Waters Micromass Q-Tof Premier Mass Spectrometer

Operator's Guide

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Customer comments

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You can reach us at tech_comm@waters.com.

Safety Information

General

The Waters Micromass Q-Tof Premier™ is designed solely for use as a mass spectrometer; any attempt to use it for any other purpose is liable to damage the instrument and will invalidate its warranty.

The mass spectrometer conforms to European standard EN61010-1:2001, Safety Requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.

The instrument has been designed and tested in accordance with recognized safety standards. If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be impaired.

Whenever the safety protection of the instrument has been compromised, disconnect the instrument from all power sources and secure the instrument against unintended operation.

The instrument must be installed in such a manner that the user can easily access and isolate the power source.

Biological Hazard

When you analyze physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in "CDC Guidelines on Specimen Handling," *CDC – NIH Manual*, 1984.

Chemical Hazard

Good Laboratory Practice should be followed when using potentially toxic, biohazardous, caustic, or flammable solvents and analytes.

Flammable Solvents Operation Hazard

Caution: If the nitrogen supply pressure falls below 4 bar, the instrument will switch off the nitrogen supply and admit air into the source. If flammable solvents are used, there is a potential ignition hazard.

If flammable solvents are used, you should ensure that the nitrogen supply pressure will not fall below 4 bar during the analysis. Also ensure that the gas

fail connection is connected to the HPLC system to ensure that the LC flow is stopped if the nitrogen supply fails.

Eye Protection

Warning: Wear suitable eye protection at all times when operating the instrument.

High Voltage Hazard

Warning: Certain areas of the instrument may have high voltages present when the instrument is in Operate. To avoid non-lethal electric shock make sure the instrument is in Standby before touching these areas.

Certain areas of the instrument may have high voltages present when the instrument is in Operate. These areas are shown in the Figure titled "Q-Tof Premier High Voltage Hazards - ESI Operation" on page v, showing the instrument configured for ESI operation.

Warning: To avoid electric shock (non-lethal), any equipment connected to the ESI probe (including columns) should be earthed.

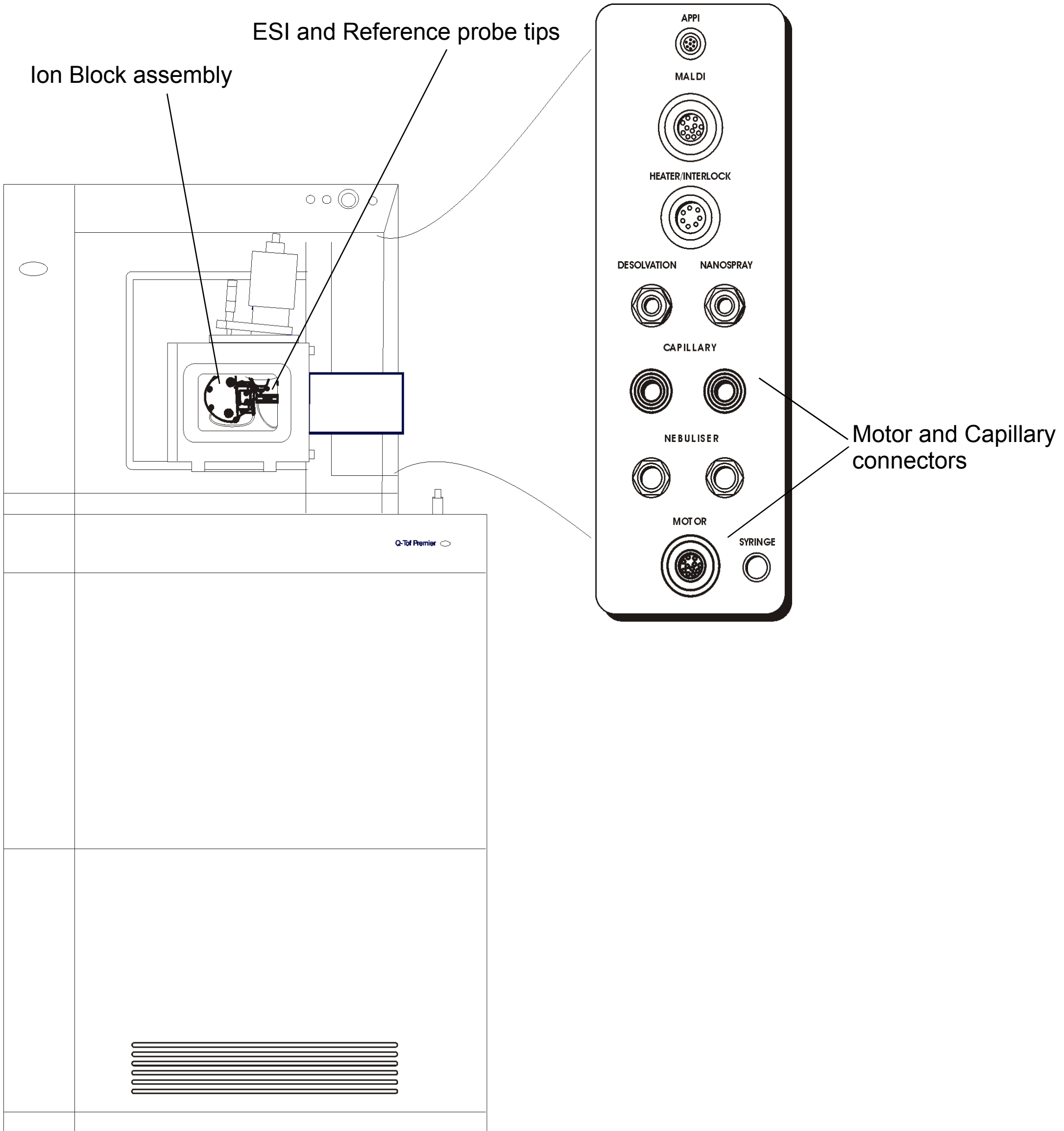
Waters recommends that any columns, T-pieces, and other metal fittings connected to the ESI probe should be earthed. Eluent (which may be highly conductive) travels from a column and/or splitter to the probe. Any splitters and columns may become live and a small static-like shock may result if the user handles them.

High temperature hazard

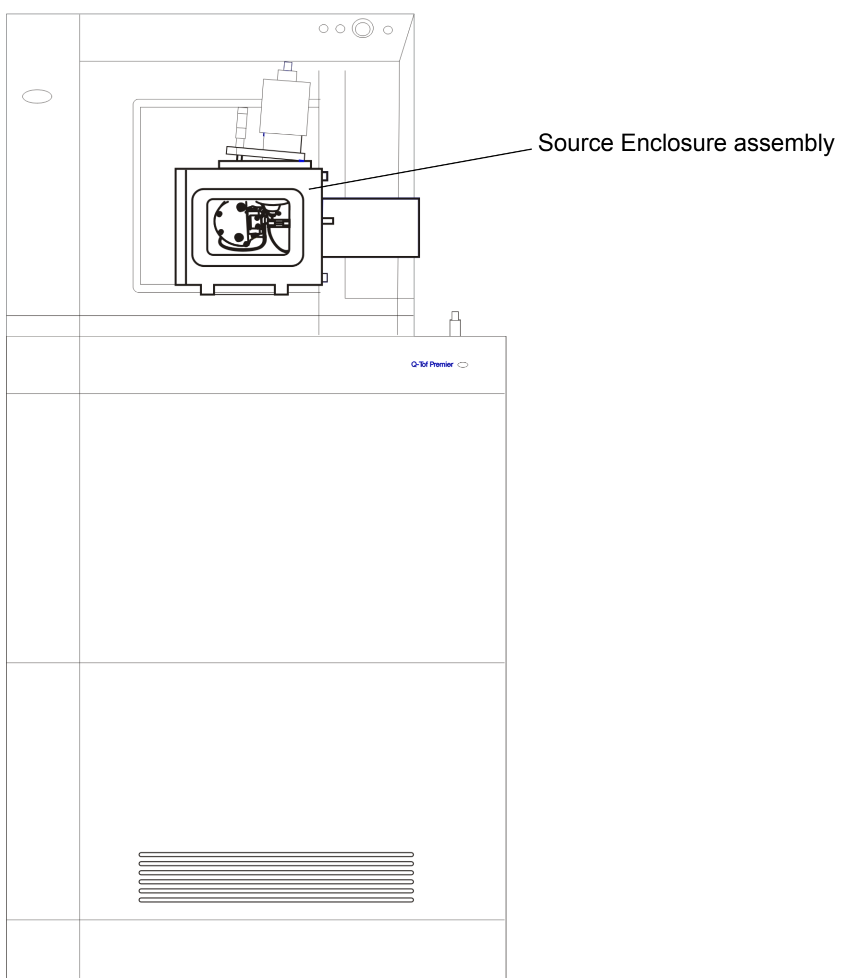
Warning: To avoid burns, take care when working with the instrument as the source enclosure assembly may be at high temperature.

The source enclosure assembly may be at high temperature, as shown in the Figure titled "Q-Tof Premier High Temperature Hazards - ESI Operation" on page vi, showing the instrument configured for ESI operation.

Q-Tof Premier High Voltage Hazards - ESI Operation



Q-Tof Premier High Temperature Hazards - ESI Operation



Safety Symbols

Warnings in this guide, or on the instrument, must be observed during all phases of service, repair, installation, and operation of the instrument. Failure to comply with these precautions violates the safety standards of the design and intended use of the instrument.

Waters Corporation assumes no liability for failure to comply with these requirements.

The following safety symbols may be used in this guide or on the instrument. A **Warning** is an instruction that draws attention to the risk of injury or death. A **Caution** is an instruction that draws attention to the risk of damage to the instrument

Consignes de sécurité

Généralités

Le Q-Tof Premier de Waters Micromass est destiné exclusivement à être utilisé comme spectromètre de masse. Tout usage détourné du Q-Tof Premier risquerait d'endommager l'instrument et invaliderait sa garantie.

Le spectromètre de masse Q-Tof Premier de Waters Micromass est conforme à la norme européenne EN61010-1 (2001) : Règles de sécurité pour appareils électriques de mesurage, de régulation et de laboratoire. - Partie 1 : prescriptions générales.

Cet instrument a été conçu et testé dans le respect de normes de sécurité approuvées. Toute utilisation de l'instrument non conforme aux instructions du fabricant risque de remettre en cause la protection assurée par l'instrument.

Dans le cas où la sécurité de l'utilisateur se trouverait compromise, débranchez le cordon d'alimentation de l'instrument et assurez-vous qu'il ne pourra être mis en marche par mégarde.

L'instrument doit être installé de façon à faciliter l'accès de l'utilisateur au bloc d'alimentation électrique.

Risques liés à l'usage de solvants inflammables

Attention: Si la pression d'alimentation en azote tombe en dessous de 4 bars, l'instrument bloque automatiquement l'arrivée d'azote et déclenche une arrivée d'air dans la source. L'usage de solvants inflammables implique l'existence d'un risque d'ignition.

Lorsque vous utilisez des solvants inflammables, assurez-vous que la pression d'alimentation en azote ne tombe pas en dessous de 4 bars en cours d'analyse. De plus, assurez-vous que la connexion Gas Fail est correctement raccordée au système HPLC, de sorte que le débit LC soit interrompu en même temps que l'alimentation en azote.

Protection des yeux

Avertissement: Portez à tout moment des lunettes de protection appropriées lorsque vous utilisez l'instrument..

Risques d'électrocution

Avertissement: Certaines parties de l'instrument peuvent être soumises à des tensions électriques élevées lorsque l'instrument est en mode de fonctionnement. Pour éviter toute électrocution accidentelle, veuillez placer l'instrument en mode veille.

Lorsque l'instrument est en mode de fonctionnement ou « Operate », certaines parties de l'instrument sont soumises à des tensions très élevées. Ces parties de l'instrument sont indiquées dans les figures 1 et 2. La Figure titled "Q-Tof Premier High Voltage Hazards - ESI Operation" on page v montre l'instrument configuré pour un usage en mode d'ionisation electrospray (ESI).

Avertissement: Pour éviter toute décharge électrique (non-mortelle), les équipements reliés à la sonde ESI (à ionisation par électrospray), colonnes y compris, devraient être mis à la terre.

Waters recommande que toutes les colonnes, raccords en T, et autres pièces métalliques reliées à la sonde ESI soient mis à la terre. L'éluant (qui peut être fortement conducteur) va d'une colonne et/ou d'un diviseur de débit à la sonde. Les diviseurs et les colonnes peuvent se charger et provoquer une petite décharge électrostatique si l'utilisateur les manipule.

Risques de brûlure

Avertissement: Pour éviter toute brûlure, faites attention lorsque vous travaillez à proximité de la source car elle peut atteindre des températures très élevées.

La source, représentée aux figures 3 et 4, peut atteindre des températures très élevées. La Figure titled "Q-Tof Premier High Temperature Hazards - ESI Operation" on page vi montre l'instrument configuré pour un usage en mode ESI.

Pictogrammes de sécurité

Les avertissements présents dans le manuel de l'utilisateur ou sur l'instrument-même doivent être scrupuleusement pris en considération, et ce à tout moment, que ce soit pendant l'entretien, la réparation, l'installation ou le fonctionnement de l'instrument. Tout défaut d'application de ces règles de sécurité serait considéré comme une violation des normes de sécurité relatives à la conception et à l'usage prévu de l'instrument.

Waters ne saurait voir sa responsabilité engagée en cas de manquement de l'utilisateur à respecter les consignes de sécurité.

Vous pourrez rencontrer les pictogrammes qui suivent dans le manuel de l'utilisateur ou sur l'instrument. Est appelée **Avertissement** toute instruction destinée à attirer l'attention de l'utilisateur sur l'existence d'un risque de blessure ou de mort. Est appelée **Attention** toute instruction destinée à informer l'utilisateur de la présence d'un risque d'endommagement de l'instrument.

Warning: This is a general warning symbol, indicating that there is a potential health or safety hazard; the user should refer to this operators guide for instructions.

Avertissement: Risque de blessure de l'utilisateur ou risque d'endommagement de l'instrument. Consultez le manuel de l'utilisateur pour instructions.

Warning: This symbol indicates that hazardous voltages may be present.

Avertissement: Présence de lignes haute tension.

Warning: This symbol indicates that hot surfaces may be present.

Avertissement: Ce pictogramme indique la presence de surfaces chaudes.

Warning: This symbol indicates that there is danger from corrosive substances.

Avertissement: Substances corrosives.

Warning: This symbol indicates that there is danger from toxic substances.

Avertissement: Substances toxiques.

Warning: This symbol indicates that there is danger from flammable substances.

Avertissement: Substances inflammables.

Warning: This symbol indicates that there is danger from laser radiation.

Avertissement: Risque de radiations laser.

Warning: This symbol indicates that there is a danger from UV radiation.

Avertissement: Risque de radiations UV.

Warning: This symbol indicates danger of contamination by a biological agent that constitutes a threat to humans.

Avertissement: Ce pictogramme indique la présence d’un risque de contamination par un agent biologique constituant un danger potentiel pour l’utlisateur.

Caution: Indicates care must be taken to avoid damaging the equipment or affecting its operation.

Attention: Utilesez l’intrument en faisant preuve de beaucoup de précaution pour éviter de l’endommager et ainsi nuire à son fonctionnement.

Symbols commonly used on the instrument

Symbol	Description
⎓	Direct current
~	Alternating current
	Protective conductor terminal
	Frame or chassis terminal
	Warning, refer to the manual
	Warning, risk of electric shock (high voltage)
	Warning, hot surface or high temperature
	Warning, risk of needle-stick puncture
UV	Warning, ultraviolet radiation
	Fuse
I	Electrical power on
0	Electrical power off
	Keep upright, handling label
	Keep dry, handling label
	Fragile, handling label

Q-Tof Premier Mass Spectrometer Information

Intended Use

The Waters Micromass Q-Tof Premier Mass Spectrometer can be used as a research tool to deliver authenticated exact mass. It is not for use in diagnostic procedures.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate a standard curve. The concentration range should cover the entire range of quality-control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that system performance is acceptable.

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1 Instrument Description

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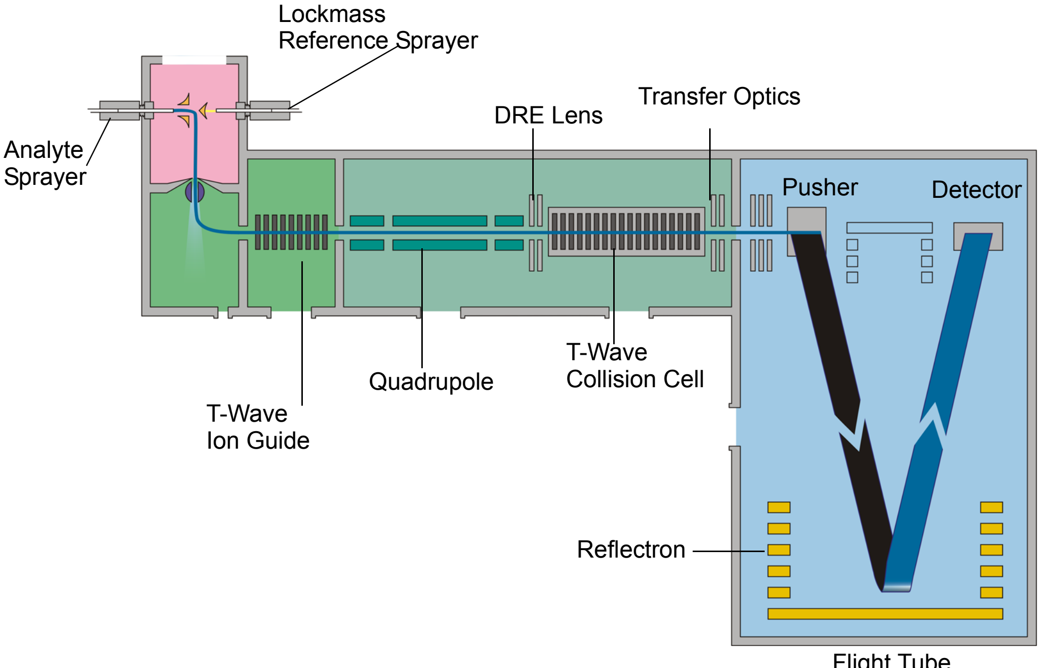
Overview

The Q-Tof Premier™ is a hybrid orthogonal acceleration Time-of Flight (oaTof) mass spectrometer that enables automated exact mass measurement of precursor and fragment ions to yield the highest confidence in structural elucidation and databank search results.



Source

The Q-Tof Premier combines the high transmission efficiency of ZSpray source technology with the latest in T-Wave ion optics. The built in LockSpray™ or optional NanoLockSpray™ capability enables routine exact mass measurement in MS and MS/MS modes. Equipped with a standard electrospray ionization (ESI) and with atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI) available as options, the Q-Tof Premier offers a wide range of atmospheric pressure ionization (API) techniques:

- ElectroSpray ionization

Electrospray ionization (ESI) takes place as a result of imparting a strong electrical charge to the eluent as it emerges from the nebulizer. An aerosol of charged droplets emerges from the nebulizer. These undergo a reduction in size by solvent evaporation until they have attained a sufficient charge density to allow sample ions to be ejected from the surface of the droplet (ion evaporation).

A characteristic of ESI spectra is that ions may be singly or multiply charged. Since the mass spectrometer separates ions according to their mass-to-charge ratio (m/z), compounds of high molecular weight can be determined if multiply charged ions are formed.

Eluent flows up to 1 mL/min can be accommodated although it is often preferable with electrospray ionization to split the flow such that 5 to 50 µL/min of eluent enters the mass spectrometer.

The optional NanoLockSpray interface allows electrospray ionization to be performed in the flow rate range 5 to 1000 nL/min.

For a given sample concentration, the ion currents observed in nanoflow are comparable to those seen in normal flow rate electrospray. Due to the great reduction in sample consumption, there are great gains in sensitivity when similar scan parameters are used.

- Atmospheric pressure chemical ionization

  APCI generally produces protonated or deprotonated molecular ions from the sample via proton transfer (positive ions) or proton abstraction (negative ions). The sample is vaporized in a heated nebulizer before emerging into a plasma consisting of solvent ions formed within the atmospheric source by a corona discharge. Proton transfer or abstraction then occurs between the solvent ions and the sample. Eluent flows up to 2 mL/min can be accommodated without splitting the flow.

- Atmospheric pressure photoionization

  APPI uses photons generated by a krypton discharge UV lamp (~10.2 eV) to produce sample ions from vaporized LC eluent. Direct photoionization of the sample molecule occurs when the photon energy is greater than the ionization potential of the sample molecule.

  The APPI source option incorporates a UV lamp. The sample is introduced into the source via the APCI IonSABRE probe. This produces a stream of sample and solvent species that undergo photon-induced ion-molecule reactions.

An electrode, known as a repeller, is used to deflect and focus the sample ions produced towards the sample cone for introduction into the mass spectrometry system for analysis.

Analyzers

Q-Tof technology provides both quadrupole (MS1) and Time-of-flight (ToF) mass analyzers, with an intermediate collision cell for fragmentation, if required. This powerful combination allows ions to be selected, individually fragmented, and then measured to a high degree of mass accuracy by the oaToF.

Quadrupole

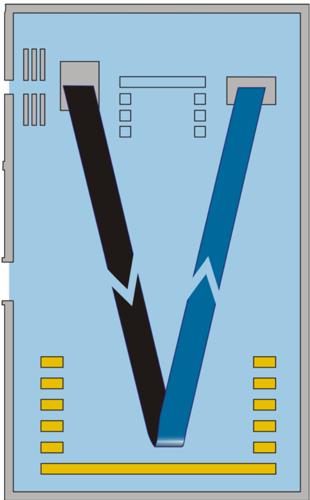
The quadrupole is available with 4, 8 and 32 kDa mass range options, and can can be operated:

- With the quadrupole resolving DC off (wide bandwidth mode). In which case ions can pass through MS1 and be accurately measured by the ToF in what is known as a Tof MS acquisition.
- With the quadrupole resolving DC switched on. The quadrupole can either be parked on one specific mass (Tof MS/MS) or can be made to scan through a wide mass range in search of candidate ions for fragmentation (parent ion scanning).
- With the instrument automatically switching between Tof MS and Tof MS/MS modes depending on the ions are detected during the Tof MS scan. This is known as data directed analysis (DDA) and is a very powerful diagnostic tool when combined with an LC system.

Tof analyzer

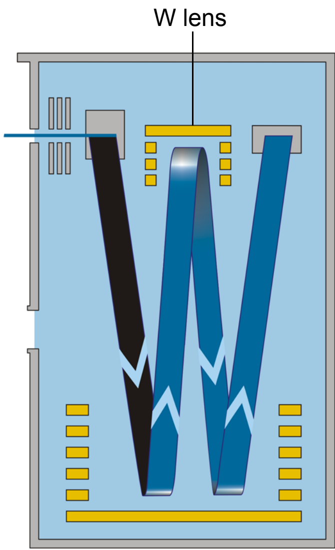
The ToF analyzer uses a high voltage pulse to orthogonally accelerate the ions down the flight tube and a reflectron to reflect them back again towards the detector (V-optics). Ions of different mass-charge ratio will have different flight times and hence a mass spectrum can be created with a resolution of 10 000 (FWHM). The mass range of the ToF analyzer is dependent on the frequency of the pusher pulse with a maximum m/z of 100 000 in V mode.

Ion Optics in V-mode:



The Q-Tof Premier Tof analyzer is also equipped with a second reflectron that enables the beam to traverse the flight tube twice. This doubling of the flight path (W-Optics) enables resolution of 17,500 (FWHM).

Ion optics in W-mode:



Detectors

The Q-ToF Premier is equipped with two detectors:

- A photomultiplier tube (PMT).

  The PMT is situated in the head behind the pusher stack and is designed to record ion arrivals from the MS1 analyzer without the ions being fired down the ToF.
- A micro-channel plate / time-to-digital converter (MCP/TDC).

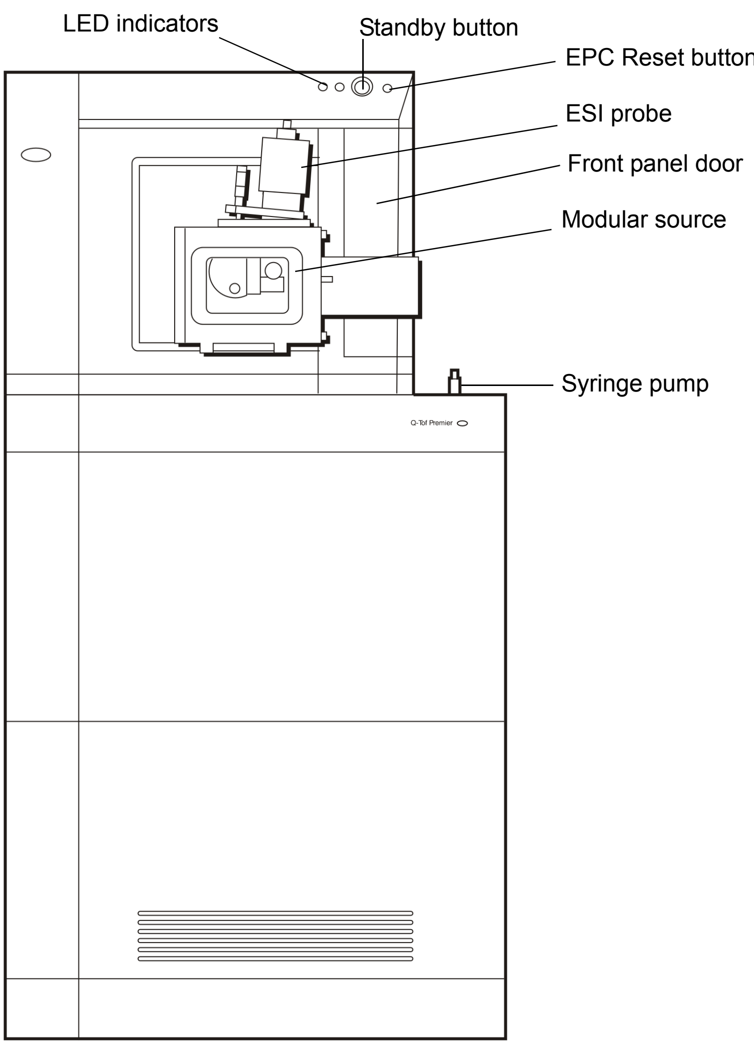
  The MCP/TDC detector accurately records the ion arrival time after they have travelled the flight tube.

Front panel

The front panel of the Q-Tof Premier includes instrument and vacuum status LED indicators. Gas connections are color-coded for easy connection. Electrical connector types differ according to function, preventing errors.

There is also an embedded PC (EPC) reset button and a Standby button.

Q-Tof Premier front view:



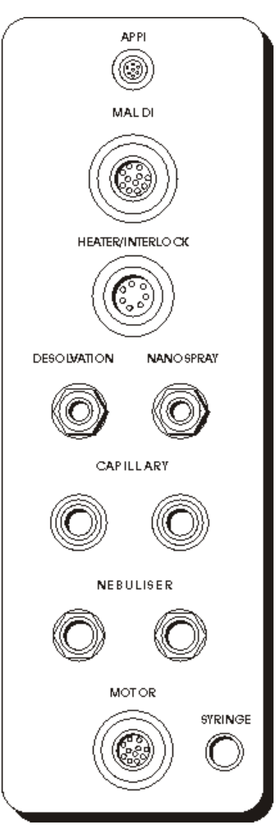
Removing instrument covers

Warning: Removing instrument covers will expose hazardous voltages. These covers should only be removed by a Waters field service engineer. No user serviceable parts are inside.

Front panel connections

The front cover connections are located on the top right of the instrument behind the Front panel door

Front panel connections:



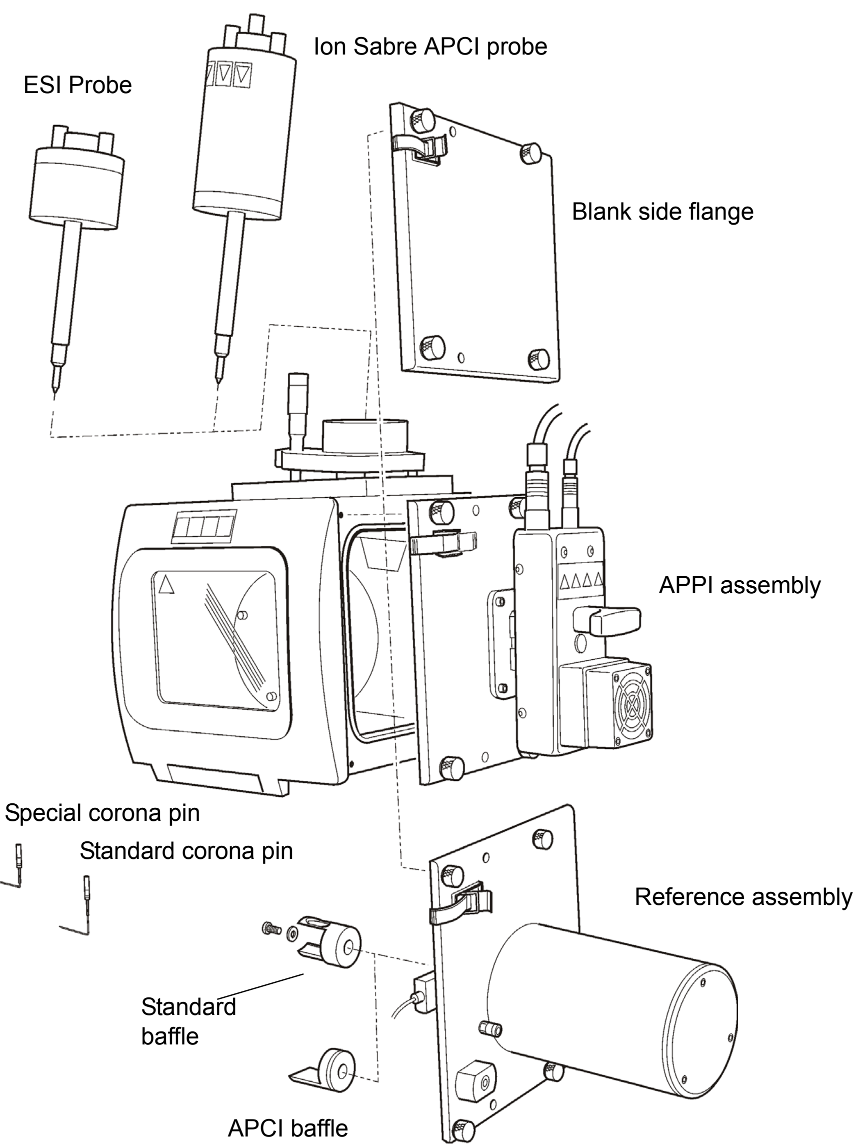
Connection	Description
Heater/Interlock	This is the desolvation heater socket and it also provides the interlock connection for source options.
Desolvation	This is the desolvation gas connector. It is color-coded yellow, and should be attached to the yellow gas line from the probe adjuster.
NanoSpray	This is the NanoFlow™ gas connector: It is color-coded blue and should be connected to the NanoFlow sprayer.
Capillary	These are the capillary voltage sockets. The cables from the ESI probe, NanoFlow sprayer LockSpray reference probe and the reference sprayer flange should be plugged in here. It is not used in APCI mode.

Connection	Description
Nebuliser	These are the nebulizer gas connections. They are color-coded red, and should be attached to the red gas line from the ESI or APCI probes. A blank plug should be inserted if the connectors are unused.
Motor	This is the motor drive socket. The cable from the Lock-Spray and NanoLockSpray motors should be plugged in here.
Syringe	This is the earthing point for the syringe. Plug the ground cable from the syringe into this socket.
APPI	This is the UV lamp socket. The cable from the UV lamp should be plugged in here. This socket is only used in APPI mode.
MALDI	This is the MALDI socket. The cable from the MALDI assembly should be plugged in here.

Modular LockSpray source

You can easily configure the LockSpray™ modular source for several source configurations.

Modular source schematic:



Modular LockSpray source configuration guide:

Ionization mode	Configuration
ESI LockSpray	ESI probe + ESI baffle + LockSpray motor
APCI LockSpray	IonSabre probe+ special corona pin + APCI baffle + LockSpray motor
APPI	IonSabre probe + UV lamp
Dual APPI	IonSabre probe + special corona pin +UV lamp
ESCi	ESI probe + standard corona pin + blank side flange

To change the source configuration:

Warning: The side flange is protected by micro-switches. These are intended to protect the operator from potential exposure to high voltages and UV radiation. Never attempt to override these micro-switches.

Caution: Check that the probe Interlock Active is not displayed in the bottom right of the Tune window when changing a source. If it is, refit each flange so that it is flush with the source housing.

1. Switch the instrument to Standby mode. Check that the operate LED turns red.
2. Switch off the API gas flow.
3. Disconnect modules from the gas and power as necessary.
4. Undo the thumbscrews and remove the appropriate modules.

   Warning: To avoid possible burns and/or contamination, be aware that modules removed from the source may be hot and/or contaminated with toxic substances.

5. Fit the required modules, by tightening thumbscrews.
6. Connect the gas and power to modules as necessary.
7. Switch on the API gas flow.
8. Switch the instrument to Operate. Check operate LED goes green.

Tip: If operate LED is flashing green, the source interlock is active. Check that all modules are securely fitted and that the source door is closed. Check that the probe adjuster is connected to the interface panel.

Installing the electrospray probe

Warning: The probe and source may be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while handling these components

Warning: The probe and source are liable to be hot. To avoid burns, take great care while working with the instrument's front access door open.

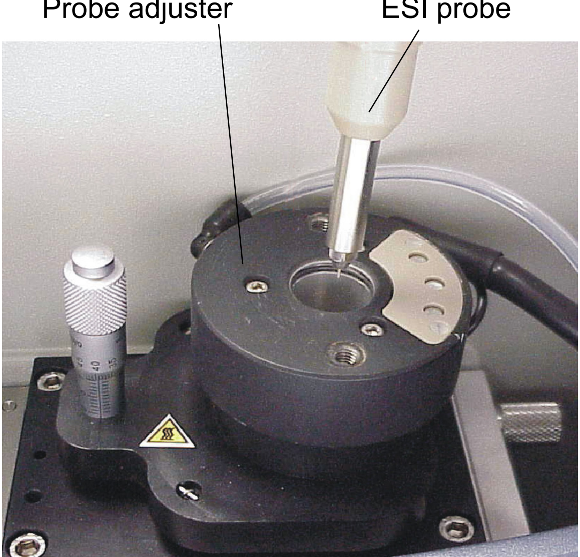
Warning: To avoid electric shock, ensure that the instrument is in standby before commencing tis procedure.

If not already fitted the instrument requires the installation of a probe. In this case we shall install the electrospray probe. The use of other probes is described in later chapters.

To install the electrospray probe:

1. Remove the protective sleeve, if fitted, from the electrospray probe tip.
2. Carefully slide the probe into the hole in the probe adjuster.

Probe adjustment flange:



3. Secure the probe by tightening the two thumbscrews.
4. Connect the probe adjustment flange electrical cable to the Heater/Interlock on the front panel.
5. Connect the probe adjustment flange PTFE tubing to the yellow Desolvation gas connection on the front panel.
6. Connect the probe PTFE tubing to the red Nebulizer gas connection on the front panel.
7. Connect the probe electrical lead to the Capillary connection on the front panel.

Status displays

Warning: A green or flashing green Operate LED indicates the presence of high voltages.

Warning: A flashing amber or flashing red Operate LED indicates an abnormal condition, where high voltages may be present.

The vacuum and operate status light emitting diodes (LEDs) are located to the right of the main panel on the instrument. The LEDs indicate the status of the pperate and the vacuum systems.

Operate LED display

Color	Status
Green	Operate.
Flashing Green	Operate, probe interlock active. Capillary voltage, cone voltage, and desolvation heater off.
Amber	Over-pressure trip. Voltages switched off because Tof vacuum is out of range.
Red	Standby.
Off	Power off.
Flashing Amber	Unexpected error - invalid state.
Flashing Red	Quadrupole RF Trip. Press standby to reset.
Flashing Red, Green	API Gas has failed, source voltages have been tripped out. Restore gas then press operate to reactivate source voltages.

Vacuum LED display

Color	Status
Green	Vacuum OK
Flashing Green	Pumped (all turbo pumps at >80%) but Tof vacuum out of range.
Amber	Turbo Pump on, one or more at <80% speed
Flashing Amber	Turbo pump off, one or more at <80% speed

Vacuum LED display (Continued)

Color	Status
Off	Vented / pumps off
Multi color	Abnormal operation

Pump-Down sequence

The Pump-down sequence as indicated by the vacuum LED lights as follows.

1. Flashing amber

   Rough pump on, soft vent off.

2. Amber

   Turbo pumps on, one or more at <80%.

3. Flashing Green

   All turbo speeds are >80%, the Tof Penning gauge turns on.

4. Green

   When the Tof pressure falls below 3.9×10^{-6} mbar

Vent sequence

The vent sequence as indicated by the vacuum LED lights is as follows.

1. Flashing Green / Amber

   Turbo pumps off, Tof Penning Gauge off.

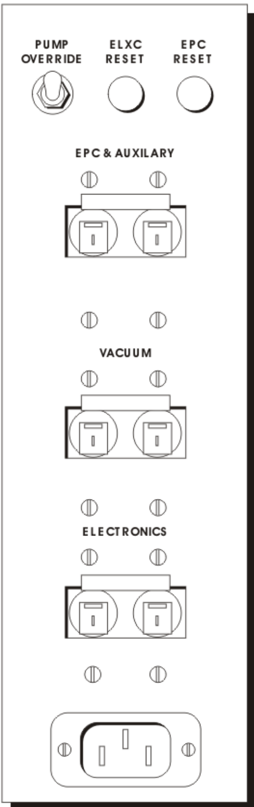
2. Off

   All Turbo speeds <80%, soft vent and rough pump off.

Rear panel

Mains power unit

Connections on mains power unit:



Vacuum

This is the vacuum power switch. When in the off position (1 = on, 0 = off), power is disconnected from vacuum pumps.

Electronics

This is the control electronics power switch. When in the off position (1 = on, 0 = off), power is disconnected from the main control electronics, the EPC, and auxiliary components.

EPC & Auxiliary

This is the EPC power switch. When in the off position (1 = on, 0 = off), power is disconnected from the embedded PC.

Pump Control

This is the pump control socket. The cable from the scroll pump should be plugged in here. This allows the instrument to switch the scroll pump on and off.

Supply Inlet

This is the mains power input socket. The cable from the laboratory mains power supply should be plugged in here.

Pump Override

This is for service use only and should always be left in the Auto position

Isolating the instrument from the mains

Before switching off the instrument it is advisable to vent the instrument in a controlled manner.

To isolate the instrument:

1. Set the Electronics and Vacuum power switches to Off.
2. Disconnect the mains supply inlet cable from the rear panel.

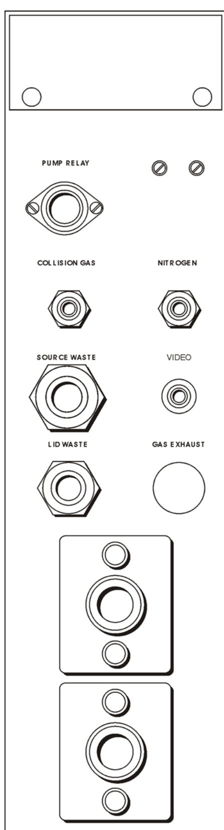
Connecting the instrument to the mains

Before connecting the instrument to the mains power, ensure that the mains supply meets the requirements on the instrument ratings plate. Connect using the appropriate supply cable to an earthed mains supply outlet.

Gas connections

All the gas connections are found on the bottom-right on the rear of the instrument.

Gas connections on rear panel:



Nitrogen Gas In

Warning: To avoid injury from high pressure nitrogen, ensure nitrogen gas is switched off when changing gas connections.

Caution: To avoid chemical contamination of the source, use only PTFE tubing, or clean metal tubing, to connect between the nitrogen supply and the instrument. Using other types of plastic tubing will result in chemical contamination of the source.

Caution: If the nitrogen supply pressure falls below 4 bar, the instrument will switch off the nitrogen supply and admit air into the source. If flammable solvents are used, there is a potential ignition hazard.

Connect the nitrogen supply (100 psi, 7 bar) to the Nitrogen Gas In push-in connector using 6-mm PTFE tubing. If necessary, this tubing can be connected to ¼-inch tubing using standard ¼-inch fittings.

Exhausts

Caution: Do not connect source and scroll pump exhausts as, in some circumstances, scroll pump exhaust could be admitted into the source chamber producing severe contamination.

Source

Warning: Due to the potential hazardous nature of the exhaust gasses, the source gas exhaust and drain, which also contains solvent vapors, should be vented via a separate fume hood, industrial vent or cold trap.

Connect the gas exhaust using 12-mm plastic tubing connected to the push-in fitting.

Source waste and LC waste

Warning: The waste liquid from the source enclosure and the LC comprises solvents and analytes. Always wear nitrile gloves while handling the container, and ensure that waste liquid is correctly disposed of according to local environmental regulations.

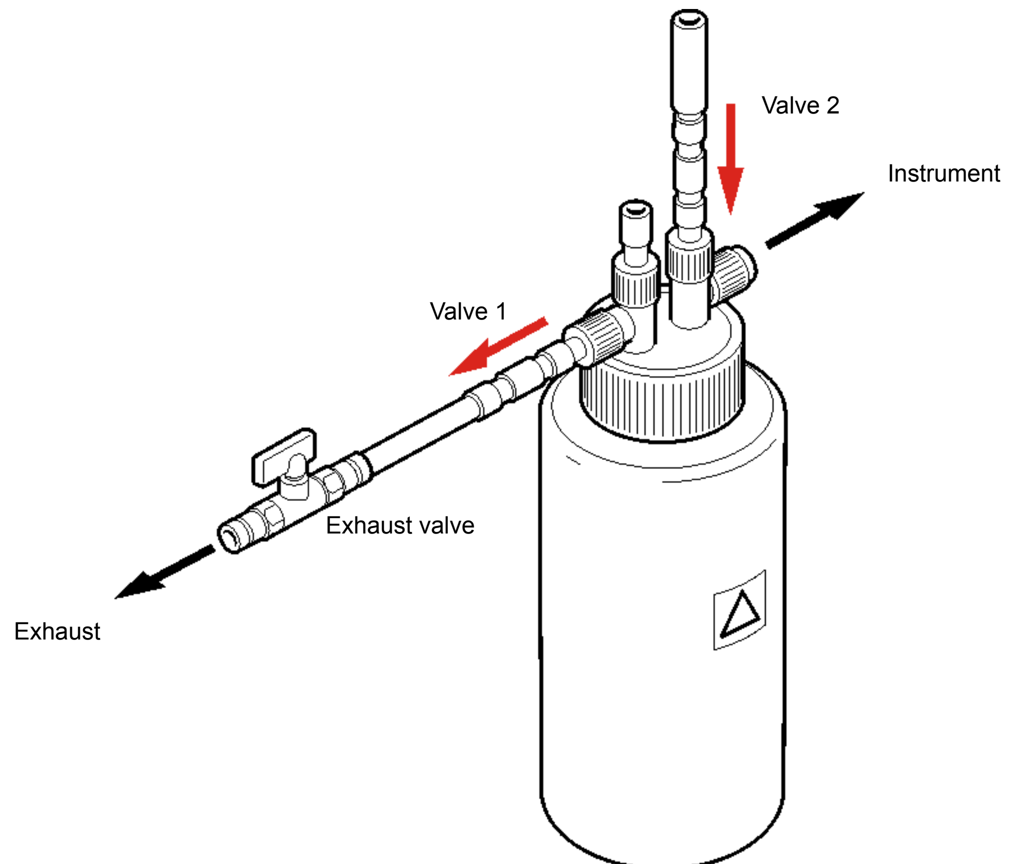
Waste liquid from the top of the instrument and the source is drained from the instrument via the Source Waste and LC Waste outlets. The liquid must be passed to a suitable container, which you must empty at regular intervals.

The 2-L waste bottle must be connected to the 12-mm Source Waste Port on the instrument rear panel to capture any liquid leaking into the source.

Connecting the waste bottle

This waste system involves the use of a single continuous waste line between the source and the source waste outlet on the instrument rear panel. This then connects directly to a solvent waste trap bottle assembly that also includes a series of one-way valves.

Waste bottle connections:



Valve 1 on the 10 mm exhaust line connects to the nitrogen exhaust. Under normal operation this valve opens to allow source gases and vapor to escape. However, when no API gas is supplied, this valve is closed and stops back flow of gas from the customer's exhaust line. There is an exhaust valve between the nitrogen exhaust and Valve 1. This is closed during source pressure testing but left open at all other times.

Valve 2 is connected so air will be admitted into the instrument waste system if a partial vacuum is formed in the source or instrument's drain. However, if the waste line is at a positive pressure, then the valve is held closed.

Scroll pump

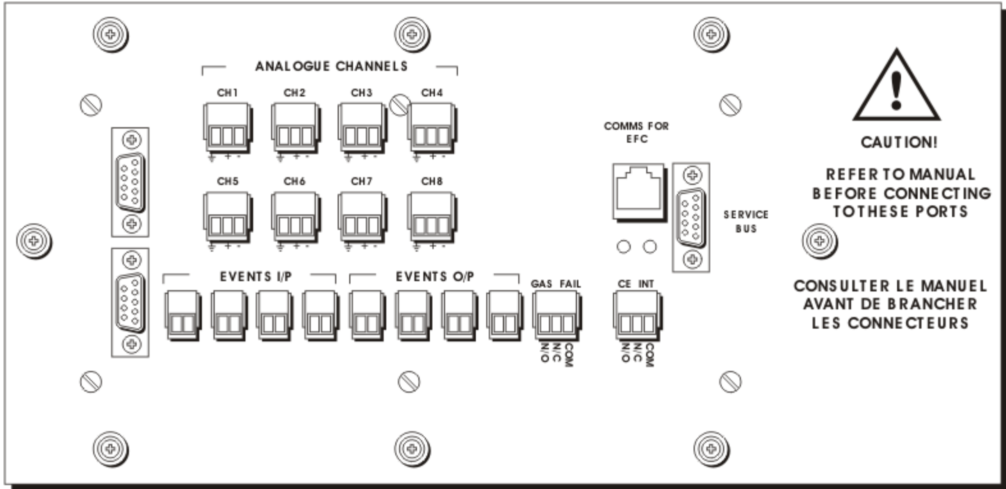
Warning: Due to the potentially hazardous nature of exhaust gasses, the exhaust from the scroll pump should be vented to atmosphere outside the laboratory.

Side panel

External IO board

The IO board is found on the left-side of the instrument.

IO board:



Analog Channels

Warning: Do not apply more than 2.5 V to any of the ANALOG CHANNELS connections.

Eight analog input channels are available, for acquiring simultaneous data such as a UV detector output. The input differential voltage is 0 to 2.5 V.

Contact Closure

Warning: To avoid electric shock and damage to the instrument, do not apply more than 5 V to any of the Events I/P connections.

Warning: To avoid electric shock and damage to the instrument, do not apply more than 25 V to any of the Events O/P connections.

Two types of contact closure are available:

- **In** – Four inputs, Events I/P, allow external devices to start acquisition. Each event input signal can be transistor-transistor logic or contact closure. The maximum voltage is 5 V.
- **Out** – Four outputs, Events O/P, allow the mass spectrometer to trigger an external event. The maximum rating is 25 V, 100 mA.

GAS FAIL

Warning: To avoid electric shock and damage to the instrument, do not apply more than 25 V to the GAS FAIL connection.

If the nitrogen pressure falls below 4 bar, while in Operate, the instrument goes into a "gas fail" state and a contact closure signal is generated. This signal can be used to stop solvent flowing into the source by connecting this GAS FAIL connection to the Stop Flow of the HPLC system. In the event of a nitrogen supply failure, any solvent from an LC will be drained automatically from the source enclosure. The maximum rating is 25 V, 0.25 A.

To return to Operate:

Restore the gas supply and click Operate.

CE Int (Capillary Electrophoresis Interlock)

Warning: To avoid electric shock and damage to the instrument, do not apply more than 25 V to the CE Int connection.

This connector interfaces with a capillary electrophoresis power supply so that the instrument is safely interlocked against high voltages. The maximum rating is 25 V, 0.25 A.

Comms for EPC Link

This RJ45 connector links the instrument’s embedded PC to the MassLynx workstation using the network cable supplied.

COM1 and COM2

These connections can be used by a Waters Field Service Engineer to communicate with the embedded PC.

Service Bus

This connection can be used by a Waters Field Service Engineer to communicate with the instrument electronics.

LEDs

Type	Description
SPEED	Green indicates normal operation. Red or Off indicates a fault
DATA	Flashes yellow during data transfers.

Top panel

Caution: Do not use the instrument top panel to store solvents.

2 Instrument Operation

This chapter will introduce the operator to the basic functional operation of the instrument; including basic software control, tuning and acquiring data. At this point the instrument should be pumped down and correctly installed.

Contents:

Topic	Page
MassLynx	2-2
Tuning	2-7
RF settings	2-21
Acquiring data from the Tune window	2-23

MassLynx

To open MassLynx and the Tune window:

1. From the Windows® desktop, double-click the MassLynx icon.
2. If applicable enter your logon name and password, and then click OK.

   Tip: The Login dialog box appears only when you enable MassLynx security. Otherwise, the MassLynx Main window appears after you click the MassLynx desktop icon. See the *MassLynx Security User's Guide* for details about enabling MassLynx security.

   Result: The MassLynx main window appears.

   Tip: After initiating, the main window displays "Instrument Present" in the status bar.
3. The shortcut bar should appear in the MassLynx main window, and Instrument should appear at its top. If it fails to appear, click Shortcut, in the toolbar, to open it. Then click Instrument, at the left edge of the shortcut bar.
4. Select MS Tune to open the Tune window.

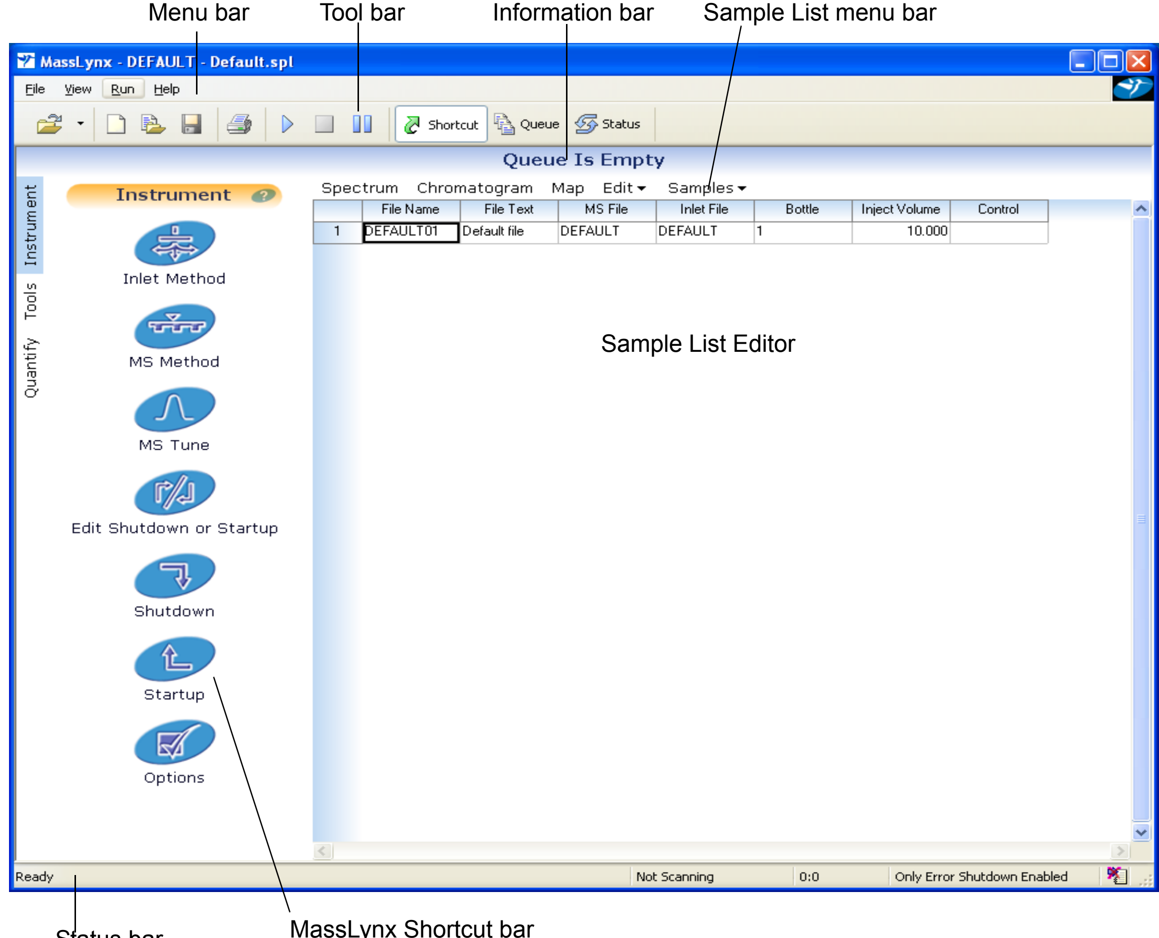
MassLynx window

The MassLynx window contains:

- A Banner, which displays the names of the current Project and Sample List.
- A top-level Menu bar.
- A Tool bar.
- An Information bar (below the Tool bar); this normally shows the status of the sample currently being acquired or processed.
- The MassLynx bar, with associated tabs, at the left-hand-side of the window. The contents of the MassLynx bar can be swapped between the Shortcut bar, Queue bar and Instrument Status bar, each of which has its own set of associated tabs and options.
- The Sample List Editor.
- A Sample List Menu bar (above the Sample List Editor), containing commands associated with the Sample List.

- A Status bar, at the bottom of the window.

MassLynx window:



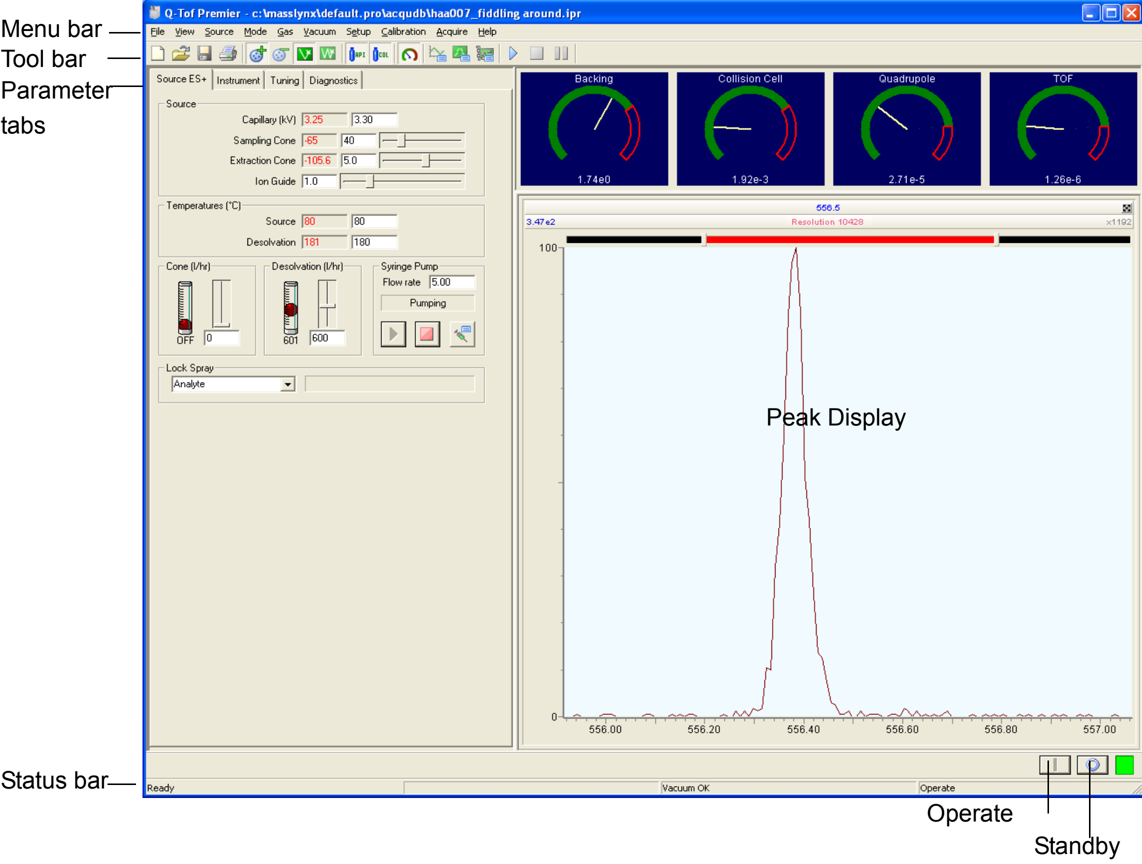
Tune window

The Tune window is the interface for instrument control, it is used for the following.

- Tuning the instrument
- Turning gases on and off
- Monitoring vacuum pressures

- Monitoring Acquisitions
- Calibrating the instrument for accurate mass

Tune window in “Tuning view”:



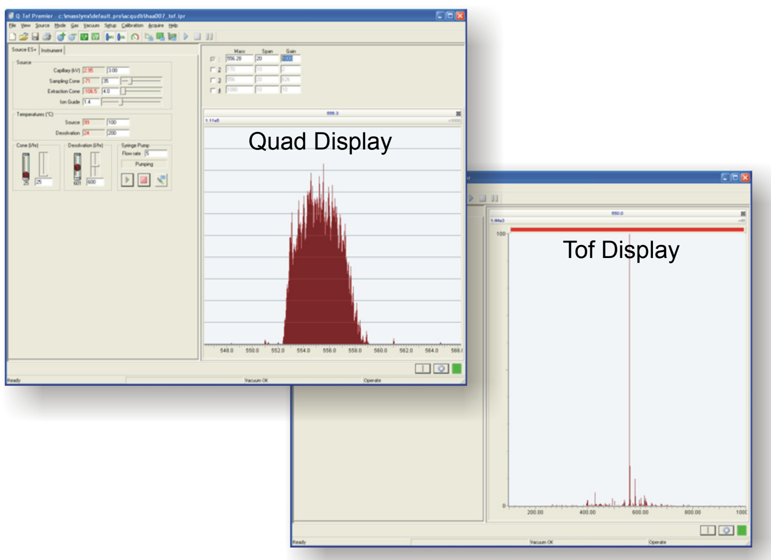
The Tune window contains:

- A Menu bar
- A tool bar
- Parameter tabs
- Status bar
- Peak display

Peak display

The peak display allows real-time spectral data to be observed without acquiring or making a data file.

Quad and Tof peak display:



There are two Peak displays:

- Quad Peak display

  This displays the signal on the photomultiplier tube using the quadrupole as the mass filter.

  Tip: This is only used for diagnostic purposes and would not normally be used by anyone other than a Waters Field Service Engineer.

- Tof Peak Display

  This displays the signal on the MCP detector using the Tof as the mass analyzer.

To switch between peak displays:

Select View > Quad Peak Display or Tof Peak Display

Resolution and ions per push

The peak display now indicates the resolution or the number of ions per push (IPP) for the largest peak in the window.

The resolution is indicated for continuum data and IPP is shown for centroid data.

Note: The resolution display will not be available until MassLynx 4.1.

Instrument control pages

Controls necessary for setting up the Q-TOF Premier are accessible in the Tune window from either toolbar or instrument control pages.

The source and instrument tabs are always visible:

- Source page - controls source voltages, temperatures, gas flows, and syringe pump control.
- Instrument page - controls the quadrupole, collision cell and detector.

Note: The Source tab is labelled with the source type and instrument polarity, e.g. ES+ means electrospray source and positive ion mode.

Additional pages are available when you, select View > Tuning controls:

- Tuning page - controls voltages that affect instrument resolution. These controls are optimized on installation, and should not require further adjustment.
- Diagnostics page - displays turbo pump information, pressures and selected instrument voltages.

Many more pages are available when you select View > Engineering controls. These additional pages are provided for use by Waters Field Service Engineers. This option is password protected and requires the password ‘access’ to be entered.

Caution: The source and instrument pages provide sufficient control for day-to-day use of the Q-Tof Premier. The adjustment of parameters in any of the additional tabs is likely to degrade instrument performance.

To change the Tune window style:

1. Click View from the Tune window.
2. Select Tuning Controls, Extended Controls or Engineer Controls.

Tuning

The following sections describe how to tune your instrument in two ways:

- Routine optimization for sensitivity using the source and instrument parameters.

  This needs to be carried out on a sample by sample basis. This assumes that the complete tuning of the Tof has already been carried out and is saved in the currently loaded tune file.

- Complete tuning of the Tof from default settings.

  Your instrument will already have been tuned optimally from the factory set default settings by the installation engineer. You should only have to tune from these settings if MassLynx has to be reinstalled.

The purpose of the following tutorials is to show you how to get a mass spectrum from each of the Q-Tof Premier's four modes using 2 standard compounds.

Important: All tuning in this chapter is done using the Tof and MCP detector. Click View > Tof Peak Display, to ensure that the Tof Peak Display is enabled.

Prerequisites: MassLynx on page 2-2.

Requirement: Ensure than analyte is selected if running in LockSpray mode.

Compounds and benchmark tests

Before using the Q-Tof Premier for routine analysis it needs to be "tuned", in all four of its operating modes, to give maximum resolution and sensitivity. It is good practice to use a sample that is representative of the samples that will be analyzed.

The Q-Tof Premier, in Tof MS and MS/MS, can operate in the following ways and consequently requires tuning in each mode:

- V positive
- W positive
- V negative
- W negative

All four modes are saved to one tune file (*.ipr).

The following procedure can be used to tune all ESI Tof MS modes of operation. It is a quick tuning method and not intended to be a performance specification. The tables below outline the compounds to use and the sensitivity and resolution that can be expected from them.

Warning: Use extreme care when working with formic acid. Use a fume hood and appropriate protective equipment.

Compounds for tuning:

Operating mode	Compound to use
V and W positive	Leucine enkephalin (m/z 556) 50 pg/µL (50:50 acetonitrile:water 0.1% formic acid)
V and W negative	Raffinose (m/z 503) 500 pg/µL (50:50 acetonitrile:water)

Sensitivity and Resolution for quick tuning:

Test	V +	V -	W +	W -
Sensitivity (cps)	200	200	50	50
Resolution (FWHM)	8000	8000	15000	15000

Routine optimization for sensitivity - Tof MS

This section is intended to demonstrate tuning for good sensitivity from a previously saved tune file.

Important: Keep the ion counts below the deadtime threshold (see Deadtime Correction on page 3-3 for more information).

