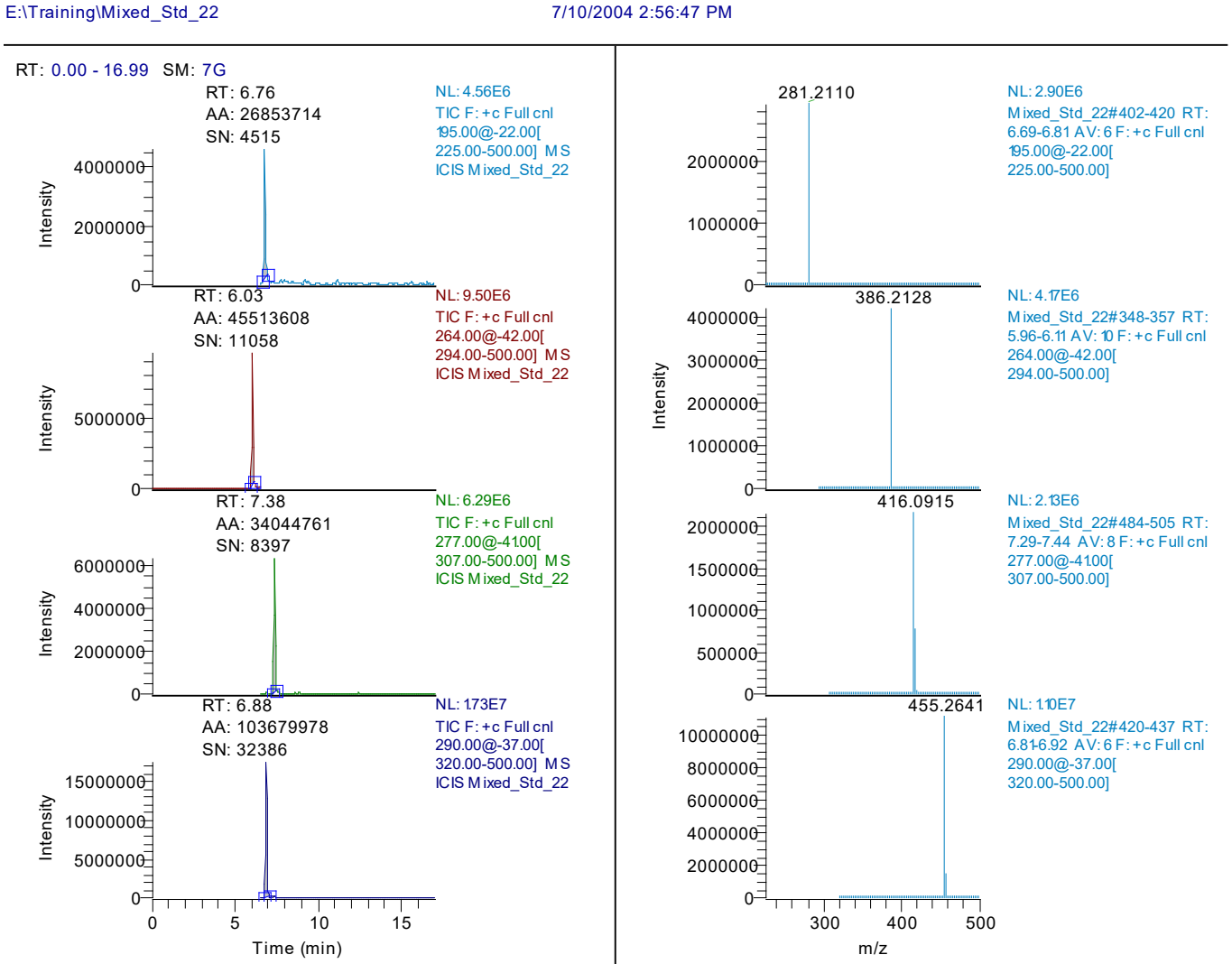
TSQ: Neutral Loss Scan (MS/MS)



Key experimental parameters:

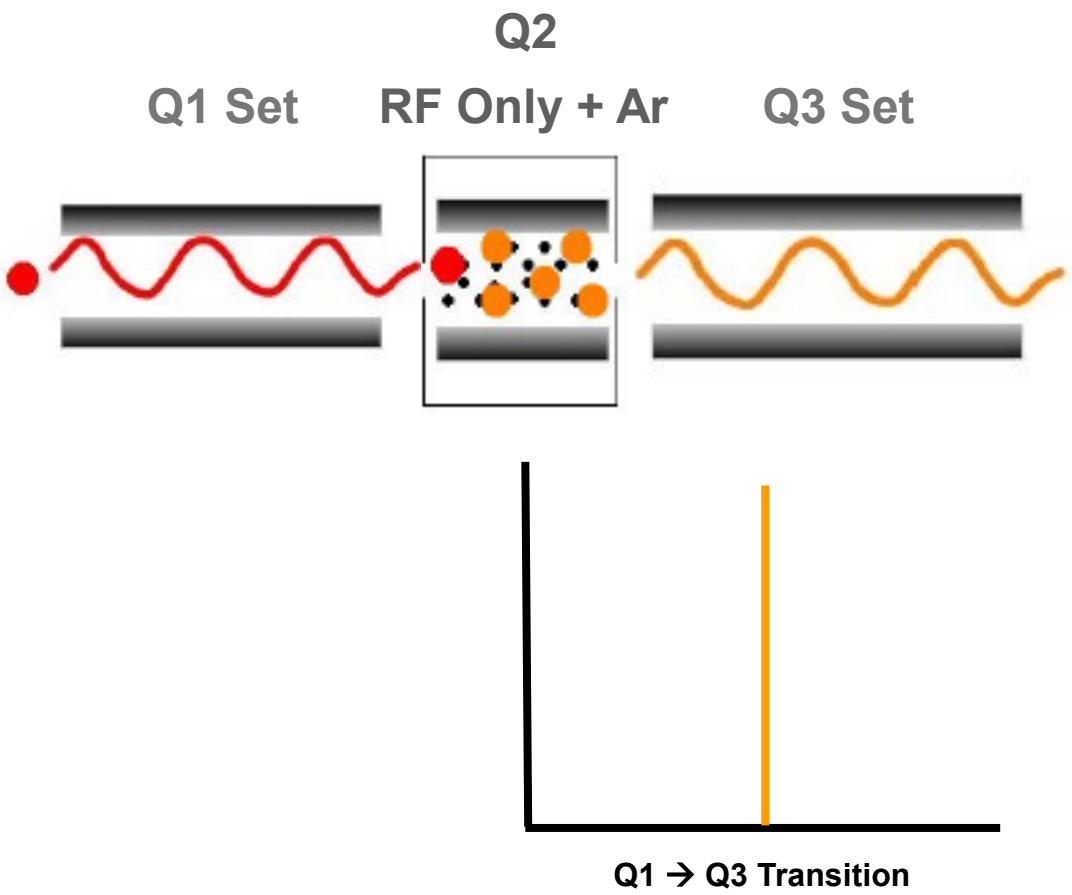
- Neutral loss mass
- Collision gas pressure
- Collision energy
- Scan range of precursor ions of interest

TSQ: Neutral Loss Scan (MS/MS)

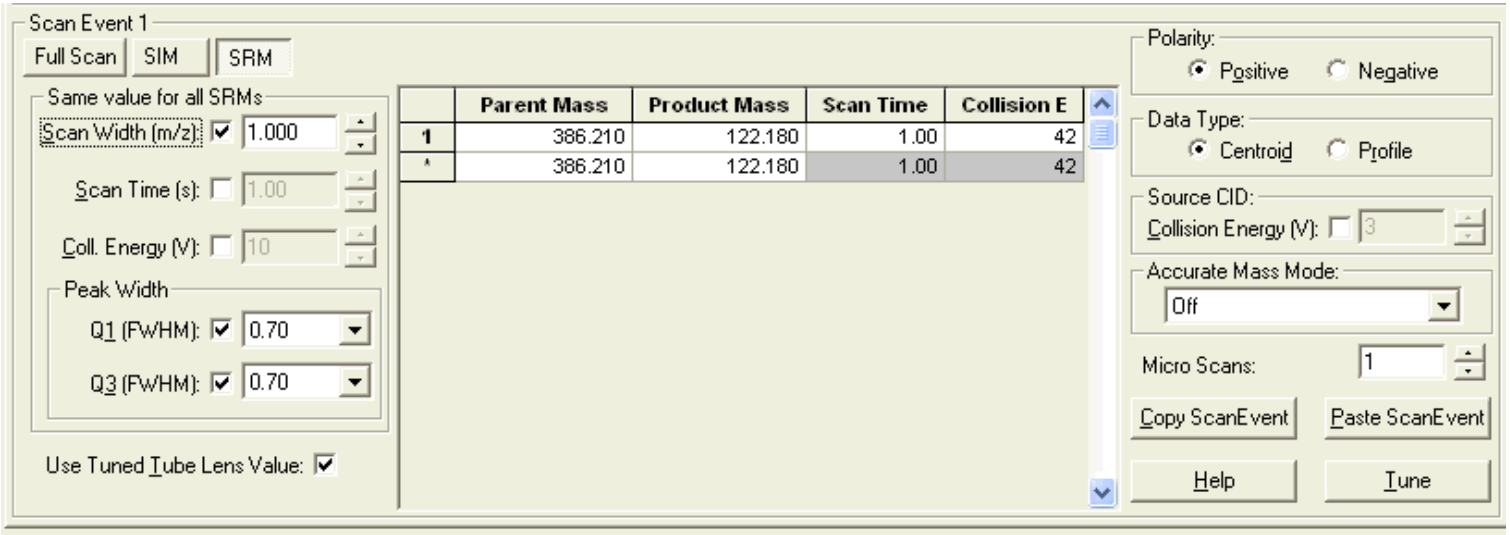


SRM (Selected Reaction Monitoring)

Purpose: Quantitation on a single product ion population



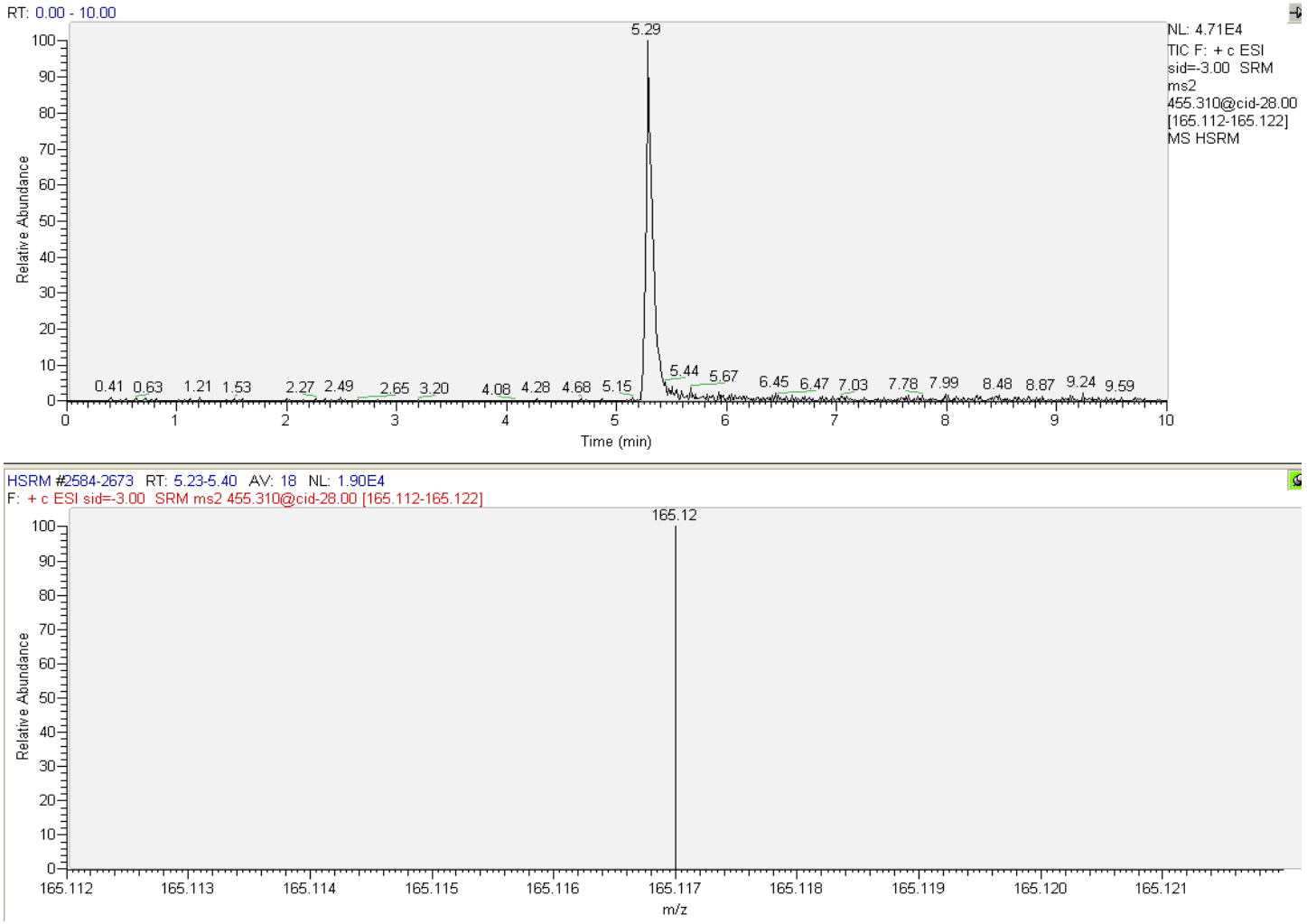
SRM (Selected Reaction Monitoring)



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)

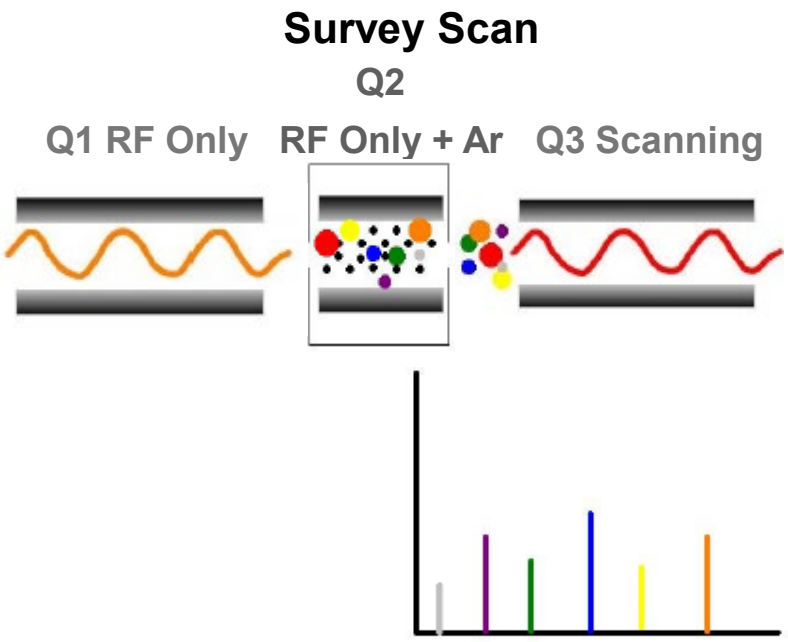


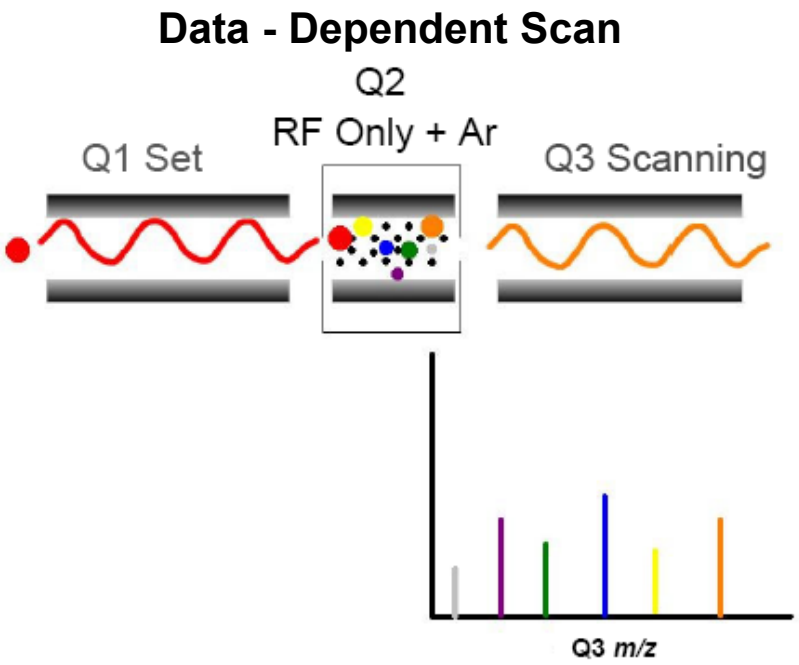
Data-Dependent Scan (Q3)

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

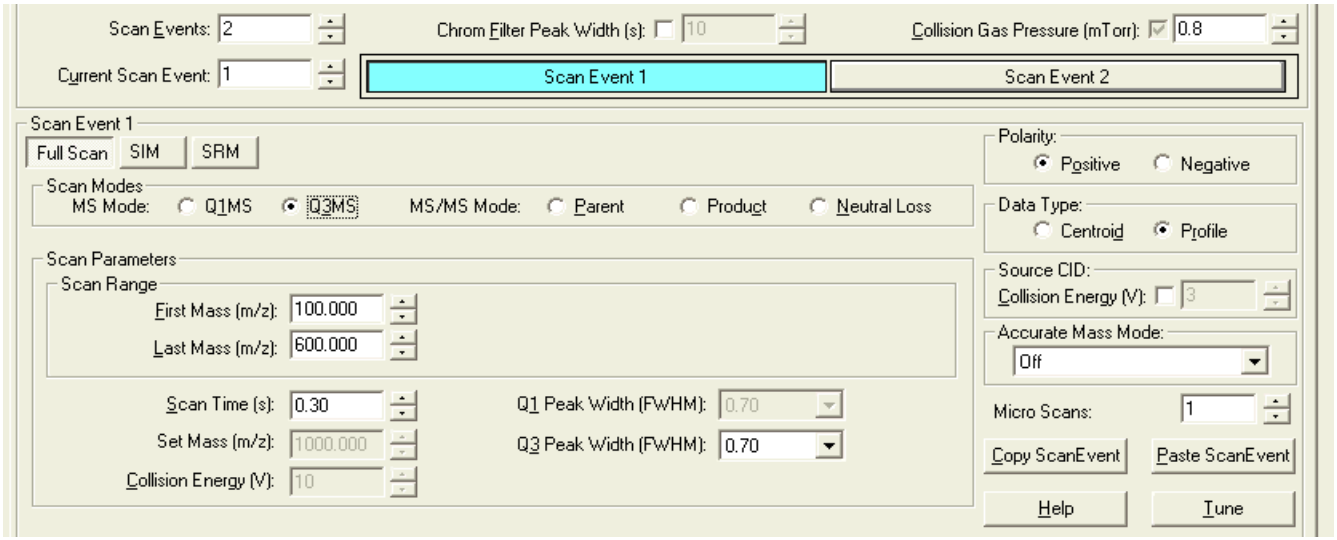
Survey Scan: **Full Scan Q3** Mode

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

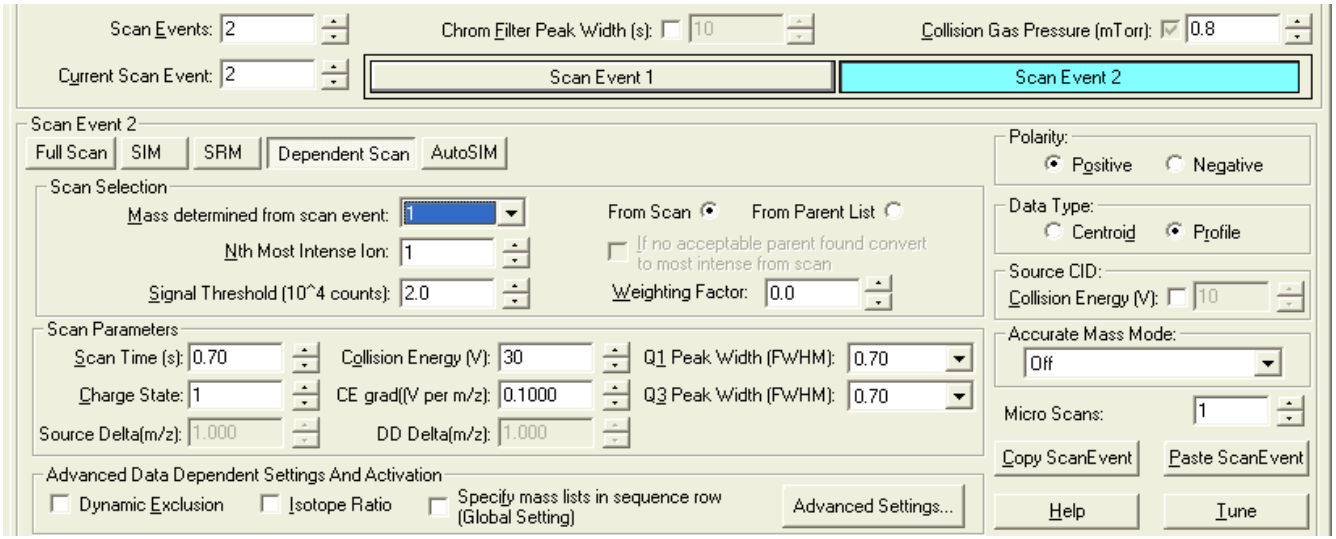




Data-Dependent Scan (Q3)

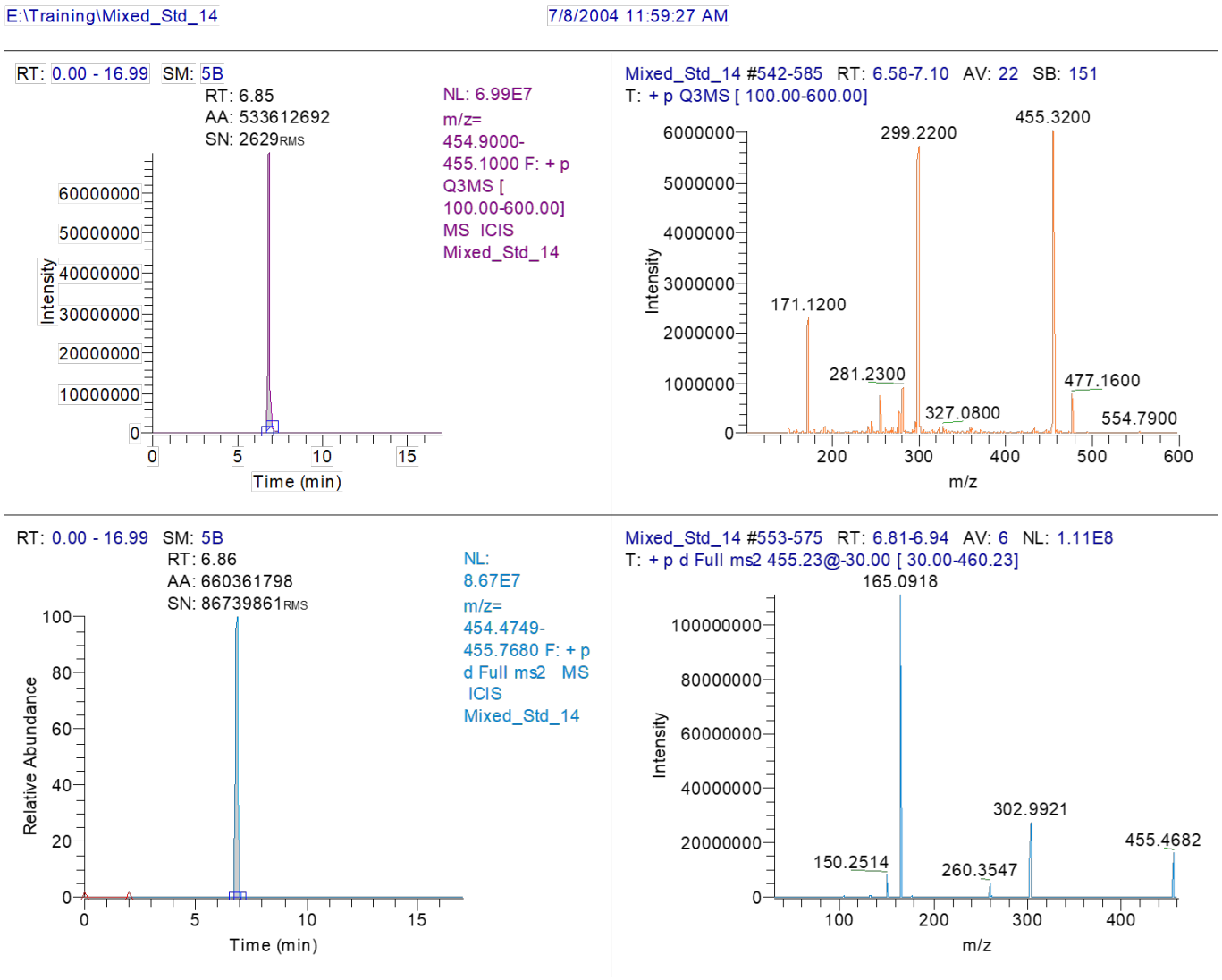


Survey Scan



Data-Dependent Scan

Data-Dependent Scan (Q3)



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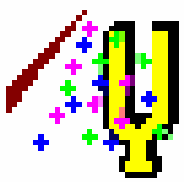
Chapter 7

Quantum Tune Page with Tuning and Calibration

TSQ Quantum Tune Page - Instrument Control



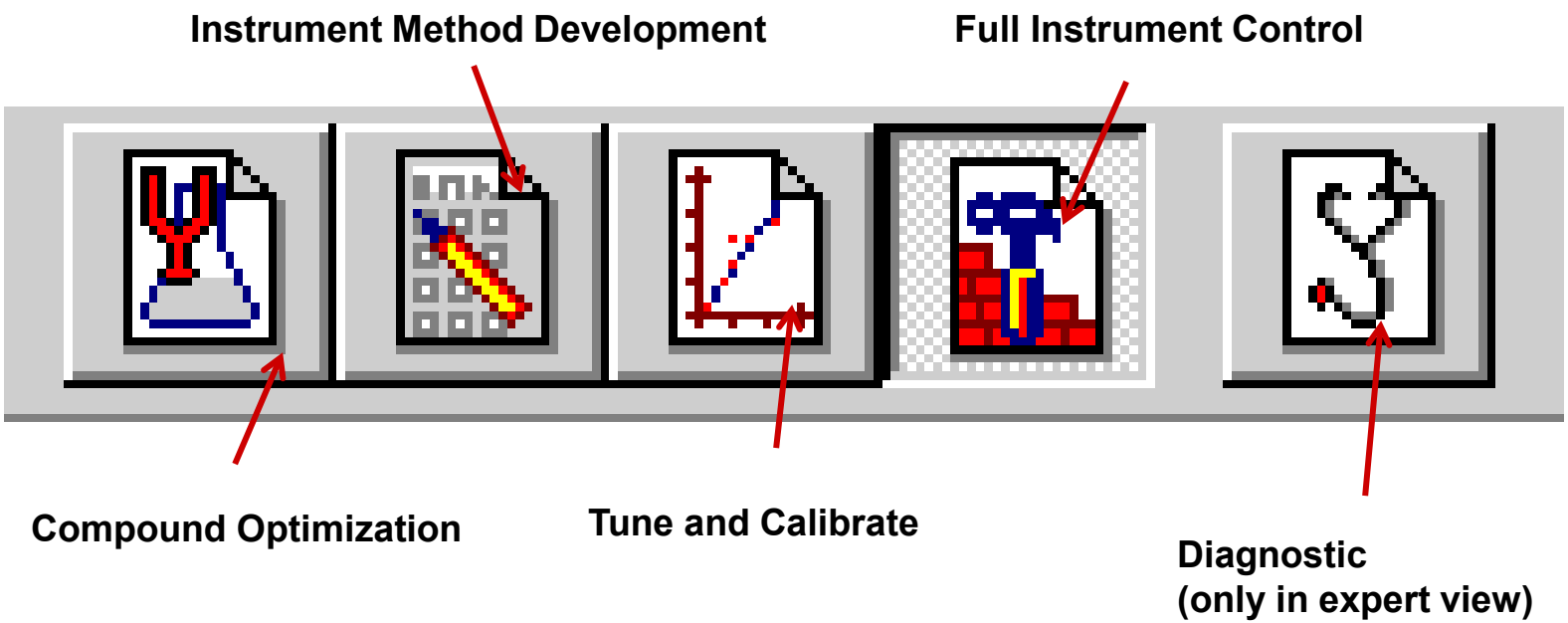
Work Space based for functional tasks:

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

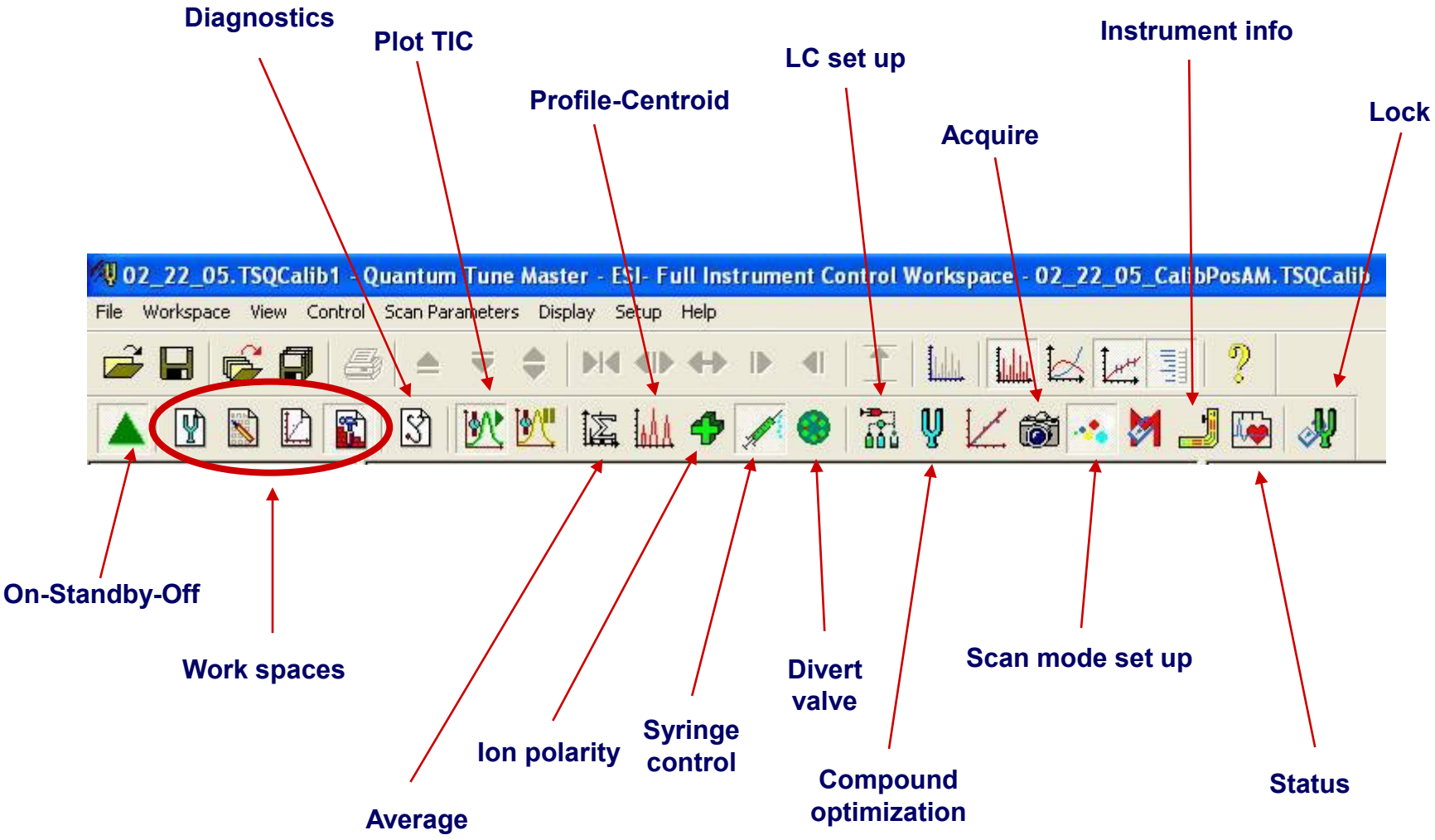
Key Features

- 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

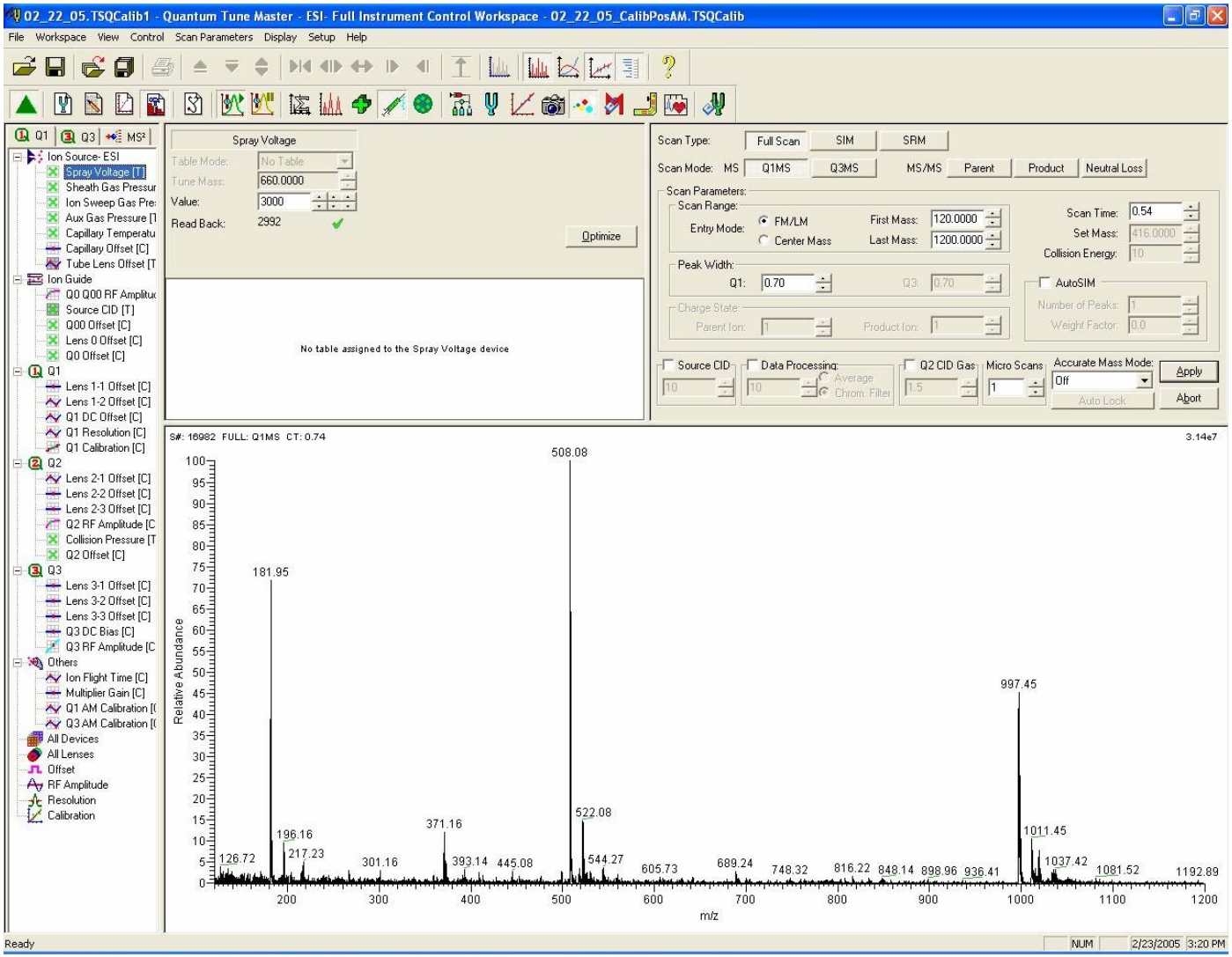
Instrument Work Spaces



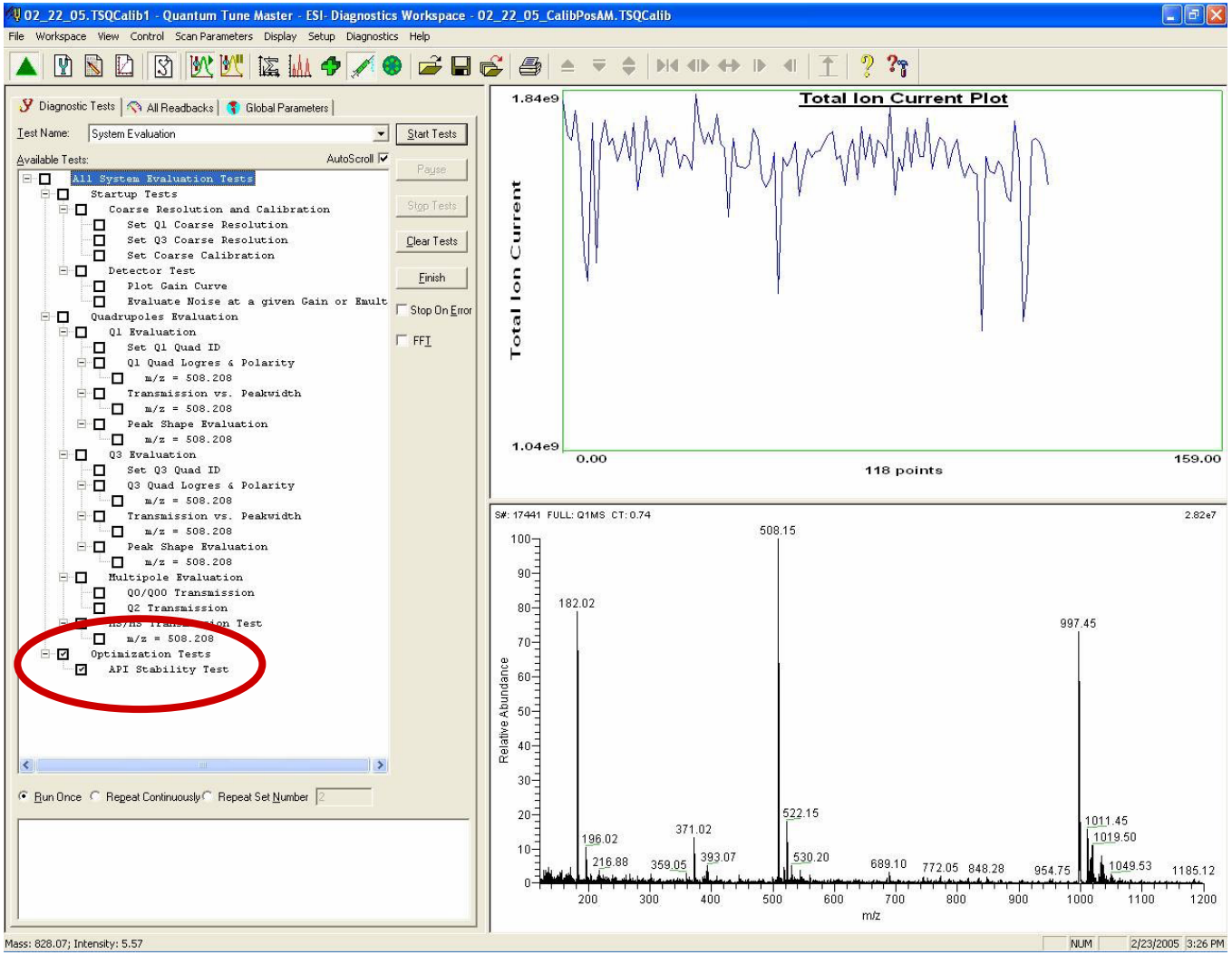
All Instrument Control Tool Bar



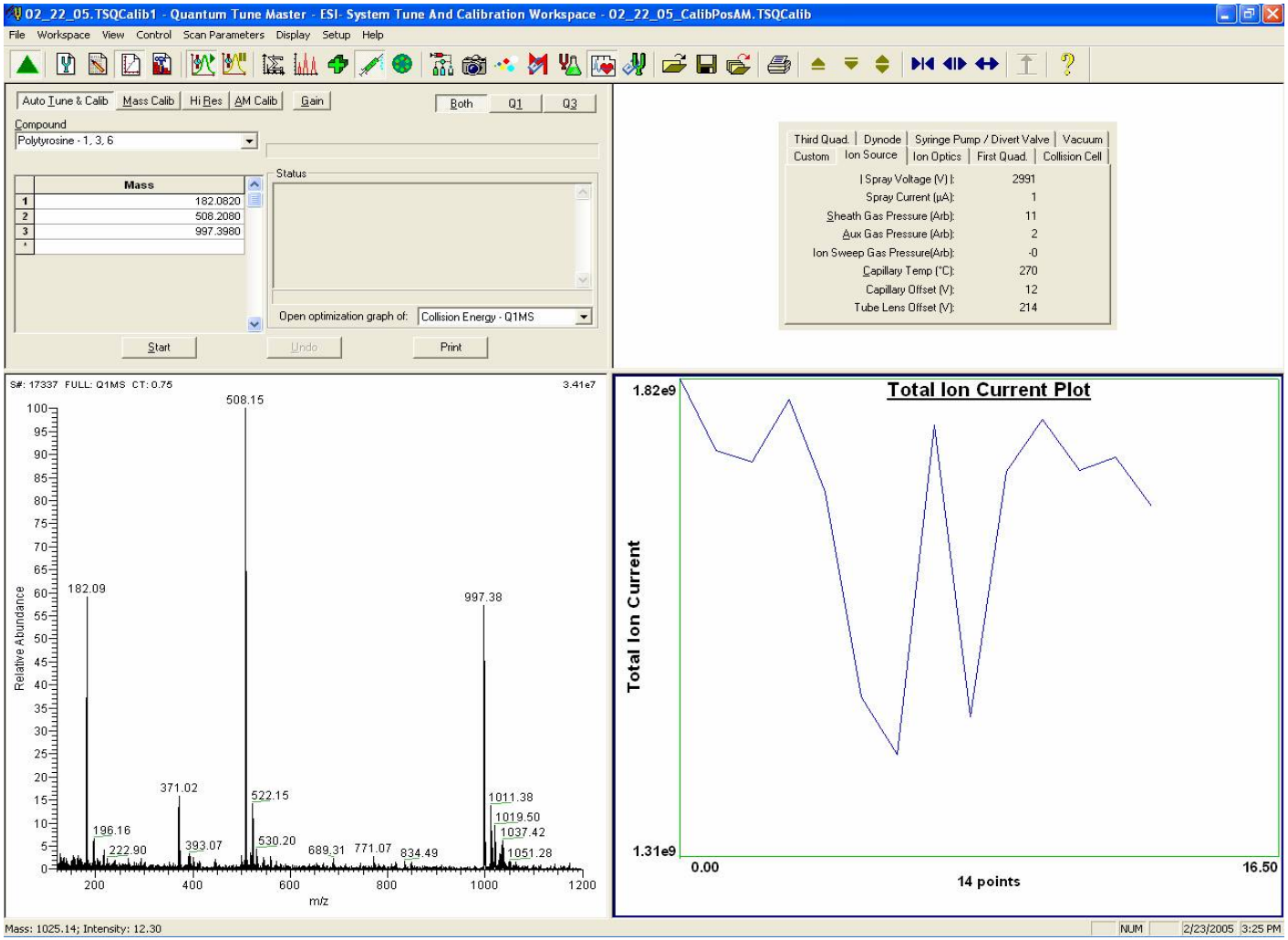
Full Instrument Control-Expert View



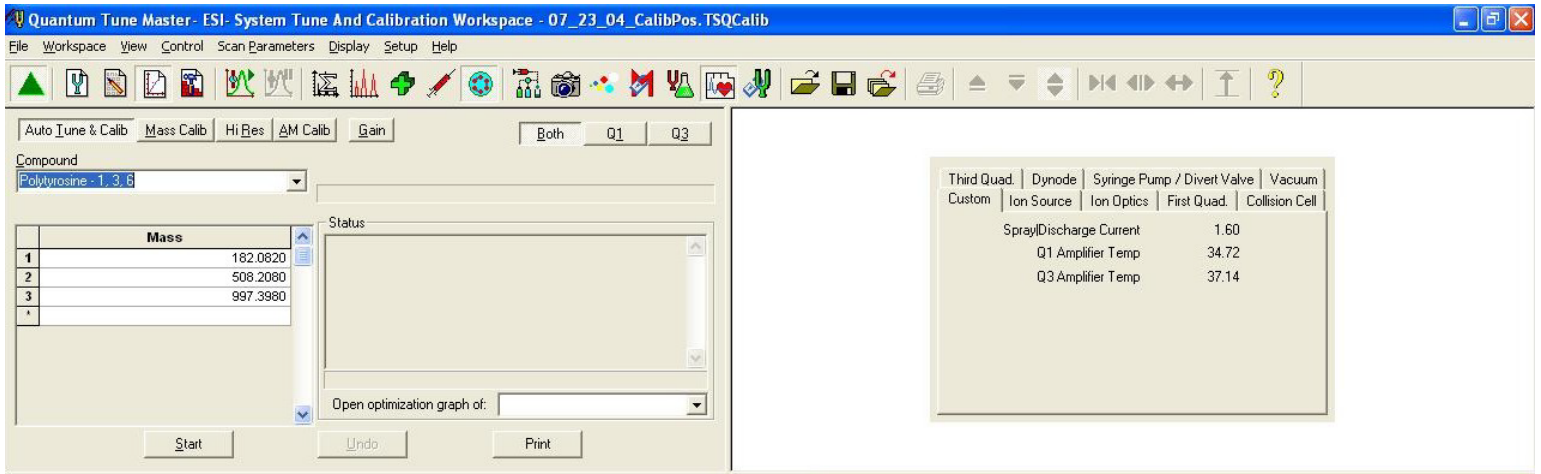
Diagnostics



Auto Tune and Calibrate



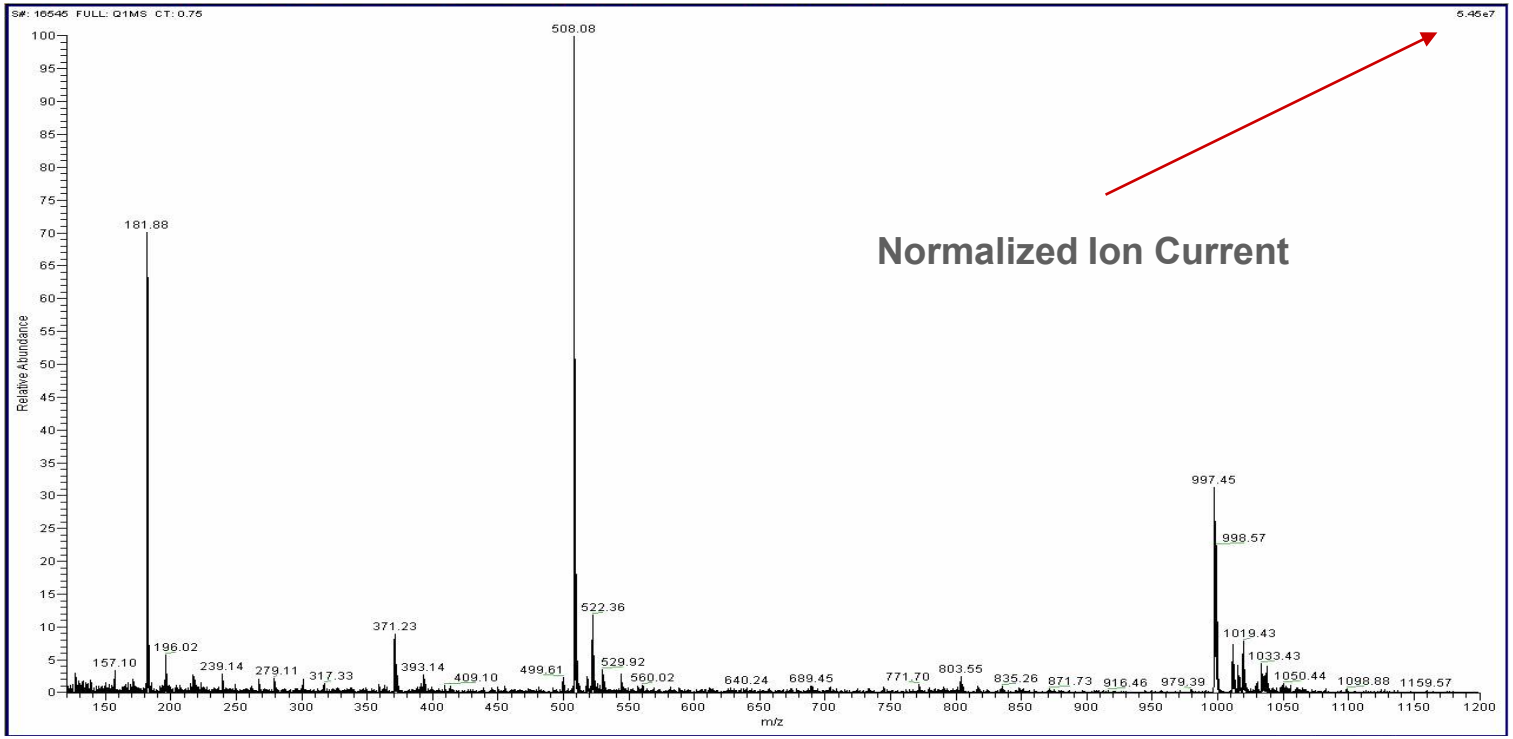
Tune and Calibrate



Pull-down calibration mass list:

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

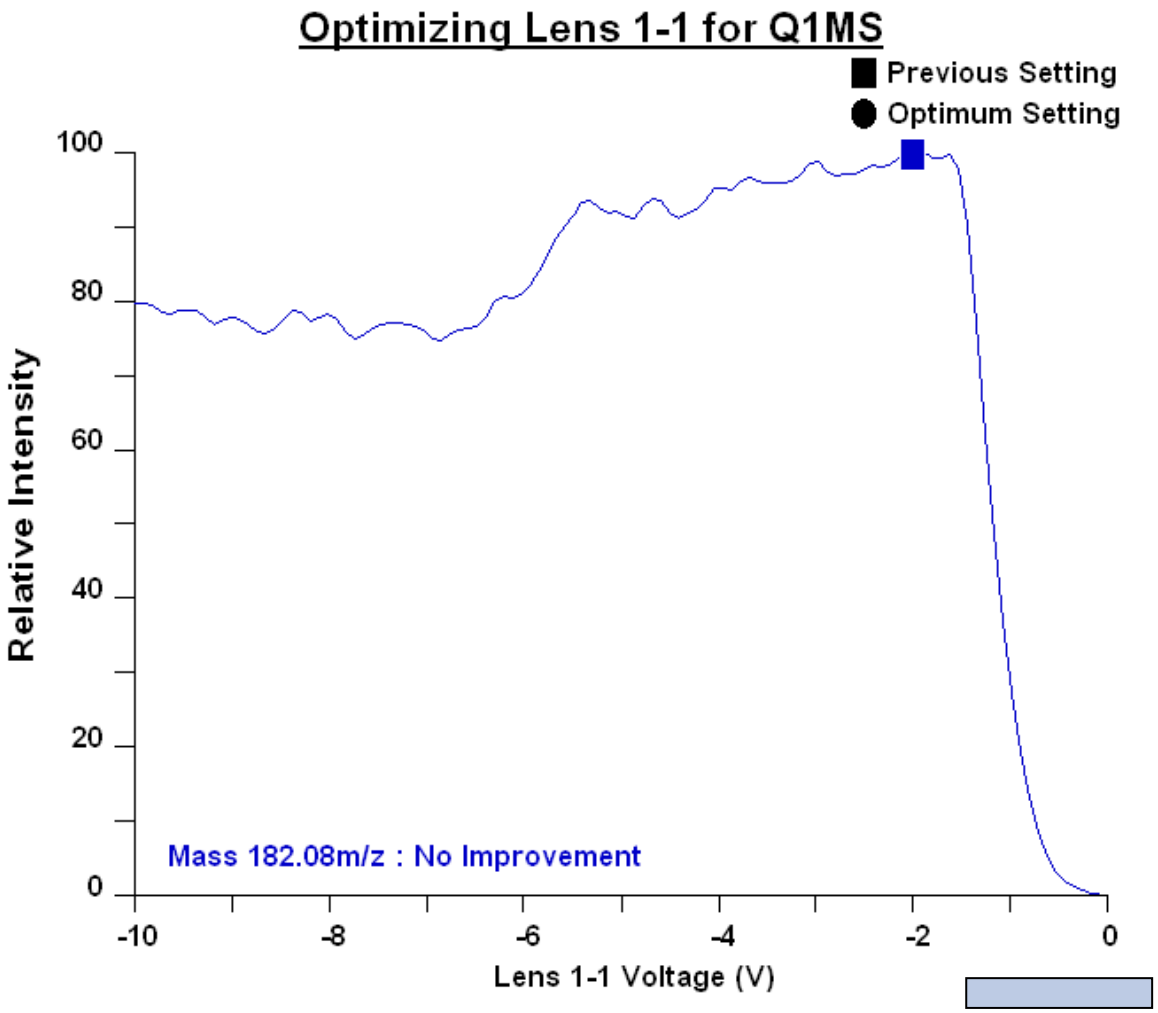
1, 3, 6 - Polytyrosine



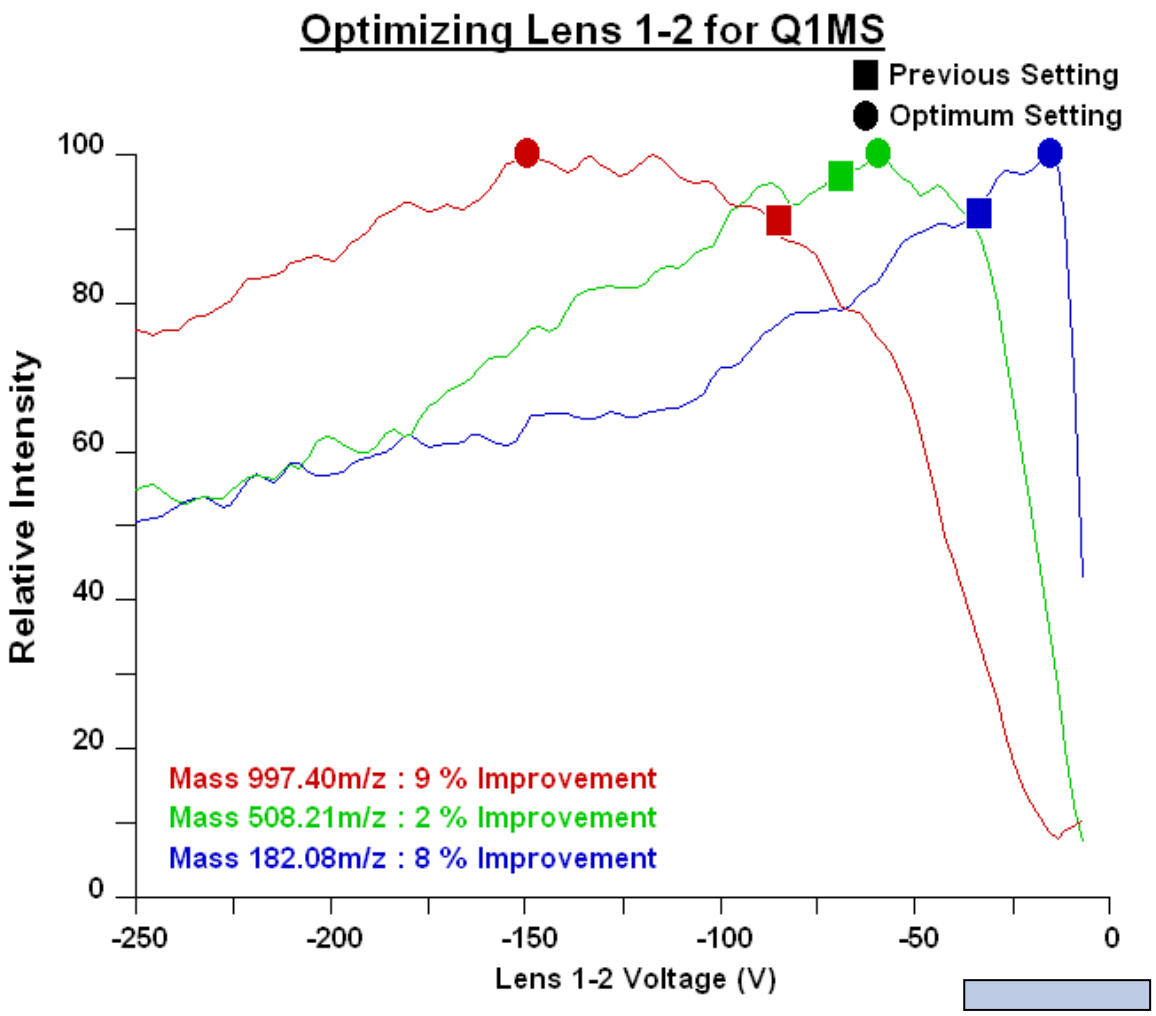
Prerequisites for successful auto-tune and calibration:

- 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)

