Introduction to LC-MS/MS technique

Waters HK Office

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- Atmospheric Pressure Ionization
  - Electrospray Ionization Theory
  - Atmospheric Pressure Chemical Ionization
- Quadrupole Theory

Mass Spectrometry Systems – Xevo TQ-S Micro

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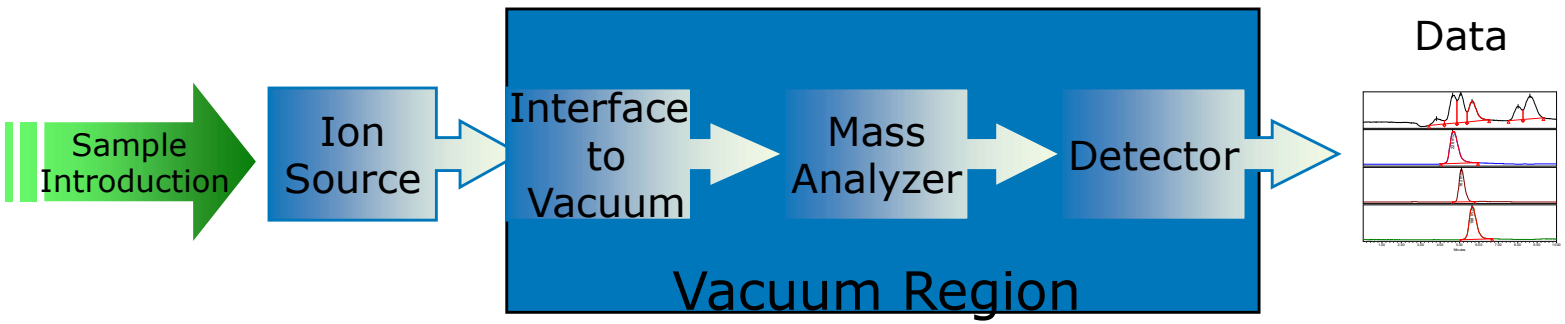
MassLynx®

Mass Spectrometer

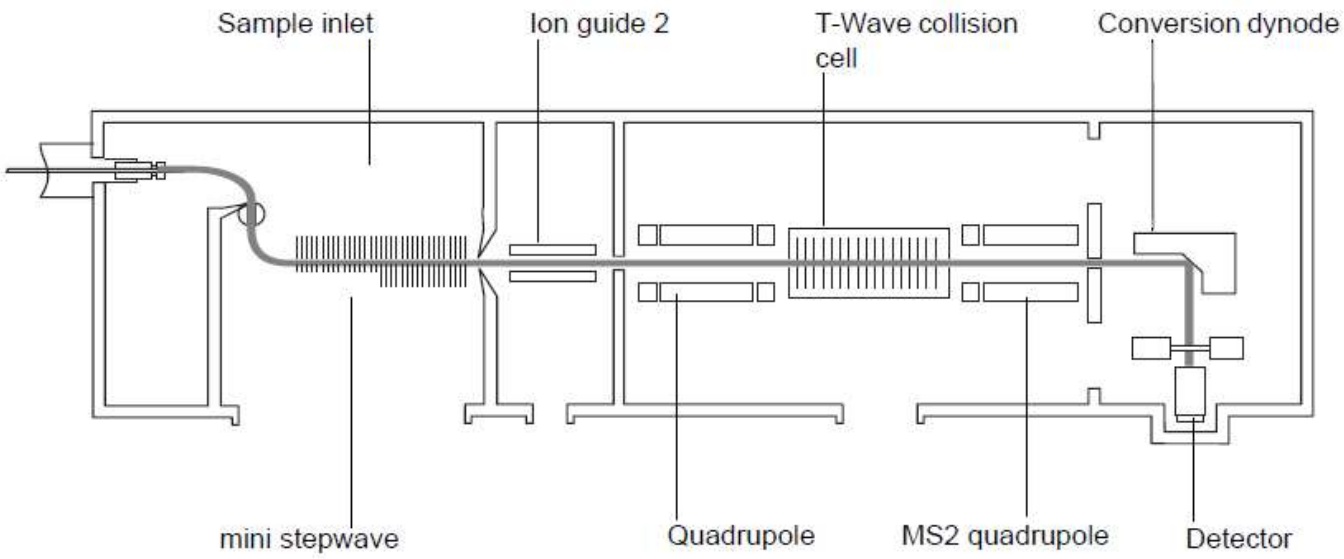
LC Inlet Acquity UPLC

MassLynx Data Acquisiton And Processing

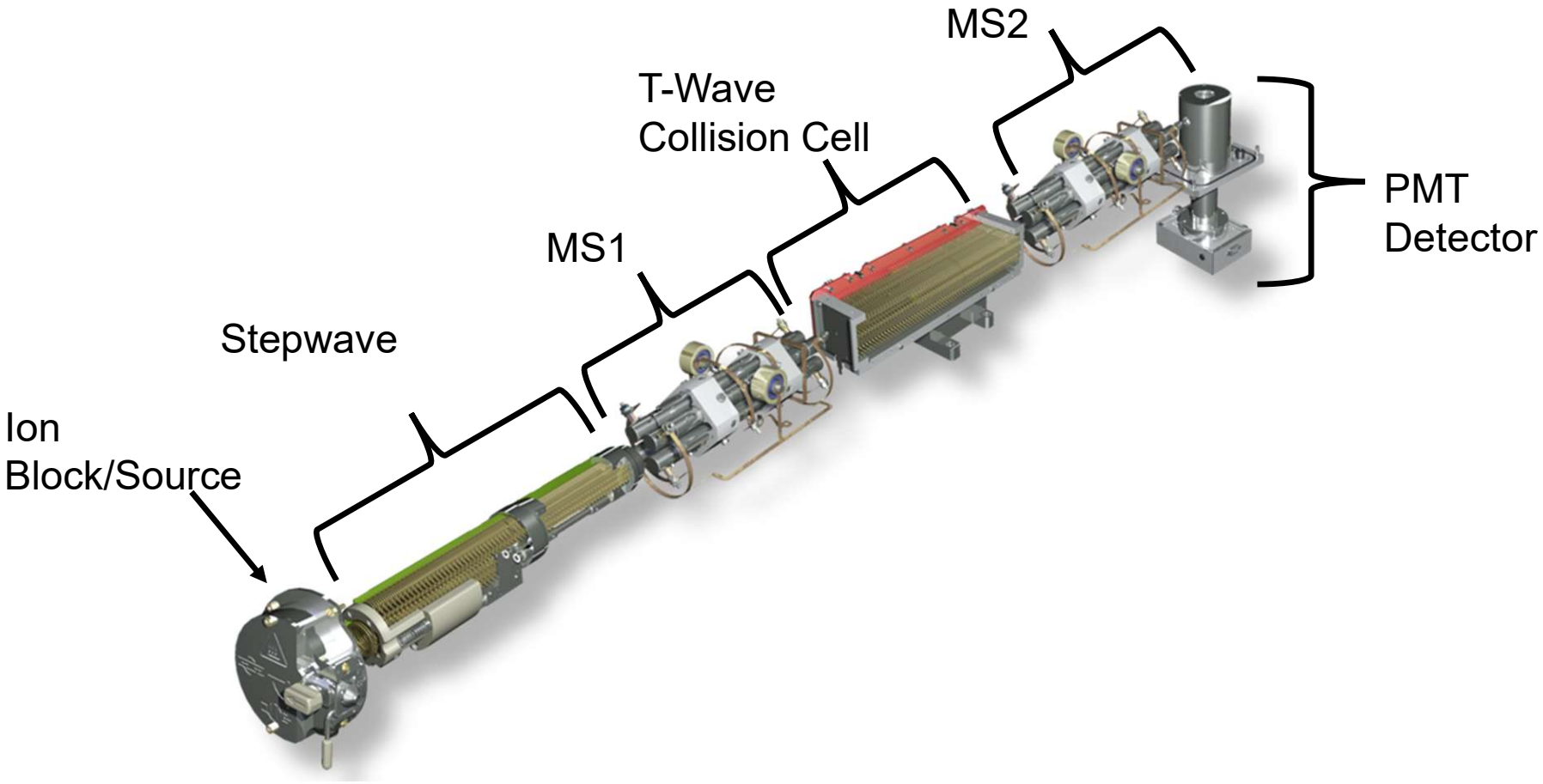
Tandem Quadrupole Instruments



Instrument Schematic – Overview



Inner diagram of Xevo TQ-S Micro Mass Analyser



Atmospheric Pressure Ionization (API)

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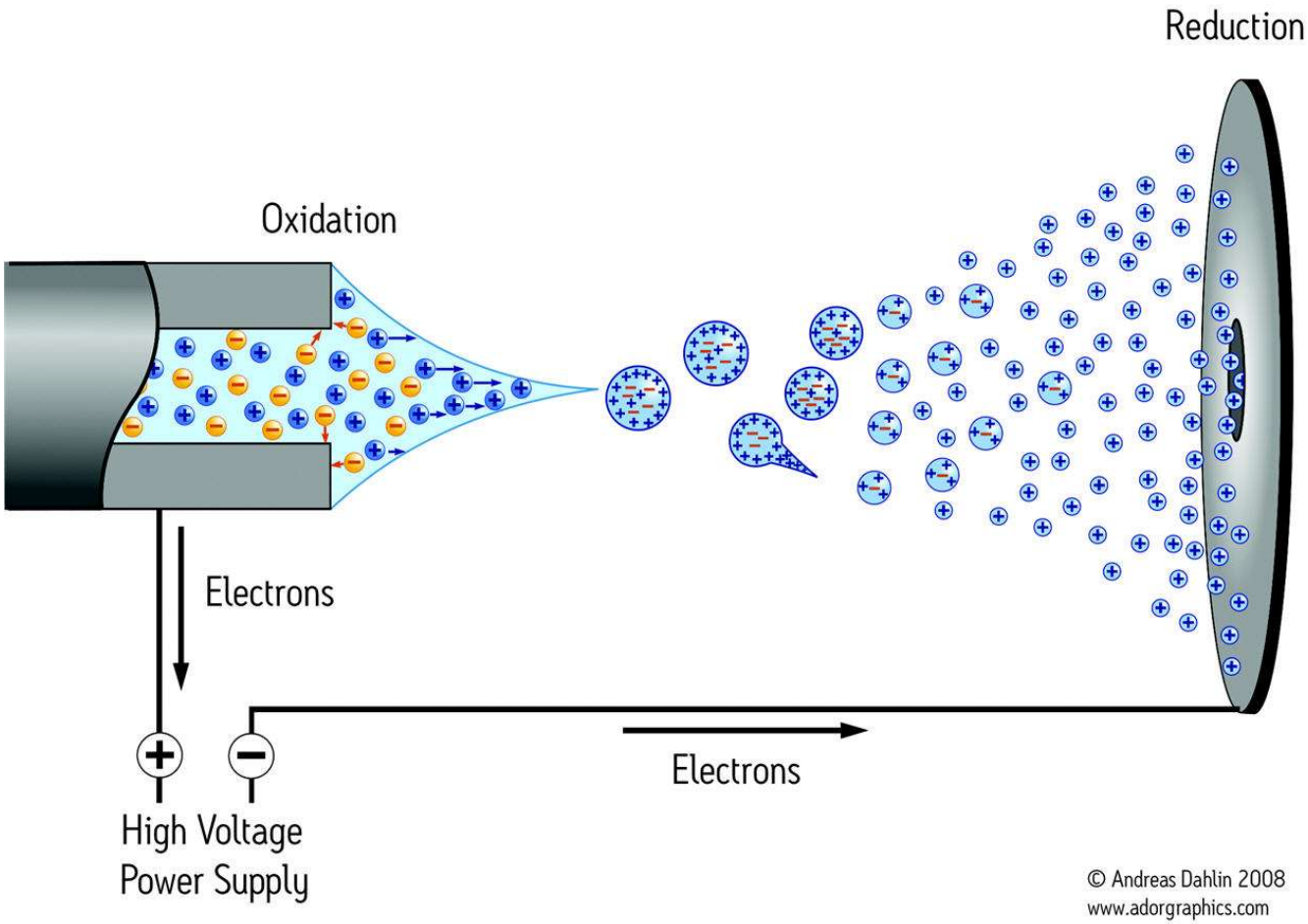
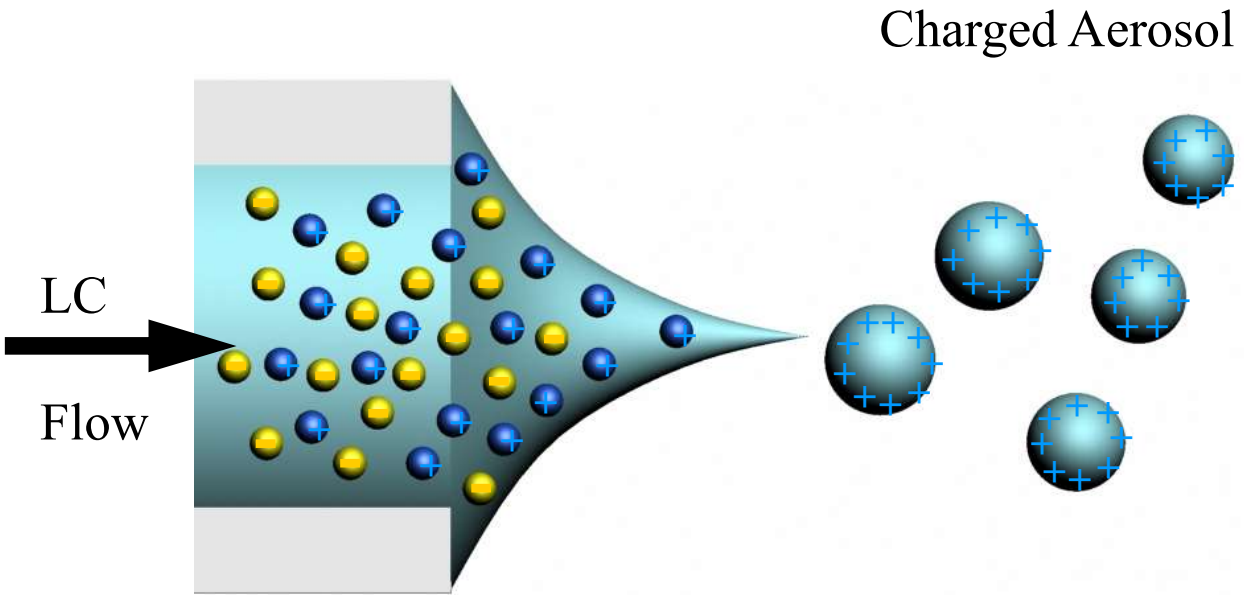
- Electrospray (ESI)
  - Liquid is sprayed from a conductive capillary, to which a high voltage is applied.
  - A spray of charged droplets forms.
  - Droplets evaporate and split until ion evaporation produces gas phase ions
- Atmospheric Pressure Chemical Ionization (APCI)
  - Liquid is passed through a heated tube to be evaporated, producing gas phase molecules.
  - Applying high voltage to a corona pin produces a cloud of ionized nitrogen atoms that ionize the solvent
  - The solvent ionizes the molecules by charge transfer.

Electrospray Events



Electrospray Ionization
Formation of charged droplets



Positive Ion Electrospray – Electrochemical Processes

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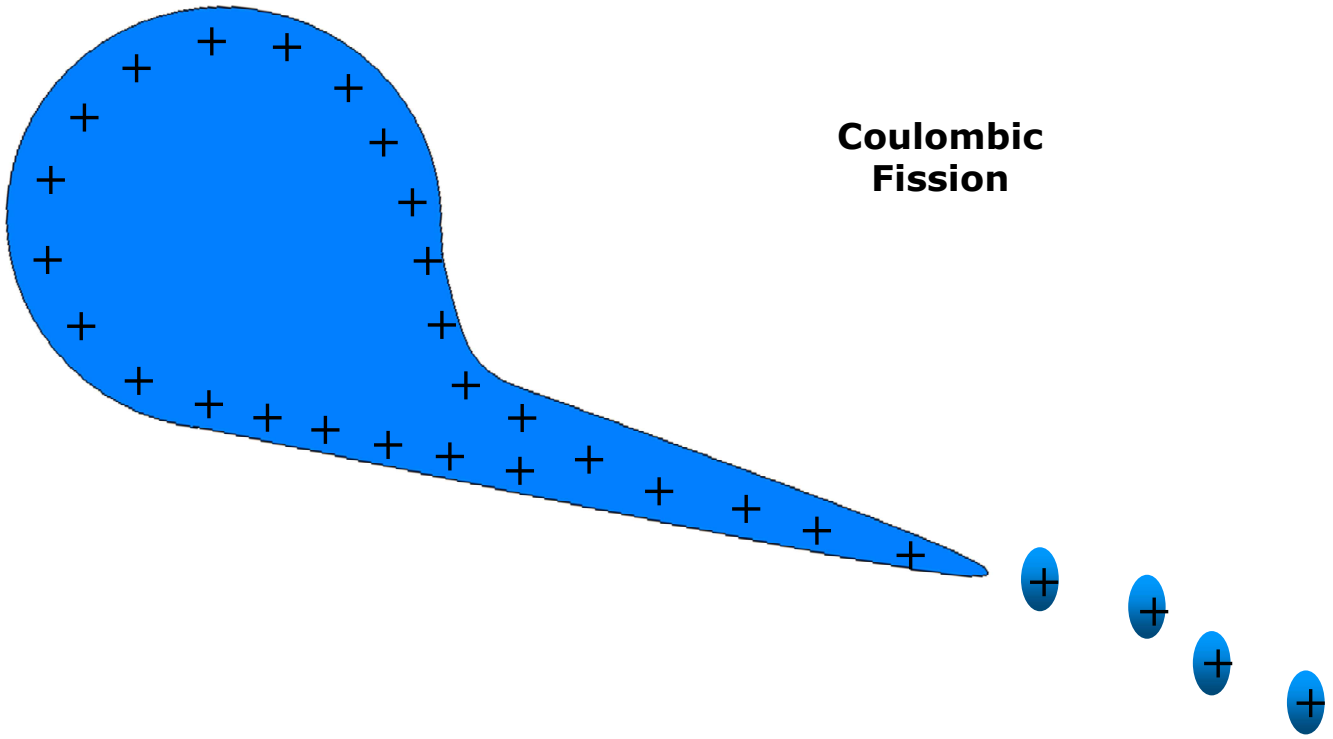
- Electrospray droplets carry positive charges away from the capillary tube.
- To balance this flow of positive charges, electrons flow out of the capillary tube.
- An electrochemical oxidation reaction produces electrons from negative ions that are close to the surface of the capillary wall.
- Electrospray can be thought of as an electrochemical process.

Electrospray Ionization
Solvent evaporation and droplet fission



Electrospray - Solutions

- Solutions should have a high percentage of organic solvents, such as acetonitrile or methanol.

- Solutions must have some aqueous content and contain ionic species such as hydronium or hydroxyl.

Formation of Gas Phase Ions

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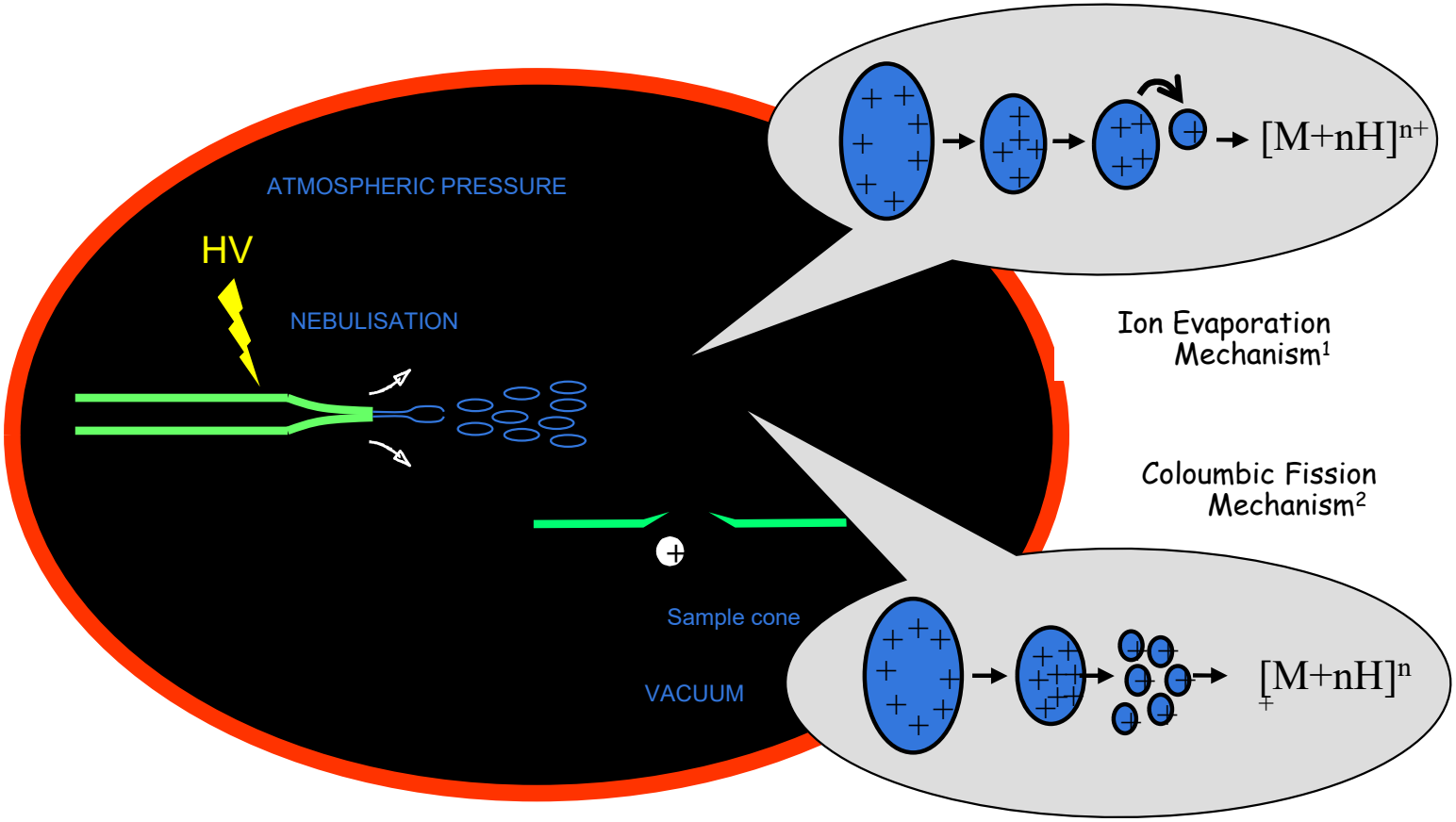
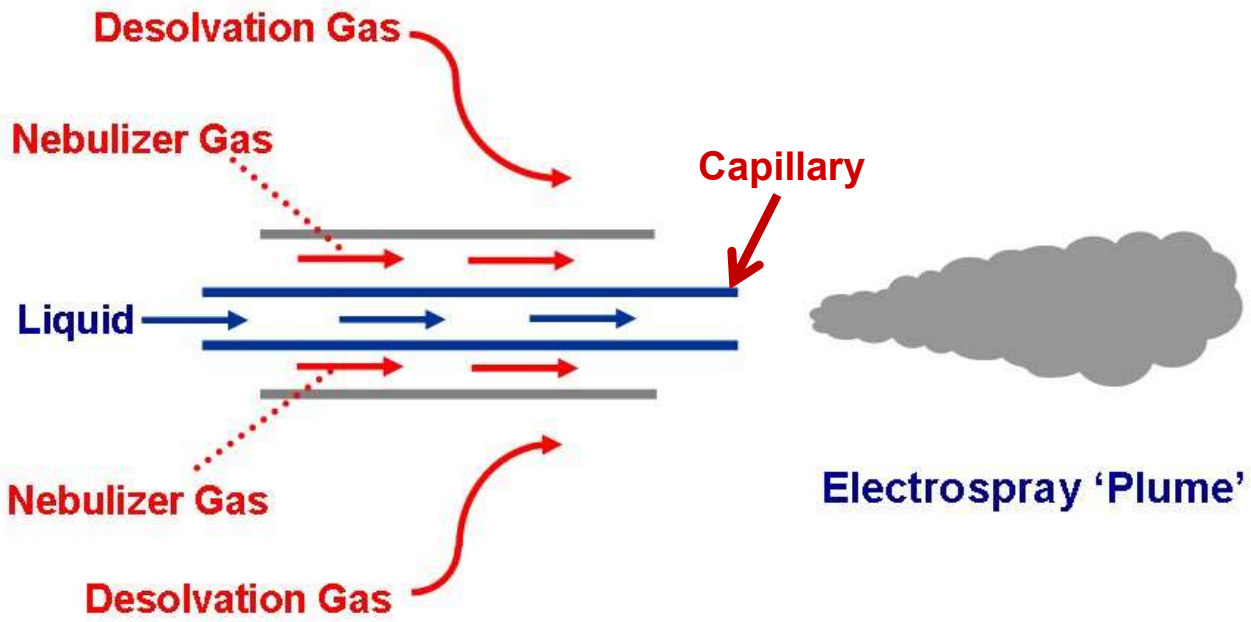
- Models for formation of gas phase ions from droplets.
  - Ion evaporation
    - Through evaporation and fission, droplets reduce in size.
    - Ions then evaporate from the surface of the droplet.
    - Molecules that are more surface active, more readily form ions in electrospray.
  - Charged residue
    - Droplets continue to lose solvent molecules through evaporation until a charged residue remains.
    - For an analyte of the form MX, the charged residue is of the form:
    - $(M+)\ n(MX)m$

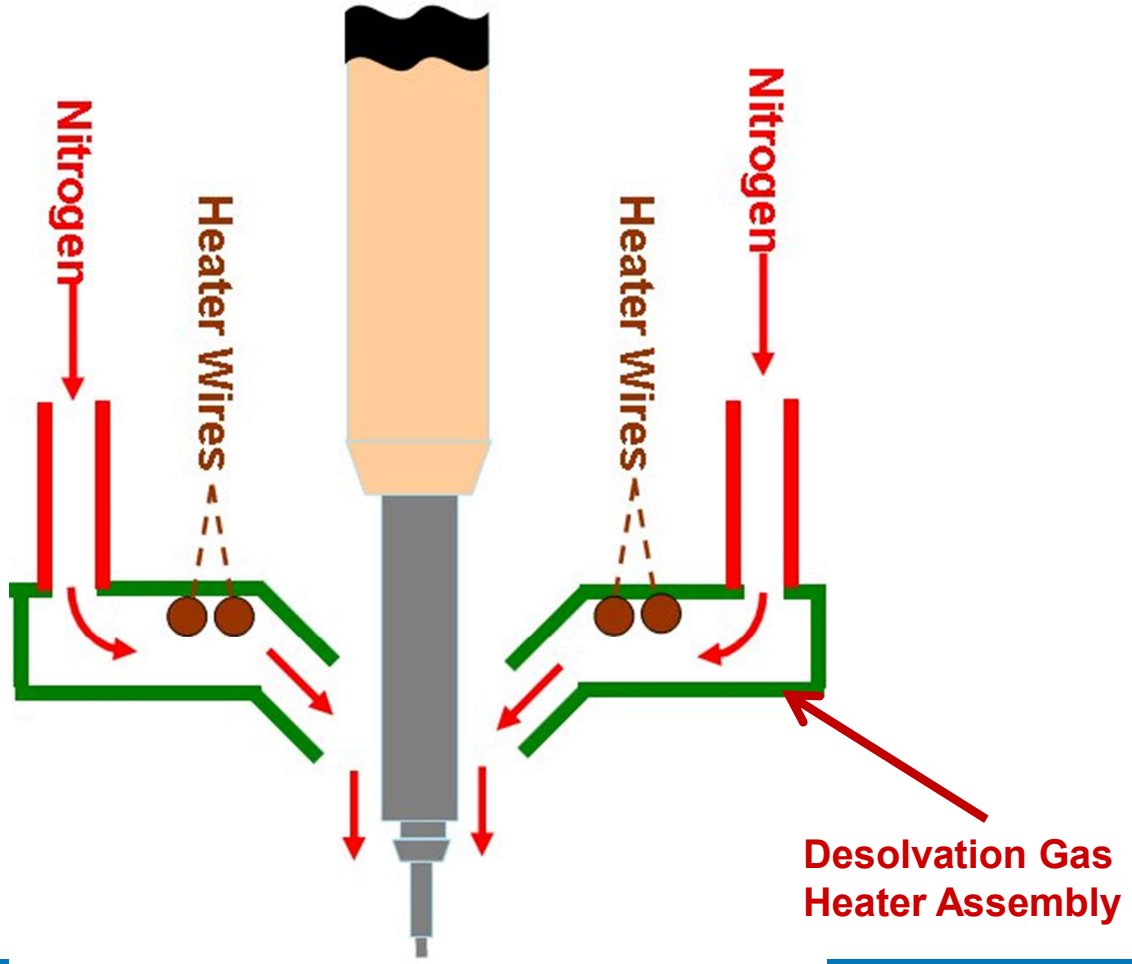
How Does Electrospray Work?



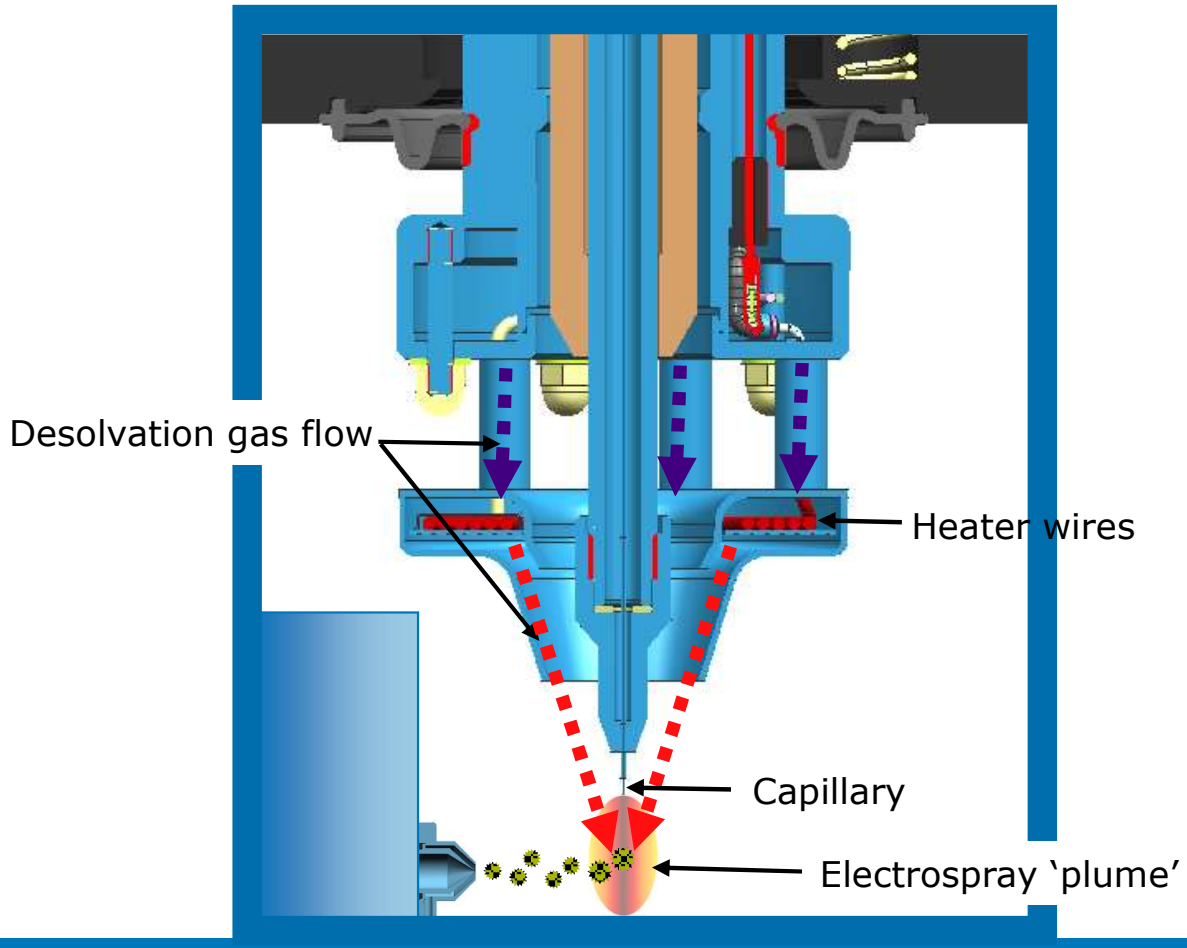
Electrospray Probe Tip



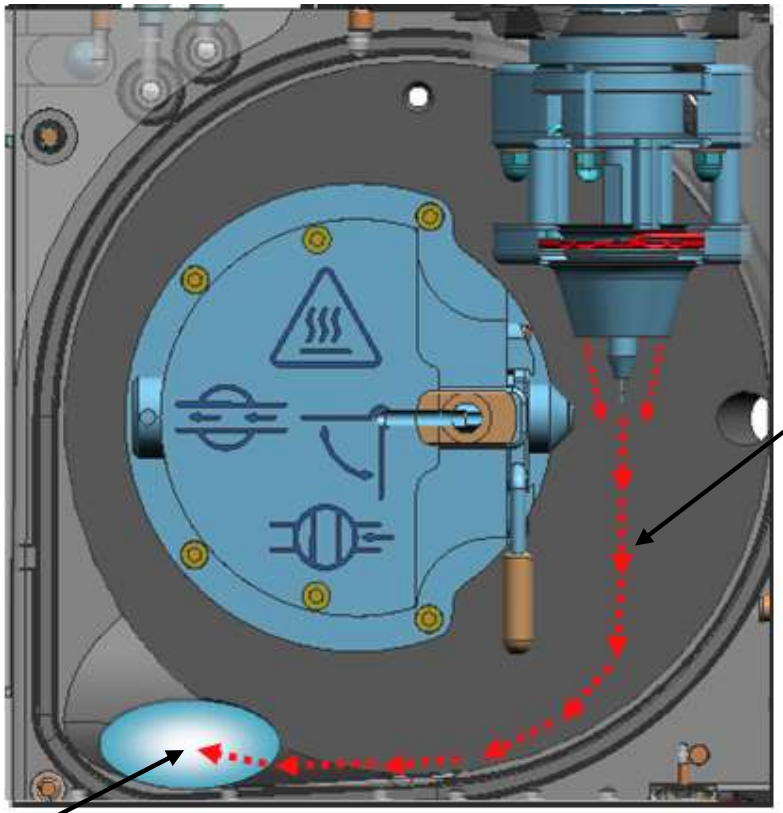
Desolvation Gas Flow



Detailed ESI Probe

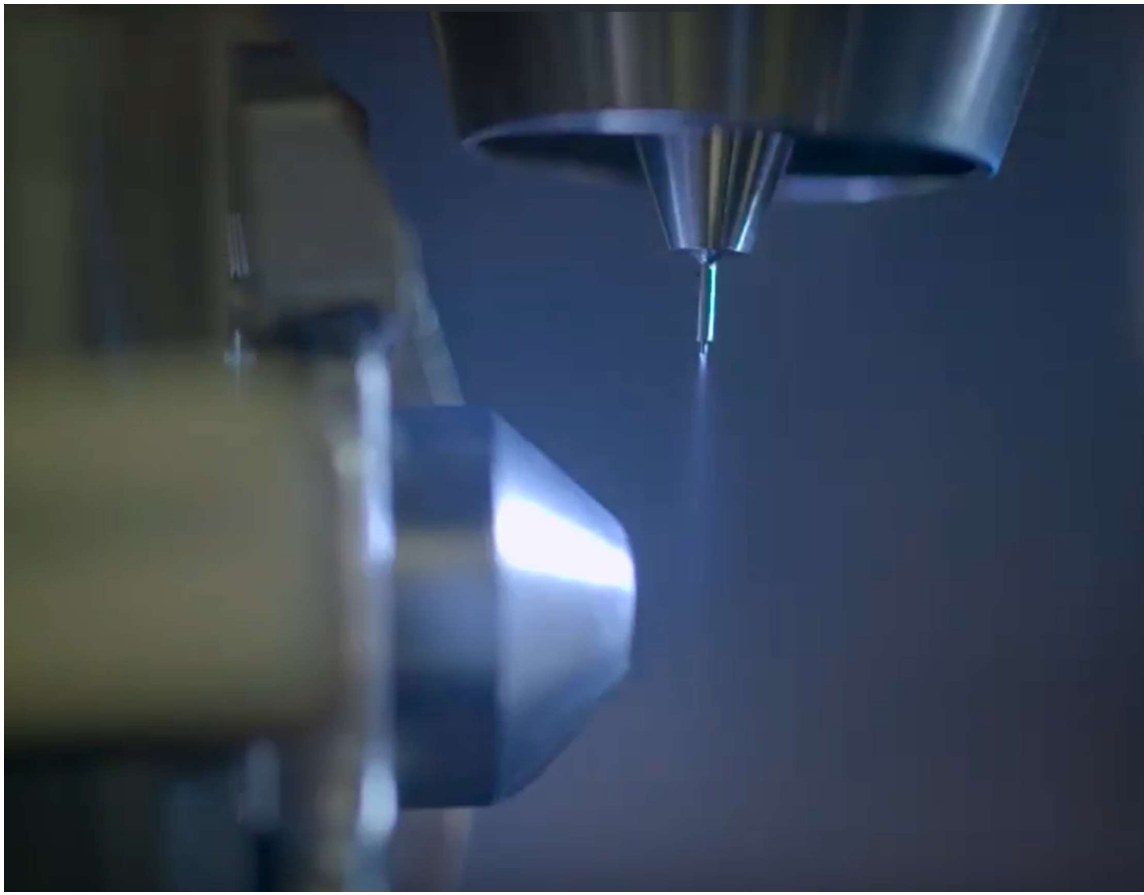


Optimizing Gas Flow Dynamics



Xevo Z-Spray

- Standard configuration across all Xevo MS detector
- Orthogonal API source for efficient removal of neutrals and maximum sampling of ions



Electrospray and Ions in Solution

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- A solution process
  - Molecules that have a greater tendency to ionize in solution have stronger electrospray signals.
    - Certain additives to mobile phases in LC/MS analyses can enhance electrospray signals.
  - For example:
    - Adding formic acid to the mobile phase in positive electrospray LC/MS analyses.
      - This often results in a stronger electrospray signal by aiding in the protonation of analytes in solution.

Electrospray Ionization - Ions Generated in Solution

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Mobile phase (volatile) additives allow ion generation with pH modification

Acid: HCOOH, low pH buffer

$$Base + H_3O^+ \longleftarrow [Base + H]^+ + H_2O$$

Base: High pH buffer, ammonium hydroxide

$$Acid + HO^- \longleftarrow [Acid - H]^- + H_2O$$

Choice of Ionization Mode

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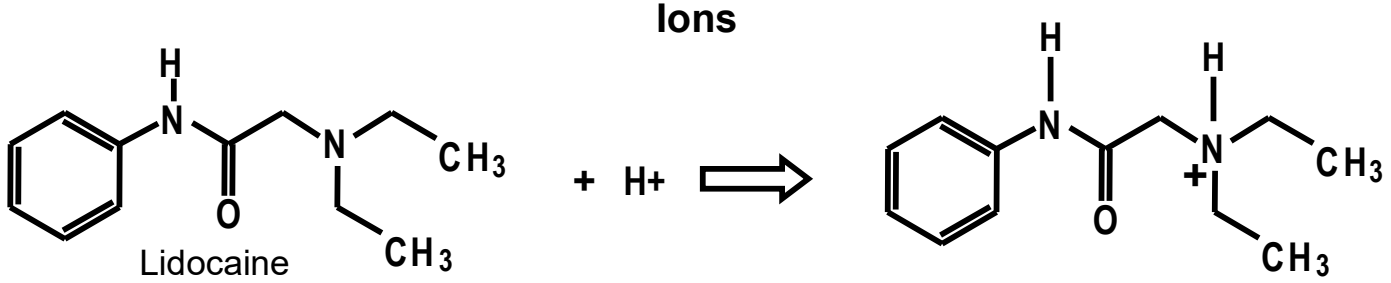
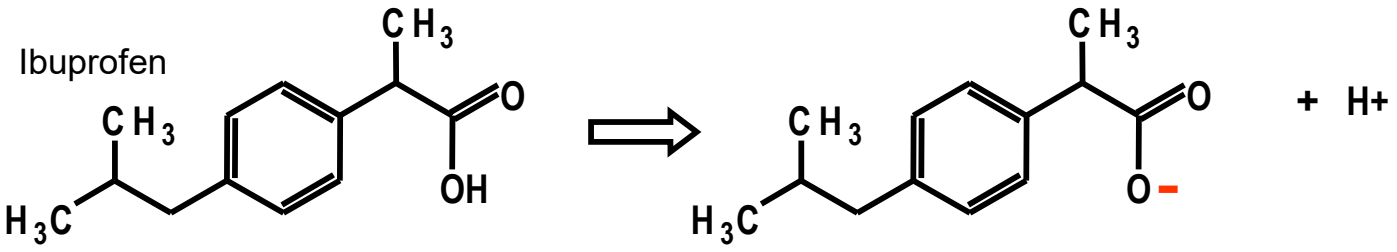
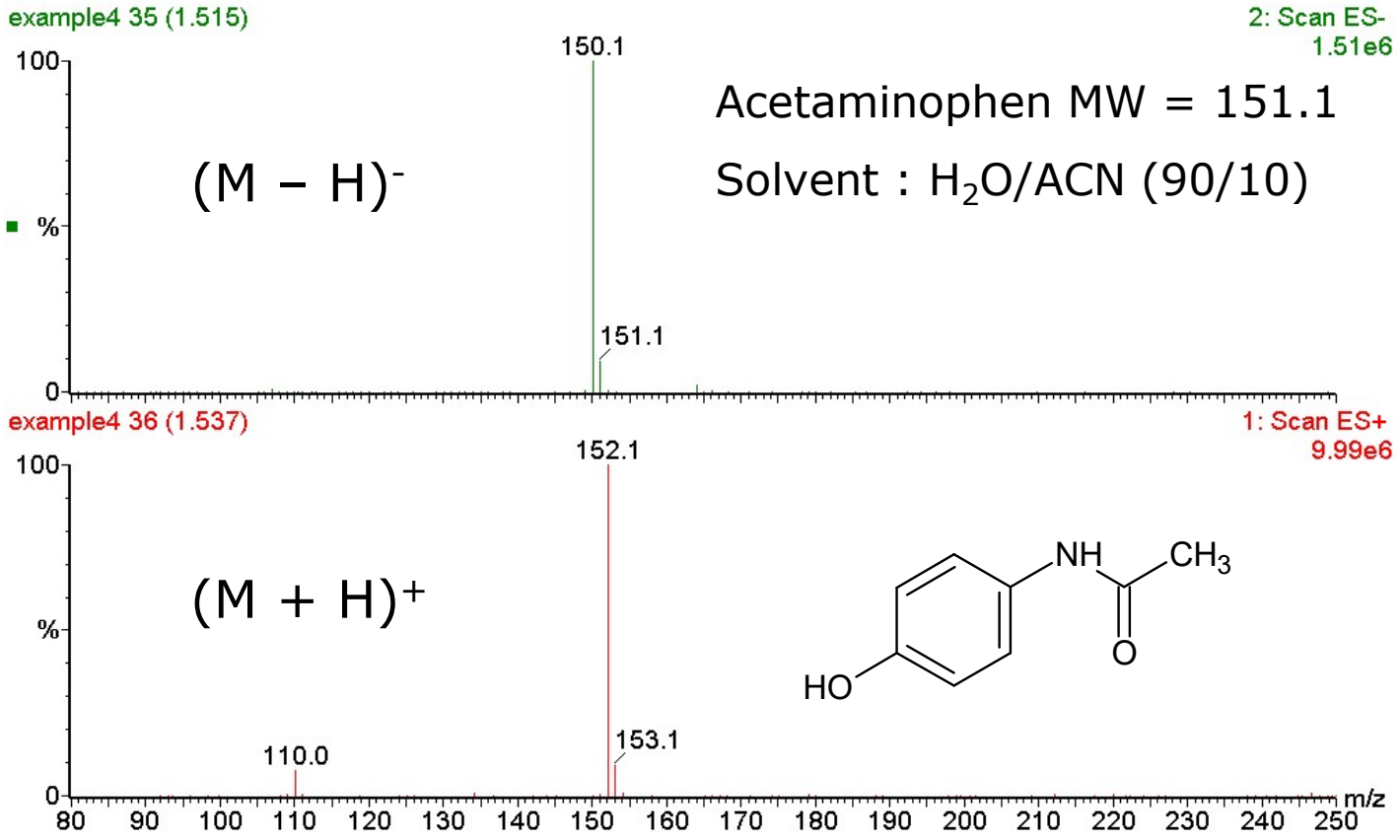
Positive Electrospray Ions



Negative ElectroSpray Ions



ESI MS Spectra Example – Positive and Negative Mode



Multiple Charging in ESI

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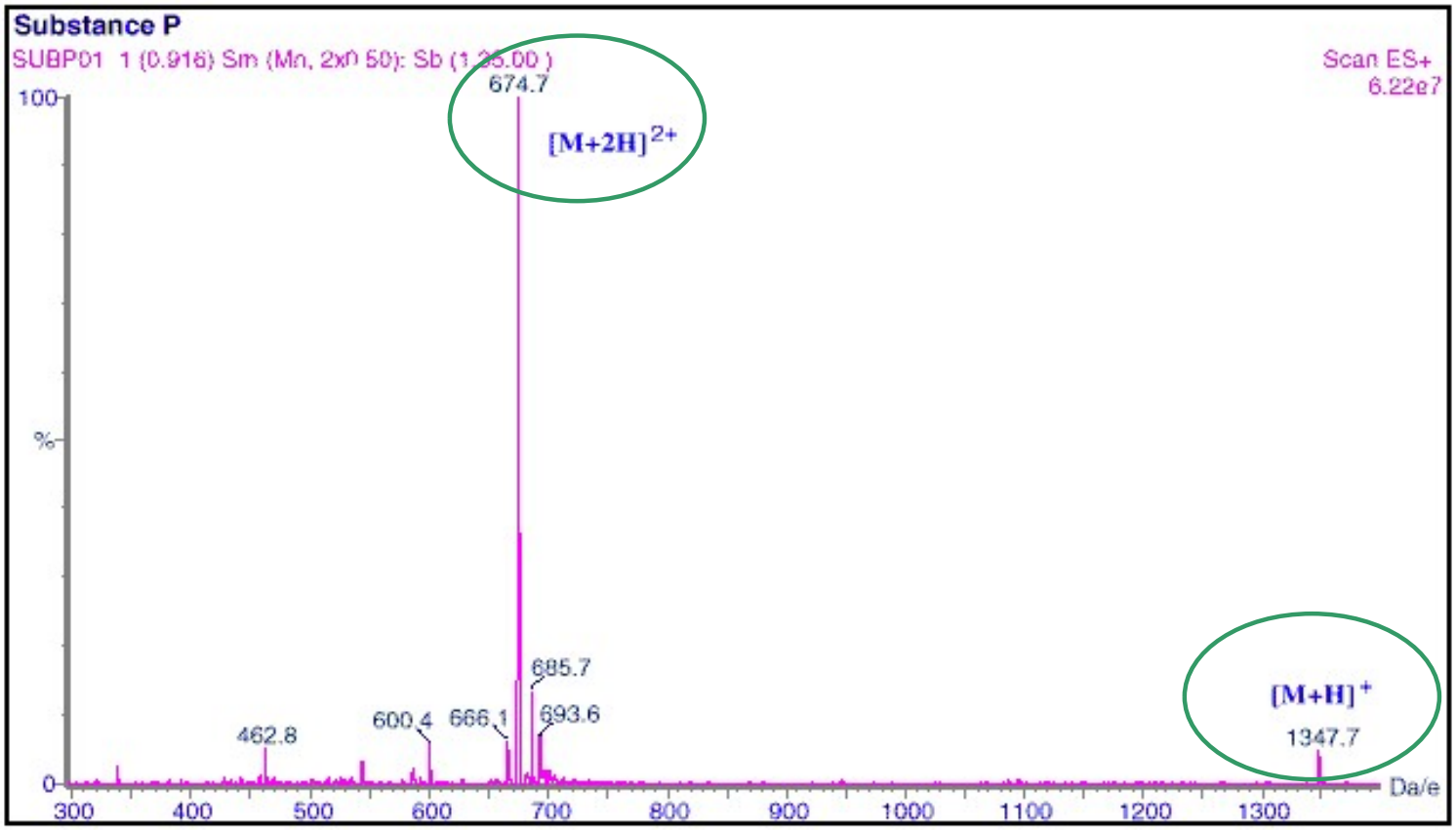
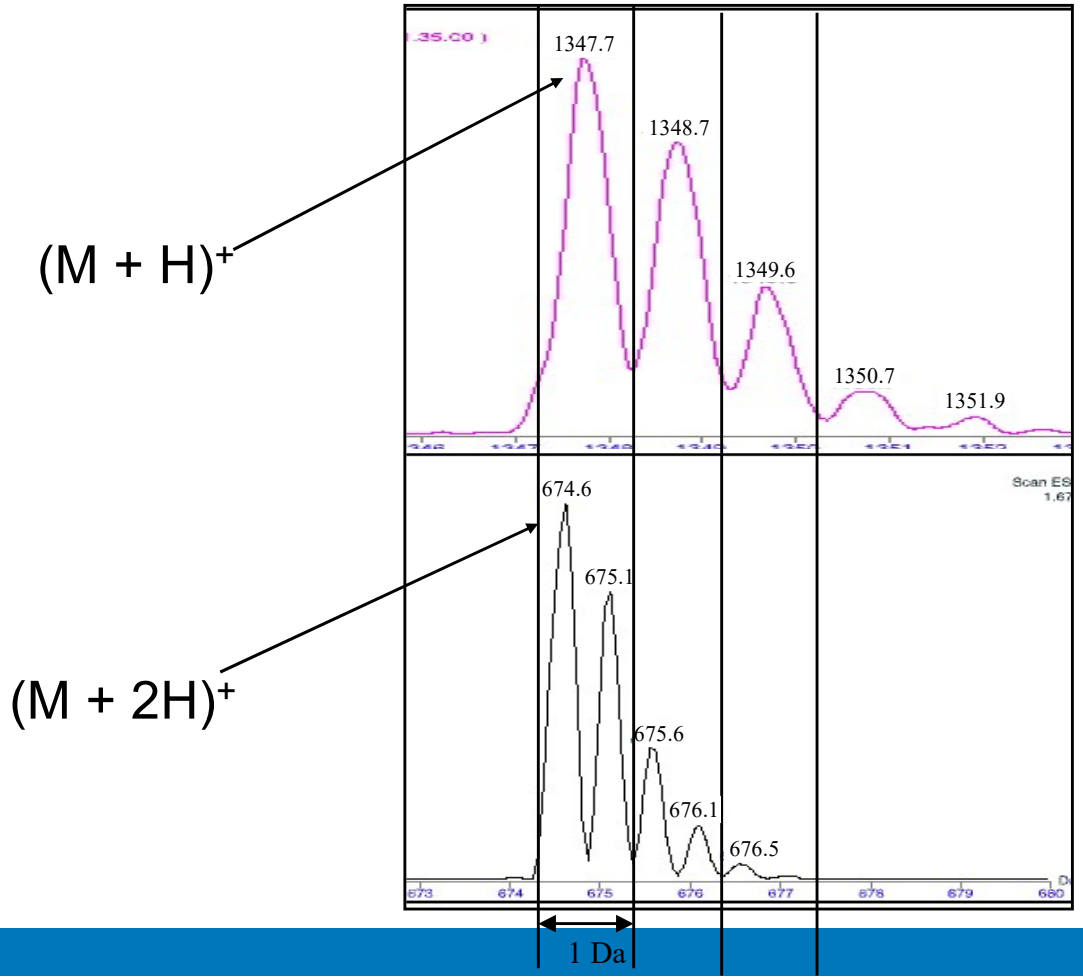
- Mass spectrometers operate on the basis of the mass-to-charge ratio (m/z).
  - Single charge m/z = (M + H+)/1z
  - Double charge m/z = (M + 2H+)/2z
  - n charge m/z = (M + nH+)/nz
- Isotope peaks of n-charged ions are separated by 1/n m/z .
- Multiple charging extends the effective mass range of the mass spectrometer.

Isotope Resolution for Multiply Charged Species

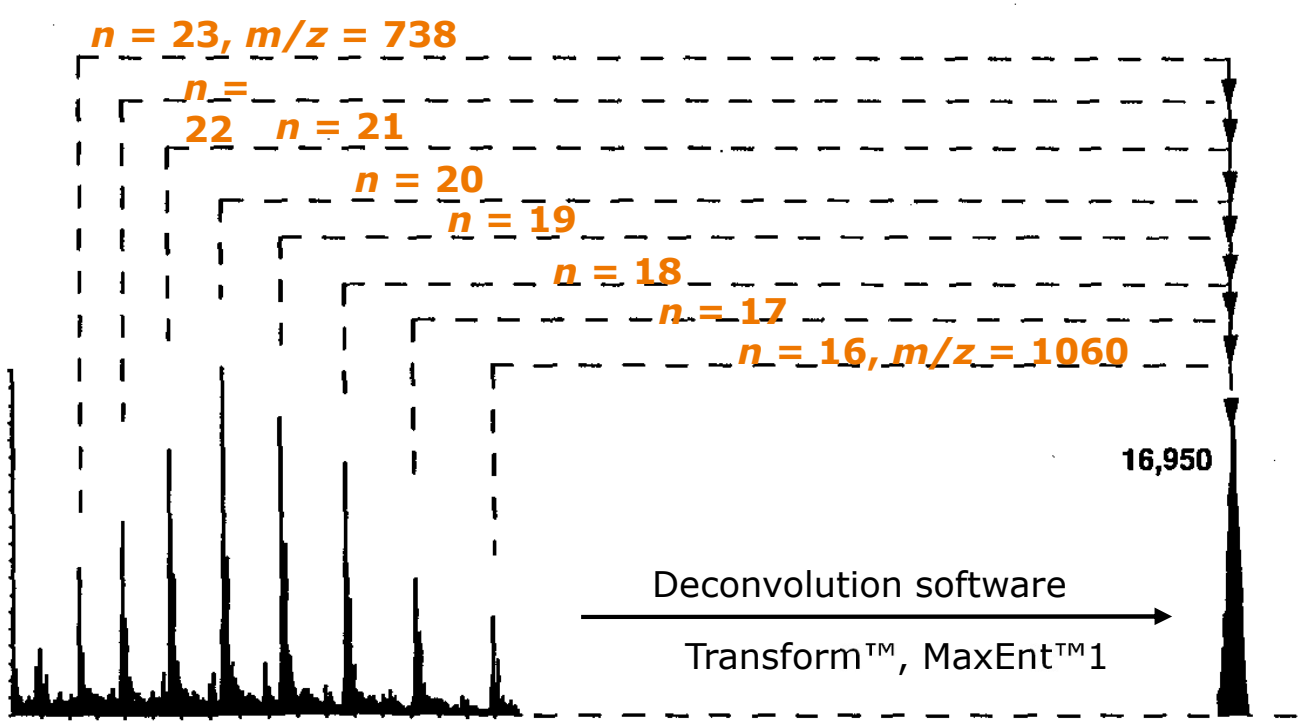


Isotope Resolution for Multiply Charged Species, expanded



Multiply Charged Distributions

Horse Heart Myoglobin



Samples Analyzed in Electrospray Mode

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- Typical positive ion samples:
  - Peptides and proteins
  - Small polar molecules (<2000 MW)
  - Drugs and their metabolites
  - Environmental contaminants
  - Dye compounds
  - Various organometallics
  - Small saccharides

- Typical negative ion samples:
  - Various proteins
  - Drug metabolites such as conjugates
  - Oligonucleotides
  - Various saccharides and polysaccharides

Effect of Solution Chemistry on Analyte Response in ESI

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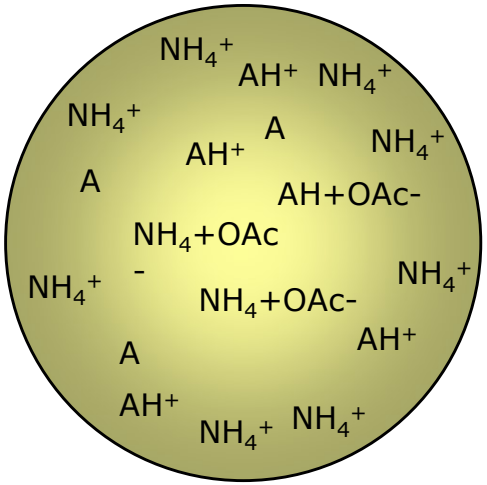
- In general, analyte response decreases with an increase in the concentration of certain mobile phase additives. This behavior is referred to as ‘ion suppression.’

- Electrolyte ions compete with analyte for charge and occupation on the droplet surface.

Effect of Matrix on Analyte Response in ESI

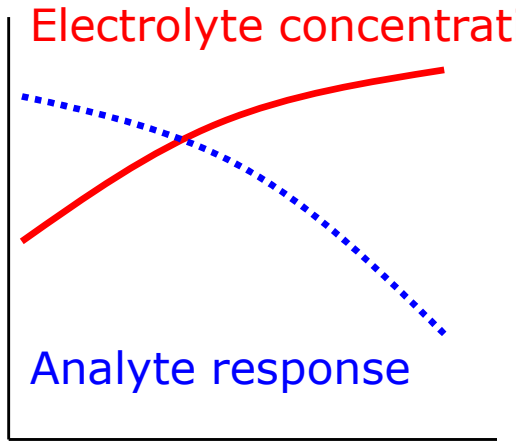
1. Surface competition



2. Charge competition

$OAc^- + AH^+ \longleftarrow HOAc + A$

Competition between analyte and electrolyte ions for conversion to gas-phase ions decreases analyte response.



ESI

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Advantages

- MW confirmation
- High MW determination
- Volatile and non-volatile solutes
- Detection of high MW compounds using multiply charged ions
- Ionic/polar analytes
- Low temperature reduces sample degradation
- Good sensitivity
- Quantitative method
- Suitable for capillary and nano LC flow rates

Disadvantages

- Must form ions in solution
- High salt conditions can suppress ionization
- LC mobile phase additives may affect ionization

Atmospheric Pressure Chemical Ionization (APCI)

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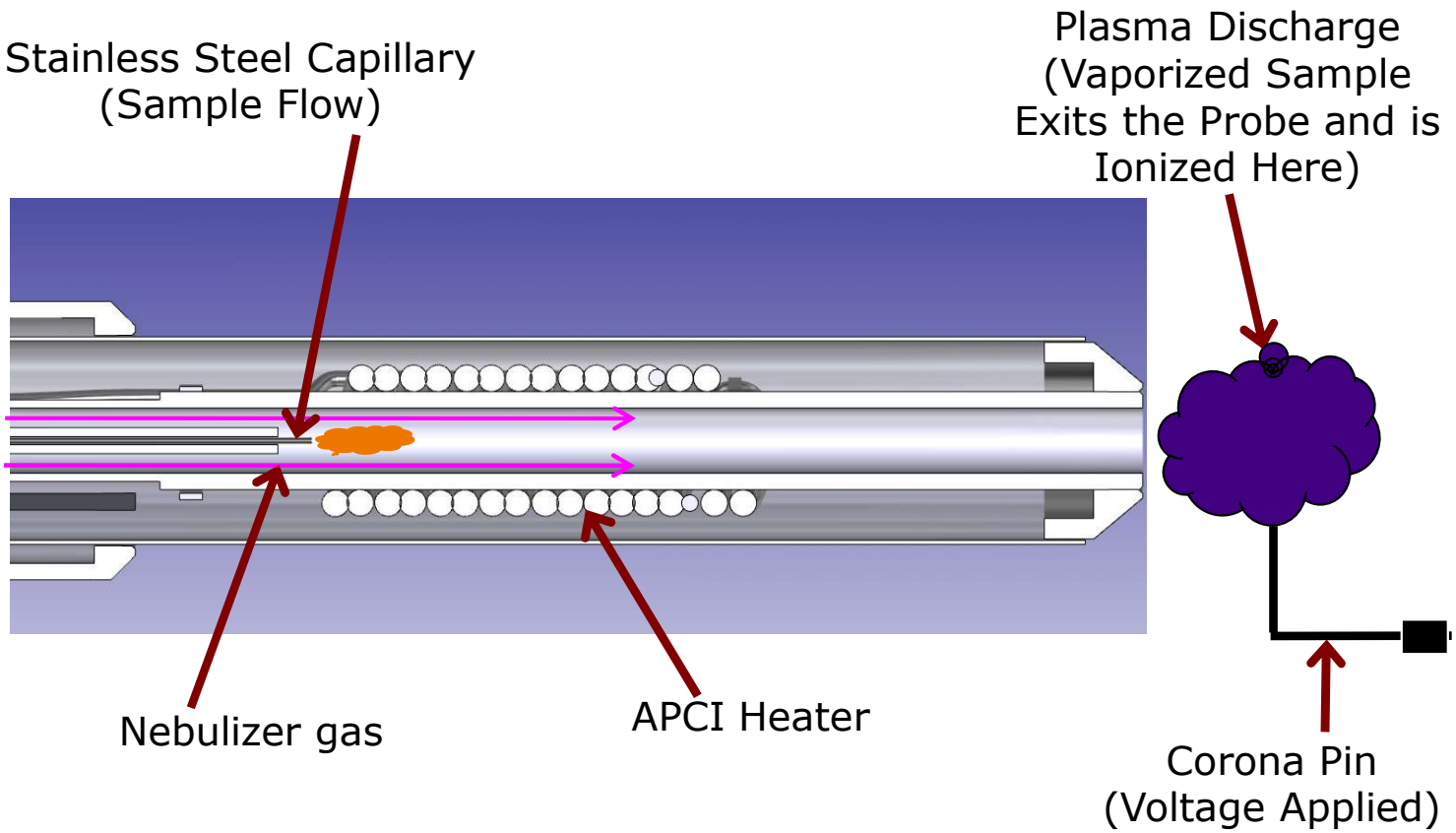
- Low molecular weight (less than 1000 Da)
- Singly-charged species
- Fragmentation, even at low cone voltages
- Mobile phase can be non-polar (normal-phase chromatography)

IonSABRE II™ APCI Probe Design



APCI Characteristics

- Higher temperature, more aggressive ionization
- Solvent and analyte molecules are in the gas phase
- Ionization takes place in the plasma
- Potentially more sensitive than electrospray with some non-polar molecules

APCI Ions

- Positive Ion APCI
  - Ions similar to those formed in positive ion electrospray are formed as:
    - $(M + H)^+$ or $(M + Na)^+$
  - Electron abstraction to form M^+, free radical cation
- Negative Ion APCI
  - $(M - H)^-$ ion formed in negative ion electrospray is also produced
  - Free electrons are formed by the corona pin
    - Certain types of molecules can pick up a free electron and become negatively charged without a change in mass. This process is sometimes referred to as M^-,free radical anion

APCI and ESI Differences

- Electrospray
  - Ionization in solution
  - Reverse phase or normal phase with post column solvent modifications
  - Ionization
    - Probe not heated
    - Capillary voltage
  - Strong mobile phase effect
  - Polar compounds
  - Suitable for thermally labile compounds

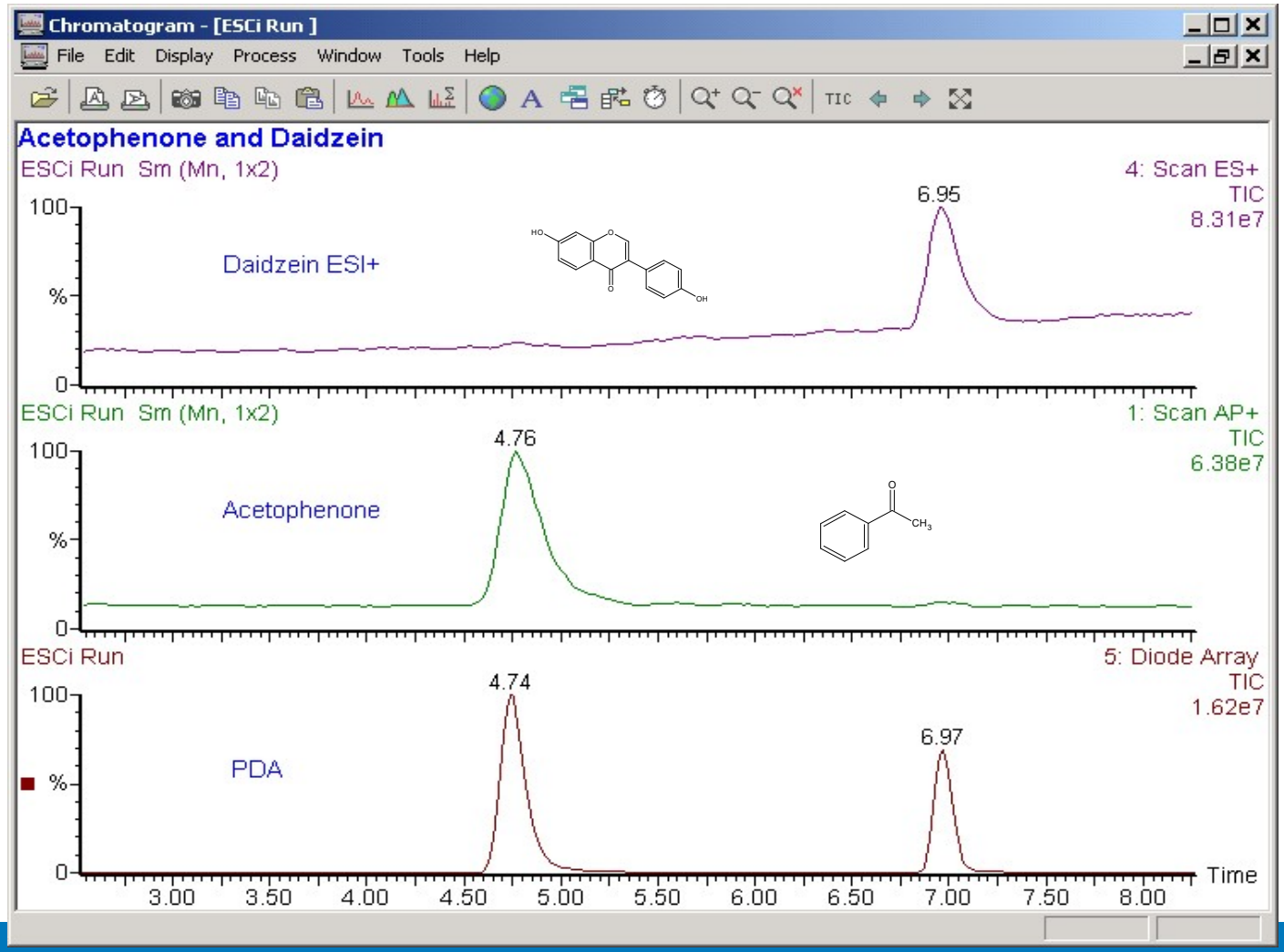
- APCI
  - Gaseous phase ionization
  - Reverse and normal phase
  - Ionization
    - Heated probe
    - Corona pin
  - Low mobile phase effect
  - Less polar compounds
  - Unsuitable for thermally labile compounds

ESCi®

Waters™

- Combines ESI and APCI capability on the Xevo TQ-S micro
- Uses existing ESI probe with the addition of the corona discharge needle
- ESCi provides a choice, through conventional methods, to alternate between ESI-, ESI+, APCI- and APCI+ modes in a single chromatographic run to reduce total analysis time.
- Information on analyte ionization is preserved, for example, when an analyte is only ionized in ESI- mode.

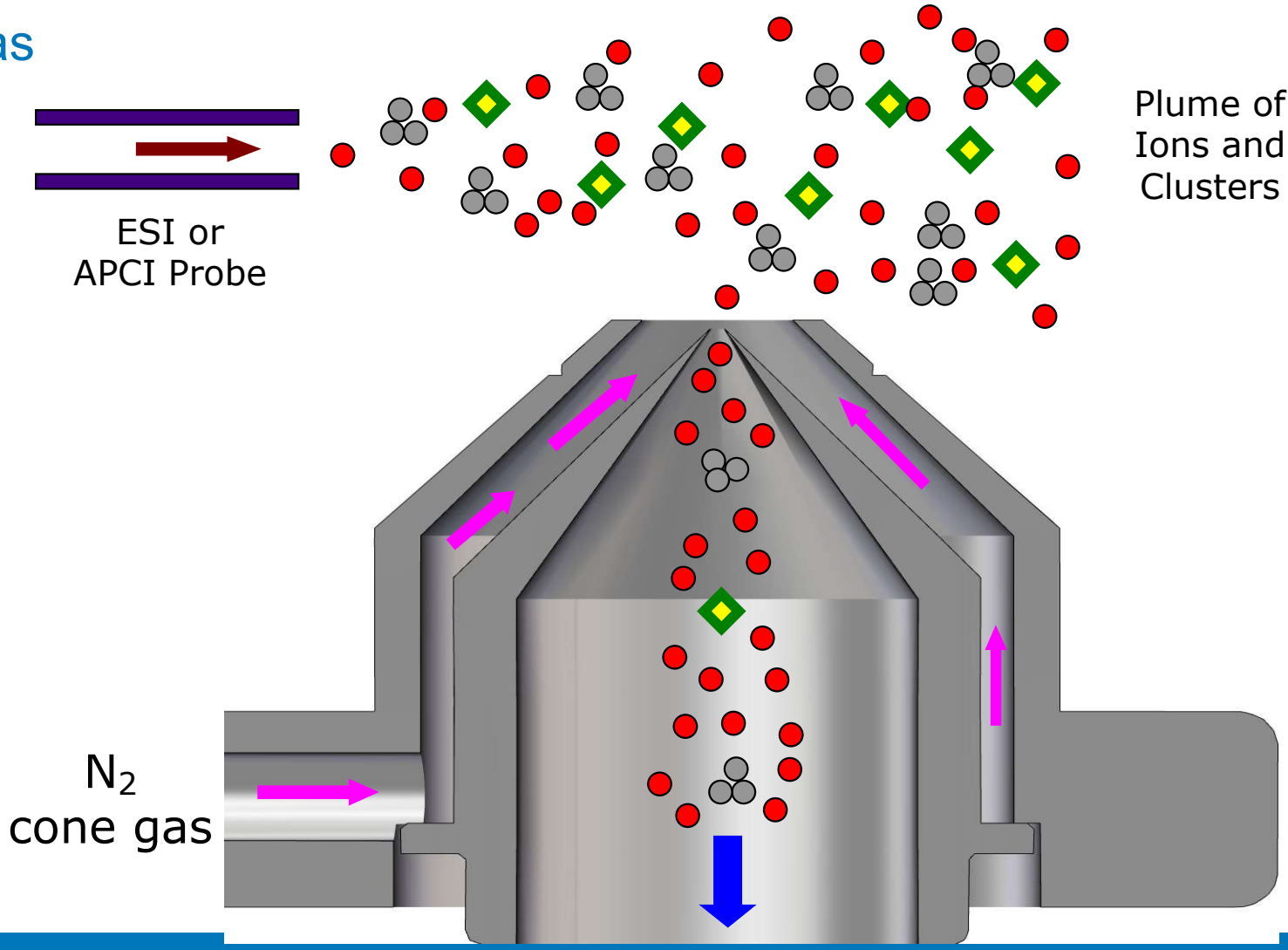
Multiple Scan Functions



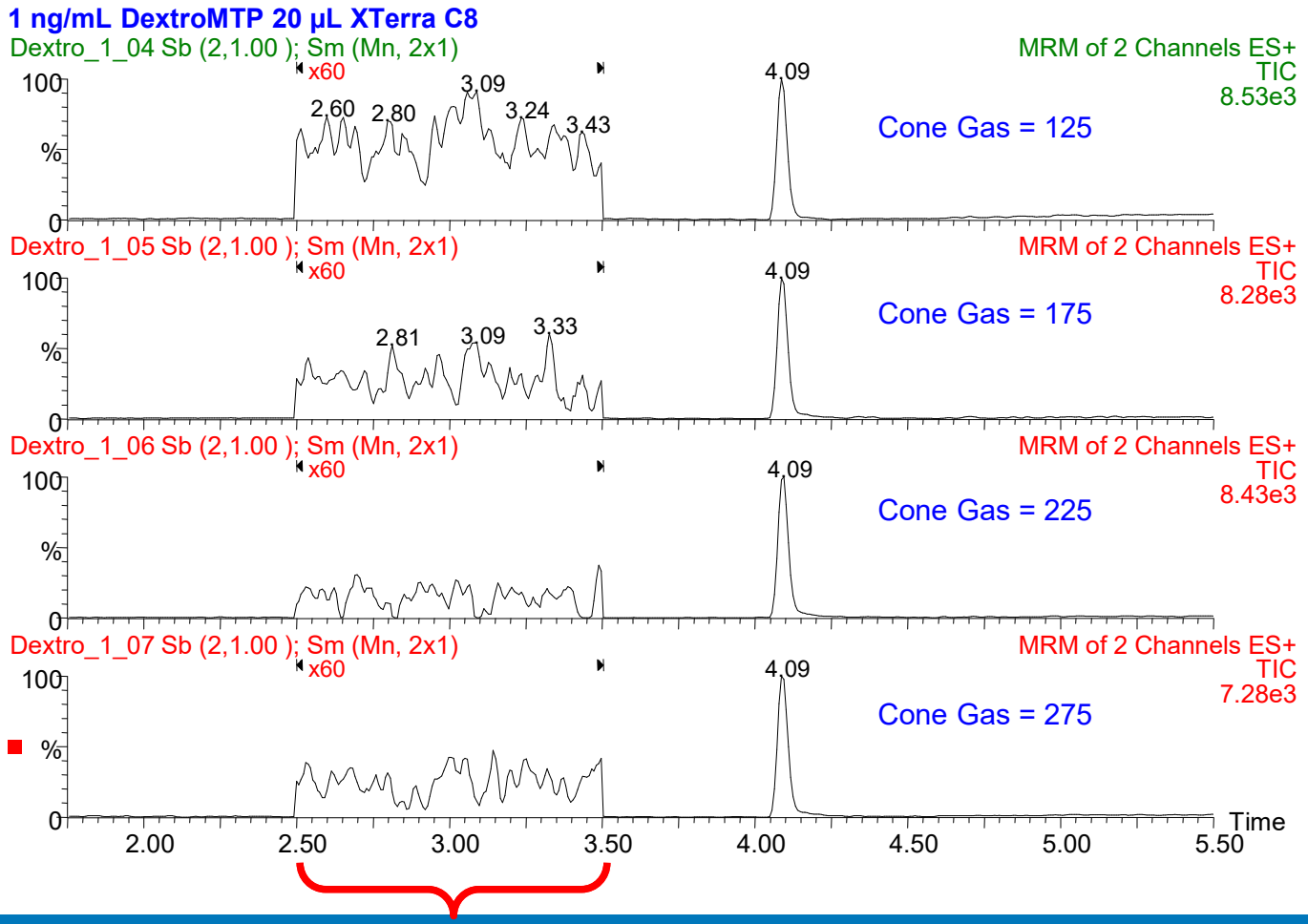
Post Ionization Events

- After ionization, the ions enter the ion block of the Xevo TQ-S micro.

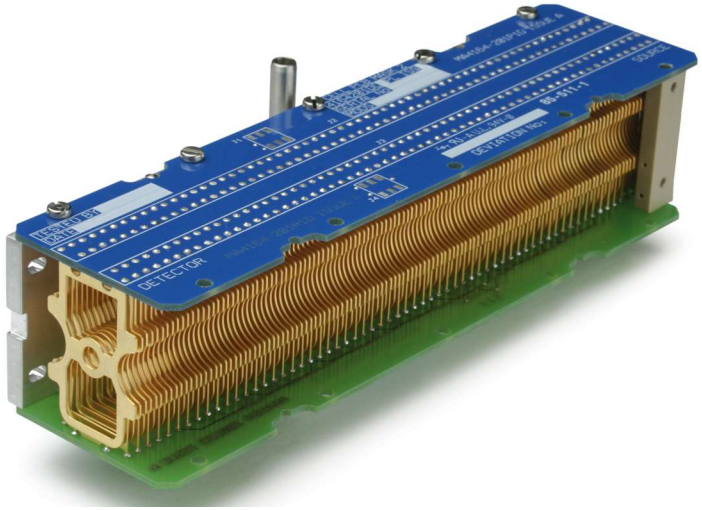
Cone Gas

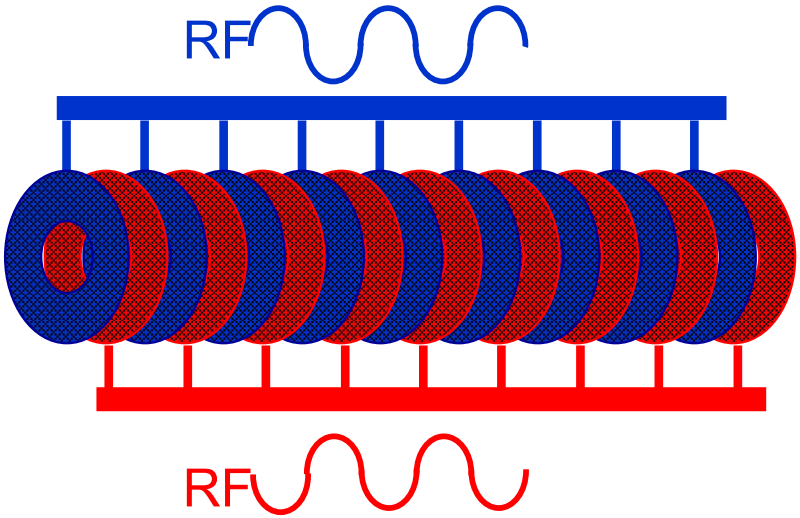


Cone Gas Example - Dextromethorphan

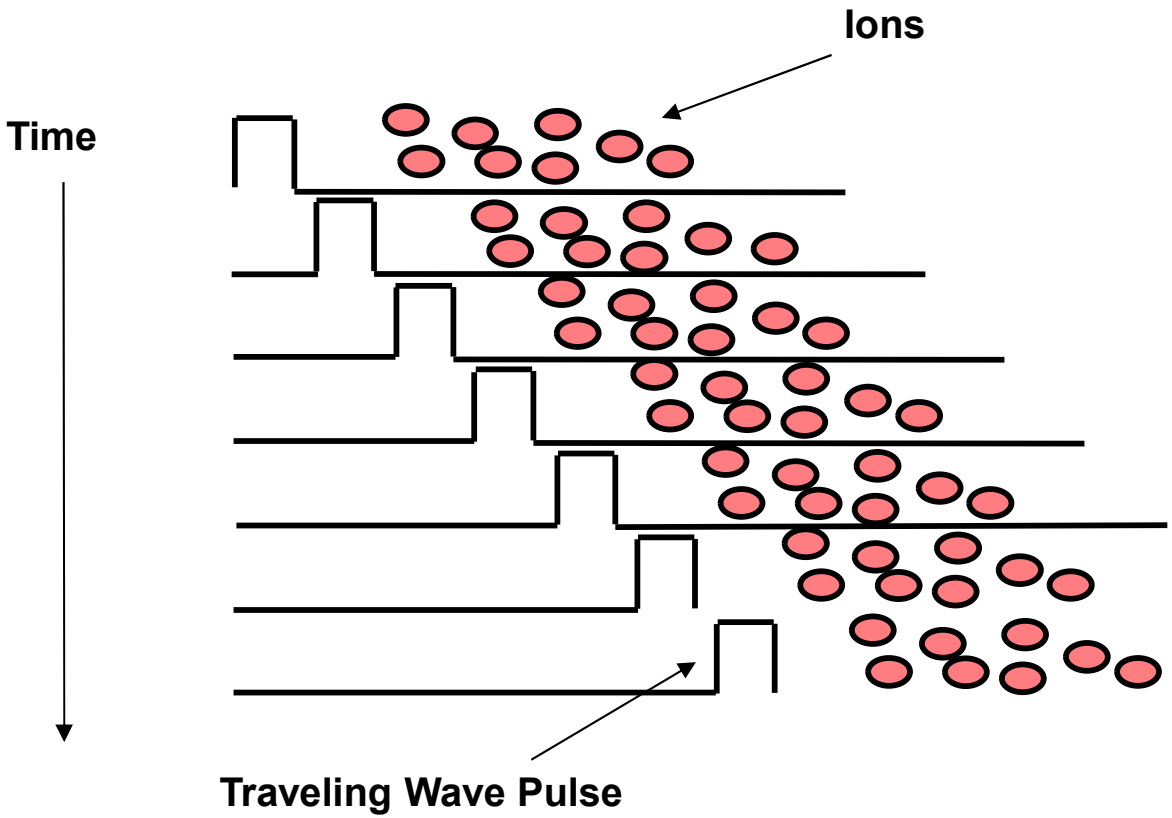


Traveling Wave Ion Transport

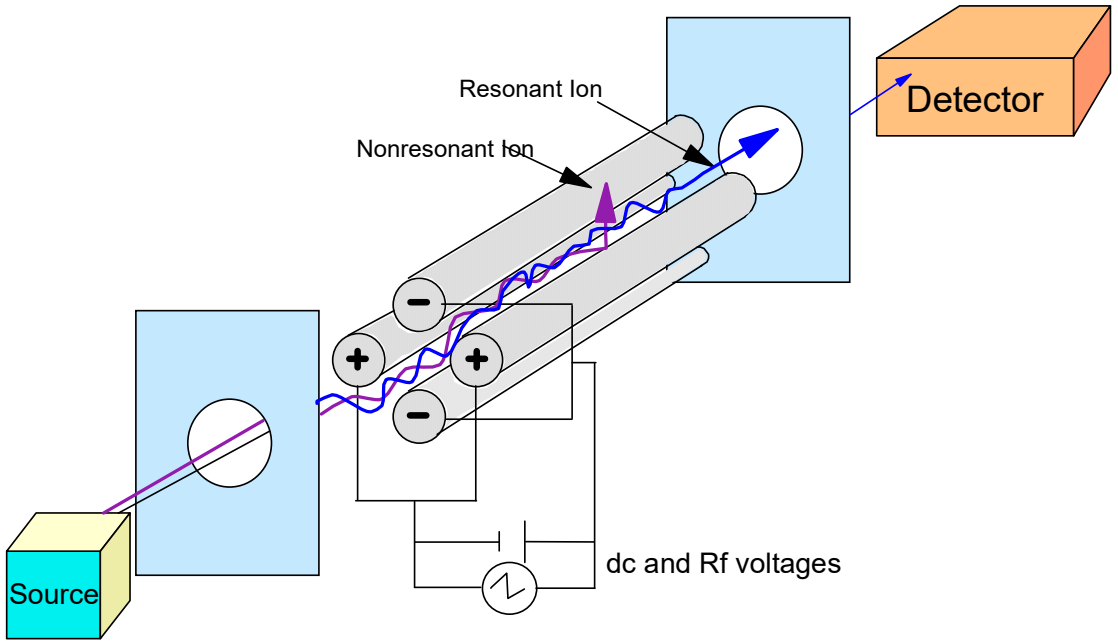




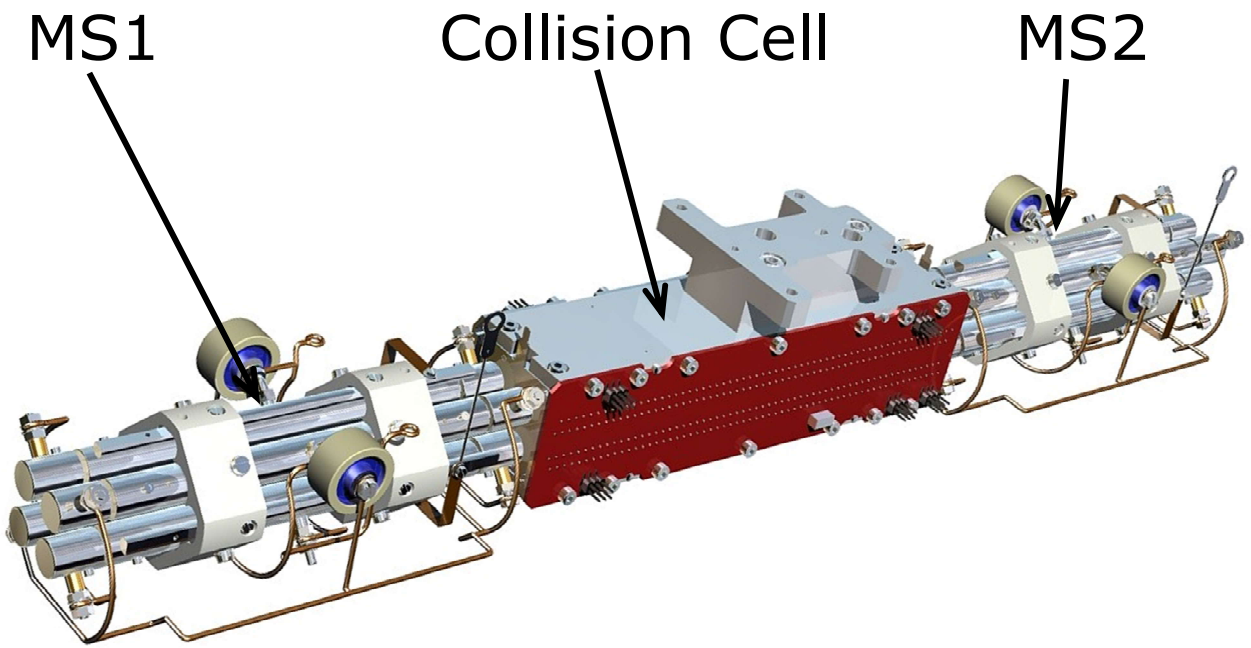
Traveling Wave Ion Transport



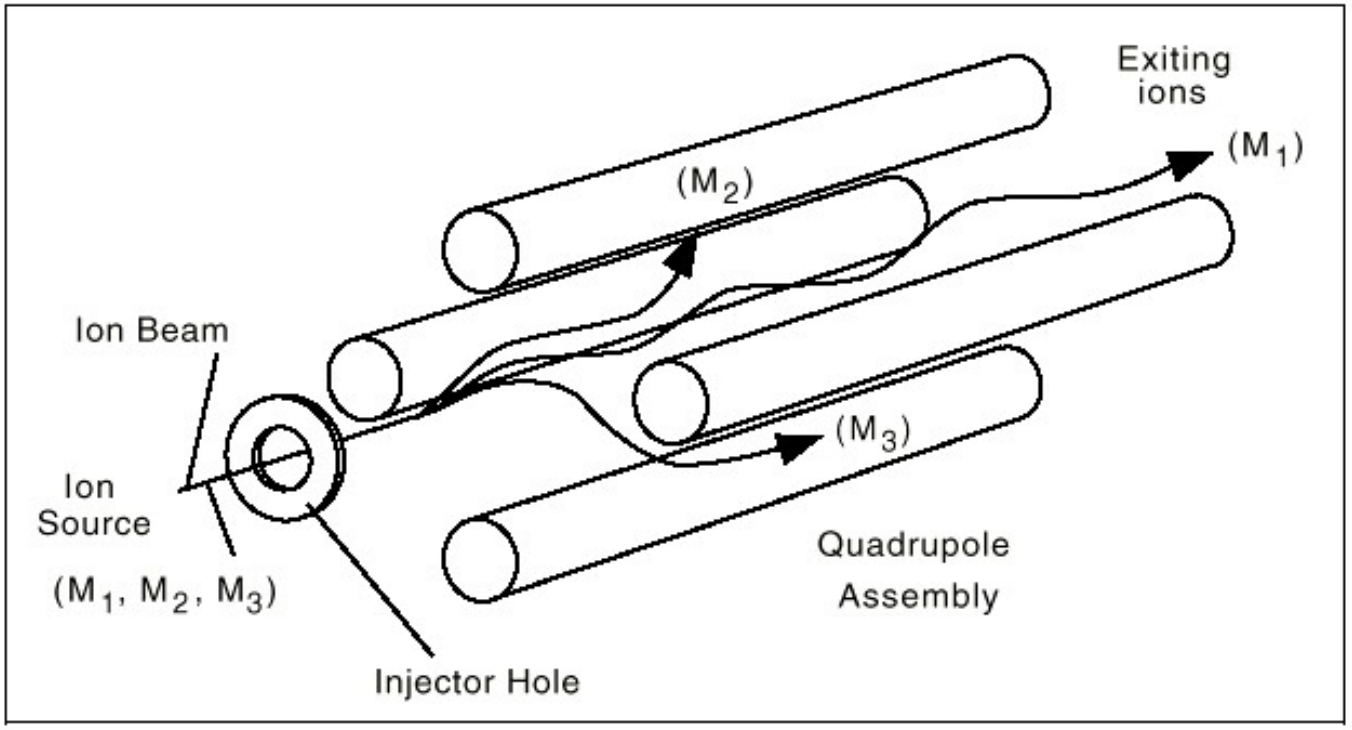
Quadrupole Schematic



Quadrupole Assembly

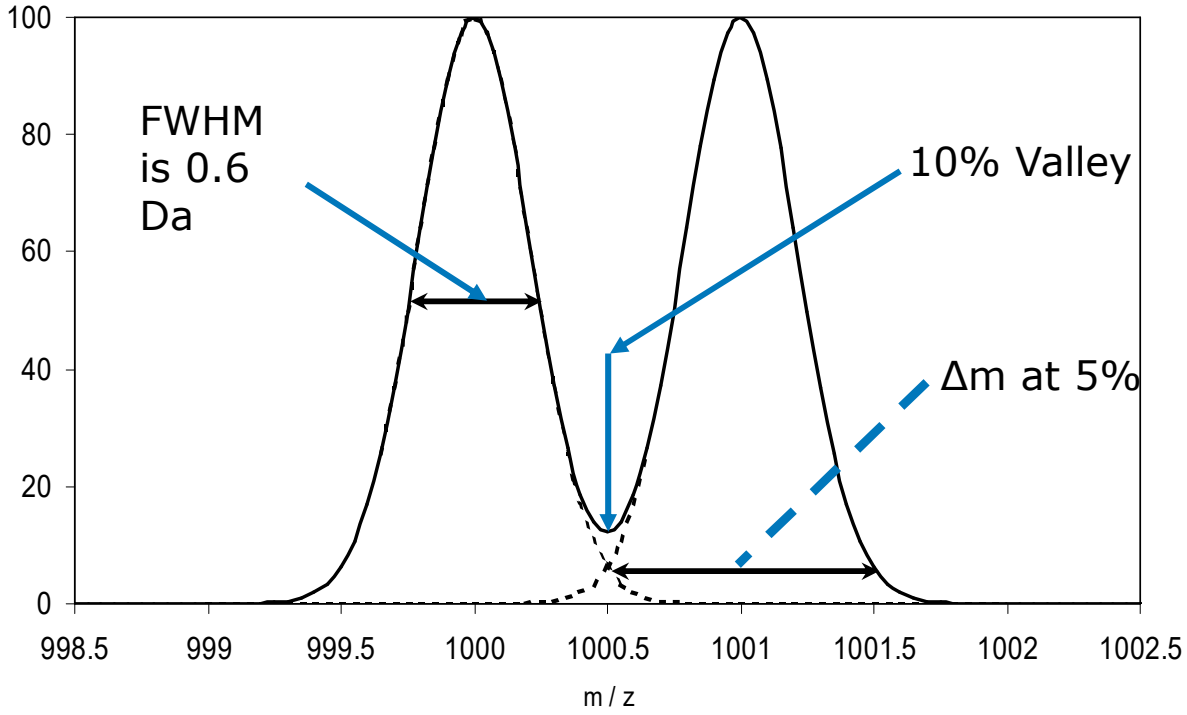


Quadrupole Theory

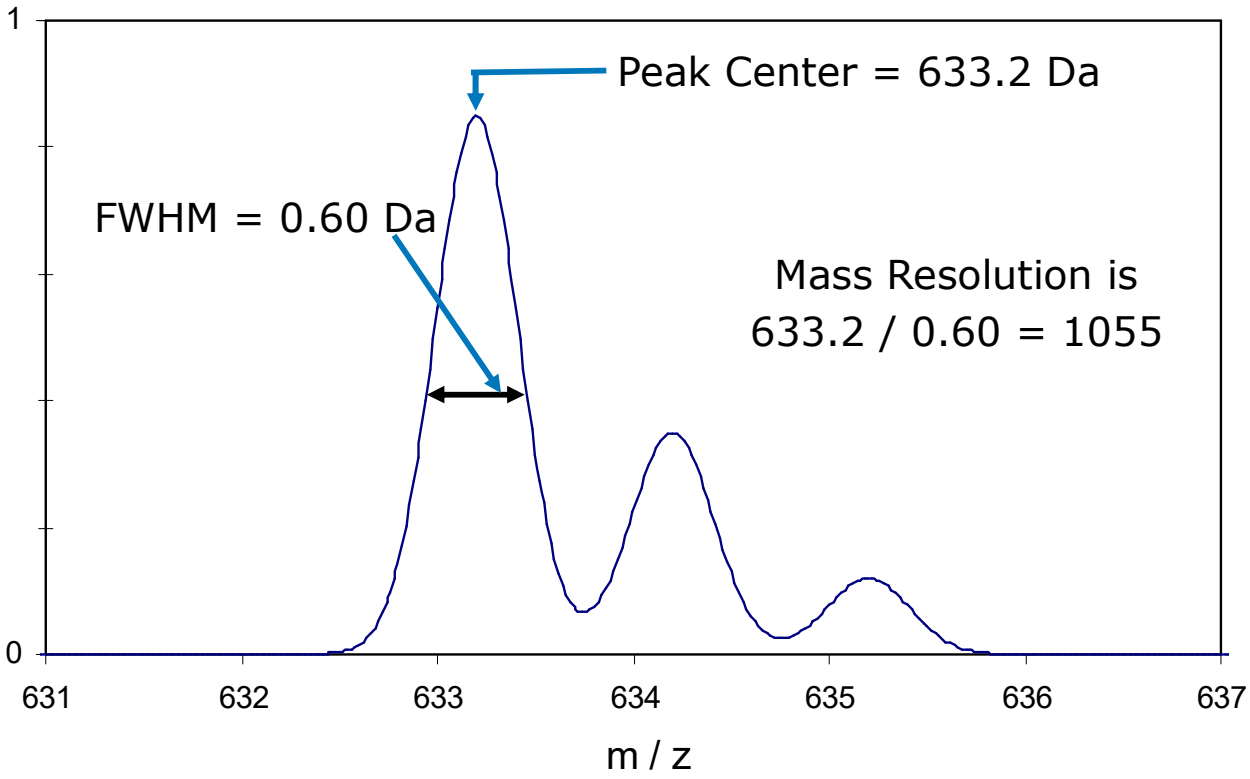


Quadrupole Example: For three ions passing through the quadrupole, m_1 has a stable trajectory while m_2 and m_3 have unstable trajectories.

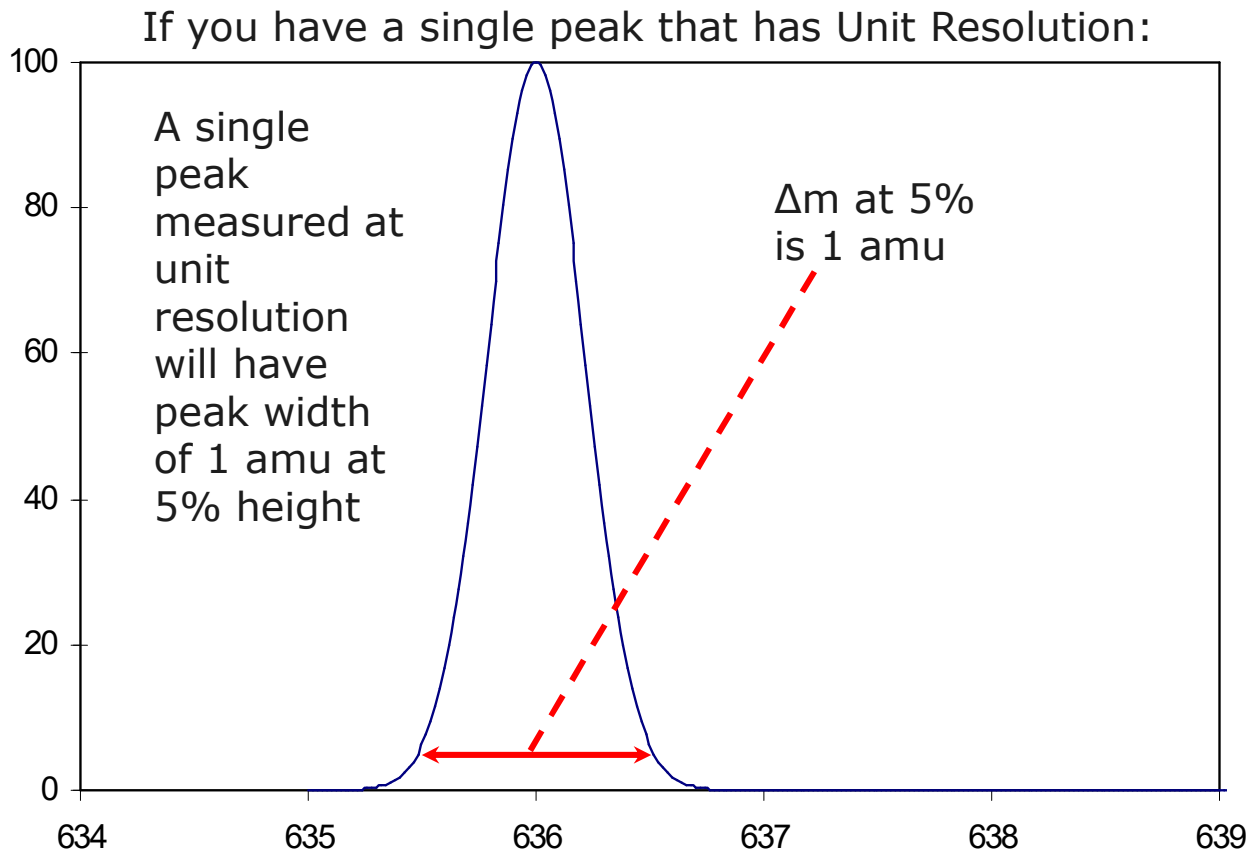
Unit Mass Resolution



Mass Resolution

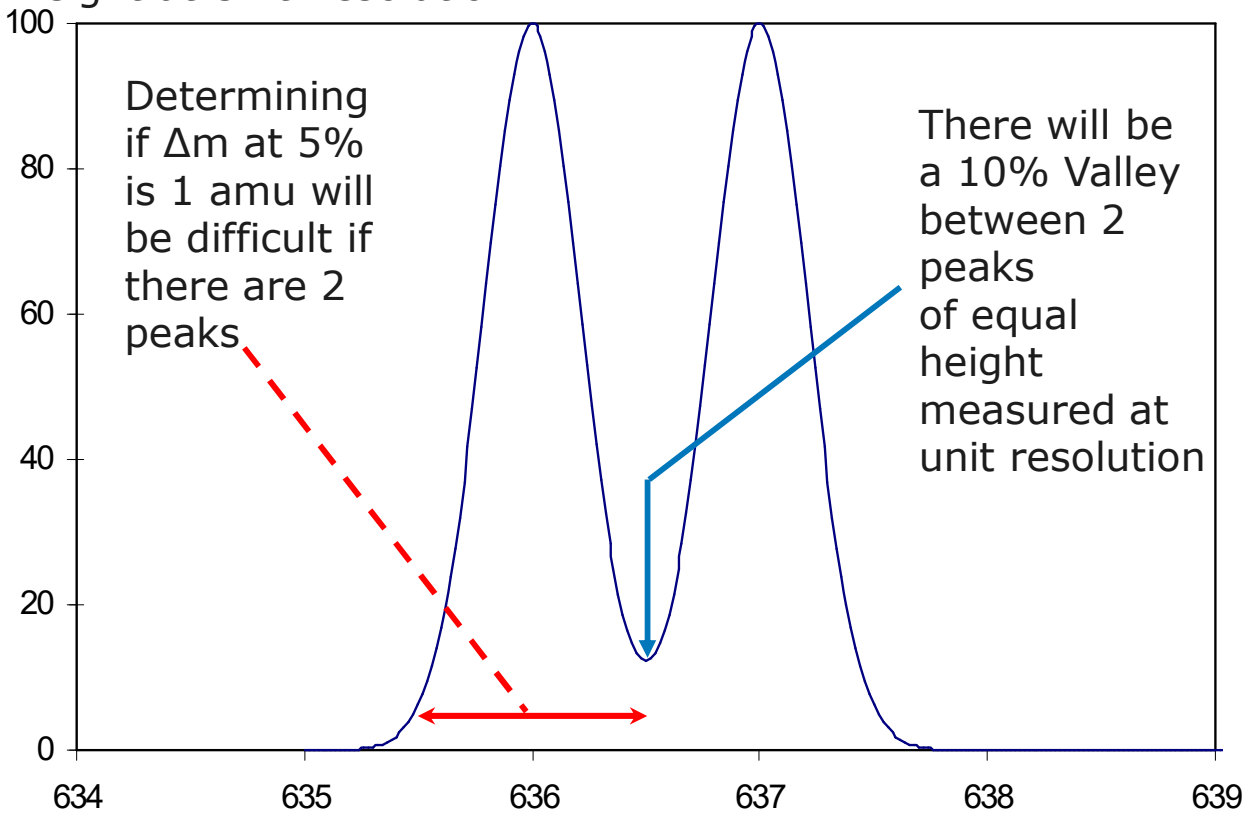


Unit Resolution



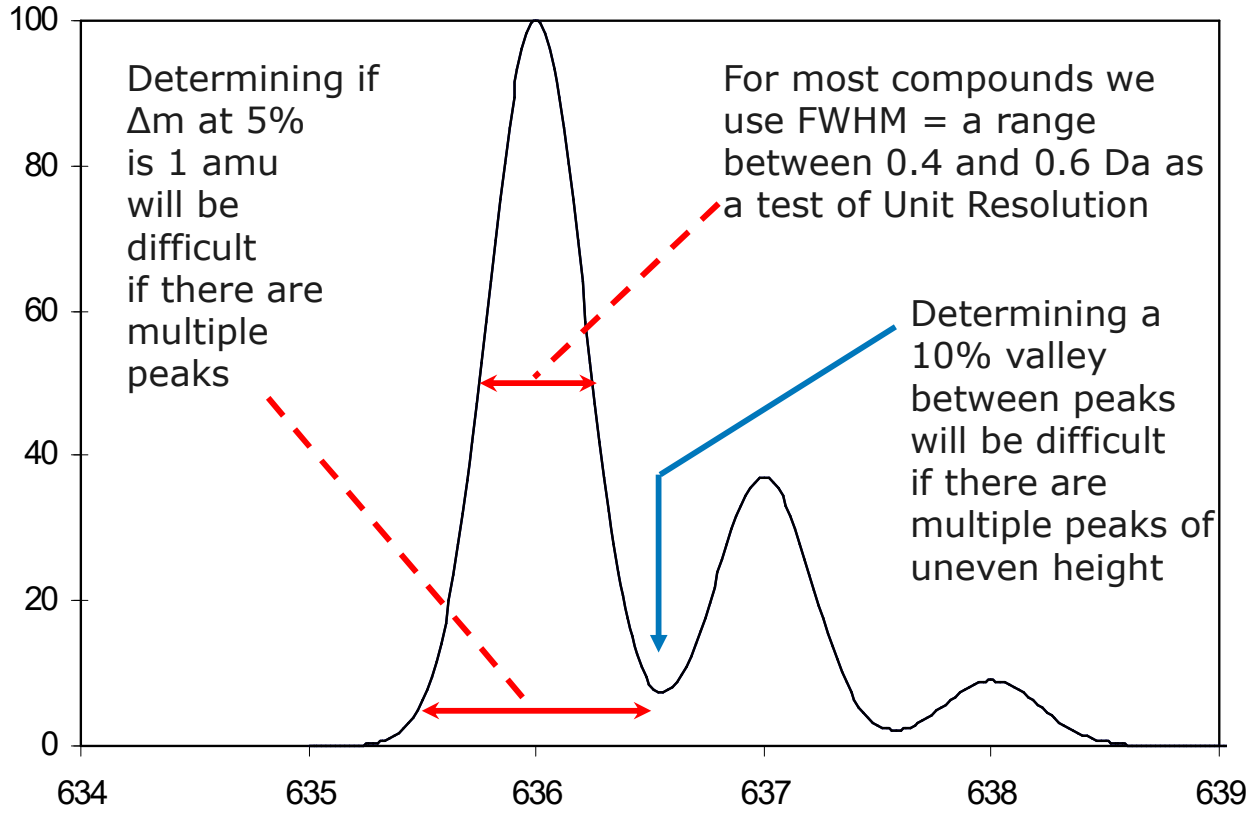
Unit Resolution

If you do not have a single peak, but have 2 peaks of equal height at Unit Resolution:



Unit Resolution

For most compounds, you do not get a single peak or 2 peaks of equal height.

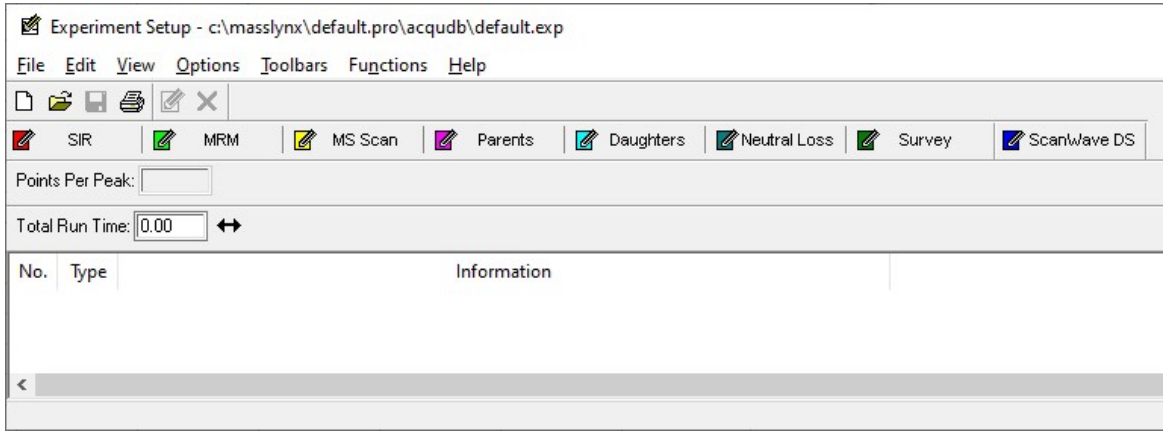


Chapter Two

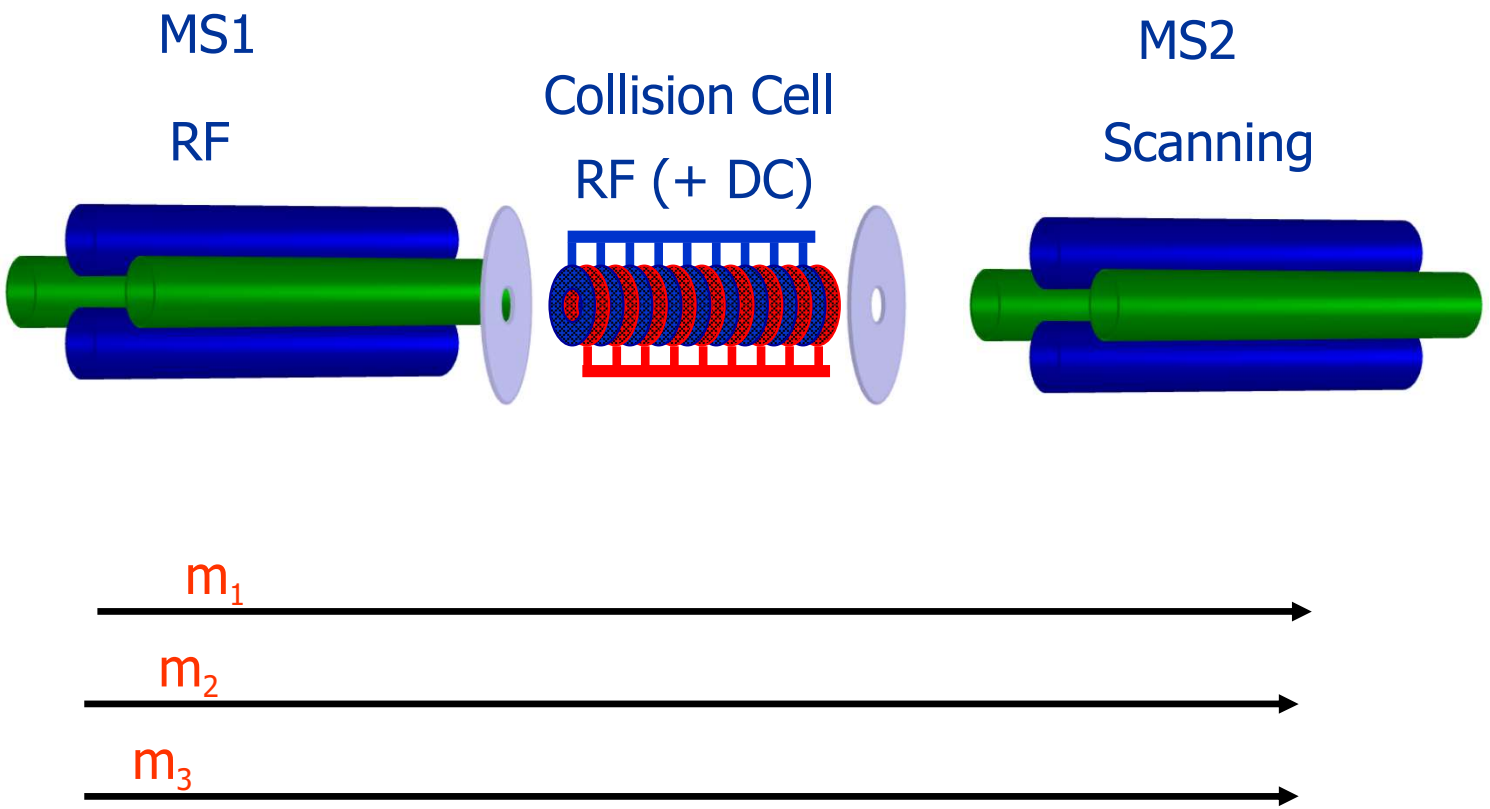
Data Acquisition Modes

Data Acquisition Modes

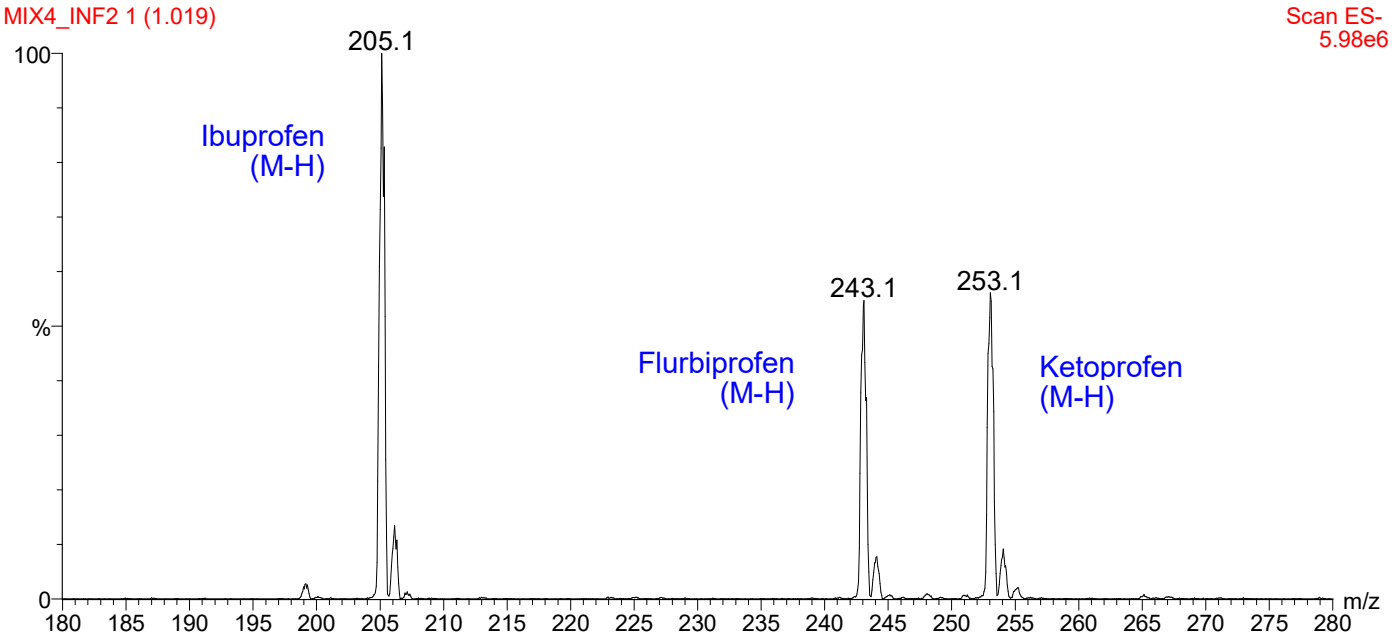
- MS Modes
  - MS Scan
  - SIR
- MS/MS Modes
  - Product Ion Analysis
  - Parent Ion Analysis
  - Multiple Reaction Monitoring
  - Neutral Loss Analysis
  - Survey Scan



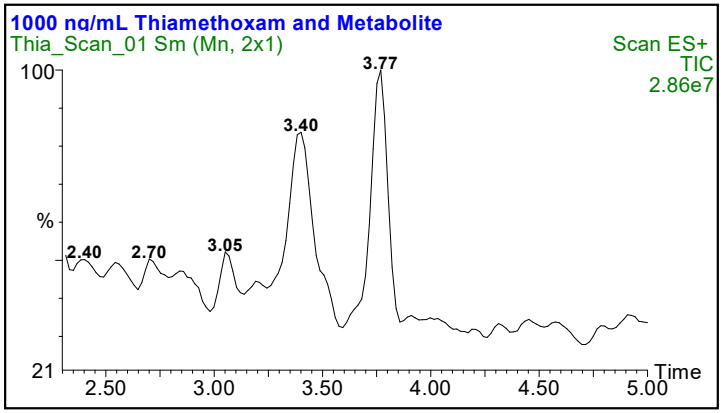
MS Scan



MS Scan

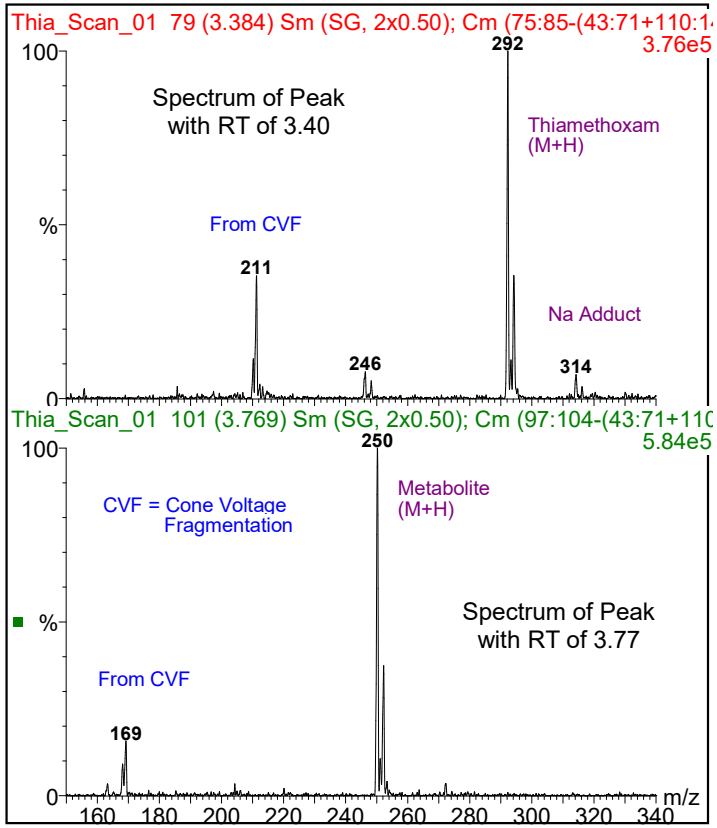


Mass Spectra of LC Peaks



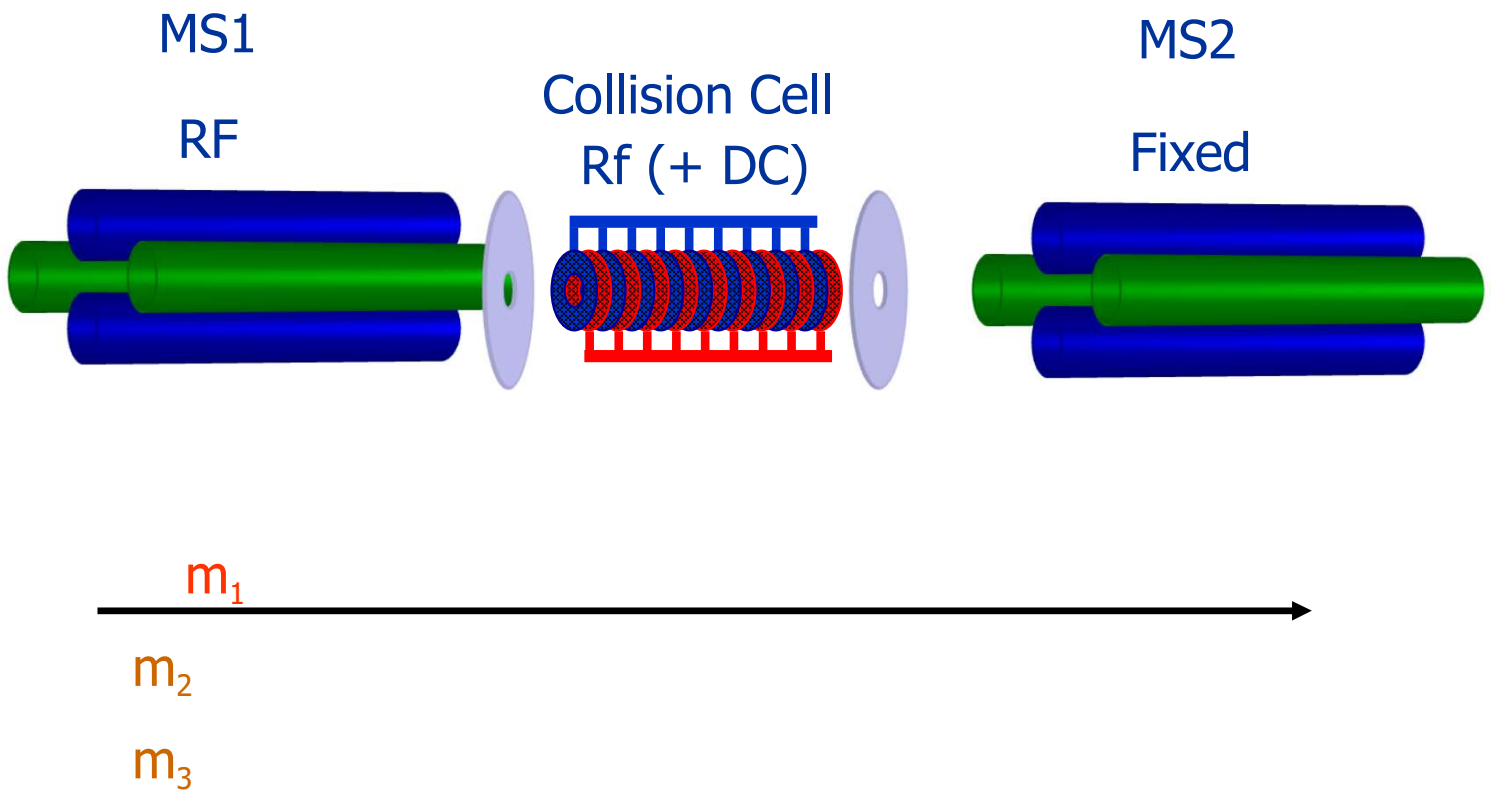
Full Scan Spectra of LC Analysis of a Standard Solution of Thiamethoxam and one of its Metabolites

Spectra taken using 1 second scan time for the range *m/z* 100 to 400

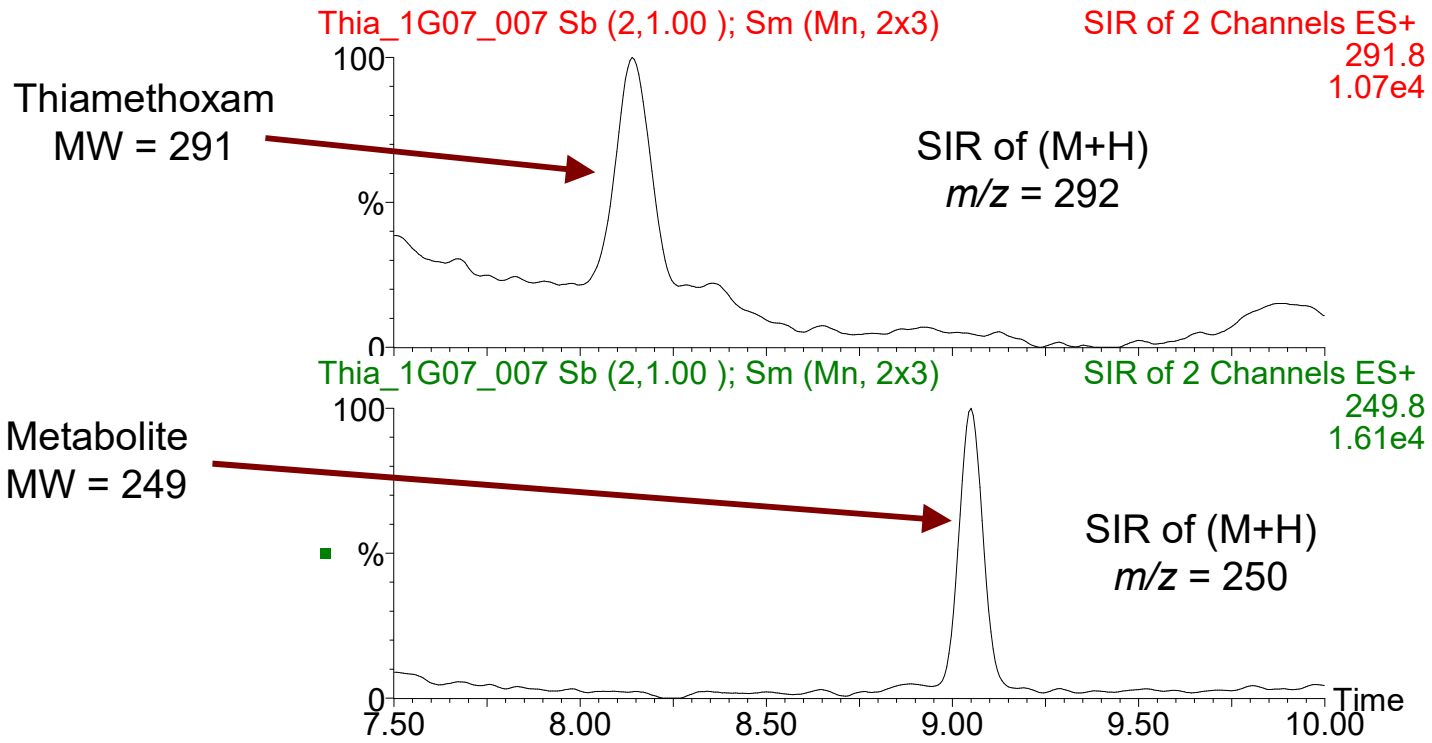


Spectra of two LC Peaks

Selected Ion Recording (SIR)



SIR Example

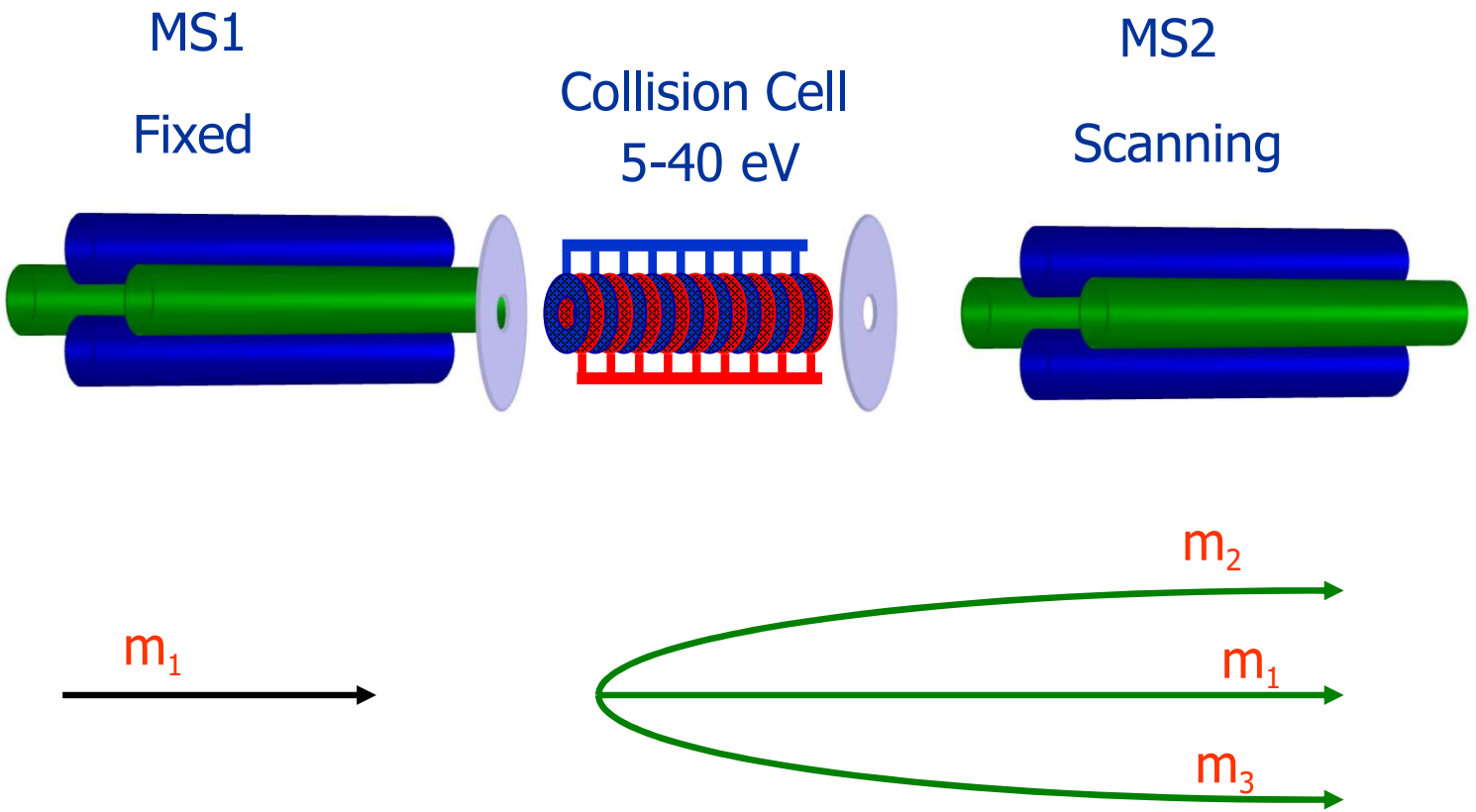


Xevo TQ-S micro MS/MS

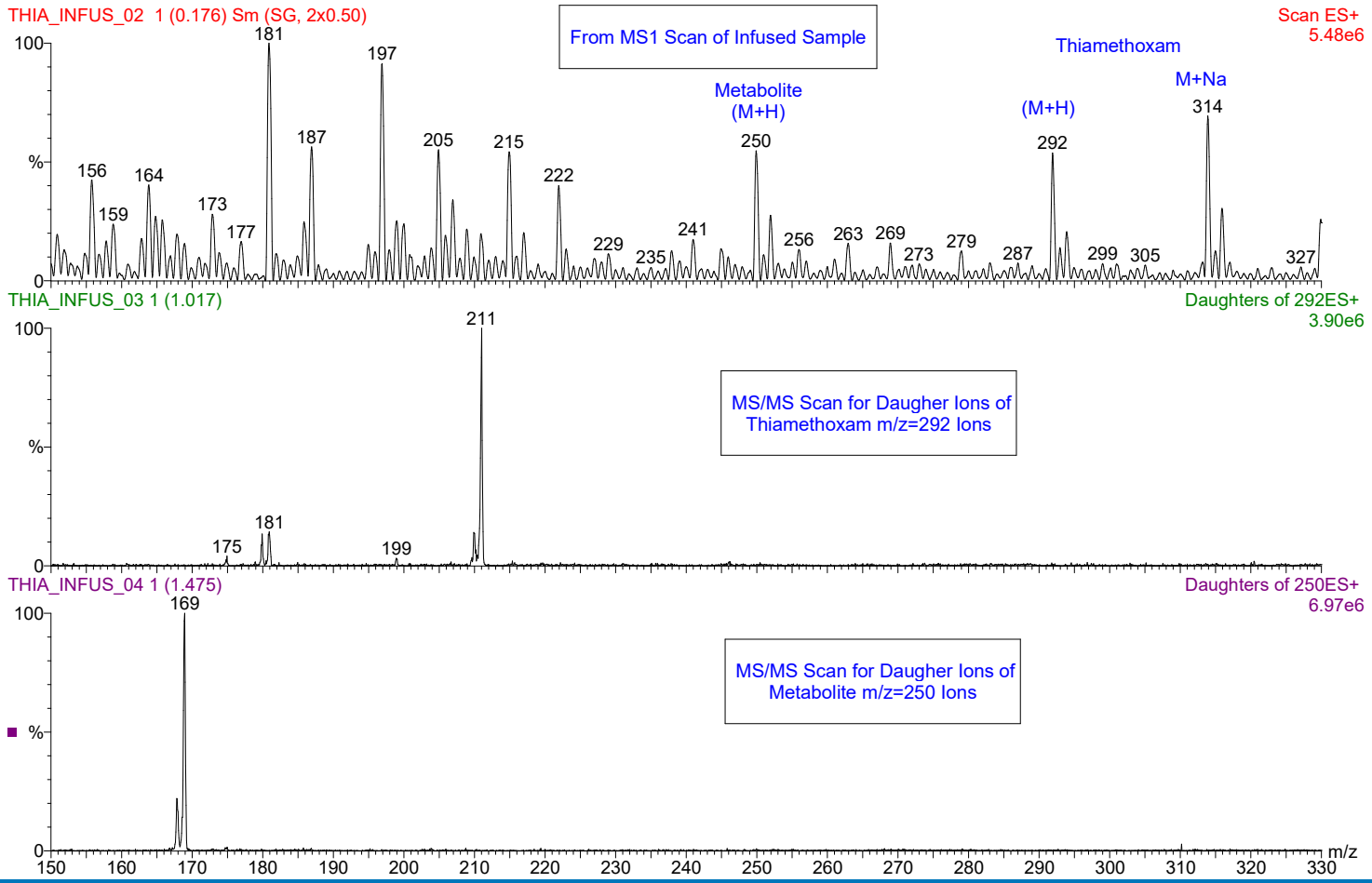
Waters™

- Low energy collisions (simple fragmentation pathways)
- Collision gas of choice is Argon
- Collision gas pressure is normally fixed while the collision energy is used to alter the degree of fragmentation. The collision energy will vary based on the compound
- Sodium and potassium adducts are normally too stable to fragment using low energy collisions.