Initial considerations

The following should be taken into account:

- Ensure that samples are fully dissolved in a suitable solvent. Either filter or centrifuge the sample to avoid blocking the transfer line.
- Syringes should be thoroughly cleaned to minimize sample carryover.
- An appropriate tune file, containing the optimized Tof parameters, should be loaded.

Initial Setup

To set up the syringe:

1. From the Source page click to open a drop down list of syringe types.
2. Select the appropriate syringe type e.g, Hamilton 250 µL.
3. Load the syringe with an appropriate compound and click to switch the syringe on.
4. Enter a syringe rate of 5 µL/min to ensure a uniform, continuous spray that will last for a relatively long period.

Important: Some level of cyclical variation, due to the syringe drive unit, in signal strength should be expected. This should be limited to less than 10%, any more is a fault and should be reported for repair.

To set source tuning parameters for sensitivity:

1. From the Tune window click to switch the instrument into Operate. Check that the adjacent instrument status indicator turns green. Ensure the syringe pump is on and the API and Collision gasses are on.

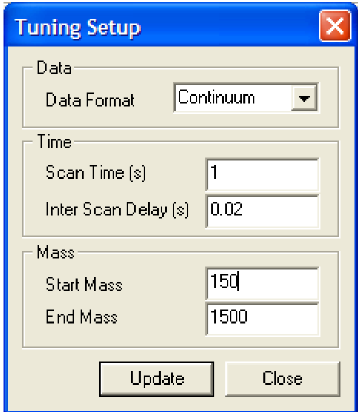
   Important: If the Q-Tof Premier has been in Standby for more than 2 hours it should be left in Operate for 1 hour to allow the instrument to stabilize.

2. Click File > Open and select a tune file that contains the optimized Tof parameters.

   Tip: The engineer will have left a tune file that contains the optimized Tof parameters, usually with the name *HAAxxx.ipr* where *xxx* is the instrument serial number

3. Specify these two settings to set the instrument mode:
   - Click Mode > Positive Ion for or Negative Ion
   - Click V or W to specify instrument mode.
4. Click to switch the API gas on.
5. Click Setup > Tuning Settings to set the scan time and mass range.
6. In the Tuning Setup dialog box, enter the following values and click Update.

Tuning Setup dialog box:



Tip: It may take a while for the sample to appear in the peak display window as any dead volume will need to be cleared first. Increase the syringe rate for a short period to push the sample through.

7. There will be a response in the peak display. Zoom in the relevant mass range, the peak display will resemble that shown in Figure titled "Tuned peak display:" on page 2-11.

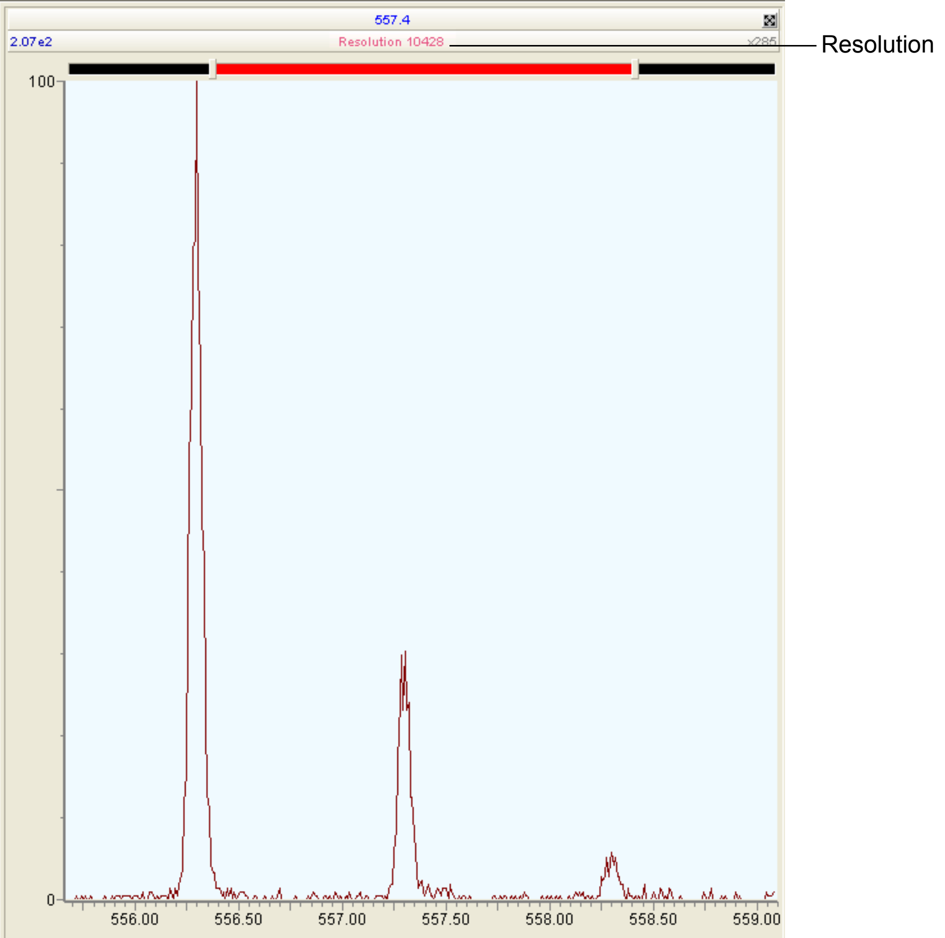
   Tip: The parameters on the Instrument page should not need changing at this time.

   Recommendation: To return the peak display to full span click .

8. Adjust the source and instrument settings up or down for maximum sensitivity. Refer to the Table titled "Source ES page parameters:" on page 2-12 and the Table titled "Instrument page parameters:" on page 2-13 for more details on each parameter. Ensure that you press enter after adjusting each parameter.

   The Peak display should resemble the following figure.

Tuned peak display:



9. Select File > Save As to save the tune settings and enter an appropriate name.

   Important: The mass position may vary since the instrument has not been calibrated.

   Tip: The tune settings are saved as a *.ipr file in the Acqudb folder of the current MassLynx project.

See also:

- A quick guide to the Source page settings on page 2-12
- Complete tuning of the Tof from default settings on page 2-15

- Acquiring data from the Tune window on page 2-23
- Calibration on page 3-1

A quick guide to the Source page settings

The tuning parameters on the Source ES window are used to optimize the ionization in the source, and control the introduction of ions into the instrument. They should be tuned for maximum sensitivity for each sample and infusion condition. These parameters do not influence Tof resolution except when the detector saturation limit has been exceeded, in which case the capillary voltage should be used to control the ion count.

Source ES page parameters:

Parameter	Description
Capillary (kV)	The voltage on the ESI probe tip. Normally tuned for maximum sensitivity. Can be used to decrease ion count to acceptable levels. Best sensitivity at 3 to 3.5 kV in positive ion mode, ~ 2.5 kV in negative ion mode.
Sampling Cone (V)	Tune for best sensitivity. Sample and charge state dependent, usually best at 25 to 50 V.
Extraction Cone (V)	Tune for best sensitivity, usually 2 to 5 V.
Ion Guide (V)	Tune for best sensitivity, usually 1 to 3 V.
Source Temperature (°C)	Usually left at 80 to 100 °C.
Desolvation Temperature (°C)	Set high enough for complete desolvation of analyte spray. Solvent flow rate and solvent dependent. For a solvent flow rate of 300 µL/min, this usually 250 to 400 °C.
Cone Gas (L/hr)	Often left at zero, but can aid desolvation in some circumstances, e.g. nanoflow. Tune for maximum sensitivity, usually 0 to 50 L/h.
Desolvation Gas (L/hr)	Used in combination with desolvation temperature to achieve complete desolvation of analyte spray. Tune for maximum sensitivity, usually 400 to 800 L/hour.
Syringe Pump Rate (µL/min)	Set to desired flow rate. For ESI reference sprayer, this is usually 5 to 20 µL/min.

A quick guide to the Instrument page settings

The tuning parameters on the Instrument window are used to optimize the passage of the ions through the rail section of the instrument. They should be tuned for maximum sensitivity and may require adjustment when moving from Tof MS to Tof MS/MS modes. These parameters do not influence Tof resolution.

Instrument page parameters:

Parameter	Description
LM Resolution	The 'low mass resolution' slider controls the width of quadrupole transmission window, i.e. it controls the selectivity of the MS1 analyser. It is only applicable in MSMS mode. It is normally set to 4.7 (4000 Da quad) or 4.9 (8000 Da quad). The transmission window will be about 4 Da. See Quadrupole resolution settings on page 2-14.
HM Resolution	The 'high mass resolution' slider controls the width of quadrupole (MS1) transmission window at higher masses. Usually left at 15.
Ion Energy (V)	Controls the energy of the ions passing through the quadrupole. Will stop beam completely if set at less than approximately -1 V. Usually set to 0 to 1 V.
Pre-Filter (V)	Tune for maximum sensitivity, usually 5 to 10 V. Higher values will decrease the mass transmission window in MS mode.
Collision Energy (V)	Controls the energy of ions entering the collision cell. In MS mode this is usually set to 5 V, in order to avoid any fragmentation. In MSMS mode increase to give optimum fragmentation, usually 20 to 30 V.
Cell Entrance (mL/min)	Always set to 2 V.
Cell Exit (mL/min)	Always set to -10 V.
Collision Cell Gas Flow (V)	Adjust to give a collision cell pressure of 4 x 10^{-3} mbar. Usually about 0.45 L/min.
Ion Guide Gas Flow (V)	Research applications only. Usually set to zero.

Instrument page parameters: (Continued)

Parameter	Description
Detector (V)	Set for optimum ion detection, see Setting the Detector Voltage on page 3-8.
Mass Range	The mass range of the TOF can be varied by changing the pusher frequency. Usually left on auto.

Quadrupole resolution settings

The 'low mass resolution' (LM Resolution) slider controls the width of quadrupole transmission window, i.e. it controls the selectivity of the MS1 analyser. It is only applicable in MSMS mode. It is normally set to 4.7 (4000 Da quad) or 4.9 (8000 Da quad). In this case the transmission window will be about 4 Da, allowing transmission of isotopes.

If a monoisotopic MSMS spectrum is required, it is necessary to increase the LM resolution setting to about 15. The exact setting required is best determined by acquiring MSMS data and adjusting the LM Resolution setting while monitoring the spectrum in real time. The actual quad resolution required to select just one isotope is a function of charge state. The resolving capability of the quadrupole is a function of mass; low mass peaks can be more easily resolved than high mass peaks.

Tuning for Tof MS/MS

During MSMS acquisitions the quadrupole selects a mass to be fragmented. Instrument tune settings are identical to MS except apart from the following:

- LM and HM Resolution controls are active, and are used to set the required selectivity
- Collision energy is increased in order to induce optimal fragmentation.

The amount of collision energy required will depend on the charge state and the stability of the ion. The Q-TOF Premier can be set to ramp between collision energy values, allowing efficient fragmentation over a range of different ions.

For details on how to set up Tof MS/MS experiments and Collision energy ramping experiments see Chapter 4 and Chapter 6.

Complete tuning of the Tof from default settings

This section is intended to demonstrate tuning for good sensitivity and resolution from a default tune file.

Important: Keep the ion counts below the deadtime threshold see Deadtime Correction on page 3-3 for more information

Initial considerations

The following should be taken into account:

- Ensure that samples are fully dissolved in a suitable solvent. Either filter or centrifuge the sample to avoid blocking the transfer line.
- Syringes should be thoroughly cleaned to minimize sample carryover.

To tune from default settings:

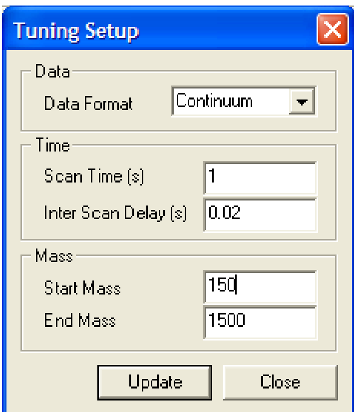
Prerequisites: Initial Setup on page 2-9

1. From the Tune window, click [button] to switch the instrument into Operate. Check that the adjacent instrument status indicator turns green. Ensure the syringe pump is on and the API and Collision gasses are on.

   Important: If the Q-Tof Premier has been in Standby for more than 2 hours it should be left in Operate for 1 hour to allow the instrument to stabilize.

2. Click File > Open and select default.ipr that contains the default Tof parameters.
3. Select Views > Tuning Controls. Specify these two settings to set the instrument mode:
   - Click Mode > Positive Ion or Negative Ion
   - Click V or W to specify instrument mode.
4. Click Setup > Tuning Settings to set the scan time and mass range.
5. In the Tuning Setup dialog box enter the following values and click Update.

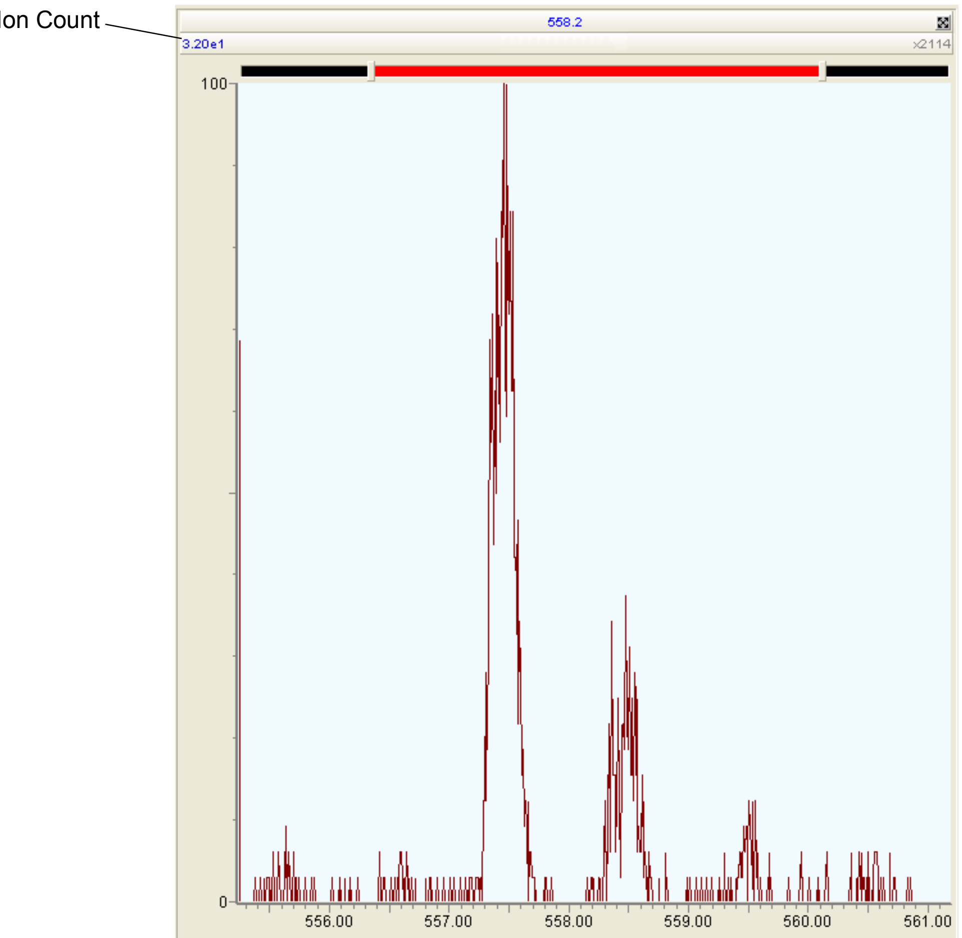
Tuning Setup dialog box:



Tip: It may take a while for the sample to appear in the peak display window as any dead volume will need to be cleared first. Increase the syringe rate for a short period to push the sample through.

6. There will be a response in the peak display. Zoom in the relevant mass range, the peak display will resemble that shown in the following figure.

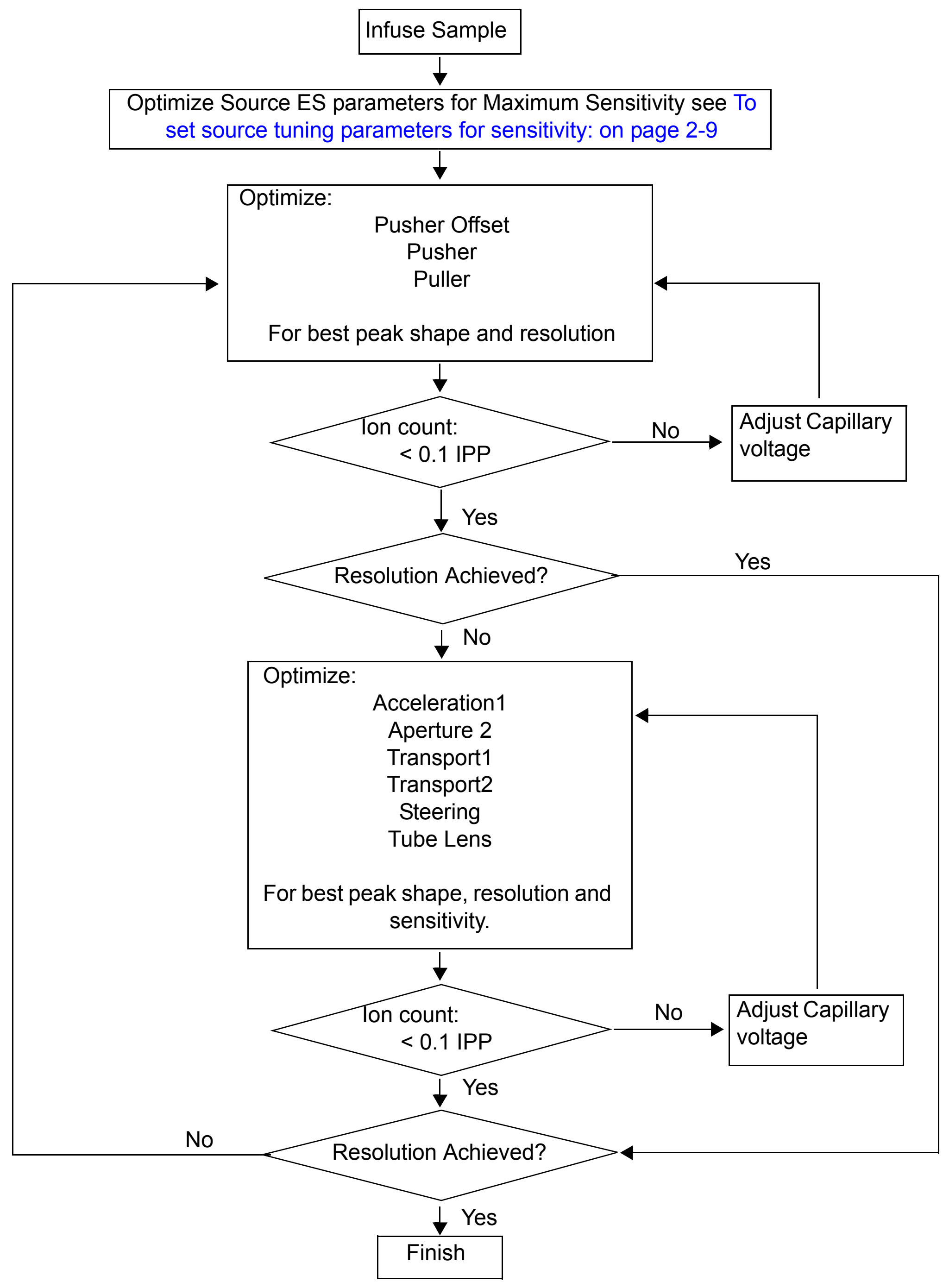
Peak display using default values:



7. With reference to the Table titled "Tuning page parameters:" on page 2-19 and the Figure titled "Tuning flow diagram:" on page 2-18 adjust the various parameters for resolution and sensitivity. Ensure that you press enter after adjusting each parameter.

   The peaks in the display should eventually resemble those shown in Figure titled "Tuned peak display:" on page 2-11.

Tuning flow diagram:



8. Select File > Save As to save the tune settings and enter an appropriate name.

See also:

- Routine optimization for sensitivity - Tof MS on page 2-8
- Tuning page parameters on page 2-19
- Acquiring data from the Tune window on page 2-23
- Calibration on page 3-1

Tuning page parameters

The Tuning page contains parameters that affect instrument resolution. These controls are optimized on installation, and should not require further adjustment. If the original settings are lost, new settings can generated from the instrument default settings. Maximum instrument resolution can often be obtained with most of these settings at their default values. The default parameter values are the same in both positive and negative ion modes.

Tip: When optimizing these parameters, start with the pusher offset control. Changing the pusher offset from 0 to about -1 V can produce a dramatic improvement.

Tuning page parameters:

Parameter	Default V Mode	Default W Mode	Description
Acceleration 1 (V)	80	50	Tune for maximum sensitivity and resolution. Should optimize within 20 V of default value.
Acceleration 2 (V)	200	100	Do not change from default value.
Aperture 2 (V)	70	30	Tune for maximum sensitivity and resolution. Should optimize within 10 V of default value.
Transport 1 (V)	60	30	Tune for maximum sensitivity and resolution. Should optimize within 10 V of default value.
Transport 2 (V)	60	30	Tune for maximum sensitivity and resolution. Should optimize within 10 V of default value.

Tuning page parameters: (Continued)

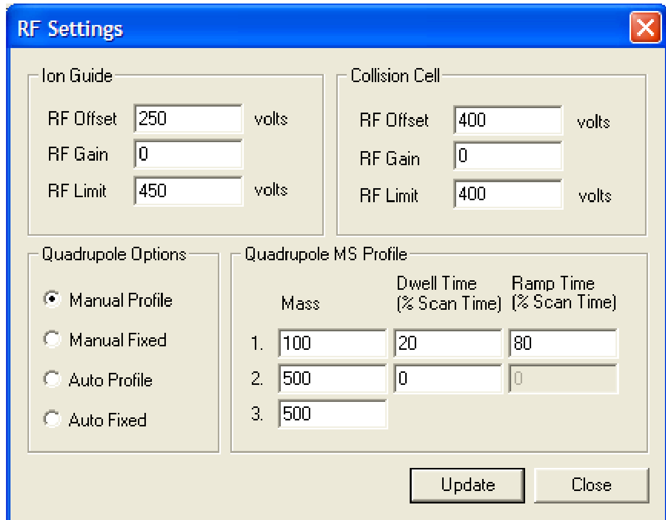
Parameter	Default V Mode	Default W Mode	Description
Steering (V)	0	0	Tune for maximum sensitivity and resolution. Should optimize between –1 and 1 V.
Tube Lens (V)	75	24	Tune for maximum sensitivity and resolution. Should optimize within 10 V of the default value.
Pusher (V)	905	925	Tune for maximum resolution. Should tune within 20 V of default.
Pusher Offset (V)	0	0	Tune for maximum resolution. Should optimize between –3 and 0 V.
Puller (V)	630	670	Tune for maximum resolution. Should tune within 50 V of default.

RF settings

The Q-TOF Premier contains three RF devices: a source ion guide, a collision cell and a quadrupole. The RF settings control the sensitivity and mass range of the Q-Tof Premier.

To open the RF settings dialog box:

1 Click [icon] to open the RF Settings dialog box.



2 Make any changes required and click Update and then Close.

Note: New settings do not take effect until Update is clicked.

Ion Guide

The default settings for the RF on the Ion Guide should be sufficient for normal mass ranges. However, if you wish to increase sensitivity for lower m/z values the RF Offset can be decreased. You will have to tune this value for your application.

Collision Cell

The default RF settings for the Collision Cell do not need to be changed.

Quadrupole options

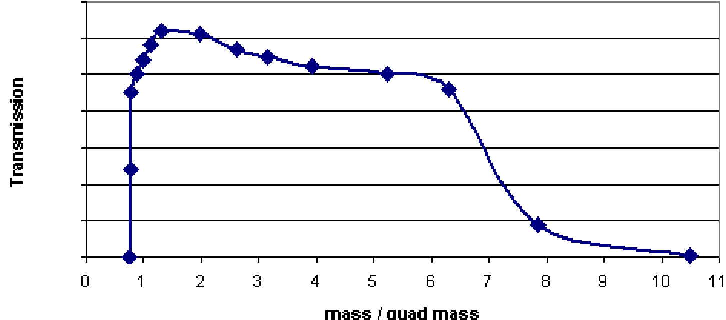
Setting quadrupole options to 'Auto Profile' allows fully automatic control of the quadrupole RF during MS acquisitions.

Option	Description
Manual Profile	This is, essentially, as described in the previous section,
Manual Fixed	Enter a m/z for maximum sensitivity at that value.
Auto Profile	This calculates the MS profile from the low m/z and high m/z that are entered as part of the acquisition.
Auto Fixed	Automatically sets a fixed quad RF dependent on the acquisition start mass.

Quadrupole MS Profile

The transmission of the quadrupole in non-resolving (MS) mode is a function of the quad RF amplitude, i.e. the 'quad mass'.

Typical transmission profile:

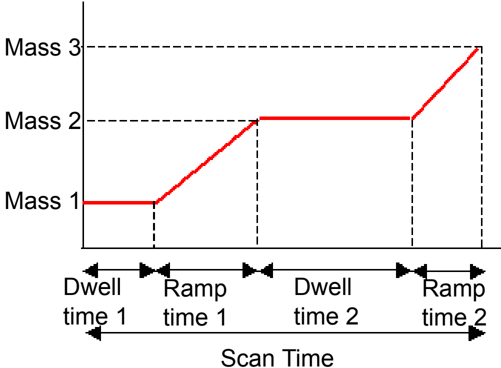


The graph defines a transmission window for the quadrupole. The size of this transmission window is a function of pre-filter voltage; use a low pre-filter voltage to get a wide transmission window.

The graph shows a sharp cut-off in transmission at 0.8. For example, if the quad mass is set to 500 Da, no ions will be transmitted below 400 Da (0.8 x quad mass). At the higher mass end the cut-off is much more gradual, and can change according to instrument conditions. For example, if the quad mass is set to 500 Da, the transmission at 2500 is still high (look at 2500/500 = 5).

The Quadrupole MS Profile settings allow the quad mass to be ramped, enabling the acquisition of mass ranges wider than the quad transmission window. Appropriate values of Mass, Dwell Time and Ramp Time must be entered to suit the acquisition mass range. Alternatively, set Quadrupole Options to Auto Profile, allowing MassLynx to calculate the optimum MS Profile.

The Quadrupole MS Parameters are defined in the following graph:



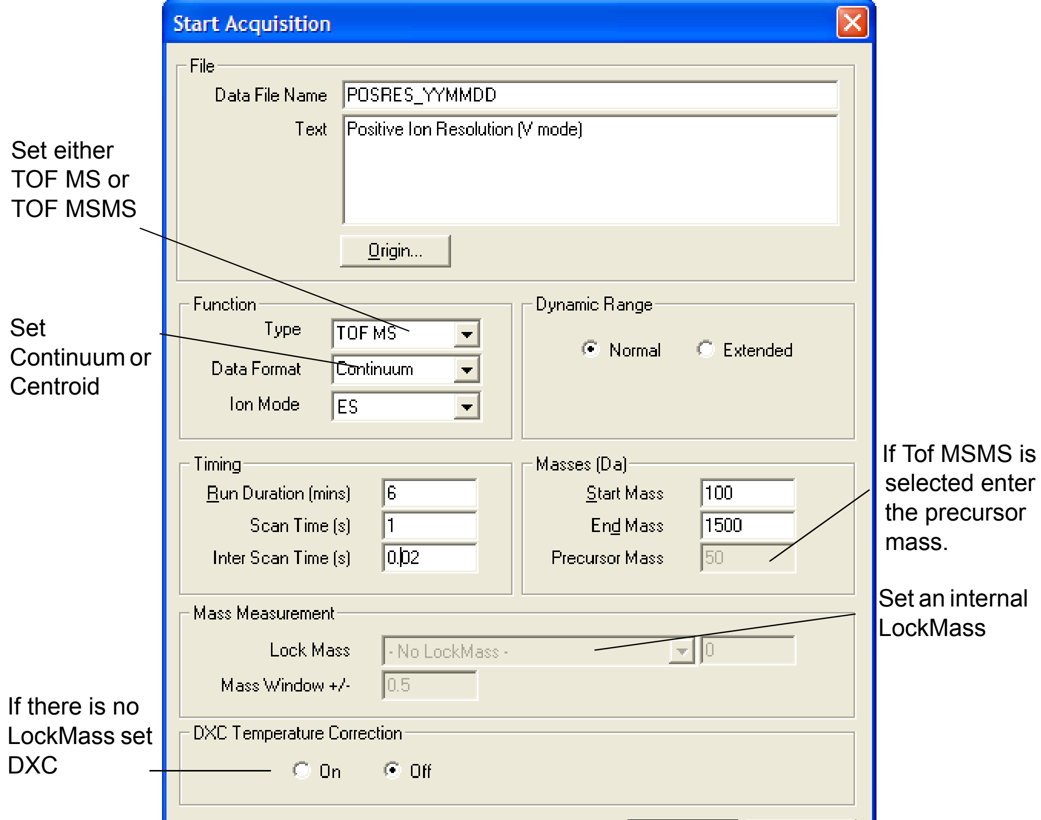
Acquiring data from the Tune window

Data can be acquired from single samples directly from the Tune window. Multiple samples need to be entered in the Sample List, this is described in Chapter 4.

To acquire data from the Tune window:

1. Click ▷ to open the Start Acquisition dialog box and enter the parameters as shown below.

Tune Acquire dialog box:



Tip: Click Origin to open a dialog box that allows you to enter your own details and details of the sample.

2. Click Start.

Result: The Q-Tof Premier begins to Acquire data and save it to disk.

Monitoring an acquisition

Tip: The progress of your data acquisition can be followed in both Spectrum and Chromatogram as well as the peak display.

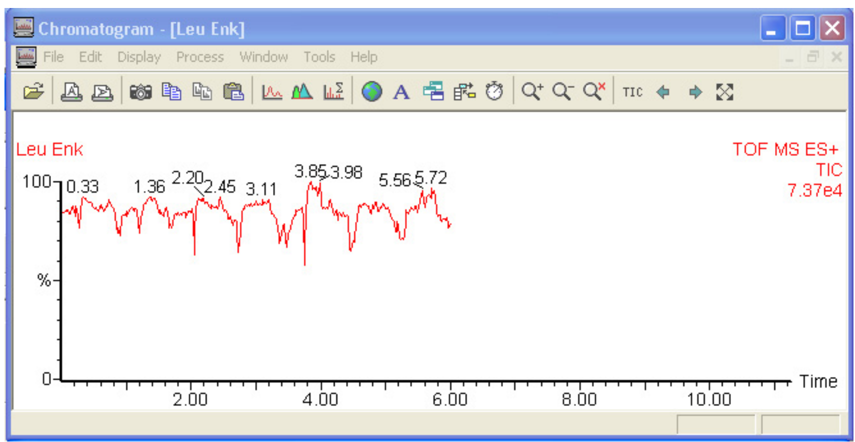
See also: MassLynx help for further details on both Spectrum and Chromatogram.

Chromatogram real-time update

To follow an acquisition in Chromatogram:

1. From the main MassLynx window, click Chromatogram from the Sample List menu bar.

   Chromatogram display during real-time update:



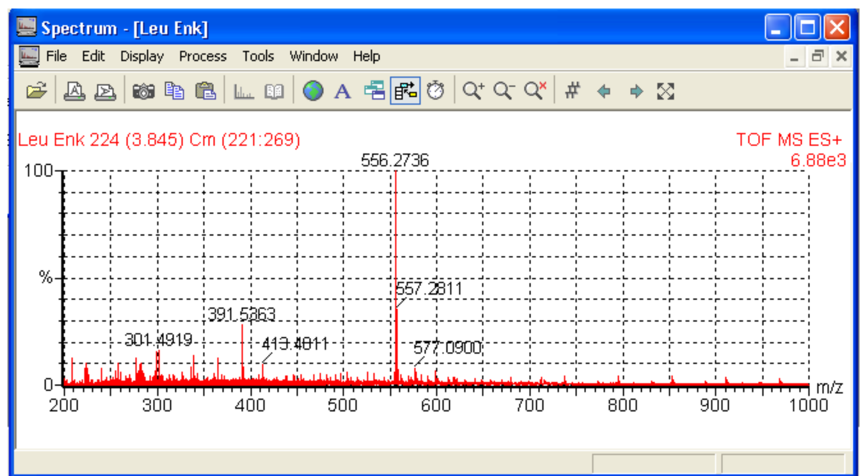
2. In Chromatogram, click ⏱ in the menu bar to update the chromatogram in real-time display as the acquisition proceeds.

Spectrum real-time update

To follow an acquisition in Spectrum:

1. From the main MassLynx window, click Spectrum from the Sample List menu bar to open Spectrum.

Spectrum display during real-time update:



2. In Spectrum, click in the menu bar to update the spectrum display in real-time as the acquisition proceeds .

   Spectrum will show each new scan.

Checking the resolution

The resolution of a peak is defined as the ratio of its mass to its peak width (measured at half height).

Use the following equation to calculate the resolution:

$$Resolution = (Mass) \div (PeakWidth)$$

The resolution in V-mode will be about 10 000 when measured on a multiply charged ion, or about 8000 when measured on a singly charged ion.

The resolution in W-mode will be about 17 500 when measured on a multiply charged ion, or about 15 000 when measured on a singly charged ion.

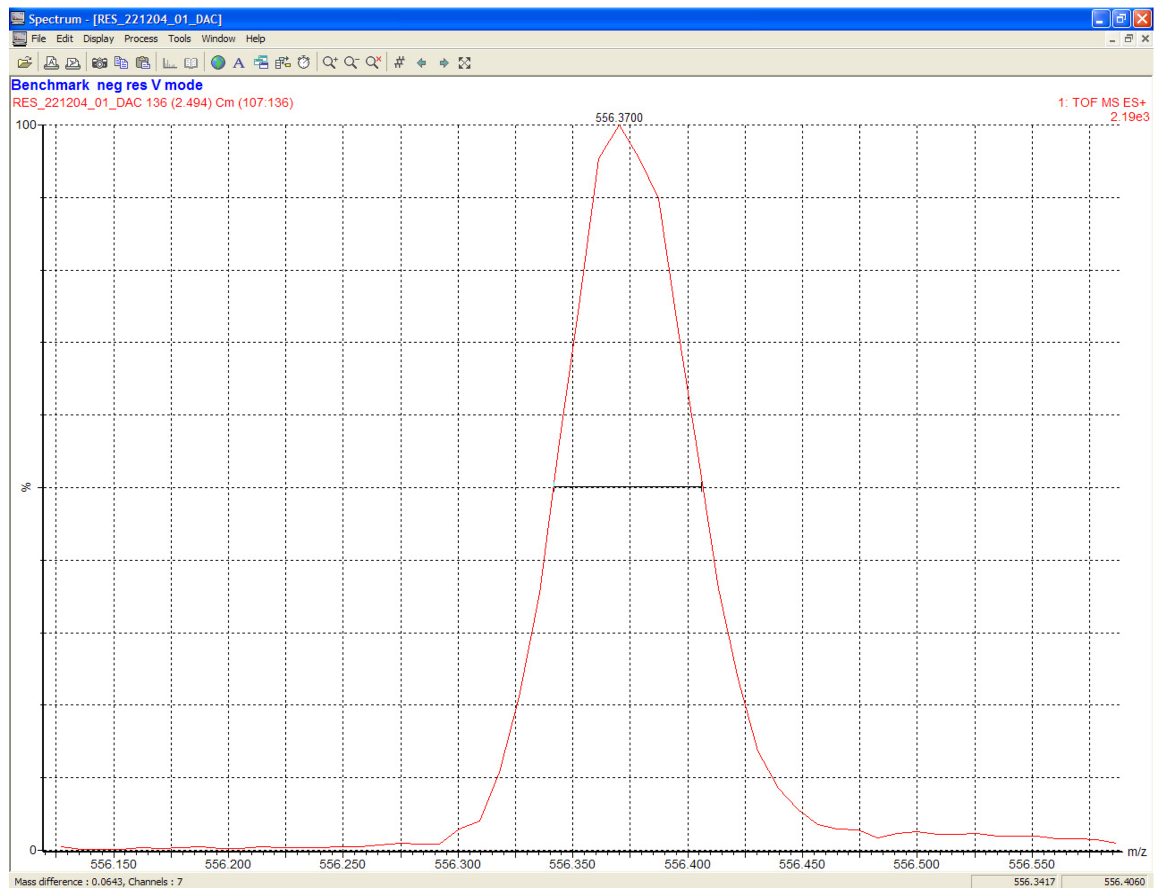
To check the resolution:

1. Acquire data as described on page 2-23.
2. Click Start.
3. Open Chromatogram from the MassLynx Sample List menu.
4. Click in the menu bar.

5. Combine data from at least 30 scans. On completion the Spectrum window will open
6. In the Spectrum window zoom in on the main peak (556 Da if running leucine enkephalin).

   Tip: To ensure that a grid is shown on the Spectrum. Select Display > View to open the Spectrum Display dialog box and then select Grid ‘Dot/Dash/Solid’.

   Rule: Select Display > Peak Annotation to open the Spectrum Peak Annotation dialog box and ensure that 4 decimal places are entered.
7. With the aid of the grid, right-click and drag the mouse pointer across the width of the peak at half maximum height. The width is shown in the bottom-left corner of the Spectrum window. Calculate the resolution using the equation at the beginning of this section.



The resolution calculation follows:

$$Resolution = (556.37/0.0643) = 8653$$

If the resolution is not achieved, optimization of tune parameters may be necessary.

Note: From MassLynx 4 the resolution will be displayed in the peak display window.

See Also:

- MassLynx Help, for details on how to use Chromatogram, Spectrum and Combine.

  See also: Acquiring data from the Tune window on page 2-23.

3 Calibration

Contents:

Topic	Page
Overview	3-2
Before calibrating	3-7
Accurate mass basics	3-10
Calibrating for accurate mass	3-11

Overview

Before using the Q-Tof Premier for exact mass measurement work the instrument requires calibrating. It is recommended that the calibration is checked regularly with an appropriate quality control standard. A new calibration should be performed after:

- Cleaning, maintenance
- Changing the Veff value
- Changing the TOF mass range
- Changing any analyzer tune settings

The Q-TOF Premier has four basic modes of operation: V+, V-, W+ and W-. Each of these has a separate TOF calibration, but all can be saved in a common calibration file.

Nominal Mass Measurement

The instrument mass scale is set up to give nominal mass measurement without a calibration. This requires the setting of the Veff and T0 parameters in the acquisition settings window.

Quad Calibration

The quad mass scale is set up via the control electronics. A separate software calibration is not always necessary. The quad mass scale has to be set up correctly in order to accurately select precursor ions. The quad calibration has no effect on the mass measurement of the Q-TOF Premier.

TOF Calibration

For all accurate mass work, calibrating the TOF is a critical part of the instrument set-up process. MassLynx uses a polynomial equation in order to calibrate precisely over a wide mass range.

Lock Mass

Temperature variations in the laboratory surroundings will cause mass shifts. This can be corrected for by the use of a single reference peak, i.e. a lock mass.

This lock mass can be either internal, i.e. in the sample solution, or external, in a separate reference spray, i.e. LockSpray.

Dynamic External Calibration

Dynamic External Calibration (DXC™) is a method of compensating for mass drift due to temperature fluctuations. It is not suitable for accurate mass work, and is only used in the absence of a suitable lock mass.

TOF Mass Range

The TOF Mass Range of the instrument is set via the instrument tab on the Tune window. It can be set either to auto, or to one of the masses on a drop down list (1000, 2000, 4000 etc.). These different upper mass limits correspond to different pusher frequencies. Doubling the mass range reduces the instrument sensitivity by a factor of $1/\sqrt{2}$ (i.e. 0.71). For optimum mass measurement performance, the TOF mass range (or pusher frequency) must be set to the same value when calibrating the instrument as used during the analysis.

In auto mode, the mass range is automatically set according to the upper mass range of the acquisition, e.g. for an acquisition high mass of between 1001 and 2000 the TOF mass range will be set to 2000.

Deadtime Correction

The data acquisition system for the instrument is a time to digital converter (TDC). This is an ion counting system which generates a mass spectrum by histograming the arrival times of ions in memory. After the arrival and registration of an ion by the TDC there is a minimum time interval before a subsequent ion arrival can be registered. This is called the 'dead time' of the TDC and is of the order of 5 ns. If more than one ion arrives in the same "bin" then only one count is registered.

At high ion currents, some of the ion arrivals are not registered, this leads to a shift to lower mass centroids, and also lower measured areas on reported peaks. However, MassLynx incorporates Digital Dead Time Correction (DDTC™), which compensates for these effects and enables accurate mass measurement and quantitation to be achieved over a large range of ion currents.

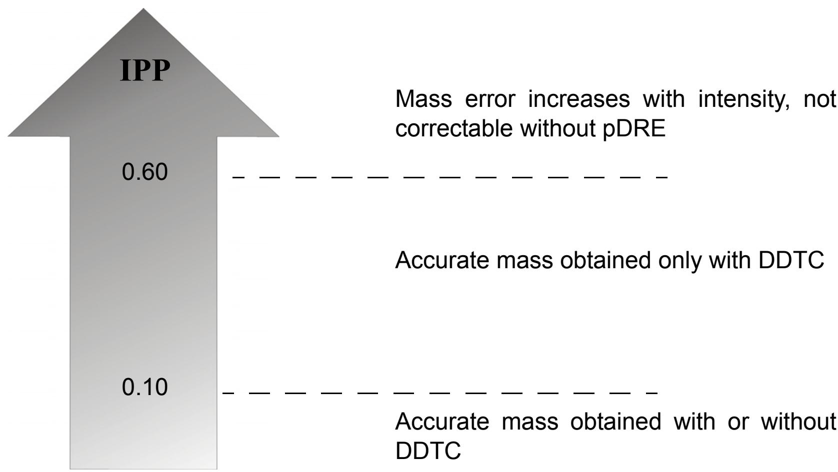
The effectiveness of DDTC does, however have its limits, and it is important to keep ion counts well below these limits when doing calibrations. The range

over which DDTC operates is defined in terms of the ions-per-push (IPP) of a peak. To find the intensity of a peak in ions-per-push, set the peak display to centroid mode. The IPP value displayed will be that corresponding to the largest peak on the display. To find the IPP value of a particular peak, zoom in on that peak until it is the largest in the displayed mass range.

The maximum peak intensity that can be corrected by DDTC corresponds to an IPP value of 0.85. Peaks with intensities in excess of this will not be corrected. Peaks with IPP values of less than 0.1 will not show any dead-time distortion effects, and the application of DDTC to such peaks will make little or no difference.

For optimum calibration accuracy, reduce peak intensities; avoiding IPP values much above 0.1.

The effect of IPP on mass accuracy:



Dynamic Range

Dynamic range should be set to normal during the acquisition of instrument calibrations. Signal intensities are always kept low while calibrating the instrument. Extended and normal dynamic range acquisitions use the same calibrations.

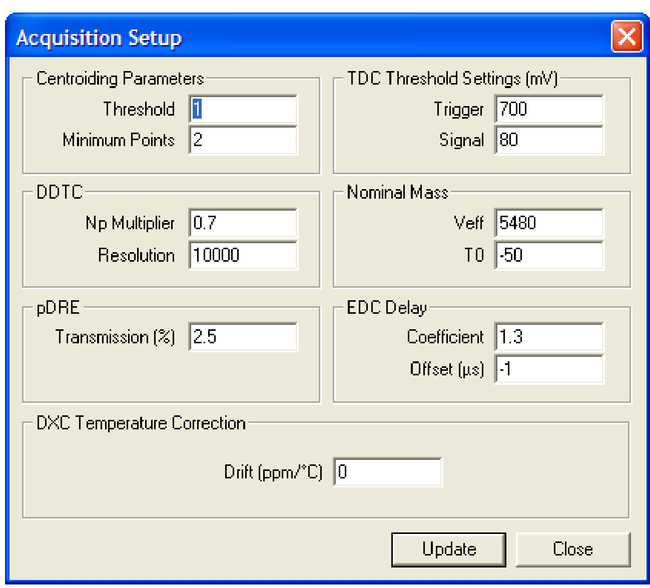
Acquisition Setup

Many of the parameters that are vital to good mass measurement are entered via the Acquisition Setup dialog box.

To open the Acquisition Setup dialog box:

From the Tune window select Setup > Acquisition Settings.

Acquisition Setup dialog box:



Parameter	Description
Centroiding Parameters (only used when collecting centroid data):	
Threshold	The intensity threshold for peak detection. Usually set to 1.
Minimum Points	Sets the minimum peak width for peak detection. Usually set within the range 2 to 6. Increase if peak splitting is observed.
DDTC:	
Np Multiplier	A value of 0.7 gives optimum mass measurement on intense peaks.
Resolution	For optimum dead time correction, set to the actual resolution of the instrument. A precision ± 500 is adequate.

Parameter	Description
pDRE:	
Transmission (%)	Sets the percentage of ions transmitted by the DRE lens while active.
DXC Temperature Compensation:	
Drift (ppm/^{o}C)	Sets the size of correction to be applied to mass measurements to compensate for the effect of temperature drift.
TDC Threshold Settings	
Trigger	Sets the trigger pulse level that will trigger the TDC. Leave at the default value of 700 mV, or that set at instrument installation, if different.
Signal	Sets the signal level that will cause the TDC to record an ion arrival event. Leave at the level set at installation, usually about 50 to 100 mV.
Nominal Mass Measurement:	
Veff	Used to set up the instrument nominal mass scale. Default values are 5840 in V-mode and 5870 in W-mode.
T0	An offset time used to calculate nominal mass. Usually left at the default value of - 50 ns.
EDC Delay:	
Coefficient	Used to calculate the correct pusher timing when in EDC mode. Usually left at default value of 1.3.
Offset	Used to calculate the correct pusher timing when in EDC mode. Usually left at default value of - 1.

Before calibrating

Before attempting to calibrate the TOF there are several tasks that have to be completed first. These are described in the following section.

Nominal Mass setup

Nominal mass accuracy can be achieved by setting up the Veff and T0 values in the Acquisition Settings dialog box. Once set, these values will not normally need to be changed. The default value for Veff is 5480 in V mode and 5870 in W mode. The default values for T0 is -50. This T0 value should not be changed. The Veff values will need to be determined for each of the four modes of operation: V+, V-, W+ and W-.

To set the Nominal Mass:

1. From the Tune window click Calibration > Calibrate TOF.
2. In the Calibration window click Calibrate> Remove Calibration.
3. Infuse leucine enkephalin (or any suitable reference compound).
4. Click to open the Start Acquisition dialog box and enter the acquisition parameters. Click Start.
5. Zoom into the leucine enkephalin molecular ion at around 556.3 Da on the peak display.
6. Click .
7. In the Acquisition Settings dialog box enter a new value of Veff and click Update.
8. Observe the peak movement and then enter another Veff value.
9. Repeat until the peak is at the correct mass (± 0.1 Da accuracy is acceptable).

Tips:

- Increasing Veff will move the peak to the right, i.e. to higher mass. Change in steps of 10 or less.
- Using a reference mass of greater than 500 Da will improve the accuracy of this determination.

- A Veff value only has to be determined once. It will not change over time.
- Changing Veff will invalidate any existing calibration files.
- Setting an accurate Veff will not improve the calibrated mass accuracy of the instrument, but will ensure the correct assignment of calibration compound peaks, allowing easier calibrating.

Setting the Detector Voltage

You should do a detector (MCP) gain test every month to check whether you are getting maximum sensitivity.

This ensures that the correct voltage is applied to the micro-channel plate (MCP) optimizing ion detection and minimizing potential electronic artefacts. You will increase the MCP voltage until a plateau is reached i.e. the ion count stops increasing. It is important for accurate mass that all ions are counted.

Prerequisite: You will need to have a suitable reference compound, in this example leucine enkephalin at 50 pg/µL is used.

To set the correct Detector voltage:

Tip: Ensure peak intensity remains below 0.1 ions per push. If this value is exceeded, adjust capillary voltage and repeat the detector setup.

1. Make a 10 minute acquisition from the Tune page with a scan time of 5 s.

   Tip: This is so you will have enough time to ramp the MCP voltage during the acquisition without running out of data.

2. Click the Instrument page and enter 1700 into the Detector text box.
3. From the main MassLynx window open Chromatogram and click real time update.
4. Right-click and drag across the acquisition to combine a minutes worth of data.

   Result: Spectrum opens with the combined data displayed.

5. In the Spectrum window zoom in on the main peak and right-click across this peak.

   Result: This gives an extracted mass chromatogram of the selected mass.

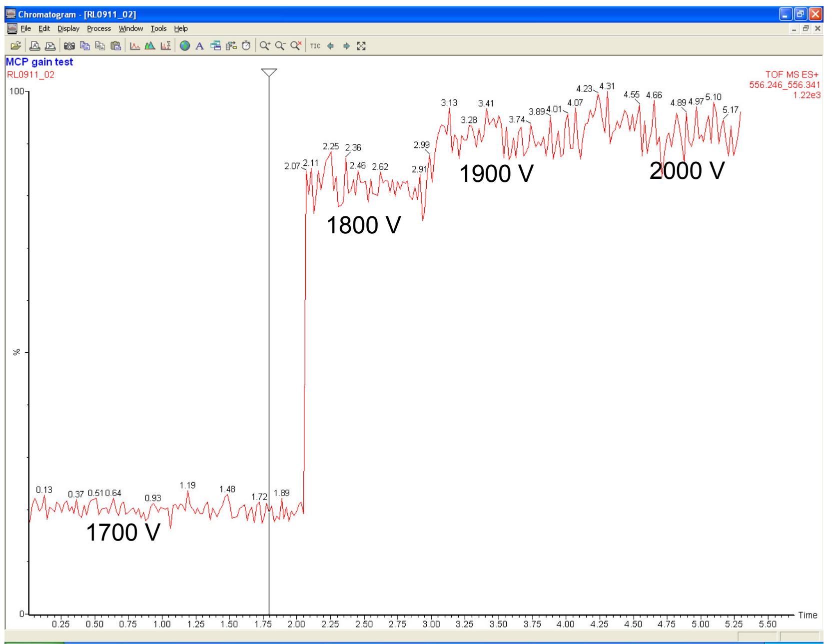
6. Increase the MCP voltage in 100-V steps, waiting one minute after each increment.

   Result: The chromatogram should plateau at, or before 2100V. The last reading before reaching this plateau provides the optimum detector voltage. In the Figure titled "MCP Gain chromatogram:" on page 3-9 you can see how the sensitivity increases sharply from 1700 to 1800 V and then begins to plateau. In this case 1800 V is the optimum detector voltage.

7. When the plateau is reached, stop the acquisition.

   Tip: The detector voltage should be set so that at least 90% of ions are detected.

MCP Gain chromatogram:



Accurate mass basics

- The Q-TOF Premier must be calibrated before accurate mass determination is possible.
- Separate calibrations are required for each instrument mode: V+, V-, W+ and W-.
- All four of these calibrations are stored in the same calibration file.
- Instrument calibration is normally done in ESI source mode; the calibration produced is valid across all source configurations.
- Accurate masses cannot be obtained outside the range of masses covered by the calibration.
- The same TOF mass range must be used for the calibration as for the subsequent mass determinations.
- Calibrations for maximum sensitivity (EDC) acquisitions must be acquired using a TOF mass range of 4000 Da.
- A LockMass is required to compensate for mass measurement drift, caused mainly by ambient temperature fluctuations.
- LockMasses can either be internal (in the analyte solution) or external (LockSpray).
- Accurate masses can only be obtained from centroid data; continuum data must be first converted to centroid.

Calibrating for accurate mass

The purpose of the following is to show you how to calibrate the instrument using sodium formate over a mass range of 100 - 1000 Da.

Prerequisites:

- The instrument is tuned to give adequate resolution
- Nominal mass is correct
- The detector voltage is set correctly
- The instrument has been in operate for at least one hour

Calibration overview:

Acquire data

1. Infuse reference compound.
2. Adjust capillary voltage to give less than 0.1 ions per push.
3. Acquire continuum data.
4. Combine and center data.
5. Save spectrum.

↓

Create calibration

1. Select reference file in calibration window.
2. Create calibration from saved spectrum.
3. Set calibration parameters and accept calibration.
4. Save calibration.

Reference files

Reference files are tab delimited files which you can edit. They are stored in the Ref folder of the main MassLynx folder. Reference files are text files containing a list of numbers in mass/intensity pairs (average or monoisotopic

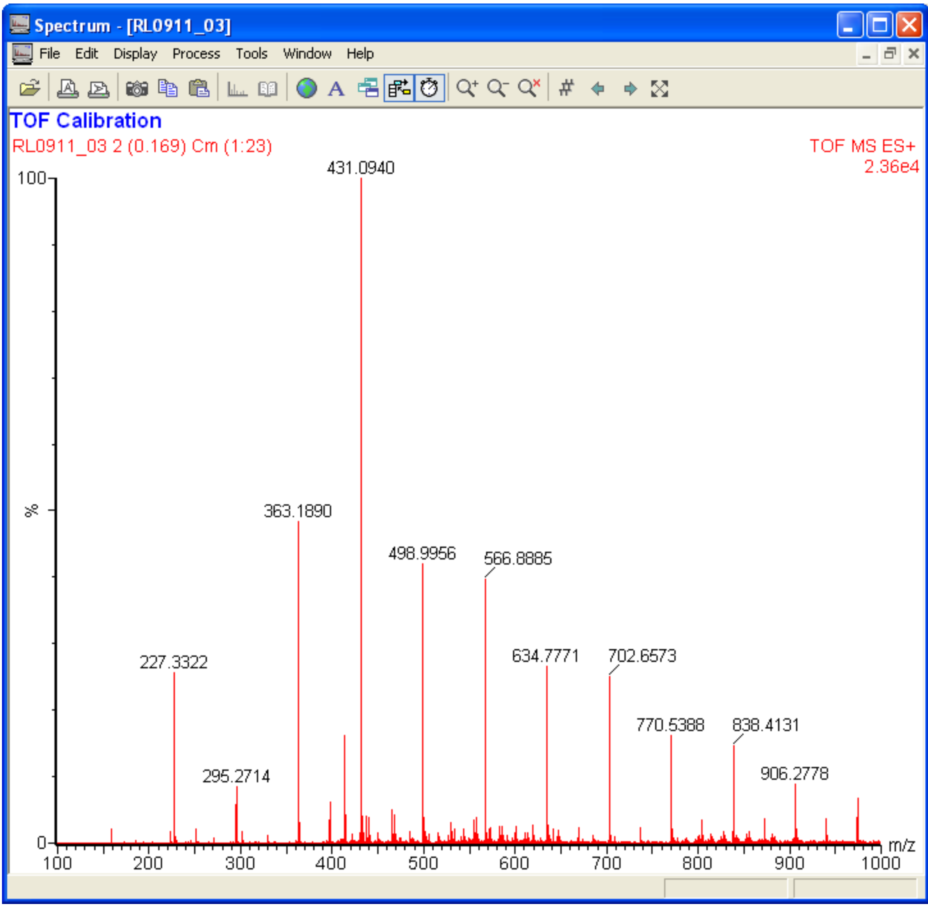
masses and relative intensities). The two columns of numbers are interpreted by MassLynx as a line graph for calibration and processing.

Acquiring data

To acquire data for calibration:

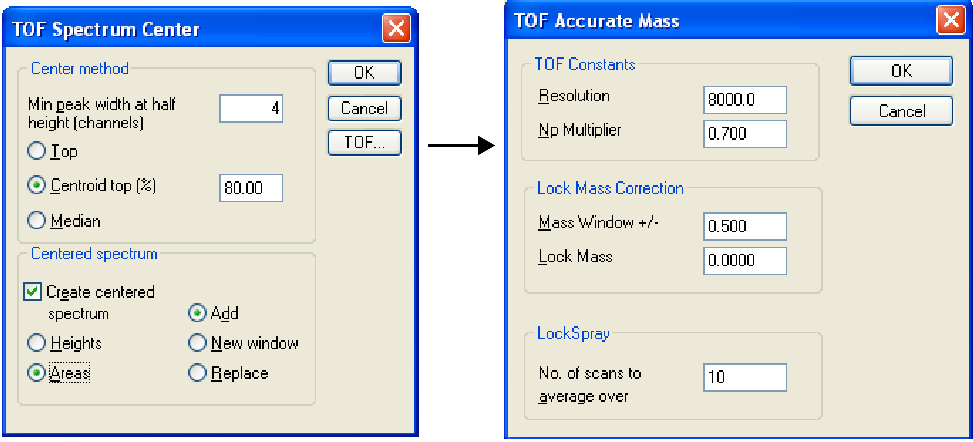
1. From the Tune window click Calibration > Calibrate Tof.
2. From the calibration window select Calibrate > Remove Calibration.
3. Infuse a solution of sodium formate (see Calibration solution on page 3-21 for preparation details).
4. Adjust the capillary voltage to give a maximum peak intensity of 0.1 ions per push (switch peak display to centroid to check this)
5. Acquire 2 minutes of MS continuum data from 60 to 1000 Da, 1 s scan, 0.02 s interscan.
6. From the main MassLynx window, open Chromatogram and click .
7. When the acquisition has finished, right-click and drag across the whole acquisition to combine the data.

Spectrum of sodium formate:



8. From the Spectrum window click Process > Center

9. From the Tof Spectrum dialog box, enter the values as shown in the following figure and click TOF. In the TOF accurate Mass dialog box enter the values as shown. The resolution should be set to the actual instrument resolution (±500). Make sure that Lock Mass is set to zero.



10. Click OK, OK to create a centroid spectrum.
11. Click File > Save to save the spectrum.

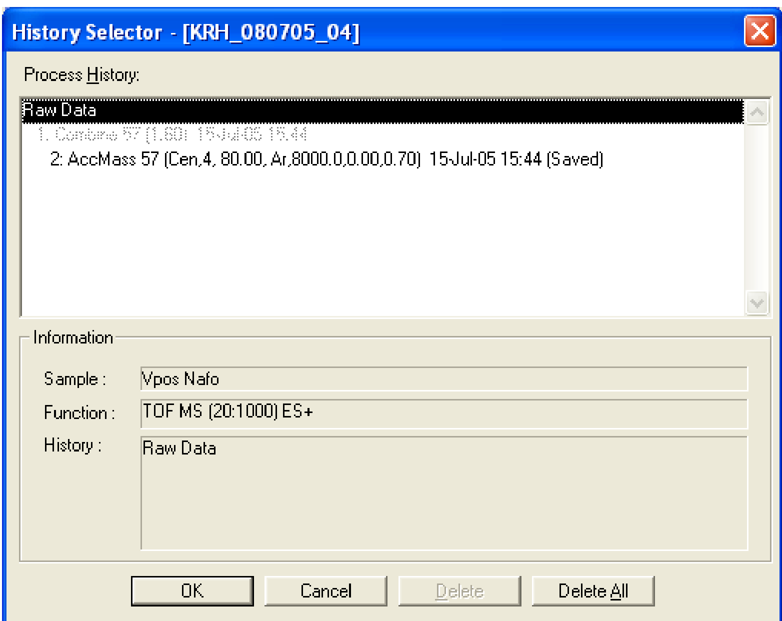
Making the calibration

To make a calibration:

1. From the Tune window click Calibrate > Calibrate Tof.
2. From the Calibration window select NaFormatePosES.ref from the reference file drop down list.
3. Click Calibrate > Create Calibration.
4. From the "Select File for Calibration dialog box" navigate to the raw data file you saved in Acquiring data on page 3-12.
5. Click History, and from the History Selector dialog box select the saved Accurate Mass centered spectrum.

   Tip: The saved spectrum will be listed in a line starting 'AccMass'.

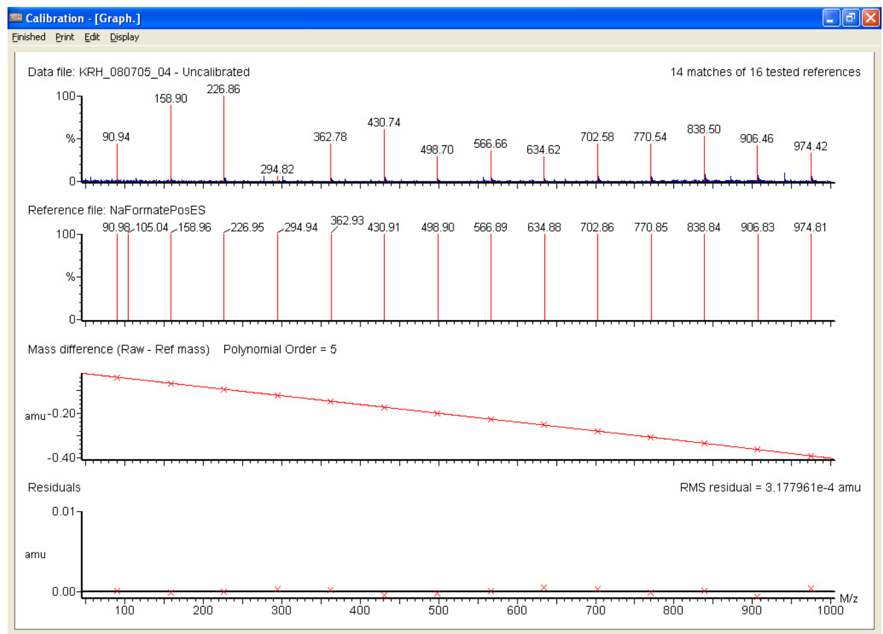
History Selector dialog box:



6. Click OK, OK.

   Result: MassLynx performs the calibration calculations. When the calibration is complete the data appears in a new Calibration window.

Calibration window showing residual errors:



7. Click Edit > Calibration Parameters and set the parameters as shown and click OK.

Hints:

- As a general rule, the residual (in mDa) on each individual calibration point should be less than 1.5 mDa. Ideally the majority of calibration points will have residuals of less than 0.5 mDa. A measure of the 'fit' of the calibration line to the experimental data is given in the error of the residual.
- The RMS residual should be less than $1x10^{-3}$ amu
- To check for outliers set the Polynomial order to 1. Outliers can be removed from the calibration curve by right-clicking the outlying peak in the reference file spectrum and right-clicking the associated peak on the data file spectrum. After removing outliers return the Polynomial order to 5.
- Click display > Switch Residuals Graph Axis to ppm for an alternative view of the residuals. The RMS residuals should be less than 1 ppm.

Caution: Always save the calibration with a new name. Do not overwrite Uncal.cal.

8. Click Finished > Accept Calibration when you are satisfied with the results. If the data is not satisfactory, slick Finished > Reject Calibration.

9. Click File > Save As, to save the calibration

   Tip: It is good practice when saving a calibration to use the date as the filename.