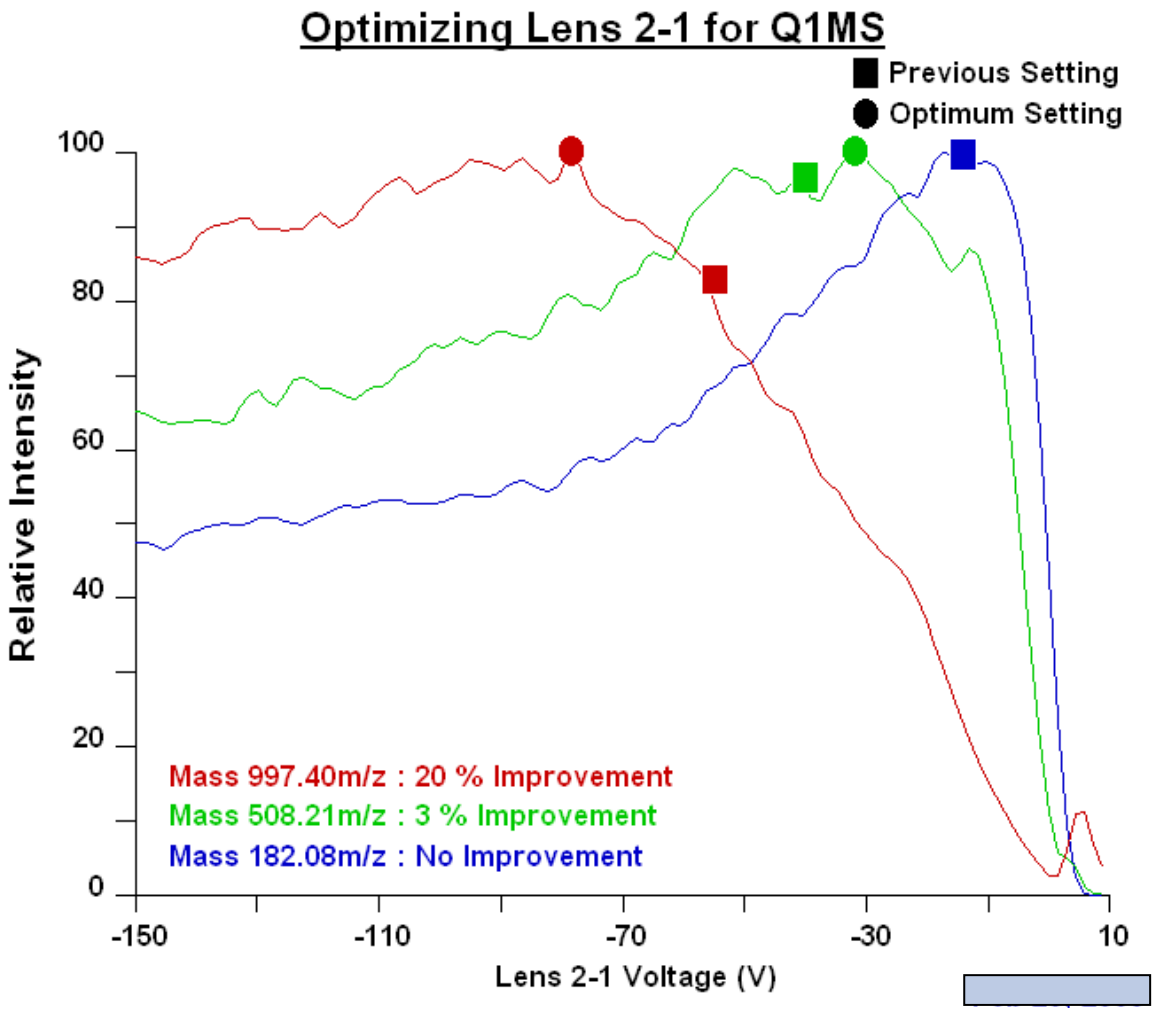
Auto Tune and Calibration



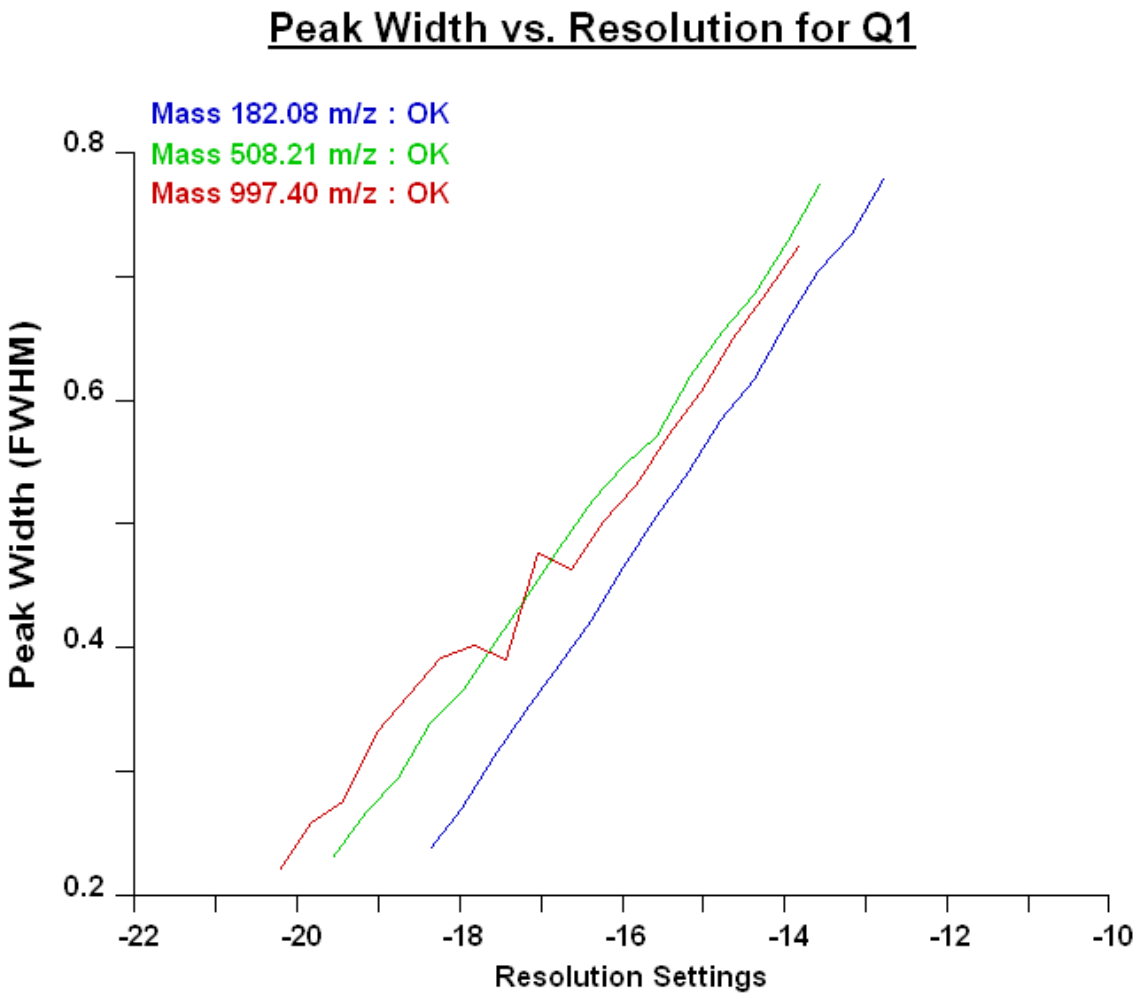
Auto Tune and Calibration



Auto Tune and Calibration

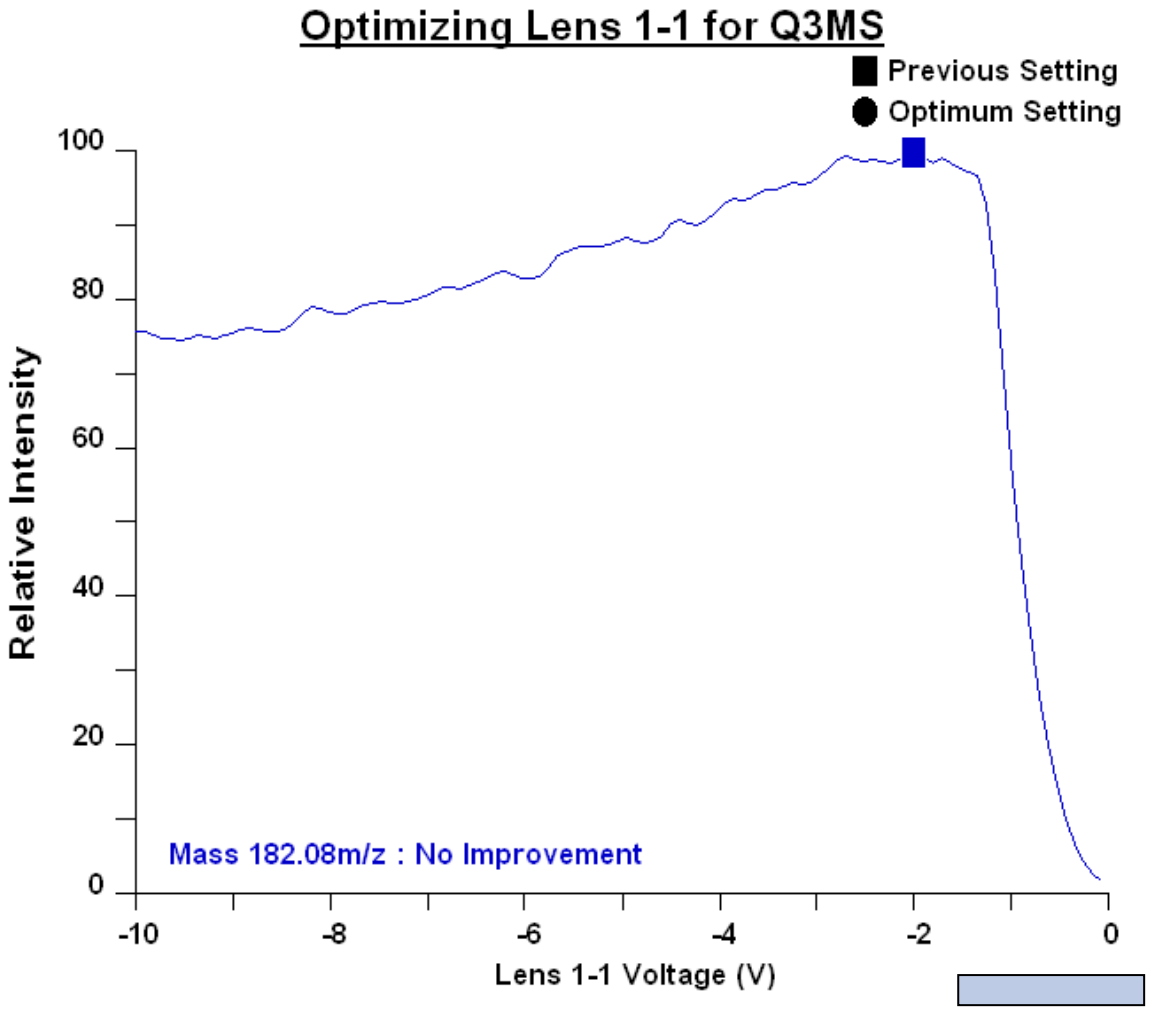


Auto Tune and Calibration

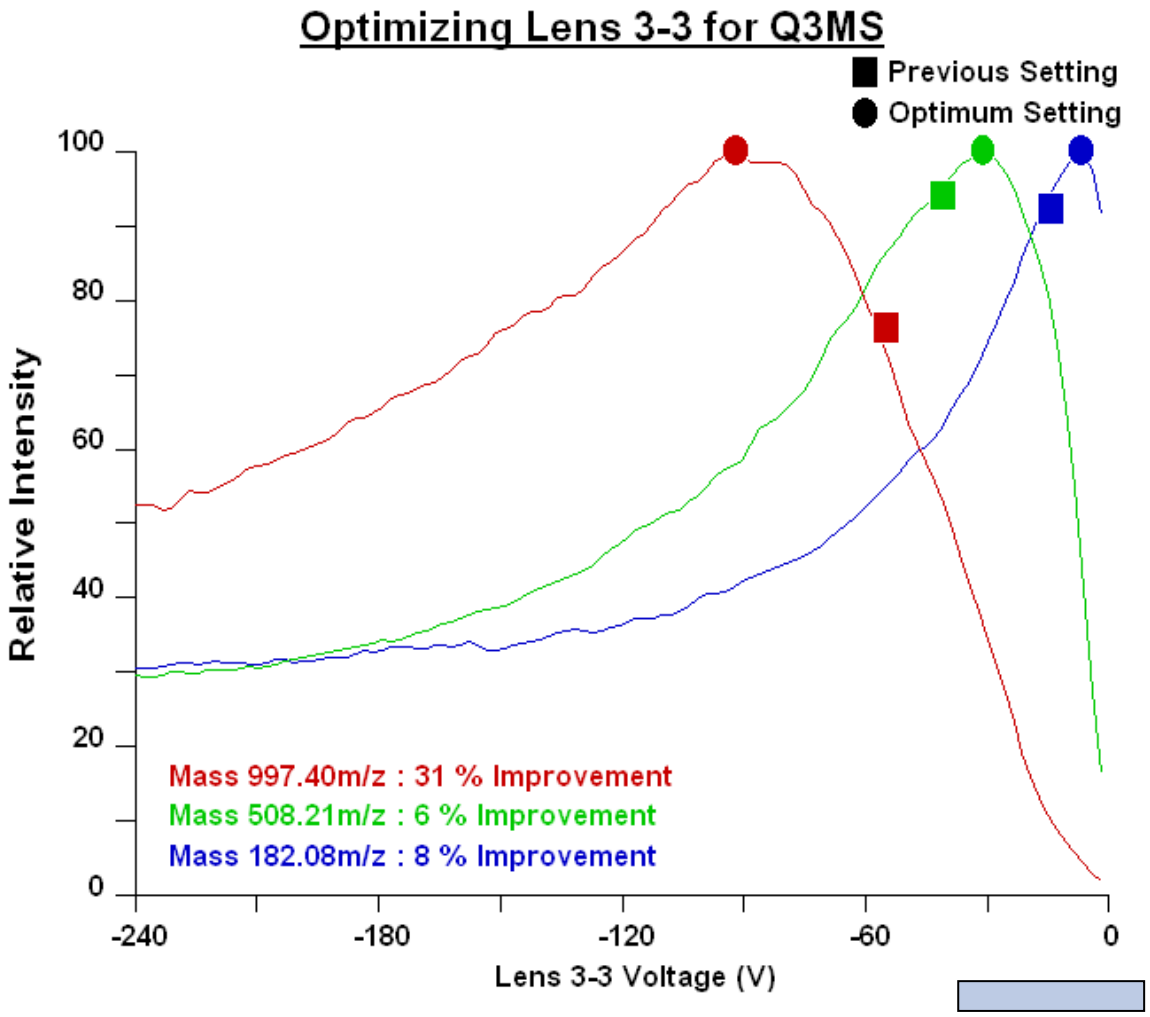


Auto Tune and Calibration

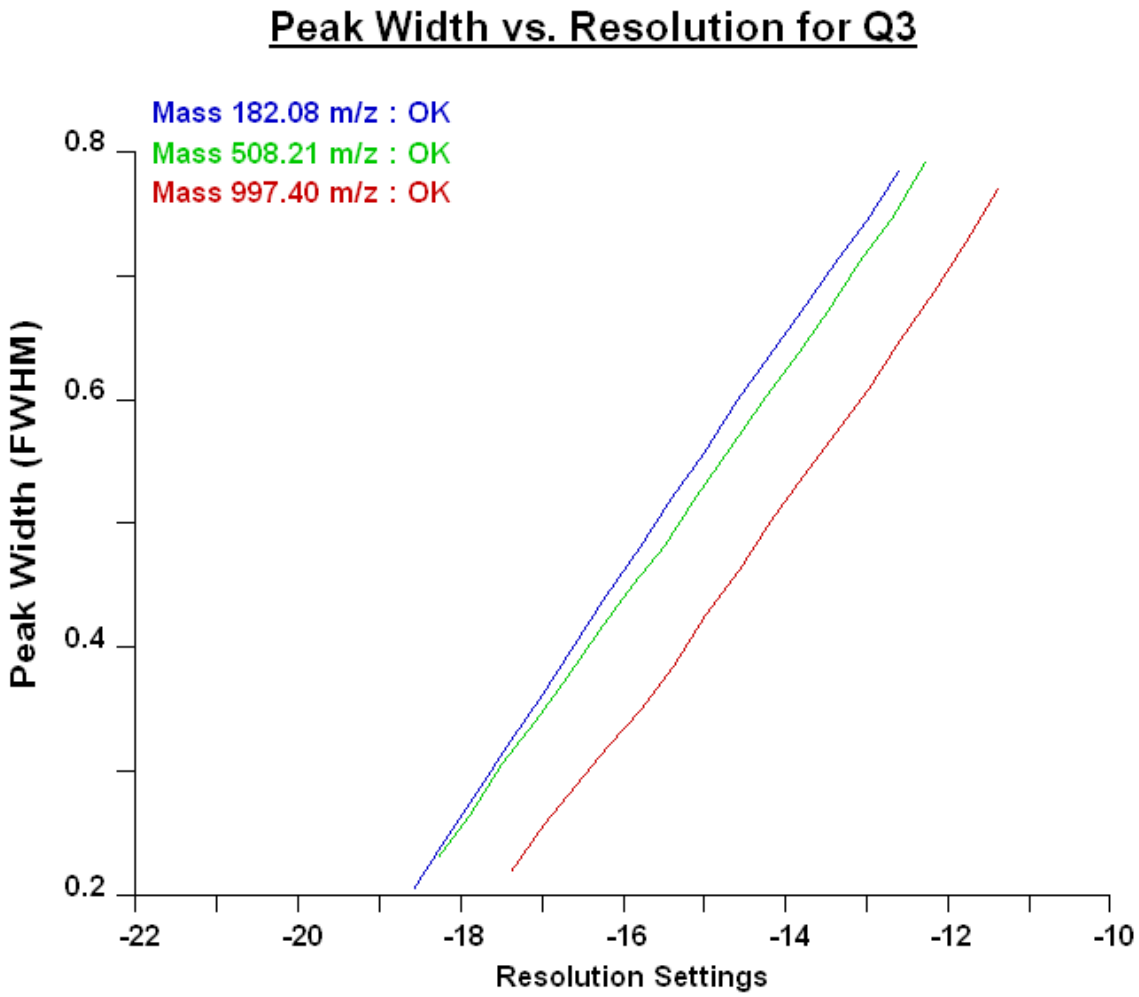
The process is repeated for Q3, one mass at a time, one parameter at a time



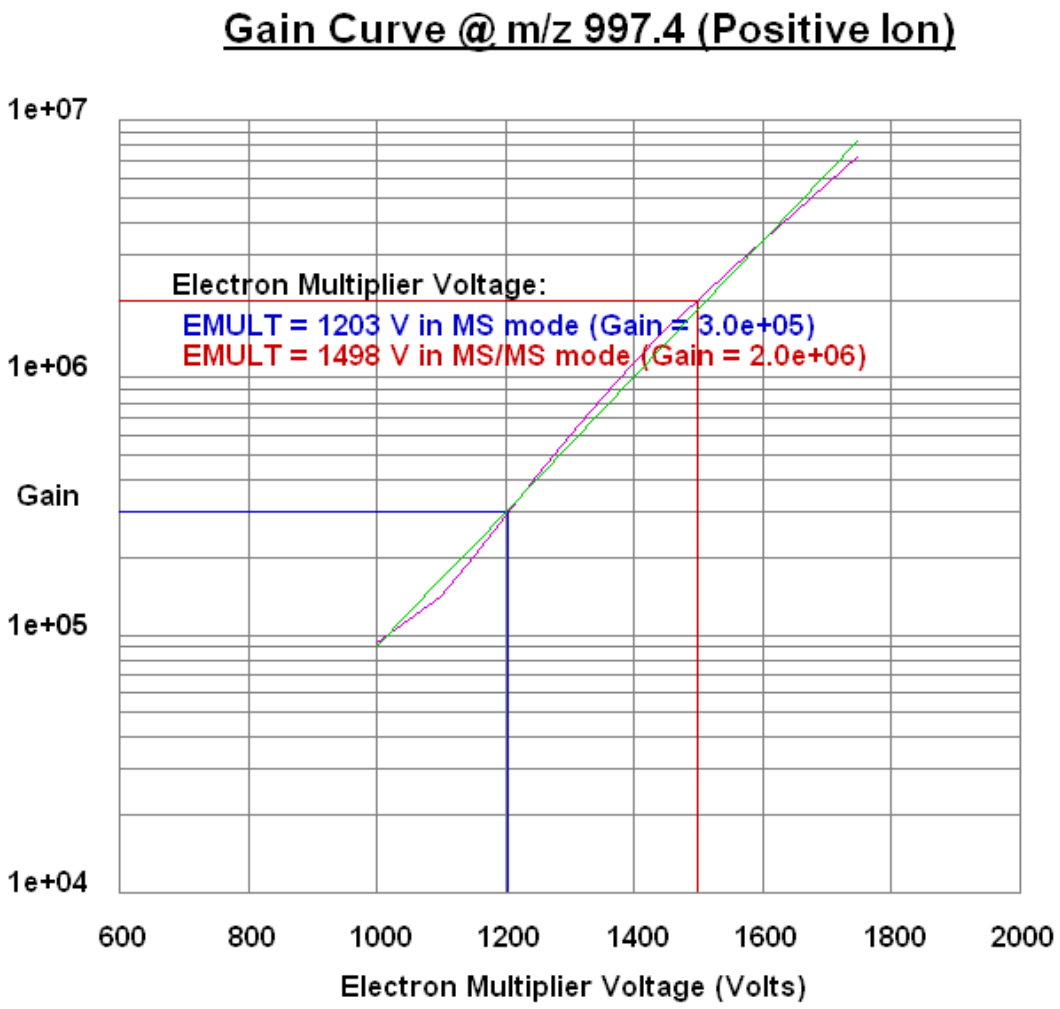
Auto Tune and Calibration



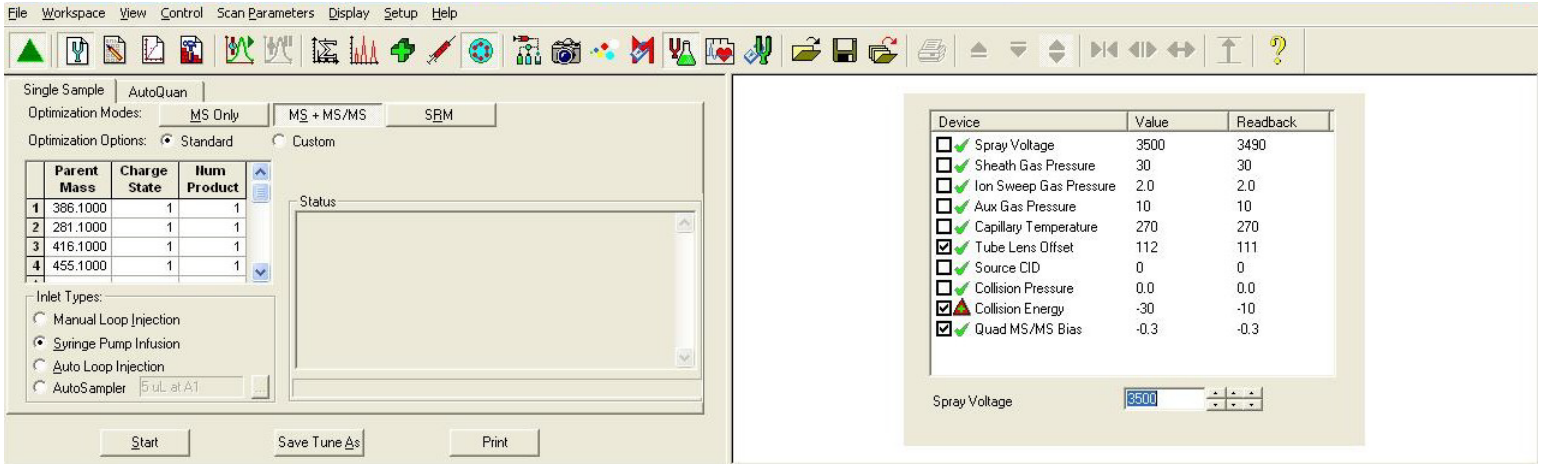
Auto Tune and Calibration



Gain Curve


Gain Curve @ m/z 997.4 (Positive Ion)
1e+07
1e+06
1e+05
1e+04
Gain
Electron Multiplier Voltage:
EMULT = 1203 V in MS mode (Gain = 3.0e+05)
EMULT = 1498 V in MS/MS mode (Gain = 2.0e+06)
600
800
1000
1200
1400
1600
1800
2000
Electron Multiplier Voltage (Volts)

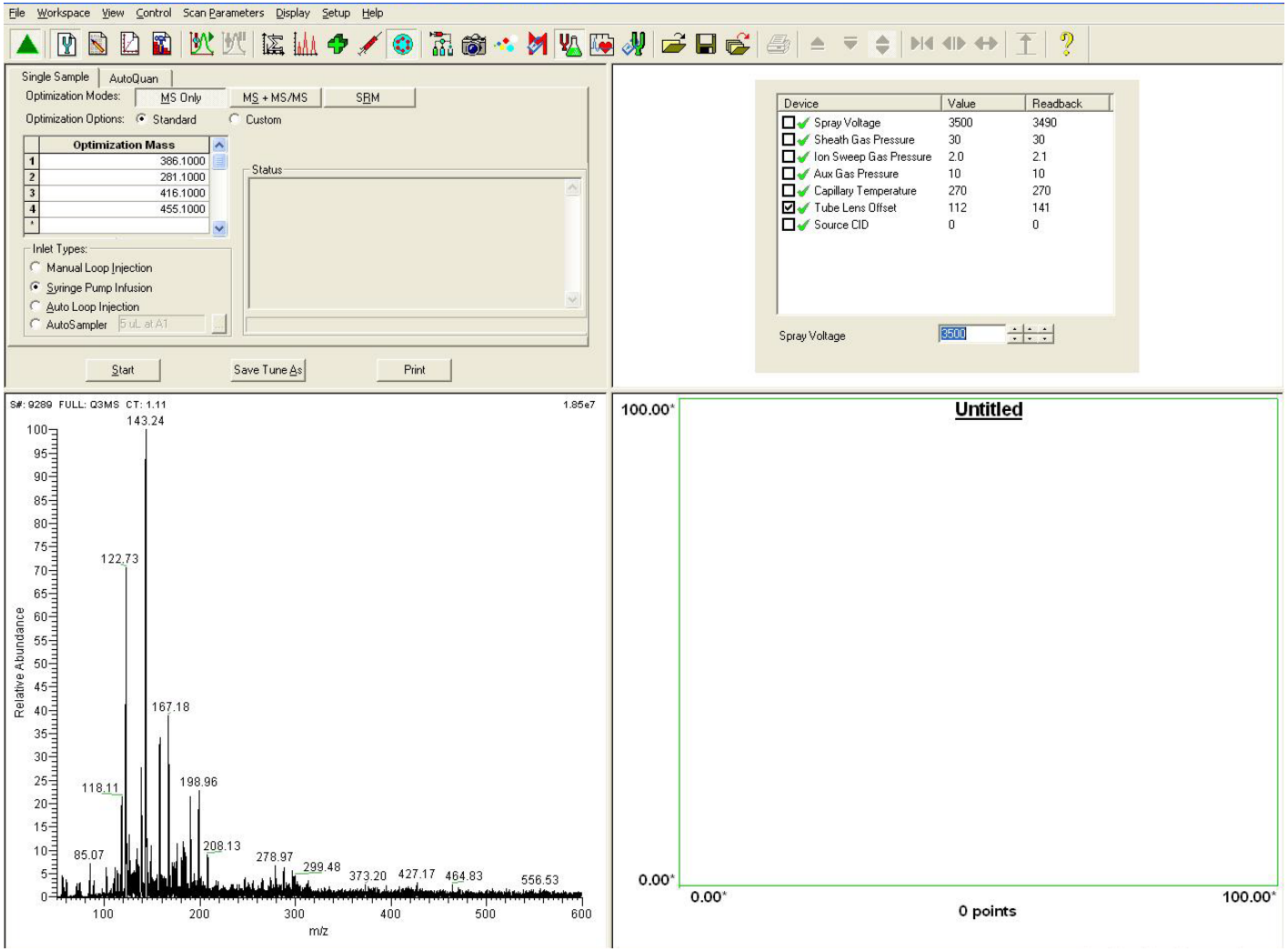
Automatic Compound Optimization



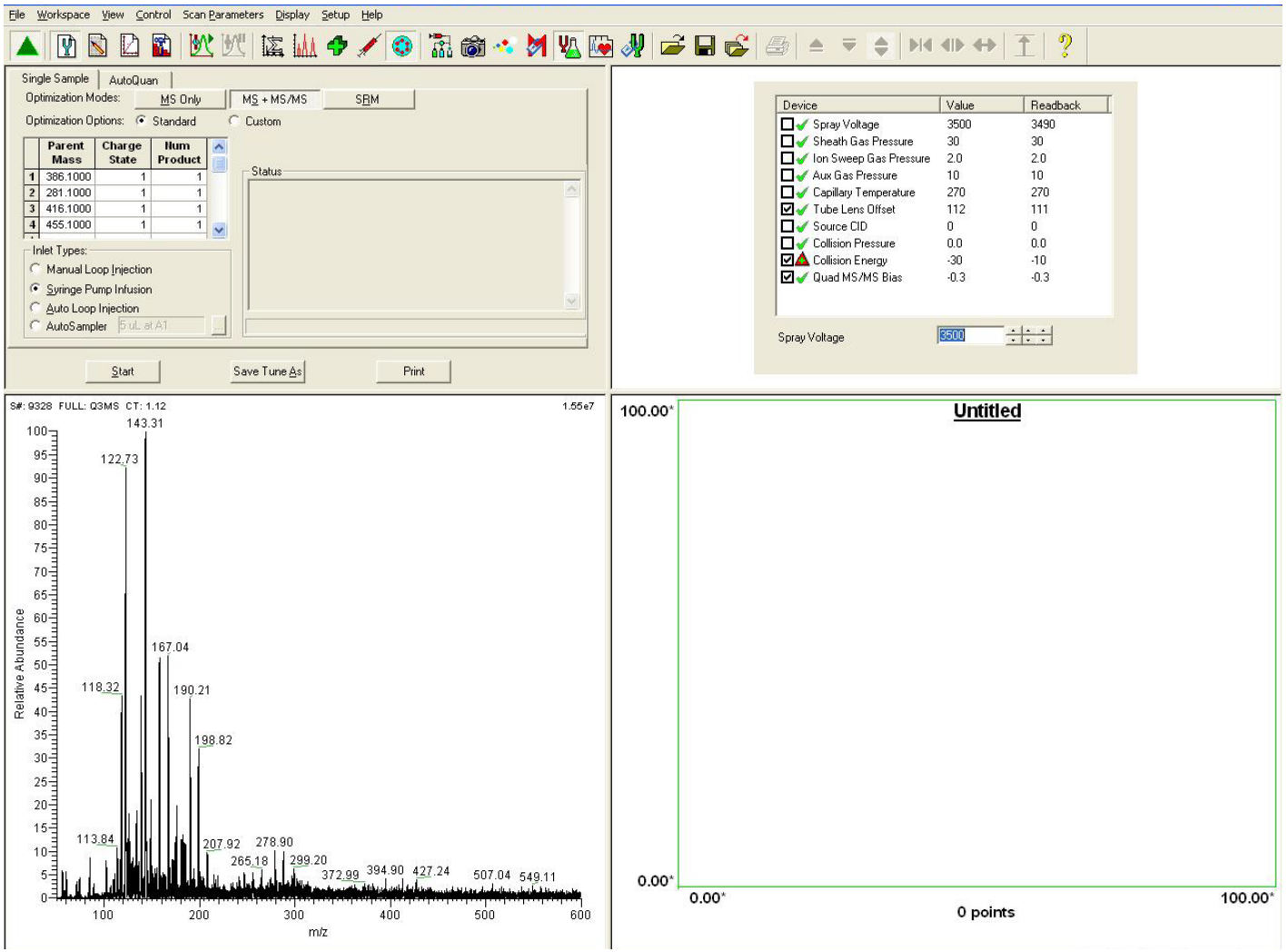
Optimization (a.k.a. “Compound tuning”) options:

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

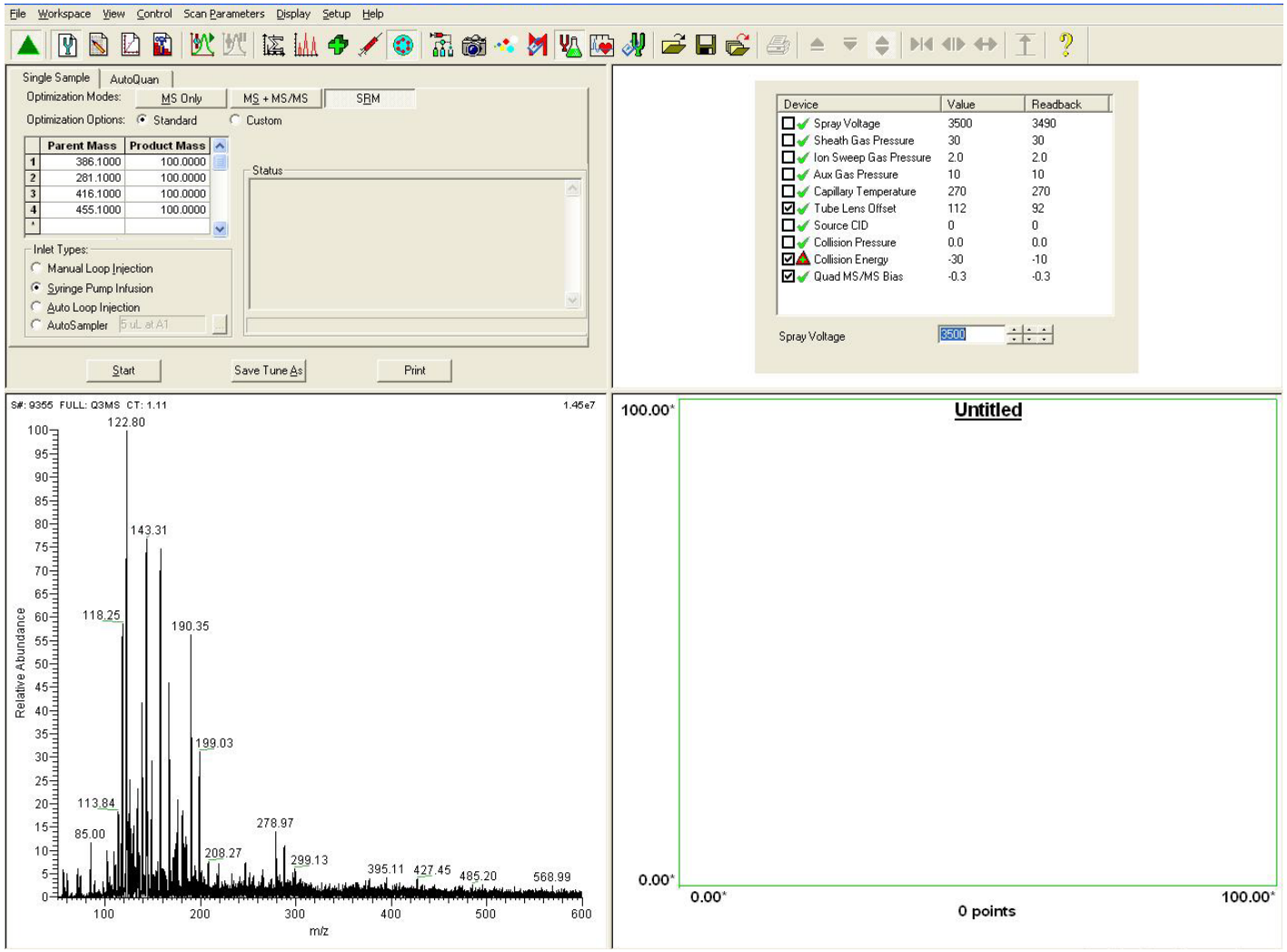
Automatic Compound Optimization - MS Only



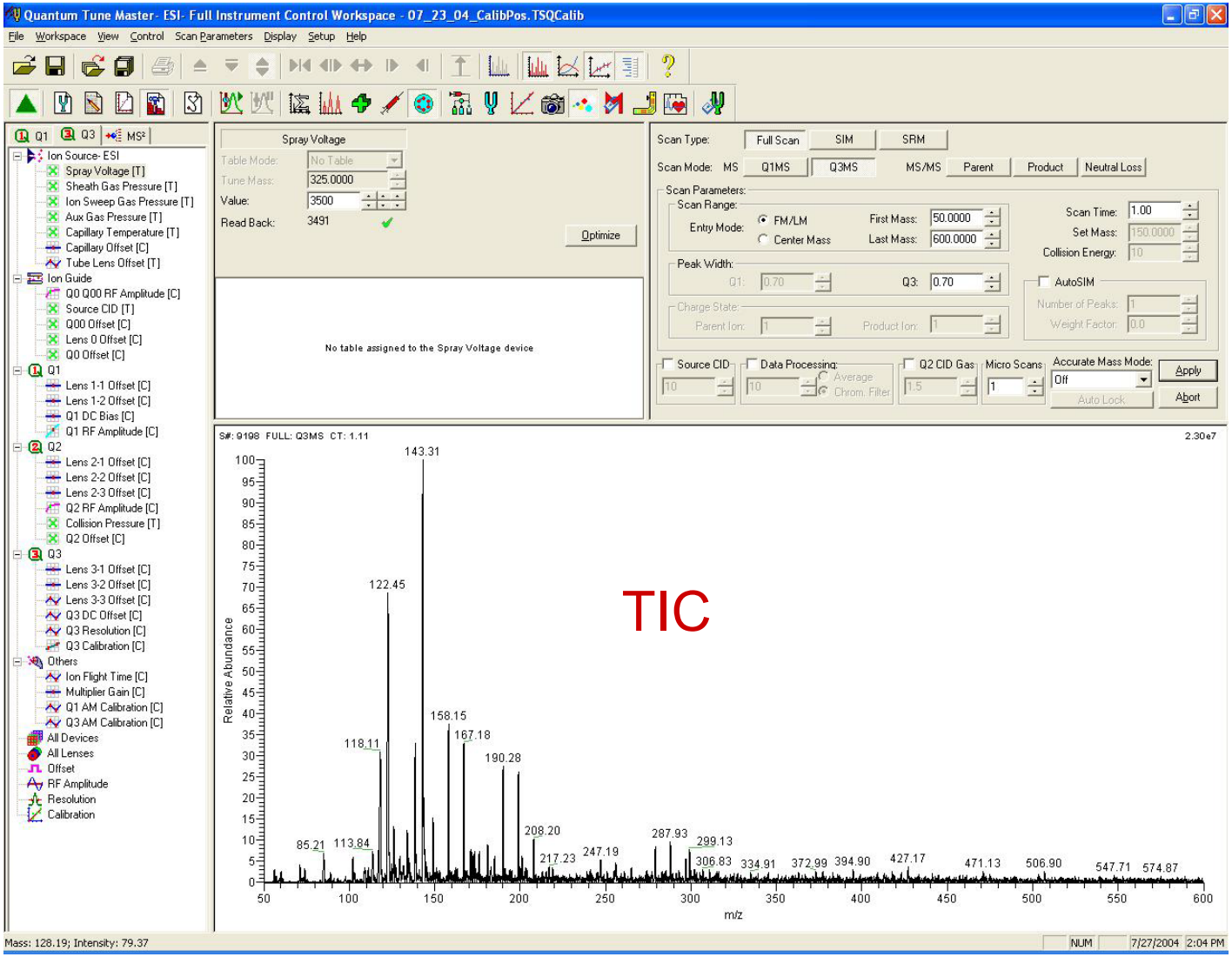
Automatic Compound Optimization - MS+MS/MS



Automatic Compound Optimization - SRM



Compound Optimization – Manual Procedure



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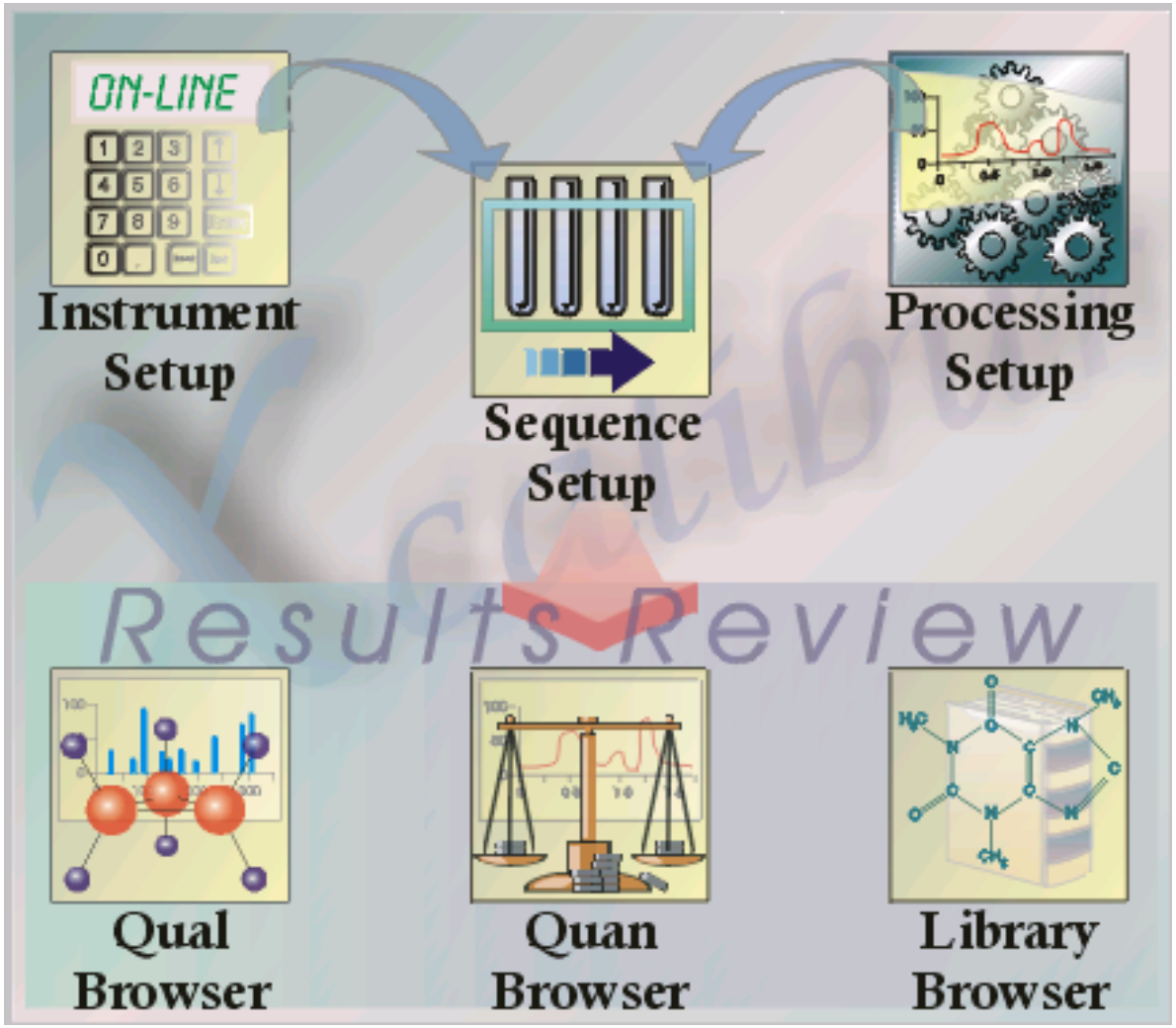
Chapter 8

Xcalibur Instrument Configuration Method Setup

Xcalibur ® 2.0


ON-LINE
Instrument Setup
Sequence Setup
Processing Setup
Results Review
Qual Browser
Quan Browser
Library Browser

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)
- a**Q**a (Navigator) / MSQ / MSQ+

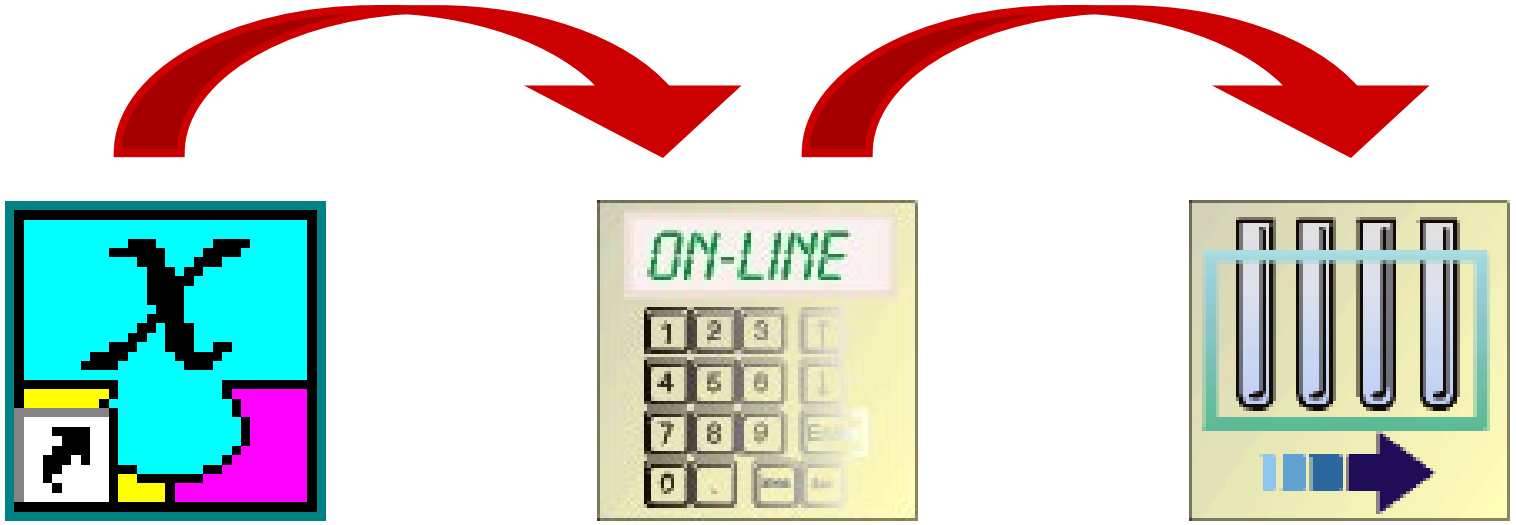
Supported LC Peripherals

- 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

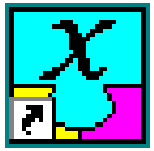
Xcalibur® File Types

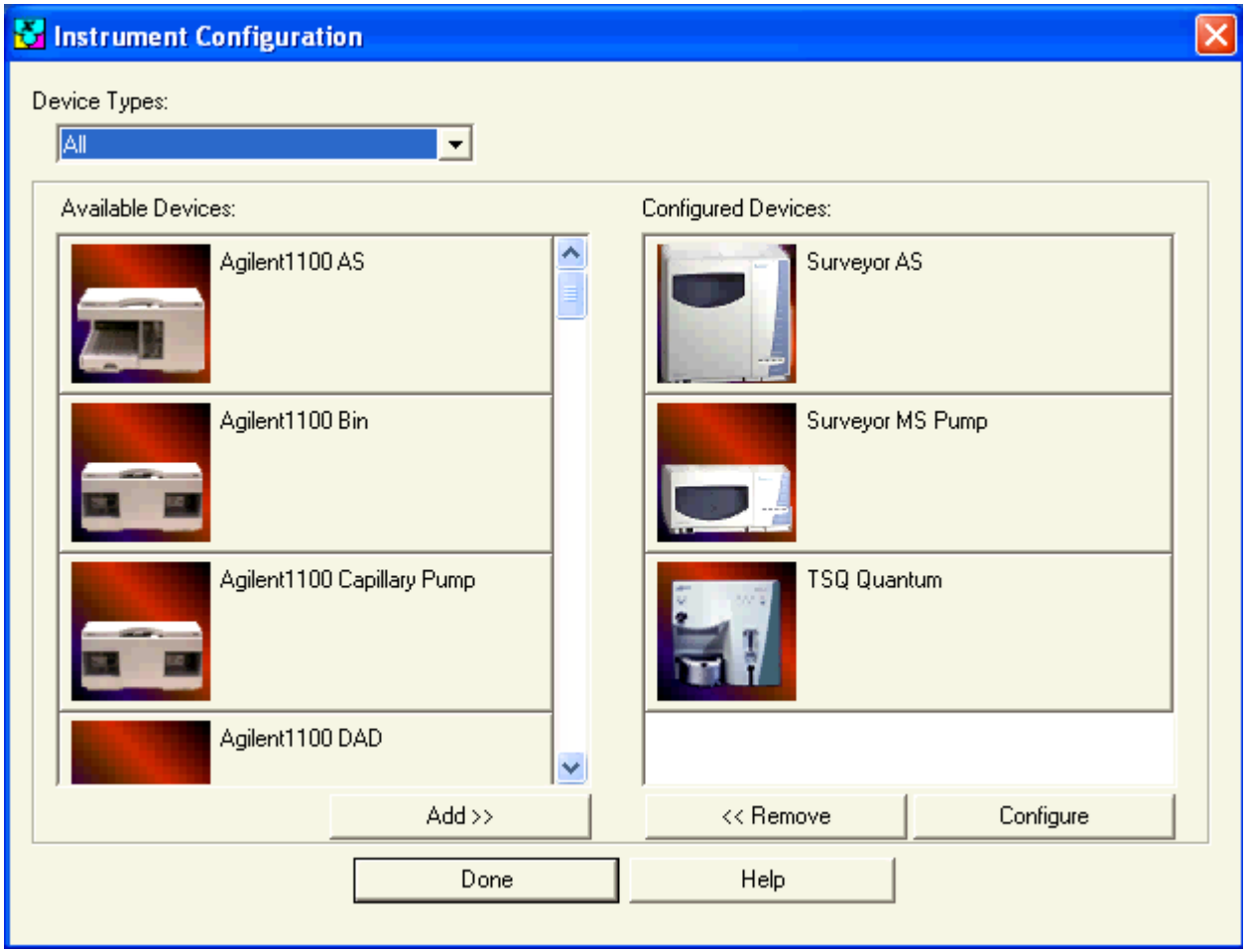
.raw	Acquired data files
.sld	Sequence setup files
.pmd	Processing setup method
.meth	Instrument setup method
.rst	Result files from quantitation
.lyt	Qual browser layout
.lqn	LCquan files
.xqn	Quan browser files
.xrt	XReport files

Instrument Configuration, Setup, and Control

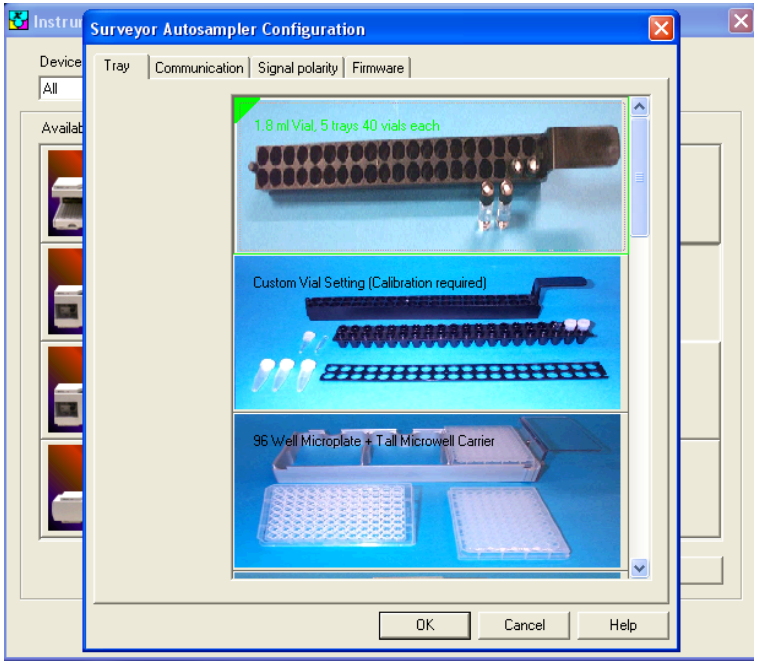


Instrument Configuration



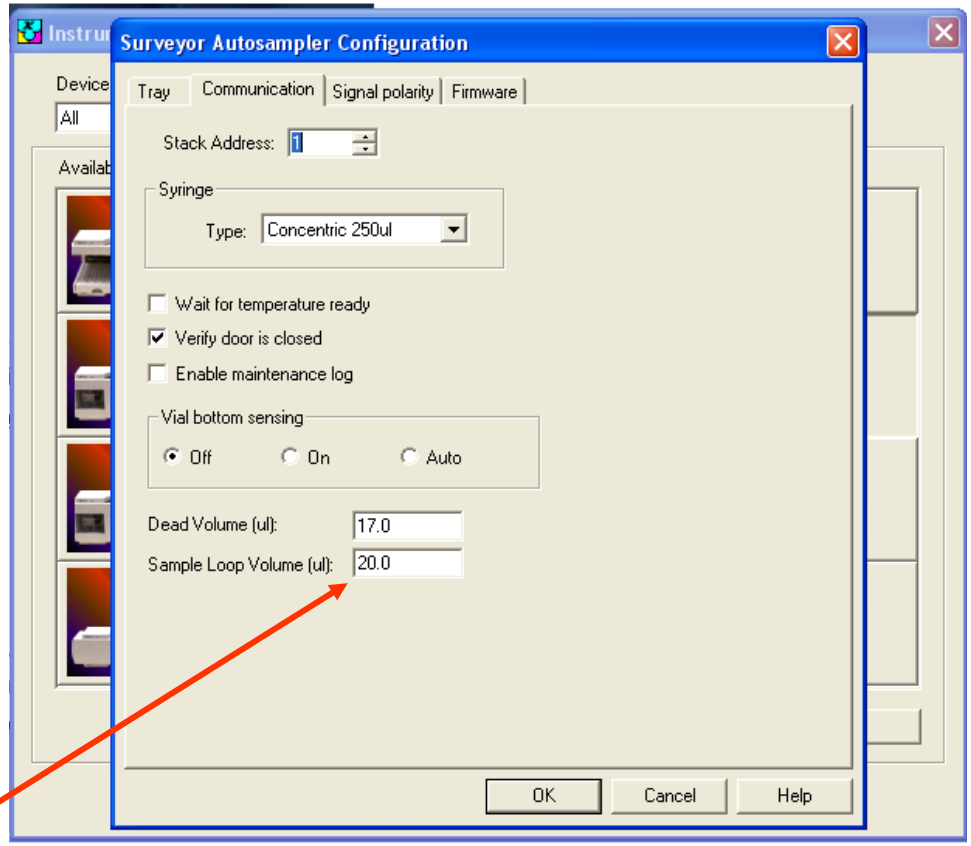


Autosampler Configuration



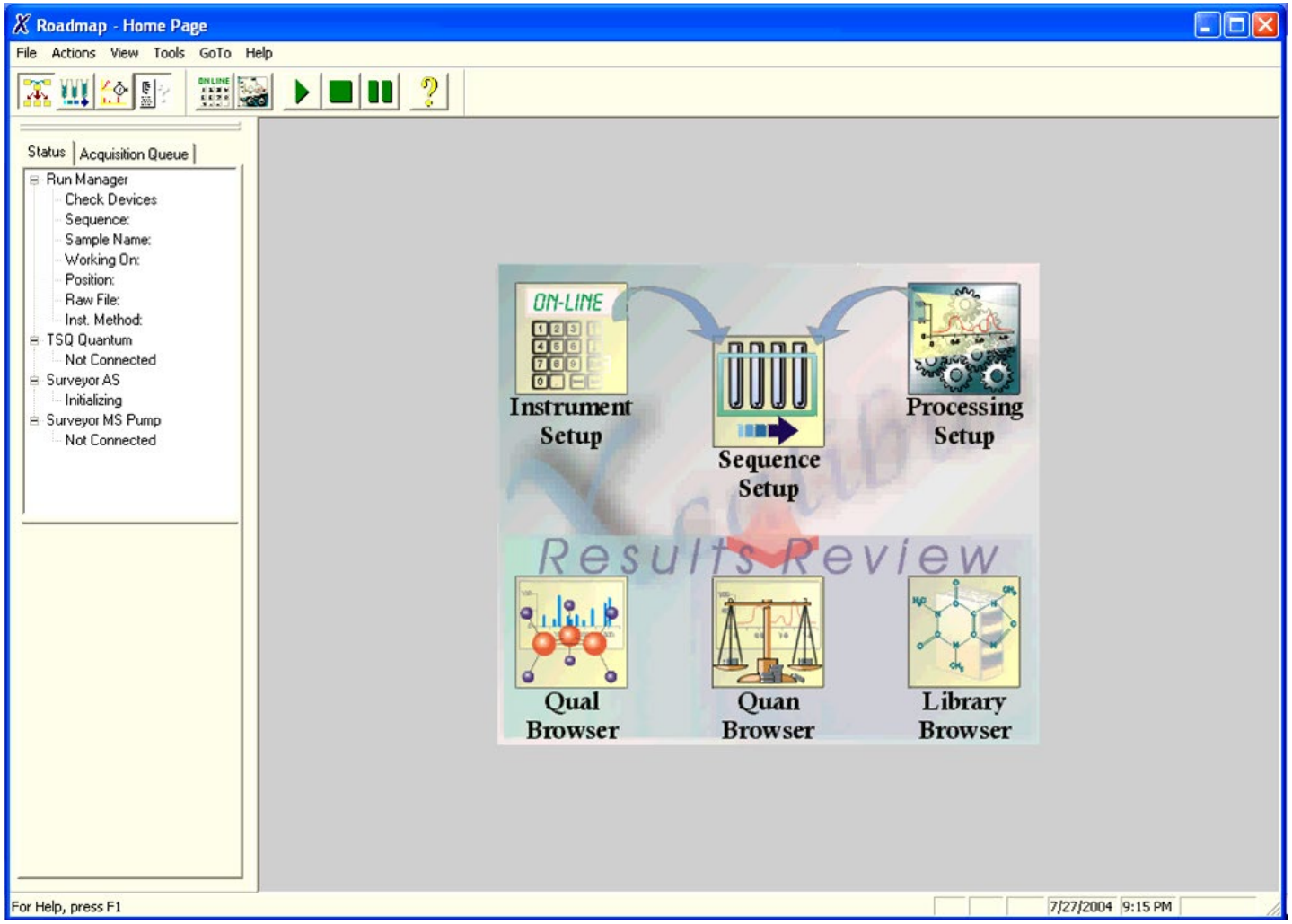
Autosampler configuration tab: Tray

Autosampler configuration tab: Communication

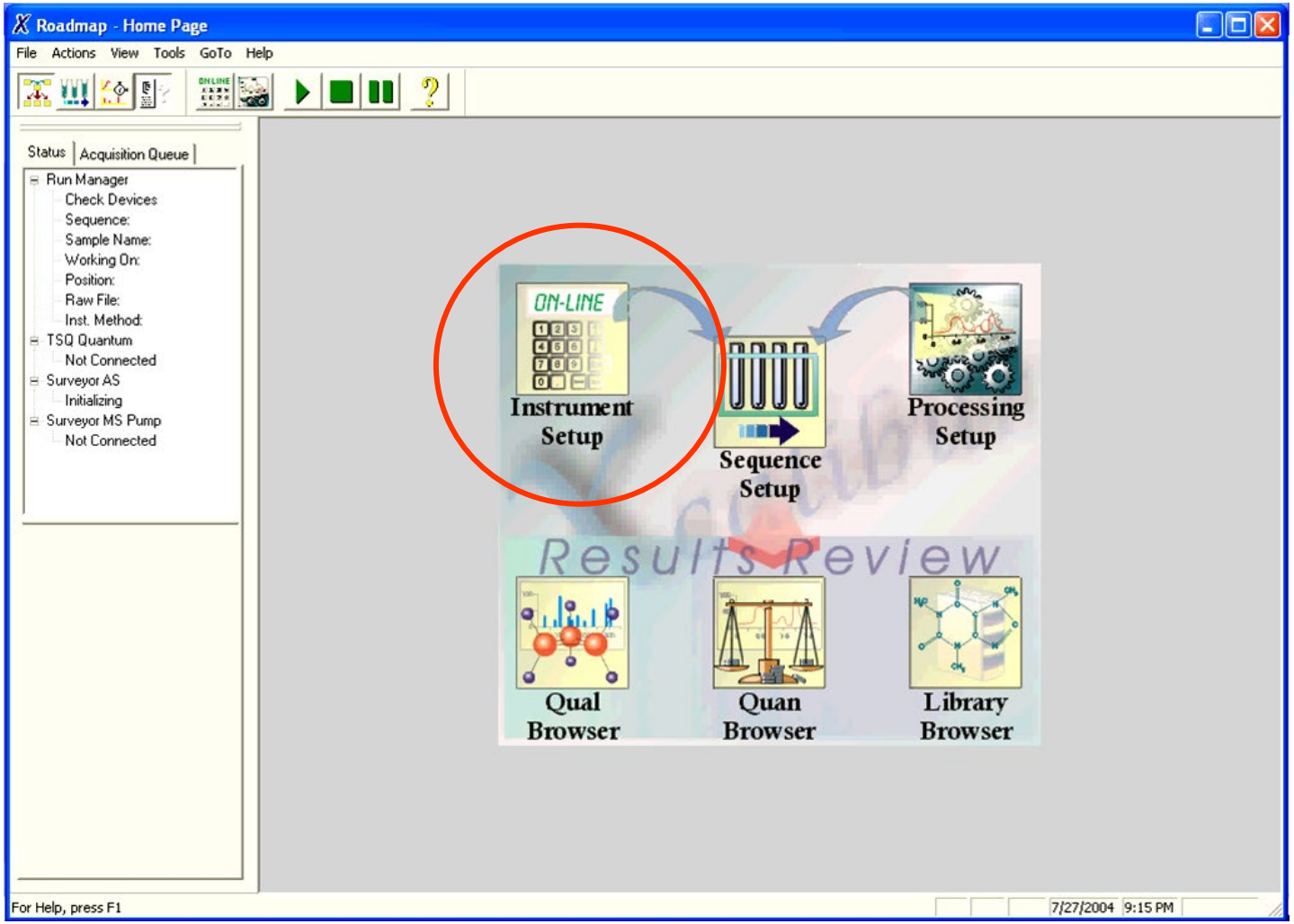


Sample Loop Volume must match loop installed for reproducible injections!!!