Product Ion Analysis (Daughter Scan)

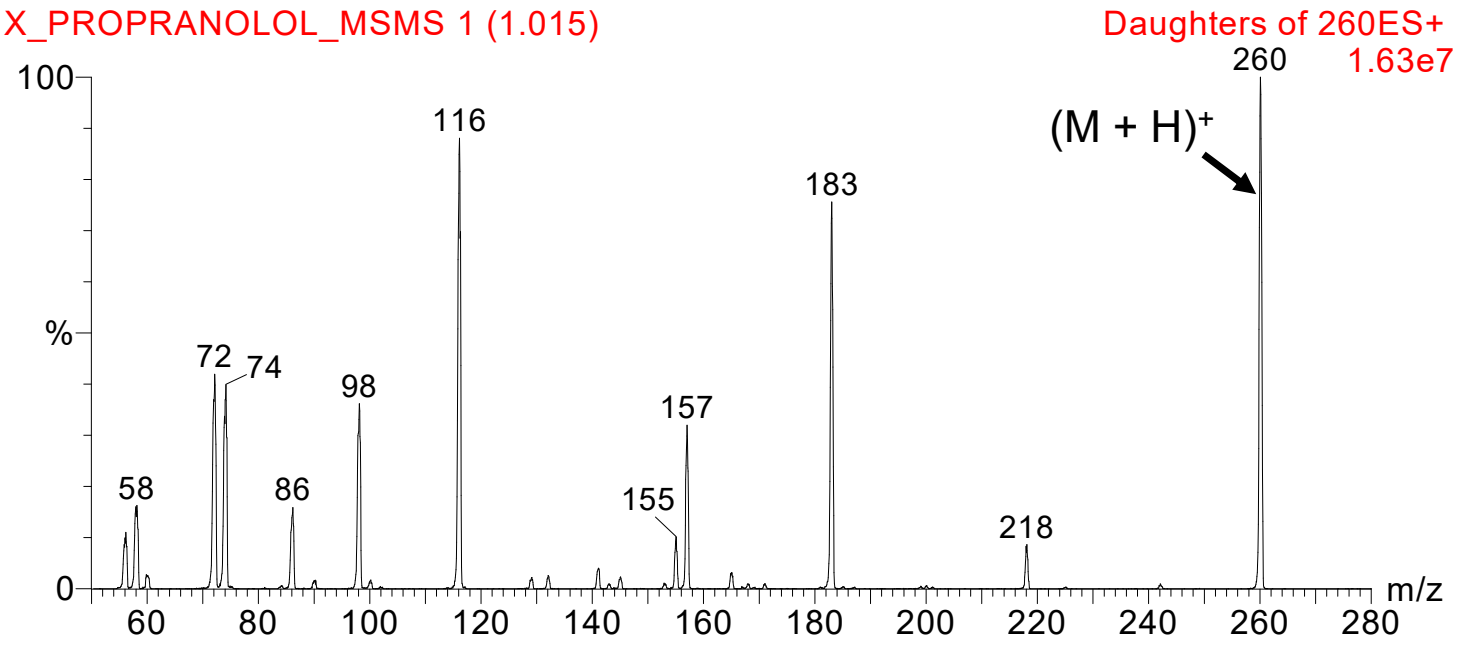


Product Ion Analysis

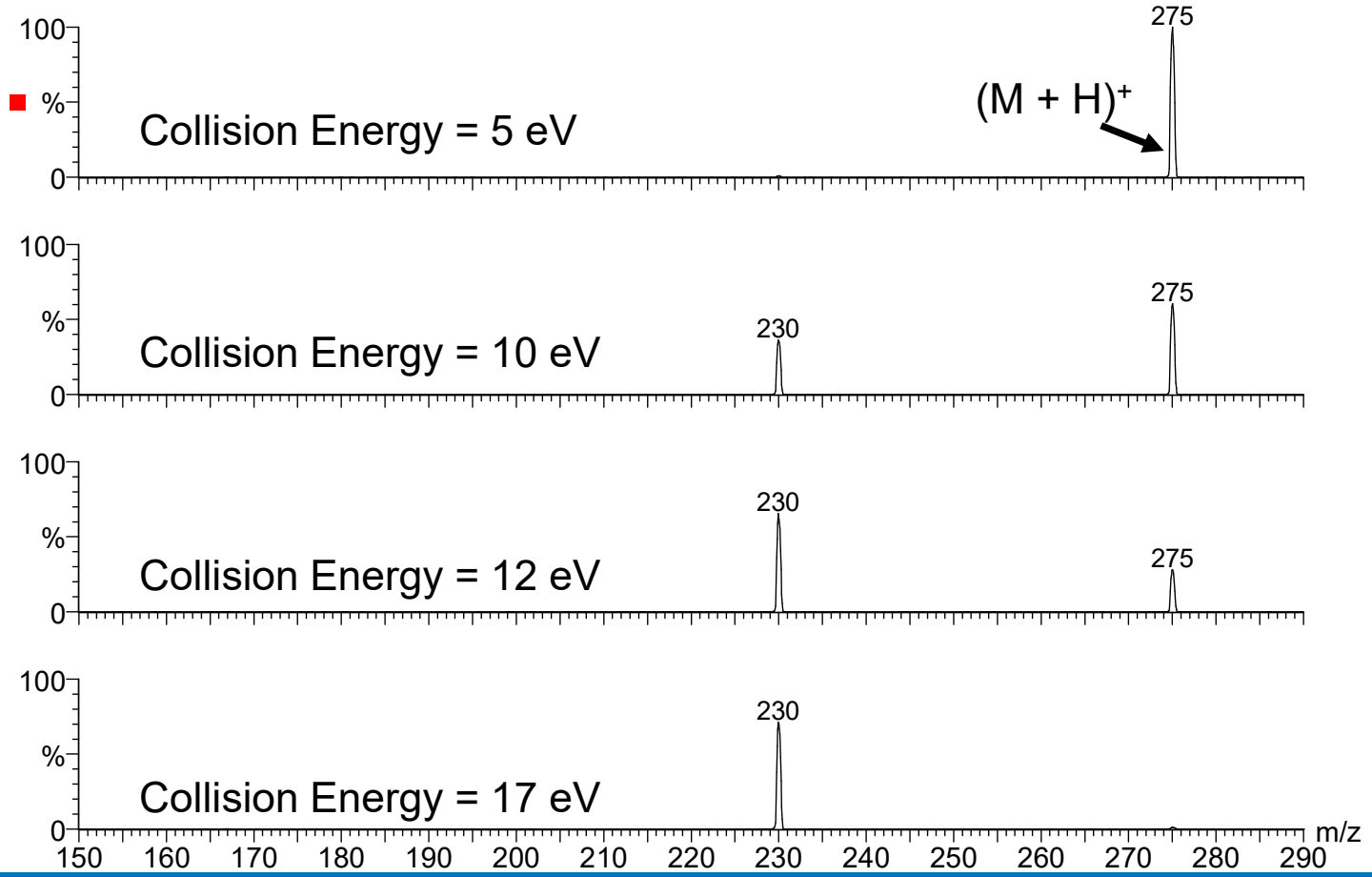


Product Ion Analysis

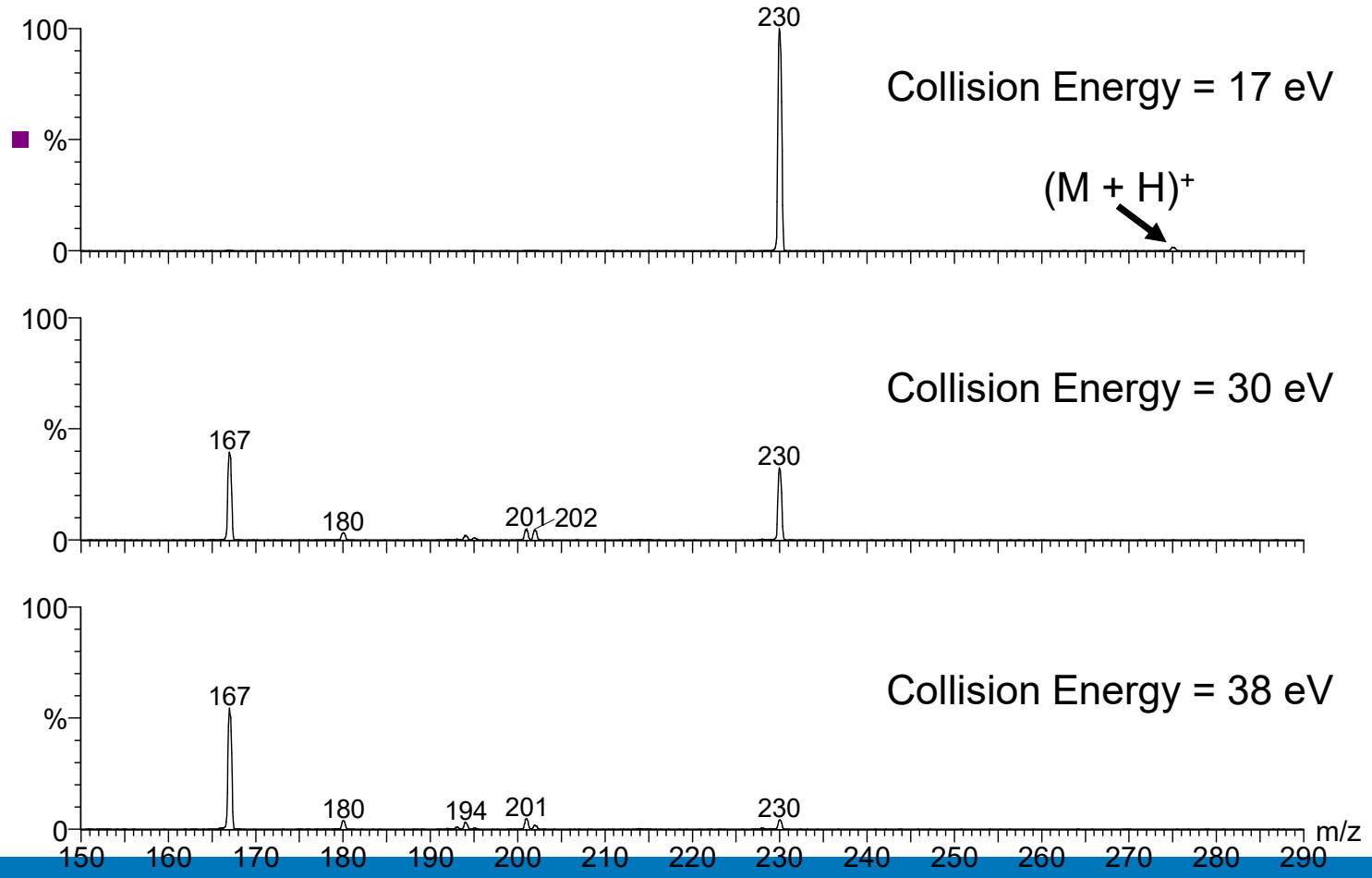
MS/MS Spectra of Propranolol (MW=259) Product Ions of *m/z*=260



Effect of Changing Collision Energy



Effect of Changing Collision Energy

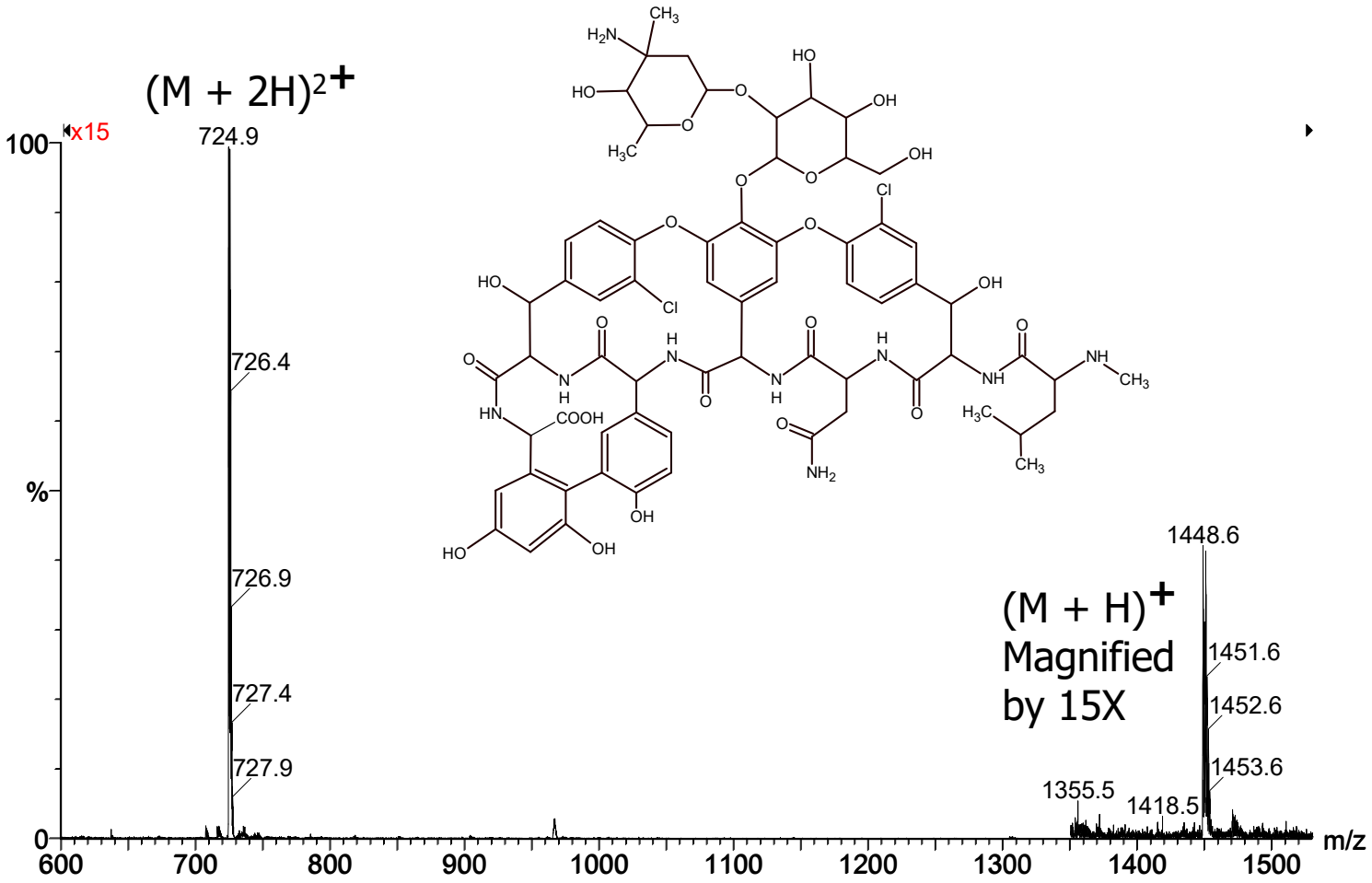


MS/MS of Multiply Charged Ions

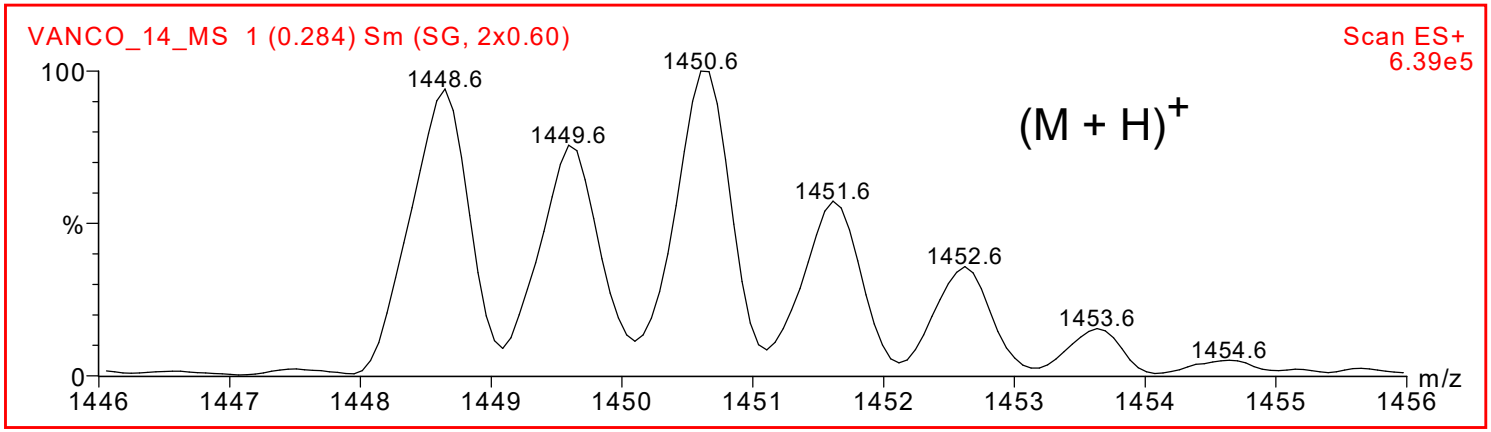
Waters™

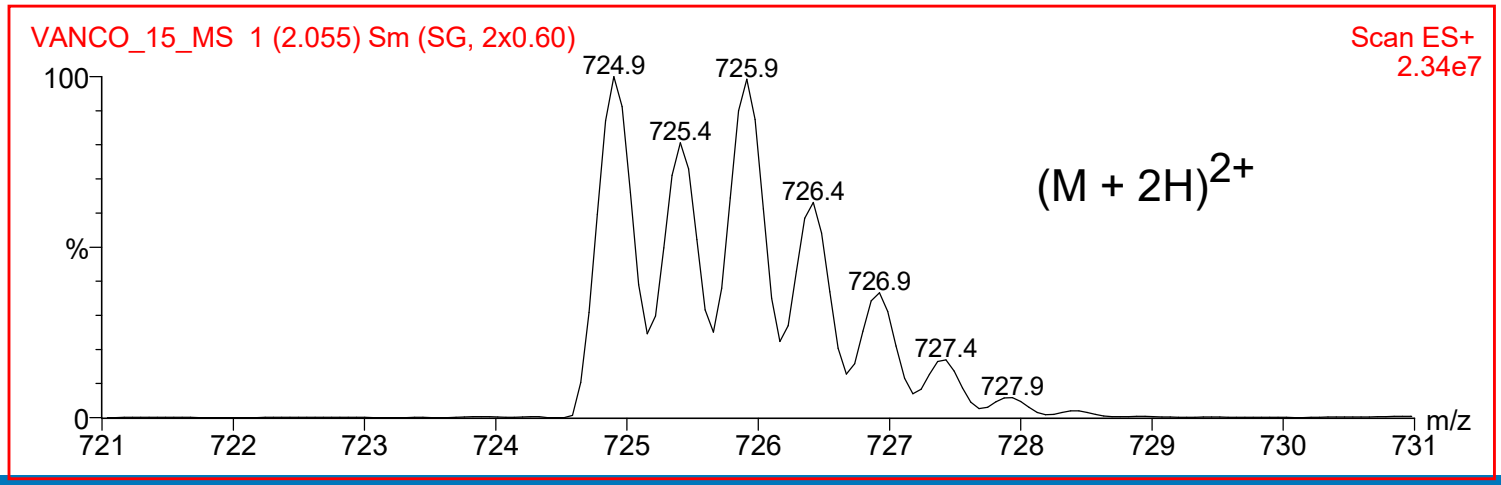
- The most intense ions are normally used for MS/MS even if they are multiply charged
- Multiply charged ions may require higher collision gas pressures than singly charged ions
- Singly-charged fragment ions may have a higher apparent mass (m/z) than their multiply-charged precursor ions

Vancomycin

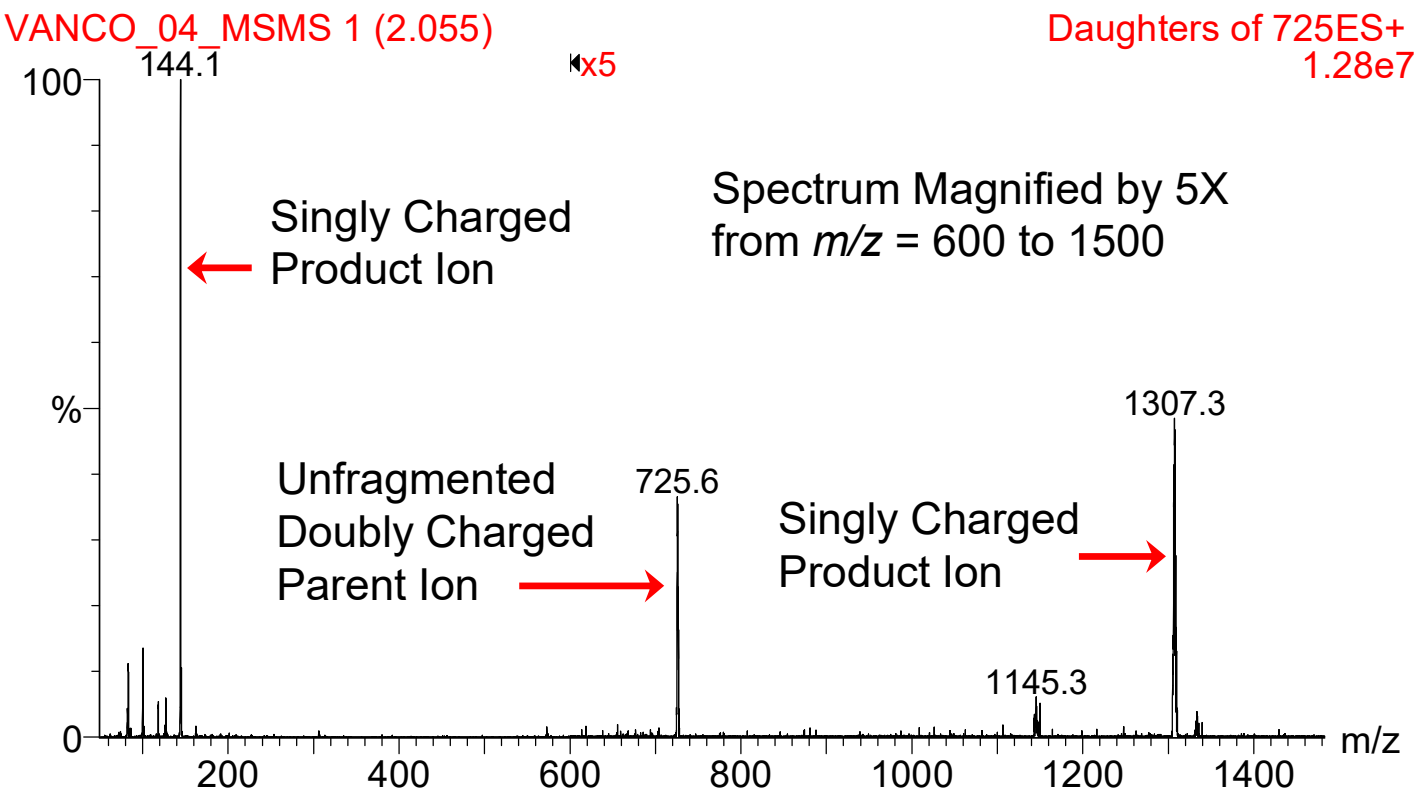


MS Spectra of Vancomycin

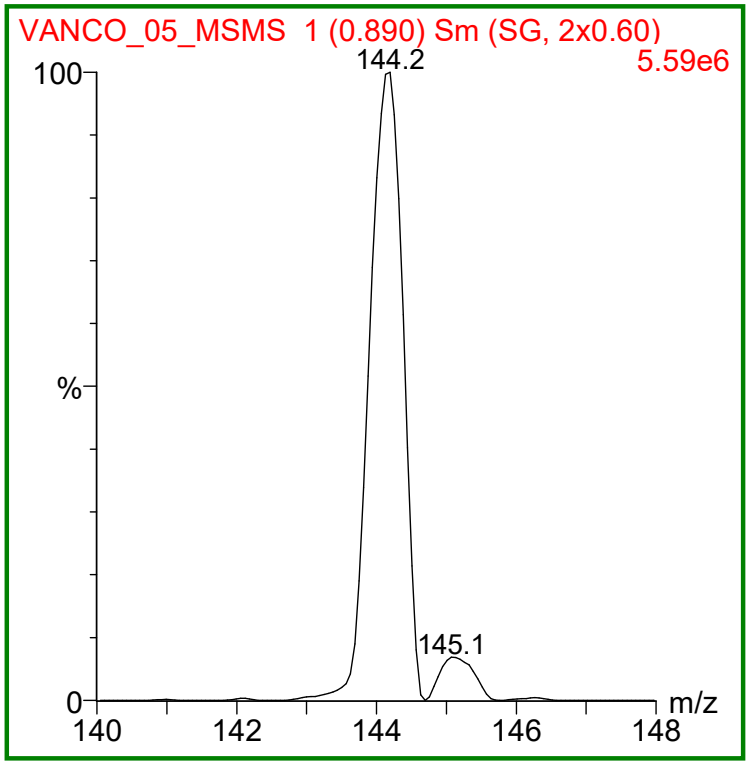


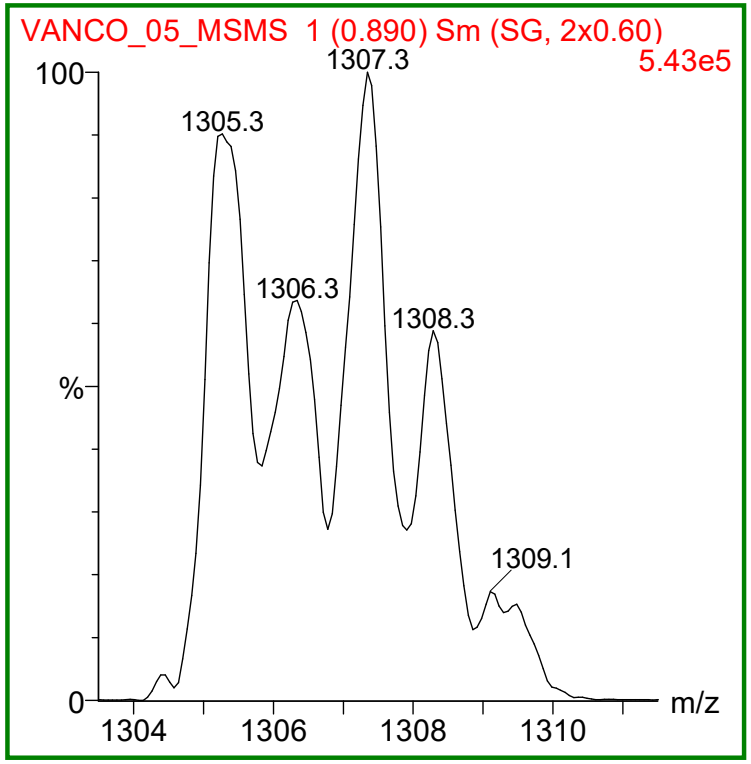


MS/MS Spectra of Vancomycin



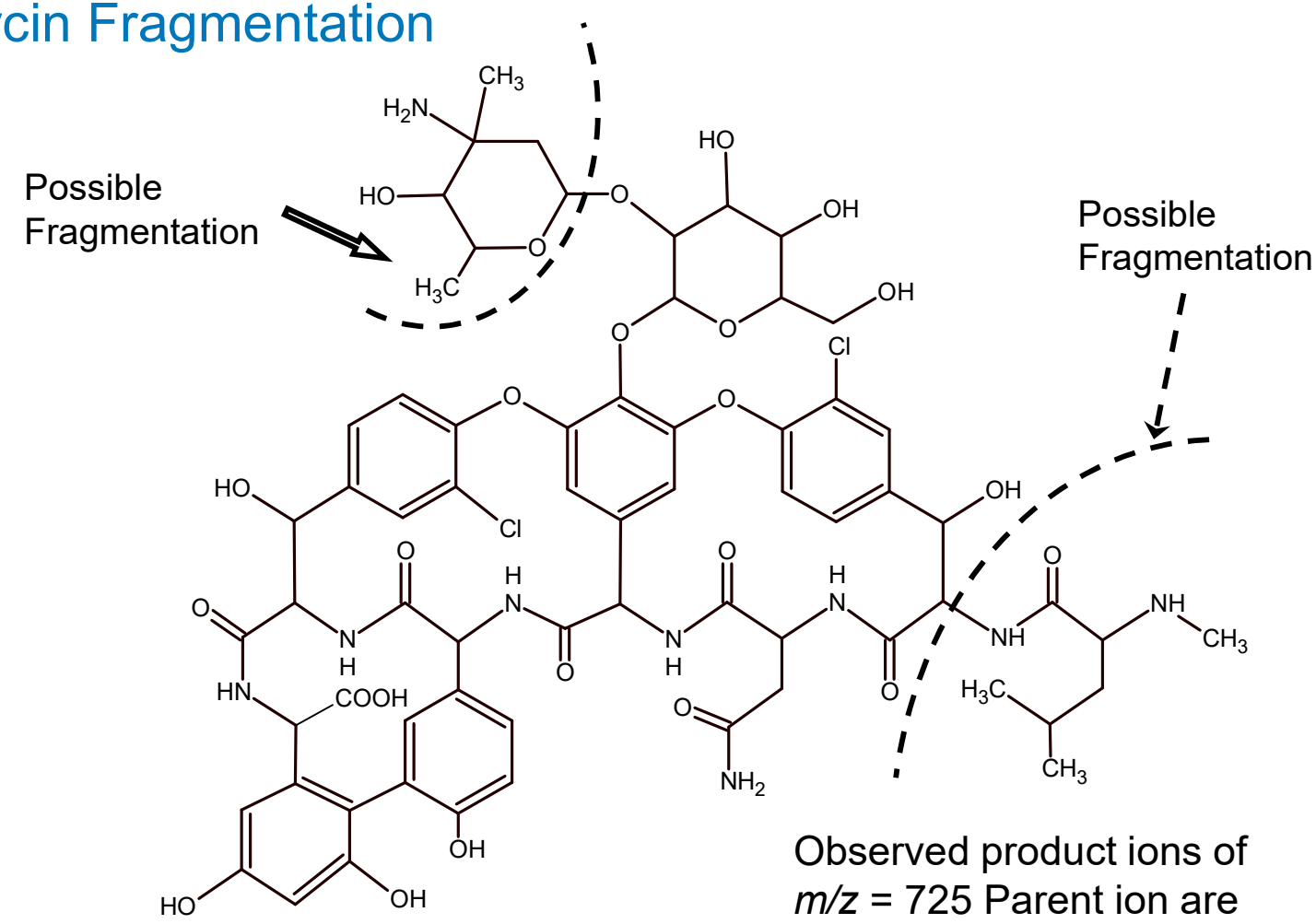
MS/MS Spectra of Vancomycin





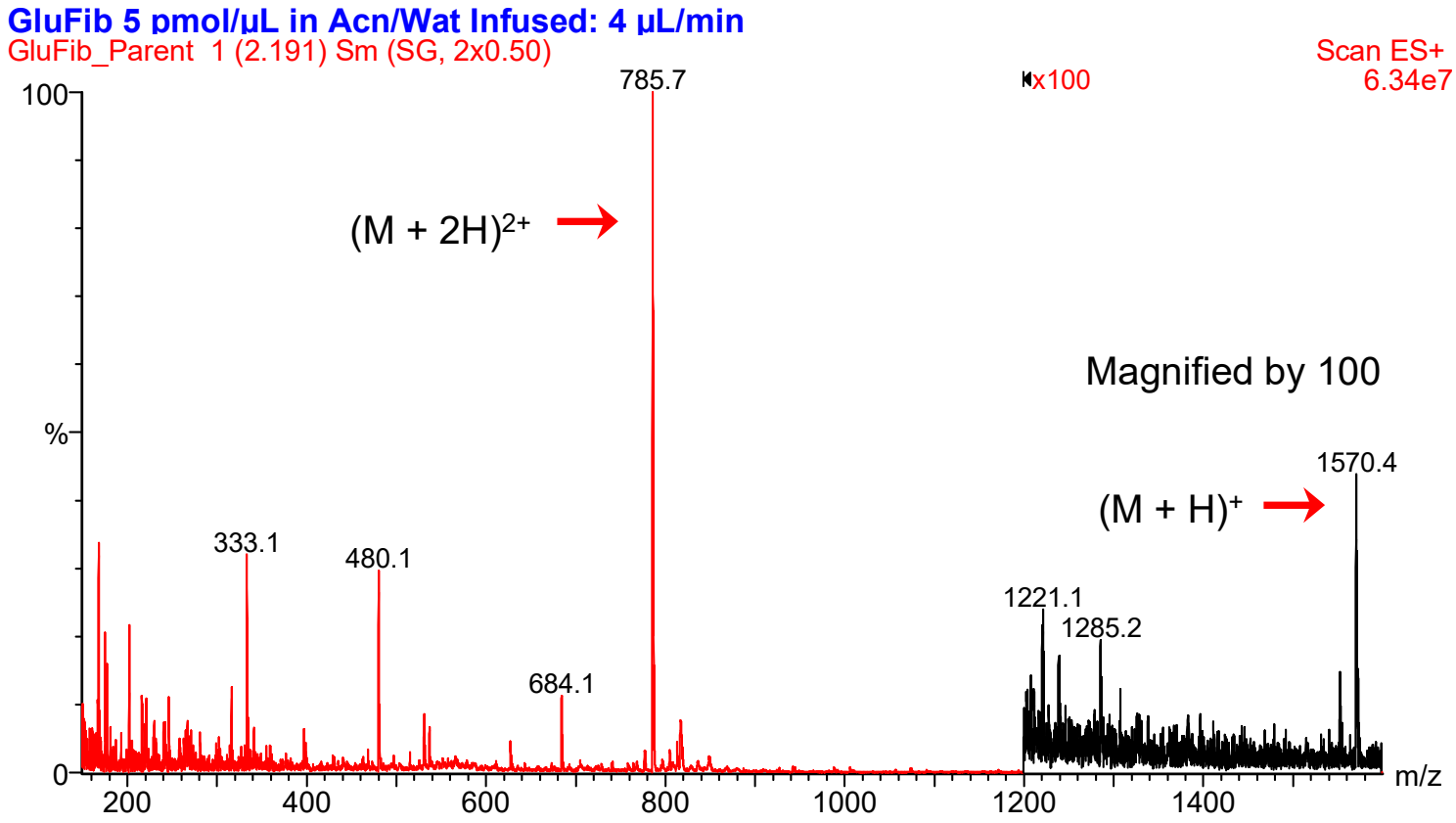
Note: LM & HM values of MS1 were lowered to pass all isotopic forms of the *m/z*=725 ion into the collision cell. 'Isotope Peaks' are 1 Da apart.

Vancomycin Fragmentation

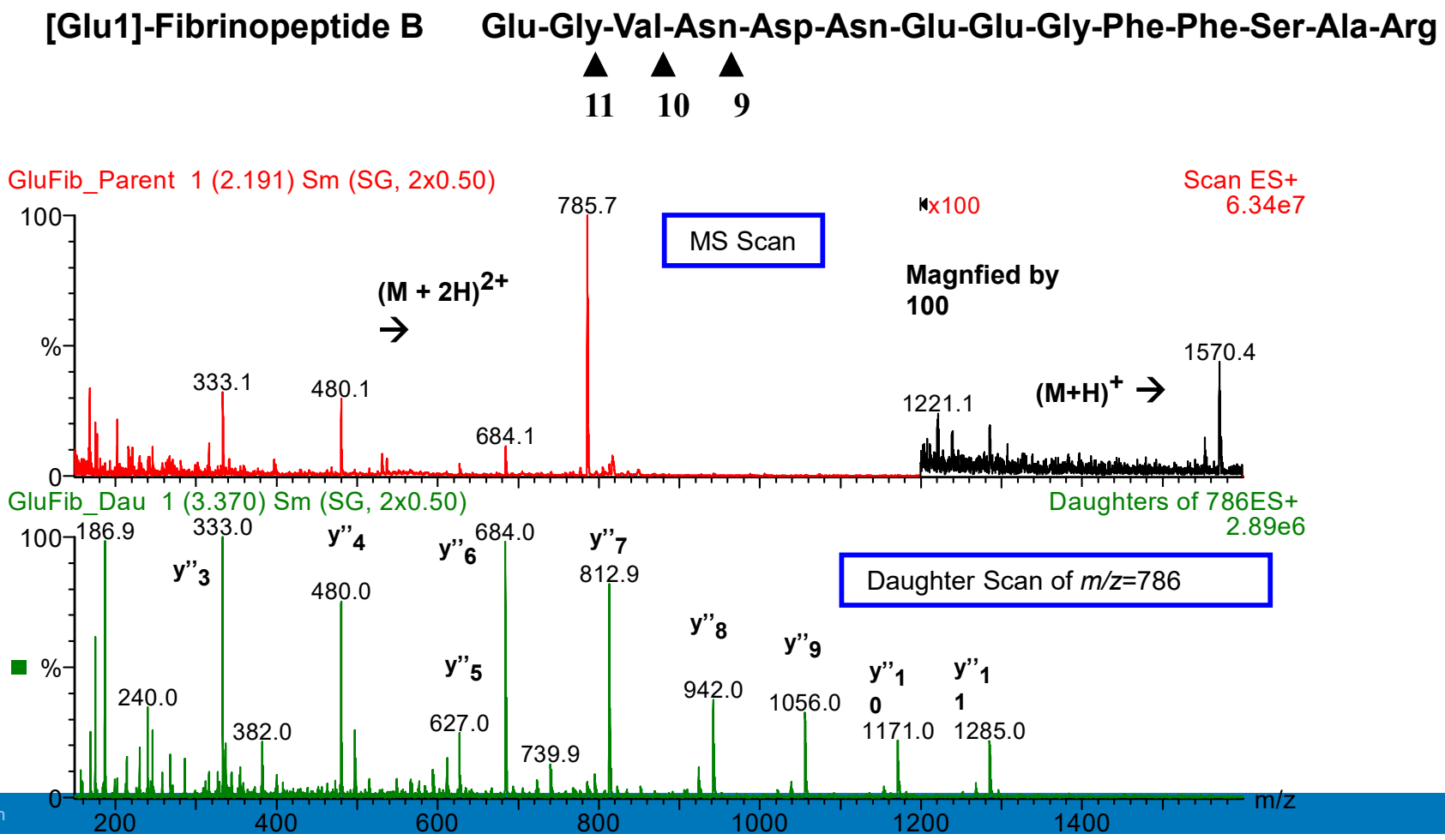


Observed product ions of *m/z* = 725 Parent ion are seen at *m/z* = 1305 and 144

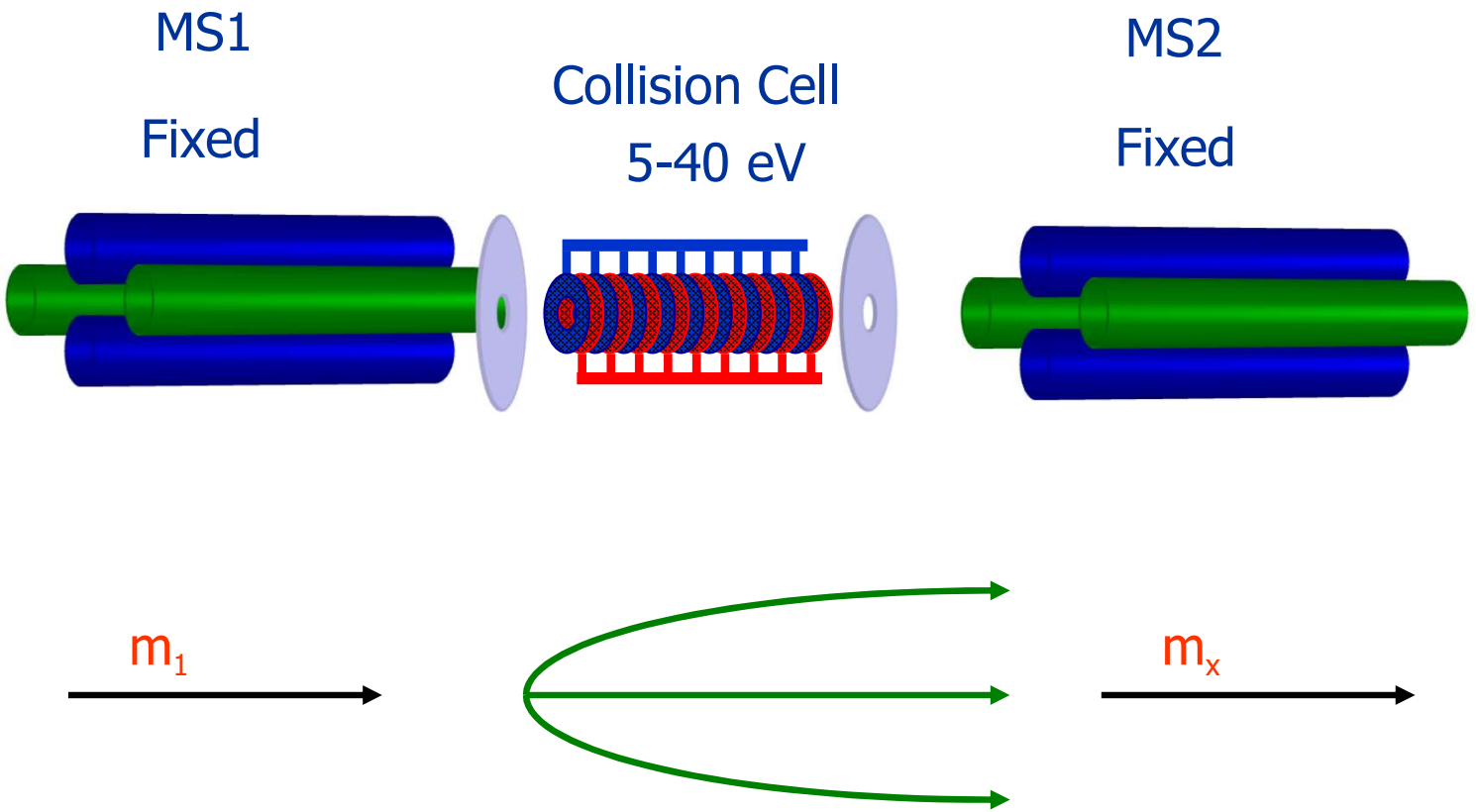
Example of a Doubly Charged Ion



Example of Product Ions from a Doubly Charged Ion

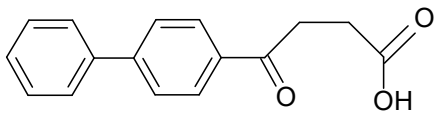


Multiple Reaction Monitoring (MRM)

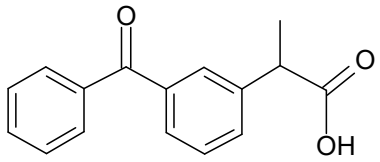


SIR of Two Compounds

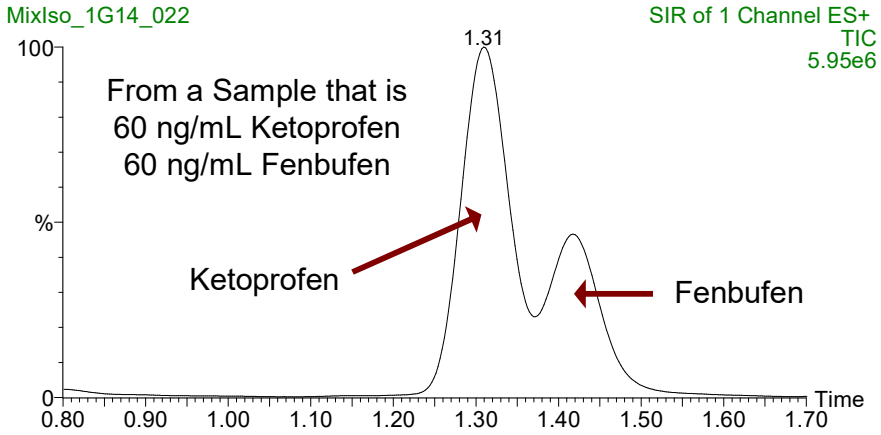
Fenbufen

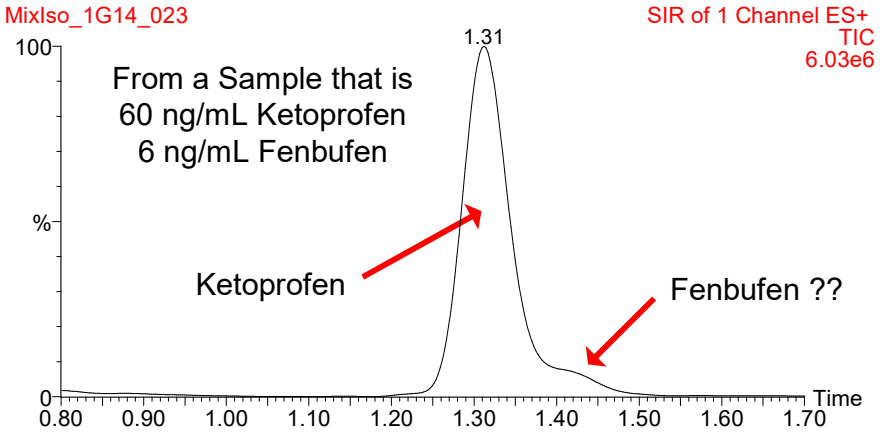


Ketoprofen

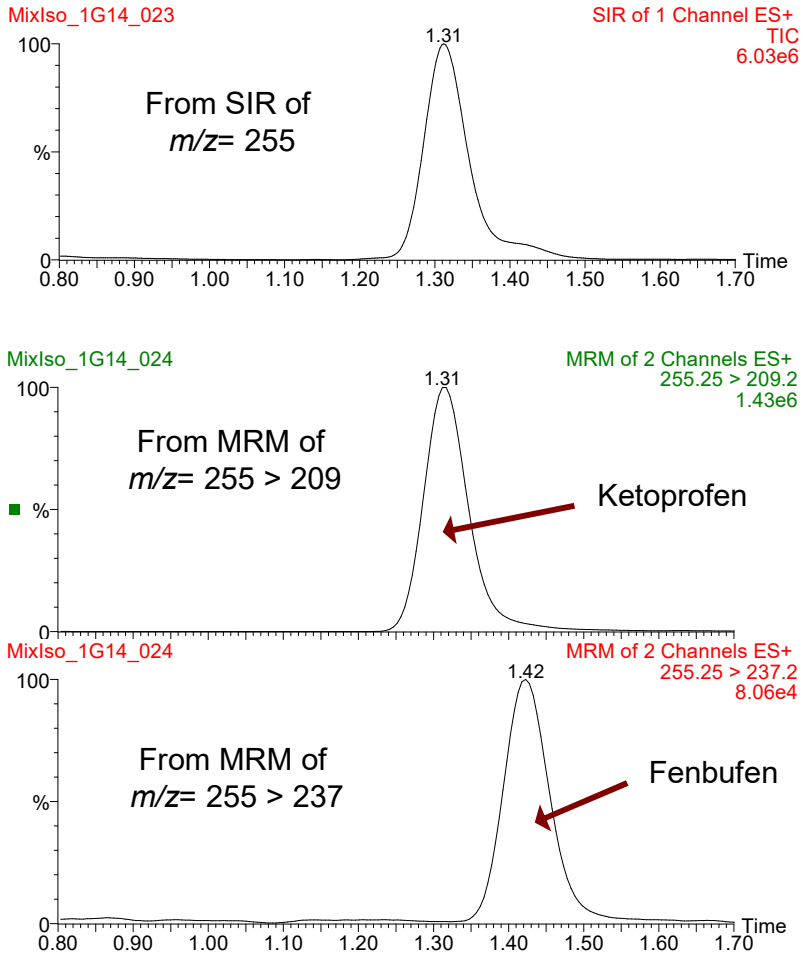


Both have a MW of 254



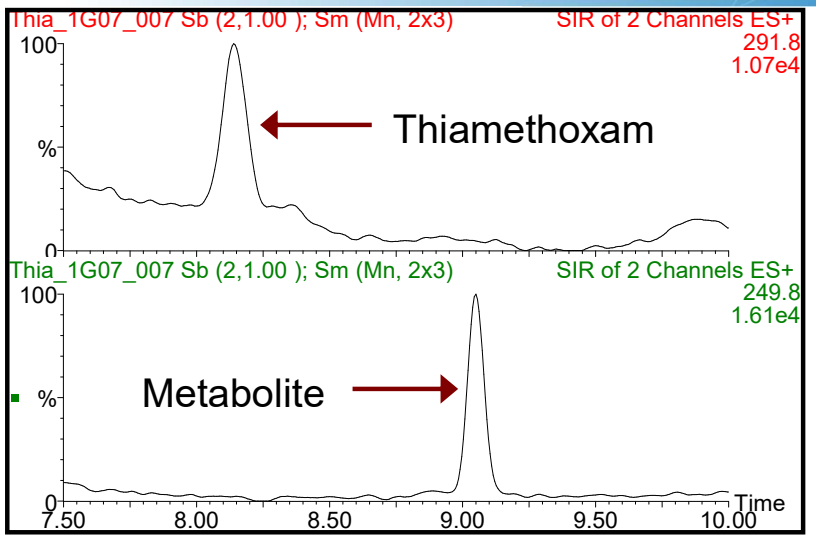


Comparing MRM and SIR

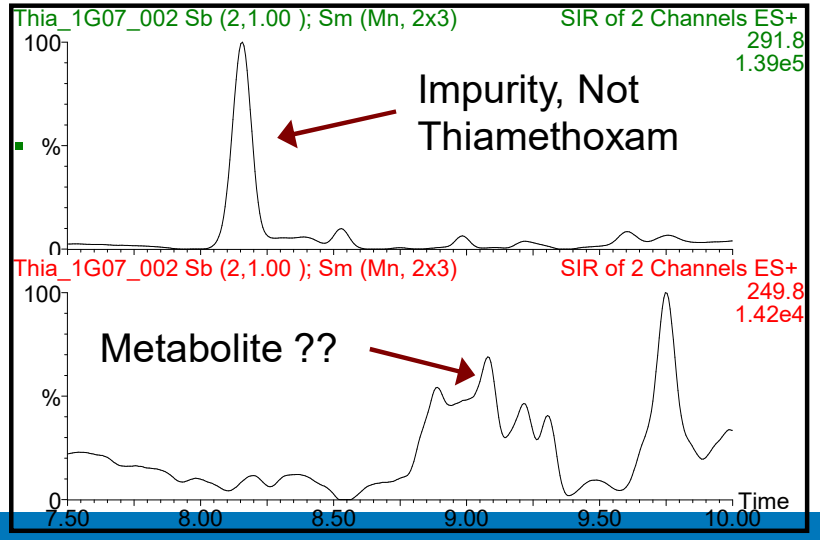


Comparing MRM and SIR

SIRs of a 10 ng/mL Standard Solution of Thiamethoxam and Metabolite



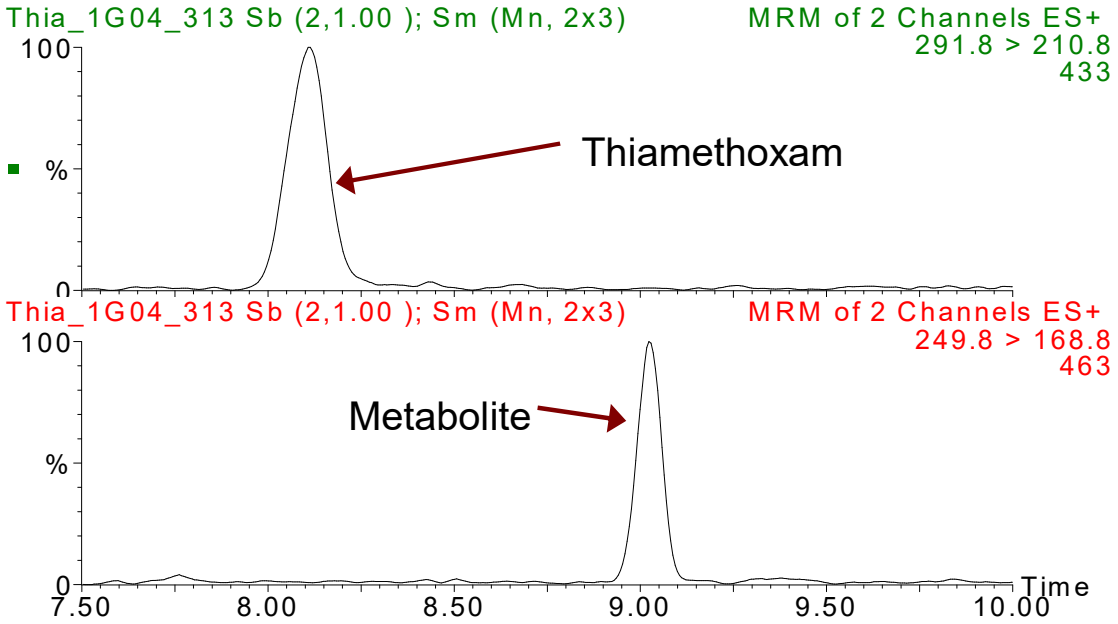
SIRs of Sample of 10 ng/mL Thiamethoxam and Metabolite in a Fruit Matrix



Peak Labeled an impurity in the fruit matrix sample is also present in blank matrix samples.

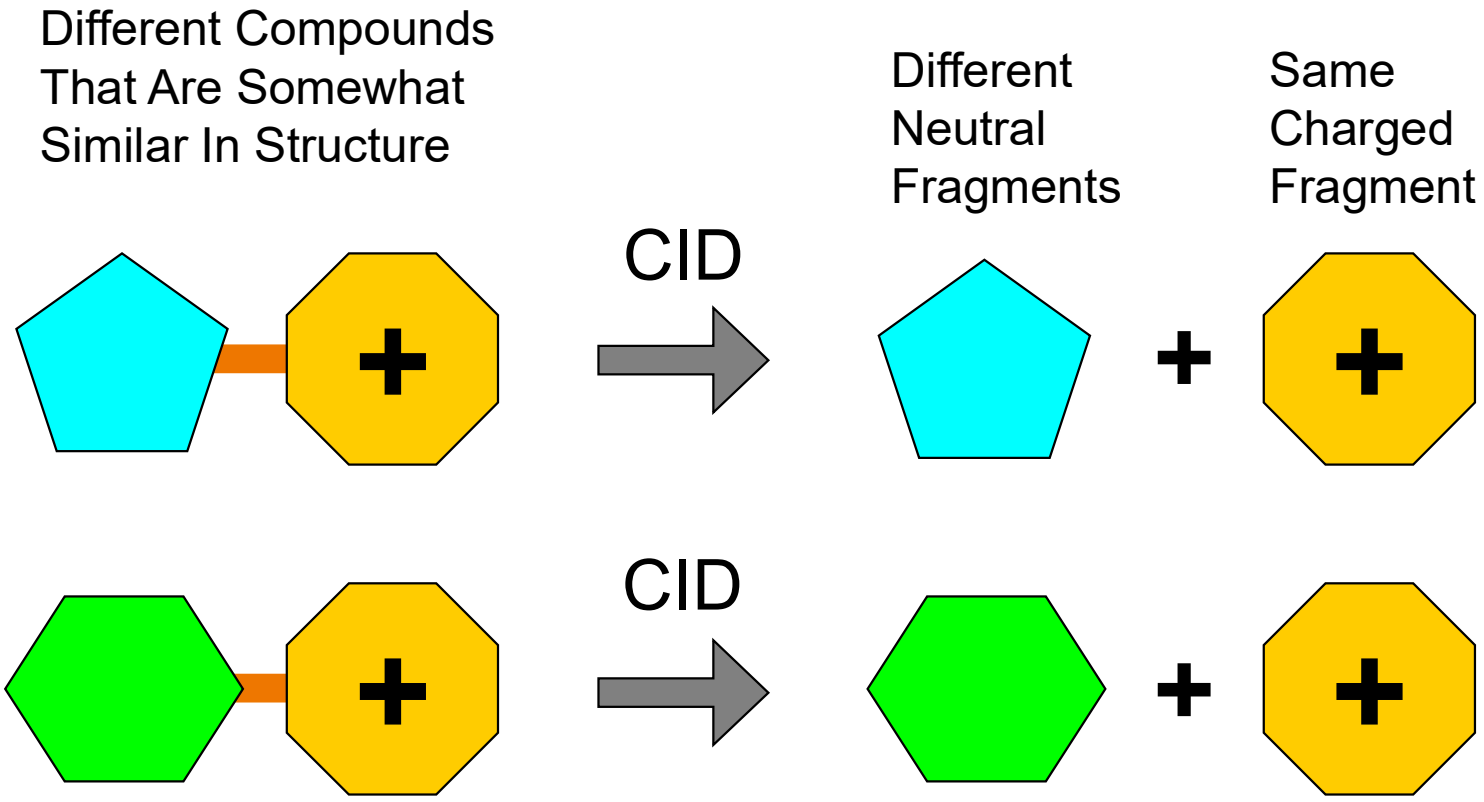
Comparing MRM and SIR

MRMs of Sample of 10 ng/mL Thiamethoxam
& Metabolite in a Fruit Matrix

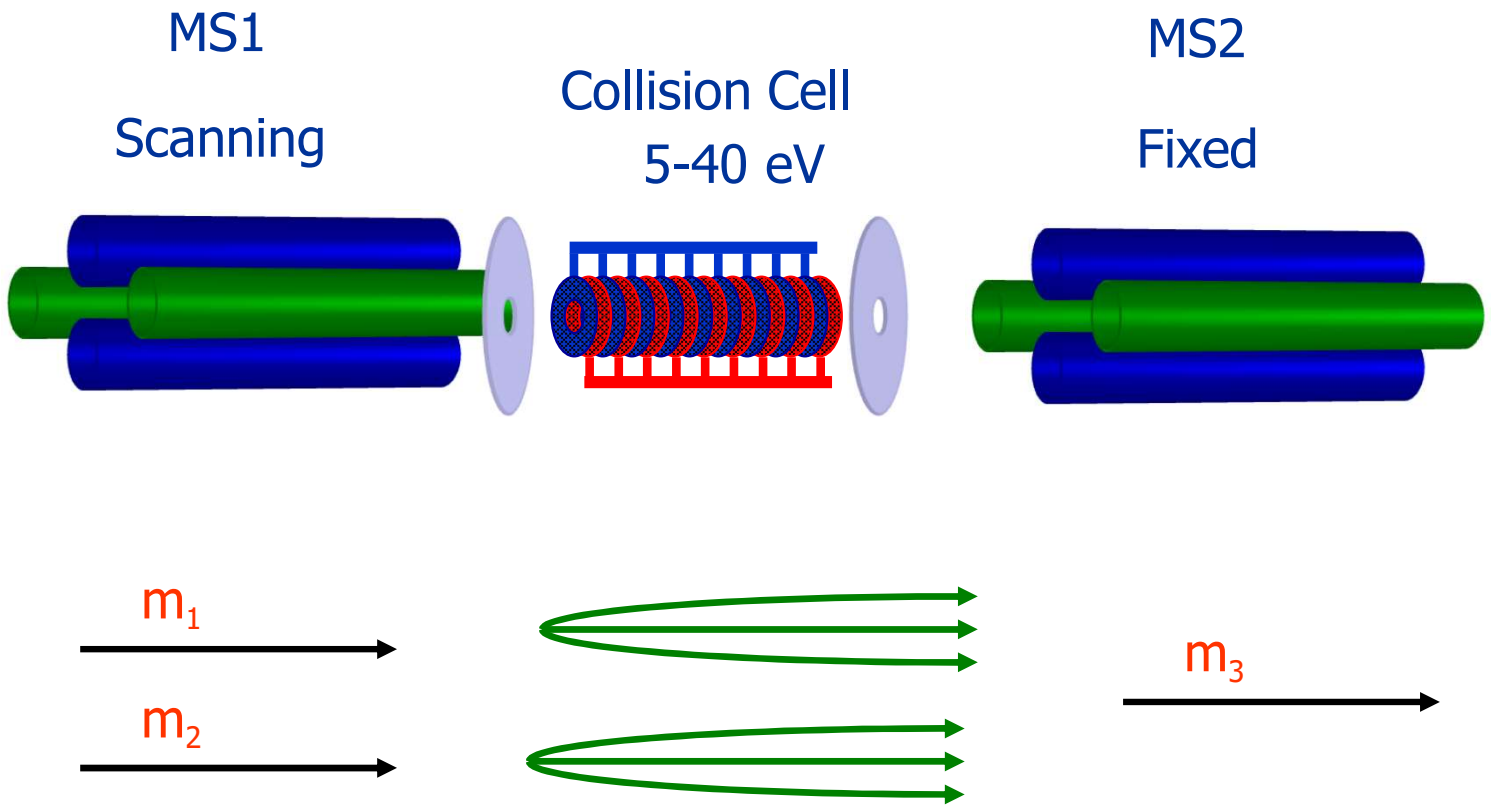


Peaks Labeled as Thiamethoxam and Metabolite are not Present in Blank Matrix Samples

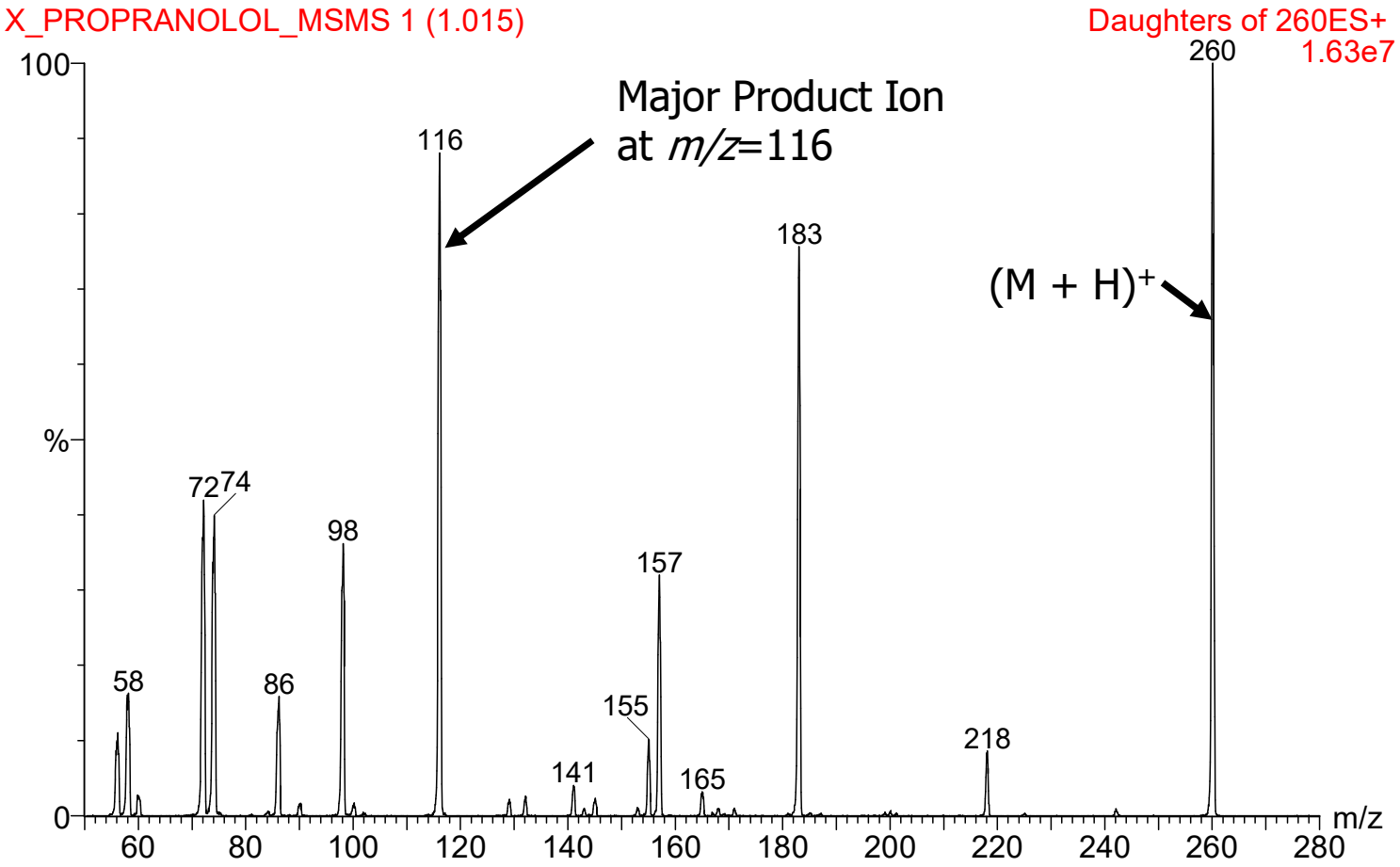
Precursor Ion Analysis (Parent Scan)



Precursor Ion Analysis

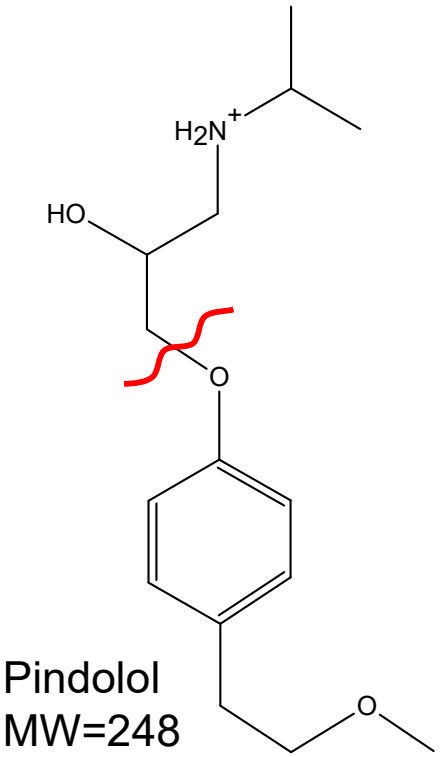


Precursor Ion Analysis Example

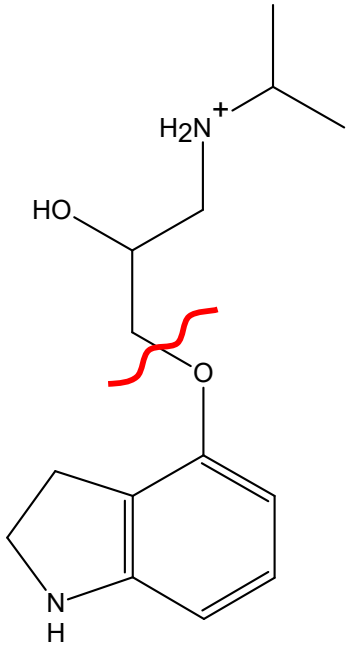


Precursor Ion Analysis Example

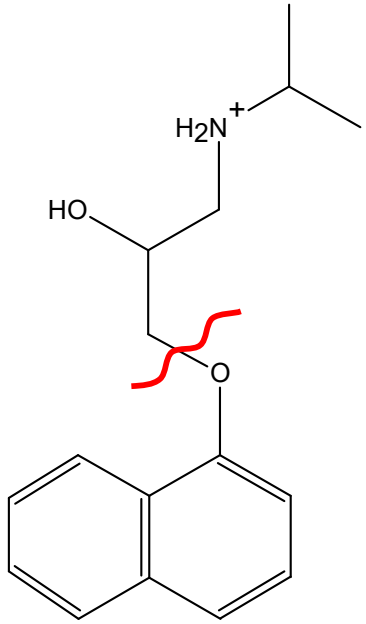
Product Ion of *m/z* = 116 produced by CID at spot indicated by line.



Pindolol
MW=248



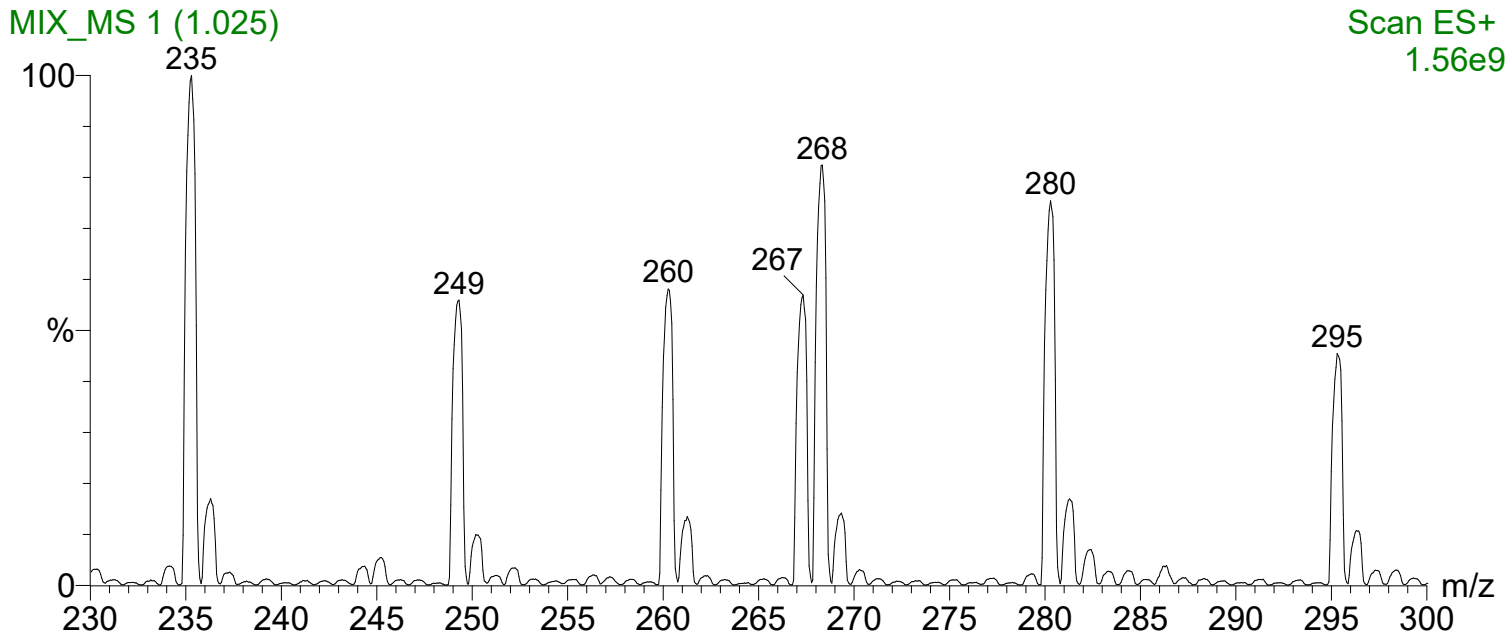
Metoprolol
MW=267



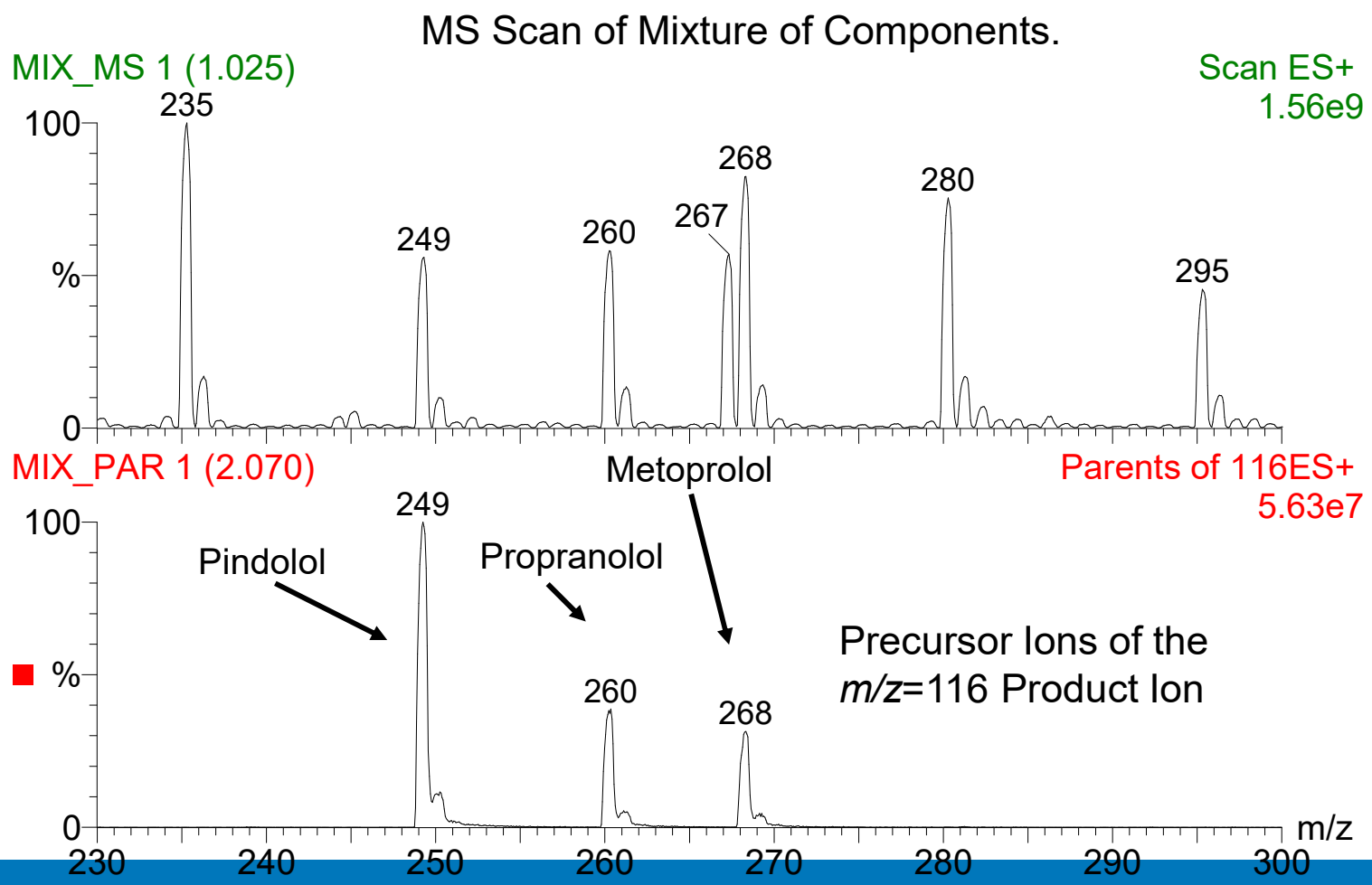
Propranolol
MW=259

Precursor Ion Analysis Example

For example, this is a MS Scan
from a mixture of components.

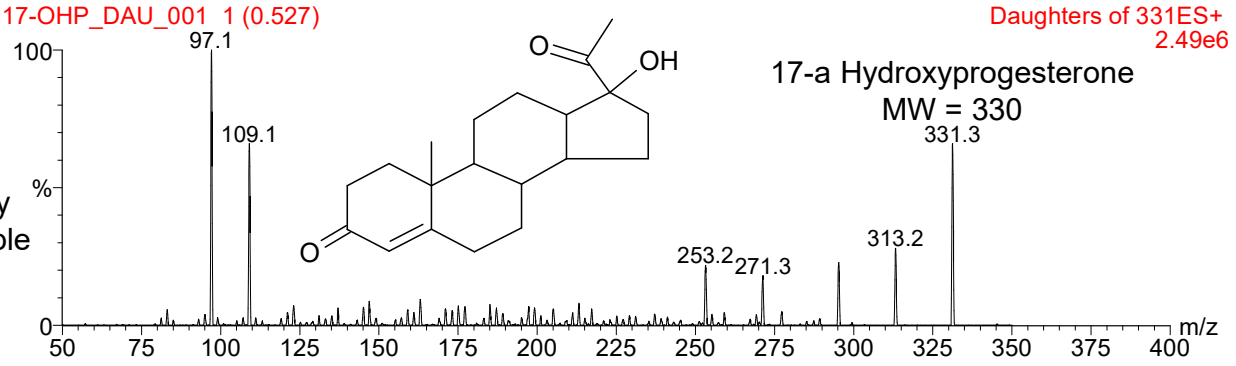


Precursor Ion Analysis Example

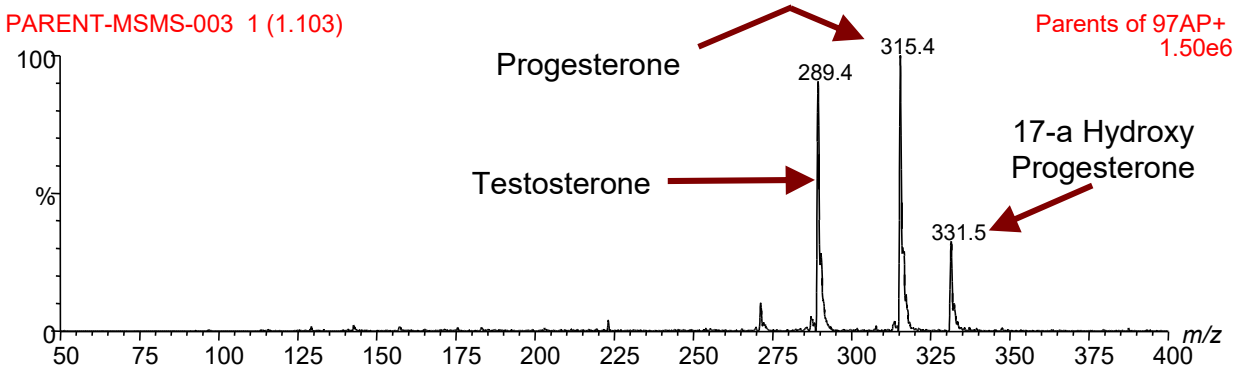


Precursor Ion Analysis Example

Product Scan
for Products
of *m/z* = 331 Ions
from a 17-a Hydroxy
Progesterone Sample



Precursor Scan
for precursors
of *m/z* = 97 ions
from a mixture
of steroids



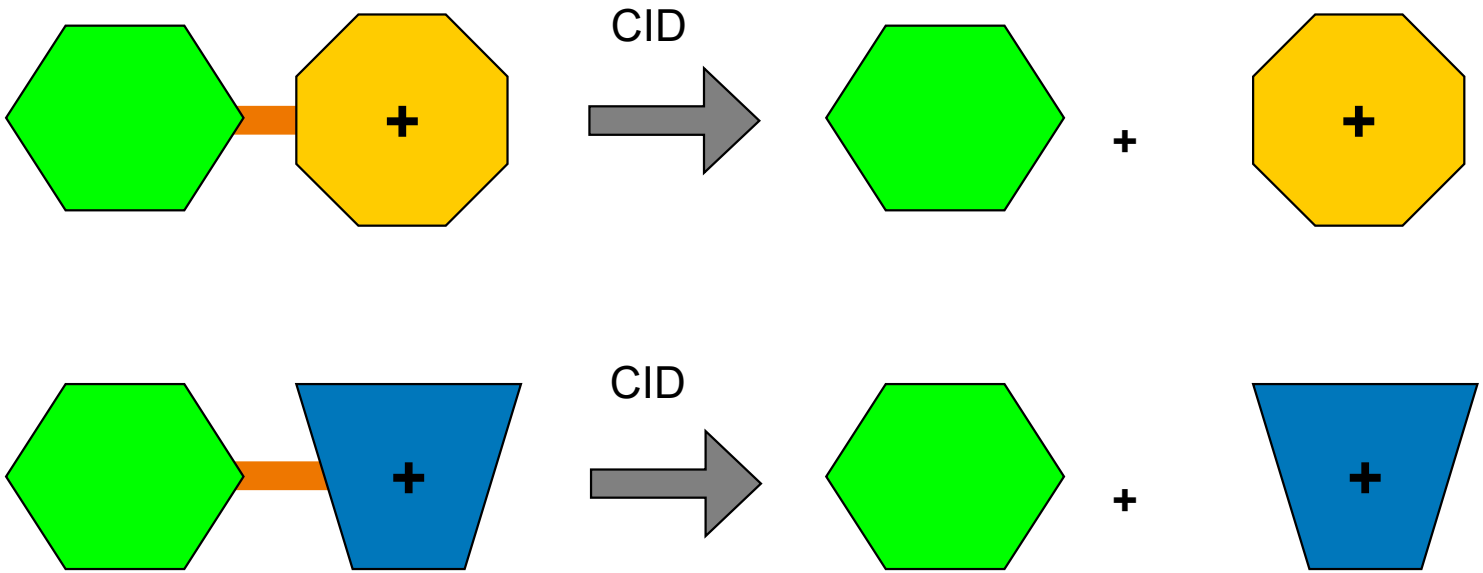
Neutral Loss Analysis

Waters™

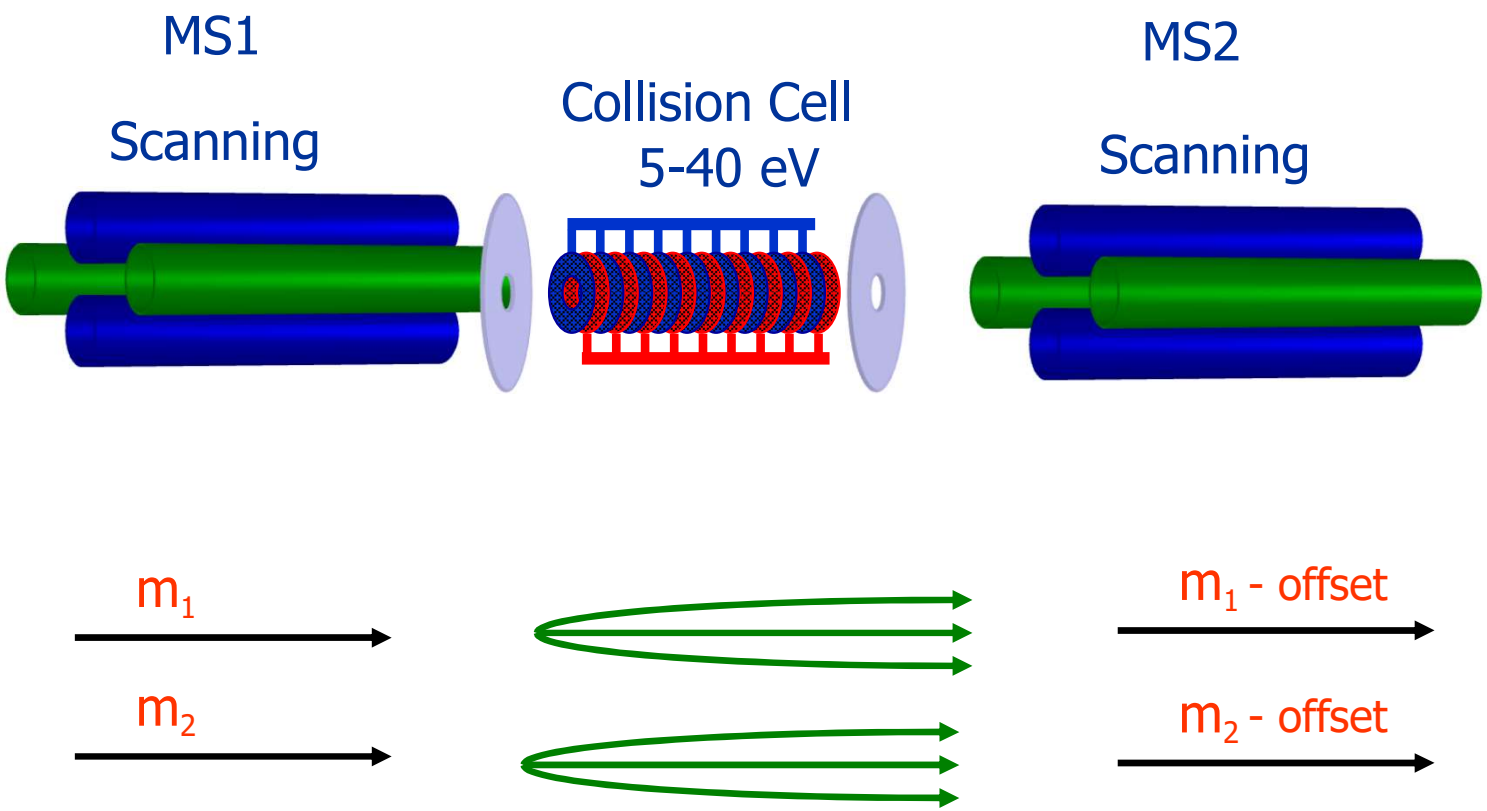
Different Compounds That Are Somewhat Similar In Structure

Same Neutral Fragment

Different Charged Fragments

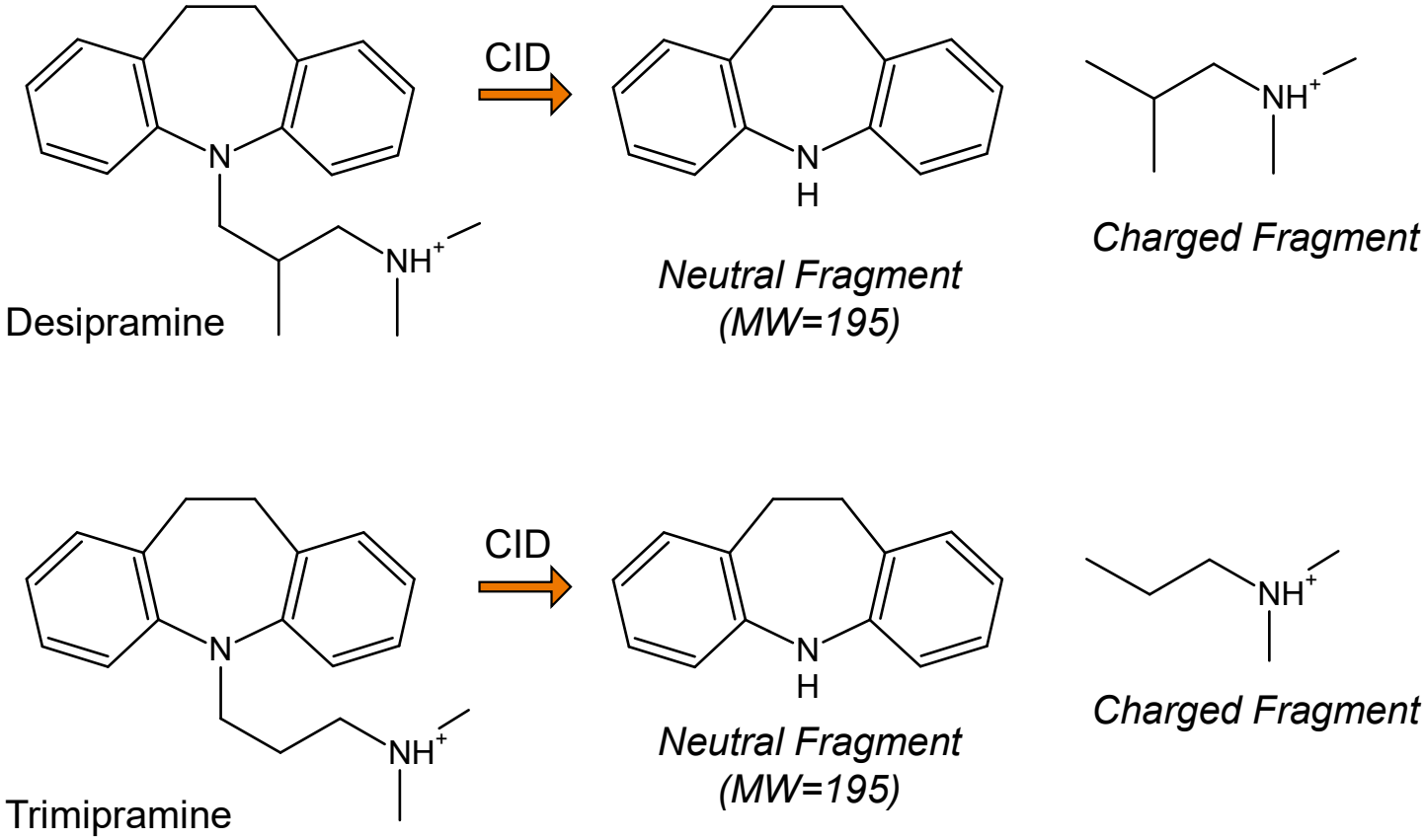


Neutral Loss Analysis

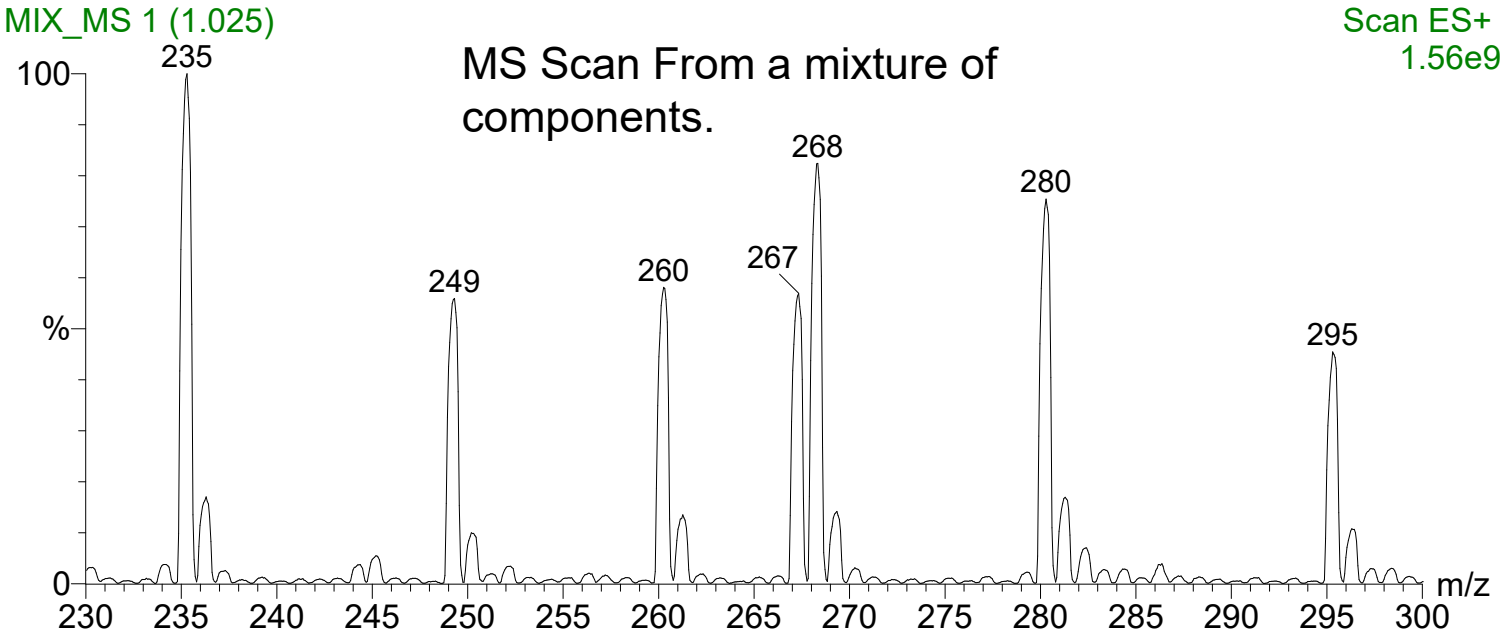


MS1 and MS2 scan together. m/z of MS2 is m/z MS1 minus an offset

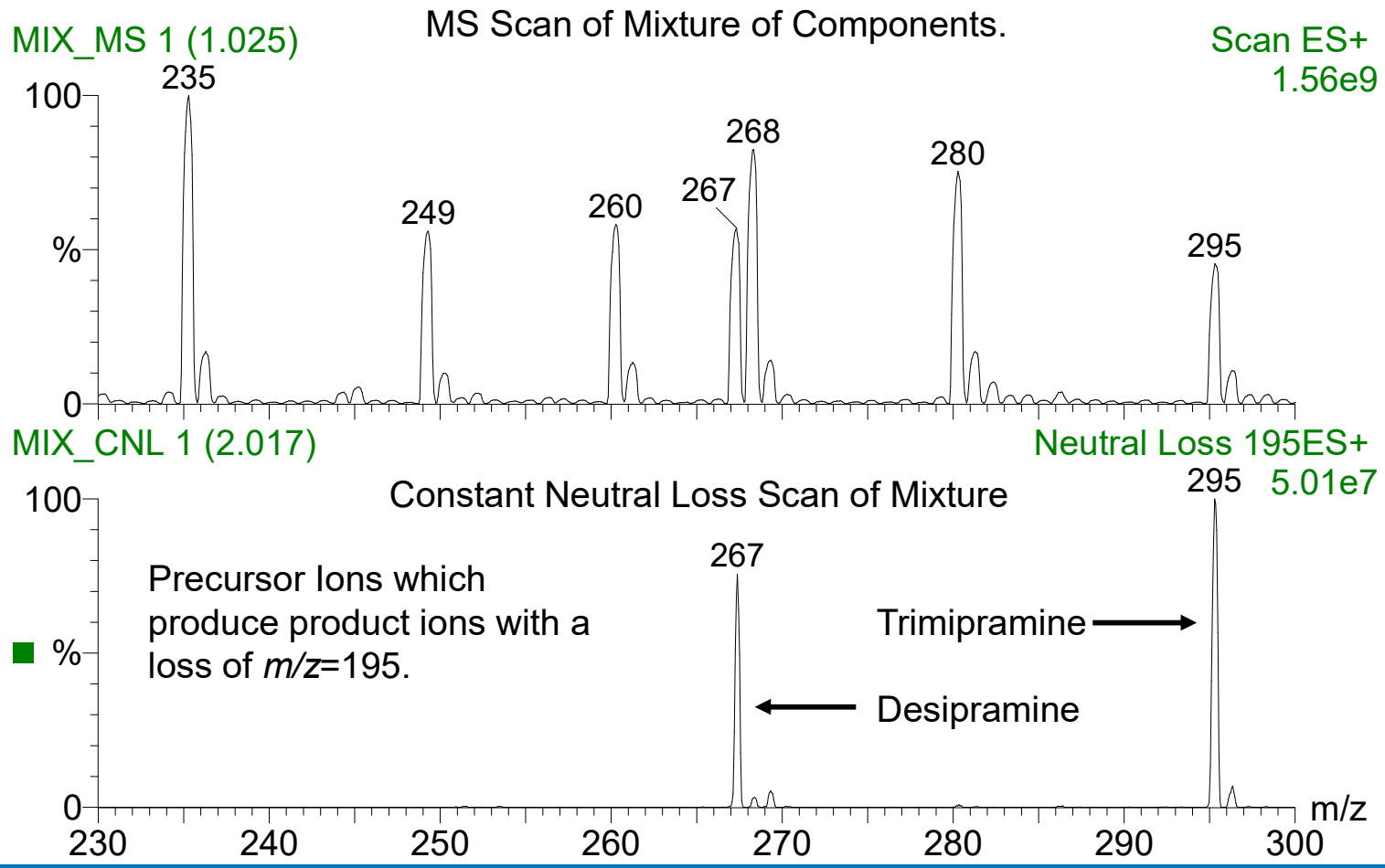
Tricyclic Antidepressants



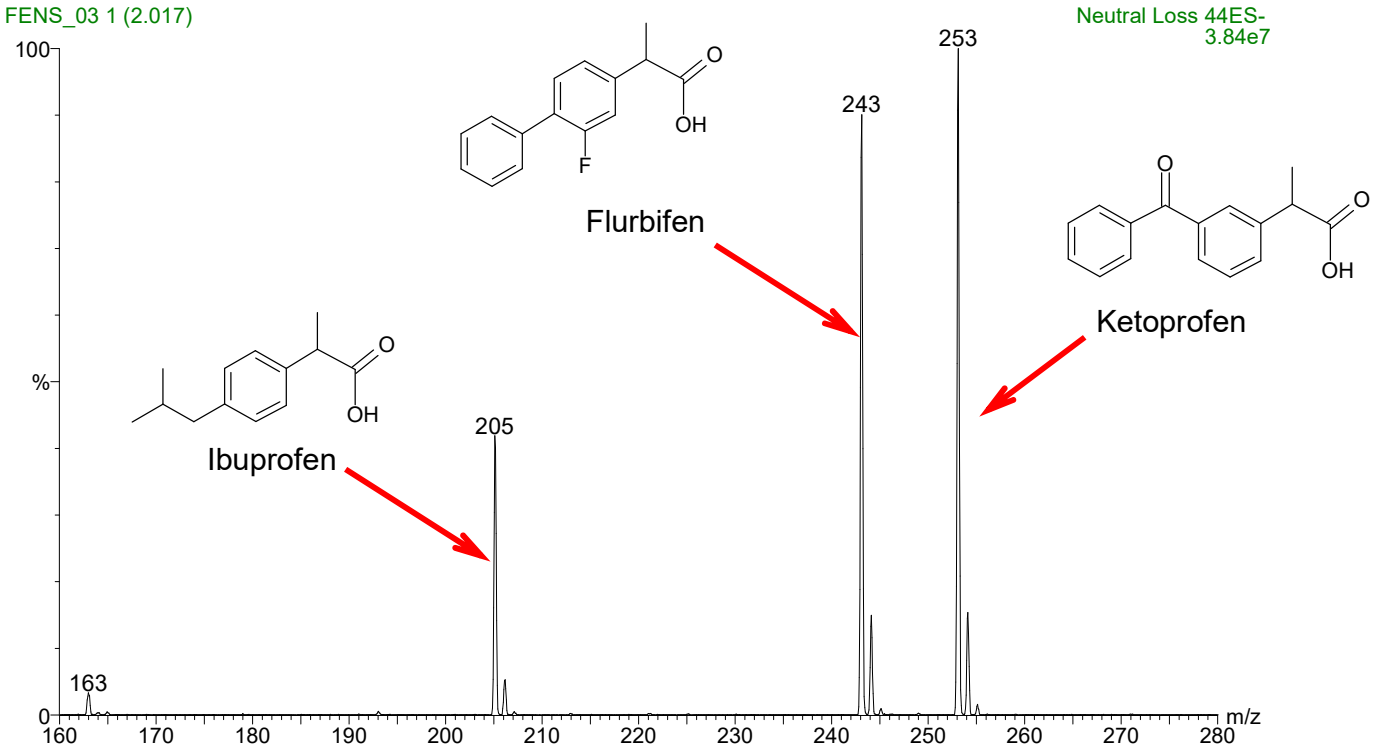
Neutral Loss Analysis Example



Neutral Loss Analysis Example

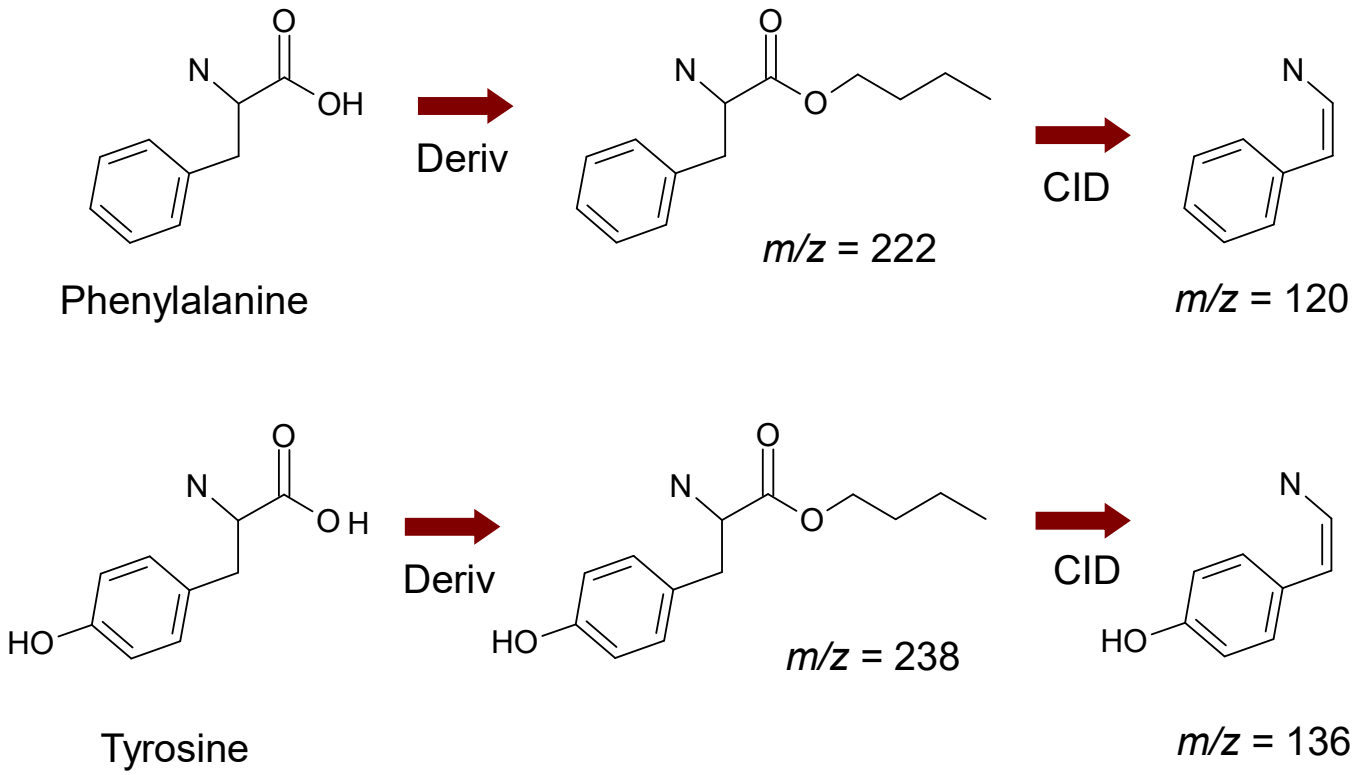


Neutral Loss Analysis Example



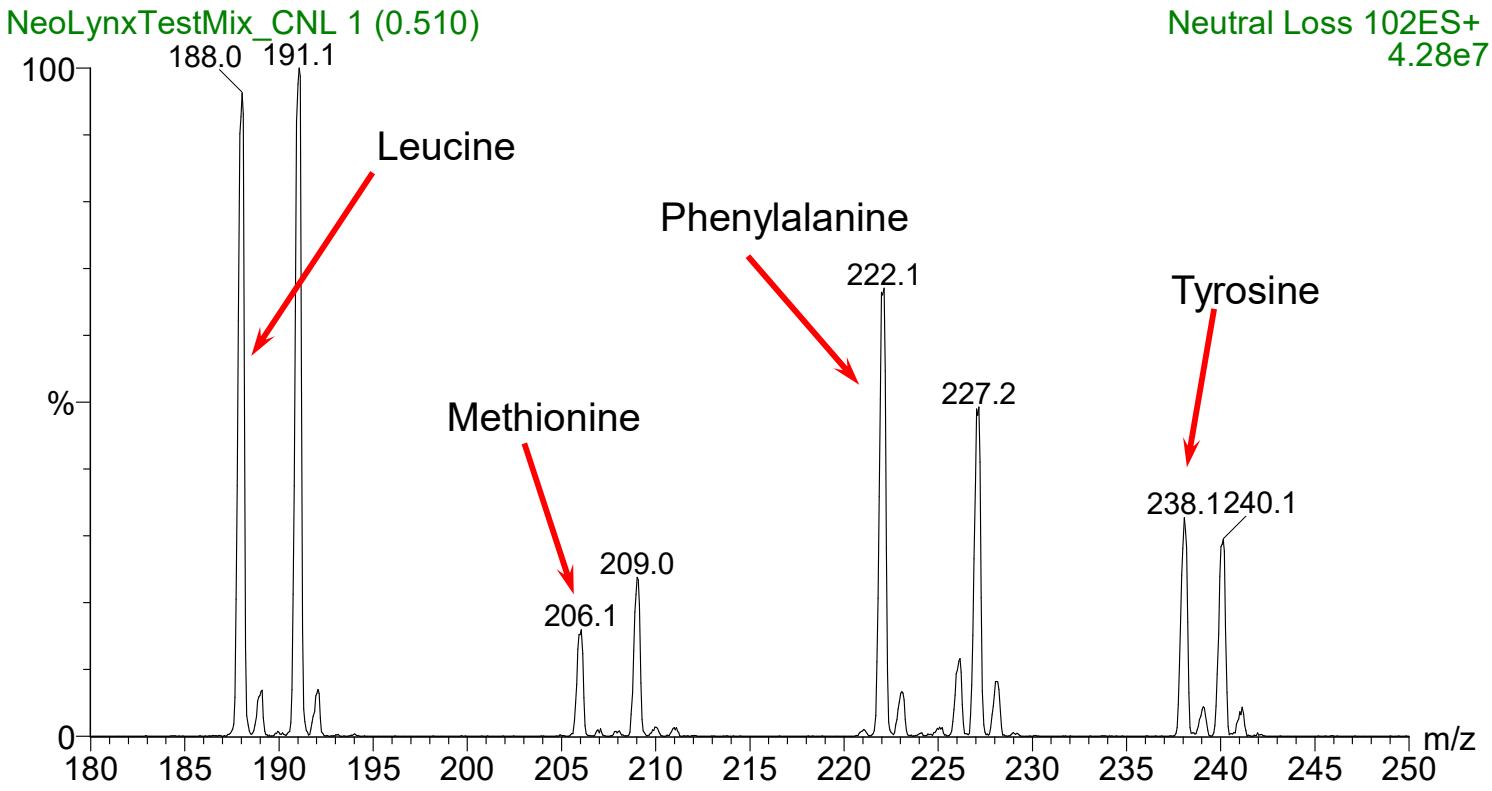
Negative Electrospray Neutral Loss Analysis for loss of *m/z* = 44 Da from a Mixture of Compounds

Neutral Loss Analysis of Amino Acids by MS/MS



CID Results in the Loss of 102 Da

Neutral Loss Analysis of Amino Acids by MS/MS



Other peaks are from deuterated forms of these amino acids

Survey Scan

Waters™

- Automatically generates product ion spectra for any components found
- Various triggers can be used
  - MS full scanning
  - Parent ion scanning
  - Neutral loss scanning
- MS and MS/MS data is provided with only one injection, usually on the column.
- MS data is collected until a parent ion of interest elutes.
- MS/MS data for that parent is recorded until the set time or the intensity threshold is reached.
- MS data is collected until another parent ion of interest elutes.

Survey Scan: Screening of Precursor Ion

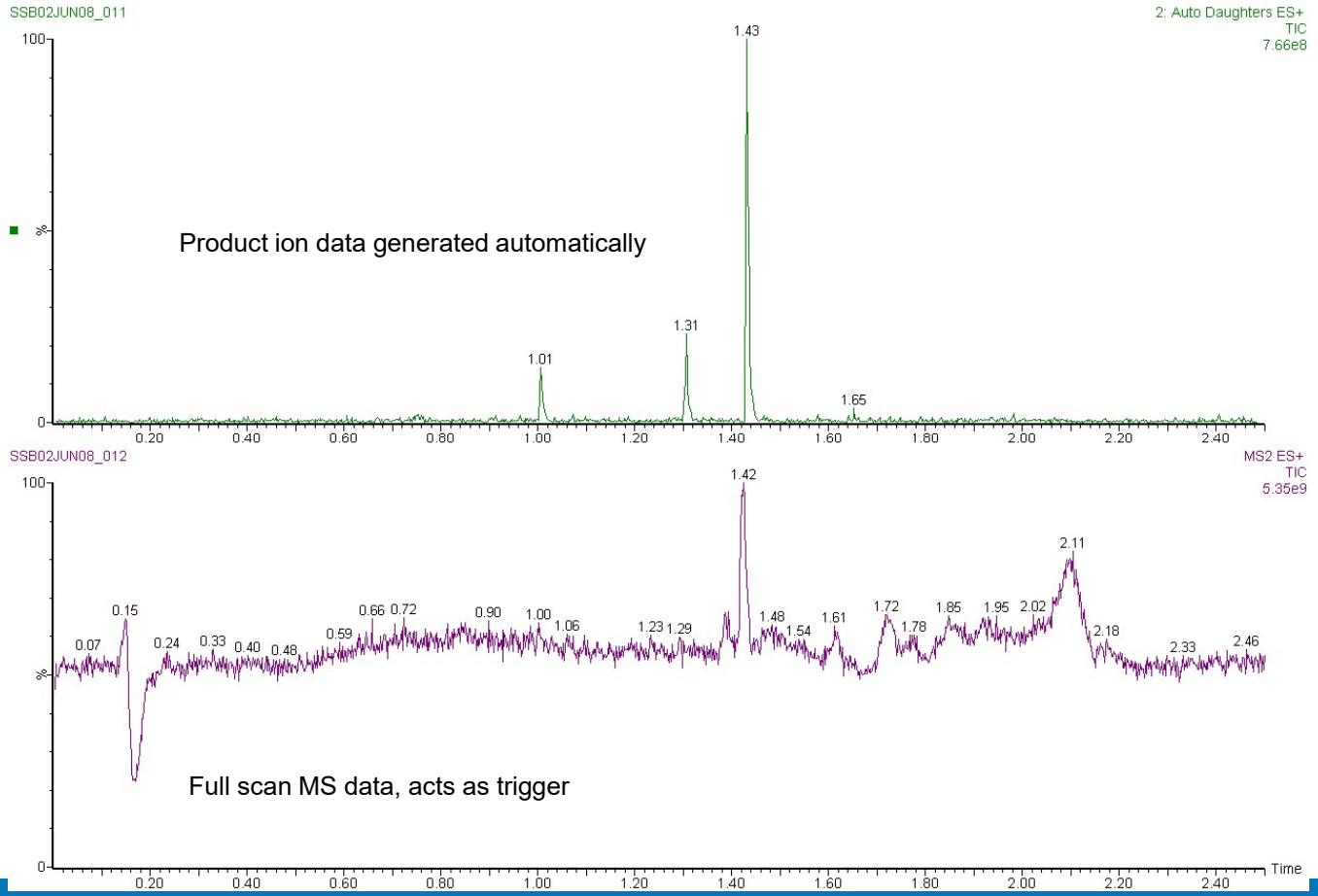
Waters™

- Screening of the parent ion fragment can be achieved by:
  - Relative or absolute intensity of a spectrum
  - Specifying included ions or an m/z range
  - Specifying excluded ions or an m/z range
  - Charge state

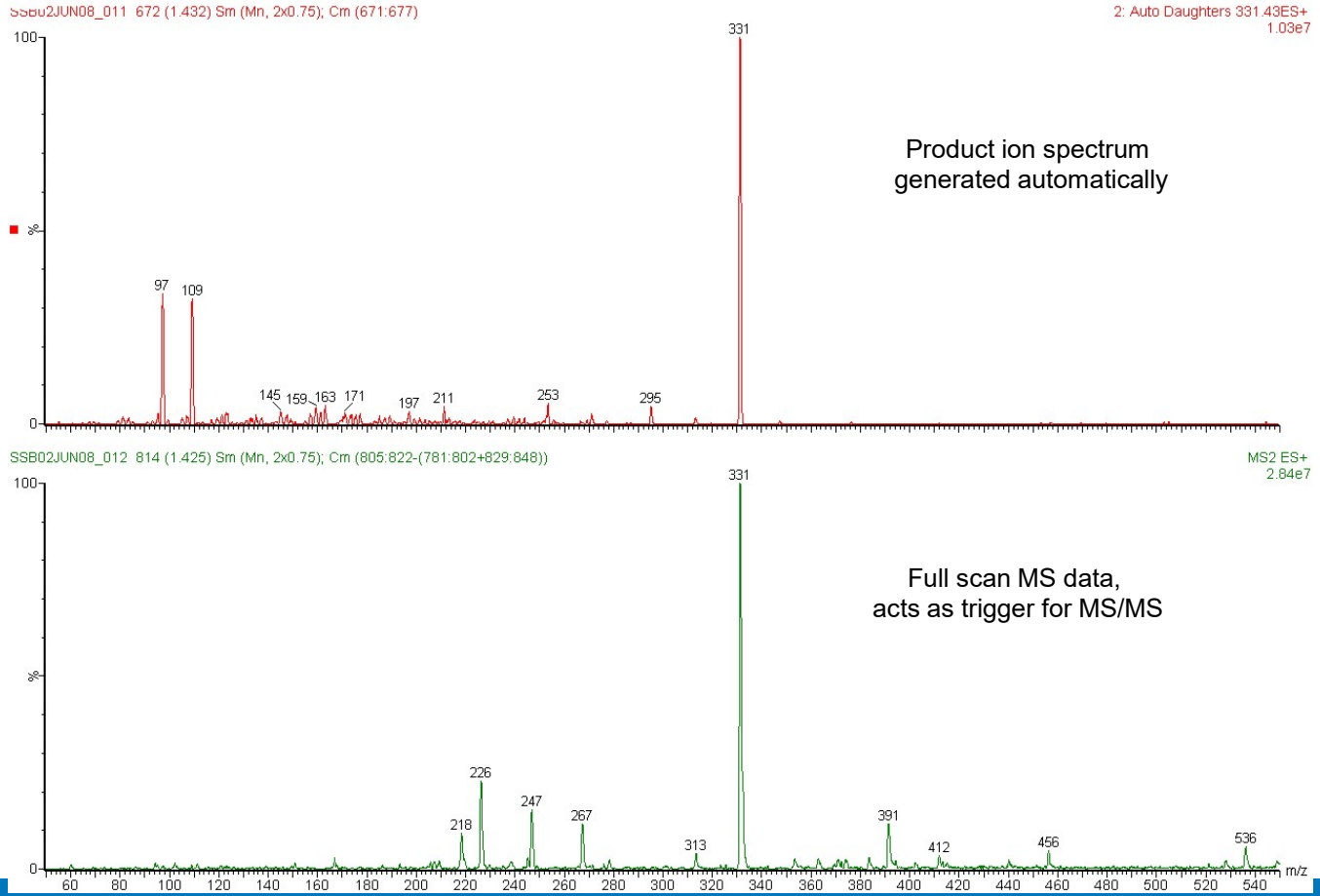
Survey Scan: Requirements

- m/z range to scan for MS analysis
- m/z range to scan for MS/MS analysis
- Any masses to include or exclude
- Collision energy required for proper fragmentation

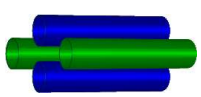
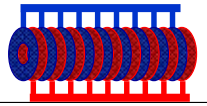
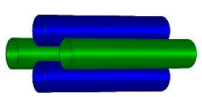
Survey Scan Example Chromatogram



Survey Scan Example Spectra



In Summary

Scan type	Instrument component: MS1	Collision cell	MS2	Data type
				
MS1 scan	Scan	Inactive	Rf only (acts as ion guide)	Qualitative
MS scan	Rf only (acts as ion guide)	Inactive	Scan	Qualitative
SIR	Mass filter	Inactive	Rf only (acts as ion guide)	Quanitative
Product ion (daughter) scan	Mass filter	Active	Scan	Qualitative
Precursor ion (parent) scan	Scan	Active	Mass filter	Usually Qualitative, sometimes Quanitative
Multiple reaction monitoring (MRM)	Mass filter	Active	Mass filter	Quanitative
Neutral loss	Scan	Active	Scan	Usually Qualitative, sometimes Quanitative

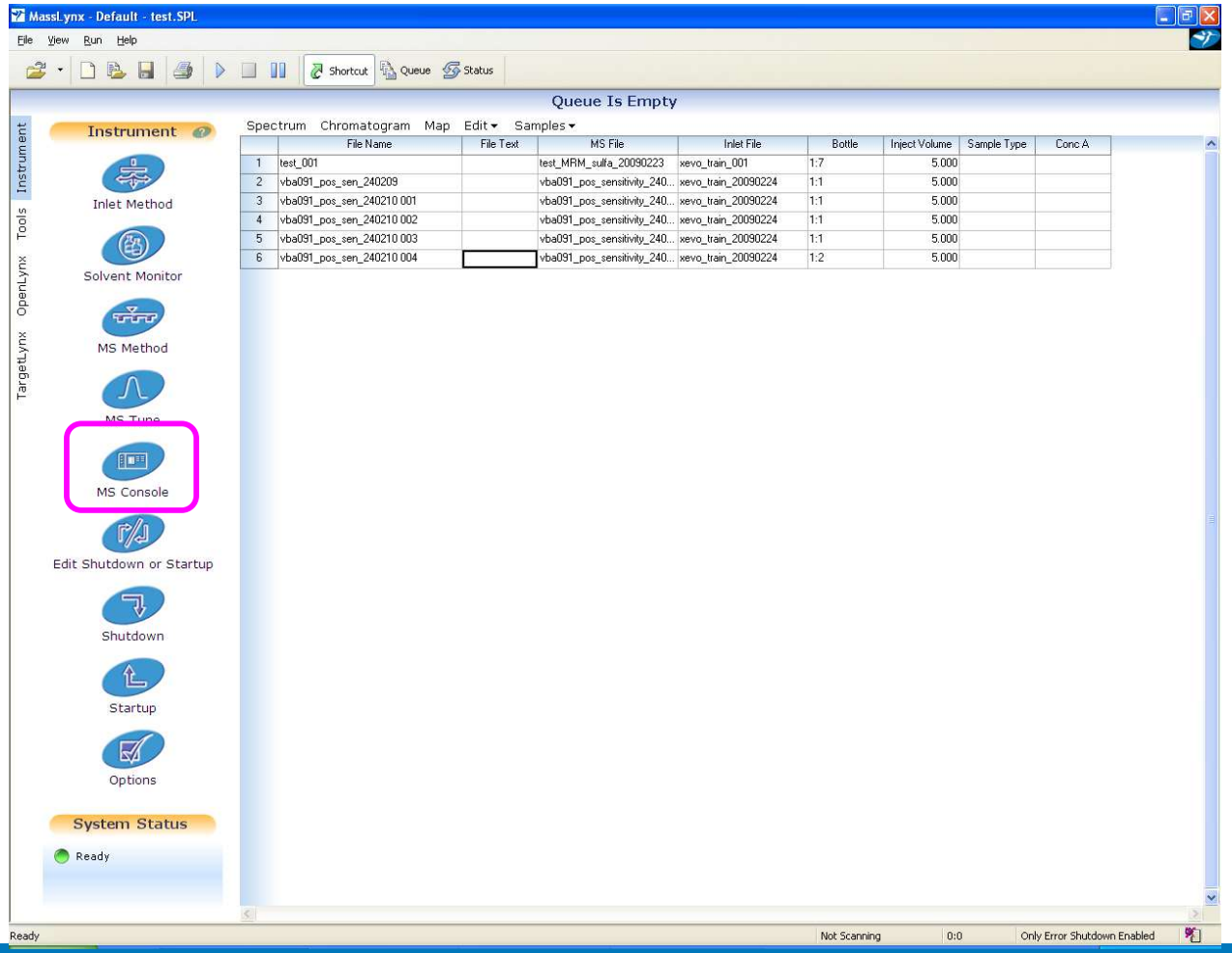
Chapter Three

IntelliStart™ Console

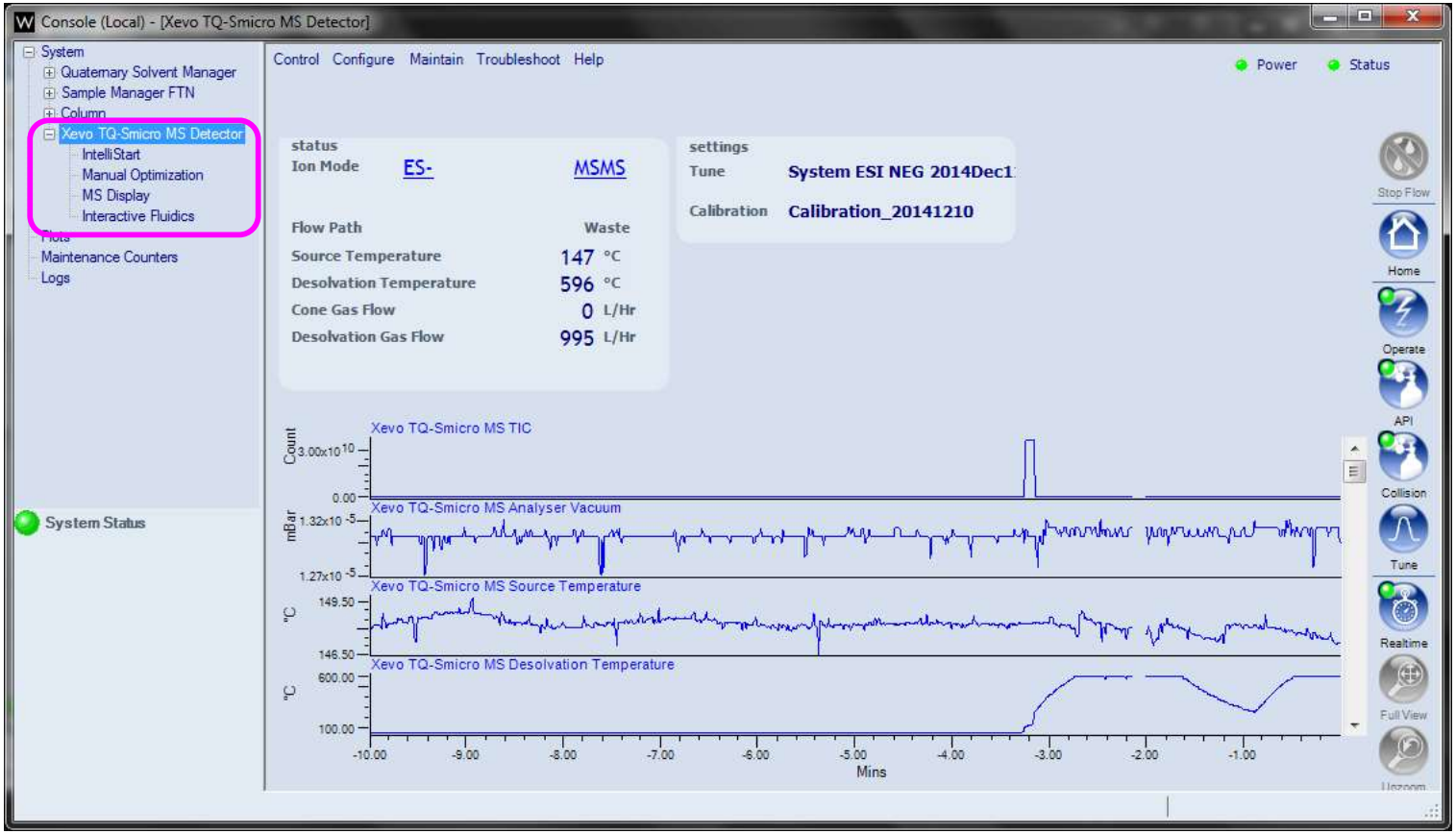
MS Console

- Xevo TQ-S micro functions
  - IntelliStart
  - Manual Optimization
  - MS Display
  - Interactive Fluidics
- Sample Tune and Develop Method
- Instrument Setup and Calibration

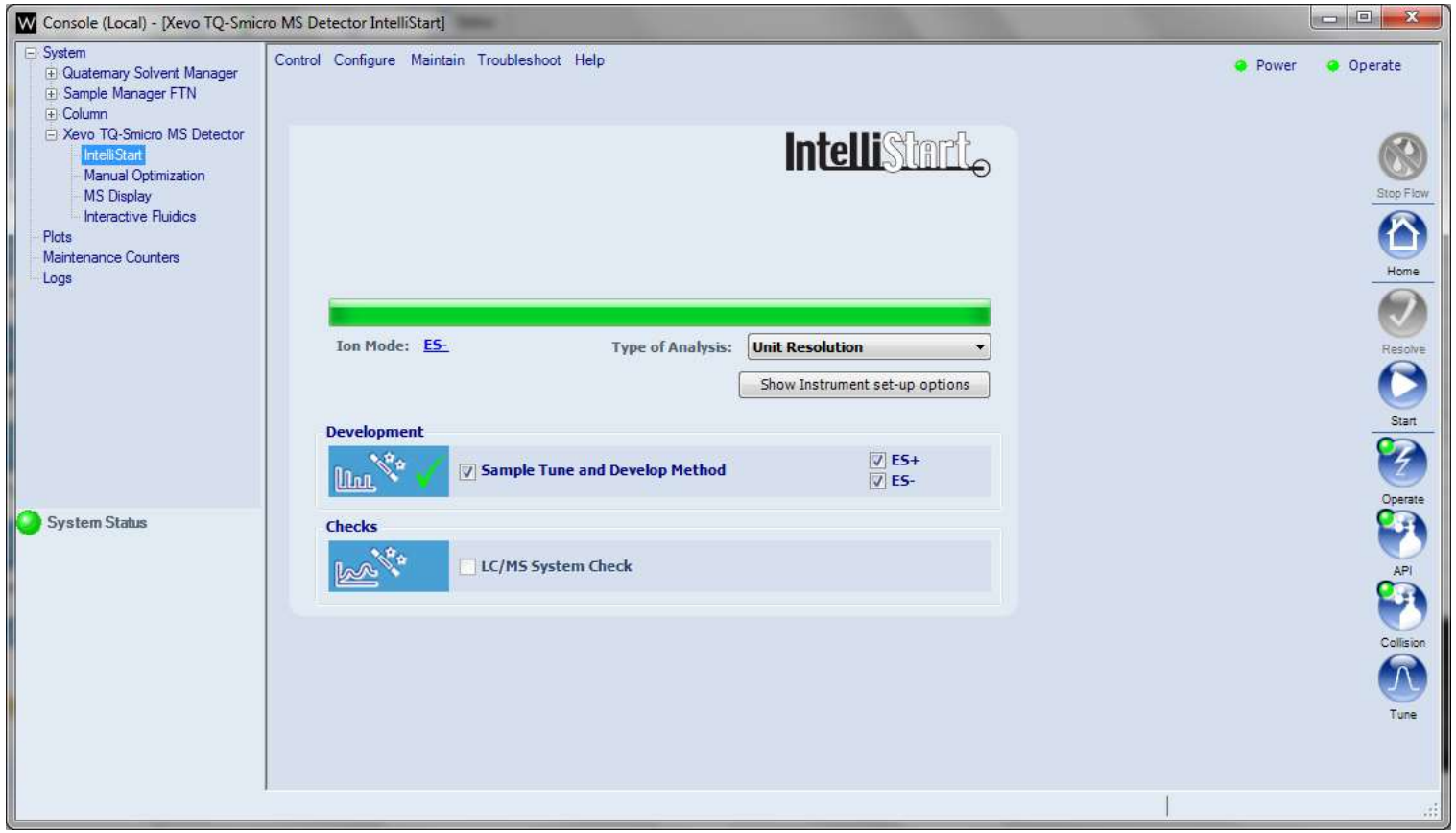
MS Console



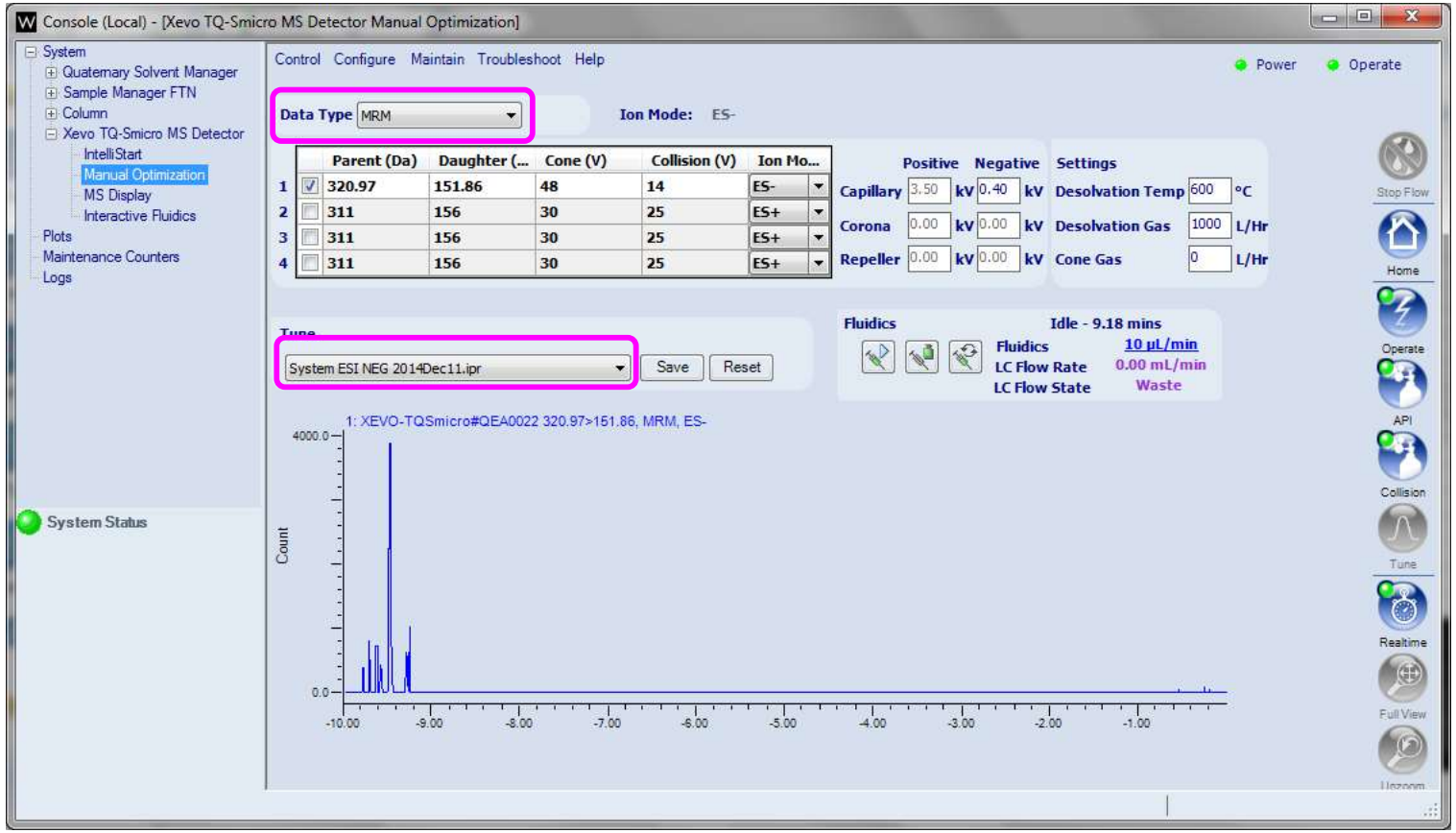
Xevo TQ-S micro Console View



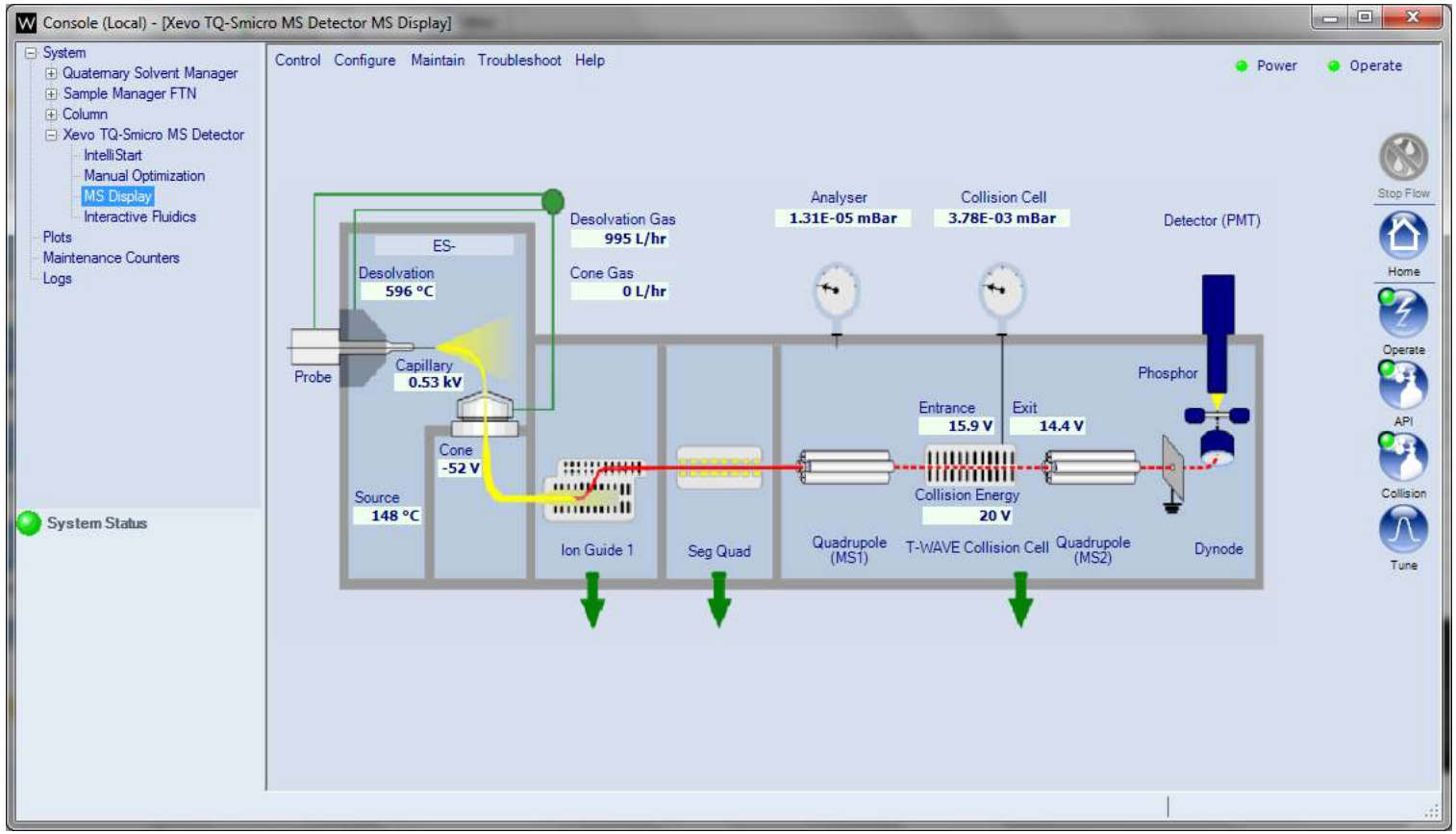
Xevo TQ-S micro – IntelliStart



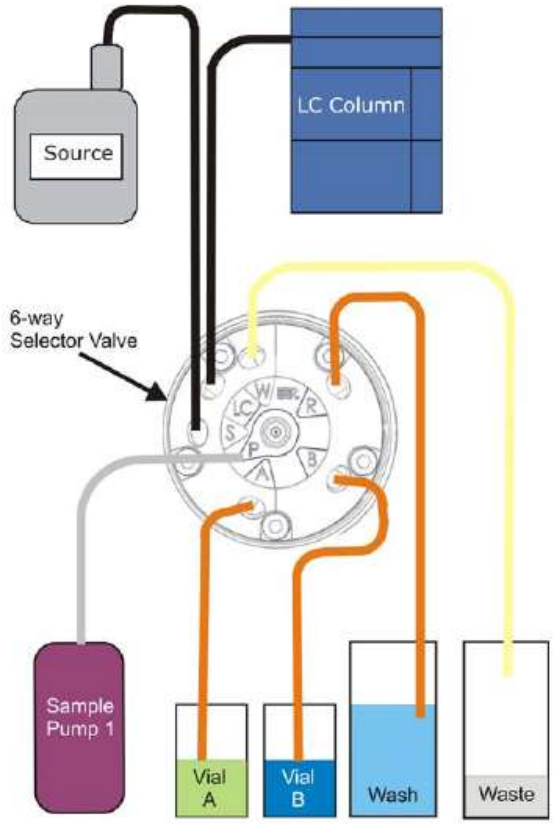
Xevo TQ-S micro – Manual Optimization



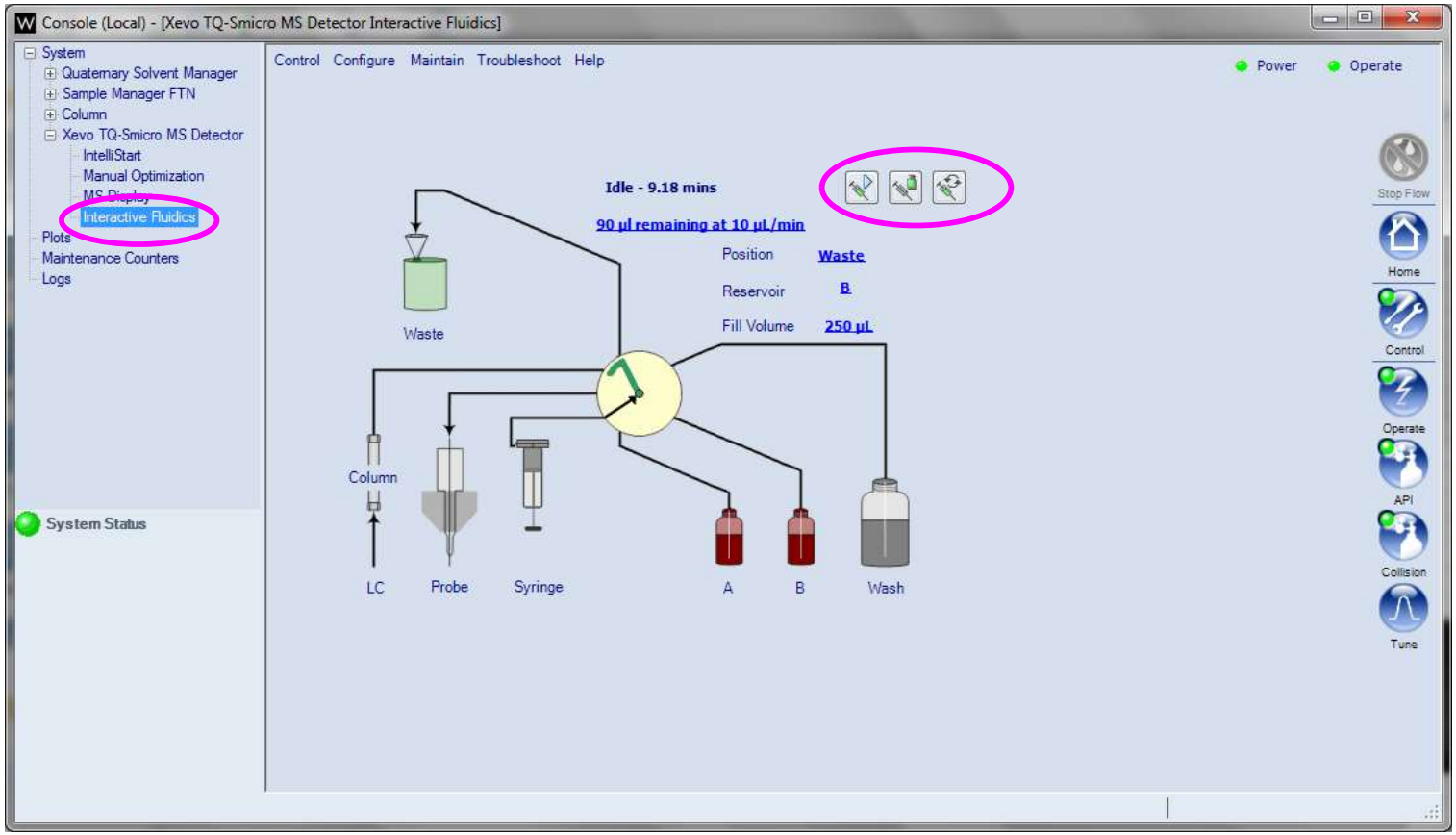
Xevo TQ-S micro – MS Display



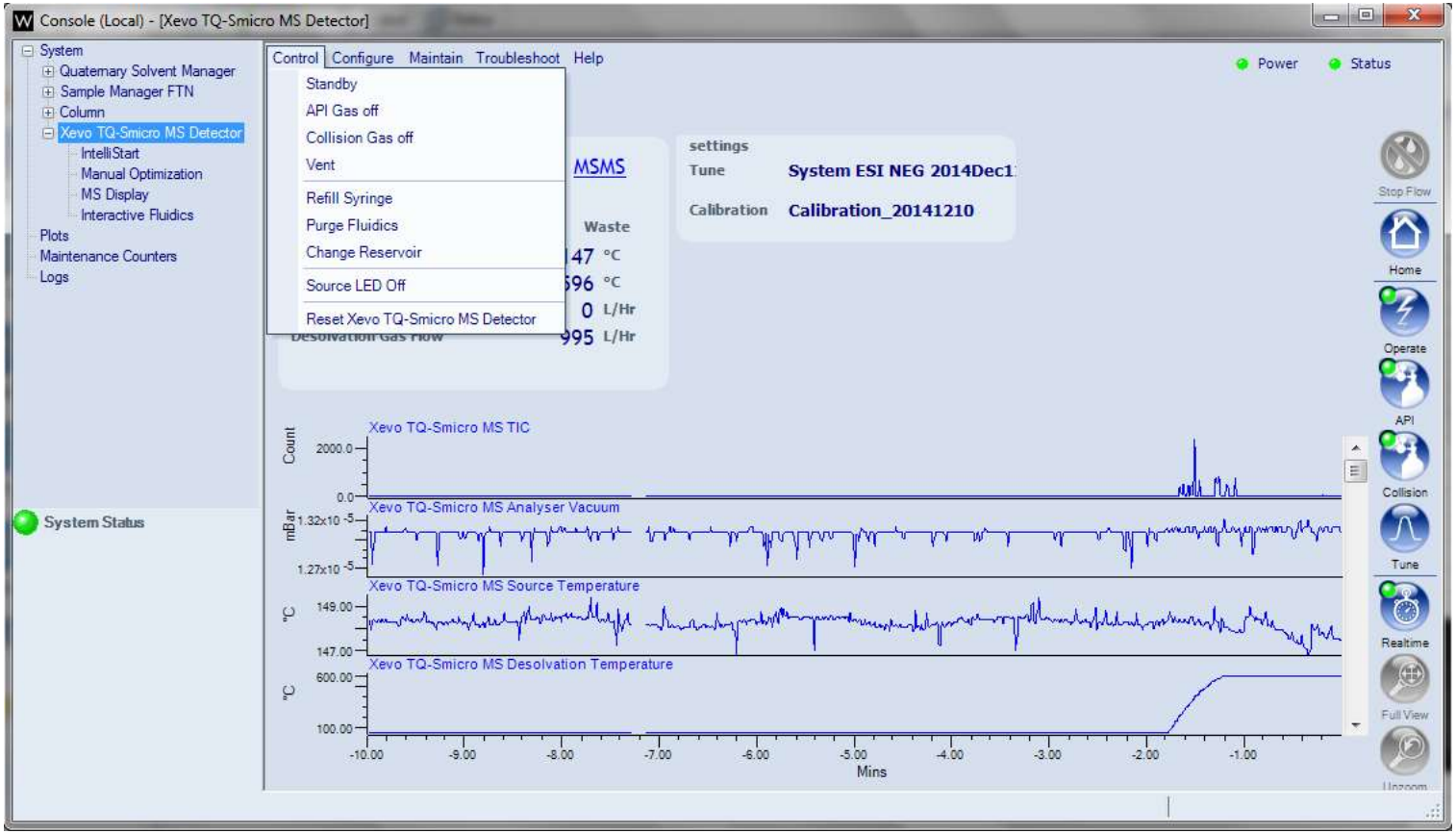
Xevo TQ-S micro Interactive Fluidics



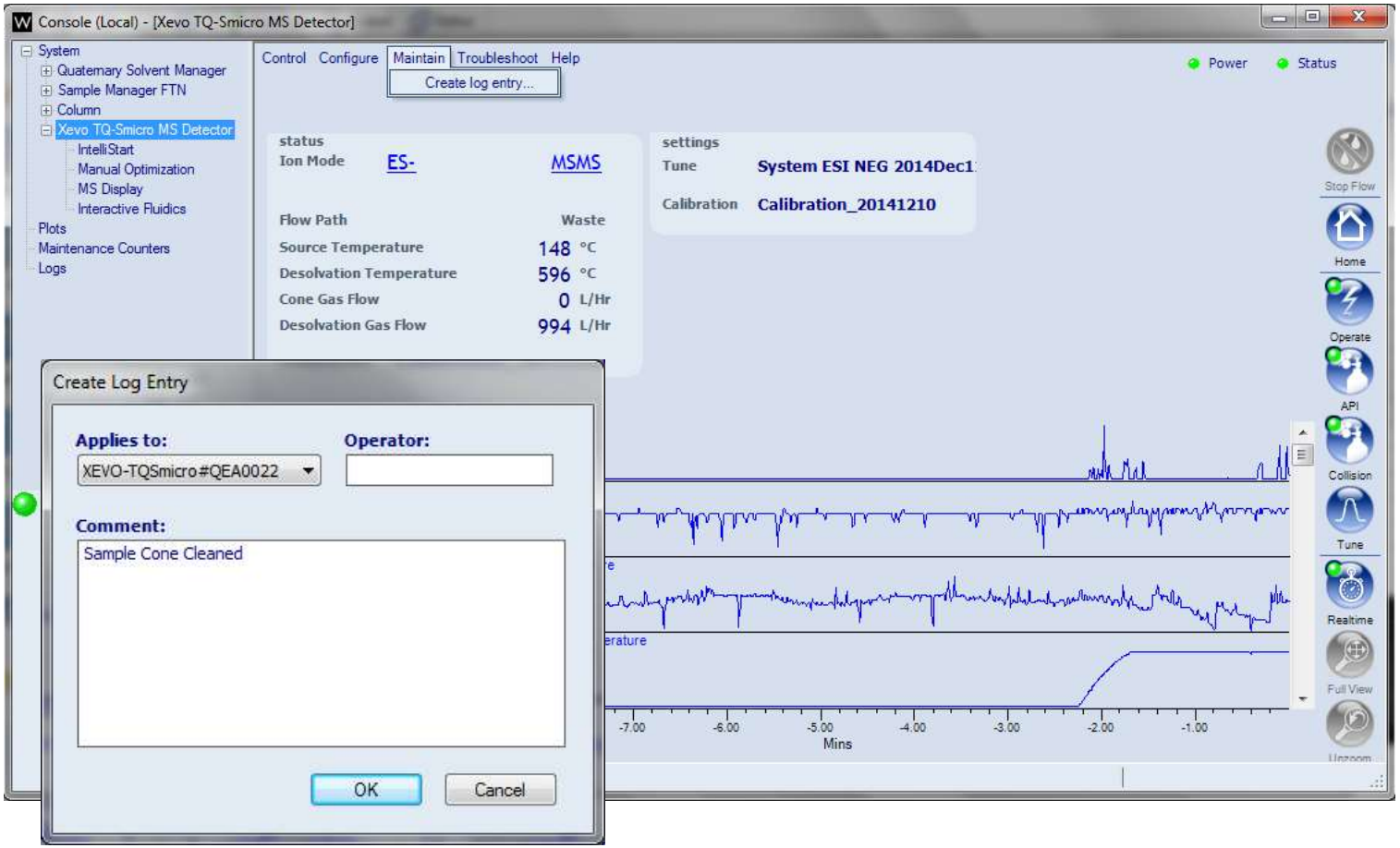
Xevo TQ-S micro – Interactive Fluidics



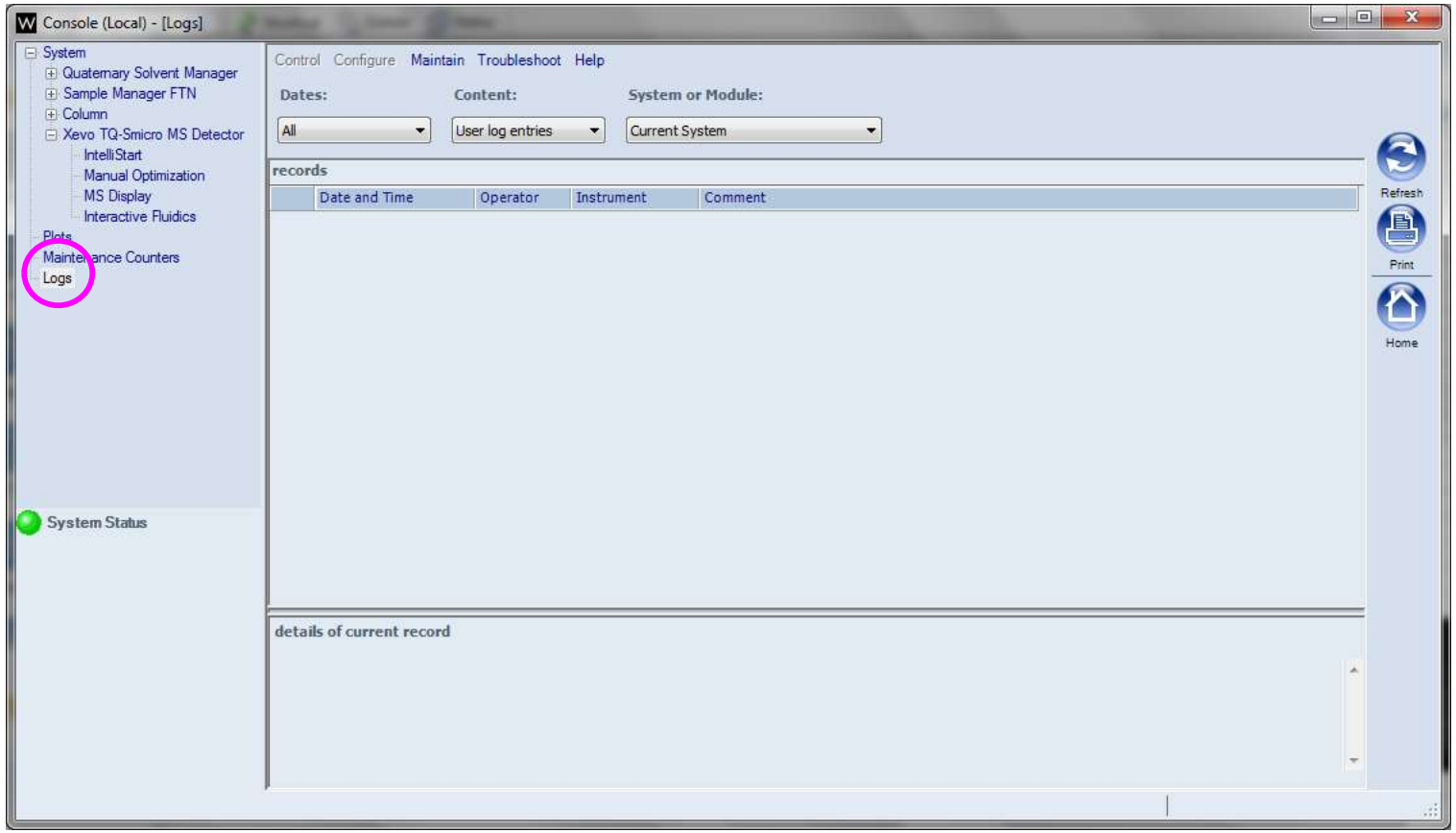
Console Control Functions



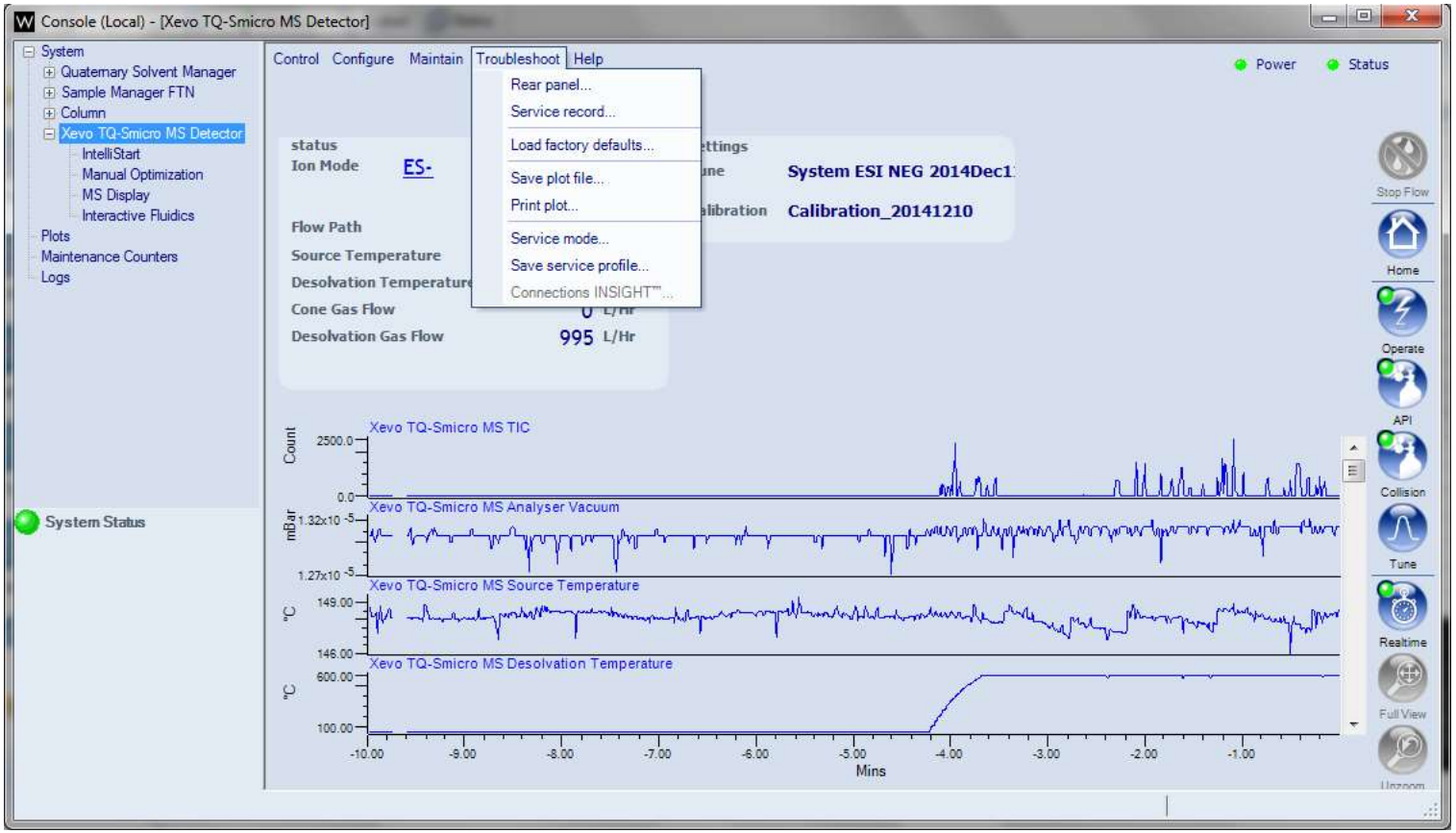
Console Log Entries



Console Log Entries



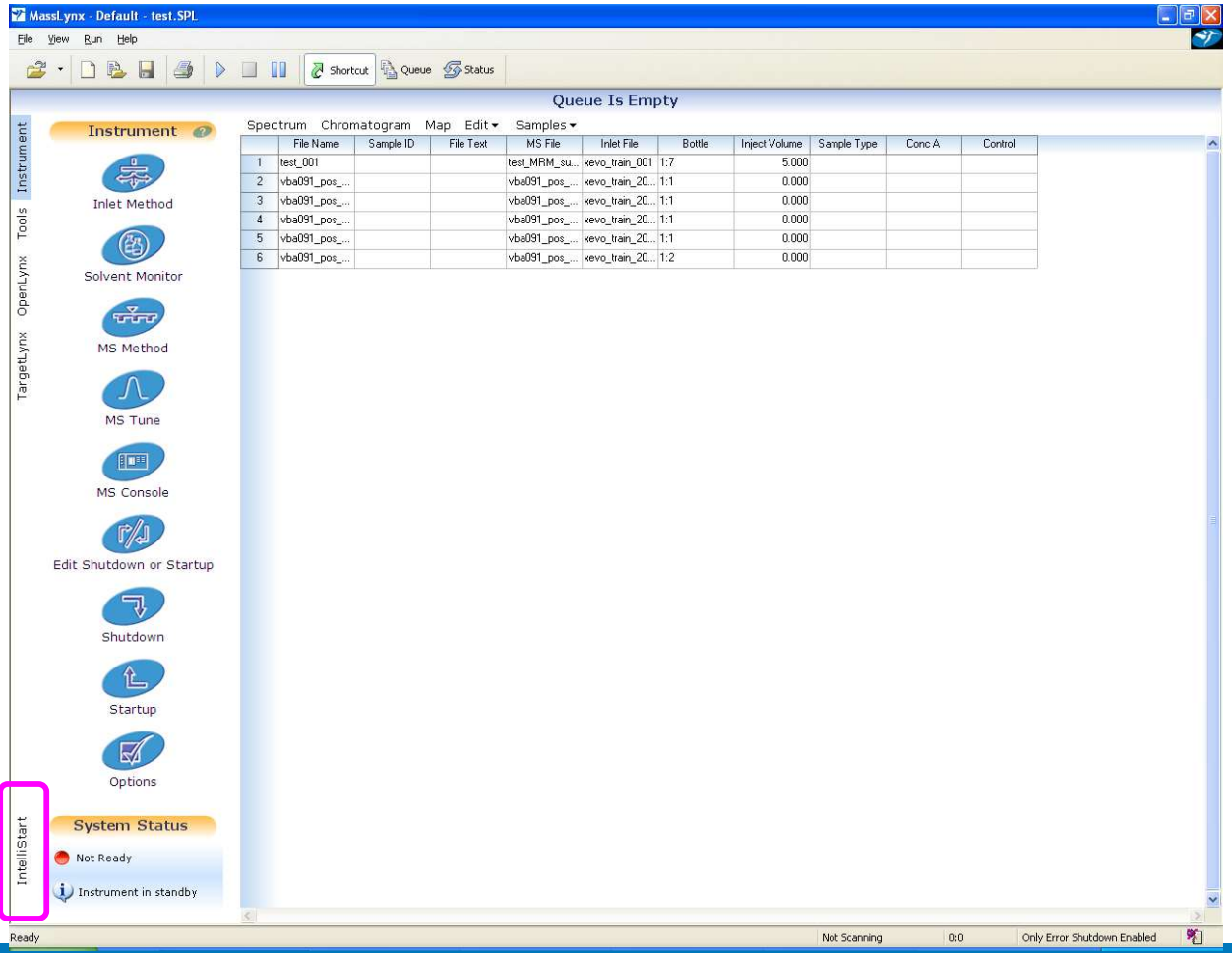
Console Troubleshooting Functions



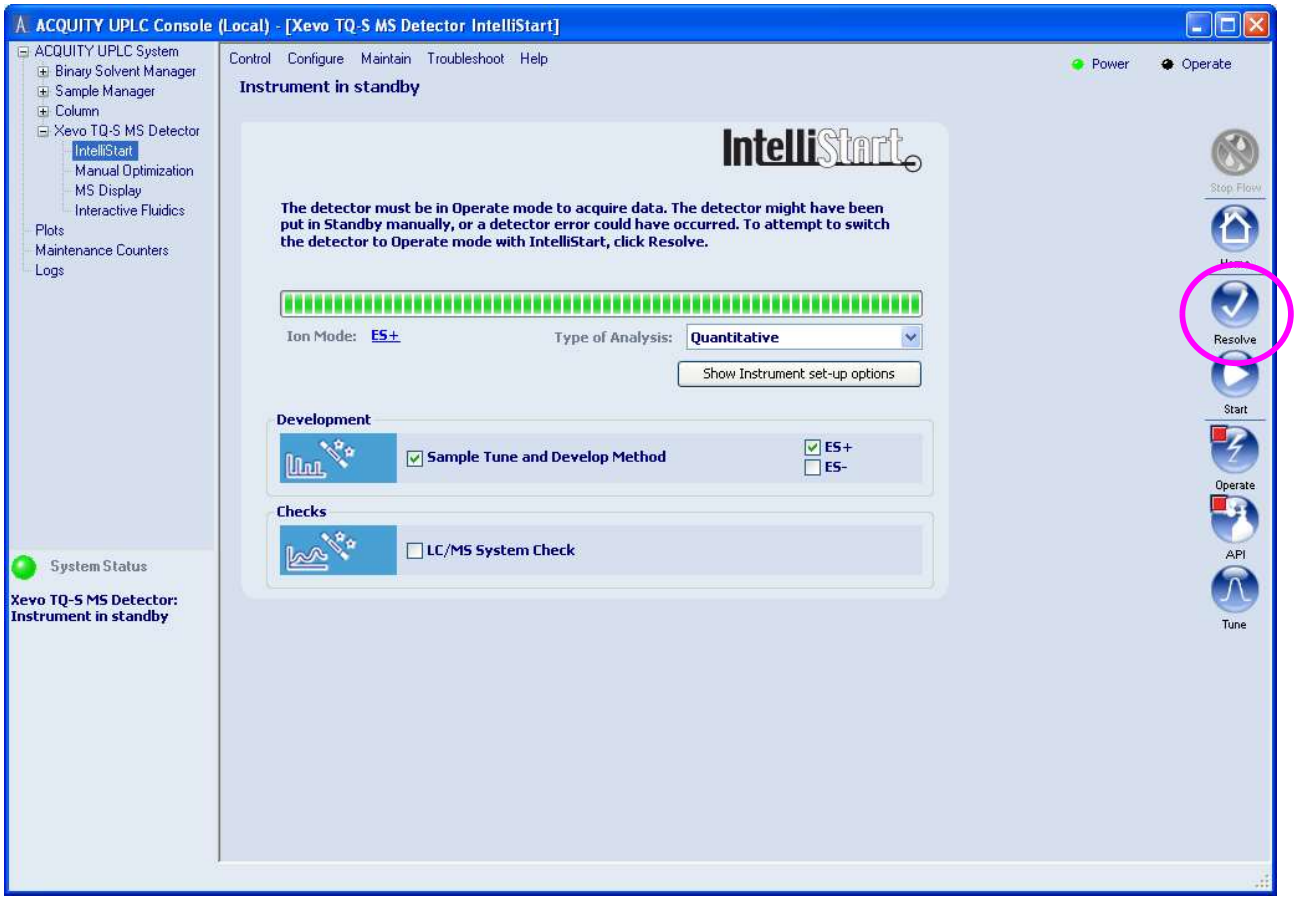
IntelliStart

- IntelliStart is designed to automatically monitor instrument readiness.
- Where possible, the system may also take corrective action to rectify any failed system checks
- IntelliStart comprises:
  - Automated system checking software
  - Diagnostic electronics
  - Integrated fluidics device
  - Three built-in vial locations for set-up/user solutions

