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The instrument is marked with this symbol where **high voltages** are present.



The instrument is marked with this symbol where **hot surfaces** are present.



The instrument is marked with this symbol where the user should refer to this *User's Guide* for instructions which may prevent damage to the instrument.

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Warnings are given throughout this manual where care is required to avoid personal injury.



If the instrument is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

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This manual is a companion to the MassLynx NT User's Guide supplied with the instrument. All information contained in these manuals is believed to be correct at the time of publication. The publishers and their agents shall not be liable for errors contained herein nor for incidental or consequential damages in connection with the furnishing, performance or use of this material. All product specifications, as well as the information contained in this manual, are subject to change without notice.

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# Instrument Description

## Introduction



The Q-Tof 2 hybrid quadrupole time of flight mass spectrometer is available with electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI).

Q-Tof 2 utilises a high performance, research grade quadrupole mass analyser, incorporating a prefilter assembly to protect the main analyser from contaminating deposits, and an orthogonal acceleration time of flight (TOF) mass spectrometer. A hexapole collision cell, between the two mass analysers, can be used to induce fragmentation to assist in structural investigations.

Ions emerging from the second mass analyser are detected by the microchannel plate detector and ion counting system. A post acceleration photomultiplier detector (Dynolite™), situated after the orthogonal acceleration cell, is used to detect the beam passing through the first stage of the instrument for tuning and optimisation.

A PC computer runs the MassLynx NT software system to control Q-Tof 2, and to acquire and process data.

## Ionisation Techniques

Using the Micromass Z-spray atmospheric pressure ionisation (API) source, two techniques are available.

### Atmospheric Pressure Chemical Ionisation

Atmospheric pressure chemical ionisation (APCI) generally produces protonated or deprotonated molecular ions from the sample via a proton transfer (positive ions) or proton abstraction (negative ions) mechanism. The sample is vapourised in a heated nebuliser before emerging into a plasma consisting of solvent ions formed within the atmospheric source by a corona discharge. Proton transfer or abstraction then takes place between the solvent ions and the sample. Eluent flows up to 2 millilitres/minute can be accommodated without splitting the flow.

### Electrospray

Electrospray ionisation (ESI) takes place as a result of imparting a strong electrical charge to the eluent as it emerges from the nebuliser. An aerosol of charged droplets emerges from the nebuliser. 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 filters ions according to their mass-to-charge ratio ( $m/z$ ), compounds of high molecular weight can be determined if multiply charged ions are formed.

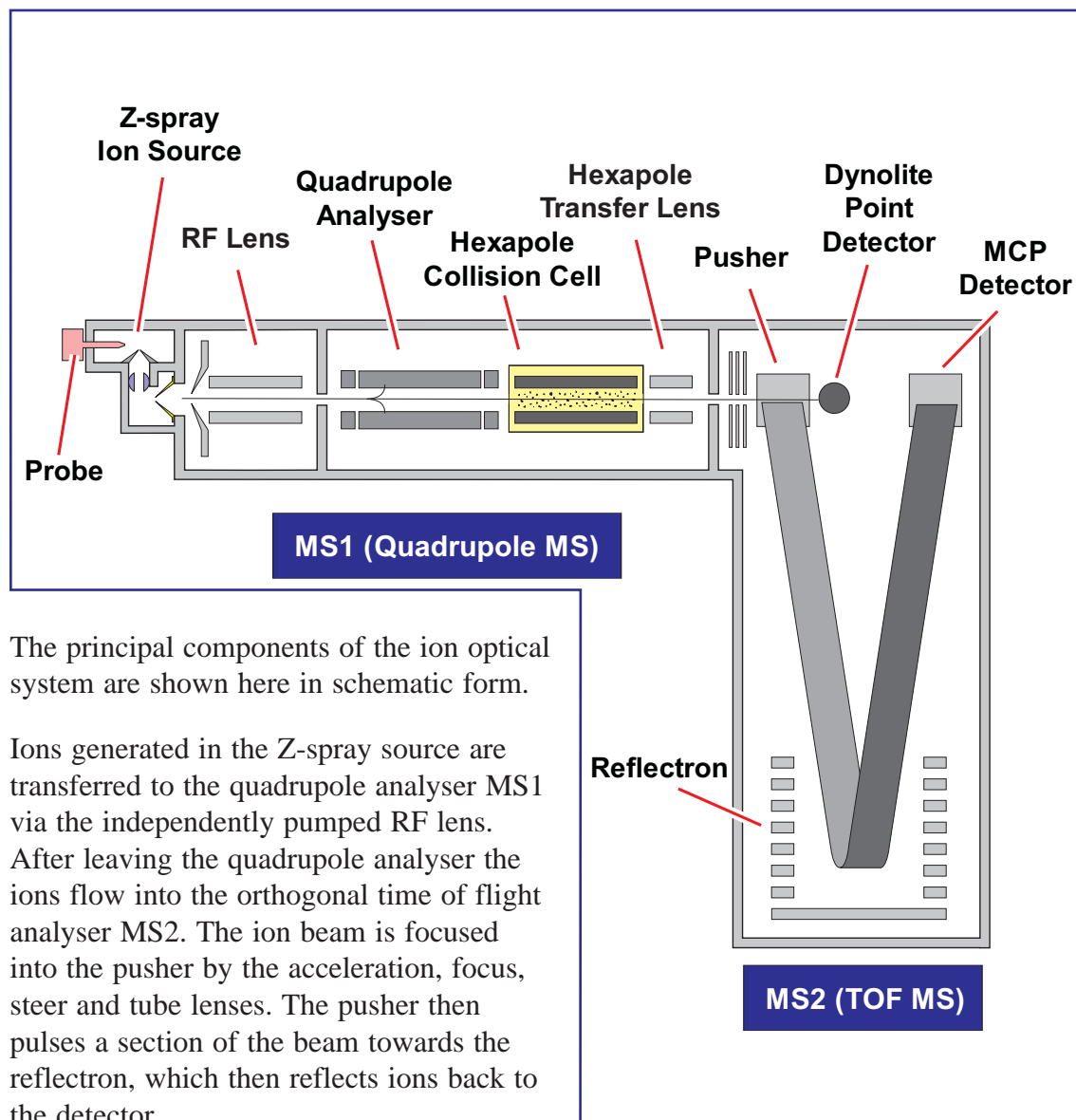
Eluent flows up to 1 ml/min can be accommodated although it is often preferable with electrospray ionisation to split the flow such that 5-50  $\mu\text{l}/\text{min}$  of eluent enters the mass spectrometer.

### Nanoflow Electrospray

The optional nanoflow interface allows electrospray ionisation to be performed in the flow rate range 5 to 1000 nanolitres per minute.

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

## Ion Optics



The principal components of the ion optical system are shown here in schematic form.

Ions generated in the Z-spray source are transferred to the quadrupole analyser MS1 via the independently pumped RF lens. After leaving the quadrupole analyser the ions flow into the orthogonal time of flight analyser MS2. The ion beam is focused into the pusher by the acceleration, focus, steer and tube lenses. The pusher then pulses a section of the beam towards the reflectron, which then reflects ions back to the detector.

As ions travel from the pusher to the detector they are separated in mass according to their flight times, with ions of the highest mass to charge ratio ( $m/z$ ) arriving later.

The pusher may be operated at repetition frequencies of up to 20 kHz, resulting in a full spectrum being recorded by the detector every 50 microseconds. Each spectrum is summed in the histogram memory of the time to digital converter until the histogrammed spectrum is transferred to the host PC.

If the user has requested an acquisition rate of 1 spectrum/second, each spectrum viewed on the host PC will be the result of summing up to 20,000 individual spectra recorded at the detector.

Unlike scanning instruments, the TOF performs parallel detection of all masses within the spectrum at very high sensitivity and acquisition rates. This characteristic is of particular advantage when the instrument is coupled to fast chromatography, since each spectrum is representative of the sample composition at that point in time, irrespective of how rapidly the sample composition is changing.

## Internal Layout

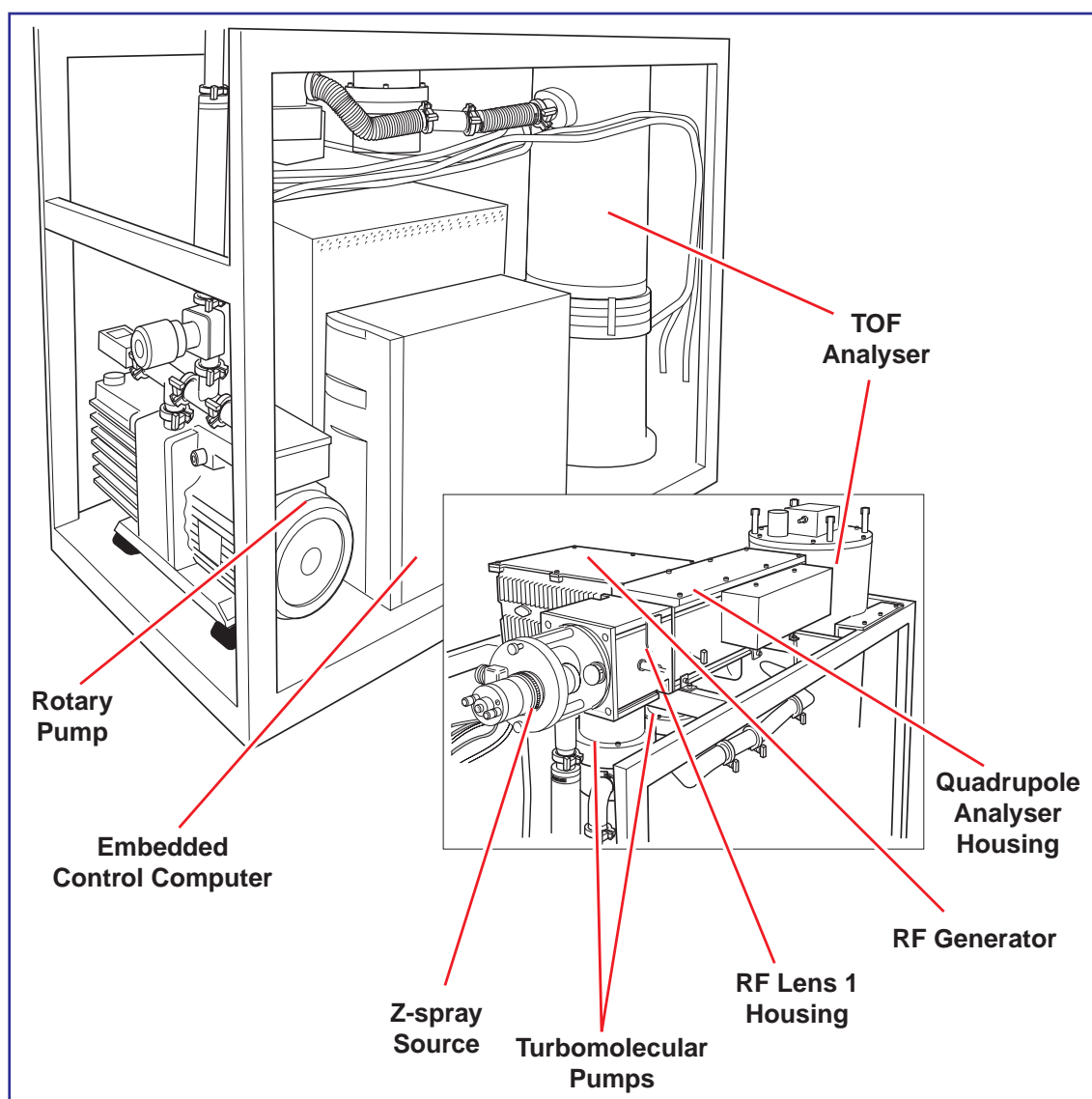


**Warning:** The covers should not be removed without first isolating the instrument at the electricity supply.



**Caution:** The internal layout is shown in the following diagrams for information only, and does not imply that labelled components are user-serviceable.

## Mechanical Components



The main internal mechanical components of the instrument are:

- The source housing, containing the RF (hexapole) lens.
- The MS1 analyser housing, containing the quadrupole analyser, hexapole collision cell and hexapole transfer lens
- The TOF analyser housing, containing the pusher, detector and reflectron assemblies.
- Three 250 litre/second turbomolecular pumps.
- Two active inverted magnetron (Penning) gauges and two Pirani gauges.

## Electronics

The main electronics unit is located in the lower section of the instrument behind the operator control panel. This contains:

- High voltage power supplies.

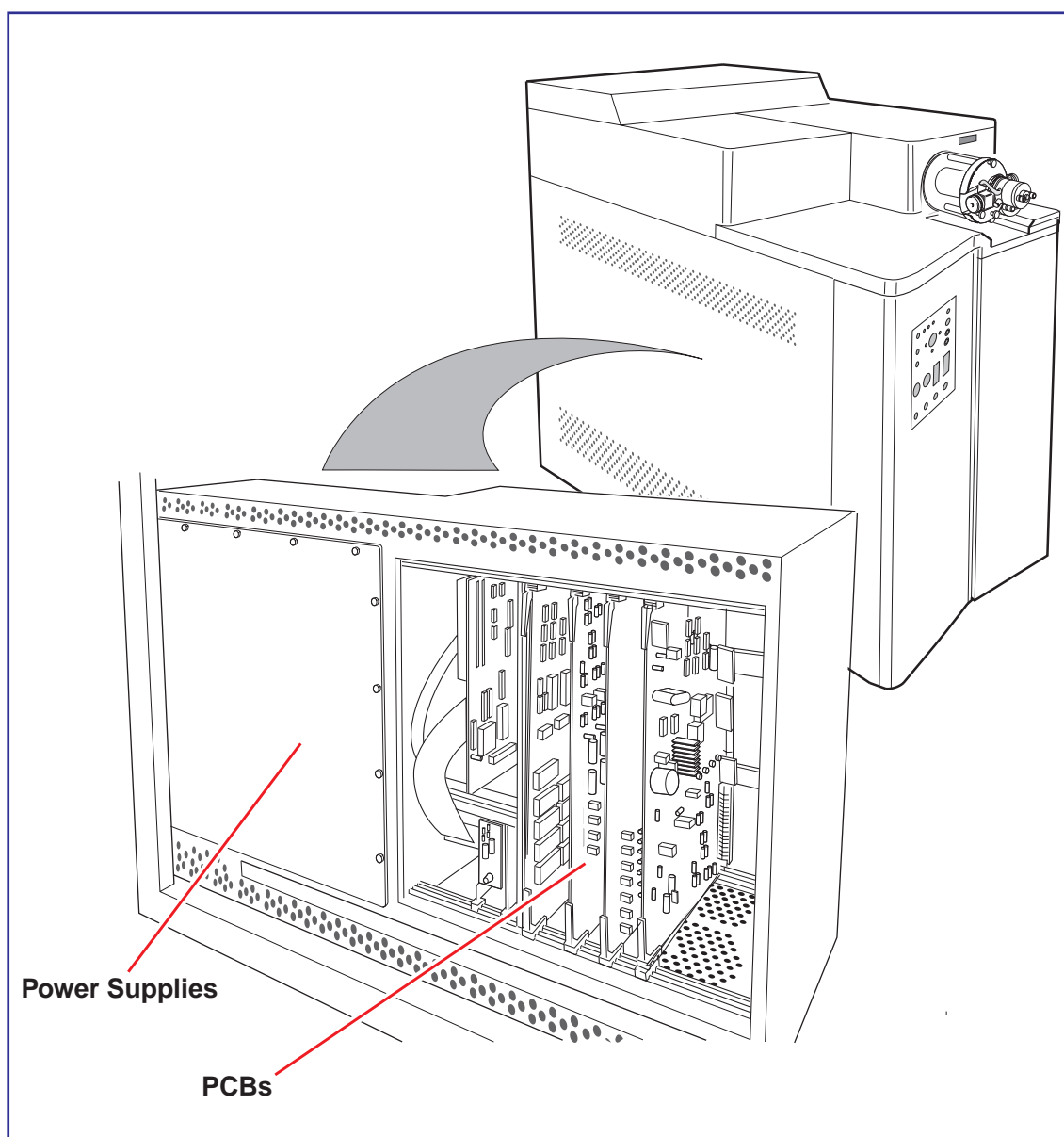
*These supply the probe or corona, Dynolite point detector, reflectron, TOF flight tube and lens circuits.*

- Low voltage power supplies.

*These supply the PCBs, high voltage supplies and turbomolecular pumps.*

- Main PCBs.

*For communications, lenses and quadrupole control.*



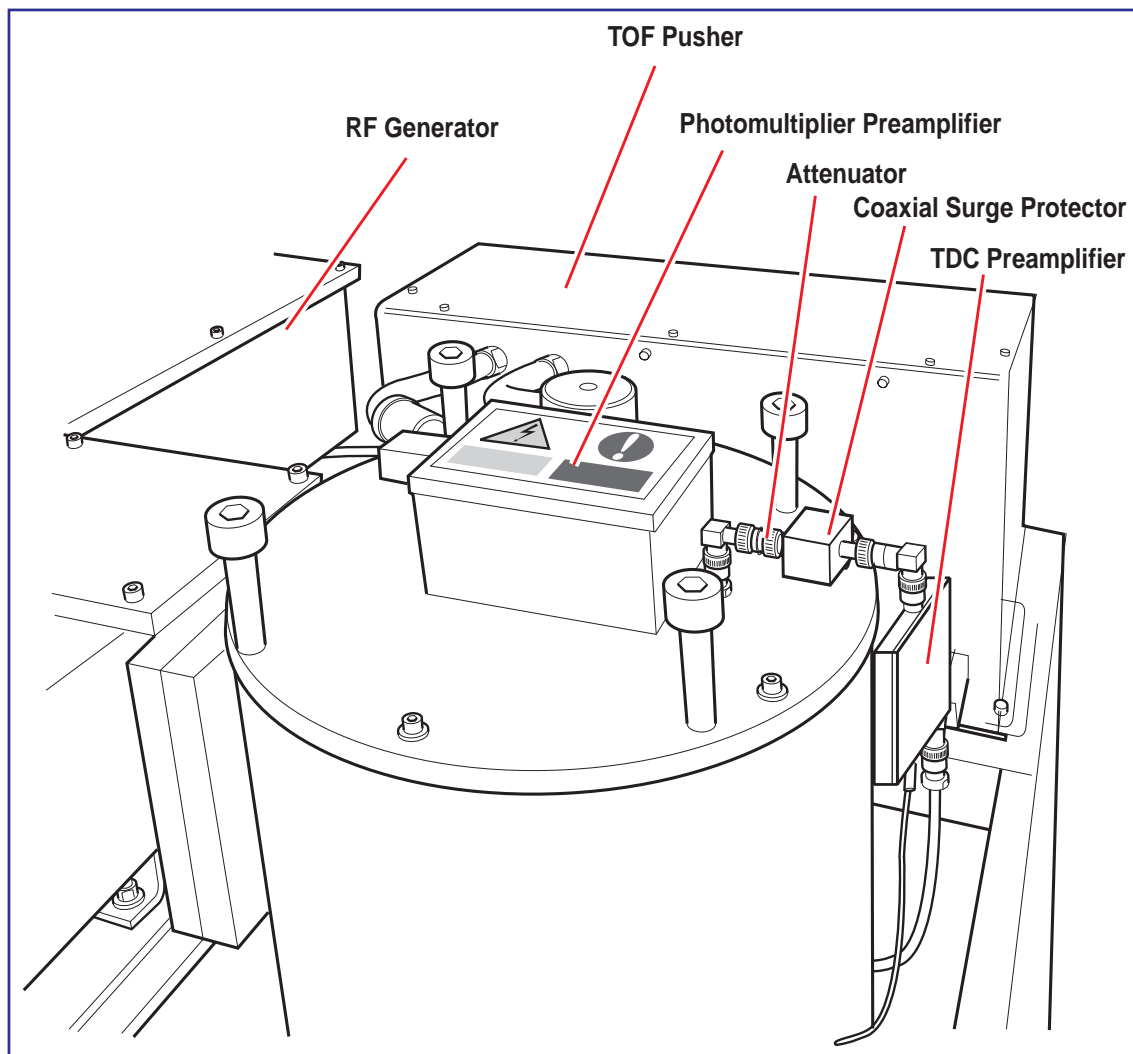
The following electronics modules are located adjacent to the TOF analyser housing:

- Pusher unit.

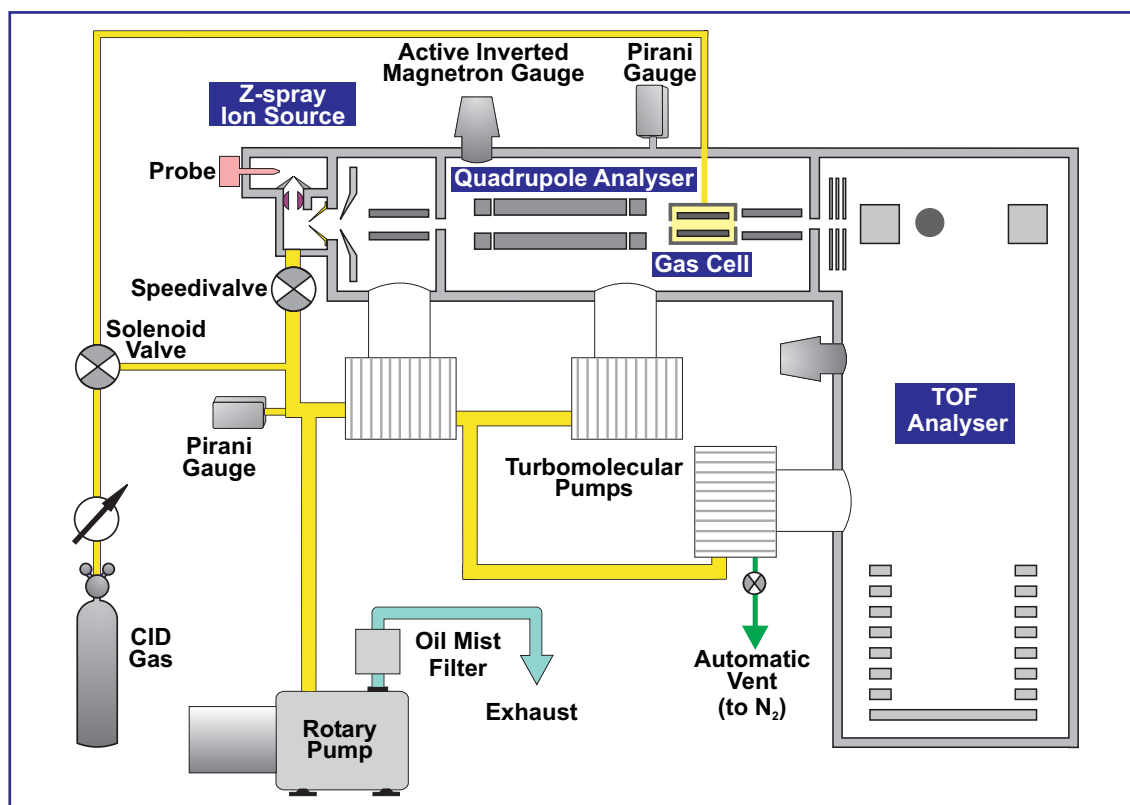
*This produces the high frequency pusher voltage, and the MCP voltage.*

- TDC preamplifier, attenuator and photomultiplier preamplifier.

*These components condition the output of the detector before the signal travels to the TDC.*



## The Vacuum System



### Fine Pumping

Q-Tof 2 is equipped with three water cooled turbomolecular pumps, providing independent fine pumping of the source hexapole, quadrupole and TOF analysers. Details of the operation and maintenance of the pumps can be found in the manufacturer's manuals provided.

### Rotary Pumping

Source pumping and turbomolecular pump backing is by a direct drive rotary pump. The rotary pump is situated at the front of the instrument. Details of the operation and maintenance of the pump can be found in the manufacturer's manual provided.

### Pressure Measurement

The backing pressure is monitored by an active Pirani gauge. The analyser and TOF pressures are monitored by active inverted magnetron (Pinning) gauges. These gauges act as vacuum switches, switching the instrument out of **Operate** mode if the pressure is too high. Pressure readings may be displayed on the MassLynx NT tune page.

The analyser Penning gauge only comes on when the vacuum display window is open. At other times the gauge is off. The analyser Pirani gauge is used when the display is off, though no pressures are shown.

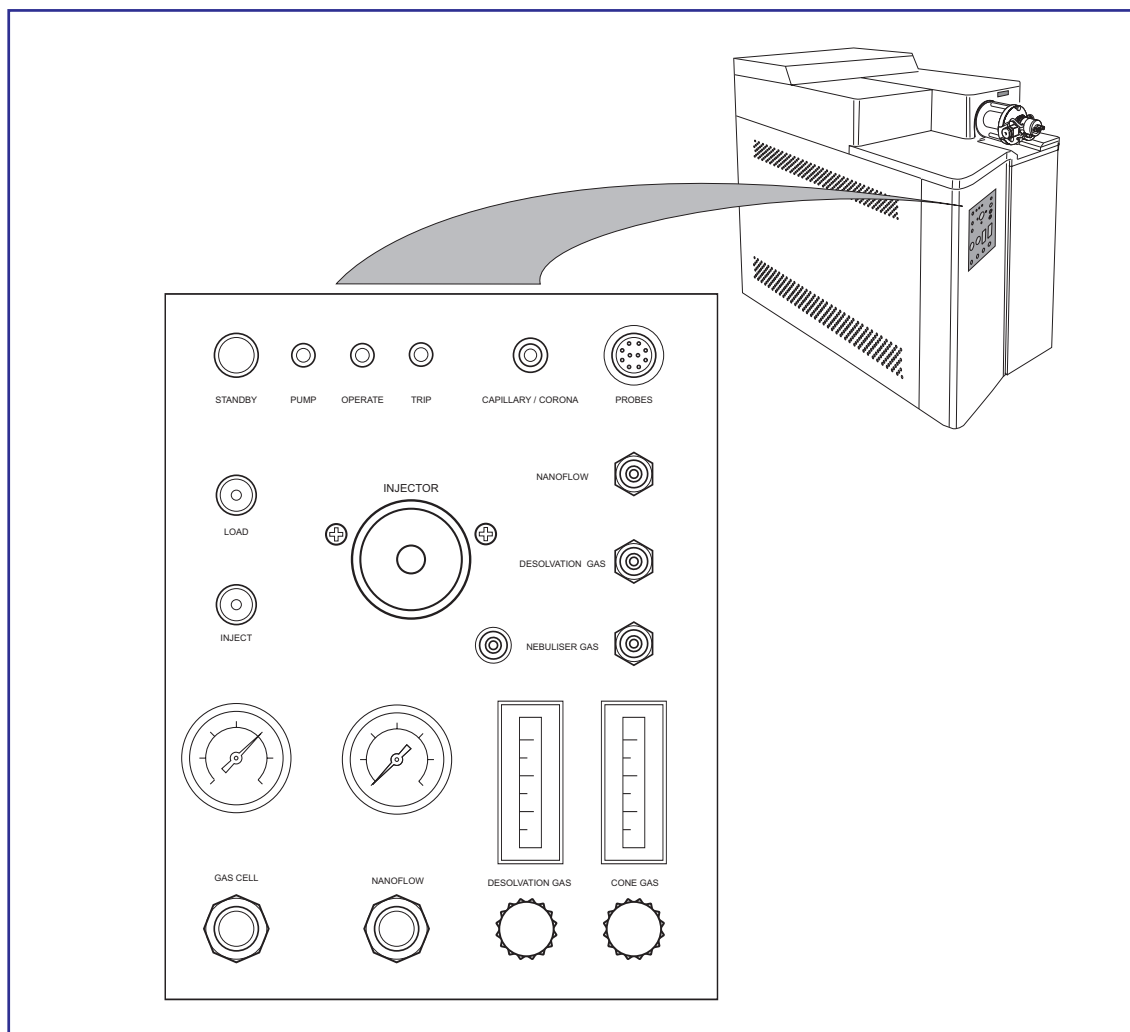


## Vacuum Protection

The vacuum system is fully interlocked to provide adequate protection in the event of:

- a fault in the vacuum system.
- a failure of the power supply.
- a failure of the water supply.
- a vacuum leak.

## Front Panel Connections



### Desolvation Gas and Probe Nebuliser Gas

The PTFE gas lines for the **Desolvation Gas** and probe **Nebuliser Gas** are connected to the front of the instrument using threaded metal fittings. **Cone Gas** is connected internally.

### High Voltage

The electrical connection for the ESI capillary or the APcI corona discharge pin is via the coaxial high voltage connector. This socket is labeled **Capillary / Corona**.

### Heaters

The electrical connection for the APcI probe or the ESI desolvation heater is via the multi-way connector labeled **Probes**. This is removed from the front panel by pulling on the metal sleeve of the plug. Both the electrospray desolvation heater and the APcI probe heater use this connector.

The power for the source block heater is permanently connected. As a consequence, the source block assembly is usually very hot, and should not be touched.

## Front Panel Controls and Indicators

### Status Display

The display on the front panel of the instrument consists of two 3-colour light emitting diodes (LEDs).

The display generated by the **Pump** LED is dependent on the vacuum status of the instrument. The **Operate** LED depends on both the vacuum status and whether the operate mode has been selected from the Data System. Further information is included in *Automatic Pumping and Vacuum Protection* (see *Routine Procedures*).

### Flow Control Valves

The **Desolvation Gas** and **Cone Gas** needle valves are five-turn valves. The flow increases as the valve is turned counterclockwise. The Nebuliser Gas valve is a four-turn valve.

### Divert / Injection Valve

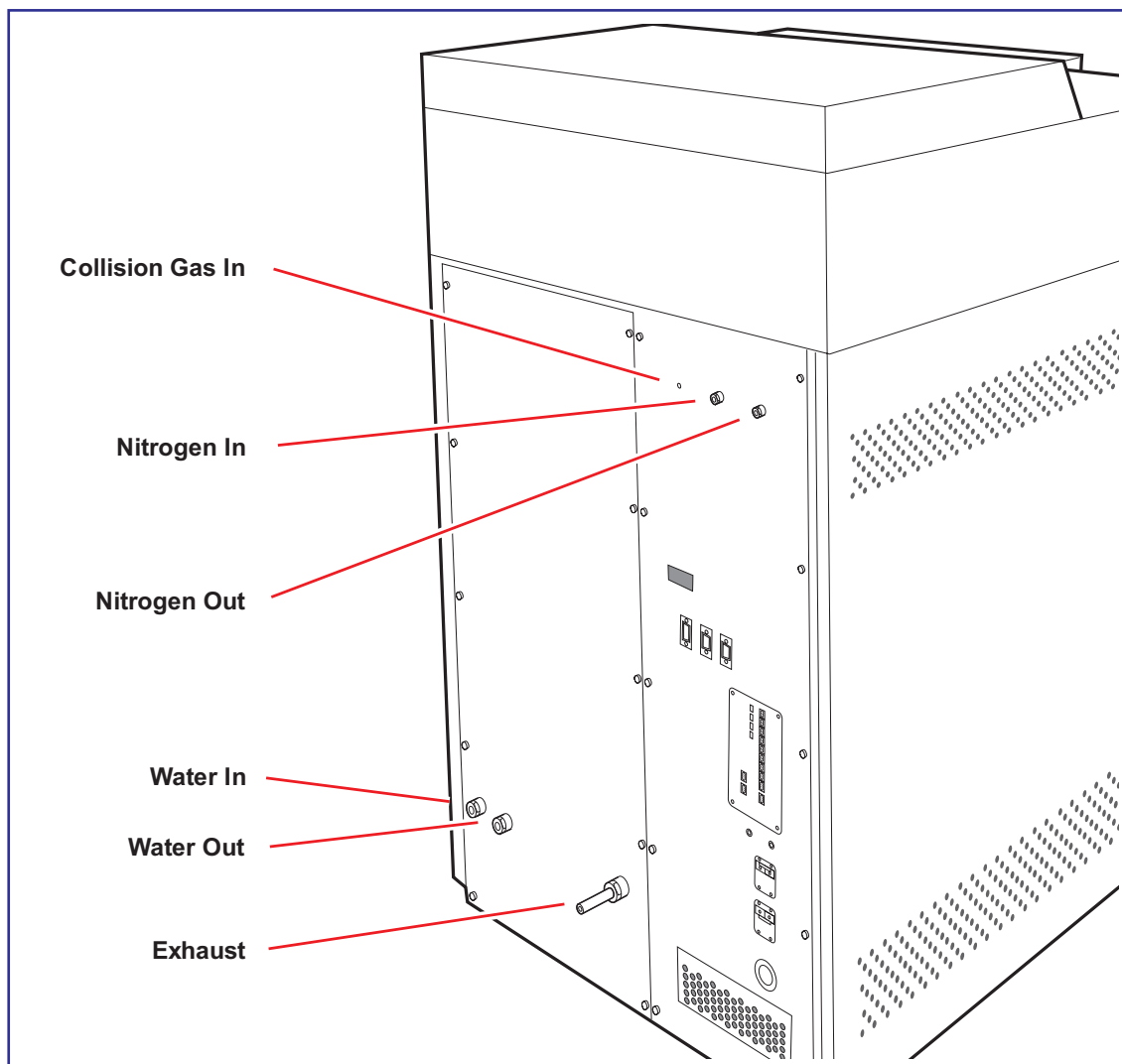
The divert / injection valve may be used in several ways depending on the plumbing arrangement:

- As an injection valve, with the needle port and sample loop fitted.
- As a divert valve, to switch the flow of solvent during a LC run.
- As a switching valve to switch, for example, between a LC system and a syringe pump containing calibrant.

This valve is pneumatically operated, using the same nitrogen supply as the rest of the instrument.

The two switches marked **Load** and **Inject** enable the user to control the valve when making loop injections at the instrument.

## Rear Panel Connections



### Water

Water is used to cool the turbomolecular pumps.

### Nitrogen Gas In

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



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

## Exhausts

The exhaust from the rotary pump should be vented to atmosphere outside the laboratory.

The gas exhaust, which also contains solvent vapours, should be vented via a separate fume hood, industrial vent or cold trap.

The gas exhaust should be connected using 10mm plastic tubing connected to the push-in fitting.



**Caution:** Do not connect these two exhaust lines together as, in the event of an instrument failure, rotary pump exhaust could be admitted into the source chamber producing severe contamination.

## Supply Inlet

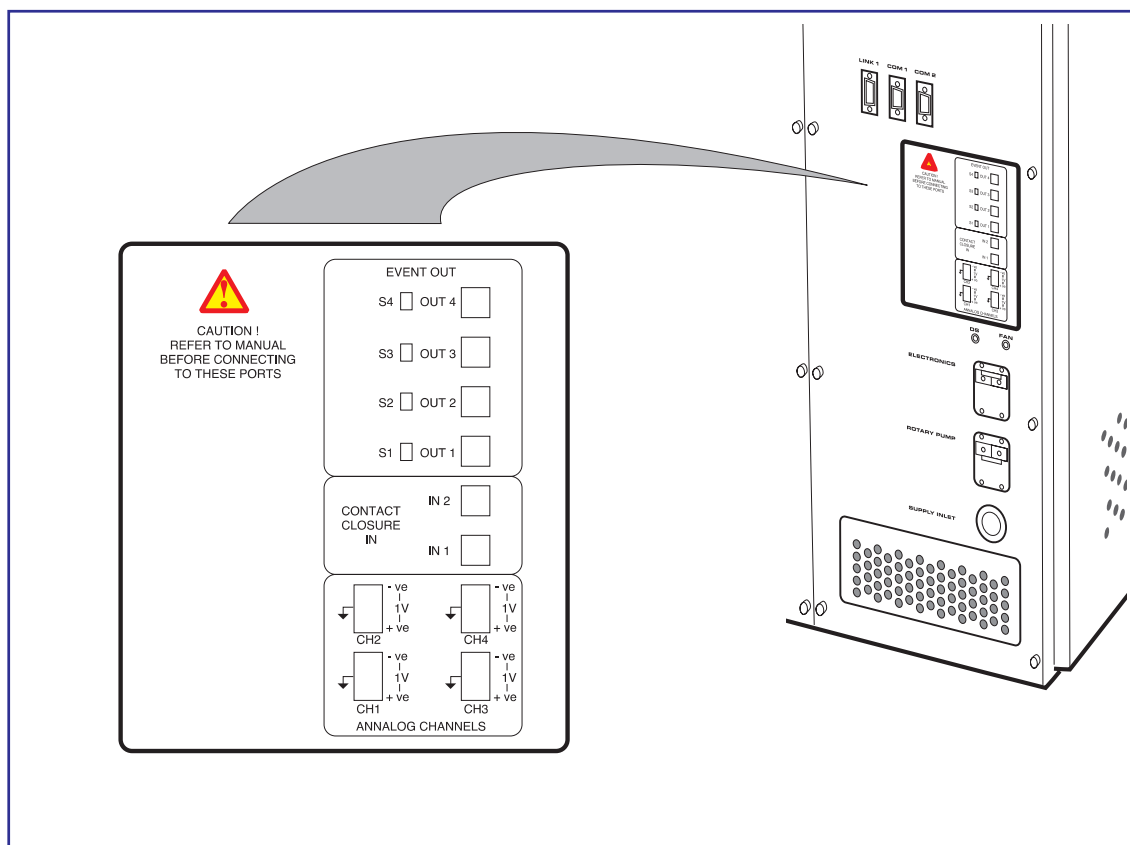
The mains power cord should be wired to a 230V mains outlet using a suitable plug, or to a transformer. For plugs with an integral fuse, the fuse should be rated at 13 amps (UK only).