10. Repeat the calibration, as desired, for all other modes.

    Note: Calibrations for all four MS modes, V +, V -, W +, and W - can be stored within one calibration file.

See also: Editing the Calibration graph on page 3-17.

Editing the Calibration graph

The Calibration graph has four sections:

- Data file
- Reference file
- Mass difference
- Residuals

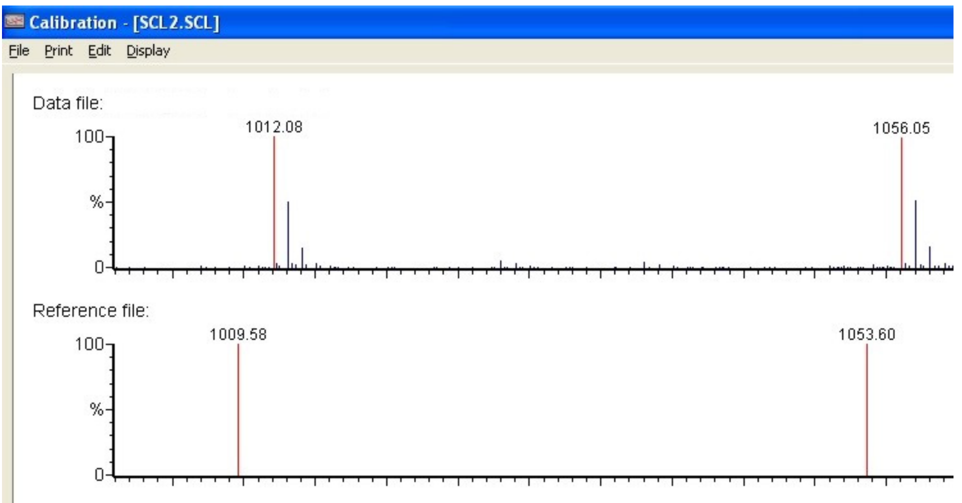
Data File and Reference File

Matched peaks are highlighted red, with the mass displayed above, other peaks are displayed in blue.

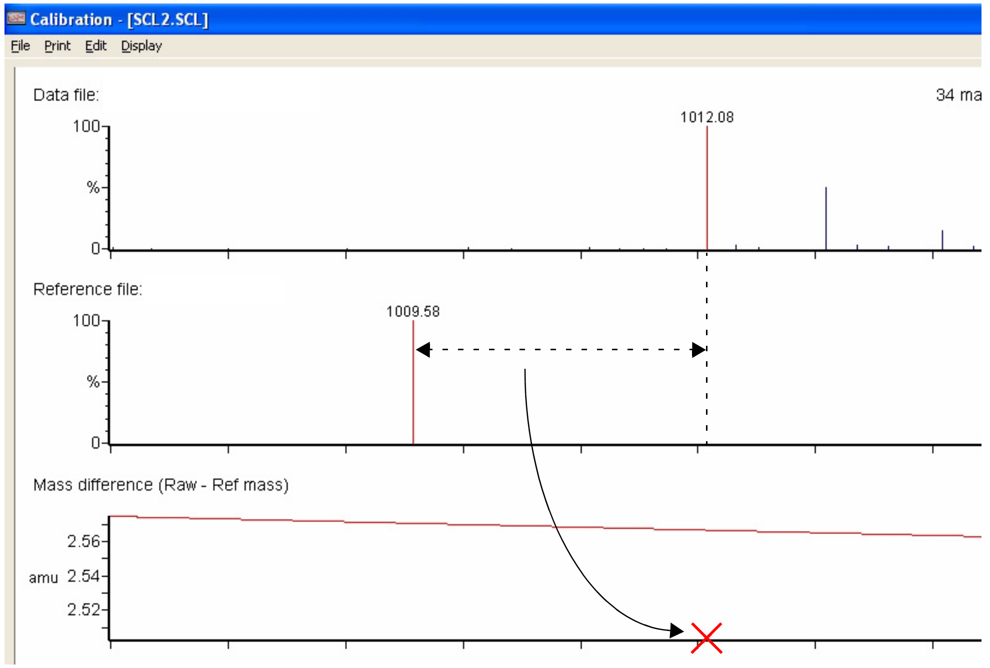
To zoom in on peaks:

1. Click and drag horizontally between the first two peaks to see a detailed view.

Calibration graph detail view:

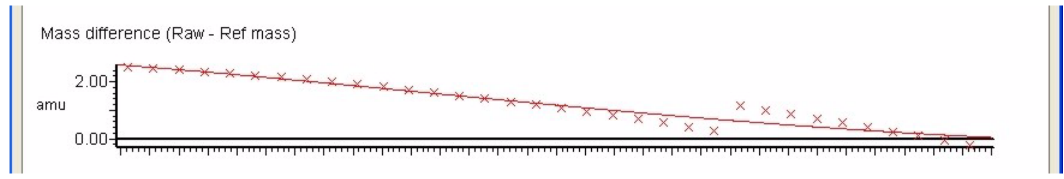


2. Zoom in closer to see the horizontal mass difference plotted on a vertical mass scale.



The following figure shows that this has been done along the mass scale.

Line of best fit:



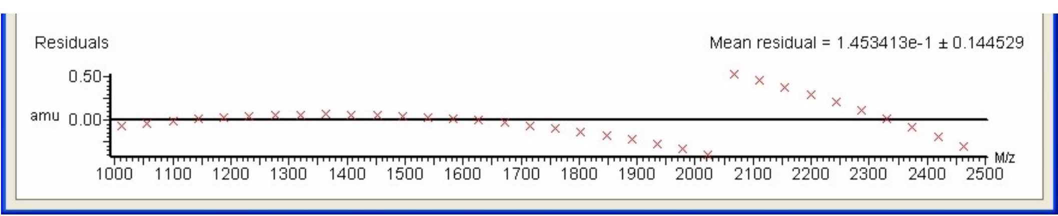
MassLynx has also drawn a 'line of best fit' through the mass difference points, but not necessarily touching any of them.

This line is the calibration, the amount by which future mass data is corrected if the calibration is accepted.

Residuals

The residuals plot (below) shows you how the reference masses appear if the acquisition is done with the current calibration in place.

Calibration residuals:

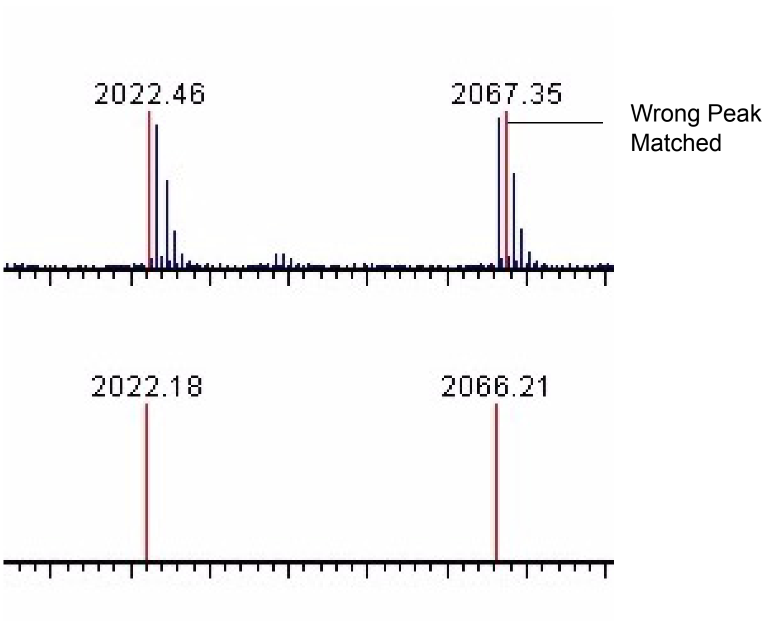


A perfect calibration puts all masses along the baseline, but any calibration is a compromise, and the mean residual mass value is displayed at the right.

Minor deviations are normal, but in this example the obvious discontinuity between mass 2000 and 2100 suggests this calibration is not satisfactory.

The following figure shows why this has happened:

Incorrect peak matching:



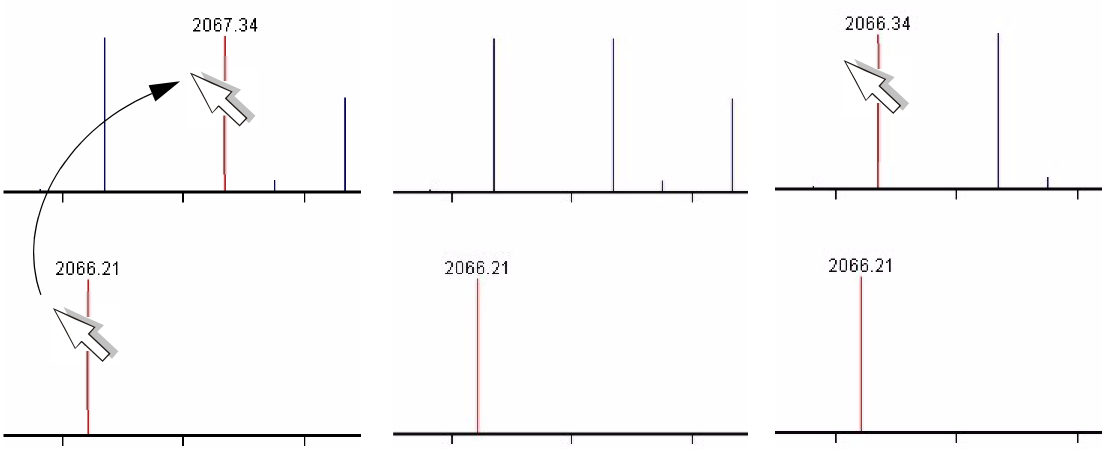
At reference mass 2022.18 MassLynx has matched the data peak 2022.46, and this is also the monoisotopic peak. However, reference mass 2066.21 has been matched with 2067.35. This is the highest peak but not the monoisotopic peak - hence the discontinuity seen in the Figure titled “Calibration residuals:” on page 3-19.

To produce an acceptable calibration all the peaks to the right of mass 2022.46 must be reassigned to the monoisotopic peaks.

Reassigning Peaks

The following figure shows how you reassign reference peaks to the correct, monoisotopic peaks.

Reassigning peaks:



To reassign peaks:

1. Right-click to the left of the reference peak, then right-click to the left of the wrongly assigned data peak.

   The peak changes from red to blue, and the mass value disappears.

2. Right-click to the left of the appropriate monoisotopic peak.

   The peak is highlighted red and the mass is displayed.

   The mass differences are now more evenly placed around the calibration curve.

Calibration solution

The sodium formate calibration solution produces cluster ions (i.e., multimers) of sodium and formic acid in electrospray mode. When compared to other similar calibration solutions, such as sodium iodide and caesium iodide, this calibration solution has a more even distribution of ion intensity and a smaller difference in mass between ions.

The distribution of higher mass cluster ions is governed mainly by the concentration of formic acid in the solution and dilution may result in the disappearance of the higher mass ions.

The instrument calibration produced from the sodium formate calibration solution is superior to sodium iodide solution over shorter mass ranges (e.g. 100 to 1000 m/z or 100 to 600 m/z) because there are a greater number of calibration points and consequently there is less chance of an interpolation error when a high-order polynomial is fitted.

To prepare the sodium formate solution:

Warning: To avoid chemical burns take great care when handling formic acid. Always add to water not water to formic acid.

The target solution constituent concentration is 0.05 M sodium hydroxide + 0.5% formic acid in 90:10 2-propanol:water.

1. Take 1 mL of formic acid and add to 9 mL of water to produce a 10% formic acid aqueous solution. Label this as Formic Acid Solution.
2. Add 500 µL of 0.1 M sodium hydroxide and 500 µL of Formic Acid Solution to 9 mL of 90:10 2-propanol:water solution. Label this as Sodium Formate Solution 1.

4 Using the Sample List, Inlet Editor, and MS Method Editor

Contents:

Topic	Page
Acquiring Accurate Mass Data with LockSpray	4-2
Demonstrating pDRE	4-8
Demonstrating EDC	4-11
Setting Up an LC Inlet	4-13
MS Method editor	4-16
Function parameters	4-18
Method Editor basic functionality	4-27

Acquiring Accurate Mass Data with LockSpray

Temperature variations in the laboratory environment can cause drifts in mass measurements of a few hundred parts per million (ppm) over the course of a day. For accurate mass work, the instrument should be kept in OPERATE at all times to enable stabilization of the power supplies. The use of a lock mass enables mass drift, from whatever source, to be accurately corrected. The Q-TOF Premier's LockSpray source enables a suitable reference compound to be introduced into the source independently of the analyte solution.

To enable LockSpray:

From the Tune window Click Mode > LockSpray On.

Result: The Lock Spray frame is enabled on the Source page.

To add a user specified lock mass:

1. From the Tune window Click Setup > Edit Lockmasses.
2. Click New to add the name and mass of the lock mass.

To use LockSpray with a Sample List acquisition:

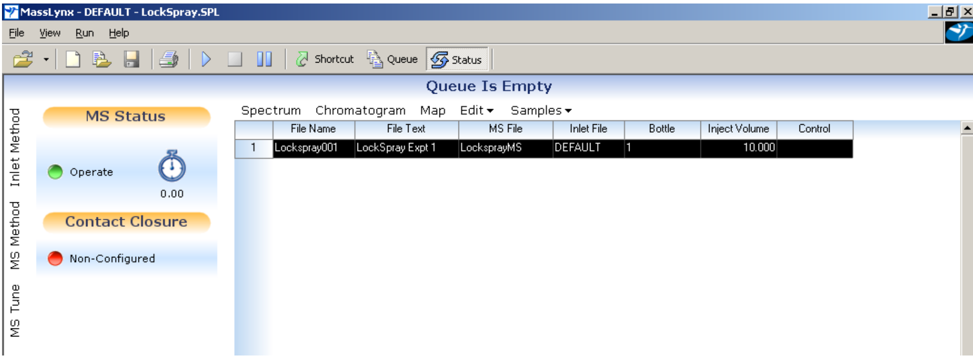
Prerequisites: You will require two syringe pumps to perform this procedure. Ensure that ESI positive, V or W mode and LockSpray are enabled.

1. Using the same Tune window settings as those in Chapter 2:
   - Infuse a solution of 50 pg/µL of leucine enkephalin at 10 µL/min into the reference sprayer. View the ion beam by selecting Reference from the Tune window.
   - Infuse a solution of 50 pg/µL of raffinose at 10 µL/min into the analyte sprayer. View the ion beam by selecting Analyte from the Tune window.

   Tip: Raffinose forms a sodium adduct in positive mode at m/z 527.

2. In the Sample List in the main MassLynx window, enter values in the File Name and File Text columns as shown below.

Sample List:



3. Right-click in the MS File column and select Edit from the pop-up menu to open the MS Method Editor.

4. Click MS Scan to open the TOF MS Scan Function editor. On the Acquisition Page enter a 10 minute acquisition in ES positive W or V mode. Leave the Sensitivity page at Normal. On the Tof MS page and LockSpray page enter the following values:

Tof MS Acquisition Parameters:

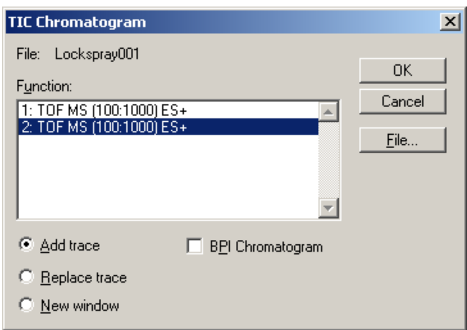
Parameter	Value
Tof MS page	
Start (m/z)	100
End (m/z)	1000
Scan Time (s)	1
Inter Scan Delay (s)	0.02
Data Format	Centroid
Cone Voltage	35
LockMass page	
Scan Time (s)	0.5
Interval (s)	10
Sampling Cone	35
Collision Energy	5 V

Tof MS Acquisition Parameters: (Continued)

Parameter	Value
Lock Mass	Leucine Enkephalin [M+H]+
Mass Window	0.5 Da
Scans to average	10

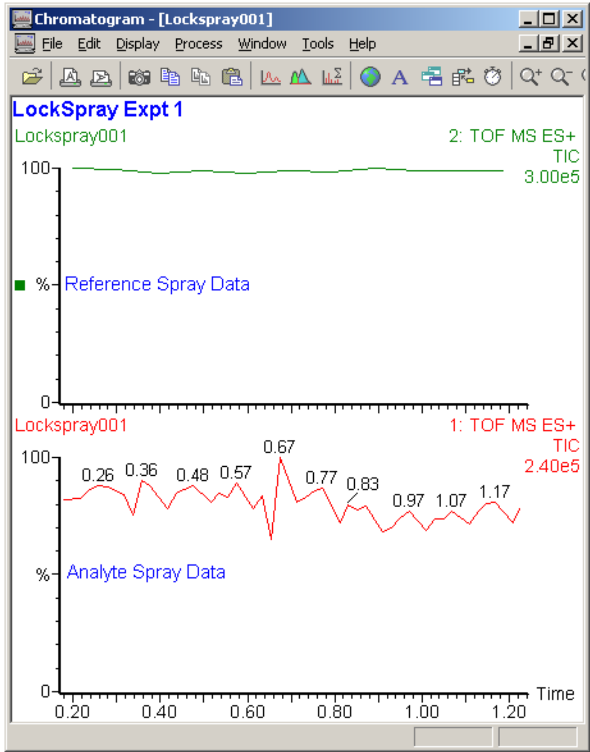
5. From the MS Method Editor select File > Save As. Choose a suitable name, e.g., LockSprayMS.
6. Select File > Exit to return to the Sample List.
7. Right-click in MS File column, select Browse from the pop-up menu and select the method file (*.exp) you just saved
8. Click to start the acquisition. This will open the Start Sample List Run dialog box. Leave the settings as they are and click OK.
9. Check that both the reference and analyte samples are acquiring data:
   – Select Chromatogram from the Sample List.
   – Select Display > TIC to open the TIC Chromatogram dialog box.

TIC Chromatogram dialog box:



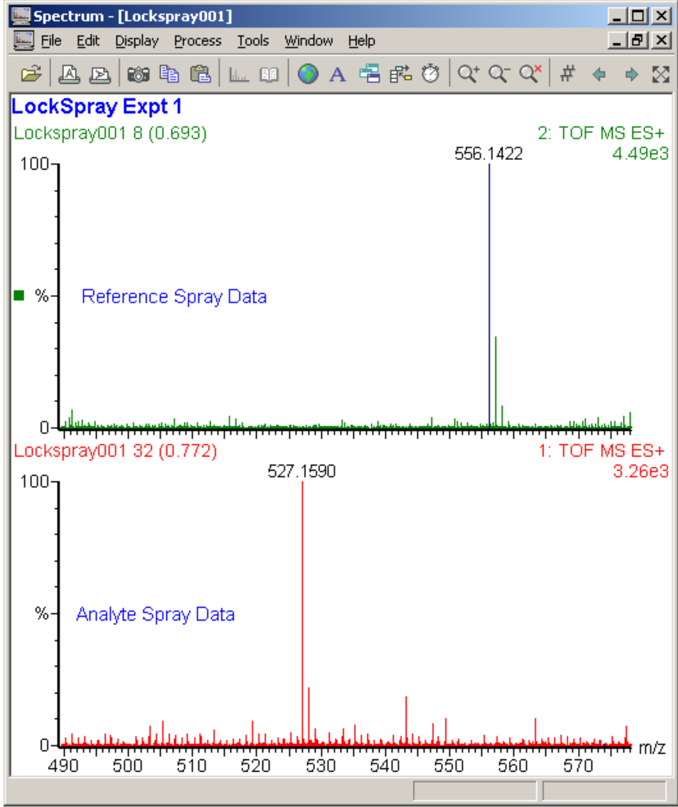
10. Select both traces to display and select for Real-Time Update.

Reference and Analyte Spray Data in the Chromatogram Window (annotated to distinguish between each trace):



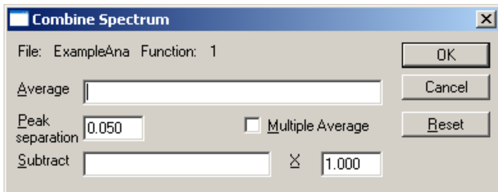
11. Double-click in a region of each chromatogram to open up a spectrum for each spray in the Spectrum window.

Analyte and Reference Spray Data in the Spectrum Window annotated to distinguish between each trace):



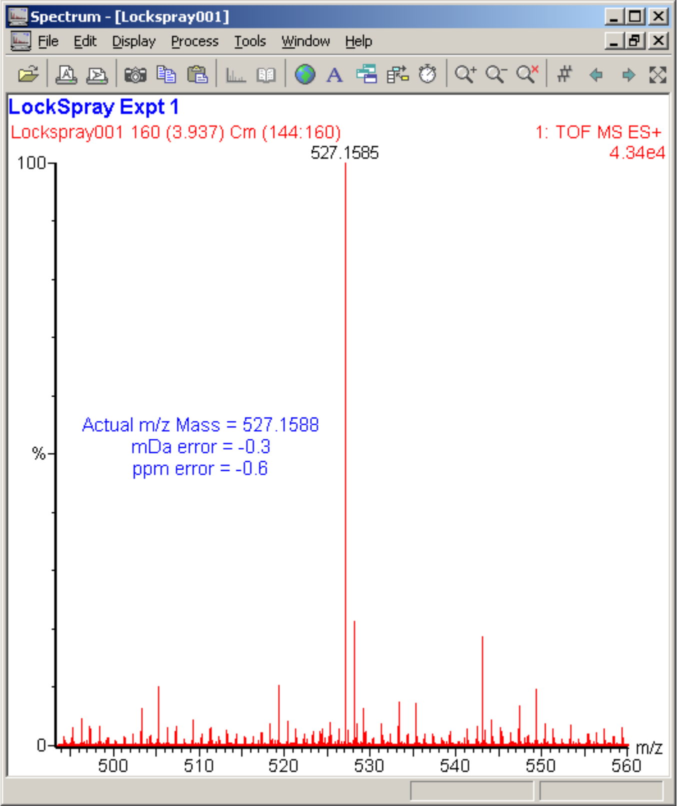
To Calculate the Mass Accuracy

1. Return to the Chromatogram window and Select Process > Combine Spectra to open the Combine Spectrum dialog box.



2. Right-click and drag across at least 30 seconds of data. The scan numbers will appear in the Average text box.

3. Click OK to open the combined spectra in the Spectrum window.



4. Use the following equation below to calculate the ppm error.

$$ppmError = \frac{m_{ind} - m_{act}}{m_{act}} \times 10^6$$

Where:

m_{ind} is the indicated mass

m_{act} is the actual mass

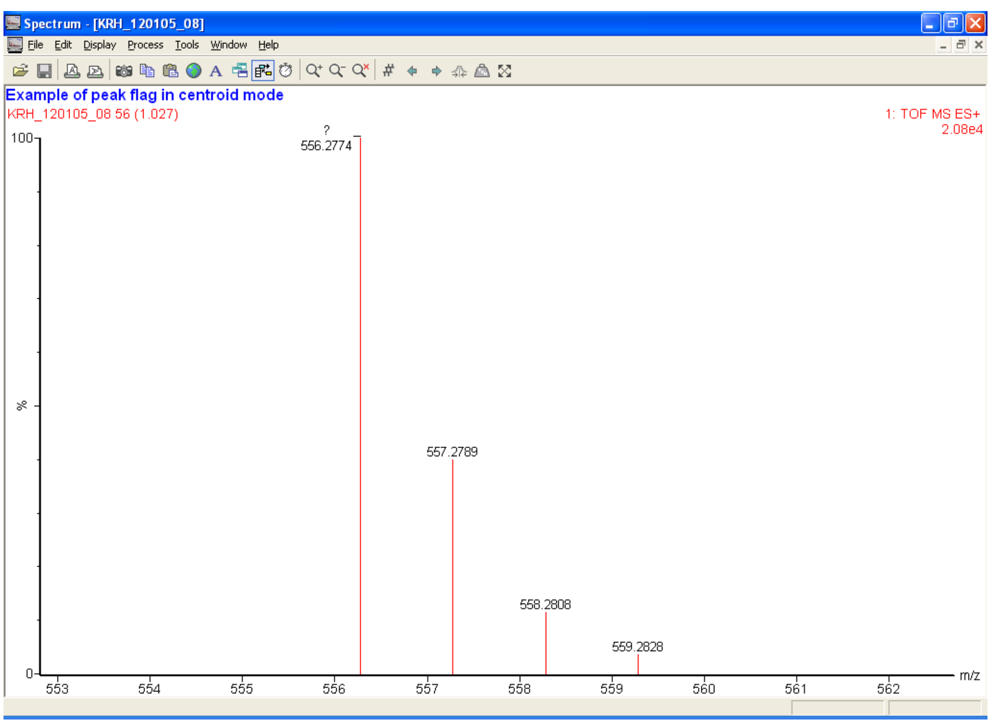
See Also: Combine in MassLynx help.

Demonstrating pDRE

The Q-Tof Premier has the ability to detect ions over four orders of magnitude when extended dynamic range is selected. The following procedure shows a method for demonstrating this.

Rationale: Without extended dynamic range switched on the maximum intensity of ions that the Q-Tof Premier will detect accurately is limited by the deadtime correction model. This varies according to the mode and mass range. Centroid peaks that fall outside the model are flagged with “?”. The figure below shows leucine enkephalin with its main peak flagged since its intensity is >20,000 cps.

Example of peak flag in centroid mode:



To demonstrate four orders of magnitude of spectral intensity:

1. Infuse a solution of 20 ng/µL leucine enkephalin at 5 µL/min.

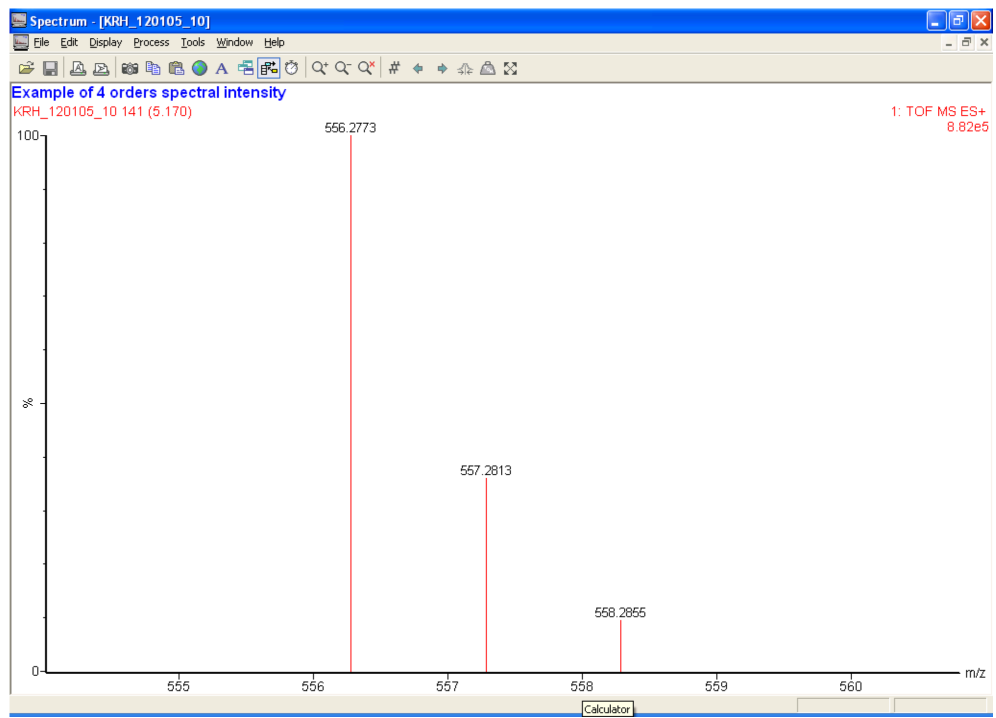
2. Tune for best sensitivity, using the relevant parameters provided in Routine optimization for sensitivity - Tof MS on page 2-8. If necessary, move the probe closer to the cone to increase sensitivity.
3. Detune the Capillary and Cone voltages to give 80 cps
4. Acquire data with the following parameters with no lock mass and select Extended Dynamic Range.

Tof MS acquisition parameters for DRE

Parameter	Value
Start (m/z)	100
End (m/z)	1000
Scan Time (s)	1
Inter Scan Delay (s)	0.02
Data Format	Centroid

5. From Chromatogram, enable real-time update and combine several scans.
6. Gradually step up the voltage combining several scans for each voltage setting. You will be able to show more than 800,000 cps before the main leucine enkephalin peak is flagged.

Leucine enkephalin peak with and ion intensity > 800,000:



Demonstrating EDC

Positive Ion Enhanced Duty Cycle (EDC) Sensitivity (V Mode)

The sample is 10 pg/µL leucine enkephalin in 50:50 ACN:water + 0.1% formic acid, infused at a flow rate of 5 µL/min.

With the mass range set to a maximum at 556 Da, the resulting singly-charged peak should display an intensity greater than 2400 cps. The parallel experiment with no EDC selected will be significantly lower.

To demonstrate EDC:

1. From the MassLynx window, click MS Method > MS Scan. Create a MS Scan Method with a total acquisition time of 10 minutes in V positive mode and normal dynamic range and the following Tof MS parameters

   Tof MS acquisition parameters for EDC:

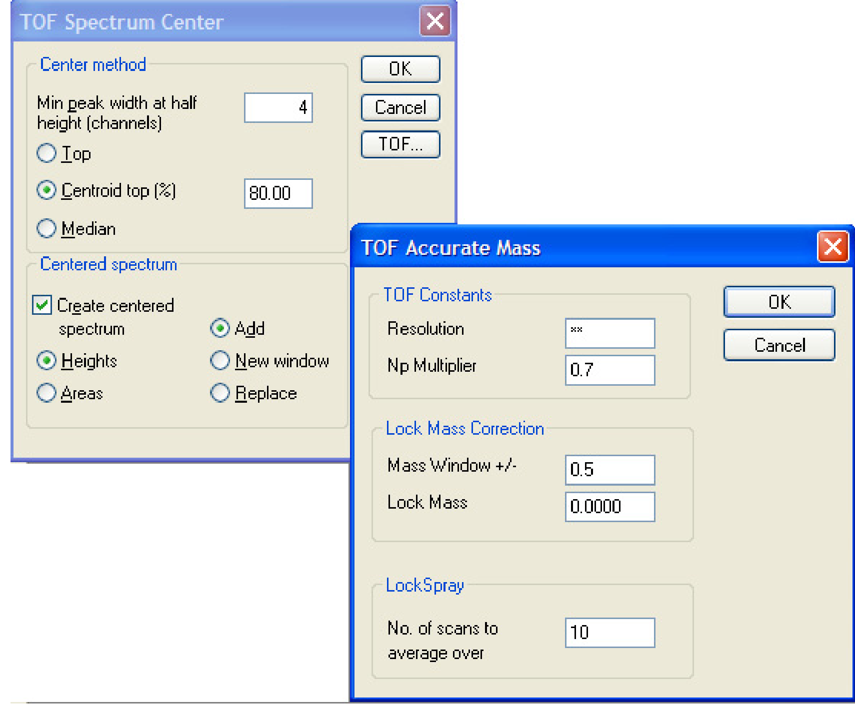
Parameter	Value
Start (m/z)	100
End (m/z)	1000
Scan Time (s)	1
Inter Scan Delay (s)	0.02
Data Format	Continuum

2. On the Sensitivity page select Maximum and type 556 into the Mass 1 maximum sensitivity box.
3. Save the experiment as edc.exp.
4. Create another with identical settings but with minimum selected on the Sensitivity page. Save this experiment as no_edc.exp.
5. From the MassLynx window, set up a sample list as shown below.

	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume
1	EDC	EDC Sensitivitiy	edc	none	1	10.000
2	No_EDC	No EDC Sensitivitiy	no_edc	none	1	10.000

6. Infuse 10 pg/µL leucine enkephalin at a flow rate of 5 µL/min.

7. Tune for best sensitivity, using the relevant parameters provided in Routine optimization for sensitivity - Tof MS on page 2-8. If necessary, move the probe closer to the cone to increase sensitivity.
8. Acquire the data files set up in the sample list.
9. For both acquisitions, combine 10 consecutive scans, calculate the resolution at 556 m/z (resolution must be greater than 8000) and center the data using the following parameters.



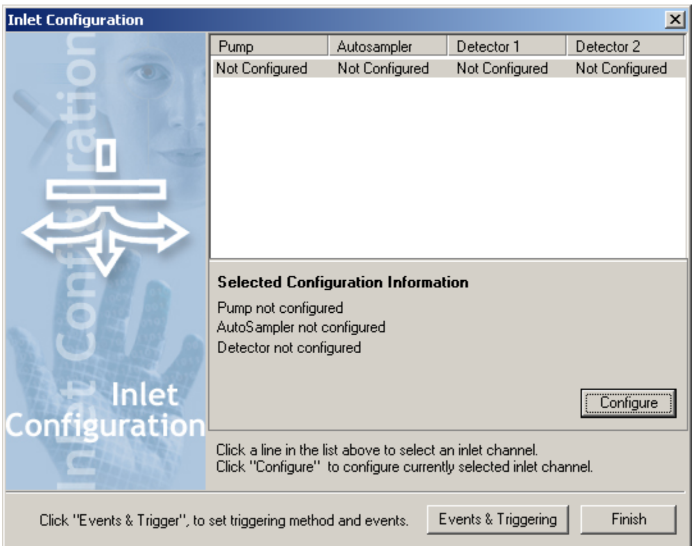
10. On the TOF Accurate Mass dialog box, in the Resolution (**) field, type the resolution value calculated from the spectra in the above step.
11. Compare the intensities of the two 556 peaks from each acquisition. The intensity of the 556 m/z peak with EDC will be significantly greater than the 556 m/z peak without EDC.

Setting Up an LC Inlet

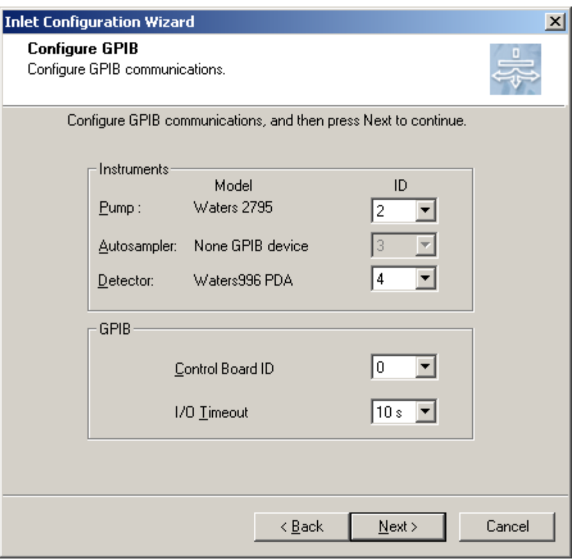
Rationale: The Q-Tof Premier will predominantly be used with an LC system, this is set up using the Inlet Configuration Wizard (see MassLynx Help for further details).

To set the instrument configuration:

1. From the MassLynx Status Shortcut bar select Inlet Method from the to open the Inlet Method Editor.
2. Click Tools > Instrument Configuration to open the Inlet Configuration dialog box.



3. Click Configure to open the Inlet Configuration Wizard.
4. Follow the on screen instructions and choose the configuration of pumps, samplers and detectors you require.
5. Configure the GPIB page (or, for Agilent instruments, HPIB page)



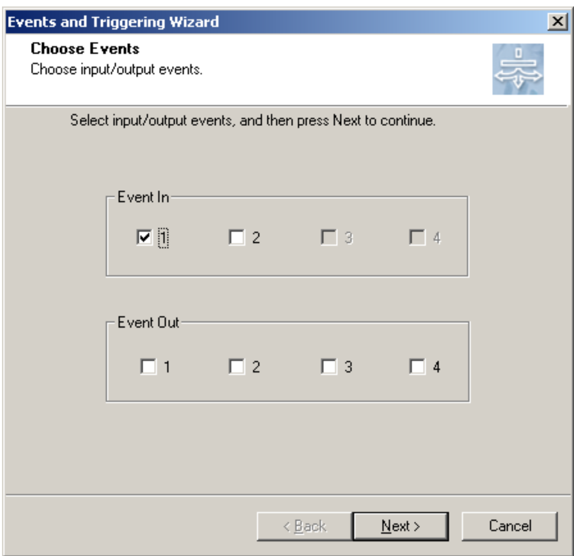
Rule: GPIB settings should not be changed if set by a Waters Field Service Engineer.

6. Close the Inlet Configuration wizard

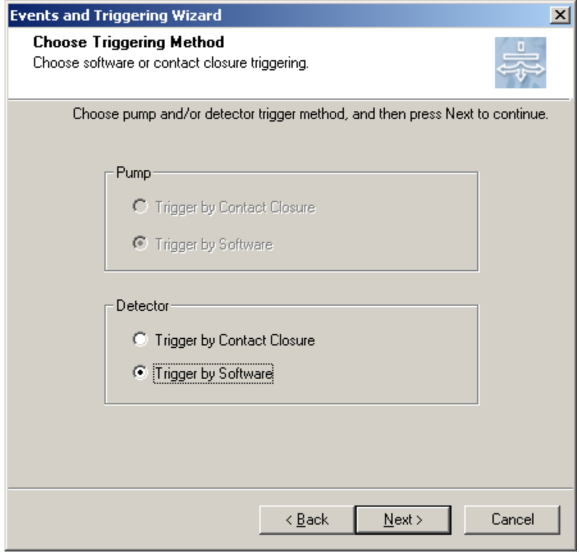
To set Events and Triggering:

Rationale: Use this wizard to configure input and output events and triggering methods for certain pumps and detectors.

1. From the Inlet Configuration dialog box click Events & Triggering to open the Events and Triggering Wizard.
2. Click Next to open the Choose Events page.
3. Click the Event In that corresponds to the HPLC cable connection on the back panel of the Q-Tof Premier (see Rear panel on page 1-17).
4. If required, select an Event Out.



5. Click Next to open the Choose Triggering Method page.



6. Click Trigger by Software and then click Next to open the Configuration Successful page.

   Tip: Triggering allows the definition of how the LC or detector run is triggered, whether by contact closure or software.

7. Click Finish to close the Events and Triggering Wizard.

8. Click Finish to close the Inlet Configuration window and return to the Inlet Method Editor.

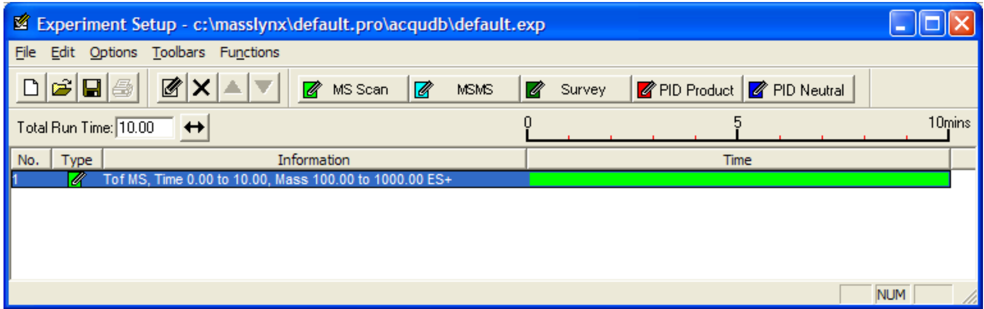
Tip: The inlet method can be chosen from the Sample List by right-clicking the Inlet File cell.

MS Method editor

The MS Method editor is the where you can set up particular types of experiment/functions that the mass spectrometer uses to scan the instrument during an acquisition. You can setup simple MS Scan and MS/MS Scan experiments (described in this chapter) and also Data directed analysis (DDA) experiments (described in Chapter 6).

A Function List is created, saved on disk, and then referenced by name in the MassLynx Sample List when an acquisition is started.

The figure below shows a simple Function List containing only one function: Tof MS scan, between 100 and 1000 amu using ES+ ionization. Immediately above the function bar display is a time scale that shows from when the function is active, and for how long it runs. In this case, the function starts after 0 minutes and then runs for 10 minutes.



The currently selected function is highlighted and enclosed in a rectangular frame. If the display shows more than one function, a different function can be selected either by clicking with the mouse or by using the keyboard arrow keys.

See also: Applying a Single-Point Lock Mass Correction on page 4-2.

Acquisition functions

There are six different acquisition function types:

Function Name	Description
MS Scan	Standard MS acquisition
MSMS	Standard MSMS acquisition
Survey	Automatic selection of precursors by MS/MS)
PID Product	Precursor Ion Discovery via product ion detection
PID Neutral	Precursor Ion Discovery via neutral loss detection
Expression	Protein Expression (an available option)

These functions are set up by entering parameters into a series of tabbed pages. Many of these pages are common to several functions.

To set up a function:

1. Click the MS Method icon in the MassLynx window to open the MS Method Editor.
2. In the MS Method Editor, click the appropriate function button or select Functions > MS Scan to open the relevant function.
3. Enter the required parameters.

   Note: Regardless of the experiment type the same basic parameters need to be set; such as the mass range, the total acquisition time, ionization mode etc.

See also:

- Acquisition page on page 4-19
- Function parameters on page 4-18
- Data-Directed Analysis on page 6-1

Types of Data Acquisition

In the Function editor, the Data format parameter specifies the type of data to be collected and stored on disk. There are two options:

- Continuum

  The TDC acquires up to 22,000 complete spectra per second. These spectra are summed in the embedded PC for a time period defined in the experiment function, the "Scan Time". The result is that one complete spectrum is saved to disk per scan. With continuum data peak shape is preserved and resolution can be calculated. Post-processing is required to determine exact mass information. A disadvantage is that file sizes can be very large.

- Centroid

  The raw spectra from the TDC are summed for the duration of the scan, and then the data is then converted to "stick" form. This preserves mass and intensity information, but peak shapes are lost. With the use of a lock mass, real-time exact mass is possible, with exact mass data being saved to disk. An advantage is that file sizes are much smaller than with continuum data.

Function parameters

Basic Function pages

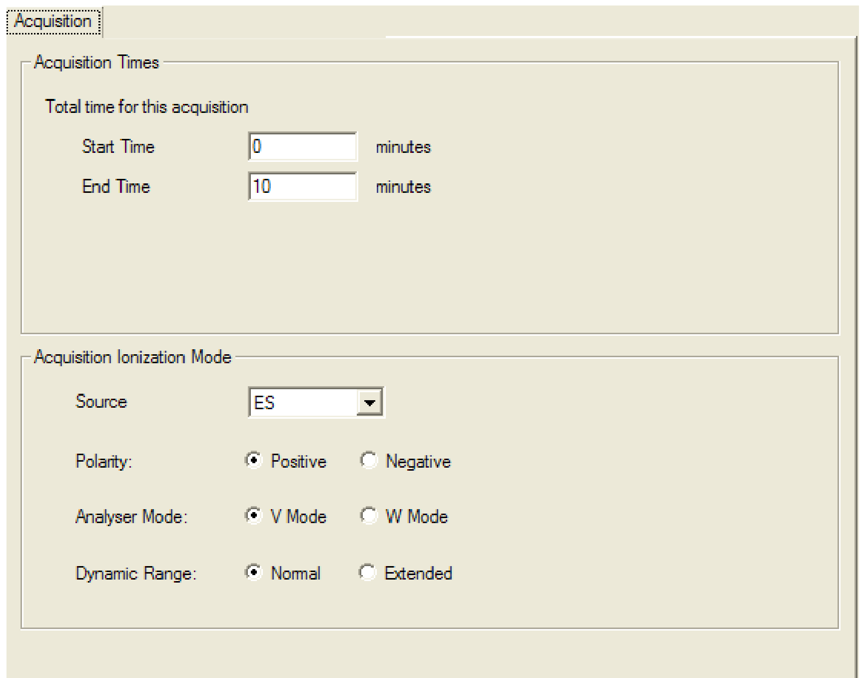
The MS Scan and MS/MS functions are set up via the following tabs:

Page	Use
Acquisition	Configures the initial acquisition parameters.
TOF MS	MS scan only.
TOF MS/MS	MS/MS only.
Collision Energy	Not usually used for MS (use default settings).
Sensitivity	Configures enhanced duty cycle.
LockMass	Configures LockSpray.

The Survey, PID Product, and PID Neutral functions are covered in Chapter 6. The Expression function is described in the *Expression System Guide*.

Acquisition page

The Acquisition page is used for setting the initial acquisition parameters for the experiment. It is common to all the acquisition functions.



Acquisition Times

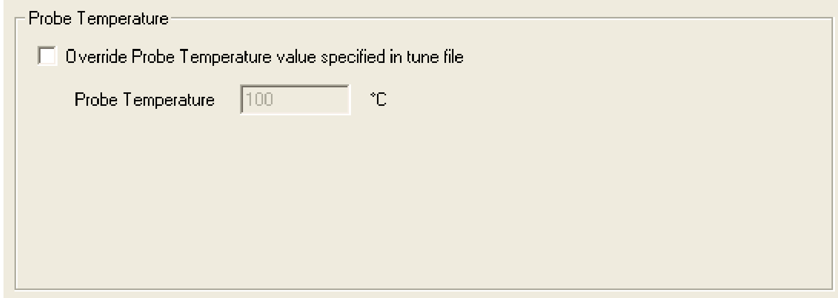
Enter the total time for the acquisition.

Acquisition Ionization Mode

Enter the source type, the type of acquisition, positive / negative, V Mode / W Mode and normal or extended Dynamic Range.

Tip: Dynamic Range is only an option for MS Scan, MS/MS Scan, and Survey Scan.

Probe Temperature



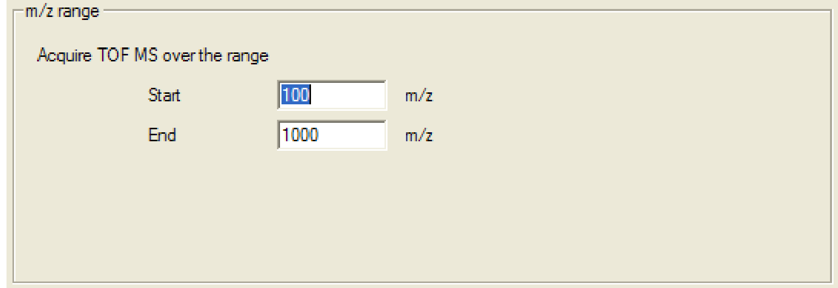
When API is chosen from the Source drop down list in the Acquisition Ionization Mode frame the Probe Temperature frame appears. You can use this frame to override the IonSabre probe temperature specified in the tune file.

Tof MS page

Using the Tof MS page, you can configure the following parameters:

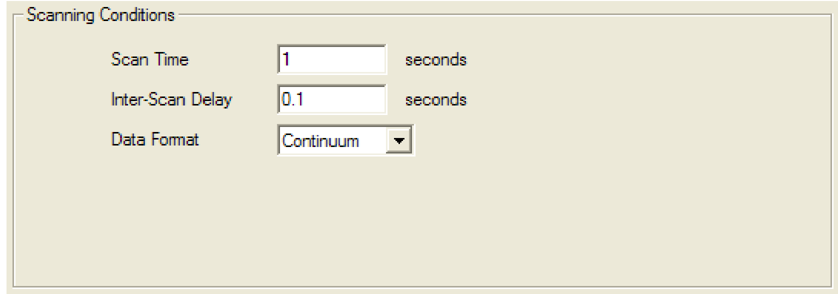
- The m/z range over which to acquire data.
- The conditions at which the instrument will scan.
- The instrument conditions

m/z range



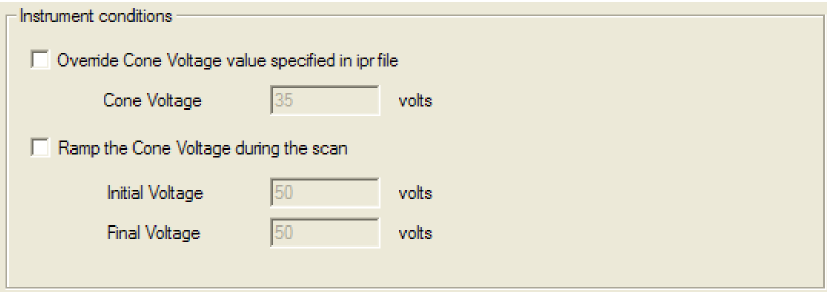
Parameter	Description
Start m/z	The start m/z value over which data is acquired.
End m/z	The end m/z value at which data acquisition stops.

Scanning Conditions



Parameter	Description
Scan Time	Specifies the duration of each scan in seconds.
Inter-Scan Delay	Specifies the time between a scan finishing and the next one starting, in seconds. No data are stored during this period. Recommended minimum inter-scan time: 0.02 s
Data Format	Specifies the type of data to be collected and stored on disk Centroid or Continuum. **See also:** Types of Data Acquisition 4-17

Instrument Conditions



Select “Override Cone Voltage Specified in ipr file” if you want to use a cone voltage other than that specified in the tune file.

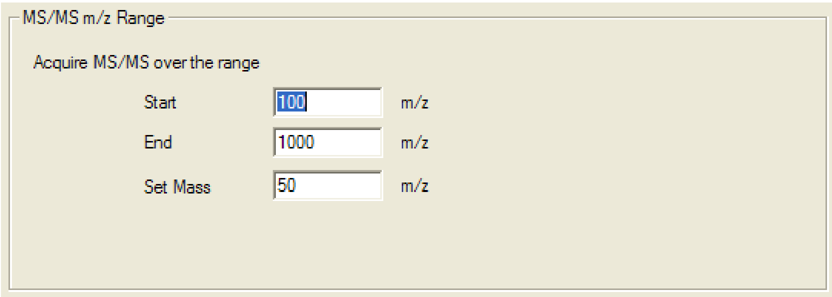
Select “Ramp the Cone Voltage during the scan” if you want to increase the Cone voltage during the scan. Enter the start and end values and MassLynx will linearly increase the voltage.

Tof MS/MS page

Using the Tof MS/MS page, you can configure the following parameters:

- The m/z range over which to acquire data (described below).
- The conditions at which the instrument will scan. These are identical to those described Scanning Conditions on page 4-21.
- The instrument conditions These are identical to those described Instrument Conditions on page 4-22.

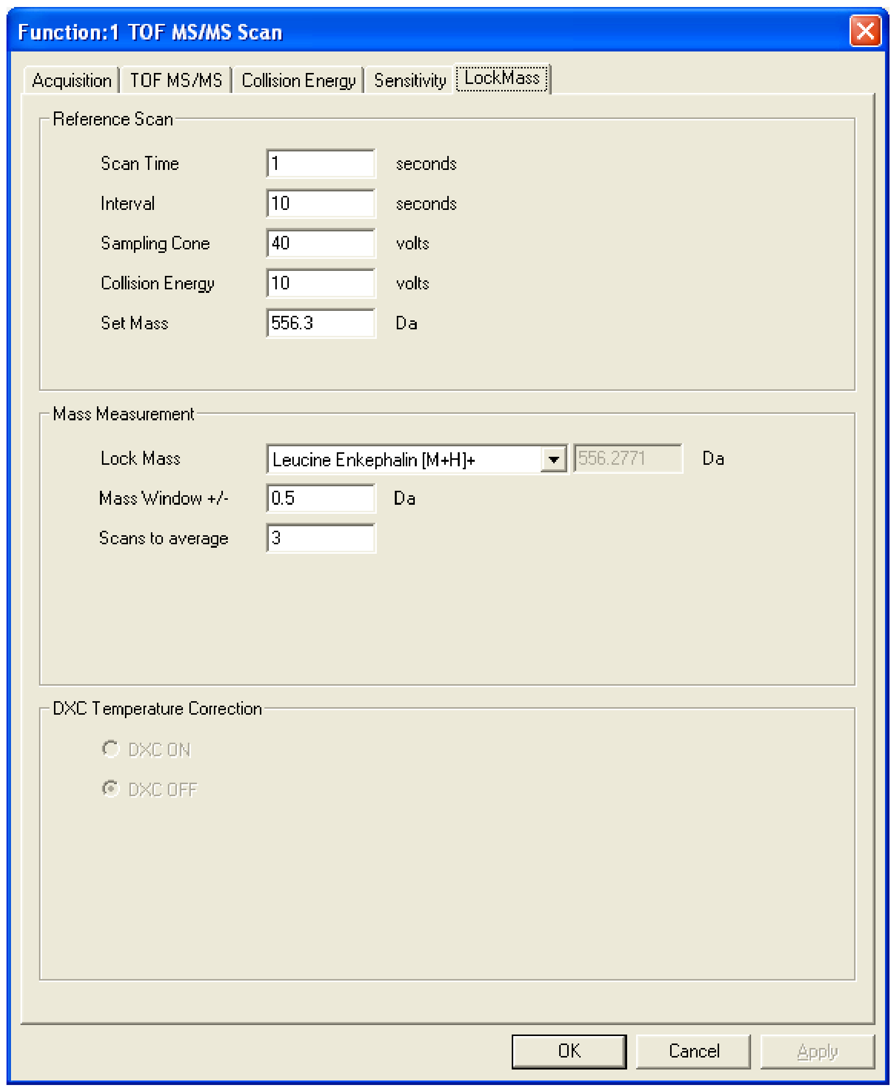
MS/MS m/z range



Parameter	Description
Start m/z	The start m/z value over which data is acquired.
End m/z	The end m/z value at which data acquisition stops.
Set Mass	Specify the precursor mass to be selected by the quadrupole for fragmentation. **Tip:** Care should be taken when ions that have a multiple charge are being analyzed, to ensure that the mass range is sufficient to include product ions with a lower charge but a higher m/z value than the precursor.

LockMass page

Using the LockMass page, you can specify the frequency of the reference data for accurate mass and the conditions at which to acquire that data.



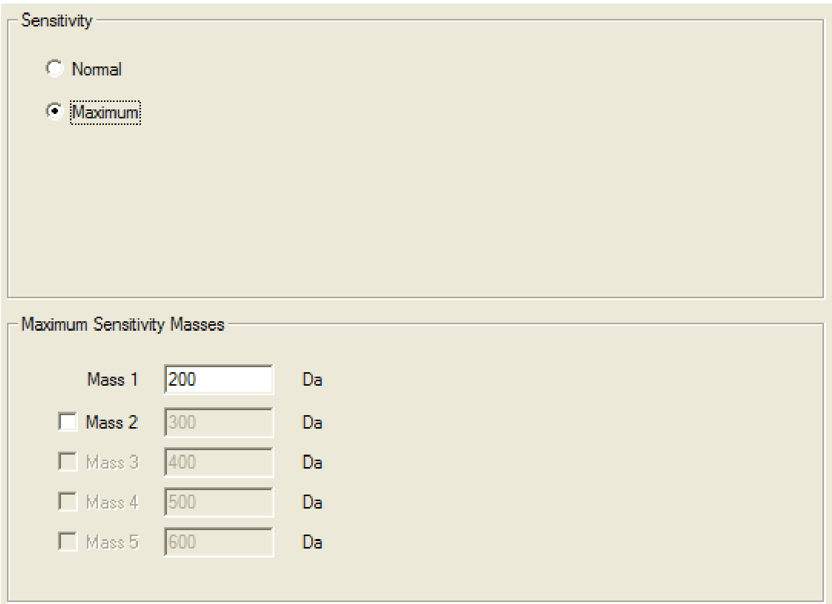
Parameter	Description
Scan Time	Sets the scan time for the reference scan. Usually set to 0.5 or 1 s.

Parameter	Description
Interval	The time period between LockSpray reference scans. Usually set to 10 s.
Sampling Cone	Sets the sampling cone voltage for the reference scan. Sample dependent, but usually 30 to 50 V.
Collision Energy	Sets the collision energy for the reference scan. Usually set to 5 V.
Set Mass	The set mass is present in MS/MS only. It sets the precursor mass for MSMS and also the maximum sensitivity mass (EDC).
Lock Mass	Select the lock mass from the drop-down list. If the required lock mass is not on the list, then add by clicking Setup > Edit Lockmasses in the Tune window. Only available if centroid data is selected.
Mass Window +/-	Sets the window size to enable the correct lock mass peak to be located in the spectrum. Usually set to 0.5 Da.
Scans to average	Sets the number of scans to be averaged before calculating the lock mass correction. Usually set to 10.

Sensitivity page

From this page you can select Normal or Maximum. Select Maximum, for Enhanced Duty Cycle, to enable Maximum Sensitivity Masses. Up to five masses can be selected at which the sensitivity will be increased.

Tip: This page is also common to MS/MS Scan.



Collision Energy page

- Use Tune Page Collision Energy

  This is the least sophisticated option since it just uses the value for the collision energy that is taken from the Tune window.

- Use a Fixed Collision Energy Value

  This allows one fixed value to be entered, that will override the collision energy set in the Tune window.

- Use Collision Energy Profile

  This option allows the selection of up to 5 collision energies. The collision energy will cycle around the selected values, using one collision energy per scan, in an attempt to produce optimal fragmentation.

- Use Collision Energy Ramp

  This allows the Collision Energy to be ramped from an Initial Energy to a Final Energy.

Method Editor basic functionality

To add a new function to the List:

1. Click one of the toolbar buttons or select the required function from the Functions menu. The editor for the function type selected opens, showing default values.
2. Make any changes required to the parameters and click OK. The new function is added to the Function List.

To modify an existing function in the list:

1. Select the function in the Function List.
2. Click or double-click on the function to open the appropriate editor for the function type and allows changes to be made.

   The Function List display is updated to show any changes.

To Copy an existing function in the list:

1. Select the function in the Function List.
2. Select Edit > Copy.
3. Select Edit > Paste. A copy of the function appears in the Function List.
4. Modify the parameters as described in To modify an existing function in the list: 4-27.

To Remove a function from the list:

1. Select the function in the Function List.
2. Click , select Edit > Delete, or press the Del key.

To change the function order in the list:

Functions appear in ascending Start Time and End Time order; this order cannot be changed. For functions with the same start and end time, you can change the order in which they are performed as follows:

1. Select the required function.
2. Repeatedly click or until the function is in the required position.

To Set the maximum retention time:

1. Enter the required value in the Total Run Time text box.
2. Click .

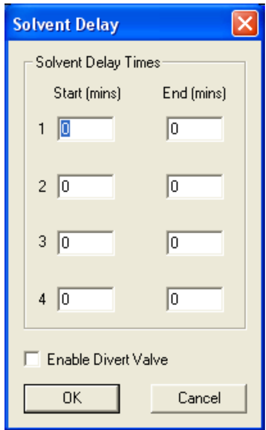
The ratio of the functions defined is maintained. For example, if two functions are defined, one from 0 to 5 minutes, and the other 5 to 10 minutes, then a Total Run Time of 10 minutes is displayed. If this value is changed to 20, the first function now runs from 0 to 10 minutes and the second from 10 to 20 minutes.

Setting a solvent delay

To set a solvent delay for a Function List, select Options > Solvent Delay on the MS Method Editor to open the Solvent Delay dialog box.

No data is stored during the solvent delay period, which means that solvent peaks that would normally be seen eluting on the TIC chromatogram are no longer seen.

Solvent Delay dialog box:



For APCI functions, the APCI probe temperature is set to the value specified in the APcI Probe Temp field for the period of the solvent delay.

To enable the divert/injector valve to be used as a divert valve, select Enable Divert Valve. This diverts the flow of solvent during a solvent delay period either to, or away from, the source for the time period shown in the solvent delay timetable.

Up to four solvent delays can be programmed.

Acquiring Analog Data

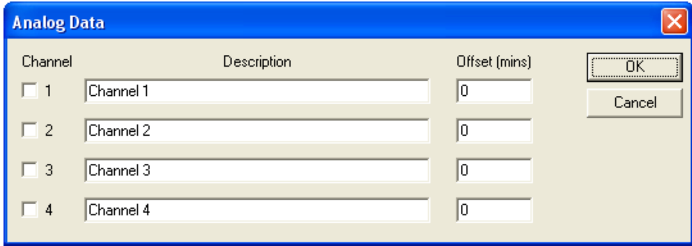
You can acquire up to eight channels of analog data, which are stored with the data acquired from the mass spectrometer.

Analog channels are typically used to collect data from external units such as UV detectors, which must be connected to the ANALOGUE CHANNEL connectors described in Analog Channels on page 1-22.

A reading is made from the external channel at the end of each scan and stored with the data for that scan. The resolution of the chromatography for an analog channel is therefore dependent on the scan speed used to acquire the mass spectrometry data.

To open the Analog Data dialog box, select Options > Analog Data on the MS Method Editor.

Analog Data dialog box:



To store data for an analog channel:

1. Select the box(es) for the channel(s) required.
2. Enter a textual description for each selected analog channel.

   This description is used on the analog Chromatogram dialog box as the channel description. See MassLynx Help.
3. Enter an Offset (minutes) to align the external unit with the mass spectrometer.
4. Click OK.

Saving and Opening a Function List

To save a Function List file:

1. On the MS Method Editor, select File > Save As to open the Save As dialog box.
2. Enter a new file name or select an existing file from the displayed list.
3. Click Save.

   If the file already exists on disk, confirmation is requested to overwrite the existing information.
4. Click Yes to overwrite the file or No to select a different name.

   When the editor closes, a prompt is displayed to save any changed Function Lists.

To open a saved Function List file:

1. From the MS Method Editor, select File > Open to open a standard Open dialog box.
2. Select the name of the Function List file to open, either by typing its name or by selecting it from the displayed list.
3. Click Open.

