

LCQ Operations Course

Thermo Fisher
SCIENTIFIC

The world leader in serving science



Thermo Scientific
- Training Institute -

LCQ Operations Course

Welcome to the Thermo Fisher Scientific LCQ Small Molecules Course. Below is a listing of important information for your future use.

Institute Registrar, Technical Support, Parts Ordering

Contract/warranty customers call: 1-877-594-3224
All other customers call: 1-800-532-4752

Thermo Scientific Training Institute
1400 Northpointe Parkway, Suite 10
West Palm Beach, FL 33407

ThermoFisher
SCIENTIFIC

The world leader in serving science

Chapter 1

Introduction

LCQ Ion Trap Instrument Evolution



Classic



Duo



Deca

LCQ CLASSIC

LCQ DUO

- 400µm capillary
- 2 octopoles
- One rotary pump

LCQ DECA

- 500µm capillary
- square quadrupole with split offset voltages
- 2 rotary pumps

Deca: approximately 10 x better signal than Duo

The Next LCQ Generation – Advantage MAX / DECA XP MAX



LCQ Advantage
MAX



LCQ DECA XP
MAX

Advantage / XP Comparisons

LCQ Advantage
MAX

- 450µm Ion Transfer Tube
- Orthogonal Probes

LCQ DECA XP
MAX

- 550µm Ion Transfer Tube
- Orthogonal Probes

XP: approximately 10 x better signal than Advantage

Practical

1. Tune and Calibrate
2. ESI compound optimization (Drug Mixture) - Infusion
3. ESI method development (Drug Mixture) - Qualitative
4. ESI data dependent MS/MS runs (Drug Mixture)
5. APCI compound optimization (Steroids) - Infusion
6. APCI method development (Steroids) - Quantitative
7. Quantitative data processing (Steroids)

ThermoFisher
SCIENTIFIC

The world leader in serving science

Chapter 2

Fundamentals of Mass Spectrometry

What is Mass Spectrometry?

“The basis in MS (mass spectrometry) is the production of ions, that are subsequently separated or filtered according to their mass-to-charge (m/z) ratio and detected. The resulting mass spectrum is a plot of the (relative) abundance of the produced ions as a function of the m/z ratio.”

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

In analysis by LC/MS, a sample is injected onto an LC column. The sample is then separated into its various components. The components elute from the LC column and pass into the MS detector where they are analyzed. Analysis by direct infusion or flow injection provides no chromatographic separation of components in the sample before it passes into the MS detector. The data from the MS detector are then stored and processed by the data system.

Mass Spectrometry “Simplified” (GMSD)

G enerate	→	Ion Production
M ove	→	Ion Optics
S elect	→	Linear Ion Trap
D etect	→	Electron Multiplier

There are four steps to mass spectrometry using the LCQ. The acronym “GMSD” - Generate, Move, Select, Detect is employed to describe this. Initially, ions are **Generated** in either the solution phase (when using electrospray) or in the gas phase (when using APCI/APPI). The difference among the three will be discussed in later slides when specifically introducing the API ionization modes. Charged ions must be **Moved** from the source to the analyzer region without contacting any of the solid internal parts of the mass spectrometer (this would neutralize the ion, losing it in mass spectrometric analysis). This is accomplished by a series of ion optics that use a combination of DC voltage, RF voltage, and a vacuum gradient. The **Selection** of ions and the scan event dynamics are completed within the Linear Ion Trap. After the ion selection occurs, ions are deflected onto the curved surface of the conversion dynode and are accelerated by a voltage gradient into the electron multiplier where they are **Detected**.

Ion Generation (API)

- **Atmospheric Pressure Ionization**
- **Source Types:**
 - Electrospray Ionization (ESI) – Solution phase process (for the most part).
 - APCI (Atmospheric Pressure Chemical Ionization) - Gas-phase process.
- **Source Purpose:**
 - Desolvate sample flow for introduction into mass spectrometer.
 - Baffle the first vacuum region of the MS from atmospheric pressure in the source.
 - Ionize the analyte or transport ion in solution to the gas phase.
 - Pump away neutrals and opposite charged ions which would otherwise interfere with the analysis of the desired polarity.

What is API?

API (Atmospheric Pressure Ionization) describes a range of three techniques of interfacing LC with mass spectrometry. Mass detectors measure mass to charge ratios of ionized entities, and all three techniques involve ionization of sample molecules at atmospheric pressure. The API techniques are Electrospray (ESI), Atmospheric Pressure Chemical Ionization (APCI), and Atmospheric Pressure Photo-Ionization.

When sampling is performed using the ESI probe, the ions are pre-formed by solution phase chemistry before the analyte ever reaches the source probe. Most commonly this is accomplished by adding a proton donor, such as acetic or formic acid, or a proton acceptor, such as ammonium hydroxide to the mobile phase. When sampling is performed using the APCI probe, the analyte reaches the probe in the neutral state, where it is protonated or de-protonated by gas-phase processes occurring across the corona discharge needle. In APPI, ions are generated from molecules when they interact with photons from a UV-light source.

Chemistry Considerations

ESI:

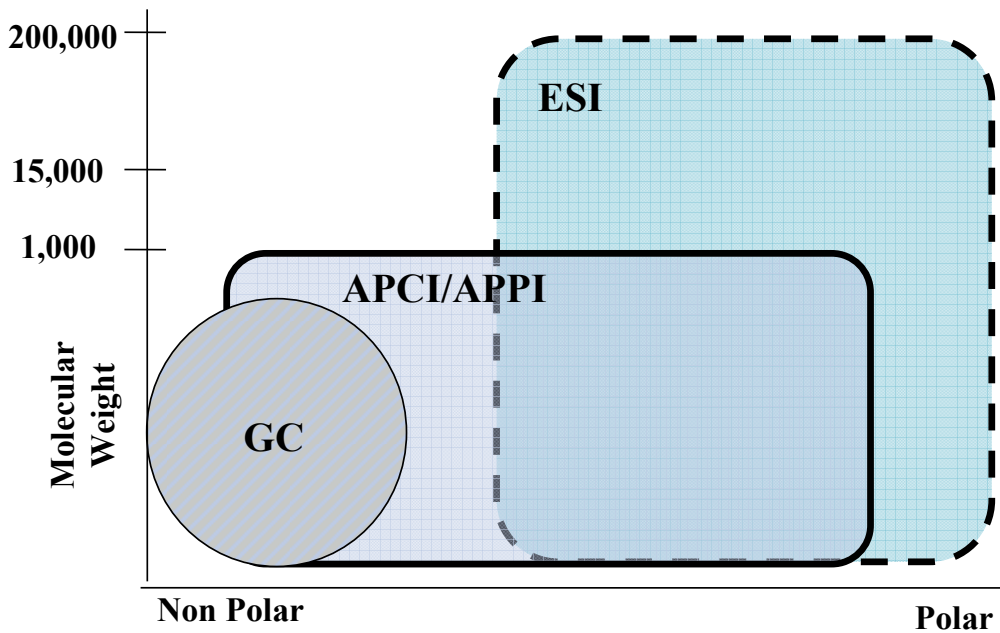
- *Ions formed by solution chemistry*
- *Good for thermally labile analytes*
- *Good for polar / semi-polar analytes*
- *Good for large molecules (proteins / peptides)*

APCI/APPI:

- *Ions formed by gas phase chemistry*
- *Good for volatile / thermally stable*
- *Good for non-polar / semi-polar*
- *Good for small molecules (steroids)*
- *Good for ions containing a chromophore (APPI)*

The ESI mode transfers ions in solution into the gas phase. Many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular mass compounds) can be analyzed by ESI. ESI can be used to analyze any polar compound that makes a preformed ion in solution. The technique is especially useful for the mass analysis of polar compounds, which include: biological polymers (for example, proteins, peptides, glycoproteins, and nucleotides); pharmaceuticals and their metabolites; and industrial polymers (for example, polyethylene glycols). Like ESI, APCI is a soft ionization technique. APCI provides molecular mass information for compounds of medium polarity that have some volatility. APCI is typically used to analyze heat-stable, small molecules. It is a robust technique that is normally not affected by changes in most variables (i.e. buffer type or buffer strength). In addition, APCI only allows for single charging due to the ionization mechanism.

Chemistry Considerations - Analyte Compatibility



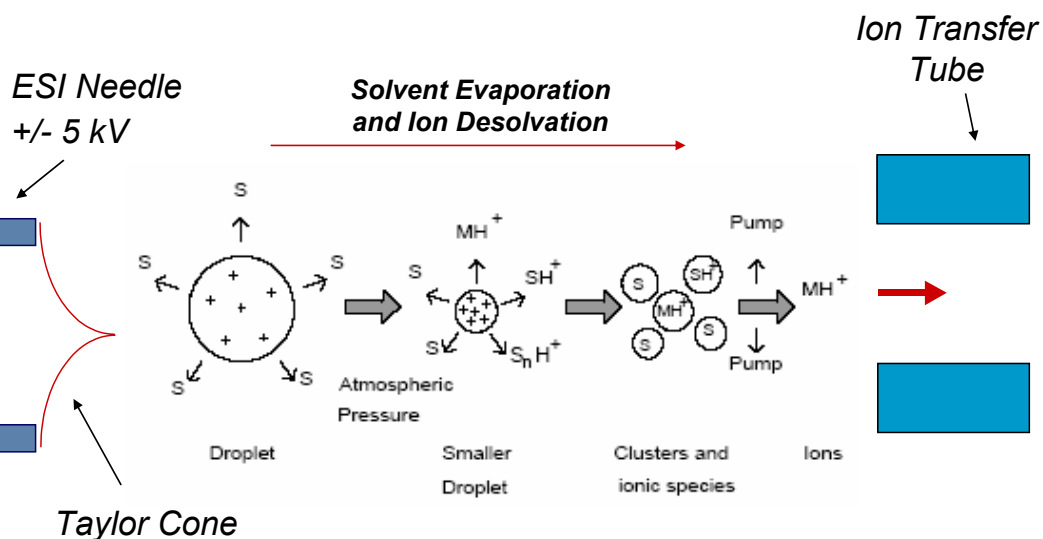
With ESI, the range of molecular weights that can be analyzed by the LCQ is greater than 100,000 Da, due to multiple charging. APCI is typically used to analyze small molecules with molecular masses up to about 2000 Da.

Positive or Negative Ionization ?



Basic compounds give rise to protonated molecular ions (positive ion), whereas acidic compounds produce de-protonated molecular ions (negative ion). Positive ion API may be seen as a general ionization mode since protons may loosely associate with a molecule, although it may not contain any basic functional groups. Negative ion API specifically requires the presence of functional groups capable of losing a proton.

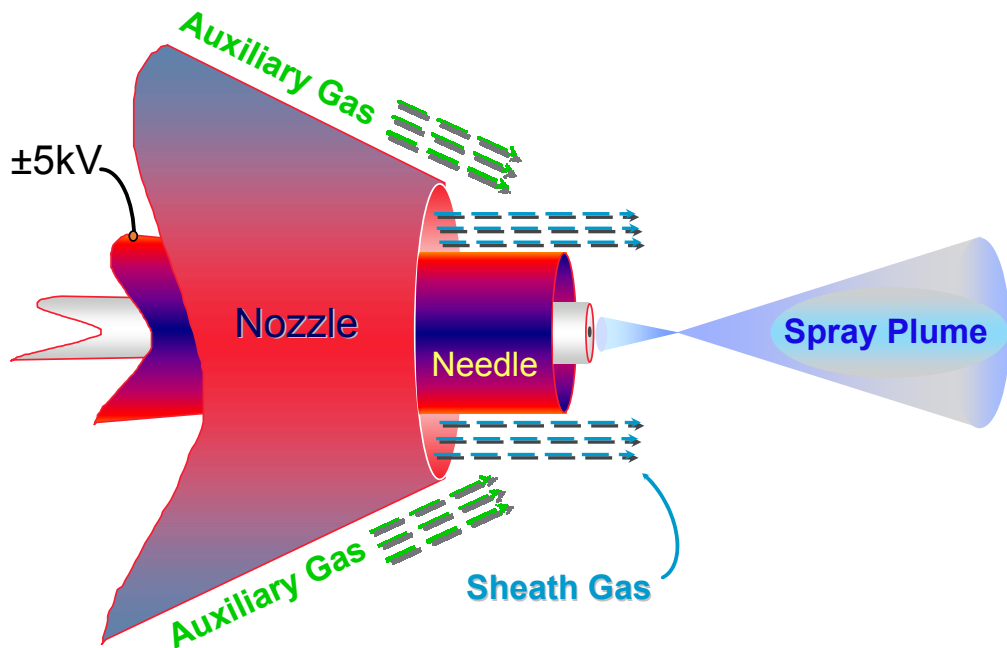
Electrospray - Basic Principle



Electrospray is a soft ionization process used to transfer ionized species from liquid solutions into the gas phase. The sample solution is sprayed from a region where it is contact with high voltage ($\pm 3 - 5$ kV, typically), where excess charges are imparted upon droplets which emerge at the end of the sample tube. The emergence of these droplets occurs at atmospheric pressure. In ESI, ions are produced and analyzed as follows:

1. The sample solution enters the ESI needle, to which a high voltage is applied.
2. The ESI needle sprays the sample solution into a fine mist of droplets that are electrically charged at their surface.
3. The electrical charge density at the surface of the droplets increases as solvent evaporates from the droplets.
4. The electrical charge density at the surface of the droplets increases to a critical point, known as the Rayleigh stability limit. At this critical point, the droplets divide into smaller droplets because the electrostatic repulsion is greater than the surface tension. The process is repeated many times to form very small droplets.
5. From the very small, highly charged droplets, sample ions are ejected into the gas phase by electrostatic repulsion.
6. The sample ions pass through an ion transfer capillary, enter the MS detector and are analyzed.

ESI Nozzle Cross Section



When sheath gas is used, nitrogen is applied as an inner coaxial gas (when used in tandem with auxiliary gas), helping to nebulize the sample solution into a fine mist as the sample solution exits the ESI or APCI nozzle. When auxiliary gas is being used, nitrogen flows through the ion source nozzle, the vapor plume is affected; the spray is focused and desolvation is improved. The auxiliary gas also helps lower the humidity in the ion source. Typical auxiliary gas flow rates for ESI and APCI are 10 to 20 units. Auxiliary gas is usually not needed for sample flow rates below 50 $\mu\text{L}/\text{min}$.

Atmospheric Pressure Chemical Ionization (APCI)

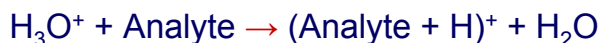
- **Gas phase ionization via a corona discharge APCI is a three step process**

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



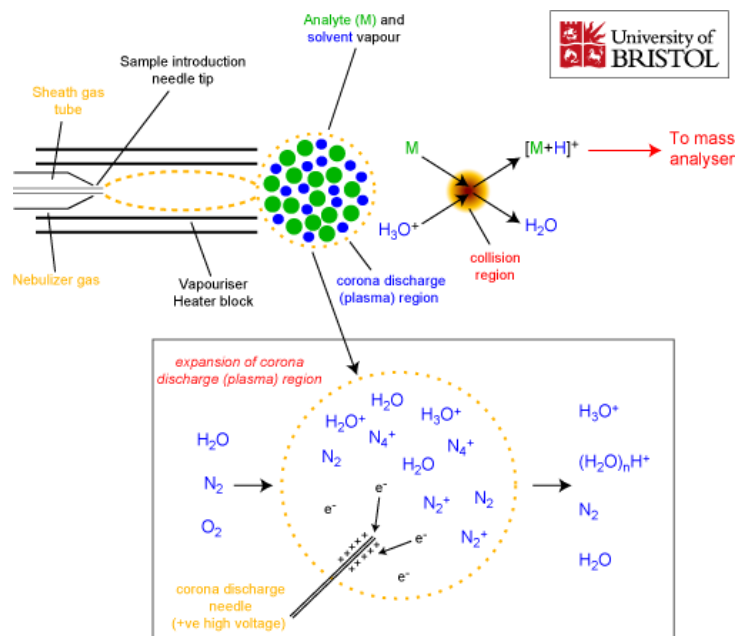
2. Through a complex series of reactions primary ions react with solvent molecules forming reagent ions, H_3O^+ and CH_3OH_2^+

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



Atmospheric Pressure Chemical Ionization (APCI) is a soft ionization technique that is used to analyze compounds of medium polarity, that have some volatility. APCI is a gas-phase ionization technique. As such, the gas-phase acidities and basicities of the solvent and analyte ions play important roles in the APCI ionization process. APCI is typically used to analyze small molecules with molecular weights up to ~ 1500 daltons. Also, APCI is an extremely robust technique and is not affected by minor changes in buffers and/or buffer strength.

Atmospheric Pressure Chemical Ionization (APCI)



© 2004 Dr. Paul Gates, University of Bristol

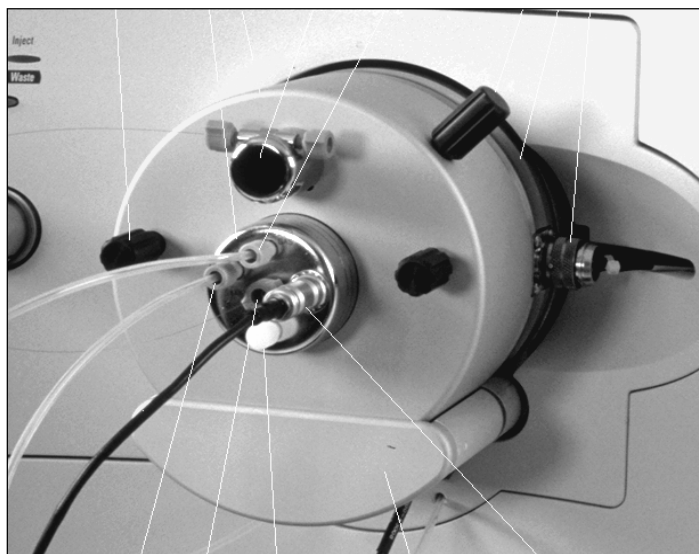
If nitrogen is utilized as the sheath and auxiliary gases with atmospheric vapor (water) present in the APCI ion source, then the type of primary and secondary reactions that occur in the plasma region are as follows:



The most abundant secondary cluster ion is (H₂O)₂H⁺, along with significant amounts of (H₂O)₃H⁺ and H₃O⁺. The reactions listed above account for the formation of these ions within the gas-plasma.

The protonated analyte ions are then formed by gas-phase ion-molecule reactions of these charged cluster ions with the analyte molecules (given in Slide #19). This results in the abundant formation of [M+H]⁺ ions.

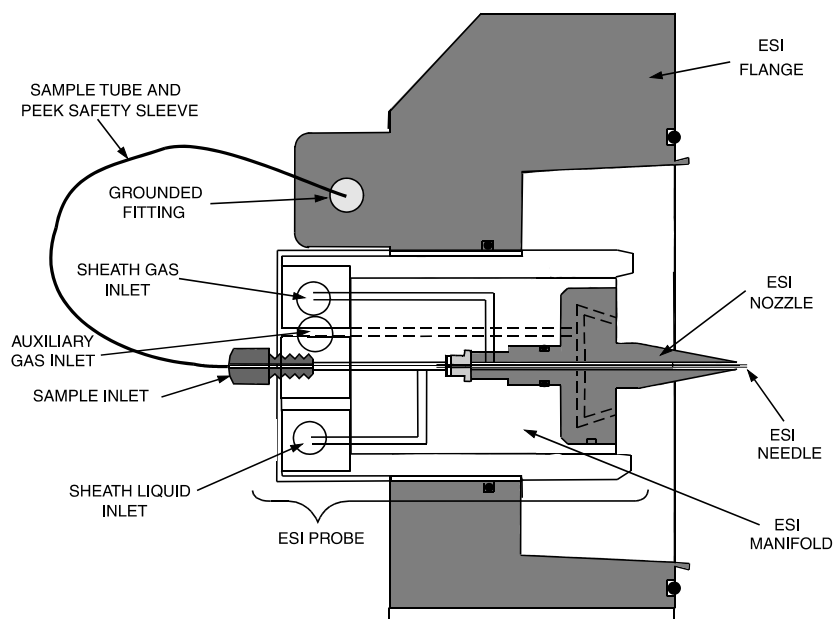
ESI Probe (API-1) Assembly – LCQ Classic



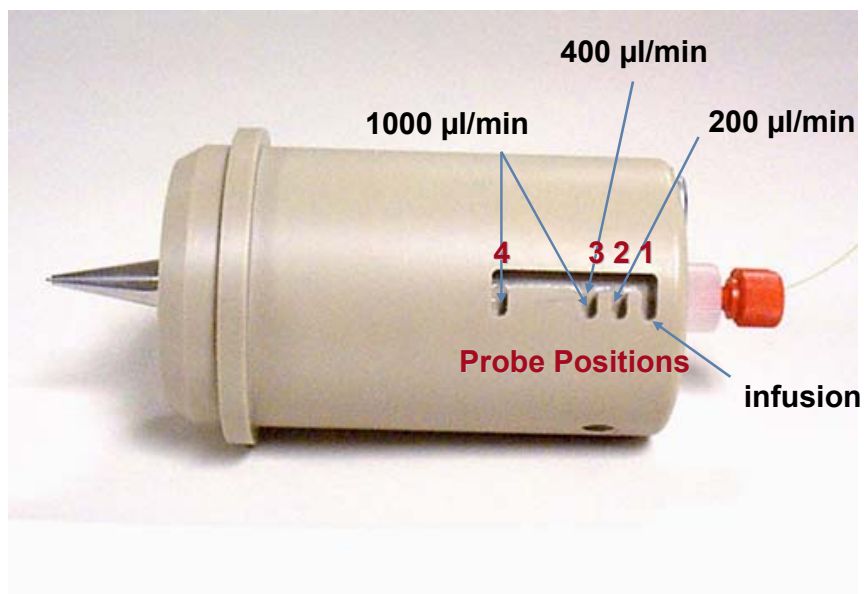
ESI Probe (API-2) Assembly – LCQ Duo / LCQ Deca



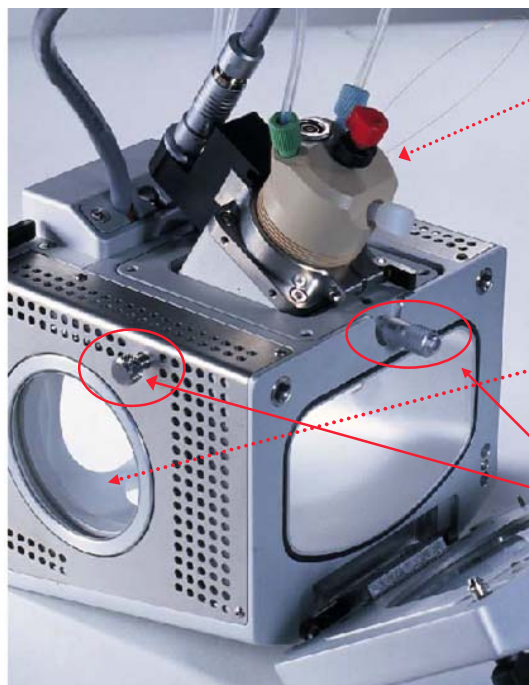
ESI Probe (API-2) Diagram



ESI Probe (API-2) Positions



Advantage MAX / DECA XP MAX Ion Max Source



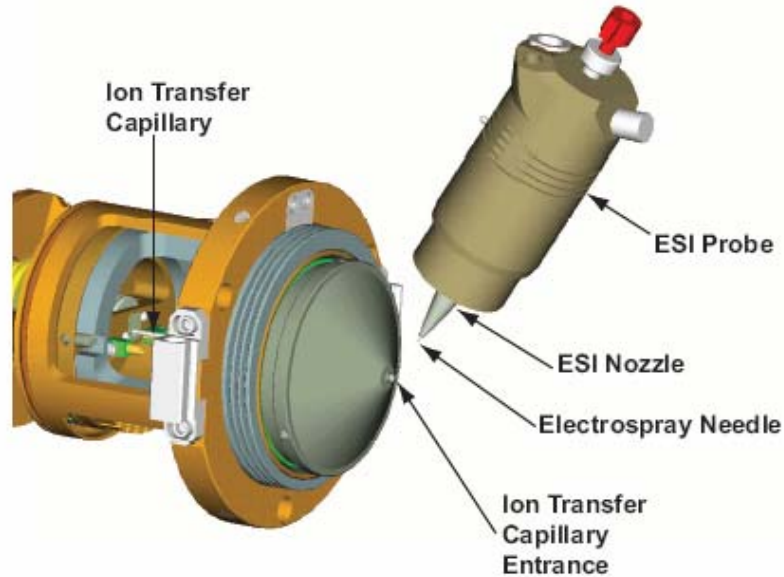
Interchangeable Source Probe
(ESI probe shown)

APPI probe inlet

Positional Adjusters for Source
Probe

The Ion Max ion source is the part of the API source that is at atmospheric pressure. The Ion Max ion source can be configured to operate in any of several API modes. The Ion Max ion source housing allows you to quickly switch between ionization modes without the need for specialized tools. The ventilation of the ion source housing ensures that the housing is always cool and easy to handle. Pressure in the ion source housing is kept at atmospheric levels, which reduces the chemical noise that can be caused by nebulized gases when they are not properly evacuated from the ion source. The probe mounting angle is fixed at the optimum angle for signal intensity and ion source robustness. Minor adjustment of the probe position in the X, Y, and Z dimensions is allowed, with marked adjustments to allow for freedom in probe position during ionization optimization. View ports are placed at the front and side of the ion source housing, which allows visual aid in positioning the probe during ESI operation, and enables easy addition of accessories.

Ion Max Source

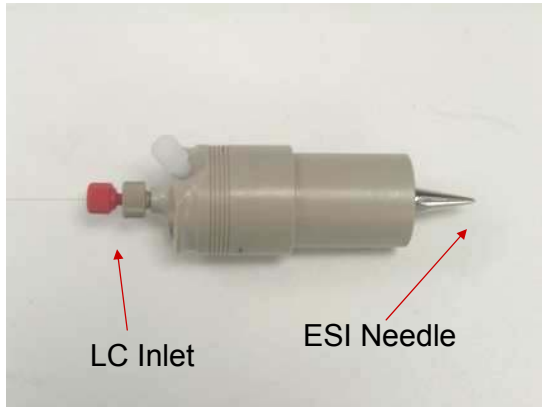


The ESI probe includes the ESI sample tube, needle, nozzle, and manifold. Sample and solvent enter the ESI probe through the sample tube. The sample tube is a short section of 0.1 mm ID fused-silica or metal capillary tubing that extends from a fitting secured to the ESI source housing, through the ESI probe and into the ESI needle, to within 1 mm from the end of the ESI needle. The ESI needle, to which a large negative or positive voltage is applied (typically ± 3 to ± 5 kV), sprays the sample solution into a fine mist of charged droplets. The ESI nozzle directs the flow of sheath gas and auxiliary gas at the droplets. The ESI manifold houses the ESI nozzle and needle and includes the sheath gas and auxiliary gas plumbing. The sheath gas plumbing and auxiliary gas plumbing deliver dry nitrogen gas to the nozzle.

Ion Max Source Design : ESI Probe

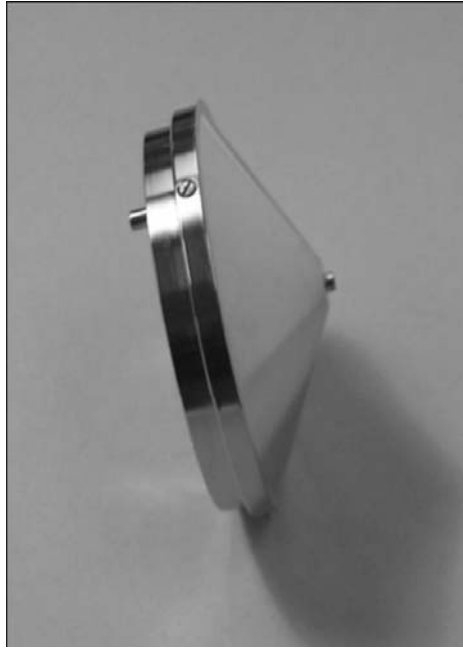
New ESI Probe features:

- *Fixed vertical spray angle (60 degrees)*
- *X,Y,Z-adjustable for further optimization*



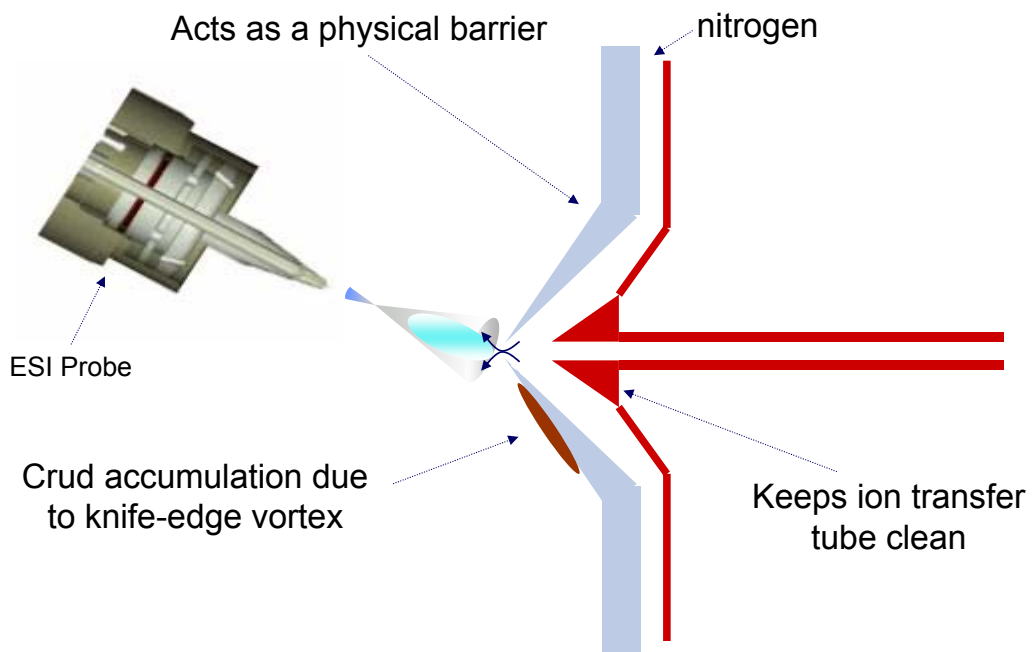
In the LCQ, the ESI needle is orthogonal to the axis of the ion transfer capillary that carries ions to the MS detector. This geometry keeps the ion transfer tube clean. The ion transfer tube assists in desolvating ions that are produced by the ESI or APCI probe. Two heater cartridges are embedded in the heater block. The heater block surrounds the ion transfer tube and heats it to temperatures up to 400 °C. A platinum probe sensor measures the temperature of the heater block. Typical temperatures of the ion transfer tube are 270 °C for electrospray and 250 °C for APCI.

Removable Ion Sweep Cone



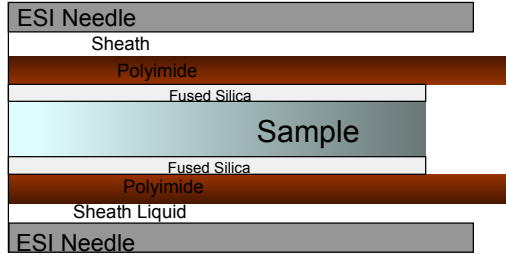
The ion sweep cone is a metallic cone located over the ion transfer tube. The ion sweep cone channels the sweep gas towards the entrance of the ion transfer tube, thereby minimizing the accumulation of endogenous or excipient materials in high-pressure region ion optics.

The Ion Sweep Gas Function

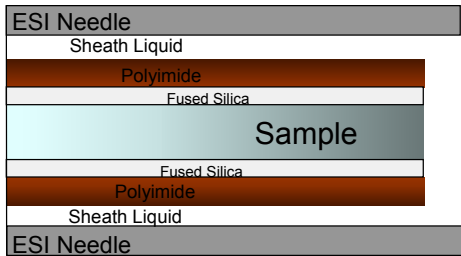


Additional nitrogen helps desolvate APCI/ESI ions that tend to re-hydrate; reduces chemical noise.

Elongation of Fused Silica Capillary Sample Tube



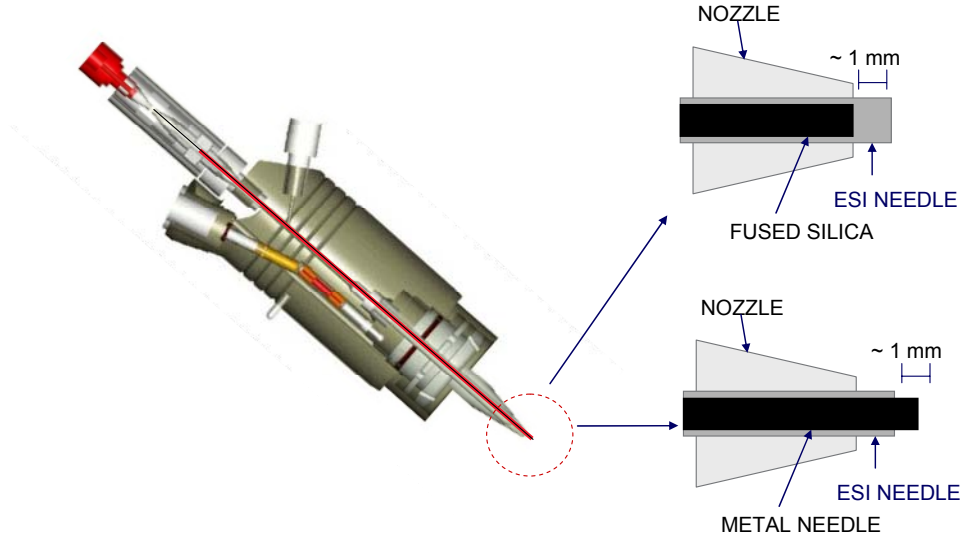
Elongation of polyimide coating occurs when specific solvents (i.e., acetonitrile) are adsorbed into the sample tube.



The sample tube must be cut square to ensure a stable spray. Best results can be achieved by positioning the sample tube about 1 mm inside the ESI needle.

When the polyimide coating on the outside of the fused silica of the sample tube elongates, the sample does not come in contact with the ESI needle and sensitivity is decreased. It is good practice to cut the fused silica on a regular basis to minimize problems associated with the elongation of the polyimide coating.

ESI Probe Cutaway



The auxiliary gas is the outer coaxial nitrogen gas that assists the sheath gas in the nebulization and evaporation of sample solutions. The auxiliary gas also helps lower the humidity in the ion source. Typical auxiliary gas flow rates for ESI and APCI are 10 to 20 units. Auxiliary gas is usually not needed for sample flow rates below 50 $\mu\text{L}/\text{min}$.

Ion Max Electrospray Source – Operational Conditions

LC Flow ($\mu\text{L}/\text{min}$)	Column Size (mm ID)	Ion Transfer Tube Temp ($^{\circ}\text{C}$)	Sheath Gas (PSI)	Aux Gas (Arb.)
≤ 10	Capillary	150 - 200	5 - 15	Off
50 - 200	1	200 - 275	20 - 40	0 - 20
100 - 500	2 - 3	250 - 350	40 - 60	0 - 20
400 - 1000	4.6	300 - 400	60 - 100	10 - 40

Spray Voltage : 3 – 4.5 kV

The numbers in the table should be taken as suggested ranges. In essence, the higher the LC flow rate, the greater the solvent evaporation conditions must be to remove the solvents, in terms of increased ion transfer tube temperature and higher sheath/auxiliary gas flow rates. Auxiliary gas is not required for flow rates from 10 $\mu\text{L}/\text{min}$. to 500 $\mu\text{L}/\text{min}$. but can help to reduce solvent background ions.

Metal Needle Kit

Stainless Steel Needle Size	Type	Solvent Flow Rate ($\mu\text{L}/\text{min}$)
34-Gauge (30 μM ID)	Low flow	0.5 - 10
32-Gauge (50 μM ID)	High flow	5 - 400

There are 2 types of metal needle kits for the ESI probe. The low flow metal needle kit is recommended for LC flow rates between 0.5 and 10 $\mu\text{L}/\text{min}$ and the high flow metal needle kit is recommended for LC flow rates between 5 $\mu\text{L}/\text{min}$ and 400 $\mu\text{L}/\text{min}$).

LCQ Advantage/XP Plus API Probes

Electrospray Ionization (ESI)

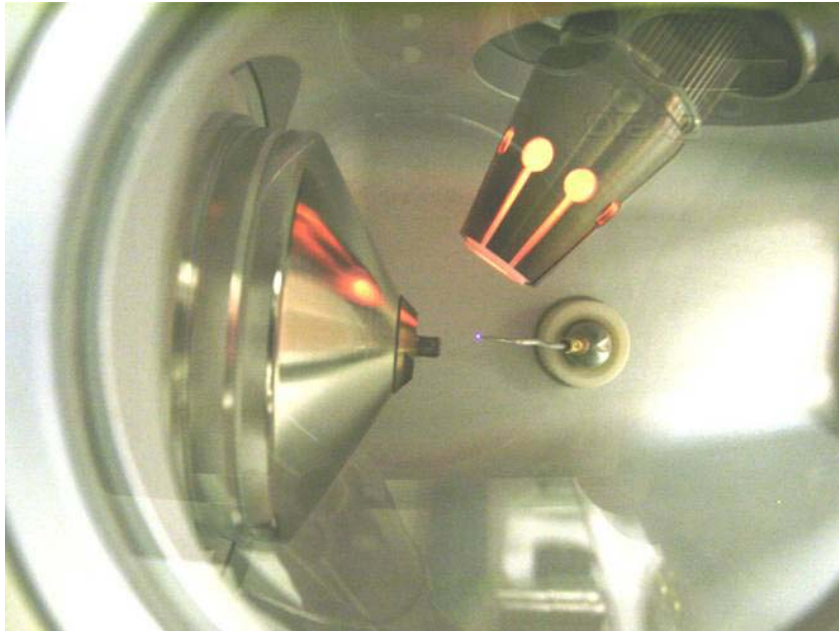


**Atmospheric Pressure
Chemical Interface (APCI)**



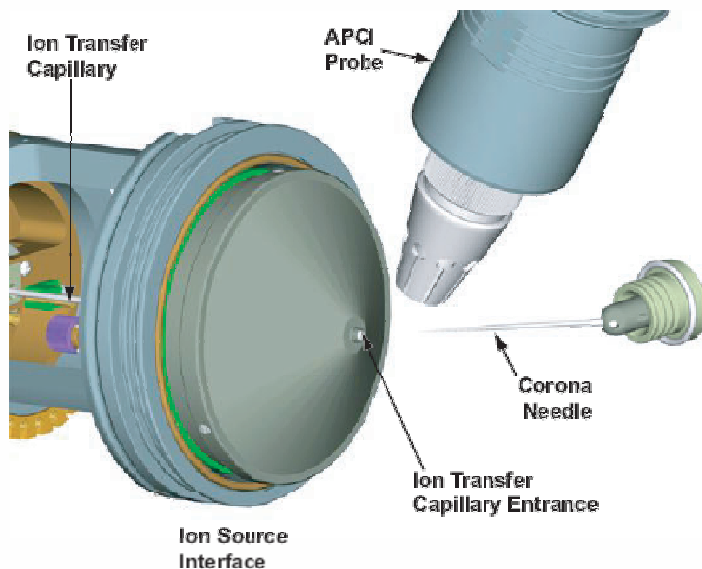
Orthogonal ESI & APCI probes

Ion Max Source Design: APCI Probe



In the LCQ, the sample tube in the APCI nozzle (as in the case of the ESI probe) is orthogonal to the axis of the ion transfer capillary that carries ions to the MS detector. This geometry keeps the ion transfer capillary clean.

Ion Max Source Design: APCI Probe



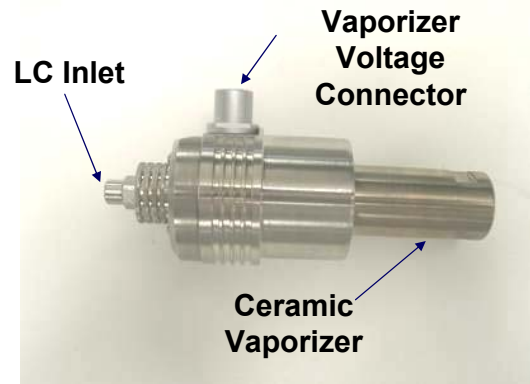
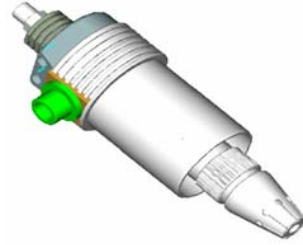
The APCI probe ionizes the sample by atmospheric pressure chemical ionization. The APCI probe accommodates liquid flows of 100 $\mu\text{L}/\text{min}$ to 2.0 mL/min without splitting. The APCI probe includes the APCI sample tube, nozzle, sheath gas and auxiliary gas plumbing, and vaporizer. Sample and solvent enter the APCI nozzle through the sample tube. The sample tube is a short section of 0.10 mm ID fused silica tubing that extends from the sample inlet to 1 mm past the end of the nozzle. The manifold houses the APCI nozzle and includes the sheath gas and auxiliary gas plumbing. The APCI nozzle sprays the sample solution into a fine mist. The sheath gas and auxiliary gas plumbing deliver dry nitrogen gas to the nozzle. The droplets in the mist then enter the vaporizer. The vaporizer flash vaporizes the droplets at temperatures up to 600 $^{\circ}\text{C}$.

The sample vapor is swept toward the corona discharge needle by the flow of the sheath and auxiliary gasses. The corona discharge needle assembly is mounted inside of the Ion Max API source housing. The tip of the corona discharge needle is positioned near the vaporizer. A high potential (typically ± 3 to ± 5 kV) is applied to the corona discharge needle to produce a corona discharge current of up to 100 μA . (A typical value of the corona discharge current is 5 μA .) The corona discharge from the needle produces reagent ion plasma primarily from the solvent vapor. The sample vapor is ionized by ion-molecule reactions with the reagent ions in the plasma.

Ion Max Source Design: APCI Probe

New APCI Probe features :

- Removable sprayer
- New ceramic heater
- Self- cleaning
 - *internal surfaces can exceed 1000 °C*
- External thermocouple
- No plastics in source housing
- Easy change nozzle assembly
- X,Y,Z adjustable



The APCI Probe has an external thermocouple for enhanced temperature feedback control. In addition, the probe contains no plastics, thereby reducing the possibility of phthalate contamination. The probe can also be adjusted in the X,Y and Z directions just as in the case of the ESI probe.

Ion Max APCI Source – Operational Conditions

Liquid Flow Rate (μL/min)	Ion Transfer Tube Temp. (°C)*	Sheath Gas Pressure (psi)	Aux Gas Flow (arbitrary units)	Vaporizer Temperature (°C)
200	250	25	5	350
1000	250	45	5	450

Corona Discharge Current : 4 μA

Although APCI can accommodate higher LC flow rates than electrospray, an increased ion transfer tube temperature is not necessary. In this case, the solvent evaporation and the ion desolvation processes are driven to completion within the APCI vaporizer tube, where the effluent is exposed to temperatures in the 400 °C to 550 °C range. Auxiliary gas is not required but can help to reduce solvent background ions.

Recommended Flow Rates

- **ESI:**
 - 3 μ L/min to 1.5mL/min
 - Optimal Flow Rate: 200 μ L/min
 - Generally, higher flow rates require higher gas flow rates and higher ion transfer tube temperatures
- **APCI:**
 - 200 μ L/min to 2mL/min
 - Optimal Flow Rate: 500 μ L/min
 - Generally, higher flow rates require higher gas flow rates but not ion transfer tube temperatures

The ESI probe can be used at flow rates down to 1.0 μ L/min, or up to a 1.0 mL/min. It is recommended that the APCI probe due to the extreme environment in the source (high gas flows and vaporizer temperatures above 400°C), should only be used for LC experiments between 200 μ L/min and 2.0 mL/min. For the APCI probe, flows below 200 μ L/min require more care to maintain a stable spray.

For both sources, as the flow rate is increased or has a higher aqueous composition, the sheath and aux gasses will optimize at higher flow rates. In the case of the ESI, a higher heated capillary temperature may also be necessary. Although for APCI, the heated capillary temperature will not alter the signal significantly since the sample is already in the gas-phase (passed through the vaporizer tube).

Divert Valve

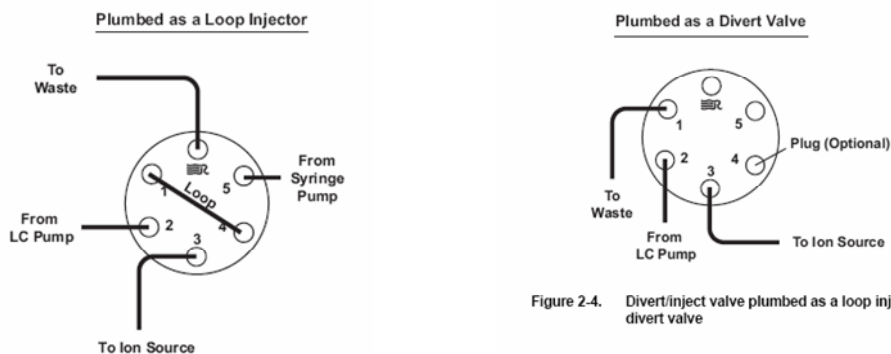


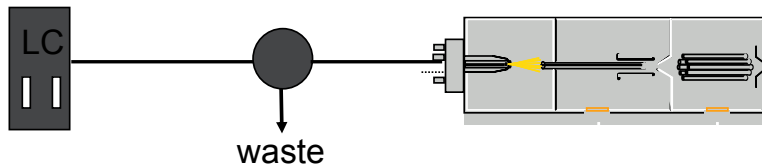
Figure 2-4. Divert/inject valve plumbed as a loop injector or divert valve

The divert valve is most commonly used to divert unwanted flow away from the detector. It is a good idea to use the divert valve whenever analyte peaks are not eluting to increase the ruggedness of the detector. If samples are particularly dirty or have been prepared or stored in an inorganic buffer or solvent, you may want to divert away a few minutes of flow at the initial LC conditions before ramping the organic phase (in reversed phase chromatography). Care should be taken to divert back to the source one to two minutes prior to elution of the first peak, to allow the spray/capillary heater to equilibrate.

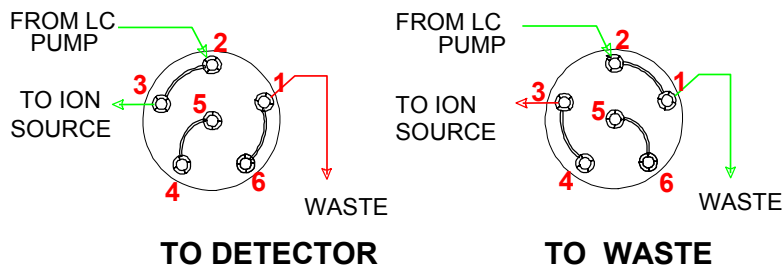
The divert valve may also be plumbed as a loop injector, and since it is dynamically controlled by Xcalibur during the run, it may be used for any custom application as well.

Divert Valve Mode

Useful for increased API source ruggedness:

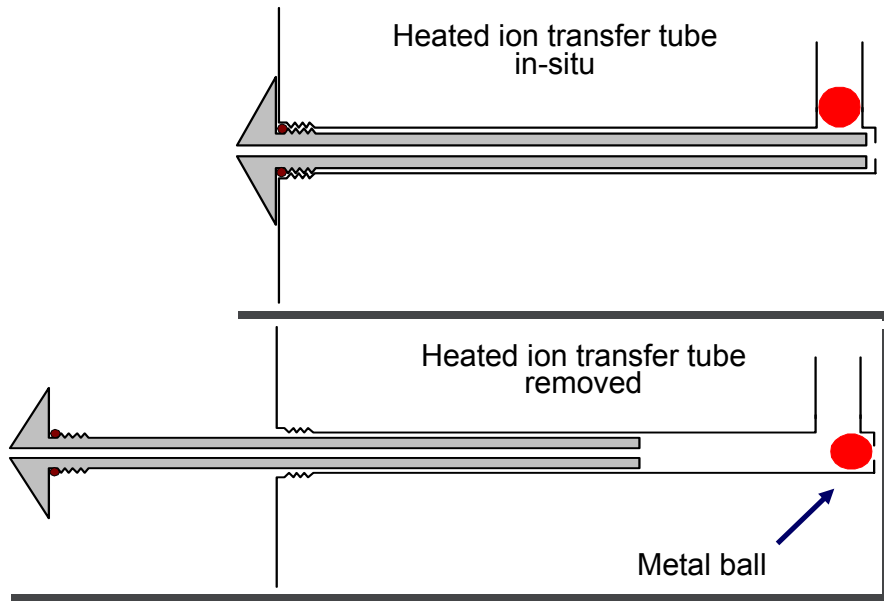


Prevents salt and protein build-up from injections of complex ("dirty") matrices
Prolongs the lifetime of API source by reducing the frequency of disassembling and cleaning



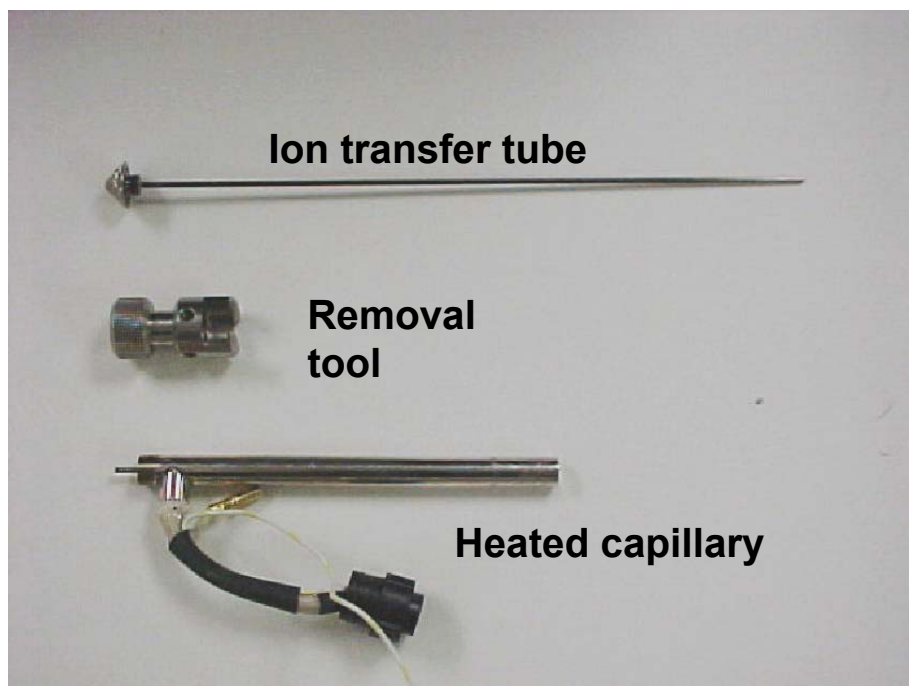
It is a good idea to use the divert valve whenever analyte peaks are not eluting to increase the ruggedness of the detector. If samples are particularly dirty or have been prepared or stored in an inorganic buffer or solvent, you may want to divert away a few minutes of flow at the initial LC conditions before ramping the organic phase (in reverse phase chromatography). Care should be taken to divert back to the source one to two minutes prior to elution of the first peak, to allow the spray/capillary heater to equilibrate.

Removable Ion Transfer Tube

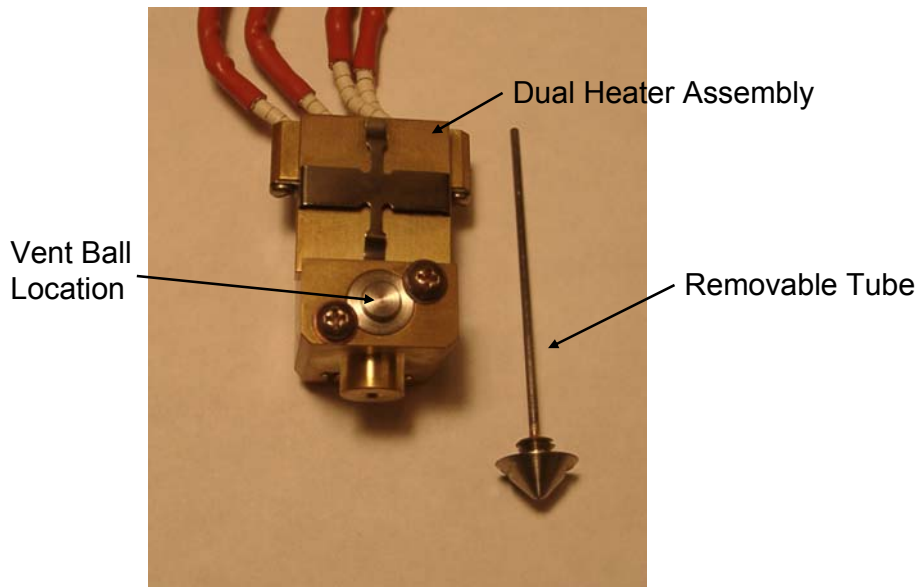


An easily removable ion transfer tube negates the need to vent the instrument in order to conduct routine maintenance. The vent prevent ball falls into the space occupied by the ion transfer tube when the tube is removed, thus preventing air from entering the vacuum manifold. The vent prevent ball allows the removal of the ion transfer tube for cleaning or exchange without venting the system.

Ion Transfer Tube and Removal Tool



Heater Assembly With Removable Ion Transfer Tube



The heated transfer capillary assembly assists in desolvating ions that are produced by the ESI or APCI probe. Ions in the gas or liquid phase are drawn into the ion transfer capillary in the atmospheric-pressure region of the API source and are transported to the capillary-skimmer region by a decreasing pressure gradient.

The ion transfer capillary is a cylindrical metal tube with a cone shaped entrance. This special entrance shape helps to reduce solvent adduction. An external heater block with two standard 60 V / 100 W cartridge heaters heats the capillary to a maximum temperature of 400 °C. Typically, an offset potential of up to ± 300 V (positive for positive ions and negative for negative ions) assists in repelling ions from the ion transfer capillary to the skimmer.

ThermoFisher
SCIENTIFIC

The world leader in serving science

Chapter 3

LC-MS Considerations

Optimal Flow Rates (Linear Velocities) for LC Columns (Standard Packing 5.0 μm)

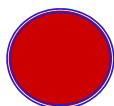
<u>Flow Rate</u>	<u>Column I.D.</u>
1.0 mL/min	4.6 mm
0.5 mL/min	3.0 mm
0.2 mL/min	2.1 mm
50 $\mu\text{L}/\text{min}$	1.0 mm
<10 $\mu\text{L}/\text{min}$	Capillary



It is important to use the correct flow rate for your HPLC column. The limiting factors in choosing a flow rate are, instrument pressure limitations, the effect on the quality of the chromatography, and time. Maintaining linear velocity is the single most important factor when trying to reproduce a chromatographic separation on columns of differing diameters.

Theoretical Increase in Response by Using Narrow Bore Columns

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



The internal diameter of an HPLC column is a critical aspect that determines the quantity of analyte that can be loaded onto the column and also influences sensitivity. The advantage of low I.D. columns is improved sensitivity and lower solvent consumption.

LC Additives

➤ Acids

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

➤ Bases

- Do not use alkali metal bases (*will 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*)

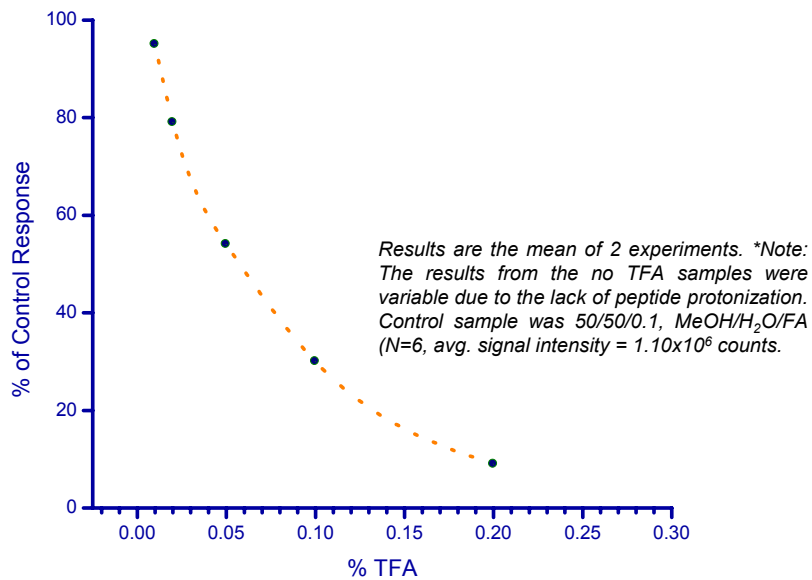
- May enhance chromatographic resolution, but causes ion suppression in **both negative and positive ion mode**

➤ Triethylamine/Trimethylamine (*TEA/TMA*)

- May enhance deprotonation for negative ion formation

This slide lists several recommendations for LC additives. One should avoid the use of inorganic acids and alkali metal bases as both will eventually lead to the damage of source hardware. Formic and acetic acids are recommended as proton donors for positive ion mode and ammonium hydroxide and ammonia solutions are recommended as proton acceptors for negative ion mode. One should avoid the use of surfactants such as Triton-X 100 for use with mass spectrometry as these detergents lead to ion suppression and coating of the ion optics. Both outcomes result in an overall loss in sensitivity. TFA is commonly used in HPLC with UV detection because of its enhancement of chromatographic resolution. Unfortunately, this additive has an adverse effect on negative and positive ion formation. Simply speaking, negative ions cannot be formed in a low pH environment, and TFA suppresses negative ion formation. Also, since TFA contains many electronegative fluorine groups, it is also a proton acceptor which leads to ion suppression in positive ion mode as well. To enhance negative ion sensitivity, or improve the chromatographic separation (as an ion-pairing reagent), the addition of TEA or TMA may be beneficial.

The effect of increasing TFA levels (in 50:50 ACN:H₂O) on MS signal intensity



*S. Baldwin, K. Stoney, K. Wheeler, I. Mychreest. "Low pH Solvent Alternatives to TFA Solvents and Their Effect on HPLC/ESI-MS of Peptides", Poster Paper Presented at ASMS 1996.

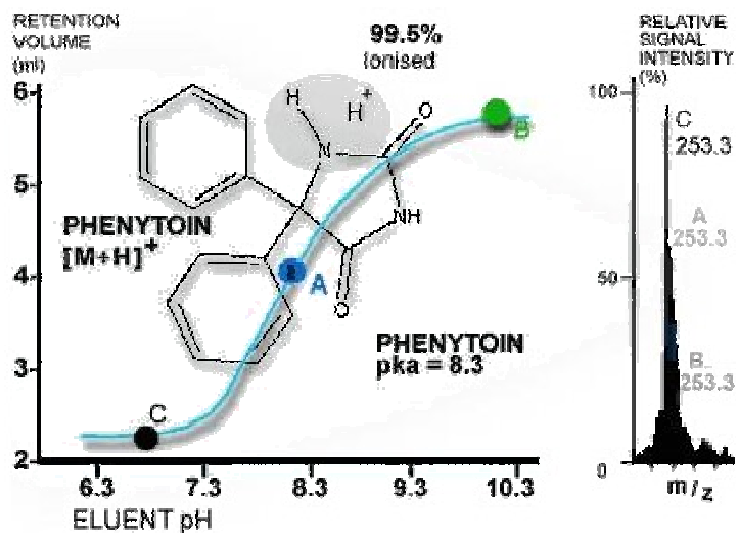
As the concentration of TFA in the mobile phase increases, there is significant loss of MS signal intensity, therefore, if TFA is necessary, it should be used at low concentrations.

Buffers

- When using non-volatile buffers, sweep cone should be in place.
- If possible, avoid using non-volatile HPLC additives such as:
 - Alkali-Metal Phosphates
 - Borates
 - Citrates
- When using buffers, more frequent cleaning of the source housing, sweep cone, ion transfer tube, skimmer, and tube lens is necessary.

If the HPLC separation requires a buffer, one should use the ion sweep cone. The ion sweep cone is a metallic cone that is installed over the ion transfer tube. The ion sweep cone channels the sweep gas towards the entrance of the capillary. This helps to keep the entrance of the ion transfer tube free of contaminants.

Buffers and pH



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

Electrospray involves the formation of $[M+H]^+$ ions in the positive ion mode and $[M-H]^-$ ions in the negative ion mode. The generation of both species is contingent upon the pK_a of the analyte and the pH of the mobile phase. Basic samples will protonate in acidic solutions, thereby becoming positively charged (the converse is true for acidic analytes in basic solutions). In-solution ionization is a competitive equilibrium process, and ionization efficiency of the analyte depends on the degree of protonation and/or deprotonation. Therefore, knowledge of the analyte's pK_a (or pK_b) is essential in determining the most favorable pH of the eluent for obtaining maximum sensitivity in LC-ESI-MS analyses.

In our Phenytoin example, if the pH of the eluent solution is adjusted to match the pK_a of the basic functional group, this group will be ionized to a 50% extent. By raising the pH by 2 units, the basic functional group will be quasi-entirely (99.5%) non-ionized (in the ion-suppressed form). Conversely, by lowering the pH by 2 units, the basic functional group will be quasi-entirely (99.5%) ionized (protonated) and will lead to higher levels of analyte detection.

LC/MS Additives and Buffers (Summary)

Acetic Acid	}	Proton Donors
Formic Acid		
Ammonium Hydroxide	}	Proton Acceptors
Ammonia Solutions		
Trichloroacetic Acid (< 0.02% v/v)	}	Chromatographic Separation
Trifluoroacetic Acid (< 0.02% v/v)		
Triethylamine (< 0.02% v/v)	}	Chromatographic Separation Negative Ion Formation
Trimethylamine (< 0.02% v/v)		
Ammonium Acetate	}	Buffers
Ammonium Formate		

Methanol
Acetonitrile
Water
Isopropanol
Dichloromethane
Chloroform
Hexane

A range of precautions is recommended, to avoid the introduction of contaminants in the system:

- 1) Use high-purity solvents, made by reputable manufacturers (i.e., J.T. Baker, VWR - Burdick & Jackson, E.M. Sciences, etc.)
- 2) Use high-purity additives (acids, bases, buffers)
- 3) Avoid transferring, degassing, and filtering the solvents; use original containers, if possible
- 4) Avoid any contact of solvents and additives with plastics (i.e., syringes)

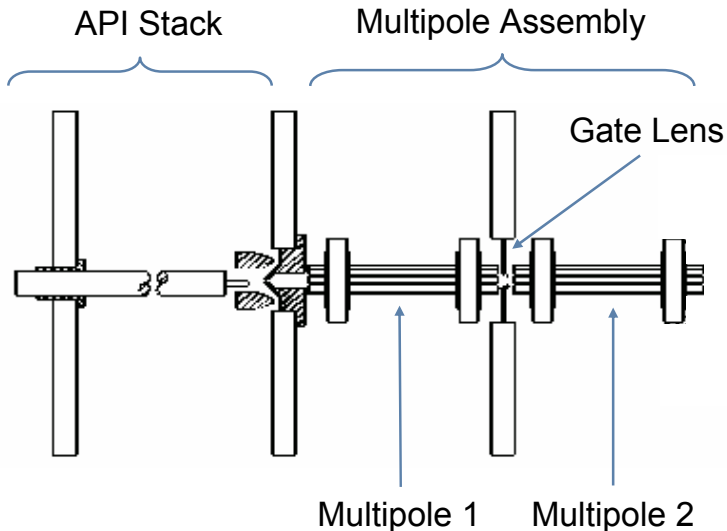
ThermoFisher
SCIENTIFIC

The world leader in serving science

Chapter 4

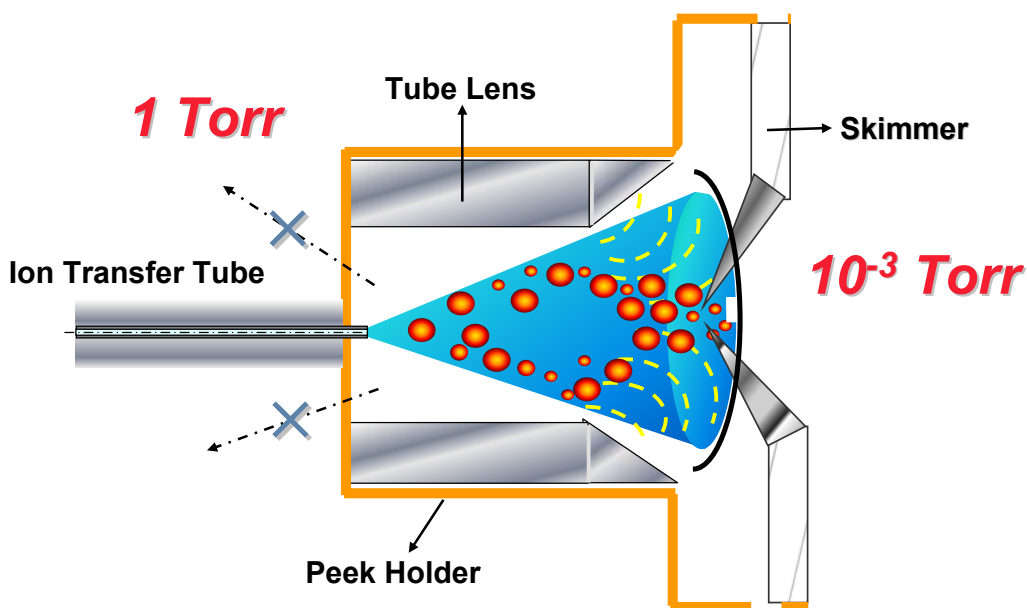
Moving Ions: The LCQ Ion Optics

LCQ Ion Optics



The ion source interface (API stack) consists of the components of the API source that are held under vacuum (except for the atmospheric pressure side of the ion sweep cone). Ions emerge from the ion transfer tube and pass through the tube lens and skimmer and then move toward multipole 1. The ion optics focus the ions produced in the API source and transmit them to the mass analyzer. Between multipole 1 and multipole 2 there is a lens called the “gate lens” to which a voltage is applied to start and stop the injection of ions into the mass analyzer. This lens is also known as the intermultipole lens or split lens.

