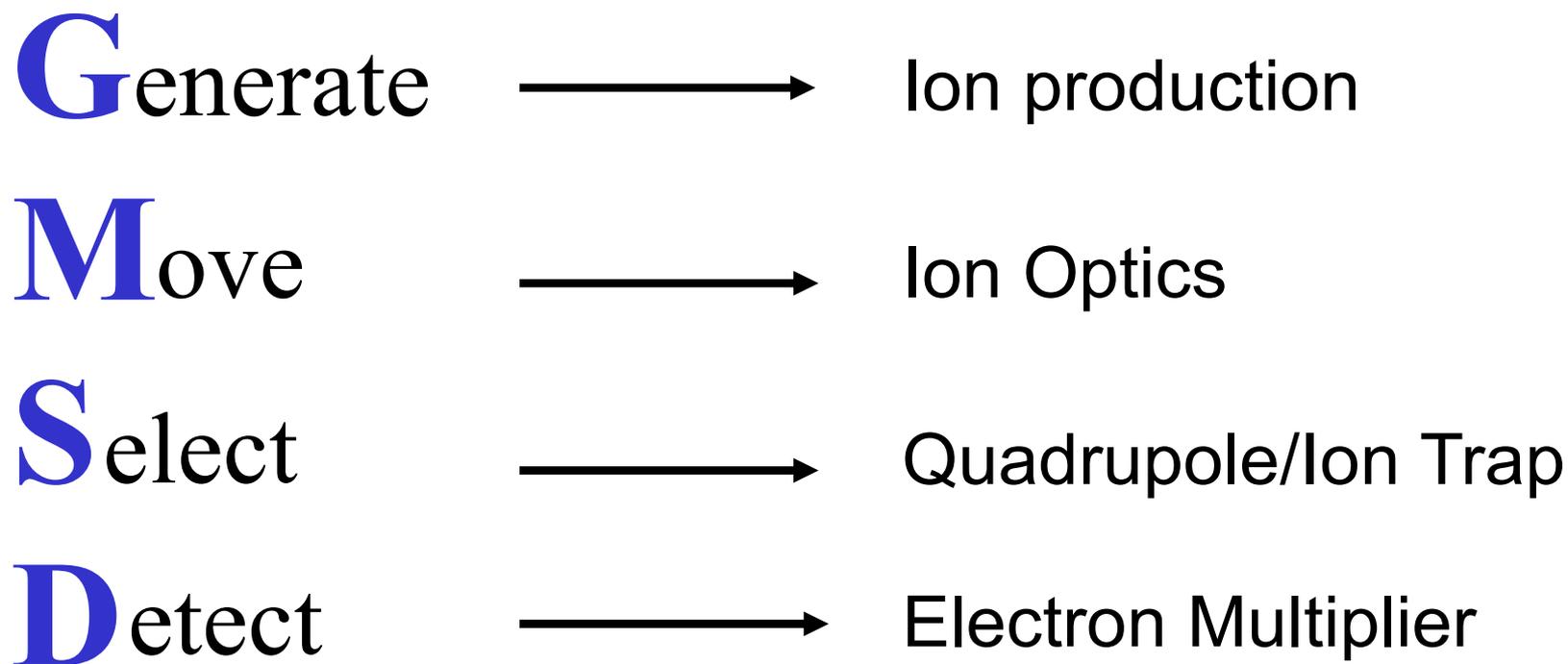
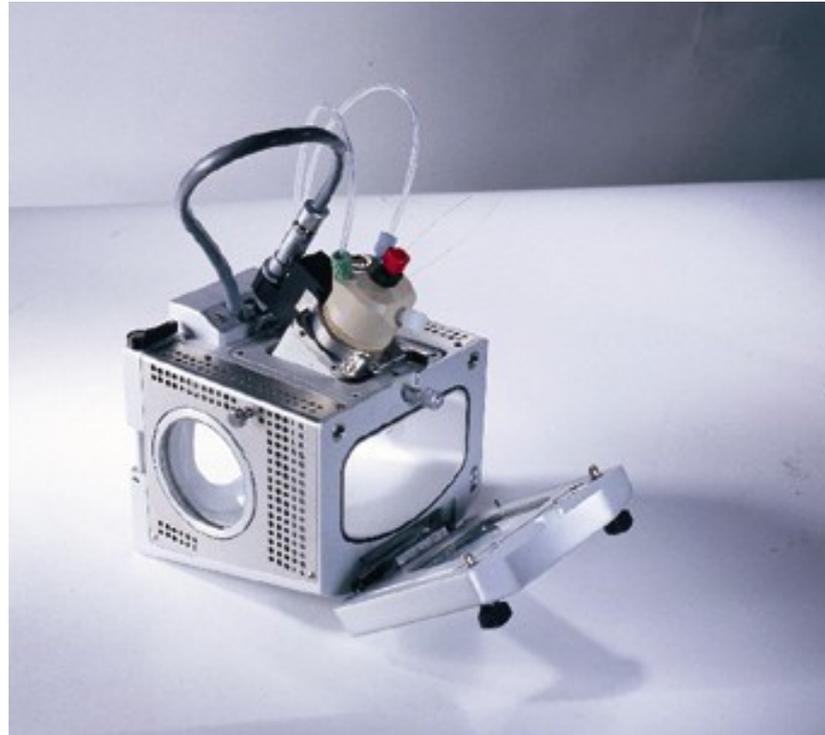


Mass Spectrometry Simplified



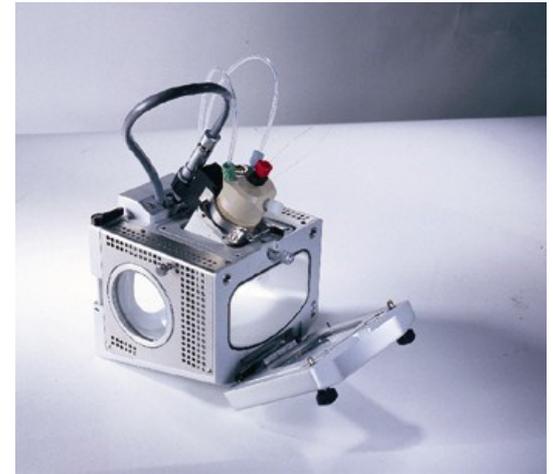
LCQ - Ion Max Source Design



LCQ - Ion Max Source Design

Source housing features:

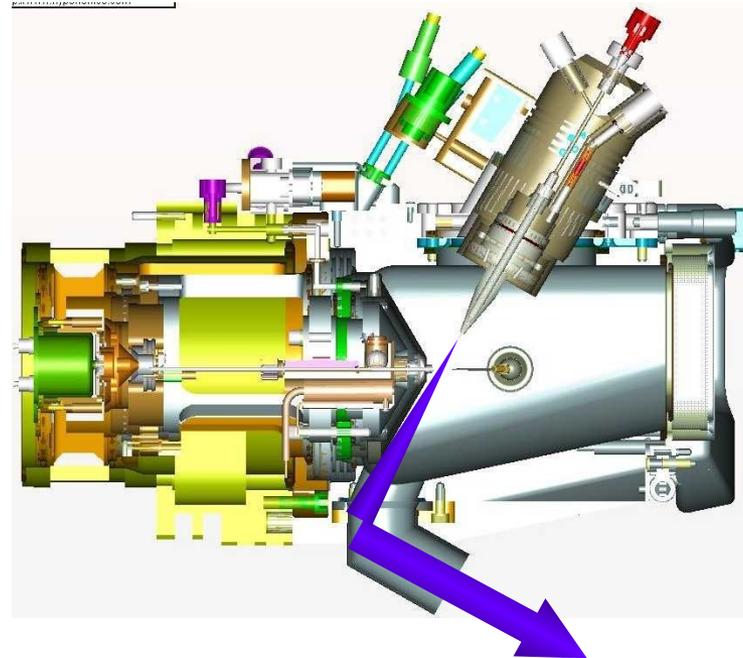
- *Plug and Play concept*
- *Fixed vertical spray angle*
- *X, Y, Z adjustments to allow flexibility for low/high flow applications*
- *Improved exhaust / drain port design*
- *Same housing for ESI and APCI probes*
- *Automatic Source Recognition*
- *APPI*
- *Unified design (Traps and Triple Quad products)*



LCQ - Ion Max Source Design

Improved Exhaust Design :

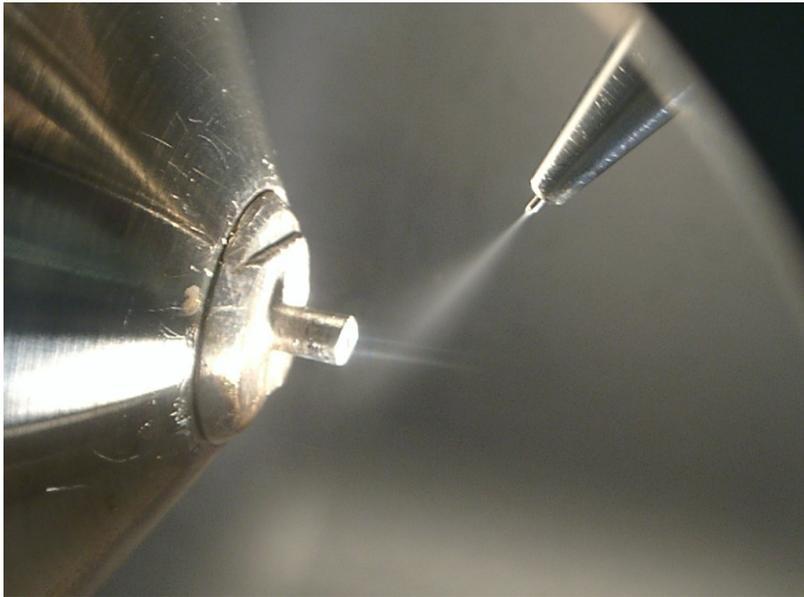
- *Prevents any accumulation of solvents in source housing*
- *Reduces chemical noise*
- *Improves **S/N!***



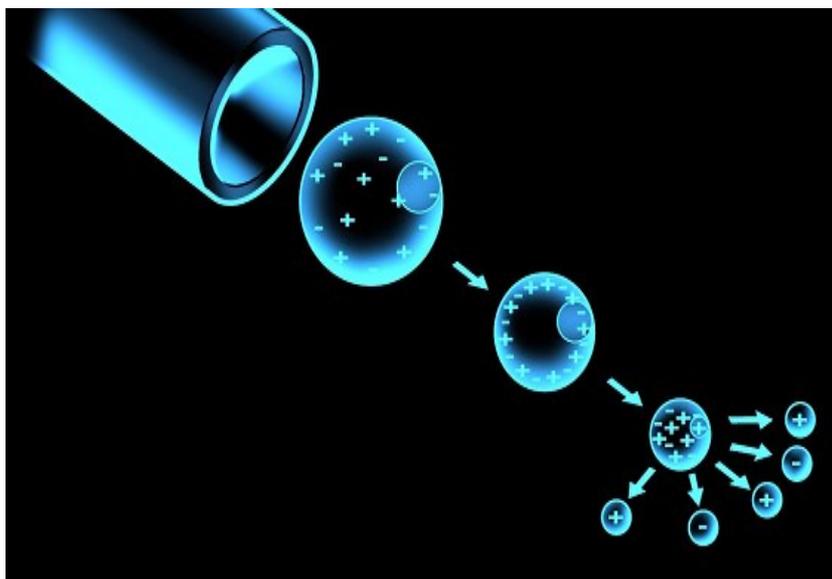
LCQ - Ion Max Source Design

ESI Probe features:

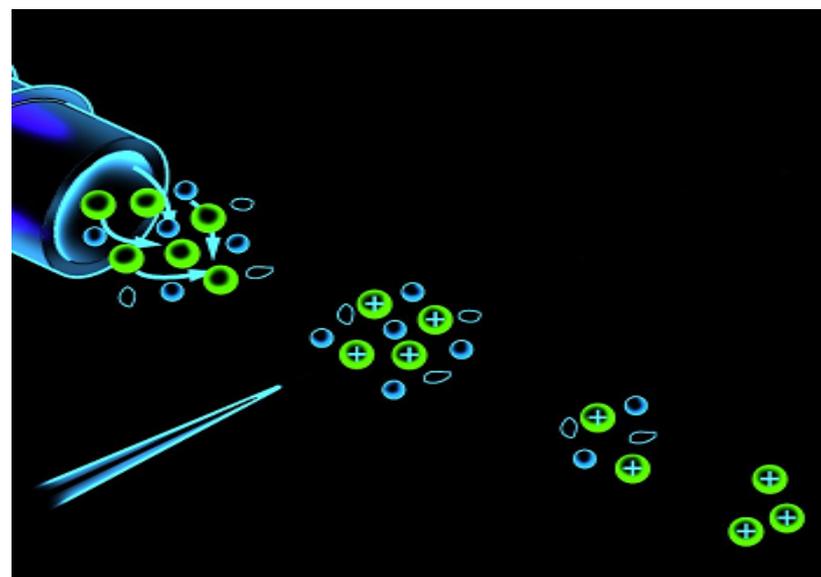
- *Fixed vertical spray angle*
- *Built in Sheath Liquid Line (accurate mass applications)*
- *X,Y,Z adjustable*



Generate Ions: Atmospheric Pressure Ionisation



**Electrospray Microspray
Nanospray Ionisation**



**Atmospheric Pressure
Chemical ionisation**

Chemistry Considerations

ESI:

- Ions formed by solution chemistry
- Good for Thermally labile analytes
- Good for Polar analytes
- Good for Large Molecules (Proteins / Peptides)

APCI:

- Ions formed by gas phase chemistry
- Good for Volatile / Thermally Stable
- Good for Non-polar analytes
- Good for Small Molecules (Steroids)

What is API

Atmospheric Pressure Ionization

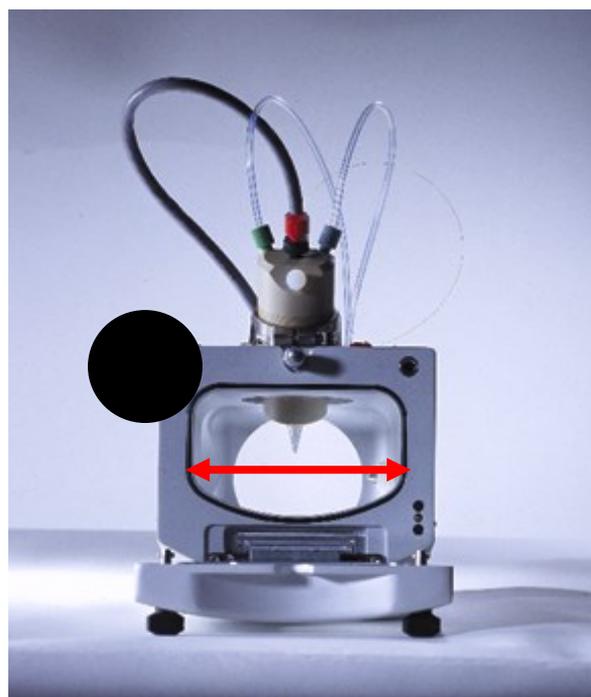
Source Types:

1. **Electrospray Ionization (ES) – Solution phase process (for the most part).**
1. **APCI (Atmospheric Pressure Chemical Ionization) - Gas-phase process.**

API Stack Purpose:

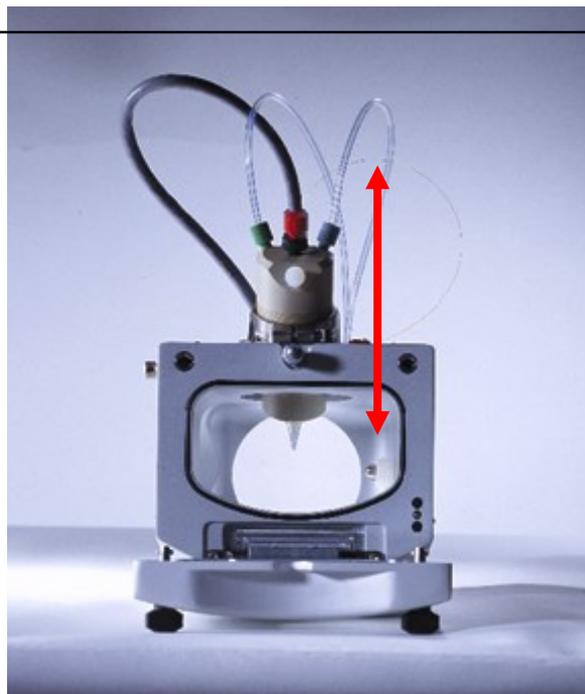
1. Ionize the analyte (APCI) or transport ion in solution to the gas phase.
2. Desolvate sample flow for introduction into mass spectrometer.
3. Pump away neutrals and opposite charged ions which would otherwise interfere with the analysis of the desired polarity.

Ion max source



Side-to-Side (+1,0,-1)

- Med impact on signal
- **Max impact on robustness**



Vertical (A,B,C,D)

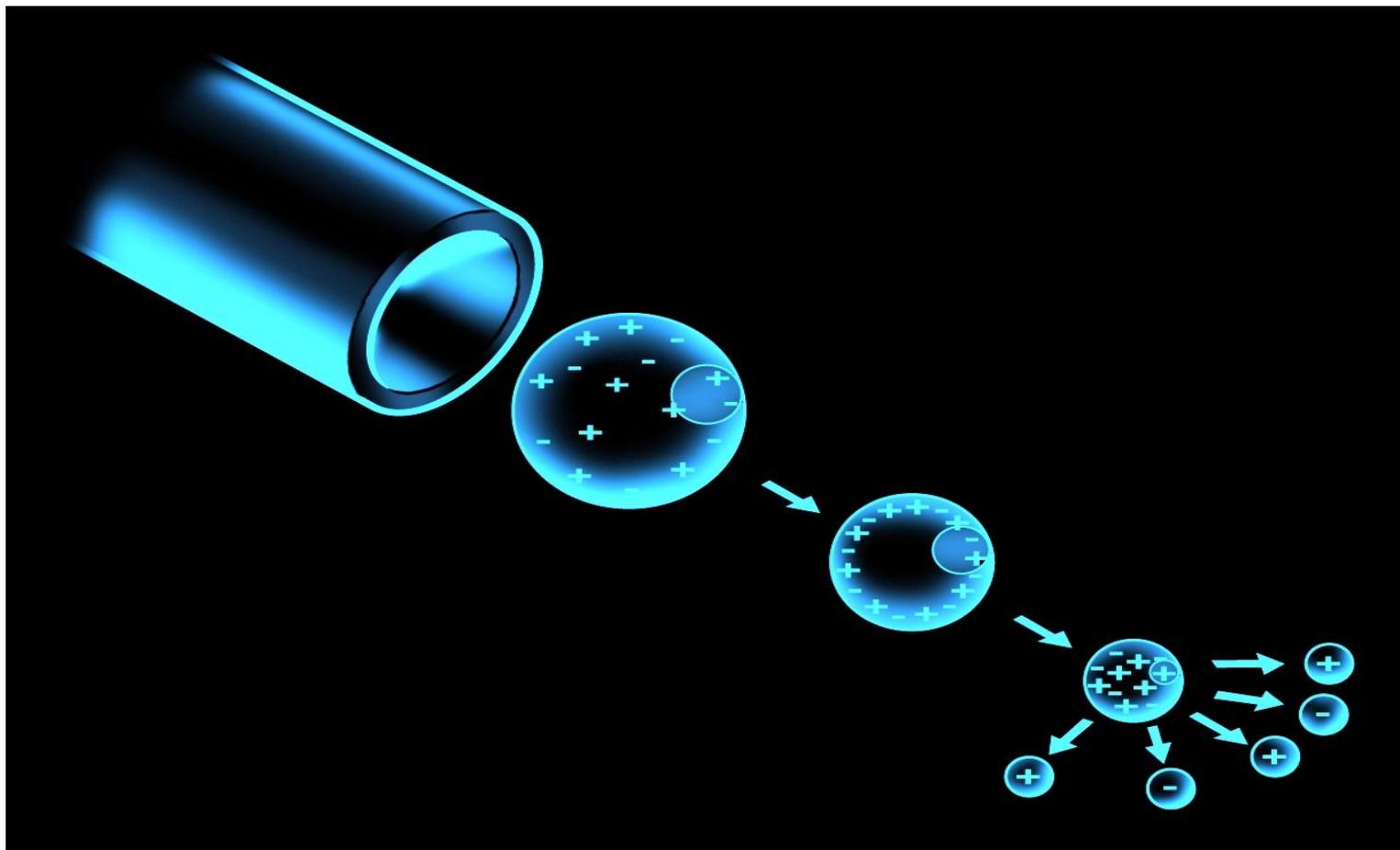
- Min impact on signal
- May impact S/N
- **Flow Rate Dependant**



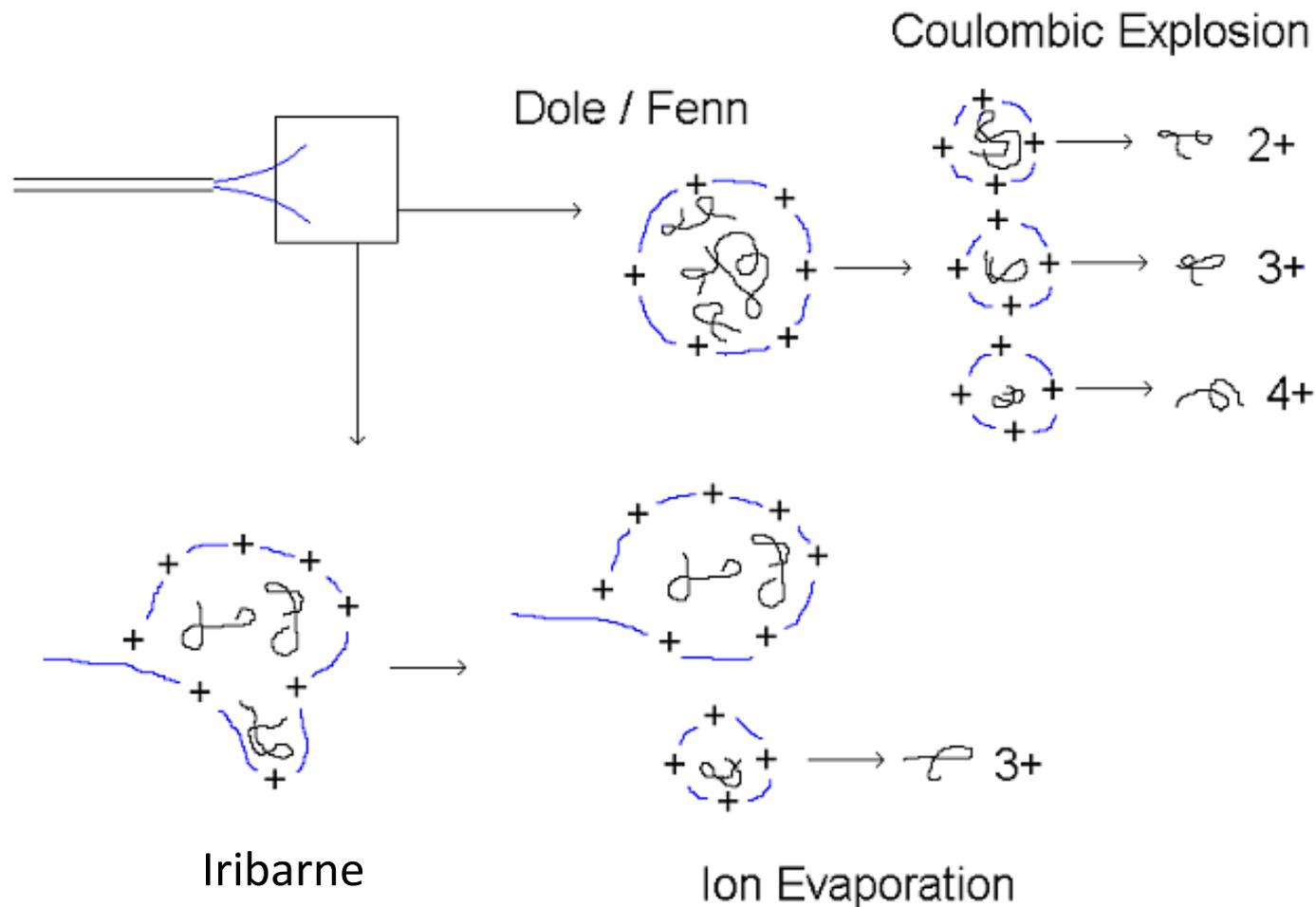
Micrometer

- **Max impact on Signal**
- Max impact on robustness

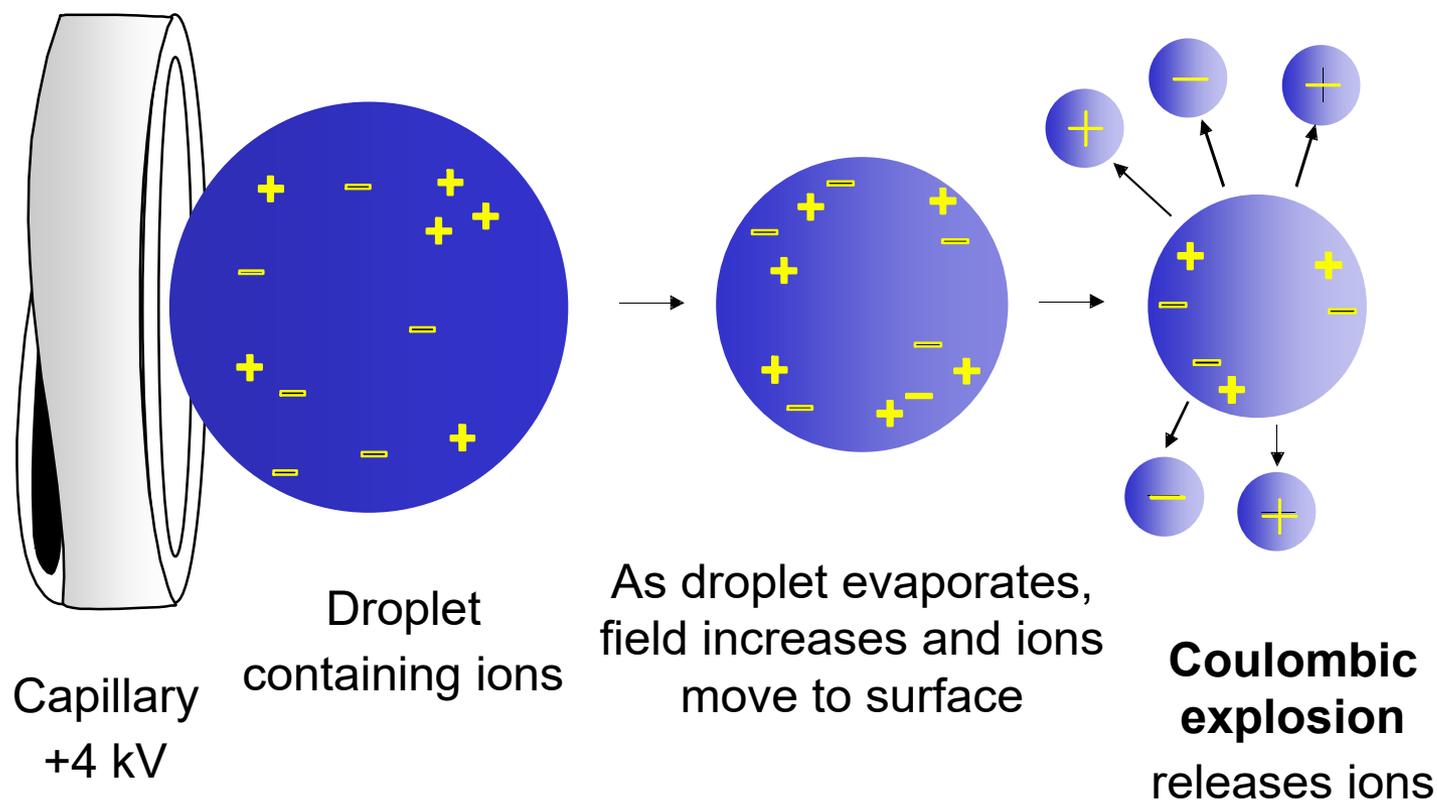
Electrospray



Alternative Theories on Electrospray Mechanisms

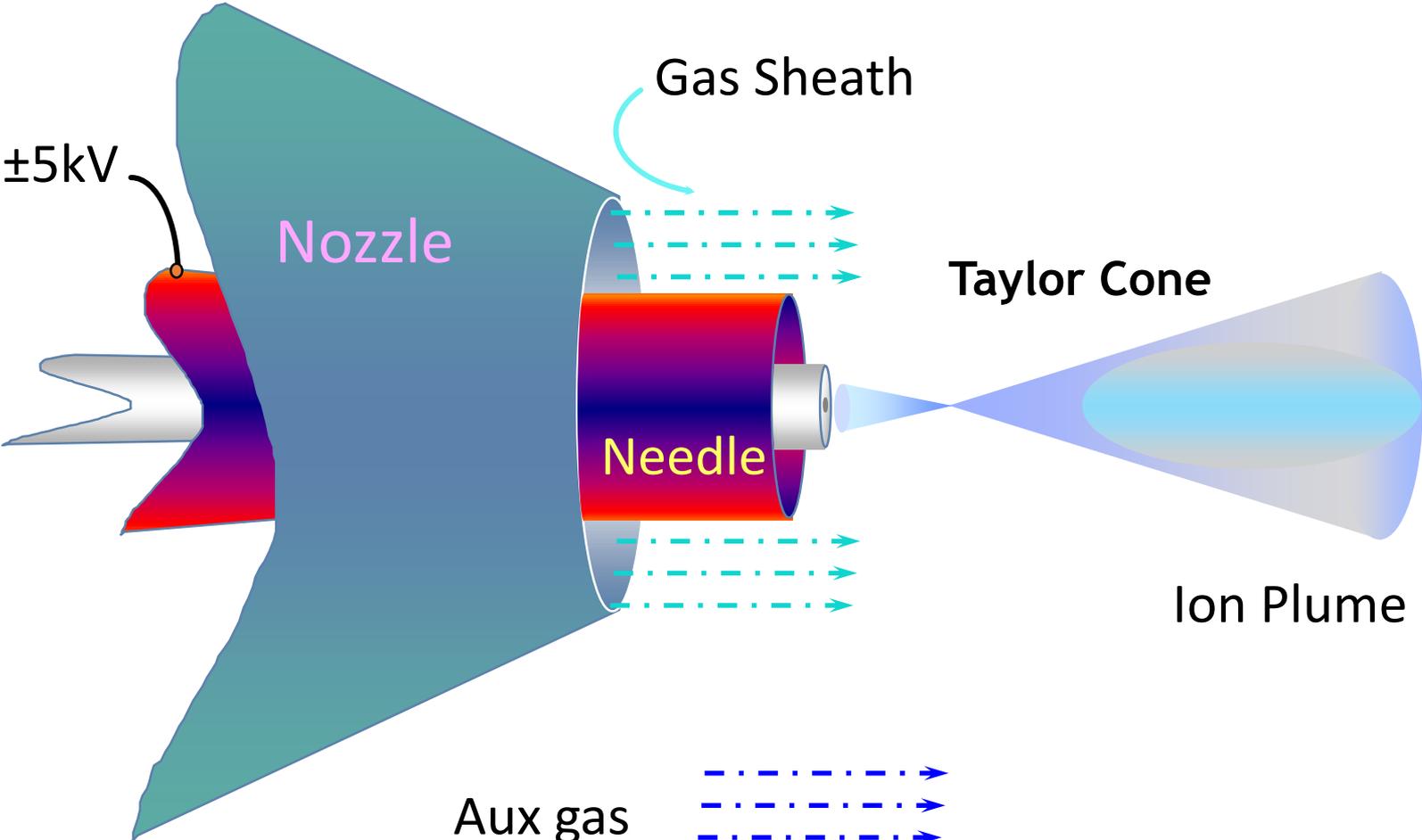


Ion Evaporation Theory



Dole, Fenn, Kebarle

Electrospray Nozzle Cross Section



Suggested source settings

Probe	Flow Rate	Micrometer	Vertical	Side-to-Side	Cap Temp	Sheath	Aux	Vap Temp
ESI	200-400uL/min	2	C-D	0	350-395C	45-60	5 to 10	
APCI	400-1000uL/min	2	C-D	0	250-300C	25-60	3 to 5	300-380C
Robustness	200-1000 ul/min	4	A-B	-1 or +1	300-395C	75	15	
Low Flow	3-6uL/min	1.5	A-B	0	300	0	0	

Column Size and Theoretical Response Increase for Analyte



$$\text{Conc}_{\text{max}} \propto \frac{D^2_{\text{Column 1}}}{D^2_{\text{Column 2}}}$$

Column Diameter

4.6 mm 3.0 mm 2.1 mm 1.0 mm 300 mm 180 mm 75 mm

Flow Rate

1.0 ml/min 0.5 ml/min 0.2 ml/min 50 μ l/min 3.0 μ l/min 700 nl/min 200 nl/min

Theoretical increase

1 2.3 5 21 235 653 3765



Multiply Charged Ions- Mass Assignments

● Single Charge

- apparent mass = $(M+H)/1$

● Double Charge

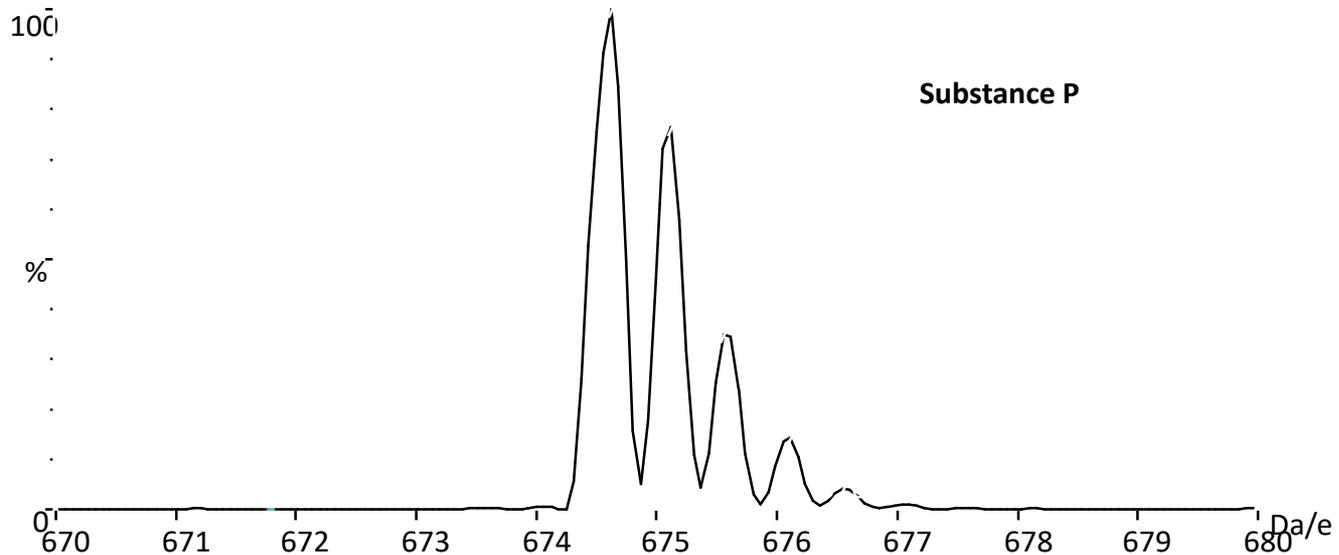
- apparent mass = $(M+2H)/2$

● Triple Charge

- apparent mass = $(M+3H)/3$

● N charges

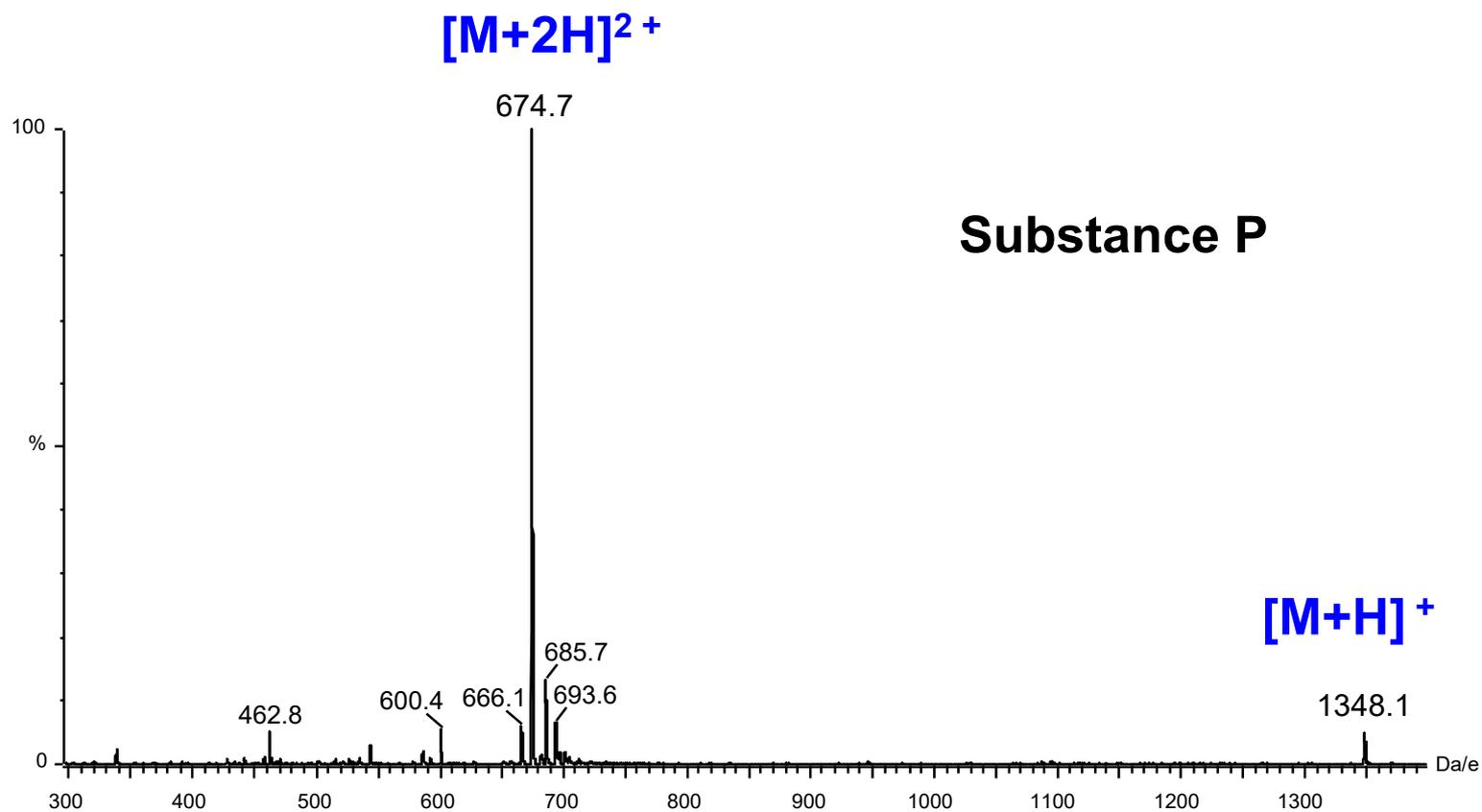
- apparent mass = $(M+nH)/n$



● The isotopes of doubly charged ions are separated by 0.5 amu

● The isotopes of triply charged ions are separated by 0.33 amu

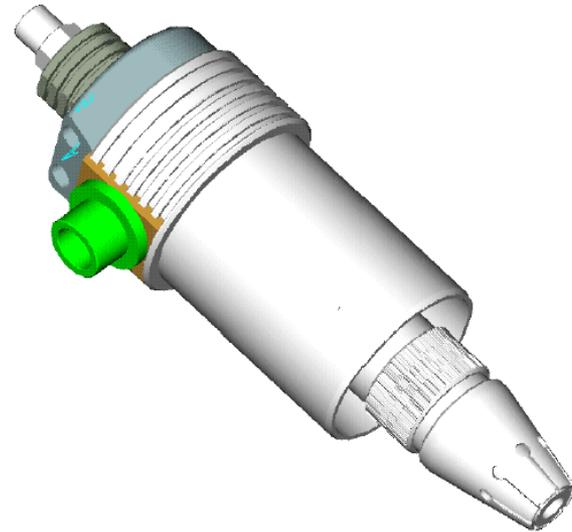
Multiply Charged Ions



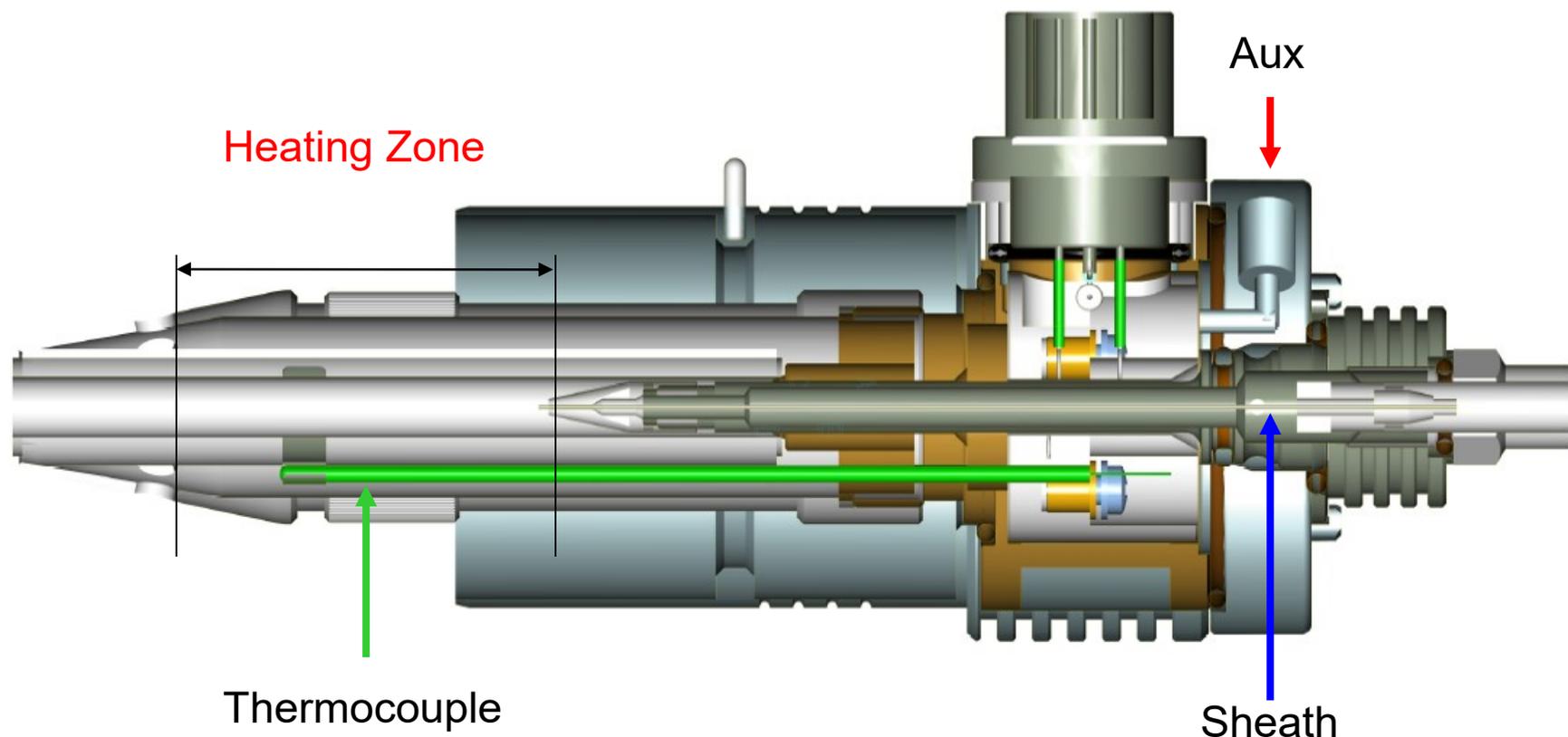
LCQ - Ion Max Source Design

APCI Probe features :

- *Removable sprayer*
- *Ceramic heater*
- *External thermocouple*
- *Self- cleaning*
- *No plastics in source housing*
- *Easy change nozzle assembly*
- *X,Y,Z adjustable*



LCQ - Ion Max Source Design



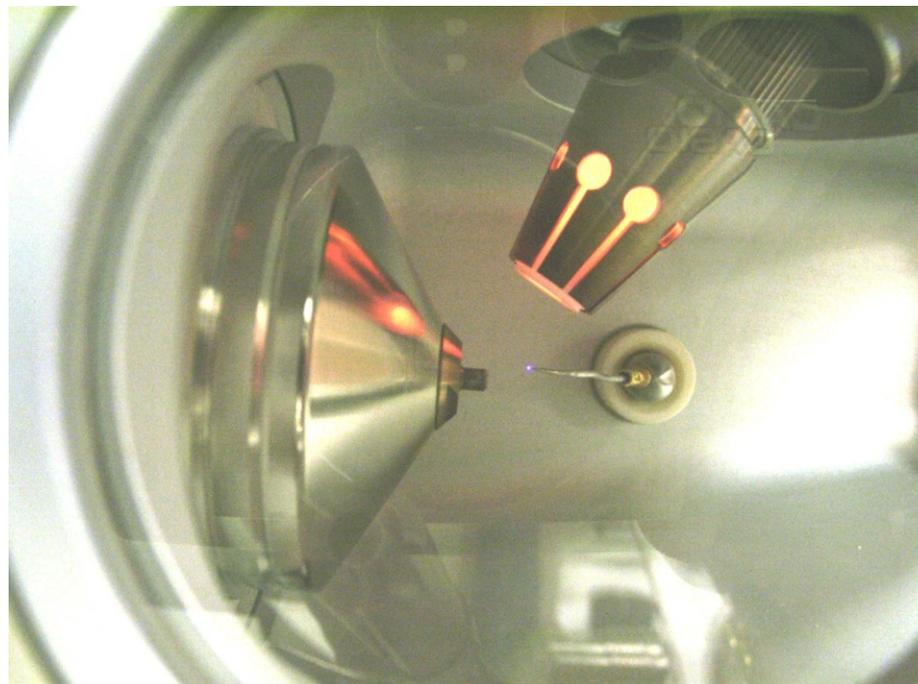
LCQ - Ion Max Source Design

Why Use Ceramic Heater Technology ?

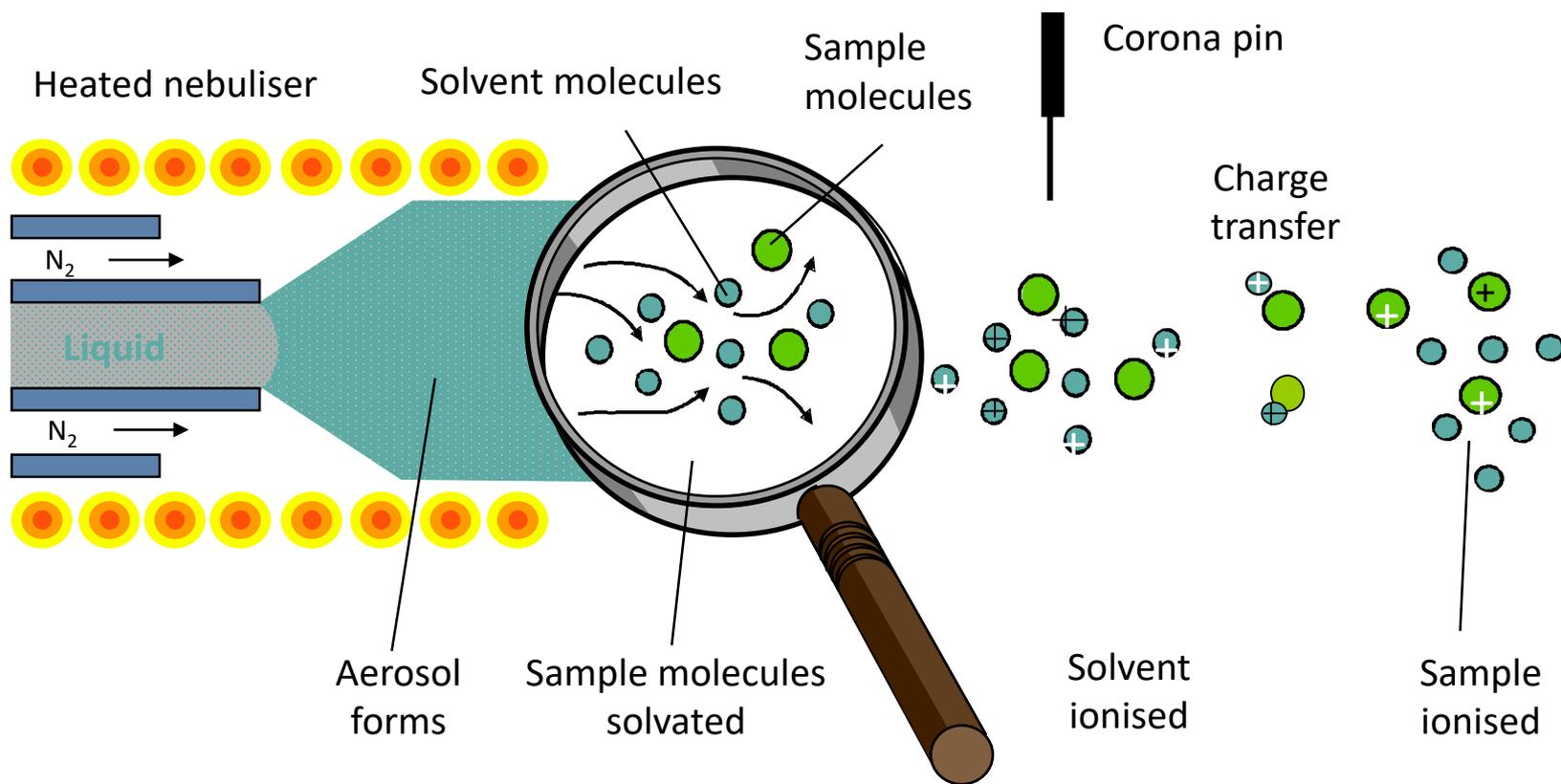
- **Direct heating**
 - *nozzle sprays directly into ceramic*
 - *thermocouple is attached externally*
 - *no plasticizer from wire leads*
 - *heating zones are controlled*
- **Rapid Heating**
- **Self-Cleaning**
 - *internal surfaces can exceed 1000 C*



LCQ - Ion Max Source Design



Mechanism of ion generation in APCI



APCI Mechanism

Primary ion formation:



Secondary ion formation:



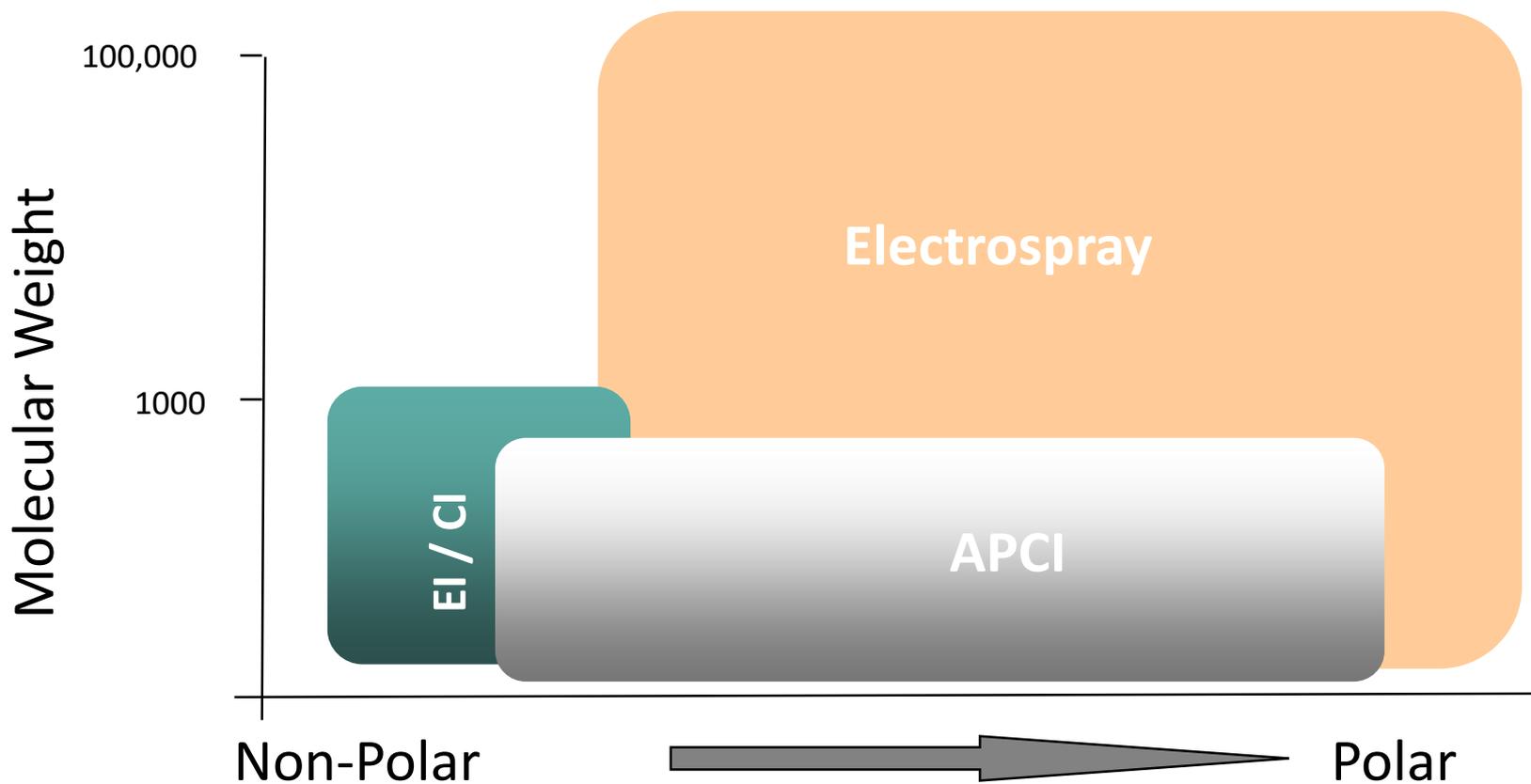
Positive analyte ion formation:



Negative analyte ion formation:



Which Ionisation Mode ?



LC Flow Rates

ESI:

- 3 $\mu\text{L}/\text{min}$ - 1mL/minute
- Optimal Flow Rate: 200 $\mu\text{L}/\text{min}$
- Generally, higher flow rates require higher heated ion tube temperatures and higher sheath and auxiliary gas flow rates.

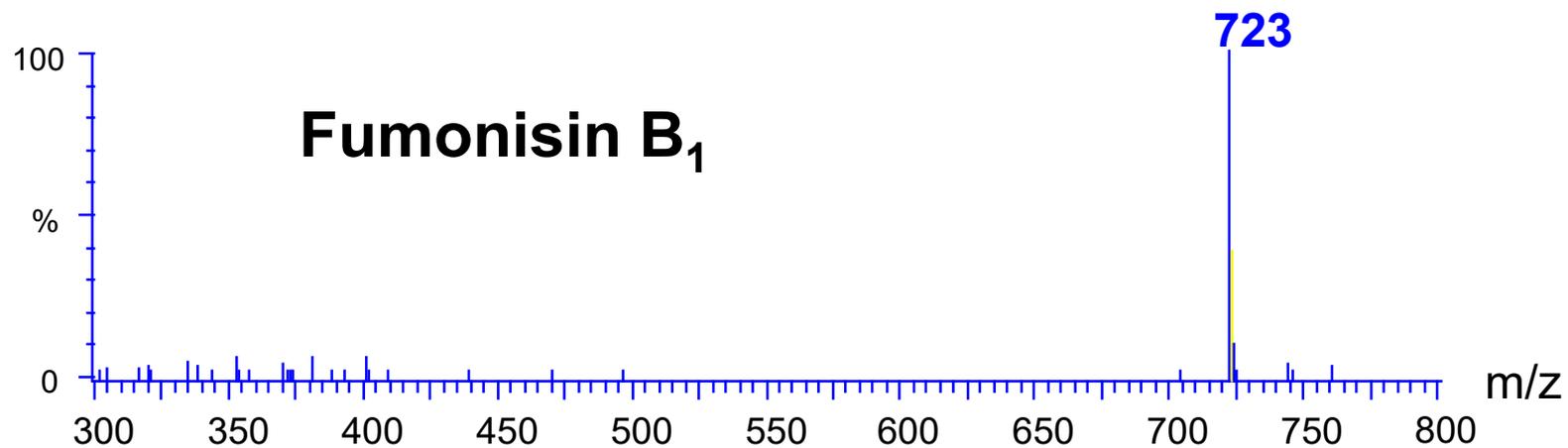
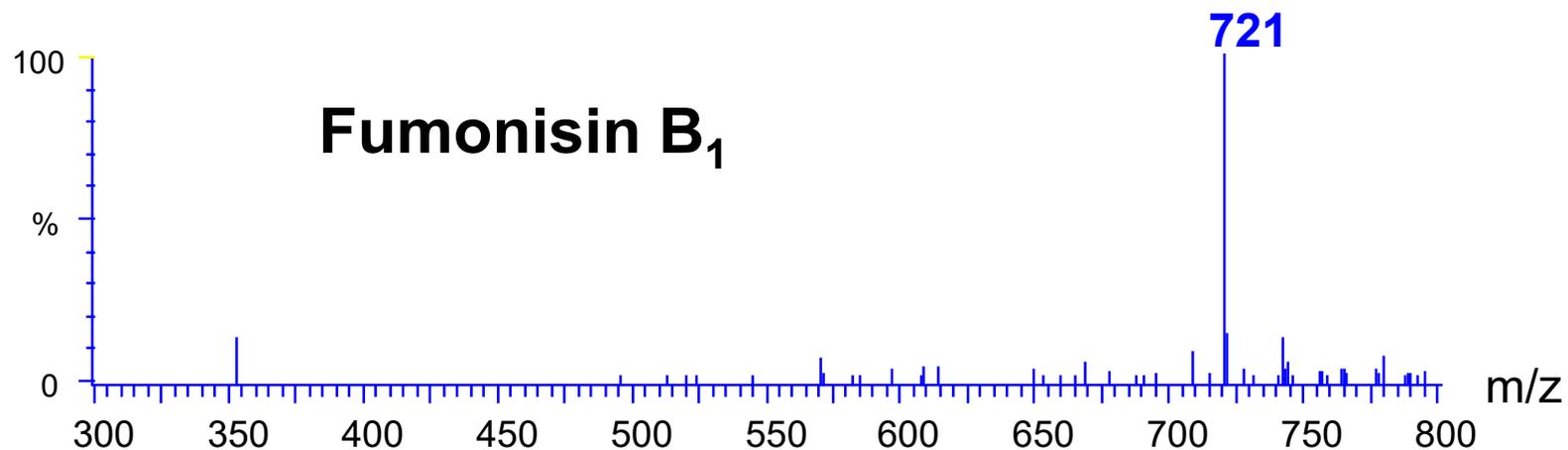
APCI:

- 200 $\mu\text{L}/\text{min}$ - 2mL/minute.
- Optimal Flow Rate: 400-500 $\mu\text{L}/\text{min}$
- Generally, higher flow rates require auxiliary gas, higher vaporizer temperatures but do not require significant changes to ion tube temperatures.

Positive or Negative Ionisation ?



Polarity switching on alternate scans



LC/MS Additives and Buffers Summary

Acetic Acid (0.1 - 1%)

Formic Acid (0.1 - 1%)

Ammonium Hydroxide

Ammonia Solutions

Trichloroacetic Acid (< 0.1% v/v)

Trifluoroacetic Acid (< 0.1% v/v)

Triethylamine (< 0.1% v/v)

Trimethylamine (< 0.1% v/v)

Ammonium Acetate (5 - 50 mM)

Ammonium Formate (5 - 50mM)

Proton Donors

Proton Acceptors

Chromatographic
Separation

Negative ion
formation

Buffers

Common LC/MS Solvents

Methanol

Acetonitrile

Water

Isopropanol

Dichloromethane

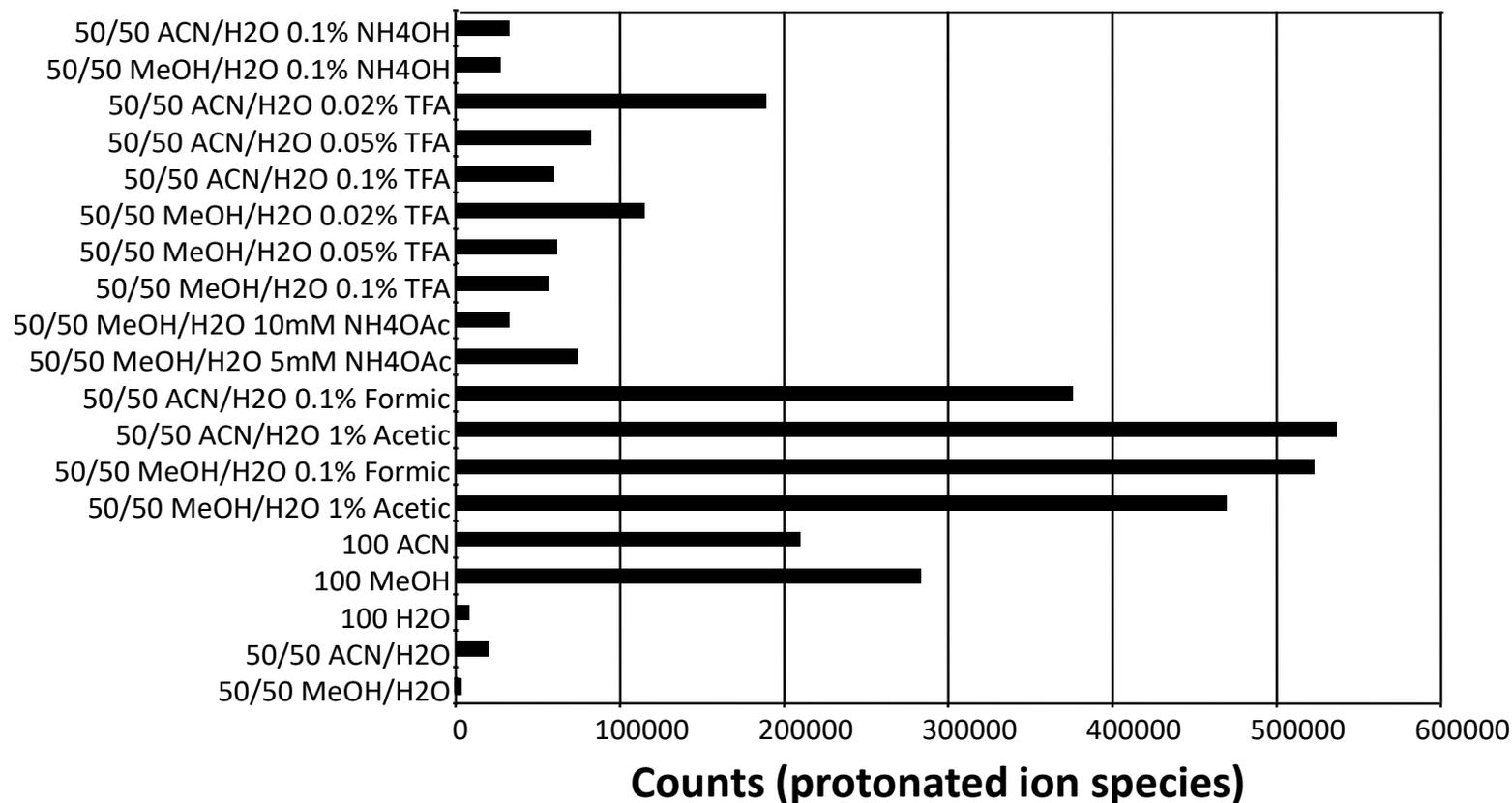
Chloroform

Hexane

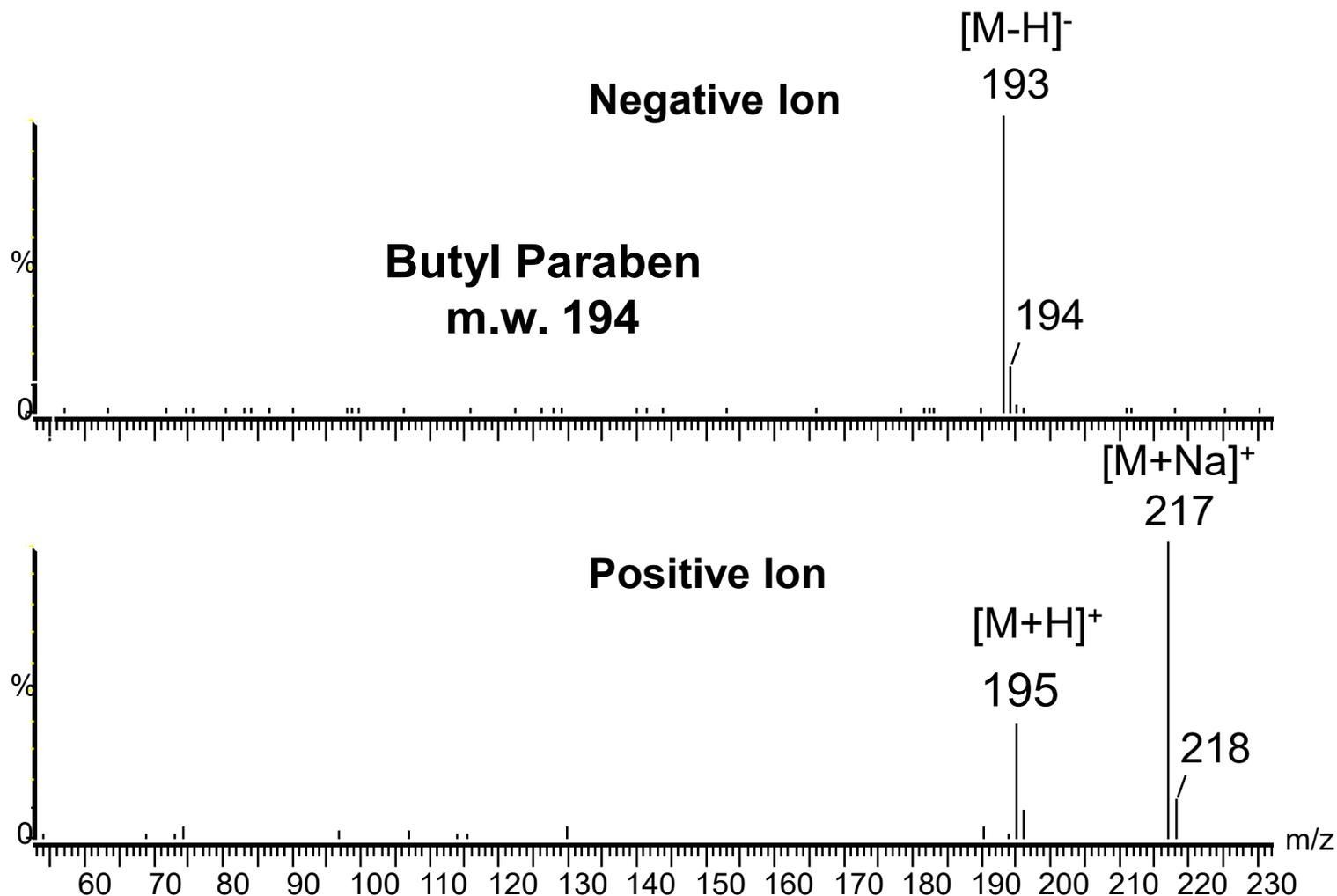
THF

General Effects of Solvents and Additives on Leucine in Positive Ionisation Electrospray

Solvent system



Polarity switching - Adduct Formation



LC Additives

➤ Acids

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

➤ Bases

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

➤ Surfactants (surface active agents)

- Detergents and other surface active agents may suppress ionization

➤ Trifluoroacetic Acid (TFA)

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

➤ Triethylamine/Trimethylamine (TEA/TMA)

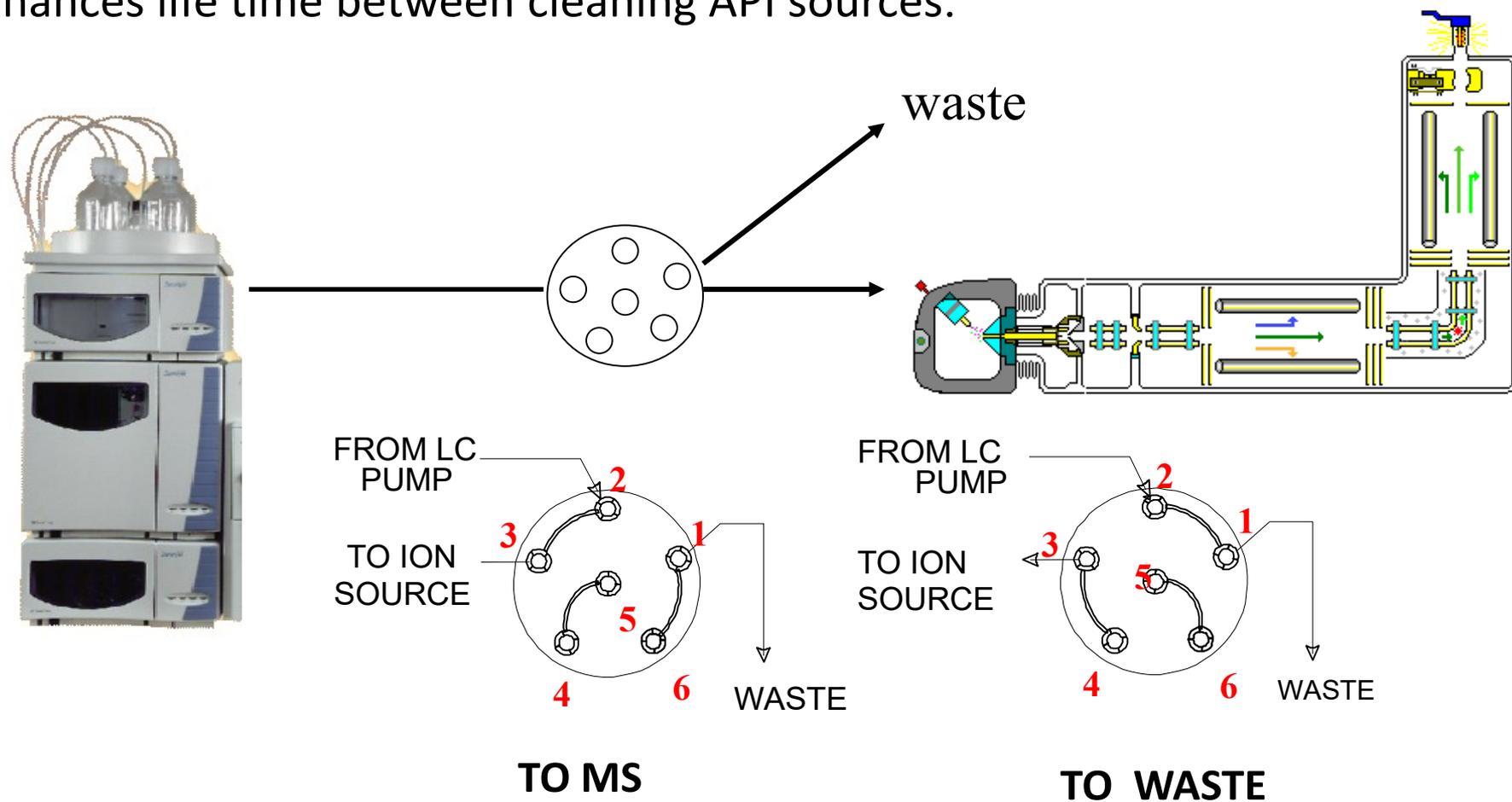
- May enhance deprotonation for Negative Ion Formation

Buffers pH

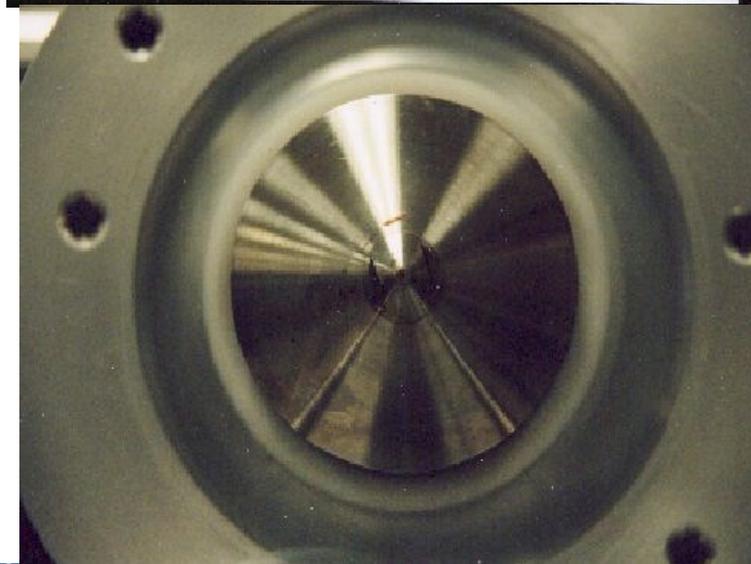
- Avoid using non-volatile HPLC additives such as:
 - Alkali Metal Phosphates
 - Borates
 - Citrates
- Keep Buffer concentrations below 20 mM using volatile salts such as ammonium acetate, ammonium formate
- When using buffers, more frequent cleaning of the heated capillary and API stack will be necessary

Use Divert Valve for Improved Ruggedness

Prevents salt and protein build up from metabolism studies.
Enhances life time between cleaning API sources.