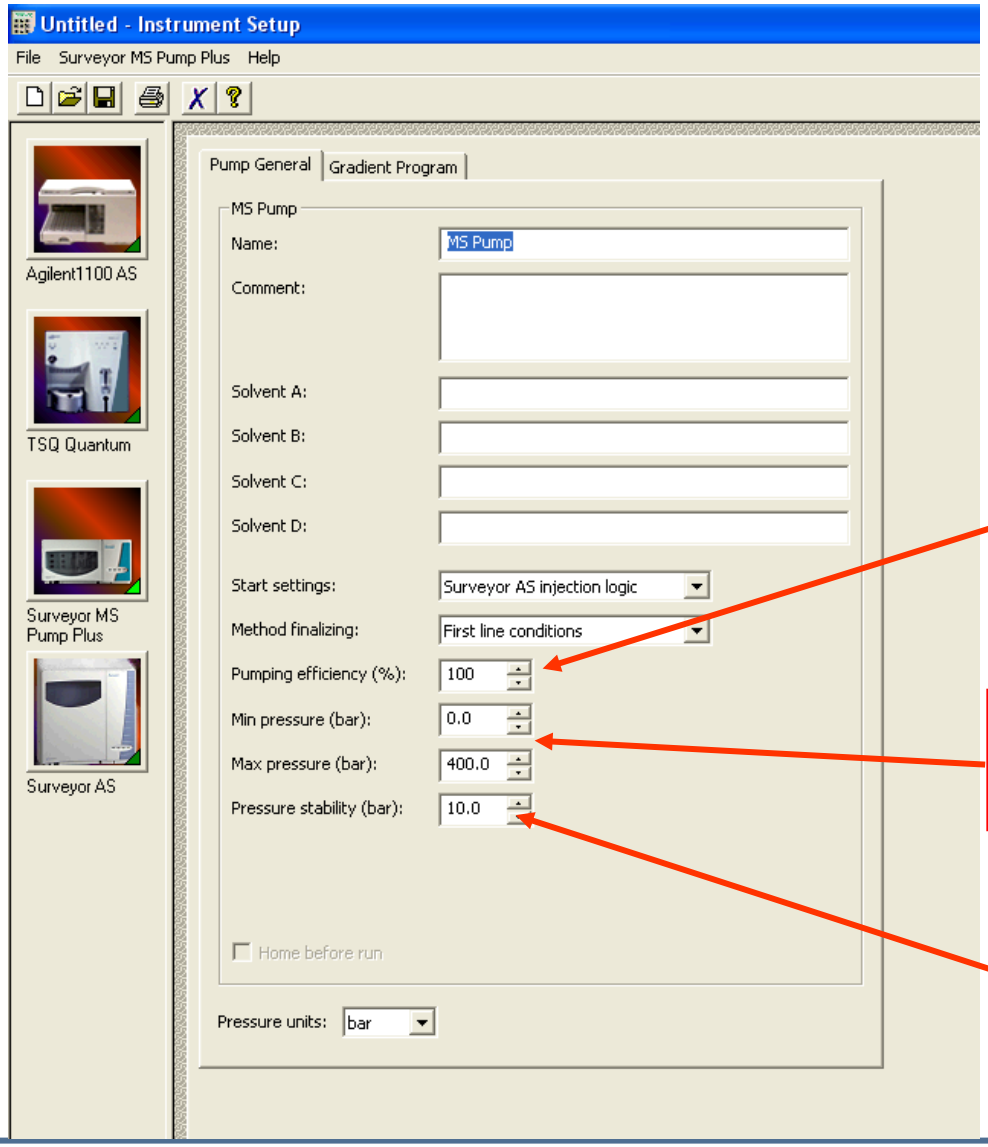
Homepage (Roadmap) – Status View



Instrument Setup



Instrument Setup – Surveyor MS Pump Plus

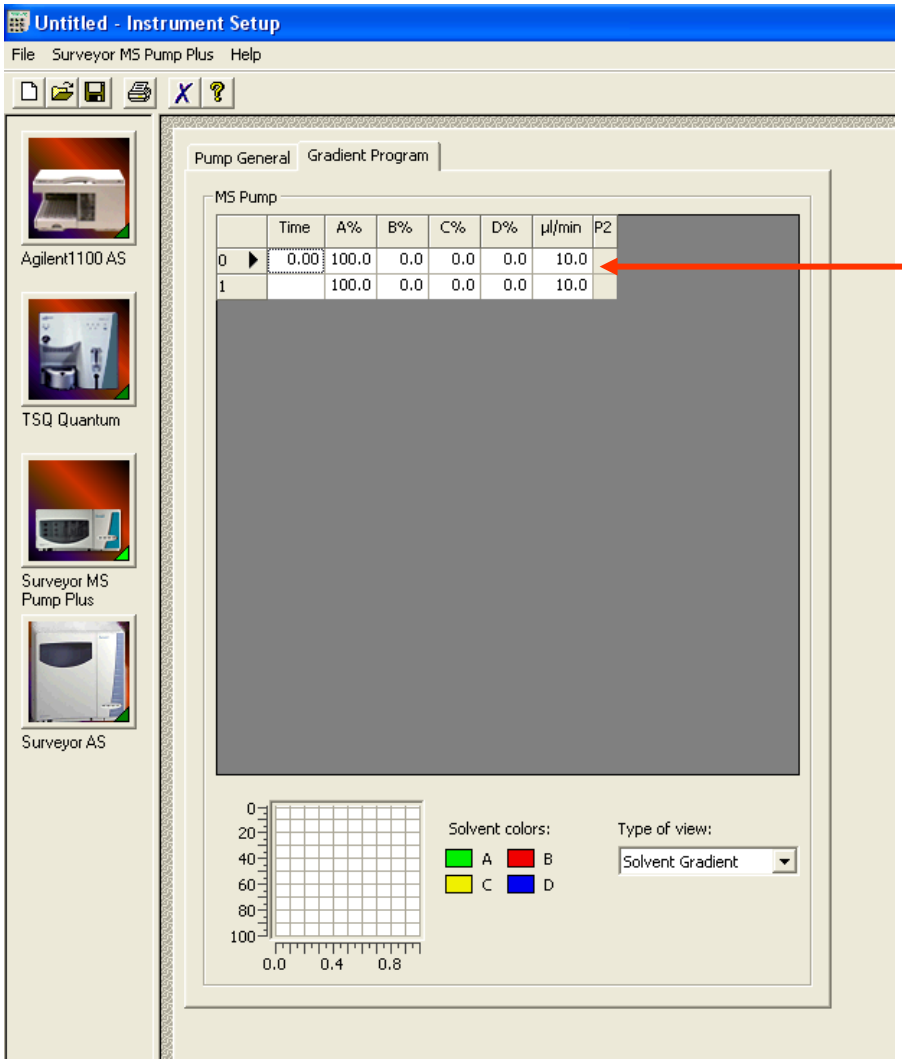


Pumping efficiency (%) takes into account solvent compressibility (for common LC solvents this can be left at 100

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

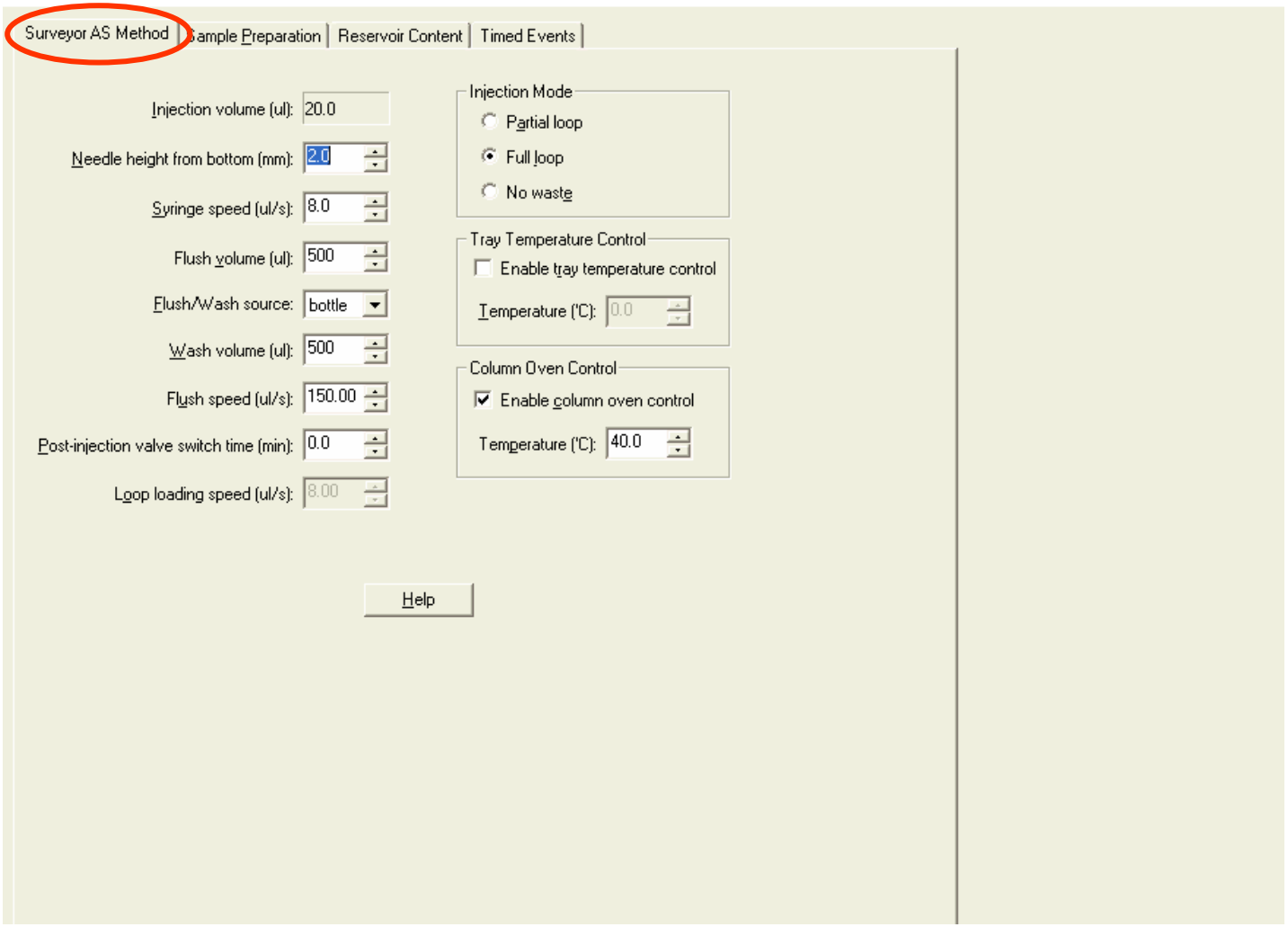
Instrument Setup – Surveyor MS Pump Plus



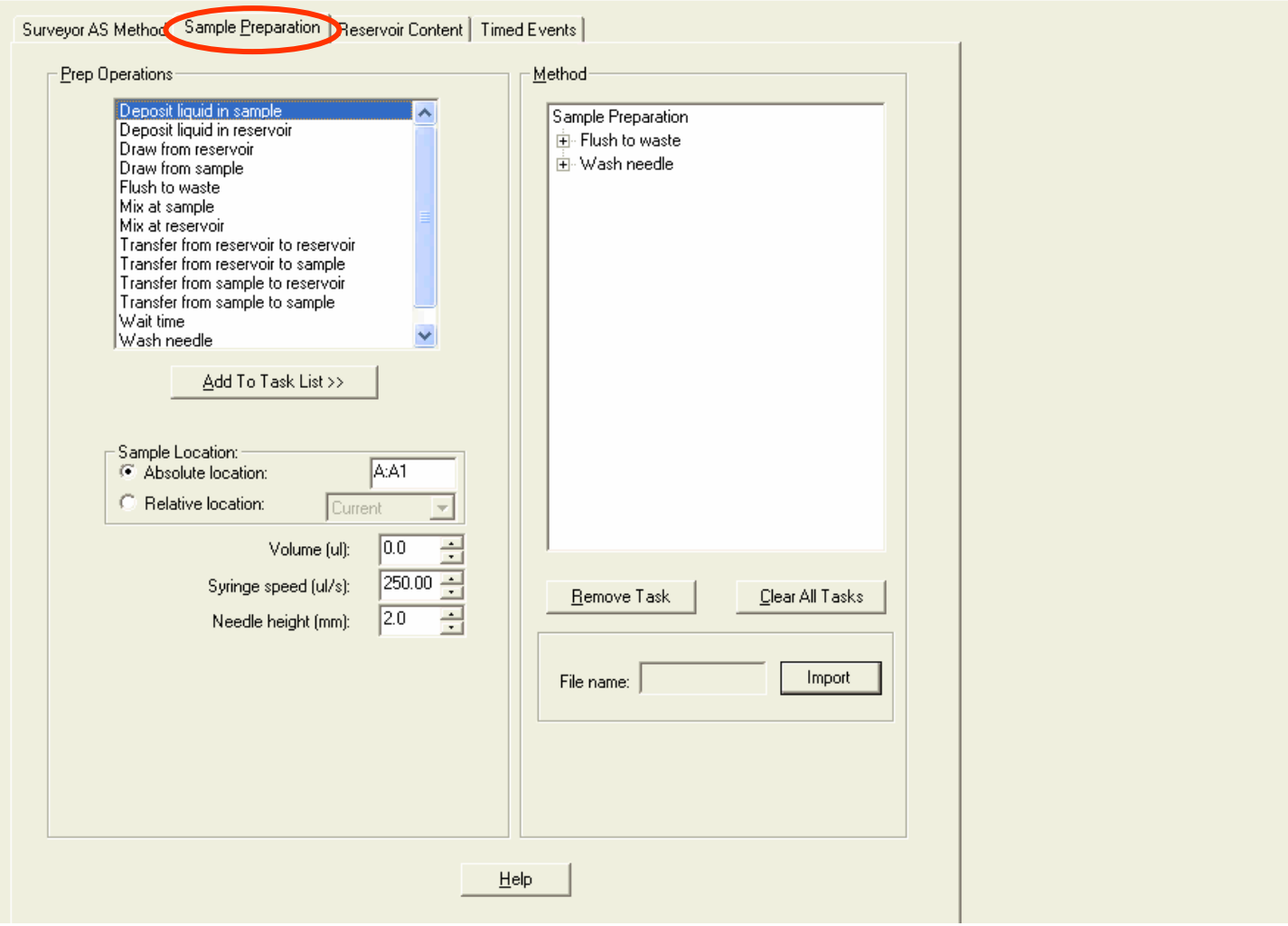
Build the gradient profile here

Note: Flow is in µL/min and there will always be an extra line that does not contribute to the pumps gradient logic

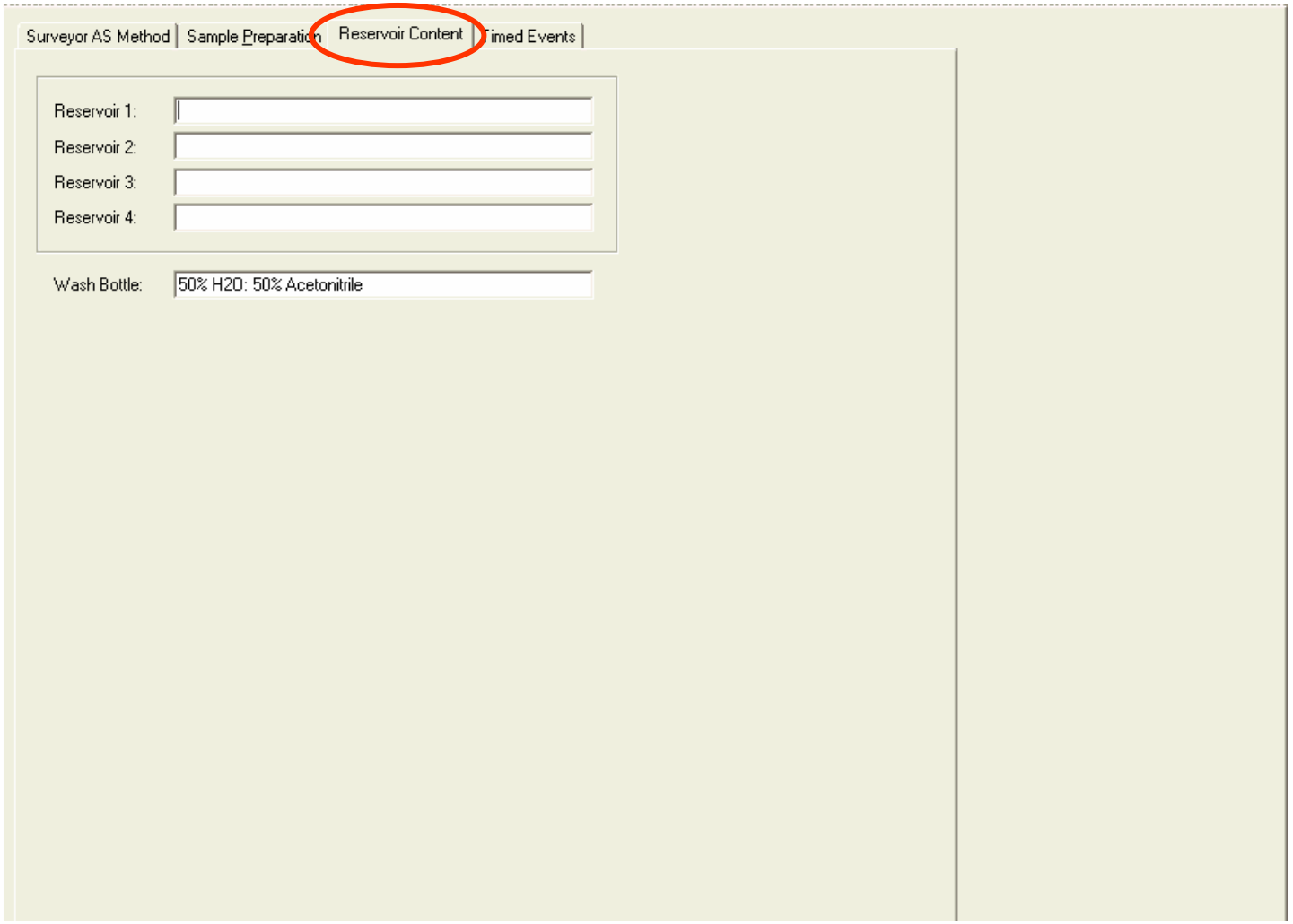
Instrument Setup – Surveyor Autosampler



Instrument Setup – Surveyor Autosampler

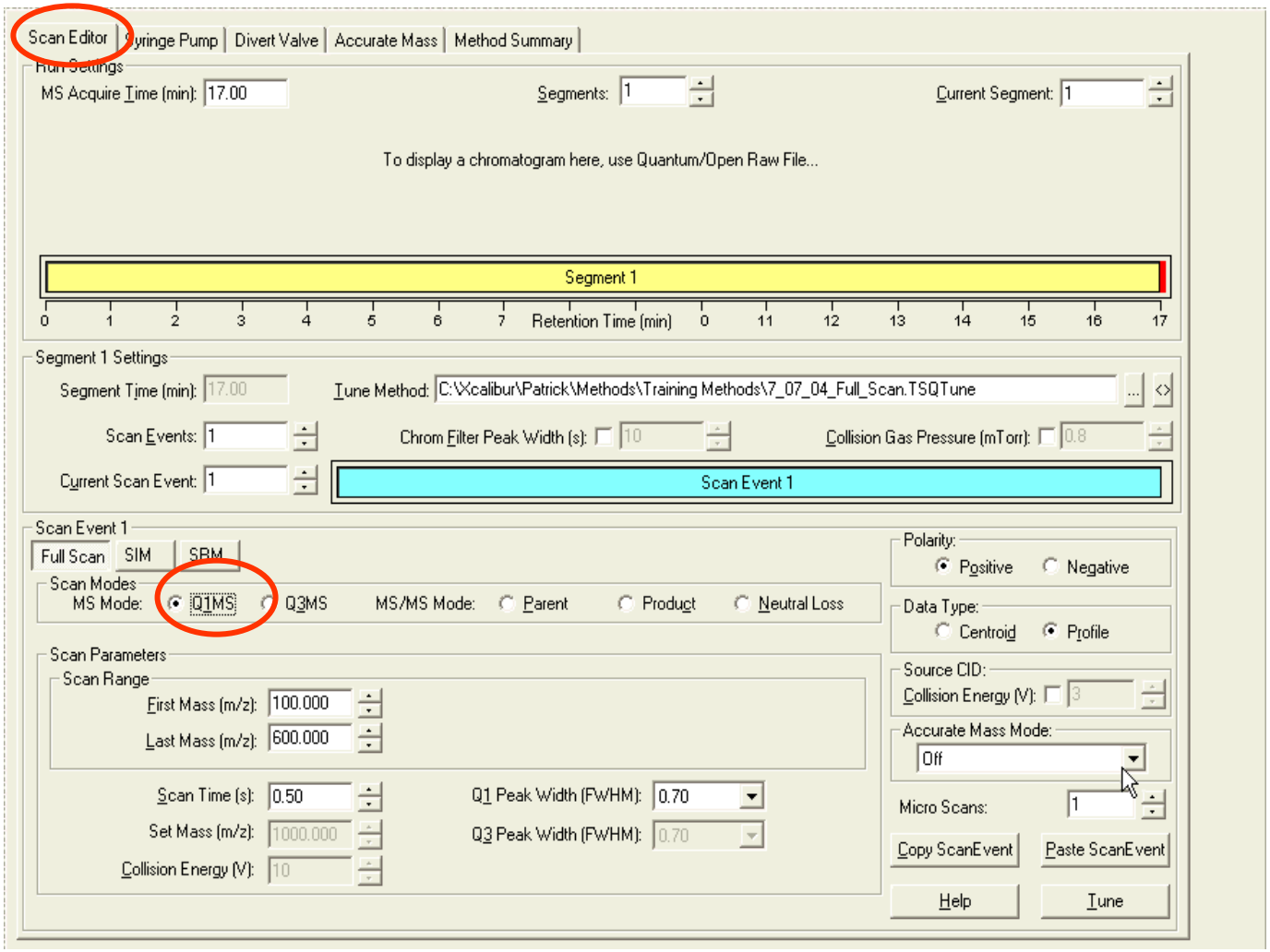


Instrument Setup – Surveyor Autosampler

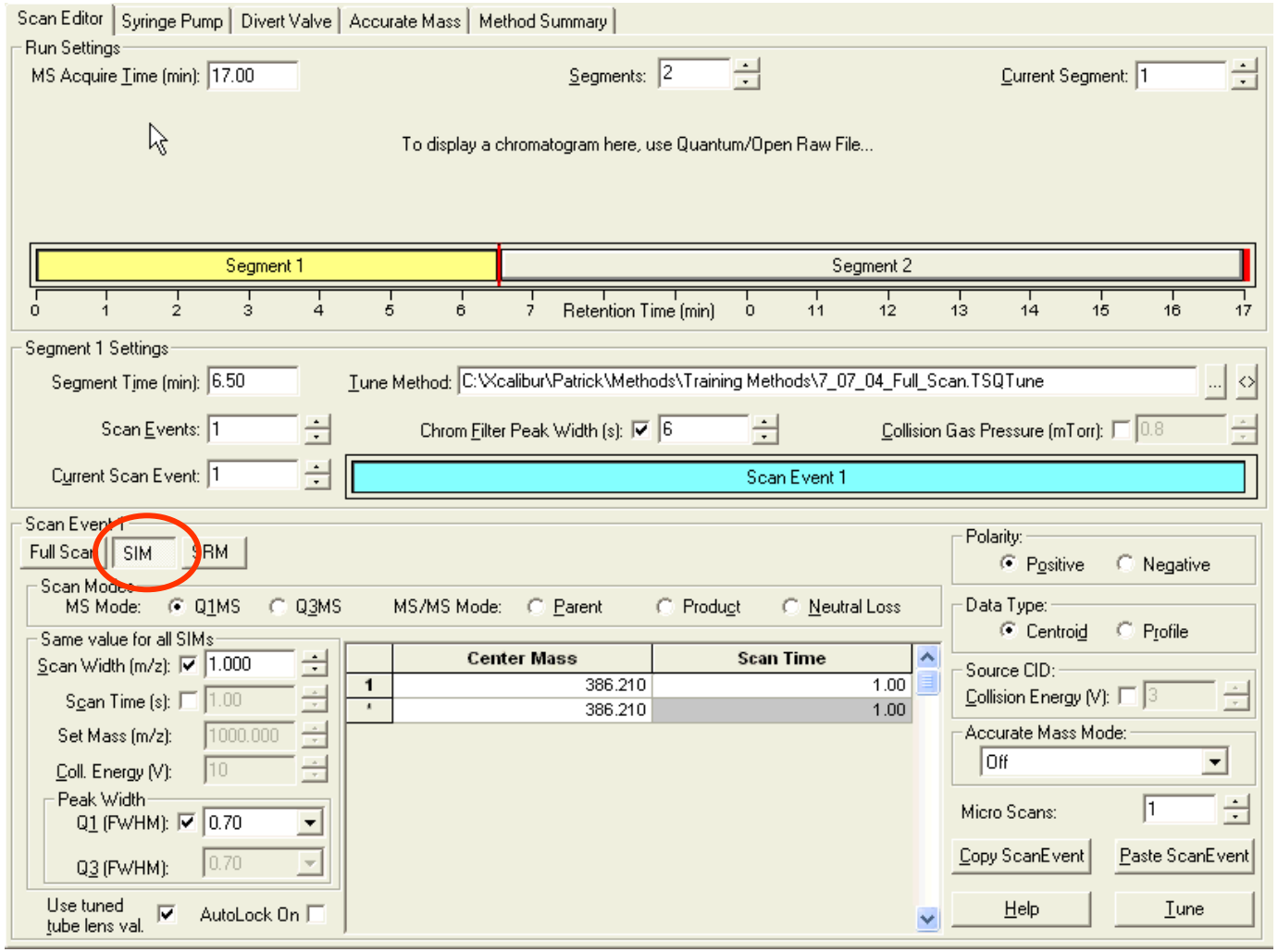


Instrument Setup – Surveyor Autosampler

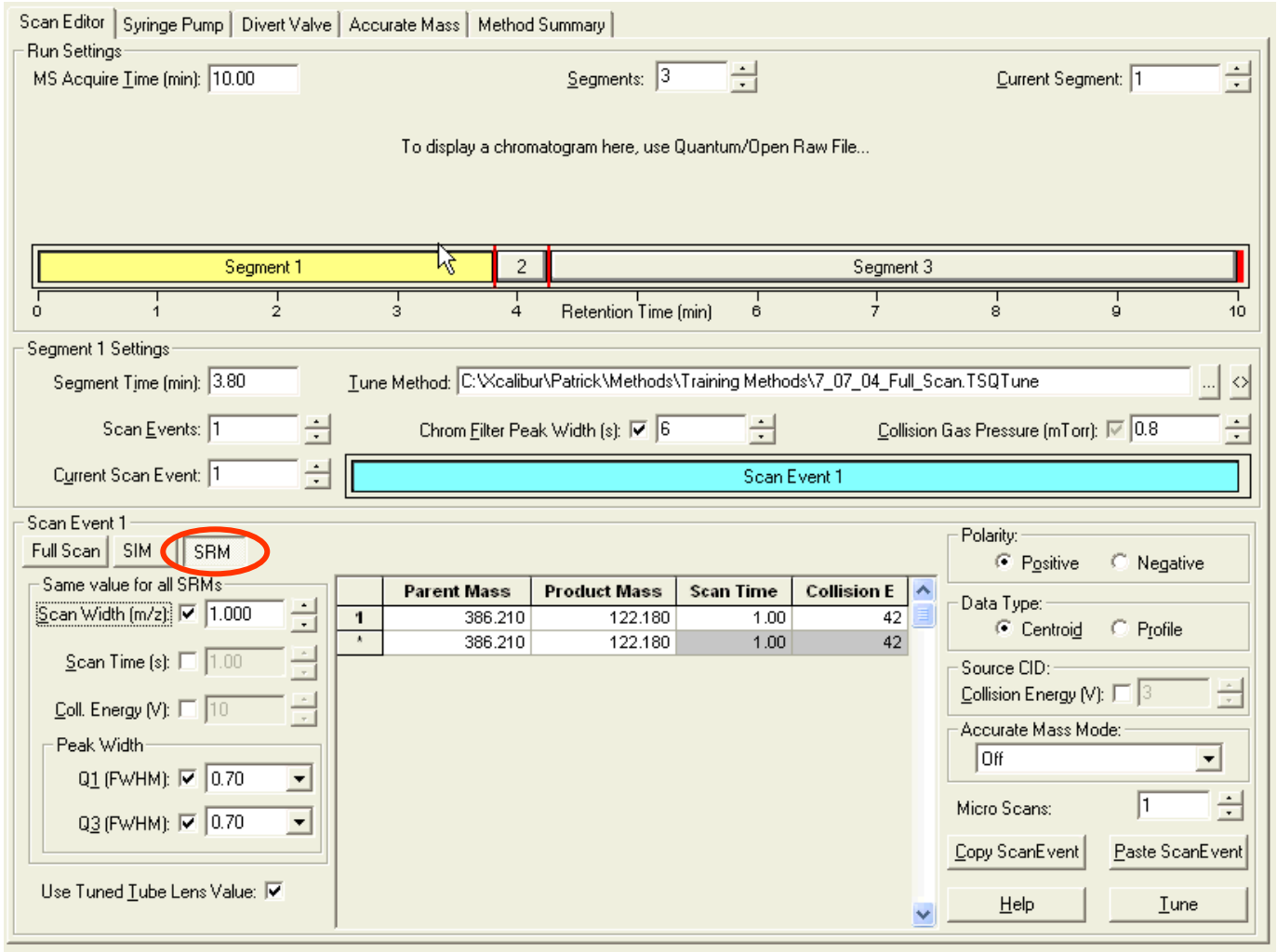
Instrument Setup - Mass Spectrometer Full Scan



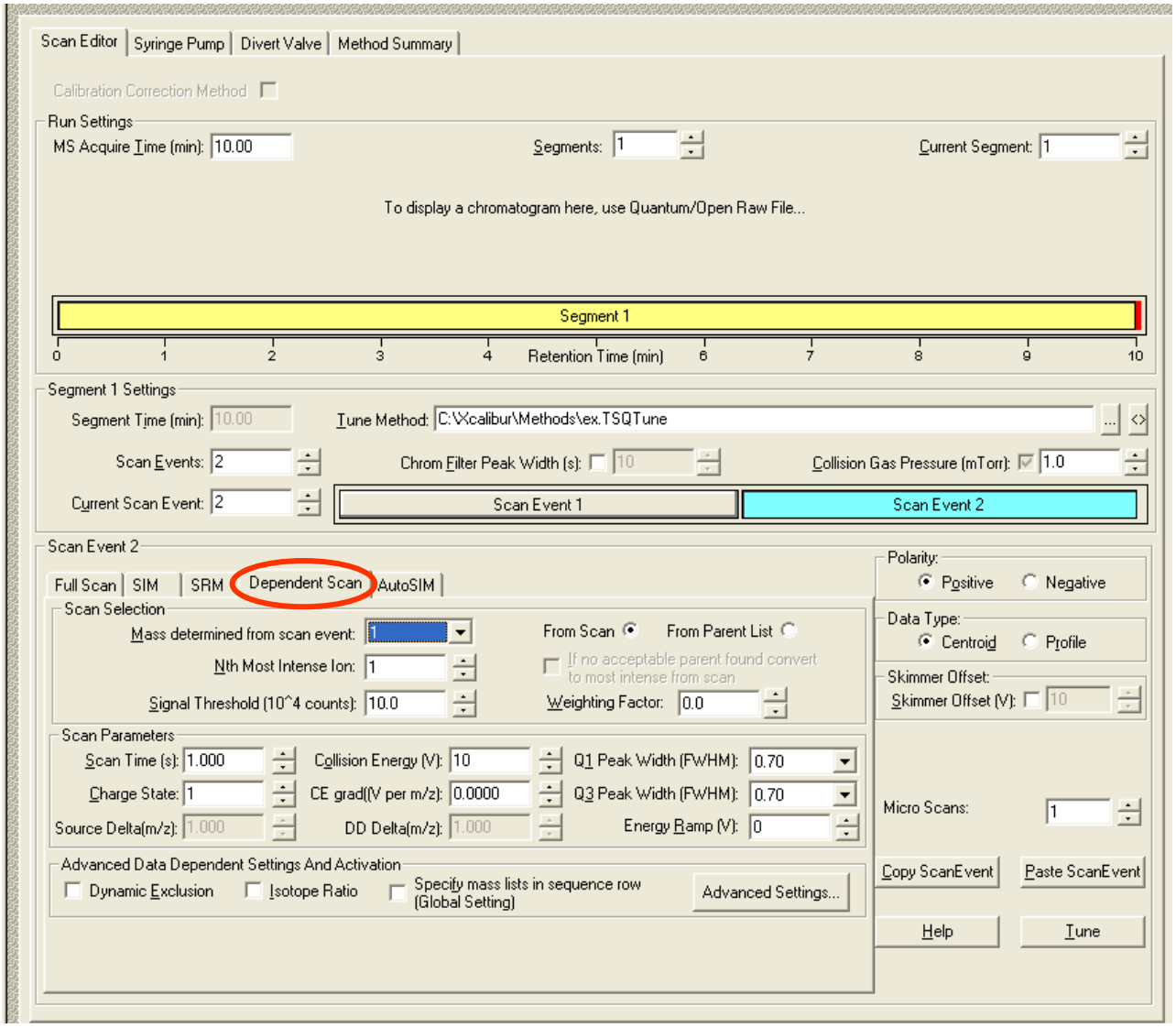
Instrument Setup - Mass Spectrometer - SIM



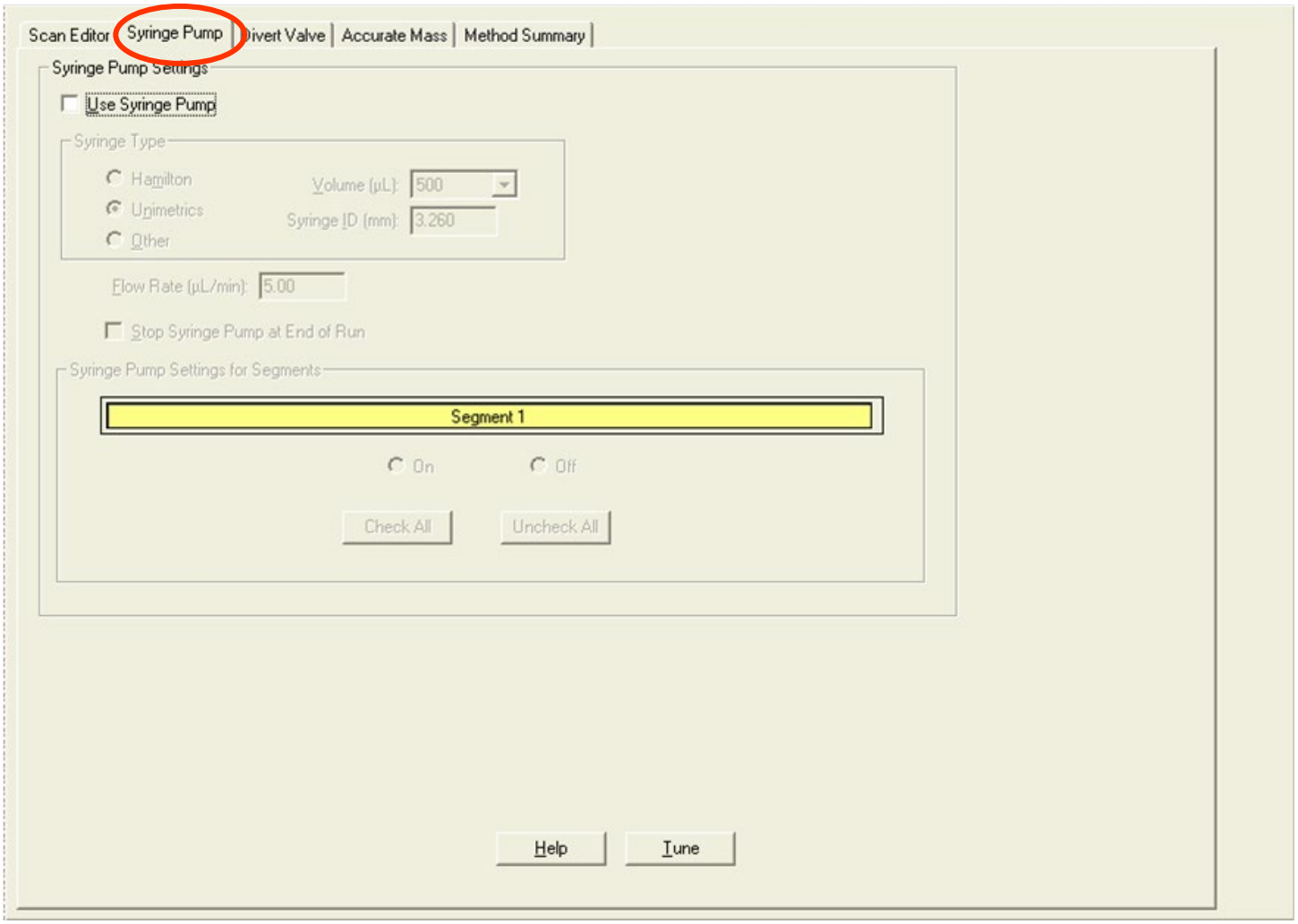
Instrument Setup - Mass Spectrometer - SRM



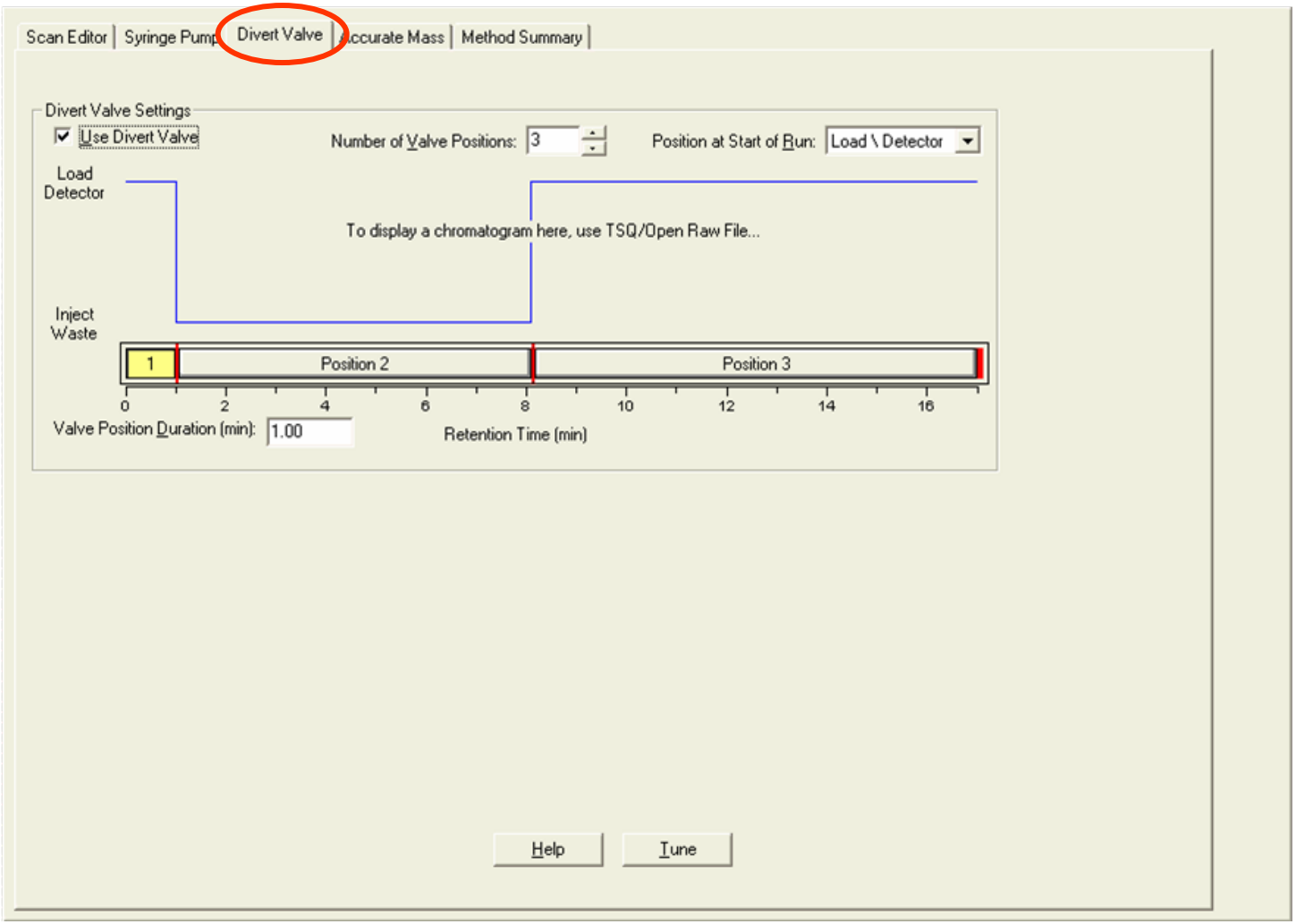
Instrument Setup – MS - Data-Dependent Scan



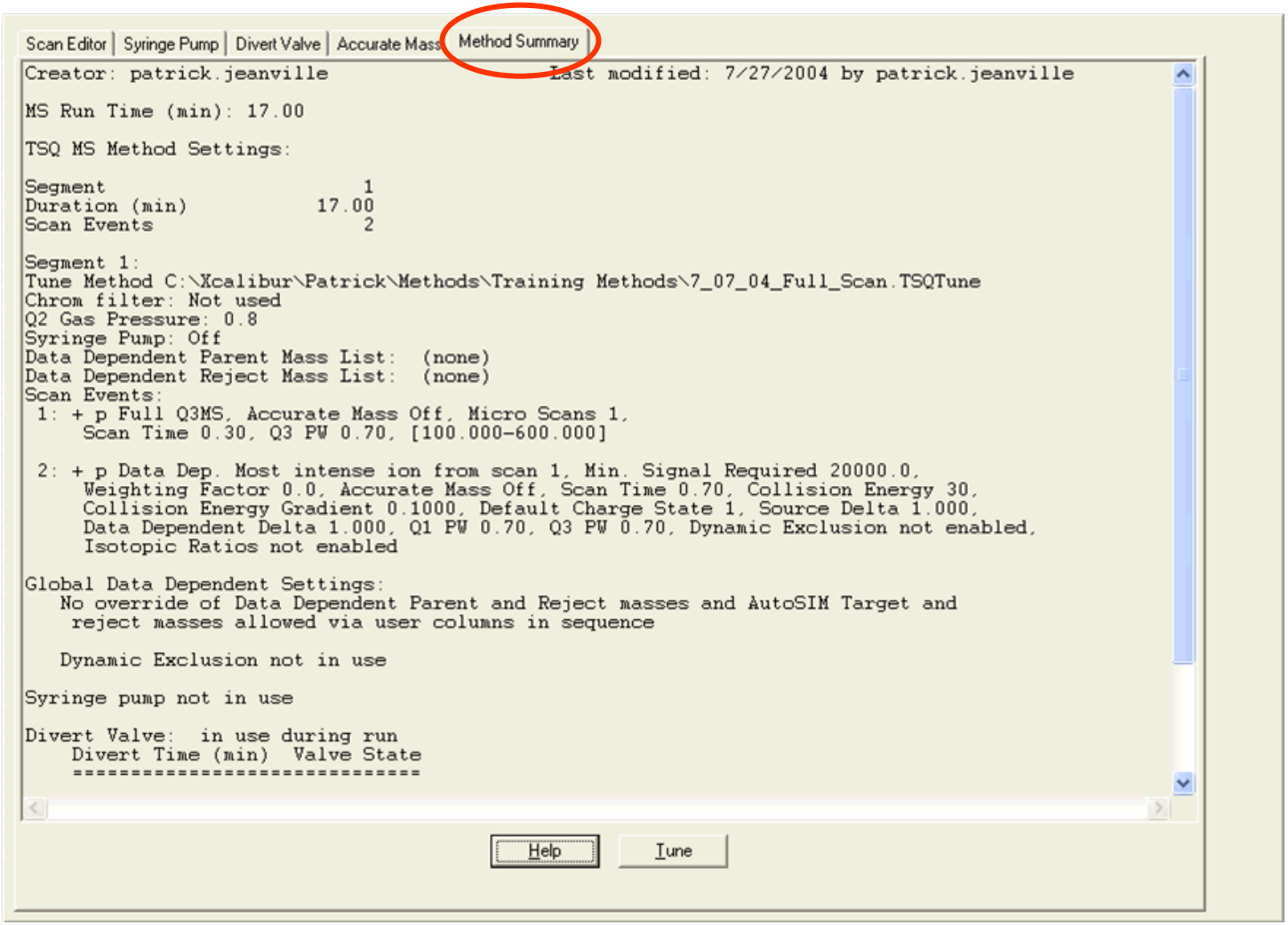
Instrument Setup - Mass Spectrometer



Instrument Setup - Mass Spectrometer



Instrument Setup - Mass Spectrometer



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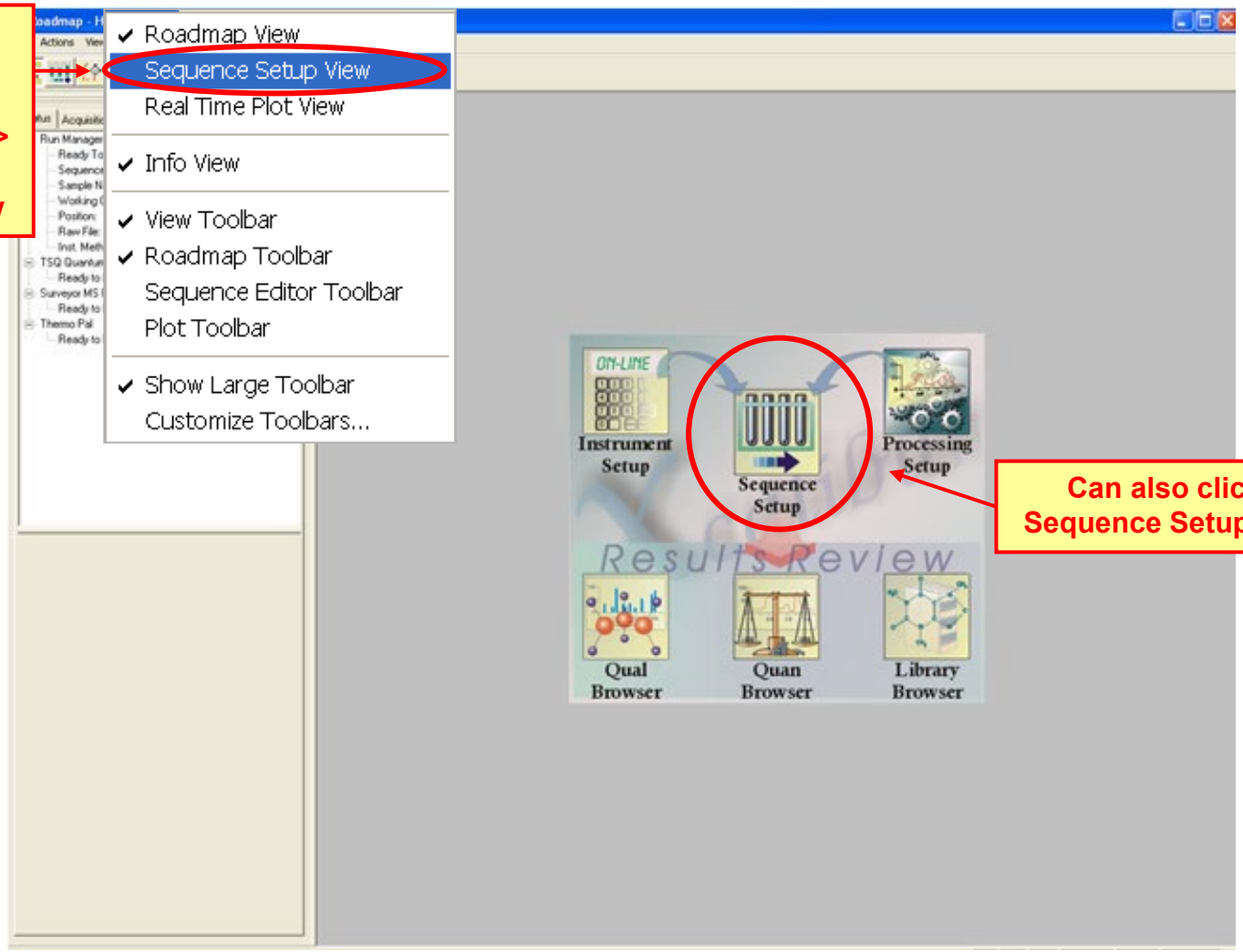
Chapter 9

Setting Up and Running Sequences

Xcalibur Home Page Sequence Setup

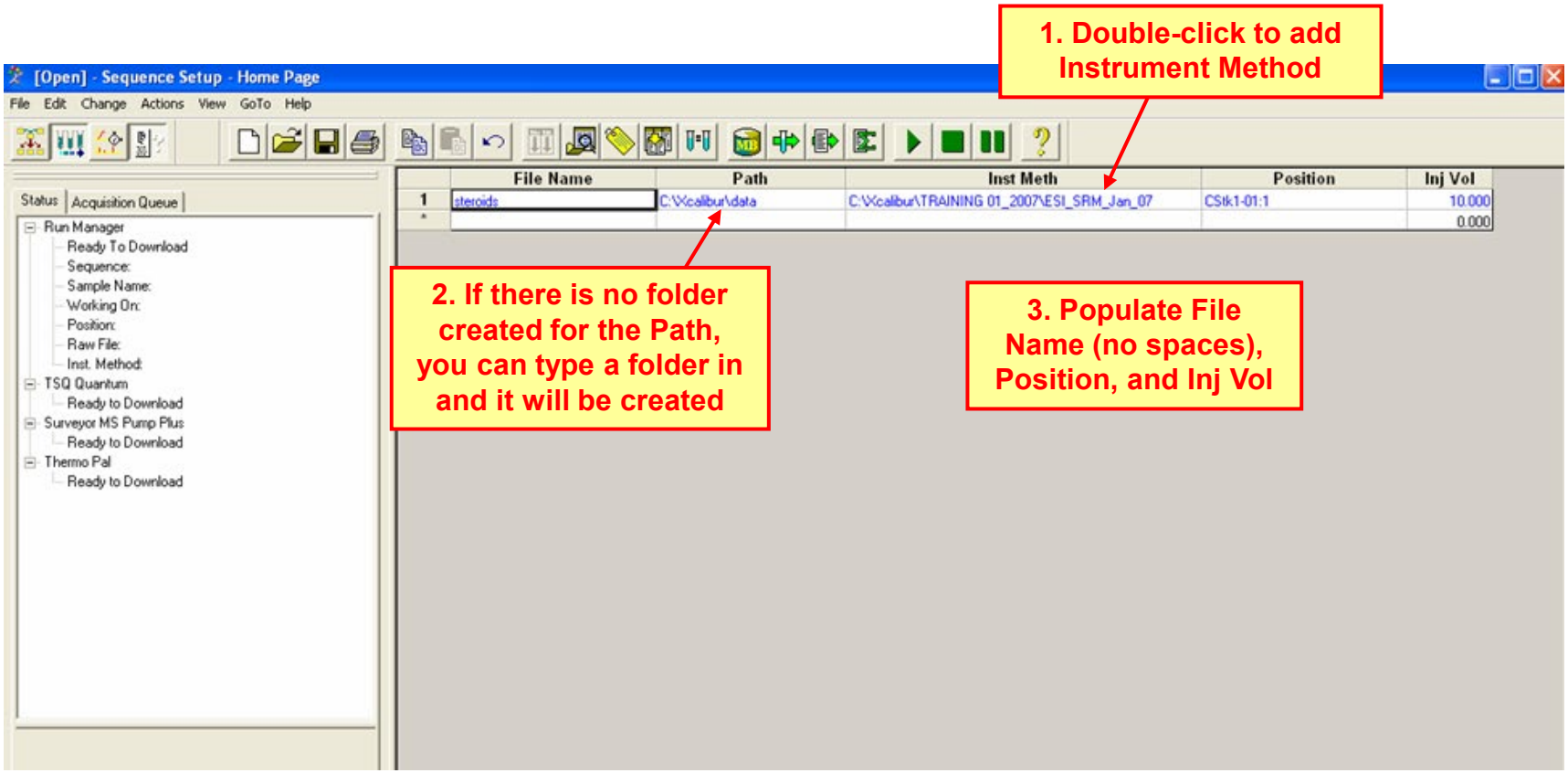
To open Sequence Setup, can click View > Sequence Setup View