Pressure Regions in the API Stack



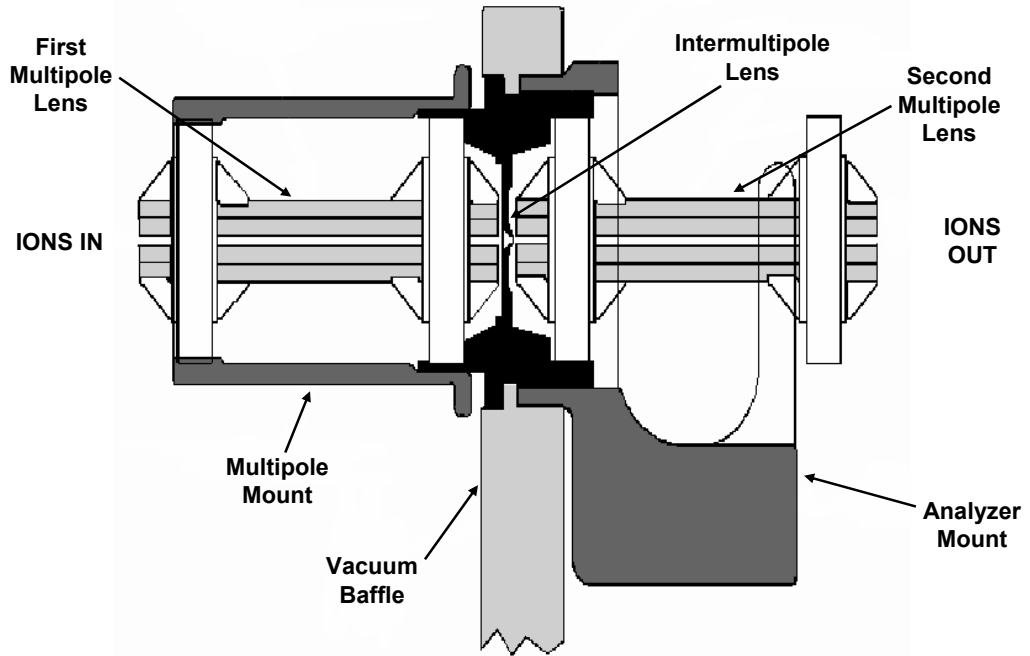
Ions in the gas or liquid phase are drawn into the heated capillary in the atmospheric-pressure region of the API source and are transported to the capillary-skimmer region by a decreasing pressure gradient. The heated capillary passes through a hole in the center of the spray shield. A potential of typically ± 25 V (positive for positive ions and negative for negative ions) assists in repelling ions from the heated capillary to the skimmer.

API Tube Lens — A lens in the API source that separates ions from neutral particles as they leave the heated capillary. The tube lens has a potential applied to it to focus the ions toward the opening of the skimmer. The tube lens also serves as a gate to terminate the injection of ions into the mass analyzer. A potential of -200 V is used to deflect positive ions toward the tube lens and away from the skimmer, and a potential of $+200$ V is used to deflect negative ions toward the tube lens and away from the skimmer.

Skimmer — The skimmer acts as a vacuum baffle between the higher pressure capillary-skimmer region (at 1 Torr) and the lower pressure first octapole region (at $10e-3$ Torr). The skimmer is at ground potential. The opening in the skimmer is offset with respect to the bore of the heated capillary to reduce the number of large charged particles that pass through the skimmer. (These large charged particles can pass through the ion optics and mass analyzer and create detector noise.)

API Capillary-skimmer region — The area between the heated capillary and the skimmer, which is surrounded by the tube lens. The capillary-skimmer region is the area where ions leave the exit end of the heated capillary, experience free-jet expansion, and are sampled by the aperture of the skimmer. It is also the area of first-stage evacuation in the API source. See also heated capillary, skimmer, API source, and atmospheric pressure ionization (API).

Ion Optics

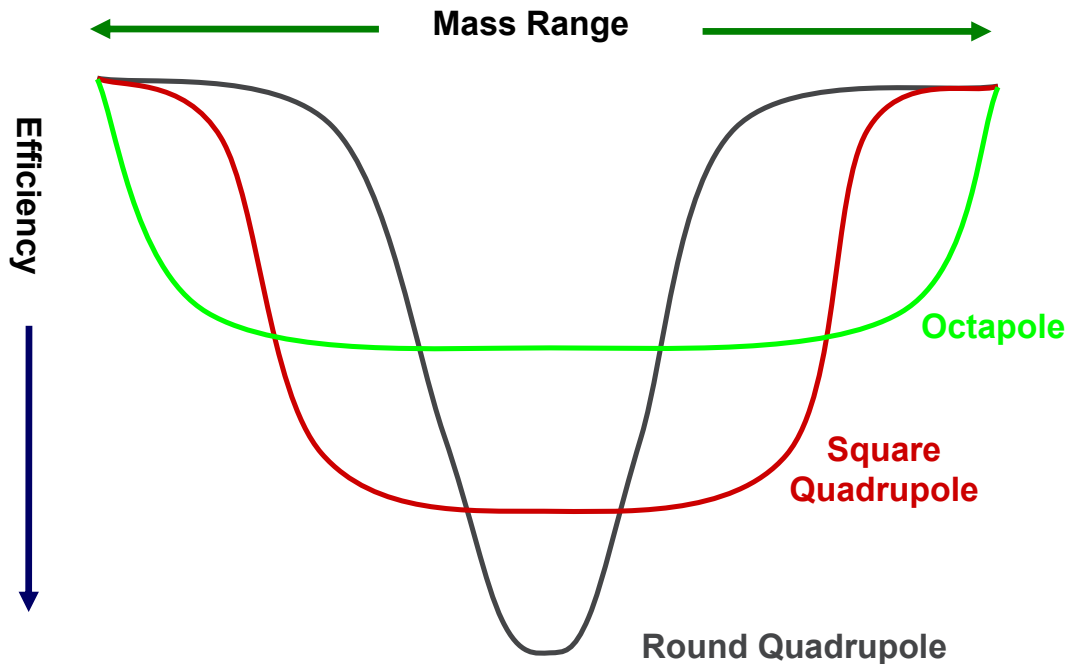


Ion Guides

- **A multipole rod assembly that is operated with only radio frequency (RF) voltage on the rods. In this type of device, virtually all ions have stable trajectories and pass through the assembly.**
- **Ion Guides focus and transfer the ion beam between the high-pressure ion source region and the mass analyzer. Also responsible for reducing kinetic energy of transmitted ions.**
- **The LCQ contains a mixture of square quadrupoles and round octopoles depending on the version of instrument.**
- **Different multipole arrangements have different transmission properties.**

The ion guides decrease the kinetic energy of the transmitted ions and ensure that ions travel in an organized, stable manner towards the ion trap. This is done in a non-selective fashion.

Multipole Potential Wells



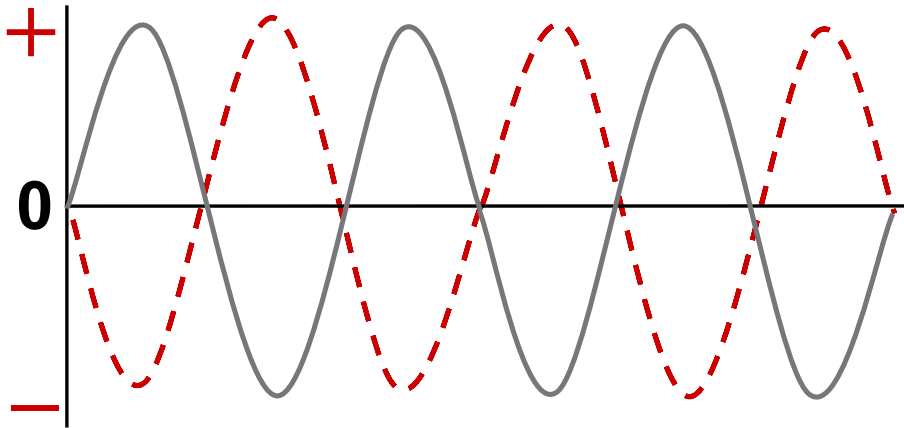
In quadrupole instruments (single or triple), the quadrupole(s) can act not only as a focusing device, but more importantly as a selection device. For that reason, it is important to obtain very good transmission efficiency for a specific mass or mass range. Thus round quadrupoles were employed as ion guides. Unfortunately, the excellent transmission efficiency of the round quadrupole does not apply across a large mass range. As a consequence, quadrupole instruments function by scanning (RF and DC) across the mass range in steps to optimize recovery.

The octapoles on the other hand, function exclusively as ion focusing devices to transmit ALL ions, and are not scanned (RF only). Therefore, it is necessary to have good efficiency across a much larger range. These poles offer good efficiency, but do not completely preclude the loss of some ions within the transmission mass range.

Recent research has shown that square quadrupoles offer the best of both worlds, a mass range similar to that of an octapole with the trapping efficiency nearing that of the round quadrupole.

What is an RF Field?

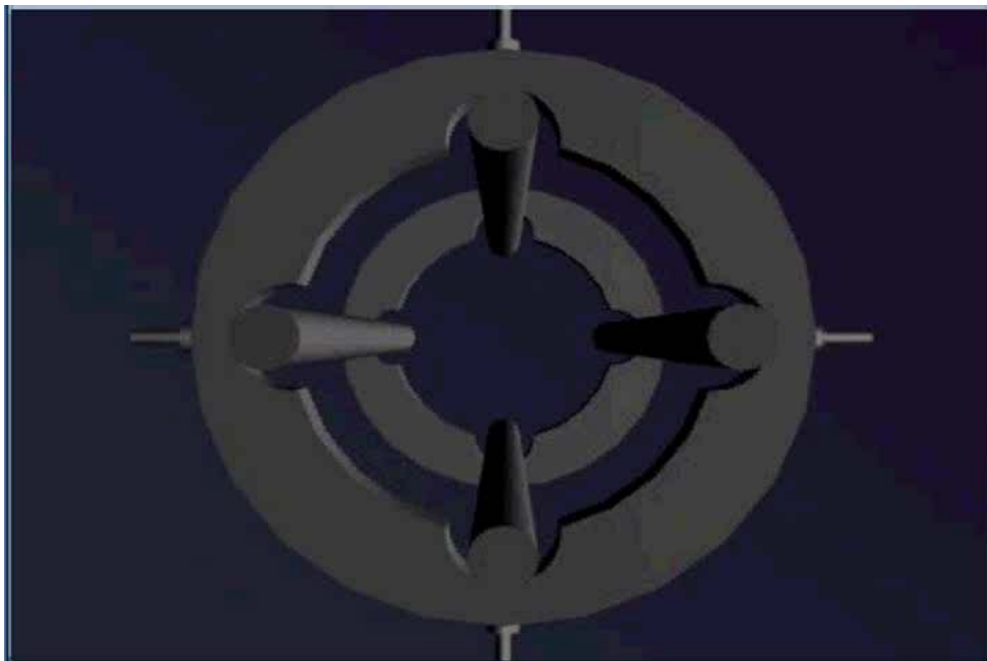
Continuously oscillating voltage of a set amplitude positive and negative relative to a center voltage. Responsible for ion movement in the X and Y directions.



The manner by which the quadrupoles focus the desired ions into a concise beam is based on the application of radio frequencies (or RF) on opposing poles.

In a quadrupole, opposite poles are connected such that the same voltage is applied to both. This voltage can be oscillated over time in a characteristic sine wave to positive, through neutral, to negative and back again (blue trace). The same exact RF oscillation can be placed on the opposing two poles 180° out of phase such that when one set is positive, the other set is negative and visa versa.

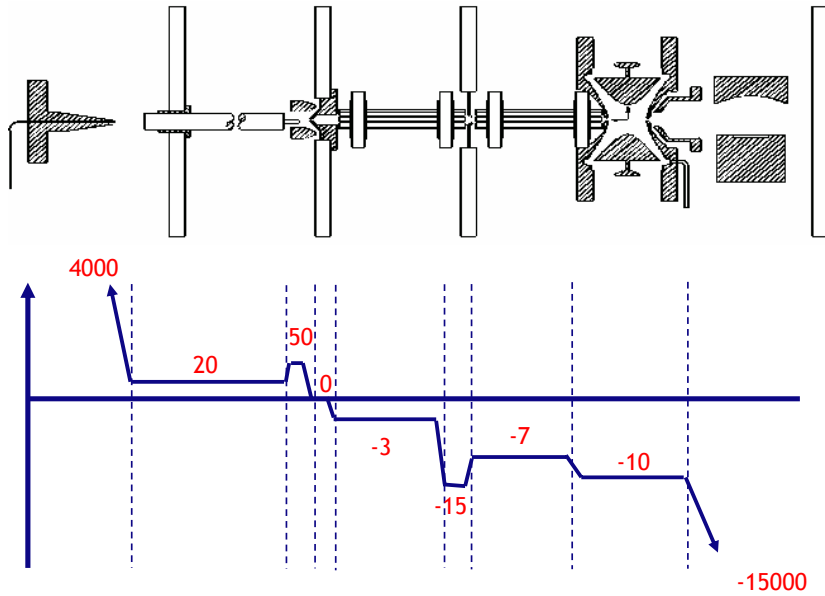
Multipole Oscillations



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

Ions are attracted to rods in the multipoles that are opposite in polarity. Since the polarity of the paired rods is alternating, ions move from rod to rod in a motion that resembles a corkscrew.

Ion Optics (DC Offsets)



There is a decreasing potential energy gradient from the front of the instrument where the ions are made to the back of the instrument where the ions are detected.

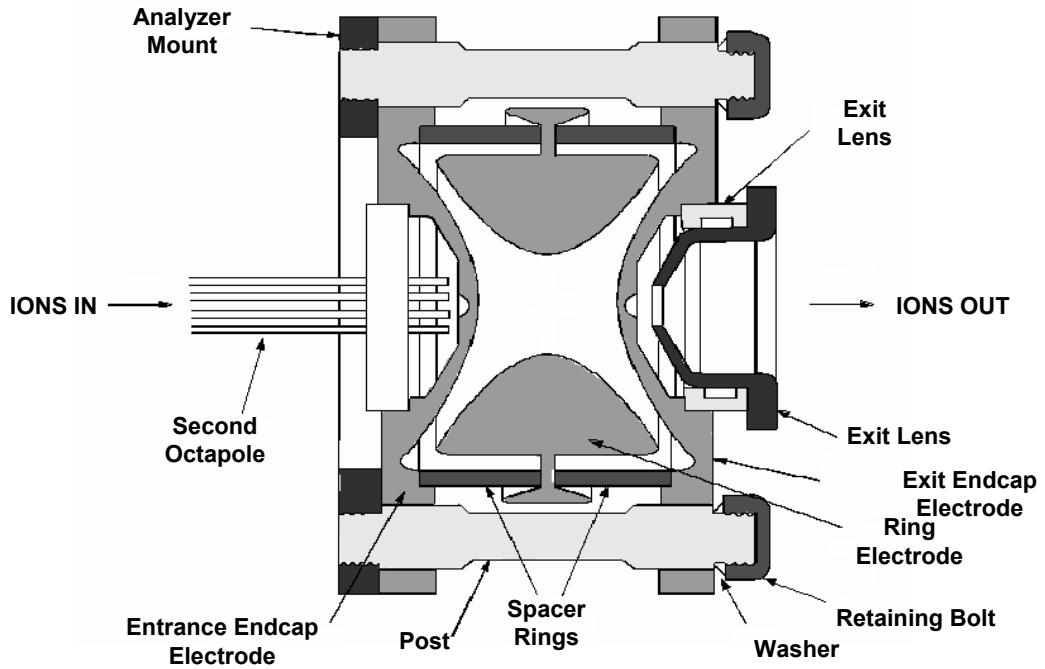
ThermoFisher
SCIENTIFIC

The world leader in serving science

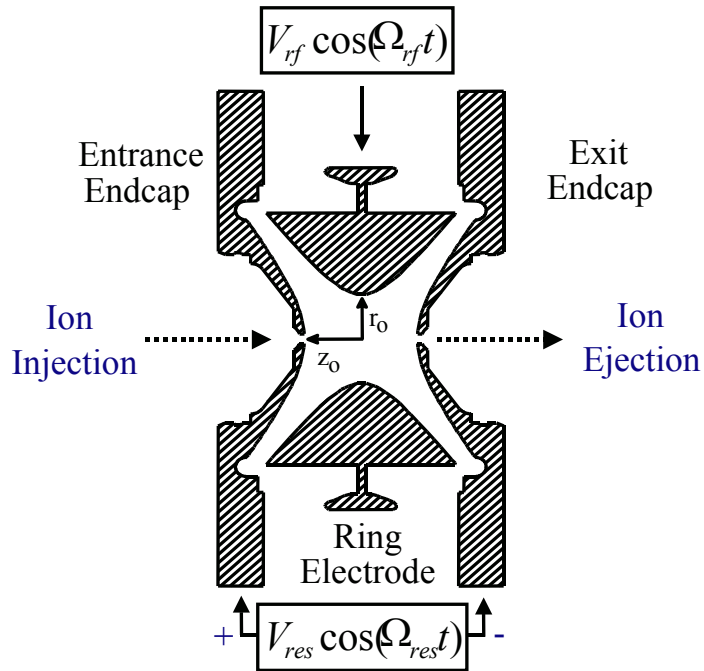
Chapter 5

Ion Trap Theory

Mass Analyzer (Ion Trap)



Basic Ion Trap Components



Steps to Ion Trap Scan Functions

- **Trapping- all scans**
- **Isolation- SIM and MSⁿ**
- **Excitation- MSⁿ**
- **Ejection- all scans**

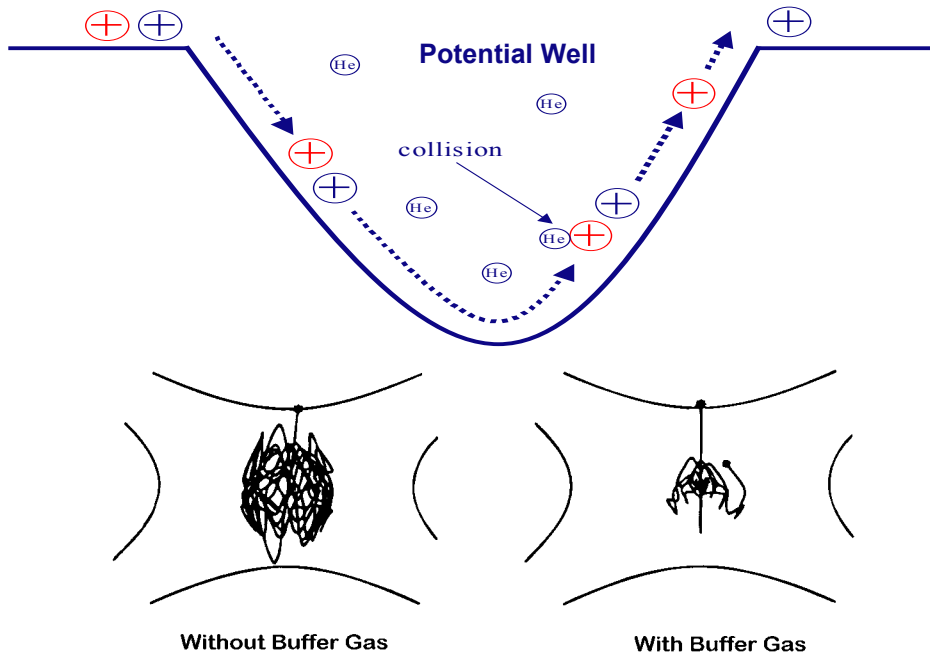
Each scan that is done on the LCQ involves trapping and ejection. In the case of SIM (selective ion monitoring), after trapping, a mass range is isolated and then the ions are scanned out (ejected). When doing an MSⁿ experiment, a mass range is isolated, fragmented and then scanned out.

Trapping of Ions

- **RF (applied to ring electrode)**
- **Helium (stabilize the ions)**

Helium is utilized when trapping to decrease the kinetic energy of the ions being trapped and stabilize the ions.

Helium's Role as a Damping Gas

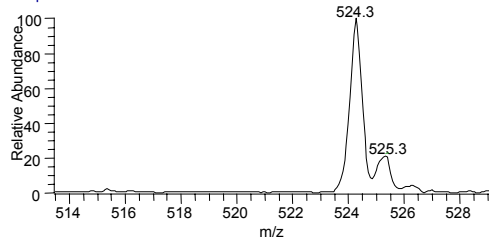


Helium is used as a dampening gas inside the ion trap, due to its ability to energetically cool the ions without inducing fragmentation. The collisions of the ions entering the mass analyzer with the helium slow the ions so that they can be trapped by the RF field in the mass analyzer. Larger gas molecules in the trap would cause collision-induced fragmentation of the ions.

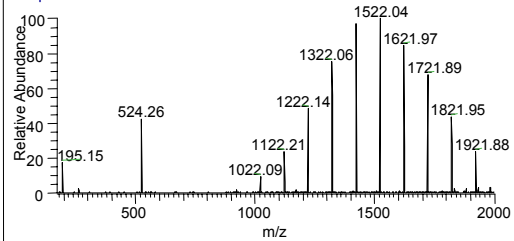
The Effects of No Helium

Helium flowing into trap

S#:1 RT:0.00 AV:1 SM7G NL:2.50E7
T: + p Full ms

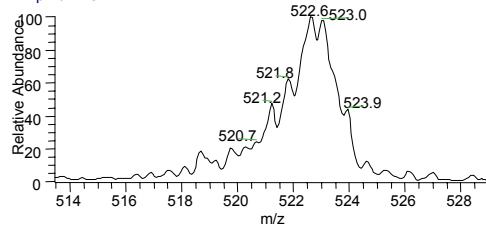


S#:23-32 RT:0.71-1.00AV:10 SM7G NL:5.61E7
T: + p Full ms

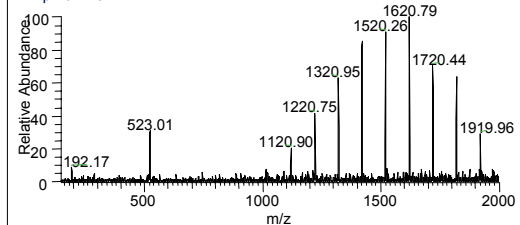


Helium shut off and not flowing into trap

S#:1 RT:0.02 AV:1 SM7G NL:9.70E6
T: + p Full ms

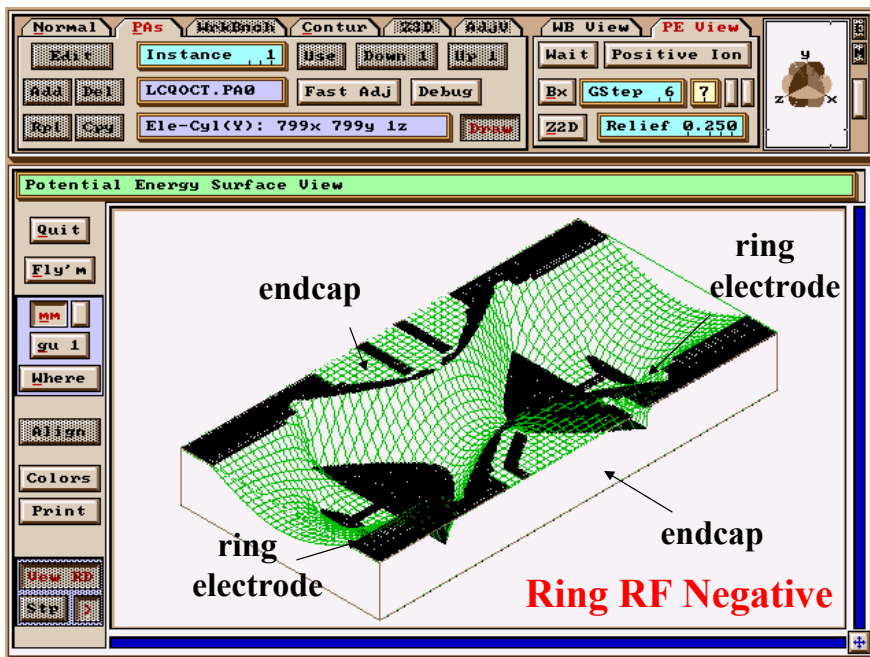


S#:23-32 RT:0.39-0.54AV:10 SM7G NL:2.80E7
T: + p Full ms

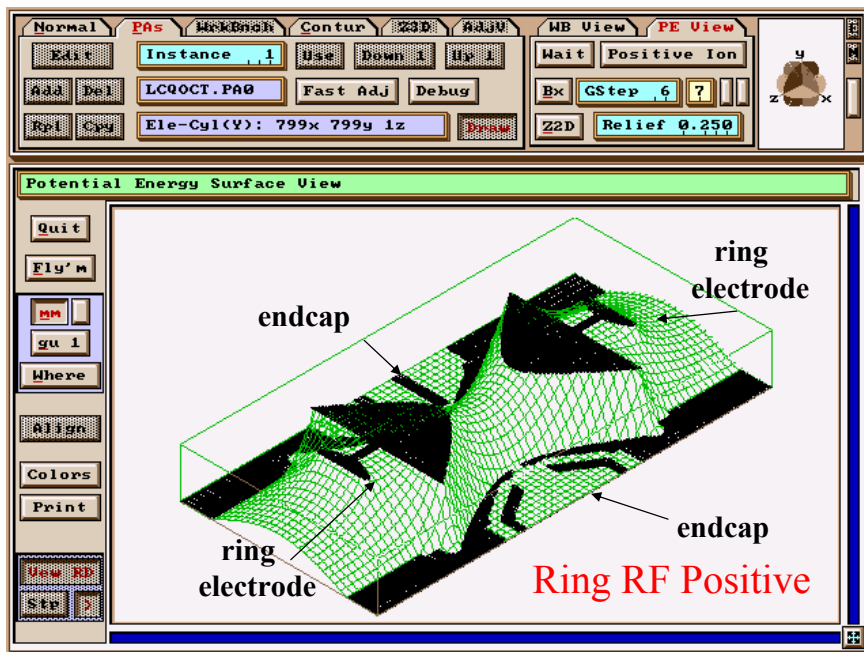


The presence of helium in the mass analyzer cavity significantly enhances sensitivity and mass spectral resolution. Before their ejection from the mass analyzer cavity, sample ions collide with helium atoms. These collisions reduce the kinetic energy of the ions, thereby damping the amplitude of their oscillations. As a result, the ions are focused to the axis of the cavity rather than being allowed to spread throughout the cavity.

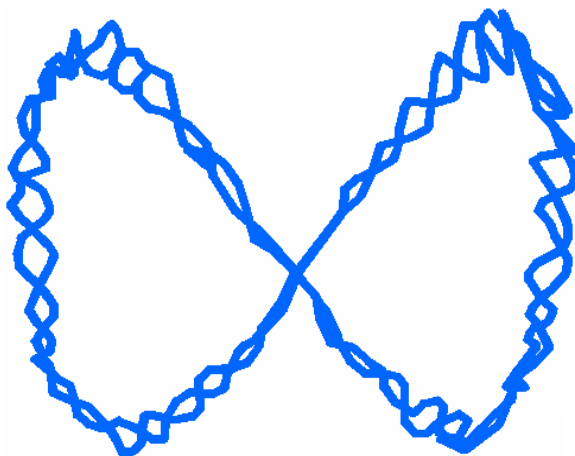
Potential Energy for Injected Ions



Potential Energy for Injected Ions



Ion Motion in the 3D Ion Trap



Ion Stability in the Trap

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

a = variable solution

q = solution

e = charge of trapped ion

U = DC Voltage

V = RF amplitude

m = mass of ion

Ω = angular frequency of rf

z_0 = distance between center of trap to either endcap

r_0 = internal radius of ring electrode

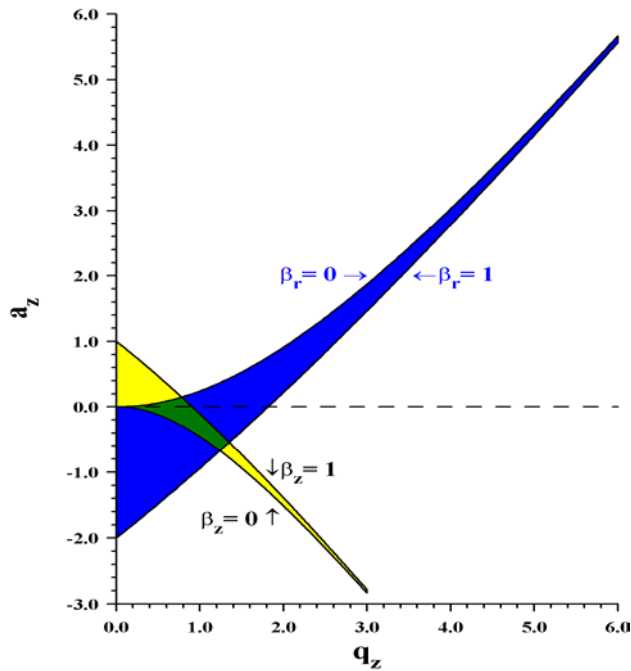
$$a_z = - \frac{16eU}{m(r_0^2 + 2z_0^2)\Omega^2}$$

$$q_z = - \frac{8eV}{m(r_0^2 + 2z_0^2)\Omega^2}$$

Ring Parameters

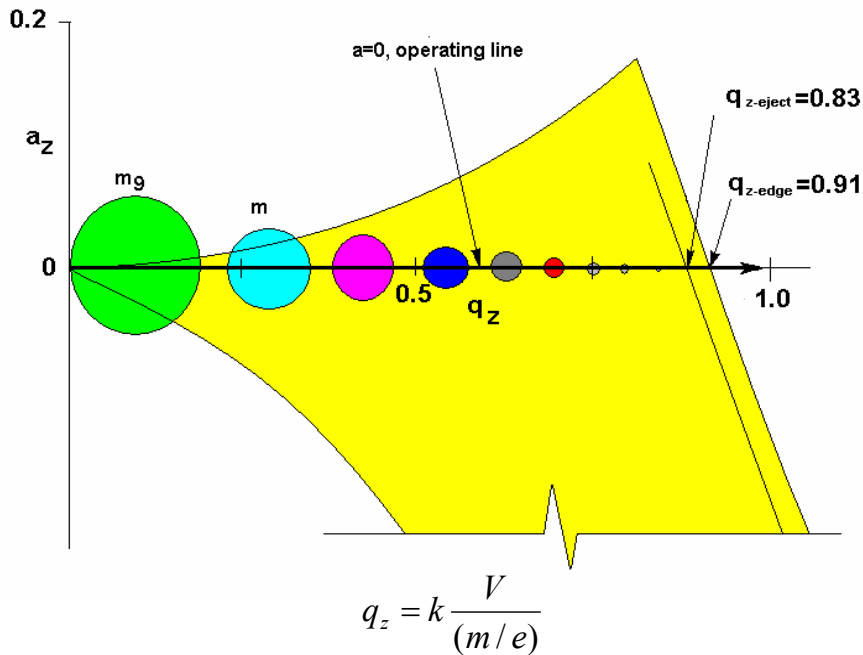
The motion of ions in quadrupole fields is described mathematically by the solutions to a second-order linear differential equation described by Mathieu in 1868. These equations can only be solved numerically, or equivalently by computer simulations. The difference between the DC voltage on the ring and endcap electrodes is 0 and therefore $a_z=0$. q_z is a calculated number which is dependent upon the difference between the RF power on the ring and endcap electrodes. Q_z is inversely related to the mass-to-charge ratio of the ion and is proportional to the RF power on the endcaps.

Ion Trap Stability Diagram



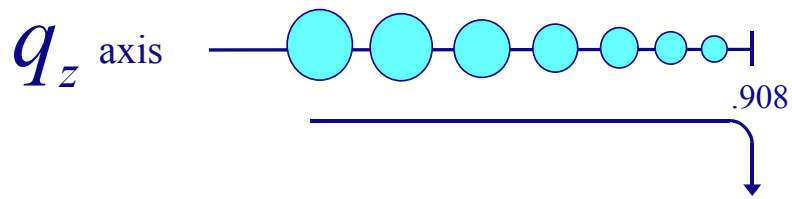
- The blue shaded region indicates (DC) and q (RF) values which provide stable trajectories in the r-direction
- The yellow shaded region indicates the z-stable a and q combinations
- The green area where the r- and z-stable regions overlap indicates the a and q combinations under which ions will be stable in the trap

Stability Diagram for Commercial Traps



The stability diagram is a graphical representation of the important aspects of the mathematics describing ion motion inside the trap. It answers the two most important questions concerning ion motion. The first being “is the ion I am interested in trapped?”. That is, does the ion have a stable trajectory inside the device or does the ion hit the endcaps? The stability diagram indicates this in terms of the parameters a and q . If the ion has an a and q value which place it inside the stability region then the ion will be trapped, and if it is outside the stability region, it will hit an endcap or be ejected through the holes in the endcap.

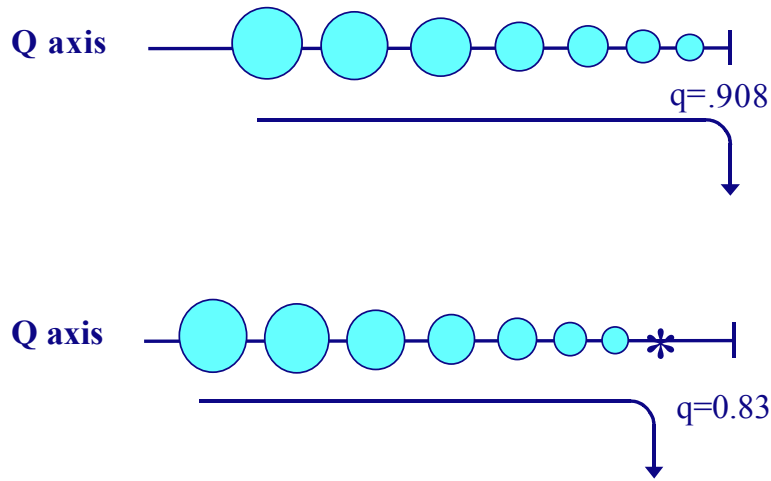
Mass Selective Instability



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

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

... with Resonance Ejection



Increases Resolution ...

The resonance ejection RF voltage is a small AC voltage, applied to the endcaps of the mass analyzer to minimize space charge effects in the mass analyzer. During mass analysis, this RF voltage facilitates the ejection of ions from the mass analyzer and thus improves mass resolution and sensitivity. The resonance ejection RF voltage is applied at a fixed frequency during the ramp of the main RF voltage. When an ion is to be ejected from the mass analyzer cavity it is brought toward resonance with the frequency of the resonance ejection RF voltage. The application of resonance ejection increases resolution because ions of a specific m/z leave the trap in a more condensed manner

LCQ Scan-Out (Ejection) Rates

Normal Scan (5500 amu/sec)

- Common full, SIM, or MSⁿ scanning
- Resolution (FWHM) = 0.50

Zoom Scan (280 amu/sec)

- Increases resolution across a narrow range (allows charge state determination)
- Resolution (FWHM) = 0.15

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

Slower scan out (ejection) rates can provide information about the charge state of one or more mass ions of interest. The data is collected by using slower scans at higher resolution. This can allow for determination of charge state, which in turn allows for the correct determination of molecular mass.

ThermoFisher
SCIENTIFIC

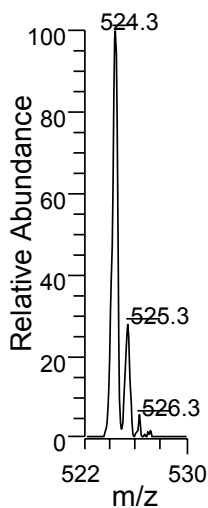
The world leader in serving science

Chapter 6

AGC – Automatic Gain Control

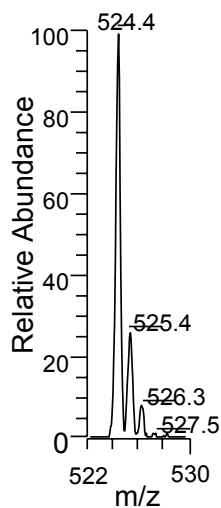
AGC (Ion Population Control)

~ 300 Ions

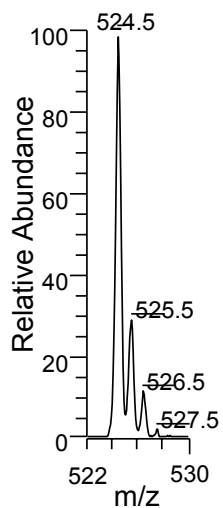


Good Resolution

~ 1500 Ions

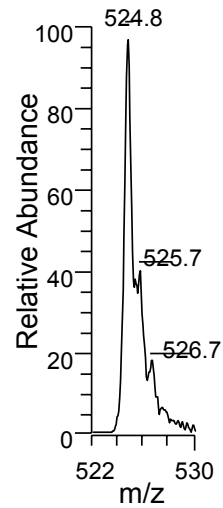


~ 3000 Ions



Poor Resolution

~ 6000 Ions



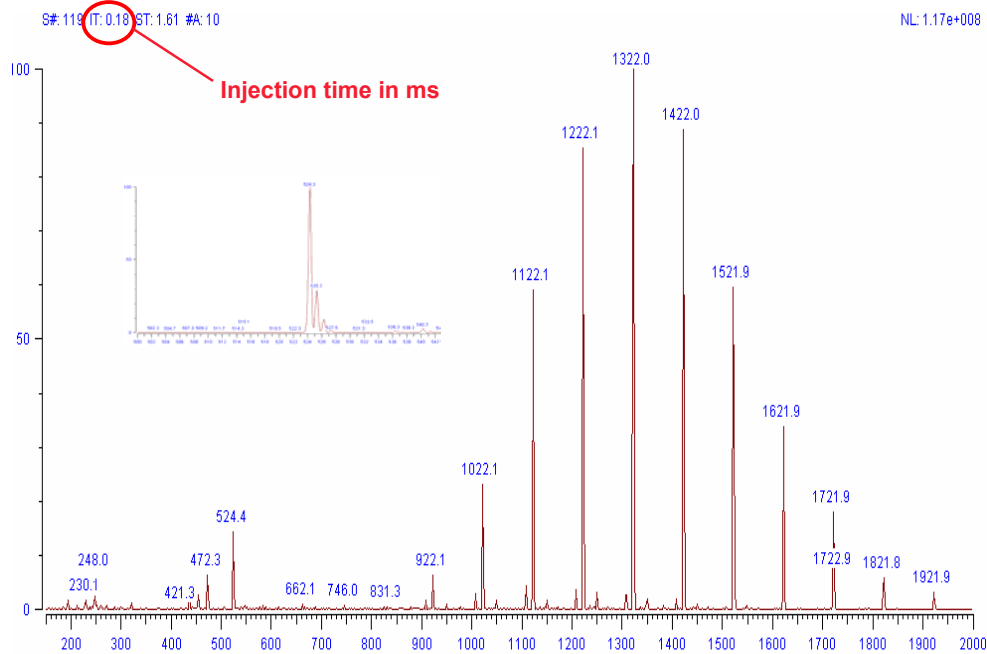
Automatic Gain Control (AGC) is utilized in the LCQ to control the number of ions in the trap at any one time. If the ion count is too high, the density of charges per unit area in the center of the trap will be too large, resulting in "space charging." This phenomena occurs when the charges of neighboring ions affect each other, causing a large decrease in resolution and mass accuracy. However, if the ion count is too low, small deviations in the ion count result in large TIC signal and spectral ratio instability from scan to scan. Therefore, it is necessary to control the number of ions present in the trap for each scan.

Prescan before the analytical scan

- Measures the # of ions in the trap for a pre-defined time (e.g. 1 ms)
- Allows software to determine optimum ion injection time

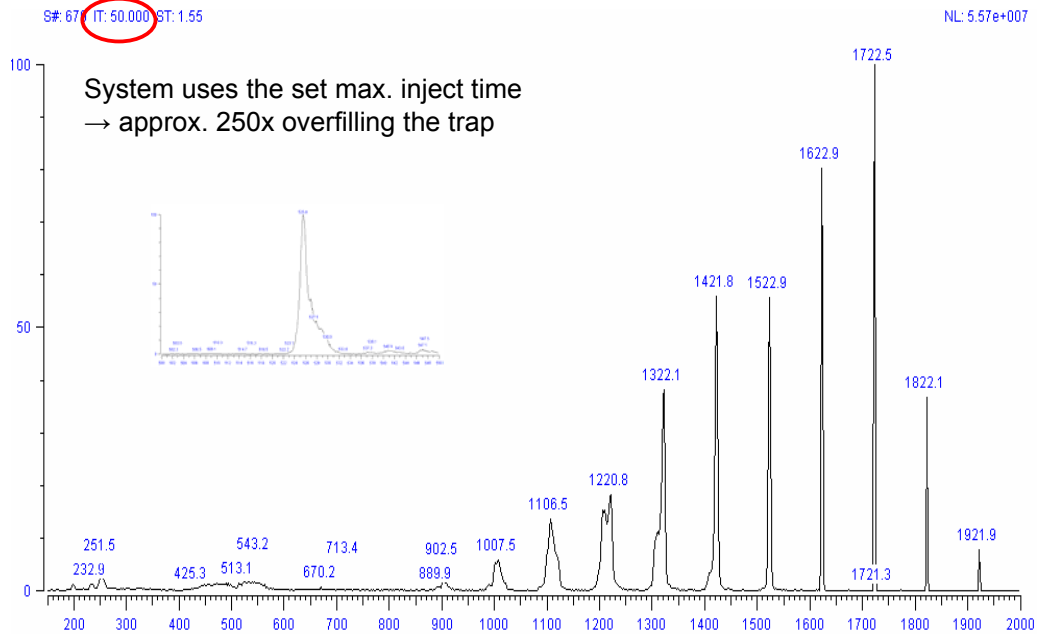
The software is used to set the ion injection time to maintain the optimum quantity of ions allowed in the trap for each scan. With AGC on, the scan function consists of a prescan and an analytical scan. The LCQ measures the flux of incoming ions during the prescan. This information allows the LCQ to determine the optimum ion injection time for the analytical scan. The ion injection time information is then used to scale the resulting values obtained by the analytical scan. AGC extends the dynamic range of the MS detector. With AGC on, the LCQ sets the ion injection time (up to a preset maximum) and thus determines the number of ions that enter the analyzer. With AGC off, the user sets the ion injection time, thus controlling the number of ions that enter the analyzer.

Spectrum of Calibration Mixture, AGC On



Injection time (IT)= 0.18 msec. The application of the automatically determined injection time (calculated during the AGC prescan) allows for enhanced resolution and more accurate m/z assignments throughout the entire mass range of interest, by not allowing the ion trap to “overcrowd” with ions, and hence to be subject to space charge distortion.

Spectrum of Calibration Mixture, AGC Off



Injection time (IT)= 50 msec. Note the loss of resolution and the m/z assignment shift, especially across the lower and medium range of the m/z scale. The space charge effects are affecting the higher m/z range to a much lesser extent. As ions leave the trap from low m/z range to high m/z range, the number of total ions in the trap decreases by the time the higher masses are ejected, making them, consequently, less prone to space charge distortion.

Calculation of Ion Time

$$\text{AGC Prescan Signal} = \text{Number of Ions} \times \text{Multiplier Gain} \times \text{Prescan Time}$$

Constant During Prescan

(3 x 10⁵ counts) (1 ms)

$$\text{Calculated Ion Time} = \frac{\text{Target Value}}{\text{ACG Prescan Signal}}$$

(how long the gate lens is "open")

The calculation above shows how the instrument determines the variable injection time for each analytical scan based on the prescan.

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

Corrected Ion Current (TIC)

$$I' \text{ (corrected ion current)} = I \left(\frac{\text{Prescan IT}}{\text{Actual IT}} \right)$$

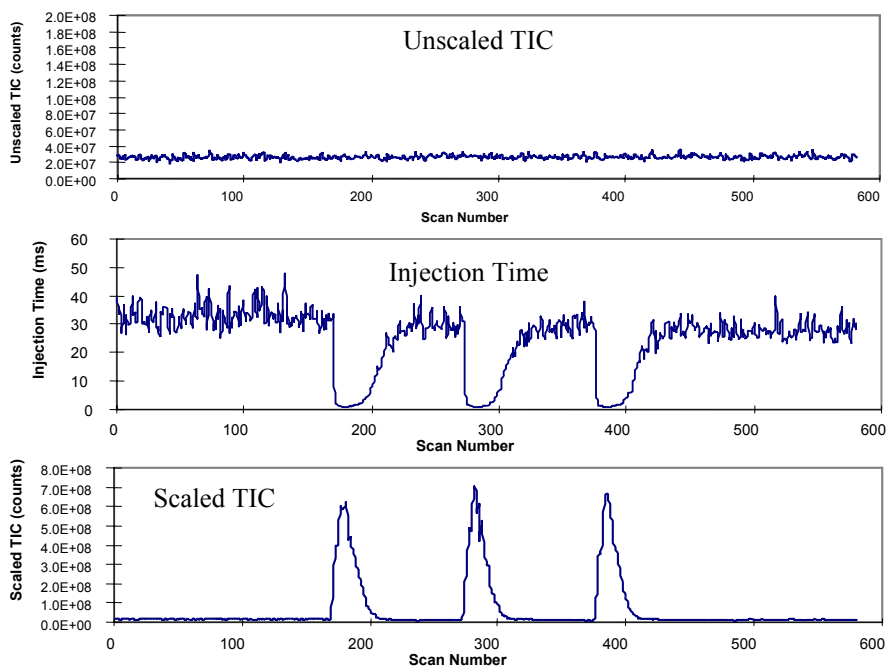
(Scaled TIC)

Example:

$$3.00 \times 10^8 = 3.00 \times 10^7 \left(\frac{1 \text{ ms}}{0.1 \text{ ms}} \right)$$

The equation used to calculate the scaled TIC value is shown above. Essentially, each scan signal (which is equal to the AGC target or the number of ions) is multiplied by the prescan IT and the inverse of the actual IT.

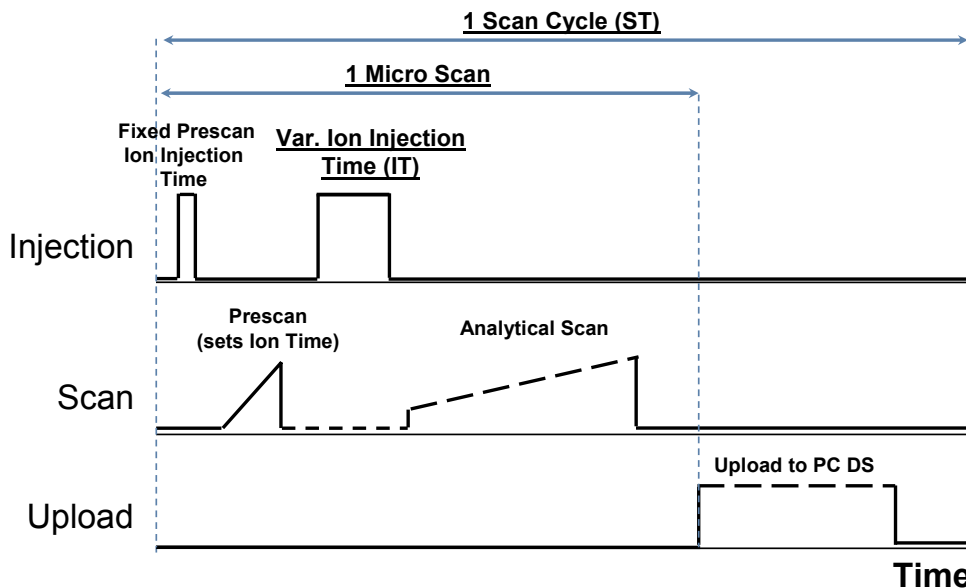
Triplicate Injection of 5 nmol of MRFA (AGC ON)



Due to AGC, the injection time decreases as the concentration of ions increases to limit the number of ions in the trap and prevent space charging. The scaled TIC is inversely related to the actual IT so after correction, the TIC seen represents the corrected ion current.

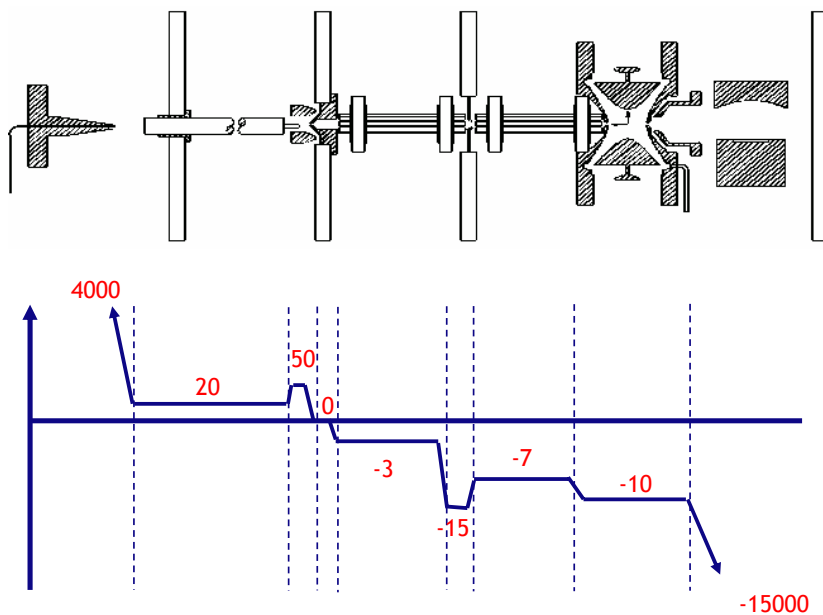
AGC Cycle – Full Scan MS Summary

Ion Time, Microscan, and Scan Cycle



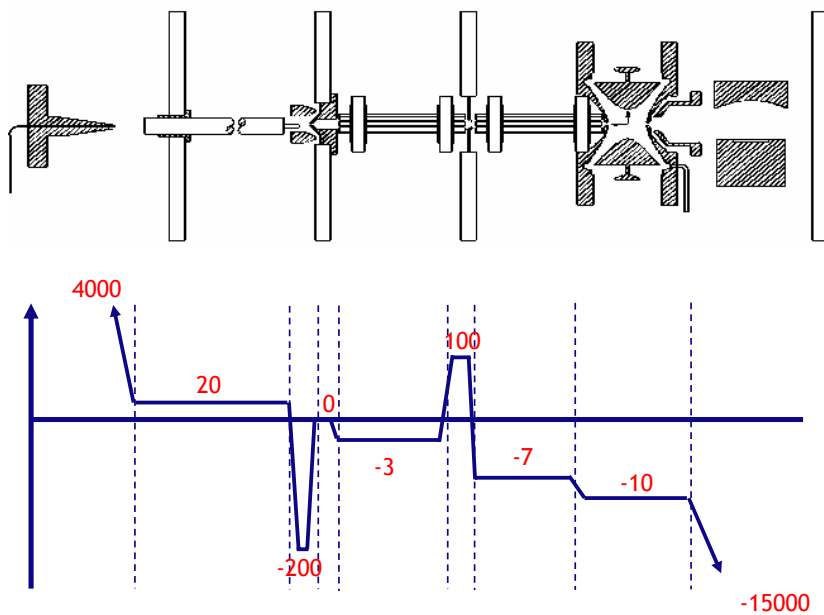
This diagram shows the LCQ in Full Scan MS mode with one microscan. The status of various elements of the LCQ during the scan is shown versus time.

Voltage Settings during Injection Time Trap Fills – ‘Gate Open’



The gate lens is used to start and stop the injection of ions into the mass analyzer. When open, it is an accelerating lens which accelerates ions towards the ion trap.

Voltage Settings during Injection Time Trap Fills – ‘Gate Closed’ (Scan Time)



When the gate lens is closed, the flow of ions is stopped electrostatically and ions are not allowed to move past the gate lens.

ThermoFisher
SCIENTIFIC

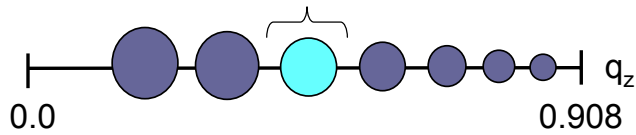
The world leader in serving science

Chapter 7

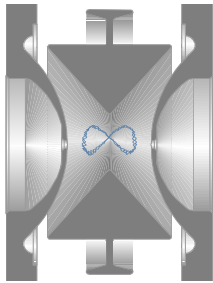
Isolation and Activation

Isolation of Ions

Ion we wish to isolate



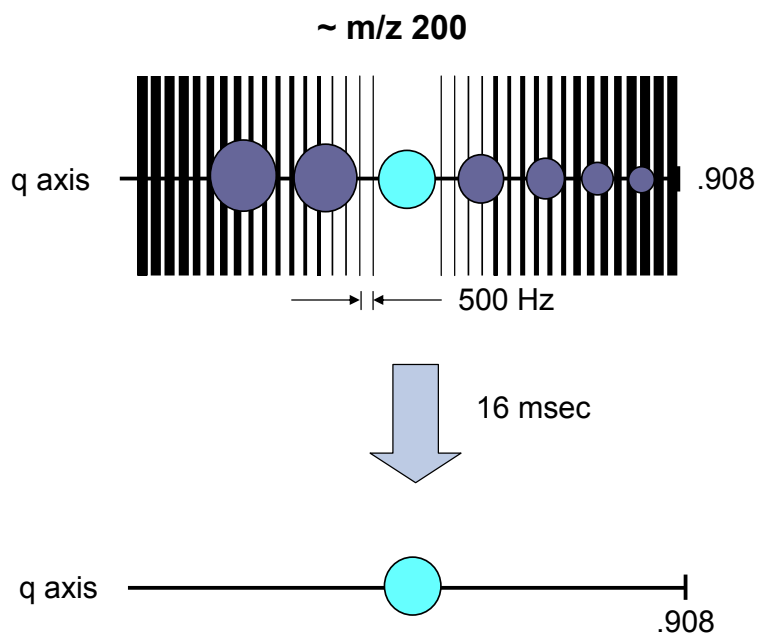
- Ions at different q_z values oscillate at different frequencies (ω_o)
- Each frequency corresponds to a unique, specific m/z value



$$\omega_o \approx \frac{q_z \Omega}{2\sqrt{2}}$$

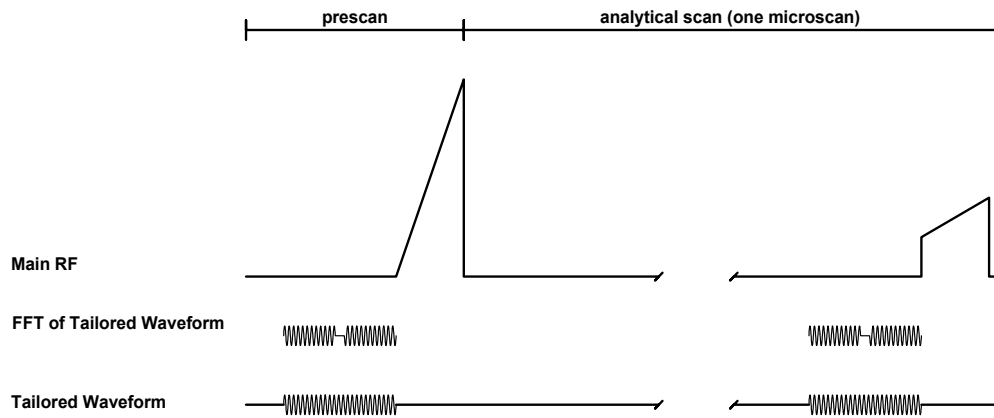
To isolate ions of a particular m/z , we take advantage of the fact that ions stored at different q values have different oscillatory frequencies in the ion trap.

Isolation Waveforms



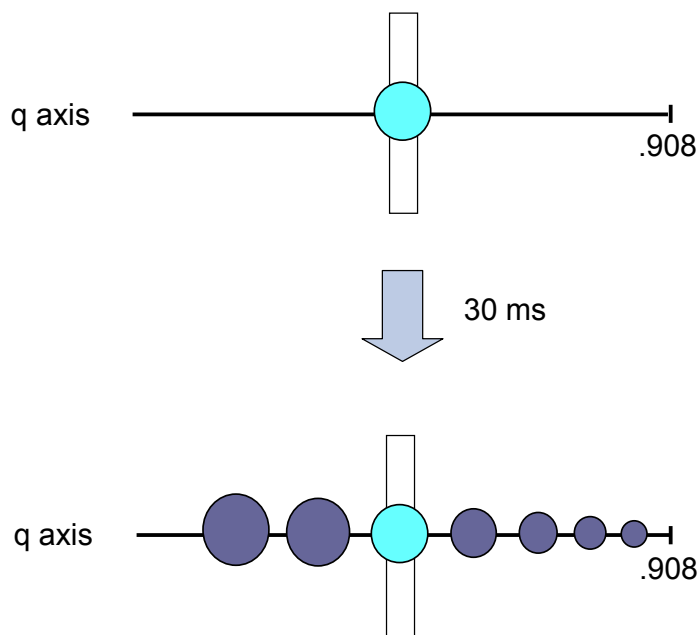
The ion isolation waveform voltage consists of a distribution of frequencies between 5 and 500 kHz containing all resonance frequencies except for those corresponding to the ions to be trapped. The ion isolation waveform voltage is applied to the endcaps, and in combination with the main RF voltage, ejects all ions except those of a selected mass-to-charge ratio or narrow ranges of mass-to-charge ratios.

Ion Isolation



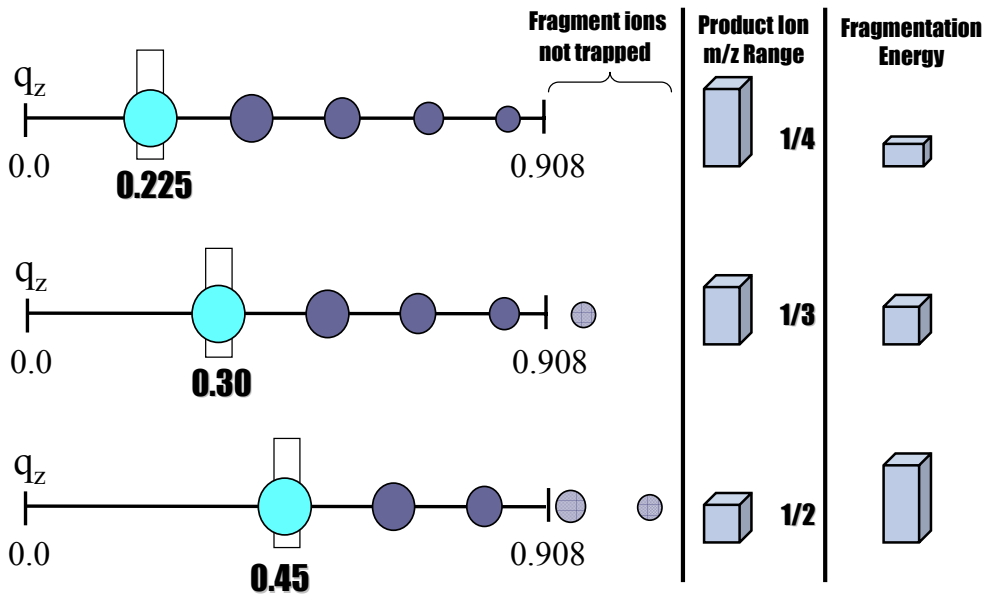
RF Power Applied at the Secular Frequencies of All Ions in the Trap BUT THE ION OF INTEREST Causes the Unwanted Ions to be Blown Out of the Trap !!!

Resonance Excitation (For Fragmentation)



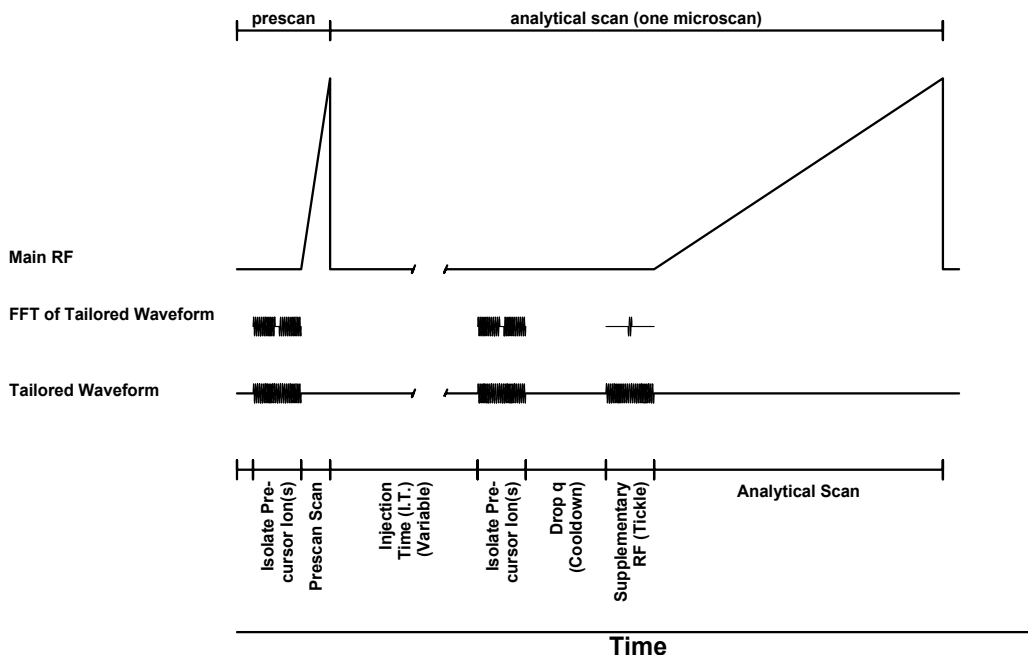
During the collision induced dissociation step of SRM, CRM, or MS_n ($n > 1$) full scan applications, the resonance excitation RF voltage is applied to the endcaps to fragment parent ions into product ions. The resonance excitation RF voltage is not strong enough to eject an ion from the mass analyzer. However, ion motion in the radial direction is enhanced and the ion gains kinetic energy. After many collisions with the helium dampening gas, which is present in the mass analyzer, the ion gains enough internal energy to cause it to dissociate into product ions. The product ions are then mass analyzed.

Resonant Excitation q_z Value



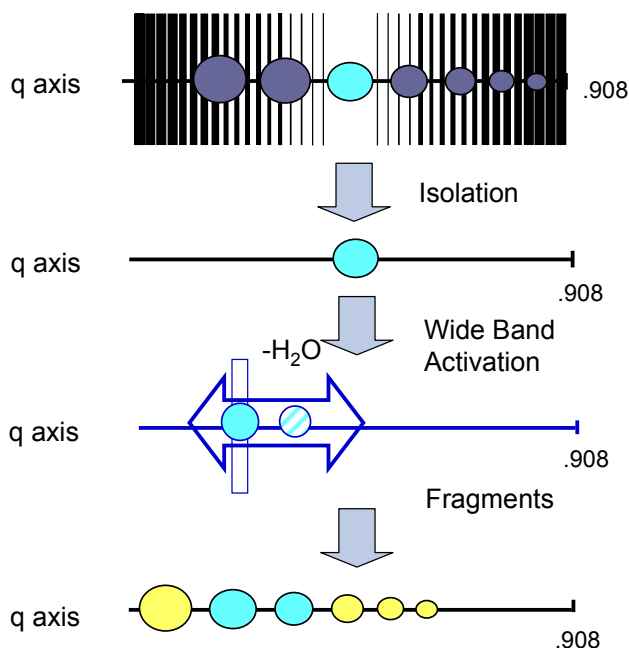
The q value ions are resonantly excited at influences the m/z range of product ions that can be trapped and the amount of kinetic energy which can be deposited. As the q value is increased, more kinetic energy is deposited and more fragmentation is observed. However, a narrower product ion m/z range results. High q values work well for fragmenting stable ions such as dioxins.

Ion Trap MS/MS Scan Function (MS²)



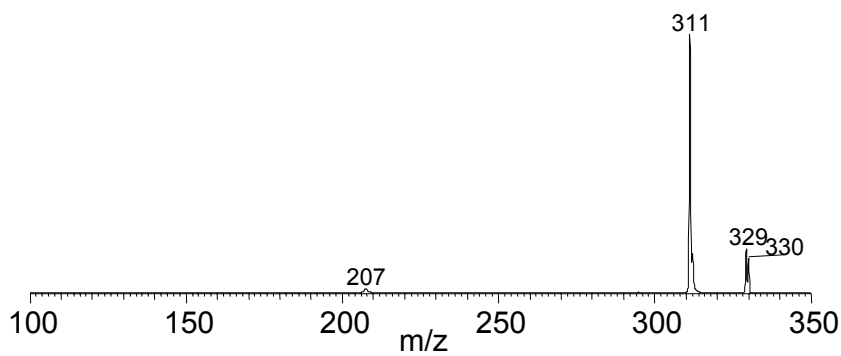
The ion trap MS² scan function starts with the isolation of a precursor ion and a prescan to assess the number of precursor ions present in the sample. Following the prescan, the precursor is isolated again and fragmented using supplementary RF applied to the endcap electrodes (tickle voltage). Finally, the main RF voltage is increased to scan out the product ion(s) to the detector.

Wideband Activation

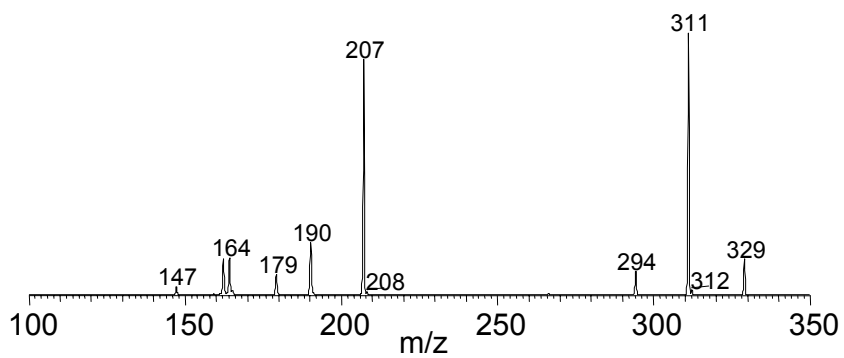


The Wideband Activation option allows the LCQ to apply collision energy to ions during MS/MS fragmentation over a fixed mass range of 20 u. This option allows the LCQ to apply collision energy to both the precursor ion, as well as to product ions created as a result of non-specific losses of water (18 u) or ammonia (17 u), for example, or to product ions formed from the loss of fragments less than 20 u. When you want enhanced structural information and you do not want to perform MS3 analysis with the LCQ, choose the Wideband Activation option for qualitative MS/MS. Because the collision energy is applied to a broad mass range, signal sensitivity is somewhat reduced when you choose this option. Therefore, increase the value of the collision energy (Activation Amplitude) to compensate somewhat for the reduction of sensitivity.