5 NanoLockSpray Interface

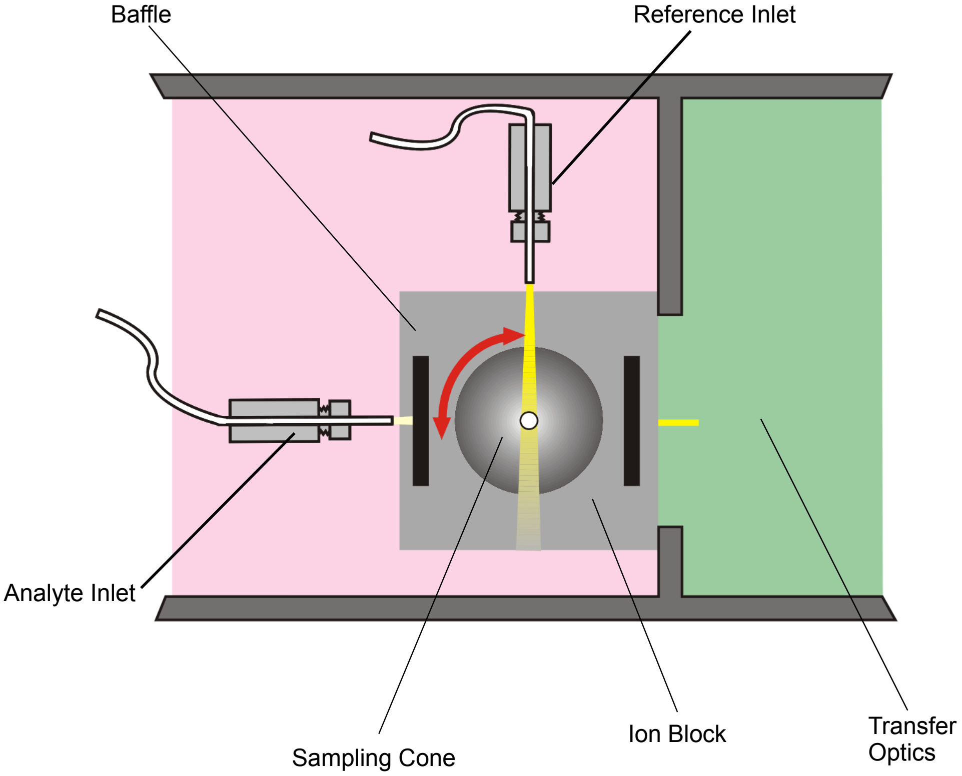
Contents:

Topic	Page
Overview of NanoLockSpray	5-2
NanoLockSpray installation	5-3
Operation overview	5-20
Adding NanoFlow Options	5-23

Overview of NanoLockSpray

The NanoLockSpray interface mounts directly onto the standard ZSpray source. It provides a low flow capability NanoSpray probe (< 2 µL/min) orthogonal to the analyte NanoSpray probe for introduction of the reference compound. This probe has the same capillary voltage applied as the standard probe and the nebulizing gas lines are coupled. Also, on the NanoLockSpray interface, is a baffle attached to a position indexed stepper motor. This admits the spray from either the standard analyte NanoSpray probe or the reference probe to the sampling cone for acquisition.

Schematic Representation of NanoLockSpray:



Spray indexing allows the analyte and reference data to be acquired into separate data files and the design of the baffle produces negligible cross talk between the two sprays. Data from the reference spray are used to calculate a correction factor for the mass scale calibration, which is then applied to the analyte data to provide exact mass information.

NanoLockSpray installation

To remove the source enclosure:

1. In the Lock Spray frame of the Source page set the baffle to Reference.
2. Disconnect the Desolvation Heater electrical connection on the instrument front panel.

Warning: The source is hot, so allow it to cool down for at least 30 minutes before proceeding.

3. Disconnect the PTFE tubing at the Desolvation gas connection on the front panel.

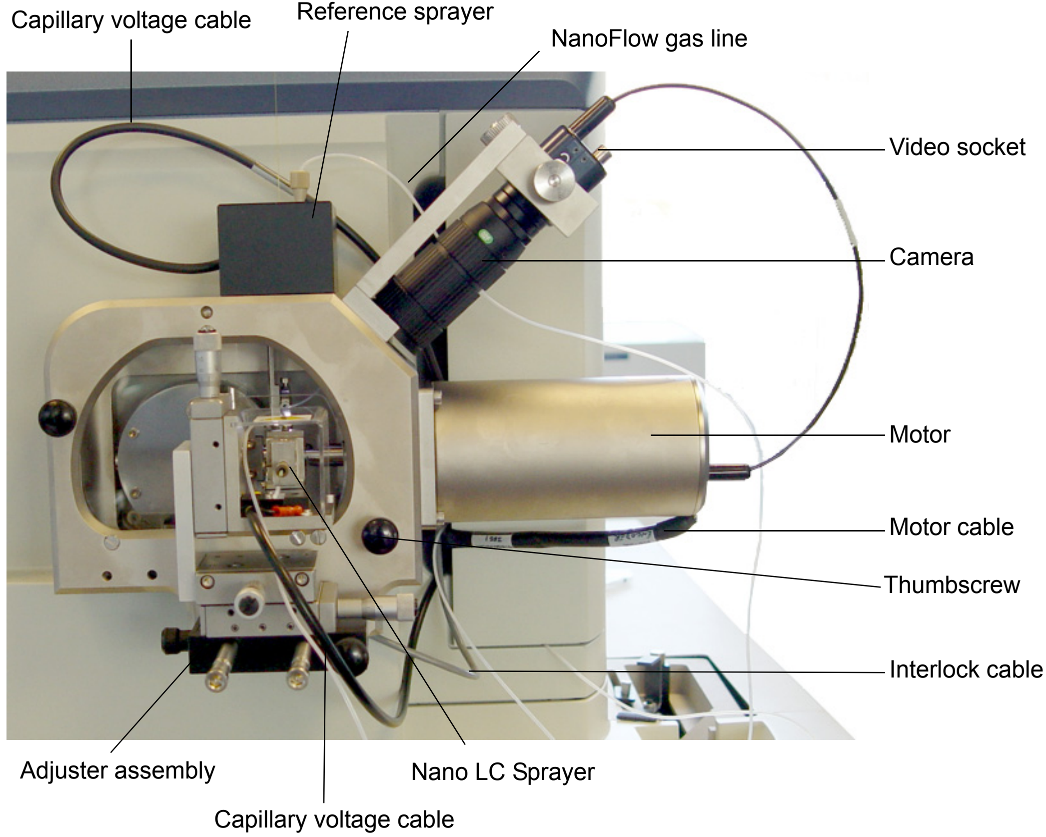
Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

4. Use an Allen key to loosen the three captive source enclosure securing screws and remove the source enclosure from the instrument.

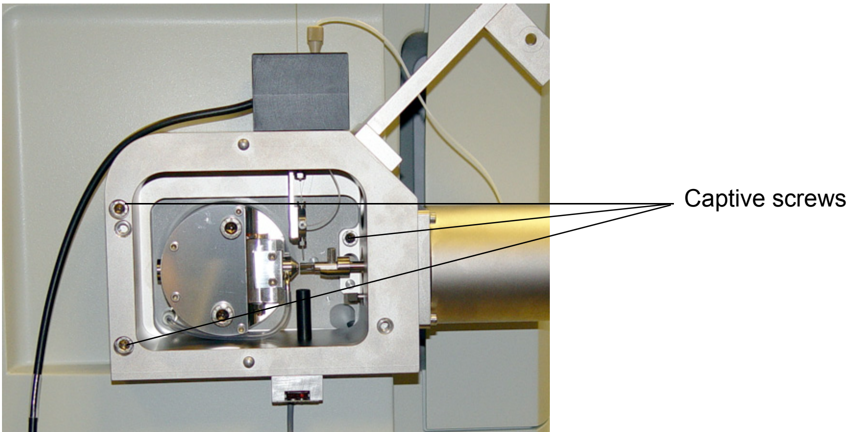
To install the NanoLockSpray interface:

Use the following figure as a guide.

NanoLockSpray connections:



1. Loosen the two thumbscrews and remove the adjuster assembly from the NanoLockSpray assembly.
2. Slot the NanoLockSpray interface on the source locating pillars and secure the three captive screws.

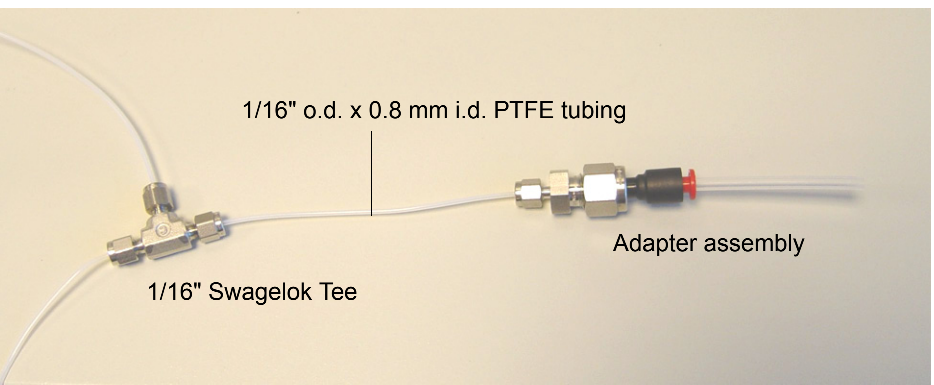


3. Refit the adjuster assembly and secure the two thumbscrews.
4. Connect the interlock cable, which hangs from the bottom of the NanoLockSpray interface, to the socket marked Heater/Interlock on the front connection panel.
5. Connect the reference probe cable to one of the sockets marked Capillary on the front connection panel.
6. Connect the sprayer platform cable to one of the sockets marked Capillary on the front connection panel.

   Tip: If a glass capillary sprayer is to be used, connect the sprayer platform cable to the NanoLockSpray Capillary Voltage Divider Box. Connect the divider box to one of the sockets marked Capillary on the front connection panel.
7. Connect the motor cable to the socket marked Motor on the front connection panel.
8. Connect the camera power cable from the socket on the end of the motor housing to the socket marked Power on the camera.
9. Connect the video cable from the socket on the camera marked Video Out to the MALDI connector on the front panel and connect the video socket on the rear of the instrument to the MassLynx PC.
10. Slide the sprayer platform out from the source, remove the sprayer cover, and fit the desired sprayer, securing with the thumbscrew under the mounting plate.

11. Connect the nebulizer gas lines from both the reference probe and analyte sprayer to the 1/16 inch Swagelock tee piece provided. Connect to the NanoSpray connector on the front connection panel, via the provided adapter assembly.

Nebulizer gas line assembly:



12. Refit the cover over the sprayer, and carefully slide the sprayer platform towards the source, making sure that the sprayer tip does not collide with the source or baffle.

13. Insert blank plugs into the two Nebuliser gas connectors on the front connection panel.

NanoLockSpray capillary voltage divider box

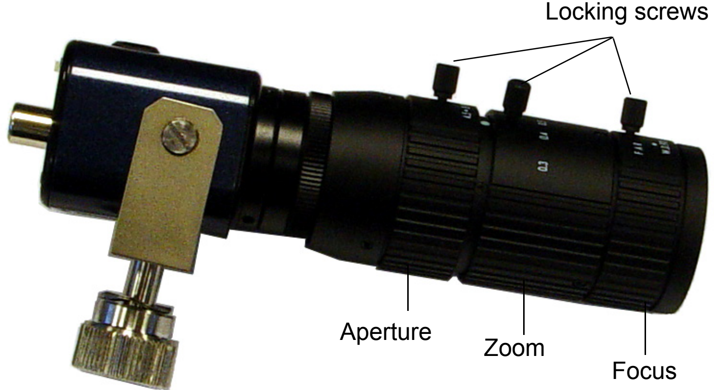
The NanoLockSpray Capillary voltage divider box is designed for use with the glass capillary option. It divides the capillary voltage applied to the glass capillary sprayer by a factor of two, e.g. if you set the capillary voltage to 2.4 kV in the tune page, the actual voltage on the glass capillary sprayer will be 1.2 kV. The voltage on the reference sprayer is unaffected. The use of this divider box is necessary as the glass capillary sprayer optimizes at a much lower voltage than the reference sprayer.

Divider box:

Adjusting the NanoLockSpray camera lens

The NanoLockSpray camera is fitted with a macro zoom lens. There are three adjustment rings on the lens barrel, each of which is fitted with a locking screw. An adjustment ring will not rotate while its locking screw is tight.

Camera controls:



Aperture

This controls the brightness of the image. It is usually set to 22.

Note: The camera has an automatic gain control that attempts to compensate for changes in image brightness.

Zoom

The lens has a x3 zoom range. Adjust the zoom to control the field of view. At the maximum zoom the sprayer tip can be examined closely. At the minimum zoom the relative positions of the sprayer, baffle and sampling cone can be observed.

Focus

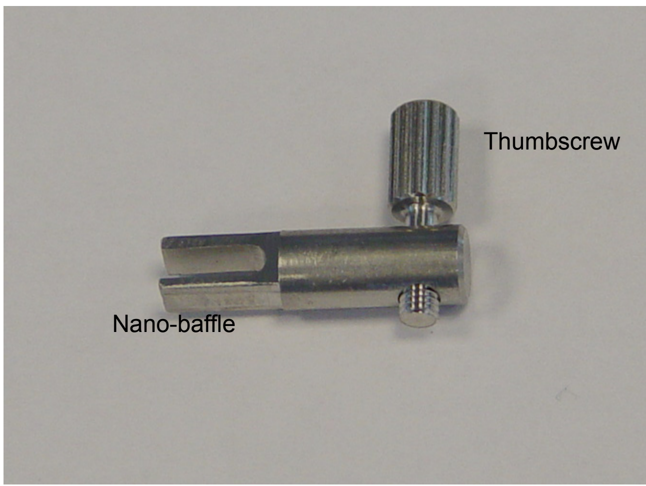
Adjust this control to focus the image. Focus on the probe tip for optimal spray imaging. The depth of field is a function of the aperture setting; a wide open aperture will produce a very small depth of field.

The NanoLockSpray baffle

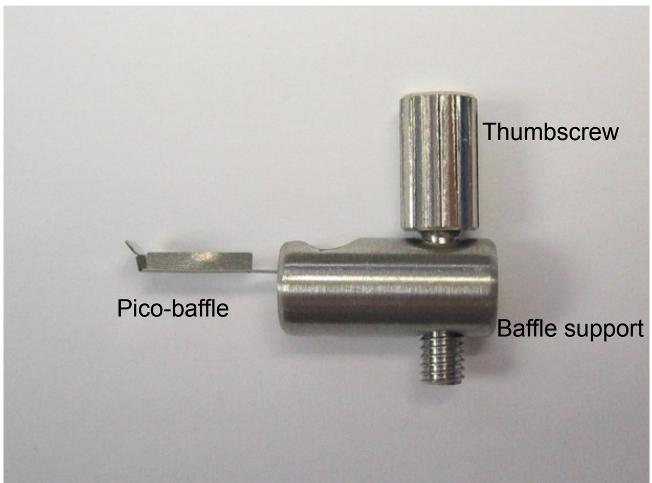
The NanoLockSpray source is supplied with two types of baffle:

- The nano-baffle - this baffle is optimized for use with the Nano-LC sprayer.
- The pico-baffle - this baffle is optimize for use with the PicoTip and glass capillary sprayers. This baffle allows the sprayer to be positioned closer to the sampling cone.

Nano-baffle:



Pico-baffle:



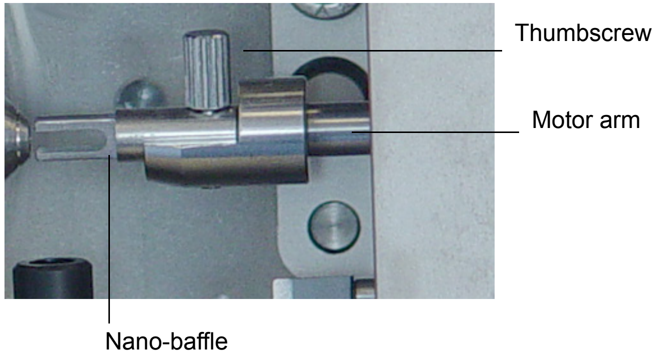
To remove a baffle:

1. Set the instrument to standby
2. Set the source temperature to 20°C. Wait for it to cool.
3. Set LockSpray to the reference position
4. Disconnect the sprayer platform cable.
5. Loosen the two thumbscrews and remove the adjuster assembly from the nanoLockSpray interface.

Warning: To avoid burns, take great care while working with the source as it is liable to be hot.

6. Loosen the thumbscrew on the baffle and remove the baffle.

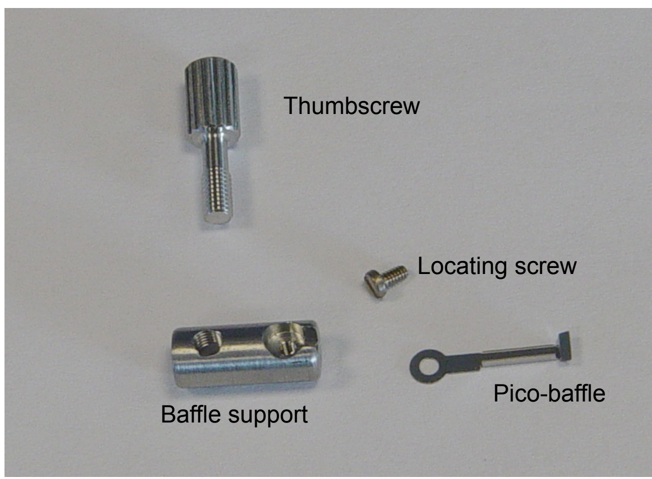
Baffle in analyte position:



To assemble the pico-baffle:

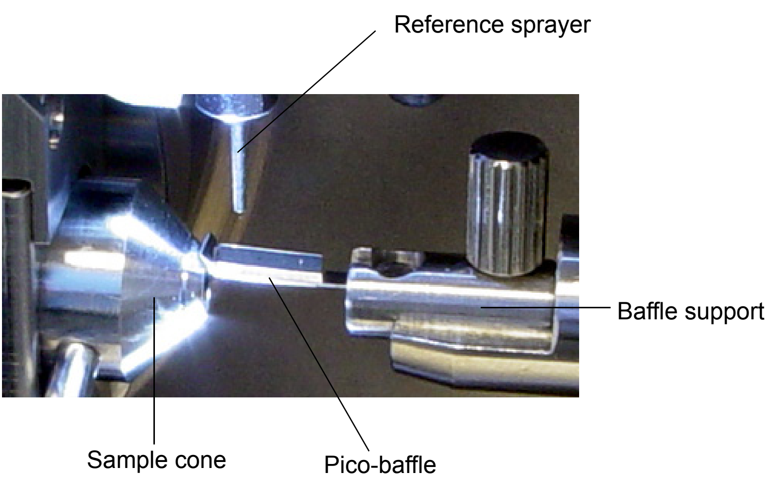
The baffle is made up of four pieces (shown below).

Pico-baffle components:



1. Attach the baffle to the baffle support with the locating screw. Observe the correct orientation: the baffle edges fold towards the screw head.
2. Insert the thumbscrew into the baffle support, from the same side as the locating screw.

Pico-baffle fitted:



Nano reference probe assembly instructions

Warning: Wear suitable eye protection at all times when handling or preparing fused silica and borosilicate tips.

Warning: To avoid injury from trace chemicals on the probe, always wear gloves.

Caution: Wear gloves whenever handling parts from the inside of the instrument. Check all of the parts are present. Refer to the shipping document for part numbers and descriptions.

To assemble the reference probe:

1. Remove the two screws holding the reference assembly to the source enclosure.
2. Carefully lift the reference assembly from the source enclosure.
3. Make a sleeve for the 20-µm fused silica (page 5-12).
4. Prepare the 20 µm fused silica (page 5-12) and the 75 µm fused silica (page 5-13).
5. Prepare the Valco tee piece (page 5-14).
6. Prepare a gas tight fitting and a nebulizing capillary (page 5-14).

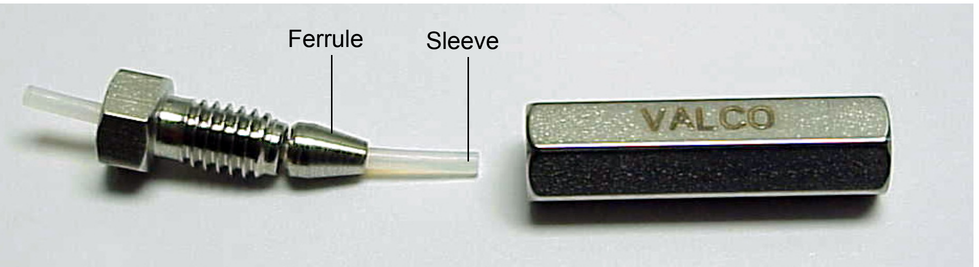
7. Prepare the nebulizing gas connector (page 5-16).
8. Thread the tube through the assembly (page 5-17).
9. Prepare the nebulizing PTFE tube (page 5-18).

To make a sleeve for the 20-µm fused silica:

1. Cut approximately 30 mm of the 1.6 x 0.12 mm PTFE tubing and insert it into one side of the Valco metal union.

   Rule: Ensure that the PTFE has been cut squarely.

2. Hand tighten and push the PTFE tube in as far as it will go.



3. To position the ferrule tighten the union nut to make the ferrule grip onto the PTFE tube, but do not overtighten
4. Remove the tube from the union and cut it down in length such that it is just flush with the edge of the union nut.

To prepare the 20-µm i.d. fused silica:

Warning: Eye protection should always be worn when cutting fused silica.

1. Using the ceramic cutter, cut approximately 15 cm of 20-µm i.d. fused silica (6028621) and thread into the sleeve made above.

2. Insert the sleeve and silica into the union.
3. Push the silica through the union and progressively tighten the union nut and test the tightness of its grip on the silica.

4. Once the grip is sufficient to restrict movement, remove the nut and move the silica so it is flush with the edge of the PTFE sleeve.
5. Tighten up the nut to lock the fused silica in place.

   Tip: This may require some force.

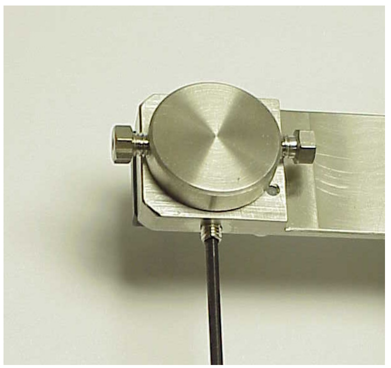
To prepare the 75-µm i.d. fused silica and make the union with the 20-µm assembly:

1. Cut a long length (>30 cm) of 75 µm i.d. fused silica (6490512) and make a sleeve using a green PEEK tubing sleeve (6070190).

2. Ensure that the silica has been cut squarely to reduce dead space. Thread the sleeve into a headless seal-tight nut and ferrule and thread the 75-µm i.d. fused silica through the sleeve making sure that the fused silica end is flush with the end of the sleeve.
3. Push the PEEK as far as it will go to ensure minimum dead space and insert the silica and nut into the other side of the Valco union. Tighten using the nut extender tool.

To prepare the Valco tee-piece:

1. Use a 1.5-mm key to loosen the grub screw locking the Valco tee-piece onto the probe assembly.



2. Remove the Valco tee-piece.

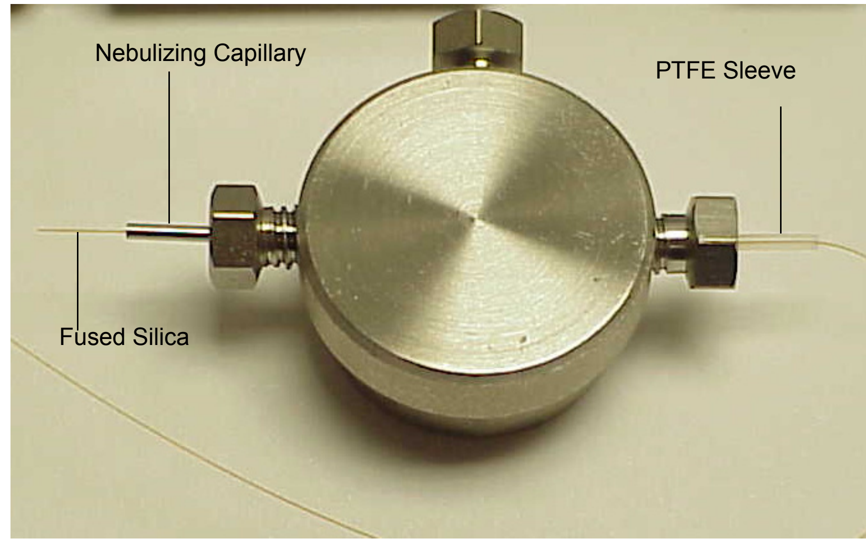
To prepare a gas-tight fitting and nebulizing capillary:

1. Take 30 mm of the 0.12 x 0.8 mm PTFE tubing and make a sleeve to form a gas tight seal at the tee piece, using the tee piece nut and ferrule.
2. Tighten the nut to make sure that the ferrule grips, taking care not to over-tighten. Remove the nut from the tee and cut down the PTFE tubing so that approximately 4 mm of sleeve protrudes from the nut of the tee.

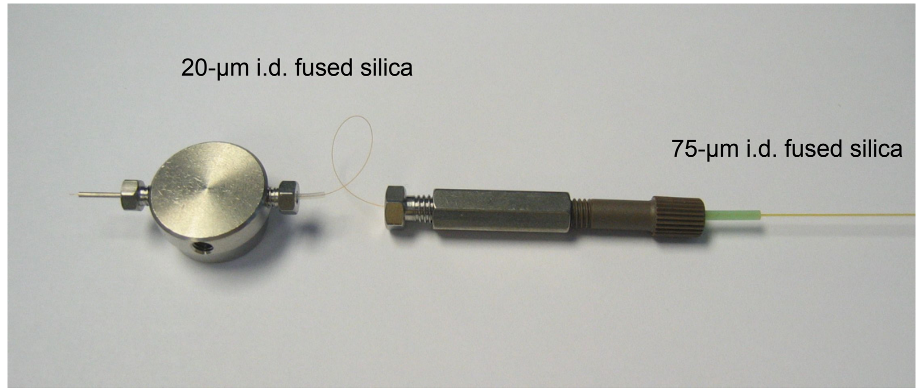
3. Attach 20-µm id fused silica by threading the 20-µm i.d. fused silica attached to the union through this nut and sleeve, but do not tighten the tee nut as the silica needs to be mobile.
4. Thread the fused silica through the tee-piece
5. Push the nebulizing tube as far as it will go into the Valco tee-piece and tighten the tee nut until the ferrule grips.

Tip: Depending on which baffle is used, there are two lengths of tube see The nebulizer tube on page 5-19.

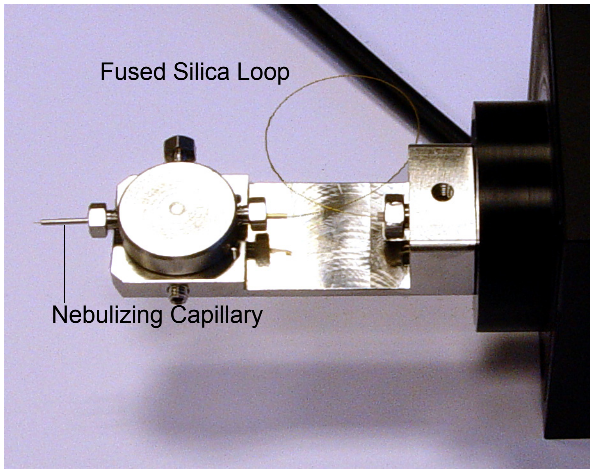
6. Thread the 20-µm i.d. fused silica through the nebulizing capillary.
7. Tighten the tee nut holding the PTFE sleeve so that it is finger tight and make sure that the fused silica is free to move though the tee.
8. Thread the fused silica through the Valco tee until the fused silica protrudes from the nebulizer tube.



9. Thread the 75-µm i.d fused silica up through the probe body and clamp the Valco union into position by tightening the grub screw behind it.



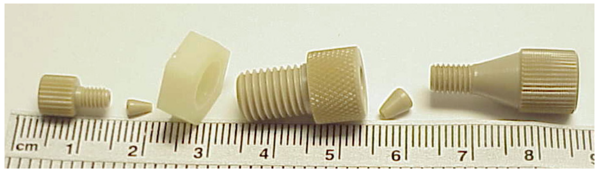
10. Clamp the Valco tee into position by tightening the grub screw. The excess length of 20-µm i.d. fused silica should form a loop.



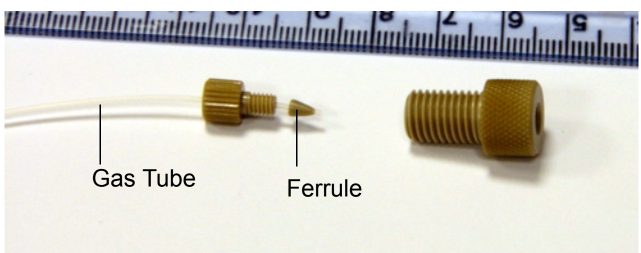
11. Adjust the length of 20-µm i.d. fused silica protruding from the nebulizer tube to between 0.5 to 1 mm, and tighten the nut on the PTFE sleeve to clamp it in position.

To prepare the nebulizing gas connection:

1. Remove the reference sprayer outer cover.
2. Remove the PEEK bulkhead union.



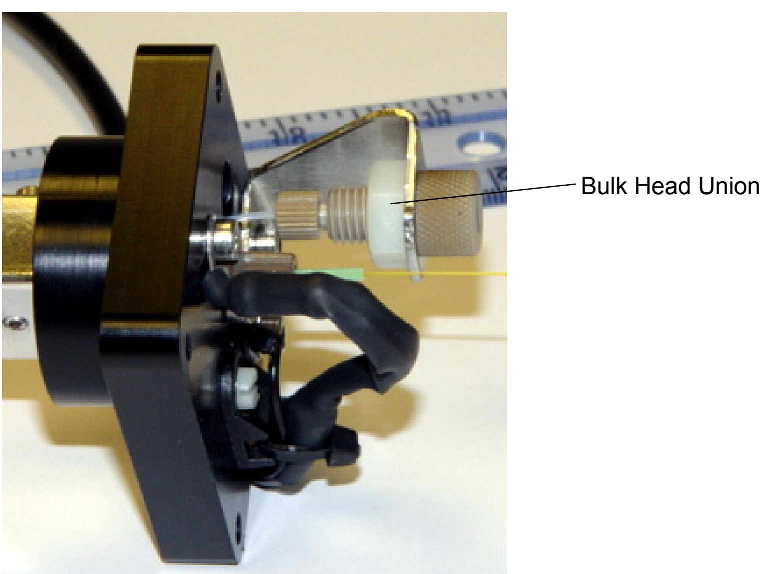
3. Cut approximately 12 cm of the PTFE gas tube (6063066).
4. Insert the nebulizing gas tube (6063066) through the small PEEK nut and ferrule and tighten until the ferrule grips.



To thread the tube through the assembly.

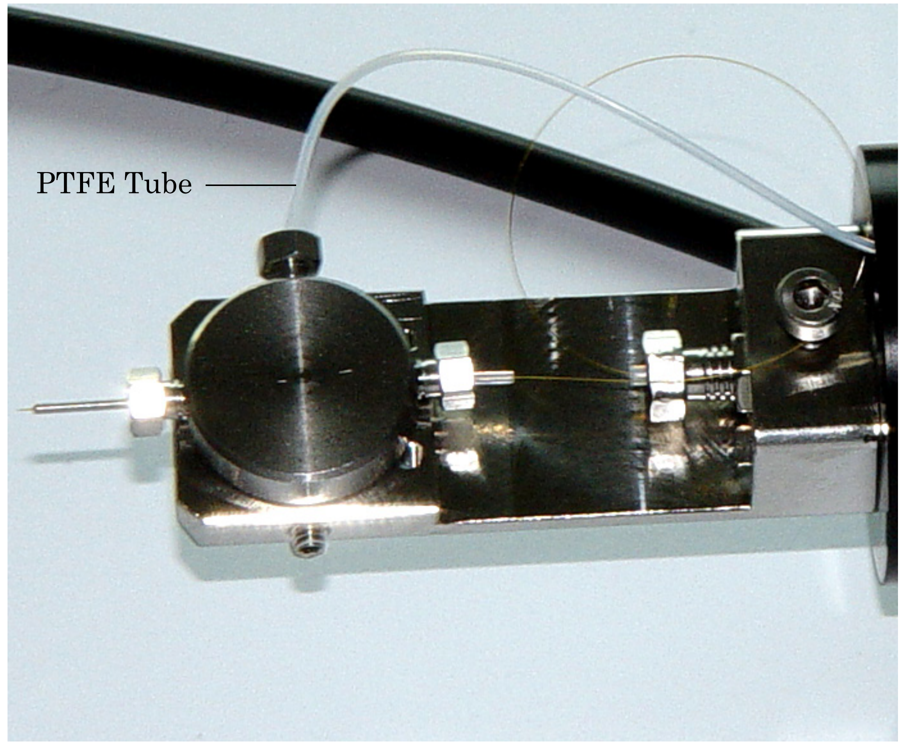
1. Thread the tube through the plastic nut and the rest of the assembly.

Threading the tube through the probe assembly



2. Tighten the nut so that the bulkhead union is held securely.

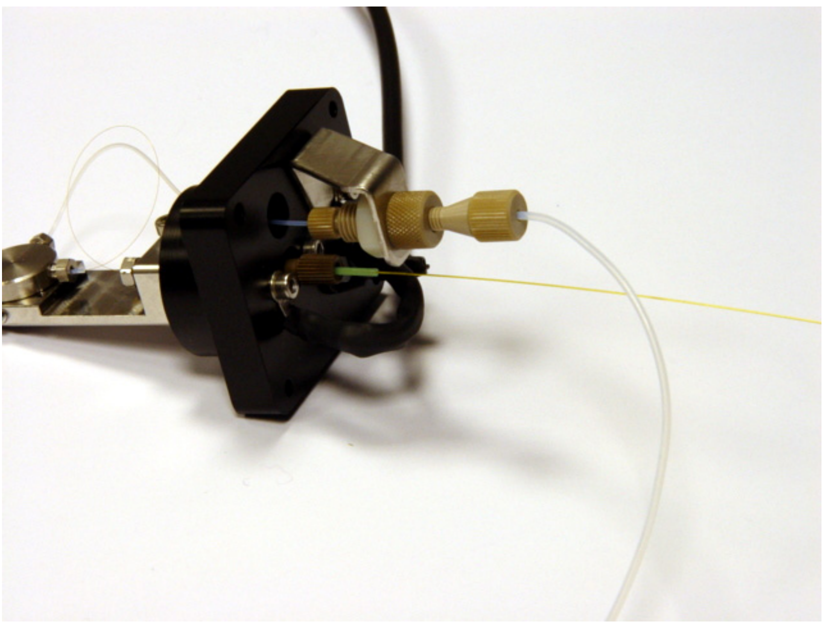
3. Bend the tube so that it fits into the tee and tighten the nut so that the ferrule grips the tube securely.



To prepare nebulizing PTFE tube:

1. Cut a length of PTFE tube and insert it into the larger of the PEEK bulkhead union nuts and tighten until the ferrule grips the tube securely.

2. Using the 1/16 inch tee split the nanoflow gas supply into two feeds. Attach the end of the reference probe PTFE tube to the tee and tighten until the ferrule grips.



3. Attach the other end of the nanoflow gas supply to the analyte probe.
4. Attach the probe casing to complete the assembly.

Warning: The Nanoflow assembly should not be operated without the probe casing attached.

The nebulizer tube

Rationale: The pico-baffle should be used in conjunction with a longer nebulizer tube. The pico-baffle nebulizer tube is 24-mm long, whereas the nano-baffle nebulizer tube is 20-mm long. The use of the correct nebulizer tube will prevent crosstalk between the reference and analyte sprays.

To install the nebulizer tube:

1. Remove the reference probe from the source.
2. Remove the Valco screw from the bottom of the Valco tee piece and withdraw the nebulizing capillary.
3. Slide the Valco nut and a ferrule over the 24-mm long nebulizer tube. Carefully slide over the 20-µm fused silica, and screw into the Valco

tee-piece, pushing the nebulizer tube in as far as it will go. Tighten the nut until the ferrule grips the nebulizer tube.

4. Loosen the nut on the top of the Valco tee-piece and adjust the length of the 20-µm fused silica so that about 0.5 to 1 mm of protrudes. Retighten the nut.

5. Refit the reference probe.

Operation overview

The operation of the nanoLockSpray source is very similar to the standard LockSpray source. See Chapter 2 for general information about how to tune the instrument, and see Chapter 4 for a description on how to acquire accurate mass data from a LockSpray source.

Source type selection

To select the source:

1. From the Tune window, click Source > Nanoflow.

2. Click Mode > LockSpray On.

Reference sprayer

The reference sprayer is normally run off the instrument's syringe pump, fitted with a 100 µL syringe, at a flow rate of 0.5 µL/min. There is no positional adjustment for the reference probe. The concentration of the reference solution should be chosen to give a suitable ion intensity.

The concentration of the reference solution should be chosen to give a suitable ion intensity, i.e. between 0.05 and 0.2 ions-per-push (IPP)

Analyte sprayer

The NanoLockSpray source can be used with a range of different nanoflow sprayers. Refer to the sprayer-specific instructions on how to set up these sprayers.

See also:

- Set capillary back to previous voltage. on page 5-33
- Glass capillary option on page 5-29

NanoSpray gas supply

The reference and analyte sprayers share a common NanoSpray gas supply. The pressure of this supply is electronically controlled over the range 0 to 3 bar. The optimum pressure is sprayer dependent, but usually lies within the range 0.5 to 1.5 bar.

Important: The Nebulizer gas connections must be blanked off, using blank plugs, while the NanoLockSpray source is in use.

Sprayer platform adjuster assembly

The sprayer platform adjuster assembly allows precise XYZ positioning of the sprayer tip. It also allows the sprayer to be withdrawn from the source to allow sprayer access.

There are two thumbscrews on the base of the adjuster assembly that are used when moving the platform in and out of the source.

To withdraw the sprayer platform from the source:

1. Unscrew the front thumbscrew.
2. Pull out the side thumbscrew while pulling the sprayer platform out from the source.
3. Release the side thumbscrew, locking the platform in the out position.
4. The Operate LED will flash green if the platform is pulled out while the instrument is in operate. The tune page will show the status message ‘Operate - probe interlock active’. The probe interlock sets the capillary voltage to zero, removing the voltage from the sprayer.

To slide the sprayer platform into the source:

1. Check the sprayer safety cover is installed
2. Pull out the side thumbscrew while pushing the sprayer platform into the source.
3. Release the side thumbscrew, locking the platform into position.
4. Tighten the front thumbscrew, securing the adjuster assembly rigidly to the source.
5. The operate LED will show steady green if the instrument is in operate.

Caution: While the operate LED is a steady green, there is a high voltage on the sprayer. The safety cover should always be installed, and care should be taken not to touch the sprayer.

Sprayer tip position

To adjust the tip position:

1. Adjust the X, Y and Z controls on the adjuster assembly in order to position the sprayer tip close to the sampling cone and baffle.
2. Adjust the height of the sprayer so that the tip is level with the center of the baffle.
3. Adjust the horizontal position of the sprayer so that the tip is towards the left end of the baffle.

Tips:

- If electrical discharge between the sprayer tip and baffle is observed, either move the tip further away from the baffle, or reduce the capillary voltage. The capillary voltage must be maintained high enough to maintain a good spray.
- The position of the sprayer should be fine-tuned while acquiring a spectrum of a standard compound. Small adjustments to the sprayer position can make large differences to the source sensitivity.

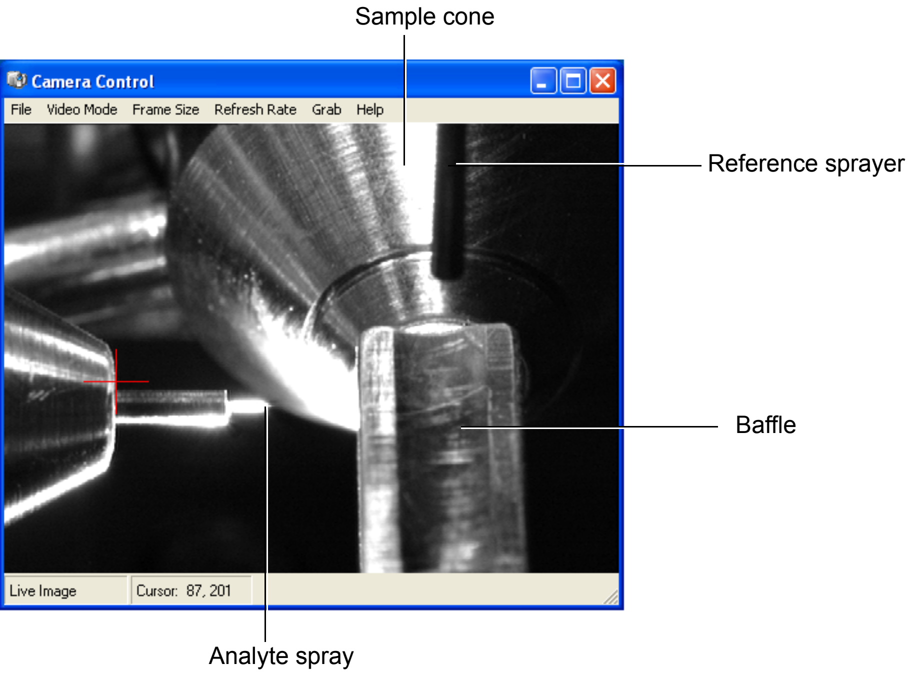
Setting up the camera

To enable the camera:

1. From the Tune window click Source > Nanoflow to enable the NanoFlow tuning parameters.
2. Click .

   Result: The Camera Control dialog box opens.

   Camera Control view of sprayers and sample cone:



3. Using the camera controls described in Adjusting the NanoLockSpray camera lens on page 5-7, focus on the analyte sprayer. If necessary manoeuvre the stage so that the camera can view the capillary tip.

Adding NanoFlow Options

You can fit the NanoLockSpray interface with several sprayer options, two of these are:

- Nano-LC Option (page 5-24)
- Glass Capillary Option (page 5-29)

Nano-LC Option

Warning: To avoid contamination from hazardous chemicals wear gloves when handling the sprayer assembly

Nano-LC Assembly

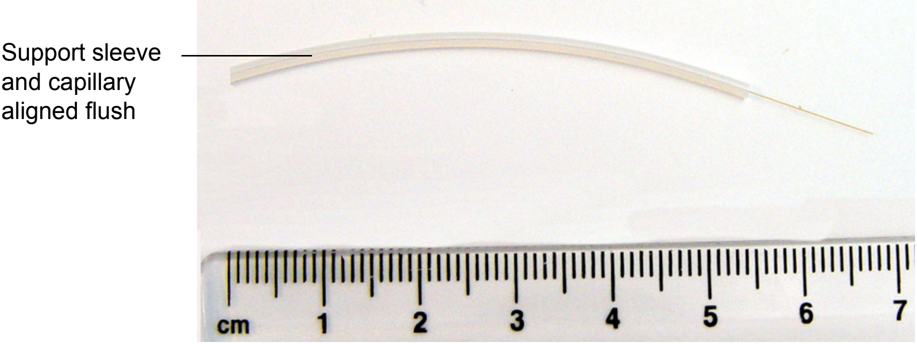
Column or silica cap from injector
Low pressure PTFE connector
Fused silica capillary
PTFE Sleeve
Microvolume insert
Make-up flow only (3-way insert required)
Valco Ferrule
Nebulizer gas
90 µm fused silica
O-ring
Nebulizer gas via NanoFlow gas regulator
Nano-LC body
Chamber
Nebulizing tip
1 mm

The sprayer assembly contains a microvolume-insert that couples the flow from an injector, syringe pump or nano-HPLC system to a length of fused silica that acts as the spray nozzle at the end of the nebulizing tip.

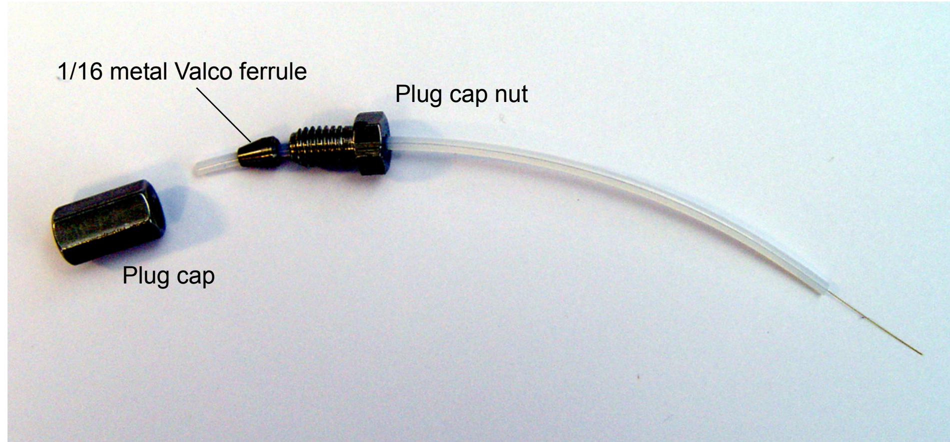
A length of PTFE tubing supports the fused silica within the chamber. The PTFE fits into the microvolume insert and is retained using a metal ferrule, along with the thread on the end of the chamber.

To assemble the fused silica and PTFE support:

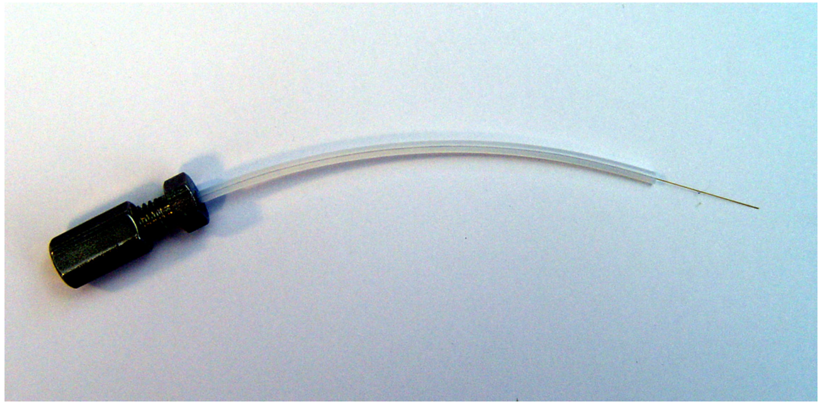
1. Cut a 50-mm length of PTFE support sleeve tubing (1/6 × 0.12).
2. Cut a 70-mm length of capillary (TSP020090).
3. Thread the capillary through the prepared length of support sleeve.
4. Align the support sleeve and capillary flush at one end.



5. Thread the supplied plug cap nut and 1/16 metal Valco ferrule over the support sleeve.



6. Fit the plug cap over the end of the support sleeve.



7. Push the support sleeve tubing to the end of the plug cap nut to set the "pilot" depth (see Figure titled "Fused Silica Capillary and PTFE Support Sleeve Assembly:" on page 5-27).

8. Engage the plug cap nut in the plug cap thread and tighten so that the 1/16 metal Valco ferrule just grips the support sleeve.

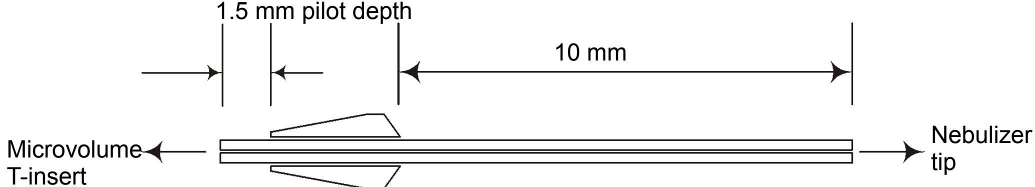
   Tip: Do not fasten the ferrule too tight at this point as you are required to re-thread the silica capillary through the PTFE support sleeve later in the procedure.

9. Unscrew the plug cap nut and remove from the support sleeve.

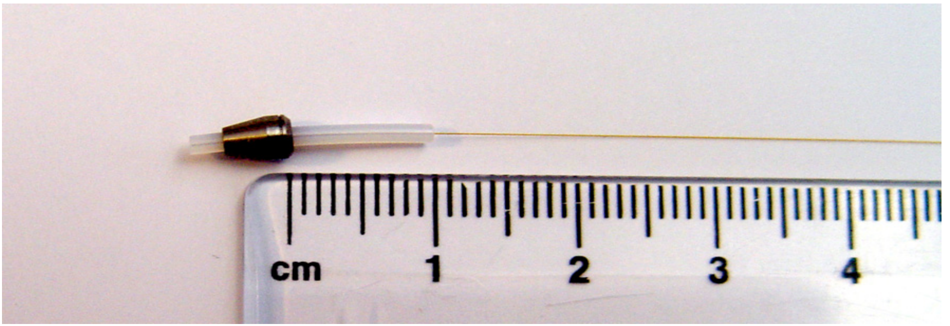
10. Remove the capillary and plug cap from the support sleeve, leaving the ferrule behind.

11. Cut the support sleeve so that only 10 mm protrudes through the back of the ferrule.

Fused Silica Capillary and PTFE Support Sleeve Assembly:



12. Re-thread the capillary through the 10-mm length of support sleeve and align flush with end of the tubing.



13. Using the plug cap and nut again, tighten the ferrule so that the capillary is firmly gripped within the support sleeve.
14. Remove the plug cap and nut from the support sleeve.

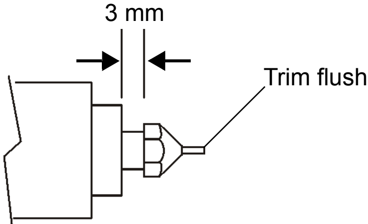
To fit the capillary in the Nano-LC chamber:

1. Thread the capillary and the PTFE support sleeve through the Nano-LC chamber.
2. Fit the microvolume insert into the Nano-LC body. Rotate the insert so that the ferrule seats line up with the column and chamber connections.
3. Fit the Nano-LC chamber into the body and tighten.
4. Pull gently on the PTFE support sleeve to confirm that the support sleeve is gripped firmly.

To fit the nebulizing tip:

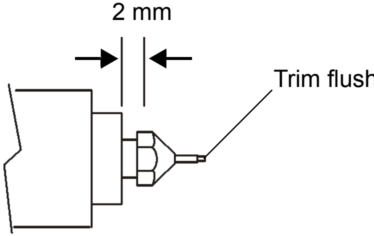
1. Slide the nebulizer tip over the capillary and tighten into the chamber, (see Figure titled "Nano-LC Assembly" on page 5-24).
2. Continue tightening until approximately 3 mm of tip protrudes.

Fitting the nebulizer tip:



3. Using a silica cutter cut the exposed silica capillary flush with the end of the nebulizer tip.
4. Continue tightening until approximately 1 mm of silica capillary protrudes through the tip.

Fitting the silica capillary:



To Operate the Nano-LC Option

The operation of the Nano-LC sprayer should be tested by infusing a known sample.

1. Set the NanoSpray pressure to about 1 bar.
2. Check for leaks of gas on the sprayer.
3. Mount the sprayer on the adjuster platform. Fit the safety cover.
4. Slide the sprayer platform into the source.

5. Adjust the sprayer position.
6. Fill a 10 mL syringe with a known sample (e.g. leucine enkephalin 50 pg/µL)
7. Set the syringe pump to 0.5 µL/min
8. Set the capillary voltage to about 3 kV.
9. Open the camera control window to view the spray. A fine spray should be observed. Try varying the nanospray pressure and the capillary voltage. It may be necessary to adjust the nebulizing tip - the sprayer will have to be removed to do this. The nebulizer tip can be screwed in or out adjusting the length a fused silica capillary protruding from the tip.
10. Carefully adjust the sprayer position to get the maximum ion count on the sample.

Glass capillary option

The glass capillary option sprayer is designed for use with metal coated borosilicate glass capillaries (nanovials). These allow extremely low flow rates of less than 100 nL/min.

Installation of pico-baffle

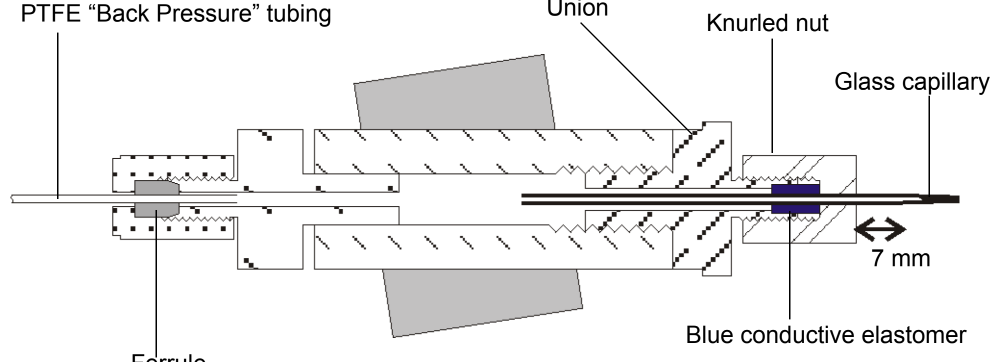
The glass capillary option should be used with the pico-baffle. See The NanoLockSpray baffle on page 5-8.

To install the glass capillary option:

1. Pull the sprayer platform assembly back from the source.
2. Remove the safety cover.
3. Place the sprayer (with gas line fitted) on the platform and secure with the thumbscrew.
4. Refit the safety cover.
5. Connect the sprayer platform cable to the nanoLockSpray capillary voltage divider box. Connect the divider box to one of the sockets marked capillary on the front connection panel.

To load the capillary:

1. Unscrew the union at the front of the sprayer, and remove the front section of the sprayer.

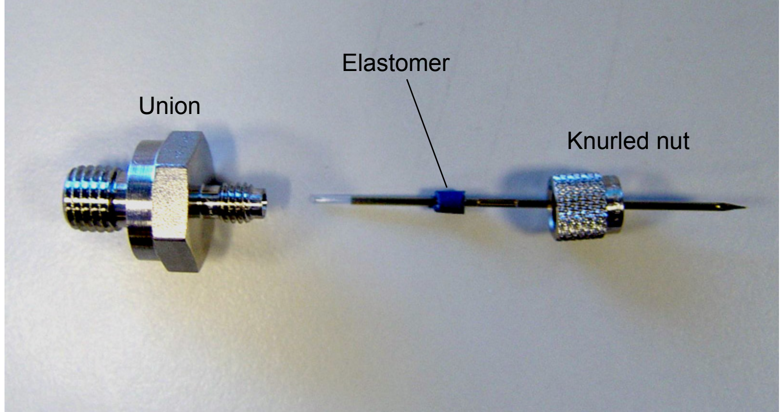


Warning: Do not touch the sharp end of the capillary. As well as the risk of injury by a sliver of glass, the capillary may contain toxic samples.

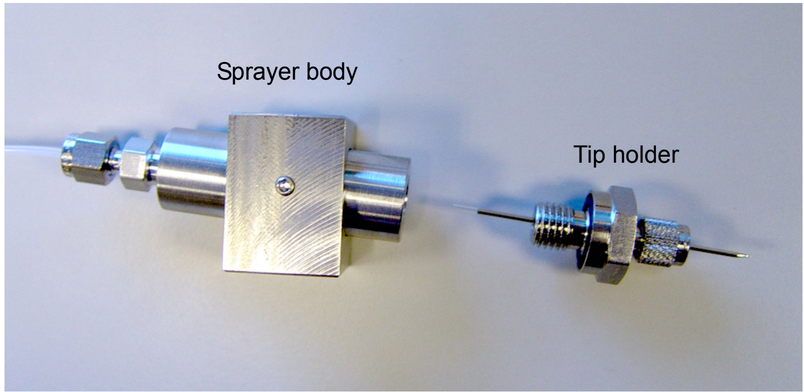
Warning: The capillaries are extremely fragile and must be handled with great care. Always handle using the square end of the capillary. The needle may become inoperable if the sharp end is touched.

2. Carefully remove the capillary from its case by lifting vertically while pressing down on the foam with two fingers.

3. Over the blunt end of the capillary, pass the knurled nut, approximately 5 mm of conductive elastomer, and finally the union.



4. Slide the glass capillary into the union and gently tighten the nut. The tip should protrude about 7 mm from the front of the knurled nut. This distance is measured from the end of the nut to the shoulder of the glass capillary.

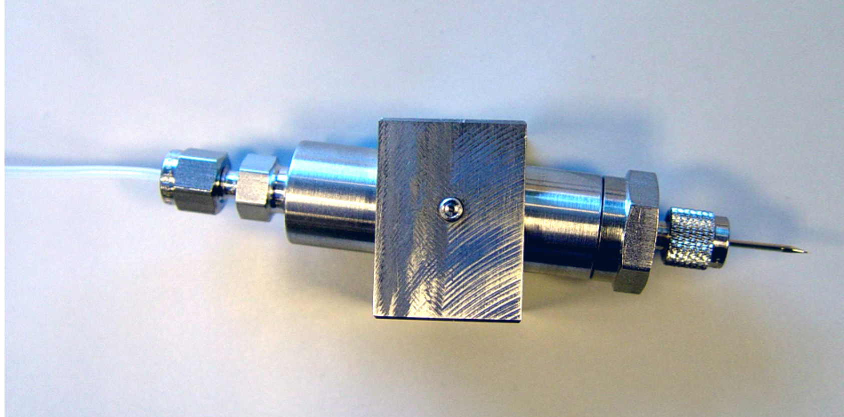


Tip: You can remove and replace the capillary without undoing the nut, just slide the old one out and carefully slide the new one in.

5. Load sample into the capillary using either a fused silica syringe needle or a GELoader tip.

Tip: Shake the loaded capillary in order to move the liquid to the tip of the sprayer

6. Screw the holder back into the assembly - finger tight is sufficient. This done when the sprayer is mounted on the adjuster platform.



7. Ensure that Capillary is set to 0 V on the Tune window.

8. Push the sprayer platform into the source.

To operate the glass capillary sprayer:

1. Set the nanospray pressure to 0.3 bar, and the cone gas to 40 L/h.

2. Check for sample flow by observing a droplet on the tip. If no droplet is observed, increase pressure briefly, up to a maximum of 1.5 bar. Return pressure to 0.3 bar.

3. If a spray is still not observed, then break off a small piece from the end of the tip, as described below in To restart the spray: on page 5-33

4. Set the capillary voltage slider to between 1.0 and 3.0 kV.

   Note: With the voltage divider box in use, a Capillary voltage slider range of 1 to 3 kV corresponds to an actual sprayer voltage of 0.5 to 1.5 kV.

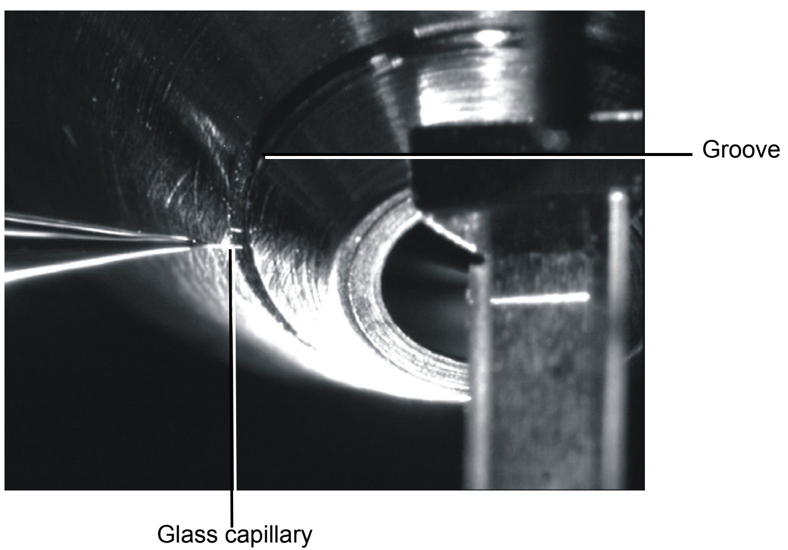
5. An ion beam should now be visible on the peak display.

6. Optimize sprayer position and capillary voltage for maximum signal intensity.

See also: Spray optimization on page 5-34.

To restart the spray:

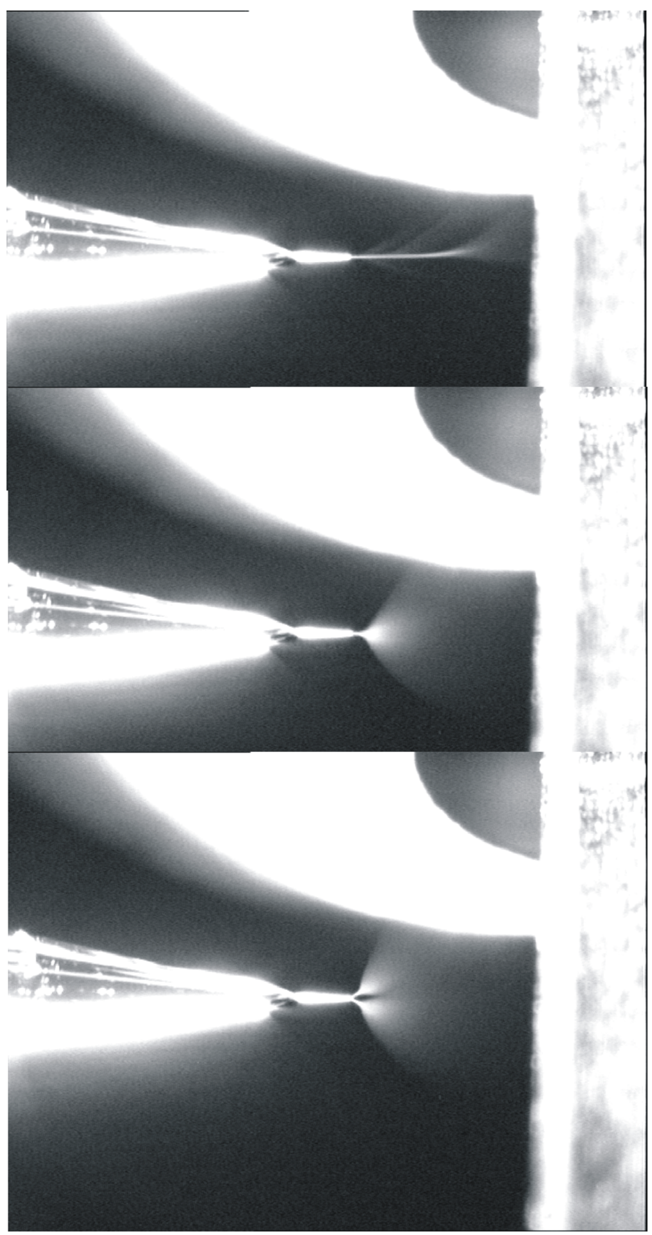
1. Set capillary voltage to zero.
2. Move the sprayer back and to the left until the tip aligns with the groove on the cone; this is best done while viewing from the front of the source.
3. While watching the camera image, carefully move the tip forwards towards the groove until it touches and a small piece of the glass hair shears off.



4. Return sprayer to previous position.
5. Set capillary back to previous voltage.

Spray optimization

The spray will optimize between 1.0 and 3.0 kV. The following diagrams indicate the shape of the spray at too low, optimal, and too high voltages.



Liquid is emitted from the tip as a narrow jet:

Capillary voltage is too low

Liquid is emitted from the tip as a wide stable spray:

Capillary voltage is optimal

Liquid is emitted from the tip as two or more unstable sprays:

Capillary voltage is too high

6 Data-Directed Analysis

Contents

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Cone Voltage page	6-25
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Product Ion page	6-38
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Overview

For the automated MS/MS analysis of unknown compounds, the Micromass Q-Tof Premier has a powerful software control system to enable the instrument to perform data-directed analysis (DDA), switching from the MS to MS/MS mode and then returning to the MS mode using data criteria. The advantage of this method is that it removes the requirement to analyze the sample in MS mode to identify the target precursor ions and then re-run the sample in MS/MS mode to acquire the MS/MS data from each of these precursors. This is particularly valuable in the analysis of unknown samples using on-line chromatography where the target precursor ions and their retention times may well be quite different for each sample.