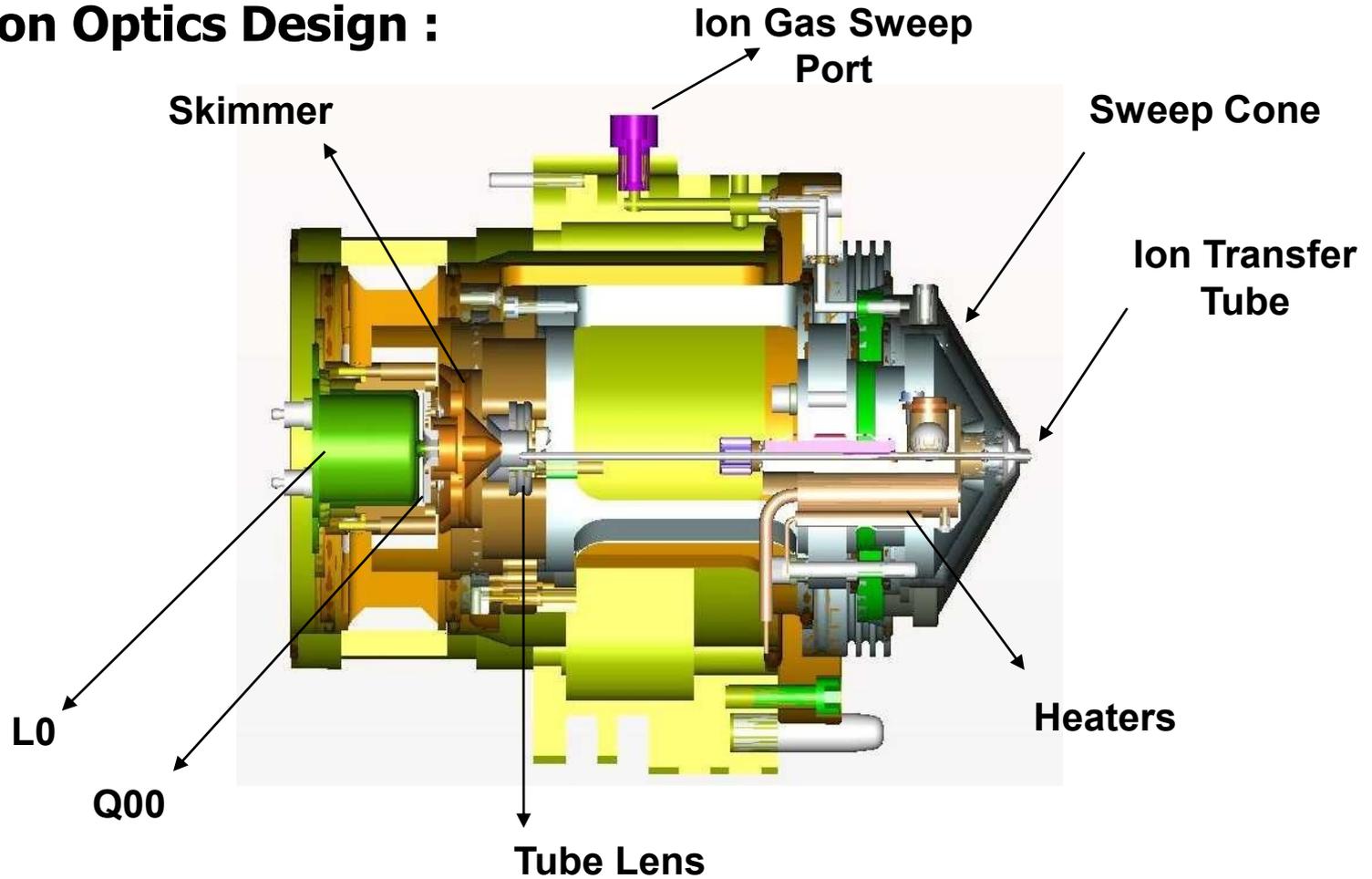


Sweep Cone for Advantage/XP: Ruggedness



LCQ - Ion Optics Design

Ion Optics Design :



LCQ - Ion Optics Design

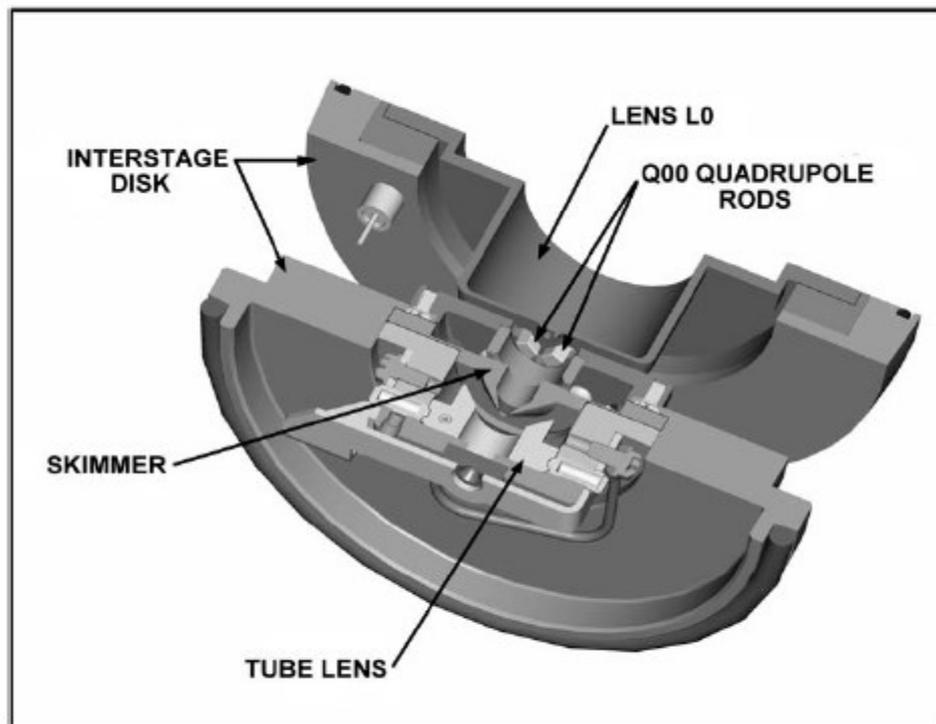


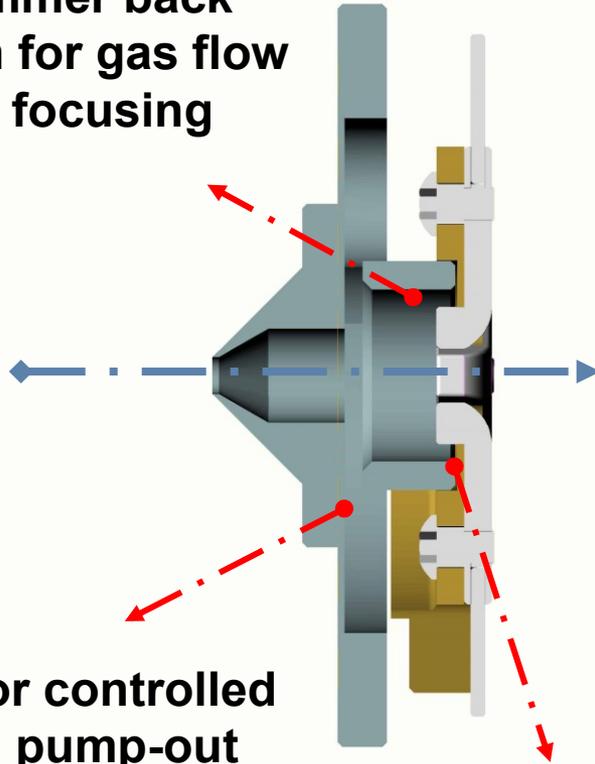
Figure 2-16. Cross sectional view of the Q00 ion guide

LCQ - Ion Optics Design

Skimmer / Q00 Ion Optics assembly :

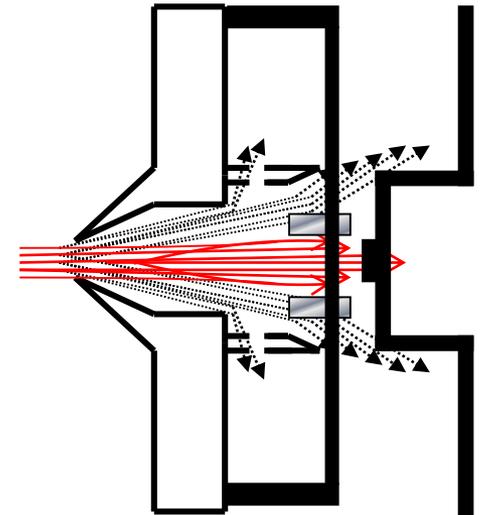


Skimmer back
design for gas flow
ion focusing

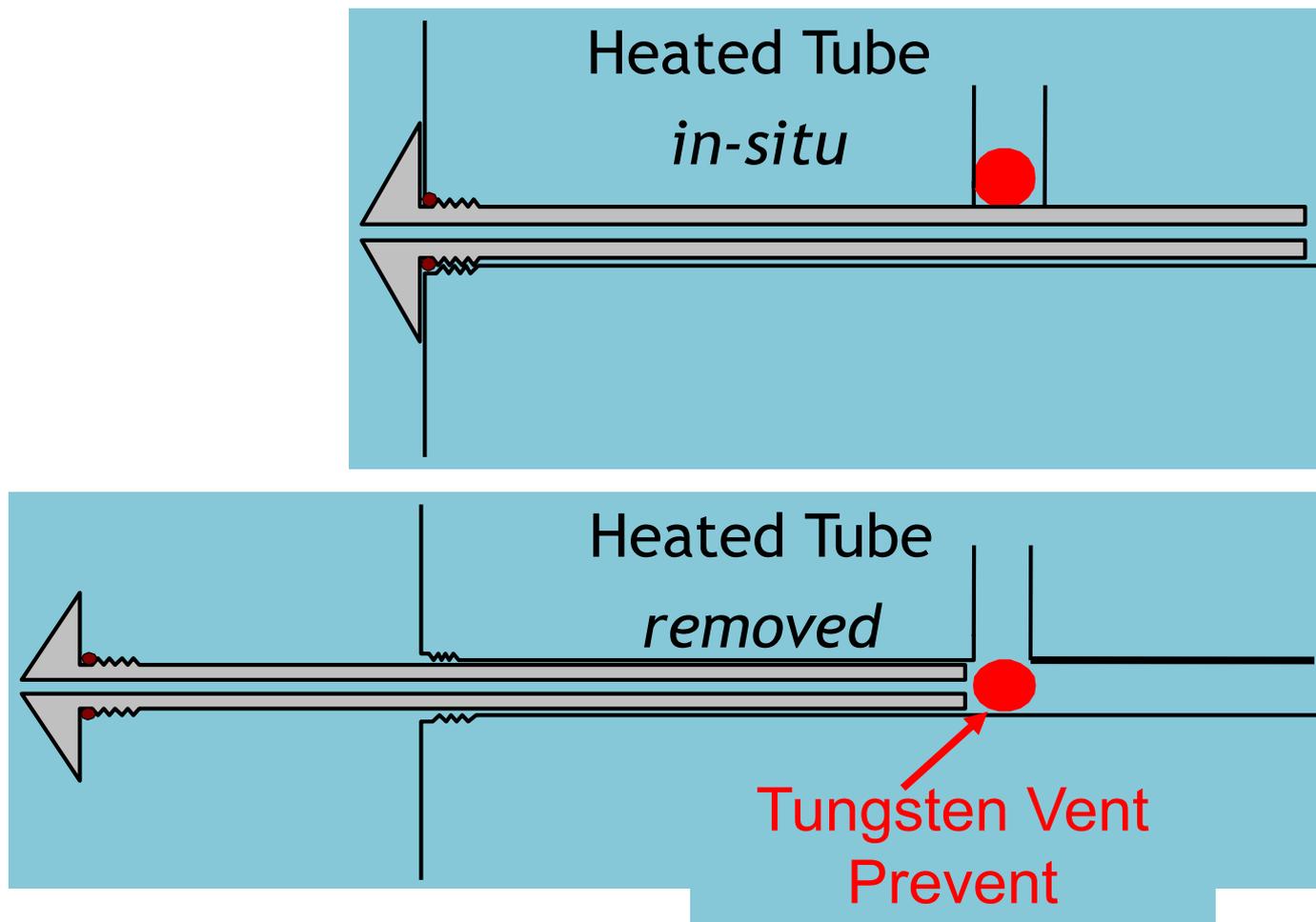


Slots for controlled
radial pump-out

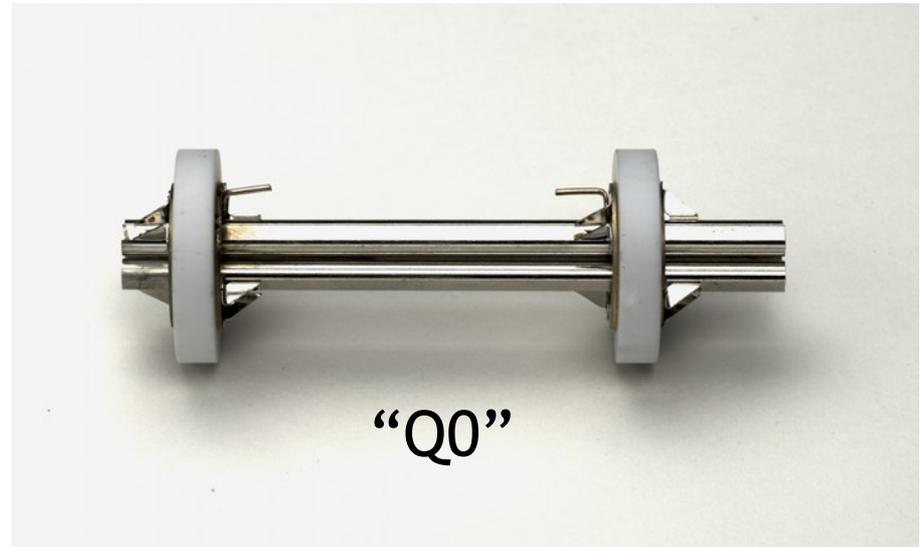
Q00 rods inserted into skimmer



Vent Prevent Mechanism on Quantum and XP

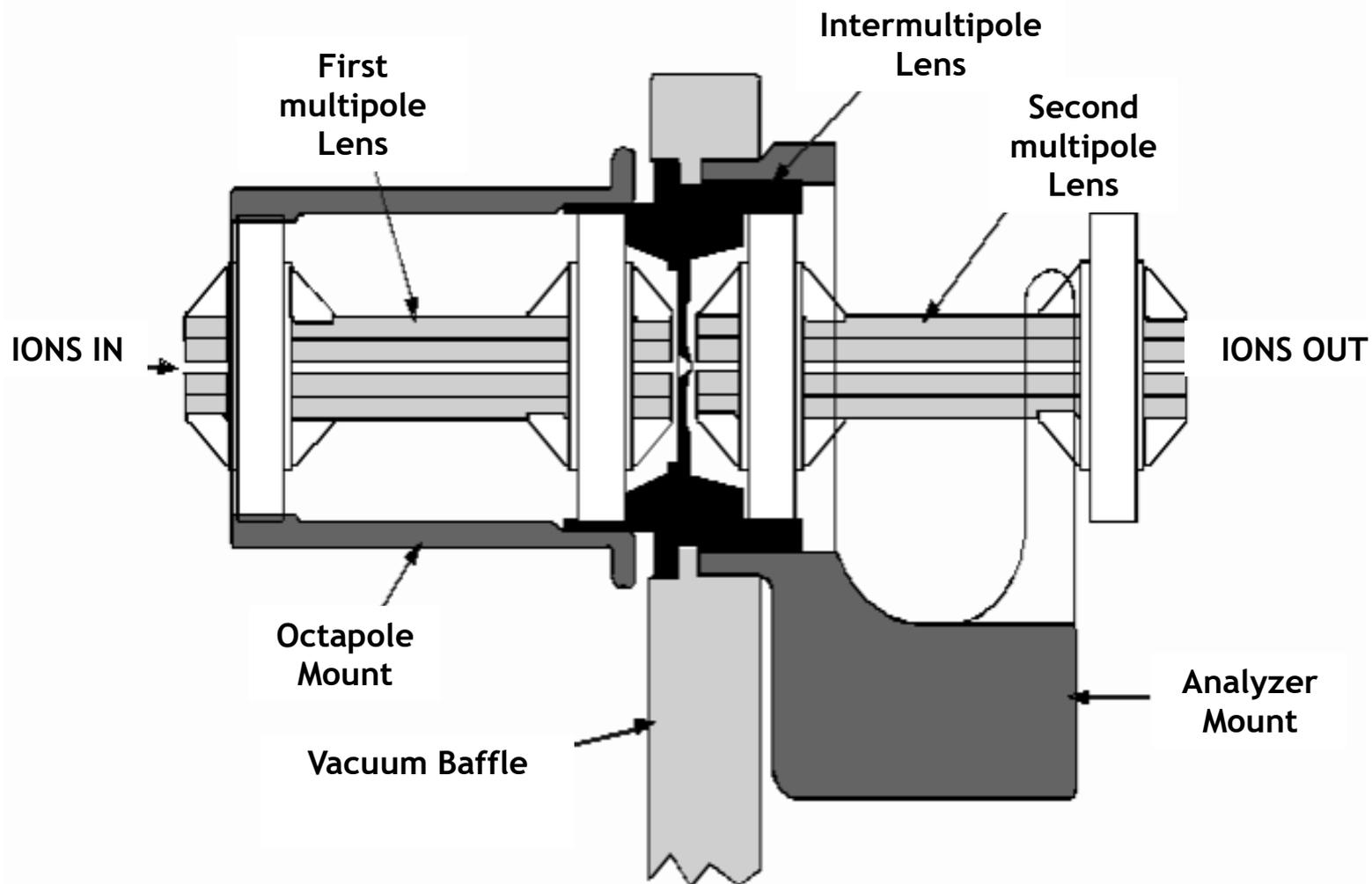


New Tandem Square Quad Ion Guides



Superior ion beam characteristics compared to conventional multipole ion guides improve sensitivity.

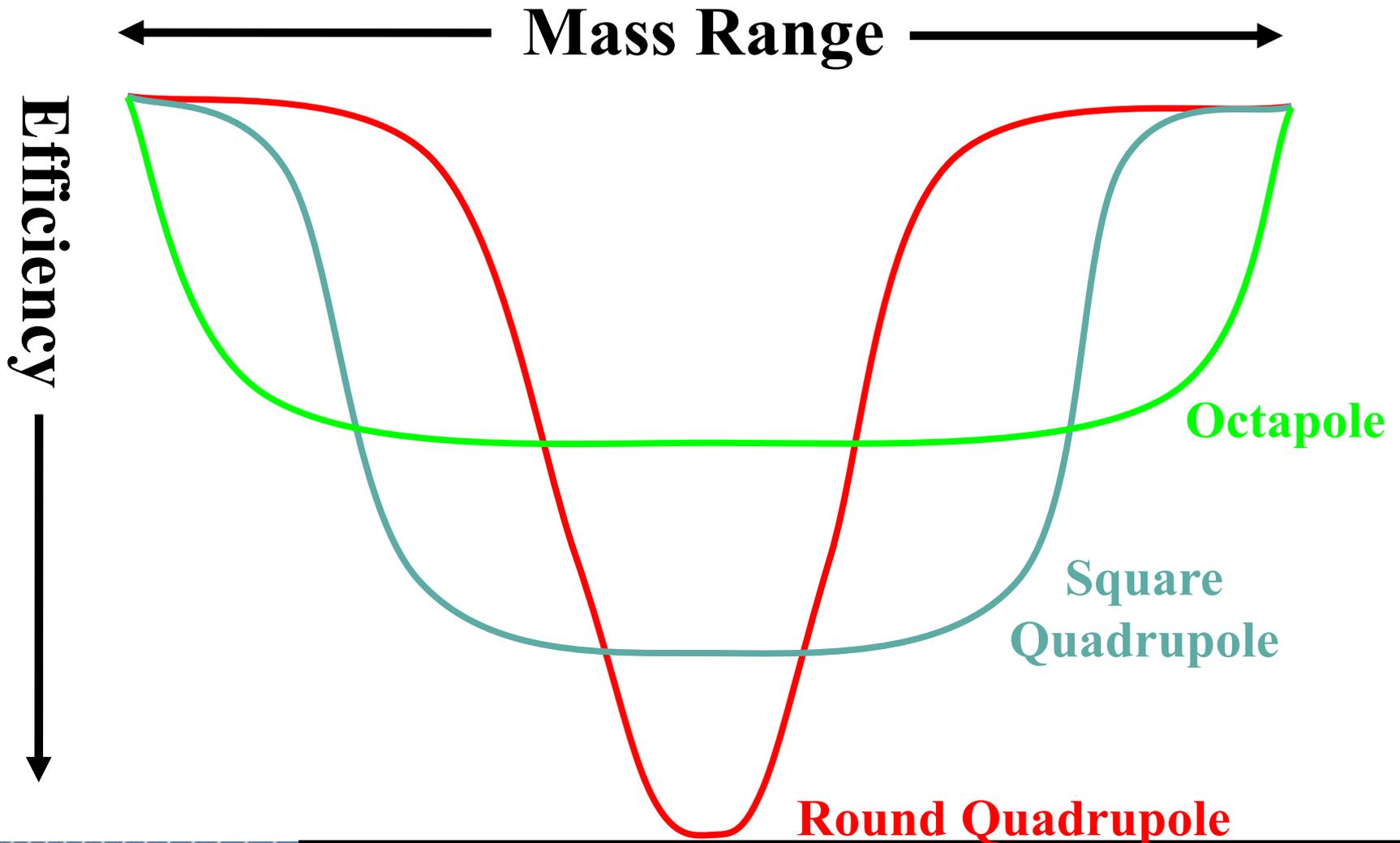
Ion Optics in the Deca



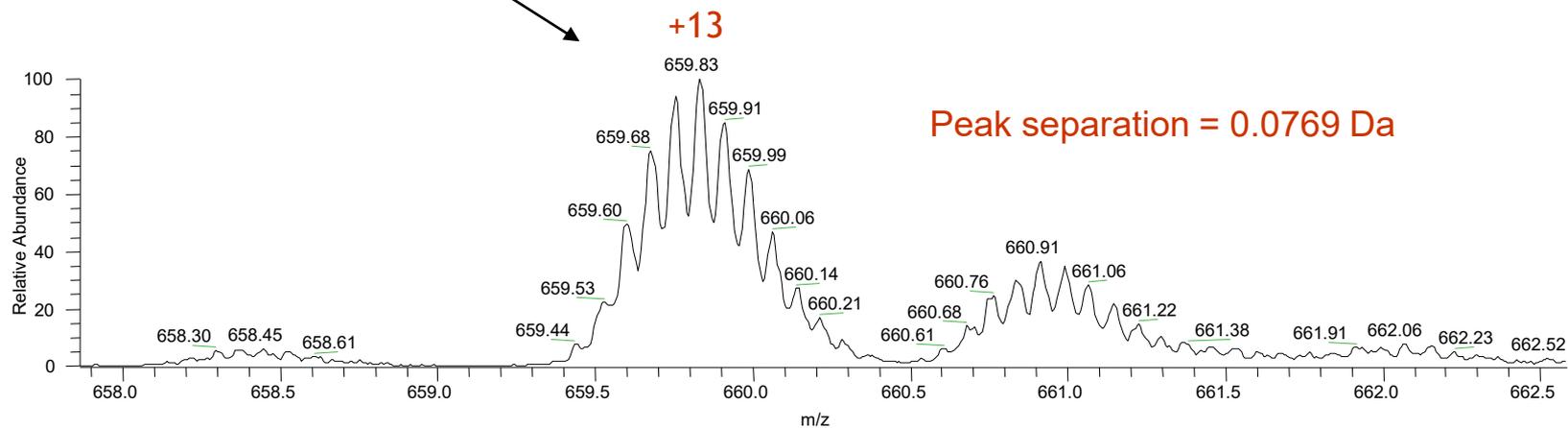
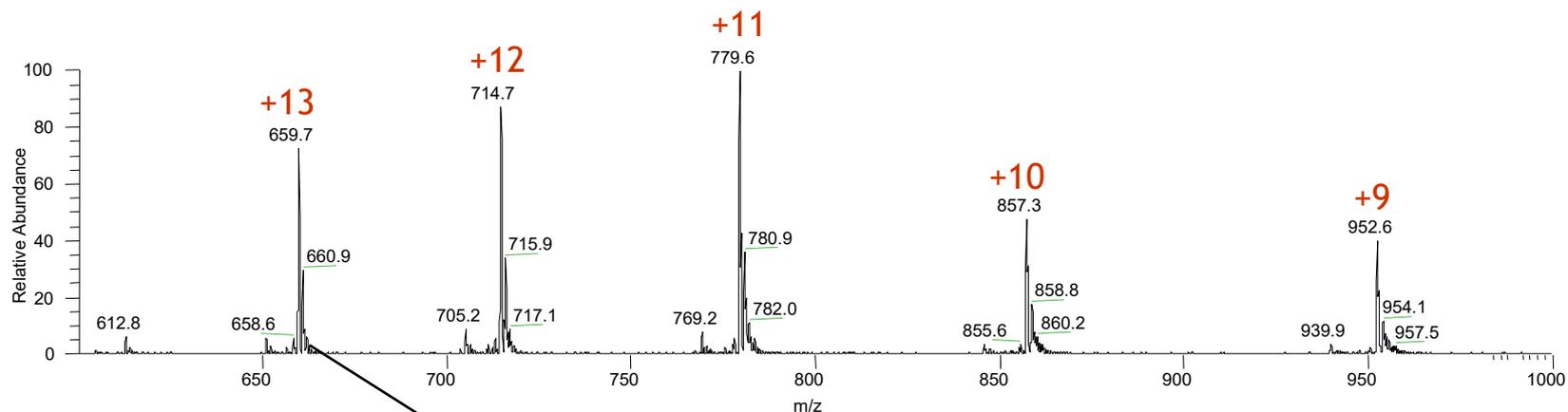
Why Use Square Quadrupoles as Ion Guides ?

- Improved transmission
- Improved ion injection into next optical element
- Improved ion acceptance
- Reduced nodding

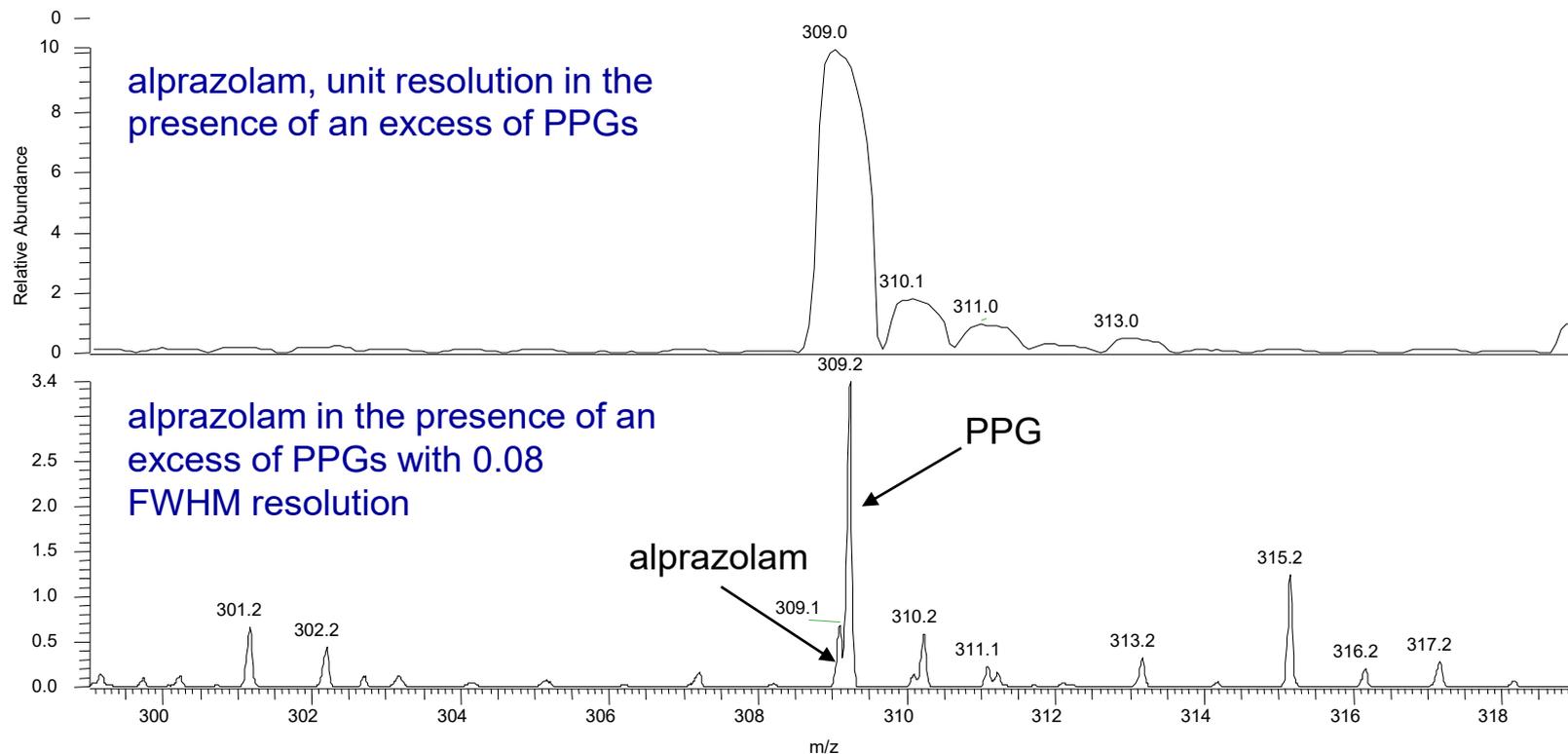
Multipole Potential Wells



Resolution For Charge State Determination



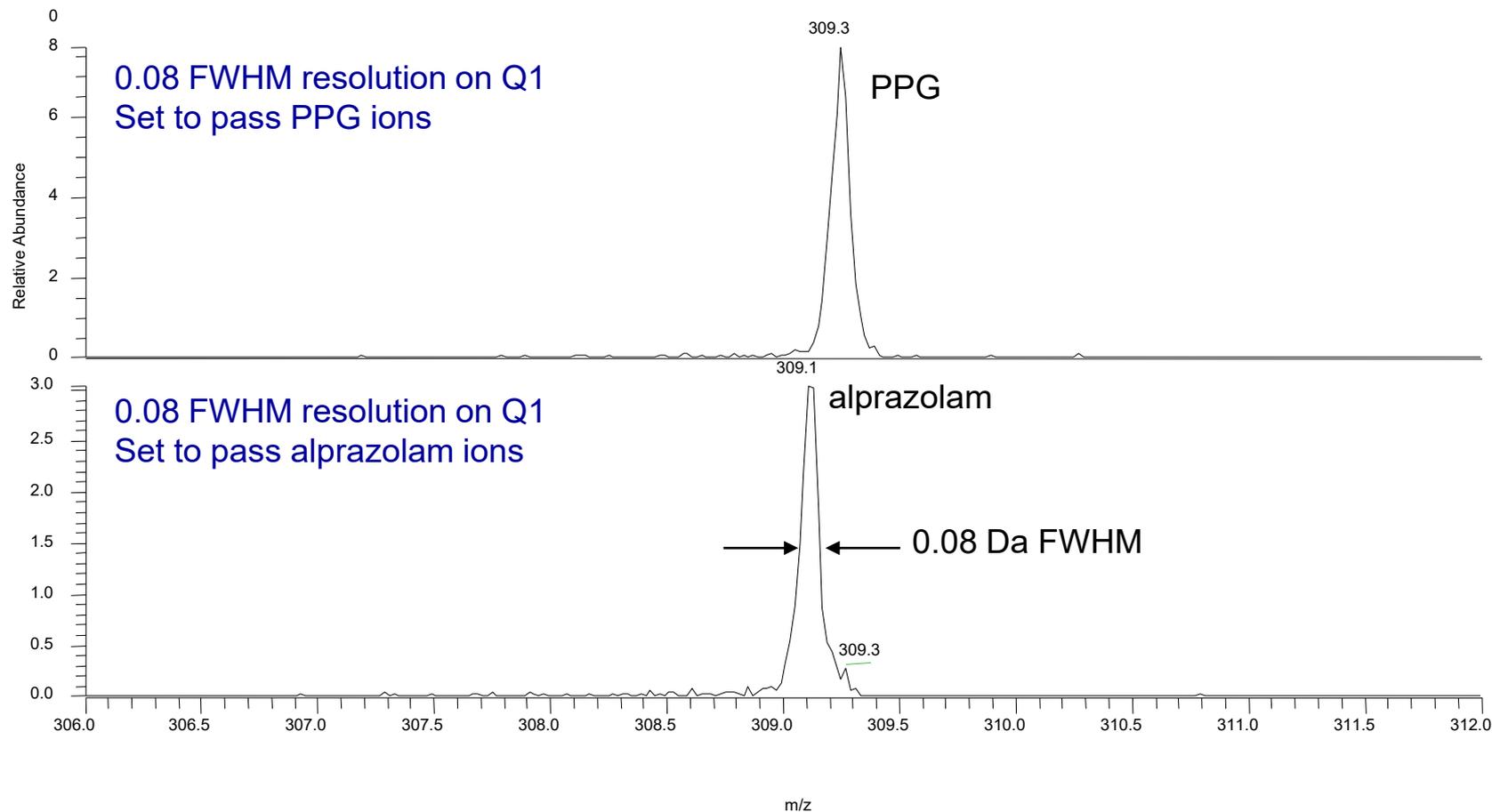
Resolving Target Compounds in the Presence of Interferences



Alprazolam resolution in the presence of PPGs
alprazolam $[M+H]^+ = 309.0907$, PPG ($n=5$) $[M+H]^+ = 309.2277$

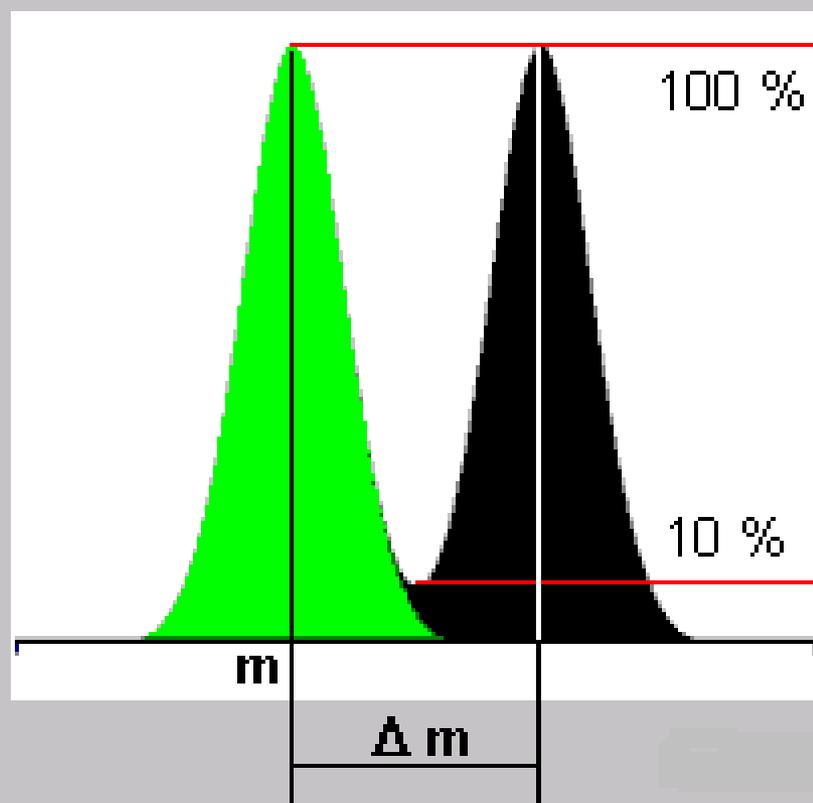
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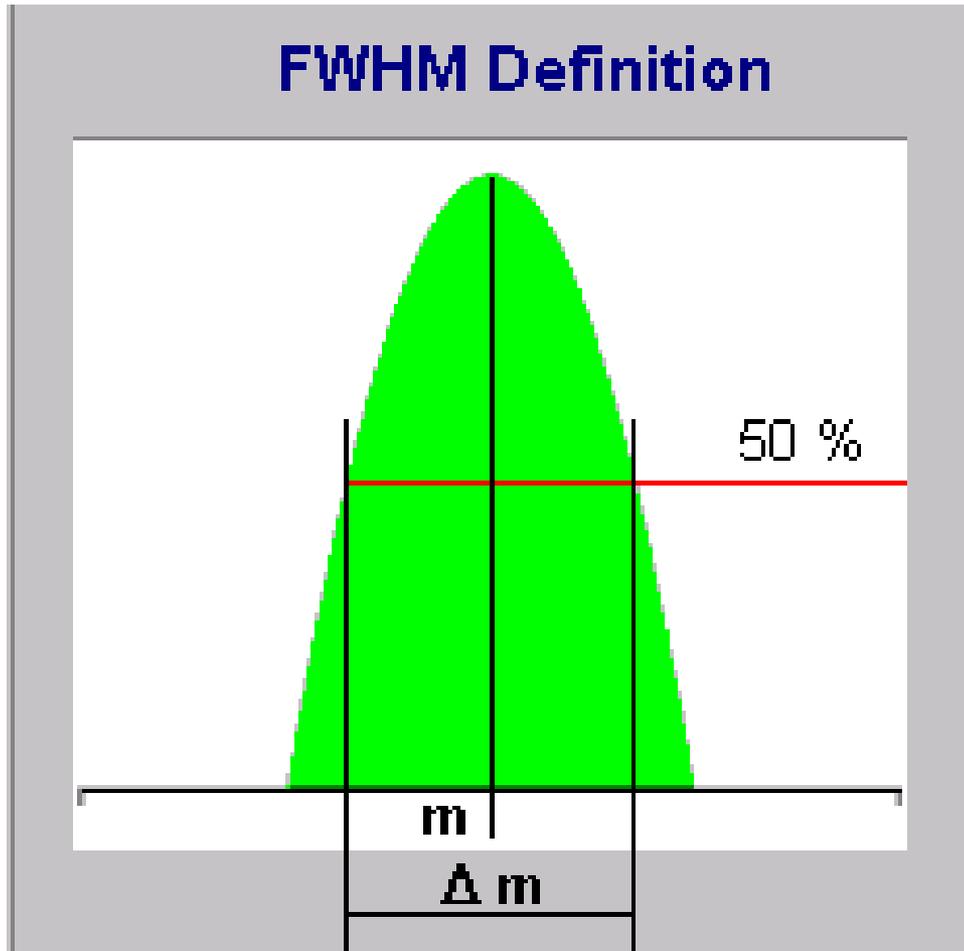
Resolution Basics

10 % Valley Definition



Magnetic Sector Instruments
Constant Resolution with mass
10% Valley Definition

Resolution Basics



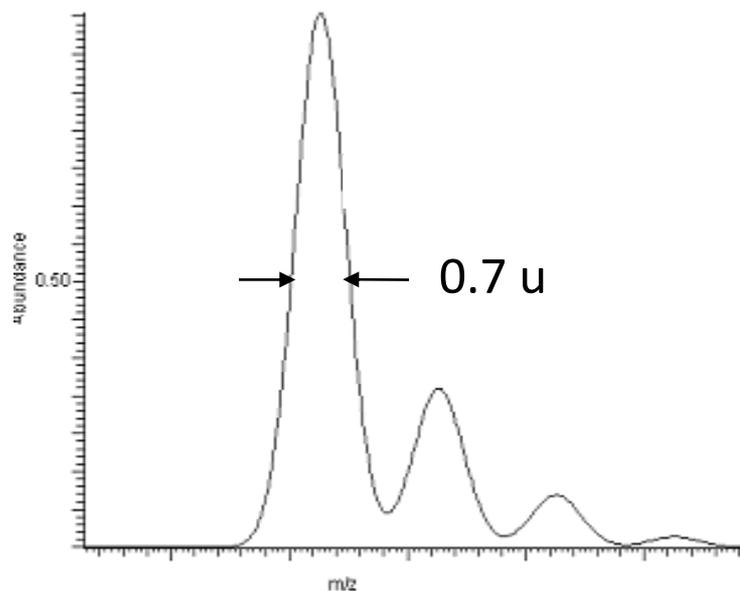
Quadrupoles Ion traps
and TOF's

Constant peak widths

Variable Resolution

FWHM Definition

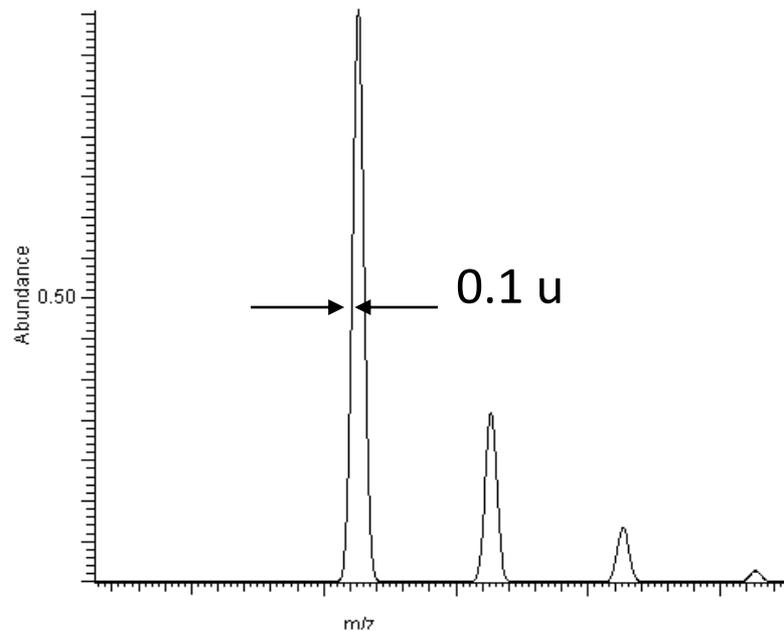
Unit mass Resolution v's 0.1 FWHM



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

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

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



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

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

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

Why Resolution ?

What is the advantage of using high resolution mass spectrometry?

The advantage of high resolution measurement is to eliminate chemical background of the same nominal mass but different accurate mass and, therefore to increase the signal to noise ratio and the sensitivity respectively.

Example:

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

Better selectivity is obtained by applying high resolution in case of isobaric compounds, i.e. two compounds of same nominal mass but different accurate mass. With low resolution only a combined Spectral result is obtained under Product ion conditions . With high resolution separate detection and therefore separate isolation and MS/MS spectra are obtained.

Example:

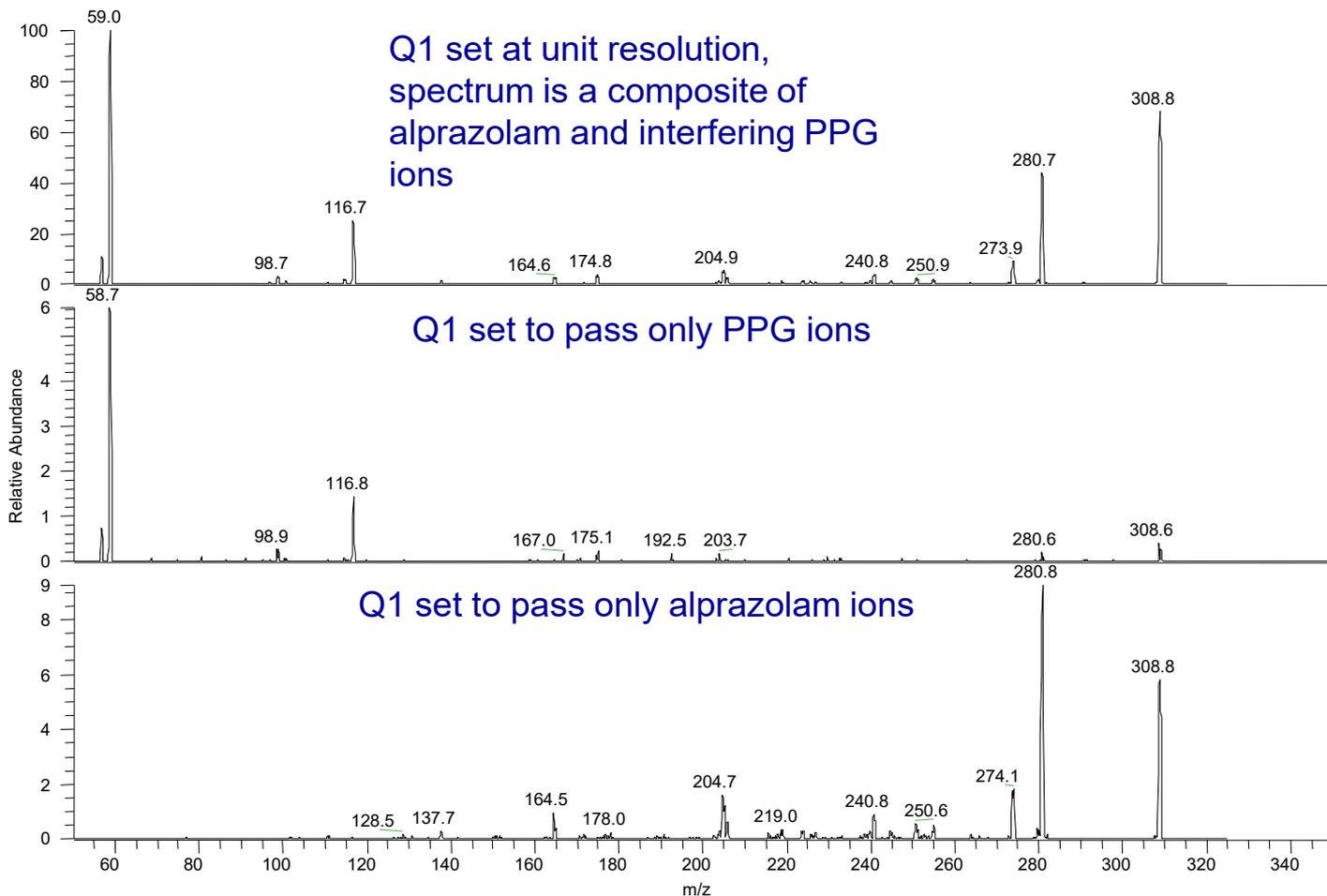
	<u>R= 1000</u>	<u>R= 5000</u>
Compound mass1: 372.351 Da	OVERLAPPING	SEPARATION
Compound mass2: 372.421 Da		

The Power of Resolution

- Unequivocal Determination of Charge State (ESI)
- High Resolution Precursor Ion Selection for MS/MS
- Separation of Ions with Same Nominal m/z Value
- Product Ion Resolution for Peptide Analysis

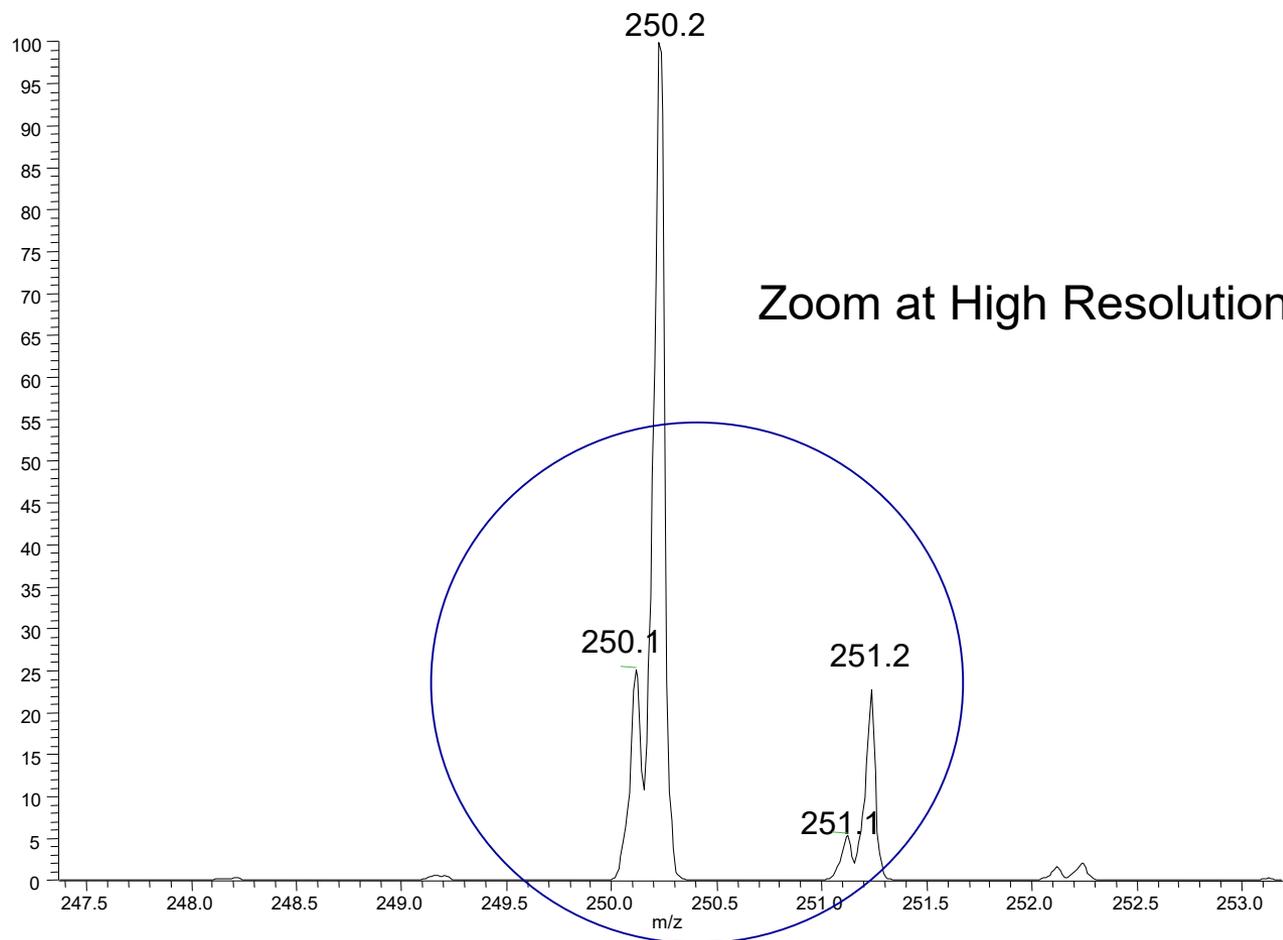
Alprazolam resolution in the presence of PPGs

alprazolam $[M+H]^+ = 309.0907$, PPG (n=5) $[M+H]^+ = 309.2277$

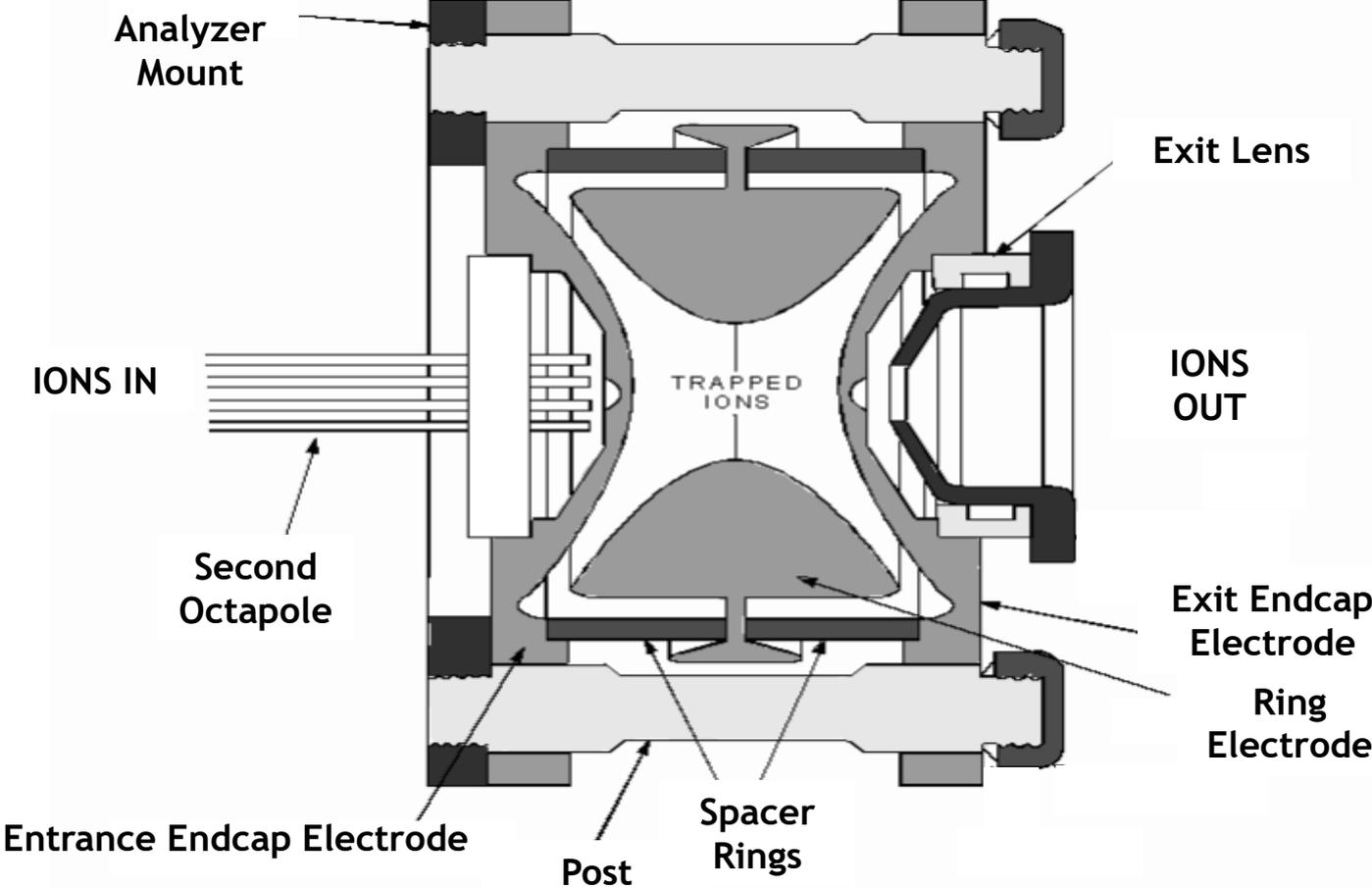


Sulfonamide resolution in the presence of an Impurity

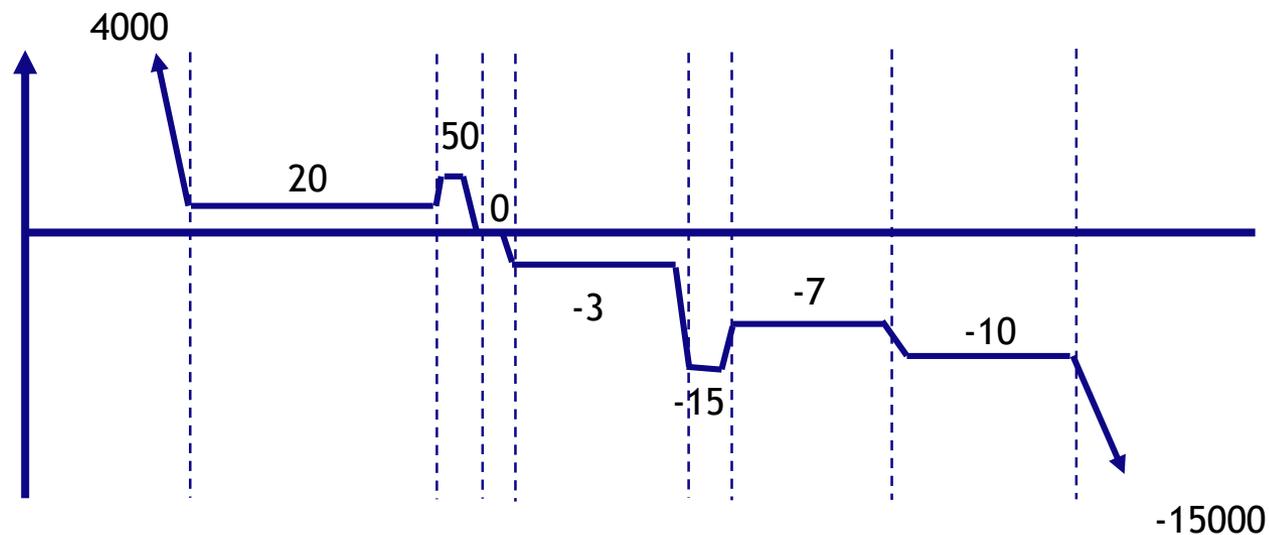
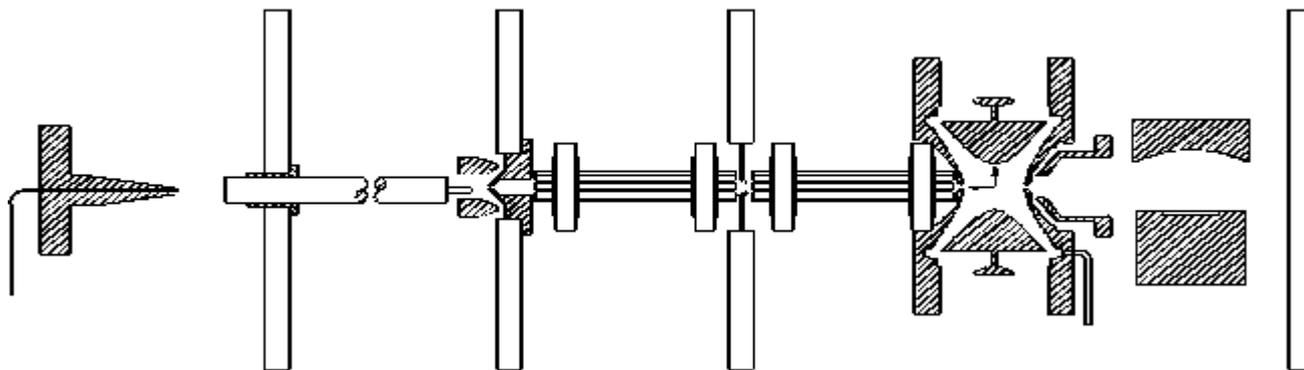
Sulfonamide $[M+H]^+ = 250.1$, Impurity $[M+H]^+ = 250.2$



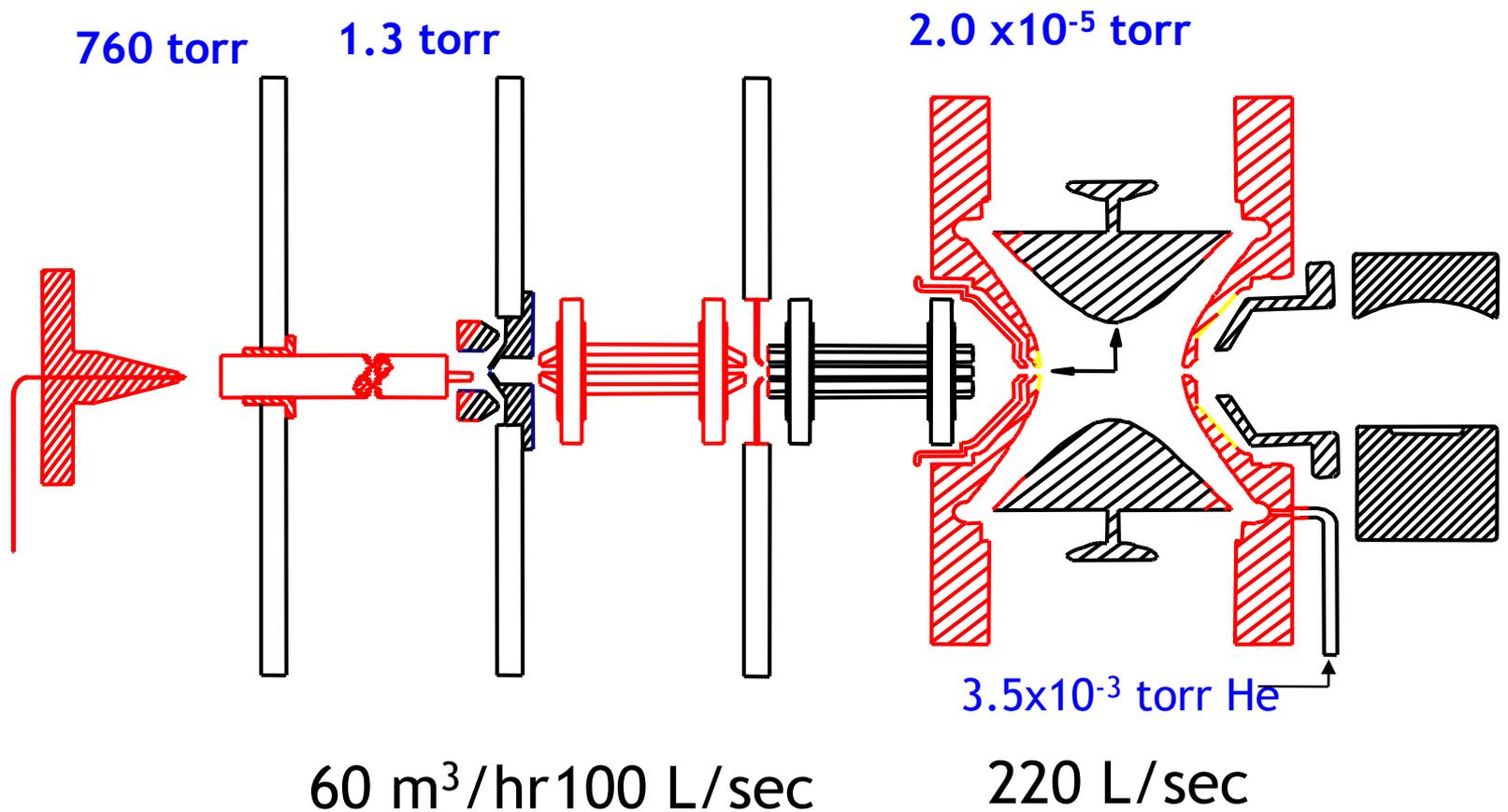
Mass Analyzer (Ion Trap)



Ion Optics (Potential Energy)

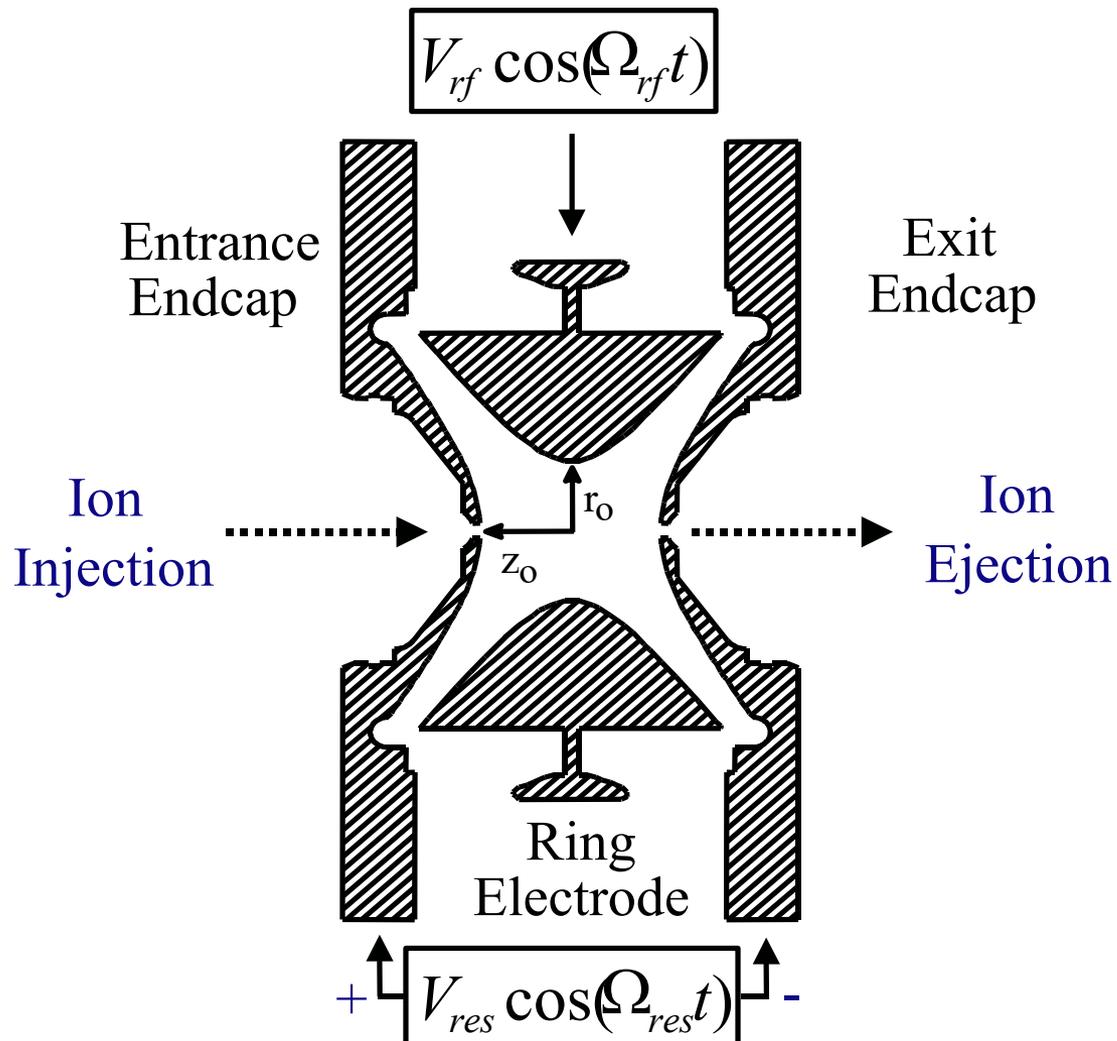


Ion Optics (Operating Pressures)



“Tandem MS/MS-in-Time”

Basic Ion Trap Components



Steps to Ion Trap Scan Functions

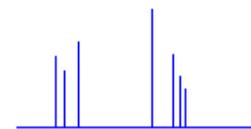
- 1) **Trapping- all scans**
- 2) **Isolation- SIM and MSⁿ**
- 3) **Excitation- MSⁿ**
- 4) **Ejection- all scans**

Typical Experiments

MS



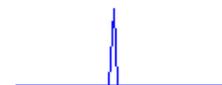
Collection Ejection



SIM



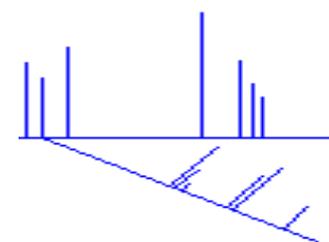
Collection Isolation Ejection



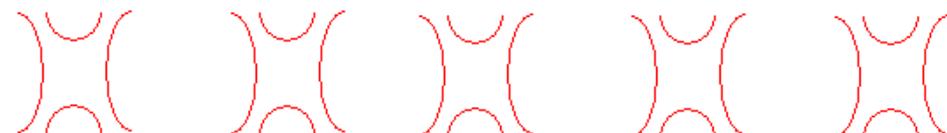
MS²



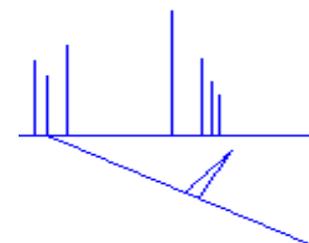
Collection Isolation Excitation Ejection