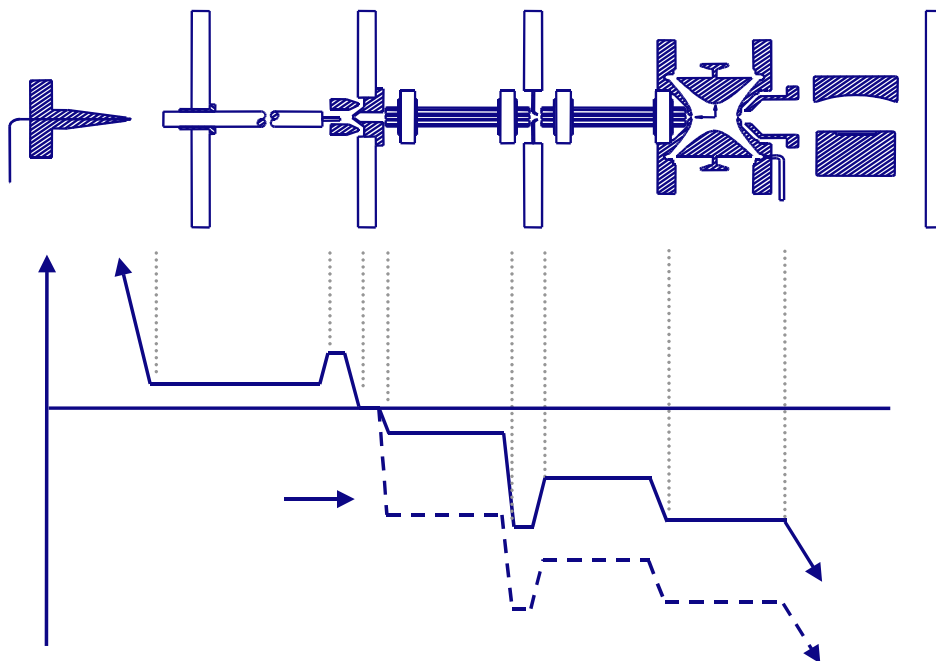
MS/MS of 329 m/z



Wideband Activation



“Source CID”



“Source CID” represents a supplemental DC voltage, added to the overall voltage gradient across the ion path throughout the instrument; it determines an additional acceleration of ions through the lowest vacuum region, inducing “preliminary”, rather non-selective, fragmentation. Employing “source CID” is beneficial when the user wants to reduce the “softness” of electrospray in that the break-up of adducts and clusters is triggered in order to increase sensitivity. “Source CID” may also be detrimental, if overestimated, as the energetically-enhanced collisions may fragment the ions of interest, reducing the sensitivity.

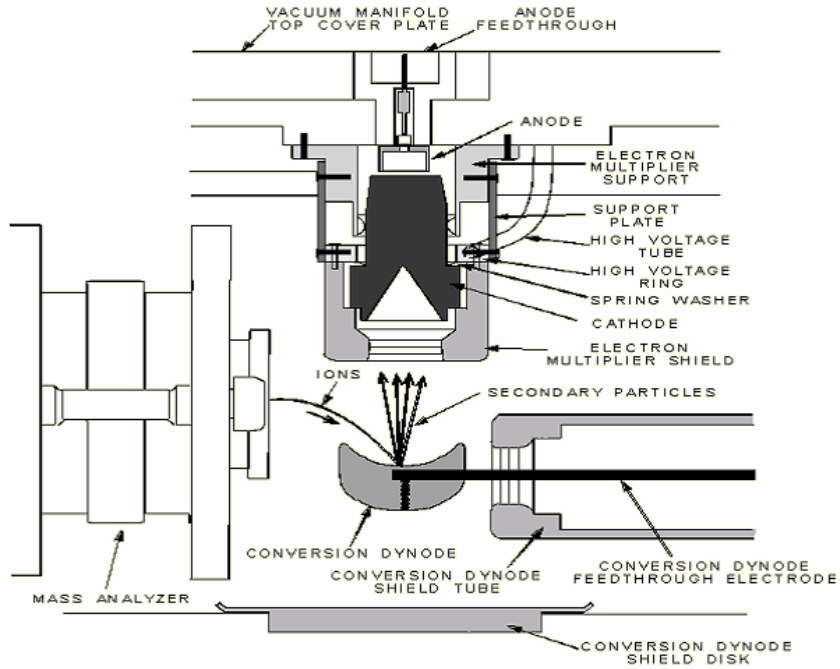
ThermoFisher
SCIENTIFIC

The world leader in serving science

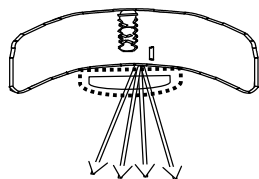
Chapter 8

Ion Detection and Summary

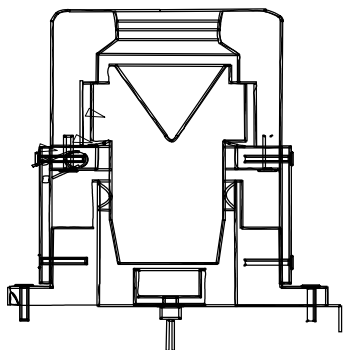
Ion Detection System



The Conversion Dynode



Positive (or negative) ions are pulled toward the conversion dynode. On impact, a proportional stream of opposite-charge particles, including electrons, are ejected.

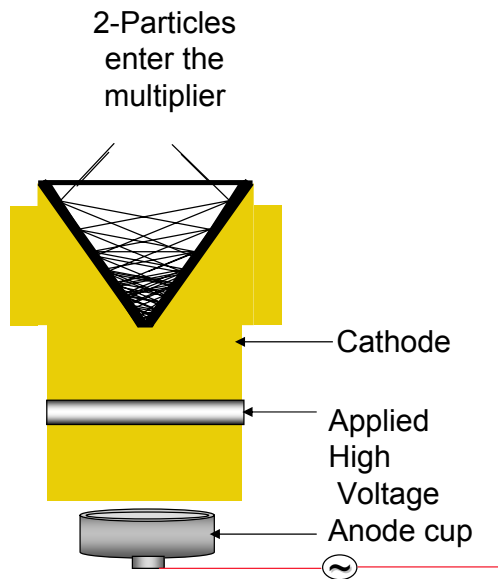


The conversion dynode operates at either of two set values:

- 15 kV (positive ion mode)
- +15 kV (negative ion mode)

The conversion dynode is a concave metal surface that is located at a right angle to the ion beam. A potential of +15 kV for negative ion detection or -15kV for positive ion detection is applied to the conversion dynode. When an ion strikes the surface of the conversion dynode, one or more secondary particles are produced. These secondary particles can include positive ions, negative ions, electrons, and neutrals. When positive ions strike a negatively charged conversion dynode, the secondary particles of interest are negative ions and electrons. When negative ions strike a positively charged conversion dynode, the secondary particles of interest are positive ions. These secondary particles are focused by the curved surface of the conversion dynode and are accelerated by a voltage gradient into the electron multiplier.

The Electron Multiplier



Each electron hits the surface of the multiplier resulting in the ejection of more electrons, according to a set amplification factor (“gain”).

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

This charge represents the signal produced by the ion.

Signal is transferred to data system.

The electron multiplier includes a cathode and an anode. The cathode of the electron multiplier is a lead-oxide, funnel-like resistor. The anode of the electron multiplier is a small cup located at the exit end of the cathode. The anode collects the electrons produced by the cathode. The anode screws into the anode feed through in the base plate.

Secondary particles from the conversion dynode strike the inner walls of the electron multiplier cathode with sufficient energy to eject electrons. The ejected electrons are accelerated farther into the cathode, drawn by the increasingly positive potential gradient. Due to the funnel shape of the cathode, the ejected electrons do not travel far before they again strike the inner surface of the cathode, thereby causing the emission of more electrons. Thus, a cascade of electrons is created that finally results in a measurable current at the end of the cathode where the electrons are collected by the anode. The current collected by the anode is proportional to the number of secondary particles striking the cathode.

1) Trapping

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

1) Trapping

For Scans: SIM, MSⁿ

By: Endcap Electrodes

2) Isolation

Method: Tailored waveform applied to all ions in the trap except ion of interest. Thus, only ions of interest remain in the trap.

3) Excitation

4) Ejection

Summary (Ion Trap Functions)

1) Trapping

For Scans: MSⁿ

By: Endcap Electrodes

2) Isolation

Method: a) Cool ion of interest back to set q value (default = 0.25).

3) Excitation

b) Apply excitation in resonance with the set q value, activation time (default = 30 msec), and optimized activation amplitude.

4) Ejection

Summary (Ion Trap Functions)

1) Trapping

2) Isolation

3) Excitation

4) Ejection

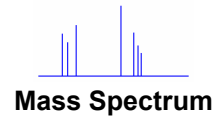
For Scans: All

By: Ring and Endcap Electrodes

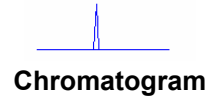
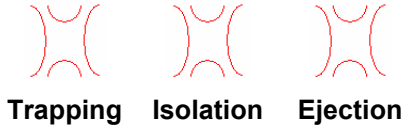
Method: Ramp the RF amplitude on the ring electrode in combination with a small AC voltage applied at a fixed frequency on the endcaps to consolidate the ions to a group (Resonance Ejection)

Experiments Available on the LCQ

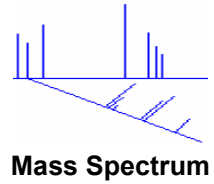
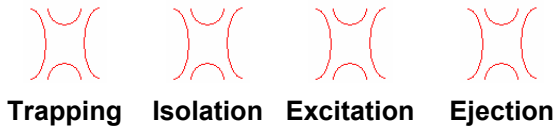
MS



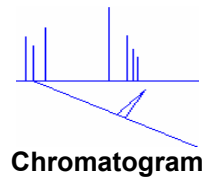
SIM



MS²



SRM



ThermoFisher
SCIENTIFIC

The world leader in serving science

Chapter 9

Tuning the LCQ Step-by-Step

First – Open a Relevant Tune File

Click to open

The screenshot shows the Thermo Tune Plus software interface. The 'File' menu is open, and the 'Open...' option is highlighted. A red arrow points to this option. Below the menu, a list of files is visible, including '1 C:\xcalbur...\Raw\angotune', '2 CALLTUNE', '3 315.3-iv1.5.ce30', '4 329.3-iv1.5.ce32', '5 363.3. -iv2.ce30', '6 C:\xcalbur...\359_iv2.5ce24', '7 APCILowFlow', '8 C:\xcalbur...\tunesteroid363', '9 416.3-iv3.0ce30', '0 455.3-iv1.5ce38', '1 C:\xcalbur...\281-iv1.5ce44', '2 C:\xcalbur...\237-iv1.5ce34', '3 C:\xcalbur...\386-iv1.5ce38', '4 C:\xcalbur...\300-iv1.5ce30', and '5 ESILowFlow'. A red arrow points to the 'Default_ESI' file in the 'Open' dialog box. The 'Open' dialog box shows the file name 'Default_ESI' and the file type 'Tune Files (*.LQTune)'. The 'Header Information' section of the dialog box contains the following text:

Created: Monday, May 16, 2005 2:47:56 PM
By: tester1, Logon ID: tester1
Times saved: 12
Last edited: Sunday, August 07, 2005 11:26:05 AM
By: tester1, Logon ID: eric.hemerway
Summary info:
Originator: Howard Tran

The main display area of the software shows a table of parameters with the following columns: 'All', 'User', and 'Value'. The table contains the following data:

Parameter	Value
pray Voltage (kV)	0.01
pray Current (µA)	0.19
Heath Gas Flow Rate:	-0.15
vac/Sweep Gas Flow Rate:	-0.16
apillary RTD OK:	Yes
apillary Voltage (V):	38.79
apillary Temp (°C):	150.00
Tube Lens (V, sp):	-30.00
vacuum	
vacuum OK:	Yes
in Gauge Pressure OK:	Yes
in Gauge:	0m
in Gauge (x10e-5 Torr):	0.67
ionvection pressure OK:	Yes
ionvection Gauge (Torr):	0.69
ibo Pump	
status:	Running
ife (hours):	10839
speed (rpm):	45000
power (Watts):	45
temperature (°C):	46.00
Optics	
ultrapole Frequency On:	Yes
ultrapole 1 Offset (V):	-11.20
ens Voltage (V):	-40.18
ultrapole 2 Offset (V):	-12.81
ultrapole RF Amp (Vp-p, sp):	400.00
entrance Lens Voltage (V):	-107.00
coarse Trap DC Offset (V):	-10.18
fine Trap DC Offset (V):	-10.21
analyzer Temperature (°C):	25.05
in RF	
reference Sine Wave OK:	Yes
standing Wave Ratio OK:	Yes
am RF DAC (steps):	63.00
am RF Detected (V):	0.01
F Detector Temp (°C):	34.33
am RF Modulation (V):	0.05
am RF Amplifier (Vp-p):	9.21
F Generator Temp (°C):	27.29
Ion Detection System	
Conversion Dynode:	Off

Take the Instrument Out of Standby Mode

Click to take out of standby

The screenshot shows the ThermoFisher Tune Plus software interface. The main window displays a large empty area with the text "No data has been received." A red arrow points from a yellow callout box to a green power button icon in the top toolbar. The right-hand panel shows a list of instrument parameters and their values.

Label	Value
ESI Source	
Ispray Voltage (kV)	0.02
Spray Current (µA)	0.24
Sheath Gas Flow Rate:	-0.00
Aux/Sweep Gas Flow Rate:	0.00
Capillary RTD OK:	Yes
Capillary Voltage (V)	15.15
Capillary Temp (°C)	205.40
Tube Lens (V, sp)	30.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	0
Ion Gauge (x10e-5 Torr):	0.69
Convectron pressure OK:	Yes
Convectron Gauge (Torr):	0.66
Turbo Pump	
Status:	Running
Life (hours):	10839
Speed (rpm):	45000
Power (Watts):	45
Temperature (°C):	46.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Dfset (V):	-5.42
Lens Voltage (V):	-16.47
Multipole 2 Dfset (V):	-7.17
Multipole RF Amp (V-p-p, sp):	400.00
Entrance Lens Voltage (V):	-60.53
Coarse Trap DC Dfset (V):	-10.40
Fine Trap DC Dfset (V):	-10.16
Analyzer Temperature (°C):	24.60
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	95.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.28
Main RF Modulation (V):	0.05
Main RF Amplifier (V-p-p):	8.97
RF Generator Temp (°C):	27.10
Ion Detection System	
Conversion Dynode:	Off

Set instrument to On/Standby mode

CAP INUM | 11/28/2006 | 11:00 AM

Turn on Syringe Pump to Infuse Sample

1. Click to open

2. Set flow rate and syringe type

Syringe Pump

Flow Control

On Flow Rate (µL/min): 5.00 Actual: 3.00

Off

Type

Hamilton Volume (µL): 250

Unimetrics Syringe ID (mm): 2.300

Other

Apply OK Cancel Help

Label	Value
ESI Source	
ISpray Voltage (kV):	5.00
Spray Current (µA):	0.10
Sheath Gas Flow Rate:	34.52
Aux/Sweep Gas Flow Rate:	0.11
Capillary RTD OK:	Yes
Capillary Voltage (V):	15.51
Capillary Temp (°C):	261.50
Tube Lens (V, sp):	30.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	On
Ion Gauge (x10e-5 Torr):	0.87
Convectron pressure OK:	Yes
Convectron Gauge (Ton):	0.65
Turbo Pump	
Status:	Running
Life (hours):	10839
Speed (rpm):	45000
Power (Watts):	43
Temperature (°C):	46.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-4.98
Lens Voltage (V):	-16.03
Multipole 2 Offset (V):	-6.81
Multipole RF Amp (Vpp, sp):	400.00
Entrance Lens Voltage (V):	-60.09
Coarse Trap DC Offset (V):	-10.25
Fine Trap DC Offset (V):	-10.21
Analyzer Temperature (°C):	24.71
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (step):	63.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.28
Main RF Modulation (V):	0.05
Main RF Amplifier (Vpp):	8.37
RF Generator Temp (°C):	27.44
Ion Detection System	
Conversion Dynode:	On

Turn on the Flow Rate from the LC Pump

1. Click to open

2. Set solvent composition and flow rate

3. Click to start pump

Inlet Direct Control

Surveyor MS Pump | Surveyor AS

Direct Control Panel

Solvents Proportions (%) and Flow Rate

A: 100 50% C: 0 0%

B: 0 50% D: 0 0%

Flow Rate, $\mu\text{l}/\text{min}$: 0 200

Pressure Status

Pressure, bar: 76.4 SD, %: 17.6

Run Time Status

RunTime, min: 0.15

Label	Value
ESI Source	
ISpray Voltage (kV)	5.00
Spray Current (μA)	0.10
Sheath Gas Flow Rate	34.67
Aux/Sweep Gas Flow Rate	-0.16
Capillary RTD OK:	Yes
Capillary Voltage (V)	15.51
Capillary Temp ($^{\circ}\text{C}$)	278.80
Tube Lens (V, sp)	30.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	On
Ion Gauge ($\times 10^{-5}$ Torr)	0.65
Convectron pressure OK:	Yes
Convectron Gauge (Torr)	0.59
Turbo Pump	
Status:	Running
Life (hours):	10839
Speed (rpm):	45000
Power (Watts):	43
Temperature ($^{\circ}\text{C}$):	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-4.91
Lens Voltage (V):	-10.03
Multipole 2 Offset (V):	-6.74
Multipole RF Amp (V _{p-p} , sp)	400.00
Entrance Lens Voltage (V):	-60.09
Coarse Trap DC Offset (V):	-10.18
Fine Trap DC Offset (V):	-10.22
Analyzer Temperature ($^{\circ}\text{C}$):	25.24
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	31.00
Main RF Detected (V):	0.01
RF Detector Temp ($^{\circ}\text{C}$):	34.57
Main RF Modulation (V):	0.05
Main RF Amplifier (V _{pp}):	6.97
RF Generator Temp ($^{\circ}\text{C}$):	30.37
Ion Detection System	
Conversion Dynode:	On

Check Source Parameters

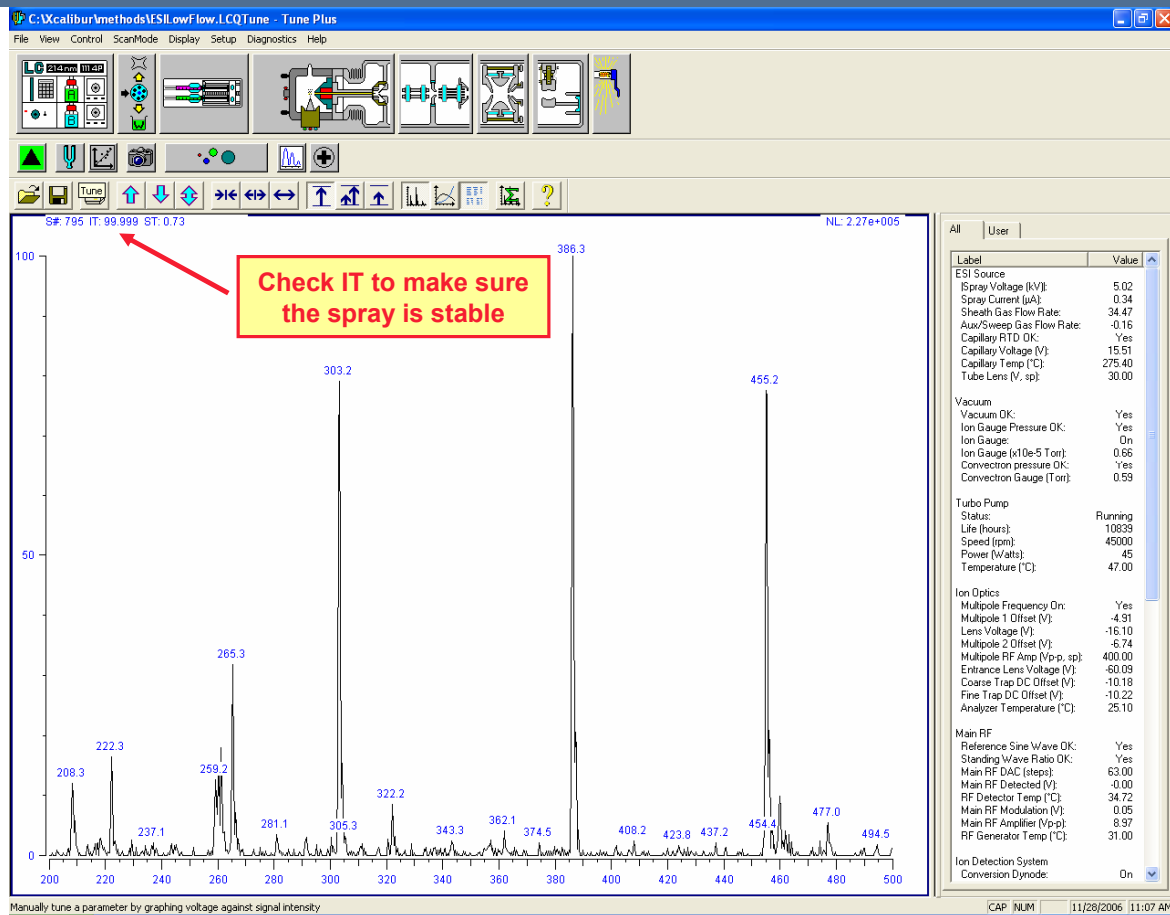
1. Click to open

The gas flows or spray voltage may need to be adjusted before starting the tuning process

Parameter	Value	Actual
Sheath Gas Flow Rate (arb)	40	40.00
Aux Gas Flow Rate (arb)	0	-0.04
Sweep Gas Flow Rate (arb)	0	-0.01
Spray Voltage (kV)	4.00	4.02
Spray Current (µA)		5.06
Capillary Temp (°C)	275.00	274.60
Capillary Voltage (V)	35.00	34.92
Tube Lens (V)	110.00	110.09

Label	Value
ESI Source	
ISpray Voltage	
Spray Current	
Sheath Gas F	
Aux/Sweep G	
Capillary RTD	
Capillary Volt	
Capillary Temp	
Tube Lens (V)	
Vacuum	
Vacuum OK:	
Ion Gauge Pre	
Ion Gauge:	
Ion Gauge (v1)	
Convection pr	
Convection G	
Turbo Pump	Running
Status:	
Life (hours):	10839
Speed (rpm):	45000
Power (watts):	45
Temperature (°C):	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-4.38
Lens Voltage (V):	-16.10
Multipole 2 Offset (V):	-6.74
Multipole RF Amp (Vpp, spl)	400.00
Entrance Lens Voltage (V):	-60.09
Coarse Trap DC Offset (V):	-10.18
Fine Trap DC Offset (V):	-10.22
Analyzer Temperature (°C):	25.00
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	63.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.67
Main RF Modulation (V):	0.05
Main RF Amplifier (Vpp):	6.97
RF Generator Temp (°C):	31.10
Ion Detection System	
Conversion Dynode:	On

Check that the Injection Time (IT) is Stable



Open the Define Scan Dialog Box and Modify Parameters

The screenshot shows the ThermoFisher Tune Plus software interface. A mass spectrum plot is visible in the background with peaks at 386.3 and 384.7. The 'Define Scan (Advanced)' dialog box is open, showing various parameters for scan configuration. Three red callout boxes provide instructions:

- 1. Click to open**: Points to the 'Define Scan' icon in the software toolbar.
- 2. Change microscans to 3 and increase max. inject time**: Points to the 'Number of Microscans' and 'Maximum Inject Time (ms)' fields.
- 3. Narrow scan ranges to range of interest**: Points to the 'Scan Ranges (m/z)' table.

From Mass	To Mass
1105.00	400.00

Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q	Activation Time (msec)
1	0.0	0.250	30.000	

Parameter	Value
Turn On	<input type="checkbox"/>
Collision Energy (V)	25.0

Parameter	Value
Number of Microscans	1
Maximum Inject Time (ms)	400.00

Parameter	Value
Mass Range	Normal
Scan Mode	MS
Scan Type	Full

Open the Tune Dialog Box

1. Click to open

The screenshot displays the Thermo Scientific Tune Plus software interface. The main window shows a mass spectrum plot with several peaks labeled with their m/z values: 208.3, 222.3, 235, 455.1, 477.1, and 499.6. The 'Tune' dialog box is open, and the 'Automatic' tab is selected. The 'What to Optimize On' section has 'Mass (m/z)' selected with a value of 386.3. The 'Status' section is empty. The background shows a mass spectrum plot with several peaks labeled with their m/z values: 208.3, 222.3, 235, 455.1, 477.1, and 499.6. A status table on the right lists various instrument parameters like ESI Source, Vacuum, Turbo Pump, and Ion Optics.

Label	Value
ESI Source	
ISpray Voltage (kV)	5.01
Spray Current (uA)	0.24
Sheath Gas Flow Rate	34.72
Aux/Sweep Gas Flow Rate	-0.16
Capillary RTD OK:	Yes
Capillary Voltage (V)	15.59
Capillary Temp (°C)	275.00
Tube Lens (V, sp)	30.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	On
Ion Gauge (x10e-5 Torr)	0.66
Convection pressure OK:	Yes
Convection Gauge (Torr)	0.59
Turbo Pump	
Status:	Running
Life (hours)	10839
Speed (rpm)	45000
Power (Watts)	43
Temperature (°C)	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V)	-4.98
Lens Voltage (V)	-16.10
Multipole 2 Offset (V)	-6.74
Multipole RF Amp (Vpp, sp)	400.00
Entrance Lens Voltage (V)	-60.09
Coarse Trap DC Offset (V)	-10.18
Fine Trap DC Offset (V)	-10.22
Analyzer Temperature (°C)	25.15
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps)	63.00
Main RF Detected (V)	0.01
RF Detector Temp (°C)	34.62
Main RF Modulation (V)	0.04
Main RF Amplifier (Vpp)	9.37
RF Generator Temp (°C)	30.52
Ion Detection System	
Conversion Dynode:	On

Automatic Tuning - Record Which Optics in the Graph View do not Give an Optimum Voltage

1. Click to open graph view

2. Type mass of ion

3. Click start

Tune
Automatic | Semi-Automatic | Manual | Collision Energy

What to Optimize On

Base Peak

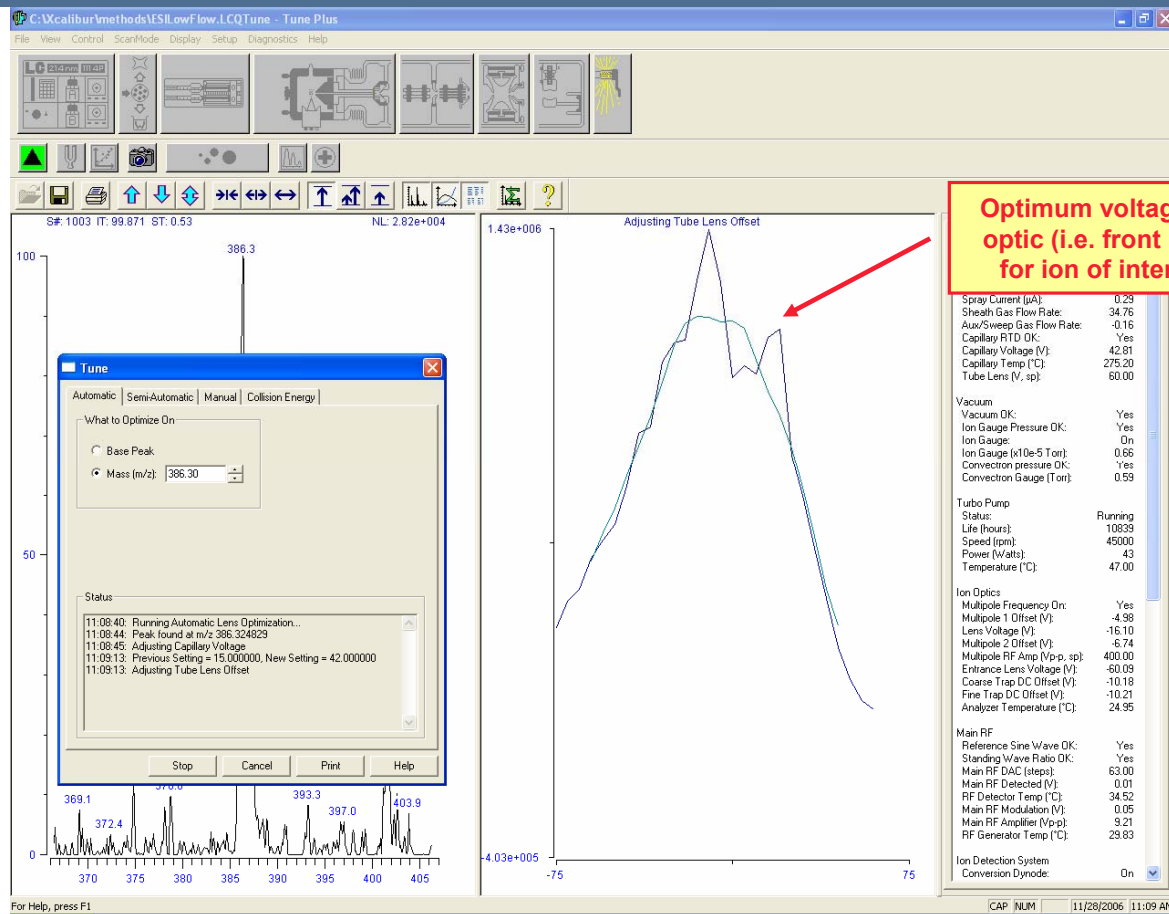
Mass (m/z): 386.3

Status

Start | Cancel | Print | Help

Label	Value
ESI Source	
ISpray Voltage (kV):	5.00
Spray Current (µA):	0.14
Sheath Gas Flow Rate:	34.96
Aux/Sweep Gas Flow Rate:	0.05
Capillary RTD OK:	Yes
Capillary Voltage (V):	15.07
Capillary Temp (°C):	275.10
Tube Lens (V, sp):	30.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	0in
Ion Gauge (x10e-5 Torr):	0.69
Convection pressure OK:	Yes
Convection Gauge (Torr):	0.57
Turbo Pump	
Status:	Running
Life (hours):	10839
Speed (rpm):	45000
Power (Watts):	43
Temperature (°C):	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-5.42
Lens Voltage (V):	-16.54
Multipole 2 Offset (V):	-7.17
Multipole RF Amp (Vp-p, sp):	400.00
Entrance Lens Voltage (V):	-60.46
Coarse Trap DC Offset (V):	-10.40
Fine Trap DC Offset (V):	-10.17
Analyzer Temperature (°C):	25.00
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	63.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.67
Main RF Modulation (V):	0.05
Main RF Amplifier (Vpp):	8.97
RF Generator Temp (°C):	30.27
Ion Detection System	
Conversion Dynode:	On

Automatic Tuning



Semi-Automatic Tuning - Change the Voltage Range to Optimize the Voltage for Each Optic

1. Choose which optics did not give an optimum in automatic tuning

2. Type mass of ion

3. Click start

Optimize the voltage range to get an optimum voltage for each optic

Tune

Automatic **Semi-Automatic** Manual Collision Energy

What to Optimize

Capillary voltage (V)

Show Advanced Settings

Optimization Range

Start: -140.00

End: 140.00

Step: 3.00

What to Optimize On

Base Peak

Mass (m/z): 386.30

Results

Initial Setting: 26.00

Best Setting: 0.00

Status

10:40:08: Previous Setting = -4.750000, New Setting = -4.500000
10:40:08: Adjusting Lens 1
10:40:17: Previous Setting = -38.000000, New Setting = -8.000000
10:40:17: Adjusting Gate Lens
10:40:24: Previous Setting = -34.000000, New Setting = -34.000000
10:40:24: Adjusting Front Lens
10:40:38: Previous Setting = -5.250000, New Setting = -5.250000
10:40:38: Optimization Complete -- change in signal = 78.927797%

ESI Source

Label	Value
ISpray Voltage (kV)	5.01
Spray Current (µA)	0.24
Spray Gas Flow Rate (L/min)	34.67
Sheath Gas Flow Rate (L/min)	-0.16
Vacuum	Yes
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	On
Ion Gauge (x10e-5 Torr)	0.66
Convectron pressure OK:	Yes
Convectron Gauge (Torr)	0.58
Turbo Pump	Running
Status:	10839
Life (hours):	45000
Speed (rpm):	45
Power (Watts):	47.00
Temperature (°C):	
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-3.95
Lens Voltage (V):	-78.31
Multipole 2 Offset (V):	-15.30
Multipole RF Amp (V-p, sp):	400.00
Entrance Lens Voltage (V):	-86.29
Coarse Trap DC Offset (V):	-10.18
Fine Trap DC Offset (V):	-10.21
Analyzer Temperature (°C):	25.15
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	63.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.57
Main RF Modulation (V):	0.05
Main RF Amplifier (V-p):	8.97
RF Generator Temp (°C):	29.59
Ion Detection System	
Conversion Dynode:	On

For Help, press F1

CAP. NUM | 11/28/2006 11:11 AM

Semi-Automatic Tuning

The screenshot displays the ThermoFisher Tune Plus software interface. The main window shows a mass spectrum plot with a peak at m/z 386.30. The 'Tune' dialog box is open, showing the 'Semi-Automatic' tab. The 'What to Optimize' section has 'Capillary voltage (V)' selected. The 'Optimization Range' is set from -132.00 to 132.00 V with a step of 5.00 V. The 'What to Optimize On' section has 'Mass (m/z)' selected at 386.30. The 'Results' section shows an initial setting of 45.00 and a best setting of 28.00. The 'Status' section shows the current setting is 28.000000. An 'Accept Optimized Value' dialog box is overlaid on the plot, with a red arrow pointing to the 'Accept' button. The dialog box contains the text: 'Semi-Automatic optimization is done. The new value is 28.00. Accept it?'. The right-hand panel shows system status information, including 'ESI Source' parameters, 'Vacuum' status, 'Turbo Pump' status, and 'Ion Optics' parameters.

Label	Value
ESI Source	
ISpray Voltage (kV):	5.01
Spray Current (µA):	0.10
Sheath Gas Flow Rate:	34.67
Aux/Sweep Gas Flow Rate:	-0.11
Capillary RTD OK:	Yes
Capillary Voltage (V):	28.69
Capillary Temp (°C):	275.00
Tube Lens (V, sp):	-5.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	On
Ion Gauge (x10 ⁻⁵ Torr):	0.66
Convection pressure OK:	Yes
Convection Gauge (Torr):	0.58
Turbo Pump	
Status:	Running
Life (hours):	10839
Speed (rpm):	45000
Power (Watts):	43
Temperature (°C):	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-6.15
Lens Voltage (V):	-60.24
Multipole 2 Offset (V):	-11.27
Multipole RF Amp (V/p.p. sp):	400.00
Entrance Lens Voltage (V):	-56.06
Coarse Trap DC Offset (V):	-10.10
Fine Trap DC Offset (V):	-10.21
Analyzer Temperature (°C):	25.39
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	31.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.57
Main RF Modulation (V):	0.05
Main RF Amplifier (V/p.p.):	8.97
RF Generator Temp (°C):	28.86
Ion Detection System	
Conversion Dynode:	On

Manual Tuning - Monitor the Effect of Changing Source Parameters on Signal Intensity

The screenshot displays the ThermoFisher Tune Plus software interface. The main window is titled "Tune" and has tabs for "Automatic", "Semi-Automatic", "Manual", and "Collision Energy". The "Manual" tab is selected and circled in red. Under "What to Graph", the "Mass(es) (m/z)" option is selected with a radio button. Four mass values are listed in a table:

Mass(es) (m/z)
1 <input checked="" type="checkbox"/> 237.10
2 <input checked="" type="checkbox"/> 281.20
3 <input checked="" type="checkbox"/> 386.30
4 <input checked="" type="checkbox"/> 455.3

A red bracket groups these four rows, with a callout box containing the text: "1. Enter mass(es) (Can tune up to 4 masses at once)". Below the table is a "Status" window showing the following text:

```
10:41:56: Adjusting Capillary Voltage  
10:42:16: Previous Setting = 26.000000, New Setting = 52.586071
```

At the bottom of the "Tune" window, there are buttons for "Start", "Cancel", "Print", and "Help". A red arrow points to the "Start" button, with a callout box containing the text: "2. Click start". The background shows a mass spectrum plot with a peak at approximately 455 m/z. On the right side of the interface, there is a "User" panel with a list of system parameters and their values, including ESI Source, Vacuum, Turbo Pump, Ion Optics, Main RF, and Ion Detection System.

Manual Tuning

1. Click to open

2. Change source parameters to get the best signal intensity for ion(s) of interest

Parameter	Setpoint	Actual
Sheath Gas Flow Rate (arb)	35	34.62
Aux/Sweep Gas Flow Rate (arb)	0	-0.16
I Spray Voltage (kV)	5.00	5.00
Spray Current (µA)		0.14
Capillary Temp (°C)	275.00	275.20
Capillary Voltage (V)	28.00	28.69
Tube Lens Offset (V)	-5.00	

Label	Value
ESI Source	
ISpray Voltage (kV)	5.00
Spray Current (µA)	0.14
Sheath Gas Flow Rate	34.62
Aux/Sweep Gas Flow Rate	-0.16
Capillary RTD OK	Yes
Capillary Voltage (V)	28.69
Capillary Temp (°C)	275.20
Tube Lens (V, sp)	-5.00
Vacuum	
Vacuum OK	Yes
Ion Gauge Pressure OK	Yes
Ion Gauge	0n
Ion Gauge (x10e-5 Torr)	0.06
Convectron pressure OK	Yes
Convectron Gauge (Torr)	0.58
Tube Base	
Running	10839
	44000
	43
	47.00
	Yes
	-6.22
	-60.24
	-11.27
	400.00
	-56.06
Loose Trap D/L Offset (V)	-10.18
Fine Trap D/L Offset (V)	-10.21
Analyzes Temperature (°C)	25.29
Main RF	
Reference Sine Wave OK	Yes
Standing Wave Ratio OK	Yes
Main RF DAC (steps)	31.00
Main RF Detected (V)	0.01
RF Detector Temp (°C)	34.57
Main RF Modulation (V)	0.04
Main RF Amplifier (V-p)	6.97
RF Generator Temp (°C)	29.44
Ion Detection System	
Conversion Dynode	On

Optimize the MS² Parameters (Isolation Width and Collision Energy)

1. Click to open

*MS² parameters are not saved so the optimum isolation width and collision energy must be recorded

Define Scan (Advanced)

Scan Description

Mass Range: Normal High Low

Scan Mode: MS MS/MS MSn

Scan Type: Full SRM ZoomScan

MSn Power: 2

TurboScan Wideband Activation

Scan Time

Number of Microscans: 2

Maximum Inject Time (ms): 400.00

Input Method: From/To Center/Width

Source Fragmentation

Turn On Collision Energy (V): 25.0

MSn Settings

Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q	Activation Time (msec)
1 386.50	5.0	0.0	0.250	30.000

Scan Ranges (m/z)

From Mass	To Mass
1 105.00	400.00

Apply OK Cancel Help

Find the Optimum Isolation Width

4. Watch the normalized level (NL) to see if there is a loss in signal intensity

***Goal: To find the narrowest isolation width while maintaining maximum signal intensity**

1. Enter mass

2. Decrease the isolation width (in steps)

3. Click to apply

Scan	Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q	Activation Time (msec)	From Mass	To Mass
1	386.50	5.0	0.0	0.250	30,000	1105.00	400.00

Label	Use
ESI Source	Yes
ISpray Vol	Yes
Spray Cur	Yes
Sheath G	Yes
Aux/Swee	Yes
Capillary F	Yes
Capillary V	Yes
Tube Len	Yes
Vacuum	Yes
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	0n
Ion Gauge (x10e-5 Torr):	0.06
Convectron pressure OK:	Yes
Convectron Gauge (Torr):	0.58
Turbo Pump	Running
Status:	Running
Life (hours):	10839
Speed (rpm):	45000
Power (Watts):	43
Temperature (°C):	47.00
Ion Optics	Yes
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-6.22
Lens Voltage (V):	-60.16
Multipole 2 Offset (V):	-11.27
Multipole RF Amp (V-p-p, sp):	400.00
Entrance Lens Voltage (V):	-55.99
Coarse Trap DC Offset (V):	-10.18
Fine Trap DC Offset (V):	-10.21
Analyzer Temperature (°C):	25.15
Main RF	Yes
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	63.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.52
Main RF Modulation (V):	0.05
Main RF Amplifier (V-p-p):	8.57
RF Generator Temp (°C):	29.34
Ion Detection System	On
Conversion Dynode:	On

Apply Collision Energy to the Isolated Ion

Define Scan (Advanced)

Scan Description

Mass Range: Normal High Low

Scan Mode: MS MS/MS MSn

Scan Type: Full SRM ZoomScan

MSn Power: 2

TurboScan Wideband Activation

Scan Time

Number of Microscans: 2

Maximum Inject Time (ms): 400.00

Input Method

From/To Center/Width

Source Fragmentation

Turn On Collision Energy (V): 25.0

MSn Settings

Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q	Activation Time (msec)
1 386.50	5.0	35.0	0.250	30.000

Scan Ranges (m/z)

From Mass	To Mass
1 105.00	400.00

Apply OK Cancel Help

Injection RF...
Scan Activation Params...

For Help, press F1

CAP NUM | 11/28/2006 11:21 AM

Automatically Optimize the Collision Energy

1. Click to open

2. Type mass of product ion

3. Click start

Collision Energy

What to Optimize On

TIC

Product Ion Mass (m/z) 122.0

Results

Initial Collision Energy: 35.00 %

Best Collision Energy: N/A %

Status

Start Cancel Print Help

Label	Value
ESI Source	
ISpray Voltage (kV)	5.00
Spray Current (µA)	0.14
Sheath Gas Flow Rate:	34.67
Aux/Sweep Gas Flow Rate:	-0.15
Capillary FID OK:	Yes
Capillary Voltage (V):	28.69
Capillary Temp (°C):	275.00
Tube Lens (V, sp):	-5.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	0n
Ion Gauge (x10e-5 Torr):	0.66
Convection pressure OK:	Yes
Convection Gauge (Torr):	0.58
Turbo Pump	
Status:	Running
Life (hours):	10833
Speed (rpm):	45000
Power (watts):	43
Temperature (°C):	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-6.22
Lens Voltage (V):	50.24
Multipole 2 Offset (V):	-11.27
Multipole RF Amp (V-p-p, sp):	400.00
Entrance Lens Voltage (V):	-55.99
Coarse Trap DC Offset (V):	-10.18
Fine Trap DC Offset (V):	-10.21
Analyzer Temperature (°C):	25.34
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	31.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.67
Main RF Modulation (V):	0.05
Main RF Amplifier (V-p-p):	8.97
RF Generator Temp (°C):	29.34
Ion Detection System	
Conversion Dynode:	On

Automatically Optimize the Collision Energy

Tune

Automatic | Semi-Automatic | Manual | Collision Energy

What to Optimize: Analyzer CID, Source CID

What to Optimize On: TIC, Product Ion Mass (m/z) 122.00

Results: Initial Collision Energy: 35.00 %, Best Collision Energy: 42.00 %

Status: 11:23:31: Optimizing Collision Energy...
11:24:01: Optimum relative collision energy for production of
11:24:01: m/z 122.000000 is 42.000000%
11:24:01: Efficiency = 10.736447%

Start | Cancel | Print | Help

Accept Optimized Value

Collision energy optimization is done.
The new value is 42.00. Accept it?

Accept | Reject | Help

Click to accept new value for collision energy

Label	Value
ESI Source	
Spray Voltage (kV)	5.00
Spray Current (µA)	0.10
Sheath Gas Flow Rate:	34.86
Aux/Sweep Gas Flow Rate:	0.05
Capillary RTD OK:	Yes
Capillary Voltage (V):	28.25
Capillary Temp (°C):	274.80
Tube Lens (V, sp):	-5.00
Vacuum	
Vacuum OK:	Yes
Ion Gauge Pressure OK:	Yes
Ion Gauge:	0 n
Ion Gauge:	0.63
Ion Gauge:	0.56
Running	
Speed (rpm):	10839
Power (Watts):	45
Temperature (°C):	47.00
Ion Optics	
Multipole Frequency On:	Yes
Multipole 1 Offset (V):	-6.66
Lens Voltage (V):	-60.68
Multipole 2 Offset (V):	-11.71
Multipole RF Amp (p-p, sp):	400.00
Entrance Lens Voltage (V):	-56.43
Coarse Trap DC Offset (V):	-10.47
Fine Trap DC Offset (V):	-10.17
Analyzer Temperature (°C):	25.58
Main RF	
Reference Sine Wave OK:	Yes
Standing Wave Ratio OK:	Yes
Main RF DAC (steps):	63.00
Main RF Detected (V):	0.01
RF Detector Temp (°C):	34.52
Main RF Modulation (V):	0.05
Main RF Amplifier (p-p):	8.57
RF Generator Temp (°C):	28.81
Ion Detection System	
Conversion Dynode:	On

Save the Tune File!

The screenshot shows the Thermo Tune Plus interface. At the top, the menu bar includes File, View, Control, ScanMode, Display, Setup, Diagnostics, and Help. The 'File' menu is open, with 'Save As...' selected. A red arrow points from the 'Save As...' menu item to the 'Save As' dialog box.

The 'Save As' dialog box is centered on the screen. It shows the following details:

- Save in: methods
- File name: Buspirone_386_IW_1.5_CE_35
- Save as type: Tune Files (*.LCQTune)
- Header Information: No file selected

In the background, a mass spectrum is visible with several peaks labeled with their retention times: 150.1, 179.8, 223.1, 222.0, 265.3, and 291.1. The x-axis represents retention time from 150 to 300, and the y-axis represents relative intensity from 0 to 50.

On the right side of the interface, there is a 'User' panel with a table of system parameters:

Label	Value
ESI Source	
ISpray Voltage (kV)	5.01
Spray Current (µA)	0.24
Sheath Gas Flow Rate	34.57
Aux/Sweep Gas Flow Rate	-0.11
Capillary RTD OK	Yes
Capillary Voltage (V)	28.69
Capillary Temp (°C)	275.00
Tube Lens (V, sp)	-5.00
Vacuum	
Vacuum OK	Yes
Ion Gauge Pressure OK	Yes
Ion Gauge	0n
Ion Gauge (x10e-5 Torr)	0.66
Convectron pressure OK	Yes
Convectron Gauge (Torr)	0.58
Turbo Pump	
Status	Running
Life (hours)	10833
Speed (rpm)	45000
Power (Watts)	43
Temperature (°C)	47.00
Ion Optics	
Multipole Frequency On	Yes
Multipole 1 Offset (V)	-6.22
Lens Voltage (V)	-60.24
Multipole 2 Offset (V)	-11.27
Multipole RF Amp (V-p, sp)	400.00
Entrance Lens Voltage (V)	-55.99
Coarse Trap DC Offset (V)	-10.18
Fine Trap DC Offset (V)	-10.22
Analyzer Temperature (°C)	25.00
Main RF	
Reference Sine Wave OK	Yes
Standing Wave Ratio OK	Yes
Main RF DAC (steps)	63.00
Main RF Detected (V)	0.01
RF Detector Temp (°C)	34.52
Main RF Modulation (V)	0.04
Main RF Amplifier (V-p)	8.74
RF Generator Temp (°C)	28.81
Ion Detection System	
Conversion Dynode	On

At the bottom of the window, the status bar shows 'CAP NUM 11/28/2006 11:28 AM'.

Acquiring a RAW File During Tuning

1. Click to open

The screenshot shows the 'Acquire Data' dialog box in the Tune Plus software. The dialog box contains the following fields and options:

- Folder: C:\Xcalibur\Data\
- File Name: Buspirone_386_MS2
- Sample Name: (empty)
- Comment: (empty)
- Use instrument method:
- Instrument Method: (empty)
- Start Mode: Immediate, Contact Closure, Divert Valve
- Acquire Time: Continuously, Scans (10), Minutes (2.50)
- Go to Standby when Finished:
- Acquisition Status: State: Idle, Time (min): 0.000

Buttons: Start, Pause, View..., Inst. Setup..., OK, Cancel, Help.

The background shows a mass spectrum plot with peaks at 148.0, 167.9, 179.9, 197.4, 223.2, 291.1, 343.6, and 386.3. The x-axis is labeled 'm/z' and the y-axis is labeled 'Intensity'.

The camera icon can be used to acquire raw files and run instrument methods directly from LCQ Tune

Typical Vacuum Settings

1. Click to open

Vacuum

Ion Trap

Ion Gauge On Off

Ion Gauge Pressure (E-5 Torr): 0.89

Convection Gauge Pressure (Torr): 0.96

Ion Trap Region (0.75 – 1.5 x 10⁻⁵)

API Stack Region (1.0 – 1.5 Torr)

Close

Help

Ion Detection System Conversion Dynode: On

Manually tune a parameter by graphing voltage against signal intensity

CAP NUM 11/28/2006 11:22 AM

ThermoFisher
S C I E N T I F I C

The world leader in serving science

Chapter 10

Xcalibur Software – Instrument Method Development

Thermo Software Standard

- TSQ Quantum Classic / Discovery / Discovery MAX / Ultra / Ultra AM / EMR
- LCQ^{Advantage} / LCQ^{Advantage} MAX / LCQ^{Deca} XP Plus / LCQ^{Duo} / LCQ^{Deca} / LCQ^{Classic}
- LXQ / LTQ / LTQ-FTMS / LTQ Orbitrap / MAT900XP / MAT900XP-Trap / MAT95XP / MAT95XP-Trap
- Tempus / PolarisQ (Polaris, GCQ)
- TraceDSQ
- TraceMS (Voyager, MD800)
- aQa (Navigator) / MSQ / MSQ+



Supported LC Peripherals

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

Xcalibur File Types

.raw	Acquired data files
.sld	Sequence setup files
.pmd	Processing Setup method
.meth	Instrument Setup method
.rst	Result files from Quantitation
.msp	Library search
.lyt	Qual Browser layout
.lqn	LCQuan files
.xqn	Quan Browser files
.xrt	XReport files

Instrument Configuration



Instrument Configuration

Device Types:
All Enable multi-user login

Available Devices:

- Agilent1100 AS
- Agilent1100 Bin
- Agilent1100 Capillary Pump
- Agilent1100 DAD
- Agilent1100 Heater

Configured Devices:

- LCQ Deca MS
- Surveyor AS
- Surveyor LC Pump
- Surveyor PDA

Buttons: Add >> << Remove Configure Done Help

Homepage – Status View

Roadmap - Home Page

File Actions View Tools GoTo Help

Status | Acquisition Queue

- Run Manager
 - Check Devices
 - Sequence:
 - Sample Name:
 - Working On:
 - Position:
 - Raw File:
 - Inst. Method:
- LOQ Decks MS
 - Initializing
- Surveyor AS
 - Initializing
- Surveyor LC Pump
 - Not Connected
- Surveyor PDA
 - Not Connected

Click to set up instrument method

ON-LINE
Instrument Setup

Sequence Setup

Processing Setup

Results Review

Qual Browser

Quan Browser

Library Browser

For Help, press F1

NUM 9/26/2007 8:58 PM

HPLC Method Setup

033104.meth - Instrument Setup

File Surveyor MS Pump Help

General Gradient Program

Solvent Descriptions

Solvent A Name:

Solvent B Name:

Solvent C Name: Water, 0.1% formic acid

Solvent D Name: Acetonitrile, 0.1% formic acid

Column Name:

Pressure Settings

Minimum Pressure:

Maximum Pressure:

Pressure Units: bar PSI

General Delivery Settings

Pumping Efficiency, % [80...120]:

Fractionations/Filling Stroke [1..4]:

Advanced Delivery Settings

Use custom stability limits

SD stability value, %:

Surveyor MS Pump

Help

Ready NOT SAVED

HPLC Method Setup

033104.meth - Instrument Setup

File Surveyor MS Pump Help

LCQ Deca

Surveyor AS

*Surveyor MS Pump

General **Gradient Program**

Gradient Table

	min	A%	B%	C%	D%	μ/min
0	0.00	0	0	95	5	150
1	30.00	0	0	30	70	150
2	40.00	0	0	0	100	150
3	45.00	0	0	0	100	150
4	45.10	0	0	95	5	150
5	60.00	0	0	95	5	150
6		100	0	0	0	150

Surveyor MS Pump

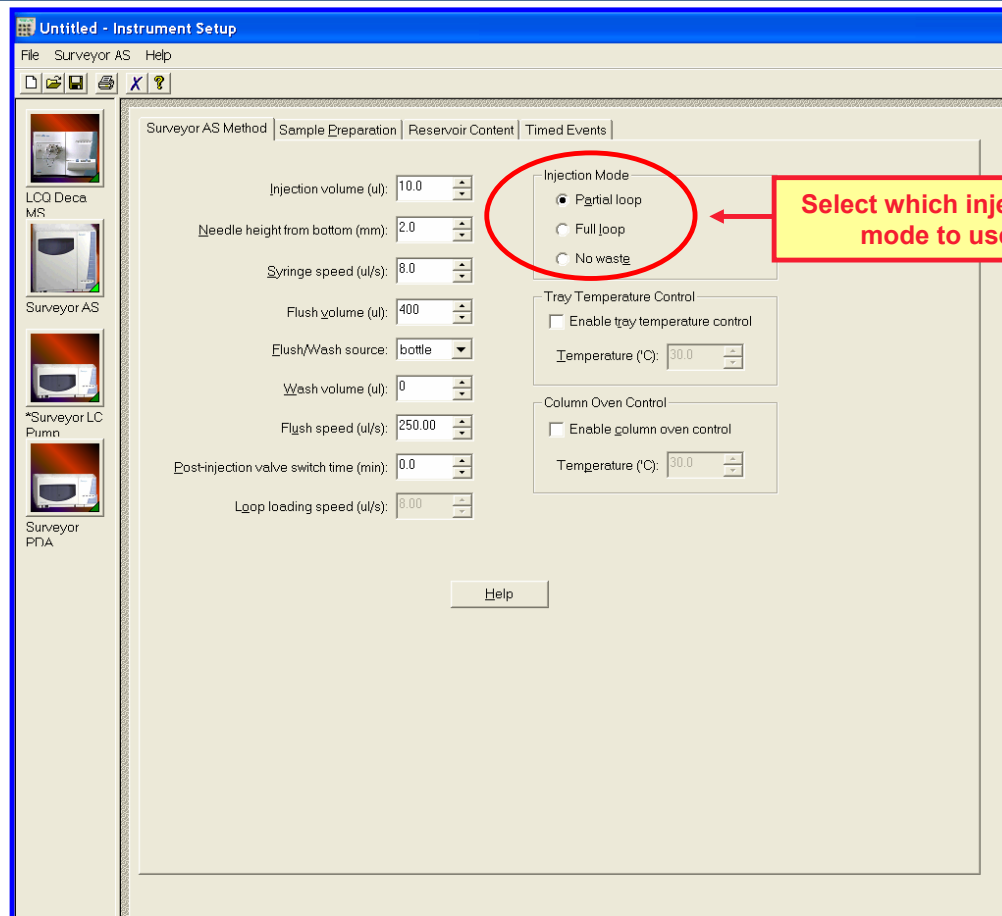
Gradient Profile

Help

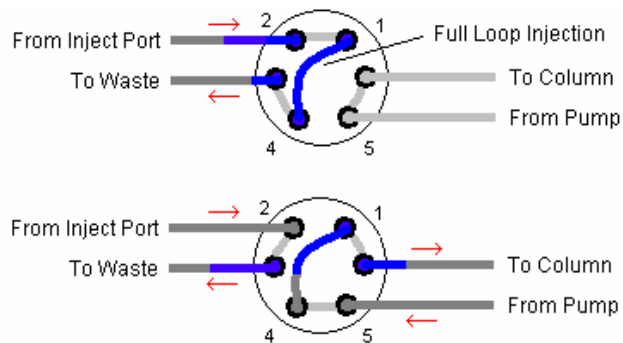
Ready

NOT SAVED

Autosampler Setup



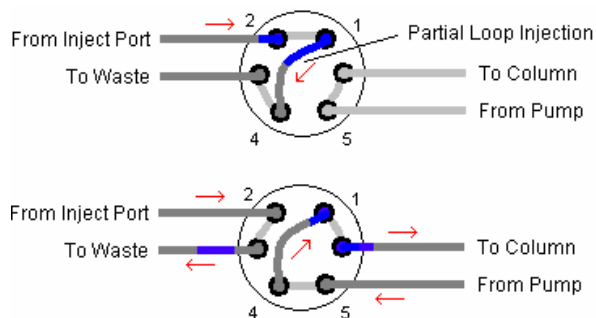
Full Loop Injection



Volume Pulled = 3 x Injection Volume + Excess volume
The excess volume is ~26 ul

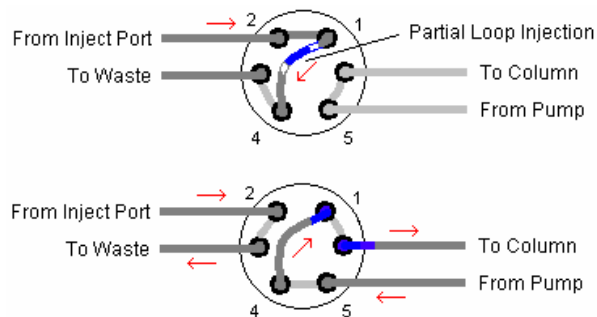
The Full Loop gives the highest injection precision

Partial Loop Injection



Total volume = Injection Volume + Excess Volume
The excess volume is 10 ul
The remaining volume is achieved using air plugs

No Waste Injection



Volume Pulled = Injection Volume

- The No Waste injection always causes 2 ul of flush solvent to be injected. This can cause poor binding of peptides if the flow rates of the system are low and the solvent is strong. Use the sample prep method if needed.
- The user should calibrate the dead volume in a no waste injection against a full loop injection of a known sample.

Flush and Wash Setup – Minimizing Carryover

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

Injection volume (ul): 10.0

Needle height from bottom (mm): 2.0

Syringe speed (ul/s): 8.0

Flush volume (ul): 400

Flush/Wash source: bottle

Wash volume (ul): 0

Flush speed (ul/s): 250.00

Post-injection valve switch time (min): 0.0

Loop loading speed (ul/s): 8.00

Injection Mode:
 Partial loop
 Full loop
 No waste

Tray Temperature Control
 Enable tray temperature control
Temperature (°C): 30.0

Column Oven Control
 Enable column oven control
Temperature (°C): 30.0

Flush- Cleans the injection port and the inside of the needle

Wash- Cleans the outside of the needle

Sample Preparation

Untitled - Instrument Setup

File Surveyor AS Help

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

Prep Operations

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

Add To Task List >>

Sample Location:

Absolute location: A:2

Relative location: Current

Volume (ul): 0.0

Syringe speed (ul/s): 8.00

Needle height (mm): 2.0

Method

Sample Preparation

- Draw from sample
 - Volume (ul) is 0.0
 - Sample absolute location is A:1
 - Syringe speed (ul/s) is 8.00
 - Needle height (mm) is 2.0
 - Air bubble volume (ul) is 3
- Deposit liquid in sample
 - Volume (ul) is 0.0
 - Sample absolute location is A:2
 - Syringe speed (ul/s) is 8.00
 - Needle height (mm) is 2.0

Remove Task Clear All Tasks

File name: Import

Help

PDA Setup

Untitled - Instrument Setup
File Surveyor PDA Help

Surveyor PDA Method

Run
Run Length (min) 10.00 Filter Rise Time (sec) 1.0 Help

Spectra
 Collect Spectral Data Wavelength Step (nm) 5
Start Wavelength (nm) 200 Sample Rate (Hz) 5.0
End Wavelength (nm) 600 Filter Bandwidth (nm) 1
Units
 Wavelength / Absorbance
 Diode / Intensity

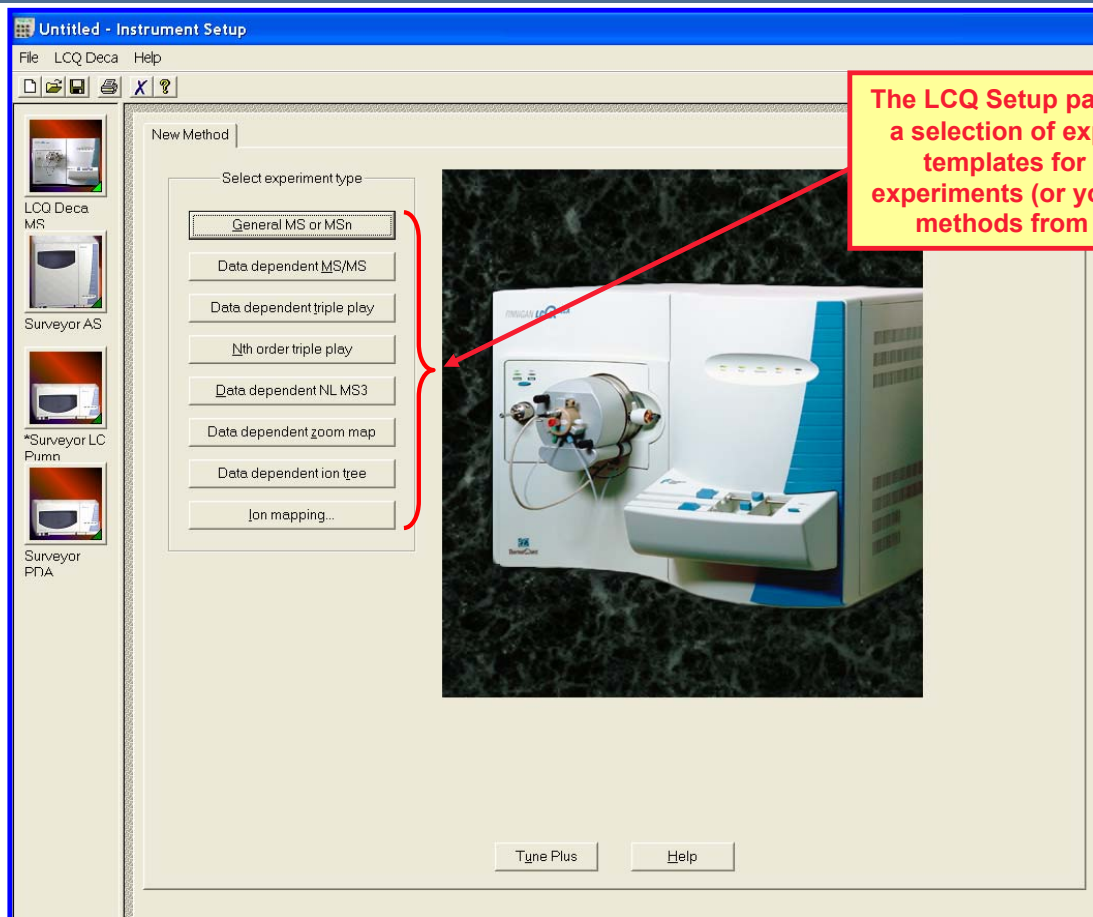
Channels
 No Channels
 One Channel
 Two Channels
 Three Channels
Sample Rate (Hz) 10.0
Channel A
Wavelength (nm) 214 Filter Bandwidth (nm) 9
Channel B
Wavelength (nm) 254 Filter Bandwidth (nm) 9
Channel C
Wavelength (nm) 280 Filter Bandwidth (nm) 9

Timed Events

Time (min)	Type	Channel	Level (mAU)	Delay (sec)
------------	------	---------	-------------	-------------

New...
Delete
Delete All

LCQ MS Setup



The LCQ Setup page provides a selection of experimental templates for popular experiments (or you can build methods from scratch)

LCQ MS Setup

Segments

1. Open a RAW file using the LCQ drop down menu to set segment time intervals
2. Type the number of segments you want in your method
3. Drag the red line to add segment time intervals
4. Can have one tune file per segment

LCQ MS Setup

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

Run settings
Acquire time (min): 10.00 Segments: 3 Start delay (min): 0.00

Retention time (min)

Segment 2 settings
Segment time (min): 3.25 Scan events: 1 Tune method: C:\Calibr\methods\AutoTune.LCQTune ...

Scan Event 1

Scan event 1 settings

Mass range: Normal High Low
Scan mode: MS MS/MS MSn Parent masses...
Scan type: Full SIM ZoomScan
Mass Range: 100.00-800.00 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

Scan Events

1. Select the number of scan events for each segment. Each scan event is essentially a different acquisition (i.e., a full scan followed by an MS/MS scan is two separate scan events).
2. Must use the same tune file for each scan event within a particular segment.

LCQ MS Setup

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

Run settings
Acquire time (min): 10.00 Segments: 3 Start delay (min): 0.00

Retention time (min)

Segment 2 settings
Segment time (min): 3.25 Scan events: 1 Tune method: C:\calibury\methods\AutoTune.LCQTune ...

Scan Event 1

Scan event 1 settings

Mass range: Normal High Low

Scan mode: MS MS/MS MSn

Scan type: Full SIM ZoomScan

Mass Range: 100.00-800.00

Polarity: Positive Negative

Data type: Centroid Profile

Source fragmentation
 Turn on Collision energy (V): 20.0

TurboScan
 Wideband activation
 Dependent scan

New method Tune Plus Help

1. Mass Range: Low, Normal or High
2. Scan Mode: MS, MS/MS, MSn
3. Scan type: Full, SRM, Zoom
5. Polarity: + or – ion mode detection
6. Data type: Centroid or Profile

Other Method Features

The screenshot displays the 'Untitled - Instrument Setup' window. The 'Run settings' section shows an acquire time of 10.00 minutes and 3 segments. A chromatogram below shows three segments: Segment 1 (0-2 min), Segment 2 (2-5 min, highlighted in yellow), and Segment 3 (5-10 min). The 'Segment 2 settings' section shows a segment time of 3.25 minutes and 1 scan event. The 'Scan event 1 settings' section includes the following options:

- Mass range: Normal (selected), High, Low
- Scan mode: MS (selected), MS/MS, MSn
- Scan type: Full (selected), SIM, ZoomScan
- Mass Range: 100.00-800.00
- Polarity: Positive (selected), Negative
- Data type: Centroid (selected), Profile
- Source fragmentation: Turn on (circled in red), Collision energy (eV): 20.0
- TurboScan:
- Wideband activation: (circled in red)
- Dependent scan:

Buttons at the bottom include 'New method', 'Tune Plus', and 'Help'.