## Electronics

This circuit breaker switches power to the electronics. In the event of the instrument drawing more than the rated current, the circuit breaker will trip.

## Rotary Pump

This circuit breaker switches power to the rotary and turbomolecular pumps. In the event of the pumps drawing more than the rated current, it will trip.



## Event Out

Four outputs, **Out 1** to **Out 4**, are provided to allow various peripherals to be connected to the instrument. Switches **S1** to **S4** allow each output to be set to be either a contact closure (upper position) or a voltage output (lower position).

**Out 1** and **Out 2**, when set to voltage output, each have an output of 5 volts. The voltage output of both **Out 3** and **Out 4** is 24 volts.

During a sample run an event output may be configured to close between acquisitions and is used typically to enable an external device to inject the next sample.

## Contact Closure In

**In 1** and **In 2** inputs are provided to allow an external device to start sample acquisition once the device has performed its function (typically sample injection).

## Analog Channels

Four analog channel inputs are available, for acquiring simultaneous data such as a UV detector output. The input differential voltage must not exceed one volt.

## MassLynx NT Data System

A PC computer runs the MassLynx NT software system to control Q-Tof 2, and to acquire and manipulate data from it. A high resolution colour monitor is also supplied.

Interaction with MassLynx NT is via the mouse and keyboard using menu-driven commands. Printing, file management and other routine procedures are performed using the appropriate Windows NT modules.

### Software

The following software packages are supplied with Q-Tof 2:

- MassLynx NT
- Screen Capture, a utility for copying user selected areas of any Windows display. The selected area can be printed directly, or saved as a bitmap file for importing into other Windows NT applications.
- DataBridge, a utility to convert other format data files into MassLynx format.
- Microsoft Windows NT graphical environment.
- Mouse configuration.

A range of optional software modules for different applications is also available.

The *MassLynx NT User's Guide* describes the many facilities of the Micromass software. Documentation for the other software is also supplied.





# Routine Procedures

## Start Up Following a Complete Shutdown

### Preparation

If the instrument has been unused for a lengthy period of time, proceed as follows:

Check the level of oil in the rotary pump sight glass. Refill or replenish as necessary as described in the pump manufacturer's literature.

Connect a supply of dry, high purity nitrogen to the connector on the service panel at the rear of the instrument. Adjust the outlet pressure to 7 bar (100 psi).

Connect the water supply to the connections at the rear of the instrument.

Check that the rotary pump exhaust is connected to a suitable vent.

Check that the exhaust gas from the instrument is connected to a suitable vent.



**Caution:** Do not connect the two exhaust lines together. In the event of an instrument failure, rotary pump exhaust could be admitted into the source chamber, producing severe contamination.

Check that the instrument, data system and other peripheral devices (LC equipment, printer etc.) are connected to suitable mains supplies.

Check that the etherlink connection is made between the control PC and the embedded PC.

Ensure that the VxWorks disk is inserted into the drive of the embedded PC.

Switch on the host PC. Log on to Windows NT and wait for the system to boot up before the Q-Tof 2 is switched on.

Switch on the mains to the mass spectrometer using the two circuit breakers situated on the service panel at the rear of the instrument.

Log on to Micromass account (password analysis).

*Windows NT and MassLynx NT are configured to prevent unauthorised access.*

On the host PC, double-click on the MassLynx icon in the Windows desktop and display the tune page.

## Pumping



**Caution:** To minimise wear to the lubricated components of the rotary pump, the manufacturers recommend that the pump is not started when the oil temperature is below 12°C.

Select **Vacuum** from the menu bar at the top of the tune page.

Click on **Pump**.

*The rotary pump and the turbomolecular pumps start simultaneously.*

*The **Vacuum** LED on the front of the instrument shows amber as the system pumps down.*

When the system has reached operating vacuum the **Vacuum** LED changes to a steady green.

If the rotary pump oil has been changed or replenished, open the gas ballast valve on the rotary pump. See the pump manufacturer's literature for details.

*Rotary pumps are normally noticeably louder when running under gas ballast.*

If opened, close the gas ballast valve when the rotary pump has run under gas ballast for 30 minutes.

## MCP Detector Conditioning

The MCP detector must be conditioned before use, by gradually increasing the applied voltage over a long time period. This is necessary to allow escape of all absorbed water from within the microchannels.

Under normal operation the analyser automatically vents to dry nitrogen. However, if the nitrogen supply was not connected to the instrument when last vented, or if the instrument has been left vented for more than one day, a significant amount of water vapour may have entered the analyser. Under these circumstances it is good practice to allow the instrument to pump for 12 hours before commencing the conditioning process.

In all cases, the TOF pressure must be 1e-6 mbar prior to commencing MCP conditioning.

MCP conditioning should be repeated after every instrument venting.

*It is not necessary to recondition the detector if the instrument has been left out of the operate mode while still under vacuum.*

MCP Conditioning

Voltage

Start (V) 100

Stop (V) 2400

Time

Duration (mins) 600

Step (mins) 5

Start

Close

*During routine cleaning of the source sample cone, the source isolation valve is closed in order to maintain analyser vacuum. It is not, therefore, necessary to recondition the detector after this procedure.*

The procedure for MCP conditioning is as follows:

Ensure that the analyser pressure is 1e-6 mbar.

Check that the **MCP Detector** voltage is set to zero on the tune page.

Switch the instrument into **Operate**.

Select **Other, MCP Conditioning** to access the MCP conditioning program.

Set **Start** to 100V, **Stop** to 2400V, **Duration** to 600 minutes and **Step** to 5 minutes.

*A 'quick condition' may be performed following brief venting, after source cleaning for example.*

Set **Start** to 100V, **Stop** to 2400V, **Duration** to 120 minutes and **Step** to 1 minute.



**Caution:** Failure to follow the recommended MCP conditioning procedure can severely reduce detector lifetime.

## Instrument Warm-up

Switch the instrument into the operate mode by selecting **Operate** on the MassLynx tune page.

For the best mass accuracy to be obtained the instrument temperature must be stabilised for a minimum of two hours after switching into operate.

*Leaving the instrument continuously in operate does not shorten the detector lifetime. It is recommended that the instrument is left in operate at all times (except of course during maintenance procedures) in order to reduce mass scale drifts due to temperature changes. Switching the instrument out of operate mode overnight is not necessary.*

## Using the Instrument

The Q-Tof 2 is now almost ready to use. To complete the start up procedure and prepare for running samples, follow the instructions in *Start Up Following Overnight Shutdown* in the following pages.

## Start Up Following Overnight Shutdown

The instrument will have been left in the operate mode under vacuum.

It is recommended that the data system is left on overnight. However, if the data system has been switched off, switch it on as described in the preceding section.

## Preparation for Electrospray Operation

If the corona discharge pin is fitted, proceed as follows:

Deselect **Operate** from the tune page to put the instrument into standby mode.

Disconnect the gas and electrical connections from the front panel.

Unscrew the probe thumb nuts and remove the probe.

Undo the three thumb screws and remove the probe adjustment flange and glass tube.



**Warning:** The ion source block can be heated to temperatures of 150°C, and will be maintained at the set temperature when the source enclosure is removed. Touching the ion block when hot may cause burns to the operator.

Disconnect the APcI high voltage cable from the socket positioned at the bottom right corner of the source flange.

Remove the corona discharge pin from its mounting contact, and fit the blanking plug.

Replace the glass tube and adjustment flange.

Ensure that the source enclosure is in place.

*The Z-spray source enclosure consists of the glass tube and the probe adjustment flange.*



**Warning:** Operating the source without the source enclosure will result in solvent vapour escape and the exposure of hot surfaces and high voltages.

With the corona discharge pin removed, the plug fitted and the source enclosure in place, proceed as follows:

Connect the source's gas line to **Desolvation Gas** on the front panel. Tighten the nut to ensure a good seal.

Check that the lead of the probe adjustment flange is plugged into the socket labelled **Probes** on the front panel.

Connect the electrospray probe's gas line to **Nebuliser Gas** on the front panel.

Connect the liquid flow of a LC system or syringe pump to the probe.

Insert the probe into the source and tighten the two thumb nuts to secure the probe firmly.

Plug the probe lead into **Capillary / Corona** on the front panel.

If necessary, change the ionisation mode using the **Ion Mode** command.

Set **Source Block Temp** to 100°C and **Desolvation Temp** to 120°C.



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

## Preparation for APcI Operation

If the corona discharge pin is not fitted, proceed as follows:

Deselect **Operate** from the tune page to put the instrument into standby mode.

Disconnect the gas and electrical connections from the front panel.

Unscrew the probe thumb nuts and remove the probe.

After a period of operation at high flow rates, allow the glass source enclosure to cool before removal.

Undo the three thumb screws and remove the probe adjustment flange and glass tube.



**Warning:** The ion source block can be heated to temperatures of 150°C, and will be maintained at the set temperature when the source enclosure is removed. Touching the ion block when hot may cause burns to the operator.

Remove the blanking plug from the discharge pin mounting contact and fit the corona discharge pin, ensuring that the tip is in line with the tip of the sample cone.

Connect the APcI high voltage cable between **Capillary / Corona** and the socket positioned at the bottom left corner of the source flange.

Replace the glass tube, adjustment flange and moulded cover.



**Warning:** Operating the source without the source enclosure will result in solvent vapour escape and the exposure of hot surfaces and high voltages.

With the corona discharge pin fitted and the source enclosure in place, proceed as follows:

Insert the APcI probe into the source and tighten up the two thumb screws.

If necessary, change the ionisation mode using the **Ion Mode** command.

Set **Source Temp** to 150°C.



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Temp** higher than 150°C.

Do not start the liquid flow until the gas flow and probe heater are switched on with the probe inserted.

## Transient Pressure Trip

The transient trip is designed to protect the instrument from potentially damaging pressure surges and operates routinely whenever the pressure rises.

Should the vacuum gauge(s) detect a pressure surge above the preset trip level (normally set at 1e-5 mbar by software) the following events occur:

- The green **Pump** lamp becomes amber.
- If in the operate mode, the system turns off the critical source, analyser and detector voltages, and the green **Operate** lamp becomes amber.
- Acquisition continues though, of course, no real data are recorded.

When the vacuum recovers:

- The amber **Pump** lamp becomes green.
- If previously in the operate mode, voltages are restored and **Operate** reverts to green.

*The period during which the trip was operative will appear in a raw total ion chromatogram as a period of reduced baseline noise.*

Further deterioration of the system pressures results in a “vacuum fault” condition and the system is shut down (see below).

## Power Failure

In the event of an unexpected failure of the electrical supply the instrument is vented safely. If power is unlikely to be restored quickly, follow the shutdown procedure described later in this chapter. When power is restored follow the startup procedure.

Should the power fail and then be restored while the instrument is unattended, the system will continue to vent, and will require to be pumped down in accordance with the start-up procedure.

## Nitrogen Supply

Replacement of nitrogen cylinders should be conducted in accordance with the operation, handling and storage instructions provided by the local gas supplier.

Toggle the API gas button to Off, to close the nitrogen inlet valve prior to disconnecting the supply.

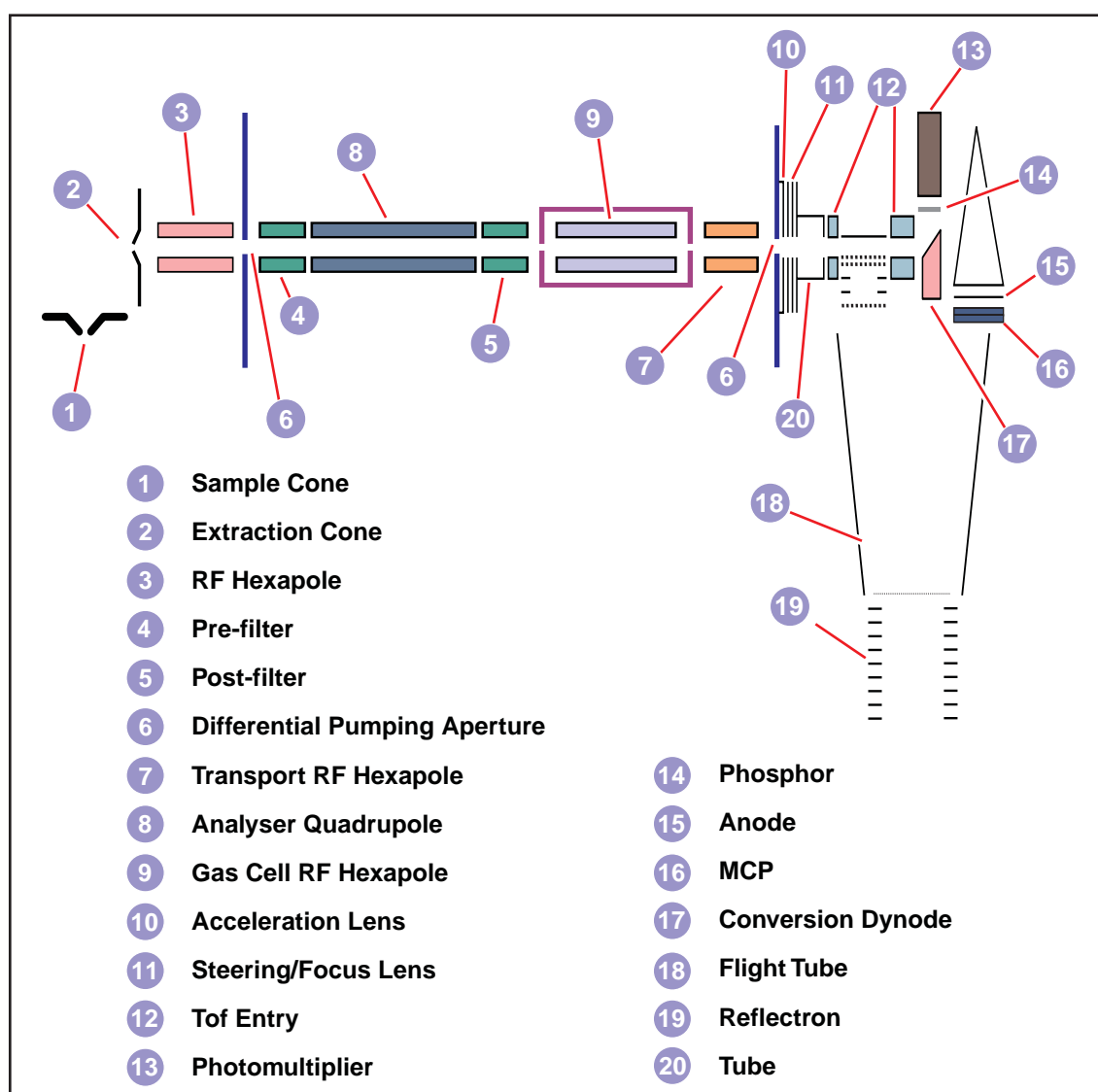
Set the nitrogen inlet pressure to 7 bar (100 psi).

***Under no circumstances*** should the nitrogen pressure exceed 10 bar (140 psi).

## Tuning

The preceding sections have outlined the software controls and connections to establish the physical conditions required as a prerequisite to mass spectrometer operation.

Within the source and analyser enclosures, electric fields controlled via MassLynx are applied to the components to manage the ion beam generated, according to the sample to be analysed, the ionisation mode and the type of information required. The ion optical system elements are indicated in the following schematic diagram.

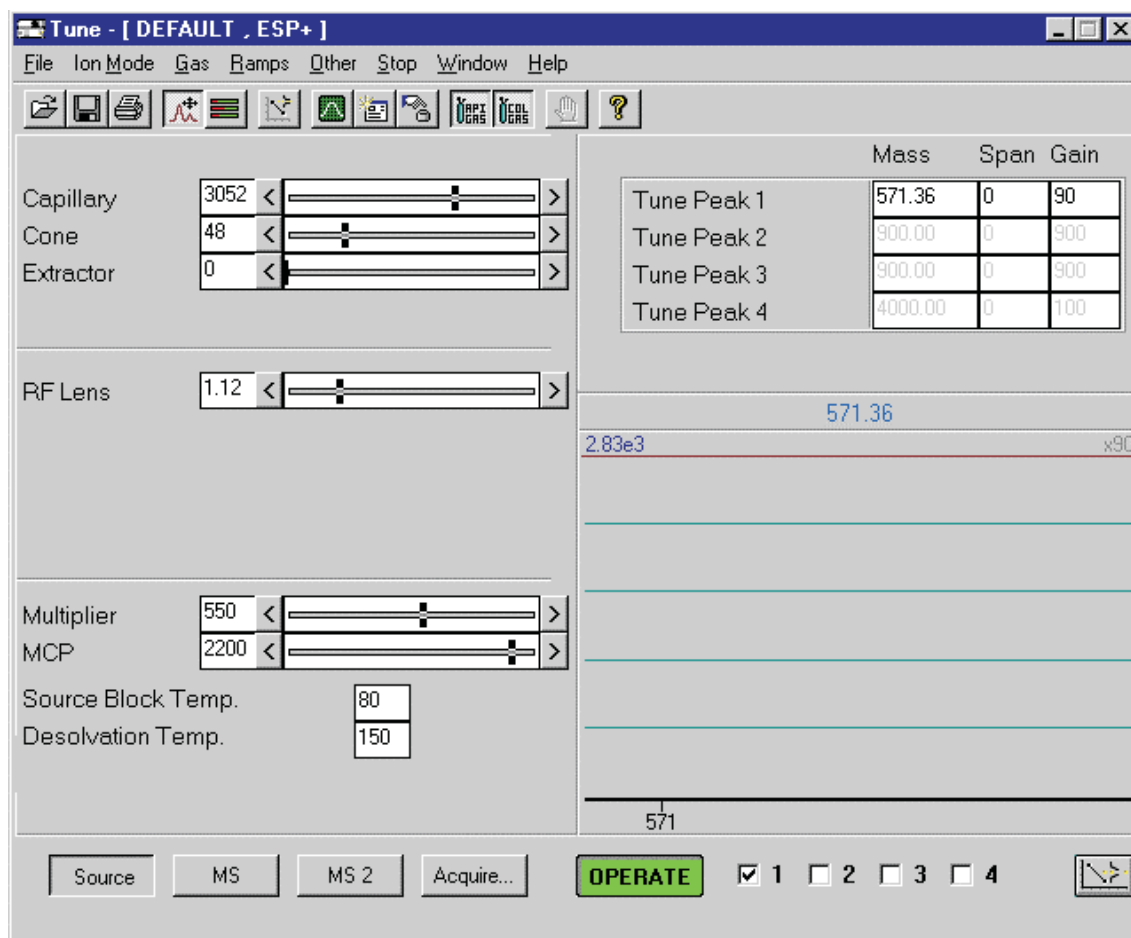


Considering the variable nature of the beam with different samples, the instrument should be tuned for signal strength and calibrated for accurate  $m/z$  using suitable reference compounds, prior to the acquisition of sample data.

Tuning parameters have been grouped in MassLynx into 3 menus as described below. Full details of source tuning procedures for electrospray, APcI and nanoflow electrospray are given in the relevant chapter of this document.



## Source Tuning Menu



The positive ion electrospray (ESP+) source tuning menu is shown. Suggested tuning parameters are as follows:

### Capillary

This sets the absolute voltage on the electrospray probe or APCI corona needle, and is typically adjusted to 3000V.

### Cone

This sets the voltage on the sampling cone relative to the extraction lens. It is dependent on compound and charge state. For multiply charged species this is set to 40 - 50eV, and higher for singly charged species. In general, higher cone voltages are needed for larger mass ions.

### Extractor

This sets the voltage on the extraction lens, and is normally set from 0 - 2V.

### RF Lens

This sets the offset voltage on the hexapole and the first differential pumping aperture. It is usually set to 1V.

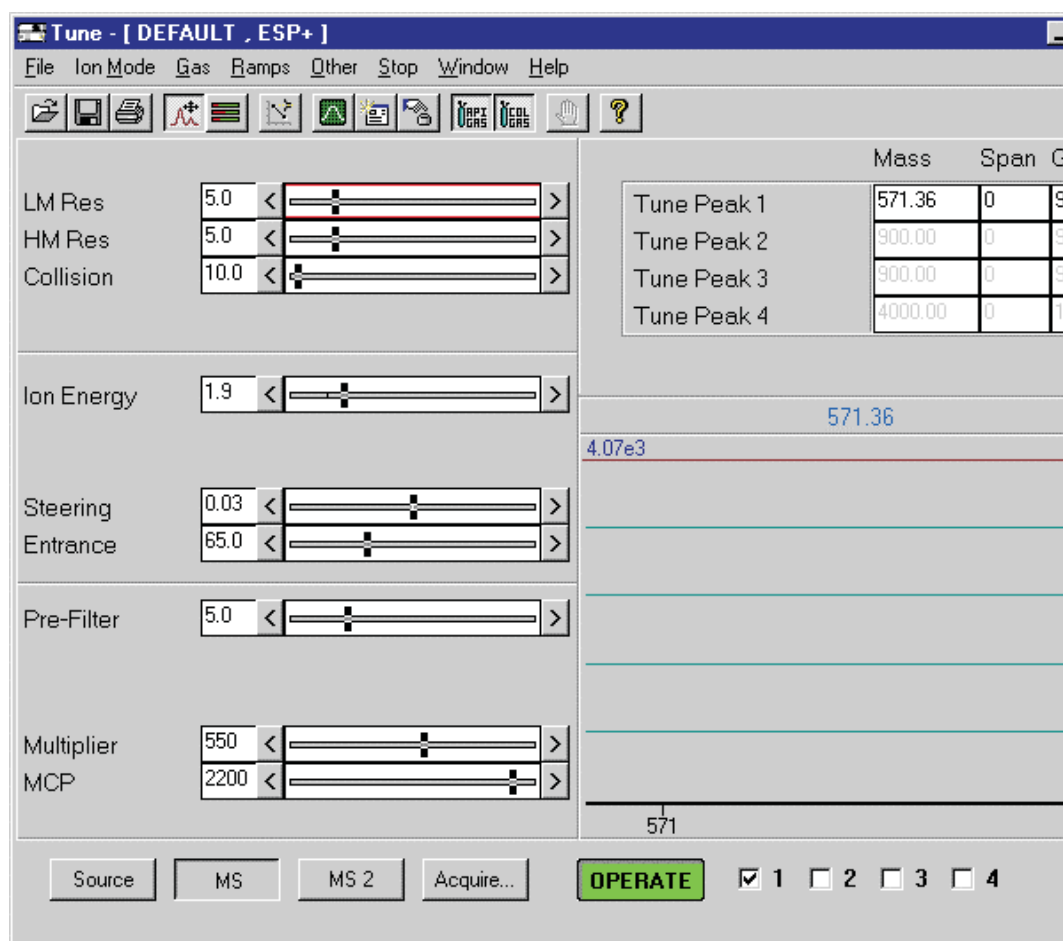
## Source Block Temp

The source block temperature is usually set to 80°C, but is increased for higher solvent flow rates.

## Desolvation Temp

This sets the temperature of the desolvation gas heater. It is usually set to 150°C, and increased for higher solvent flow rates.

## Analyser Tuning Menu



## LM Res and HM Res

These set the resolving DC on the quadrupoles. The two sliders are set to give constant resolution across the mass range. When the quadrupoles have been set up, settings of 15 on both sliders should give unit resolution at 20% peak height.

## Collision

This sets the collision energy of the ions when they reach the collision cell. The cell itself is grounded but the collision energy voltage is simultaneously applied to all the optical elements preceding it. i.e cone, extraction lens, source hexapole, differential pumping aperture, and quadrupole. In Q-Tof 2 gas is always introduced into the cell, affording collisional cooling and consequently higher resolution. The collision energy is set to 10eV for MS mode to maximise ion transmission but produce little or no fragmentation. When in the MS/MS mode the collision energy is adjusted to give the best fragmentation pattern for the selected parent.

## Ion Energy

This sets the quadrupole offset DC with respect to the collision energy of the ions travelling between the quadrupoles. This should be set between 1 - 1.8V.

## Steering

This adjusts the voltage difference between the top and bottom half plates of the steering/focus lens. It acts as a y-deflector, directing the beam into the pusher. A setting close to zero should produce an optimum beam. Better sensitivity can be achieved, however, with a voltage (positive or negative) close to zero, e.g. +1.0, -0.5 etc., and not more than  $\pm 2V$ .

## Entrance

This sets the voltage on the pusher entrance and exit, and defines the axial speed of the ions through the TOF. The theoretical value is 65eV when the ion beam should be central to the detector. It should be optimised when looking at a TOF beam, not on the first detector. It should be possible to correctly tune the instrument using this value.

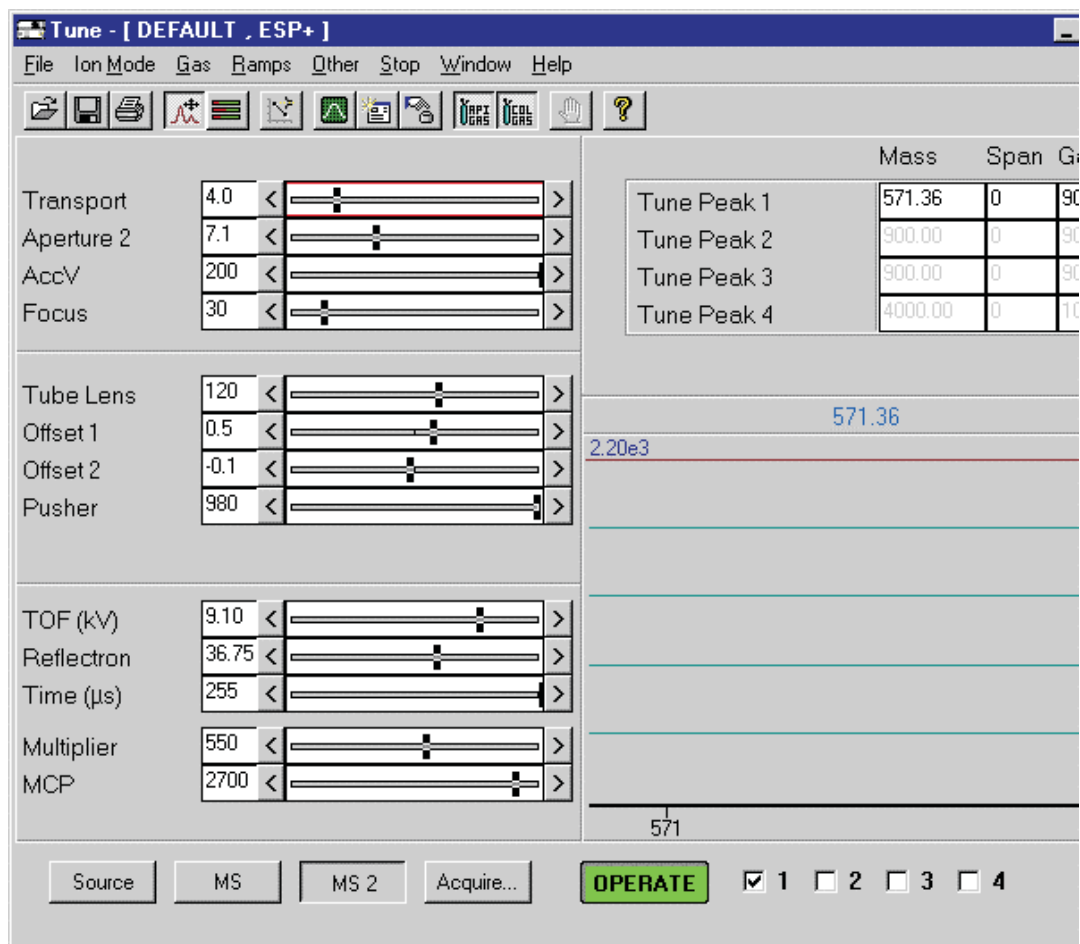
## Multiplier

This sets the photomultiplier voltage, and should typically be set to 550V.

## MCP

This sets the voltage on the TOF detector. The voltage across the MCP itself is limited to 270V less than this value. MCP must be conditioned before applying high voltage. The TOF analyser is usually operated with this detector set at 2200V.

## MS2 Tuning Menu



### Transport

This sets the offset DC on the transport hexapole, and the DC on the apertures of the gas cell. The optimum setting is usually between 2 - 4V.

### Aperture 2

Operating Aperture 2 at 5V generally gives best resolution, but at some cost to sensitivity. For maximum sensitivity it should be set to around 15V.

### AccV

Usually set to maximum voltage (200V) in all modes of operation.

### Focus

This adds an equal voltage to both the top and bottom steering/focus lens halfplates. The optimum setting for the first detector is typically 80 - 130V, but for maximum TOF resolution it is set to zero.

## Tube Lens

This is set to its optimum value in the factory and by the engineer at installation. It helps shape the ion beam on entry into the pusher and so has a large effect on resolution and peak shape.

The factory setting should be noted, before attempting to improve resolution by changing its voltage.

## Offset 1

This determines the voltage difference between the two plates of the first acceleration region of the ToF (Pusher and Grid3). It is used as a fine tuning control to optimise resolution.

The optimum value for this element may change with pusher frequency. The pusher frequency is dependent on mass range when in automatic pusher mode and takes one of six discrete values. See pusher rates on *Page 38*.

## Offset 2

This determines the voltage difference between the two plates of the second acceleration region of the ToF (G3 and puller). It is normally set around zero volts and does not need to be adjusted.

## Pusher

This sets the amplitude of the pusher pulse and is normally set to 980V.

## TOF

This sets the flight tube voltage. It is always set to 9.1kV. Adjusting this will change the peak position and resolution.

## Reflectron

This is set expressed as a percentage of the flight tube voltage and its value is 2150V or about 36.0%. Adjusting this will change the peak position and resolution.

## Prefilter

This sets the offset voltage on the quadrupole pre and post filters. The optimum beam is usually obtained at a setting of 5 - 7V.

*It is recommended that a record of these values is kept for future reference.*

## Manual Pusher

If the **Manual Pusher** box is selected from the **Other** sub-menu then the repetition frequency of the pusher pulse is determined by the **Time** entered.

**Time** may be set between 30  $\mu\text{sec}$  and 255  $\mu\text{sec}$ . Setting **Time** at less than 30  $\mu\text{sec}$  defaults to 30  $\mu\text{sec}$ . Entering 0  $\mu\text{sec}$  switches the pusher off.

Increasing the flight time reduces the duty cycle (sampling efficiency) of the TOF analyser resulting in decreased sensitivity.

For the best mass accuracy the instrument should be re-calibrated if the flight time is changed.

If **Manual Pusher** is not selected then the repetition frequency of the pusher pulse is determined automatically according to the highest  $m/z$  requested in the acquisition range, as shown in the table below.

Maximum Flight Time	Highest $m/z$
44 $\mu\text{sec}$	$\leq 1000$
62 $\mu\text{sec}$	1001 - 2000
88 $\mu\text{sec}$	2001 - 4000
124 $\mu\text{sec}$	4001 - 8000
176 $\mu\text{sec}$	8001 - 16000
246 $\mu\text{sec}$	16001 - 32000

## Other Tune Page Settings

### TDC Settings

To access the TDC (time to digital converter) settings:

Select **Other, TDC settings**.

Section	Parameter	Value
Signal	Start (mV)	1000
	Stop (mV)	150
Data	Threshold	0
	Lteff	6760
	Bunching	1
Centroid	First Diff Width	2
	Second Diff Width	2
	Min peak height	1
	Min points in peak	1
	Np	1.00
	Resolution	10000
	Lock Mass	0.0000
	Lock Mass Window	0.00

#### Start

This is the size of the trigger signal that is necessary to trigger the TDC (start the clock). The start signal is derived from the pusher voltage itself, and a typical value is 800mV. This voltage may be different in negative ion mode.

#### Stop

This is the size of pulse needed to register as being an ion, so stopping the clock. It is usually set at 100mV, a value high enough to prevent electronic noise being detected as ions.

#### Threshold

This parameter should normally be set to zero. Setting to 1 will cause all peaks in the spectrum with one count to be thresholded out.

#### Bunching

This facility can reduce data file size, although at the expense of resolution. For most analyses it is generally set to 1.

## Lteff

This is used to make the TOF mass measurement nominally correct without a calibration. The default value is 1800.

To adjust the nominal mass scale (as displayed on the tune page without calibration):

Acquire a TOF spectrum of a standard compound with **Lteff** set to 1800.

Calculate the new value of **Lteff** from the relation:

$$\text{Lteff} = 1800\sqrt{(m_{\text{ind}}) \div (m_{\text{act}})}$$

where:

$m_{\text{ind}}$  = indicated  $m/z$ .

$m_{\text{act}}$  = actual  $m/z$ .

Enter the new **Lteff** value in the tune page.

Once the new **Lteff** value has been entered all subsequent acquisitions should nominally be correctly mass measured.

## Centroid

If data is acquired in centroid format, the right-hand section of the dialog displays the settings generated during the instrument calibration, described in *Calibration and Accurate Mass* later in this document. This feature was introduced with MassLynx Version 2.0 and is absent from previous versions of MassLynx.

## Calibration

Information concerning the calibration of Q-Tof 2 is provided in *Calibration and Accurate Mass* later in this document and in the *Guide to Data Acquisition*.

## Data Acquisition

The mechanics of the acquisition of sample data are comprehensively described in the *Guide to Data Acquisition*. Refer to that publication for full details.

## Data Processing

The processing of sample data is comprehensively described in the *MassLynx NT User's Guide*. Refer to that publication for full details.



## Shutdown Procedures

### Emergency Shutdown

In the event of having to shut down the instrument in an emergency, proceed as follows:

Switch off the power at the wall mounted isolation switch(es), if fitted. If not, switch the power off at the rear of the instrument and switch off all peripherals.

*A loss of data is likely.*

Disconnect any LC systems to prevent solvent flowing into the source.

### Overnight Shutdown

When the instrument is to be left unattended for any length of time, for example overnight or at weekends, proceed as follows:

Switch off the LC pumps.

Set **Capillary** to 0V.

Undo the finger-tight connector on the probe to release the tubing leading from the LC system.

*Before disconnecting the probe, it is good practice to temporarily remove the probe and flush it of any salts, buffers or acids.*

If APcI is being used, reduce **APcI Probe Temp** to ambient temperature.



**Caution:** Leaving the APcI probe hot with no gas or liquid flow will shorten the lifetime of the probe heater.

Deselect **API Gas** to turn off the supply of nitrogen gas.

If the instrument is not to be used for a long period of time:

Reduce **Source Block Temp** to 60°C.

*It is not necessary to turn the instrument out of the operate mode.*

### Complete Shutdown

If a power cut is expected, or if the instrument is to be moved, proceed as follows:

Switch off the LC pumps.

On the tune page, deselect **Operate** to put the instrument in standby mode.

*The tune page indicator changes to red, indicating that the instrument is no longer in the operate mode.*

Undo the finger-tight connector on the probe to release the tubing leading from the LC system.

*Before disconnecting the probe, it is good practice to temporarily remove the probe and flush it of any salts, buffers or acids.*

If APcI is being used, reduce **APcI Probe Temp** to ambient temperature.



**Caution:** Leaving the APcI probe hot with no gas or liquid flow will shorten the lifetime of the probe heater.

Deselect **API Gas** to turn off the supply of nitrogen gas.