SRM



Collection Isolation Excitation Isolation Ejection



Step 1: TRAPPING OF IONS

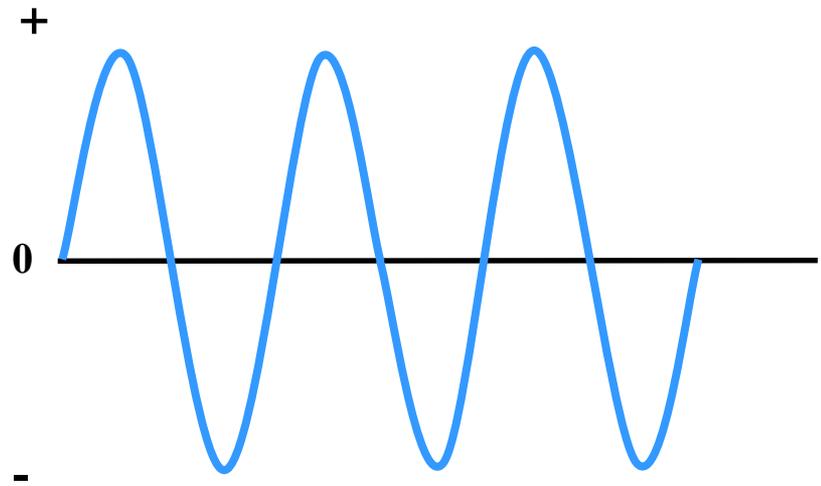
- Rf (applied to ring electrode)
- Helium (stabilize the ions)

Ring Electrode RF

Radio-Frequency Alternating Voltages

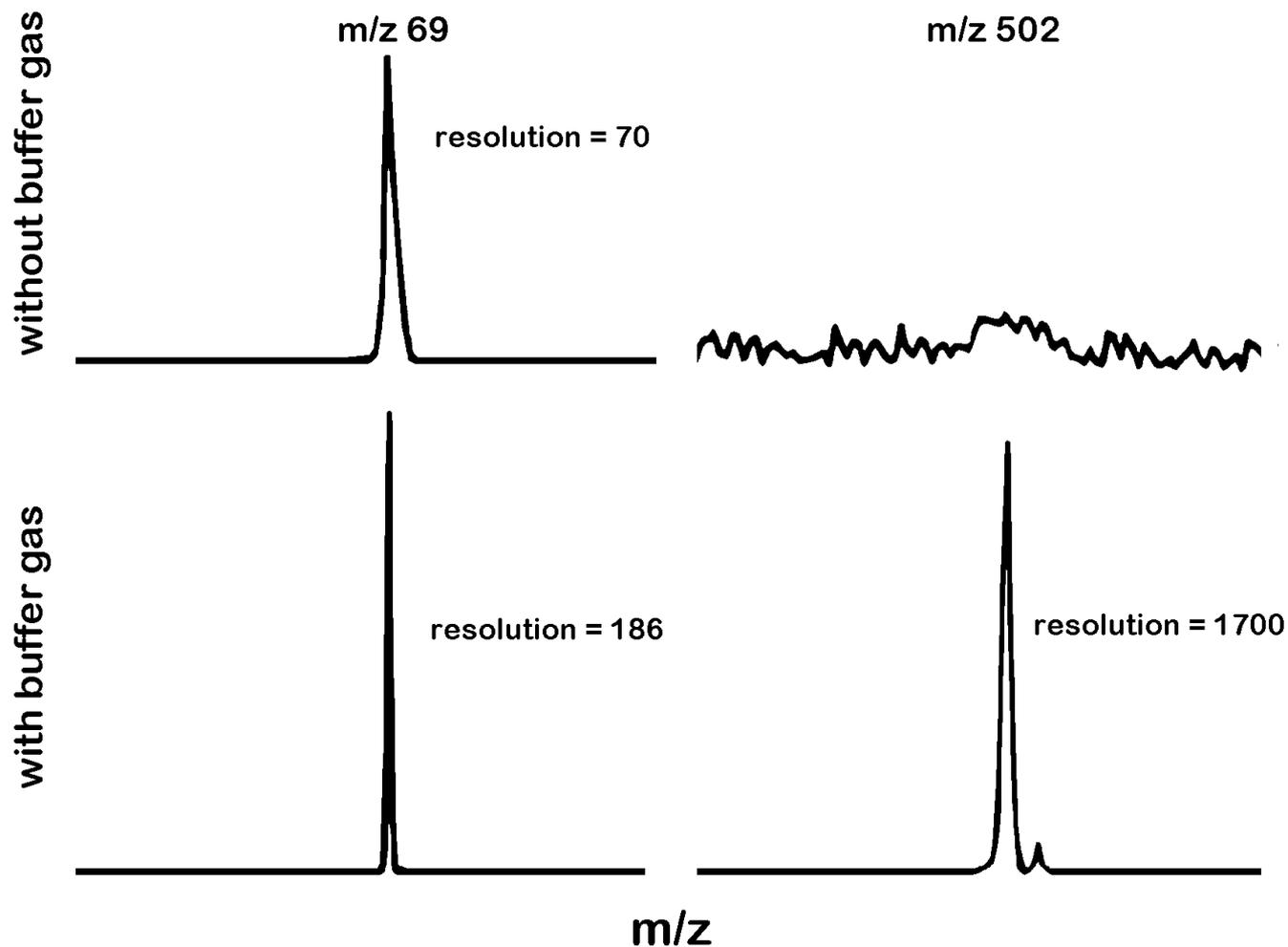
RF Has Two Components:

- Frequency (cycles/second)
- Power (amplitude of wave)



Rf of Trap = 760 KHz

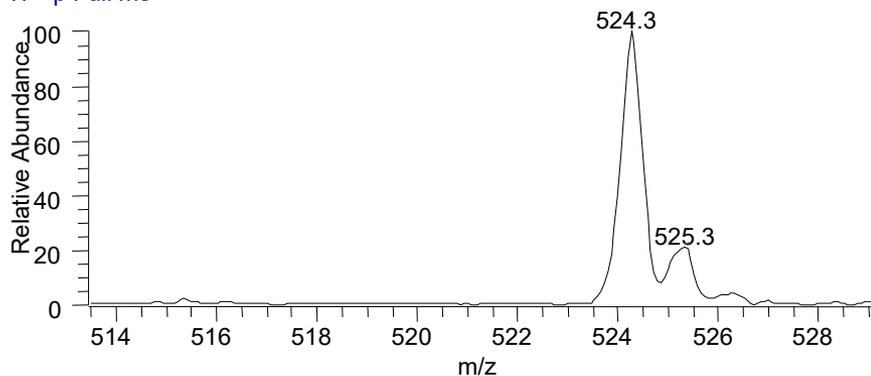
Discovery of Helium as Trapping Gas



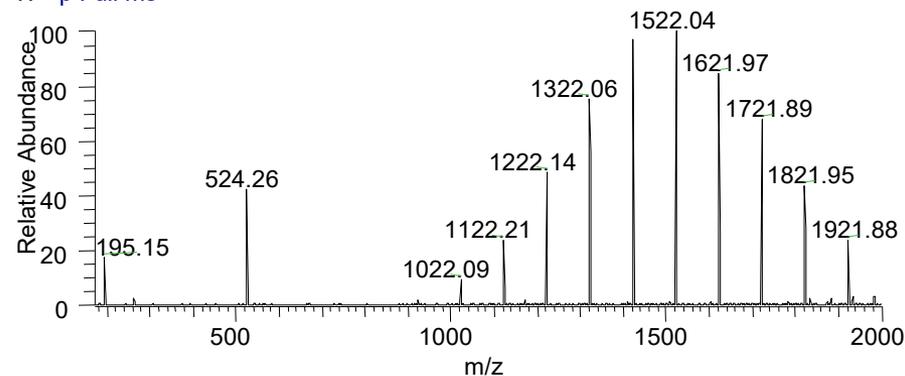
Effect of Helium

Helium flowing into trap

S#: 1 RT: 0.00 AV: 1 SM: 7G NL: 2.50E7
T: + p Full ms

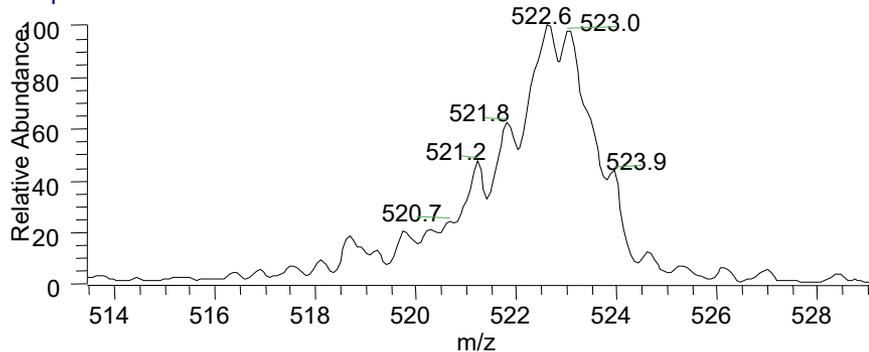


S#: 23-32 RT: 0.71-1.00 AV: 10 SM: 7G NL: 5.61E7
T: + p Full ms

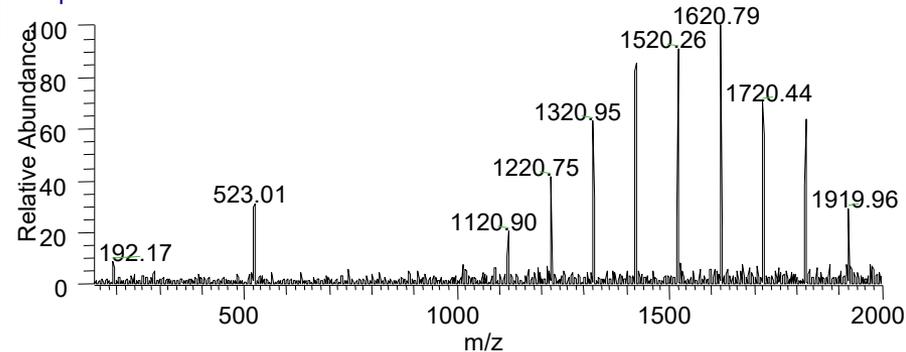


Helium shut off and not flowing into trap

S#: 1 RT: 0.02 AV: 1 SM: 7G NL: 9.70E6
T: + p Full ms



S#: 23-32 RT: 0.39-0.54 AV: 10 SM: 7G NL: 2.80E7
T: + p Full ms



Step 4: EJECTION OF IONS

Dependent upon Ion Instability in the Trap

Ion Stability in the Trap

Controlled by a culmination of differential equations termed
Reduced Mathieu Equations:

$$a_z = - \frac{16eU}{m(r_0^2 + 2z_0^2) \text{[?]}}$$

DC on the Ring

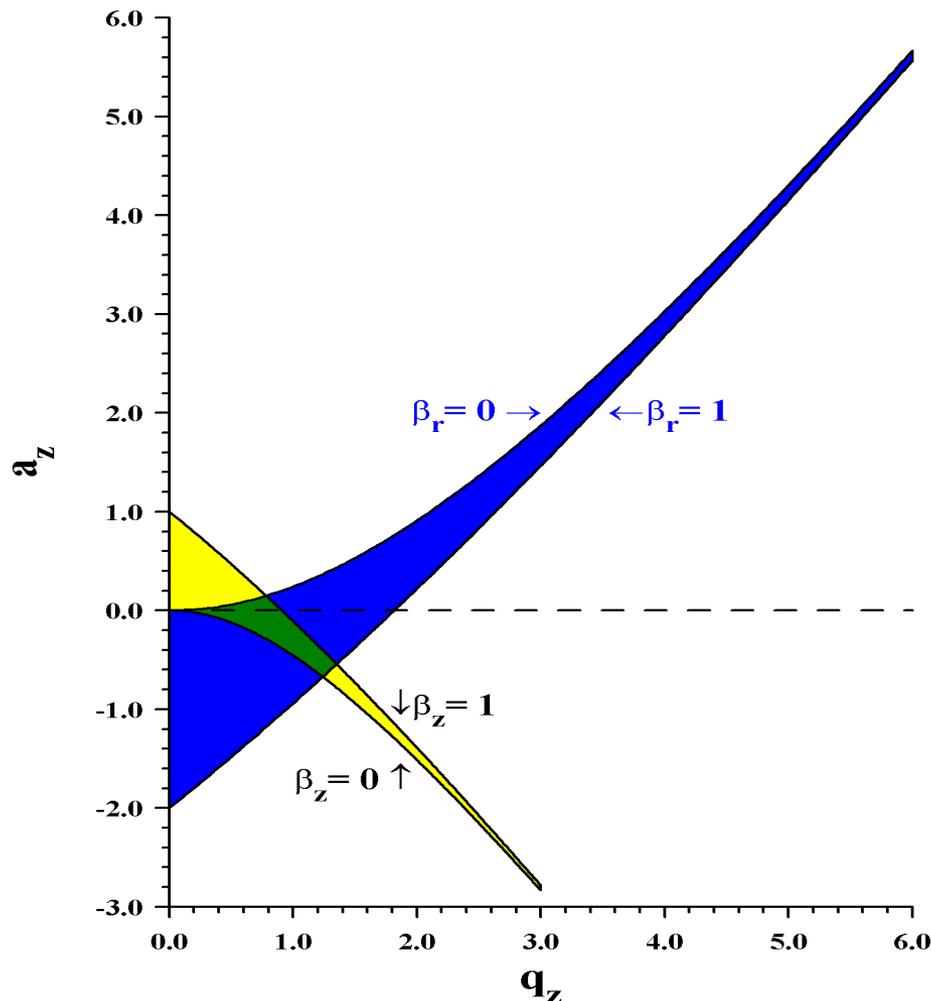
$$q_z = - \frac{8eV}{m(r_0^2 + 2z_0^2) \text{[?]}}$$

RF on the Ring

Mass

Ring Parameters

Ion Stability Diagram

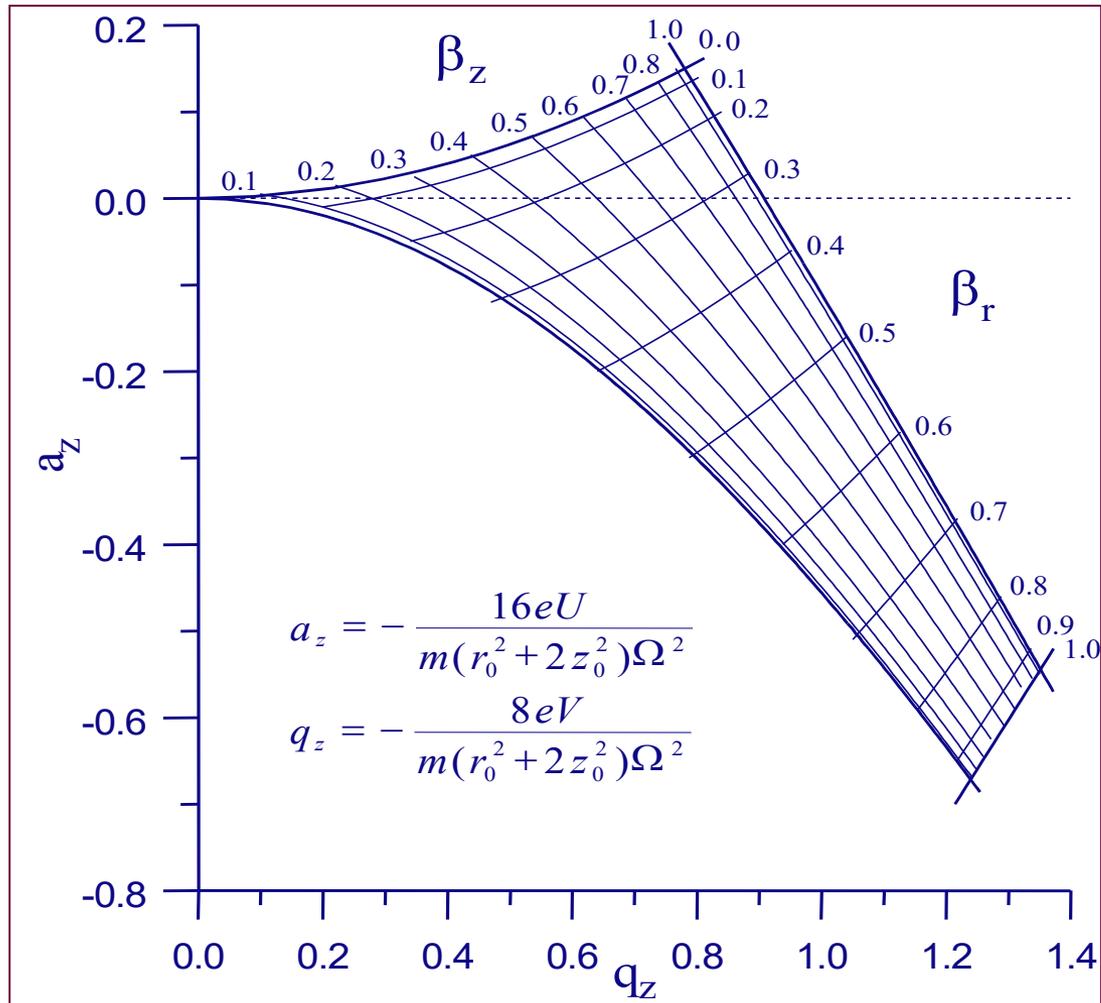


The region shaded **blue** indicates **a** (DC) and **q** (RF) values which provide stable trajectories in the **r-direction**

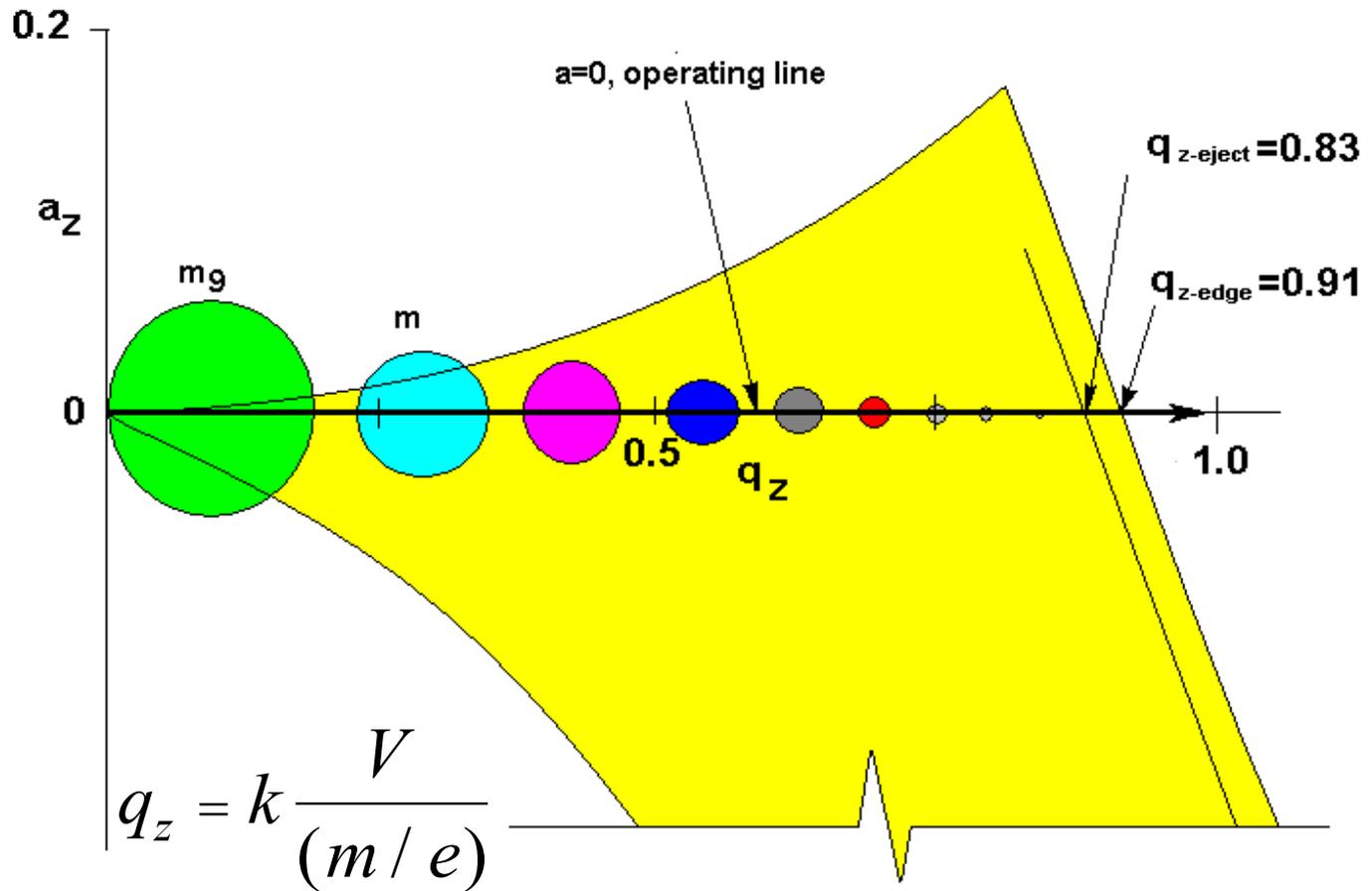
The region shaded **yellow** indicates the **z-stable a** and **q** combinations

The **green** area where the **r- and z-stable regions** overlap indicates the **a** and **q** combinations under which ions will be stable in the trap

Ion Stability Diagram (Overlap Region)

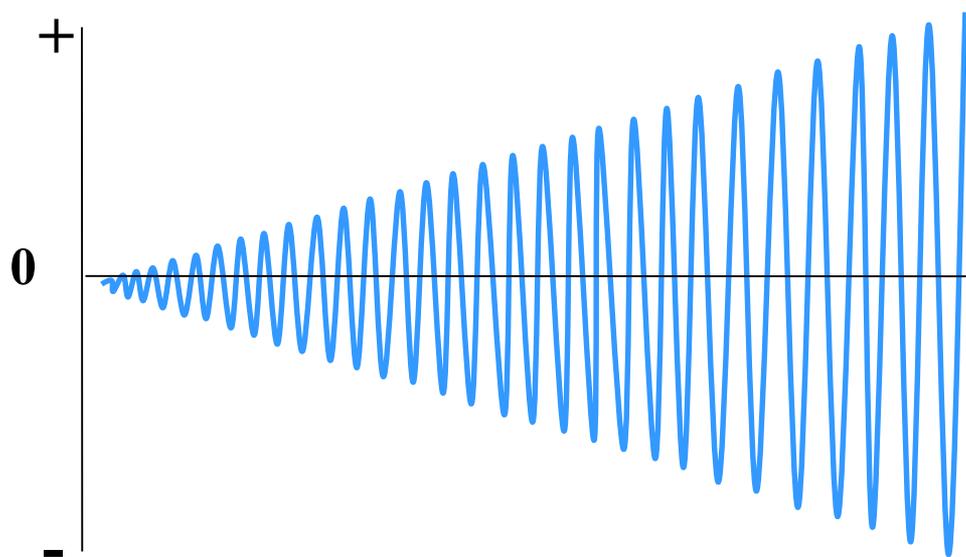


Stability Diagram for Commercial Ion Traps



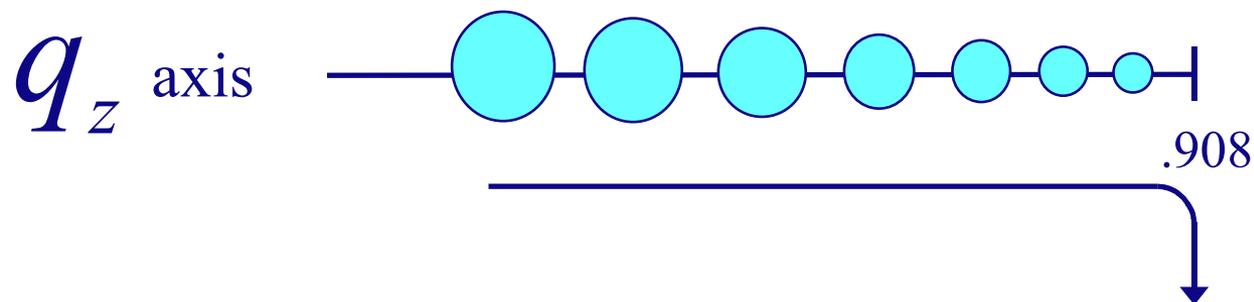
Ejection – Ring Electrode RF

Radio-Frequency Alternating Voltages



RF Power Can be Changed Over Time
(Ramping RF)

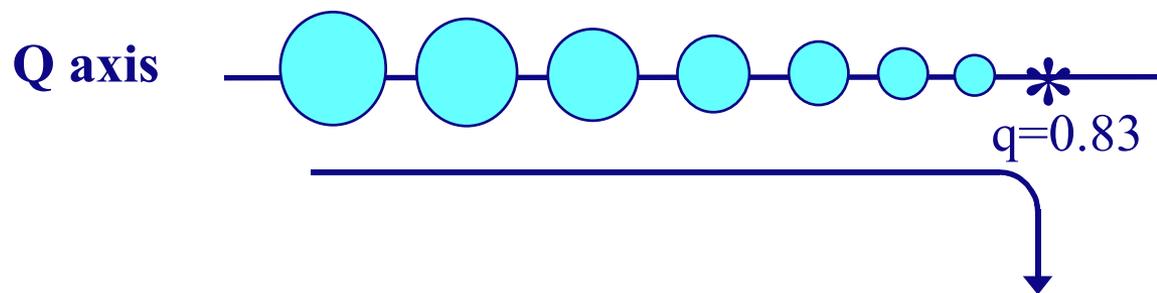
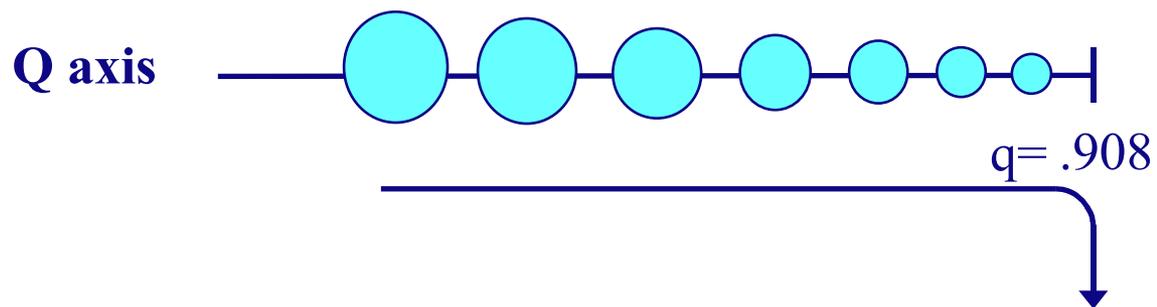
Mass Selective Instability



$$q_z = k \frac{V}{(m/e)}$$

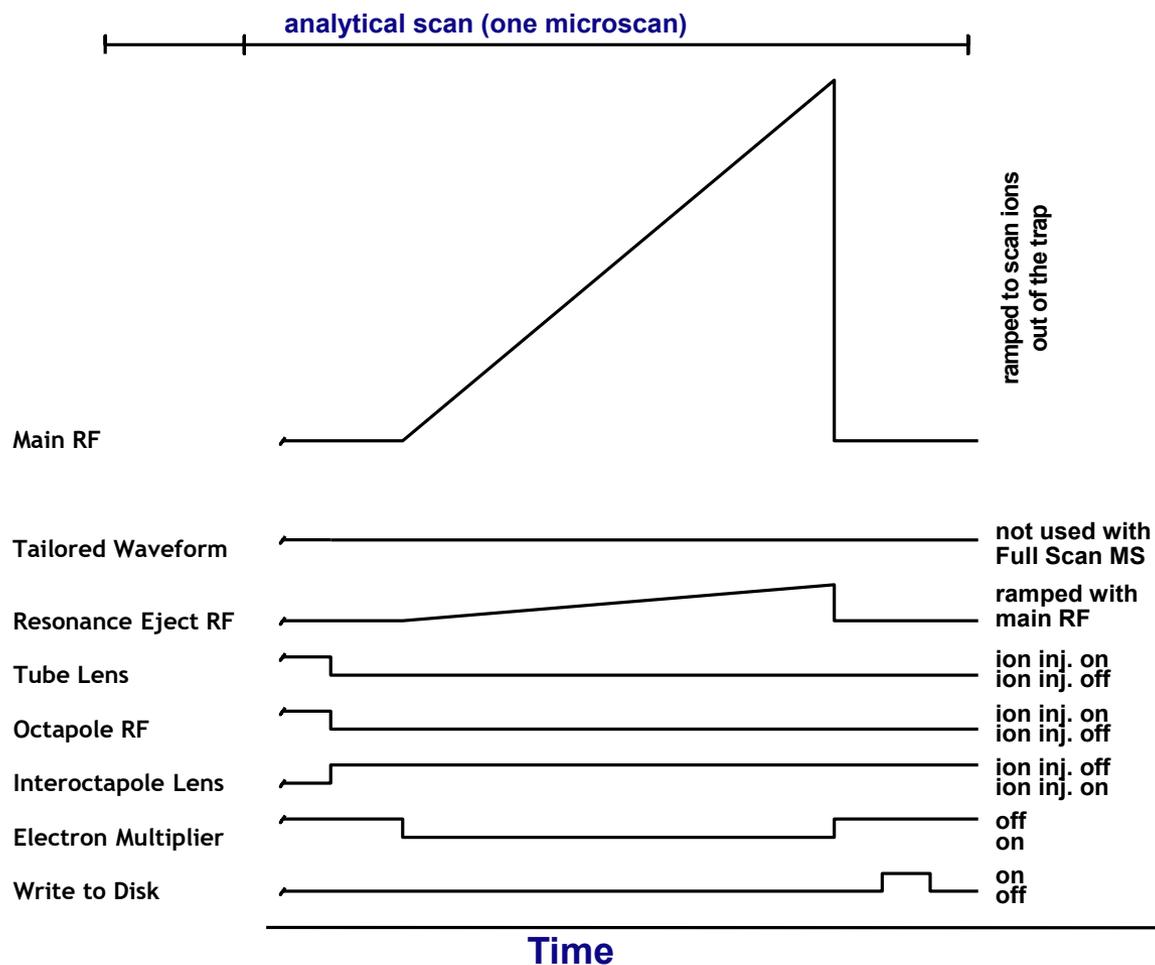
“Ramp RF, Ions Leave Low m/z to High m/z ”

... with Resonance Ejection



“Increases Resolution ...”

Ion Trap Full Scan Function



LCQ Scan-Out (Ejection) Rates

- **Normal Scan (5500 amu/sec)**

Common full, SIM, or MS^n scanning

Resolution (FWHM) = 0.50, Mass Accuracy = ± 0.05

- **Zoom Scan (280 amu/sec)**

Increases resolution and mass accuracy across a narrow range (allows charge state determination)

Resolution (FWHM) = 0.15, Mass Accuracy = ± 0.02

- **Turbo Scan (55,000 amu/sec)**

Decreases total scan time of a full scan, thus increasing number of scans across a chromatographic peak

Resolution (FWHM) = 3.0, Mass Accuracy = ± 0.5

Resolution in a Trap

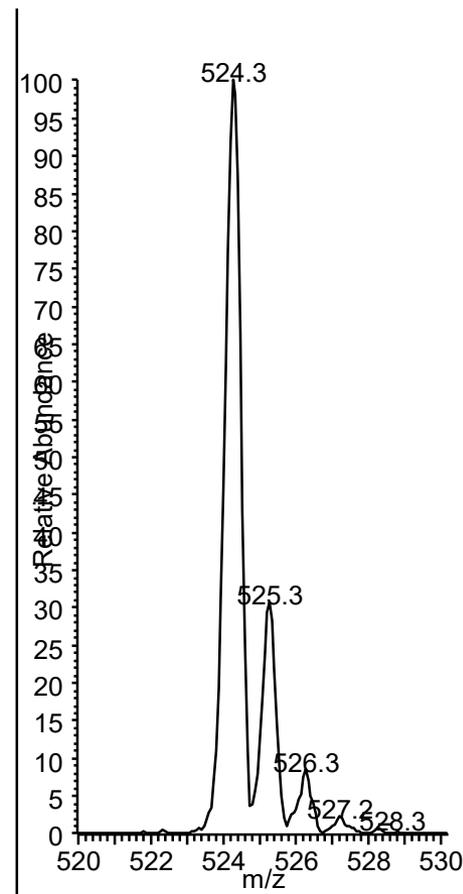
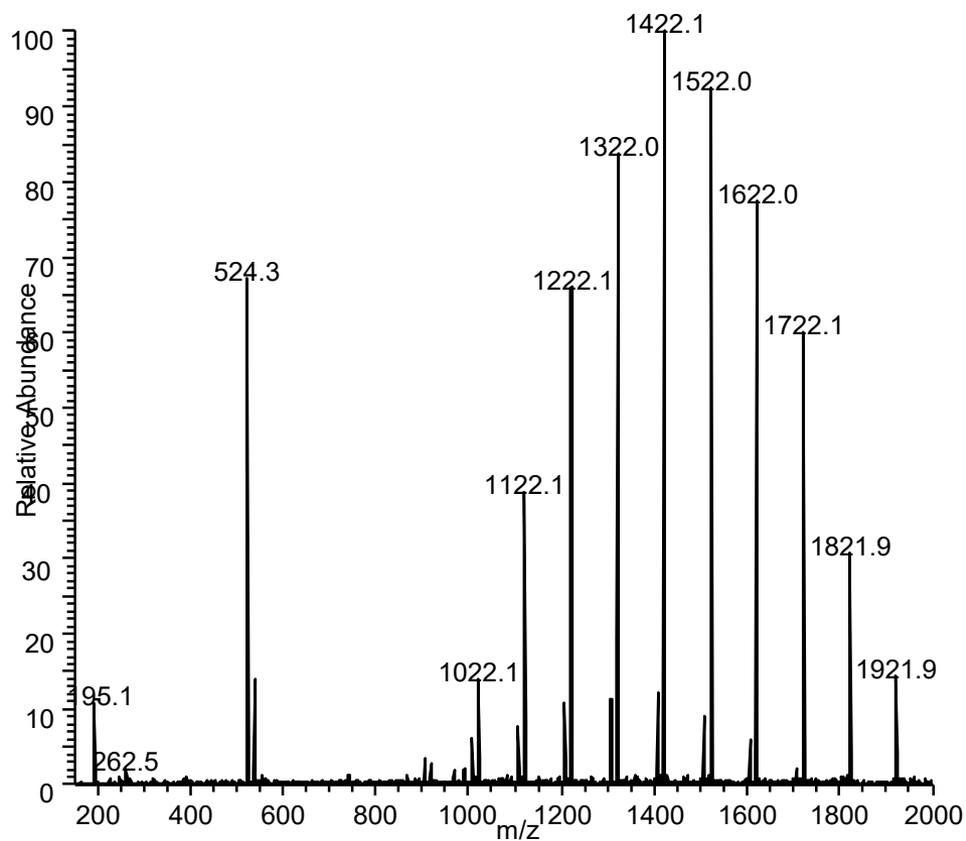
In an ion trap the resolution is a function of the number of ions in the trap and the speed at which they are scanned out. In “normal mode” the LCQ is operated such that the resolution is 0.7μ FWHM. This value can be considered fixed and is not adjustable by the user. The LCQ can be operated in a higher resolution mode, the ZoomScan. This traps ions over a 10μ range and scans them out at a slower speed. The ZoomScan can resolve the isotopes of 4^+ charged ion. The LCQ can also be used in a lower resolution mode the TurboScan which operates at slightly less than unit resolution.

Automatic Gain Control

Prescan before the analytical scan

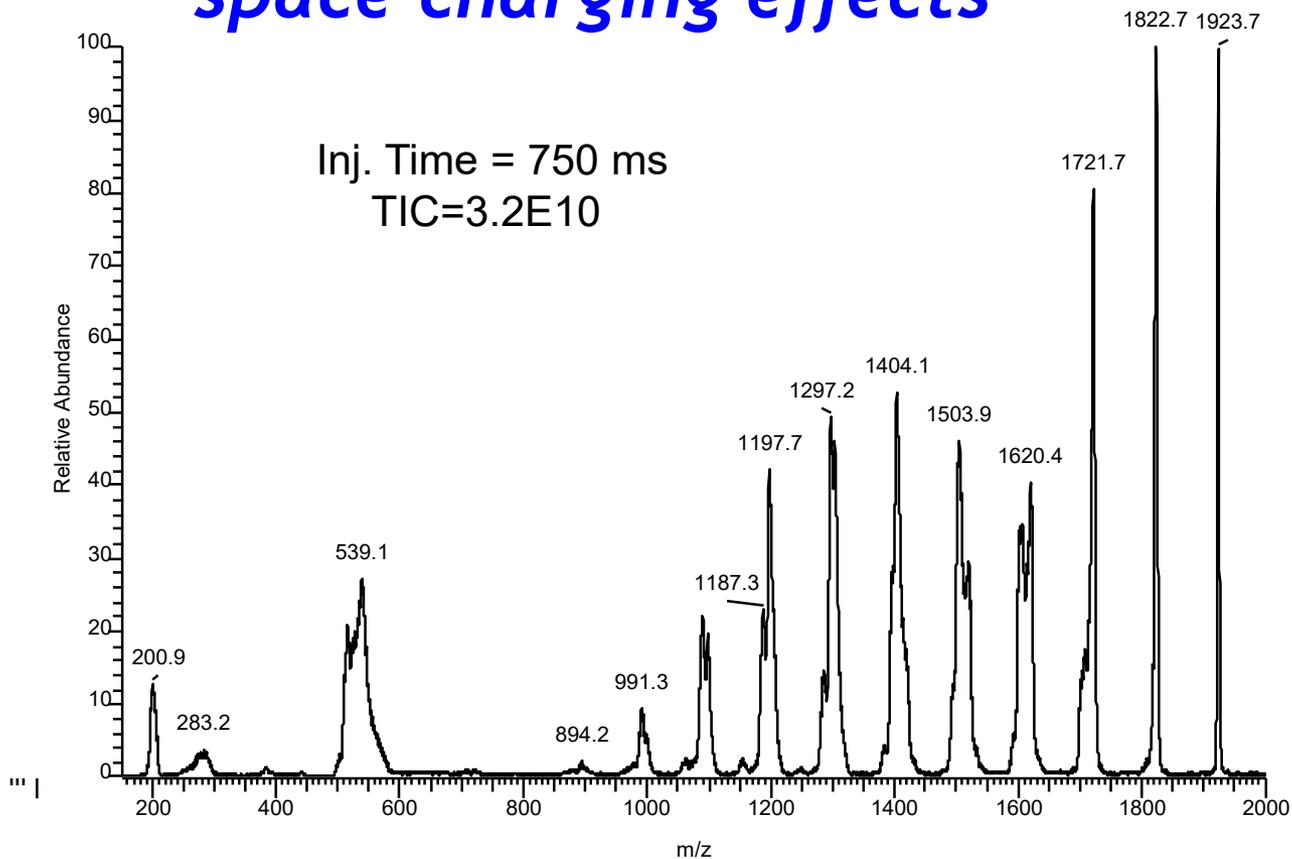
- Measures the number of ions in the trap for a pre-defined time (10 ms)
- Allows software to determine optimum ion injection time (IT)

Calibration Mixture Spectra using AGC



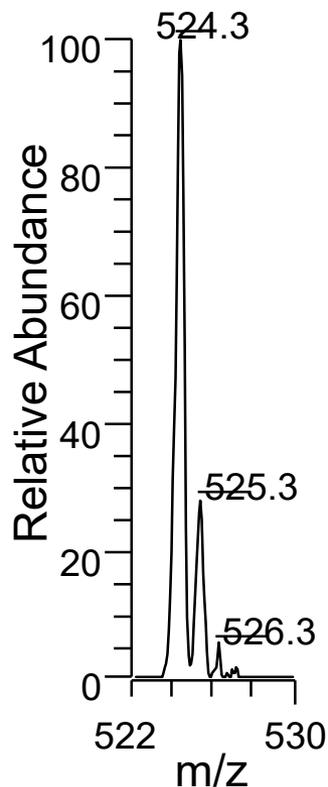
Calibration Mixture Spectra no AGC

space charging effects



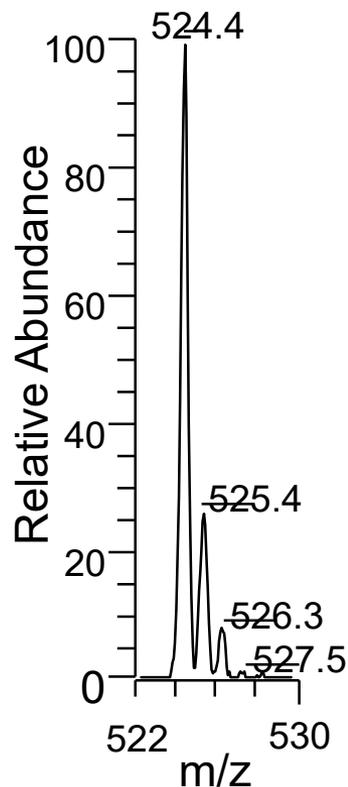
AGC (Ion Population Control)

~ 300 Ions

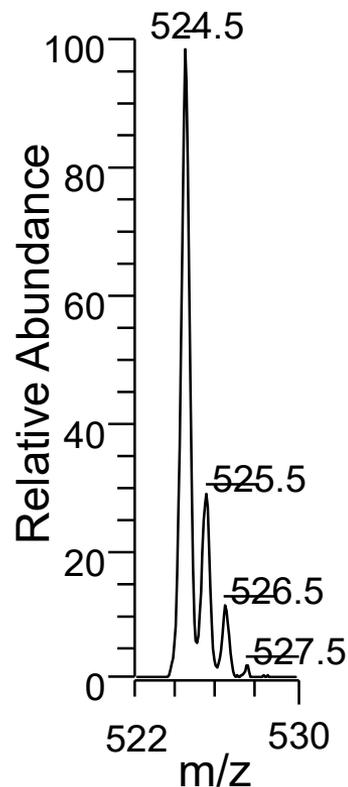


Good Resolution

~ 1500 Ions

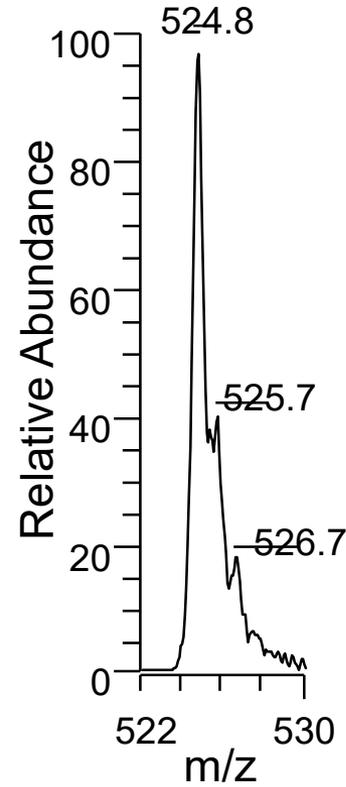


~ 3000 Ions



Poor Resolution

~ 6000 Ions



AGC Calculation

Step 1: Calculate and set injection time to fill ion trap to capacity (prescan)

$$\text{Injection Time} = \frac{\text{AGC Target}}{\text{Prescan TIC}} * \text{Prescan Injection Time}$$

Step 2: Scale intensity data (analytical scan)

$$I'(m) = I(m) * \frac{\text{Normalization Injection Time}}{\text{Injection Time}}$$

Note: The maximum injection time is set by the user!

AGC Calculation (example)

Step 1: Calculate Injection Time

$$IT \text{ (ms)} = \frac{5 \times 10^7}{5 \times 10^8} * 10 \text{ ms}$$

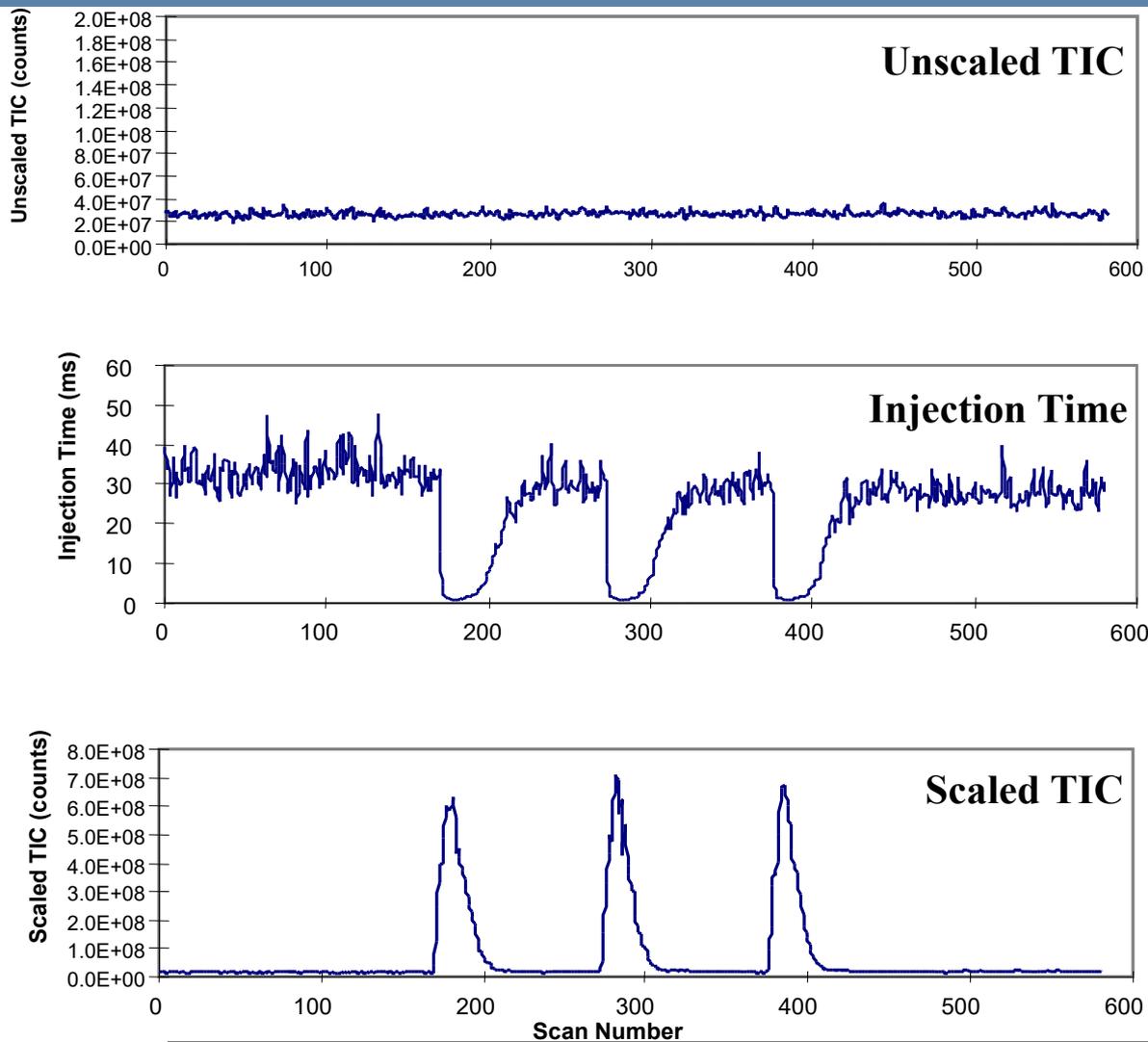
$$IT \text{ (ms)} = 1$$

Step 2: Scale intensity data

$$\text{Corrected TIC (I')} = \text{Sample TIC (} 3.15 \times 10^7 \text{)} * \frac{10 \text{ ms}}{IT \text{ (1 ms)}}$$

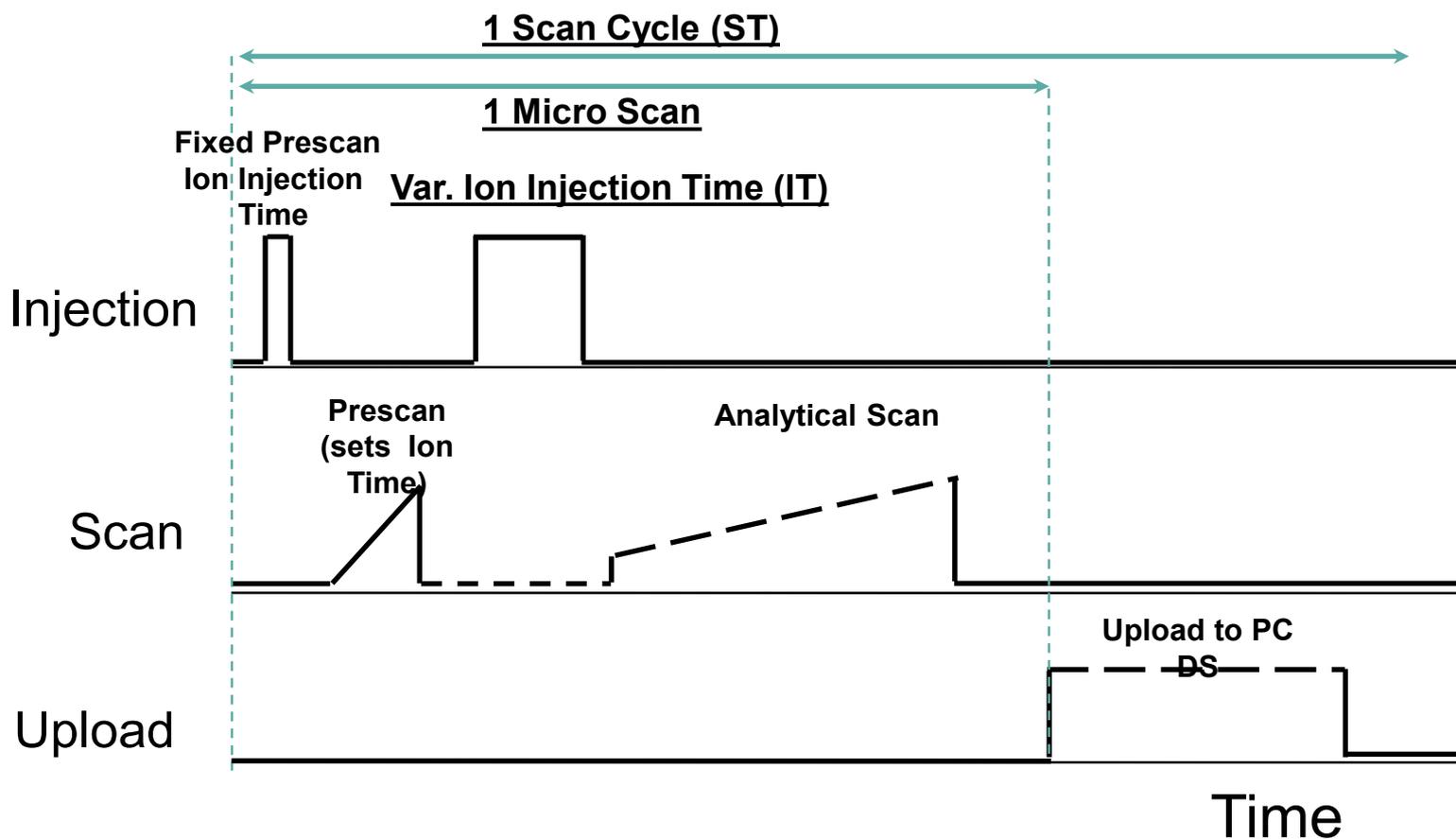
$$I' = 3.15 \times 10^7$$

TIC Plot – Triplicate Injection of MRFA

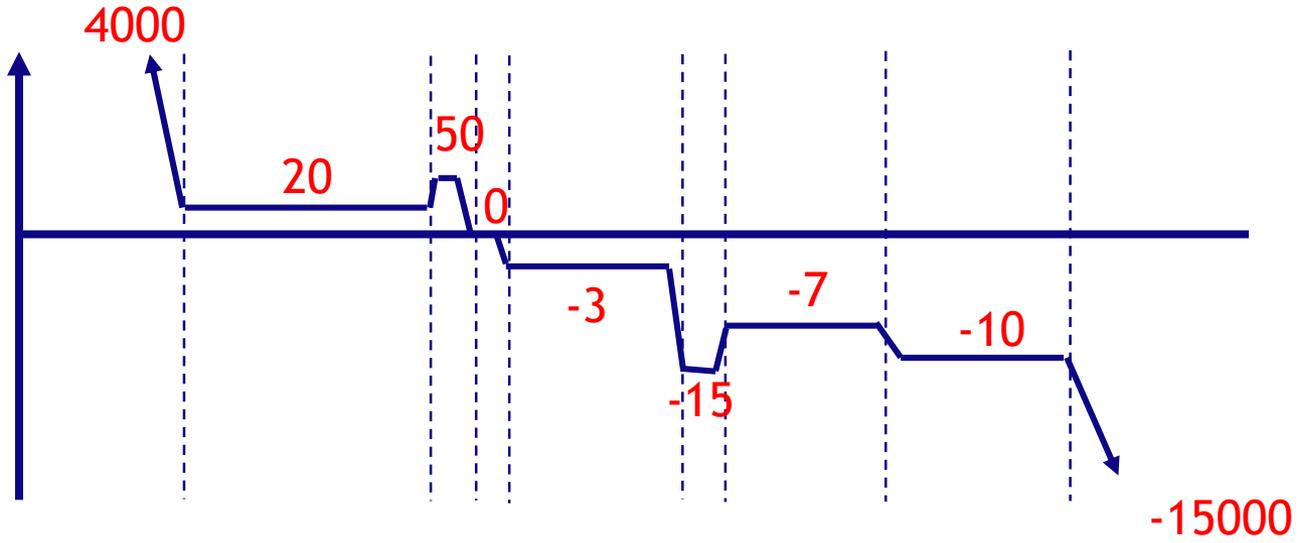
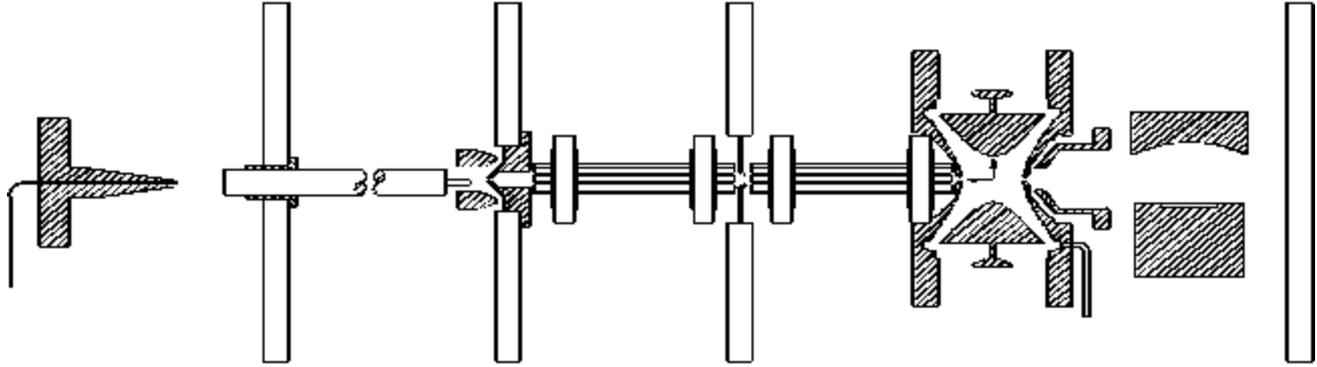


AGC Cycle – Summary full scan MS

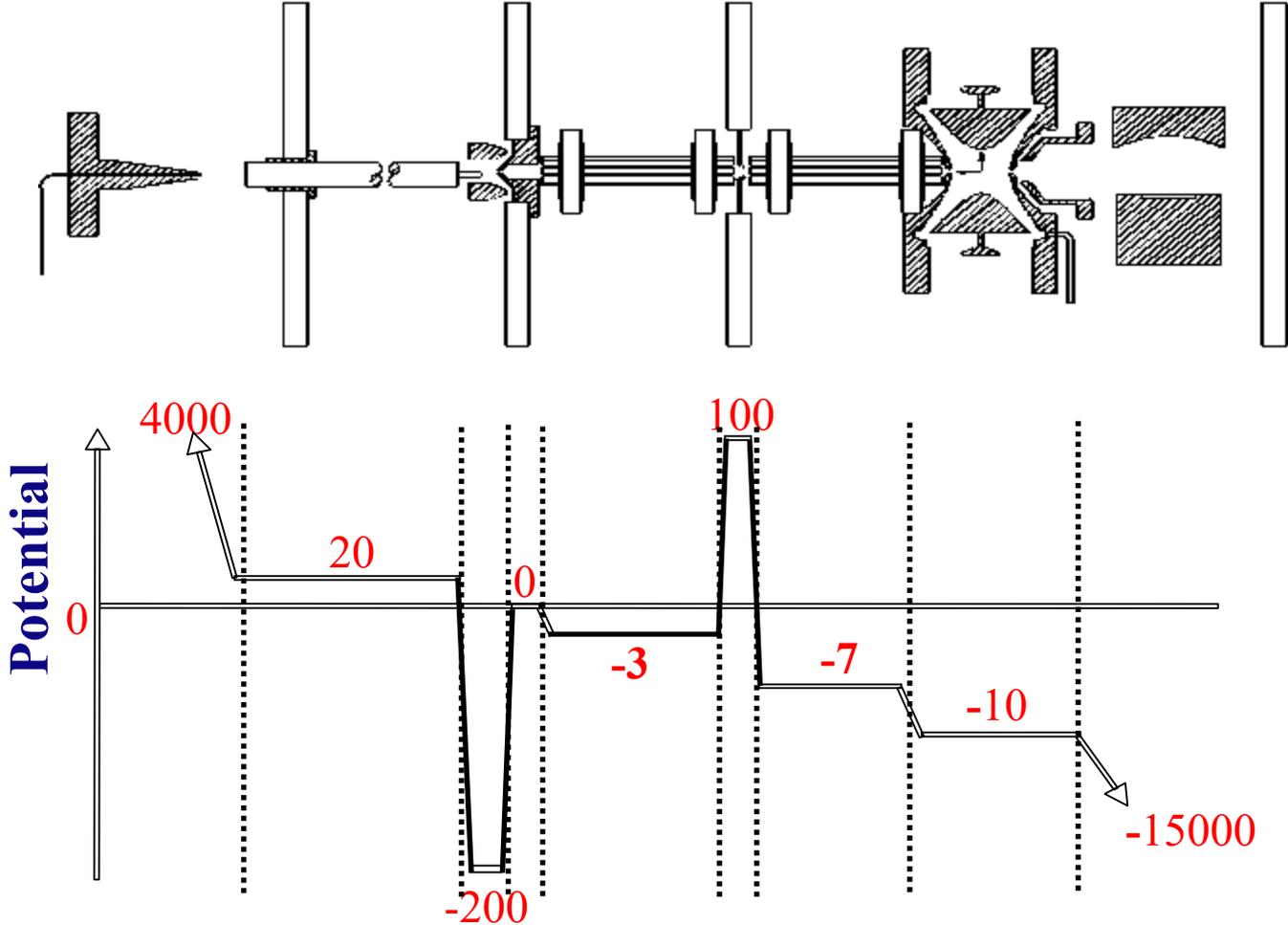
Ion Time, Microscan, and Scan Cycle



“Gate Lens” Open

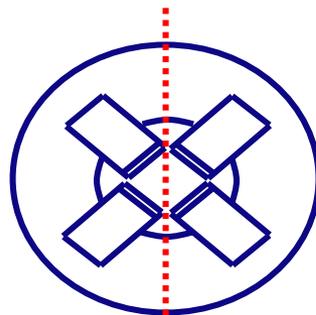


“Gate Lens” Closed

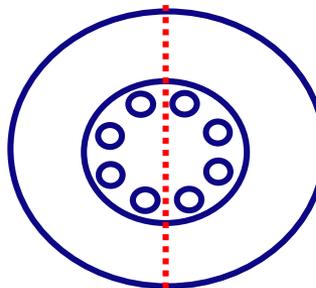


Gating the Ions (Split Multipole)

Split Square
Quadrupole



Split Octopole



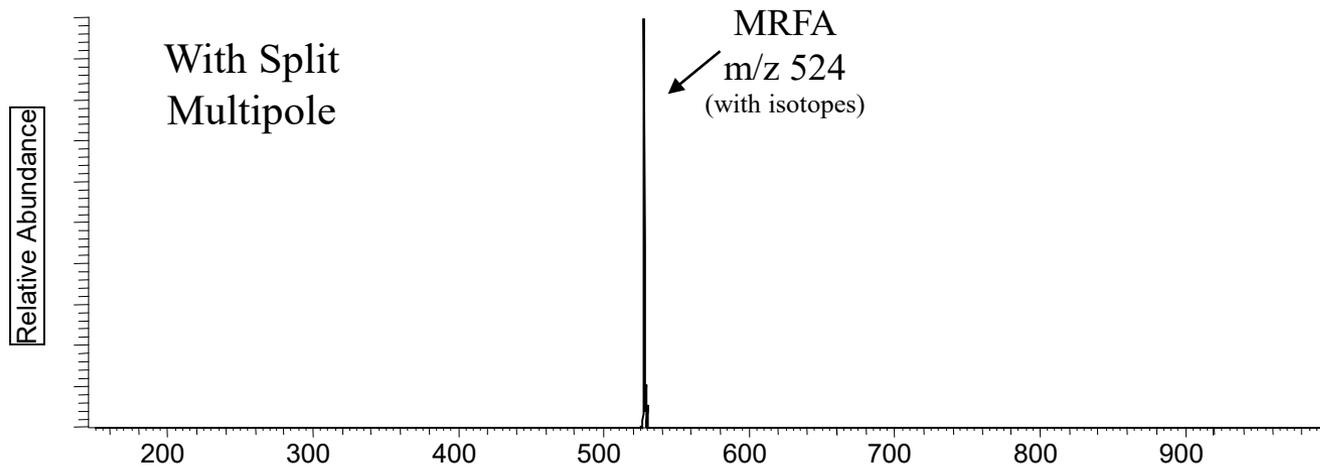
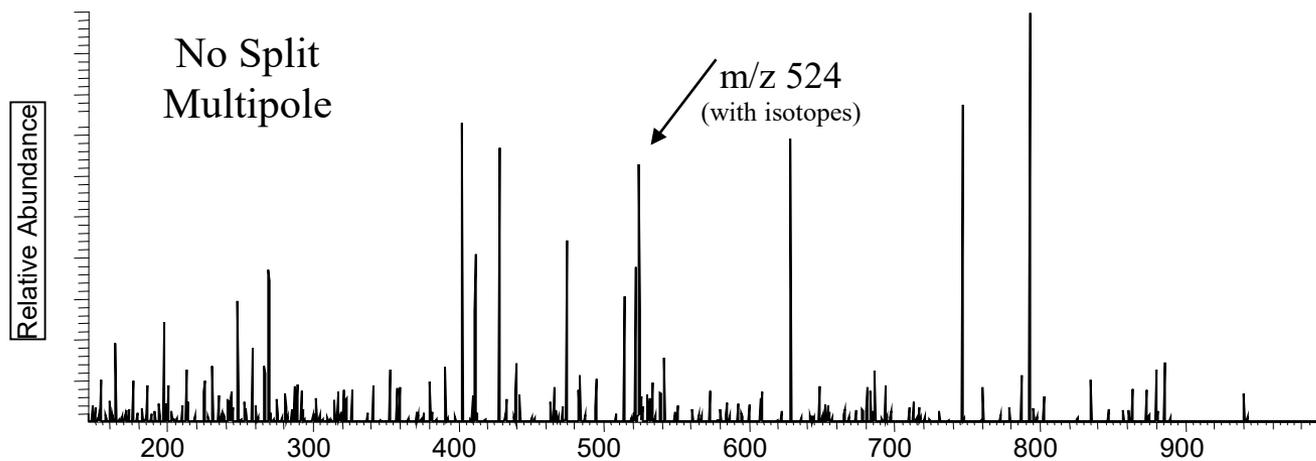
Deflection - set to +132



Deflection - set to -132

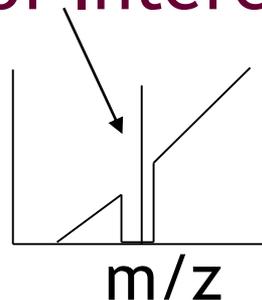
Transmit - both set to Offset Value

Effect of Split-Multipole



Step 2: ION ISOLATION (SIM and MSⁿ)

Ion of Interest

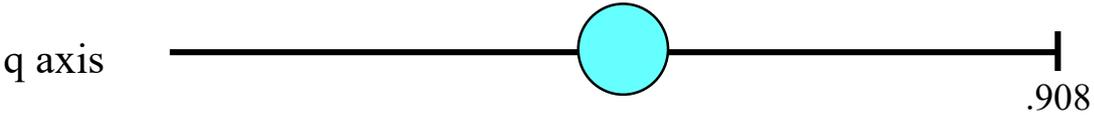
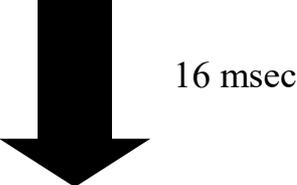
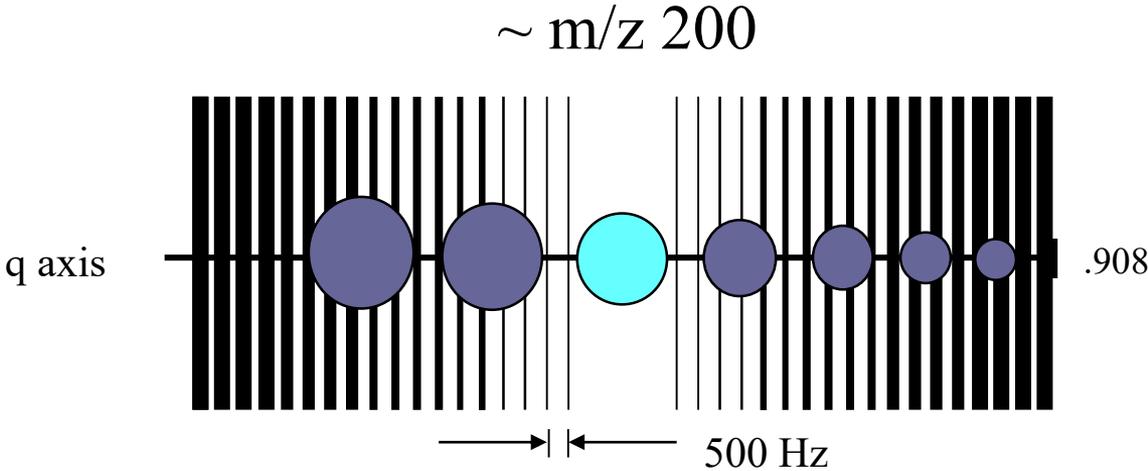


(Secular Frequencies)

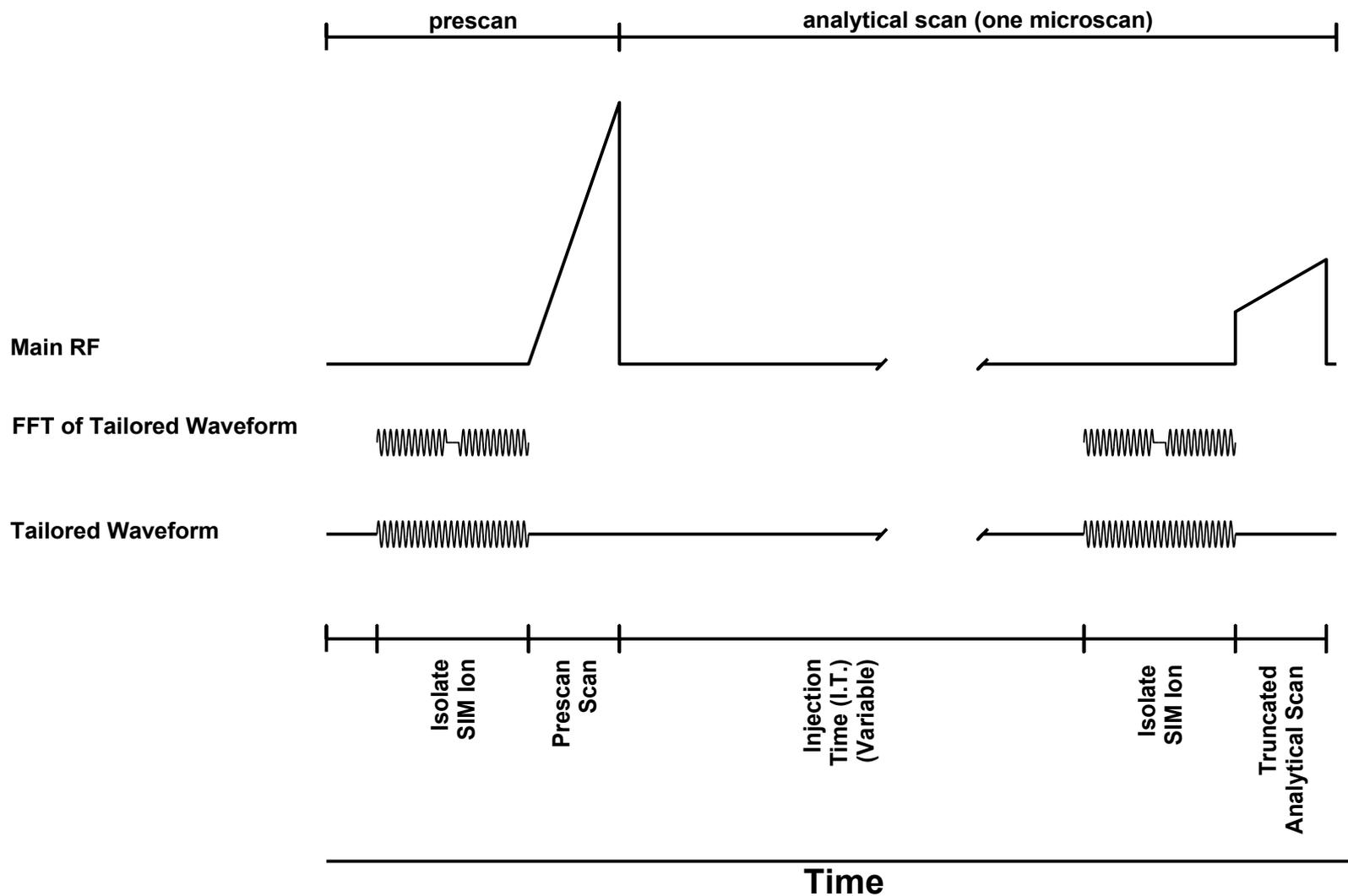
Tailored RF Waveform Applied to Endcaps

RF Power Applied at the Secular Frequencies of All Ions in the Trap BUT THE ION OF INTEREST Causes the Unwanted Ions to be Blown Out of the Trap !!!