1. Select Source Fragmentation
On if in-source CID is desired
2. Wideband activation will be active if an MS/MS or MSn scan event is selected

Selected Ion Monitoring

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

Run settings
Acquire time (min): 10.00 Segments: 2

Segment 2 settings
Segment time (min): 3.25 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): 100.00-800.00
Polarity: Positive Negative
Data type: Centroid Profile

SIM Ranges

Input method:
 Center/width
 From/to

Num ranges: 1

	Center Mass:	Width:
1	329.30	2.00
2	329.00	2.00
3	300.00	2.00
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

Buttons: OK, Cancel, Help

Buttons: Setup ranges..., Turn on, TurboScan, Wideband detection, Dependent scan, Settings...

Product Ion MS/MS

The screenshot displays the 'Instrument Setup' interface for an MS Detector. The 'Run settings' section shows an acquire time of 10.00 minutes and 3 segments. A chromatogram shows a peak highlighted in yellow. The 'Scan event 1 settings' section is configured for MS/MS with a mass range of 80.00-350.00. A 'Set Parent Mass' dialog box is open, showing the following parameters:

Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q	Activation Time (msec)
329.30	1.5	32.0	0.250	30.000

The 'Scan event 1 settings' section includes options for Mass range (Normal, High, Low), Scan mode (MS, MS/MS, MSn), Scan type (Full, SRM, ZoomScan), Mass Range (80.00-350.00), Polarity (Positive, Negative), and Data type (Centroid, Profile). The 'Parent masses...' button is highlighted with a red circle, and a red dotted line connects it to the 'Set Parent Mass' dialog box.

Data Dependent Acquisition

Untitled - Instrument Setup

File LCQ Deca Help

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

Run settings

Acquire time (min): 10.00 Segments: 1 Start delay (min): 0.00

To display a chromatogram here, use LCQ Deca/Open raw file...

Segment 1

Retention time (min)

Segment 1 settings

Segment time (min): 10.00 Scan events: 2 Tune method: C:\Xcalibur\methods\AutoTune.LCQTune ...

Scan Event 1 Scan Event 2

Scan event 1 settings

Mass range: Normal High Low

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

Scan type: Full SIM ZoomScan

Mass Range: 100.00-800.00 Setup ranges...

Polarity: Positive Negative

Data type: Centroid Profile

Source fragmentation

Turn on Collision energy (V) 20.0

TurboScan

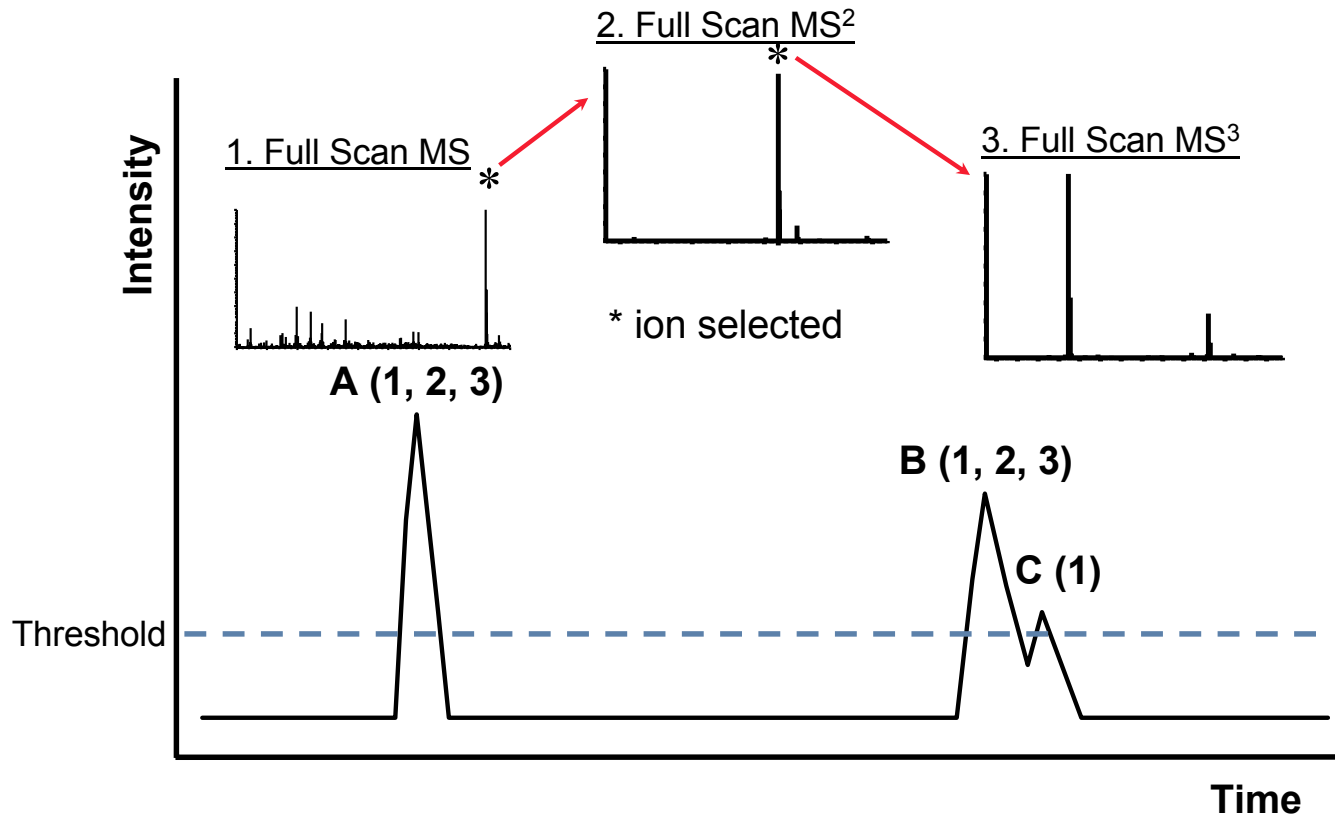
Wideband activation

Dependent scan Settings...

New method Tune Plus Help

1. First scan event is the trigger scan
2. All subsequent scans events may be dependent on Scan event 1

Data Dependent Scanning



Building Double Play (Data Dependent MS/MS)

- Steps:
 1. Full MS
 2. Data Dependent (dd) MS² on the largest ion from the Full MS spectrum

- Pros/Cons:
 1. Provides MS/MS (structural) information
 2. Misses co-eluting peaks

Building Double Play: Scan Event One

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

Run settings

Acquire time (min): 10.00 Segments: 1 Start delay (min): 0.00

To display a chromatogram here, use LCQ Deca/Open raw file...

Segment 1

Retention time (min)

Segment 1 settings

Segment time (min): 10.00 Scan events: 2 Tune method: C:\Xcalibur\methods\AutoTune.LCQ Tune ...

Scan Event 1 Scan Event 2

Scan event 1 settings

Mass range: Normal High Low

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

Scan type: Full SIM ZoomScan

Mass Range: 100.00-800.00 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

1. Add two scan events, set the Acquire time and Tune method
2. Scan event one needs to have the Mass Range set

Building Double Play: Scan Event Two

The screenshot shows the 'Untitled - Instrument Setup' window. The 'Run settings' section includes 'Acquire time (min): 10.00', 'Segments: 1', and 'Start delay (min): 0.00'. Below this is a chromatogram showing 'Segment 1' from 0 to 10 minutes. The 'Segment 1 settings' section shows 'Segment time (min): 10.00', 'Scan events: 2', and 'Tune method: C:\Xcalibur\methods\AutoTune.LCQTune ...'. The 'Scan Event 2' bar is highlighted with a red oval. The 'Scan event 2 settings' section includes 'Mass range: Normal', 'Scan mode: MS/MS', 'Scan type: Full', 'Polarity: Positive', and 'Data type: Centroid'. The 'Source fragmentation' section has 'Turn on' checked and 'Collision energy (V): 20.0'. The 'Wideband activation' section has 'Dependent scan' checked and highlighted with a red box, with a 'Settings...' button next to it. At the bottom are 'New method', 'Tune Plus', and 'Help' buttons.

1. Check the box next to Dependent scan
2. Click on Settings

Building Double Play: Scan Event Two

Data Dependent Settings

Global | **Segment** | Scan event

Current Segment | Charge State | Add/Sub

Parent masses: 386.30, 281.20 Import..

Reject masses: 391.10, 371.10 Import..

Most intense if no Parent Masses found

Normalized collision energy (%): 35.0

Activation Q: 0.250

Activation time (msec): 30.000

Default charge state: 1

Min MS signal (10⁴ counts): 10.0

Min MSn signal (10⁴ counts): 0.50

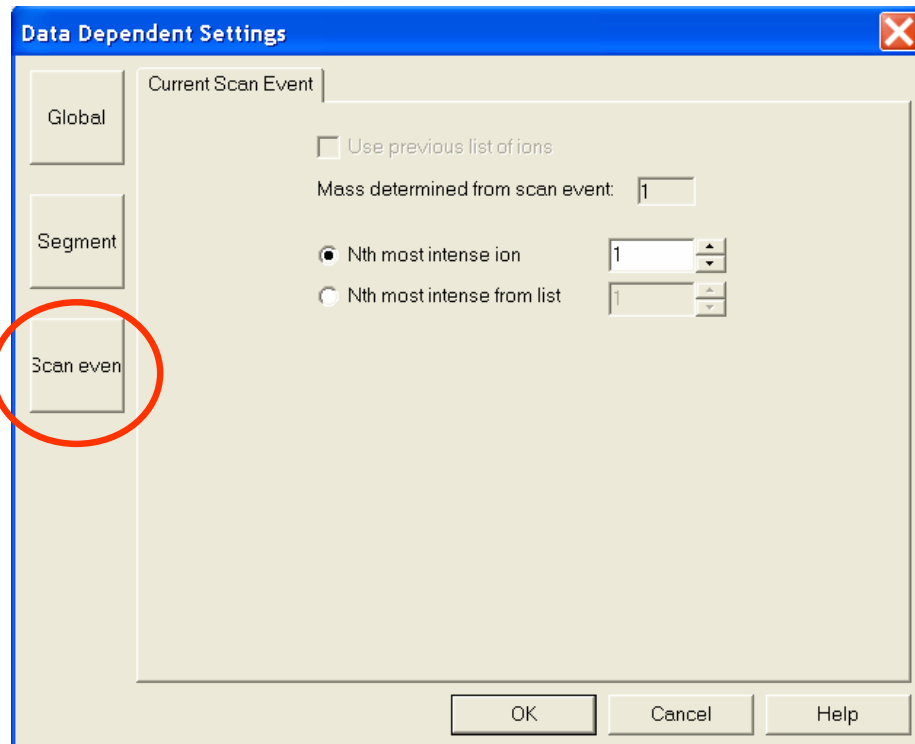
Isolation width (m/z): 2.0

OK Cancel Help

Segment Settings

1. Enter Reject masses (These should be found by examining a blank run in Qual Browser using a Full MS scan)
 2. Set the Normalized Collision Energy, Default Charge State, Min. MS signal (Threshold) and Isolation Width
- Enter Parent masses (if desired)

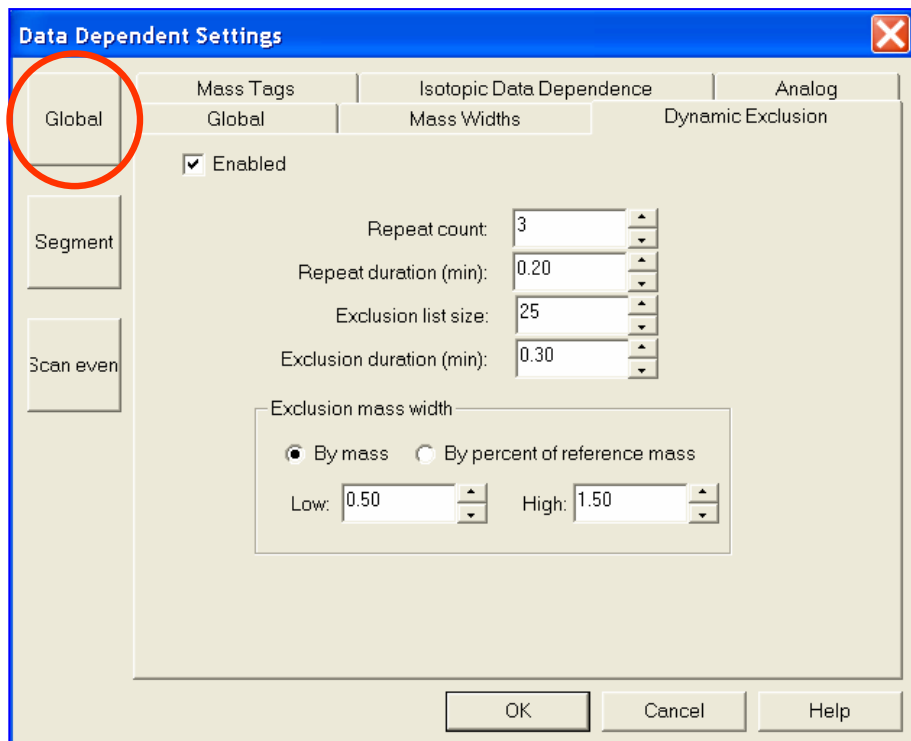
Building Double Play: Scan Event Two



Scan Event Settings

- Nth most intense ion = 1
- Use Nth most intense from list when parent masses are specified in the Segment settings

Dynamic Exclusion



1. Choose Global > Dynamic Exclusion
 2. Set the Repeat count, Repeat duration, Exclusion list size, Exclusion duration, and Exclusion mass width
- ***These settings cause three MS/MS events happening within 0.2 min. to trigger the exclusion of the mass for the next 0.3 minutes***
 - ***The mass widths can be set asymmetrically to account for isotopes***

Divert Valve Operation

Untitled - Instrument Setup

File LCQ Deca Help

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

Divert Valve Settings

Use divert valve Number of valve positions: 3 Position at start of run: To Waste

To source

To waste

Waste 1 Source 2 Waste 3

Valve position duration (min): 8.03 Retention time (min)

New method Tune Plus Help

Experiment Summary

Untitled - Instrument Setup
File LCQ Deca Help

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

Method summary:

Segment 1 Information

Duration (min): 10.00
Number of Scan Events: 2
Tune Method: AutoTune

Scan Event Details:

1: + c norm o(100.0-800.0)
2: + c norm Dep MS/MS Most intense ion from (1).

Data Dependent Settings:

Parent Mass List:	281.20	386.30
Reject Mass List:	371.10	391.10
Default Charge State:	1	
Default Isolation Width:	2.00	
Normalized Collision Energy:	35.0	
Activation Q:	0.250	
Activation Time:	30.000	
Min. Signal Required:	100000	
Min. MSn Signal Required:	5000	
Most intense if no parent masses found not enabled		
Add/subtract mass not enabled		
Charge state screening not enabled		
Charge state rejection not enabled		

Global Data Dependent Settings:

Exclusion mass width by mass	
Exclusion mass width low:	0.50
Exclusion mass width high:	0.50
Reject mass width by mass	
Reject mass width low:	0.50
Reject mass width high:	0.50
ZoomScan mass width by mass	
ZoomScan mass width low:	5.00
ZoomScan mass width high:	5.00

New method | Tune Plus | Help

Common Data Dependent LCQ Experiments

Big 3:

- **Steps:**
 1. Full MS
 2. Data Dependent (dd) MS² of the Largest, dd MS² of 2nd Largest, dd MS² of 3rd Largest
- **Pros/Cons:**
 1. High ratio of time spent doing MS²
 2. Hits peak apex

Double Play with Dynamic Exclusion:

- **Steps:**
 1. Full MS
 2. Data Dependent (dd) MS² of the Largest with Dynamic Exclusion
- **Pros/Cons:**
 1. Adds opportunity to analyze coeluting peaks
 2. May miss peak apex

ThermoFisher
S C I E N T I F I C

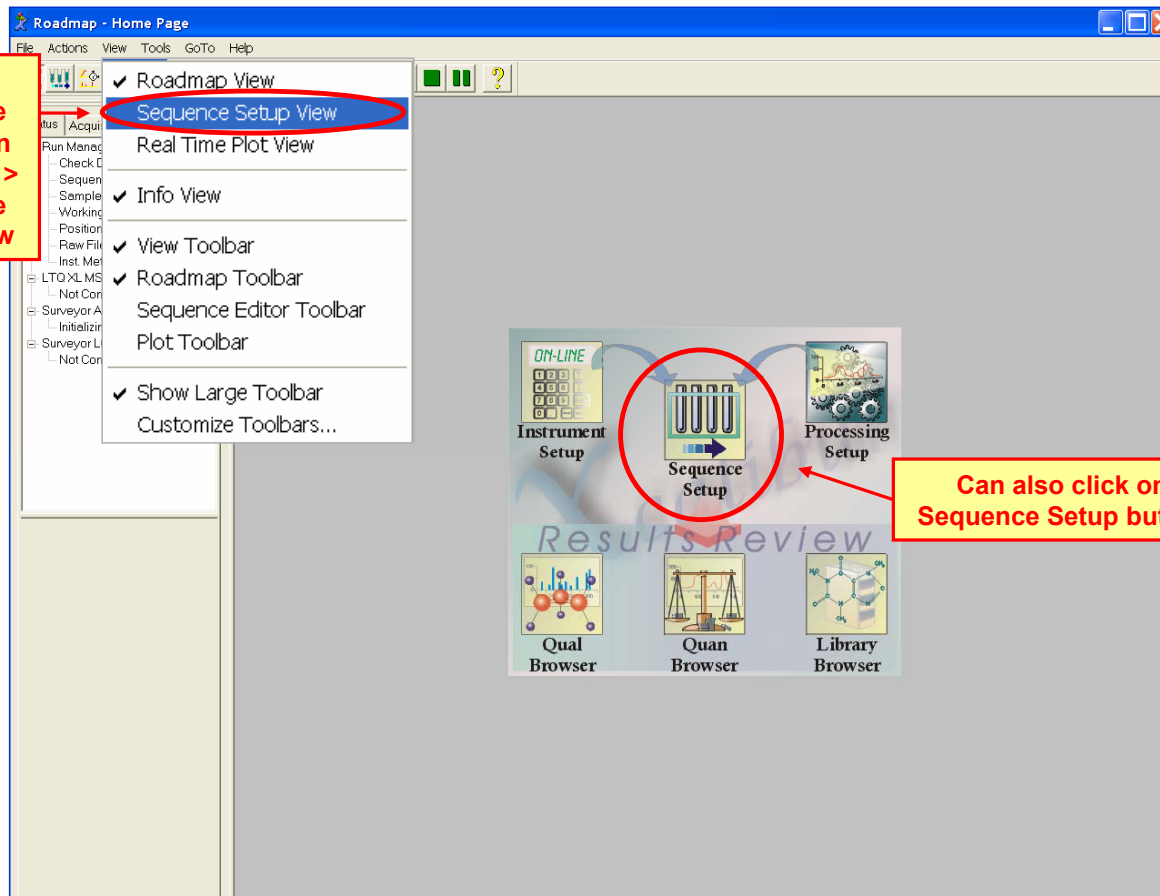
The world leader in serving science

Chapter 11

Setting Up and Running Sequences

Xcalibur Home Page Sequence Setup

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



Can also click on Sequence Setup button

Creating a Sequence

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

1. Double-click to add Instrument Method

	File Name	Path	Inst Meth	Position	Inj Vol
1	Microsomes	C:\Xcalibur\Data\Metabolism	C:\Xcalibur\methods\Test	A:1	10.0
*					0.1

2. If there is no folder created for the Path, you can type a folder in and it will be created

3. Populate File Name (no spaces), Position, and Inj Vol

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

Creating a Sequence

The screenshot shows the 'Sequence Setup - Home Page' window. The main area contains a table with the following data:

	File Name	Path	Inst Meth	Position	Inj Vol
1	Microsomes	C:\Xcalibur\Data\Metabolism	C:\Xcalibur\methods\Test	A:1	10.0
*					0.1

Annotations in red boxes provide instructions:

- A yellow box with red text: "Hotkey F2 puts the cursor in the boxes and makes the fields editable".
- A white box with red text: "To open the Inst Meth from the sequence, right-click and select Open File". An arrow points from this box to the 'Open File' option in a right-click context menu.

The context menu also includes options: Browse, Paste Cells..., and Insert Row...

Creating a Sequence Using the New Sequence Template

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

1. Click New

The screenshot shows the 'Sequence Setup - Home Page' window. The 'File' menu is open, and the 'New...' option is circled in red. A red arrow points from the 'New...' option to the 'New Sequence Template' dialog box. The dialog box has several sections: 'General' with fields for Base File Name, Path, Instrument Method, Processing Method, and Calibration File; 'Samples' with fields for Number of Samples, Tray Type, Injections per Sample, Initial Vial Position, and Base Sample ID; 'Bracket Type' with radio buttons for None, Open, Non-Overlapped, and Overlapped; 'Calibration' with checkboxes for Add Standards, Add Blanks, and Fill in Sample ID for Standards; and 'QC' with checkboxes for Add QCs, Add Blanks, and Fill in Sample ID for QCs. At the bottom are buttons for OK, Cancel, Save As Default, and Help.

New Sequence Template

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

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

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

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

2. Enter the number of unknown samples

3. Select the Initial Vial Position

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

New Sequence Template

	File Name	Path	Inst Meth	Position	Inj Vol
1	Steroids01	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.1	10.0
2	Steroids02	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.2	10.0
3	Steroids03	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.3	10.0
4	Steroids04	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.4	10.0
5	Steroids05	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.5	10.0
6	Steroids06	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.6	10.0
7	Steroids07	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.7	10.0
8	Steroids08	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.8	10.0
9	Steroids09	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.9	10.0
10	Steroids10	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.10	10.0
*					0.1

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

New Sequence Template

If you want to type a new File Name:

2. Select Edit and click Fill Down

1. Type File name

The screenshot shows the 'Sequence Setup' software interface. The main window displays a table with columns: File Name, Path, Inst Meth, Position, and Inj Vol. The table contains 10 rows of data, with the first row highlighted. The 'Edit' menu is open, showing options: Undo (Ctrl+Z), Clear (Ctrl+X), Copy (Ctrl+C), Paste (Ctrl+V), Insert Row... (Ins), Delete Row... (Del), Go to Row..., Fill Down... (circled in red), and Browse File name... (circled in red). A red arrow points from the 'Fill Down...' option to the first row of the table. Another red arrow points from the '1. Type File name' text to the first cell of the 'File Name' column in the table.

	File Name	Path	Inst Meth	Position	Inj Vol
1	Steroids_New 01	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:1	10.0
2	Steroids02	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:2	10.0
3	Steroids03	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:3	10.0
4	Steroids04	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:4	10.0
5	Steroids05	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:5	10.0
6	Steroids06	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:6	10.0
7	Steroids07	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:7	10.0
8	Steroids08	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:8	10.0
9	Steroids09	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:9	10.0
10	Steroids10	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:10	10.0
*					0.1

Changing the Sequence Column Arrangement

1. Select Change and click Column Arrangement

Column Arrangement...

Path	Inst Meth	Position	Inj Vol
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.1	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.2	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.3	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.4	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.5	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.6	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.7	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.8	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.9	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.10	10.0

Column Arrangement

Available Columns

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

Displayed Columns

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

3. Click Add

2. Select which columns to add from the available columns

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

Changing the User Labels

1. Select Change and click User Labels

Path	Inst Meth	Position	Inj Vol
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:1	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:2	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:3	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:4	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:5	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:6	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:7	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:8	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:9	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:10	10.0

Specify your own labels for the five Comment Columns

NUM | 9/5/2007 | 3:17 PM

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

Changing the Tray Name

1. Select Change and click Tray Name

The screenshot shows the 'Test [Open] - Sequence Setup - Home Page' window. The 'Change' menu is open, and 'Tray Name...' is selected. A 'Tray Selection' dialog box is open, showing a dropdown menu with '96 Well Microplate + Tall Microwell Carrier' selected. A table of sequence data is visible in the background.

	Path	Inst Meth	Position	Inj Vol
7	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A1	10.0
8	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A2	10.0
9	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A3	10.0
10	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A4	10.0
x	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A5	10.0
	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A6	10.0
	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A7	10.0
	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A8	10.0
	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A9	10.0
	C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A10	10.0
				0.1

Change tray name which vial positions are to be validated against. NUM 9/6/2007 3:25 PM

2. Select tray to use

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

Exporting a Sequence to Excel

1. Select File and click Export Sequence

The screenshot shows the 'Test [Open] - Sequence Setup - Home Page' application. The 'File' menu is open, and 'Export Sequence...' is highlighted with a red circle. A red arrow points from the instruction box to this menu item. Another red arrow points from the 'Export Sequence...' menu item to the 'Export Sequence' dialog box. The dialog box shows 'Export to File:' with a 'Browse' button and a 'Select Columns to Export' section with various checkboxes. The background shows a table with columns: Path, Inst Meth, Position, and Inj Vol.

Path	Inst Meth	Position	Inj Vol
C:\XCALIBUR(DATA)\	C:\Xcalibur\methods\Test	A:1	10.0
C:\XCALIBUR(DATA)\	C:\Xcalibur\methods\Test	A:2	10.0
C:\XCALIBUR(DATA)\	C:\Xcalibur\methods\Test	A:3	10.0
C:\XCALIBUR(DATA)\	C:\Xcalibur\methods\Test	A:4	10.0

Export Sequence dialog box options:

- Export to File: [] Browse
- Select Columns to Export:
 - Sample Type
 - Sample Name
 - File Name
 - Sample ID
 - Path
 - Instrument Method
 - Processing Method
 - Calibration File
 - Position
 - Injection Volume
 - Level
 - Sample Weight
 - Sample Volume
 - ISTD Corr Amt
 - Dil Factor
 - Study
 - Client
 - Laboratory
 - Company
 - Phone
 - Comment
- Buttons: All, Clear, OK, Cancel, Help

Exporting a Sequence to Excel

The image shows two dialog boxes from a software application. The first dialog, titled 'Export Sequence', has a 'Browse' button circled in red. A red arrow points from a yellow callout box to this button. The callout box contains the text: '1. Select which columns to export and Click Browse'. Below the 'Browse' button is a list of columns with checkboxes, including 'Sample Type', 'Sample Name', 'File Name', 'Sample ID', 'Path', 'Instrument Method', 'Processing Method', 'Calibration File', 'Position', 'Injection Volume', 'Level', 'Sample Weight', 'Sample Volume', 'ISTD Corr Amt', 'Dil Factor', 'Study', 'Client', 'Laboratory', 'Company', 'Phone', and 'Comment'. A second dialog, titled 'Select CSV Sequence', shows a file explorer view with a 'File name:' field and a 'Files of type:' dropdown set to 'CSV File (*.csv)'. A red arrow points from a yellow callout box to the 'File name:' field. The callout box contains the text: '2. Give a name to your sequence that will be exported'. Another red arrow points from a yellow callout box to the 'Files of type:' dropdown. The callout box contains the text: '3. The sequence is exported as a .csv file'. Both callout boxes have a red border and a yellow background.

Example of an Exported Sequence

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

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

Importing a Sequence from Excel

1. Select File and click Import Sequence

The screenshot shows the 'Test [Open] - Sequence Setup - Home Page' window. The 'File' menu is open, and 'Import Sequence...' is highlighted with a red oval. A red arrow points from the text '1. Select File and click Import Sequence' to this menu item. The 'Import Sequence' dialog box is open, showing 'Import from File: C:\Documents and Settings\...\a' and a 'Browse' button. Below this, the 'Select Columns to Import' section has a list of 17 items, all of which are checked with a red checkmark. A red arrow points from the text '2. Select which columns to import and Click Browse to find the modified sequence' to the 'Browse' button. The dialog box has 'OK', 'Cancel', and 'Help' buttons at the bottom.

Path	Inst Meth	Position
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:1
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:2
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:3
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:4
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:5
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:6
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A:7

Import Sequence

Import from File: C:\Documents and Settings\...\a Browse

Select Columns to Import

- Sample Type
- Sample Name
- File Name
- Sample ID
- Path
- Instrument Method
- Processing Method
- Calibration File
- Position
- Injection Volume
- Level
- Sample Weight
- Sample Volume
- ISTD Corr Amt
- Dil Factor
- Study
- Client
- Laboratory
- Company
- Phone
- Comment

All Clear

OK Cancel Help

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

Running the Sequence

1. Can either
Run One
Sample or Run
Sequence

The screenshot displays the 'Run Sequence' dialog box in the foreground, overlaid on the 'Sequence Setup - Home Page' window. The dialog box contains the following settings:

- Acquisition Options:**
 - Instrument: Surveyor AS, Start Instrum...: Yes
 - Surveyor AS, LTQ XL MS, Surveyor LC Pump
 - Start When Ready, Change Instruments...
- Instrument Method:**
 - Start Up: [text box], Browse...
 - Shut Down: [text box], Browse...
- Programs:**
 - Pre Acquisition: [text box], Browse...
 - Post Acquisition: [text box], Browse...
- Run Synchronously:**
 - Pre Acquisition, Post Acquisition
- After Sequence Set System:**
 - On, Standby, Off
- User:** [text box]
- Run Rows:** 2-3
- Priority Sequence
- Processing Actions:**
 - Quan
 - Qual
 - Reports
 - Programs
 - Create Quan Summary

Buttons at the bottom of the dialog box are OK, Cancel, and Help.

In the background, the 'Sequence Setup - Home Page' window shows a table with columns: Path, Inst Meth, Position, and Inj Vol. The table contains six rows of data. A menu is open, showing options: 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, and Reinstate Warnings. A red circle highlights 'Run This Sample...' and 'Run Sequence...', with a red arrow pointing from the text box to the 'Run Sequence...' option.

Path	Inst Meth	Position	Inj Vol
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.1	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.10	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.2	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.3	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.4	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.5	10.0
C:\XCALIBUR\DATA\	C:\Xcalibur\methods\Test	A.6	10.0

Running the Sequence

The screenshot shows the 'Run Sequence' dialog box with the following sections and controls:

- Acquisition Options:** A table listing instruments and their status.
- Start When Ready:** A checked checkbox.
- Instrument Method:** Fields for 'Start Up' and 'Shut Down' with 'Browse...' buttons.
- Programs:** Fields for 'Pre Acquisition' and 'Post Acquisition' with 'Browse...' buttons.
- Run Synchronously:** Checked checkboxes for 'Pre Acquisition' and 'Post Acquisition'.
- After Sequence Set System:** Radio buttons for 'On', 'Standby', and 'Off'.
- Processing Actions:** A group of unchecked checkboxes: 'Quan', 'Qual', 'Reports', 'Programs', and 'Create Quan Summary'.
- Priority Sequence:** An unchecked checkbox.
- Run Rows:** A text box containing '2-3'.
- User:** A text box.

Annotations with red arrows point to specific elements:

- 'Displays all instruments that have been configured using Instrument Configuration' points to the instrument list table.
- 'If not checked, the sequence will not go into the queue until you click Actions > Start Analysis' points to the 'Start When Ready' checkbox.
- 'Can specify Instrument Method to run before or after the sequence' points to the 'Start Up' and 'Shut Down' fields.
- 'Make sure these are the rows to run' points to the 'Run Rows' text box.
- 'Select if you want to run sequence ASAP' points to the 'Priority Sequence' checkbox.
- 'Allows you to process samples automatically' points to the 'Processing Actions' group of checkboxes.

The Info View

Status

The screenshot shows the 'Status' tab with a tree view under 'Run Manager'. The tree includes 'Check Devices', 'Sequence:', 'Sample Name:', 'Working On:', 'Position:', 'Raw File:', 'Inst. Method:', 'LTQ XL MS', 'Surveyor AS', and 'Surveyor LC Pump'. Below the tree are tabs for 'Source', 'Vacuum', 'Syringe', 'Analog', and 'State'. The 'State' tab is active, showing parameters like Vaporizer Temp. (°C), Sheath Flow Rate, Aux Flow Rate, Current (uA), Voltage (kV), Capillary Temp. (°C), and Capillary Voltage (V).

The Status tab shows the status of all configured instruments

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

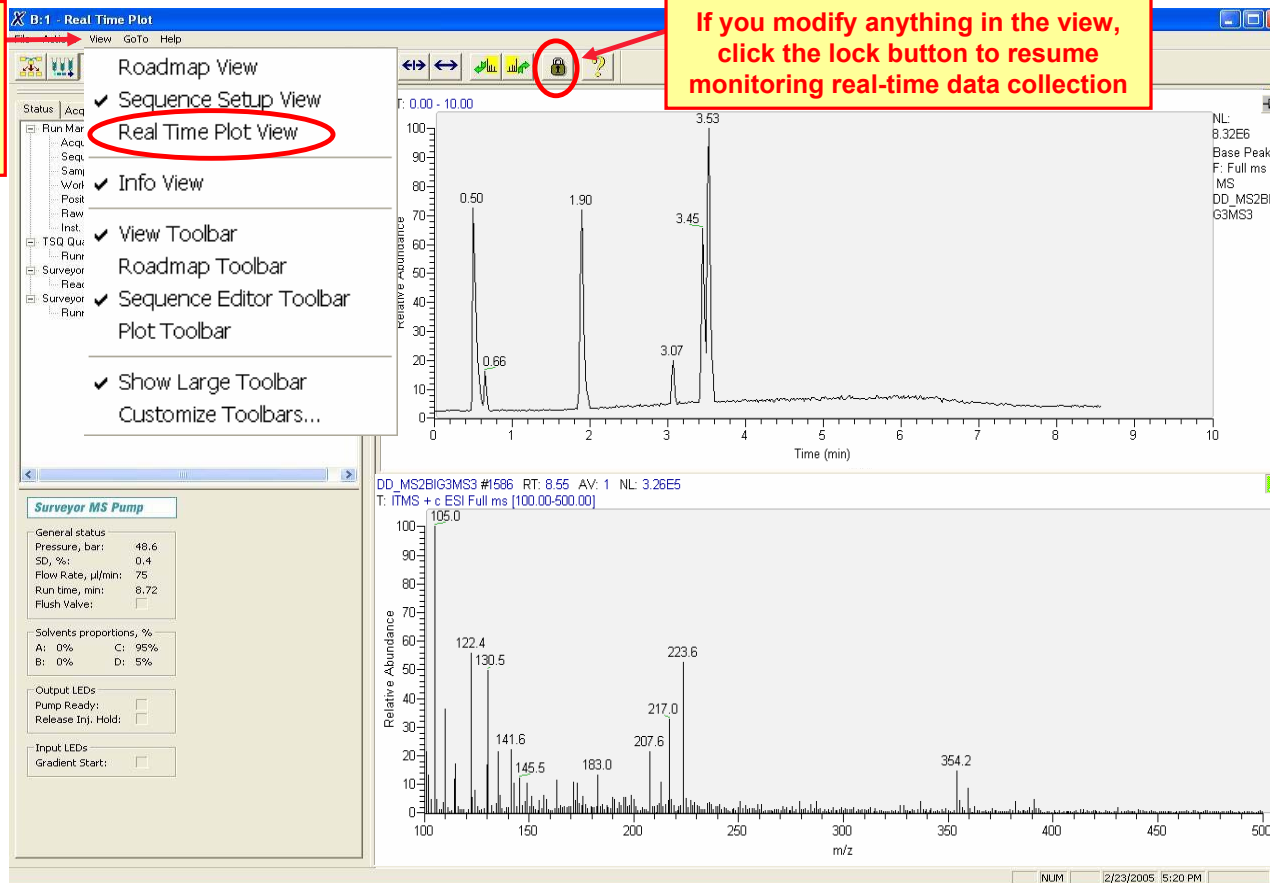
Acquisition Queue-Sequence Progress

The screenshot shows the 'Acquisition Queue' tab with a tree view. The tree includes 'All Sequences' and '[HOMEPAGE] - C:\Xcalibur\Patrick\Methods\test.sld'. Under the sequence, there are 'Sequence Row #15' and 'Sequence Row #16'. A green progress bar is visible next to 'Sequence Row #15'.

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

Real Time Plot View

1. Select View and click Real Time Plot View



ThermoFisher
S C I E N T I F I C

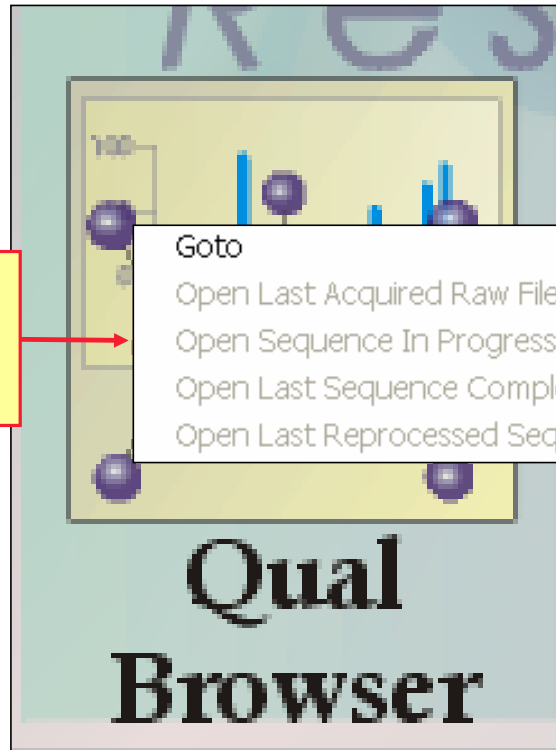
The world leader in serving science

Chapter 12

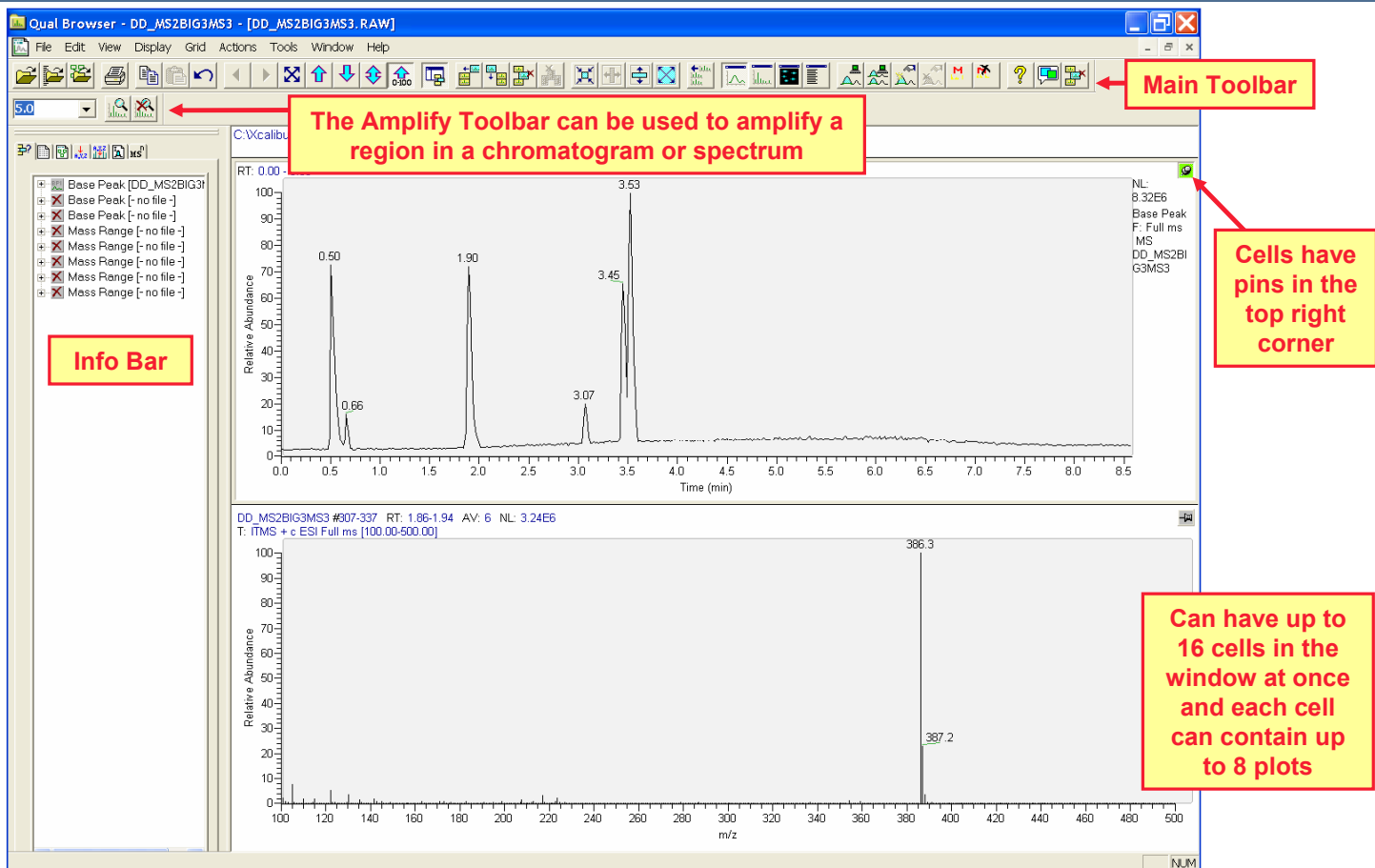
Qual Browser

Opening Qual Browser

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



Qual Browser Main View



Opening Data in Qual Browser

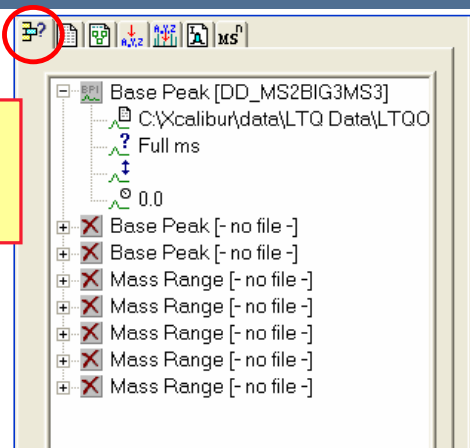
1. Click File and select Open (can also open sequences or result files)

The screenshot shows the Qual Browser interface with the File menu open. The 'Open...' option is highlighted. The 'Open Raw File' dialog box is displayed, showing a file list with 'Imipramine_Tripleplay' selected. The dialog box has a 'Replace' section with radio buttons for 'Window', 'Cell', and 'Plot', and a 'Default Layout' dropdown menu. A red box highlights the 'Replace' section and the 'Default Layout' dropdown.

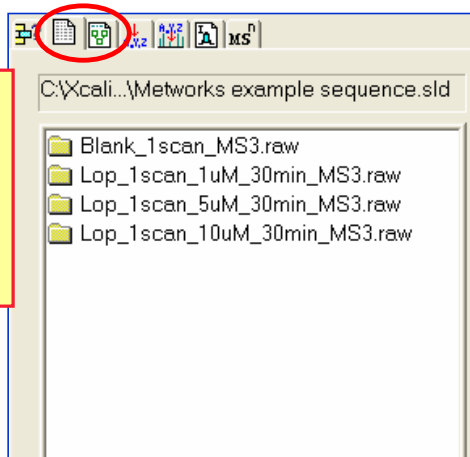
2. Select to replace (the current window, cell or plot), add a new window or plot (by default a new window will be added), and choose which layout to use

The Info Bar

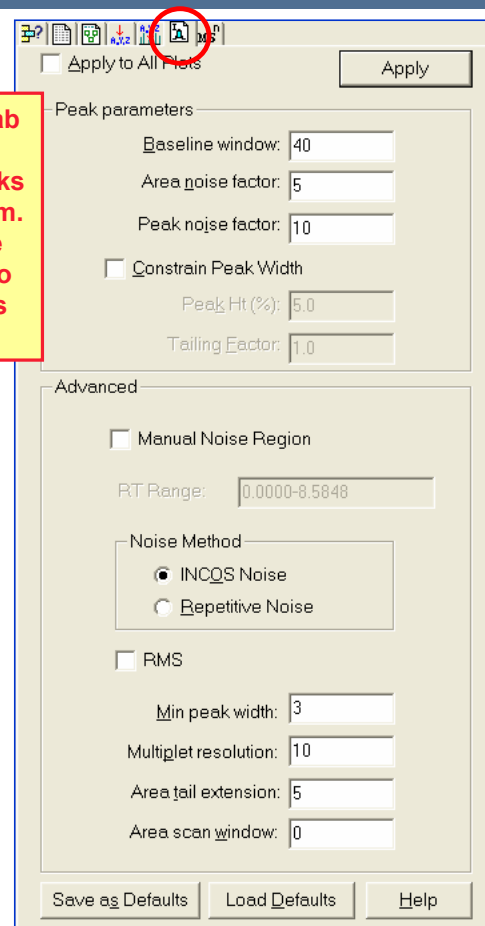
The Cell Info tab gives info about each plot within a cell



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



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



The Info Bar – Elemental Composition



The Elemental Composition page of the Info Bar allows you to calculate the "best matching" chemical formula for a mass (from a spectrum)

1. Type mass value or right-click on a mass in the spectrum and select 'Generate Formula from Mass'

Elemental Composition

Single mass

Mass: 386.25085

Max. results: 10

Calculate

Idx	Formula	RDB	Delta amu
1	¹² C ₂₁ ¹ H ₃₂ ¹⁶ O ₂ ¹⁴ N ₅	8.5	-0.004
2	¹² C ₂₁ ¹ H ₃₈ ¹⁶ O ₆	3.0	-0.015
3	¹² C ₂₁ ¹ H ₃₀ ¹⁶ O ₃ ¹⁴ N ₄	9.0	0.020
4	¹² C ₂₁ ¹ H ₃₄ ¹⁶ O ₁ ¹⁴ N ₆	8.0	-0.028
5	¹² C ₂₁ ¹ H ₄₀ ¹⁶ O ₅ ¹⁴ N ₁	2.5	-0.039
6	¹² C ₂₁ ¹ H ₂₈ ¹⁶ O ₄ ¹⁴ N ₃	9.5	0.043
7	¹² C ₂₁ ¹ H ₃₆ ¹⁴ N ₇	7.5	-0.052
8	¹² C ₂₁ ¹ H ₂₂ ¹⁴ N ₈	15.0	0.055
9	¹² C ₂₁ ¹ H ₄₂ ¹⁶ O ₄ ¹⁴ N ₂	2.0	-0.063
10	¹² C ₂₁ ¹ H ₂₆ ¹⁶ O ₅ ¹⁴ N ₂	10.0	0.067

File... List Simulate

Limits

Charge: 1

Nitrogen-Rule: Do not use

Mass tolerance: 0.10 amu

RDB equiv: -1.0-100.0

Elements in use

Isotope	Min	Max	DB eq.	Mass
14 N	0	10	0.5	14.003
16 O	0	15	0.0	15.995
12 C	21	21	1.0	12.000
1 H	0	60	-0.5	1.008

Load... Save as... Apply Help

2. Set limits for the formulas

4. Click Calculate and the formulas appear in the table

3. To change elements in use, right-click and go to Add Isotopes. Select isotope from periodic table.

The Info Bar – Isotope Simulator



The Isotope Simulator allows you to create a simulated spectrum for a chemical formula entered

1. Enter a chemical formula

2. Select the parameters

3. Click new

4. The exact mass appears here and the simulation appears in a new dialog box

Isotope simulation

New Insert Replace

Chemical formula

Peptide/Protein

Plus H₂O 386.2550518 amu

Change mixture...

Adduct

Identity

Concentration

Charge distribution

Most abundant:

Half width:

Output style

Pattern

Profile

Samples/peak:

Centroid

Resolution

0.7 Daltons

50 PPM

20000 Resolving pwr

Valley

FWHM

10%

5%

C21H31O2N5 +H: C21 H32 O2 N5 p(gss, s/p:40) Chrg 1...

Relative Abundance

100

95

90

85

80

75

70

65

60

55

50

45

40

35

30

25

20

15

10

5

0

384 386 388 390 392 394

m/z

386.26969

387.34360

388.39555

The Info Bar – MSⁿ Browser



The MSⁿ Browser of the Info Bar allows you to display and analyze MSⁿ experimental data

1. If data does not show up in the MSⁿ browser, pin the spectrum

2. Click to expand

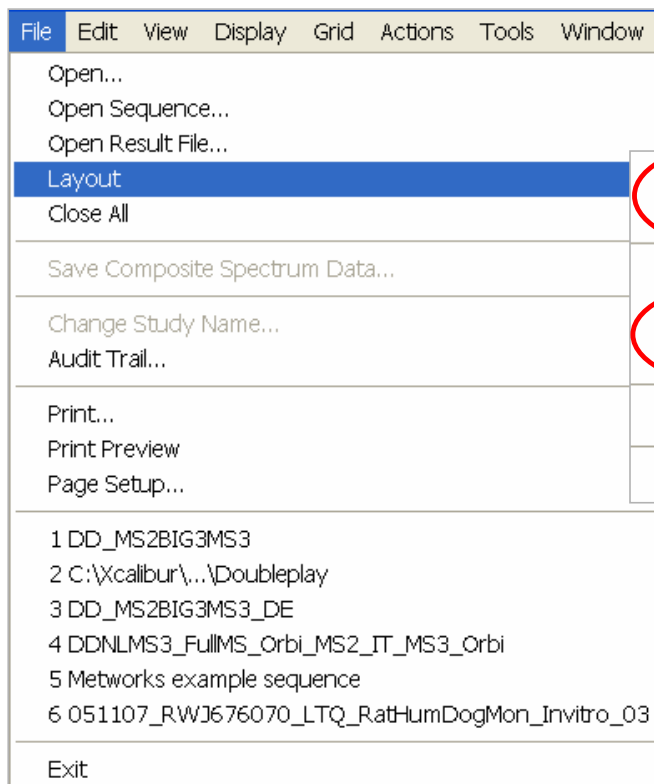
4. Can also right-click and select to Include individual scans

3. Can double-click to get either the Average or Composite spectrum

The screenshot shows the MSⁿ Browser interface with the following elements:

- MSⁿ Browser Header:** Includes a toolbar with icons for help, refresh, zoom, and a red circle around the MSⁿ icon.
- Search Parameters:** Time range (min): 0.01-8.58, Mass range: *, Mass tolerance: 0.50, and a checkbox for "Track".
- Normalization:** A checkbox for "Normalize composite spectrum".
- Tree View:** A list of MSⁿ precursor and composite/average spectra. The "MS2 precursor 386.26" node is expanded, showing sub-nodes for "MS3 precursor 122.02", "Composite spectrum MS3 386.26,122.02", "Average spectrum MS3 386.26,122.02 (311-366)", "Average spectrum MS2 386.26 (310-368)", and "MS2 precursor 455.27".
- Context Menu:** A right-click context menu is open over the "Average spectrum MS3 386.26,122.02 (311-366)" node, with "Include individual scans" circled in red.

Qual Browser Layouts

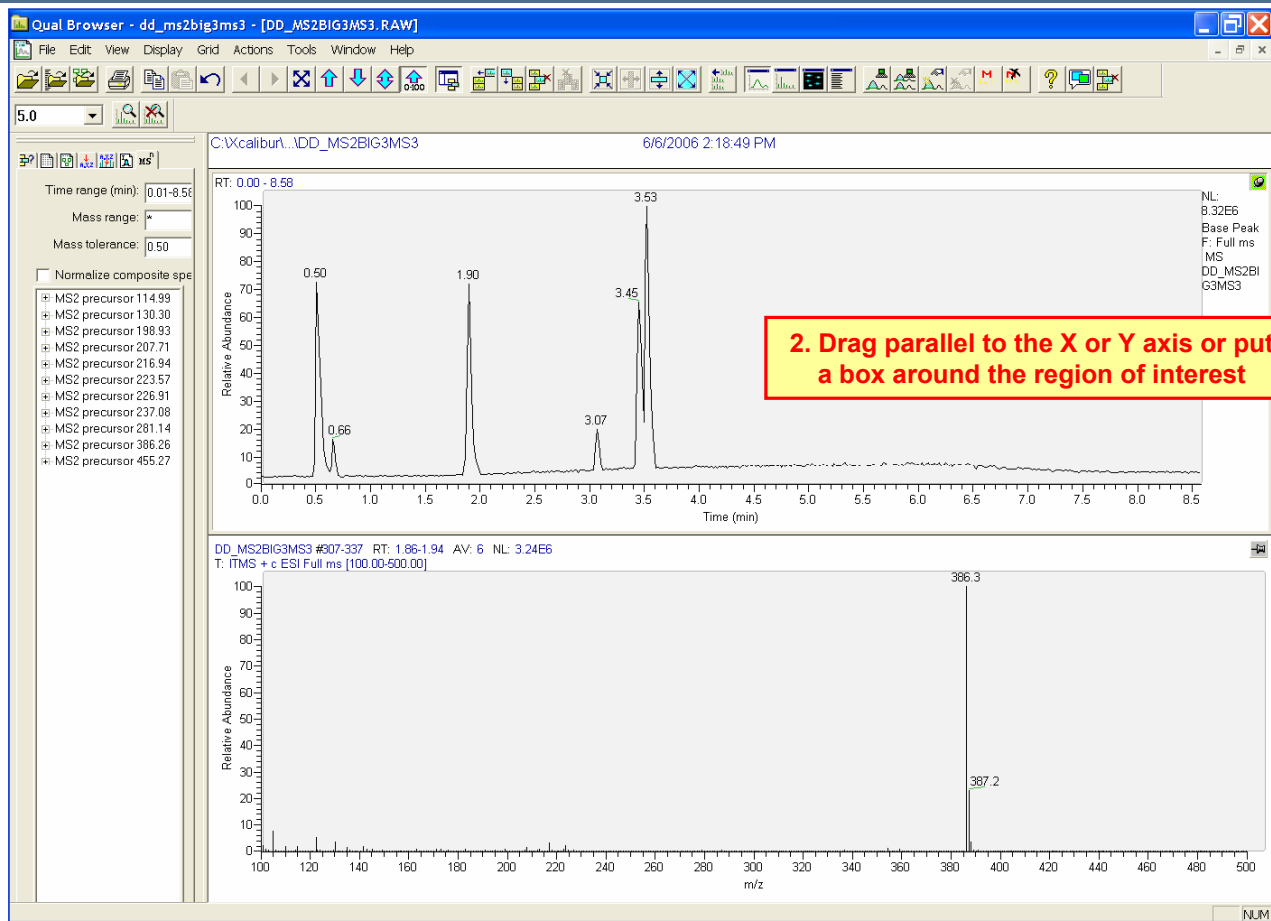


1. Set the cells, plots, integration, etc. to your specifications

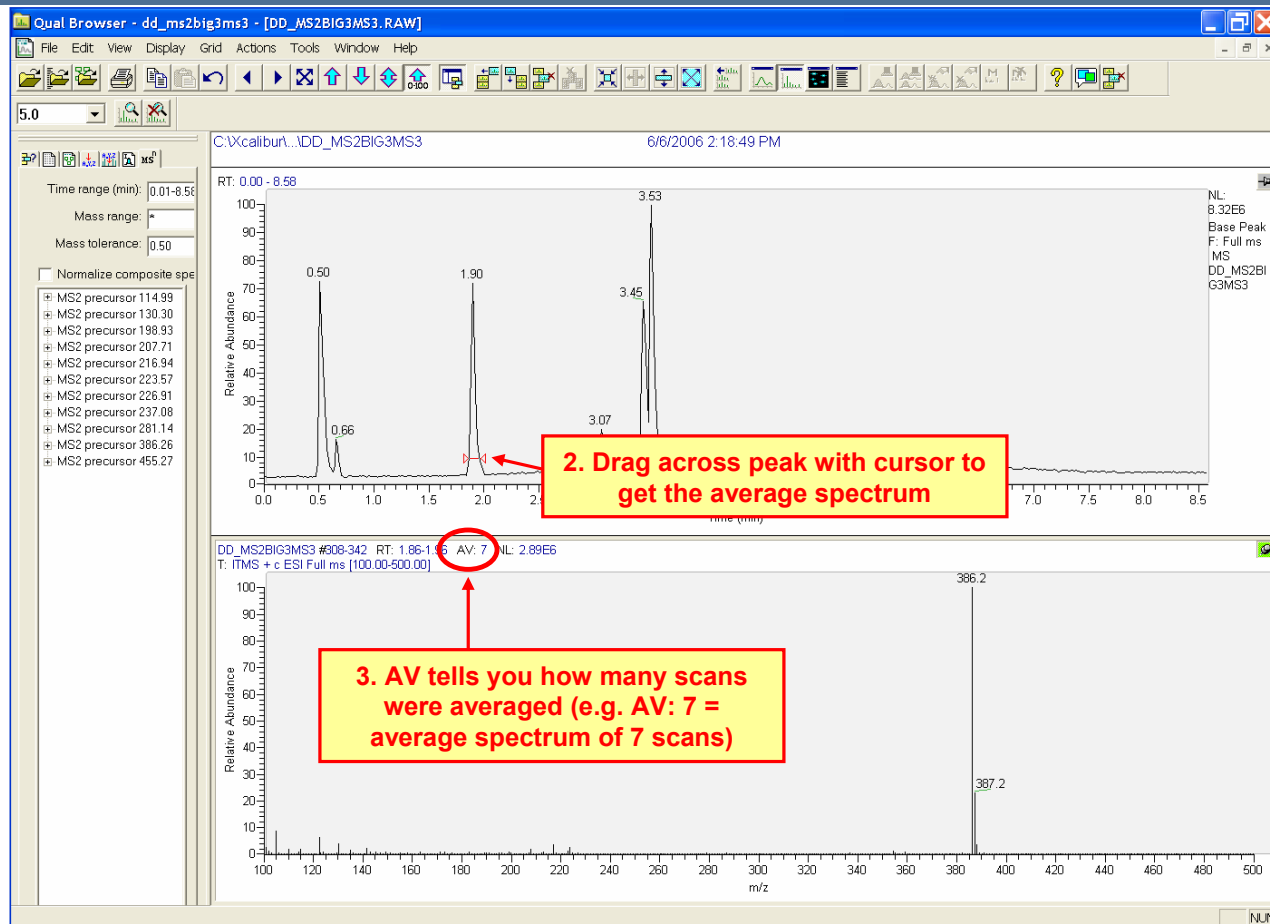
3. Apply the layout to subsequent samples

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

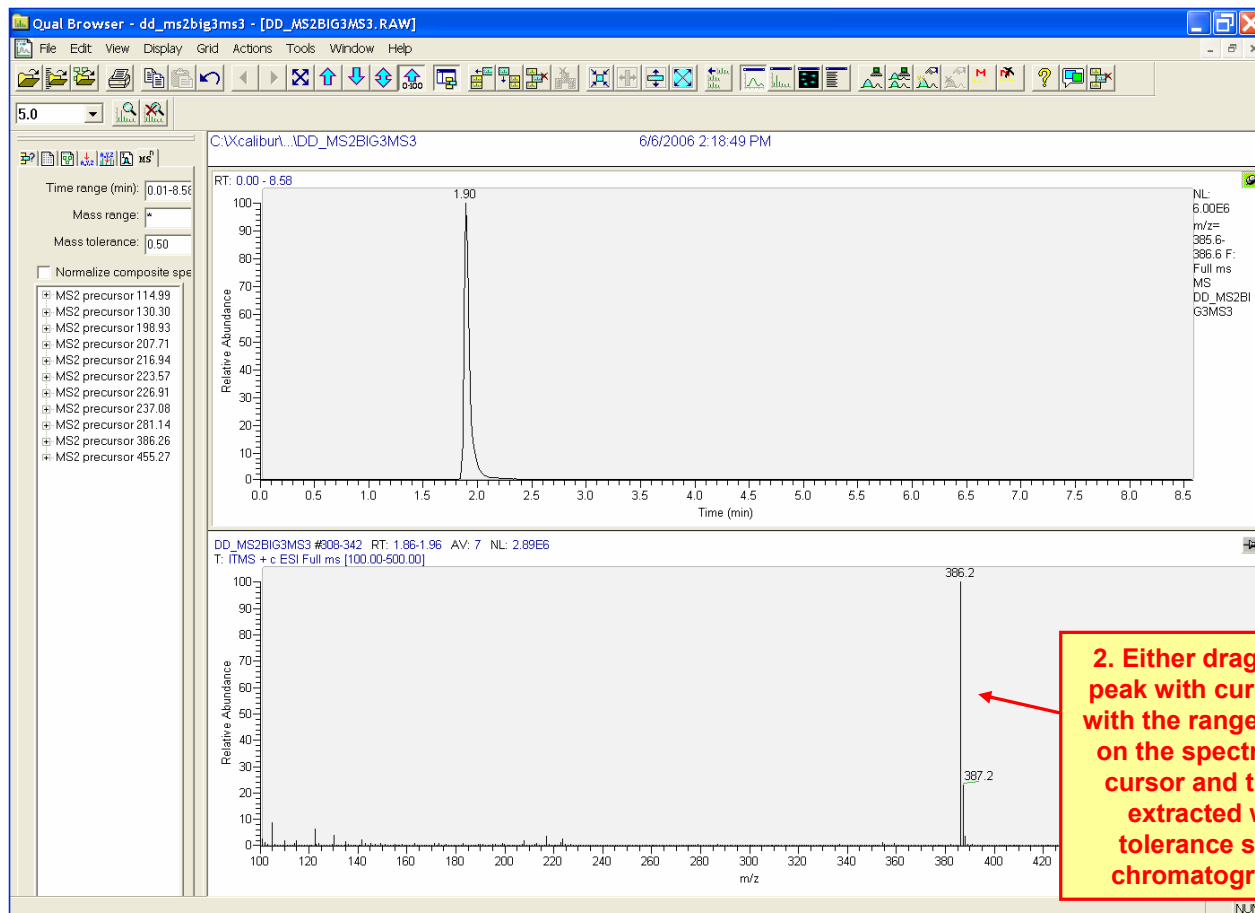
To Zoom In...



Getting an Average Spectrum of a Peak in the Chromatogram



Extracting an Ion from the Chromatogram



1. Pin chromatogram

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

Chromatogram Right-Click Menu – Peak Detection

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

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

Manual Noise Region

RT Range: 0.0000-8.5848

Noise Method

INCQS Noise

Repetitive Noise

RMS

Min peak width: 3

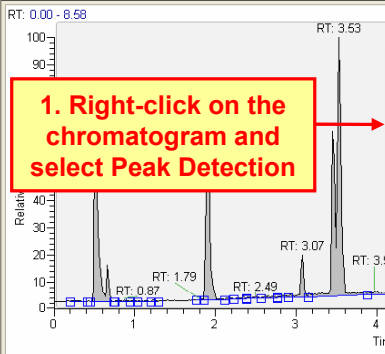
Multiplet resolution: 10

Area tail extension: 5

Area scan window: 0

Save as Defaults Load Defaults Help

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



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

View

Plot

Peak Detection

Peak Purity...

Library

Export

AutoFilter

Ranges...

Display Options...

Settings...

Toggle Detection in This Plot

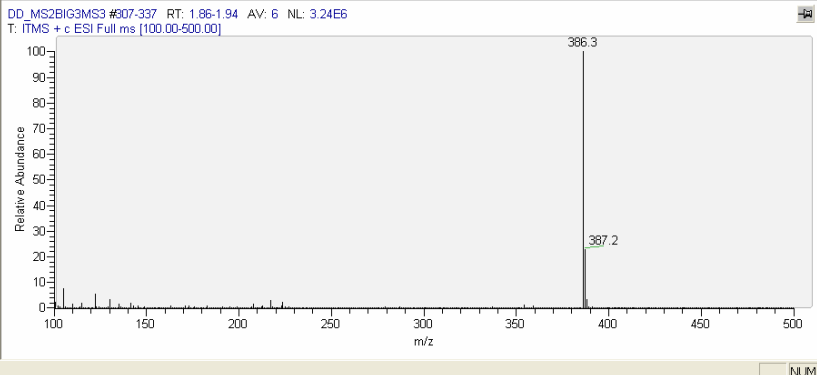
Toggle Detection in All Plots

Set Peak Detection Algorithm and Detect in this Plot

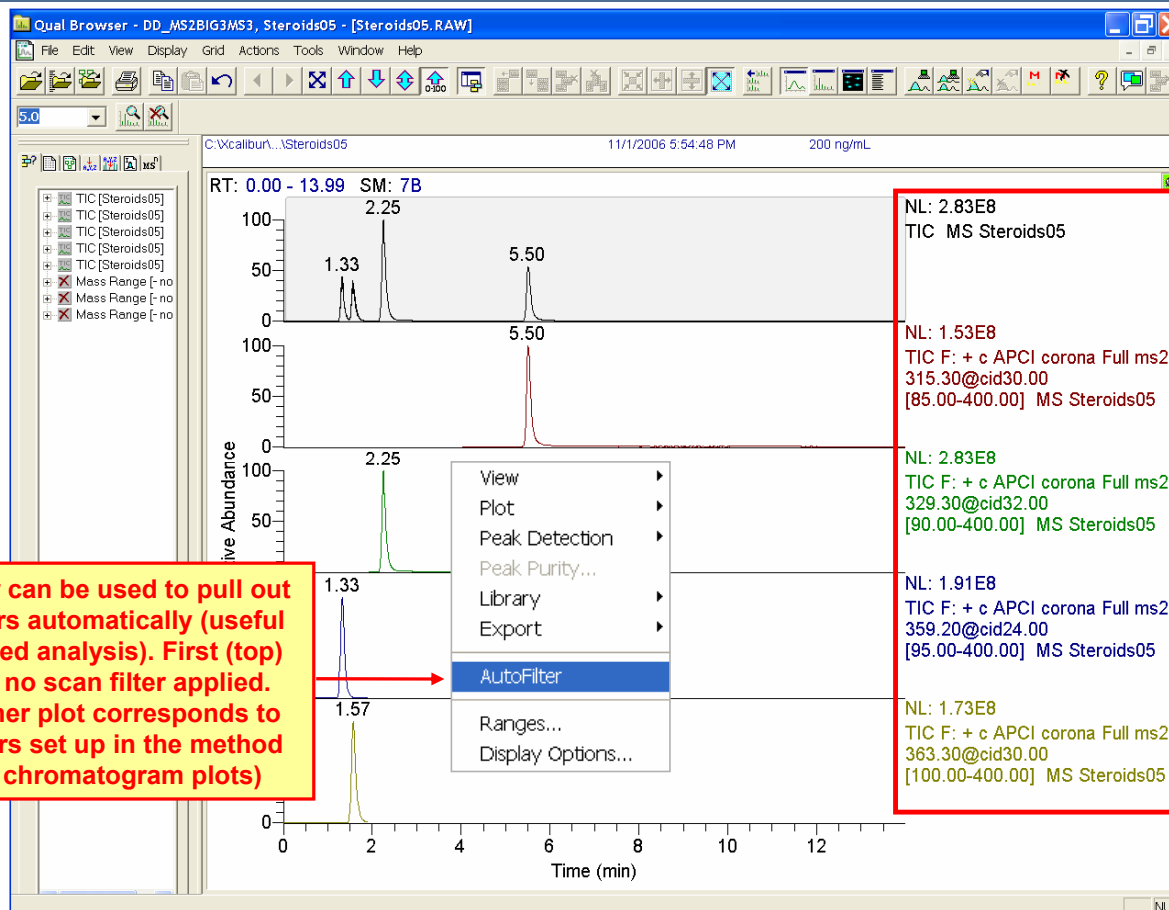
Set Peak Detection Algorithm and Detect in all Plots

Add Peaks

Delete Peaks



Chromatogram Right-Click Menu – AutoFilter



Chromatogram Right-Click Menu – Chromatogram Ranges

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

The screenshot shows the Qual Browser interface. The main window displays a chromatogram with a peak at 3.53 minutes. A right-click context menu is open over the peak, with the 'Ranges...' option selected. The 'Chromatogram Ranges' dialog box is open, showing a table of ranges and plot properties.