Select **Other** from the menu bar at the top of the tune page. Click on **Vent**.

*The turbomolecular pumps switch off.*

*When the turbomolecular pumps have run down to 80% of their normal operating speed the vent valve opens and the instrument is automatically vented to dry nitrogen.*

*The rotary pump switches off.*

Exit MassLynx.

Shut down the host PC.

Switch off all peripherals.

Switch off the power to the instrument using the circuit breakers on the rear panel of the instrument.

Switch off power at the wall mounted isolation switch, or remove the plug.

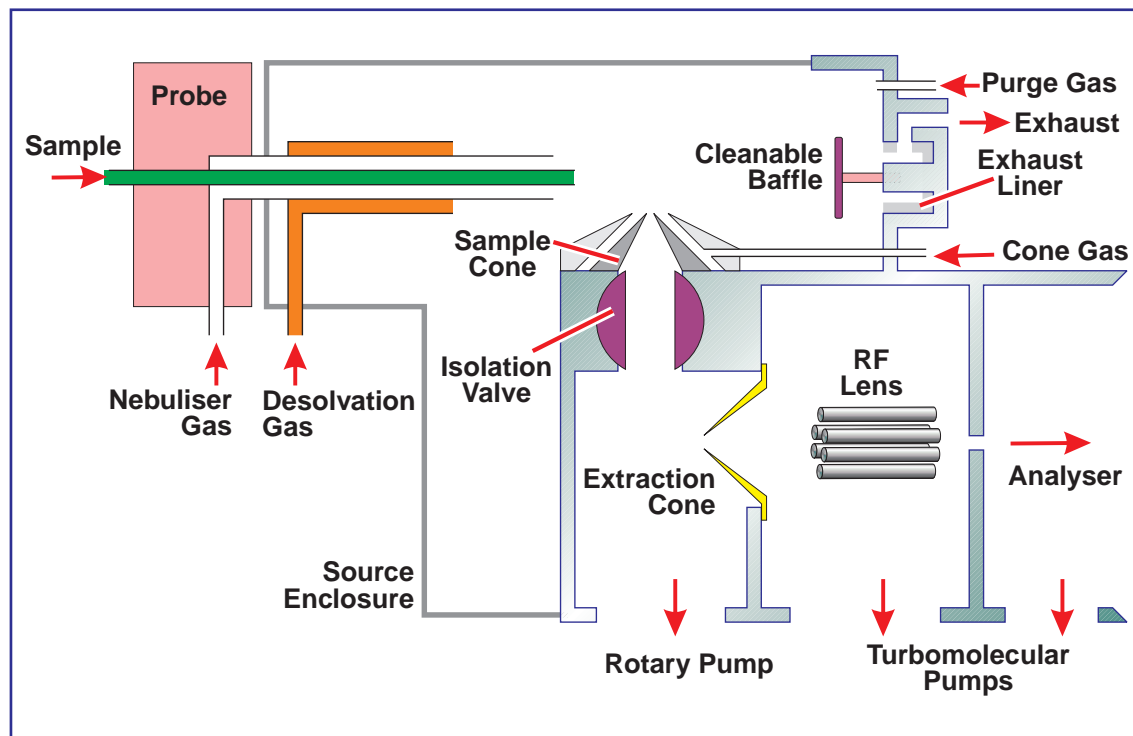
If the instrument is to be switched off for more than one week:

Drain the oil from the rotary pump according to the manufacturer's instructions.

Refill the rotary pump with new oil.

# Electrospray

## Introduction



The ESI interface consists of the standard Z-spray source fitted with an electrospray probe. See the following chapter for additional information concerning the optional nanoflow interface.

Mobile phase from the LC column or infusion pump enters through the probe and is pneumatically converted to an electrostatically charged aerosol spray. The solvent is evaporated from the spray by means of the desolvation heater. The resulting analyte and solvent ions are then drawn through the sample cone aperture into the ion block, from where they are then extracted into the analyser.

The electrospray ionisation technique allows rapid, accurate and sensitive analysis of a wide range of analytes from low molecular weight (less than 200 Da) polar compounds to biopolymers larger than 100 kDa.

Generally, compounds of less than 1000 Da produce singly charged protonated molecules ( $[M+H]^+$ ) in positive ion mode. Likewise, these low molecular weight analytes yield ( $[M-H]^-$ ) ions in negative ion mode, although this is dependent upon compound structure.

High mass biopolymers, for example peptides, proteins and oligonucleotides, produce a series of multiply charged ions. The acquired data can be transformed by the data system to give a molecular weight profile of the biopolymer.

The source can be tuned to fragment ions within the ion block. This can provide valuable structural information for low molecular weight analytes.

The most common methods of delivering sample to the electrospray source are:

- **Syringe pump and injection valve.**

A flow of mobile phase solvent passes through an injection valve to the electrospray source. This is continuous until the pump syringes empty and need to be refilled. Sample is introduced through the valve injection loop (usually 10 or 20 µl capacity) switching the sample plug into the mobile phase flow. Tuning and acquisition are carried out as the sample plug enters the source. (At a flow rate of 10 µl/min a 20 µl injection lasts 2 minutes.)

- **Reciprocating pump and injection valve.**

A flow of mobile phase solvent passes through an injection valve to the electrospray source. Sample injection and analysis procedure is the same as for the syringe pump. The pump reservoirs are simply topped up for continuous operation. The most suitable reciprocating pumps for this purpose are those which are specified to deliver a flow between 1 µl/min and 1 ml/min. A constant flow at such rates is more important than the actual flow rate. The injection valve on reciprocating pumps may be replaced by an autosampler for unattended, overnight operation.

- **Infusion pump.**

The pump syringe is filled with sample in solution. The infusion pump then delivers the contents of the syringe to the source at a constant flow rate. This arrangement allows optimisation and analysis while the sample flows to the source at typically 5-30 µl/min. Further samples require the syringe to be removed, washed, refilled with the next sample, and replumbed.

A 50:50 mixture of acetonitrile and water is a suitable mobile phase for the syringe pump system and the reciprocating pump systems. This is appropriate for positive and negative ion operation.

Positive ion operation may be enhanced by 0.1 to 1% formic acid in the sample solution.

Negative ion operation may be enhanced by 0.1 to 1% ammonia in the sample solution. Acid should not be added in this mode.

These additives should not be used in the mobile phase for flow injection analysis (FIA) studies, to allow easy change over between positive and negative ion analysis.

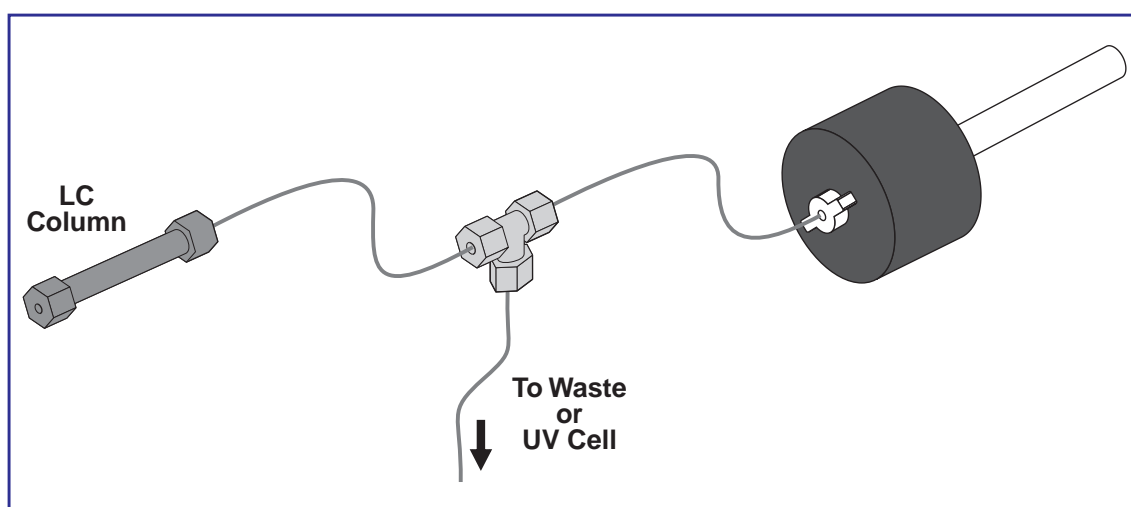
Degassed solvents are recommended for the syringe and reciprocating pumps. Degassing can be achieved by sonification or helium sparging. The solvents should be filtered, and stored under cover at all times.

It is wise periodically to check the flow rate from the solvent delivery system. This can be carried out by filling a syringe barrel or a graduated glass capillary with the liquid emerging from the probe tip and timing a known volume, say 10  $\mu$ l. Once the rate has been measured and set, a note should be made of the back pressure readout on the pump as fluctuation of this reading can indicate problems with the solvent flow.

## Post-column Splitting

Although the electrospray source can accommodate flow rates up to 1 ml/min, it is recommended that the flow is split post-column to approximately 200  $\mu$ l/min. Also, even at lower flow rates, a split may be required to save valuable samples.

The post-column split consists of a zero dead-volume tee piece connected as shown.



The split ratio is adjusted by increasing or decreasing the back pressure created in the waste line, by changing either the length or the diameter of the waste tube. A UV cell may also be incorporated in the waste line, avoiding the requirement for in-line, low volume “Z cells”. As the back pressure is varied, the flow rate at the probe tip should be checked as described above.

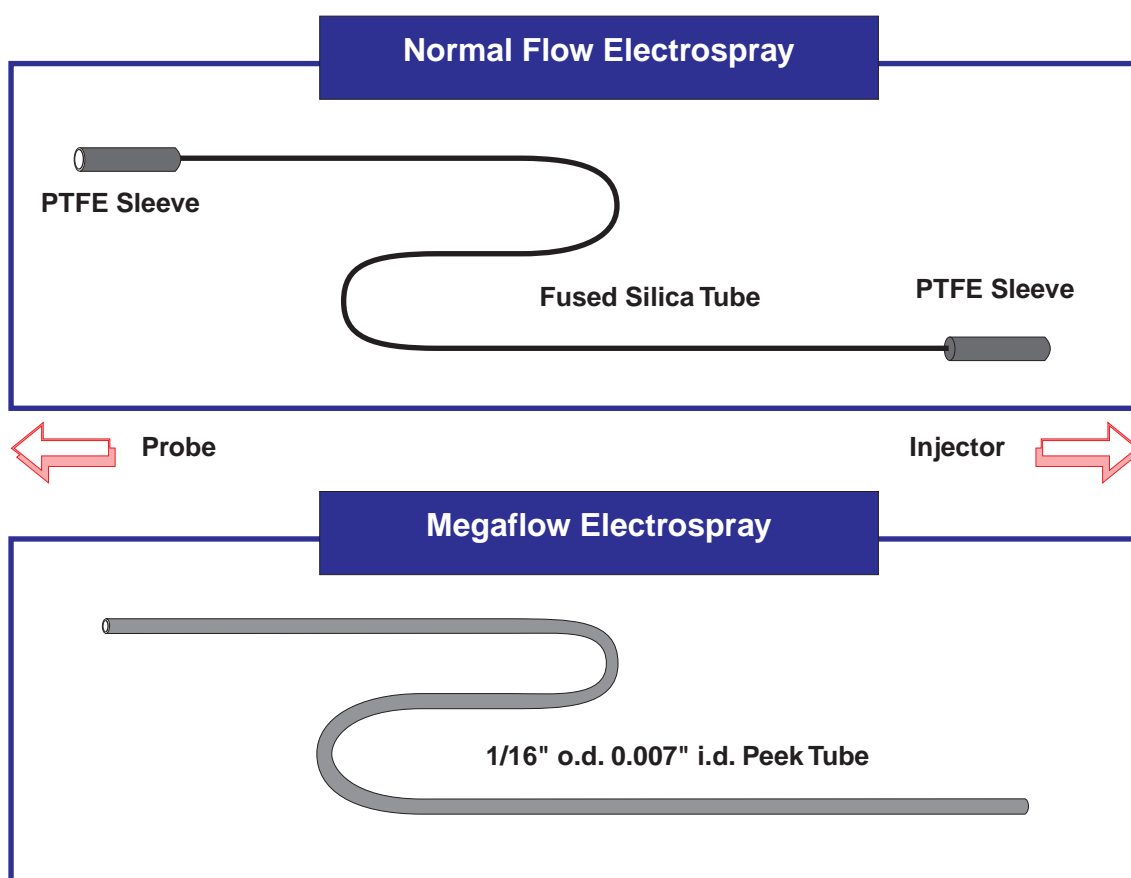
These principles apply to splitting for both megafLOW and normal flow electrospray.

## Megaflow

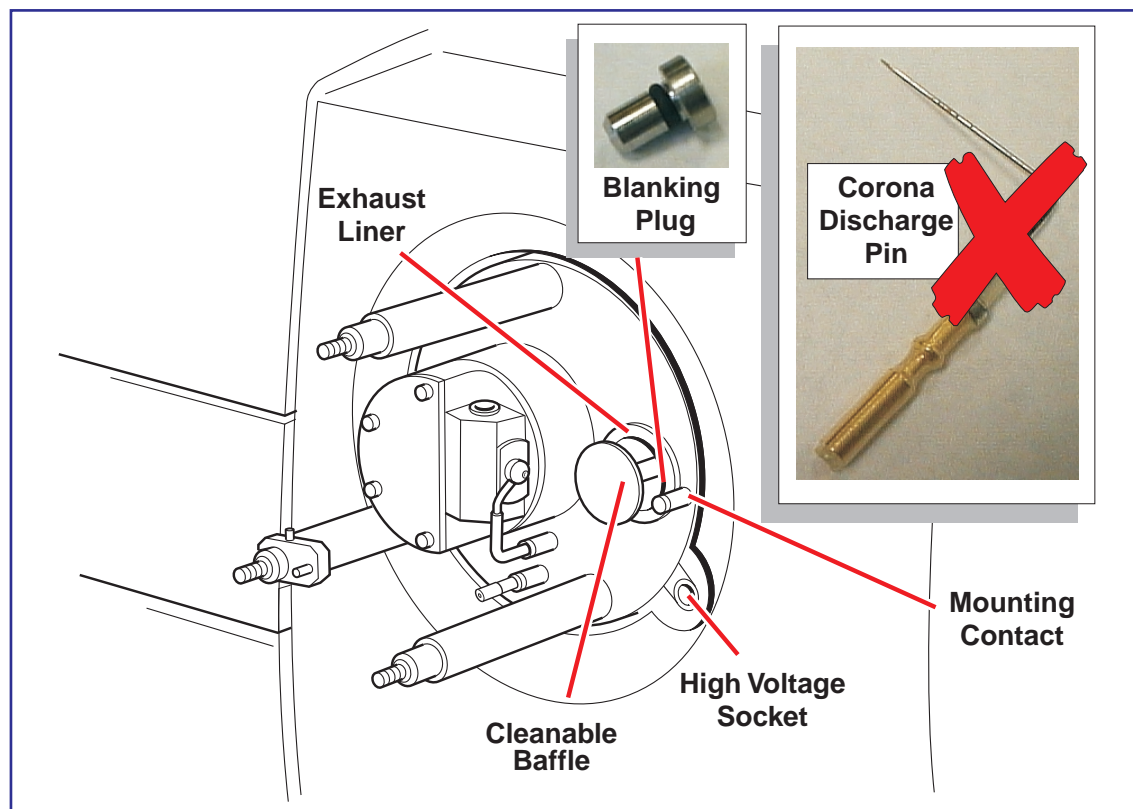
Megaflow electrospray enables flow rates from 200  $\mu\text{l}/\text{min}$  to 1  $\text{ml}/\text{min}$  to be accommodated. This allows microbore (2.1mm) or 4.6mm diameter columns to be interfaced without splitting.

### Changing Between Flow Modes

When changing between megaflow and standard electrospray operation, it is essential that the correct tubing is used to connect the probe to the sample injector. For megaflow operation  $1/16"$  o.d., 0.007" i.d. peek tubing, easily identified by its yellow stripe, is used. This replaces the standard fused silica tube, together with the PTFE sleeves.



## Operation



**Warning:** The probe tip is sharp, and may be contaminated with harmful and toxic substances. Always take great care when handling the electrospray probe.

Ensure that the source is assembled as described in *Maintenance and Fault Finding*, and that the instrument is pumped down and prepared for electrospray operation as described in *Routine Procedures*.

Ensure that a supply of nitrogen has been connected to the gas inlet at the rear of the instrument and that the head pressure is between 6 and 7 bar (90-100 psi).

Ensure that the exhaust liner and the cleanable baffle are fitted to the source.

*This is important for optimum electrospray intensity and stability when operating at low flow rates.*

## Checking the ESI Probe

Connect the electrospray probe to a pulse free pump.

*Solvent should be degassed to prevent beam instabilities caused by bubbles.*

Connect the PTFE tubing of the electrospray probe to **Nebuliser Gas** on the front panel. Secure with the nut provided.

With the probe removed from the source, turn on the liquid flow at 10 µl/min and check that liquid flow is observed at the tip of the capillary.

*To avoid unwanted capillary action effects, do not allow liquid to flow to the probe for long periods without the nitrogen switched on.*

Turn on **Nitrogen** by selecting **API Gas**, and check that a nebuliser flow of less than 100 litres/hour is registered.

Check that there is gas flow at the probe tip and ensure that there is no significant leakage of nitrogen elsewhere.

Adjust the probe tip to ensure complete nebulisation of the liquid.

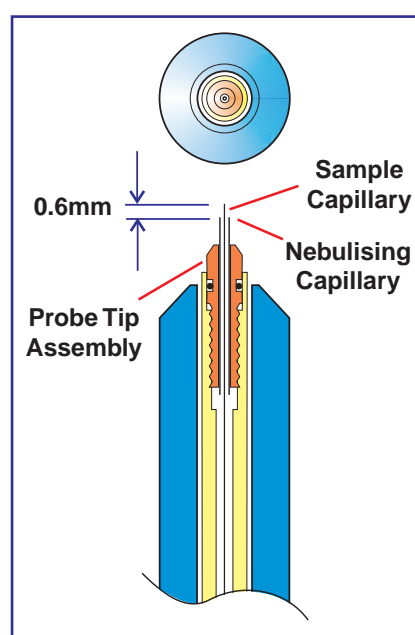
*There should be approximately 0.5mm of sample capillary protruding from the nebulising capillary.*

*The tip of the electrospray probe can influence the intensity and stability of the ion beam. A damaged or incorrectly adjusted probe tip will lead to poor electrospray performance.*

Using a magnifying glass ensure that both inner and outer stainless steel capillaries are straight and circular in cross-section.

Ensure that the inner stainless steel capillary is coaxial to the outer capillary.

*If the two capillaries are not coaxial, it is possible to bend the outer capillary slightly using thumbnail pressure.*



Insert the probe into the source and tighten the two thumb screws.

Plug the probe high voltage cable into **Capillary / Corona** on the front panel.

## Obtaining an Ion Beam

If necessary, change the ionisation mode using the **Ion Mode** command.

Using the needle valves on the front panel, set the **Desolvation Gas** flow rate to 300 litres/hour and the **Cone Gas** flow to 50 litres/hour.

Turn on the liquid flow at 10 µl/min and set **Desolvation Temp** to 150°C.

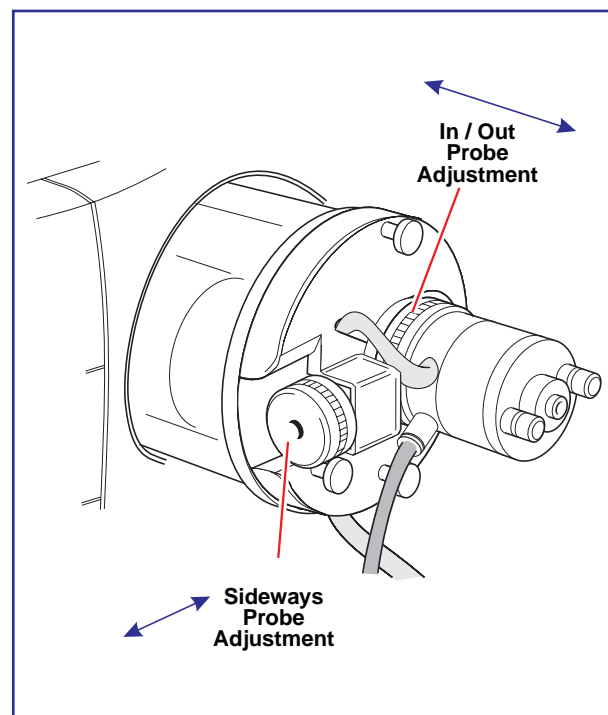
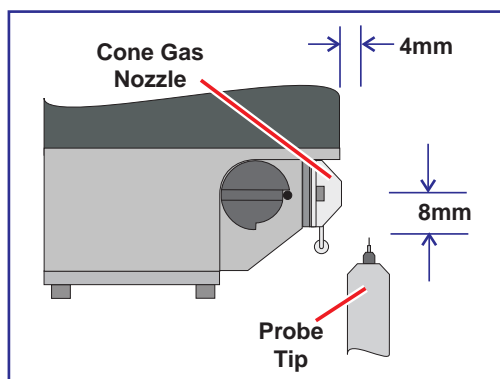


## Tuning and Optimisation

The following parameters, after initial tuning, should be optimised using a sample representative of the analyte to be studied. It will usually be found, with the exception of the sample cone voltage, that settings will vary little from one analyte to another.

### Probe Position

The position of the probe is adjusted using the probe adjustment collar (in and out) and the adjustment knob (sideways) located to the left of the probe. The two screws can be adjusted singly or simultaneously to optimise the beam. The position for optimum sensitivity and stability for low flow rate work (10  $\mu\text{l/min}$ ) is shown.



Small improvements may be gained by varying the position using the sample and solvent system under investigation. The following information should be considered when setting the probe position:

- 10mm of movement is provided in each direction, with 1.25mm of travel per revolution of the probe positioning controls.
- At higher liquid flow rates the probe tip should be positioned further away from the sample cone to achieve optimum stability and sensitivity. The position is less critical than at lower flow rates.

### Nebuliser Gas

Optimum nebulisation for electrospray performance is achieved with a nitrogen flow between 10 and 20 litres per hour. This can be achieved by fully opening the **Nebuliser Gas** flow control valve, which is situated on the instrument's front panel.

### Desolvation Gas

The desolvation gas, also nitrogen, is heated and delivered as a coaxial sheath to the nebulised liquid spray by the desolvation nozzle.

*The position of the desolvation nozzle heater is fixed relative to the probe tip and requires no adjustment.*

The **Desolvation Gas** flow rate is adjusted by the control value situated on the instrument's front panel. The optimum **Desolvation Temp** and flow rate is dependent on mobile phase composition and flow rate. A guide to suitable settings is given below.

*The **Desolvation Gas** flow rate indicated on the flow meter represents total drying flow, that is desolvation gas + cone gas (nanoflow only) + purge gas (if enabled).*

Solvent Flow Rate µl/min	Desolvation Temp °C	Desolvation Gas Flow Rate litres/hour
<10	100 to 120	200 to 250
10 to 20	120 to 250	200 to 400
20 to 50	250 to 350	200 to 400
>50	350 to 400	500 - 750

Higher desolvation temperatures give increased sensitivity. However increasing the temperature above the range suggested reduces beam stability. Increasing the gas flow rate higher than the quoted values leads to unnecessarily high nitrogen consumption.

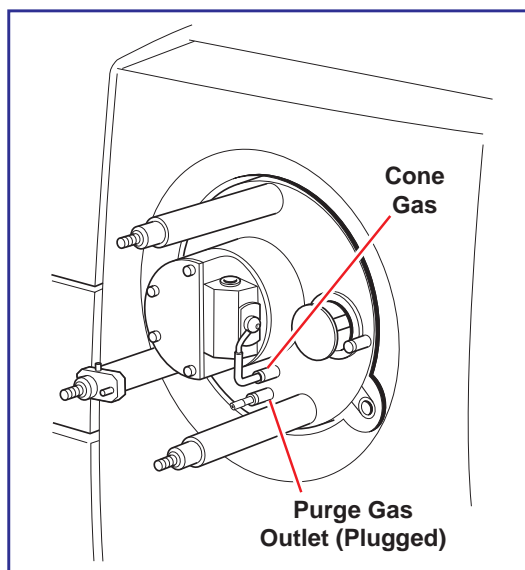


**Caution:** Do not operate the desolvation heater for long periods of time without a gas flow. To do so could damage the source.

## Cone Gas

The cone gas reduces the intensity of solvent cluster ions and solvent adduct ions. The cone gas flow rate should be optimised by increasing until solvent cluster ions and / or adduct ions are reduced as much as possible without diminishing the intensity of the ion of interest, normally  $(M+H)^+$ .

Typical cone gas flow rates are in the range 100 to 300 litres per hour.



## **Purge Gas**

The purge gas is not necessary for most ESI applications. It may be useful for megafLOW operation where an analyte is susceptible to acetonitrile adducting.

Purge gas is enabled simply by removing the blanking plug from the outlet situated within the source enclosure.

Purge gas flow rate is a constant fraction (30%) of the total desolvation gas flow.

## Source temperature

100°C is typical for 50:50 CH<sub>3</sub>CN:H<sub>2</sub>O at solvent flow rates up to 50 µl/min. Higher source temperatures, up to 150°C, are necessary for solvents at higher flow rates and higher water content.



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Temp** higher than 150°C.

## Capillary Voltage

**Capillary** usually optimises at 3.0kV, although some samples may tune at values above or below this, within the range 2.5 - 4.0kV for positive electrospray. For negative ion operation a lower voltage is necessary, typically between 2.0 - 3.5kV.

At high flow rates this parameter may optimise at a value as low as 1kV.

## Sample Cone Voltage

A **Cone** setting between 25V and 70V will produce ions for most samples, although solvent ions prefer the lower end and proteins the higher end of this range. Whenever sample quantity and time permit, **Cone** should be optimised for maximum sensitivity, within the range 15V to 150V. Increasing **Cone** will increase ion fragmentation within the source.

## Extraction Cone Voltage

**Extractor** optimises at 0 - 5V. Higher values may induce ion fragmentation of low molecular weight samples.

## Megaflow Hints

With this high flow rate technique the setup procedure involves making the following adjustments:

- Increase **Desolvation Gas** flow to 500 litres/hour.
- Increase **Desolvation Temp** to 400°C.
- Increase **Source Block Temp** to 150°C.
- Move the probe further away from the sample cone.

*When changing from electrospray to megaflow operation it is not necessary to adjust any source voltages.*



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Cluster ions are rarely observed with Z-spray. However solvent droplets may form within the source enclosure if the source and desolvation temperatures are too low.

Refer to the previous section on operating parameters for typical desolvation gas flow rates.

Purge gas can be used during megaflow operation to stop the source enclosure from overheating. This is also beneficial when the analyte is susceptible to acetonitrile adducting. Purge gas is enabled by removing the blanking plug from the outlet situated within the source enclosure.

If the sample is contained within a 'dirty matrix' the probe may be moved away from the sample cone to extend time between source cleaning operations. This may incur a small loss in sensitivity.



**Warning:** It is normal for the source enclosure, the glass tube and parts of the probe mounting flange, to get hot during prolonged megaflow operation. Care should be taken when handling source components during and immediately after operation.

*The source enclosure will run cooler if purge gas is used.*



**Warning:** For health and safety reasons always ensure the exhaust line is vented outside the building or to a fume hood.



**Warning:** Ensure that a plastic bottle is connected in the exhaust line to collect any condensed solvents.

## Removing the Probe

To remove the probe from the source proceed as follows:

On the tune page deselect **Operate** to put the instrument into standby mode.

Switch off the liquid flow and disconnect from the probe.

Deselect **API Gas** and turn off **Nitrogen**.

Disconnect the probe cable from the instrument.

Disconnect the nebulising gas supply from the instrument.

## Sample Analysis and Calibration

### General Information

Care should be taken to ensure that samples are fully dissolved in a suitable solvent. Any particulates must be filtered to avoid blockage of the transfer line or the probe's capillary. A centrifuge can often be used to separate solid particles from the sample liquid.

There is usually no benefit in using concentrations greater than 20 pmol/μl for biopolymers or 10 ng/μl for low molecular weight compounds.

Higher concentrations will not usually improve analytical performance. Conversely, for biopolymers, lower concentrations often yield better electrospray results. Higher levels require more frequent source cleaning and risk blocking the transfer capillary.

Optimisation for low molecular weight compounds may usually be achieved using a concentration of 1ng/μl.

Samples with phosphate buffers and high levels of salts should be avoided. Alternatively, at the expense of a small drop in sensitivity, the probe can be pulled away from the sample cone to minimise the deposit of involatile material on the cone.

To gain experience in sample analysis, it is advisable to start with the qualitative analysis of known standards. A good example of a high molecular weight sample is horse heart myoglobin (molecular weight 16951.48) which produces a series of multiply charged ions that can be used to calibrate the  $m/z$  scale from 800-1600 in either positive ion or negative ion mode.

Polyethylene glycol mixtures, for example 300 / 600 / 1000, are low molecular weight samples suitable for calibrating the  $m/z$  scale from approximately 100 to 1200 in positive ion mode. A mixture of sugars covers the same range in negative ion mode.

Alternatively, sodium iodide or caesium iodide can be used for calibration.

### **Typical ES Positive Ion Samples**

- Peptides and proteins.
- Small polar compounds.
- Drugs and their metabolites.
- Environmental contaminants (e.g. pesticides, pollutants).
- Dye compounds.
- Some organometallics.
- Small saccharides.

### **Typical ES Negative Ion Samples**

- Some proteins.
- Some drug metabolites (e.g. glucuronide conjugates).
- Oligonucleotides.
- Some saccharides and polysaccharides.



## Chromatographic Interfacing

Electrospray ionisation can be routinely interfaced to reversed phase and normal phase chromatographic separations. Depending on the LC pumping system, chromatography column and setup, there are some basic options:

- Microbore and capillary chromatography separations employing 1mm diameter (and smaller) columns can be interfaced directly to the electrospray probe. Typical flow rates for such columns may be in the region of 3-50  $\mu\text{l}/\text{min}$ . It is suggested that a syringe pump is used to deliver these constant low flow rates through a capillary column. Alternatively, accurate pre-column splitting of higher flow rates from reciprocating pumps can be investigated.

In all cases, efficient solvent mixing is necessary for gradient elution separations. This is of paramount importance with regard to low flow rates encountered with capillary columns. HPLC pump manufacturers' recommendations should be heeded.

- 2.1mm diameter reversed phase columns are gaining popularity for many separations previously addressed by 4.6mm columns. Typically flow rates of 200  $\mu\text{l}/\text{min}$  are used, allowing direct coupling to the electrospray source. The increased sample flow rate requires increased source temperature and drying gas flow rate.

A UV detector may be placed in-line to the probe, provided that the volume of the detector does not significantly reduce the chromatographic resolution. Whenever a UV detector is used, the analog output may be input to MassLynx NT for chromatographic processing.

- The interfacing of 4.6mm columns to the electrospray source can be achieved either by flow splitting or by direct coupling. In both cases an elevated source temperature and drying gas flow rate are required. In general, the best results are obtained by splitting after the column using a zero dead volume tee piece so that 200-300  $\mu\text{l}/\text{min}$  is transferred to the source.



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Conventional reverse phase and normal phase solvent systems are appropriate for LC-electrospray.

Involatile buffers may be used but prolonged periods of operation are not recommended. When using involatile buffers the probe should be moved as far away from the sample cone as possible. This may reduce sensitivity slightly, but will reduce the rate at which involatile material will be deposited on the sample cone.

Trifluoroacetic acid (TFA) and triethylamine (TEA) may be used up to a level of 0.05%. If solvents of high aqueous content are to be used then tuning conditions should be appropriate for the solvent composition entering the source.

Higher source temperatures (150°C) are also recommended for high aqueous content solvents. Tetrahydrofuran (THF) should **not** be used with peek tubing.

## LC-MS Sensitivity Enhancement

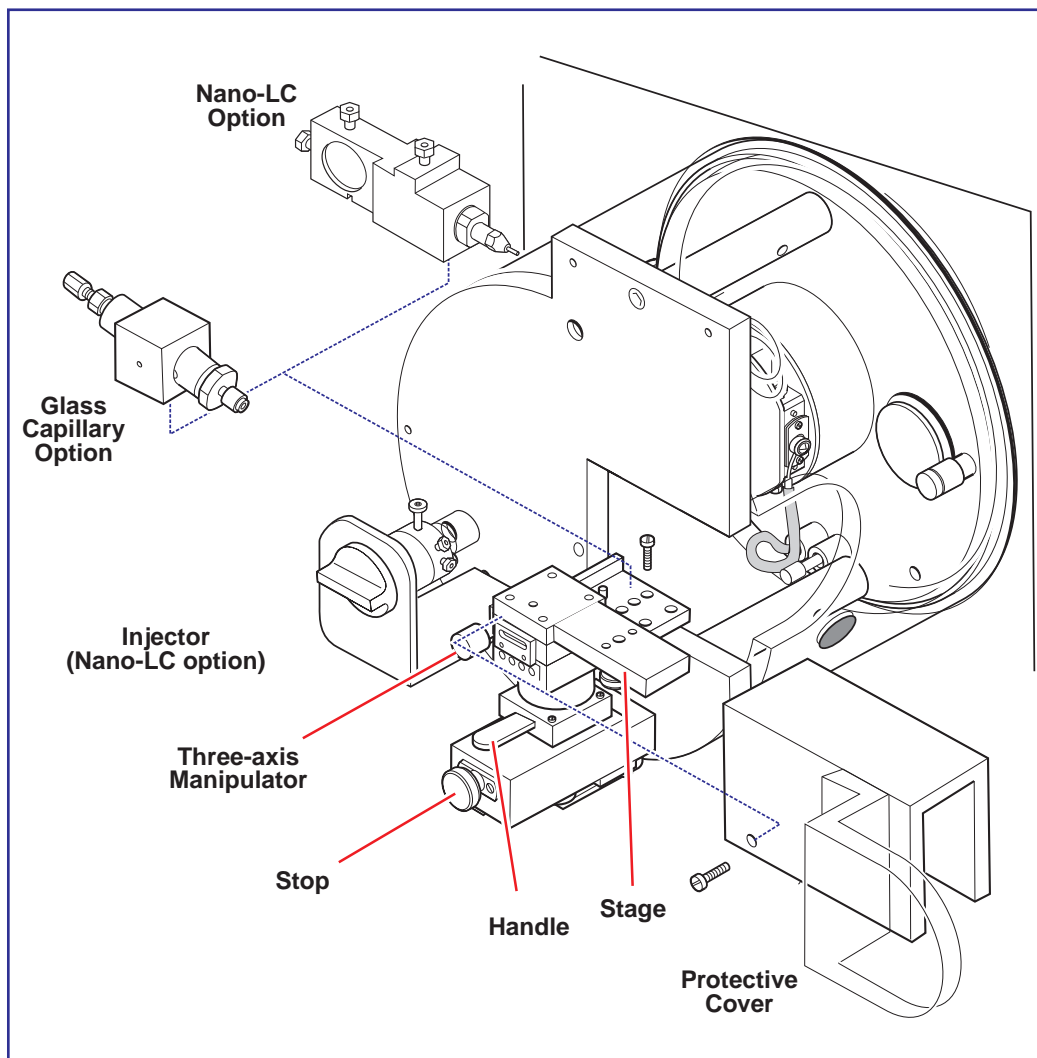
The sensitivity of a LC-MS analysis can be increased or optimised in a number of ways, by alterations to both the LC operation and the MS operation.

In the LC area some examples include the use of high resolution columns and columns with fully end capped packings. For target compound analysis, techniques such as trace enrichment, coupled column chromatography, or phase system switching can have enormous benefits.

Careful choice of the solvent, and solvent additives or modifiers, may also prove important.

# Nanoflow Electrospray

## Overview



The optional nanoflow interface allows electrospray ionisation to be performed in the flow rate range 5 to 1000 nanolitres per minute. There are two options for the spraying capillary, which can be alternately fitted to the interface:

- Borosilicate metal coated glass capillary.