Can also click on Sequence Setup button

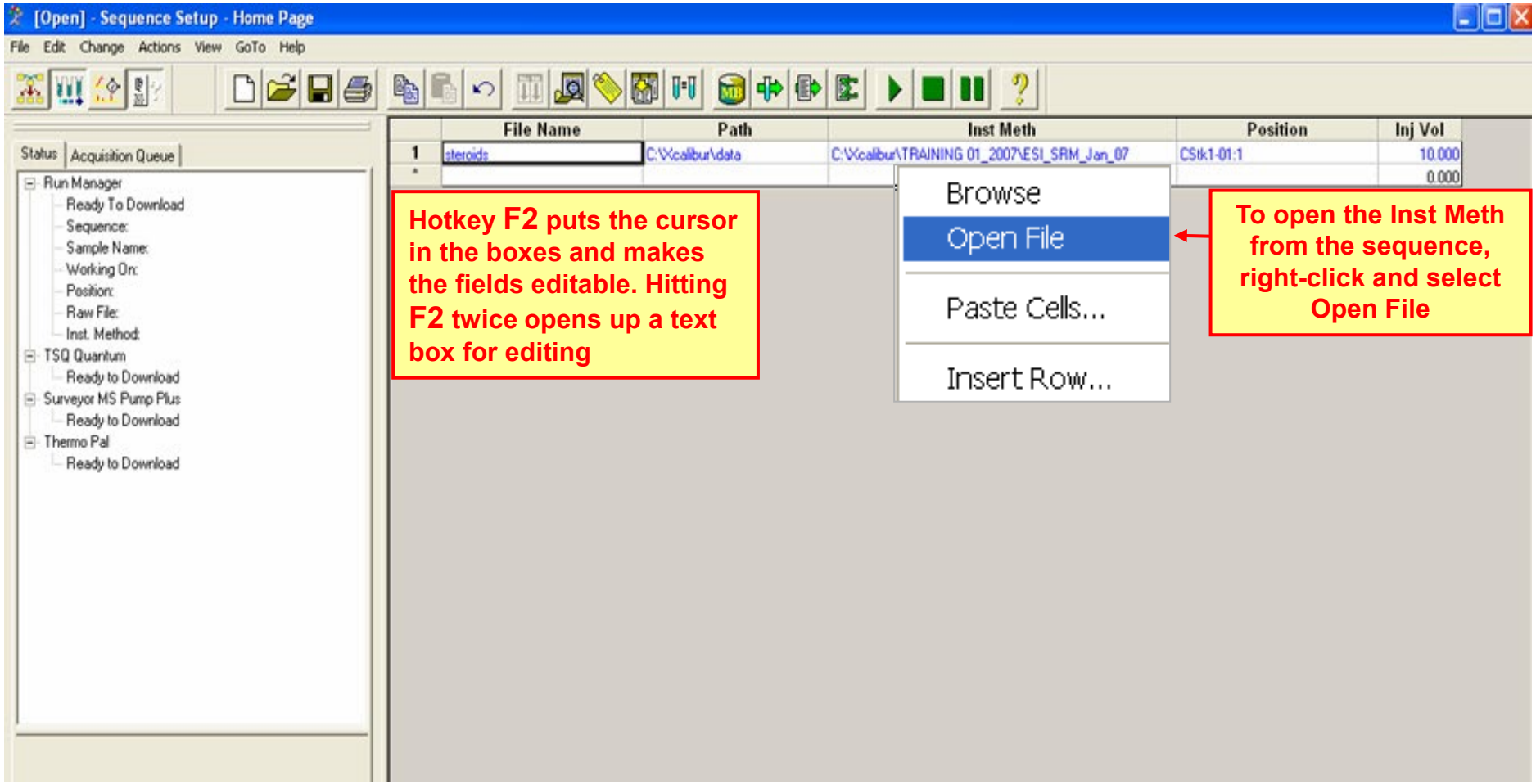
Creating a Sequence

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



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

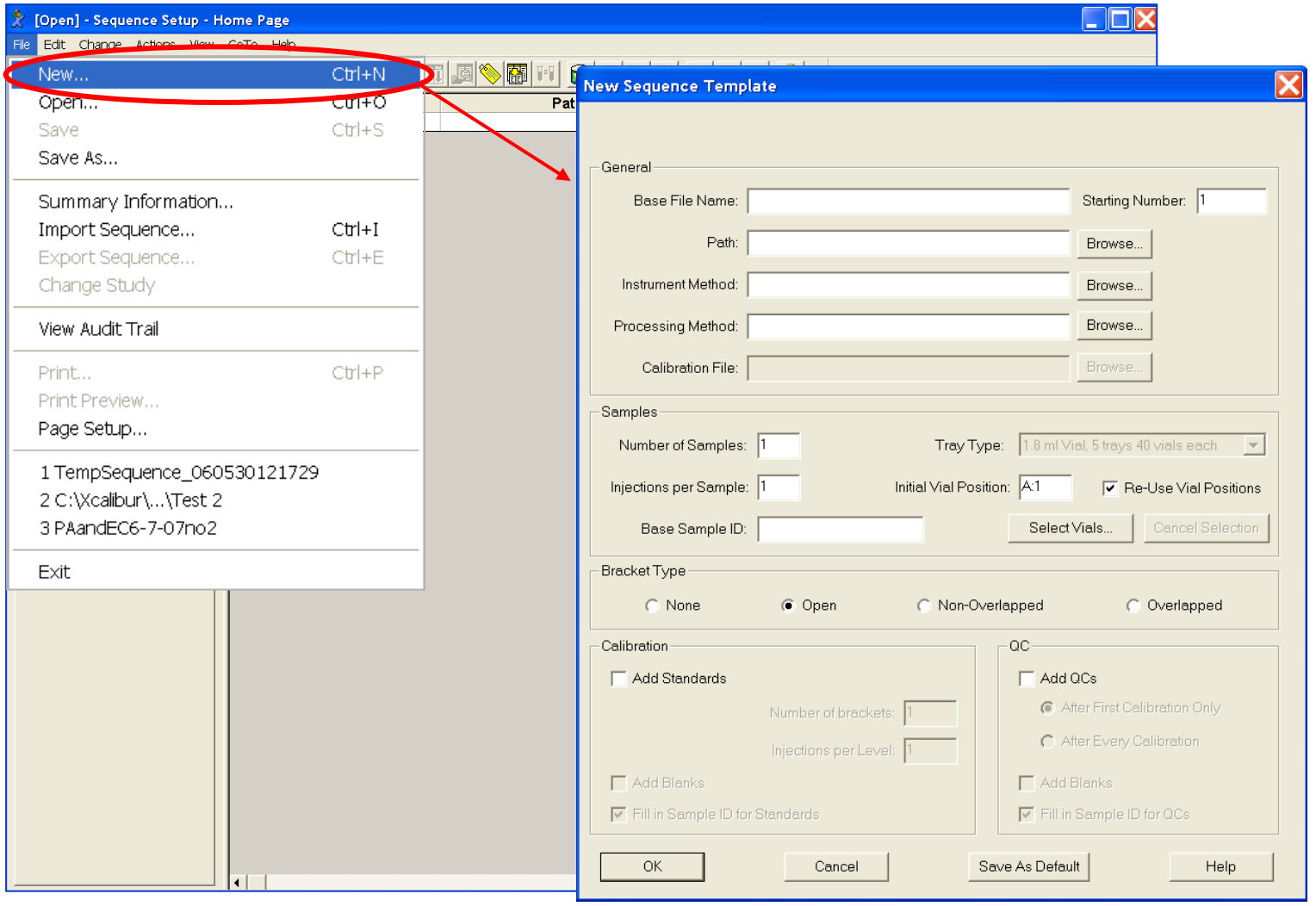
Creating a Sequence



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



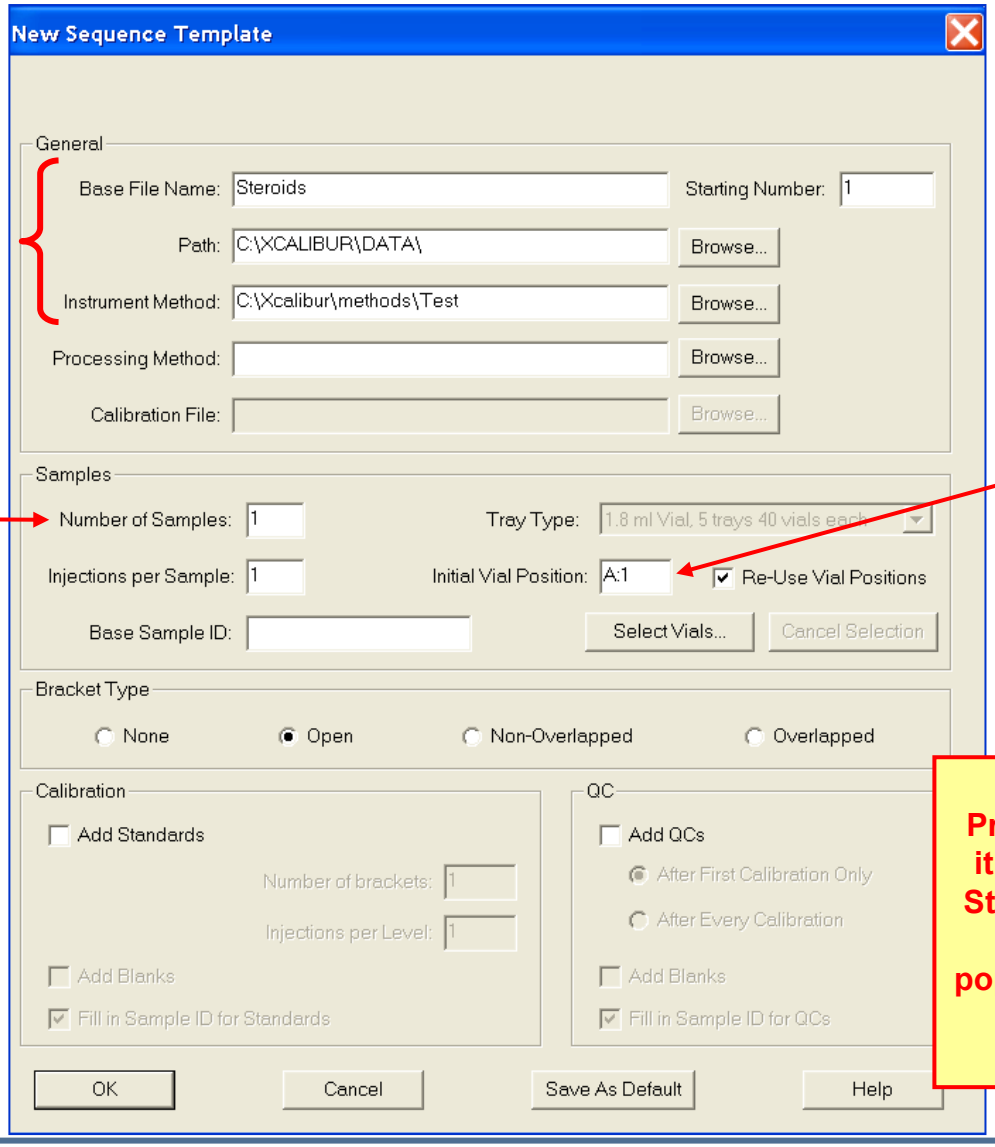
New Sequence Template

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

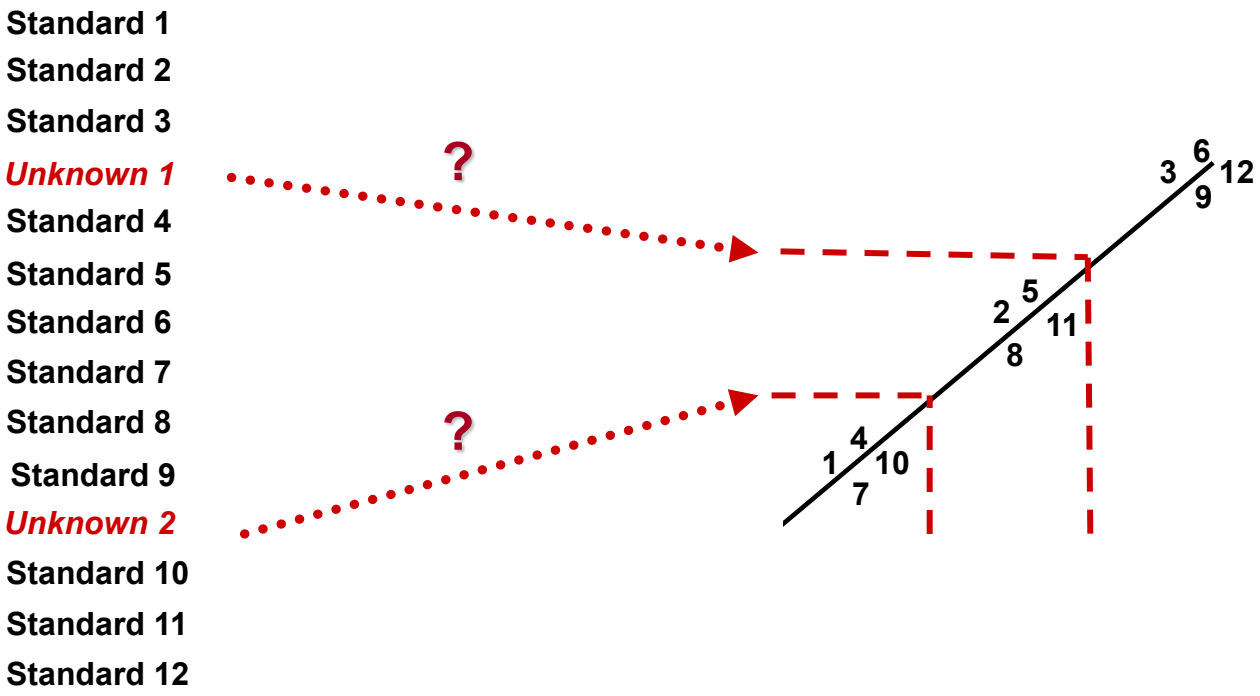
2. Enter the number of unknown samples

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.



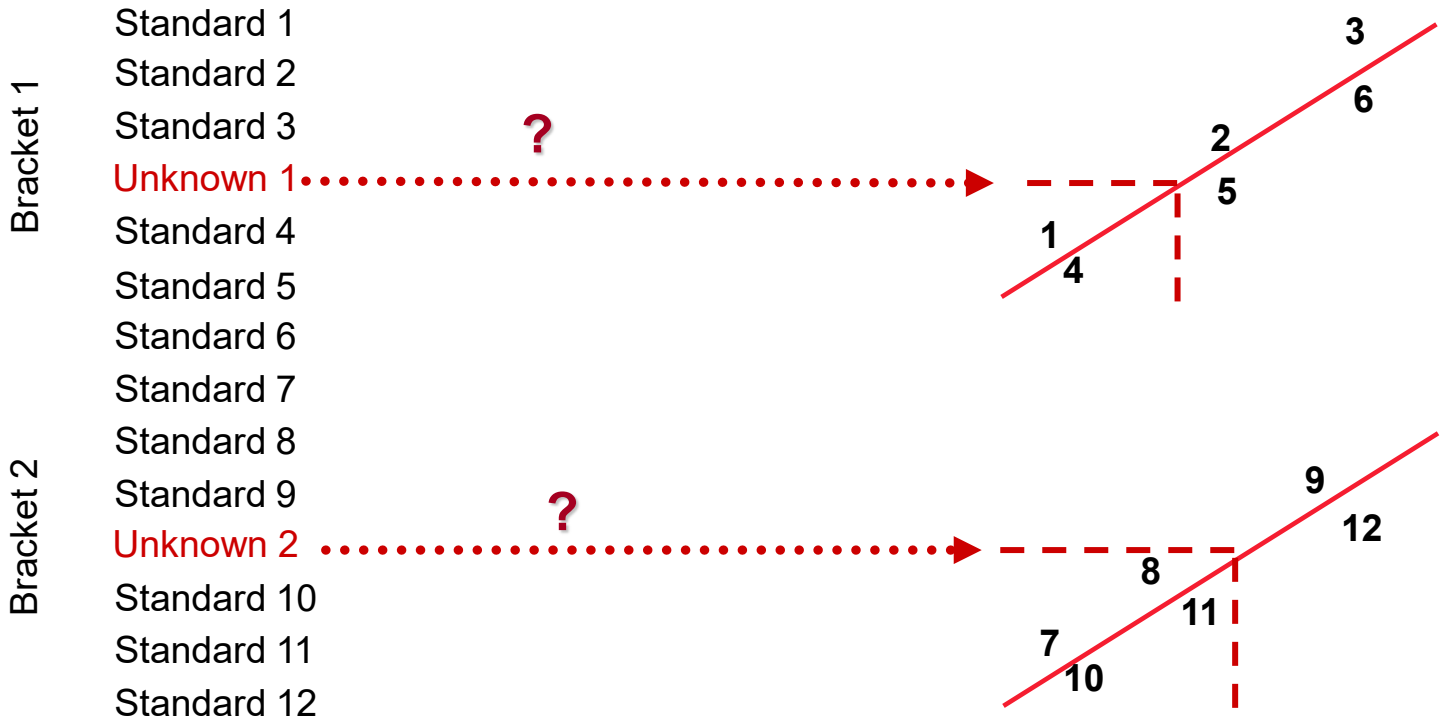
Open Bracket



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

All standards are equally involved in interpolation

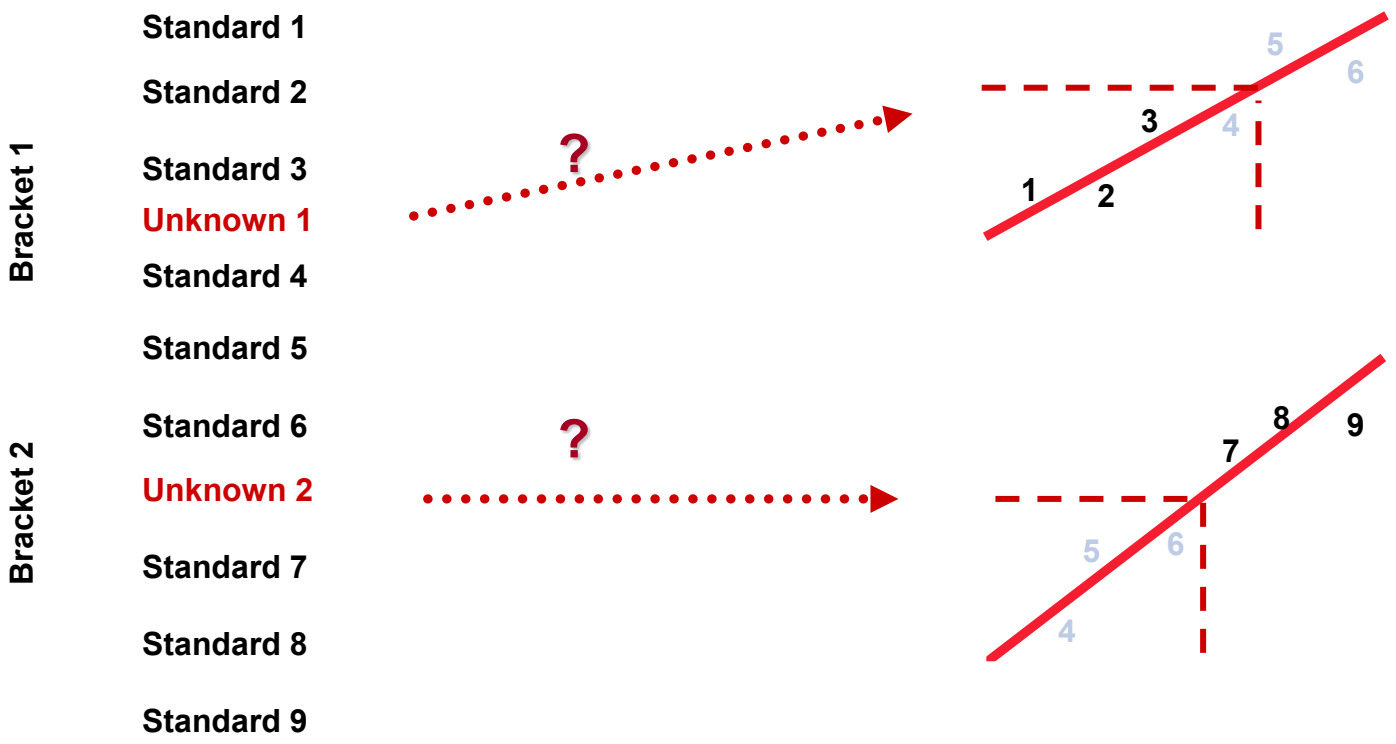
Non-Overlapping Bracket



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



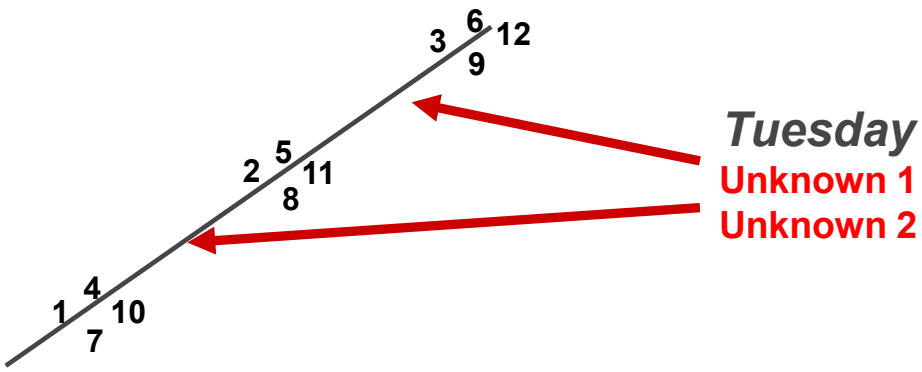
Separate calibration curves for each unknown (**1** or **2**) (or group of unknowns)

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)

Bracket None

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

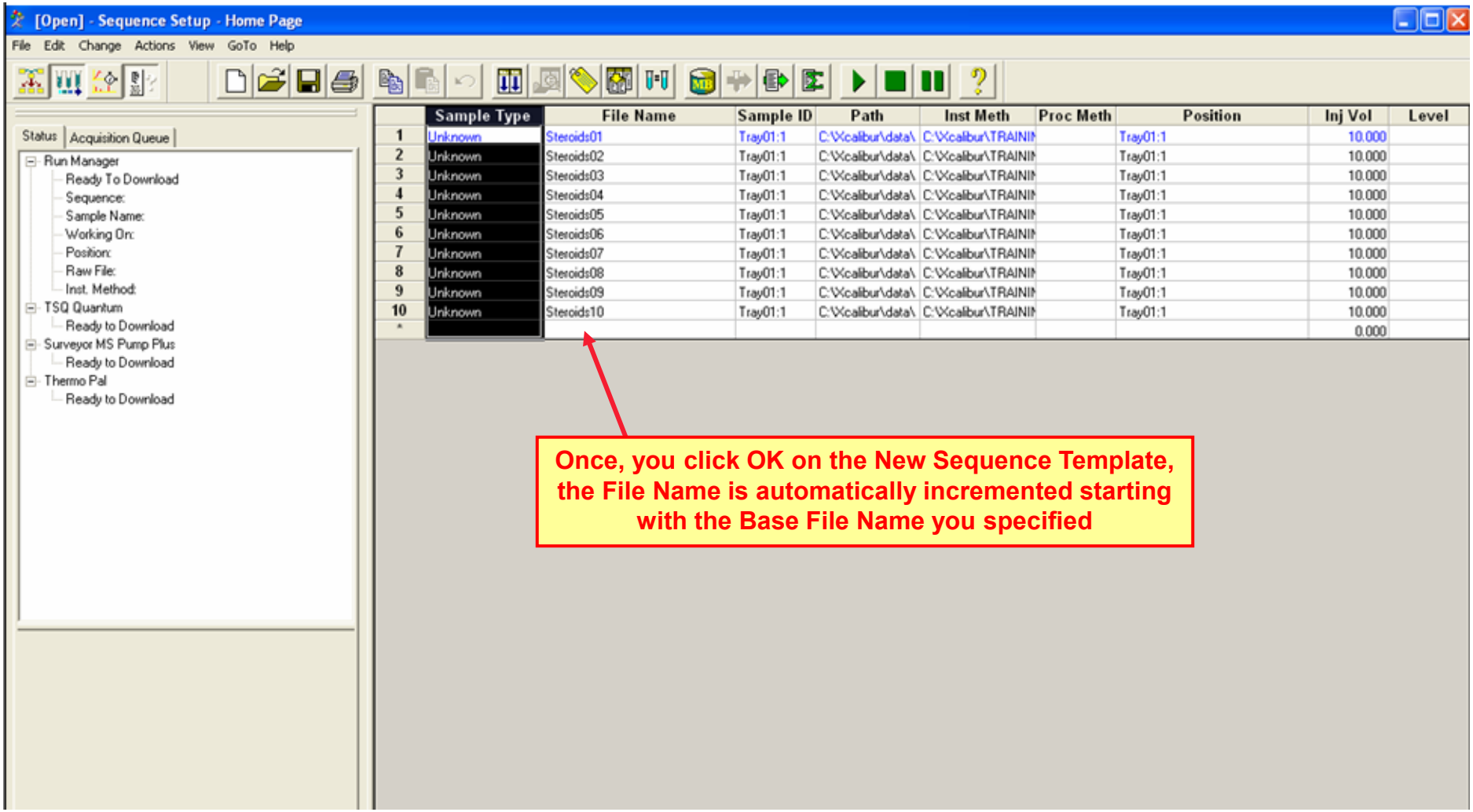


Two types of designated standards:

Standard Clear (kept from a previous experimental batch)

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

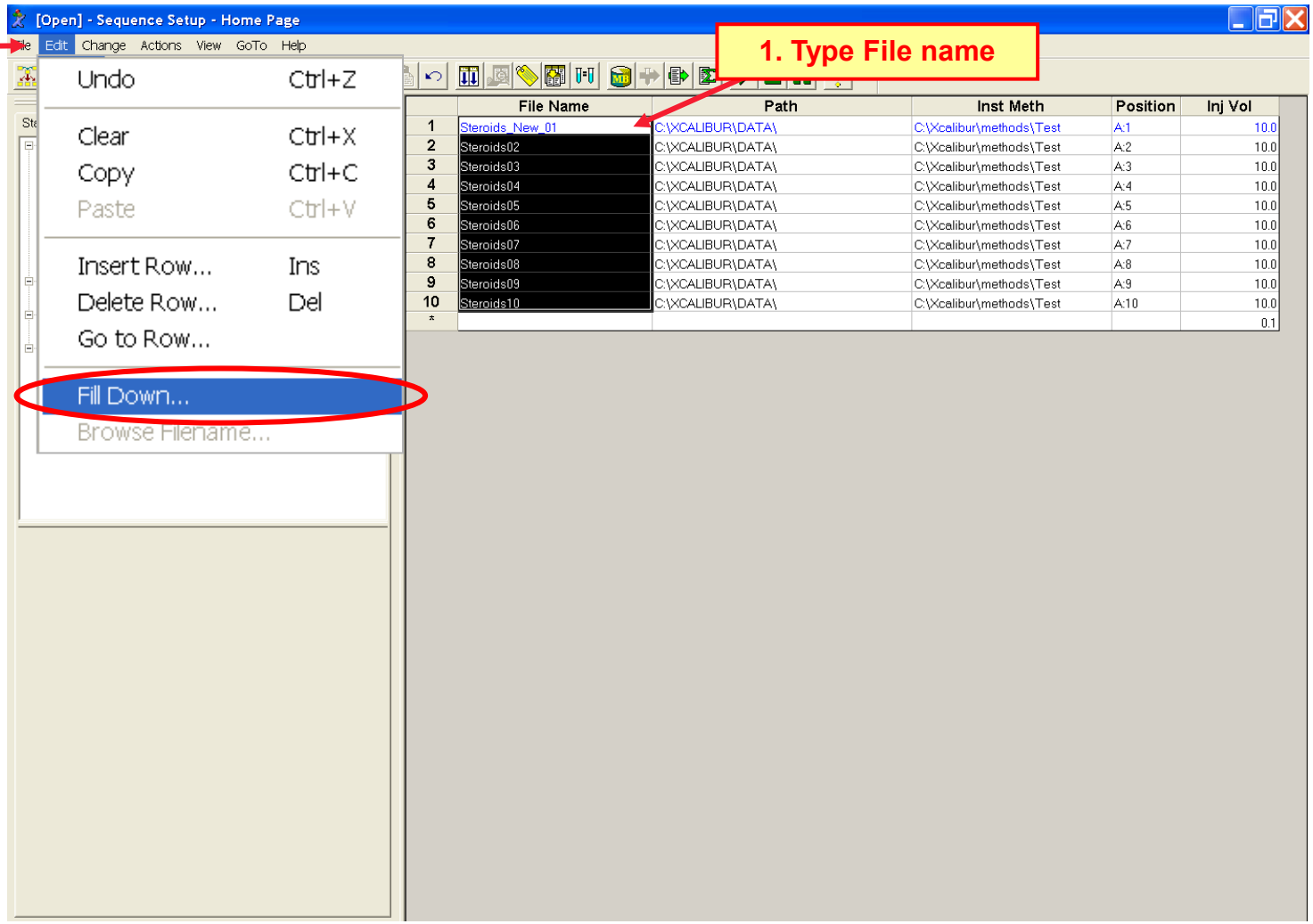
New Sequence Template



New Sequence Template

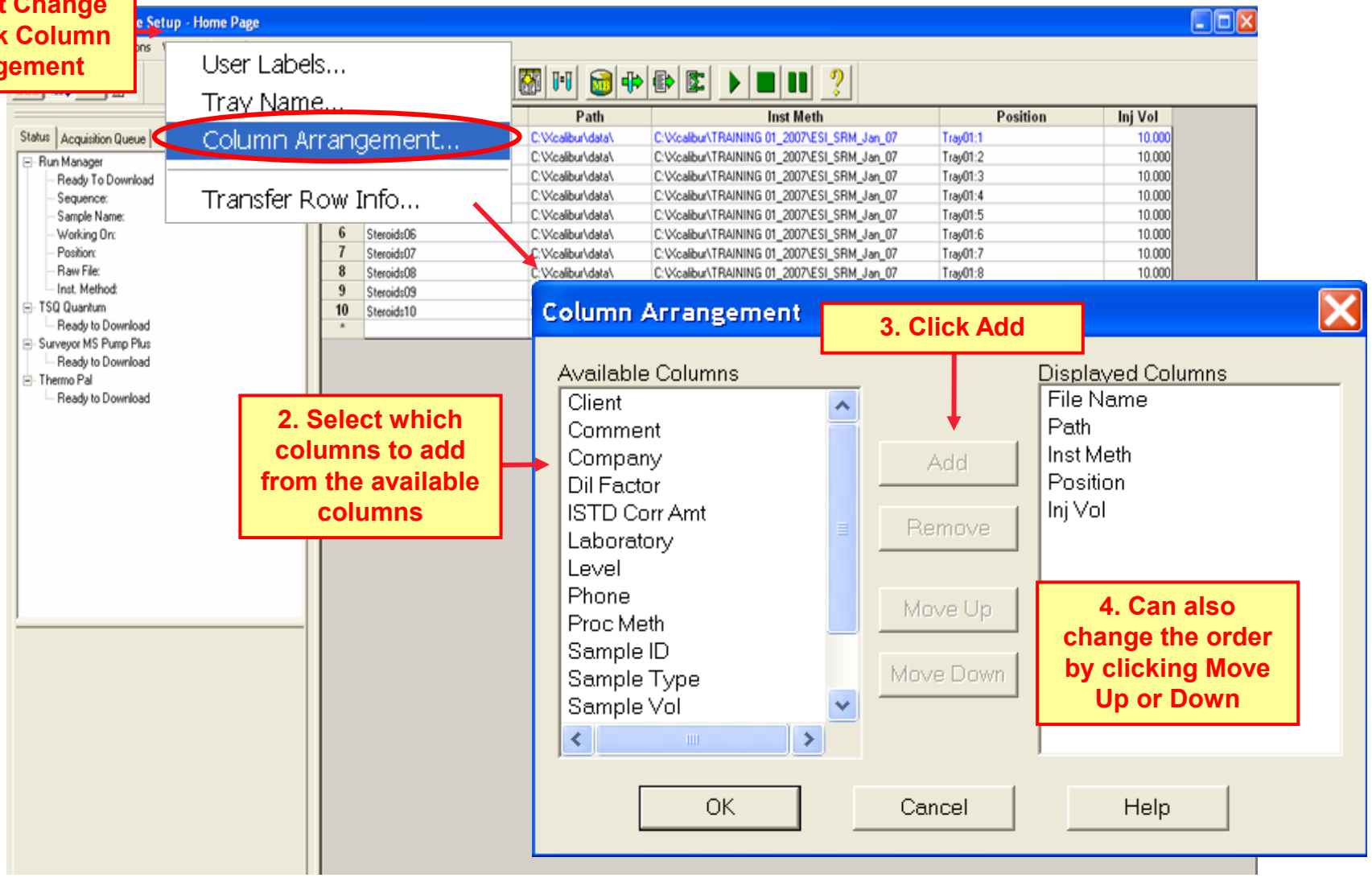
If you want to type a new File Name:

2. Select Edit and click Fill Down



Changing the Sequence Column Arrangement

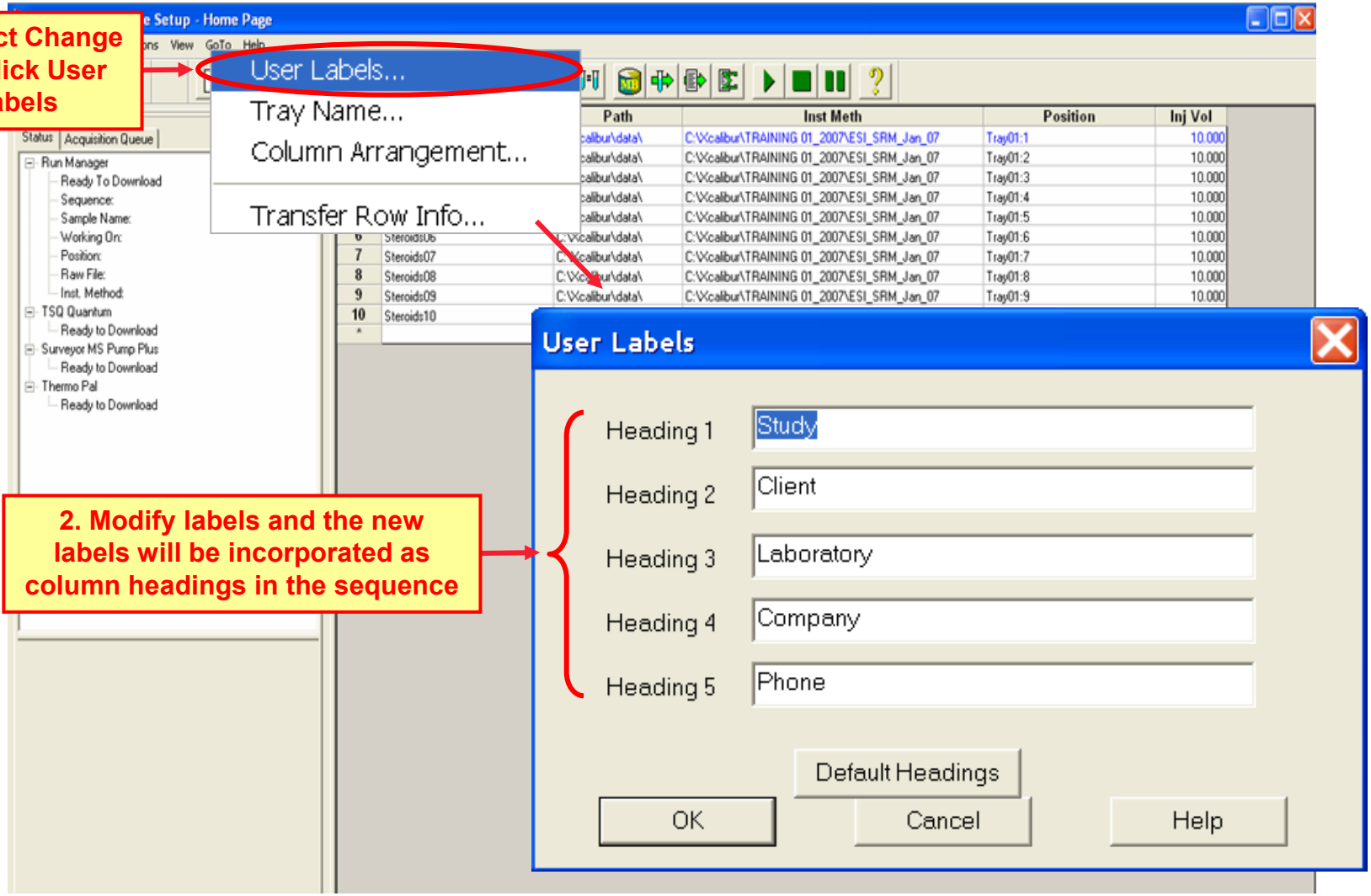
1. Select Change and click Column Arrangement



2. Select which columns to add from the available columns

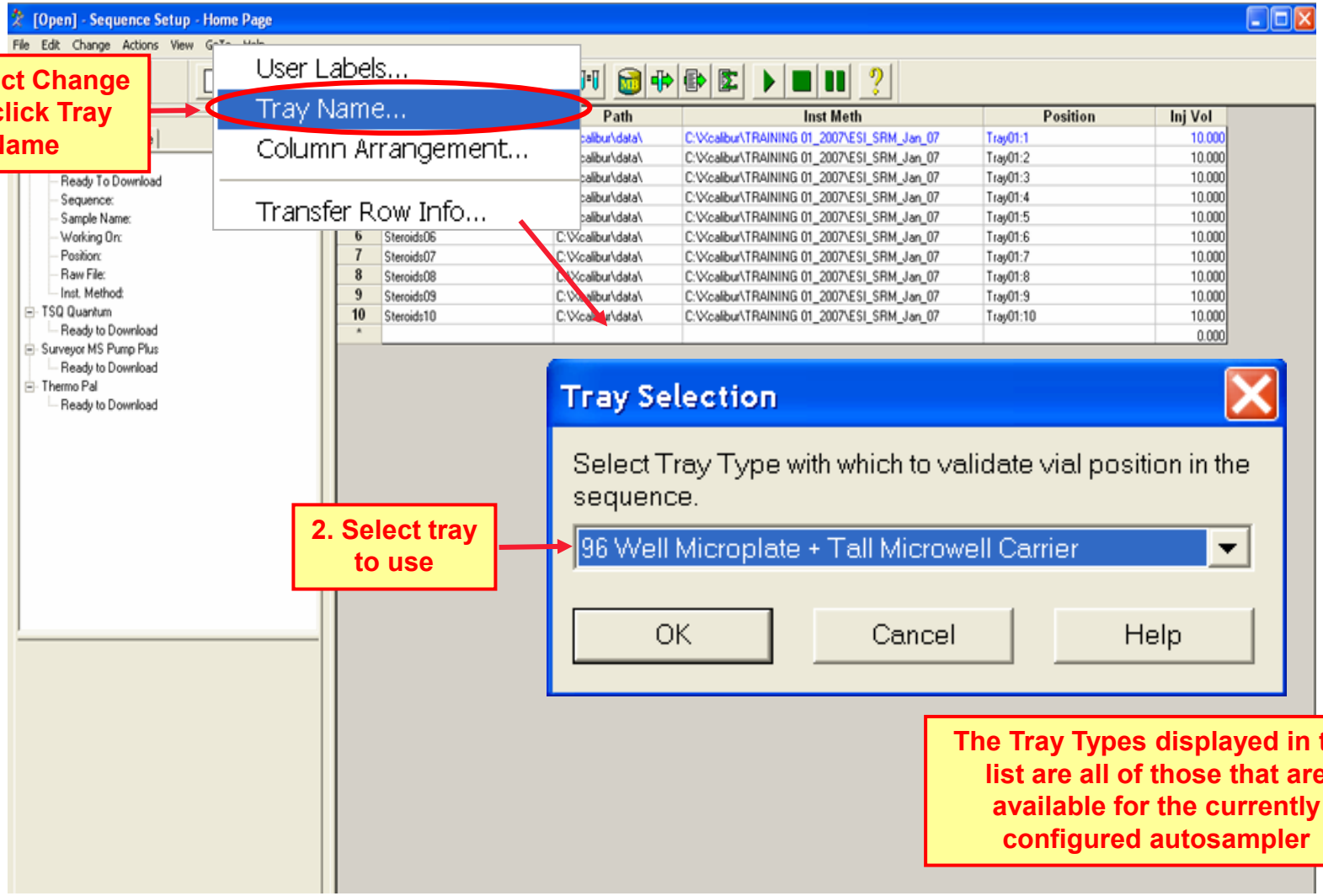
Changing the User Labels

1. Select Change and click User Labels



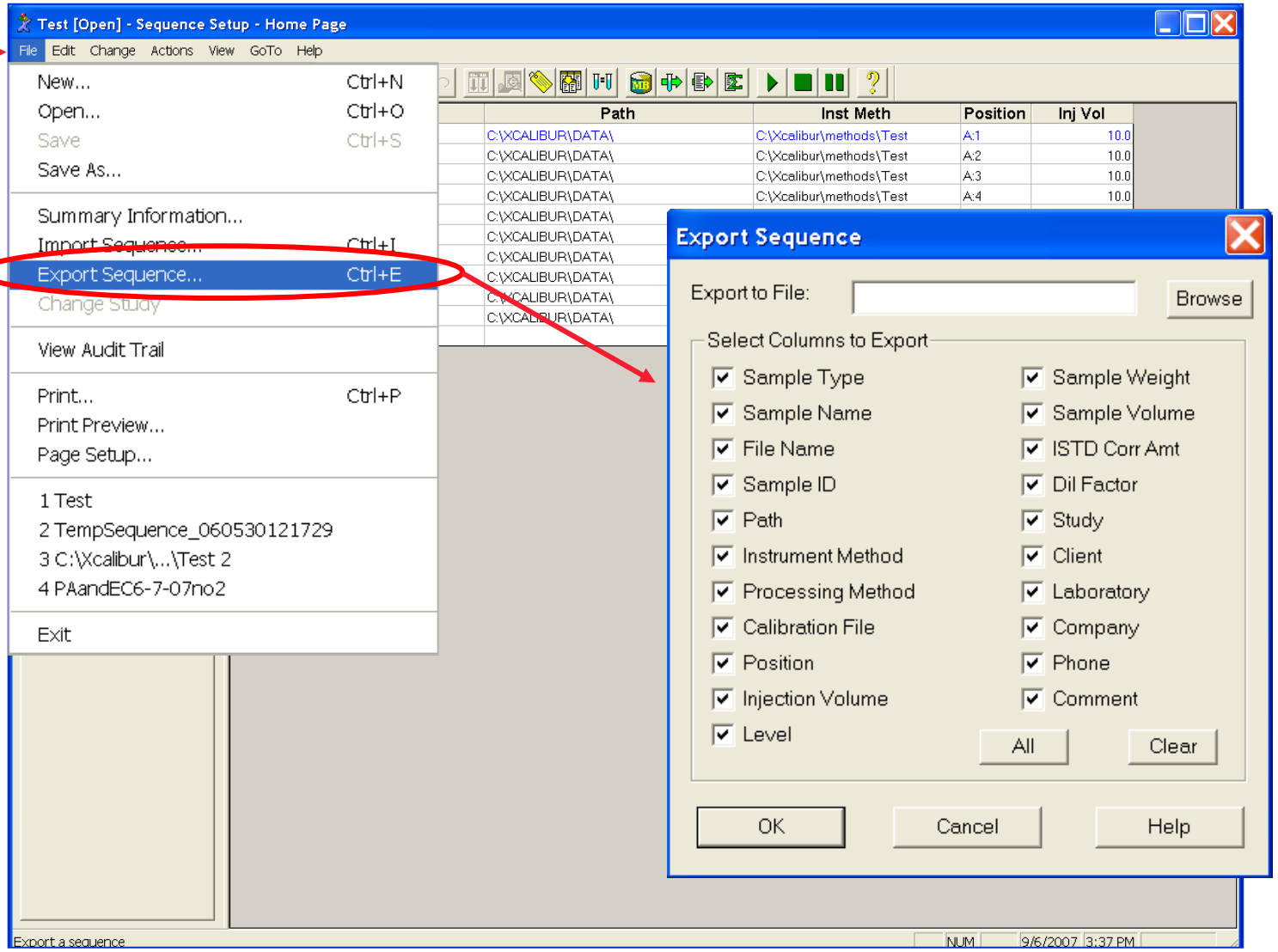
2. Modify labels and the new labels will be incorporated as column headings in the sequence

Changing the Tray Name

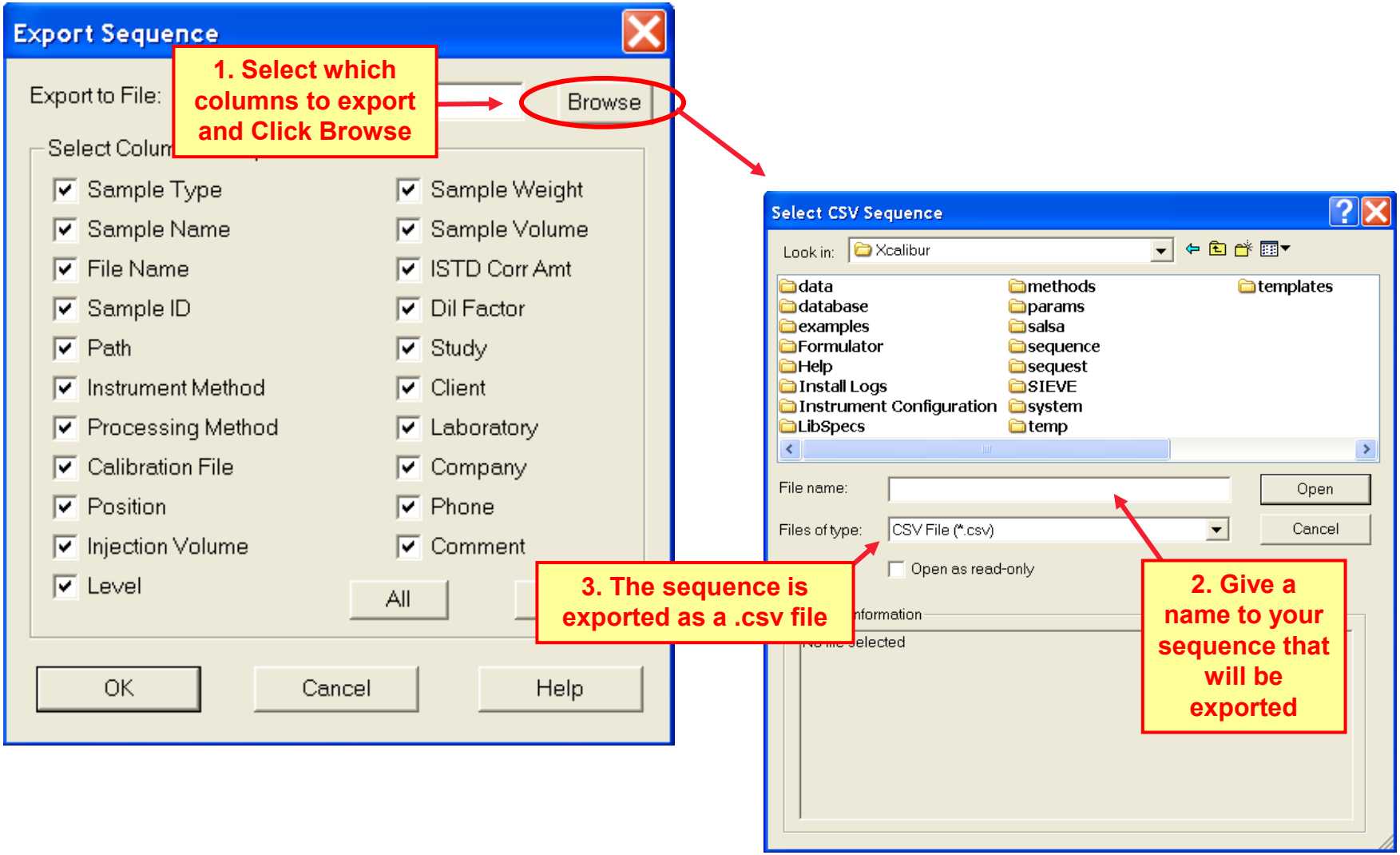


Exporting a Sequence to Excel

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

Microsoft Excel - Test

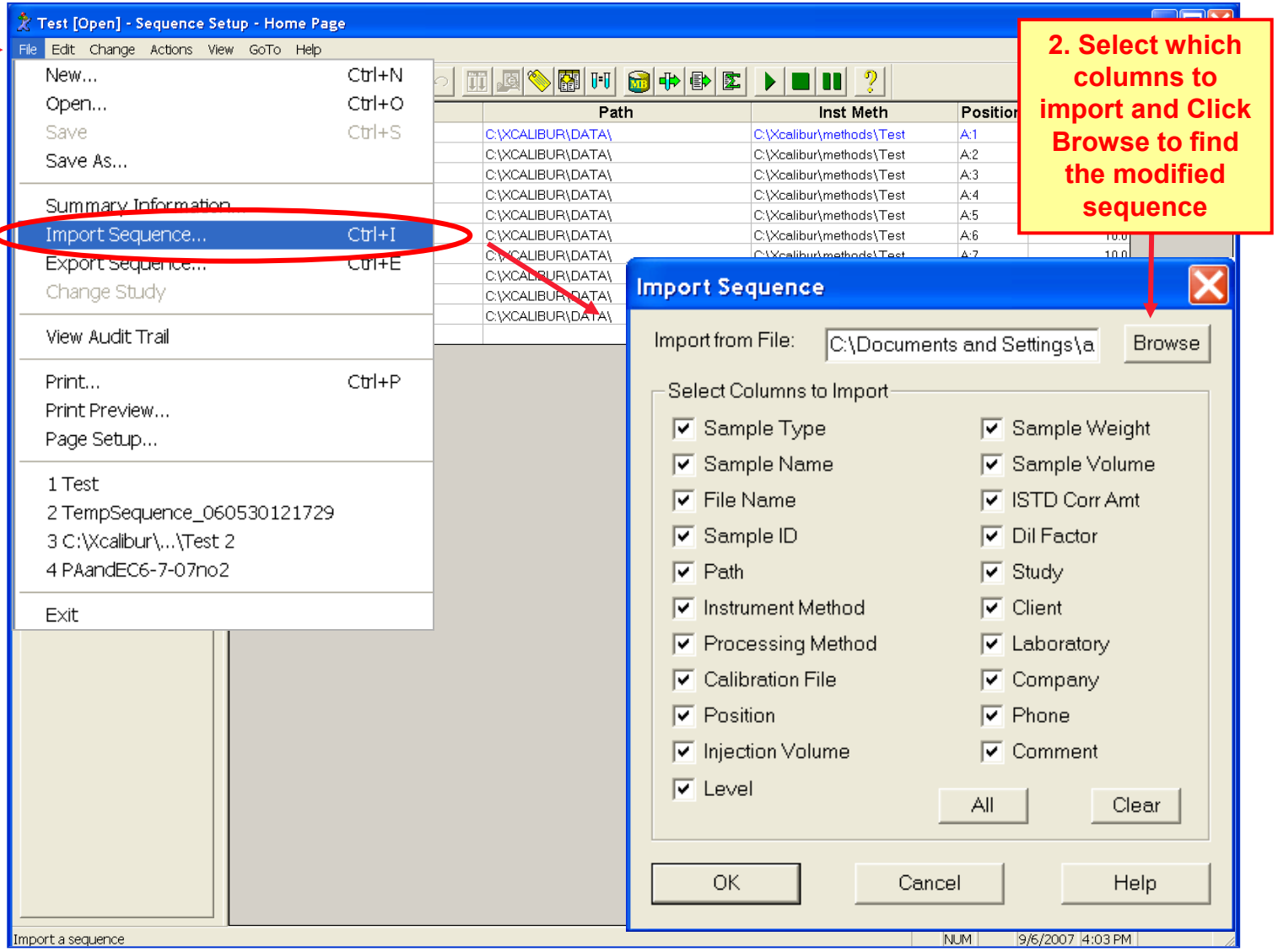
File Edit View Insert Format

A1 Bracket Type=4

	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

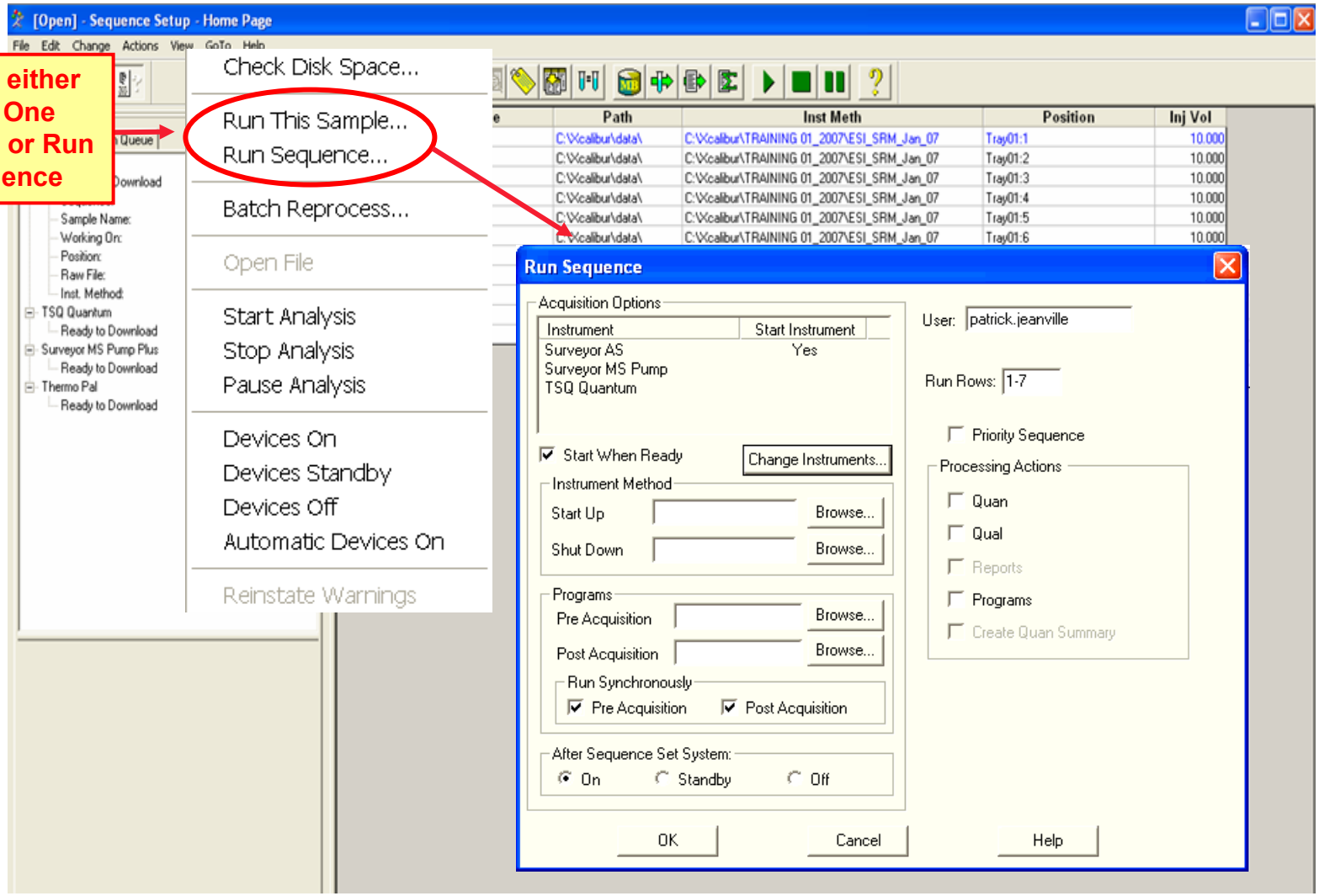
1. Select File and click Import Sequence



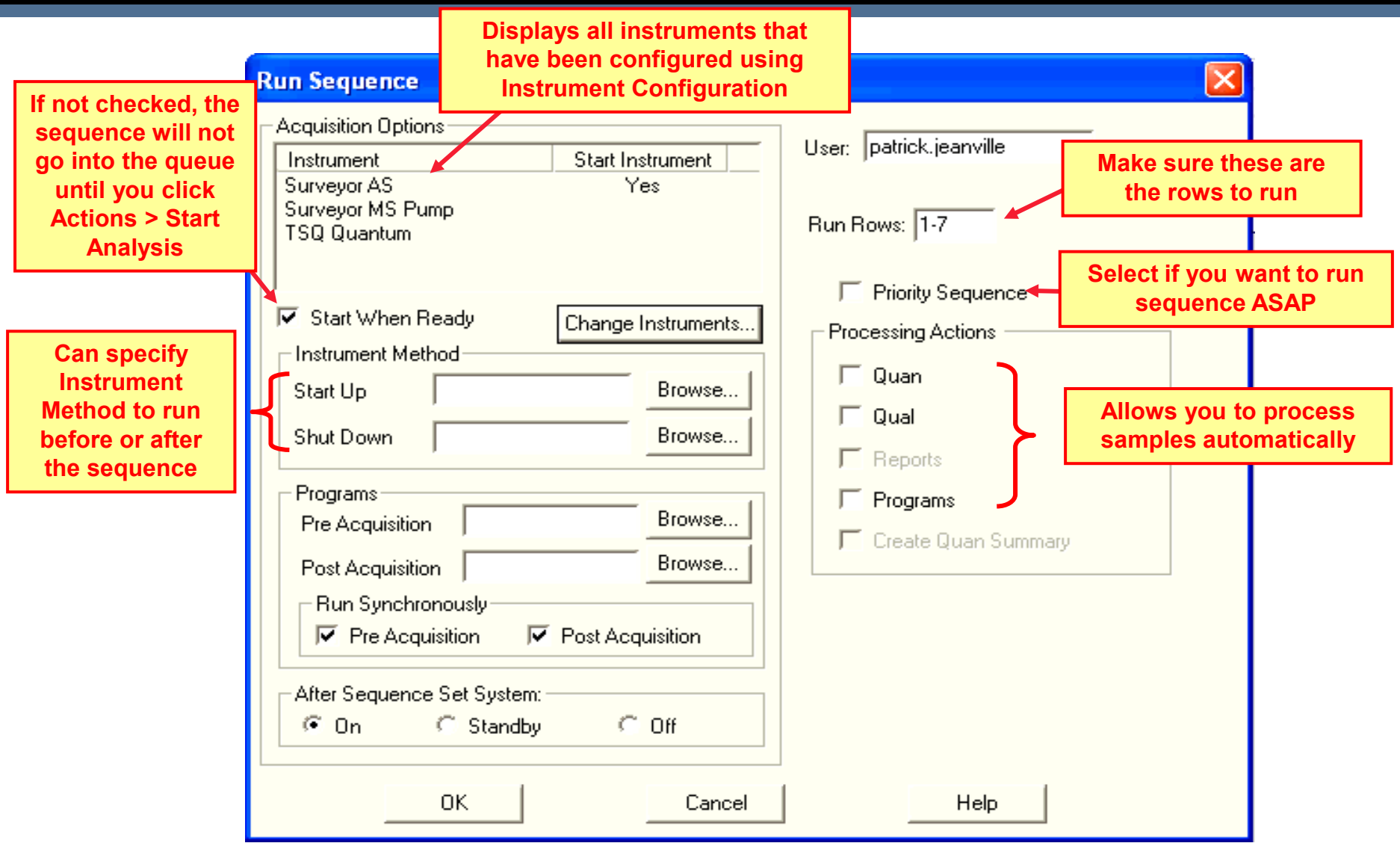
2. Select which columns to import and Click Browse to find the modified sequence