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

Plot properties:

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

Chromatogram Right-Click Menu – Chromatogram Ranges

Chromatogram Ranges

Ranges | Automatic processing

Range

Time range (minutes): Fixed scale

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

Plot properties

Raw file: ... Detector:

Scan filter: Peak algorithm:

Plot type: Delay (min):

Range(s): Fix scale to:

OK Cancel Help

Check to add plots (8 max)

Click to change the raw file name

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

Chromatogram Ranges – Scan Filter

Chromatogram Ranges

Ranges | Automatic processing

Range

Time range (minutes): Fixed scale

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

Plot properties

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

Scan filter: Full ms

Plot type: ITMS + c ESI Full ms [100.00-500.00]

Range(s): ITMS + c ESI d Full ms2 114.77@cid35.00 [50.00-125.00]
ITMS + c ESI d Full ms2 130.15@cid35.00 [50.00-145.00]
ITMS + c ESI d Full ms2 130.30@cid35.00 [50.00-125.00]
ITMS + c ESI d Full ms2 130.35@cid35.00 [50.00-120.00]
ITMS + c ESI d Full ms2 130.37@cid35.00 [50.00-125.00]
ITMS + c ESI d Full ms2 130.37@cid35.00 [50.00-145.00]
ITMS + c ESI d Full ms2 130.39@cid35.00 [50.00-130.00]
ITMS + c ESI d Full ms2 130.39@cid35.00 [50.00-120.00]
ITMS + c ESI d Full ms2 130.60@cid35.00 [50.00-130.00]
ITMS + c ESI d Full ms2 130.60@cid35.00 [50.00-145.00]

Detector: MS

Peak algorithm: ICIS

Delay (min): 0.00

Cancel Help

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

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

Chromatogram Ranges – Plot Types

Chromatogram Ranges

Ranges | Automatic processing

Range

Time range (minutes): * Fixed scale

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

Plot properties

Raw file: c:\xcalibur\data\tq_data\tqopsjune06\dd_ms2big3ms3.raw ... Detector: MS

Scan filter: Full ms Peak algorithm: ICIS

Plot type: Base Peak

Range(s):

- Mass Range
- TIC
- Base Peak
- Neutral Fragment

Delay (min): 0.00

OK Cancel Help

1. Click to change the Plot type

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

Chromatogram Ranges – Extracted Ion Chromatogram

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

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

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

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

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

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

Chromatogram Ranges – Neutral Fragment

The Neutral Fragment Plot type will plot any ions that have a specific neutral loss that you specify (from the MS to the MS²)

Chromatogram Ranges

Ranges Automatic processing

Range

Time range (minutes): Fixed scale

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

Plot properties

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

Scan filter: Peak algorithm: ICIS

Plot type: Neutral Fragment Delay (min): 0.00

Mass: 176.03 Fix scale to: 1000000.00

OK Cancel Help

1. Delete Scan filter

2. Select Neutral Fragment

3. Type Neutral Fragment mass

Chromatogram Ranges – Automatic Processing tab

1. Can enable smoothing for the chromatogram plot

The screenshot shows the 'Chromatogram Ranges' dialog box with the 'Automatic processing' tab selected. The 'Smoothing' section is highlighted with a red box. Inside this section, the 'Enable' checkbox is checked, the 'Type' dropdown is set to 'Boxcar', and the 'Points' field contains the value '7'. A red callout box points to the 'Points' field with the text 'Smoothing points must be an odd number'. Other sections include 'Baseline subtraction' (disabled), 'Mass tolerance' (500.0, units: mmu), and 'Mass precision' (2 decimals). The 'Include peaks' section has the 'Reference and exception peaks' checkbox unchecked. The dialog has 'OK', 'Cancel', and 'Help' buttons at the bottom.

Smoothing points must be an odd number

Chromatogram Right-Click Menu - Display Options

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

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

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

Display Options

Style | Color | Labels | Axis | Normalization

Plotting: Point To Point, Stick

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

3D: Elevation: 0 30 60, Skew: 0 0 45

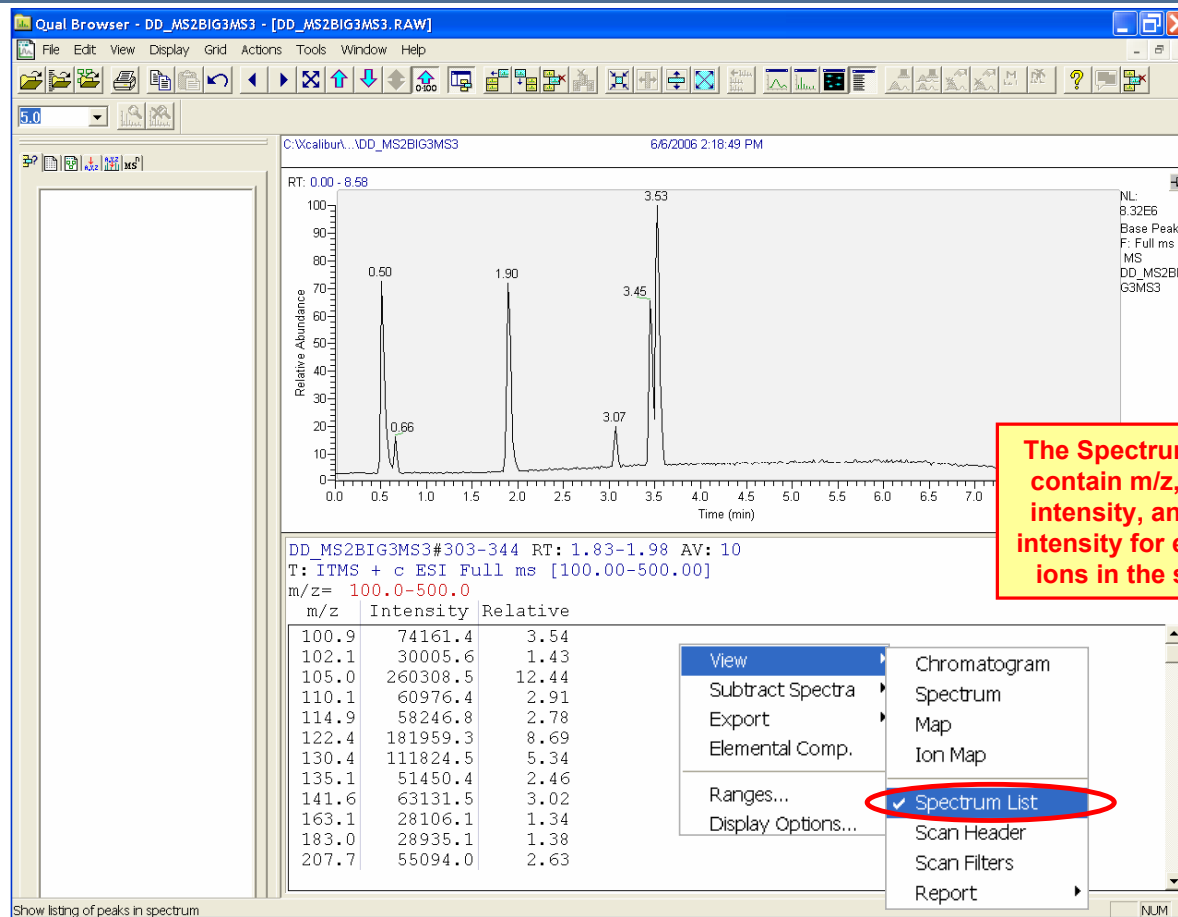
Draw Backdrop

RT: 0.00 - 8.58

Relative Abundance vs Time (min)

OK Cancel Help

Spectrum Right-Click Menu – Spectrum List



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

Spectrum Right-Click Menu – Scan Header

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

The screenshot displays the Qual Browser interface. At the top, the window title is "Qual Browser - DD_MS2BIG3MS3 - [DD_MS2BIG3MS3.RAW]". The main area shows a chromatogram with relative abundance on the y-axis (0 to 100) and time in minutes on the x-axis (0.0 to 8.5). Several peaks are labeled with their retention times: 0.50, 0.66, 1.90, 3.07, 3.45, and 3.53. The scan header for scan 303 at RT 1.83 is displayed below the chromatogram, showing scan mode (ITMS + c ESI Full ms) and various parameters. A right-click context menu is open over the scan header, with the "Scan Header" option highlighted by a red circle.

Qual Browser - DD_MS2BIG3MS3 - [DD_MS2BIG3MS3.RAW]

File Edit View Display Grid Actions Tools Window Help

5.0

C:\xcalibur\..._DD_MS2BIG3MS3 6/6/2006 2:18:49 PM

RT: 0.00 - 8.58

Relative Abundance

Time (min)

NL: 8.32E6
Base Peak
F: Full ms
MS
DD_MS2BI
G3MS3

DD_MS2BIG3MS3#303 RT: 1.83

Scan Mode: ITMS + c ESI Full ms [100.00-500.00]

LTQ Data:
=====

AGC: On

Micro Scan Count: 1

Ion Injection Time (ms): 6.065

Scan Segment: 1

Scan Event: 1

Master Index: 0

Elapsed Scan Time (sec): 0.09

API Source CID Energy: 0.00

Average Scan by Inst: No

Charge State: 0

Monoisotopic M/Z: 0.0000

View

Chromatogram

Subtract Spectra

Spectrum

Export

Map

Elemental Comp.

Ion Map

Ranges...

Spectrum List

Display Options...

Scan Header

Scan Filters

Report

Show statistics and genealogy for current scan

Spectrum Right-Click Menu – Tune and Instrument Methods

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

Qual Browser - DD_MS2BIG3MS3 - [DD_MS2BIG3MS3.RAW]

File Edit View Display Grid Actions Tools Window Help

C:\caliburA..._DD_MS2BIG3MS3 6/6/2006 2:18:49 PM

RT: 0.00 - 8.58

Relative Abundance

Time (min)

View

- Chromatogram
- Spectrum
- Map
- Ion Map
- Spectrum List
- Scan Header
- Scan Filters
- Report
- Tune Method**
- Instrument Method**
- Sample Information
- Status Log
- Error Log

Segment 1 Information

Duration (min): 10.00

Number of Scan Events: 5

Tune Method: hydrocortisone

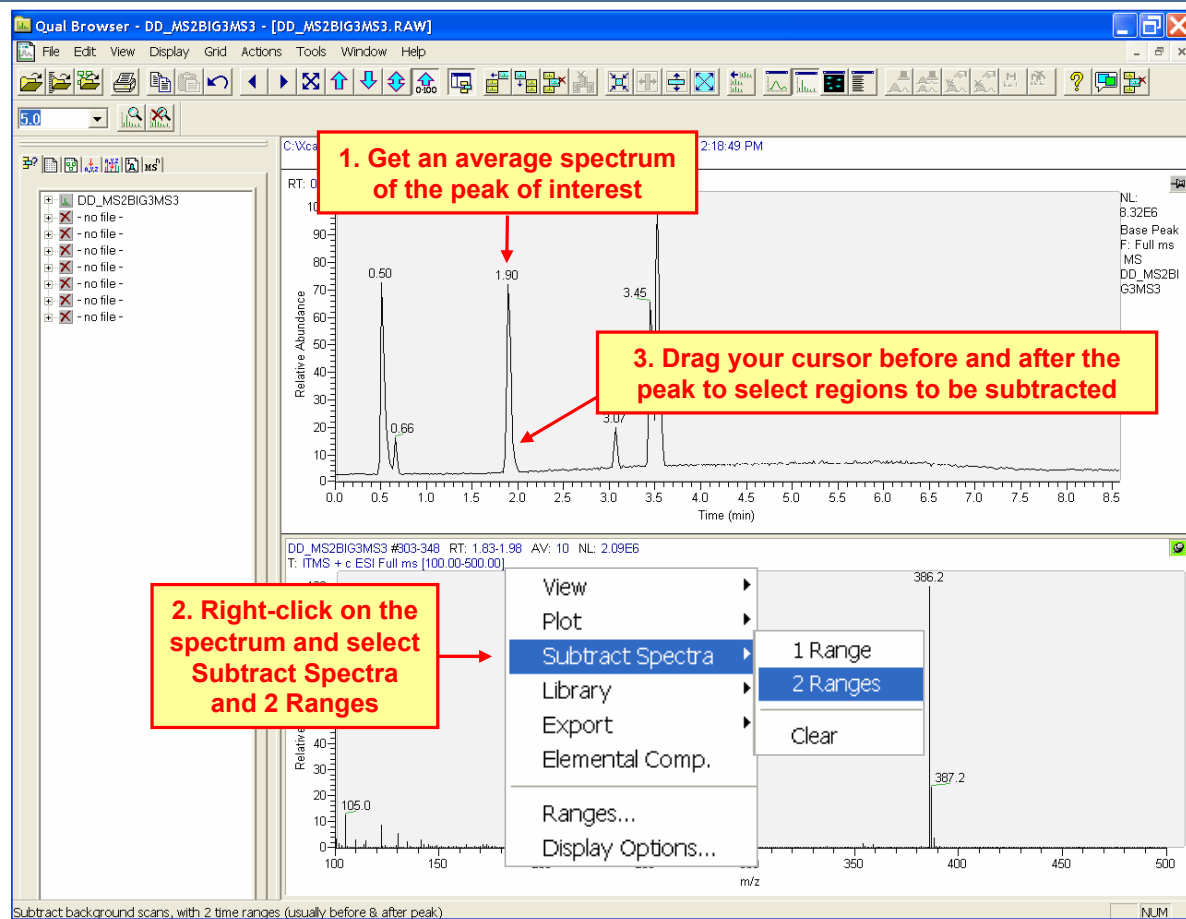
Scan Event Details:

- 1: ITMS + c norm o(100.0-500.0)
- 2: ITMS + c norm Dep MS/MS Most intense ion fr
- 3: ITMS + c norm Dep MS3 Most intense ion fr
- 4: ITMS + c norm Dep MS/MS 2nd most intense i
- 5: ITMS + c norm Dep MS/MS 3rd most intense i

Data Dependent Settings:

Display the instrument method used to collect this raw file

Spectrum Right-Click Menu – Spectral Subtraction



Spectrum Right-Click Menu - Spectrum Ranges

The screenshot shows the Qual Browser interface with a mass spectrum plot. The plot displays Relative Abundance on the y-axis (0 to 100) and m/z on the x-axis (0.0 to 1.5). A peak is labeled at 0.50. The plot title is 'C:\calibur_DD_MS2BIG3MS3' and the retention time range is 'RT: 0.00 - 8.58'. A right-click context menu is open over the spectrum, listing options: View, Plot, Subtract Spectra, Library, Export, Elemental Comp., **Ranges...**, and Display Options... A red box with the text '1. Right-click on the spectrum and select Ranges...' has an arrow pointing to the 'Ranges...' option. The 'Spectrum Ranges' dialog box is also open, showing the 'Automatic Processing' tab. The 'Range' section has 'Mass range: 1.00 0-500.0', 'Average' checked, and 'Fix scale: 1000000.0'. The table below shows one entry: Time: 1.83-1.99, Filter: Full ms, Raw File: C:\calibur\data\LTQ Data..., Subtract 1: -, Subtract 2: -. The 'Plot properties' section shows 'Detector: MS', 'Time: 1.83-1.99', 'Filter Type: Scan', and 'Filter: Full ms'. The 'Background Subtraction' section has 'Time range 1: 0.01' and 'Time range 2: 0.01'. The 'Raw file' is 'c:\calibur\data\lq_data\lqopsjune06\dd_ms2big3ms3.r'. The dialog has 'OK', 'Cancel', and 'Help' buttons.

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

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

Spectrum Right-Click Menu - Spectrum Ranges

Spectrum Ranges [Close]

Ranges | Automatic Processing

Range

Mass range: Average Fix scale:

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

Plot properties

Detector: Time:

Filter Type: Scan Process

Filter:

Raw file: ...

Background Subtraction

Time range 1:

Time range 2:

Simulation

OK Cancel Help

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

Presentation

1. To add text, click Display and select Annotate

3. Click on pinned chromatogram to annotate

2. Type Annotation

Annotation text: Boxed Rotated Pointer

Test

Marked position is:
 Left Center Right

Height drawn:
 Just above graph
 Above marked position
 Below marked position

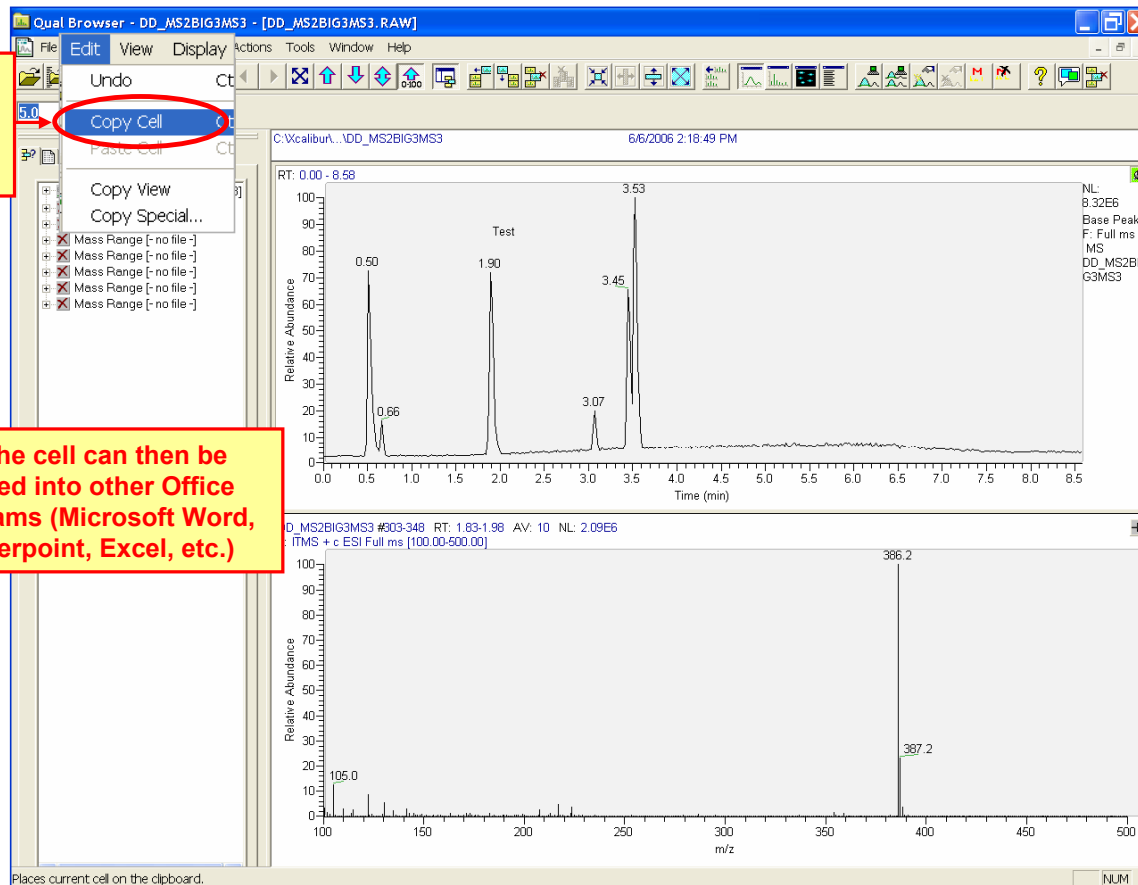
Multiple lines aligned:
 Left Center Right

OK Cancel Help

Chromatogram Capture

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

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



ThermoFisher
S C I E N T I F I C

The world leader in serving science

Chapter 13

Quantitative Processing

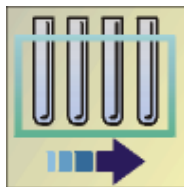
Quantitative Processing

1. Processing Setup



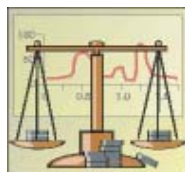
Input known compounds for identification
Set up peak detection/integration parameters
Choose calibration/QC type, levels, weighting
Select advanced chromatographic processing

2. Sample Processing/Reprocessing



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

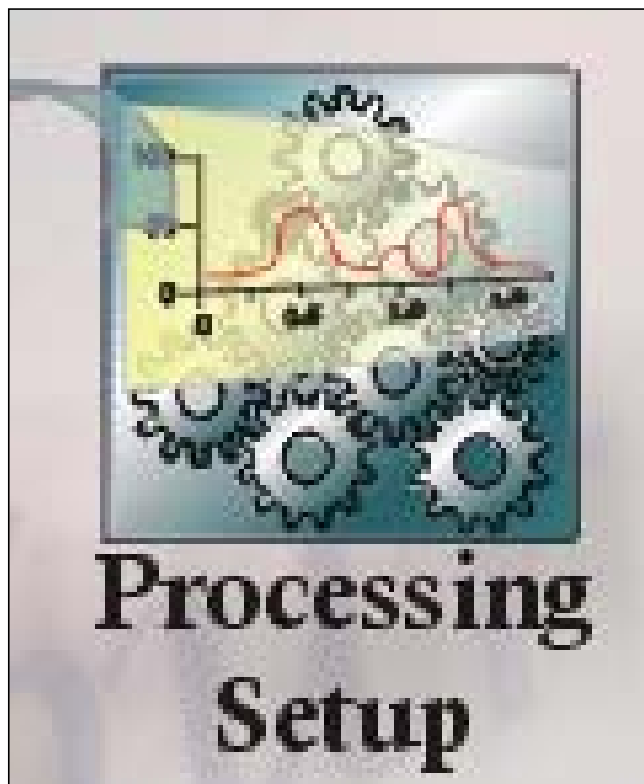
3. Quan Browser



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

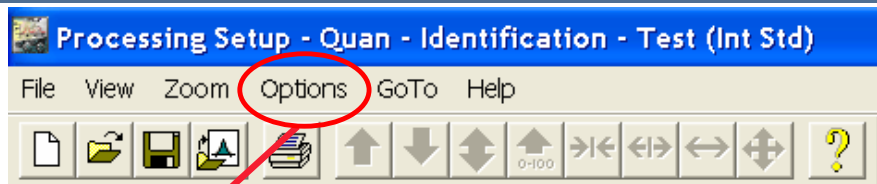
Quan Processing Setup

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

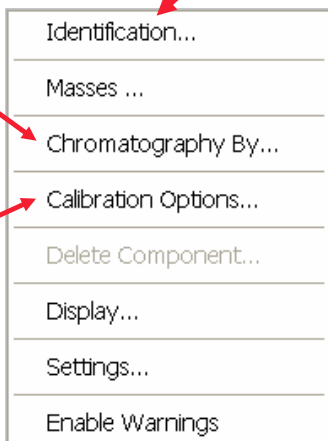


Quantitation Options

1. Click Options

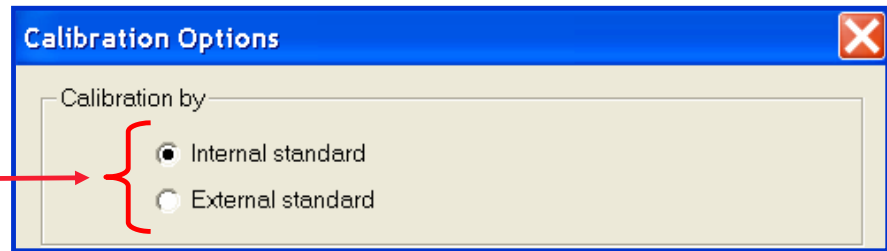
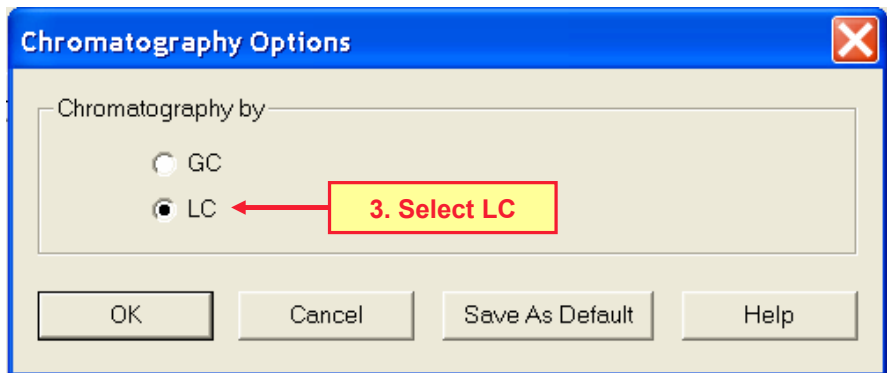


2. Click Chromatography By...



4. Select Calibration Options...

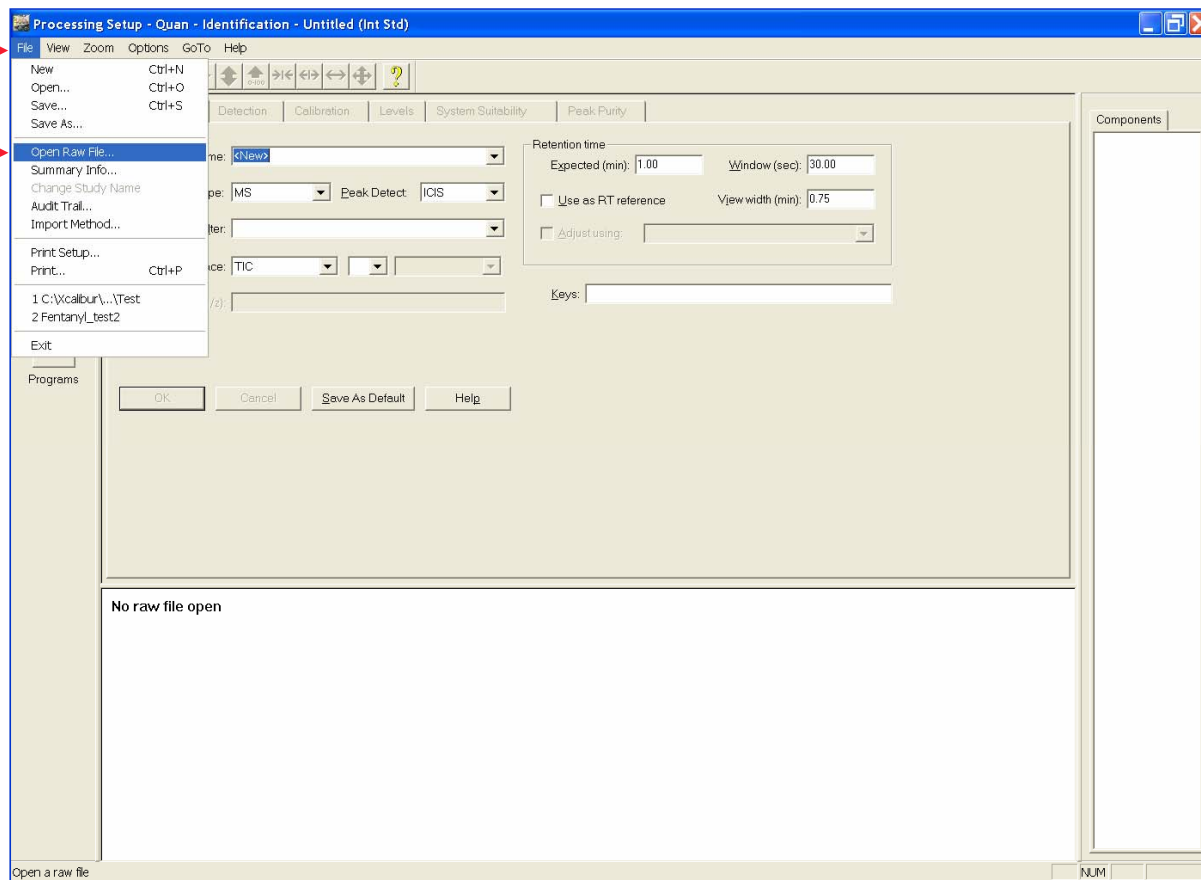
5. Choose whether to use an Internal or External standard



Open a Raw File to Set Up the Processing Method

1. Click File

2. Click Open Raw File...



Quan Processing – Identification Tab

1. Select <New> and type name of component

2. Select Detector type and Peak Detection algorithm

3. Select scan filter and trace type

4. Click OK to update chromatogram

Chromatogram changes to reflect scan filter and trace type selected

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

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Name: Steroid_X

Detector type: MS Peak Detect: IOS

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

Trace: TIC

Retention time: Expecte

Use a Adjusting

Keys:

OK Cancel Save As Default Help

Components

Steroid_X

Ready

NUM NOT SAVED

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

RT: 0.00 - 9.99 SM: 1G

8.04

NL: 6.61E5

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

Relative Abundance

297.20245

279.25766

215.14893

171.14845

96.94240

Time (min)

m/z

Quan Processing – Identification Tab

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

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Retention time

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

Use as RT reference Viewwidth (min): 0.75

Adjust using: _____

Keys: _____

Trace: TIC

Mass (m/z)

Reports

ms

OK Cancel Save As Default Help

Ready

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

RT: 0.00 - 9.99 SM: 1G

8.04

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

Relative Abundance

Time (min)

3.22 4.34 4.75 5.59 5.99 7.24 8.83 9.57

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

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

Relative Abundance

m/z

96 171 215 279 297

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

Quan Processing – Detection Tab

1. Click on Detection tab

2. Modify parameters to achieve satisfactory peak integration

3. Click OK for changes to update on chromatogram

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

File View Zoom Options GoTo Help

Detection Calibration Levels System Suitability Peak Purity

ICIS Peak Integration

Smoothing points: 7

Baseline window: 150

Area noise factor: 5

Peak noise factor: 10

Constrain peak width

Peak height (%): 5.0

Tailing factor: 1.0

ICIS Peak Detection

Highest peak

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

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

RT: 7.05 - 9.05 SM: 7G

RT: 8.05

Relative Abundance

Time (min)

NL: 6.45E5

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

Relative Abundance

m/z

96.94200 108.94489 173.11150 215.23209 279.26367 297.23602

Ready NUM NOT SAVED

Quan Processing - Calibration Tab Internal Standard Setup

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

File View Options GoTo Help

1. Click on Calibration tab

2. Select ISTD

3. Type Amount and Units for ISTD

Target compounds: ISTD: [dropdown] Isotope %: [dropdown]

Target compound
 ISTD

Integration curve: Average RF

Units: [dropdown]

ISTD:

Amount: 5.000
Units: ng/mL

Origin:
 Ignore
 Forge
 Include

Response:
 Area
 Height

Components:
Steroid_Y
Steroid_X

OK Cancel Save As Default Flags... Help

Ready NUM NOT SAVED

Quan Processing - Calibration Tab Target Compound Setup

The screenshot shows the 'Calibration' tab of the 'Processing Setup' dialog. The 'Component type' section has 'Target compound' selected. The 'Target compounds' list shows 'Steroid_X' selected. The 'Weighting' section has 'Equal' selected. The 'Origin' section has 'Ignore' selected. The 'Response' section has 'Area' selected. The 'Calibration curve' is set to 'Linear'. Three red callout boxes provide instructions: 1. '1. Select Target compound' points to the 'Target compound' radio button. 2. '2. Select how to weight the calibration curve (if at all)' points to the 'Weighting' options. 3. '3. Select what to do with the origin on the calibration curve' points to the 'Origin' options. A fourth callout box, 'If using ISTD, select ISTD here', points to the 'ISTD' radio button.

1. Select Target compound

2. Select how to weight the calibration curve (if at all)

If using ISTD, select ISTD here

3. Select what to do with the origin on the calibration curve

Quan Processing – Levels Tab

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

File View Options GoTo Help

Contribution Levels System Suitability Peak Purity

Units:

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

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

2. Enter name for each calibration level

3. Enter amount for each calibration level

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

OK Cancel Save As Default Help

Ready NUM NOT SAVED

Thermo SCIENTIFIC

224

ThermoFisher SCIENTIFIC

Copying Levels to All Target Compounds

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

File View Options GoTo Help

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

Units:

Cal Level	Amount	QC Level	Amount	% Test	
1	2 ng/mL	1	QC1	5.000	20.00
2	10 ng/mL				
3	50 ng/mL				
4	200 ng/mL				
5	1000 ng/mL				

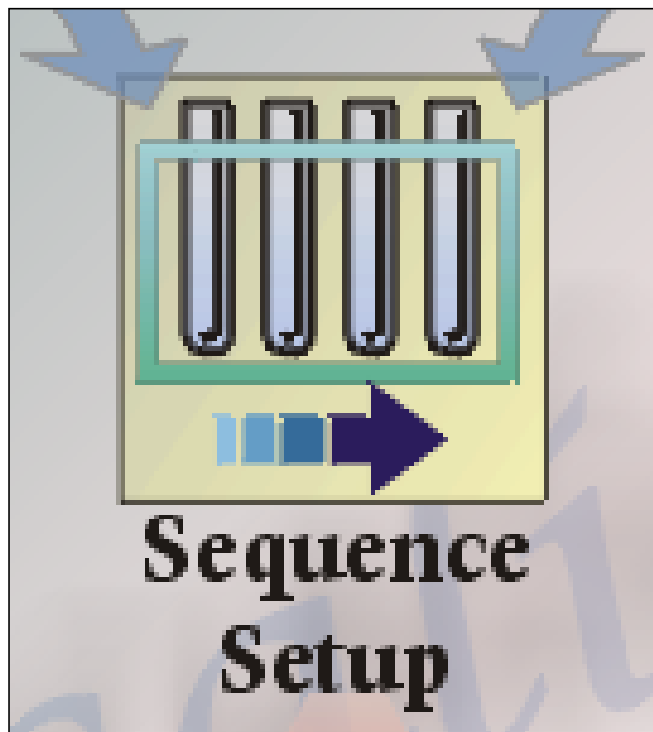
Components
Steroid_Y
Steroid_X

Ready NUM

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

Quan Processing/Reprocessing

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



Open the Sequence and Add Extra Columns

1. Click Change and select Column Arrangement

TempSequence_060530121729 [Open] - Sequence Setup - Home Page

File Edit Change Actions View GoTo Help

User Labels...
Tray Name...
Column Arrangement...

Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol	Level
1	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A1	20.0	
2	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A2	20.0	Cal1
3	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A3	20.0	Cal2
4	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A4	20.0	Cal3
5	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A5	20.0	Cal4
6	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A6	20.0	Cal5
7	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A1	20.0	
8	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A8	20.0	Low
9	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1B1	20.0	Mid
10	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1B2	20.0	High
11	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1A1	20.0	
12	C:\Xcalibur\Data\Orbitrap Data\SteroidQuantite	C:\Xcalibur\methods\Amber\SteroidQuan_IT	C:\Xcalibur\data\Ort	1B4	20.0	
*					0.1	

Column Arrangement

Available Columns

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

Displayed Columns

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

Buttons: Add, Remove, Move Up, Move Down, OK, Cancel, Help

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

Enter Information into the Sequence

TempSequence_060530121729 [Open] - Sequence Setup - Home Page

File Edit Change Actions View GoTo Help

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

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

Batch Reprocessing Quantitative Data

1. Click Actions and select Batch Reprocess

2. Check Quan, Peak Detection & Integration and Quantitation

3. Click OK to process

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

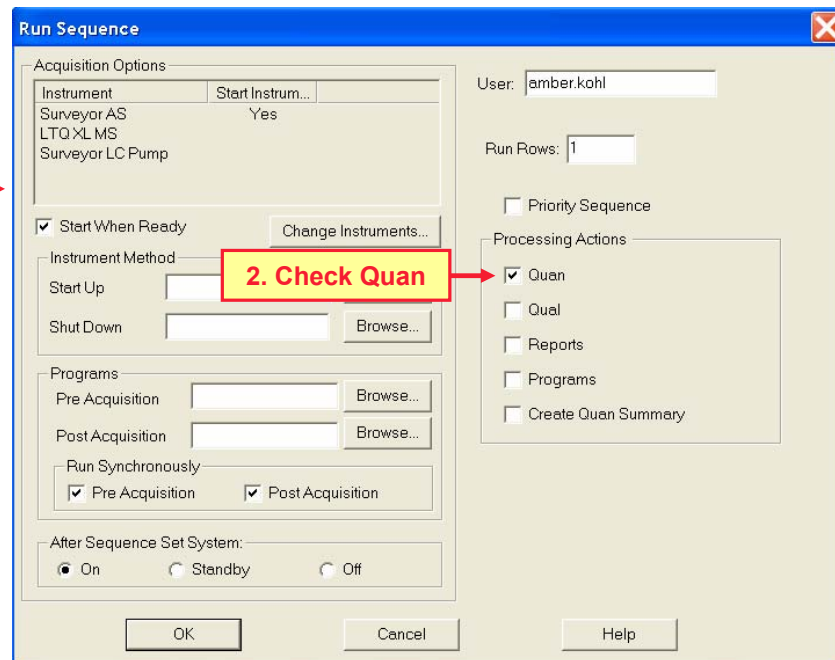
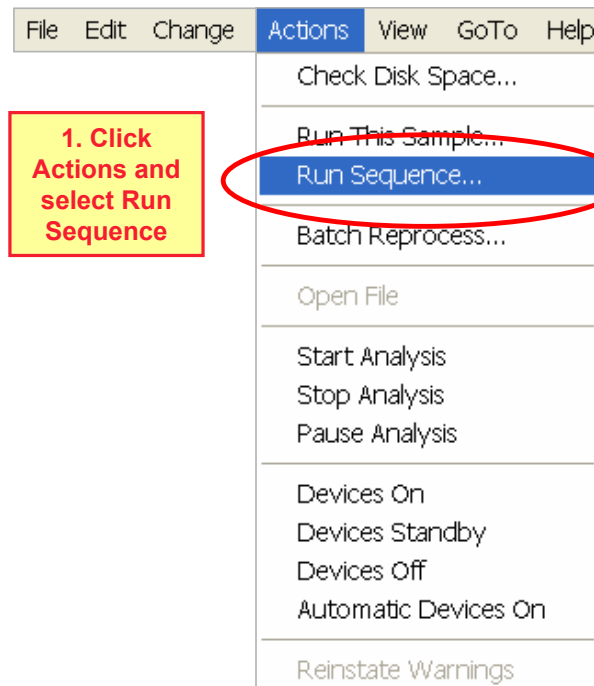
Advanced Options

- Replace Sample Info

Process Rows: 1-12

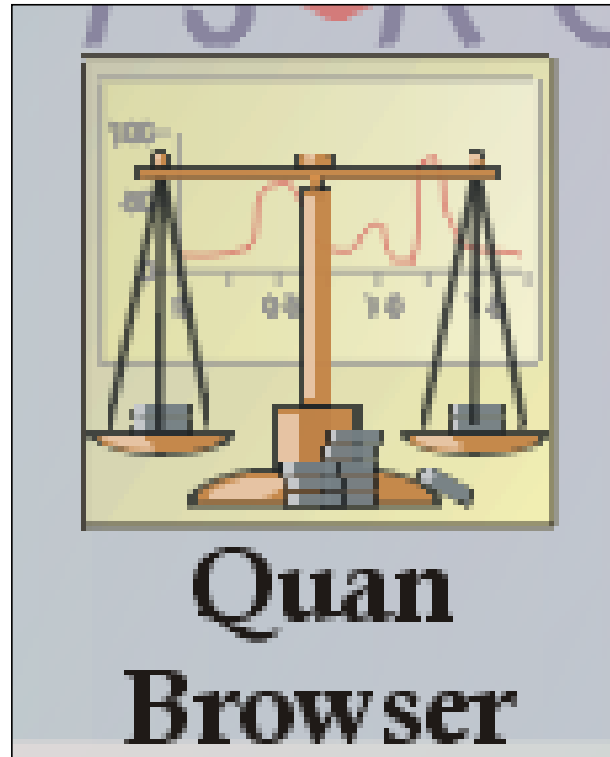
OK Cancel Help

Enabling Quantitative Processing During Acquisition



Quan Browser

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



Quan Browser Main View

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

File View Zoom Options GoTo Help

Bracket in use: Bracket 1 Calibration File: Embedded Calibration

	File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specified Am
1	SteroidQuantitation-5-30-0602	Standard		Method Settings	16175	NA	NA	
2	SteroidQuantitation-5-30-0603	Standard		Method Settings	96664	NA	NA	11
3	SteroidQuantitation-5-30-0604	Standard		Method Settings	493705	NA	NA	5
4	SteroidQuantitation-5-30-0605	Standard		Method Settings	2414778	NA	NA	20
5	SteroidQuantitation-5-30-0606	Standard		Method Settings	6904492	NA	NA	100

Results Grid

You can display either All, Standards, QCs, Blanks, Unknowns

Steroid_Y
Steroid_X

Select which component to view the data for that component

RT: 1.94 - 2.69 SM: 7G
RT: 2.31
NL: 8.39E4
TIC F: ITMS + c APCI
corona Full.ms2
329.30@cid35.00
[90.00-340.00] MS ICIS
SteroidQuantitation-5-30-0604

Chromatogram

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

Area

Calibration Curve

NUM 8/29/2007 7:46 AM

Changing the Results Grid Display

The screenshot displays the 'Quan Browser - Manual.QXN (Bracket 1, View All)' window. The main Results Grid shows a table with columns: File Name, Sample Type, Sample Name, and Integration Type. A context menu is open over the grid, with 'Columns...' and 'Set Sorting Order...' highlighted. The 'Result List Column Hiding' dialog is open, showing a list of columns with checkboxes for selection. The 'Quantitation Results Sorting Order' dialog is also open, showing sorting options for columns.

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

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

Can change the columns that are displayed in the Results Grid

Can change the sorting order of the columns

RT: 1.94 - 2.69 SM: 7G
RT: 2.31
NL: 8.39E4
TIC F: ITMS + c APCI
corona Full.ms2
329.30@cid35.00
[90.00-340.00] MS ICIS
SteroidQuantitation-5-30-0604

Relative Abundance
Time (min)

Area

NUM 8/29/2007 7:46 AM

Changing Peak Detection/Integration Parameters

4. Integration Type changes to User Integration

File Name	Sample Type	Sample Name	Integration Type
1			Method Settings
2			Method Settings
3			User Integration
4			Method Settings
5			Method Settings

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

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

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

User Identification Settings

Identification | Detector | ICIS Integration | ICIS Advanced | Flags

Name: Steroid.Y
Detector: MS
Plot Type: TIC
Scan Filter: ITMS + c APCI corona Full ms2 329.30@cid35
Mass (m/z):
Keys:
Retention Time: Expected (min): 2.29 Window (sec): 30.00
 Use as RT Reference View Width (min): 0.75
 Adjust Using

User Identification Settings

Identification | Detector | **ICIS Integration** | ICIS Advanced | Flags

Smoothing Points: 0
Baseline Window: 80
Area Noise Factor: 5
Peak Noise Factor: 10
 Constrain Peak Width
Peak Ht (%): 5.0
Tailing Factor: 1.0

Changing Peak Integration Parameters Manually

The screenshot displays the Thermo Quan Browser interface. At the top, a table lists integration parameters for several peaks. A red box highlights the 'Integration Type' column, with a red arrow pointing to the 'Manual Integration' entry for peak 3. Below the table, two chromatograms are shown. The left chromatogram shows a peak at RT: 2.31 with a blue box at its base. A red box with an arrow points to this box, labeled '1. Drag blue squares with cursor'. The right chromatogram shows a linear regression plot for 'Steroid_Y' with the equation $Y = 148400 + 8848.01 \cdot X$ and $R^2 = 0.9946$. A red box with an arrow points to the data points on the plot, labeled '2. Integration Type changes to Manual Integration'.

File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specified Am
			Method Settings	16175	NA	NA	
			Method Settings	96684	NA	NA	11
			Manual Integration	478265	NA	NA	8
			Method Settings	2414778	NA	NA	20
			Method Settings	8904492	NA	NA	100

RT: 1.94 - 2.69 SM: 7G
RT: 2.31
NL: 6.39E4
TIC F: ITMS + c APCI
corona Full ms2
329.30@cid35.00
[90.00-340.00] MS
SteroidQuantitation-5-30-0604

$Y = 148400 + 8848.01 \cdot X$ $R^2 = 0.9946$ W: Equal

NUM 8/29/2007 7:56 AM

Changing Back to Original Integration (Method Settings)

2. Integration Type changes back to Method Settings

File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specified Am
			Method Settings	16175	NA	NA	
			Method Settings	96684	NA	NA	11
			Method Settings	493705	NA	NA	9
			Method Settings	2414778	NA	NA	20
			Method Settings	8904492	NA	NA	100

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

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

Relative Abundance vs Time (min) chromatogram showing a peak at 2.13 min. The x-axis ranges from 2.0 to 2.6 minutes, and the y-axis ranges from 0 to 90. A peak is labeled with its retention time, 2.13.

Linear regression plot for Steroid_Y: $175+8843.08 \cdot X$ $R^2 = 0.9946$ W: Equal. The x-axis ranges from 0 to 1000, and the y-axis ranges from 0 to 90. Two data points are plotted, and a line of best fit is shown.

NUM 8/29/2007 8:51 PM

Changing Calibration Parameters

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

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

RT: 1.94 - 2.69 SM: 7G

Relative Abundance

Time (min)

2.00 2.13

2.0 2.1 2.2 2.3 2.4 2.5 2.6

Area

0 500 1000

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

Calibration Settings

Type Curve Levels Isotope% Flags

Calibration Curve

Linear

Origin

Ignore

Force

Include

Response

Area

Height

Weighting

Equal

1/X

1/X^2

1/Y

1/Y^2

1/s^2

Units:

OK Cancel Apply Help

Calibration Settings

Type Curve Levels Isotope% Flags

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

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

Units:

OK Cancel Apply Help

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

Ways to Exclude a Calibration Curve Point

The screenshot displays the 'Quan Browser - Manual.XQN (Bracket 1, View All)' interface. At the top, there is a menu bar (File, View, Zoom, Options, GoTo, Help) and a toolbar. Below the menu, a dropdown shows 'Bracket in use: Bracket1' and a text field for 'Calibration File: Embedded Calibration'. The main area contains a table with the following data:

	File Name	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	% Diff	Level	RT	Exclude
1	SteroidQuantitation-5-30-0602	NA	NA	2.000	-15.492	-874.62	2 ng/mL	2.29	<input type="checkbox"/>
2	SteroidQuantitation-5-30-0603	NA	NA	10.000	-6.368	-163.88	10 ng/mL	2.29	<input type="checkbox"/>
3	SteroidQuantitation-5-30-0604	NA	NA	50.000	-39.908	-22.98	50 ng/mL	2.29	<input checked="" type="checkbox"/>
4	SteroidQuantitation-5-30-0605	NA	NA	200.000	255.748	27.87	200 ng/mL	2.27	<input type="checkbox"/>
5	SteroidQuantitation-5-30-0606	NA	NA	1000.000	989.624	-1.04	1000 ng/mL	2.29	<input type="checkbox"/>

Below the table, a navigation bar shows 'All Standards QCs Blanks Unknowns'. The bottom section features a chromatogram plot of 'Relative Abundance' vs 'Time (min)'. A peak is visible at RT 2.31. A context menu is open over the plot, showing options: 'Exclude', 'Calibration Settings...', 'Exclusion List...', 'Show Spectrum Plot', 'Reset Scaling', and 'Copy Graph'. The 'Exclude' and 'Exclusion List...' options are circled in red.

Annotations in red boxes provide instructions:

- To exclude a calibration curve point, you can check to exclude in the Results Grid** (points to the 'Exclude' column in the table).
- To exclude a calibration curve point, right-click on the calibration curve** (points to the 'Exclude' and 'Exclusion List...' options in the context menu).
- Use Exclude to exclude one point at a time or the Exclusion List to exclude more than one point at a time** (points to the 'Exclude' and 'Exclusion List...' options in the context menu).

Showing the Spectrum Plot Instead of the Calibration Curve

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

File View Zoom Options GoTo Help

Bracket in use: Bracket1 Calibration File: Embedded Calibration

	File Name	Sample Type	Sample Name	Integration Type	Area	ISTD Area	Area Ratio	Specified Am
1	SteroidQuantitation-5-30-0602	Standard		Method Settings	16175	NA	NA	
2	SteroidQuantitation-5-30-0603	Standard		Method Settings	96684	NA	NA	11
3	SteroidQuantitation-5-30-0604	Standard		Method Settings	493705	NA	NA	5
4	SteroidQuantitation-5-30-0605	Standard		Method Settings	2414778	NA	NA	20
5	SteroidQuantitation-5-30-0606	Standard		Method Settings	8904492	NA	NA	100

Steroid_Y
Steroid_X

RT: 1.94 - 2.69 SM: 7G
RT: 2.31
NL: 8.39E4
TIC F: ITMS + c APCI
corona Full ms2
329.30@cid35.00
[90.00-340.00] MS ICIS
SteroidQuantitation-5-30-0604

Y = 153175+8843.08*X

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

To show the spectrum at the cursor position, right-click on the calibration curve and select Show Spectrum Plot

NUM 8/29/2007 9:02 PM

Exporting Data to Excel and Printing Reports

The screenshot displays the Quan Browser software interface. The top menu bar includes File, View, Zoom, Options, GoTo, and Help. The main window is divided into several sections:

- File Menu:** Open... (Ctrl+O), Save (Ctrl+S), Save As..., Save All, Export Method..., Export data to Excel, Summary Information..., Change Dataset Name..., Audit Trail..., Print Setup..., Print, 1 Manual, 2 TempSequence_060530121729, 3 FT_List_061018172746, 4 Steroid Quantitation Sequence, Exit.
- Table:** A table with columns: ISTD Area, Area, Specified Amount, Calculated Amount, and Exclude. The table contains several rows of data, with one row highlighted in blue. A red circle highlights the 'Export Short Excel report' button, and a red arrow points to a yellow box containing the text 'Click to export data to Excel'. Another red circle highlights the 'Reports Dialog...' button, and a red arrow points to a yellow box containing the text 'See next slide'.
- Print Menu:** Print All Enabled Reports, Print Enabled Sample Reports, Print Enabled Summary Reports.
- Chromatograms:** Two chromatograms are shown. The left one is a Total Ion Chromatogram (TIC) showing a single peak at RT: 2.31. The right one is a mass spectrum showing relative abundance versus m/z, with several peaks labeled with their m/z values.

Printing Reports

Reports

Sample Reports - 0 selected samples

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

Summary Reports

Enabled	Save As	Report Template Name
*	None	

Include Sample Reports Include Summary Reports

1. Click to enable reports

2. Select report template to use

3. Click to select samples

Selecting Samples to Include in the Report

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

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

Raw File	Sample Type
SteroidQuantitation...	Standard
SteroidQuantitation...	Standard
SteroidQuantitation...	Standard
SteroidQuantitation...	Standard
SteroidQuantitation...	Standard
SteroidQuantitation...	QC
SteroidQuantitation...	QC
SteroidQuantitation...	QC
SteroidQuantitation...	Blank
SteroidQuantitation...	Blank
SteroidQuantitation...	Blank
SteroidQuantitation...	Unknown

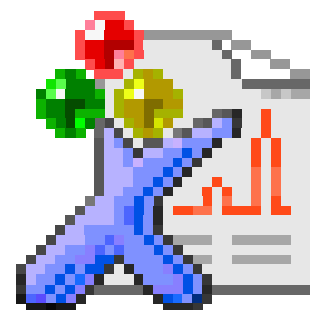
Raw File	Sample Ty...
SteroidQuantitatio...	Standard

Buttons: Add >>, << Remove, Add All >>, << Remove All, OK, Cancel, Help

ThermoFisher
SCIENTIFIC

The world leader in serving science

XReport 1.0



XReport 1.0 :

The Reporting Application for Xcalibur 2.0

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

Drag and Drop Interface: Quan Peak Results Canned Template

Available Sections and Objects

Report Template

Report Outline

The screenshot displays a software interface for creating a report template. On the left, a panel titled "Available Sections and Objects" contains various icons for report elements: "Non-Repeating", "Quan Repeating", "Qual Repeating", "Annotation", "Bitmap", "Page Break", "Columns", "Electronic Signature", "Chromatogram", "Component Calcurve", "Spectrum", "Avalon Quan Events Table", "Component Cal Level Table", "Component Ion Ratio Table", and "Component QC Level Table".

The central area, labeled "Report Template", shows a preview of a report section. At the top is a table with columns: Component Name[A], Component Name[C], Area[D], and Peak Status[E]. Below this is a "Section End" marker. The main content area is divided into two columns, each containing a chromatogram plot and a data table. The plots show "Relative Abundance" vs "Time (min)" with a peak at RT: 1.99. The data tables list parameters such as NL: 9.60E4, m/z: 266.5-267.5+, and Full MS: 203.30 | 100.00-310.00] MS steroid92.

On the right, a "Report Outline" panel shows a vertical stack of sections: "HEAD" (Header Section), "NR" (Non-Repeating Section), "QN" (Quan Repeating Section), and "FOOT" (Footer Section). The "QN" section is highlighted, indicating it is the active section being edited.

At the bottom right of the interface, the status bar shows "NUM | 5/12/2004 | 11:19:56 AM".

Steps to XReport Reporting

1. XReport



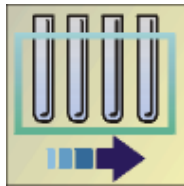
- Open XReport 1.0
- Drag and Drop required items into appropriate fields
- Specify Data Sources to view example report
- Save Report Template

2. Processing Setup



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

3. Reprocess Selected Files



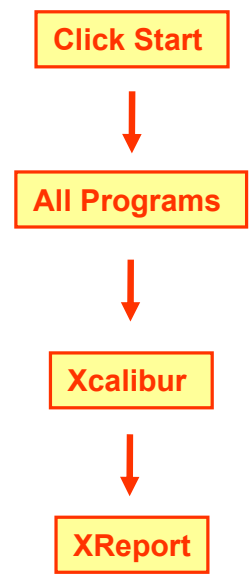
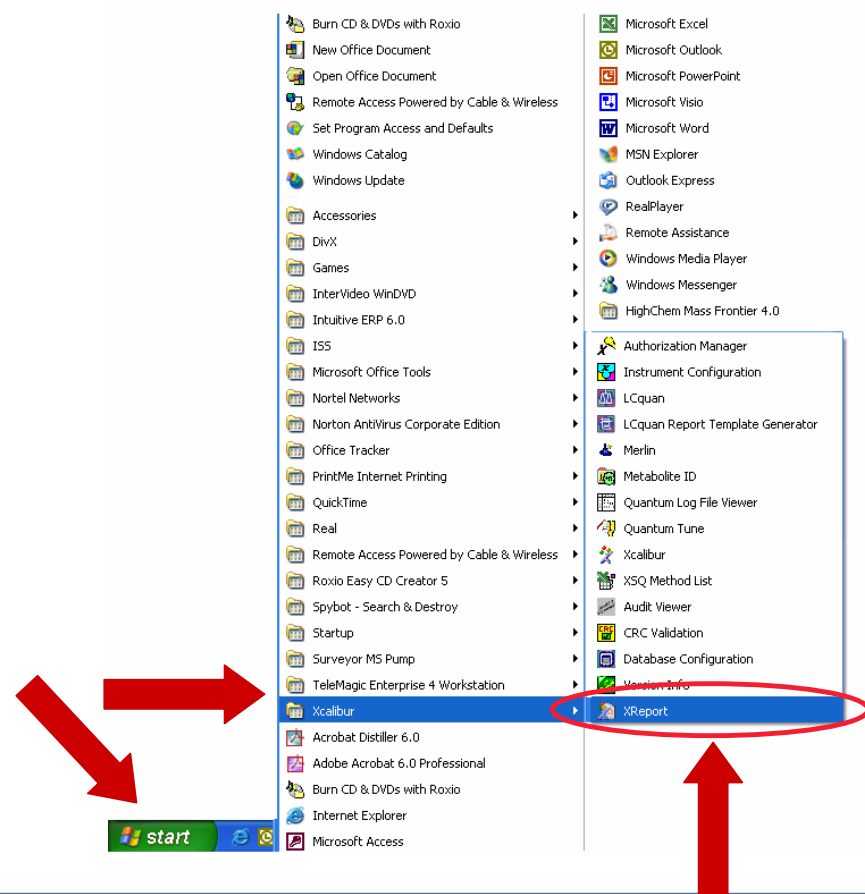
- Go to Home Page - Sequence Setup
- Open/Make a Sequence
- Click Actions : Batch Reprocess...
- Check Reports and Print Reports Boxes

Before you Start



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

Open XReport



Specify Data Sources...

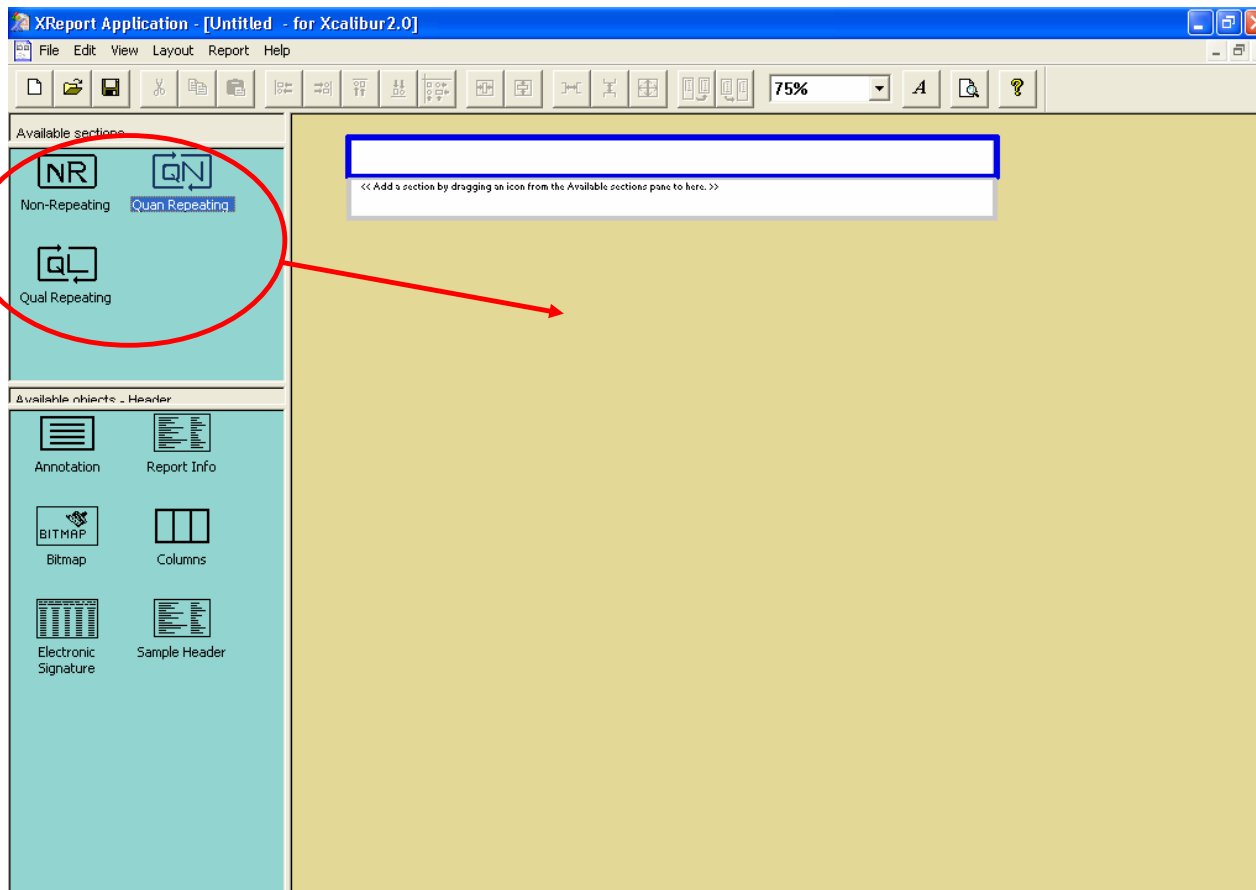
The screenshot shows the XReport Application interface. A yellow callout box with the text "1. Click Report and select Data Sources" points to the "Data Sources..." menu item in the Report menu. A second yellow callout box with the text "2. Specify Data Sources" points to the "Data Sources" dialog box. The dialog box contains several fields for specifying data sources:

- Calibration File: [Empty field]
- Processing Method File: C:\Xcalibur\examples\methods\steroid.pmd
- Raw Data File: C:\Xcalibur\examples\data\steroids13.raw
- Result File: C:\Xcalibur\examples\data\steroids13.RST
- Sequence List File: C:\Xcalibur\examples\methods\steroid.sld

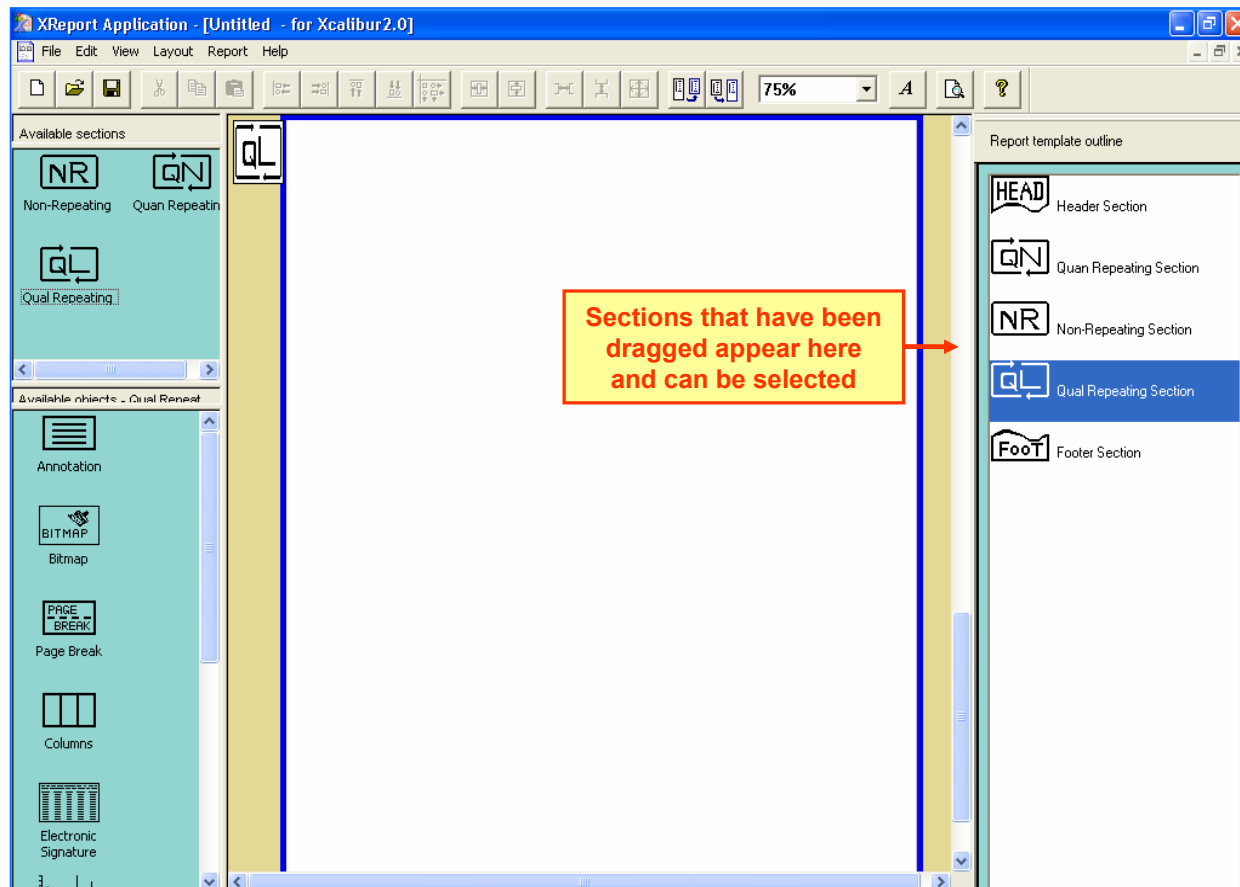
The background shows a chromatogram and a mass spectrum. The chromatogram has peaks at 2.33 and 2.33 minutes. The mass spectrum has peaks at 220.0 and 356.3 m/z. Below the mass spectrum is a table with the following data:

Component	
Cal Level [A]	Amount [B]

Drag and Drop Sections...



Drag and Drop Sections...



Drag and Drop Individual Objects...

XReport Application - [Untitled - for Xcalibur 2.0]

File Edit View Layout Report Help

75%

Available sections

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

Available objects - Quan Repeating

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

Report template outline

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

The screenshot shows a report template with several data visualization objects. Red arrows indicate the drag-and-drop process from the 'Available objects' panel to the report area:

- A chromatogram object is dragged from the 'Available objects' panel to the top-left plot area.
- A spectrum object is dragged from the 'Available objects' panel to the bottom-left plot area.
- A bitmap object is dragged from the 'Available objects' panel to the bottom-right plot area.
- A 'QN' section object is dragged from the 'Available sections' panel to the top of the report area.