*Metal coated glass capillaries allow the lowest flow rates to be obtained although they are used for one sample only and must then be discarded.*

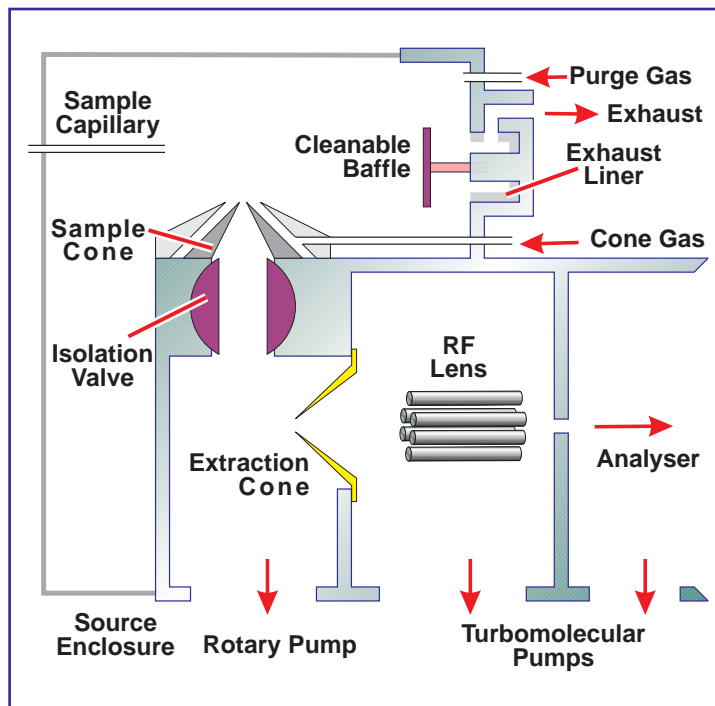
- Nano-LC.

*This option is suitable for flow injection analyses or for coupling to nano-HPLC, and uses a pump to regulate the flow rate down to 100 nl/min. If a syringe pump is to be used, a gas-tight syringe is necessary to obtain correct flow rates without leakage. A volume of 25µl is recommended.*

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

The nanoflow end flange consists of a three-axis manipulator, a stage, a protective cover and a stop / handle arrangement for rotation of the manipulator and stage.

The manipulator and stage are rotated by 90 degrees to change option or, in the glass capillary option, to load a new nanovial.



**Caution:** Failure to use the stop and handle to rotate the stage can result in permanent damage to the three-axis manipulator.

## Installing the Interface

To change from the normal electrospray interface and install the nanoflow interface:

If fitted, remove the probe.

Remove the moulded cover from around the source.

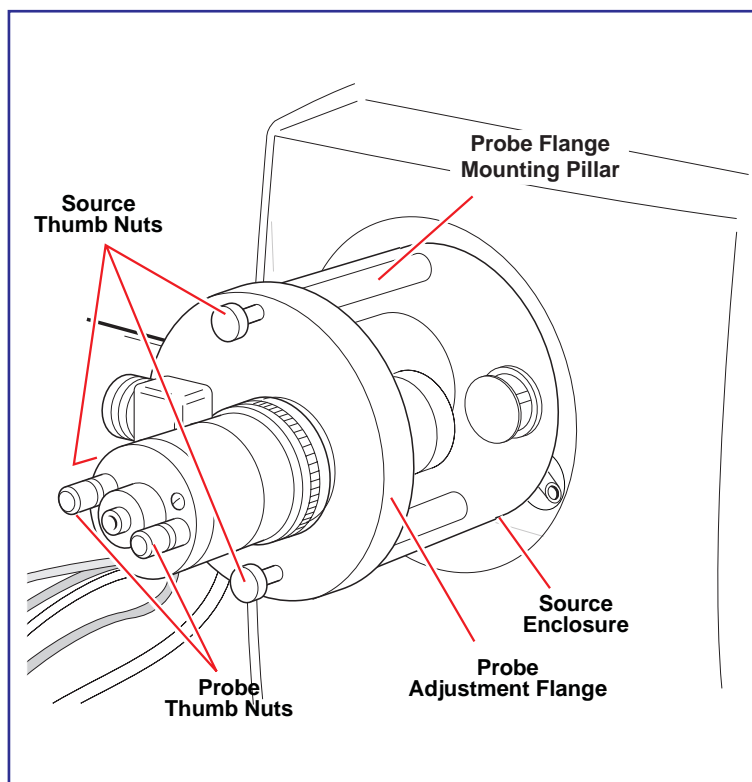
Undo the three thumb screws and withdraw the probe adjustment flange assembly and glass tube.

Place the glass tube, end on, on a flat surface and place the probe support flange assembly on top of the glass tube.

Remove the PTFE encapsulated source O ring.



**Warning:** When the source enclosure has been removed the ion block heater is exposed. Ensure that the source block heater has been switched off and has cooled before proceeding. Observe the **Source BlockTemp** readback on the tune page.



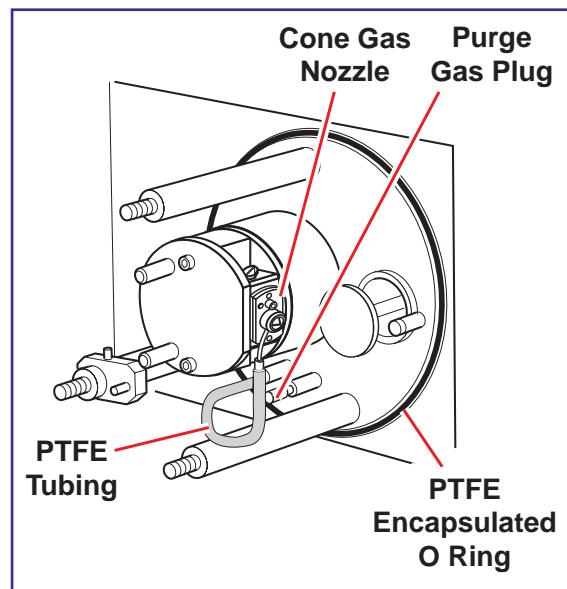
Unscrew the three probe flange mounting pillars, using the holes to obtain the necessary leverage.

If the cone gas nozzle is not in place, close the sample cone isolation valve. Remove the two screws that secure the sample cone and fit the cone gas nozzle.

Replace the two screws.

Connect the cone gas outlet to the cone gas nozzle using the PTFE tubing provided. Open the sample cone isolation valve.

*The cone gas flow rate is set at 30% of the total desolvation gas flow.*



Ensure that the purge gas is plugged (disabled).

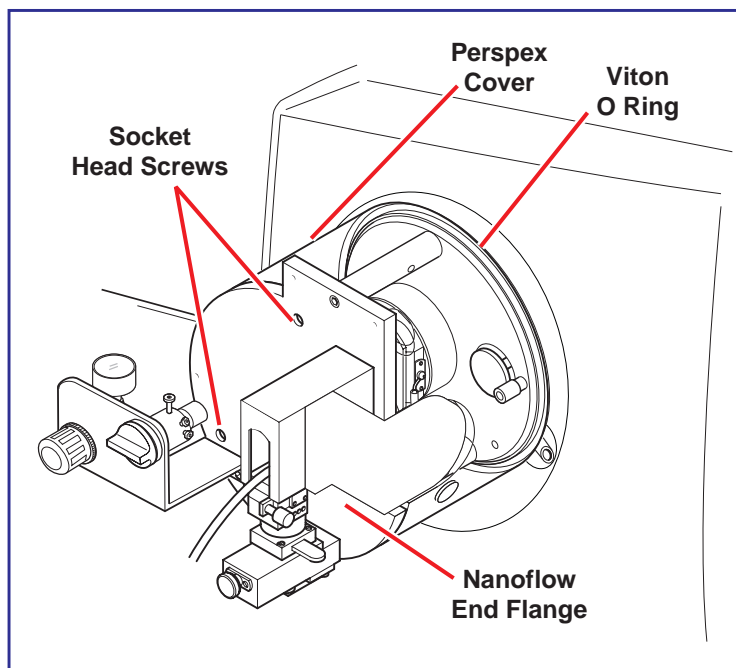
Ensure that the cleanable baffle, the exhaust liner and the corona discharge pin blanking plug are fitted.

Fit a viton O ring and the three shorter nanoflow pillars.

Install the perspex cover and the nanoflow end flange, securing this with socket head screws.

*Do not attempt to refit the moulded cover.*

If not already in place, attach the microscope or camera brackets using the screw hole and dowels at the top of the bracket.



Insert the flexible light guide into the grommet at the base of the perspex cover.

Set the light source to its brightest.

Block the **Desolvation Gas** outlet on the instrument's front panel.

Close the nebuliser needle valve.

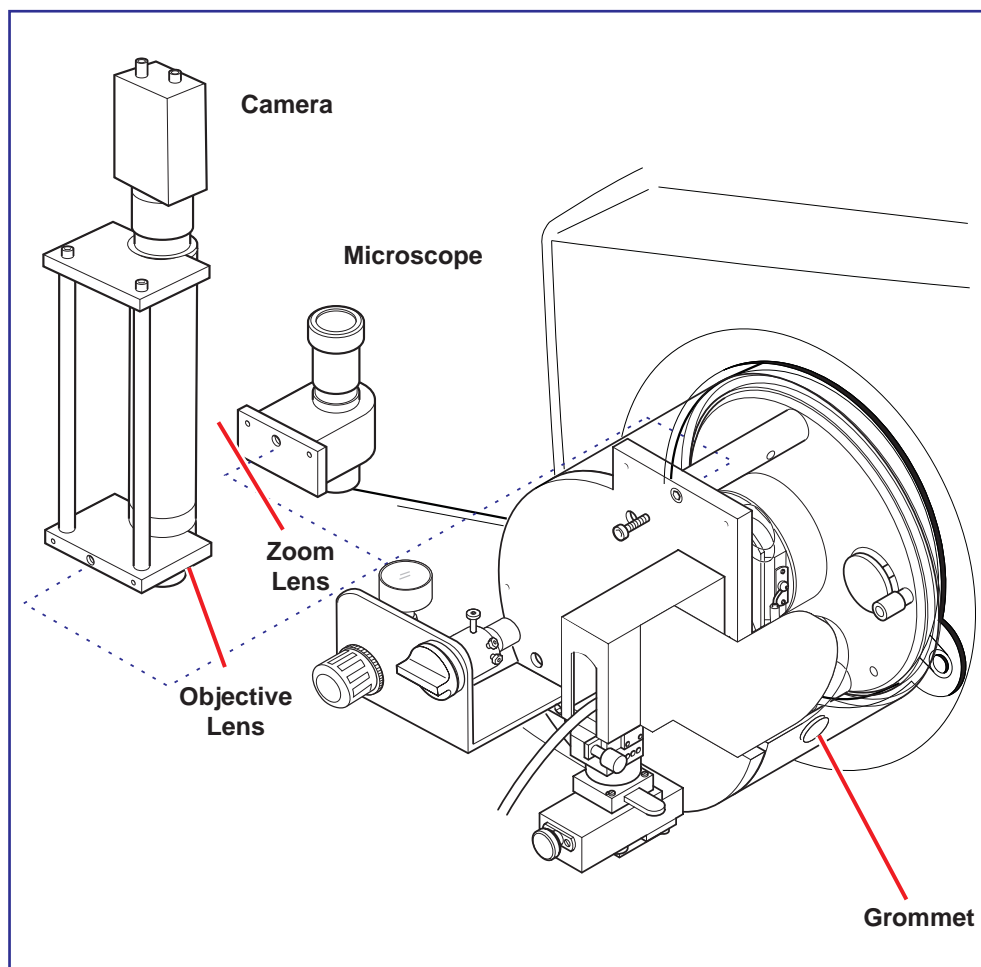
*The cone gas is split from the desolvation gas internally.*

Attach the two cables to the sockets marked **Capillary / Corona** and **Probes** on the front panel of the instrument.

Set **Source Block Temp** to approximately 80°C.



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.



## Operation of the Camera System

Magnification is controlled by the zoom lens. A fine focus can be achieved by rotating the objective lens.

## Using the Microscope

Focusing is adjusted by rotating the top of the microscope.

## Glass Capillary Option

### Installation

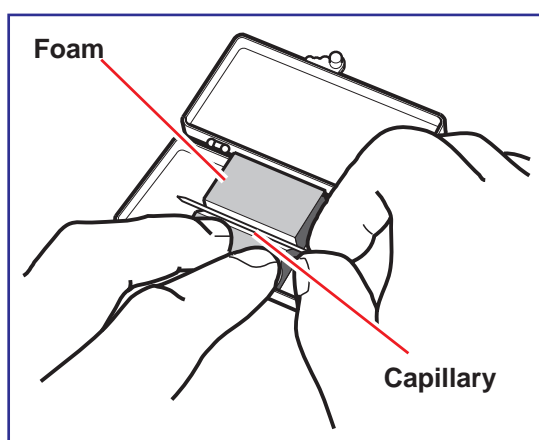


**Warning:** Do not touch the sharp end of the capillary. As well as the risk of injury by a sliver of glass, the capillary may contain toxic samples



**Caution:** 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.

With the stage rotated outwards, unscrew the union from the end of the assembly.



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

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

Tighten the nut (finger tight is sufficient) so that 5mm of glass capillary is protruding from the end of it. This distance is measured from the end of the nut to the shoulder of the glass capillary.

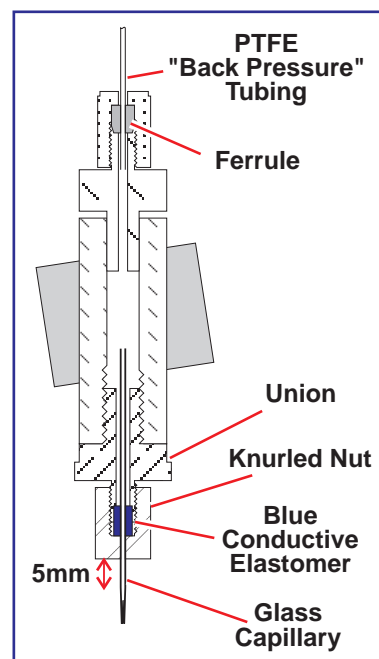
Load sample into the capillary using either a fused silica syringe needle or a GELoader tip.

Screw the holder back into the assembly - finger tight is sufficient.

Ensure that **Capillary** is set to 0V on the tune page.

Rotate the stage back into the interface using the stop and handle.

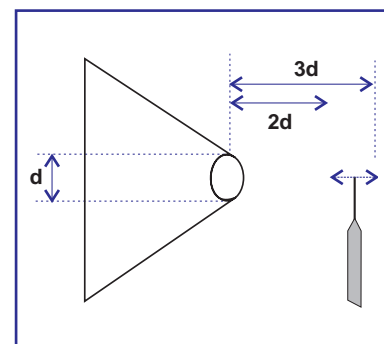
When using a GELoader tip, break the nanovial in half, by scoring with a fused silica cutter. This enables the GELoader to reach the tip of the nanovial





## Nanovial Tip Position

The position of the nanovial is adjusted as shown. The tip is in line with the centre of the sample cone, at a distance between two and three times the diameter of the cone orifice, as observed through the microscope.



## Operation

Manoeuvre the stage so that the microscope or camera can view the capillary tip.

Using the nanoflow regulator, apply pressure to the back of the tip until a drop of liquid is seen.

On the tune page, select **APIGas** to turn on **Nitrogen**.

Select **Operate**.

Set **Capillary** between 600 and 1.0kV.

Adjust **Desolvation Gas** flow to 100 litres/hour using the knob on the front panel of the instrument.

*An ion beam should now be visible on the tune page.*

Tune the source voltages, adjust the gas flow and adjust the three-axis manipulator for maximum ion current.

*The ion current may change dramatically with very slight changes of position but the high resolution of the threads in the manipulator allows very fine tuning.*

## Restarting the Spray

Should the spray stop, it is possible to restart it by adjusting the three-axis manipulator so that, viewed under magnification, the capillary tip touches the sample cone and a small piece of the glass hair shears off. Set the capillary to zero when doing this.

It may also be necessary to apply some back pressure to the holder to force a drop of liquid from the capillary. Up to 1.4 bar (20 psi) can be applied and, with this pressure, a drop should be visible unless the capillary is blocked.

## Nano-LC Option

### Installation

With the sprayer assembly removed from the stage:

Cut approximately 25mm of the red stripe peek tubing and, using the plug cap and a Valco nut, set a ferrule to the correct position on the tubing.

*At this stage the ferrule is required only to grip the tubing lightly, and should not be too tight.*

Cut the peek such that 10mm of the peek protrudes from the back of the ferrule.

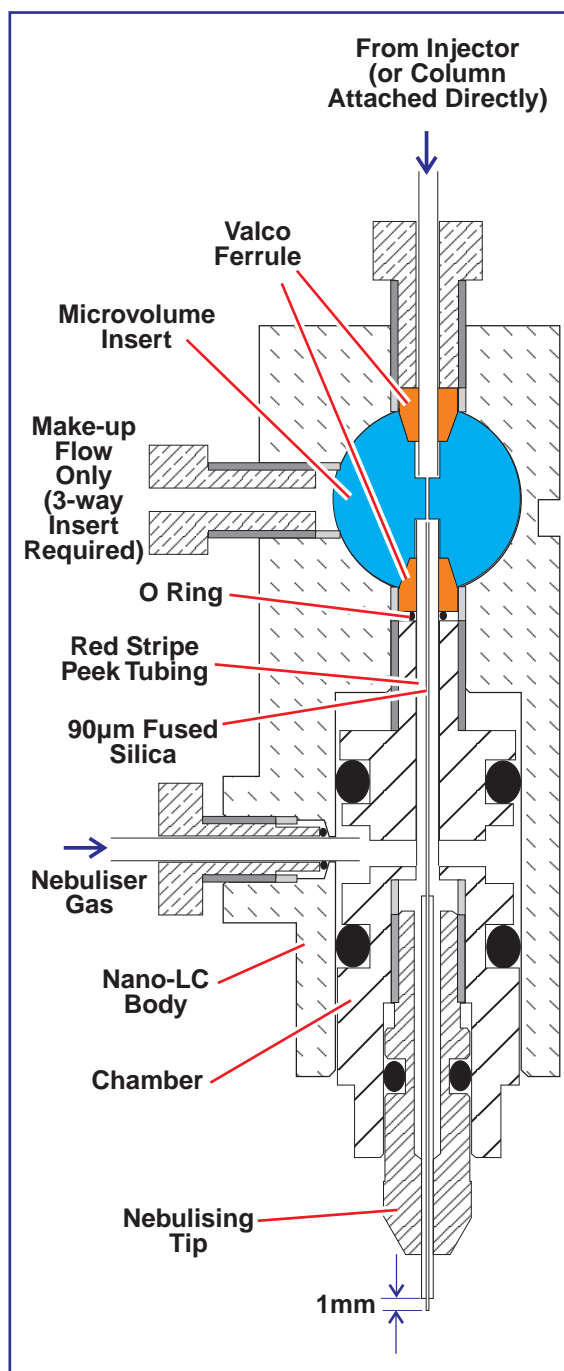
Thread approximately 70mm of the 90 micron o.d. fused silica through the new fitting.

Ensure that the fused silica is flush with the peek sleeve.

Again using the plug cap, tighten the nut further to ensure that the fused silica is gripped. Some force may be required to do this.

Remove the sleeved fused silica from the plug cap and remove the Valco nut.

Place an O ring onto the peek tube, using tweezers if necessary.



*The O ring is required to seal the region between the ferrule and the end of the thread on the nano-LC chamber.*

Thread the sleeved fused silica through the nano-LC chamber.

Rotate the microvolume union in the body such that the ferrule seat is aligned correctly.

Insert the chamber into the nano-LC body and tighten using a pair of spanners.

*The capillary can now be checked for flow by connecting the output from a Harvard syringe pump to the other side of the union and setting the flow to 1  $\mu\text{L}/\text{min}$ , using a micropipette to measure the flow. It is recommended that a syringe with a volume of no more than 50 millilitres is used.*

Thread the fused silica through the nebulising tip and screw in the nano-LC chamber such that it is screwed in approximately half way.

Cut the fused silica using a tile cutter and adjust the nebulising tip further, such that 1mm of fused silica protrudes from the tip.

Attach the nebulising gas tubing to the sprayer using an O ring and the special screw.

Attach the sprayer assembly to the stage.

*It may be necessary to alter the position of the thumbscrew underneath the baseplate to attach the sprayer correctly.*

Swing the stage into the interface using the stop and handle.

## Operation

For tuning purposes it may be useful to infuse a known sample in 95% water using a Harvard syringe pump.

Set the liquid flow to about 200 nl/min.

Switch on **Gas** at the MassLynx tune page.

Set the pressure of the gas on the regulator to approximately 0.5 bar (7 psi).

Ensure there are no leaks of gas at the sprayer, particularly where the PTFE tubing is connected to it.

By viewing under magnification, the spray emanating from the capillary may be examined and tuned by altering the nebulising tip such that a fine spray is observed. Altering the gas slightly may also help in this tuning process.

Swing the stage back out of the source and place the cover over the sprayer ensuring that the tubing coming from the sprayer is threaded correctly through it.

Lock the cover in place with two screws.

Swing the stage back into the source and alter the translation stage (in / out direction) such that the capillary is approximately 5mm from the cone.

Select **Operate** and set **Capillary** to approximately 2.5kV.

*An ion beam should now be present.*

Optimise the ion beam by altering the position of the spray using the controls of the translation stage.

The sprayer can now be connected to the HPLC system. The injection valve is plumbed as follows:

- P from the pump.
- C to the column (or to the union).
- S is the sample port, attach a VISF sleeve here.
- W is a waste port.

*A short tail of fused silica, attached to the entrance port of the union, and the use of low pressure PTFE connectors will remove the need to move the stage. This will prevent accidental alteration of the sprayer's position when changing between tuning and HPLC operation.*

## Changing Options

To change between the glass capillary and the nano-LC options:

Rotate the stage outwards.



**Caution:** Failure to use the stop and handle to rotate the stage can result in permanent damage to the three-axis manipulator.

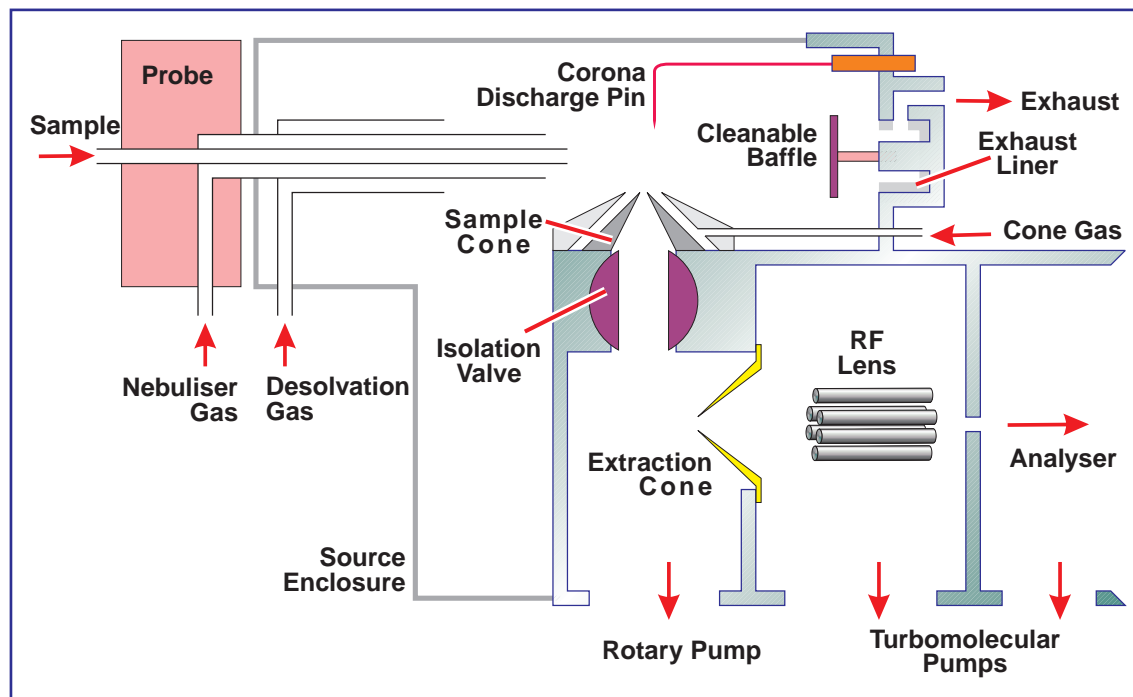
Remove the protective cover and release the captive screw located underneath the stage.

Lift off the holder and replace it with the alternative holder, securing it with the captive screw.

Replace the protective cover, ensuring that either the PTFE back pressure tubing (glass capillary option) or the fused silica transfer line is fed through the slot in the back of the protective cover along with the HV cabling.

# Atmospheric Pressure Chemical Ionisation

## Introduction



Atmospheric Pressure Chemical Ionisation (APCI) is an easy to use LC-MS interface that produces singly-charged protonated or deprotonated molecules for a broad range of involatile analytes.

The ability to operate with 100% organic or 100% aqueous mobile phases at flow rates up to 2 ml/min makes APCI an ideal technique for standard analytical column (4.6mm i.d.) normal phase and reverse phase LC-MS.

The APCI interface consists of the standard Z-spray source fitted with a corona discharge pin and a heated nebuliser probe. Mobile phase from the LC column enters the probe where it is pneumatically converted into an aerosol and is rapidly heated and converted to a vapour / gas at the probe tip. Hot gas from the probe passes between the sample cone and the corona discharge pin, which is typically maintained at 2.5kV. 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 positive ion mode) or deprotonated (in the negative ion mode). The sample and reagent ions pass through the sample cone into the ion block prior to being extracted via the extraction cone into the RF lens.

Changeover between electrospray and APcI operation is simply accomplished by changing the probe and installing the corona discharge pin within the source enclosure.

For APcI operation, the desolvation gas is not heated in the desolvation nozzle. However, it is important that desolvation gas is used throughout.

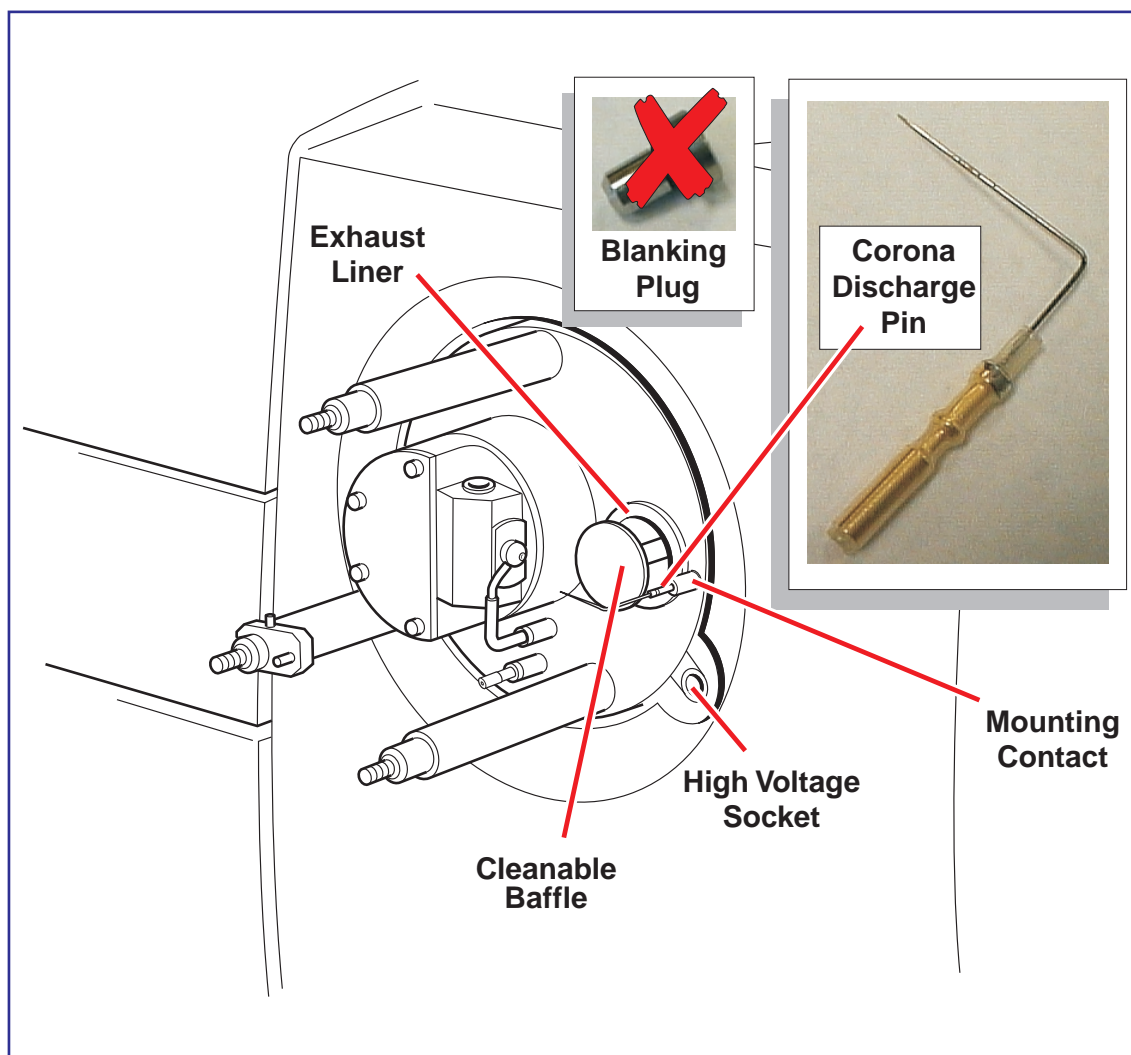
The background spectrum for 50:50 acetonitrile:water is dependent upon the settings of **Cone** and **Extractor**. The main reagent ions for typical sample cone and extraction cone voltages of 40V and 10V respectively are 42, 56, 83 and 101.

The transmission of these ions will be dependent on the setting of **RF Lens**. A lower **RF Lens** voltage is required for optimum transmission at lower  $m/z$ .

Acetonitrile adducting may be minimised by optimisation of the probe position, as described in the chapter entitled *Electrospray*.

## Preparation

Ensure that the source is assembled as described in *Maintenance and Fault Finding*, and that the instrument is pumped down and prepared for APcI operation as described in *Routine Procedures*.



*APcI may be operated with or without the cleanable baffle fitted.*

Ensure that a supply of nitrogen has been connected to the gas inlet at the rear of the instrument and that the head pressure is between 6 and 7 bar (90-100 psi).

## Checking the Probe

Ensure that the probe heater is off.

Unplug the probe from the instrument's front panel and remove the probe from the source.

Connect the PTFE tube to the **Nebuliser Gas** outlet on the front panel.

Remove the probe tip assembly by carefully loosening the two grub screws.

Disconnect the heater from the probe body by pulling parallel to the axis of the probe.

Ensure that 0.5 to 1mm of fused silica is protruding from the stainless steel nebuliser tube.

Connect the LC pump to the probe with a flow of 50:50 acetonitrile:water at 1 ml/min.

Check that the liquid jet flows freely from the end of the capillary and that the LC pump back pressure reads 250 to 400 psi.

Check that the nitrogen supply pressure is 6 to 7 bar (90 to 100 psi).

Select **API Gas** and turn on **Nitrogen**.

Check that the liquid jet converts to a fine uniform aerosol.

Switch off the liquid flow.

Deselect **API Gas** and turn off **Nitrogen**.

Reconnect the probe tip assembly.

Insert the APcI probe into the source and secure it by tightening the two thumb screws.

Connect the probe cable to **Probes** on the instrument's front panel.



## Obtaining an Ion Beam

Ensure that the corona discharge pin is fitted as described in *Routine Procedures, Preparation for APcI Operation* and that the pin is connected using the APcI HV cable.

Ensure that the APcI probe is fitted as described above, that the desolvation gas tube is connected to the front panel, and that the cone gas and purge gas outlets are plugged.

If necessary, change the ionisation mode using the **Ion Mode** command.

Set **Source Block Temp** to 150°C.



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Set **APcI Probe Temp** to 20°C with no liquid flow and **Nitrogen** off.

Initially set **Corona** to 2.5kV and **Cone** to 30V.

When **Source Block Temp** reaches 150°C:

Select **API Gas** to switch on the nitrogen gas.

Using the valves on the front of the instrument, adjust **Desolvation Gas** to 150 litres/hour and set **Nebuliser Gas** to its maximum setting.

Set one of the peak display boxes to show masses down to at least 100 Da.

Select **Operate**.

Set **APcI Probe Temp** to 350°C.

When **APCI Probe Temp** reaches 350°C:

Start the LC pump at a flow of 1 ml/min.

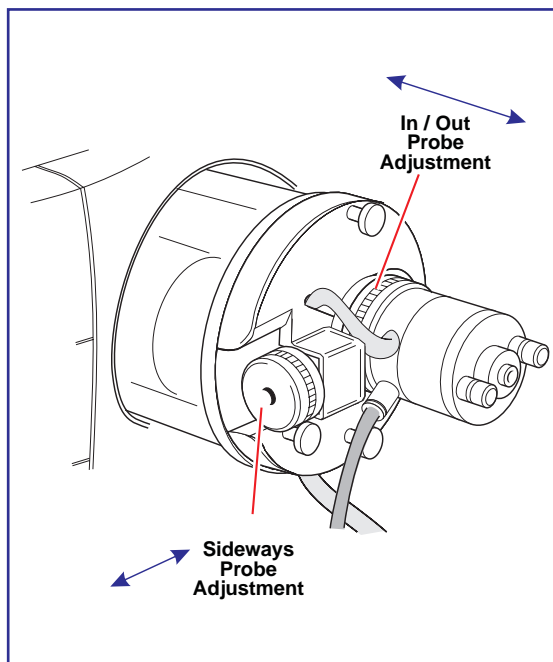
Optimise **Corona** so that the peaks reach maximum intensity.

Optimise the probe position for intensity and stability.

*The two screws can be adjusted singly or simultaneously to optimise the beam.*

*The position of the probe will affect sensitivity. However, if the sample is contained in a 'biological matrix' or is contained in an involatile solvent the probe should be moved away from the sample cone and towards the corona discharge pin.*

*The tune page shows a typical reagent ion spectrum for a methanol / water mobile phase.*



**Warning:** It is normal for the source enclosure, the glass tube and parts of the probe adjustment flange to reach temperatures of up to 60°C during prolonged APcI operation. Care should be exercised when handling source components immediately after operation.



**Warning:** Switch off the liquid flow and allow the probe to cool (<100°C) before removing it from the source.



**Caution:** Failure to employ a desolvation gas flow during APcI operation may lead to heat damage to the source.

## Hints for Sample Analysis

### Tuning

- Start by tuning on the solvent ions.
- It is generally found that the most significant analyte tuning parameter to adjust following tuning on the solvent ions is **Cone**.
- Fine tuning on the analyte of interest can be performed either by large loop injections (100µl) or by constant infusion in the mobile phase typically at analyte concentrations of a few ng/µl.
- 10µl loop injections can be monitored using real time chromatogram updates.

### Mobile Phase

- The choice of mobile phase is an important compound specific factor in APcI. For example, steroids prefer methanol:water mixtures as opposed to acetonitrile:water.
- Analyte sensitivity is also dependent on mobile phase composition, which can be varied from 100% aqueous to 100% organic for any particular mixture.

### Probe Temperature

This can be a critical factor for some analytes.

- Involatile samples (for example steroids) generally require high probe temperatures (>400°C).
- Volatile samples (for example pesticides) can be analysed with low probe temperatures (<400°C).
- In some cases, too high a probe temperature can lead to thermal degradation of labile samples.

### Desolvation Gas

Although a **Desolvation Gas** flow of approximately 150 litres/hour is typical for most samples, this flow rate should be tuned for maximum sensitivity while ensuring that the flow rate is not decreased below 100 litres/hour.

## Removing the Probe

After a session of APcI operation:

Turn off the LC flow.

Set **APcI Probe Temp** to 20°C.

Deselect **Operate** to put the instrument in standby mode.