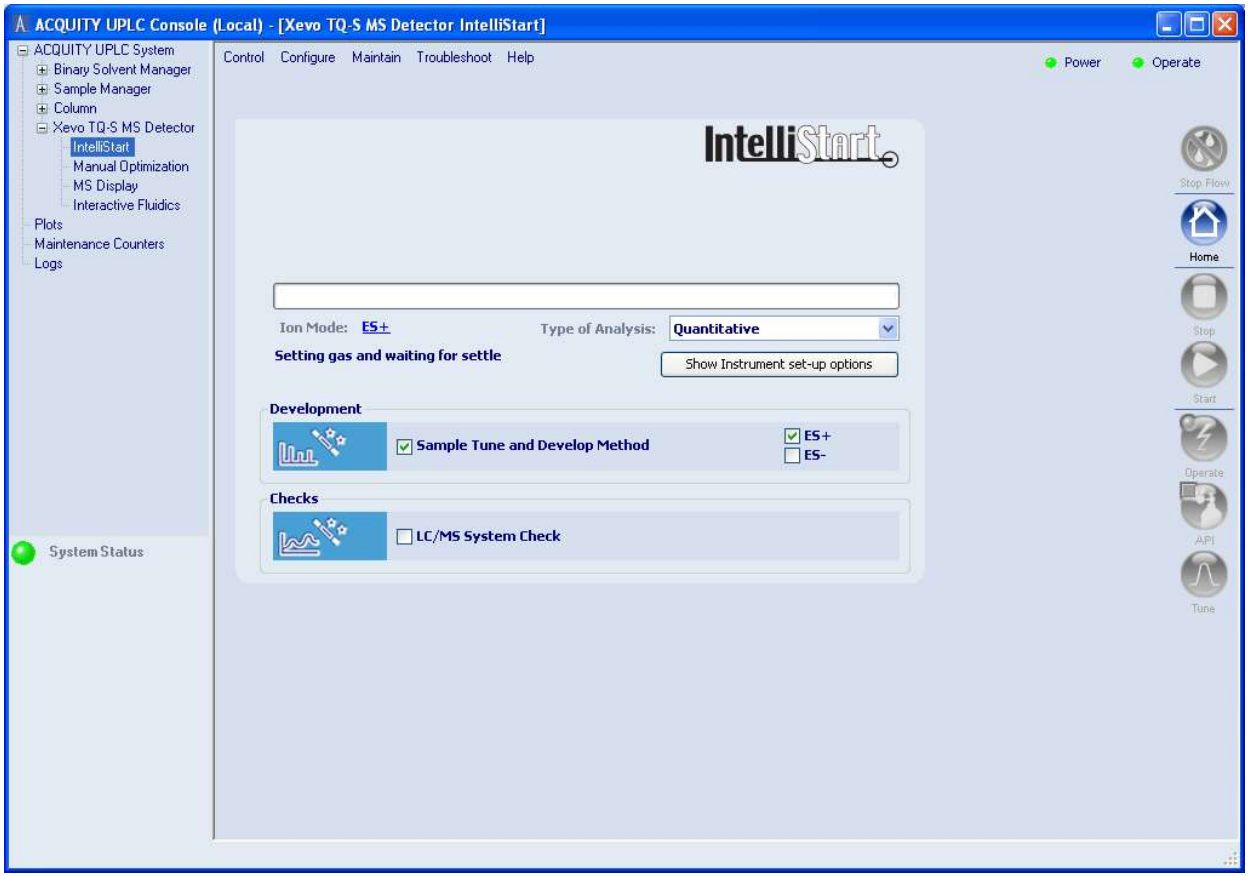
Using IntelliStart to Monitor the Xevo TQ-S micro



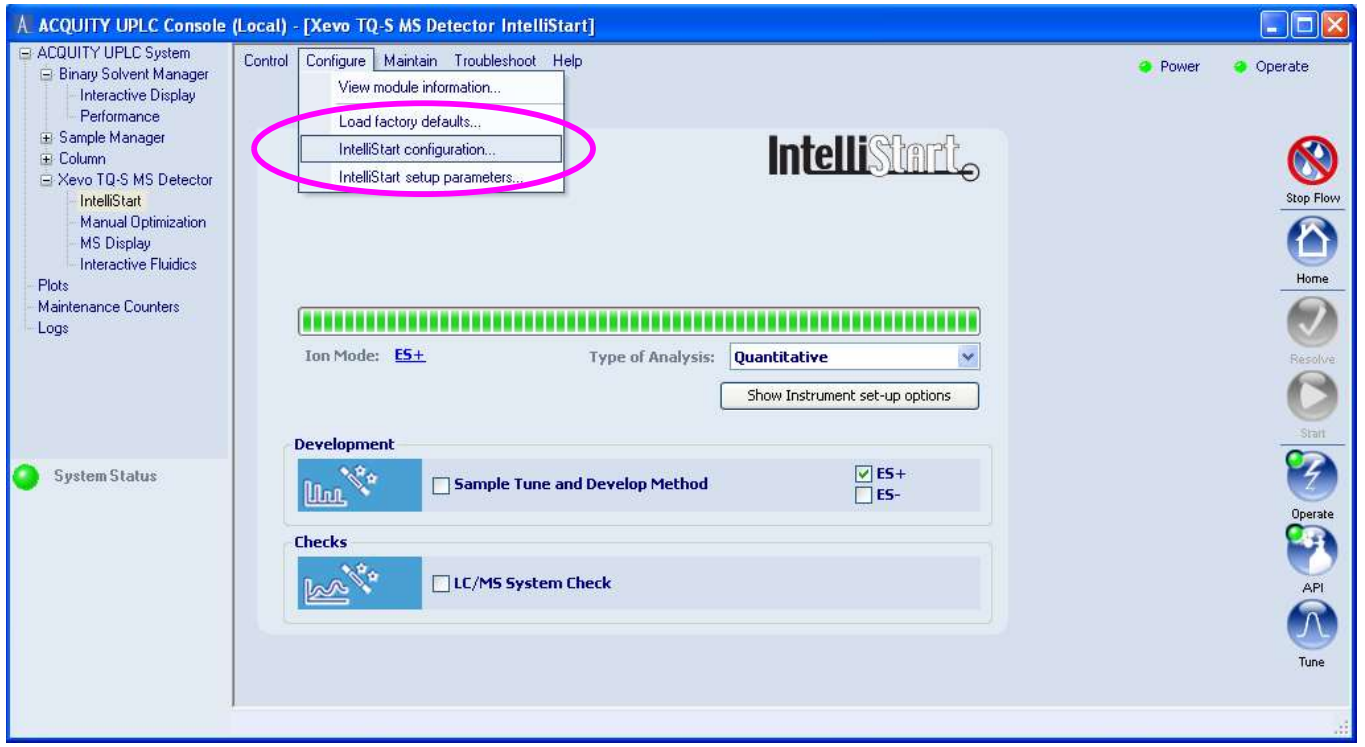
Using IntelliStart to Resolve “Not Ready” Conditions



Using IntelliStart to Resolve “Not Ready” Conditions

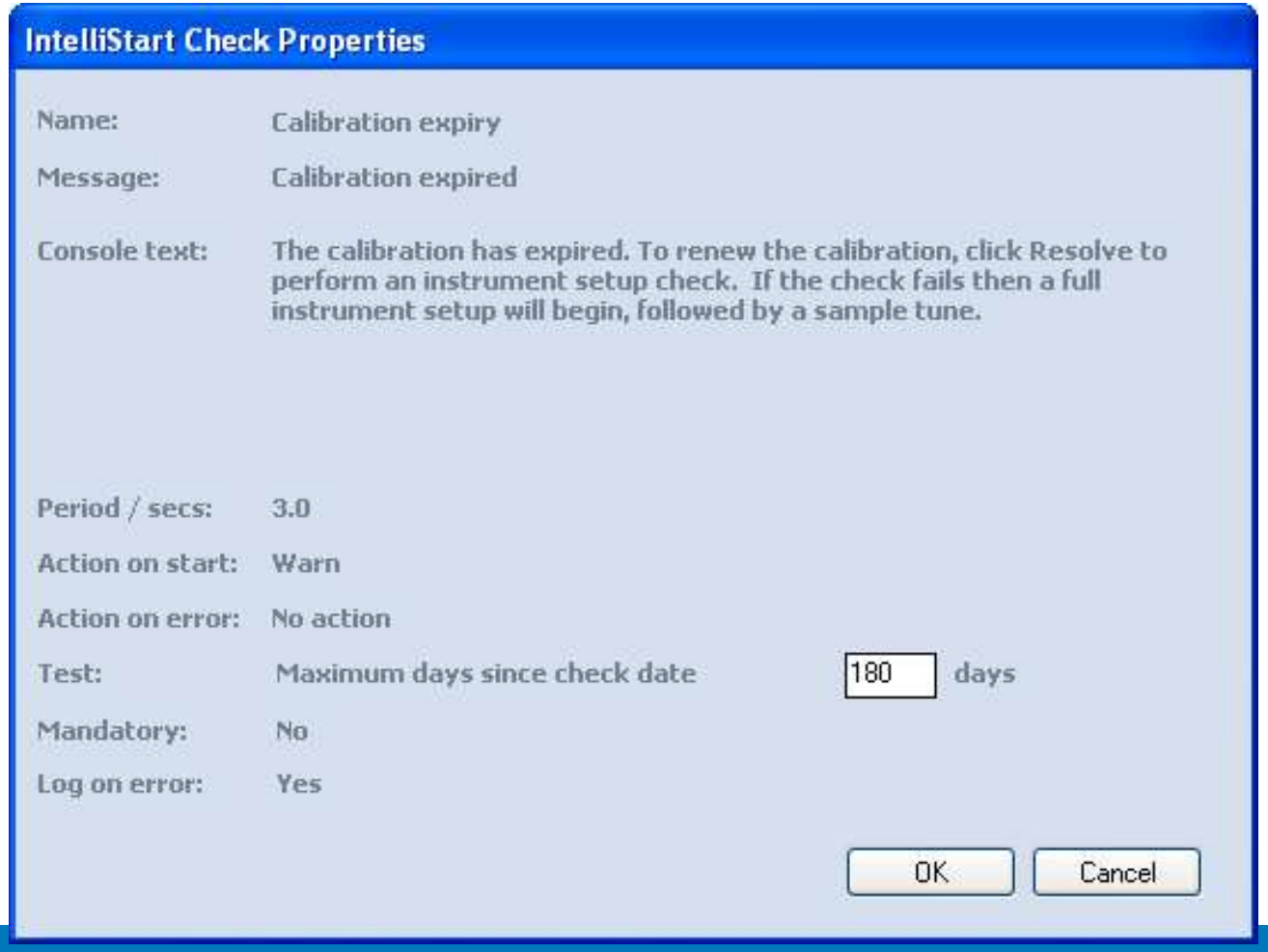


IntelliStart Configuration



IntelliStart Configuration

IntelliStart Configuration Properties

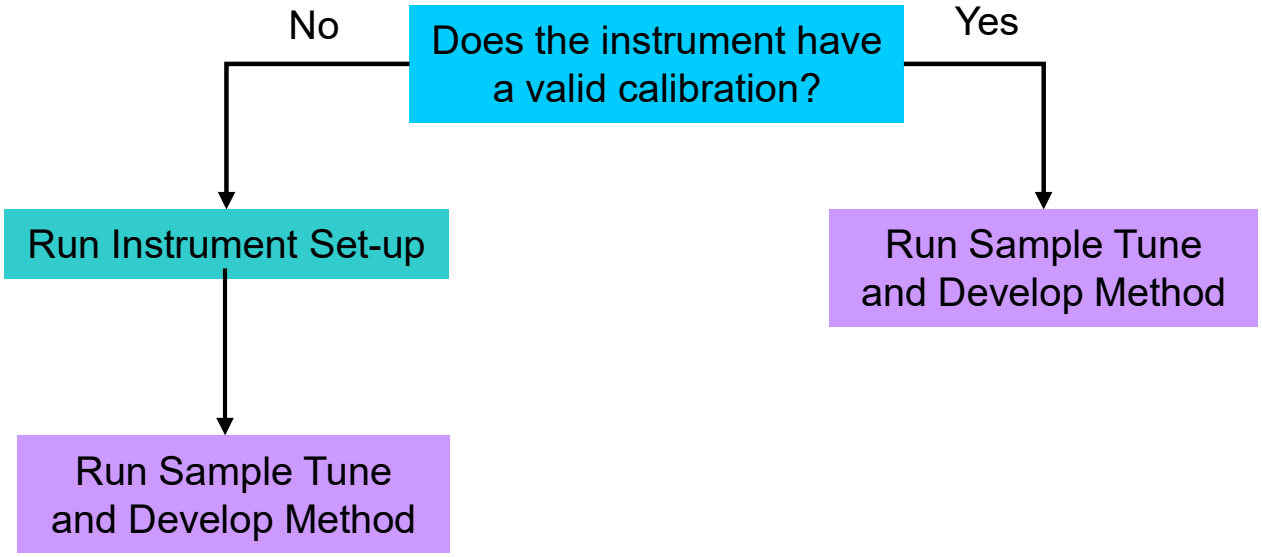


IntelliStart Functions

- Instrument Set-up
  - Instrument Resolution
  - Instrument Calibration
- Development
  - Sample Tune
  - Develop Method
- Checks
  - LC/MS System Check

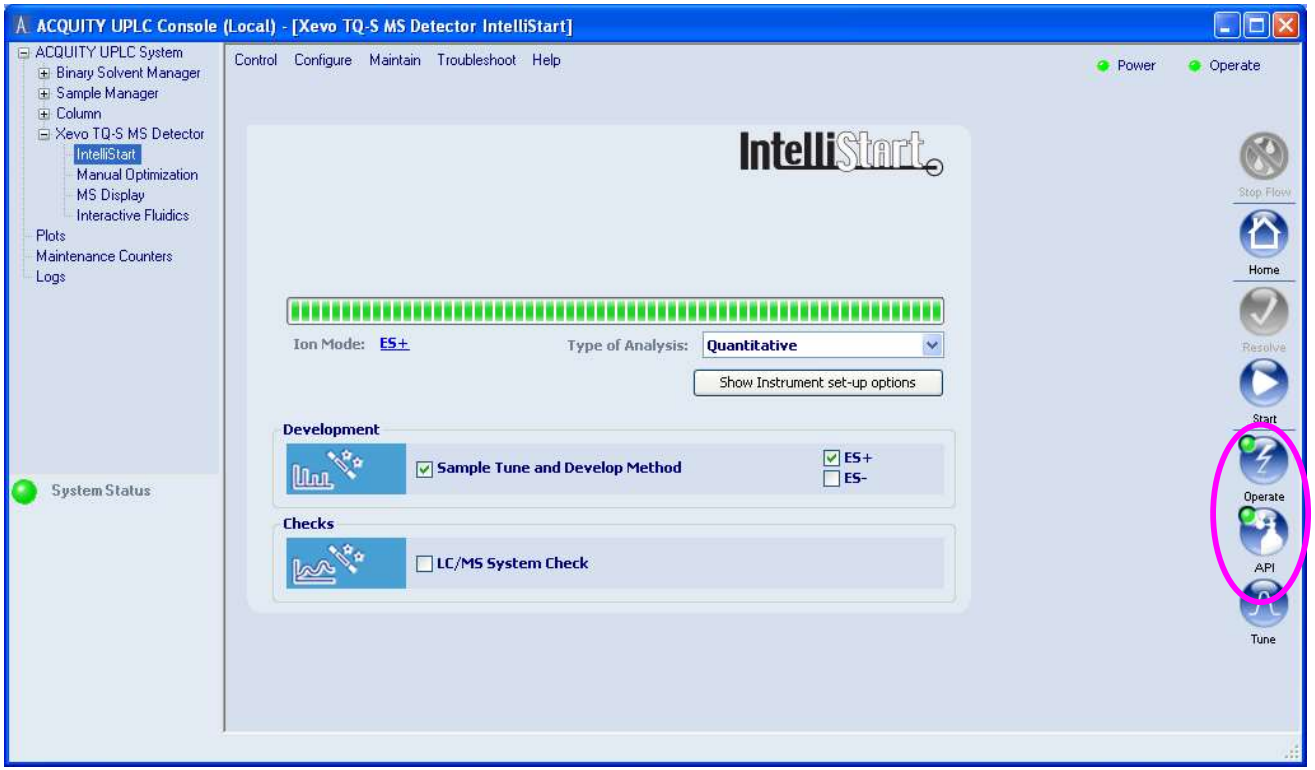
Xevo TQ-S micro IntelliStart Function Flowchart

- When the instrument is installed, the engineer will create a valid calibration for each of the four analysis types. Recalibrate the instrument as required.

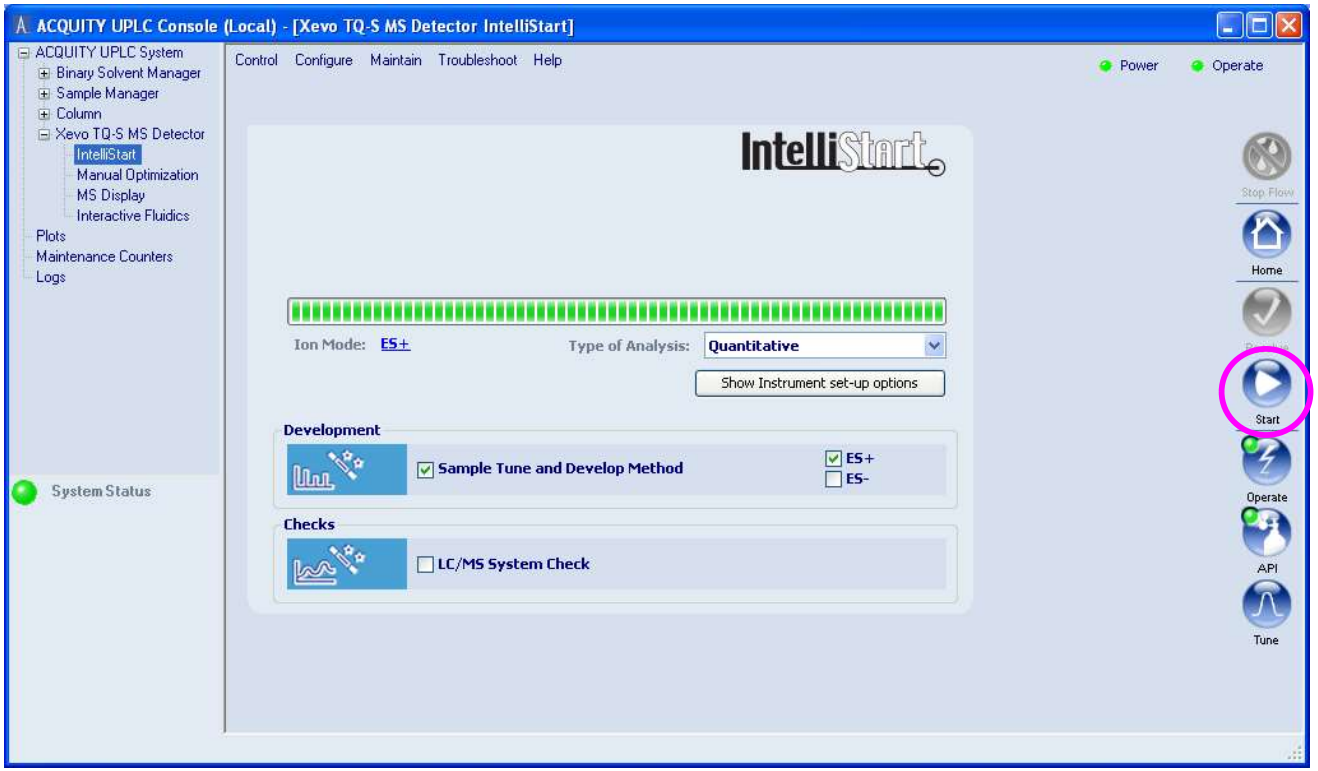


Sample Tune and Develop Method

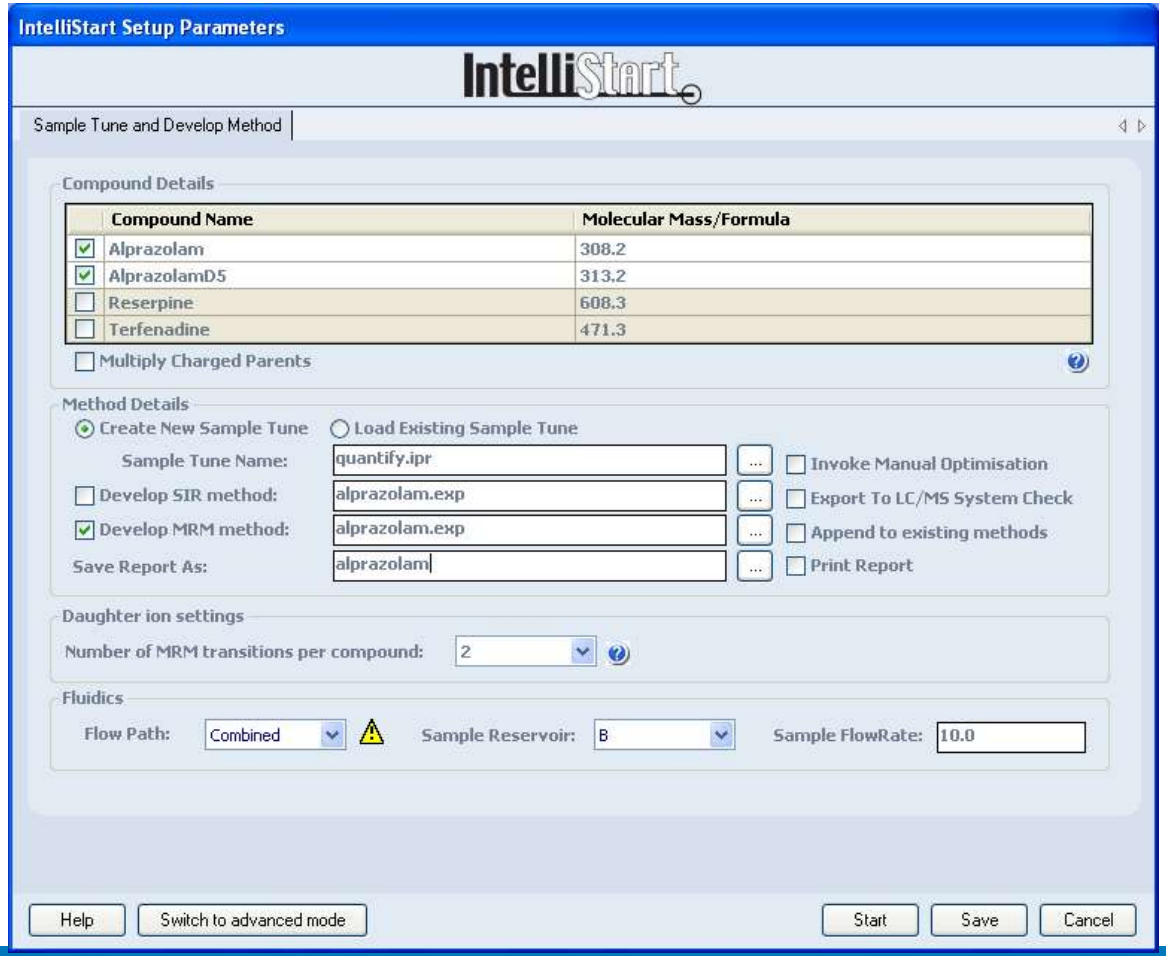
- Using IntelliStart for Sample Tuning.



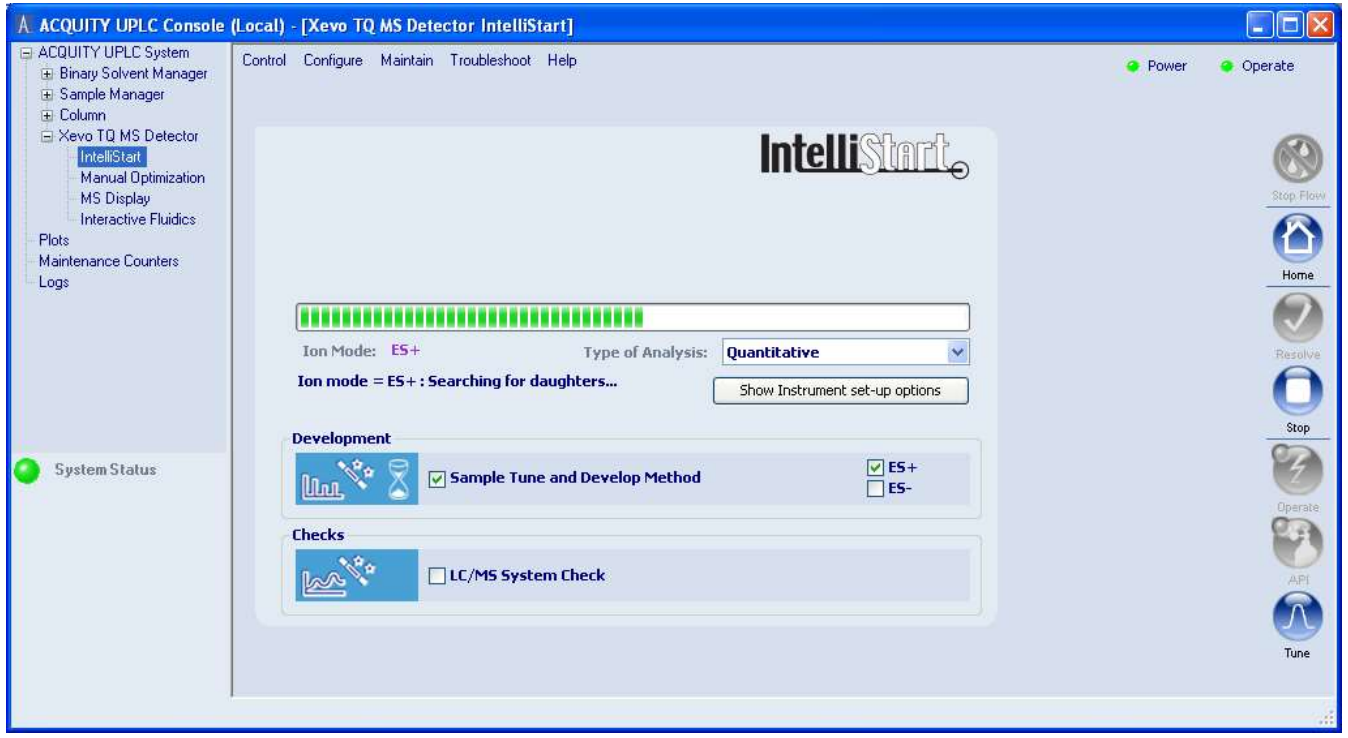
Sample Tune and Develop Method



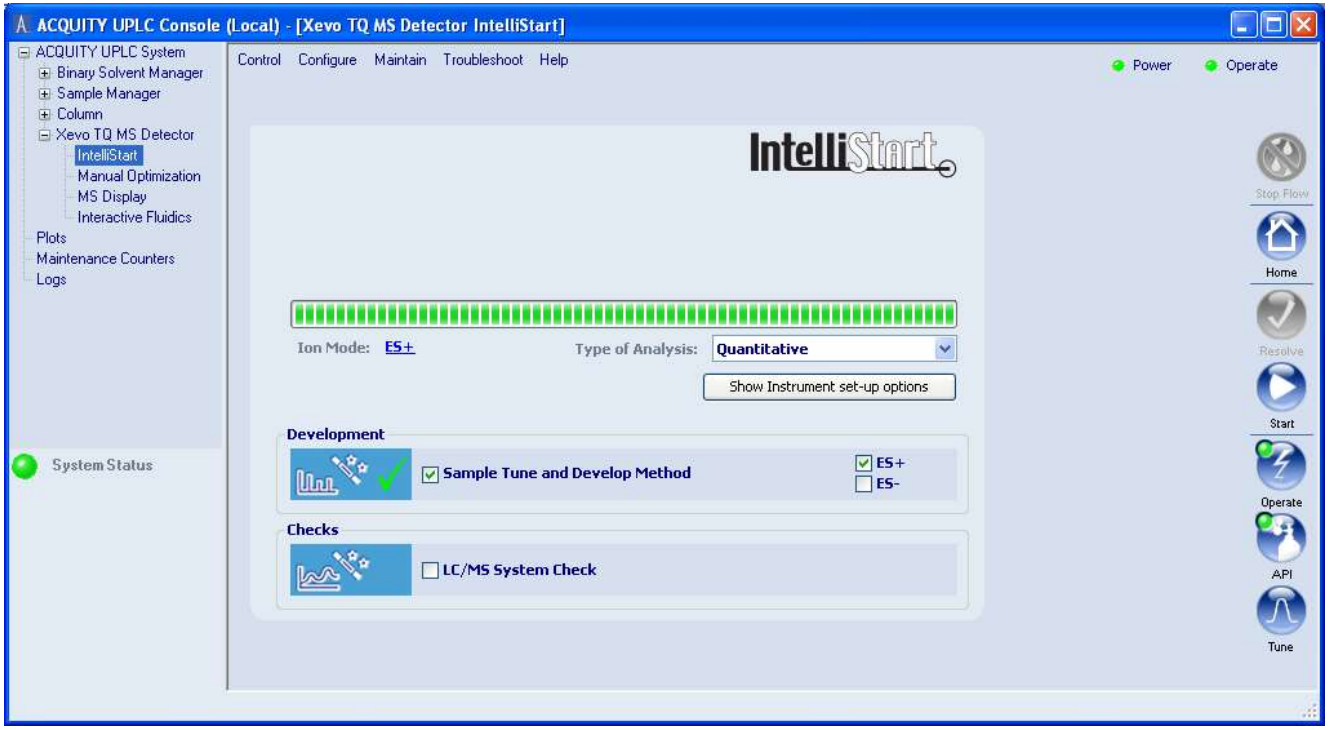
Sample Tune and Develop Method – Basic Mode



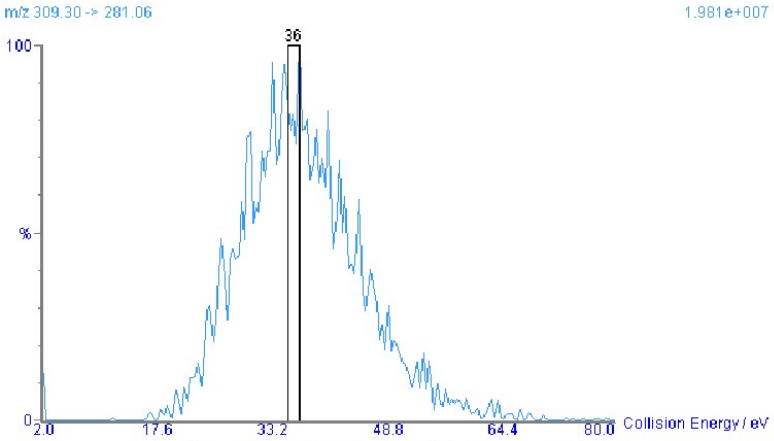
Sample Tune & Develop Method – In Progress



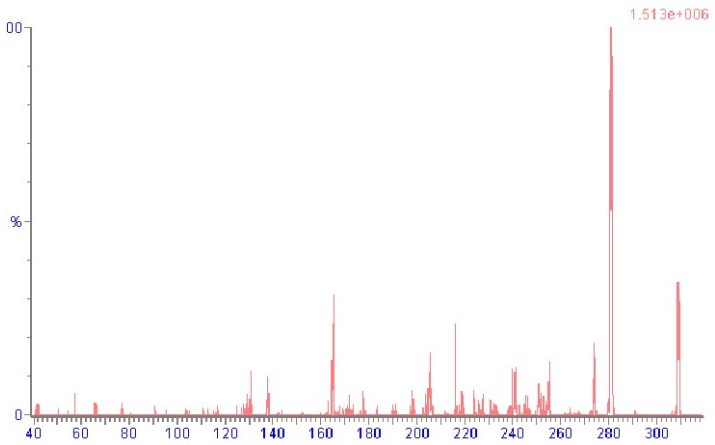
Sample Tune & Develop Method – Complete



Sample Tune & Develop Method – Report



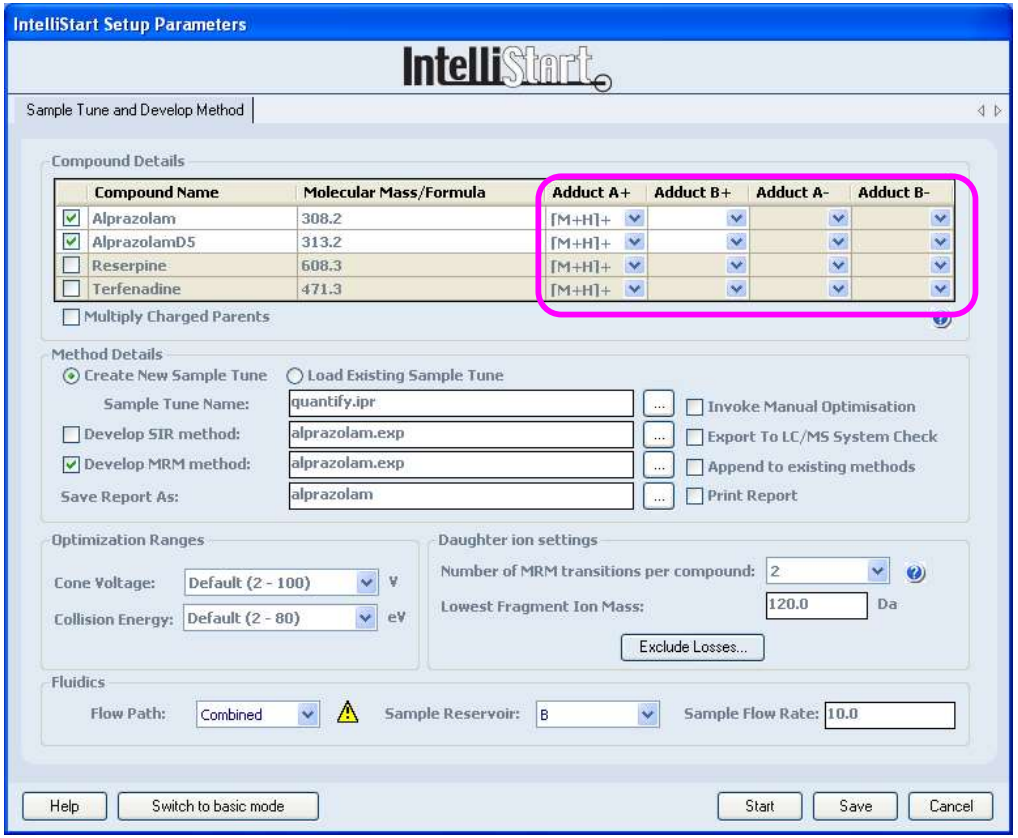
Collision Energy Optimization (m/z 309.30 -> 281.06)



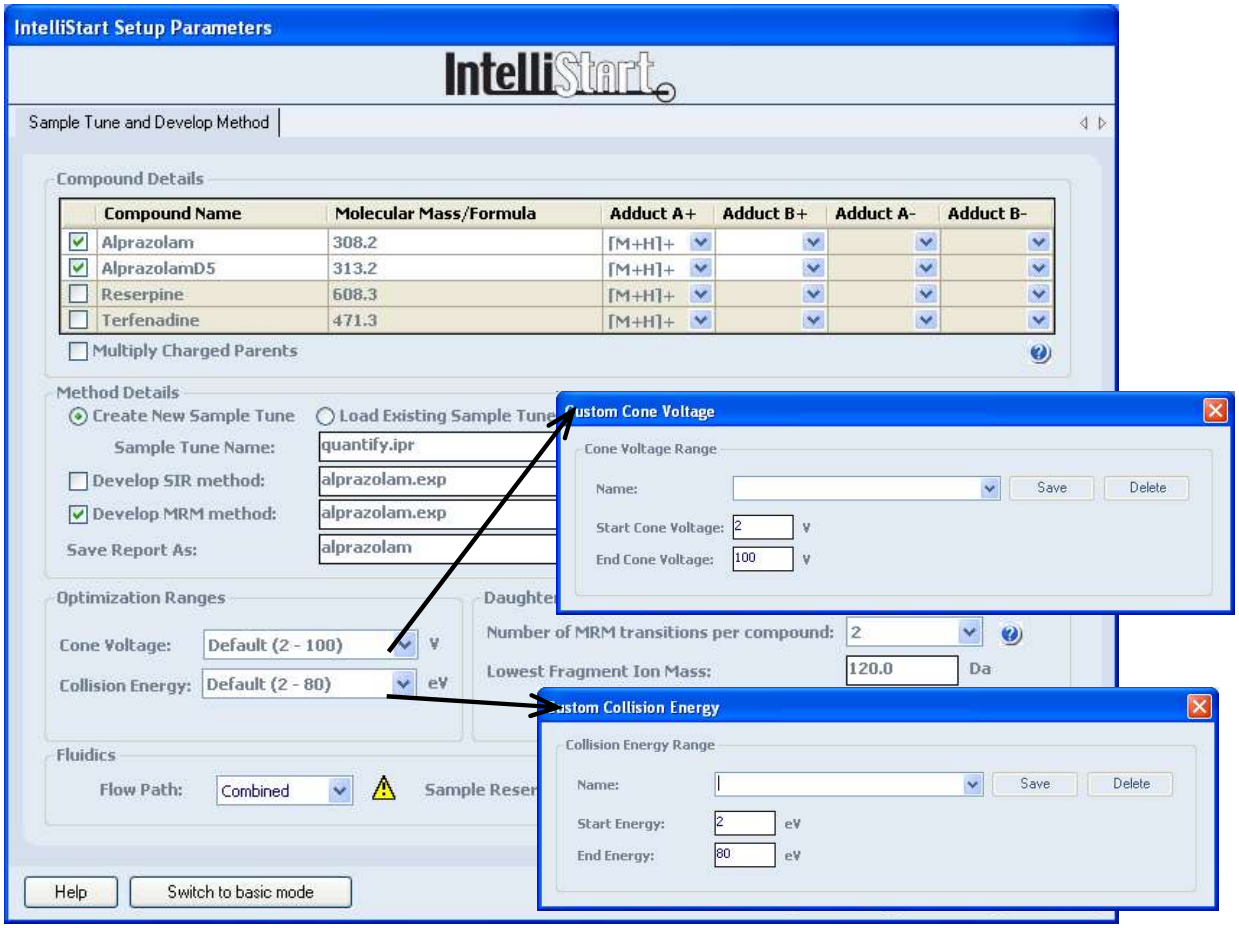
Optimized Daughter Spectrum (at Collision Energy 36eV)

Sample Tune & Develop Method – Advanced Mode

- Advanced Sample Tune Options
- System Setup Using Intellistart



Sample Tune & Develop Method – Custom Cone Voltage



Sample Tune & Develop Method – Exclude Losses Options

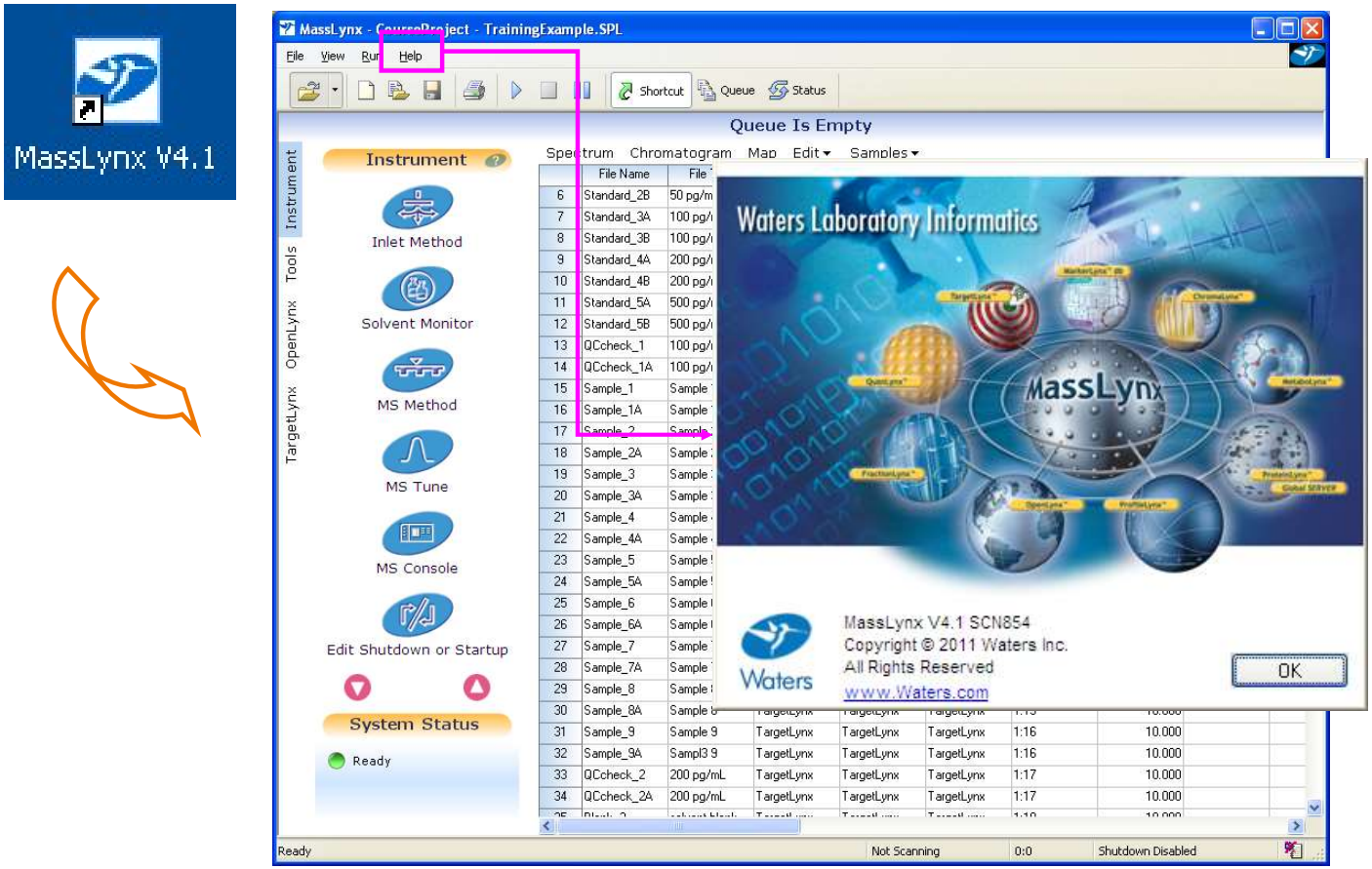
Chapter Four

Data Acquisition

MassLynx Overview

- MassLynx Structure
- Automated data acquisition
  - Projects
  - Creating a Sample list
  - Queue properties
  - Real time data
  - Shutdown methods

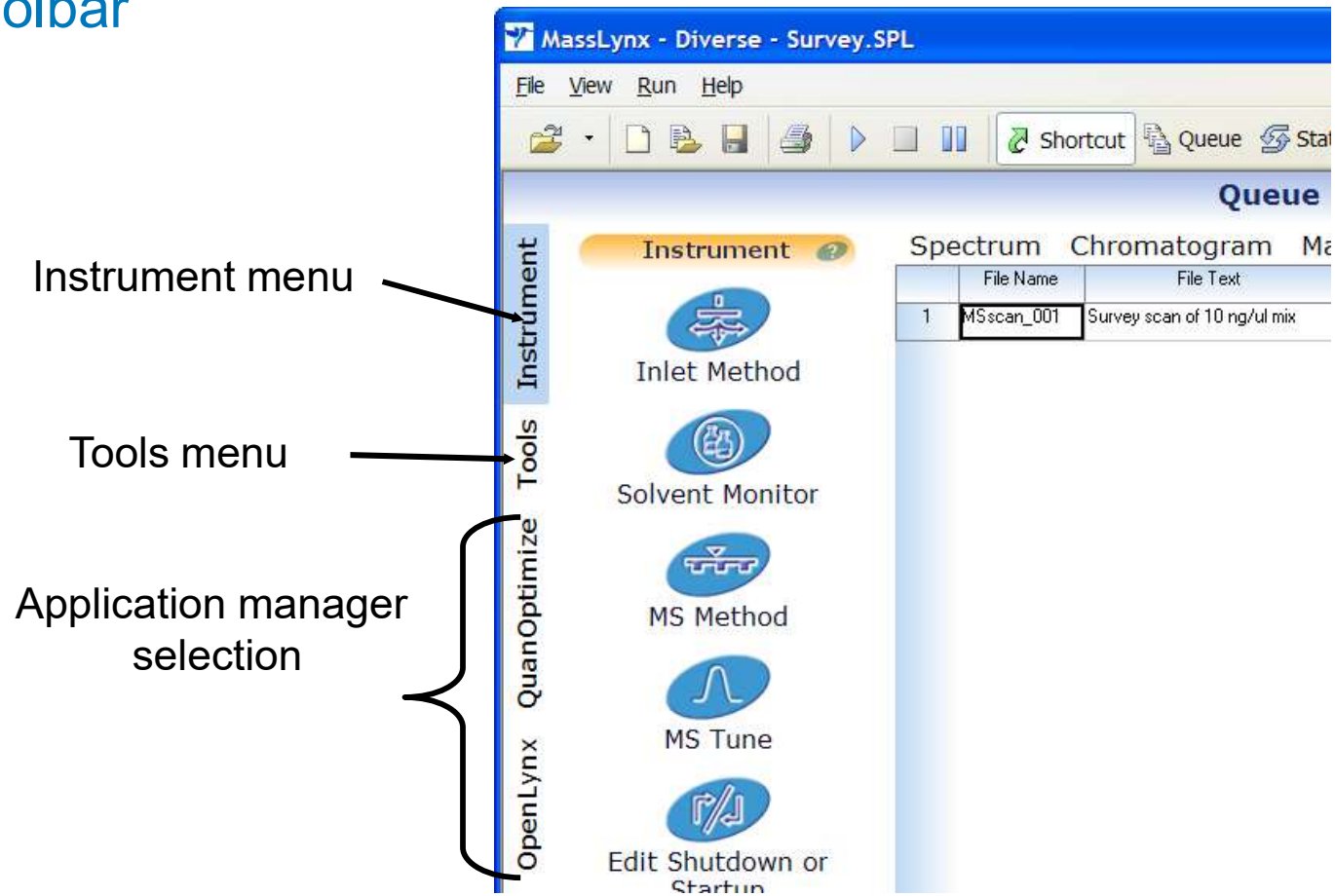
MassLynx Architecture - Main Window



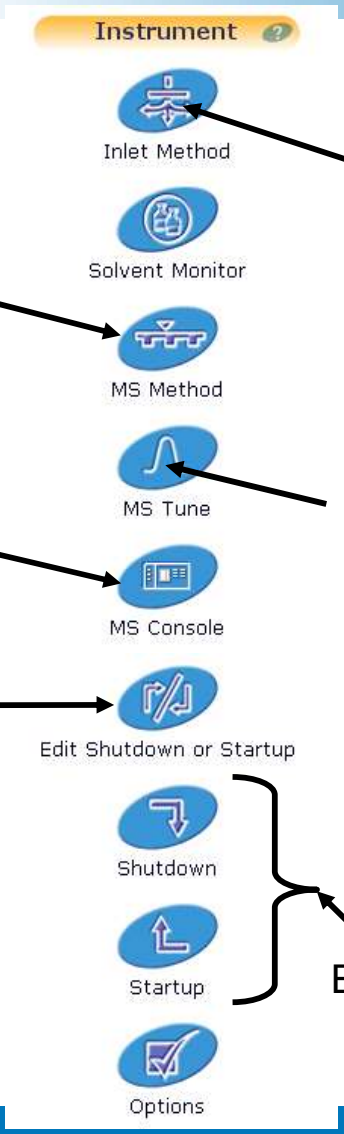
Project Sub-Folders

- Acqudb - Acquisition files
  - Tune files (.ipr)
  - MS (instrument) calibration files (.cal)
  - LC method files (.wat or other)
  - MS method files (. exp)
- Curvedb - Quantify calibration curves (.cdb)
- Data - Raw data files (.raw)
- Methdb - Quantify methods (.mdb)
- Peakdb - Peak lists (.pdb)
- Sampledb - Sample lists (.spl)

MassLynx Toolbar



Main Page Instrument Menu



Open LC method editor

MS method building

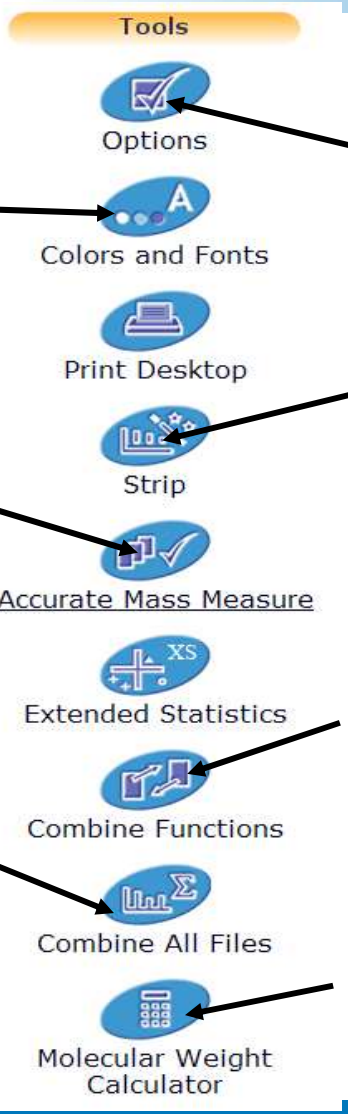
MS Console/ Intellistart

Open Xevo TQ-S micro tune page

Shutdown/Start-up method building

Execute Shutdown/ start-up process

Main Page Tools Menu



Change colors of chromgatograms and font sizes and styles

Configure Options for displaying mass spectra

Options for processing entire data files

For use only with instruments capable of accurate mass measurements

Options for processing all scan functions in

Options for combining entire raw data files

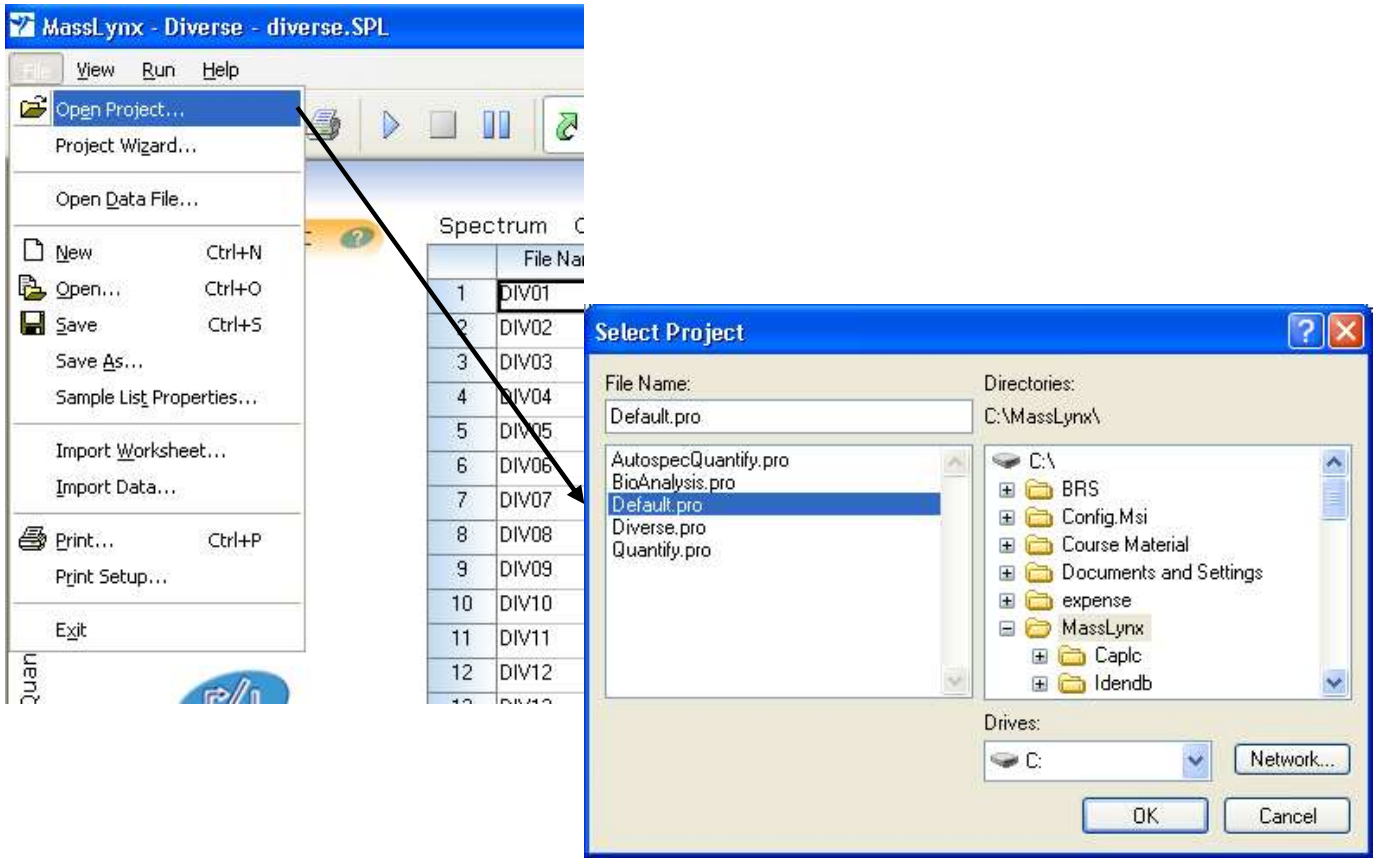
Calculate the average or monoisotopic MW using an empirical formula

Creating a Sample List

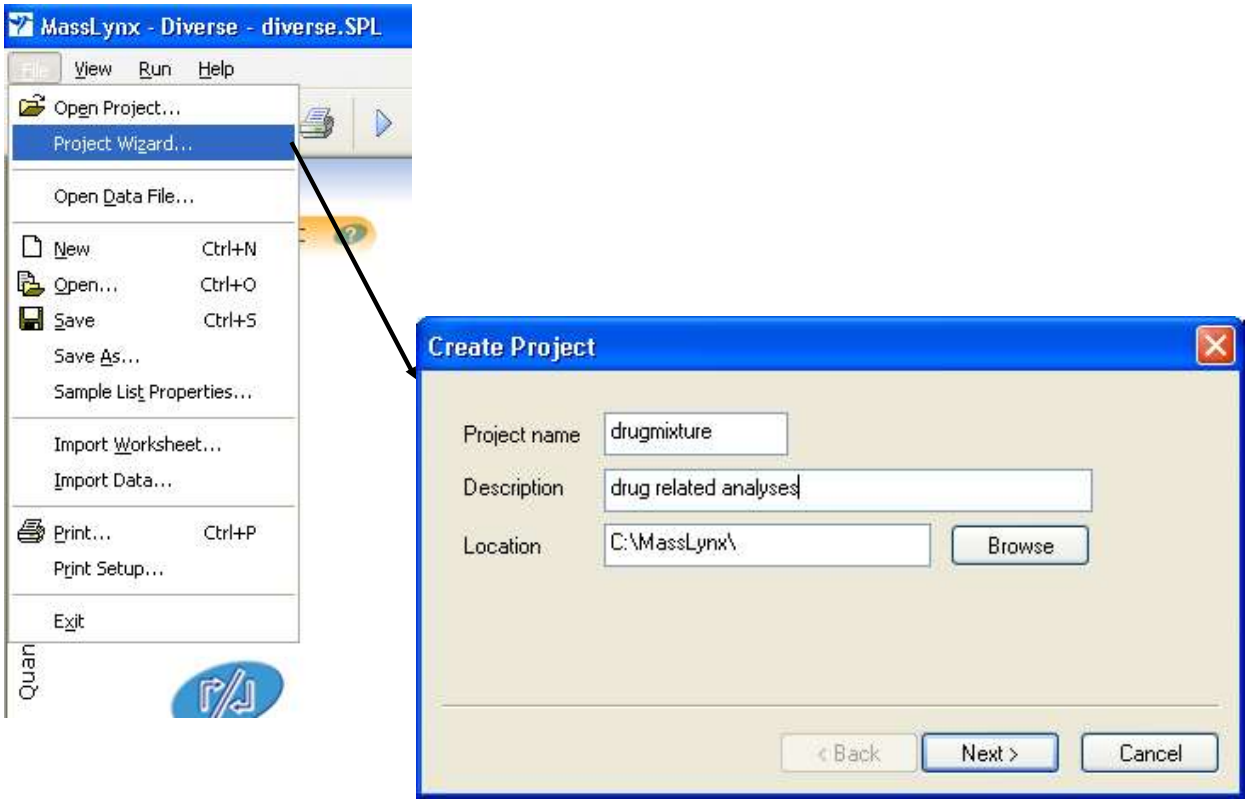
Waters™

- Specify a project where the data will be collected
  - Open an existing project OR
  - Create a new project
- Build the sample list
  - File Name
  - MS File
  - Inlet (LC) file
  - Bottle (sample location)
  - Injection Volume

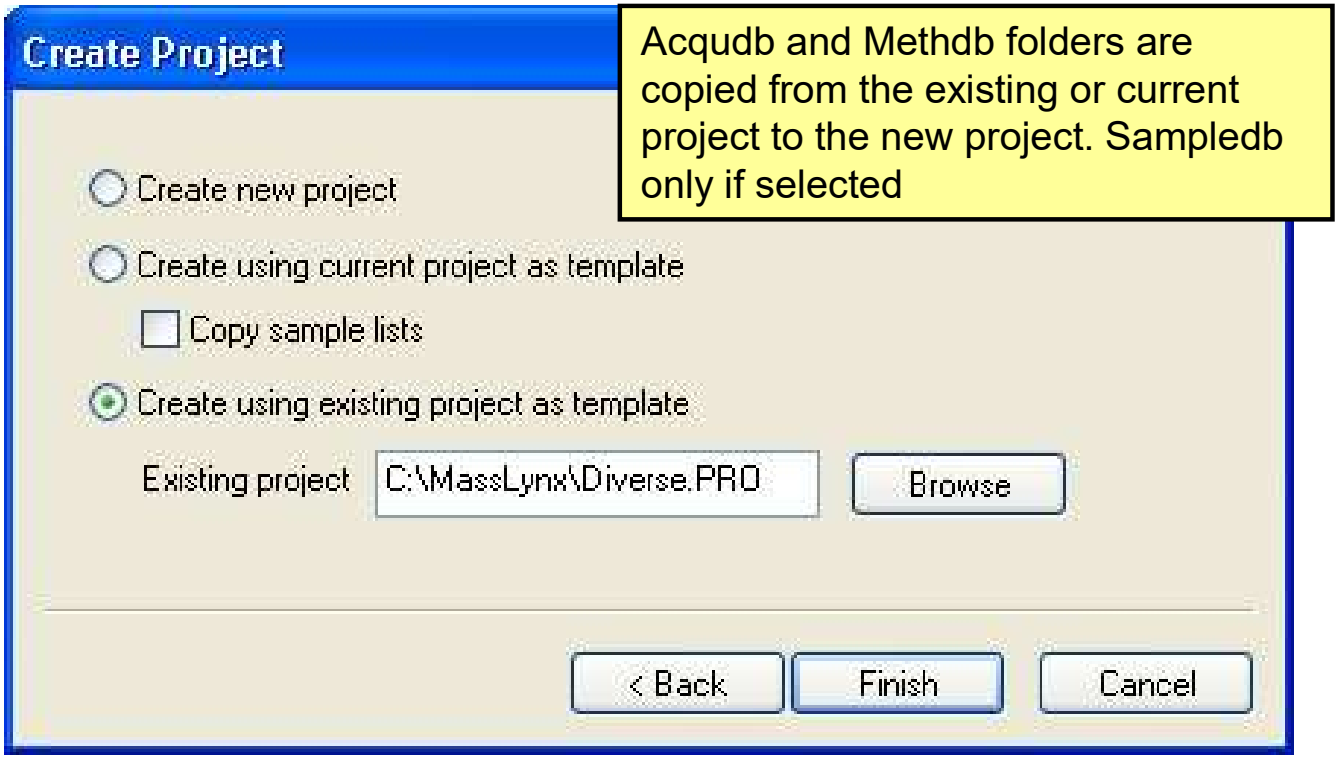
Open Existing Project



Create New Project

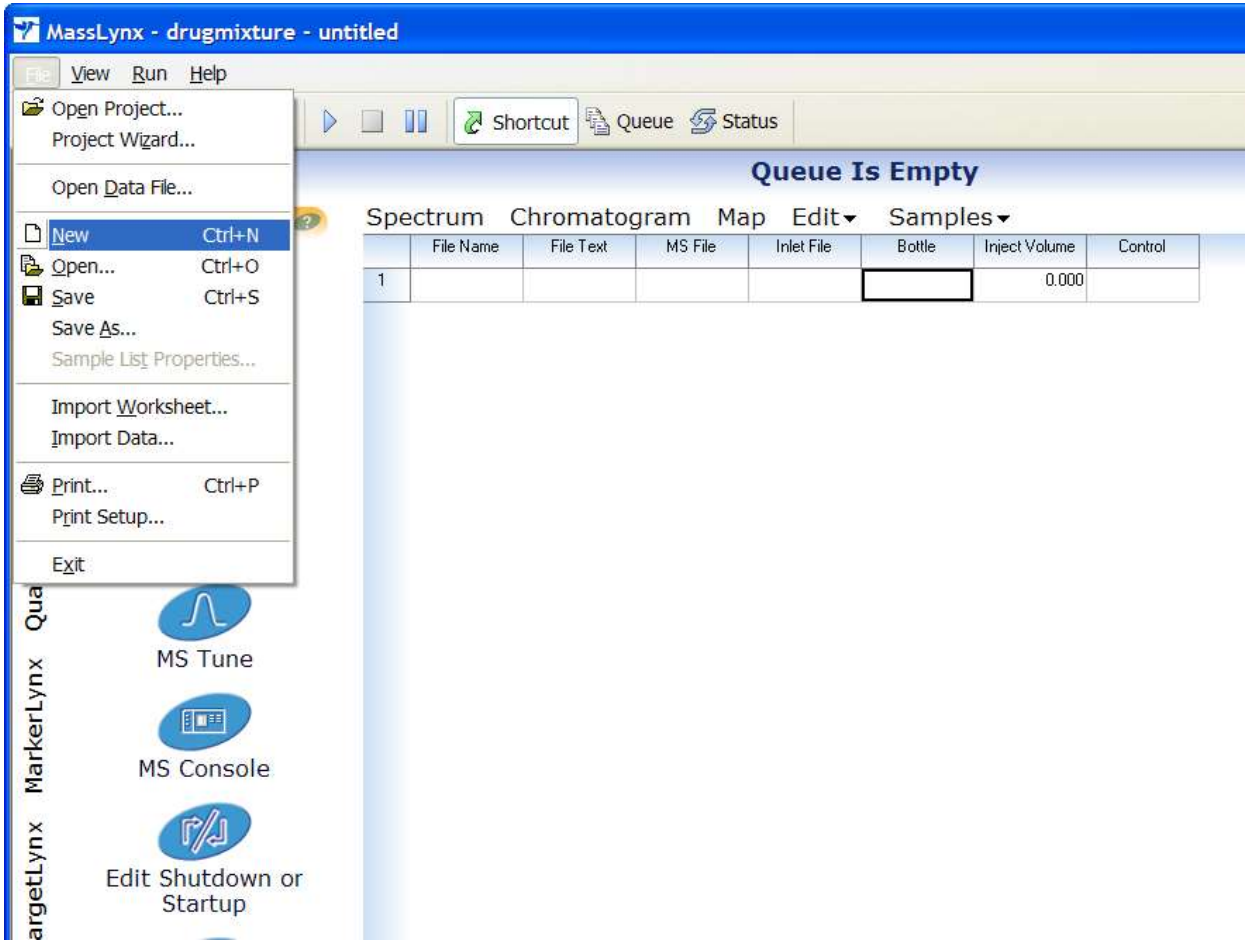


Project Wizard

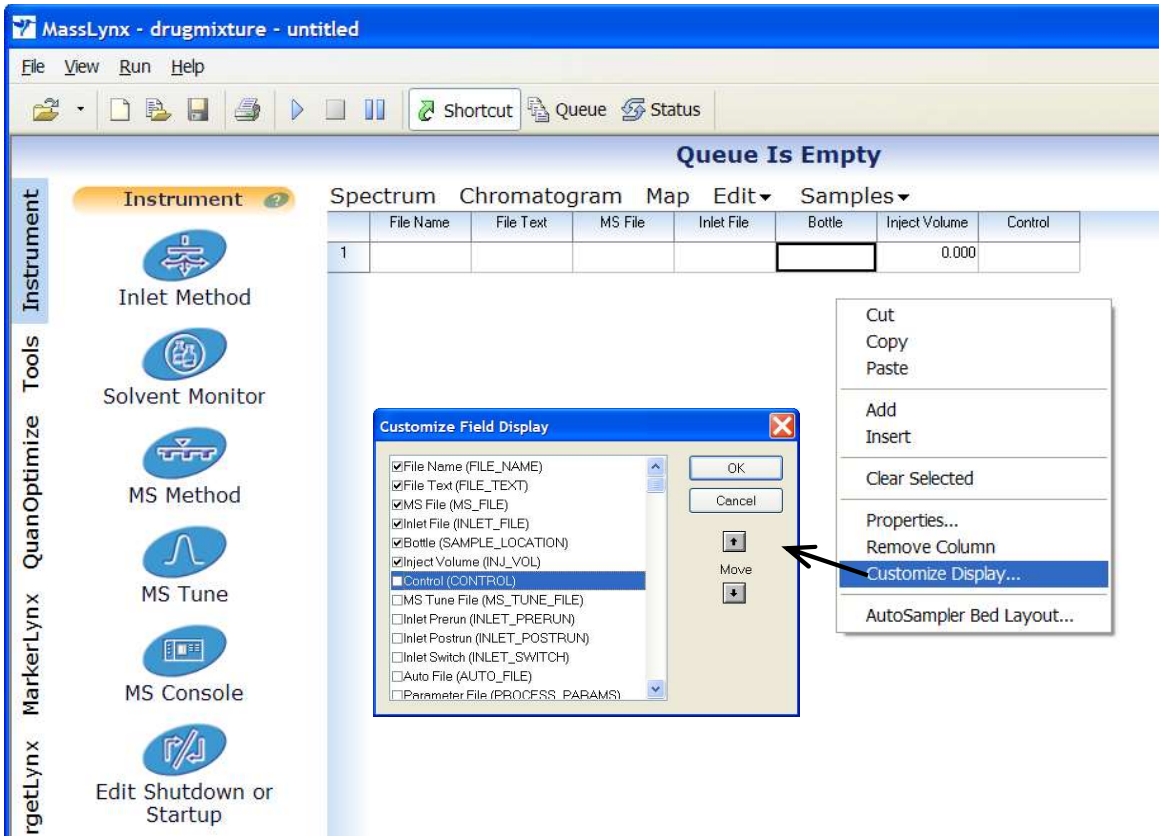


Acqudb and Methdb folders are copied from the existing or current project to the new project. Sampledb only if selected

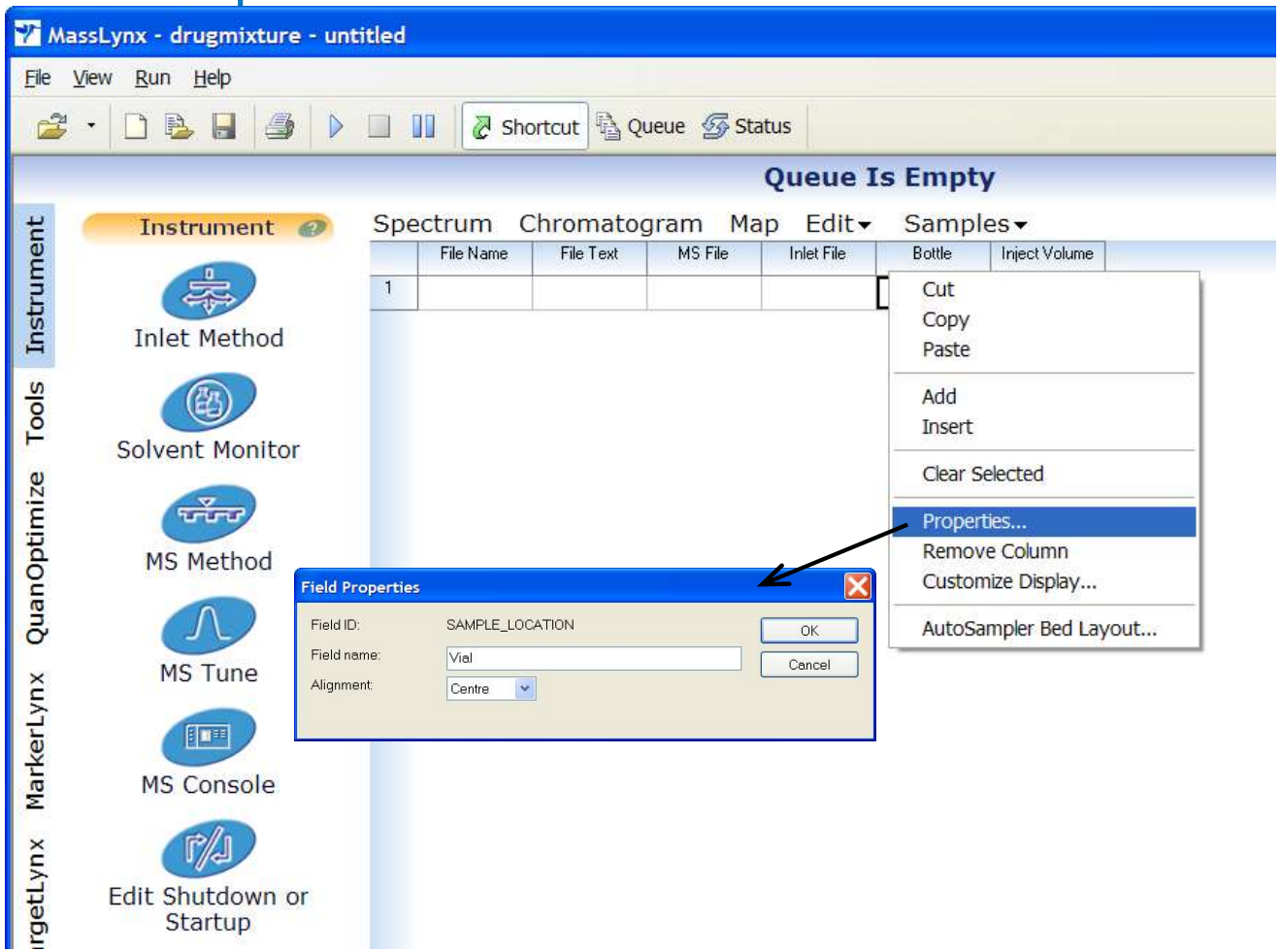
New Sample List



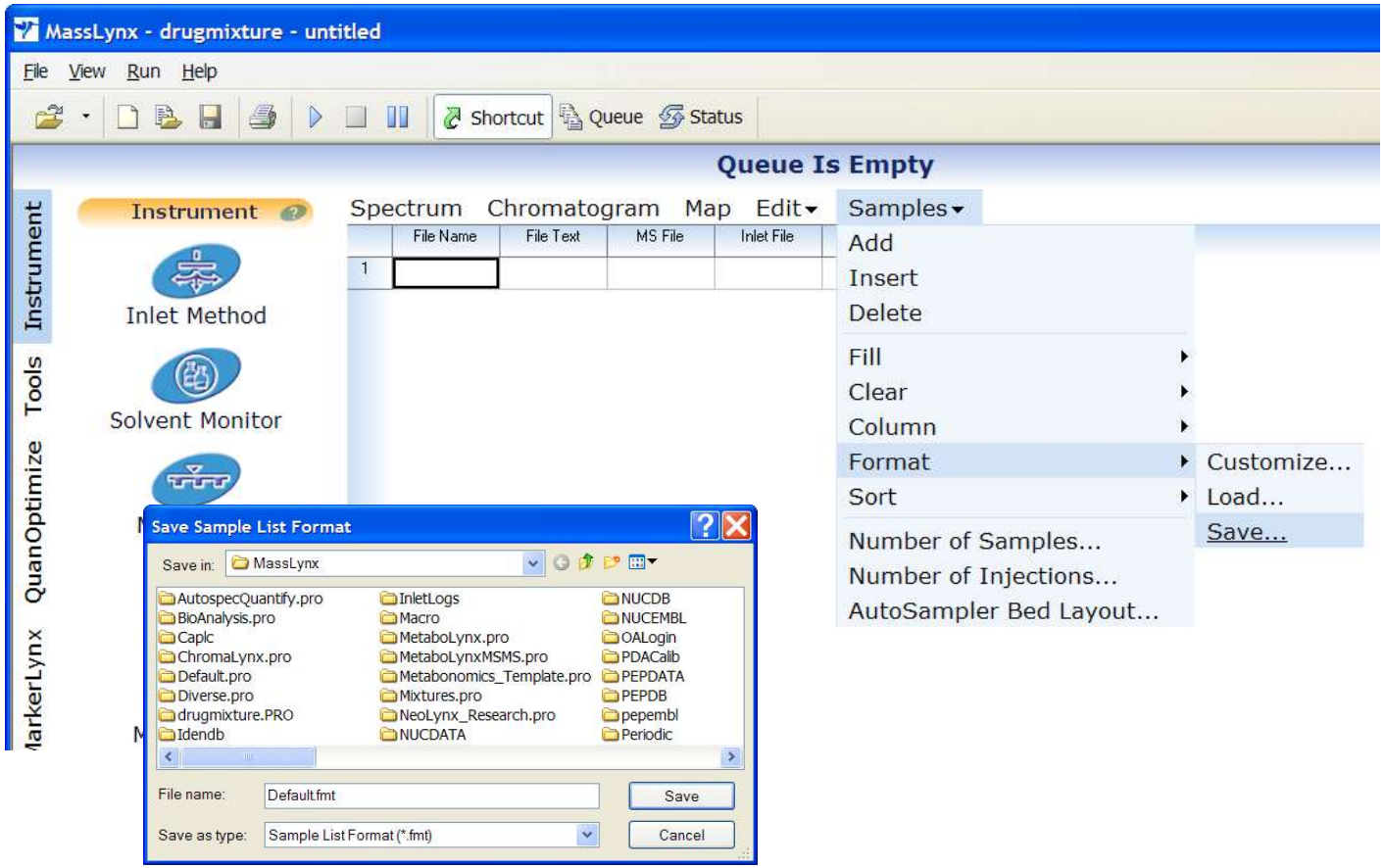
Customize Sample List Format



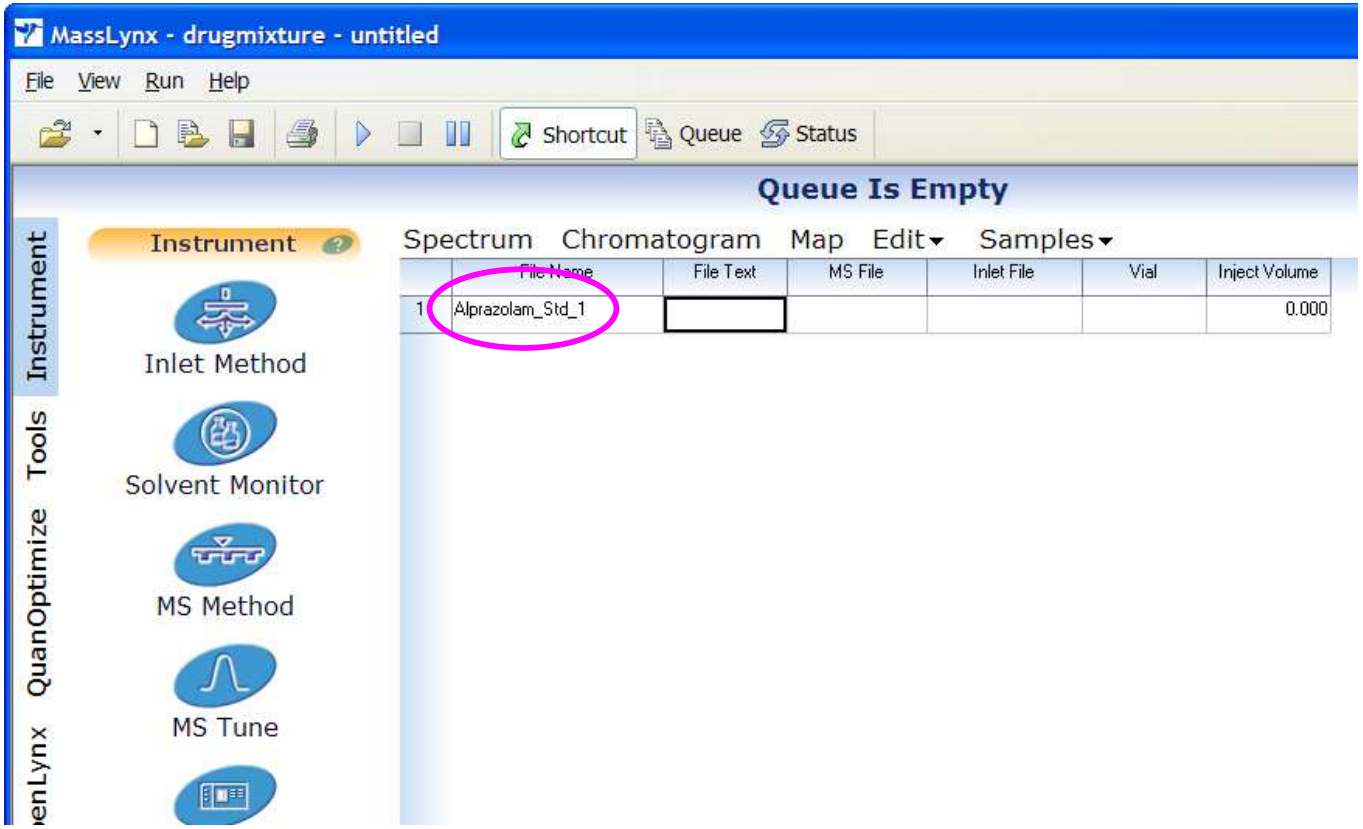
Changing Column Properties



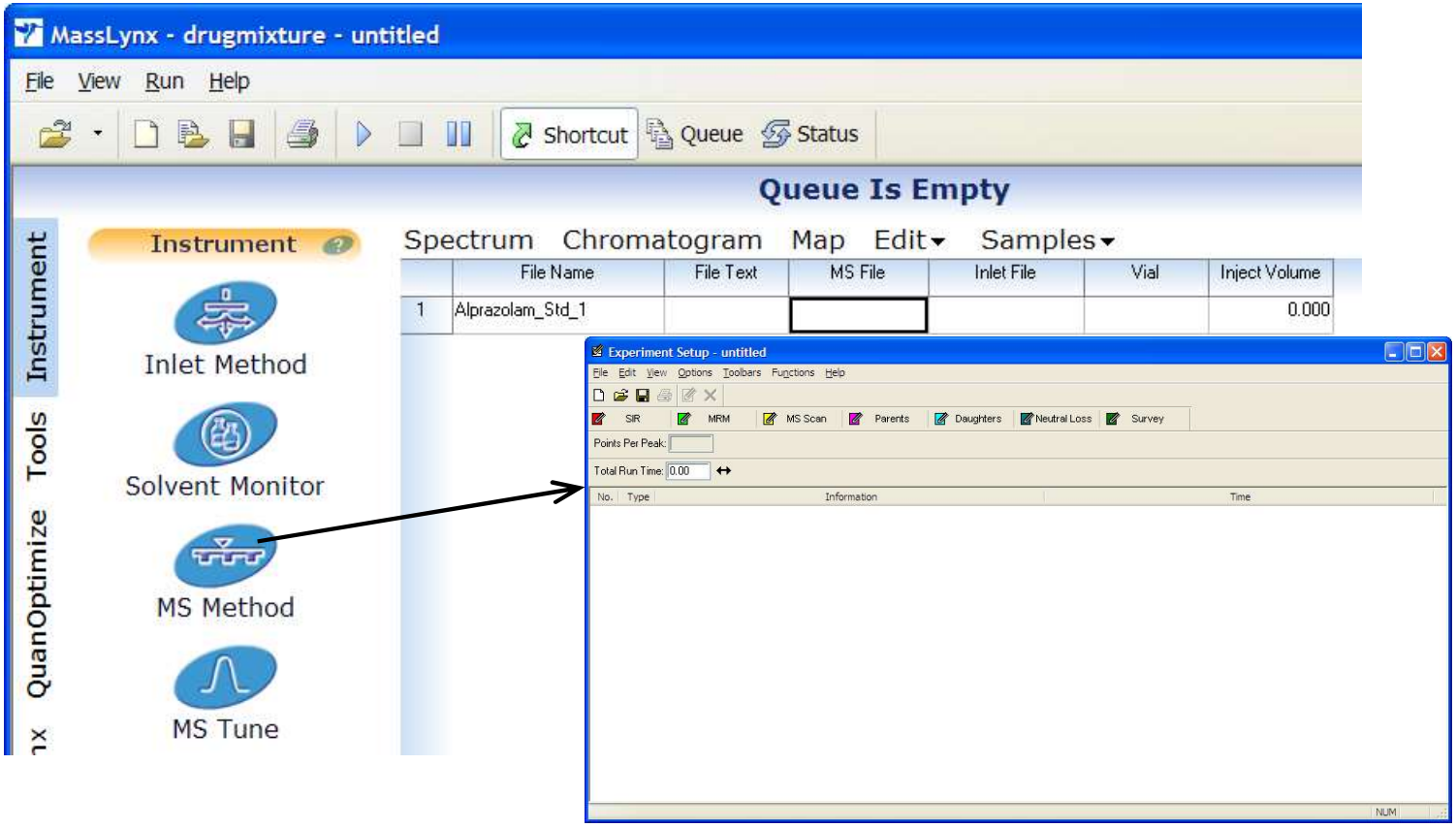
Saving the Sample List Format



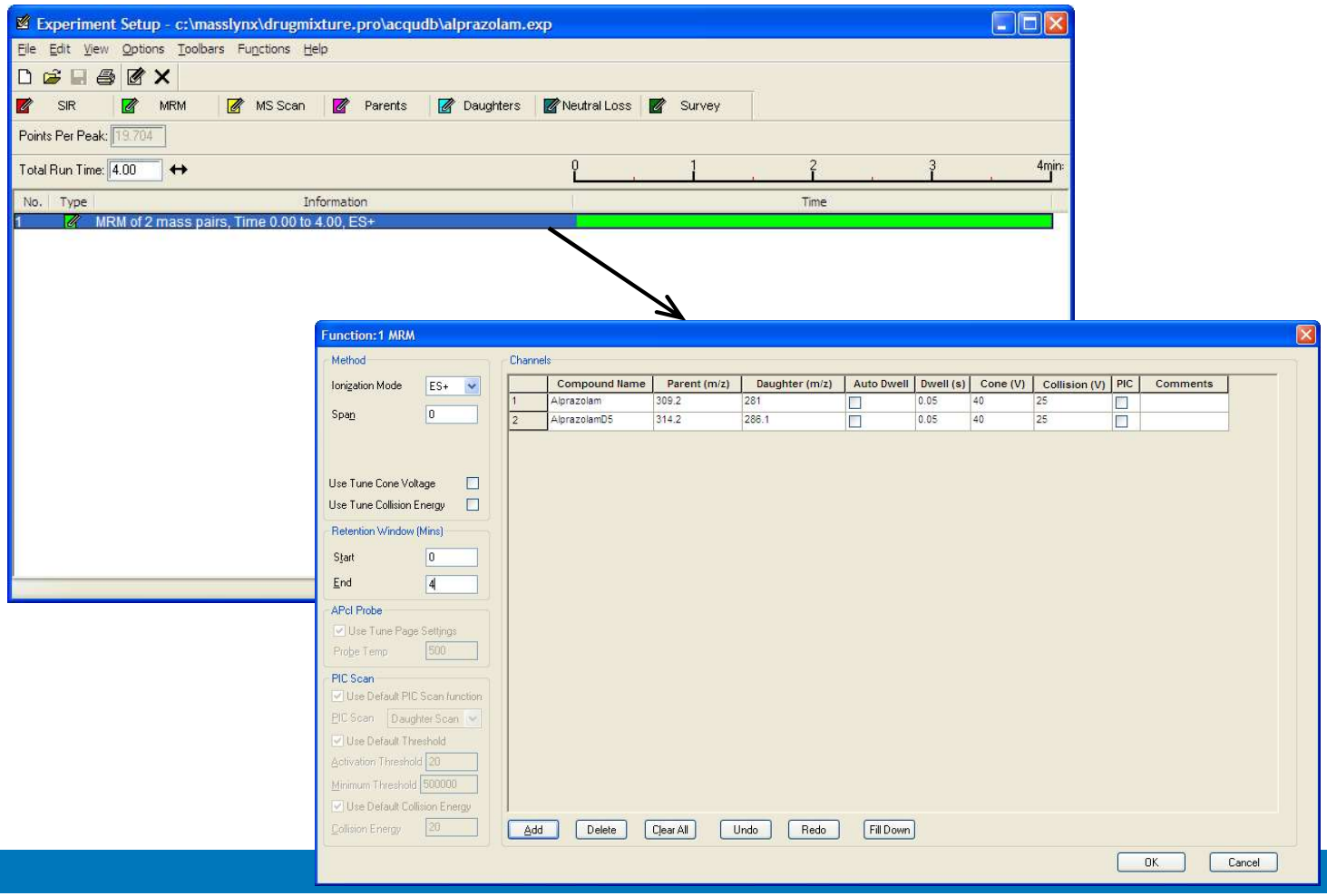
Enter File Name



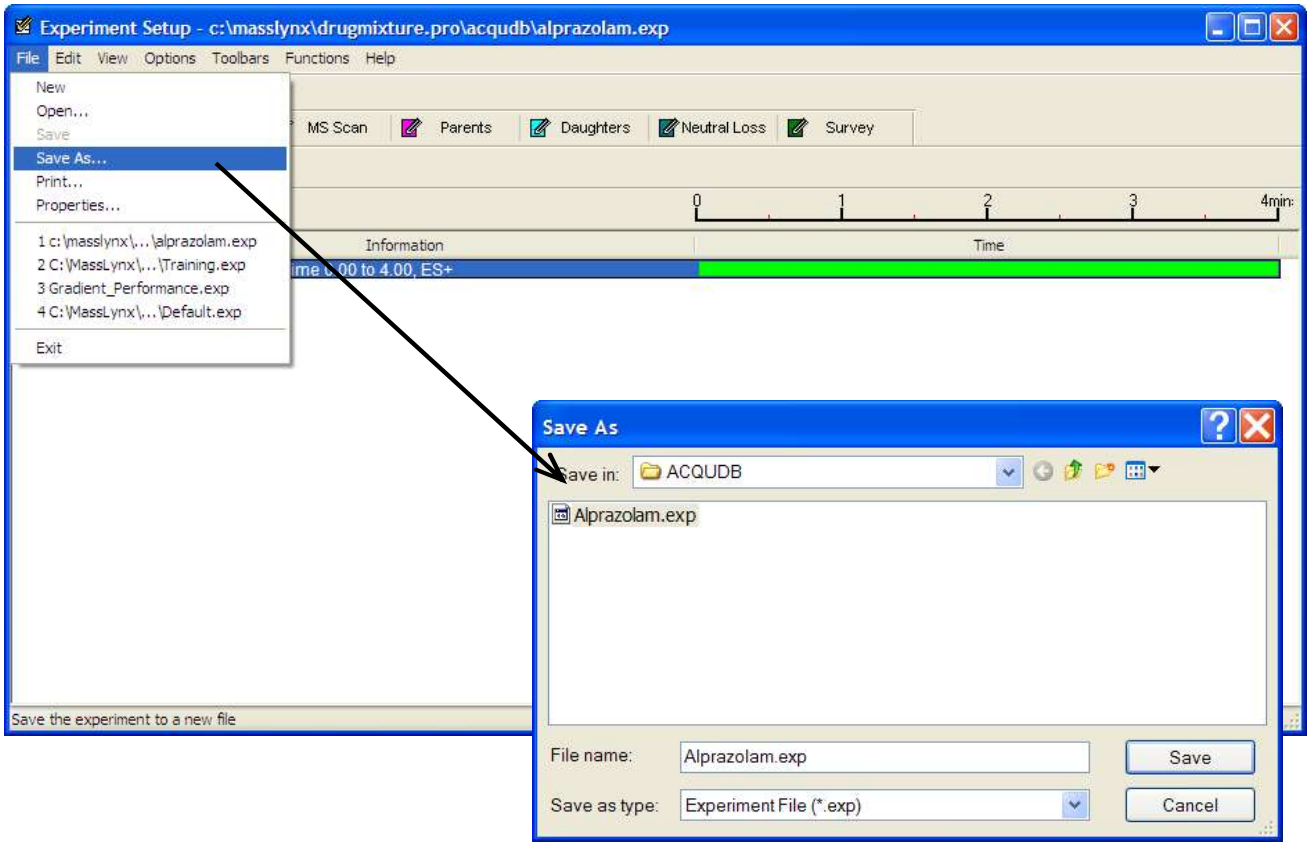
Create an MS Method



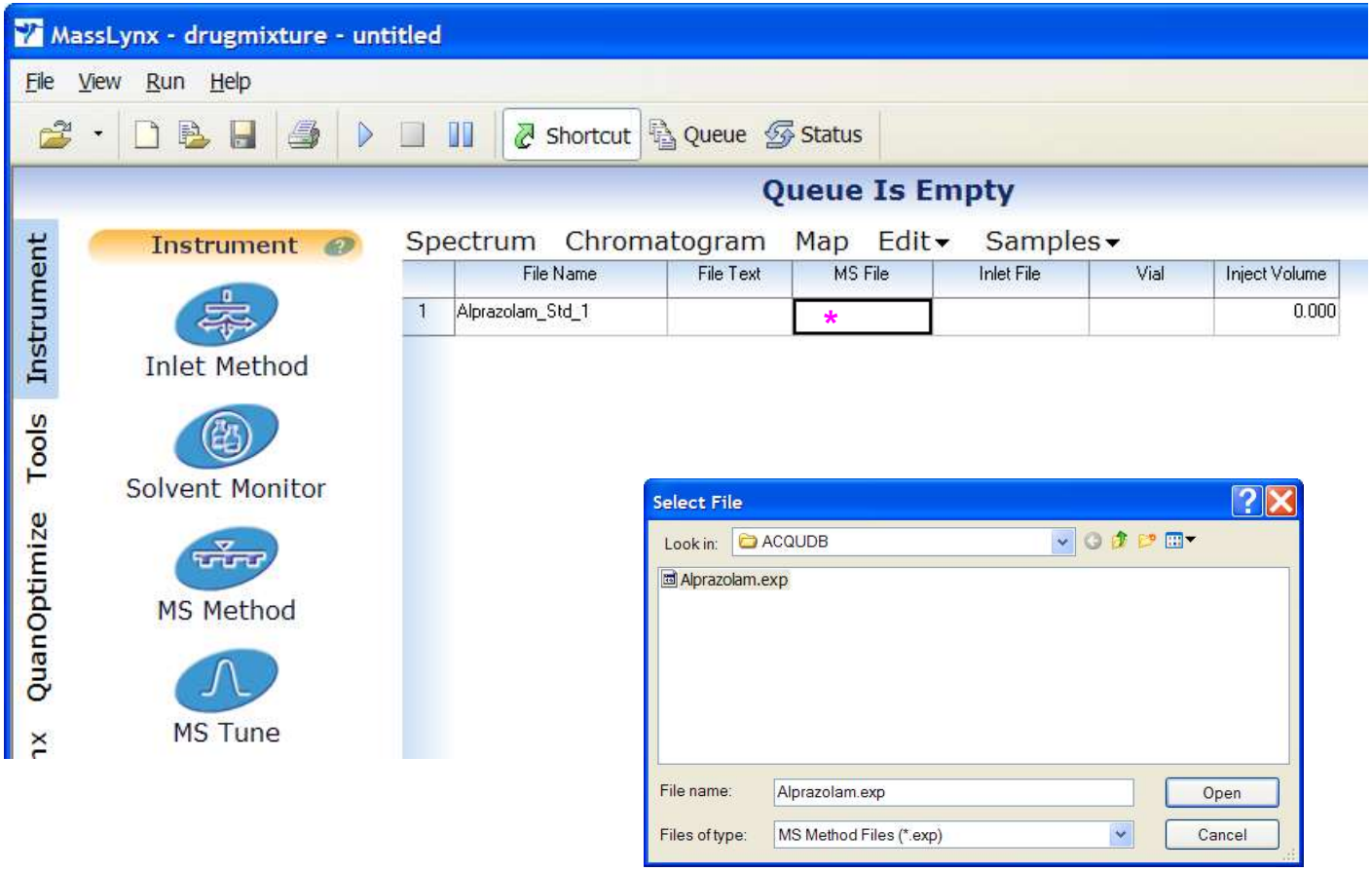
Editing the MS Method



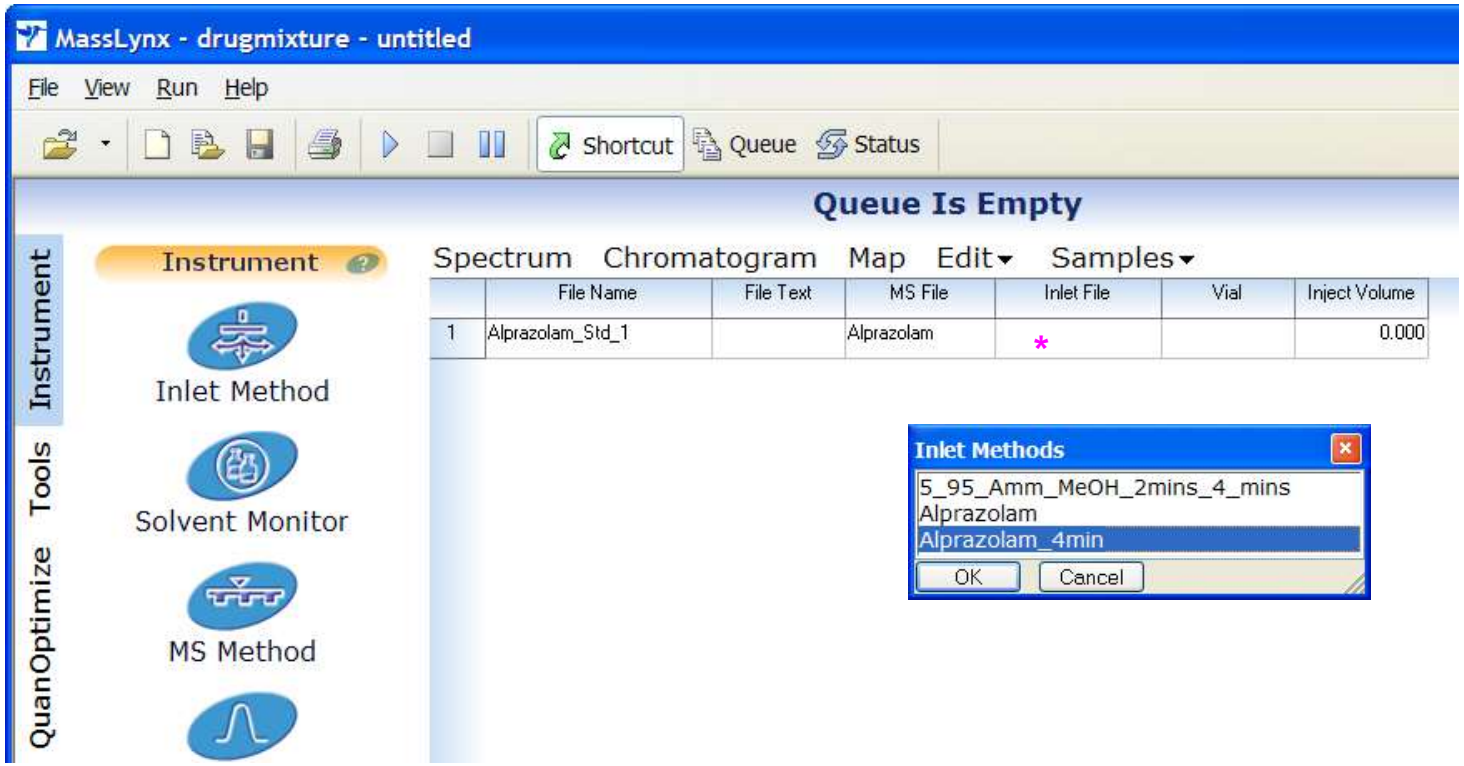
Saving the MS Method



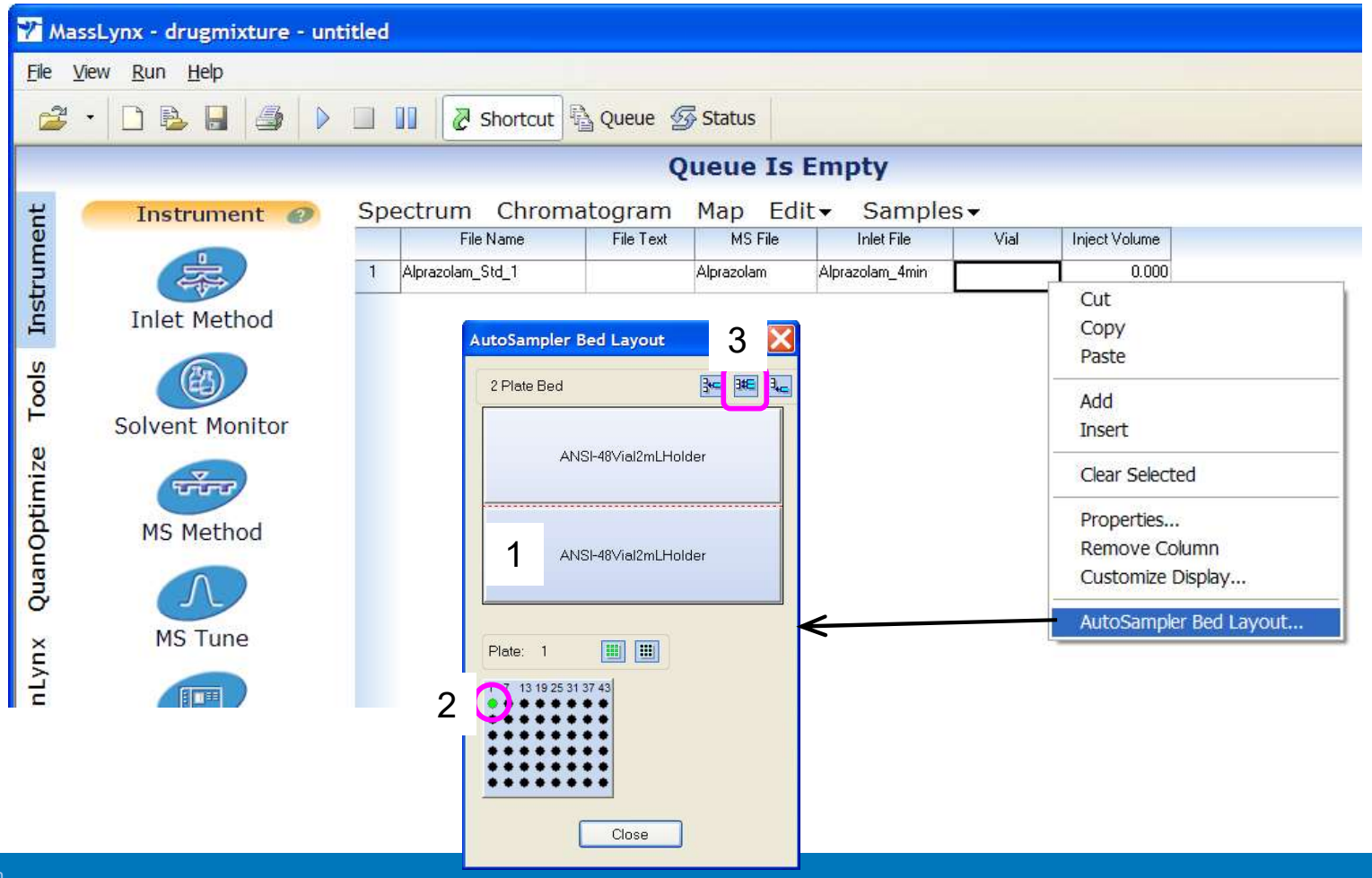
Select MS Method



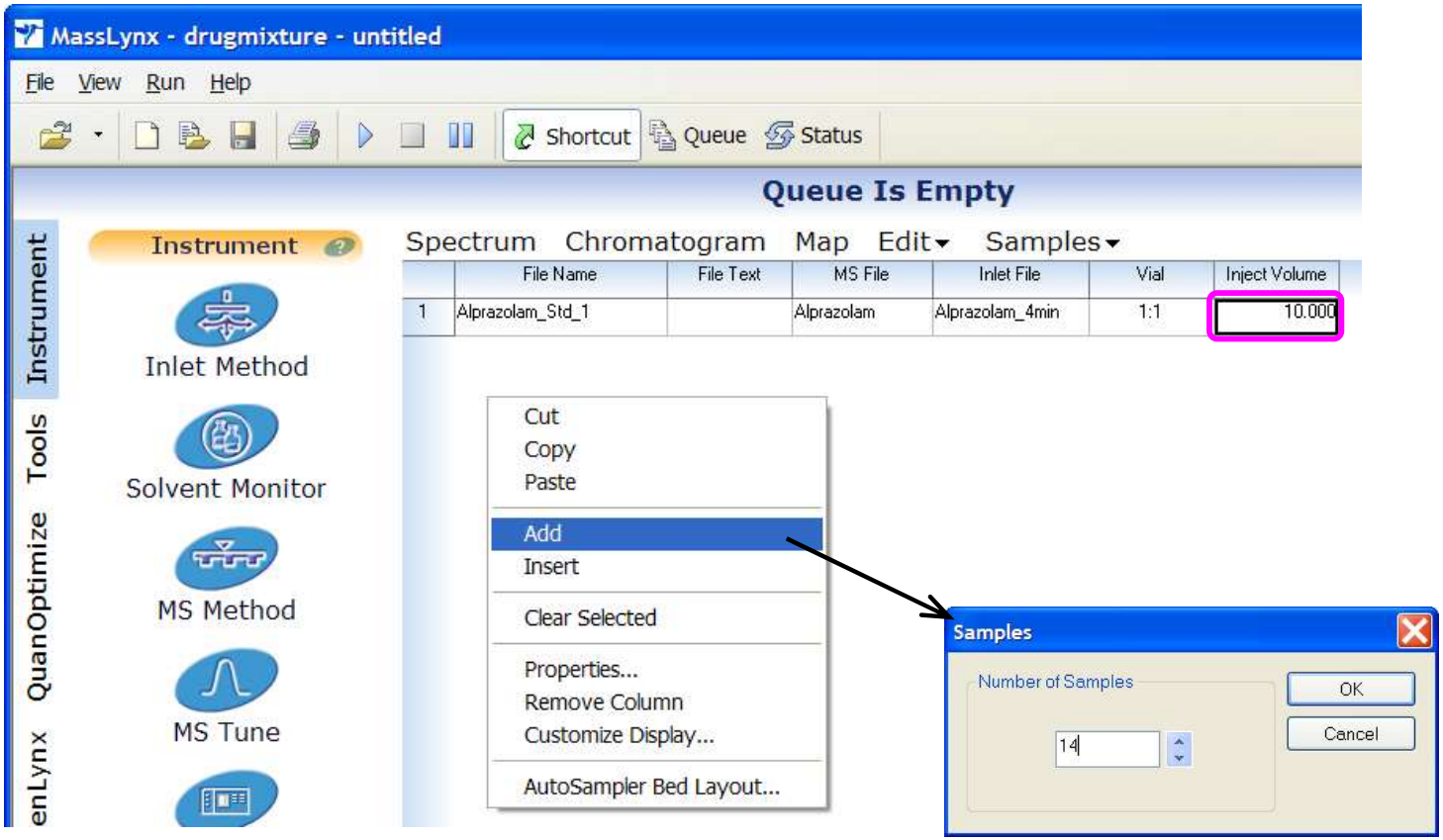
Selecting the ACQUITY Method



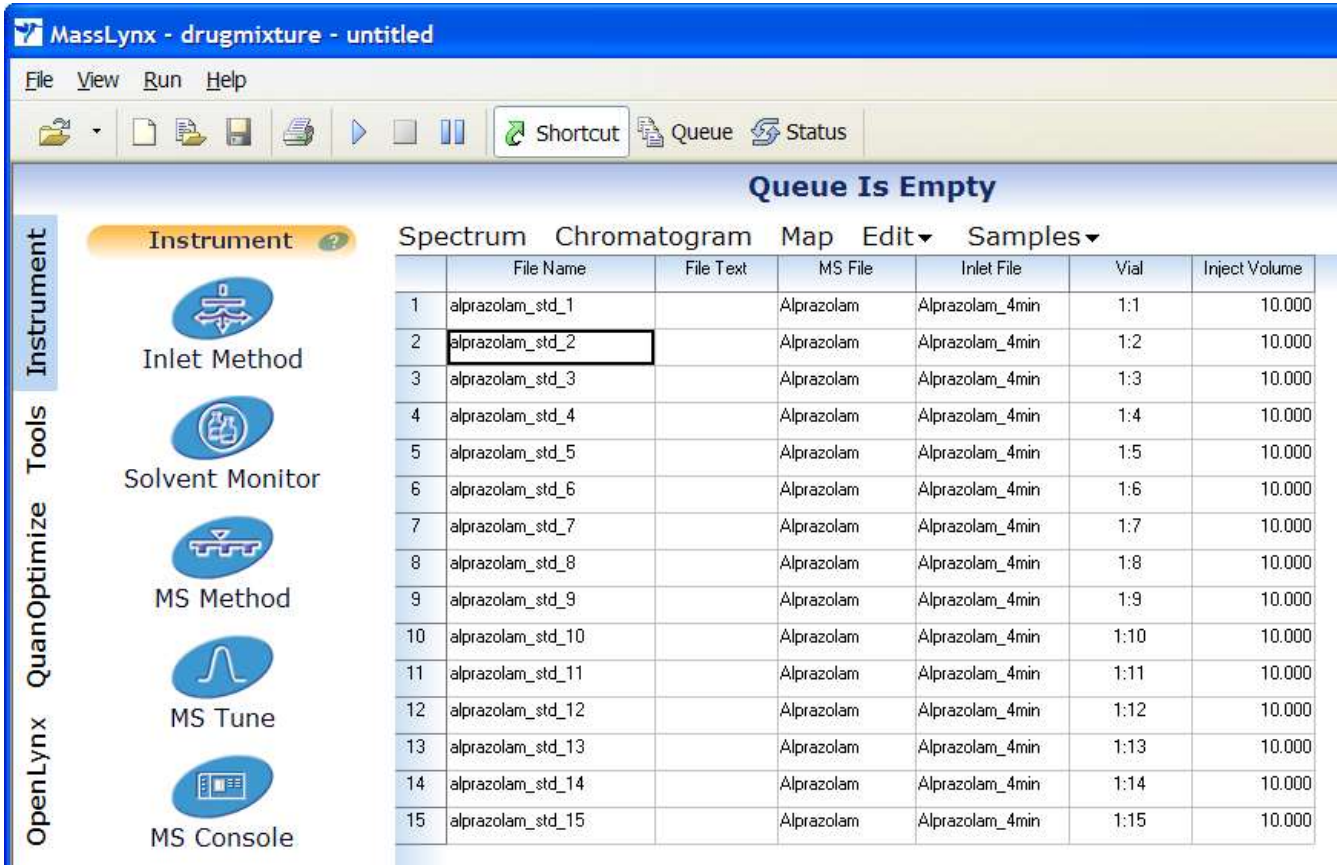
Sample Location



Adding Samples to the Sample List

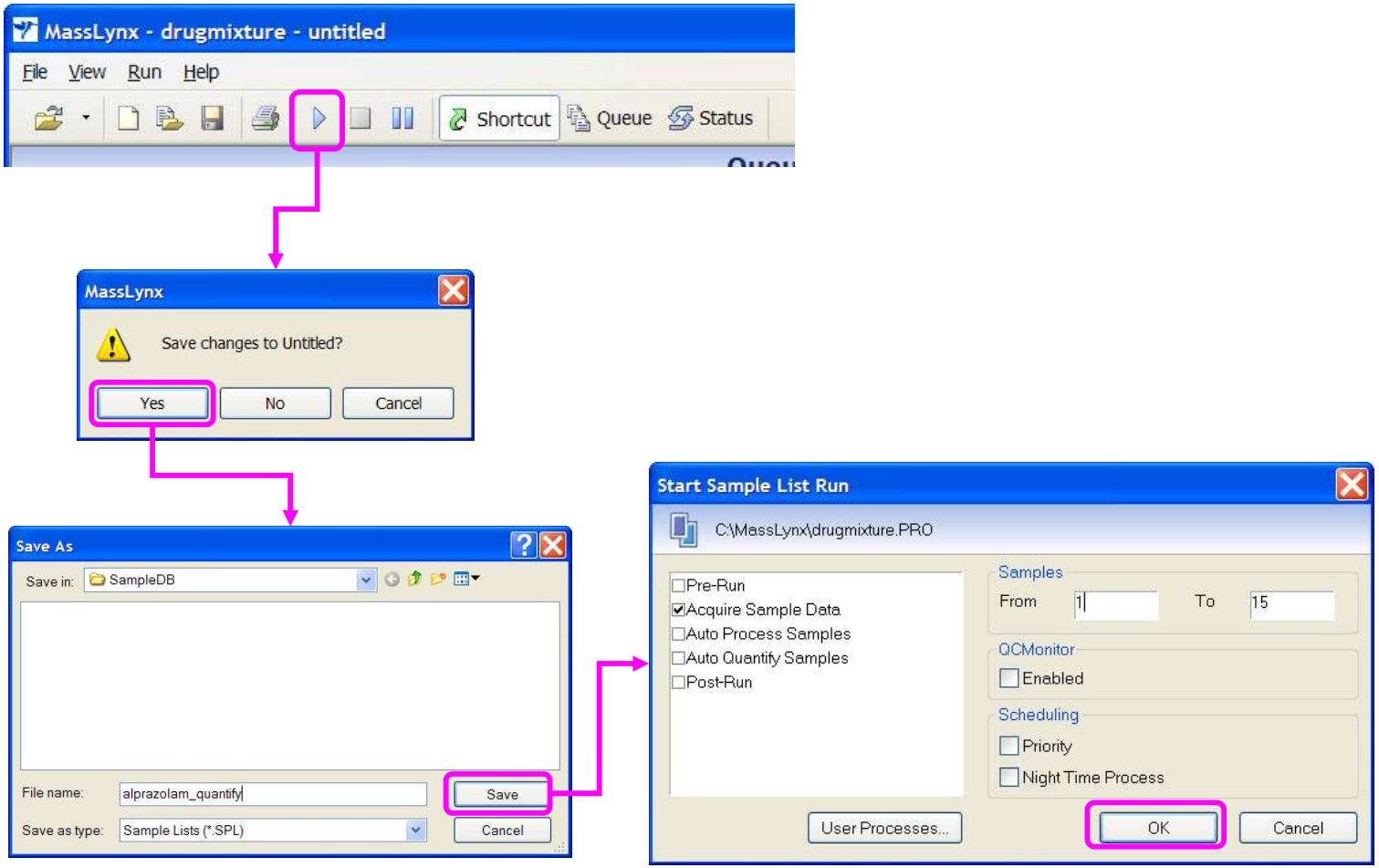


Complete Sample List

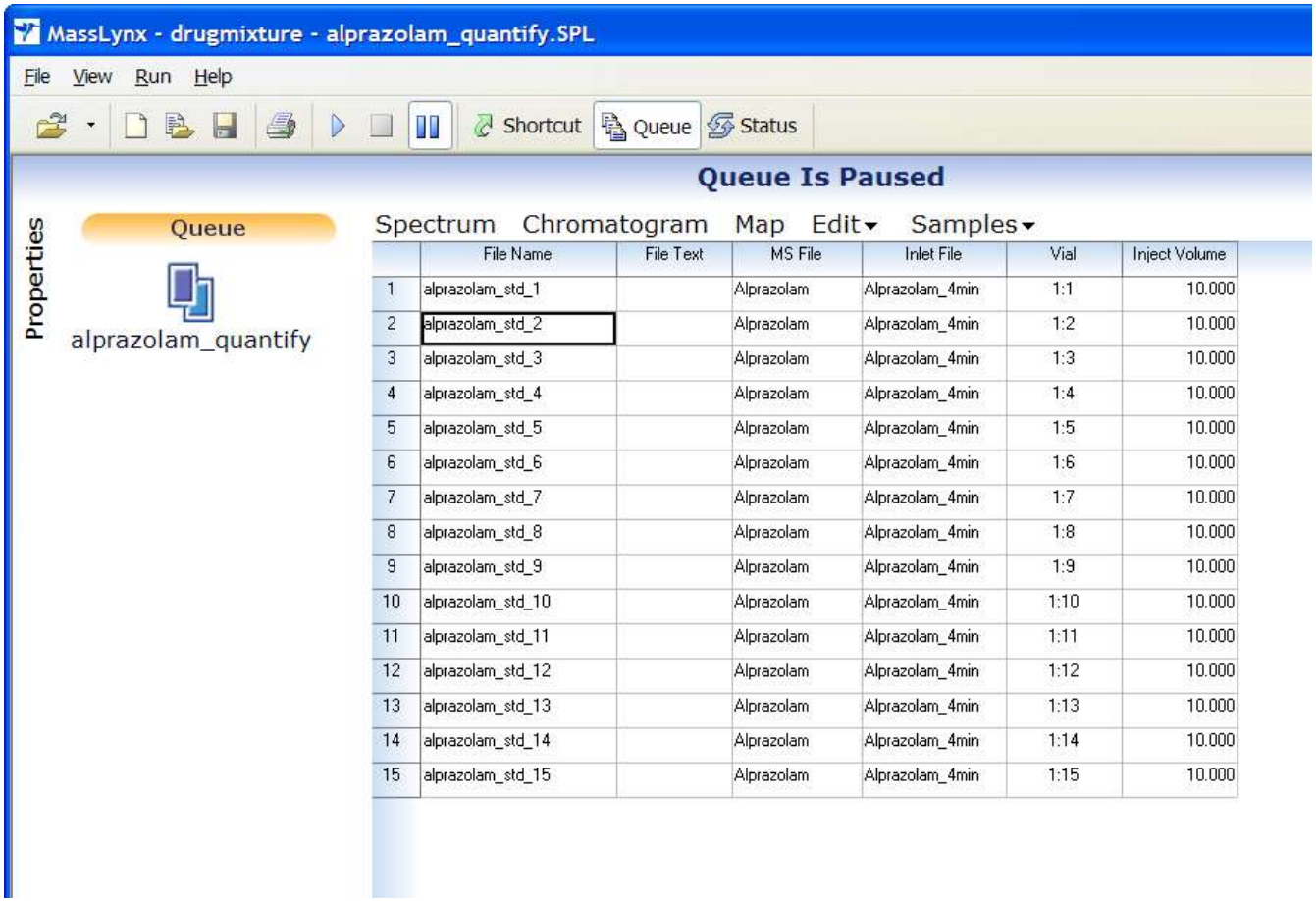


	File Name	File Text	MS File	Inlet File	Vial	Inject Volume
1	alprazolam_std_1		Alprazolam	Alprazolam_4min	1:1	10.000
2	alprazolam_std_2		Alprazolam	Alprazolam_4min	1:2	10.000
3	alprazolam_std_3		Alprazolam	Alprazolam_4min	1:3	10.000
4	alprazolam_std_4		Alprazolam	Alprazolam_4min	1:4	10.000
5	alprazolam_std_5		Alprazolam	Alprazolam_4min	1:5	10.000
6	alprazolam_std_6		Alprazolam	Alprazolam_4min	1:6	10.000
7	alprazolam_std_7		Alprazolam	Alprazolam_4min	1:7	10.000
8	alprazolam_std_8		Alprazolam	Alprazolam_4min	1:8	10.000
9	alprazolam_std_9		Alprazolam	Alprazolam_4min	1:9	10.000
10	alprazolam_std_10		Alprazolam	Alprazolam_4min	1:10	10.000
11	alprazolam_std_11		Alprazolam	Alprazolam_4min	1:11	10.000
12	alprazolam_std_12		Alprazolam	Alprazolam_4min	1:12	10.000
13	alprazolam_std_13		Alprazolam	Alprazolam_4min	1:13	10.000
14	alprazolam_std_14		Alprazolam	Alprazolam_4min	1:14	10.000
15	alprazolam_std_15		Alprazolam	Alprazolam_4min	1:15	10.000

Starting the Sample List

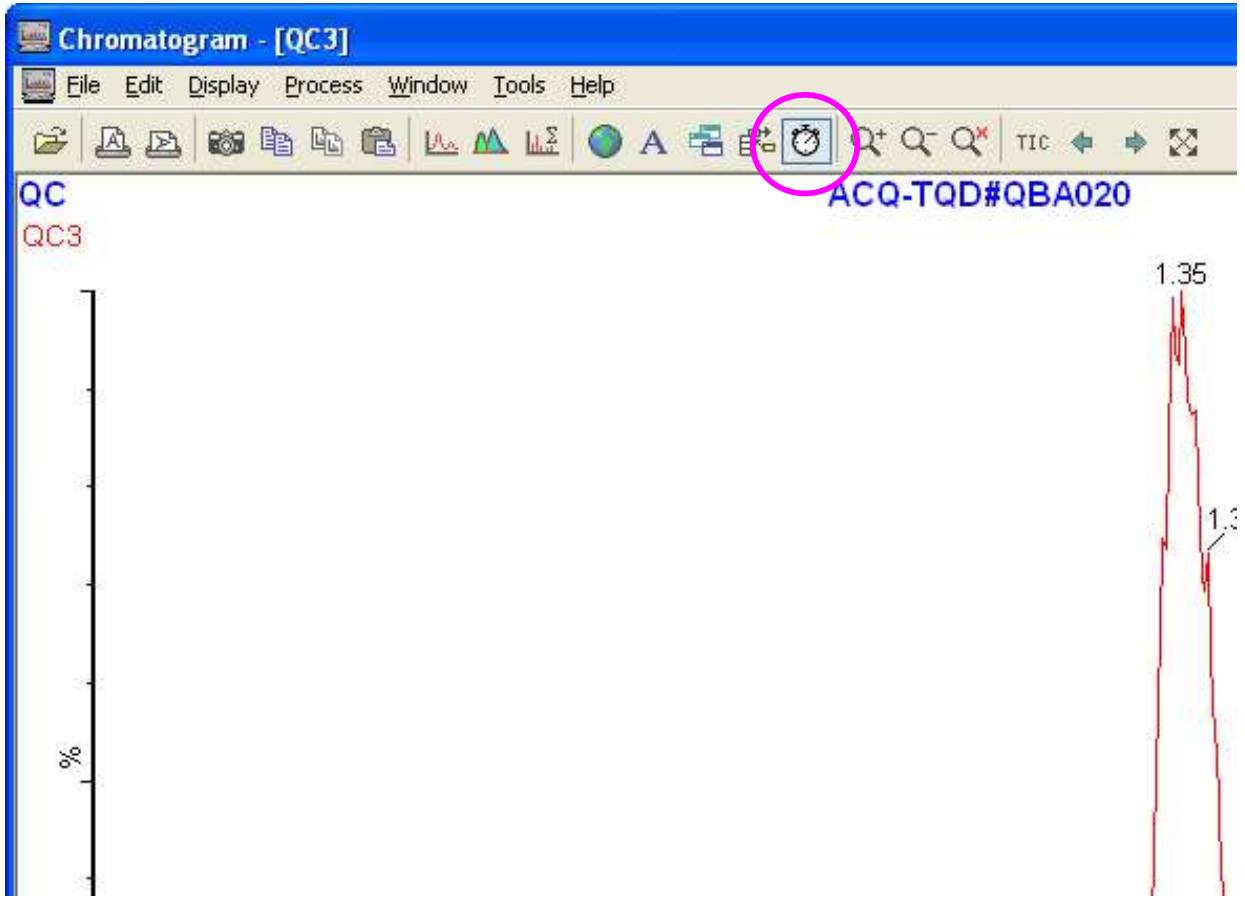


Sample List in Progress

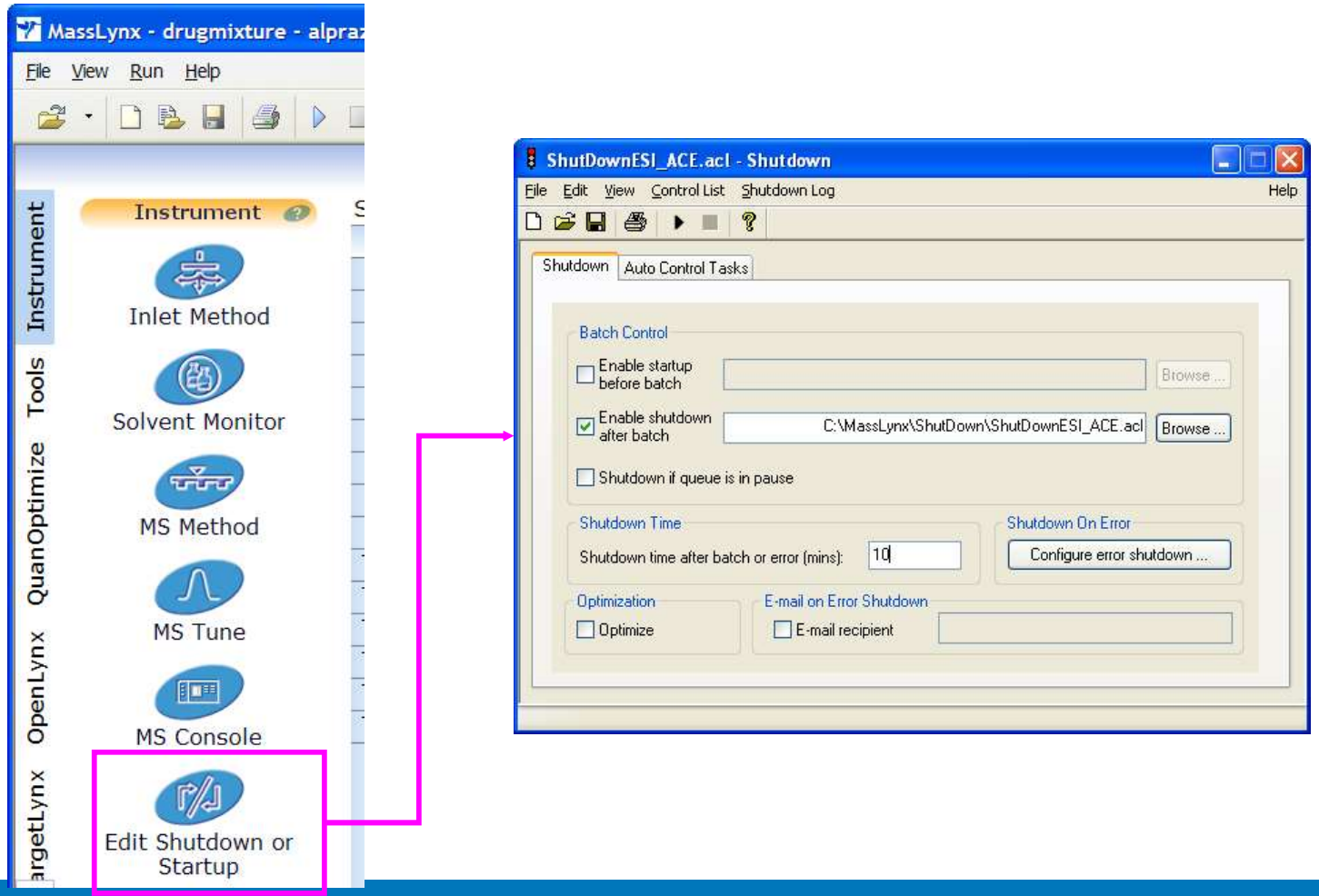


	File Name	File Text	MS File	Inlet File	Vial	Inject Volume
1	alprazolam_std_1		Alprazolam	Alprazolam_4min	1:1	10.000
2	alprazolam_std_2		Alprazolam	Alprazolam_4min	1:2	10.000
3	alprazolam_std_3		Alprazolam	Alprazolam_4min	1:3	10.000
4	alprazolam_std_4		Alprazolam	Alprazolam_4min	1:4	10.000
5	alprazolam_std_5		Alprazolam	Alprazolam_4min	1:5	10.000
6	alprazolam_std_6		Alprazolam	Alprazolam_4min	1:6	10.000
7	alprazolam_std_7		Alprazolam	Alprazolam_4min	1:7	10.000
8	alprazolam_std_8		Alprazolam	Alprazolam_4min	1:8	10.000
9	alprazolam_std_9		Alprazolam	Alprazolam_4min	1:9	10.000
10	alprazolam_std_10		Alprazolam	Alprazolam_4min	1:10	10.000
11	alprazolam_std_11		Alprazolam	Alprazolam_4min	1:11	10.000
12	alprazolam_std_12		Alprazolam	Alprazolam_4min	1:12	10.000
13	alprazolam_std_13		Alprazolam	Alprazolam_4min	1:13	10.000
14	alprazolam_std_14		Alprazolam	Alprazolam_4min	1:14	10.000
15	alprazolam_std_15		Alprazolam	Alprazolam_4min	1:15	10.000