Isolation Waveforms



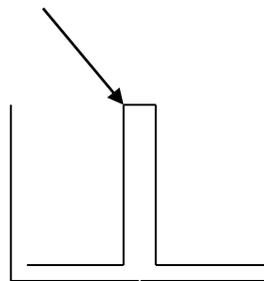
Ion Trap SIM Scan Function



Step 3: EXCITATION (MSⁿ)

(Excitation, CAD, CID, Tickle Voltage)

Ion of Interest



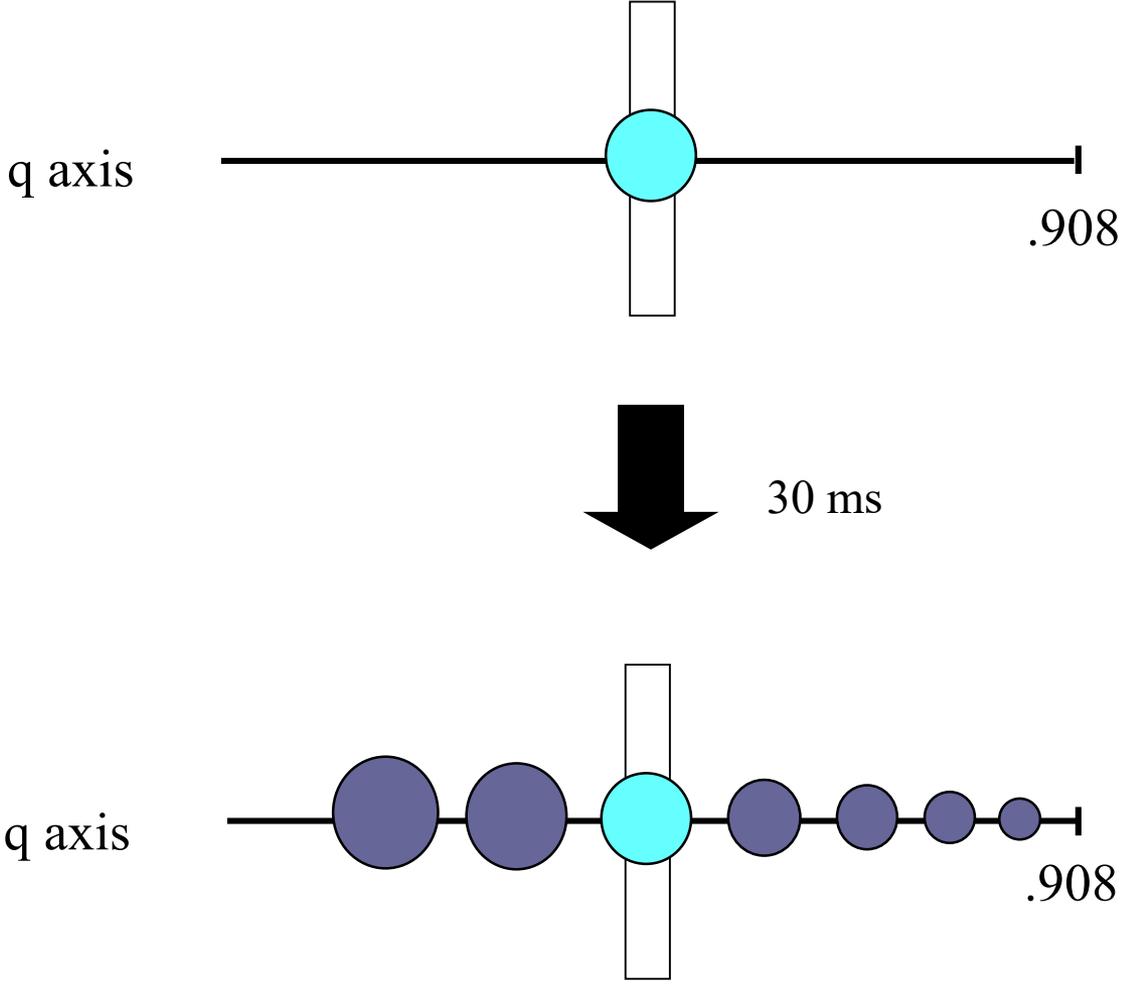
m/z

(Secular Frequencies)

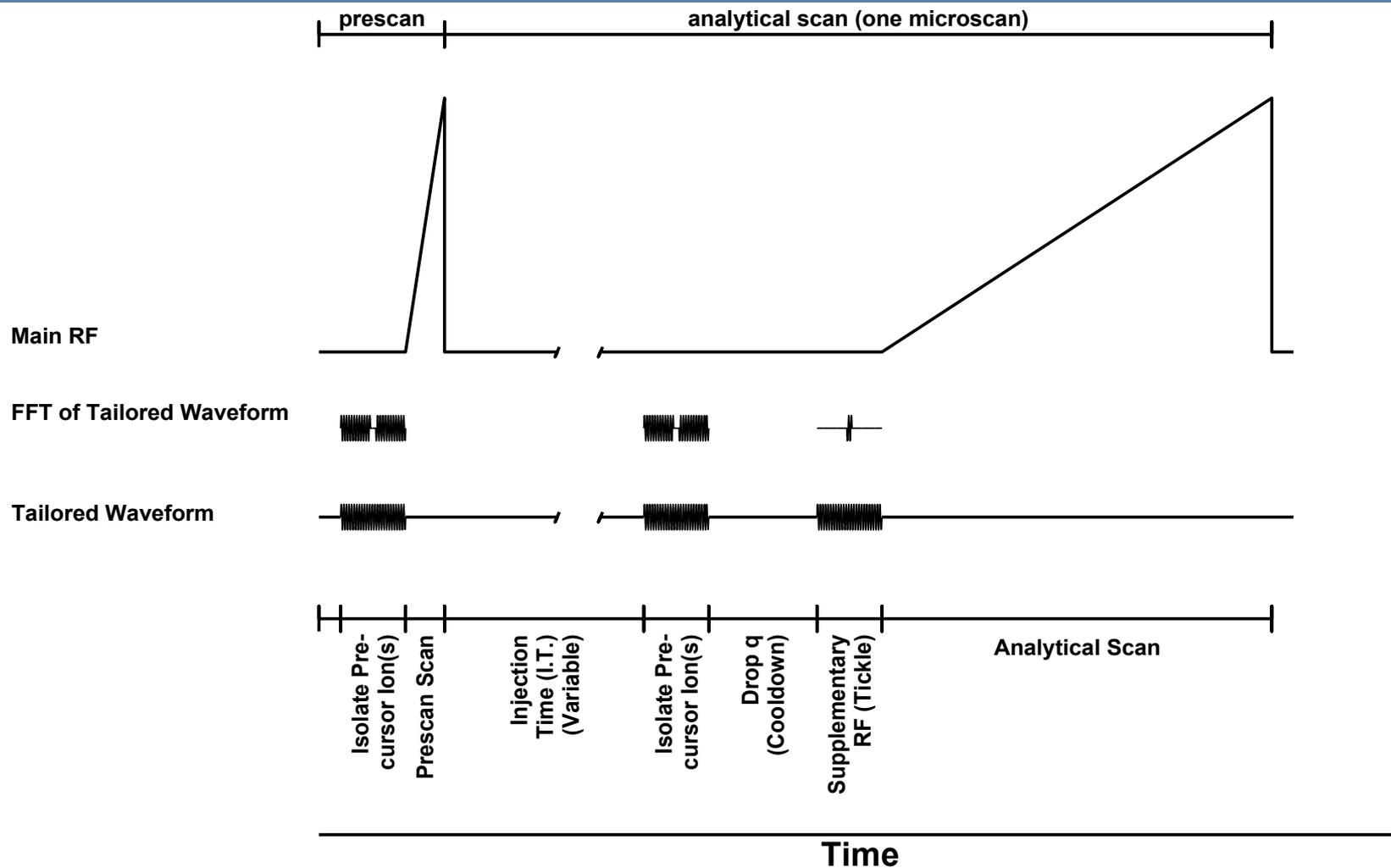
Tailored RF Waveform Applied to Endcaps

***RF Power is Applied Only to the Ion of Interest !!!
The User can Control the Amount (Collision Energy)
and the Width (Isolation Width) !!!***

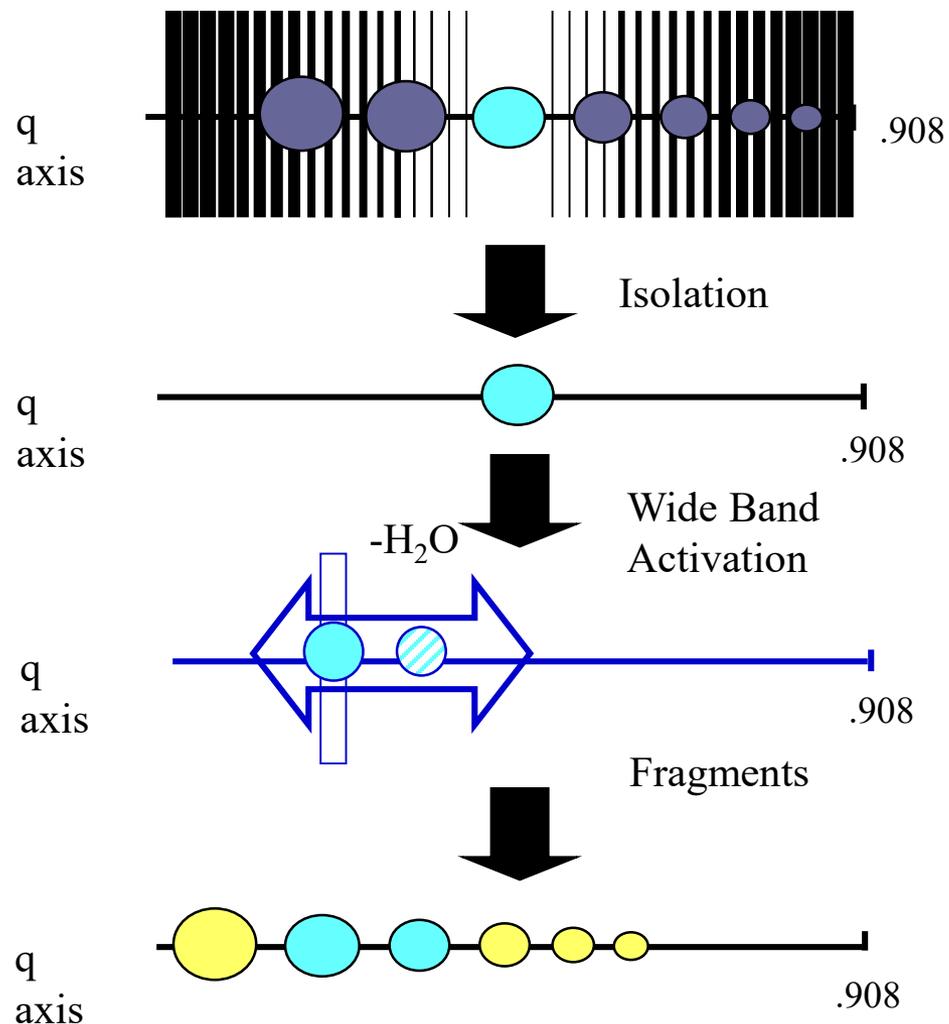
Resonance Excitation



Ion Trap MS/MS Scan Function (MS²)

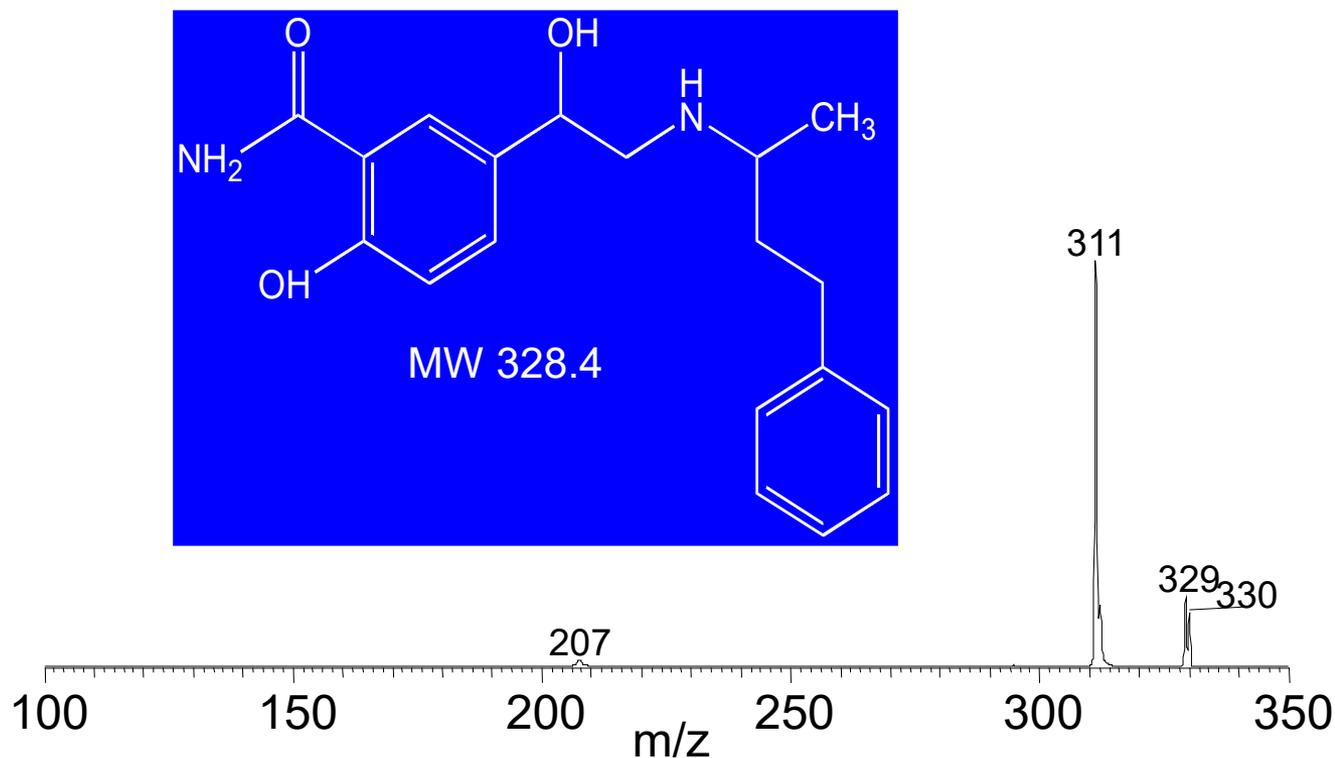


Wideband Activation



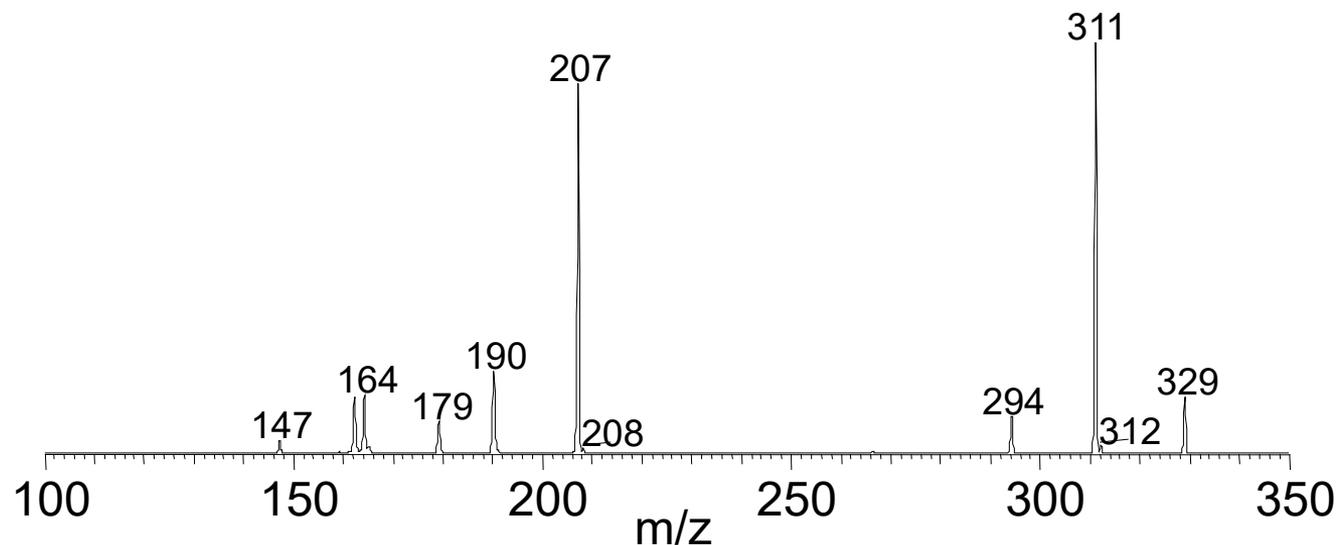
Labetalol $C_{19}H_{24}N_2O_3$ - M.W. = 328.18 Da

MS/MS of 329 m/z



Labetalol $C_{19}H_{24}N_2O_3$ - M.W. = 328.18 Da

Wideband Activation



Summary (Ion Trap Functions)

1) Collection

For Scans: All

2) Isolation

By: Ring Electrode

3) Excitation

Method: Alternating RF frequency (760 kHz) at a set amplitude along with He dampening gas traps and cools the ions to the center of the trap.

4) Ejection

Summary (Ion Trap Functions)

1) Trapping

For Scans: SIM, MSⁿ

2) Isolation

By: Endcap Electrodes

3) Excitation

Method: a) Tailored waveform applied to all ions in the trap except ion of interest

4) Ejection

b) Thus, only ions of interest remain in the trap.

Summary (Ion Trap Functions)

1) Trapping

For Scans: MS^n

2) Isolation

By: Endcap Electrodes

3) Excitation

Method: a) Cool ion of interest back to set q value (default = 0.25). b) Apply custom RF waveform in resonance with the set q value, activation time (default = 30 msec), and optimized activation amplitude.

4) Ejection

Summary (Ion Trap Functions)

1) Trapping

For Scans: All

By: Ring Electrode

2) Isolation

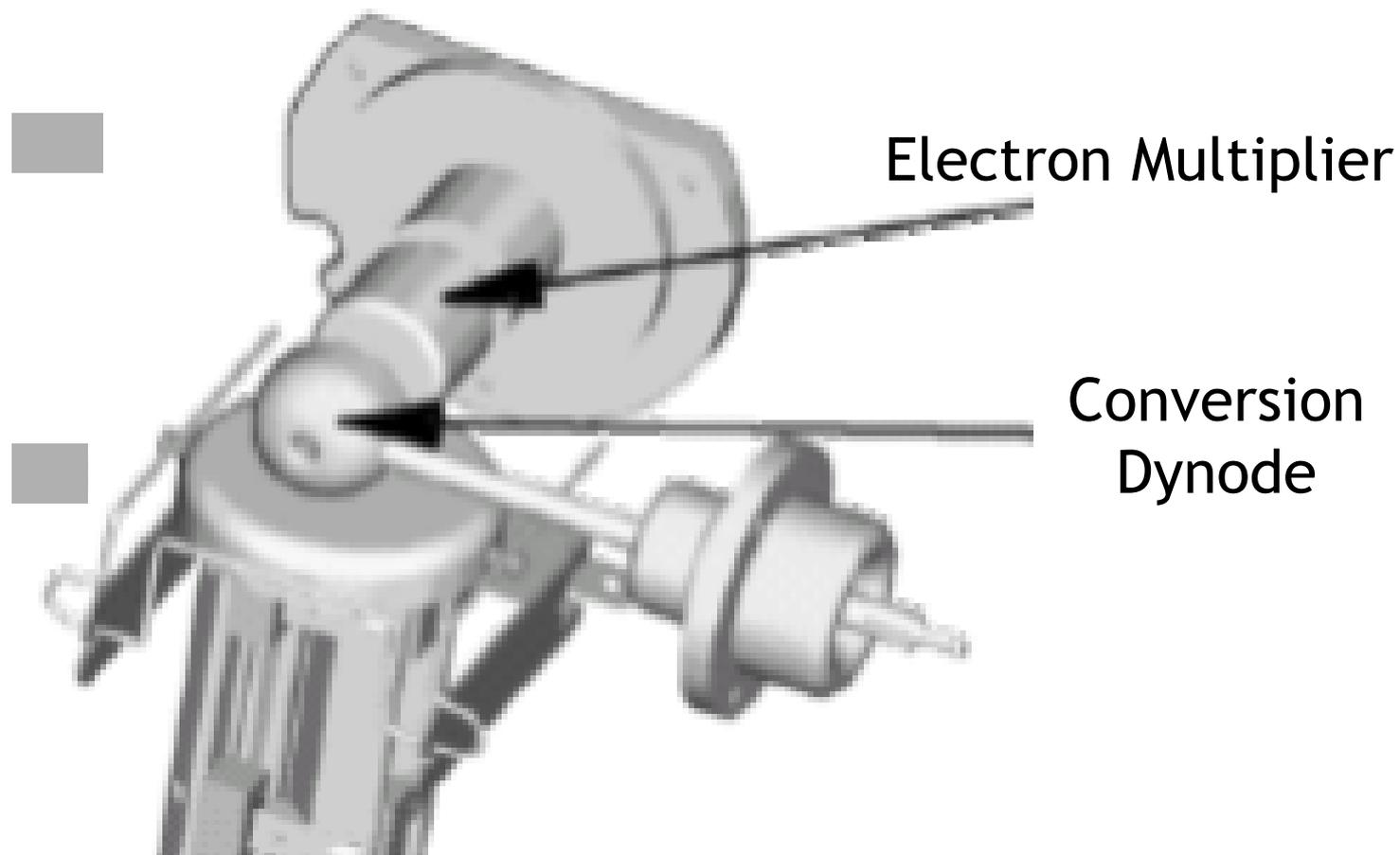
Method: Ramp ring RF power to increase the q values of all ions in desired scan range, low mass to high mass. (i.e. Mass Selective Scanning)

3) Excitation

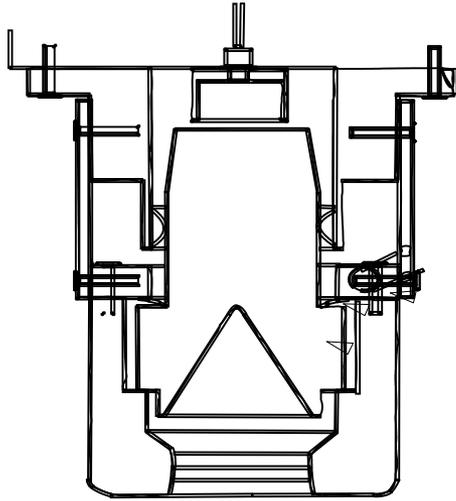
4) Ejection

Also, ramp the RF amplitude on the endcap electrodes to consolidate the ions to a group (Resonance Ejection)

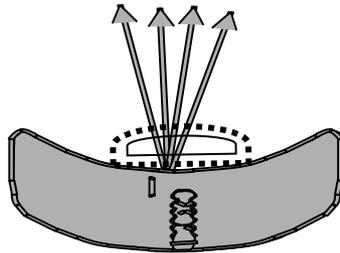
Detect



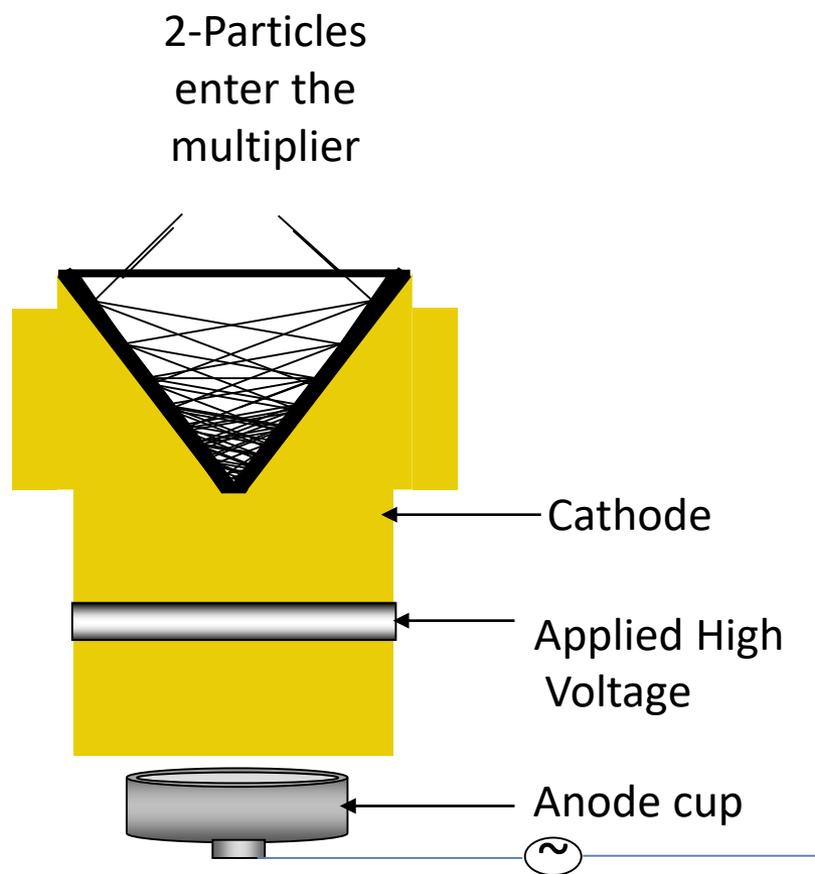
Conversion Dynode



Ions exiting the Quad hit the dynode to produce secondary particles. Dynode particles then enter the multiplier.



Electron multiplier



Each particle hits the surface of the multiplier resulting in the ejection of more particles.

The cascading effect of this process will produce a charge on the anode cup.

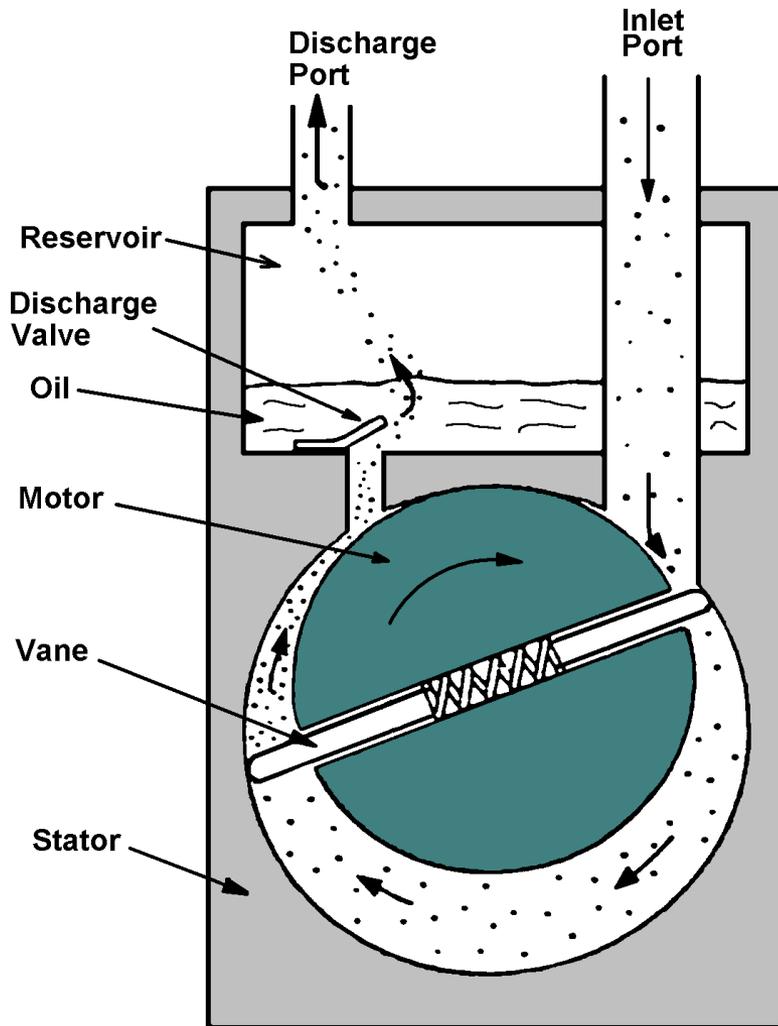
This charge represents the signal produced by the ion.

Signal to Data system.

Vacuum System

- Every mass analyzer must operate under vacuum in order to minimize both ion/molecule and molecule/molecule collisions.
- At atmospheric pressure, the mean free path of a typical ion is only ca. 52 nm; at 1 mTorr, it is 40 mm; and at 1 μ Torr, it is 40 m.
- Without vacuum, the ions produced in the source won't make it to the detector.
- Two vacuum pump types are used in the Quantum.
 - Rotary vane
 - Triple-inlet turbomolecular pump

Rough Pump (Forepump)



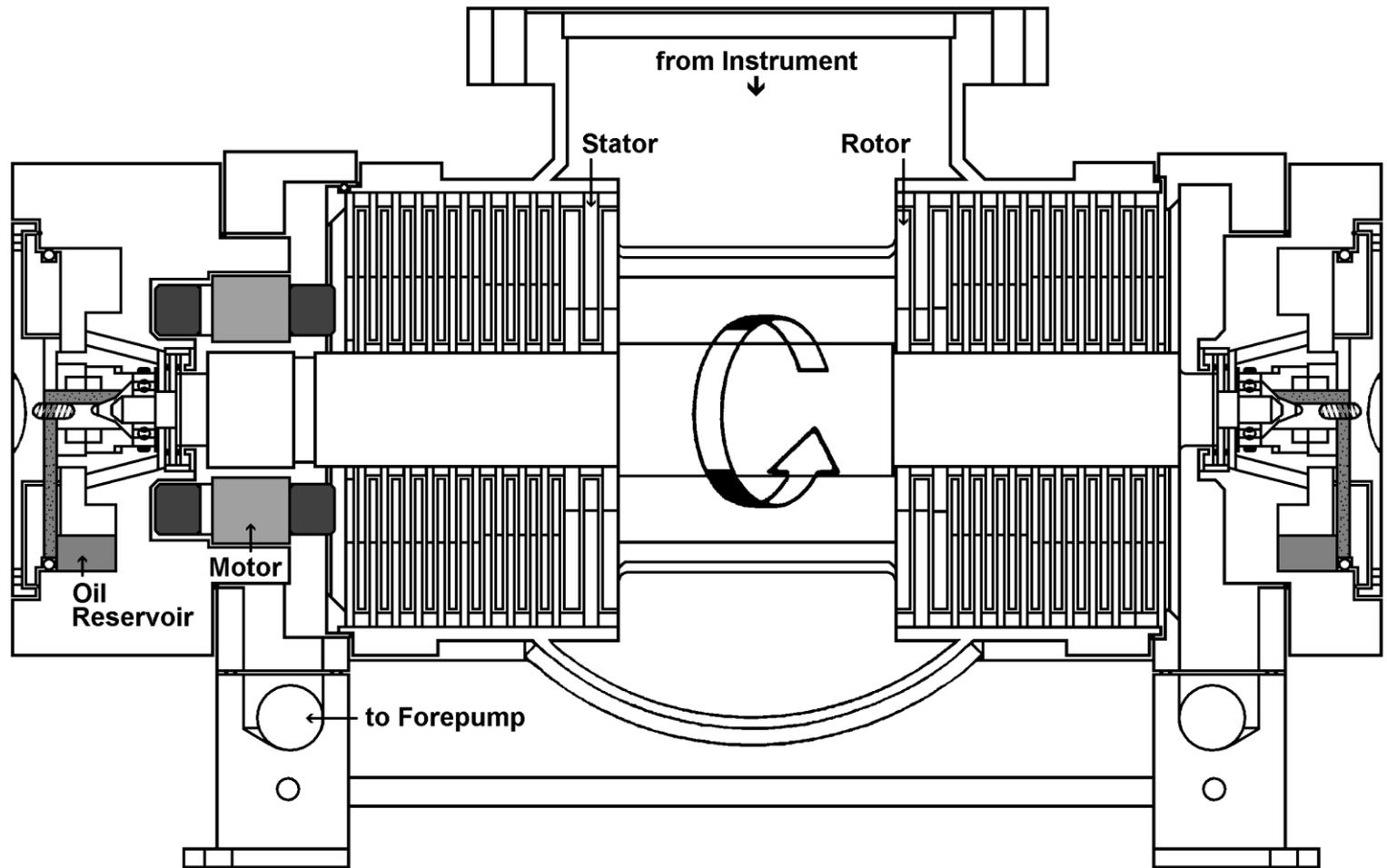
–Provides initial vacuum (ca. 1 torr), backup for higher vacuum pumps, operates inlet valves, etc.

–Low maintenance

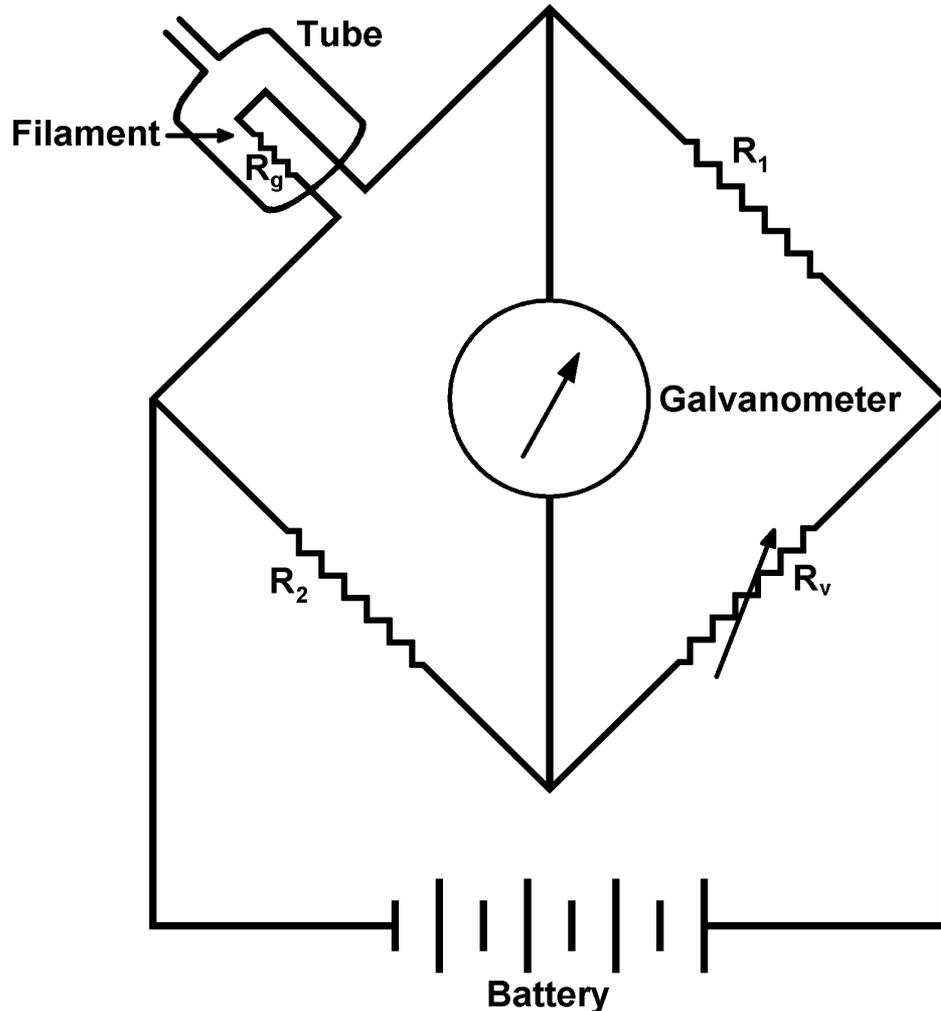
–Pump failure most often due to failure to change pump oil regularly

–Much of the sample is dissolved in the oil which becomes hazardous waste

Turbomolecular Pump



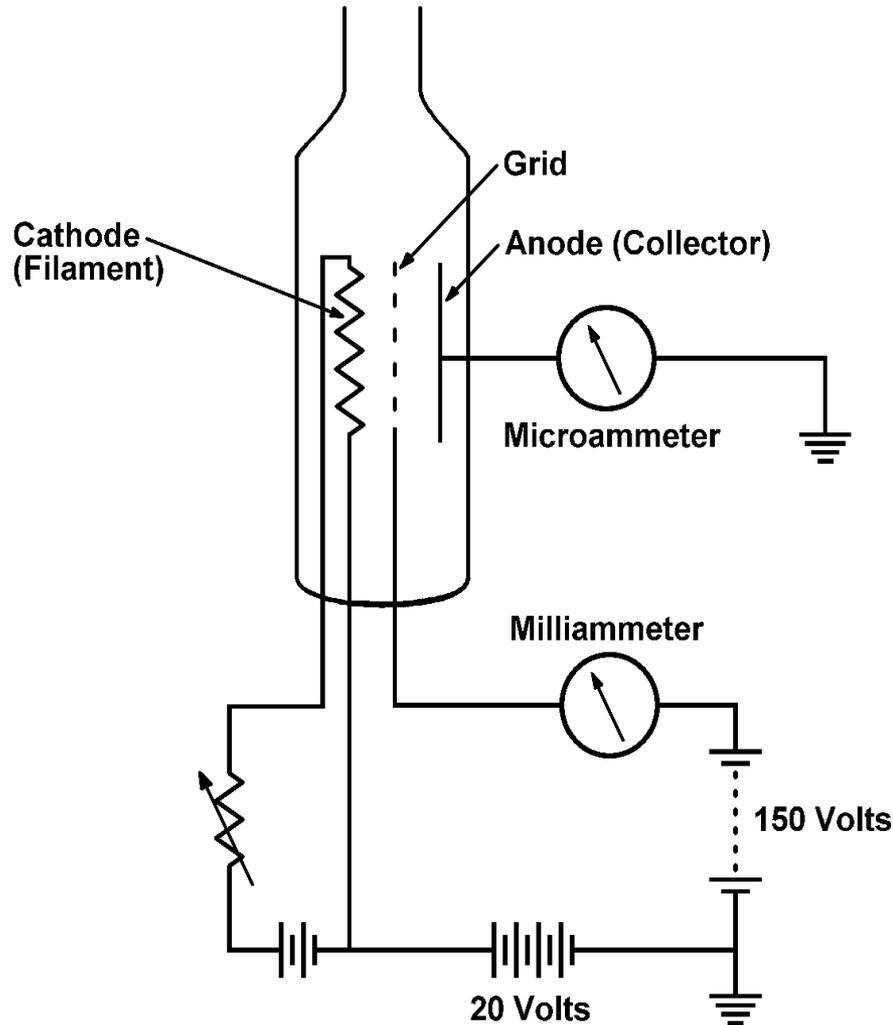
Pirani or Convectron™ Gauge



- Low vacuum measurement
- Operates on principle of the Wheatstone bridge (R_g is a thermistor)
- Rugged
- Low cost
- Convectron gauge uses operational amplifiers with large dynamic range in place of resistors

*Convectron™ is a registered trademark of Granville-Phillips

Ion Gauge

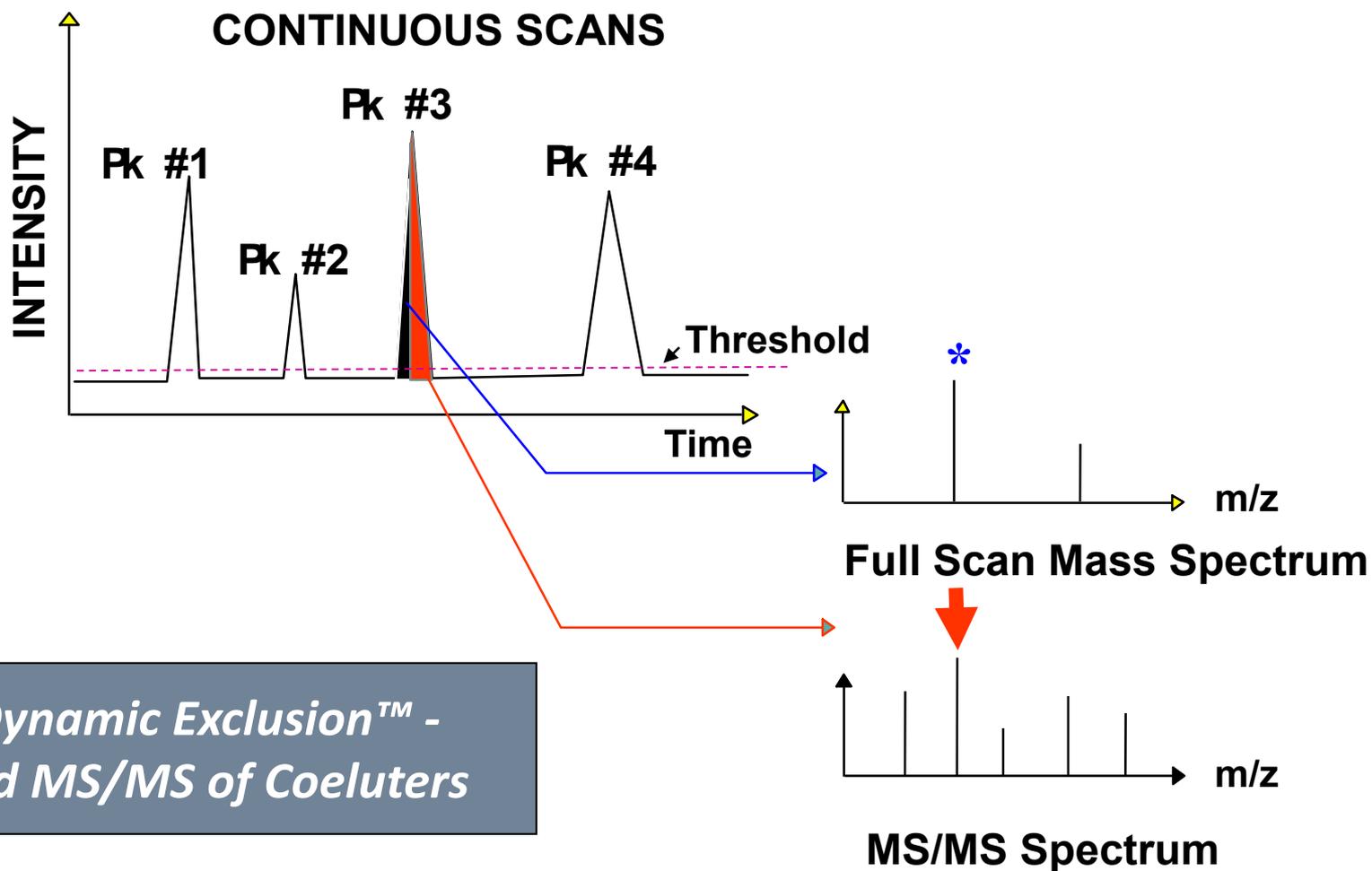


- High vacuum measurement
- Expensive
- Outgasing used to dispel contaminants
- Ions are formed at the filament and attracted toward collector
- Ions striking grid generate current

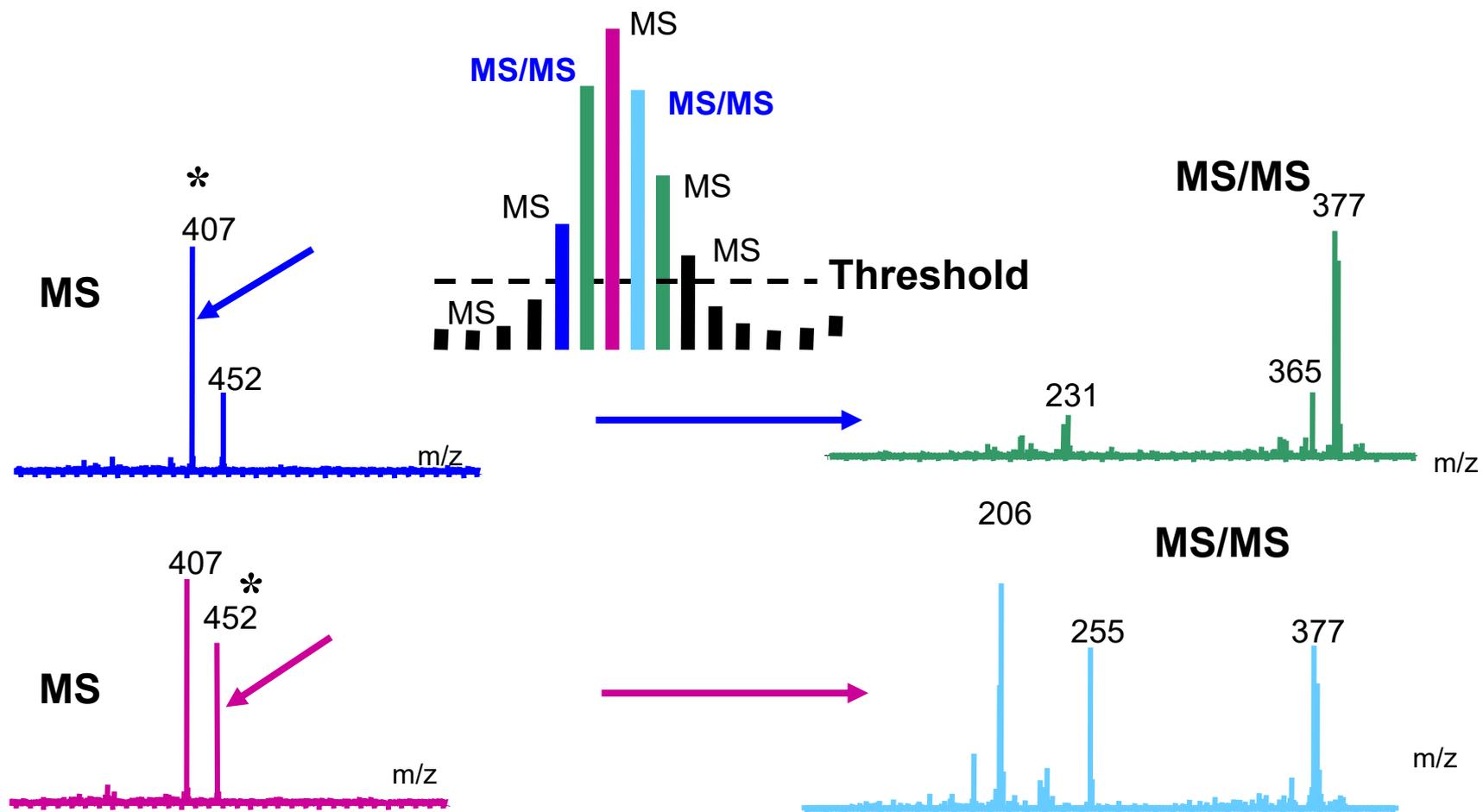
Advanced LCQ Experiments

- Data Dependent Analysis
- Dynamic Exclusion –LCQ only at present

Automated Data-Dependent Analysis



Explaining dynamic Exclusion



Maximising Counts

- Big Three Experiment:
 - Full MS with Reject List
 - Data Dependent MS² of Biggest Full MS Peak
 - Data Dependent MS² of Second Biggest Full MS Peak
 - Data Dependent MS² of Third Biggest Full MS Peak
- Turn on Dynamic Exclusion:
 - Repeat Count = 1 Repeat Duration = LC Peak Width
 - Exclusion Duration = LC Peak Width
- Set Threshold Just Above Noise Level

Maximising Scan Quality

- Use Multiple Data Dependent MS² Scans
- Set Threshold to Just Above Noise Level
- Turn Off Dynamic Exclusion
 - Guarantees the Apex of the Peak Will be Scanned When the Trap is Best Filled

Understanding Maximum IT and Microscans

● Maximum Injection Time

When a chromatographic peak is just rising off the baseline longer injection times will increase the ion population in the trap and therefore increase the spectral quality

Longer injection times may limit the amount of coeluters seen, injection times can be found determined in Qual Browser

● Microscans

Making use of more microscans can increase both the spectral quality and the scan time length

Using more Microscans can be more efficient than signal averaging

Isotopic Data Dependent MS/MS- LCQ

Compounds containing 2 chlorines

Data Dependent Settings

Global | Mass Widths | Dynamic Exclusion

Isotopic Data Dependence | Analog

Enabled

Match tolerance: 0.050

	Mass Difference	Expected Ratio
1	2.00	0.65
2		
3		
4		
5		
6		
7		
8		
9		
10		

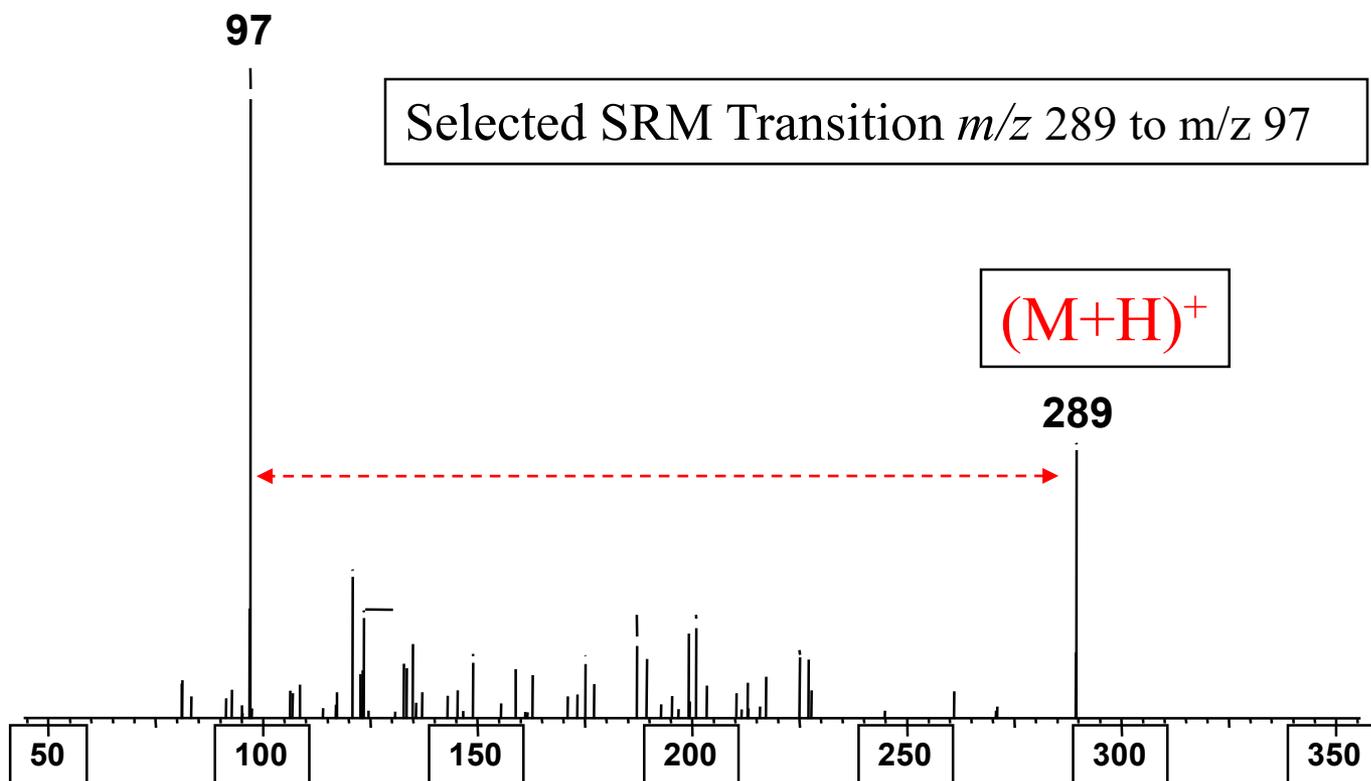
OK | Cancel | Help

Factors that effect Sensitivity

- Chromatographic conditions/Column diameters
- Source Tuning
- Molecule Fragmentation
- Collision Gas Pressure
- Collision Energy
- Resolution of Q1 and Q3
- Scan Speeds
- Internal standard type: Co-elution/Non Co-elution
- Matrix effects
- Multiplier Gain
- Number of ions being monitored
- Product ion window

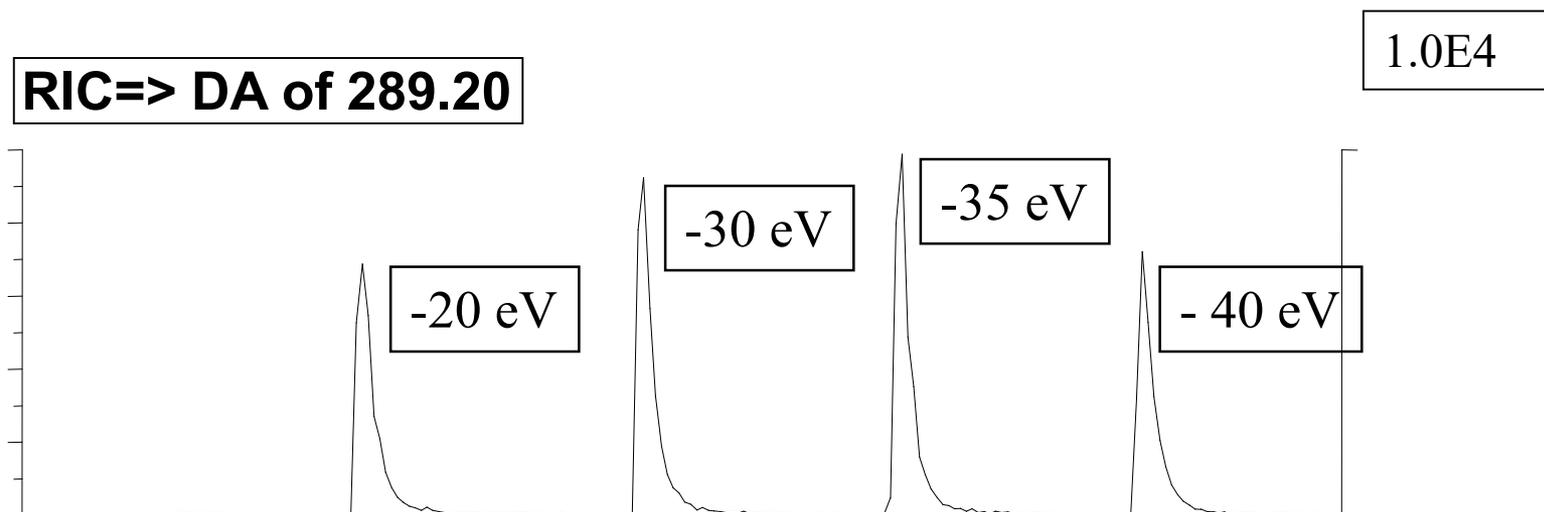
Determine the Transition

Full scan MS/MS of m/z 289 2.5 mtorr -35 eV



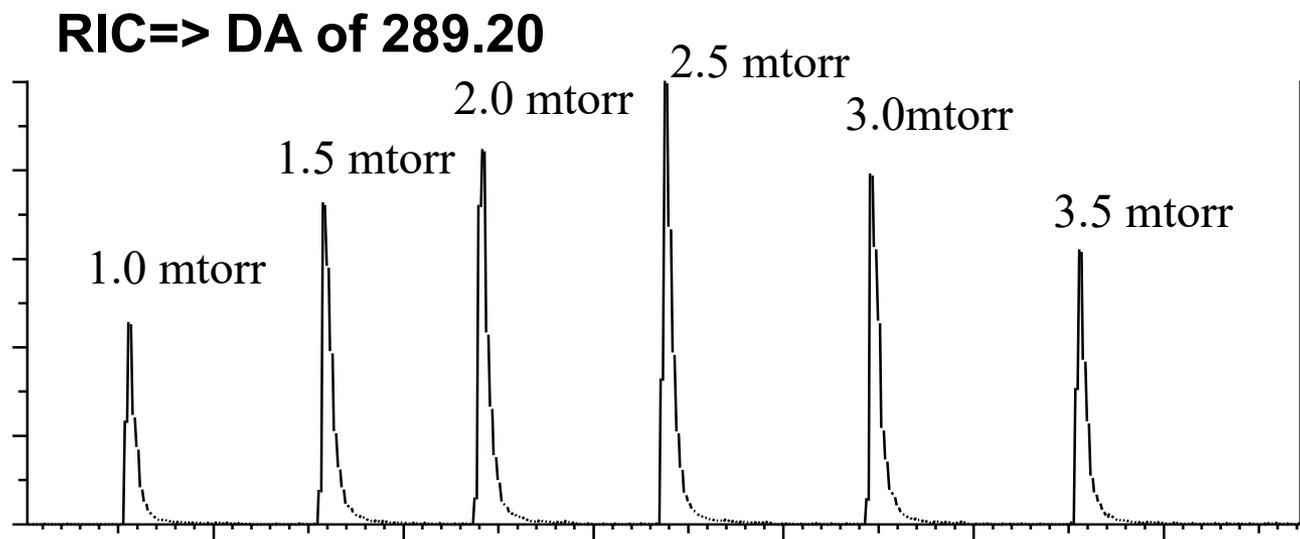
Effect of Collision Energy

**Effect of collision energy on transmission
for the m/z 289 to m/z 97 transition**



Effect of Collision Pressure

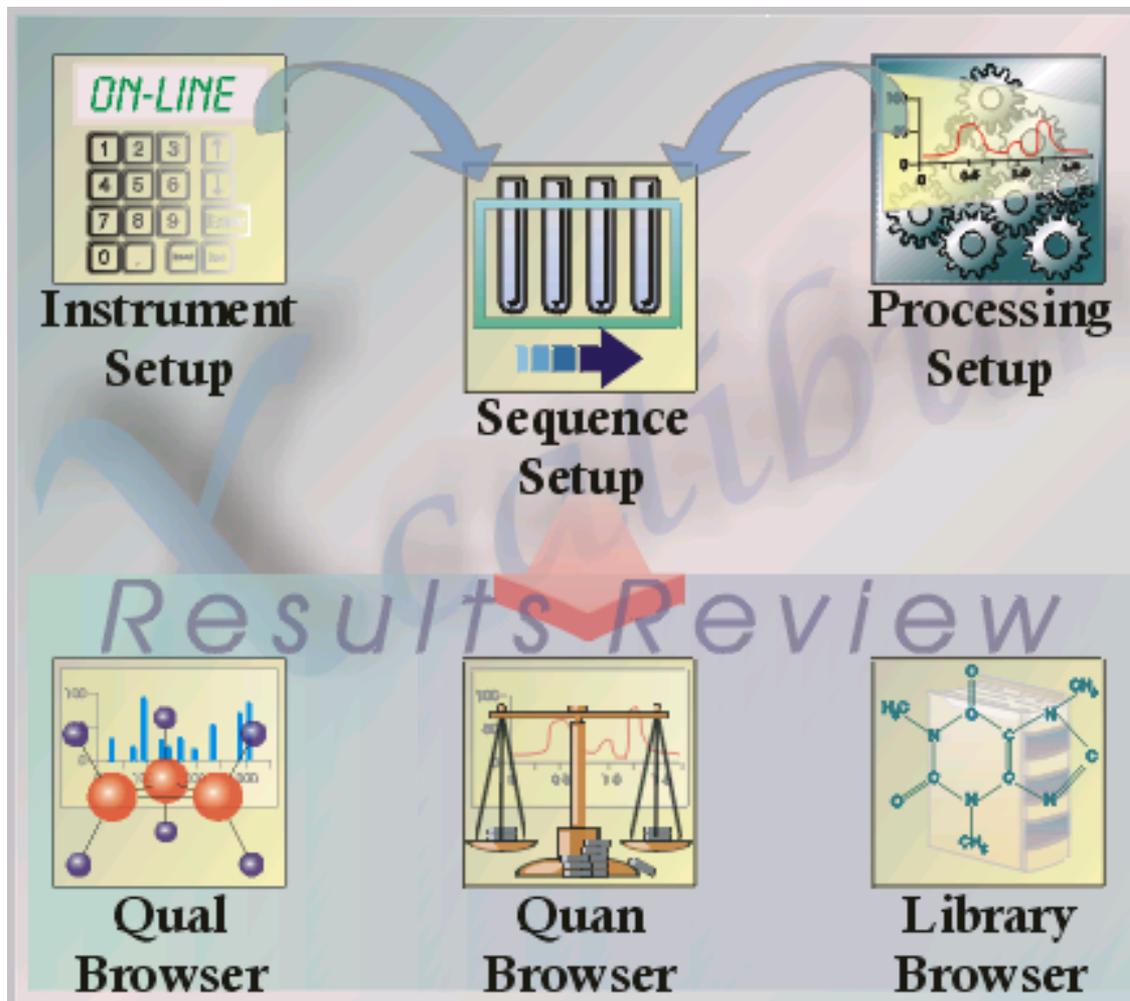
Effect of collision cell pressure on transmission at - 35 eV for the m/z 289 to m/z 97 transition



Chrom Filter

- **What is the Chrom Filter?**
- The Chrom Filter is a sophisticated noise reduction algorithm that runs in real time on the acquisition DSP on the System Control PCB. The default state on the TSQ Quantum is “on”.

Xcalibur Software

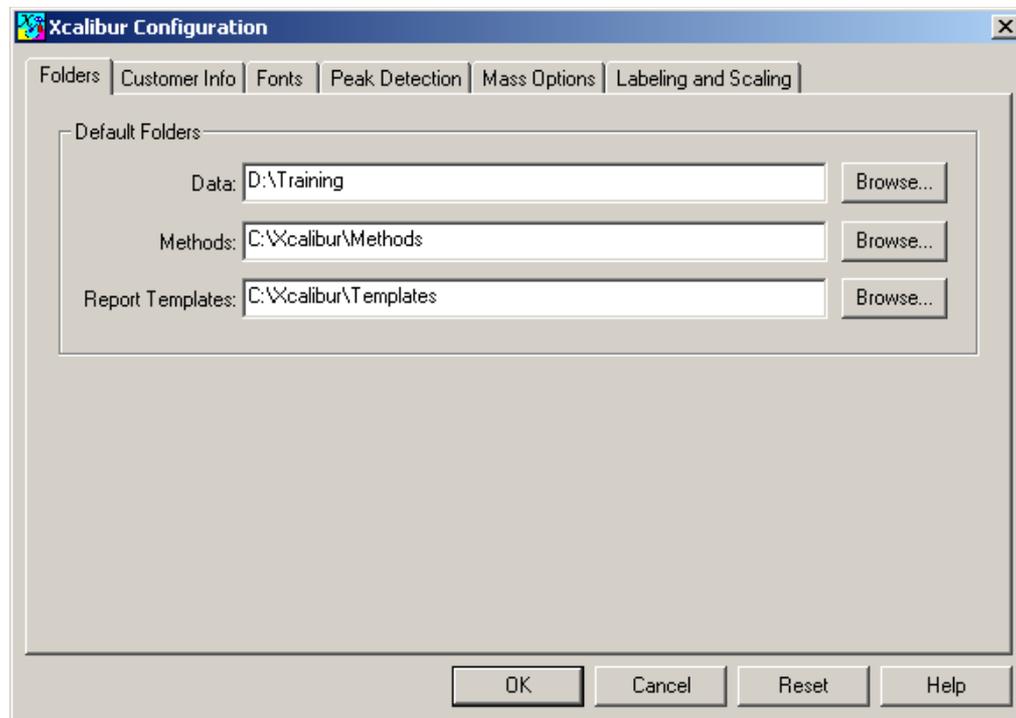
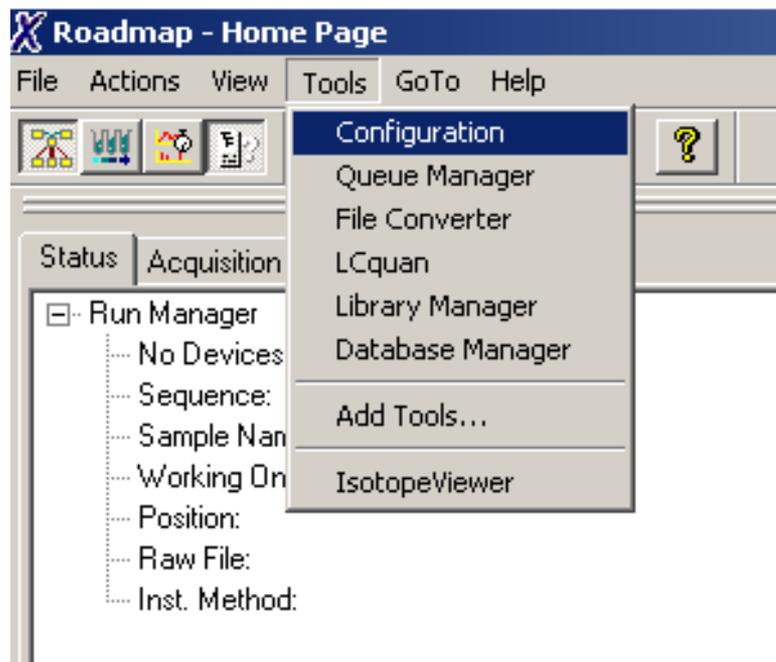


Xcalibur File Types- Reference Notes



.raw	Acquired data files
.sld	Sequence setup files
.pmd	Processing Setup method
.meth	Instrument Setup method
.rst	Result files from Quantitation
.msp	Library search Hit List and Clipboard
.lyt	Qual Browser layout
.lqn	LCQuan files
.xqn	Quan Browser files
.xcal	Non Bracket Calibration file
.doc	Word file (Merlin report)

Xcalibur Configuration



Homepage- Status View



Roadmap - Home Page

File Actions View Tools GoTo Help

Status Acquisition Queue

- Run Manager
 - Ready To Download
 - Sequence:
 - Sample Name:
 - Working Dir:
 - Position:
 - Raw File:
 - Inst. Method:
- TSQ MS
 - Ready to Download

ON-LINE

Instrument Setup

Sequence Setup

Processing Setup

Results Review

Qual Browser

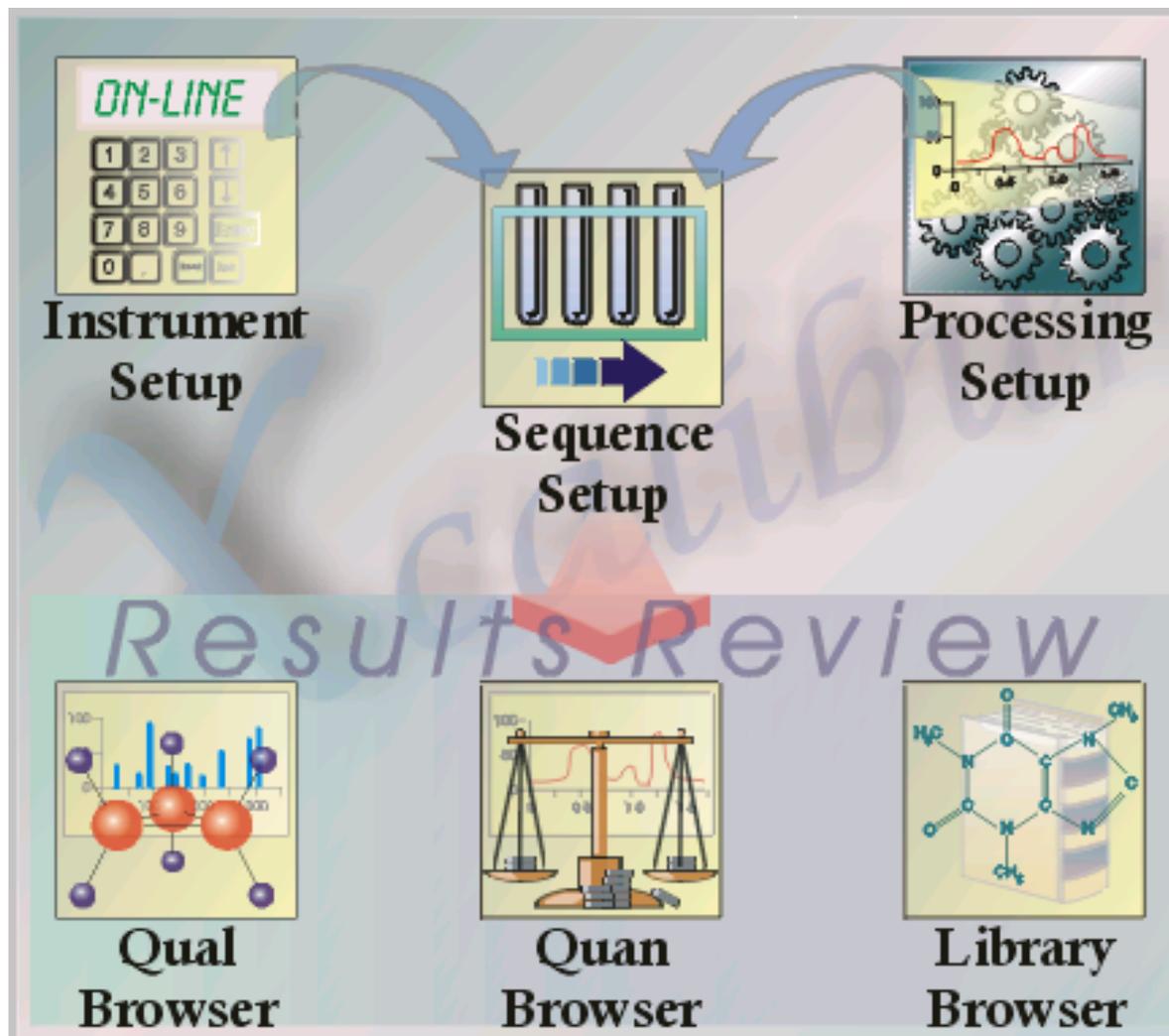
Quan Browser

Library Browser

For Help, press F1

NUM 06/19/2001 3:05 PM NOT SAVED

Xcalibur Software



Xcalibur File Types



- .raw** Acquired data files
- .sld** Sequence setup files
- .pmd** Processing Setup method
- .meth** Instrument Setup method
- .rst** Result files from Quantitation
- .msp** Library search
- .lyt** Qual Browser layout
- .lqn** LCQuan files
- .xqn** Quan Browser files
- .doc** Word file (Merlin report)

Instrument Configuration



Instrument Configuration [X]

Device Types:
All

Available Devices:	Configured Devices:
 Surveyor AS	 Surveyor AS
 Surveyor MS Pump	 Surveyor MS Pump
 Surveyor PDA	 TSQ Quantum
 TSP Autosampler	

Buttons: Add >> << Remove Configure

Buttons: Done Help

Homepage – Status View



Roadmap - Home Page

File Actions View Tools GoTo Help

Status Acquisition Queue

- Run Manager
 - Ready To Download
 - Sequence:
 - Sample Name:
 - Working On:
 - Position:
 - Raw File:
 - Inst. Method:
- TSQ MS
 - Ready to Download

Instrument Setup (ON-LINE keypad icon) → **Sequence Setup** (test tubes icon) → **Processing Setup** (gears icon)

Results Review

- Qual Browser** (molecular model icon)
- Quan Browser** (scales icon)
- Library Browser** (chemical structure icon)

For Help, press F1 | NUM | 06/19/2001 | 3:05 PM | NOT SAVED

Instrument Set-up



The screenshot displays the "Roadmap - Home Page" software interface. The main workspace contains a workflow diagram with the following components:

- Instrument Setup**: A keypad icon, circled in purple, representing the configuration of the instrument.
- Sequence Setup**: An icon showing test tubes, representing the setup of the analysis sequence.
- Processing Setup**: An icon showing a chromatogram and gears, representing the configuration of data processing parameters.
- Results Review**: A large, semi-transparent text overlay across the bottom of the workflow area.
- Qual Browser**: An icon showing a bar chart and molecular structures, representing the qualitative analysis results browser.
- Quan Browser**: An icon showing a balance scale, representing the quantitative analysis results browser.
- Library Browser**: An icon showing a chemical structure and a barcode, representing the library of reference compounds.