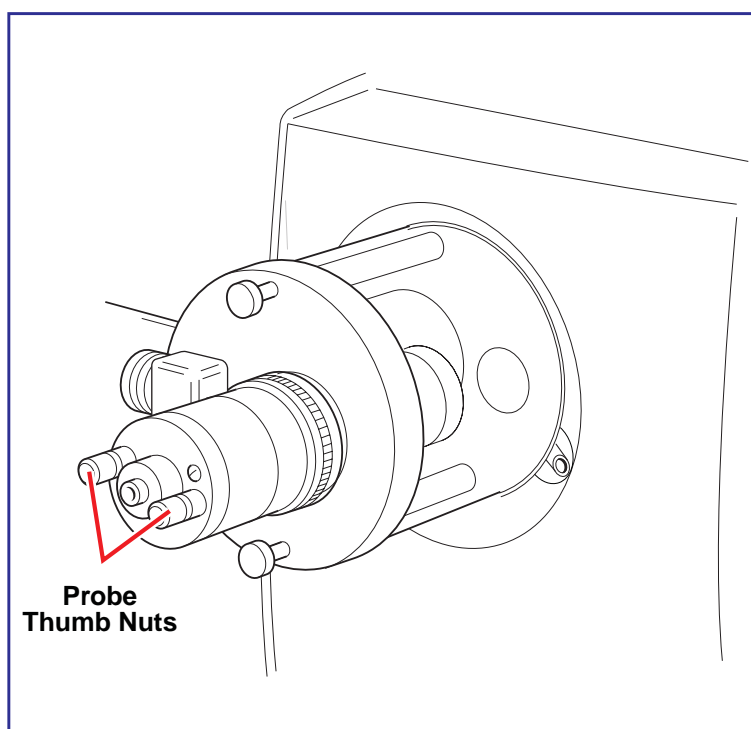
When the probe temperature falls below 100°C:

Deselect **API Gas** and turn off **Nitrogen**.

Undo the two thumb nuts and remove the probe from the source.

**Warning:** Take care when removing the APcI probe. There is a risk of burns to the operator.

**Caution:** Removal of the APcI probe when hot will shorten the life of the probe heater.



If the instrument is not to be used for a long period of time the source temperature should be reduced to 60°C.

# Calibration and Accurate Mass

## Introduction

Extremely accurate mass measurements can be performed with the Q-Tof 2, due to the elevated resolution and inherent stability of the calibration law of orthogonal TOF instruments.

The basic time of flight calibration from mass (m) to time (t) is of the form:

$$\sqrt{m/z} = Q + Pt$$

where:

the term P represents the resultant gain from the instrument geometry (pathlengths and voltages).

Q is an offset, arising from propagation delays through the electronics (detector rise time and delays of trigger signals through cables).

If a data file is acquired from the instrument with no calibration applied, then it is assumed that the offset is zero and the gain P is calculated from the instrument geometry.

## Nominal Mass Accuracy

The pathlengths should be set up to give at least nominal mass accuracy. Nominal mass measurement is achieved on the Q-Tof 2 by adjustment of the **Lteff** factor, a term which quantifies the difference between the indicated and actual mass.

A TOF spectrum of a standard compound is acquired with **Lteff** set to its default value of 1800.

A new value of **Lteff** can be calculated from the following relation:

$$L_{\text{teff}} = 1800 \sqrt{(m_{\text{ind}}) \div (m_{\text{act}})}$$

where  $m_{\text{ind}}$  = indicated m/z

and  $m_{\text{act}}$  = actual m/z

Enter this new value under **TDC Parameters**; all subsequent mass measurements will be nominally correct.

## Calibration

With no calibration applied, the spectral data in MassLynx is merely a set of mass intensity pairs {Mn, In} based upon instrument geometry.

The inherent relationship between mass and time shown above makes it prudent to generate higher order calibration coefficients that are applied to the square root of the nominal masses {Mn}:

$$\sqrt{m}_c = A + B\sqrt{m}_n + CM_n + DM_n^{3/2}$$

where:

the terms A, B, C, D.... are calculated by fitting a polynomial to the acquired mass spectral data.

Mc is the calibrated displayed mass.

*If a polynomial of order 1 is requested, the values for A & B are calculated, and the higher terms are set to zero.*

*For a polynomial of order 5 (the highest supported in MassLynx) there will be six terms generated.*

When calibrating over a large mass range (>500 Da) it is advisable to use a higher order polynomial, as the deviations from the straight line fit become more appreciable.

Once a calibration has been generated from a reference compound such as PEG it should be used as an 'instrument calibration' to be applied to all subsequently acquired data.

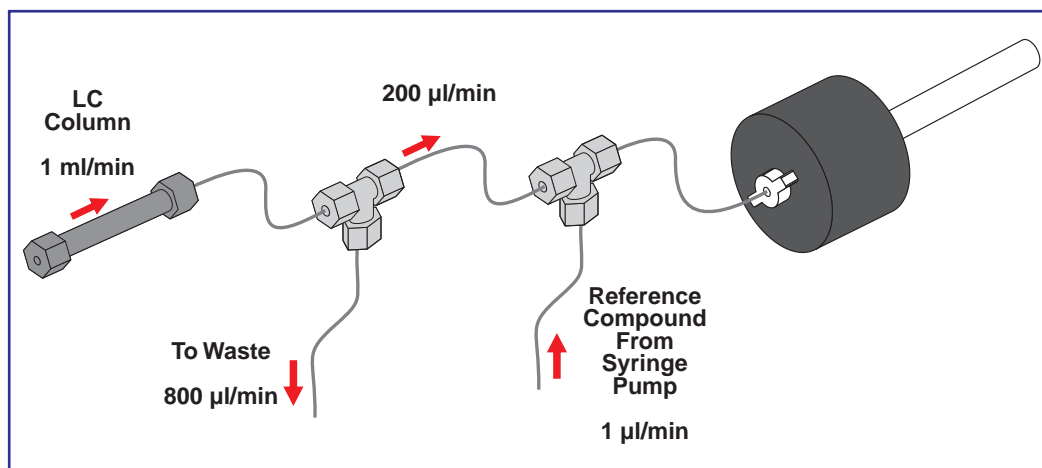
The procedure for this is described below.

## Lock Mass

Temperature variations in the environment and in the instrument power supplies can cause drifts in 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 stabilisation of the power supplies.

Users can compensate for instrument drift by applying a single point lock mass correction that recalculates the term B in the above equation.

The lock mass reference compound must be internal to the sample, and may be introduced by 'teeing in' post column on an LC system. The following diagram shows a typical arrangement:



## Dead Time 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 storing the arrival times of ions in a histogram 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 nanoseconds.

At high ion currents some of the ions generated are not registered, leading to a shift to lower mass centroids, with consequently lower measured areas on reported peaks.

However, the MassLynx software incorporates a correction facility which allows for accurate mass measurements to be achieved over a large range of ion currents, and the use of dead time correction is described below.

## Generation of an Instrument Calibration

Make up an analyte solution consisting of:

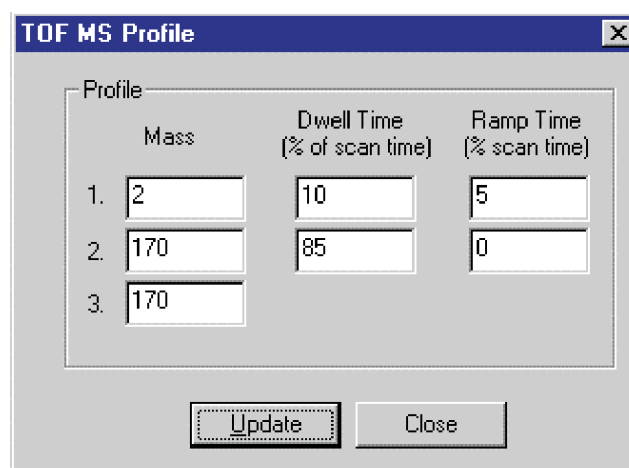
PEG 300 (10 nanolitres/millilitre),

PEG 600 (10 nanolitres/millilitre),

in a stock solution of 2mM ammonium acetate in 50/50 acetonitrile/water.

Dilute this by a factor of ten (with the stock solution) and introduce this to the instrument using a syringe pump operating at 5 microlitres per minute.

The TOF MS Profile should be set up as below. This will give coverage of masses from 150 to 1000 Da, albeit with some compromise to sensitivity at the higher masses.



The TOF MS Profile dialog box contains a table with three columns: Mass, Dwell Time (% of scan time), and Ramp Time (% scan time). It lists three profiles. Profile 1 has a mass of 2, dwell time of 10, and ramp time of 5. Profile 2 has a mass of 170, dwell time of 85, and ramp time of 0. Profile 3 has a mass of 170, dwell time of 85, and ramp time of 0. At the bottom are 'Update' and 'Close' buttons.

	Mass	Dwell Time (% of scan time)	Ramp Time (% scan time)
1.	2	10	5
2.	170	85	0
3.	170	85	0

On the tune page, choose **RF Settings** then set **Offsets** of 0.5 and **Gains** of zero.

A **Cone** voltage of 35 volts will give a good distribution of PEG peaks.

Acquire data for one minute over the range 100 to 1000 Da, with a scan time of 1 second. Combine at least 30 scans of data, and check that the signal is not too intense - center the data using the Spectrum Center menu as set up below:

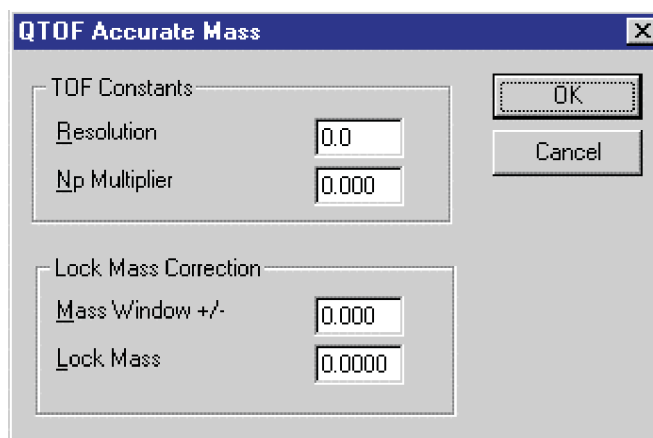


The QTOF Spectrum Center dialog box has two main sections. The 'Center method' section includes a text box for 'Min peak width at half height (channels)' set to 5, and three radio buttons: 'Top', 'Centroid top (%)' (selected), and 'Median'. The 'Centroid top (%)' is set to 80.00. The 'Centered spectrum' section has a checked 'Create centered spectrum' checkbox, and four radio buttons: 'Add' (selected), 'Heights', 'Areas', 'New window', and 'Replace'. On the right are 'OK', 'Cancel', and 'QTOF...' buttons.

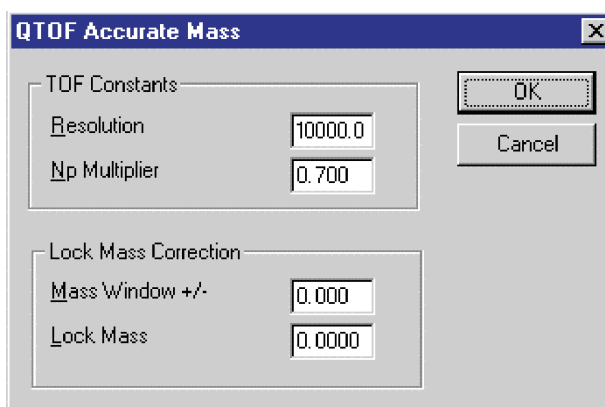
The data acquired should not be above 500 counts/sec or deadtime distortion will occur.



Check that no deadtime correction is employed at this stage (**Resolution** = 0 and **Np Multiplier** = 0).



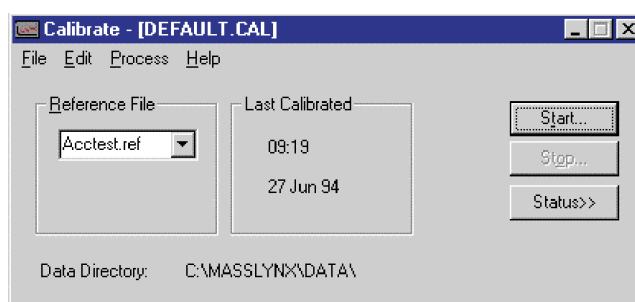
Re-center the data using **Resolution** = 10000 and **Np Multiplier** = 0.7 as below:



Check that any differences in the centroided masses of the two centred spectra are less than 1 mDa.

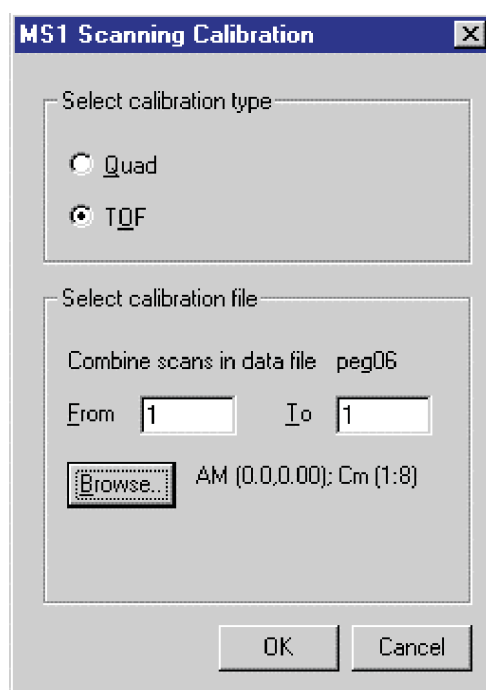
If this is not the case then repeat the acquisition with less intense peaks, either by sample dilution or by de-tuning of the electrospray needle.

Once a satisfactory centered spectrum has been acquired, save it in the spectrum history. Go to the **Control Panel** menu, select **Instrument**, then **Calibrate**, to obtain the following box:

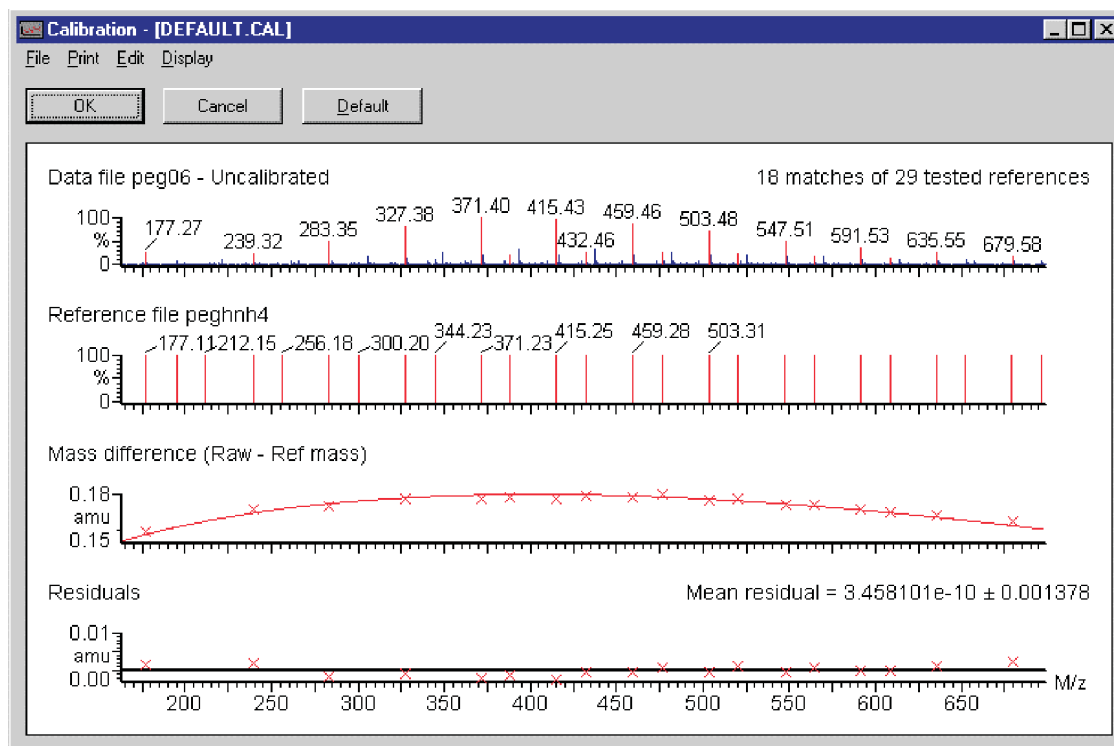


Select the reference file to use (pehnh4.ref will give the correct masses with the sample prepared as described above).

From **Process** select **Calibration from file**:



Use the **Browse** option to select the centred spectrum which has been previously saved. In the above example box it is from a data file peg06. Once the spectrum has been selected click on **OK**:

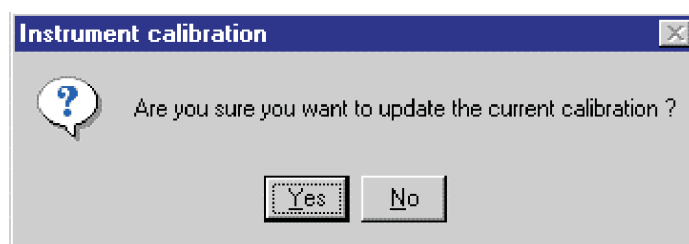


Make sure that the residual errors are all less than 3 mDa. The calibration parameters can be altered by clicking on **Edit** to reveal the box below:

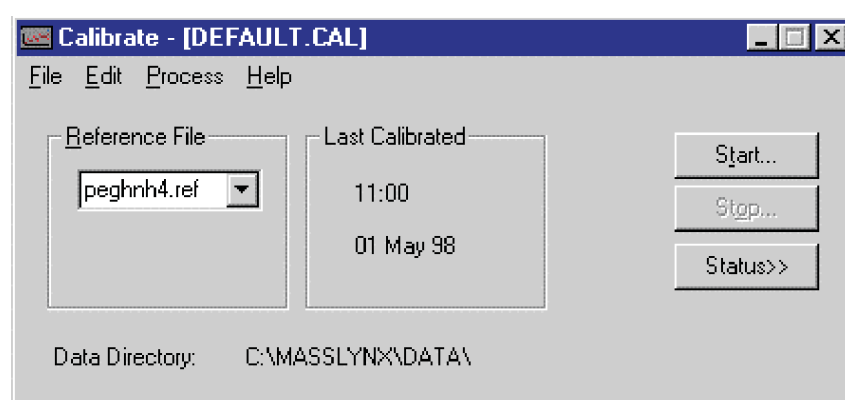
The figure shows the 'Calibration Parameters' dialog box. It has a title bar 'Calibration Parameters' and two buttons on the right: 'OK' and 'Cancel'.


- Peak Match section:**
  - ☒ Perform auto peak matching
  - Peak window (Da) +/-:
  - Initial error (Da):
  - Intensity threshold:
- Curve Fit section:**
  - Polynomial order:
  - ☐ Intensity weighting
- Display section:**
  - ☐ Calibrate display

A fifth order polynomial has been chosen here, as a large mass range is being covered by the calibration. After these parameters have been set, exit the calibration window. The following prompt appears:



Choose **Yes**, and the calibration will be updated with the time of update displayed in the box as shown below:

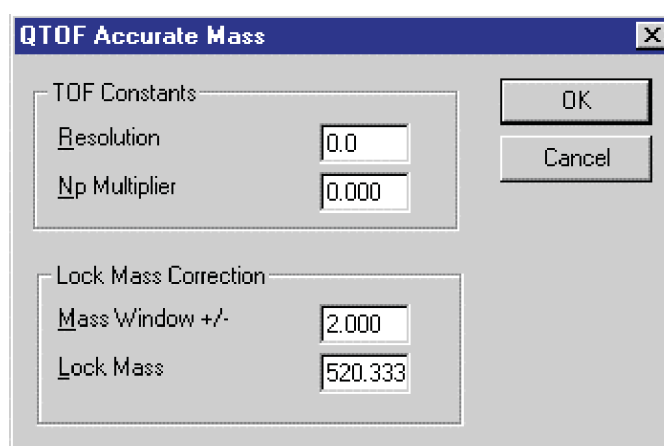


Click  to dismiss this box, and all subsequent acquisitions will now be tagged with the newly generated calibration.

## Lock Mass Correction

The application of a single point lock mass correction will now correct for subsequent instrument drift and bring masses back to within 5 ppm RMS, on the condition that there is no isobaric chemical interference with either lock mass or analyte peaks.

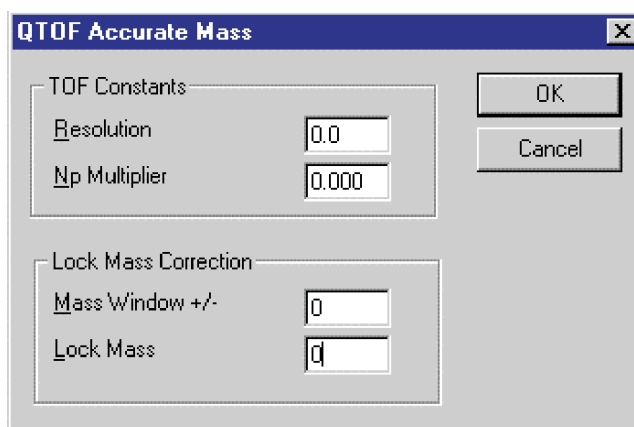
The lock mass is found under the TOF Spectrum Center menu when **QTOF** is clicked:



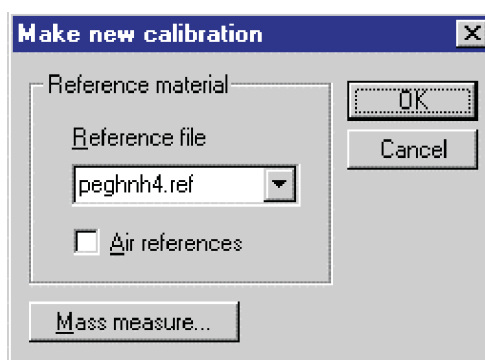
Centering the spectrum with these parameters will force the peak at 520 to be 520.333 exactly and recalibrate the entire mass spectrum.

## Deadtime Correction

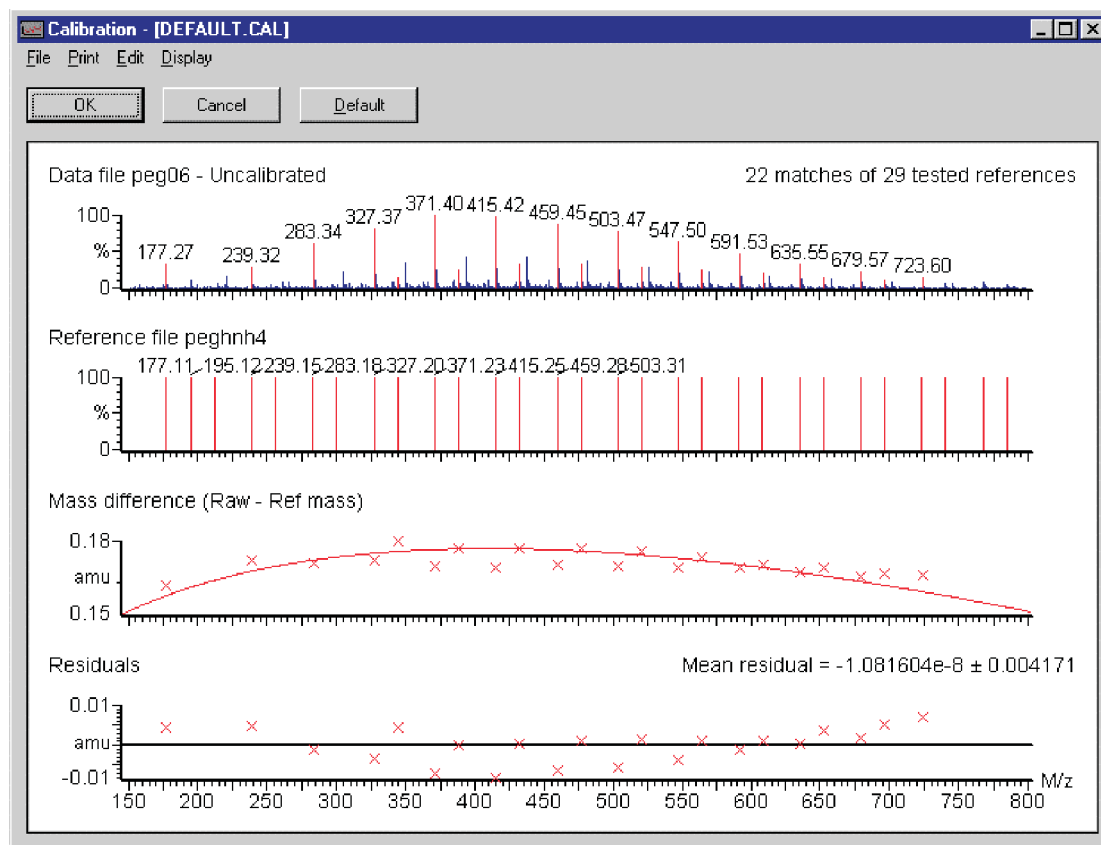
Acquire a spectrum of PEG at the higher concentration of 10 nl/ml and create a centered spectrum with no lock mass and no correction. (All parameters set to zero.)



Now go to **Tools, Make calibration** and choose the same pegnh4 reference file:



Click on **OK** to reveal the residual errors, whilst making sure that a polynomial of order1 has been selected. The deadtime distortion is revealed as the observed difference between the high and low intensity peaks:



Do **not** click on **OK** now, as this would erase the instrument calibration.

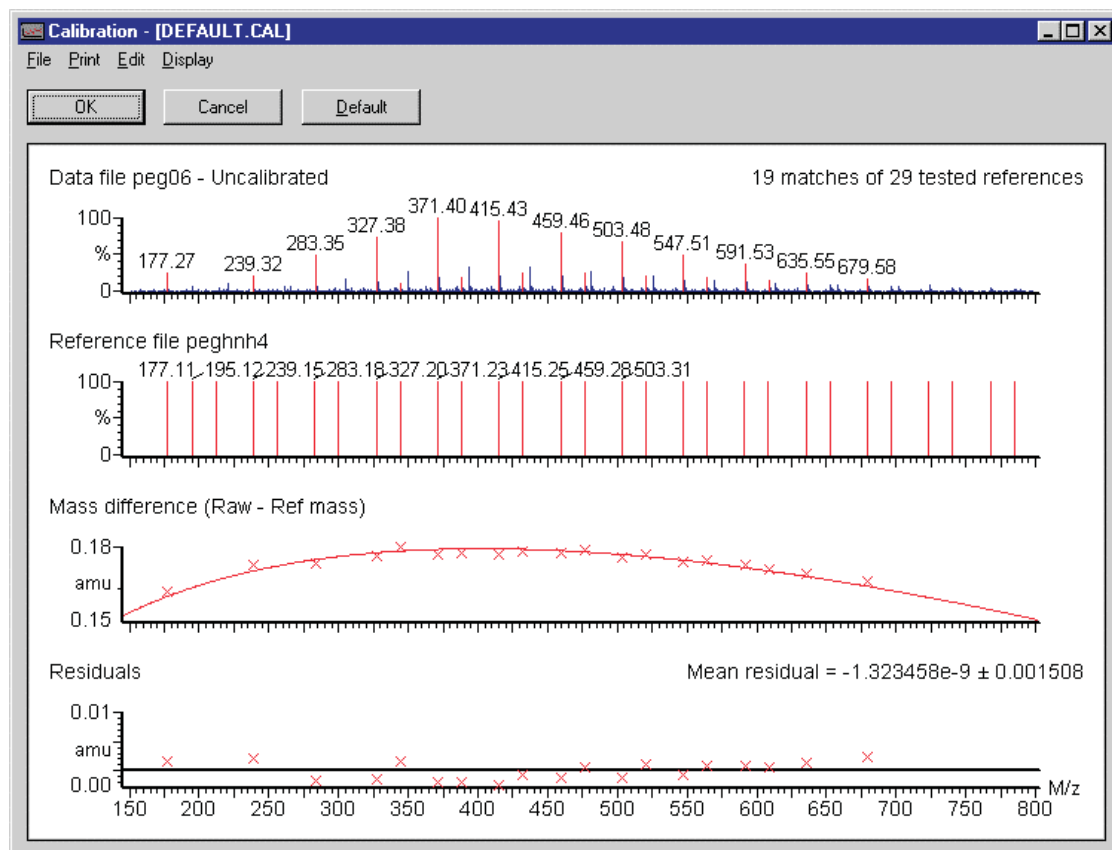
*This menu is being used only to set up the deadtime correction, and **not** to create a new calibration.*

Click on **Cancel**, and re-center the data using a **Resolution** of 5000 and **Np Multiplier** of 1.

*There should now be a reduction in the mass deviation observed between adjacent peaks of high and low intensity.*

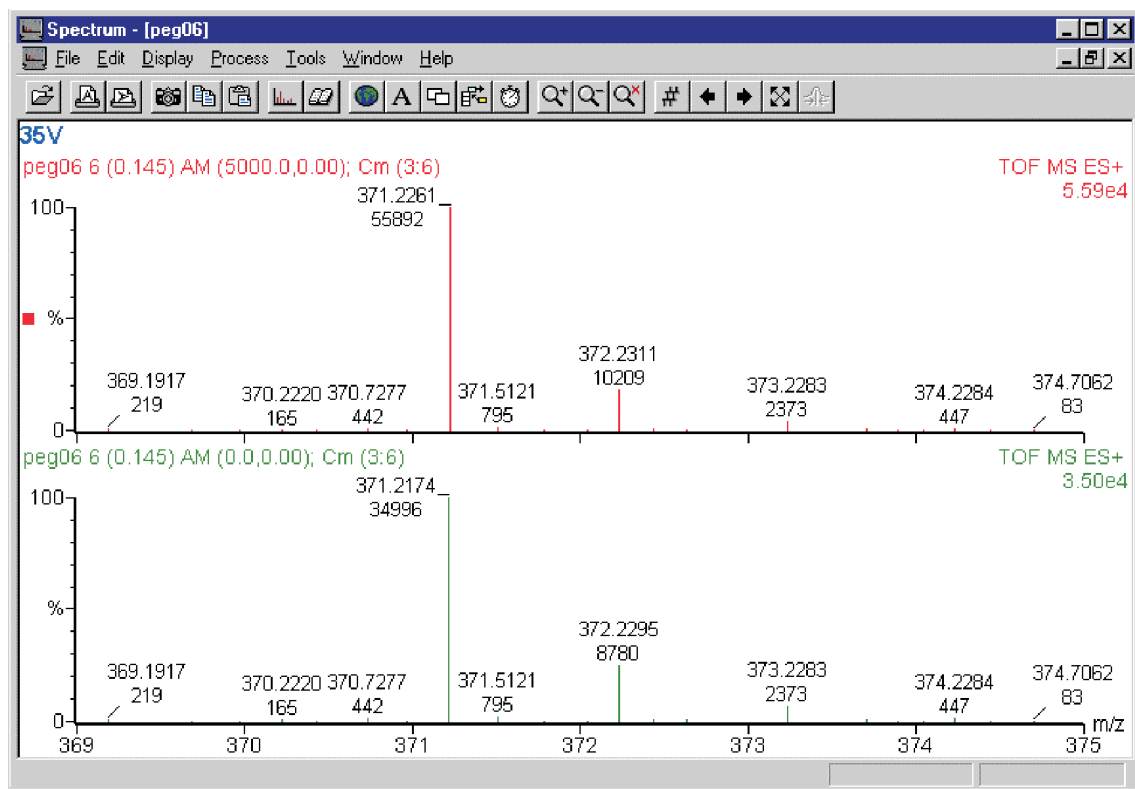
Repeat this process at subsequently lower values of Np until the deviation is minimised.

*At this point the peaks should lie close to the axis - the line of best fit:*



Typical values for **Np Multiplier** will be about 0.7, and the **Resolution** used should be as measured on the instrument at mass 500.

Now that the deadtime correction has been set up, the difference in reported areas and masses between corrected and uncorrected centered data can be seen. In the example below, the 371 peak has been shifted by 8.7 mDa:



The model successfully corrects for deviations of up to about 15 mDa at mass 500, at which point the limits of the model are reached, with no correction applied at higher ion current.

Users should familiarise themselves with the ion current range over which successful mass measurements can be made. Once the **Resolution** and **Np Multiplier** figures have been evaluated they can be left active in the menu without affecting centered data in any adverse way.

The procedure involved using the **Spectrum, Make Calibration** commands is a post-processing calibration, producing a data file of a particular format and with the extension **.cal**.

The file resulting from the commands **Instrument Calibrate, Save Calibration** has the same file extension **.cal**, but it should be noted that because it is an instrument calibration file, it is of an incompatible format to that described above.

Folders and naming conventions should be appropriately assigned.

Information concerning the calibration of Q-Tof 2 is provided in the *MassLynx NT Guide to Data Acquisition*.



## Exact Mass Measurement: Additional Hints

- Best results are obtained if the lock mass gives an intensity of approximately 100 to 200 counts per second as shown on the real time tune display.
- The lock mass should be chosen to be at the upper end of the mass range used.
- Do not change transfer lens voltages without re-calibrating the instrument.

*Changing **Cone** will not change the calibration.*

- Always be aware of possible chemical interference problems, either on the sample or the lock mass peak.
- When performing a base calibration (using PEG, for example) better results may be obtained by using a stronger solution and moving the probe off axis to limit the ion current, rather than using a lower concentration.

*This has the effect of minimising any possible chemical interference from background ions.*

- Always check the stability of the spray (see the instructions for the setting of the probe tip in *Maintenance and Fault Finding*).

*Short term variations in the spray produce fluctuations in the number of ions per peak per pusher pulse, giving rise to errors in the deadtime correction calculations. The number of ions per peak per pusher pulse is calculated from the ion current integrated over a period of time thus only giving an average value.*

- To obtain the best deadtime correction, only combine scans of a similar intensity, either at the top of a chromatographic peak or in the tail of a chromatographic peak.

*This ensures the number of ions per peak per push is calculated correctly.*

- The deadtime correction algorithm can only correct for ion intensities and  $m/z$  shifts up to a limit. If the ion current approaches approximately 10,000 counts per peak (without correction) in one second then the limits of the model are being reached.

*If the limits of the model are exceeded no correction is applied - the same result will be obtained by centring the data with **Resolution** and **Np Multiplier** set to zero.*

- If the limits of the deadtime correction algorithm are exceeded it may be possible to use the  $C^{13}$  isotope instead.
- For the best mass accuracy when mass measuring doubly charged ions, it is advisable to use a doubly charged lock mass peak.

- To obtain the true number of ions per peak, areas must be selected on the peak centre menu.
- The standard deviation in the determination of the mass (strictly  $m/z$ ) centroid of a triangular-shaped peak ( $\sigma_{\text{ppm}}$ ) due to ion statistics alone is given by the equation below.

*A triangle is assumed to be a close enough approximation to the shape of the mass spectrometer peak for the equation to be valid.*

$$\sigma_{\text{ppm}} = 10^6 \Delta M / M(24N_p)^{0.5} \text{ ppm}$$

where:

$\Delta M$  is the width ( $m/z$ ) of a triangular peak across the base.

$M$  is the  $m/z$  value of the peak.

$N_p$  is the number of ions per peak.

Using the above equation we can calculate the number of ions per peak required to give a standard deviation of 5 ppm when measuring a peak at 500  $m/z$ .

Assuming 10000 (FWHM) resolution, so  $\Delta M = 0.1 m/z$  (width at base = twice width at half height), then:

$$N_p = [5 \times 10^{-6} \times 500 \div 0.1 \times (24^{0.5})]^{-2} = 67 \text{ ions per peak.}$$

Thus standard deviations of less than 5 ppm cannot be expected unless the number of ions per peak is greater than 267.

# Parent Ion Scanning

## Introduction

Identification of the components of a mixture sharing a common structural motif by precursor scanning MS/MS is a well-recognised analytical strategy. For example, phosphopeptides may be selectively detected in protein digest mixtures by producing precursor scans for  $m/z79^-$  (SA Carr, MJ Huddleston and RS Annan, *Anal. Biochem.*, 1996, 239, 180-192.) and similarly glycopeptides from scans of  $m/z204^+$  and  $366^+$  (SA Carr, MJ Huddleston and MF Bean, *Protein Science*, 1993, 2, 183-196.).

More recently it has been used, to good effect, to locate the peptide protonated molecular ions from the nano-electrospray analysis of low level protein digests in a chemical background present at a high level (M. Wilm, G. Neubauer and M. Mann, *Anal. Chem.*, 1996, 68, 527-533.). In this case precursor scans of  $m/z86$ , the immonium ion from commonly occurring Leu/Ile, were used.