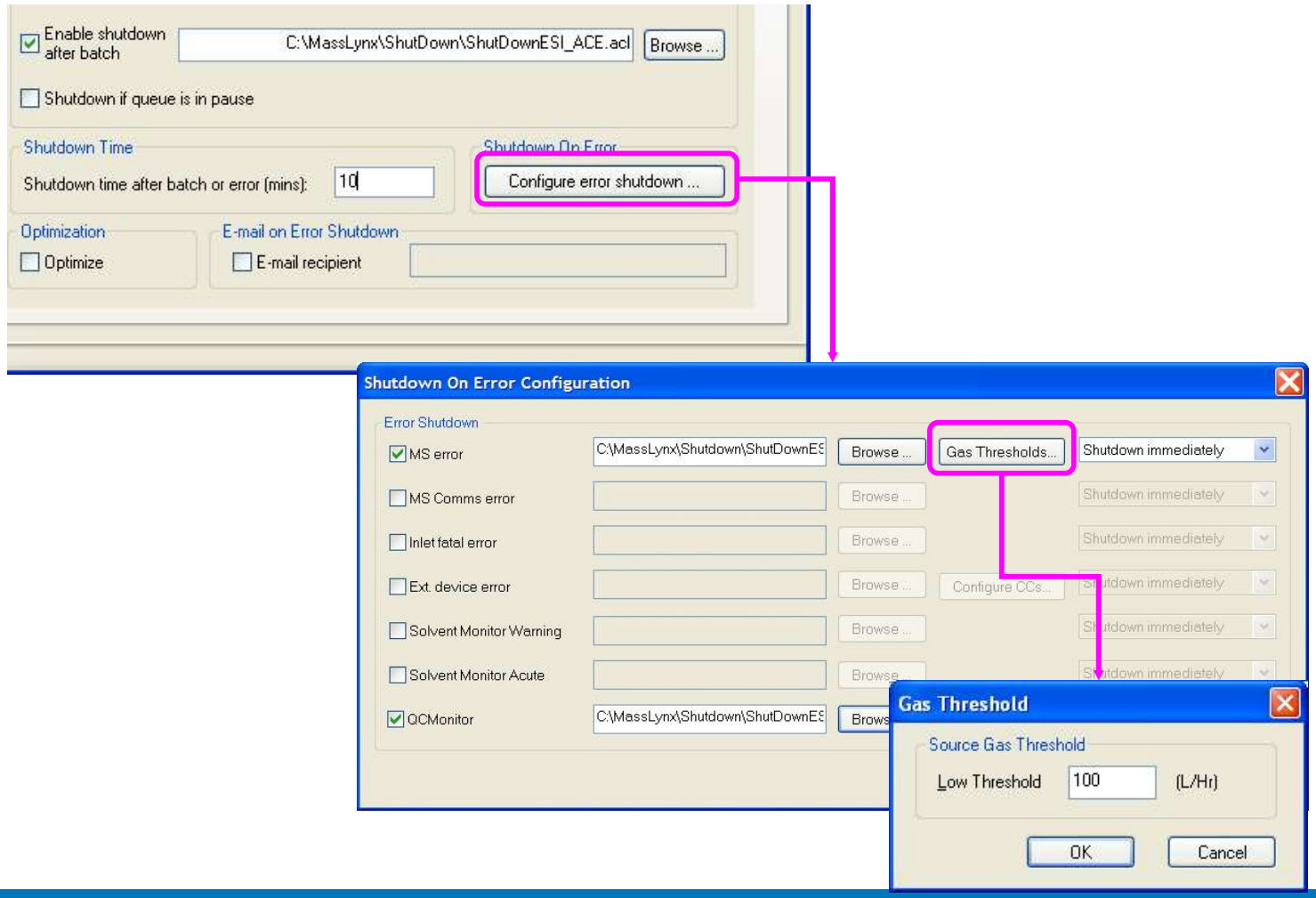
Real Time Data Viewing



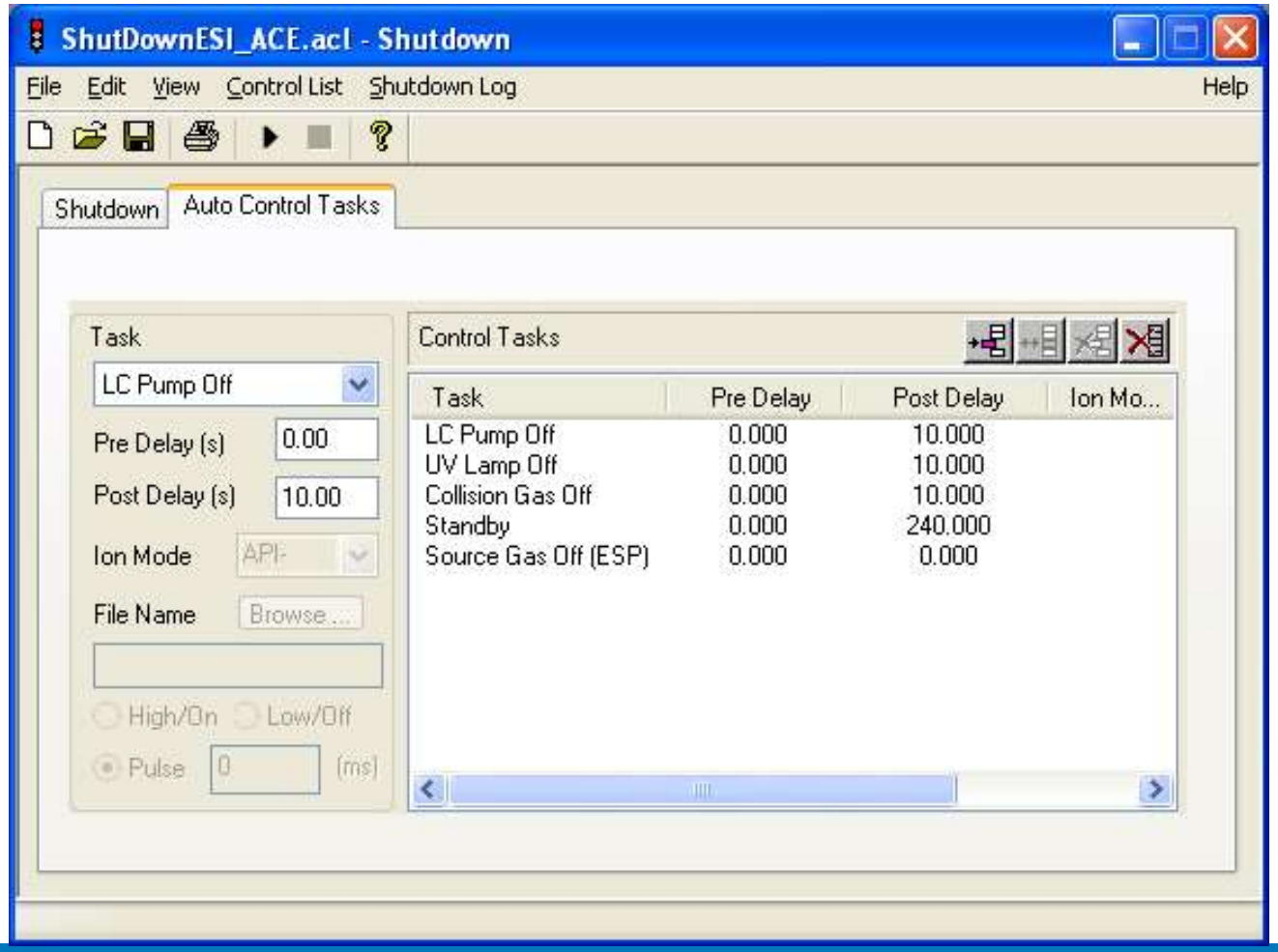
Automatic Shutdown



Automatic Shutdown



Shutdown Tasks



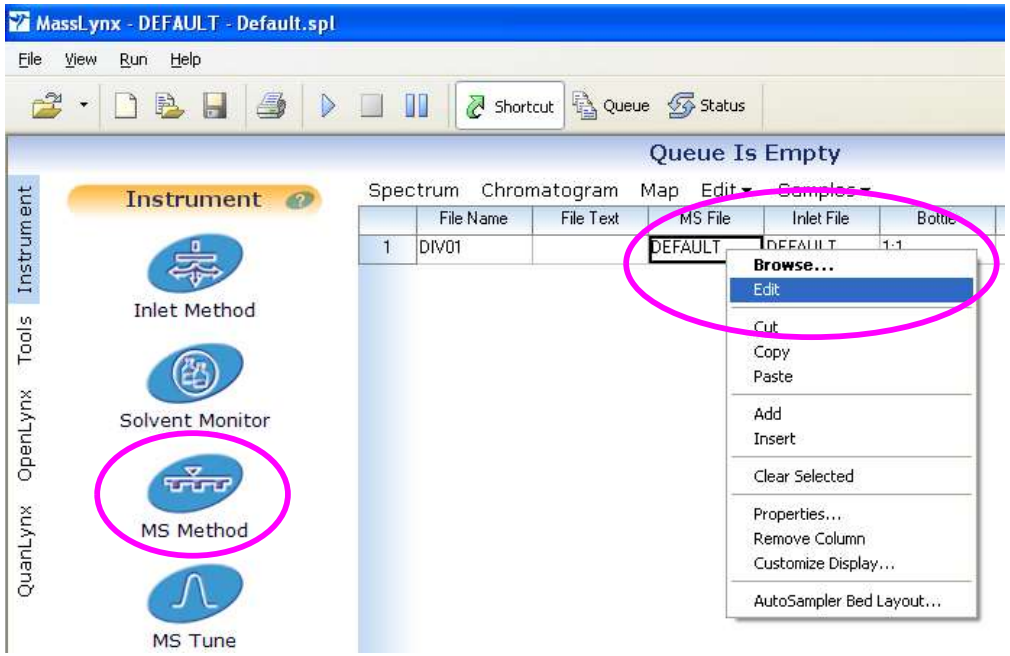
Chapter Five

Programming Instrument Methods

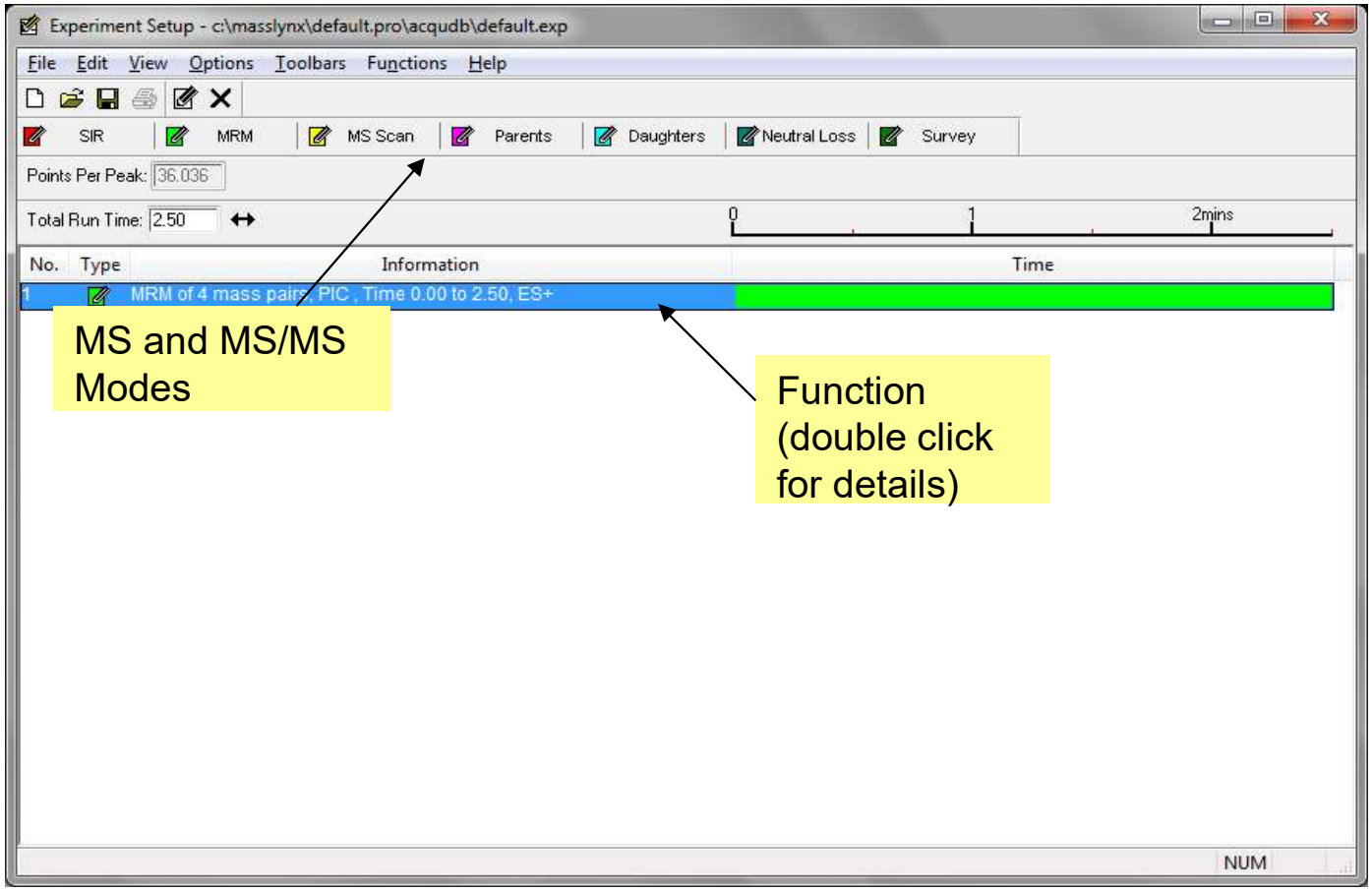
Programming Instrument Methods

- Scanning Methods
- Product (daughter) Ion Scan
- Precursor (parent) Ion Scan
- Multiple Reaction Monitoring
- Neutral Loss
- Survey Scan

Accessing MS Method Editor



MS Method Editor

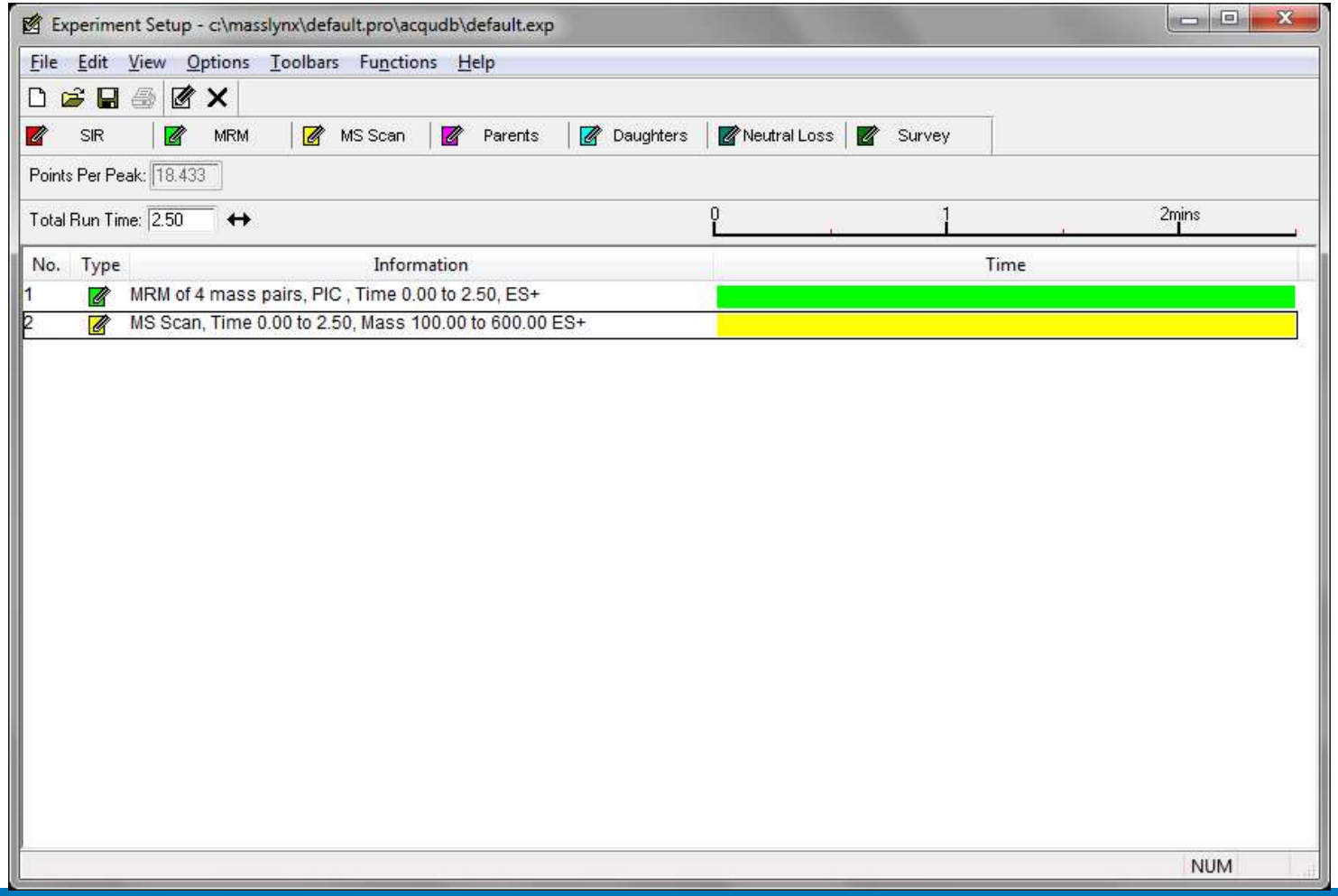


RADAR™

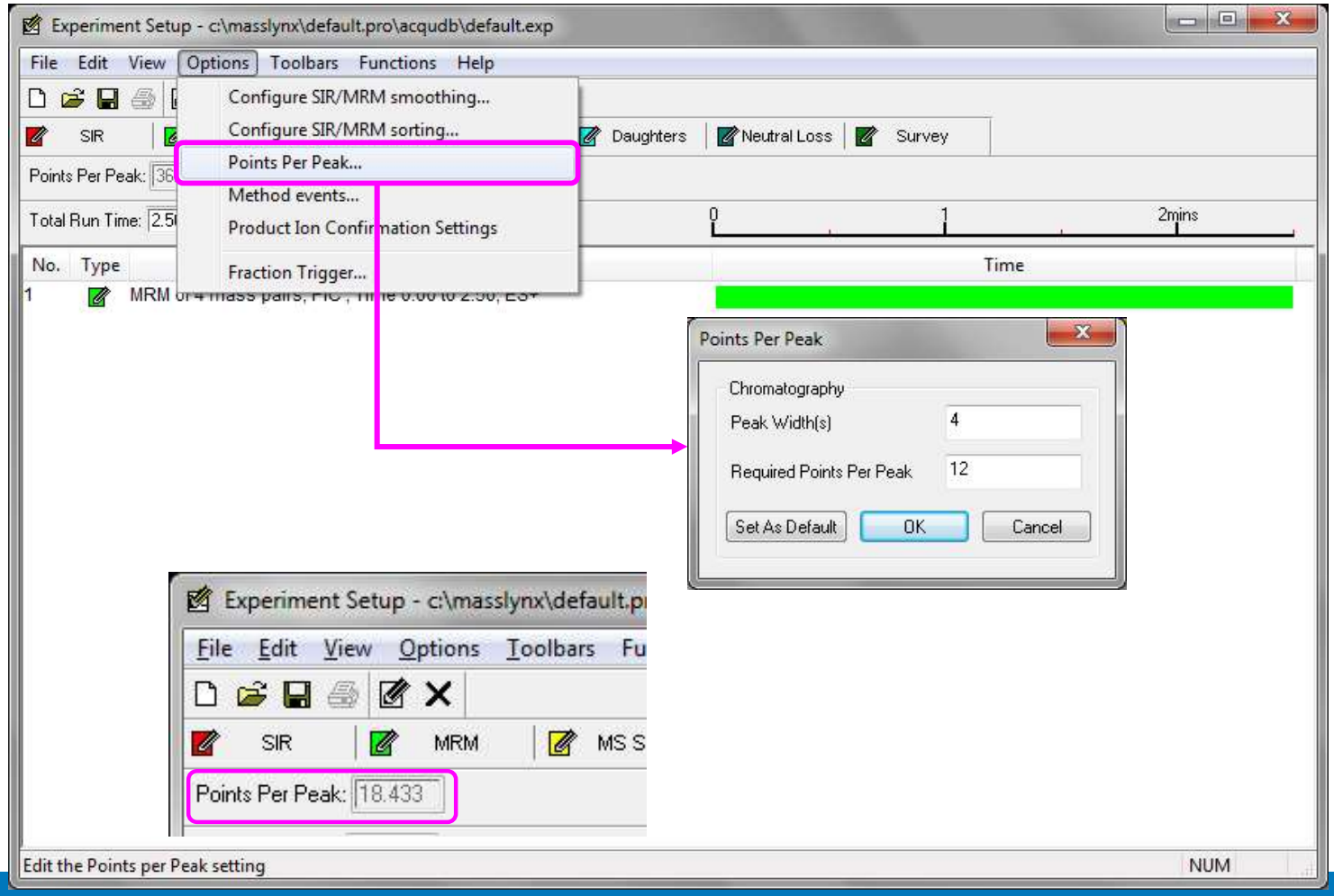
Waters™

- Collision cell is permanently filled with collision gas
- Allows MRM data to be collected in parallel to the collection of spectral MS data.
- No appreciable impact on sensitivity

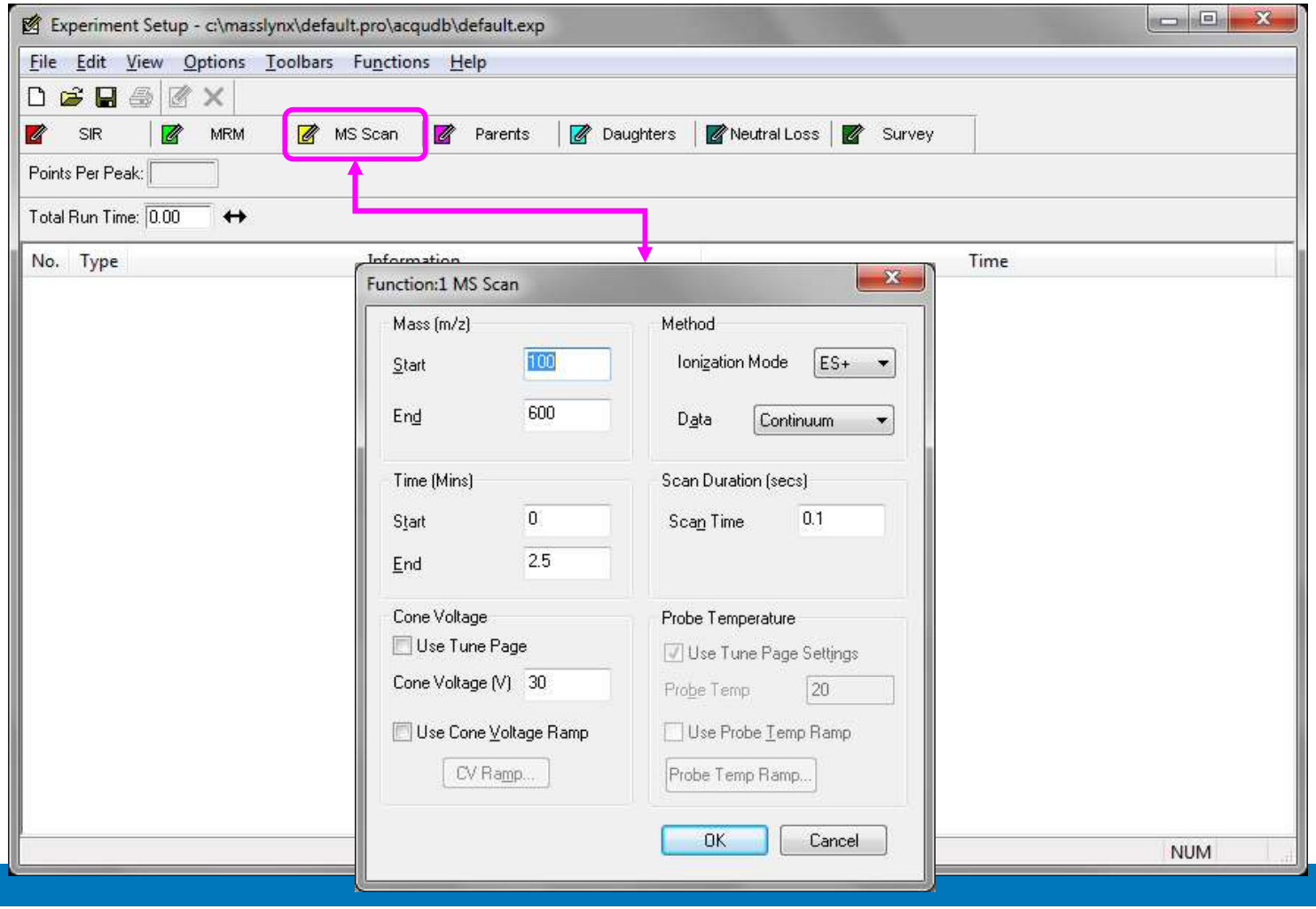
RADAR



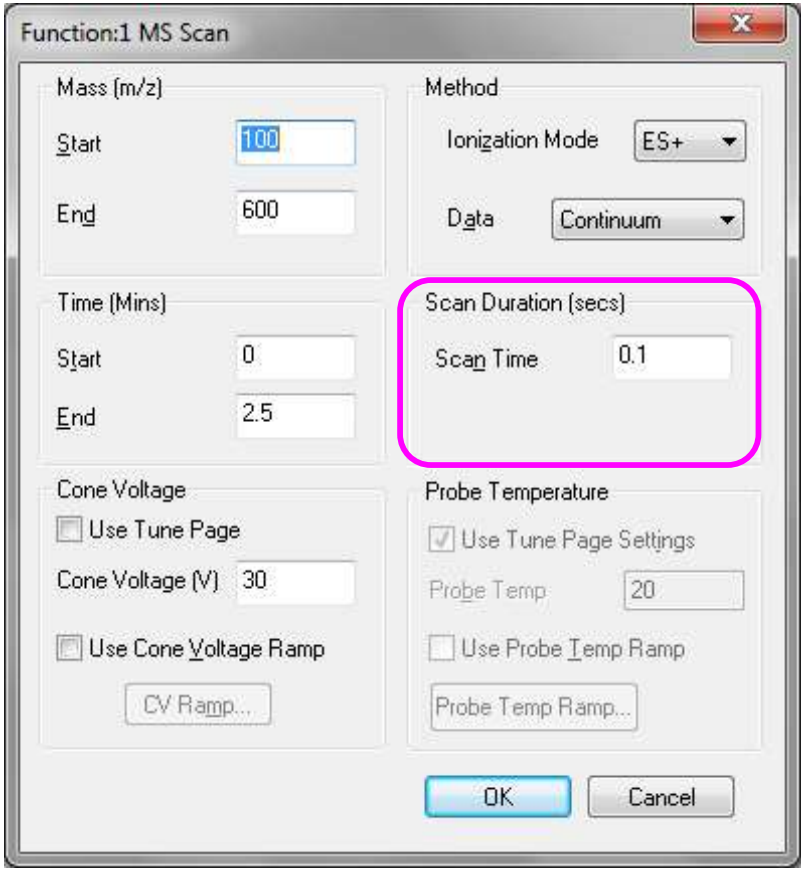
Data Acquisition – Points Per Peak



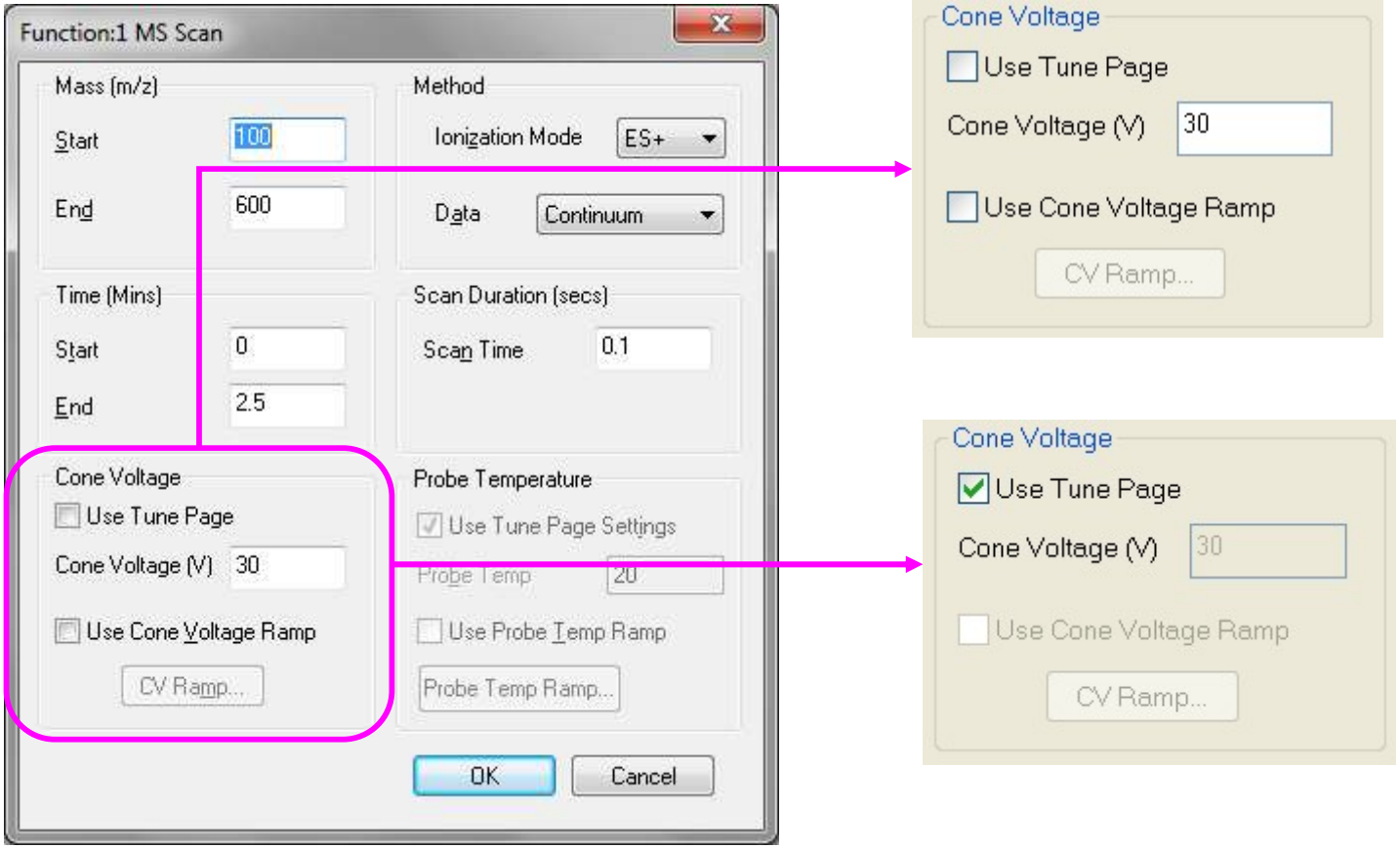
MS Method Editor - Full Scan Function



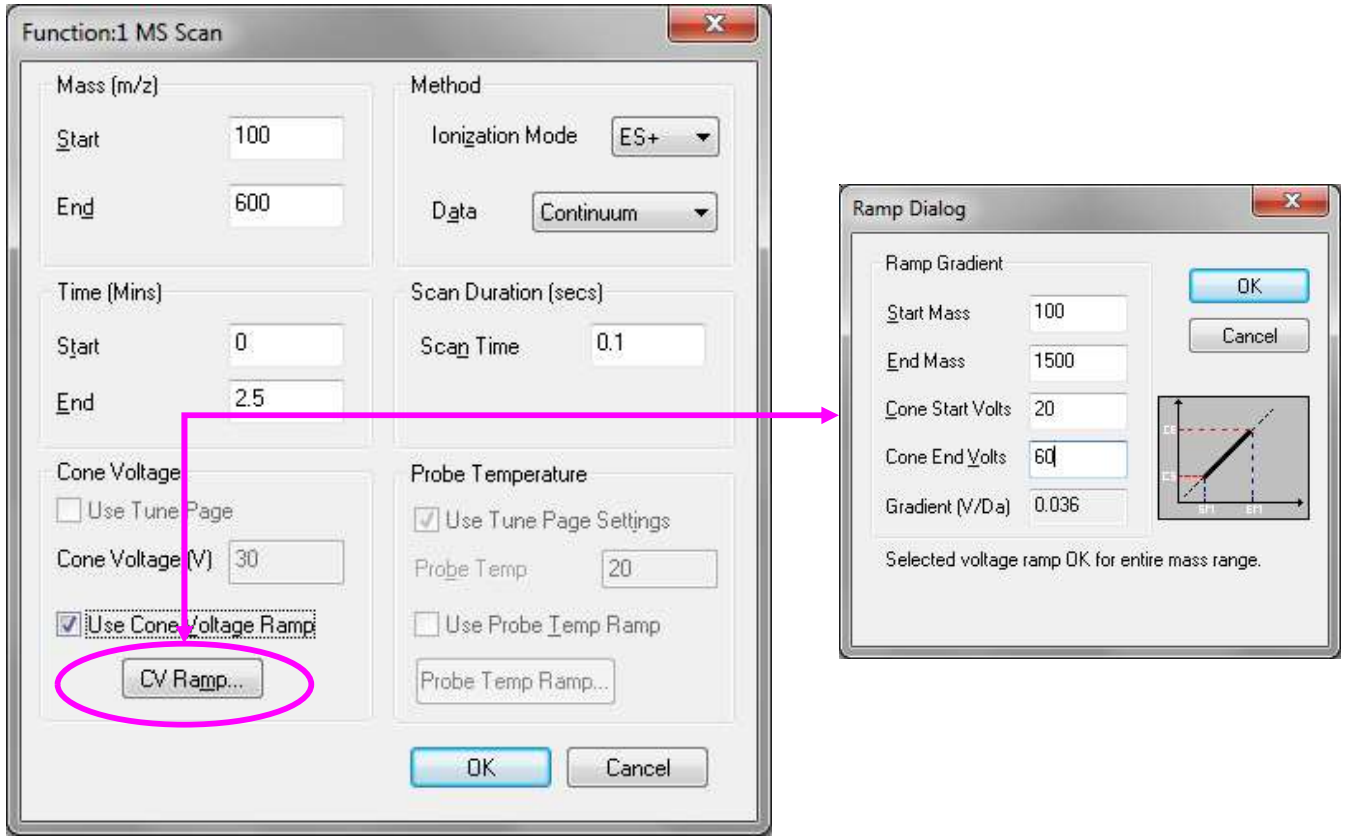
Setting the Scan Duration



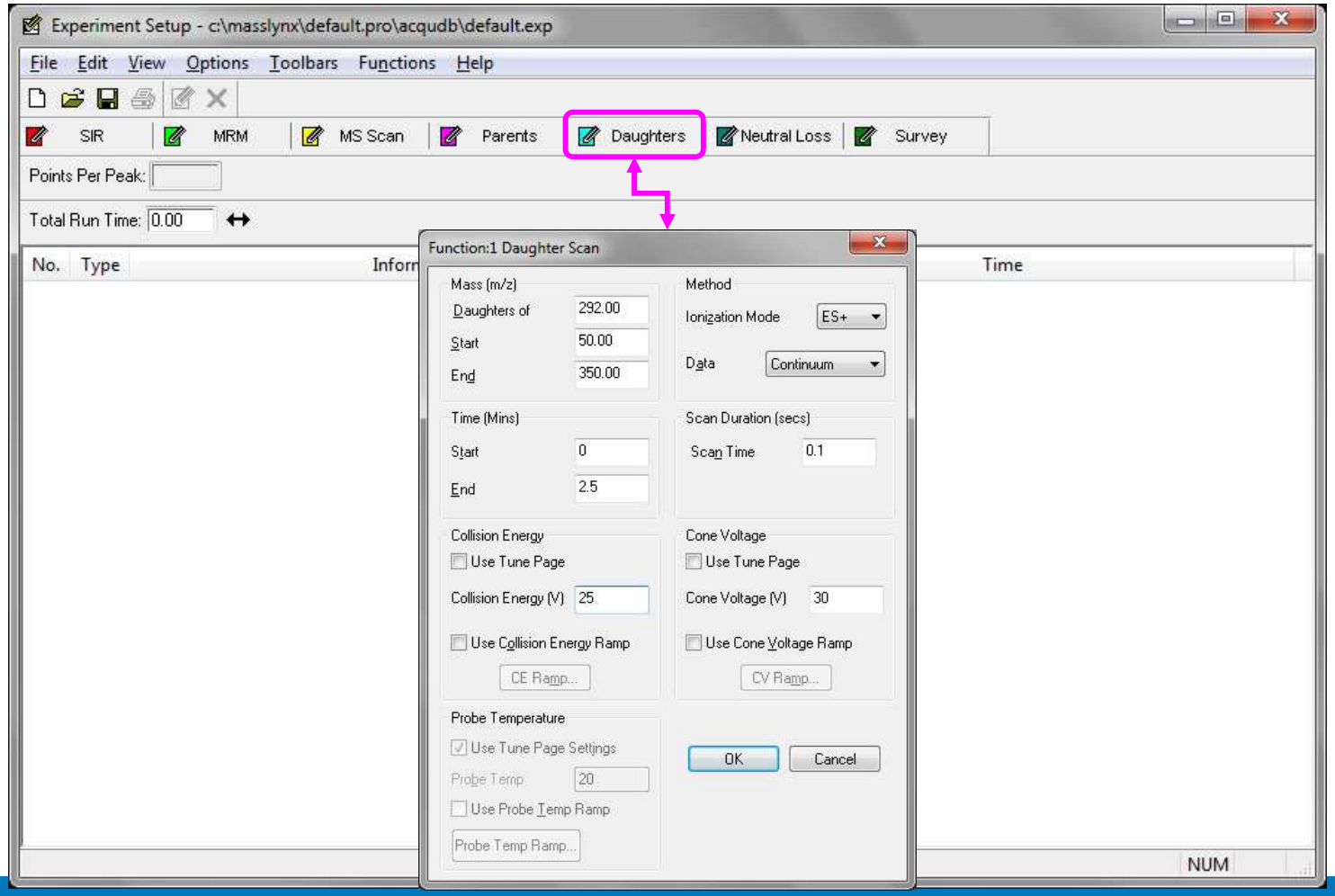
Setting the Cone Voltage



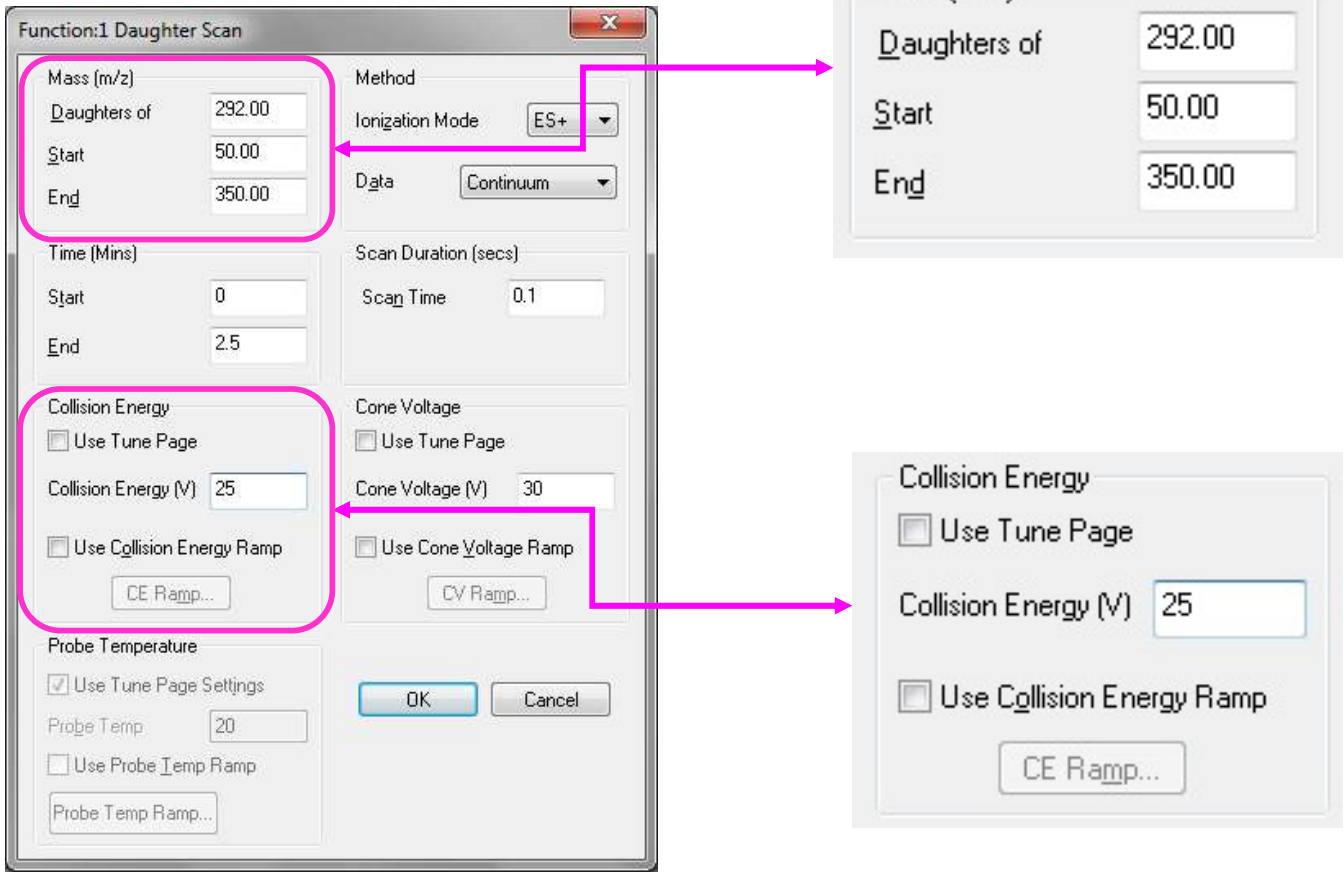
Setting the Cone Voltage Ramp



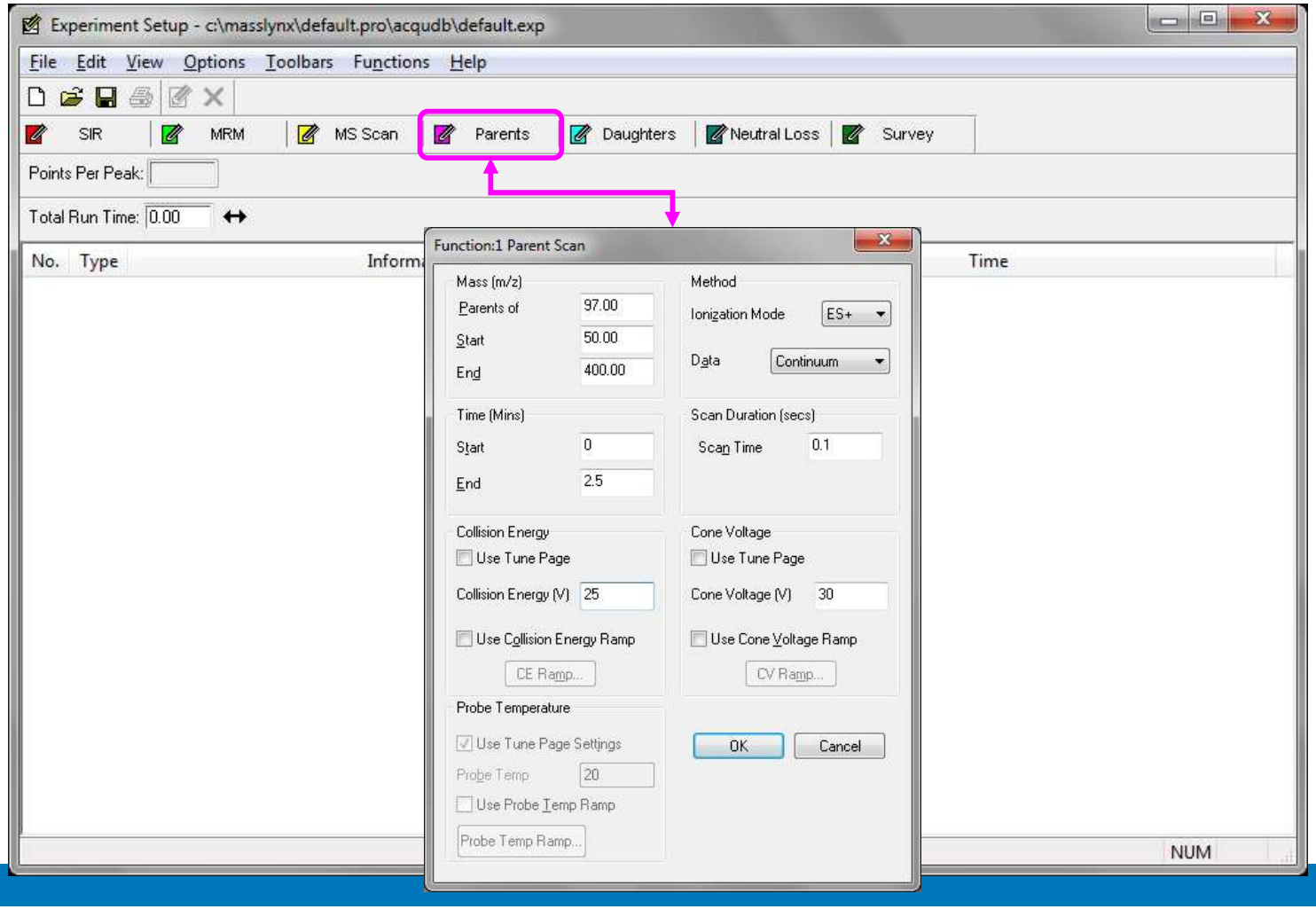
Product Ion Analysis (Daughter Scan)



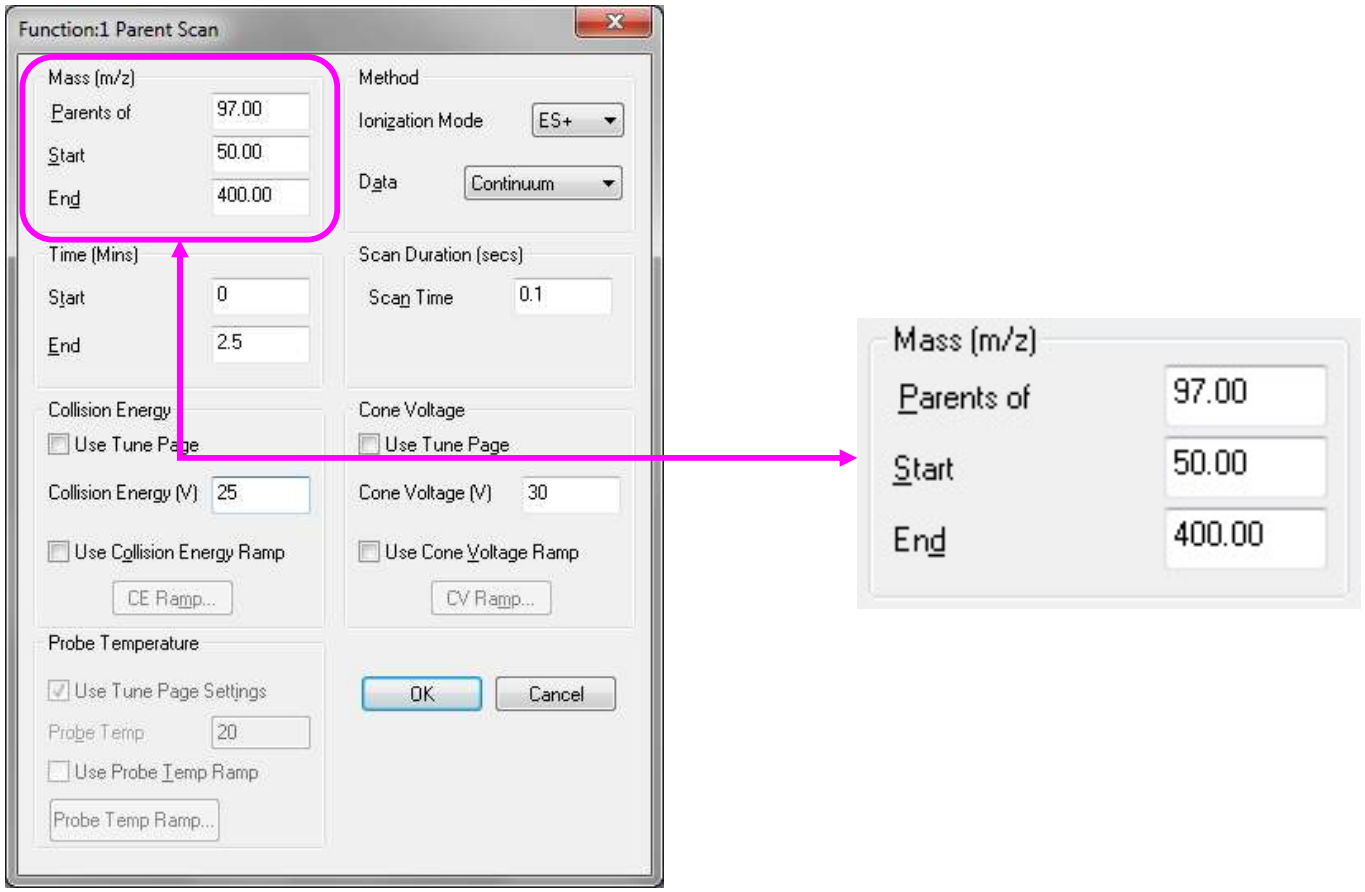
Mass Range and Collision Energy



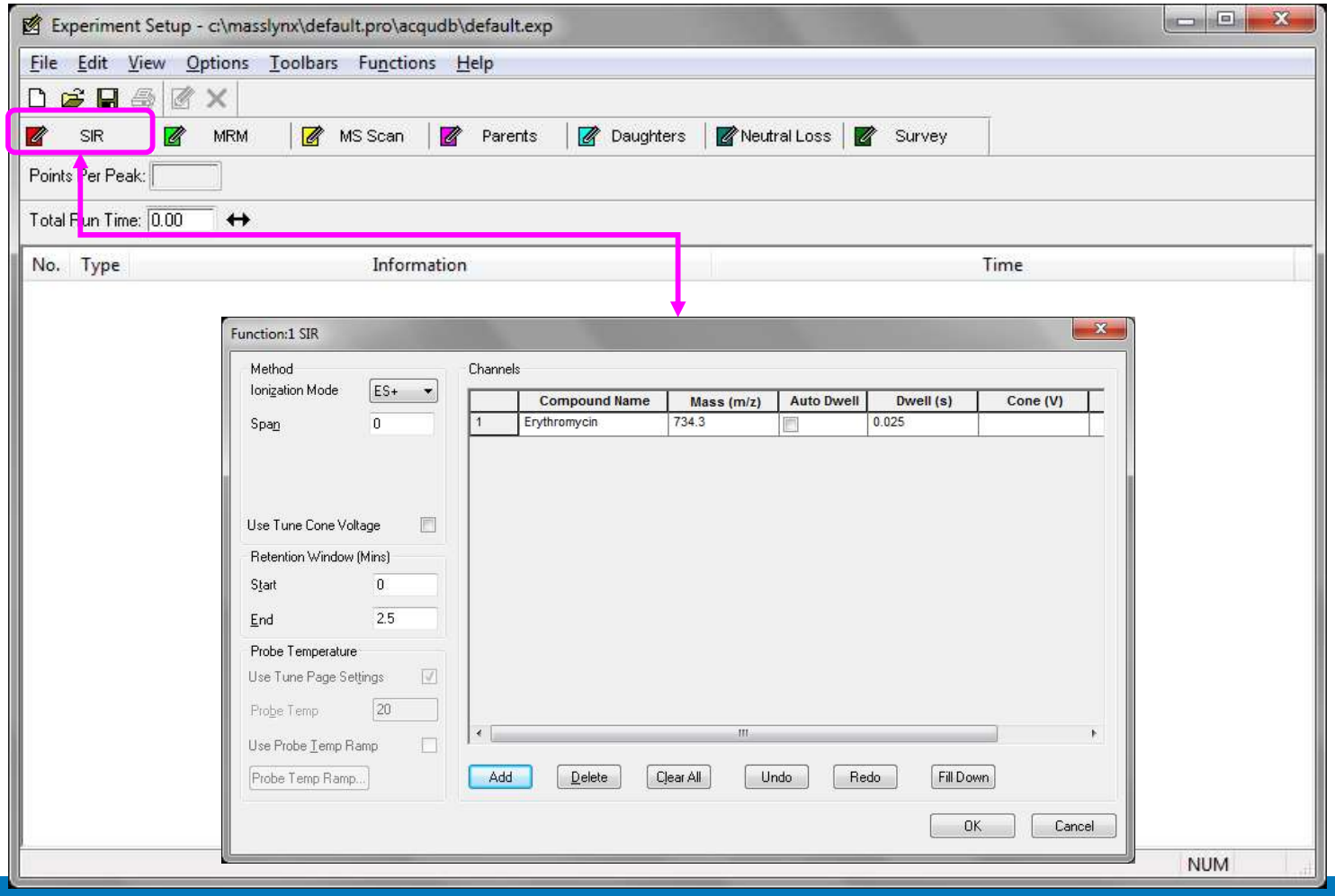
Precursor Ion Analysis (Parent Scan)



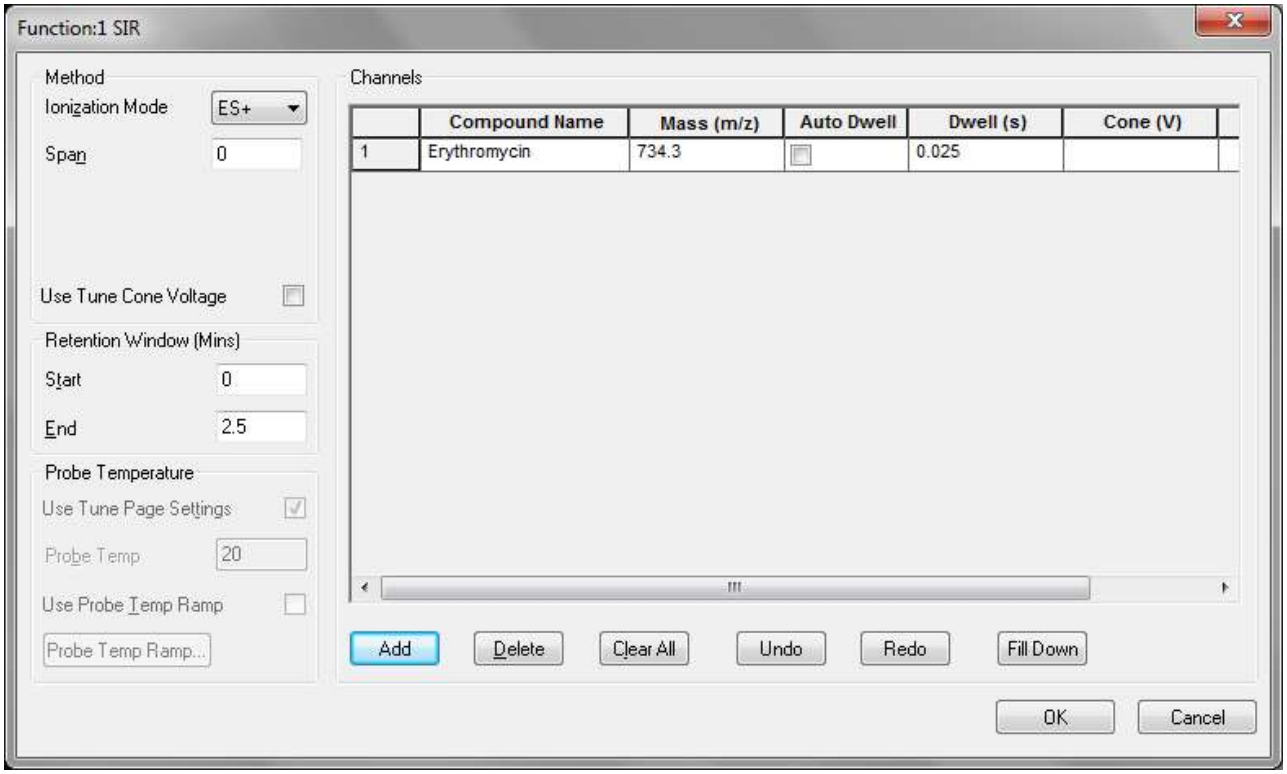
Setting the Mass Range



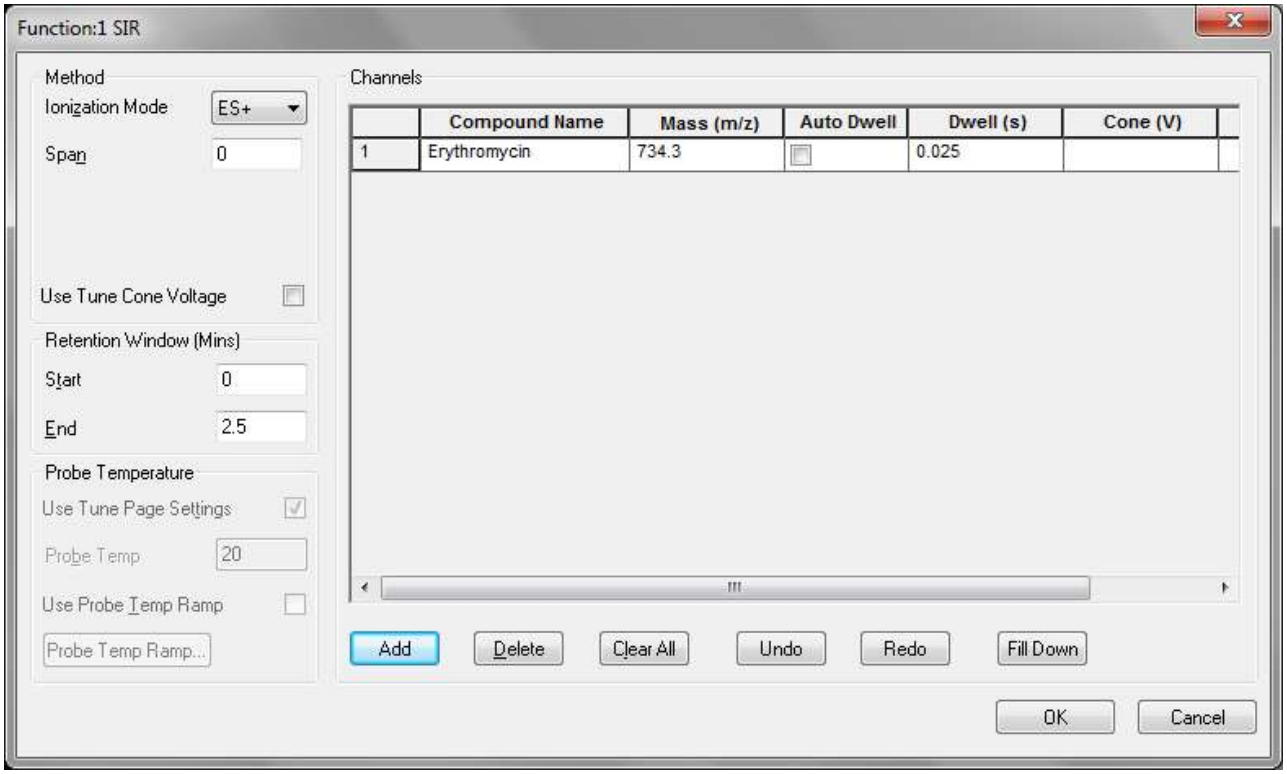
SIR Function



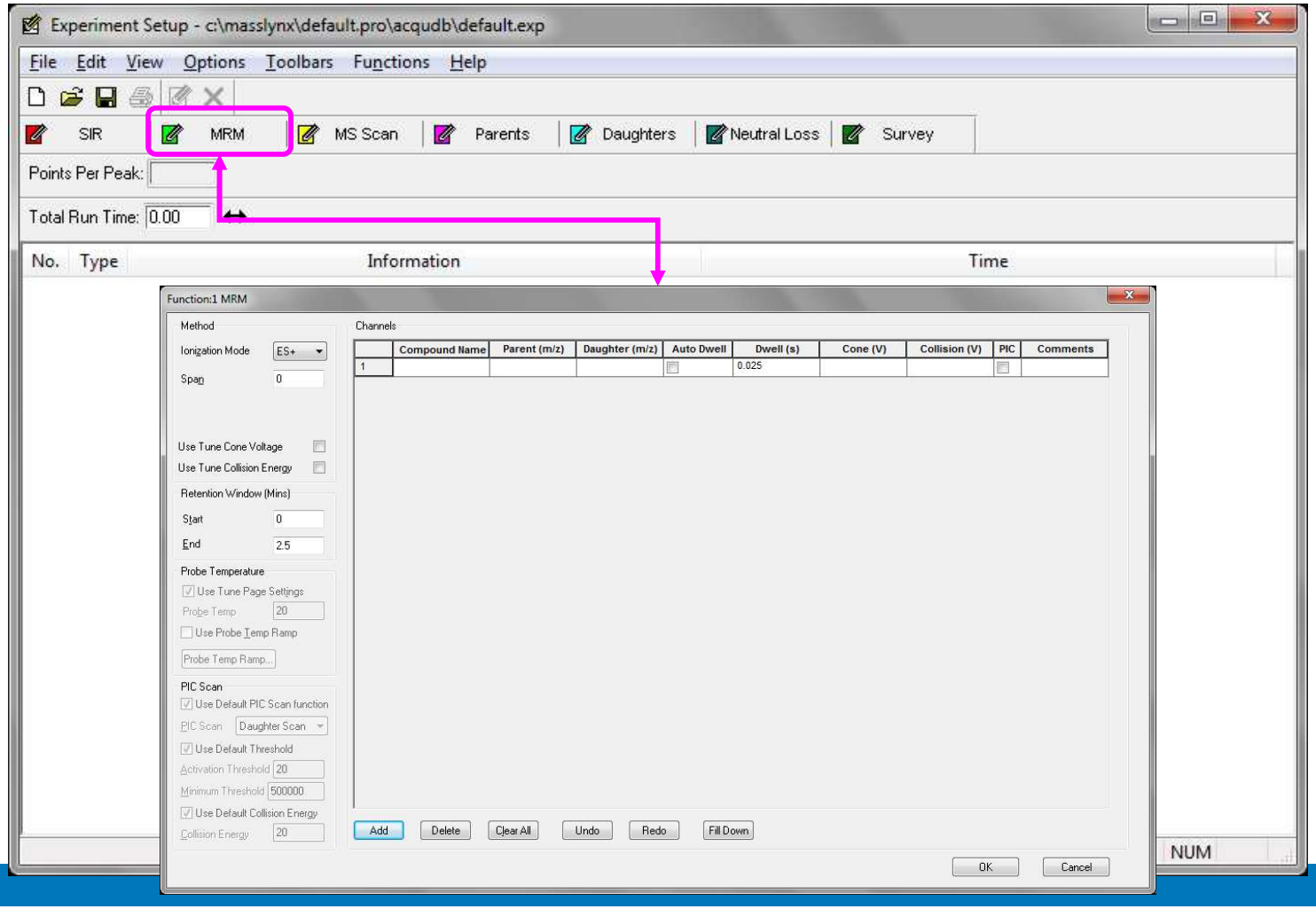
Monitored Masses and Dwell Time



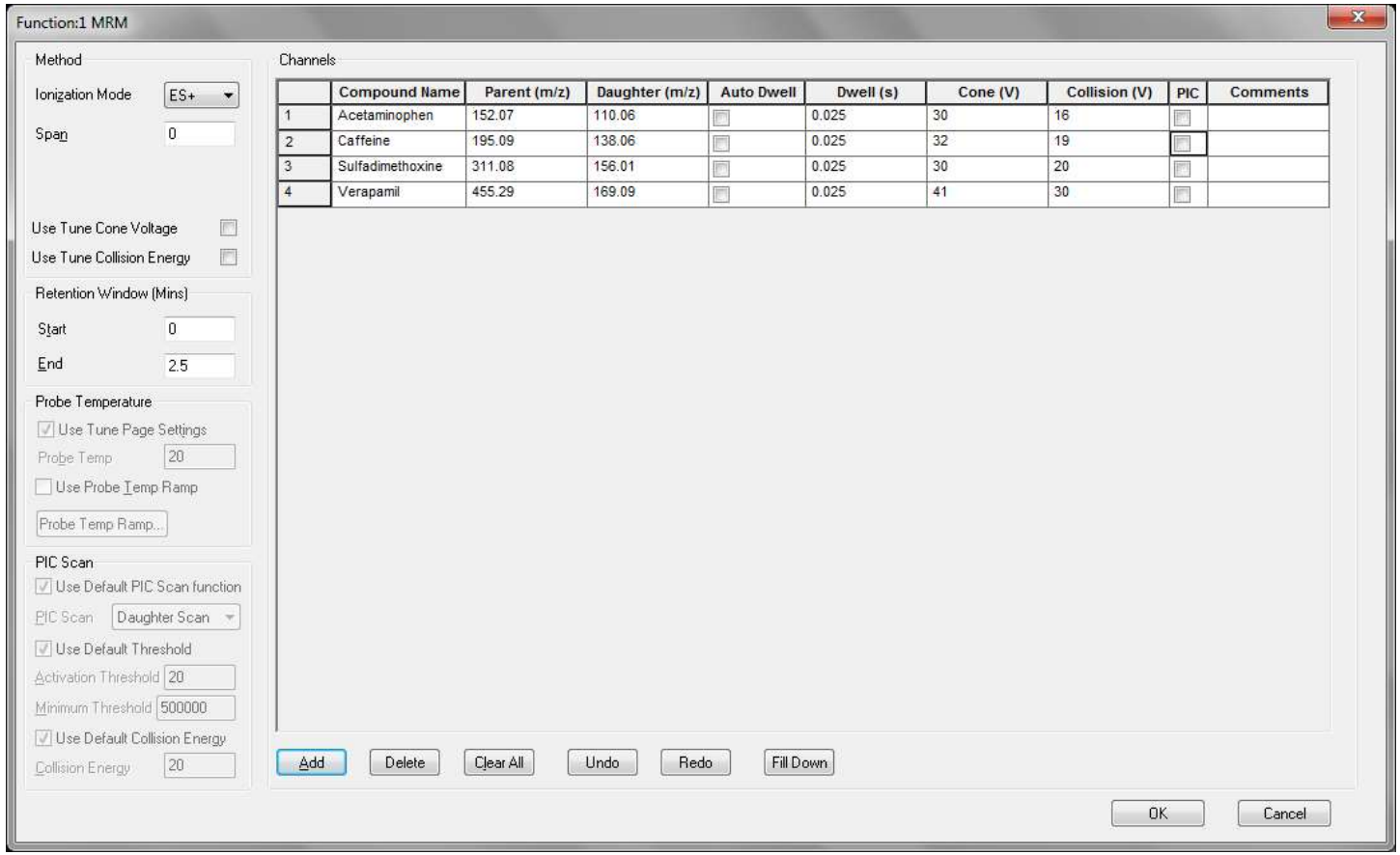
Entering Masses



Multiple Reaction Monitoring



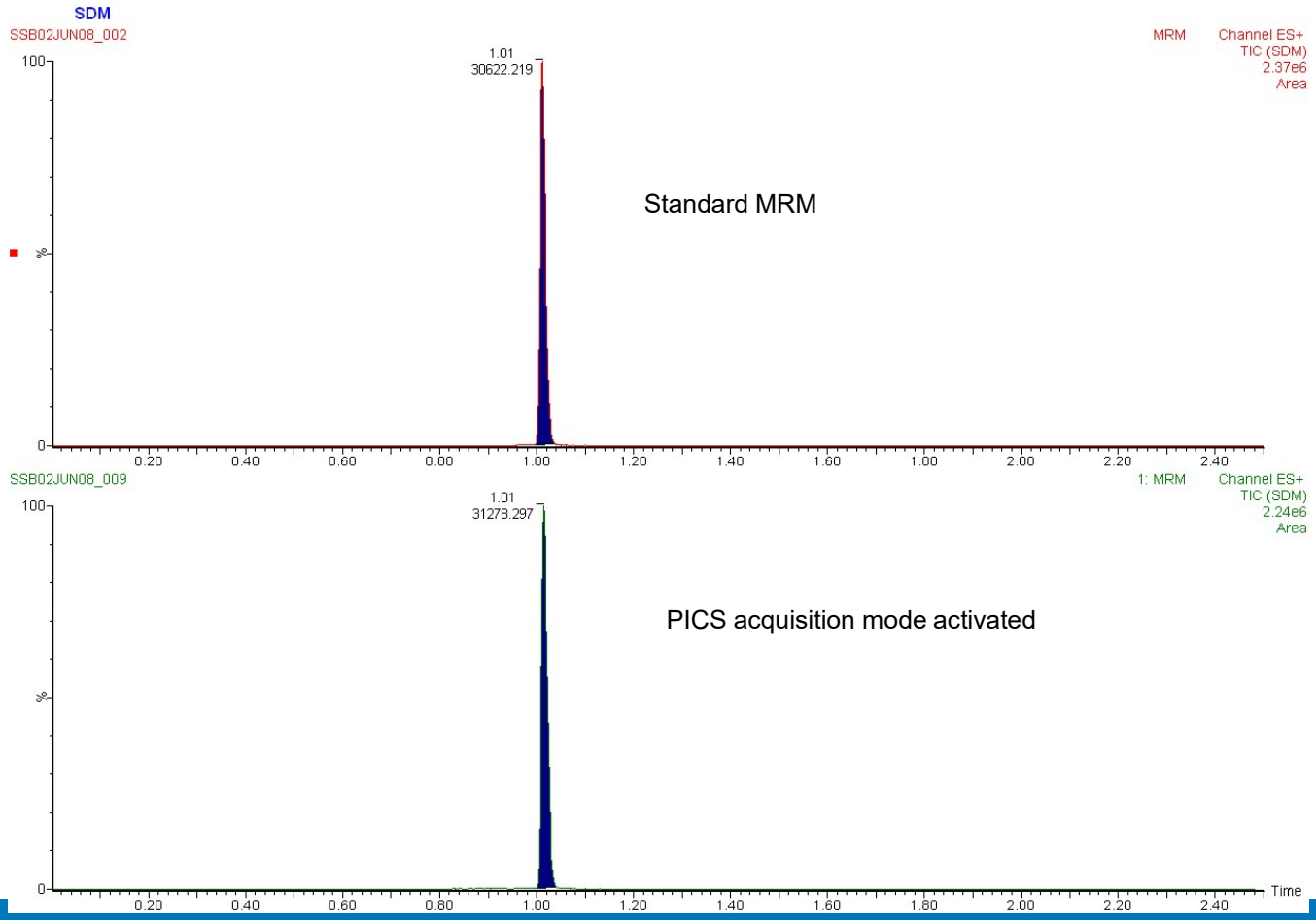
Entering Masses



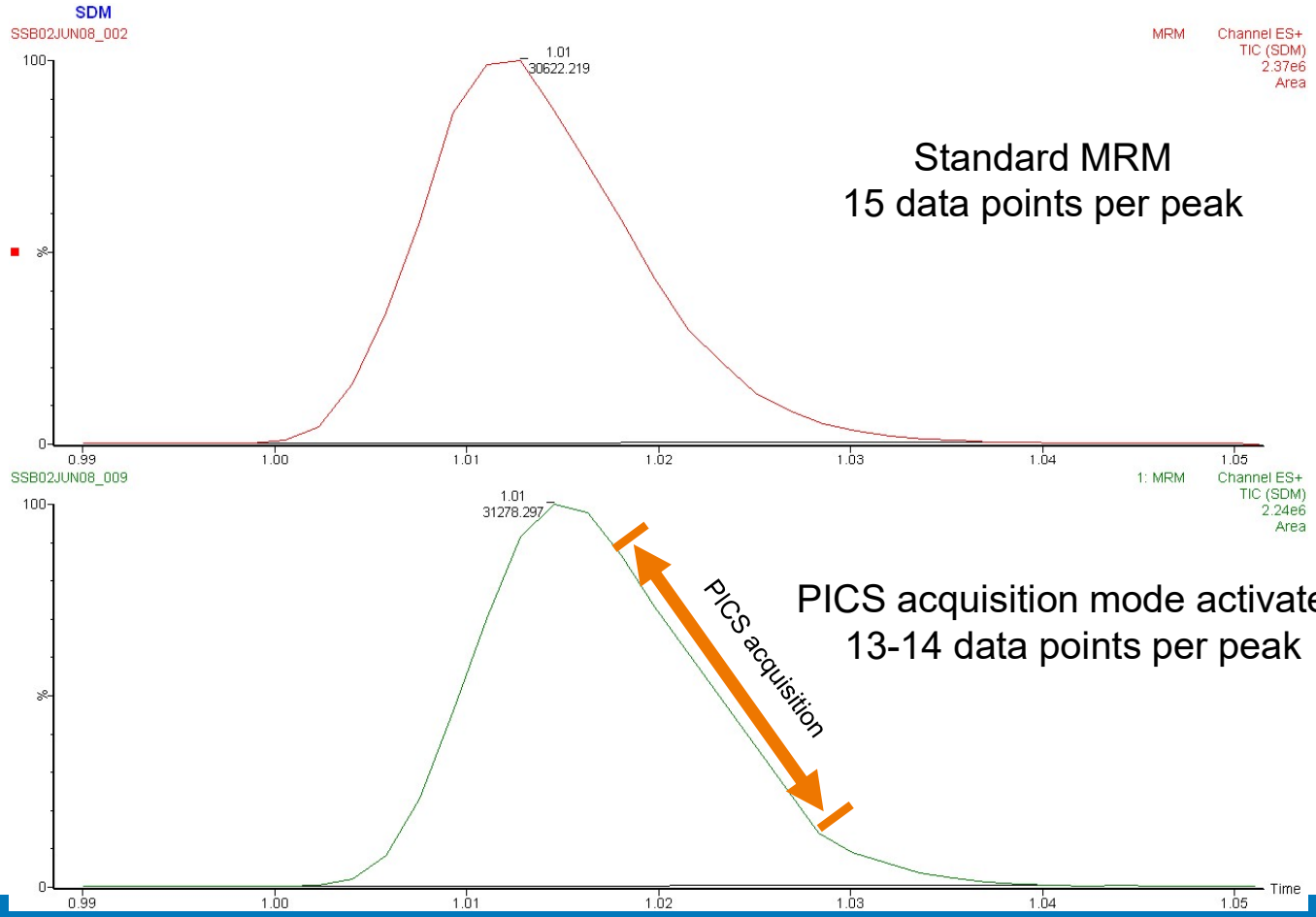
PICS Acquisition Mode

- Acquire quantitative MRM
- Use MRM data as a specific trigger for the acquisition of a product ion spectrum
- This spectral data could provide useful extra information about a suspect MRM quantification result

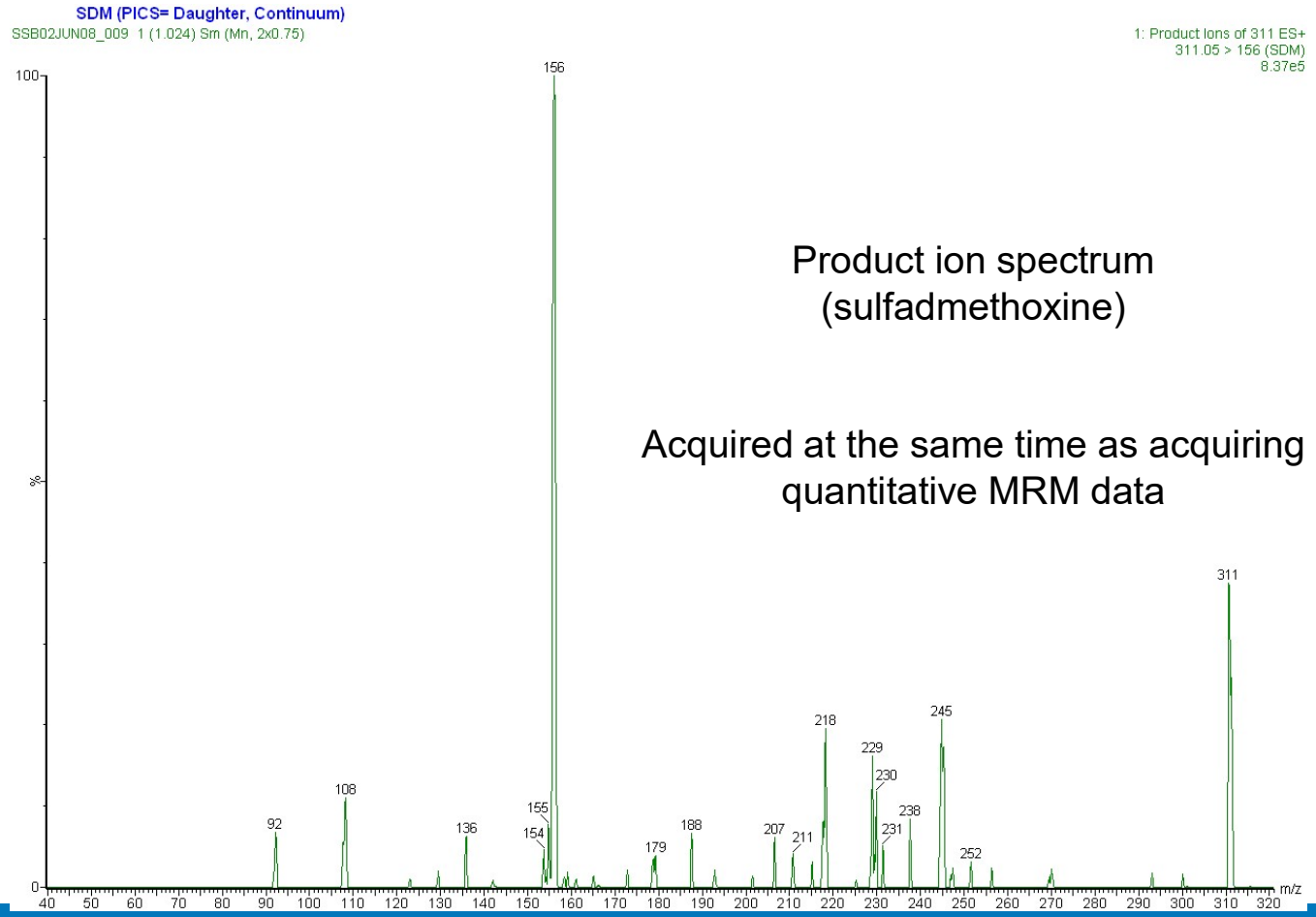
PICS Example Data: (UPLC/MS/MS of Sulfadimethoxine)



PICS Example Data: (UPLC/MS/MS of Sulfadimethoxine)



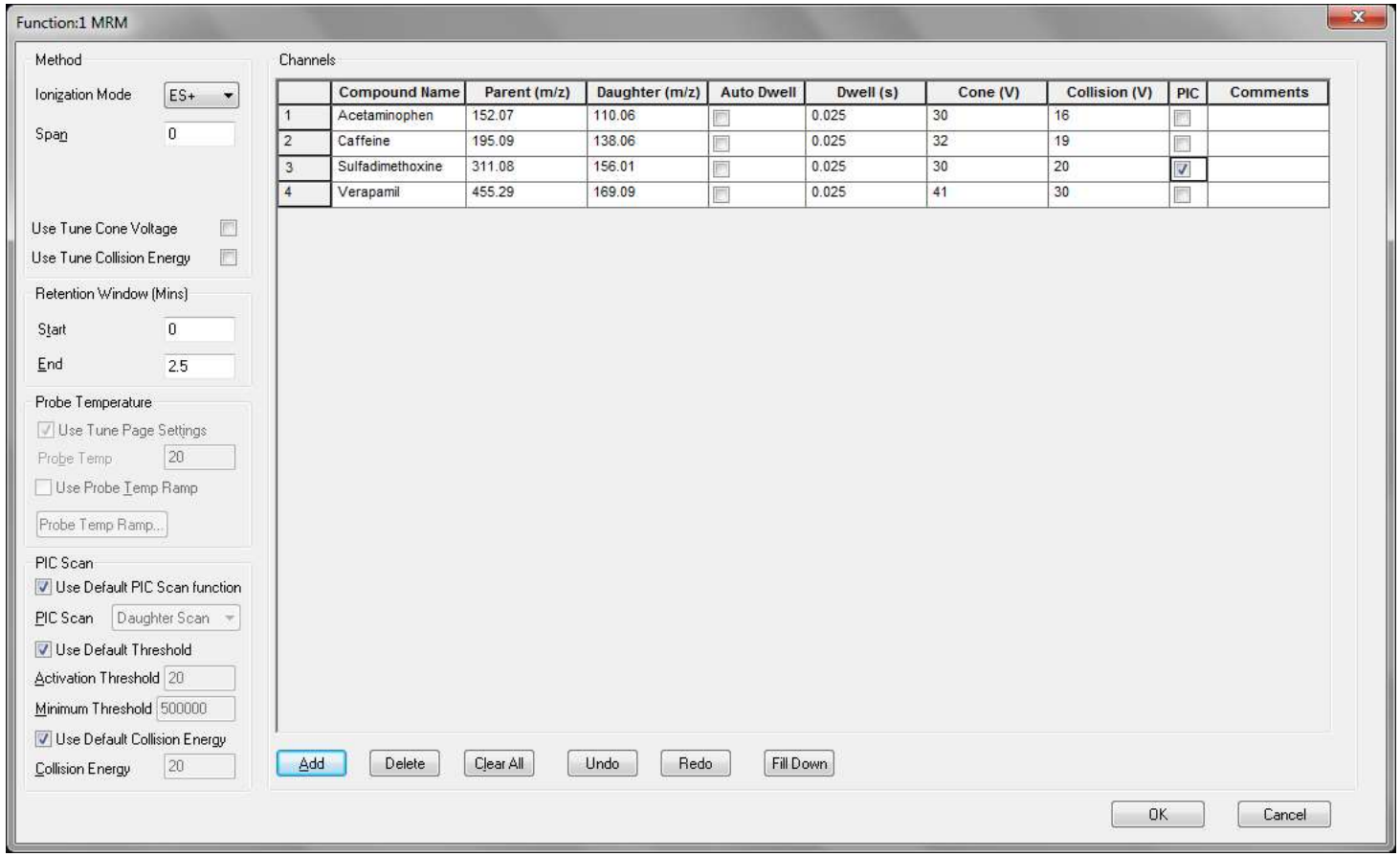
PICS Example Data: (UPLC/MS/MS of Sulfadimethoxine)



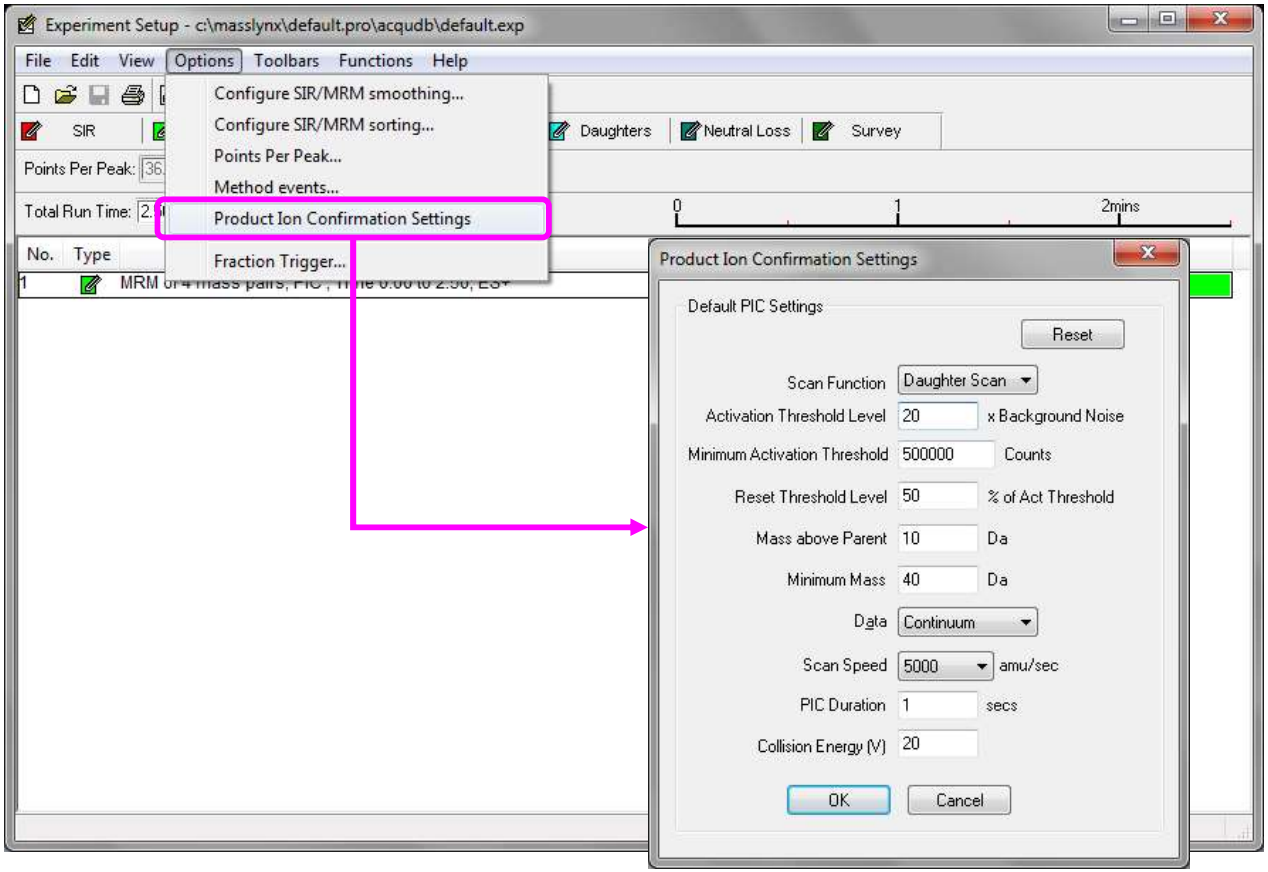
Product ion spectrum (sulfadmethoxine)

Acquired at the same time as acquiring quantitative MRM data

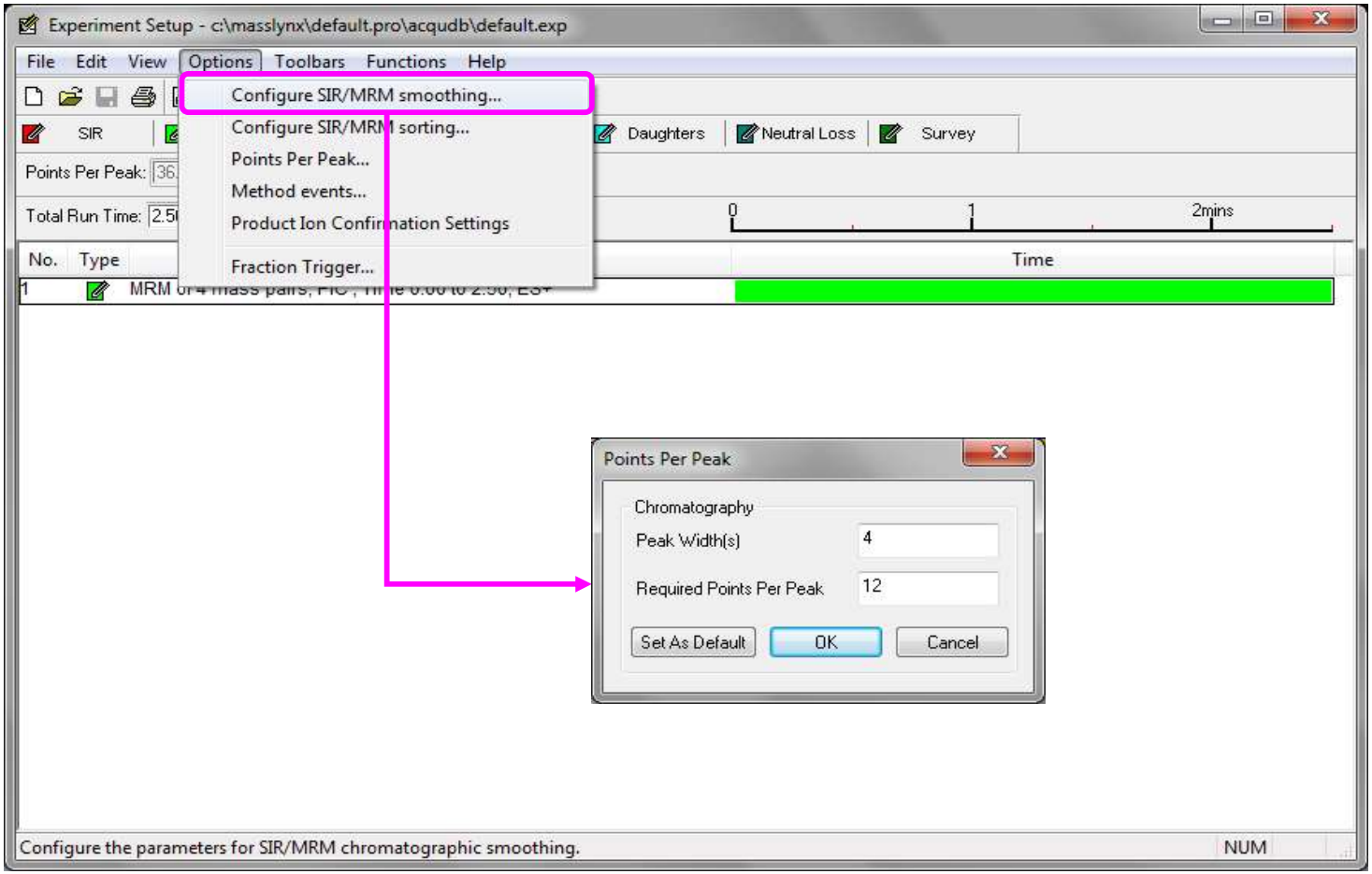
Product Ion Confirmation Setup



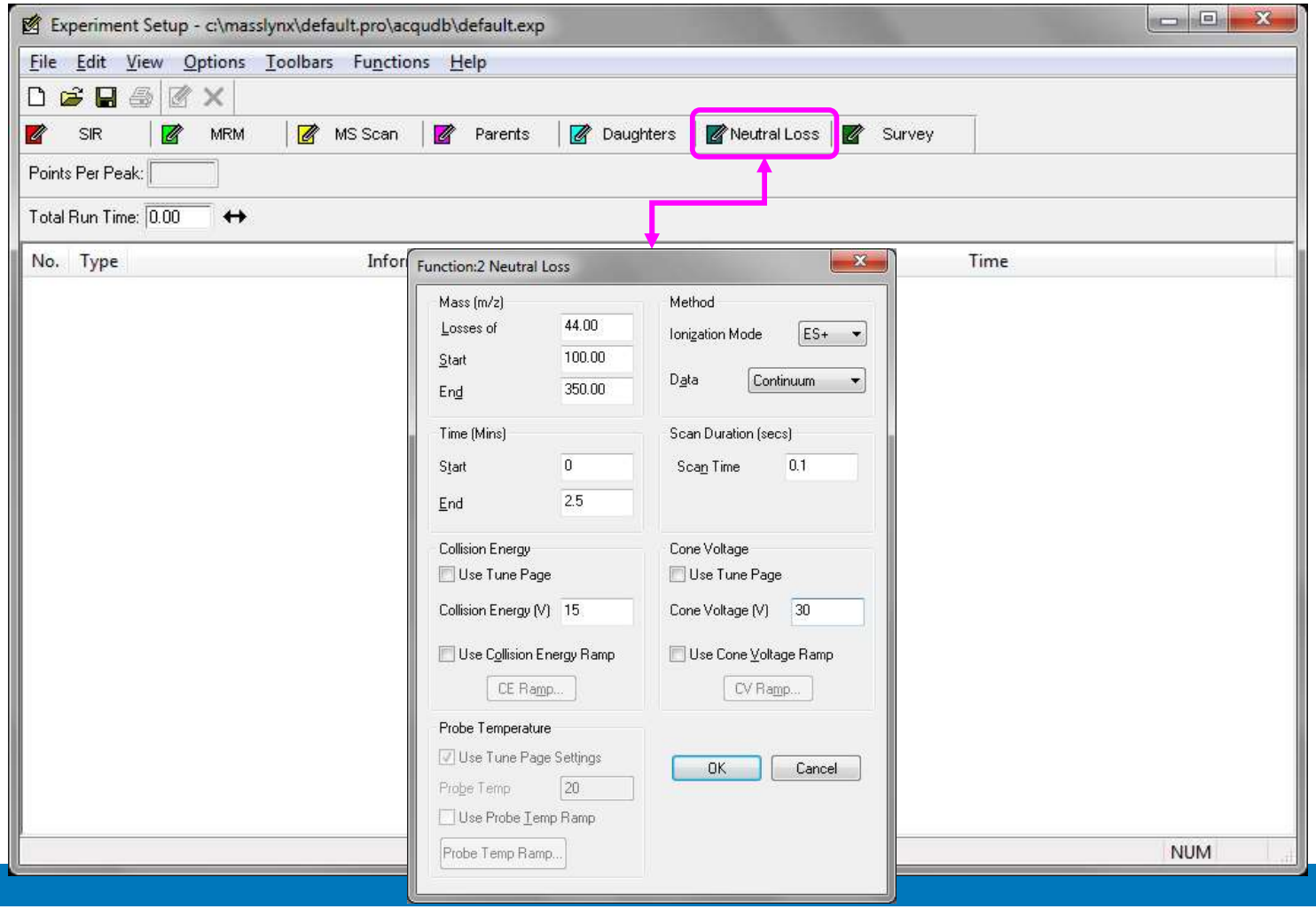
Product Ion Confirmation Setup



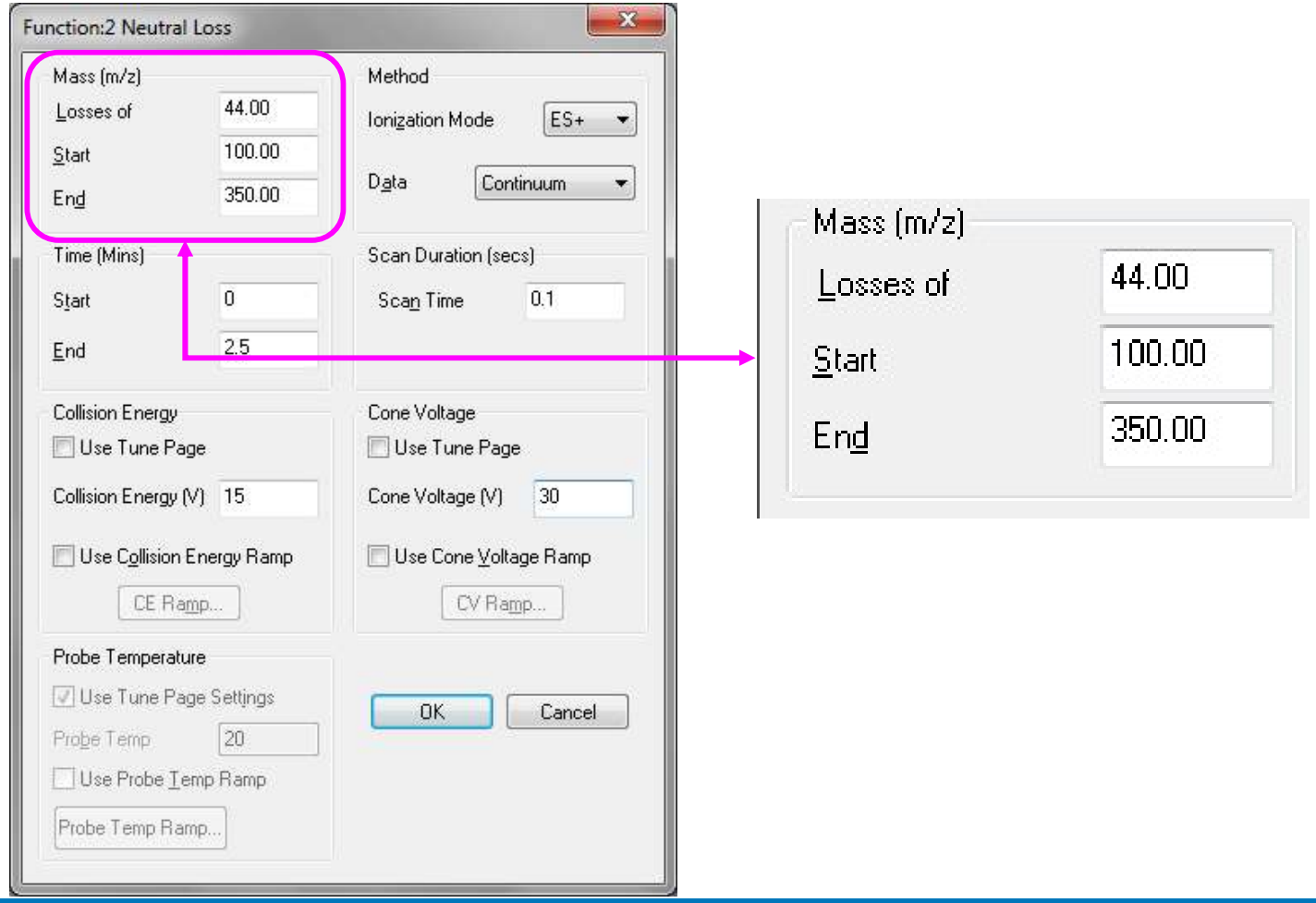
SIR/MRM Chromatogram Smoothing



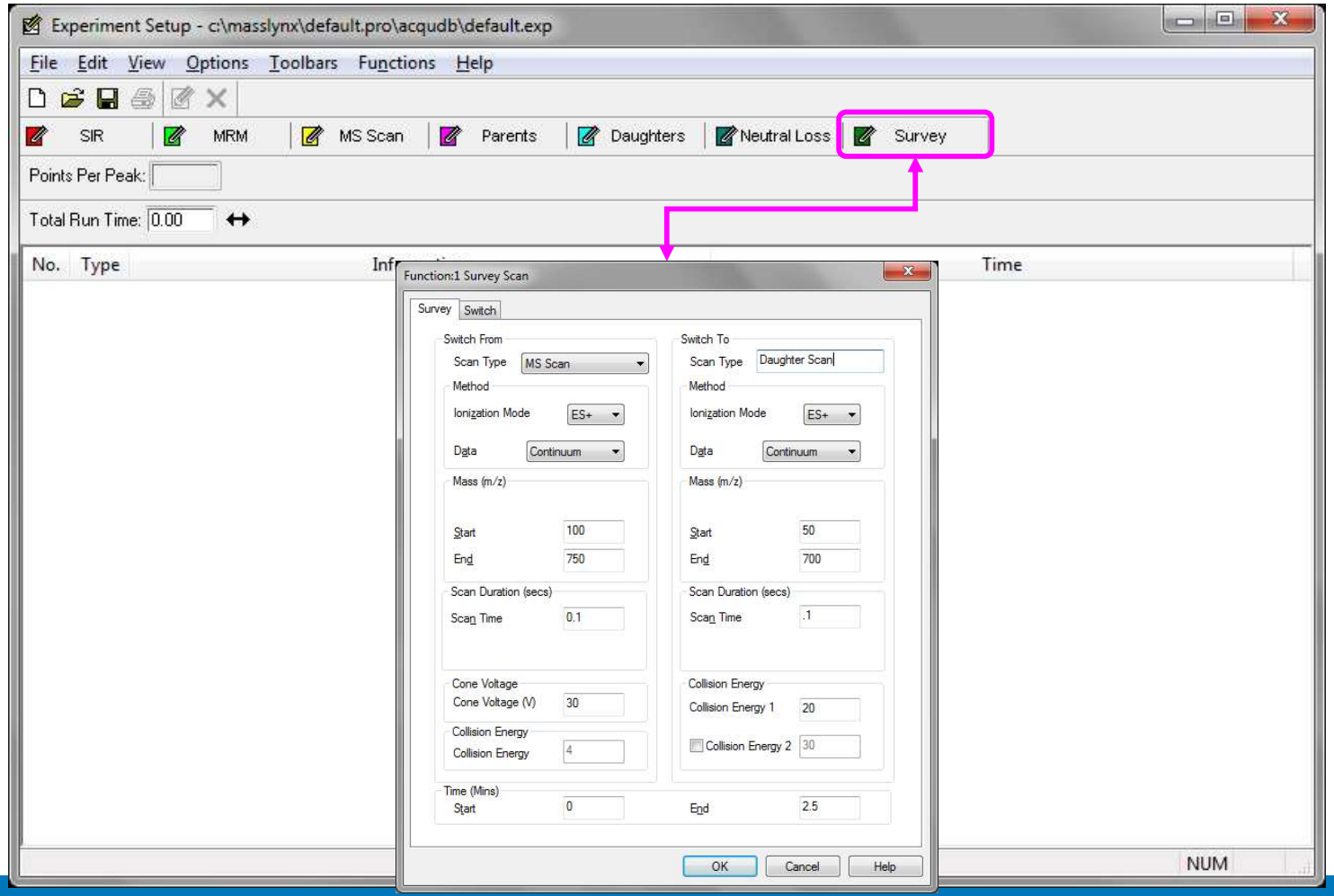
Neutral Loss



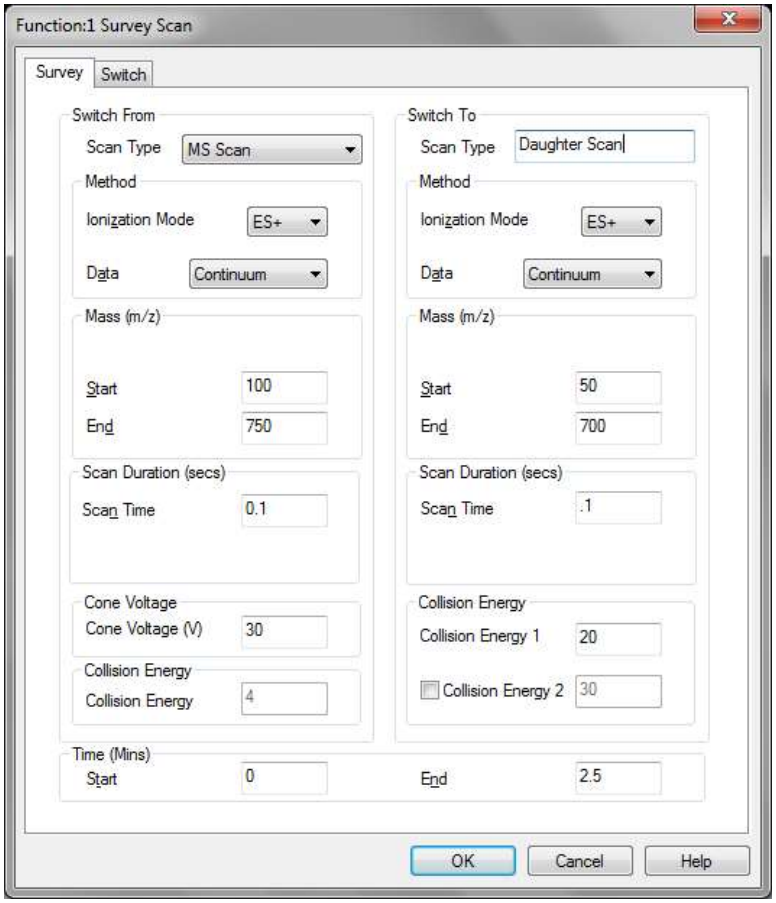
Setting the Mass Range



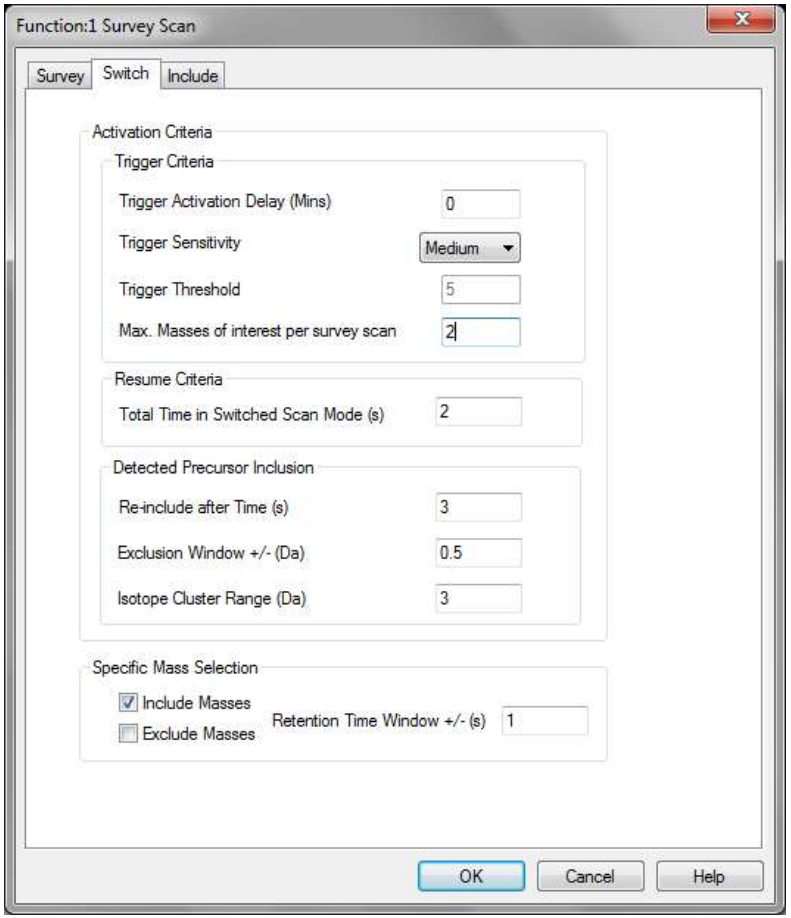
Survey Scan Method Editor



Survey Scan Method Editor: Survey Tab



Survey Scan Method Editor: Switch Tab



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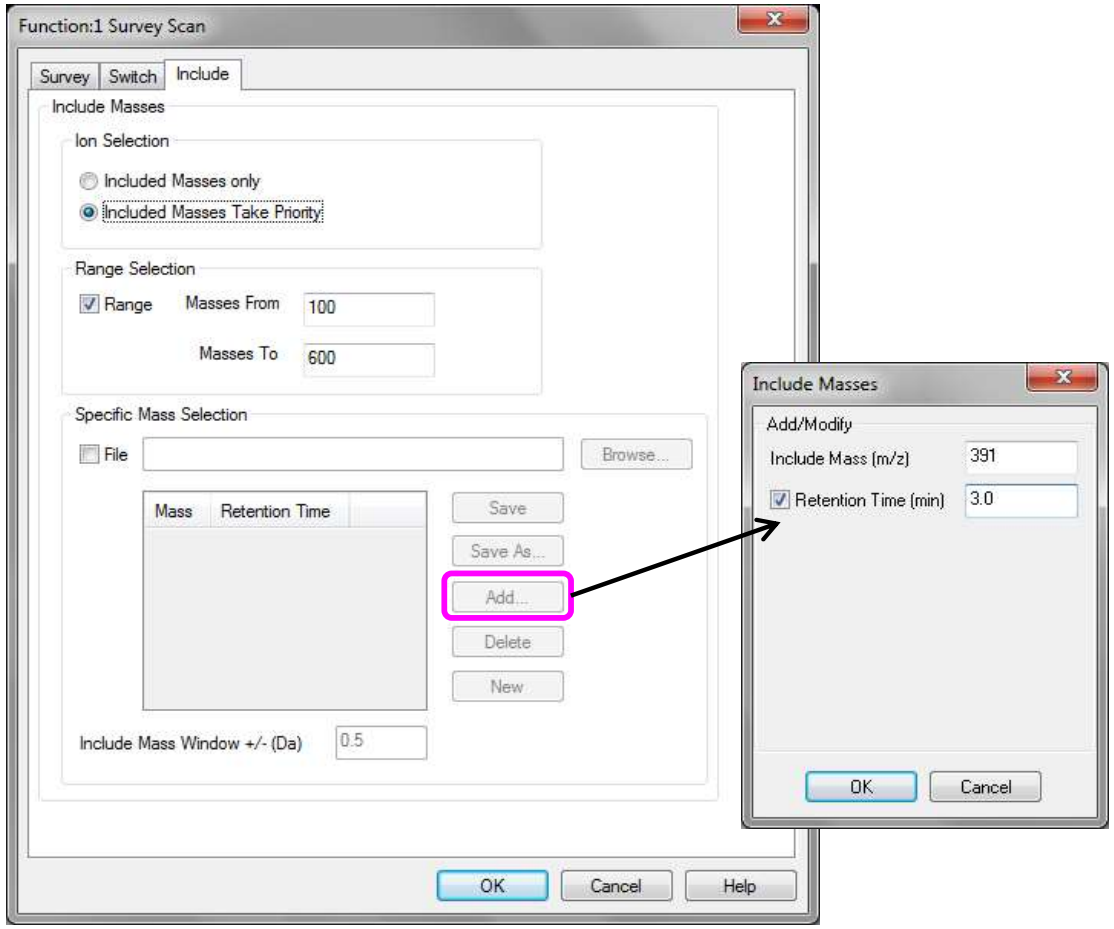

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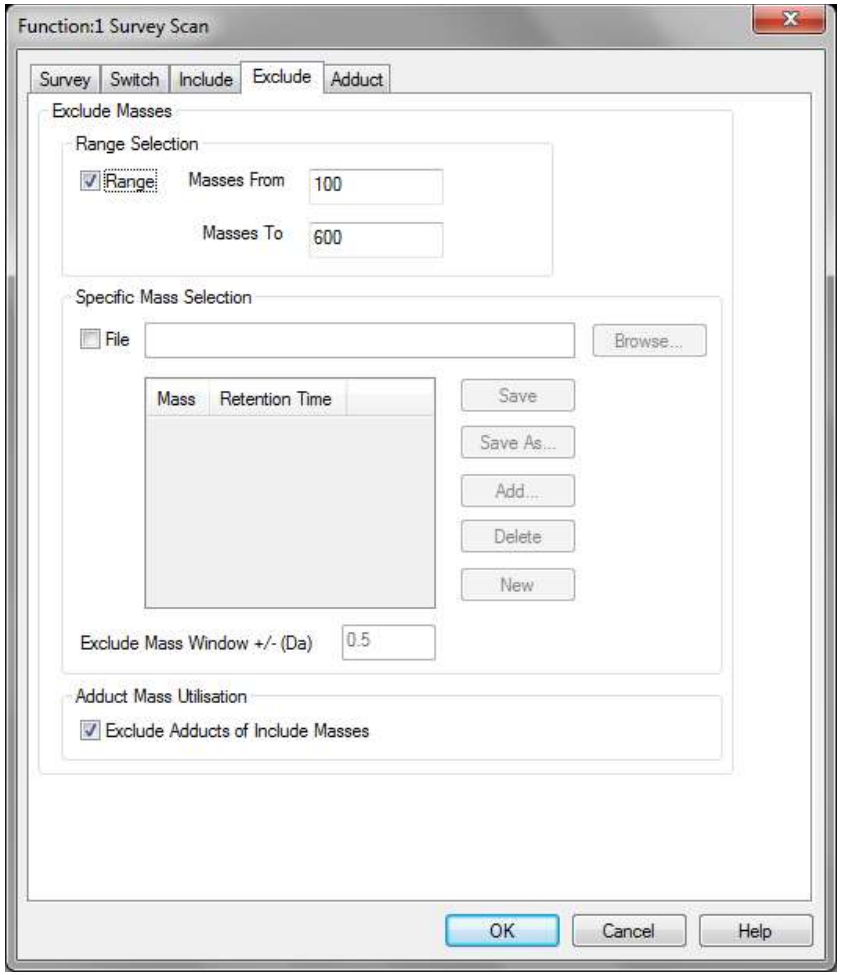
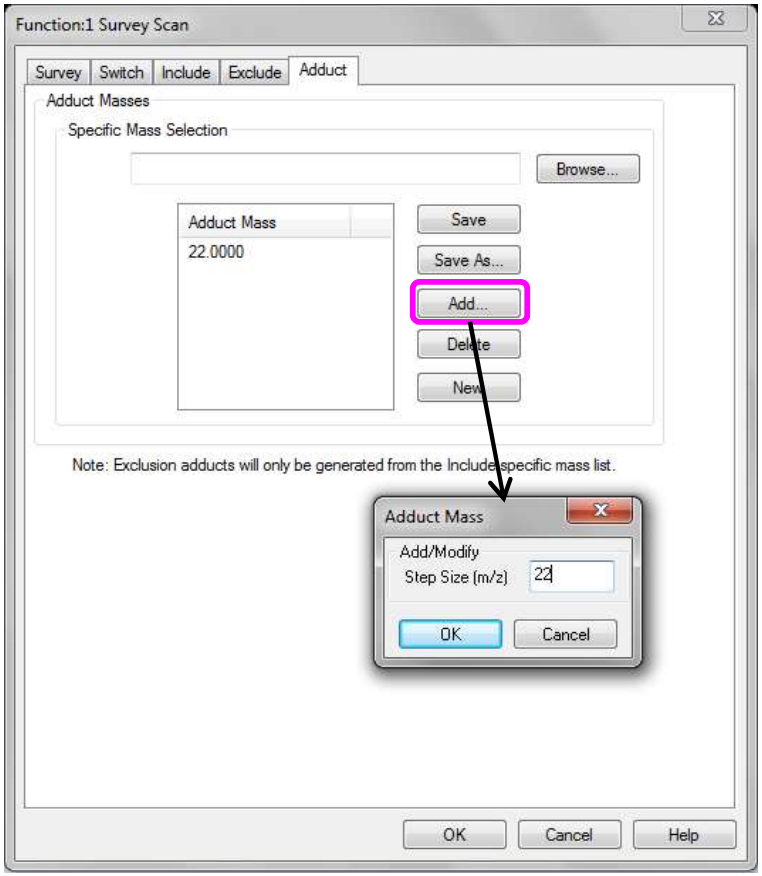
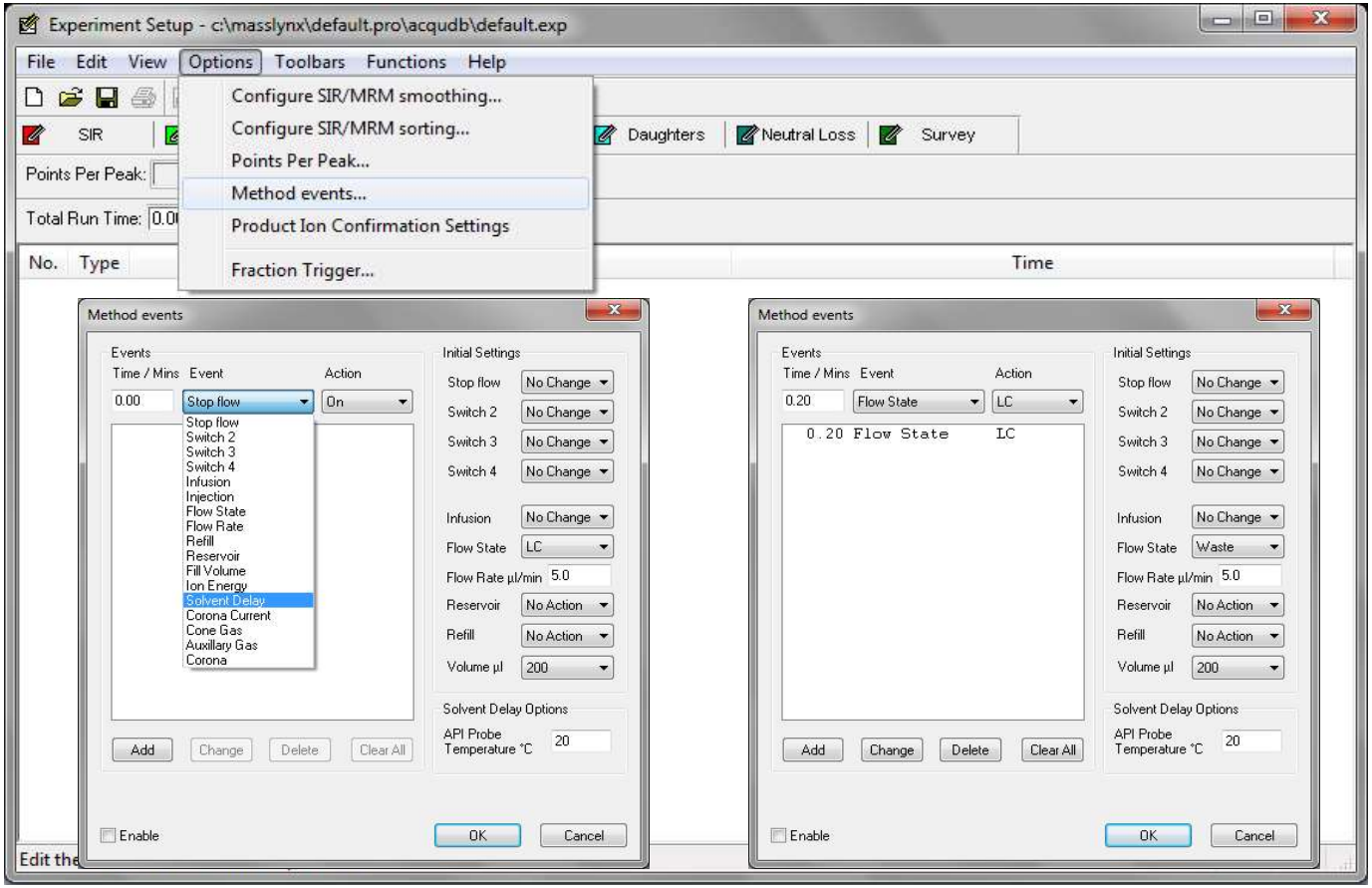


Survey Scan Method Editor: Adduct Tab



MS Method Events



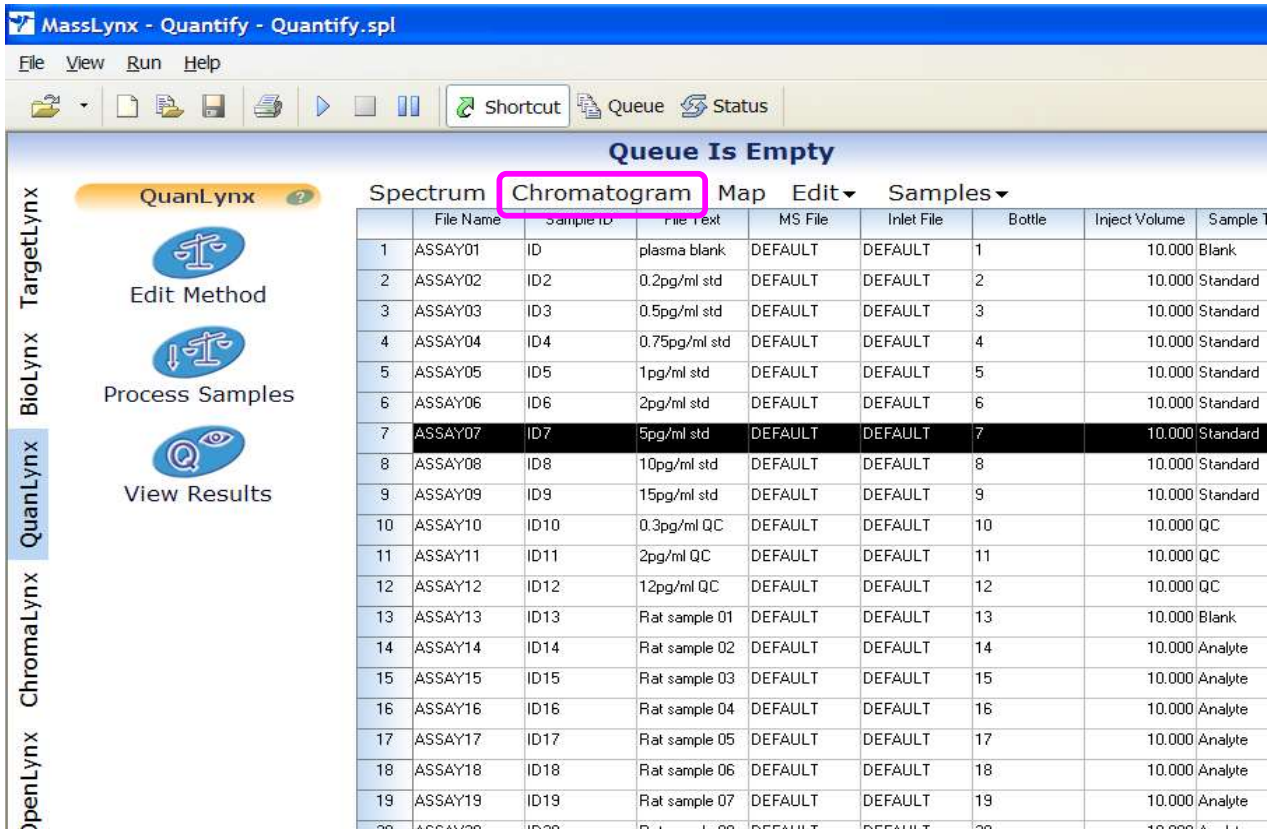
Chapter Six

Data Processing

Data Processing

- Displaying and Processing Chromatograms
- ApexTrack™ Peak Integration
- Integrating and Editing Peaks
- Signal-to-Noise
- Displaying and Processing Spectra

Creating a Chromatogram



	File Name	Sample ID	File Text	MS File	Inlet File	Bottle	Inject Volume	Sample T
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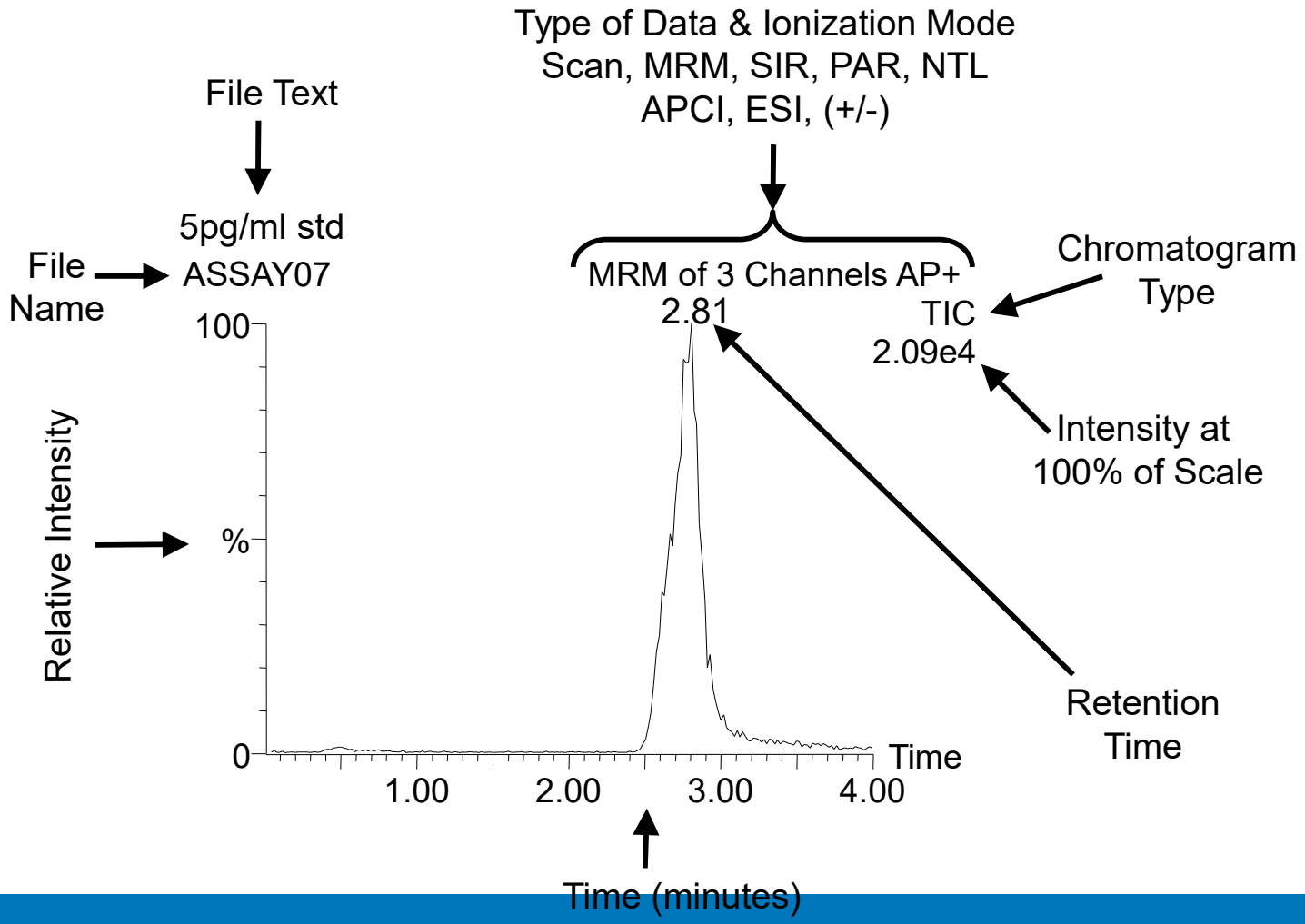
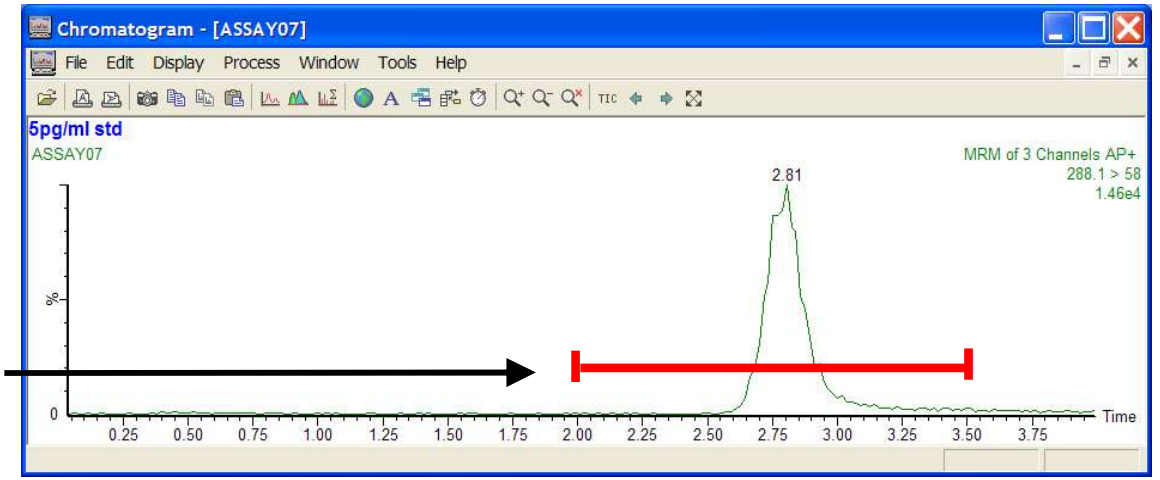
2	ASSAY02	ID2	0.2pg/ml std	DEFAULT	DEFAULT	2	10.000	Standard
3	ASSAY03	ID3	0.5pg/ml std	DEFAULT	DEFAULT	3	10.000	Standard
4	ASSAY04	ID4	0.75pg/ml std	DEFAULT	DEFAULT	4	10.000	Standard
5	ASSAY05	ID5	1pg/ml std	DEFAULT	DEFAULT	5	10.000	Standard
6	ASSAY06	ID6	2pg/ml std	DEFAULT	DEFAULT	6	10.000	Standard
7	ASSAY07	ID7	5pg/ml std	DEFAULT	DEFAULT	7	10.000	Standard
8	ASSAY08	ID8	10pg/ml std	DEFAULT	DEFAULT	8	10.000	Standard
9	ASSAY09	ID9	15pg/ml std	DEFAULT	DEFAULT	9	10.000	Standard
10	ASSAY10	ID10	0.3pg/ml QC	DEFAULT	DEFAULT	10	10.000	QC
11	ASSAY11	ID11	2pg/ml QC	DEFAULT	DEFAULT	11	10.000	QC
12	ASSAY12	ID12	12pg/ml QC	DEFAULT	DEFAULT	12	10.000	QC
13	ASSAY13	ID13	Rat sample 01	DEFAULT	DEFAULT	13	10.000	Blank
14	ASSAY14	ID14	Rat sample 02	DEFAULT	DEFAULT	14	10.000	Analyte
15	ASSAY15	ID15	Rat sample 03	DEFAULT	DEFAULT	15	10.000	Analyte
16	ASSAY16	ID16	Rat sample 04	DEFAULT	DEFAULT	16	10.000	Analyte
17	ASSAY17	ID17	Rat sample 05	DEFAULT	DEFAULT	17	10.000	Analyte
18	ASSAY18	ID18	Rat sample 06	DEFAULT	DEFAULT	18	10.000	Analyte
19	ASSAY19	ID19	Rat sample 07	DEFAULT	DEFAULT	19	10.000	Analyte

Information Layout

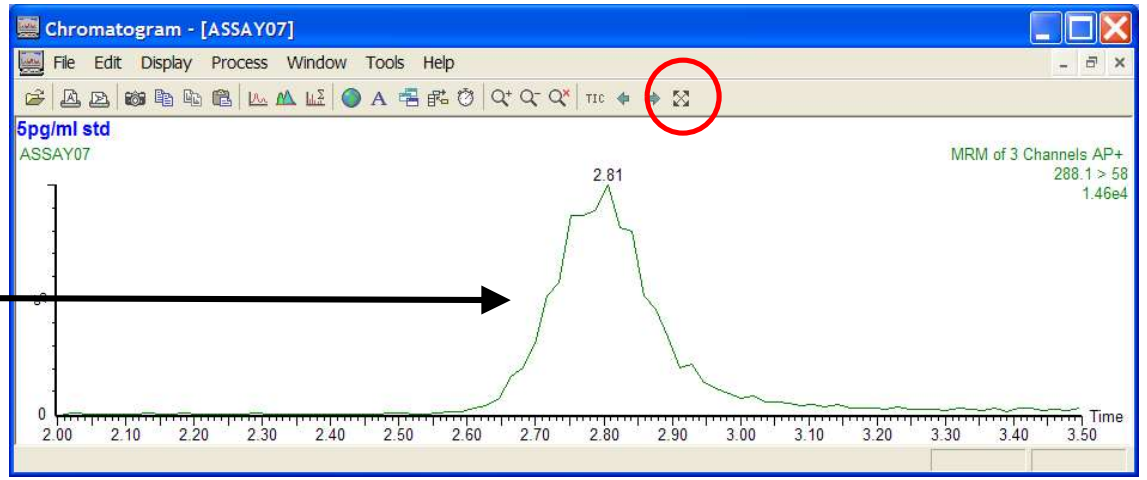


Zooming Functions

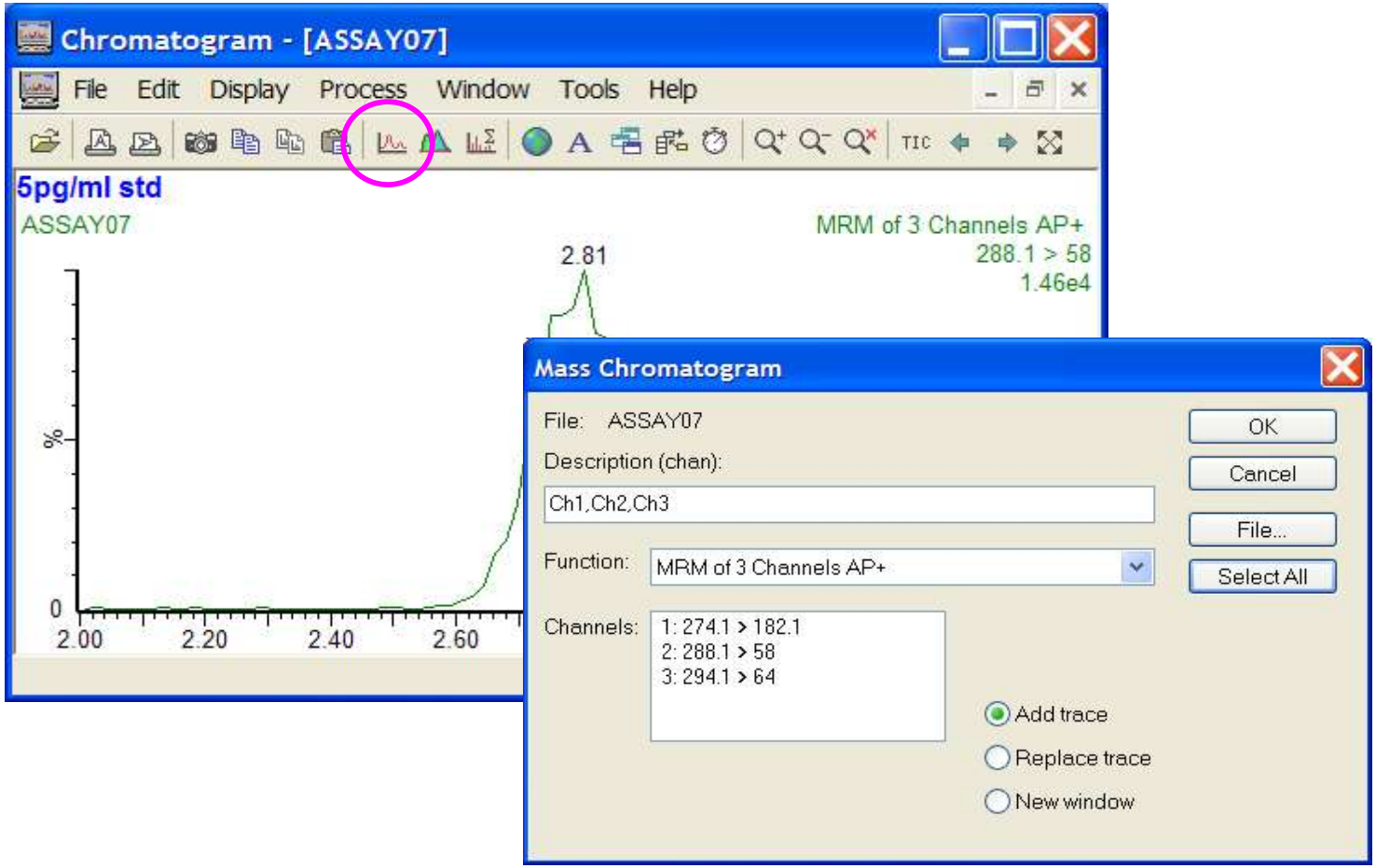
Left-Click and Drag to Expand



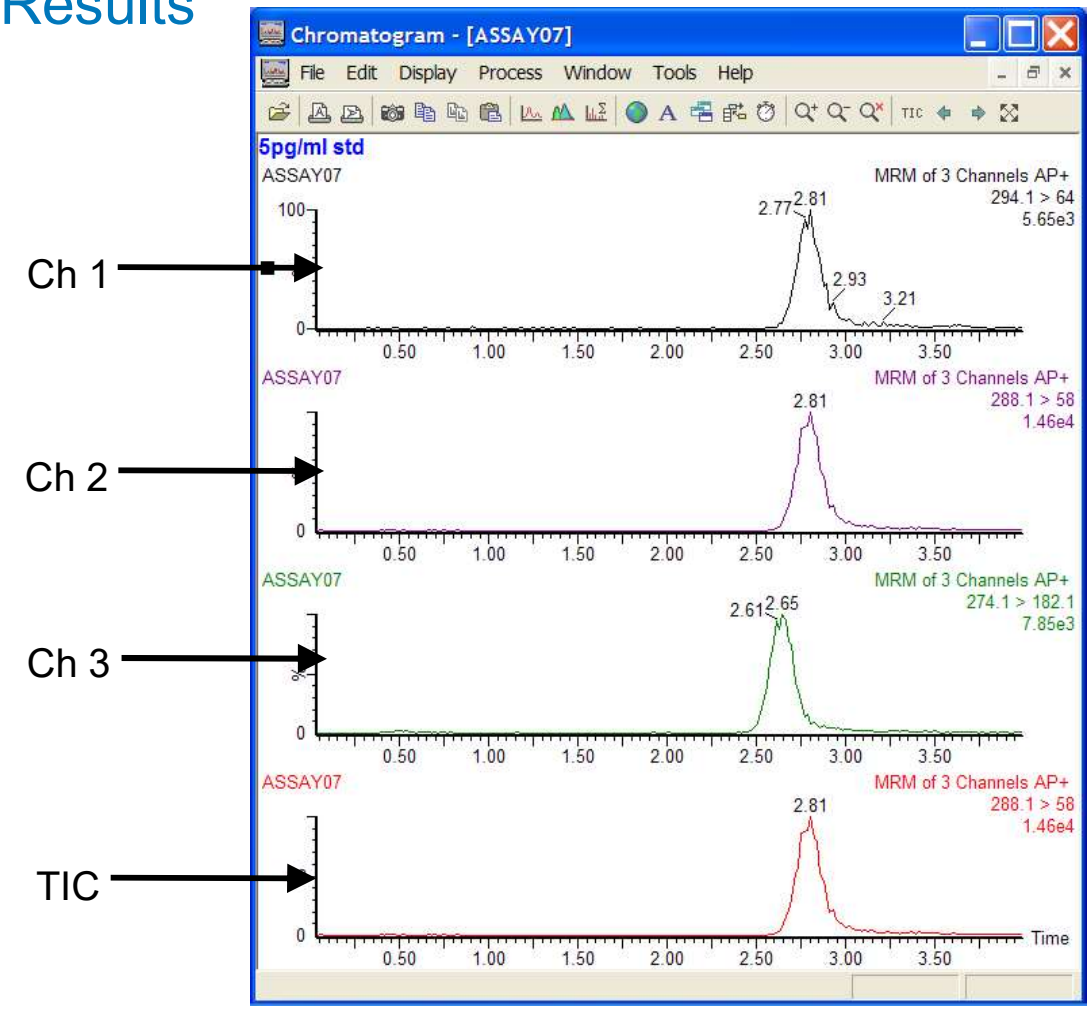
Results of Expansion



Extracting Data from Ion Chromatograms



Channel Selection Results



Deleting a Chromatogram

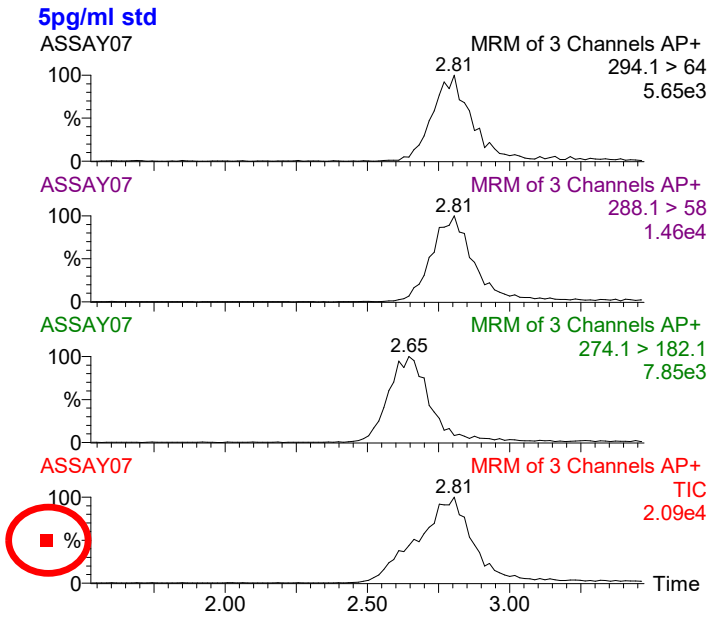


Figure 1

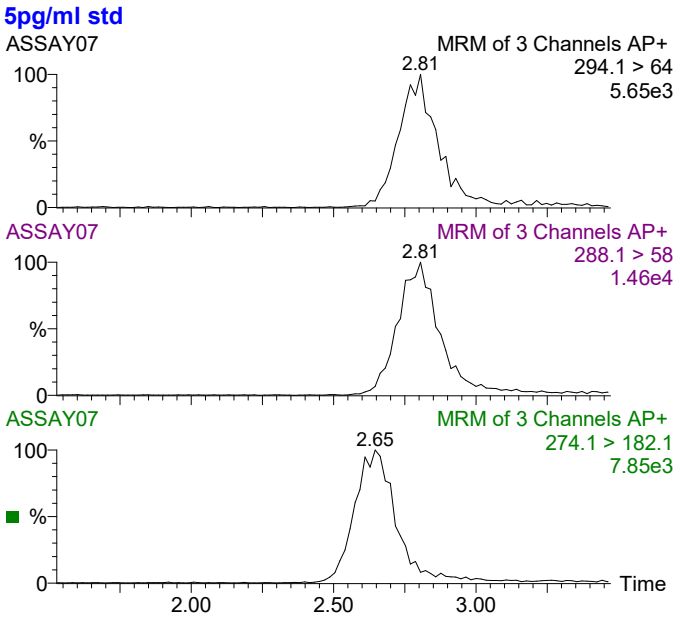


Figure 2

Changing Trace Order

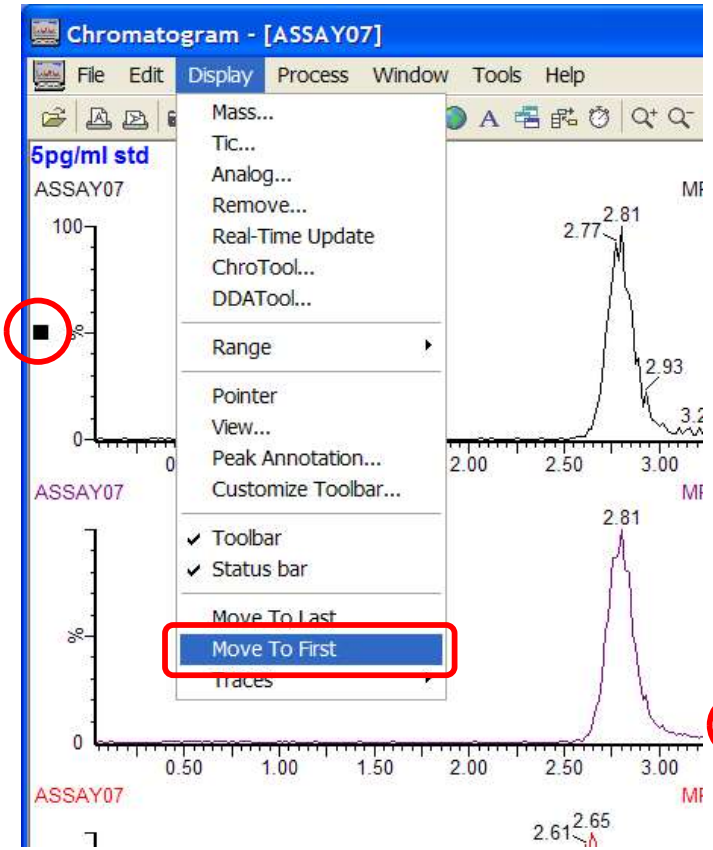


Figure 3

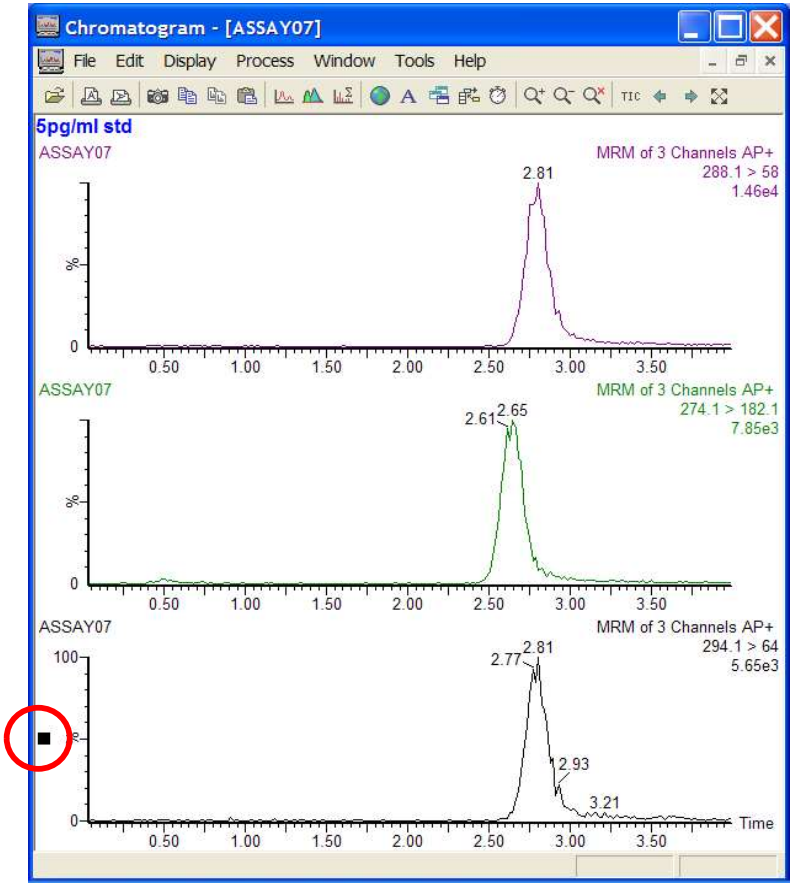
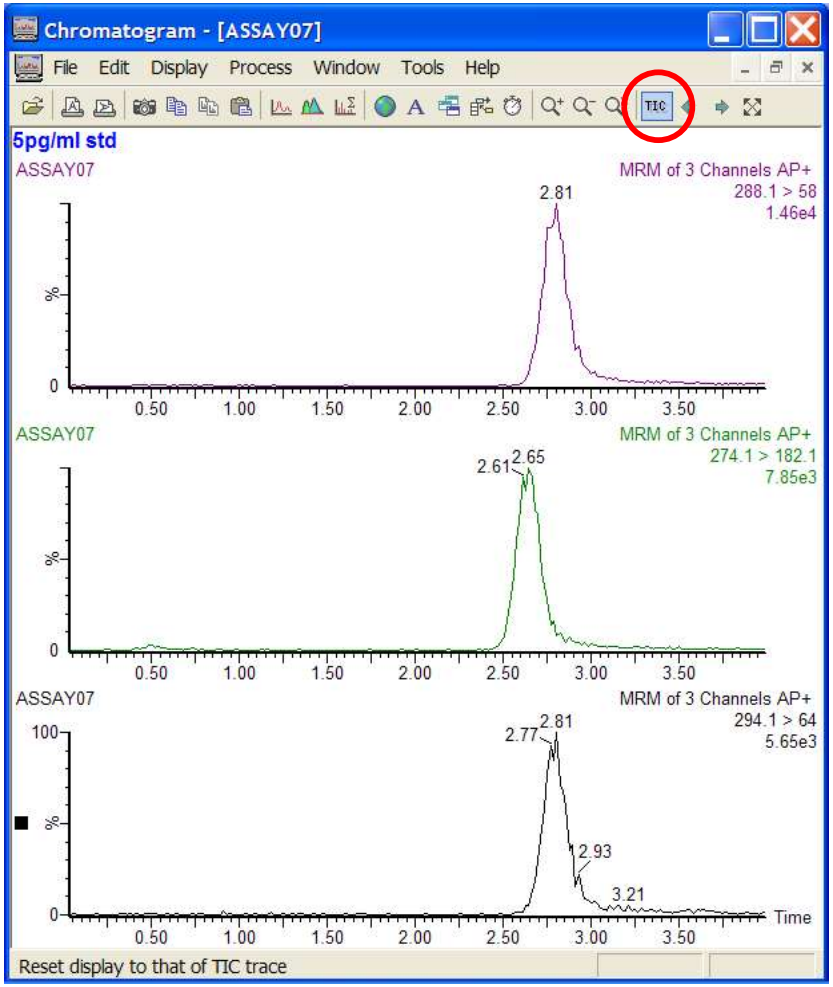
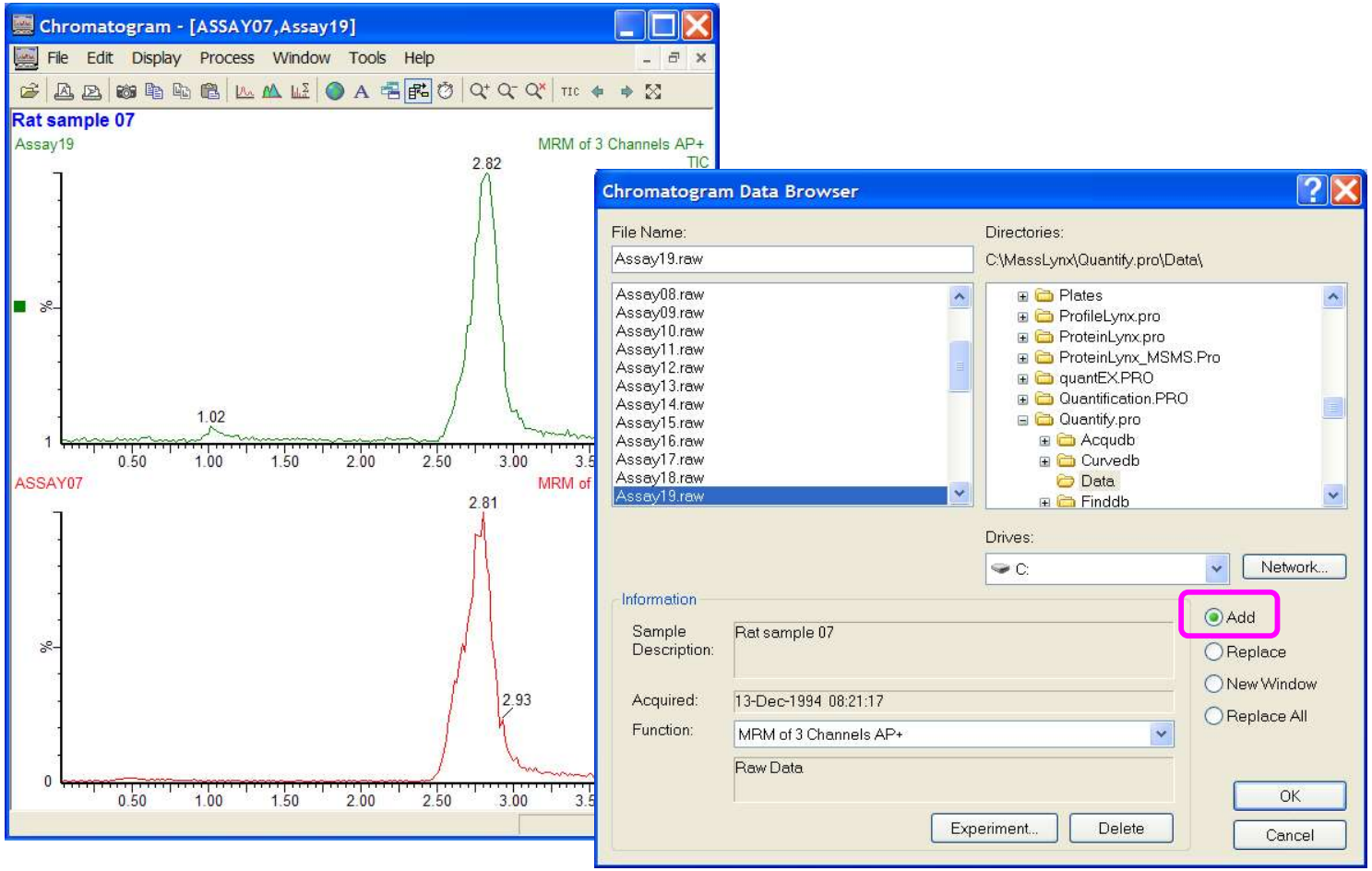