During acquisition, the instrument is controlled by the MS file in the MassLynx Sample List. DDA can only be acquired using the Sample List and may not be started by acquiring from the Tune window.

Precursor Ion Discovery

Precursor ion discovery allows specific targeting of molecules containing a common structural motif, e.g. post translationally modified peptides. It allows both identification and structural information to be obtained during the course of an HPLC experiment on the Q-Tof Premier. During the HPLC run the instrument is switched alternately at one-second intervals between low and high collision energy with argon in the collision cell. The quadrupole, MS1 is not mass selective, operating in the rf only mode. The first data set at low energy (4 eV) shows only the normal pseudo molecular ions. The second at higher energy shows their fragments. Wherever a precursor ion of interest occurs in the high-energy data, all its possible precursors are revealed by the corresponding 4 eV data.

Since the two data sets contain the entire set of product and precursor ions that can be formed, it is also clearly possible to generate the equivalent of a constant neutral loss scan.

Example: This is invaluable in the case of phosphorylated peptides where the neutral loss of 98da (H_3PO_4) occurs via ß-elimination from the phosphoserine and phosphothreonine residues. This allows the Q-Tof Premier to switch from the MS mode to the MS/MS mode of operation when a potential pseudo molecular ion exhibits a neutral loss of 98 Da between the high energy and low energy data sets. The precursor ion MS/MS spectrum can then be acquired on the phosphorylated precursor ion.

Setting-up an Experiment

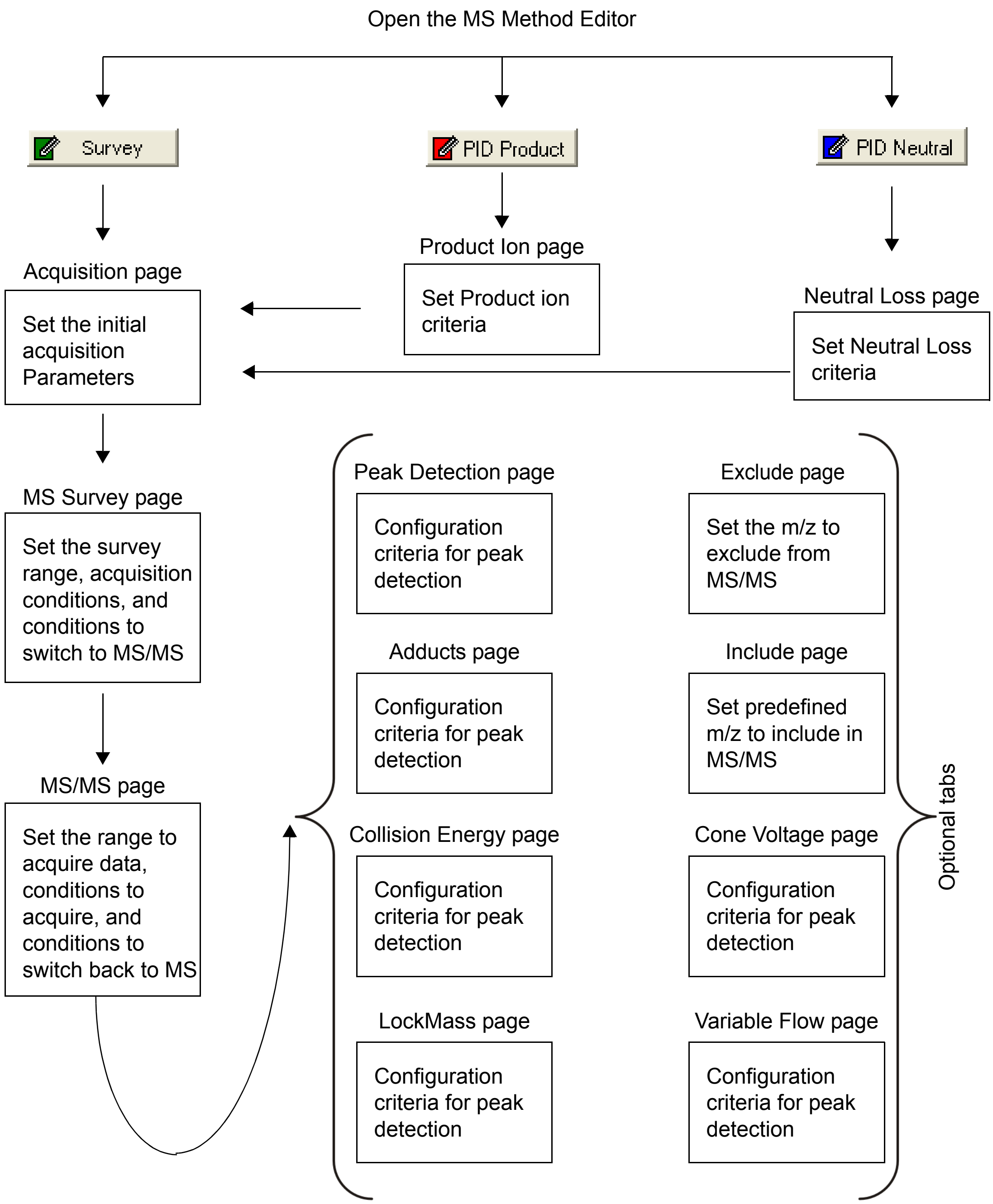
DDA experiments are set up from the MS Method editor. There are three different types od DDA acquisitions:

- Survey - Automatic selection of precursors for MSMS
- PID Product - Precursor Ion Discovery by product ion detection
- PID Neutral - Precursor Ion Discovery by neutral loss detection

Experiment parameters and MS > MSMS > MS switching criteria are entered into a series of pages that are largely common to all three DDA experiment types. These are shown diagramatically in the Figure titled "DDA flow diagram:" on page 6-4.

The simplest Survey experiment can be set up using just the Acquisition page, MS Survey page and MS/MS page. More advanced selection criteria can be entered on other pages. For PID and PID Neutral further criteria have to be entered into Product Ion page and Neutral Loss page respectively.

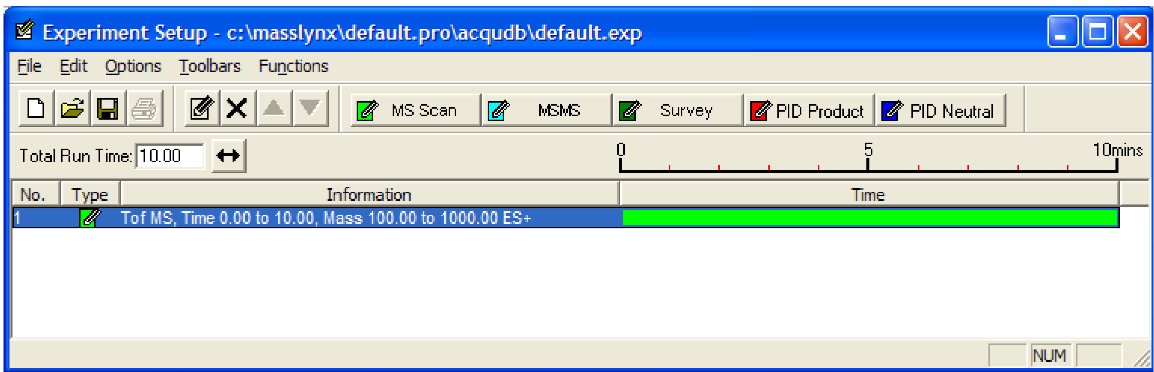
DDA flow diagram:



To Create a DDA Experiment:

1. From the MassLynx window click the MS Method icon to open the MS Method Editor.

   MS Method Editor:



2. Select the default entry that appears in the MS Method Editor.
3. Click , to delete the selected entry.
4. Select the type of experiment you want to perform, click either:
   - Survey
   - PID Product
   - PID Neutral

   Result: The relevant DDA dialog box opens.
5. Set the experiments parameters on the pages.
6. Save the *.exp method file.

Acquisition page

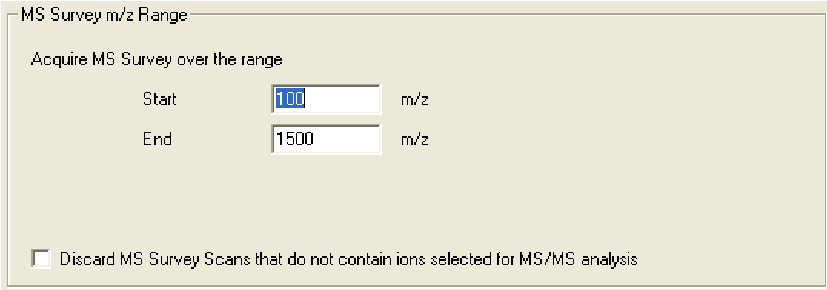
The Acquisition page is used for setting the initial conditions for the experiment. It is common to all Function scans and is described in Chapter 4 on page 4-19.

MS Survey page

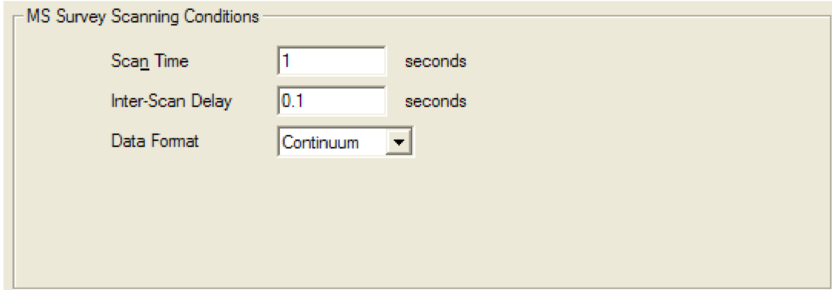
Using the MS Survey page, you can configure the following parameters:

- The m/z range over which to acquire data. (MS Survey m/z Range on page 6-6)
- The conditions that the data will be acquired (MS Survey Scanning Conditions on page 6-7).
- The conditions at which the survey scan is stopped and the acquisition switches to MS/MS mode (Stop the MS Survey on page 6-8).

MS Survey m/z Range

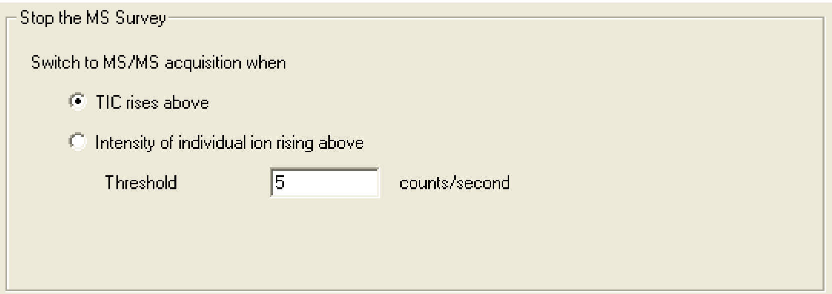


MS Survey Scanning Conditions



Parameter	Description
Inter-Scan Delay	Normally set to 0.02 s.
Data Format	The data format used to save the scans: • Centroid • Continuum **Important:** MaxEnt3 and PLGS require data in continuum format. **See also:** Types of Data Acquisition on page 4-17.

Stop the MS Survey



Parameter	Description
TIC rising above threshold	When selected, the MS scan stops and the MS/MS scan starts when the Total Ion Current (TIC) of the scan rises above the specified threshold value.
Intensity of individual ion rising above	When selected, the MS scan stops and the MS/MS scan starts when the intensity of an individual ion is above the specified threshold value.
Threshold	The value at which the switch to MS/MS acquisition is made.

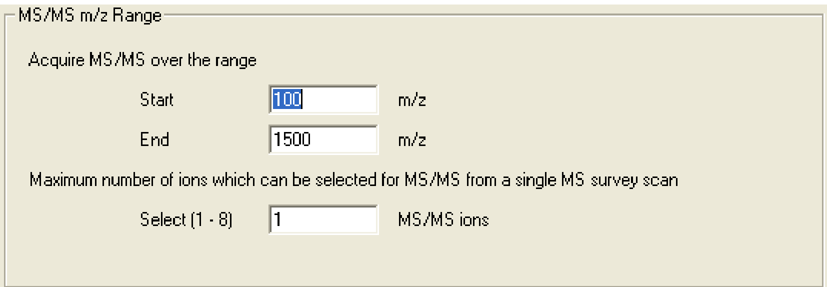
MS/MS page

Using the MS/MS page, you can configure the following parameters:

- The m/z range over which to acquire data. (MS/MS m/z Range on page 6-9)
- The conditions that the data will be acquired (Scan rates and Instrument Conditions for MS/MS on page 6-9).
- The conditions at which the MS/MS acquisition is stopped and the acquisition switches back to MS Survey (Stop MS/MS (Survey experiment) on page 6-10).

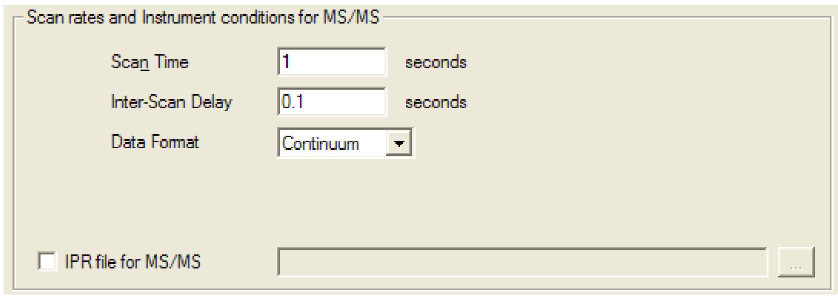
Tip: The parameters that are used to stop the acquisition are different depending whether you are doing a Survey or PID experiment

MS/MS m/z Range



Parameter	Description
Acquire MS/MS over the range	
Start m/z	The mass at which the MS/MS scan starts.
End m/z	The mass at which the MS/MS scan stops.
Maximum number of ions which can be selected for MS/MS from a single MS survey scan	
Select (1-8)	Specifies the maximum number of simultaneous MS/MS functions.

Scan rates and Instrument Conditions for MS/MS

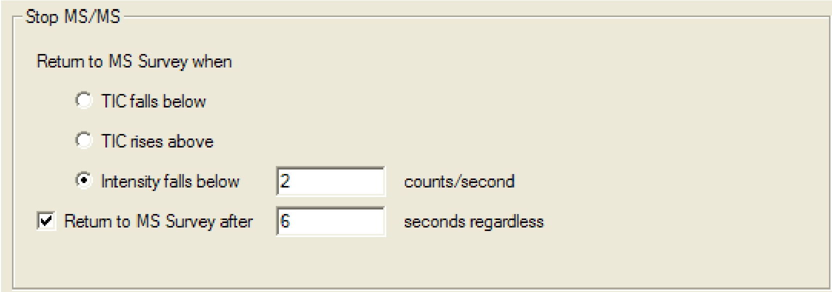


Parameter	Description
Scan Time	The scan duration of the MSMS scans.
Inter-Scan Delay	Normally set to 0.02 s.

Parameter	Description
Data Format	The data format used to save the scan in: • Centroid • Continuum See also: Types of Data Acquisition on page 4-17.
IPR file for MS/MS	When selected, the cone voltage defined in the tune file in the adjacent box will be used. Type the file name in the box or use [...] to browse for a file.

Stop MS/MS (Survey experiment)

The decision to Stop MS/MS can be made using the TIC or individual ion intensity. Since multiple precursors may be selected for concurrent MS/MS experiments it is usual to set this to Intensity and a “Return to MS Survey” after value.



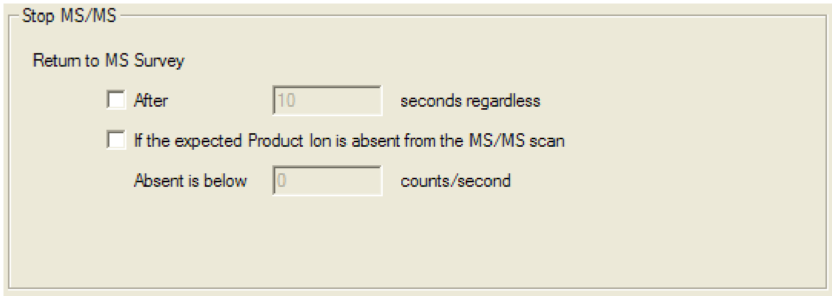
In the figure above the switch would occur after 6 seconds assuming the intensity of the base peak in the MS/MS spectrum had not fallen below 2 cps. These rules apply on a per precursor basis.

Example: If MS/MS were concurrently running on 3 precursors and one MS/MS spectrum fell below 2 cps then MS/MS would be terminated on that precursor. The MS/MS on the remaining 2 precursors would continue until either they fell below the 2 cps threshold or each MS/MS precursor ion had been acquired for 6 seconds. If the intensity threshold were set to 0 cps then

MS/MS acquisition would continue on all three functions until a total of 18 seconds had elapsed (6 seconds for each precursor)

Parameter	Description
TIC falls below	When selected, the MS/MS scan stops and switches to MS scan when the TIC of the MS/MS scan falls below the specified threshold value.
TIC rises above	When selected, the MS/MS scan stops and switches to MS scan when the TIC of the MS/MS scan rises above the specified threshold value.
Intensity falls below	When selected, the MS/MS scan stops and switches to MS scan when the Base Peak Intensity (BPI) falls below the specified value.
Return to MS Survey after	When selected, specifies a time, (in seconds) after which, if non of the threshold criteria are met, the MS/MS scan stops and returns to the MS scan. Typical values are from 3 s upwards, depending upon the chromatography and number of concurrent MS/MS spectra.

Stop MS/MS (PID Experiment)



Parameter	Description
After	Select to specify the maximum time that will pass before returning to MS survey

Parameter	Description
If the expected Product Ion is absent from the MS/MS scan	Select this to return to the MS scan if the product ion is not found. **Tip:** For a Neutral Loss experiment this parameter is the same but “Product Ion” is replaced with “Neutral Loss”.
Absent is below	Enter the number of counts per second at which the software will consider an ion absent.

Include page

The Include tab allows a number of predefined m/z values to be included in the decision making process. Once enabled you can either:

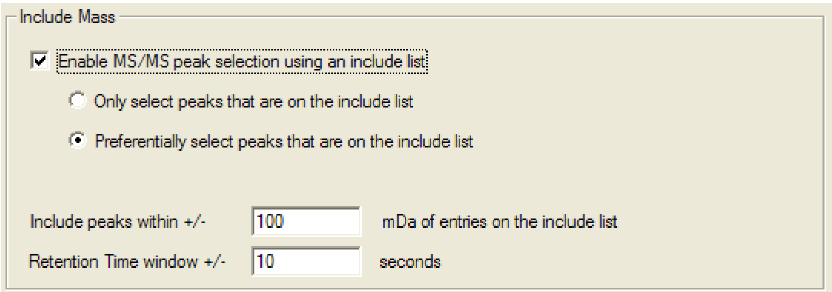
- Only select ions that are on the list

  Or

- Select the ions preferentially

Tip: The selection criteria entered here only apply to Survey experiments. This page is not available for PID experiments.

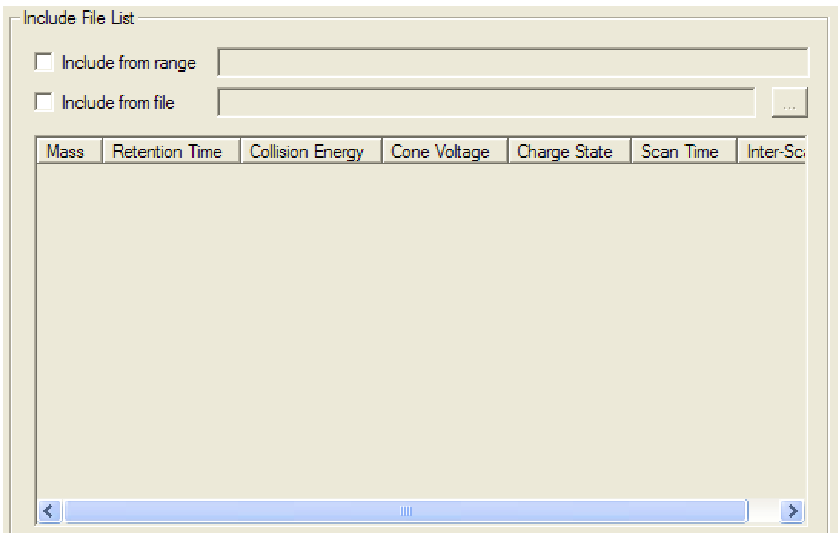
Include Mass



Parameter	Description
Enable MS/MS peak selection using an include list	When selected, allows a number of predefined mass values to be used as switching criteria from MS to MS/MS. There are two distinct modes of operation.

Parameter	Description
Only select peaks that are on the include list	When selected, only masses on the Include list will be selected for MS/MS analysis.
Preferentially select peaks that are on the include list	When selected, masses include on the Include list have priority over any other mass detected for MS/MS.
Include peaks within +/-	Specifies the mass tolerance value to be applied to all include masses.
Retention Time Window +/- ...mDa of entries on the include list	Specifies a tolerance to apply to the retention times in the include list.

Include File List



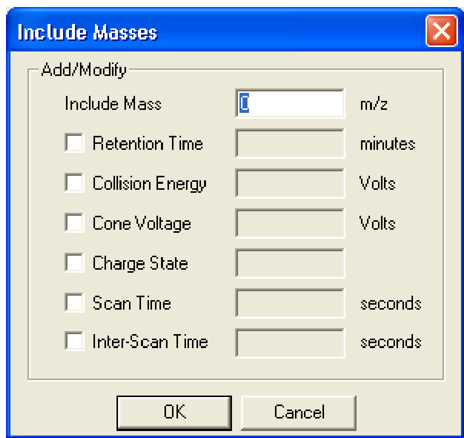
Parameter	Description
Include from range	Specifies the required masses, or range of masses, to be used from those in the Include List. Use commas to separate individual masses. Use an underscore to denote a range of masses, for example, 510, 520, 550_600, 700.
Include from file	When selected, the name of an existing Include List file can be entered in the adjacent text box. Alternatively, use ... to browse for an existing file. Specific parameters (Retention Time, Collision Energy, Cone Voltage, Charge State) can be associated with these m/z values, that will override parameters set elsewhere. **See Also:** Include File List on page 6-14
New	Resets all the options and clears the Include List.
Add	Opens the Include Masses dialog box, which allows the Include List to be edited. If Add is not enabled, select Include from Range or Include of File. **See Also:** Include File List on page 6-14
Delete	Deletes the selected entry from the list.

Parameter	Description
Save	Saves the Include List details to an existing file.
Save As	Save the Include List to a new file.

To edit the Include list

1. Click Add to open the Include Masses dialog box.

   Include Masses dialog box:

   Tip: To edit an existing entry in the Include List, double-click it.

2. Select the check box for the required option and enter the value in the adjacent text box.

   Tip: The text box becomes available only when you select an option.

   The Include Masses dialog box parameters are described below.

Parameters	Description
Include Mass	Specifies the include mass to be added to the mass list.
Retention Time	Specifies the associated retention time, in minutes. If the specified mass elutes at the specified Retention Time and is within the Include tab Retention Time Window (+/-) value, the mass is considered to be on the Include List. This allows masses eluting from a column to be included as a mass of interest.

Parameters	Description
Collision Energy	Specifies the collision energy used to fragment the detected mass for the MS/MS scan.
Cone Voltage	Specifies the cone voltage value applied during the MS/MS scans.
Charge State	Include the mass by the charge state.
Scan Time	The scan time, in seconds, used during MS/MS scans for the detected mass.
Inter-Scan Time	The inter-scan time, in seconds, used during MS/MS scans for the detected mass.

Collision Energy page

Using the Collision Energy tab, you can configure the collision energy parameters that are used during the experiment. There are three possible options:

- Use Tune Page Collision Energy (see page 6-16)

  This is the least sophisticated option since it just uses the value for the collision energy that is taken from the Tune window.

- Use Collision Energy Profile (see page 6-17)

  The collision energy profile option allows a range of different collision energies to be used depending on the m/z value of the selected precursor. In addition from 1 to 5 collision energies may be used for each precursor m/z to attempt to produce optimal fragmentation.

- Use Charge State Recognition (see page 6-20)

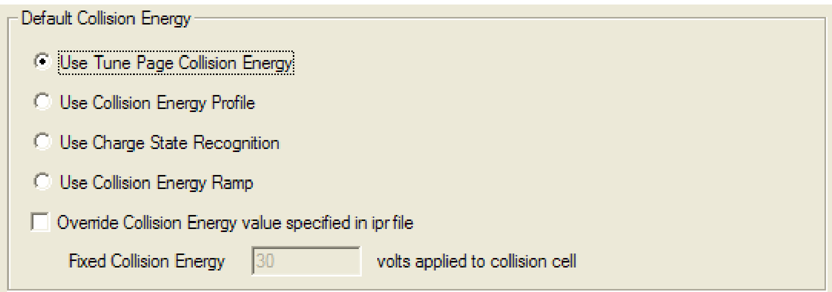
  This is the most sophisticated option. The decision that the software makes, on what collision energy to use, is based upon the m/z and the charge state.

  This mode will allow the collision energy to be set according the charge state (z) and m/z of the precursor as determined by the software. Collision energy files for charge states from 1 to 6 may be created and selected. The files specify a list of m/z values and associated collision energies. The applied energy is interpolated linearly between the specified masses within the file, so each m/z across the mass range will have different energies applied

Rationale: For peptide analysis collision energy profiling by cycling around a number of different energies is less likely to be required.

- Use Collision Energy Ramp (see page 6-24).

Default Collision Energy pane:



Parameter	Description
Override collision energy value specified in ipr file	When selected specifies the collision energy to be used

Collision Energy Profile parameters

Using the Collision Energy tab, Collision Energy Profile parameters, you can set up a list of collision energy profiles for a range of m/z values.

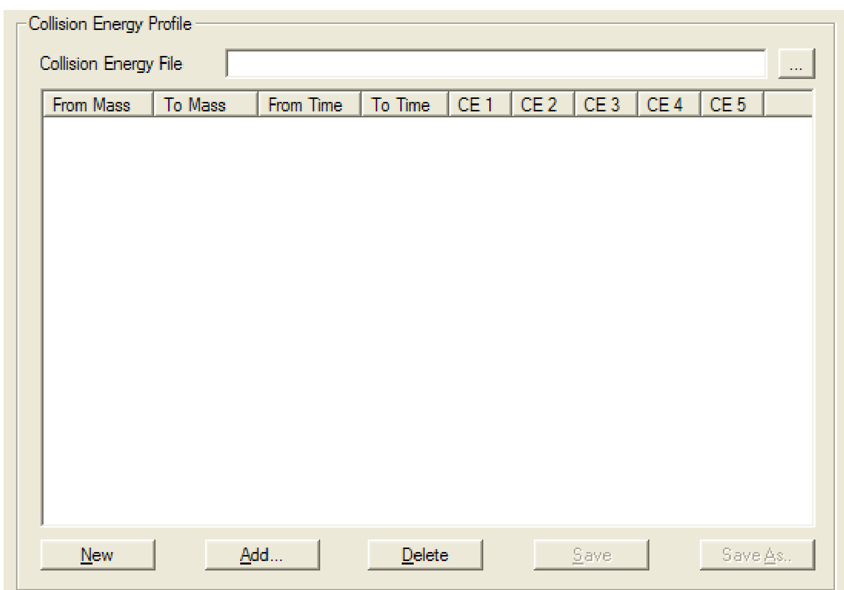
This option allows a range of different collision energies to be used depending of the m/z value of the selected precursor. In addition 1 to 5 collision energies may be used for each precursor to attempt to produce optimal fragmentation.

Example: If 4 collision energies are selected, the scan pattern for a single MSMS function is as follows:

Scan	1	2	3	4	5	6	7	8	9	10	11
CE	1	2	3	4	1	2	3	4	1	2	3

To enable the Collision Energy Profile Parameters:

Select the “Use Collision Energy Profile” option.

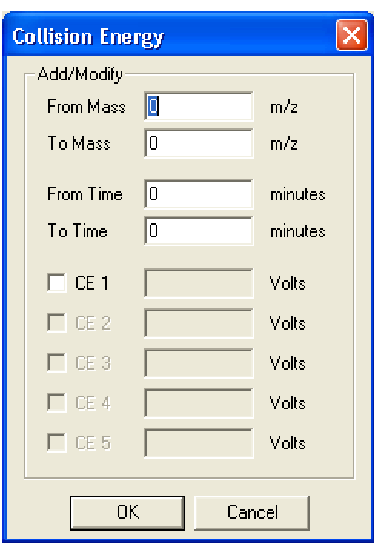


Parameter	Description
Collision Energy File	Enter the file name of the Collision Energy Profile file or click ... to browse for a file.
New	Resets all the options and clears the Collision Energy Profile list.
Add	Opens the CE Profile dialog box which allows collision energy profile lists to be created for masses that are being switched on.
Delete	Deletes the selected entry from the list.
Save	Saves the Collision Energy Profile list to an existing file.
Save As	Saves the Collision Energy Profile list to a new file.

To create a Collision Energy Profile list

Using the CE Profile dialog box, you can create new collision energy profiles. The CE Profile dialog box parameters are described below.

CE Profile dialog box:



Parameter	Description
From Mass (m/z)	Start mass for the mass range in which the collision energy profile is to be used.
To Mass (m/z)	End mass for the mass range in which the collision energy profile is to be used.
From Time (min)	Start time for the time range in which the collision energy profile is to be used.
To Time (min)	End time for the time range in which the collision energy profile is to be used.
CE1 - CE5	The five allowed collision energy values, which will be applied to a relevant mass at a relevant time during MS/MS scanning. Selecting the check box for one value enables the check box for the following value.
OK	Closes the Collision Energy dialog box and enters the selected values in the CE Profile dialog box Collision Energy Profile list.

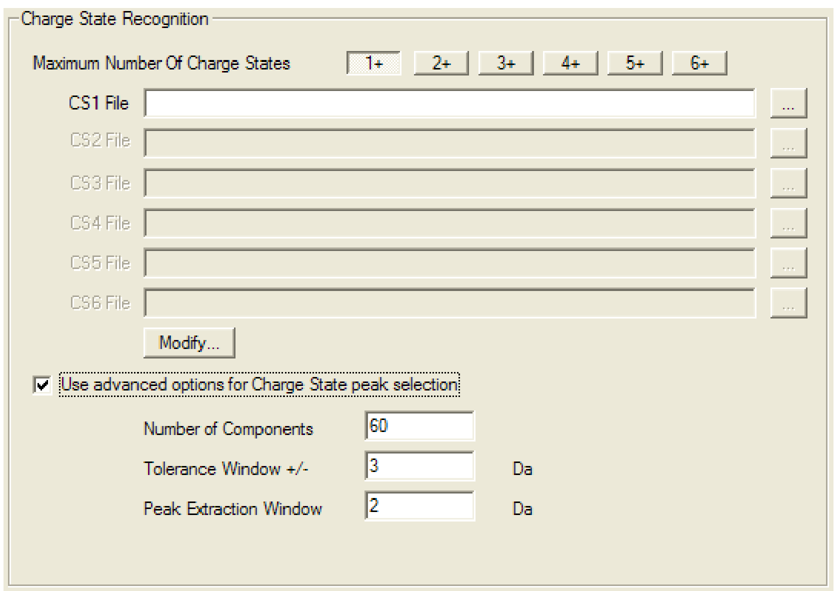
Charge State Recognition parameters

Using the Collision Energy page Charge State Recognition parameters, a mass and it’s charge state can be used to obtain a collision energy value from the Charge State list.

Example: When a mass of interest is detected in an MS experiment, its charge state is calculated. Using the mass and its charge state, a Collision Energy value can be obtained from the entered charge state table. The value is then used in the MS/MS experiment.

The Include page’s Include by Charge State and the Collision Energy page’s Use Charge State Recognition functionalities can be used independently, or both can be configured to combine their individual functionalities.

See also: Charge State Recognition functionality on page 6-23.



Parameter	Description
CS1 File - CS6 File	Enter the file name of the required charge state file, or click ... to browse for a file.
Modify	Displays the Modify Charge State dialog box; this is used to create, or modify charge state files.

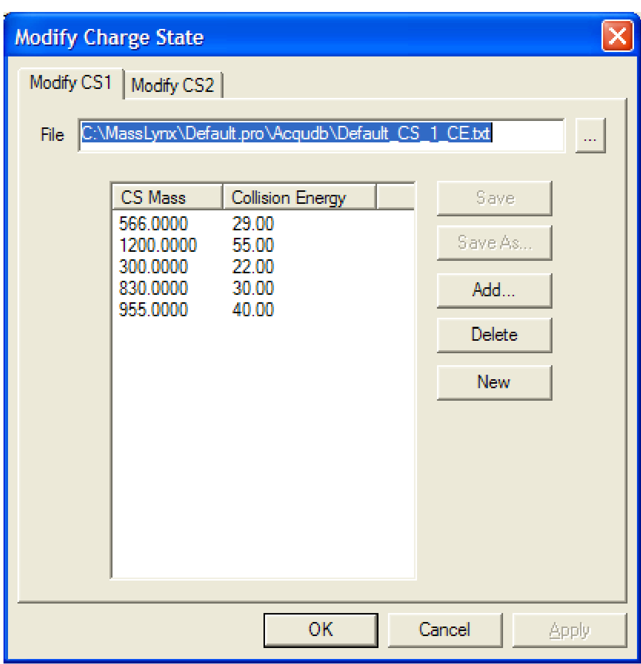
Parameter	Description
Use advanced options for Charge State peak selection	When selected, allows you to specify the following advanced settings. If advanced options are not selected, the default values are used. See Charge State Peak Selection Parameters on page 6-32 for more details.

Creating or modifying Charge State files

Using the Modify Charge State dialog box, you can create or modify charge state files. The examples shown are a set of suggested collision energy profiles for peptides or protein digest applications when selecting M^{2+} ions for MS/MS analysis.

Tip: Default files are included with MassLynx, which you can edit.

Modify Charge State dialog box:



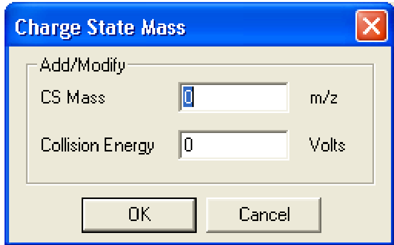
To open the modify charge state dialog box

1. On the Survey Scan Collision Energy tab, select Use Charge State Recognition.
2. Enter the required Maximum Number of Charge States.
3. Click Modify to open the Modify Charge State dialog box.

   Tip: The number of Modify CS tabs displayed in this dialog box corresponds with the Maximum Number of Charge States selected in step 2.

To add a new Charge State:

1. From the Modify Charge State dialog box, click Add to open the Charge State Mass dialog box.



Charge State Mass parameters

Button	Description
CS mass (m/z)	Specifies the mass of interest.
Collision Energy	Sets the correct collision energy value for the mass of interest.
OK	Closes the Charge State Mass dialog box and enters the selected values in the Modify Charge State dialog box Mass List.

2. Enter a charge state mass value.
3. Enter a collision energy value.
4. Click OK. The new charge state mass is added to the list in the Modify Charge State dialog box.

Charge State Recognition functionality

You can apply charge state recognition functionalities from the Survey Scan Include and Collision Energy tabs. The functionalities, Include by Charge State and Use Charge State Recognition, can operate independently or together.

For peptide work the Use Charge State Recognition option is most useful as the diversity of peptide charge states and masses requires application of a large variation of collision energies. Importantly, the precursor isolated switches after each scan, in contrast to the Collision Energy Profile. This provides a more efficient profiling of the chromatographic peak.

Using Charge State Recognition with Include by Charge State disabled

This allows the software to switch on any peak of interest, but a collision energy value will be calculated only for those masses with a charge state that matches those set up in the Charge State Recognition frame. For all other masses, the default collision energy will be applied; this is either the Tune window value, or the value specified in the Survey Scan dialog box MS/MS Template tab.

For example, if the Include by Charge State option is disabled, and the Charge State Recognition has been configured for Charge States 1 and 2, any detected mass of interest switches on in the normal way. Masses switched on with a charge state of 1 or 2 will have a collision energy value calculated from the Charge State Recognition table. All other masses use the specified default collision energy value.

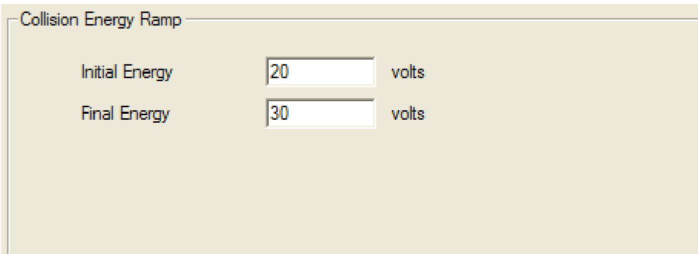
Using Charge State Recognition with Include by Charge State enabled

This restricts the masses that are switched on to those with the correct charge state. A collision energy value will be calculated for those masses with a charge state that is configured in both Include by Charge State and Charge State Recognition. For masses with a charge state which is only on the Include by Charge State list, a default collision energy value will be used.

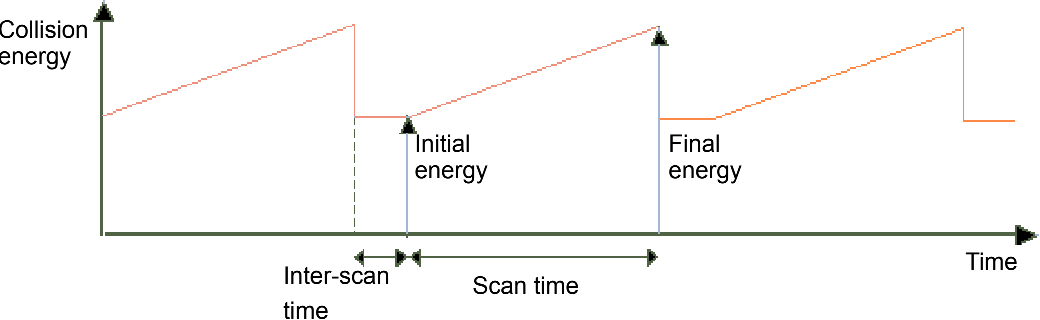
For example, if the Include by Charge State section is configured to allow charge states of 2 and 3, and the Charge State Recognition section is configured for Charge States 1 and 2, only masses with a charge state of 2 or 3 are switched on. Masses switched on with a charge state of 2 will have a collision energy value calculated from the Charge State Recognition table.

Masses with a charge state of 3 will use the specified default collision energy value.

Collision Energy Ramp



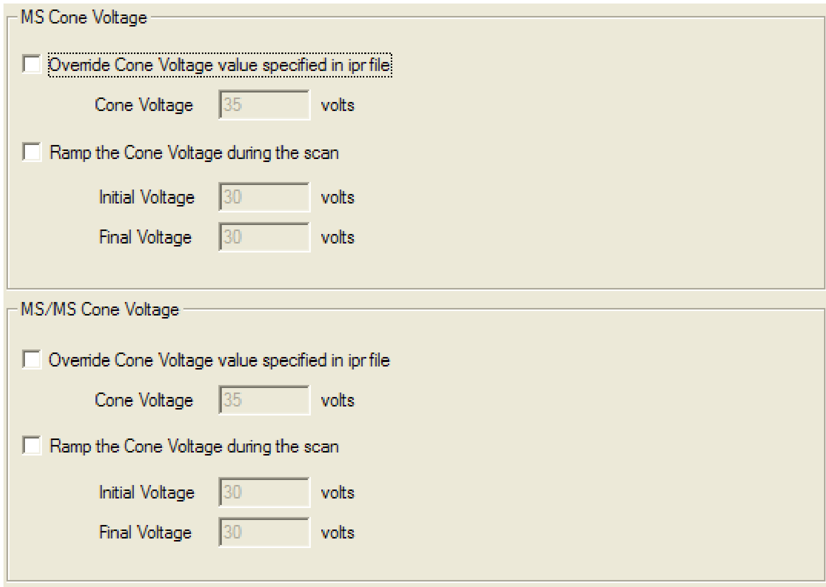
This function ramps the collision energy between two user-specified limits during each individual scan.



To ramp the collision energy:

Enter an initial voltage and a final voltage.

Cone Voltage page



For both MS and MS/MS mode the cone voltage specified in the tune file can be over ridden by checking the box and entering a value.

In addition the cone voltage can be ramped during each scan from an initial low value to a final higher value, for both MS and MS/MS

To ramp the cone voltage:

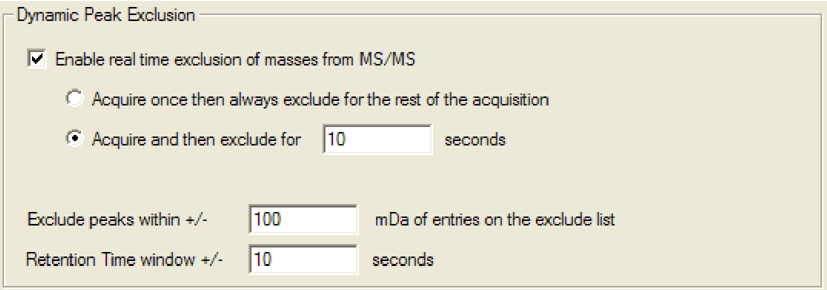
1. Check "Ramp the Cone Voltage during scan"
2. Enter an initial voltage and a final voltage.

Exclude page

The Exclude tab allows predefined masses to be excluded from selection for MS/MS analysis. There are two types of peak exclusion:

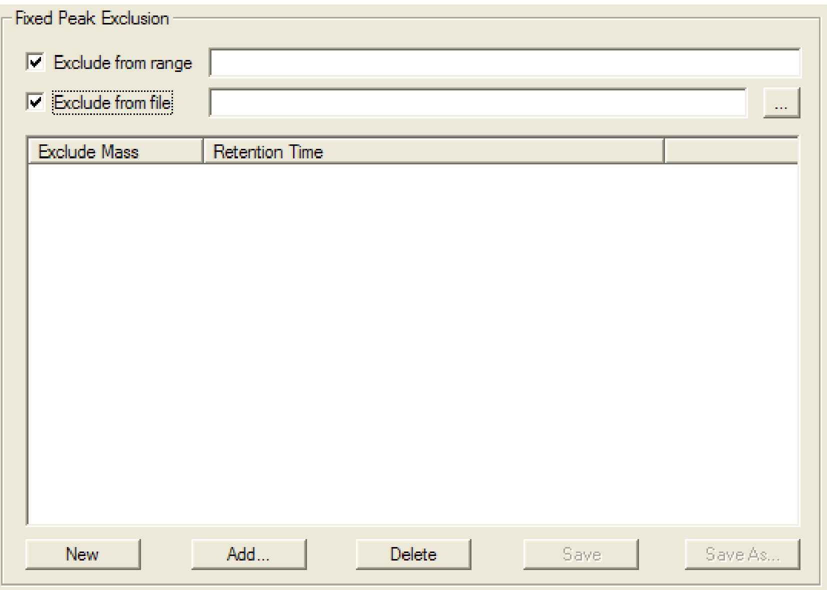
- Dynamic Peak Exclusion on page 6-26
- Fixed Peak Exclusion on page 6-27

Dynamic Peak Exclusion



Parameter	Description
Enable real time exclusion of masses from MS/MS	When selected, enables real time exclusion option.
Acquire once then always exclude for the rest of the acquisition	When selected, automatically excludes masses from MS/MS scans after their first acquisition.
Acquire and then exclude for (seconds)	When selected, allows masses to be excluded for a specific amount of time. Using the Exclude Mass dialog box, you can specify the amount of time for which the mass is excluded.
Exclude peaks with +/- ...mDa of entries on the exclude list	Specifies a mass tolerance value which will be applied to all exclude masses.
Retention Time window +/- (seconds)	Specifies a tolerance to apply to the retention times in the exclude list.

Fixed Peak Exclusion



Parameter	Description
Exclude from range	When selected, allows mass ranges to be specified so that any masses detected within these mass ranges will be excluded. Separate individual masses with commas. Use an underscore to denote a range of masses, for example, 510, 520, 550_600, 700. **Tip:** This is only suitable for a small number of components. For larger numbers use "Exclude from file"
Exclude from file	When selected, specifies the exclude mass file name or click ... to browse for a file. **Tip:** This is particularly useful for large numbers of components, particularly for components or impurities not permanently present.
New	Resets all the options and clears the Exclude List.

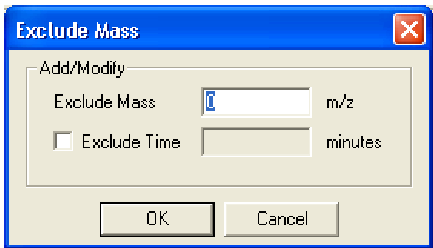
Parameter	Description
Add	Opens the Exclude Mass dialog box, which allows the Exclude List to be edited. **See also:** To edit the Exclude list on page 6-28.
Delete	Deletes the selected entry from the list.
Save	Saves the Exclude List details to an existing file.
Save As	Saves the Exclude List details to a new file.

To edit the Exclude list

1. On the Survey Scan dialog box Exclude page, click Add to open the Exclude Mass dialog box.

   Tip: To edit an existing entry in the Exclude List, double-click an entry.

2. Enter the values as required.



The parameters in the Exclude Mass dialog box are described below.

Parameter	Description
Add/Modify	
Exclude Mass (m/z)	Specifies mass to exclude from the acquisition.
Exclude Time	When selected, specifies the exclude time in minutes. If a mass is within the Exclude tab Retention Time Window value, it will be excluded from the acquisition.

Adducts page

If enabled, adduct masses are excluded from the MS/MS experiment.

If a mass of interest (target mass) on the Include List is detected, the mass spectrometer is switched to MS/MS operation and that mass is added to the Exclude List, so that it is ignored if detected again. Also, when the target mass is detected, any mass in the Adduct page adduct list is added to that target mass, and the resulting mass added to the Exclude List, so that the adduct is also ignored if it is detected.

Example: If the molecular target mass is (M), the target mass (m/z) on the Include List is (M + H+). When detected, a switch to MS/MS operation is triggered and the mass is added to the Exclude List.

A typical adduct is sodium (Na, mass 23), for which the target adduct mass is M + Na+. Hence the value to be added to the Adduct page adduct list is (M + Na+) - (M + H+) = (223 - 201) = 22.

Similarly, if the adduct is ammonium (NH4, mass 18), the target adduct mass is (M + NH4+). Hence the value to be added to the Adduct page adduct list is (M + NH4+) - (M + H+) = (218 - 201) = 17.

When a mass of interest is found, and an Adduct list exists, the function switching software generates adduct masses on the Exclude list. This is to stop the adducts of a mass being switched on in future MS/MS scans.

To add adduct masses to the Adduct List:

1. Select Use Adduct masses to enable the Adduct List
2. Click Add, or double-click an existing mass in the Adduct list to open the Adduct Mass dialog box.
3. Enter the required value in the Add/Modify, Step Size (m/z) text box.
4. Click OK to add the value to the Adduct list on the Adduct tab.

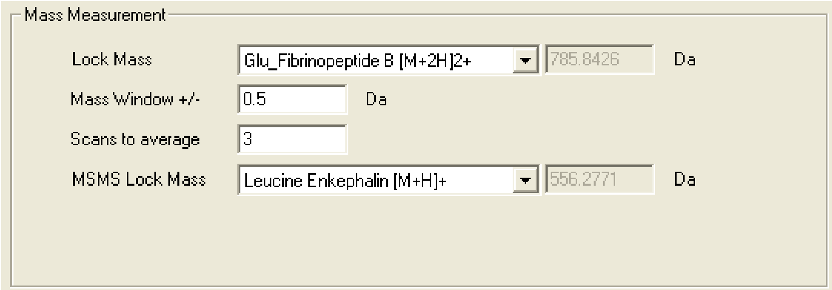
LockMass page

Using the LockMass page, you can specify the frequency of the reference data for accurate mass and the conditions at which to acquire that data. The LockMass page Reference Scan parameters are described in LockMass page on page 4-24. In addition to the Mass Measurement parameters described earlier there is the ability to set dual lock masses

Dual lock masses

For best mass accuracy, singly-charged peaks should be lock-massed using a singly-charged reference, and multiply charged peaks should be lock-massed using a multiply-charged reference.

For example, when analyzing multiply-charged peptides, set the survey lock mass to a doubly-charged peak such as Glu-Fibrinopeptide m/z 785.8, and set the MSMS lock mass to a singly-charged peak such as leucine enkephalin m/z 556.3. This ensures optimum mass accuracy on the singly-charged peptide.



Parameter	Description
Lock Mass	Use this lock mass for survey scans
MSMS Lock Mass	Us this lock mass for MS/MS scans

Peak Detection page

Using the Peak Detection tab you can specify additional selection criteria.

Caution: These parameters should only be used by advanced users. They are advanced options and would normally be left at the default settings

Peak Selection

Apply no criteria other than intensity (as specified on MS Survey tab) for peak selection

Charge State Peak selection

Isotope Pattern selection

Deisotope Peak selection

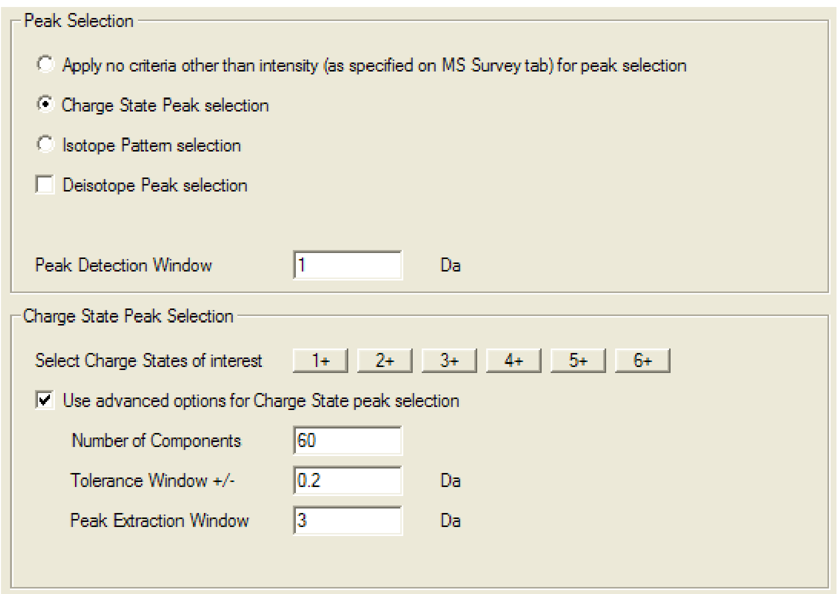
Peak Detection Window 1 Da

Parameter	Description
Apply no criteria other than intensity (as specified on the MS Survey tab) for peak selection	The default setting. peak selection is by intensity only.
Charge State Peak selection	When selected, the Charge State Peak detection parameters will be used to find peaks of interest. **See also:** • Charge State Peak Selection Parameters on page 6-32. • How peak detection works on page 6-34
Isotope Pattern selection	When selected, Isotope Pattern detection algorithms will be used to find peaks of interest in the MS scan. **See also:** • Isotopic Pattern selection on page 6-34. • How peak detection works on page 6-34
Deisotope Peak selection	When selected, a Deisotope Peak filter will be added to the Isotopic Pattern algorithm. **See also:** • Deisotope Peak Selection on page 6-33. • How peak detection works on page 6-34

Parameter	Description
Peak Detection Window (Da)	The window size to be used to detect peaks. Typical values for peptides are 2 to 3 Da. **See also:** How peak detection works on page 6-34

Charge State Peak Selection Parameters

The Charge State Peak selection parameters are only available when the Charge State Peak selection radio button is selected. These are typically used for multiply charged proteolytic peptide mixtures.

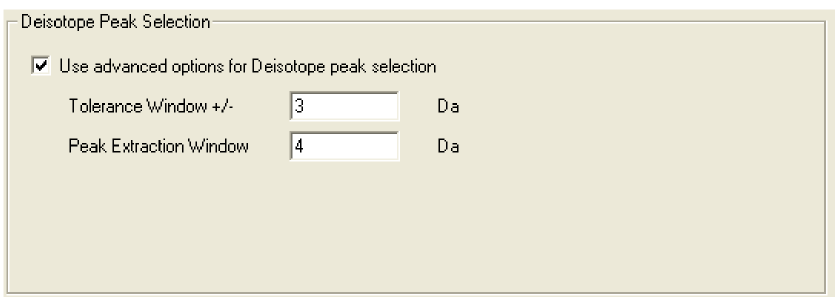


Parameter	Description
Select Charge State of interest	Select the required charge states.
Use advanced options for Charge State peak selection	When selected, enables the advanced parameters. When cleared, the default values are used.

Parameter	Description
Number of Components	Specifies the maximum number of components that are allowed to be identified, from a single MS survey spectrum. Typically a value such as 60 is used.
Tolerance Window +/-	Specifies the tolerance window for charge state determination, typical value of 0.2 Da is used.
Peak Extraction Window	For peptide work a value of 3 Da is recommended.

Deisotope Peak Selection

The Deisotope Peak selection parameters are only available when either “Apply no criteria other than intensity for peak selection” or “Charge State Peak” selection is selected.



Parameter	Description
Use advanced options for Deisotope peak selection	When selected, enables the advanced parameters. When cleared, the default values are used.
Tolerance Window +/-	Specifies the tolerance window for finding the best fit for the isotopes to determine their charge state. Usually set at half the extraction window i.e., 3.5 Da.
Peak Extraction Window	For Deisotope Peak Selection a typical value is 7 Da (half the overall peak cluster size).

How peak detection works

To prevent the selection of more than one isotope peak from a single chromatogram, a peak detection window is set. The size of this window will determine which peak of an isotopic cluster is marked as a potential candidate for an MS/MS experiment. Using a small peak detection window i.e., 0.5 Da, in the case of a doubly charged species, all the peaks in a cluster can be individually selected. If the peak window is increased, only the most abundant one within the window will be selected for MS/MS. Typical values for peak detection window for peptides are 2 to 3 Da.

When Deisotope Peak Selection is selected, an area of spectra is extracted and passed to the Charge State Recognition routine. The Extraction Window needs to be wide enough to extract all the peaks within the cluster or a false answer could be produced. The minimum and maximum number of charges to be considered is set by selecting the appropriate Charge State buttons. For proteolytic peptides, 2X, 3X and 4X charged components are normally considered. The charge state algorithm firstly calculates theoretical isotope distributions for the identified component mass for all charge states that have to be considered.

In the case of Deisotope Peak Selection the Peak Extraction Window should cover all the peaks in the cluster. If the extraction window is too small the Deisotope routine would return an incorrect mass.

Isotopic Pattern selection

The Isotopic Pattern parameters allow you to specify the Isotopic peak detection algorithm to be used to detect peaks of interest from the MS Survey.

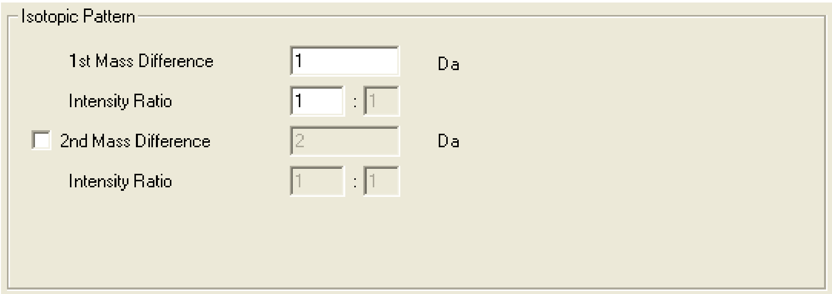
There are two frames of parameters:

- Isotopic Pattern
- Detection Parameters

In the case of Isotope Pattern selection, the peaks are detected as previously described and the Peak Detection Window should be set to 2 Da as before. A specific isotope pattern can be specified in the Isotopic Pattern field and thus only components fulfilling these criteria will be selected for an MS/MS experiment.

Tip: Isotope Pattern selection is particularly useful in case the targeted precursors exhibit a specific distribution, such as chlorinated or brominated molecules.

Isotopic Pattern parameters



Parameter	Description
1st Mass Difference	Specifies the first mass difference for isotopic selection.
Intensity Ratio	Specifies the intensity ratio for the first mass difference.
2nd Mass Difference	Specifies the second mass difference for isotopic selection.
Intensity Ratio	Specifies the intensity ratio for the second mass difference

Detection Parameters

The parameters are only available when the Isotope Pattern selection radio button is selected. The Detection Parameters allow the tolerances, of the following parameters, be used to detect the presence of the specified isotope pattern and subsequently switch to perform an MS/MS experiment.

Detection Parameters

Only consider peaks that have

Intensity above 10 counts/second

Mass within +/- 100 mDa

☑ Only consider peaks within a ratio of

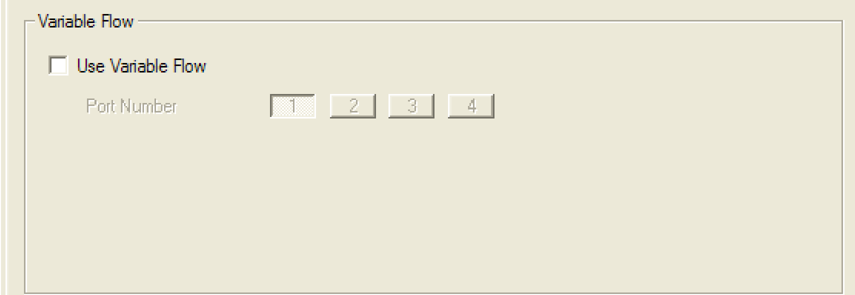
Intensity Ratio Tolerance 30 %

Parameter	Description
Intensity above	Considers peaks that have an intensity above the specified value.
Mass within +/-	Considers peaks that have a mass within the specified range.
Only consider peaks within a ratio of	When selected, enables the Intensity Ratio Tolerance parameter.
Intensity Ratio Tolerance	Considers peaks within the specified intensity ratio tolerance range.

Variable Flow page

Using the Variable Flow tab, you can define the port which is used to connect the instrument to the LC. When configured, a pulse is sent to the selected port, enabling switching to start and stop.

Variable Flow page:

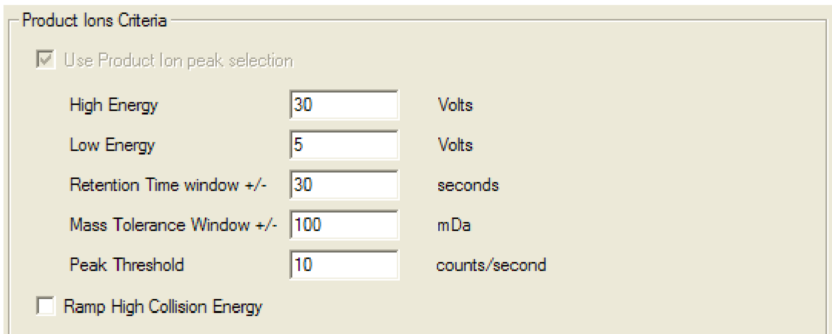


Parameter	Description
Use Variable Flow	When selected, allows you to select a port number.
Port Number	Specifies the port number to be used for variable flow during acquisition, when a scan switch occurs.

Product Ion page

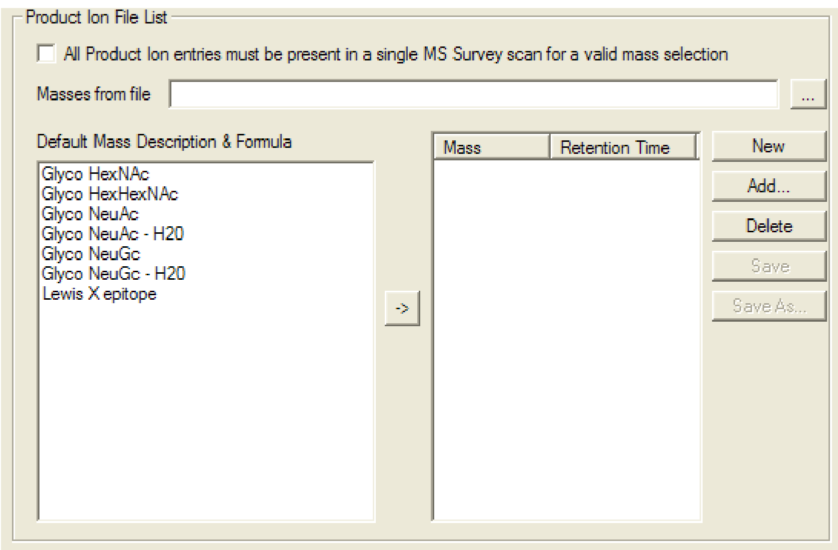
This page is only present in PID experiments.

Product Ions Criteria



Parameter	Description
High Energy (volts)	Specifies the collision energy to be used during the low energy survey scan.
Low Energy (volts)	Specifies the collision energy to be used during the high energy survey scan.
Retention Time Window +/- (seconds)	Specifies a tolerance to apply to the retention times in the product ion list.
Mass Tolerance Window +/- (mDa)	Specifies the mass tolerance to be placed around each mass on the product ion list.
Peak Threshold	The minimum peak intensity that will be selected for MSMS.
Ramp High Collision Energy	Ramps the collision energy in the high energy survey scan between two specified values.