Chromatogram Data:

Retention Time (min)	Relative Abundance
1.49 - 2.49 <td>~100</td>	~100
1.99 <td>~100</td>	~100
2.33 <td>~20</td>	~20

Spectrum Data:

m/z	Relative Abundance
220	~20
281.4	~100
356.3	~40

Area Plot Data:

Area (ng)	Relative Abundance
~0.7	~700,000

Sample Table (Quan Results)

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

Formatting Objects...

The screenshot shows the XReport Application interface. On the left, there are panels for 'Available sections' (NR, QN, QL) and 'Available objects - Quan Repeating' (Annotation, BITMAP, Page Break, Columns, Electronic Signature, Chromatogram, Component Calcurve, Spectrum, Avalon Quan Events Table, Component Cal Level Table). The main workspace contains a chromatogram with a peak at 1.99 minutes. A context menu is open over this peak, listing options: Cut (Ctrl+X), Copy (Ctrl+C), Paste (Ctrl+V), Delete (Del), Edit Object (Ctrl+E), Properties... (Alt+Enter), and Delete Section. The 'Properties...' option is circled in red. Below the chromatogram is a 'Sample Table (Quan Results)' with columns for Sample ID [A], Data File Name [B], and Area [C]. On the right, the 'Report template outline' shows sections: Header Section, Quan Repeating Section (highlighted), Non-Repeating Section, Qual Repeating Section, and Footer Section.

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

Viewing the Report...

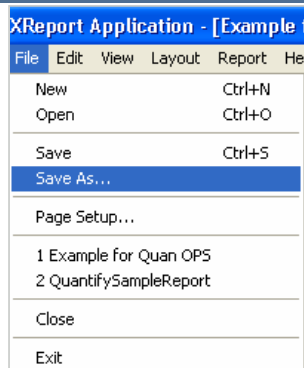
The screenshot displays the XReport Application interface. The main window shows a report page with the following components:

- Top Menu:** File, Edit, View, Layout, Report, Help.
- Toolbar:** Includes icons for file operations, navigation, and a zoom level set to 75%.
- Report Content:**
 - Top Left:** A chromatogram plot showing a single sharp peak.
 - Top Right:** A calibration curve plot with the equation $y = 0.037617x - 0.0003$ and $R^2 = 0.9993$.
 - Bottom Left:** A multi-peak chromatogram plot.
 - Bottom Right:** An image of a laboratory instrument.
 - Bottom Center:** A data table with columns for Sample ID, Date File Name, and Area.

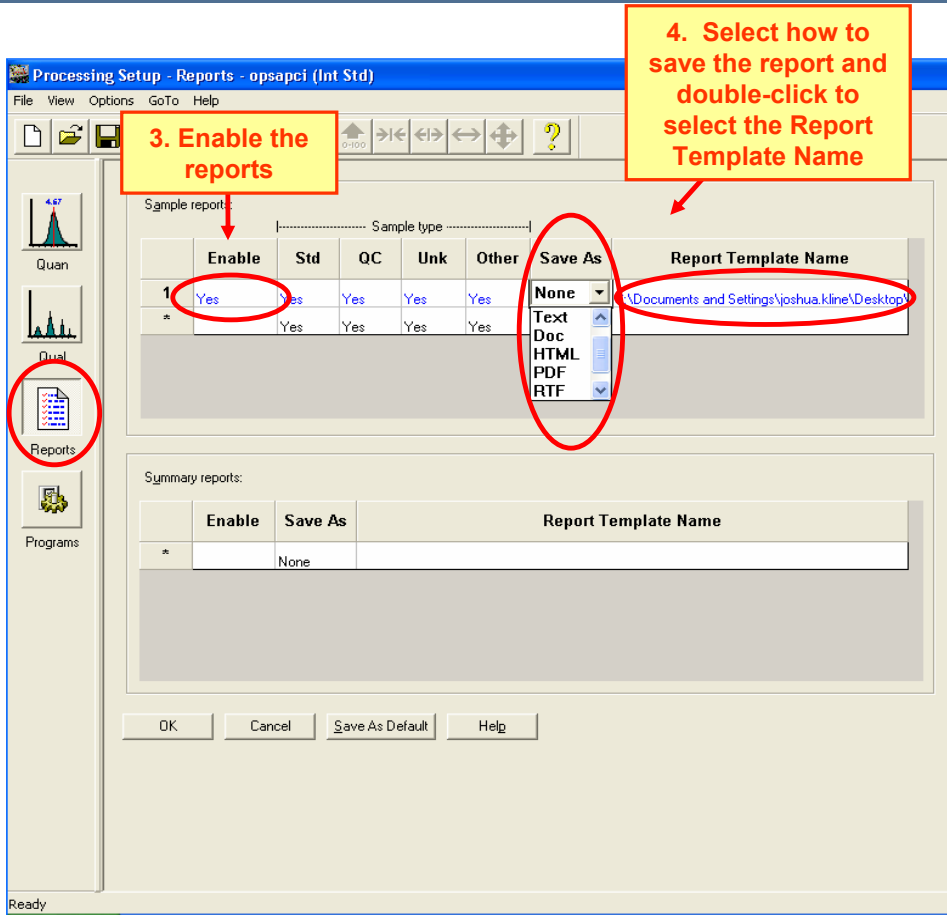
Sample ID	Date File Name	Area
Sample02	Sample02	1892942.73
Sample03	Sample03	501543.72
Sample04	Sample04	211847.95
Sample05	Sample05	243328.14
Sample08	Sample08	1842820.33
Sample09	Sample09	263011.26
Sample10	Sample10	3433747.72
Sample11	Sample11	278701.72
Sample12	Sample12	489259.88
Sample13	Sample13	1839442.73
Sample14	Sample14	884512.77
Sample15	Sample15	313633.84
Sample16	Sample16	4429485.34

Save, Insert, Use...

1. Click to save the report



2. In Processing Setup, click Reports



Save, Insert, Use...

Run Sequence

Acquisition Options

Instrument: Surveyor AS, LTQ XL MS, Surveyor LC Pump

User: []

Run Rows: 1

Priority Sequence

Processing Actions

- Quan
- Qual
- Reports
- Programs
- Create Quan Summary

1. Click to enable reports when you run the sequence

OK Cancel Help

Batch Reprocess Setup

Processing Actions

Process Rows: 1

- 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

2. Click to enable reports after the sequence has run

OK Cancel Help

ThermoFisher
S C I E N T I F I C

The world leader in serving science

Chapter 14

Calibration and Maintenance

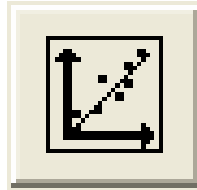
Calibration

- Calibration should be performed approximately every 3 to 6 months or after the multipoles and the trap have been removed.
- It is not usually necessary to calibrate more frequently and unnecessary calibration will lead to Ultramark contamination.
- The best procedure is to calibrate before maintaining the stack but after the multipoles and the trap have been cleaned.

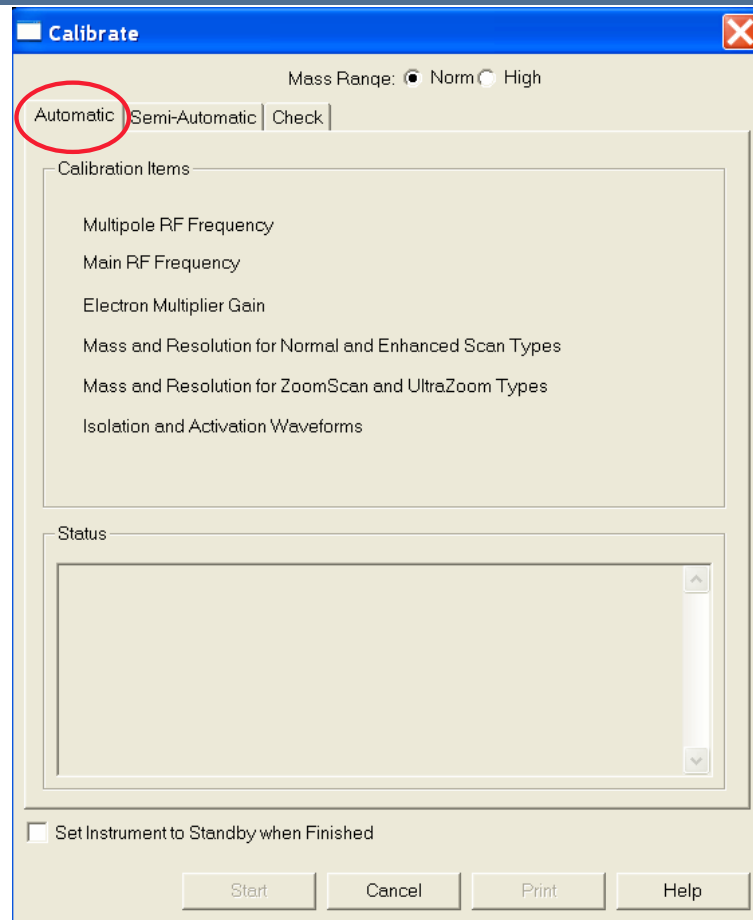
First Make Sure...

- Your calibration solutions are fresh
- Your calibration solution contains all calibrant masses
- Your ion source settings are optimized for the flowrate used
- You have a stable spray: Focus on the injection time (IT) and on the normalized level (NL) in the Tune Page scan header

Automatic Calibration

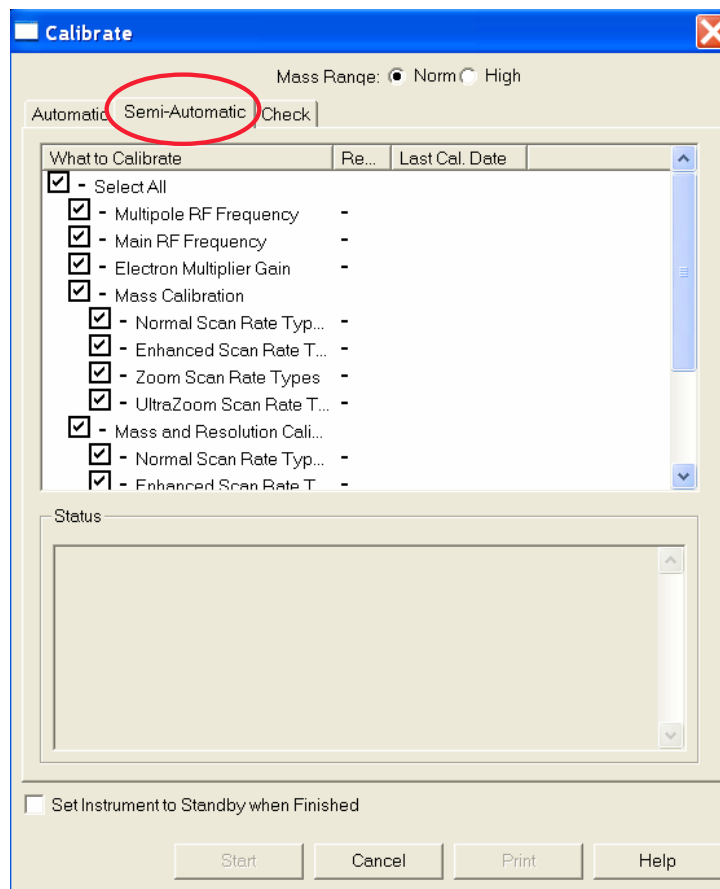


Start the automatic calibration procedure (takes about 40 min.)



Semi-Automatic Calibration

Use the semi-automatic calibration procedure to calibrate parameters that did not pass during automatic calibration.



LCQ Recommended Maintenance Schedule

Daily:

- Check the convectron and ion gauge pressures. Make sure the vacuum system is operational. Typical LCQ values are: a) convectron pressure 0.9 – 1.1 Torr; b) ion gauge pressure $1.0 - 1.5 \times 10^{-5}$ Torr.
- If bottled nitrogen gas is being used, check nitrogen gas pressure. The recommended value for the LCQ is 80 – 120 psig.
- Check the ESI fused silica sample tube. Make sure the fused silica is not elongated. Trim the tube if necessary.
- Remove the APCI needle (if switching from APCI to ESI).
- Check HPLC solvent levels.
- After completion of analysis place system in stand-by mode.

LCQ Recommended Maintenance Schedule

Weekly:

- Check the mechanical pump oil level.
- Fill the mechanical pump as needed.
- Ballast the mechanical pump.
- Replace LC solvents to maintain low background levels.

LCQ Recommended Maintenance Schedule

Monthly:

- Check the helium gas tank pressure. The recommended value for the LCQ is 30 – 50 psig. Replace the tank as needed.
- Check the nitrogen gas supply. Replace or refill as needed.
- Trim / Exchange ESI fused silica sample tube.
- Clean / Replace ion transfer tube. Optional cleaning procedure is sonication for 15 min in methanol or isopropanol.
- Optional (at least quarterly):
 - Calibrate LCQ.
 - Clean the API stack. Wipe tube lens and skimmer clean with methanol or isopropanol. **Do not sonicate the tube lens and skimmer in acidic solutions!**
 - Tune instrument after cleaning the API stack.

LCQ Recommended Maintenance Schedule

Quarterly:

- Calibrate LCQ.
- Clean/replace ion transfer tube.
- Clean API stack.
- Check/replace mechanical pump oil.
- Clean air filter pad in the back of the instrument.
- Back up data and defragment PC.
- Check the Thermo website for available software updates / fixes:
 - Thermo Fisher Scientific Fast LC and LC/MS Support
<http://www.thermo.com/> => Search for “Fast LC and LC/MS”
 - Thermo Fisher Scientific Knowledge Base
<http://198.173.130.188/thermofinnigandb/thermo.nsf>
 - Thermo Fisher Scientific Customer Download Site
<http://mssupport.thermo.com/>

LCQ Recommended Maintenance Schedule

Annual:

- Clean oil traps on mechanical pumps.
- Clean ion optics and the ion trap.
- Change oil filter pads on turbomolecular pumps (if present).

Notes:

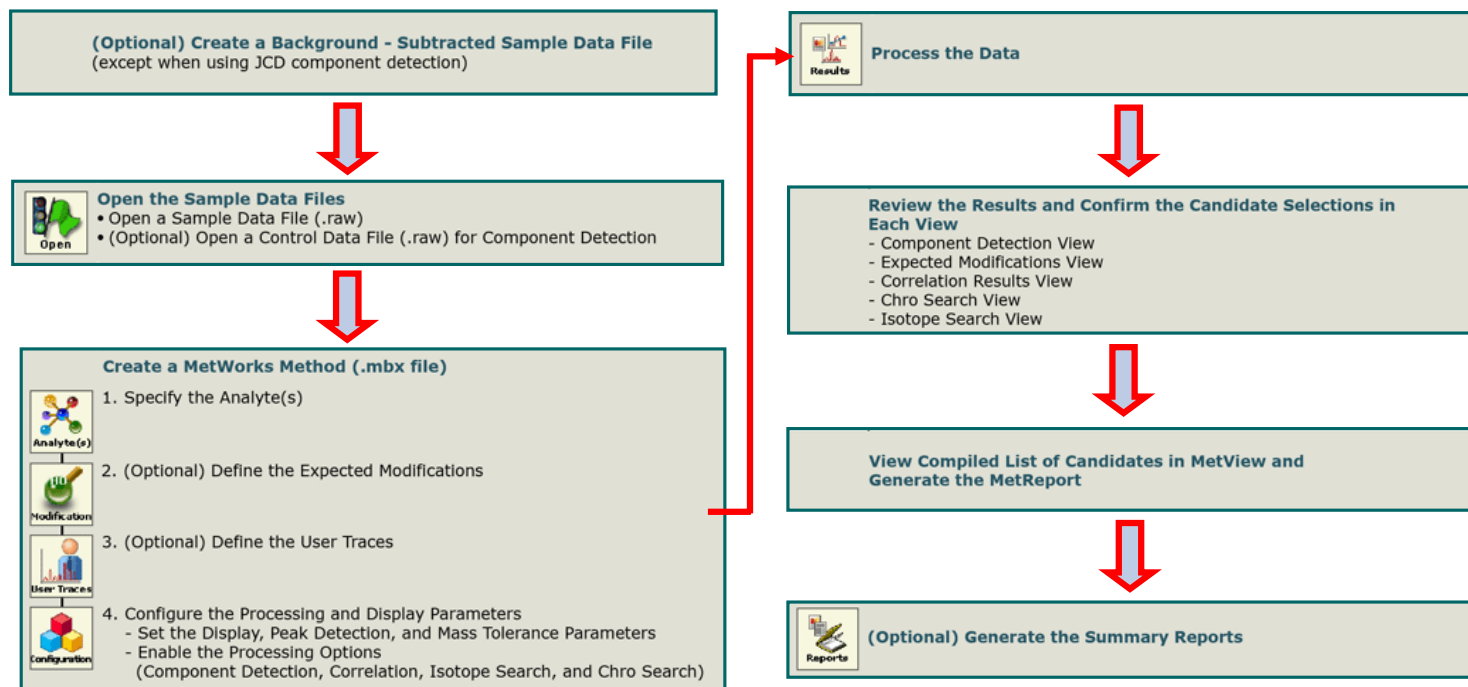
- Heavy use and dirty samples may require more frequent cleaning of the ion transfer tube, the API stack and the analyzer.
- If the sensitivity starts to drop off and can not be restored by replacing the ion transfer tube, clean the API stack (and analyzer) as needed.

ThermoFisher
SCIENTIFIC

The world leader in serving science

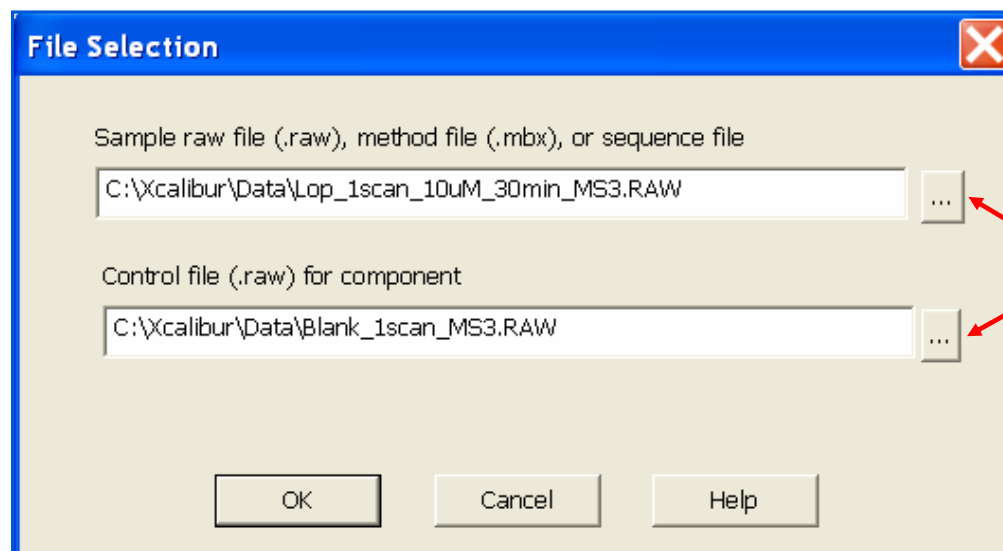
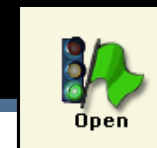
MetWorks

Workflow for a Data Dependent Experiment (to process one file at a time)



To process the acquired sample data one file at a time, you can first create a background subtracted file to use for your sample data file (optional). The next step is to open a sample data file and a control data file for component detection. To create the MetWorks method, specify your parent drug, define the modifications you expect to your parent drug, define any additional traces you wish to view in the results and configure the processing and display parameters. Click the Results button to process the data and then review the results and confirm the components that are of interest in each of the MetWorks views. After selecting the components of interest from each view, a summary of these components can be viewed in MetView and a report generated using MetReport. There are also other types of summary reports that can be created by clicking on the Reports button.

Opening the Raw Data Files



Click to
open raw files

To open a raw file, on the Info bar Button page, click the Open button and then select the sample data file (.raw) to be processed using the MetWorks method you are creating. For component detection, you can also select a control file (.raw) that will be compared to the sample file. Alternatively, you can choose to open a previously created MetWorks method (.mbx file).

MetWorks Window After Opening the Raw Data Files (General Trace View)

The screenshot displays the MetWorks software window titled "Untitled [Lop_1scan_10uM_30min_MS3.raw] - MetWorks". The interface includes a menu bar (File, View, Display, Options, Tools, Help) and a toolbar with various icons. On the left, a vertical toolbar contains icons for "Open", "Analyte(s)", "Modification", "User Traces", "Configuration", "Results", and "Reports".

The main display area shows three stacked plots:

- Total ion chromatogram:** The top plot shows Relative Abundance vs. Time (min) from 0.5 to 8.0. It features a noisy baseline with several peaks. A red arrow points to this plot with the label "Total ion chromatogram".
- Base peak chromatogram:** The middle plot shows Relative Abundance vs. Time (min) from 0.5 to 8.0. It displays a clean baseline with two prominent peaks at 3.60 and 3.82 minutes. A red arrow points to this plot with the label "Base peak chromatogram".
- Spectrum at cursor position:** The bottom plot shows Relative Abundance vs. m/z from 150 to 1000. It displays a mass spectrum with a base peak at m/z 477.23 and other significant peaks at 479.29 and 480.32. A red arrow points to this plot with the label "Spectrum at cursor position".

Below the mass spectrum plot, there are two arrow buttons (left and right) and the text "Use arrows to scroll thru the scans". A red arrow points from this text to the arrow buttons. At the bottom right of the plot area, there is a "NUM" field.

At the bottom of the window, the Thermo Scientific logo is on the left, the page number "270" is in the center, and the ThermoFisher Scientific logo is on the right.

After opening the raw data files, the MetWorks window changes to display the total ion chromatogram and base peak chromatogram of the sample raw file and the mass spectrum of the sample raw file at the current cursor position below in the General Trace view. The arrow buttons above the spectrum can be used to scroll thru the scans in the chromatograms.

Entering the Analyte

1. Type a name for the list then click new item

2. Type information for analyte

3. Click to enter structure

4. Click to add new analyte to Working analyte list

One way to specify an analyte for a MetWorks method is to simply Enter a New Analyte. After clicking on the Analyte(s) button, the Analyte(s) Manager dialog box opens. First, type a name for your new Working analyte list and then click New Item to add an analyte to the list. In the New Analyte dialog box, type the name, mass and select the polarity. To enter a structure, click the button next to the Structure file (mol) box. **IMPORTANT:** MetWorks uses a precursor mass tolerance of ± 0.2 m/z to determine whether an MS_n spectrum for a particular mass is from a precursor ion. If you do not specify the mass with the necessary precision, MetWorks cannot display the appropriate MS_n indicators (blue tic marks) above the analyte chromatogram in the Expected Modifications view or locate a reference scan for the spectral cross-correlation. Click OK to add the new analyte to the Working analyte list.

Saving a Working Analyte List

The screenshot shows the 'Analyte(s) Manager' dialog box. On the left, the 'Master analyte list' contains 'Loperamide (user)'. In the center, the 'Working' list contains 'Loperamide' with sub-items '477', '+ polarity', 'No Filter', and 'loperamide.mol'. On the right, there are buttons for 'OK', 'Cancel', 'Save', 'Import...', and 'Help'. Two red callout boxes with arrows point to the 'OK' and 'Save' buttons. The 'OK' callout says 'Click "OK" to use Working analyte list only for current MetWorks method'. The 'Save' callout says 'Click "Save" to save working analyte list in the master analyte list for future use'. Below the buttons is a 'Structure preview' section showing the chemical structure of loperamide.mol.

Master analyte list
Name: Loperamide (user)

Working
Name: Loperamide

Click "OK" to use Working analyte list only for current MetWorks method

Click "Save" to save working analyte list in the master analyte list for future use

Structure preview: loperamide.mol

CN1C(=O)C(C1)(C2=CC=CC=C2)CCN3CCCCC3C4=CC=C(C=C4)O

To use the newly created Working analyte list for only the current MetWorks method, click OK. To use the newly created Working analyte list for future analysis as well as the current analysis, save the Working analyte list before clicking OK. When Save is clicked, you will be prompted to save as list type: Current user only or Shared with all users. Select whether to make the list available for the current user or for all users. You can define the current user from Windows. You might change the current user identity, for example, if you run MetWorks using a computer network. For details, see the Windows documentation.

Defining the Expected Modifications

1. Type name for new list or keep current name

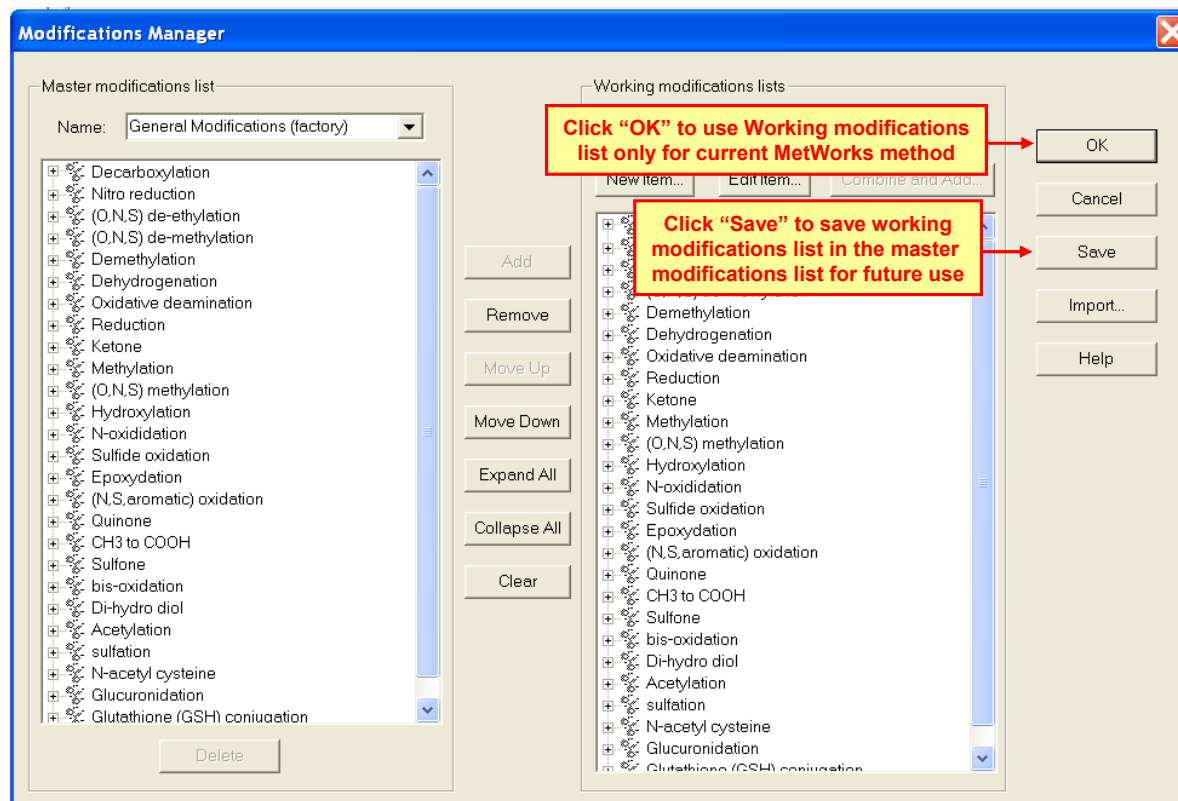
2. Create a new modification for the list or edit an existing modification

3. Enter information

4. Click for changes to appear in list

To create or edit a Working modifications list, either type a name for the new list or keep the existing name. Then, create a new modification for the list or edit an existing modification. In the Edit Modification dialog box, enter the name for the modification and the mass shift. For the mass shift, to calculate the m/z , you can enter the mass shift directly or click the Formula button to open the Enter Elemental Formula dialog box. In the Formula box, type the formula, and then press Update. Click OK and the calculated m/z appears in the Mass shift (m/z) box. Click OK again for the changes to appear in the list.

Saving a Working Modifications List



To use the newly created Working modifications list for only the current MetWorks method, click OK. To use the newly created Working modifications list for future analysis as well as the current analysis, save the Working modifications list. When Save is clicked, you will be prompted to save as list type: Current user only or Shared with all users. Select whether to make the list available for the current user or for all users.

Defining the User Traces (Optional)



User Trace Manager 1. Check box to add trace

Type	Range	Scan filter	Delay (...)	SM	Radio	Title
<input checked="" type="checkbox"/> TIC	-	-	0	1	-	-
<input checked="" type="checkbox"/> Base Peak	-	ITMS + c ESI Full ms [150.00-1000.00]	0	1	-	-
<input checked="" type="checkbox"/> Base Peak	477.00	ITMS + c ESI Full ms [150.00-1000.00]	0	1	-	-
<input checked="" type="checkbox"/> Base Peak	-	ITMS + c ESI d Full ms2 477.23@cid35.00 [120.00-490.00]	0	1	-	-
<input checked="" type="checkbox"/> Base Peak	-	ITMS + c ESI d Full ms3 477.36@cid35.00 411.51@cid35...	0	1	-	-
<input checked="" type="checkbox"/> Total Scan	-	-	0	1	-	PDA Trace
<input checked="" type="checkbox"/> Analog 1	-	-	0	3	x	Radioactivity Trace
<input type="checkbox"/> TIC	-	-	0	1	-	-
<input type="checkbox"/> -	-	-	-	-	-	-
<input type="checkbox"/> -	-	-	-	-	-	-

2. Select detector type (MS, Analog, A/D Card, PDA, or UV) and delay if any

3. Select plot type and plot properties

Check box if trace is for a radioactively labeled sample

Plot properties:

Title: Radioactivity Trace

Plot type: Analog 1

Scan filter:

Range(s):

Detector: Analog

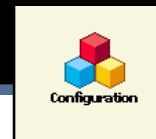
Delay (min): 0

Smoothing points: 3

Radioactive label trace:

OK Cancel Help

To define a user trace, click on the User Traces button to open the User Trace Manager dialog box and check a box in the Type column to add the trace. Select whether the data was acquired from an MS, Analog, A/D Card, PDA or UV detector and also select the plot type and properties. If one of the traces is a radioactively labeled trace, check the box next to Radioactive label trace. After processing, the custom plots can appear in the Expected Modifications view and the Component Detection view. Up to 10 chromatograms, including one trace from a radioactivity detector, can appear in addition to the modification chromatograms in the Expected Modifications view. One trace from a radioactivity detector can appear in addition to the chromatograms for the sample and the control in the Component Detection view. When you process the data, the software filters the radioactively labeled sample data and retains only the results that correspond in time to a detected peak in the radioactively labeled trace.



- **The Configuration button allows you to configure settings that MetWorks uses to display and process your data. The processing modes include :**
 1. **Display (Expected Modifications view)**
 - Specifies the types of peak labels, number of traces to display, and how MetWorks displays the MSⁿ scans in the spectrum panes of the workspace.
 2. **Peak Detection (Expected Modifications, Chro Search Results, Isotope Search Results view)**
 - Sets the peak detection algorithm and integration parameters.
 3. **Correlation (Spectral Correlation view)**
 - Searches for potential components based on the correlation with the MS² or MSⁿ spectrum from the analyte (or parent drug).
 4. **Chro Search (Chro Search Results view)**
 - Searches for potential components by sorting the extracted-ion chromatograms (EIC) based on peak intensity after removing all EICs for the expected modifications.
 5. **Component Detection (Component Detection view)**
 - Detects chromatographic components in complex LC/MS data and extracts the mass spectral signals from closely co-eluting components (deconvolution).
 6. **Isotope (Isotope Search Results view)**
 - Searches for compounds that have specific isotopic patterns.
 7. **Mass Tolerance**
 - Allows you to set the parameters for accurate mass data.

By clicking on the Configuration button, the Default Display and Processing Settings dialog box opens which allows you to configure settings used to process your data. There are 7 tabs in the Default Display and Processing Settings dialog box and most of these pages contain parameters to configure settings for the 8 different views in MetWorks. The Display page allows you to specify how MetWorks displays the data in the Expected Modifications view. The Peak Detection page allows you to set the peak detection and integration parameters for the Expected Modifications, Chro Search Results and Isotope Search Results view. The Spectral Correlation view searches for potential components based on the correlation of potential components with the MS² or MSⁿ spectrum of the analyte (or parent drug) and the Correlation page allows you to configure how the data is displayed in this view. With the Chro Search page, you can configure how your data is displayed in the Chro Search Results view which searches for potential components by displaying the most intense EICs based on base peak intensity after removing the EICs from the Expected Modifications view. The Component Detection page allows you to configure parameters for the Component Detection view which detects chromatographic components in complex LC/MS data and groups the mass spectra from closely eluting components. Using the Isotope Search page, you can configure parameters for the Isotope Search Results view which searches for compounds that have isotopic patterns that you specify. The Mass Tolerance page allows you to set the parameters for accurate mass data for all of the views in MetWorks. You can configure MetWorks to use your display preferences and to perform only the types of data processing required for your experiments. MetWorks stores the current configuration settings in an .mbx file when you save a MetWorks method.

Display

The screenshot shows the 'Default Display and Processing Settings' dialog box with the 'Display' tab selected. The dialog has several sections and controls:

- Number of traces to display during review:** A text box containing the value '3'.
- MS order to display:** A dropdown menu currently set to 'ms'.
- Display options (checkboxes):**
 - Show modification names
 - Show MSn scans
 - Show closest MS/MS scan
- Label Peaks with (checkboxes):**
 - Retention time
 - Scan number
 - Base peak
 - Signal to noise
 - Flags
 - Area
 - Height
- Label threshold (%):** A text box containing the value '0'.

At the bottom of the dialog are buttons for 'OK', 'Cancel', 'Set As Default', and 'Help'.

1. Enter # of traces and which MS order that will appear in the Expected Modifications view

2. Select check boxes of display options you want to include in results

3. Select peaks labels and enter label threshold (percent of the base peak height above which MetWorks applies any labels to the peaks)

Thermo SCIENTIFIC 277 ThermoFisher SCIENTIFIC

You can use the Display page to configure default display preferences for the modification chromatogram plots (EICs) in the Expected Modifications view. These parameters only affect how your data is displayed in the Expected Modifications view and do not affect the actual processing of the data. First, enter the Number of traces to display during review which are the number of traces to appear in the window at one time in this view and which MS order to display. Always select an MS order that corresponds to the lowest MSn order acquired. For example, if Data Dependent MS/MS data (MS and MS2) are present in the experiment, select MS as the default display parameter. Next, select the check boxes of the display options you want to include in the results. Show modification names displays the modification names in the results views. Show MSn scans displays blue tic marks above the extracted ion chromatogram (EIC). The tick marks correspond to the sites of MSn data and allow for fast searching of MSn data. Show closest MS/MS scan displays a spectrum in the spectrum pane, instead of the message "No MS/MS Data", when there is no MS/MS data at the given retention time. MetWorks displays the MS/MS scan closest to the current retention time within the integrated peak. Finally, select peak labels and enter a label threshold which is the percent of base peak height above which labels are applied to the peaks.

Peak Detection

1. Select detector type (MS, Analog, A/D Card, PDA, or UV), peak detection algorithm (ICIS or Avalon), and the # of Gaussian smoothing points you want MetWorks to apply to the data

2. Modify parameters to achieve satisfactory peak detection and integration

Sets the same peak detection and integration parameters for all detector types in list

You can use the Peak Detection page to configure the default peak detection and integration settings for the Expected Modifications view, Chro Search Results view, and Isotope Search Results view. For most analyses, the default parameters are sufficient. At the top of the Peak Detection page, select the detector type, the peak detection algorithm and the number of Gaussian smoothing points to apply to the data. For the peak detection algorithm, ICIS provides superior peak detection efficiency at low MS signal levels for MS data, but, you can use ICIS for all detector types. If you want to set the same peak detection and integration parameters for all detector types in the list click the Set same button. Then, modify the parameters to achieve satisfactory peak detection and integration. To improve the peak detection results for selected chromatograms after the processing, you can reset the peak detection parameters by right-clicking on the chromatogram.

Correlation

Default Display and Processing Settings

Display | Peak Detection | **Correlation** | Chro Search | Component Detection | Isotope | Mass Tolerance

Enable

Normalize spectra

Detect mass-shifted fragments

High highlight threshold: 0.9

Low highlight threshold: 0.4

Correlation cutoff: 0.15

Minimum Intensity Cutoffs

Absolute counts: 3000

Relative to basepeak: 0.05

OK Cancel Set As Default Help

Correlates fragments that are shifted in mass relative to the corresponding fragments in the reference scan

Normalizes each spectrum to a common base peak intensity

Adds a green (high) or yellow (low) highlight to any scan in the Spectral Correlation table with a cross-correlation factor greater than the specified value

Specifies the minimum cross-correlation factor required before a scan appears in the Spectral Correlation table

Peak intensity must meet or exceed cutoffs before being displayed in the Spectral Correlation table

Parameters in the Correlation page affect how your data is displayed in the Spectral Correlation view. MetWorks quantifies the cross-correlation of modifications to an analyte by MS₂ or MS_n spectral comparison using cross-correlation factors. Metabolites generally give MS/MS spectral patterns similar to their parent drug, but are mass-shifted by their metabolic modifications. Compared to conventional library searches, the results of cross-correlation analyses between parent drugs and the metabolite MS/MS spectra provide scores called cross-correlation factors with much higher confidence levels. If you want to enable Spectral Correlation, check the box next to Enable. A check in the box next to Normalize spectra will normalize each spectrum to a common base peak intensity. To correlate fragments that are shifted in mass relative to the parent data (reference scan), check the box next to Detect mass-shifted fragments. The High and Low highlight thresholds as well as the Correlation cutoff have to do only with how your data is displayed in the Spectral Correlation table on the left hand side of the Spectral Correlation view. Any scan that has a cross-correlation factor above the high or low highlight threshold will be highlighted either green (high) or yellow (low), respectively, and any scan that has a cross-correlation factor below the correlation cutoff will not be included in the table. You can also specify a Minimum Intensity Cutoff in either absolute counts or relative to the base peak intensity so that a peak intensity must meet or exceed these values to be displayed in the Spectral Correlation table.

Chro Search

Default Display and Processing Settings

Display | Peak Detection | Correlation | Chro Search | Component Detection | Isotope | Mass Tolerance

Enabled

Threshold: 1000

Max chros: 10

Time range (min): *

Filter: Full ms

Mass range(s): *

OK Cancel Set As Default Help

Specifies the maximum number of extracted-ion chromatograms (EICs) MetWorks returns

Only the chromatograms with peaks that meet or exceed the threshold are included in the search results

Generally, use Full MS

Chro Search searches for potential components by sorting the extracted-ion chromatograms (EICs) based on peak intensity after removing all EICs from the Expected Modifications view. Use the Chro Search page to configure the parameters for a chromatogram (Chro) search. After processing the data, MetWorks displays the results in the Chro Search Results view. To activate a chromatogram search, check the box next to Enabled. When you type a number in for the Threshold, only the chromatograms with peaks that meet or exceed the threshold are included in the results. Max chros specifies the maximum number of EICs MetWorks returns. Generally, for the Filter, use Full ms (default), or you can choose to specify a different scan filter for the data. Also, you can specify a Time range and/or a Mass range to look for EICs. Typing in values here can help to more specifically narrow down the EICs in the Chro Search results to chromatograms that are of interest.

Component Detection - JCD Algorithm

1. Select Enable and choose the JCD algorithm

of scans used to identify a potential component

Any peaks less than this intensity will be rejected as noise

Specifies limit for the time difference of ion profile maxima

Check box to merge wide components

Specifies degree of similarity of ion profile shapes

Thermo SCIENTIFIC

281

ThermoFisher SCIENTIFIC

On the Component Detection page, if you want to perform Component Detection using the JCD algorithm, check the box beside Enable and choose the JCD Algorithm. The Average peak width specifies the peak width (in # of scans) that the JCD algorithm uses to identify a potential component. It is best to start with a lower value and adjust based on the results. Too high of a value for the Average peak width can result in the loss of narrow peaks and too low of a value can split a real component into two different components. For the Baseline threshold, any peaks less than this value will be rejected as noise. The Merging factor specifies a limit for the time difference of the ion profile (chromatogram) maxima. A high value of the Merging factor can cause the merging of randomly coeluting components and a low value can split a component into more false-positive components. The Sharpness tolerance specifies the degree (%) of similarity of ion profile shapes. The Wide component merge mode can be selected to avoid splitting a component into ion peaks that are detected as redundant components in chromatograms with wide peaks.

Component Detection - TECD Algorithm

1. Select Enable and choose TECD

Specifies min. # of tree sections created from the initial spectral tree

Compares spectral trees up to the distance specified by the Allowed gap value

2. Select Enable and adjust the threshold to be near the baseline for your data

3. Enter time or m/z range for component detection, if any

Min. % two trees must match before being considered the same component

Max. distance over which to compare trees for merging

Thermo SCIENTIFIC

282

ThermoFisher SCIENTIFIC

On the Component Detection page, if you want to perform Component Detection using the TECD algorithm, check the box beside Enable and choose the TECD Algorithm. To use TECD based on your Chromatogram Info, check the box next to Enable and adjust the threshold to be near the baseline for your data. The threshold is an important parameter to adjust to be close to the noise level in your data. Max Chros specifies the maximum number of most intense extracted ion chromatograms (EIC) MetWorks can use in component detection. Each EIC can contain several detected peaks, thus the number of components MetWorks detects can be larger than the value you specify for Max Chros. You can also enter a time or mass range to narrow down the number of components generated. If you want to use the TECD algorithm based on General parameters, you can adjust these parameters if necessary. Minimal tree depth specifies the minimum number of tree sections TECD creates from the initial spectral tree. This determines the MS stage where a division takes place. Tree match factor specifies the minimum percentage that two spectral trees within adjacent tree sections must match before TECD can consider the two spectral trees as the same component. Matching spectral trees are defined as having identical precursors up to the level specified by the Minimal Tree Depth value and a Tree Match Factor value that exceeds the specified value. Wide component merge mode activates a comparison of the spectral trees for potential matching and merging, not only in adjacent sections, but also in sections up to the distance specified by the Allowed gap value. The Allowed gap specifies the maximum distance between nonadjacent tree sections over which to compare the spectral trees for potential merging.

Isotope

1. Click to activate an isotope search

2. Select Elements

3. Type single # or range of Br and/or Cl combinations only

Displays a preview of the pattern selected from the Isotope Pattern Preview list

	Element	Number
1	Br	0
2	Cl	1
3		
4		
5		
6		
7		
8		
9		
10		

Use the Isotope page to configure and preview the settings for an isotope search. After you process the data, you can view and edit the isotope search results. First, click the box next to Enabled to activate an isotope search. Under Input, you can specify various parameters for the isotope search. Time range (min) is the range to specify the search window. To search over the entire time range, type: *. Tolerance is the percentage that the found clusters are allowed to differ from the theoretical clusters in the search. Threshold is the threshold to apply for including found clusters in the search results. Mass range(s) is the mass range or ranges that you want to search. To search the entire mass range, type: *. To enter multiple ranges, separate each range with a comma, for example: 243-265,354-423. Most intense specifies the number of isotopes to include in the search results based on intensity. Next, select Elements and in the Elements table, type a single number or range of Br and/or Cl combinations. The Elements table uses combinations of Cl and Br isotopic clusters only to specify the types of combinations of isotopic atoms. On the right-hand side under Isotope Pattern Preview, this displays a preview of the pattern selected from the Isotope Pattern Preview List.

Defining a Custom Isotope Pattern

Default Display and Processing Settings

Display | Peak Detection | Correlation | Chro Search | Component Detection | Isotope | Mass Tolerance

Enabled

Input

Time range [min]: 2.00-5.00 Tolerance %: 0.1

Threshold: 1000 Mass range(s): *

Most intense: 10

Elements Custom

<New pattern name> (Current)

	Delta	Rel. abund
1	0	100
2	2	31.978
3		
4		
5		
6		
7		

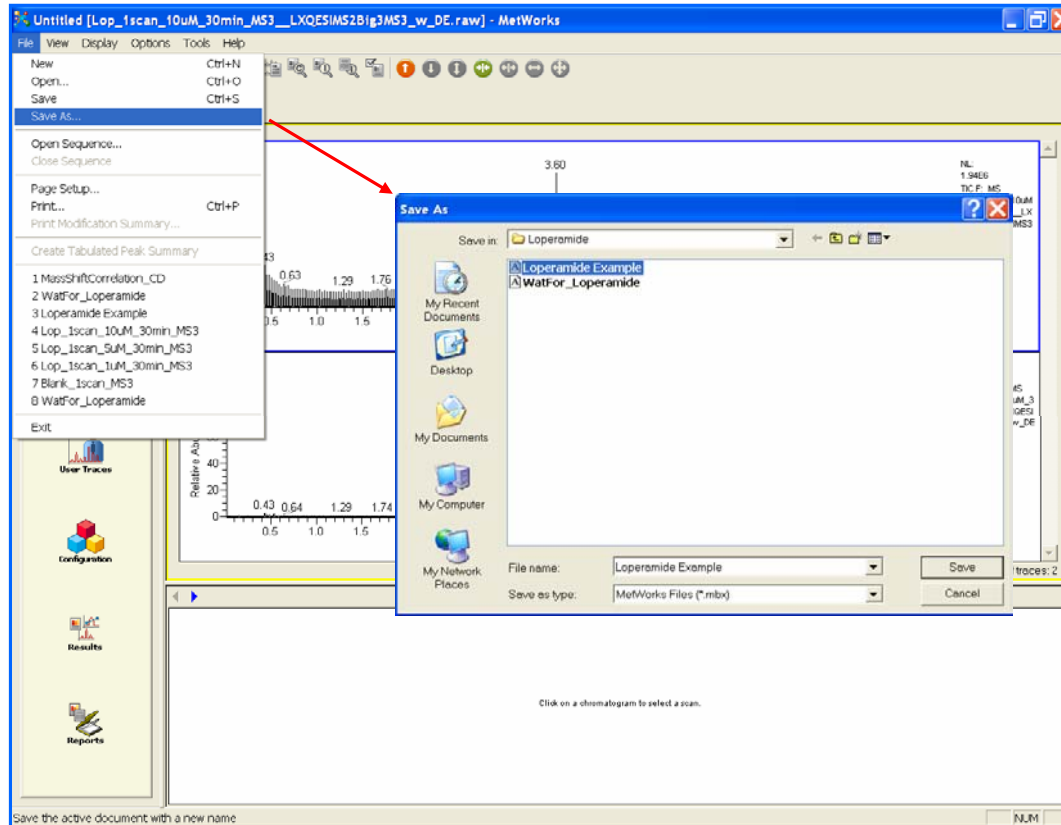
Add Delete

OK Cancel Set As Default Help

Isotope Pattern Preview

If you want to specify an isotope pattern to search for components with isotopes other than Br and Cl, you can define a custom isotope pattern using the Custom table. First, click the box next to Enabled to enable the isotope search. Click on the dot next to Custom to define a custom isotope pattern. Then, type a name for the custom isotope pattern over <New Pattern Name>. Specify the difference in mass (delta) and the relative abundance of your custom isotope. Finally, click the Add button to save the pattern in the list. The Delete button displays the Custom Pattern Deletion dialog box, which allows you to delete the saved patterns.

Save the MetWorks Method

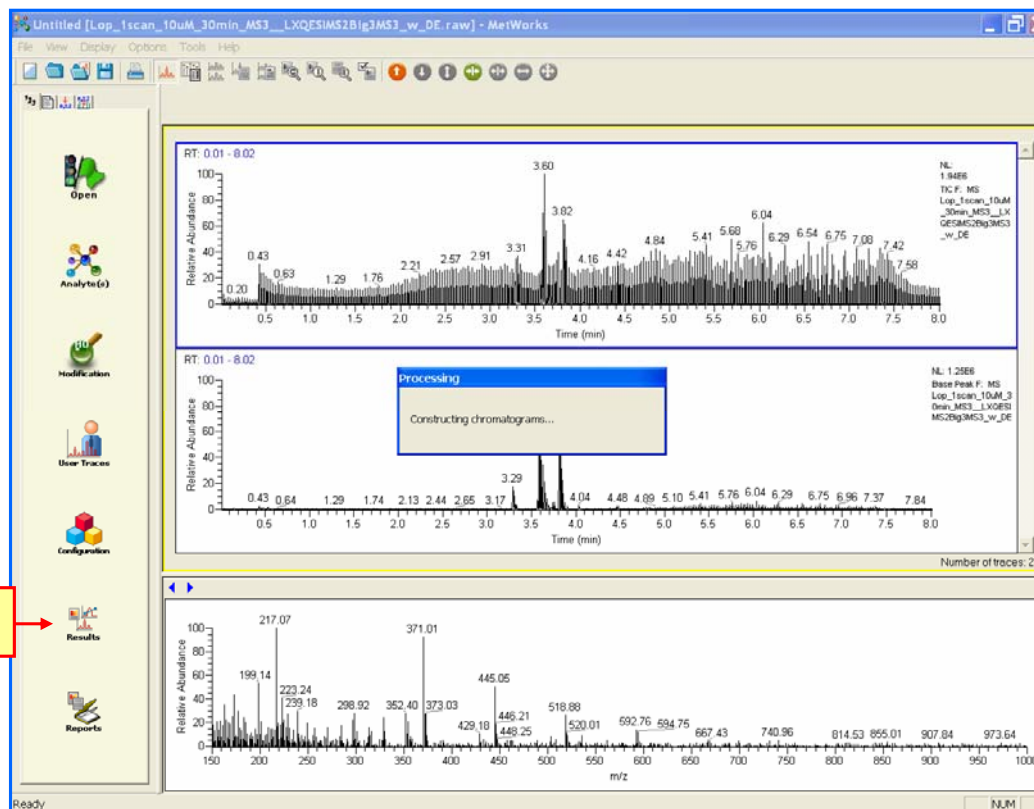


To save the settings you specified in a MetWorks method, click on File from the dropdown menu and choose Save As. In the Save As dialog box, type a file name, and then click Save to save the MetWorks method file (.mbx).

Processing the Data



Results



Click Results to process the data

Thermo
SCIENTIFIC

286

ThermoFisher
SCIENTIFIC

Click the Results button on the left-hand side to process the data. When MetWorks finishes the processing, the results appear in the MetWorks workspace. IMPORTANT: Avoid using software applications other than MetWorks when you perform component detection. MetWorks works interactively with Mass Frontier to detect the components and requires significant computer processing resources.

Component Detection View

▼ = Sample component

▲ = Control component



	M/Z	Intensity	Incl.
1	703.11	10187.832	☑
2	481.25	51095.555	☑
3	482.31	18558.747	☑
4	479.16	133603.694	☑
5	333.14	11796.821	☑
6	252.15	44388.630	☑
7	463.26	894215.029	☑
8	465.31	348290.305	☑
9	466.26	92297.881	☑
10	467.21	20245.644	☑
11	471.20	36245.908	☑
12	473.23	12539.757	☑
13	282.13	11972.037	☑
14	491.31	14439.828	☑
15	271.20	70080.430	☑
16	272.21	10545.656	☑
17	293.06	71924.617	☑
18	315.25	11868.074	☑
19	441.36	11306.993	☑
20	562.66	17764.387	☑
21	504.99	14865.428	☑
22	266.25	21113.290	☑
23	479.29	177051.035	☑
24	477.23	485132.514	☑
25	245.09	11926.839	☑
26	337.31	10990.021	☑
27	437.30	18336.562	☑
28	238.12	17374.400	☑
29	332.08	13173.250	☑
30	337.30	36537.845	☑
31	361.28	11406.650	☑
32	526.25	25978.137	☑
33	548.30	27441.649	☑
34	54.89	11638.934	☑
35	570.28	12976.407	☑
36	961.65	22229.617	☑

The first results view in MetWorks is the Component Detection view. You can also display this view by clicking on the Component Detection button on the toolbar. This view allows you to review and confirm the list of components that MetWorks determined to be unique to the sample data. The Component Detection view displays the Sample TIC on the top right, the Control TIC in the middle, and the Potential Modification TIC as the third trace which shows components unique to the sample. You can click anywhere on these chromatograms and get a comparison of the sample and control spectra at the bottom of the MetWorks window. MetWorks uses different colored triangles on the chromatograms and color coding in the Component Detection table to indicate status of various components. The Green triangles on the chromatograms and in the Component Detection table are components the software determined to be unique to the sample. The Red triangles on the chromatograms and in the Component Detection table are components the software detected in the control as well as the sample (this is not applicable if you did not associate a control with your sample). The Yellow highlighted components in the Component Detection table are components that you manually included or excluded from the list of selected components. If you defined a custom trace from a radioactivity detector, the software uses the trace to filter the sample data and retains only the results that correspond in time to a detected peak in the radioactively labeled trace. The Potential Modification Components chromatogram displays only the components that both pass the radioactive-label trace filter and are unique to the sample.

Displaying Spectra for a Selected Component

Components in Sample RT: 3.20 - 4.26

NL: 1.94E6
TIC F: ms MS
Lop_1scan_10uM_30mi
n_MS3_LXGESIMS2Bi
g3MS3_w_DE

Click above triangle to put red cursor on component and display spectrum

Components in Control RT: 3.20 - 4.26

NL: 1.23E6
TIC F: ms MS
BlankLop_1scan_MS
3_LXGESIMS2Bi
MS3_w_DE

Potential Modification Components RT: 3.20 - 4.26

NL: 1.94E6
TIC F: ms MS
Lop_1scan_10uM_30mi
n_MS3_LXGESIMS2Bi
g3MS3_w_DE

Use arrows to scroll thru components or scans and click "I" or "E" to include or exclude components

Number of traces: 3

I E C A 1 2 3 n

#24 RT: 3.832 NL: 7.27E4 (Sample Average)
F: ITMS + c ESI Full ms [150.00-1000.00]

Relative Abundance

477.27
479.28
293.12 463.25 562.80 706.67 743.04 961.20

m/z

Click numbers to display selected MS order and "C" or "A" to display the composite or averaged spectrum

Click on a control component to view spectrum

To display the spectrum for a component, click above a triangle in a chromatogram to put the red cursor on a component or a click a row in the Component detection table. If the component was detected in the sample only, the corresponding spectrum appears in the bottom left spectrum pane. If the component was detected in the sample and control, a spectrum appears in both the spectrum panes. Using the toolbar above the spectrum pane you can display the composite, averaged or single spectrum. Click "C" to display the Composite spectrum, which combines all MSn stages for the component. Click "A" to display the Averaged spectrum and then click 2, 3, or n to display the average of the MS2, MS3, or MSn, respectively, for the component. To display a Single spectrum, click "A" or "C", if selected, so that neither is selected. Click the 1, 2, 3, or n to display the spectrum of the scan for the selected MS order. Click the arrow buttons to display the spectrum for the previous or next component (composite or averaged spectrum) or the previous or next scan (single spectrum) and "I" or "E" to include or exclude components.

Editing the Component Selections

Component Detection View

	M/Z	Intensity	Incl
1	703.11	10187.832	<input checked="" type="checkbox"/>
2	481.25	51095.555	<input checked="" type="checkbox"/>
3	482.31	16556.747	<input checked="" type="checkbox"/>
4	479.16	133603.694	<input checked="" type="checkbox"/>
5	333.14	11795.621	<input checked="" type="checkbox"/>
6	252.15	44356.530	<input checked="" type="checkbox"/>
7	463.26	894215.029	<input checked="" type="checkbox"/>
8	465.31	348250.306	<input checked="" type="checkbox"/>
9	466.26	92297.881	<input checked="" type="checkbox"/>
10	467.21	20245.644	<input checked="" type="checkbox"/>
11	471.20	35245.988	<input checked="" type="checkbox"/>
12	473.23	12539.757	<input checked="" type="checkbox"/>
13	502.13	14979.618	<input checked="" type="checkbox"/>
14	491.31	14439.928	<input checked="" type="checkbox"/>
15	271.20	70080.430	<input checked="" type="checkbox"/>
16	272.21	10545.656	<input checked="" type="checkbox"/>
17	293.06	71924.617	<input checked="" type="checkbox"/>
18	315.25	11666.074	<input checked="" type="checkbox"/>
19	441.36	11305.993	<input checked="" type="checkbox"/>
20	552.66	17764.387	<input checked="" type="checkbox"/>
21	584.99	14965.428	<input checked="" type="checkbox"/>
22	266.25	21113.360	<input checked="" type="checkbox"/>
23	479.29	177051.836	<input checked="" type="checkbox"/>
24	477.23	485132.514	<input checked="" type="checkbox"/>
25	245.09	11926.839	<input checked="" type="checkbox"/>
26	337.31	10998.021	<input checked="" type="checkbox"/>
27	437.30	16335.562	<input checked="" type="checkbox"/>
28	239.12	17374.400	<input checked="" type="checkbox"/>
29	332.08	13173.250	<input checked="" type="checkbox"/>
30	337.30	36527.846	<input checked="" type="checkbox"/>
31	361.28	11406.650	<input checked="" type="checkbox"/>
32	526.25	25978.197	<input checked="" type="checkbox"/>
33	548.30	27441.649	<input checked="" type="checkbox"/>
34	549.39	11638.934	<input checked="" type="checkbox"/>
35	570.28	12976.407	<input checked="" type="checkbox"/>
36	961.65	22229.617	<input checked="" type="checkbox"/>

Component Detection View

Components in Sample RT: 3.20 - 4.26

Components in Control RT: 3.20 - 4.26

Potential Modification Components RT: 3.20 - 4.26

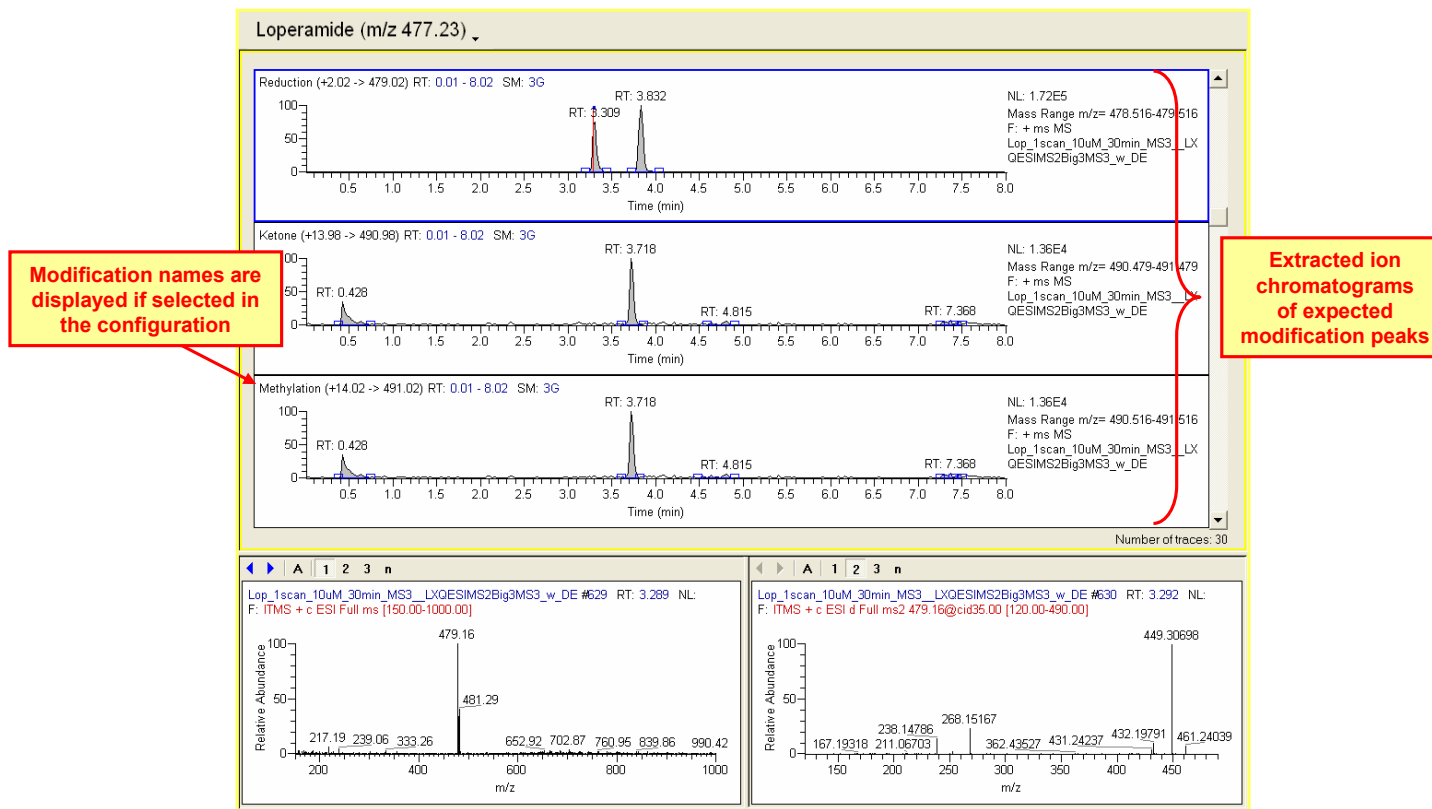
Sample Spectrum: #23 RT: 3.832 NL: 7.28E4 (Sample Average)
F: ITMS + c ESI Full ms [150.00-1000.00]

Callouts:

- Click boxes to include or exclude components
- Triangles of excluded components turn clear
- Can also right-click and select exclude to exclude component

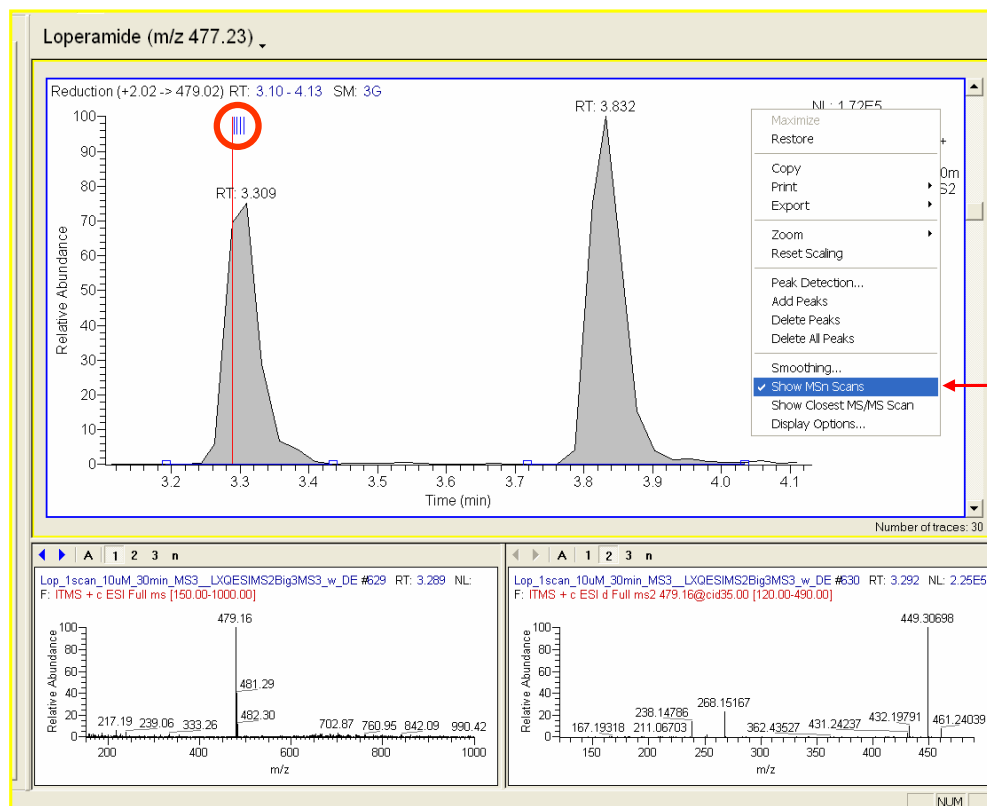
There are various ways to include components of interest or exclude components that are not of interest. MetWorks updates the list of candidates you included or excluded in MetView. In the Component detection table, select the check boxes of the components you want to include in the MetView list and clear the check boxes of the components you want to exclude from the candidate list. In the Potential Modification Components chromatogram, MetWorks removes the green triangle and in the sample trace, the triangle turns clear. Alternatively, you can right-click the green triangle, and then choose Exclude to remove a component from the candidate list. Right-click the green triangle, and then choose Include to add a component to the candidate list. Components can also be included or excluded by clicking the "I" or "E" button above the spectrum pane. Choose File > Save to save your edits.

Expected Modifications View



The second MetWorks view is the Expected Modifications view and you can display this by clicking on the Expected Modifications button on the toolbar. If you defined expected modifications for an analyte when you set up the MetWorks method, the Expected Modifications view displays the extracted ion chromatograms for which MetWorks detected peaks. MetWorks also adds the detected peaks to the list of candidates in MetView. The extracted ion chromatogram (EIC) of the analyte is displayed as the first (top) trace, the modification chromatograms following and User Traces that were set up in the workflow are displayed last (after all of the expected modification chromatograms). If you selected Show modification names in the Display page when configuring the MetWorks method, the modification names are displayed on the top of all of the traces.

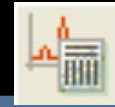
Displaying the Chromatograms



To display blue tick marks (MSⁿ scan indicators), make sure "Show MS Scans" is highlighted and zoom in on chromatogram

In the Expected Modifications view, any MSⁿ scans that were acquired are displayed with blue tick marks above the peaks in the chromatograms. When setting up the MetWorks method, check the box next to Show MSⁿ Scans in the Display page of the Default Display and Processing Settings. If this was not set up in the MetWorks method, right-click a chromatogram, and then choose Show MSⁿ Scans from the shortcut menu to view the MSⁿ scan indicators (blue tick marks).