Running the Sequence

1. Can either Run One Sample or Run Sequence

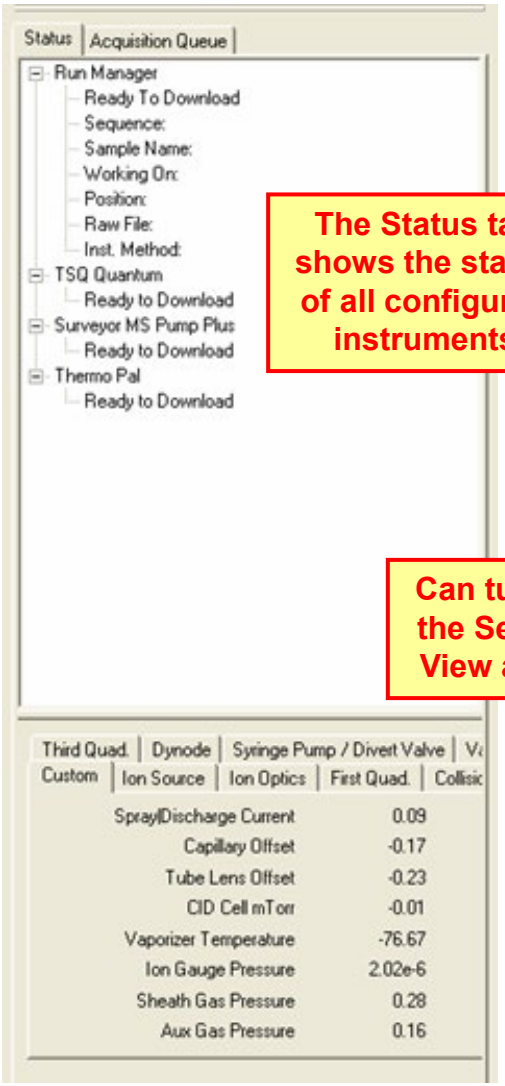


Running the Sequence



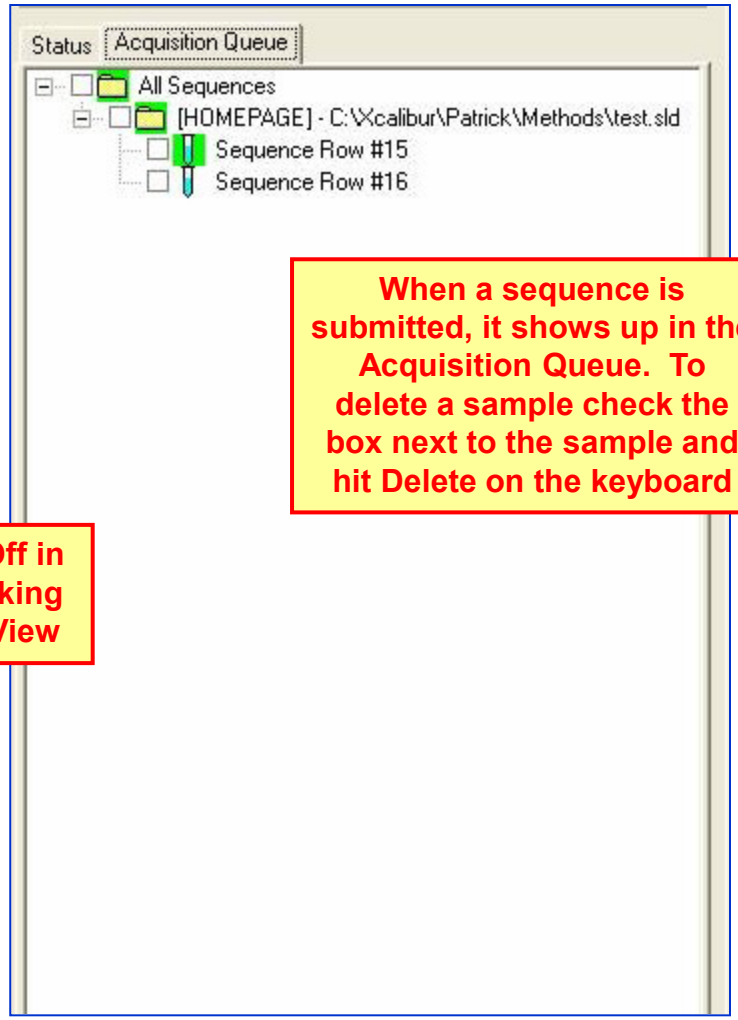
The Info View

Status



The Status tab shows the status of all configured instruments

Acquisition Queue-Sequence Progress

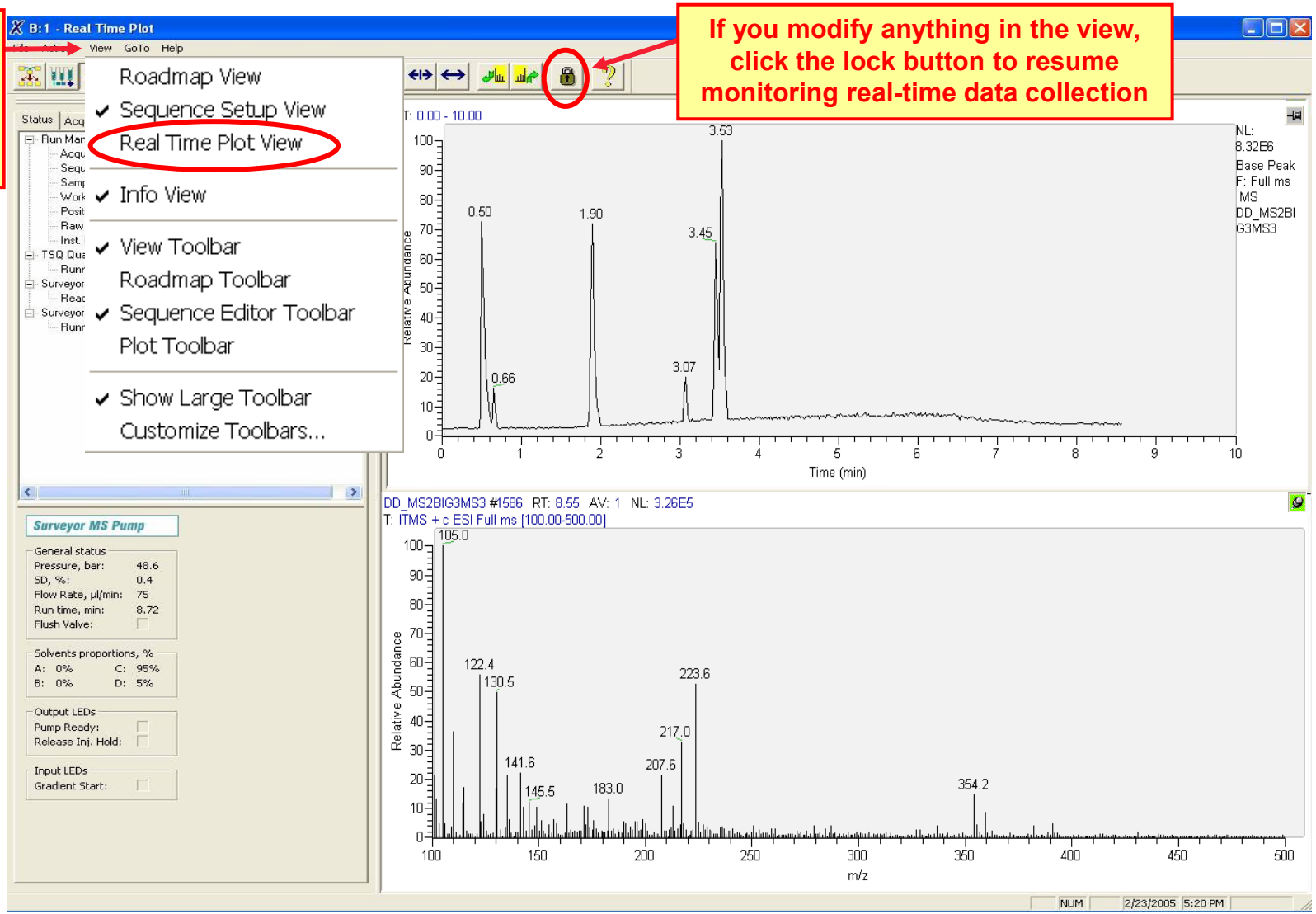


When a sequence is submitted, it shows up in the Acquisition Queue. To delete a sample check the box next to the sample and hit Delete on the keyboard

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

Real Time Plot View

1. Select View and click Real Time Plot View



If you modify anything in the view, click the lock button to resume monitoring real-time data collection

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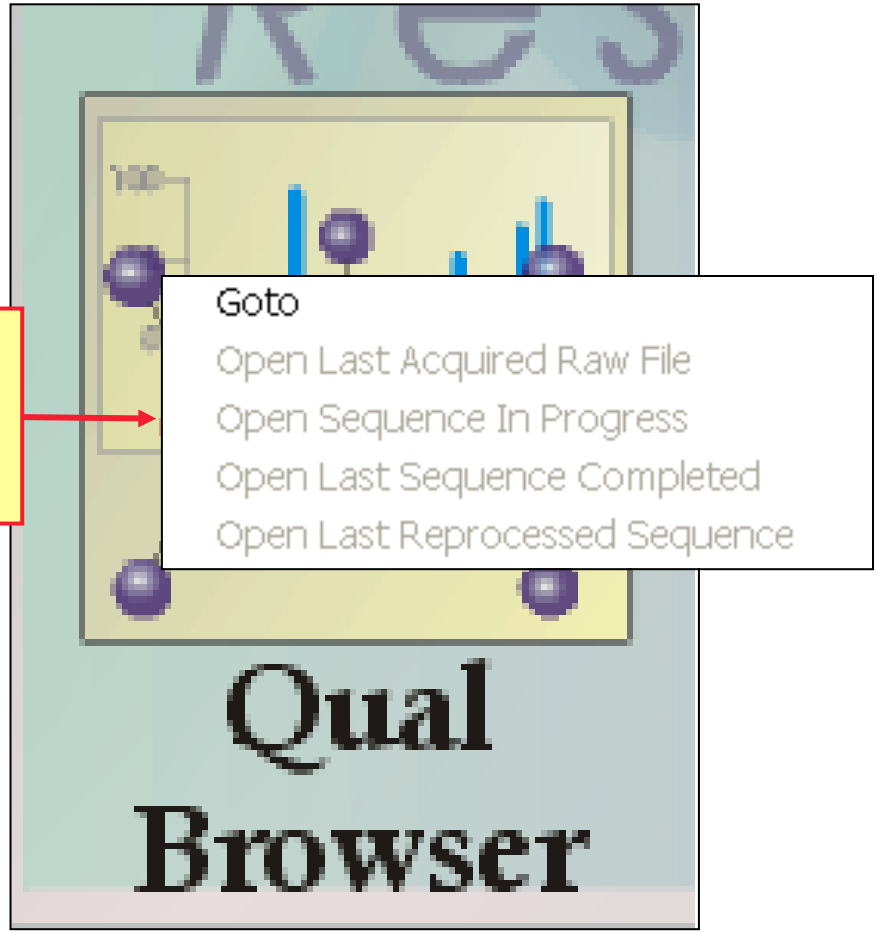
Chapter 10

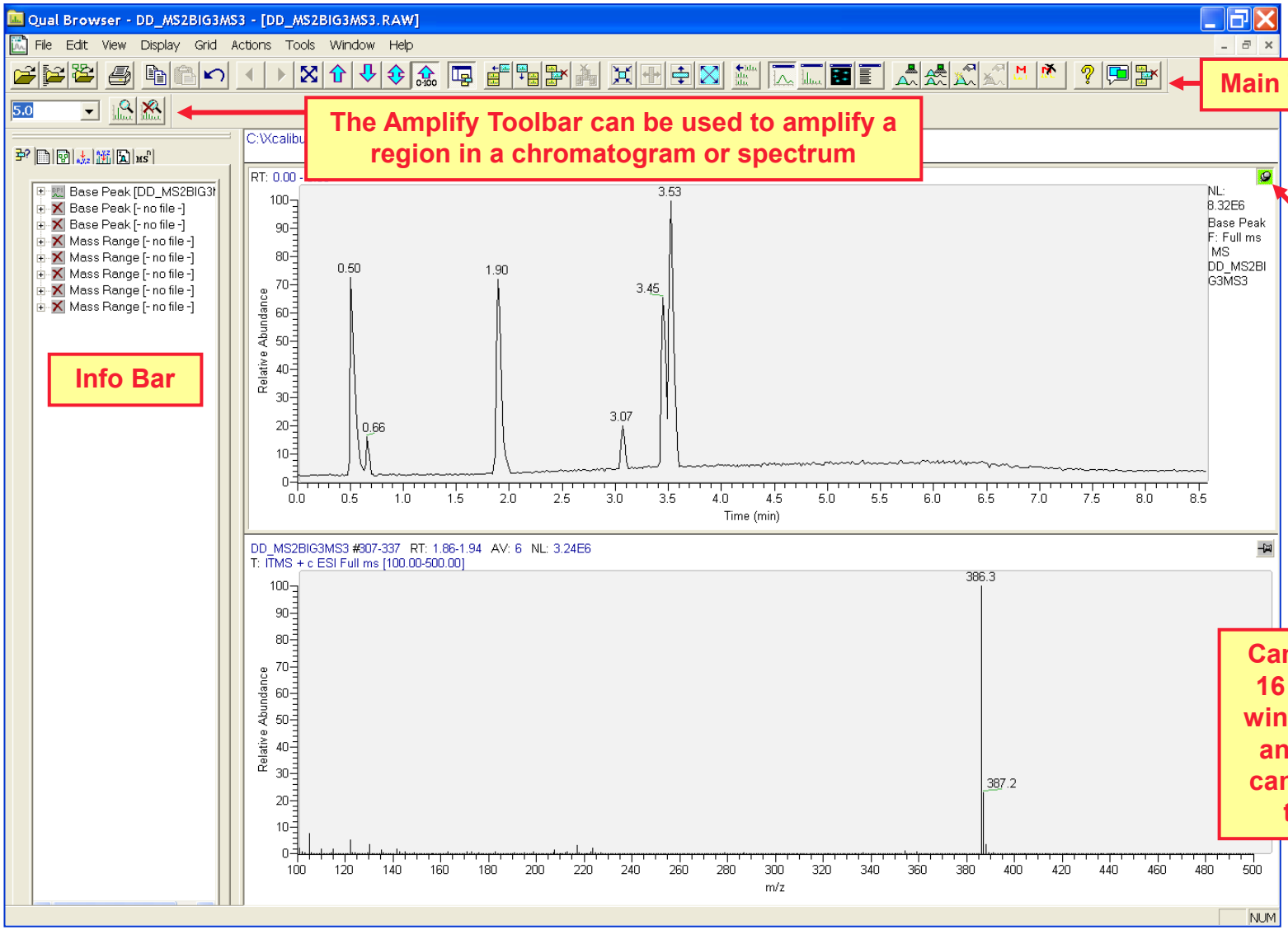
Qual Browser

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

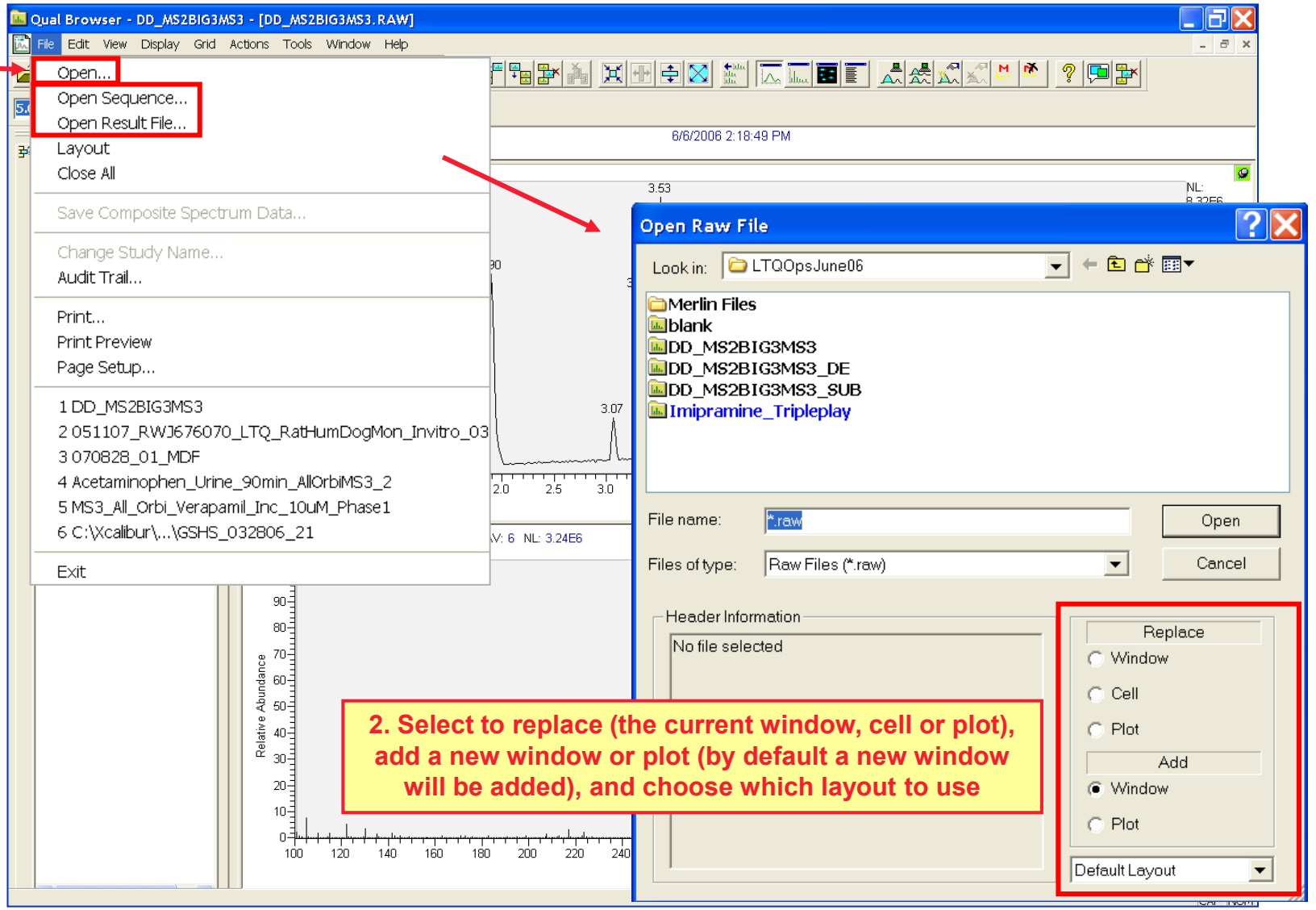


Qual Browser Main View



Opening Data in Qual Browser

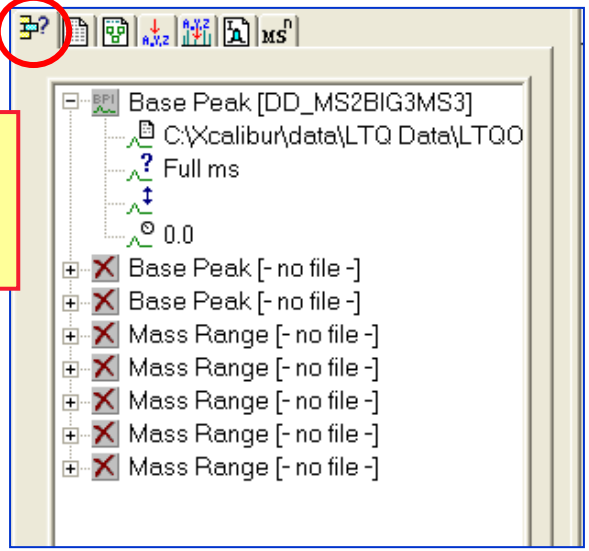
1. Click File and select Open (can also open sequences or result files)



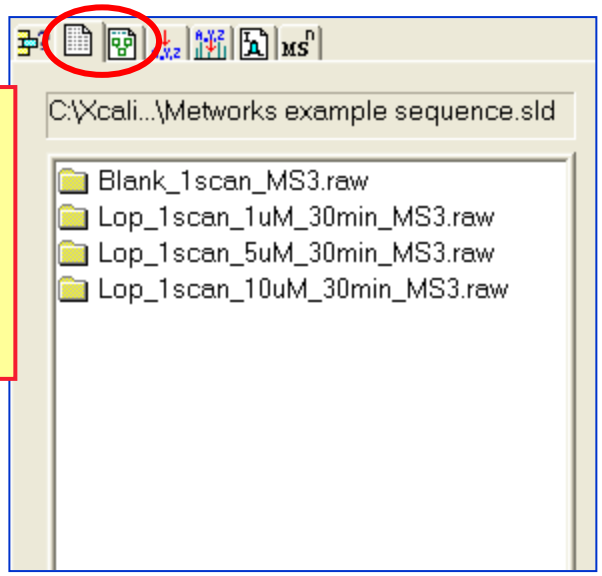
2. Select to replace (the current window, cell or plot), add a new window or plot (by default a new window will be added), and choose which layout to use

The Info Bar

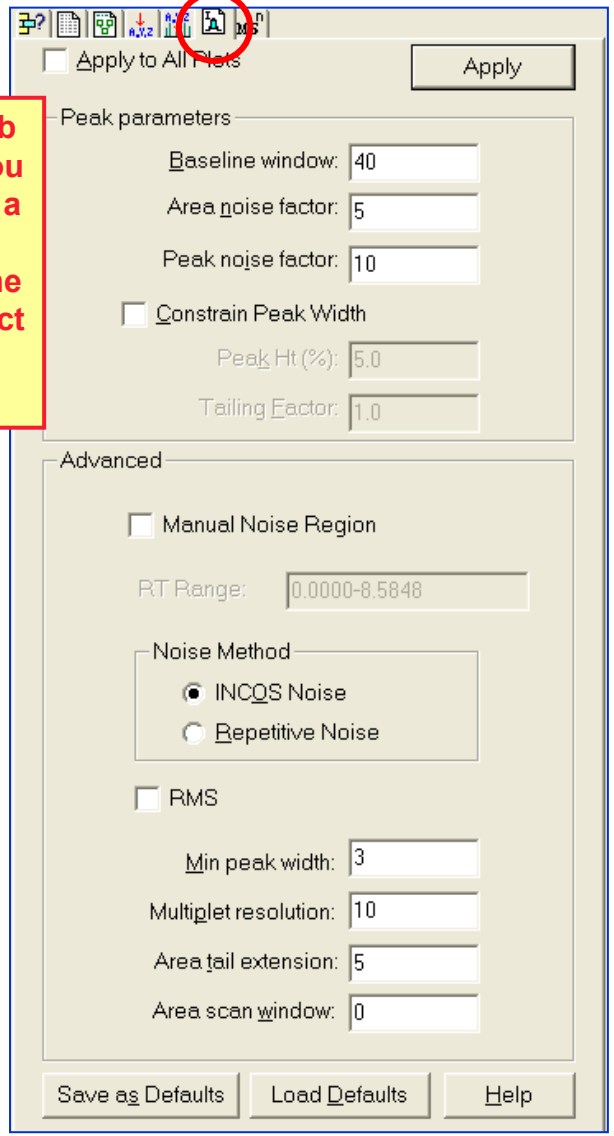
The Cell Info tab gives info about each plot within a cell



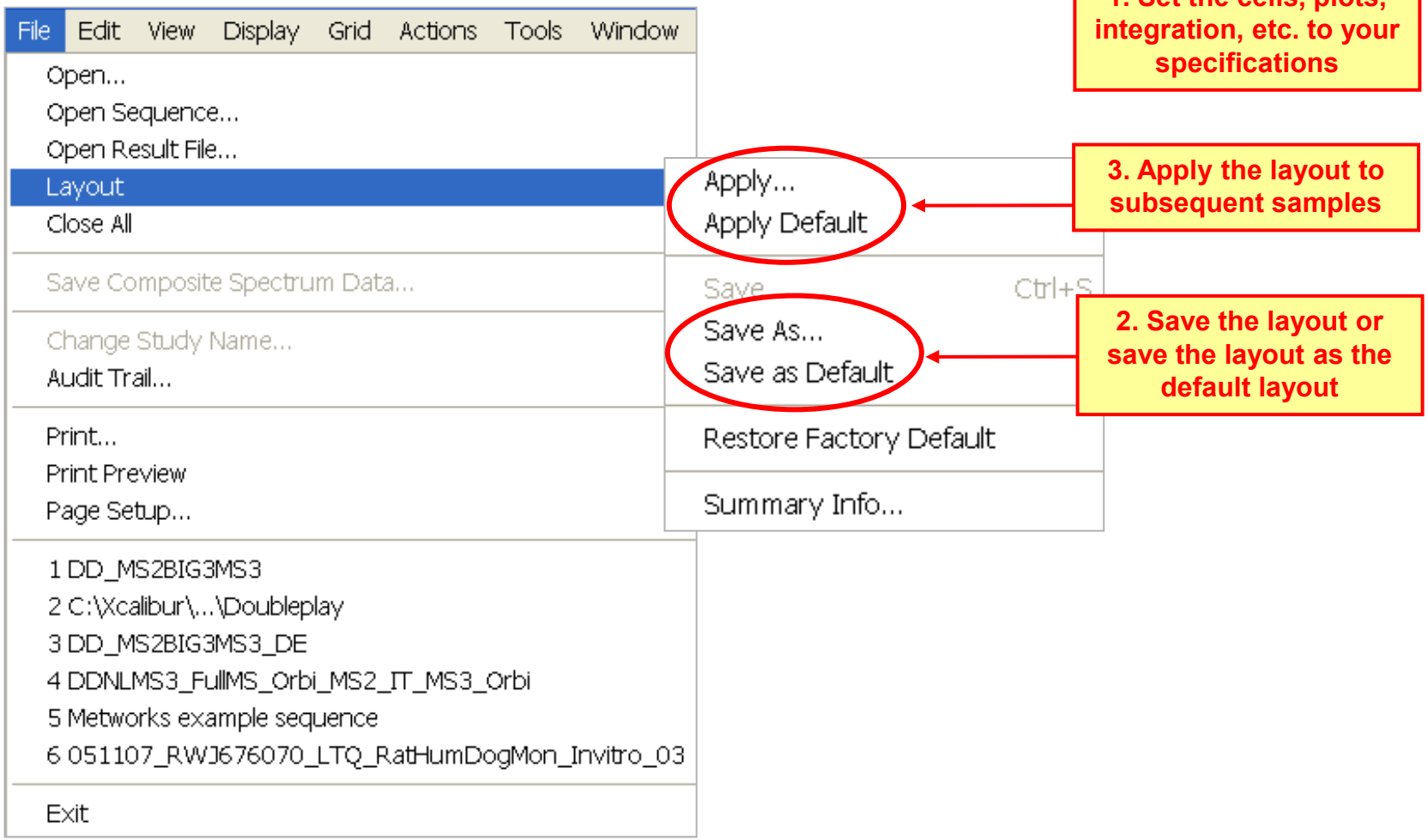
If you open a sequence or result files, they appear in the second and third tabs of the Info Bar, respectively



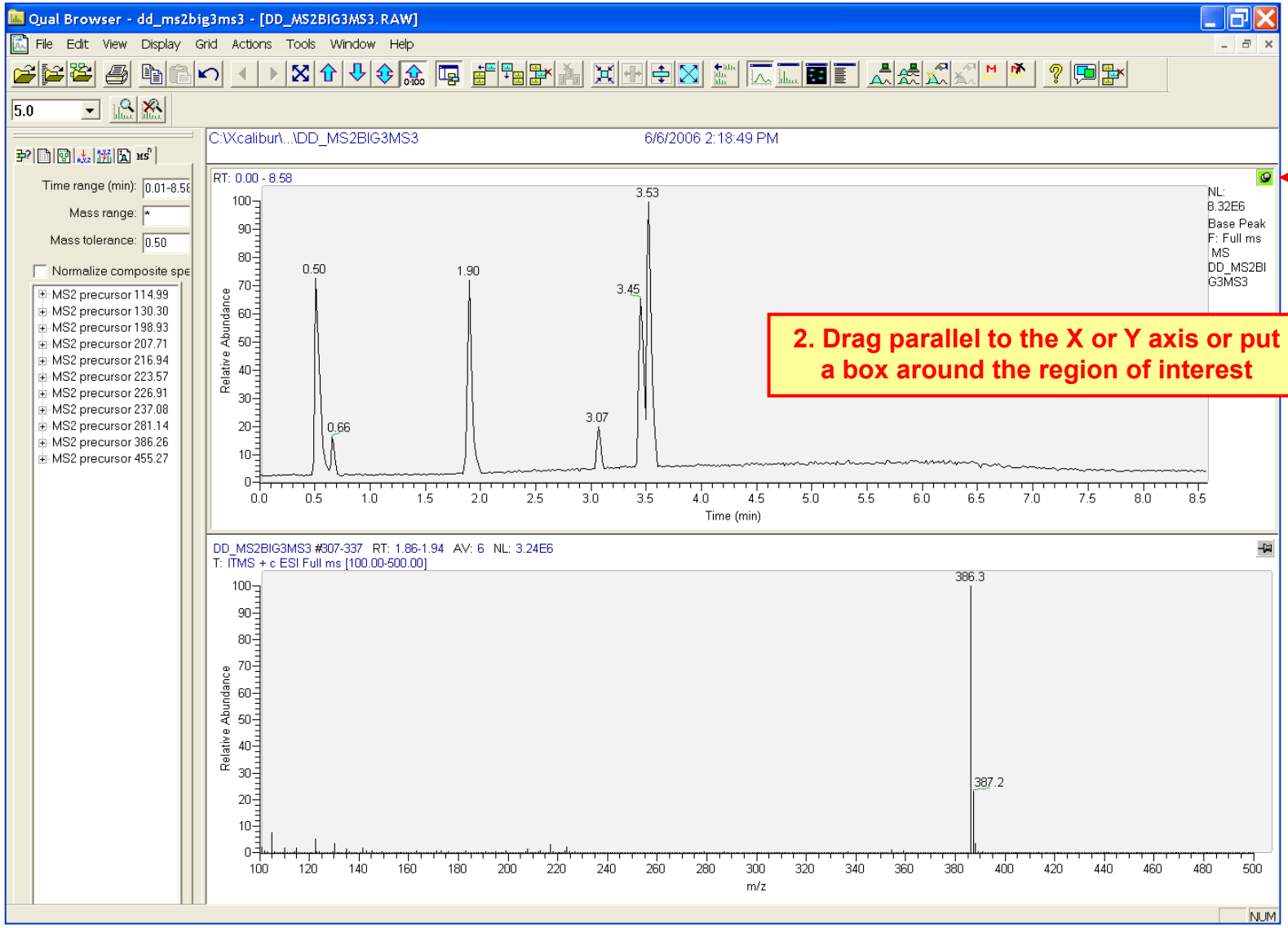
The Integration tab shows up when you integrate peaks in a chromatogram. You can change the parameters to affect how peaks are integrated.



Qual Browser Layouts



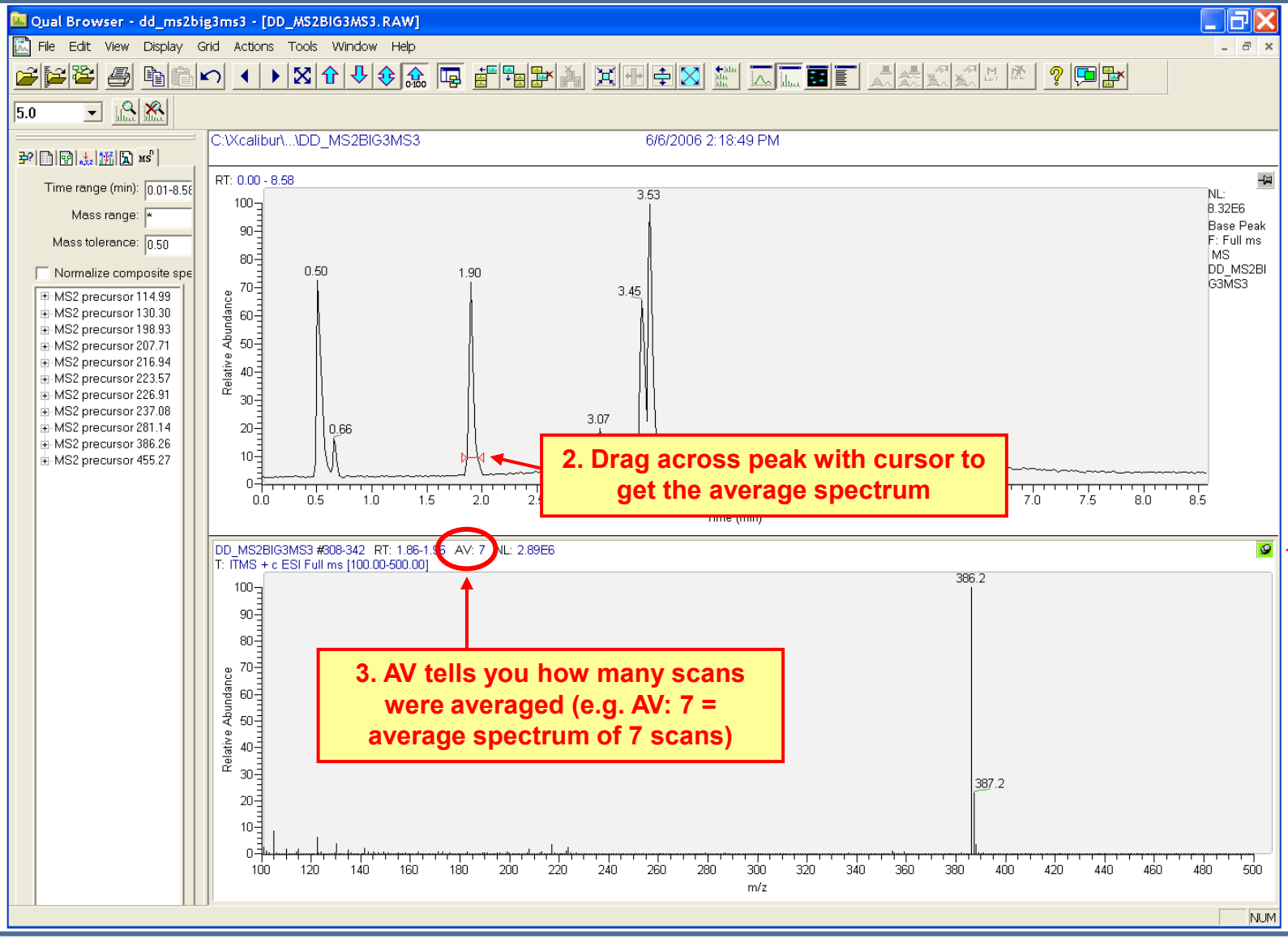
To Zoom In…



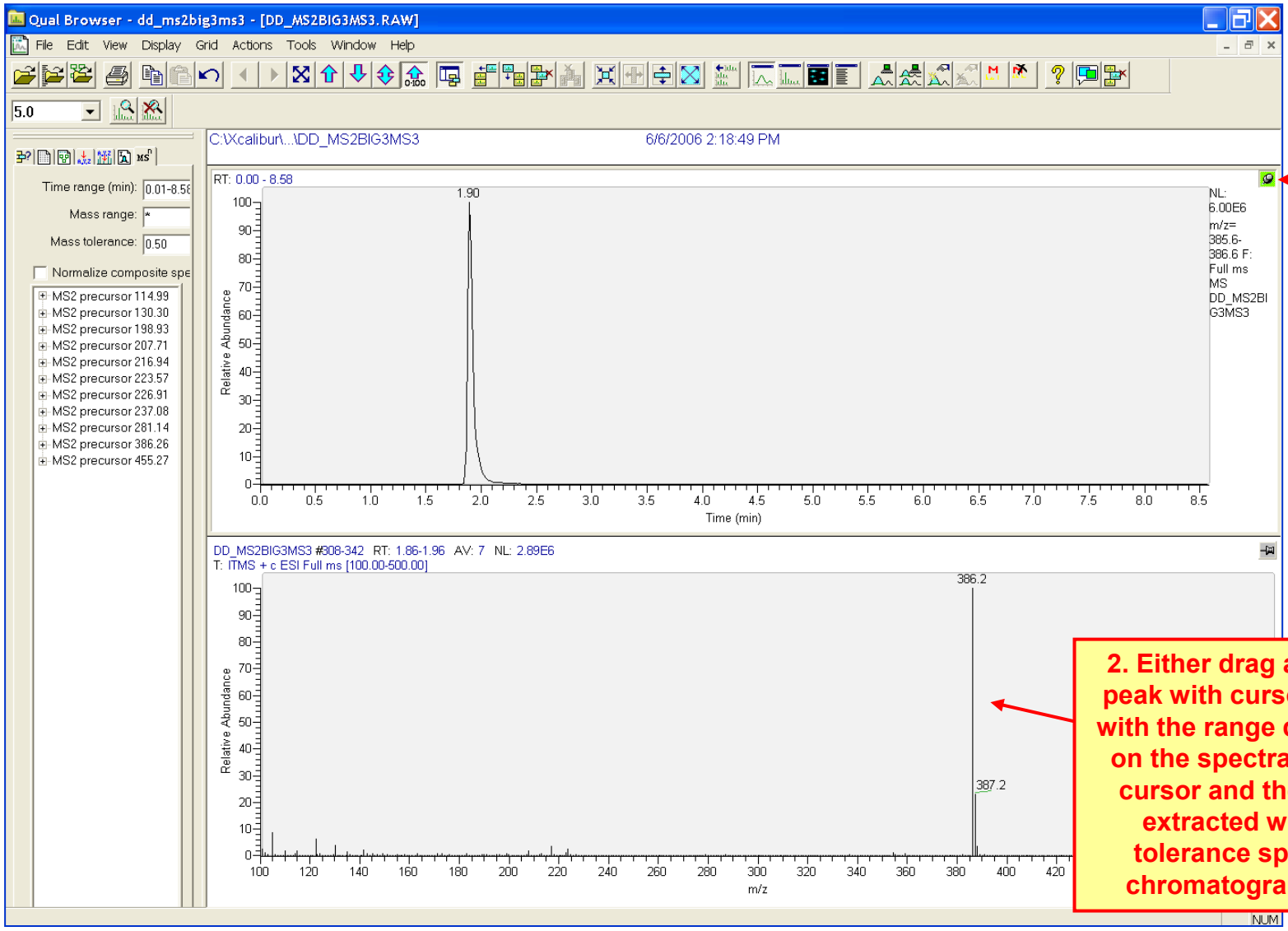
1. Pin cell

2. Drag parallel to the X or Y axis or put a box around the region of interest

Getting an Average Spectrum of a Peak in the Chromatogram



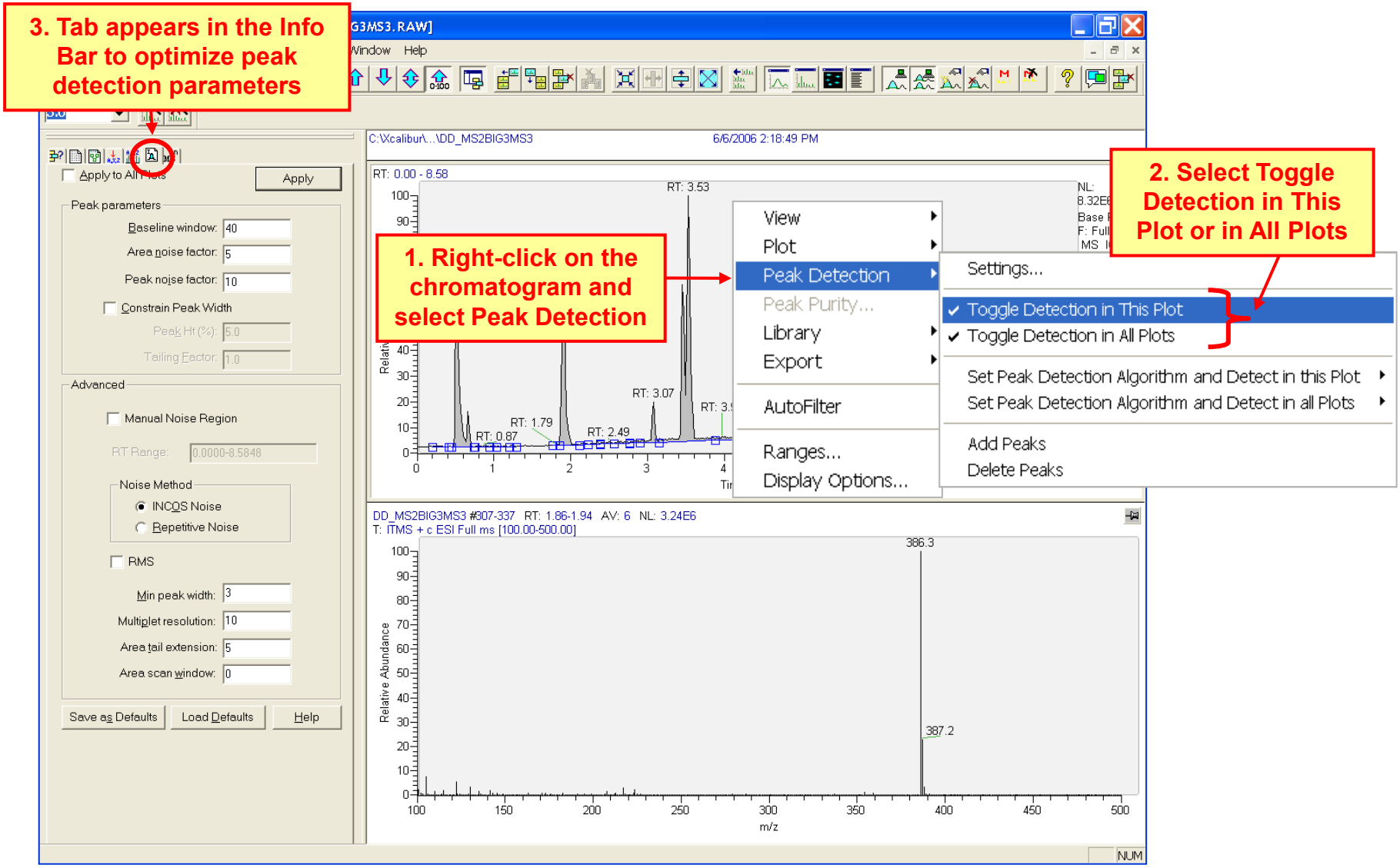
Extracting an Ion from the Chromatogram



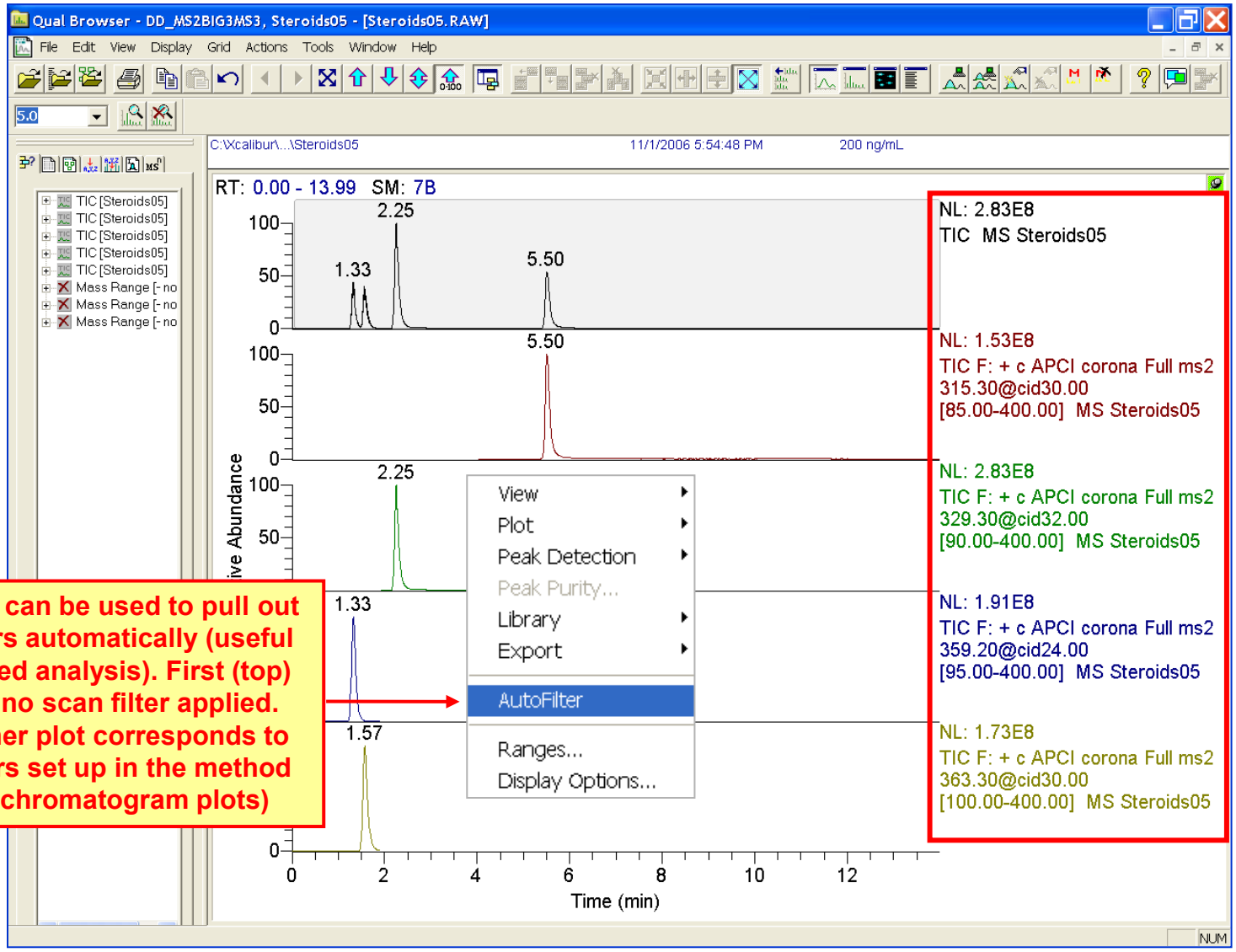
1. Pin chromatogram

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

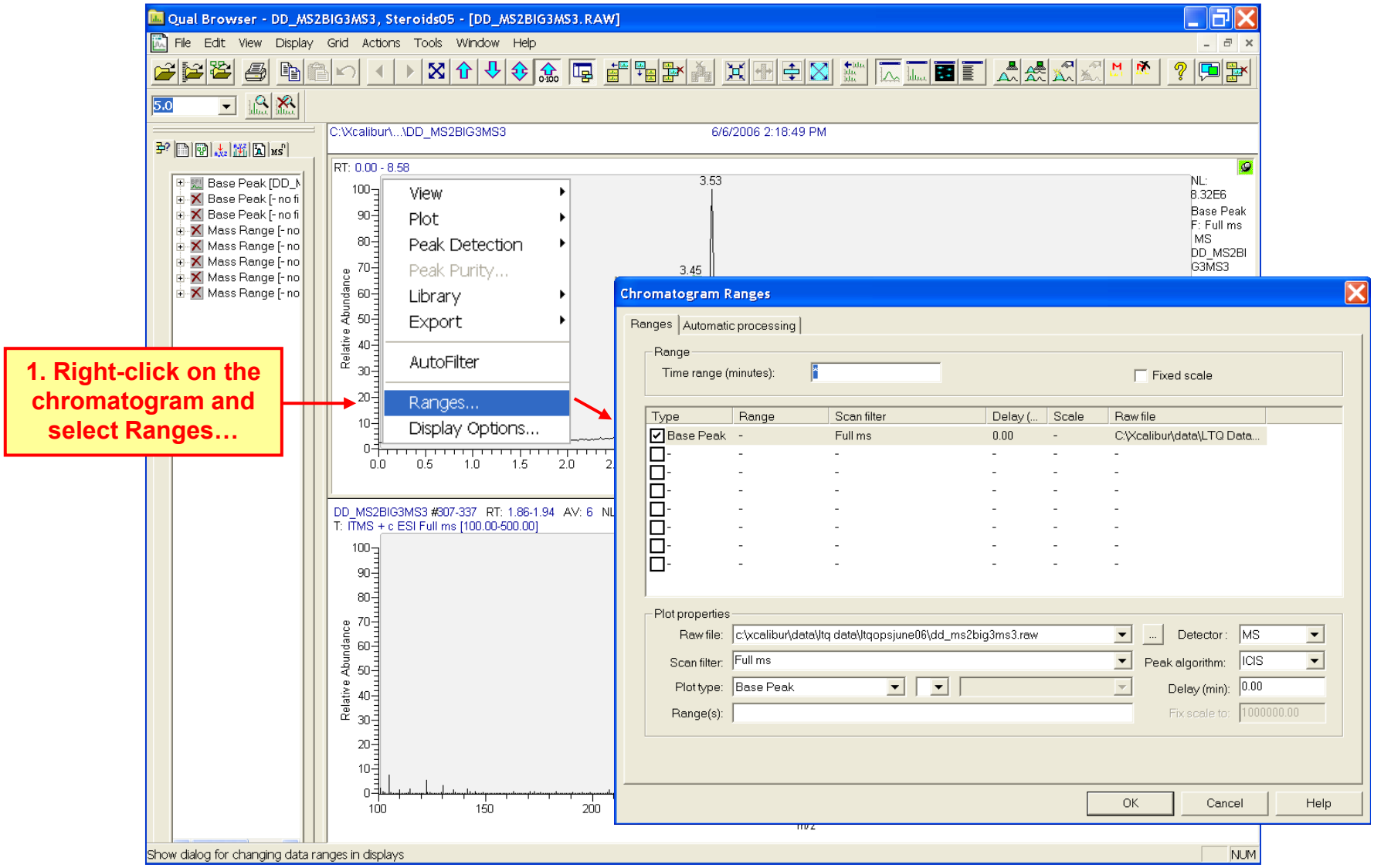


Chromatogram Right-Click Menu – AutoFilter

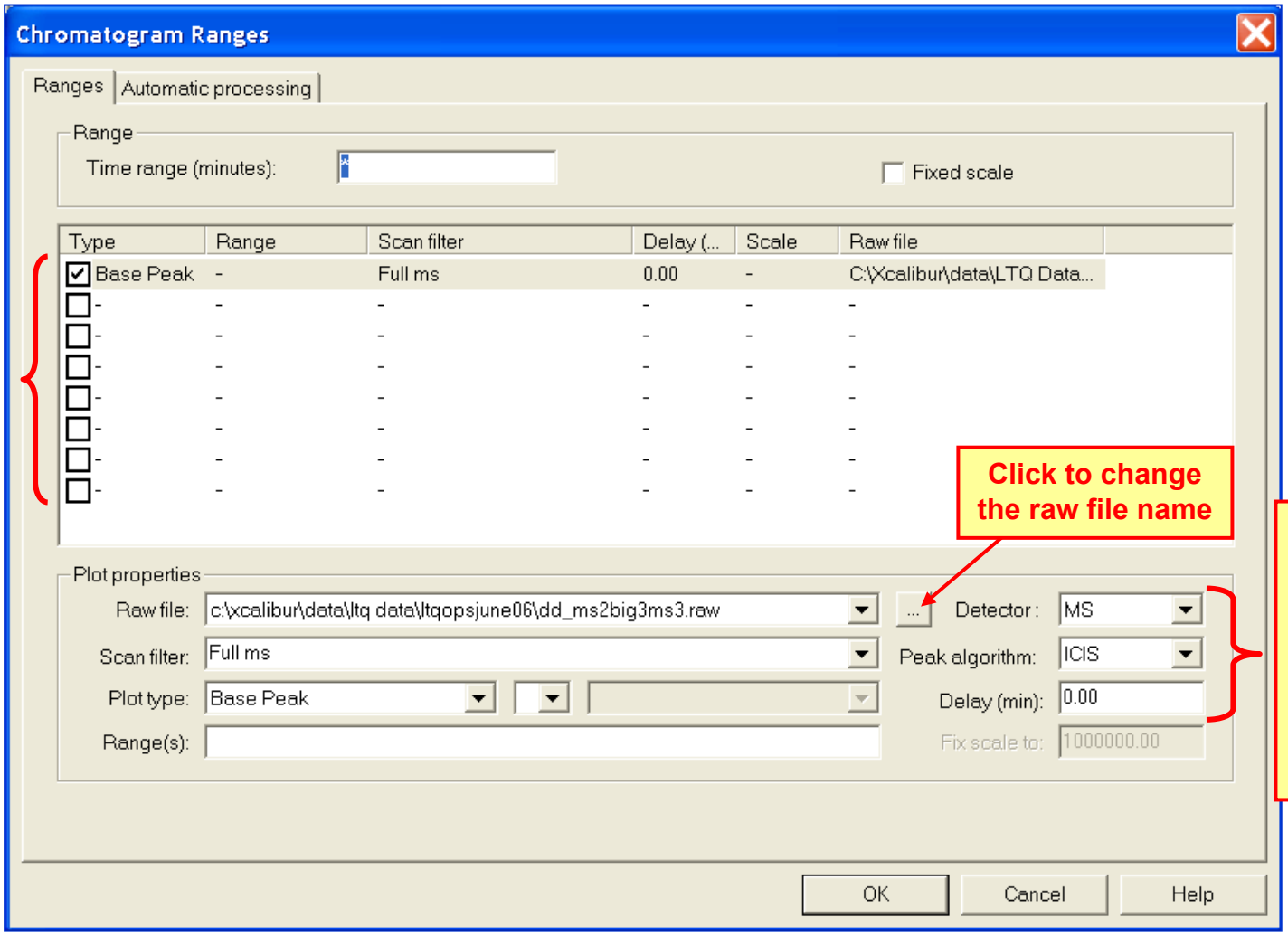


AutoFilter can be used to pull out scan filters automatically (useful for targeted analysis). First (top) plot has no scan filter applied. Every other plot corresponds to scan filters set up in the method (max. 8 chromatogram plots)

Chromatogram Right-Click Menu – Chromatogram Ranges



Chromatogram Right-Click Menu – Chromatogram Ranges

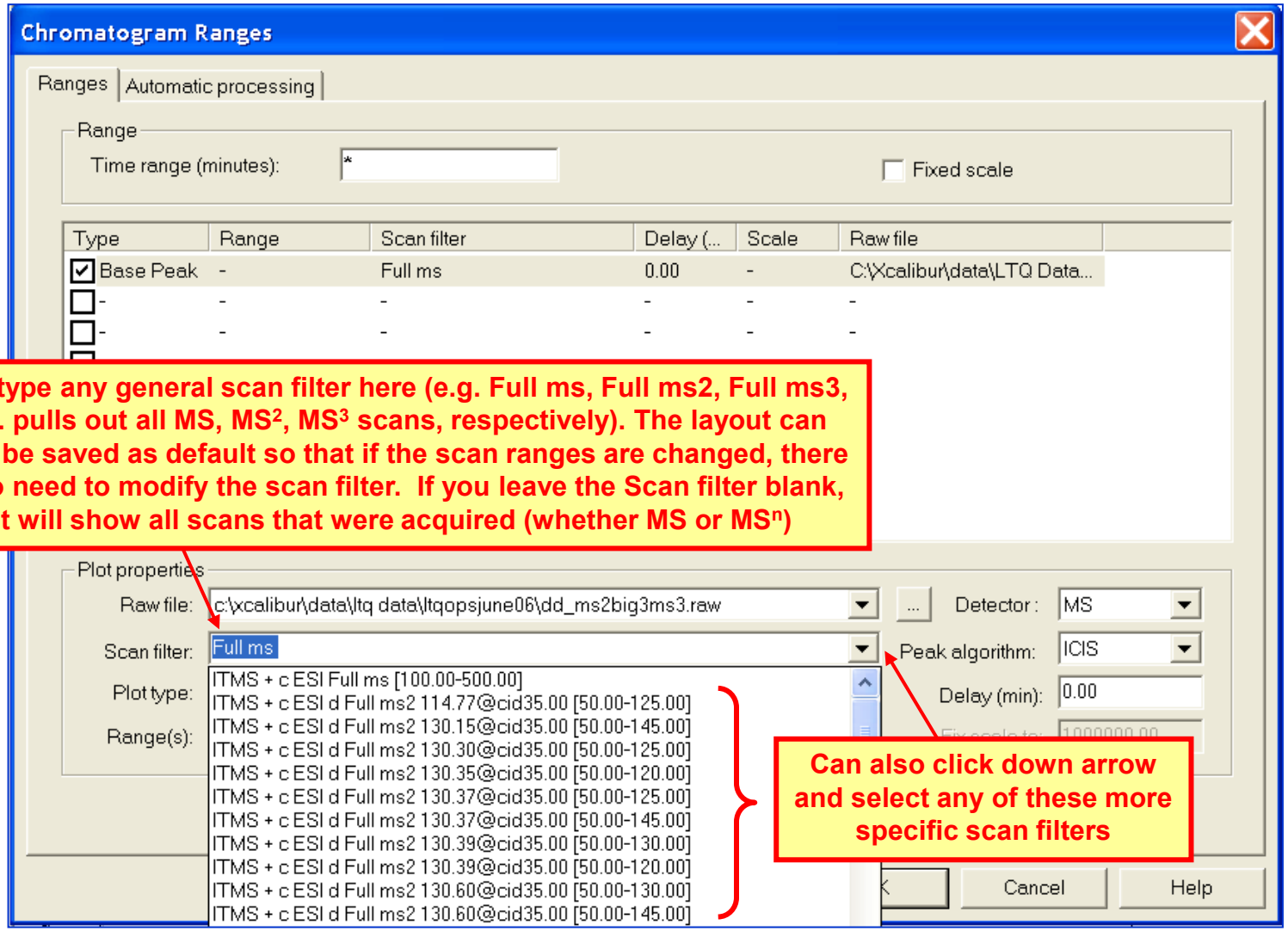


Check to add plots (8 max)

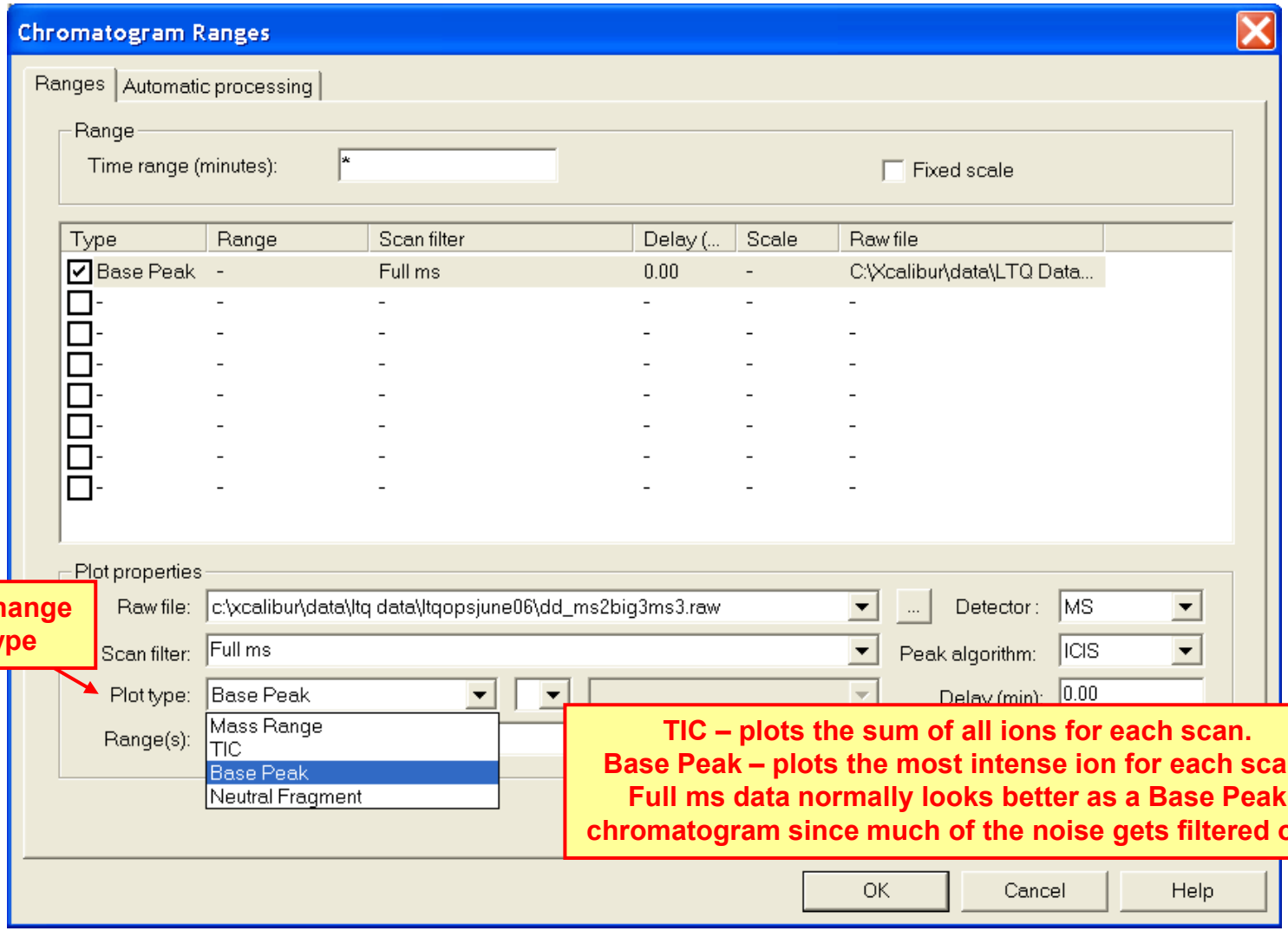
Click to change the raw file name

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

Chromatogram Ranges – Scan Filter



Chromatogram Ranges – Plot Types

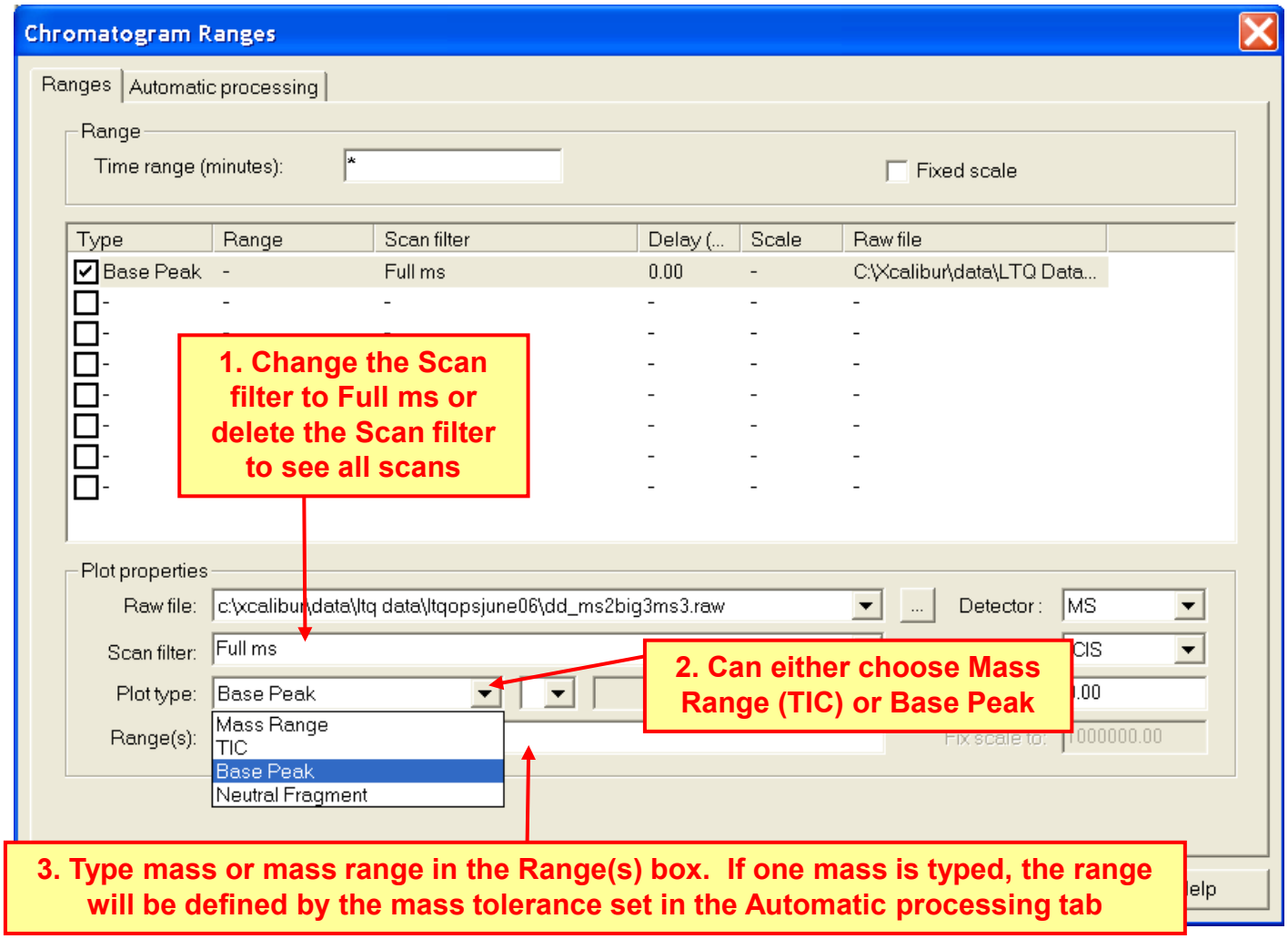


1. Click to change the Plot type

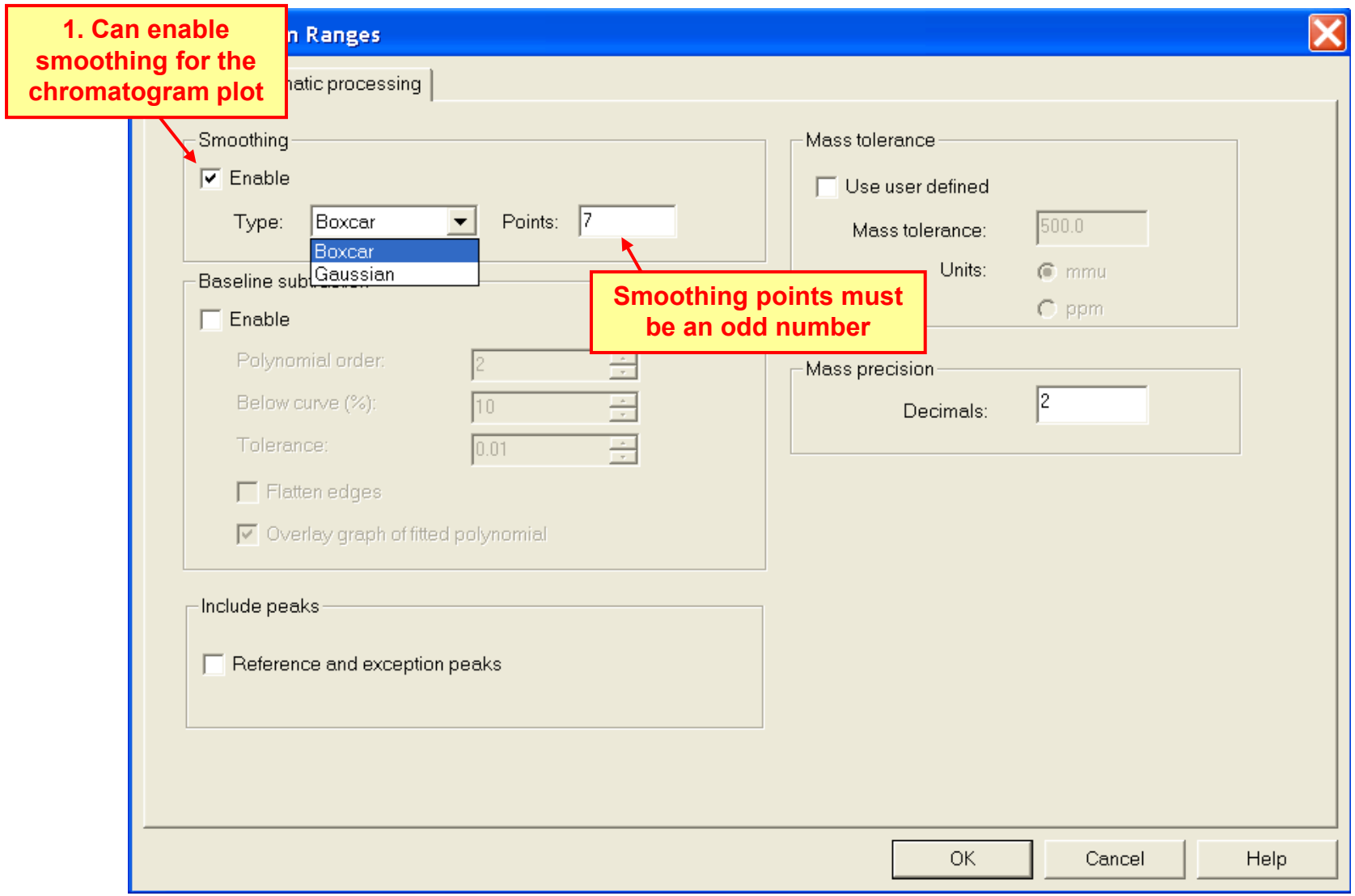
TIC – plots the sum of all ions for each scan.
Base Peak – plots the most intense ion for each scan.
Full ms data normally looks better as a Base Peak chromatogram since much of the noise gets filtered out.