On a triple quadrupole instrument MS2 is set to transmit the mass of the chosen product ion for which the precursors are sought and MS1 is scanned over the range of masses for which precursors are sought. Any mass transmitted by MS1 which decomposes in the gas cell to form the mass being transmitted by MS2 is recorded as a precursor and it follows from this that each precursor scan must be a separate experiment.

In the Q-ToF 2 mass spectrometer MS2 is a time of flight mass spectrometer. In this configuration since MS2 is no longer mass selective, as MS1 is scanned (or stepped) over a range of masses, full product spectra can be recorded for any species decomposing in the gas cell.

In this mode MS2 acquires product mass spectra over a selected mass range (e.g. from  $m/z50$  - 2000). MS1 is then stepped over the mass range for which precursors are sought (e.g. from  $m/z300$  - 1500) in steps of 1Da interval. The data set that is produced may be used to construct precursor scans, or constant neutral loss scans, for any mass within the scan range of MS1 and MS2, with the advantage that multiple precursor scans may be simultaneously displayed from the data obtained in a single experiment.

If only a single mass is used for the construction of the precursor scan then the sensitivity is, in theory, substantially (about 1/50) lower for Q-ToF 2 relative to the triple quadrupole. However, by using several to many ions to construct the scan this can be recovered to a large extent. Under some circumstances the full product spectrum generated in this mode is sufficient for structure interpretation, however, at the lowest sample levels it may be necessary to return to the appropriate precursor and acquire more data in product mode to improve the quality of the product spectrum.

When the number of components in the sample is small then it may be more efficient to break the experiment into several steps. By surveying the mass scale in large (e.g. 20Da) intervals and then flagging interesting segments for further study at, say 5Da and then 1 Da intervals the experiment time can be significantly reduced.

The parent scanning facility is automatically available in MassLynx 3.4, in previous versions it may need to be unlocked.

## Unlocking the Parent Scan Facility

In this case the MassLynx.ini file must be modified to unlock the parent facility.

Close down all MassLynx windows including the tune page and exit MassLynx.

In the MassLynx root directory find the **MassLynx.ini** file and double click it to open it up in Notepad.

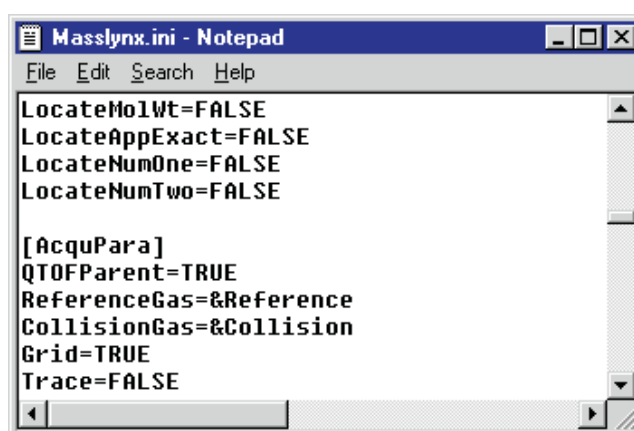
Use the **Find** facility (under **Search** in the top banner) to move to the top of the section headed **[AcquPara]**. Beneath this entry add as the next line the following:

```
QTOFParent=TRUE
```

Save the file and exit.

When a user logs into MassLynx, the MassLynx.ini file is used to create a specific ini file for that user and, therefore, there will also be a file named user.ini in the MassLynx root directory. (Often users log into MassLynx as Administrators, in which case the user.ini file will be called Administrator.ini). For the parent scan to be unlocked, this user.ini file will need either to be deleted (so that it is re-created at login), or the same changes will need to be made as in the MassLynx.ini file.

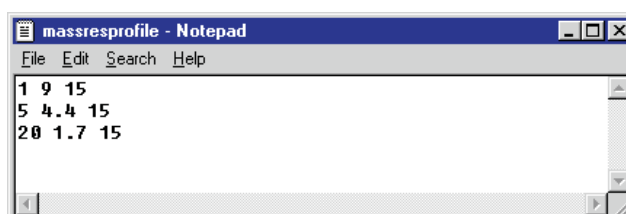
**NB** If neither of these are done the parent facility will not be unlocked.



This is an example of how the MassLynx.ini file appears after successful modification.

## Setting up the Mass Transmission of the Quadrupole

In the multi step mode of acquisition (see next section) the data is acquired using quadrupole mass transmission windows of differing widths. The widths of these windows are controlled using the information in the **massresprofile** file in the MassLynx root directory. This file should have been created during the instrument installation.

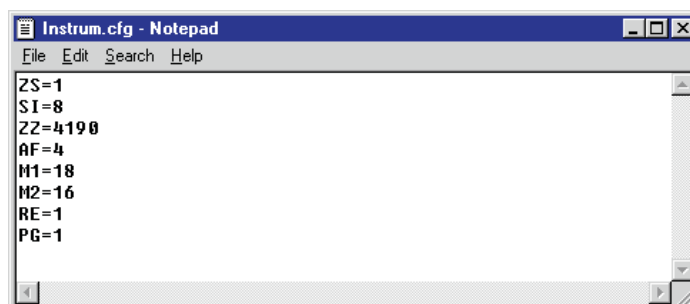


The left most number is the transmission window of the quadrupole followed by the **LM** and **HM Resolution** settings.

**NB** It is important that these numbers are not separated by commas or any character other than a space, and that there is not a carriage return following the final number.

If this file does not exist, it must be created. The transmission at various resolution settings can be easily determined by using the interactive acquisition mode. A section of the mass spectrum containing only background is most useful, and the collision energy should be set to that required for MS mode (4eV for Q-Tof and 10eV for Q-Tof 2). The **HM** setting should be the same for all transmission windows as shown in the example above. Determine three sets of conditions giving a transmission of 20, 5 and 1Da.

To allow the multi step method to operate requires the appropriate hardware. This is standard on later instruments, but may be retrofitted to earlier models. If it has been fitted then the instrument configuration file should have the line RE=1 as shown in the example. To test if in doubt, if the hardware is in place and optimised the transmission on MS1 in the MS/MS mode with the HM and LM sliders set to 0 should be about 30 – 35Da. If this transmission window cannot be set to greater than 20Da then the instrument cannot be operated in the multi step mode without modification.



## Setting up a Scan Function for Precursor Scanning

There are two modes of data acquisition:

### (i) Multi Step Mode

The multi step mode is of most use when there are only a small number of components in the mixture. For example, proteins are usually phosphorylated at only one or two sites. A digest of a phosphorylated protein will consequently contain only one or two phospho peptides. If precursor scanning is being used to find these phospho peptides, by looking in the negative ion MS/MS mode for the characteristic  $m/z$  78.9<sup>-</sup> signal for  $PO_3^-$ , then only one or two masses in the spectrum will produce this fragment.

In this case the data can be acquired in a survey mode to try to home in to the regions of the precursor spectrum containing this characteristic signal in as short a time as possible. To do this the data can be acquired in a three tier hierarchical fashion using wide (20Da) windows to rapidly survey the mass spectrum to find the areas of interest. Any of these survey windows which contain the signal of interest can be re-evaluated using 5Da windows. As a final step the 5Da windows are re-evaluated using 1Da windows to locate the precursor to within +/- 0.5Da.

### (ii) Single Step Mode

Single step mode is most useful when there are large numbers of components and there would, consequently, be no great advantage in using the multi step approach. In the single step mode, the experiment is set-up to step the quad over a given mass range in steps of constant width, typically 1Da, and acquire the MS/MS spectrum at each step.

## Multi Step mode

After the MassLynx.ini file has been modified, log into MassLynx and open up the Scan Function editor.

This is accessed either by opening up the MS File in the Sample List window, or by selecting **Set Up Scan** which is under the **Acquire** option in the top banner of the Acquisition Control Panel (see Control Panel under the Run command in the Sample List window).

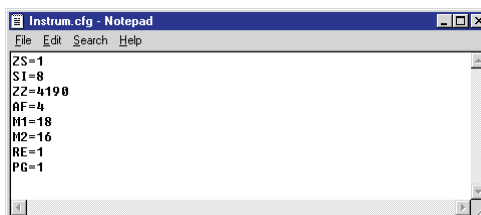
The Scan Functions window should now appear.

In the top banner select **File** and **New** to open up a new Scan Function file. Click on the **Parents** button to add a Parents function to the editor.

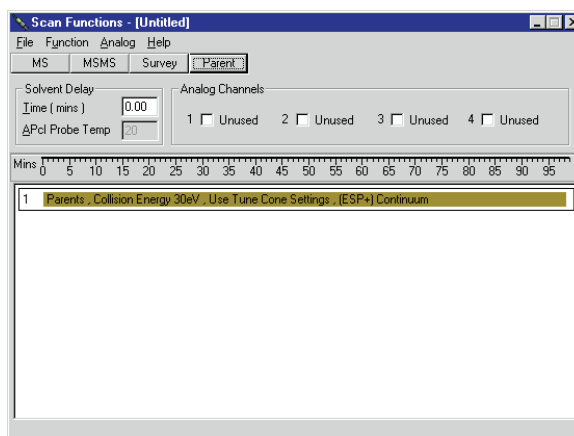
In the Function 1 Parent Scan window that appears, modify the parameters accordingly. In the example shown here, function 1 will produce a precursor scan using 20Da windows and cover the mass range  $m/z$  400 – 1000.

**NB** The mass range that is set must be an integer multiple of the step width otherwise acquisition will not proceed. In this case if the mass range were set to be  $m/z$  390 – 1000, this will not divide exactly into an integer number of 20Da windows and the experiment will not run.

The **Collision Energy** is set to 30eV and the **Cone Voltage** is the same as that in the tune page. The **Spectrum Integration Period** is 0.9sec with 0.1sec **Inter-Spectrum Period** to transfer the data to the host PC. The experiment will cycle for up to 99 minutes unless terminated interactively.



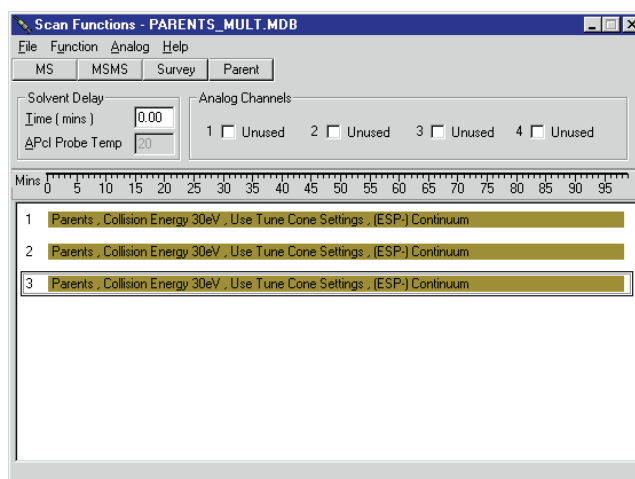
When **OK** is selected this function will be added to the Scan Function window as shown below:



A second function is added by selecting the **Parent** button and the Function 2 Parent Scan window appears. All parameters in this window should be set to be the same as in function 1 except the **Step Mass**, which should be set to 5Da.

The third function is added in the same way and the step mass parameter is set to 1Da. Thus the final experiment should appear as shown below. There are three functions that differ only in the Step Mass parameters. All other aspects of the three functions are the same.

**NB** The step mass in function 1 must be an integer multiple of the step mass in function 2 which must be an integer multiple of the step mass in function 3. For example the windows may be set to 20Da, 5Da and 1Da as shown. Failure to observe this requirement will prevent the experiment from proceeding and no data will be acquired.

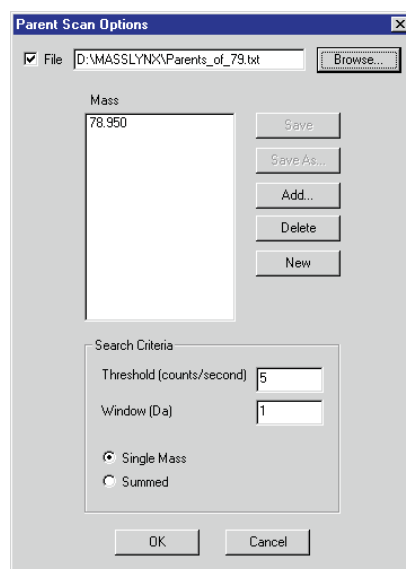


Before the experiment can be performed a file containing the required product ion, for which the precursors are sought (e.g.  $\text{PO}_3^-$ ,  $m/z$  78.9) must be created.

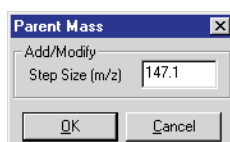
## Parent Scan Options

In the Scan Function window select the **Function** tab in the top banner and then select **Parent Scan Options** to open up the Parent Scan Options window.

If a file containing target product ions for which the precursors are being sought has already been created then it can be found using the browse facility.



If not, or if a different set of products is to be specified, then a new file can be created using the **New** and **Add** facilities.





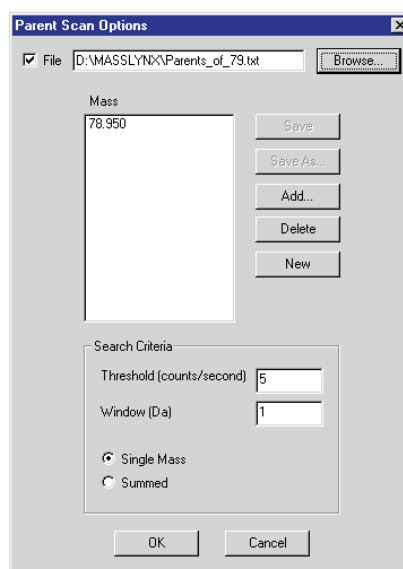
Selecting **New** will clear the Parent Scan Options window and **Add** will select the Parent Mass window.

Enter the mass of the product for which precursors are sought and select **OK** to add to the list in the Parent Scan Options window.

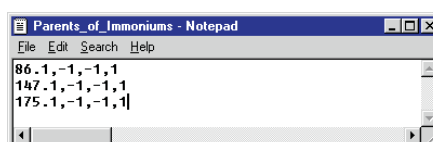
When the list is complete, a value for the threshold above which the precursor must appear for a window to be flagged for the next stage and a mass tolerance within which the precursor must appear should be entered.

In single mass mode if any of the masses is detected above the threshold then that segment will be flagged. In summed mode the intensities of all the ions are added together and if the sum is above the threshold then that segment will be flagged.

Select **OK** and as a final step save the experiment using the **File Save As** command in the top banner.



An alternative way to create the file is to use Notepad and make a text file using the format shown.



The cursor must be at the end of the last text line (i.e. the last character in the file must not be a carriage return). If precursors are being sought for more than a single product ion then a new line is added for each using the same format as shown. It might be useful to create a Folder in the MassLynx root directory (e.g. Parents) in which to place these files.

## Single Step Mode

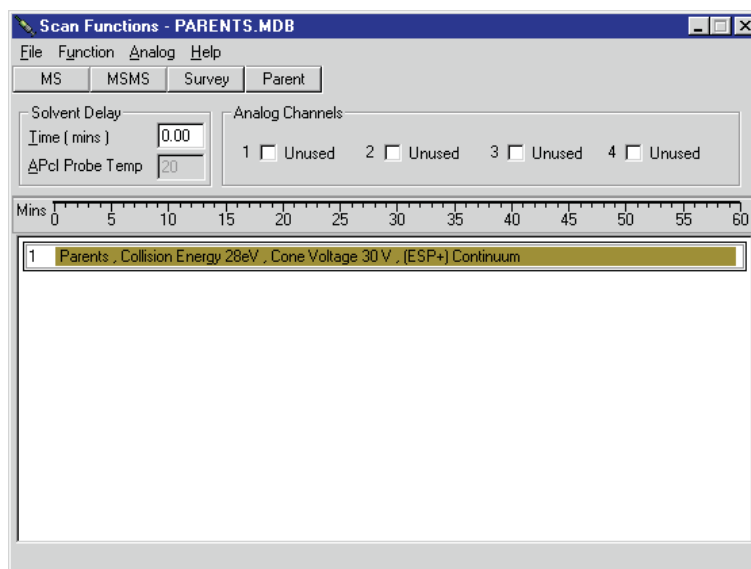
After the MassLynx.ini file has been modified, log into MassLynx and open up the Scan Function editor.

This is accessed either by opening up the **MS File** in the Sample List window, or by selecting Set Up Scan which is under the Acquire option in the top banner of the Acquisition Control Panel (see Control Panel under the Run command in the Sample List window).

This Scan Functions window should now appear:

In the top banner select **File** and **New** to open up a new Scan Function file. Click on the **Parents** button to add a Parents function to the editor.

In the Function 1 Parent Scan window that appears modify the parameters accordingly.



In this example MS1 would begin at  $m/z$ 300 and step in 1Da intervals up to  $m/z$ 1000. The spectrum integration time for each step would be 0.9 sec and each step would take 1.0 sec (i.e. 0.9 sec + 0.1 sec). 700 steps at 1 sec/step = 11.66 minutes so the retention time is set to 11.7 min to terminate acquisition at the end of the cycle. (If the retention time is set to a longer period, the cycle will begin again at  $m/z$ 300 and continue until the retention time is reached.)

The collision energy would be fixed and is set here to 28V. (Collision energy ramping will be available in a future version of MassLynx to allow the collision energy to be increased as MS1 moves to higher  $m/z$ .)

At the completion of this experiment the data file should contain 700 scans of MS/MS data.

Function : 1 Parent Scan

Mass (m/z)

Step Mass: 1

Start: 300

End: 1000.0

Method

Ionization Mode: ESP

Data: Continuum

Repeats: 1

Scans/Spectrum: 0

Retention Window (mins)

Start Time: 0.00

End Time: 99.00

Scan Duration (secs)

Scan Time: 1.00

Inter-Scan Delay: 0.10

Collision Energy

☐ Use Tune Page Settings

Collision Energy: 28

☐ Use Collision Energy Ramp

OE Ramp...

Cone Voltage

☒ Use Tune Page Settings

Cone Voltage: 30

APol Probe

☒ Use Tune Page Settings

Probe Temp: 20

OK Cancel

## Acquiring the Data

**NB** The data in the multi step mode must be acquired from the Sample List. If the experiment is launched from the Acquisition Control Panel it will not proceed and no data will be acquired.

Data may be acquired in single step mode by launching the experiment either from the Acquisition Control Panel or from the Sample List.

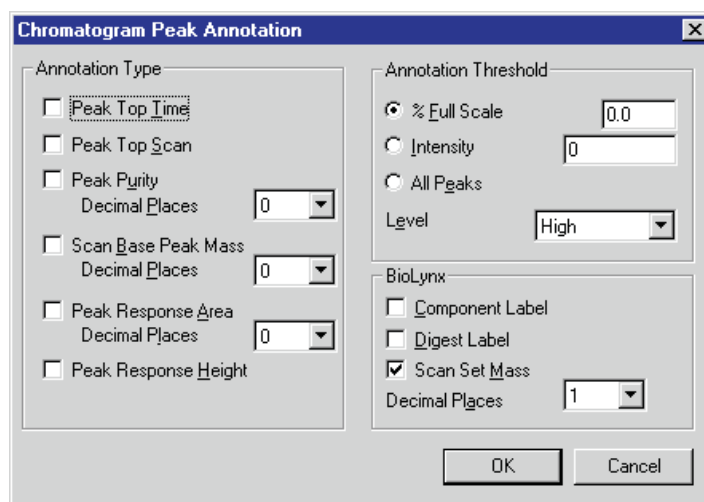
The intensity of these low mass fragments will be enhanced if the RF levels on the gas cell and transport lens are reduced from the normal values (0.3 – 0.5) to about 0.1 for the precursor scanning experiment.

## Displaying the Data

### The Precursor Scan

The precursor scan data is viewed in the chromatogram window, since a precursor scan is really a display of the intensity of a mass (or number of masses) displayed as a function of the mass transmitted by MS1.

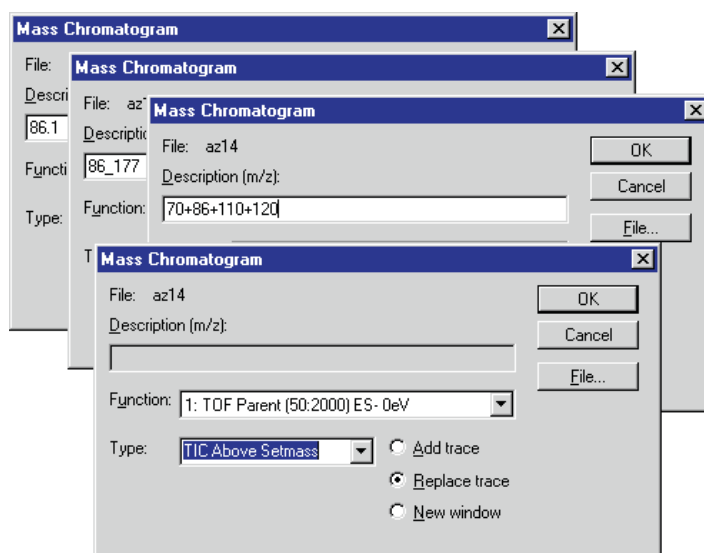
In chromatogram set the **Peak Annotation Type** to **Scan Set Mass** so that the peaks will be annotated, at the top, with the mass being transmitted by MS1.

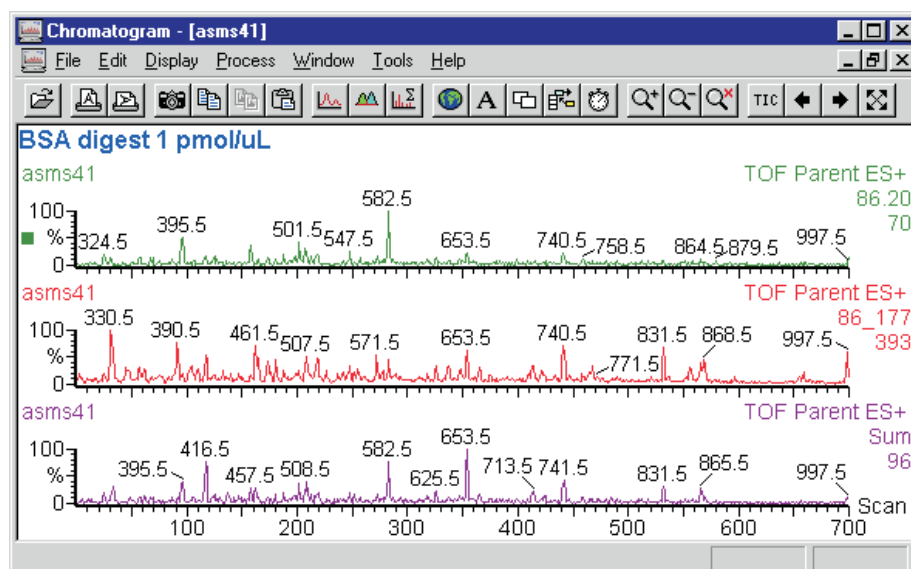


If the axis is also set to Scan then in this example scan 100 is equivalent to  $m/z$ 400, scan 200 equivalent to  $m/z$ 500 and so on. (X-axis annotation with mass rather than Scan or Time will be available in a later version of MassLynx).

The TIC display is of little value since this also includes any undissociated precursor ion coming through the gas cell.

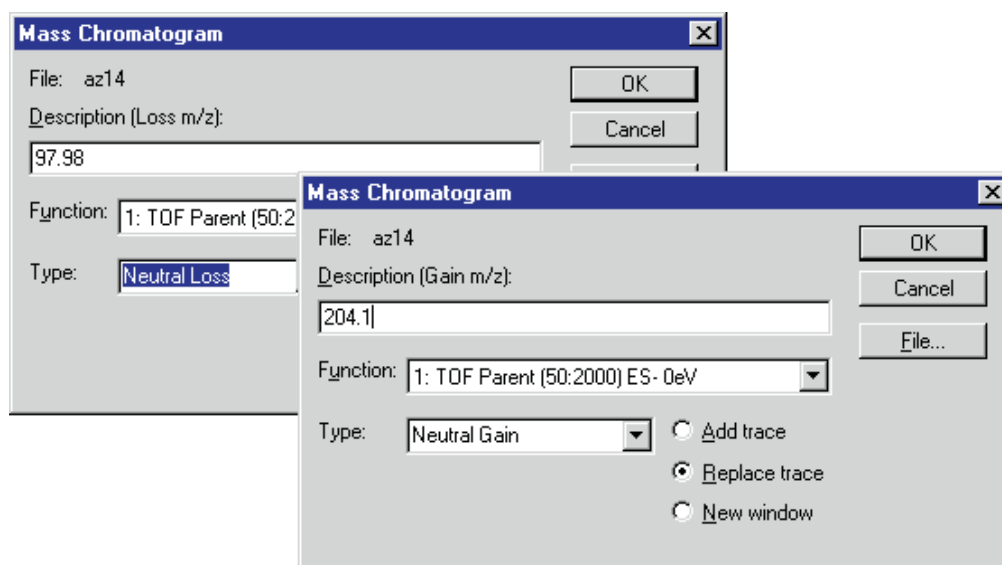
Use the **Mass Chromatogram** facility under **Display** in the top banner of the Chromatogram window to display either a single mass (e.g. 86) or range of masses (e.g. 86\_177) or a sum of masses (e.g. 70+86+110+120) or the total ion current above the parent mass (TIC Above Setmass) as shown below:





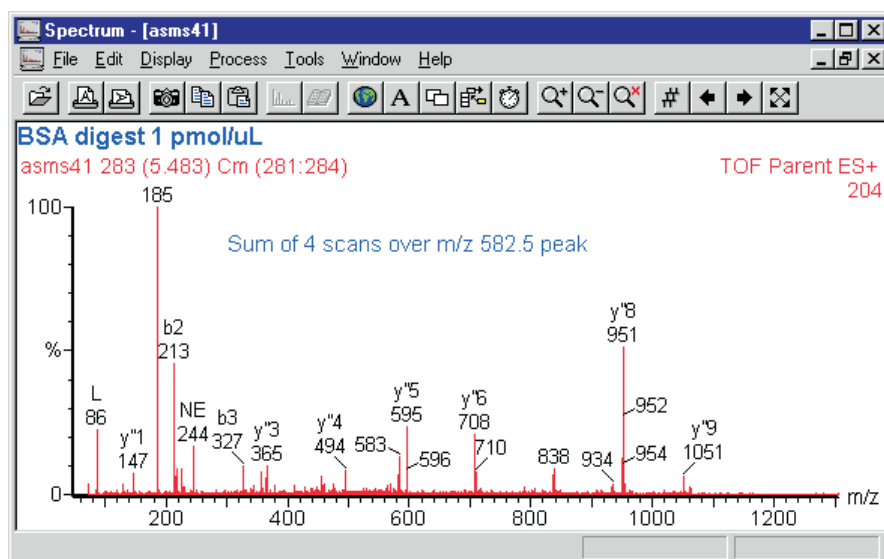
## Constant Neutral Loss or Gain Scans

Data acquired using the single step method can also be used to display constant neutral loss or constant neutral gain data.



## The Product Scan

The product scan data is displayed in the Spectrum window by using the pointer and right mouse button (in the usual way) to select a single scan or several scans over the range of interest. In the example shown below, four scans over the  $m/z$  582.5 peak have been summed together and annotated with the BioLynx package.

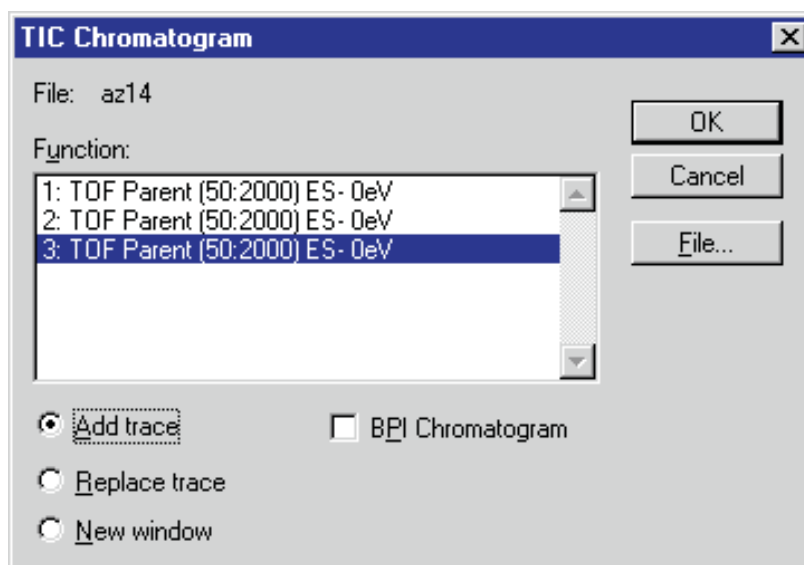


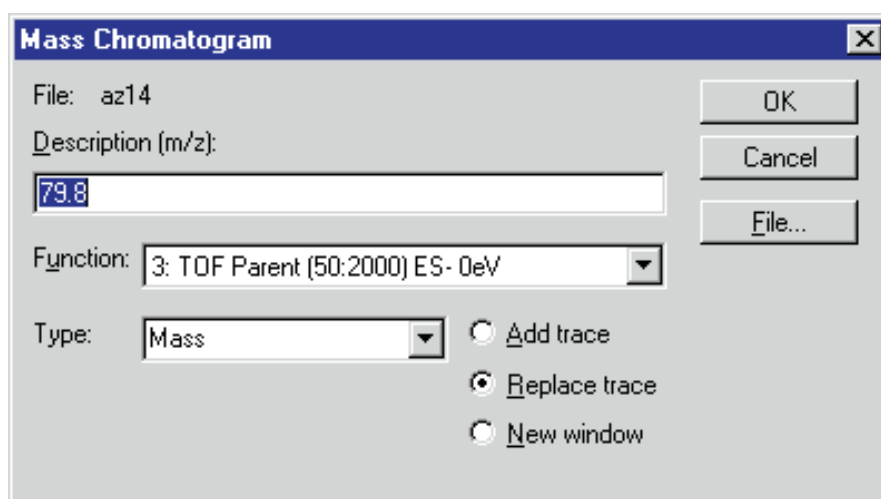
At low levels where precursor scanning of this type would be most valuable, data of this quality is unlikely to be produced with only a few seconds acquisition time for a precursor ion and a product spectrum would need to be acquired for longer with a separate experiment.

## The Precursor Scan Multi Step Mode

In multi step mode the display of the precursor scan data is slightly more complex since there is a data set for each of the different window widths used in the experiment.

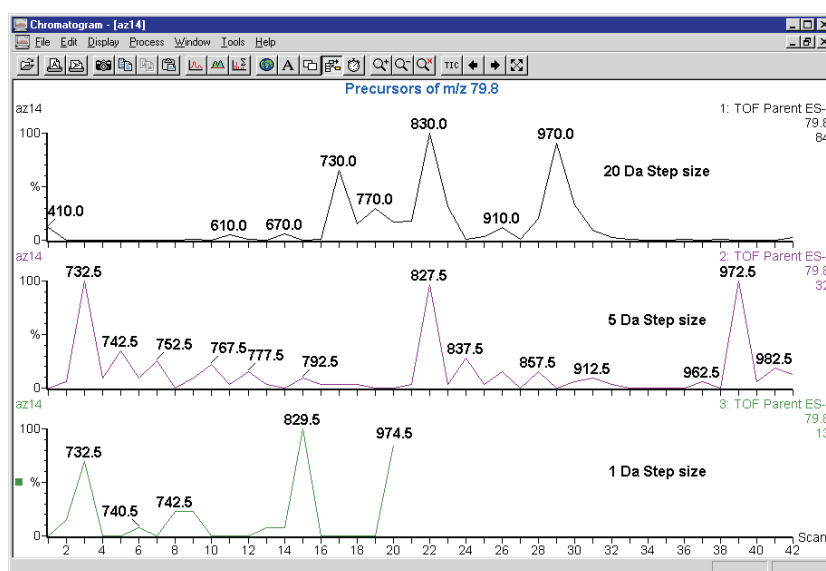
In the Chromatogram top banner select the **TIC option** under **Display** to open up the TIC Chromatogram window.





From this window display all the functions using add mode. Then using the **Mass Chromatogram** facility under **Display** in the chromatogram top banner replace each trace with the mass(es) used in the experiment.

This should give a picture similar to the example shown where a precursor scan of  $m/z$  79.8 was carried out on a protein digest containing some phosphopeptides. In this example the phosphopeptides produced  $(M-2H)^{2-}$  ions at  $m/z$  828.9 and  $m/z$  974.5. The parent at  $m/z$  733 produced a product peak at  $m/z$  79.8, but was not in this case phosphorylated.







# Maintenance and Fault Finding

## Introduction

Cleanliness and care are of the utmost importance whenever internal assemblies are removed 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 fibres in sensitive areas.
- ✗ Avoid touching sensitive parts with fingers.
- ✗ Do not use rubber gloves.
- ✓ Before reassembling and replacing dismantled components, inspect O rings and other vacuum seals for damage. Replace with new 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 of all to ensure that this part has been correctly refitted and / or adjusted and that adjacent components have not been inadvertently disturbed.



**Warning:** Many of the procedures described in this chapter involve the removal of possibly toxic contaminating deposits using flammable or caustic agents. Personnel performing these operations should be aware of the inherent risks, and should take the necessary precautions.

## Removal and Replacement of Outer Panels



**Warning:** There are high voltages and hot surfaces present throughout the mass spectrometer. The instrument outer panels must not be removed unless the instrument has been electrically isolated at the power outlet.



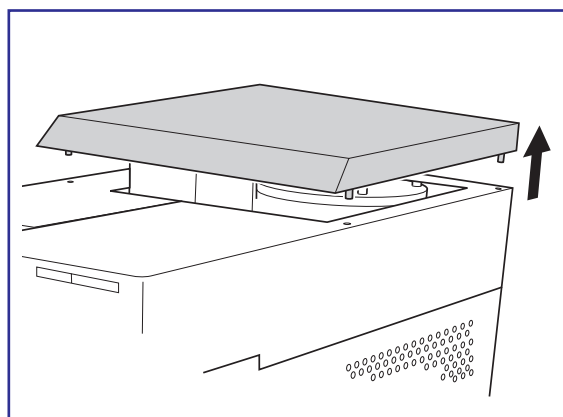
**Caution:** Ensure that each panel is adequately supported during removal. Damage to internal electronic components could result if the panel is allowed to drop.

To remove or replace the instrument panels for maintenance purposes, follow in order the procedures described below.

### Panel 1

Panel 1 covers the top of the TOF analyser MS2 and the associated housing containing the pusher electronics. Remove by lifting vertically.

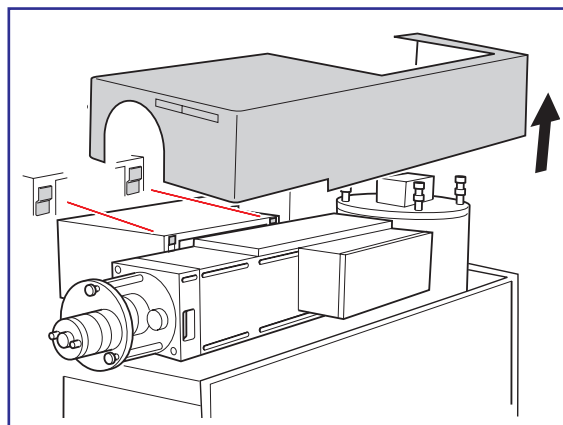
To replace, lower the panel into position, aligning the attached pins with the corresponding holes in the panels beneath.



### Panel 2

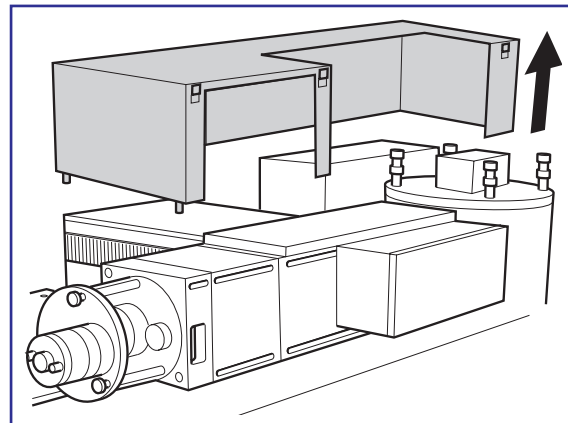
Panel 2 encloses the quadrupole analyser MS1, with cut-outs at the front for the source and at the rear for the TOF analyser MS2. This panel is also removed by lifting vertically.