Modification Peak Summary View



Loperamide (m/z 477.23) ▾

Peak detection summary:

2	3.815	540204	47.528	607303
---	-------	--------	--------	--------

User trace 3 summary

Peak#	RT (min)	Area	%Area	Height
1	3.832	1679438	100.000	315422

Chro_Search 1 (-11.76379 -> 465.23621) summary

Peak#	RT (min)	Area	%Area	% of Analyte Area	Height
1	3.604	973296	100.000	57.952	332372

Chro_Search 2 (+171.55908 -> 648.55908) summary

Peak#	RT (min)	Area	%Area	% of Analyte Area	Height
1	4.945	181327	6.642	10.797	19027
2	5.178	224602	8.228	13.373	22646
3	5.407	318356	11.662	18.956	41789
4	5.559	136635	5.005	8.136	24832
5	5.834	223951	8.204	13.334	27466
6	6.037	279128	10.225	16.620	69470
7	6.135	126197	4.623	7.514	39813
8	6.263	201683	7.388	12.009	27649
9	6.571	386100	14.143	22.989	34774
10	6.753	140558	5.149	8.369	50839
11	6.932	193606	7.092	11.528	26480
12	7.083	175234	6.419	10.434	32730
13	7.213	142509	5.220	8.485	22162

Chro_Search 3 (-183.84900 -> 293.15100) summary

Peak#	RT (min)	Area	%Area	% of Analyte Area	Height
1	0.478	227007	48.147	13.516	15964
2	3.761	244462	51.853	14.557	68956

Chro_Search 4 (-205.82599 -> 271.17401) summary

Peak#	RT (min)	Area	%Area	% of Analyte Area	Height
1	3.761	221244	100.000	13.173	66269

Chro_Search 5 (+484.00116 -> 961.00116) summary

Peak#	RT (min)	Area	%Area	% of Analyte Area	Height
1	5.049	231637	8.467	13.792	20673
2	5.332	291407	10.652	17.351	40073
3	5.533	134159	4.904	7.988	20561

Context menu options:

- Copy All
- Print...
- Create Excel Summary
- Show included Chro Search Only

Can select to view only included Chro Search data

The Modification Peak Summary can be accessed by clicking on the Modification Peak Summary button. This displays the peak summary of the chromatographic results from the Expected Modifications view. The Modification Peak Summary displays the overall summary of the analyte and the expected modifications you defined, the summary for the analyte chromatogram, the summary for each modification (including any User Traces that were specified as part of the workflow), the summaries from the results of the Chro Search and the summary for each custom trace. You can use the Modification Peak Summary to print or export a peak summary to Excel by right-clicking on the page and selecting Print or Create Excel Summary. There is also the option of selecting to view only data from the Chro Search Results view that you have chosen to include.

Spectral Correlation View



Spectral Correlation View

Analyte M/Z	RT (min)	Correlation	Shift	Incl.
646.59	7.060	1.069	410.888	<input type="checkbox"/>
646.78	6.445	1.025	411.027	<input type="checkbox"/>
266.25	3.812	1.020	0.00000	<input checked="" type="checkbox"/>
266.17	4.072	1.017	0.00000	<input checked="" type="checkbox"/>
477.23	3.832	1.000	0.00000	<input checked="" type="checkbox"/>
646.80	5.938	0.970	410.814	<input type="checkbox"/>
722.05	2.987	0.969	485.889	<input type="checkbox"/>
961.44	6.487	0.956	620.961	<input type="checkbox"/>
646.56	6.214	0.952	411.033	<input type="checkbox"/>
646.60	6.959	0.951	411.002	<input type="checkbox"/>
648.53	5.834	0.932	121.079	<input checked="" type="checkbox"/>
646.64	5.407	0.916	410.864	<input type="checkbox"/>
646.62	5.834	0.899	411.115	<input checked="" type="checkbox"/>
645.00	6.014	0.894	410.006	<input type="checkbox"/>
648.51	4.945	0.882	63.2503	<input checked="" type="checkbox"/>
646.65	6.112	0.875	411.113	<input type="checkbox"/>
646.63	5.074	0.859	224.825	<input checked="" type="checkbox"/>
646.64	5.684	0.842	410.853	<input type="checkbox"/>
646.71	6.651	0.838	411.029	<input type="checkbox"/>
958.74	5.432	0.836	398.126	<input checked="" type="checkbox"/>
958.37	6.533	0.828	513.947	<input type="checkbox"/>
721.95	2.884	0.820	292.926	<input type="checkbox"/>
646.77	6.755	0.818	410.842	<input type="checkbox"/>
960.96	6.677	0.810	457.049	<input type="checkbox"/>
445.05	0.210	0.801	148.146	<input type="checkbox"/>
961.04	5.332	0.750	671.028	<input checked="" type="checkbox"/>
961.05	5.049	0.745	605.158	<input checked="" type="checkbox"/>
961.03	5.935	0.742	654.968	<input checked="" type="checkbox"/>
958.79	6.149	0.739	340.857	<input type="checkbox"/>
961.06	5.533	0.733	684.139	<input checked="" type="checkbox"/>
960.69	6.573	0.732	456.150	<input type="checkbox"/>
646.58	6.858	0.730	410.962	<input type="checkbox"/>
961.09	6.888	0.721	612.911	<input type="checkbox"/>
645.01	4.688	0.700	409.049	<input type="checkbox"/>
961.01	4.623	0.695	620.964	<input type="checkbox"/>
648.60	5.935	0.695	343.964	<input checked="" type="checkbox"/>
648.81	5.407	0.695	141.082	<input checked="" type="checkbox"/>
645.21	2.340	0.693	410.063	<input type="checkbox"/>
961.10	6.265	0.685	642.047	<input type="checkbox"/>
648.59	6.600	0.679	199.113	<input type="checkbox"/>

Modification summary for Loperamide RT: 0.01 - 8.02

Correlation summary for Loperamide RT: 0.01 - 8.02

Lop_1scan_10uM_30min_MS3_LXQESIMS2Big3MS3_w_DE #43 RT: 3.828 NL: 2.64E3
F: ITMS + c ESI d Full ms3 477.23@cid35.00 238.21@cid35.00 [55.00-250.00]

Lop_1scan_10uM_30min_MS3_LXQESIMS2Big3MS3_w_DE #92 RT: 4.072 NL: 3.45E2
F: ITMS + c ESI d Full ms3 266.17@cid35.00 238.15@cid35.00 [55.00-250.00]

Spectral Correlation Results Table

Modification Summary Chromatogram

Correlation Summary Chromatogram

Reference Scan

Spectrum at Cursor Position

The Spectral Correlation view can be accessed by clicking on the Spectral Correlation button. The Spectral Correlation view provides the results of the cross-correlation between the MS/MS or MSn spectral patterns in the data to the reference scan which is an MS2 or MSn scan from your analyte or parent drug. This view includes the Spectral Correlation Results table which displays the correlation values from the cross-correlation analysis. MetWorks highlights the rows with either green or yellow depending on the high and low highlight thresholds you set in the Default Display and Processing Settings dialog box. This view also incorporates the Modification Summary chromatogram which displays the total extracted ion chromatogram for all modification chromatograms associated with the analyte and the Correlation Summary chromatogram which graphs the cross-correlation factors that MetWorks calculates by comparing the scans in the data to the reference MS2 or MSn scan. MetWorks graphs the same correlation values in the Correlation Summary chromatogram that appear in the Spectral Correlation results table. The reference scan from your parent drug is displayed beneath the Correlation Summary chromatogram. Click on the Modification Summary Chromatogram or the Correlation Summary Chromatogram to display the corresponding spectrum in the bottom pane and you can compare this to the reference scan.

Viewing the Results and Selecting the Peaks of Interest

Spectral Correlation View

Click to expand and show MSⁿ data associated with m/z

Analyte M/Z	RT (min)	Correlation	Shift	Incl.
646.58	7.060	1.069	410.886	<input type="checkbox"/>
646.78	6.445	1.025	411.027	<input type="checkbox"/>
266.25	3.812	1.020	0.00000	<input checked="" type="checkbox"/>
266.25	3.865	1.020	0.00000	<input type="checkbox"/>
266.25	3.859	0.481	0.00000	<input type="checkbox"/>
266.25	3.854	0.481	0.00000	<input type="checkbox"/>
266.17	4.072	1.017	0.00000	<input type="checkbox"/>
477.23	3.832	1.000	0.00000	<input checked="" type="checkbox"/>
646.60	5.938	0.970	410.814	<input type="checkbox"/>
722.05	2.987	0.969	485.689	<input type="checkbox"/>
961.44	6.487	0.956	620.961	<input type="checkbox"/>
646.56	6.214	0.952	411.033	<input type="checkbox"/>
646.60	6.959	0.951	411.002	<input type="checkbox"/>
648.53	5.834	0.932	121.079	<input type="checkbox"/>
646.64	5.407	0.916	410.864	<input checked="" type="checkbox"/>
646.62	5.834	0.899	411.115	<input type="checkbox"/>
646.62	5.834	0.894	410.006	<input type="checkbox"/>
646.71	6.651	0.836	398.126	<input checked="" type="checkbox"/>
958.74	5.432	0.828	513.947	<input type="checkbox"/>
958.37	6.533	0.820	292.926	<input type="checkbox"/>
721.95	2.884	0.820	410.942	<input type="checkbox"/>
646.77	6.755	0.818	410.942	<input type="checkbox"/>
960.96	6.677	0.810	457.049	<input type="checkbox"/>
445.05	0.210	0.801	148.146	<input type="checkbox"/>
961.04	5.332	0.750	671.028	<input type="checkbox"/>
961.05	5.049	0.745	605.158	<input type="checkbox"/>
961.03	5.935	0.742	654.968	<input type="checkbox"/>
958.79	6.149	0.739	340.857	<input type="checkbox"/>
961.06	5.533	0.733	684.139	<input type="checkbox"/>
960.69	6.573	0.732	456.150	<input type="checkbox"/>
646.58	6.858	0.730	410.962	<input type="checkbox"/>
961.09	6.888	0.721	612.911	<input type="checkbox"/>
645.01	4.688	0.700	409.049	<input type="checkbox"/>
961.01	4.623	0.695	620.964	<input type="checkbox"/>
648.60	6.935	0.695	343.954	<input type="checkbox"/>

Sort: By Retention Time, By Analyte Mass, By Correlation Value, By Mass Shift

Right-click to sort table rows

Modification summary for Loperamide RT: 0.01 - 8.02 NL: 1.02E6

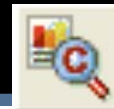
Correlation summary for Loperamide RT: 0.01 - 8.02 NL: 1.07

Lop_1scan_10uM_30min_MS3_LXQESIMS2Big3MS3_w_DE #743 RT: 3.828 NL: 2.64E3
F: ITMS + c ESI d Full ms3 477.23@cid95.00 238.21@cid95.00 [55.00-250.00]

Lop_1scan_10uM_30min_MS3_LXQESIMS2Big3MS3_w_DE #430 RT: 7.331 NL: 2.34E2
F: ITMS + c ESI d Full ms3 314.03@cid95.00 286.02@cid95.00 [65.00-300.00]

After importing the reference scan, select the check boxes of the candidates you want to include in the MetView list and clear the check boxes of the candidates you want to exclude. You can click on the + box next to the candidate to expand and show MSⁿ data associated with the candidate. To set the sorting order of the table, right-click on the table, and then choose to sort by Retention Time, Parent Mass, Correlation Value or Mass Shift. Use the vertical scroll bar in the table to scroll through the results. The green and yellow color highlights help locate the peaks with correlation scores that match the thresholds you specified in the Correlation page of the Default Display and Processing Settings dialog box. Green color highlights scans with a high cross-correlation factor and yellow highlights scans with a medium cross-correlation factor. No color highlights the scans with cross-correlation factors that fall below the Low highlight threshold, but above the Correlation cutoff threshold. Choose File > Save to save your edits as part of the MetWorks method.

Chro Search Results View

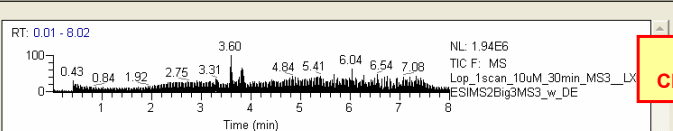


Loperamide (m/z 477.23)

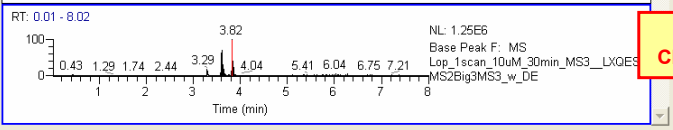
Number of chro search results: 9

	M/Z	Shift	Intensity	Incl
1	463.34	-13.89	894215.1	<input checked="" type="checkbox"/>
2	465.24	-11.99	348250.3	<input type="checkbox"/>
3	479.29	2.06	177051.8	<input checked="" type="checkbox"/>
4	648.96	171.33	98044.3	<input type="checkbox"/>
5	293.15	-184.08	71924.6	<input type="checkbox"/>
6	271.17	-206.06	70080.4	<input type="checkbox"/>
7	961.00	483.77	67792.7	<input type="checkbox"/>
8	646.48	169.25	62208.6	<input type="checkbox"/>
9	958.49	481.26	59048.3	<input type="checkbox"/>

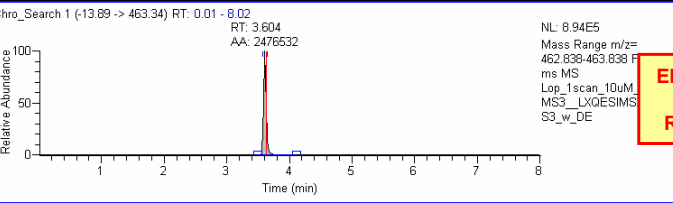
Results Table



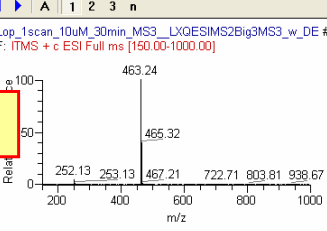
Total Ion Chromatogram



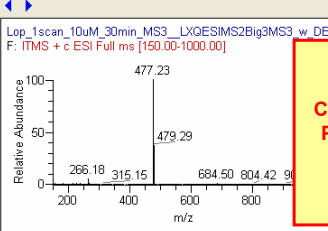
Base Peak Chromatogram



EIC of Selected m/z from Results Table



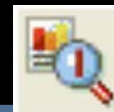
Spectrum at EIC Cursor Position



Spectrum at TIC or Base Peak Chromatogram Cursor Position or Toggle to Display Reference Scan from Spectral Correlation View

The Chro Search Results view can be displayed by clicking the Chro Search button. For unknown drugs it is not always possible to decide what the modifications are before you analyze them. The Chro Search Results view can help with this and identifies the extracted ion chromatograms for each of the most intense ions that have an intensity above a specific threshold. MetWorks compares the results of the search to the chromatograms generated from the expected modifications and eliminates any duplicate chromatograms. In the Chro Search Results table, you review the chromatograms and select the masses of interest to be included in the MetView candidate list. Also included in this view is the total ion chromatogram, the base peak chromatogram and the EIC of the selected candidate from the Chro Search Results table. You can click on these chromatograms to obtain the spectrum at the cursor position in the EIC in the bottom left-hand pane. In the bottom right-hand pane, you can either select to view the spectrum at the TIC or base peak chromatogram cursor position or right-click to toggle and display the reference scan.

Isotope Search Results View



Isotope Search View

Number of isotopes found: 10

Formula	RT (min)	M/Z	Intensity	Incl
C11	3.604	463.34	894215.1	<input checked="" type="checkbox"/>
C11	3.832	477.26	485132.4	<input type="checkbox"/>
C11	3.832	478.37	170025.1	<input type="checkbox"/>
C11	6.037	645.01	51312.7	<input type="checkbox"/>
C11	6.037	960.74	36932.3	<input type="checkbox"/>
C11	3.692	471.13	35245.9	<input checked="" type="checkbox"/>
C11	6.957	958.24	28207.3	<input type="checkbox"/>
C11	6.037	629.37	23960.2	<input type="checkbox"/>
C11	5.609	646.64	22594.1	<input type="checkbox"/>
C11	4.815	524.17	20271.1	<input type="checkbox"/>

Results Table

Total Ion Chromatogram

Base Peak Chromatogram

EIC of Selected m/z from Results Table

Spectrum at Cursor Position

The Isotope Search Results view can be accessed by clicking on the Isotope Search button. An isotope search allows you to search for compounds that have specific isotope ratios you specified during the setup of the MetWorks method. MetWorks can search for multiple labels for a single atom (for example, multiple Cl atoms) and multiple labels for different atoms (for example, multiple Cl and Br atoms). In the Results table, select the check boxes of the candidates you want to include in the MetView list and clear the check boxes of the candidates you want to exclude. The Isotope Search Results view also displays the total ion chromatogram, the base peak chromatogram, the EIC of the selected m/z from the Results table and the spectrum at the cursor position at the bottom.

Isotope Peak Summary View



Isotope Peak Summary View

Peak detection summary:

Isotope 1 (m/z 463.33844) summary (Included)

Peak#	RT (min)	Area	%Area	Height
1	3.604	2466695	100.000	851769

Isotope 2 (m/z 477.25861) summary

Peak#	RT (min)	Area	%Area	Height
1	3.832	1608438	100.000	470268

Isotope 3 (m/z 479.33044) summary

Peak#	RT (min)	Area	%Area	Height
1	3.309	450141	42.931	129296
2	3.832	598371	57.069	172020

Isotope 4 (m/z 645.00690) summary

Peak#	RT (min)	Area	%Area	Height
1	5.074	50096	3.798	9800
2	5.280	184221	13.966	15287
3	5.533	79421	6.021	12418
4	5.834	101127	7.666	10916
5	6.037	229638	17.409	43980
6	6.287	94030	7.128	14778
7	6.416	140903	10.682	18861
8	6.545	90712	6.877	26764
9	6.753	64935	4.923	22440
10	6.957	101251	7.676	10679
11	7.057	88241	6.689	13885
12	7.213	94526	7.166	21322

Isotope 5 (m/z 646.64337) summary (Included)

Peak#	RT (min)	Area	%Area	Height
1	5.074	117070	6.498	11743
2	5.230	89867	4.988	22622
3	5.407	213462	11.848	21678
4	5.684	119517	6.634	27801
5	5.834	90127	5.002	16094
6	6.037	359154	19.934	51397
7	6.287	143026	7.938	22840
8	6.545	291698	16.190	27865
9	6.753	93619	5.196	26463

- Copy All
- Print
- Create Excel Summary
- Show Included Isotopes Only

Right-click to select to view included isotopes only

The Isotope Peak Summary displays a peak detection summary for each of the isotopes from the Isotope Search Results view. To display the data for only the included isotopes, right-click the view, and then choose Show Included Isotopes Only from the shortcut menu. Included isotopes are the rows for which you select the Incl check box in the Isotope Search Results view. Each peak detection summary table contains columns for Peak #, RT (min.), Area, % Area, Height.

Specifying the MetView Columns and the MetReport Format

1. Click Options > MetReports

2. Select MetView Columns and MetReport Format

3. Click OK

Candidate	m/z	Include in
Loperamide_1	477.00	
CompDet 2	479.16	
CompDet 3	401.25	
CompDet 4	482.31	
CompDet 5	333.14	
CompDet 6	252.15	
CompDet 7	463.26	
CompDet 8	465.31	
CompDet 9	466.26	
CompDet 10	467.21	
CompDet 11	282.13	
CompDet 12	200.00	
CompDet 13	200.00	
CompDet 14	200.00	
CompDet 15	200.00	
CompDet 16	200.00	
CompDet 17	200.00	
CompDet 18	315.25	
CompDet 19	441.36	
CompDet 20	562.66	
CompDet 21	584.99	
CompDet 22	266.25	
CompDet 23	477.23	
CompDet 24	479.29	
CompDet 26	397.31	
CompDet 34	54.00	
CompDet 35	57.00	
CompDet 39	96.00	
CompDet 40	648.51	
CompDet 41	961.05	
CompDet 42	646.63	
CompDet 43	626.83	
CompDet 44	640.59	
CompDet 45	626.10	
CompDet 46	646.63	
CompDet 47	958.23	
CompDet 48	645.07	
CompDet 49	950.06	

To specify the MetView columns and the MetReport format, choose Options > MetReports to open the MetView Reports Configuration dialog box. Under MetView, select the check boxes of the columns you want to include in the MetView table. The options include Retention time, Structure, Delta, Number of fragments, Type of modification, Modification name, Elemental composition, Present in (which views included the candidate). Under MetReport, select the check boxes of any traces you want to include in MetReport. Under Layout, select how to display the traces. You can choose either Side by side or Stacked. To close the dialog box, click OK.

Specifying the MetView Columns and the MetReport Format

1. Click for new settings to update

Right-click and select Import to add structure

Indicates views where each candidate was selected

The candidate column orders components by first displaying the analyte and then candidates that were selected in: Component Detection, Expected Modifications, Spectral Correlation, Isotope Search, and Chro Search Views

Loperamide (m/z 477.23)													
MetView: Update Create this report													
Candidate	m/z	Structure	RT	Delta	F1	F2	PresView	Elem. Comp.	Include in MetReport				
Loperamide_1	477.00		3.83	0.00	0.00000	0.00000	P	C ₂₈ H ₄₁ O ₂ N ₅	<input checked="" type="checkbox"/>				
CompDet 2	479.16		3.31	2.16	449.30691	268.16040	CD,EM,Cor	C ₂₈ H ₄₁ N ₅	<input checked="" type="checkbox"/>				
CompDet 3	481.25		3.31	4.25	461.17258	450.23895	CD,Cor,Chro	C ₂₇ H ₄₀ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 4	482.31		3.31	5.31	0.00000	0.00000	CD	C ₂₇ H ₄₀ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 5	333.14		3.33	-143.86	315.11877	297.20721	CD	C ₁₂ H ₁₇ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 6	252.15		3.60	-224.85	224.14244	196.13542	CD,Cor	C ₁₈ H ₂₁ N ₅	<input checked="" type="checkbox"/>				
CompDet 7	463.26		3.60	-13.74	445.22096	252.13966	CD,EM,Cor,Iso	C ₁₈ H ₂₁ O ₂ N ₇	<input checked="" type="checkbox"/>				
CompDet 8	465.31		3.60	-11.69	253.11136	252.14056	CD,Cor,Chro	C ₂₇ H ₄₀ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 9	466.26		3.60	-10.74	0.00000	0.00000	CD	C ₁₂ H ₁₈ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 10	467.21		3.60	-9.79	253.14484	252.16486	CD,Cor	C ₁₈ H ₂₁ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 11	282.13		3.69	-194.87	0.00000	0.00000	CD	C ₈ H ₁₆ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 12	471.20		3.69	-5.80	282.15152	252.16470	CD,Cor	C ₂₄ H ₃₂ O ₂ N ₅	<input checked="" type="checkbox"/>				
CompDet 13	473.23		3.69	-3.77	283.21335	282.17725	CD,Cor	C ₂₈ H ₄₀ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 14	491.31		3.72	14.31	0.00000	0.00000	CD,EM	C ₁₈ H ₂₄ O ₂ N ₅	<input checked="" type="checkbox"/>				
CompDet 15	271.20		3.76	-205.80	171.91551	155.05356	CD	C ₁₂ H ₁₈ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 16	272.21		3.76	-204.79	0.00000	0.00000	CD	C ₁₈ H ₂₈ O ₁	<input checked="" type="checkbox"/>				
CompDet 17	293.06		3.76	-183.94	219.95224	194.12064	CD	C ₇ H ₁₁ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 18	315.25		3.76	-161.75	0.00000	0.00000	CD	C ₁₂ H ₂₀ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 19	441.36		3.76	-35.64	0.00000	0.00000	CD	C ₂₇ H ₄₀ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 20	562.66		3.76	85.66	494.97906	427.23431	CD,Cor		<input checked="" type="checkbox"/>				
CompDet 21	584.99		3.76	107.99	0.00000	0.00000	CD	C ₂₂ H ₃ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 22	266.25		3.81	-210.75	211.17020	210.18367	CD,Cor	C ₁₇ H ₂₂ O ₂ N ₄	<input checked="" type="checkbox"/>				
CompDet 23	477.23		3.83	0.23	266.18025	210.25462	CD,Cor	C ₁₈ H ₃₂ O ₈ N ₆	<input checked="" type="checkbox"/>				
CompDet 24	479.29		3.83	2.29	267.24976	266.16806	CD,EM,Cor	C ₁₃ H ₂₀ O ₈ N ₁₀	<input checked="" type="checkbox"/>				
CompDet 26	337.31		4.16	-139.69	319.15601	301.33678	CD,Cor	C ₂₂ H ₄₁ O ₂	<input checked="" type="checkbox"/>				
CompDet 34	549.39		4.79	72.39	0.00000	0.00000	CD	C ₂₇ H ₄₀ O ₄ N ₆	<input checked="" type="checkbox"/>				
CompDet 35	570.28		4.79	93.28	0.00000	0.00000	CD	C ₁₈ H ₄₀ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 39	958.26		4.87	481.26	853.73345	795.65769	CD,Cor,Iso		<input checked="" type="checkbox"/>				
CompDet 40	648.51		4.95	171.51	389.49721	273.42628	CD,Cor		<input checked="" type="checkbox"/>				
CompDet 41	961.05		5.05	484.05	893.86791	679.63124	CD,Cor		<input checked="" type="checkbox"/>				
CompDet 42	646.63		5.07	169.63	621.57560	447.27594	CD,Cor,Iso,Chr		<input checked="" type="checkbox"/>				
CompDet 43	625.83		5.10	148.83	0.00000	0.00000	CD		<input checked="" type="checkbox"/>				
CompDet 44	648.58		5.18	171.58	468.30320	447.40129	CD,Cor		<input checked="" type="checkbox"/>				
CompDet 45	626.10		5.20	149.10	0.00000	0.00000	CD	C ₂₄ H ₃₉ O ₂ N ₆	<input checked="" type="checkbox"/>				
CompDet 46	646.63		5.23	169.63	621.57019	447.19897	CD,Cor,Iso,Chr		<input checked="" type="checkbox"/>				

In MetView, click the Update button for the new configuration settings to update. The results appear for the preferences that you specified in the MetView Reports Configuration dialog box. If you want to import a structure, right-click and select Import to add a structure to the Structure column. The PresView column indicates the views where each candidate was selected. At a minimum, the MetView table includes Candidate, m/z and Include in MetReport columns. The Candidate column lists the analyte followed by the potential modifications selected from each of the MetWorks results views. The candidate column orders components by displaying the analyte and then candidates that were selected in the Component Detection, Expected Modifications, Spectral Correlation, Isotope Search, and Chro Search views. The m/z column lists the mass (m/z) of the peak of interest. The Include in MetReport column contains check boxes so that you can select the candidates that you want to include in the MetReport. By default all the candidates are selected. Clear the check boxes of any candidates you do not want to include in the MetReport.

Creating a MetReport

Loperamide (m/z 477.23)

MetView: Update Create this report

1. Click to create report

Candidate	m/z	Structure	RT	Delta	...	Elem. Comp.	Include in MetReport		
Loperamide_1	477.00		3.83	0.00	0.00000	0.00000	P	C ₂₆ H ₃₇ O ₂ N ₅	<input checked="" type="checkbox"/>
CompDet 2	479.16		3.31	2.16	449.30691	268.16040	CD,EM,Cor	C ₂₆ H ₃₇ N ₅	<input checked="" type="checkbox"/>
CompDet 3	481.25		3.31	4.25	451.17258	450.23895	CD,Cor,Chro	C ₁₉ H ₂₇ O ₁₆ N ₄	<input checked="" type="checkbox"/>
CompDet 4	482.31		3.31	5.31	0.00000	0.00000	CD	C ₂₇ H ₄₀ O ₂ N ₆	<input checked="" type="checkbox"/>
CompDet 5	333.14		3.33	-143.86	315.11877	297.20721	CD	C ₁₇ H ₂₁ O ₇ N ₄	<input checked="" type="checkbox"/>
CompDet 6	252.15		3.60	-224.85	224.14244	196.13542	CD,Cor	C ₁₆ H ₁₈ N ₃	<input checked="" type="checkbox"/>
CompDet 7								N ₇	<input checked="" type="checkbox"/>
CompDet 8								N ₆	<input checked="" type="checkbox"/>
CompDet 9								N ₆	<input checked="" type="checkbox"/>
CompDet 10								N ₆	<input checked="" type="checkbox"/>
CompDet 11								N ₆	<input checked="" type="checkbox"/>
CompDet 12								N ₆	<input checked="" type="checkbox"/>
CompDet 13								N ₆	<input checked="" type="checkbox"/>
CompDet 14								N ₆	<input checked="" type="checkbox"/>
CompDet 15								N ₆	<input checked="" type="checkbox"/>
CompDet 16								N ₆	<input checked="" type="checkbox"/>
CompDet 17								N ₆	<input checked="" type="checkbox"/>
CompDet 18								N ₆	<input checked="" type="checkbox"/>
CompDet 19								N ₆	<input checked="" type="checkbox"/>
CompDet 20								N ₆	<input checked="" type="checkbox"/>
CompDet 21								N ₆	<input checked="" type="checkbox"/>
CompDet 22								N ₆	<input checked="" type="checkbox"/>
CompDet 23								N ₆	<input checked="" type="checkbox"/>
CompDet 24								N ₆	<input checked="" type="checkbox"/>
CompDet 25								N ₆	<input checked="" type="checkbox"/>
CompDet 26								N ₆	<input checked="" type="checkbox"/>
CompDet 27								N ₆	<input checked="" type="checkbox"/>
CompDet 28								N ₆	<input checked="" type="checkbox"/>
CompDet 29								N ₆	<input checked="" type="checkbox"/>
CompDet 30								N ₆	<input checked="" type="checkbox"/>
CompDet 31								N ₆	<input checked="" type="checkbox"/>
CompDet 32								N ₆	<input checked="" type="checkbox"/>
CompDet 33								N ₆	<input checked="" type="checkbox"/>
CompDet 34								N ₆	<input checked="" type="checkbox"/>
CompDet 35	570.28		4.79	93.28	0.00000	0.00000	CD	C ₁₈ H ₃₄ O ₁₅ N ₆	<input checked="" type="checkbox"/>
CompDet 39	958.26		4.87	481.26	853.73345	795.65769	CD,Cor,Iso		<input checked="" type="checkbox"/>
CompDet 40	648.51		4.95	171.51	389.49721	273.42628	CD,Cor		<input checked="" type="checkbox"/>
CompDet 41	961.05		5.05	484.05	893.86791	679.53124	CD,Cor		<input checked="" type="checkbox"/>
CompDet 42	646.63		5.07	169.63	621.57550	447.27594	CD,Cor,Iso,Chr		<input checked="" type="checkbox"/>
CompDet 43	625.83		5.10	148.83	0.00000	0.00000	CD		<input checked="" type="checkbox"/>
CompDet 44	648.58		5.18	171.58	468.30320	447.40129	CD,Cor		<input checked="" type="checkbox"/>
CompDet 45	626.10		5.20	149.10	0.00000	0.00000	CD	C ₂₄ H ₃₈ O ₁₃ N ₆	<input checked="" type="checkbox"/>
CompDet 46	646.63		5.23	169.63	621.57019	447.19897	CD,Cor,Iso,Chr		<input checked="" type="checkbox"/>
CompDet 47	958.23		5.23	481.23	795.74770	679.50204	CD,Cor,Iso		<input checked="" type="checkbox"/>
CompDet 48	645.07		5.28	168.07	0.00000	0.00000	CD	C ₂₂ H ₃₅ O ₁₅ N ₆	<input checked="" type="checkbox"/>
CompDet 49	958.66		5.33	481.66	853.76788	737.71136	CD,Cor,Iso		<input checked="" type="checkbox"/>

2. Select Print

MetReport Format

Report output

Enable

Print PDF Word Excel

C:\Documents and Settings\amber.kohl\Desktop\Networks re ...

2. Or select path

3. Click OK

OK Cancel Help

The MetReport is a customizable report that compiles the data for the candidates (potential metabolites) you selected from all the other results views into a single report. To generate a MetReport, click Create this report. In the MetReport Format dialog box, select the Enable check box. To print the report without creating a new file, select Print, and then click OK. In the Print dialog box, select the print options, and then click OK. To output to a file, select the PDF, Word, or Excel option. Click to display the Save As dialog box. Select the destination folder, type a file name, and click Save.

Creating Additional Summary Reports (Optional)



Reports Configuration and Generation

Chro/Spec Summary | Tabulated Peak Summary | Custom Excel Report

Report Summary | Chromatogram Summary | Modification Summary | Peak Summary

Report settings

Result type

- Display analytes and modifications
- Display chro search results
 - Display included chro search only
- Display isotope search results
 - Display included isotope search only

Layout

Number per page:

- Show modification names
- Show MSn Scans

Orientation

Portrait Landscape

Margins (inches)

Left: Right:

Top: Bottom:

Click to print report →

Select which result types to include

Click to print report

Typically, you use MetView and MetReport to create customized reports for your selected candidates. However, you can also create additional summary reports by using the Reports Configuration and Generation dialog box by clicking on the Reports button at the end of the MetWorks workflow. If you want to change the settings for a given report, click the tab to display the corresponding reports page. The additional reporting options include a Chromatogram Summary, Modification Summary, Peak Summary, Chromatogram/Spectrum Summary, Tabulated Peak Summary and Custom Excel Report. On the specific page from which you want to generate the report, select which result types to include and then click Create this report to print the report.

ThermoFisher
SCIENTIFIC

The world leader in serving science

Mass Frontier 5.0

Database Manager

The Database Manager is a module for managing spectral and structural information in Microsoft SQL Desktop Engine (MSDE) database. This module provides powerful library maintenance utilities that enable you to create and organize spectral and chromatographic libraries with chemical structures. In addition, since the program supports ion structures and tree spectra representation you can also create true MSn libraries. Advanced library query and search features provide access to the information needed for compound identification and can help you interpret unknown spectra. A flexible set of search restrictions is available to target your search results, especially useful when you are dealing with large libraries.

Database Manager



All spectra organizing and manipulation is carried out in the **Database Manager**

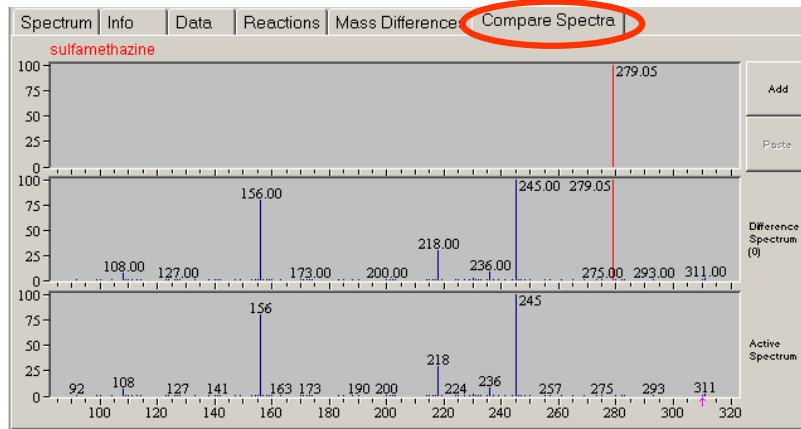
The screenshot displays the Database Manager software interface. The main window is titled "Database Manager: 3" and features a toolbar with various icons. Below the toolbar, there are several tabs: "Spectrum", "Info", "Data", "Reactions", "Mass Differences", and "Compare Spectra". The "Spectrum" tab is active, showing a mass spectrum plot with a base peak at 311.05. To the left of the plot, there are three smaller plots labeled "File MS1 #370", "Avg. MS2 311.05", and "File MS3 156.15 #372". To the right of the plot, there is a chemical structure of sulfadimethoxine. Below the plot, there is a "Spreadsheet" tab with a "Structures" sub-tab. The spreadsheet shows two records:

	ID Num.	Mol. Mass	Formula	Name
1	1	310.074	C ₁₂ H ₁₄ N ₄ O ₄ S	sulfadimethoxine
2	2	278.084	C ₁₂ H ₁₄ N ₄ O ₂ S	sulfamethazine

An arrow points from the "Structures" sub-tab to a window showing the chemical structures of sulfadimethoxine (1) and sulfamethazine (2).

In the Database Manager, each record includes additional information that is arranged in different tabs. The mass spectrum of the selected tree section can be viewed in the Spectrum tab. By clicking on the Structures tab, the record structures can be viewed instead of the record information displayed in the Spreadsheet tab.

Database Manager

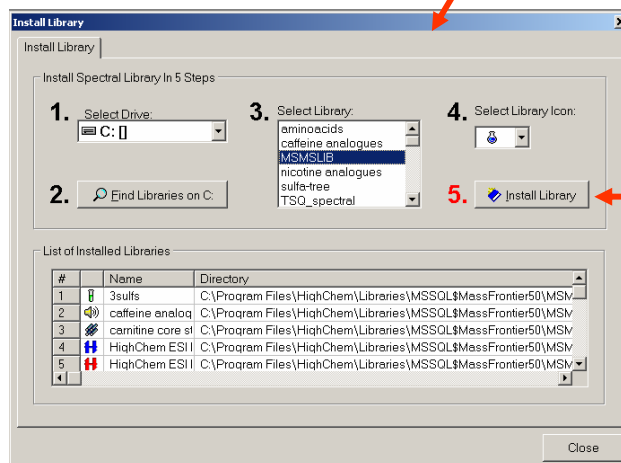
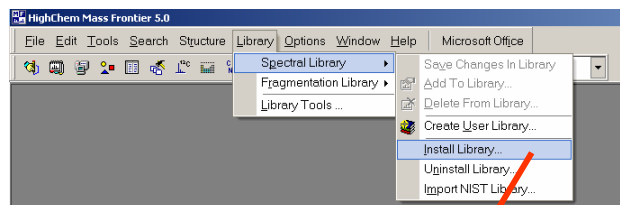


The Compare Spectra tab allows spectra comparisons to be carried out. The bottom spectrum is from the active record in the spreadsheet, while the top spectrum is updated with the active record by pressing the Add or Paste button. The middle spectrum displays the difference between the top and bottom spectrum.

After a spectrum search has been carried out, the query spectrum is automatically pasted to the top spectrum in the Compare Spectra tab to allow viewing of the peak differences of spectra in the hit list and query spectrum.

Commercially Available Libraries

If a commercially available library is in SQL format, then it can be directly **installed** in Mass Frontier.

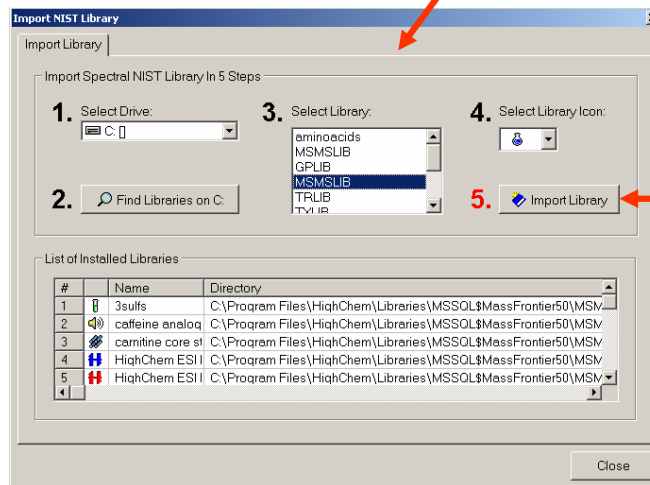
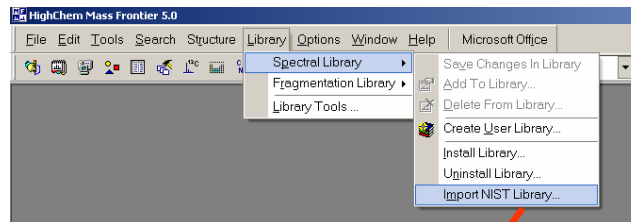


Follow steps 1-5 to install your library

Mass Frontier library utilities are based on the Microsoft SQL Server 2000 Desktop Engine (MSDE 2000)®. MSDE 2000 is a data engine built and based on core SQL server technology. It is a reliable storage engine and query processor for desktop applications. MSDE 2000 is Mass Frontier's background application. Library utilities are seamlessly integrated in a graphical interface and the user does not need to directly interact with the database engine. database engine allows the creation of databases with a maximum file size of 2 GB. Therefore, libraries created in Mass Frontier cannot exceed this file size. If you try to store data above this limit, Mass Frontier will inform you that this action cannot be completed and you should store the extra data in a new library.

Commercially Available Libraries

If in a different format, then the library has to be imported.

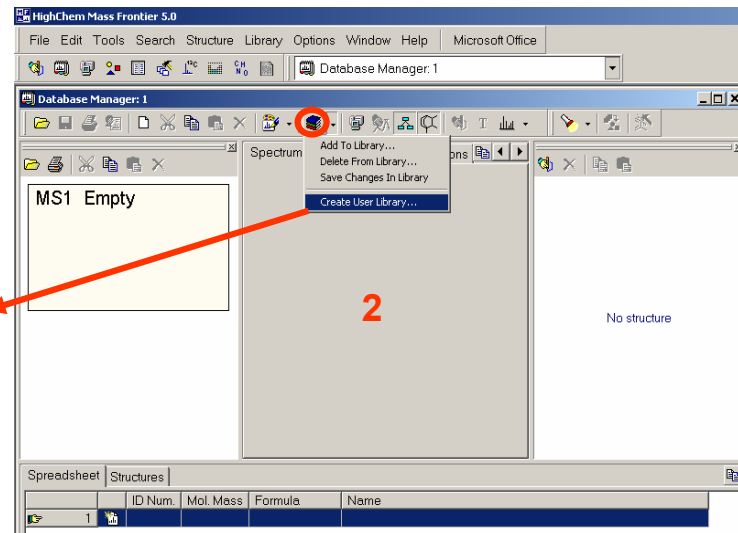
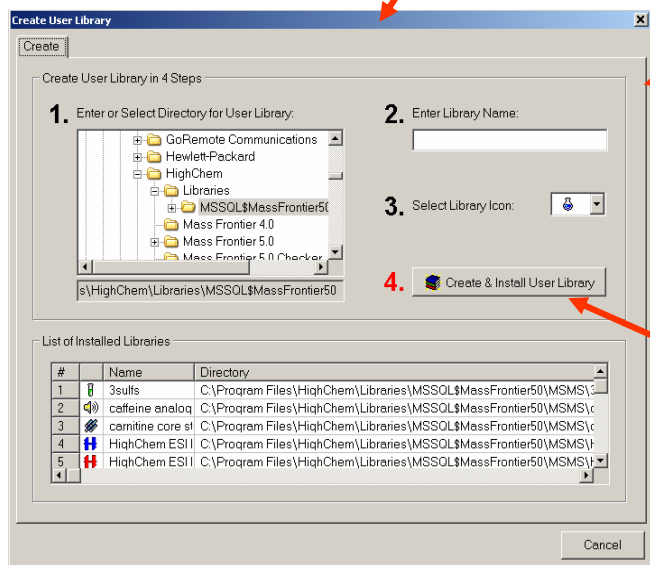
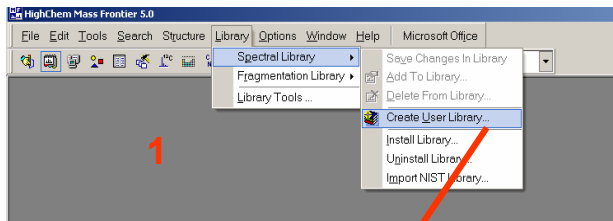


Follow steps 1-5 to import your library

When a library is imported, the software converts all the data to the SQL format.

Creating User Libraries

User libraries can be created in 2 ways:



Follow steps 1-4 to create and install a user library

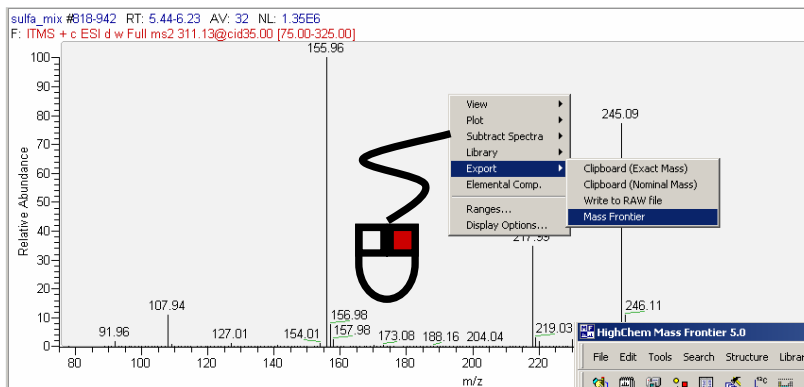


Access to all library functions is gained by clicking on this button from the Database Manager

An empty library is first created and installed. An unlimited number of libraries can be created, but no more than 255 libraries may be installed in Mass Frontier at the same time. To view different libraries than the ones displayed, you would need to uninstall some of them. If you uninstall a library, the library is not deleted, only the library reference is removed from the program without loss of any structural or spectral information. An uninstalled library can be reinstalled at any time.

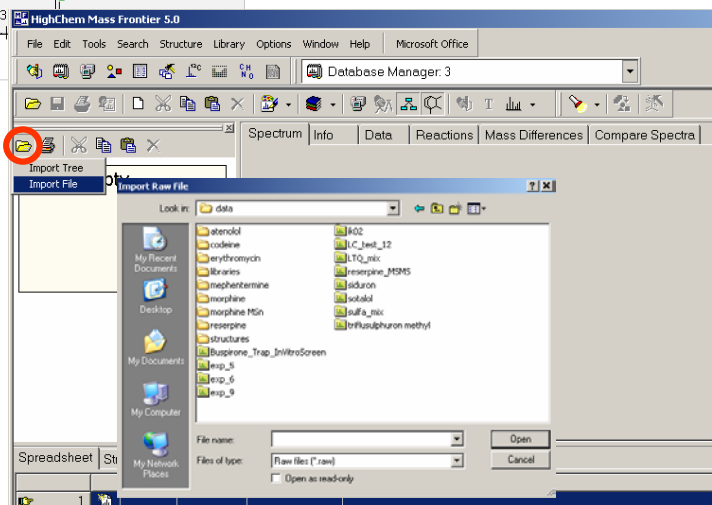
Building Libraries in the Database Manager

Mass spectra can be opened in the Database Manager in 2 ways:



1. Open the file in Qual Browser and export spectrum to Mass Frontier

2. Open the file directly into the Database Manager



Mass Frontier supports three file formats for spectra: JCAMP, MSP and Xcalibur® RAW format. The first two formats are ACSII files. Mass spectra in table format or spectral trees stored in Excel can also be imported to Database Manager via the Clipboard. If the spectral table is vertical in Microsoft Excel, the first column must be the m/z value and the second must be abundance. Click the Copy button in Excel and Paste the data into the Database Manager window. More than one spectrum can be imported at a time. In this case your first column, must be the m/z values and all the other columns must be abundance.

Building Libraries in the Database Manager

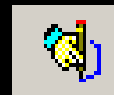
Adding Structures

Structures can be added to an entry in the Database Manager in 3 ways:

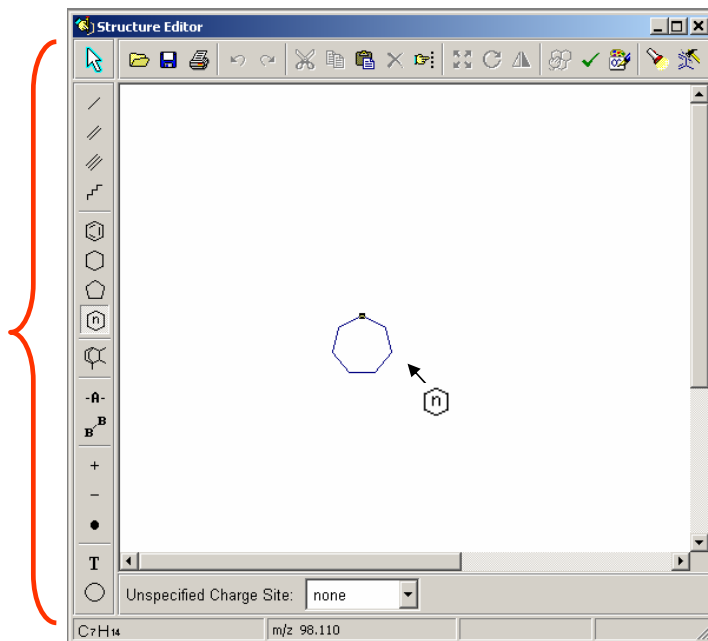
- By opening the Structure Editor directly from the structure pane and drawing a new structure.
- By copying and pasting a structure in the Database Manager from anywhere in the program.
- By loading a structure from an external file, in .mol or .sdf format.

You can copy a structure you wish to paste into Database Manager, from anywhere in the program. To paste a structure into Database Manager, use the Paste Structure button in the top right corner. The Copy and Paste buttons on the button bar are intended for records, not for single structures. Structures can be imported to Database Manager by loading them from an external MOL-file or SDF-file. In contrast to the pasting of a structure, when you load from an external file you can add structures to more than one record at a time. When adding structures to a spectral tree, different structures can be added for various nodes of the tree (make sure to have the node of interest selected).

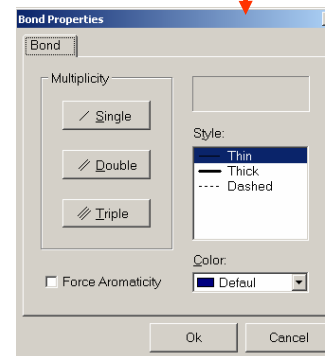
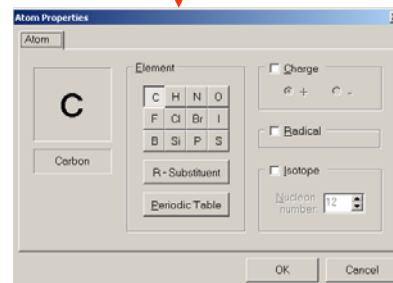
Structure Editor



To begin drawing a chemical structure, click on one of the buttons on the vertical toolbar. The shape of the cursor will change to visually represent the engaged drawing mode.



Atom **A** and Bond **B** properties can be edited in these windows.



The Structure Editor is a full-featured structure drawing tool for editing, importing, exporting and checking chemical structures. Structure Editor is the gateway to four other modules in this program: Database Manager, Fragments and Mechanisms, Fragmentation Libraries, and Isotope Pattern.

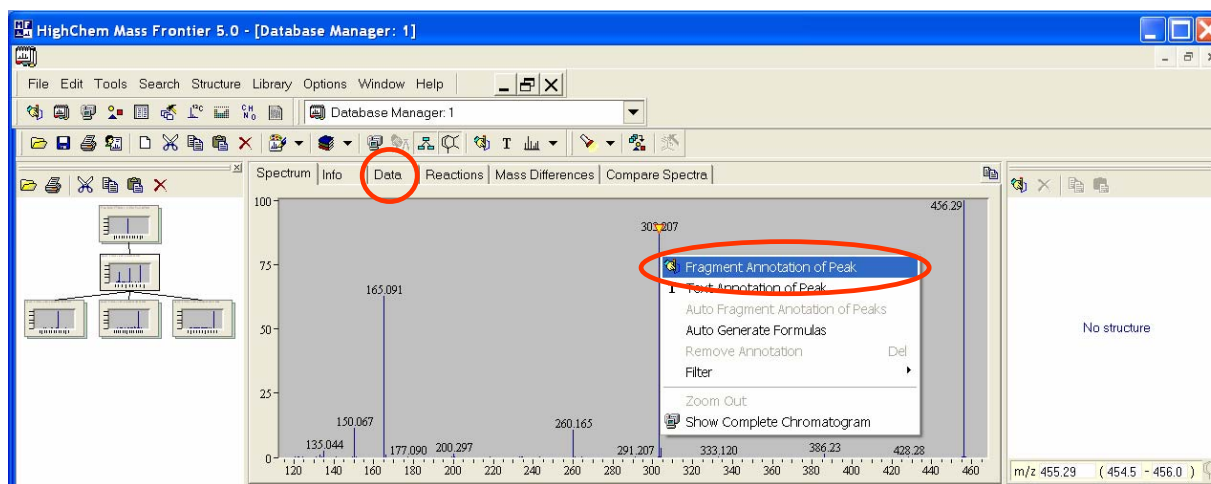
Building Libraries in the Database Manager

Adding Structures



Chemical structures can also be added to any ion in the spectrum. To add a chemical structure to an ion, right-click on the spectral peak and select **Fragment Annotation of Peak**.

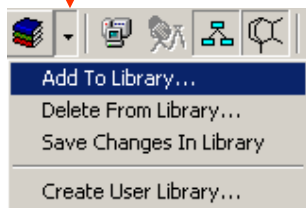
A structure can also be assigned to a peak using the **Data** tab in the Database Manager window.



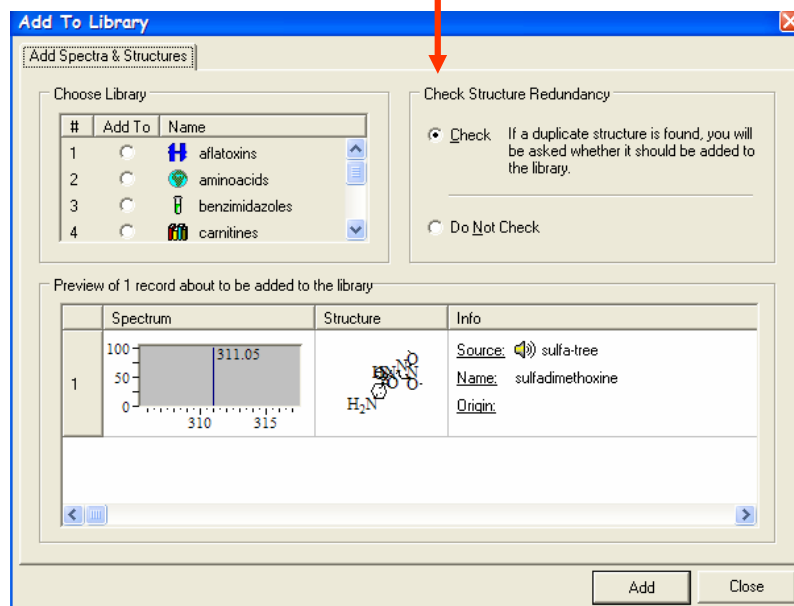
Chemical structures can be added to any peak in the spectrum. After you finish drawing, the program will automatically attempt to connect your structure with a spectral peak according to the fragment's m/z value. If, for whatever reason, you need to connect the drawn fragment to a different peak, simply drag the connecting circle with the mouse to the required peak. The drawn fragment can be resized. Fragments generated by Fragments & Mechanisms window can be assigned automatically to peaks in a spectrum. A structure can also be assigned to a peak using the Data page of the Database Manager window, where all peaks in the spectrum are listed. Click on the line where the peak is listed, then click on the button in the Fragment column and Structure Editor will open.

Adding Entries to User Libraries

1. Click to add to library



Mass Frontier allows you to check whether a structure is already present in a library before adding a new spectrum/structure pair.



Only SELECTED records in a Database Manager Spreadsheet can be added to a user library. Any record information can be changed and saved in a library. If anything is changed in the record, a small circle is displayed in the Database Manager in the ID Number column in the spreadsheet. To save any changes, go to Library > Spectral Library > Save Changes in Library. If you decide to delete a library record, information will be irreversibly lost. This process can be carried out using again the appropriate Library buttons. If you delete a record from a library, the ID numbers will change so that there are no ID gaps.

Searching Libraries

The screenshot shows the Database Manager software interface. A callout box points to the search menu with the text: "1. Click to search a library". The search menu includes options like "Search (Sub)Structure...", "Search Name...", "Search Molecular Mass...", "Search Formula...", "Search ID Number...", "Search CAS Number...", and "Search Retention Time...". A chemical structure of caffeine is displayed in the main window. A second callout box points to the "Substructure" option in the search menu with the text: "A (Sub)structure search is the most common type of search in Mass Frontier. It retrieves compounds that contain a common structural subset." The "Library Search" dialog box is open, showing the "Structure" tab. The "Search Type" section has "Identity" and "Substructure" radio buttons, both circled in red. The "Substructure" option is selected. Below this, there are checkboxes for "Ignore Charges, Radicals and Adducts" (checked), "Ignore Isotopes" (checked), "Substructure Best Match" (unchecked), and "Substructure Match Ring Bonds" (unchecked). The "Libraries" section shows a list of libraries with checkboxes for selection. The "Search in MS^n Trees" section has "Top Level Only" (unchecked) and "Everywhere" (checked) radio buttons. The "Constraints" section has "On" (checked) and "Off" (unchecked) radio buttons, and an "Edit" button. At the bottom, there is a checkbox for "Merge results into active Database Manager window" and "OK" and "Cancel" buttons.

MS1 Empty

1. Click to search a library

A (Sub)structure search is the most common type of search in Mass Frontier. It retrieves compounds that contain a common structural subset.

An Identity search provides an exact match of query and library search

Library Search

Structure

Search Type

Identity Substructure

Ignore Charges, Radicals and Adducts

Ignore Isotopes

Substructure Best Match

Substructure Match Ring Bonds

Libraries

#	Act...	Name
1	<input type="checkbox"/>	3sults
2	<input type="checkbox"/>	caffeine analogues
3	<input type="checkbox"/>	caffeine core structures
4	<input type="checkbox"/>	HighChem ESI Neg Total
5	<input type="checkbox"/>	HighChem ESI Pos Total

Search in MSⁿ Trees

Top Level Only

Everywhere

Constraints

On Off Edit

Merge results into active Database Manager window

OK Cancel

Thermo SCIENTIFIC

314

ThermoFisher SCIENTIFIC

Tree Search - searches for the library spectral tree most closely matching an unknown spectral tree or sub-tree.

Spectrum Search - searches for the library spectra most closely matching an unknown spectrum.

(Sub)structure Search - searches for an exact match for the structure (structure search) or searches for an exact match for the structure subset (substructure search).

Name Search - incremental name search.

Molecular Mass Search - searches for compounds with a given molecular mass.

Formula Search - searches for compounds with a given molecular formula.

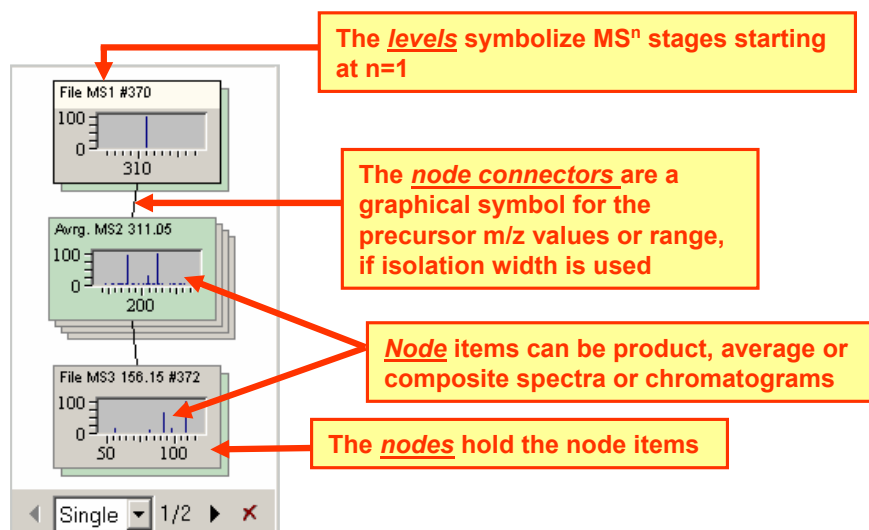
ID Number Search - searches for library entries with a given ID number.

CAS Number Search - searches for compound(s) with a given Chemical Abstract Service registry number.

Retention Time Search - searches for library entries with a range of given retention times.

Spectral Trees

A spectral tree consists of levels, nodes, node connectors and node items.



Mass Frontier uses spectral tree representations for MSⁿ spectra. Complete trees can be stored in a library and updated at any time. Any complementary information associated with a single stage spectrum or a chromatogram can be associated with a node spectrum or node chromatogram.

Creating Spectral Trees

Reconstruction from raw files

The tree reconstruction feature reads raw files stored in a directory and automatically creates a tree according to the precursor m/z and isolation width values.

1. Click on Open and Import Tree

2. Select the directory that contains the files that are to be used to create the tree

3. Add the directory by clicking on Add directory. Your directory will be added to the window on the right hand side

4. Click Import. The tree will appear in the Database Manager window

ID Num.	Mol. Mass	Formula	Name
1			

All spectra in a directory must come from an identical chemical entity. One directory, per tree, per compound. Each file is considered as a single parallel spectrum. This feature works for files that have been acquired using a single compound. The tree reconstruction feature not only creates levels, nodes and node connections but also automatically assigns node spectra to specific nodes.

Creating Spectral Trees

Spectral tree deconvolution from a chromatogram in the Chromatogram Processor

The image displays two overlapping windows from the ThermoFisher Mass Frontier 5.0 software. The top window, titled 'Chromatogram Processor MS2Big3MS3_FT_FT_1', shows a chromatogram with a prominent peak at approximately 100% relative intensity. A red box labeled '2. Click Copy' points to the 'Copy' icon in the software's toolbar. A red arrow labeled '1. Select component' points to the peak in the chromatogram. The bottom window, titled 'HighChem Mass Frontier 5.0 - [Database Manager: 1]', shows a 'Spectrum' view. A red box labeled '3. Open the Database Manager and paste the tree to import' points to the 'Paste Tree' option in a context menu that is open over the 'MS1' spectrum. The 'Paste Tree' option is circled in red. The left sidebar of the bottom window lists various precursor and component m/z values.

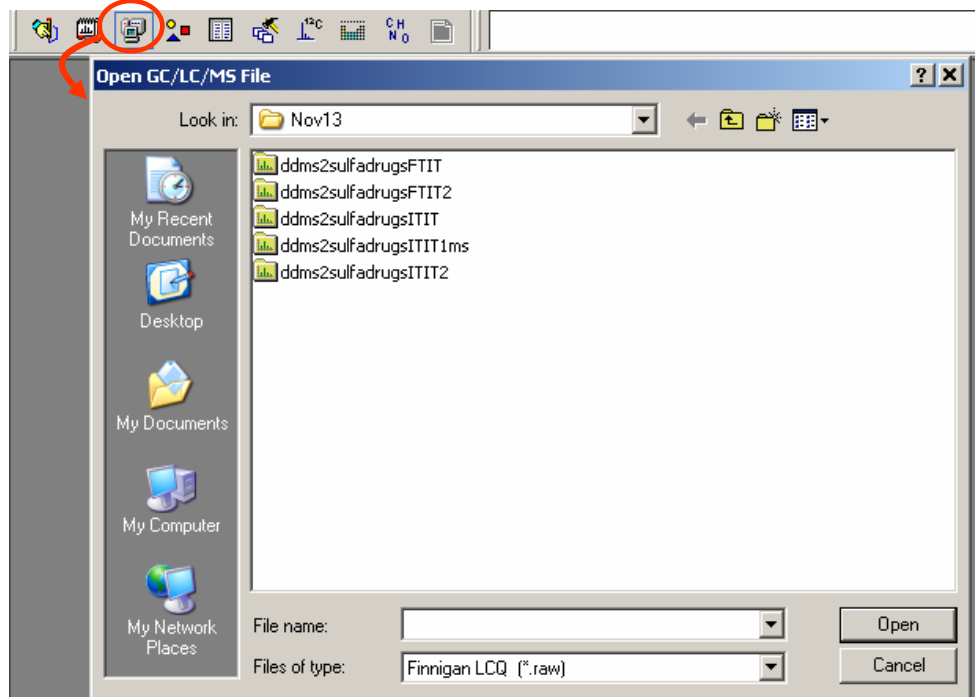
Mass Frontier allows the exchange of trees between windows, records, chromatographic components or programs (e.g. Excel). This can be done using standard copy and paste commands. When using the paste command, you must distinguish between a single spectrum and a tree so be sure to use the correct paste option when exchanging trees.

Chromatogram Processor

Chromatogram Processor



To open a raw file in the Chromatogram Processor, click on the Chromatogram Processor button. Select file and click open.



Mass Frontier supports various data file formats for importing GC-MS and LC-MS files: Xcalibur® RAW files (MS and MSn), Finnigan LCQ™, GCQ™, ITS40 and Magnum, Varian Saturn, HP ChemStation, JACAMP (DOS, Windows and UNIX). These files can be imported to Chromatogram Processor but cannot be exported. Single scans can be saved in JACAMP or MSP format. Mass Frontier supports centroid type data for mass spectra. Centroid mass spectra are displayed as a bar graph. Profile type data is not supported.

Chromatogram Processor

Automatic Components Detection and Spectra Deconvolution



This module of the software is designed to automatically detect the components present in complex mixtures analyzed mainly by data dependent analysis.

Mass Frontier identifies components using the following algorithms:

- RCD (Rapid Component Detection)
- JCD (Joint Component Detection)
- TECD (Total Extraction Component Detection)
- Direct Infusion

Mass Frontier incorporates an advanced automated system for detecting chromatographic components in complex GC/MS or LC/MS runs and extracting mass spectral signals from closely coeluting components (deconvolution). The Components Detection and Spectra Deconvolution system works fully automatically. The system is designed for broad types of chromatographic runs, for both GC/MS, LC/MS and GC/LC/MSn analyses, for clean and noisy signals, and for simple and for more complex chromatograms. However, some parameter changes may be needed to optimize the system for specific applications. This automated procedure is designed for small and medium size organic compounds and should not be used for the processing of proteins, peptides, oligonucleotides, or other biomolecules.

Chromatogram Processor

To perform component detection (spectra deconvolution):

1. Click the arrow beside the button and select the component detection algorithm to use

The screenshot displays the Chromatogram Processor software interface. At the top, a toolbar contains several icons, with a red circle highlighting the 'Components Detection and Spectra Deconvolution' icon. A dropdown menu is open, listing four options: RCD (highlighted in blue), JCD, TECD, and Direct Infusion. A red arrow points from this menu to the 'Deconvolution' dialog box on the right. The dialog box has a 'Calculate' button highlighted with a red box and a red arrow. The dialog box contains the following settings:

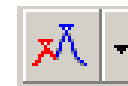
- Component Parameters: Threshold of T, Minimum Mode
- Minimum Component Spacing (TR): 0 sec.
- Smoothing: Smoothing Factor: 0 (0-99)
- Spectra Difference Factor: 500 (1-999)
- Scale Component Profiles
- Retention Time Range: Start: 0 min, End: 22 min
- m/z Range: Start: 49 min, End: 1500 min
- Background Subtraction: Automatic Manual None
- Precursor Ion Subtraction: Yes No
- Spectra Deconvolution: Sharp Soft
- Restore Defaults when Program Starts

The main window shows a chromatogram with several peaks. Below it is a mass spectrum plot with a peak at m/z 311.081. The x-axis is labeled 'm/z' and the y-axis is labeled 'Spectrum'. The status bar at the bottom shows: Scan TR: 7.1825 min., Scan No.: 941, SIC: m/z = 279.092 m/z = 251.060 m/z = 311.081 m/z = 254.060 m/z = 265.076 m/z = 271.032 | Filter: FTMS + p ESI Full ms [200.00-500.00]

To start automated component detection and the spectra deconvolution procedure, click on the Components Detection and Spectra Deconvolution button. When the parameters setup dialog window appears, change the settings if required, and then click the OK button.