Chromatogram Ranges – Extracted Ion Chromatogram

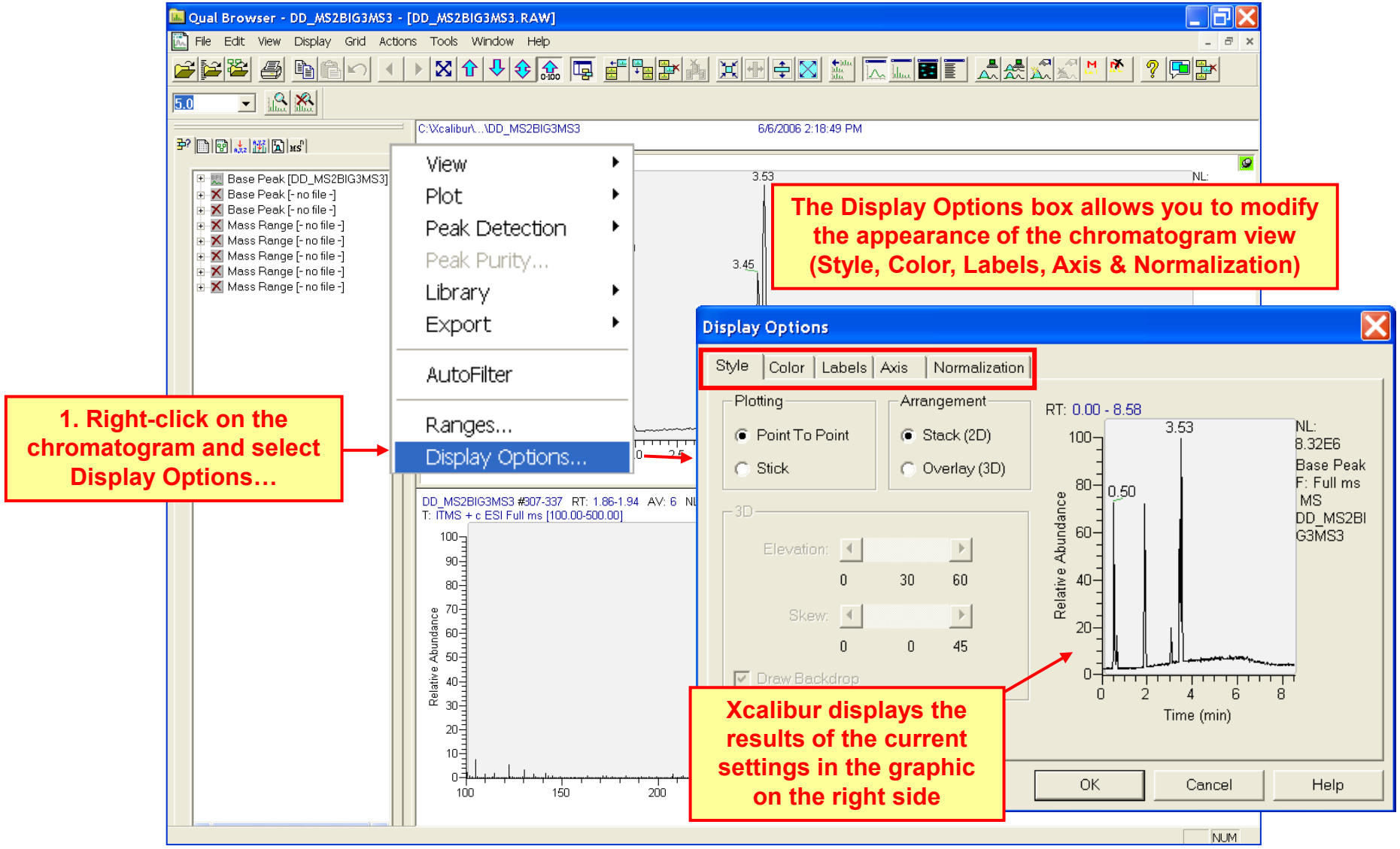
There are different ways to extract an ion in your chromatogram using the Chromatogram Ranges box:



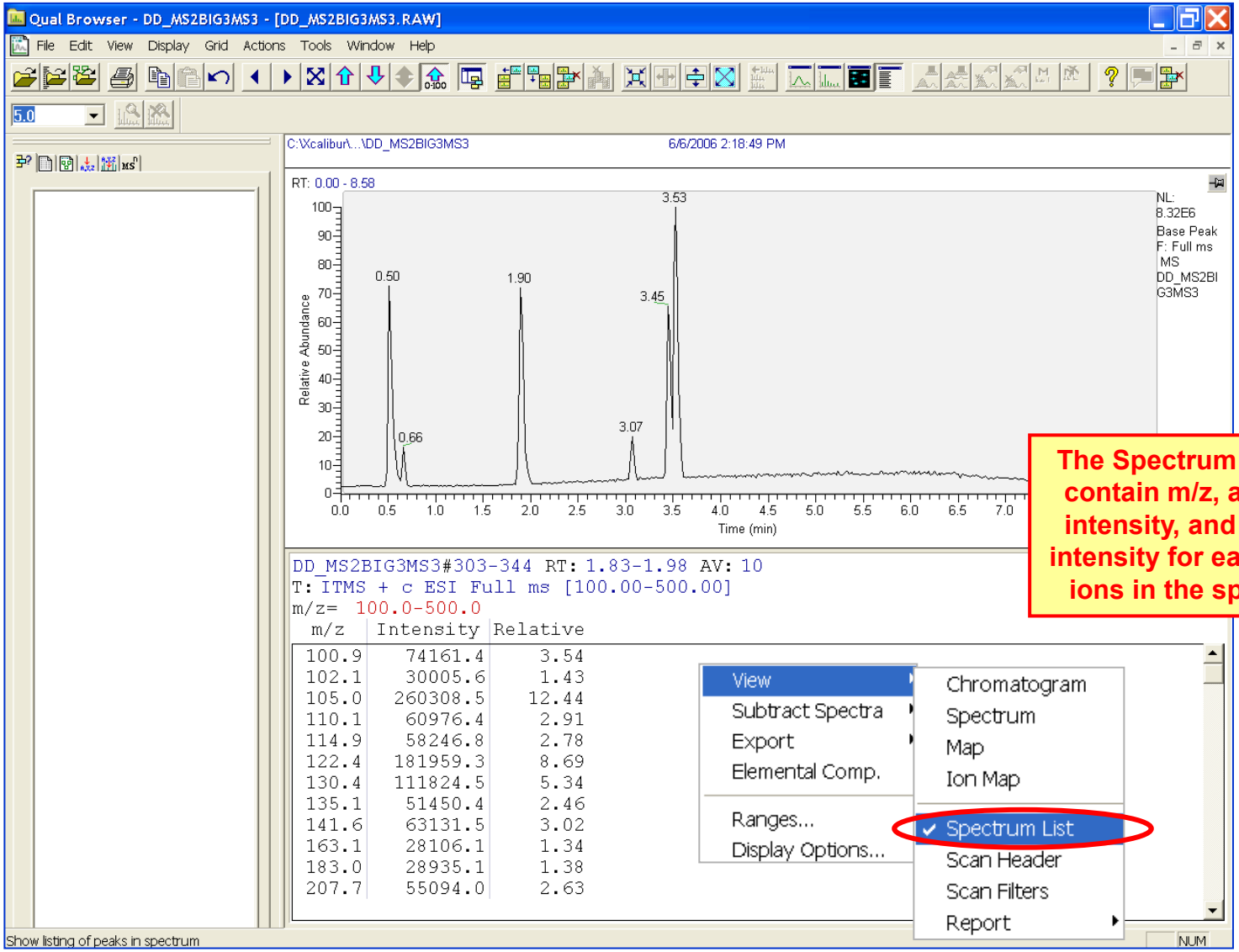
Chromatogram Ranges – Automatic Processing tab



Chromatogram Right-Click Menu - Display Options



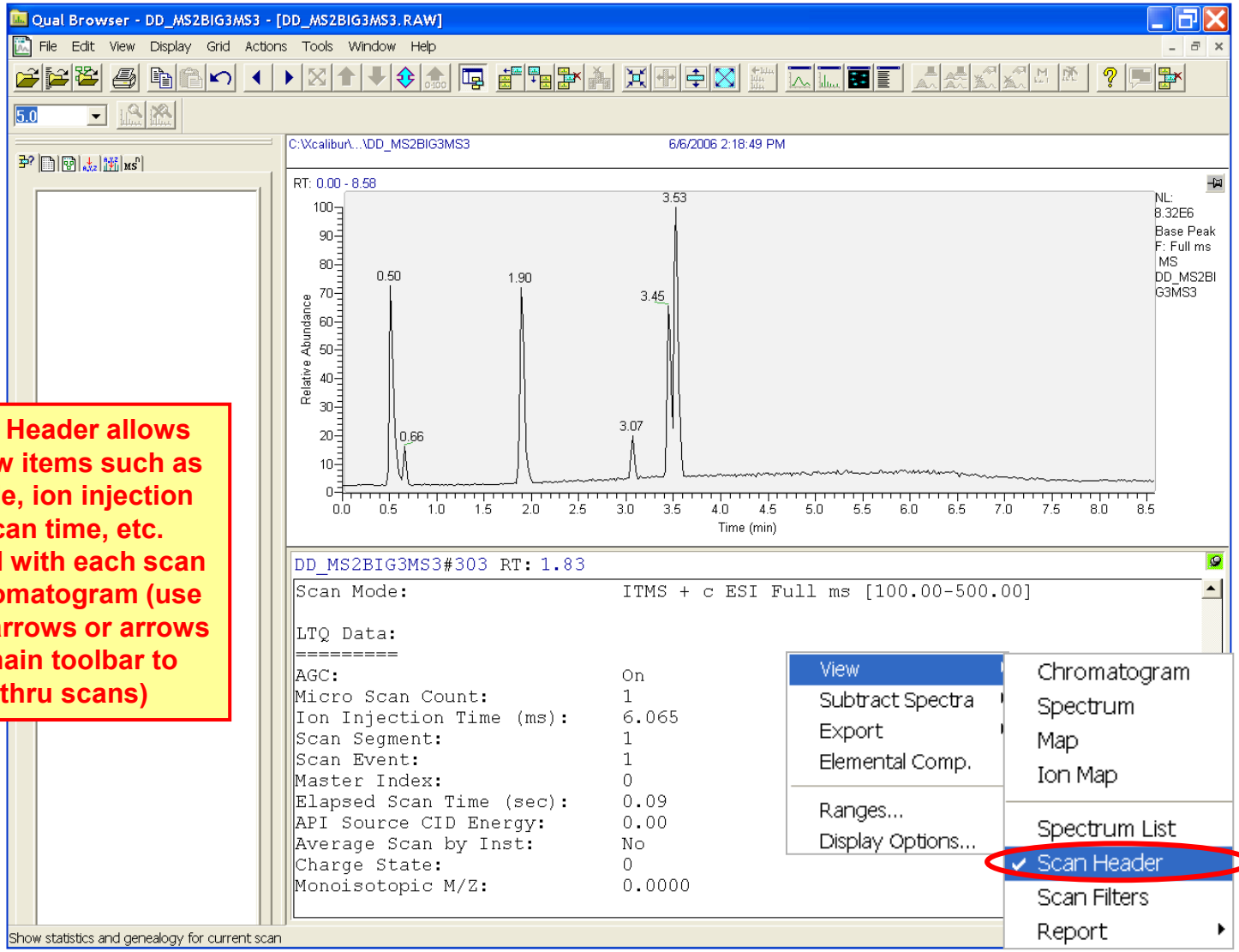
Spectrum Right-Click Menu – Spectrum List



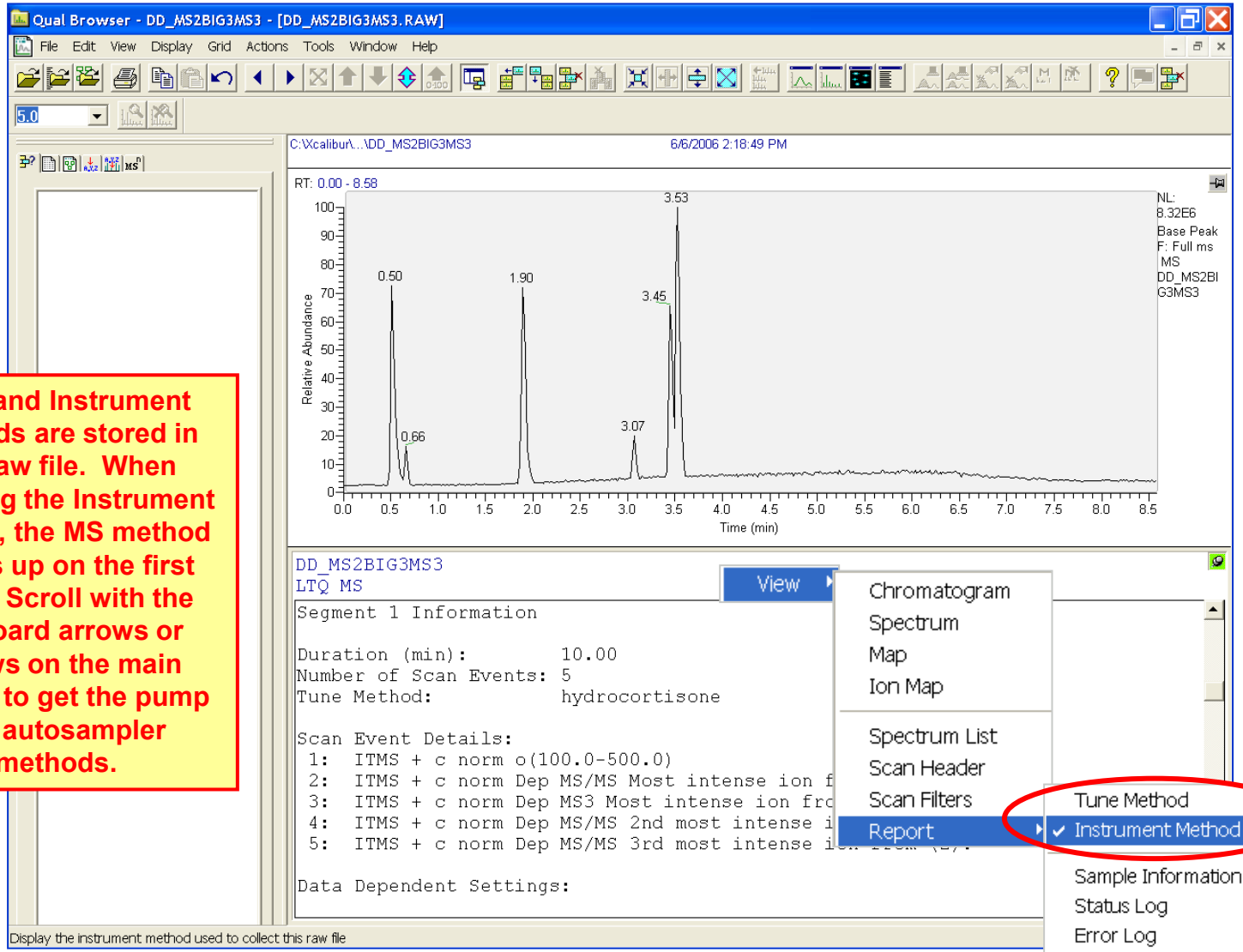
The Spectrum List can contain m/z, absolute intensity, and relative intensity for each of the ions in the spectrum

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)

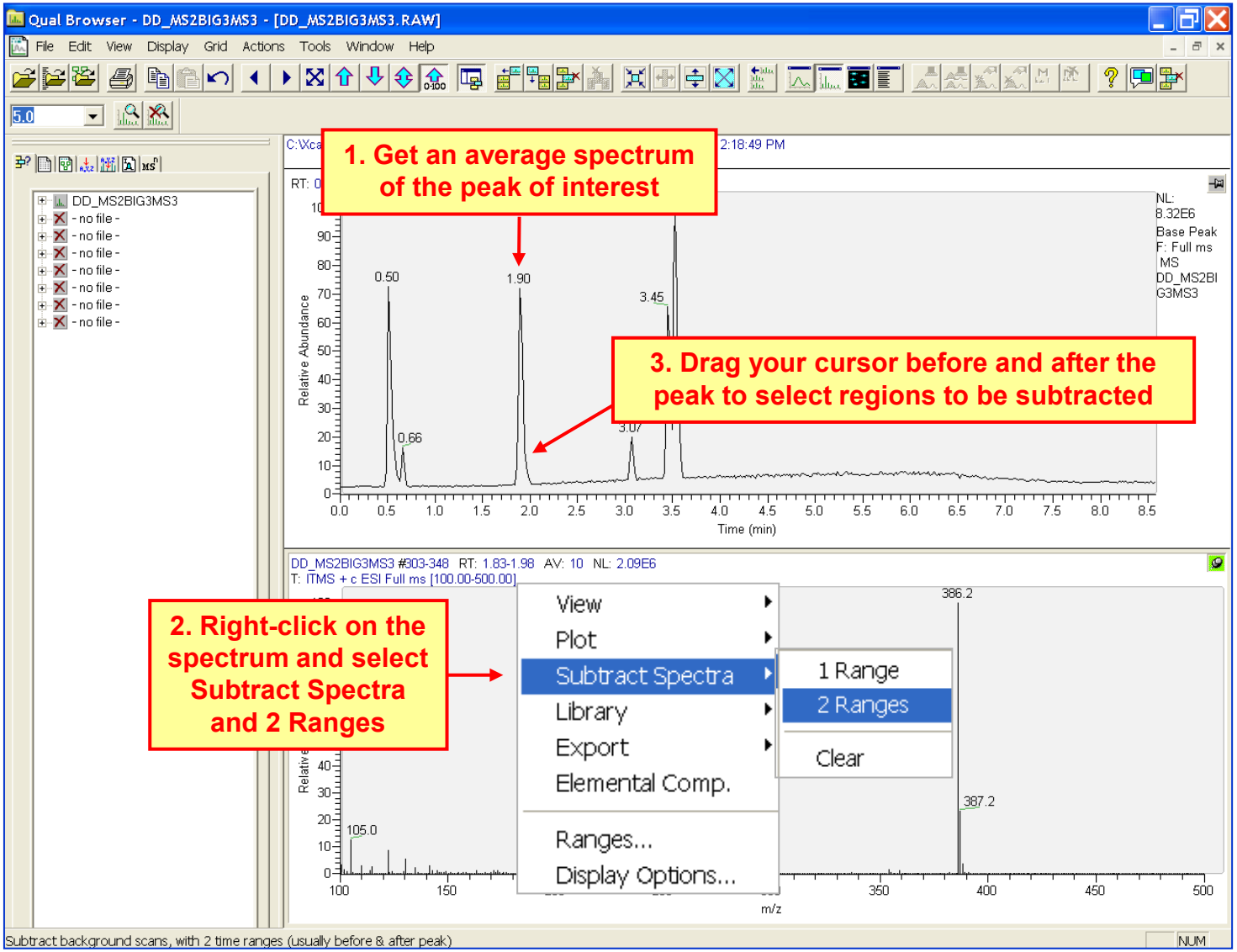


Spectrum Right-Click Menu – Tune and Instrument Methods

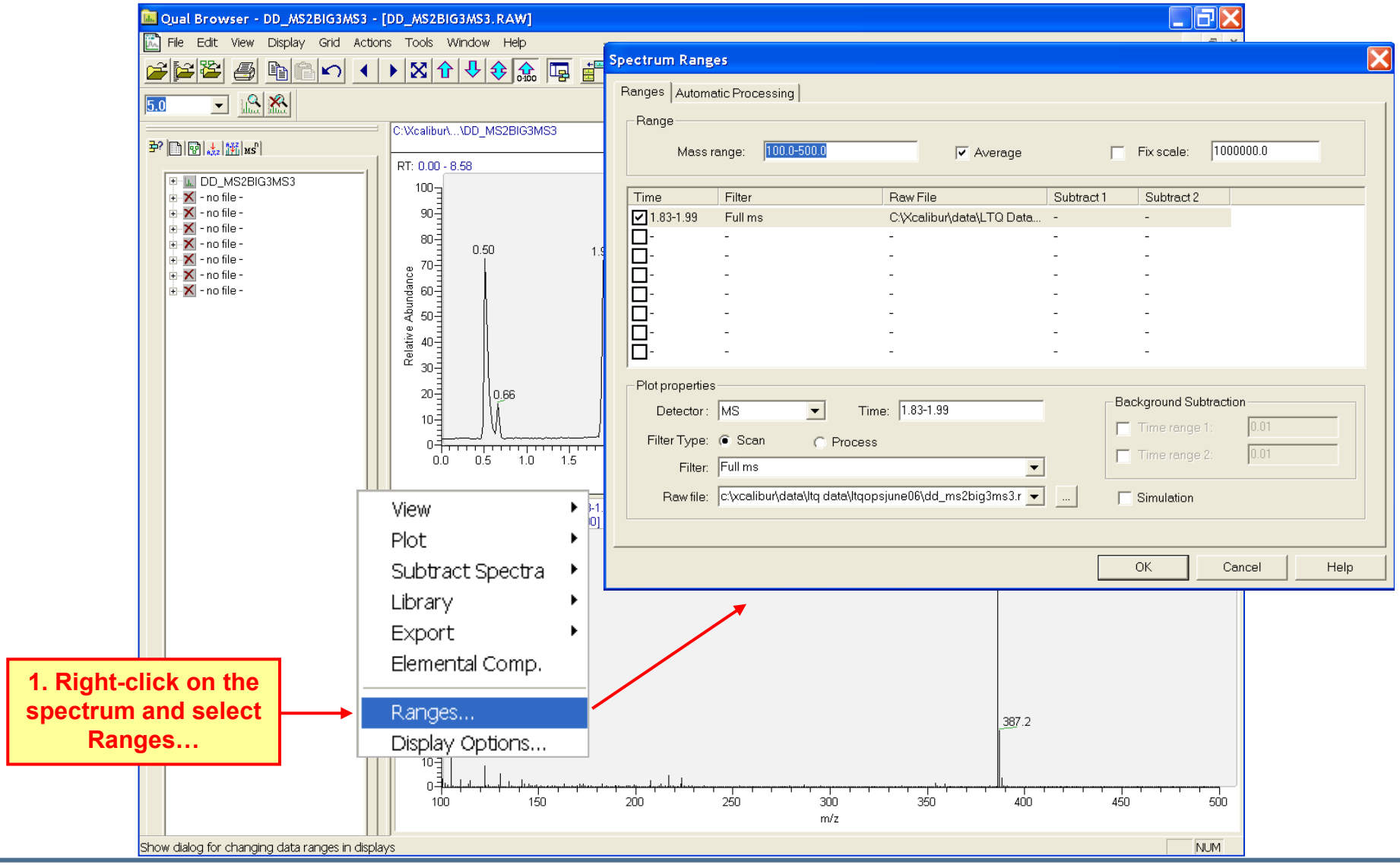


Tune and Instrument Methods are stored in the raw file. When selecting the Instrument Method, the MS method shows up on the first page. Scroll with the keyboard arrows or arrows on the main toolbar to get the pump and autosampler methods.

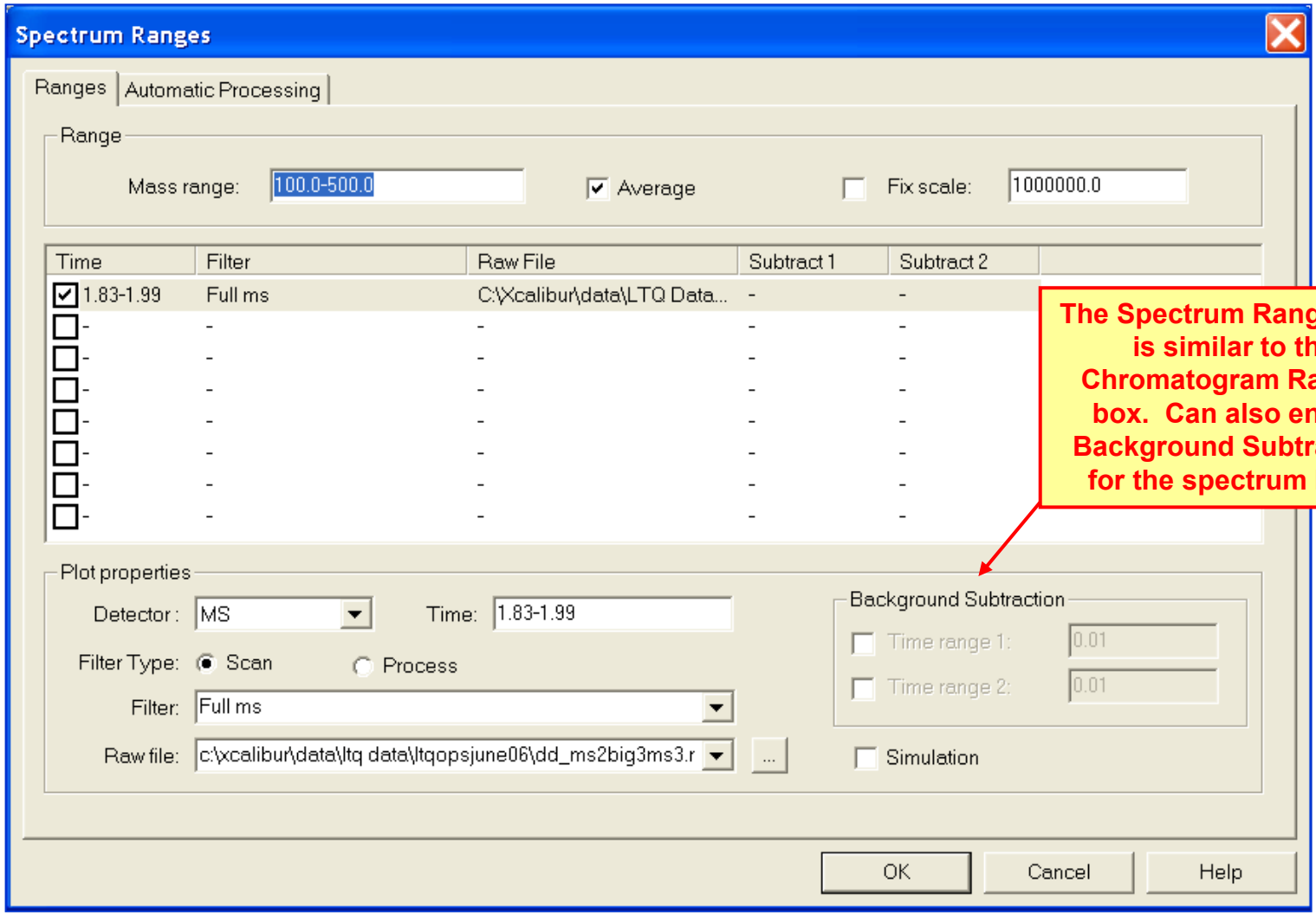
Spectrum Right-Click Menu – Spectral Subtraction



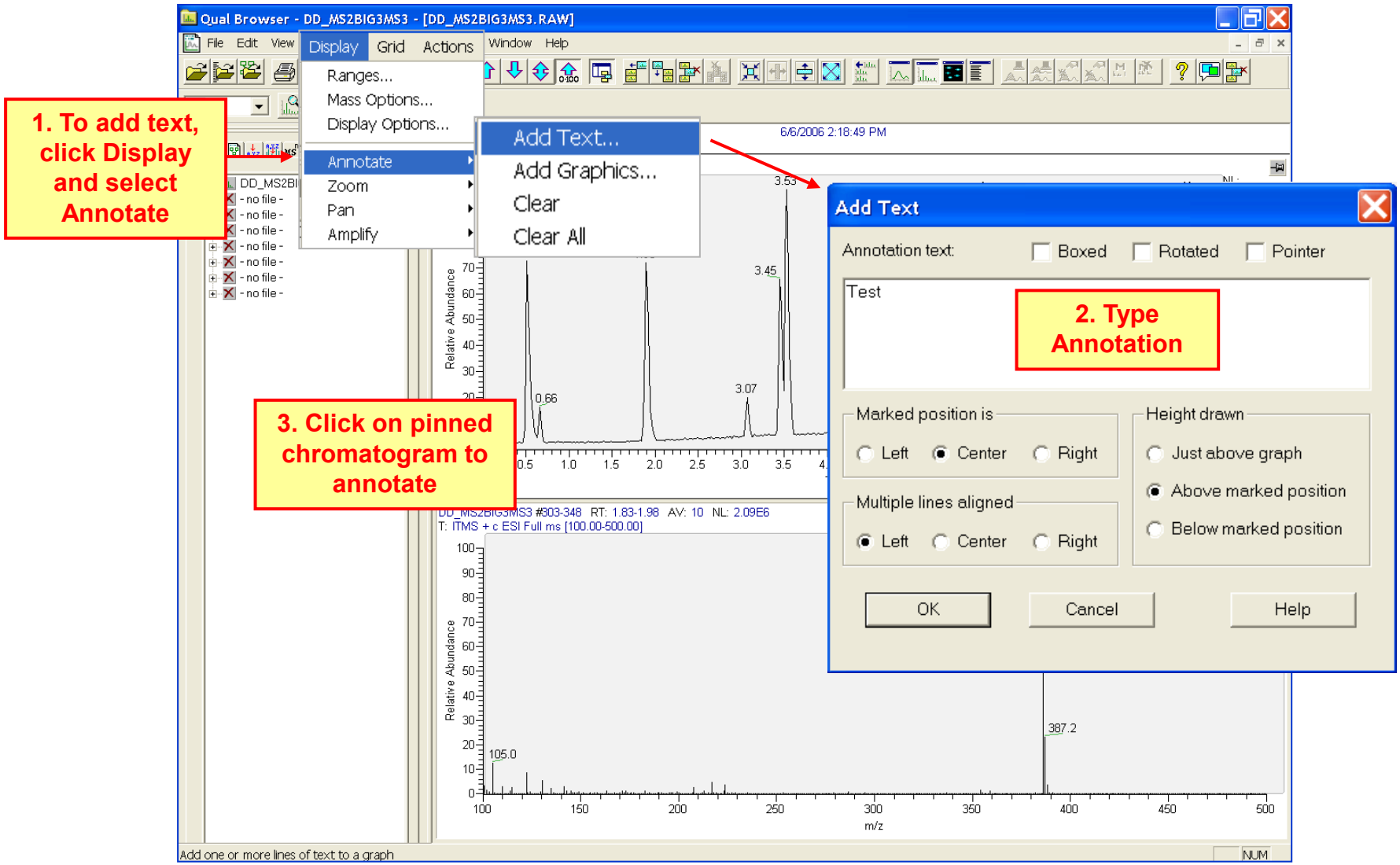
Spectrum Right-Click Menu - Spectrum Ranges



Spectrum Right-Click Menu - Spectrum Ranges



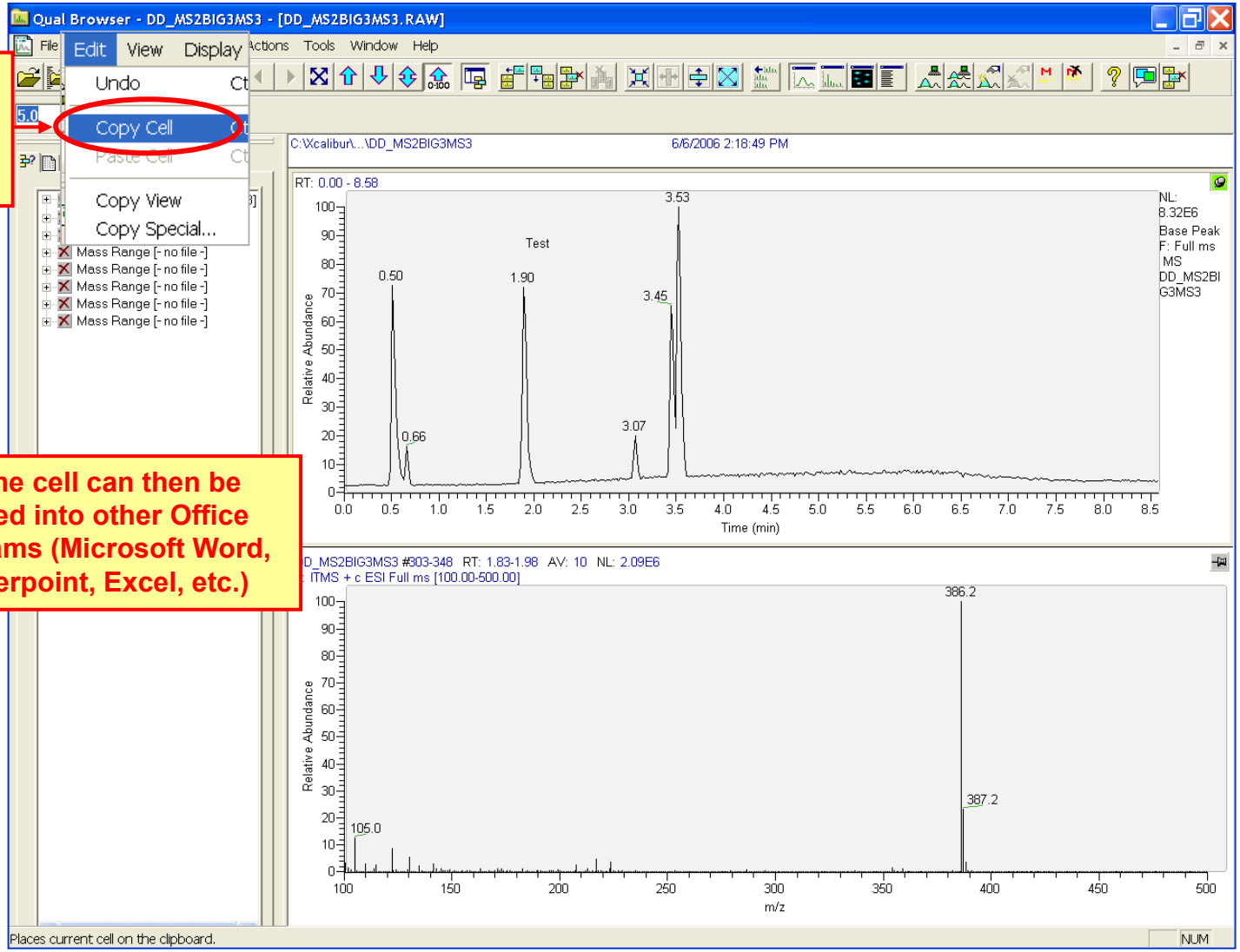
Presentation



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

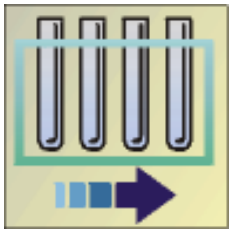
Quantitative Processing

Quantitative Processing

1. Processing Setup

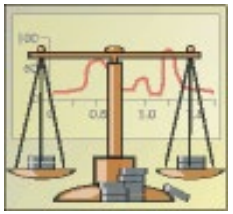
Input known compounds for identification
Set up peak detection/integration parameters
Choose calibration/QC type, levels, weighting
Select advanced chromatographic processing

2. Sample Processing/Reprocessing



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

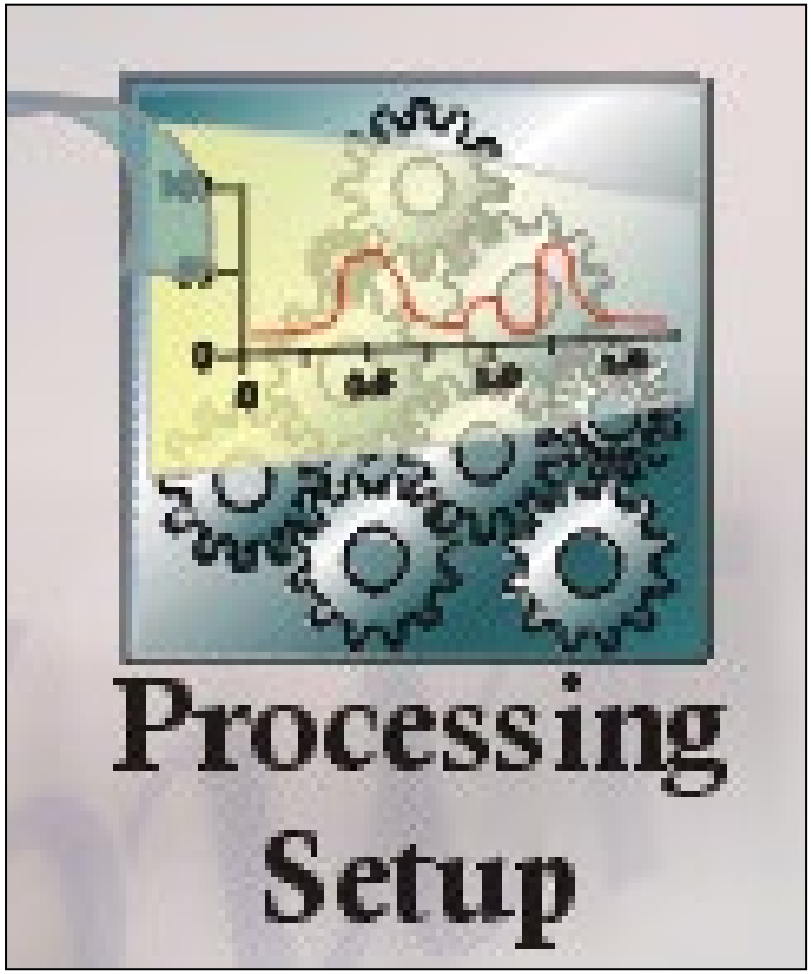
3. Quan Browser



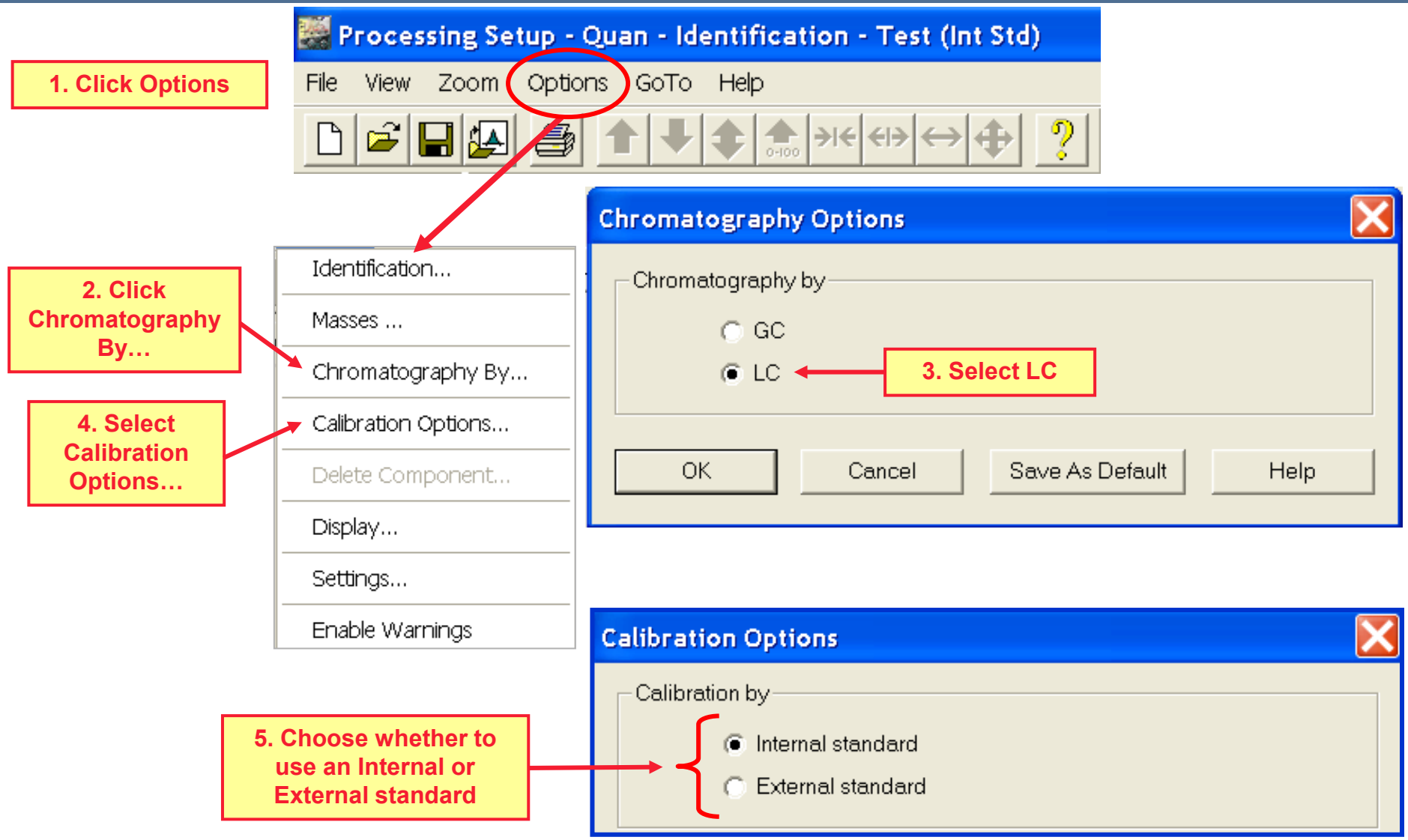
View quantitative results
Evaluate standard curve, QCs, and flags
Recalculate peaks with different parameters
Analyze detailed quantitation information

Quan Processing Setup

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



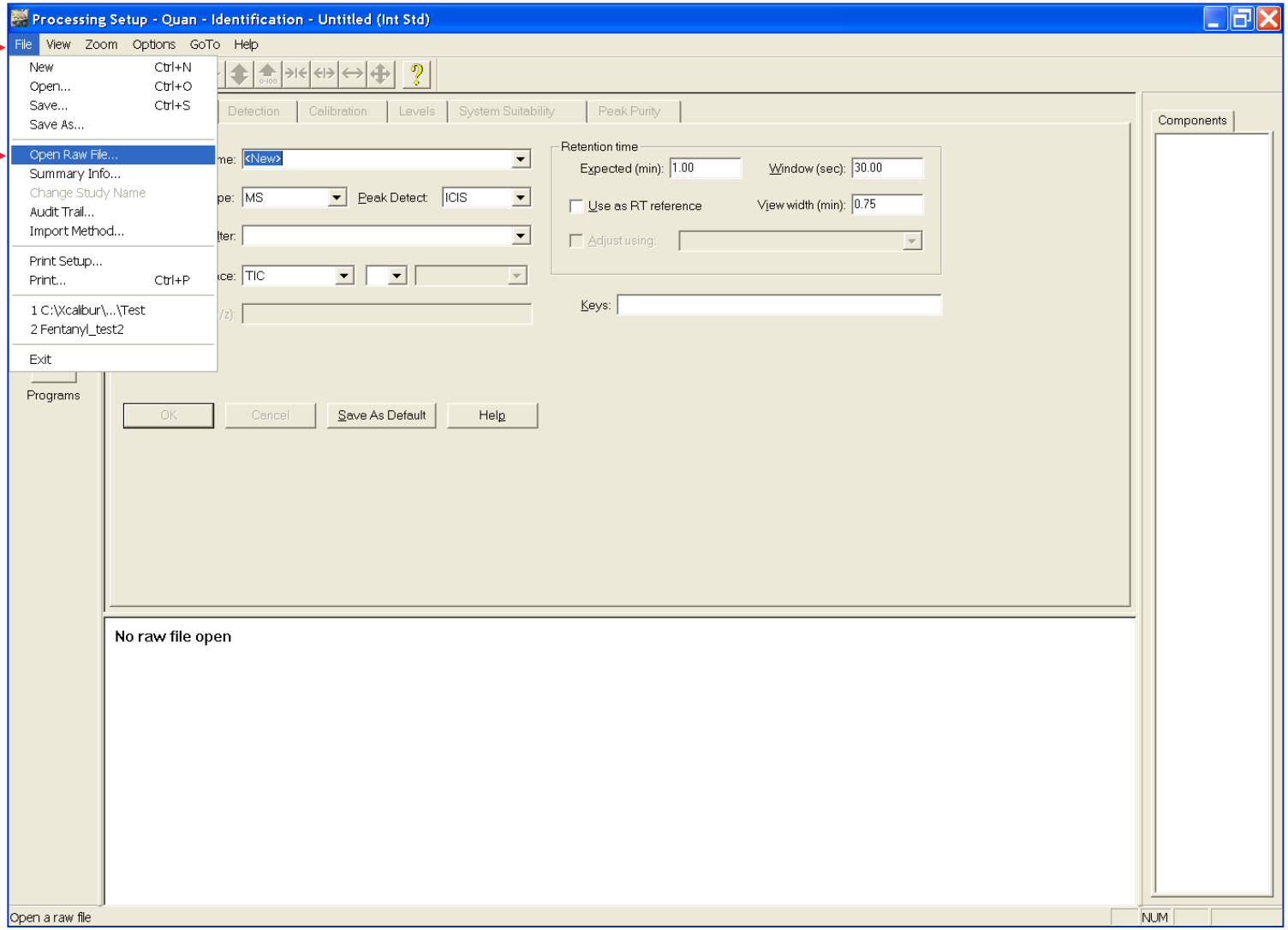
Quantitation Options



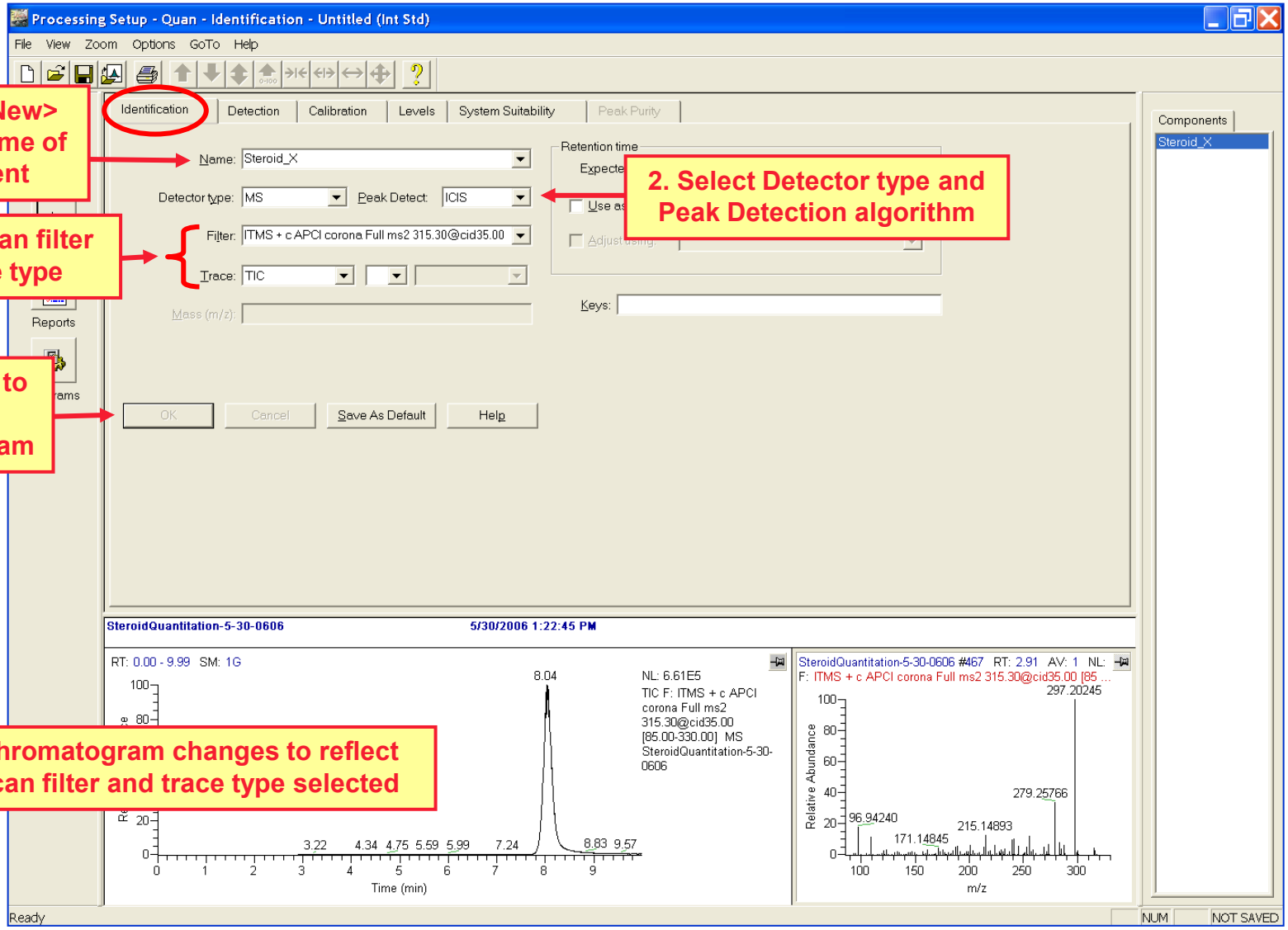
Open a Raw File to Set Up the Processing Method

1. Click File

2. Click Open Raw File…



Quan Processing – Identification Tab



1. Select <New> and type name of component

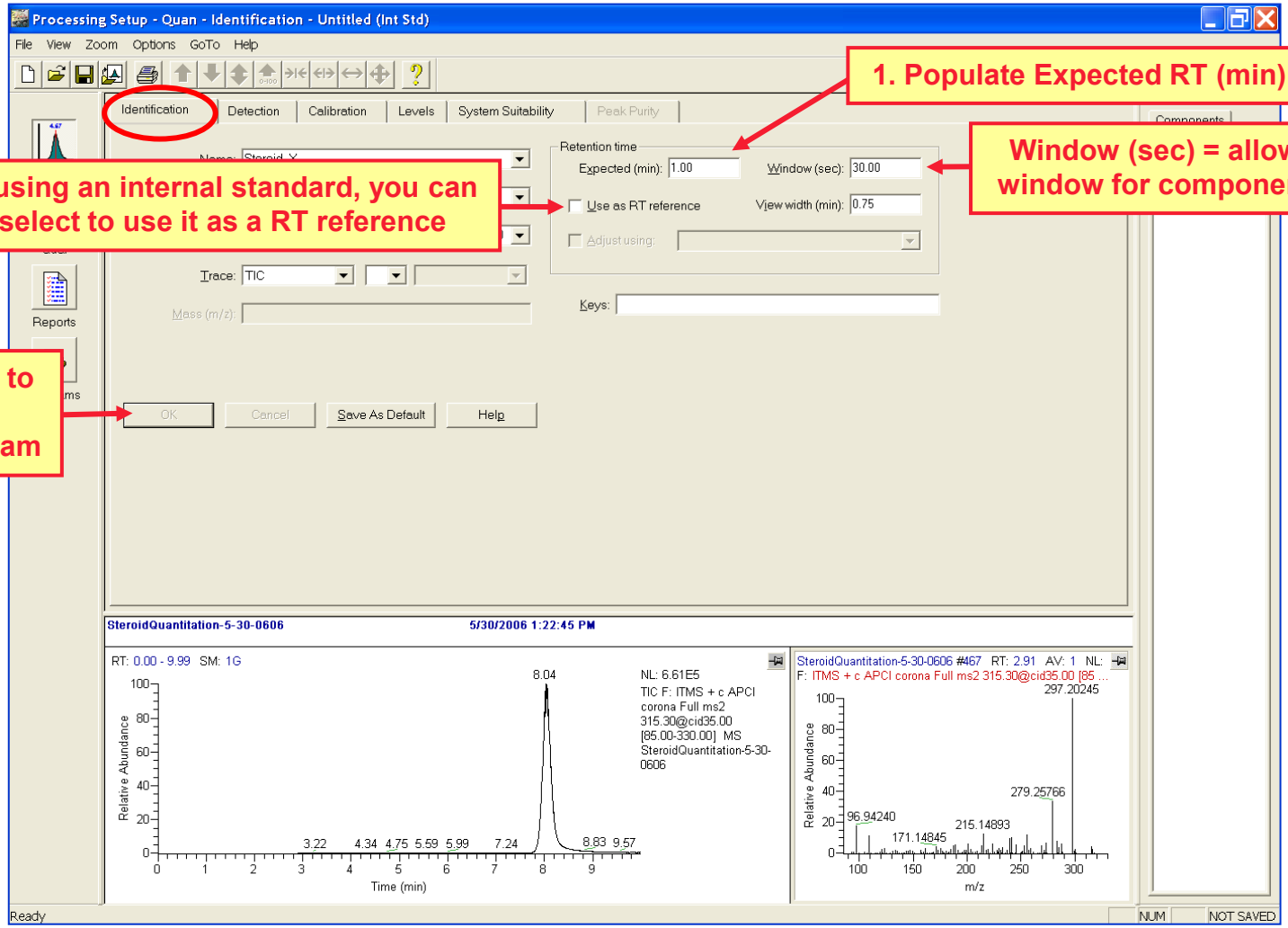
2. Select Detector type and Peak Detection algorithm