Figure 4

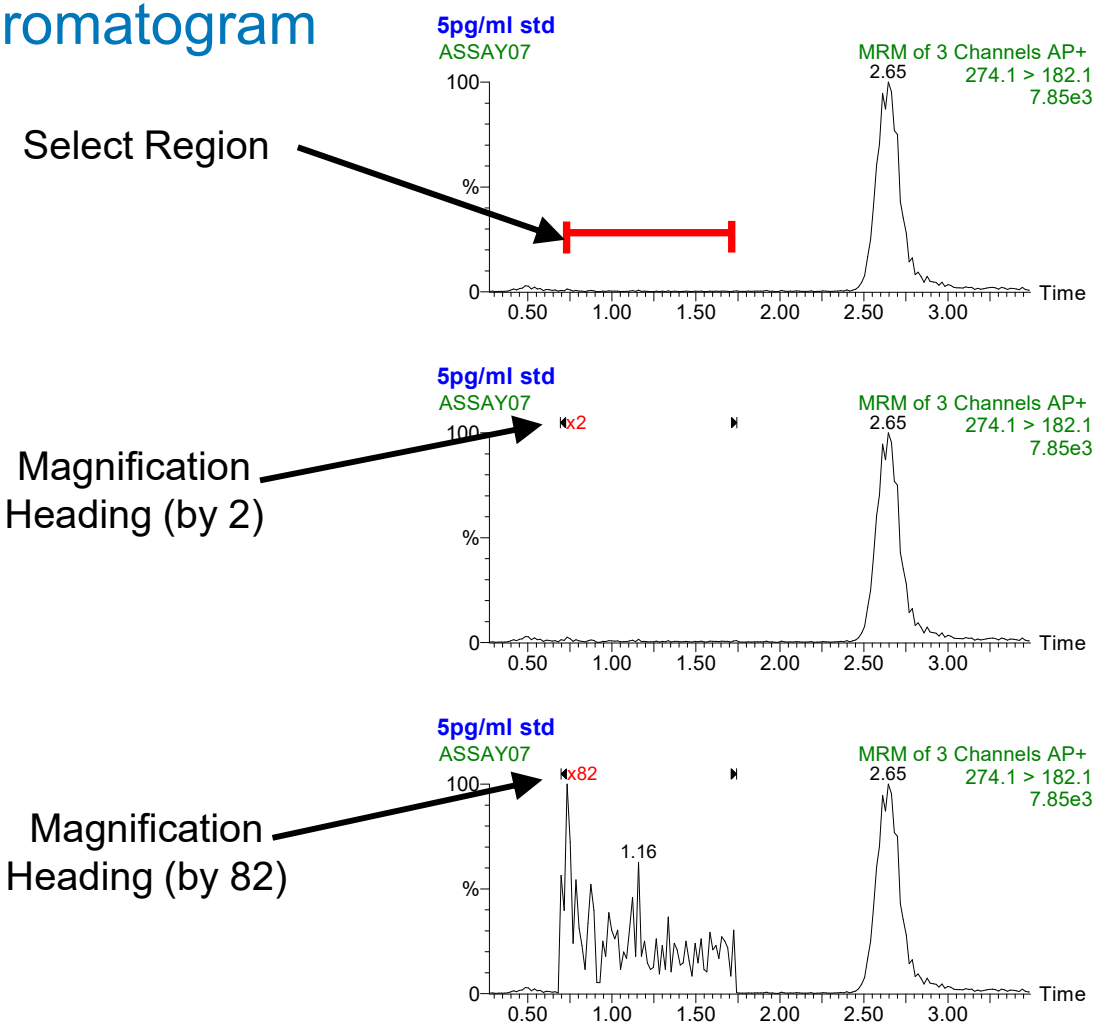
Restoring Full View of TIC



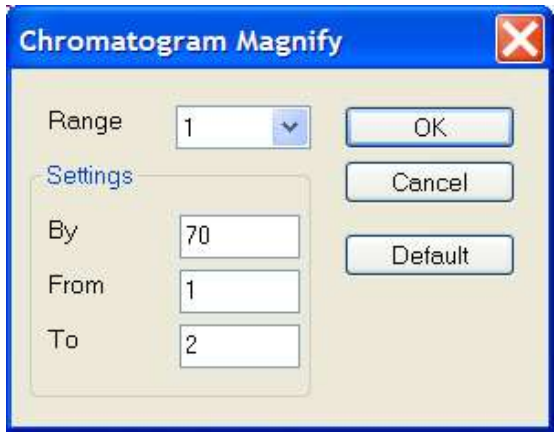
Display Chromatograms from Different Runs



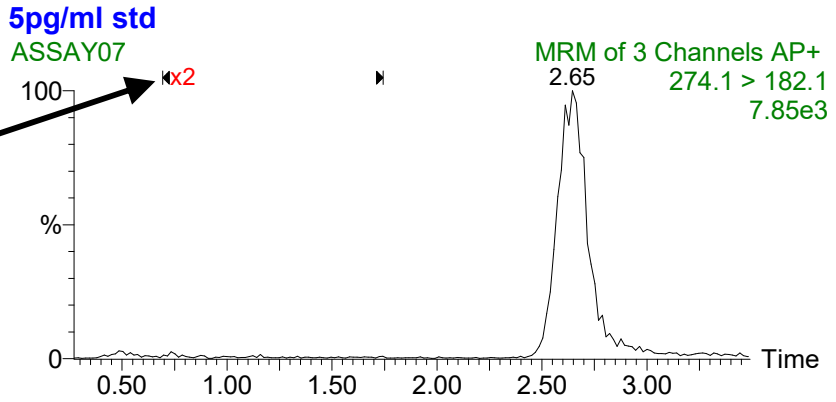
Magnifying the Chromatogram



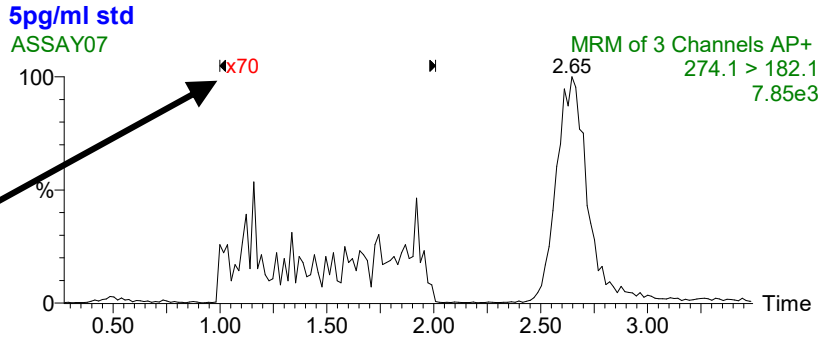
Fine Tuning the Magnification



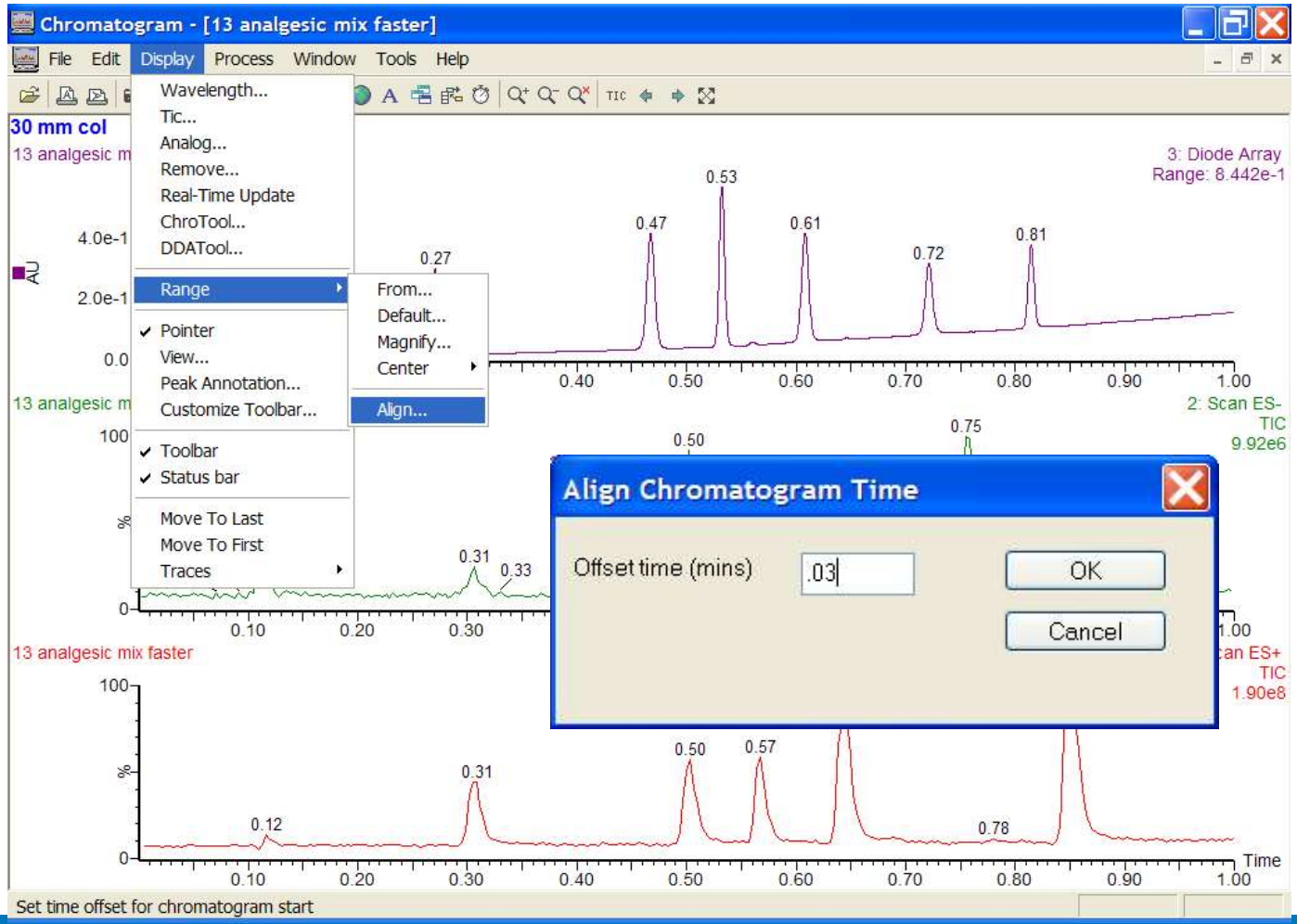
Magnification Heading



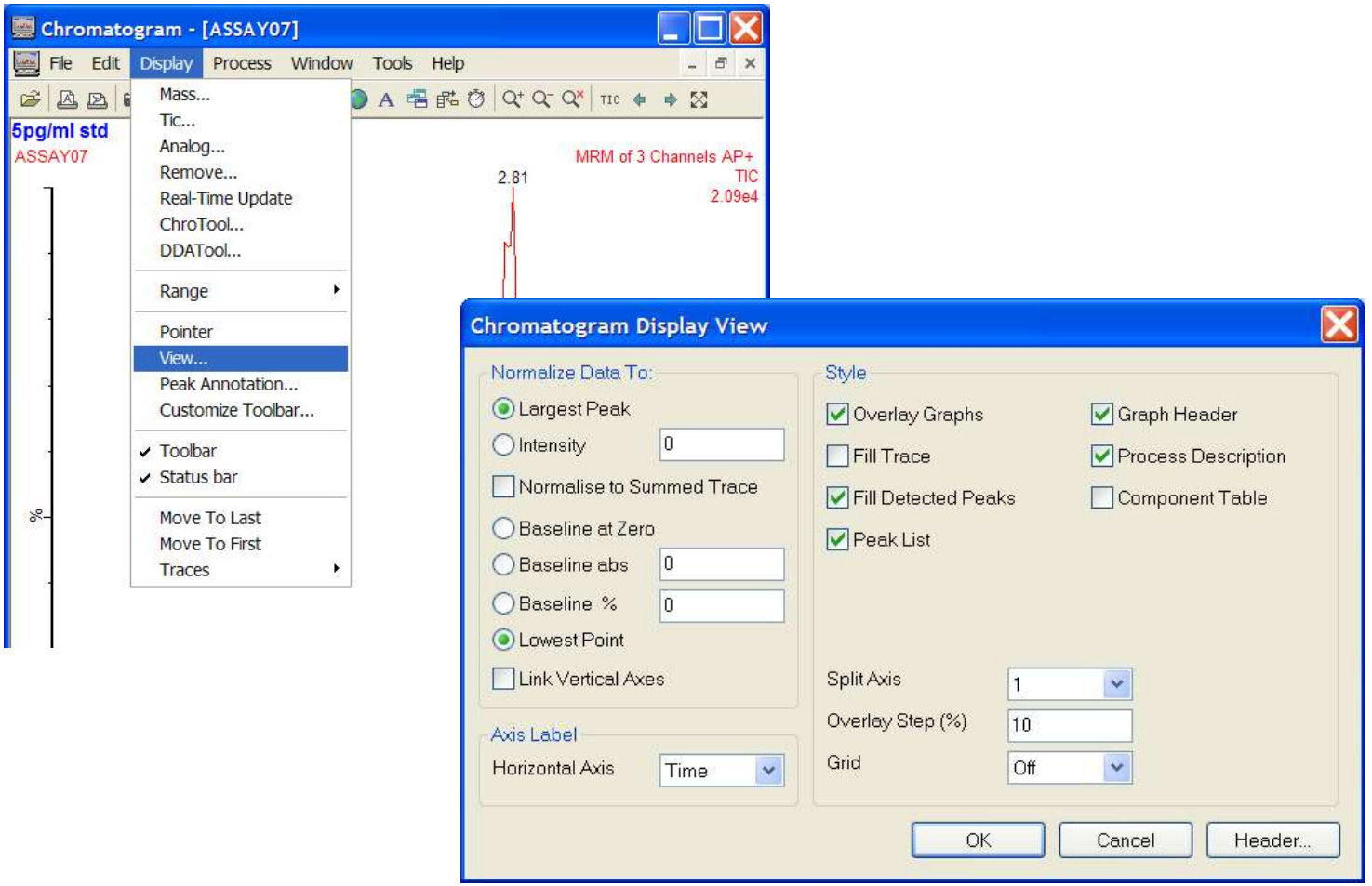
Magnification Results



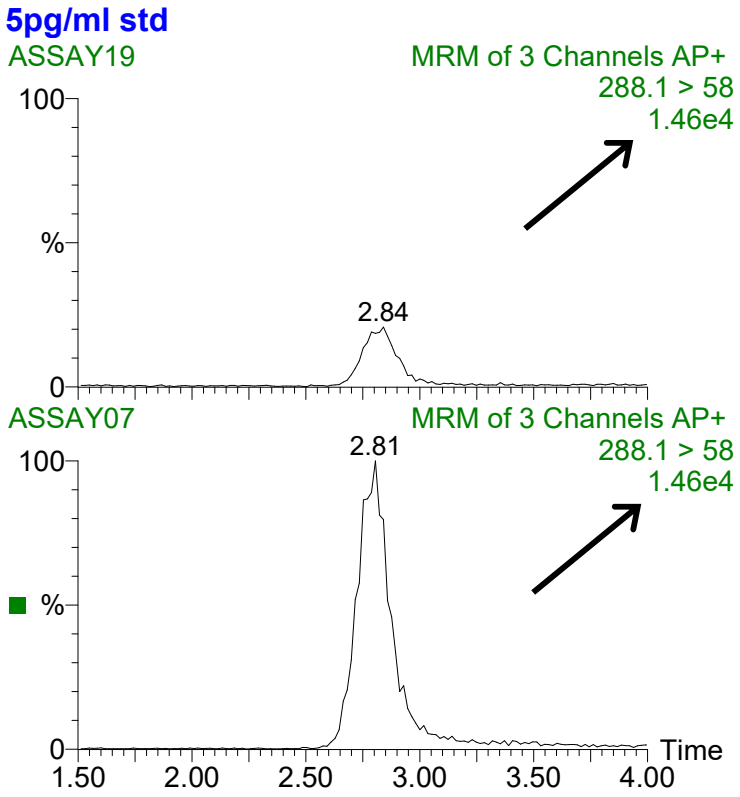
Align Chromatogram Time

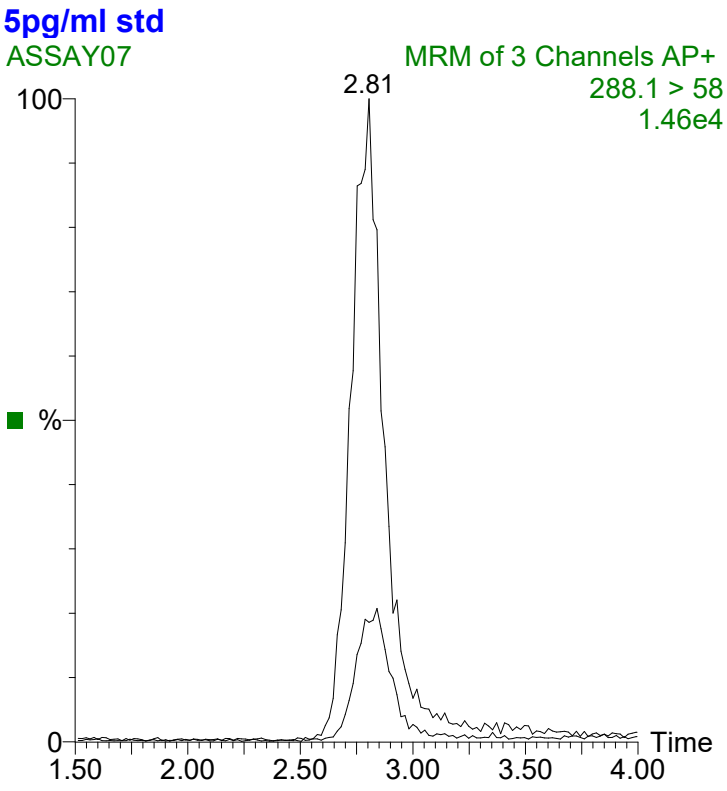


Altering Display Properties

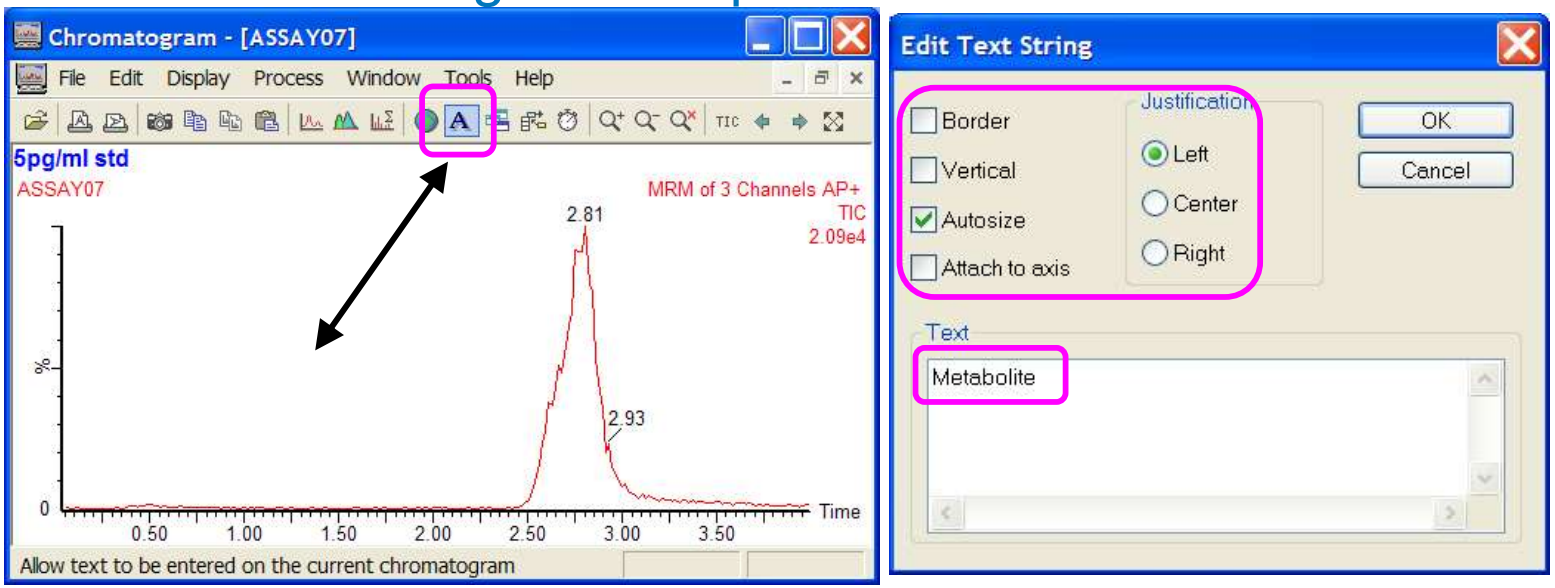


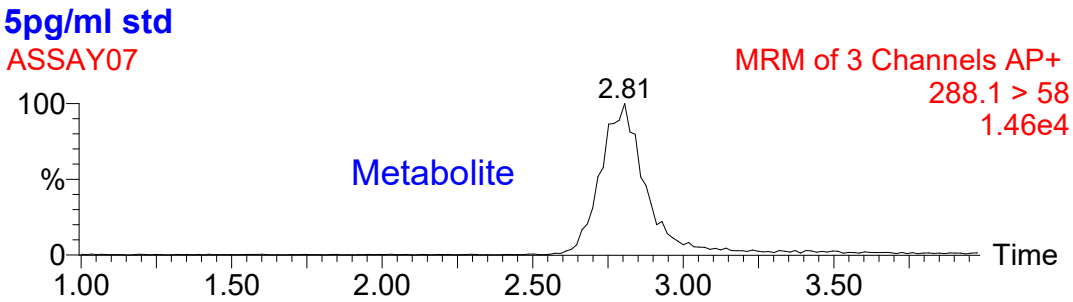
Link Vertical Axes





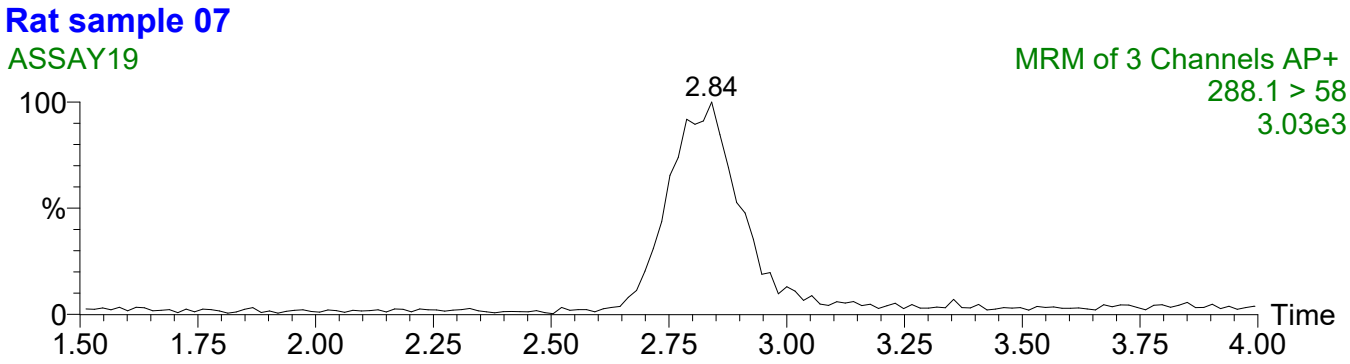
Adding Text to a Chromatogram or Spectrum

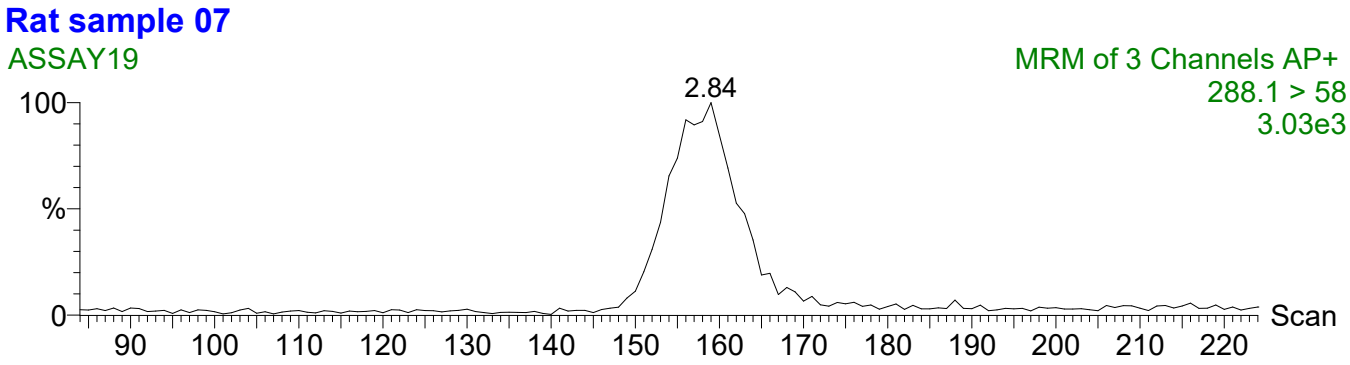




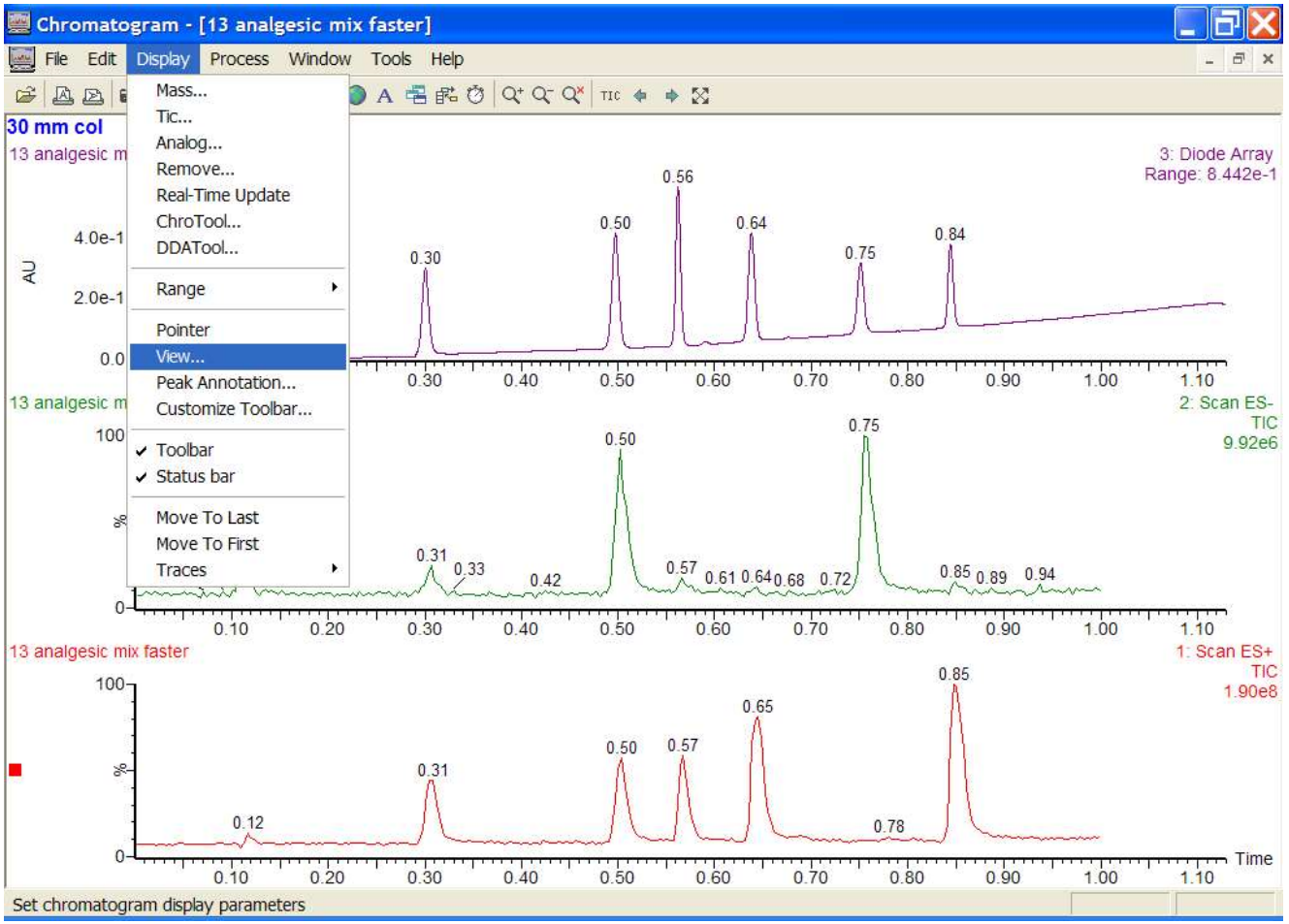
Chromatogram with Text

Display X Axis





Adding Header Information



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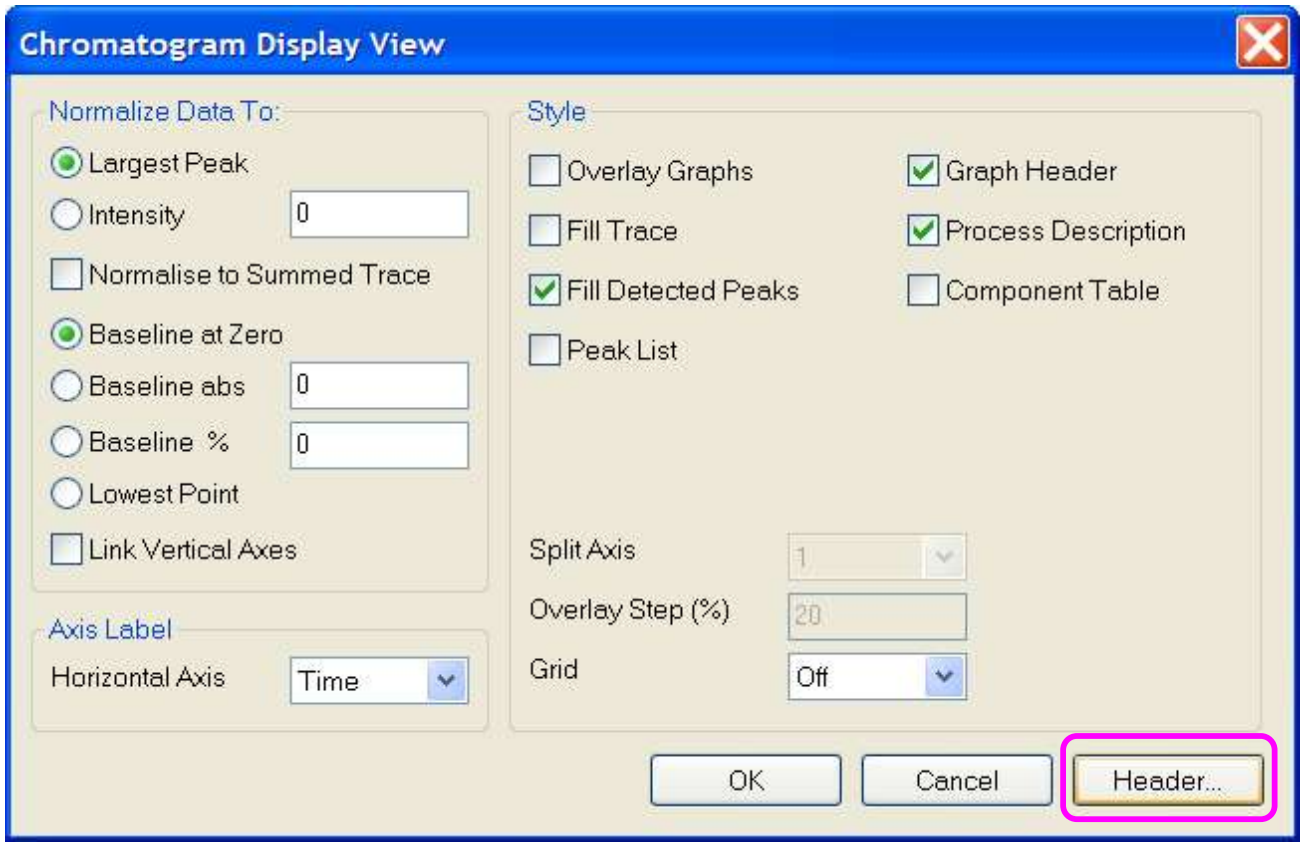

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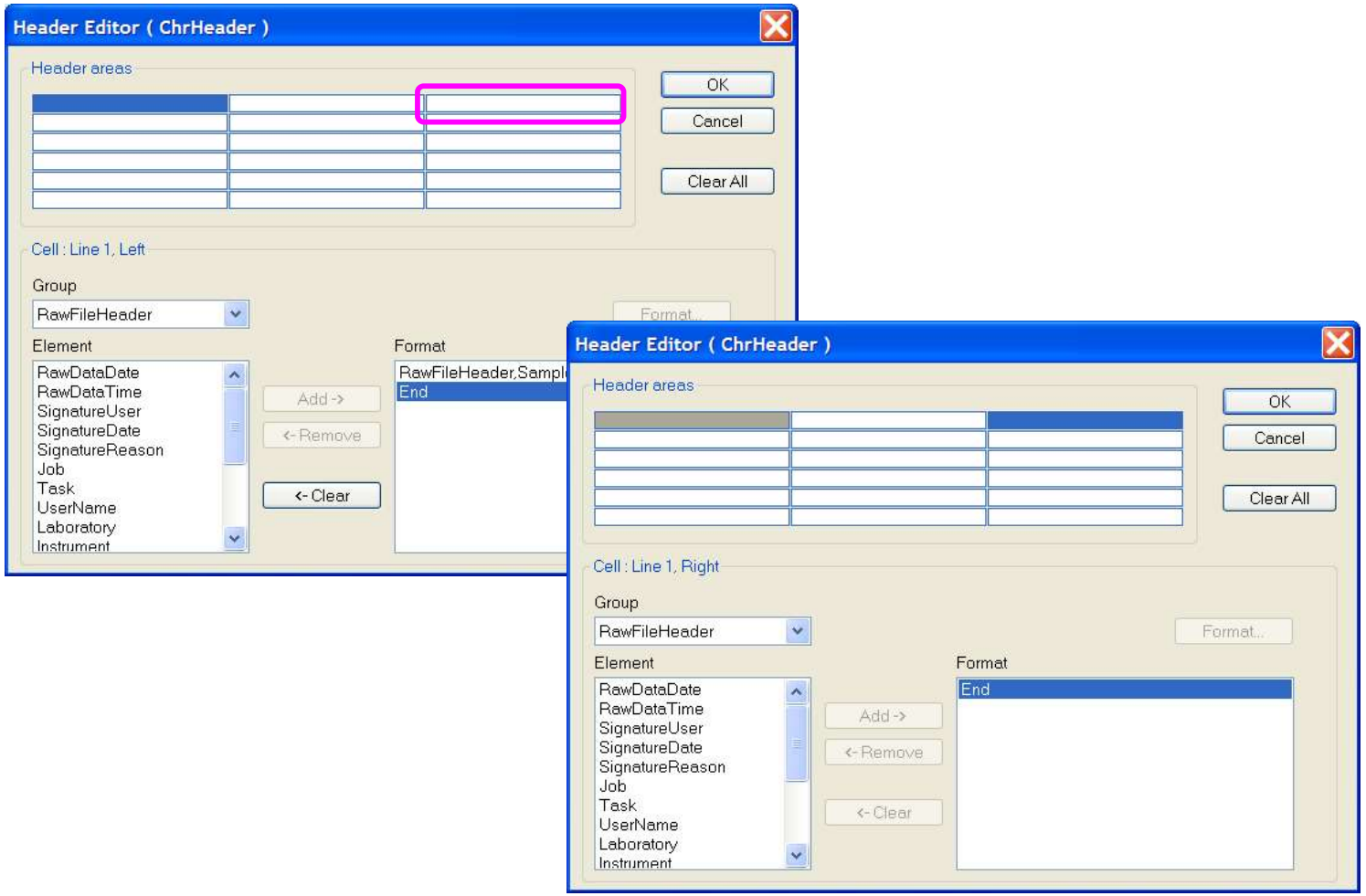

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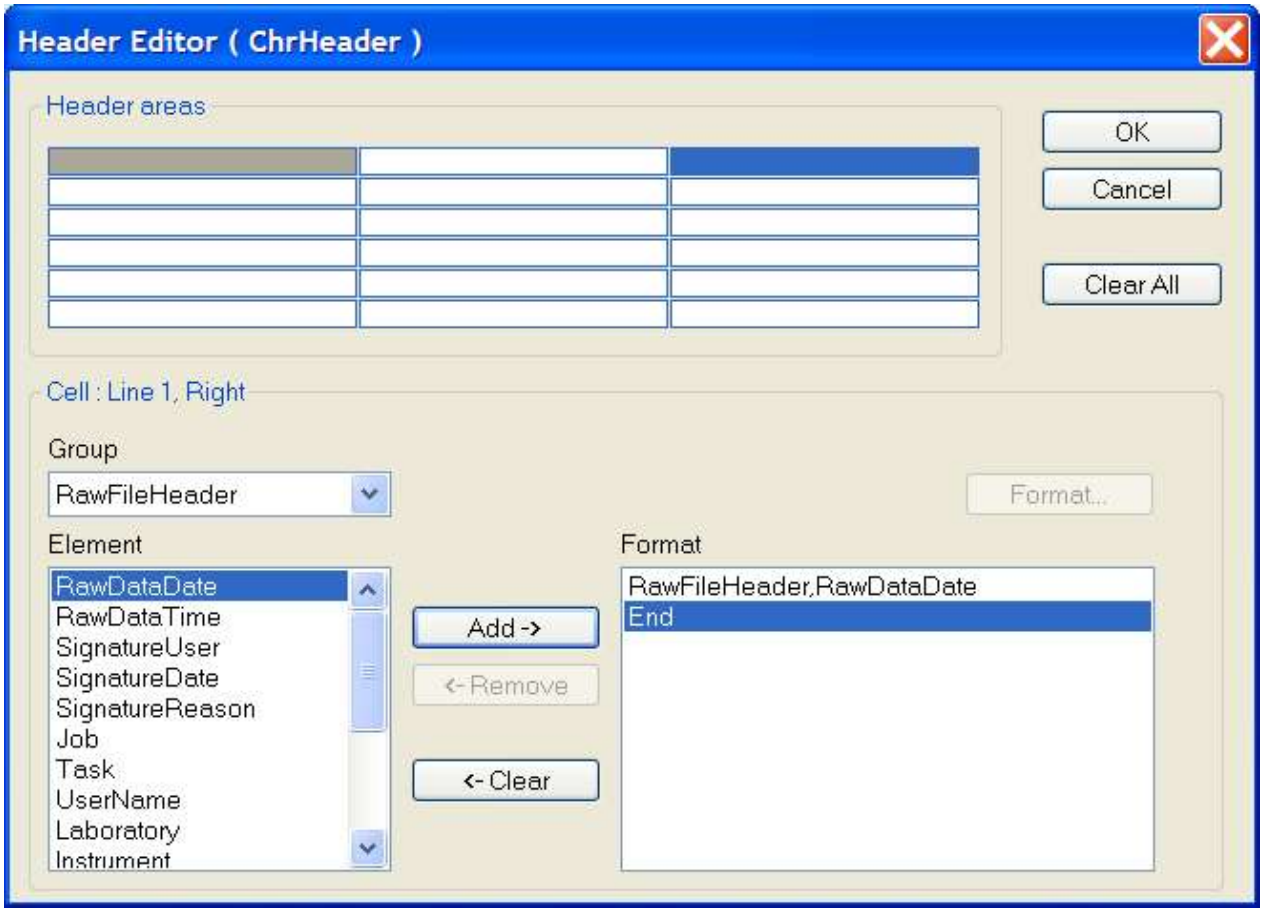

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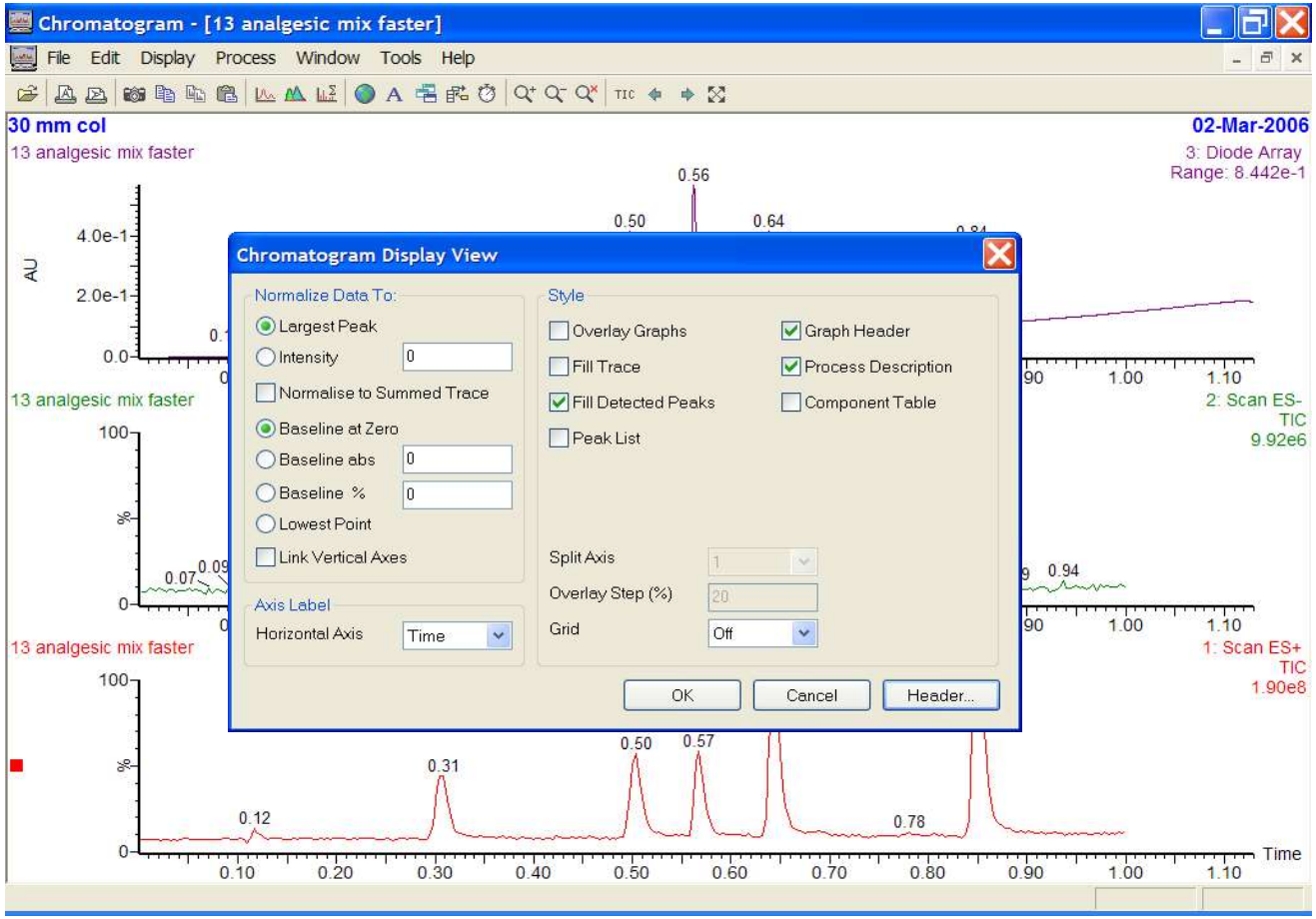
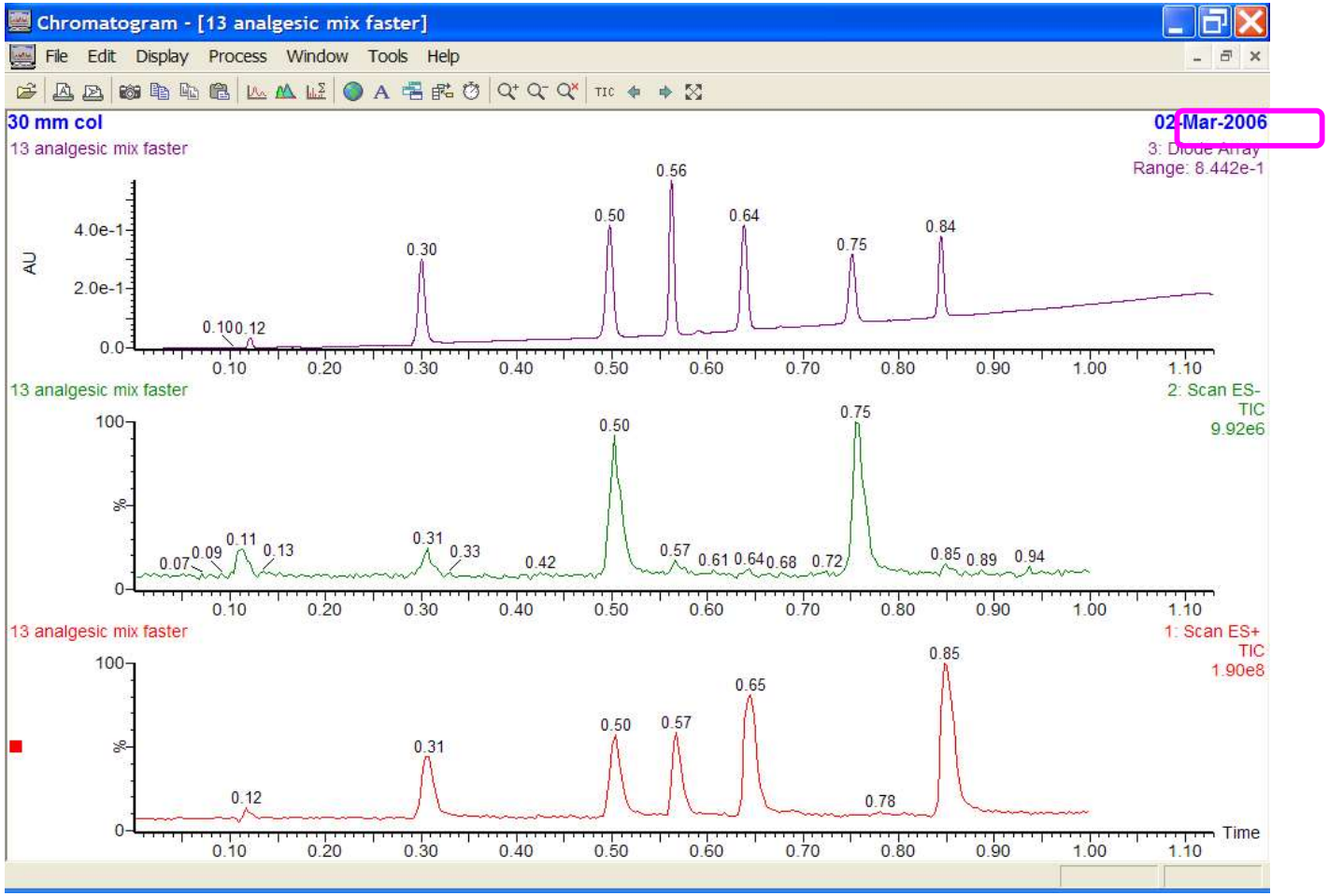


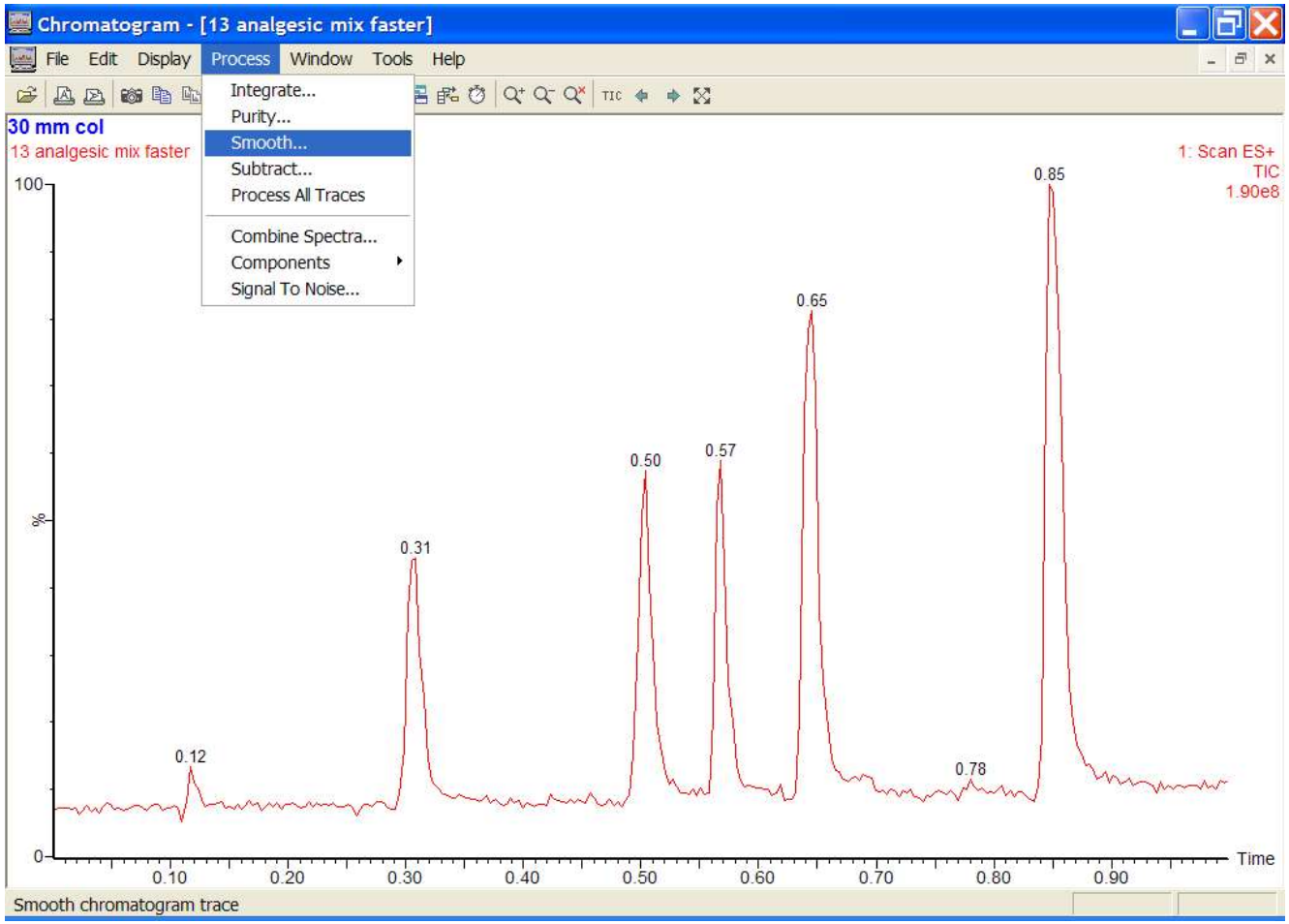
Adding Header Information



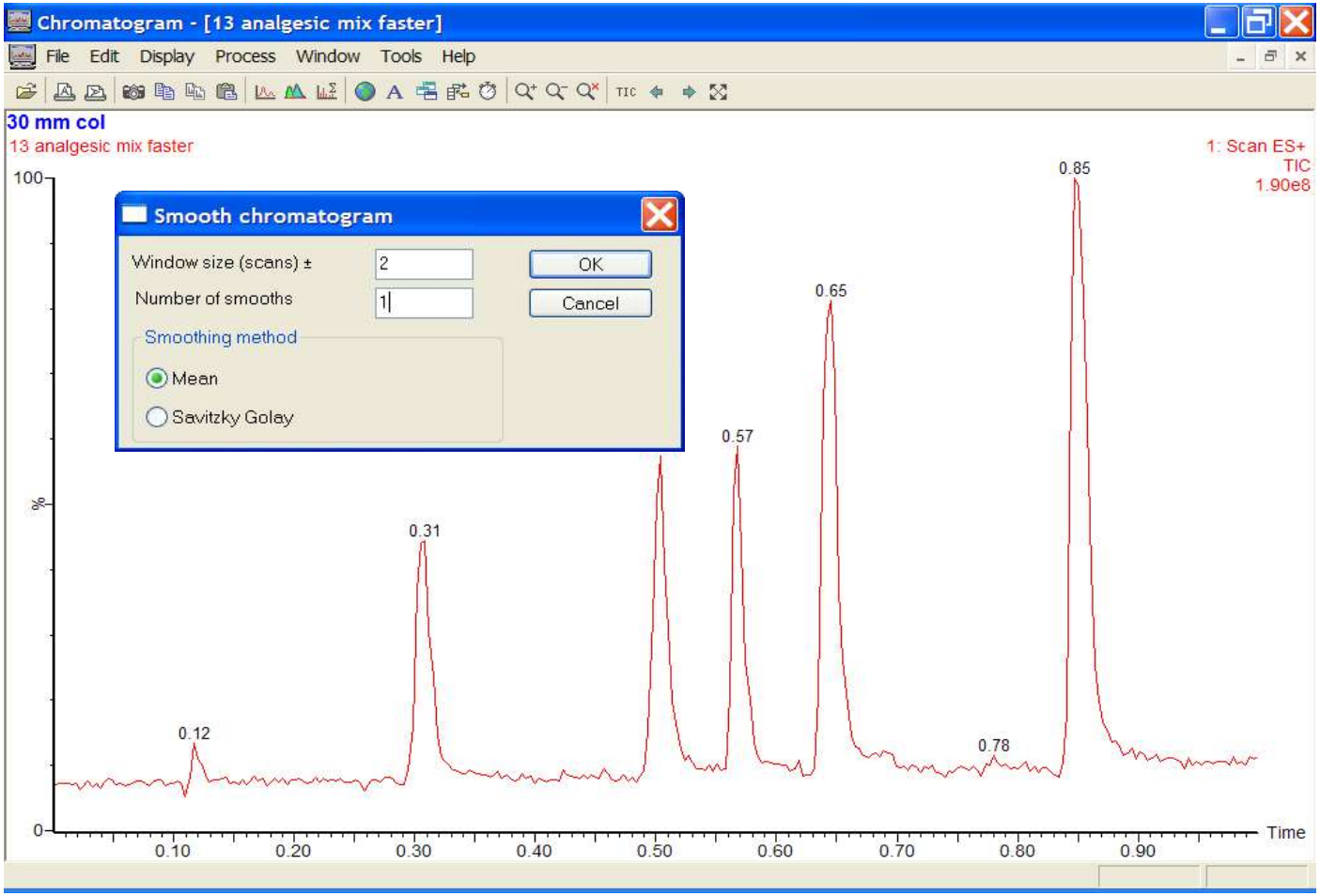
Chromatogram Processing

- Smoothing
- Background (Baseline) Subtraction
- Integration
  - Setting integration parameters
    - Baseline Defined Peak Integration
    - Apex Track Peak Integration
  - Integrating a selected chromatogram region
  - Editing detected peaks
  - Peak annotation
- Signal-to-Noise

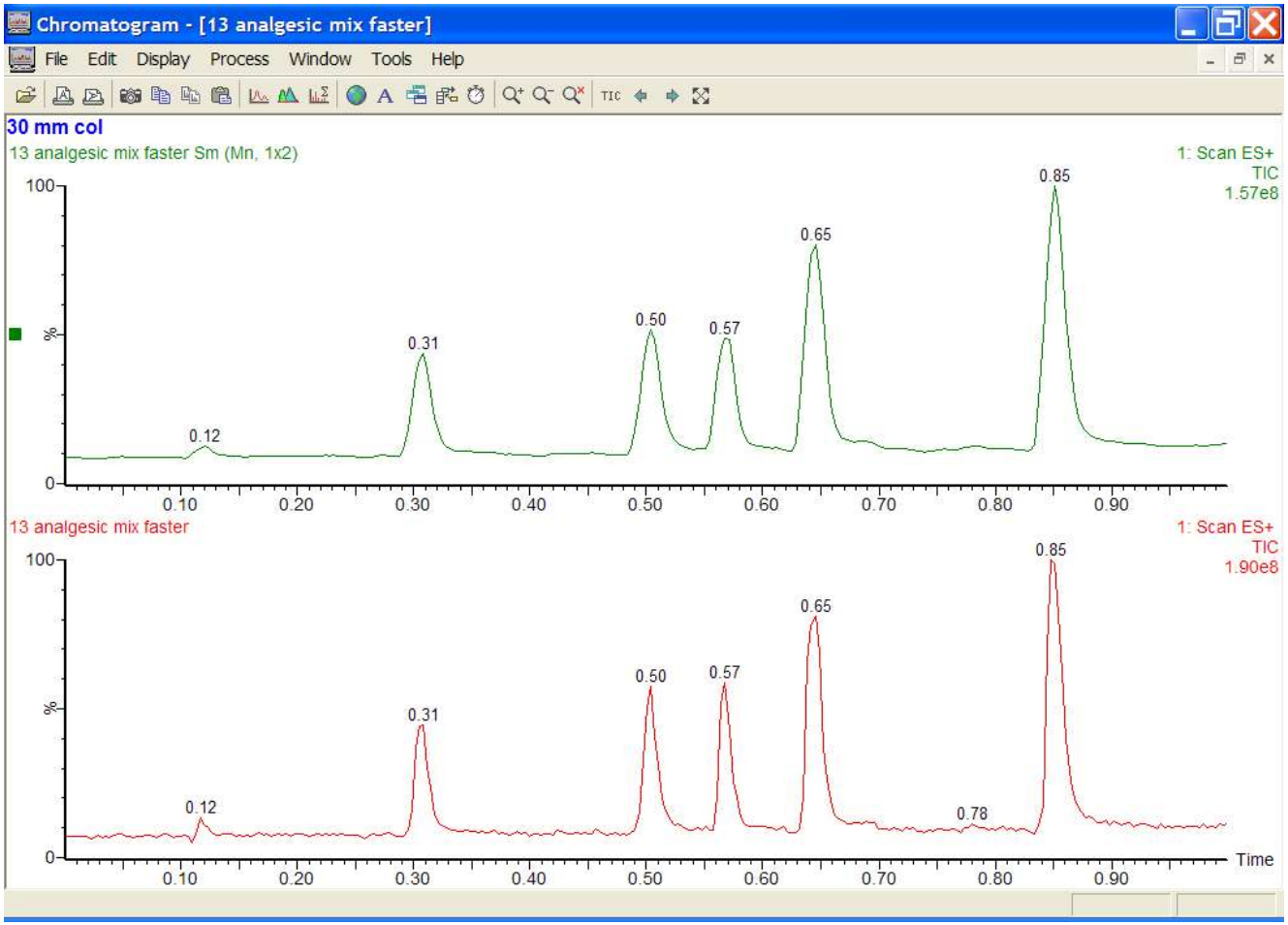
Smoothing a Chromatogram



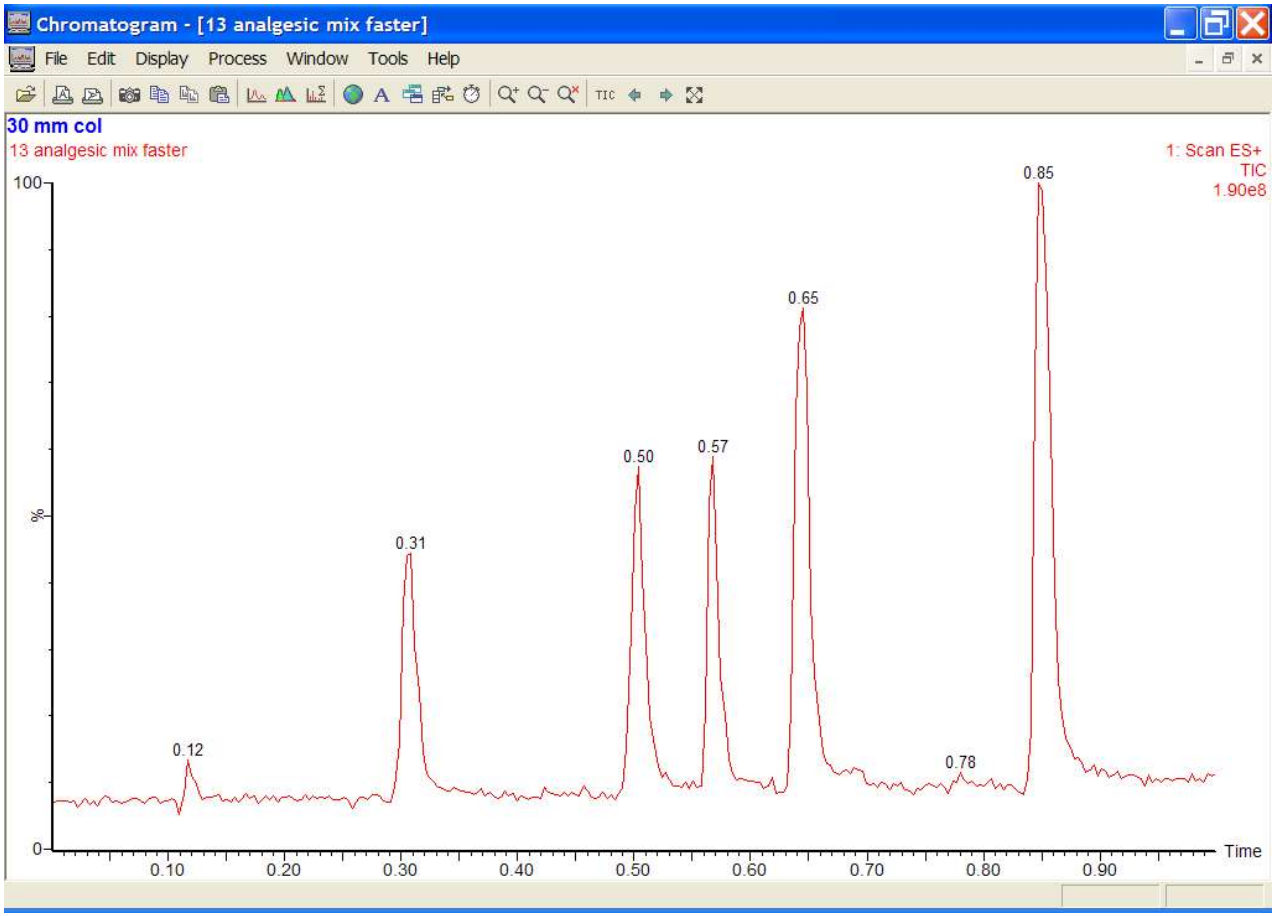
Smoothing a Chromatogram



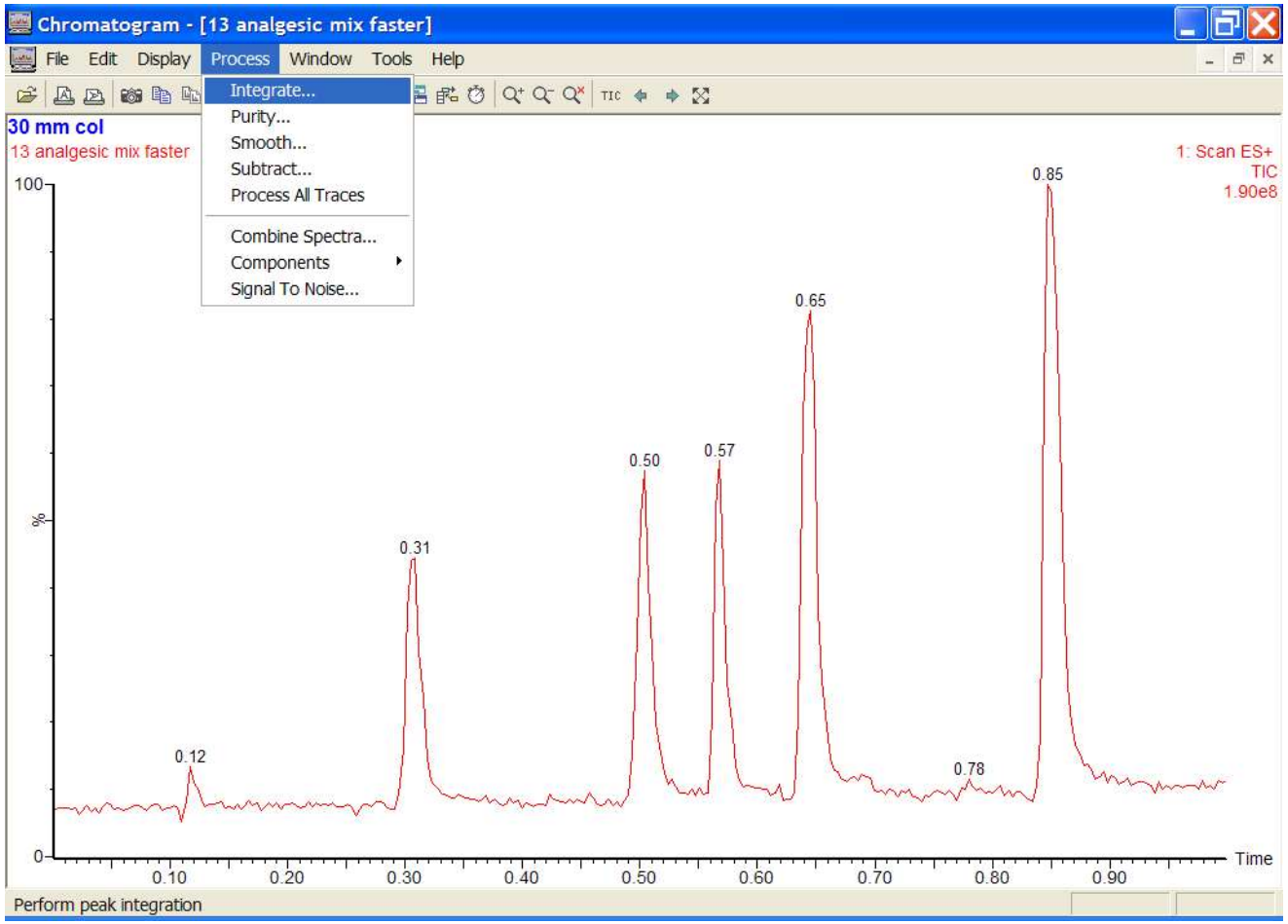
Smoothing a Chromatogram



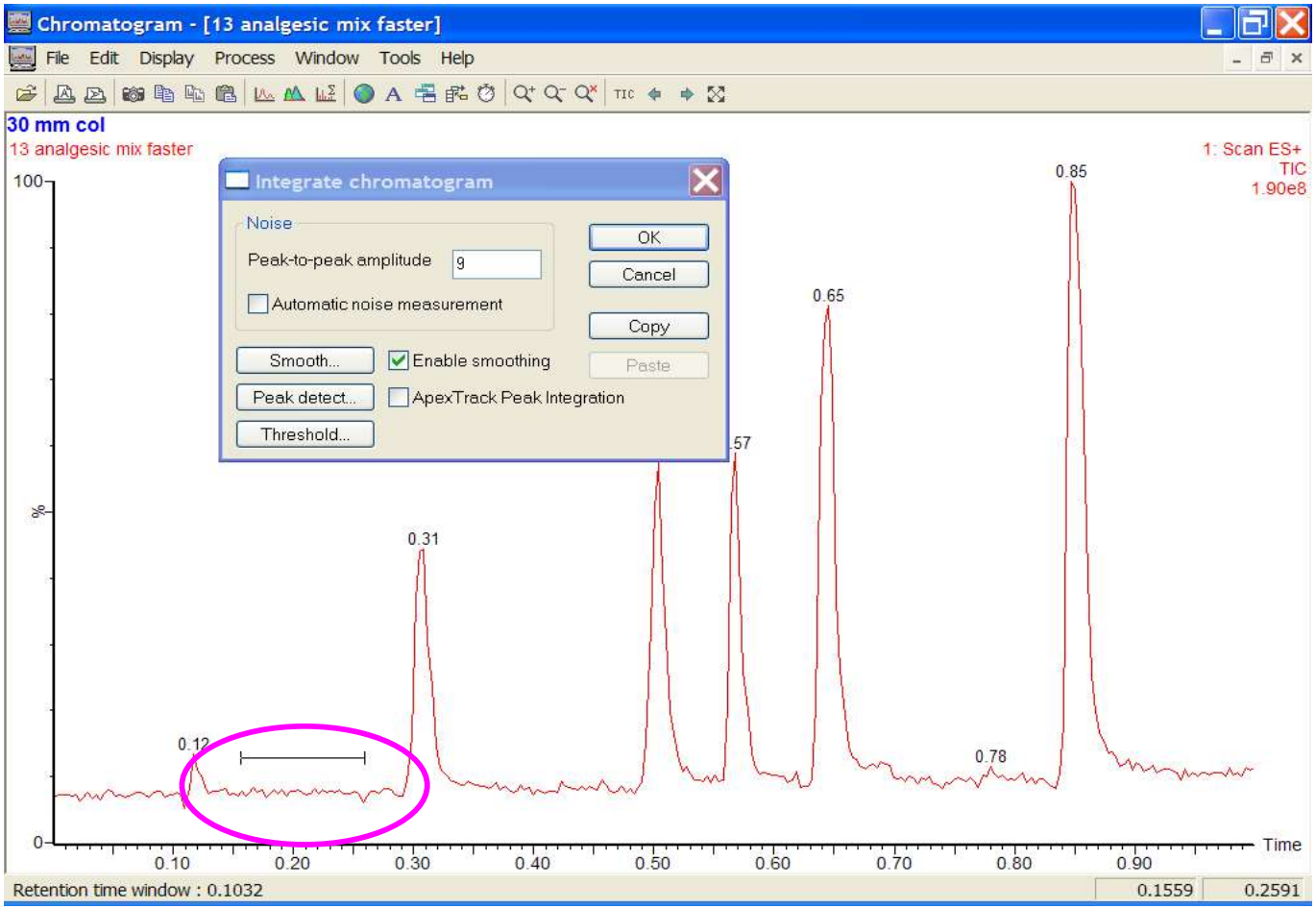
Integrating a Chromatogram



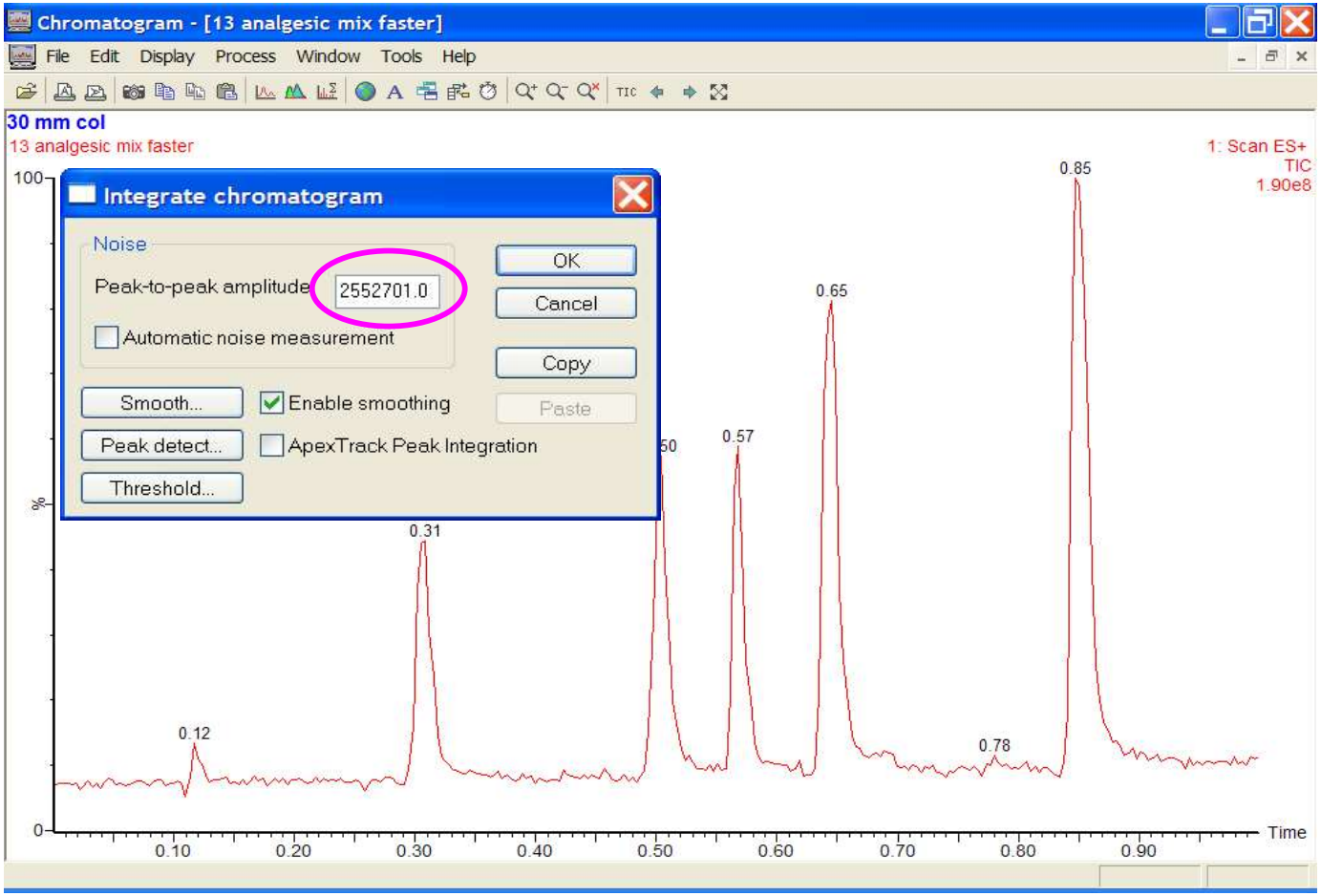
Integrating a Chromatogram



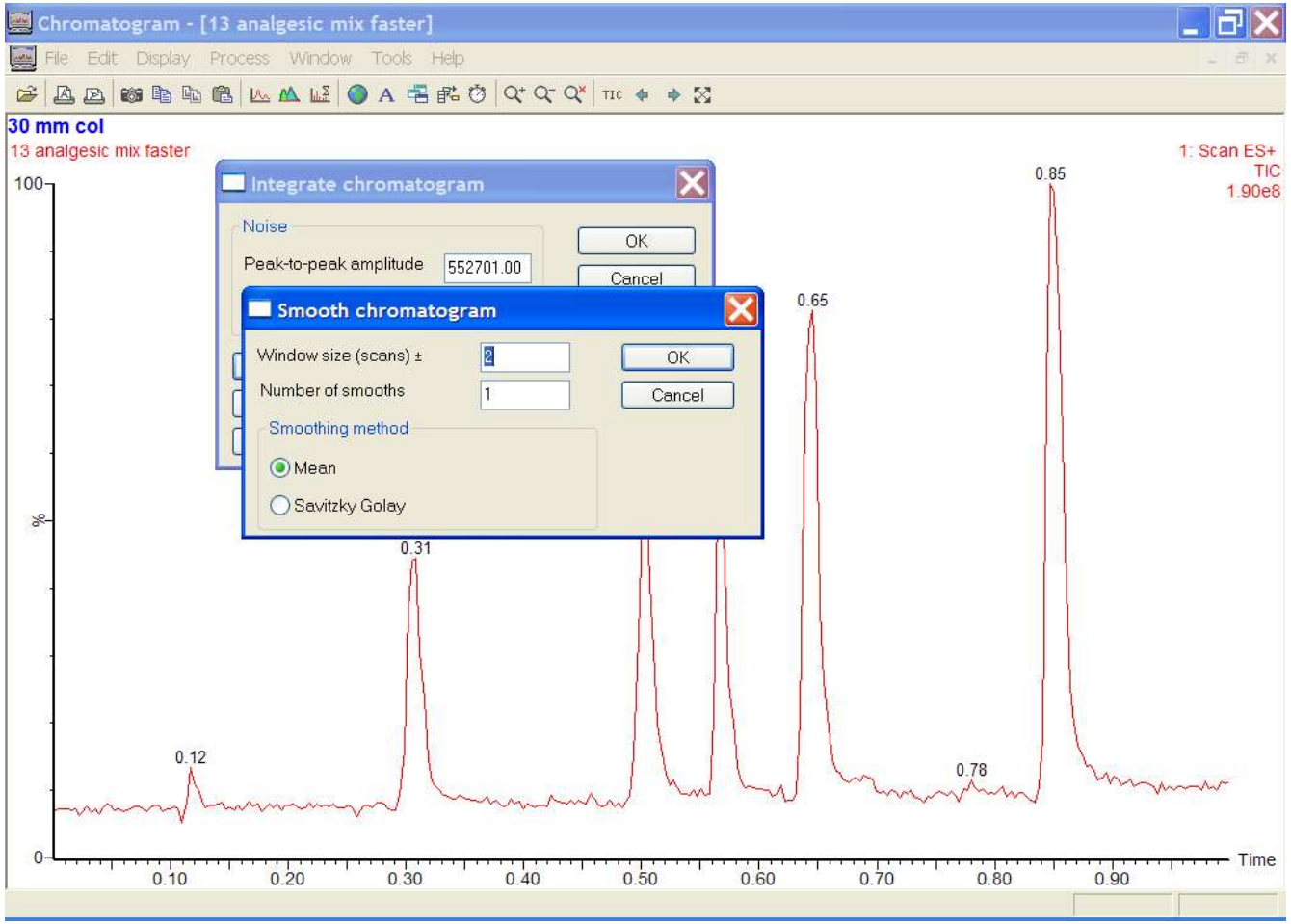
Integrating a Chromatogram



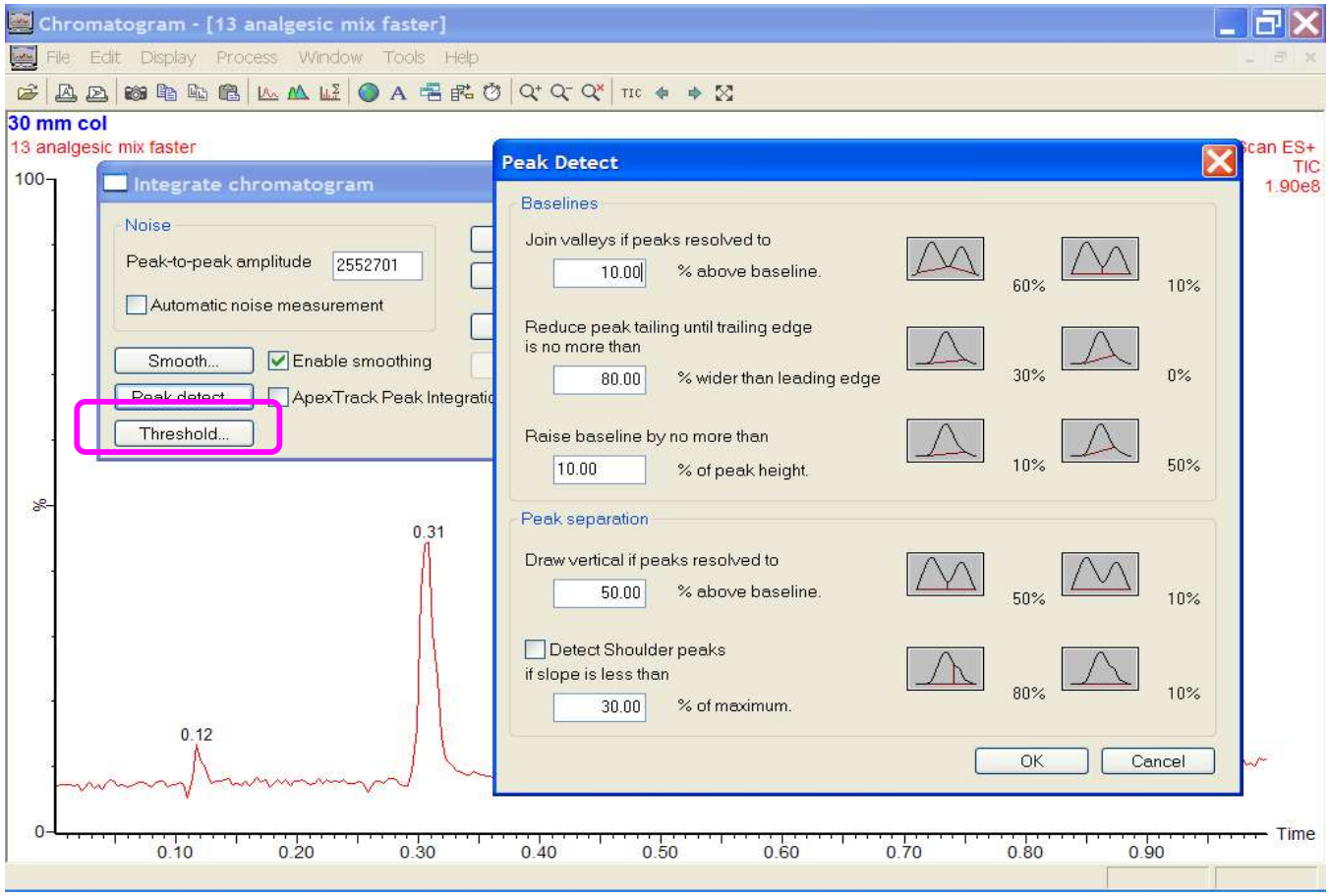
Integrating a Chromatogram



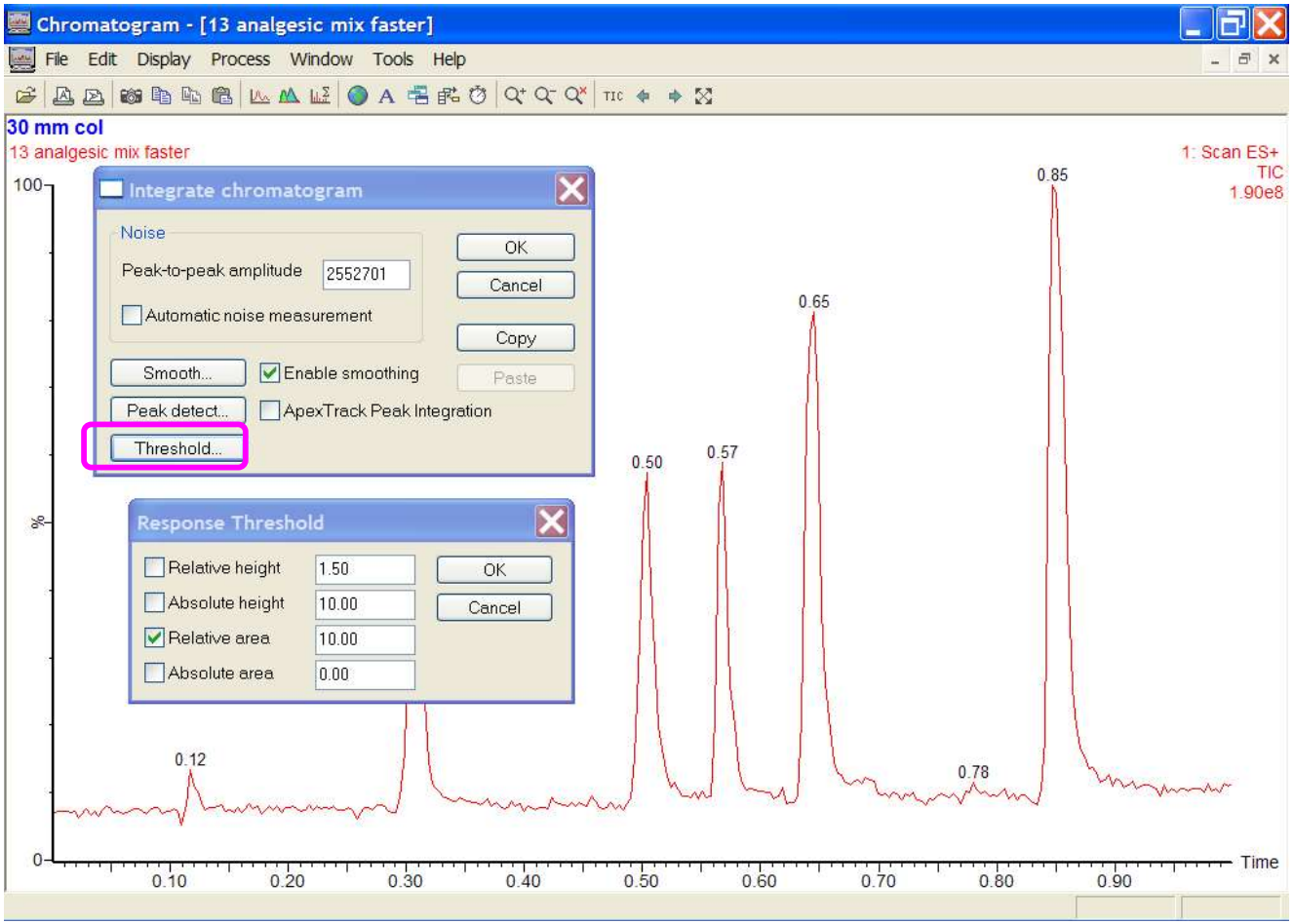
Integrating a Chromatogram



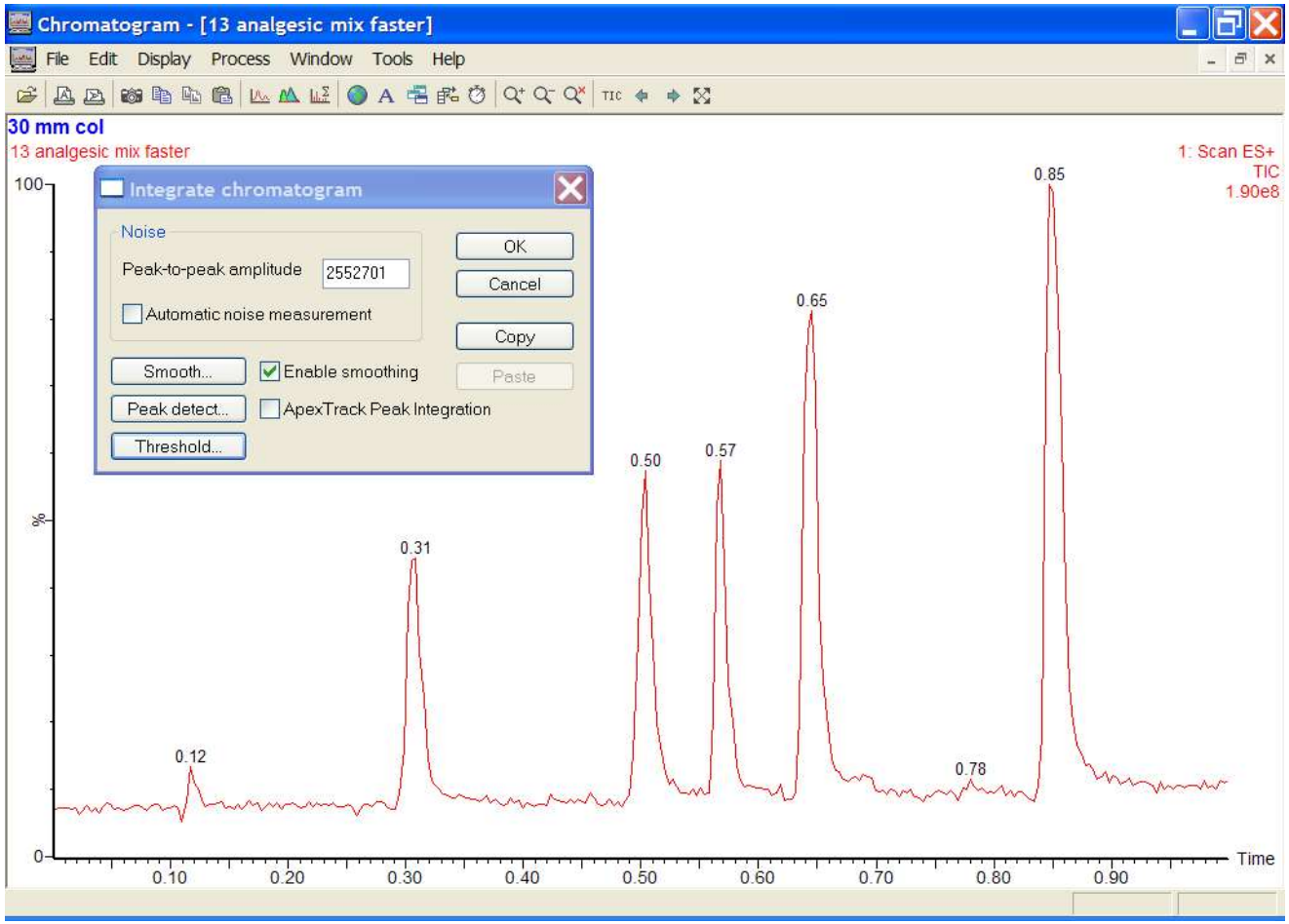
Integrating a Chromatogram



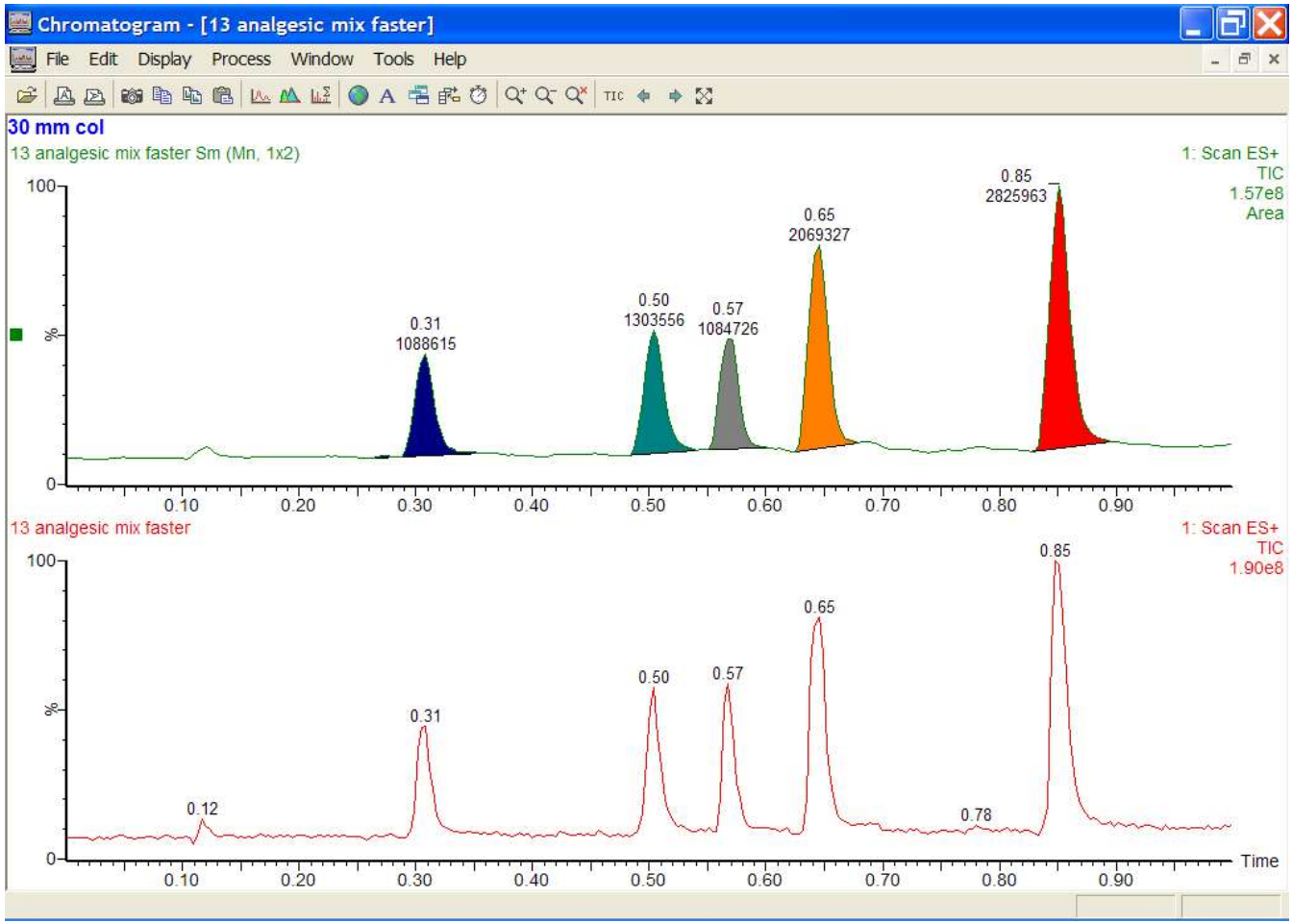
Integrating a Chromatogram



Integrating a Chromatogram



Integrating Chromatograms

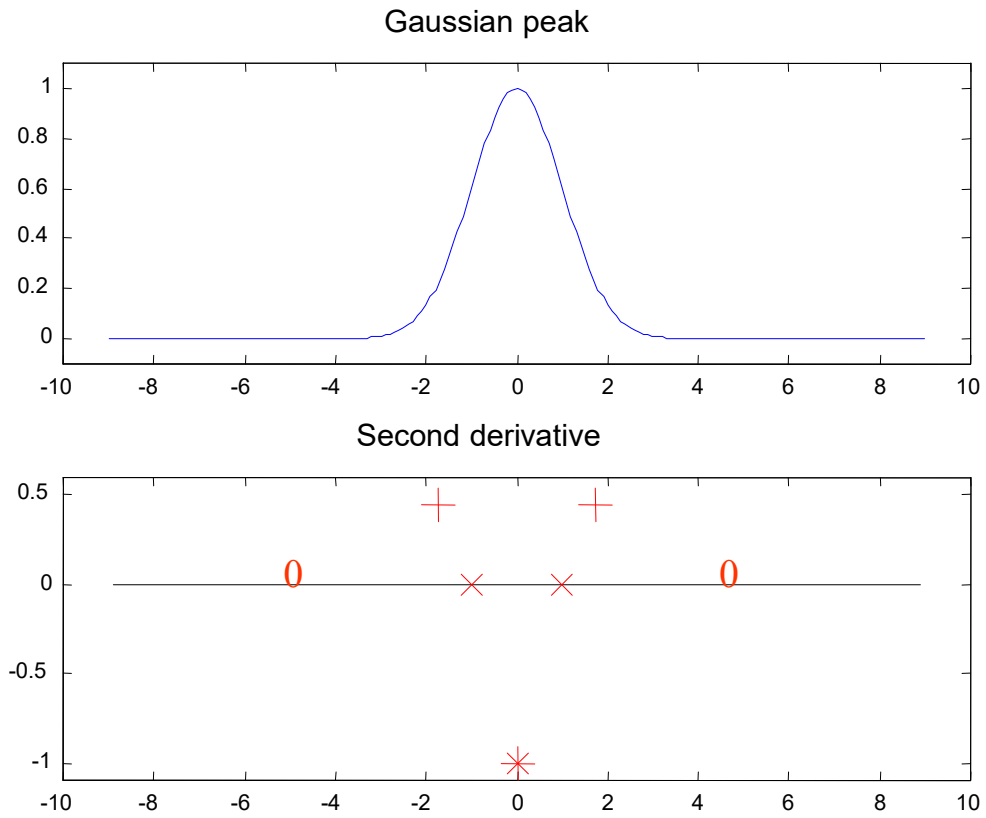


ApexTrack Peak Integration

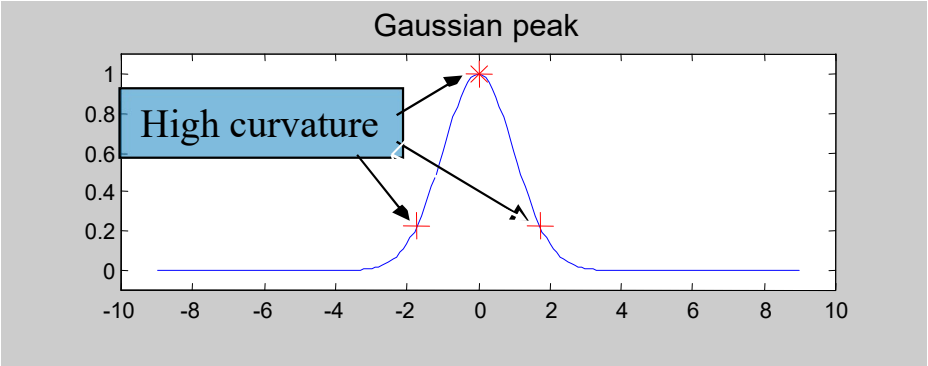
Waters™

- Major Advantages
  - Peak apices detected by curvature
  - Apex detection independent of baseline determination
  - Reliable shoulder detection
  - Adjustable baseline criterion

Curvature of a Peak



Curvature of a Peak



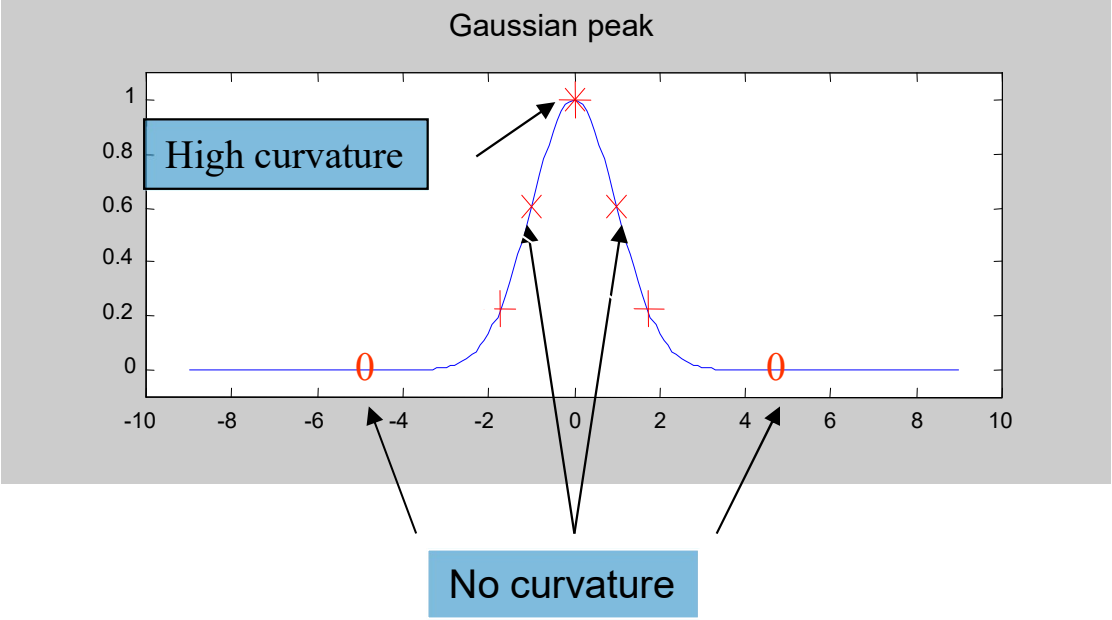
Apex has high curvature.

Inflection points have no curvature.

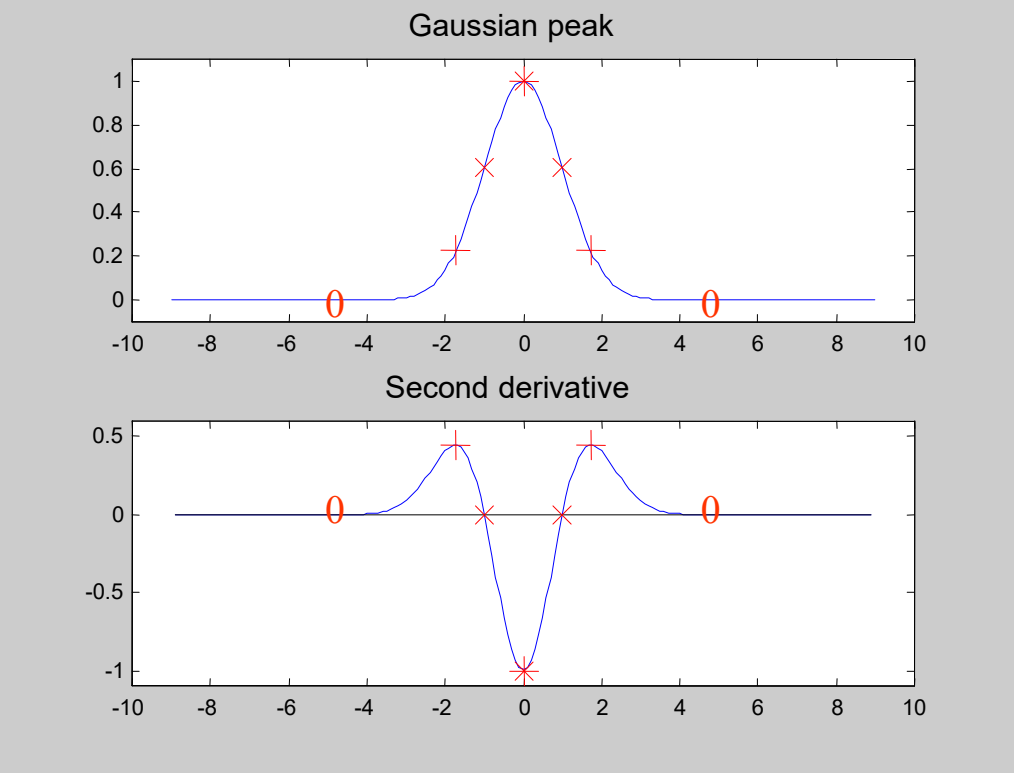
Upslope/downslope has high curvature.

Baseline has no curvature.

Curvature of a Peak



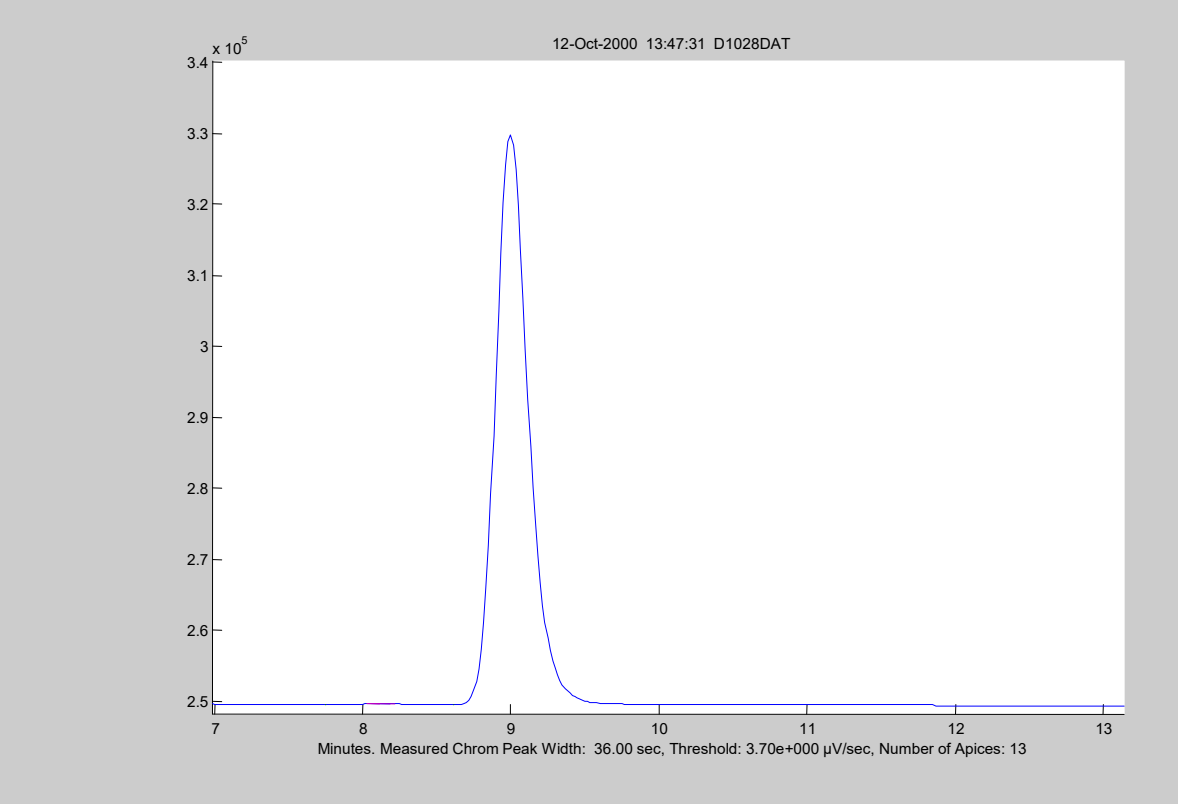
The Complete Second Derivative



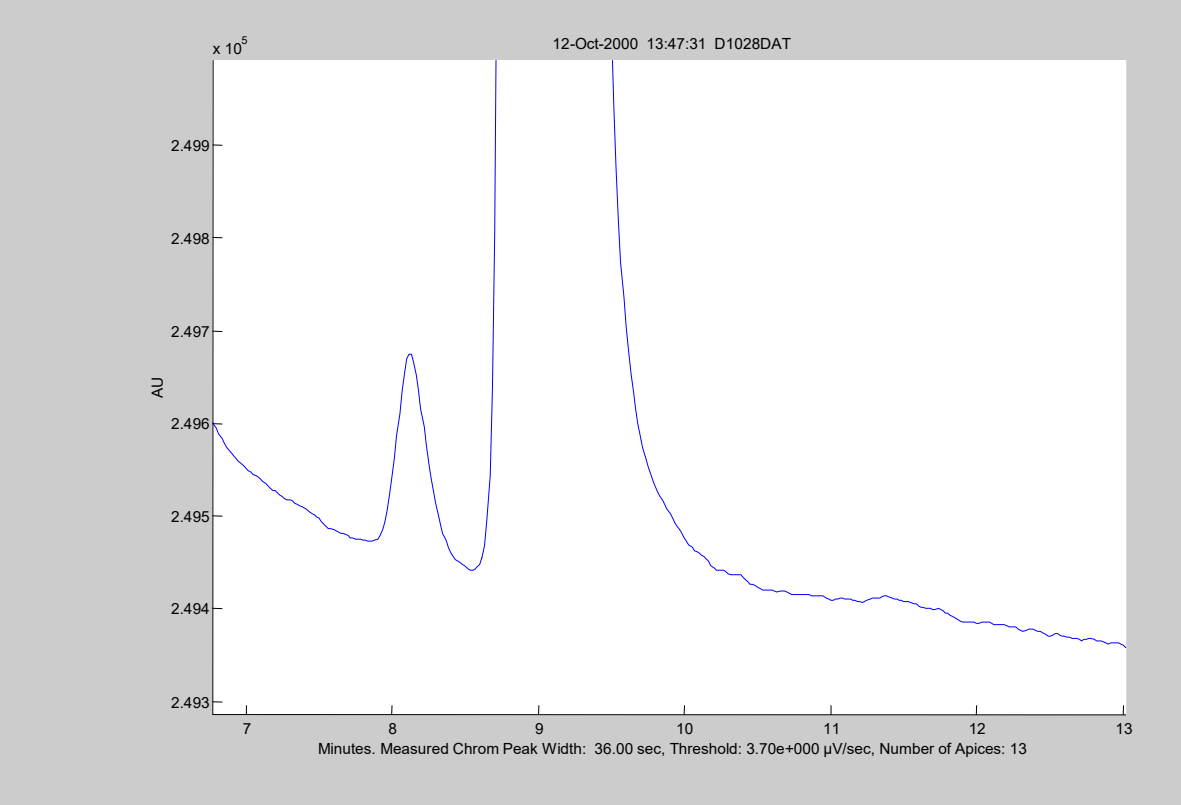
Apex Detection

- Computation of second derivative
  - Automatic Determination of Peak Width
  - Automatic Determination of Apex Detection Threshold
- Detection of peak apices
  - Identification of Inflection points

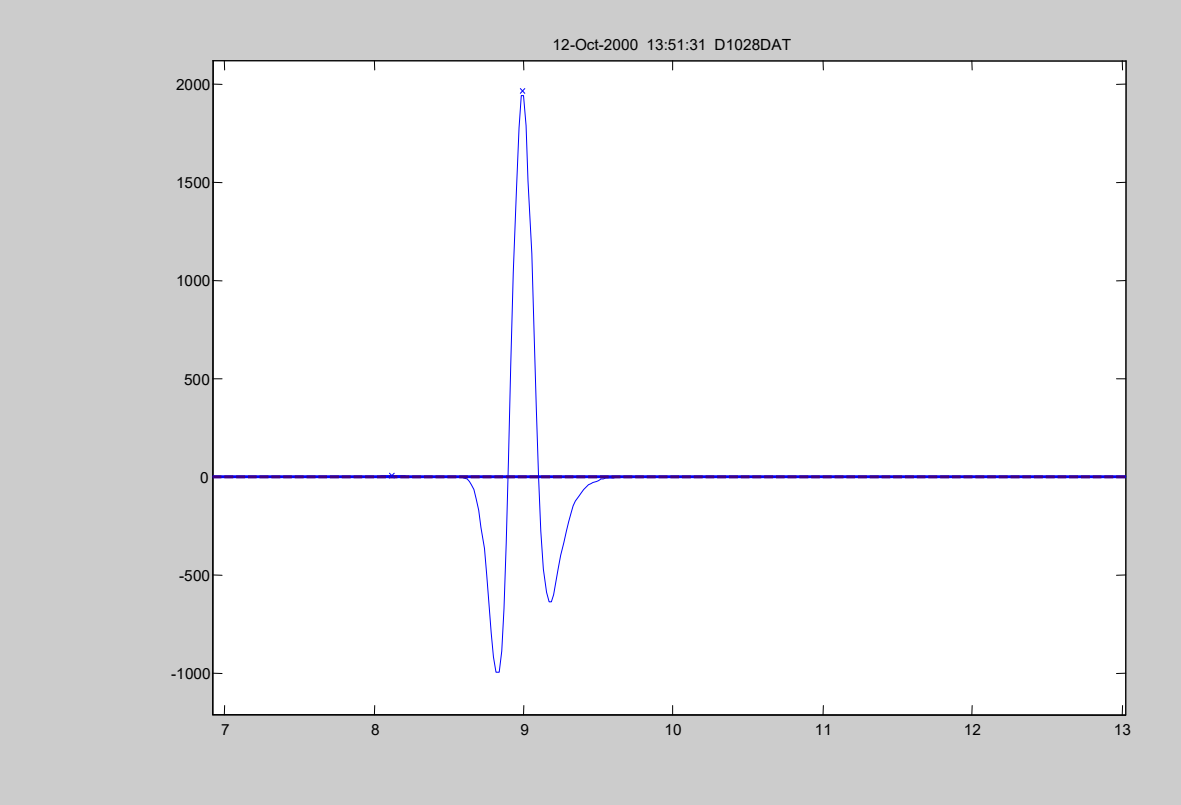
Two Peaks



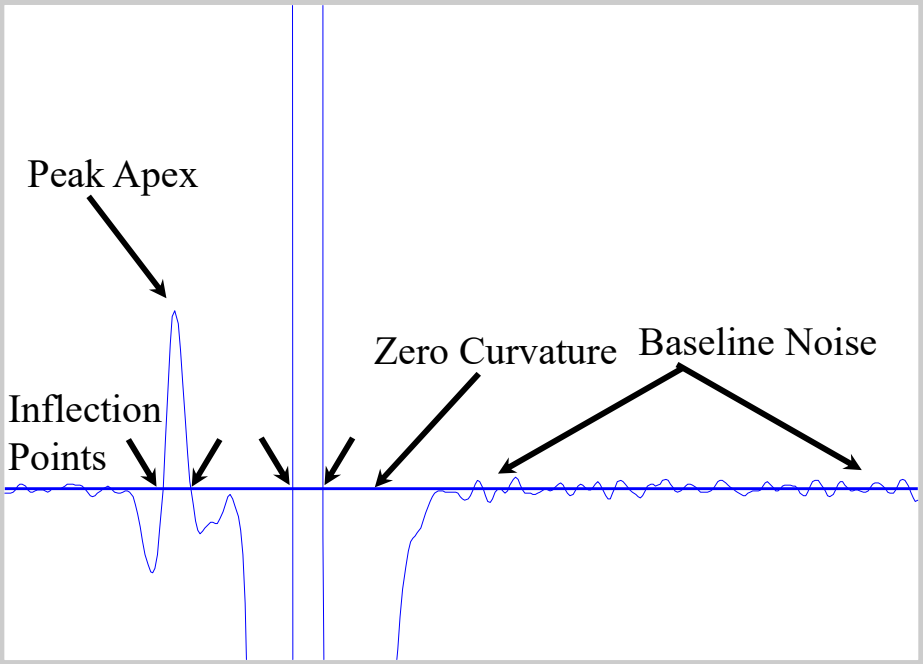
Zoom in on Baseline



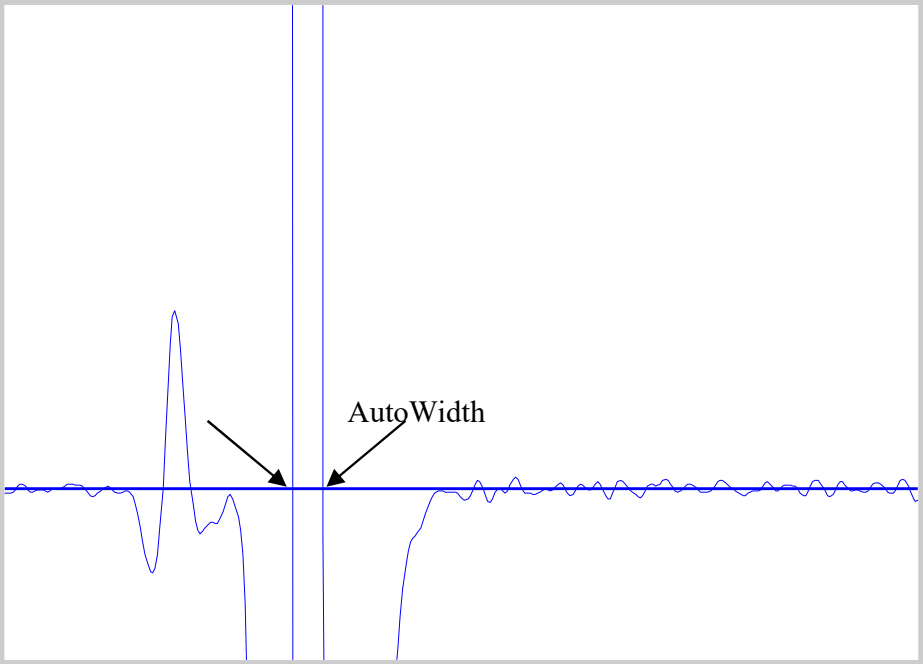
Second Derivative



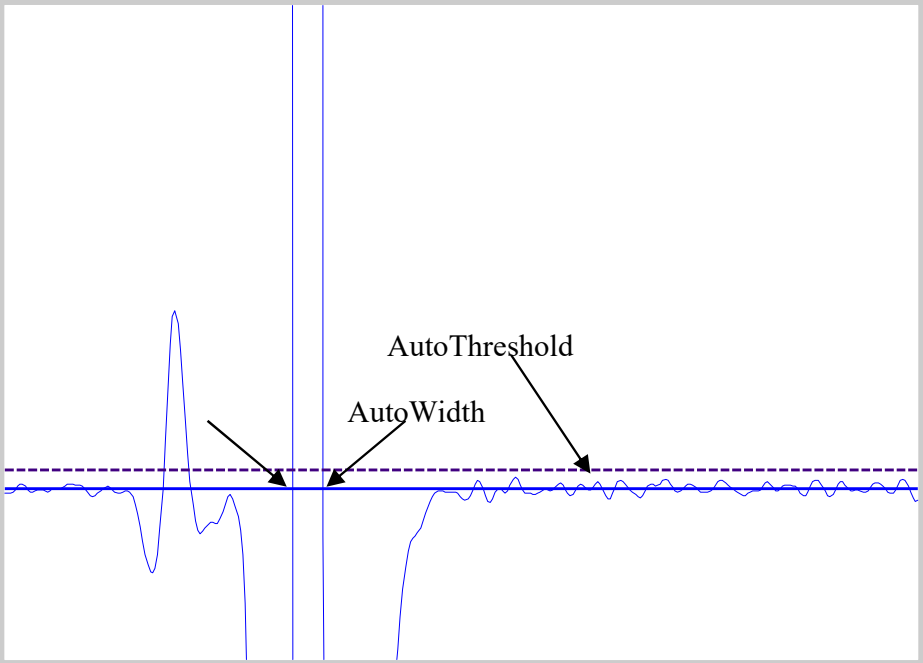
Zoom in on Second Derivative



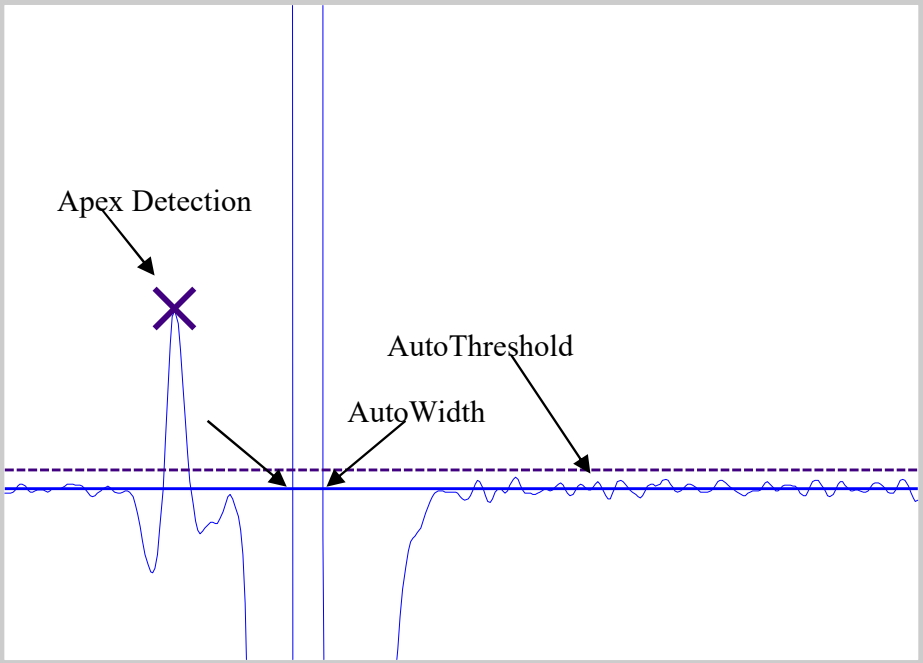
AutoWidth



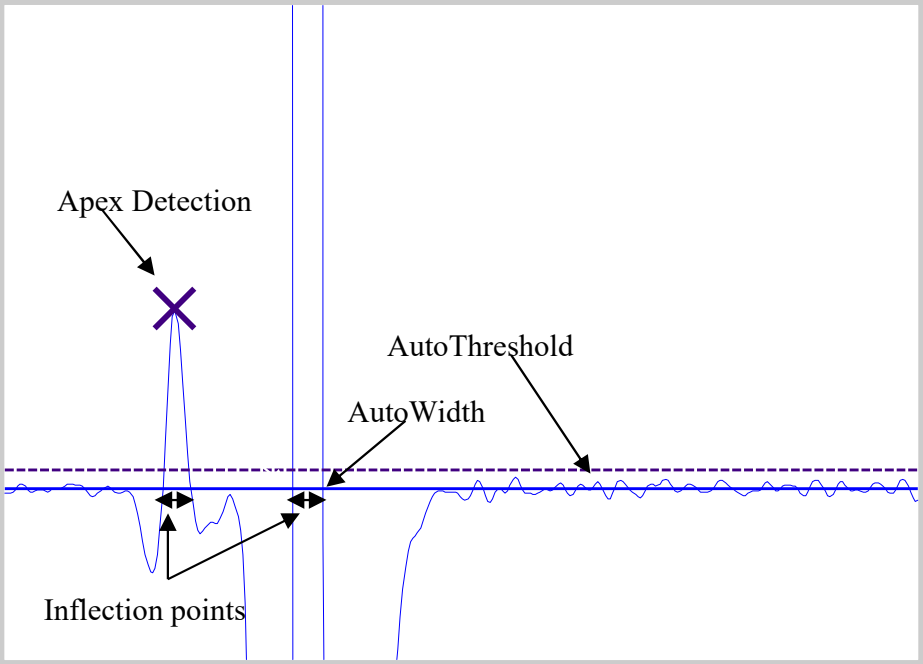
AutoThreshold



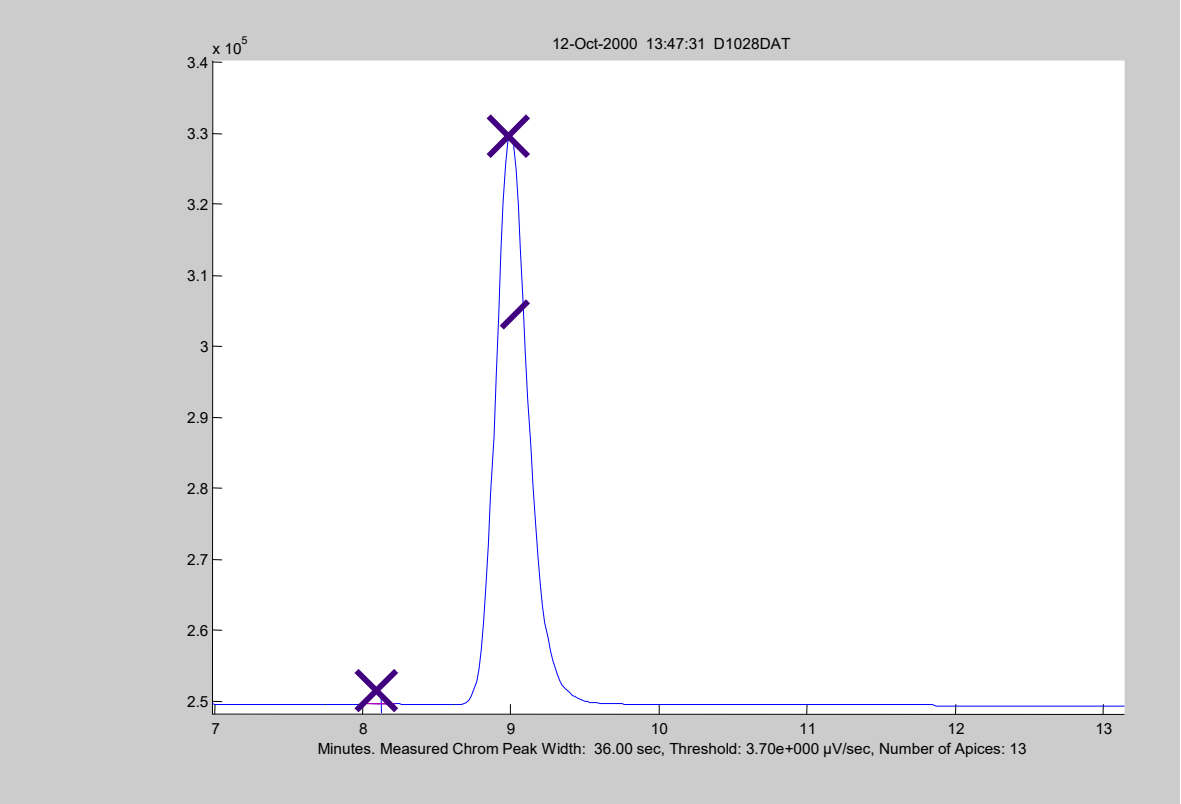
Apex Detection



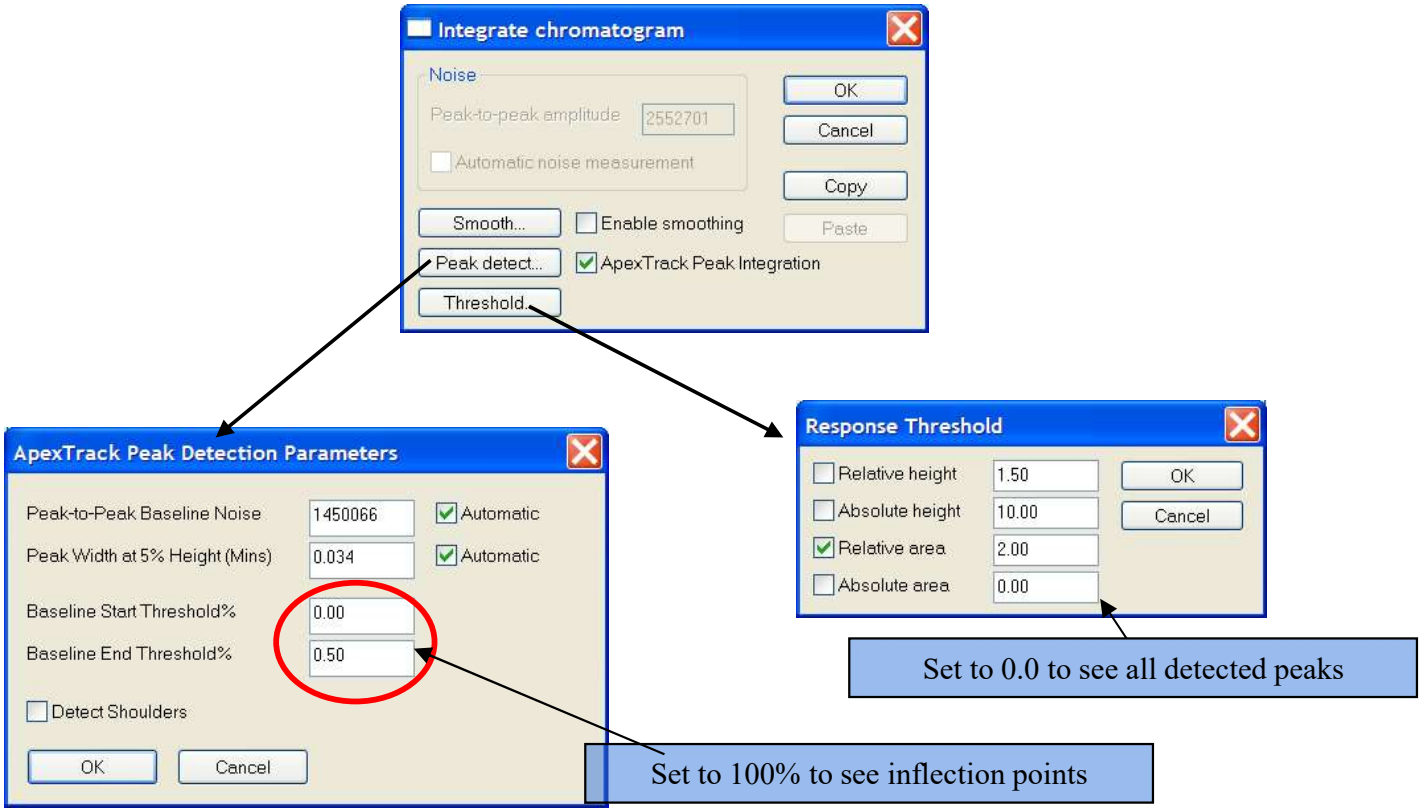
Identify Inflection Points



Mark up Chromatogram



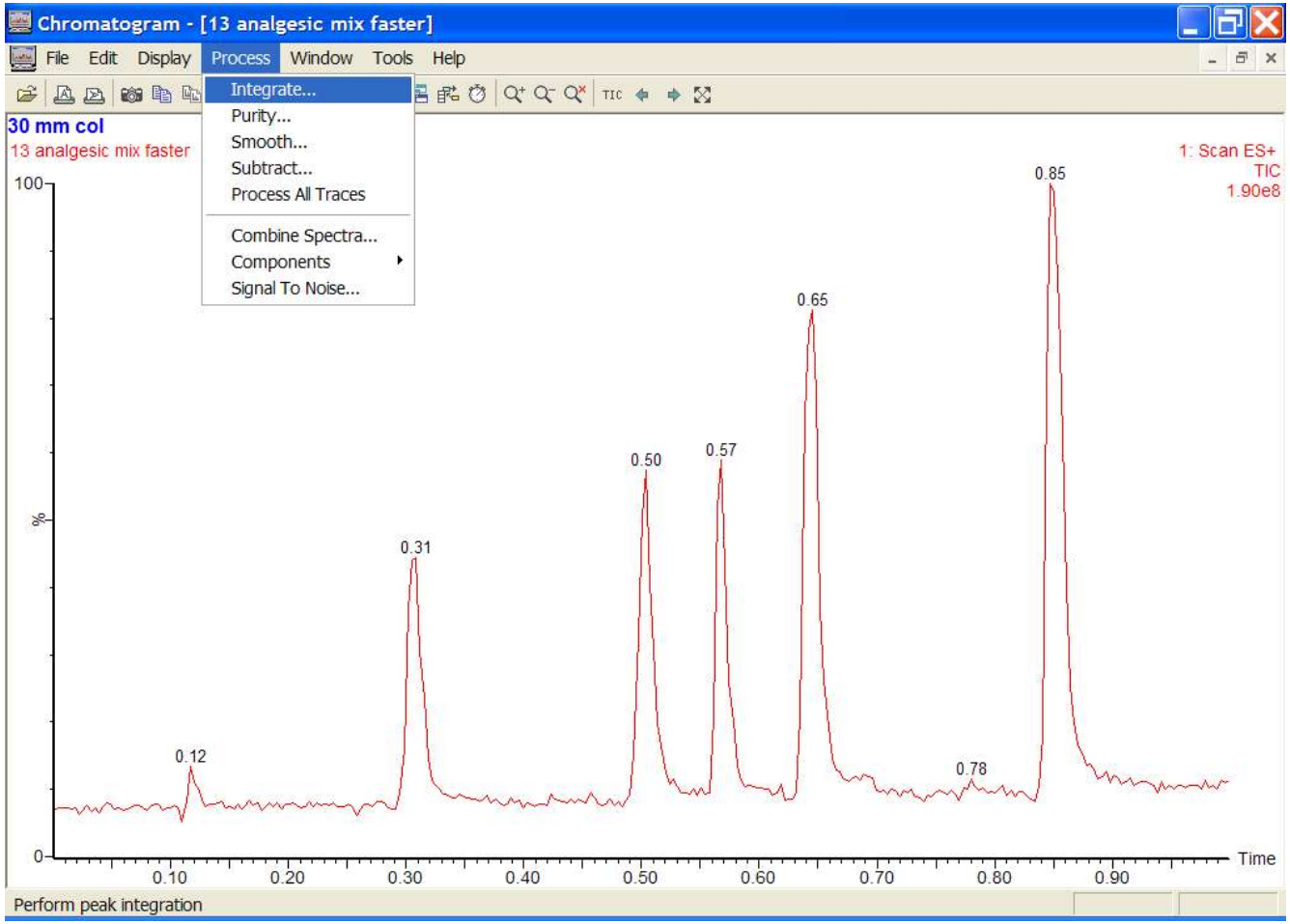
Apices and Inflection Points



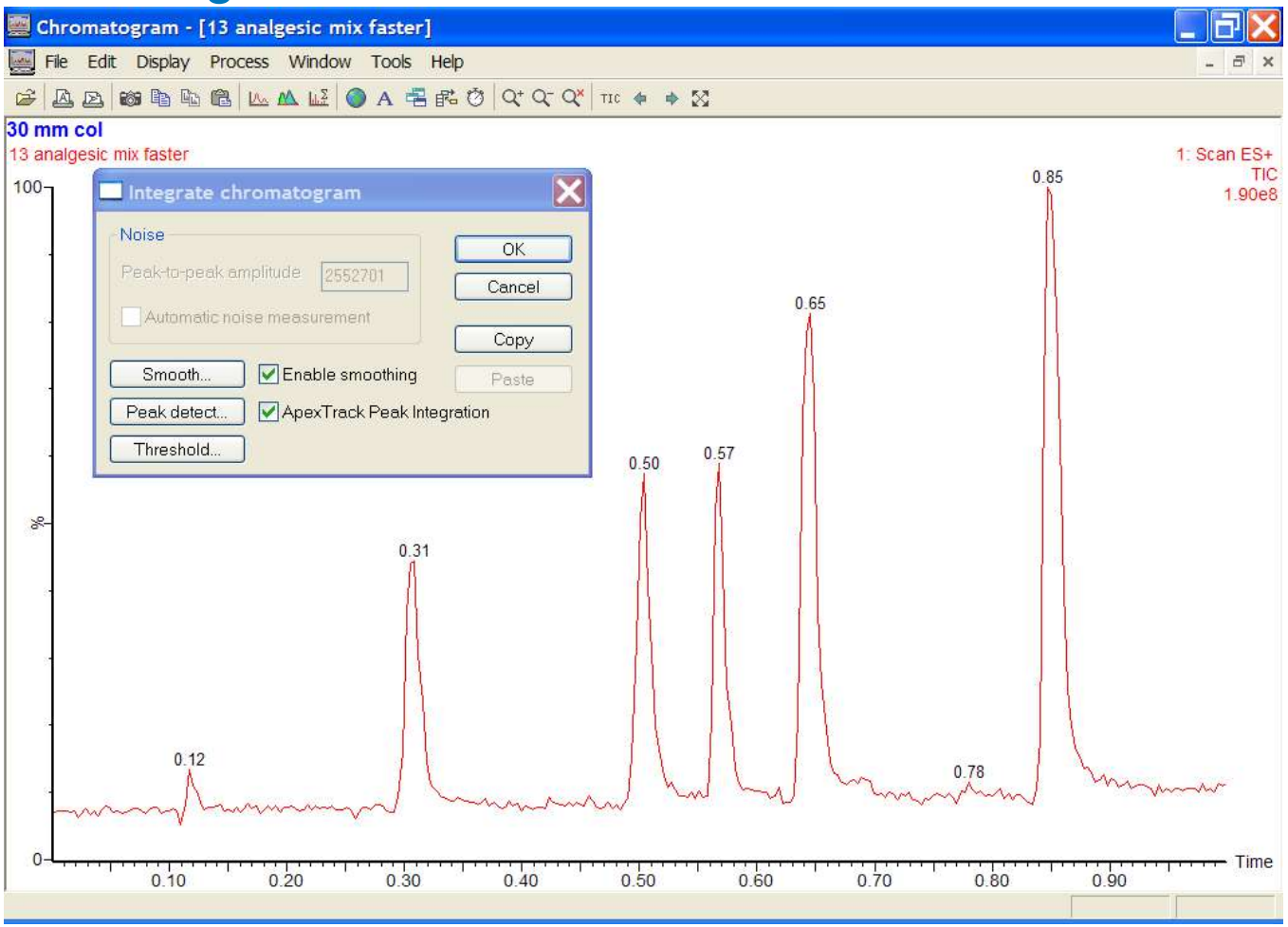
Baseline Determination

- Connect inflection points
  - Inflection points define initial baseline!
  - Final baselines satisfies % slope criterion.
- Form cluster baseline
  - From adjacent peaks
- Identify shoulders
- Compute Height, Area, Retention Time