It must be lowered into position with care, and engaged with each of the locating tabs indicated.



### Panel 3

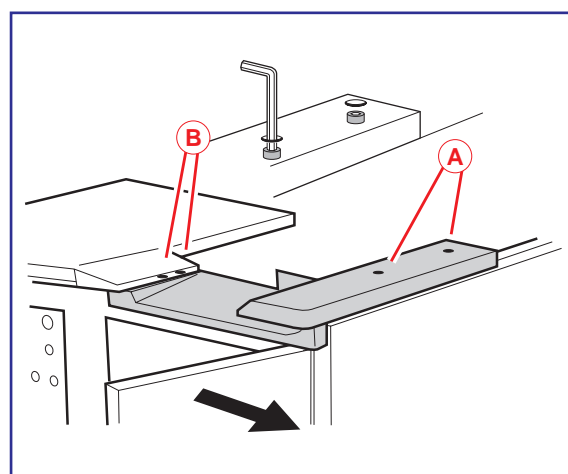
Panel 3 covers the RF generator for the quadrupole analyser MS1. It is raised and lowered vertically in the same way as panel 1, with lateral location similarly provided by six pins.



### Panel 4

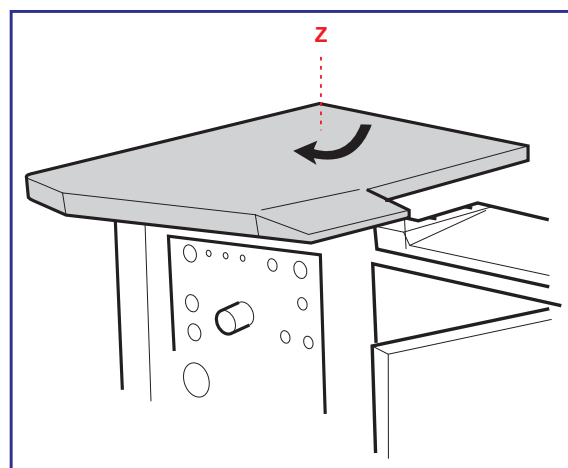
Panel 4 is attached by four 3mm Allen screws. Those marked A in the diagram must be removed completely.

Those marked B need only be loosened. The panel must then be tilted slightly, to enable it to be pulled clear of the chassis rail.



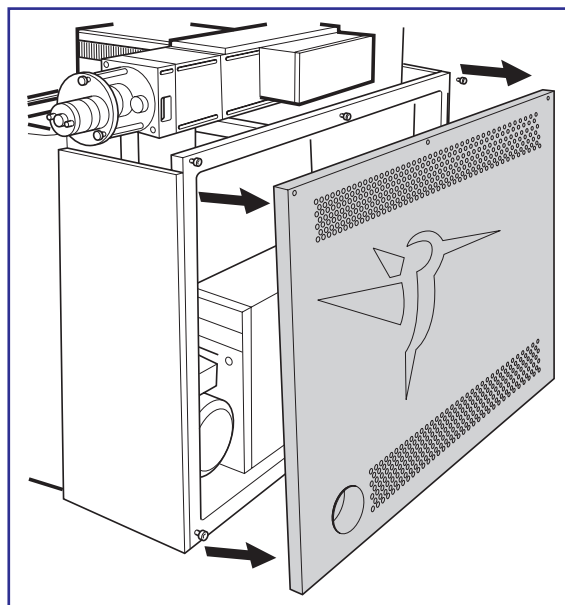
### Panel 5

Panel 5 is above the front operator control panel. In addition to the Allen screws securing this panel to panel 4, two concealed screws engage with slotted holes in the chassis. Once panel 4 has been removed, panel 5 may be rotated about the vertical axis z and then pulled forward.



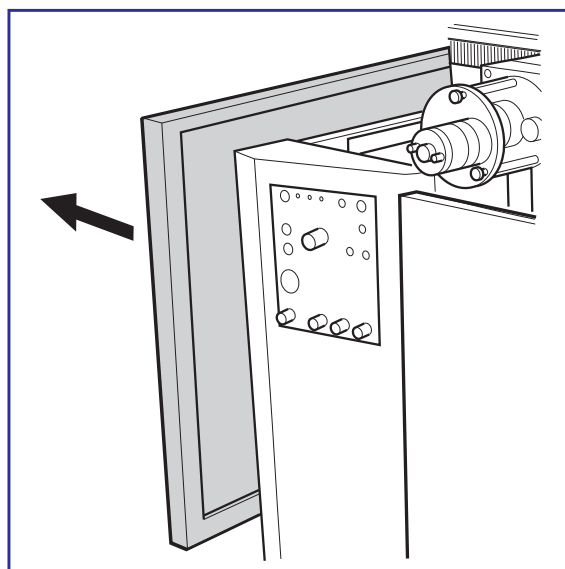
## Panel 6

Panel 6 covers the embedded PC, the rotary pump and the main body of the TOF analyser housing. It is held in position by six Allen screws, which must only be loosened for the panel to be lifted, clearing the locating slots.



## Panel 7

Panel 7 covers some of the electronics systems and the lower air filter tray. It is attached in exactly the same way as panel 6, and subsequently the same procedure should be followed for removal and replacement.



## Electronics Maintenance

Removal of the left side instrument cover gives access to the main electronics unit. On the top of this unit is a toggle switch, marked PUMPS. In normal operation this switch should be in the AUTO position.

For electronic maintenance and fault finding this switch may be set to the ON position which will allow the electronics circuit breaker to be switched off without venting the vacuum system.



**Caution:** Do not leave the instrument unattended with this switch in the ON position as a reduced level of vacuum protection is in operation.

Return the switch to the AUTO position at the earliest opportunity.

## Cooling Fans and Air Filters

Always ensure that none of the cooling fans is obstructed. It is essential that the fan filter is checked and cleaned at regular intervals, and replaced if there is any doubt about its effectiveness.

## The Vacuum System

The performance of the mass spectrometer will be severely impaired by the lack of a good vacuum in the ion transfer (hexapole) region or the analyser.

- An excessive analyser pressure results in a general loss in performance indicated by a loss of resolution and an increase in the background noise.
- As the vacuum deteriorates, the vacuum becomes insufficient to maintain the instrument in the operate mode.

Before suspecting a leak, the following points should be noted:

- The turbomolecular pumps will not operate if the rotary pump has failed.
- If the rotary pump is not maintained, the oil may become so contaminated that optimum pumping speed is no longer possible. Initially, gas ballasting may clean the oil. If the oil in the rotary pump has become discoloured, then it should be changed according to the pump manufacturer's maintenance manual.
- The turbomolecular pumps switch off if an over temperature is detected. This could be due to poor backing vacuum, failure of the water supply or a leak in the source or analyser.

## Vacuum Leaks

If a leak is suspected, the following basic points may help to locate it:

- Leaks very rarely develop on an instrument that has been fully operational. Suspect components that have recently been disturbed.

*Leaks on flanges can usually be cured by further tightening of the flange bolts or by replacing the seal.*

- All seals are made using O rings. When refitting flanges pay attention to the condition of O rings. Any that are cut or marked may cause a leak. The O rings should be clean and free from foreign matter.

*A hair across an O ring is sufficient to prevent the instrument pumping down.*

- Source components that operate at, or slightly above, atmospheric pressure are not susceptible to vacuum leaks.

In the unlikely event of a leak on a feedthrough, then the unit should be replaced or returned to Micromass for repair.

## Pirani Gauge

The Pirani gauge head does not require routine maintenance.

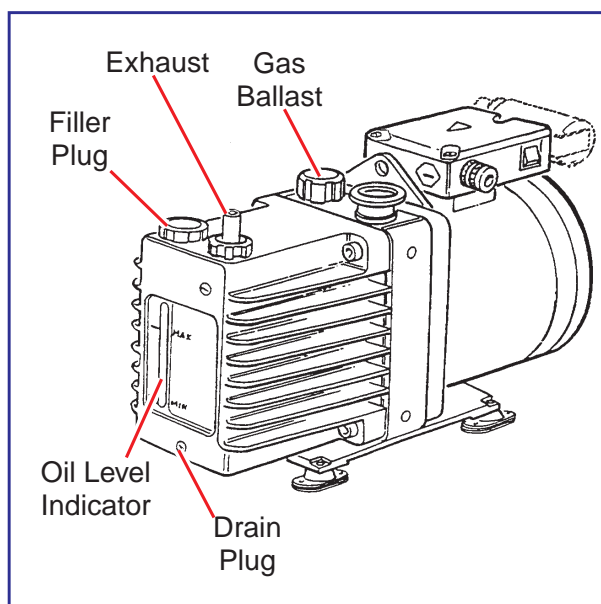
## Active Inverted Magnetron Gauge

In particular, the quadrupole analyser gauge requires regular maintenance. For information on cleaning the active inverted magnetron (Penning) gauge, refer to the Edwards literature supplied with the instrument.

## Gas Ballasting

Gas ballasting serves two important purposes:

- When rotary pumps are used to pump away solvent vapours, the solvent vapour can become dissolved in the pump oil causing an increase in backing line pressure. Gas ballasting is a method of purging the oil to remove dissolved contaminants.
- Oil mist expelled from the rotary pump exhaust is trapped in the oil mist filter. This oil is returned to the rotary pump during gas ballasting.



Gas ballasting should be performed routinely on a weekly basis for 30 minutes. If the source is used in the APcI or megafLOW electrospray modes, more frequent gas ballasting is recommended.

Gas ballasting is performed on the E2M28 pump by rotating the gas ballast valve 5 to 6 turns in a counterclockwise direction.

*It is normal for the rotary pump to make more noise when the gas ballast valve is open.*



**Caution:** Failure to gas ballast the rotary pump frequently leads to shortened oil lifetime which in turn may shorten rotary pump lifetime.

## Oil Mist Filter

The E2M28 rotary pump is fitted with an Edwards EMF20 oil mist filter which traps oil vapour from the rotary pump exhaust. The trapped oil is then returned to the rotary pump during routine gas ballasting. The oil mist filter contains two elements; the odour element need not be changed, but the mist element must be changed every time the rotary pump oil is changed.

- To change the element follow the instructions in the Edwards manual.

## Rotary Pump Oil

The oil in the rotary pump should be maintained at the correct level at all times. Check the oil level at weekly intervals, topping up if necessary.

It is important to monitor the condition of the oil regularly. Replace the oil when it has changed to a noticeable reddish brown colour, or routinely at 4 month intervals (3000 hours operation). At the same time, replace the oil mist filter's mist element (see above).

Change the oil in the rotary pump as follows:

Gas ballast lightly for 30 to 60 minutes.

Vent and shut down the instrument as described in *Routine Procedures*.

*It will be found easier to drain the oil while the pump is still warm.*

Drain the oil through the drain hole situated near the oil level sight glass.

Flush the pump, then replace the drain plug and refill the pump with the correct grade oil to the correct level.

Gas ballast lightly for 30 to 60 minutes.

For further servicing information refer to the manufacturer's manual.

## Foreline Trap

This is used to protect against the chance of pump oil backstreaming into the collision gas solenoid and/or the turbo pumps. The activated alumina should be changed according to the manufacturer's instructions.

## The Source

### Overview

The Z-spray source is a robust assembly requiring little maintenance. The source consists of three basic parts:

- The probe adjustment flange.
- The glass tube.
- The source flange assembly.

The probe adjustment flange and the glass tube can be readily removed, without venting the instrument, to gain access to the source block and sample cone. This allows the following operations to be performed:

- Wiping the sample cone.
- Removing the sample cone.
- Fitting or removing the APcI corona discharge pin.
- Fitting or removing the exhaust liner and cleanable baffle.
- Fitting or removing the nanoflow electrospray interface.
- Enabling or disabling the purge gas.

The sample cone may be cleaned in situ, by gentle wiping with a cotton swab or lint tissue soaked with 50:50 acetonitrile:water. More thorough cleaning of the sample cone may be achieved by removing it from the source. This may also be done without venting the instrument, by closing the isolation valve located on the ion block. Less frequently it may be necessary to clean the ion block, the extraction cone and the hexapole lens, in which case the instrument must be vented. This should only be done when the problem is not rectified by cleaning the sample cone or when charging effects are apparent.

*Charging is evidenced by a noticeable progressive drop in signal intensity, often resulting in a complete loss of signal. Switching the instrument out of and back into operate causes the beam momentarily to return.*

The hexapole transfer lens should not require frequent cleaning. If it is suspected that the lens does need cleaning it may be withdrawn from the front of the instrument after removing the ion block support.



**Warning:** Cleaning the various parts of the source requires the use of solvents and chemicals which may be flammable and hazardous to health. The user should take all necessary precautions.



## Cleaning the Sample Cone in Situ

This may be necessary due to lack of sensitivity or fluctuating peak intensity, or if deposited material is visible on the outside of the sample cone. Proceed as follows:

On the MassLynx top-level window, launch the tune page.

Deselect **Operate** to put the instrument in standby mode

Switch off the LC pumps.

Disconnect the liquid flow at the rear of the probe.

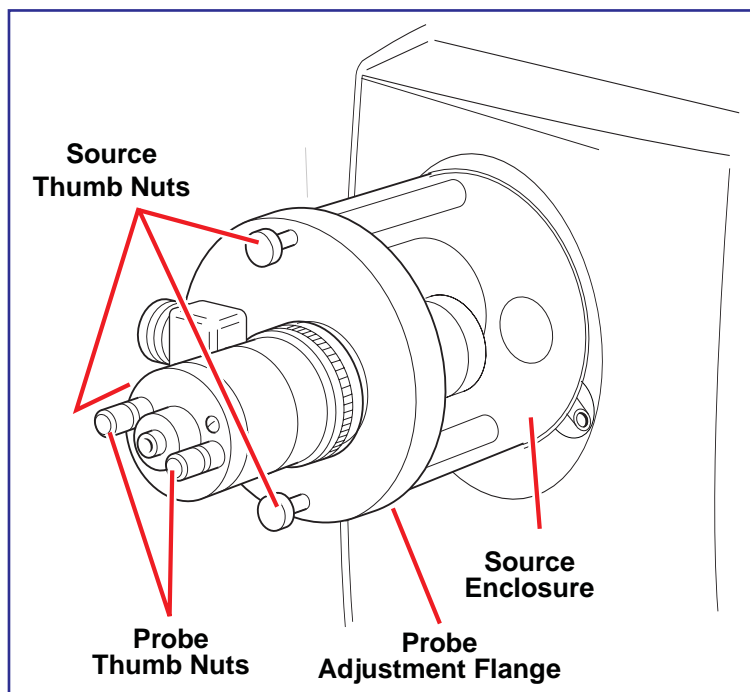
Set **Source Block Temp** and either **APCI Probe Temp** or **Desolvation Temp** to 20°C to switch off the heaters.



**Warning:** Removal of the APCI probe or desolvation nozzle when hot may cause burns.

**Caution:** Removal of the APCI probe when hot will shorten the probe heater's life.

*The cooling time will be significantly shortened if the API gases are left flowing.*



When **APCI Probe Temp** or **Desolvation Temp** has cooled below 100°C:

Deselect **API Gas** to switch off the nitrogen supply.

Disconnect both gas lines from the front panel by undoing the knurled nuts.

Disconnect both electrical connections by pulling back on the plug sleeves to release the plugs from the sockets on the front panel.

Undo the two knurled thumb nuts that retain the probe and withdraw it from the source. Place it carefully to one side.

Undo the three thumb screws and withdraw the probe adjustment flange and glass tube. Place the glass tube, end on, on a flat surface and place the probe adjustment flange on top of the glass tube.



**Warning:** When the source enclosure has been removed the source block is exposed. Ensure that the source block heater has cooled before proceeding.

If fitted, remove the APcI corona discharge pin.

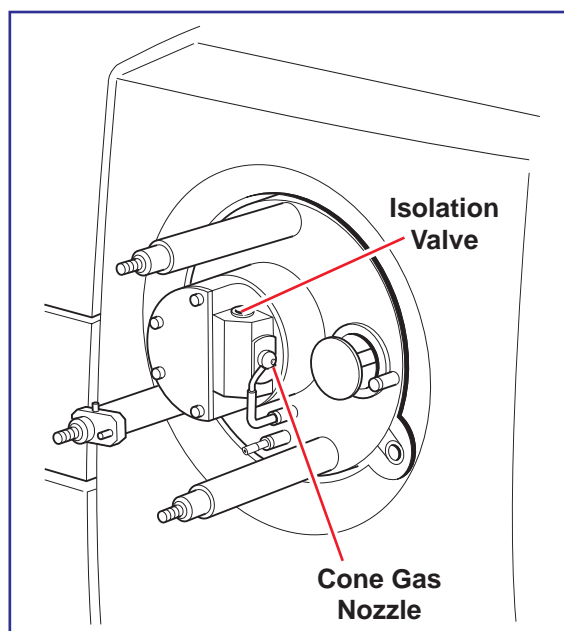
*The sample cone is now accessible.*

Using a suitable flat blade screwdriver rotate the isolation valve by 90° into its fully anticlockwise position.

*A small improvement in the analyser vacuum may be observed as a result of this operation.*

*The isolation valve is closed when the slot is perpendicular to the direction of flow.*

Carefully wipe the sample cone with a cotton swab or lint free tissue soaked in 50:50 acetonitrile:water or 50:50 methanol:water.



**Caution:** Do not attempt to remove any obstruction by poking. This may result in damage to the sample cone.

Dry the cone using nitrogen.

If the sample cone is still not clean, or if the aperture is partially blocked, proceed to the following section. Otherwise, when the cone is clean and dry:

Open the isolation valve.

Replace all removed components, following in reverse order the removal procedures.

## Removing and Cleaning the Sample Cone



**Caution:** The sample cone is a delicate and expensive component and should be handled with extreme care.

It is not necessary to vent the instrument to remove the sample cone. The source block incorporates an isolation valve for this purpose. To remove the sample cone proceed as follows:

Follow the procedure in the previous section, to gain access to the sample cone.

Using a suitable flat blade screwdriver rotate the valve by 90° into its fully anticlockwise position.

*A small improvement in the analyser vacuum may be observed as a result of this operation.*

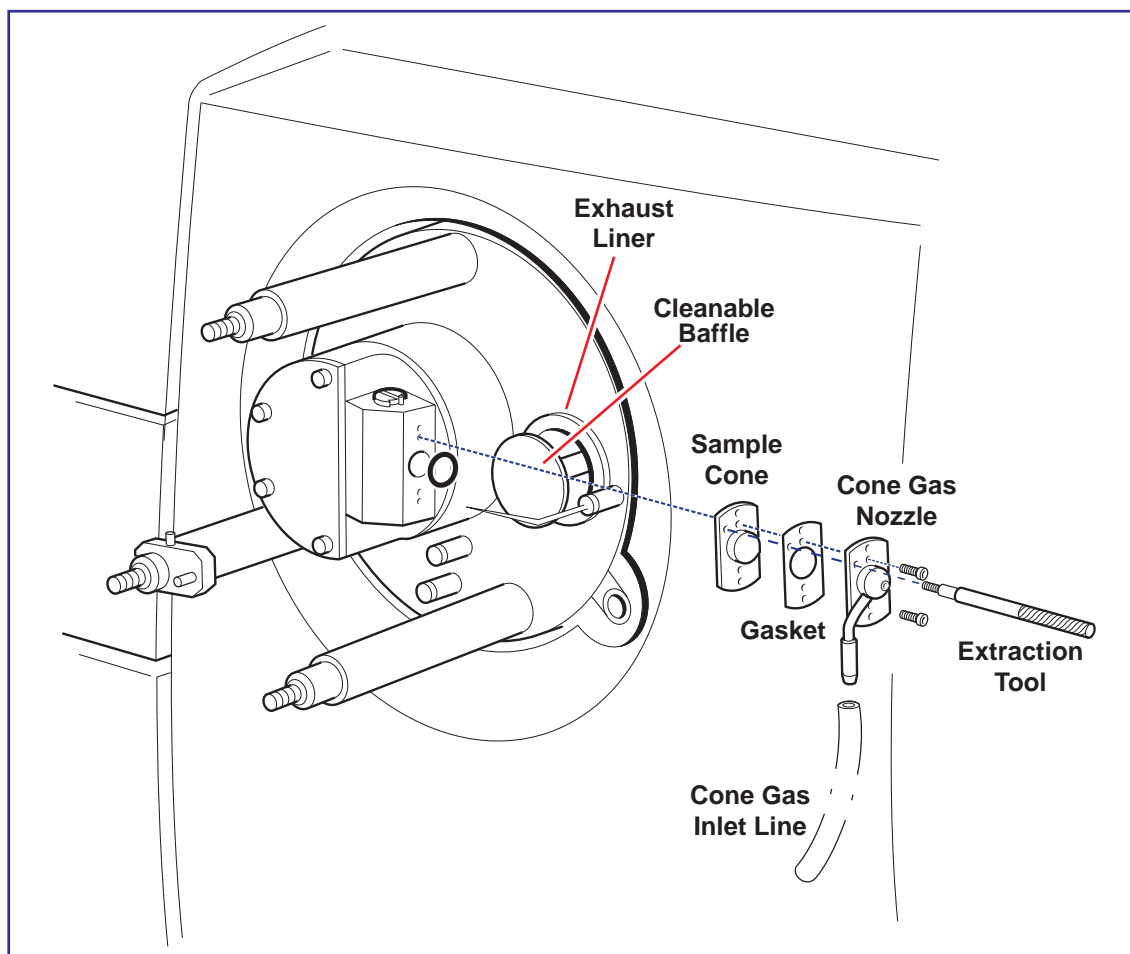
*The isolation valve is in the closed position when the slot is perpendicular to the direction of flow.*

Disconnect the cone gas inlet line (if fitted).

Take the sample cone extraction tool supplied in the source spares kit and screw it to the flange of the sample cone.

Remove the two sample cone retaining screws using a 1.5mm Allen key and withdraw the sample cone and cone gas nozzle (if fitted) from the ion block.

Remove the extraction tool, and separate the sample cone from the cone gas nozzle. Place both components in an ultrasonic bath containing 40:40:10 acetonitrile:water:formic acid or 40:40:10 methanol:water:formic acid. Rinse and sonicate with 50:50 acetonitrile:water or methanol:water.



Dry the cone and nozzle using nitrogen.

*To minimise down time fit a spare sample cone, obtainable from Micromass, at this stage.*

If material has built up on the exhaust liner and cleanable baffle:

Remove the cleanable baffle and the exhaust liner.



**Caution:** Do not attempt to remove the baffle without first removing the sample cone.

Clean these components, or obtain replacements.

Fit the cleaned (or the replacement) exhaust liner and cleanable baffle to the ion block.

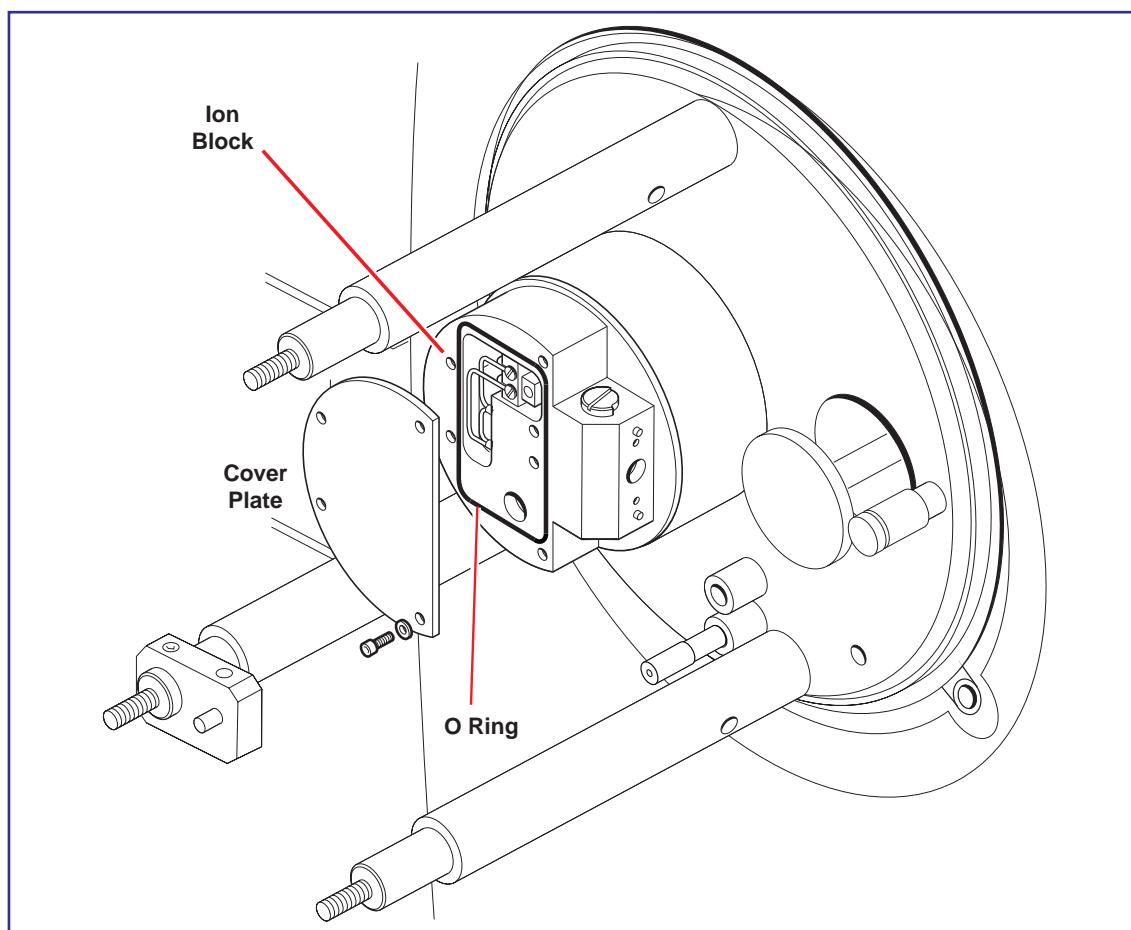
Refitting the sample cone is a reversal of the removal procedure.

## Removing and Cleaning the Source Block and Extraction Cone

On the tune page select **Other** from the menu bar at the top of the tune page. Click on **Vent**.

*The rotary pump and the turbomolecular pumps switch off. The turbomolecular pumps are allowed to run down to 50% speed after which a vent valve automatically admits dry nitrogen.*

Remove the source enclosure and the sample cone as described in the previous section.



When the instrument has vented:

Remove the two screws which secure the ion block and remove the ion block heater and the ion block.

Separate the extraction cone and the PTFE insulating ring from the ion block.

Remove the plug and the PTFE sealing washer.

Remove the sample cone as described above.

Leaving the valve stem in place, immerse the ion block in an ultrasonic bath containing 50:50 acetonitrile:water or 50:50 methanol:water, followed by 100% methanol.

Clean the sample cone and the extraction cone using in turn:

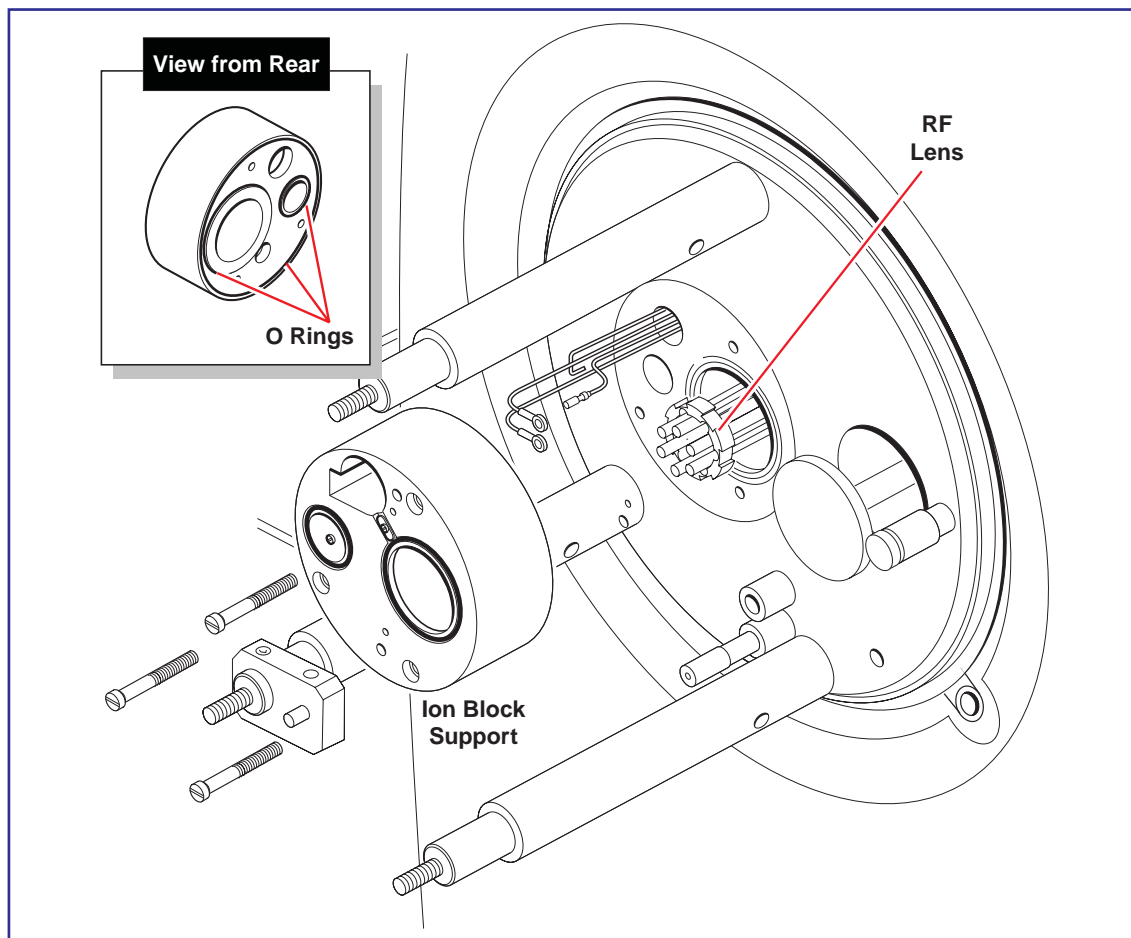
- concentrated formic acid.
- 50:50 acetonitrile:water or 50:50 methanol:water.
- 100% methanol.



**Warning:** Strong acid causes burns. Carry out this procedure in a fume cupboard using protective equipment.

Dry all components using a flow of nitrogen, or place them in a warm oven.

## Removing and Cleaning the RF Lens Assembly



To remove the RF hexapole transfer lens assembly, proceed as follows:

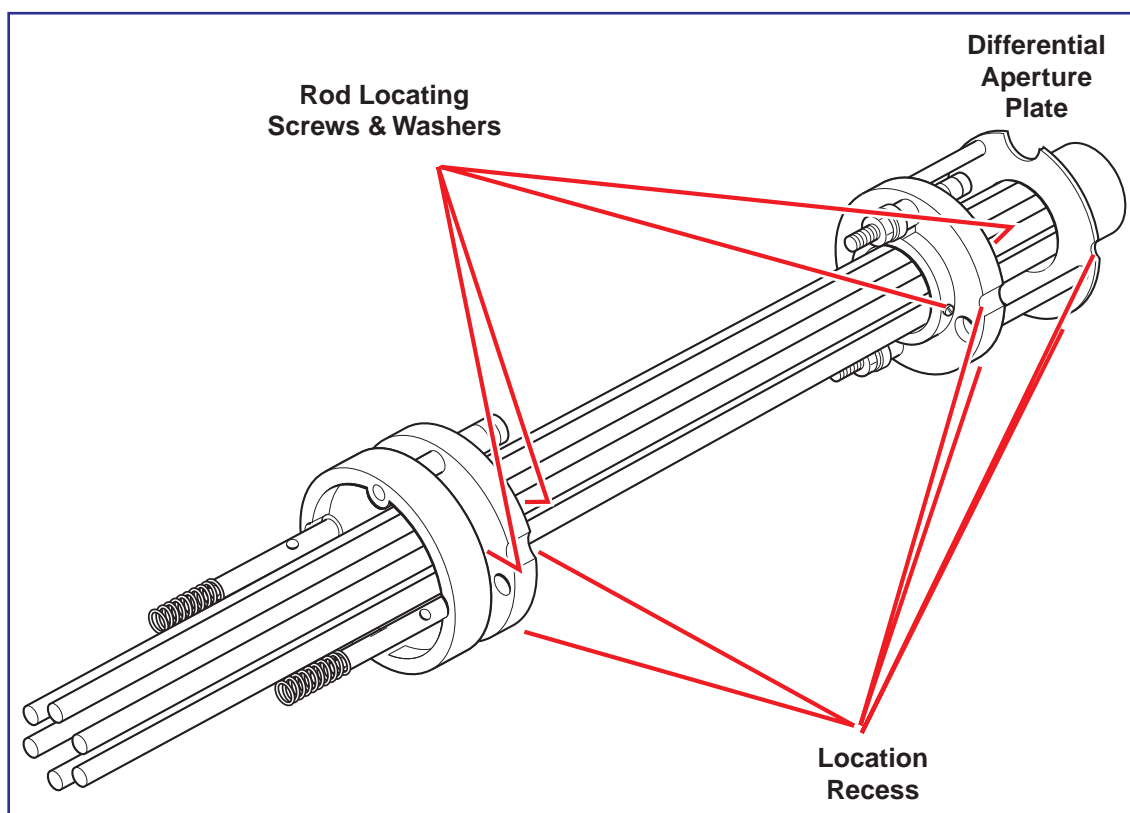
Remove the ion block, as described above.

Remove the three screws retaining the ion block support and carefully withdraw it, together with the support liner and O rings, from the pumping block.

Using a lint free tissue to gently grasp the hexapole, carefully withdraw it.



**Caution:** Take care not to scratch the internal bore of the pumping block as the hexapole lens assembly is withdrawn.



To clean the hexapole transfer lens proceed as follows:

Immerse the complete assembly in a suitable solvent (100% methanol) and sonicate in an ultrasonic bath.

Thoroughly dry the assembly using a flow of nitrogen.

In severe cases:

Remove, clean, dry and replace each rod separately (one at a time).

Reassemble the assembly with extreme care, checking the assembly against the diagram.



## Reassembling and Checking the Source

Feed the hexapole transfer lens into the instrument, allowing the recesses in the differential aperture plate to locate onto the two support rails within the analyser assembly. Ensure that the assembly is pushed fully in.

Check the condition of the O rings on the ion block support. Replace them if necessary.

Replace the ion block support, pushing it in against the springs of the hexapole assembly.

Replace the three retaining screws.

Fit the plug and sealing ring to the ion block.

Fit the insulating ring and extraction cone.

Offer the ion block up to the peek ion block support, locate the two dowels and push firmly.

Replace the ion block heater.

Replace and firmly tighten the two retaining screws taking care not to over-tighten the screws.

On the tune page select **Other** and click on **Pump**.

Replace the PTFE exhaust liner and cleanable baffle, if removed.

Replace the sample cone and, if the nanoflow option is to be used, the cone gas nozzle on the ion block.

Reconnect the cone gas supply (nanoflow operation only).

Plug the purge and cone gas outlets and fit the APcI corona discharge pin.

Fit the source enclosure and the probe adjustment flange.

Insert the APcI probe and connect the **Nebuliser Gas** line.

Select **Gas** and turn on **Nitrogen**. Fully open the **Nebuliser Gas** valve.

Set **Desolvation Gas** to read back 400 litres/hour (monitored on the tune page).

Check for gas leaks using soap solution.

Reduce **Desolvation Gas** to 150 litres/hour.

Set **Source Block Temp** to 150°C, and **APcI Probe Temp** to 20°C



**Caution:** The maximum operating temperature for the source heater is 150°C. Do not set **Source Block Temp** higher than 150°C.

Select **Operate** on the tune page.

With **Corona** set to zero, check that the **Cone** readback is reading the correct set value.

Set **Corona** to 4.0kV.