Arrows indicate a sequential flow from Instrument Setup to Sequence Setup, then to Processing Setup, and finally to the Results Review section. A red circle highlights a button in the top toolbar, and a red line connects it to the Instrument Setup icon.

At the bottom of the window, the status bar shows: "For Help, press F1" on the left, and "NUM | 10/02/2000 | 8:00 PM" on the right.

LCQ MS Editor



Untitled - Instrument Setup

File LCQ Deca XP Help

MS Detector Setup | Syringe Pump | Divert Valve | Contact Closure | Summary

Run settings

Acquire time (min): 4.00 Segments: 4 Start delay (min): 0.00

Open Raw File

Retention time (min)

Segment 1 Segment 2 Segment 3 Segment 4

Segment 2 settings

Segment time (min): 0.58 Scan events: 1 Tune method: C:\Xcalibur\methods\AutoTune.LCQT1...

Scan Event 1

Scan event 1 settings

Mass range: Normal High Low

Scan mode: MS MS/MS MSn Parent masses...

Scan type: Full SRM ZoomScan

SRM Range(s): 300.00-2000.00 Setup ranges...

Polarity: Positive Negative

Data type: Centroid Profile

Source fragmentation

Turn on Collision energy (V): 20.0

TurboScan

Wideband activation

Dependent scan Settings...

New method Tune Plus Help

Ready

NOT SAVED

Scan Events - Pos/Neg Switching



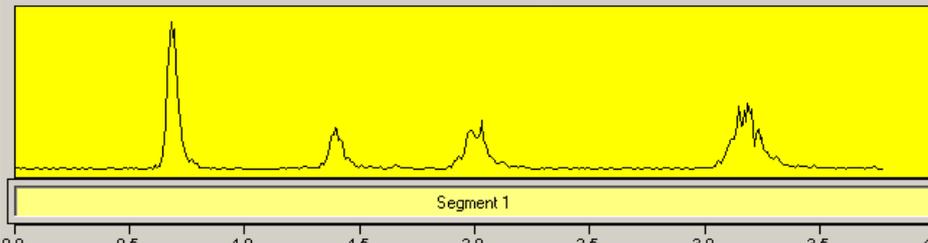
Untitled - Instrument Setup

File LCQ Deca XP Help

MS Detector Setup | Syringe Pump | Divert Valve | Contact Closure | Summary

Run settings

Acquire time (min): 4.00 Segments: 1 Start delay (min): 0.00



Retention time (min)

Segment 1 settings

Segment time (min): 4.00 Scan events: 2 Tune method: C:\Xcalibur\methods\AutoTune.LCQT...

Scan Event 1 Scan Event 2

Scan event 2 settings

Mass range: Normal High Low

Scan mode: MS MS/MS MSn

Scan type: Full SIM ZoomScan

Mass Range: 300.00-2000.00

Polarity: Positive Negative

Data type: Centroid Profile

Source fragmentation

Turn on Collision energy (V): 20.0

TurboScan

Wideband activation

Dependent scan

Ready

NOT SAVED

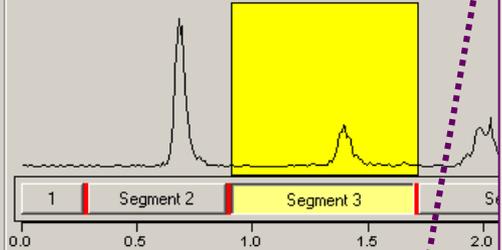
Selected Ion Monitoring



Untitled - Instrument Setup
File LCQ Deca XP Help

MS Detector Setup | Syringe Pump | Divert Valve | Contact Closure

Run settings
Acquire time (min): 4.00 Segments: 5



Retention time

Segment 3 settings
Segment time (min): 0.81 Scan events: 1

Scan event 1 settings
Mass range: Normal High Low
Scan mode: MS MS/MS MSn
Scan type: Full SIM ZoomScan
SIM Range(s): 300.00-2000.00
Polarity: Positive Negative
Data type: Centroid Profile

Parent masses...
Setup ranges...

Turn on Collision energy (V): 20.0
 TurboScan
 Wideband activation
 Dependent scan Settings...

New method Tune Plus Help

Ready

NOT SAVED

SIM Ranges

Input method
 Center/width
 From/to

Num ranges: 1

	Center Mass:	Width:
1	279.00	2.00
2	300.00	1.00
3	300.00	1.00
4	300.00	1.00
5	300.00	1.00
6	300.00	1.00
7	300.00	1.00
8	300.00	1.00
9	300.00	1.00
10	300.00	1.00

OK Cancel Help

Product Ion MS/MS



Untitled - Instrument Setup

File LCQ Deca XP Help

MS Detector Setup | Syringe Pump | Divert Valve | Contact Closure | Summary

Run settings

Acquire time (min): 4.00 Segments: 5 Start delay (min): 0.00

Segment 1 Segment 2 Segment 3

Segment 3 settings

Segment time (min): 0.81 Scan event 1

Scan Event 1 settings

Mass range: Normal High Low

Scan mode: MS MS/MS MSn Parent masses...

Scan type: Full SRM ZoomScan

Mass Range: 80.00-1000.00 Setup ranges...

Polarity: Positive Negative

Data type: Centroid Profile

Source fragmentation

Turn on Collision energy (V): 20.0

TurboScan

Wideband activation

Dependent scan Settings...

Set Parent Mass

Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q	Activation Time (msec)
279.00	2.0	30.0	0.250	30.000

OK Cancel Help

Ready

NOT SAVED

Selected Reaction Monitoring



Untitled - Instrument Setup

File TSQ Help

SRM Table

	Parent Mass	Center Mass	Width	Scan Time	Collision Energy
1	232.00	150.00	0.60	0.10	25.00
2	345.00	212.00	0.60	0.10	30.00
3	453.00	97.00	0.60	0.10	28.00
*	15.00	15.00	0.60	1.00	25.00

Segments: 1

Segment 1

Time (min)

Scan Type
 Full SIM Table SRM Table

Event 1

Polarity: Positive Negative
Data Type: Centroid Profile

MS/MS Mode: Product Parent Neutral Set Mass: 500.00

Mass Range: 150.00-550.00

Scan Time(sec): 1.00

Collision Energy(V): 35.00

Source Fragmentation
 Turn On

Collision Energy (V): 10.00

Dependent Scan Settings..

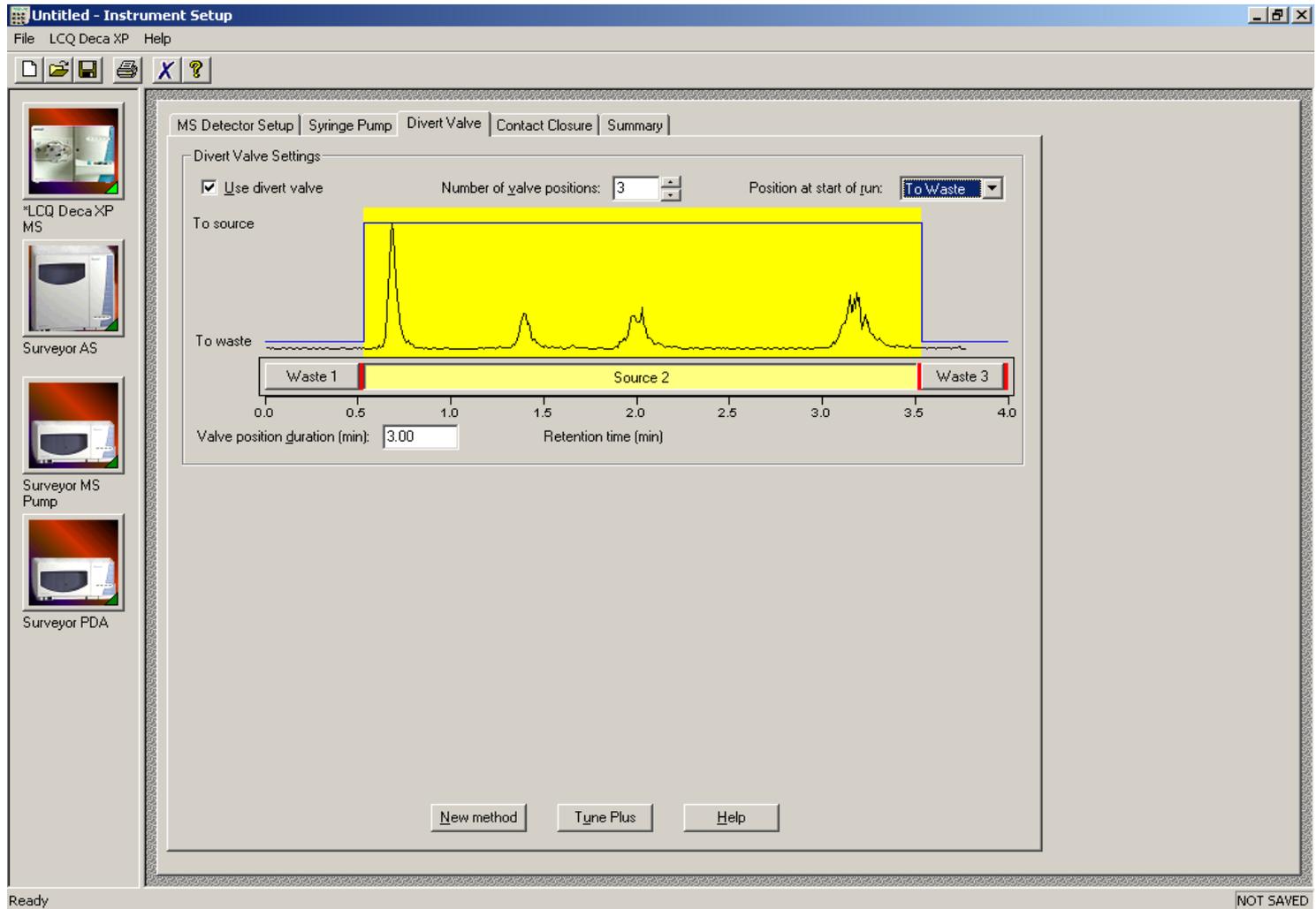
SRM Table

Help

Ready

NOT SAVED

Divert Valve Operation



Experiment Summary

The screenshot displays the 'Untitled - Instrument Setup' window with a menu bar (File, LCQ Deca XP, Help) and a toolbar. The main area is divided into a left sidebar and a central content pane. The sidebar contains icons for 'LCQ DecaXP MS', 'Surveyor AS', 'Surveyor MS Pump', and 'Surveyor PDA'. The central pane has tabs for 'MS Detector Setup', 'Syringe Pump', 'Divert Valve', 'Contact Closure', and 'Summary'. The 'Summary' tab is active, showing a 'Method summary' section with the following details:

Method summary:
Tune Method: AutoTune

Scan Event Details:
1: + c norm o(300.0-2000.0)

Segment 3 Information
Duration (min): 0.81
Number of Scan Events: 2
Tune Method: AutoTune

Scan Event Details:
1: + c norm o(279.0)->o(75.0-500.0)
MS/MS: Amp. 30.0% Q 0.250 Time 30.000 IsoW 2.0
2: + c norm o(300.0)->o(80.0-2000.0)
MS/MS: Amp. 20.0% Q 0.250 Time 30.000 IsoW 1.0

Segment 4 Information
Duration (min): 0.91
Number of Scan Events: 1
Tune Method: AutoTune

Scan Event Details:
1: + c norm o(300.0-2000.0)

Segment 5 Information
Duration (min): 1.37
Number of Scan Events: 1
Tune Method: AutoTune

Scan Event Details:

At the bottom of the central pane are buttons for 'New method', 'Tune Plus', and 'Help'. The status bar at the bottom left shows 'Ready' and the bottom right shows 'NOT SAVED'.

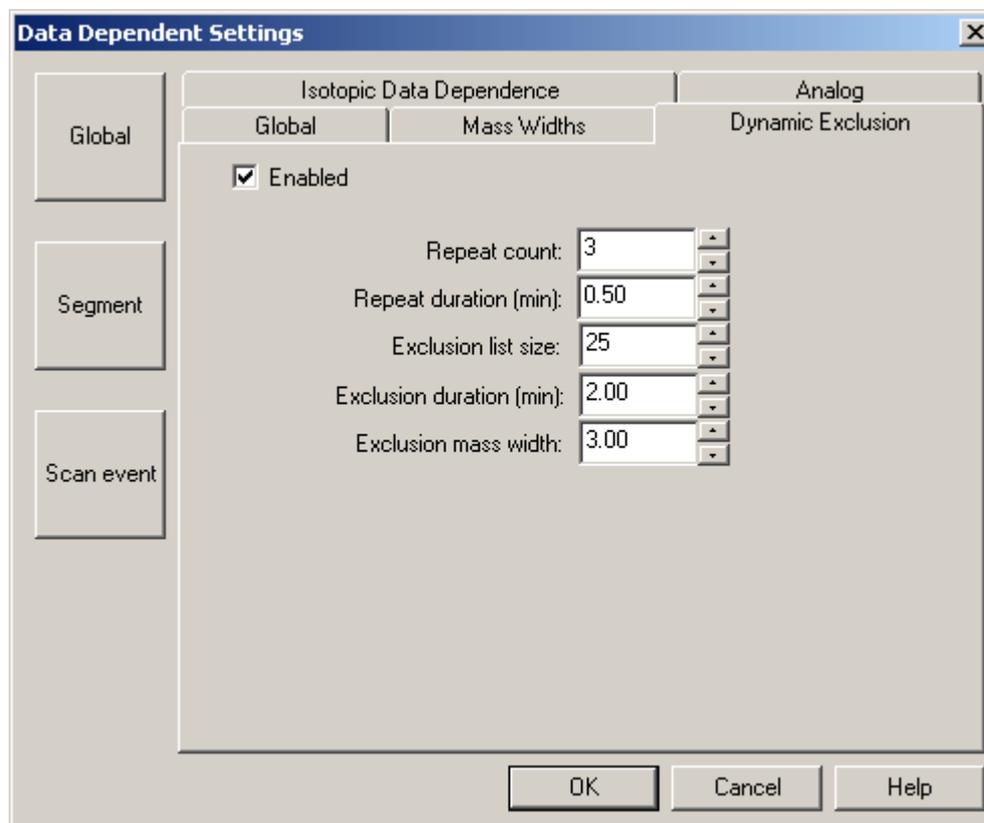
Xcalibur Data-Dependent Method Set-Up

The screenshot displays the 'Untitled - Instrument Setup' window for an LCQ Deca XP MS. The interface is divided into several sections:

- Run settings:** Acquire time (min): 10.00, Segments: 1, Start delay (min): 0.00.
- MS Detector Setup:** Syringe Pump, Divert Valve, Contact Closure, Summary.
- Segment 1 settings:** Segment time (min): 10.00, Scan events: 2, Tune method: C:\Xcalibur\methods\AutoTune.LCQT...
- Scan Event 2 settings:** Scan mode: MS/MS, Scan type: Full, Mass Range: 300.00-2000.00, Polarity: Positive, Data type: Centroid. The 'Dependent scan' checkbox is checked and circled in blue.
- Data Dependent Settings (Global):** Current Scan Event: Mass determined from scan event: 1, Nth most intense ion: 1.
- Data Dependent Settings (Segment):** Current Segment: Add/Sub, Parent masses, Reject masses, Normalized collision energy (%): 25.0, Default charge state: 1, Min MS signal (10⁴ counts): 10.0, Isolation width (m/z): 2.0.

Buttons at the bottom include 'New method', 'Tune Plus', 'Help', 'OK', 'Cancel', and 'Help'. The status bar shows 'Ready' and 'NOT SAVED'.

Using Dynamic Exclusion



Isotopic Data Dependent MS/MS

Compounds containing 2 chlorines

Data Dependent Settings

Global | Mass Widths | Dynamic Exclusion

Isotopic Data Dependence | Analog

Enabled

Match tolerance: 0.050

	Mass Difference	Expected Ratio
1	2.00	0.65
2		
3		
4		
5		
6		
7		
8		
9		
10		

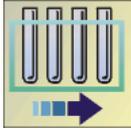
OK | Cancel | Help

Xcalibur Homepage- Sequence Set Up



The screenshot displays the 'Roadmap - Home Page' software interface. The window title bar reads 'Roadmap - Home Page' and includes standard window controls. The menu bar contains 'File', 'Actions', 'View', 'Tools', 'GoTo', and 'Help'. The toolbar features icons for file operations and execution. The main workspace contains a central menu with six options: 'Instrument Setup', 'Sequence Setup', 'Processing Setup', 'Qual Browser', 'Quan Browser', and 'Library Browser'. The 'Sequence Setup' icon, which depicts a vial rack, is circled in purple. A purple line originates from the top-left 'X' and points to the 'Sequence Setup' icon. The text 'Results Review' is faintly visible in the background. The status bar at the bottom shows 'For Help, press F1', a 'NUM' indicator, and the date and time '10/02/2000 | 8:00 PM'.

Sequence Set Up

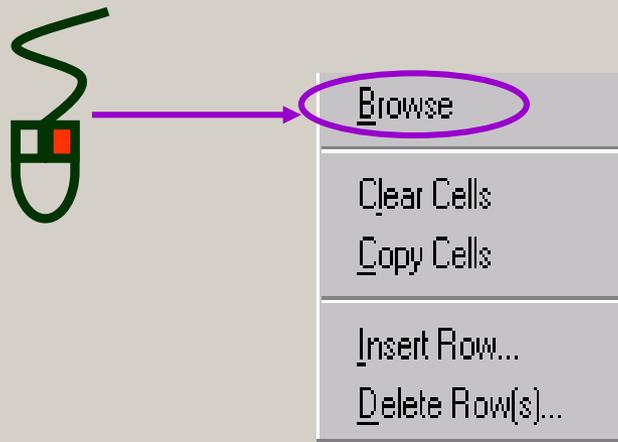


Untitled [Open] - Sequence Setup

File Edit Change Actions View GoTo Help

Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	osittio	Inj Vol	Level
*							0.1	

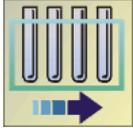
F2 or



For Help, press F1

20/06/2001 09:16

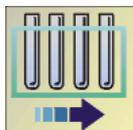
Sequence Set Up



	File Name	Path	Inst Meth	Inj Vol	Position	Comment
1	file01	D:\Alprazolam\April201	D:\Alprazolam\April2001\Alpr.	10.00	1	sample 1
2	file02	D:\Alprazolam\April201	D:\Alprazolam\April2001\Alpr.	10.00	2	sample 2
3	file03	D:\Alprazolam\April201	D:\Alprazolam\April2001\Alpr.	10.00	3	sample 3
4	file04	D:\Alprazolam\April201	D:\Alprazolam\April2001\Alpr.	10.00	4	sample 4
5	file05	D:\Alprazolam\April201	D:\Alprazolam\April2001\Alpr.	10.00	5	sample 5
*				0.00		

- Setup Sample Properties
 - Name, type, ID, lab info, and any comments
- Instrument Setup
 - Instrument settings, sample location, and injection volume
- Data processing
 - Post acquisition processing, std concentrations, calibration

New Sequence Template



File Edit Change Actions View GoTo Help

- New... Ctrl+N
- Open... Ctrl+O
- Save Ctrl+S
- Save As...
- Summary Info...
- Import Sequence... Ctrl+I
- Export Sequence... Ctrl+E
- Print... Ctrl+P
- Print Preview...
- Print Setup...
- 1 Demo_Sequence.sld
- 2 temp.sld
- Exit

New Sequence Template

General

Base File Name: Starting Number:

Path:

Instrument Method:

Processing Method:

Calibration File:

Samples

Number of Samples: Tray Type:

Injections per Sample: Initial Vial Position: Re-Use Vial Positions

Base Sample ID:

Bracket Type

None Open Non-Overlapped Overlapped

Calibration

Add Standards
Number of brackets:
Injections per Level:

Add Blanks

Fill in Sample ID for Standards

QC

Add QCs

After First Calibration Only

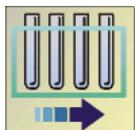
After Every Calibration

Add Blanks

Fill in Sample ID for QCs

	Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth
1	Unknown	Qualmix01	1	C:\Xcalibur\Data	C:\Xcalibur\methods\training	C:\Xcalibur\methods\qual-training
2	Unknown	Qualmix02	2	C:\Xcalibur\Data	C:\Xcalibur\methods\training	C:\Xcalibur\methods\qual-training
3	Unknown	Qualmix03	3	C:\Xcalibur\Data	C:\Xcalibur\methods\training	C:\Xcalibur\methods\qual-training
4	Unknown	Qualmix04	4	C:\Xcalibur\Data	C:\Xcalibur\methods\training	C:\Xcalibur\methods\qual-training
5	Unknown	Qualmix05	5	C:\Xcalibur\Data	C:\Xcalibur\methods\training	C:\Xcalibur\methods\qual-training

Sequence Column Arrangement



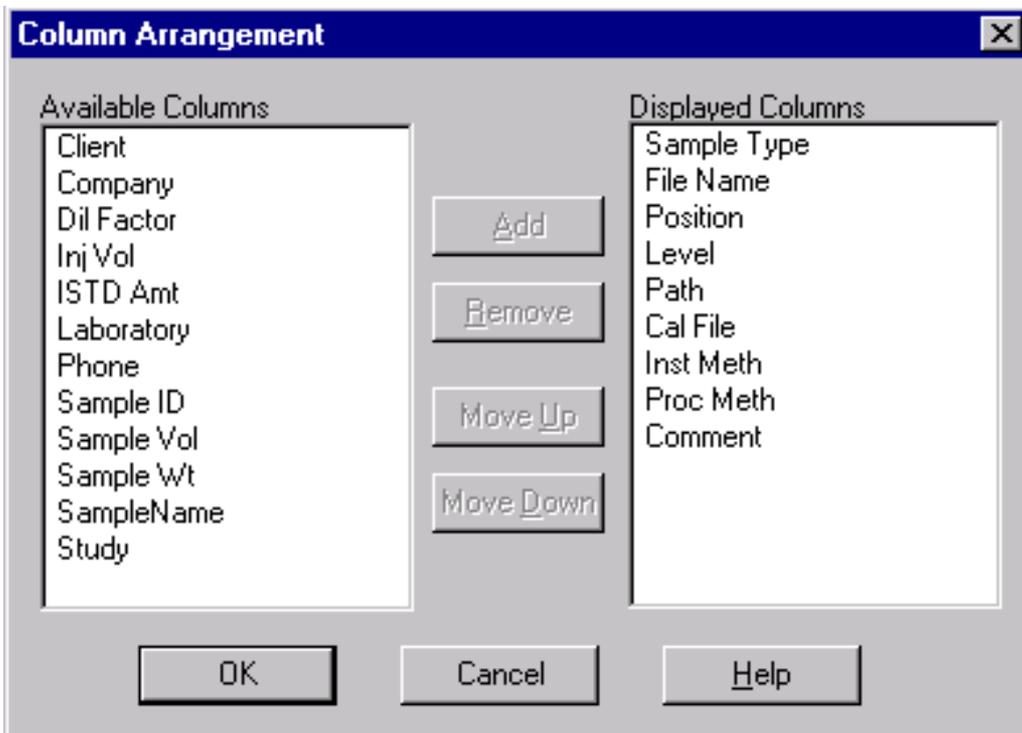
File Edit Change Actions View GoTo Help

User Labels...

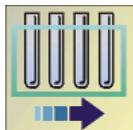
Tray Name...

Column Arrangement...

Transfer Row Info...



Changing User Labels



File Edit Change Actions View GoTo Help

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

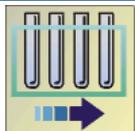
User Labels [X]

Heading 1	PCBs in soil
Heading 2	EnviroCorp
Heading 3	London
Heading 4	Dr J Smith
Heading 5	0579 362984

Default Headings

OK Cancel Help

Running the Sequence



Autosampler

Run Sequence

Acquisition Options

Instrument: TSQ MS, Surveyor AS, Surveyor MS Pump

Start Instrument: Yes

User: Administrator

Run Rows: 1-13

Start When Ready

Change Instruments...

Instrument Method

Start Up: [] Browse...

Shut Down: [] Browse...

Processing Actions

Priority Sequence

Quan

Qual

Reports

Programs

Create Quan Summary

Programs

Pre Acquisition: [] Browse...

Post Acquisition: [] Browse...

Run Synchronously

Pre Acquisition Post Acquisition

After Sequence Set System:

On Standby Off

OK Cancel Help

Manual/contact closure

Run Sequence

Acquisition Options

Instrument: TSQ MS, Surveyor MS Pump

Start Instrument

User: Administrator

Run Rows: 1-13

Priority Sequence

Processing Actions

Quan

Qual

Reports

Programs

Create Quan Summary

Programs

Pre Acquisition: [] Browse...

Post Acquisition: [] Browse...

Run Synchronously

Pre Acquisition Post Acquisition

After Sequence Set System:

On Standby Off

OK Cancel Help

Change Instruments In Use

Instrument	In Use	Start Instrument
TSQ MS	Yes	
Surveyor AS		Yes
Surveyor MS Pump		Yes

OK Cancel Help

The Info Bar



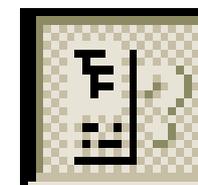
Status

The 'Status' window displays system information and operational parameters. It is divided into several sections:

- Run Manager:** Shows details for the current run, including the sequence file path, sample name, working directory, position, raw file path, and instrument method.
- Hardware Status:** Lists the AS2000 Autosampler (Ready for Run) and the TRACE GC 2000 (Preparing for Run).
- Temperature, Flow, Pressure:** A sub-panel with a 'General' section showing 'Status: Not ready'.
- Run:** Displays 'Elapsed time: 0.00' and 'Remaining time: 13.67'.
- Emergency conditions:** A list of checkboxes for 'Emergency shutdown', 'Over temperature', 'Shorted RTD', and 'Shorted oven RTD', all currently unchecked.
- Keypad...:** A button at the bottom for accessing the keypad.

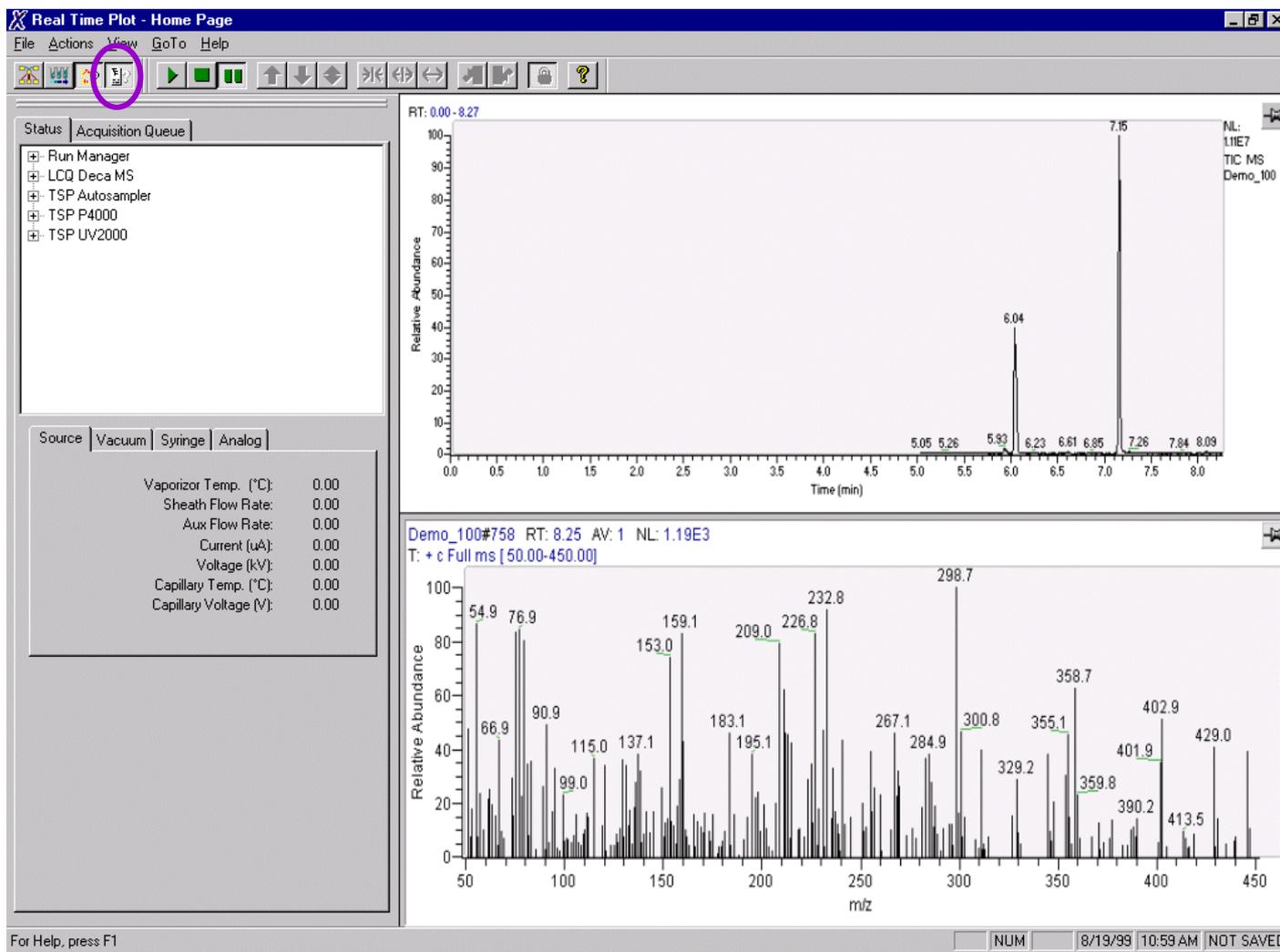
Sequence Progress

The 'Sequence Progress' window shows the status of the acquisition queue. It features a tree view under 'All Sequences' with a sub-entry for 'Temporary sequence'. Below this, five 'Sequence Row' entries are listed, each with a progress indicator (a vertical bar) and a status icon (a small blue and red bar). The first row, 'Sequence Row #1', is highlighted in yellow, indicating it is the current or next row to be processed.

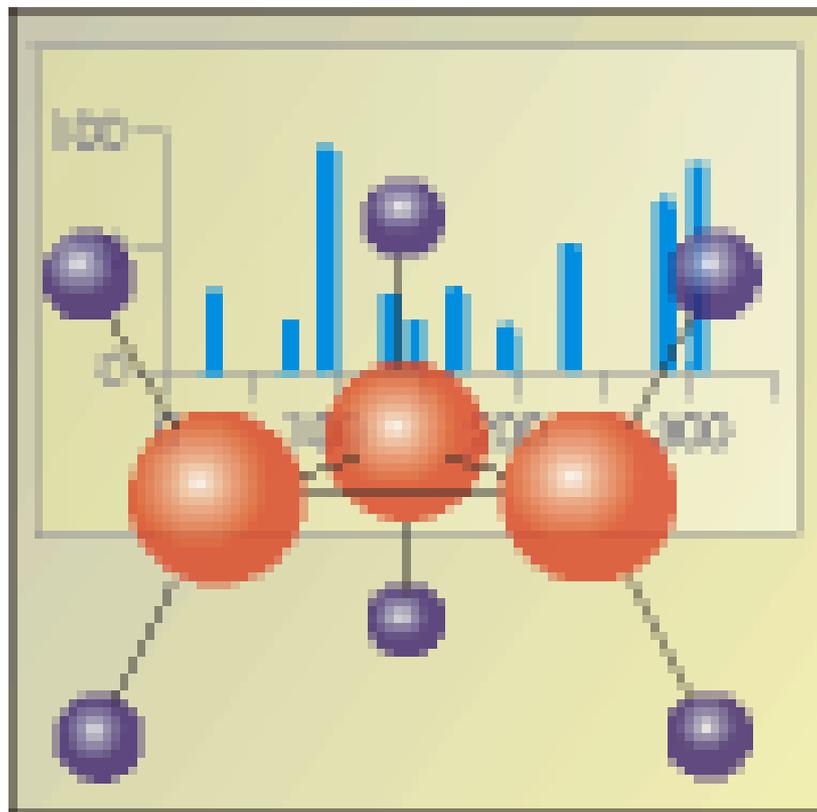


Info On/Off

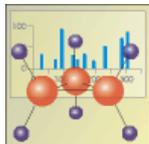
Homepage- Real Time plot Display



Qual Browser



Qual Browser



The screenshot shows the 'Roadmap - Home Page' software interface. The window title is 'Roadmap - Home Page' and the menu bar includes 'File', 'Actions', 'View', 'Tools', 'GoTo', and 'Help'. The main area contains a central diagram with the following components:

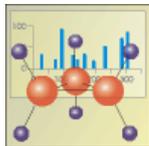
- Instrument Setup**: A keypad icon with 'ON-LINE' text above it.
- Sequence Setup**: An icon of a test tube rack with a blue arrow pointing right.
- Processing Setup**: An icon of a chromatogram with gears.
- Results Review**: A large, semi-transparent text overlay in the center.
- Qual Browser**: An icon of a chromatogram with a peak highlighted.
- Library Browser**: An icon of a chemical structure.

A purple arrow points from the left towards the 'Qual Browser' icon, which has a context menu open over it. The menu items are:

- Open Last Acquired Raw File
- Open Sequence In Progress
- Open Last Sequence Completed
- Open Last Reprocessed Sequence

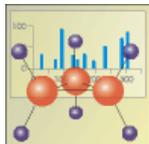
At the bottom of the window, there is a status bar with the text 'Open the last acquired raw file in Qual Browser'. The taskbar shows the Start button, the 'Roadmap - Home Page' window, and the system tray with the date '06/20/2001', time '10:01 AM', and 'NOT SAVED'.

Qual Browser Functions



- Peak Integration
- View Spectra
- View MSⁿ Browser data- option
- Submit Spectrum for Library Search
- Create and Save Layouts
- Annotate and Print Chromatograms/Spectra

Qual Browser Tool Bars



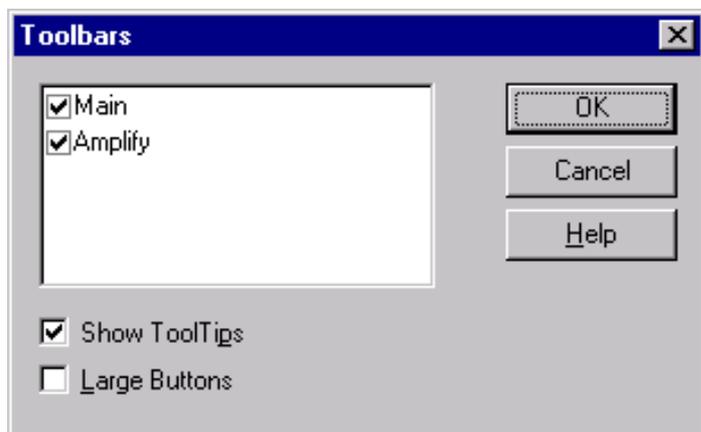
Main Toolbar



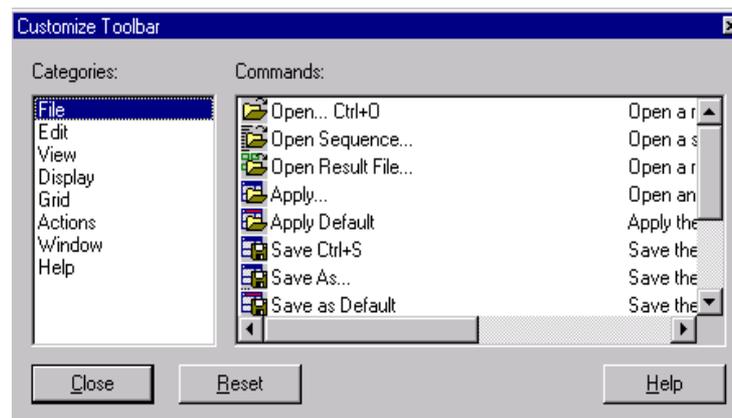
Amplify Toolbar



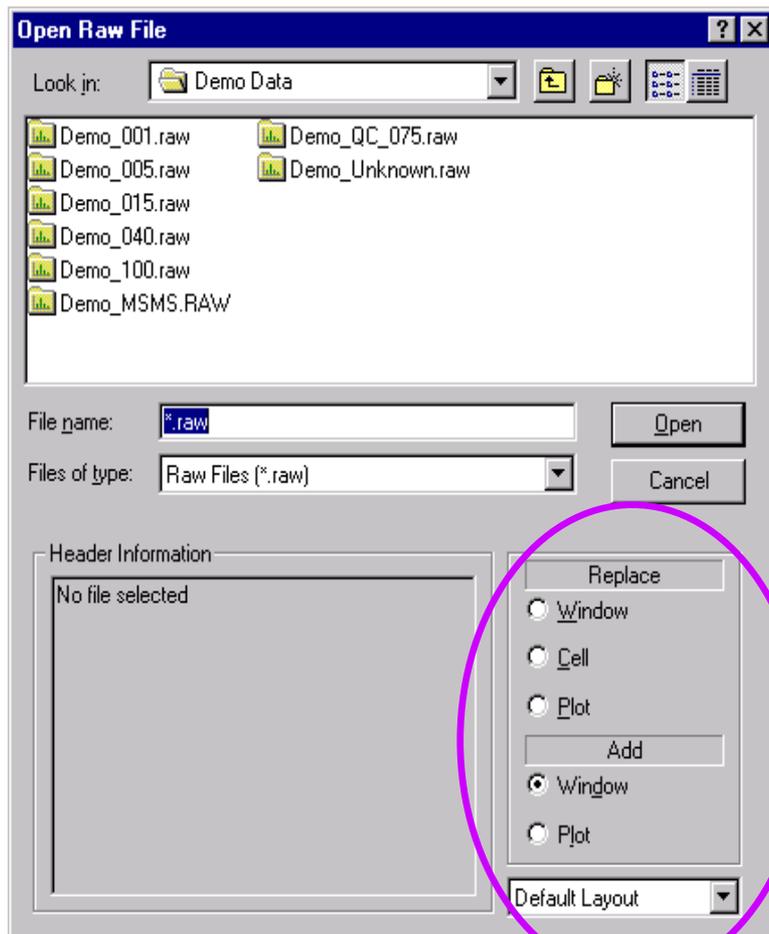
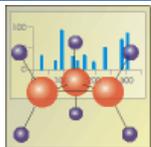
View: Toolbars



View: Customise Toolbars

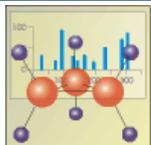


Opening Raw Files

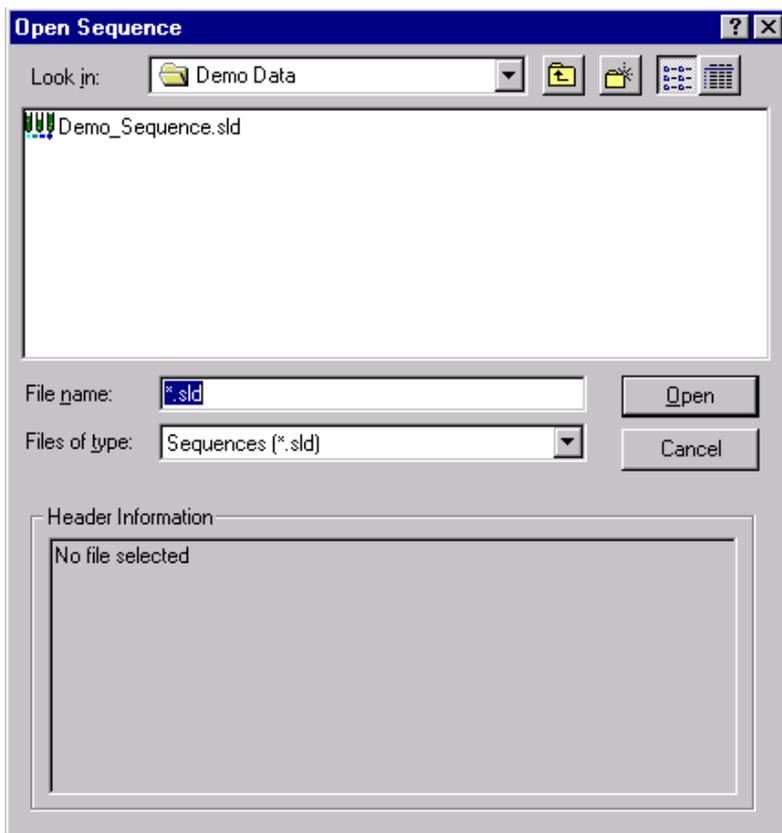


- **Replace:**
Select to replace the active **Window, Cell, or Plot** in the **Grid** with the new data
- **Add:**
Select to add a new **Window or Plot** to the **Grid** containing the new data
- **Default:**
Default or Current layout

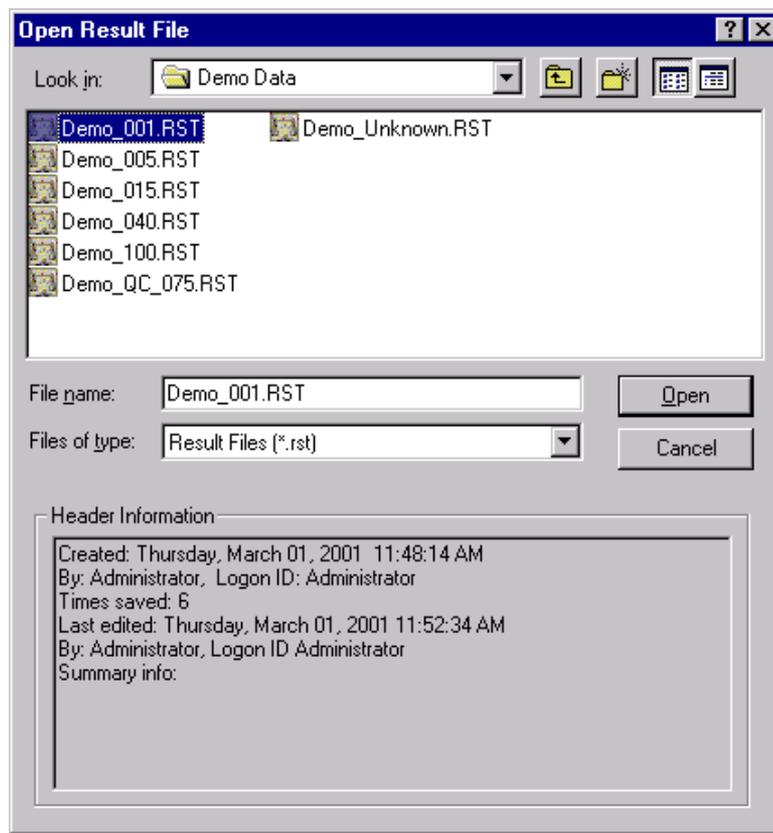
Other Qual Browser File Types



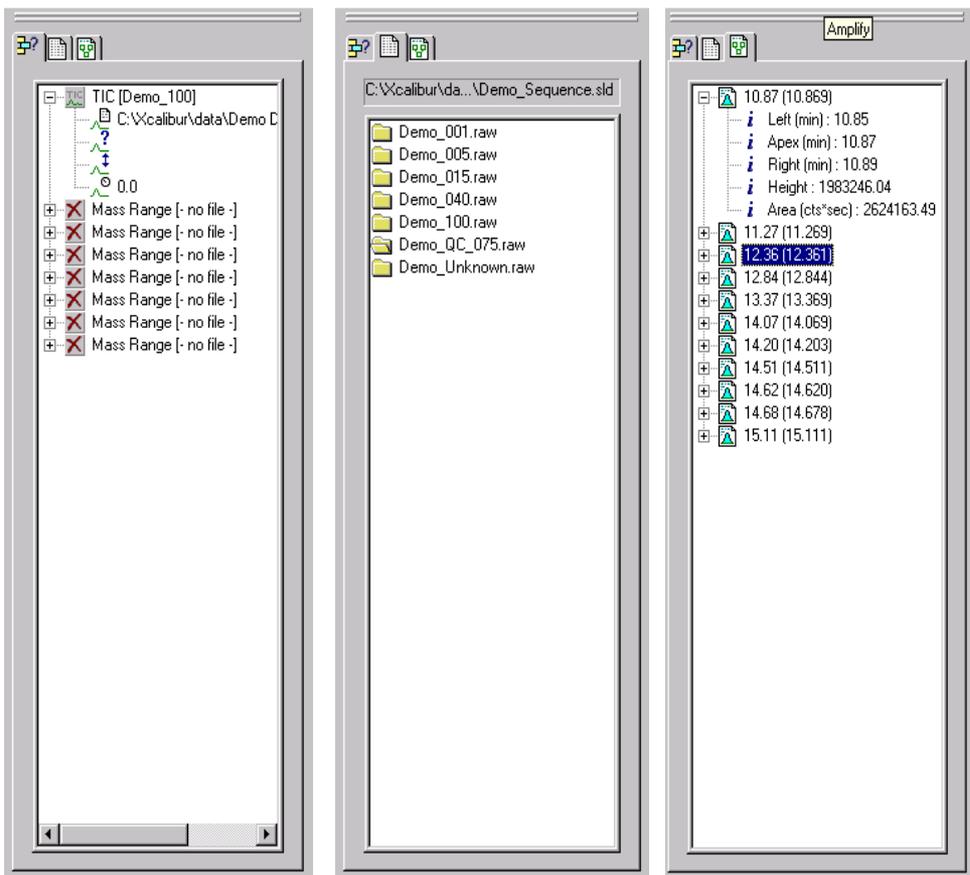
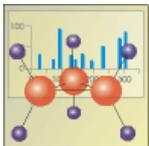
Sequence (.sld)



Result (.rst)



Info Bar



Cell Info

Contains information for each of the plots within the active grid cell

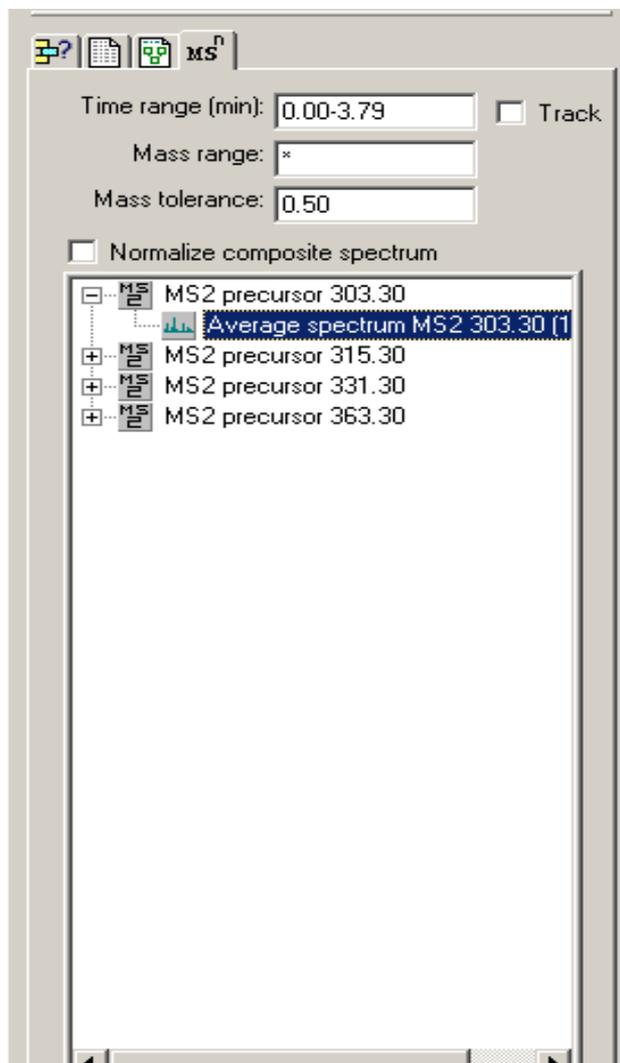
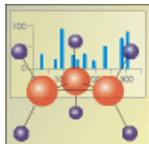
Sample Sequence

Contains a pointer to all raw files contained in the opened sequence

Result File

Contains information about each auto-detected peak in the chromatogram after being processed

Info Bar: MSⁿ Browser



The screenshot shows the MSⁿ Browser interface with the following settings and data:

- Time range (min): 0.00-3.79 Track
- Mass range: *
- Mass tolerance: 0.50
- Normalize composite spectrum
- Tree view:
 - MS2 precursor 303.30
 - Average spectrum MS2 303.30 [1]
 - MS2 precursor 315.30
 - MS2 precursor 331.30
 - MS2 precursor 363.30

MSⁿ Browser

View MSⁿ experiment filters and spectra

Info Bar: Peak Integration Parameters

The screenshot shows a dialog box titled "Peak Integration Parameters" with a toolbar at the top containing icons for help, print, save, and a file icon. The dialog is divided into two main sections: "Peak parameters" and "Advanced".

Peak parameters section:

- Apply to All Plots** (with an **Apply** button to its right)
- Baseline window:
- Area noise factor:
- Peak noise factor:
- Constrain Peak Width**
- Peak Ht (%):
- Tailing Factor:

Advanced section:

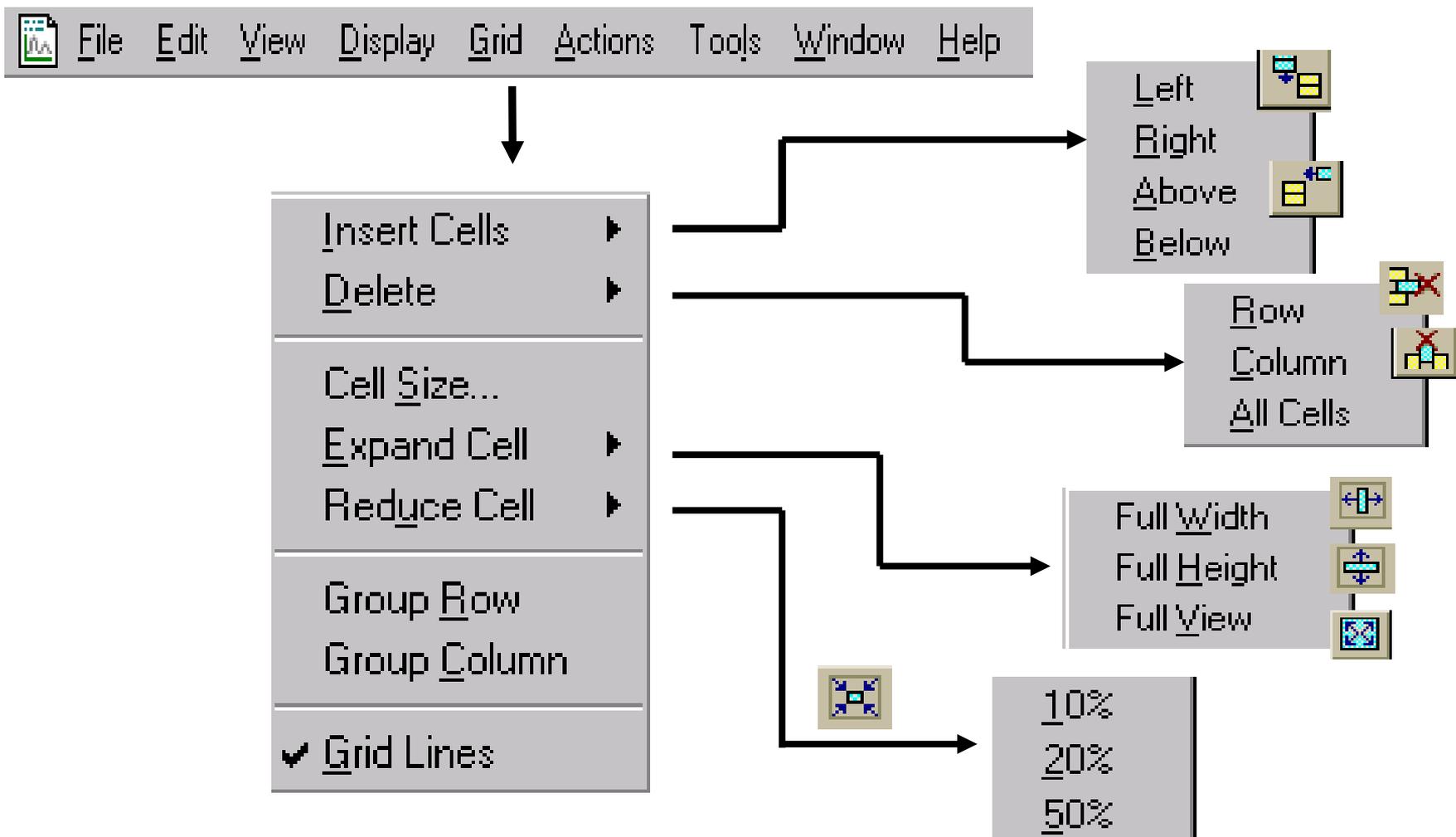
- Noise Method** (radio buttons):
 - INCQS Noise**
 - Repetitive Noise**
- Min peak width:
- Multiplet resolution:
- Area tail extension:
- Area scan window:

At the bottom of the dialog are three buttons: **Save as Defaults**, **Load Defaults**, and **Help**.

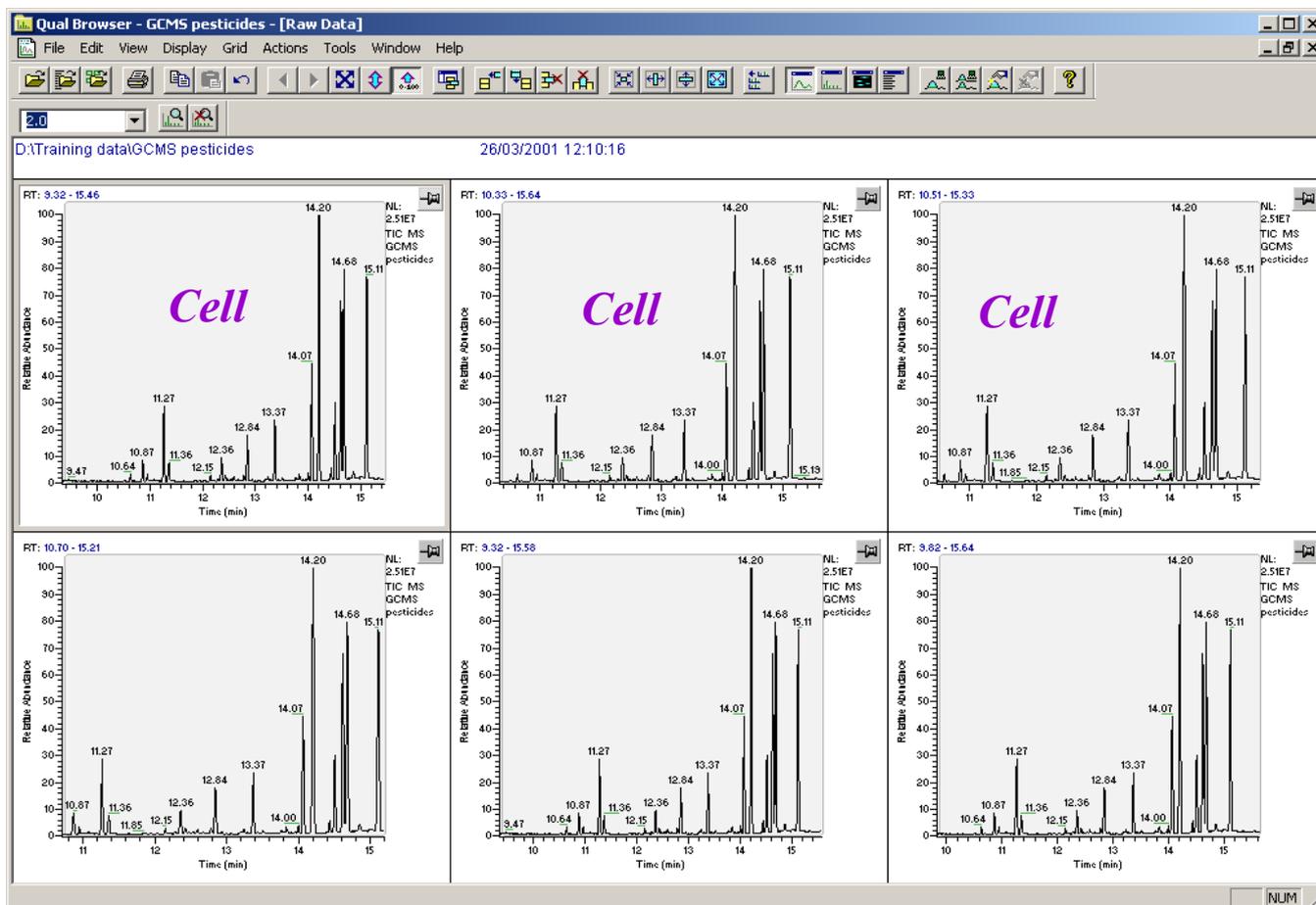
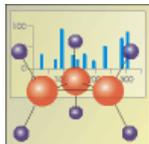
Allows User to set and modify peak integration parameters.

Three integration algorithms are available:
GENESIS; ICIS and AVALON

Cell Manipulation

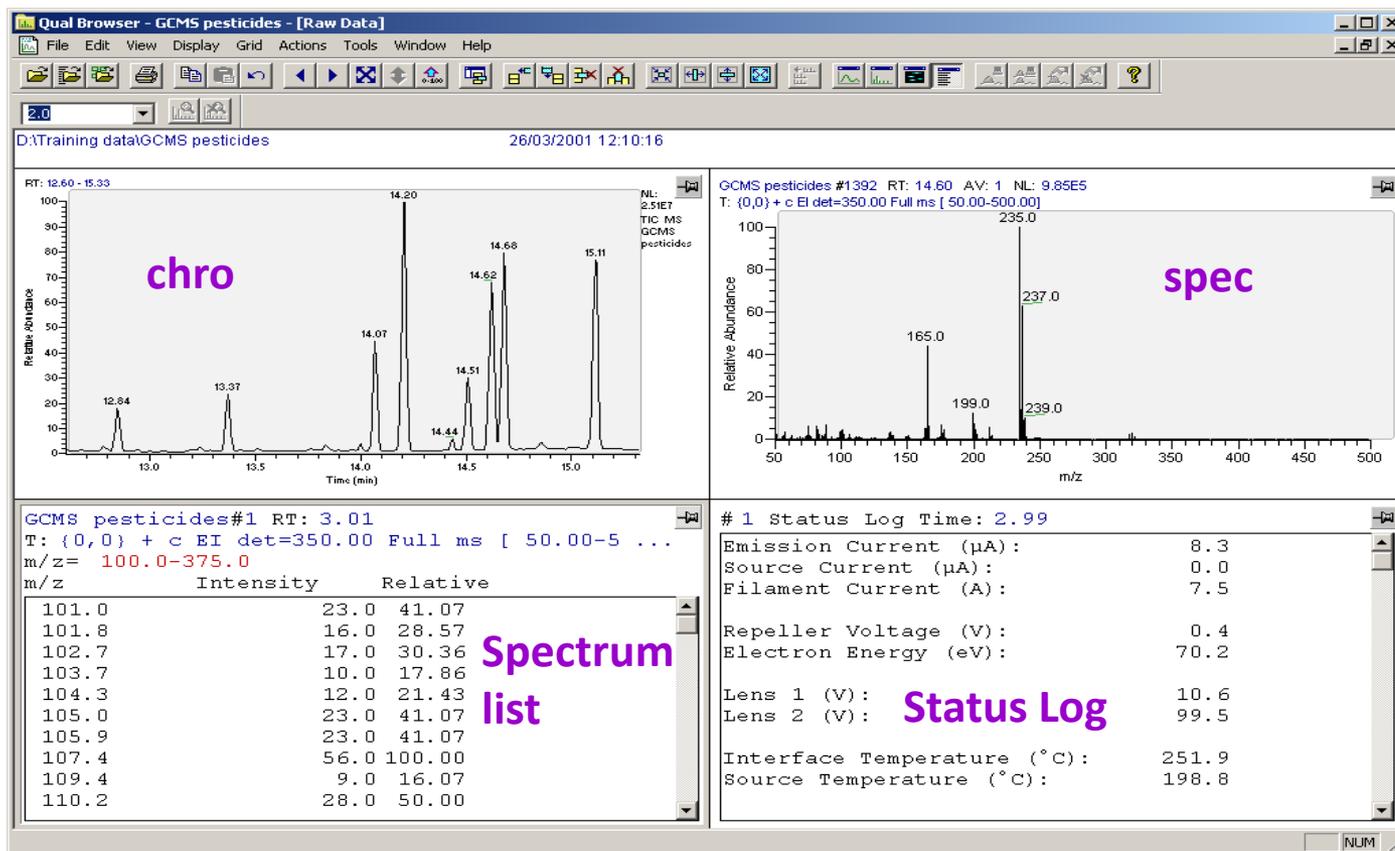
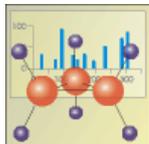


Object Definitions



- Window -contains a *Grid* of up to 16 *Cells*
- Cell -contains *View*

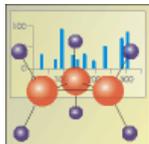
Object Definitions



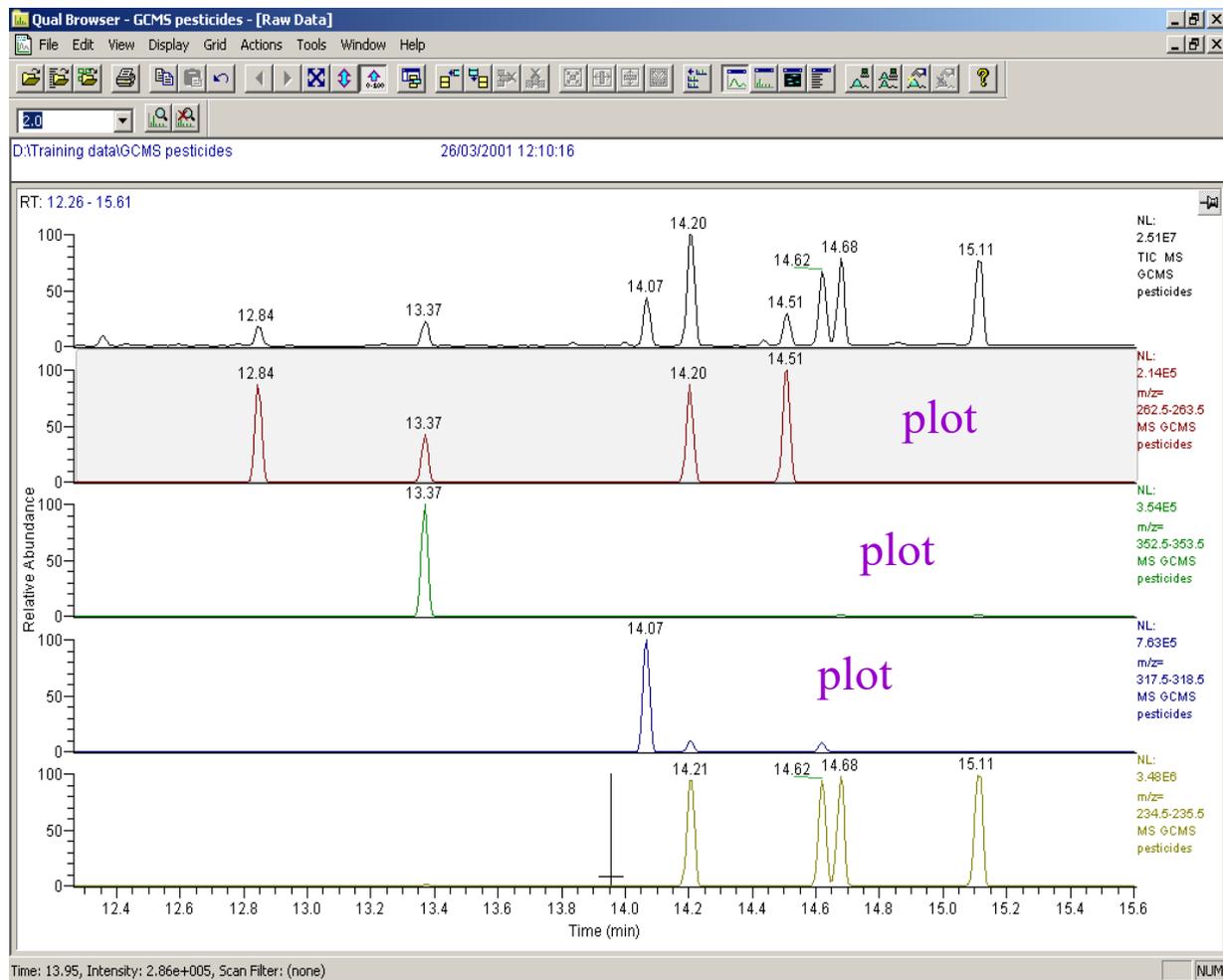
View Options

chro, spec, mass map, spectrum list, scan header, scan filter list, tune method, instrument method, sample info, status log, error log

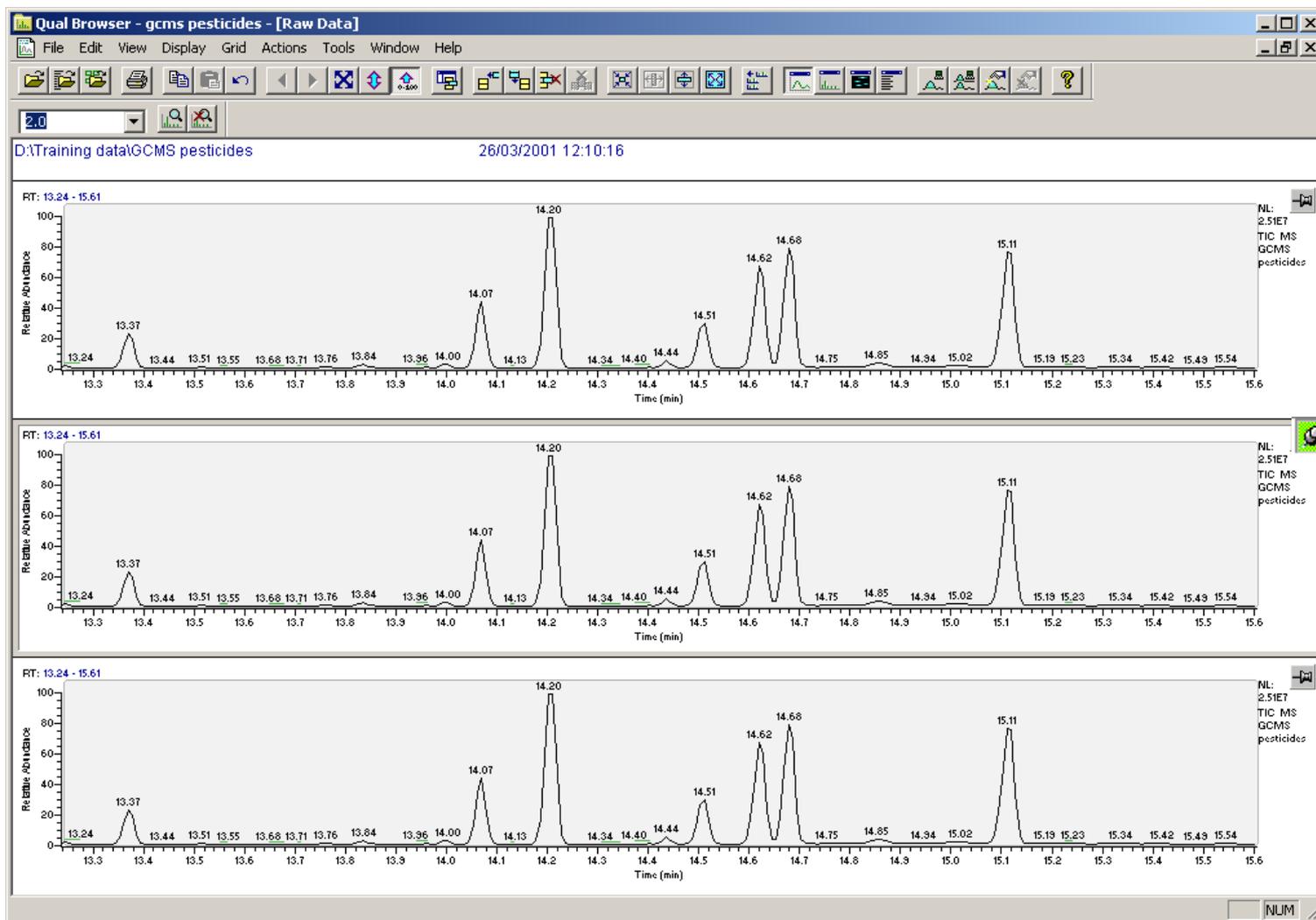
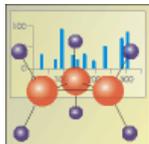
Object Definitions