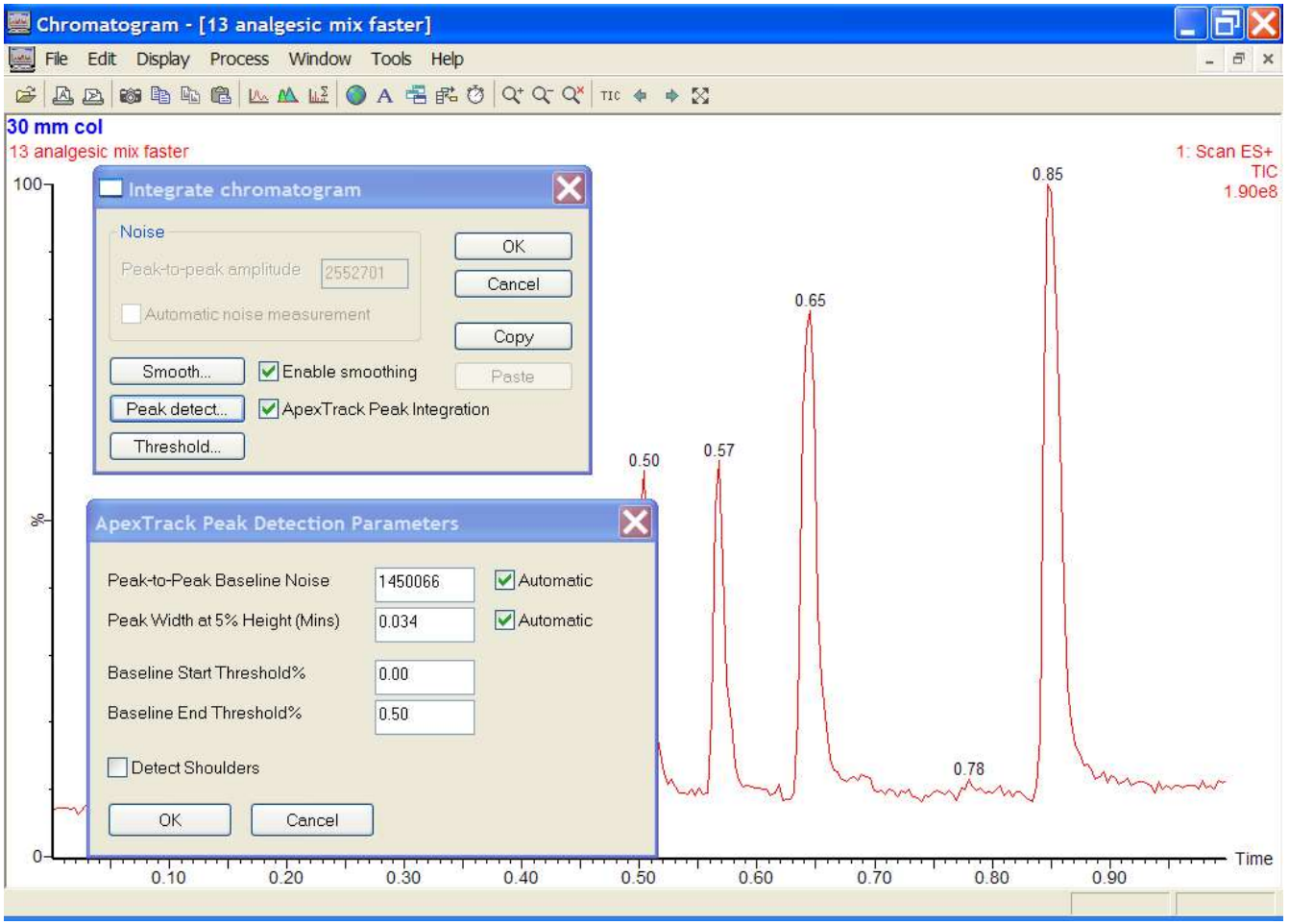
ApexTrack Peak Integration



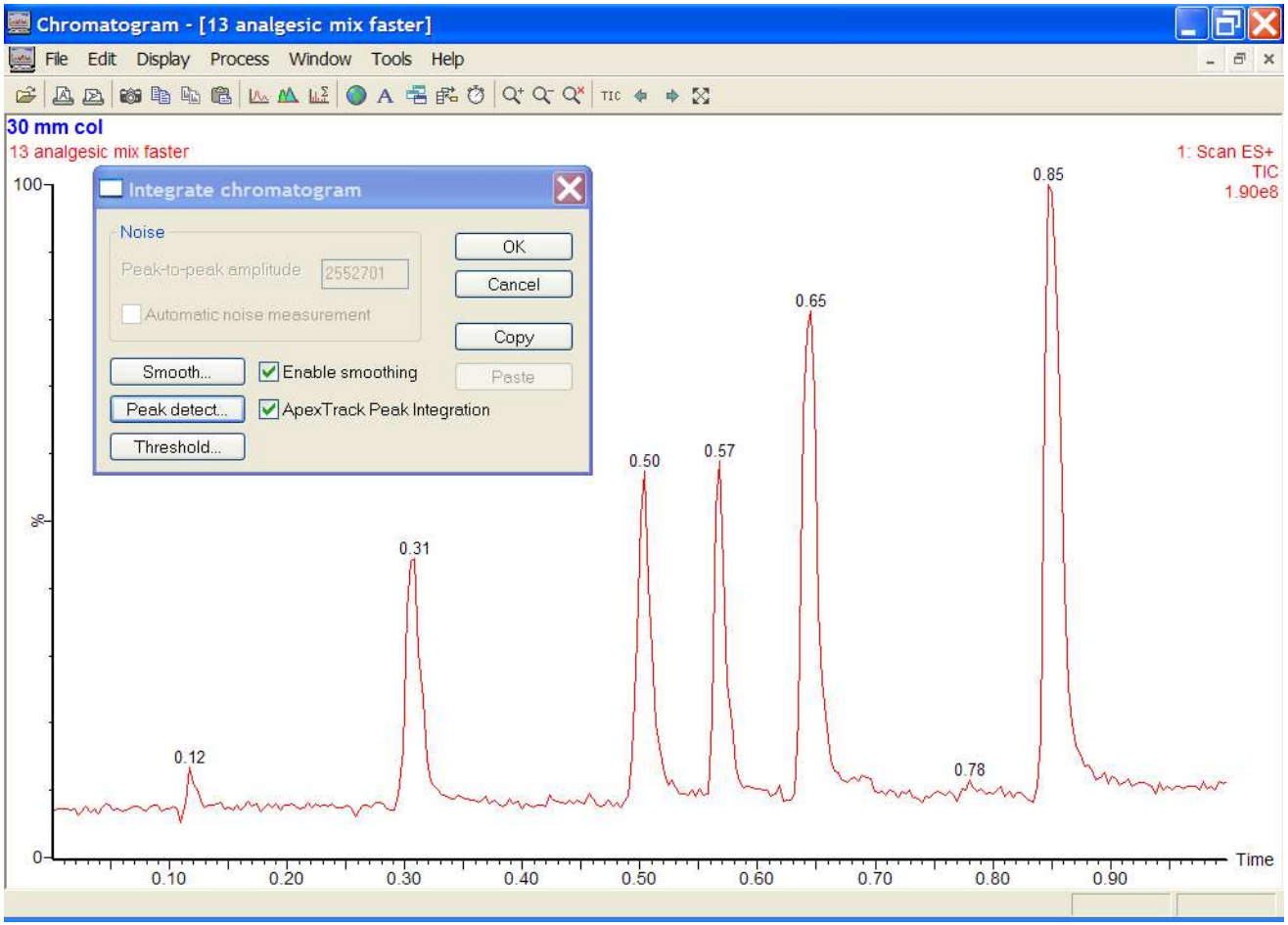
ApexTrack Peak Integration



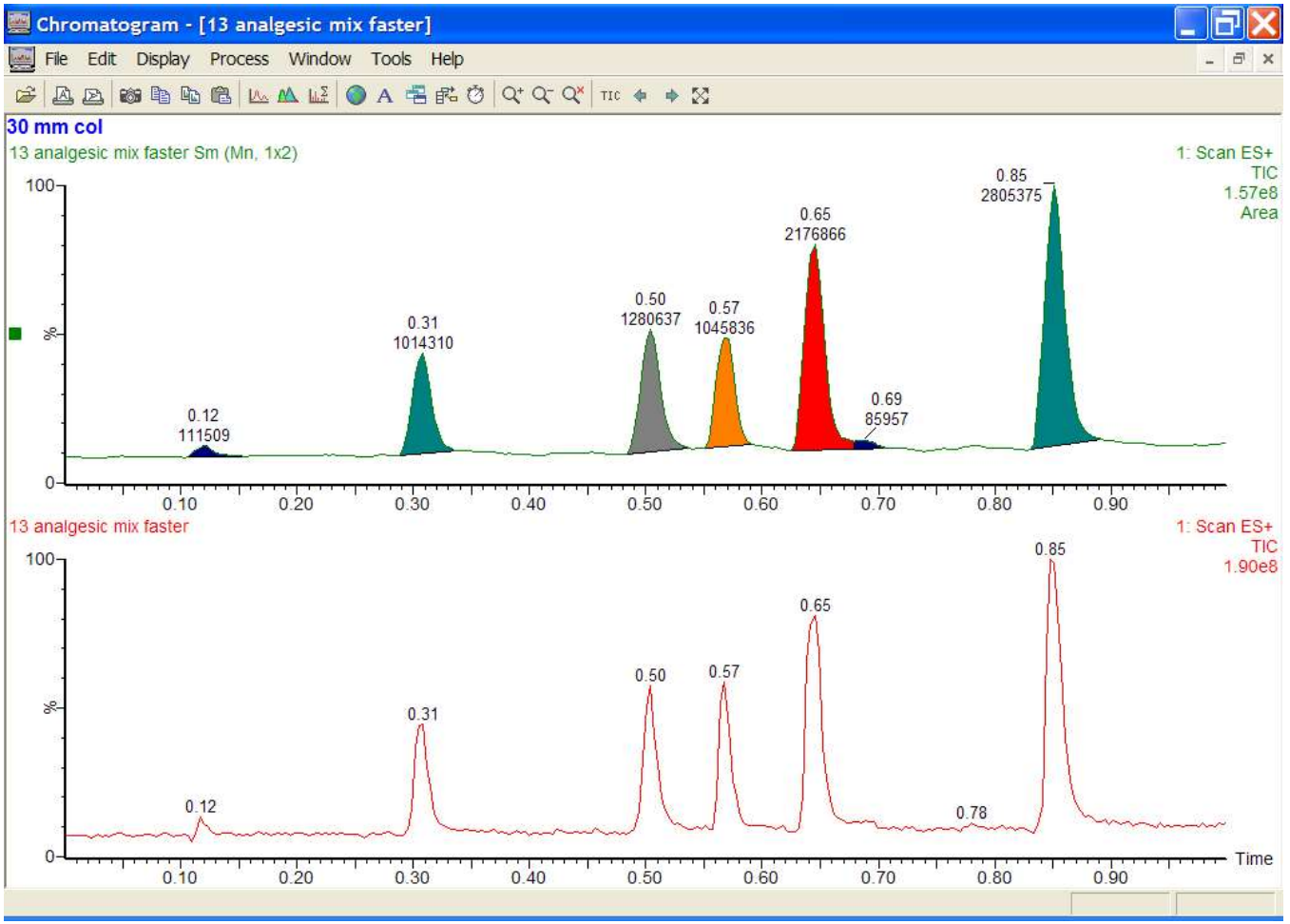
ApexTrack Peak Integration



ApexTrack Peak Integration



ApexTrack Peak Integration

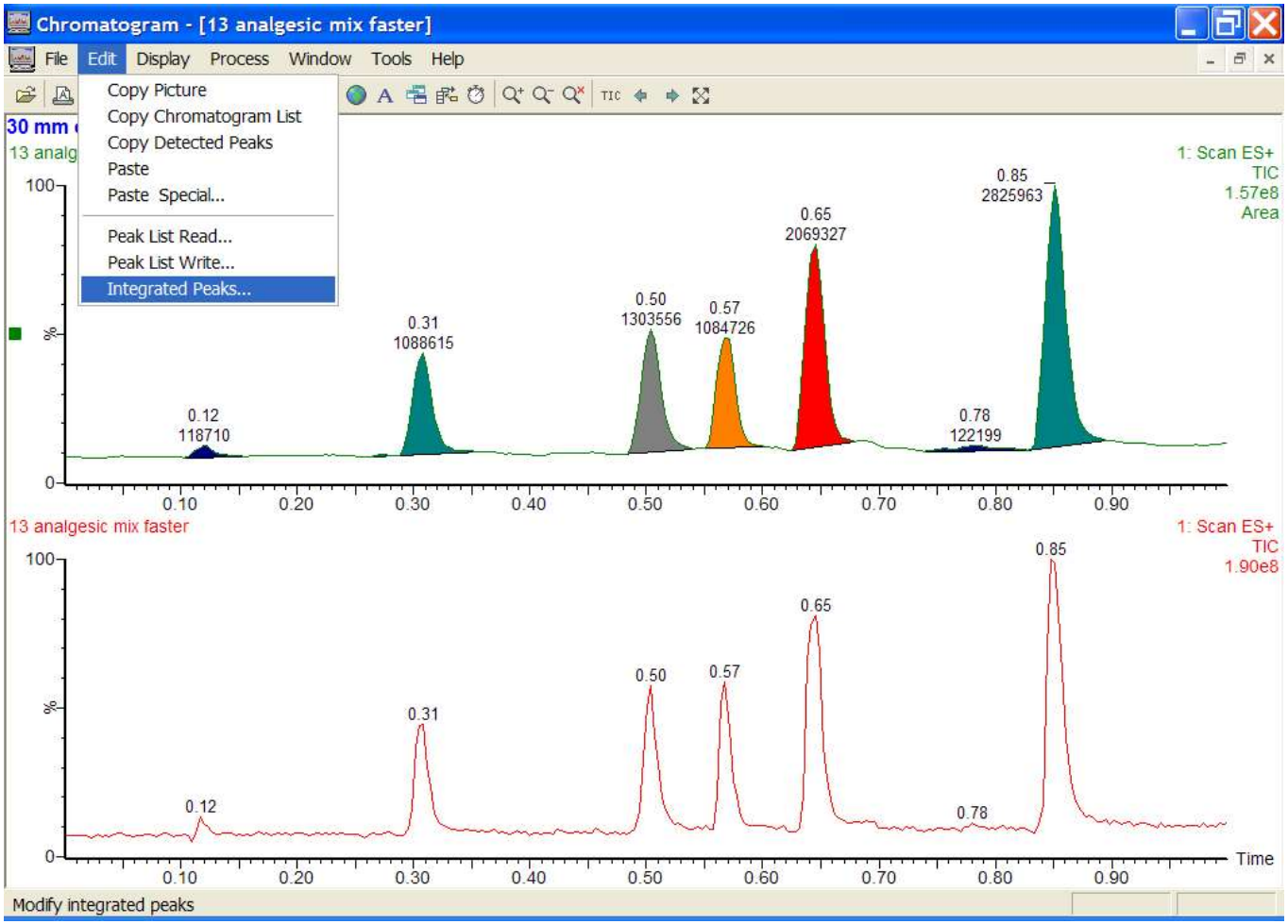


ApexTrack Peak Integration Summary

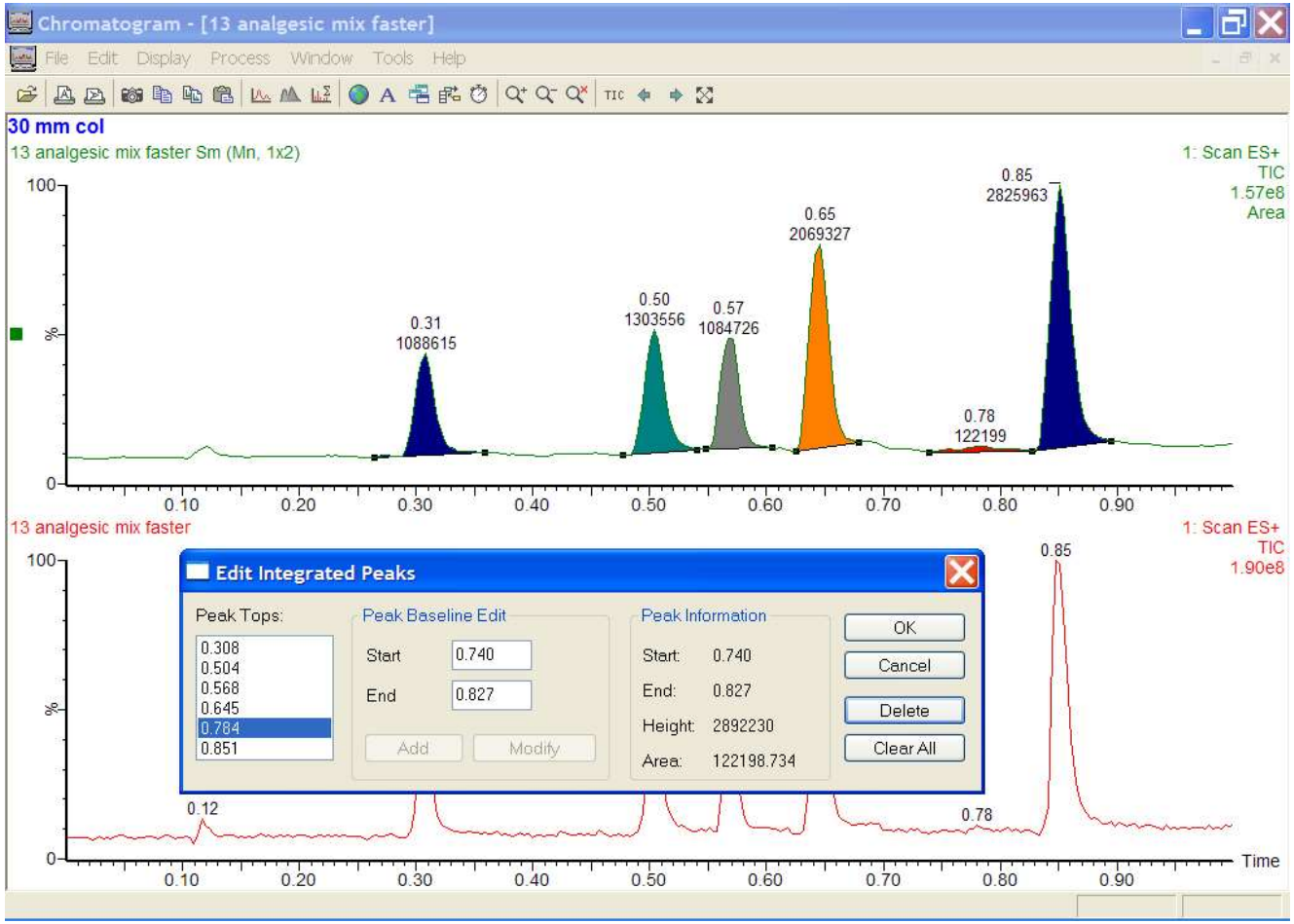
Waters™

- Peak detection separate from baseline determination
- Robust shoulder detection
- Automatic parameter determination
- Rapid method development

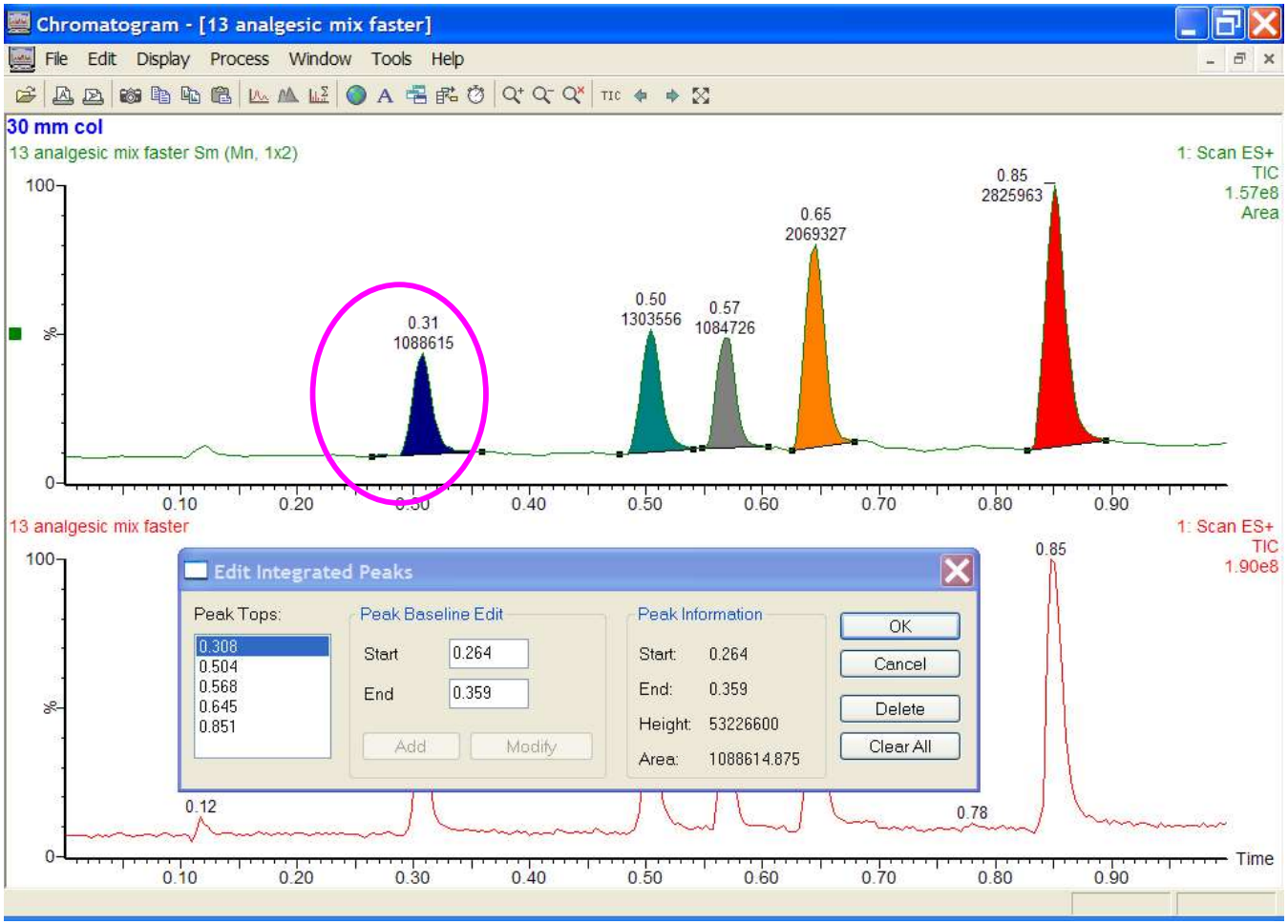
Editing Detected Peaks



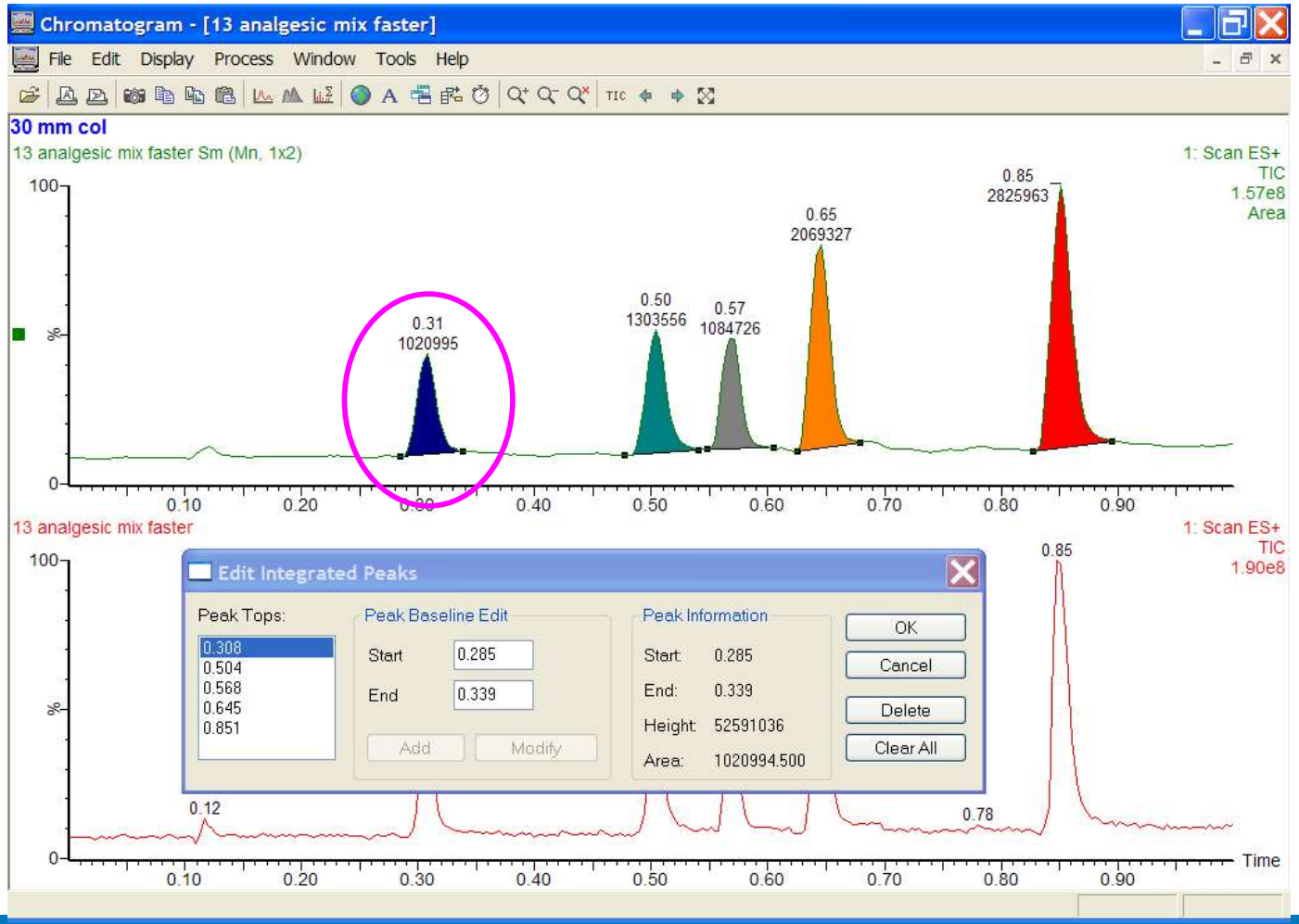
Editing Detected Peaks



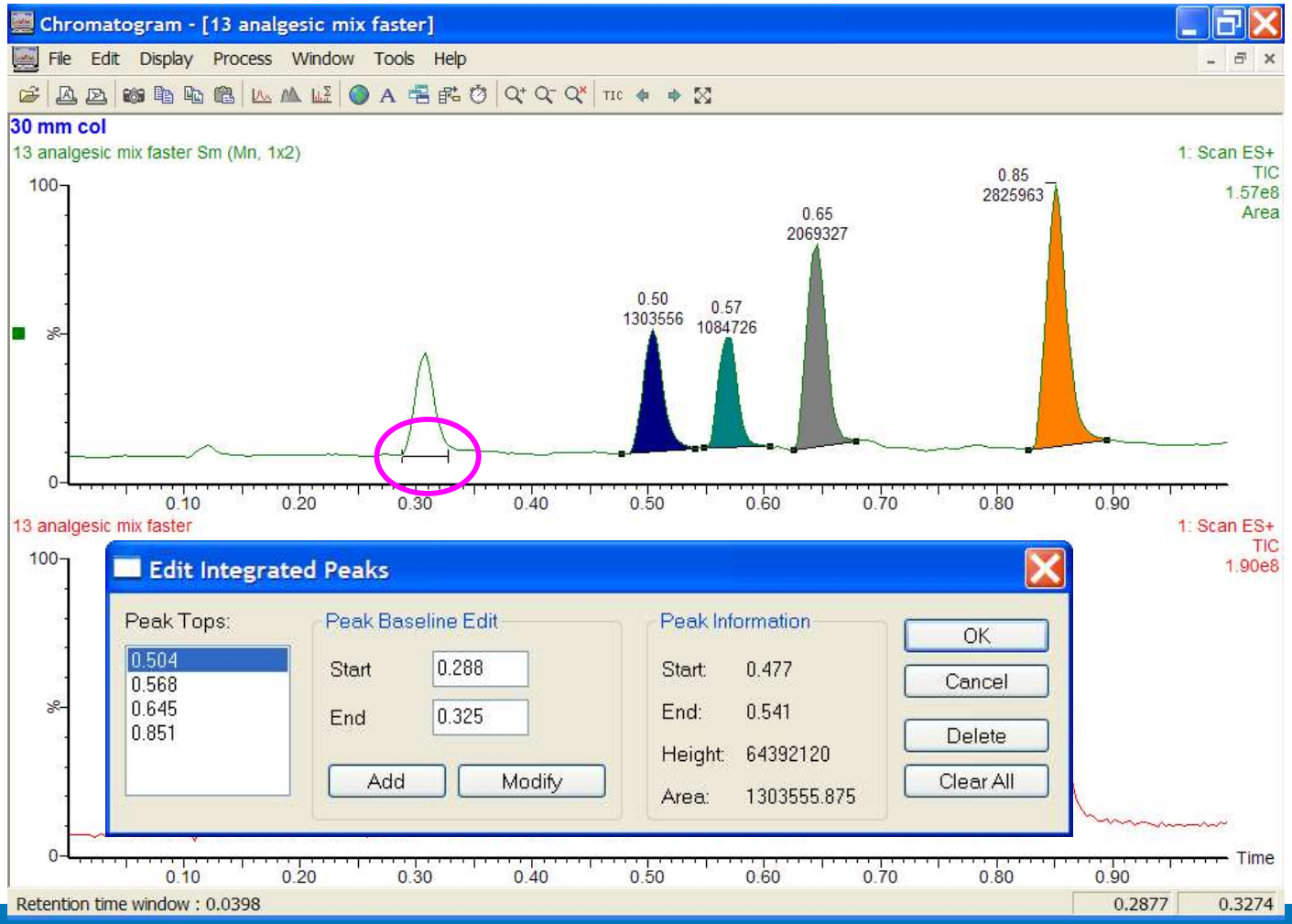
Editing Detected Peaks



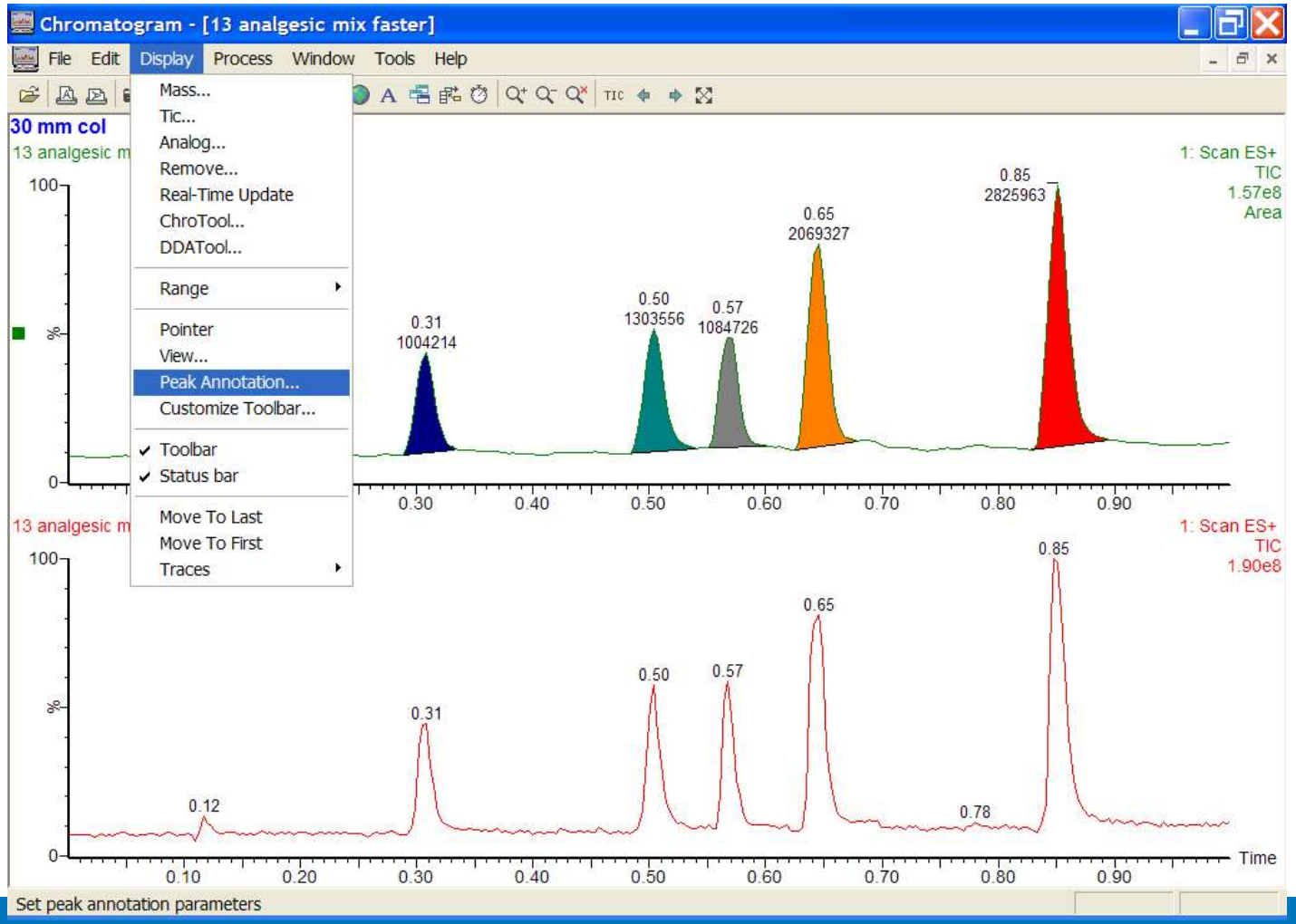
Editing Detected Peaks



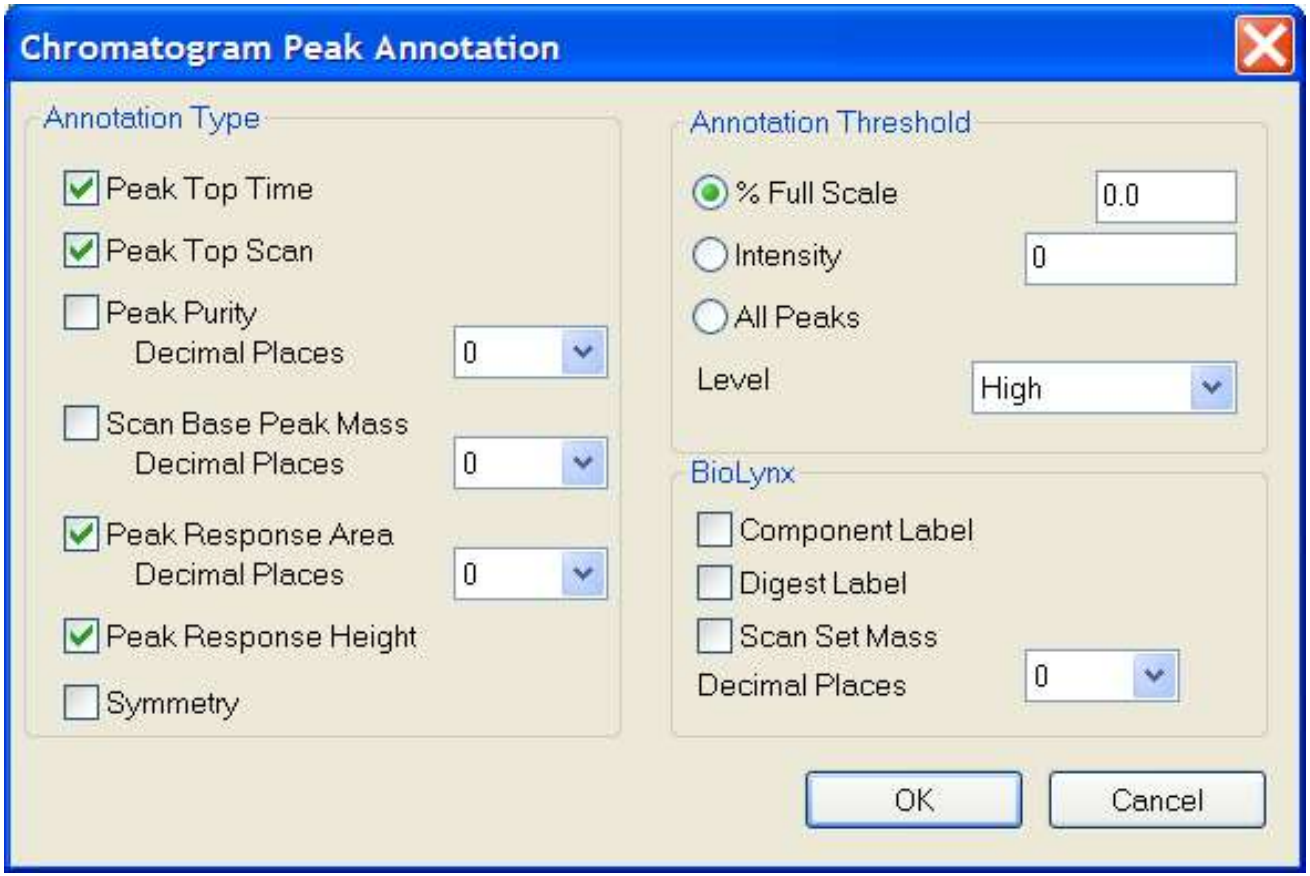
Editing Detected Peaks



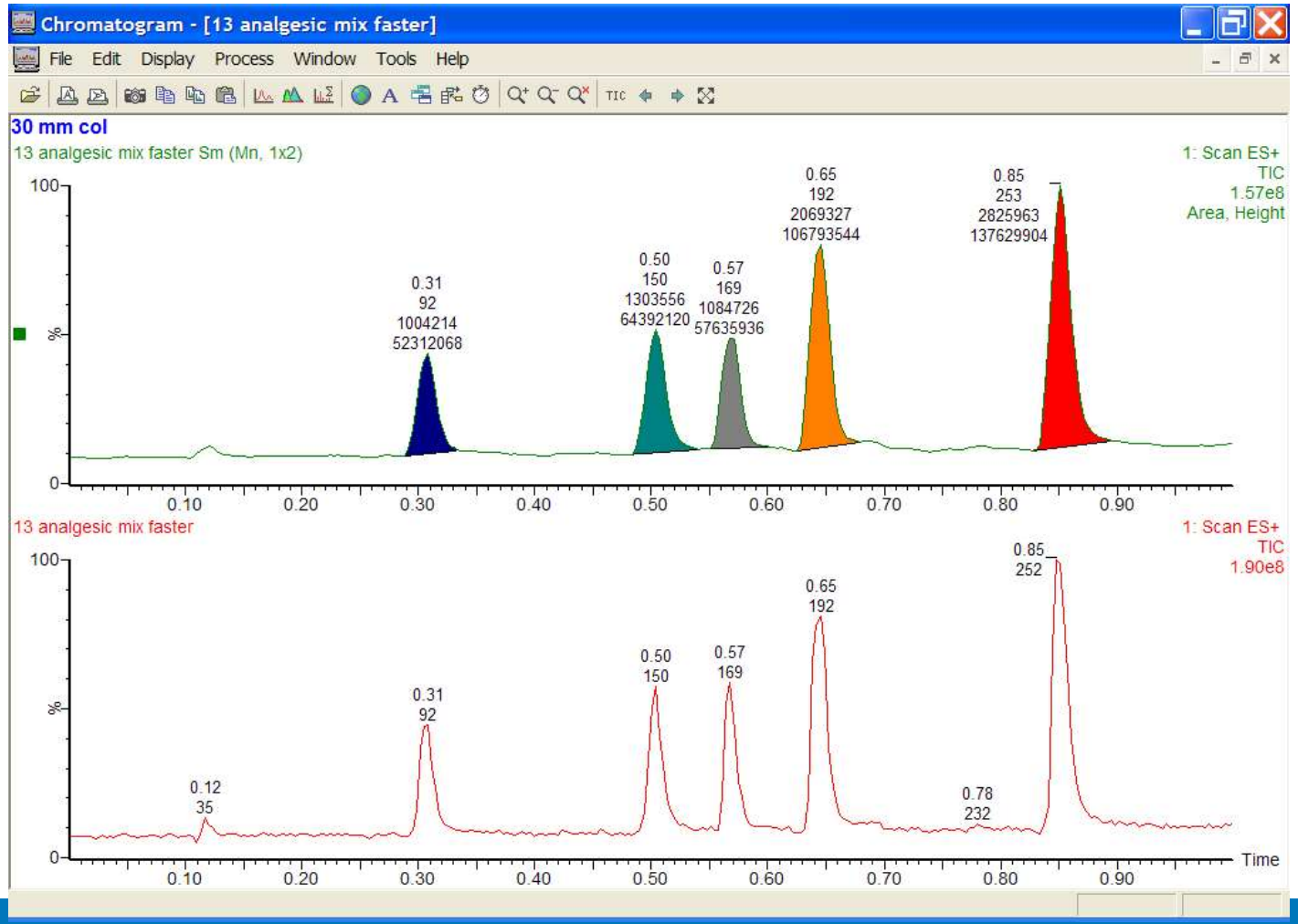
Peak Annotation



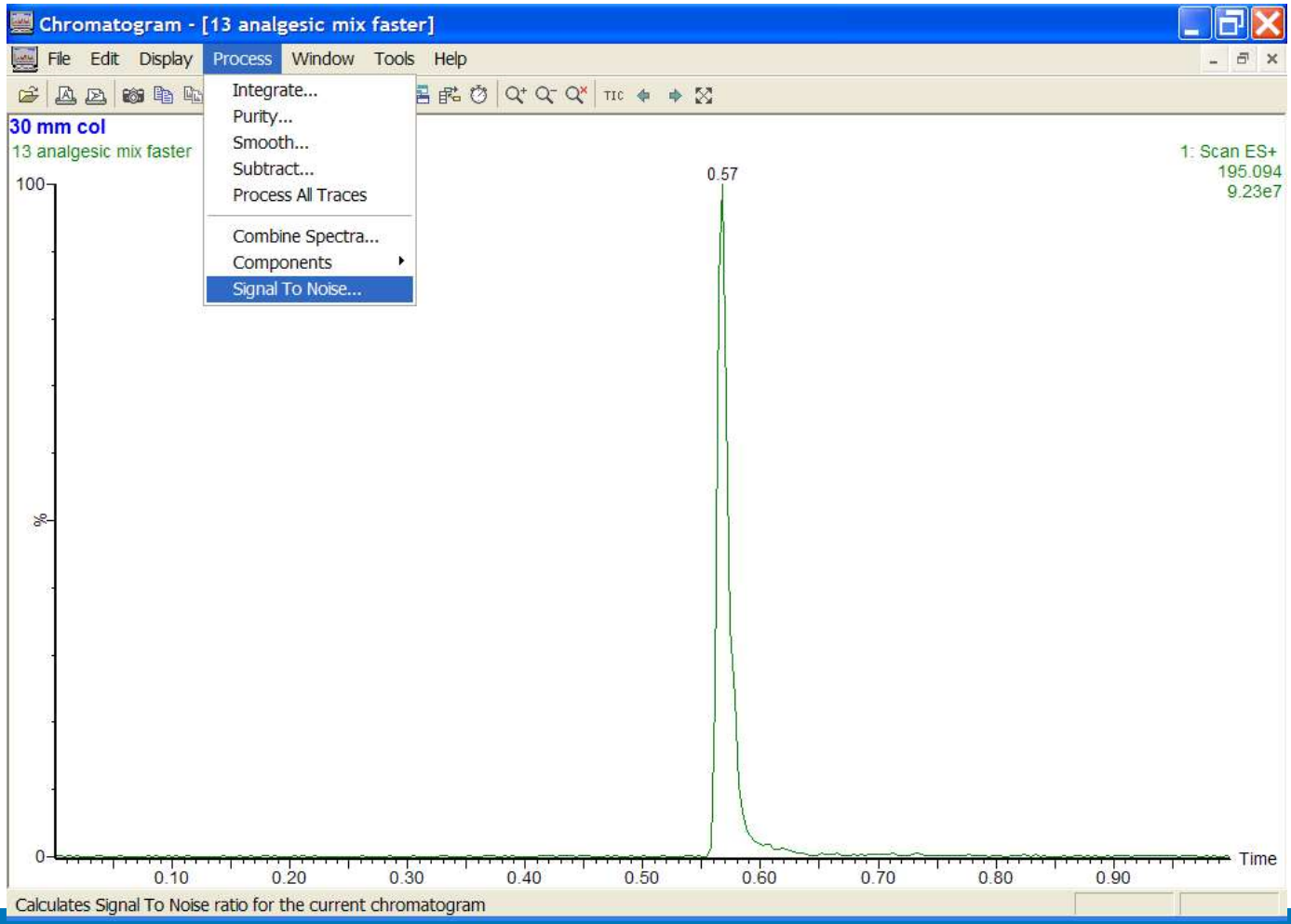
Peak Annotation



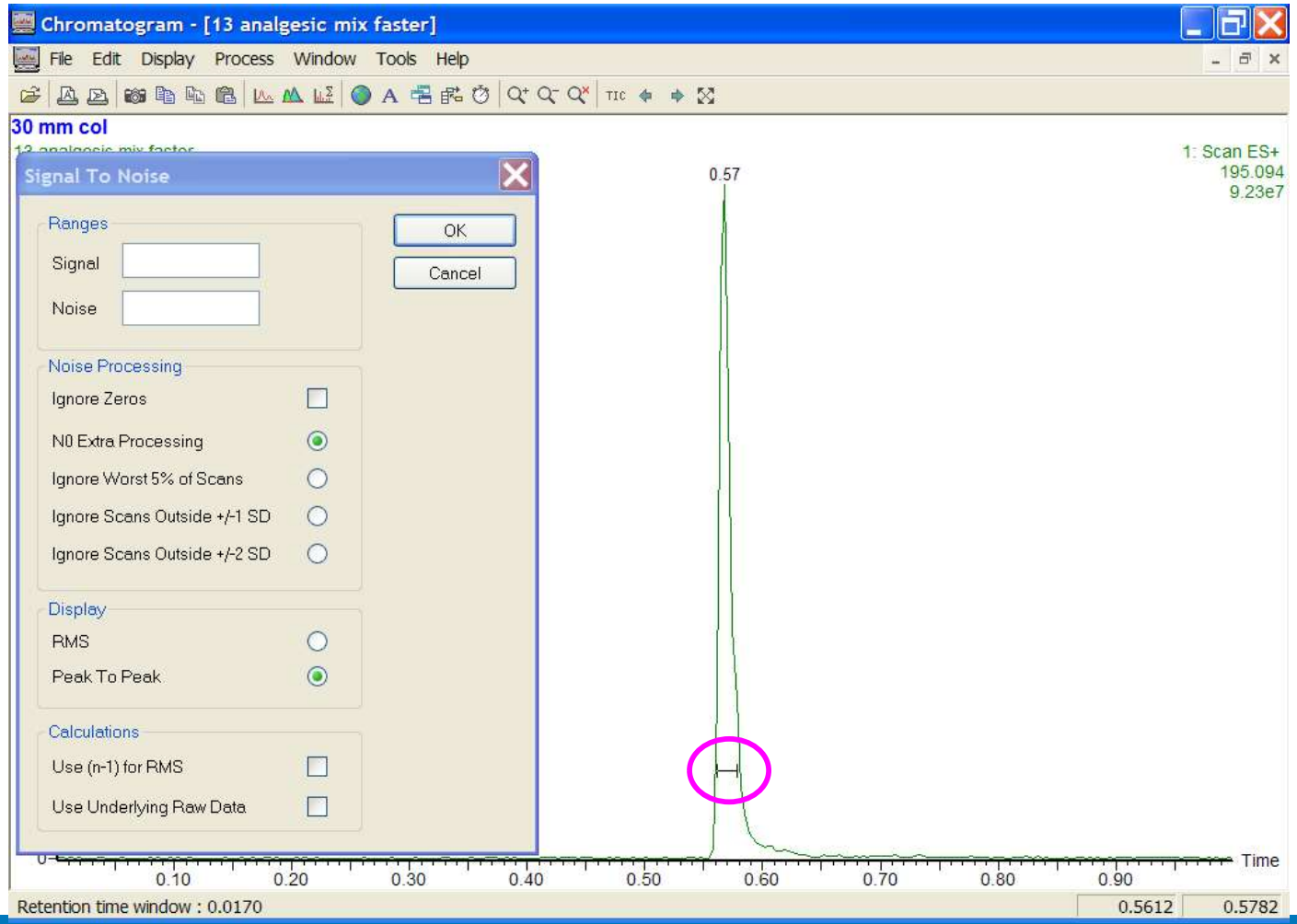
Peak Annotation



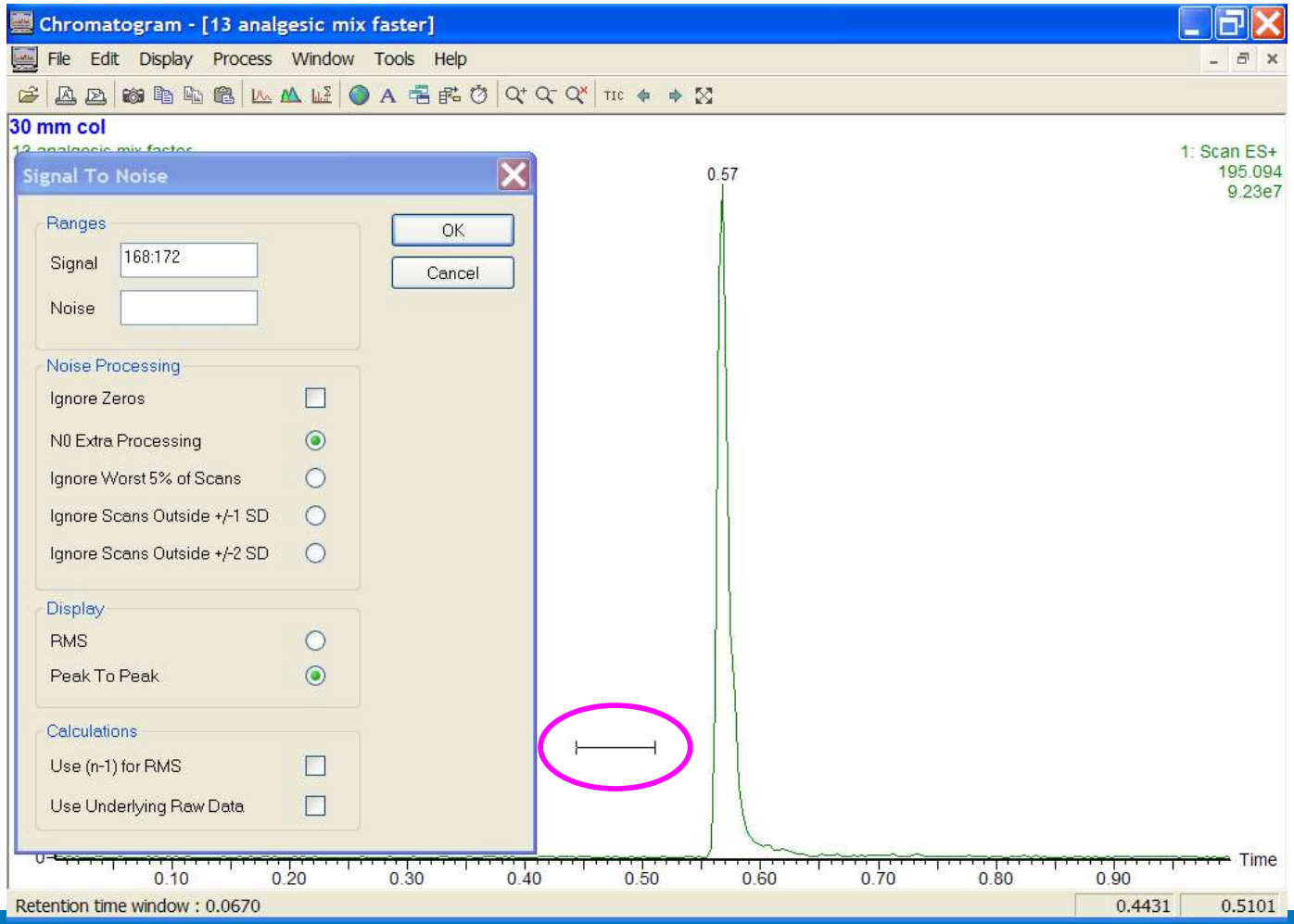
Signal to Noise



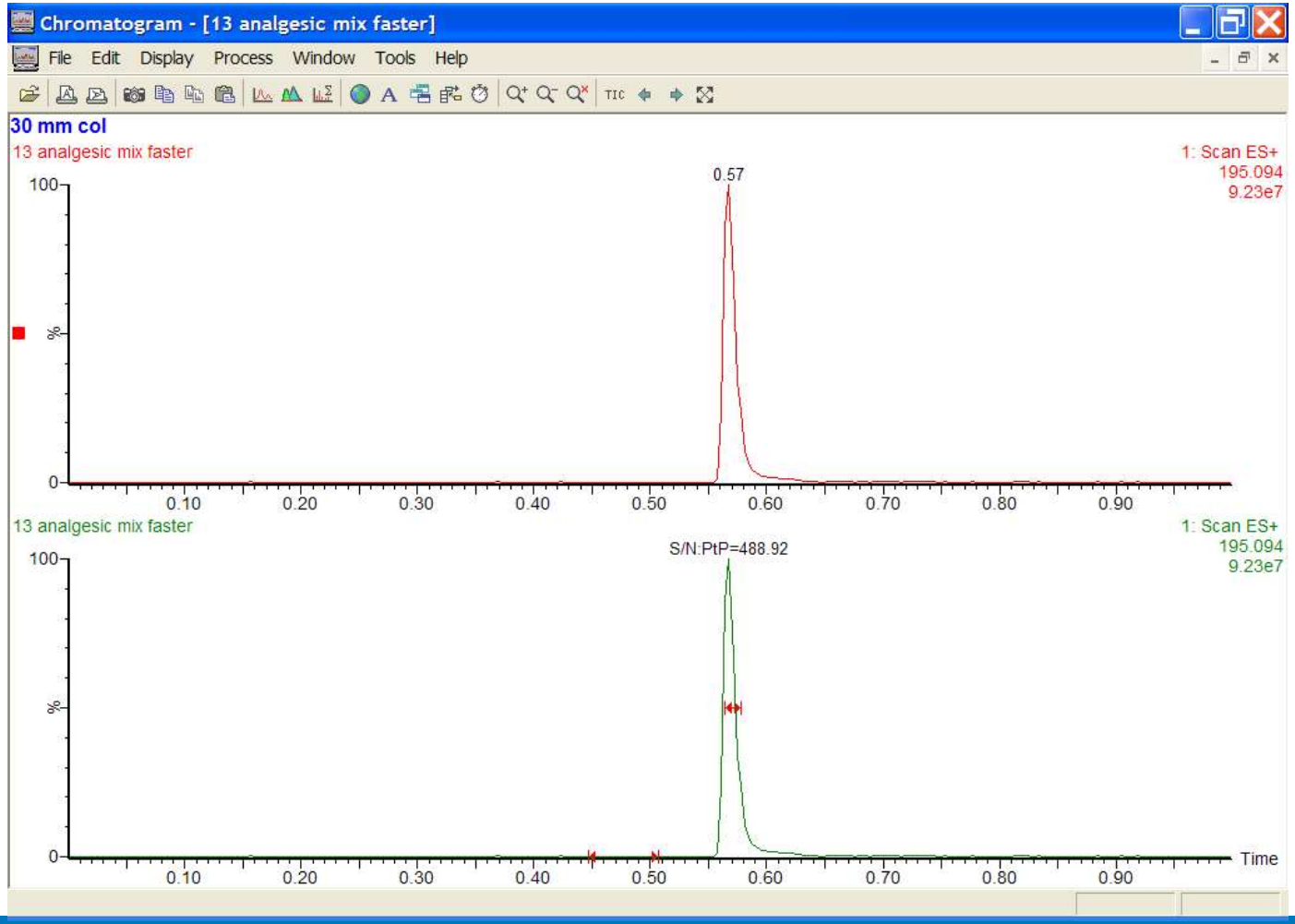
Signal to Noise



Signal to Noise

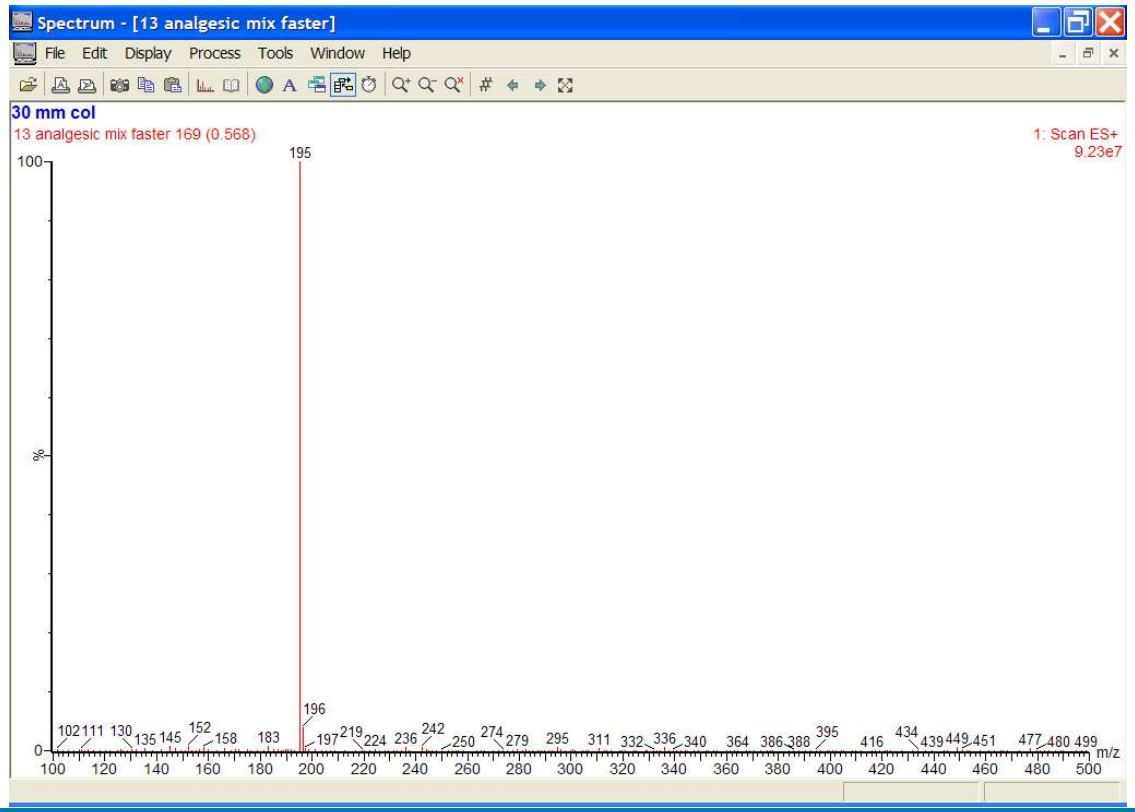


Signal to Noise



Spectrum

- The Spectrum window is accessed from the MassLynx window by selecting spectrum from the sample list menu bar

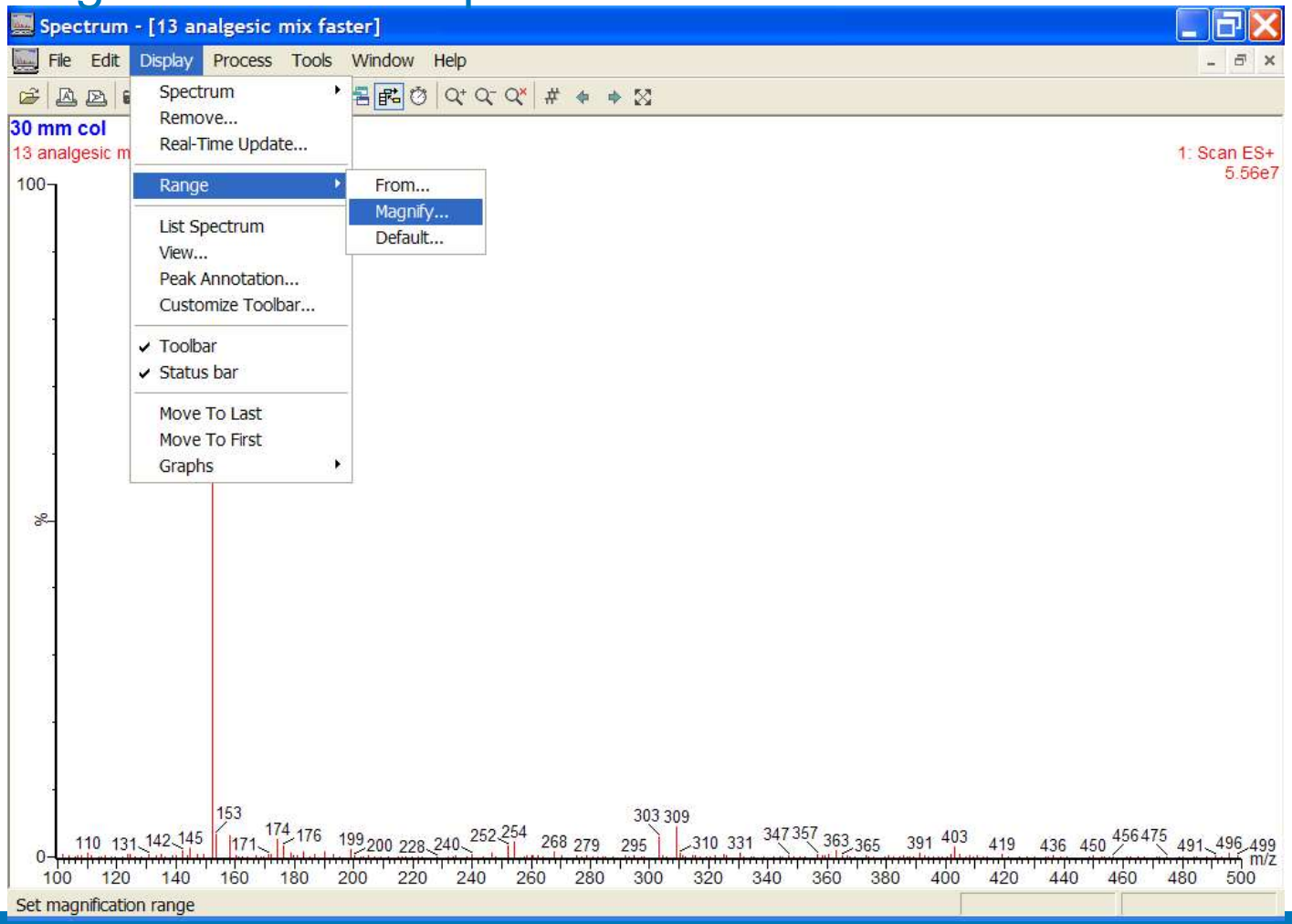


Displaying Spectra

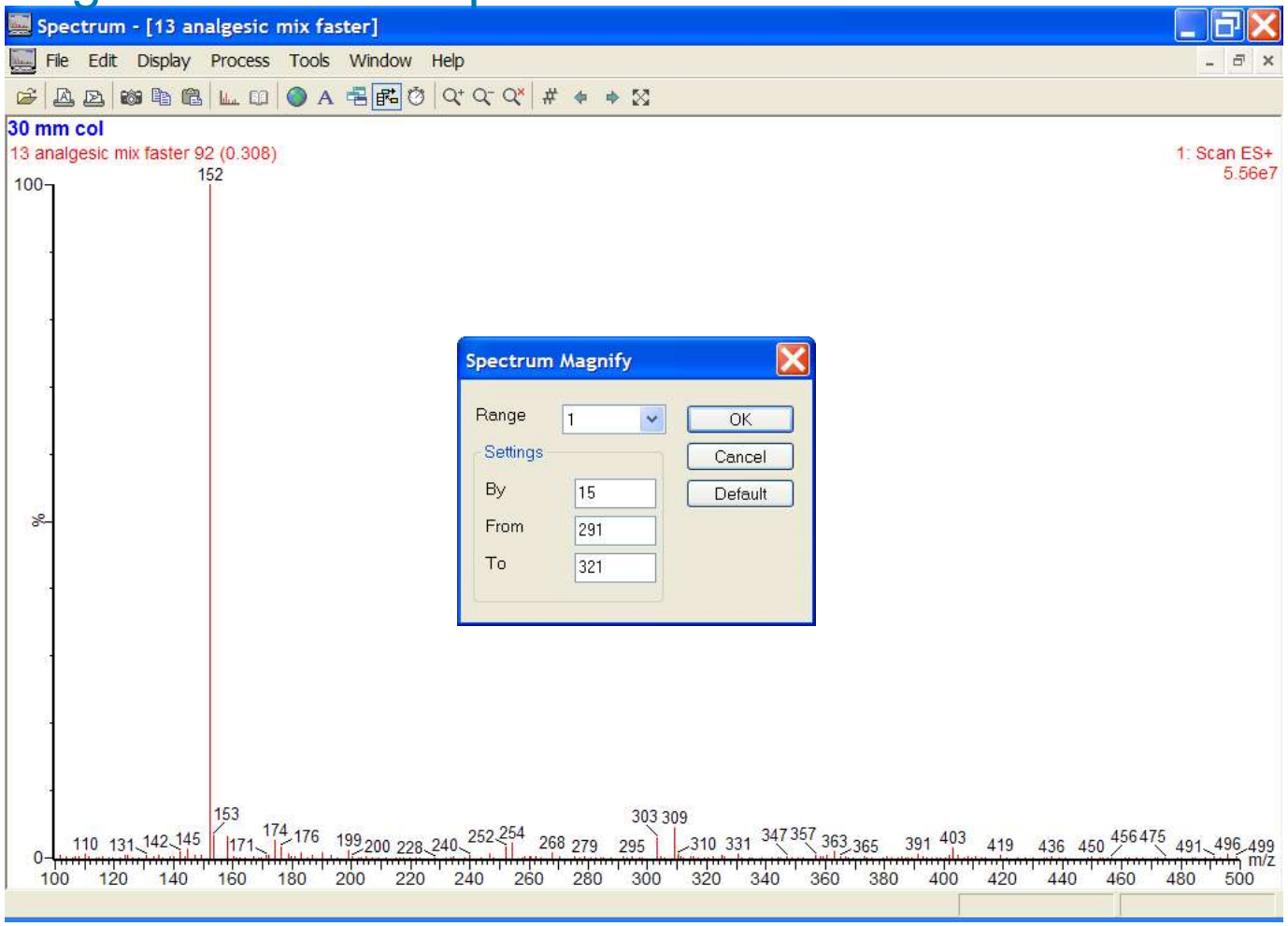
- Setting magnified ranges
- Peak annotation
  - Changing the number of decimal places for mass labeling
  - Labeling peaks relative to a reference peak
  - Setting the threshold for annotation
- Adding text to a spectrum
- Displaying a list spectrum

Magnifying Regions Within a Spectrum

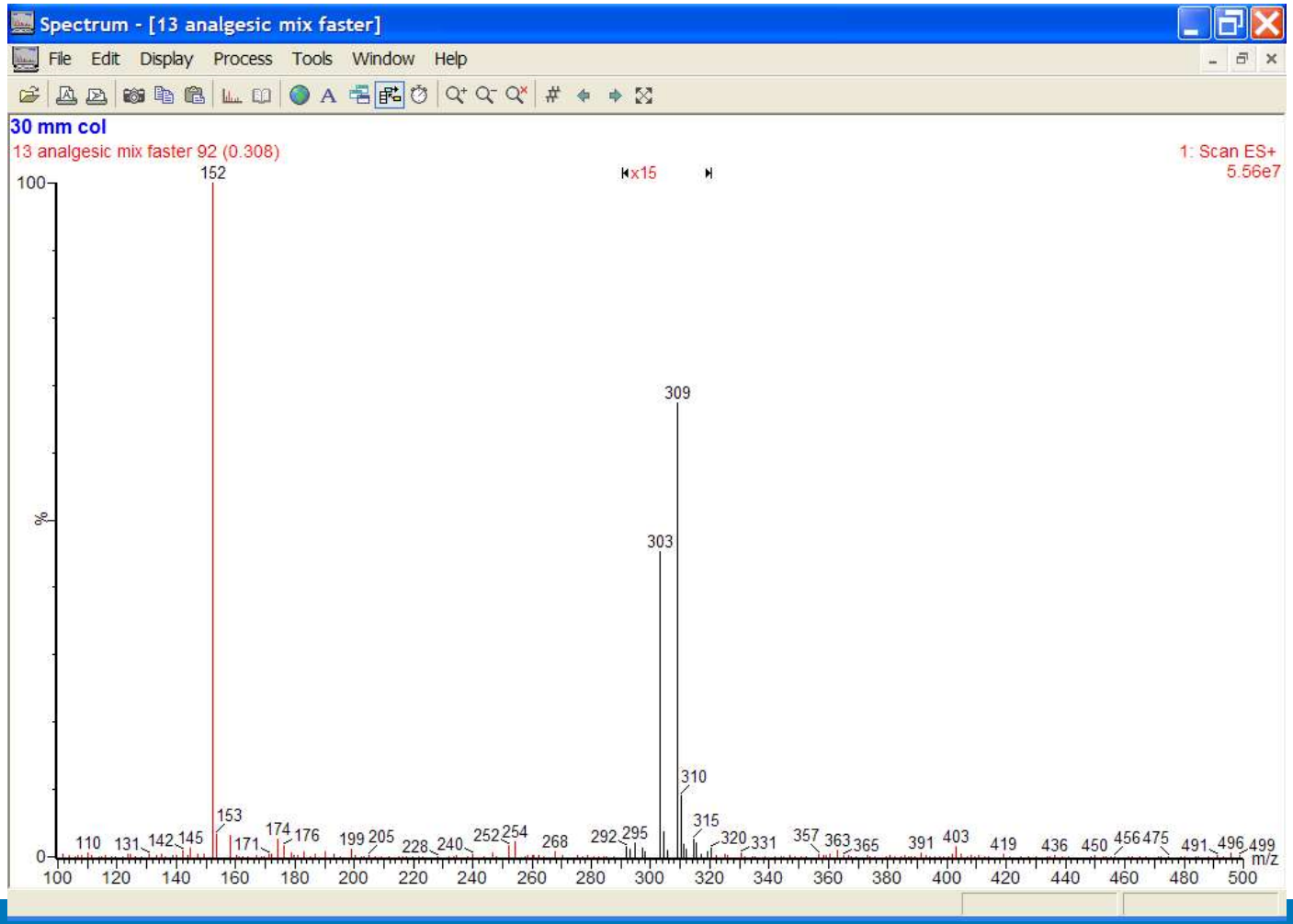
Magnifying Regions Within a Spectrum



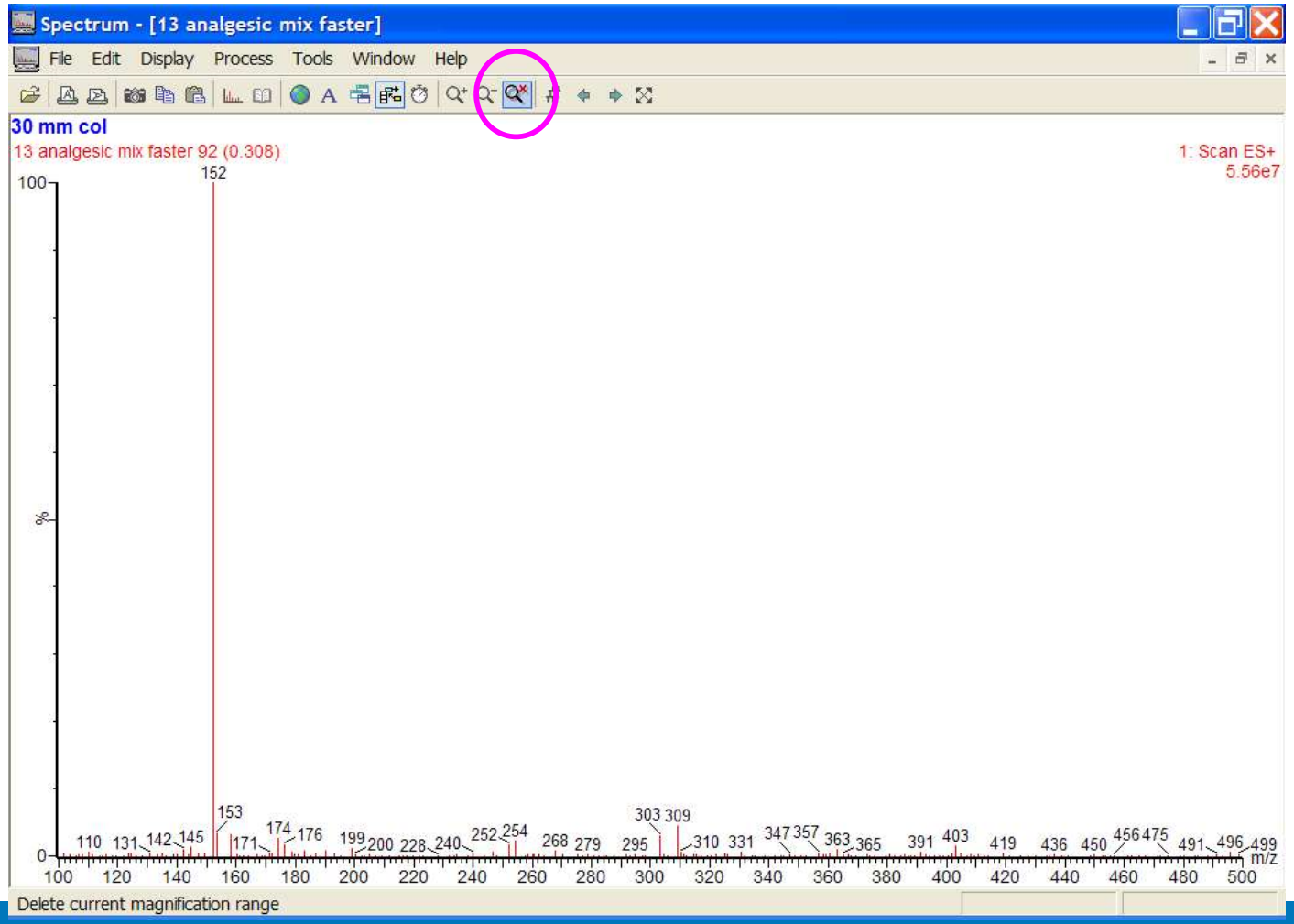
Magnifying Regions Within a Spectrum



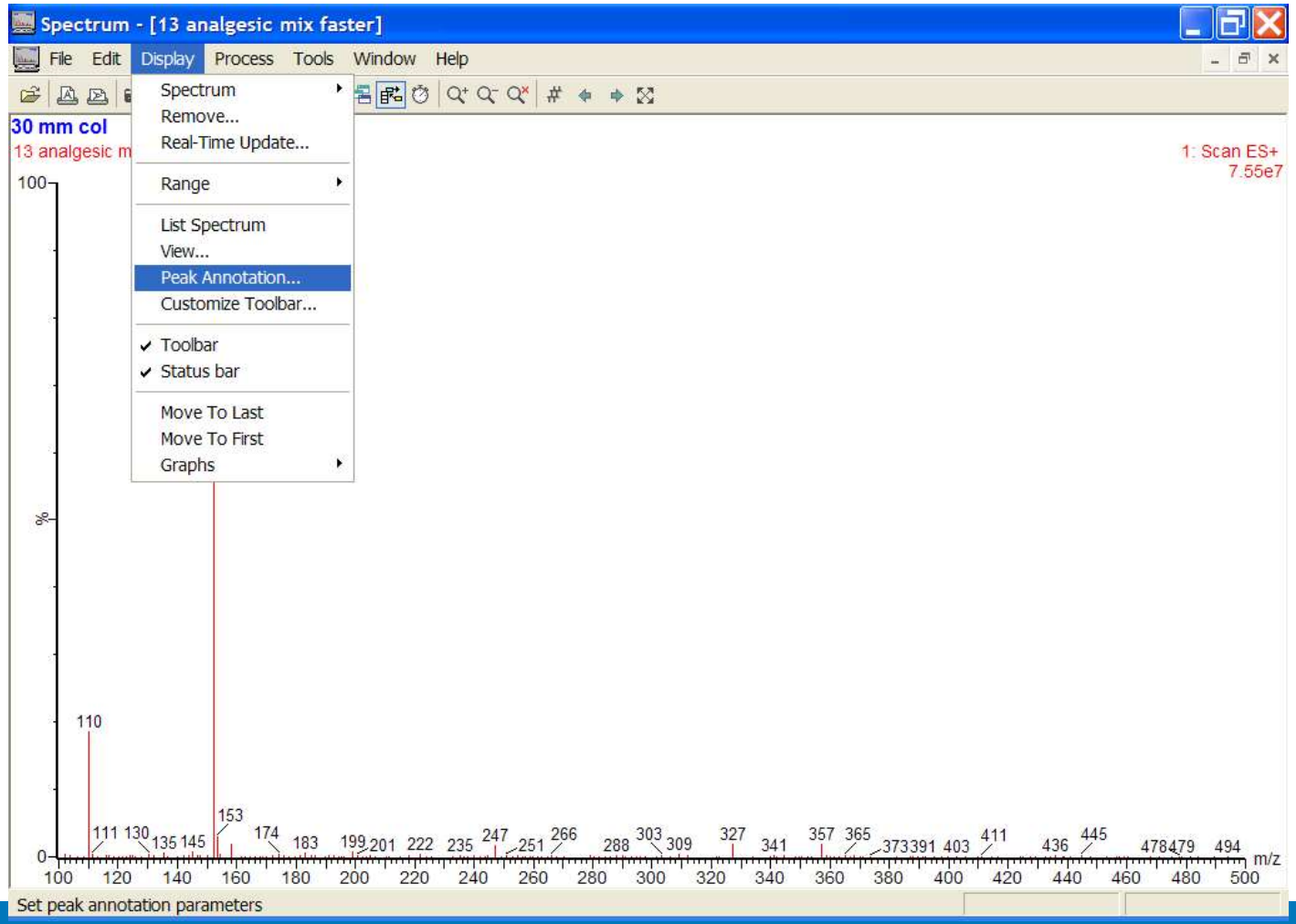
Magnifying Regions Within a Spectrum



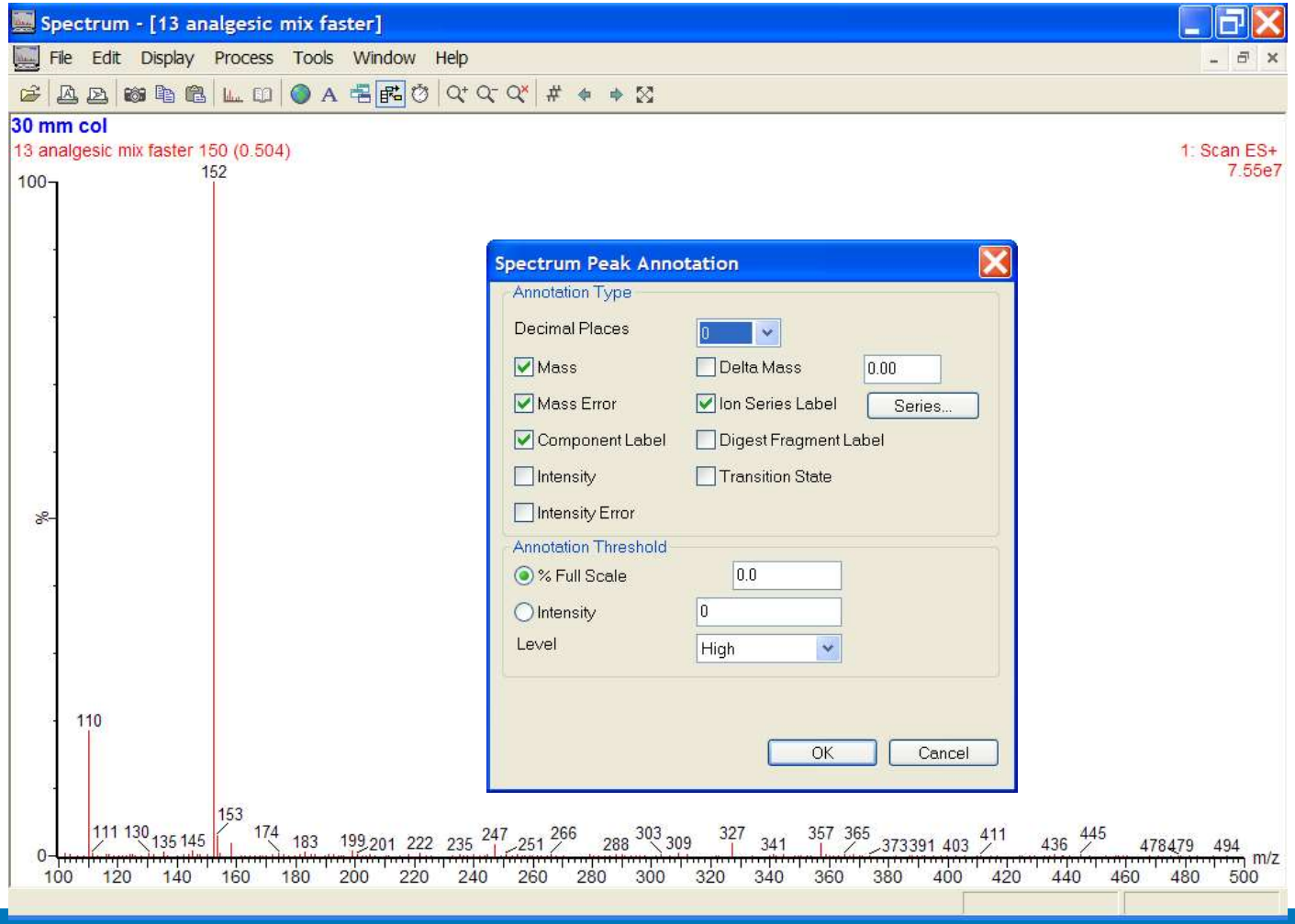
Magnifying Regions Within a Spectrum



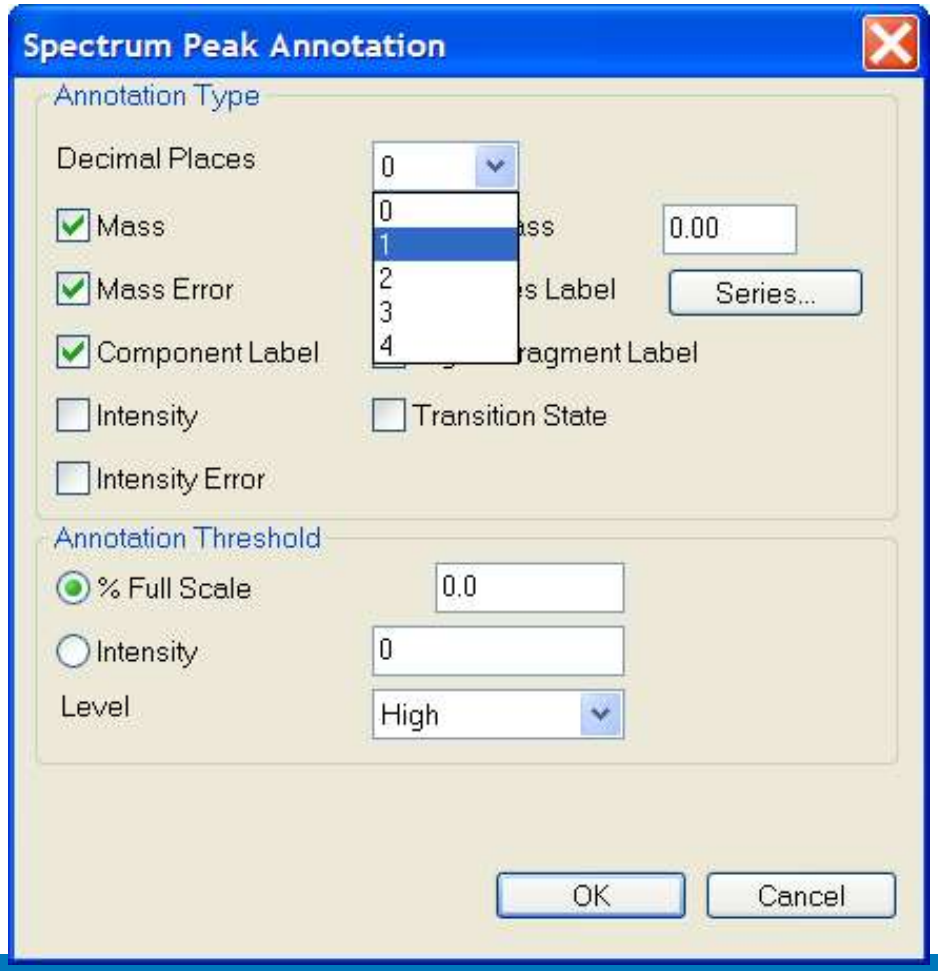
Spectrum Annotation



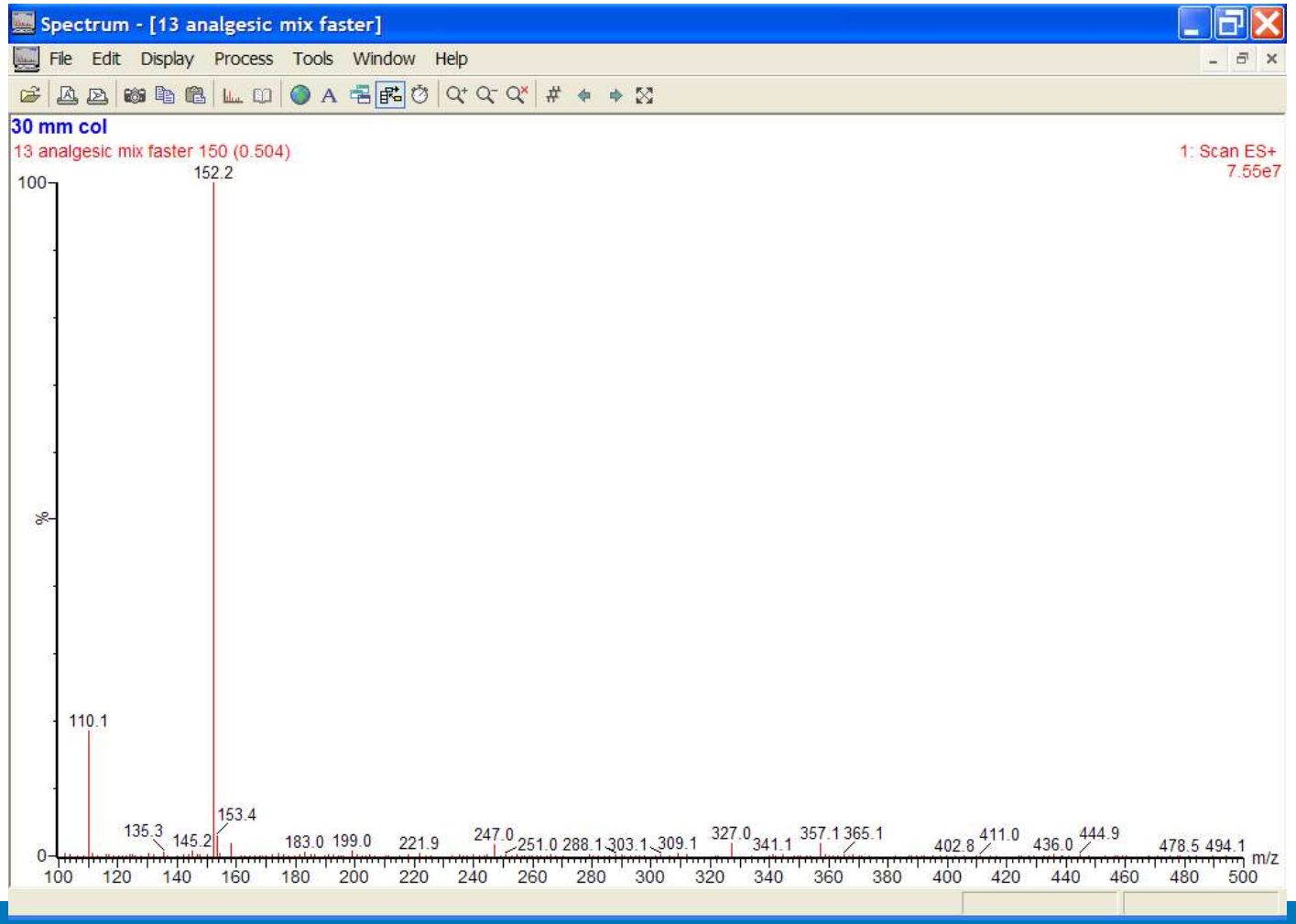
Spectrum Annotation



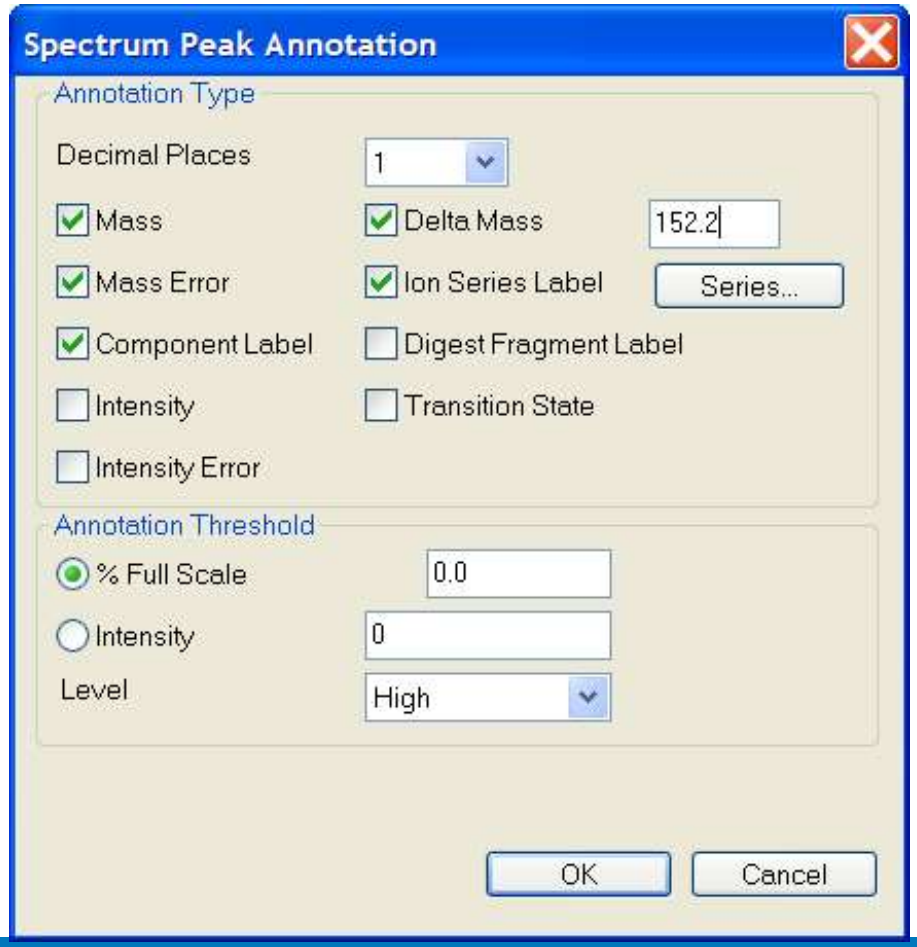
Spectrum Annotation



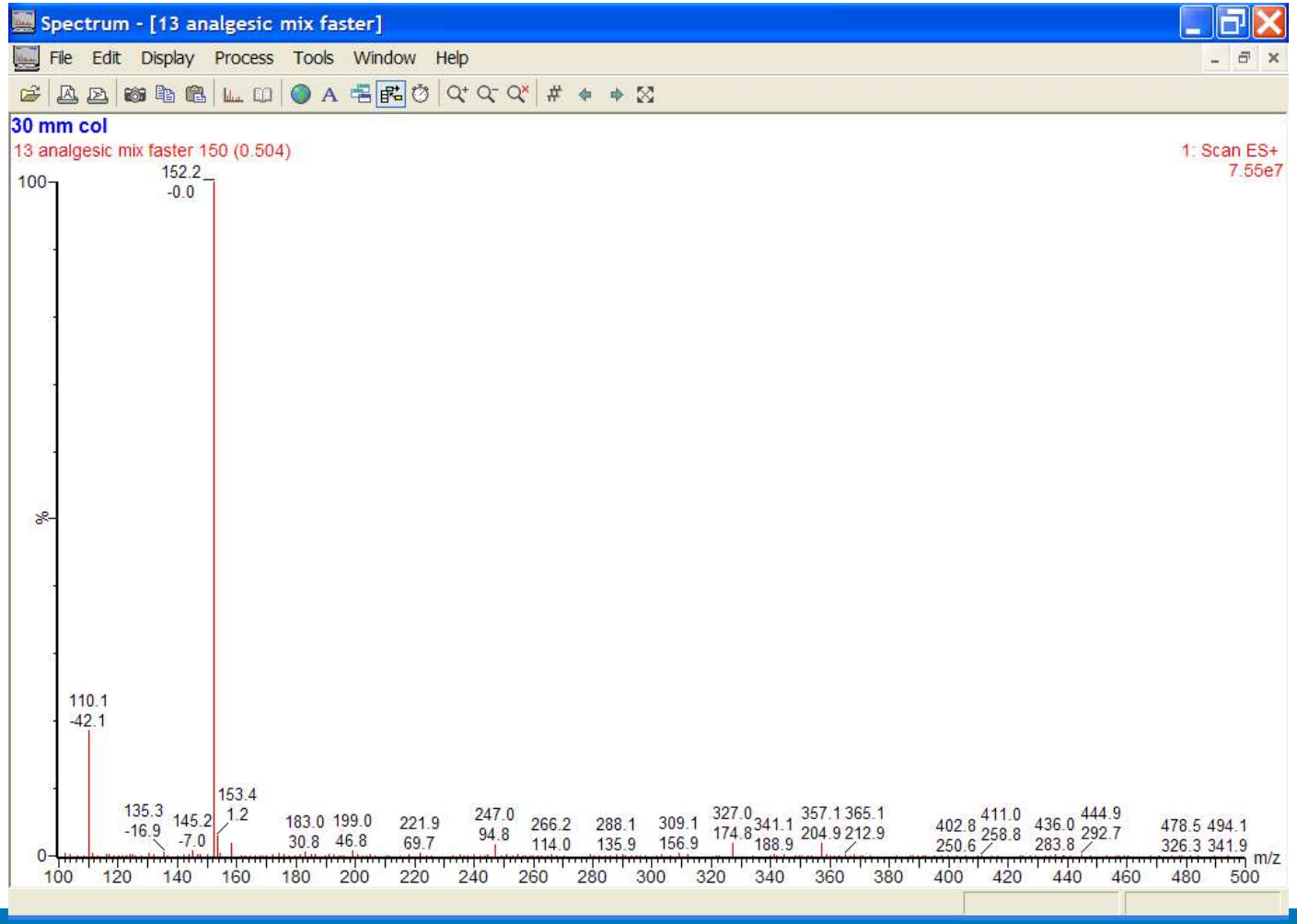
Spectrum Annotation



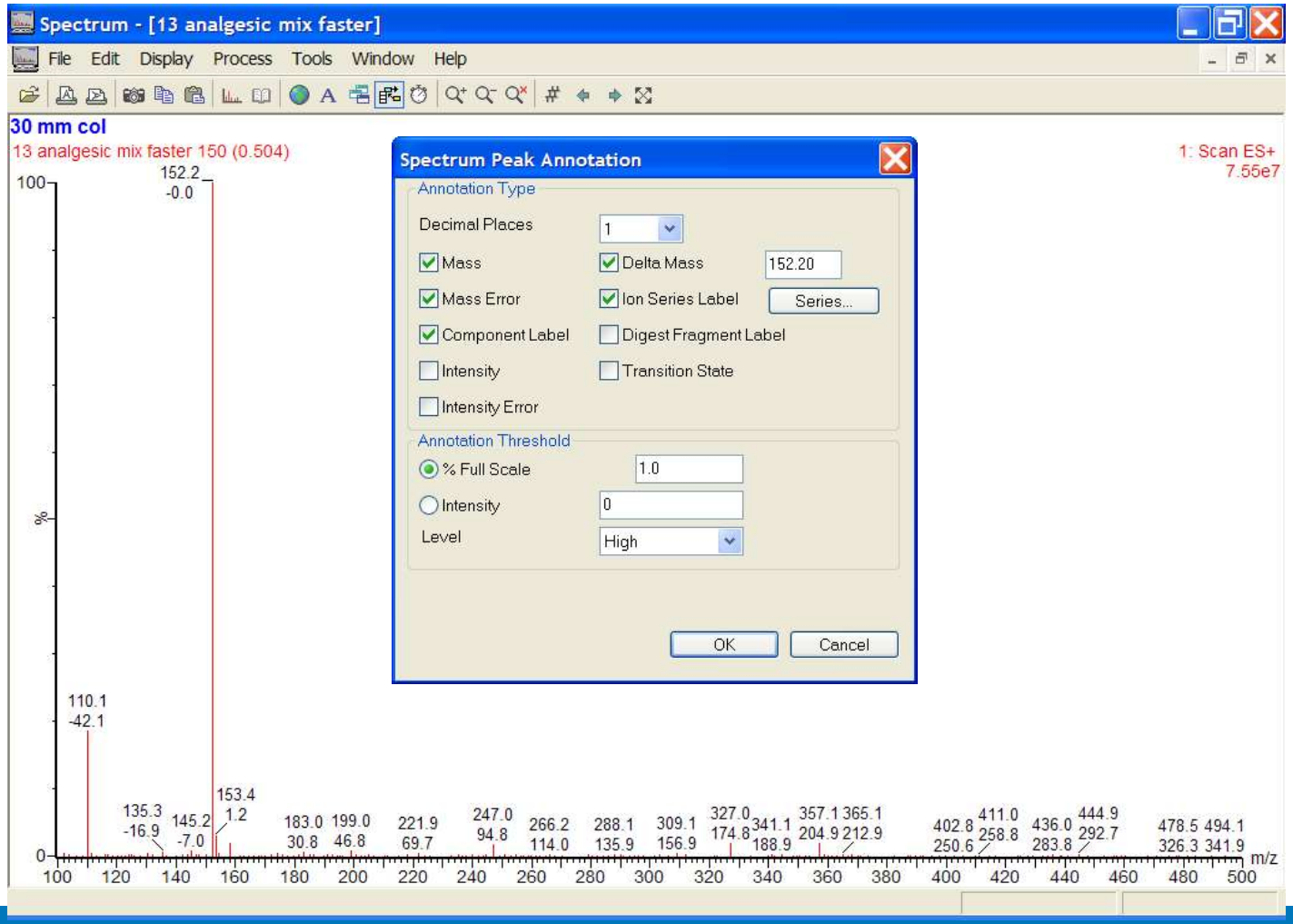
Spectrum Annotation



Spectrum Annotation

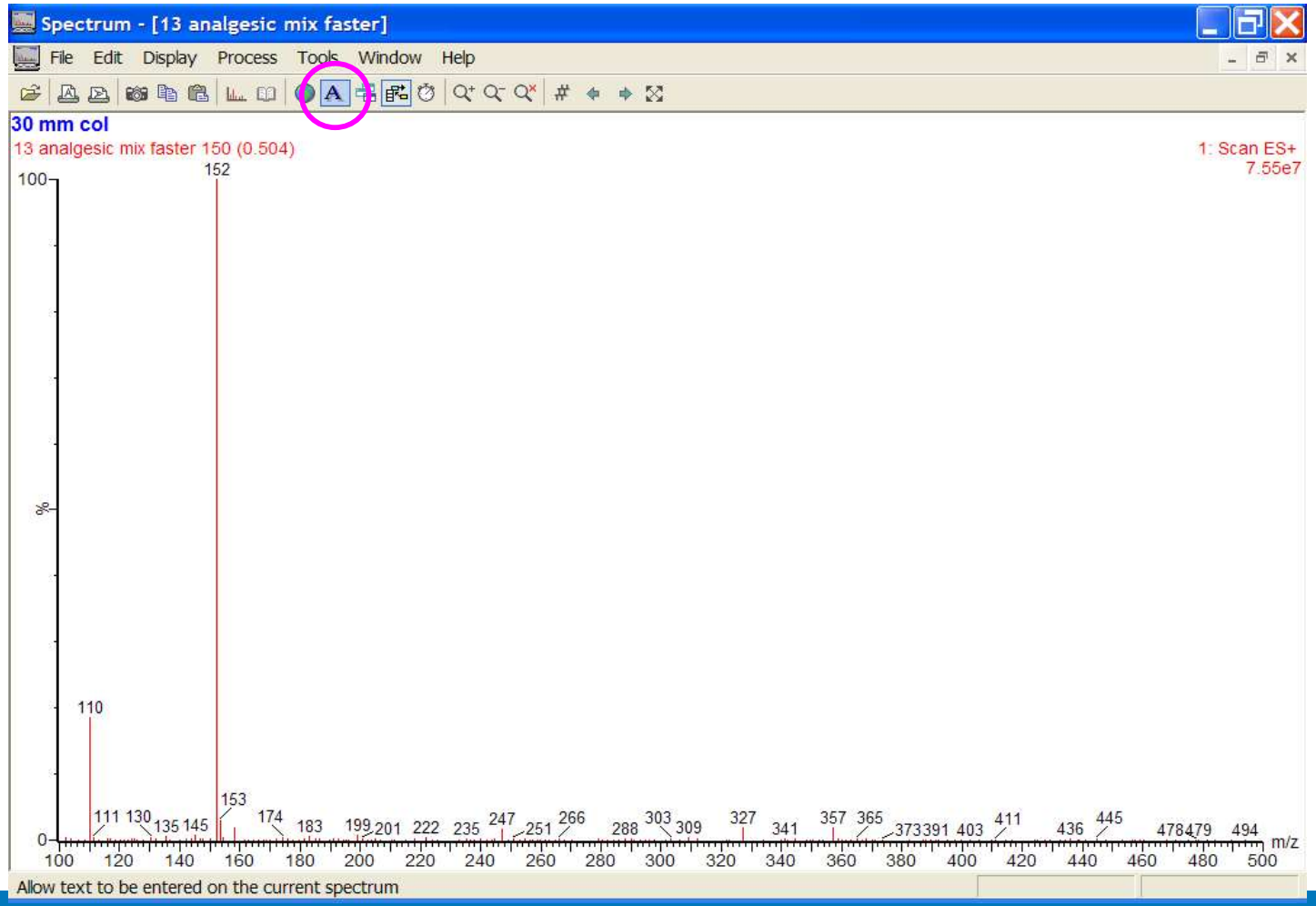


Spectrum Annotation



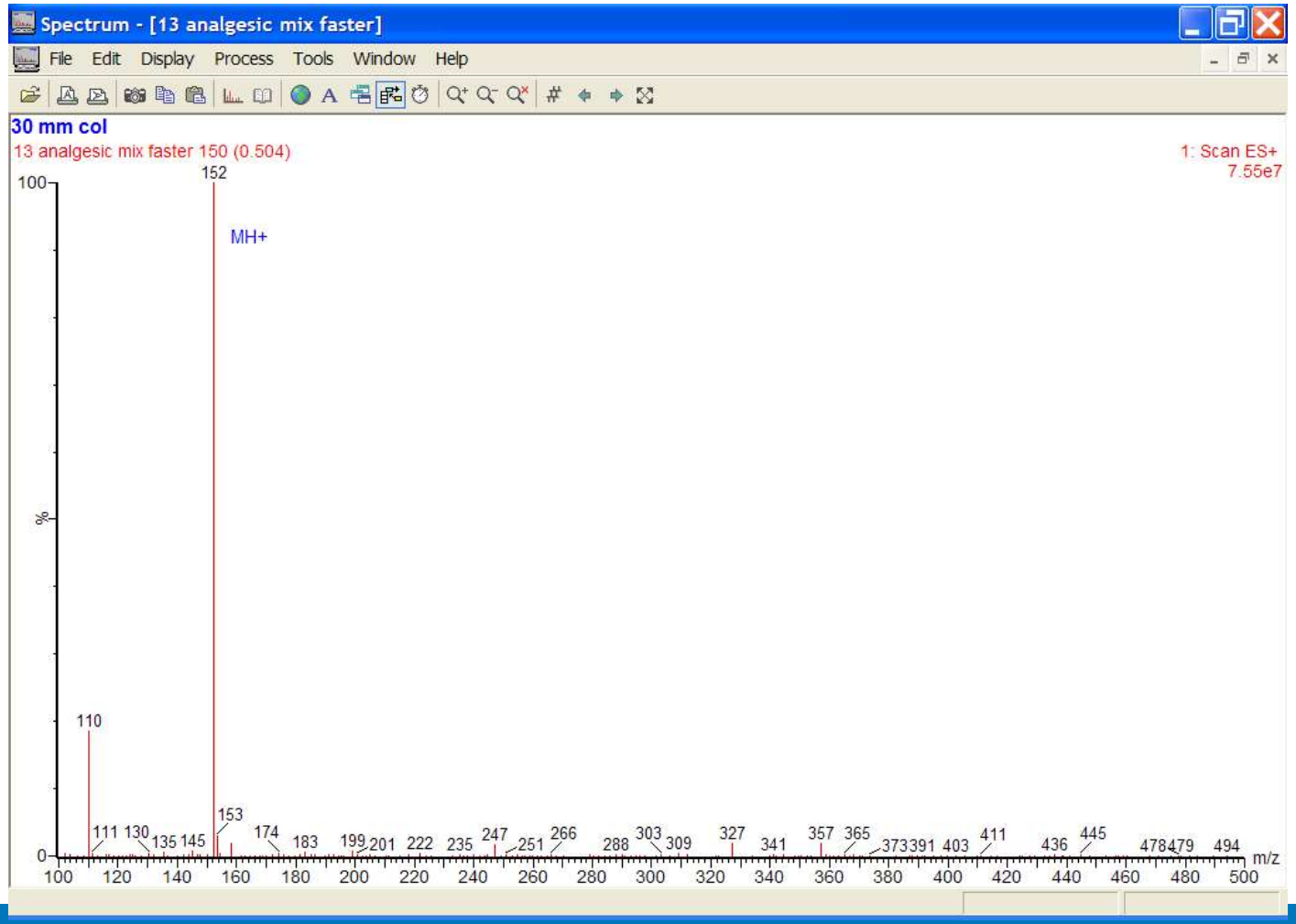
Spectrum Annotation

Adding Text to a Spectrum



Adding Text to a Spectrum

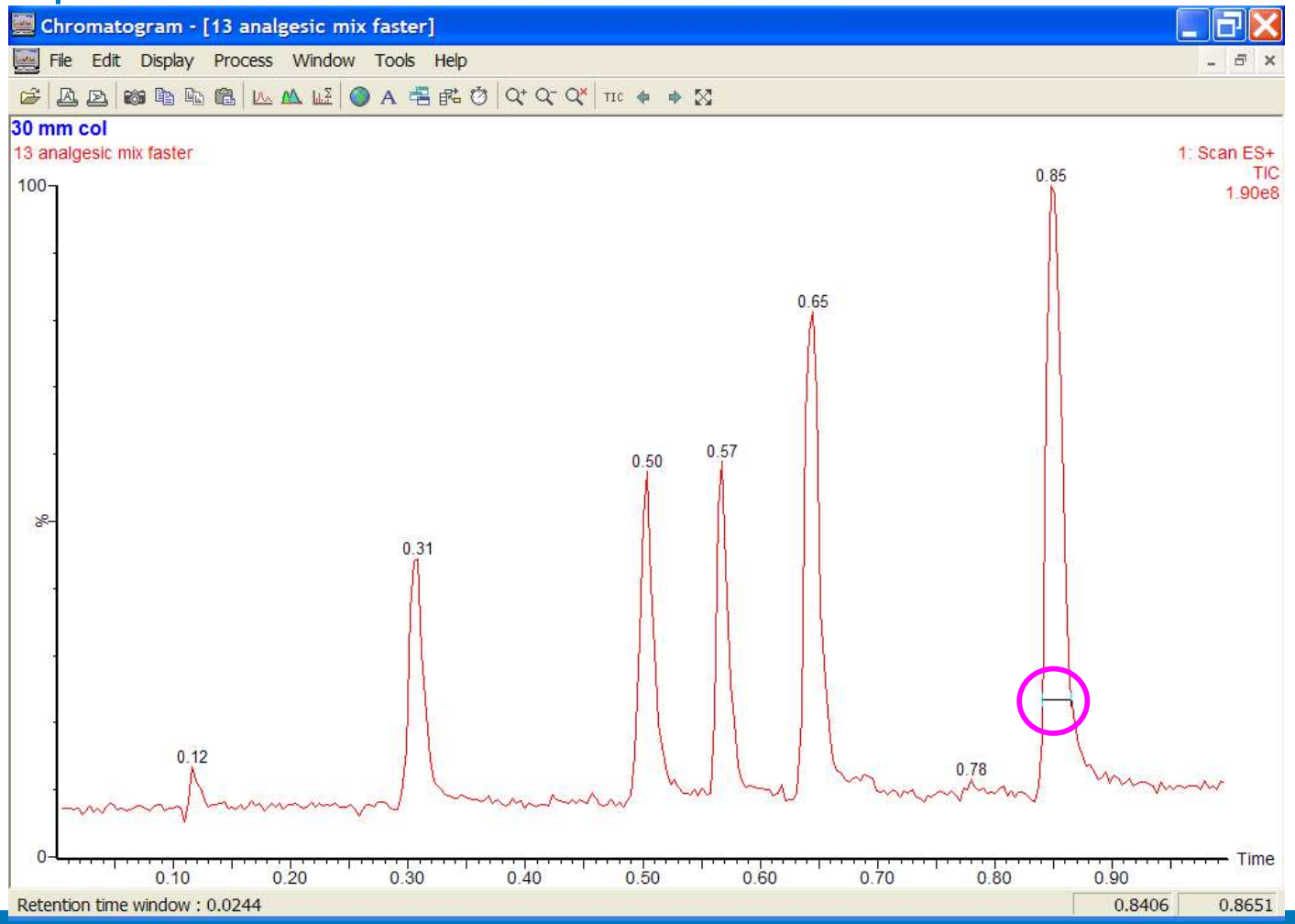
Adding Text to a Spectrum



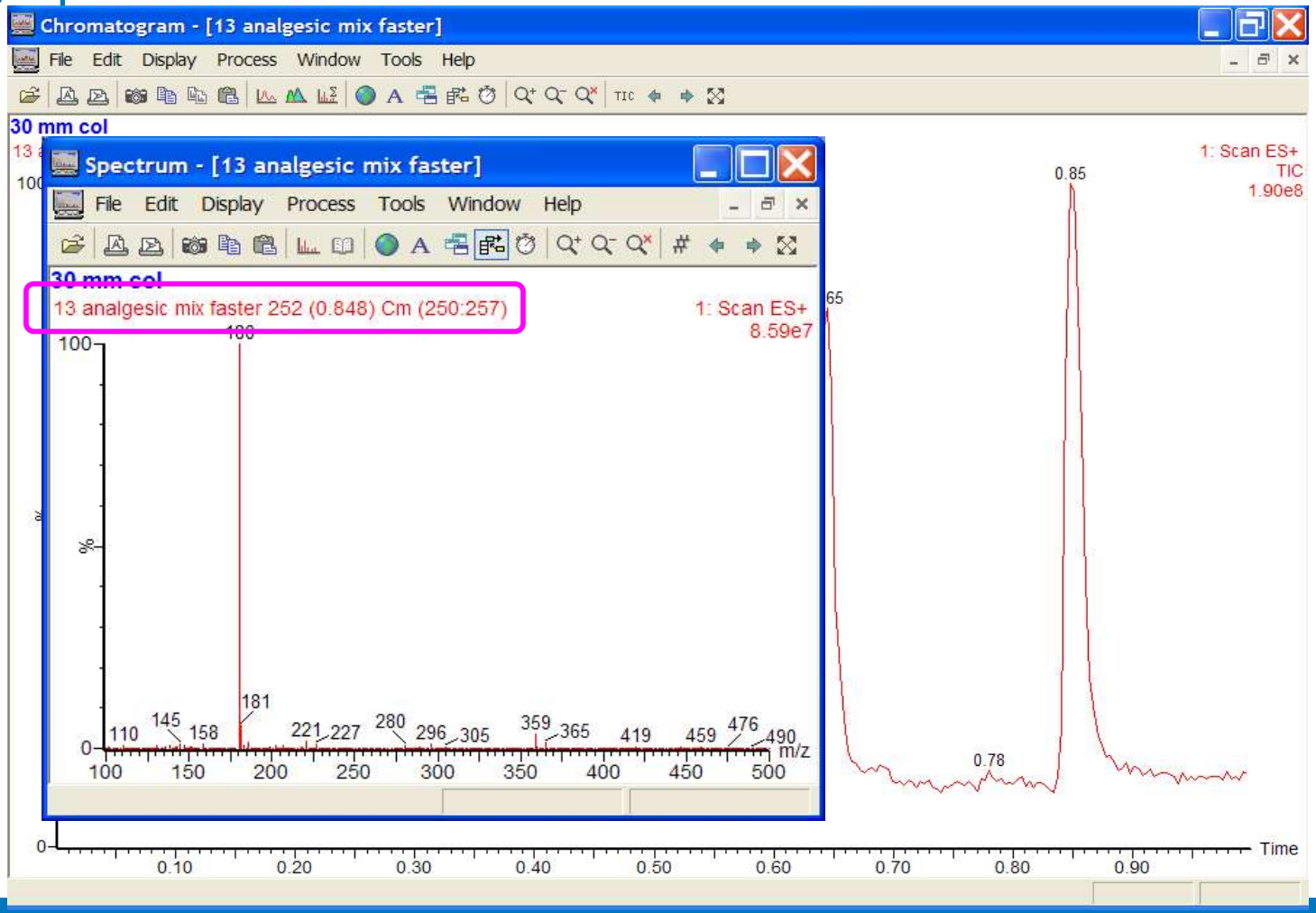
Processing Spectra

- Combined spectra
- Combined spectra with background subtraction

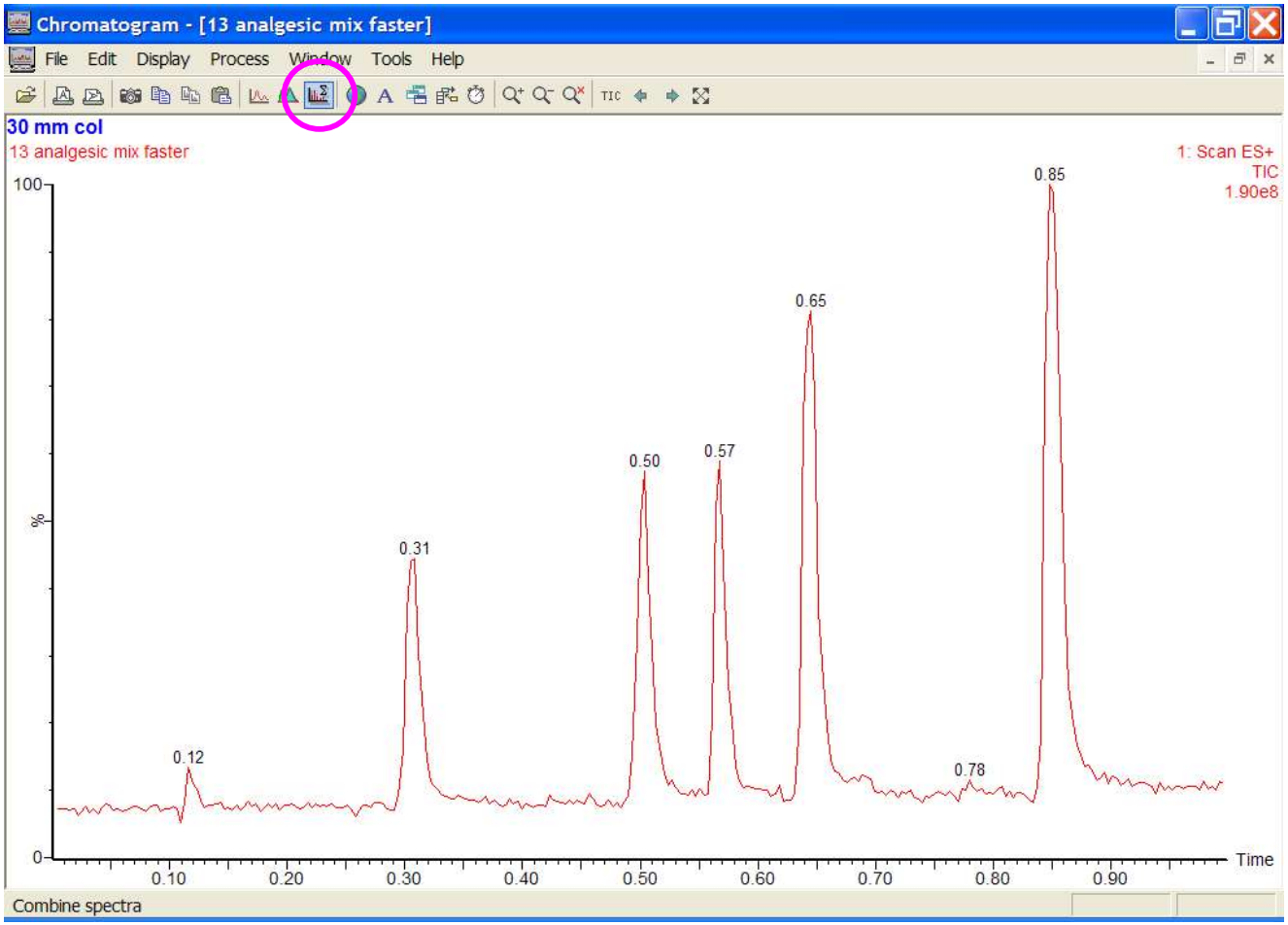
Combining Spectra



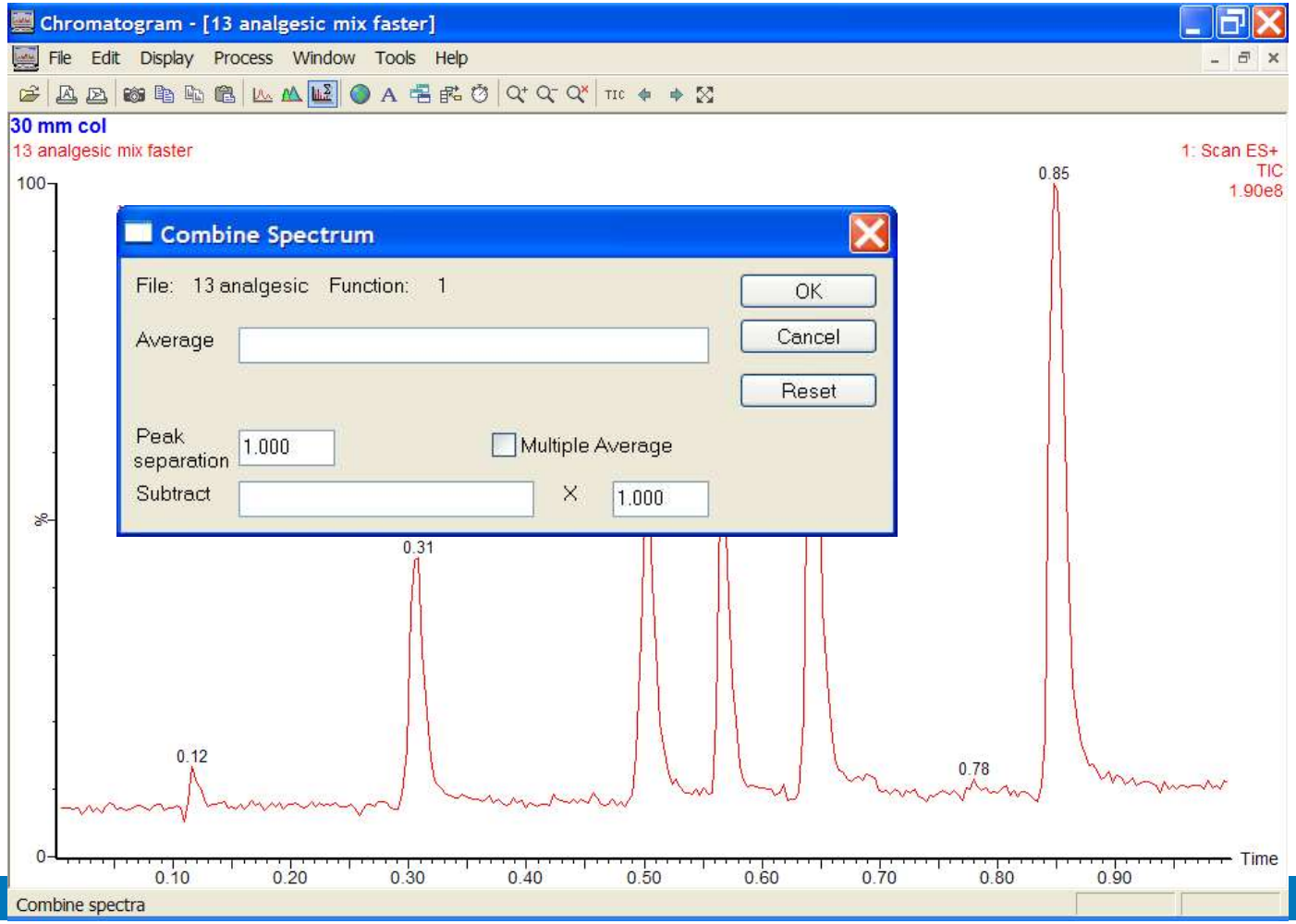
Combining Spectra



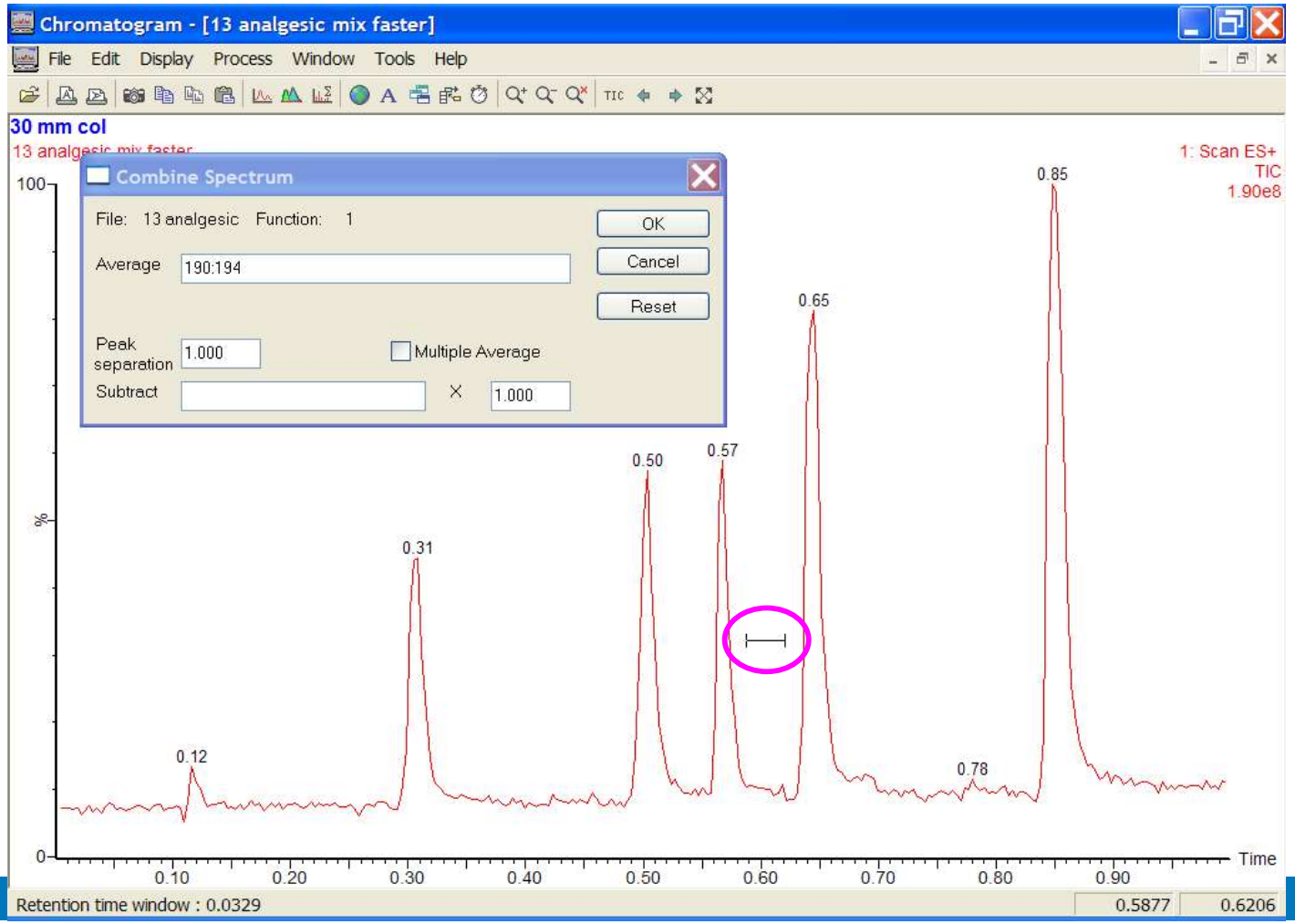
Combining Spectra with Background Subtraction



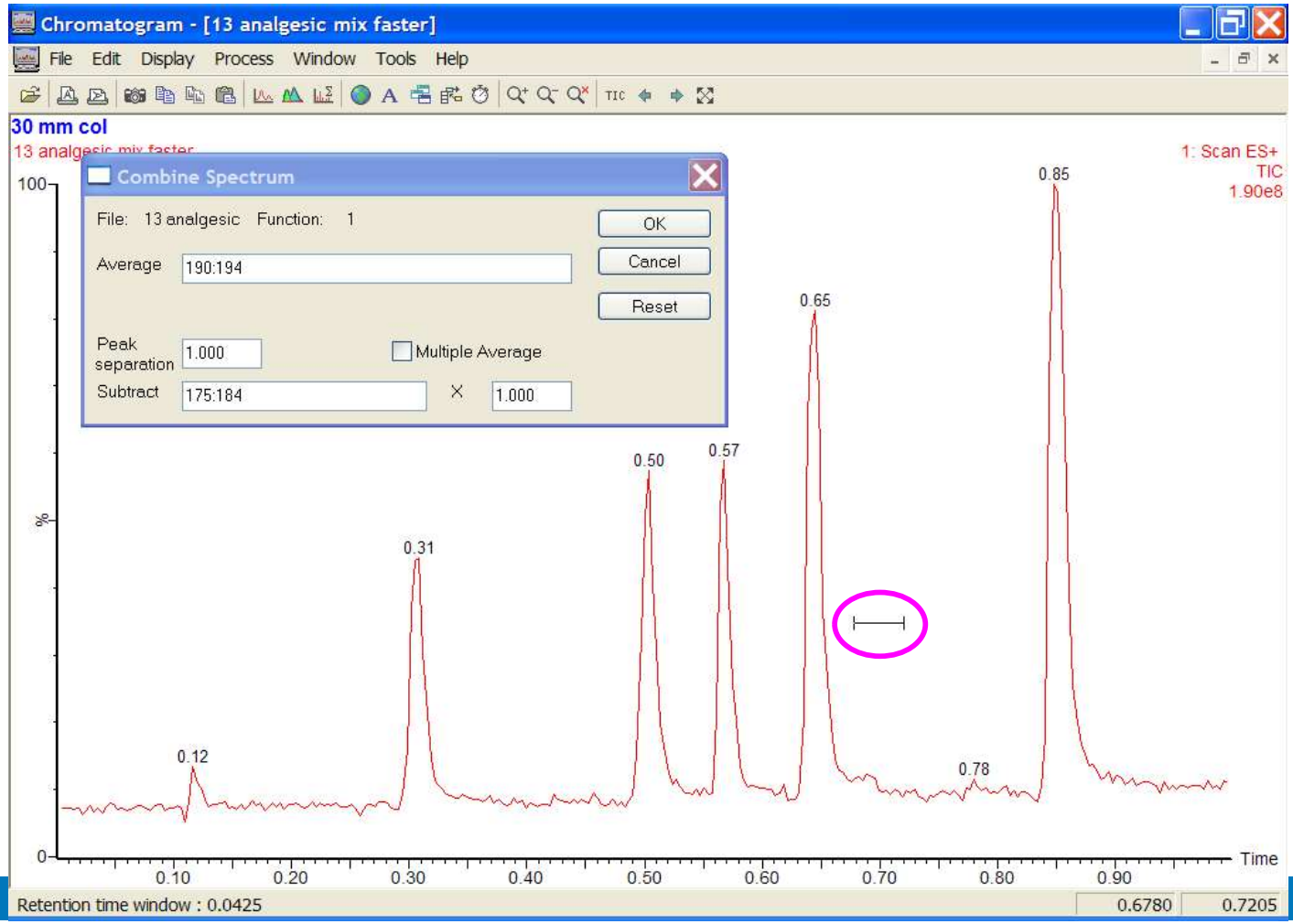
Combining Spectra with Background Subtraction



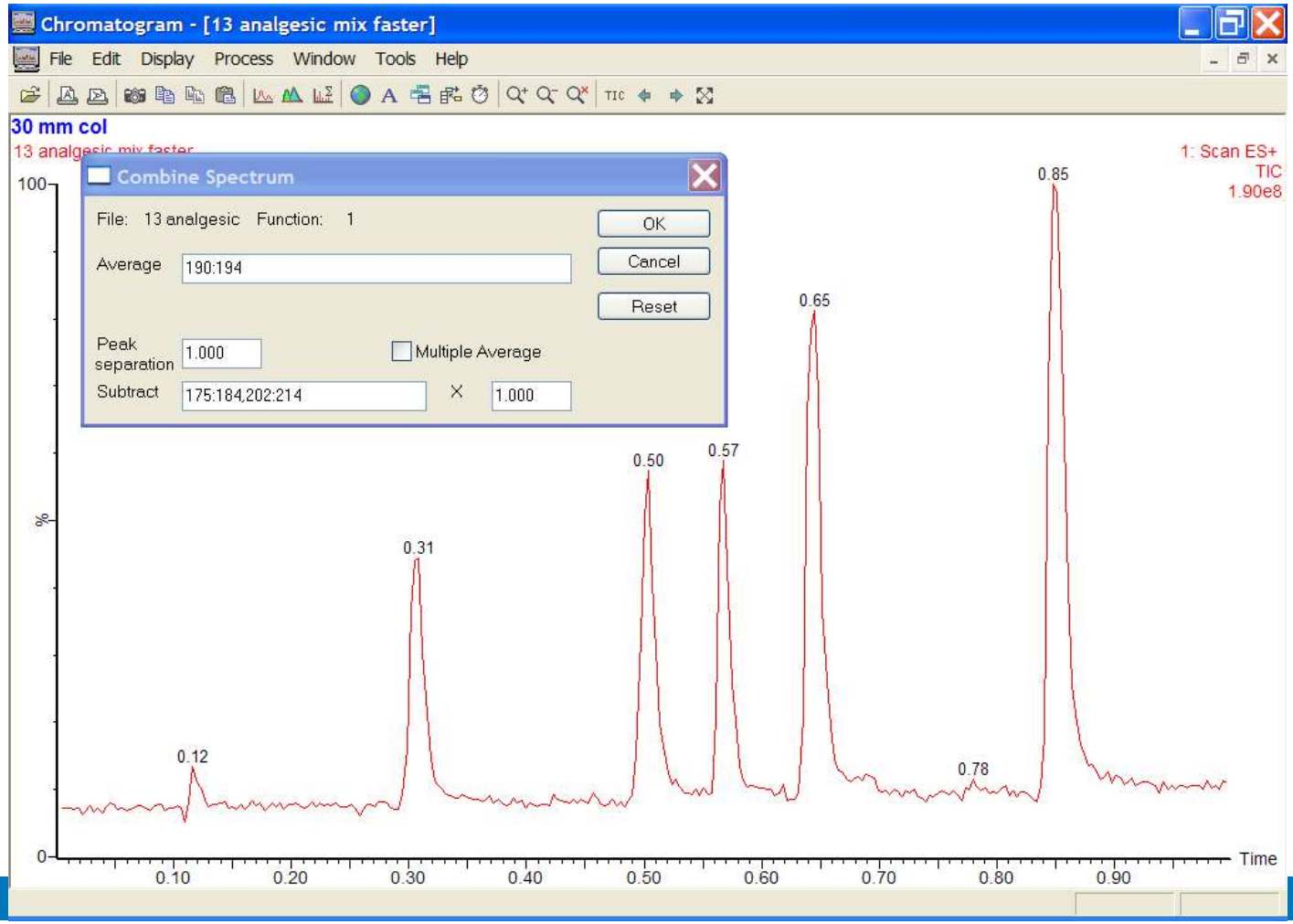
Combining Spectra with Background Subtraction



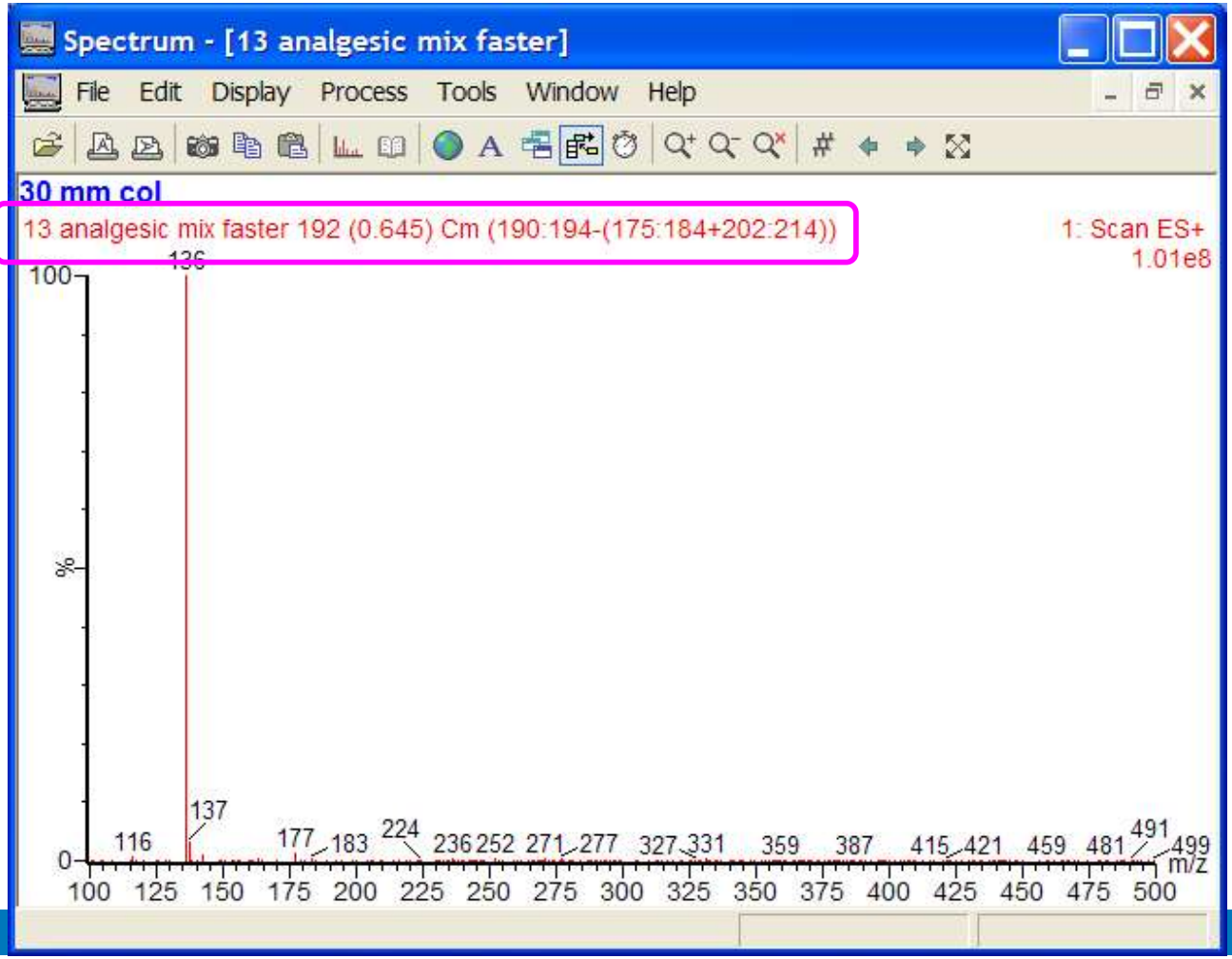
Combining Spectra with Background Subtraction



Combining Spectra with Background Subtraction



Combining Spectra with Background Subtraction



Chapter Seven

TargetLynx™

Quantitation

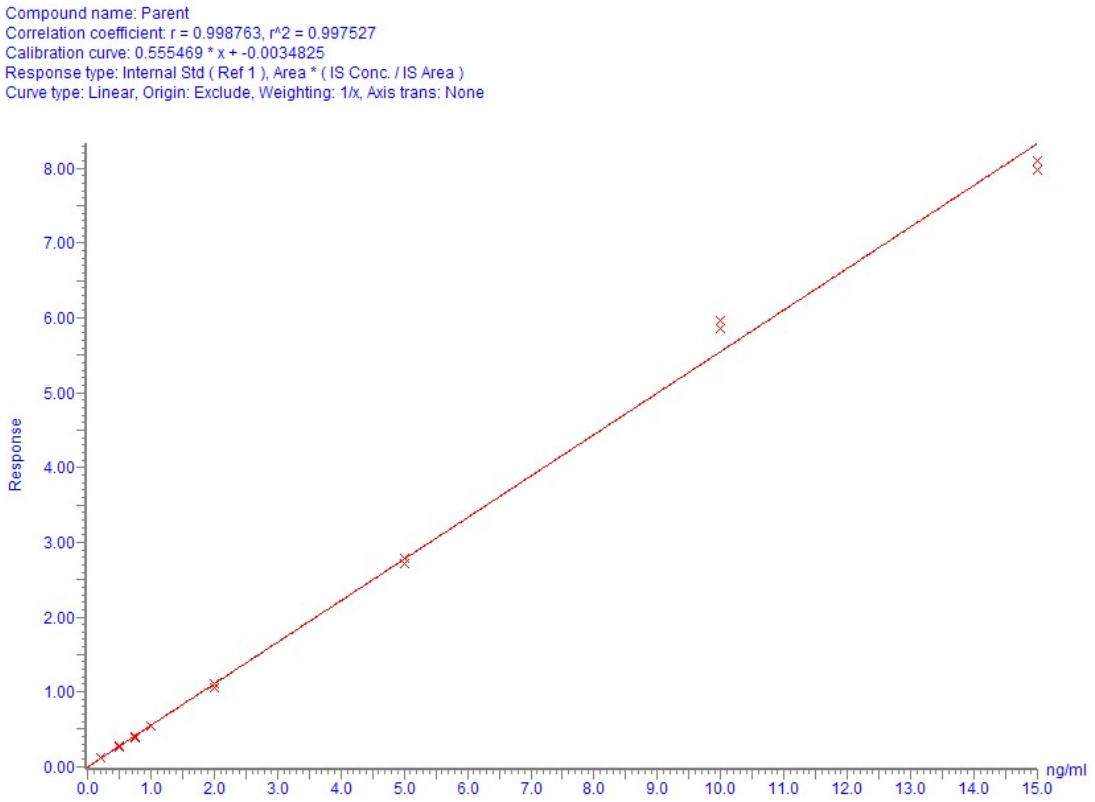
- Determines the concentration of specific analytes within a sample
- Can be done on data acquired through a variety of Acquisition Modes:
  - Multiple Reaction Monitoring (MRM)
  - Single Ion Recording (SIR)
  - Full Scan Acquisition

How Do We Quantitate?

- In addition to unknown samples, a set of standards is also run to form a calibration curve.
- MassLynx analyzes the response of unknown samples and compares their response to that indicated by the calibration curve, then calculates the concentrations of the unknowns.

Calibration Curve

Waters™



More On How Do We Quantitate?