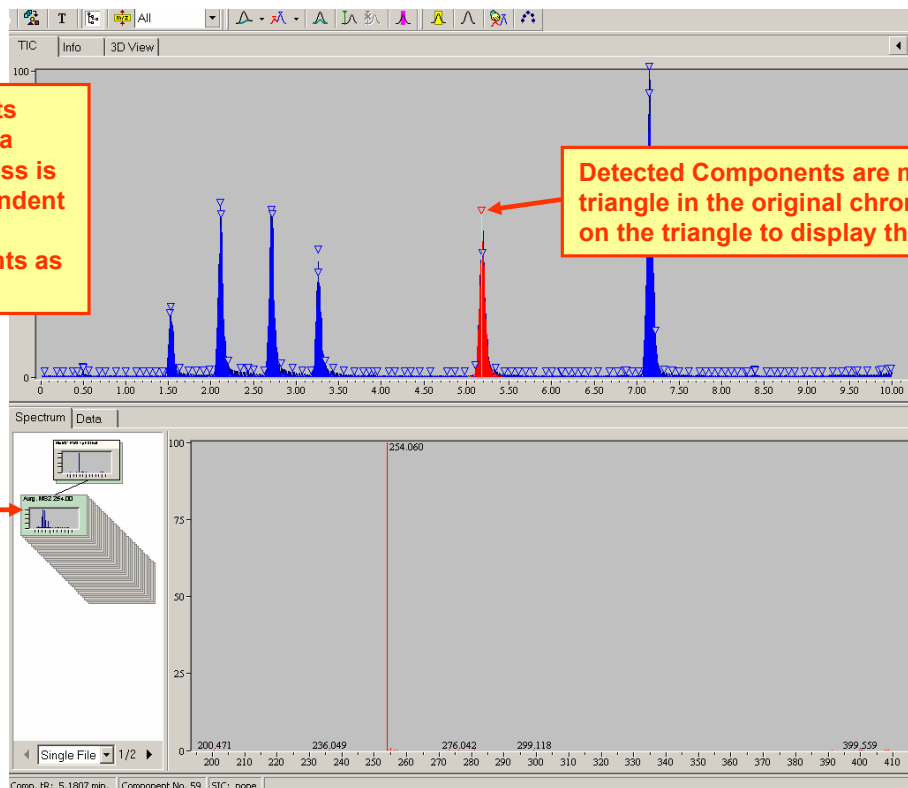
Chromatogram Processor

Automatic Components Detection and Spectra Deconvolution



Once the components detection and spectra deconvolution process is applied to data dependent scans, the program generates components as spectral trees

Detected Components are marked with a triangle in the original chromatogram. Click on the triangle to display the spectral tree.



Chromatogram Processor

To Import the Component (Spectral Tree) into the Database Manager from the Chromatogram Processor:

The image shows two overlapping software windows. The top window is the Chromatogram Processor, displaying a mass spectrum plot with a list of peaks on the left. A red box labeled "2. Click Copy" points to the Copy icon in the toolbar. A red arrow labeled "1. Select component" points to a peak in the spectrum. The bottom window is the HighChem Mass Frontier 5.0 Database Manager, showing a context menu with options: "Paste Tree", "Paste Parallel Spectrum", and "Paste Chromatogram". A red box labeled "3. Open the Database Manager and paste the tree to import" points to the "Paste Tree" option.

A convenient way to detect components, generate spectral trees and store them is to first perform component detection and spectral deconvolution on a data dependent experiment. Components and spectral trees can then be copied and pasted into the Database Manager for editing and storing the data.

Chromatogram Processor

Components Editor

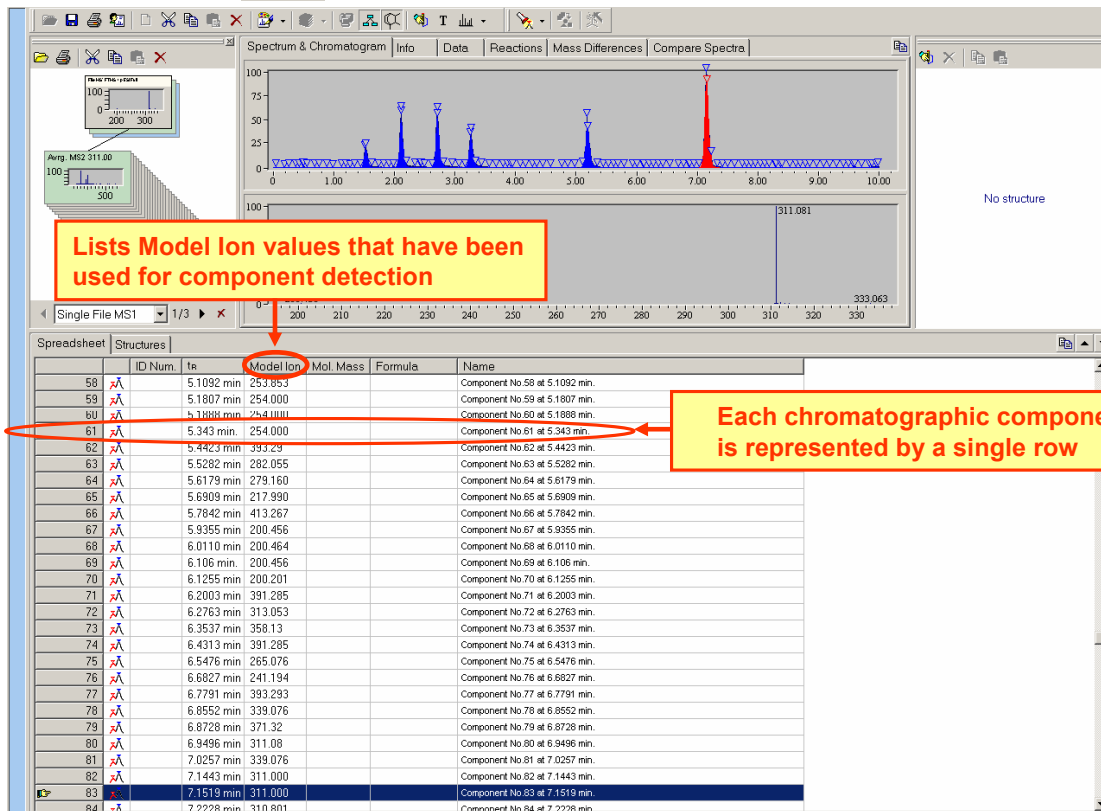


- The **Components Editor** allows the user to edit, search and organize chromatographic components found using the automated detection and deconvolution process.
- This module presents users with a complete set of management tools to delete unrelated components, add chemical structures, edit extensive data fields, process spectral trees, annotate spectral peaks and sort search hit lists for every component in a processed chromatogram.
- Components Editor is accessible from the Database Manager or Chromatogram Processor window using Components Editor button.

The Components Editor closely resembles visually the Database Manager module, however, the processing item is a chromatographic component rather than a database record. To ensure the modules are easy to tell apart, Components Editor has a light blue bar on the left side of the window. Both modules handle almost identically, so please refer to the Database Manager description for an explanation of module functionality.

Chromatogram Processor

Components Editor



Each chromatographic component is represented by a single row in the Components Editor. The columns contain component supplementary information. One of the columns lists Model Ion values that have been used for component detection. These values will help you quickly orient yourself and find components of interest. In most cases the model ion is the base peak in the full scan spectrum, however, if closely coeluting components have isobaric base peaks the algorithms select different model ions to distinguish the components.

Chromatogram Processor

Components Editor

Click to search selected components or all components for library matches

ID Num	Ret	Model Ion	Mol. Mass	Formula	Name
58	5.1092 min	253.853			Component No.58 at 5.1092 min.
59	5.1807 min	254.000			Component No.59 at 5.1807 min.
60	5.1898 min	254.000			Component No.60 at 5.1898 min.
61	5.343 min	254.000			Component No.61 at 5.343 min.
62	5.4423 min	393.29			Component No.62 at 5.4423 min.
63	5.5282 min	282.055			Component No.63 at 5.5282 min.
64	5.6179 min	279.160			Component No.64 at 5.6179 min.
65	5.6909 min	217.990			Component No.65 at 5.6909 min.
66	5.7842 min	413.267			Component No.66 at 5.7842 min.
67	5.9355 min	200.456			Component No.67 at 5.9355 min.
68	6.0110 min	200.464			Component No.68 at 6.0110 min.
69	6.106 min	200.456			Component No.69 at 6.106 min.
70	6.1255 min	200.201			Component No.70 at 6.1255 min.
71	6.2003 min	391.285			Component No.71 at 6.2003 min.
72	6.2763 min	313.053			Component No.72 at 6.2763 min.
73	6.3537 min	358.13			Component No.73 at 6.3537 min.
74	6.4313 min	391.285			Component No.74 at 6.4313 min.
75	6.5476 min	265.076			Component No.75 at 6.5476 min.
76	6.6827 min	241.194			Component No.76 at 6.6827 min.
77	6.7791 min	393.293			Component No.77 at 6.7791 min.
78	6.8552 min	339.076			Component No.78 at 6.8552 min.
79	6.8728 min	371.32			Component No.79 at 6.8728 min.
80	6.9496 min	311.08			Component No.80 at 6.9496 min.
81	7.0257 min	339.076			Component No.81 at 7.0257 min.
82	7.1443 min	311.000			Component No.82 at 7.1443 min.
83	7.1519 min	311.000			Component No.83 at 7.1519 min.
84	7.2228 min	310.801			Component No.84 at 7.2228 min.

The Components Editor allows the user to search one selected subset or all the chromatographic components in the spectral or chromatographic libraries at once. To search selected components, select components in the Spreadsheet and click the Search button in the toolbar and then choose the Search Selected Components item from the pop-up menu. To search all the components from the processed chromatogram, click the Search button and choose the Search All Components pop-up menu item.

Chromatogram Processor

Components Editor



The screenshot displays the Chromatogram Processor software interface. The main window shows a chromatogram with several peaks. A table below the chromatogram lists components with their retention times, model numbers, molecular masses, and formulas. A red box highlights components with library matches, such as component 83 (Sulfadimethoxine). A Hit Selector window is open, showing a list of matches for the selected component. A red box highlights the Hit Selector window, and another red box highlights the glasses icon in the component list, with an arrow pointing to the Hit Selector window. A chemical structure of Sulfadimethoxine is shown in the Hit Selector window.

Match	ID Num.	Mol. Mass	Formula	Name
1	972	447	C ₈ H ₁₀ N ₄ O ₄ S	Amino-N-(2,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide
2	765	448	C ₈ H ₁₀ N ₄ O ₄ S	4-Amino-N-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide
3	919	470	C ₈ H ₁₀ N ₄ O ₄ S	SULFADIMETHOXINE
4	919	2	C ₈ H ₁₀ N ₄ O ₄ S	SULFADIMETHOXINE
5	983	1	310.074	C ₈ H ₁₀ N ₄ O ₄ S sulfadimethoxine
6	919	9	310.074	C ₈ H ₁₀ N ₄ O ₄ S SULFADIMETHOXINE

Match	ID Num.	ts	Model Ion	Mol. Mass	Formula	Name
		5.343 min.	254.000			Component No.61 at 5.343 min.
		5.4423 min	393.29			Component No.62 at 5.4423 min.
		5.5292 min	282.055			Component No.63 at 5.5292 min.
		5.6179 min	279.160			Component No.64 at 5.6179 min.
		5.6909 min	217.990			Component No.65 at 5.6909 min.
		5.7842 min	413.267			Component No.66 at 5.7842 min.
		5.9355 min	200.456			Component No.67 at 5.9355 min.
		6.0110 min	200.464			Component No.68 at 6.0110 min.
		6.106 min.	200.456			Component No.69 at 6.106 min.
		6.1255 min.	200.201			Component No.70 at 6.1255 min.
		6.2003 min	391.285			Component No.71 at 6.2003 min.
		6.2763 min	313.053			Component No.72 at 6.2763 min.
		6.3537 min	358.13			Component No.73 at 6.3537 min.
		6.4313 min	391.285			Component No.74 at 6.4313 min.
						75 at 6.5476 min.
						76 at 6.6827 min.
						77 at 6.7791 min.
						78 at 6.8552 min.
						79 at 6.8728 min.
80		6.9496 min	311.08			Component No.80 at 6.9496 min.
81		7.0257 min	339.076			Component No.81 at 7.0257 min.
82		7.1493 min	311.000			Component No.82 at 7.1493 min.
83	919	7.1519 min	311.000	310.074	C ₈ H ₁₀ N ₄ O ₄ S	SULFADIMETHOXINE
84		7.2080 min	310.001			Component No.84 at 7.2080 min.
85		7.328 min.	353.092			Component No.85 at 7.328 min.
86		7.4180 min	393.293			Component No.86 at 7.4180 min.
87		7.5023 min	311.081			Component No.87 at 7.5023 min.

Components with library matches are highlighted in red

Click on the glasses to view the Hit Selector showing all the matches for a component

The Hit Selector window lists the best matches found during the library search. The match factor describes the similarity of the match to your component. If at least one hit was found, the text in the name field of the component row will appear in red. To process the hit list of a component, select the component row and open the Hit Selector window by clicking on the Hit Selector button (glasses). In Hit Selector you can review the hit list and accept a library record that correspond to the component by selecting the hit and clicking OK button. If you accept a library record for a component, all relevant information (structure, name, mol. mass, ion types etc.) will be adopted and entered in the component fields.

Fragments and Mechanisms

Fragments and Mechanisms

The **Fragments and Mechanisms** module is based on a system which uses a mathematical approach for the simulation of unimolecular ion-decomposition reactions. Two different methods are used:

1. General Fragmentation and Rearrangements rules

- The software generates fragment ions using straight cleavages and hydrogen rearrangements only.
- Bond creation is not supported with the exception of H bonds and thus, ring contractions, cyclizations and non-hydrogen rearrangements are not supported.
- Compound specific mechanisms that cannot be applied generally are also not included in this feature.

2. Fragmentation Library

- “Intelligent” fragmentation mechanism database.
- The software contains an expert system that automatically extracts a decomposition mechanism for each fragmentation reaction in the database and determines the compound class that the mechanism can be applied to.

One of Mass Frontier’s most outstanding features is the automated generation of possible fragments at an expert level, including complete fragmentation and rearrangement mechanisms, starting from a user-supplied chemical structure. The Fragments & Mechanisms module provides information about basic fragmentation and rearrangement processes that may occur in a mass spectrometer. Mass Frontier comes complete with almost 5,000 fragmentation schemes that contain around 19,000 reactions collected from mass spectrometry literature.

Fragments and Mechanisms

Fragmentation and rearrangement pathways can be generated from any user-supplied structure using the hammer button

Click the hammer button

Reaction Restrictions

Base | Ionization & Cleavage | H-Rearrangement | Resonance | Additional | Sizes

Knowledge Base

General Fragmentation Rules Fragmentation Library Both

Selected Fragmentation Library

#	Active	Library Name
1	<input type="checkbox"/>	HighChem
2	<input type="checkbox"/>	Training

Fragmentation Library Options

Active records only

Library Ionization only

Ignore General Frag. Rules in library reactions

Charge Localization Concept only

Display this window every time Generation of Fragments & Mechanisms is started

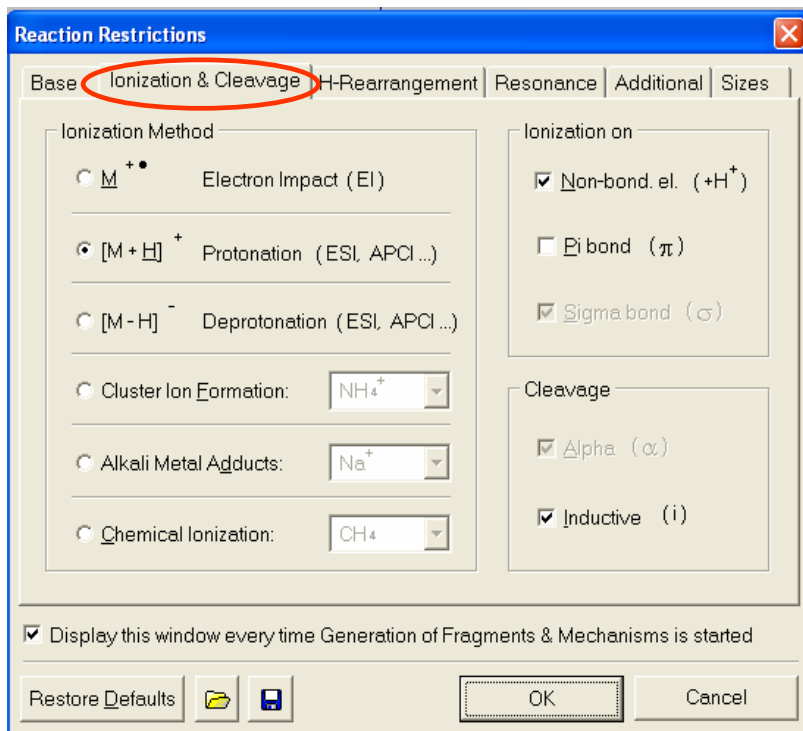
OK Cancel

Click to Generate Fragments and Mechanisms

You can specify the way the system will generate the various fragmentation pathways. The options are: General Fragmentation Rules, Fragmentation Library or Both.

If you choose the Fragmentation Library, you can select which library should be used from the list of libraries. Please note that the preinstalled HighChem Fragmentation Library™ contains around 19,000 mechanisms, and so calculation times will be significantly longer when it is used.

Fragments and Mechanisms



You can choose between the various ionization modes available. The Deprotonation option is not supported by general fragmentation rules and can only be used in connection with the Fragmentation Library.

When comparing generated fragments and mechanisms with a mass spectrum, you should always choose the correct ionization method. The program will show a warning message if the reaction restrictions are set for protonation techniques or chemical ionization and you are attempting to compare generated fragments with a spectrum from the NIST library which contains EI spectra only.

Fragments and Mechanisms

Reaction Restrictions

Base | Ionization & Cleavage | H-Rearrangement | Resonance | Additional | **Sizes**

Reaction Steps

Max. Number: 3

Resonance reactions are not included in this number

Mass Range

From: 30 m/z

To: 3000 m/z

Reactions Limit

Value: 10000

Reactions limit means number of temporary generated internal reactions. You can reasonably increase this number for larger input structures.

Display this window every time Generation of Fragments & Mechanisms is started

Restore Defaults | [Folder Icon] | [Save Icon] | OK | Cancel

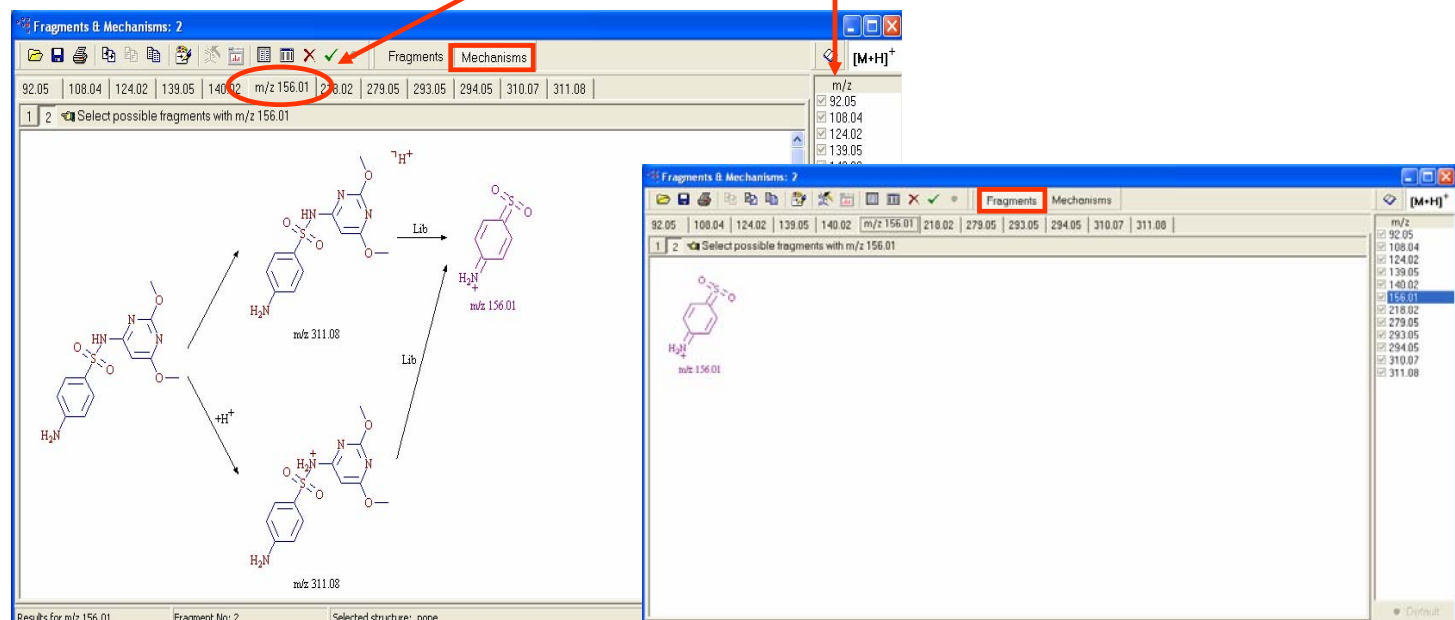
The Sizes page allows the user to limit the size and the complexity of a reaction pathway generation.

The Reaction Steps Max Number box gives the number of cascaded fragment ion reactions. Increasing this number could exponentially increase the number of fragment ions produced for a given reaction path. Generally, this number should be kept small and if additional fragments need to be generated, individual fragments can be selected by the user and used as starting points for additional reactions.

Fragments and Mechanisms

The Fragments and Mechanisms window displays either the complete mechanisms or the resulting fragment ions only.

To display the desired view for a certain m/z value, choose the appropriate m/z tab or select the ion from the m/z list on the right



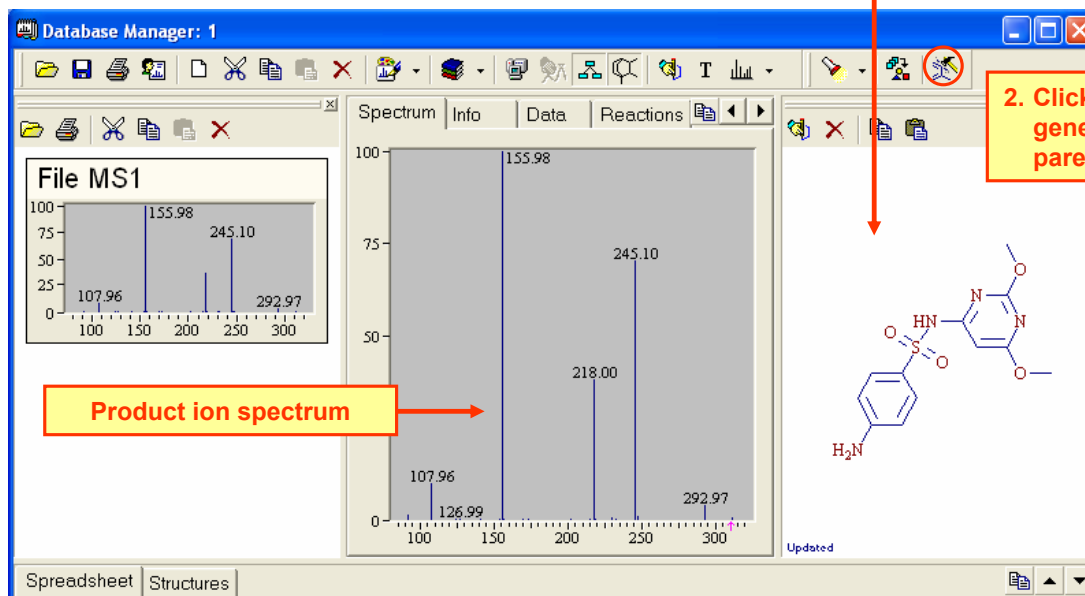
Once the reaction-generating process has finished, a Fragments & Mechanisms window is displayed. A Fragments & Mechanisms window allows the display of either complete mechanisms of ion-decomposition reactions, or of resulting fragments only. To display the desired mechanism, or fragment for a particular m/z value, choose the appropriate m/z tab. If you do not like the tab control you can replace it with a combo box in the Options > Reaction Layout dialog window. Several possible isobaric fragments can be generated for a particular m/z value. The isobaric fragments, with their corresponding mechanisms, can be displayed by clicking the numbered buttons next to the hand pointer. The fragments are sorted according to the simplicity of their production mechanism (with #1 being the simplest, shortest mechanism).

Fragments and Mechanisms

To link generated fragments to spectral data:

1. Input parent m/z into the Structure Editor window

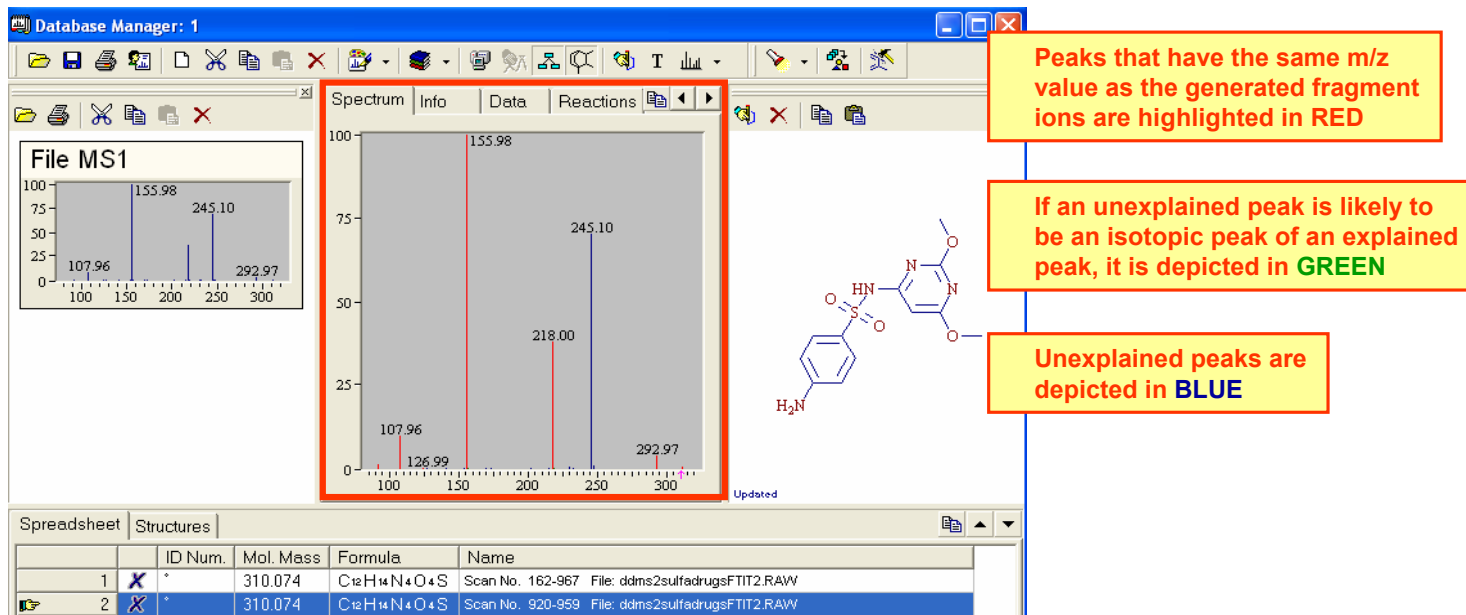
2. Click on the Hammer button to generate fragments of this parent



Mass Frontier offers the ability to link generated fragments with a mass spectrum. If you start a generation of fragments and mechanisms from Database Manager, the generated fragments are automatically linked with peaks in spectrum according to their m/z values. So Mass Frontier helps you to explain peaks in spectrum. While a generation is in progress the Reactions Limit bar gives you an approximate indication of how many temporary internal reactions have been generated from a particular structure. If the reactions limit is reached a message will appear informing you of the fact.

Fragments and Mechanisms

If the generation of fragment ions started from the Database Manager window, the program automatically links the generated fragment ions with the corresponding spectrum in the Database Manager window.



**Right-click on the spectrum and select 'Auto Fragment Annotation of Peaks' to display simulated fragments on the spectrum*

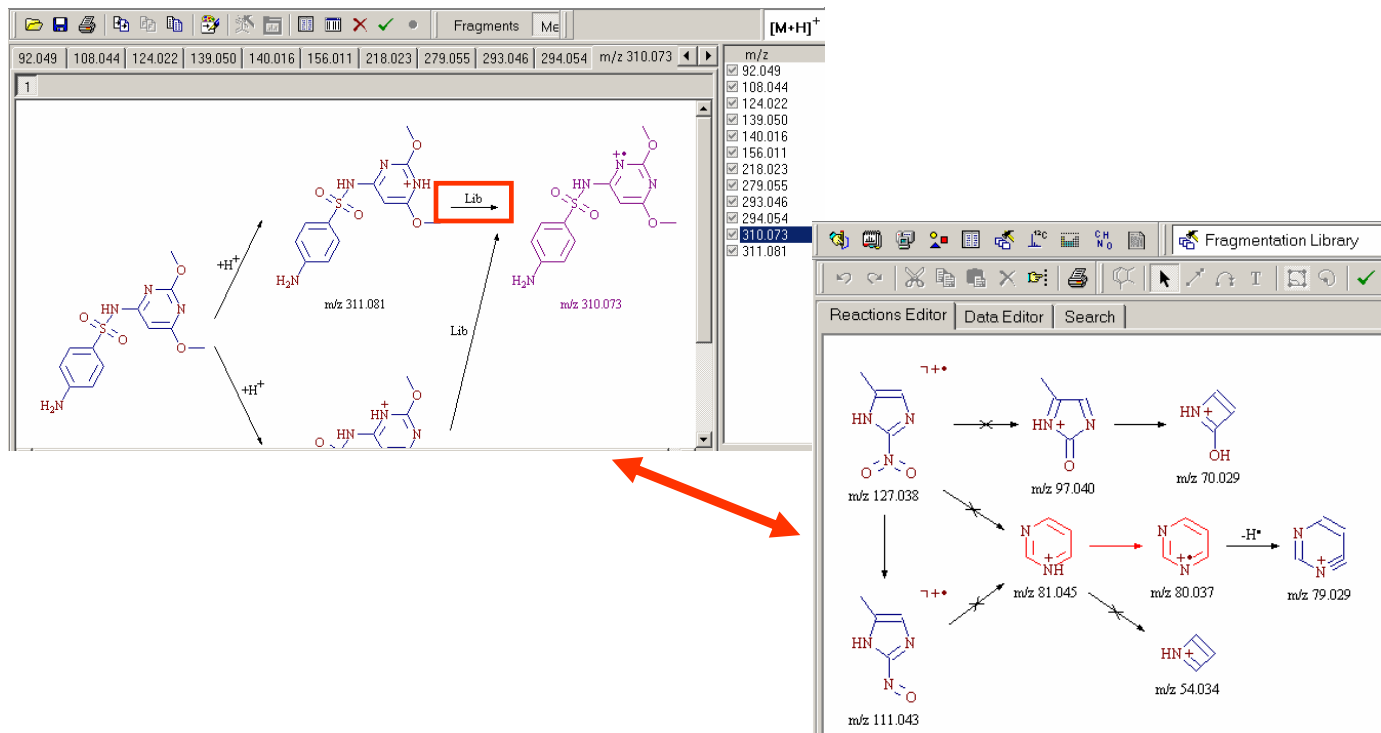
After a generation, highlighted ("explained") peaks are displayed in a different color (by default red) in the original mass spectrum. Selecting a highlighted peak reveals all the mechanisms leading to it. In addition, generated fragments (a corresponding Fragments & Mechanisms window must be open) can be assigned automatically to peaks in a spectrum in the Database Manager by right-clicking on the spectrum and choosing Auto Fragment Annotation of Peaks.

Fragmentation Libraries

Creating Fragmentation Libraries



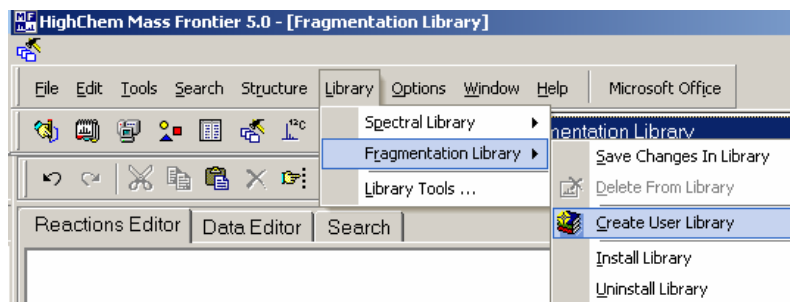
If a fragmentation reaction is predicted using a library reaction, you can double click on the **Lib** arrow to see the corresponding mechanism.



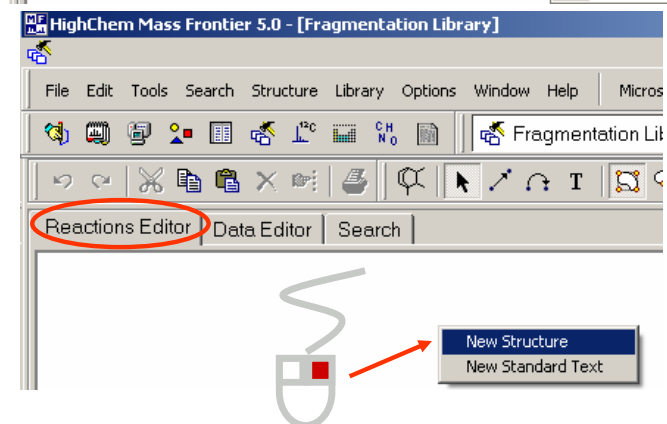
After double-clicking Lib in the Fragments and Mechanisms module, the Fragmentation Library opens in a new window. The Fragmentation Library™ module allows the creation and management of fragmentation mechanism databases. This module contains a full-featured graphical editor for entering fragmentation reactions, which can be stored in a database, together with complementary information for the reaction. All the fields of the database can be queried, for example: authors, ionization method, or mass analyzer. All the library structures from the reactions are also fully searchable.

Creating Fragmentation Libraries

For creating and editing fragmentation libraries, the **Reactions Editor** in the Fragmentation Library module should be used.



1. Create fragmentation library



2. Open that fragmentation library and either copy and paste the mechanisms from the Fragments and Mechanisms window OR add a new structure

The structures in a reaction scheme must be properly connected by arrows. The system considers standalone structures and disconnected arrows as errors and ignores them. To make a change permanent in a Fragmentation Library, it must be saved. To save changes in the Reaction Editor or Data Editor:

Click on any prior or following record to the edited record in the record grid

Or, click the line up or line down key

Or, click on the Library button and choose Save Record To Library

Report Creator



A report can be created as follows:

- Open one or more modules with the corresponding data you wish to report
- Click on the Report Creator button on the main tool bar, or choose **Tools > Report Creator** from the main menu

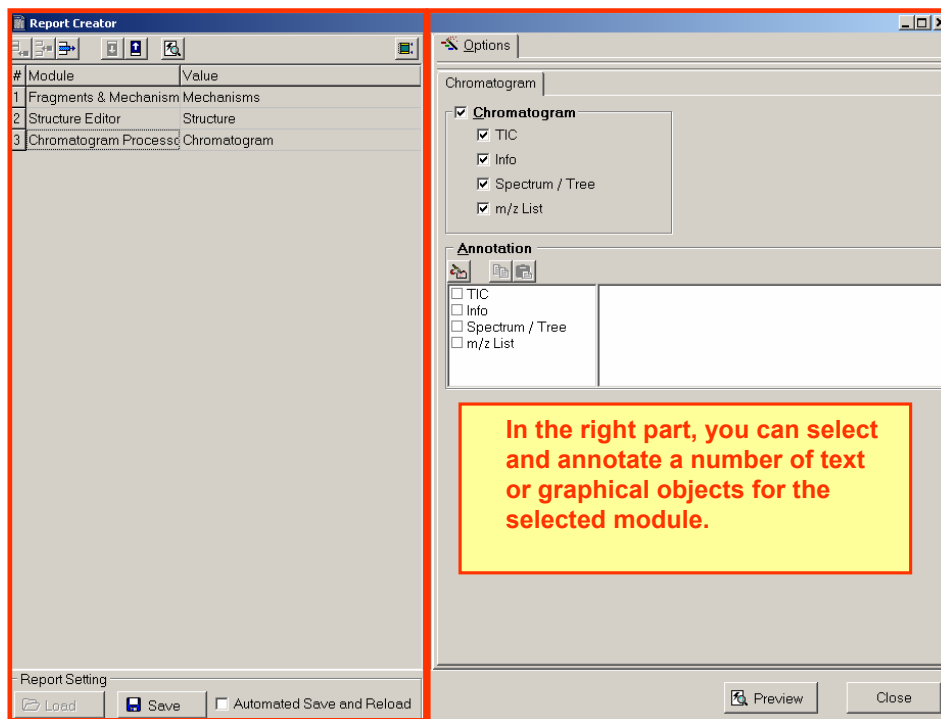
Report Creator allows you to create customizable reports from modules displayed on the screen. Reports can be printed or exported as pdf files. Reports can only be created using Report Creator from windows which are open. You cannot report data that is stored in Mass Frontier, but does not appear on the screen when Report Creator is launched.

Creating Reports

The Report Creator window is divided into two parts:

The left part allows you to manage the modules which you wish to create a report from. You can select a module by highlighting its name and its corresponding customizable items will be displayed on the right side.

The left part of the Report Creator window allows you to add or remove a module from reporting or change the order in which they will appear in the report.

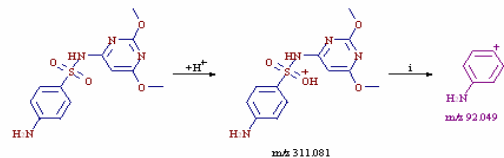


In the right part, you can select and annotate a number of text or graphical objects for the selected module.

When the Report Creator opens, all the modules available for reporting are listed on the left side. If you click on a module name, options and annotation fields specific for the selected module will appear on the right side. To change the general report settings, e.g. header, footer, separation lines, page breaks and orientation, click on the Report Layout button in the Report Creator window.

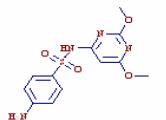
Report Preview

Fragments & Mechanisms: 1

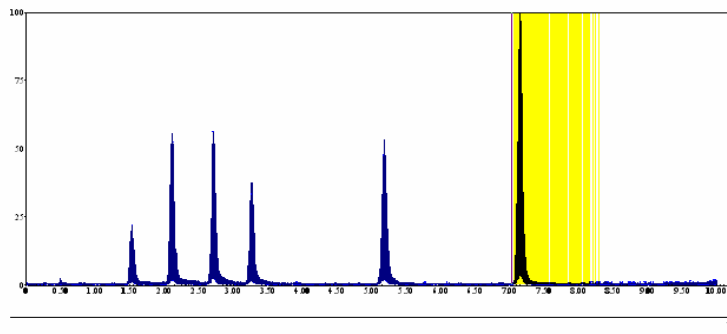


#	m/z
1	82.049
2	139.050
3	156.011
4	279.055
5	294.054
6	311.081

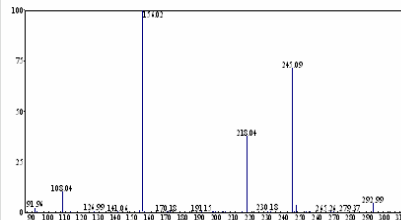
Structure



Chromatogram: ddms2sulfadrgsFTIT2.RAW



LowestMass	85
HighestMass	835
Start Time	0.012525
End Time	10.0076
Max. Integ. Intensity	243026112
Resolution Source	Acquired from File
Mass Resolution	Unit Resolution



#	m/z	Abundance	RelAbundance
1	81.96	209.4642	2.10
2	83.19	3.1257	0.03
3	84.81	2.8237	0.03
4	86.98	4.9103	0.05
5	87.93	2.9065	0.03
6	88.94	5.4663	0.05
7	105.58	2.1074	0.02
8	108.04	996.6072	9.99
8	110.48	2.4476	0.02
10	111.11	2.4969	0.03
11	113.08	12.3447	0.12
12	114.21	4.3446	0.04
13	123.03	2.1752	0.02
14	123.98	17.2369	0.17
15	126.39	62.5292	0.63
16	128.39	2.7959	0.03
17	131.18	1.1695	0.01
18	137.07	3.3430	0.03
19	139.07	7.4793	0.07
20	141.08	35.5298	0.37
21	148.19	3.6624	0.04
22	149.27	3.4507	0.03
23	154.04	74.0740	0.74
24	156.02	9980.2157	100.00
25	158.94	20.1973	0.20
26	157.58	3.1724	0.03
27	158.25	8.0757	0.08
28	163.11	1.1691	0.01
29	164.89	1.8194	0.02
30	168.36	6.6698	0.07
31	170.18	26.3486	0.26
32	171.02	1.7119	0.02
33	172.06	14.0764	0.14
34	172.92	8.5061	0.09
35	184.31	0.9514	0.01

#	m/z	Abundance	RelAbundance
36	189.13	0.8396	0.01
37	190.37	2.1747	0.02
38	191.15	5.5960	0.04
38	197.25	2.3094	0.02
40	198.20	4.6668	0.05
41	199.37	1.8470	0.02
42	202.25	10.9075	0.11
43	203.08	5.4263	0.05
44	204.19	8.1093	0.08
45	205.21	1.9261	0.02
46	206.71	4.1678	0.04
47	210.37	10.5665	0.11
48	212.03	4.7296	0.05
49	213.21	1.7057	0.02
50	215.20	40.9421	0.41
51	218.04	8776.1497	87.84
52	219.10	1.7126	0.02
53	227.33	3.0713	0.03
54	230.18	89.5985	0.90
55	232.27	12.8175	0.13
56	236.10	4.4397	0.04
57	236.83	2.2011	0.02
58	239.23	0.9480	0.01
59	245.09	1146.0655	11.60
60	246.00	11.2413	0.11
61	247.14	620.7234	6.21
62	247.77	5.6460	0.04
63	253.39	5.2302	0.05
64	255.36	1.8204	0.02
65	260.76	3.1644	0.03
66	265.26	28.8415	0.29
67	267.05	10.4625	0.11
68	267.70	5.8038	0.04
69	269.83	13.2406	0.13
70	270.55	2.6573	0.03

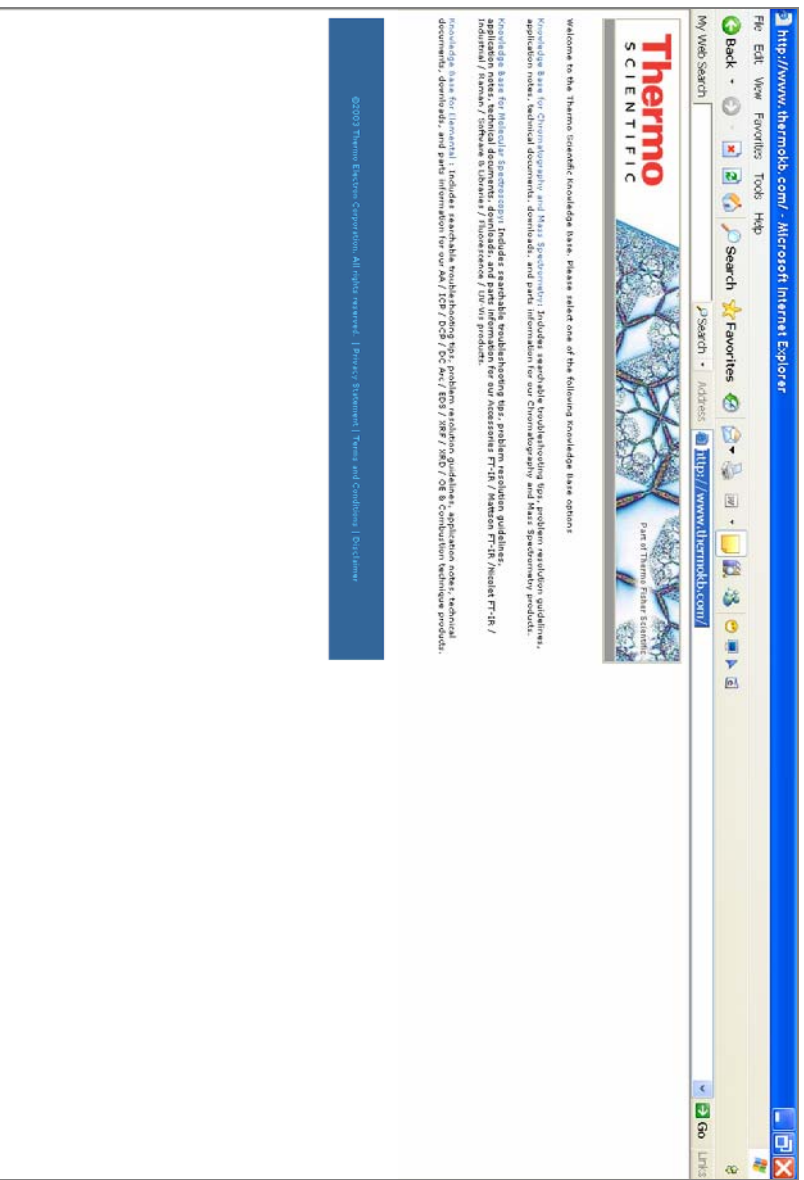
Once the modules and their objects have been selected and annotated, you can generate a report preview by clicking on the Preview button.

Procedure for Getting into the Technical Support Website

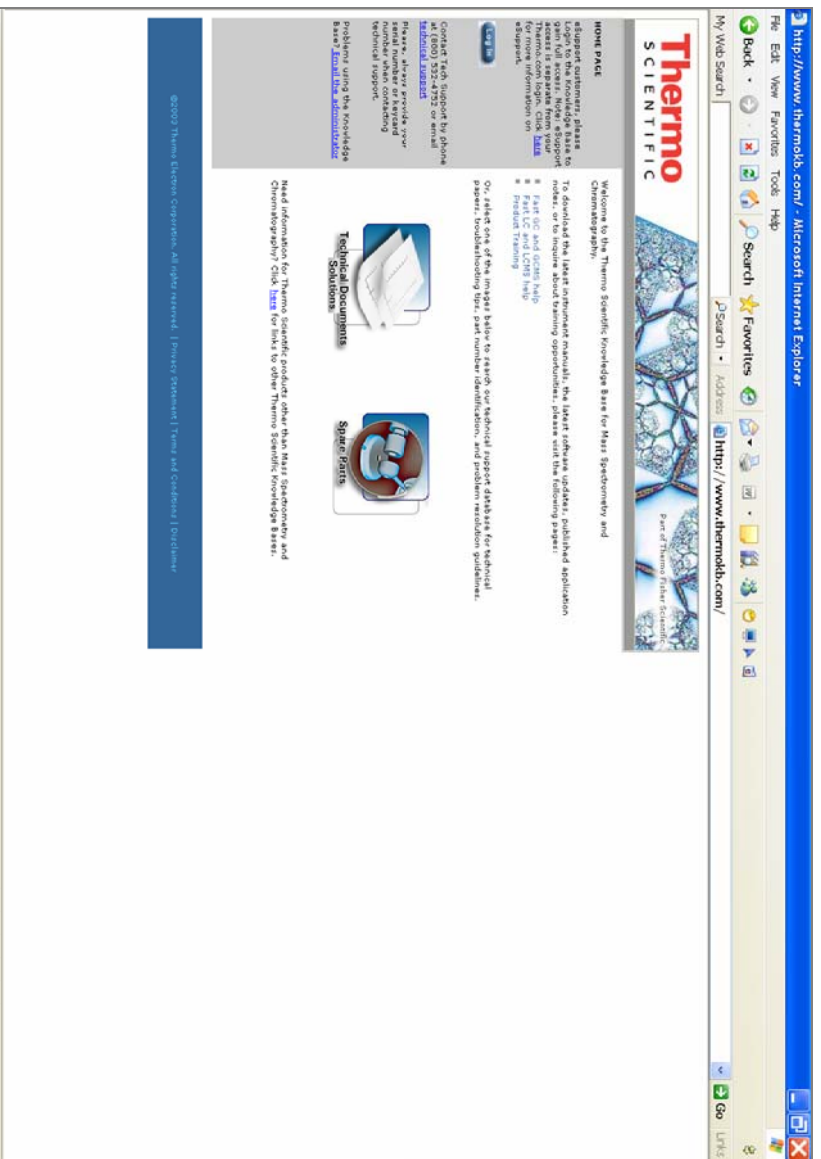
1. In Microsoft Internet Explorer or your internet browser, type the following in the “Address” box.

<http://www.thermokb.com>

(This will get you into the main Thermo Scientific Knowledge Base page)



2. Then, click on “Knowledge Base for Chromatography and Mass Spectrometry” which is located on the top of the page.



3. If you are under warranty or have a support plan, you have access to a greater number of technical documents. For access to the maximum number of technical documents, **Login** using your Key Card Number (Equipment Number) for both the Username and Password.

Server Login

Please enter your Username and Password

Username

Password

LC-MS CONTAMINATION

General Discussion

Chemical contamination is one of the more common problems with LC/MS. Therefore, please use the highest purity chemicals available.

- Try Burdick and Jackson solvents and water. These seem to be uniformly better than other suppliers. Switch to this brand if you have contamination problems.
- Additionally, use high purity acetic acid: Aldrich p/n 38,012-1 double distilled in Teflon bottle \$175 for 500 ML.

Chemical contamination problems (with specific mass peaks) are rarely attributed to problems deep inside the MS. Prioritize like this:

1. HPLC pre or trap column
2. HPLC column
3. HPLC hardware
4. Chemicals
5. ESI or APCL probe
6. Spray shield area
7. Ion Transfer Tube
8. Tube lens/Skimmer
9. Q00/Lens 0/Q0
10. Analyzer Quads
11. MS Dynode

Generally, if 8 – 11 are dirty you will see random peaks not specific masses.

Identifying the source of contamination

1. Is the contamination always there or does it elute as specific peaks during the HPLC run? If the later, the problem is most likely with the HPLC.
2. Turn off flow. If the noise disappears take the column out of the flow path and test again. If the noise is still there try these items, go to step #3.
3. If you suspect that there is chemical contamination from the HPLC mobile-phase, use a clean syringe and tubing to infuse the mobile-phase components individually into the MS (e.g. water, then water + acid, then methanol or acetonitrile. Generally, the acid is the source of contamination.

LC-MS CONTAMINATION

HPLC Troubleshooting

Many times contaminant peaks occur during a gradient. Try this procedure to locate the source of the contamination:

- Inject a solvent blank.
- Make a zero volume injection
- Run a gradient with the injection valve or autosampler out of the liquid flow.

Contamination in the MS

If the noise disappears when HPLC flow is turned off and you have eliminated contamination in the mobile phase:

- Trim or replace the sample tube and set it to the correct distance within the needle.
- Clean the ESI spray nozzle (cone and needle).
- Replace the Teflon needle seal behind the needle
- If still noisy, the cause could be neutral chemical noise (non-ionized materials). Change source conditions (e.g. increase heated capillary temperature 10 or 20°C, increase Sheath gas flow 10 or 20 units, increase Auxiliary gas flow 10 units).

Other possibilities

- Solvent reservoir pickup filters are common sources of contamination. Usually the aqueous phase one will be the first to go.
- Offline solvent filters are common sources of contamination. Serious contamination has been seen with the Nylon 0.22 µm filters used for buffer solutions.
- Address the containers used for storing your acetonitrile. A similar problem occurs when using glass scintillation vials with polypropylene insert caps to makeup solvent.

Phthalate Contamination

- 391 protonated diisooctyl phthalate [M+H]⁺
- 413 sodium adduct of diisooctyl phthalate [M+Na]⁺
- 798 ammoniated dimer of diisooctyl phthalate [2M+NH₄]⁺
- 803 sodiated dimer of diisooctyl phthalate [2M+Na]⁺

- This is usually from contaminated solvents. It can concentrate on the column and elute during a gradient. Follow the normal solvent contamination checking procedures.

LC-MS CONTAMINATION

Glassware cleaned by means of a “dishwasher” often picks up phthalate contamination. Remove this with a rinse of 30% nitric acid, followed by a rinse with 2M NH₄OH. Please keep in mind, that the APCI probe can retain this. However, baking the APCI will eliminate this problem. Try 600°C for 15 min.

+44 Series Ions Possible Polymer Contamination

If you have analyzed detergent containing samples on the system, that could explain the +44 series background problem. Also, PEGs and other ethoxylated polymers give +44 ion series. The PEG's could originate from the water, or extracted polymers from plastic ware and/or silicon coatings.

Background Ramps Up With Gradient

Background can originate from contaminated organic phase or the high organic content maybe eluting retained materials from the column. Substitute with Burdick and Jackson organic phase and a new column.

+59 Series Ions

59 Da is the mass of the acetate ion (*observed when employing ammonium acetate or acetic acid*). Is it possible to have acetate polymerization? There is another explanation for the +59 ion series. Polymers of +59 might be iron in some form, presumably leaching from the steel in acid.

+74 Series Ions

Peaks at 536, 610 and 684 Da have been seen with LCQ DecaXP and LCQ Advantage and TSQ Quantum. The contamination came from connecting the opaque silicon tube to the API source housing. The proper tubing is Tygon. The instrument parts kits contain both types of tubing. The TSQ Quantum contains a 12-foot length of clear Tygon tube and a 2.5-foot length of opaque silicon tube. The intended function of the silicon tube is to connect from the waste container (P/N 00301-57020) to the fume exhaust system. If the silicon tube is instead connected to the API source housing, background ions at m/z 536, 610, and 684 might be observed. Solution: Remove all silicon tubing from the solvent waste system.

+77 Series Ions

+77 Da clusters, mostly across the mass range, can be attributed to the ion-transfer tune and Kalrez o-ring. One could postulate that the worn o-ring was allowing leakage into the tube lens skimmer area and the leak was also leaching something out of the o-ring. Normally the o-ring is a total block and any polymers in it would not enter the MS.

LC-MS CONTAMINATION

+ 615.7 and 1229.8 Chaps Ions

615.7 Da is $[M+H]^+$ of CHAPS and 1229.8 Da is $[2M+H]^+$ in the sample. Removal can be extremely difficult. Acetone precipitation removes the excess CHAPS, but there may still be an appreciable amount remaining (determined by the above ions in the mass spectrum). CHAPS won't kill your SCX chromatography, but it will chromatograph nicely on reversed phase and you'll get an intense ion at 615 m/z $[M+H]^+$.

+136 Series Ions

This is exhibited by background (contamination) that has a repeating sequence of 136 amu (TFA sodium salt is 136 Da). Remove TFA from the sample and/or mobile-phase. TFA may be difficult to remove from the column, so take off the column and flow mobile-phase directly into the MS. Identify the source by infusing methanol with a clean syringe and a new piece of tubing.

Pentafluoropropionic Acid

May stick to PEEK tubing and fittings. This contamination is usually associated with areas that are poorly swept by the flowing liquid (e.g. unions). If you have concerns about the pentafluoropropionic acid, then use fused-silica and steel unions. The ferrules should be Kel-F, and PEEK tube nuts should be ok as they do not touch the liquids.

Water

If any contamination is seen, use high-grade bottled water (Burdick & Jackson HPLC grade.) Avoid ANY nanoPure or MilliQ water!

+798 and +803 Contamination

Both ions are diisooctyl phthalate analytes derived from plasticizers. Sometimes, their origins can be really hard to track down. If the source is your HPLC, these species can concentrate on your column until the gradient is ramped and the compounds eluted. Check the HPLC solvents by infusion to determine if it's coming from your HPLC or if it's in one of your reagents (e.g. TFA). In the later case, clean the HPLC reservoir bottles. These could be cleaned by rinsing well with 30% Nitric Acid followed, after a water rinse, then by 2M NH_4OH .

+453.3, +679.5 and +905.7 Contamination

Nylon HPLC solvent filters can produce nylon (6,6monomer) peaks at masses of 226 Da, a dimer 452 Da, trimer 678 Da and tetramer 905 Da. The contaminant is very hard to get rid of since it binds very well to C18.

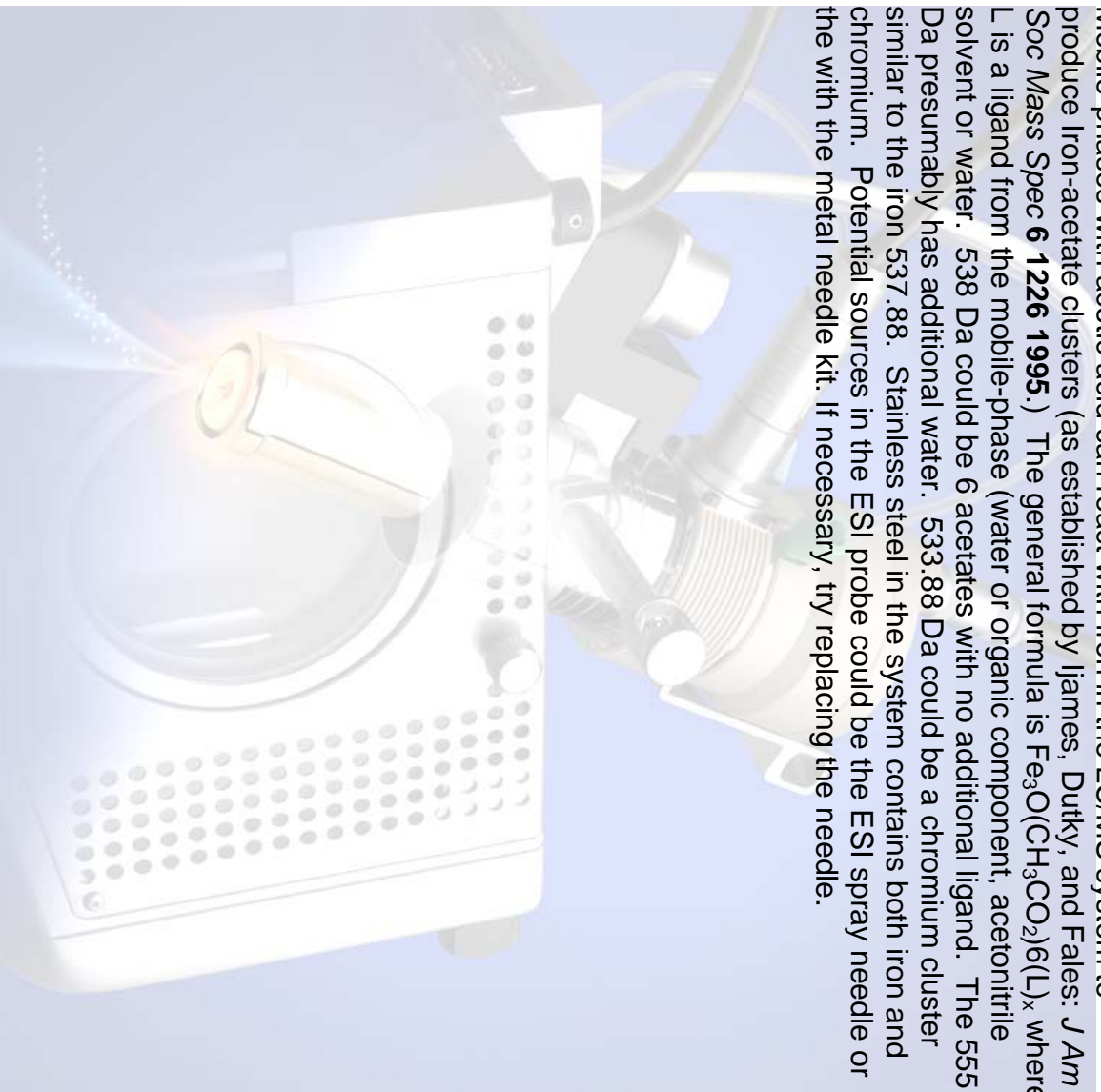
LC-MS CONTAMINATION

Peak Clusters at +21, -17, -35, -52

+21 is sodium, -17, -35, -52 are various losses of ammonia and water (if you look at the -17 ion you will probably see a small amount of the -18, as well).

Iron-Acetate clusters @ +538, +555 and +534

Mobile-phases with acetic acid can react with iron in the LC/MS system to produce Iron-acetate clusters (as established by James, Dutky, and Fales: *J Am Soc Mass Spec* **6** 1226 1995.) The general formula is $\text{Fe}_3\text{O}(\text{CH}_3\text{CO}_2)_6(\text{L})_x$ where L is a ligand from the mobile-phase (water or organic component, acetonitrile solvent or water. 538 Da could be 6 acetates with no additional ligand. The 555 Da presumably has additional water. 533.88 Da could be a chromium cluster similar to the iron 537.88. Stainless steel in the system contains both iron and chromium. Potential sources in the ESI probe could be the ESI spray needle or the with the metal needle kit. If necessary, try replacing the needle.



COMMON BACKGROUND IONS FOR ELECTROSPRAY (Positive Ion)

m/z	Ion	Analyte	m/z	Ion	Analyte
101	[M+Na] ⁺	DMSO	267	[M+H] ⁺	Tributylphosphate
102	[M+H] ⁺	Triethylamine	273	M ⁺	Momomethoxytrityl cation
104/106	[M+Cu] ⁺	Acetonitrile	279	[M+H] ⁺	Dibutylphthalate
105	[2M+Na] ⁺	Acetonitrile	282		Plasticizer in Polyethylene
120	[M+Na+CH ₃ CN] ⁺	DMSO	301	[M+Na] ⁺	Dibutylphthalate
122	[M+H] ⁺	TRIS	317	[M+K] ⁺	Dibutylphthalate
123	[M+H] ⁺	Dimethylaminopyridine	336	[M+H] ⁺	Tributyl tin formate
130	[M+H] ⁺	Diisopropylethylamine	338	[M+H] ⁺	Erucamide
137	[M+CAN+NH ₄] ⁺	DMSO	360	[M+Na] ⁺	Erucamide
144	[M+H] ⁺	TPA	371	[M+H] ⁺	Polysiloxane, followed by 388
145/147	[2M+Cu] ⁺	Acetonitrile	391	[M+H] ⁺	Diisooctyl phthalate
146	[3M+Na] ⁺	Acetonitrile	413	[M+Na] ⁺	Diisooctyl phthalate
150	[M+H] ⁺	Phenyldiethylamine	425	[M+Na] ⁺	Unknown plasticiser
153	[M+H] ⁺	1,8-Diazabicyclo[5,4,0]undec-7-ene(DBU)	429	[M+K] ⁺	Diisooctyl phthalate
157	[2M+H] ⁺	DMSO	445	[M+H] ⁺	Polysiloxane, followed by 462
159	[M+Na] ⁺	Sopdium trifluoroacetate	449	[2M+H] ⁺	Dicyclohexyl urea (DCU)
179	[2M+Na] ⁺	DMSO	454	[M+Na+ACN] ⁺	Diisooctyl phthalate
186	[M+H] ⁺	Tributylamine	522		Unknown
214		Unknown Surfactant	550		Unknown
217		Unknown Contaminant	798	[2M+NH ₄] ⁺	Diisooctyl phthalate
225	[M+H] ⁺	Dicyclohexyl Urea (DCU)	803	[2M+Na] ⁺	Diisooctyl phthalate
231	[M+NH ₃] ⁺	Unknown	PEG polymers exhibit peaks spaced @ 44 amu intervals		
239/241	[(M.HCl) ₂ -Cl] ⁺	Triethylamine	<h3 style="text-align: center;">Common Adducts</h3>		
242	M ⁺	Tetrabutylammonium (C ₄ H ₉) ₄ N ⁺			
243	M ⁺	Trityl cation			
257	[3M+H] ⁺	DMSO			
			[M+NH ₄] ⁺	[M+18]	
			[M+Na] ⁺	[M+23]	
			[M+K] ⁺	[M+39]	

COMMON FRAGMENT ION AND NEUTRAL FRAGMENTS

Common Small Ions		Common Neutral Fragments	
m/z	Composition	m/z loss	Composition
15	CH ₃	1	H
17	OH	15	CH ₃
18	H ₂ O	17	OH
19	H ₃ O, F	18	H ₂ O
26	C ₂ H ₂ , CN	19	F
27	C ₂ H ₃	20	HF
28	C ₂ H ₄ , CO, H ₂ CN	27	C ₂ H ₃ , HCN
29	C ₂ H ₅ , CHO	28	C ₂ H ₄ , CO
30	CH ₂ NH ₂	30	CH ₂ O
31	CH ₃ O	31	CH ₃ O
33	SH, CH ₂ F	32	CH ₄ O, S
34	H ₂ S	33	CH ₃ + H ₂ O, HS
35(37)	Cl	33	H ₂ S
36(38)	HCl	35(37)	Cl
39	C ₃ H ₃	36(38)	HCl
41	C ₃ H ₅ , C ₂ H ₃ N	42	C ₃ H ₆ , C ₂ H ₂ O, C ₂ H ₄ N
42	C ₃ H ₆ , C ₂ H ₂ O, C ₂ H ₄ N	43	C ₃ H ₇ , CH ₃ CO
43	C ₃ H ₇ , CH ₃ CO	44	CO ₂ , CONH ₂
44	C ₂ H ₄ O	45	C ₂ H ₅ O
46	NO ₂	55	C ₄ H ₇
56	C ₄ H ₈	57	C ₄ H ₉
57	C ₄ H ₉	59	C ₂ H ₃ O ₂
60	CH ₄ CO ₂	60	C ₂ H ₄ O ₂
79(81)	Br	64	SO ₂
80(82)	HBr	79(81)	Br
91	I	80(82)	HBr
127	HI	91	I
		127	HI

Notes Pages

Notes Pages

Notes Pages

Notes Pages

Notes Pages