3. Select scan filter and trace type

4. Click OK to update chromatogram

Chromatogram changes to reflect scan filter and trace type selected

Quan Processing – Identification Tab



1. Populate Expected RT (min)

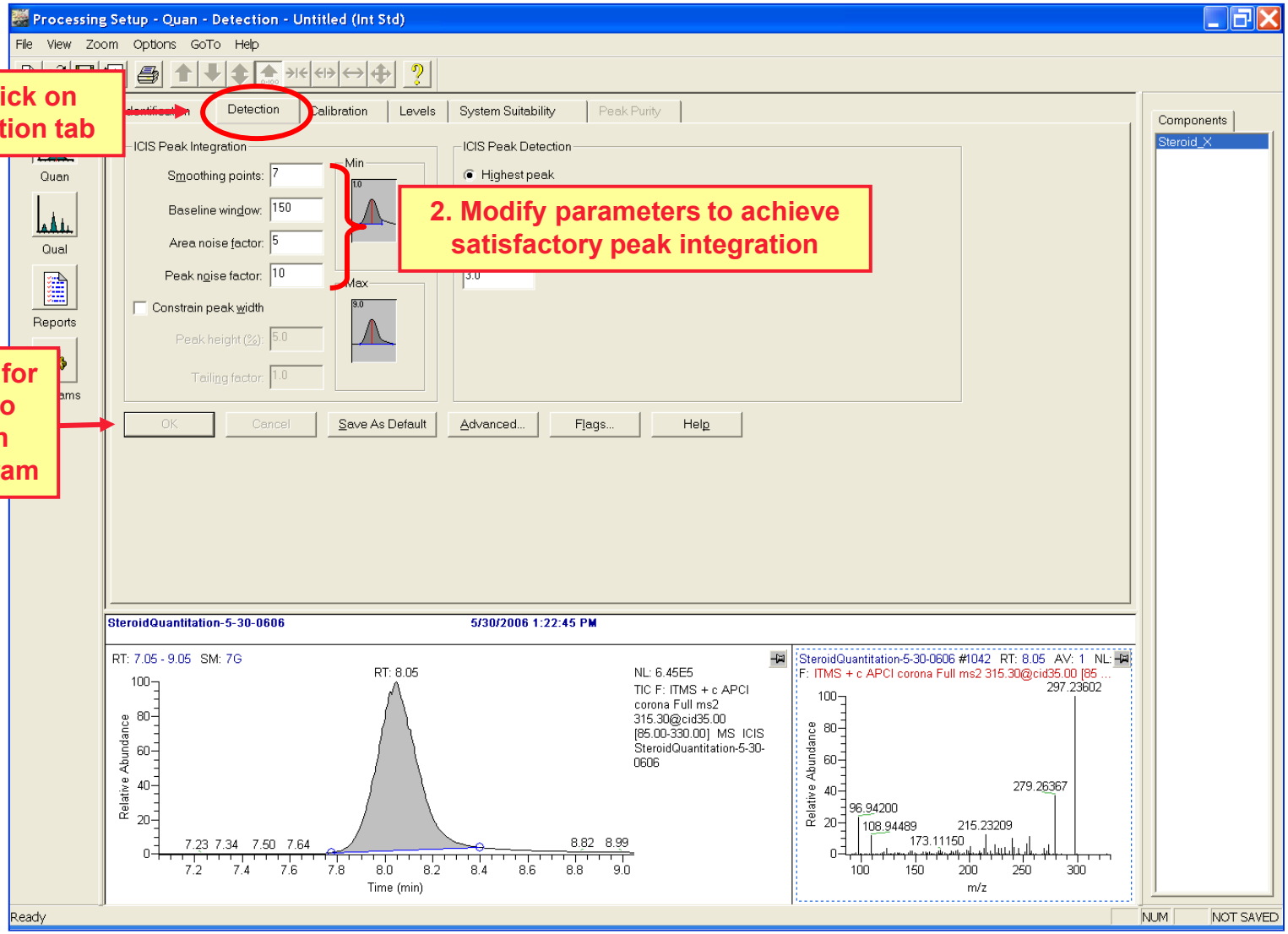
Window (sec) = allowable RT window for component elution

If using an internal standard, you can select to use it as a RT reference

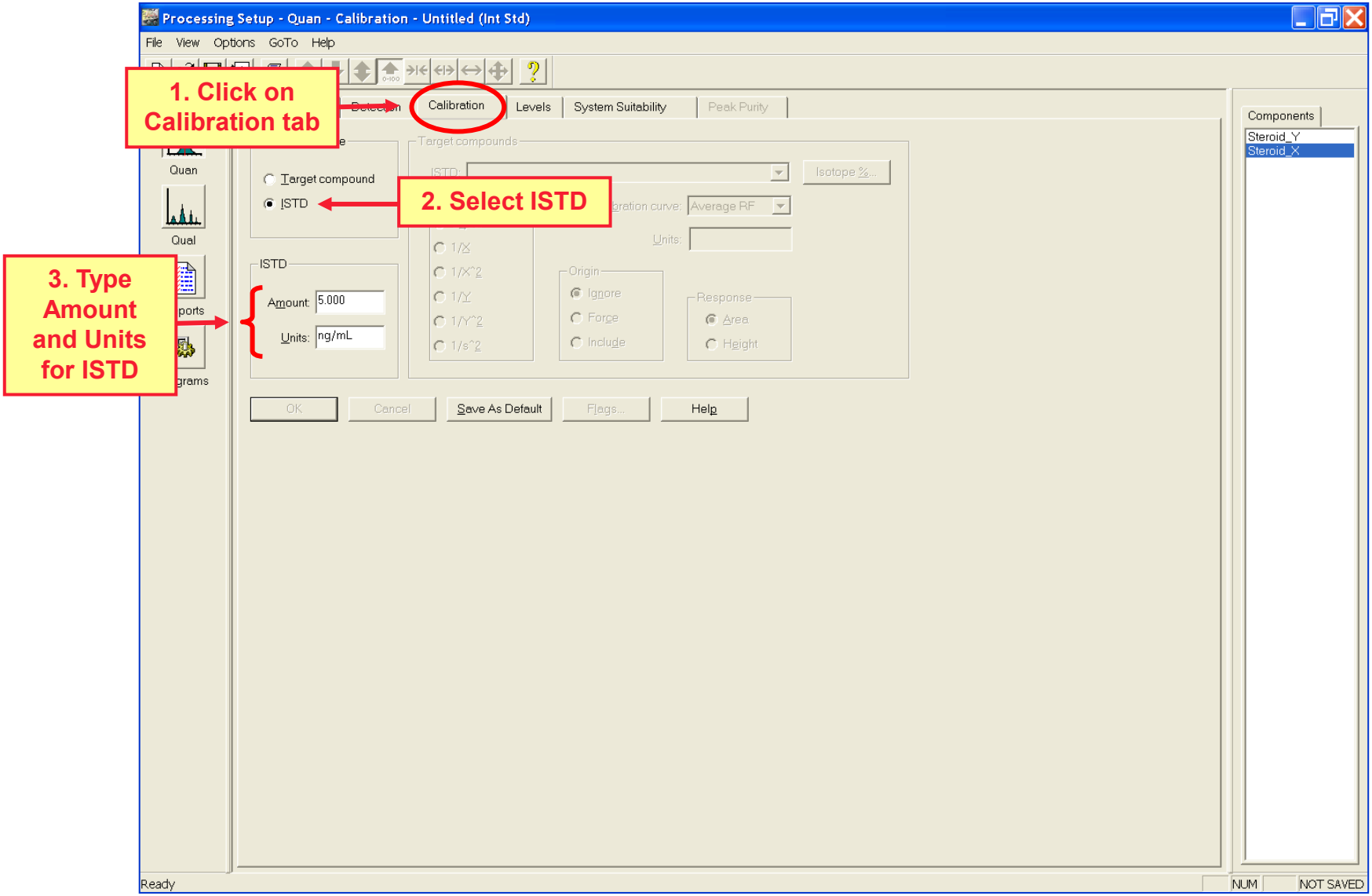
2. Click OK to update chromatogram

*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.

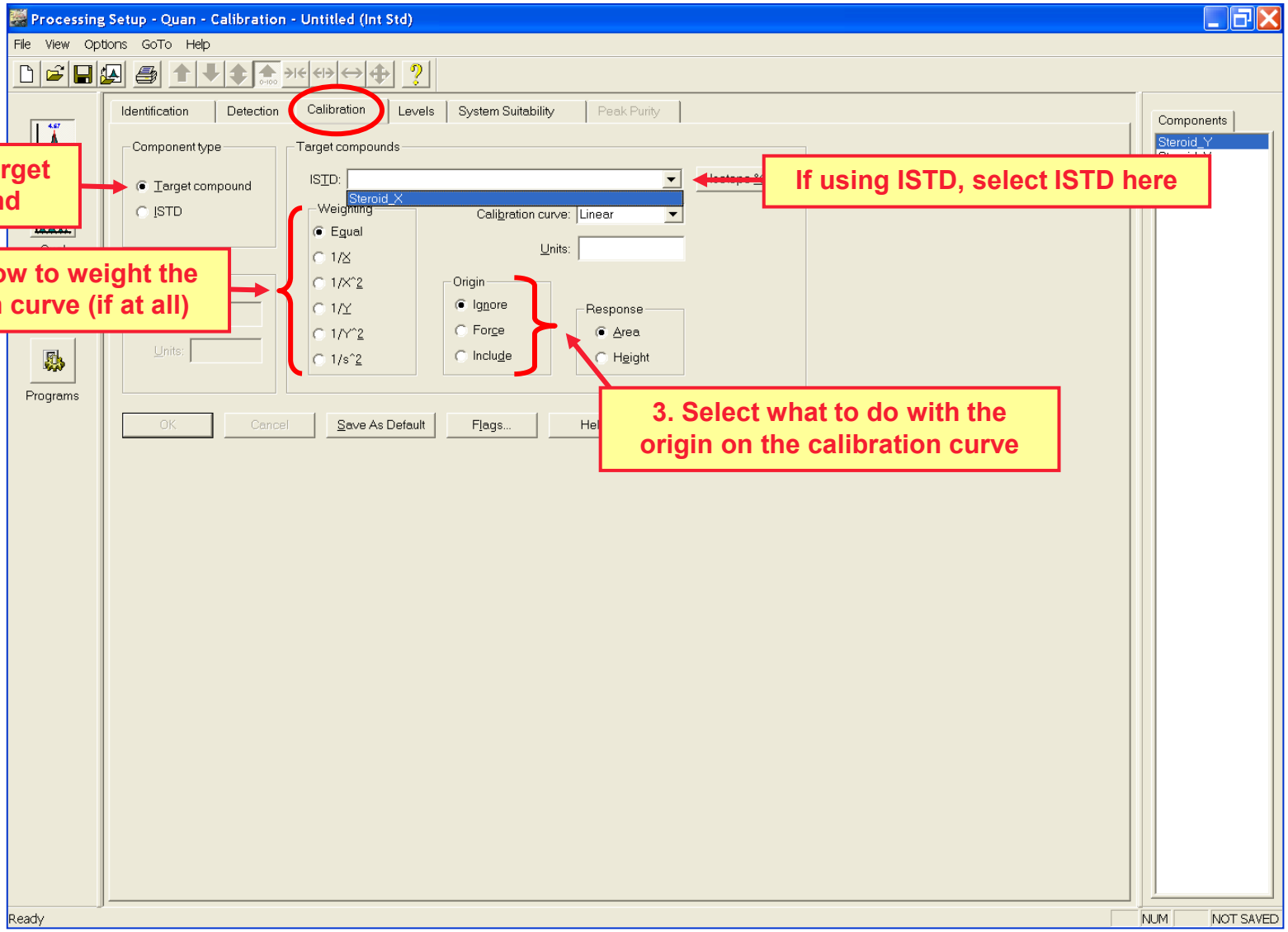
Quan Processing – Detection Tab



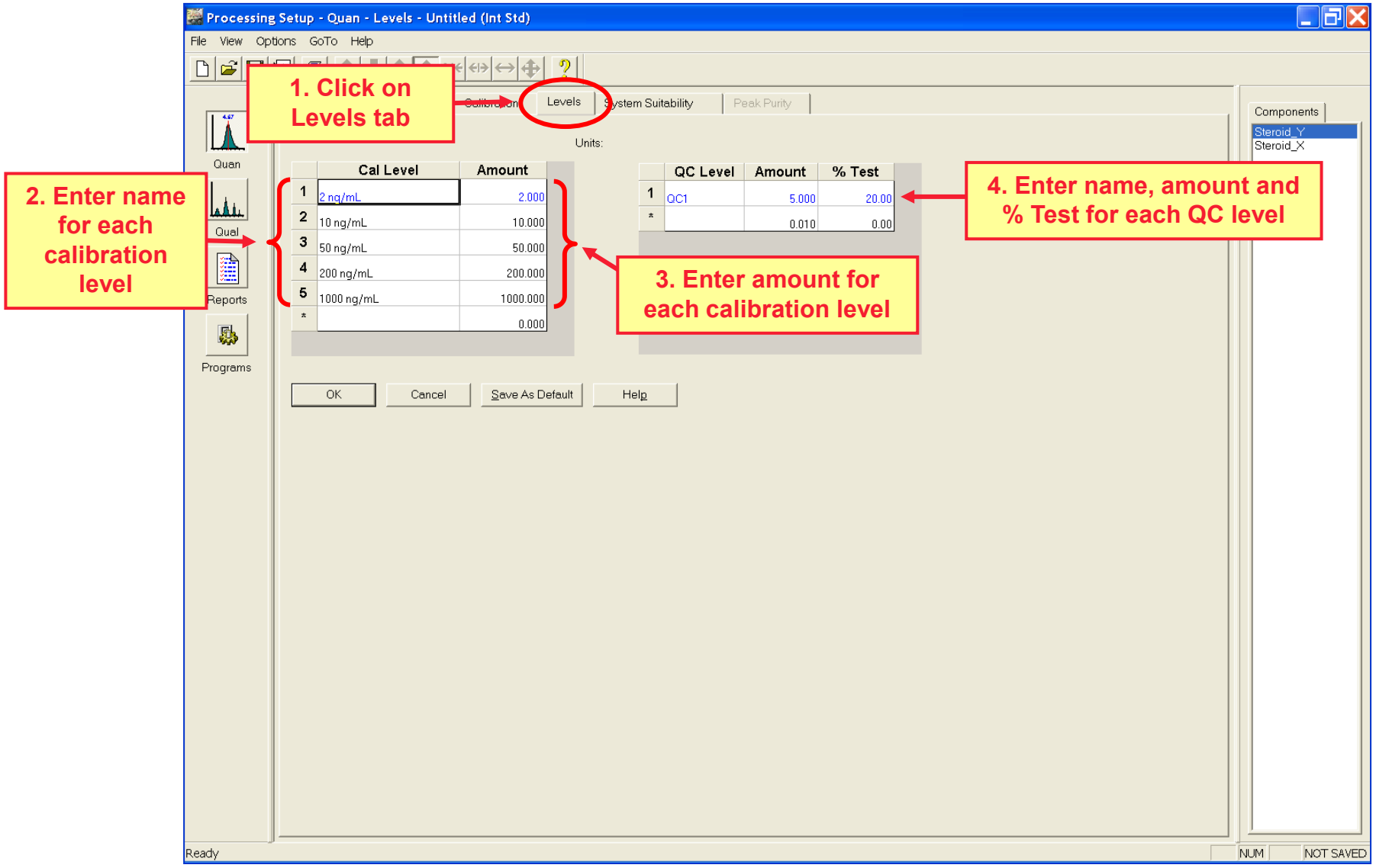
Quan Processing - Calibration Tab
Internal Standard Setup



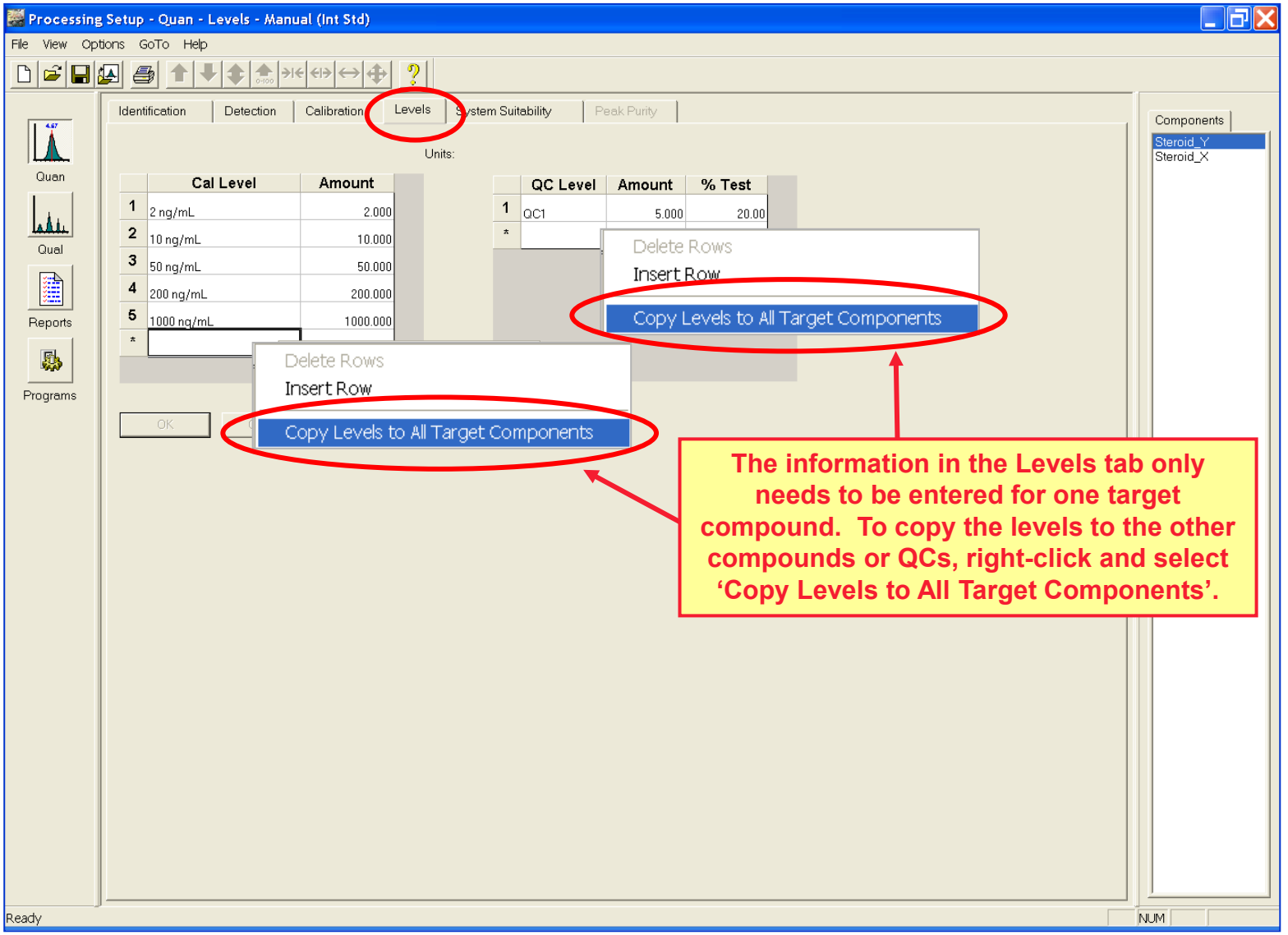
Quan Processing - Calibration Tab
Target Compound Setup



Quan Processing – Levels Tab

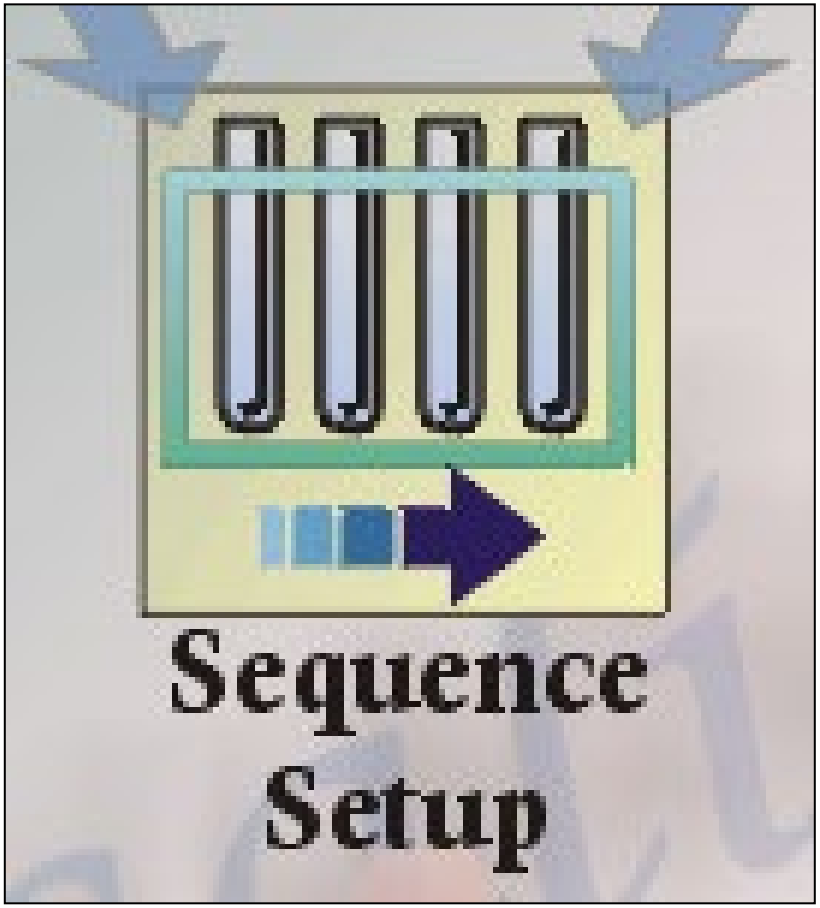


Copying Levels to All Target Compounds



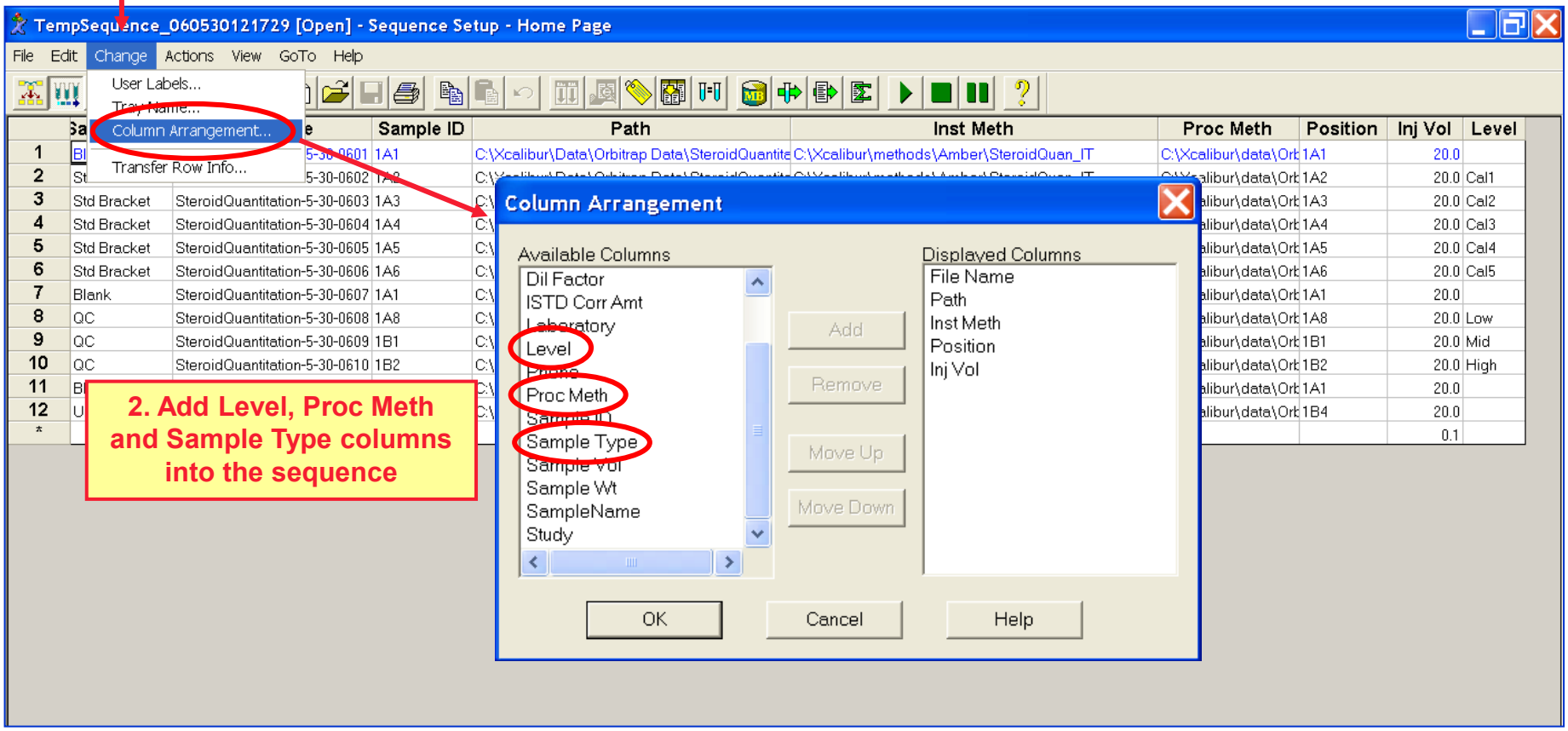
Quan Processing/Reprocessing

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



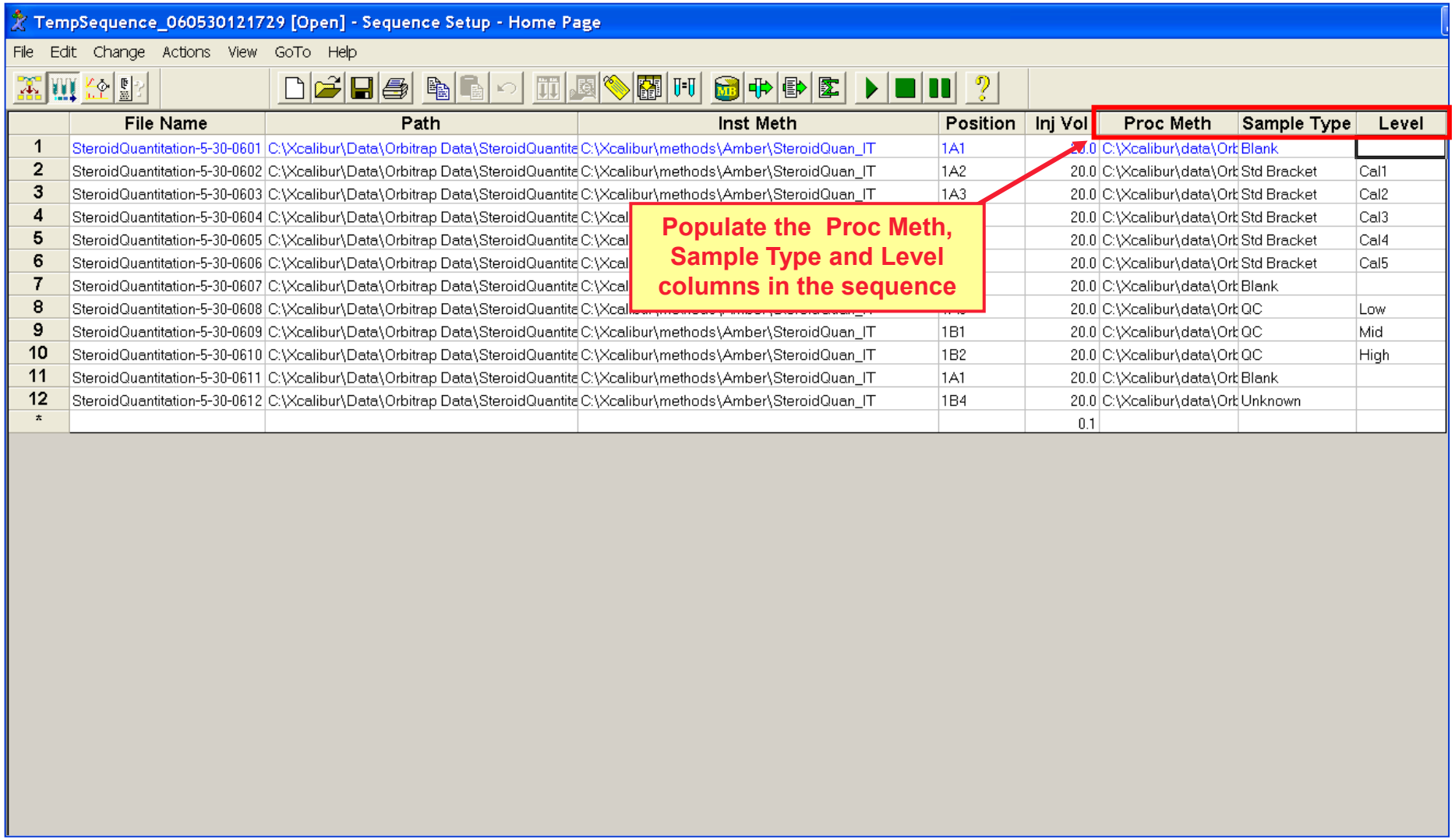
Open the Sequence and Add Extra Columns

1. Click Change and select Column Arrangement



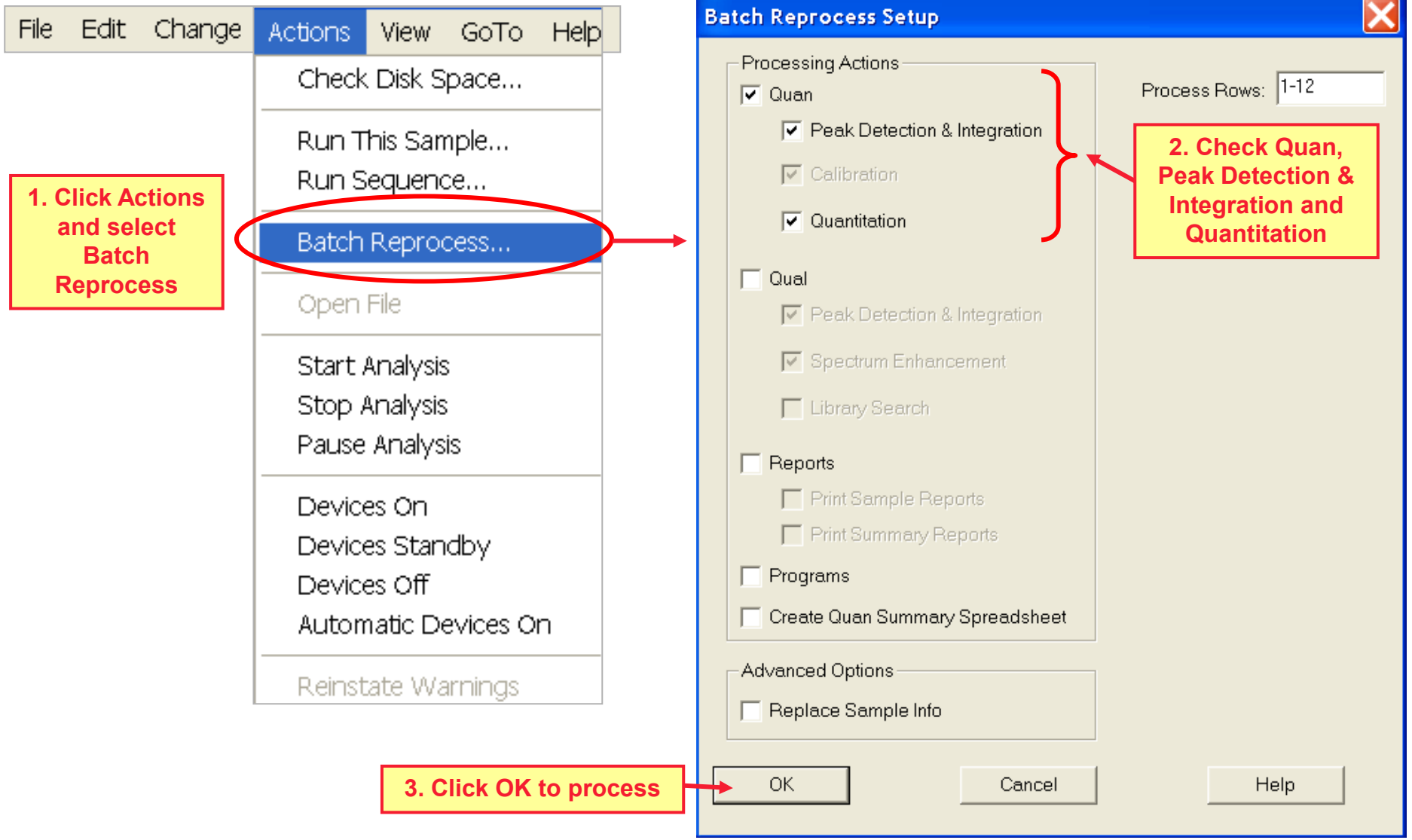
2. Add Level, Proc Meth and Sample Type columns into the sequence

Enter Information into the Sequence

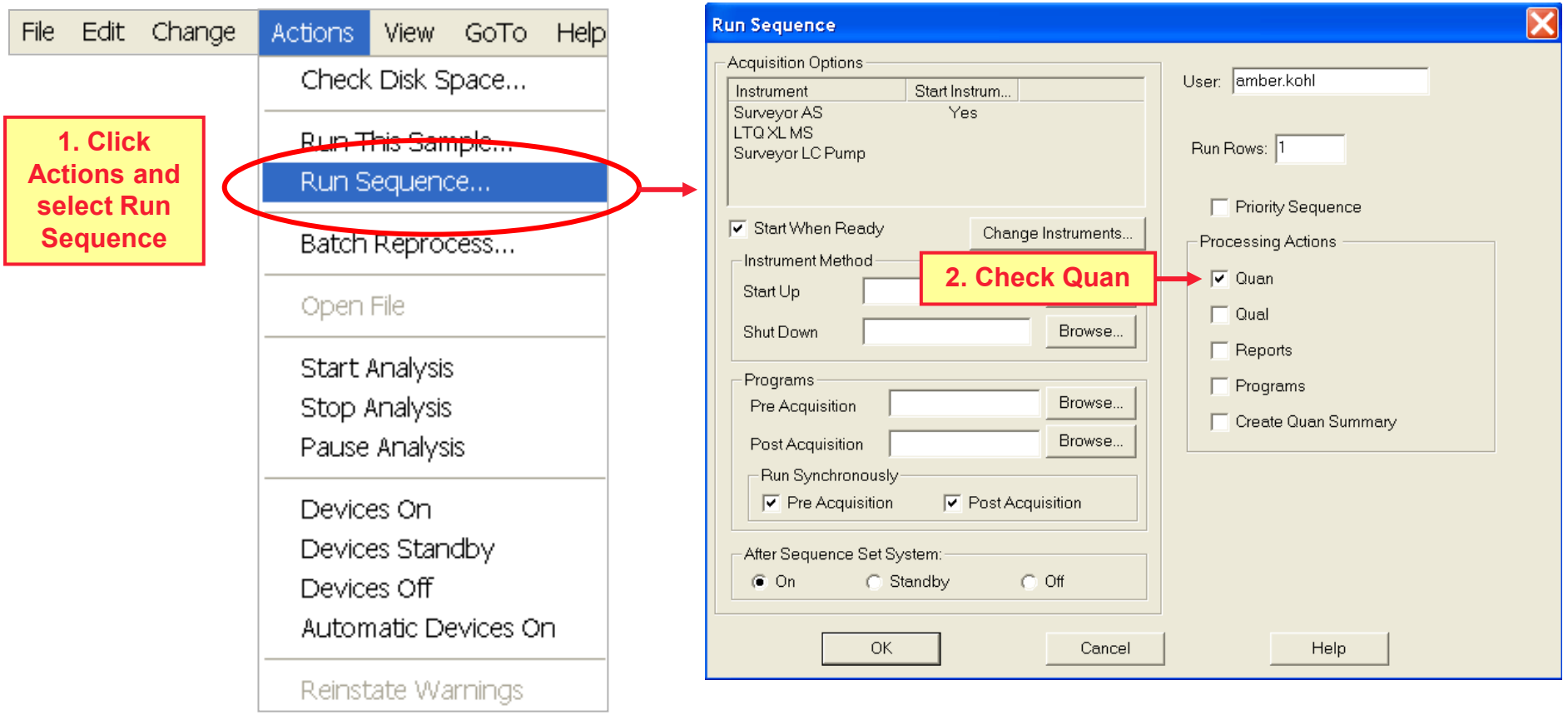


	File Name	Path	Inst Meth	Position	Inj Vol	Proc Meth	Sample Type	Level
1	SteroidQuantitation-5-30-0601	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	1A1	20.0	C:\Xcalibur\data\Orb	Blank	
2	SteroidQuantitation-5-30-0602	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite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

Batch Reprocessing Quantitative Data

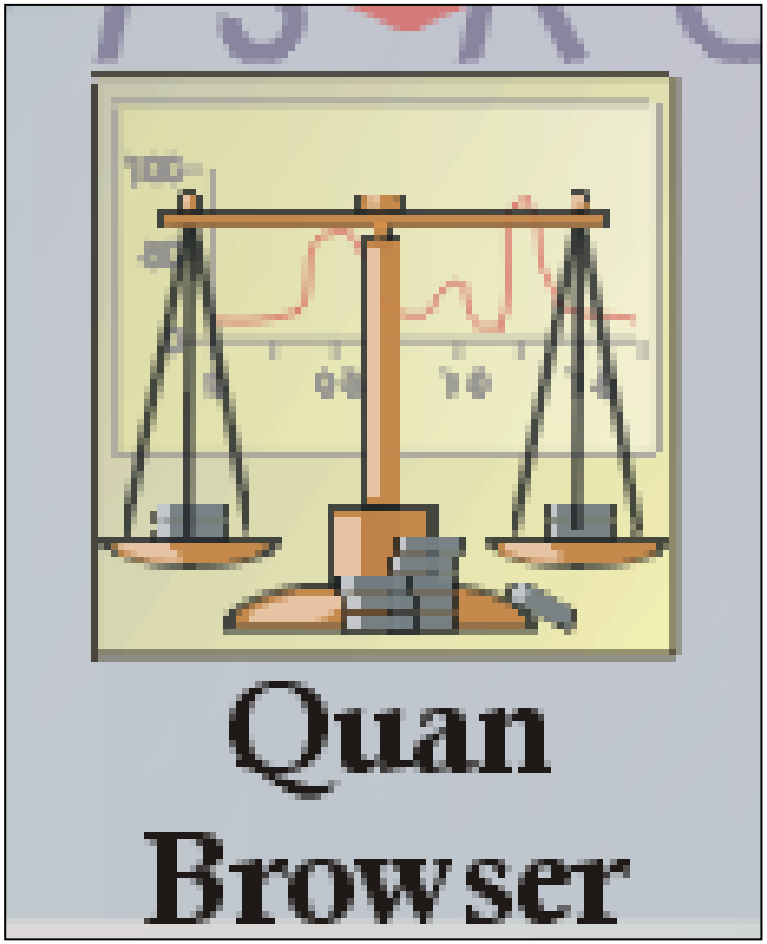


Enabling Quantitative Processing During Acquisition

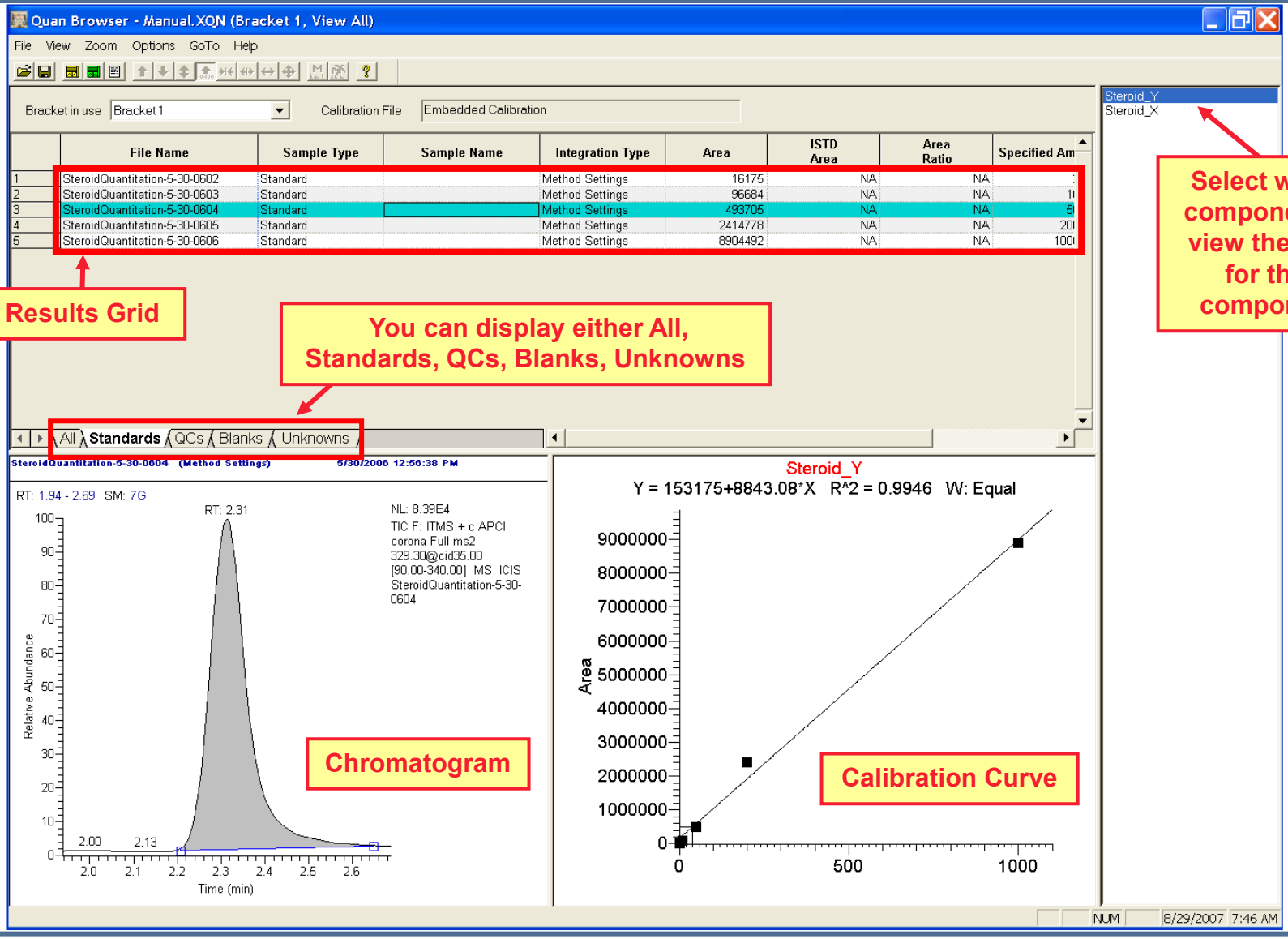


Quan Browser

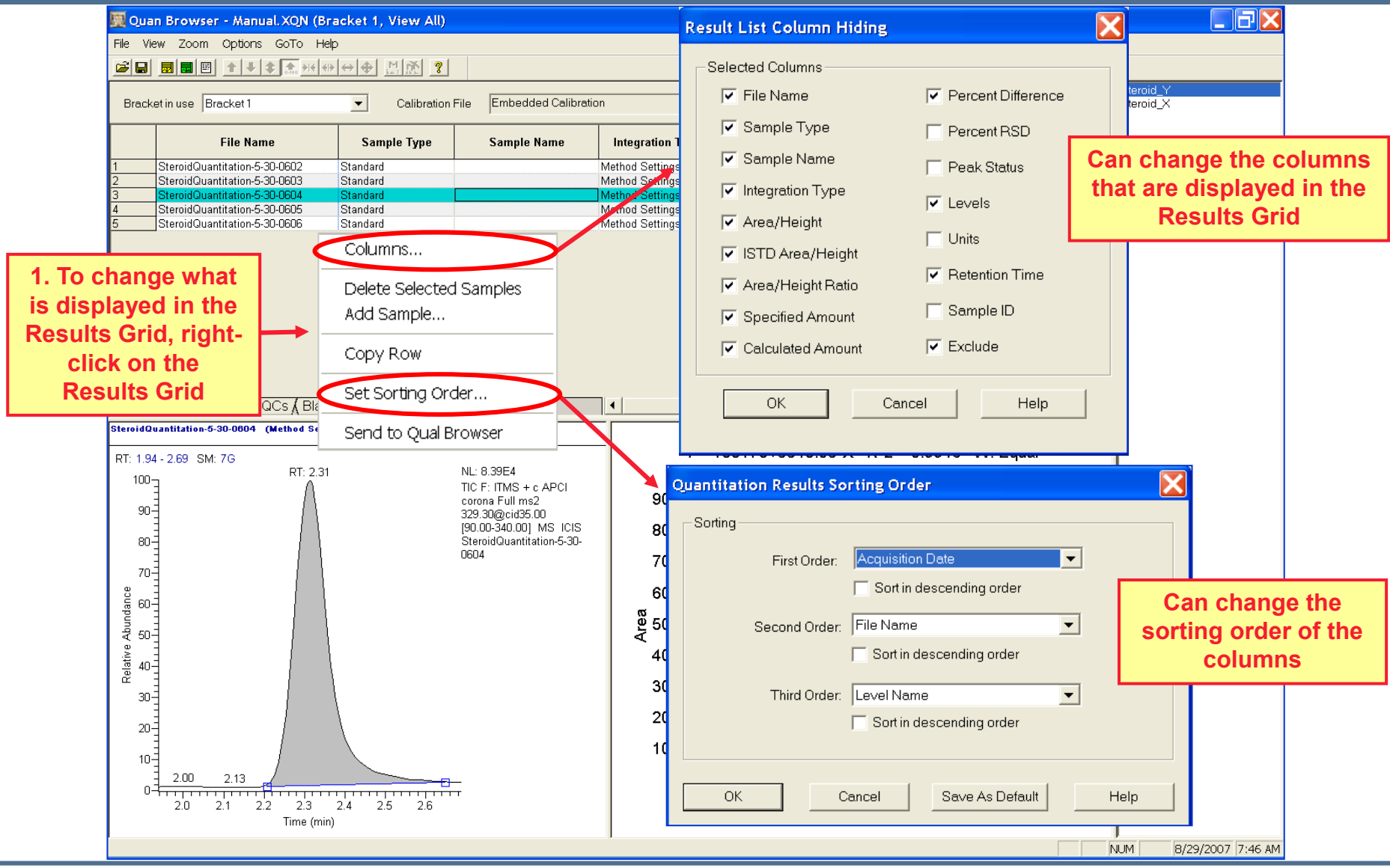
To view the processed data, click on the Quan Browser button on the Xcalibur Homepage



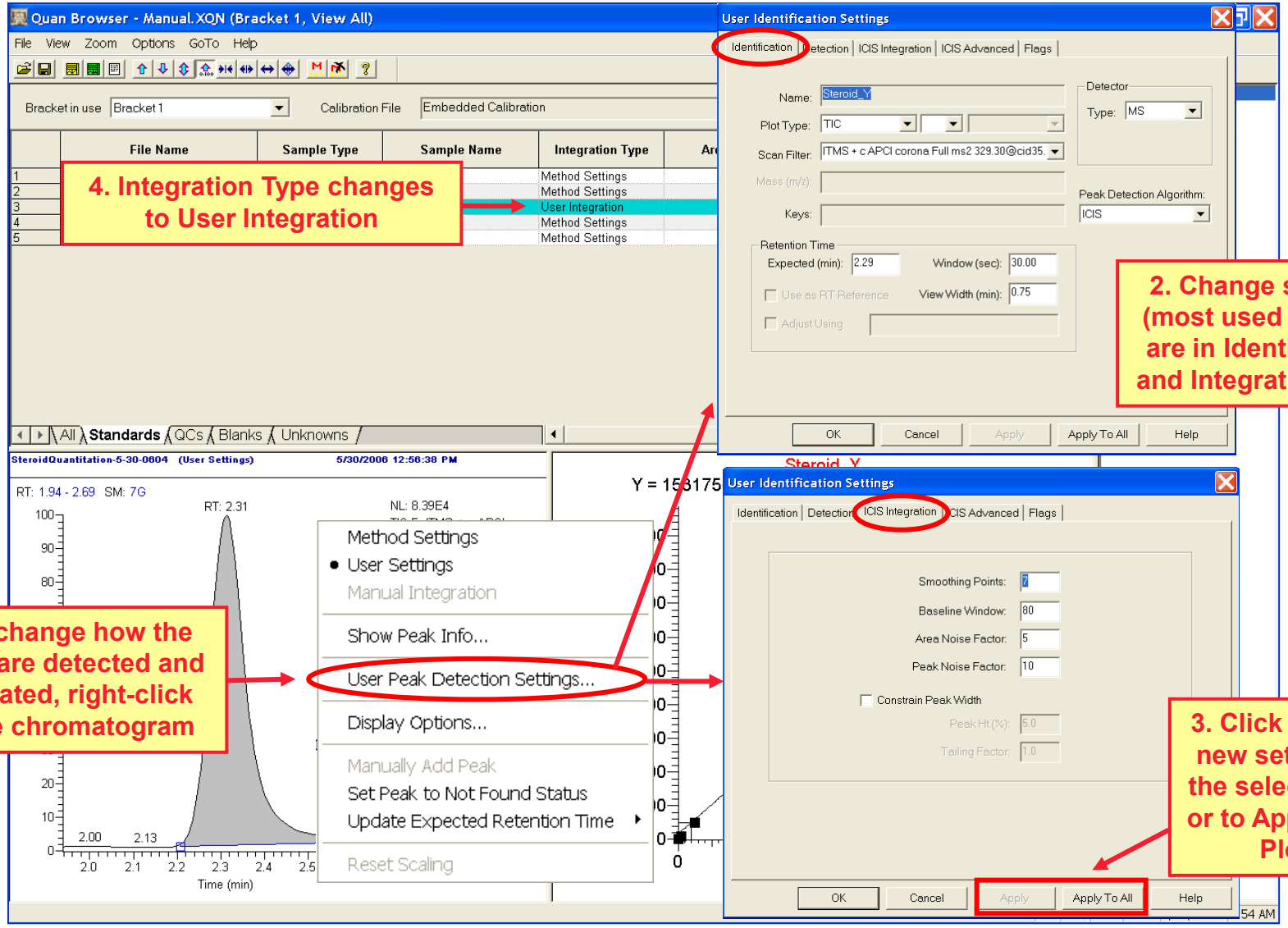
Quan Browser Main View



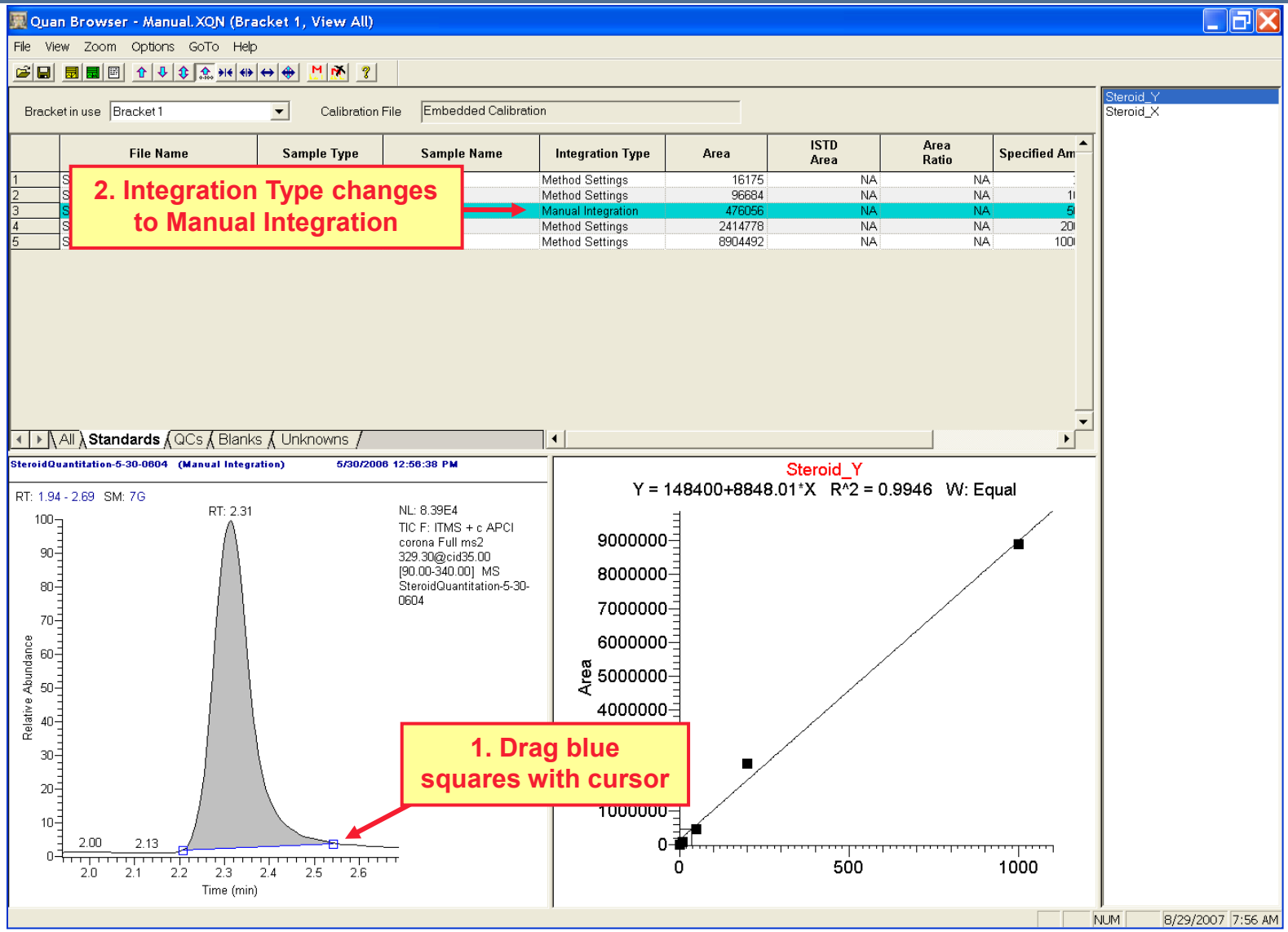
Changing the Results Grid Display



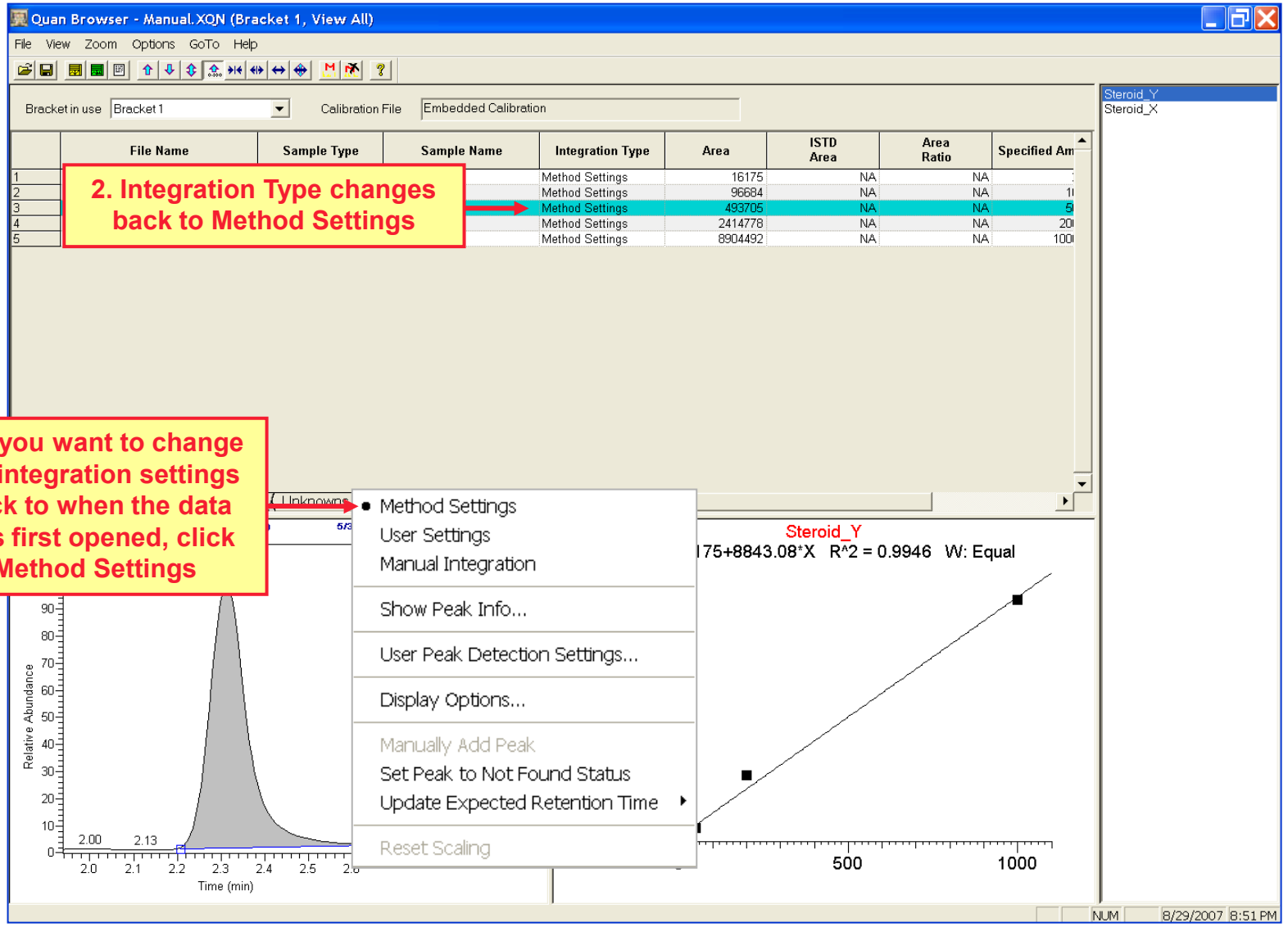
Changing Peak Detection/Integration Parameters