Waters™

- Steps in Creation of a Calibration Curve for Quantitation:
  - In each chromatogram, determine the location of the peak relating to a specific compound
  - Integrate peaks in chromatograms
  - Calculate response factor for the located peak
  - Create a calibration curve for that compound

Quantification Example

- Set of analyses on samples using an MS method that has 2 MRM channels
  - Alprazolam (309.2 > 281)
  - Alprozolam-D5 (314.2 > 286.1)

Internal Standards

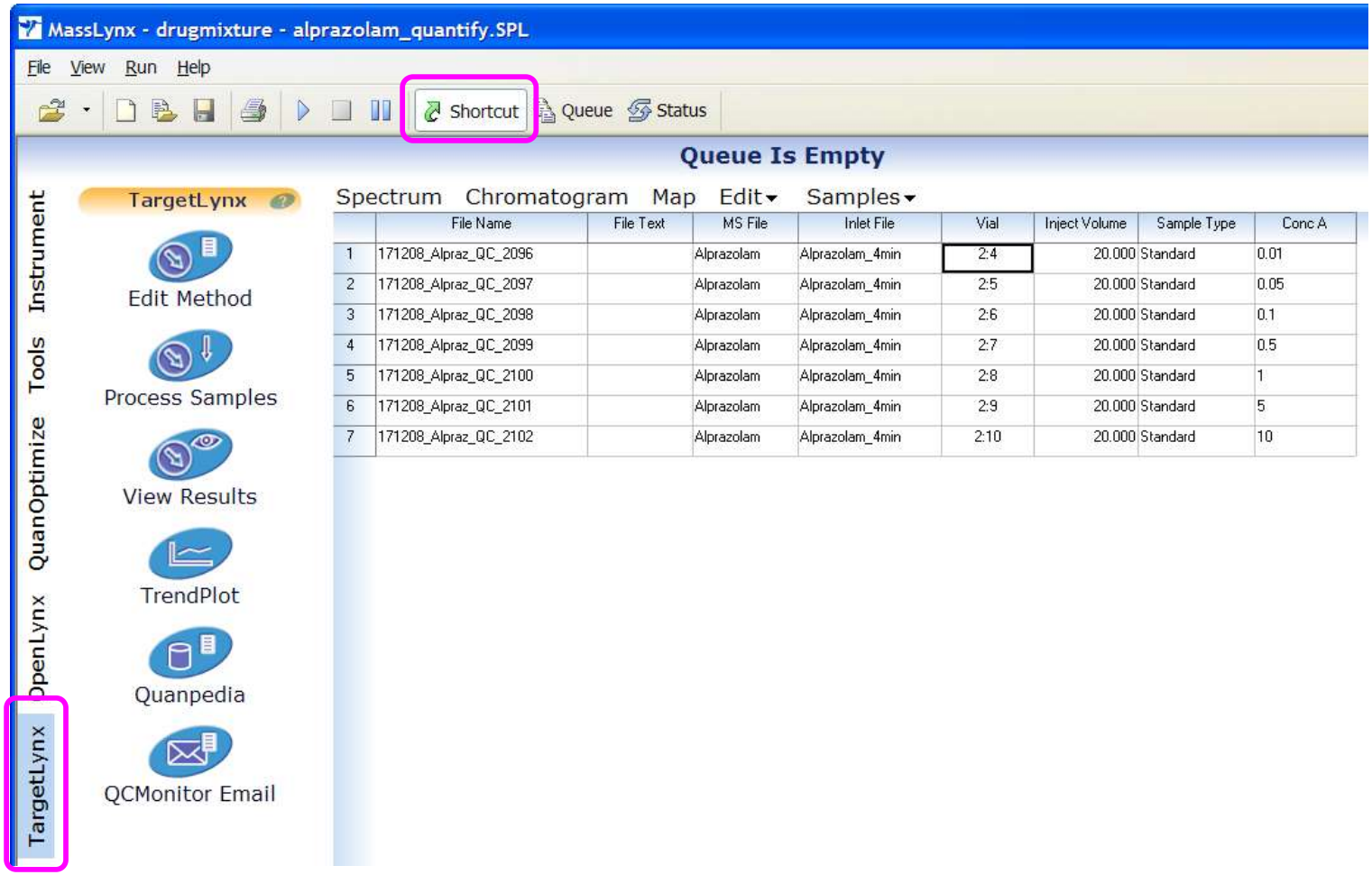
- Used to account for experimental drift
- Can be added at various points in the analysis
  - In the original sample
  - Before injection by the LC
- Response of analyte in a sample is:

$$\frac{\textbf{(Peak Area of Analyte)}}{\textbf{(Peak Area of I.S.) / (Conc of I.S.)}}$$

Quantification Steps

- Enter sample types & concentrations into sample list
- Determine correct integration parameters for chromatogram peaks
- Create Quantification Method
- Process samples
- Check results – adjust if needed
- Print out results – save results in report File

Set up Sample List

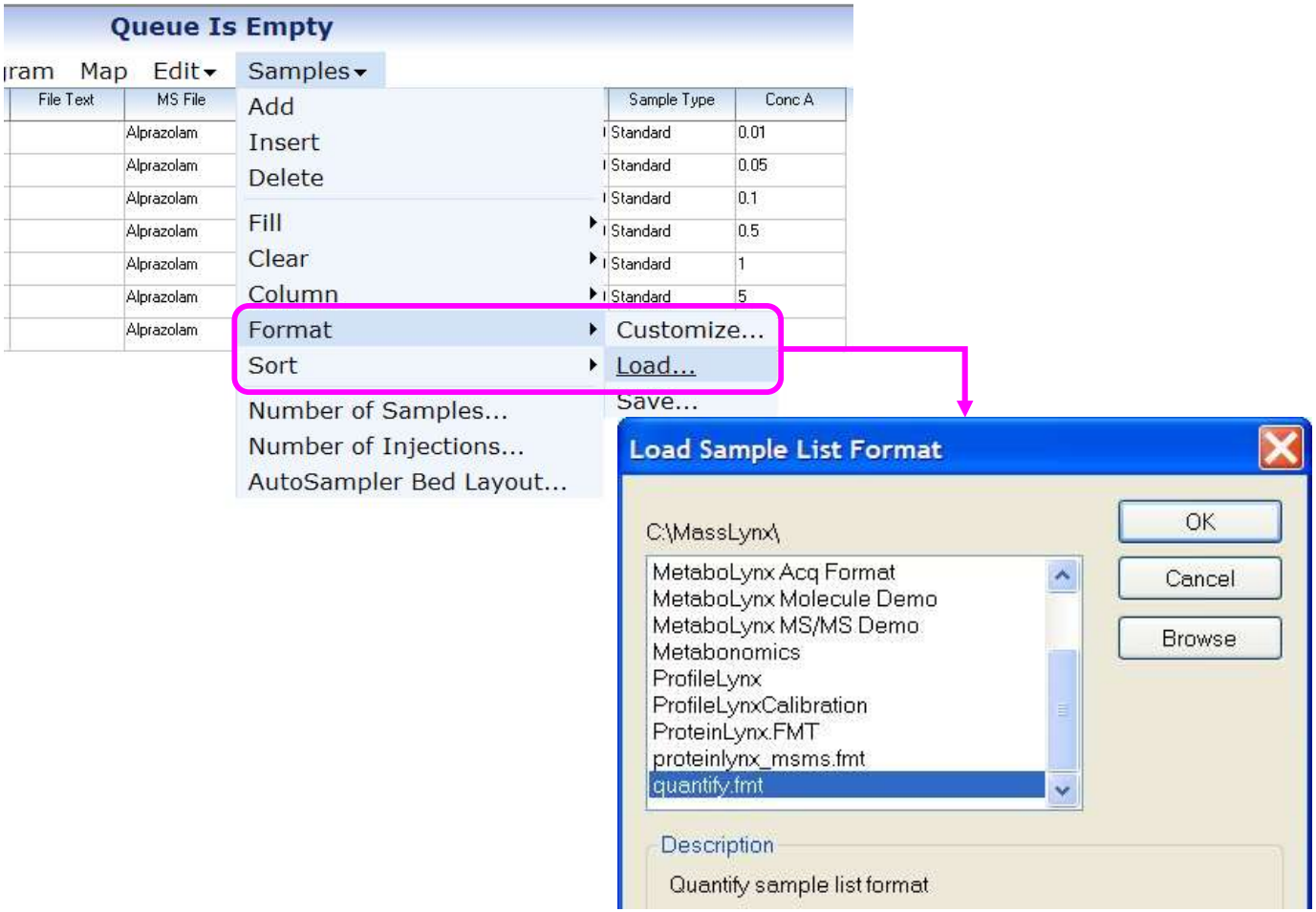


Set up Sample List

- Standard Sample list plus two additional categories:
  - Sample Type
  - Concentration A (B, C, D….)

	File Name	File Text	MS File	Inlet File	Vial	Inject Volume	Sample Type	Conc A
1	171208_Alpraz_QC_2096		Alprazolam	Alprazolam_4min	2:4	20.000	Standard	0.01
2	171208_Alpraz_QC_2097		Alprazolam	Alprazolam_4min	2:5	20.000	Standard	0.05
3	171208_Alpraz_QC_2098		Alprazolam	Alprazolam_4min	2:6	20.000	Standard	0.1
4	171208_Alpraz_QC_2099		Alprazolam	Alprazolam_4min	2:7	20.000	Standard	0.5
5	171208_Alpraz_QC_2100		Alprazolam	Alprazolam_4min	2:8	20.000	Standard	1
6	171208_Alpraz_QC_2101		Alprazolam	Alprazolam_4min	2:9	20.000	Standard	5
7	171208_Alpraz_QC_2102		Alprazolam	Alprazolam_4min	2:10	20.000	Standard	10

Set up Sample List – Load Format

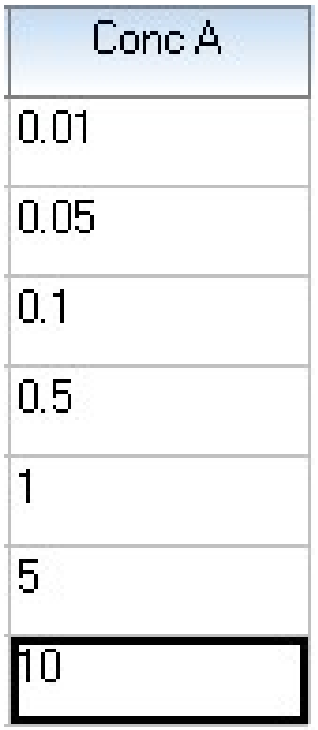


Review of Sample Types

- Blank - Ensures that system is clean and/or shows endogenous material in sample.
- Solvent – Similar to Blank sample type. This setting is used with the QC monitor feature.
- Standard - Sample of a known concentration, used to form calibration curve.
- Analyte - Sample of unknown concentration.
- QC - Quality Control - Known concentrations, used to test the validity and accuracy of the calibration curve.

Specify Sample Types and Concentrations

- Pull Down menu within the sample list. Specify whether the sample is a Blank, Standard, Analyte, Solvent or QC.



- The known concentrations of Standards or QC's must be entered into this column.

Reference Samples

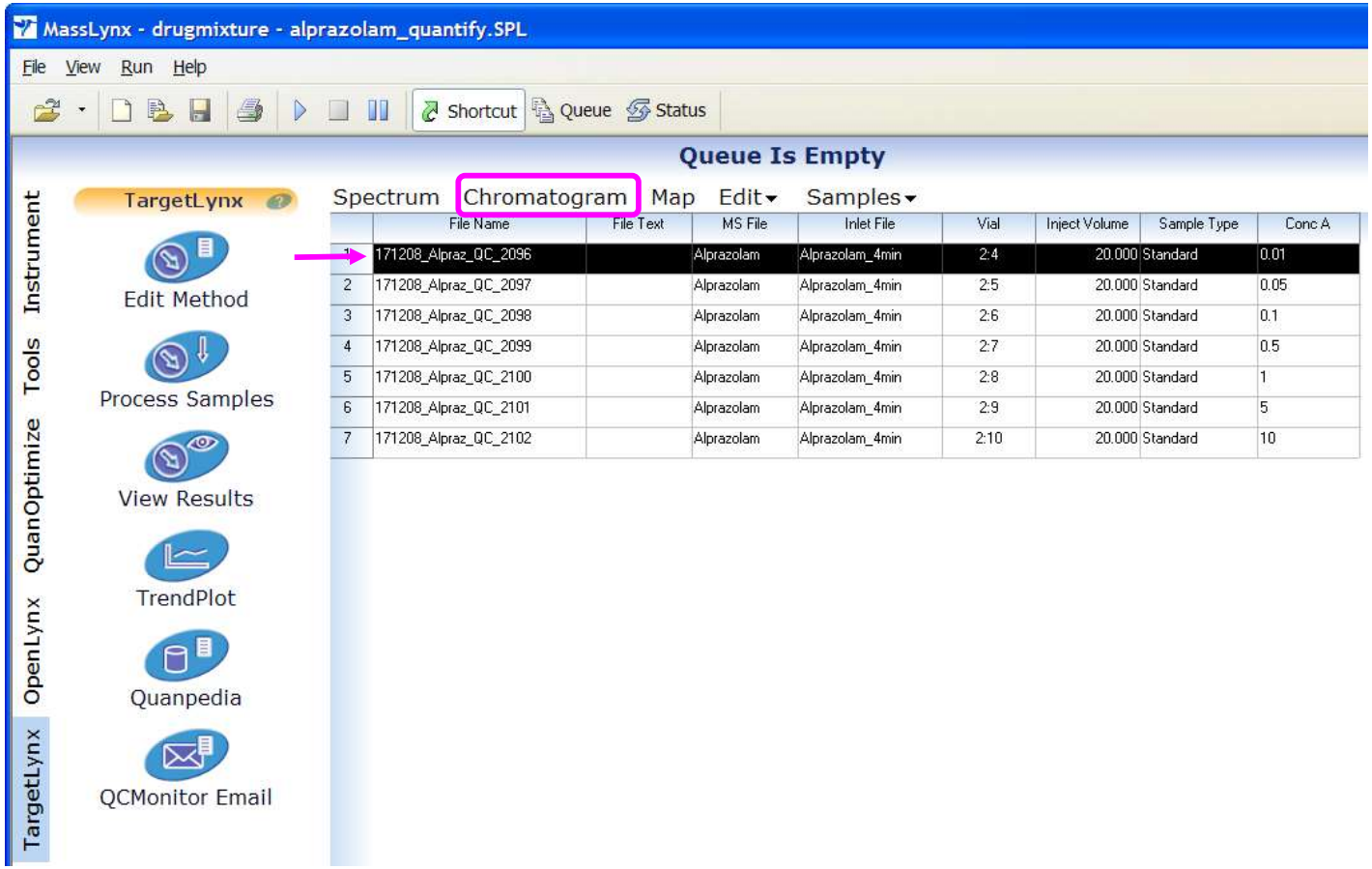
Waters™

Queue Is Empty

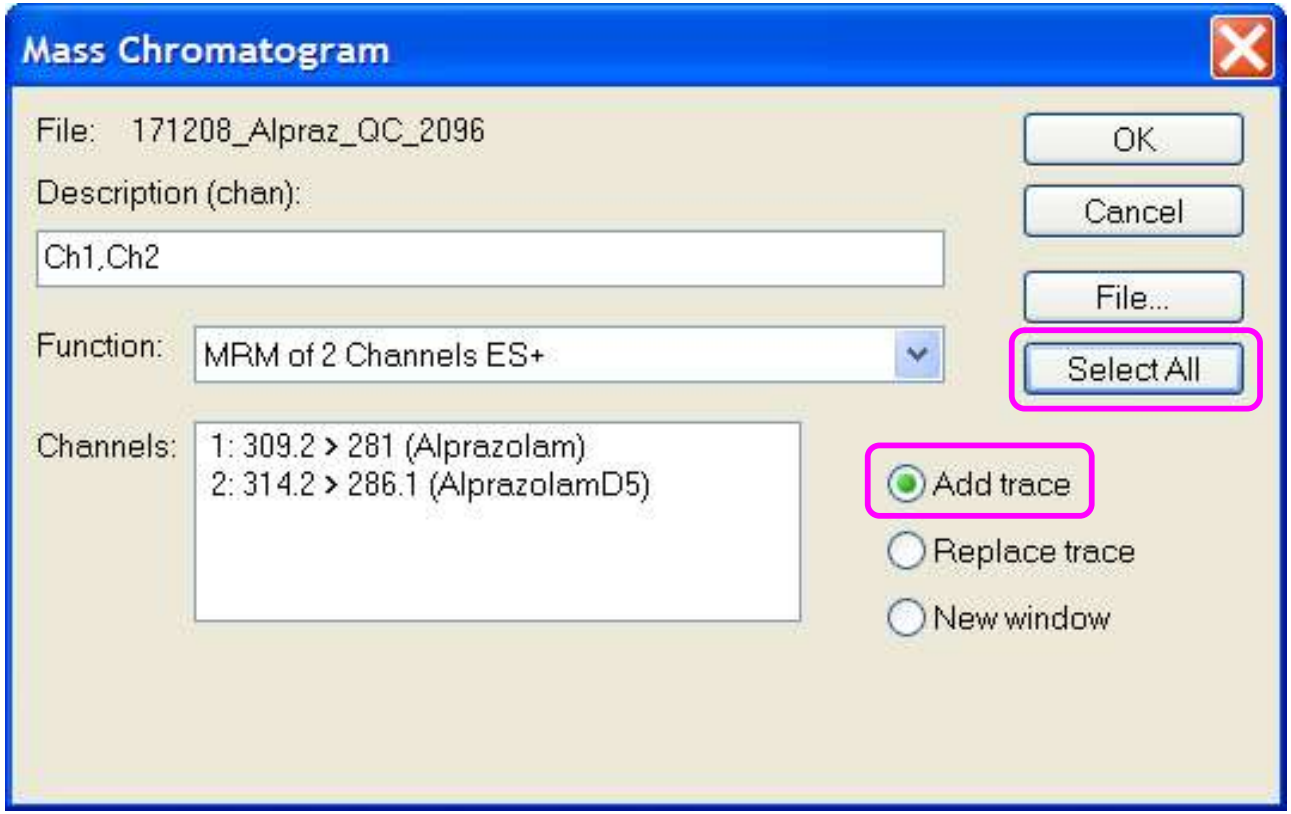
Spectrum Chromatogram Map Edit Samples

	File Name	File Text	MS File	Inlet File	Vial	Inject Volume	Sample Type	Conc A	Quan Referen...
1	171208_Alpraz_QC_2096		Alprazolam	Alprazolam_4min	2:4	20.000	Standard	0.01	
2	171208_Alpraz_QC_2097		Alprazolam	Alprazolam_4min	2:5	20.000	Standard	0.05	
3	171208_Alpraz_QC_2098		Alprazolam	Alprazolam_4min	2:6	20.000	Standard	0.1	
4	171208_Alpraz_QC_2099		Alprazolam	Alprazolam_4min	2:7	20.000	Standard	0.5	
5	171208_Alpraz_QC_2100		Alprazolam	Alprazolam_4min	2:8	20.000	Standard	1	*
6	171208_Alpraz_QC_2101		Alprazolam	Alprazolam_4min	2:9	20.000	Standard	5	
7	171208_Alpraz_QC_2102		Alprazolam	Alprazolam_4min	2:10	20.000	Standard	10	

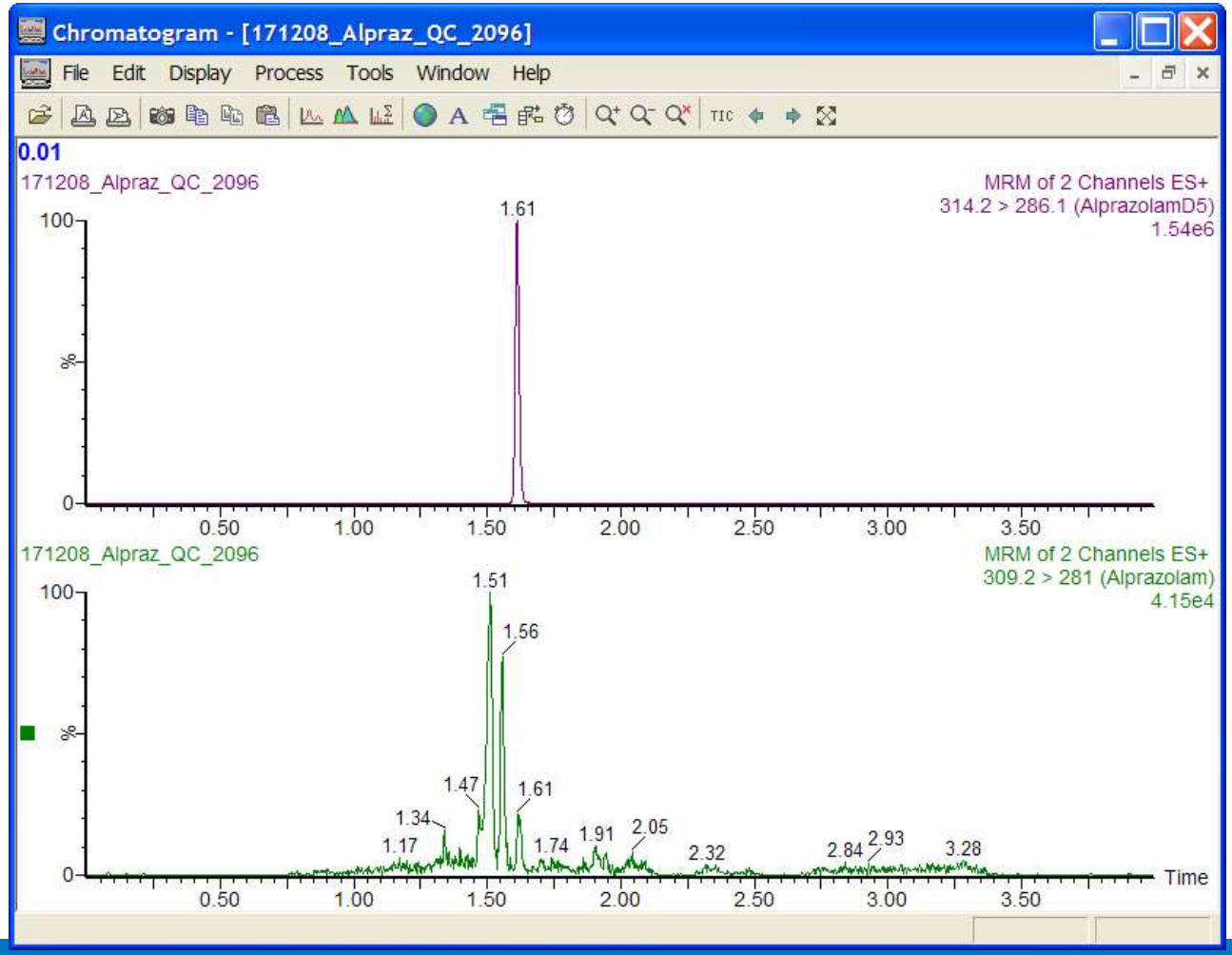
Determining Integration Parameters



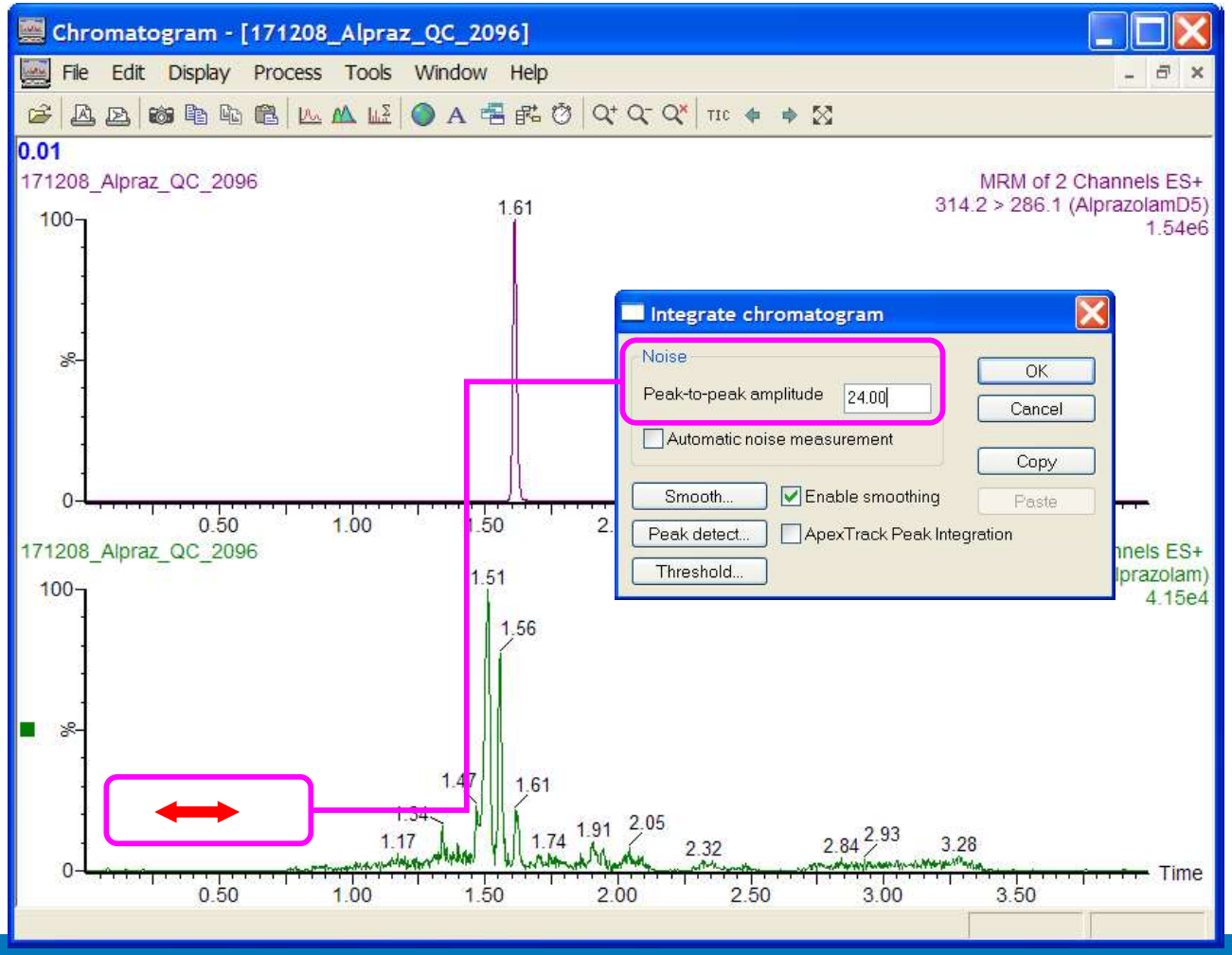
Peak Integration - Display All Traces



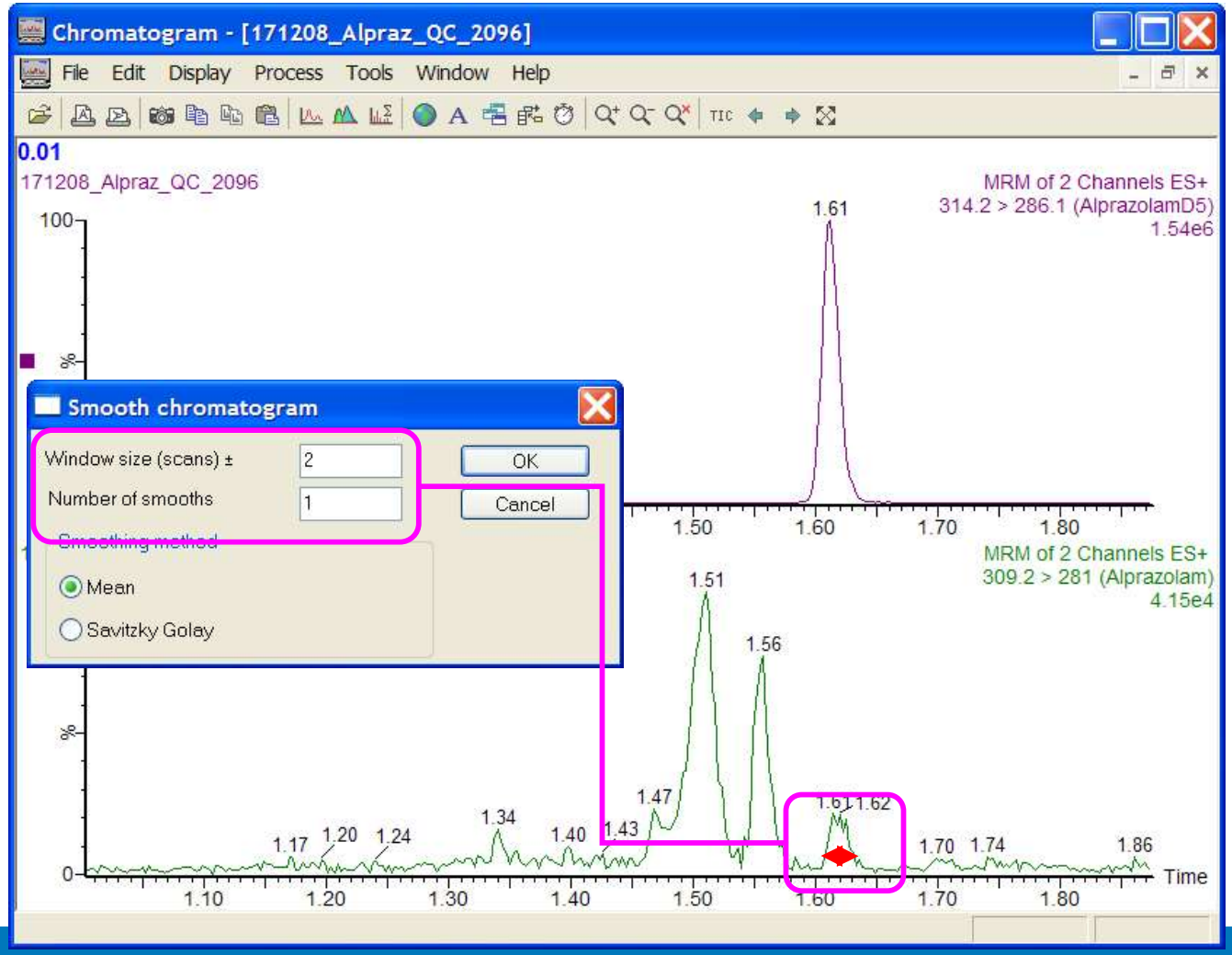
Peak Integration - Display All Traces



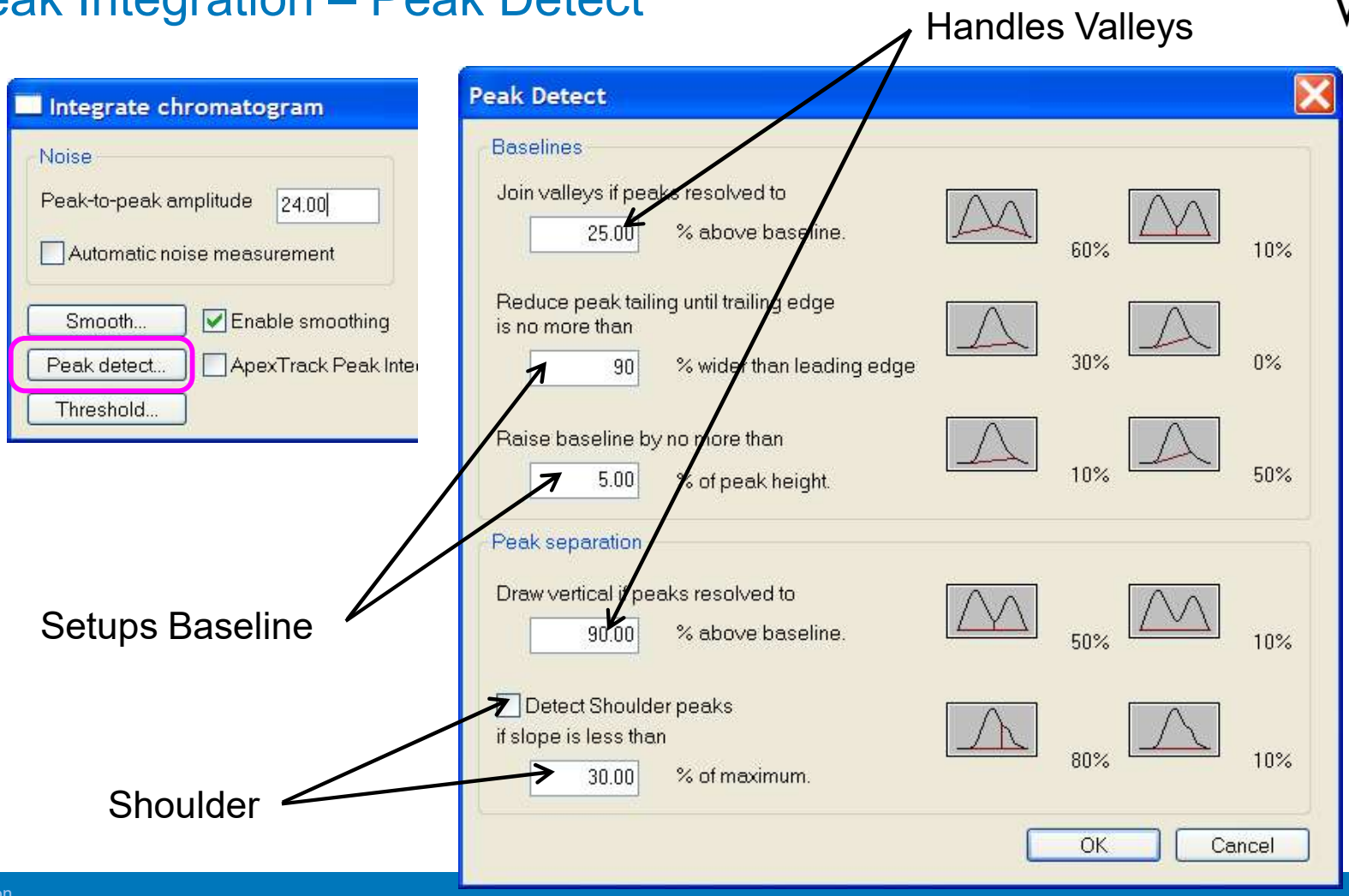
Setup Peak Integration - Noise



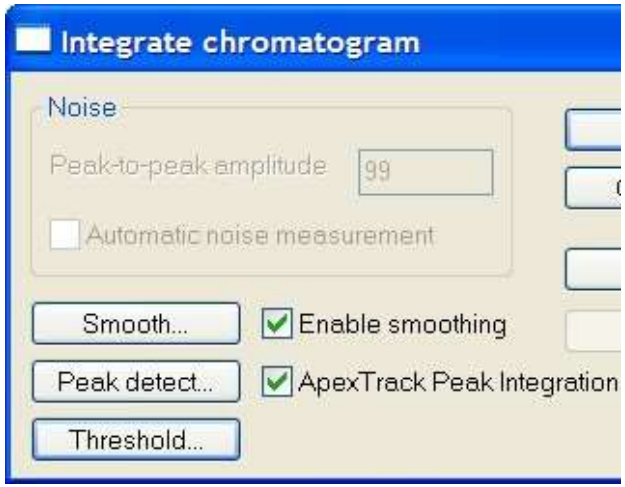
Setup Peak Integration-Smoothing

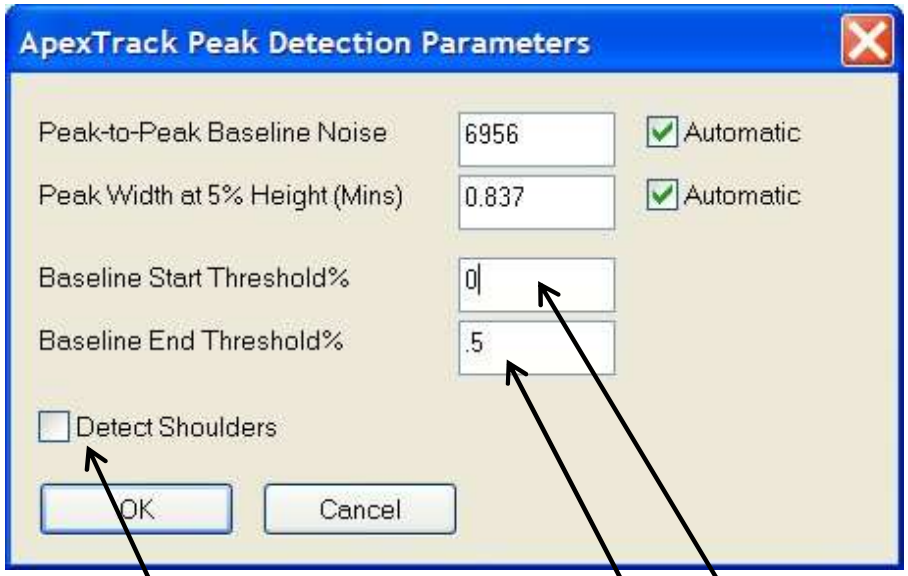


Setup Peak Integration – Peak Detect



Peak Detection with Apex Integration

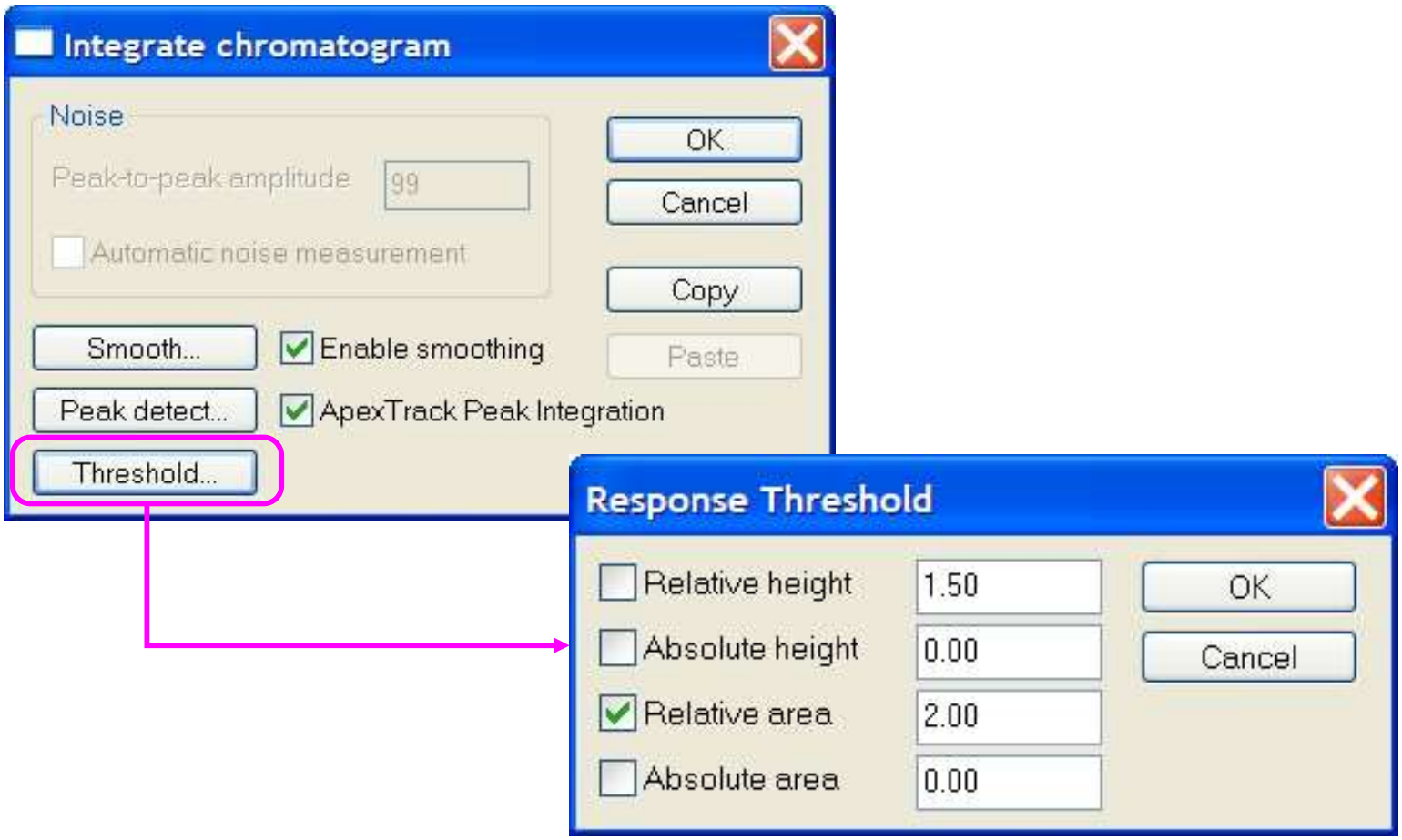




'Check' if you want Shoulders detected

Set starting and ending Baseline levels

Response Threshold

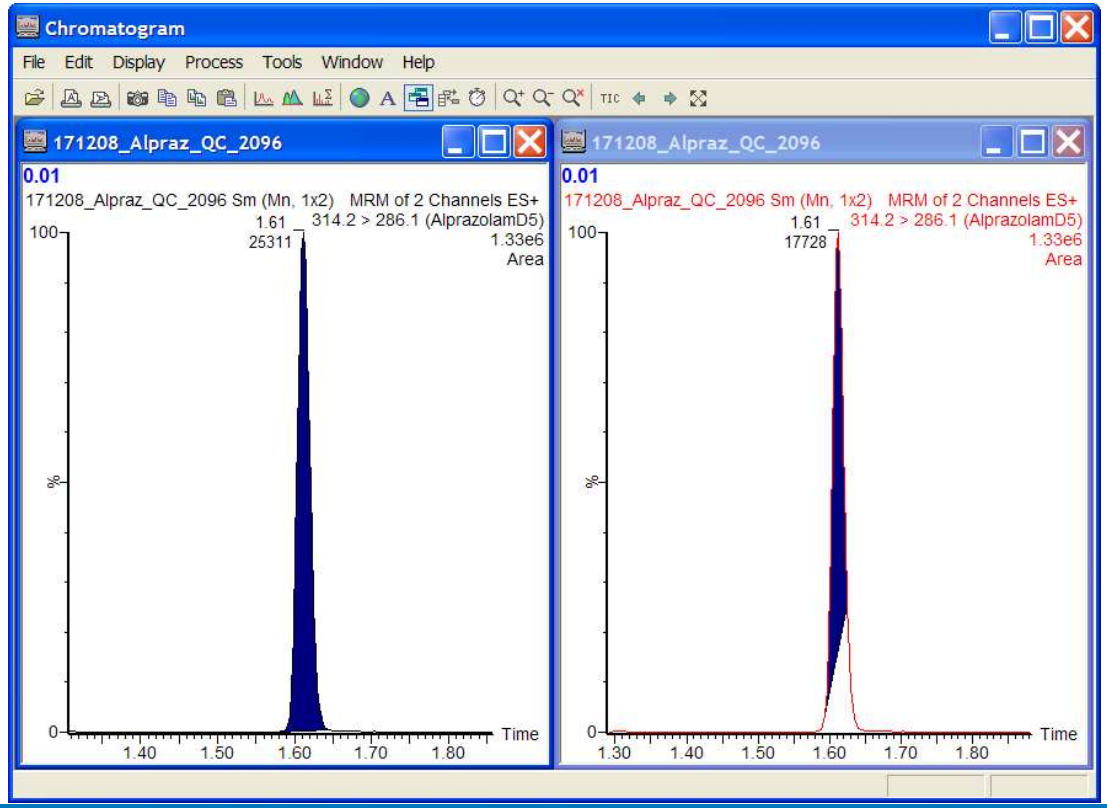


Setup Peak Integration Parameters

- Click **OK**, the peak of interest will be integrated.
- Review the integration - is it acceptable? If not, repeat the integration with different parameters (noise, peak detect, thresholding) until satisfactory results are obtained.
- Once an acceptable integration is attained, you may want to test it on a low range standard and a high range standard to insure that parameters are adequate for the full range of response.

Review Peak Integration

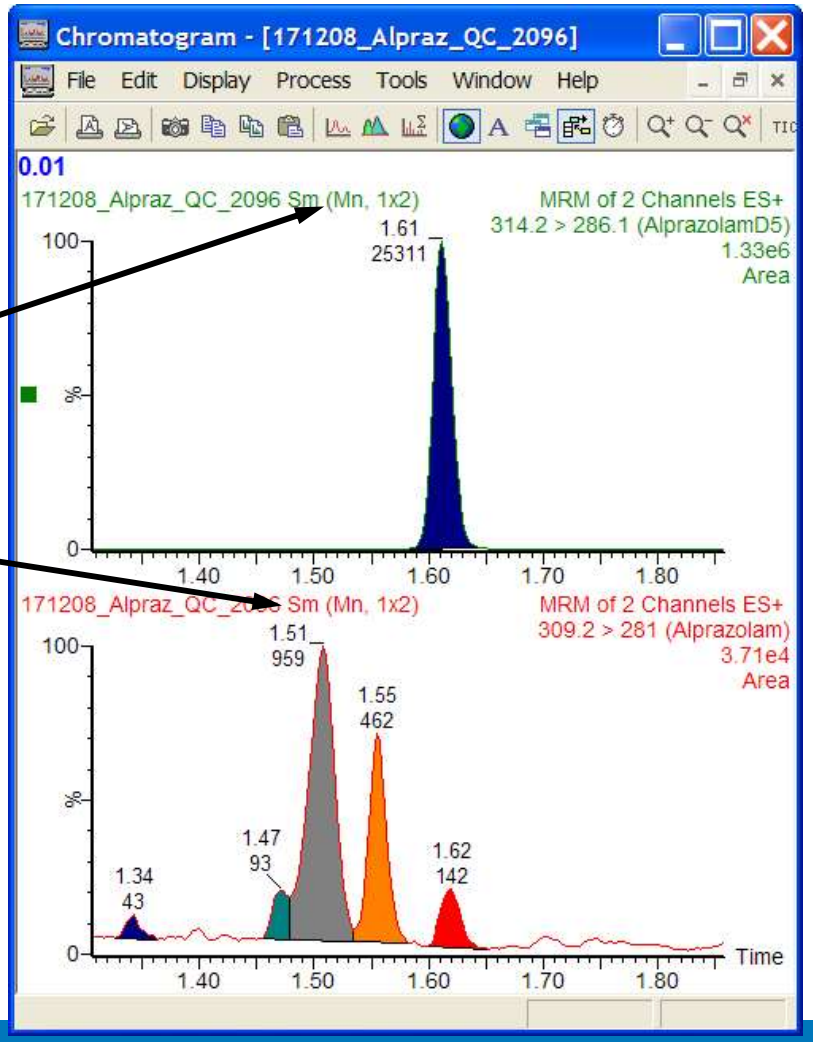
- This is an example of a well integrated peak (left) and a poorly integrated peak (right).



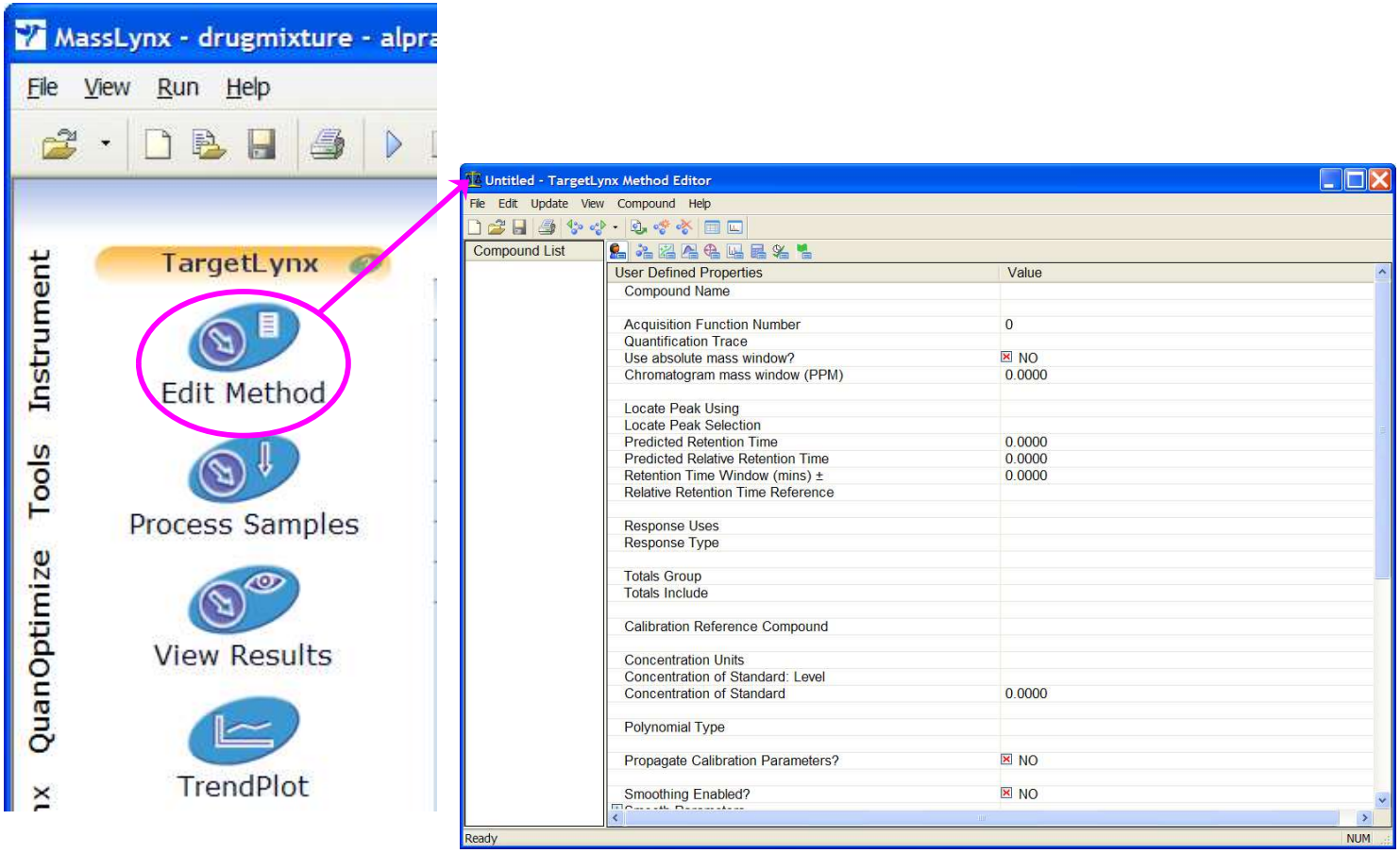
Review Peak Integration – Example

Example of Peak Integration

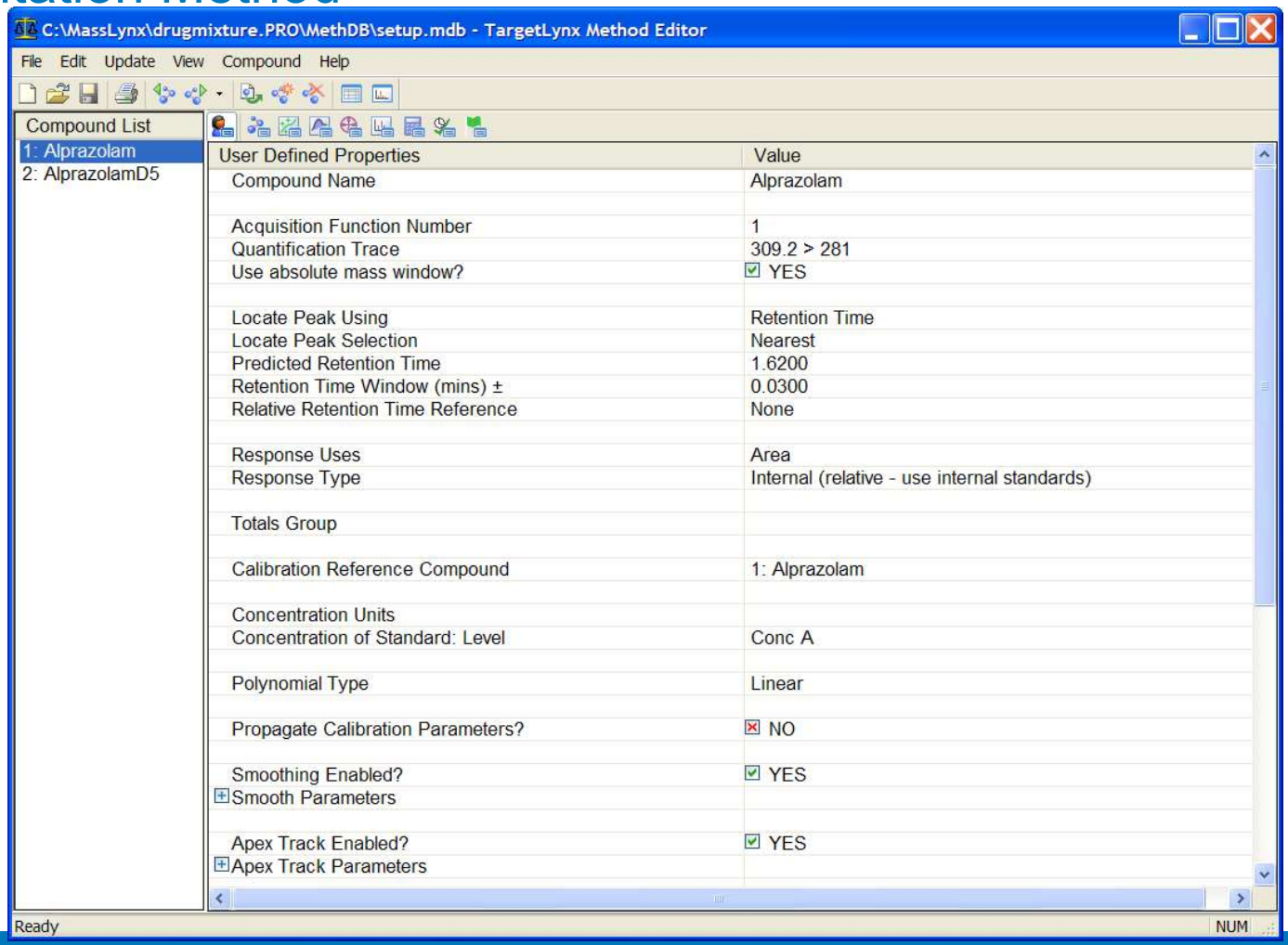
Note Smoothing Parameters for each transition



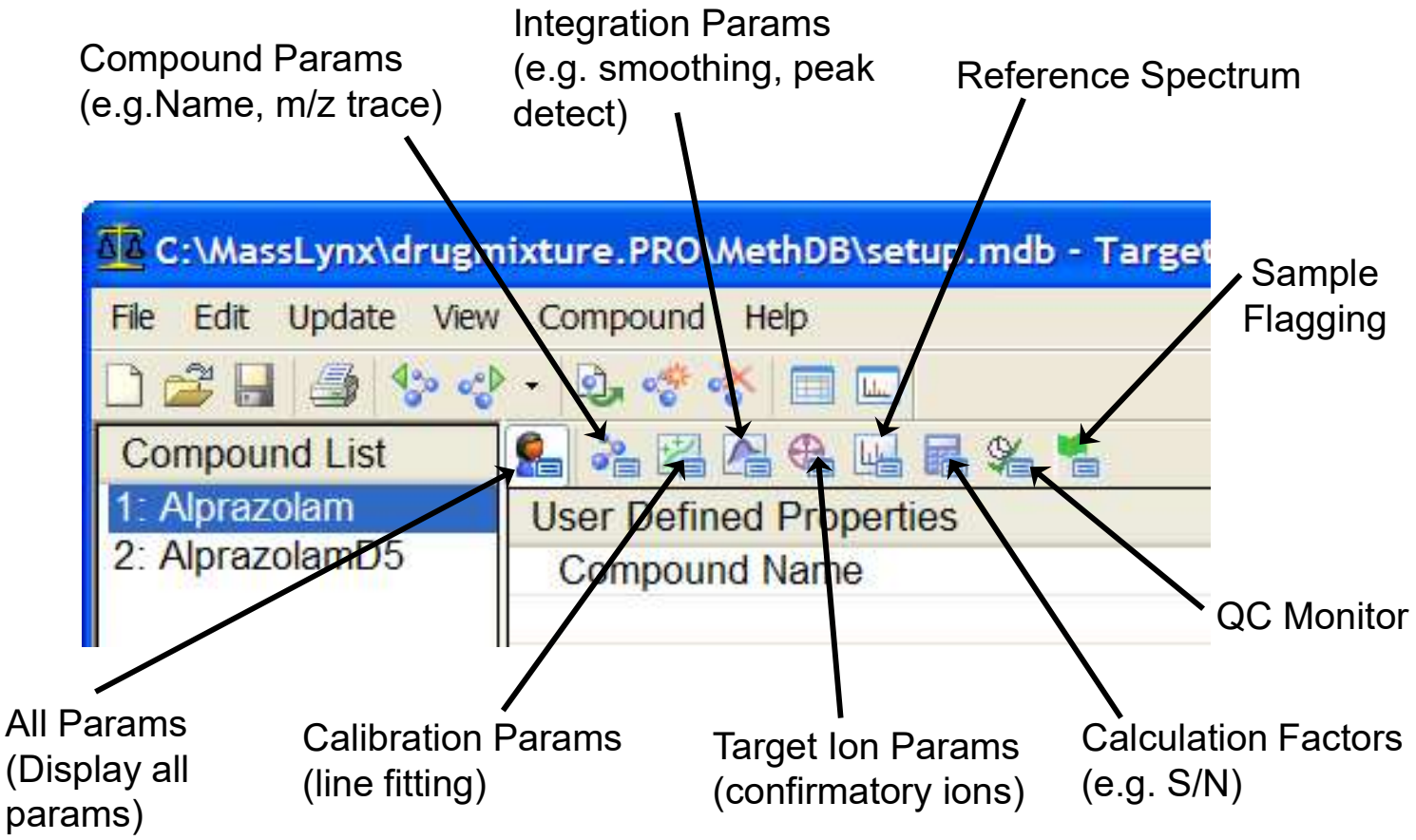
Build Quantitation Method



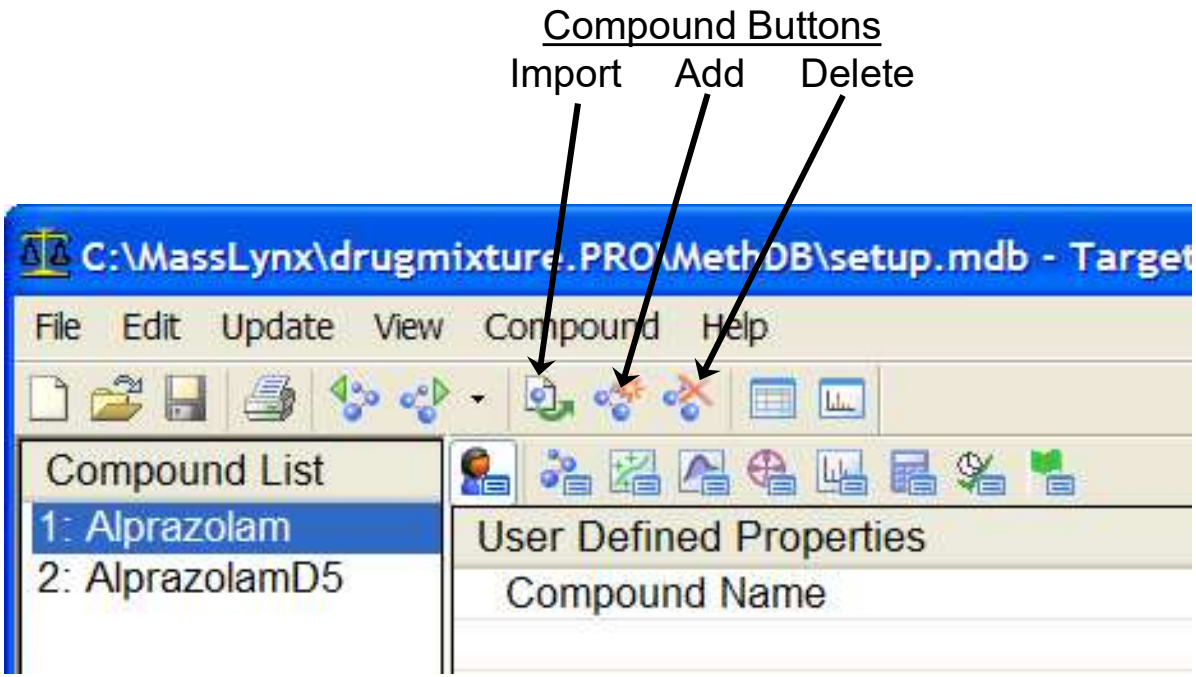
Build Quantitation Method



Build Quantitation Method



Build Quantitation Method



Quant Method Editor – Add Info on Compounds

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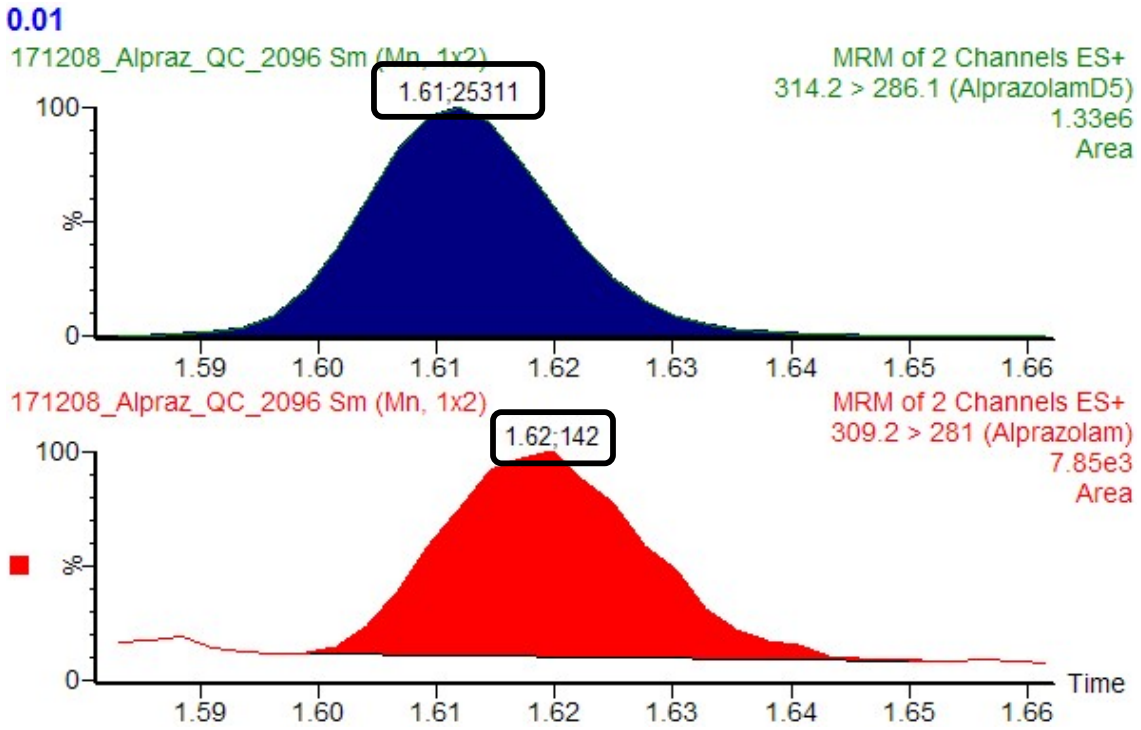
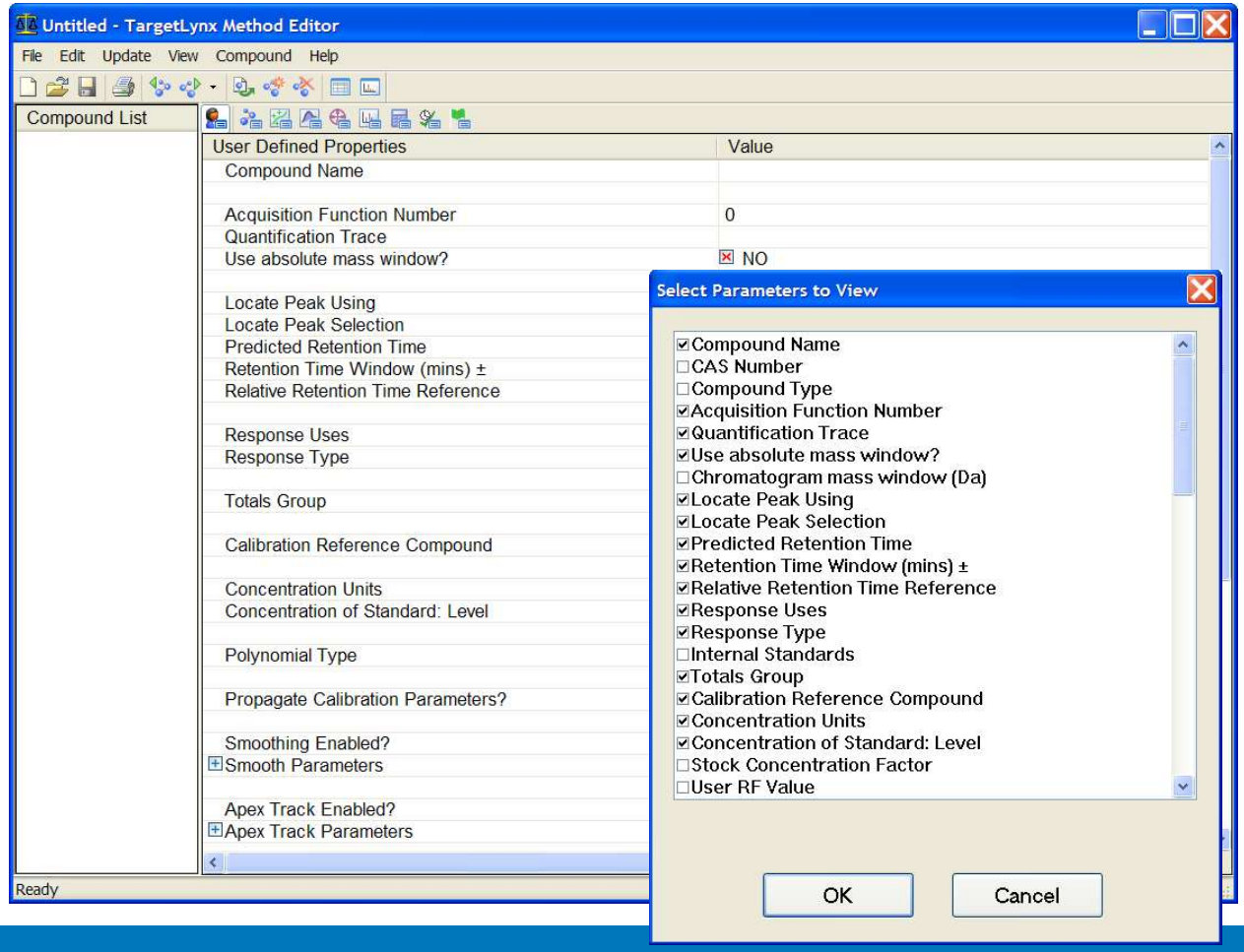
Internal Std

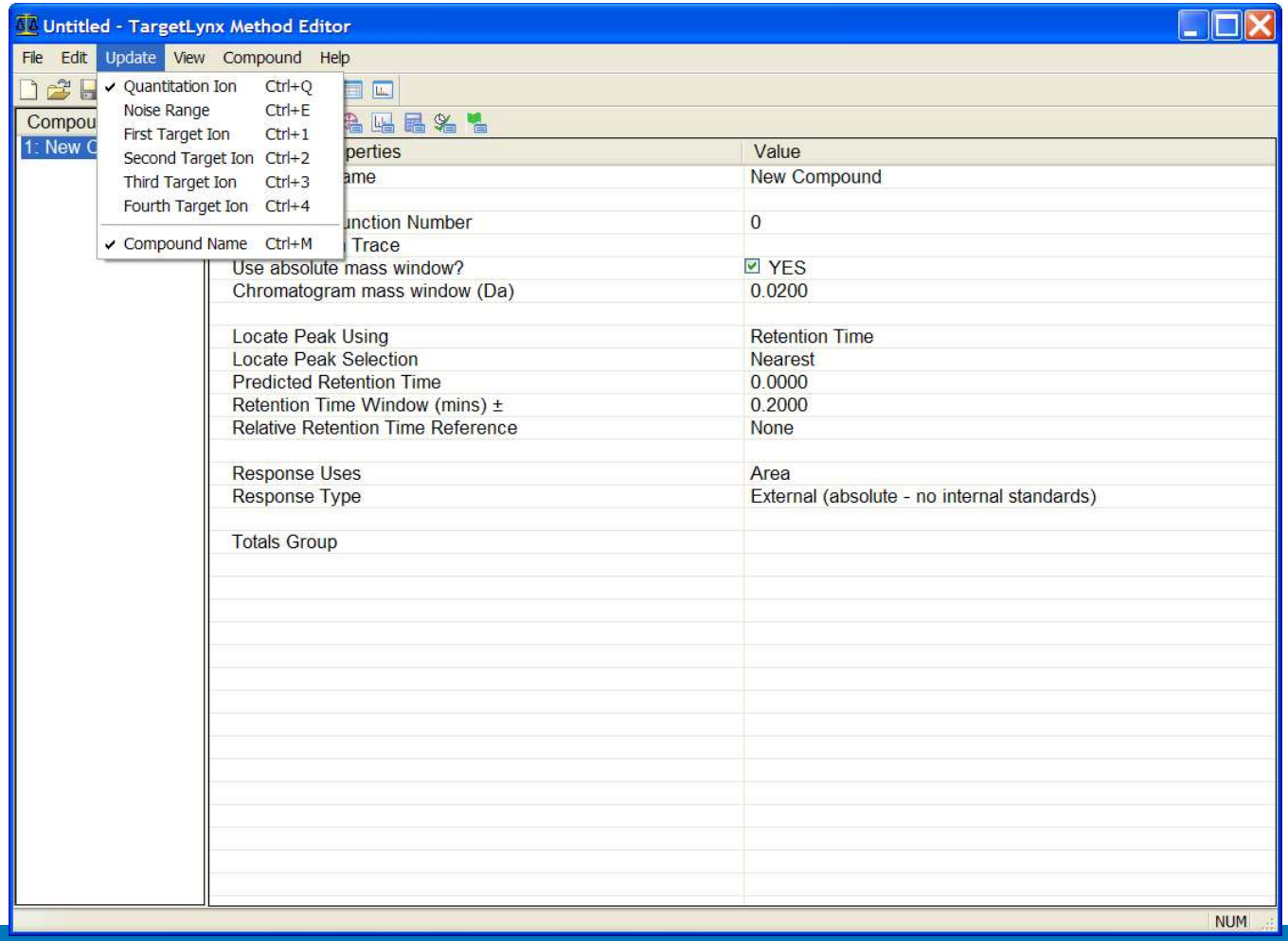
Alprazolam



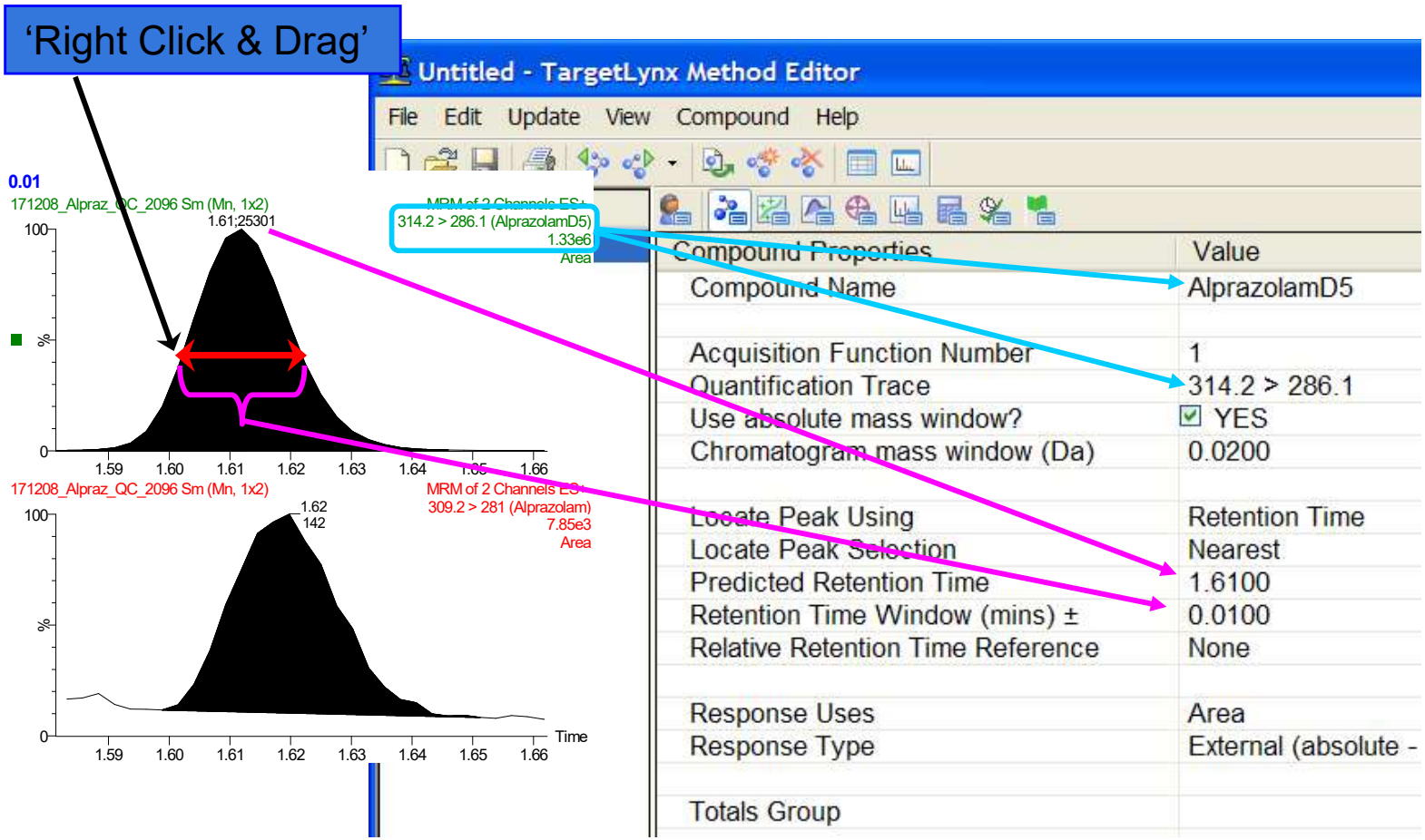
Build Quantitation Method



Build Quantitation Method



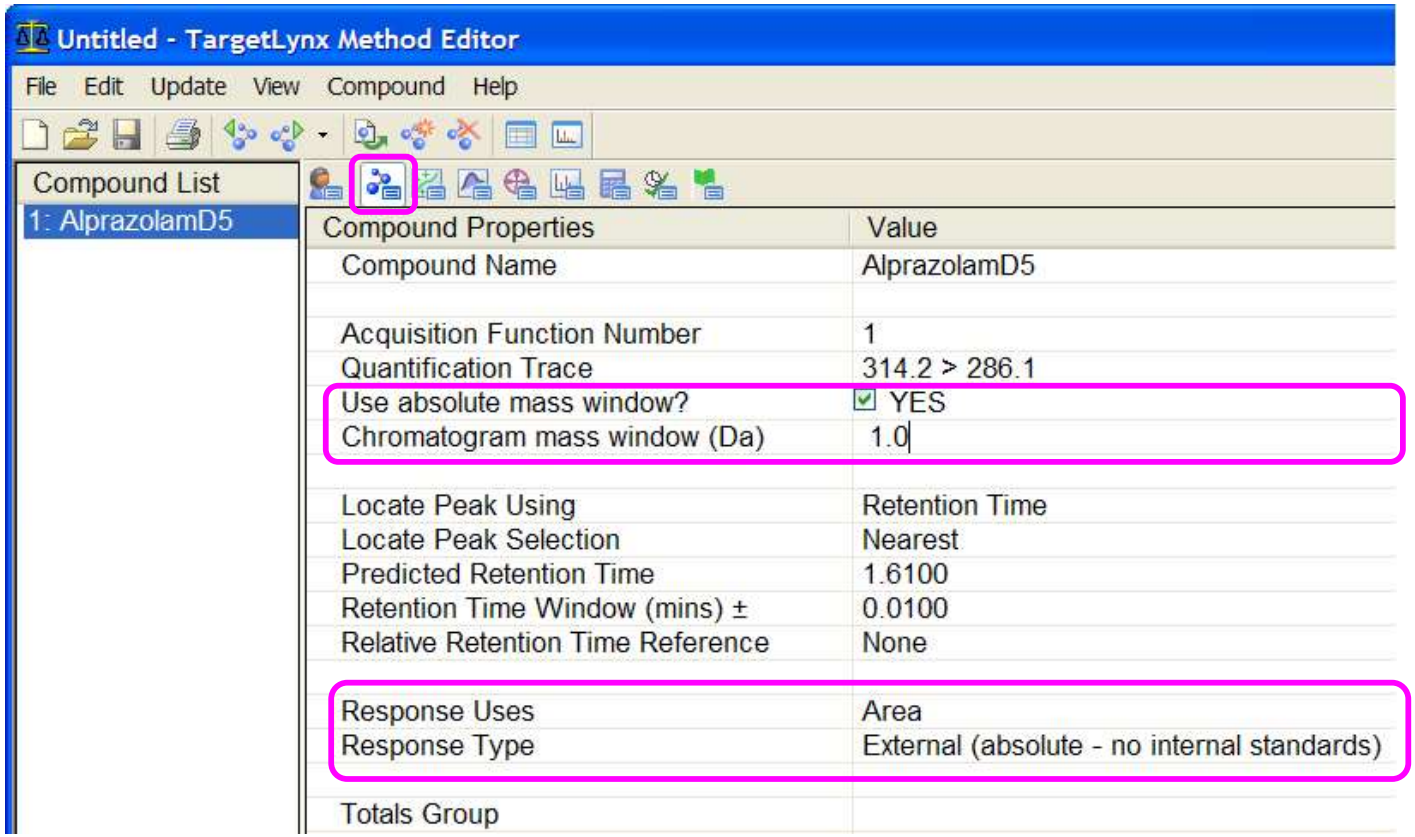
Build Quantitation Method



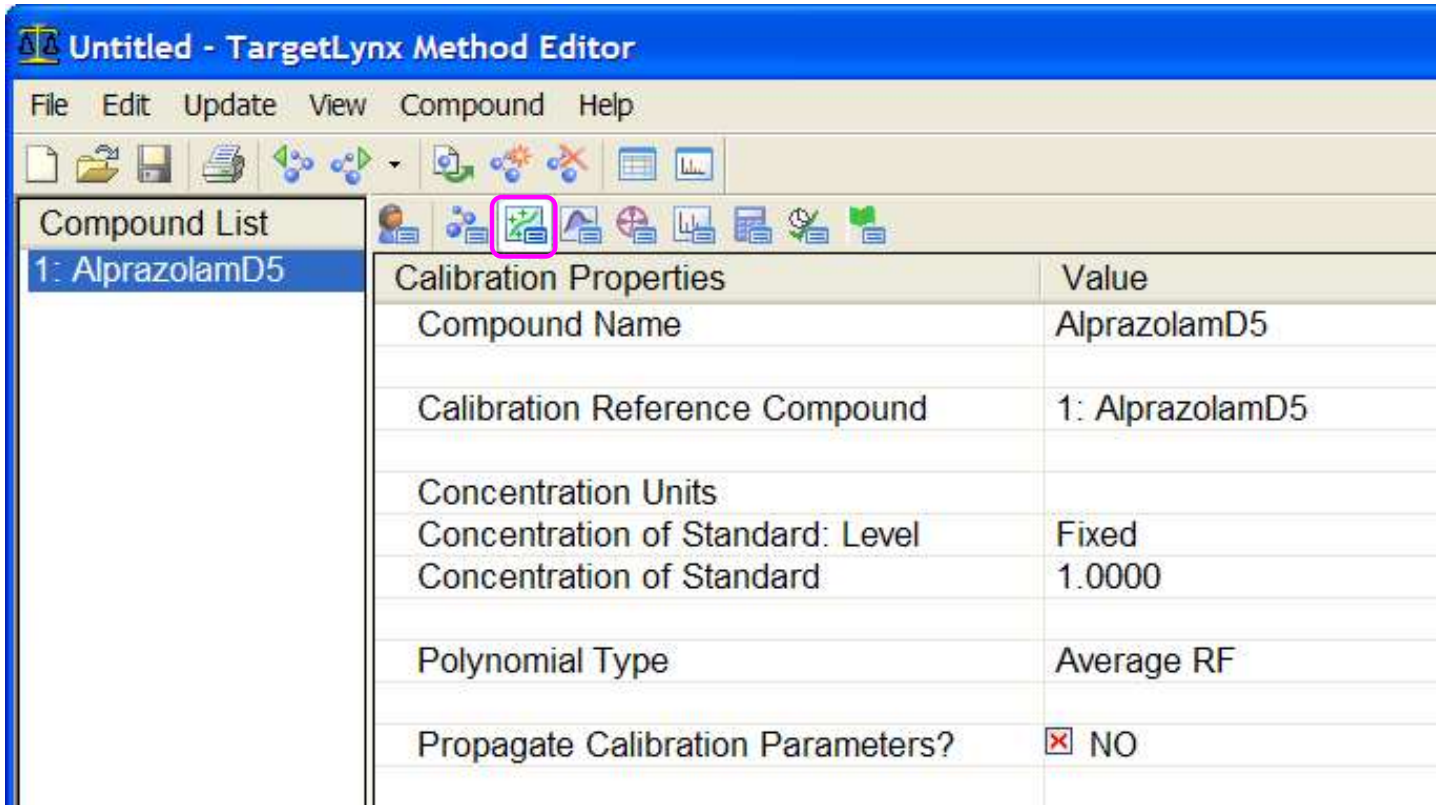
Quantify Trace

- A single decimal number (m/z) for mass chromatograms from SIR or Full Scan data
- Two single decimal numbers separated by a ">" for an MRM function (e.g. 609.2 > 195.1)
- TIC for total ion current chromatograms
- BPI for base peak intensity chromatograms
- An1, An2, An3, or An4 for analog data
- The wavelength for DAD data
- Ch1, Ch2, etc. for SIR data to use one quantify method with multiple SIR functions. Ch1 is the first mass in the list, Ch2 is the second etc

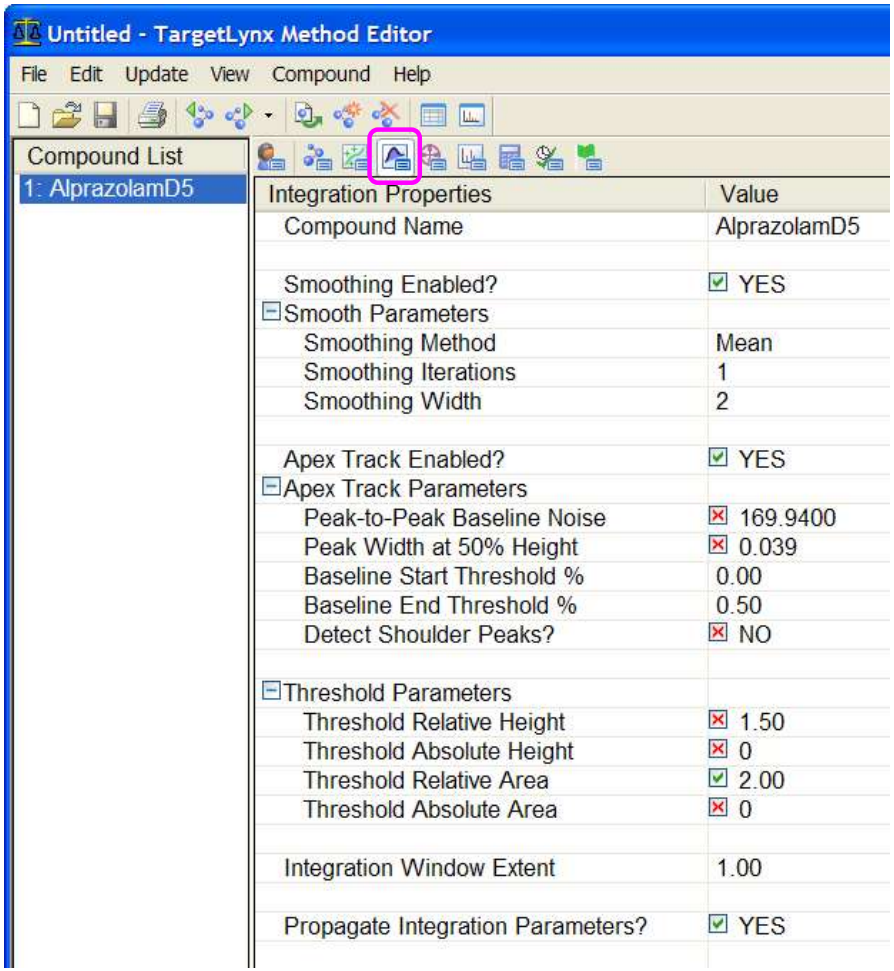
Enter Compound Properties (Internal Standard)



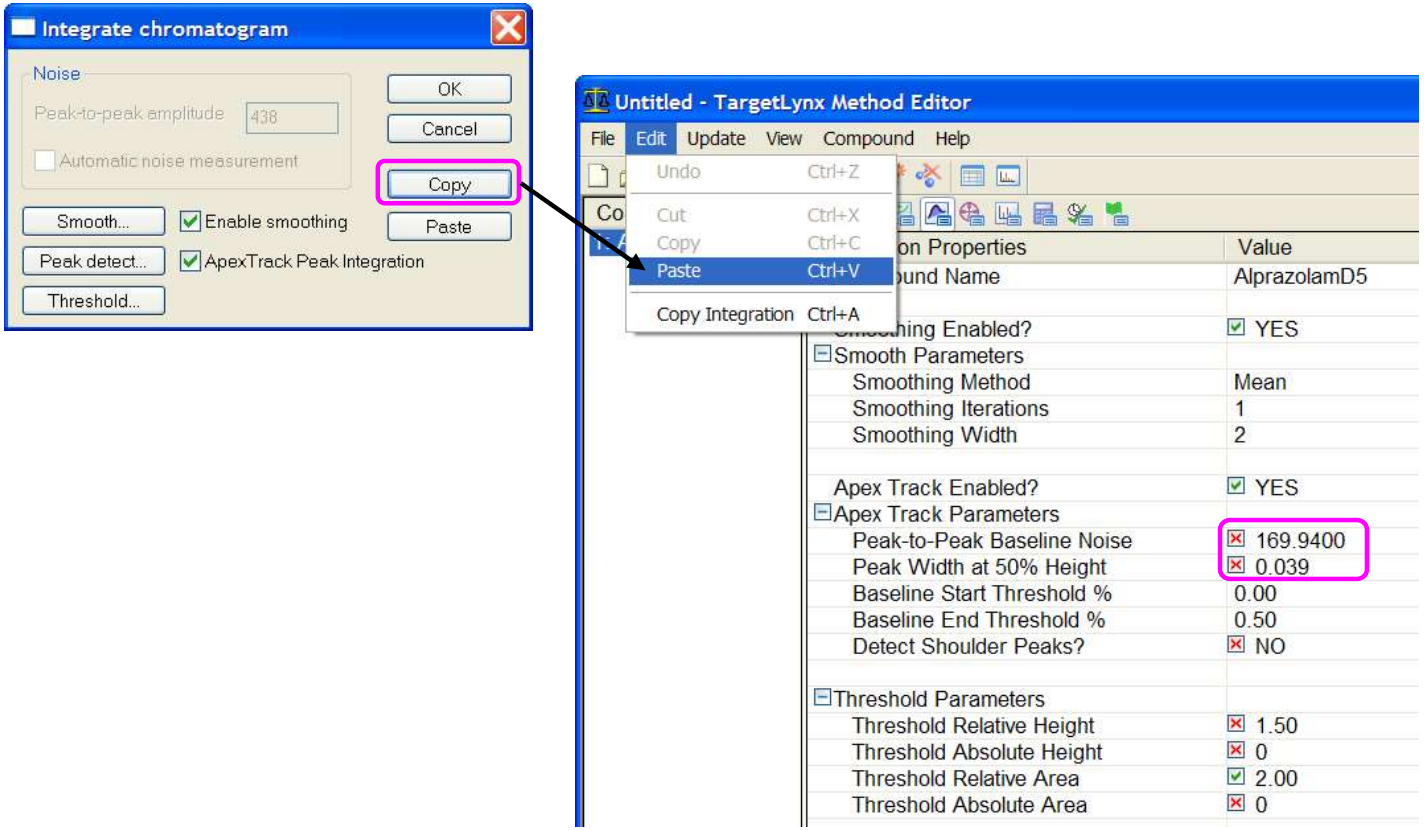
Enter Calibration Parameters (Internal Standard)



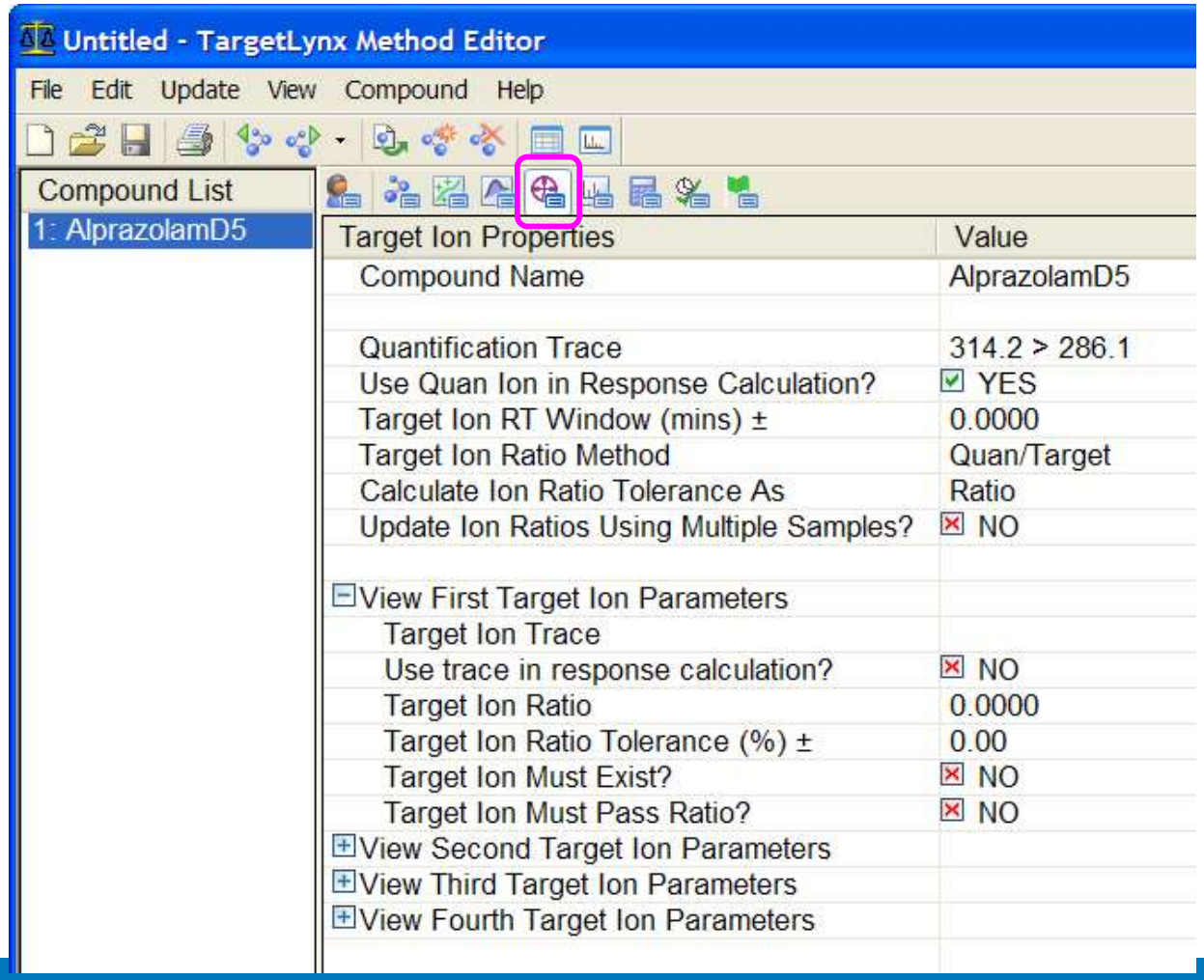
Enter Integration Properties



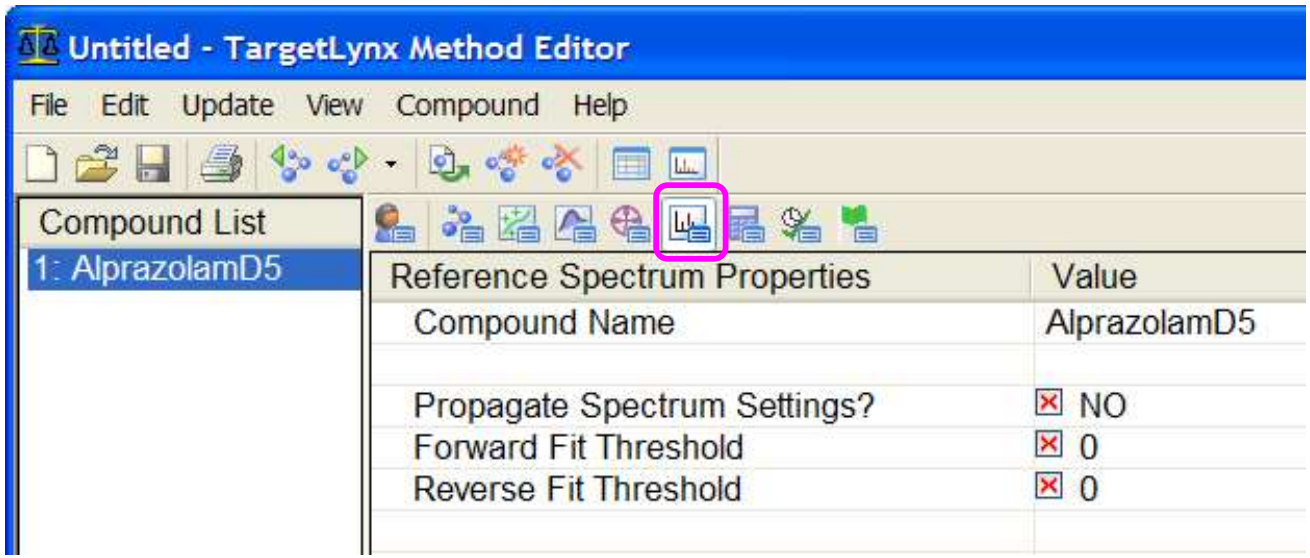
Enter Integration Properties



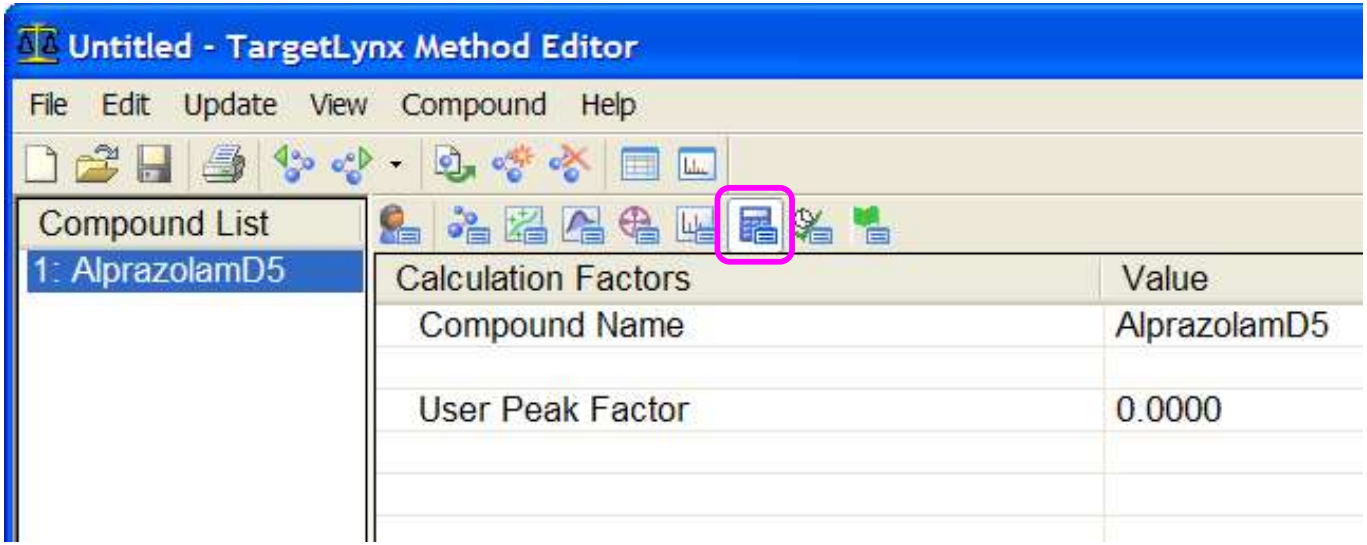
Enter Target Ion Parameters



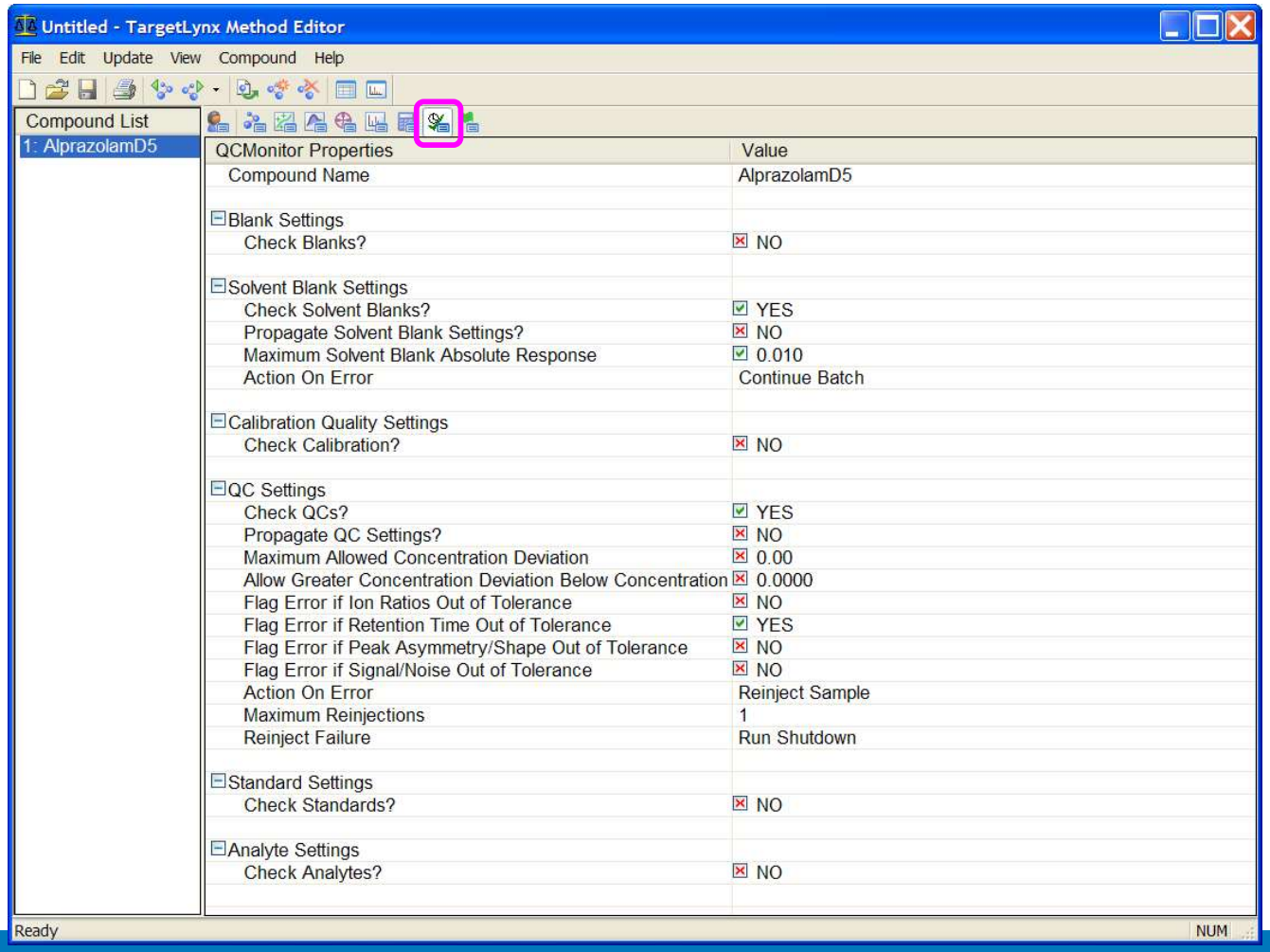
Reference Spectrum



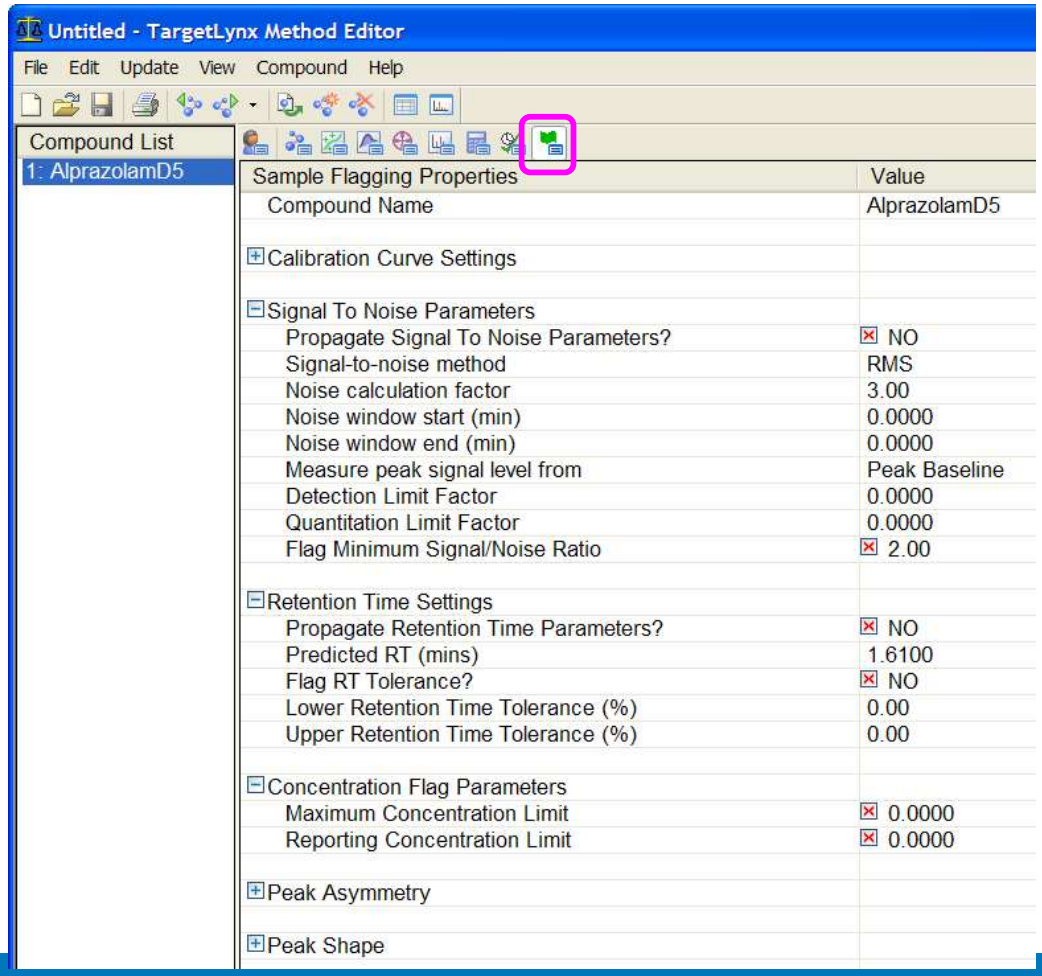
Enter Calculation Factors



QC Monitor Page



Sample Flagging Page

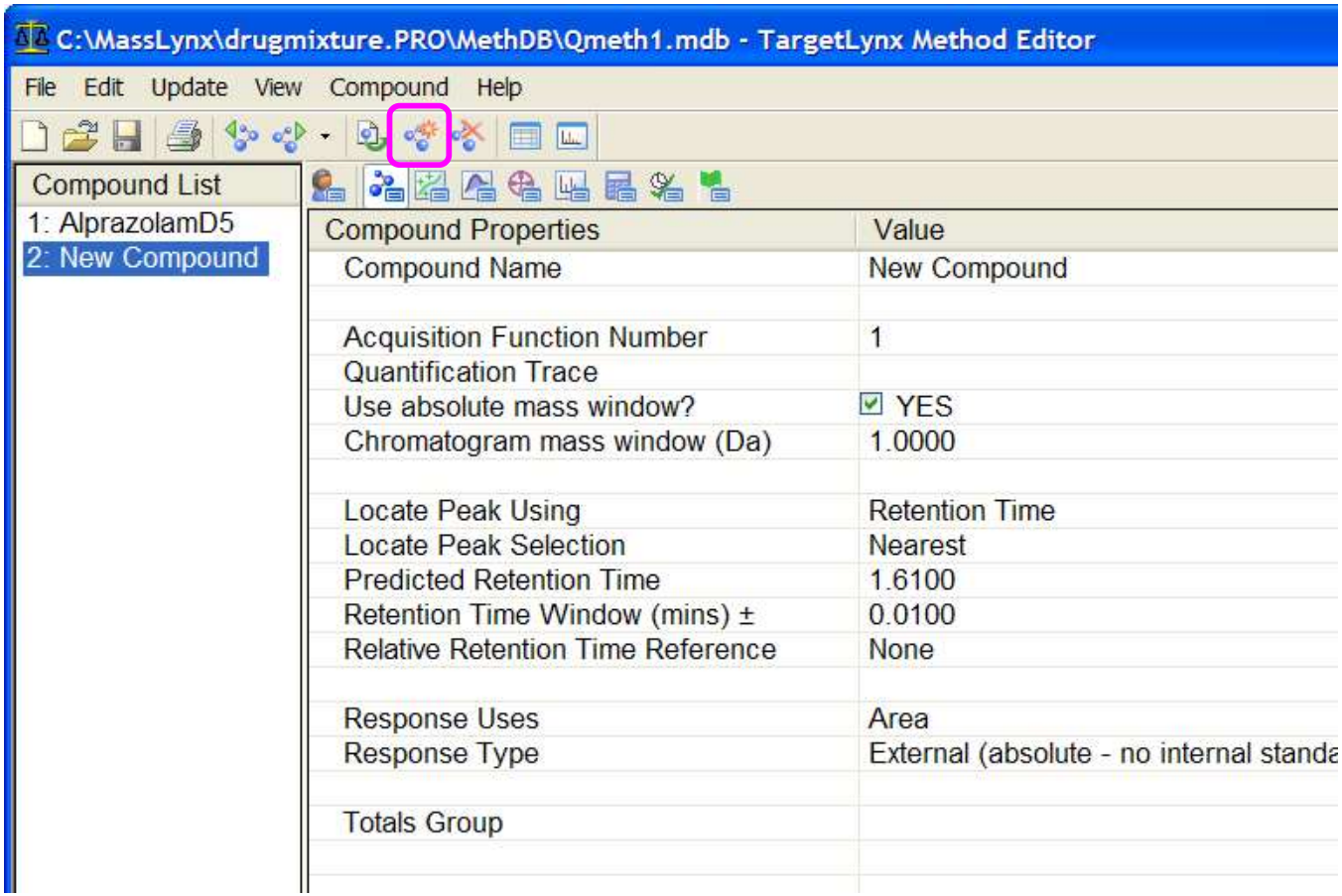


Adding to the TargetLynx Method

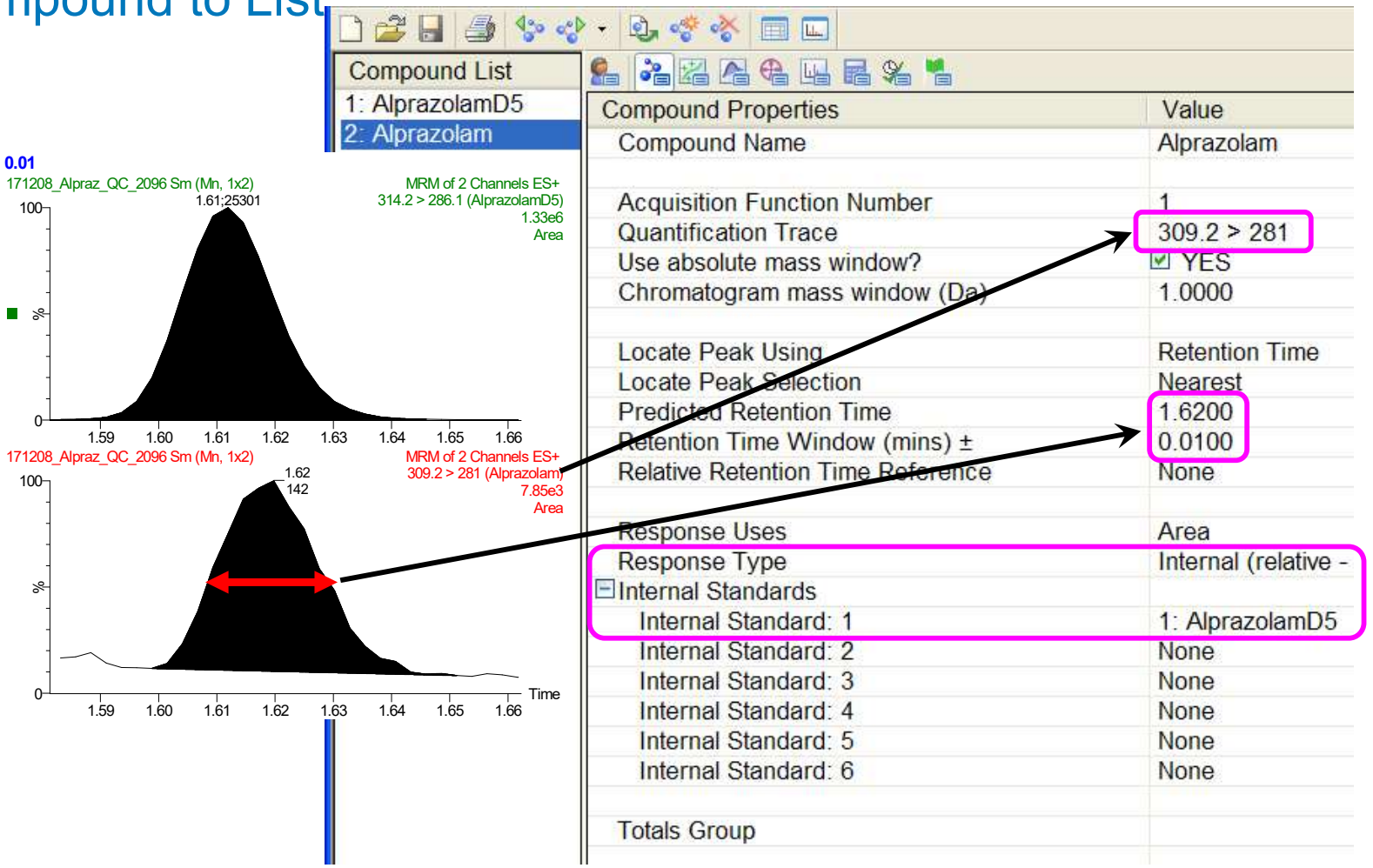
Waters™

- This entire process now needs to be repeated for the two other compounds in the example.
- Parameters that may differ between compounds:
  - Name
  - Transition (Quantify Trace)
  - Integration Parameters
  - Internal Reference (Selecting an Internal Standard if used)
  - Concentration of Standards
  - Retention Time
  - Time Window
  - Response Type in General Parameters Window
  - Polynomial Type in the General Parameters Window

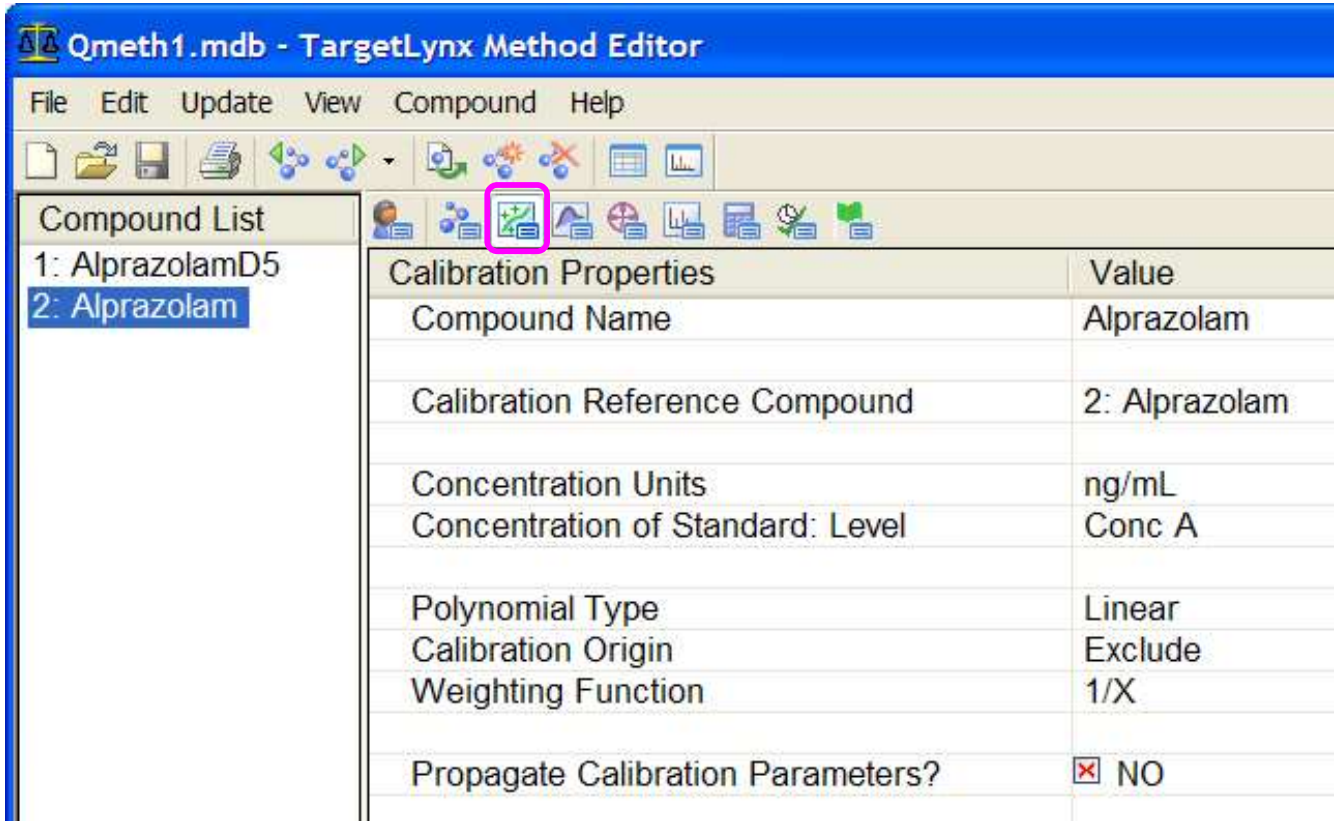
Add Compound to List



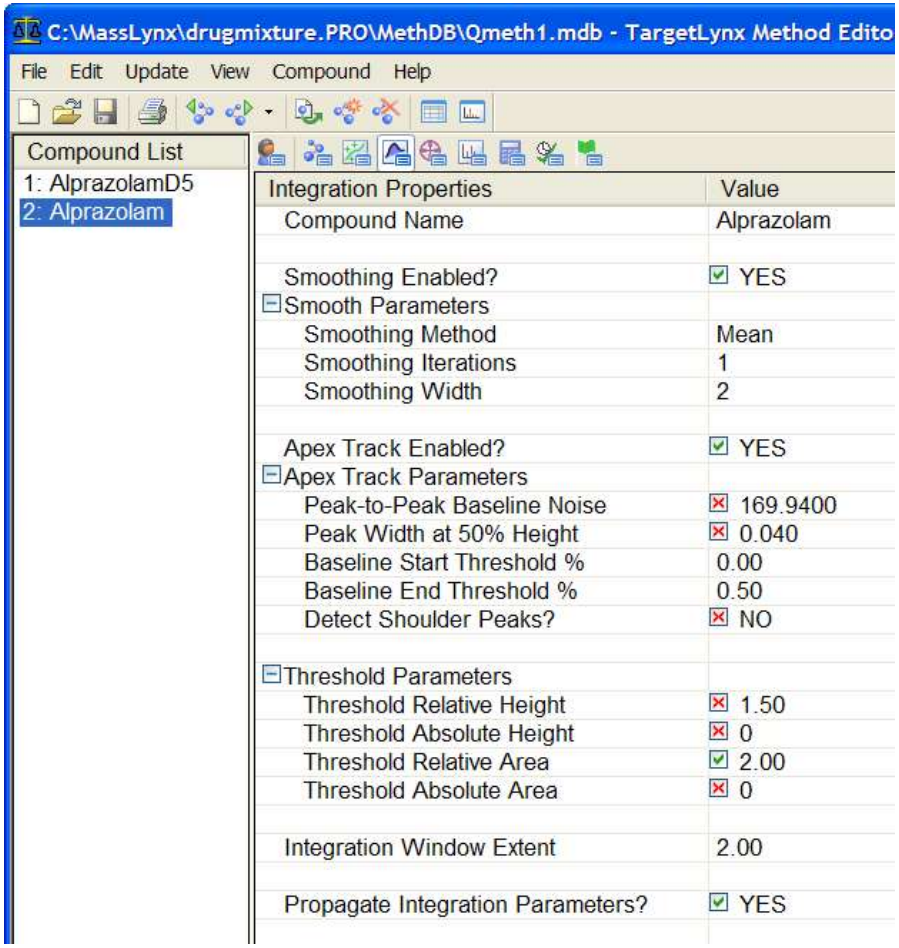
Add Compound to List



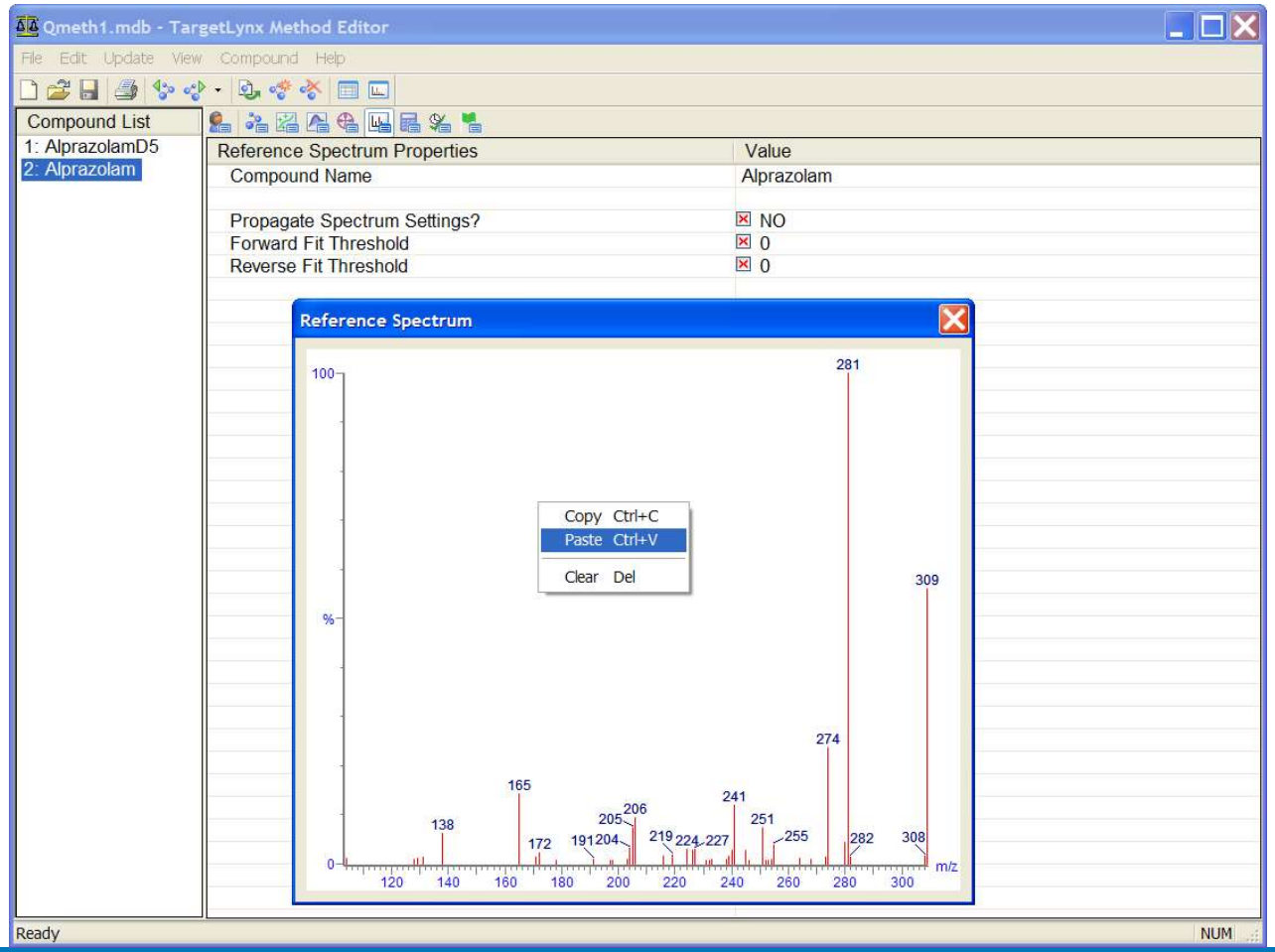
Enter Calibration Properties (Analyte)



Enter Integration Properties (Analyte)



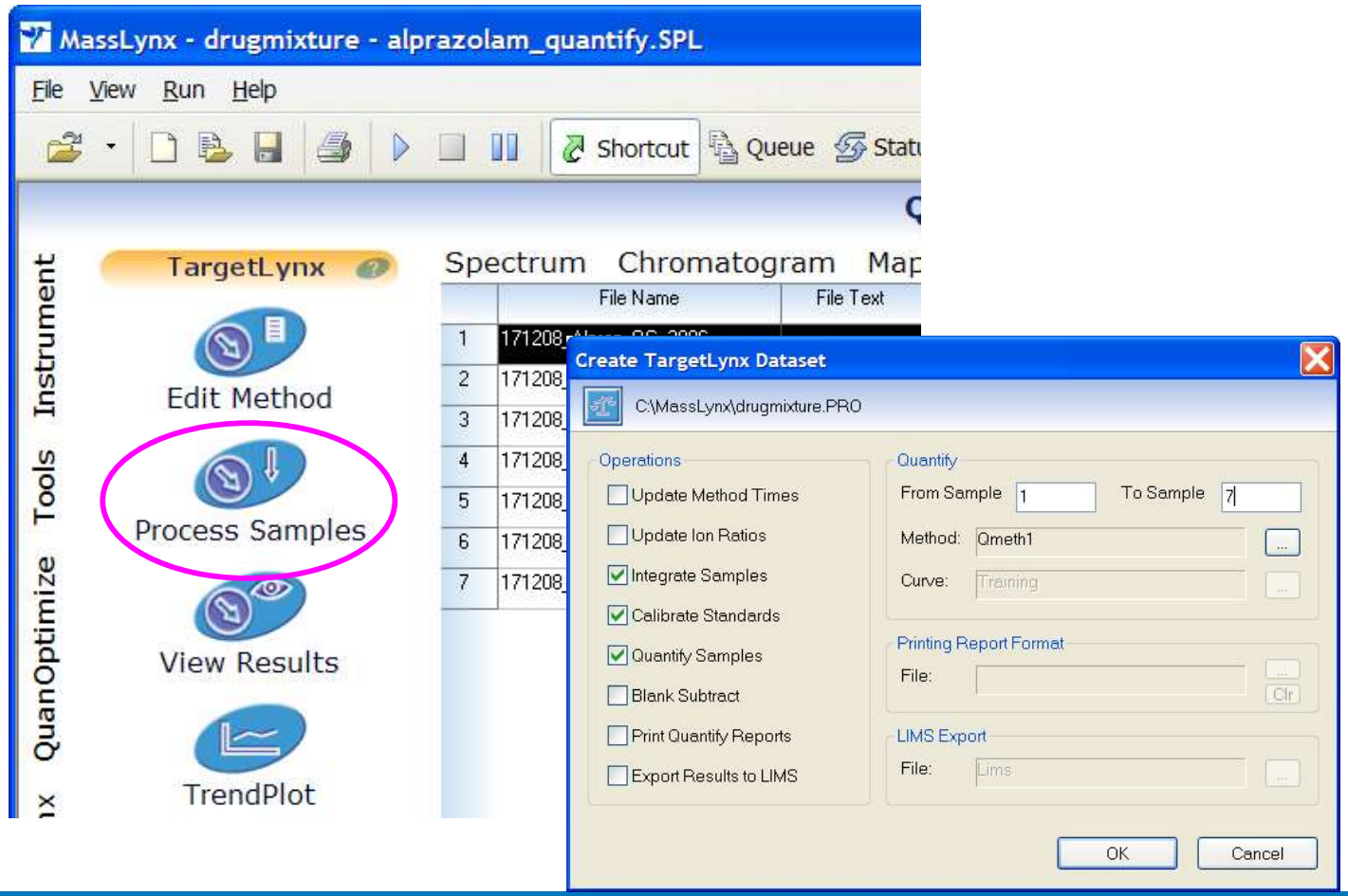
Reference Spectrum



Processing Samples

- Repeat the previous steps for additional compounds that would be present in your method.
- Once the entire method is built, it is time to process the samples.
- Highlight the samples to quantitate. If the entire sample list is to be processed, click on the upper left box to activate the entire sample list.
- Click **TargetLynx** then click **Process Samples**.

Processing Samples with TargetLynx



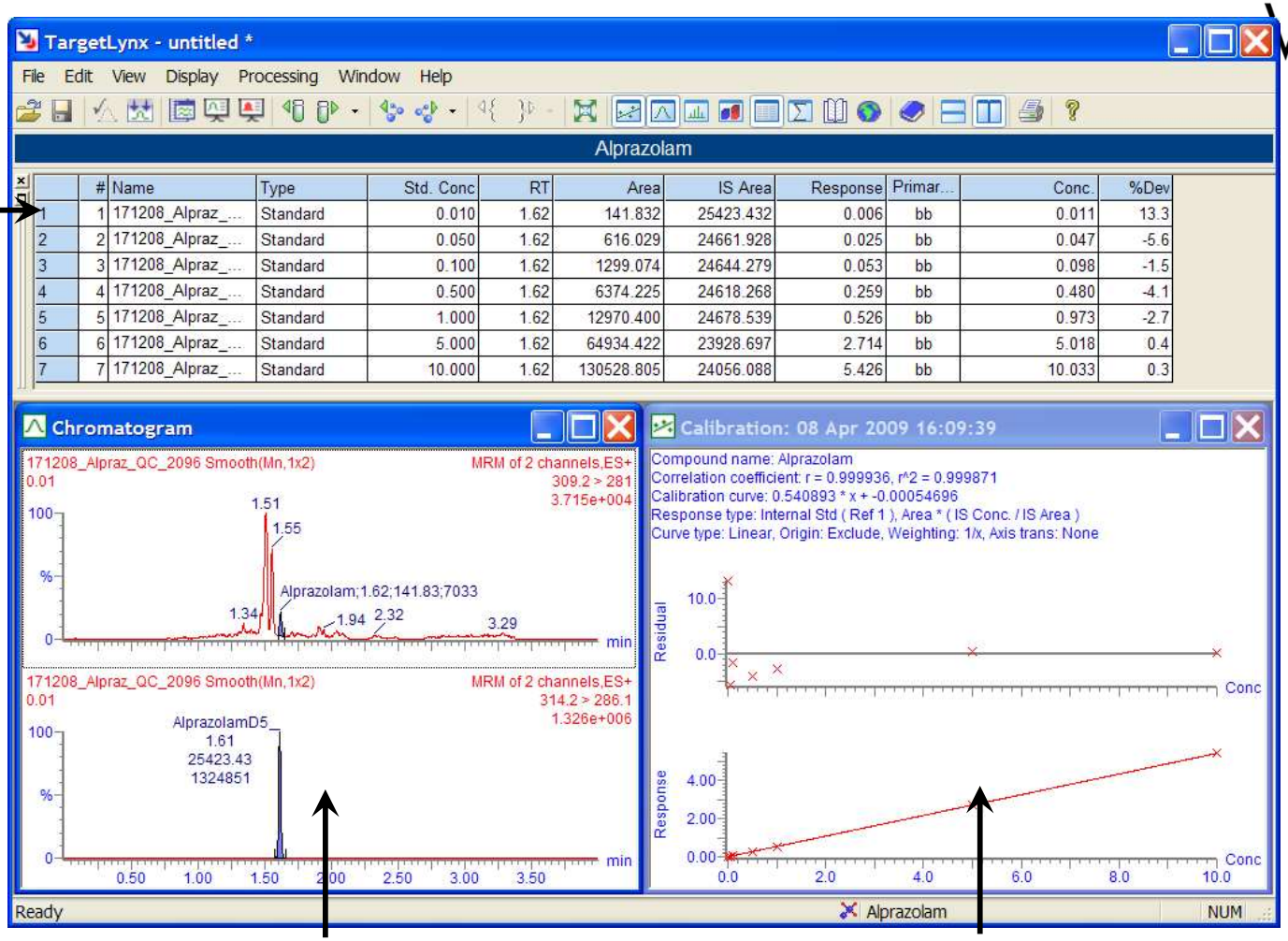
TargetLynx Browser

Waters™

- The TargetLynx Browser is used to create and view TargetLynx datasets, print results or export results to other applications.
- A dataset contains the extracted raw data, methods, calibrations and results associated with a processed set of samples. All required processing can be performed from the TargetLynx Browser.
- The TargetLynx Browser file is automatically displayed when a Sample List is processed from MassLynx.