Product Ions File List

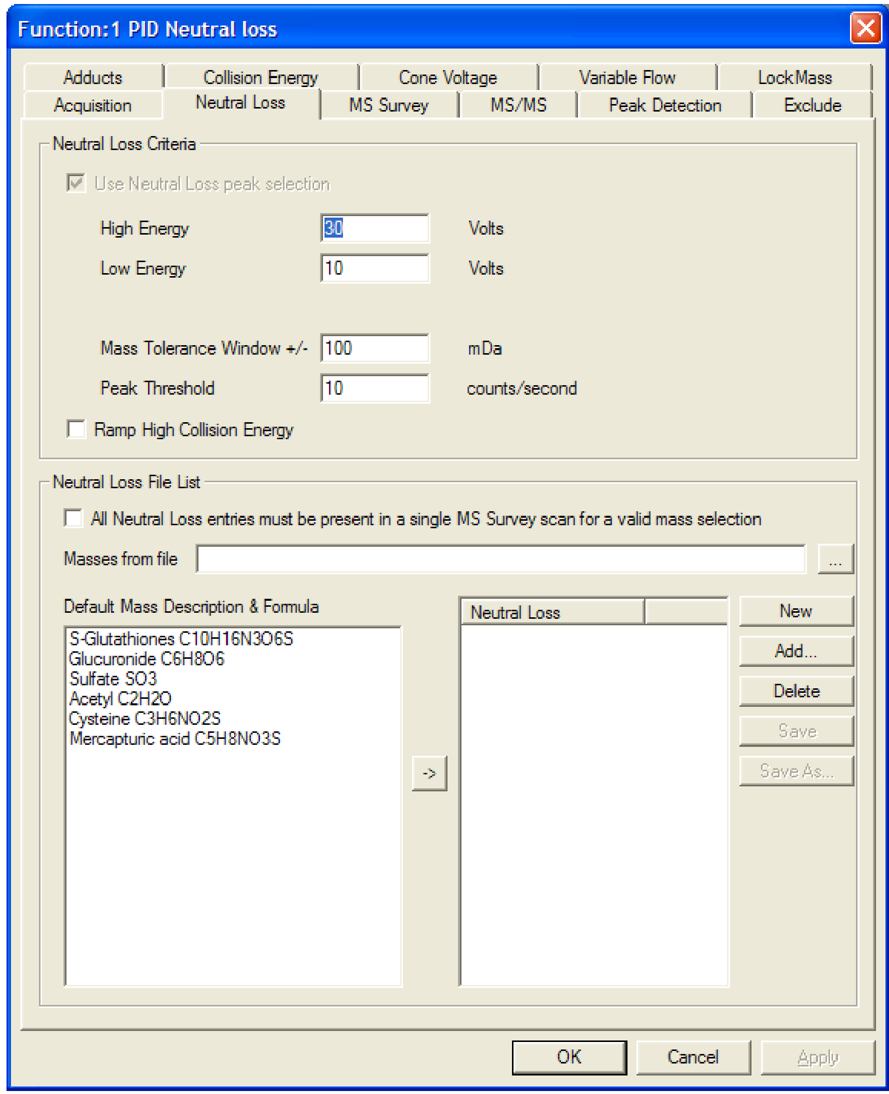


Parameter	Description
All product ion entries must be present in a single MS Survey can for a valid mass selection	Specifies that all masses in the Product list must appear in a single spectrum for a switch to MS/MS to occur.
Masses from File	Enter the file name or use ... to browse for a file.
New	Resets all the options and clears the Product Ion list.
Add	Opens the Product Ion dialog box, which allows the list to be edited.
Delete	Deletes the selected entry from the list.
Save	Saves the Product Ion details to an existing file.
Save As	Saves the Product Ion details to a new file.

Neutral Loss page

This page is only present in PID neutral experiments.

Neutral Loss page:



There are ten tabs in the Neutral Loss dialog box, which allow you to access all the parameters for the analysis experiment. The Neutral Loss dialog box

comprises the same tabs as the Product Ions dialog box, with the exception of the Neutral Loss page.

Using the Neutral Loss tab, you can configure a list of neutral loss masses to search for during the parent switch experiment. The Neutral Loss page parameters are described in the following tables.

Neutral Loss Criteria

Parameter	Description
Use Neutral Loss peak selection	When selected, allows product ions to be used during the acquisition. **Tip:** When not selected all the parameters described below are greyed out.
High Energy (volts)	Specifies the collision energy to be used during the low energy survey scan.
Low Energy (volts)	Specifies the collision energy to be used during the high energy survey scan.
Mass Tolerance Window +/- (mDa)	Specifies a tolerance to apply to the retention times in the product ion list.
Peak Threshold (counts/seconds)	The minimum peak intensity that will be selected for MSMS.
Ramp High Collision Energy	Ramps the collision energy in the high energy survey scan between two specified values.

Neutral Loss File List

Parameter	Description
All Neutral Loss entries must be present in a single MS Survey scan for a valid mass selection	Specifies that all masses in the Neutral Loss list must appear in a single spectra for a switch to MS/MS to occur.
Masses from File	Specifies the include mass file name to be used during the acquisition.
New	Resets all the options and clears the Neutral Loss List.

Parameter	Description
Add	Opens the Neutral Loss dialog box, which allows the Exclude List to be edited. **See also:** To edit the Neutral Loss list on page 6-42).
Delete	Deletes the selected entry from the list.
Save	Saves the Neutral Loss details to an existing file.
Save As	Saves the Neutral Loss details to a new file.

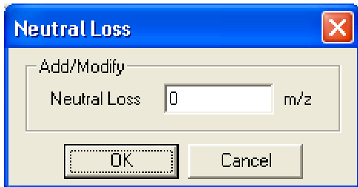
To edit the Neutral Loss list

1. On the Neutral Loss dialog box, Neutral Loss tab, click Add, to open the Neutral Loss dialog box.

   Tip: To edit an existing entry in the Exclude List, double-click an entry.

2. Enter the values as required.

   The table below describes the parameters in the Neutral Loss dialog box.



7 APCI, APPI and ESCi

The Q-TOF Premier Modular LockSpray source can be configured to operate in three different ionization modes: ESI, APCI and APPI. In addition it is capable of two combined ionization modes: ESI / APCI (ESCi) and APCI / APPI (Dual APPI).

ESI operation is described earlier; this chapter covers the other options.

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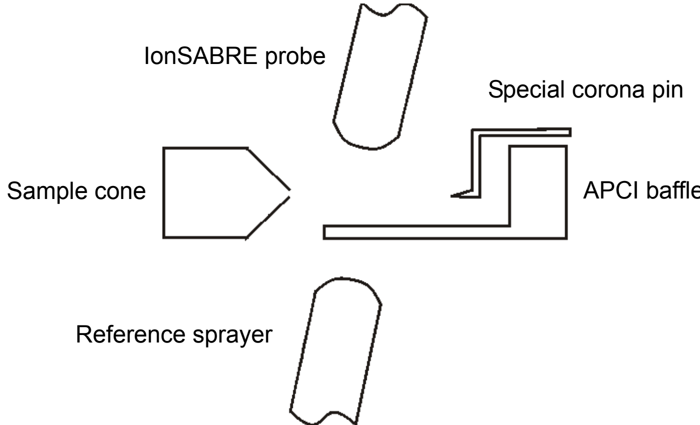
About APPI mode	7-3
About dual APPI	7-4
About ESCi LockSpray	7-5
APPI and dual APPI operation	7-17
ESCi operation	7-26
Creating MS method files for dual mode ionization	7-30

About IonSABRE APCI mode

Atmospheric Pressure Chemical Ionization (APCI) produces singly-charged protonated or deprotonated molecules from a broad range of non-volatile analytes.

The Q-TOF Premier modular LockSpray source is configured for IonSabre LockSpray APCI by the addition of an IonSabre probe, a special corona pin and an APCI baffle. Mobile phase from the LC column enters the probe, where it is pneumatically converted into an aerosol, rapidly heated, and converted to a vapor/gas at the probe tip.

APCI mode:



Hot gas from the probe passes between the sample cone and the corona discharge pin, which is typically operated with a discharge current of 5 µA. Mobile phase molecules rapidly react with ions generated by the corona discharge to produce stable reagent ions. Analyte molecules introduced into the mobile phase react with the reagent ions at atmospheric pressure and typically become protonated (in the positive ion mode) or deprotonated (in the negative ion mode). The sample and reagent ions pass through the sample cone and into the ion block before entering the analyzer.

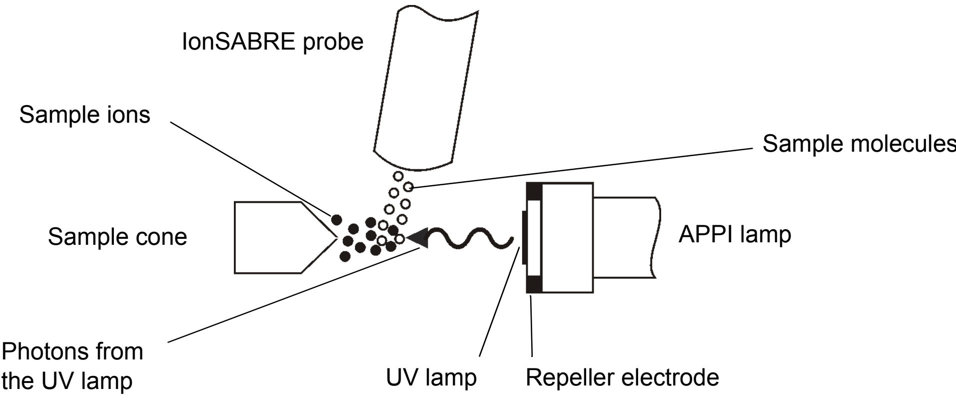
About APPI mode

Atmospheric Pressure Photoionization (APPI) uses photons generated by a krypton discharge lamp (~10.2 eV) to produce sample ions from vaporized LC eluent. Direct photoionization of the sample molecule occurs when the photon energy exceeds the ionization potential of the sample molecule.

The Q-TOF Premier modular LockSpray source is configured for APPI by the addition of an IonSabre probe and a UV lamp. The sample is introduced into the source via the IonSabre probe producing a stream of sample and solvent species that undergo photon-induced, ion-molecule reactions.

Inside the source, a repeller electrode deflects and focuses the resulting sample ions toward the sample cone for introduction into the mass spectrometer.

APPI mode:

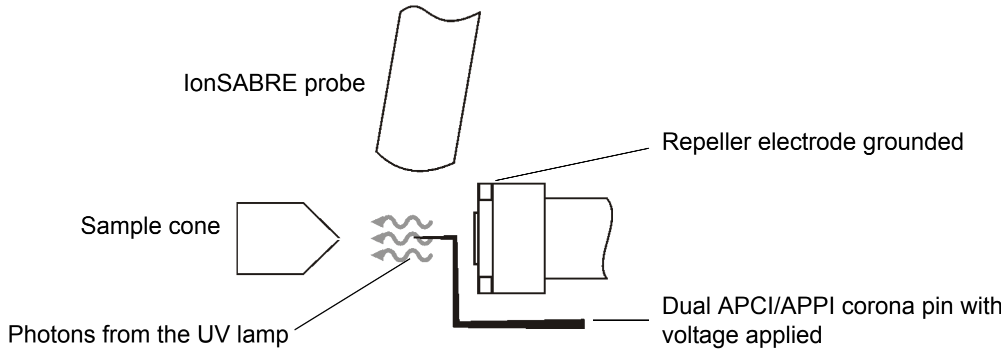


About dual APPI

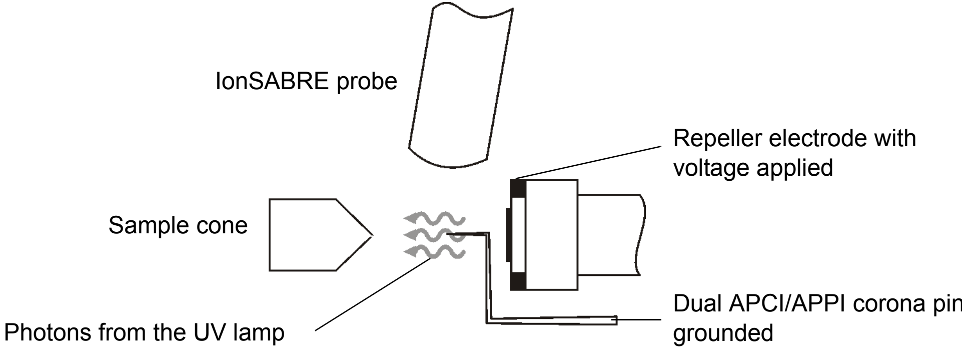
The Q-TOF Premier modular LockSpray source is configured for Dual APPI by the addition of an IonSabre probe, a special corona pin and a UV lamp.

Dual APPI/APCI mode enables rapid switching between ionization modes and facilitates high throughput (for example, for sample screening).

APPI dual mode (repeller electrode grounded):



APPI dual mode (repeller electrode on):

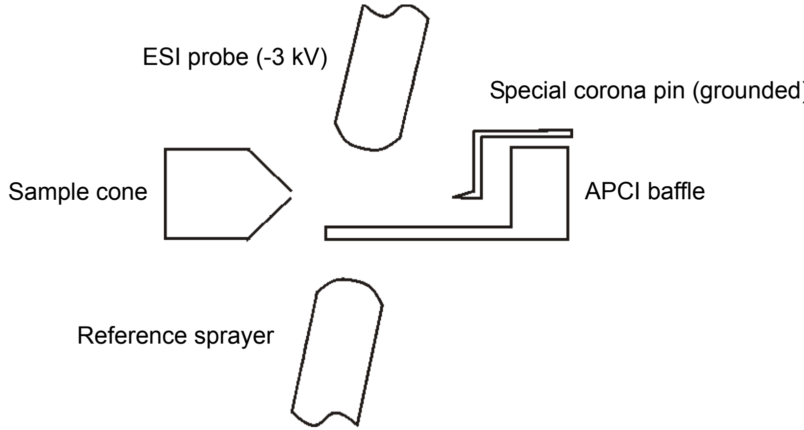


About ESCi LockSpray

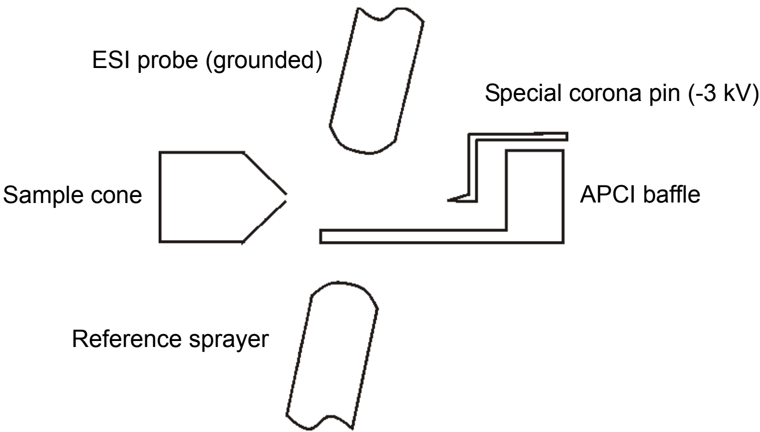
The Q-Tof Premier modular LockSpray source is configured for ESCi LockSpray by the addition of a special corona pin. ESCi mode allows the simultaneous acquisition of ESI and APCI data, facilitating high throughput. A standard ESI probe is used.

The ESCi configuration enables ESI and APCI, as illustrated in the following diagrams:

ESI mode:



APCI mode:



IonSABRE APCI operation

Installing IonSABRE LockSpray

This procedure assumes, as a starting position, that the instrument is in ESI LockSpray mode.

To install IonSABRE LockSpray:

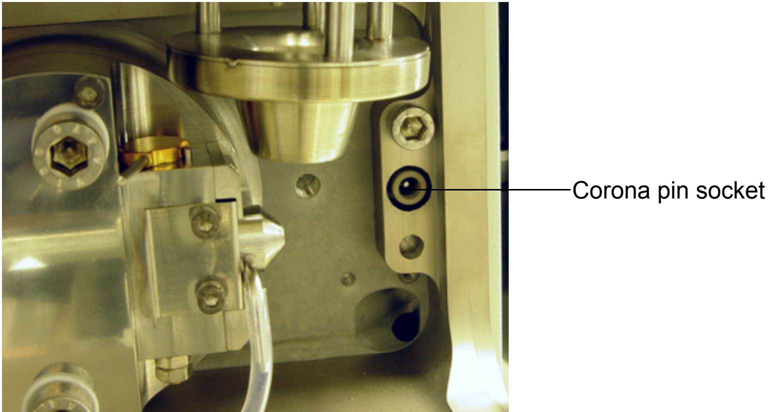
1. Click to put the instrument into standby. Ensure that the adjacent instrument status indicator turns red.
2. Click to turn off the nitrogen gas flow.
3. Disconnect the LC system from the ESI probe
4. Disconnect and remove the ESI probe
5. Install the IonSabre probe, and plug into the heater/interlock socket on the front connection panel

   Note: The cable from the probe adjuster is not used while the IonSabre probe is in use.

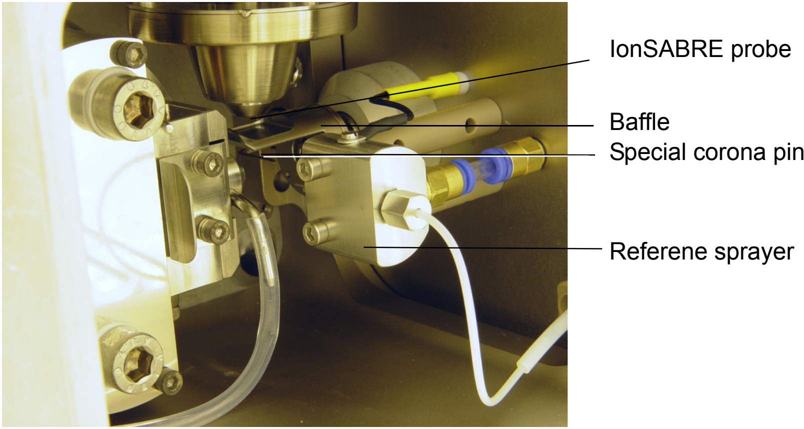
   Warning: To avoid burns, take great care while working with the instrument's source cover removed as the probe and source may still be hot.

6. Open the source door and, from the Tune window Source page, set the LockSpray baffle to the analyte position.
7. Disconnect and remove the LockSpray motor assembly
8. Remove the corona pin socket blanking plug and fit the special corona pin.

Corona pin socket:



9. Replace the standard baffle with the APCI baffle (see APCI baffle installation on page 7-8).



10. Refit the LockSpray motor assembly and close the source door.

11. In the Tune window click Source > IonSabre to open the IonSabre Source page.

See also:

- Modular LockSpray source on page 1-10

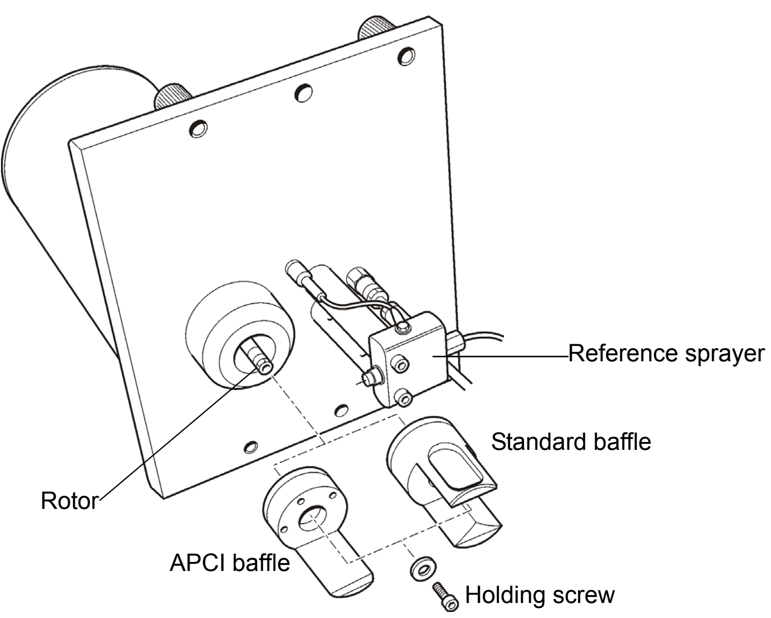
APCI baffle installation

The LockSpray baffle is attached to the rotor shaft by a single screw. Changing between standard and APCI baffles is therefore straightforward, but pay careful attention to the orientation of the baffle.

Tips:

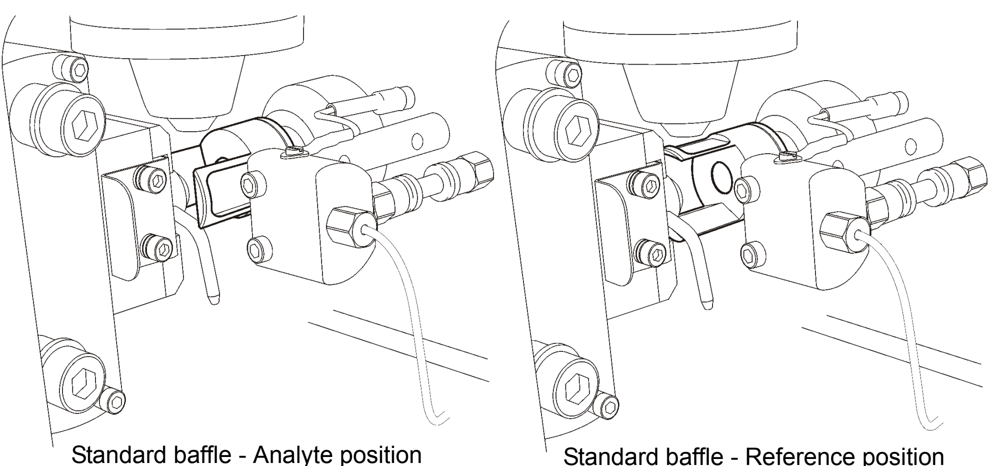
- Always set the baffle to the analyte position (from the software) before disconnecting the motor assembly cable.
- When you attach the new baffle to the square-ended rotor shaft, ensure the baffle is facing towards the reference sprayer before you place it over the end of the shaft. Avoid rotating the rotor shaft.
- After you have fitted the baffle and tightened its holding screw, you should plug the motor into the front panel motor socket. Do this before you install the motor assembly back onto the source enclosure. Set the baffle to the reference position, then set back to the analyte position. The baffle may reset itself, doing a full rotation at this point. Now you can install the motor assembly onto the source enclosure.
- If the baffle resets itself (does a full rotation) while installed in the source, it will push the corona pin to one side. This may happen if the embedded PC or instrument electronics are reset. If this occurs, the motor assembly will have to be removed, to allow access to adjust the corona pin position back to normal.

LockSpray motor assembly:



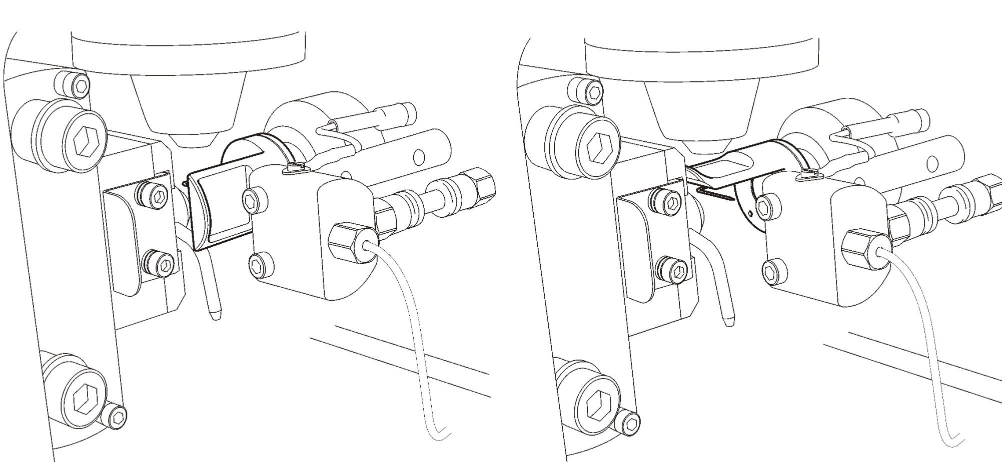
The orientations of the two baffles are illustrated in the following diagram:

Baffle positions:



Standard baffle - Analyte position

Standard baffle - Reference position



APCI baffle - Analyte position

APCI baffle - Reference position

Obtaining an ion beam and source optimization for APCI

Warning: To avoid possible high-pressure liquid jet spray, wear safety goggles when making the connections between the HPLC pump, LC column, syringe pump, and APCI probe.

Warning: To avoid the possibility of electric shock, ensure that the instrument is in standby mode before making the connections between the HPLC pump, LC column, syringe pump, and APCI probe.

In this example, a sample 17 α hydroxyprogesterone (concentration 10 ng/µL, in 70:30 acetonitrile:water) will be teed into an LC flow of 500 µL/min (70:30 acetonitrile:water) in APCI positive V mode. You will need to carry out the following procedures:

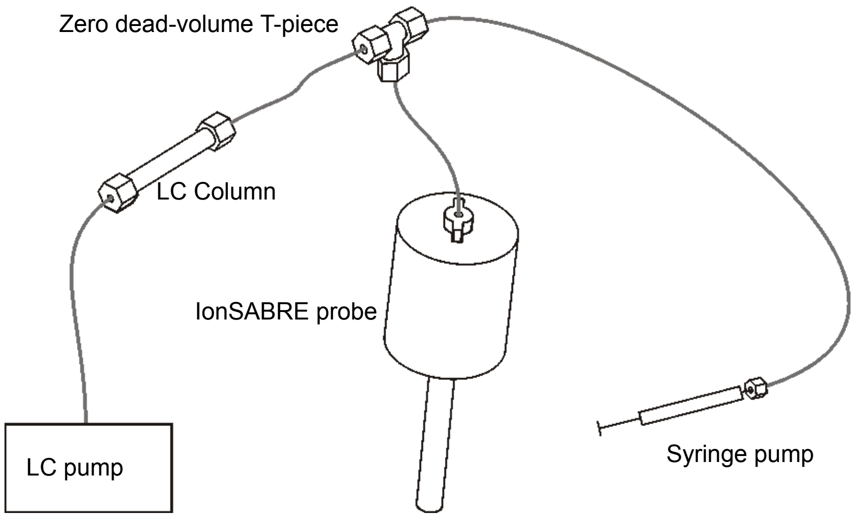
To obtain an ion beam and optimize the source (APCI):

Important: If the Q-Tof Premier has been in Standby for more than 2 hours it should be left in Operate for 1 hour to allow the instrument to stabilize.

Tip: Use real-time chromatogram to monitor the acquisition (see To follow an acquisition in Chromatogram: on page 2-25).

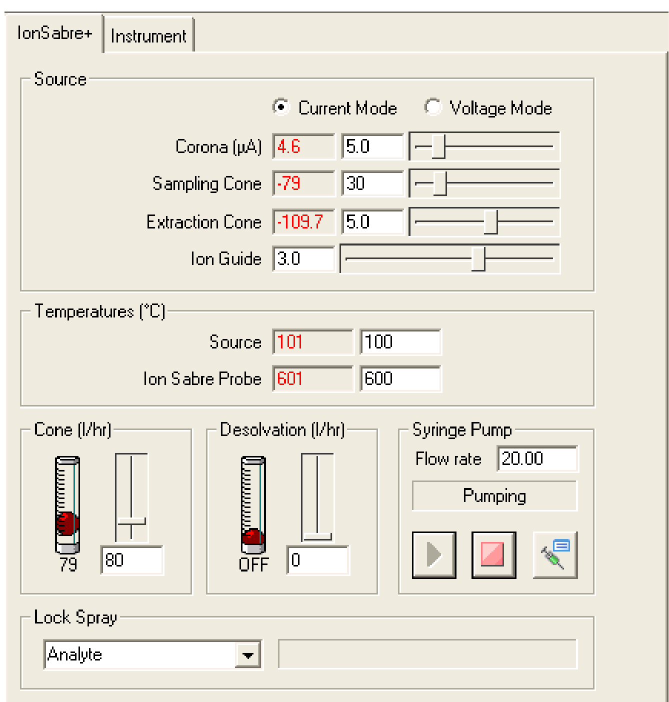
1. Make connections between the HPLC pump, syringe pump, and IonSABRE probe.

Pump and column connections to the IonSABRE probe:



2. Click Source > IonSabre.
3. Click and for positive ion in V mode.
4. From the IonSabre Source page, set the corona current to 5 µA, Source Temperature to 100 °C and Desolvation gas flow to 100 L/h

IonSabre+ Source page tune parameters:



Tip: For most compounds, Current Mode should be selected so as to maintain a constant current. For some compounds, Voltage Mode may provide better results.

5. Click and check that the adjacent instrument status indicator turns green.
6. Click to turn on the nitrogen gas flow.
7. Set the Ion Sabre probe temperature to 500°C and check that the readback reaches and stabilizes at this temperature.

8. Set the LC system to run at 500 µL/min and turn it on. Check that its pressure is stable. Allow 15 minutes for the LC column to equilibrate or until background levels are constant.

9. Infuse 17 α hydroxyprogesterone (concentration 10 ng/µL, in 70:30 acetonitrile:water) at 20 µL/min and observe the 17 α hydroxyprogesterone peak at m/z 331.2 ($M+H^+$) displayed on the Tune window.

   Tip: For negative ion, sulfadimethoxine could be used.

10. Starting with the probe tip midway between the cone and the end of the corona pin, use the probe adjuster to maximize the displayed peak intensity.

11. Use the probe adjuster to move the probe tip as far away from the cone as possible, without losing more than 20% of the maximum displayed peak intensity obtained in step 10. This will minimize source contamination.

12. Use the nebulization adjuster on the probe to give the best displayed peak intensity and stability.

13. On the Tune window IonSabre+ Source tab, optimize the Corona (µA) current to the value giving the maximum displayed peak intensity. See To maximize the Corona current: on page 7-14.

14. Starting from a value of 0, increase the Cone (L/hr) gas flow in increments of 50. Allow the pressure to stabilize after each adjustment. Set the gas flow to the highest value that does not significantly reduce the peak intensity. This minimizes solvent ion cluster formation.

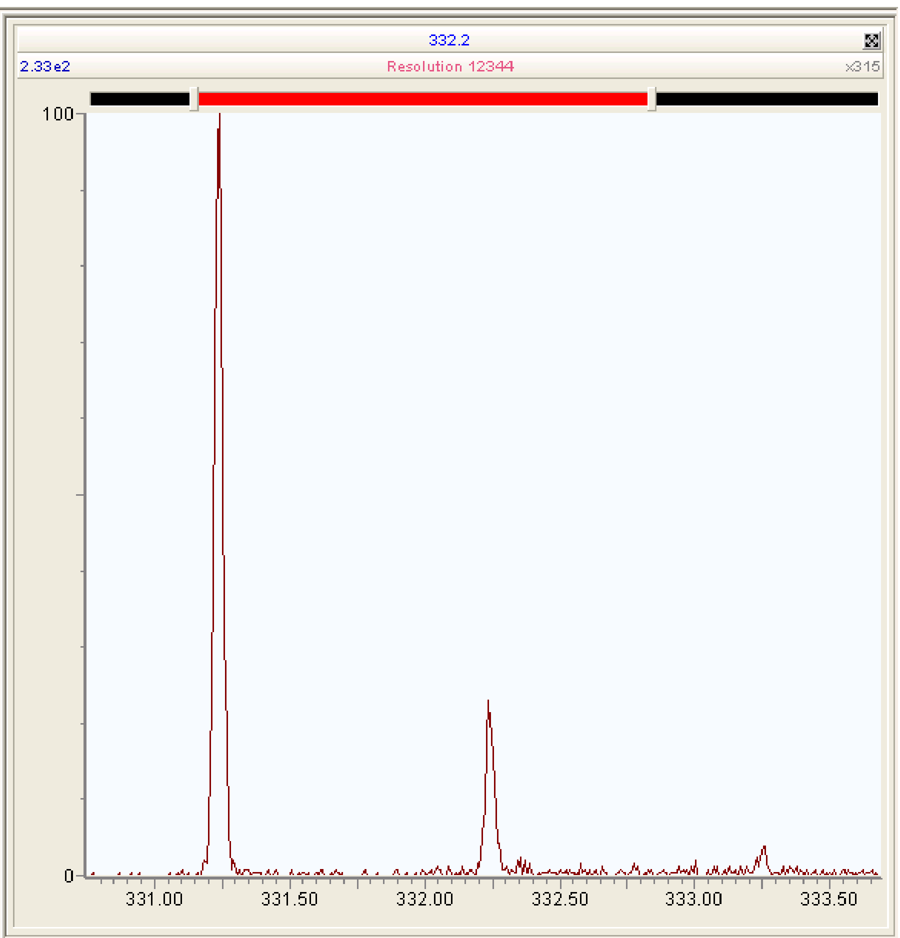
15. Starting from a value of 15, increase the Sample Cone voltage in increments of 5. Set the cone voltage to the value giving the highest displayed peak intensity. Record this value.

16. Reduce the Ion Sabre Probe Temp (°C) to 300, and then increase its value in increments of 50. Allow the temperature readback to stabilize after each adjustment. Set the temperature to the highest value that does not decrease the displayed peak intensity.

    If the probe temperature is too low, the mobile phase may not be efficiently desolvated. This can result in chromatographic peak tailing.

17. With the Sample Cone, Corona Cone Gas, and IonSabre probe temperature now at their optimum values, the displayed peak should be similar to that shown in the following figure.

Peak display for IonSABRE APCI:



To maximize the Corona current:

1. Starting with a value of 5, decrease the value to 1, in decrements of 1.
2. Decrease the value to 0, in decrements of 0.1.
3. Reset the value to 5, and then increase the value in increments of 5.
4. Set the Corona (µA) current to the value giving the maximum displayed peak intensity.

Tip: Typical values will be 5 µA for positive ion and 15 µA for negative ion

IonSABRE LockSpray reference setup

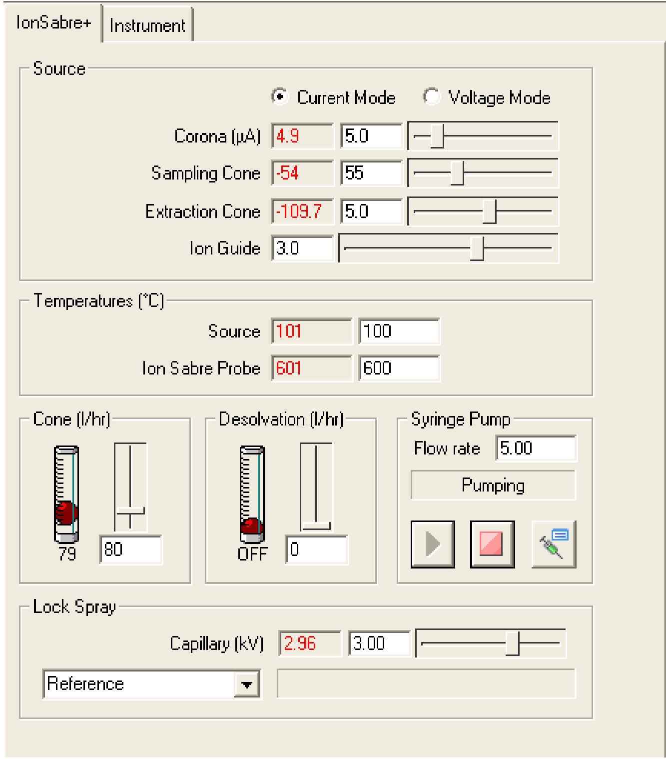
For IonSABRE LockSpray, a reference solution suitable for electrospray ionization is required. Leucine enkephalin is often the preferred choice

To tune the reference solution:

1. On the Tune window IonSabre page select Reference.

   Result: The baffle rotates into the reference position. Capillary voltage is available in the Lock Spray frame.

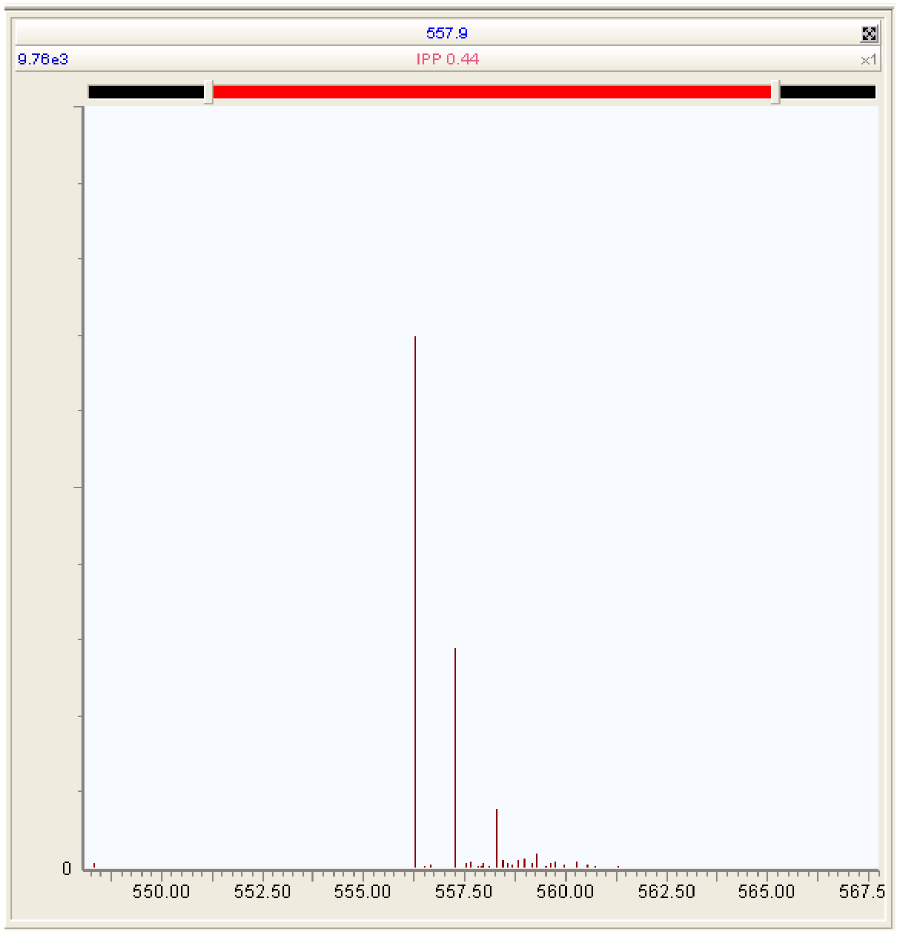
   IonSabre+ Source page with Reference selected:

2. Click and change the data format to Centroid.
3. Infuse 2 ng/µL leucine enkephalin at 5 µL/min.
4. Tune on the 556 peak using the Lock Spray Capillary voltage and the Sample Cone voltage to give 0.5 ions per push (IPP). This gives a balance between ion statistics and detector saturation.

Tip: Write down the value of the Cone Voltage so that you can enter it in the MS Method editor when required.

Centroid peak display for leucine enkephalin:

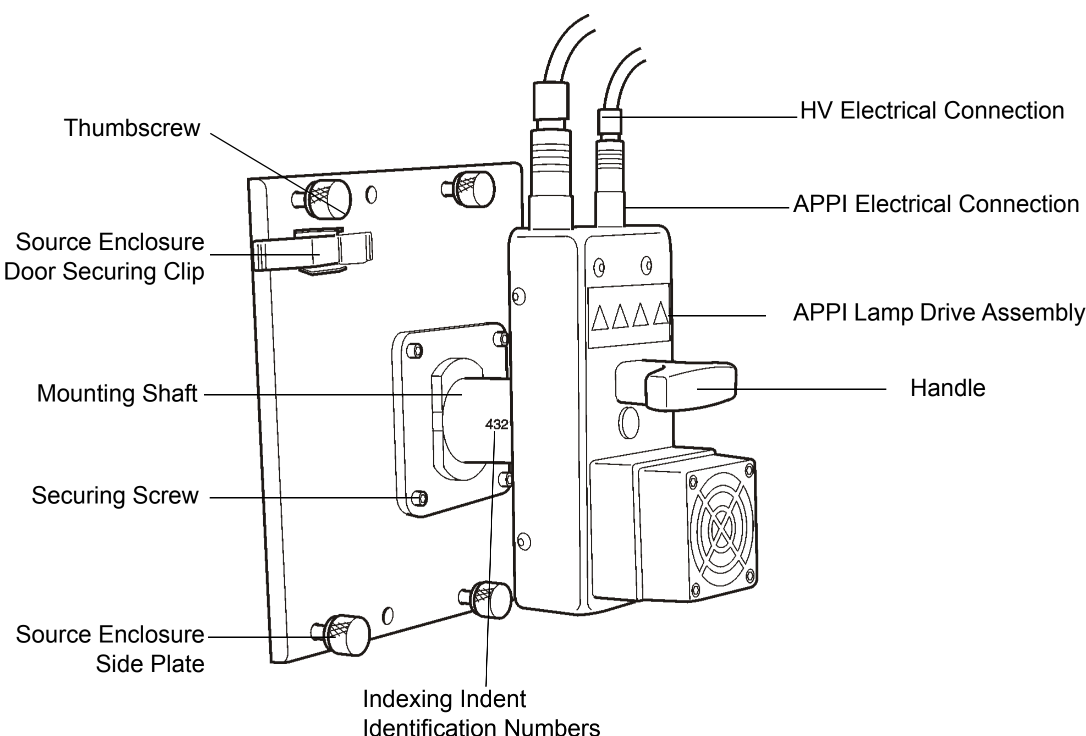


APPI and dual APPI operation

For APPI and dual APPI/APCI, both the APPI lamp drive assembly and IonSabre probe are required.

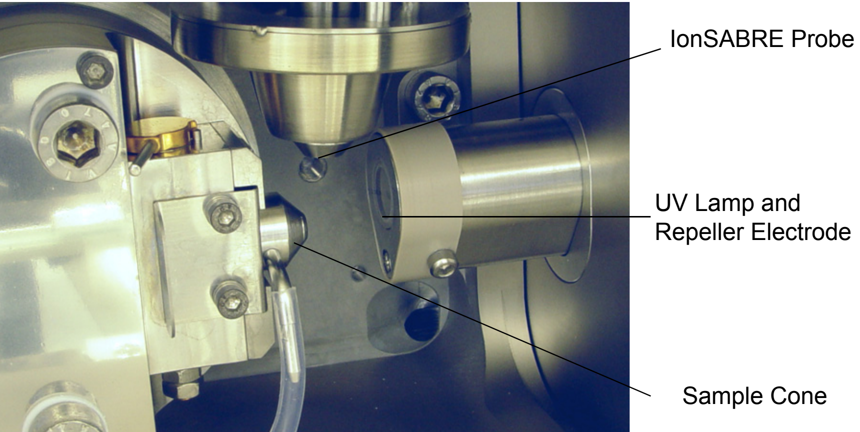
APPI lamp assembly

APPI lamp assembly and side plate:



Switching the UV lamp on provides a constant photon output. The intensity of incident radiation up the sample molecules is varied by manually adjusting the distance between the lamp and probe tip, using the handle on the APPI lamp drive assembly to move the whole assembly on its mounting shaft. The mounting shaft's four numbered indexing indents aid in setting this distance.

APPI lamp drive assembly fitted to the source enclosure:



To install APPI / dual APPI:

This procedure assumes, as a starting position, that the instrument is in ESI LockSpray mode.

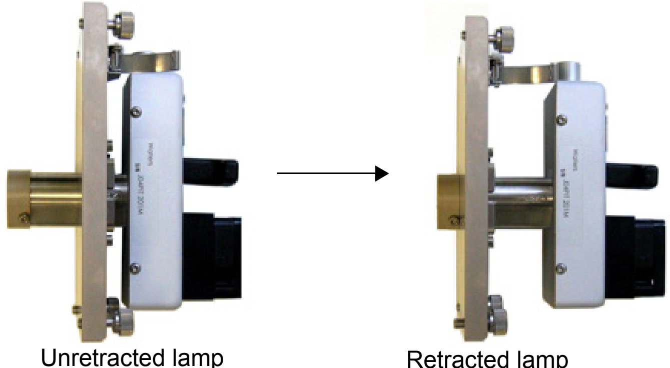
1. Click to put the instrument into standby. Ensure that the adjacent instrument status indicator turns red.
2. Click to turn off the nitrogen gas flow.
3. Disconnect the LC system from the ESI probe
4. Disconnect and remove the ESI probe
5. Install the IonSabre probe, and plug into the heater/interlock socket on the front connection panel

   Note: The cable from the probe adjuster is not used while the IonSabre probe is in use.

   Warning: To avoid burns, take great care while working with the instrument's source cover removed as the probe and source may still be hot.

6. Open the source door.
7. Disconnect and remove the LockSpray motor assembly.
8. Remove the corona pin socket blanking plug and fit the special corona pin (Dual APPI only)

9. Fully retract the lamp’s mounting shaft in the APPI lamp drive assembly.

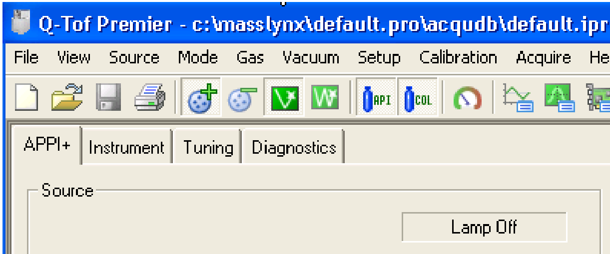


10. Fit the UV lamp assembly onto the source enclosure and secure using the four thumbscrews (see Figure titled “APPI lamp drive assembly fitted to the source enclosure:” on page 7-18).
11. Connect the UV lamp assembly to the front panel APPI and capillary sockets
12. Close the source door.
13. In the Tune window click Source > APPI, or Dual APPI.

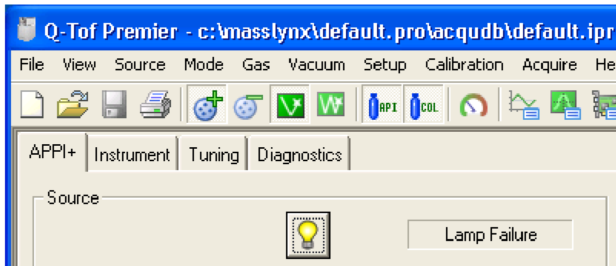
Lamp status

The APPI lamp can be switched on and off from the APPI and the Dual APPI source pages. There are four states:

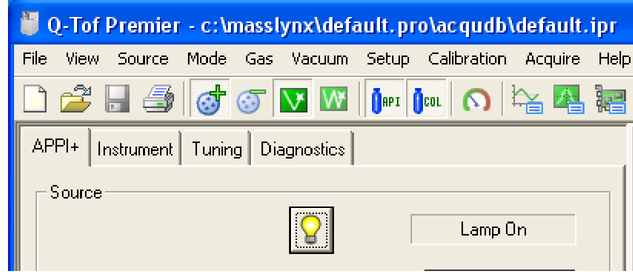
The lamp button is not visible in standby:



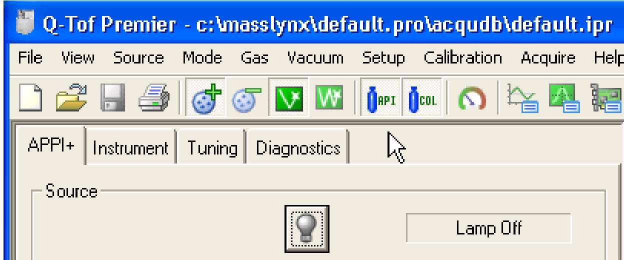
The lamp button shows ‘On’ with read-back ‘Lamp Failure’ if the lamp is requested while the lamp is disconnected:



Lamp button shows ‘On’ with read-back ‘Lamp On’ when lamp switched on:



Lamp button shows off with read-back ‘lamp off’ when lamp is switched off.



Obtaining an ion beam and source optimization (APPI)

Warning: To avoid possible high-pressure liquid jet spray, wear safety goggles when making the connections between the HPLC pump, LC column, syringe pump, and IonSABRE probe.

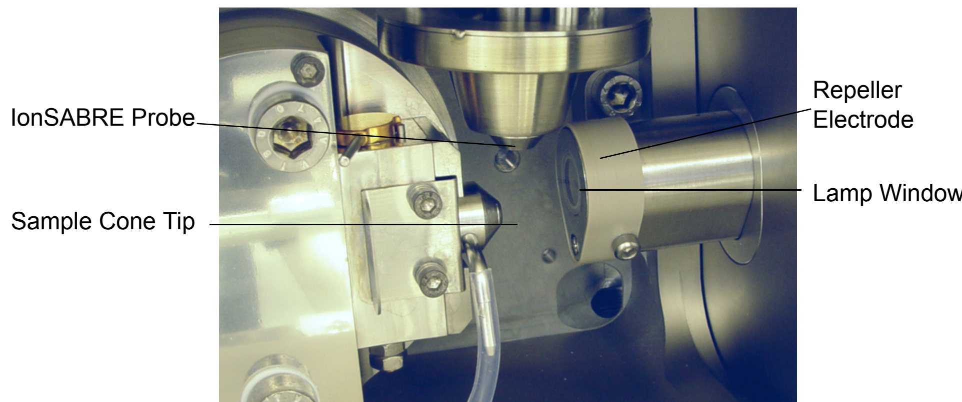
Warning: To avoid the possibility of electric shock, ensure that the instrument is in standby mode before making the connections between the HPLC pump, LC column, syringe pump, and IonSABRE probe.

In this example a sample of cholesterol (concentration 10 ng/µL, in 100% methanol) will be teed into an LC flow of 500 µL/min in APPI positive V mode.

To obtain an ion beam and optimize the source for APPI:

1. Make connections between LC system, syringe pump and IonSabre probe
2. Fill syringe with 10 ng/µL cholesterol in 100% methanol and set-up an LC pump with 100% methanol
3. Click Source > APPI.
4. Click and for positive ion in V mode.
5. Manually advance the lamp to notch 3 on the mounting shaft. This position gives the best compromise when first obtaining an ion beam.

APPI lamp position:



6. From the IonSabre Source page set source temperature to 100 °C, probe temperature to 600 °C and desolvation gas flow to 100 L/h.
7. Click to switch the instrument into Operate. Check that the adjacent instrument status indicator turns green.
8. Click to turn on the nitrogen gas flow.

9. On the Tune window APPI+ Source page, click [lamp icon], and confirm the Lamp is on.

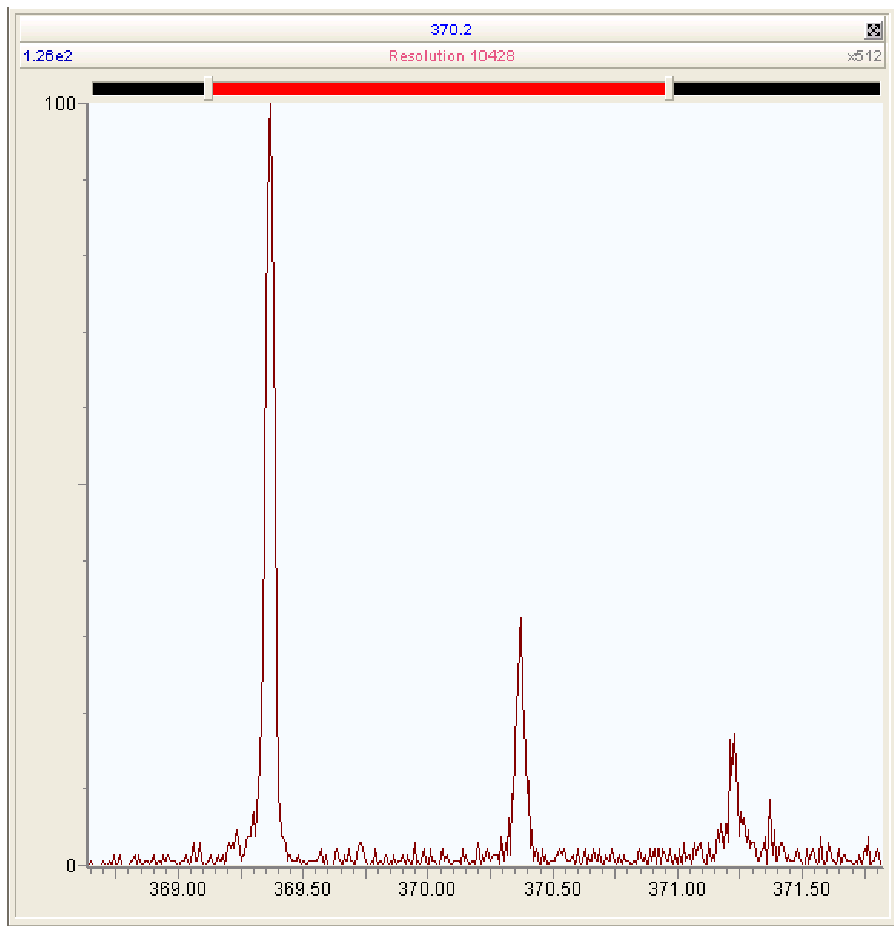
10. Start the LC flow (500 µL/min) and the syringe pump (20µL/min)

11. Observe the cholesterol peak at m/z 369.0 (M(-H2O)+H+) on the peak display and optimize the lamp position, probe position, probe temperature, repeller voltage and sampling cone voltage, to give the maximum peak intensity

    Caution: Do not to touch the probe with the repeller electrode:

    - A short circuit to the electrode would prevent proper ion detection.
    - The probe is hot and may damage the lamp assembly.

Peak display with Cholesterol Peaks



Dual APPI operation

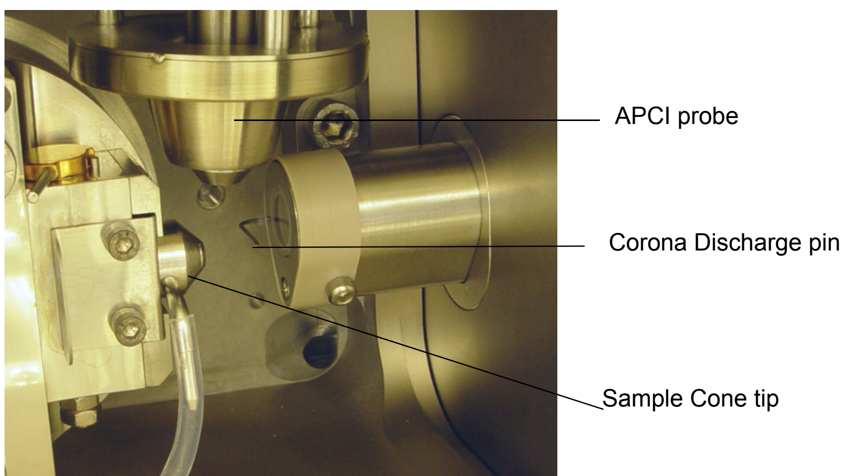
In this example a sample of cholesterol (concentration 10 ng/µL, in 100% methanol) will be teed into an LC flow of 500 µL/min in APPI positive V mode.

To obtain an ion beam and optimize the source for dual APPI:

1. Make connections between HPLC pump, syringe pump and IonSabre probe
2. Fill syringe with 10 ng/µL cholesterol in 100% methanol and setup an LC pump with 100% methanol

3. Click Source > APPI.
4. Click [icon] and [icon] for positive ion in V mode.
5. Manually advance the lamp to notch 3 on the mounting shaft. This position gives the best compromise when first obtaining an ion beam.

Combined APPI/APCI Corona Discharge Pin Alignment:

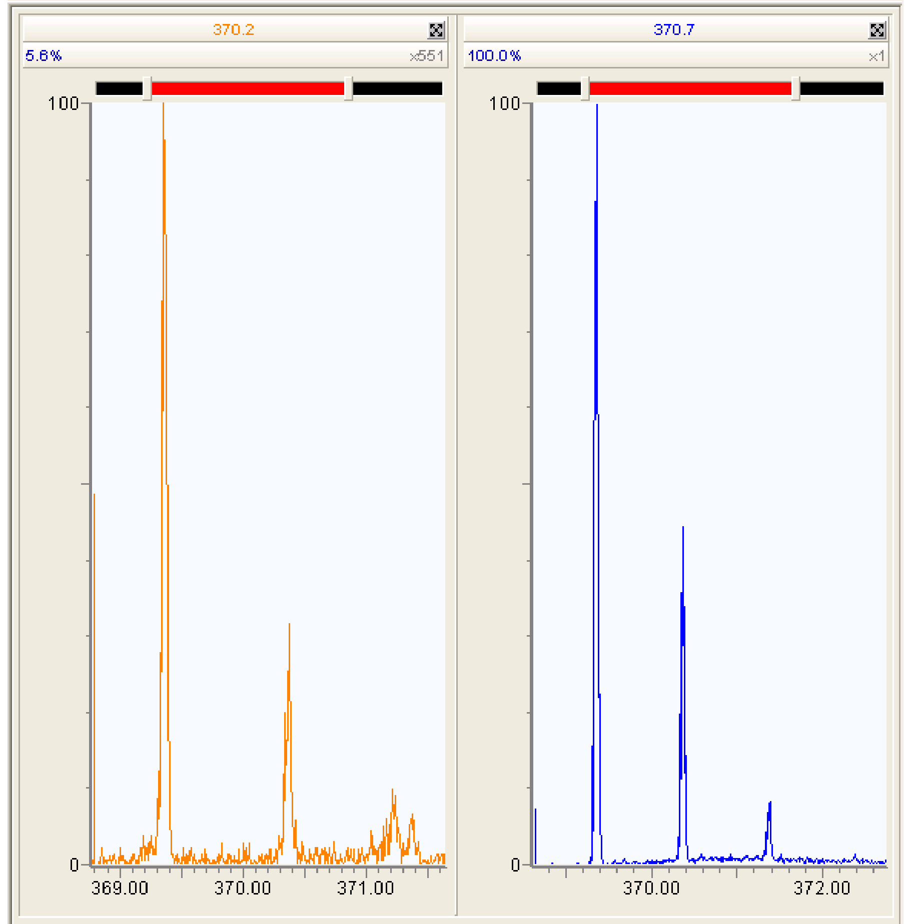


6. From the IonSabre Source page set source temperature to 100 °C, probe temperature to 600 °C and desolvation gas flow to 100 L/h.
7. Click [icon] to switch the instrument into Operate. Check that the adjacent instrument status indicator turns green.
8. Click [icon] to turn on the nitrogen gas flow.
9. On the Tune window APPI+ Source page, click [icon], and confirm the Lamp is on.
10. Start the LC flow (500 µL/min) and the syringe pump (20µL/min)
11. Observe the cholesterol peak at m/z 369.0 (M(-H2O)+H+) on the peak display and optimize the lamp position, probe position, probe temperature, repeller voltage and sampling cone voltage, to give the maximum peak intensity.

    Note: The APPI spectrum is displayed in the left pane and is colored orange. The APCI spectrum is displayed in the right pane and is colored blue.

The repeller voltage will only affect the APPI spectrum. The corona current will only affect the APCI spectrum.

Dual mode APCI/APPI Peal display:



ESCi operation

The Q-Tof Premier, with the ESI probe installed and corona discharge pin fitted, can alternate between acquiring data in ESI and APCI modes. The software switches the voltage to the ESI capillary and the current to the APCI corona discharge pin continuously.

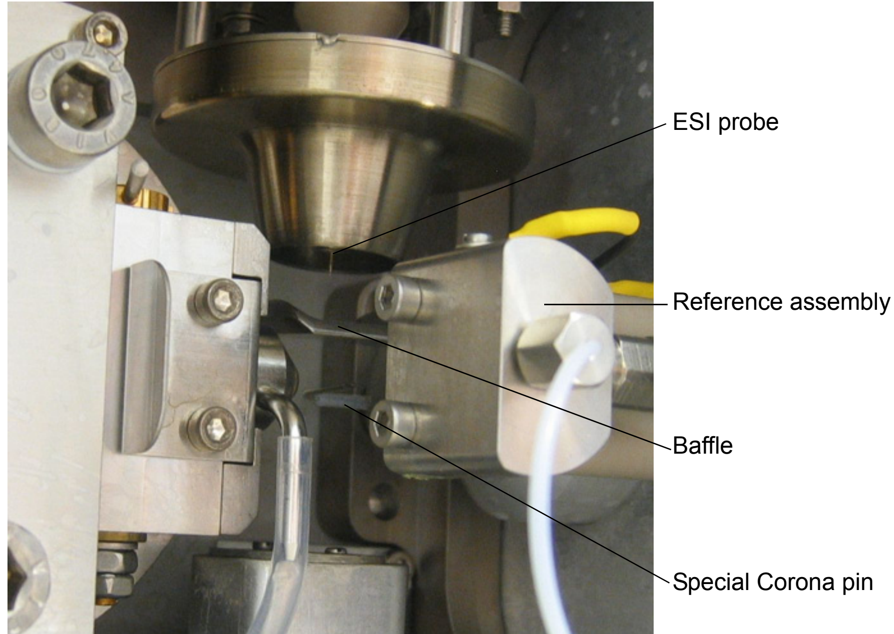
To install LockSpray for ESCi:

This procedure assumes, as a starting position, that the instrument is in ESI LockSpray mode.

1. Click to put the instrument into standby. Ensure that the adjacent instrument status indicator turns red.
2. Click to turn off the nitrogen gas flow.
3. Open the source door.

Warning: To avoid burns, take great care while working with the instrument's source cover removed as the probe and source may still be hot.

4. From the Tune window Source page, set the LockSpray baffle to the analyte position.
5. Disconnect and remove the LockSpray motor assembly.
6. Remove the corona pin socket blanking plug and fit the special corona pin.



7. Replace the standard baffle with the APCI baffle (see APCI baffle installation on page 7-8).
8. Refit the LockSpray motor assembly.
9. Close the source door.
10. On the Tune window click Source > ESCi.

To obtain an ion beam and optimize the source tune for ESCi:

Warning: To avoid possible high-pressure liquid jet spray, wear safety goggles when making the connections between the HPLC pump, LC column, syringe pump, and ESI probe.

Warning: To avoid electric shock, ensure that the instrument is in standby mode before making the connections between the HPLC pump, LC column, syringe pump, and ESI probe.

1. Make connections between HPLC pump, syringe pump and IonSabre probe
2. Fill syringe with 10 ng/µL cholesterol in 100% methanol and set-up an LC pump with 100% methanol

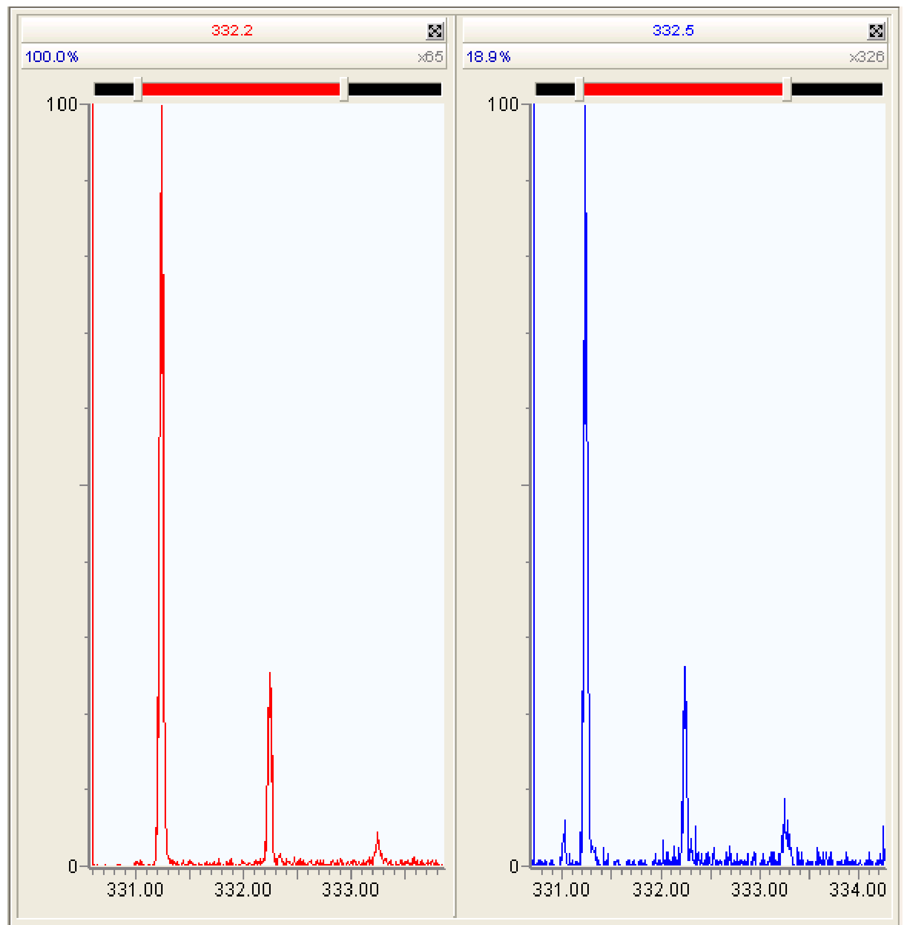
3. Click Source > ESCi.
4. Click and for positive ion in V mode.
5. Click to switch the instrument into Operate. Check that the adjacent instrument status indicator turns green.
6. Click to turn on the nitrogen gas flow.
7. Start the LC flow (300 µL/min) and the syringe pump (20 µL/min)
8. Observe the cholesterol peak at m/z 369.0 (M(-H2O)+H+) on the peak displays

   Note: The ESI spectrum is displayed in the left pane and is colored red. The APCI spectrum is displayed in the right pane and is colored blue.