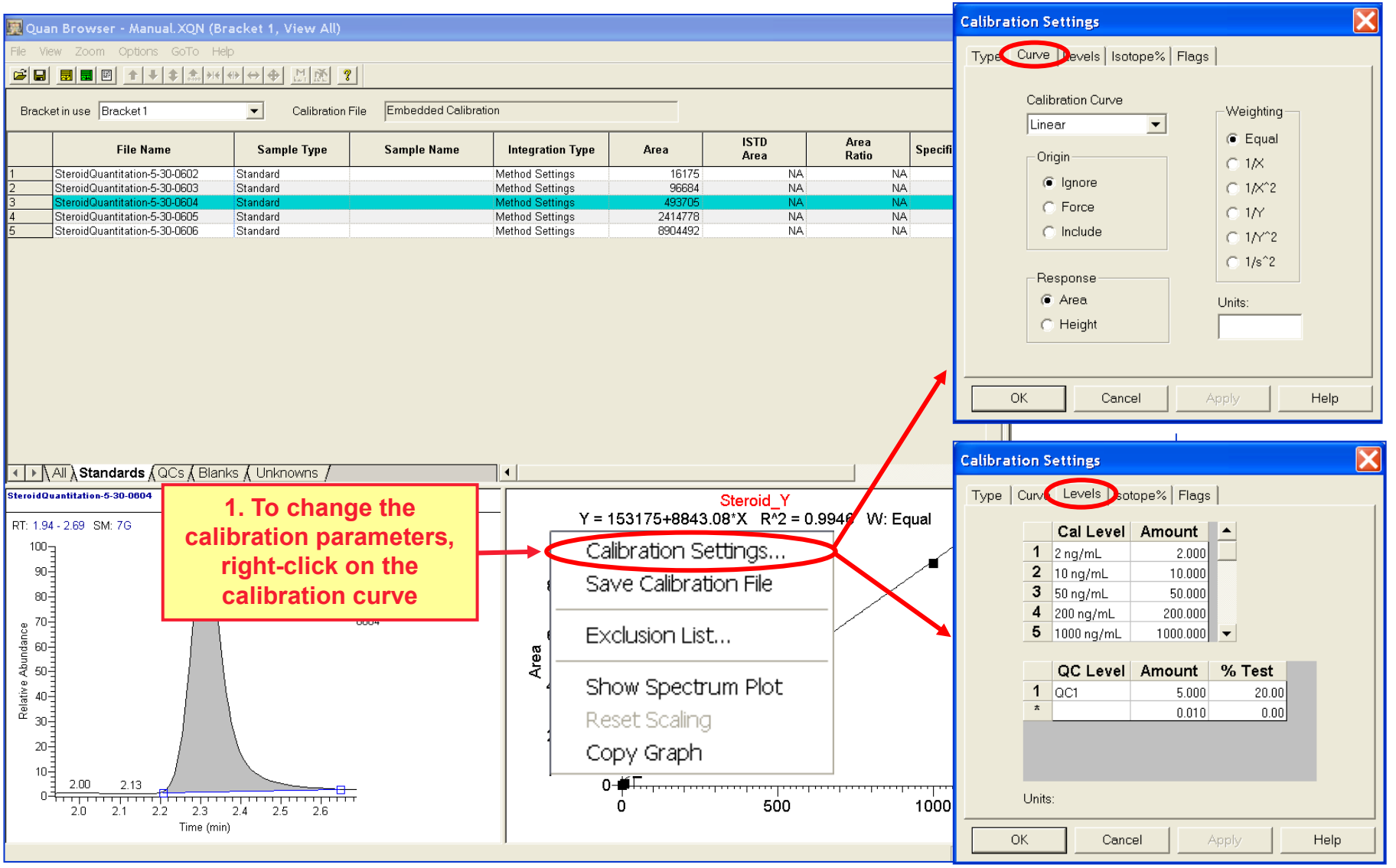
Changing Peak Integration Parameters Manually



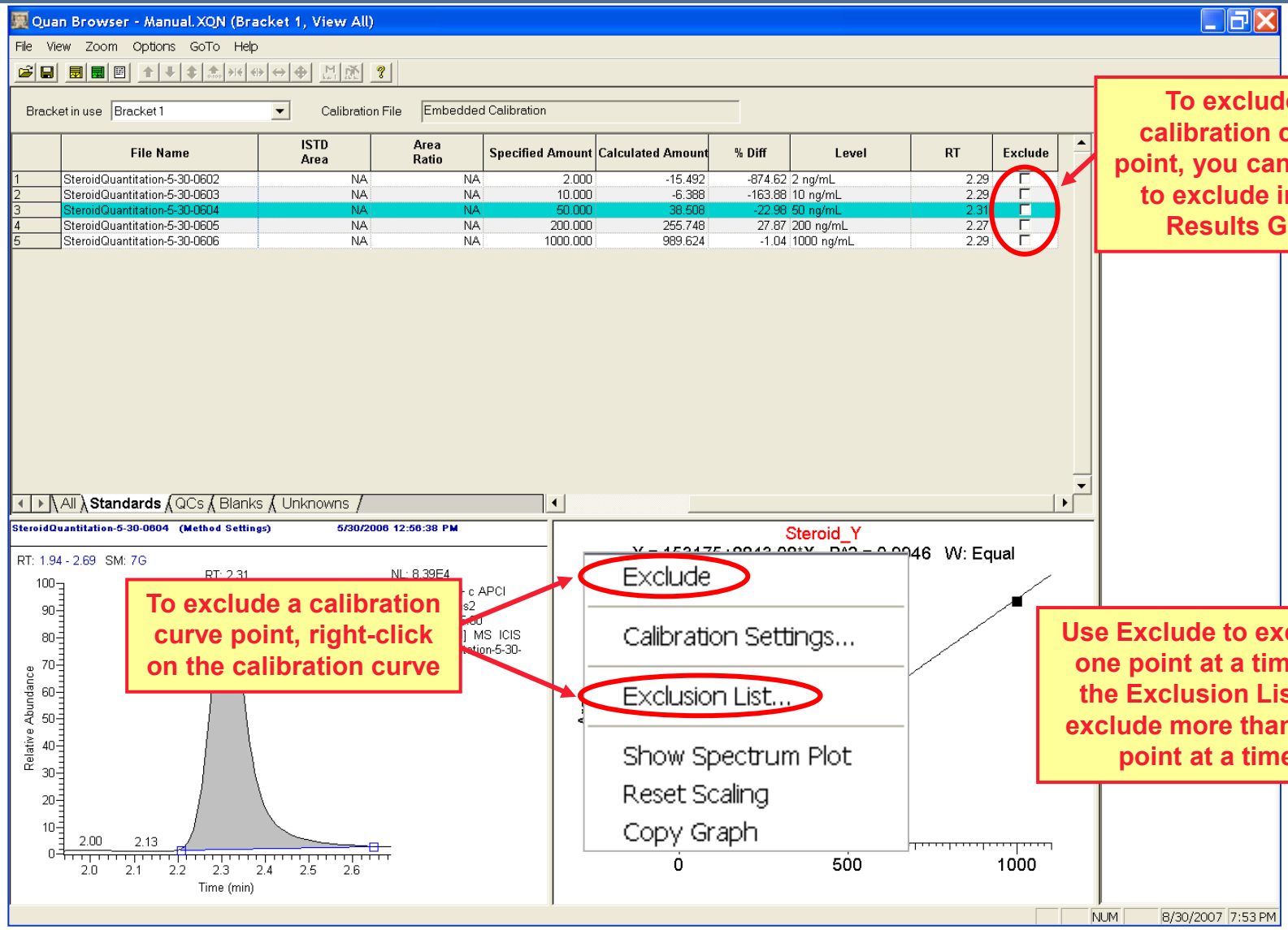
Changing Back to Original Integration (Method Settings)



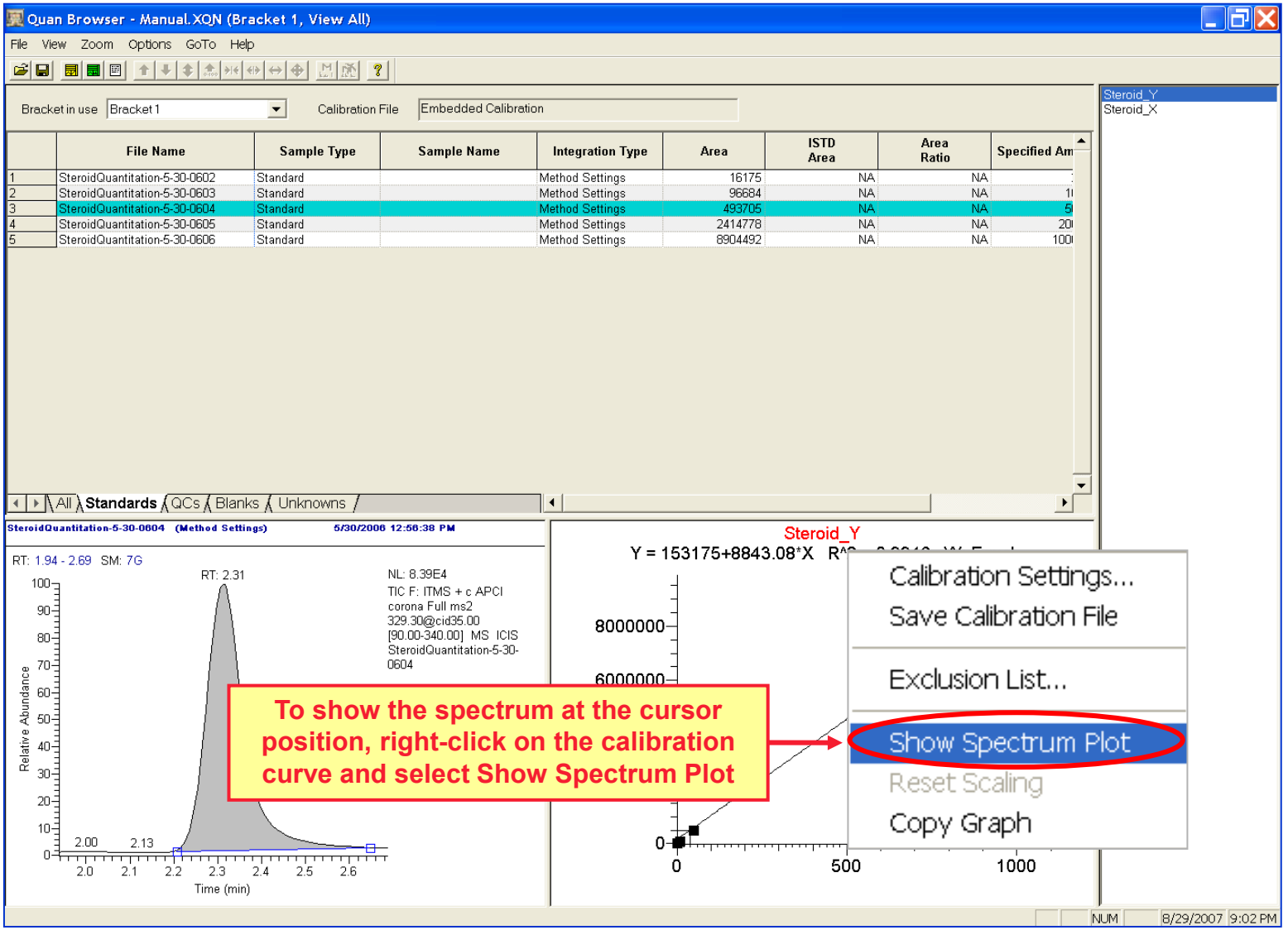
Changing Calibration Parameters



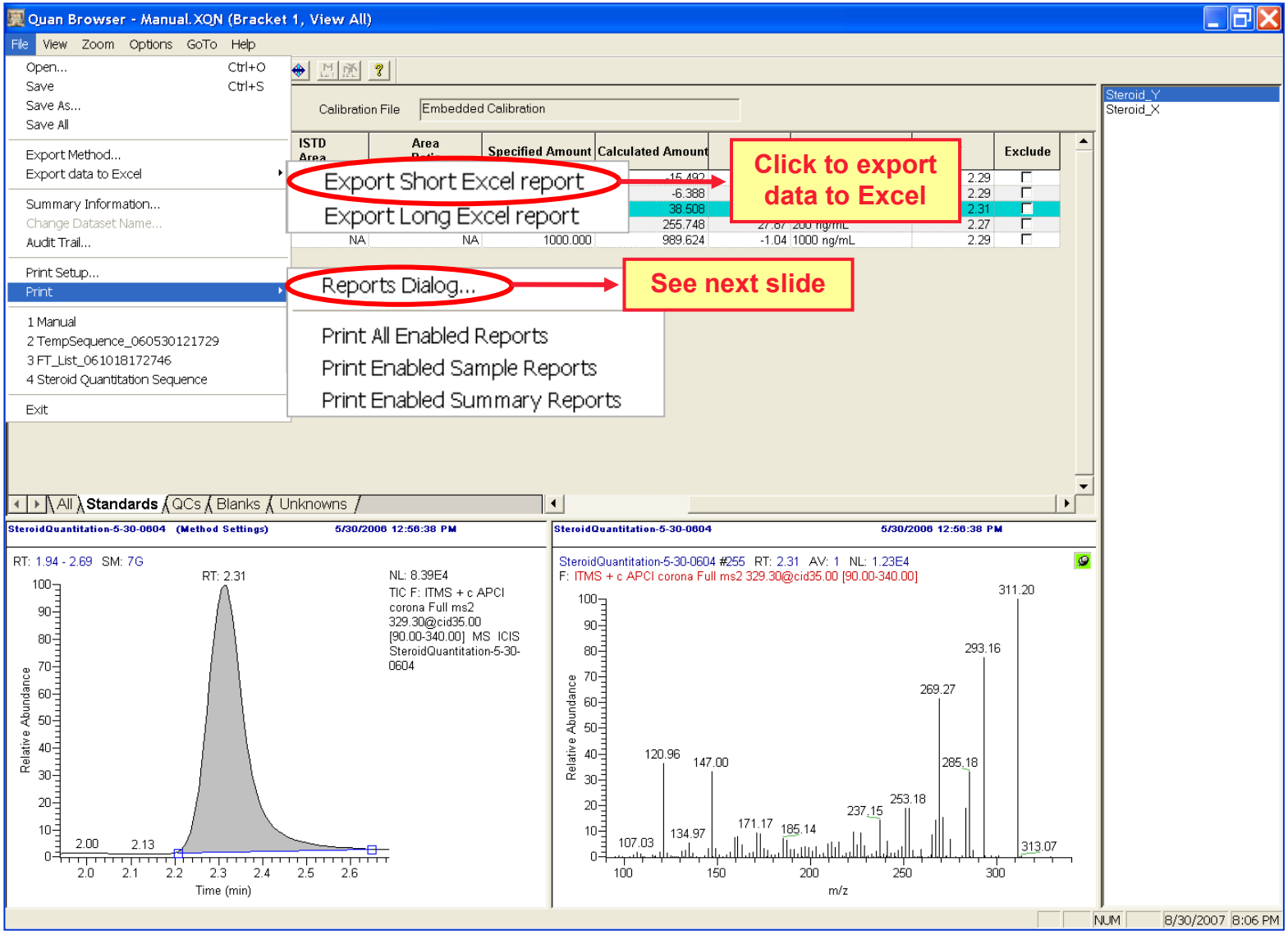
Ways to Exclude a Calibration Curve Point



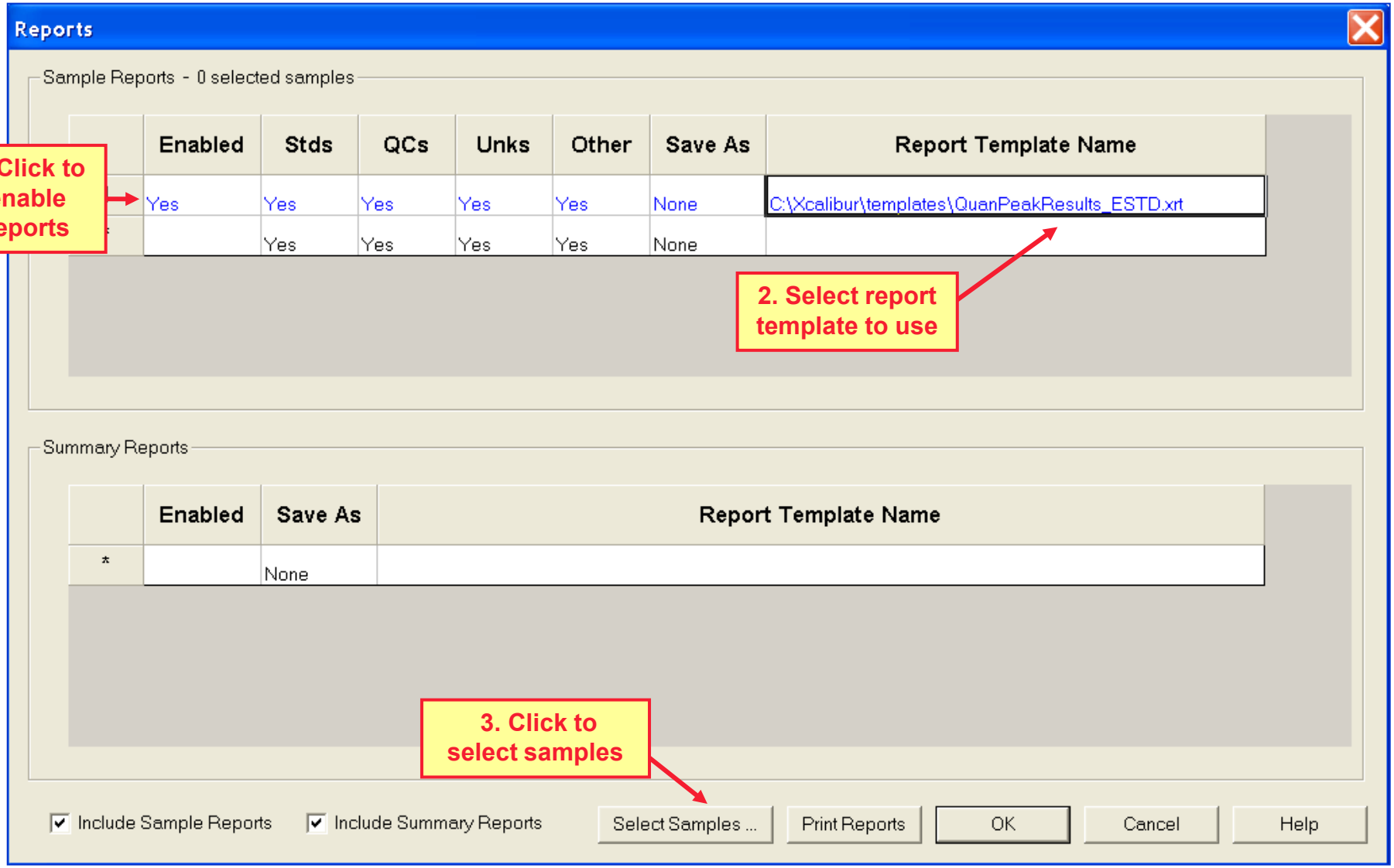
Showing the Spectrum Plot Instead of the Calibration Curve



Exporting Data to Excel and Printing Reports



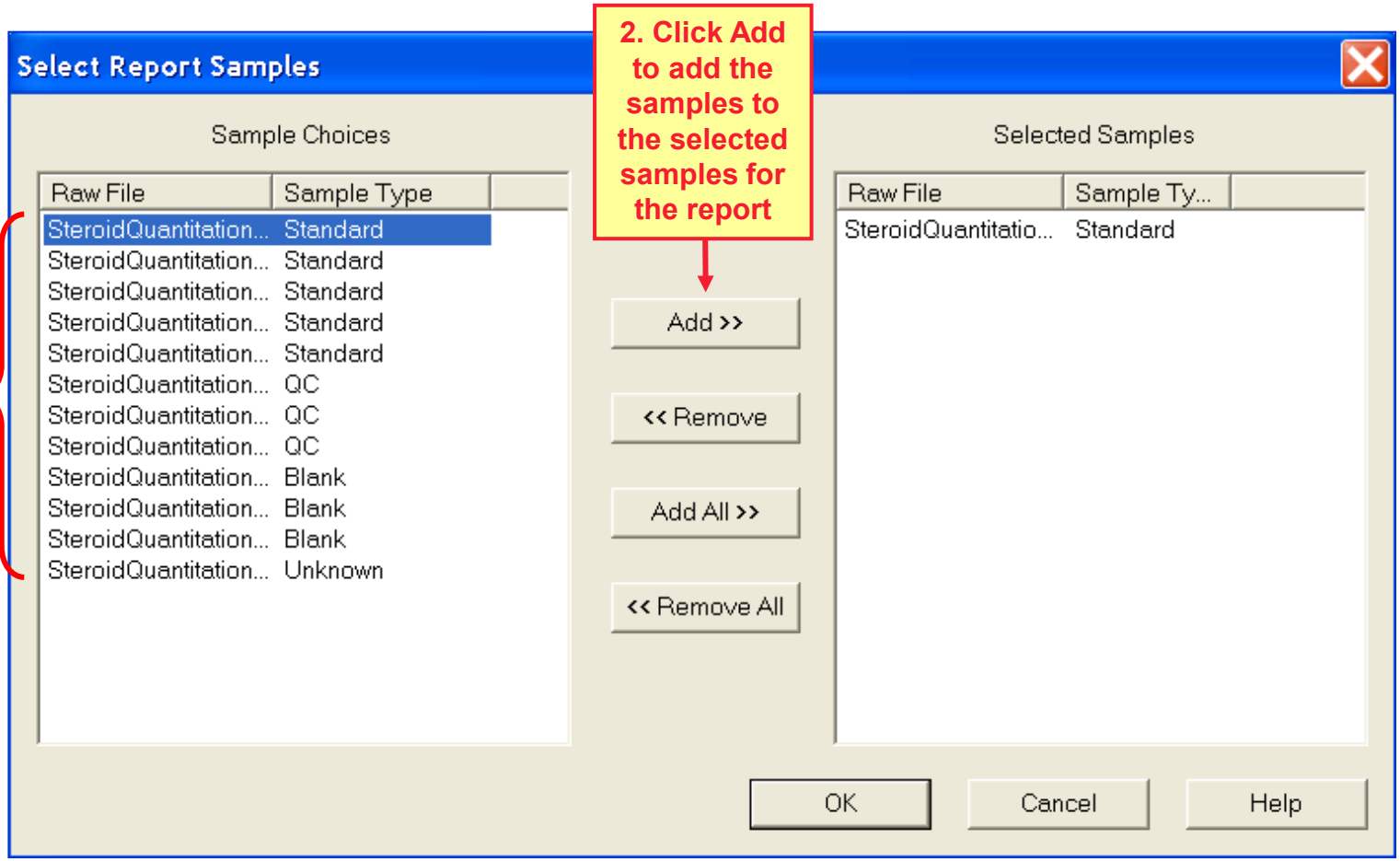
Printing Reports



Selecting Samples to Include in the Report

1. Select which samples to include from the sample choices (hold CTRL or SHIFT to select multiple samples)

2. Click Add to add the samples to the selected samples for the report



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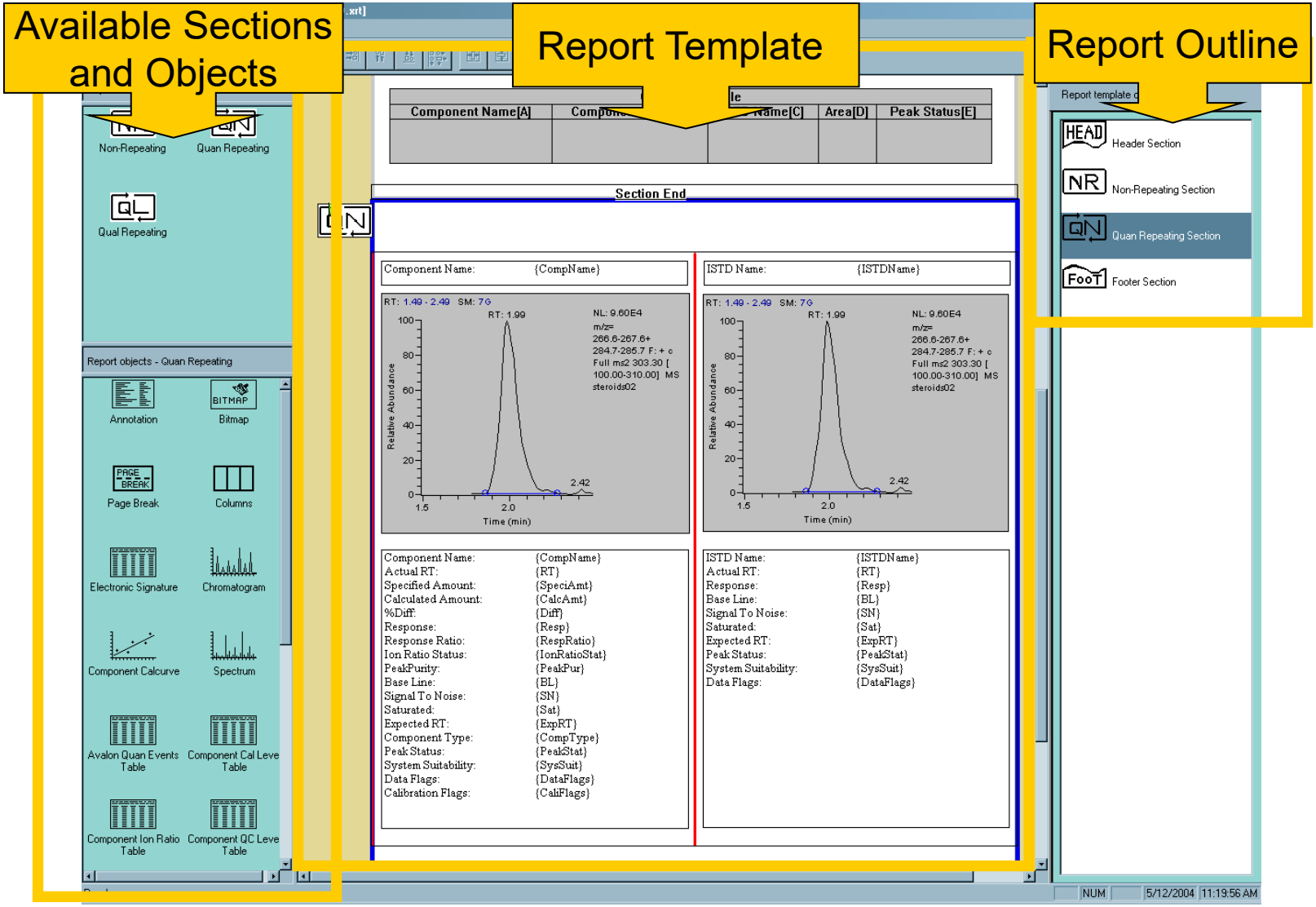
XReport 1.0

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



Steps to XReport Reporting

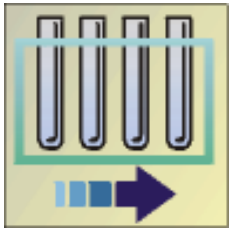
1. XReport

- 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

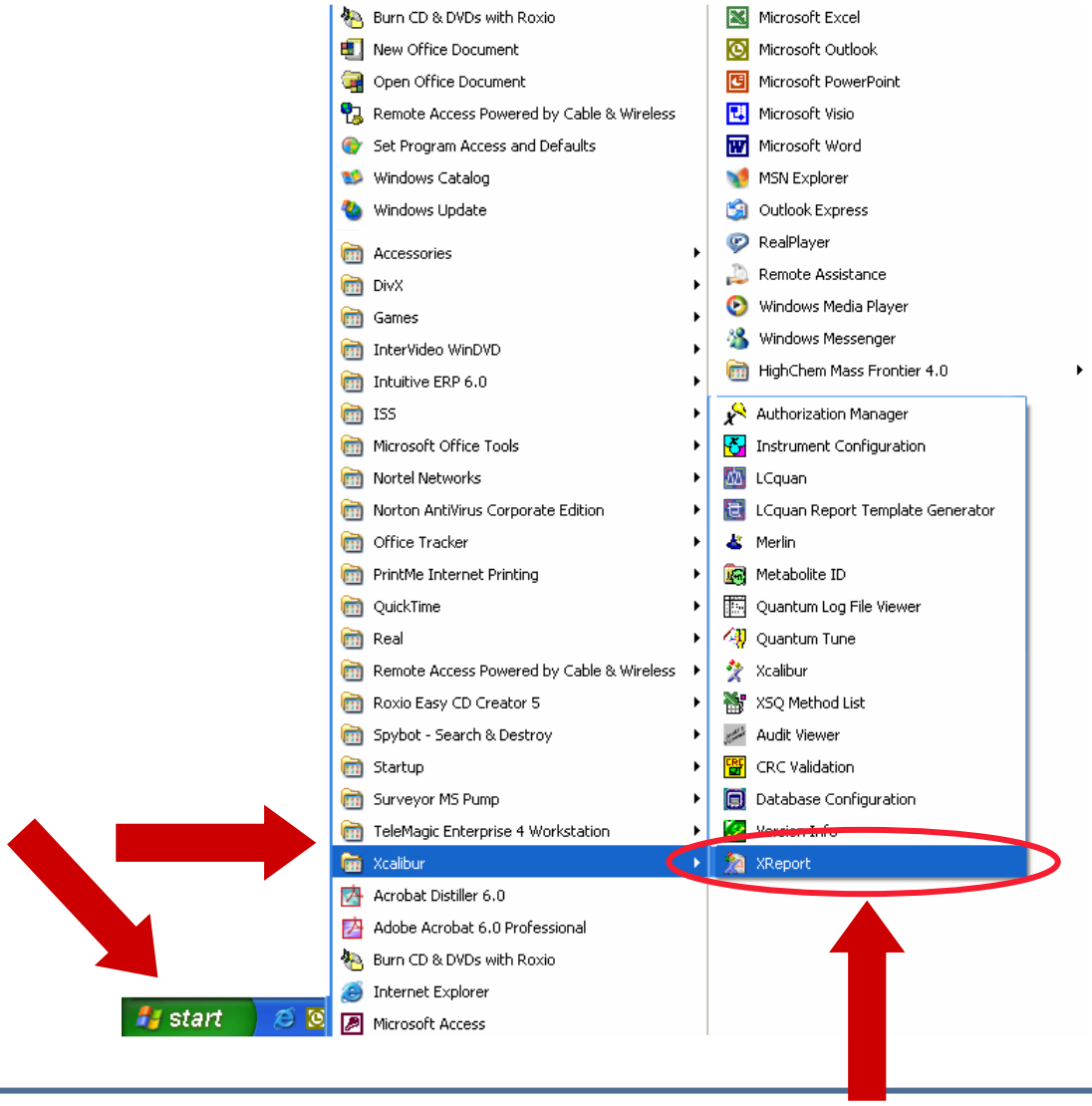


- Go to Home Page - Sequence Setup
- Open/Make a Sequence
- Click Actions : Batch Reprocess...
- Check Reports and Print Reports Boxes

Before you Start

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

Open XReport



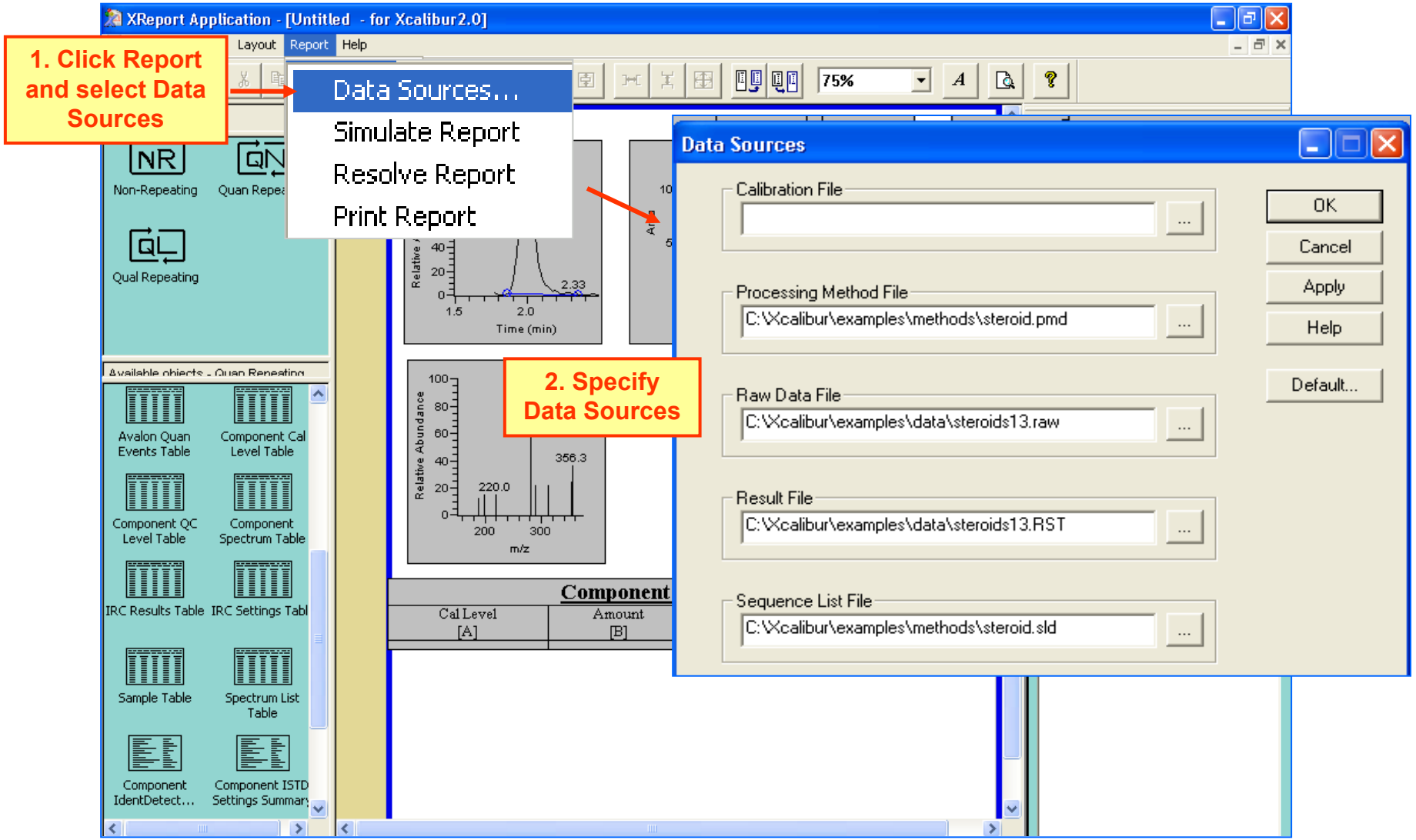
Click Start

All Programs

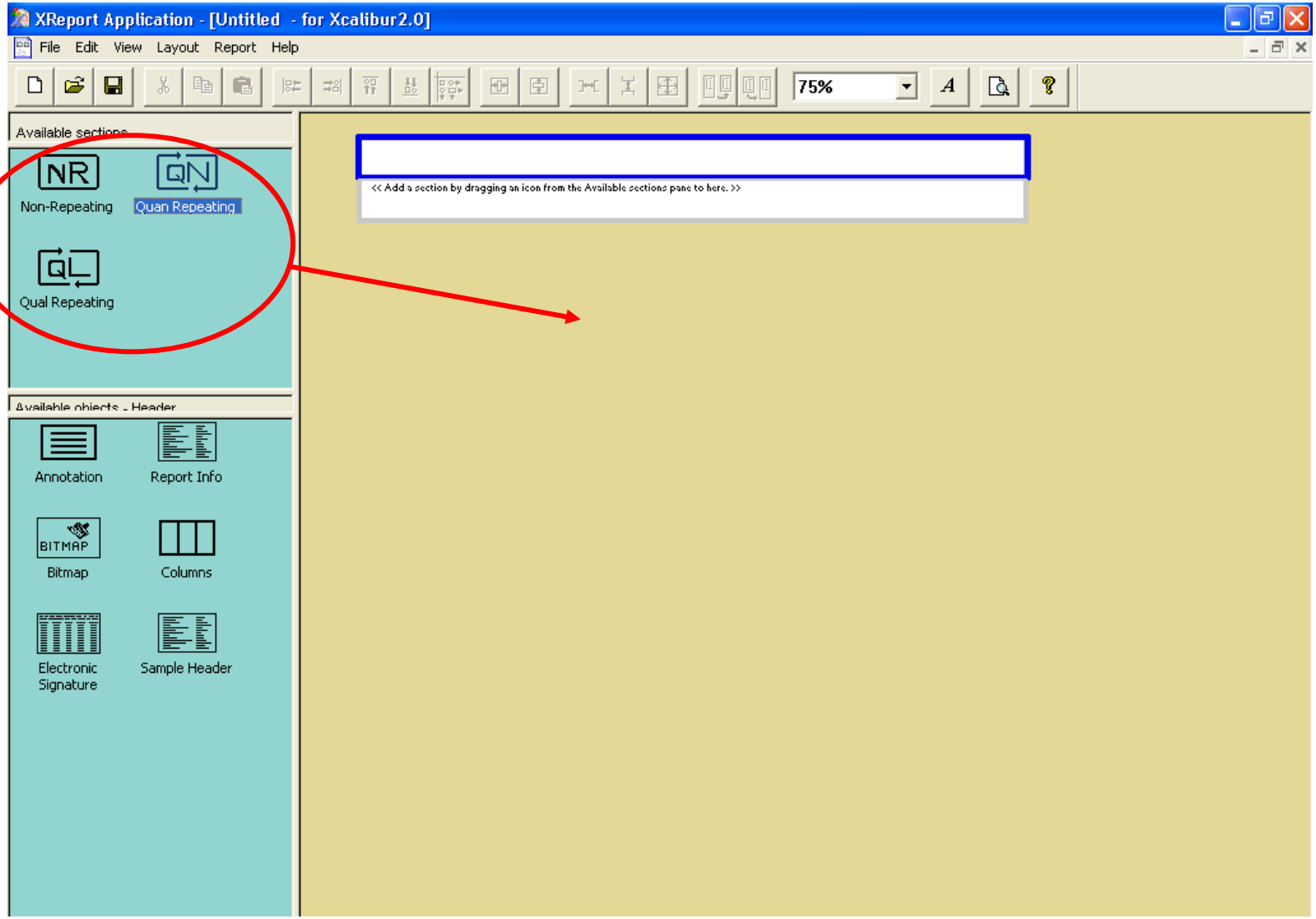
Xcalibur

XReport

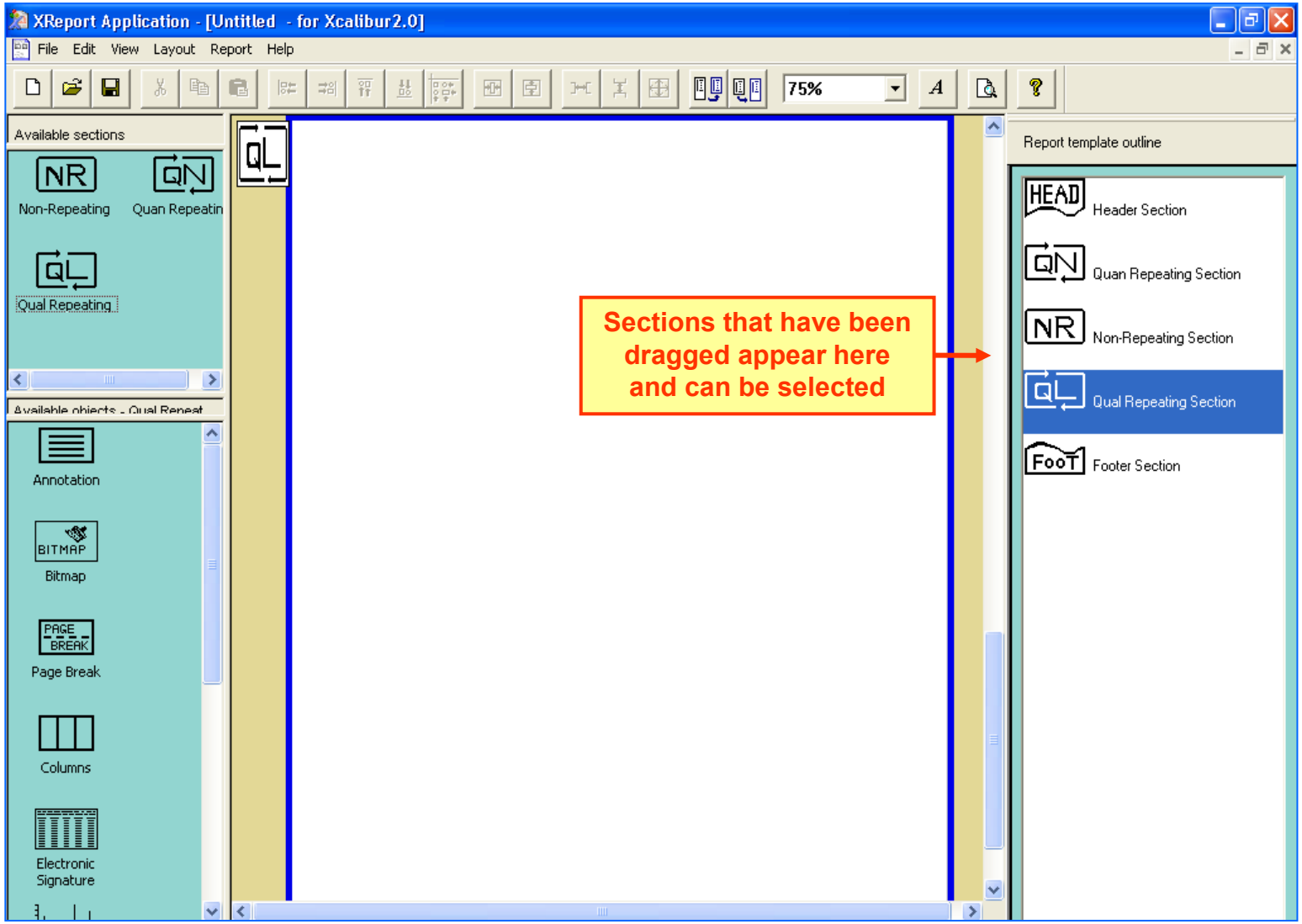
Specify Data Sources…



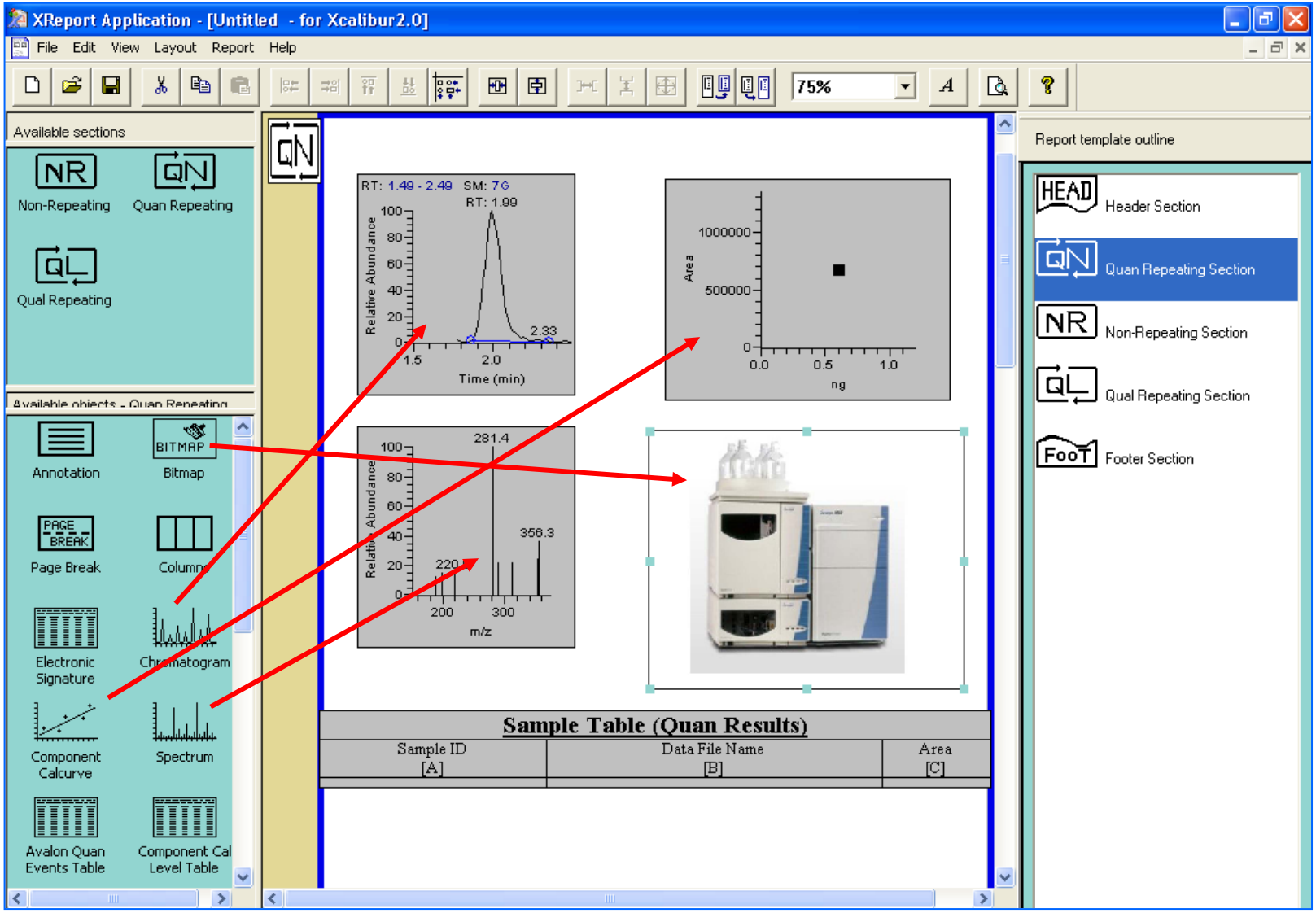
Drag and Drop Sections…



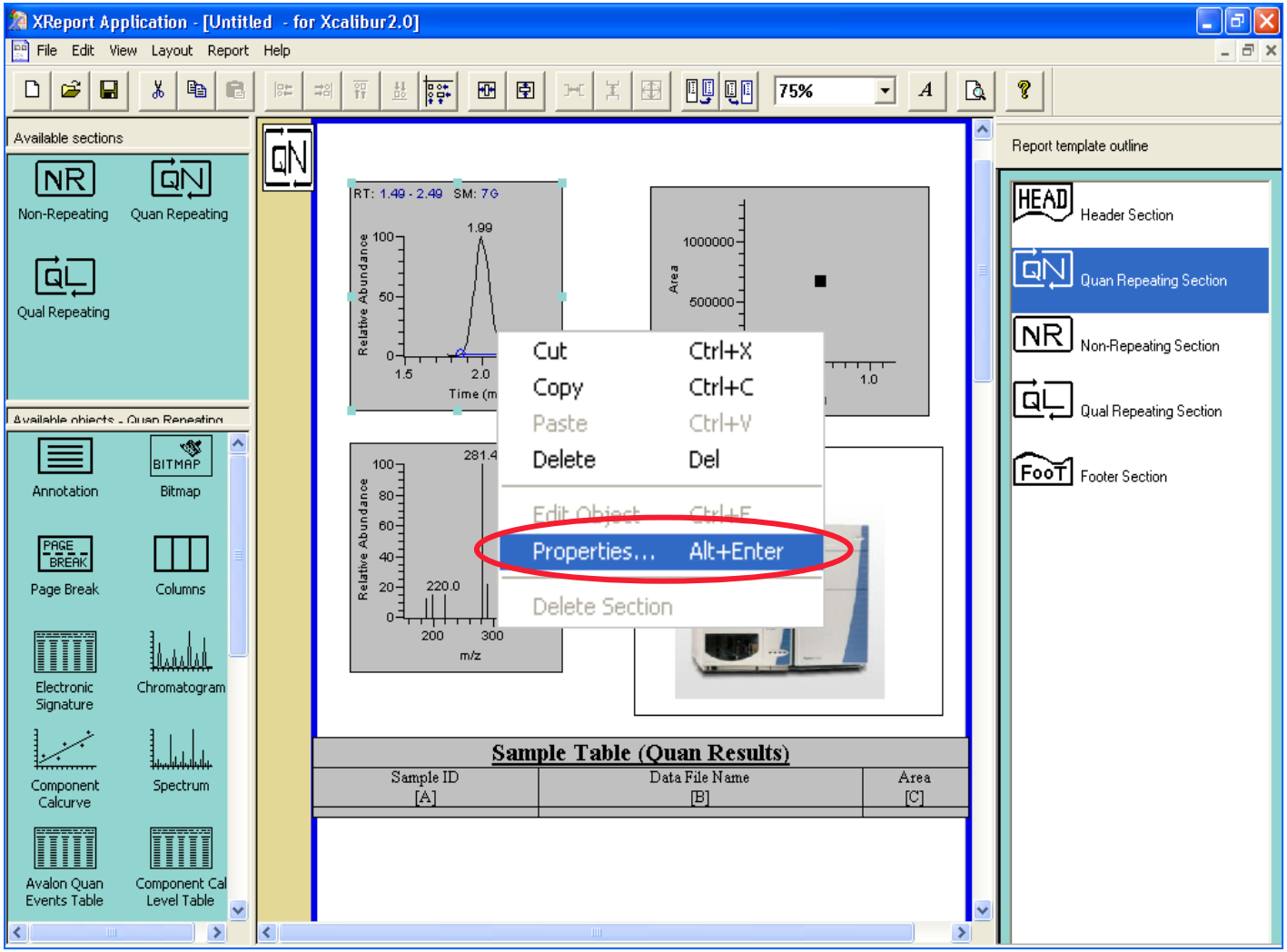
Drag and Drop Sections…



Drag and Drop Individual Objects…

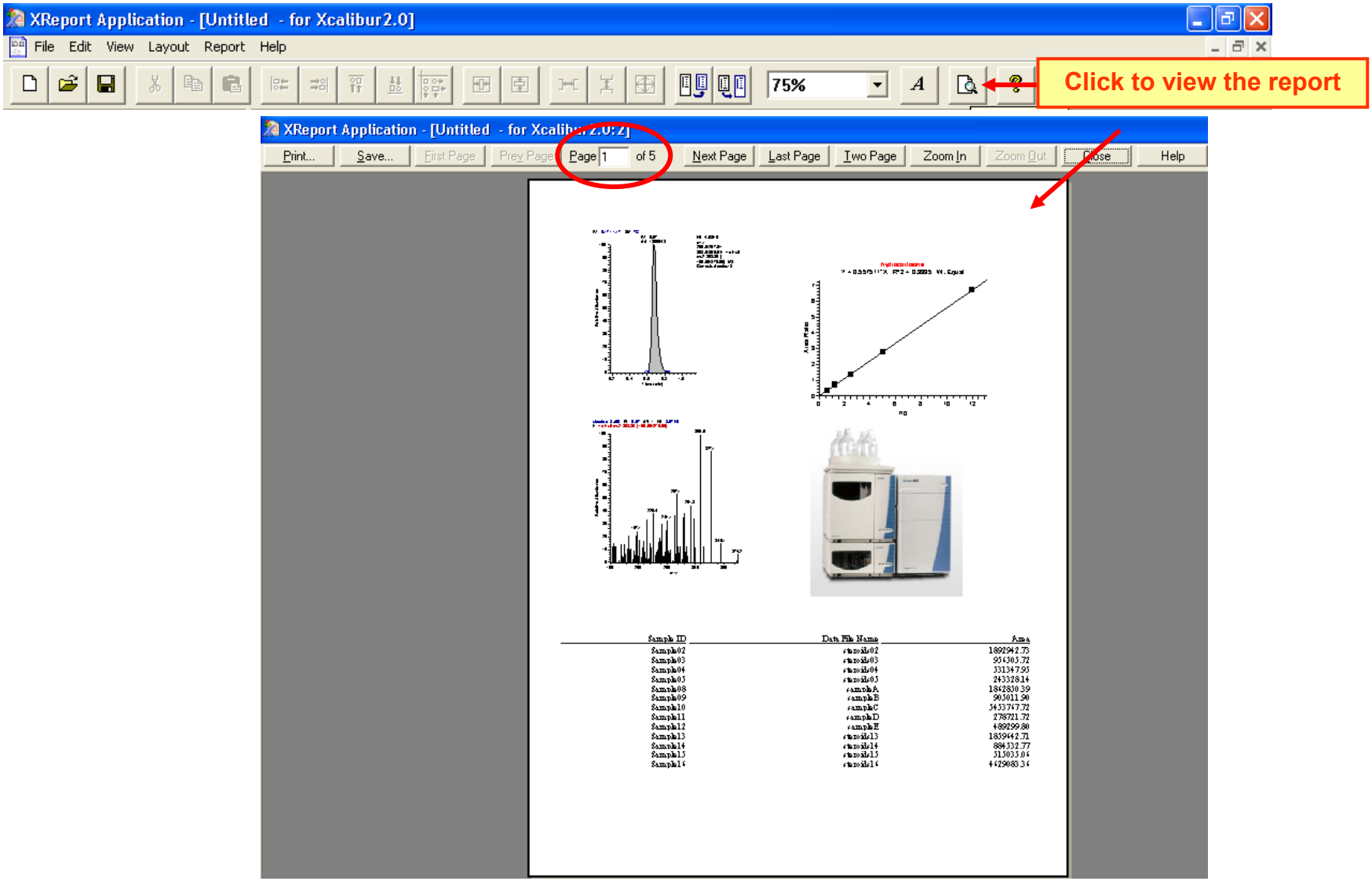


Formatting Objects…

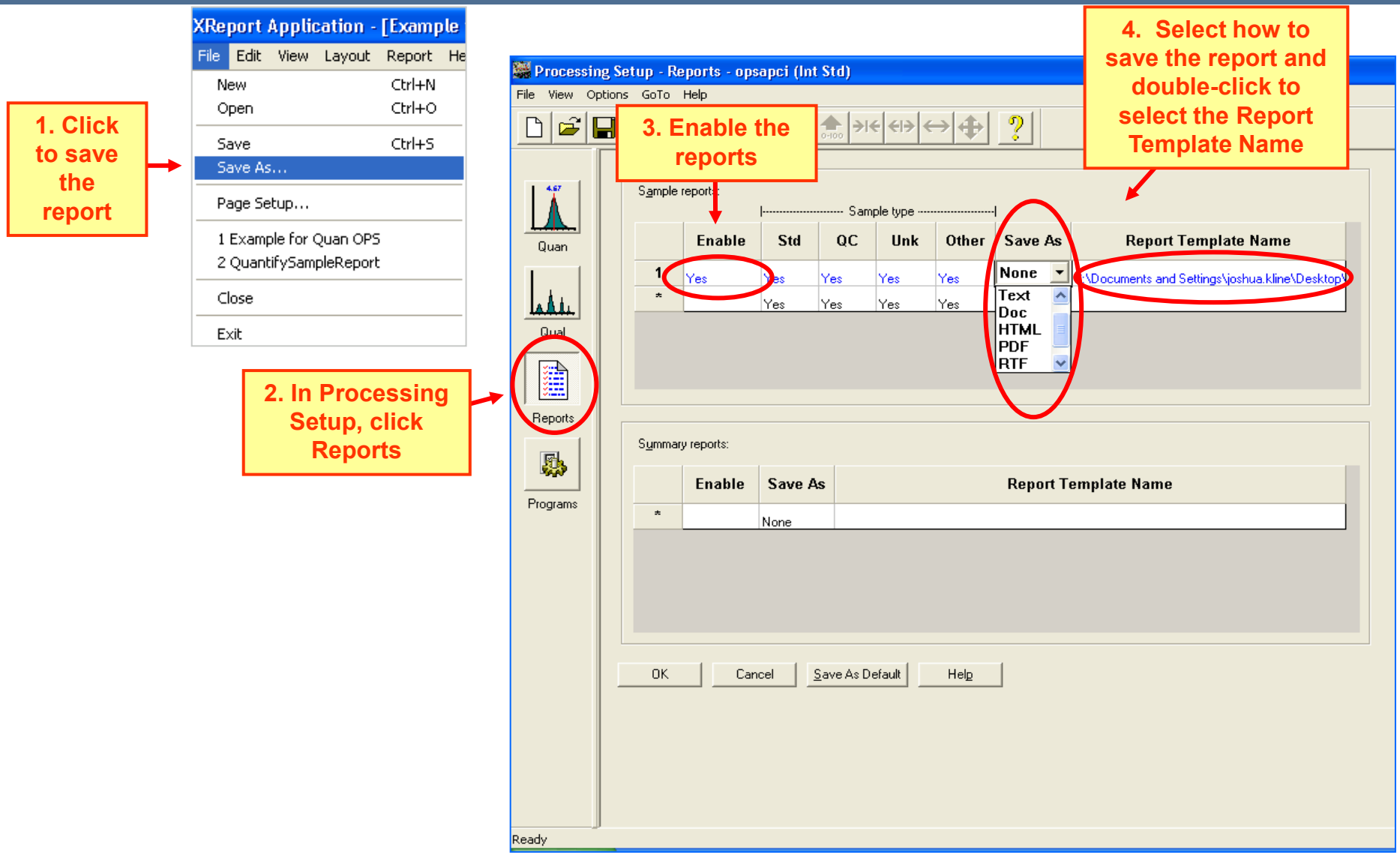


- Any inserted object can be formatted to some degree
- Right Click
- Select Properties
- Follow the Instructions

Viewing the Report…



Save, Insert, Use…



Save, Insert, Use…