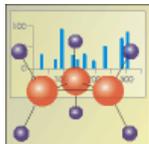
- **Chro/Spec View**
contains up to 8 *Plots*
- **Layout**
arrangement of *Views* in
a *Cell* and *Cells* in a
Window Grid



Chromatogram View with Pinned Cell

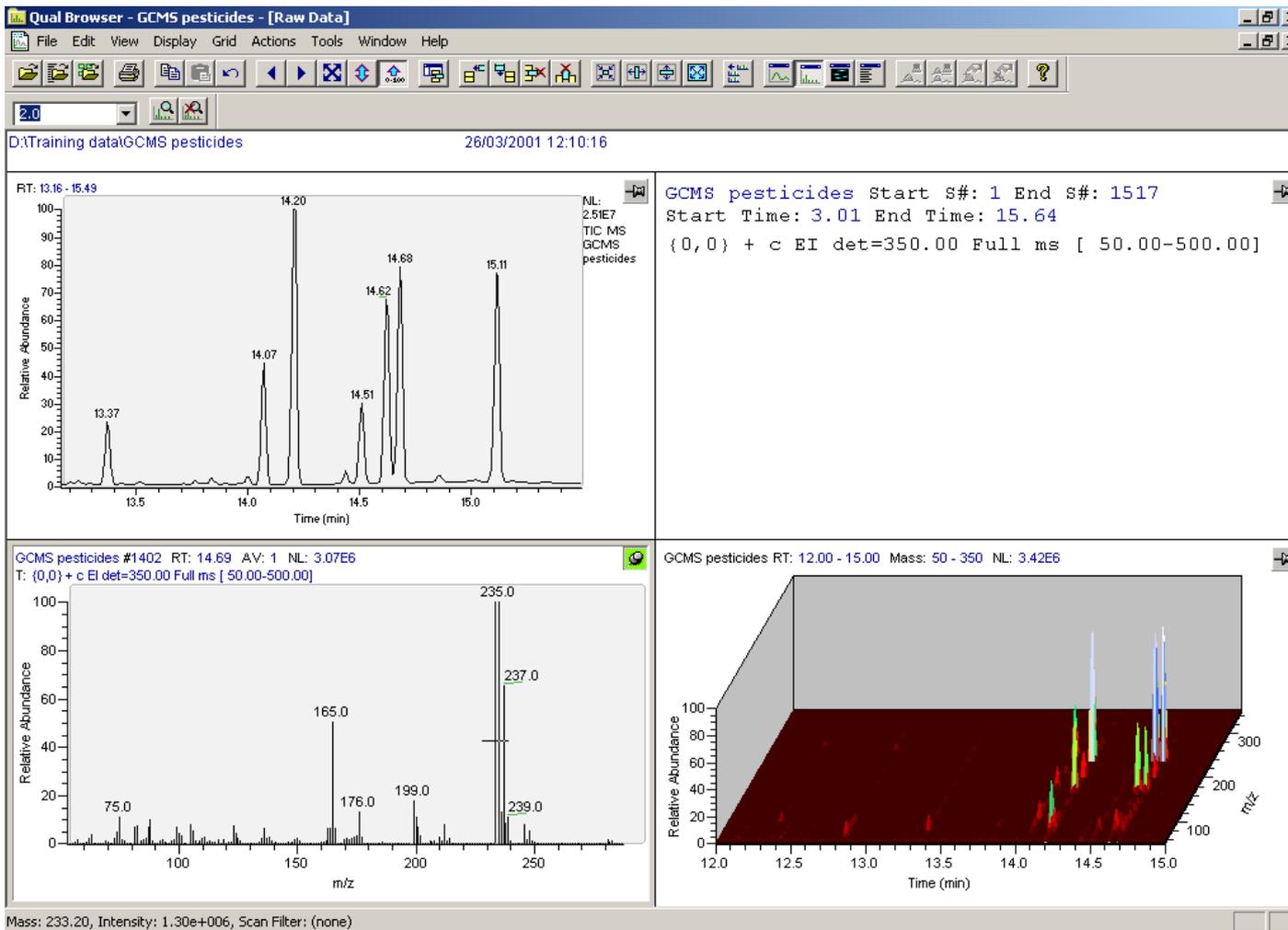
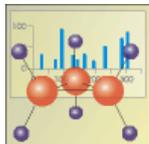


Cursor Actions

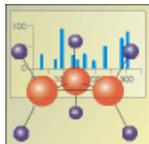


<i>Cursor Action</i>	<i>Effects on pinned cell</i> 
Click	Makes view active
Drag parallel to X-axis	Rescale graph showing selected X range only. The Y range may rescale depending on the selected Normalisation
Drag parallel to Y-axis	Rescale graph showing selected Y range only, same X range.
Dragged box area	Rescale graph showing selected X & Y ranges only

Pinned Cells

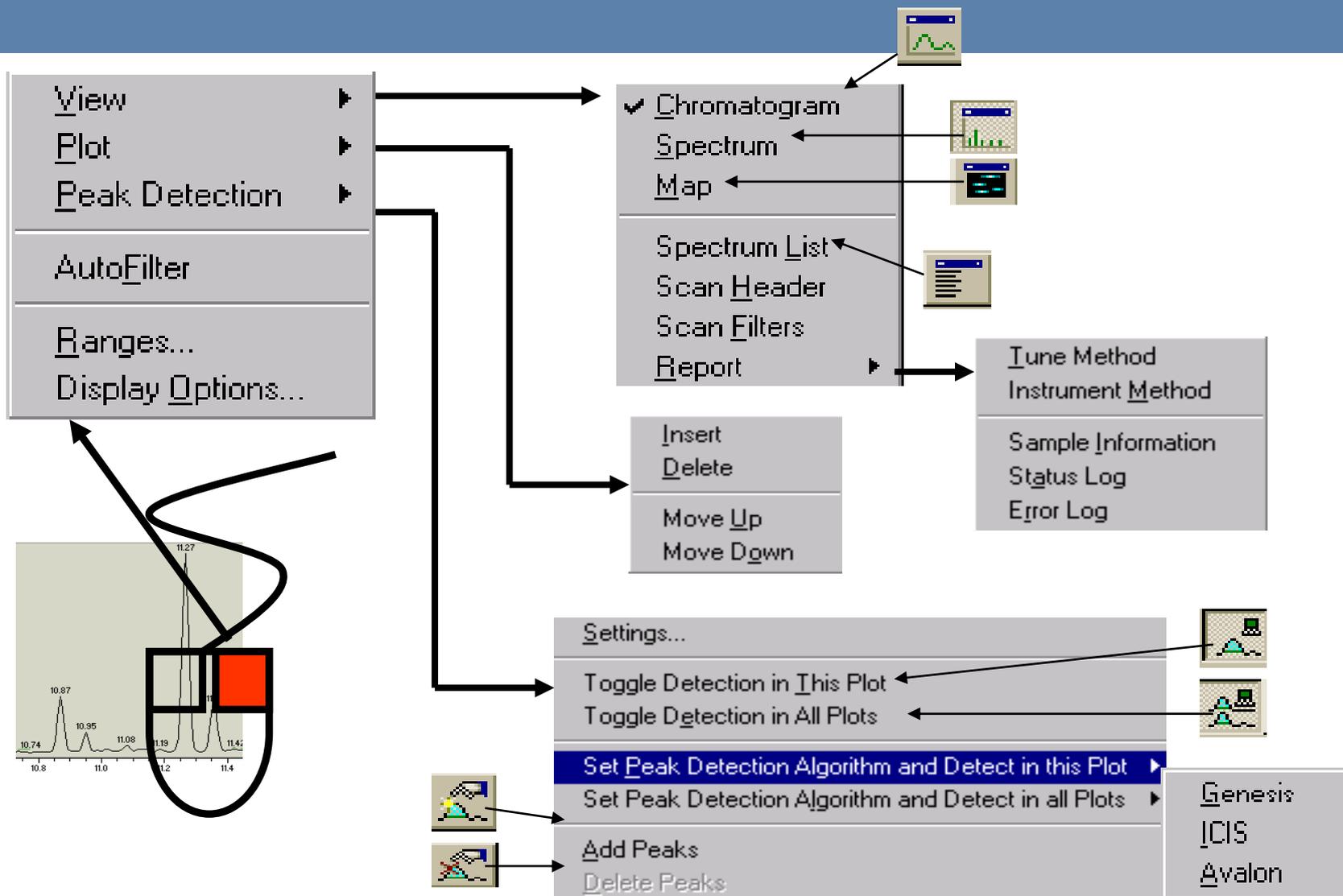
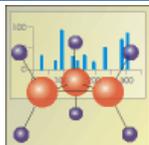


Pinned Cells

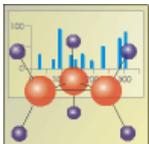


Pinned Cell 	Use Cursor in this view	Cursor Action	Effect on active view in pinned cell 
Spectrum	Chromatogram	Click on RT	Mass spectrum of RT
Status log	Chromatogram	Click on RT	Status log for RT
Scan filter	Chromatogram	Click on RT	Status log for RT
Spectrum	Chromatogram	Drag between RT_1 - RT_2	Average spectrum for RT_1 - RT_2
Chromatogram	Spectrum	Drag between m/z_1 - m/z_2	Ion Chromatogram for range m/z_1 - m/z_2
Chromatogram	Map	Drag area RT_1 - RT_2 and m/z_1 - m/z_2	Ion Chromatogram for range m/z_1 - m/z_2 between RT_1 & RT_2

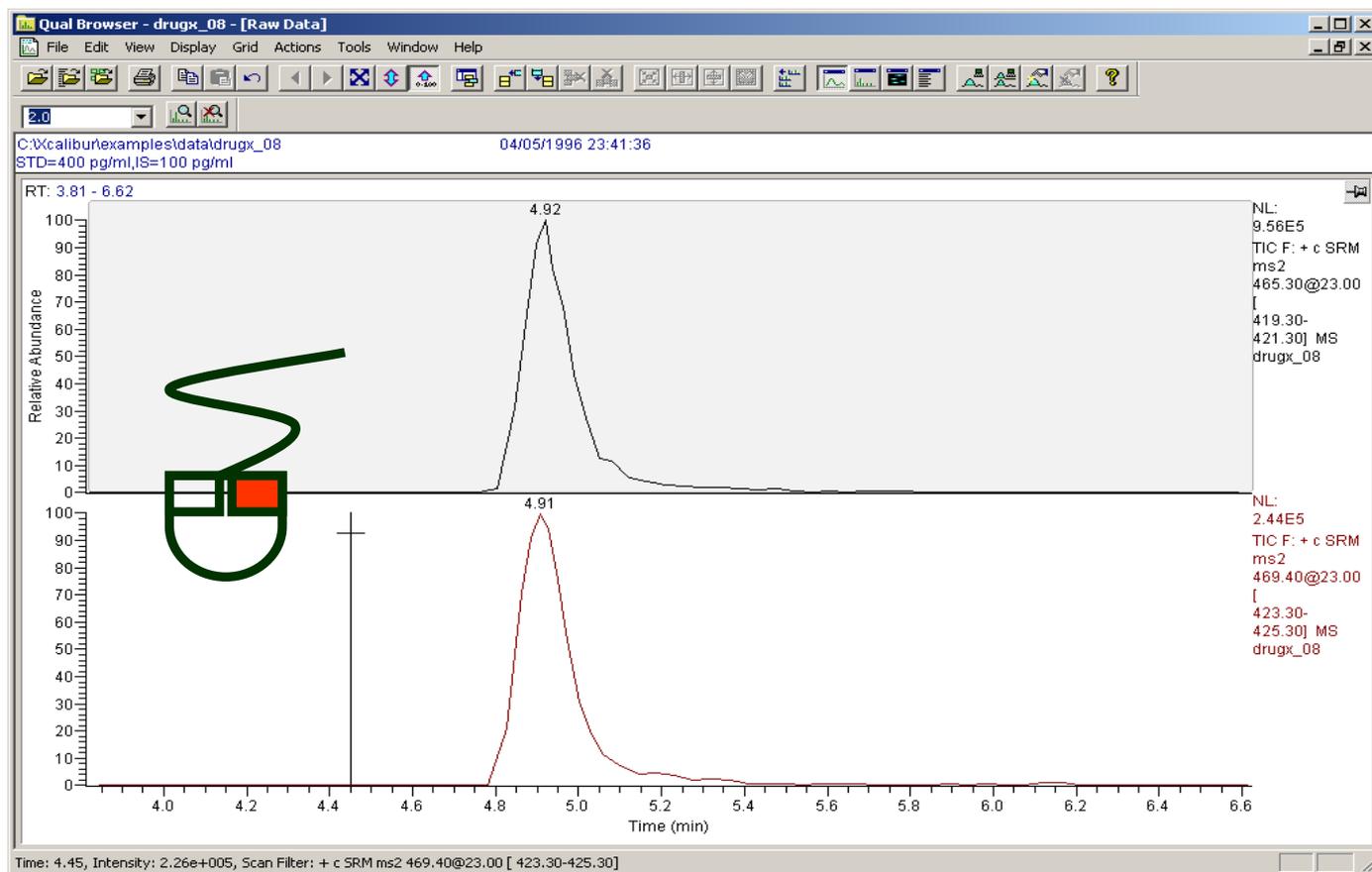
Chromatogram- Right View Click



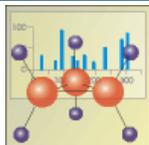
Autofilter feature



Automatically adds a plot for each filter that was used to acquire the chromatogram



Chromatogram Ranges: (Extracted Ion)



Chromatogram Ranges

Ranges | Automatic Processing

Range
Time Range (minutes): * Fixed Scale

Type	Range	Scan Filter	Delay (min)	Scale	Raw File
<input checked="" type="checkbox"/> TIC	-	.	0.00	-	c:\xcalibur\data\demo data\dem...
<input checked="" type="checkbox"/> Mass Range	235.00	.	0.00	-	C:\Xcalibur\data\Demo Data\De...
<input checked="" type="checkbox"/> Mass Range	235.00	.	0.00	-	c:\xcalibur\data\demo data\dem...
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

Plot Properties

Raw File: c:\xcalibur\data\demo data\demo_100.raw Detector: MS

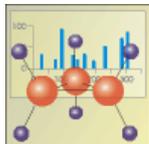
Scan Filter:

Plot Type: TIC Delay (min): 0.00

Range(s) Fix scale to: 1000000.0

OK Cancel Help

Chromatogram Processing



Chromatogram Ranges [X]

Ranges Automatic Processing

Smoothing

Enable

Type: Points:

Baseline Subtraction

Enable

Polynomial Order:

Below Curve (%):

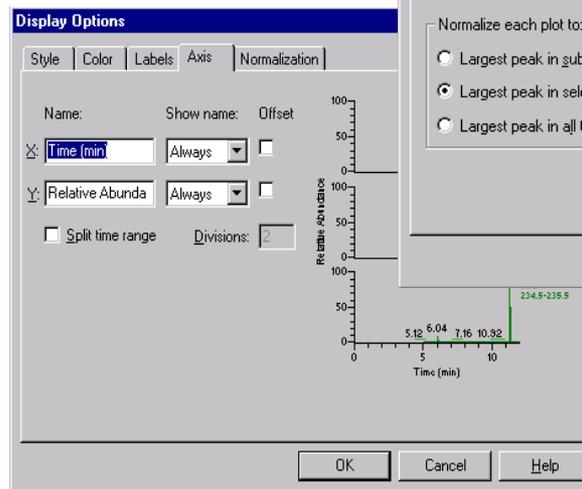
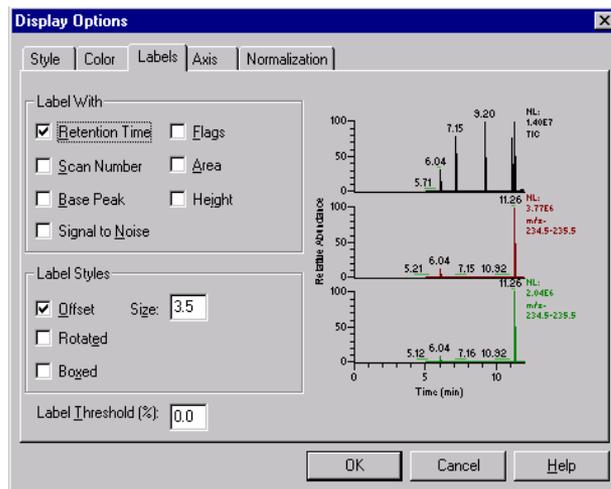
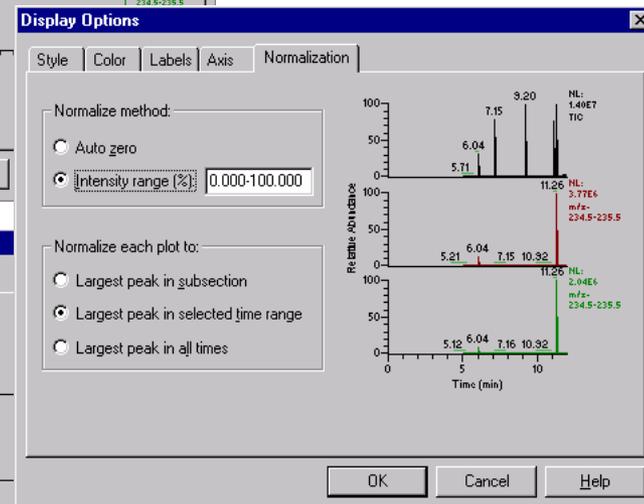
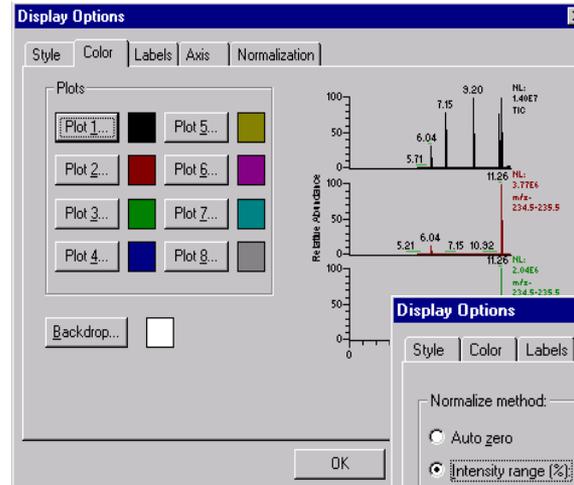
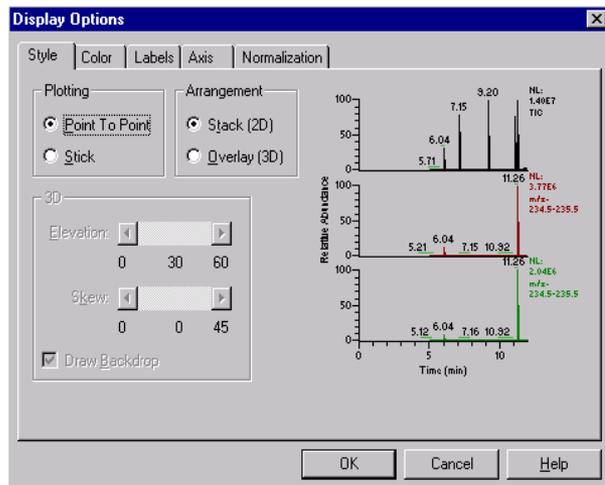
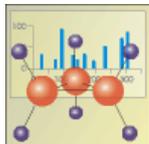
Tolerance:

Flatten Edges

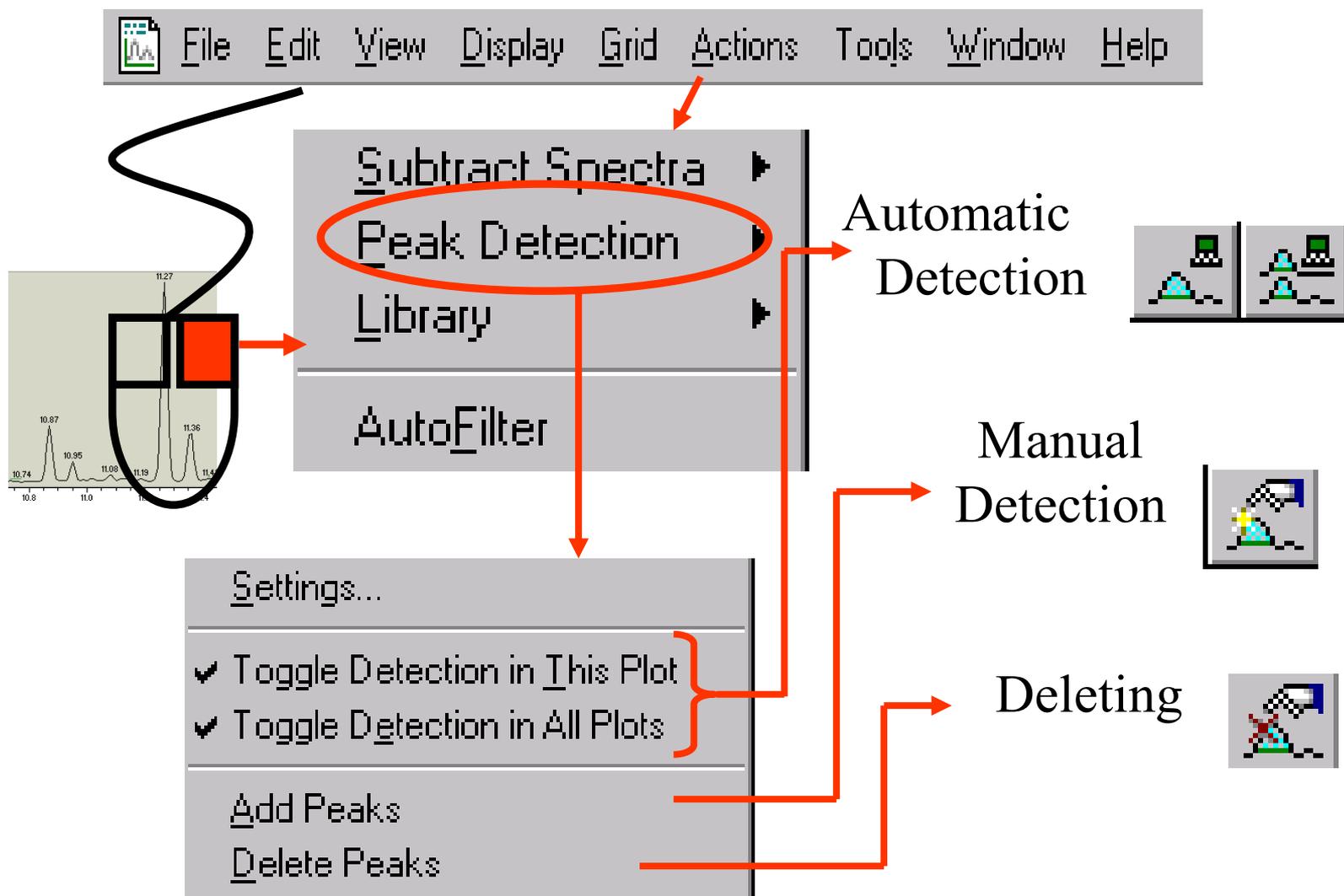
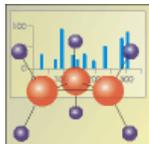
Overlay Graph of Fitted Polynomial

OK Cancel Help

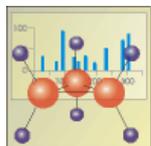
Chromatogram Display Options



Peak Detection



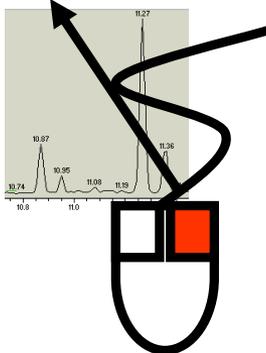
Peak Detection Algorithm Options



- View
- Plot
- Peak Detection
- AutoFilter
- Ranges...
- Display Options...

- Settings...
- Toggle Detection in This Plot
- Toggle Detection in All Plots
- Set Peak Detection Algorithm and Detect in this Plot
- Set Peak Detection Algorithm and Detect in all Plots
- Add Peaks
- Delete Peaks

- Genesis
- ICIS
- Avalon



Apply

Apply to All Plots

Peak parameters:

- Percent of Highest Peak: 10.0
- Minimum Peak Ht (S/N): 2.0
- S/N Threshold: 0.5
- Valley Detection Enabled
- Expected Width (sec): 0.0
- Constrain Peak Width
- Peak Ht (%): 5.0
- Tailing Factor: 1.0

Advanced:

- Baseline Noise Tolerance (%): 10.0
- Min Number of Scans in Baseline: 16
- Baseline Noise Rejection Factor: 2.0
- Peak S/N Cutoff: 200.0
- Rise Percentage: 10.0
- Valley S/N: 1.0
- Background Recomputation (min): 5.0
- Number of Scans in Background: 5

Save as Defaults Load Defaults Help

Genesis

Apply

Apply to All Plots

Peak parameters:

- Baseline window: 40
- Area noise factor: 5
- Peak noise factor: 10
- Constrain Peak Width
- Peak Ht (%): 5.0
- Tailing Factor: 1.0

Advanced:

Noise Method

- INCOIS Noise
- Repetitive Noise

- Min peak width: 3
- Multiplet resolution: 10
- Area tail extension: 5
- Area scan window: 0

Save as Defaults Load Defaults Help

ICIS

Apply to all plots

Auto Calc Initial Events

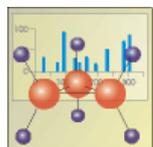
Time	Event	Value
Initial Value	Start Threshold	10000.000
Initial Value	End Threshold	10000.000
Initial Value	Area Threshold	10000.000
Initial Value	P-P Threshold	1.000
Initial Value	Bunch Factor	1.000
Initial Value	Negative Peaks	Off
Initial Value	Tension	1.000

Time (Min): 0.000 Event: Tension Value: 1.000

Add Delete Change Help

Avalon

Spectrum- Right Click Options



- View
- Plot
- Subtract Spectra
- Library
- Export

- Ranges...
- Display Options...

- Chromatogram
- ✓ Spectrum
- Map
- Ion Map

- Spectrum List
- Scan Header
- Scan Filters
- Report

- Iune Method
- Instrument Method

- Sample Information
- Status Log
- Error Log

- 1 Range
- 2 Ranges

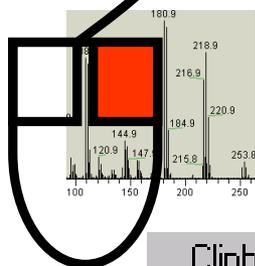
- Clear

- Insert
- Delete

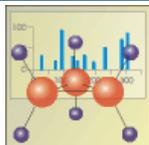
- Move Up
- Move Down

- Search
- Export to Library Browser
- Options...

- Clipboard (Exact Mass)
- Clipboard (Nominal Mass)
- Mass Frontier



Spectrum Ranges



Spectrum Ranges [X]

Ranges | Automatic Processing

Range

Mass Range: Average Fix scale:

Time	Scan Filter	Raw File	Subtract 1	Subt
<input checked="" type="checkbox"/> 5.04	.	C:\xcalibur\data\Demo Data\De...	-	-
<input checked="" type="checkbox"/> 5.04	.	C:\xcalibur\data\Demo Data\De...	-	-
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

Plot Properties

Detector: Time:

Scan Filter:

Raw File: ...

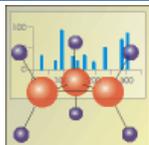
Background Subtraction

Time Range 1:

Time Range 2:

OK Cancel Help

Spectrum Processing



Spectrum Ranges [X]

Ranges | Automatic Processing

Smoothing

Enable

Type: Points:

Refine

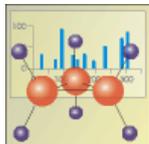
Enable

Window Size (sec.):

Noise Threshold:

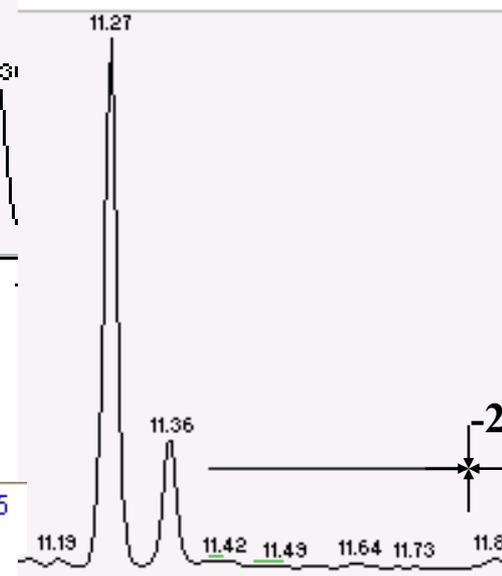
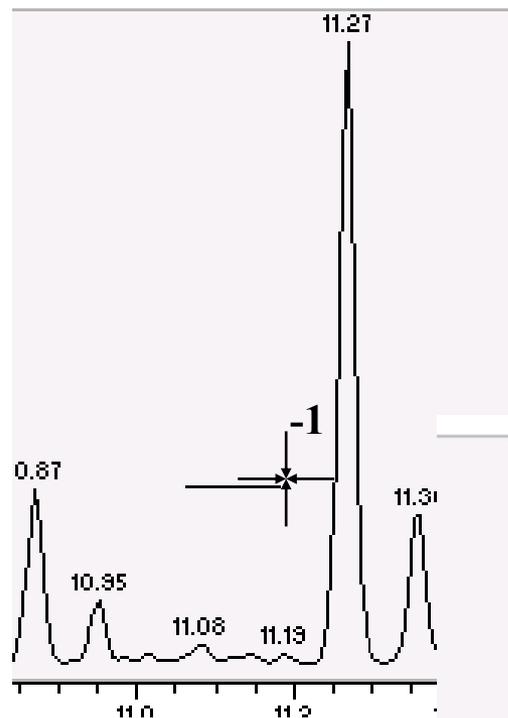
OK Cancel Help

Spectrum Subtraction



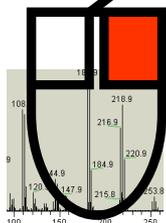
qual01 #991 RT: 11.26 AV: 1 NL: 5.03E5
T: {0,0} + c EI det=350.00 Full ms [50.00-500.00]

- View
- Plot
- 1 Range**
- Library 2 Ranges
- Export Clear
- Ranges...
- Display Options...

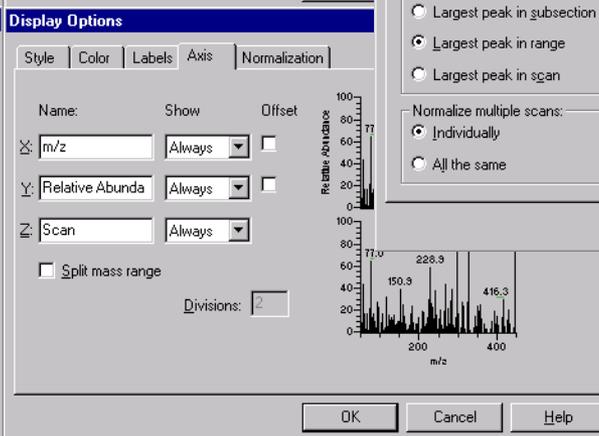
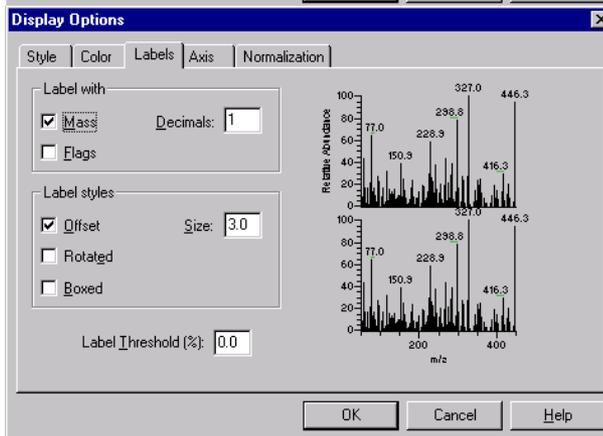
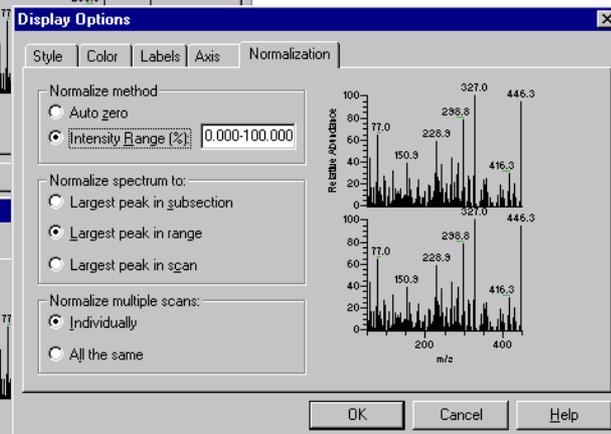
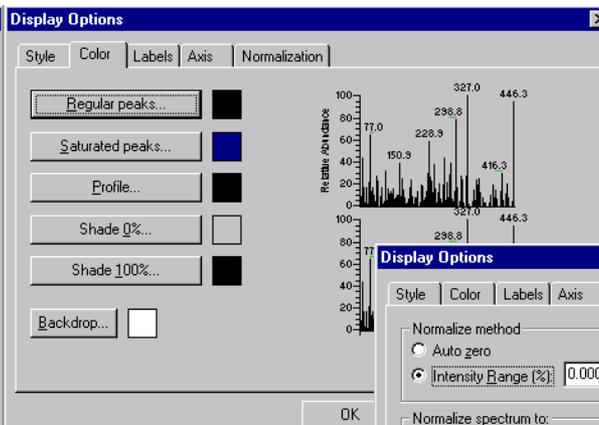
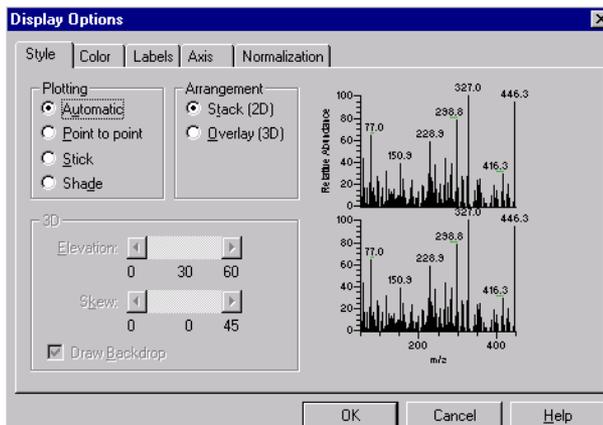
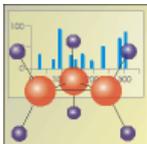


**Left Click & Drag Ranges
to be subtracted on Chromatogram**

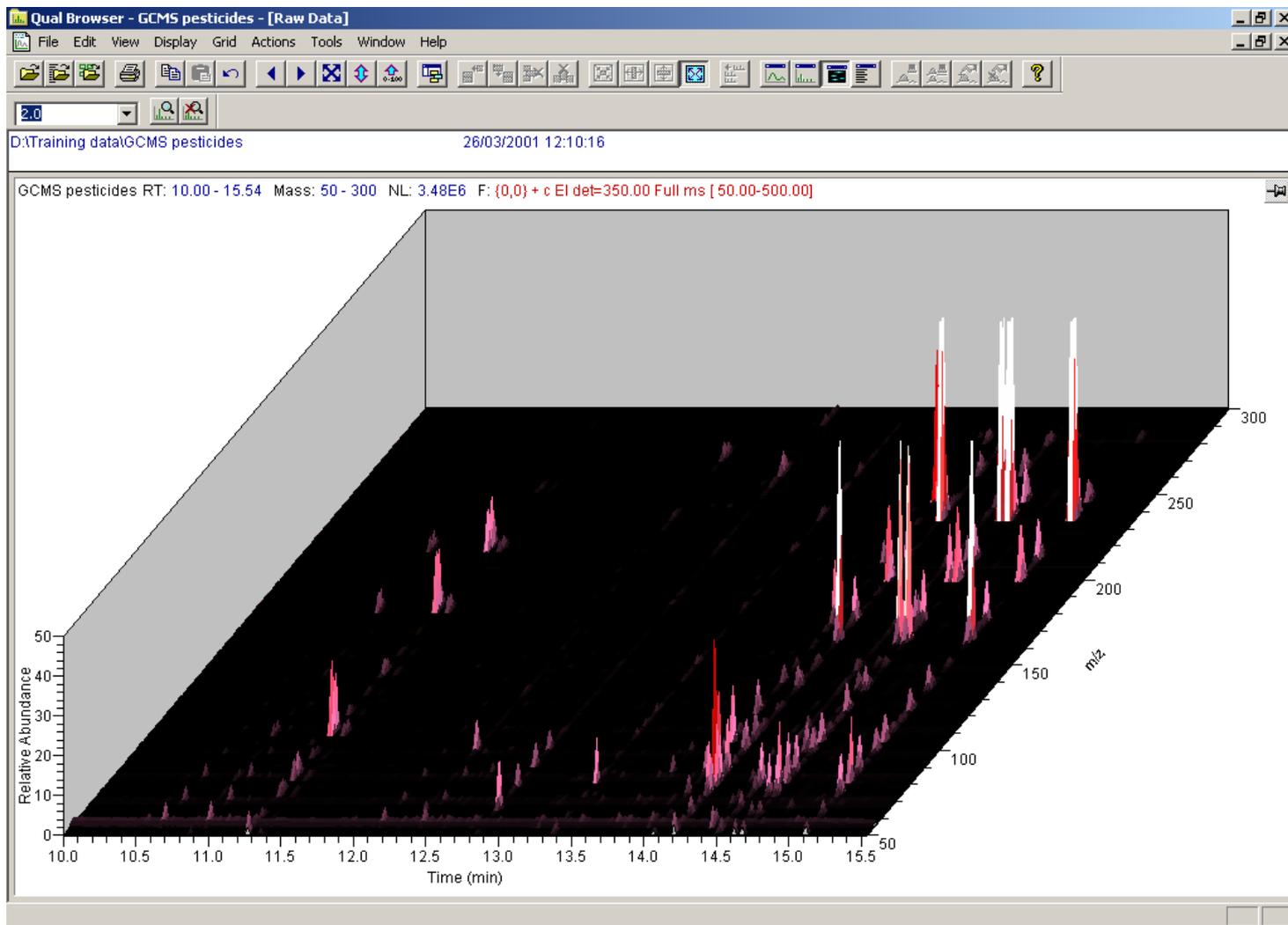
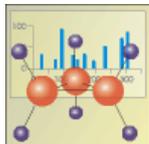
qual01 #991 RT: 11.26 AV: 1 SB: 57 11.12-11.22, 11.42-11.78 NL: 5.02E5
T: {0,0} + c EI det=350.00 Full ms [50.00-500.00]



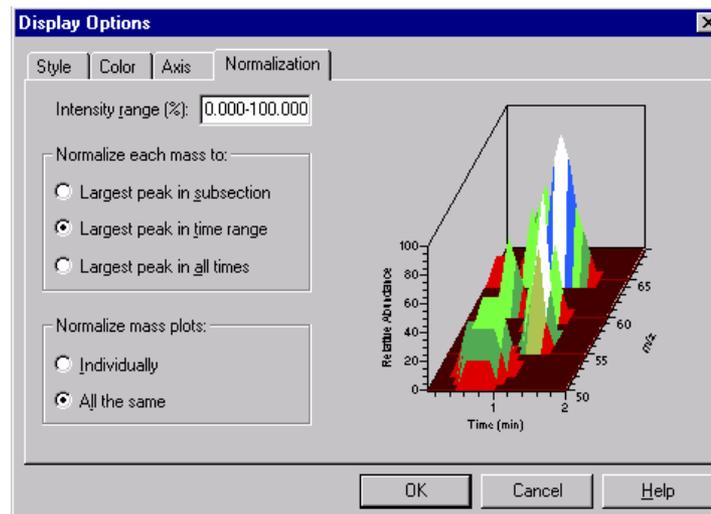
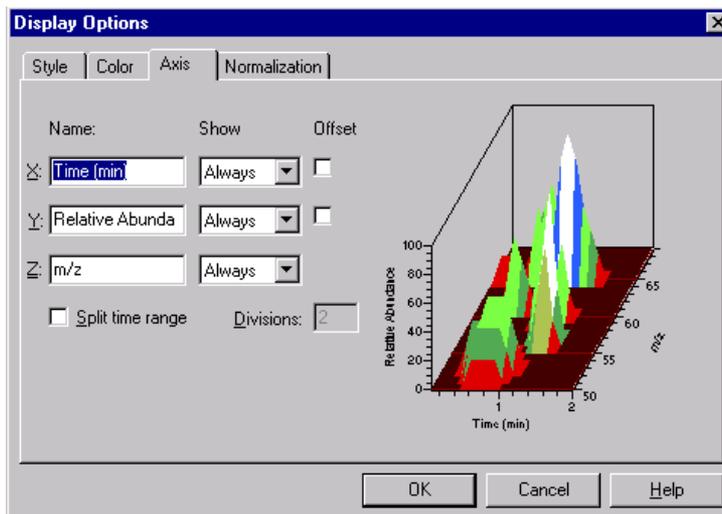
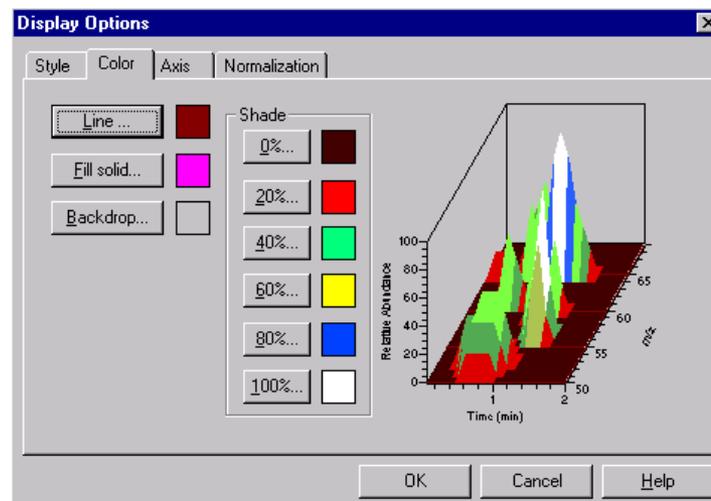
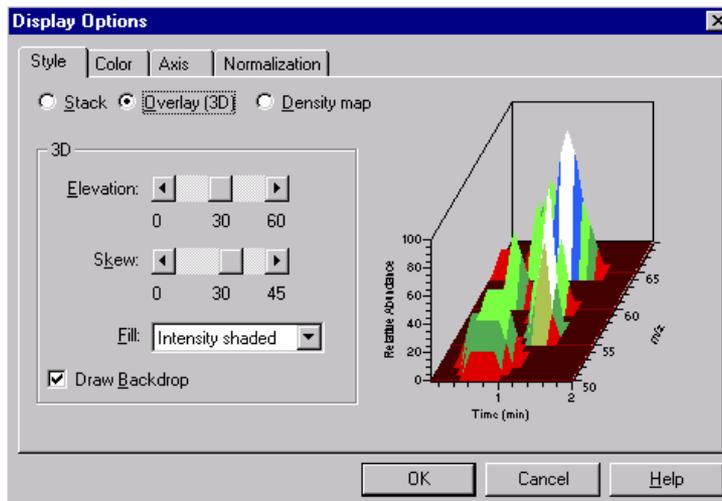
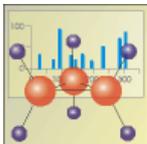
Spectrum Display Options



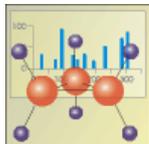
Map View



Map Display Options



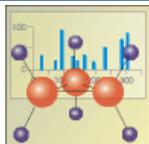
Spectrum List



Demo_100#1 RT: 5.04
T: + c Full ms [50.00-450.00]

m/z	Intensity	Relative
50.15	24.0	0.82
50.87	2064.0	70.11
51.87	256.0	8.70
52.87	72.0	2.45
54.92	1284.0	43.61
56.07	77.0	2.62
56.96	555.0	18.85
58.96	362.0	12.30
60.87	485.0	16.47
62.92	100.0	3.40
64.92	510.0	17.32
66.10	214.0	7.27
67.00	382.0	12.98
68.85	64.0	2.17
70.83	82.0	2.79
72.95	646.0	21.94
74.11	350.0	11.89
74.96	1003.0	34.07
76.19	35.0	1.19
76.96	1916.0	65.08
77.99	394.0	13.38
79.02	201.0	6.83
81.04	498.0	16.92
81.85	54.0	1.83
82.86	222.0	7.54
85.29	118.0	4.01
86.19	35.0	1.19
86.97	302.0	10.26

Spectrum List Ranges



Spectrum List Ranges [X]

Mass: 49.6-500.4 Detector: MS

Time: 14.19-14.22

Scan filter: []

Smoothing

Enable:

Type: Boxcar

Points: 7

Refine

Enable

Window size (sec.): 6.00

Noise threshold: 3

Background subtraction

Time range 1: 13.50-13.70

Time range 2: 14.30-14.40

Mass tolerance

Use user defined

Mass tolerance: 0.5

Units: mmu ppm

Include peaks

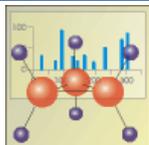
Reference and exception peaks

Mass Precision

Decimals: 1

OK Cancel Help

Spectrum List Display Options



Display Options

Style | Normalization

Intensity range (%):

Normalize list to:

- Largest peak in subsection
- Largest peak in range
- Largest peak in scan

m/z	Intensity	Relative
50.15	24.0	0.82
50.87	2064.0	70.11
51.87	256.0	8.70
52.87	72.0	2.45
54.92	1284.0	43.61
56.07	77.0	2.62
56.96	555.0	18.85
58.96	362.0	12.30
60.87	485.0	16.47
62.92	100.0	3.40
64.92	510.0	17.32
66.10	214.0	7.27
67.00	382.0	12.98
68.85	64.0	2.17
70.83	82.0	2.79
72.95	646.0	21.94
74.11	350.0	11.89
74.96	1003.0	34.07

OK Cancel Help

Display Options

Style | Normalization

All peaks

Order by:

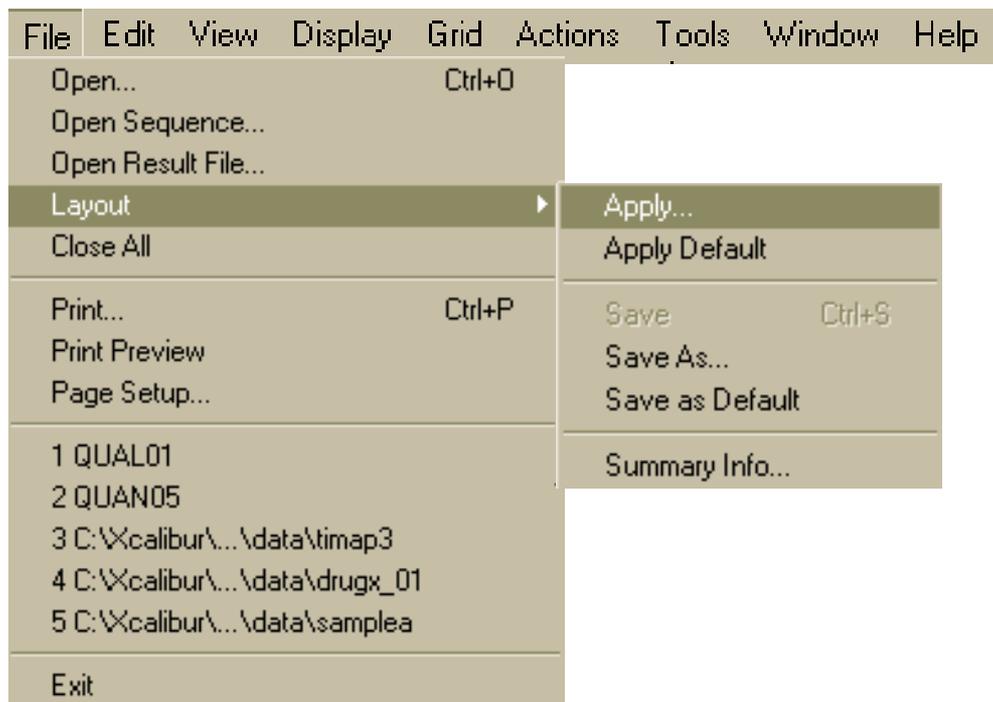
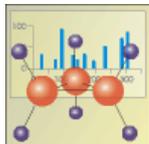
- Mass
- Intensity

Top:

m/z	Intensity	Relative
50.15	24.0	0.82
50.87	2064.0	70.11
51.87	256.0	8.70
52.87	72.0	2.45
54.92	1284.0	43.61
56.07	77.0	2.62
56.96	555.0	18.85
58.96	362.0	12.30
60.87	485.0	16.47
62.92	100.0	3.40
64.92	510.0	17.32
66.10	214.0	7.27
67.00	382.0	12.98
68.85	64.0	2.17
70.83	82.0	2.79
72.95	646.0	21.94
74.11	350.0	11.89
74.96	1003.0	34.07

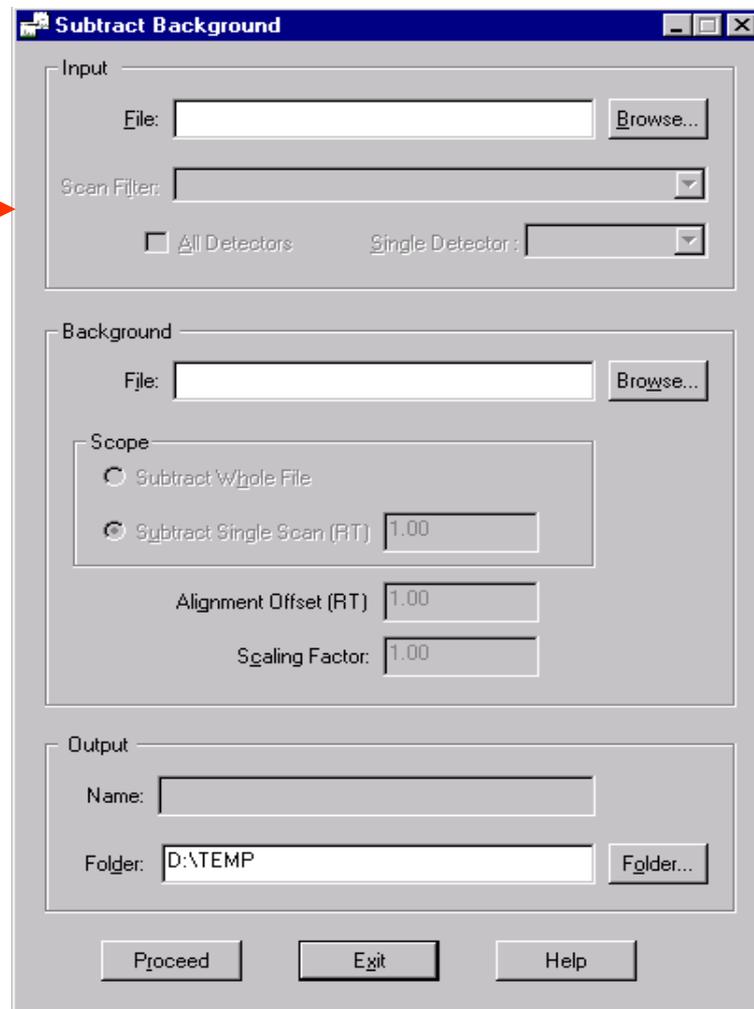
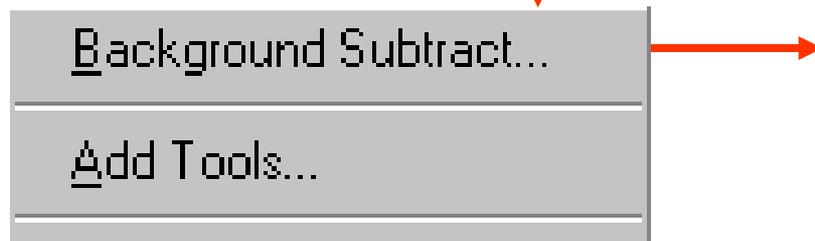
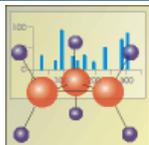
OK Cancel Help

Layouts- Saving and Applying



- Set cells/views/plots/integration etc. to user specifications
- Save layout: *Save As... *.lyt/default*
- Apply layout to subsequent samples: *Apply... *.lyt/default*

Background Subtract File



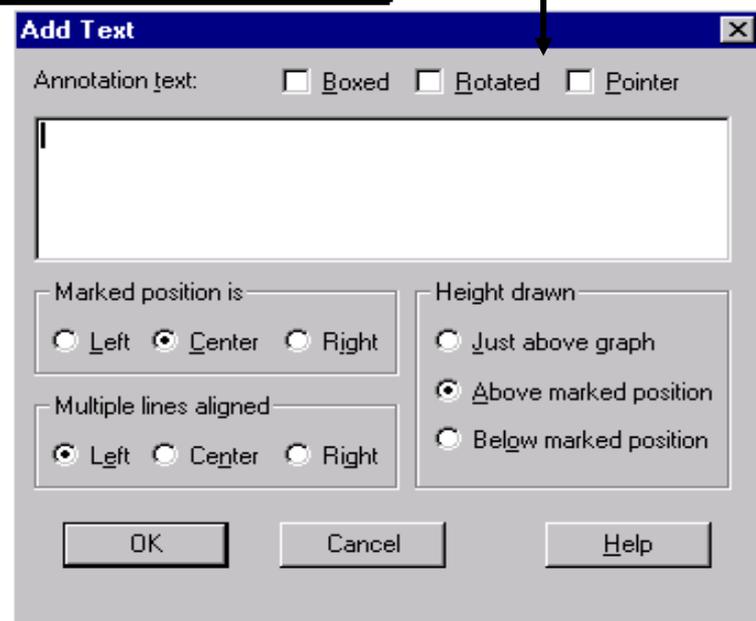
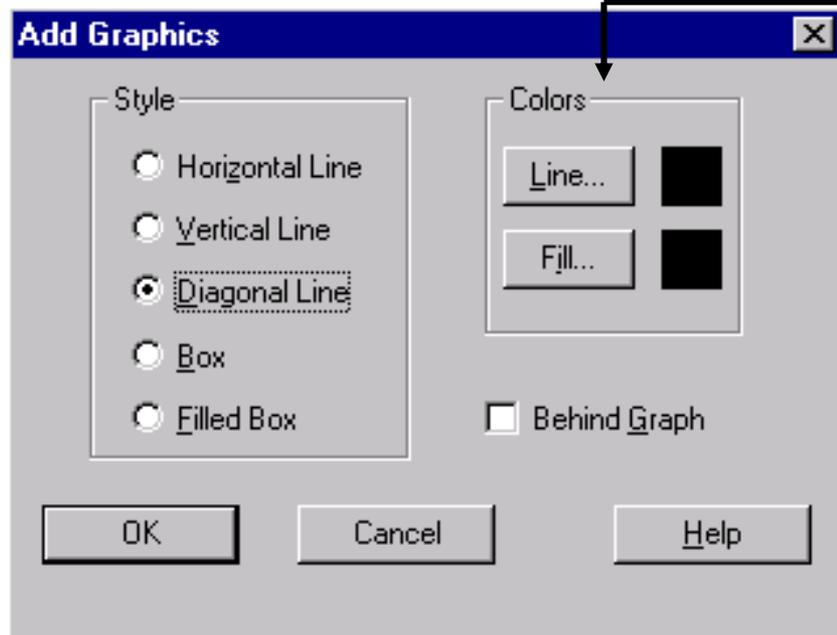
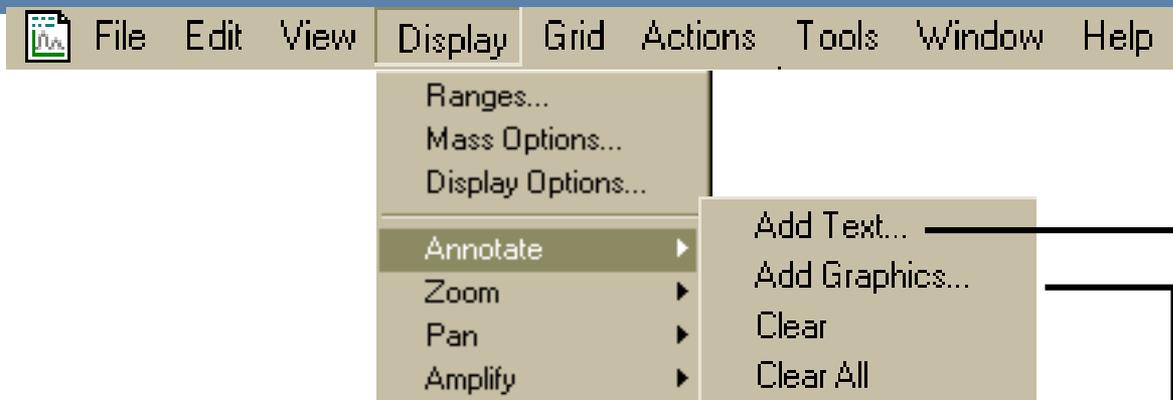
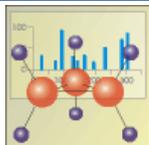
3 parts:

–*Input*

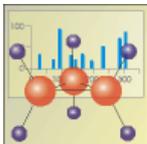
–*Background*

–*Output*

Data Presentation



Chromatogram Capture



Qual Browser - belg04, BACquan04 - [Raw Data : 2]

File Edit View Display Grid Actions Tools Window Help

Undo Ctrl+Z
Copy Cell Ctrl+C
Paste Cell Ctrl+V
Copy View
Copy Special...

Apply to All Plots Apply

Peak parameters:
Baseline window: 40
Area noise factor: 5
Peak noise factor: 10
 Constrain Peak Width
Peak Ht (%): 5.0
Tailing Factor: 1.0

Advanced:
Noise Method:
 INCOS Noise
 Repetitive Noise
Min peak width: 3
Multiplet resolution: 10
Area tail extension: 5
Area scan window: 0

Save as Defaults Load Defaults Help

RT: 0.00 - 4.99

1.35
2.12
2.15
2.56
1.01 1.14 1.23 1.47 1.85 2.00 2.35 2.76 2.98 3.10 3.31 3.56 3.71 3.81 4.04 4.18 4.23 4.50 4.64 4.79 4.95

NL: 1.89E7
TIC MS
BACquan04

BACquan04 #453 RT: 3.93 AV: 1 NL: 7.16E4
T: (0,0) + c EI det=350.00 Full ms [45.00-450.00]

207.1
55.1 73.0 97.1 133.1 177.1 191.0 208.1 213.2 253.1 281.1 282.1 327.1 355.1 405.1 429.1

Relative Abundance

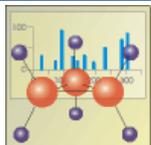
Time (min)

m/z

Slide 174 of 248 Template

Start untyped - Paint Roadmap - Home ... Trace MS basic o ... TRaCE MS Basic ... Qual Browser - ... 2:34 AM

Library Interaction



- Subtract Spectra ▶
- Peak Detection ▶
- Library ▶
 - Search
 - Export to Library Browser
 - Options...
- AutoFilter

Search Properties

Search List | Search Parameters

Available libraries:

Selected libraries:
NISTDEMO

Add >> << Remove

Top Up

OK Cancel

Search Properties

Search List | Search Parameters

Search type

Identity Similarity

Normal Simple

Quick Hybrid

Penalize rare compounds Neutral loss

Options

Search with MW= 0

Reverse search

Mass defect

Enable

These values are set using the Xcalibur Configuration utility. You may enable or disable their use for Library searches by Qual Browser here.

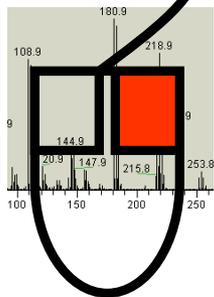
Defect (mmu) at mass (amu)

0 1.0

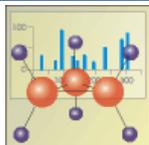
Defect (mmu) at mass (amu)

300 1000.0

OK Cancel Apply Help



Library Search Results



Qual Browser - qual01 - [Library Search Results]

File Edit View Display Grid Actions Tools Window Help

2.0

Hit	SI	RSI	Name
1	785	929	1,2-Bis(2-chlorophenyl)-1,2-bis(3-chlorophenyl)ethane
2	917	922	Mitotane
3	909	914	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane
4	882	906	m,p'-DDD
5	832	861	1-Chloro-2,2-Bis(p-chlorophenyl)ethane
6	718	852	9H-Carbazole, 3,6-dichloro-
7	790	849	2,2-Bis(p-chlorophenyl)ethanol
8	768	822	Benzene, 1-chloro-2-(or 4)-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]-
9	765	818	Benzene, 1,1'-(chloromethylene)bis[4-chloro-
10	771	800	3-Butanone, 1,1-bis(4-chlorophenyl)-2,2-dimethyl-
11	796	799	Technical chlorophenothane
12	787	793	Chlorophenothane
13	787	792	o,p'-DDT
14	722	788	2,2-Bis(4-chlorophenyl)acetic acid
15	714	750	Ethane, 1,1,1-trichloro-2,2-bis(chlorophenyl)-
16	677	725	Ethane, 1,1-bis(p-chlorophenyl)-
17	681	717	Benzene, 1,1'-(2,2,2-trifluoroethylidene)bis[4-chloro-

1,2-Bis(2-chlorophenyl)-1,2-bis(3-chlorophenyl)ethane
Formula C₂₆H₁₈Cl₄, Mw 470, CAS# NA, Entry# 30532

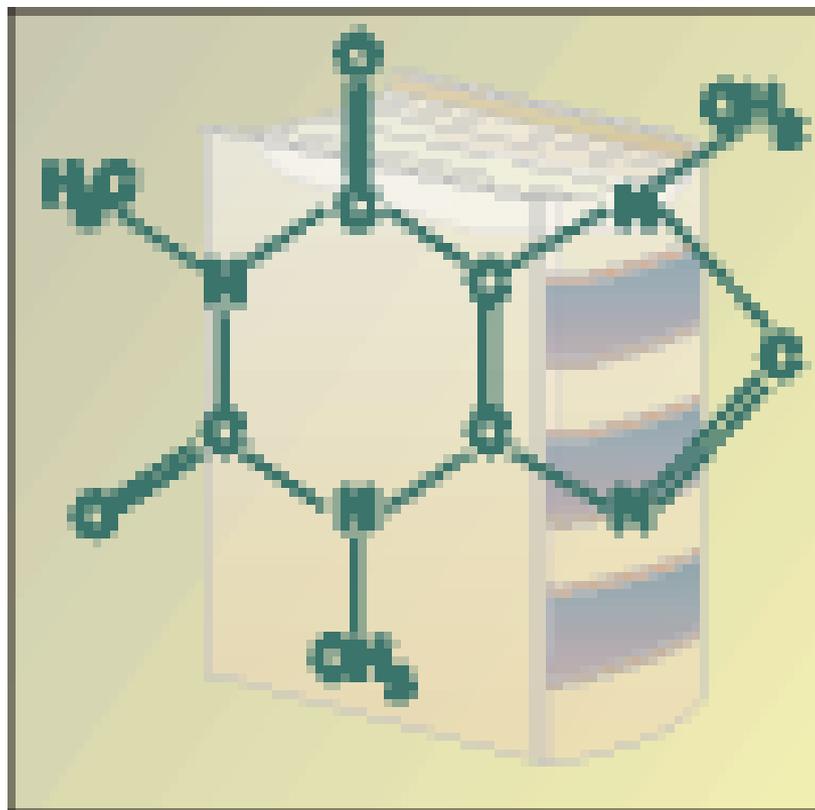
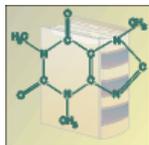
Raw data - Library entry

qual01 #1394 RT: 14.62 AV: 1
NL: 3.28E6 T: [0,0] + c. EI
det=350.00 Full ms |
50.00-500.00

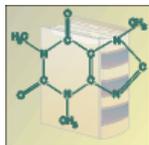
SI 785, RSI 929, MAINLIB, Entry#
90532, CAS# NA,
1,2-Bis(2-chlorophenyl)-1,2-
bis(3-chlorophenyl)ethane

Ready

Library Browser

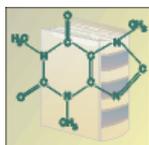


Purpose



- Search imported spectra against NIST or other libraries fully adjustable search parameters
- Create a unique user library

Library Browser Desktop



NIST MS Search

File Edit Search Desktop Tools Help

Incremental Name Search

ACENAPHTHENE

a-z only, no prefix Clear

Library: nistdemo

A 8729

Acenaphthene

Acenaphthene-d10

Acenaphthylene

Acenaphthylene, 1,2-dihydro-5-nitro-

Acetaldehyde

Acetaldehyde, chloro-

Acetaldehyde, trichloro-

Acetamide

Acetamide, 2-fluoro-

Expanded Plot

(U)Acenaphthene

Structure

Hit List, 1 spectrum

1 ? qual01#1002 RT: 11.35 AV: 1 NL: 1.42E5

1 (U) Acenaphthene

Compare with difference

(U)Acenaphthene

Text Info

Acenaphthene

Formula: C₁₂H₁₀

MW: 154 CAS#: 83-32-9

NIST#: NA ID#: 362

DB: nistdemo

47 Masses and Abundances

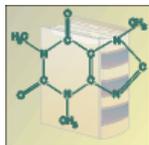
14	6	75	101	115	9
27	10	76	295	122	5
28	6	77	97	125	12
32	6	85	8	126	48
38	10	86	24	127	30
39	25	87	32	128	14
50	42	88	8	139	7
51	58	89	11	149	10

Clipboard, 1 spectrum

1 (U) Acenaphthene

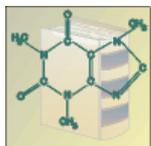
Previous Next Repl. UserSpec StructInfo Constraints AdjustWin QuickPrint SwitchBack

Desktop Elements



- **Incremental Name Search** is the text search utility of compound names within the NIST Libraries.
- **Compare** can compare 2 or 3 spectra, or 2 spectra with difference.
- **Plot** is the graphical display of the mass spectrum.
- **Text Info** is the text display of compound identification and mass spectrum, including synonyms.
- **Structure** is the graphical display of the molecular structure.
- **Hit List** is the text of graphical display of results of a search.
- **Clipboard** is a storage area for library hits, or spectra imported from Qual Browser.

Creating a User Library



File Edit Search Desktop **Tools** Help

Librarian...

Update User Lib Names...

MS Interpretation...

M/W estimation

Cl and/or Br estimation

Substructure information

Isotope Calculator

Mass Spectrum Interpreter

User Library Manager

Source: Name of Compound:

TextFile Demo_100#975 RT: 9.19 AV: 1 NL: 2.38E6

Lib Maintenance

Add

Move

Delete

DelLib

Files

Import

Export

New

Edit

SelAll

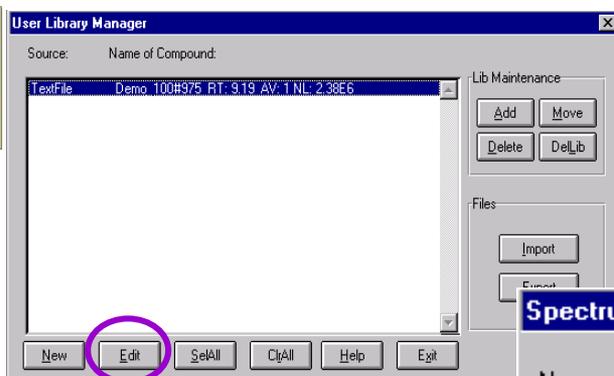
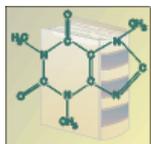
ClrAll

Help

Exit

- User Library Manager lists clipboard entries
- Enables Editing
- Enables Library Creation

Creating a User Library



- Select Spectrum to Edit
- Edit Information and Spectrum
- Save Spectrum

- Append Structure to Library Entry

Spectrum Information

Name: Demo_100#975 RT: 9.19 AV: 1 NL: 2.3

Chem. Formula:

Other Names:

Comments: T: + c Full ms [50.00-450.00]

Mol. Weight:

CAS Number:

ID Number: 0

Peaks Count: 195

Structure: No structure

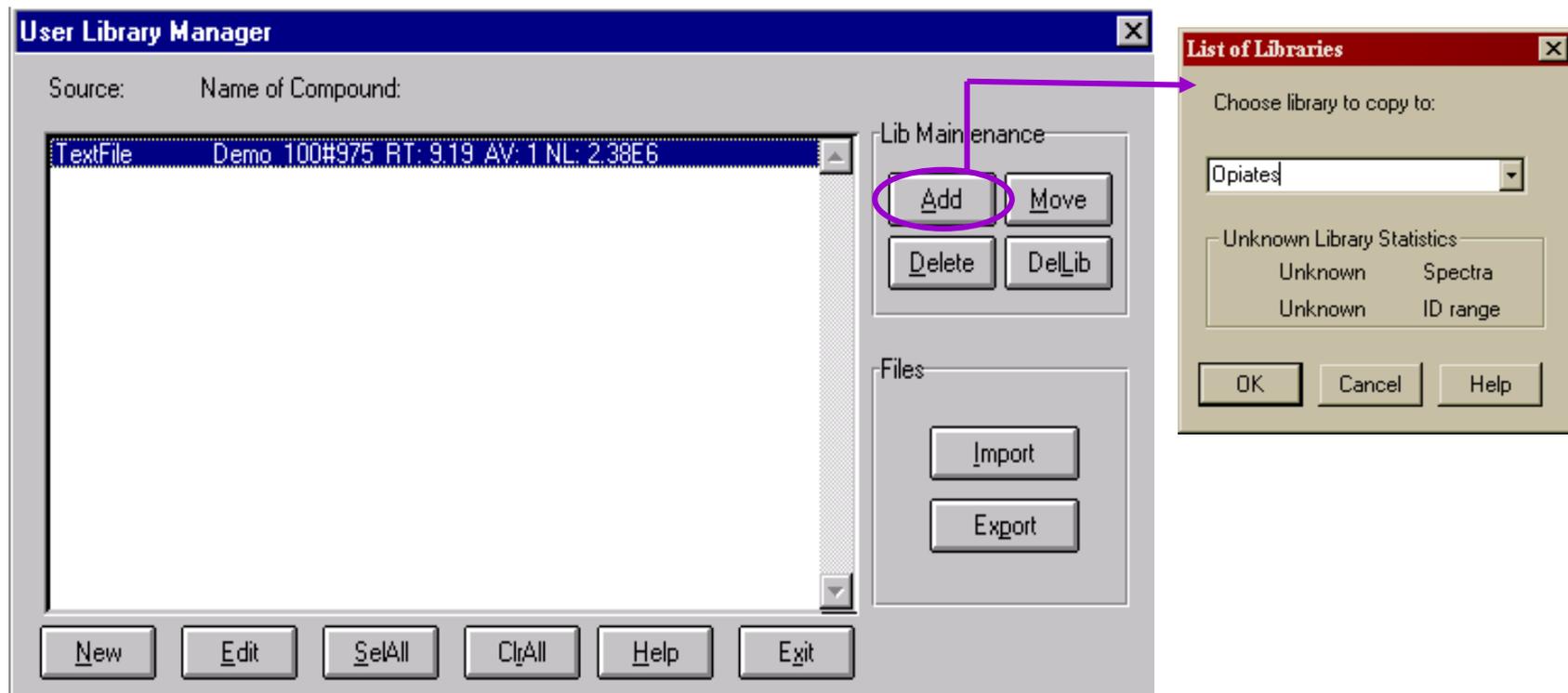
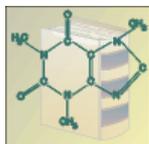
Save, Cancel, Help buttons are also visible.