9. From the IonSabre Source page optimize the ESI probe position, corona current and sampling cone voltage, to give the maximum peak intensity.

Observe the two peaks in the Tune window. The following figure illustrates how the peak display should appear when tuned correctly.

ESCi Peak display:



Creating MS method files for dual mode ionization

Dual APPI and ESCi data can only be acquired from the sample list. Create an MS method file consisting of two parallel functions, differing only in source type. Mixed polarity (+/-), analyzer mode (V/W) or data type (centroid/continuum) functions are not permitted.

For Dual APPI, select source types API and APPI.

For ESCi, select source types ES and API.

To create dual mode ionization method files:

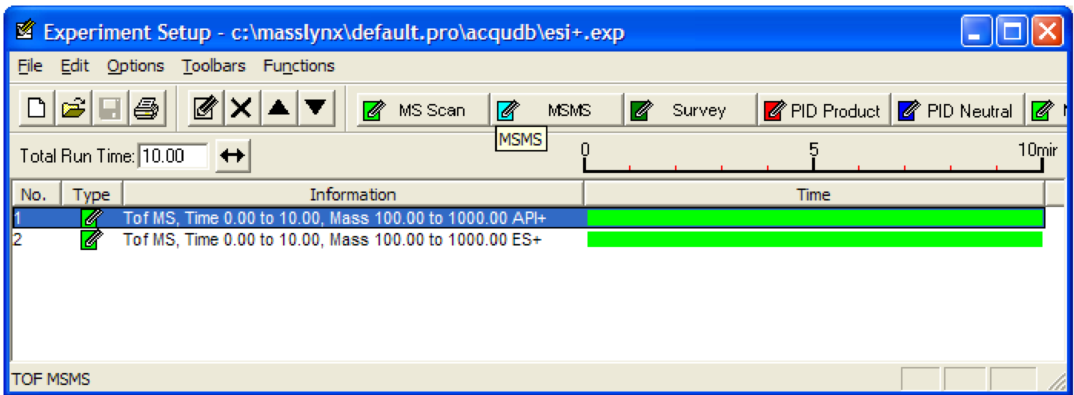
1. From the MassLynx window, click the MS Method icon to open the MS Method Editor.
2. Click ✖ to delete the current entry.
3. Click MS Scan to open the MS Scan Function Editor.
4. On the Acquisition page set the required Source mode (ES, API or APPI).
5. Click OK.

   Result: The MS Function Editor closes, and the parameters are now included as the APPI/ES/API entry in the MS Method Editor.
6. Add a second MS function this time selecting a different Source mode.
7. Save the method using a suitable name.

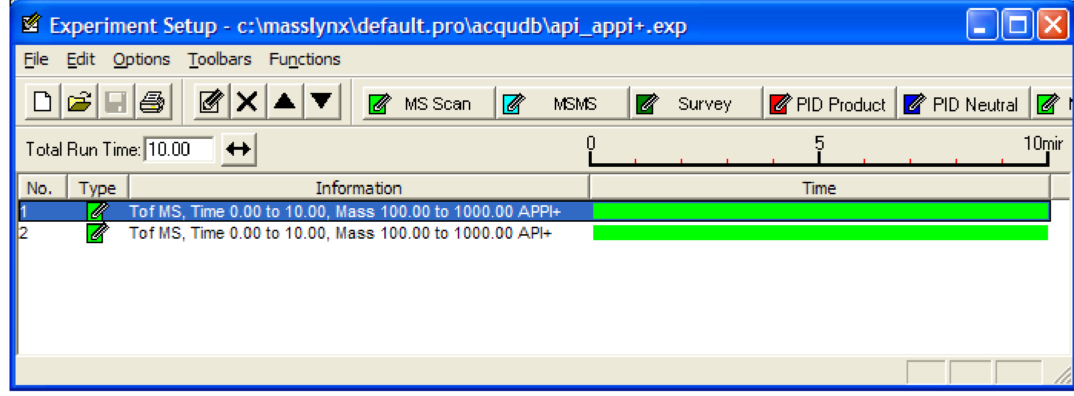
   Important:

   - The parameter values entered in the Acquisition Time frame must be identical for both functions.
   - LockSpray parameters are common to each function. LockSpray data will always be acquired in ESI mode regardless of the function source type.

ESCi method:



Dual APPI method:



8 Preventative Maintenance

Contents

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Maintenance schedule

The table below lists periodic maintenance schedules that, when followed, ensure optimum instrument performance.

The maintenance frequencies shown apply to instruments that normally receive moderate use.

Maintenance schedule:

<table>
<tr><th>Procedure</th><th>Frequency</th></tr>
<tr><td>Replace the scroll pump seals</td><td>Annually (see the Edwards document XDS 35i Instruction Manual A730-01-880)</td></tr>
<tr><td>Clean the source assembly (see Cleaning the source ion guide on page 8-20).</td><td>When sensitivity decreases to unacceptable levels.</td></tr>
<tr><td>Clean the ESI probe tip (see Cleaning or replacing the ESI probe tip on page 8-26).</td><td>When sensitivity decreases to unacceptable levels.</td></tr>
<tr><td>Clean the ion block assembly (see Cleaning the Source components on page 8-5).</td><td>When it is visibly fouled.
When background or high peak contaminants are unacceptably high.</td></tr>
<tr><td>Clean all source components (see Cleaning the Source components on page 8-5).</td><td>When sensitivity decreases to unacceptable levels.
When cleaning the cone gas cone, sample cone fails to improve analytical results.</td></tr>
<tr><td>Replace the ESI probe capillary (see Replacing the ESI probe capillary on page 8-31).</td><td rowspan="3">When sensitivity decreases to unacceptable levels.</td></tr>
<tr><td>Perform LockSpray Maintenance (see Performing LockSpray maintenance on page 8-52)</td></tr>
<tr><td>Perform NanoLockSpray Maintenance (see Performing NanoLockSpray maintenance on page 8-58)</td></tr>
</table>

Safety and handling

Bear in mind the following safety considerations when performing maintenance procedures.

Warning: To avoid injury, ensure the jack feet are in the lock position.

Warning: To avoid possible electric shock, do not remove the instrument panels. There are no user-serviceable items inside.

Warning: To avoid possible electric shock, ensure that the instrument is in standby mode before commencing any maintenance.

Warning: To prevent injury, always observe good laboratory practices when handling solvents, changing tubing, or operating the Q-Tof Premier. Know the physical and chemical properties of the solvents used. See the Material Safety Data Sheets for the solvents in use.

Warning: To avoid burns, take great care while working with the probe and source as they are liable to be hot.

Warning: The instrument components are liable to be contaminated with biologically hazardous and toxic materials. Wear rubber gloves at all times while handling the components.

Cleanliness and care are very important whenever you remove internal assemblies from the instrument.

- Always prepare a clear clean area in which to work.
- Make sure that any tools or spare parts that may be required are close at hand.
- Obtain some small containers in which screws, washers, spacers etc. can be stored.
- Use tweezers and pliers whenever possible.
- If nylon or cotton gloves are used, take care not to leave fibers in sensitive areas.
- Avoid touching sensitive parts with fingers.

- Before reassembling and replacing dismantled components, inspect O-rings and other vacuum seals for damage. Replace with new ones if in doubt.

Should a fault occur soon after a particular part of the system has been repaired or otherwise disturbed, it is advisable first to ensure that this part has been correctly refitted and / or adjusted, and that adjacent components have not been inadvertently disturbed.

Cleaning the Source components

Overview

Clean the source components (sample cone and cone gas nozzle when:

- They are visibly fouled.
- LC and sample-related causes for decreased signal intensity have been ruled out.

If cleaning these parts fails to increase signal sensitivity, also clean the extraction cone, source ion guide, and ion block.

To clean the source components:

1. Disassemble the source components and source ion guide assembly (see To remove the probe from the source on page 8-6 to To disassemble the ion block: on page 8-13).
2. Clean the source components and source ion guide assembly (see Cleaning the sample cone and cone gas nozzle on page 8-19 to Cleaning the isolation valve stem on page 8-21).
3. Reassemble the source ion guide assembly and the source components (see Reassembling the source ion block on page 8-22 to Fitting the sample cone on page 8-24).

Required materials

- Rubber gloves
- Needle-nose pliers
- Set of Allen keys (hex wrench) including 2.5-mm and 6-mm
- Jeweller’s screwdriver
- Large, flat-blade screwdriver
- Glass-fiber pen
- Appropriately sized glass vessels, in which to completely immerse components when cleaning. Use only glassware not previously cleaned with surfactants.
- HPLC-grade methanol

- HPLC-grade water
- Formic acid
- Ultrasonic bath
- Source of oil-free, inert gas (nitrogen or helium) for drying (air-drying optional).
- Lint-free paper towels

To remove the probe from the source

1. Disconnect the LC system from the probe.
2. On the MassLynx window, click and ensure that the adjacent instrument status indicator turns red.
3. Wait for 3 minutes to allow the desolvation gas flow to cool the probe and source.
4. Click to turn off the nitrogen gas flow.

Warning: To avoid burns take care when handling the probe and source as they are liable to be hot.

5. Disconnect the electrical connection(s) on the instrument front panel.
6. Disconnect the PTFE tubing at the Nebulizer gas connection on the front panel.
7. Undo the two thumbscrews securing the probe to the probe adjuster.

Warning: To avoid contamination from previously sprayed sample, wear gloves at all times when handling the probe. This also prevents finger-grease contaminating the capillary.

Caution: To avoid damaging the probe seals when removing an APCI probe, ensure that the probe temperature is less than 150 °C [as displayed on the Tune windows's APcI Probe Temp (°C) readback] before removing the probe.

8. Carefully remove the probe from the probe adjuster.

To remove the sample cone

Warning: The source components are liable to be contaminated with toxic and biohazardous materials. Wear rubber gloves at all times while handling the components.

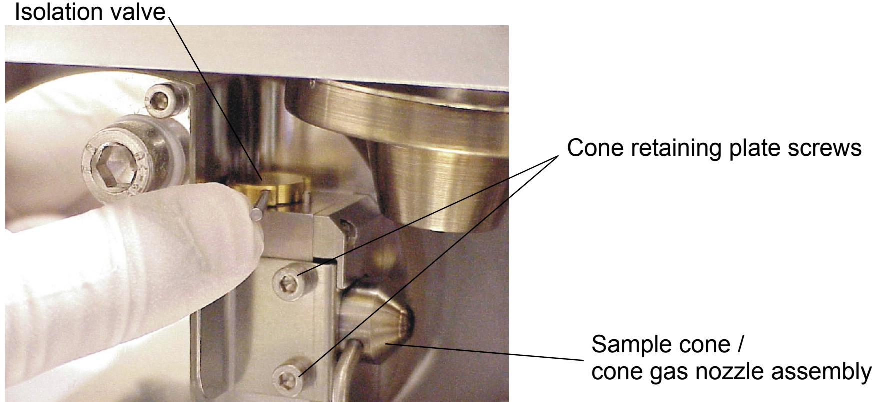
Warning: To avoid possible electric shock, ensure that the instrument is in standby mode before commencing this procedure.

Warning: The source is liable to be hot. To avoid burns, take great care while working with this item.

1. Switch the instrument to Standby
2. Set the Source temperature to 20 °C.
3. Wait for the Source temperature to fall below 50 °C.
4. Switch off the API gas flow.
5. Unfasten the source enclosure door's securing clips and open the door.
6. Remove the baffle and motor assembly by taking off the right-hand flange of the Source enclosure.

   If using an Ion Sabre APCI probe, carefully remove the corona discharge pin.
7. Close the isolation valve by moving its lever fully to the right.

   Caution: Failure to close the isolation valve before removing the sample cone is liable to damage the instrument.



8. Pull out the PTFE tube attached to the cone gas nozzle.

   Tip: Use a flat-blade screwdriver to push the collar back while removing the tube.

9. Use an Allen key to remove the two cone retaining plate screws.

10. Remove the cone retaining plate.

11. Carefully remove the sample cone/cone gas nozzle assembly from the Source ion block.

12. Use a jeweller's screwdriver to carefully remove the O-ring from the sample cone/cone gas cone assembly.

   Caution: The sample cone is very fragile. To avoid damage, never place the sample cone on its tip; always place it on its flanged base.

13. Separate the sample cone and cone gas nozzle.

   The Figure titled "Sample cone/cone gas nozzle assembly components" on page 8-9 shows the sample cone, cone gas cone, and O-ring.

Sample cone/cone gas nozzle assembly components

14. Clean the sample cone and cone gas nozzle as described in "Cleaning the Sample Cone and Cone Gas Cone" on page 1-24.

Ion source enclosure and ion block removal

Warning: To avoid possible electric shock, ensure that the instrument is shut down and is isolated from the mains power supply.

To remove the ion source and ion block:

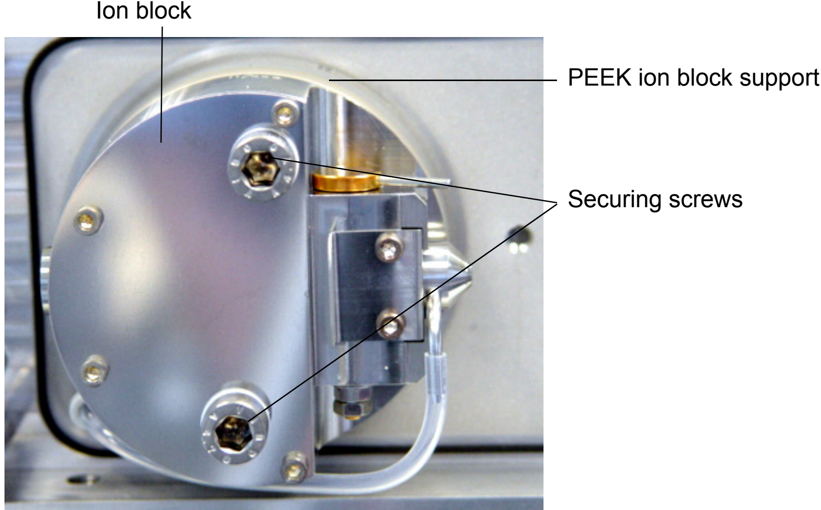
1. Vent the instrument as described in Appendix A.
2. Disconnect the Desolvation Heater electrical connection on the instrument front panel.

   Warning: The source is hot, so allow it to cool down for at least 30 minutes before proceeding.

3. Disconnect the PTFE tubing at the Desolvation gas connection on the front panel.

   Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

4. Use an Allen key to loosen the three captive source enclosure securing screws and remove the source enclosure from the instrument.
5. Use an Allen key to remove the two ion block securing screws.



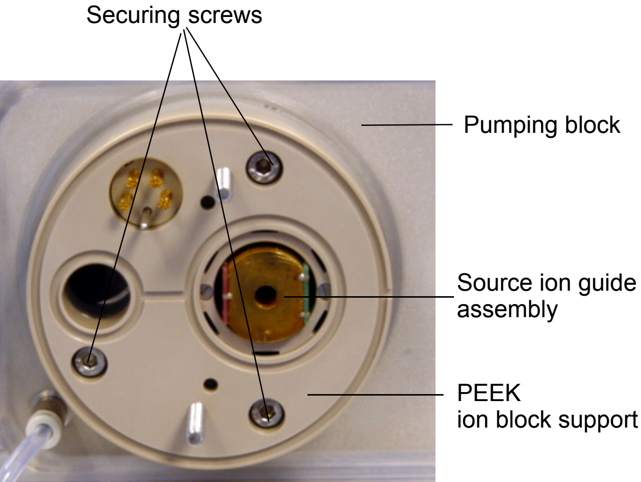
6. Remove the ion block from the PEEK ion block support.

Source ion guide removal

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

To remove the source assembly from the instrument:

1. Use a hex Allen key to remove the three PEEK ion block support securing screws.

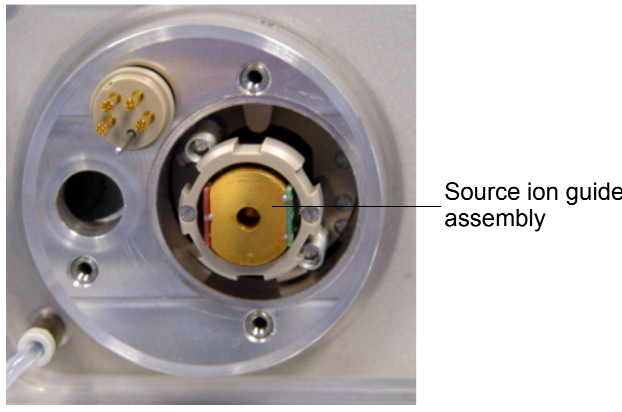


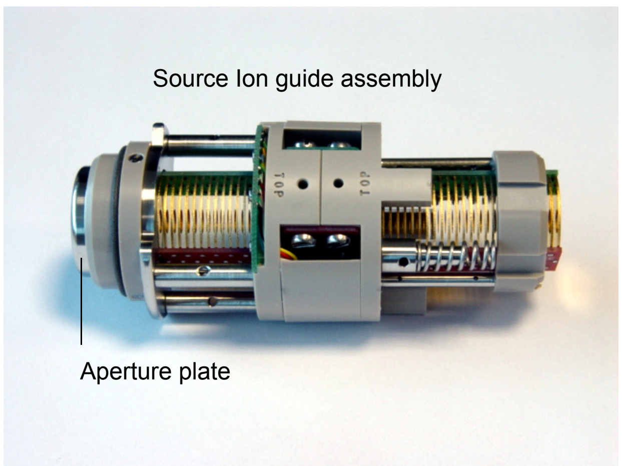
2. Remove the PEEK ion block support from the pumping block.

   Tip: Ensure that the three O-rings remain in position on the rear face of the support.

   Caution: Take care not to scratch the internal bore of the pumping block, as the ion tunnel assembly is withdrawn.

3. Carefully remove the source T-Wave assembly from the pumping block.



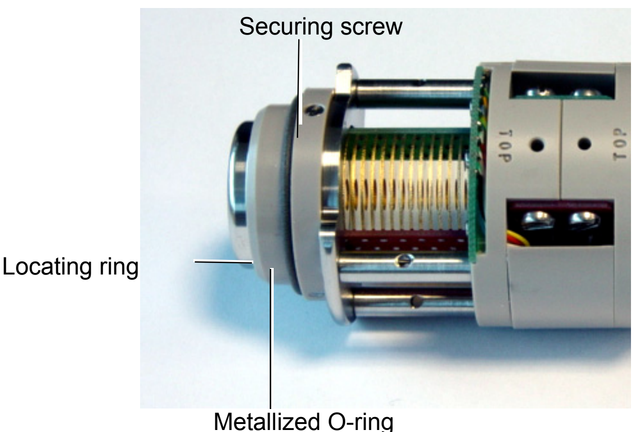


Disassembling the source T-Wave assembly

Warning: The source ion guide components may be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while performing this procedure.

To disassemble the source T-Wave assembly:

1. Use the jeweller's screwdriver to remove the three screws securing the locating ring to the source T-Wave assembly.

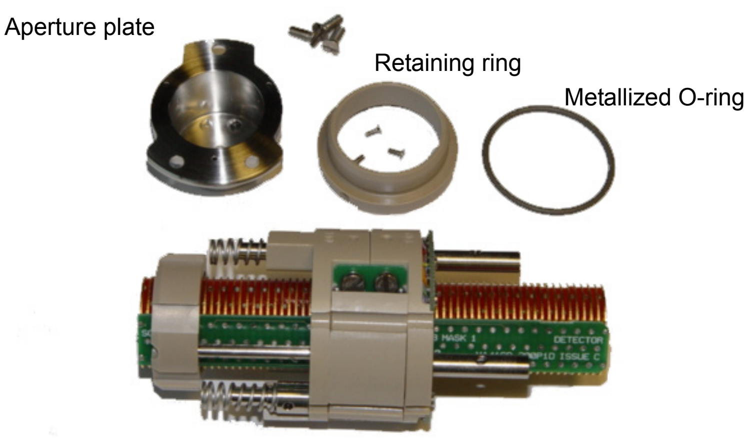


2. Remove the metallized O-ring and locating ring from the assembly.

3. Remove the three screws securing the aperture plate to the assembly.
4. Remove the aperture plate from the assembly. The source ion guide assembly components are shown in the figure below.

   Result: No further disassembly of the source ion guide assembly is required.

Source ion guide assembly components



5. Clean the source ion guide assembly and differential aperture plate.

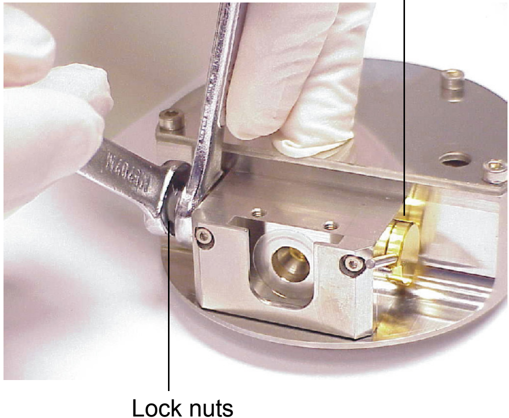
See also: Cleaning the source ion guide on page 8-20

Disassembling the source ion block

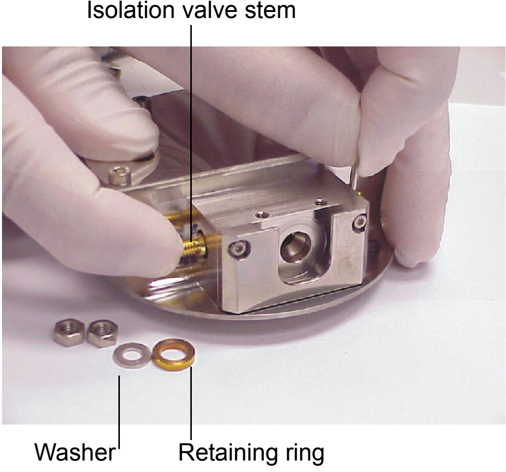
Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

To disassemble the ion block:

1. Remove the two lock nuts from the bottom of the isolation valve body.



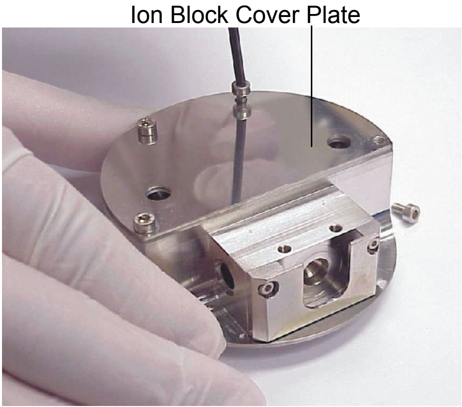
2. Remove the washer and retaining ring from the isolation valve stem.



3. Remove the isolation valve stem from the isolation valve body (push the stem while repeatedly opening and closing the isolation valve).

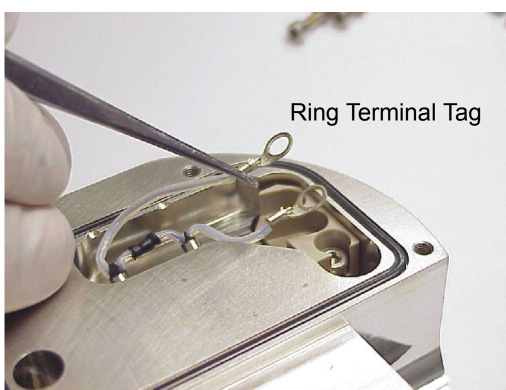
The isolation valve stem components are shown below:

4. Clean the isolation valve stem as described in (see Cleaning the isolation valve stem on page 8-21).
5. Use an Allen key to remove the four ion block cover plate securing screws.

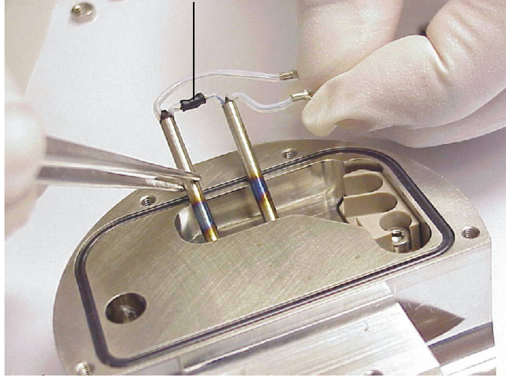


6. Remove the ion block cover plate.
7. Use an Allen key to remove the two screws securing the heater cartridge wires to the PEEK terminal block.

8. Use the needle-nose pliers to carefully swing the ring terminal tags out of the terminal block.

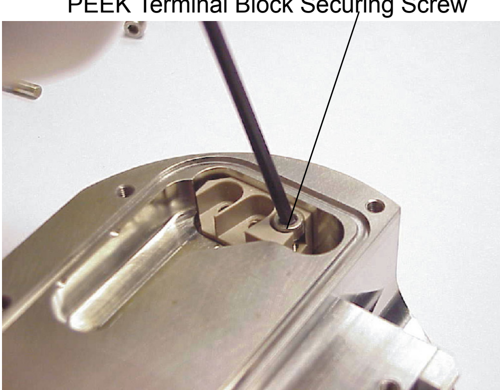


9. Use the needle-nose pliers to gently slide the heater cartridge assembly out of the ion block.

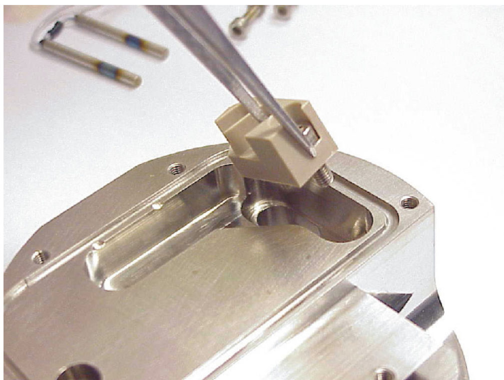


10. Use a jeweller’s screwdriver to carefully remove the D-shaped seal from the ion block.

11. Use an Allen key to remove the PEEK terminal block securing screw.



12. Use the needle-nose pliers to remove the PEEK terminal block from the ion block.



13. Use a wrench to remove the blanking plug.
14. Clean the ion block as described in (see Cleaning the ion block on page 8-21).
15. Reassemble the ion block as described in (see Reassembling the source ion block on page 8-22).

Cleaning the sample cone and cone gas nozzle

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

Caution: The sample cone is very fragile; to avoid damage, never place the sample cone on its tip, always place it on its flanged base.

To clean the sample cone and cone gas cone:

1. Use a glass-fiber pen to remove gross contamination from the cone gas cone surface by gentle abrasion.

   Warning: Use extreme care when working with formic acid. Use a fume hood and appropriate protective equipment.

2. If the sample cone contains debris, place a drop of formic acid on its orifice.
3. Immerse the sample cone and cone gas nozzle in separate glass vessels containing methanol:water (1:1).

   Tip: If the parts are obviously contaminated, use a mixture of 45% methanol, 45% water, and 10% formic acid.

   Warning: Handle the container carefully. The exothermic reaction caused by the addition of formic acid to water may have caused the container to heat up.

4. Place the vessels in the ultrasonic bath for 30 minutes.

   If formic acid was used in the cleaning solution:

   – Rinse the parts by immersing them in separate glass vessels containing water and placing the vessels in the ultrasonic bath for 20 minutes, to remove the formic acid.

   – Displace the water by immersing the parts in separate glass vessels containing methanol and placing the vessels in the ultrasonic bath for 10 minutes.

5. Carefully remove the parts from the vessels and blow-dry them using inert, oil-free gas.

Alternatively, the parts may be placed on lint-free towels and allowed to air dry. Wipe off any water spots with a lint-free cloth.

Cleaning the source ion guide

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

To clean the aperture plate:

Use a glass-fiber pen to gently remove ion burn marks; pay particular attention to the inner surfaces of the aperture plate.

Caution: Use only methanol or water as solvents when cleaning the source assembly. Use of acetone, chlorinated solvents, or acid will damage the assembly.

Caution: Take great care not to damage the assembly's plates when using a wire brush for cleaning.

To clean the source ion guide:

1. Flush-out the assembly, using methanol from a wash-bottle.
2. Immerse the assembly in a glass vessel containing methanol:water (1:1).
3. Place the vessel in the ultrasonic bath for 30 minutes.

   Caution: Do not dry the source assembly by any method other than blow-drying, otherwise reintroduced contamination may lead to difficulty in pumping down the instrument.

4. Carefully remove the assembly from the vessel and blow-dry it using inert, oil-free gas.
5. Visually inspect the assembly to ensure that no fibers from the wire brush are lodged in the assembly. If fibers are present, repeat the procedure from step 1 onwards.

Cleaning the ion block

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

Warning: Use extreme care when working with formic acid. Use a fume hood and appropriate protective equipment.

To clean the ion block:

1. Immerse the ion block in a glass vessel containing methanol:water (1:1).

   Tip: If the components are obviously contaminated, use a mixture of 45% methanol, 45% water, and 10% formic acid.

2. Place the vessels in the ultrasonic bath for 30 minutes.
3. If formic acid was used in the cleaning solution:
   - Rinse the ion block by immersing them in separate glass vessels containing water and placing the vessels in the ultrasonic bath for 20 minutes to remove the formic acid.
   - Displace the water by immersing the ion block in separate glass vessels containing methanol and placing the vessels in the ultrasonic bath for 10 minutes.
4. Carefully remove the ion block from the vessel and blow-dry them using inert, oil-free gas.

   Alternatively, place the components on lint-free towels and allow to air dry. Wipe off any water spots with a lint-free cloth.

Cleaning the isolation valve stem

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

To clean the isolation valve stem:

1. Use a glass-fiber pen to remove carbon deposits by gentle abrasion.

2. Rinse the valve stem by placing it in a vessel containing methanol:water (1:1) and placing the vessel in an ultrasonic bath for 20 minutes.

Reassembling the source ion block

Tip: Wear clean rubber gloves when reassembling the source components.

To reassemble the source ion block:

1. Check the condition of all O-rings and seals; if damaged, replace with new items.
2. Fit the ion block blanking plug to the ion block and tighten.
3. Fit the PEEK terminal block to the ion block and fit and tighten the PEEK terminal block securing screw.
4. Fit the D-shaped seal to the ion block, ensuring that it is correctly seated.
5. Use the needle-nose pliers to slide the new heater cartridges into the ion block.
6. Position the two heater cartridge ring tags onto the PEEK block terminals.
7. Use an Allen key to fit and tighten the two screws securing the heater cartridge wires to the PEEK terminal block.
8. Refit the ion block cover plate.
9. Fit and tighten the four ion block cover plate securing screws.
10. Fit the isolation valve stem to the isolation valve body.
11. Fit the washer and retaining ring to the bottom of the isolation valve stem.
12. Fit and tighten the two lock nuts on the bottom of the isolation valve stem.

Reassembling the source ion guide assembly

To reassemble the T-Wave assembly:

1. Fit the aperture plate to the source ion guide assembly.

2. Fit and tighten the three screws that secure the differential aperture plate to the source ion guide assembly.
3. Fit the locating ring to the assembly.
4. Check the condition of the metallized O-ring; if it is damaged, replace it.
5. Fit the metallized O-ring to the assembly.
6. Fit and tighten the three screws that secure the locating ring to the source ion guide assembly.

Fitting the source ion guide assembly to the instrument

To fit the ion guide assembly:

1. Ensuring that the Top labels (stamped on the assembly's PEEK supports) are upper most, carefully slide the source ion guide assembly into the pumping block.
2. Fit the PEEK ion block support to the pumping block.
3. Fit and tighten the three PEEK ion block support securing screws.

Fitting the ion block and ion source enclosure

To fit the ion block and source enclosure:

1. Check the condition of all O-rings; if damaged, replace with new items.
2. Ensure that all the O-rings are in position on the ion block.
3. Fit the ion block to the PEEK ion block support.
4. Fit and tighten the two ion block securing screws.
5. Fit the source enclosure to the instrument housing.
6. Tighten the three source enclosure securing screws.
7. Connect the Desolvation Heater electrical connection on the instrument front panel.
8. Connect the PTFE tubing at the Desolvation gas connection on the front panel.

Fitting the sample cone

Caution: The sample cone is very fragile. To avoid damage, never place the sample cone on its tip; always place it on its flanged base.

To fit the sample cone:

1. Fit the sample cone into the cone gas cone.
2. Check the condition of the sample cone/cone gas cone assembly O-ring; if damaged, replace with a new item.
3. Fit the O-ring to the sample cone/cone gas cone assembly.
4. Fit the sample cone/cone gas cone assembly to the side of the isolation valve body.
5. Fit the cone retaining plate.
6. Fit and tighten the two cone retaining plate securing screws.
7. Connect the PTFE tube to the cone gas cone.
8. Open the isolation valve by moving its lever fully to the left.
9. Refit the LockSpray motor and baffle.
10. If an IonSabre probe is to be used, fit the corona discharge pin.
11. Close the source enclosure door and fasten the clips.

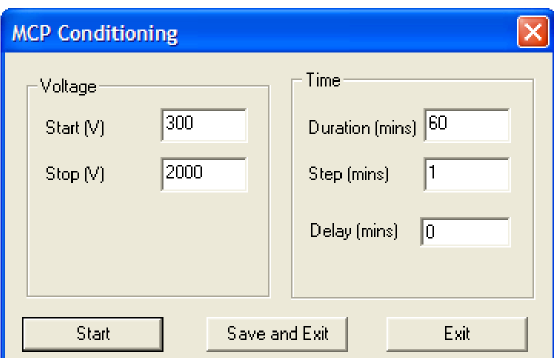
Detector conditioning

Caution: When the instrument has been vented, the detector should be reconditioned. Ensure that the Tof vacuum has been at < 3e-6 mBar for at least 1 hour before conditioning the detector.

To condition the detector:

1. Select V mode.
2. Click the Instrument tab in the Tune window and set Detector voltage to 0.
3. Switch to Operate.
4. Click Setup > Detector Conditioning.

5. In the MCP Conditioning dialog box set the following parameters and Click Start.



Cleaning or replacing the ESI probe tip

Clean the ESI probe tip if a blockage occurs in the internal metal sheathing through which the stainless steel capillary passes.

Replace the ESI probe tip if the threads are damaged.

Replace the O-ring if gas leaks from the O-ring.

Required Materials

- Rubber gloves.
- ¼-inch (6-mm) Allen key (hex wrench).
- Appropriately-sized glass vessels, in which to completely immerse components when cleaning.
- Use only glassware not previously cleaned with surfactants.
- HPLC-grade methanol.
- HPLC-grade water.
- Ultrasonic bath.
- Source of oil-free, inert gas (nitrogen or helium) for drying (air-drying optional).

To replace the ESI Probe tip:

Warning: To avoid contamination from previously sprayed sample, wear gloves at all times when handling the probe. This also prevents finger-grease contaminating the capillary.

Caution: Perform all work on the probe on a clean work bench.

1. Remove the probe from the source (see page 8-6).
2. Use the ¼-inch (6-mm) wrench to unscrew and remove the probe tip.
3. If the probe tip is damaged, replace with a new tip; alternatively clean the probe tip.
4. If necessary, remove the O-ring and fit a new O-ring.
5. Fit and tighten the probe tip to the probe.

6. Adjust the probe tip so that the fully extended capillary (when the probe nebulizer adjuster knob is fully screwed down) protrudes by approximately 1 to 1.5 mm.
7. Refit the probe to the source.

See also:

- To remove the probe from the source on page 8-6
- To clean the probe tip: on page 8-27

To clean the probe tip:

1. Immerse the probe tip in a glass vessel containing methanol:water (1:1).
2. Place the vessel in the ultrasonic bath for 20 minutes.
3. Carefully remove the probe tip from the vessel and blow-dry using inert, oil-free gas.

Replacing the ion block cartridge heater

Replace the cartridge heater if it fails to heat the source ion block.

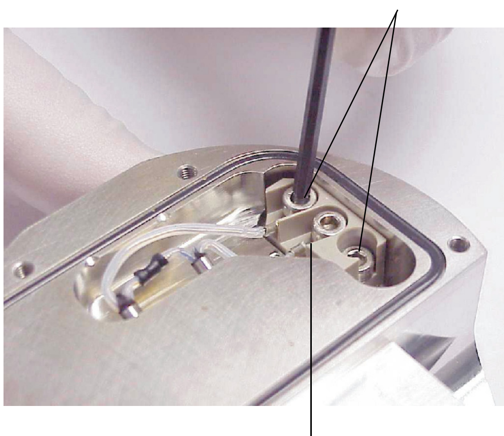
Required Materials

- Rubber gloves
- 3-mm hex wrench
- 1.5-mm hex wrench
- Flat-blade screwdriver
- Needle-nose pliers

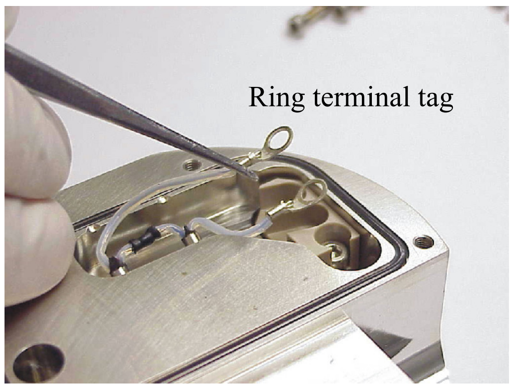
To remove the heater cartridge assembly:

Prerequisite: Ion source enclosure and ion block removal on page 8-9

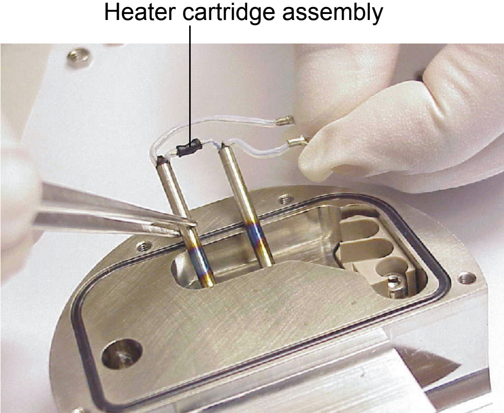
1. Use an Allen key to remove the four ion block cover plate securing screws.
2. Remove the ion block cover plate.
3. Use an Allen key to remove the two screws securing the heater cartridge wires to the PEEK terminal block.



4. Use the needle-nose pliers to carefully swing the ring terminal tags out of the terminal block.



5. Use the needle-nose pliers to gently slide the heater cartridge assembly out of the ion block.



6. Use the needle-nose pliers to slide the new heater cartridges into the ion block.
7. Position the two heater cartridge ring tags onto the PEEK block terminals.
8. Use an Allen key to fit and tighten the two screws securing the heater cartridge wires to the PEEK terminal block.

To refit the ion block:

Prerequisites:

- Ion source enclosure and ion block removal on page 8-9

1. Refit the ion block cover plate.
2. Fit and tighten the four ion block cover plate securing screws.
3. Check the condition of all O-rings; if damaged, replace with new items.
4. Ensure that all the O-rings are in position on the ion block.
5. Fit the ion block to the PEEK ion block support.
6. Fit and tighten the two ion block securing screws.
7. Fit the source enclosure to the pumping block.
8. Fit and tighten the three source enclosure securing bolts.
9. Connect the PTFE tube to the cone gas cone.
10. If using an APCI probe, carefully refit the corona discharge pin.
11. Connect the Probe electrical connection on the instrument front panel.
12. Connect the PTFE tubing at the Desolvation gas connection on the front panel.
13. Fit the probe on the source.

Replacing the ESI probe capillary

The stainless steel sample capillary in the ESI probe must be replaced if it becomes blocked and cannot be cleared, or if it becomes contaminated or damaged.

Required materials

- 7-mm hex wrench
- ¼-inch (6-mm) hex wrench
- 5/16-inch wrench
- Needle-nose pliers

To remove the existing capillary:

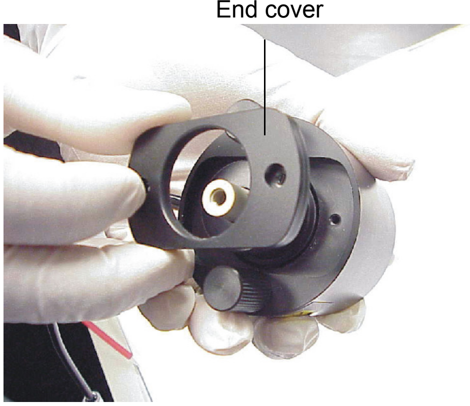
Warning: To avoid contamination from previously sprayed sample, always wear gloves when handling the probe. This also prevents finger-grease contaminating the capillary.

Caution: Perform all work on the probe on a clean work bench.

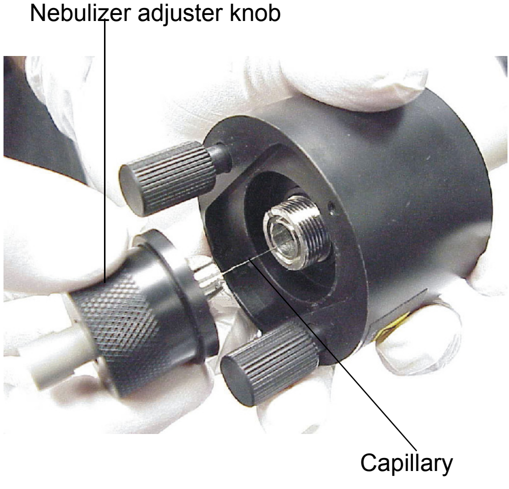
Prerequisite: To remove the probe from the source on page 8-6.

1. Use an Allen key to remove the two probe end cover retaining screws.

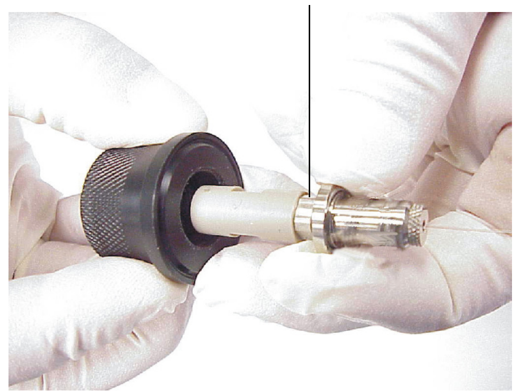
2. Remove the end cover.



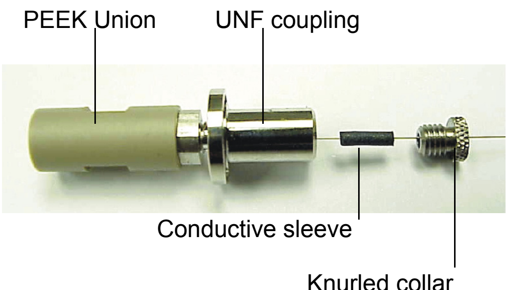
3. Unscrew and remove the nebulizer adjuster knob to reveal a PEEK union/UNF coupling assembly and the capillary.



4. Remove the nebulizer adjuster knob, PEEK union/UNF coupling assembly and capillary from the probe.
5. Remove the PEEK union/UNF coupling assembly and capillary from the nebulizer adjuster knob.

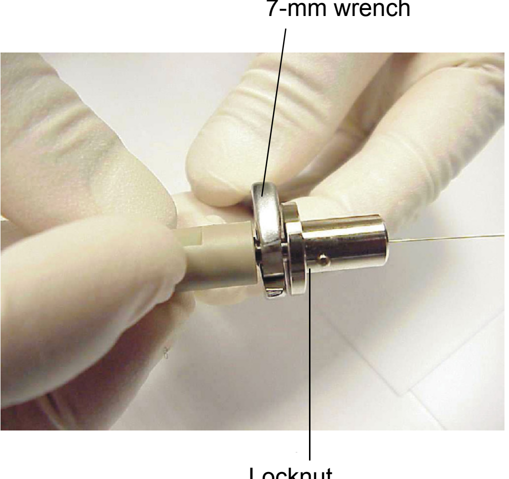


6. Unscrew and remove the knurled collar from the UNF coupling to reveal a conductive sleeve on the capillary.

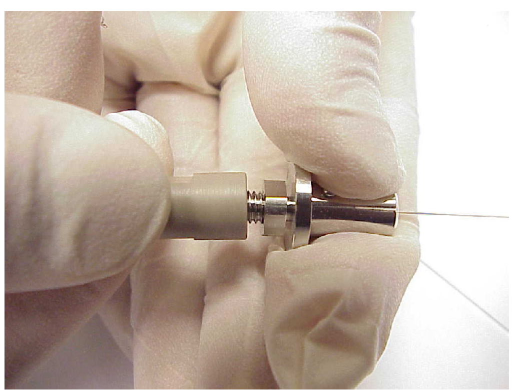


7. Remove the knurled collar and conductive sleeve from the capillary.

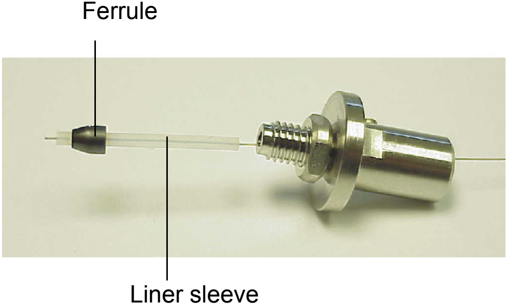
8. A locknut is used to secure the PEEK union and UNF coupling. Use the 7-mm wrench to loosen the locknut.



9. Unscrew the PEEK union from the UNF coupling (this connection is finger-tight only).



This reveals a ferrule and liner sleeve:

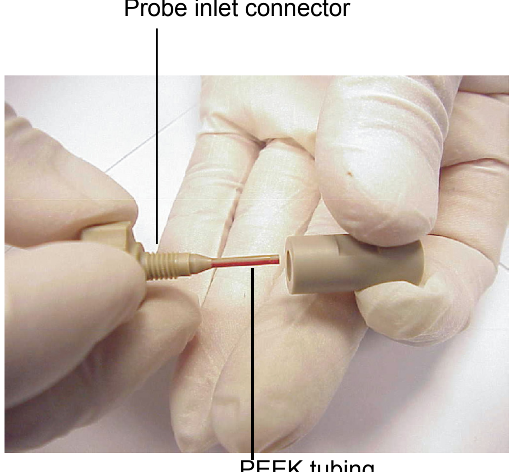


10. Remove the ferrule and liner sleeve from the capillary.
11. Remove the capillary from the UNF coupling.

Installing the new capillary

To install the new capillary:

1. Insert a square-cut length of red PEEK tubing in the probe inlet connector and screw the connector finger-tight into the PEEK union. This ensures a minimum dead volume when fitting the capillary.

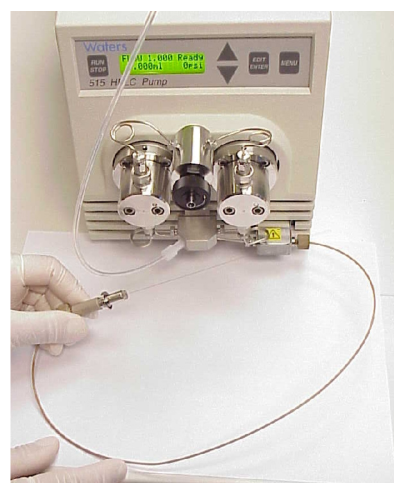


2. Fit the UNF coupling to the new capillary.
3. Use the needle-nose pliers to slide a new liner sleeve and ferrule onto the capillary.

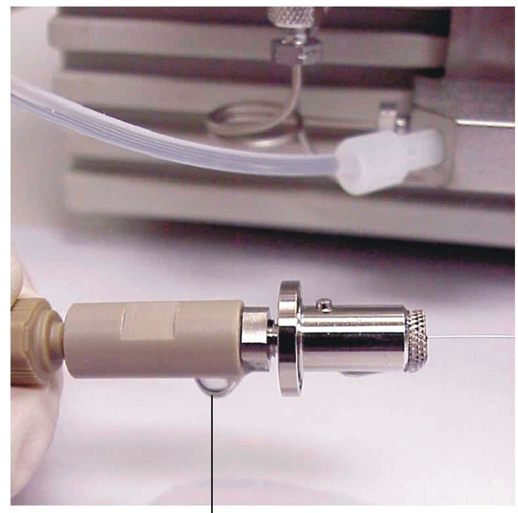
4. Insert the capillary in the PEEK union and ensure that it is fully seated.
5. Screw the UNF coupling into the PEEK union, finger-tight only.
6. Pull on the capillary gently, testing to ensure that it stays in place.
7. Use the 7-mm wrench to tighten the locknut against the PEEK union until the union can no longer be twisted.
8. Slide a new conductive sleeve and the knurled collar over the capillary.
9. Tighten the knurled collar to the UNF coupling.

Warning: To avoid injury, from possible high-pressure liquid jet spray, wear safety goggles when performing the leak test.

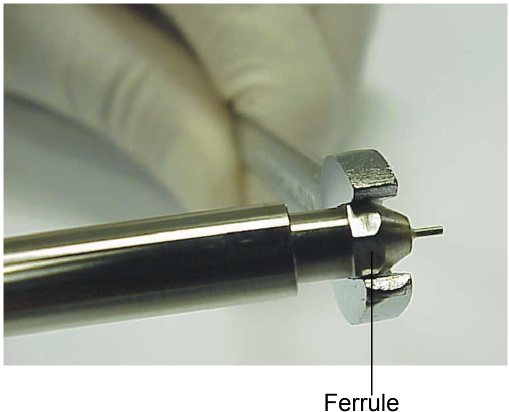
10. Check for leaks in the assembly by attaching the free end of the PEEK tubing to an LC pump and pumping through 50:50 acetonitrile:water at 1 mL/min.



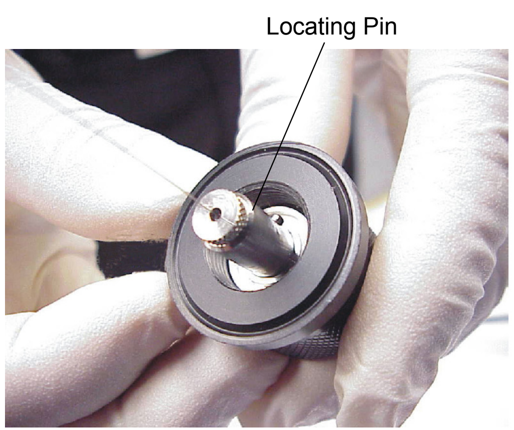
If leakage occurs, the connection must be disassembled and remade, and the leak test repeated.

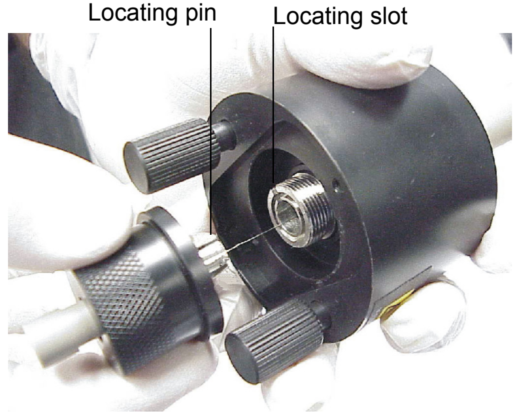


11. When performing the leak test, check the backpressure on the LC pump. This will be high if the capillary is blocked. If this is the case, replace the capillary with a new one.
12. When the leak test has been performed successfully, disconnect the PEEK tubing from the LC pump.
13. Remove the probe inlet connector and PEEK tubing from the PEEK union.
14. Refit the PEEK union/UNF coupling assembly to the nebulizer adjuster knob.
15. Use the ¼-inch (6-mm) wrench to remove the probe tip from the probe.

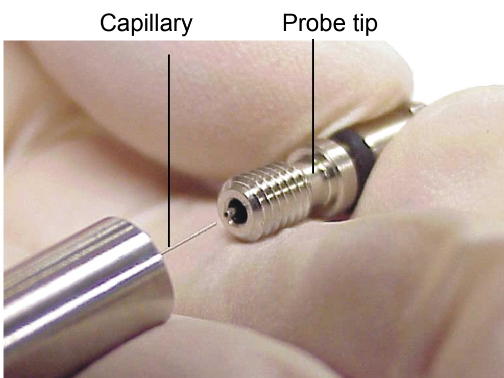


16. Carefully thread the capillary through the probe assembly.
17. Ensuring that the locating pin on the UNF coupling engages with the slot in the head of the probe assembly, fit and screw the nebulizer adjuster knob and PEEK union/UNF coupling assembly to the probe assembly. Do not tighten the knob fully.





18. Refit the probe end cover to the probe assembly.
19. Refit and tighten the two end cover securing screws.
20. Refit the probe tip over the capillary and screw the tip onto the probe assembly.



21. If necessary, adjust the probe tip so that the fully extended capillary (when the nebulizer adjuster knob is fully screwed down) protrudes by approximately 1 to 1.5 mm.
22. Use the nebulizer adjuster knob to adjust the capillary so that the capillary protrudes by approximately 0.5 mm from the end of the probe.
23. Attach the nebulizer gas connection and turn on the nitrogen, by clicking [icon] on the MassLynx Tune window.

24. Check the probe tip for nitrogen leaks. If a leak is found, replace the probe tip assembly and its O-ring (see Cleaning or replacing the ESI probe tip on page 8-26).
25. Refit the probe to the instrument.

IonSABRE probe maintenance

Cleaning or replacing the corona discharge pin

Clean the corona discharge pin if it appears to be corroded or black, or when the signal intensity weakens.

Required materials

- Rubber gloves
- Needle-nose pliers
- Lapping film
- HPLC-grade methanol
- Lint-free tissue

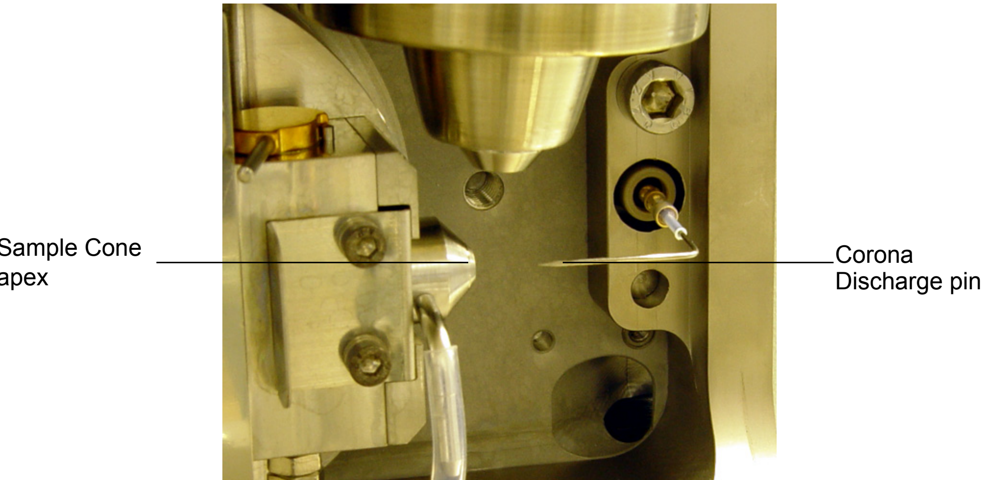
To clean or replace the corona discharge pin:

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

Warning: To avoid possible electric shock, ensure that the instrument is in standby mode before commencing this procedure.

Warning: The probe and source are liable to be hot. To avoid burns, take great care while working with these items.

1. Remove the probe from the source, (see To remove the probe from the source on page 8-6).
2. Unfasten the source enclosure door’s securing clips and open the door.
3. Remove motor assembly or UV lamp if fitted.
4. Use the needle-nose pliers to remove the corona discharge pin from the source, pulling it straight out.
5. Clean and sharpen the tip of the new pin with the lapping film, then wipe it clean with a methanol-saturated tissue. Replace the pin by a new pin if it is deformed or otherwise damaged.
6. Reinstall the pin with the tip pointing toward the sample cone apex.



7. Close the source enclosure door and fasten the clips.
8. Refit the probe.
9. Reconnect the front panel gas and electrical connections.

Cleaning the IonSABRE Probe Tip

If a reduction in sensitivity, or an increase in chemical background is noticed, the probe tip should be cleaned by running at maximum temperature without a liquid flow.

To clean the IonSABRE probe tip:

1. The instrument should be in operate with the LC liquid flow off
2. Set desolvation gas flow to 500 L/h
3. Set IonSabre probe temperature to 650 °C
4. Wait for 10 minutes. This will remove any chemical contamination from the probe tip.
5. Return IonSabre probe temperature to its previous value
6. Set the desolvation gas flow rate to 100 L/h

Replacing the IonSABRE Probe Capillary

Replace the stainless steel sample capillary in the APCI probe if it becomes blocked and cannot be cleared, or if it becomes contaminated or damaged.

Required Materials

- 7-mm hex wrench
- ¼-inch (6-mm) hex wrench
- 5/16-inch wrench
- Needle-nose pliers

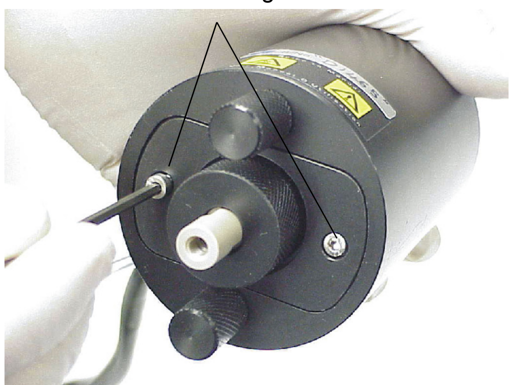
Removing the Existing Capillary

Warning: To avoid contamination from previously sprayed sample, always wear gloves when handling the probe. This also prevents finger-grease contaminating the capillary.

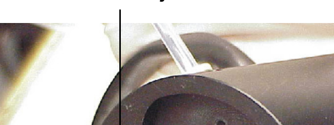
Caution: Perform all work done on the probe on a clean work bench.

To remove the existing capillary:

1. Remove IonSABRE probe from source and allow to cool completely.
2. Use the Allen key to remove the two probe end cover retaining screws and remove the end cover.

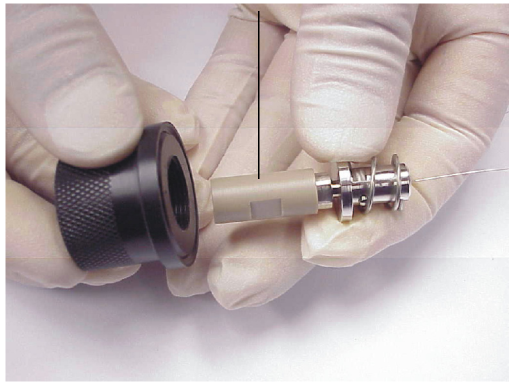


3. Unscrew and remove the nebulizer adjuster knob to reveal a PEEK union/UNF coupling assembly, compression spring, and the capillary.

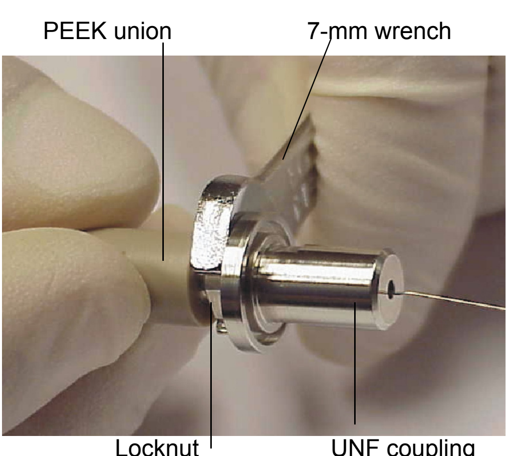


4. Remove the nebulizer adjuster knob, PEEK union/UNF coupling assembly, compression spring, and capillary from the probe.

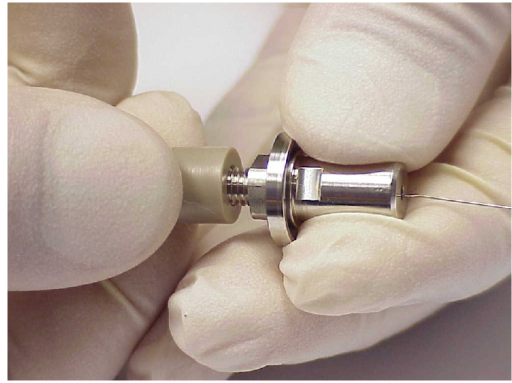
5. Remove the PEEK union/UNF coupling assembly, compression spring, and capillary from the nebulizer adjuster knob.



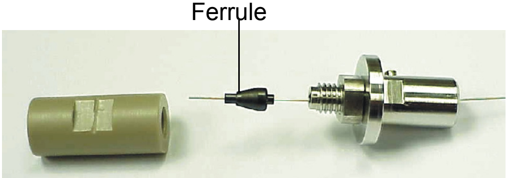
6. Remove the compression spring from the PEEK union/UNF coupling assembly and capillary.
7. A locknut is used to secure the PEEK union and UNF coupling. Use the 7-mm wrench to loosen the locknut.