Viewing Results

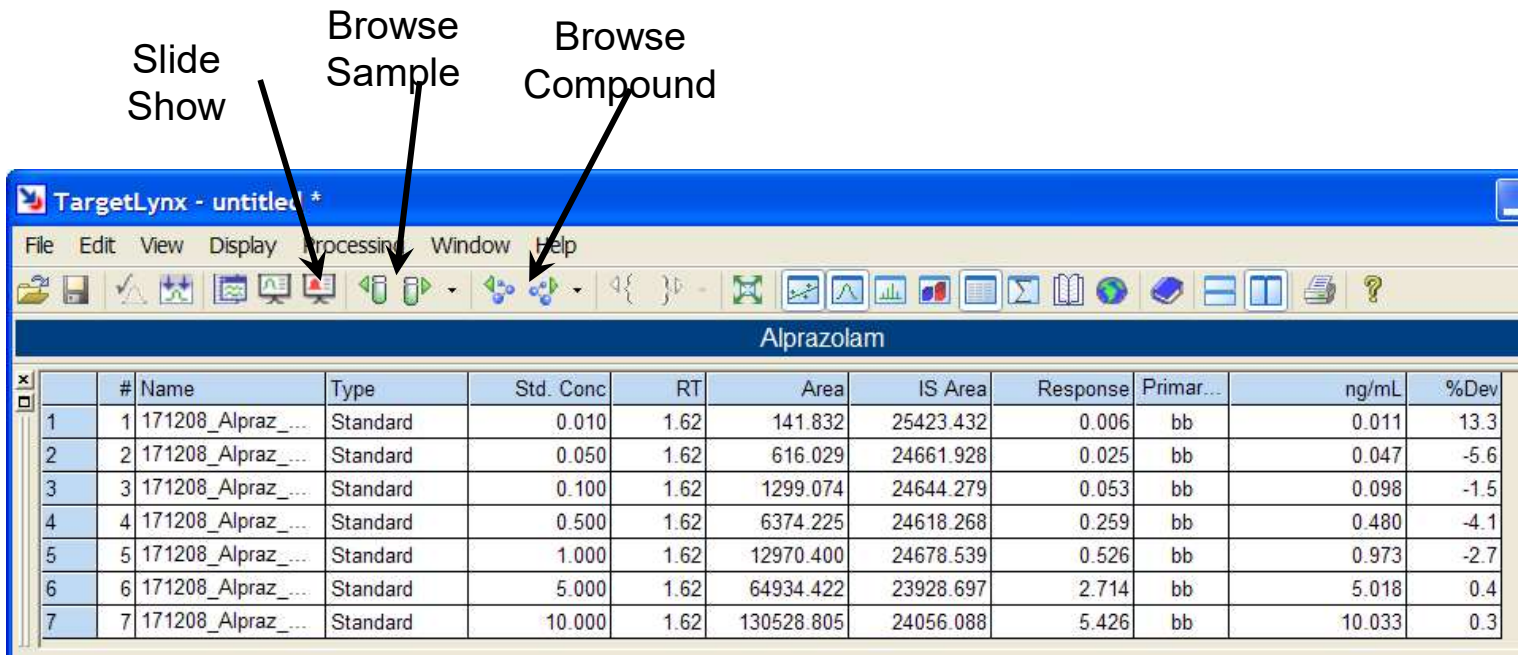
Summary Window



Analyte Chromatogram

Calibration Curve

Results Window and Shortcuts



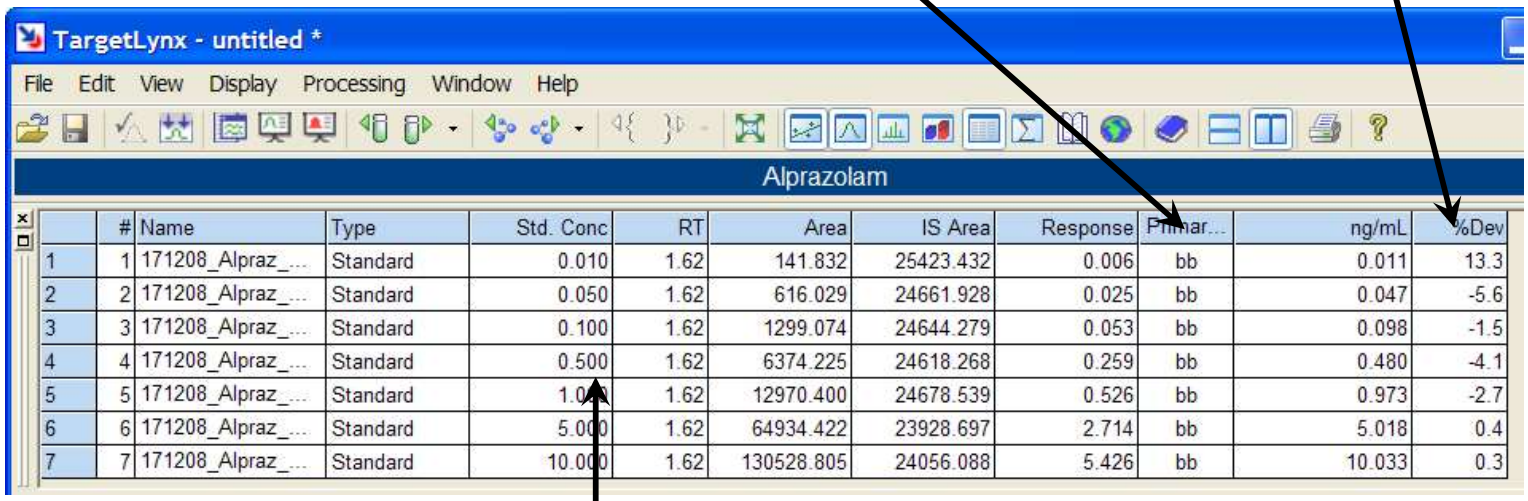
	#	Name	Type	Std. Conc	RT	Area	IS Area	Response	Primar...	ng/mL	%Dev
1	1	171208_Alpraz_...	Standard	0.010	1.62	141.832	25423.432	0.006	bb	0.011	13.3
2	2	171208_Alpraz_...	Standard	0.050	1.62	616.029	24661.928	0.025	bb	0.047	-5.6
3	3	171208_Alpraz_...	Standard	0.100	1.62	1299.074	24644.279	0.053	bb	0.098	-1.5
4	4	171208_Alpraz_...	Standard	0.500	1.62	6374.225	24618.268	0.259	bb	0.480	-4.1
5	5	171208_Alpraz_...	Standard	1.000	1.62	12970.400	24678.539	0.526	bb	0.973	-2.7
6	6	171208_Alpraz_...	Standard	5.000	1.62	64934.422	23928.697	2.714	bb	5.018	0.4
7	7	171208_Alpraz_...	Standard	10.000	1.62	130528.805	24056.088	5.426	bb	10.033	0.3

Summary Window

Waters™

Flags Refer to Integration:
d=dropped baseline
b=baseline
X=excluded from calibration line
M=manual modification

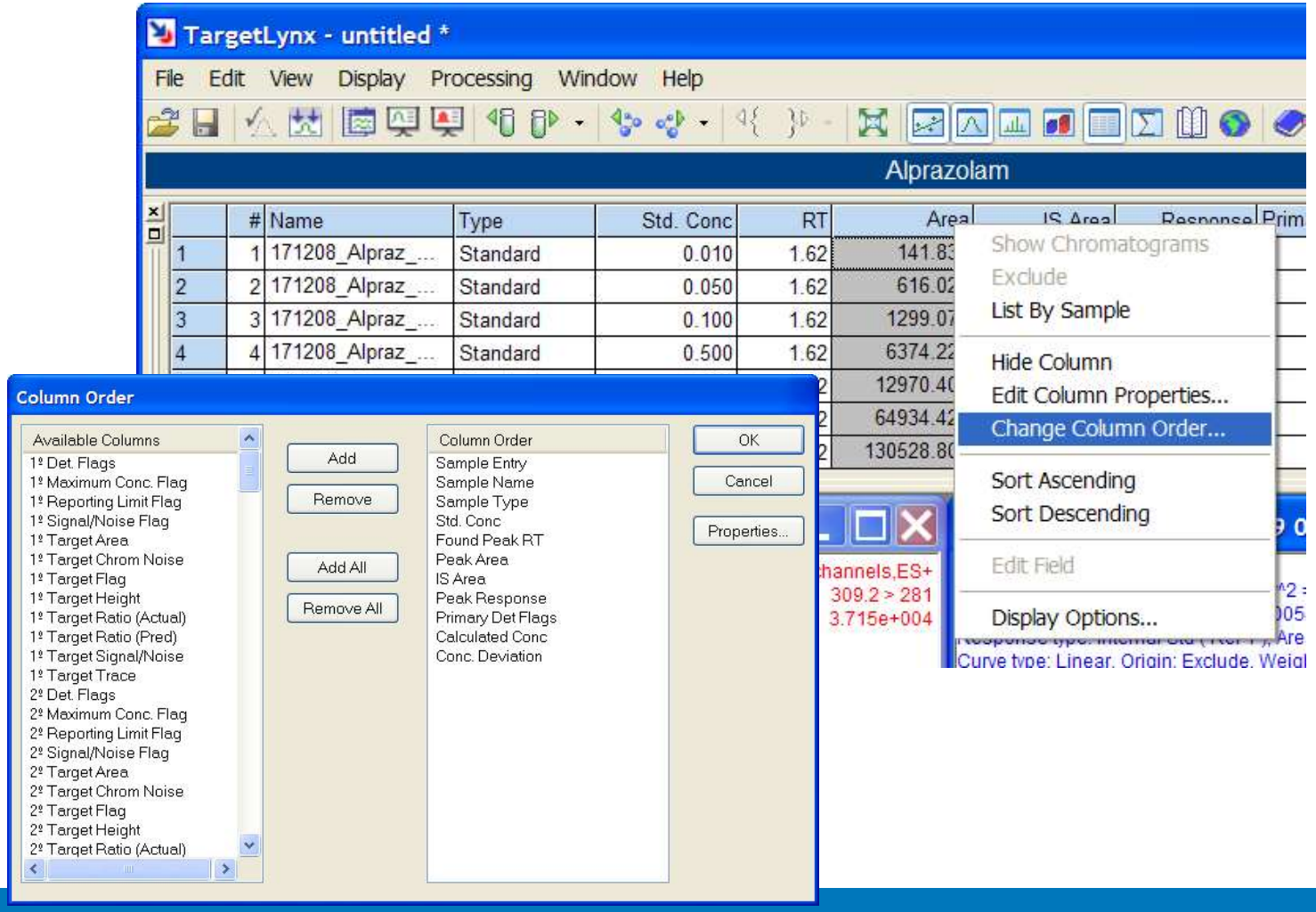
% Deviation from Calculated Calibration Line



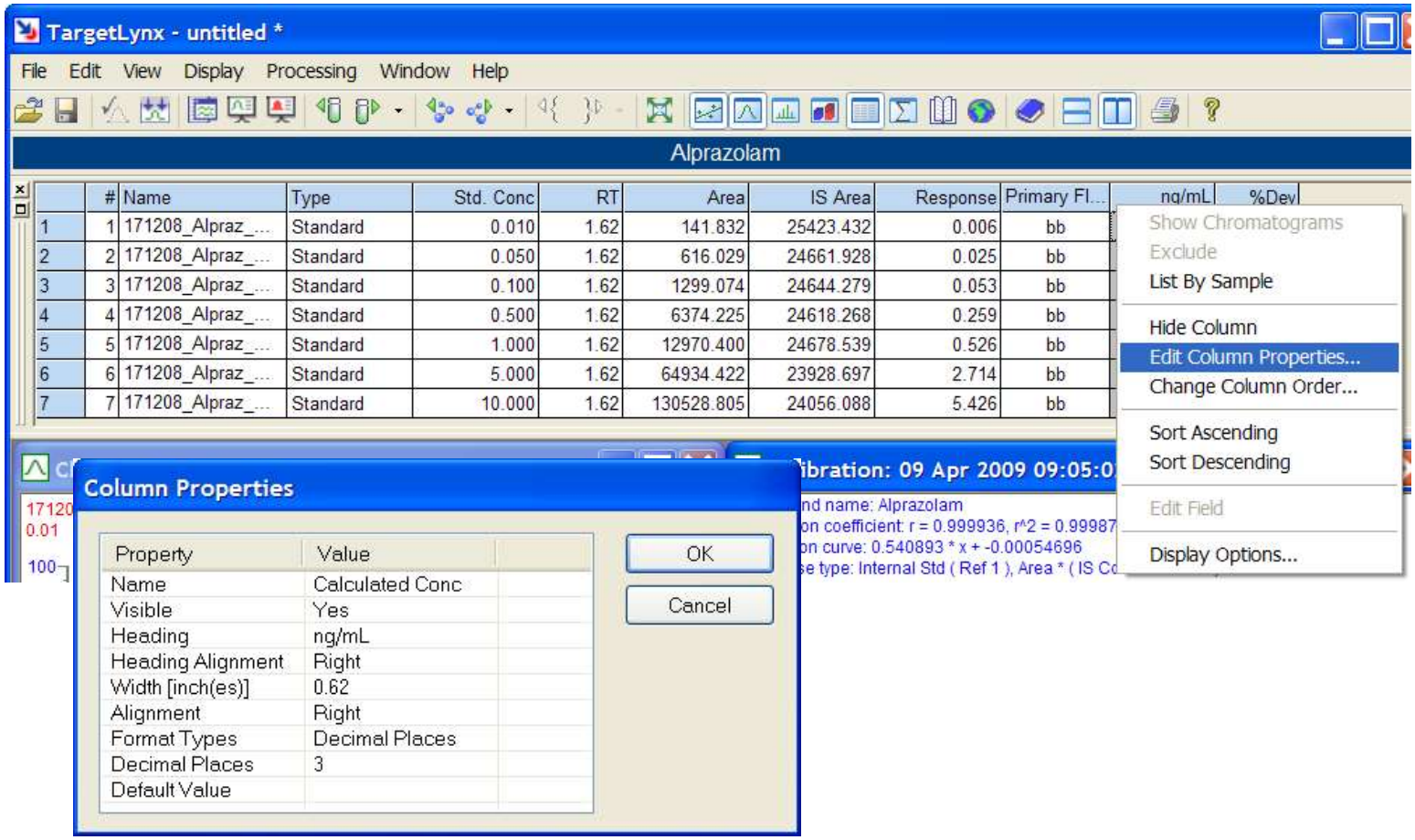
	#	Name	Type	Std. Conc	RT	Area	IS Area	Response	Primar...	ng/mL	%Dev
1	1	171208_Alpraz_...	Standard	0.010	1.62	141.832	25423.432	0.006	bb	0.011	13.3
2	2	171208_Alpraz_...	Standard	0.050	1.62	616.029	24661.928	0.025	bb	0.047	-5.6
3	3	171208_Alpraz_...	Standard	0.100	1.62	1299.074	24644.279	0.053	bb	0.098	-1.5
4	4	171208_Alpraz_...	Standard	0.500	1.62	6374.225	24618.268	0.259	bb	0.480	-4.1
5	5	171208_Alpraz_...	Standard	1.000	1.62	12970.400	24678.539	0.526	bb	0.973	-2.7
6	6	171208_Alpraz_...	Standard	5.000	1.62	64934.422	23928.697	2.714	bb	5.018	0.4
7	7	171208_Alpraz_...	Standard	10.000	1.62	130528.805	24056.088	5.426	bb	10.033	0.3

Std. Conc. = Concentration from Sample List

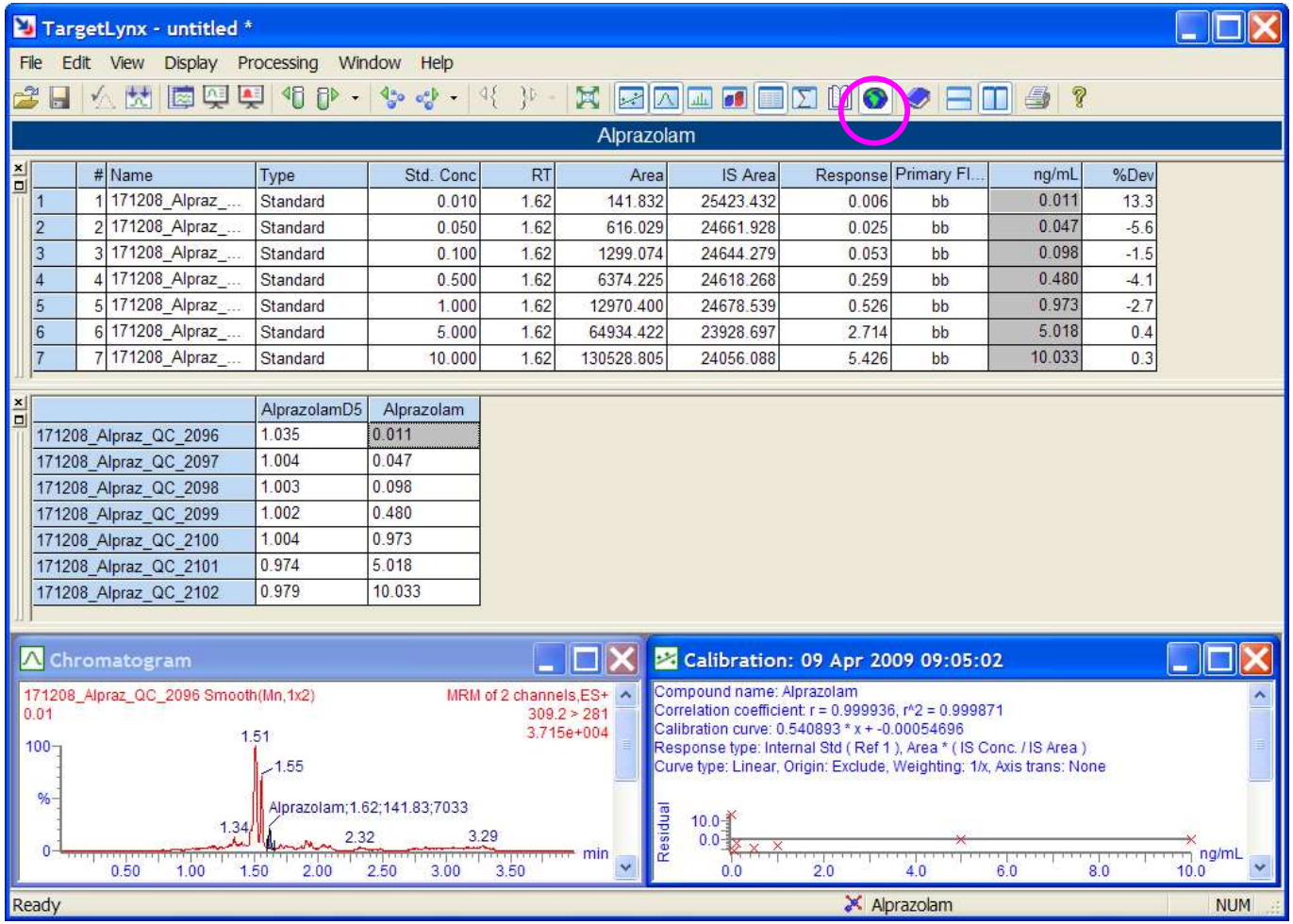
Change Column Order



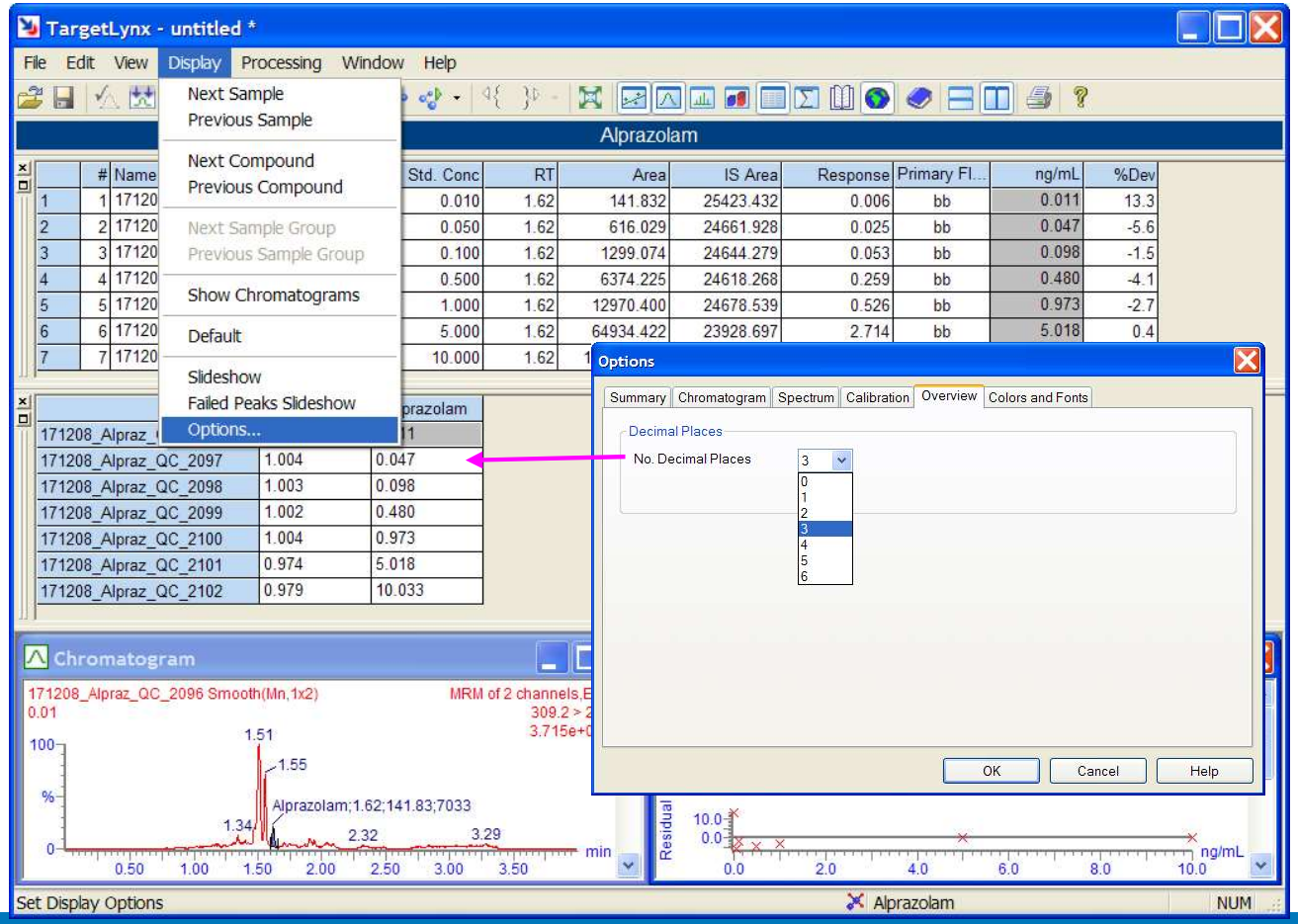
Edit Column Properties



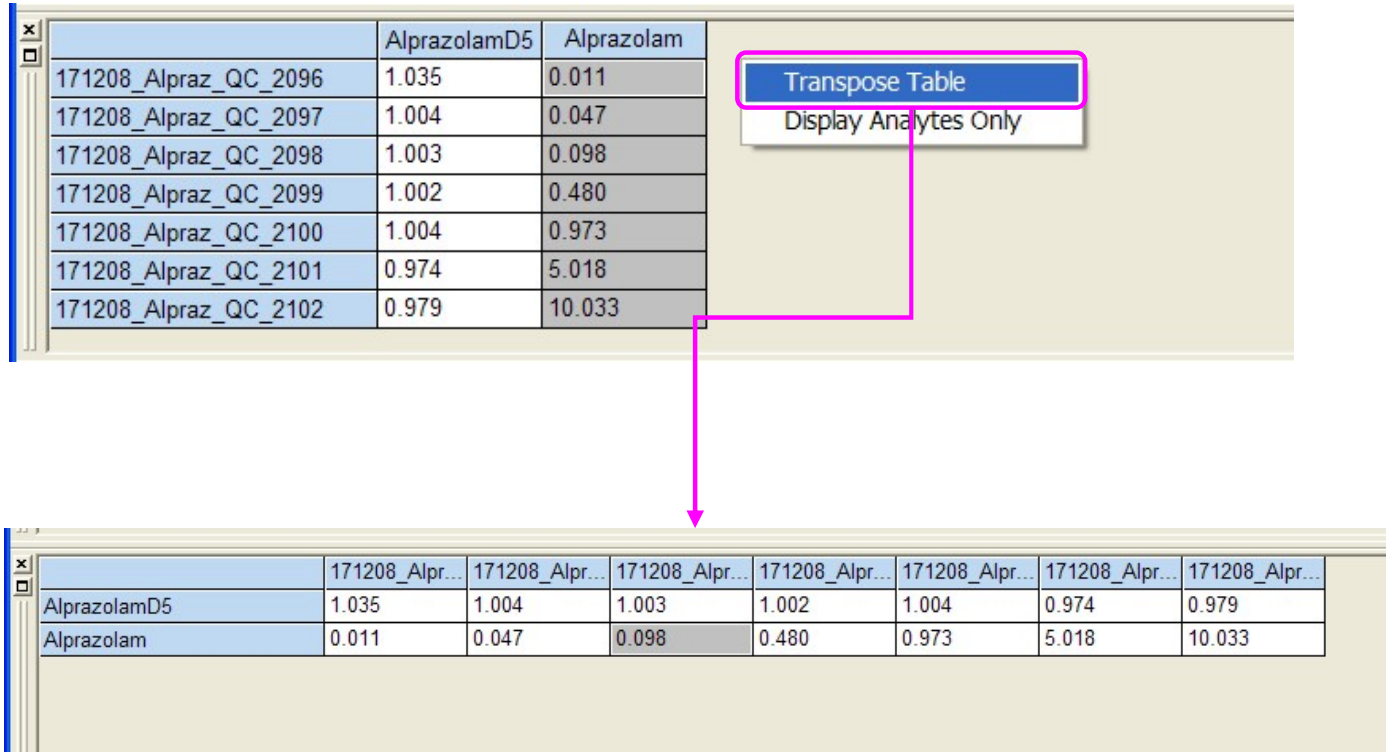
Overview Table



Overview Window Options



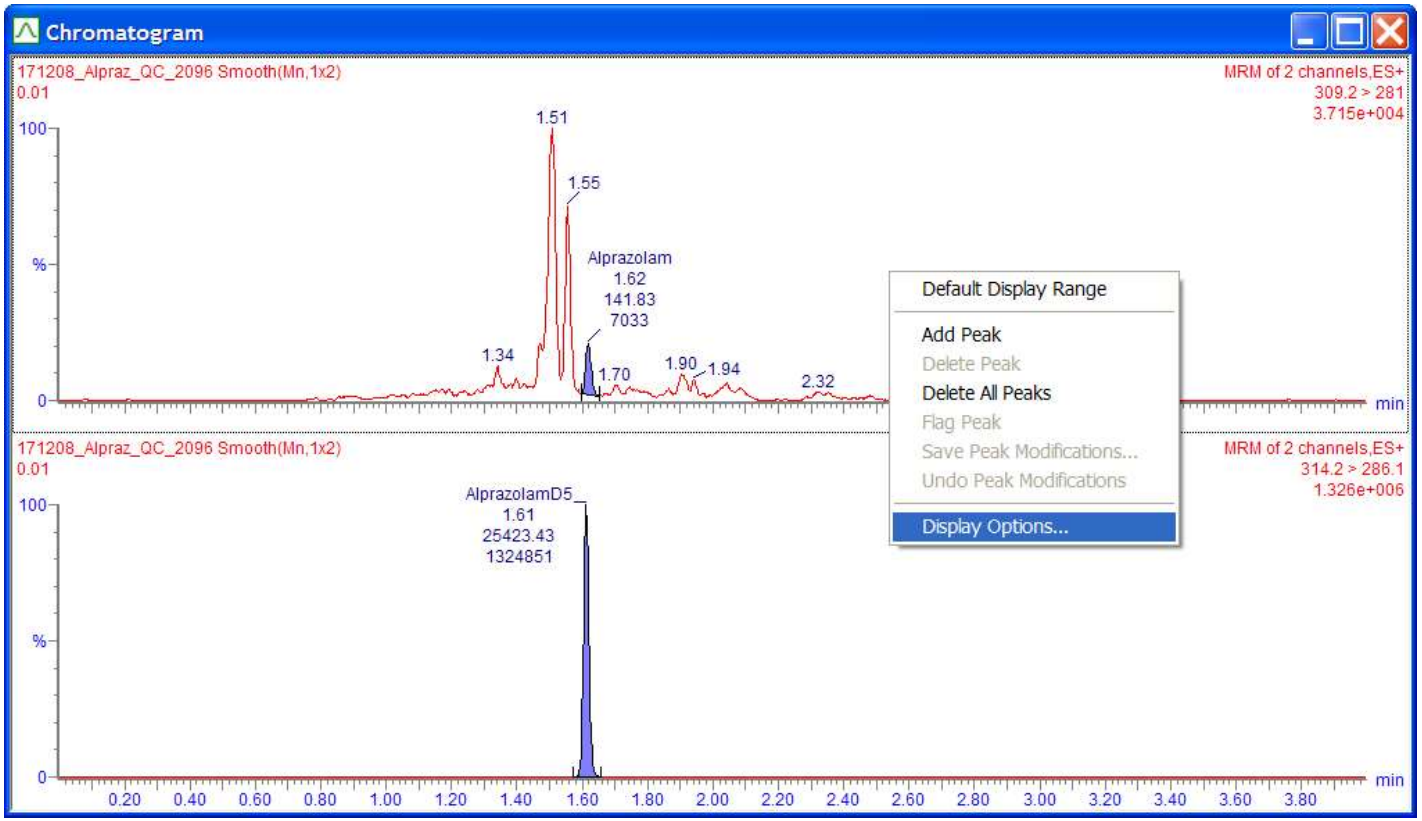
Transpose Overview Table



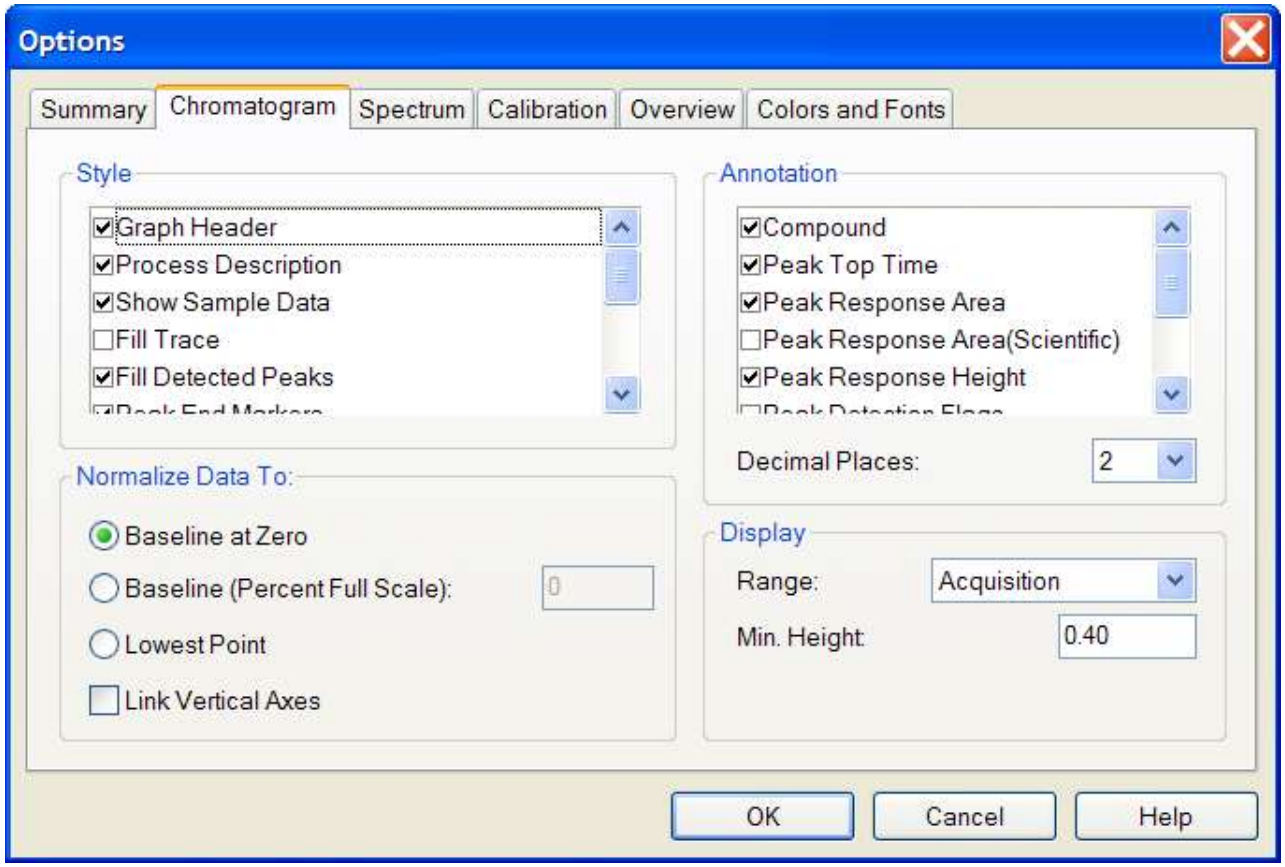
	AlprazolamD5	Alprazolam
171208_Alpraz_QC_2096	1.035	0.011
171208_Alpraz_QC_2097	1.004	0.047
171208_Alpraz_QC_2098	1.003	0.098
171208_Alpraz_QC_2099	1.002	0.480
171208_Alpraz_QC_2100	1.004	0.973
171208_Alpraz_QC_2101	0.974	5.018
171208_Alpraz_QC_2102	0.979	10.033

	171208_Alpr...	171208_Alpr...	171208_Alpr...	171208_Alpr...	171208_Alpr...	171208_Alpr...	171208_Alpr...
AlprazolamD5	1.035	1.004	1.003	1.002	1.004	0.974	0.979
Alprazolam	0.011	0.047	0.098	0.480	0.973	5.018	10.033

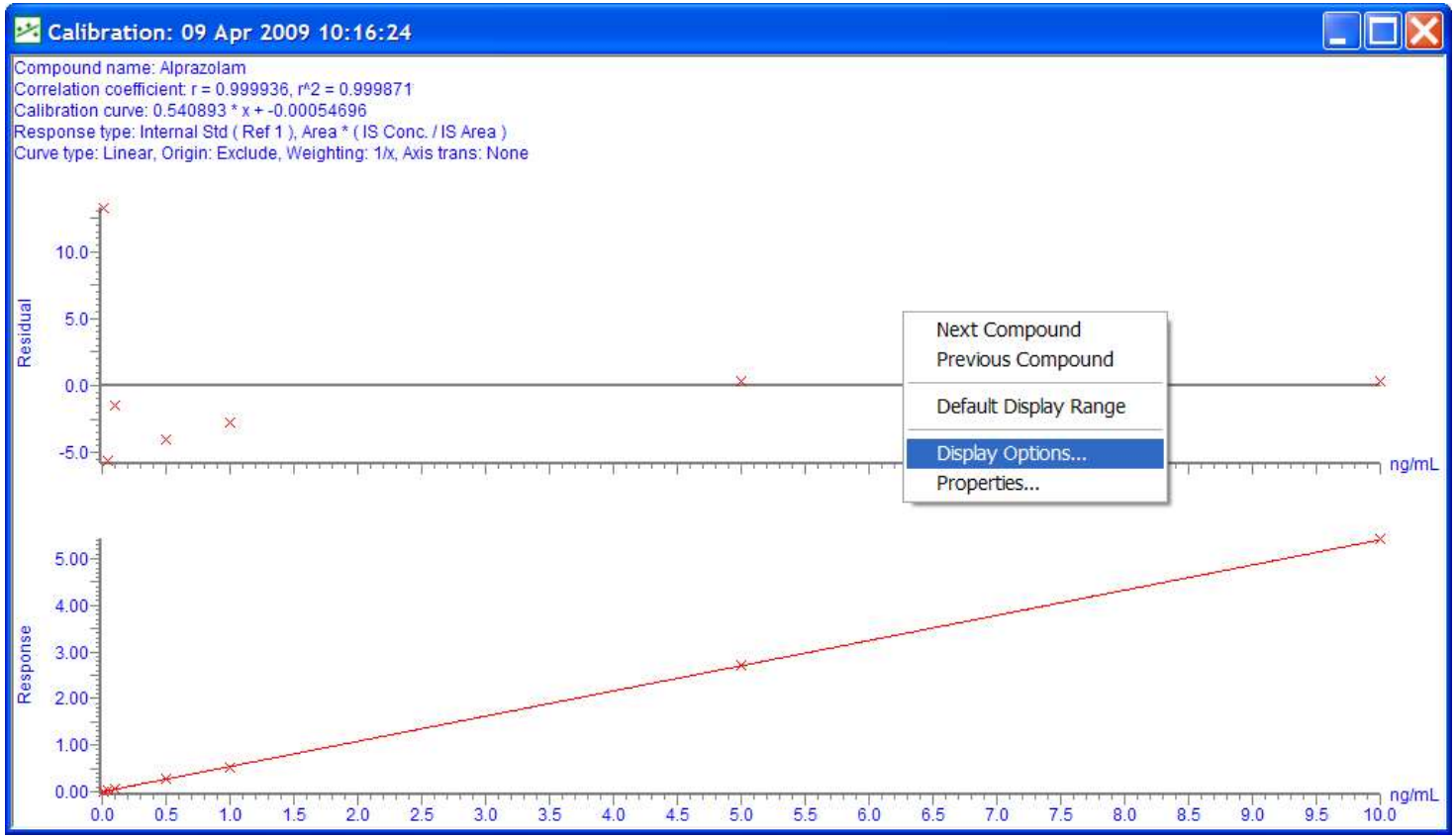
Chromatogram Window – Display Options



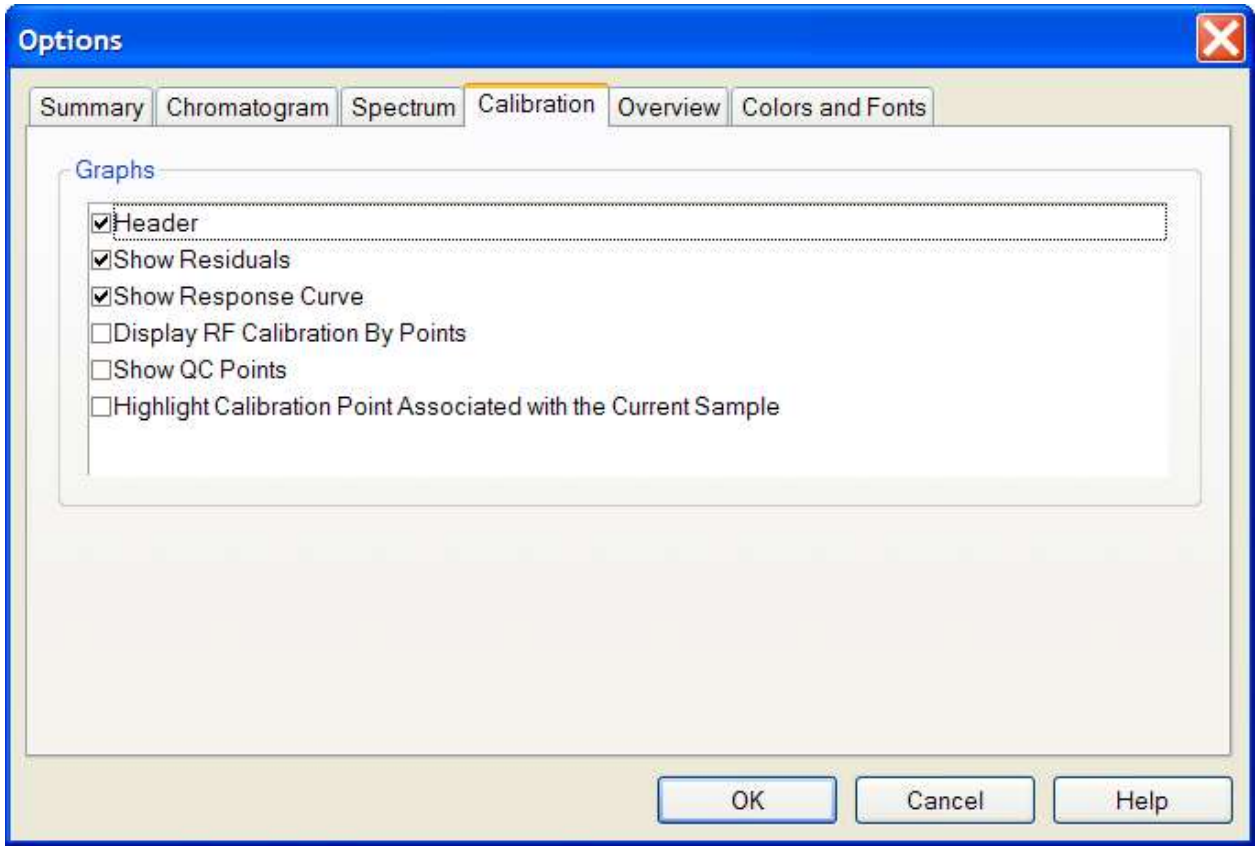
Chromatogram Window – View Options



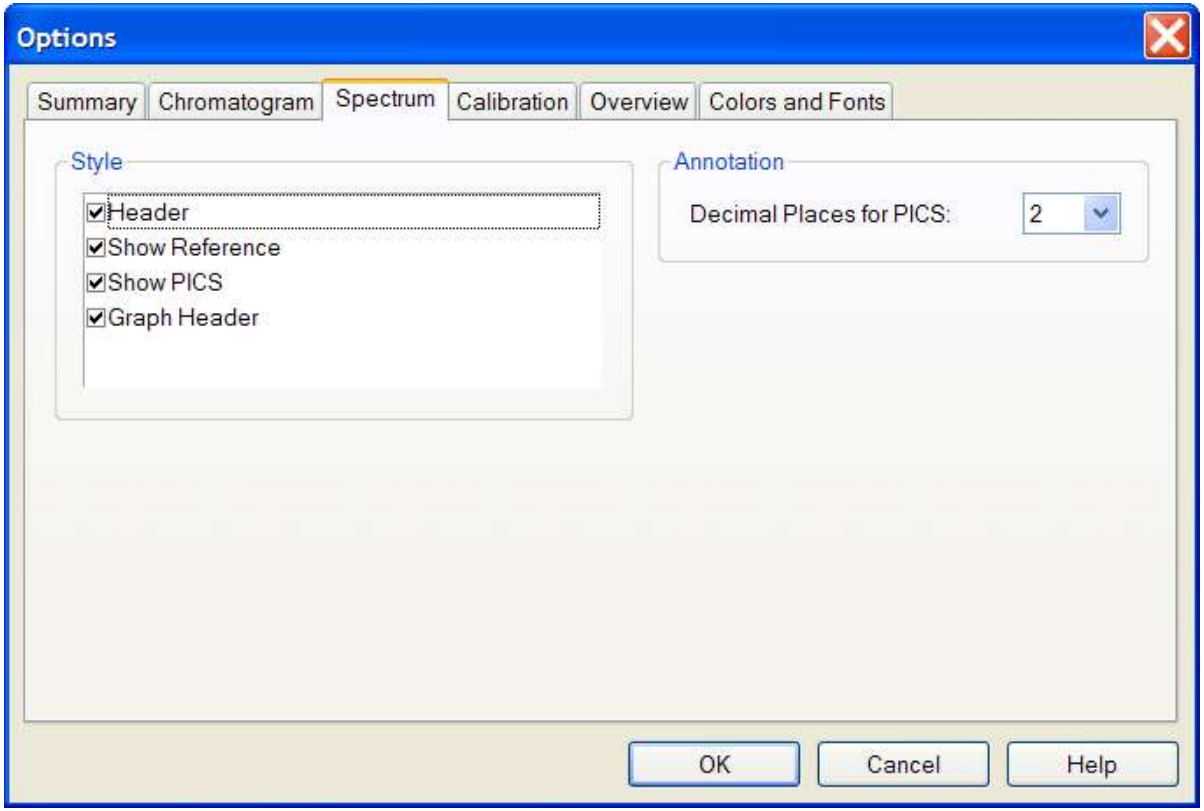
Calibration Window



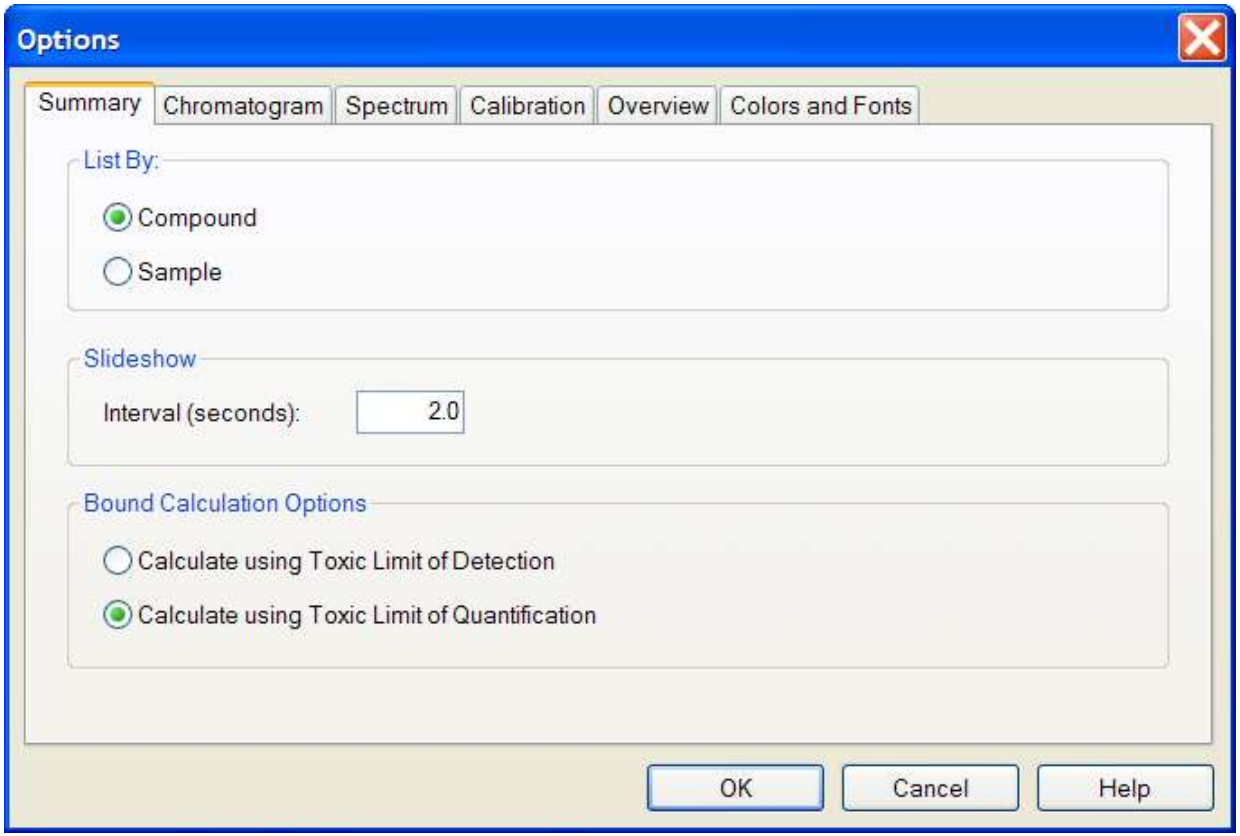
Calibration Window – View Properties



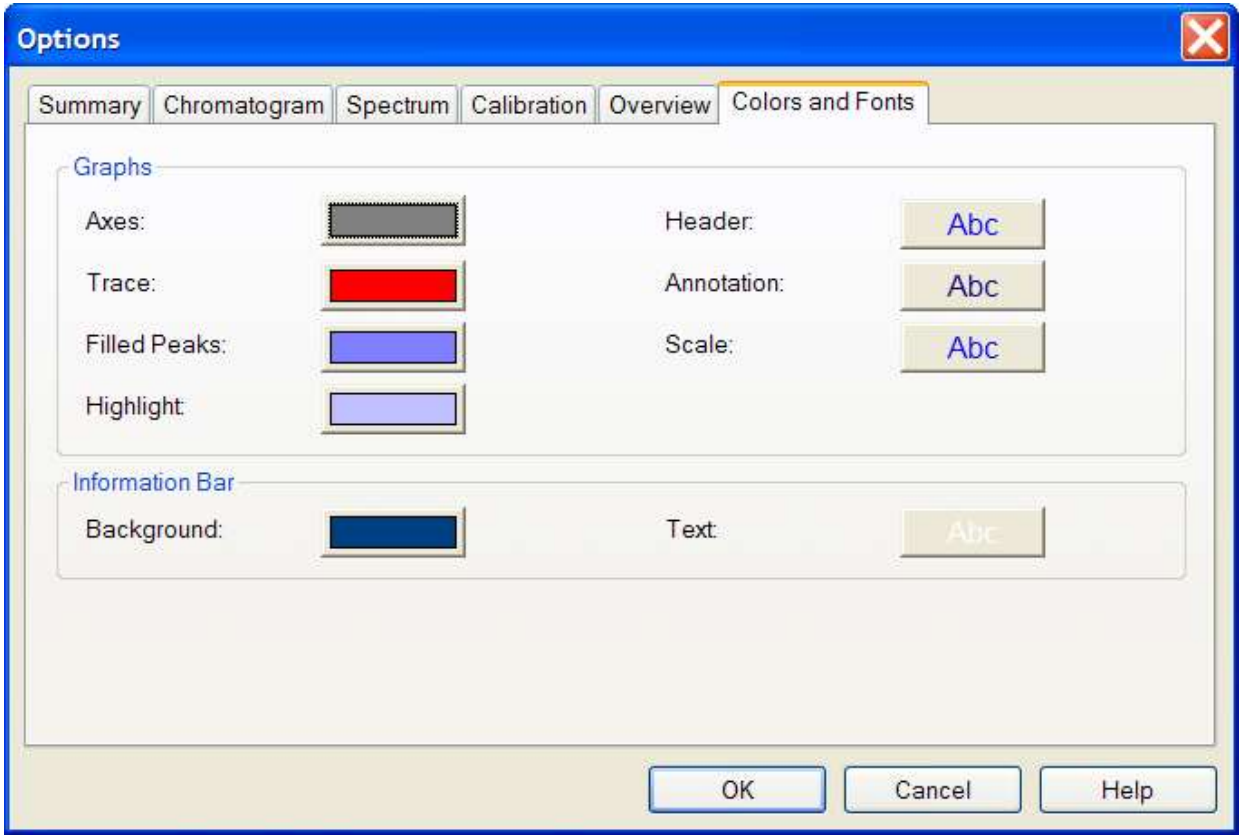
Spectrum Window – View Properties



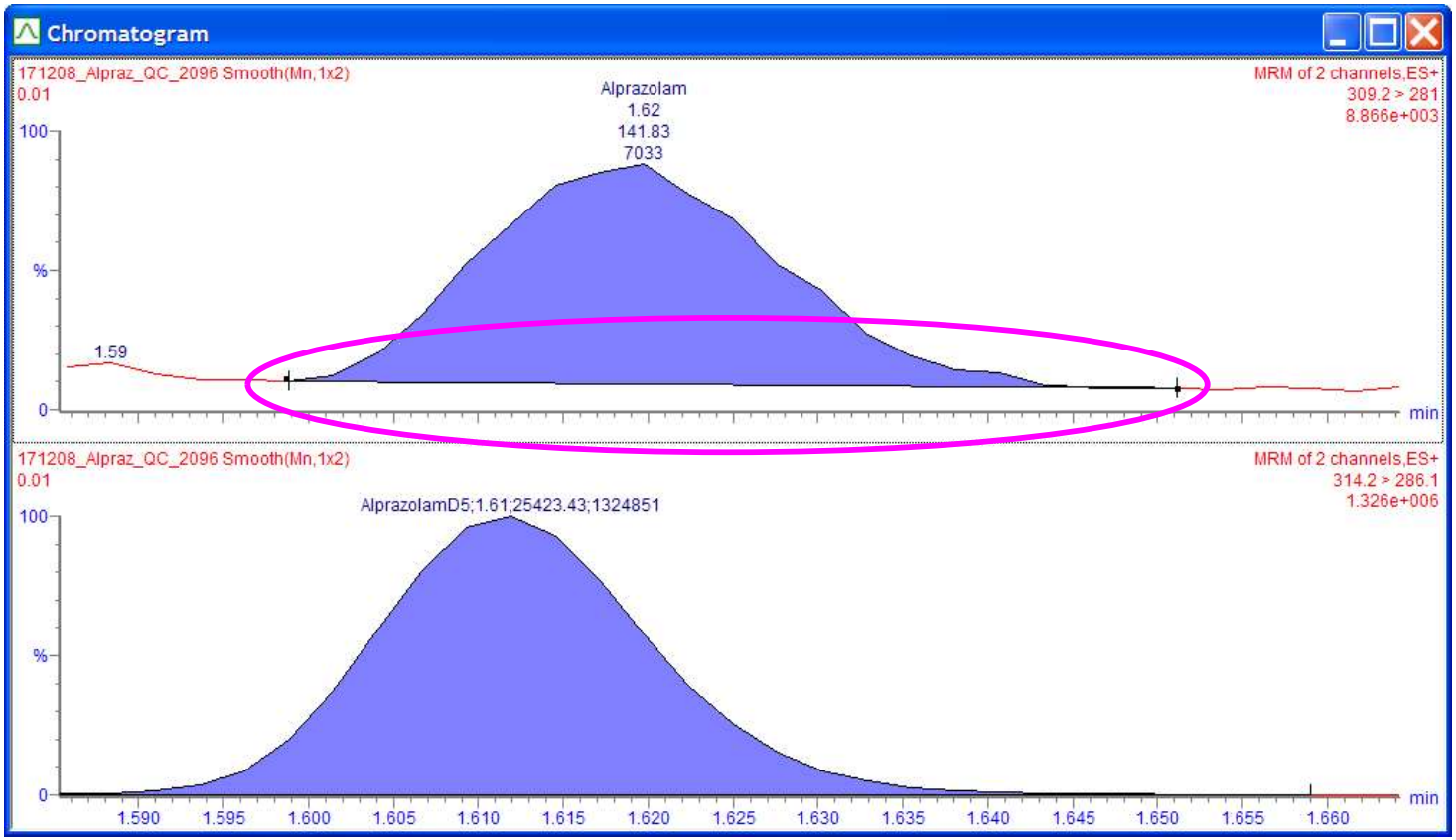
Summary Window Properties



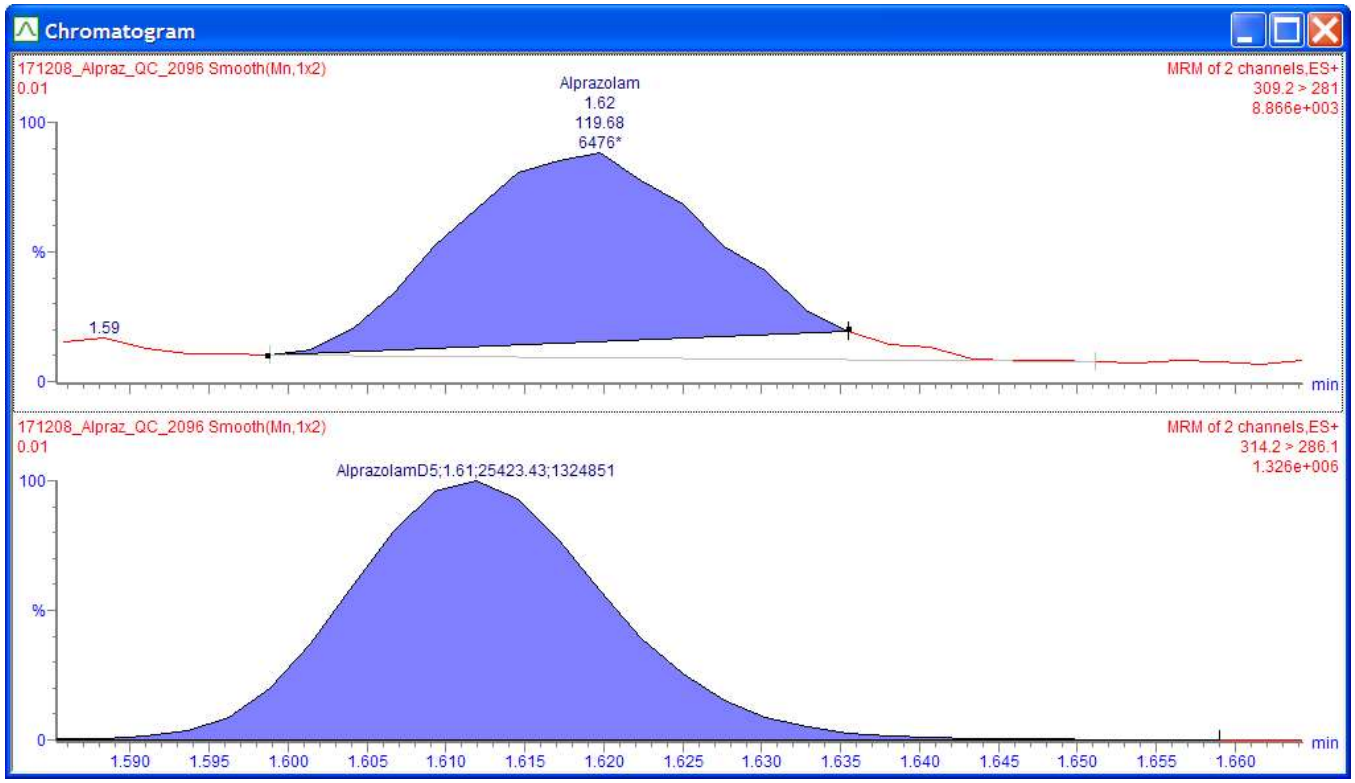
Color and Fonts Window



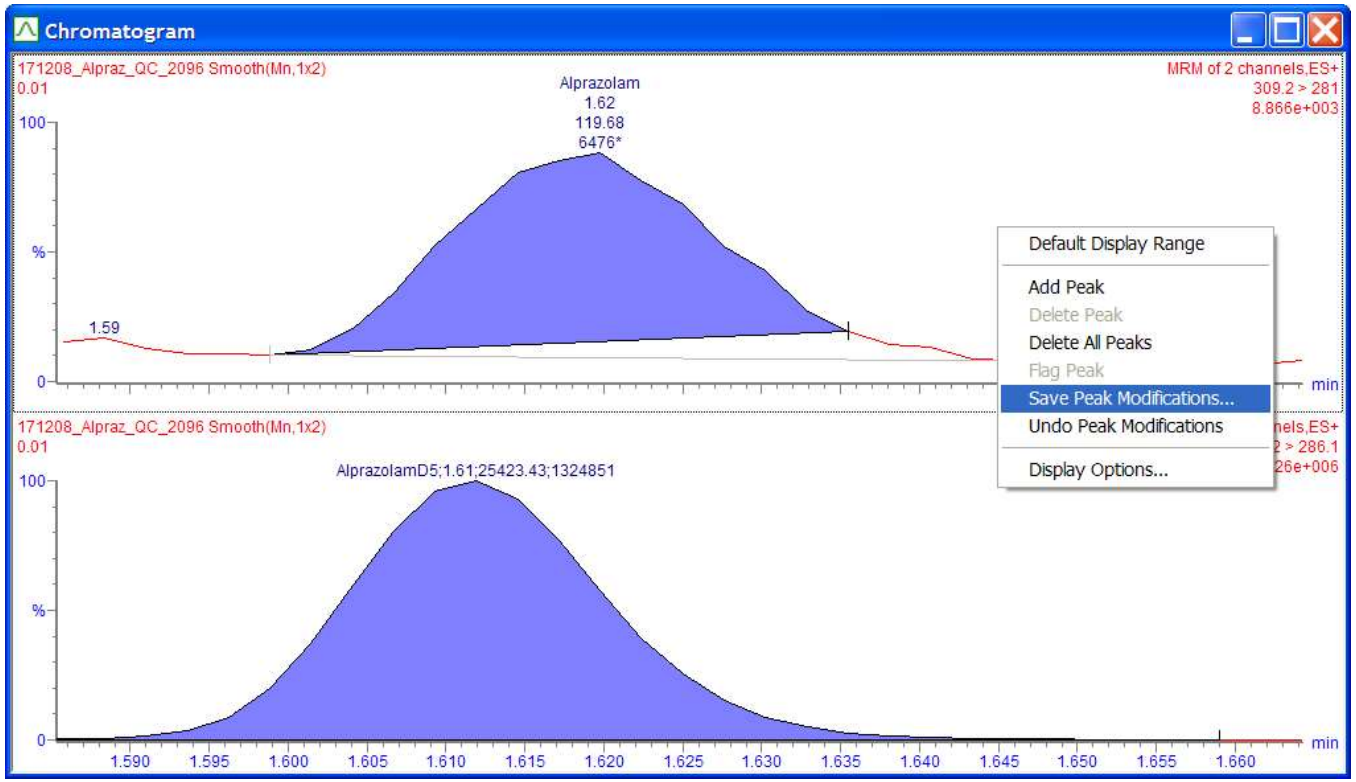
Manual Integration



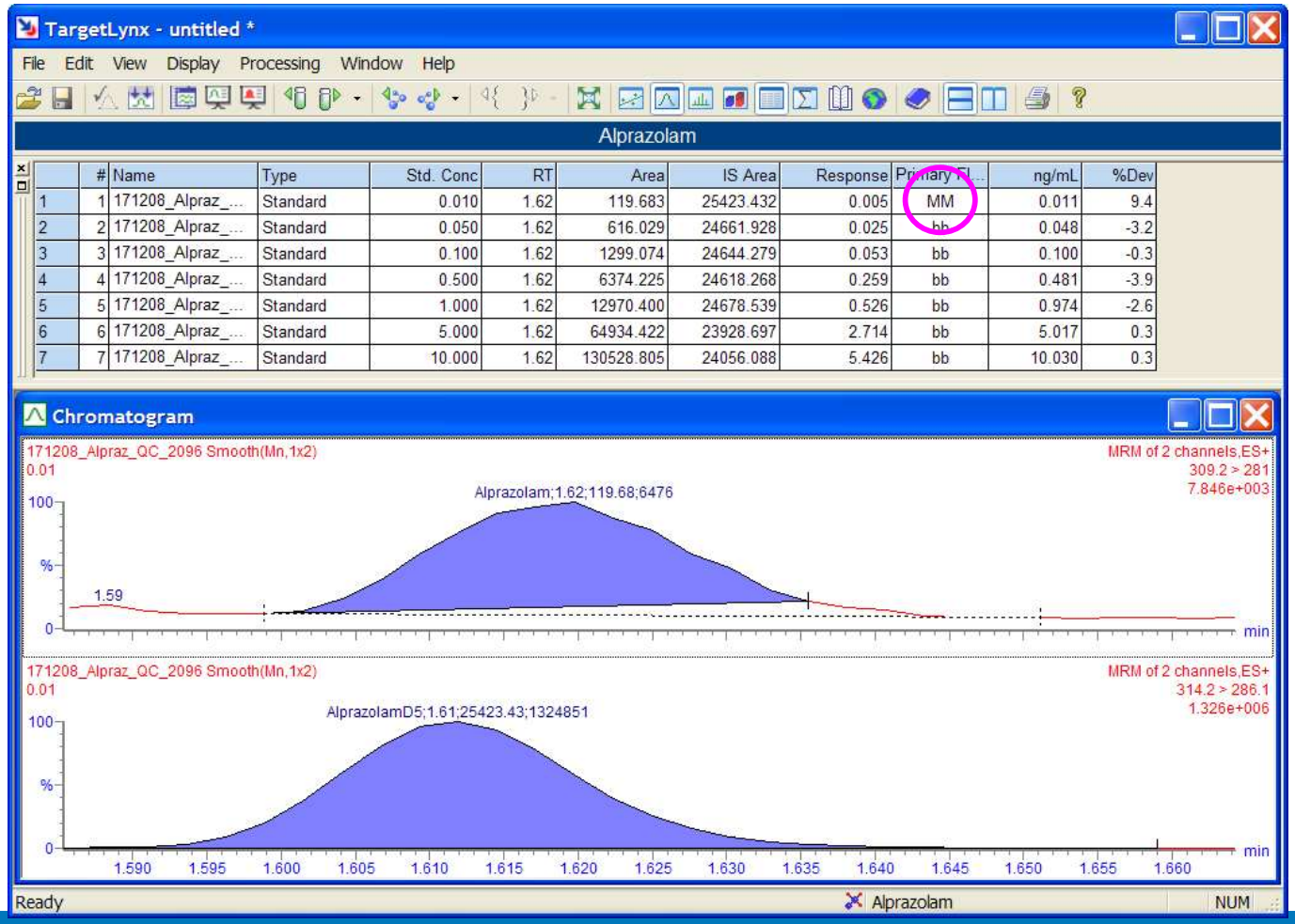
Manual Integration



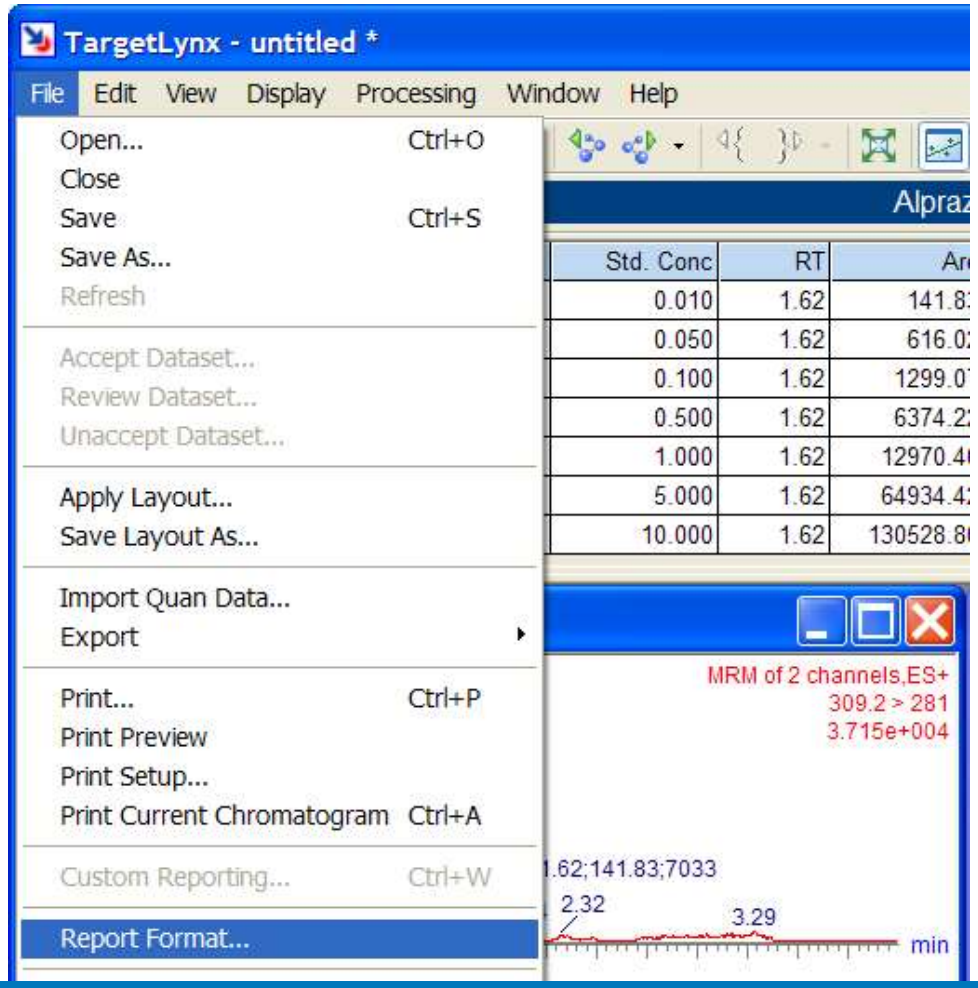
Manual Integration



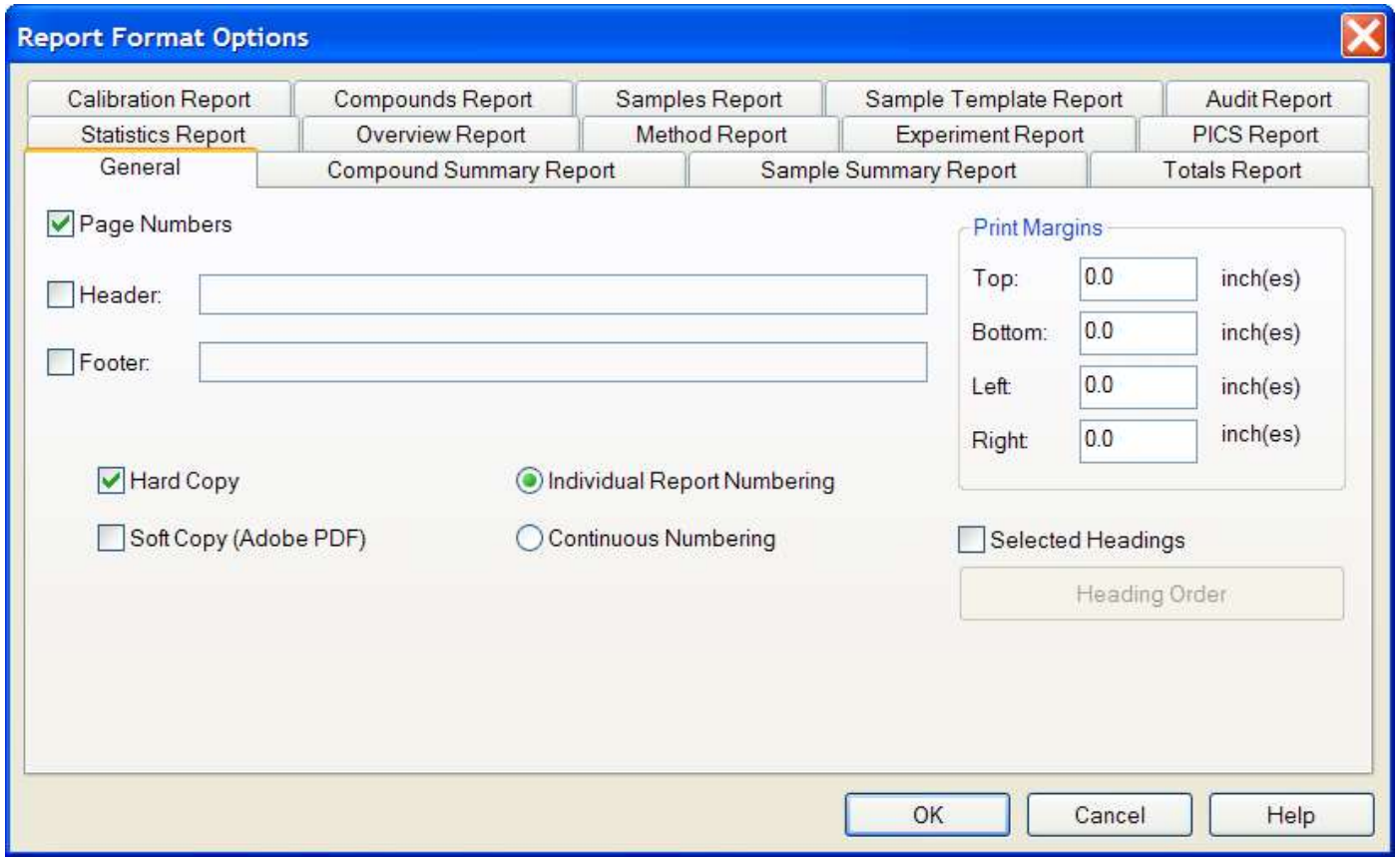
Manual Integration



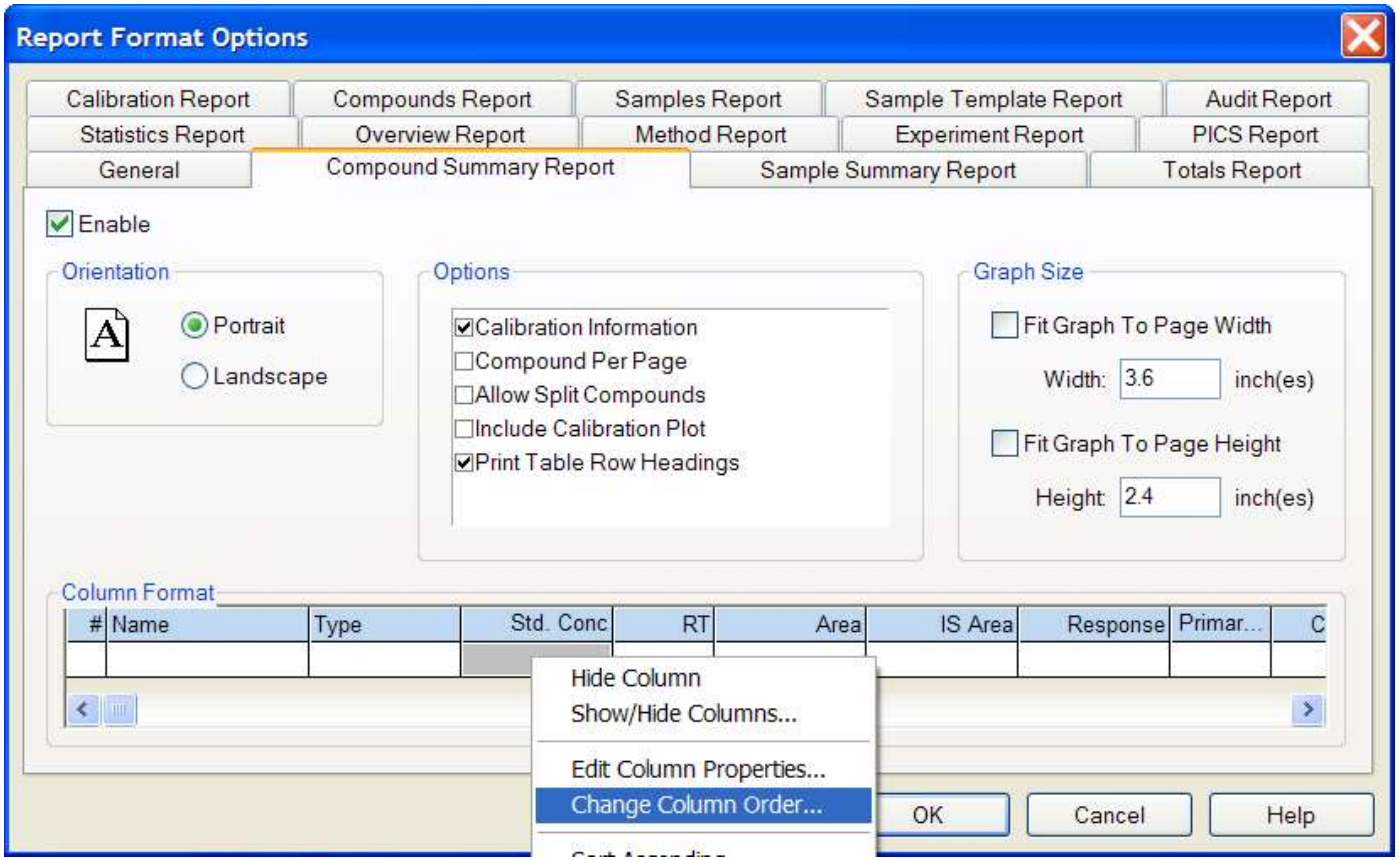
Report Format



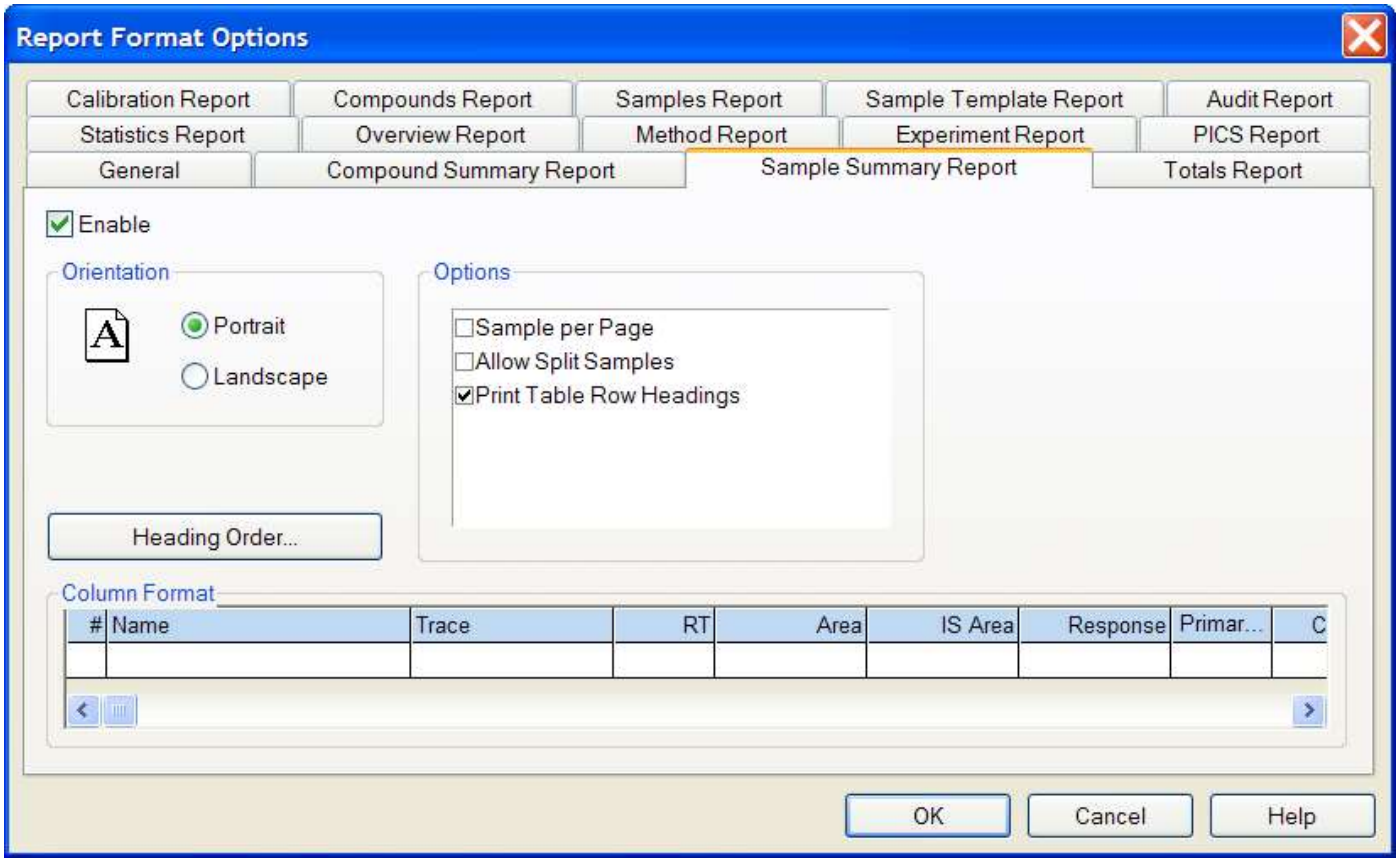
Report Format Options – General Tabbed Page



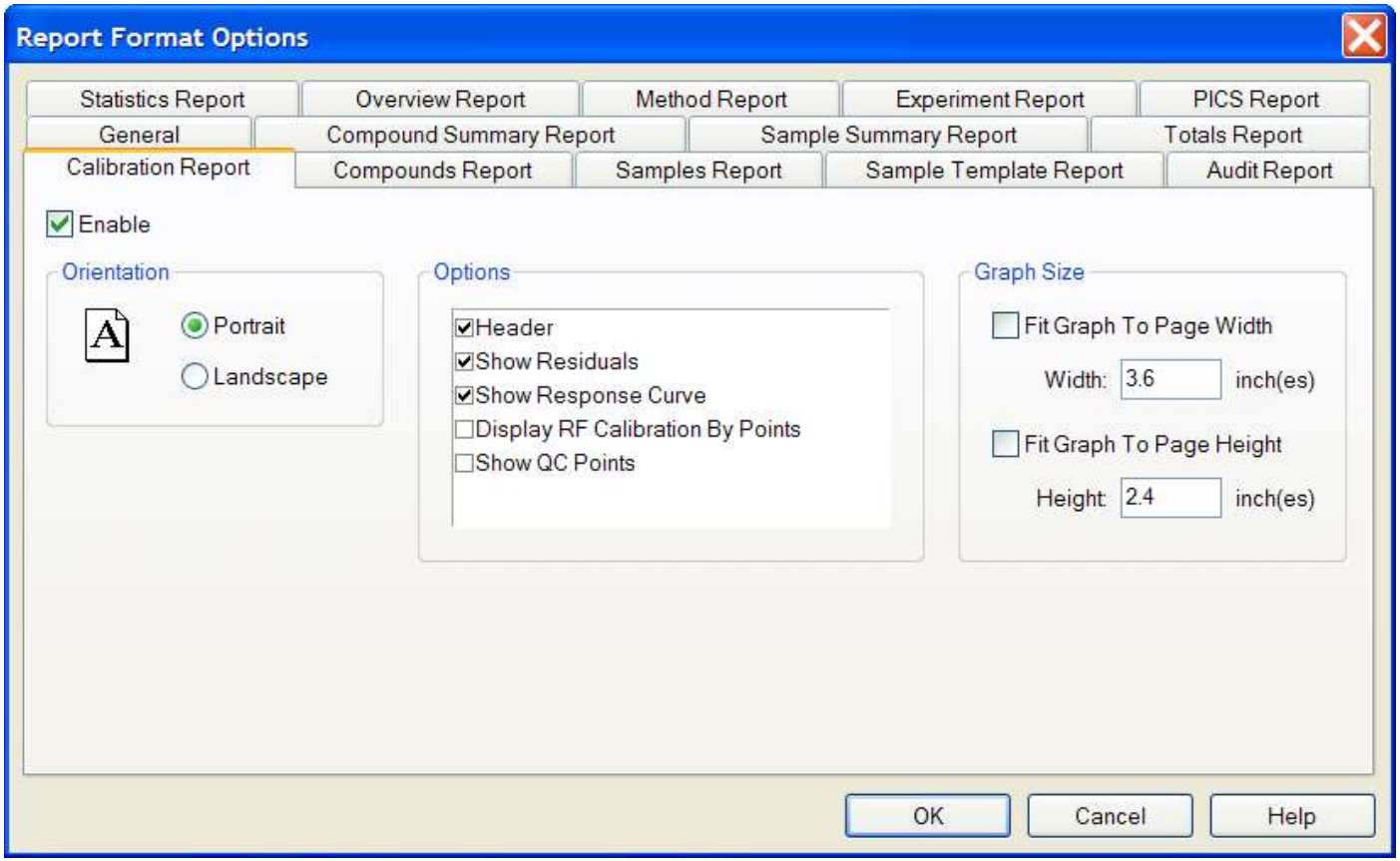
Report Format Options – Compound Summary Report Tabbed Page



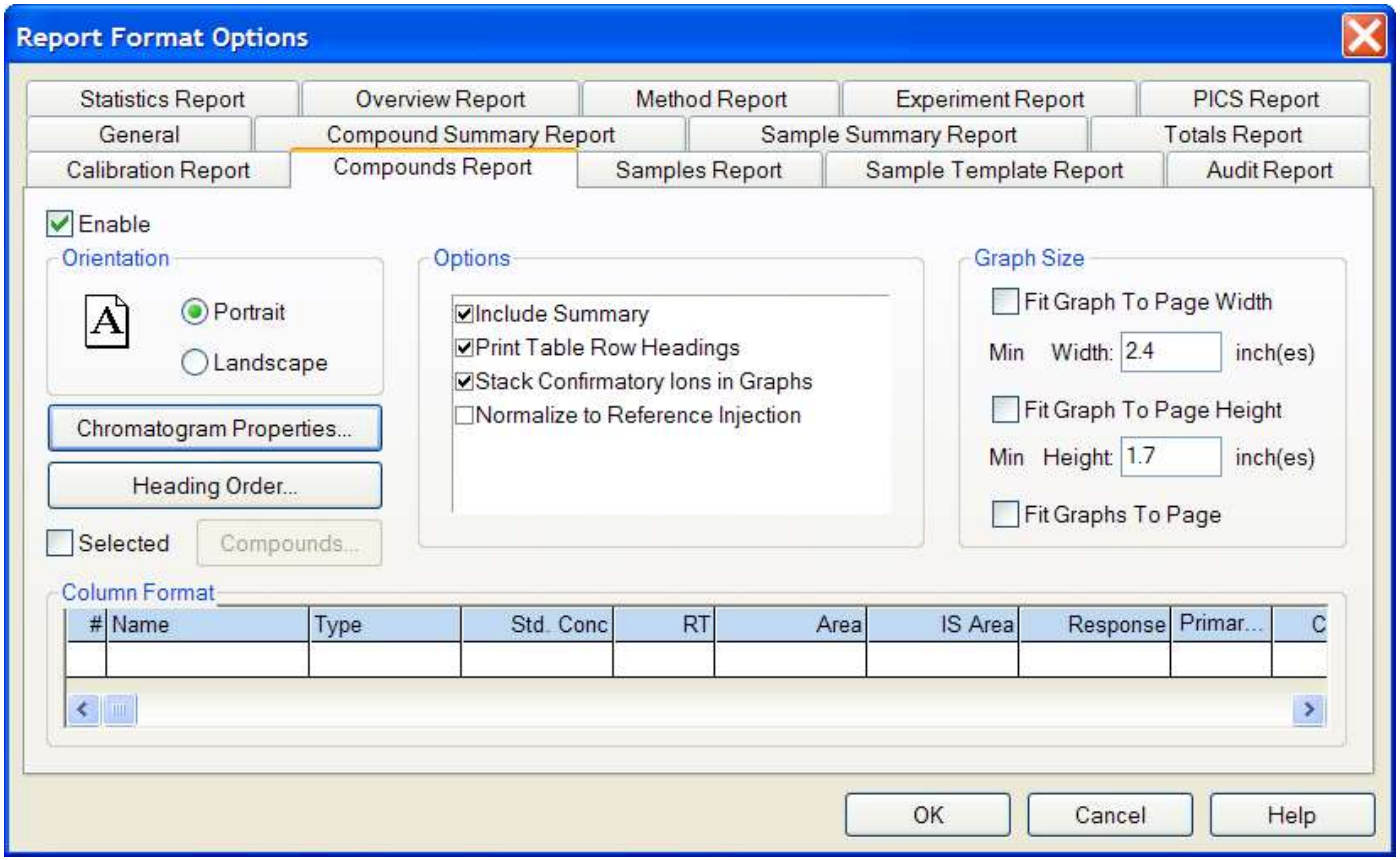
Report Format Options – Sample Summary Report



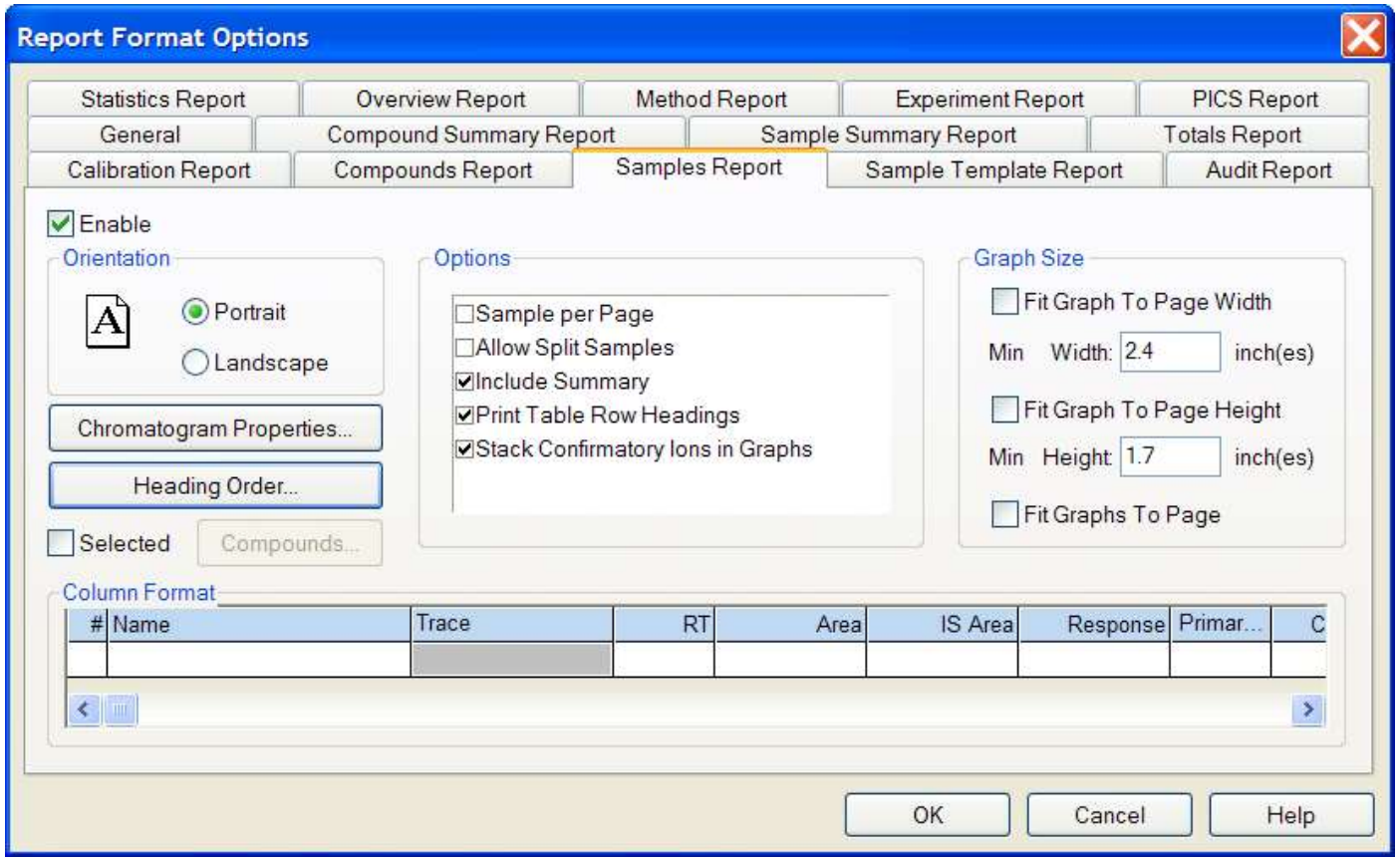
Report Format Options – Calibration Report Tabbed Page



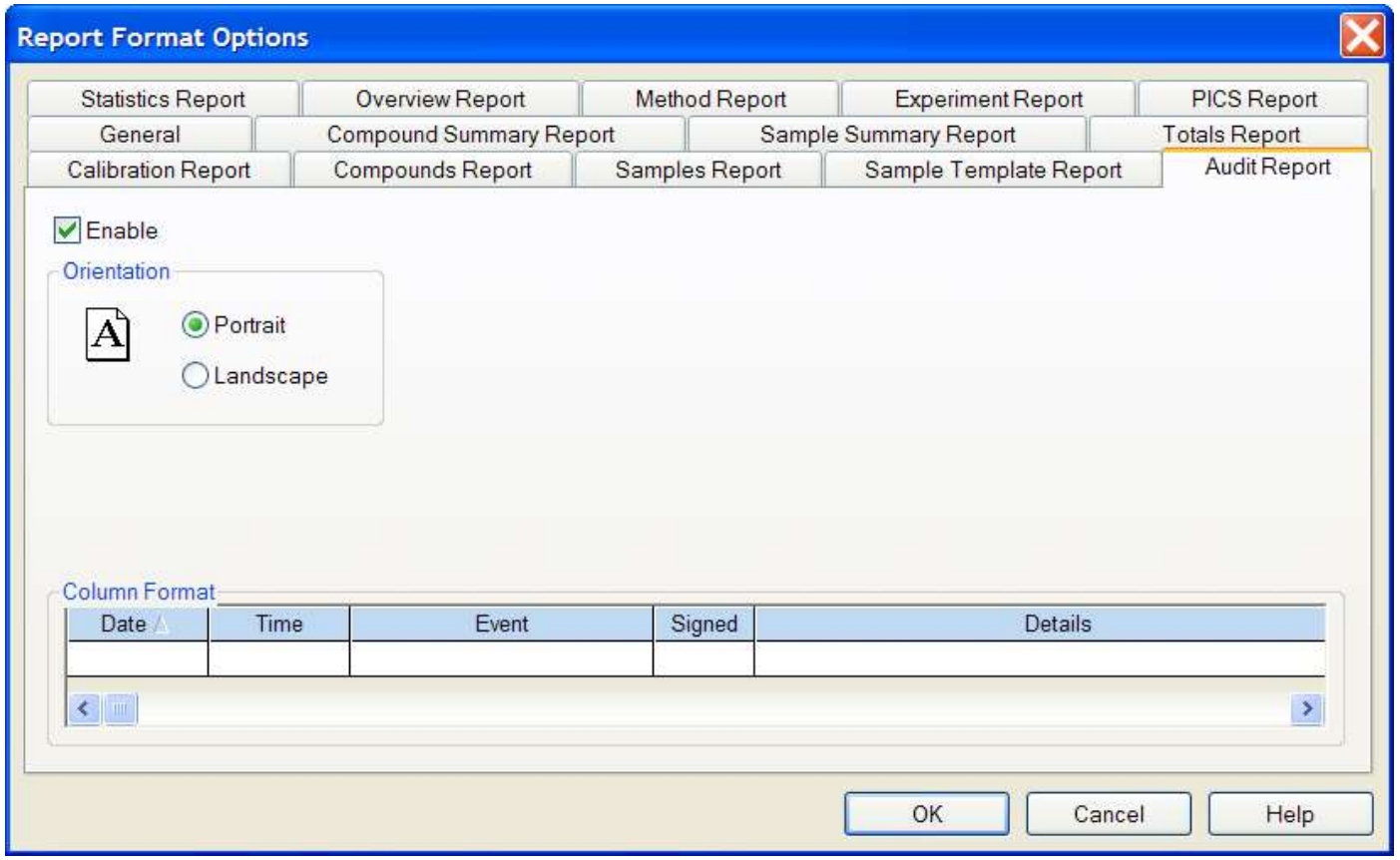
Report Format Options – Compounds Report



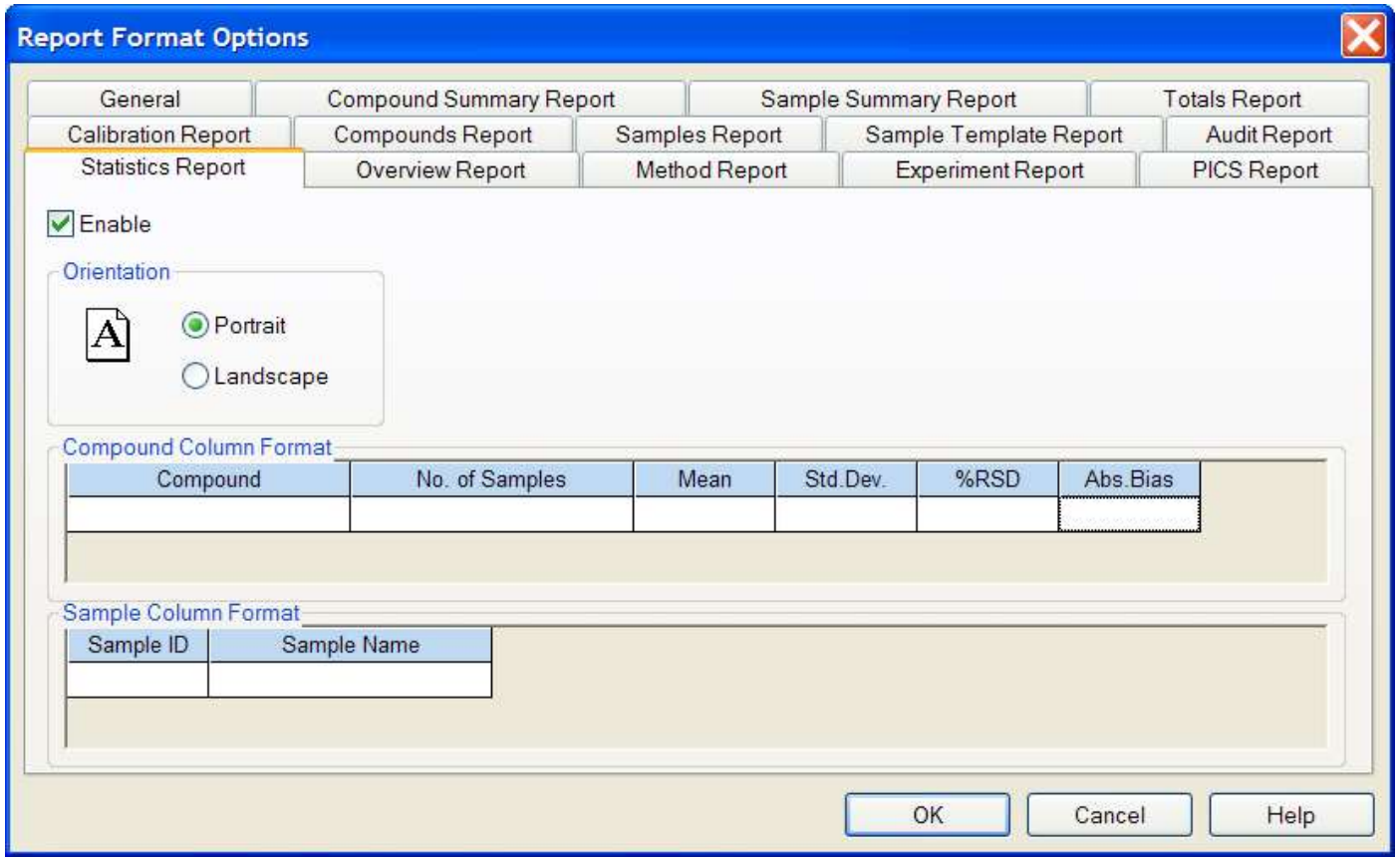
Report Format Options – Samples Report Tabbed Page



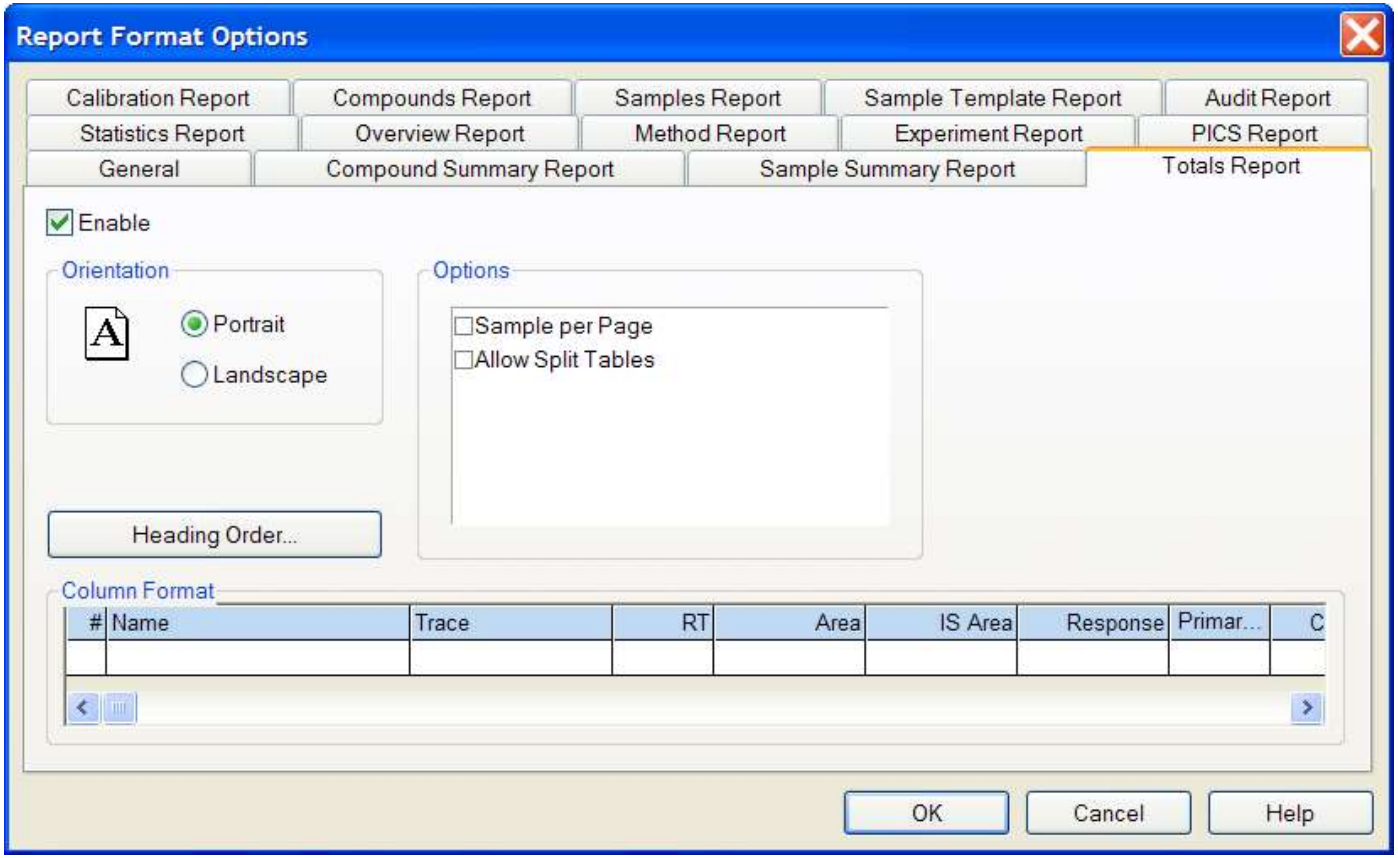
Report Format Options – Audit Report Tabbed Page



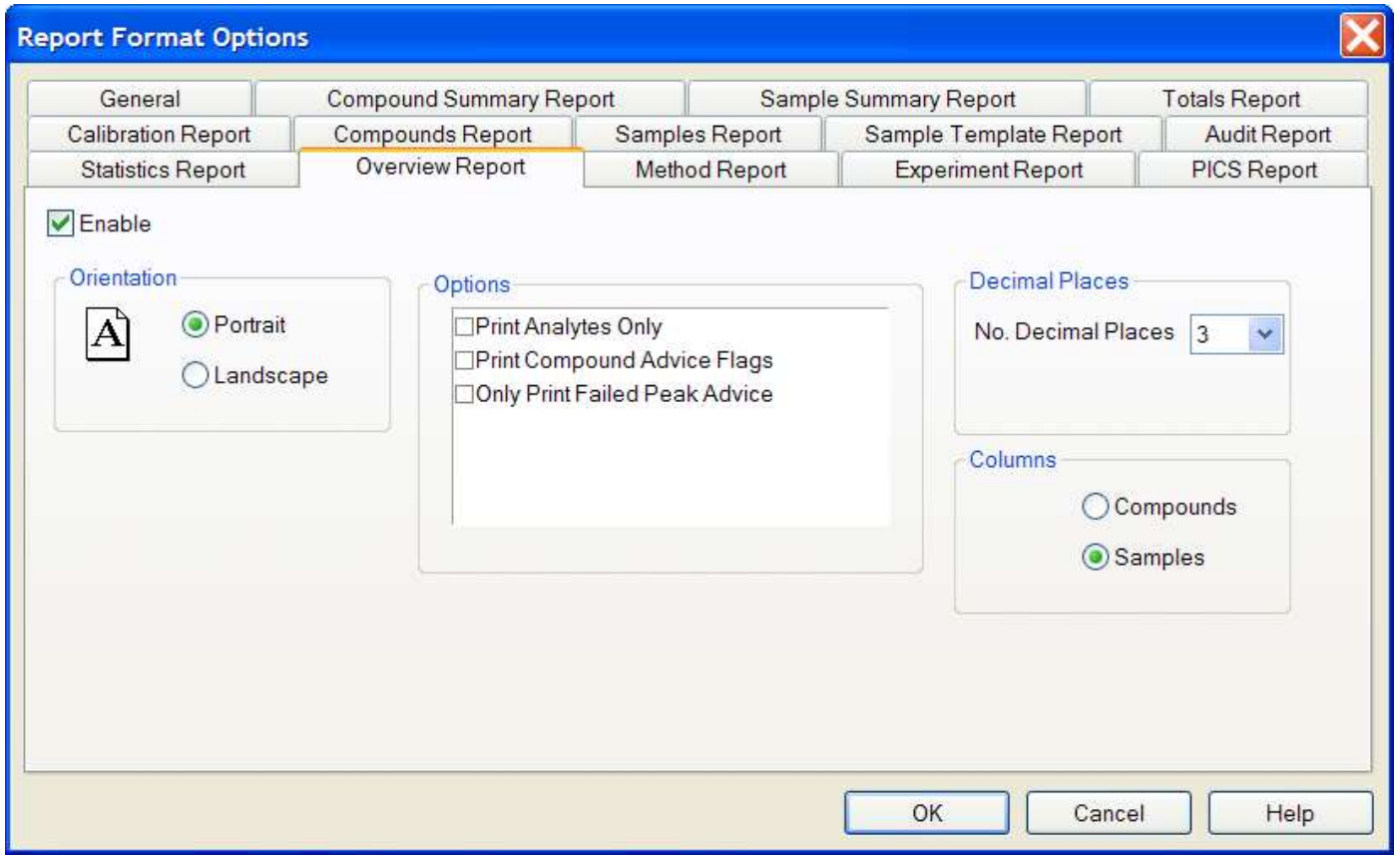
Report Format Options – Statistics Report Tabbed Page



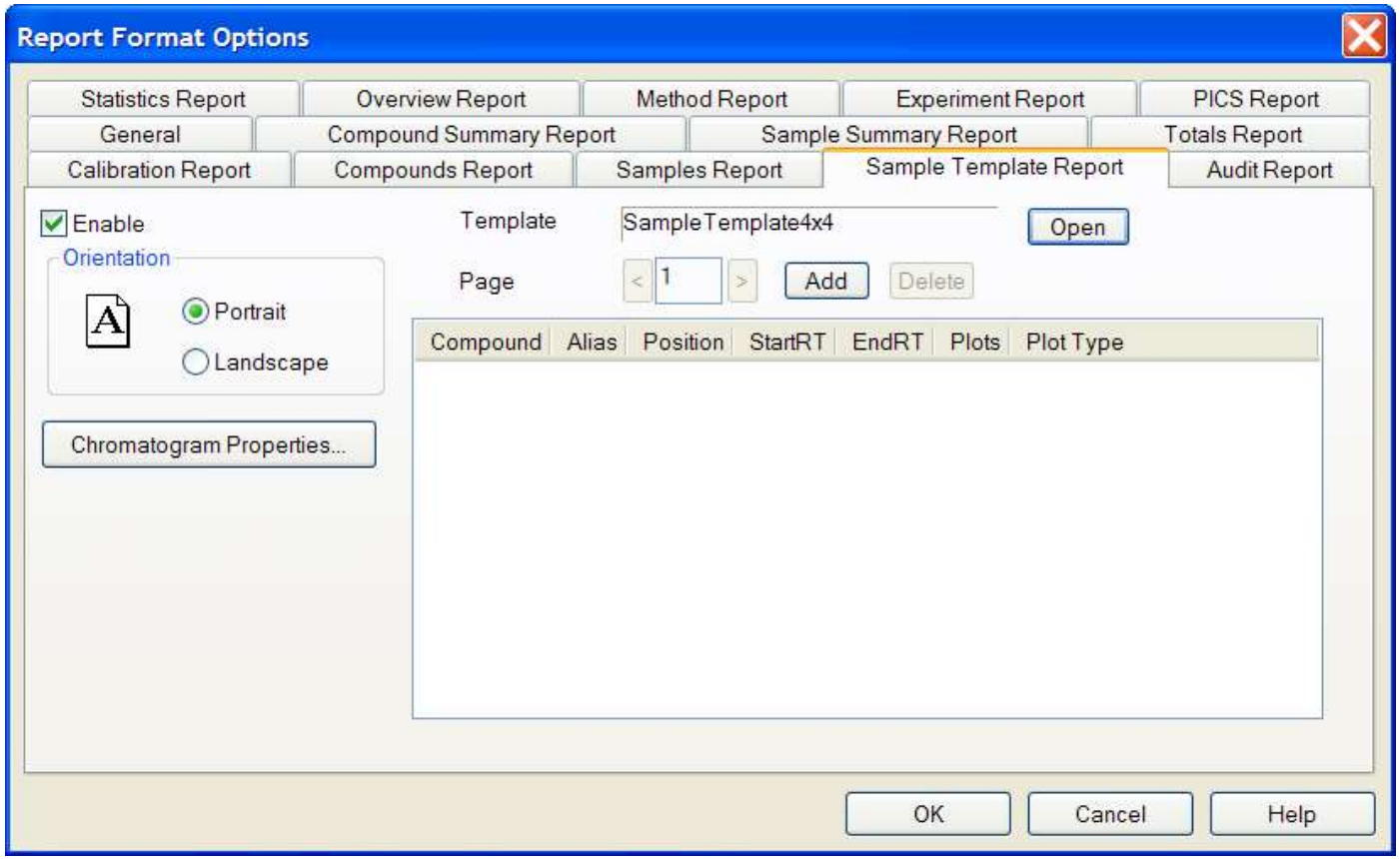
Report Format Options – Totals Report Tabbed Page



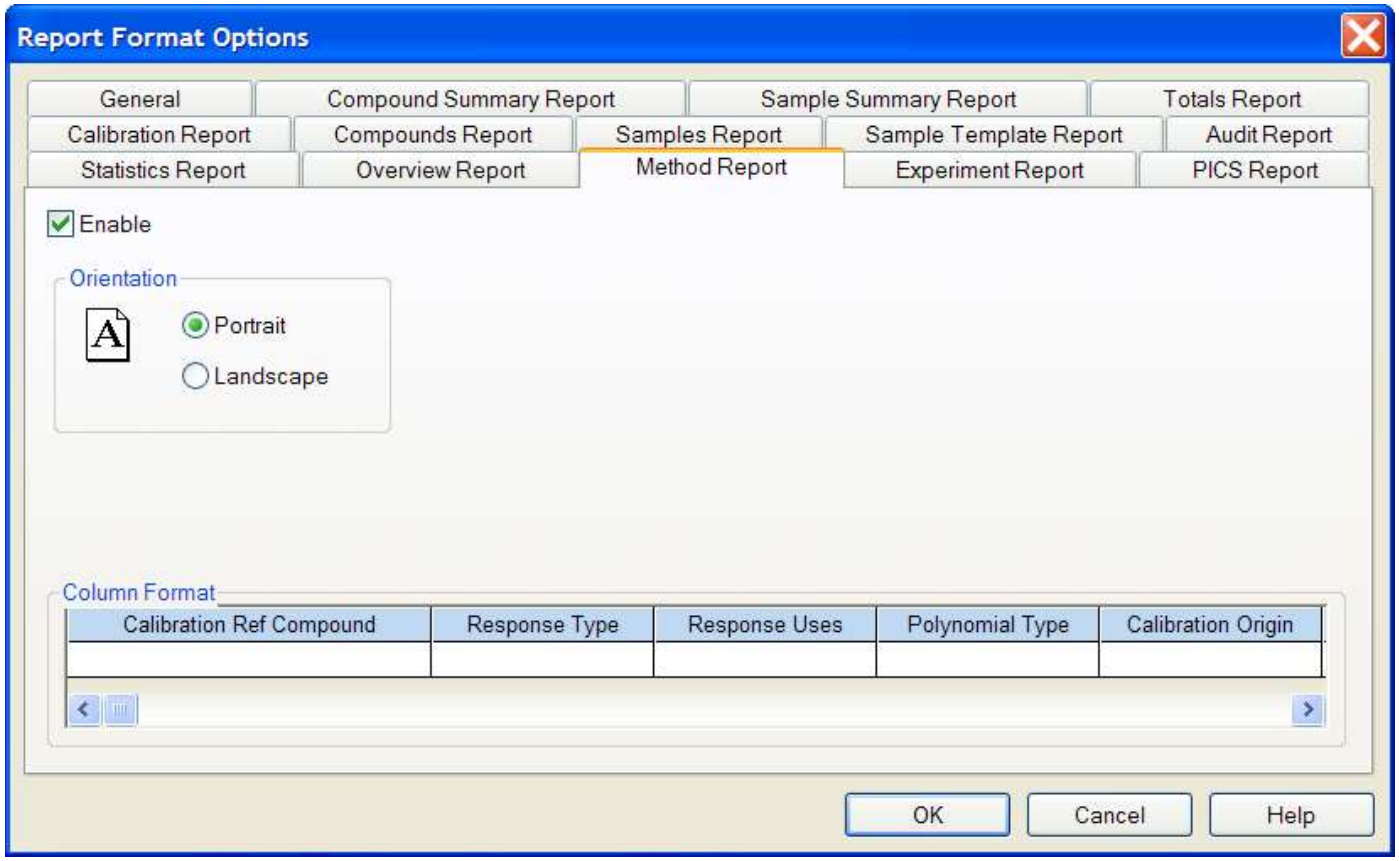
Report Format Options – Overview Report



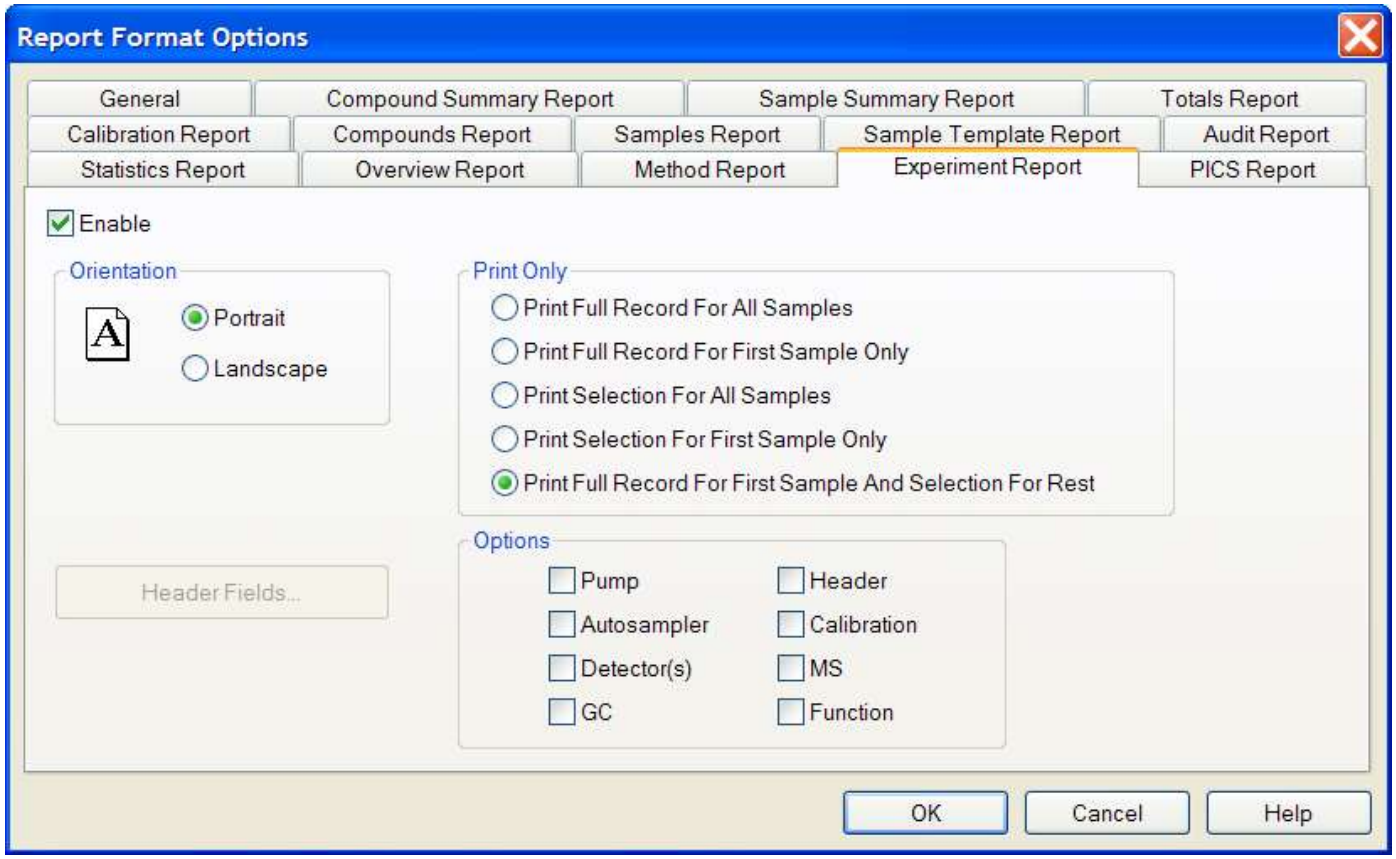
Report Format Options – Sample Template Report



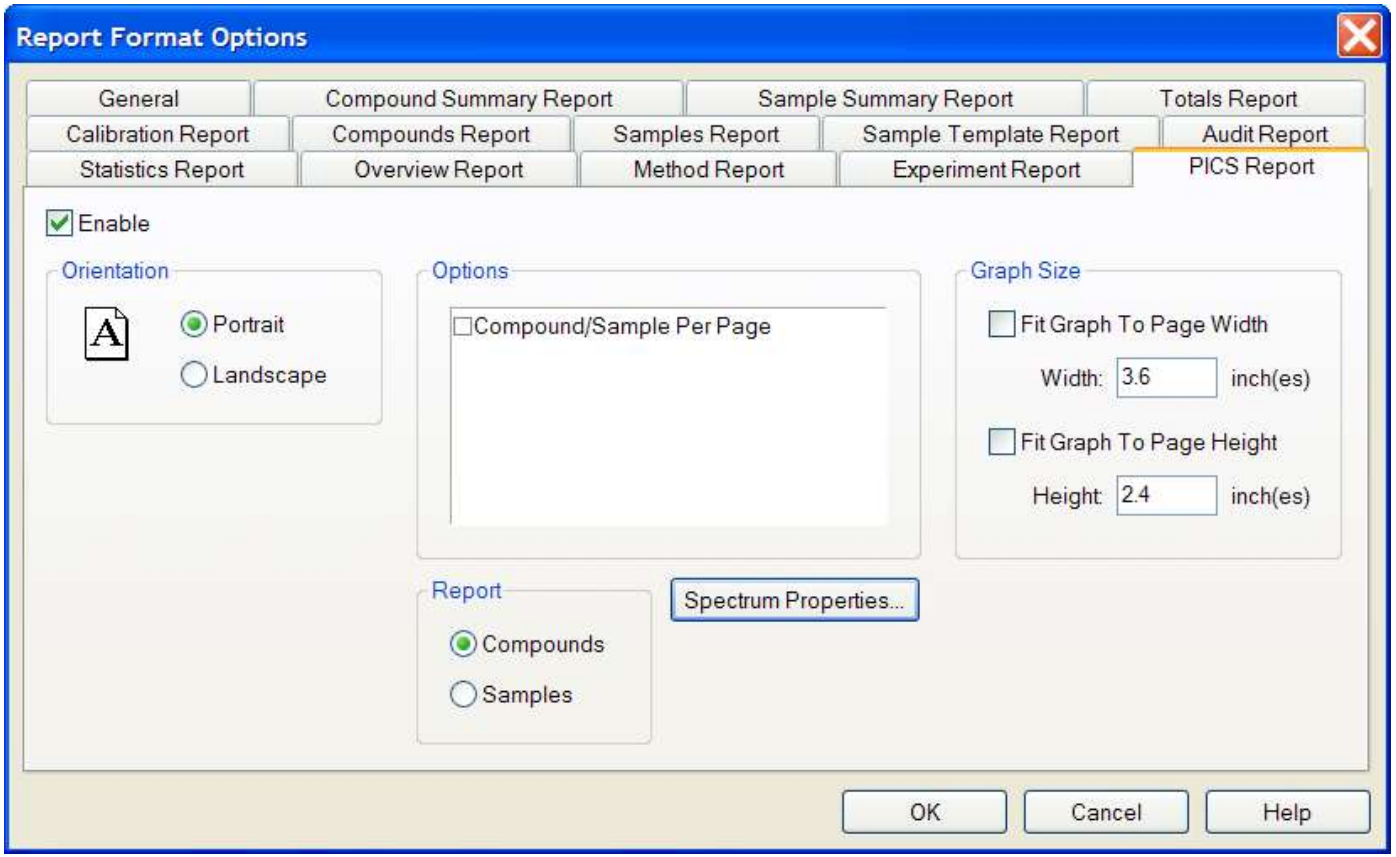
Report Format Options – Method Report



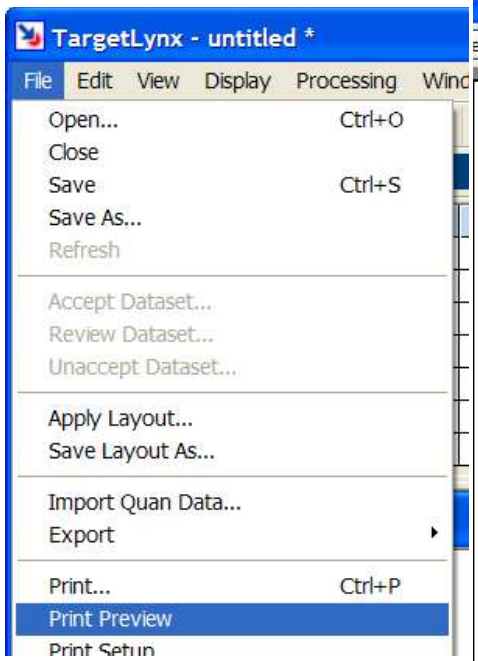
Report Format Options – Experiment Report

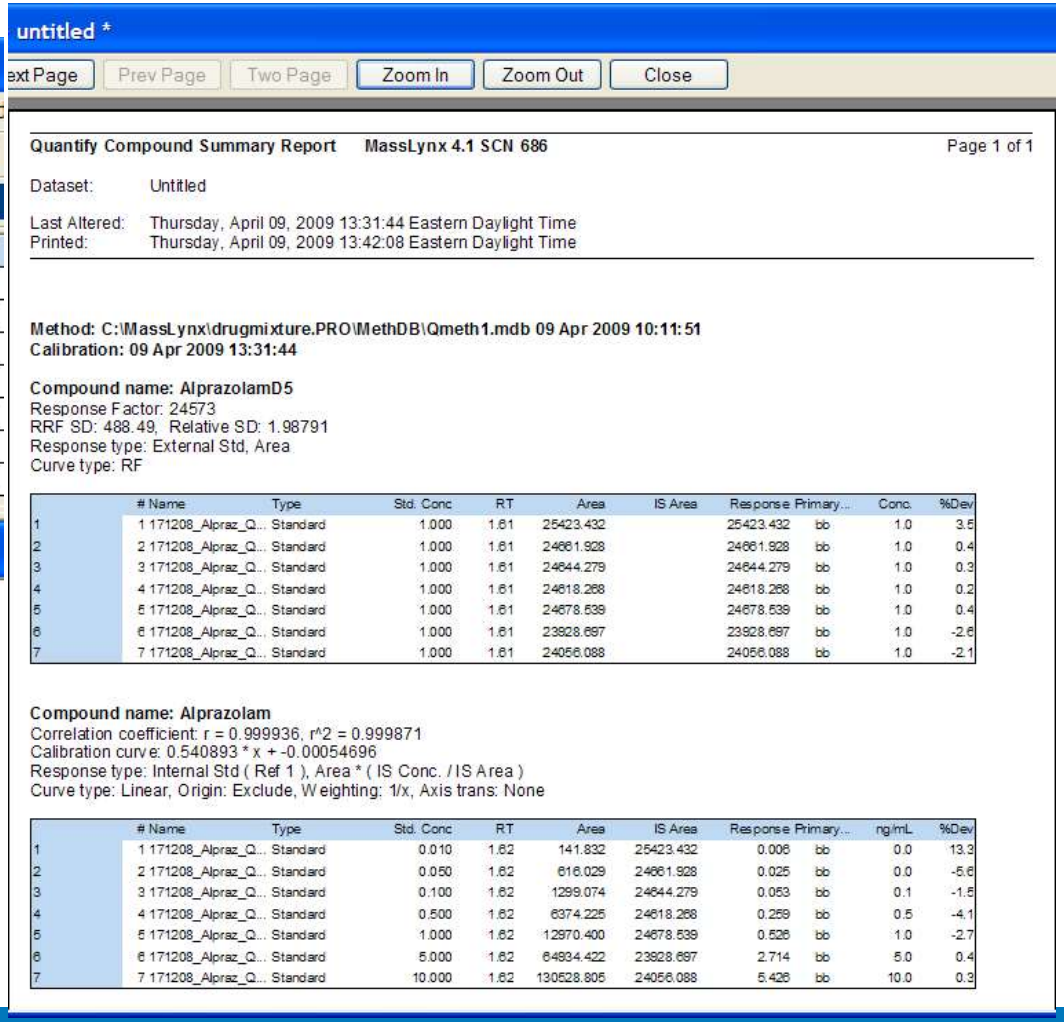


Report Format Options – Experiment Report

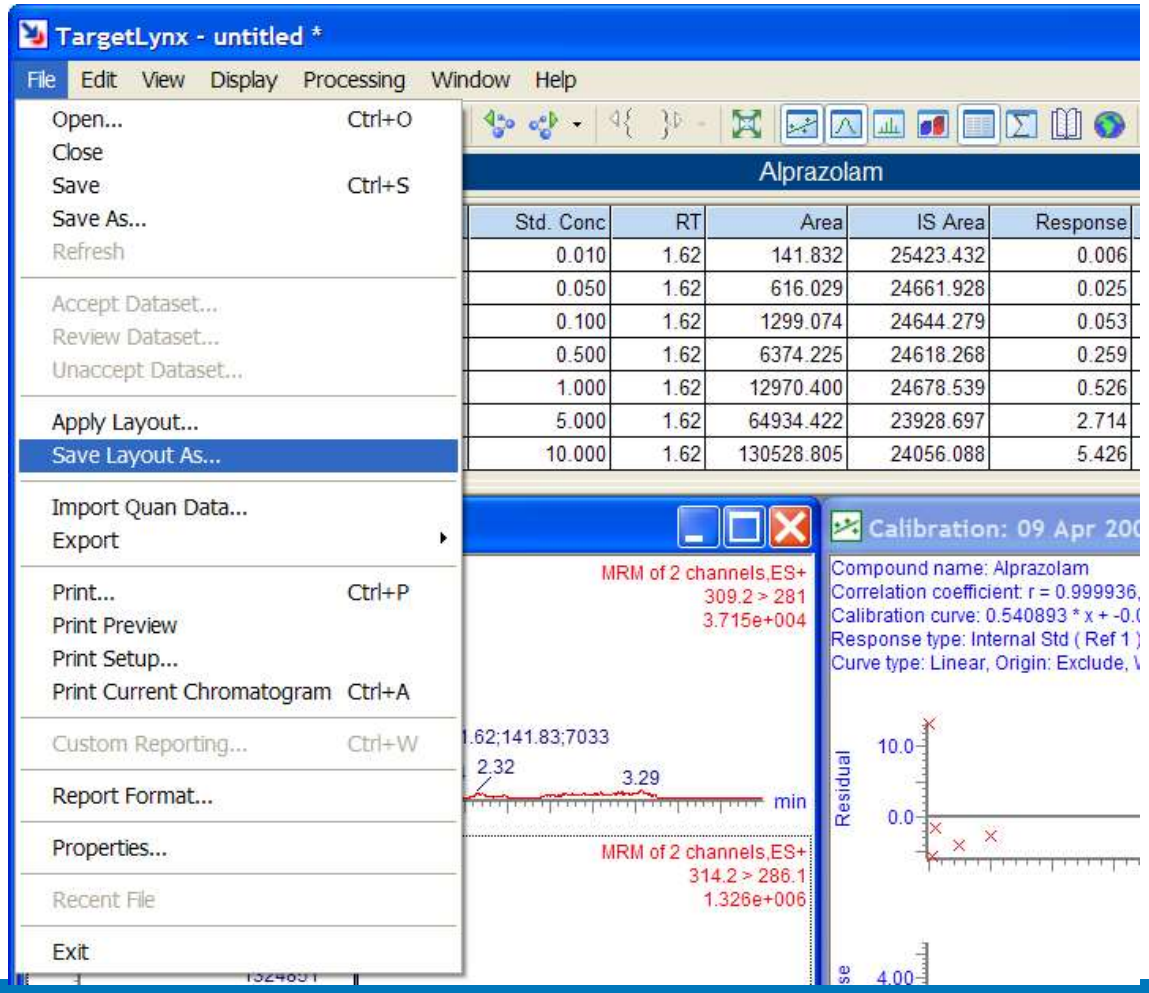


Print Preview

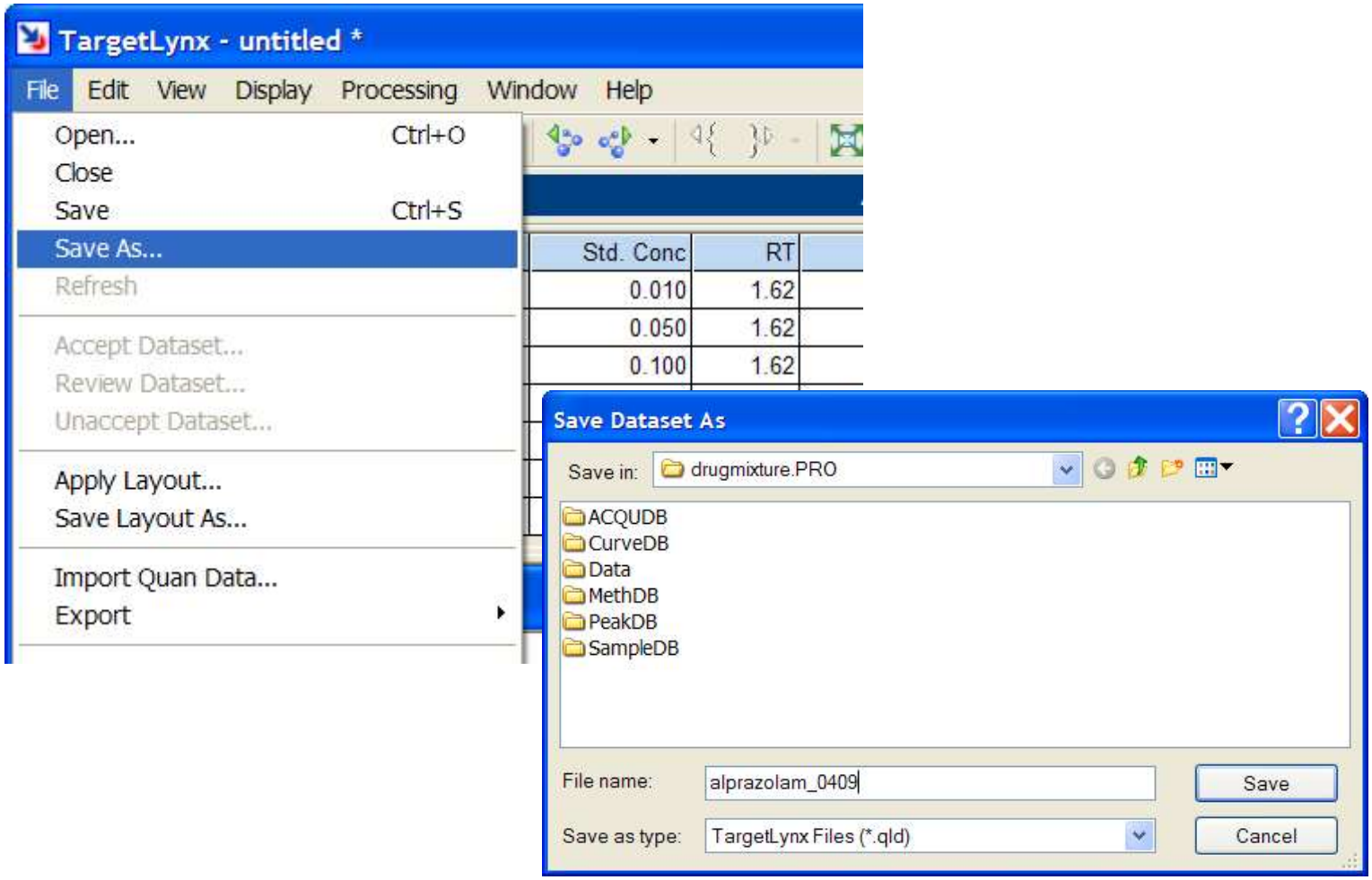




Saving the Layout



Saving Results



More on TargetLynx Quantification

Waters™

- Printing Reports (File, Print Report). Besides a full report, results from a set range of samples can be printed.
- Screen and Report Format. A customized format can be saved in a file for later use.
- The TargetLynx method used with a report can be changed using (Edit, Quantify Method)
- Editing of Calibration Curve (Edit, Calibration Curve) allows excluding of specific data points. (‘Right Click’ on a point in a Calibration Curve and select ‘Exclude Point’).
- Reprocessing samples after editing the TargetLynx method (Process, Calculate)

Saving Results

- Everything is in one file
- This file can be viewed and reports printed at a later date without reprocessing data
- This file will contain:
  - Compound and Sample Summaries
  - Calibration Curves
  - Chromatograms
  - Experimental Record for Each Analysis Run
  - Quantitation Method

Titles Are Initial Capitalization Style, Blue Font, 20 Point Size

- Bullets are Arial font, 18 point, and black
- This slide master is called “Title and Content”
- It does not have a subtitle

Titles Are Initial Capitalization Style, Blue Font, 20 Point Size

Subtitle: Font is Arial, 16 point and text color is gray

- Bullets are Arial font, 18 point, and text color is black
- This slide master is called “Title, Sub, and Content”
- Click to edit Master text styles
  - Second level
    - Third level
      - Fourth level
        - Fifth level

Two Column Master Slide/Two Line Header Slide: Titles Are Initial Capitalization Style, Blue Font, 20 Point Size

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- Bullets are Arial font, 18 point, and text color is black
- This slide master is called "Two Content"
- It does not have a subtitle
- Click to edit Master text styles
  - Second level
    - Third level
      - Fourth level
        - Fifth level
- Second column starts here
- And so on

Chapter Eight

Xevo TQ-S micro Maintenance

System Maintenance

- What to use and not to use with MS detector
- Good practices
- Troubleshooting
- Source Maintenance
  - Cleaning the Sample Cone and Cone Gas Cone
  - Cleaning the Source and Ion Block
- Probe Maintenance
  - Replacing the ESI Probe Stainless Steel Capillary

What to use with MS detector

- List of compatible acids, bases and organic solvent
  - Acids: Formic, acetic, trifluoroacetic acid (caution)
  - Base: Ammonium hydroxide
  - Organic: Methanol, Acetonitrile, Propan-2-ol, Acetone
- Preferred/compatible buffer salts
  - Formates, Acetates, Carbonates
  - Keep below 10mM
- Ion pairing reagent can be used but risk prolonged ion suppression
  - TFA, DFA, HFIP can be used
  - Must no use sulfonate salts

What not to use with MS detector

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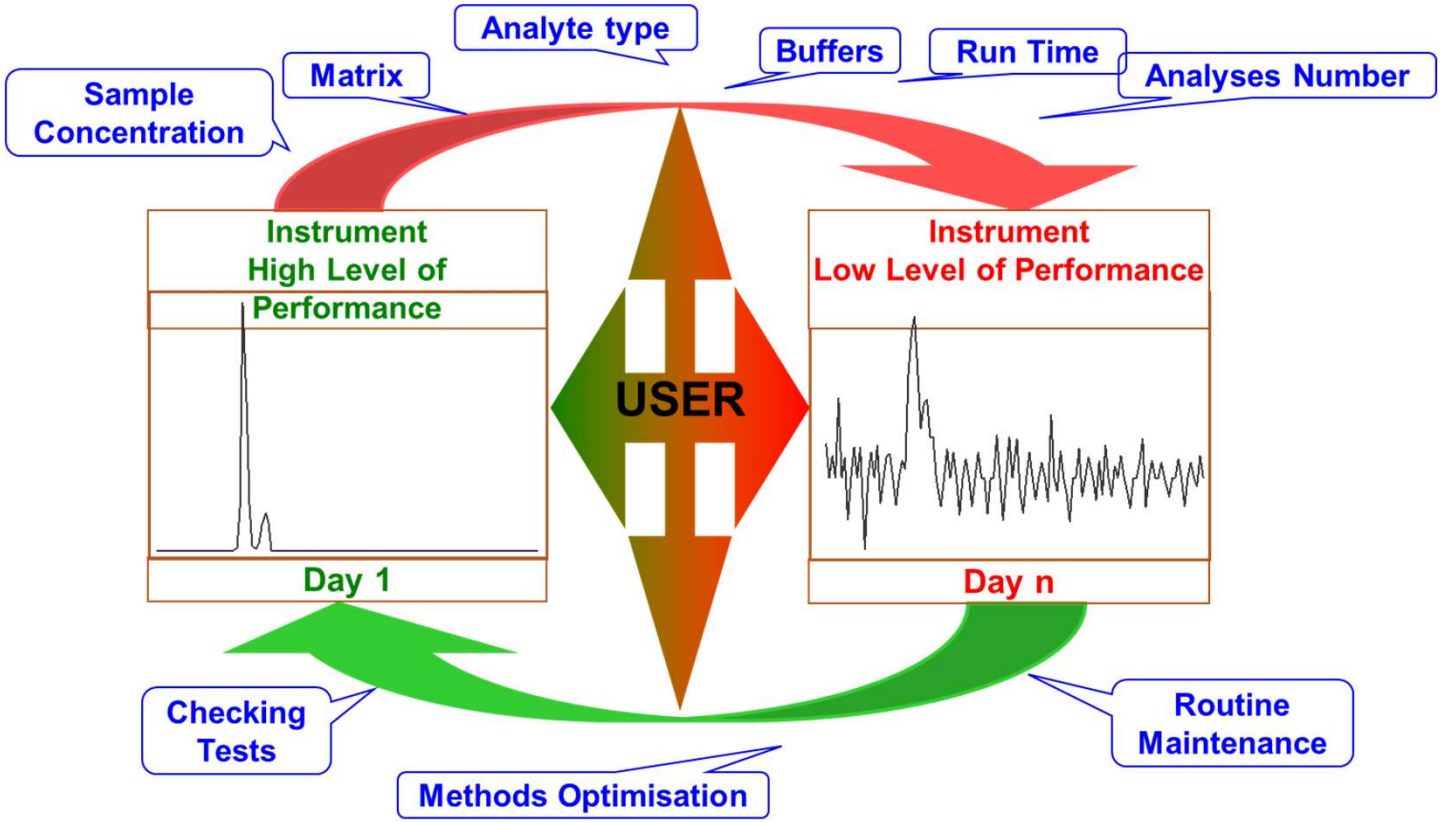
- Must not use non-volatile salts
  - Phosphates, nitrates
- Must remove as much detergents as possible from sample
  - SDS, PEG, TWEEN, Triton etc
  - Will result in ion suppression and persistent peak in chromatogram
- Chlorinated solvents, non-volatile organics and those not listed as usable in previous slide
- Strong acids and bases
- MS cleaning solution
- Concentrated buffered mobile phase
  - With the exception of HILIC, consult Waters before using

Good practices with MS

- Use clean solvents and fresh mobile phases
  - Prepare daily
  - Wash bottle thoroughly
  - Discard mobile phase if appears cloudy or particulates seen floating
- Respect the MS max capillary voltages
  - Positive 3.5kV, Negative 2.5 kV
  - Always start low and go higher for better signal intensity
- Inject samples previously cleaned up or treated
  - Dilute and shoot will contaminate your MS faster than you would expect
  - Unfiltered samples will clog your LC system and column
  - Incompatible solvents will cause LC relevant issues
- Make use of timed events, diverting LC flow to waste in between analyte detection window

Factors Affecting Performance



Checking the Instrument Performance

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- Why - In order to insure optimal performance of your system.
- How - By injecting a test compound to check the parameters of your API/MS system

- Types of crucial parameters

Mass position:	Mass Calibration
Resolution:	Quad.
Sensitivity:	Ion source

Corrective Actions

- Poor sensitivity
  - Main Cause : Dirty (polluted) optics (source)
    - Solution : Source and optics cleaning
- Inaccurate mass position
  - Main cause : Inadequate operating mass range or bad choice of calibration ions
    - Solution : Mass scale calibration
  - Do not attempt calibration unless you know how or engineer recommended it

Cleaning the Source Components

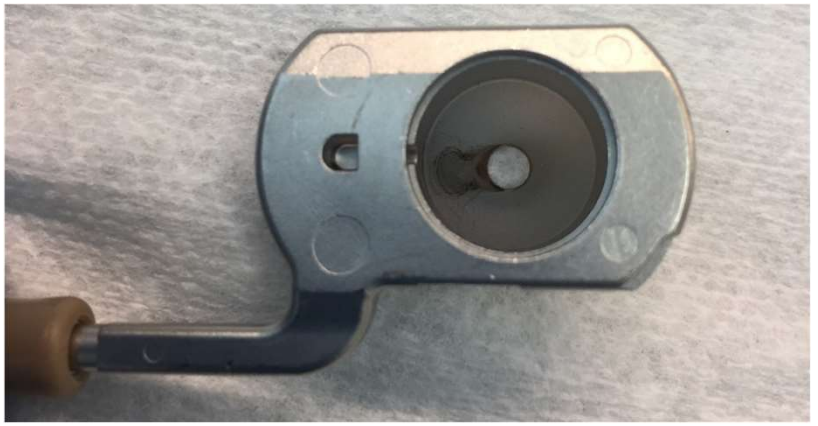
- When there is a noticeable drop in signal intensity that is not due to the sample or LC components of the system, clean the sample cone and the cone gas cone.
- The source block incorporates an isolation valve that lets you service the sample cone without venting the system.
- If cleaning the sample cone and cone gas cone fails to increase signal sensitivity, also clean the ion guide and ion block.
- The illustrated maintenance procedure can be found in the Xevo TQ-S micro operator's guide.

Hints and tips for sample cone cleaning

- There is no special cleaning required for the cone.
- 50:50 MeOH:Water 10% formic sonicate for 30 minutes then sonicate
- Discard and rinse then fill with water, sonicate for a couple of minutes
- Discard and cover with methanol sonicate for a couple of minutes
- Blow the methanol off with clean air or nitrogen ensuring no solvent staining

NB – Later work has demonstrated that in some instances cleaning inside the cone is difficult using formic acid and 10% Nitric acid has been used as an alternative

Its not uncommon to get a bias in the location of build-up



Replacing the Tool Free ESI Probe

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- The stainless steel capillary should be replaced when:
  - There is a noticeable drop in signal intensity or
  - Lack of signal accompanied by an increase in backpressure, not related to the LC components of the system.
- The illustrated maintenance procedure can be found in the Xevo TQ-S micro operator's guide.

Xevo TQ-S micro: Troubleshooting

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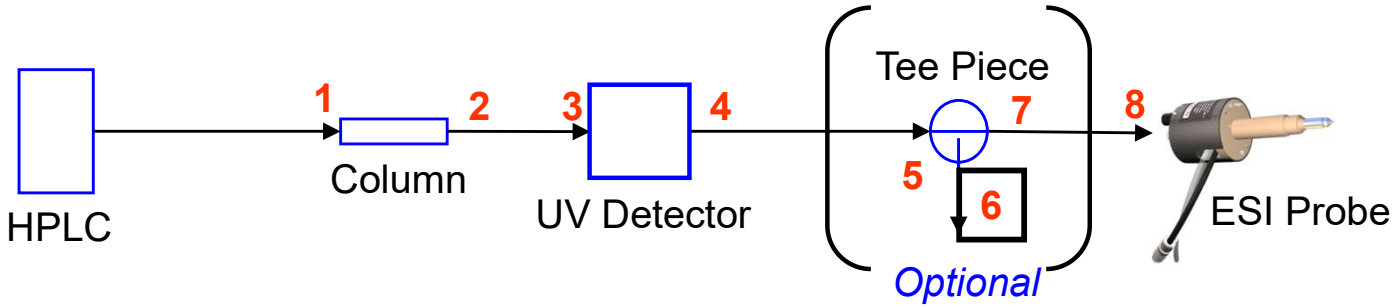
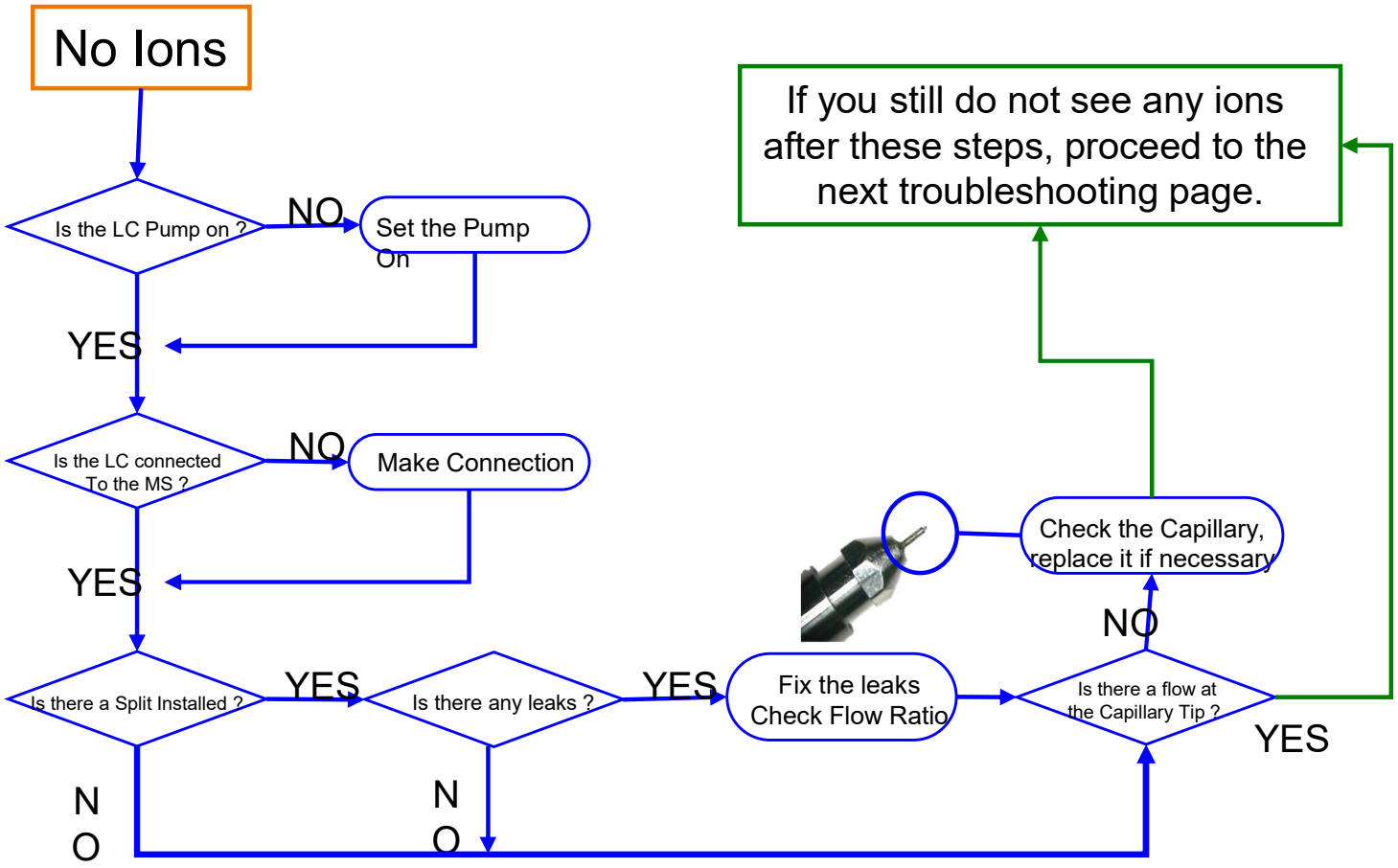
- Source Drawings
  - No signal in the Tune Page
  - Basic Troubleshooting: Fluidics and MS default parameters
  - Advanced Troubleshooting: The Diagnostic Page
- Miscellaneous Analytical Related Problems
  - Poor Sensitivity
  - Poor Resolution
  - Unstable Signal
- Miscellaneous Instrument Hardware Related Problems
  - Poor Vacuum
  - Heating Issues
  - Unstable Gas Flow and related issues (Nitrogen)

Xevo TQ-S Micro Basic Troubleshooting Guide: No Ions

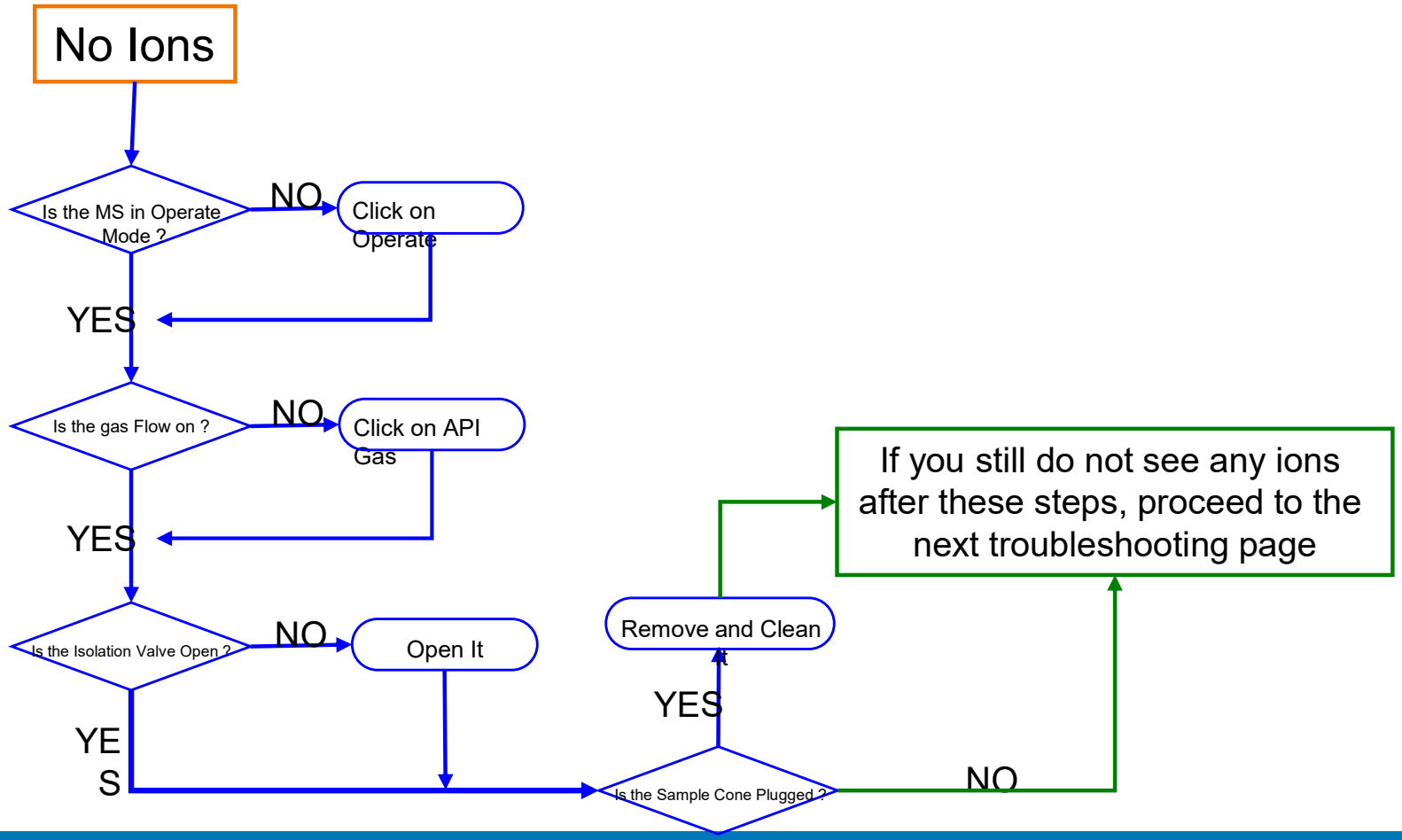


- Basic Control of the Fluidics
  - Check that the isolation valve is open on the source
  - Check all connections **(from 1 to 8)**, check the UV flow cell
  - Disconnect at a specific location to see if there is a liquid coming out
  - Change any suspect parts of tubing or/and damaged nuts
  - Check to see if liquid is coming out at the tip of the ESI capillary

Xevo TQ-S Micro Basic Troubleshooting Guide: No Ions



Xevo TQ-S Micro Basic Troubleshooting Guide: No Ions Fluidics



Miscellaneous Analytical Related Problems

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- Poor sensitivity
  - Dirty Source
    - Clean the Sample Cone and eventually the Ion Block
  - Leak located at the ESI/APCI connection (sample lost)
    - Fix the leak
  - Resolution too high

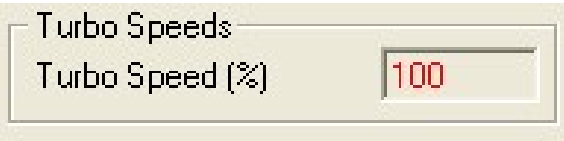
- Poor Resolution (poor peak shape)
  - Resolution settings too low and/or ion energy too high
    - Dirty optics
    - Clean source ion guide and collision cell

Instrument Hardware Related Problems

- Poor Vacuum
  - Leak between the rough pump and the MS
    - Check the vacuum hose connections at the back of the MS and at the top of the pump
  - Leak located in the source area
    - Occurs most of the time when the source has been replaced after a cleaning procedure
    - Dismantle the source and carefully check that all O-rings are well fitted
    - Leak at Ion Block Plug

Instrument Hardware Related Problems

- Poor Vacuum (cont')
  - Poor efficiency of the primary pump
    - Ballast pump for 1 hour, change the oil if necessary
  - Turbo speed too slow
    - Occurs when there is a leak located at the source or in between the MS and the rough pump



Always between 97 and 100%

Instrument Hardware Related Problems

Waters™

- Unstable Gas Flow
  - No gas flow or very low flow into the MS
    - Leak located on the tubing between the gas delivering system and the MS nitrogen inlet
    - The gas regulators utilized in the Xevo TQ-S micro require at least 7 bars/100 psi to work correctly and accurately deliver stable gas flow rates. Check the pressure on the gas cylinder.
    - If you use a gas generator, check that the air compressor delivers the requested flow to the generator
  - Excessive nitrogen consumption
    - Check for leaks the entire gas line
    - Check if the nebulization and desolvation gas inlets on the source have not been reversed

Summary

Waters™

- Routine maintenance is required to keep the instrument working properly and acquiring quality data.
- Frequency of source cleaning will be due to numerous factors, including mobile phase composition, sample type, and number of injections.
- Clean the source when proper resolution and signal intensity cannot be achieved for a known reference compound.
- Visit https://support.waters.com/ for learning more about errors seen on instrument or MassLynx
- Read Overview and Maintenance Guide https://www.waters.com/webassets/cms/support/docs/715004599v04.pdf for more detailed information on the MS system
- Comprehensive guides https://www.waters.com/nextgen/hk/en/education/primers.html