Peaks Info

m/z	Abundance
50	0
51	3
52	9
53	8
54	80
55	228
57	0
58	0
59	0
62	0
63	0
65	1
66	7
67	112

Accept, Delete buttons are also visible.

Creating a User Library



User Library Manager

Source: Name of Compound:

TextFile	Demo 100#975 RT: 9.19 AV: 1 NL: 2.38E6
----------	--

Lib Maintenance

Add **Move**

Delete **DelLib**

Files

Import

Export

New **Edit** **SelAll** **ClrAll** **Help** **Exit**

List of Libraries

Choose library to copy to:

Opiates

Unknown Library Statistics

Unknown Spectra

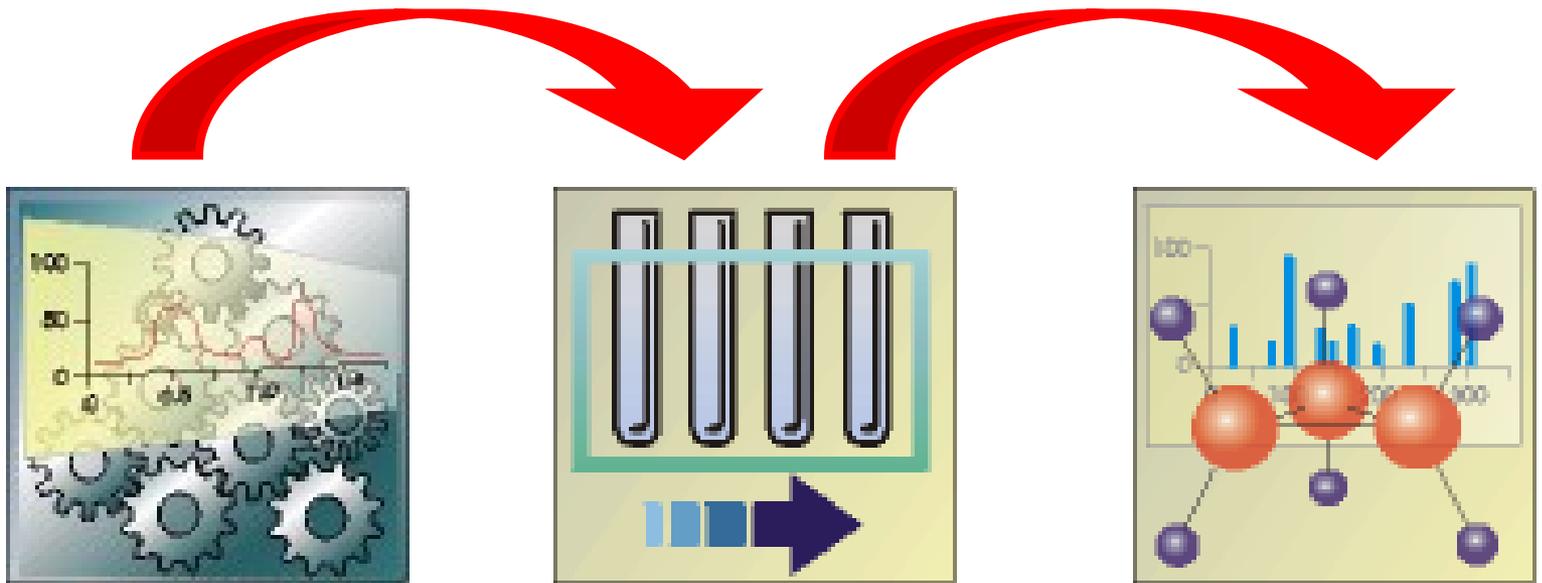
Unknown ID range

OK **Cancel** **Help**

- List Of Libraries

- Select Library to Add Spectrum to
- Type Name to Create New Library

Qual Processing



Qual Processing - Identification



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

File View Zoom Options GoTo Help

Identification | Spectrum Enhancement | Library Search Options | Library Search Constraints | Peak Purity

Detector: Type: MS Peak Detect: ICIS Delay (min): Genesis ICIS Avalon

Filter: TIC Trace: Mass Range TIC Base Peak Selected retention time window Range (min): 10.2-15.6

ICIS Peak Integration: Smoothing points: 15 Baseline window: 40 Area noise factor: 5 Peak noise factor: 10 Constrain peak width: Peak height (x): 5.0 Tailing factor: 1.0

Limit peaks: Select top peaks: Enable Select by area Select by height Num to select: 10 Rel peak height threshold: Enable % of highest peak: 10

OK Cancel Save As Default Advanced... Help

C:\Xcaliber\data\qual01 26/03/2001 12:10:16

RT: 10.41 - 15.64 SM: 15G

Relative Abundance vs Time (min)

RT: 10.64, 10.87, 11.27, 11.65, 12.15, 12.36, 12.84, 13.01, 13.37, 13.84, 14.07, 14.51, 14.67, 14.86, 15.11, 15.54

NL: 1.03ET TIC: MS ICIS: qual01

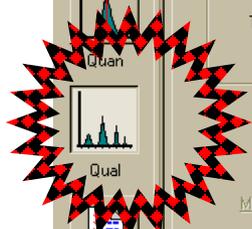
qual01 #14 RT: 3.12 P: + NL: 2.48E5 T: 10.00 + <E1 det=350.00 Full m/z [50.00-500.00]

Relative Abundance vs m/z

70.08, 98.06, 163.15, 206.39, 281.07, 365.02, 461.29

Ready NOT SAVED

Start Microsoft PowerPoint - [...] Roadmap - Home Page Processing Setup - Qual... 08:43



Qual Option

Open Raw File



Qual Processing - Integration

The screenshot displays the 'Processing Setup - Qual - Identification - Untitled (Int Std)' window. The 'ICIS Peak Integration' section is highlighted with a purple oval around the 'Baseline window' field, which is set to 2.0. A purple arrow points from this field to the 'Advanced...' button. Below the main window, a chromatogram plot shows 'Relative Abundance' vs 'Time (min)' with several peaks labeled with retention times (RT): 10.87, 10.95, 11.27, 11.36, 11.44, 11.85, 12.15, 12.36, 12.78, 12.84, 12.94, 13.24, 13.37, 13.84, 14.00, 14.44, 14.5, 14.20, and 14.44. The 'Advanced...' button is also circled in purple.

The 'ICIS Advanced Parameters' dialog box is open, showing the following settings:

- Noise Method: INCOS Noise, Repetitive Noise
- Peak Parameters:
 - Min peak width: 3
 - Multiplet resolution: 3 (circled in purple)
 - Area tail extension: 5
 - Area scan window: 0

Qual Processing – Spectrum Refine



Processing Setup - Qual - Spectrum Enhancement - Demo_ProcMeth.pmd (Int Std)

File View Zoom Options GoTo Help

Identification Spectrum Enhancement Library Search Options Library Search Constraints

Enhancement options:

- Enable
- Refine
- Combine
- Threshold

Refine

Window size (sec): 6.00 Noise threshold: 3

OK Cancel Save As Default Help

C:\Xcalibur\data/Demo Data/Demo_005 07/27/1999 11:58:23 AM

RT: 0.00 - 12.00 SM: 3G

Relative Abundance

Time (min)

RT: 6.04 RT: 7.16 RT: 9.19 RT: 11.26

NL: 7.24E6
TIC F: + c
Full mol
50.00-
450.00]
MS
Demo_005

Demo_005#239 RT: 6.04 P: + TH: 0.00 NL: 3.06E4

F: + c Full ms [50.00-450.00]

Relative Abundance

m/z

70.3 94.9 117.1 129.3 164.9 203.1 236.3 271.8 275.8 358.5 415.0 446.2

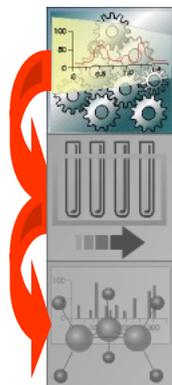
NUM NOT SAVED

“Refine” Algorithm



- Examines the chromatogram of each ion contributing to the scan
 - Discards ions without a peak maximum within ± 1 scan of the target chromatogram peak apex
 - Searches for a minimum within the specified Window Size range (half on either side of the peak) - Start and end of peak
 - Measures background noise level from scan beyond peak edges
 - Extrapolates the contribution of noise to the scan apex and adjusts the mass intensity of the apex scan accordingly
 - Uses Noise Threshold value to determine whether the adjusted intensity is significant in comparison to the background noise
 - The mass is discarded from the final spectrum if:
$$\text{Adjusted Intensity} < \text{Noise Intensity} \times \text{Background Noise}$$

Qual Processing – Spectrum Combine



Processing Setup - Qual - Spectrum Enhancement - Demo_ProcMeth.pmd (Int Std)

File View Zoom Options GoTo Help

Identification Spectrum Enhancement Library Search Options Library Search Constraints

Enhancement options

- Enable
- Refine
- Combine
- Threshold

Combine

Background subtraction left region

Region width (points): 5

Peak top region

Width (points): 4

Background subtraction right region

Region width (points): 5

Region end

- Peak start
- Points before peak top: 4

Region start

- Peak end
- Points after peak top: 6

OK Cancel Save As Default Help

C:\Xcalibur\data/Demo Data/Demo_005 07/27/1999 11:58:23 AM

RT: 0.00 - 12.00 SM: 3G

Relative Abundance

Time (min)

RT: 6.04 RT: 7.16 RT: 9.19 RT: 11.26

NL: 7.24E6
TIC F: + c
Full ms [50.00- 450.00]
MS
Demo_005

Demo_005#239 RT: 6.04 P: + TH: 0.00 NL: 3.06E4
F: + c Full ms [50.00-450.00]

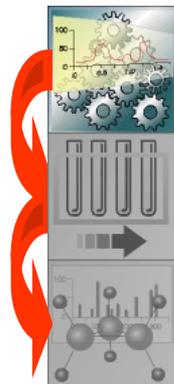
Relative Abundance

m/z

70.9 94.9 117.1 123.9 164.9 203.1 236.9 271.8 275.8 358.5 415.0 446.2

Ready NUM NOT SAVED

Qual Processing – Spectrum Threshold



Processing Setup - Qual - Spectrum Enhancement - Demo_ProcMeth.pmd (Int Std)

File View Zoom Options GoTo Help

Identification Spectrum Enhancement Library Search Options Library Search Constraints

Enhancement options

- Enable
- Refine
- Combine
- Threshold

Threshold

Cutoff threshold (%): 0.00

OK Cancel Save As Default Help

C:\Xcalibur\data/Demo Data/Demo_005 07/27/1999 11:58:23 AM 1:20

RT: 0.00 - 12.00 SM: 3G

Relative Abundance

Time (min)

RT: 6.04 RT: 7.16 RT: 9.19 RT: 11.26

NL: 7.24E6
TIC F: + c
Full ms [50.00 - 450.00]
MS
Demo_005

Demo_005#233 RT: 6.04 P: + TH: 0.00 NL: 3.06E4
F: + c Full ms [50.00-450.00]

Relative Abundance

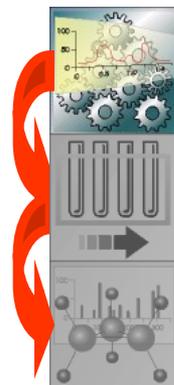
m/z

70.3 34.9 117.1 129.9 164.3 203.1 236.3 271.8 275.8 358.5 415.0 446.2

Ready

NUM NOT SAVED

Qual Processing – Library Search Options



Processing Setup - Qual - Library Search Options - Demo_ProcMeth.pmd (Int Std)

File View Options GoTo Help

Identification | Spectrum Enhancement | **Library Search Options** | Library Search Constraints

Search type

- Identity
- Similarity
- Normal
- Simple
- Quick
- Hybrid
- Penalize rare compounds
- Neutral loss

Options

Maximum number of hits:

Reverse search

Search with MW =

Append to user library

Enable

User library:

Thresholds

Match factor:

Reverse match factor:

Probability (%):

OK Cancel Save As... **Search List...** Help

Search List

Available libraries:

- REPLIB
- toxlib

Selected libraries:

- MAINLIB
- NISTDEMO

Add >>

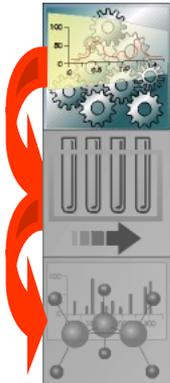
<< Remove

Top Up Down Bottom

OK Cancel Save As Default Help

Ready NUM NOT SAVED

Qual Processing – Search Constraints



Processing Setup - Qual - Library Search Constraints - Demo_ProcMeth.pmd (Int Std)

File View Options GoTo Help

Identification Spectrum Enhancement Library Search Options Library Search Constraints

Molecular weight

Enable

Range: 1-999999

Other databases

Enable

Fine NIH

TSCA EINECS

RTECS IR

EPA

USP

HODOC

Name fragment

Enable

Name:

Element constraints

Enable

Individual element

	Element	Condition	Value
*	<input type="text"/>	>	<input type="text" value="0"/>

Elements in compound

Elements:

All Some

Mass spectral peak constraints

Enable

	Type	m/z	From	To
*	<New>	<input type="text" value="0"/>	<input type="text" value="1"/>	<input type="text" value="1"/>

Absolute Relative

Ready NUM NOT SAVED

Enabling Qual Processing

The screenshot shows the 'Run Sequence' dialog box with the following settings:

- Acquisition Options:**
 - Instrument: GCQ Mass Spectrometer
 - Start Instrument: AS2000 Autosampler, TRACE GC 2000
 - Start When Ready:
 - Change Instruments...: [Button]
- Instrument Method:**
 - Start Up: [Text Field] [Browse...]
 - Shut Down: [Text Field] [Browse...]
- Programs:**
 - Pre Acquisition: [Text Field] [Browse...]
 - Post Acquisition: [Text Field] [Browse...]
 - Run Synchronously:
 - Pre Acquisition:
 - Post Acquisition:
- After Sequence Set System:**
 - On: Standby: Off:
- Processing Actions:**
 - Quan:
 - Qual:**
 - Reports:
 - Programs:
 - Create Quan Summary:

Buttons: OK, Cancel, Help

Reprocessing Data in Qual

The screenshot illustrates the process of reprocessing data in Qual. The 'File' menu is open, and the 'Batch Reprocess...' option is selected. The 'Batch Reprocess Setup' dialog box is displayed, showing the 'Processing Actions' section. The 'Qual' option is checked and circled, indicating that the data will be reprocessed using the Qual method. Other options in the 'Processing Actions' section include 'Peak Detection & Integration', 'Spectrum Enhancement', and 'Library Search', all of which are also checked. The 'Process Rows' field is set to '1-7'. The 'Advanced Options' section is also visible, with the 'Replace Sample Info' option unchecked. The 'OK', 'Cancel', and 'Help' buttons are at the bottom of the dialog box.

File Edit Change Actions View GoTo Help

Check Disk Space...
Run This Sample...
Run Sequence...
Batch Reprocess...
Open File
Start Analysis
Stop Analysis
Pause Analysis
Devices On
Devices Standby
Devices Off
Automatic Devices On
Reinstate Warnings

Batch Reprocess Setup

Processing Actions

- Quan
- Peak Detection & Integration
- Calibration
- Quantification
- Qual
 - Peak Detection & Integration
 - Spectrum Enhancement
 - Library Search
- Reports
 - Print Sample Reports
 - Print Summary Reports
- Programs
- Create Quan Summary Spreadsheet

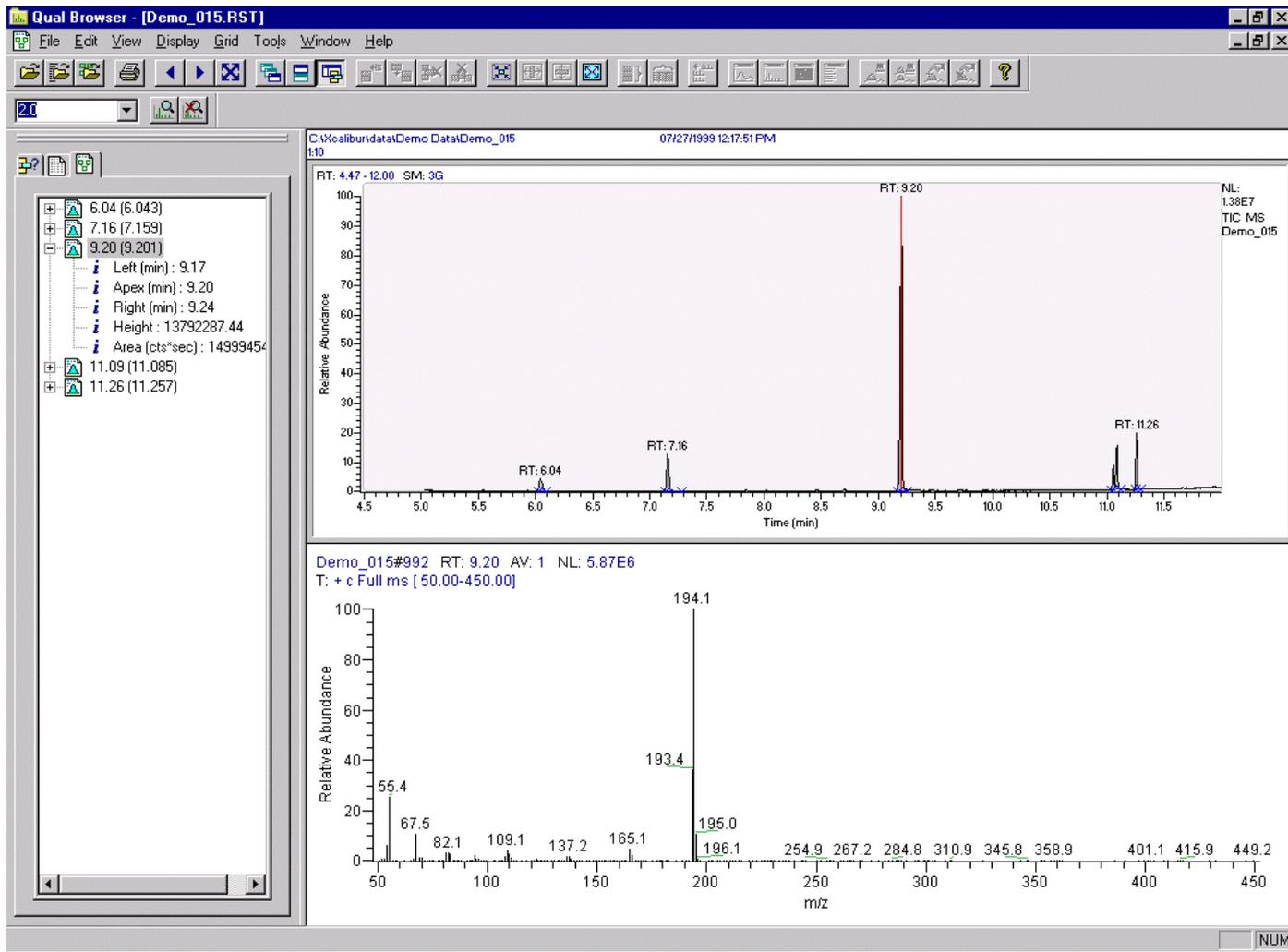
Process Rows: 1-7

Advanced Options

- Replace Sample Info

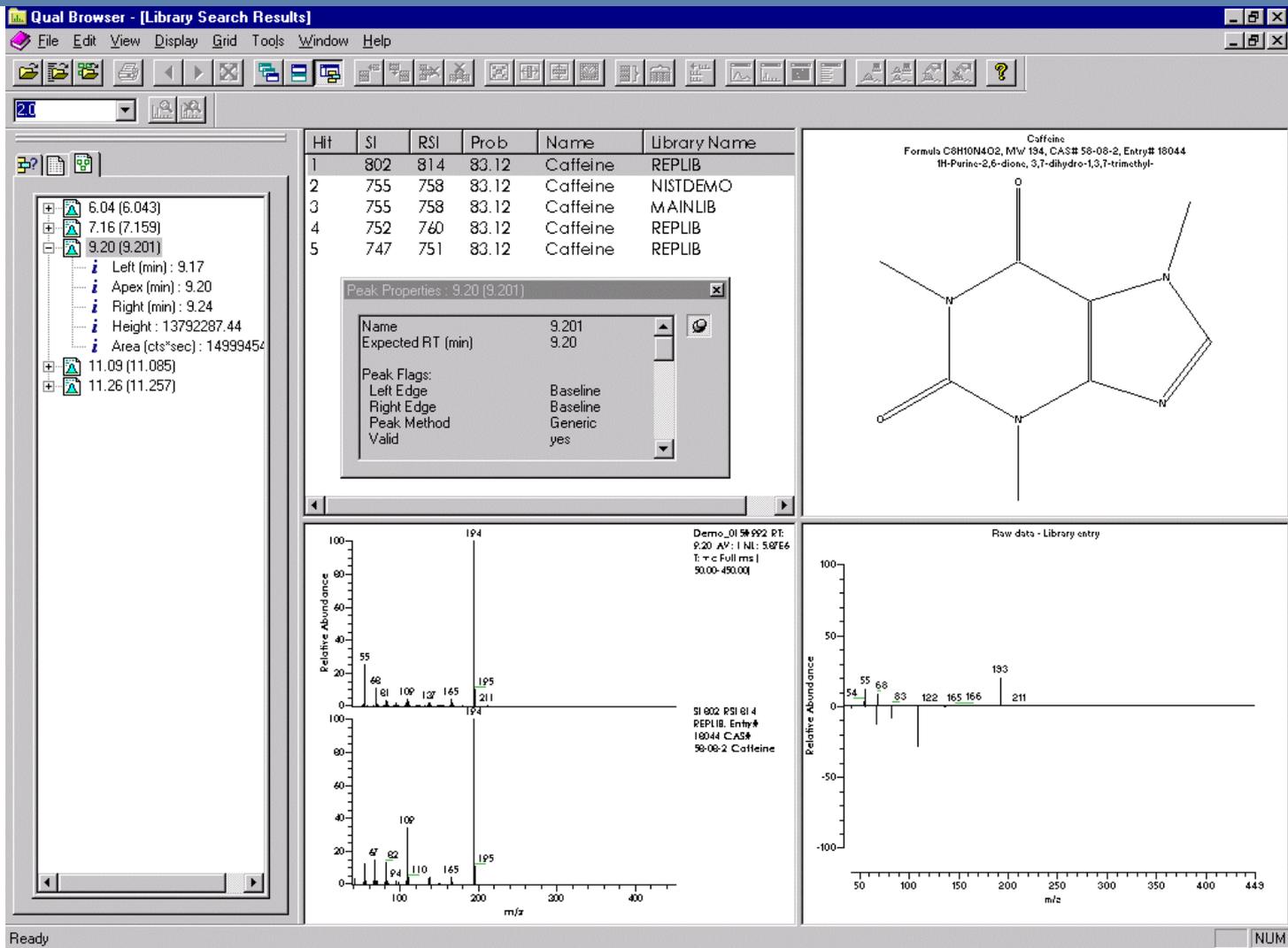
OK Cancel Help

Qual Browser - Results File - Spectrum



Open Result
File

Qual Browser - Results File – Library Search



Library Search Results

Qual Browser
File Edit View Display Grid Tools Window Help

21

Library Search Results

Hit	SI	RSI	N...	Library Name
1	929	939	1,4...	

11.27 (11.269)
12.36 (12.361)
12.84 (12.844)
Left (min): 12.81
Apex (min): 12.84
Right (min): 12.90
Height: 4235565.99
Area (cts*sec): 6654729.03
13.37 (13.369)
14.07 (14.069)
14.20 (14.203)
14.51 (14.511)
14.62 (14.620)
14.68 (14.678)
15.11 (15.111)

Peak Properties: 12.84 (12.844)

Name	12.844
Expected RT (min)	12.84
Peak Flags:	
Left Edge	Baseline
Right Edge	Baseline
Peak Method	Generic
Valid	no

Library Search Results

Hit	SI	RSI	N...	Library Name
1	929	939	1,4...	

qual01*1181 RT: 12.84 AV: 1 NI: 41265T: (0 0) r
c El det=250.00 Full ms | 50.00-500.00

SI 929 RSI 939 MAIN LIB. Entry# 20977. CAS#
124-96-9: 1,4-Dichloronaphthalene
1,2,3,4-tetrahydro-1,4-dichloro-1,4-dihydro-
hexahydro-

Raw data - Library entry

Relative Abundance

m/z

qual01

C:\Xcalibur\data\qual01 26/03/2001 12:10:16

RT: 12.36 RT: 12.84 RT: 13.37

Time (min)

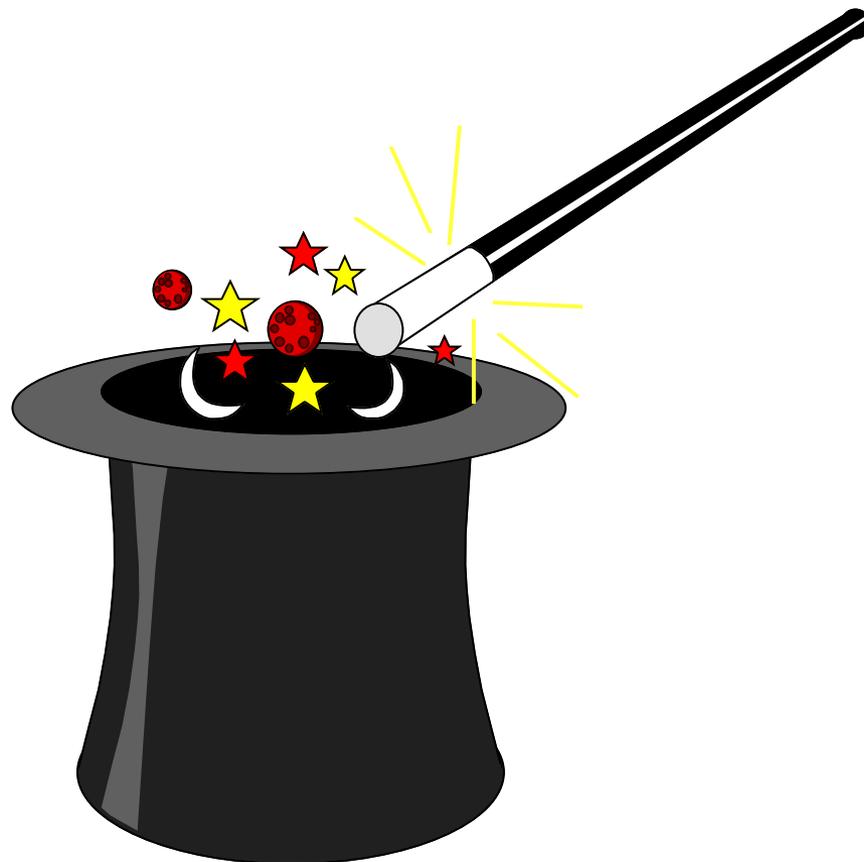
Relative Abundance

m/z

Open Results
File

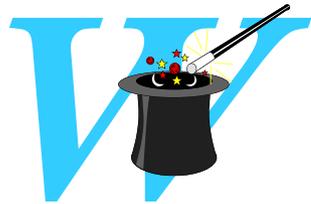


Merlin Reporting



Steps to Merlin Reporting

1. Merlin Report Template (MS Word)



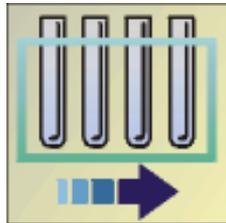
Open Microsoft Office
Click on **File : New**
Double Click on **Merlin.dot**
Create and Save Report Template

2. Processing Setup



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

3. Reprocess Selected Files



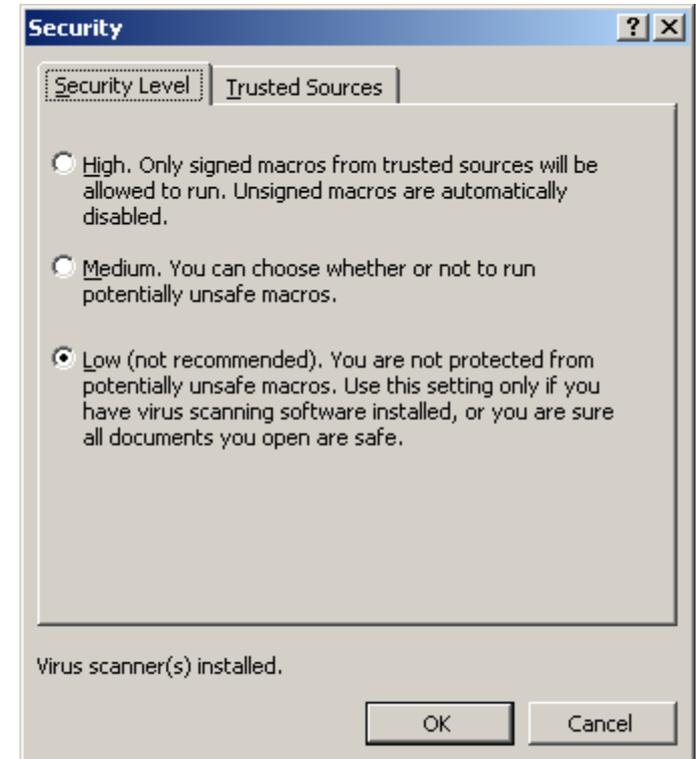
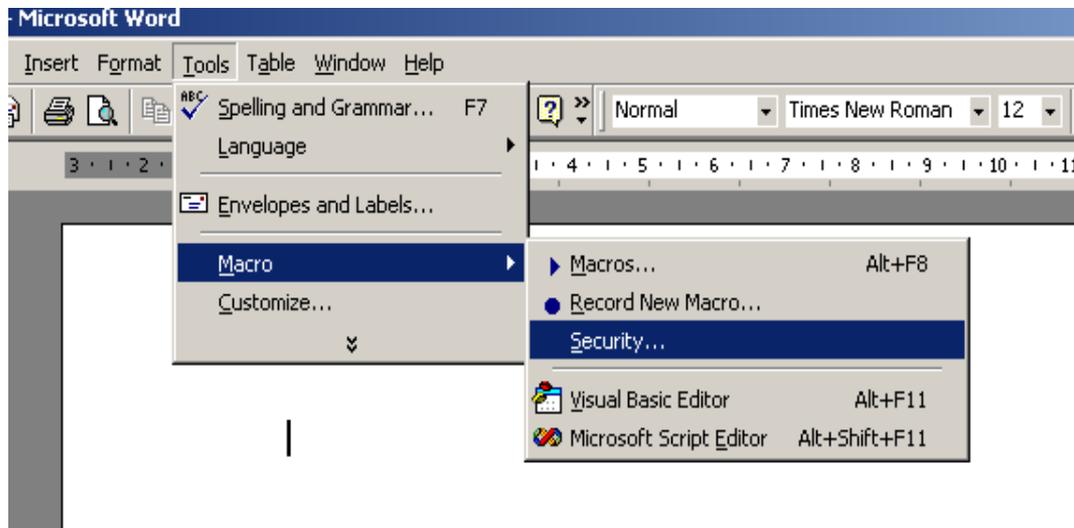
Goto **Home Page - Sequence Setup**
Open/Make a Sequence
Click **Actions : Batch Reprocess...**
Check **Reports** and **Print Reports** Boxes

Before you Start



Decide what objects you want on the report and how they should be laid out to avoid excessive manipulation once in Merlin

Macro Security in Word



Example Report Outline



- Sample Information
- Chromatogram
 - TIC, full scale, peaks (w/ RT, height, area)
- Spectrum for each detected peak
- Qualitative Results Table
 - retention time, peak height, and peak area
- Library Search Results
 - retention time, component name, and probability

Open Merlin



The image shows a screenshot of the Microsoft Word 97 interface. The menu bar at the top includes File, Edit, View, Insert, Format, Tools, Table, Window, and Help. The 'File' menu is open, showing options like New... (Ctrl+N), Open... (Ctrl+O), Close, Save (Ctrl+S), Save As..., Versions..., Page Setup..., Print Preview, Print... (Ctrl+P), Send To, Properties, and Exit. A red arrow points from the 'File' menu to the 'New' dialog box. The 'New' dialog box has tabs for Reports, Web Pages, and Office 97 Templates. Under 'Office 97 Templates', there are sub-tabs for General, Legal Pleadings, Letters & Faxes, Memos, Other Documents, and Publications. The 'General' tab is selected, showing icons for Blank Document, Web Page, E-mail Message, and BaseGrd.dot. The 'Merlin.dot' icon is circled in red. On the right side of the dialog, there is a 'Preview' section with a preview window that says 'Preview not available.' At the bottom, there are radio buttons for 'Create New' with 'Document' selected and 'Template' unselected. 'OK' and 'Cancel' buttons are at the bottom right.

Merlin Wizard



Merlin Report Structure Wizard - Step 1 of 4 : Select Report

Which type of REPORT would you like to construct?

Sample: Summary: Method:

Quan By Sample Quan Processing

Qual

Quan & Qual

A sample report allows you to present information relating to a particular Xcalibur sample 's raw file and the results created when processing it.

Qual reports utilize the results generated using the Qual section of a Processing Method. These reports are typically used to present a chromatogram plot with detected peaks shown, the spectra associated with the tops of those detected peaks and, if appropriate, the results of library seraching of thos spectra. This report type does not offer any of the results created using Quan processing.

< Back Next > Cancel

Select Repeat and Non-Repeating Sections



Merlin Report Structure Wizard - Step 2 of 4 : Select Sections

-> Sample Qual Report

- Non Repeating Qual Section
- Qual Repeating Section

Which SECTIONs would you like in the Sample Qual Report?

Sections available:

- Non Repeating Qual Section
- Qual Repeating Section

Sections added:

- Non Repeating Qual Section
- Qual Repeating Section

Add >

< Remove

<< Remove All

Move Up **Move Down**

Description of section:

A Qualitation Repeating Section is used to display details relating to a single Qual Peak within data. The section is then repeated for all Qual peaks found

< Back **Next >** **Cancel**

Select Objects



Merlin Report Structure Wizard - Step 3.1 of 4 : Select Objects

Which OBJECTs would you like in **the Non Repeating Qual Section?**

Objects available:

- Avalon Qual Events Table
- Chromatogram
- Page Break
- Processing Method - Qual
- Qual Peak Table
- Sample Header
- Spectrum
- Text Label

Objects added:

- Sample Header
- Chromatogram
- Qual Peak Table

Buttons: Add >, < Remove, << Remove All, Move Up, Move Down

Description of report object:
Displays details of the Avalon peak detection algorithm events configured in the qual section of a processing method

Merlin Report Structure Wizard - Step 3.2 of 4 : Select Objects

Which OBJECTs would you like in **the Qual Repeating Section?**

Objects available:

- Chromatogram
- Lib Search Graph Table
- Lib Search Results Table
- Mass Spectrum List Table
- Page Break
- Spectrum
- Text Label

Objects added:

- Spectrum
- Lib Search Graph Table
- Lib Search Results Table

Buttons: Add >, < Remove, << Remove All, Move Up, Move Down

Description of report object:
Displays an explicitly specified spectrum from a RAW file in a non-repeating section or either a quan or qual peak spectrum inside the appropriate repeating section

< Back Next > Cancel

Confirm Report Summary Options



Merlin Report Structure Wizard - Step 4 of 4 : Confirm Report

The following report structure will be generated:

- Non Repeating Qual Section
 - Sample Header
 - Chromatogram
 - Qual Peak Table
- Qual Repeating Section
 - Spectrum
 - Lib Search Graph Table
 - Lib Search Results Table

< Back Finish Cancel

Format Chromatogram



Select Chromatogram Type

A chromatogram control can be used to display raw data explicitly or processed raw data in the form of a results file. When results are being displayed, these results can be specifically QUAN or QUAL results.

Chromatogram Type

Select the type of chromatogram control you want to add to the report template:

- Raw file based chromatogram
- Quan results based chromatogram
- Qual results based chromatogram
- Show all qual peaks in result file

< Back Next > Cancel

Select Demo File

A demo file can be associated with a chromatogram control. This demo file allows the control to show the current settings for the chromatogram which will look when applied to real data.

Use demo file

Demo File

C:\xcalibur\training\Quan21.RST

< Back Next > Cancel

Configure How The Peak Window Will Look

When a peak from a result file is being displayed it is possible to configure whether baselines are shown and also the width of the window to display.

Show Baselines

Set Custom Window Width

Custom width (min): 1.75

< Back Next > Cancel

Select Chromatogram Style

Select the style to use when plotting the chromatogram trace. The arrangement is only really applicable when there will be more than one trace on the chromatogram.

Plotting

- Point To Point
- Stick

Arrangement

- Stack (2D)
- Overlay (3D)

Elevation: 0 45 90

Skew: 0 90 45

Draw Backdrop

< Back Next > Cancel

Select The Peak Labelling

Configure how peaks in the chromatogram trace will be labelled and the style of these labels.

Label With

- Retention Time
- Scan Number
- Base Peak
- Signal to Noise
- Flags
- Area
- Height

Label Styles

- Offset
- Rotated
- Boxed

Size: 35

Label Threshold (%): 5

< Back Next > Cancel

Select How The Chromatogram Axes Will Be Configured

It is possible to configure how the axis are labelled and whether they are offset. It is also possible to split the X axis into several divisions and the units on the Y axis can be made absolute or relative.

X-Axis

Name: Time (min) Offset:

Split time range: Divisions: 2

Y Axis

Label: From detector Absolute Custom

Units: Absolute Relative

Name: Relative Abundance Offset:

< Back Next > Cancel

Configure Normalization For The Chromatogram

Configure how the chromatogram display will be normalized. The normalization method will be applied to each trace in the chromatogram individually.

Normalize method

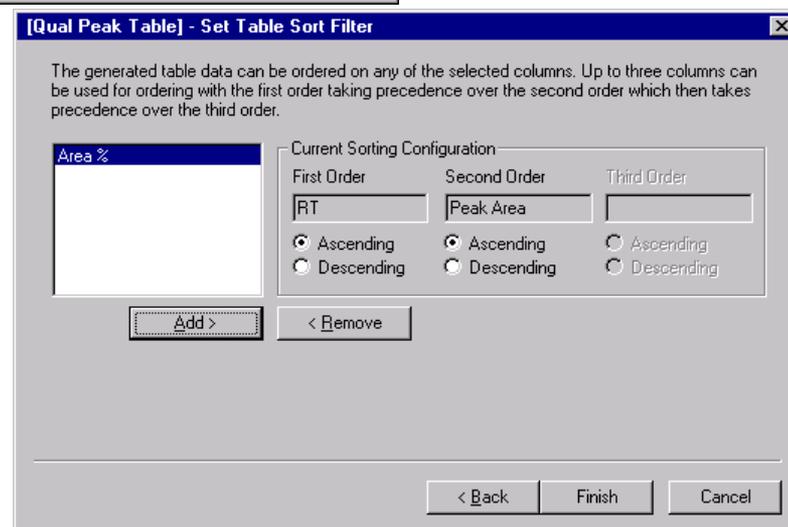
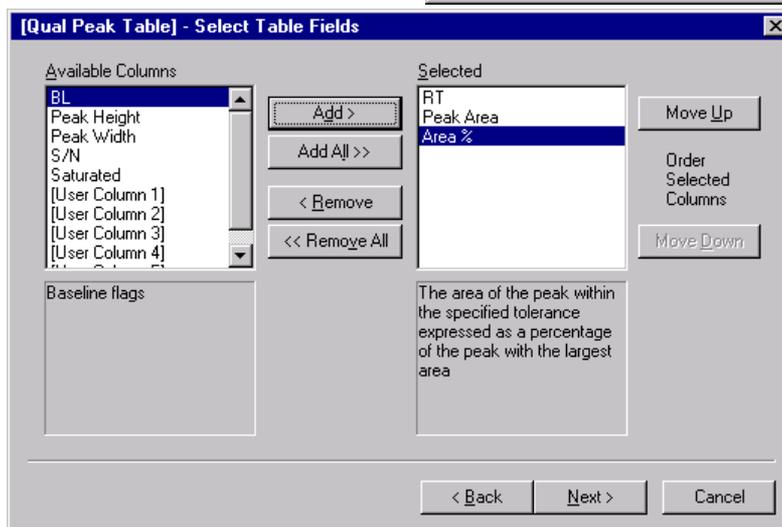
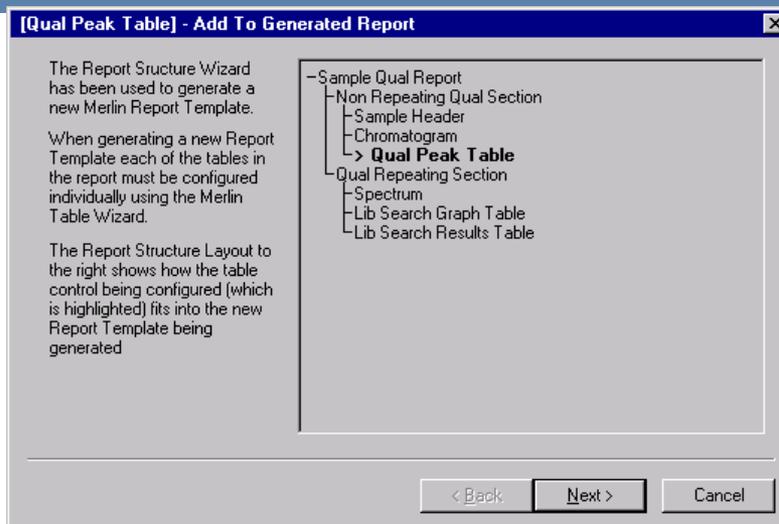
- Auto zero
- Intensity Range (%) 0.0-100.0

Normalize to

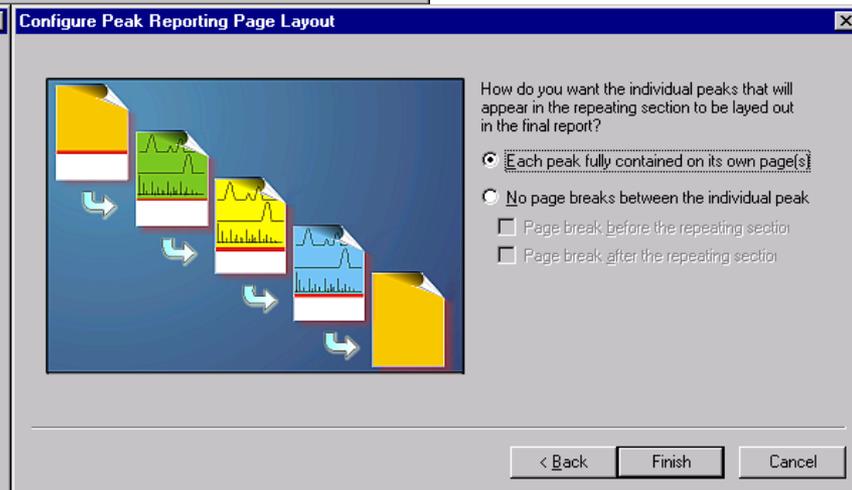
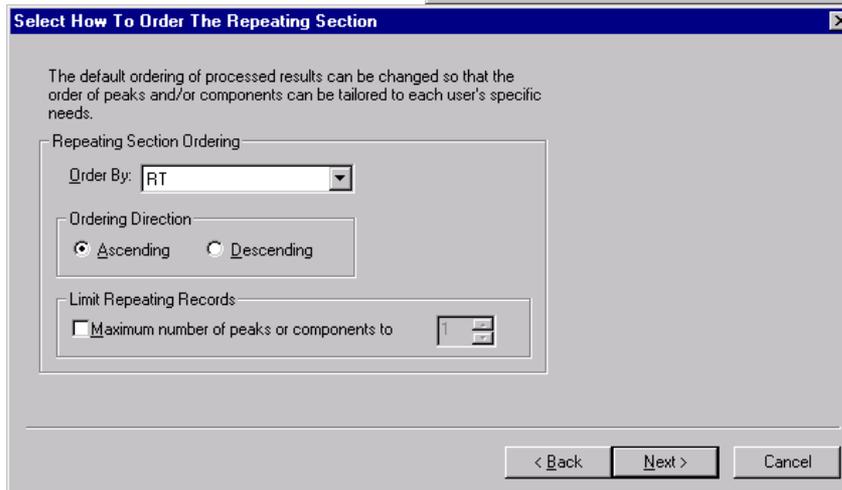
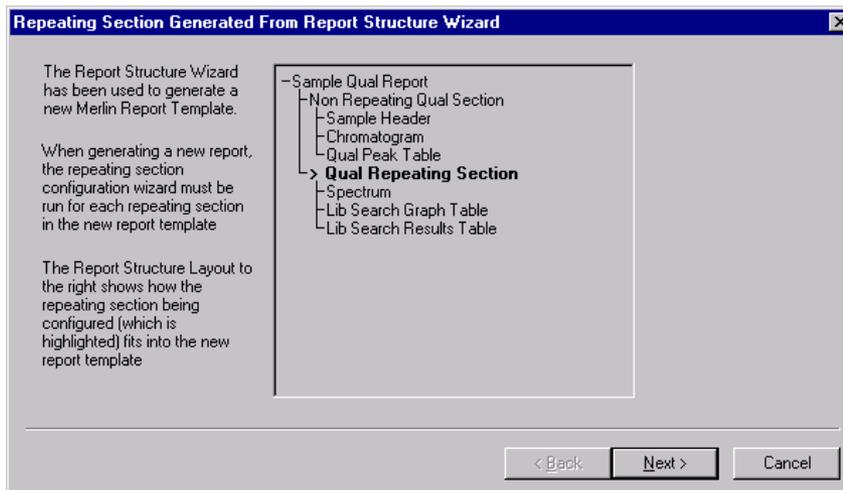
- Largest peak in subsection
- Largest peak in range
- Largest peak in scan

< Back Finish Cancel

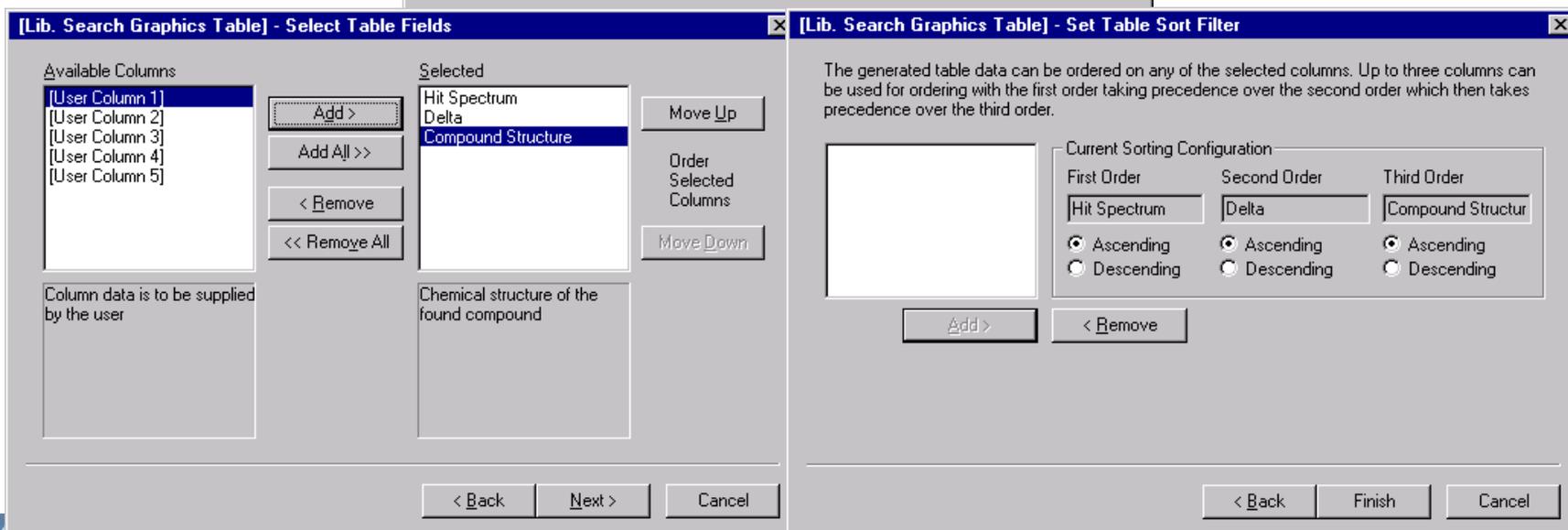
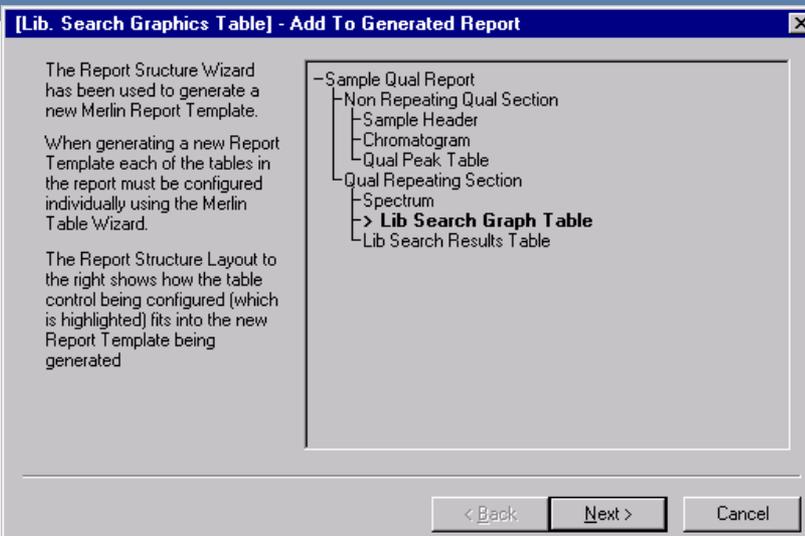
Format Qual Peak Table



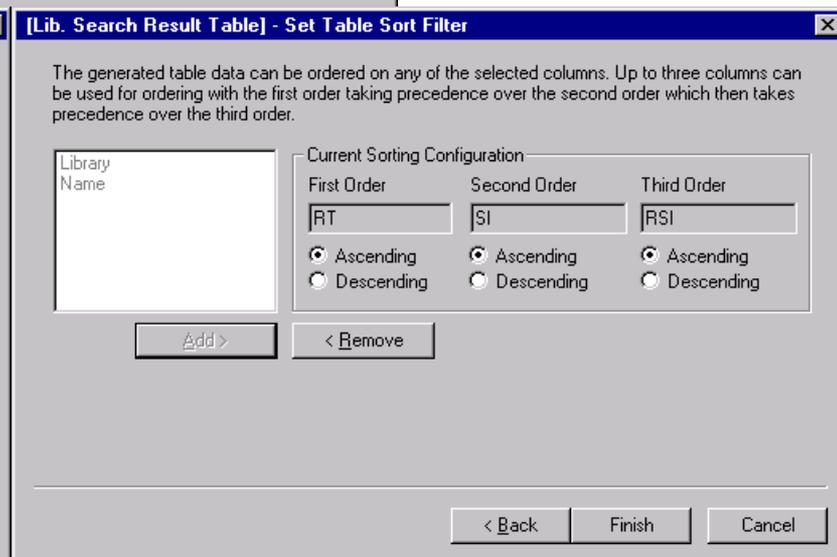
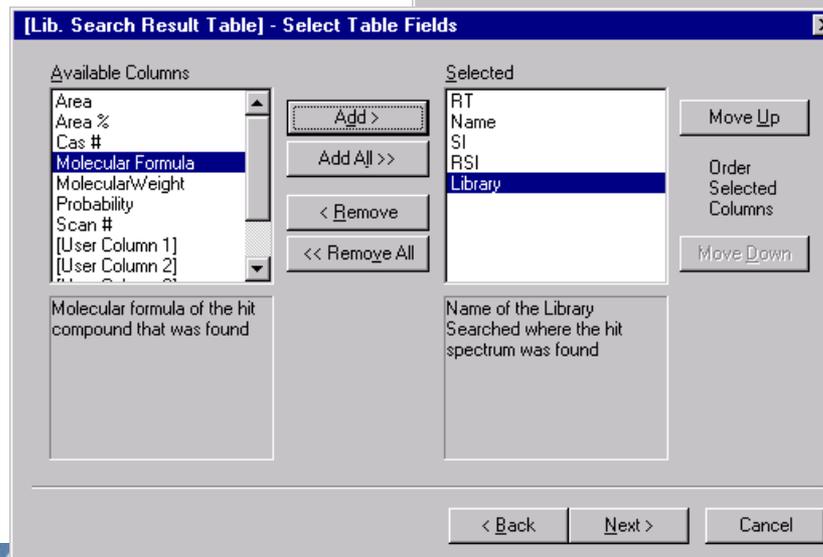
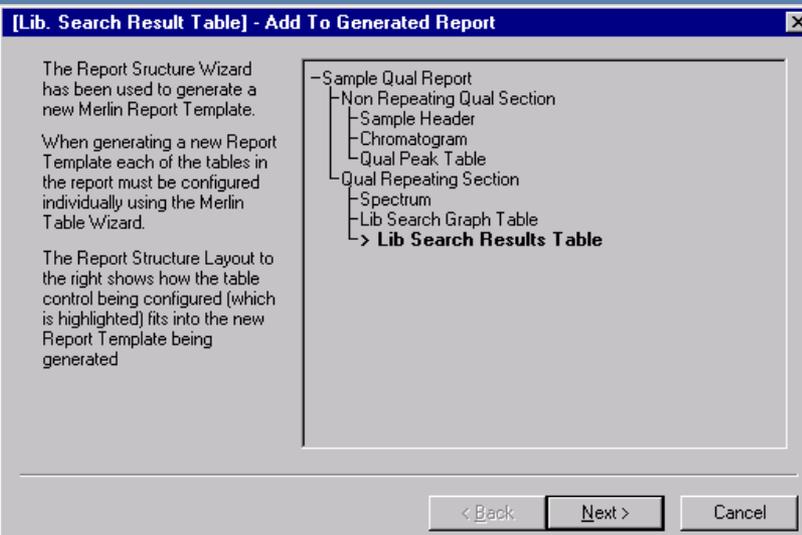
Format Qual Repeating Section



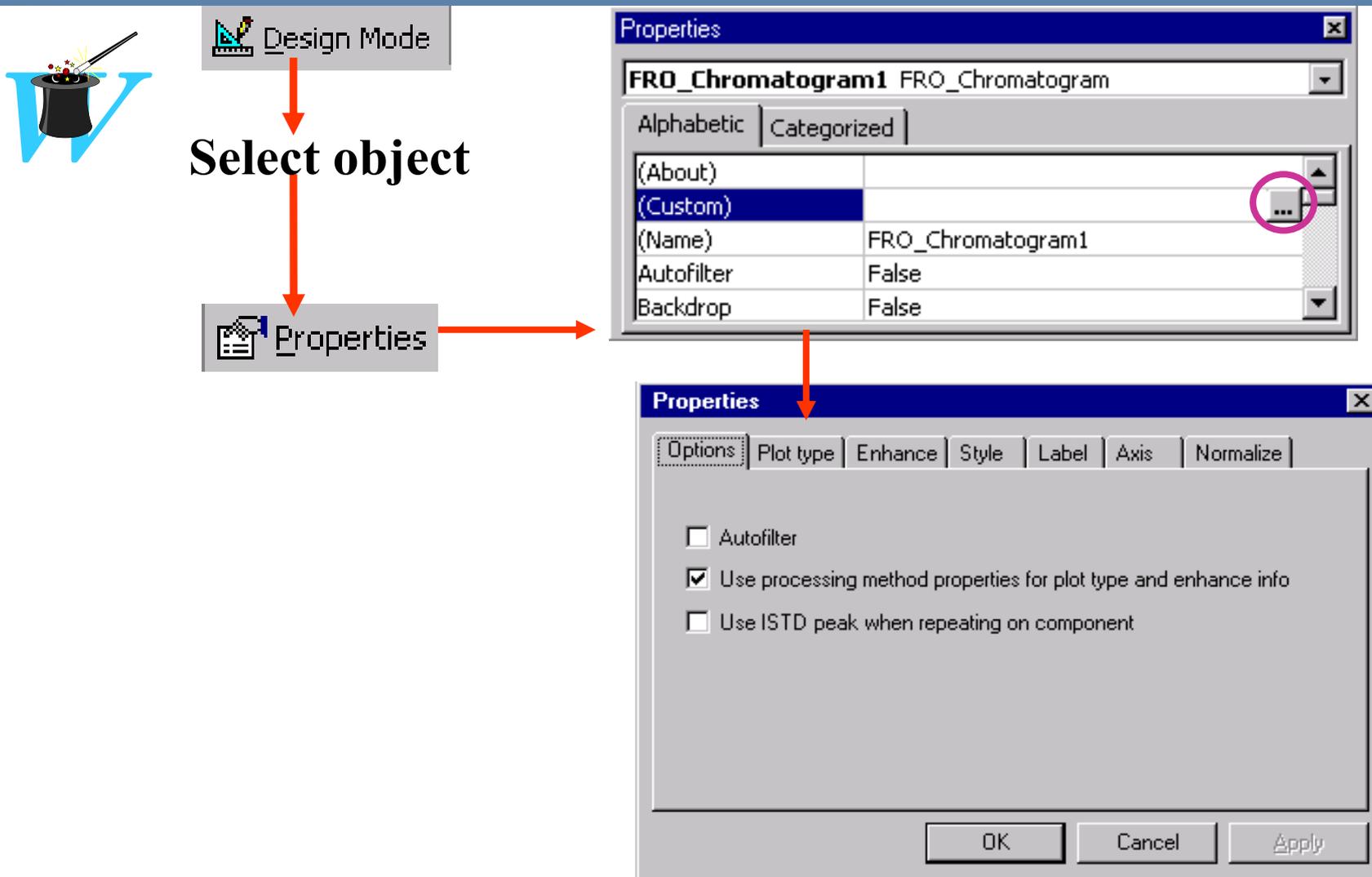
Format Library Search Graph Table



Format Library Search Results Table



Post Wizard: Format Objects



Post Wizard – Insert New Objects



- Component Ident/Detect/Cal Settings
- Component Settings Summary
- Component Sys Suit/Flags Settings
- Proc. Method General/Qual
- Quan Peak Summary
- Sample Header

Text Objects ▾ Table Objects ▾ Graphic Objects ▾

- Chromatogram
- Component Cal Curve
- Spectrum

- Calibration File Table
- Component Cal Level Table
- Component Ion Ratio Confirmation Table
- Component QC Level Table
- Component Spectrum Table
- Dilution Factor Table
- Ion Ratio Confirmation Results Table
- Lib. Search Graph Table
- Lib. Search Result Table
- Proc. Method Audit Trail Table
- Proc. Method Programs Table
- Proc. Method Sample Reports Table
- Proc. Method Summary Reports Table
- Qual Peak Table
- Quan Peak Table
- Sample Table (Quan Results)
- Sequence Table
- Spectrum Candidate Results Table
- Spectrum List Table
- Use Another Table For Values

Add Repeating Section

Finishing the Template



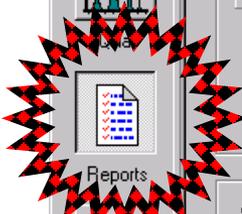
- Exit Design Mode
- Close all bookmarks
- Save the report to the template directory
- Build one section at a time
- Test as you go

The screenshot shows the Merlin Operations menu with the following options: Generate Report, Show Required Data Sources, and Clean Document. An arrow points from the 'Generate Report' option to a dialog box titled 'Merlin Data Sources - Select Xcalibur Data File to Generate Report'. The dialog box contains the text 'An Xcalibur Result file is required to generate the report' and a 'Result File' field with the path 'C:\Xcalibur\data\qual01.RST'. The 'OK' button is circled in pink, and a pink circle highlights the browse button (three dots) next to the file path. Below the dialog box, the text 'Browse for *.RST' is written.

Input Merlin Report into Processing Method



Report
Option



Processing Setup - Reports - quan.pmd (Int Std)

File View Options GoTo Help

Sample reports:

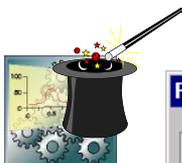
	Enable	Std	QC	Unk	Other	Save As	Report Template Name
1	Yes	Yes	Yes	Yes	Yes	Doc	C:\Xcalibur\templates\LibrarySearchReport2
*		Yes	Yes	Yes	Yes	None Text Doc HTML	

Summary reports:

	Enable	Save As	Report Template Name
*		None	

Ready NUM

Enabling Reports



The image shows two overlapping dialog boxes from a software interface. The 'Run Sequence' dialog box is on the left, and the 'Batch Reprocess Setup' dialog box is on the right. Both boxes have a blue title bar and a close button in the top right corner. The 'Run Sequence' dialog box has several sections: 'Acquisition Options' with a table of instrument details, 'Start When Ready' checked, 'Instrument Method' with 'Start Up' and 'Shut Down' fields, 'Programs' with 'Pre Acquisition' and 'Post Acquisition' fields, 'Run Synchronously' with 'Pre Acquisition' and 'Post Acquisition' radio buttons, and 'After Sequence Set System' with 'On', 'Standby', and 'Off' radio buttons. The 'Batch Reprocess Setup' dialog box has a 'Process Rows' field set to '1-10' and a 'Processing Actions' list. In both dialog boxes, the 'Reports' checkbox is highlighted with a pink oval. The 'Batch Reprocess Setup' dialog box also has 'Print Sample Reports' and 'Print Summary Reports' checkboxes checked, and 'Replace Sample Info' unchecked.

Run Sequence

Acquisition Options

Instrument	Start Instrument
TRACE MS	
AS2000 Autosampler	Yes
TRACE GC 2000	

Start When Ready Change Instruments...

Instrument Method

Start Up: [] Browse...

Shut Down: [] Browse...

Programs

Pre Acquisition: [] Browse...

Post Acquisition: [] Browse...

Run Synchronously

Pre Acquisition Post Acquisition

After Sequence Set System:

On Standby Off

OK Cancel Help

Batch Reprocess Setup

Process Rows: 1-10

Processing Actions

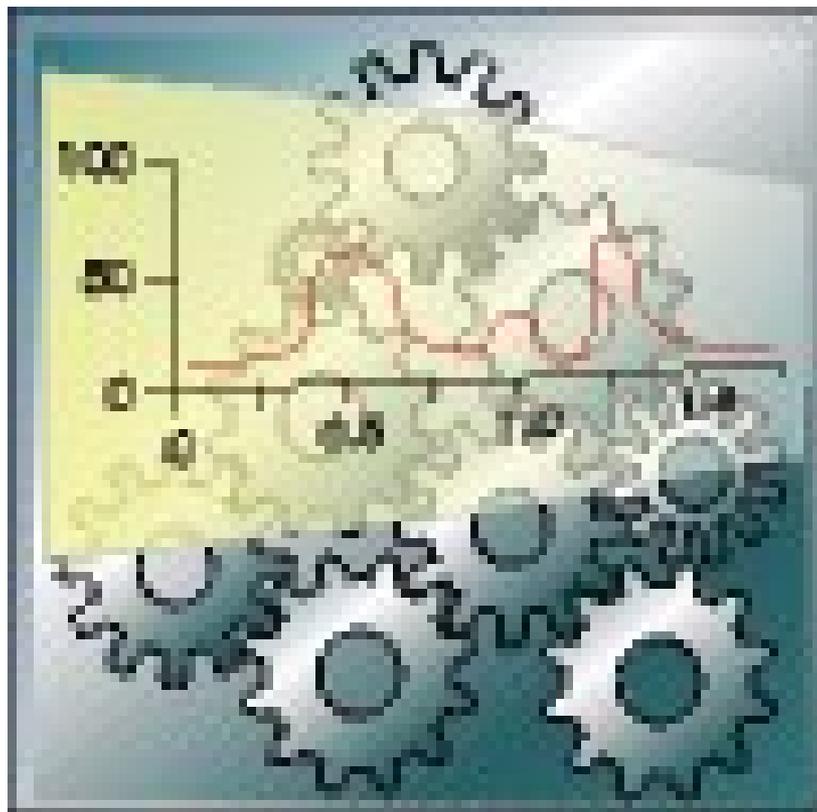
- Quan
- Peak Detection & Integration
- Calibration
- Quantitation
- Qual
 - Peak Detection & Integration
 - Spectrum Enhancement
 - Library Search
- Reports
 - Print Sample Reports
 - Print Summary Reports
- Programs
- Create Quan Summary Spreadsheet

Advanced Options

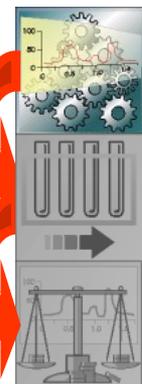
Replace Sample Info

OK Cancel Help

Quan Processing Set Up



Quan Options



File View Zoom Options GoTo Help

- Identification...
- Chromatography By...
- Calibration By...
- Delete CAFFEINE_(IS)...
- Display...
- Settings...
- Enable Warnings

Chromatography Options [X]

Chromatography by

GC

LC

OK Cancel Save As Default Help

Calibration Options [X]

Calibration by

Internal standard

External standard

OK Cancel Save As Default Help

Identification Tab

Processing Setup - Quan - Identification - quan.pmd (Int Std)

File View Zoom Options GoTo Help

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

Name: CAFFEINE

Retention time: Expected (min): 11.18 Window (sec): 30.0

Detector type: MS Peak Detect: ICIS

Filter: _____

Trace: Mass Range

Mass (m/z): 194.0

Use as RT reference View width (min): 0.75

Adjust using: _____

Keys: _____

OK Cancel Save As Default Help

Qual

4.67

Components

- benzophenone
- Hexachlorohexan
- CAFFEINE

C:\Xcaliber\training1\Quan24 03/27/01 06:51:25 PM

RT: 10.80 - 11.55 SM: 1G

Relative Abundance

Time (min)

RT: 11.18
AA: 61141751

NL: 4.51E7
m/z: 193.5-194.5 MS
ICIS
Quan24

Quan24 #476 RT: 11.18 P: + NL: 4.51E7
T: [0,0] + c EI det=350.00 SIM ms [104.95-105.05, 108.95-109.05, ...]

Relative Abundance

m/z

181.0 194.0 290.0

Ready

NUM NOT SAVED

Peak Detection Tab

Processing Setup - Quan - Detection - quan.pmd (Int Std)

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Quant Qual Reports

Genesis Peak Integration

Smoothing points: 1

S/N threshold: 0.5

Enable valley detection

Expected width (sec): 2.0

Constrain peak width

Peak height (%): 5.0

Tailing factor: 1.0

Genesis Peak Detection

Ion ratio confirmation

Enable

Ion ratio using: Area

m/z	Target Ratio (%)	Window (±%)
*	0.0	0

Minimum peak height (S/N): 1.0

Window %

Relative

Absolute

Qualifier ion coelution: 0.025 min

ICIS Peak Integration

Smoothing points: 1

Baseline window: 50

Area noise factor: 2

Peak noise factor: 10

Constrain peak width

Peak height (%): 2.0

Tailing factor: 4.0

Avalon Peak Integration

Smoothing points: 1

Time	Event	Value
Initial Val	Start Threshold	10000.000
Initial Val	End Threshold	10000.000
Initial Val	Area Threshold	10000.000
Initial Val	P-P Threshold	1.000
Initial Val	Bunch Factor	1.000
Initial Val	Negative Peaks	Off
Initial Val	Tension	1.000

Auto Calculate Initial Events

03/27/01 06:51:25 PM

RT: 11.18
AA: 57243785

NL: 4.51E7
m/z: 193.5-
194.5 MS
Genesis
Quan24

Relative Abundance

Time (min)

Components

- benzophenone
- Hexachlorohexan
- CAFFEINE

Ion Ratio Confirmation Option

Processing Setup - Quan - Detection - quan.pmd (Int Std)

File View Zoom Options GoTo Help

Quantification icons: Quan, Qual, Reports, Programs

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

Genesis Peak Integration

Smoothing points: 1
S/N threshold: 0.5
 Enable valley detection
Expected width (sec): 2.0
 Constrain peak width
Peak height (x): 5.0
Tailing factor: 5.0

Genesis Peak Detection

Ion ratio confirmation (highlighted)
 Enable
Ion ratio using: Area

	m/z	Target Ratio (%)	Window (±%)
1	109.0	50	20
*	0.0	0	5

Minimum peak height (S/N): 1.0

Window %:
 Relative
 Absolute

Qualifier ion coelution: 0.025 min

Buttons: OK, Cancel, Save As Default, Advanced..., Flags..., Help

Components

- benzophenone
- Hexachlorohexan
- CAFFEINE

Chromatogram

RT: 10.80 - 11.55 SM: 1G
RT: 11.18
AA: 53897146
NL: 4.51E7
m/z= 193.5-194.5 MS
Genesis
Quan24

Mass Spectrum

Quan24 #476 RT: 11.18 P: NL: 4.51E7
T: 0.00 • c EI det=350.00 SIM m/z [104.95-105.05, 108.95-109.05, ...]