8. Unscrew the PEEK union from the UNF coupling (this connection is finger-tight only).



This reveals a ferrule:

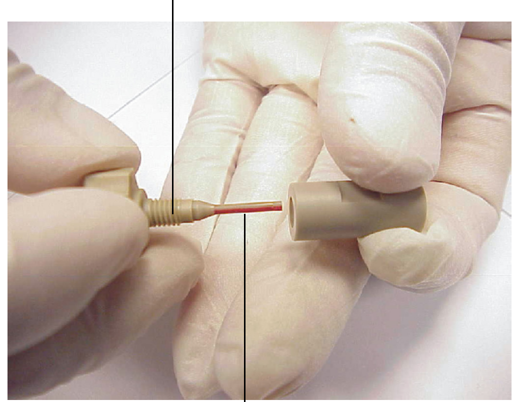


9. Remove the ferrule from the capillary.
10. Remove the capillary from the UNF coupling.

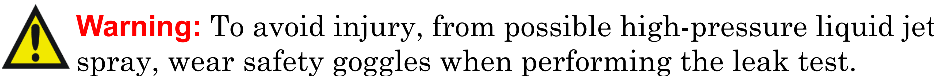
Installing the New Capillary

To install the new capillary:

1. Insert a square-cut length of red PEEK tubing in the probe inlet connector and screw the connector finger-tight into the PEEK union. This ensures a minimum dead volume when fitting the capillary.

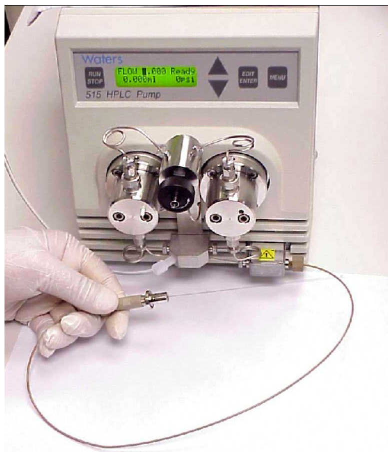


2. Fit the UNF coupling to the new capillary.
3. Use the needle-nose pliers to slide a new ferrule onto the capillary.
4. Insert the capillary in the PEEK union and ensure that it is fully seated.
5. Screw the UNF coupling into the PEEK union, finger-tight only.
6. Pull on the capillary gently, testing to ensure that it stays in place.
7. Use the 7-mm wrench to tighten the locknut against the PEEK union.



Warning: To avoid injury, from possible high-pressure liquid jet spray, wear safety goggles when performing the leak test.

8. Check for leaks in the assembly by attaching the free end of the PEEK tubing to an LC pump and pumping through 50:50 acetonitrile:water at 1 mL/min.



If leakage occurs, the connection must be disassembled and remade, and the leak test repeated.

9. When performing the leak test, check the backpressure on the LC pump. This will be high if the capillary is blocked. If this is the case, replace the capillary with a new one.

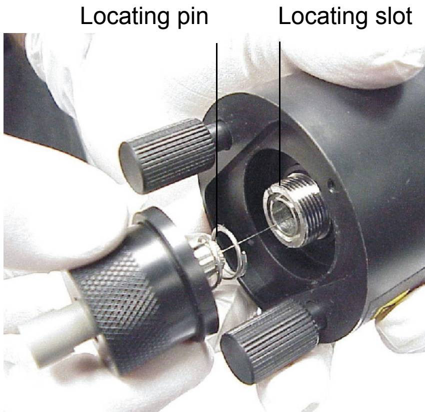
10. When the leak test has been performed successfully, disconnect the PEEK tubing from the LC pump.

11. Remove the probe inlet connector and PEEK tubing from the PEEK union.

12. Pull the probe heater off.

Caution: Take great care not to damage the probe heater electrical wiring either when removing the probe heater cover or while the probe heater is exposed.

13. Refit the compression spring to the capillary and PEEK union/UNF coupling assembly.
14. Carefully thread the capillary through the probe assembly.
15. Ensuring that the locating pin on the UNF coupling engages with the slot in the head of the probe assembly, fit and screw the nebulizer adjuster knob and PEEK union/UNF coupling assembly to the probe assembly. Do not tighten the knob fully.



16. Refit the probe end cover to the probe assembly.
17. Refit and tighten the two end cover securing screws.
18. Carefully slide the probe heater over the capillary sleeve, align the probe heater connections with the probe body, and then push all the way on.
19. Use the nebulizer adjuster knob to adjust the capillary so that the capillary protrudes by approximately 0.5 mm from the end of the probe.
20. Refit the probe to the instrument.

Replacing the IonSABRE Probe Heater

Replace the IonSABRE probe heater if it fails to heat.

To replace the probe heater:

1. Disconnect and remove the probe from the source
2. Pull the probe heater off the probe (pull straight off, do not rotate).
3. Carefully slide the new probe heater over the capillary sleeve, align the probe heater connections with the probe body, and then push all the way on.

Maintaining the APPI Lamp

Changing the lamp bulb

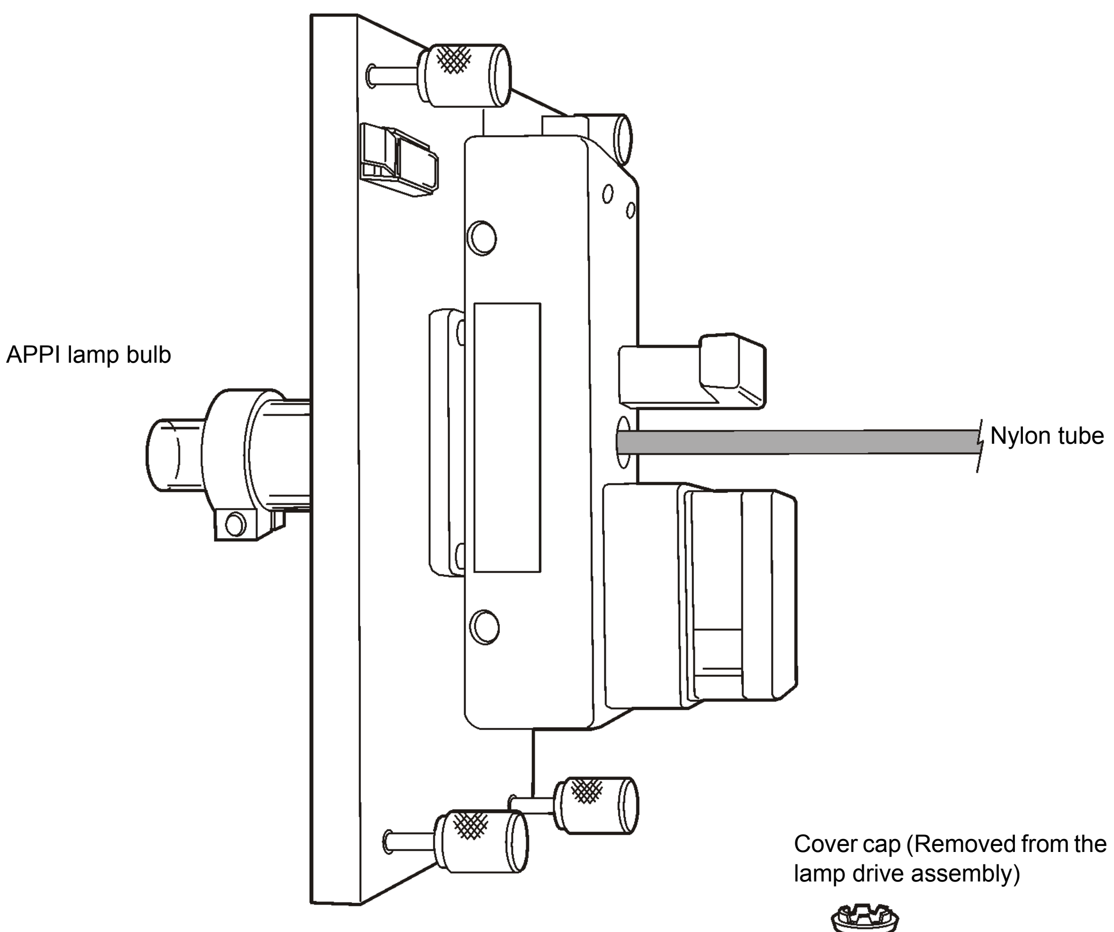
Warning: To avoid electric shock, ensure that the instrument is in standby mode before commencing this procedure.

Warning: To avoid contamination with toxic and biohazardous materials, wear rubber gloves at all times while handling the components.

To change the lamp bulb:

1. Click [button] and confirm the adjacent instrument status indicator shows red
2. Disconnect and remove the UV lamp assembly from the source enclosure
3. Remove the cover cap from the back of the lamp drive assembly (Figure titled "Changing the APPI lamp bulb:" on page 8-51).
4. Insert an appropriate tool (for example, a length of nylon tube) through the back of the lamp drive assembly, and push the bulb forward.
5. Withdraw the bulb from the lamp drive assembly.
6. Insert the new bulb into the lamp drive assembly.
7. Refit the cover cap to the lamp drive assembly.

Changing the APPI lamp bulb:



Performing LockSpray maintenance

Cleaning the oscillating baffle

The baffle will need removing and cleaning at regular intervals particularly if you can see that it is dirty. The frequency that you will have to clean the baffle will depend on your application.

To clean the oscillating baffle:

1. Unplug the LockSpray motor from the instrument.
2. Remove the LockSpray motor from the source.
3. Put the baffle in a beaker of 50:50 acetonitrile:water or 50:50 methanol:water with 1% formic acid and place in an ultrasonic bath for 15 minutes. Rinse with water after cleaning.
4. When replacing the baffle, ensure it locates correctly on the square end of the shaft and check that it is oriented such that the reference and analyte sprays are selected correctly from the Tune window.

Replacing the reference probe capillary

If the LockSpray reference probe is blocked, replace its stainless steel capillary.

Before carrying out this operation:

1. Switch the instrument into Standby.

   Warning: Always remove the high voltage connection before attempting any probe maintenance.

2. Disconnect the high voltage cable from the probe.

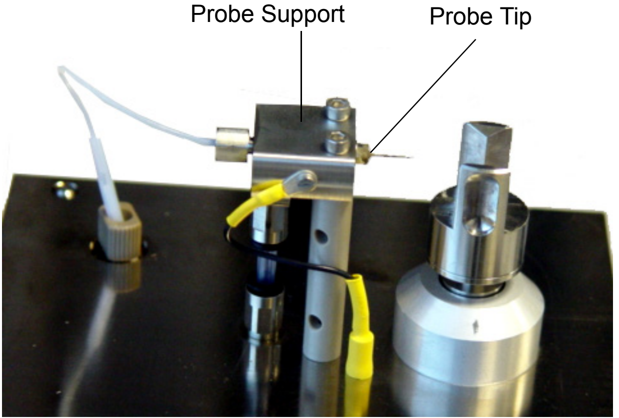
   Warning: Allow the probe to cool down before working on it.

3. Disconnect the nebulizer gas line, and remove the probe from the source.

To replace the reference spray probe tip:

1. Remove the reference sprayer from the source enclosure.

2. Using the wrench provided, unscrew and carefully remove the probe tip from the probe support segment



3. Carefully slide the new electrospray probe tip over the stainless steel capillary and fit it to the probe support segment.
4. Adjust the probe tip so that the sample capillary is protruding by approximately 0.5 mm from the end of the tip.
5. Refit the probe to the source enclosure.

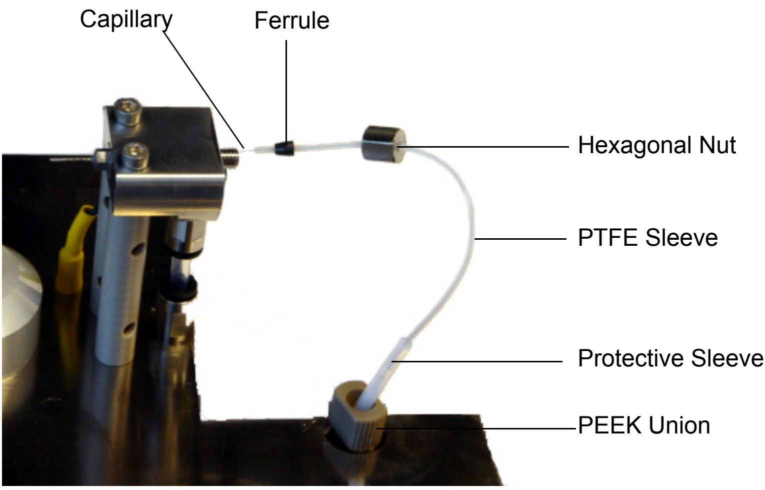
   Tip: Tweezers may be used to apply light pressure to the capillary to adjust its position.

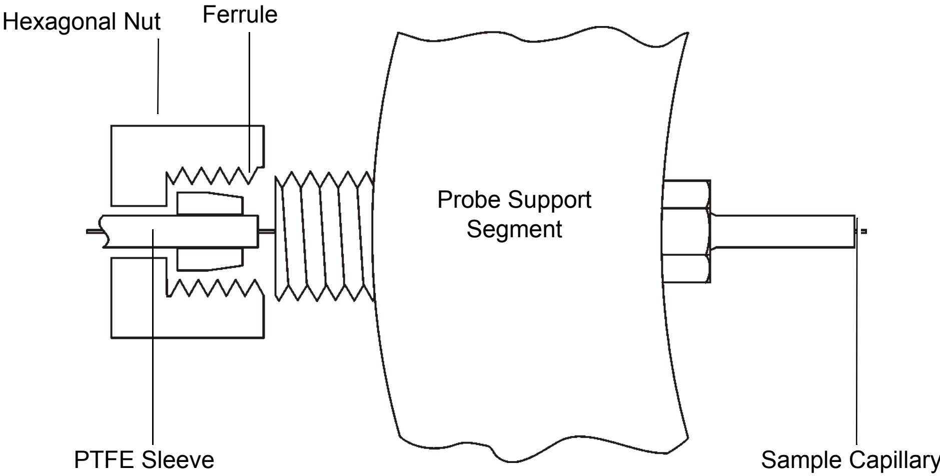
To replace the sample capillary:

Warning: The interface may be contaminated with biologically hazardous materials. Always wear rubber gloves while handling the components.

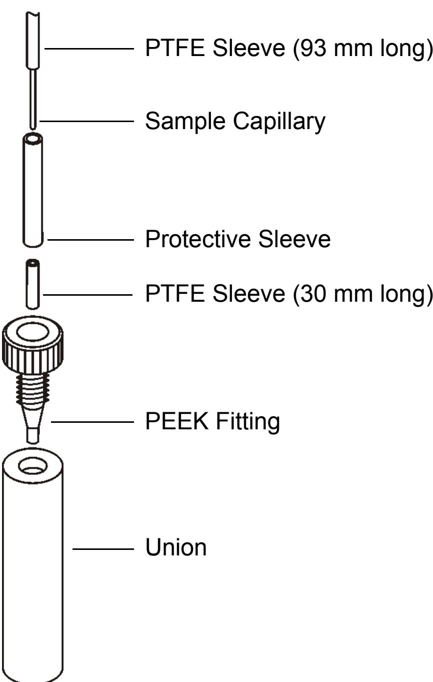
If the sample capillary becomes blocked, or is damaged, it may be replaced as follows:

1. Remove the reference sprayer from the source enclosure
2. Unscrew the hexagonal nut from the probe support segment.





3. Unscrew the finger-tight PEEK fitting from the union passing through the reference flange.
4. Remove the PEEK fitting and PTFE protective sleeve from the sample capillary.



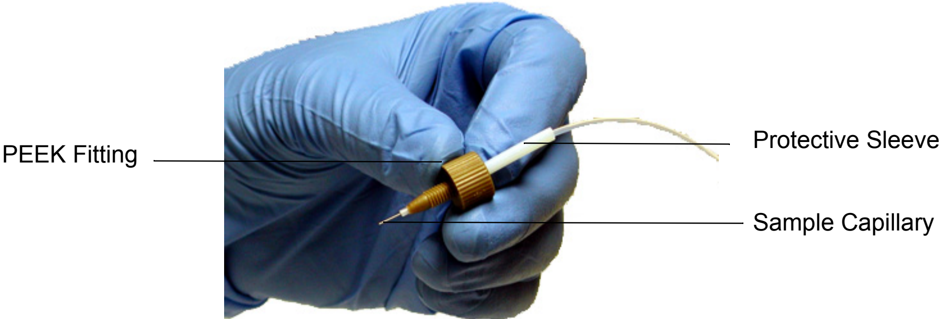
5. Slide the sample capillary out of the PTFE sleeve seated in the probe support segment.
6. Slide the PTFE sleeve out of the probe support segment.

   Caution: If using a tool to remove the ferrule from the PEEK fitting, take care not to damage the fitting.

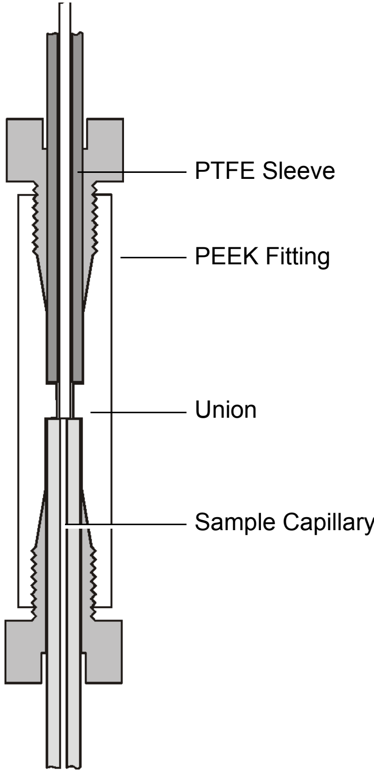
   Tip: If the graphite ferrule is stuck in the probe support segment, use a screwdriver, or similar object, to lever it out.
7. Feed the new sample capillary through the probe tip. Continue feeding the capillary through until approximately 0.5 mm of capillary is left protruding from the probe tip.
8. Slide a 93 mm length of 1/16 inch o.d., 0.010 inch i.d. PTFE sleeve over the free end of the sample capillary. Feed this into the probe support segment until it is firmly located in the segment.
9. Slide a graphite ferrule and the hexagonal nut over the PTFE sleeve.

   Caution: Ensure that the ferrule is not over-compressed when tightening the hexagonal nut onto the probe support segment.

10. Ensure that approximately 0.5 mm of sample capillary is protruding from the probe tip, then tighten the nut onto the probe support segment sufficiently to grip the ferrule onto the sample capillary.
11. Slide the PTFE protective sleeve over the sample capillary and PTFE sleeve.
12. Ensure that the 30 mm long 1/16 inch o.d., 0.010 inch i.d. PTFE sleeve is located in the PEEK fitting.
13. Slide the PEEK fitting over the capillary.
14. Slide the protective sleeve over the joint between the two PTFE sleeves (this prevents the sample capillary kinking at this point) and ensure the sample capillary protrudes a few millimeters through the PEEK Union.



15. Locate the PEEK fitting within the union on the reference flange, making sure the sample capillary still protrudes through the union.
16. To ensure that the sample capillary is fully butted to the union passing through the reference flange, use another PEEK fitting from the other side to locate the capillary precisely.



17. Fit and finger-tighten the PEEK fitting into the union.
18. Refit the reference flange to the source enclosure.

Performing NanoLockSpray maintenance

Cleaning the oscillating baffle

The baffle will need removing and cleaning at regular intervals particularly if you can see that it is dirty. The frequency that you will have to clean the baffle will depend on your application.

To clean the oscillating baffle:

1. Unplug the motor cable from the front panel.
2. Unplug the HV and Interlock cables from the front panel and remove the analyte adjuster assembly.
3. Undo the thumbscrew holding it to the shaft. In order to gain access to the screw with the NanoLockSpray source still mounted on the instrument, the NanoLockSpray should be set in the Analyte position.
4. Put the baffle in a beaker of 50:50 acetonitrile:water or 50:50 methanol:water with 1% formic acid and place in an ultrasonic bath for 15 minutes. Rinse with water after cleaning.
5. When replacing the baffle, ensure it locates correctly in the baffle holder on the end of the shaft.

Replacing the reference probe capillary

If the silica reference capillary is blocked, or if it is contaminated or damaged, replace it.

In most cases, use the ceramic cutter to remove the end of the capillary and feed more of the capillary loop through the Valco tee-piece. When there is no loop remaining you will have to replace the whole assembly

To replace the reference probe capillary:

1. Disconnect the probe.
2. Remove the nebulizing gas line from the tee (page 8-59).
3. Build the sprayer as described in (Chapter 5).

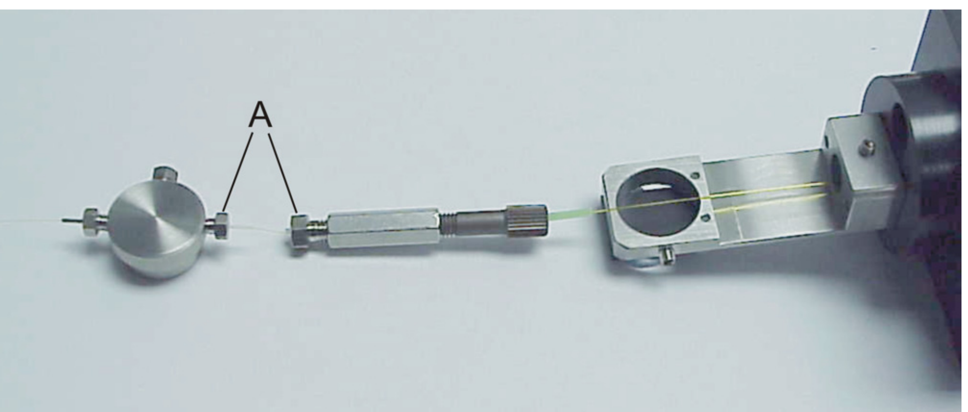
To disconnect the probe:

Warning: Always remove the high voltage connection before attempting any probe maintenance.

1. Disconnect the reference line from the probe and remove the finger-tight nut.
2. Disconnect the HV cable from the reference probe at the instrument front panel.
3. Disconnect the HV cable from the analyte probe at the instrument front panel.
4. Disconnect the reference probe nebulizing gas tube.
5. Remove the reference probe cover and undo the two bolts holding the reference probe to the NanoLockSpray housing and carefully withdraw the probe.

To remove the nebulizing gas line from the tee:

1. Loosen the grub screws that secure the Tee and Union, remove the tee and union from the probe holder.



2. Undo the tee nut and union nut marked A and remove the fused silica and the PTFE sleeves.
3. Remove the headless sealtight nut and ferrule and 75-µm i.d. fused silica.
4. Discard both pieces fused silica.

9 Fault Finding

Most faults can be traced to a malfunction of the ion source or inlet system. On systems equipped with more than one source, this can often be confirmed by changing sources to see if the fault moves with the source.

Should a fault occur soon after a part of the system has been repaired or otherwise disturbed, first ensure that this part has been correctly refitted and adjusted, and that adjacent components have not been inadvertently disturbed.

Contents

Topic	Page
Instrument stops responding to MassLynx	9-2
No Beam	9-4
Unsteady beam	9-4
High back-pressure	9-5
Loss of sensitivity	9-6
Incorrect isotope distributions	9-6
High noise levels	9-6
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Instrument stops responding to MassLynx

If the instrument stops responding to commands from MassLynx, the first thing to try is to exit MassLynx and then restart the application. If this fails, a reboot will be necessary.

There are three levels of reboot, with the levels required being dependent on the nature of the problem. Before rebooting you must determine whether a communications (comms) link exists between the MassLynx PC and the embedded PC (EPC)

Tip: It iss best to exit MassLynx before doing a reboot.

Testing Communications between MassLynx PC and the EPC

From Windows, select Start > Run and type *ping -t epc* in the Run dialog box.

The following messages appear if the comms link is OK.

```
C:\WINDOWS\System32\ping.exe

Pinging epc [64.1.1.2] with 32 bytes of data:

Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
Reply from 64.1.1.2: bytes=32 time<1ms TTL=64
```

The following messages will appear if there is no PC to EPC comms. This indicates a test failure.

```
C:\WINDOWS\System32\ping.exe

Pinging epc [64.1.1.2] with 32 bytes of data:

Request timed out.
Request timed out.
Request timed out.
Request timed out.
```

Tip: The comms test will fail during an EPC reboot. The comms link will recover with the completion of the reboot. If the comms link does not recover check the network cable linking the MassLynx PC with the PC Link socket at the rear of the instrument.

Rebooting

There are three level of reboot that you can try.

- From Telnet
- By resetting the EPC
- Switching the instrument off.

However, success depends on the nature of the problem. You should therefore try all three in the order listed here, until you succeed

To reboot from TELNET:

1. From Windows, select Start > Run and type *telnet epc* in the Run dialog box.
2. Type reboot at the –> prompt in the telnet epc window. This will reload the EPC software.

   If the –> prompt is absent, then an embedded PC reset will be necessary.

Tip: The full reboot will take about 3 minutes to complete.

To reboot by resetting the EPC:

Push the EPC reset button on the front of the instrument. This will reboot the EPC operating system and reload the EPC software.

The full reboot will take about 3 minutes to complete.

If this doesn't fix the problem, then a full power-off reboot will probably be necessary

To reboot by power off:

1. Switch the Pump override switch, on the rear of the instrument, to the down position.
2. Switch off the electronics power at the rear of the instrument. Wait 5 seconds, and switch back on. This will reboot both the embedded PC and the control PCB.

   The full reboot will take about 3 minutes to complete.
3. Open MassLynx and the Tune window before resetting the Pump Override switch to the up position.

No Beam

Consult the relevant chapters of this manual and ensure the system meets these conditions:

- Normal tuning parameters are set and, where applicable, readback values are acceptable.
- All necessary cables have been correctly attached to the source and probe.
- Solvent is reaching the probe tip and the solvent flow rate is as required.

  Tip: For solvent flow rates below 100 µL/min, it may be necessary to temporarily turn off the nebulizing gas and remove the probe from the source to allow the solvent to be seen at the probe tip.
- The desolvation and nebulizer gas are flowing and set according to the correct flow rates.
- The source has been assembled correctly and is clean.
- The source isolation valve is open.

If, after performing the above checks, the beam is still absent contact Waters.

Unsteady beam

Refer to the relevant chapters of this manual and check that:

- Capillary (electrospray) and Sample Cone are tuned correctly.
- The capillary is not protruding too far from the end of the probe.
- The probe is not too far into the source.
- The flow of solvent from the HPLC pump is correct and steady.

  Note: To do this, remove the probe, degas the solvent, increase the flow rate for several minutes to purge any trapped air then reset and re-measure the flow rate.
- Solvents have been adequately degassed.
- The nitrogen flow of desolvation and nebulizer gas is steady. The nitrogen supply pressure should be 7 bar (100 psi) ±10%.
- Desolvation and Source temperatures are not set too high or low for the liquid flow rate used.

Tip: High temperatures can vaporize solvent within the electrospray probe.

Should the preceding checks fail to reveal the cause of the problem, proceed to the following section.

High back-pressure

For electrospray, a higher than normal back-pressure readout on the HPLC pump, together with a slowing of the actual solvent flow at the probe tip, can indicate that there is a blockage in the capillary transfer line or injection loop due to particulate matter from the sample.

To clear the blockage:

Remove the probe from the source and increase the solvent flow to 50 µL/min to remove the blockage.

Tip: Often, injections of neat formic acid help to redissolve any solute which has precipitated out of solution.

If the blockage cannot be cleared in this fashion:

1. Remove the finger-tight nut and tubing from the back of the probe.
2. If the back pressure remains high, replace the tubing with new tubing (or first try removing both ends of the tube).
3. If the back pressure falls, replace the stainless steel sample tube inside the probe (or try reversing the tube to blow out any blockage).
4. Reconnect the tubing to the probe.

The solvent flow can now be readjusted and the probe replaced into the source.

Tip: To check the flow rate from the solvent delivery system, time how long a known volume takes to emerge from the probe tip. Once the rate has been measured and set, note the back pressure readout on the pump, as fluctuation of this reading can indicate problems with the solvent flow.

For APCI, a higher than normal back pressure readout on the HPLC pump can imply that, after a long period of use, the filter pad requires replacement.

Loss of sensitivity

As the ion source becomes dirty after prolonged use, the performance will degrade.

Unstable or reduced ion currents indicate that the source needs cleaning. The usual remedy is to clean the source as described in Cleaning the Source components on page 8-5.

An increase in the analyzer pressure above 4e-6 mbar can also cause loss of sensitivity, although the pressure at which this occurs will be sample dependent.

Incorrect isotope distributions

Incorrect isotope distributions can be caused by:

- The TDC Signal threshold (mV) threshold being set too high
- A faulty MCP
- Saturated Signal
- A faulty attenuator

High noise levels

High noise levels can either be chemical or electronic in nature.

Chemical noise

Chemical noise usually originates from contaminated samples, solvents, or source gases.

It can be distinguished from electronic noise simply by stopping source ionization. If no liquid or gases are entering the source and all the source voltages are set to zero, then the remaining noise will be electronic in nature.

Electronic noise

Electronic noise can be caused by setting the signal threshold too low. The microchannel plate detector can be damaged by failure to properly condition the detector after venting of the system to atmosphere. If the detector is

producing micro discharges, excessive noise is apparent on the baseline of mass spectra in the absence of any ion beam. Reducing the detector voltage will reduce the number of discharges and also reduce the noise.

LockSpray fault finding

The following faults and solutions apply to LockSpray and NanoLockSpray:

- If the Tune window "times out" when attempting to find either the analyte or reference spray, ensure that the motor cable is connected.
- If the signals from either spray are unstable, or the signal changes when operating, it is possible that the baffle may need cleaning (see Cleaning the oscillating baffle on page 8-52) or that the sprayer is discharging to the baffle(this indicates the need to reduce the capillary voltage).

General maintenance and fault finding for a ZSpray source operating in the electrospray mode are found in the appropriate sections of this chapter.

Contacting Waters

You can easily correct many problems with the Q-Tof Premier. However, if this is not the case, you must contact Waters.

Customers in the USA and Canada should report maintenance problems they cannot resolve to Waters Technical Service (800 252-4752). All others should visit http://www.waters.com and click Offices, or phone their local Waters subsidiary or Waters corporate headquarters at 34 Maple Street, Milford, MA 01757, USA.

When contacting Waters, have the following information available:

- The nature of the symptom
- The Q-Tof Premier serial number

Depending on the nature of the fault, it may also be useful to have the following information available:

- Details about the flow rate, mobile phases, and sample concentrations
- Tune window settings
- The Software version update reference

Starting Up and Shutting Down

Contents:

Topic	Page
Starting the Q-Tof Premier	A-2
Shutting down the Q-Tof Premier	A-3
Automatic startup and shutdown	A-5

Starting the Q-Tof Premier

To startup the Q-Tof Premier:

1. Turn on the following Vacuum, electronics Embedded PC, and auxiliary breakers on the rear of the instrument. Allow 3 minutes for the embedded PC to initialize.
2. Start the MassLynx software. The MassLynx window appears and the word Ready appears in the status bar at the bottom of the window.
3. Click the Instrument shortcut bar MS Tune icon to open the Tune window.
4. Select Vacuum > Pump.
5. Click the Diagnostics tab.

   Tip: If the Diagnostics tab is not present, select View > Extended Controls.
6. Monitor the Turbo Speeds. These parameters should reach 98 to 100% within approximately 5 minutes of selecting Options > Pump.
7. Ensure that the instrument has pumped sufficiently such that the Vacuum LED on the front panel is steady green. The mass spectrometer is sufficiently evacuated to enable operation in three hours.

See also:

- Front panel on page 1-7

Shutting down the Q-Tof Premier

Emergency instrument shutdown

Warning: The power switch does not isolate the instrument from the mains power supply. Unplug the mains power from the rear of the instrument to isolate the instrument from the mains power supply.

In the event of having to shut down the instrument in an emergency, switch off the power at the wall mounted isolation switch(es), if fitted. If not, switch off the System Power switch located on the rear of the instrument and turn off all peripherals.

Important: A loss of data is likely.

Tip: If control of the instrument is lost through MassLynx, you can place it in Standby by pressing the Standby switch on the front of the instrument.

See also:

- Rear panel on page 1-17
- Front panel on page 1-7

Overnight instrument shutdown

It is not necessary to switch the instrument out of Operate mode. However, this is acceptable provided that the instrument warm-up time is considered when restarting analysis.

To shutdown overnight:

When the instrument is to be left unattended for a long time, for example, overnight or at weekends, proceed as follows:

1. On the MassLynx Tune window Source tab, switch off the API gas and stop the syringe.
2. If required, click Press for Standby to switch the instrument out of Operate mode.

To completely shutdown:

1. On the Tune window, click Press for Standby.

2. Select Vacuum > Vent.
3. Select Vent Instrument, a message confirms the vent command.
4. Click OK. The turbomolecular pump is switched off. When the turbomolecular pump has run down to half its normal operating speed, the vent valve is opened and the instrument is automatically vented. The Vacuum LED will switch off after changing from green to amber.
5. Exit MassLynx.
6. Shut down the PC.
7. Switch off all peripherals.
8. Switch off the vacuum, electronics, Embedded PC / auxiliary breakers located on the rear panel.

See also: Rear panel on page 1-17

Automatic startup and shutdown

MassLynx has automatic Startup and Shutdown files. They are found in the C:\MassLynx\Shutdown directory and are called ShutDownxxx.acl and StartUpxxx.acl, where xxx refers to the instrument configuration. E.g. ShutDownESI_ACE.acl for an instrument configured as an ACE system.

Shutdown editor

The Shutdown Editor allows automatic startup and shutdown procedures to be modified or created. These can be run automatically before or after a batch if you select the relevant check boxes in the Batch Control frame of the Shutdown Page.

Select Edit Shutdown or Startup from the MassLynx Instrument Shortcut Bar to open the Shutdown Editor. It contains the Shutdown page and the Auto Control Tasks page.

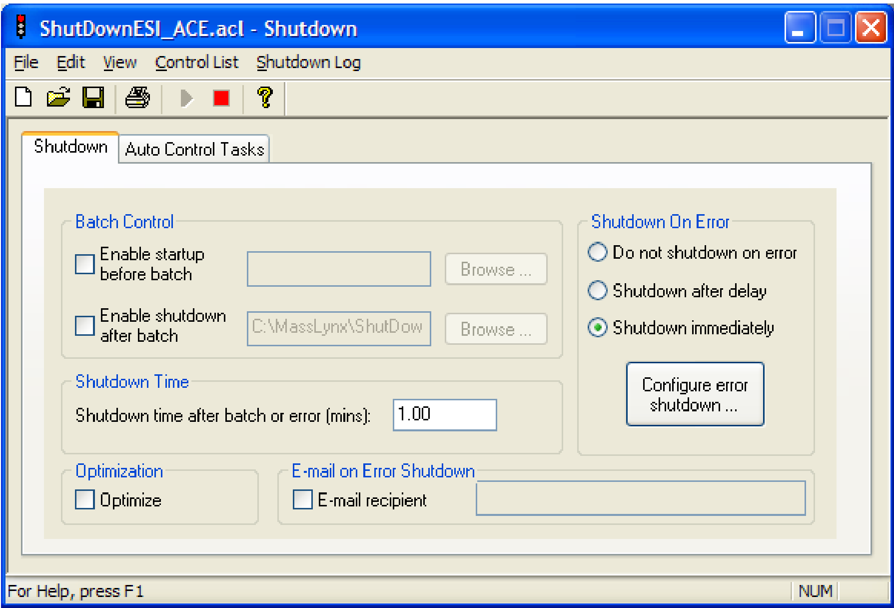
See also:

- Shutdown page on page A-5
- Auto Control Tasks page on page A-10

Shutdown page

Click Edit Shutdown or Startup from the MassLynx Instrument Shortcut bar.

Shutdown page:



By default the Shutdown page appears with the following parameters.

Shutdown page parameters:

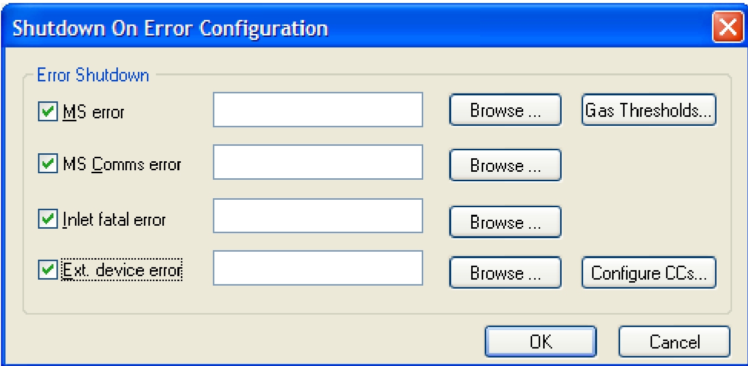
Parameter	Description
Enable startup before batch	Enables/disables the running of a task file before the start of a batch of samples. Startup Enabled will appear on the right of the Status bar on the bottom of the MassLynx window when selected.
Startup file (text box)	The file name of the task file that is run before the start of a batch.
Browse (startup file)	Displays a file dialog allowing you to select a task file for the startup before batch procedure.
Enable shutdown after batch	Enables/disables the running of a task file after the end of a batch of samples. Shutdown Enabled will appear on the right of the status bar on the bottom of the MassLynx window when selected.
Shutdown file (text box)	The file name of the task file that is run after the end of a batch.

Shutdown page parameters: (Continued)

Parameter	Description
Browse (shutdown file)	Displays a file dialog box where you select allowing a task file for the shutdown after batch procedure.
Shutdown Time	The delay (in minutes) between the batch finishing and the shutdown procedure initiating.
Shutdown On Error	Allows shutdown on error to be enabled/disabled and to determine whether the shutdown tasks should be initiated immediately, or after the time entered in the Shutdown Time edit box.
Configure Error Shutdown	Displays up the Shutdown on Error Configuration dialog. **See also:** Configure Shutdown on Error on page A-7.
Optimization	Optimizes the procedure.
E-mail on Error Shutdown	Enter the e-mail address to send the shutdown information.

Configure Shutdown on Error

Clicking the Configure Error Shutdown button displays the Shutdown on Error Configuration dialog box.

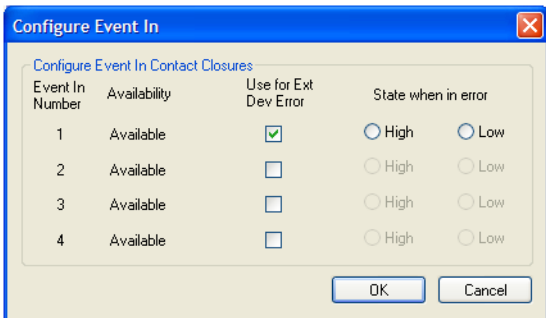


Parameter	Description
MS error	Enables/disables running a task file when an MS error occurs.

Parameter	Description
MS error shutdown file	The task file to run on an MS error.
MS Comms Error	Enables/disables running task file when an MS comms error occurs
MS comms error shut-down file	The task file to run on an MS comms error.
Inlet fatal error	Enables/disables running a task file when an LC error occurs.
Inlet error shutdown file	The task file to run when an LC error occurs.
Ext. device error	Enables/disables running a task file when an external device error occurs.
Ext. device shutdown file	The task file to run when an external device error occurs.
Gas Threshold	Opens the Gas Threshold dialog. **See also:** Gas thresholds on page A-9.
Configure CCs	Opens the Configure Event In dialog. **See also:** Configure contact closures on page A-8).

Configure contact closures

Clicking on the Configure CCs displays the following dialog.



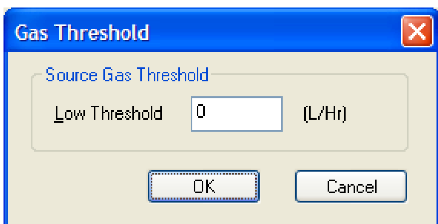
Use this dialog to configure the Event In contact closures on the back of the MS. These can be used to detect errors in external devices enabling the External device error flag to be set. Only contact closures not used to signal

the completion of an injection, or not used by MUX systems, are enabled. Each available CC has the following controls.

Parameter	Description
Event In Number	Relates to the Event In number on the rear of the MS.
Availability	This can either be Used By Inlet, Available, Used by MUX or Not Configured. The controls are only enabled if the CC is Available.
Use For Ext Dev Error	Enables/disables the use of this contact closure to signal an error in an external device.
State when in error	Determines what state the CC will be in when an error is present in an external device.

Gas thresholds

Clicking Gas Thresholds in the Configure Shutdown on Error dialog box opens the Gas Threshold dialog box.

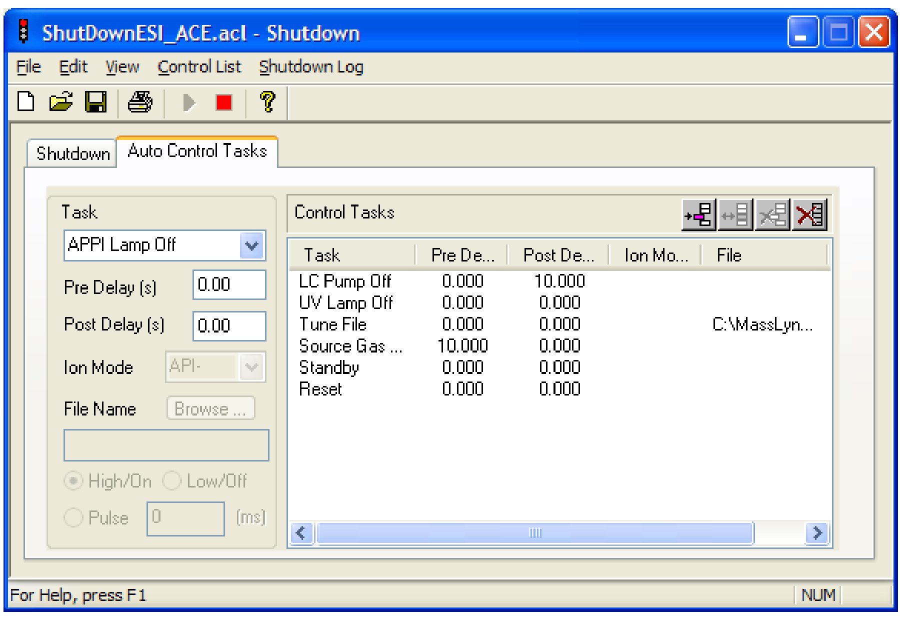


An MS error is flagged if the source gas flow rate falls below the value entered in the Low Threshold text box.

Tip: The Gas Thresholds button is available only when the MS error option is selected.

See also: Shutdown page on page A-5

Auto Control Tasks page



Parameter	Description
Task	Lists all the available tasks.
Pre-Delay	The length of time that will elapse before the current task is performed
Post-Delay	The length of time that will elapse after the current task has been completed and before the next task is started. E.g. a Post delay of 60s, in the Tune File task above, means that there will be a delay of 60 seconds before the next task is started, to allow the machine to stabilize with the new Tune window settings.
Ion Mode	Lists all the available ionization modes.
File Name	The name of the Tune file to be used. The file name can be typed in, including the full path name, or selected from the browser displayed when the Browse button is clicked.

To add a task:

1. Select a task from the Task list.
2. Enter the required parameters.
3. Click the add button.

Tip: If this is a new task timetable the task will be added to the end of the list. If a task has been inserted into the task timetable then all subsequent tasks will be added after the inserted task. To add a task to the end of the timetable after inserting a task, double click below the last entry in the timetable and then add the new task.

To insert a task:

1. Click the entry in the task timetable before which you want to insert the new task.
2. Select a task from the drop down Task list box.
3. Enter the required parameters.
4. Click the add button. The task will be inserted before the selected entry.

To modify a task:

1. Click the entry in the task timetable. The details for the task will be displayed in the fields on the left of the screen.
2. Change the required parameters.
3. Click the modify button. The details will change in the task timetable.

To delete a task:

1. Click the entry in the task timetable. The details for the task will be displayed in the fields on the left of the screen.
2. Click the add button. The task selected will be deleted from the task timetable.

To delete all Tasks

Click the add button. All tasks will be deleted from the task timetable.

Running startup and shutdown files

If Startup or Shutdown is selected from the MassLynx Shortcut bar or from the Shutdown editor Control List menu, then the automatic startup and shutdown files are run.

To run a different startup or shutdown file:

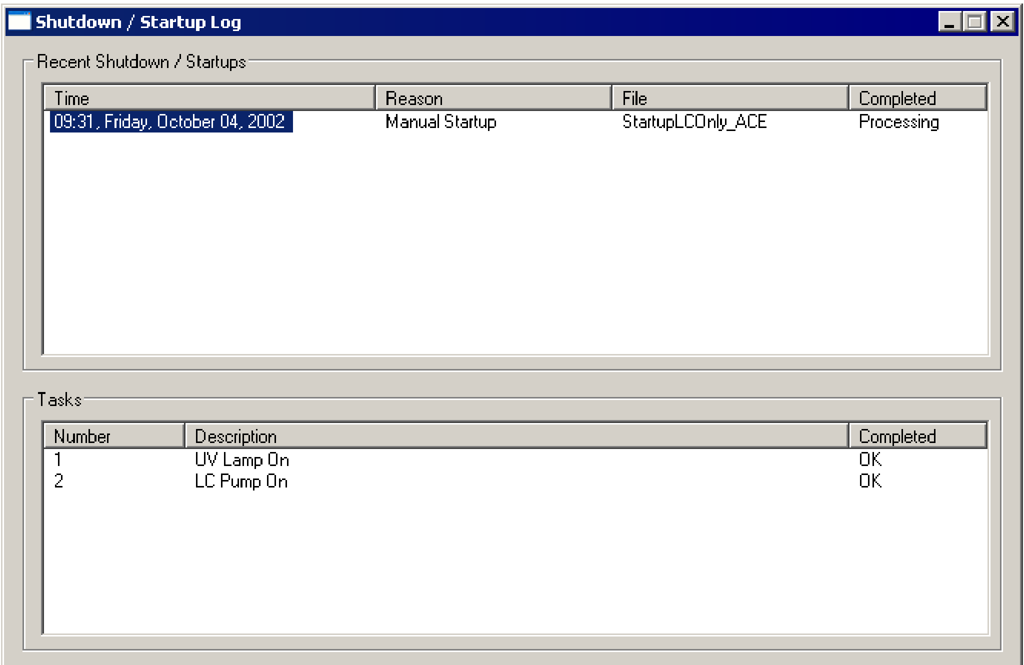
1. Open the required file in the Shutdown Editor and click the toolbar button or select Run List from the Shutdown Editor Control List menu.
2. Click the toolbar button or select Stop List from the Shutdown editor Control List menu to stop running this file.

Alternatively if the Enable startup before batch or Enable shutdown after batch options are selected on the Shutdown Page, the files will be run before or after a Sample List run.

See also: Shutdown page on page A-5.

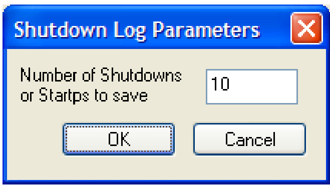
Shutdown / Startup Log

The Shutdown / Startup Log keeps a record of the most recent startups and shutdowns. Select Shutdown Log > Recent Shutdowns and Startups opens the Shutdown Startup Log. Selecting the most Recent Shutdowns and Startups in the top pane will show the tasks carried out in the bottom pane.



Log parameters

Selecting Shutdown Log > Log Parameters from the main menu bar opens the Shutdown Log Parameters dialog box. You can alter the number of startups and shutdowns recorded in the Startup / Shutdown Log by changing the number in the text box.



B Tune Window

This appendix adds more detail to the Tune window already described in Instrument Operation on page 2-1 and Chapter 3, Calibration.

Contents:

Topic	Page
Tune window basics	B-2
Controlling the display of readback windows	B-4
Changing Tune parameter settings	B-5

Tune window basics

Selecting the operating mode

The various operating modes are selected from the Mode menu. The currently selected modes have a check mark next to them.

Controlling gas flows

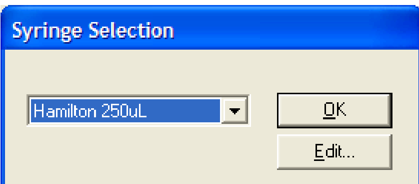
To toggle the nebulizer, desolvation, and cone gasses on and off, click .

Controlling the syringe pump

To toggle the syringe pump on and off, click .

To select the syringe type

1. Click to open the Syringe Selection dialog box.

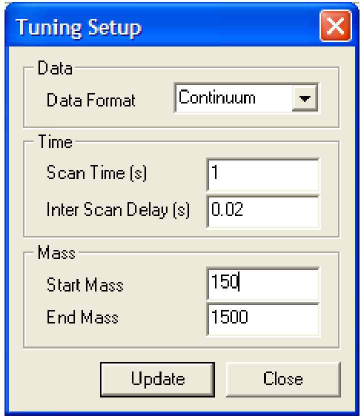


2. Select the syringe type currently being used from drop-down list.
3. If the required syringe type is not available, click Edit to open the syringe list, and then add the required details.
4. Click OK.

Tuning setup

To set parameters for tuning:

1. Click or select Setup > Acquisition Settings to open the Tuning Setup dialog Box.



2. Enter the Start Mass and End Mass.
3. Enter the Scan Time and Inter Scan Delay.

   Tip: Scan Time (s) and Inter Scan Delay (s) control the speed with which the tune peak display is updated.Tuning is more responsive when these parameters are set to low values.

4. Select a data format.

Controlling the display of readback windows

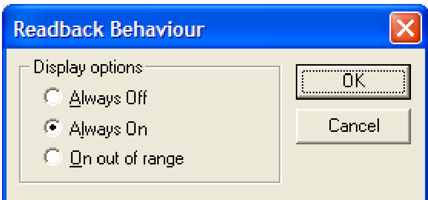
There are three options for displaying system readbacks on the Tune window:

- Readbacks displayed continuously (Always On)
- Readbacks hidden (Always Off)
- Readbacks displayed only when differing from their defined values by more than 10% (On out of range)

A number of the readbacks are for diagnostic purposes only, their function being to confirm that a voltage is present. The acceptable variation between the set value and the readback value varies depending on the particular tune parameter. If you are concerned about any reading, contact Waters for advice (see Section 9.4, Contacting Waters).

To change the readback behavior:

1. Select View > Readback Behavior to open the Readback Behavior dialog box.



2. Select one of the three options.

Changing Tune parameter settings

You can modify most parameters in the following ways:

- Drag the slider bar using the mouse.
- Click the slider bar and use the left and right arrow keys to change the value by one increment.
- The edit window updates as the slider bar is activated.
- Type a new value into the edit window.

Other parameters have only an edit window and are changed by direct typing.

The speed with which the system responds to changes depends on the speed with which the peak display refreshes. For the fastest response, set the Tuning Setup dialog box Scan Time (s) and Inter Scan Delay (s) values to be as short as possible.

Instrument parameter files

Instrument tuning parameters can be saved in an instrument parameter file (*.ipr), which can be recalled later.

An instrument parameter file contains all the parameters for all supported ionization modes, not just the ionization mode currently selected. Instrument parameter files also contain settings for the analyzer, inlet set points, and peak display.

Printing tune information

To print a report containing a copy of the tune peak information displayed on the screen and with a record of each parameter setting, click or select File > Print.

You cannot configure this report.

Selecting the span of a displayed peak

To select the span of a peak:

1. Click and drag the mouse horizontally from one end to the other of the region of interest.

As the mouse is dragged, a “rubber band” stretches out to indicate the selected range.

Do not go beyond the bounds of the axis.

2. Release the mouse button to redisplay the selected range so that it fills the current window.

   You can repeat this as often as required.

   Clicking once, displays the previous magnification range, clicking it again returns to the default settings.

To select the span of a peak using the Span box:

1. Enter a value in the Span box for the required peak.
2. Click Return.

   This becomes the default, so if the range is altered with the mouse and you click twice, Span returns to this value.

Changing the gain of a displayed peak

To change the gain of a peak, either:

1. Double-click the line displaying the gain value (e.g., 2×) above the peak, to double the gain applied to that peak.
2. Double-click below the peak display to halve the gain.

Or:

1. Click and drag the left mouse button vertically from one end to the other of the region of interest.

   As the mouse is dragged, a “rubber band” indicates the selected range.

   Do not go beyond the bounds of the axis.

2. Release the mouse button to redisplay the selected range so that it fills the current window.

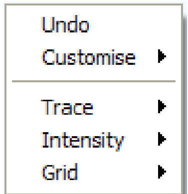
Customizing the peak display

Opening the Peak display menu

Use the Peak display menu to customize the peak display. Open it by right-clicking in the appropriate peak display window on the Tune window.

Tip: You can customize the display window for each peak customized, e.g., the peak color for peak 1 can be red, for peak 2 green, etc.

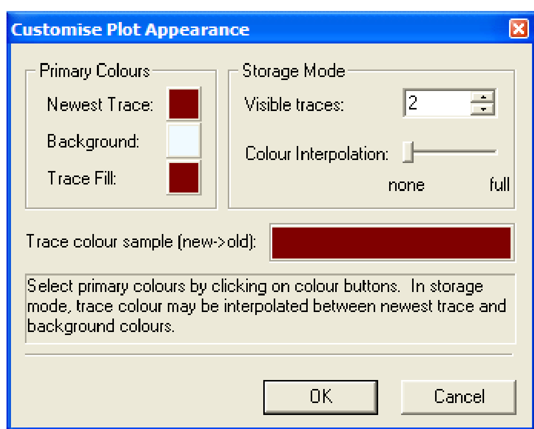
Peak display menu:



Customizing the colors and numbers of displayed traces

To change the color of the background and traces, and to change the number of traces displayed, select Customise > Plot Appearance from the Peak Display pop-up menu to open the Customise Plot Appearance dialog box.

Customise Plot Appearance dialog box



To change the colors on the display:

Click the color box adjacent to Newest Trace, Background, or Trace Fill as required, and select a new color from the Color dialog box.

To change the number of displayed traces:

Enter the required value in the Visible traces box, within the range 2 to 20.

If more than one trace is displayed, the older traces can be displayed in color shades different to the newer ones. Drag the Colour Interpolation slider toward the full position. The colors of the older traces appear in the Trace colour sample (new->old) field.

Customizing the peak trace line appearance

Each trace may be displayed as an outline, with the area below the line filled, or as maximum and minimum points only.

To display the peak outline only, select Trace > Outline from the Peak Display pop-up menu.

To display the filled trace, select Trace > Fill from the Peak Display pop-up menu.

To display the trace as minimum and maximum data points only, select Trace > Min/Max from the Peak Display pop-up menu.

The currently selected option has a check mark displayed next to it in the Peak Display pop-up menu.

Customizing the peak intensity display

To display the peak intensities as absolute values (cps), select Intensity > Absolute Intensity from the Peak Display pop-up menu.

To display the peak intensities as percentage values relative to the intensity of the highest peak, select Intensity > Relative Intensity from the Peak Display pop-up menu.

The Peak Display pop-up menu Intensity > Normalise Data option can be selected in conjunction with either of the above options. It controls the way in which the peak display is scaled. When enabled, the display scales to the value of the intensity of the highest peak; when disabled, the display scales to the default value set in the MassLynx software. It is recommended that the Intensity > Normalise Data option is normally enabled.

The currently selected options have a check mark displayed next to them in the Peak Display pop-up menu.

Customizing the peak display grid

The Peak Display vertical and horizontal grid lines may be independently displayed or hidden.

To display the horizontal grid lines:

Select Grid > Horizontal from the Peak Display pop-up menu.

To display the vertical grid lines:

Select Grid >Vertical from the Peak Display pop-up menu.

The currently selected options have a check mark displayed next to them in the Peak Display pop-up menu.

Selecting the instrument name

To select the instrument name:

1. Select Options > Instrument Name to open the Instrument Name dialog box.
2. Enter the required name in the ID text box.
3. Click OK.

C Setting-Up the Syringe Pump

This appendix describes how to set-up the syringe pump, which is used for infusing the standard solution during the tuning process.

To setup the syringe pump:

Warning: To avoid electric shock (non-lethal), the syringe must be grounded.

1. Plug the syringe grounding cable into the socket marked ‘syringe’ on the front connection panel.
2. Mount the syringe onto the pump
3. Attach the syringe grounding cable to the syringe needle
4. Connect the syringe pump to the required probe using a fused silica syringe line
5. Before using the syringe pump, ensure that the type of syringe used is selected in the MassLynx software (see Appendix B)

To prepare the fused silica:

1. Screw the Valco syringe adapter into the peek union.
2. Slide a syringe into the syringe adapter.
3. Tighten the syringe adapter nut until it grips the syringe.
4. Pull out the syringe.
5. Use a ceramic silica cutter to make a square, even cut on both ends of the sample capillary (supplied in the ESI probe installation kit) before installing. Examine new cuts for squareness using and eye glass. When cutting the capillary, allow enough length to form loops at angles and corners.
6. Connect the capillary to the PEEK union, using Upchurch® Scientific nut, ferrule and PTFE tubing.

D Materials of Construction and Compliant Solvents

Warning: To avoid possible excessive leakage of solvent into the laboratory atmosphere, you must address any safety issues raised by the contents of this Appendix.

Contents:

Topic	Page
Items exposed to solvent	D-2
Common ingredients used to prepare mobile phases	D-3

Items exposed to solvent

The items detailed in Table titled "Items exposed to solvent" on page D-2 may be exposed to solvent; you must evaluate the safety issues involved if the solvents used in your application differ from the solvents normally used with these items. See Common ingredients used to prepare mobile phases on page D-3 for details of the most common ingredients used to prepare mobile phases.

Items exposed to solvent

Item	Material
O-rings	Viton or PTFE-encapsulated Viton
Gas tubes	PTFE
Ion block	Stainless steel
Ion block support	PEEK
Corona discharge pin mounting contact	PEEK
Gas exhaust port	Aluminium
Isolation valve	Gold-plated aluminium/bronze
Push-in gas fittings	Nickel/brass
Source enclosure	Alochromed aluminium
Source enclosure view port	Toughened plate glass
Probe adjustment flange	Anodized aluminium, glass filled acetal, and stainless steel
Probe shaft	PEEK
Probe adjuster bellows	PTFE
Waste bottle	Polypropylene

Common ingredients used to prepare mobile phases

The following lists the most common ingredients used to prepare mobile phases for reverse-phase LC/MS (API):

- Water
- Methanol
- Acetonitrile
- Formic acid (<0.1%)
- Acetic acid (<1%)
- Trifluoroacetic acid (<0.1%)
- Ammonium acetate (<10 mM)
- Ammonium formate (<10 mM)

These solvents are not expected to cause any problems with the materials identified in Items exposed to solvent on page D-2.

Glossary

AFAMM	All File Accurate Mass Measure.
API	Atmospheric Ionization
APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photoionization
Atomic mass	The mass of an atom, or isotope, usually expressed in atomic mass units
Atom mass unit (amu)	A unit of mass 1/12 of the mass of the most abundant isotope of carbon, carbon 12, which is assigned a mass of 12. Also called Dalton.
Atomic number	The number of protons in an atomic nucleus.
Average mass	The mass of an ion calculated using the relative average isotopic mass of each element (where C=12.0111, H=1.00797, O=15.9994, etc.).
Base peak	The peak in a mass spectrum corresponding to the m/z value that has the greatest intensity.
BPI	Base Peak Intensity.
CID	Collision Induced Dissociation.
cps	Counts per second.
CVF	Cone Voltage Fragmentation.
Dalton	See Atomic mass unit.
DDA	Data Directed Analysis.
Dead Time	The data acquisition system for the instrument is a Time-to-Digital Converter (TDC). This is an ion counting system that generates a mass spectrum by storing the ion arrival times in a histogram memory. After the arrival and registration of an ion by the TDC there is am minimum time interval before a subsequent ion arrival can be registered. This is called the “dead time” of the TDC and is of the order of 5 nanoseconds.
Desolvation	The action by which a nebulized (or sprayed) sample is dried to remove the solvent component.

DXC	Dynamic eXternal Calibration. On a time-of-flight mass spectrometer, if the temperature of the lab changes, the mass position of a peak detected also changes due to expansion of the flight tube. To compensate for this, Waters instruments can be equipped with DXC technology.
EPC	Embedded PC; the computer inside the instrument that connects to the control PC and the instrument electronics.
EPCAS	Embedded Personal Computer Acquisition System.
ESCi	ElectroSpray Chemical Ionization
ESI	ElectroSpray Ionization.
FWHM	Full Width at Half Maximum.
GFP	Glu^{1}-FibrinoPeptide B.
HPLC	High Performance Liquid Chromatography
Ion	An atom or a group of atoms, that has acquired a net electric charge by gaining or losing one or more electrons.
IPP	Ions Per Push
Isotope	One of two or more atoms having the same atomic number but different atomic masses.
Isotopic abundance	The naturally occurring distribution of the same element with different masses, e.g. ^{12}C-12.0000=98.89%, ^{13}C=13.0034=1.1%.
Inlet file	Stored in the AcquDb folder of the MassLynx project, the inlet file stores the parameters for the inlet system of your choice.
Inlet Method Editor	Accessed from the MassLynx instrument shortcut bar or the Sample List, it is used to create and edit the Inlet file.
LED	Light-Emitting Diode
LC	Liquid Chromatography.
MALDI	Matrix-Assisted Laser Desorption Ionization
MCP	Micro-channel Plate (ion collector).
Method Editor	Accessed from the MassLynx instrument shortcut bar or the Sample List, it is used to create MS method files.

MRM Multiple Reaction Monitoring.

MS Mass Spectrometer.

Nebulization The process by which the sample solution is transformed into a fine mist in the source. The sample mist is also referred to as the spray.

Nominal Mass The mass of an ion calculated using the integer mass of the most abundant isotope of each element (i.e. it neglects the mass defect, where H=1, C=12, O=16 etc.).

oa-Tof Orthogonal acceleration Time-of-flight.

Operate mode (Of an instrument), the instrument mode in which high voltages are switched on. Data can be acquired in this mode. The instrument maybe left in this mode.

PEG Polyethylene glycol.

Penning gauge Gauge used to measure pressures from approximately 10^{-3} to approximately 10^{-8} mbar, the range in which the turbomolecular pumps operate.

Pirani gauge Gauge used to measure pressures from approximately atmospheric to approximately 10^{-3} mbar, the range in which the roughing pumps (rotary pumps and scroll pumps) operate.

Sample List The area in the main MassLynx window by which all the necessary parameters (MS method, Inlet, Tune, etc.) are defined for a list of samples.

Standby mode (Of an instrument), the instrument mode in which high voltages are switched off. Data cannot be acquired in this mode.

TDC Time-to-Digital Converter.

TIC Total Ion Count.

Tof Time-of-flight.

Tune file Stored in the AcquDb folder of the MassLynx project, the tune file stores the parameters for the mass spectrometer.

Tune window The MassLynx module where the mass spectrometer is controlled. Tune parameters can be set and the instrument calibrated for exact mass.

UPLC Ultra Performance Liquid Chromatography.

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