Relative Abundance vs. m/z

Ready | NUM | NOT SAVED

Spectrum Option

Processing Setup - Quan - Detection - quan.pmd (Int Std)

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Genesis Peak Integration

Smoothing points: 1

S/N threshold: 0.5

Enable valley detection

Expected width (sec): 2.0

Constrain peak width

Peak height (%): 5.0

Tailing factor: 5.0

Genesis Peak Detection

Spectrum

Highest peak

Nearest BT

Minimum peak height (S/N): 1.0

m/z	Intensity (%)
43	165.2 4.70
44	166.2 1.98
45	167.3 0.18
46	179.2 0.26
47	193.4 32.93
48	194.1 100.00
49	195.1 10.78

Thresholds

Forward: 700

Reverse: 700

Match: 75

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

C:\Xcaliber\data1\Demo Data1\Demo_100.t1 07/27/99 12:56:44 PM

RT: 8.83 - 9.58 SM: 1G

Relative Abundance

Time (min)

RT: 9.20
AA: 6553916

NL: 5.75E6
m/z: 193.5
194.5 MS
Genetic
Demo_100

Relative Abundance

m/z

Demo_100 #377 RT: 9.20 P: NL: 4.51E6
T: + c Full ms [50.00-450.00]

NUM NOT SAVED

Detection Flags



Processing Setup - Quan - Detection - quan.pmd (Int Std)

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability

Genesis Peak Integration

Smoothing points: 1

S/N threshold: 0.5

Enable valley detection

Expected width (sec): 2.0

Constrain peak width

Peak height (x): 5.0

Tailgng factor: 5.0

Genesis Peak Detection

Spectrum

Highest peak

Nearest RT

Minimum peak height (S/N): 1.0

43	166.2	1.98	Integration
44	167.3	0.18	
45	179.2	0.26	Match: 75
46	193.4	20.93	
47	194.1	100.00	
48	195.1	10.78	

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

C:\Xcaliber\data\Demo Data\Demo_100 07/27/99 12:56:44 PM

RT: 6.83 - 9.58 SM IC

RT: 3.20 AA: 6553916

NL: 5.75E6
m/z: 194.1
194.1 MS
Genesis
Demo_100

Peak Amplitude

Time (min)

Peak Amplitude

m/z

Ready NUM NOT SAVED

Data Flags

Area threshold: 0.0

Height threshold: 0.0

OK Cancel Save As Default Help

Calibration Tab

Processing Setup - Quan - Calibration - Demo_ProcMeth.pmd (Int Std)

File View Options GoTo Help

Quantification icons: Quan, Qual, Reports, Programs

Calibration Tab Settings:

- Component type: Target compound, ISTD
- ISTD: Amount: 1.000, Units: []
- Target compounds: ISID: CAFFEINE_(IS), Isotope %: []
- Weighting: Equal, 1/X, 1/X², 1/Y, 1/Y², 1/s²
- Calibration curve: Linear
- Units: UNITS
- Origin: Ignore, Forge, Include
- Response: Area, Height

Buttons: OK, Cancel, Save As Default, Flags..., Help

Components List:

- HEXACHLOROPENTADIENE
- ACENAPHTHENE
- CAFFEINE_(IS)
- AMITRIPTYLINE
- COCAINE
- 4,4_DDD

Ready NUM

Calibration Flags



Processing Setup - Quan - Calibration - Demo_ProcMeth.pmd (In Std)

File View Options GoTo Help

Identification Detection Calibration Levels System Suitability

Component type

Target compound

ISID: CAFFEINE_IS

Weighting

Calibration curve: Linear

Units: UNITS

Response

Area

Height

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

Calibration and Quantitation Flags

Calibration flag

R-squared: 0.995

Quantitation flags

Detection limit: 0.5 Linearity limit: 100.0

Quantitation limit: 0.5 Carry over limit: 100.0

Detection limit <= Quantitation limit < Linearity limit <= Carry over limit

OK Cancel Save As Default Help

Calibration Levels Tab

Processing Setup - Quan - Levels - Demo_ProcMeth.pmd (Int Std)

File View Options GoTo Help

Identification Detection Calibration Levels System Suitability

Units: UNITS

	Cal Level	Amount
1	Cal 1	1.000
2	Cal 2	5.000
3	Cal 3	15.000
4	Cal 4	40.000
5	Cal 5	100.000
*		0.000

	QC Level	Amount	% Test
1	QC 1	75.000	20.00
*		0.010	0.00

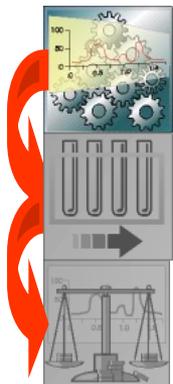
Components

- HEXACHLOROPENTADIENE
- ACENAPHTHENE
- CAFFEINE_(IS)
- AMITRIPTYLINE
- COCAINE
- 4,4_DDD

Ready

NUM

Copying Levels to Target Compound List



Units: ug/ml

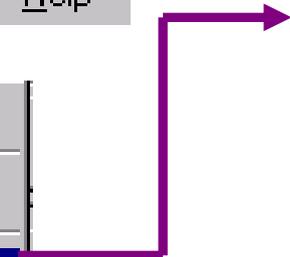
	Cal Level	Amount	nt	% Test
1	1	0.100	0.010	0.00
2	2	0.500		
3	3	1.000		
4	4	5.000		
*		0.000		

Context menu options:

- Delete Rows
- Insert Row
- Copy Levels to All Target Components

Buttons: OK, Cancel, Save As Default, Help

Using the Standard Dilution Option



The image illustrates the process of using the Standard Dilution option in a software application. It shows the menu path and the resulting dialog box.

Menu Path: File View **Options** GoTo Help

Options Menu:

- Identification...
- Masses ...
- Standard Dilution...**
- Chromatography By...
- Calibration By...
- Delete benz...
- Settings...
- Enable Warnings

Standard Dilution Dialog Box:

Target compound components: 2 of 3
Selected components: 2 of 3

Base amounts

Target compound components:

Component	Amount
hch	100.000
benz	200.000

Dilution factors

	Cal Level	Dilution
1	1	1.00000000
2	2	0.50000000
3	3	0.20000000
*		1.00000000

Buttons: OK Cancel Help

System Suitability Tab

Processing Setup - Quan - System Suitability - Demo_ProcMeth.pmd (Int Std)

File View Options GoTo Help

Resolution parameters

- Enable
- Resolution threshold (%): 90
- 70% 100%

Symmetry parameters

- Enable
- Peak height (%): 50
- Symmetry threshold (%): 90
- 30% 50%

Peak classification parameters

- Enable
- Detect peak width
 - Peak height (%): 50
 - Min peak width (sec): 1.80
 - Max peak width (sec): 3.60
 - 30% 50%
- Detect tailing
 - Peak height (%): 10
 - Failure threshold: 2.0
 - Tailing factor is defined as RHS/LHS at specified peak height
 - 10% 30%
- Detect column overload
 - Peak height (%): 50
 - Failure threshold: 1.5
 - Overload factor is defined as LHS/RHS at specified peak height
 - 30% 50%
- Detect baseline clipping
 - Number of peak widths for noise detection: 1.0
 -
- Detect minimum Signal-to-noise ratio
 - Signal-to-noise ratio: 3
 -

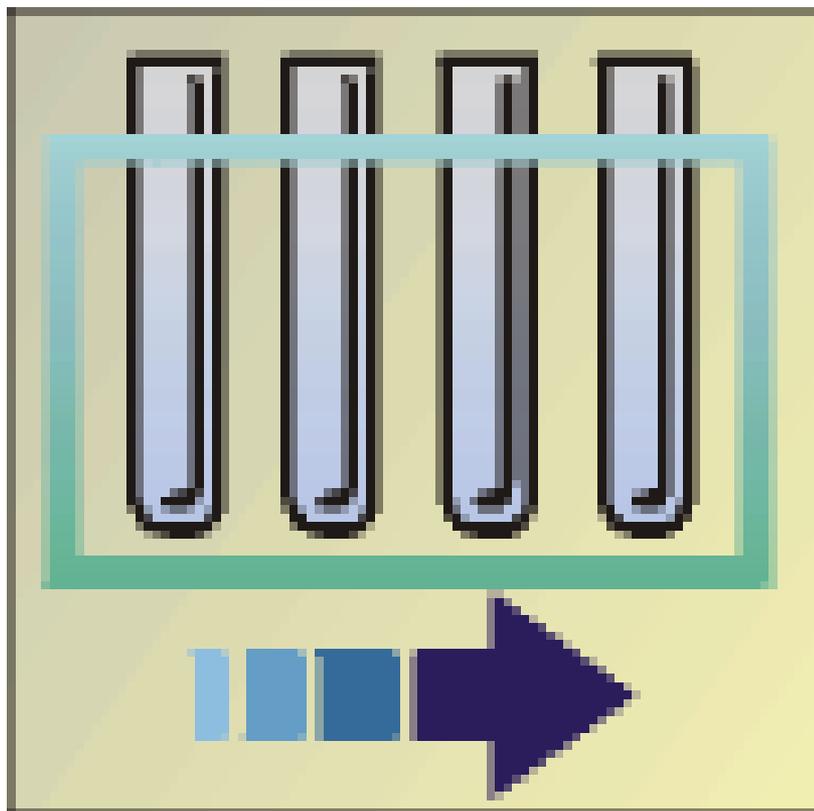
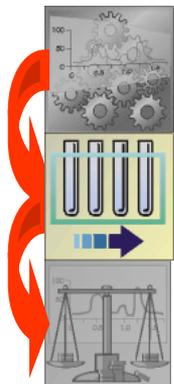
Components

- HEXACHLOROPENTADIENE
- ACENAPHTHENE
- CAFFEINE (IS)
- AMITRIPTYLINE
- COCAINE
- 4,4_DDD

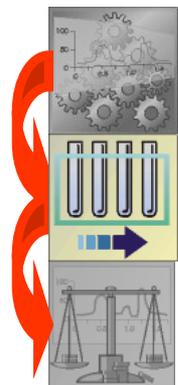
Ready

NUM

Sequence Set Up



Bracket Type Options



File Edit Change Actions View GoTo Help

New... Ctrl+N
Open... Ctrl+O
Save Ctrl+S
Save As...

Summary Info...
Import Sequence... Ctrl+I
Export Sequence... Ctrl+E

Print... Ctrl+P
Print Preview...
Print Setup...

1 Demo_Sequence.sld
2 temp.sld

Exit

New Sequence Template

General

Base File Name: Starting Number:

Path:

Instrument Method:

Processing Method:

Calibration File:

Samples

Number of Samples: Tray Type:

Injections per Sample: Initial Vial Position: Re-Use Vial Positions

Base Sample ID:

Bracket Type

None Open Non-Overlapped Overlapped

Calibration

Add Standards
Number of Calibration Sets:
Injections per Level:

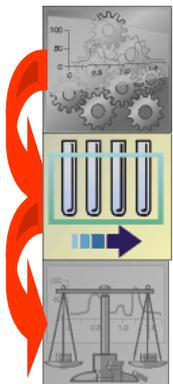
Add Blanks
 Fill in Sample ID for Standards

QC

Add QCs
 After First Calibration Only
 After Every Calibration

Add Blanks
 Fill in Sample ID for QCs

Open Bracket Type



Standard 1

Standard 2

Standard 3

Unknown 1

Standard 4

Standard 5

Standard 6

Standard 7

Standard 8

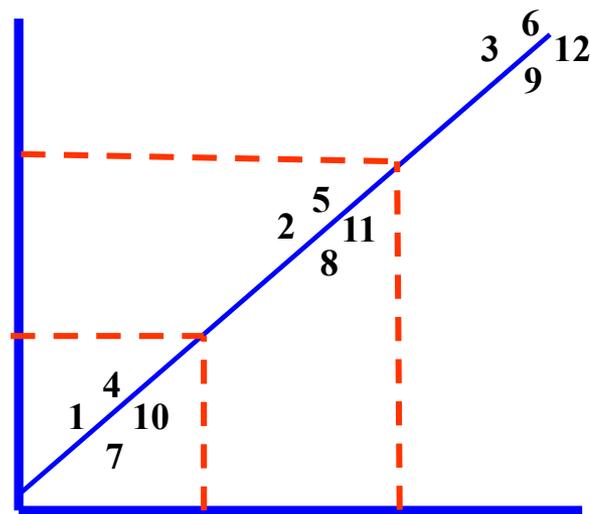
Standard 9

Unknown 2

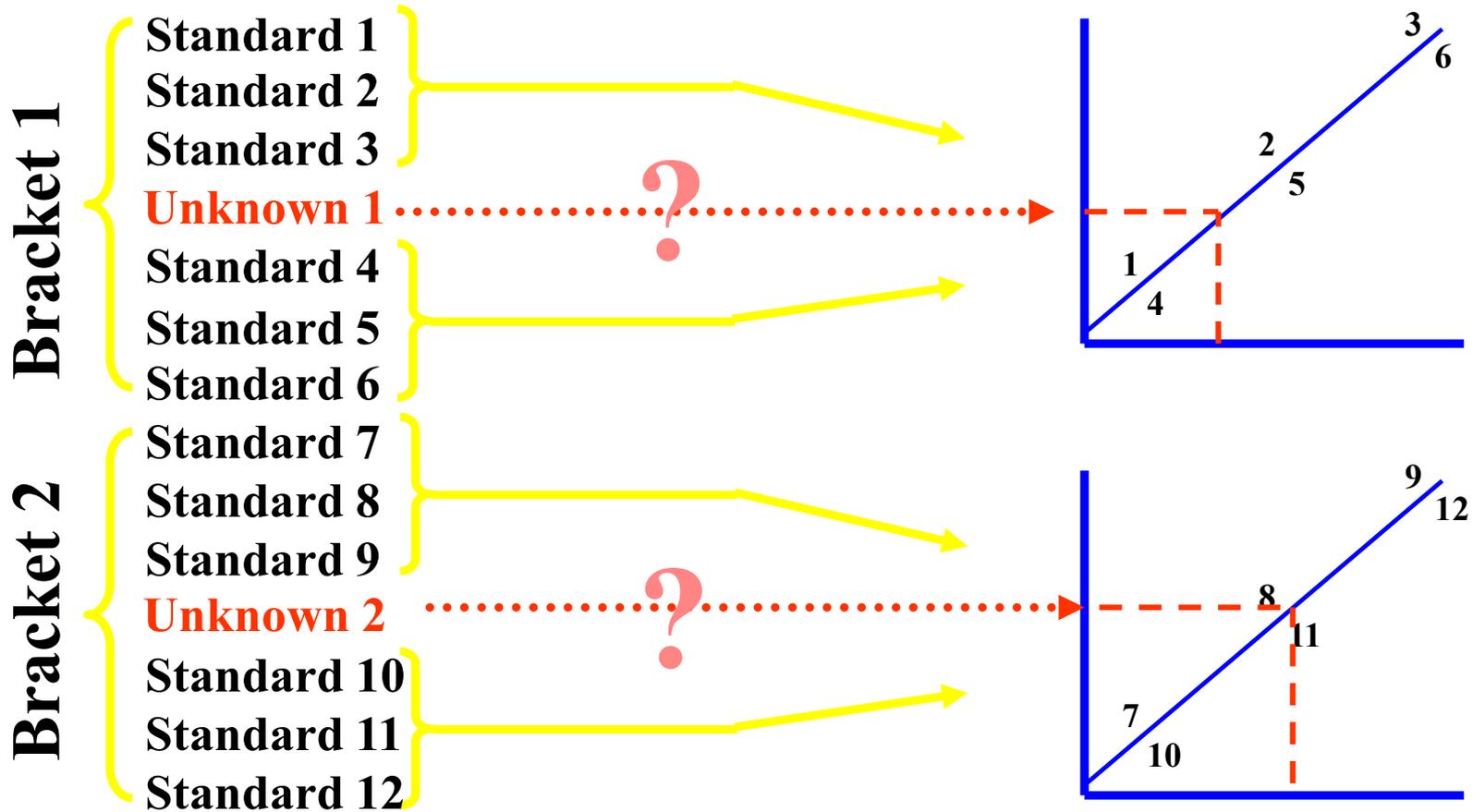
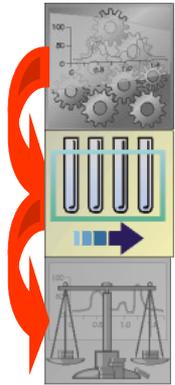
Standard 10

Standard 11

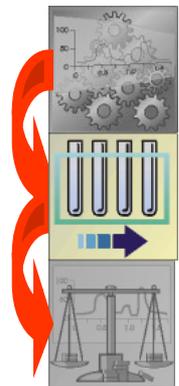
Standard 12



Non-Overlapping Bracket Type



Overlapping Bracket Type



Bracket 1
Bracket 2

Standard 1

Standard 2

Standard 3

Unknown 1

Standard 4

Standard 5

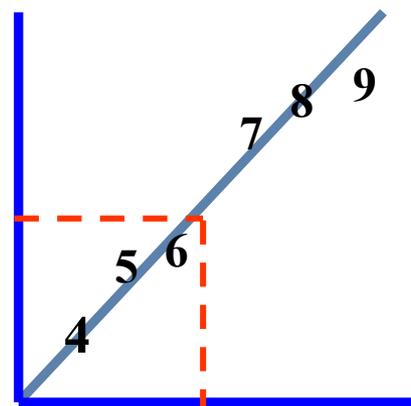
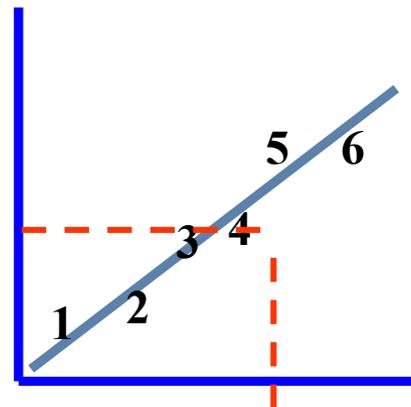
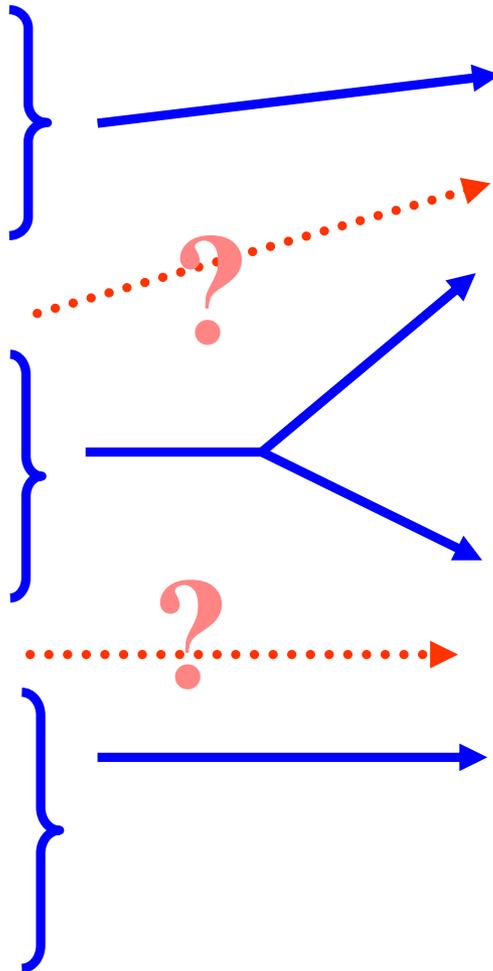
Standard 6

Unknown 2

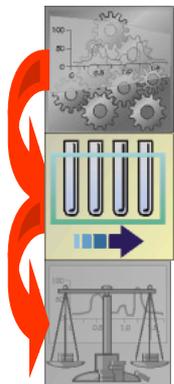
Standard 7

Standard 8

Standard 9



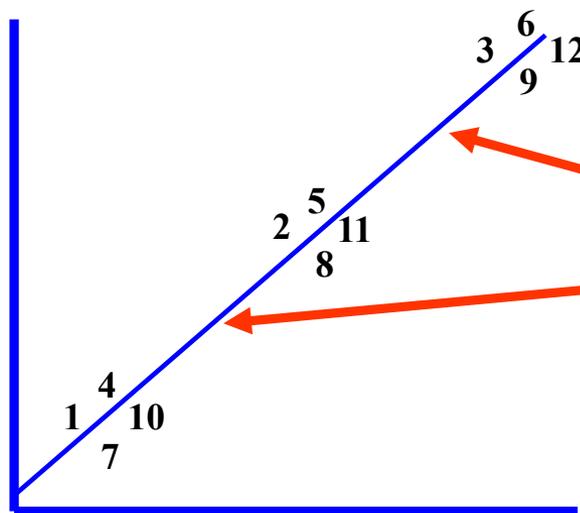
No Bracketing



Monday

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

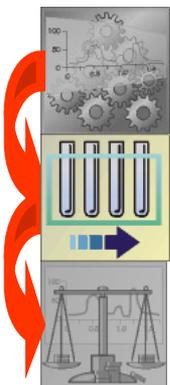
Calibration curve is saved



Tuesday

- Unknown 1
- Unknown 2

Enabling Quan Processing



File Edit Change Actions View GoTo Help

Check Disk Space...

Run This Sample... 

Run Sequence... 

Batch Reprocess...

Open File

Start Analysis

Stop Analysis

Pause Analysis

Devices On

Devices Standby

Devices Off

Automatic Devices On

Reinstate Warnings

Run Sequence

Acquisition Options

Instrument	Start Instrument
GCQ Mass Spectrometer	Yes
AS2000 Autosampler	
TRACE GC 2000	

Start When Ready Change Instruments...

Instrument Method

Start Up Browse...

Shut Down Browse...

Programs

Pre Acquisition Browse...

Post Acquisition Browse...

Run Synchronously

Pre Acquisition Post Acquisition

After Sequence Set System:

On Standby Off

User:

Run Rows:

Priority Sequence

Processing Actions

Quan

Qual

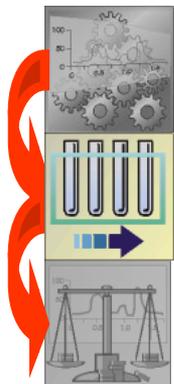
Reports

Programs

Create Quan Summary

OK Cancel Help

Batch Reprocessing of Data



File Edit Change Actions View GoTo Help

- Check Disk Space...
- Run This Sample...
- Run Sequence...
- B**atch Reprocess... 
- Open File
- Start Analysis
- Stop Analysis
- Pause Analysis
- Device On
- Device Standby
- Device Off
- Automatic Device On
- Reinstall Warnings

Batch Reprocess Setup

Process Rows: 1-7

Processing Actions

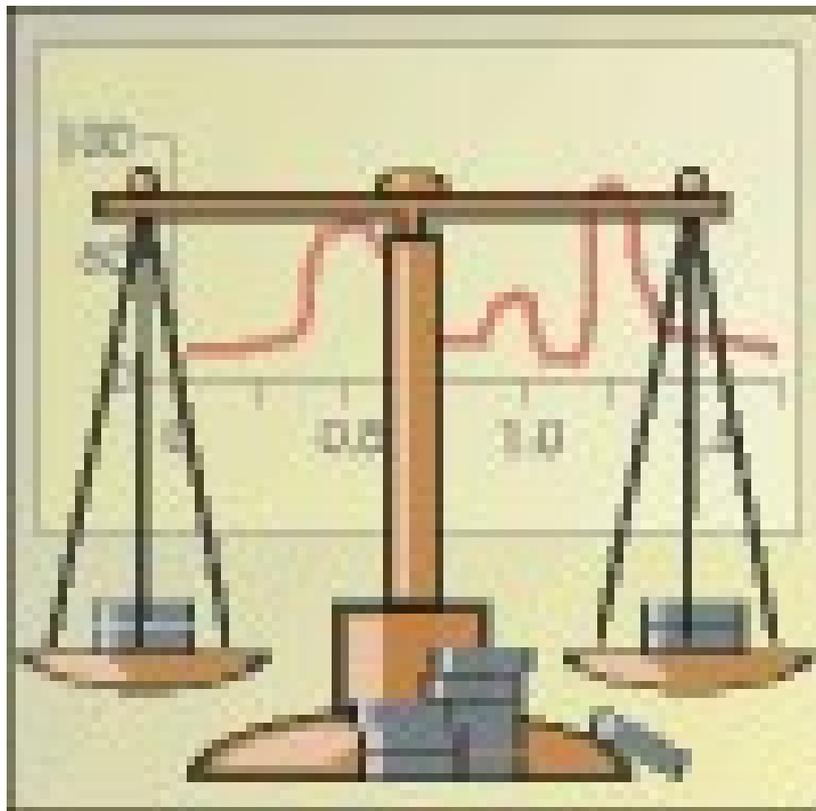
- Q**uan
 - Peak Detection & Integration
 - Calibration
 - Quantitation
- Q**ual
 - Peak Detection & Integration
 - Spectrum Enhancement
 - Library Search
- R**eports
 - Print Sample Reports
 - Print Summary Reports
- P**rograms
 - Create Quan Summary Spreadsheet

Advanced Options

- Replace Sample Info

OK Cancel Help

Quan Browser



Quan Browser View



Quan Browser - Browser - Demo_Sequence.sld (Group 1, View All)

File View Zoom Options GoTo Help

Group in use: Group 1 Calibration File: Cal File Unavailable - Using Embedded Calibration

	File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio
1	Demo_001	Standard		Method Settings	2738	5953210	0
2	Demo_005	Standard		Method Settings	13797	3466756	
3	Demo_015	Standard		Method Settings	46521	6082725	
4	Demo_040	Standard		Method Settings	137871	7270832	
5	Demo_100	Standard		Method Settings	450623	6606543	

HEXACHLOROPENTADIENE
ACENAPHTHENE
CAFFEINE_(IS)
AMITRIPTYLINE
COCAINE
4,4_DDD

All Standards QCs Blanks Unknowns

Demo_001 (Method Settings)
t:100

RT: 5.67 - 6.42 SM: TG

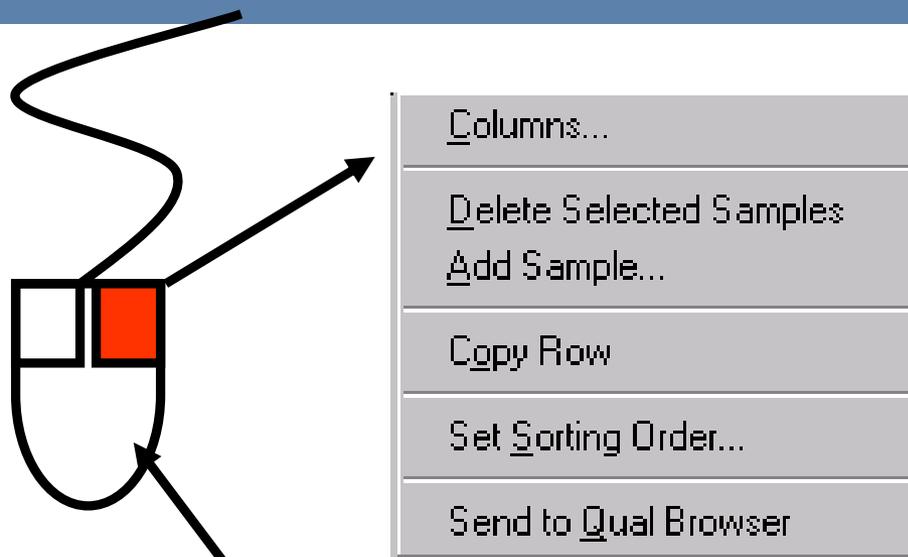
NL: 1.25E3
mics: 2115-
212.5 MS
Demo_001

HEXACHLOROPENTADIENE

$Y = -0.00145423 + 0.000670708 * X$ $R^2 = 0.9835$ W: Equal

x: 103.35, y: 0.00 NUM 08/19/1999 4:00 PM

The Results Grid



Group in use: Calibration File:

	File Name	Sample Type	Integration Type	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	% Diff	Level
1	Demo_001	Standard	Method Settings	2738	5953210	0.000	1.000	2.854	185.40	Cal 1
2	Demo_005	Standard	Method Settings	13797	3466756	0.004	5.000	8.102	62.04	Cal 2
3	Demo_015	Standard	Method Settings	46521	6082725	0.008	15.000	13.571	-9.53	Cal 3
4	Demo_040	Standard	Method Settings	137871	7270832	0.019	40.000	30.440	-23.90	Cal 4
5	Demo_100	Standard	Method Settings	450623	6606543	0.068	100.000	103.865	3.86	Cal 5
6	Demo_QC_075	QC	Method Settings	289610	5864487	0.049	75.000	75.797	1.06	QC 1
7	Demo_Unknown	Unknown	Method Settings	5162	6399916	0.001	0.000	3.371	0.00	

Navigation:

Column Arrangement and Sorting



Columns...

Delete Selected Samples

Add Sample...

Copy Row

Set Sorting Order...

Send to Qual Browser

Result List Column Hiding

Selected Columns

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

OK Cancel Help

Group in use: Group 1 Calibration File: Cal File Unavailable - Using Embedded Calibration

	File Name	Sample Type	Integration Type	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	% Diff	Level
1	Demo_001	Standard	Method Settings	2738	5953210	0.000	1.000	2.854	185.40	Cal 1
2	Demo_005	Standard	Method Settings	13797	3466756	0.004	5.000	8.102	62.04	Cal 2
3	Demo_015	Standard	Method Settings	46521	6082725	0.008	15.000	13.571	-9.53	Cal 3
4	Demo_040	Standard	Method Settings	137871	7270142	0.019	40.000	30.440	-23.90	Cal 4
5	Demo_100	Standard	Method Settings	450623	6606147	0.022	100.000	103.865	3.86	Cal 5
6	Demo_QC_075	QC	Method Settings	289610	586417	0.049	75.000	75.797	1.06	QC 1
7	Demo_Unknown	Unknown	Method Settings	5162	639991	0.001	0.000	3.371	0.00	

All Standards QCs Blanks Unknowns

Quantitation Results Sorting Order

Sorting

First Order: Sample Type

Second Order: File Name

Sort in descending order

Third Order: Level Name

Sort in descending order

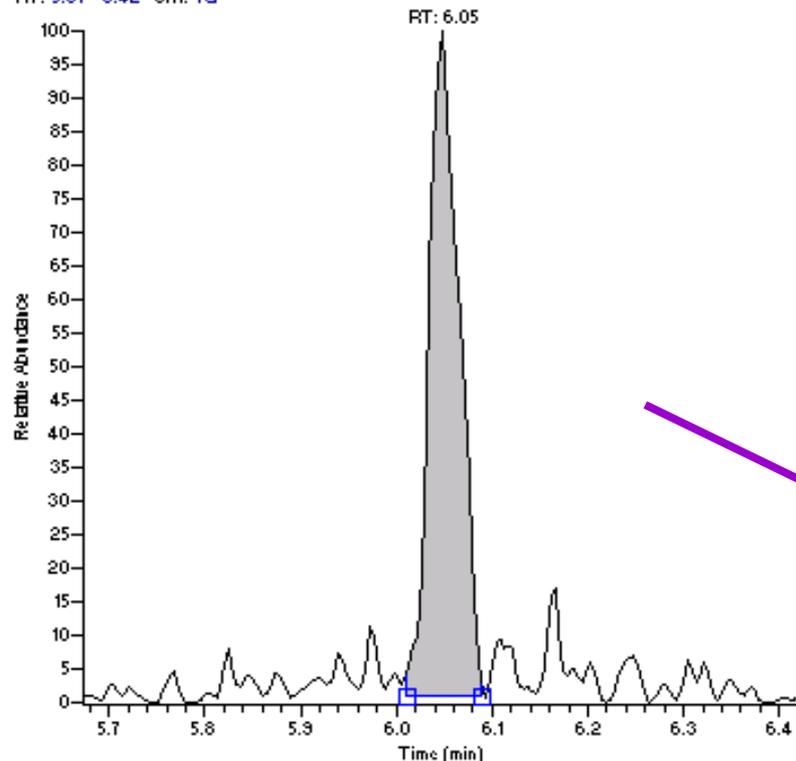
OK Cancel Save As Default Help

Chromatogram plot



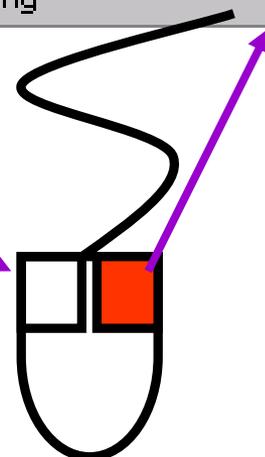
Demo_001 (Method Settings)
1:100

RT: 5.67 - 6.42 SM: TG

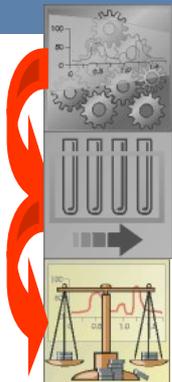


NL:
1.25E3
m/z=
271.5-
272.5 MS
Demo_001

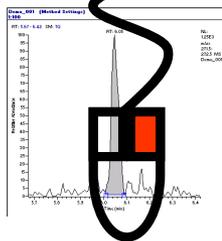
- Method Settings
- User Settings
- Manual Integration
- Show Peak Info...
- User Peak Detection Settings...
- Display Options...
- Manually Add Peak
- Set Peak to Not Found Status
- Update Expected Retention Time ▶
- Reset Scaling



Resetting Peak Detection Settings



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



User Identification Settings

Identification | Detection | Integration | Advanced | Flags

Name: COCAINE

Plot Type: Mass Range

Scan Filter:

Mass (m/z): 182.0

Keys:

Retention Time

Expected (min): 11.10 Window (sec): 30.0

Use as RT Reference View Width (min): 0.75

Adjust Using CAFFEINE_(9)

Detector Type: MS

OK Cancel Apply Apply To All Help

User Identification Settings

Identification | Detection | Integration | Advanced | Flags

Spectrum

Highest Peak

Nearest RT

Min Pk Ht (S/N): 3.0

Ion Ratio Confirmation

Enabled

	m/z	Target %	Window %
1	0.500	0	5

Window %

Relative

Absolute

Qualifier resolution: 0.025 min

OK Cancel Apply Apply To All Help

User Identification Settings

Identification | Detection | Integration | Advanced | Flags

Smoothing Points: 3

S/N Threshold: 2.0

Valley Detection Enabled

Expected Width (sec): 4.0

Constrain Peak Width

Peak Ht (%): 5.0

Tailing Factor: 2.0

OK Cancel Apply Apply To All Help

User Identification Settings

Identification | Detection | Integration | Advanced | Flags

Rise Percentage: 10.0

Valley S/N: 2.0

Peak S/N Cutoff: 200.0

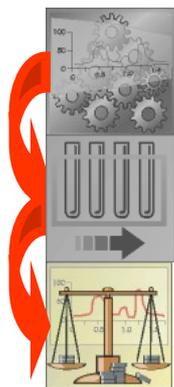
Baseline Noise Tolerance (%): 10.0

Min Number of Scans in Baseline: 16

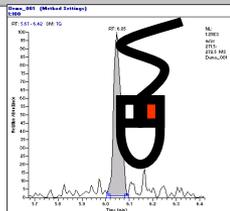
Number of Background Scans: 3

OK Cancel Apply Apply To All Help

Chromatogram Peak Info



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



Peak Information - COCAINE

Info | Flags | More Flags | Suitability | Spectrum

Left (min)	11.07	Base Peak (m/z)	182.12
Apex (min)	11.08	Signal to Noise	1340.31
Right (min)	11.11	Expected RT (min)	11.10
Height	10776.38	ISTD Response	5953209.72
Area (cts-sec)	11330.24	Response Ratio	0.00
Baseline	2.26	Calculated Amount	0.00

Close Help

Peak Information - COCAINE

Info | Flags | More Flags | Suitability | Spectrum

Integration info

Detected By: Nearest RT Valid

Left Edge Type: Stripe Right Edge Type: Stripe

Flags

<input checked="" type="checkbox"/> Saturated	<input type="checkbox"/> QC Failed	<input checked="" type="checkbox"/> Response OK
<input checked="" type="checkbox"/> Calculated Amount	<input type="checkbox"/> RT Ref OK	<input checked="" type="checkbox"/> Response Low
<input checked="" type="checkbox"/> Valley Detect		<input type="checkbox"/> Response High

Close Help

Peak Information - COCAINE

Info | Flags | More Flags | Suitability | Spectrum

Suitability Flags

Symmetrical	Passed
Resolution	Passed
Peak Width	Failed
Tailing	Passed
Column Overload	Passed
Baseline Clipping	Passed
Signal-to-noise Ratio	Passed
Concave	Passed
Saturation	Passed

Close Help

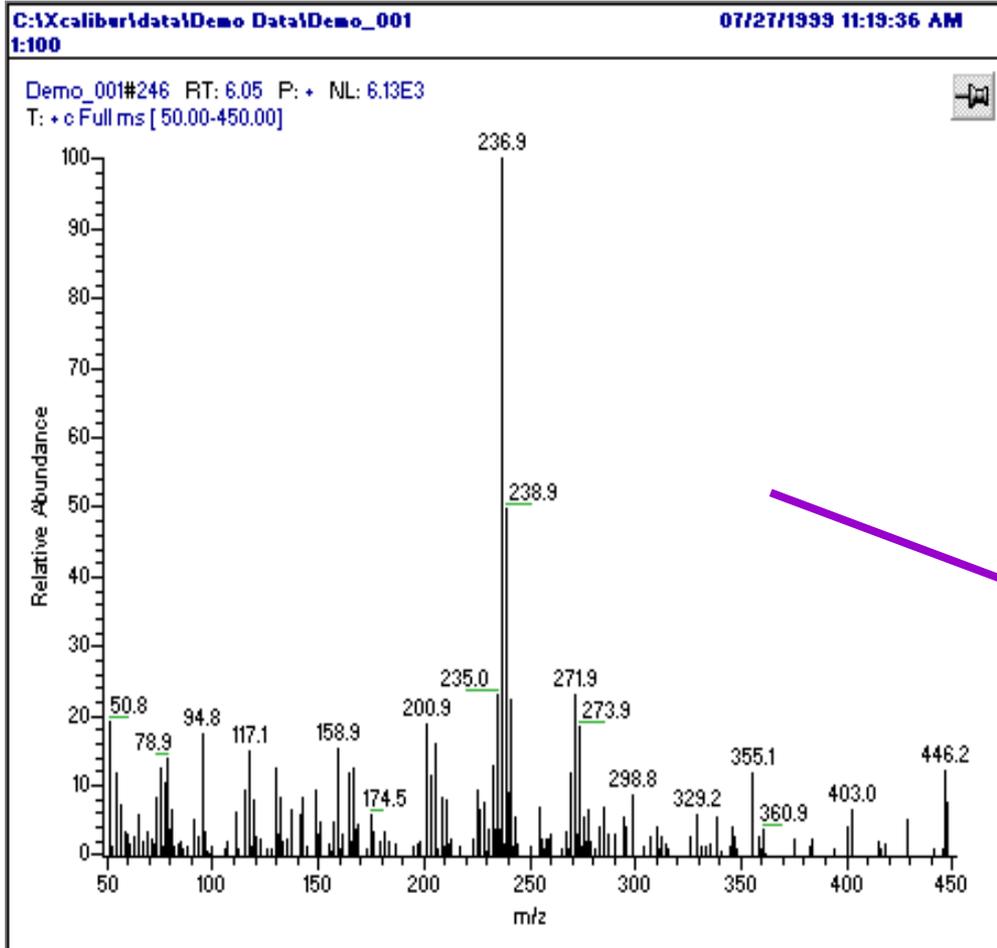
Peak Information - COCAINE

Info | Flags | More Flags | Suitability | Spectrum

Demo_001#1454 RT: 11.08 P: + NL: 2.35E4
T: + c Full ms [50.00-450.00]

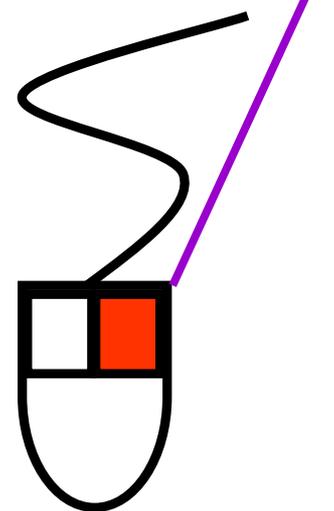
Close Help

Spectrum Plot Options

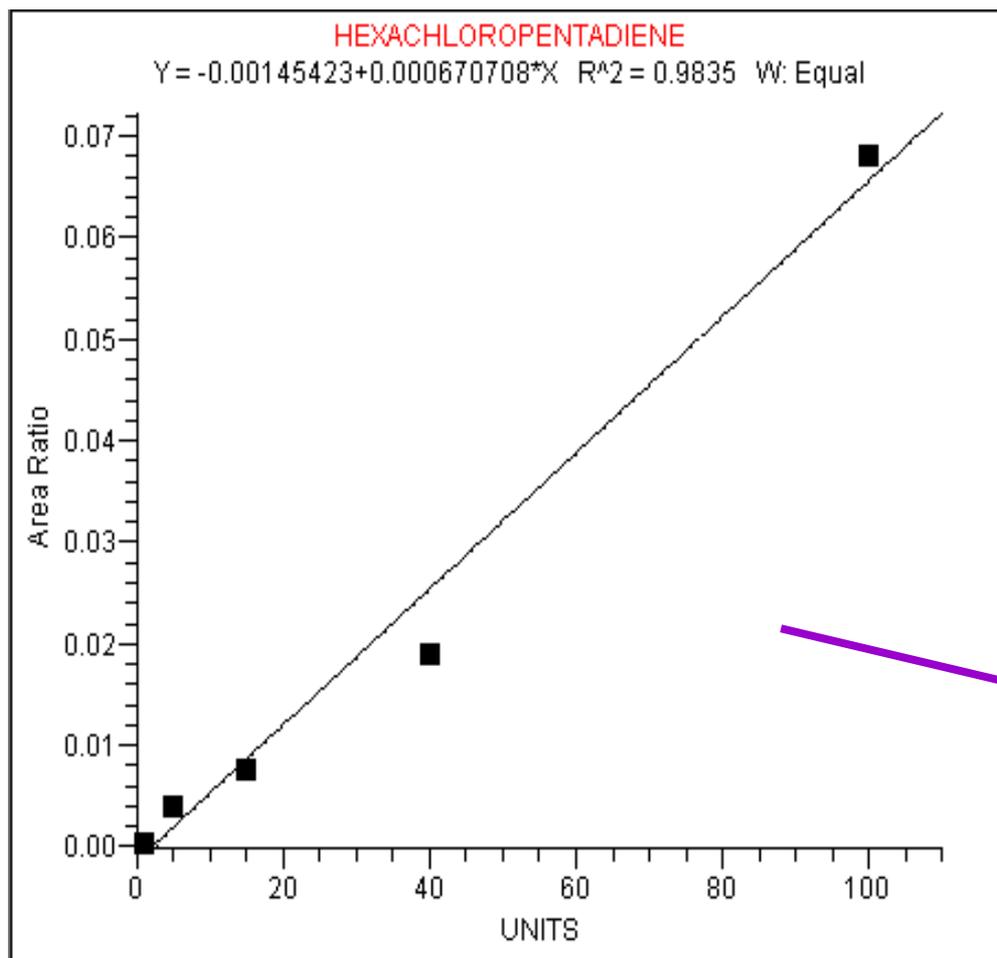


Spectrum at Peak Apex
Spectrum at Peak Left Edge
Spectrum at Peak Right Edge

Show Calibration Curve
Reset Scaling



Calibration Plot Options



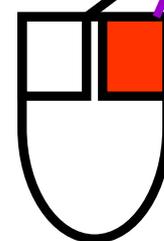
Calibration Settings...

Exclusion List...

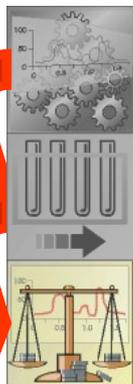
Show Spectrum Plot

Reset Scaling

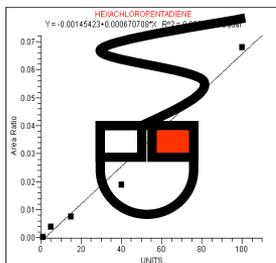
Copy Graph



Resetting the Calibration Options



- Calibration Settings...
- Exclusion List...
- Show Spectrum Plot
- Reset Scaling
- Copy Graph



Calibration Settings

Type | Curve | Levels | Isotope% | Flags

Component Type

Target Compound

ISTD

ISTD:

CAFFEINE (IS)

OK Cancel Apply Help

Calibration Settings

Type | Curve | Levels | Isotope% | Flags

Calibration Curve

Linear

Origin

Ignore

Force

Include

Response

Area

Height

Weighting

Equal

1/X

1/X^2

1/Y

1/Y^2

1/s^2

Units:

UNITS

OK Cancel Apply Help

Calibration Settings

Type | Curve | Levels | Isotope% | Flags

	Cal Level	Amount
1	Cal 2	5.000
2	Cal 1	1.000
3	Cal 3	15.000
4	Cal 4	40.000
5	Cal 5	100.000

	QC Level	Amount	% Test
1	QC 1	10.000	20.00
*		0.010	0.00

Units: UNITS

OK Cancel Apply Help

Calibration Settings

Type | Curve | Levels | Isotope% | Flags

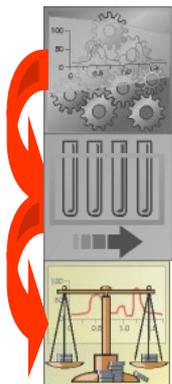
Contribution of

ISTD to Target Compound (%): 0.0

Target Compound to ISTD (%): 0.0

OK Cancel Apply Help

Calibration Curve Exclusion List



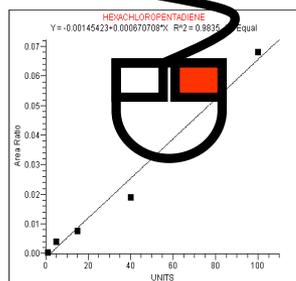
Calibration Settings...

Exclusion List...

Show Spectrum Plot

Reset Scaling

Copy Graph

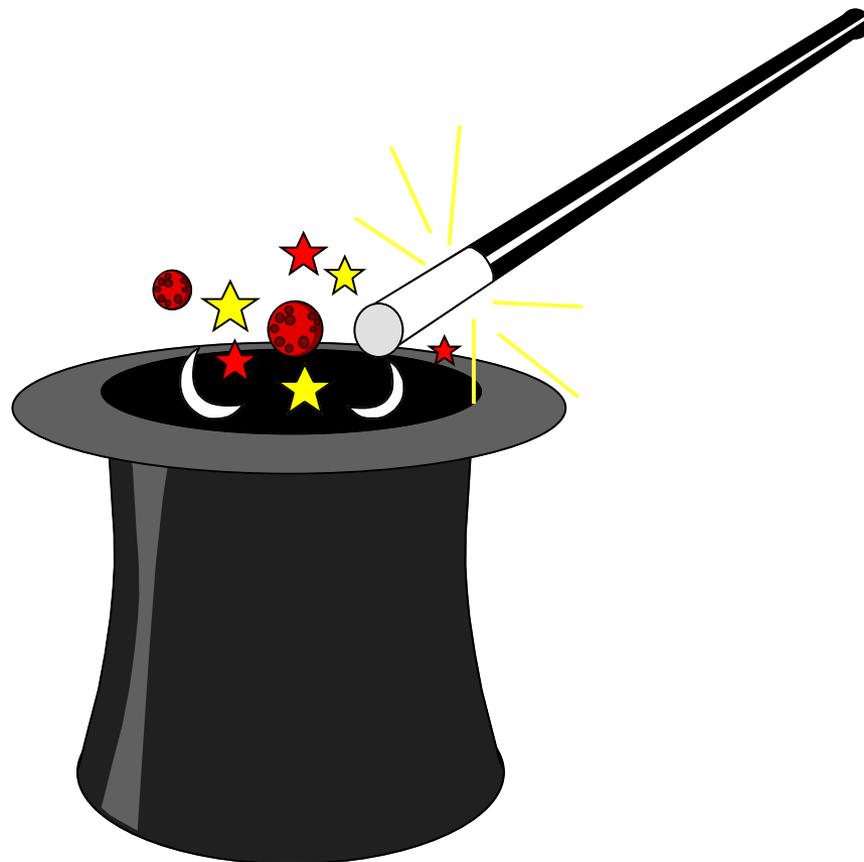


Cal Exclusion List - COCAINE

Level	Expected	%Diff	Exclude
Cal 2	5.000 UNITS	-13.155	
Cal 1	1.000 UNITS	-207.621	
Cal 3	15.000 UNITS	13.717	
Cal 4	40.000 UNITS	12.133	
Cal 5	100.000 UNITS	-2.196	

OK Cancel Apply Help

Merlin Reporting Quantitation



Quan Merlin Report

Merlin Report Structure Wizard - Step 1 of 4 : Select Report

Which type of REPORT would you like to construct?

Sample:

- Quan
- Qual
- Quan & Qual

Summary:

- By Sample Quan

Method:

- Processing

A sample report allows you to present information relating to a particular Xcalibur sample's raw file and the results created when processing it.

Quan reports utilize the results generated using the Quan section of a Processing Method. There are many report options, but typical reports may include a calibration curve plot for each component, chromatogram plots showing the integrated peaks used to create that curve and textual quantitative peak information relating to those peaks. This report type does not offer any of the results created using Qual processing.

< Back Next > Cancel

Add Repeating and Non-Repeating Sections

Merlin Report Structure Wizard - Step 2 of 4 : Select Sections

-> Sample Quan Report

- Non Repeating Quan Section
- Quan Repeating Section
- Non Repeating Quan Section

Which SECTIONs would you like in the Sample Quan Report?

Sections available:

- Non Repeating Quan Section
- Quan Repeating Section

Sections added:

- Non Repeating Quan Section
- Quan Repeating Section
- Non Repeating Quan Section

Add >

< Remove

<< Remove All

Move Up **Move Down**

Description of section:

A Non-Repeating Section will usually display static details associated with either data or method that apply once only and globally within the system

< Back **Next >** **Cancel**

Chose Non-Repeating Objects

Merlin Report Structure Wizard - Step 3.1 of 4 : Select Objects

-Sample Quan Report
 -> **Non Repeating Quan S...**
 └ Sample Header
-Quan Repeating Section
-Non Repeating Quan Section

Which OBJECTs would you like in the Non Repeating Quan Section?

Objects available:

- Calibration File Table
- Chromatogram
- Component Cal Curve
- Dilution Factor Table
- Page Break
- Quan Peak Table
- Sample Header**
- Spectrum
- Spectrum Candidate Results Table

Objects added:
Sample Header

Add >
< Remove
<< Remove All

Move Up **Move Down**

Description of report object:
Displays static details associated with a raw data file i.e. Data File Name, Acquisition Time, Run Tim in Minutes, Scans, etc.

< Back **Next >** **Cancel**

Chose Repeating Objects

Merlin Report Structure Wizard - Step 3.2 of 4 : Select Objects

- Sample Quan Report
├─ Non Repeating Quan Section
│ └─ Sample Header
├─ **> Quan Repeating Section**
│ └─ Component Cal Curve
│ └─ Chromatogram
└─ Non Repeating Quan Section

Which OBJECTs would you like in the Quan Repeating Section?

Objects available:

- Avalon Quan Events Table
- Chromatogram**
- Component Cal Curve
- Component Cal Level Table
- Component ISTD Settings Summary
- Component Ident/Detect/Cal Settings
- Component Ion Ratio Confirmation Ta
- Component QC Level Table
- Component Settings Summary

Objects added:

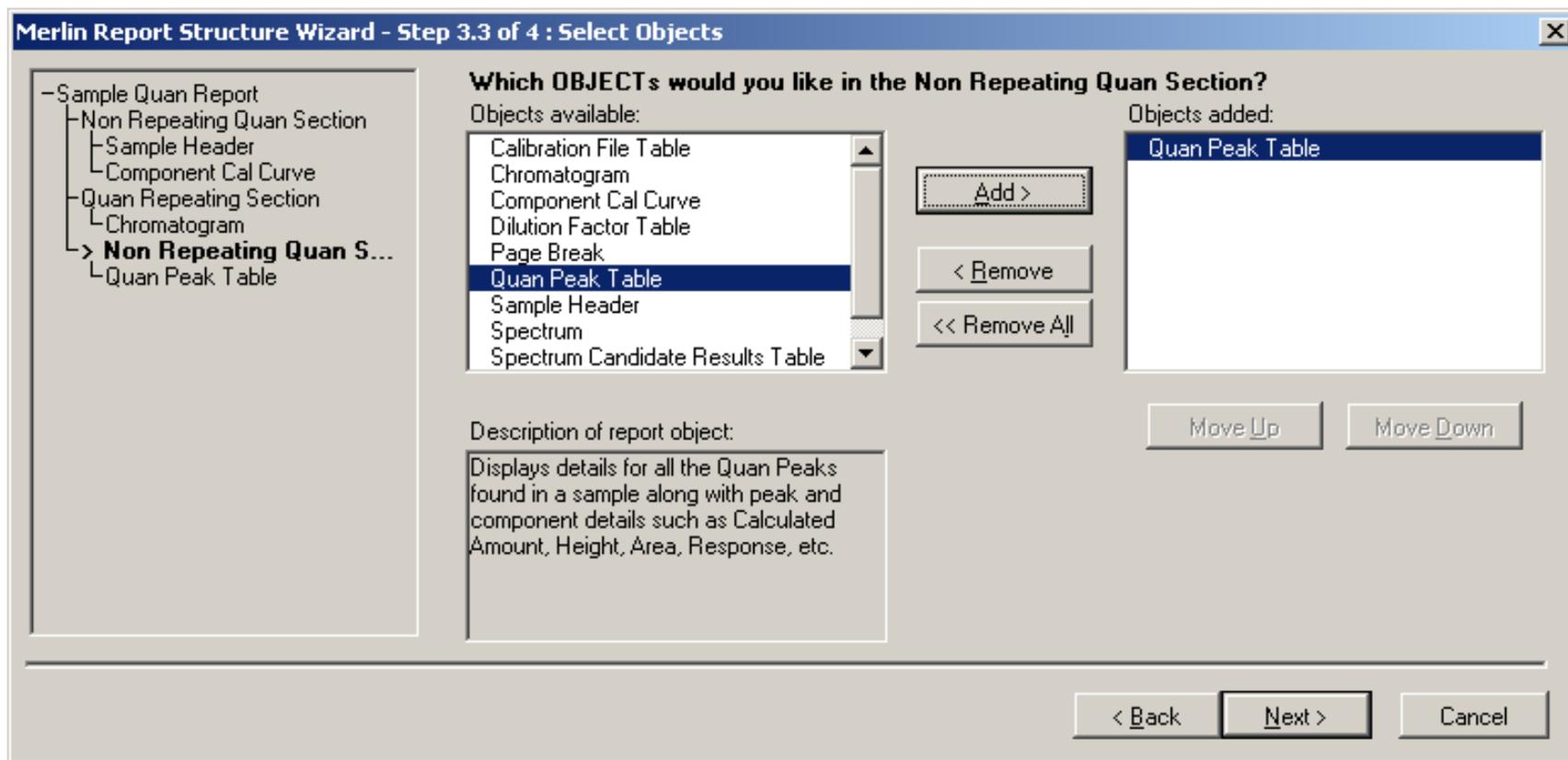
- Component Cal Curve
- Chromatogram**

Buttons: Add >, < Remove, << Remove All, Move Up, Move Down

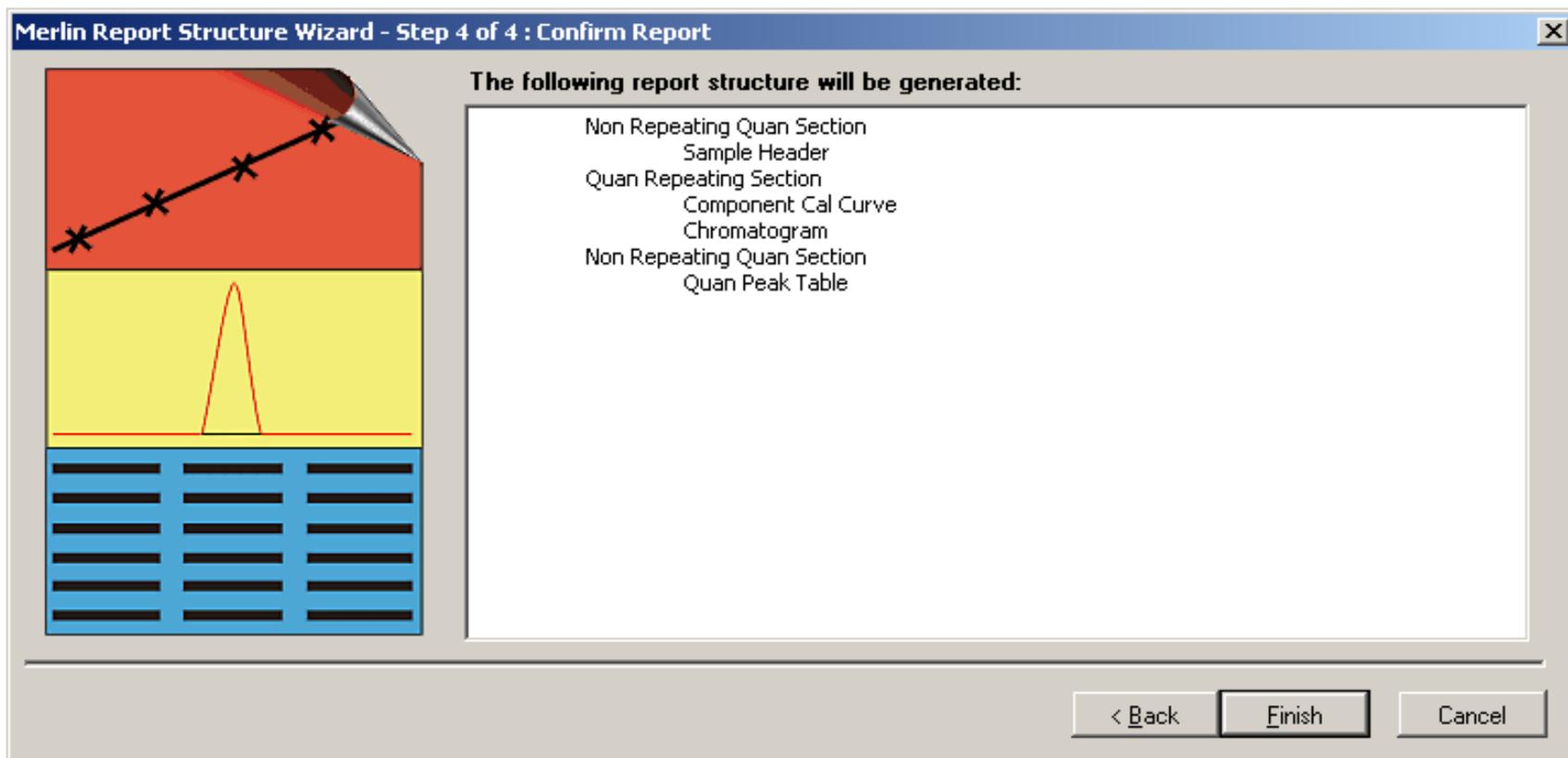
Description of report object:
Displays a configured chromatogram (usually TIC) in a non-repeating section or either a quan or qual peak inside the appropriate repeating section

Navigation: < Back, Next >, Cancel

Chose 2nd Set of Non Repeating Objects



Report Content Summary



Component Calibration Curve

Calibration Curve Generated From Report Structure Wizard

The Report Structure Wizard has been used to generate a new Merlin Report Template.

When generating a new Report Template each of the calibration curves in the report must be configured individually using the Merlin Cali Curve Wizard.

The Report Structure Layout to the right shows how the calibration curve control being configured (which is highlighted) fits into the new Report Template being generated.

- Sample Quan Report
 - Non Repeating Quan Section
 - Sample Header
 - > Component Cal Curve**
 - Quan Repeating Section
 - Chromatogram
 - Non Repeating Quan Section
 - Quan Peak Table

< Back Next > Cancel

What to Include in the Chromatogram

Repeating Section Generated From Report Structure Wizard

The Report Structure Wizard has been used to generate a new Merlin Report Template.

When generating a new report, the repeating section configuration wizard must be run for each repeating section in the new report template

The Report Structure Layout to the right shows how the repeating section being configured (which is highlighted) fits into the new report template

Property Pages

Exclude | Ordering | Page Breaks

The Repeating Section type is:

Repeating Section Excludes

When processing Quan configurations or results it is possible to exclude specific elements from the process

Exclude Component Type: **Internal Standards**

Show only found components

It is also possible to discard any components that haven't been found.

Select How To Order The Repeating Section

The default ordering of processed results can be changed so that the order of peaks and/or components can be tailored to each user's specific needs.

Repeating Section Ordering

Order By: **RT**

Ordering Direction

Ascending Descending

Limit Repeating Records

Maximum number of peaks or components to

Configure Peak Reporting Page Layout

How do you want the individual peaks that will appear in the repeating section to be laid out in the final report?

Each peak fully contained on its own page(s)

No page breaks between the individual peak

Page break before the repeating sector

Page break after the repeating sector

< Back Finish Cancel

Dictate How the Chromatogram Looks

Use Container Supplied Quan Peak

When a chromatogram control is in a section that automatically supports a quan peak (e.g Quan Repeating Section) then the default behaviour is to implicitly display the section specific peak when generating a report.

Implicit quan peak options

It is possible to display the details of the Internal Standard associated with the implicit quan peak.

Use quan peak's associated Internal Standard

Explicit chromatogram type

The implicit quan peak supplied by the container can be ignored and the chromatogram type explicitly specified.

Explicitly specify the chromatogram type

Select Mass Spectrum Quan Traces To Display

The quan traces properties allow confirmation ion traces in Mass Spectrum data to be plotted. This section is only applicable to MS data and will be ignored for any other detector types.

When a quan component is being displayed in a chromatogram control, there is potentially six traces associated with that component - the quan mass (QM) trace and up to five confirmation ion traces.

It is possible to select any of these six traces to be displayed in the control, or alternatively all of the component's available traces can be displayed.

Quan Traces

Quan Mass (QM) Trace

< Back Next > Cancel

Select Demo File

A demo file can be associated with a chromatogram control to allow the control to show how the current settings look when applied to real data.

Use demo file

Demo File

C:\Xcalibur\examples\data\steroids05.RST

When using result data for the chromatogram demo you must explicitly specify the peak to demo.

Demo Peak

Quan component: methyltestosterone

< Back Next > Cancel

Find your result file

Modify how the Chromatogram is Presented

Configure How The Peak Window Will Look

When a peak from a result file is being displayed it is possible to configure whether baselines are shown and also the width of the window to display.

Show Baselines
 Set Custom Window Width
Custom width (min):

RT: 1.51 - 2.51 SM: TG
RT: 2.01 NL: 9.63E4
m/z: 266.6-267.6+
284.7-285.7 F: + c
Full ms: 2 303.30 [100.00-310.00] MS
steroids:05

< Back Next > Cancel

Select The Peak Labelling

Configure how peaks in the chromatogram trace will be labelled and the style of these labels.

Label With:
 Retention Time Flags
 Scan Number Area
 Base Peak Height
 Signal to Noise

Label Styles:
 Offset Size:
 Rotated
 Boxed

Label Threshold (%):

RT: 1.51 - 2.51 SM: TG
RT: 2.01 NL: 9.63E4
m/z: 266.6-267.6+
284.7-285.7 F: + c
Full ms: 2 303.30 [100.00-310.00] MS
steroids:05

< Back Next > Cancel



Select Chromatogram Style

Select the style to use when plotting the chromatogram trace. The arrangement is only really applicable when there will be more than one trace on the chromatogram.

Plotting:
 Point To Point
 Stick

Arrangement:
 Stack (2D)
 Overlay (3D)

3D:
Elevation:
Skew:
 Draw Backdrop

RT: 1.51 - 2.51 SM: TG
RT: 2.01 NL: 9.63E4
m/z: 266.6-267.6+
284.7-285.7 F: + c
Full ms: 2 303.30 [100.00-310.00] MS
steroids:05

< Back Next > Cancel

Configure Normalization For The Chromatogram

Configure how the chromatogram display will be normalized. The normalization method will be applied to each trace in the chromatogram individually.

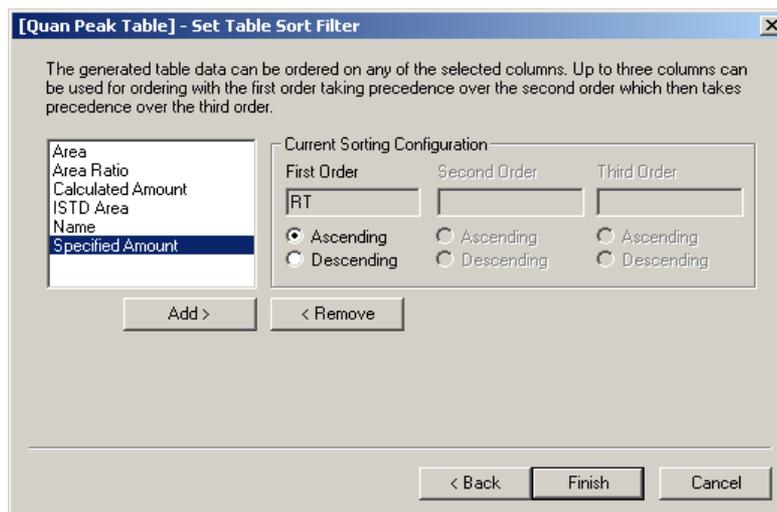
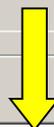
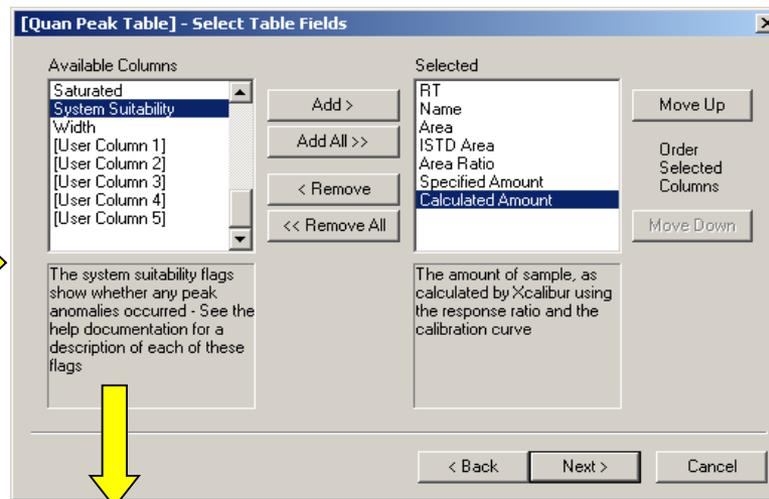
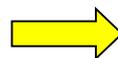
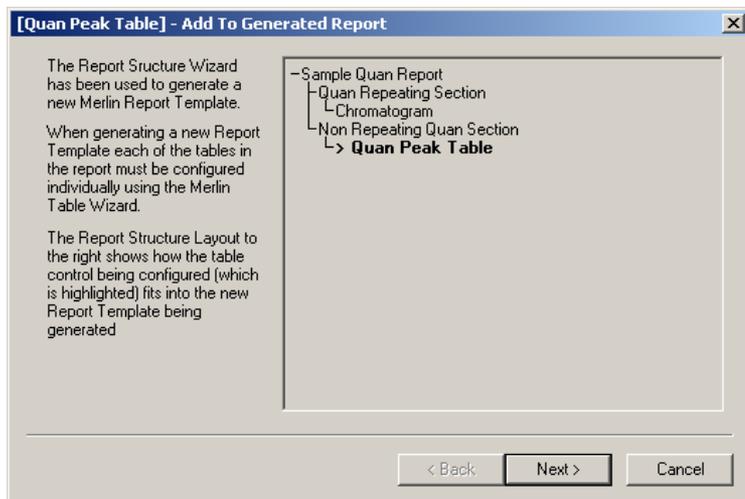
Normalize method:
 Auto zero
 Intensity Range (%):

Normalize to:
 Largest peak in subsection
 Largest peak in range
 Largest peak in scan

RT: 1.51 - 2.51 SM: TG
RT: 2.01 NL: 9.63E4
m/z: 266.6-267.6+
284.7-285.7 F: + c
Full ms: 2 303.30 [100.00-310.00] MS
steroids:05

< Back Finish Cancel

Quan Peak Summary Table



Advanced Report Options

The screenshot shows a Microsoft Word document titled "Document1 - Microsoft Word". The document content includes a red line labeled "Begin Repeating Section 1.", followed by a "Quan Repeating Section" containing a chromatogram. The chromatogram displays a major peak at RT: 1.97 and several smaller peaks at RT: 2.17, 2.32, and 2.42. The y-axis is labeled "Relative Abundance" and the x-axis is "Time (min)". To the right of the chromatogram, the following data is listed:

NL: 9.77E4
m/z:
266.6-267.6+
284.7-285.7 F: + c
Full ms2 303.30 [
100.00-310.00] MS
steroids16

Below the chromatogram is a red line labeled "End Repeating Section". A yellow arrow points from the "End Repeating Section" line to the "Insert Table" dialog box. The dialog box is titled "Insert Table" and has the following settings:

- Table size: Number of columns: 2, Number of rows: 2
- AutoFit behavior: Fixed column width: 6.0|cm, AutoFit to contents, AutoFit to window
- Table format: (none) with an "AutoFormat..." button
- Set as default for new tables
- Buttons: OK, Cancel

The status bar at the bottom of the Word window shows: Page 1, Sec 1, 1/1, At 3.8cm Ln 3 Col 2, REC, TRK, EXT, OVR, English (U.S).

Insert table here

Insert Chromatogram Object into Right Column

Begin Repeating Section 1.

Quan Repeating Section

{#Component Name}	Internal Standard
<p>RT: 0.19 - 1.19 SM: 7G</p> <p>RT: 0.63</p> <p>NL: 4.32E5 m/z: 266.6-267.6+ 308.6-309.6 F: + c Full ms2 363.30 [150.00-375.00] MS sampleA</p>	

End Repeating Section

Resize Column width

Page 1 Sec 1 1/1 At 5.4cm Ln 7 Col 1 REC TRK EXT OVR English (U.S)