Check that the **Corona** readback is 4.0 kV and that the **Cone** readback is still reading the same set value.

Check that all other readbacks on the tune page agree with the set values.

## The Corona Discharge Pin

If the corona discharge pin becomes dirty or blunt:

Remove it from the source.

Clean and sharpen it using 600 grade emery paper.

If the needle becomes bent or otherwise damaged it should be replaced.

# The Electrospray Probe

## Overview



**Warning:** The probe tip is sharp, and may be contaminated with harmful and toxic substances. Always take great care when handling the electrospray probe.

Indications that maintenance is required to the electrospray probe include:

- An unstable ion beam.

*Nebulising gas may be escaping from the sides of the probe tip.*

Ensure that the probe tip O ring is sealing correctly.

*The probe tip setting may be incorrect.*

Adjust the probe tip setting as described in *Electrospray*.

*The probe tip may be damaged.*

Replace the probe tip.

*There may be a partial blockage of the sample capillary or the tubing in the solvent flow system.*

Clear the blockage or replace the tubing.

- Excessive broadening of chromatogram peaks.

*This may be due either to inappropriate chromatography conditions, or to large dead volumes in the transfer capillaries between the LC column or probe connection.*

Ensure that all connections at the injector, the column, the splitting device (if used) and the probe are made correctly.

- High LC pump back pressure.

*With no column in line and the liquid flow set to 300  $\mu$ l/min the back pressure should not exceed 7 bar (100 psi). Pressures in excess of this indicate a blockage in the solvent flow system.*

*Samples containing particulate matter, or those of high concentrations, are most likely to cause blockages.*

Check for blockages at the tube connections and couplings to the injector, the column and, if used, the flow splitter.

Concentrated formic acid can be injected to clear blockages. Rinse thoroughly afterwards.

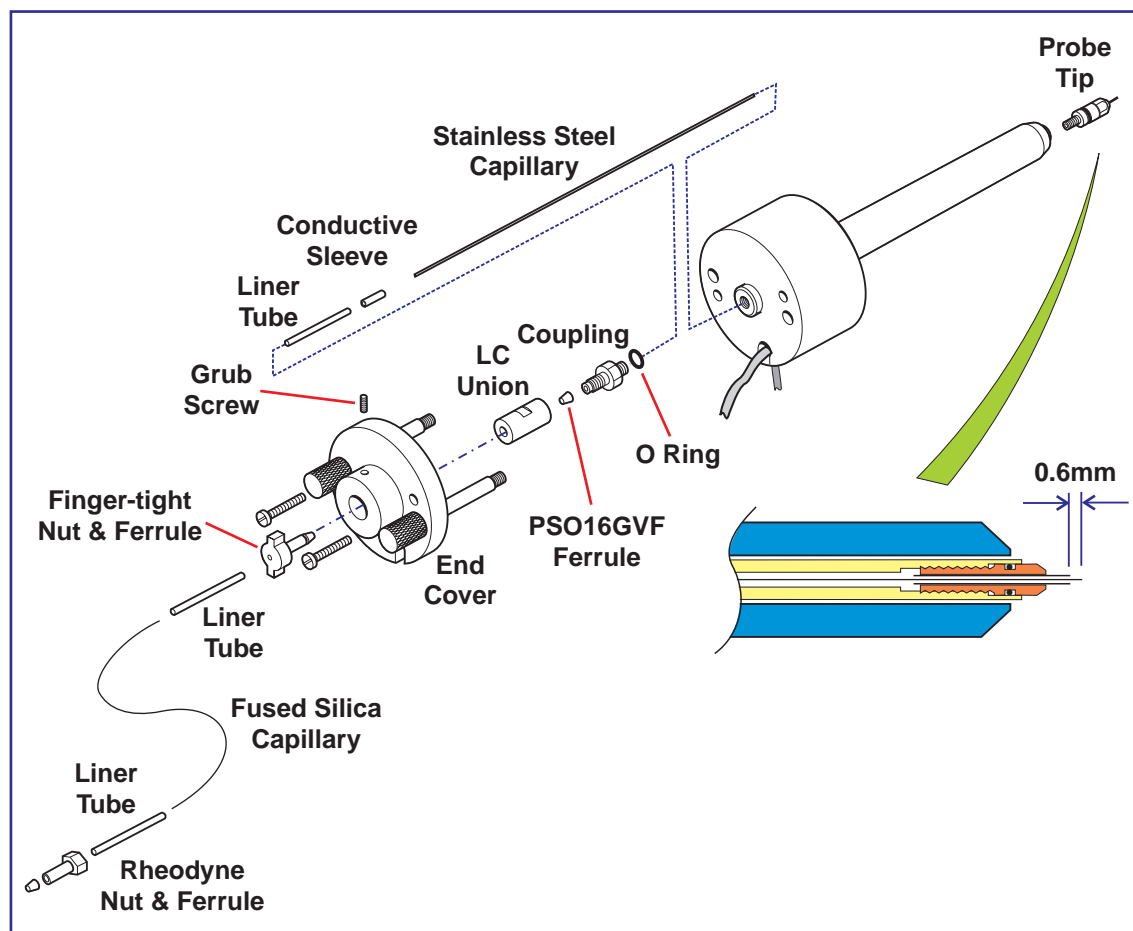
*Blockage of the stainless steel sample capillary may occur if the desolvation heater is left on without liquid flow. This is particularly relevant for samples contained in involatile solvents or high analyte concentrations. To avoid this problem it is good practice to switch off the heater before stopping the liquid flow, and flush the capillary with solvent.*

A blocked stainless steel sample capillary can often be cleared by removing it and reconnecting it in the reverse direction, thus flushing out the blockage.

- Gas flow problems

Check all gas connections for leaks using soap solution, or a suitable leak searching agent such as Snoop.

## Replacement of the Stainless Steel Sample Capillary



If the stainless steel sample capillary cannot be cleared, or if it is contaminated or damaged, replace it as follows:

Remove the probe from the source.

Disconnect the LC line from the probe and remove the finger-tight nut.

Loosen the grub screw retaining the LC union.

Remove the two probe end cover retaining screws, and remove the probe end cover.

Unscrew and remove the probe tip.

Remove the LC union and adapter nut. Withdraw and discard the stainless steel sample capillary.

Remake the LC connection to the LC union.

Sleeve one end of new sample capillary with the PTFE liner tube.

Using a GVF/16 ferrule and the adapter nut, connect the sample capillary to the LC union, ensuring that both the liner tube and sample capillary are fully butted into the LC union.

Disconnect the LC connection and feed the sample capillary through the probe, ensuring that a 0.3mm graphitised vespel ferrule (GVF/003) is fitted.

Using a Rheodyne spanner, gently tighten the adapter nut onto the probe.

Replace the probe tip and adjust so that 0.5mm of sample capillary protrudes from the probe tip.

Replace the probe end cover and tighten the grub screw to clamp the LC union.

## The APcI Probe

Indications that maintenance to the APcI probe is required include:

- The probe tip assembly becomes contaminated, for example by involatile samples if the probe temperature is too low during operation (300°C).
- The appearance of chromatogram peak broadening or tailing.

*Samples that give rise to a good chromatogram peak shape in APcI (for example reserpine and common pesticides) should display peak half widths of the order 0.1 minutes for 10µl loop injections at a flow rate of 1 ml/min. The appearance of significant peak broadening or tailing with these compounds is most likely to be due to a broken fused silica capillary or probe tip heater assembly.*

- Low LC pump back pressure.

*For 50:50 acetonitrile:water at a flow rate of 1 ml/min, a LC pump back pressure less than 14 bar (200 psi) is indicative of a broken fused silica capillary or a leaking connector.*

- High LC pump back pressure.

*For 50:50 acetonitrile:water at a flow rate of 1 ml/min, a LC pump back pressure above 35 bar (500 psi) is indicative of a blockage or partial blockage in the fused silica capillary, in a LC connector or in the filter. It is advisable to change the inner filter pad on a regular basis (see “Replacing the Fused Silica Capillary” in the following pages).*

- Gas flow problems.

Check all gas connections for leaks using soap solution, or a suitable leak searching agent such as Snoop.

## Cleaning the Probe Tip

Remove any visible deposits on the inner wall of the probe heater with a micro-interdental brush (supplied in the spares kit) soaked in methanol:water.

Before starting an analysis:

With the probe out of the instrument, connect the nebulising gas supply line.

Select **API Gas** and turn on **Nitrogen**.

Allow the gas to flow for several seconds to clear any debris from the heater.

Turn off **Nitrogen**.

Insert the probe into the source.

Select **API Gas** and turn on **Nitrogen**.

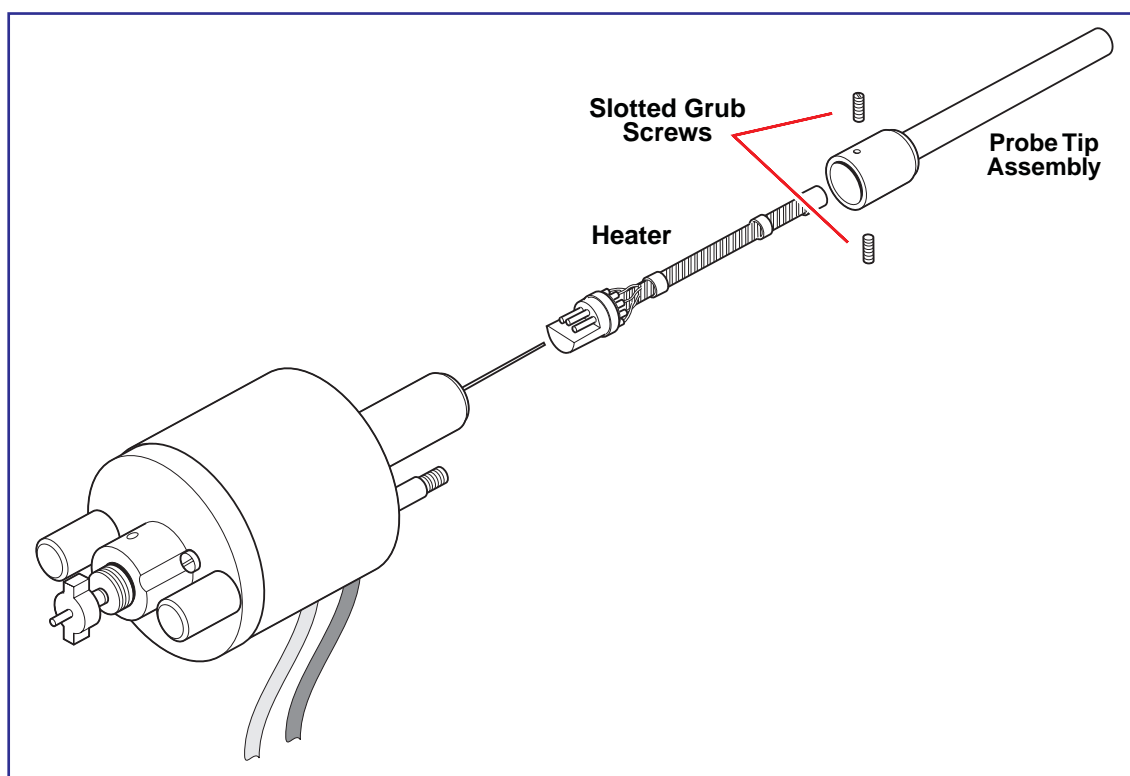
Raise **APcl Heater** gradually, starting at 100°C and increasing in 50°C intervals to 650°C over a period of 10 minutes.



**Caution:** Do not set **APcl Heater** to 650°C immediately as this may damage the probe heater.

*This procedure should remove any chemical contamination from the probe tip.*

## Replacing the Probe Tip Heater



Remove the probe tip assembly by carefully loosening the two grub screws.

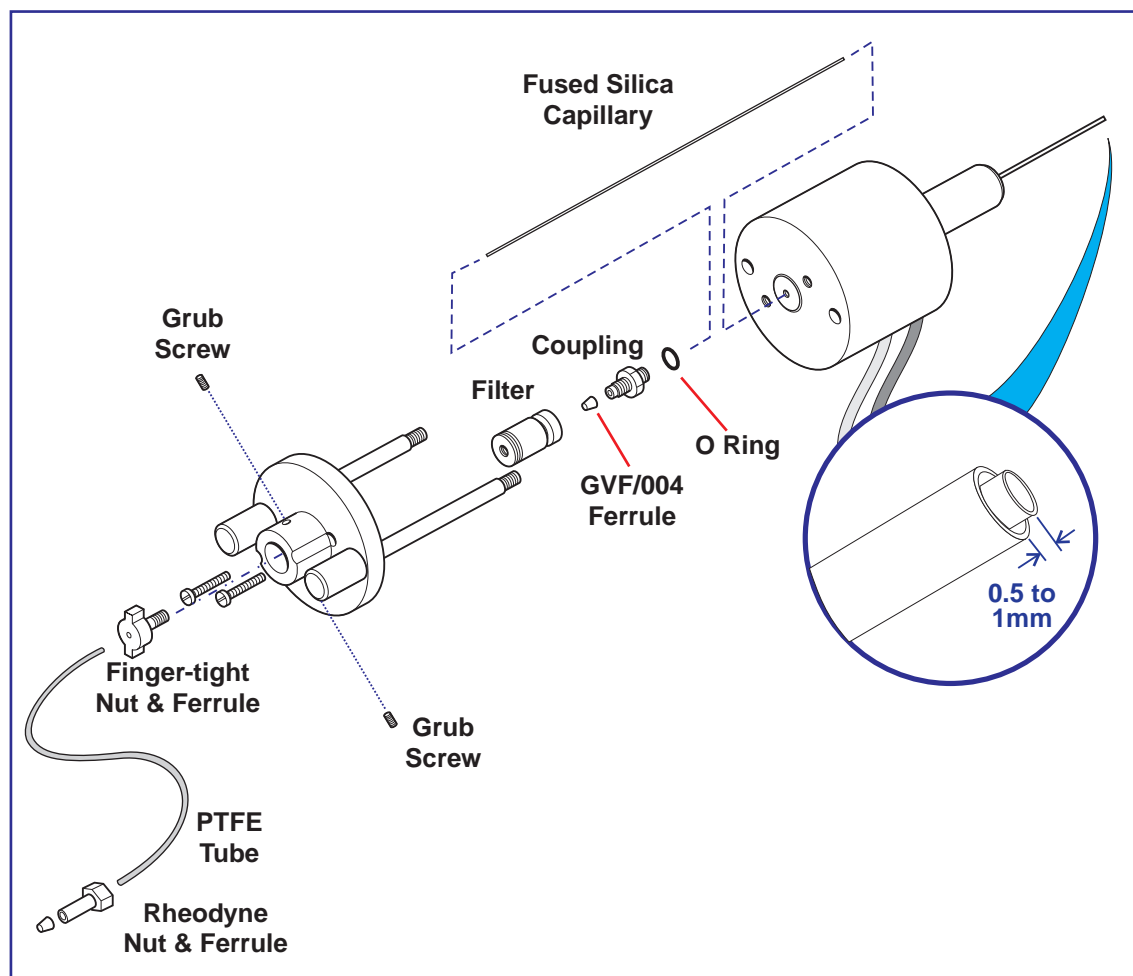
Disconnect the heater from the probe body by pulling parallel to the axis of the probe.

Fit a new heater assembly.

Reconnect the probe tip assembly.



## Replacing the Fused Silica Capillary



With the probe removed from the source proceed as follows:

Remove the probe tip assembly and the heater, as described in the preceding section.

Remove the probe end cover by removing the two screws and the grub screws that retain the LC filter.

Loosen the filter from the adapter nut.

Unscrew the adapter nut from the probe.

Remove and discard the fused silica capillary.

Using a ceramic capillary cutter, cut a new length of  $300\mu\text{m o.d.} \times 100\mu\text{m i.d.}$  fused silica capillary, about 1 centimetre excess in length.

Using a GVF/004 ferrule and the adapter nut, connect the capillary to the filter ensuring that the capillary passes through the ferrule but stops short of the filter.

Feed the sample capillary through the probe, ensuring that a 0.4mm graphitised vespel ferrule (GVF/004) is fitted.

Using a ceramic capillary cutter, cut the capillary at the nebuliser so that between 0.5 and 1.0mm of capillary is protruding from the nebuliser.

*It is important to cut the capillary square. This should be examined using a suitable magnifying glass.*

Undo the adapter nut from the probe and withdraw the capillary from the probe.

Remove 20mm of polyamide coating from the end of the capillary using a flame and clean with a tissue saturated with methanol.

Carefully re-feed the sample capillary through the probe ensuring that the graphitised vespel ferrule is still fitted.

Using a Rheodyne spanner, gently tighten the adapter nut to the probe.

Replace the probe end cover and retaining screws.

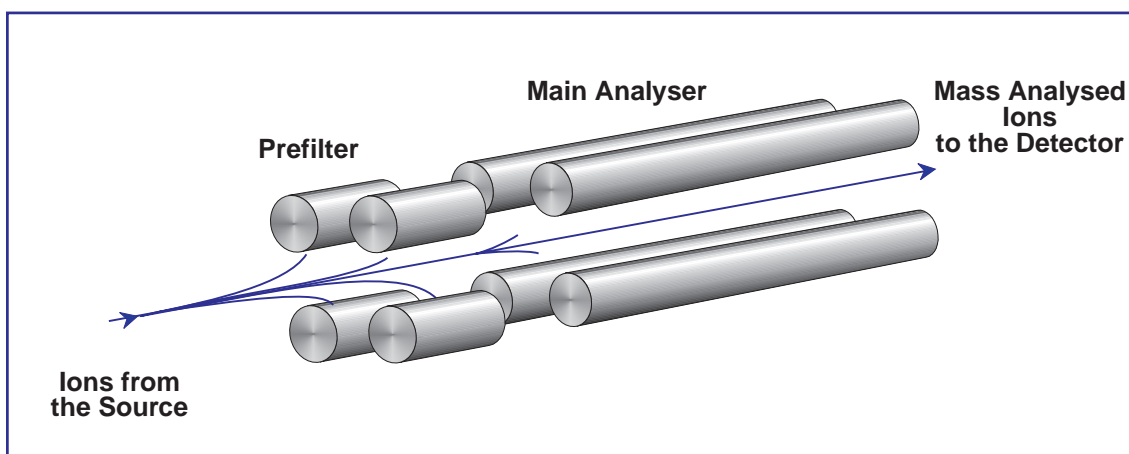
Using a 1.5mm Allen key, tighten the grub screw in the probe end cover to clamp the filter.

Replace the heater and probe tip assembly.

## The Analyser

The analyser element of any high performance quadrupole mass spectrometer is, of necessity, a precisely machined and aligned assembly.

Q-Tof 2 is fitted with a prefilter assembly designed to protect the main analyser by absorbing the majority of any contamination. The prefilter is not as mechanically critical as the main rods, as it has only RF applied and is, therefore, not resolving. It does, however, act as a high pass filter, and will reject ions of low mass before they enter the main rods.



The need to clean the prefilter rods is usually indicated by poor peak shape or loss of resolution (in tuning mode), although other more likely causes, such as contamination of the source should be eliminated first.



**Caution:** Cleaning the analyser on site is not a task to be undertaken lightly. It should be done only when there is clear evidence that the analyser is the source of lack of performance. The main rods of the analyser are precision ground, as are the ceramics in which they are mounted. ***Under no circumstances should any of the analyser rods be removed from the ceramic mountings.***

It is unlikely that there will be any means on site for measuring the assembly to the micron level required. If analyser charging effects cannot be resolved by the techniques below, it is almost certain that the analyser will have to be returned to Micromass for refurbishment.

## Removing the MS1 and Collision Cell Optical Bench Assembly

Vent the system as described at the beginning of this chapter.

Remove the analyser housing top plate.

Working inside the MS1 analyser housing proceed as follows:

Disconnect the two push-on connectors which take the analyser RF through to the RF lens at the source end of the analyser.

Disconnect the three push-on connections bringing RF and DC voltages to the gas cell (analyser housing right hand side).

Disconnect the three push-on connections bringing RF and DC voltages to the hexapole transfer lens following the gas cell (analyser housing right hand side).

Remove the gas line connection to the gas cell where it enters the gas cell.

Disconnect the push-on DC (pole bias) connection to the main filter.

Disconnect the heavy gauge copper RF/DC main filter supply from the feedthroughs on the left side of the analyser housing.

*All connections to the ion optical bench (main filter assembly/gas cell/RF lens assembly) should now have been removed.*

At the front and rear of the optical bench, remove the two 4mm Allen socket screws that secure the bench to the mounting flanges.



**Caution:** Before removing the optics from the housing prepare a clean area where it may be placed in preparation for removing and cleaning the prefilter rods.

Holding the optical bench assembly, using two of the clamps that secure the optical elements to the aluminium base plate, lift the rear end upwards to clear the vacuum housing and then withdraw the optical bench from the housing.

Once removed replace the vacuum housing lid to prevent the ingress of dust into the housing.

## Dismantling and Cleaning the Entrance Prefilter

Dismantling and cleaning of the entrance prefilter is a skilled procedure which should be entrusted only to a Micromass engineer.

## Cleaning the MS1 Analyser Assembly



**Caution:** Under no circumstances should any of the analyser rods be removed from the ceramic mountings.

Having removed the optical bench assembly as described above, remove the two top clamps that secure the main filter (including pre and post-filter assemblies) to the optical bench.

Roll up a narrow strip of absorbent lint-free paper. Pass one end through the gap between two adjacent main filter rods and back through the next gap so that the paper is wrapped one half turn around a rod.

Wet the paper with a solvent such as methanol, and move the strip up and down along the analyser rod.

Remove the strip and use dry nitrogen gas from a cylinder (**not** compressed air) to blow out any dust or particles.



**Caution:** Tools, carborundum paper or micromesh must not be used to remove contamination from the main filter rods.

It is not anticipated that the gas cell or hexapole transfer lens will require cleaning.

## Replacing the MS1 and Gas Cell Optical Bench Assembly

Reassembly is the reverse of the appropriate dismantling procedure. Take extra care to ensure that all electrical connections are correctly and securely made, and that the various mechanical assemblies are accurately aligned within the housing on their locating dowels.

## The MS2 Analyser and Detector Assembly

The tuning detector, orthogonal acceleration cell, reflectron and microchannel plate assemblies are separated from the MS1 and gas cell region by a differential pumping orifice. It is anticipated that this region of the instrument will not require routine maintenance under normal operating conditions.

The tuning detector system has been designed for trouble-free operation over many years. The sealed photomultiplier is safe from contamination and pressure surges (the traditional enemies of mass spectrometer multipliers) and the conversion dynodes are tolerant of ion burn. The phosphor has also proved to be reliable and long-lasting. No routine maintenance of this detector is therefore required.

The microchannel plate system is at risk from failure properly to condition the detector following venting of the system to atmosphere or vacuum failure.



**Caution:** It is strongly recommended that assistance is sought from Micromass if maintenance to any of the components within the TOF analyser housing are thought to be necessary (e.g. excessive noise, spikes, loss of detector gain or abnormal TOF peak shapes).

## Fault Finding

### Introduction

The majority of faults that occur 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, it is advisable first of all to ensure that this part has been correctly refitted and adjusted, and that adjacent components have not been inadvertently disturbed.

### No Beam

Refer to the relevant chapters of this manual and check the following:

- The tune page real time display is activated by pressing the appropriate button on the tool bar of the tune page.
- Normal tuning parameters are set and, where appropriate, 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.

*For solvent flow rates below 100 µl/min it may be necessary temporarily to turn off the nebulising gas and remove the probe from the source to allow the solvent to be seen at the probe tip.*

- The flows of desolvation gas and nebuliser gas are on and are set 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:

Acquire TOF data with a mass range extending down to  $m/z$  20.

Check that there is an interference ‘peak’ at approximately  $m/z$  28 due to the pusher pulse being switched off.

*If this interference peak is not present, either the pusher is not pulsing or the output from the detector is not reaching the TDC (time to digital converter).*

*The most likely cause of an absent pusher interference pulse is a faulty attenuator.*

*If the pusher interference peak is not present no data will be acquired.*

## 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.

*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 gas and nebuliser gas is steady. The nitrogen supply pressure should be 7 bar (100 psi)  $\pm 10\%$ .
- **Desolvation Temp** is not set too high for the liquid flow rate used.

*High temperatures can vapourise 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 imply 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  $\mu\text{l}/\text{min}$  to remove the blockage.

*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:

Remove the finger-tight nut and tubing from the back of the probe.

If the back pressure remains high:

Replace the tubing with new tube (or first try removing both ends of the tube).

If the back pressure falls:

Replace the stainless steel sample tube inside the probe (or try reversing the tube to blow out any blockage).

Reconnect the tubing to the probe.

The solvent flow can now be readjusted and the probe replaced into the source.

*To check the flow rate from the solvent delivery system, fill a syringe barrel or a graduated glass capillary with the liquid emerging from the probe tip, and time a known volume, say 10  $\mu\text{l}$ .*

*Once the rate has been measured and set, a note should be made of 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 are indicators that the source needs cleaning. The usual remedy is to clean the source as described earlier in this chapter.

An increase in the analyser pressure above  $4\text{e-}6$  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 **Stop (mV)** threshold being set too high.

Refer to the tune page settings section of *Routine Procedures* for information regarding the setting of this parameter.

- A faulty attenuator.

*Attenuators can fail so that they are open circuit (no beam or pusher interference 'peak' present), or they can fail such that they stop attenuating. The latter failure mode gives rise to incorrect isotope distributions.*

When the attenuator fails in this way the TDC **Stop (mV)** threshold can be increased to a significantly higher value than that used previously without reducing the beam intensity.

*In normal operation setting the TDC threshold above 200 or 250mV will start to reduce the beam intensity. If the attenuator has failed the TDC threshold can be increased to 500mV or higher before the beam intensity is reduced.*

## 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.

Chemical noise can be distinguished from electronic noise simply by stopping source ionisation. 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 TDC **Stop (mV)** threshold too low. Refer to the tune page settings section of *Routine Procedures* for information regarding the setting of this parameter.

The microchannel plate detector can be damaged by failure to properly condition the detector following venting of the system to atmosphere. If the detector is producing microdischarges, excessive noise will be 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 reduce the noise.



**Caution:** It is strongly recommended that assistance is sought from Micromass if maintenance to the detector system is thought necessary.



**Caution:** Assistance from Micromass should be sought if, due to symptoms such as excessive noise, spikes, loss of detector gain or abnormal peak shapes, maintenance to any of the components within the TOF analyser housing is thought to be necessary.

## Poor Analyser Vacuum

Before suspecting a pump fault or vacuum leak (see *Vacuum System* earlier in this chapter) it is worth checking the inverted magnetron (Penning) gauge. If this gauge has become dirty it will indicate a poor vacuum, or even fail to register at all.

For information on cleaning the gauge, refer to the Edwards literature supplied with the instrument.



**Warning:** The instrument must be vented and electrically isolated at the supply outlet before removing the instrument's covers to gain access to the active inverted magnetron gauge.

Note that if the instrument has been vented to atmosphere (instead of dry nitrogen) it may take one to two days before reaching the vacuum levels obtained prior to venting.

## Cleaning Materials

It is important when cleaning internal components to maintain the quality of the surface finish. Deep scratches or pits can cause loss of performance. Where no specific cleaning procedure is given, fine abrasives should be used to remove dirt from metal components. Recommended abrasives are:

- 600 and 1200 grade emery paper.
- Lapping paper (produced by 3M).

After cleaning with abrasives it is necessary to wash all metal components in suitable solvents to remove all traces of grease and oil. The recommended procedure is to sonicate the components in a clean beaker of solvent and subsequently to blot them dry with lint-free tissue. Recommended solvents are:

- Isopropyl Alcohol (IPA)
- Methanol
- Acetone

Following re-assembly, components should be blown with oil-free nitrogen to remove dust particles.



**Warning:** Many of the procedures described in this chapter involve the removal of possibly toxic contaminating deposits using flammable or caustic agents. Personnel performing these operations should be aware of the inherent risks, and should take the necessary precautions.

## Preventive Maintenance Check List

- ✗ Avoid venting the instrument when the rotary pump is gas ballasting.
- ✗ Do not gas ballast the rotary pump for more than 2 hours under any circumstances.

*For full details of the following procedures, consult the relevant sections of this chapter and / or refer to the manufacturer's literature.*

### Daily

- Gas ballast the rotary pump for 30 minutes at the end of a day's megafLOW or APcI operation.

*It is normal for the rotary pump noise level to increase during gas ballasting.*

### Weekly

- Gas ballast for at least 30 minutes by rotating the gas ballast knob anticlockwise by 5 to 6 turns.

When gas ballast is complete, check the rotary pump oil level and colour.

*Oil that has become noticeably red in colour should be replaced.*

- Check the water chiller level and temperature (if fitted).

### Monthly

- Check all cooling fans and filters.

### Four-Monthly

- Change the mist element in the oil mist filter.
- Change the oil in the rotary pump.

*Gas ballast lightly for 30 to 60 minutes both before and after changing oil.*



# Reference Information

## Overview

The reference files listed in this chapter have all ion intensities set to 100%. Actual ion intensities are not, of course, all 100%, but the calibration software does not take account of the ion intensities and this is a convenient way to store the reference files in the required format.

Most samples can be purchased from the Sigma chemical company. To order, contact Sigma via the internet, or by toll-free (or collect) telephone or fax:

### Internet:

<http://www.sigma.sial.com>

*This site contains a list of worldwide Sigma offices, many with local toll-free numbers.*

### Toll-free telephone:

USA & Canada 800-325-3010

Outside USA & Canada ++1 314-771-5750 (call collect)

### Toll-free fax:

USA & Canada 800-325-5052

Outside USA & Canada ++1 314-771-5750  
(call collect and ask for the fax machine)

### Direct fax:

Outside USA & Canada ++1 314-771-5757 (this is a toll call)

## Positive Ion

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
UBQ	Bovine Ubiquitin [U6253]	8564.85	650-1500	General
HBA	Human $\alpha$ globin [H753]	15126.36	700-1500	Hb analysis
SOD	Superoxide dismutase [S2515]	15591.35	900-1500	Hb (internal cal.)
HBB	Human $\beta$ globin [H7379]	15867.22	800-1500	Hb analysis
MYO	Horse heart myoglobin [M1882]	16951.48	700-1600	General
PEGH1000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000		80-1000	ES+ and APcI+ calibration
PEGH2000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000+1450		80-2000	ES+ calibration
NAICS	Sodium Iodide / Caesium Iodide mixture		20-4000	General, ES+ calibration
NAIRB	Sodium iodide / Rubidium Iodide mixture		20-4000	ES+ calibration



## Horse Heart Myoglobin

Reference File: MYO.REF  
Molecular Weight: 16951.48

Charge State	Calculated m/z Value	Charge State	Calculated m/z Value	Charge State	Calculated m/z Value
28 <sup>+</sup>	606.419	21 <sup>+</sup>	808.222	13 <sup>+</sup>	1304.969
	616.177	20 <sup>+</sup>	848.583	12 <sup>+</sup>	1413.633
27 <sup>+</sup>	628.841	19 <sup>+</sup>	893.192	11 <sup>+</sup>	1542.053
26 <sup>+</sup>	652.989	18 <sup>+</sup>	942.758	10 <sup>+</sup>	1696.158
25 <sup>+</sup>	679.068	17 <sup>+</sup>	998.155	9 <sup>+</sup>	1884.508
24 <sup>+</sup>	707.320	16 <sup>+</sup>	1060.477	8 <sup>+</sup>	2119.945
23 <sup>+</sup>	738.030	15 <sup>+</sup>	1131.108	7 <sup>+</sup>	2422.651
22 <sup>+</sup>	771.531	14 <sup>+</sup>	1211.829		

## Polyethylene Glycol

PEG + NH<sub>4</sub><sup>+</sup>

Reference Files: PEGH1000.REF, [PEGH2000.REF](#)

Calculated m/z Value					
63.04	459.28	855.52	<a href="#">1251.75</a>	<a href="#">1647.99</a>	
107.07	503.31	899.54	<a href="#">1295.78</a>	<a href="#">1692.01</a>	
151.10	547.33	943.57	<a href="#">1339.80</a>	<a href="#">1736.04</a>	
195.12	591.36	987.60	<a href="#">1383.83</a>	<a href="#">1780.07</a>	
239.15	635.39	<a href="#">1031.62</a>	<a href="#">1427.86</a>	<a href="#">1824.09</a>	
283.18	679.41	<a href="#">1075.65</a>	<a href="#">1471.88</a>	<a href="#">1868.12</a>	
327.20	723.44	<a href="#">1119.67</a>	<a href="#">1515.91</a>	<a href="#">1912.15</a>	
371.23	767.46	<a href="#">1163.70</a>	<a href="#">1559.94</a>	<a href="#">1956.17</a>	
415.25	811.49	<a href="#">1207.73</a>	<a href="#">1603.96</a>	<a href="#">2000.20</a>	

## Sodium Iodide and Caesium Iodide Mixture

Reference File: NAICS.REF

Calculated m/z Value					
22.9898		772.4610		1671.8264	
132.9054		922.3552		2571.1918	
172.8840		1072.2494		2721.0861	
322.7782		1222.1437		2870.9803	
472.6725		1372.0379		3020.8745	
622.5667		1521.9321		3170.7688	
				3320.6630	

## Sodium Iodide and Rubidium Iodide Mixture

Reference File: NAIRB.REF

Calculated m/z Value					
22.9898		772.4610		1671.8264	
84.9118		922.3552		2571.1918	
172.8840		1072.2494		2721.0861	
322.7782		1222.1437		2870.9803	
472.6725		1372.0379		3020.8745	
622.5667		1521.9321		3170.7688	
				3320.6630	

## Negative Ion

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
MYONEG	Horse heart myoglobin [M1882]	16951.48	700-2400	General
SUGNEG	Sugar mixture of: maltose [M5885] raffinose [R0250] maltotetraose [M8253] corn syrup [M3639]		100-1500	Low mass range
NAINEG	Sodium Iodide / Caesium Iodide (or Rubidium Iodide) mixture		200-3900	ES-calibration

### Horse Heart Myoglobin

Reference File: MYONEG.REF

Calculated m/z Value				
891.175		1209.812		1882.490
940.741		1302.952		2117.927
996.138		1411.615		2420.632
1058.460		1540.036		
1129.091		1694.140		

### Mixture of Sugars

Reference File: SUGNEG.REF

Calculated m/z Value				
179.06		665.21		1151.37
341.11		827.27		1313.42
503.16		989.32		1475.48

Sodium Iodide and Caesium Iodide (or Rubidium Iodide) Mixture

Reference File: NAINEG.REF

Calculated m/z Value					
126.9045		1026.2699		1925.6353	
276.7987		1176.1641		2075.5296	
426.6929		1326.0584		2225.4238	
576.5872		1475.9526		2375.3180	
726.4814		1625.8469		2525.2123	
876.3757		1775.7411		2675.1065	
				2825.0008	
				2974.8950	
				3124.7892	
				3274.6835	
				3424.5777	
				3574.4719	
					3724.3662
					3874.2604

## Preparation of Calibration Solutions

### PEG + Ammonium Acetate for Positive Ion Electrospray and APcl

Prepare a solution of polyethylene glycols at the following concentrations:

PEG 200	25 ng/μl
PEG 400	50 ng/μl
PEG 600	75 ng/μl
PEG 1000	250 ng/μl

Use 50% acetonitrile and 50% water containing 2 mmol ammonium acetate.

Use reference file PEGH1000.REF.

### PEG + Ammonium Acetate for Positive Ion Electrospray (Extended Mass Range)

Prepare a solution of polyethylene glycols at the following concentrations:

PEG 200	25 ng/μl
PEG 400	50 ng/μl
PEG 600	75 ng/μl
PEG 1000	250 ng/μl
PEG 1450	250 ng/μl

Use 50% acetonitrile and 50% water containing 2 mmol ammonium acetate.

Use reference file PEGH2000.REF.

## Sodium Iodide Solution for Positive Ion Electrospray

### Method 1

Prepare a solution of sodium iodide at a concentration of 2 µg/µl (micrograms per microlitre) in 50:50 propan-2-ol (IPA):water with no additional acid or buffer.

Add caesium iodide to a concentration of 0.05 µg/µl.

*The purpose of the caesium iodide is to obtain a peak at  $m/z$  133 ( $\text{Cs}^+$ ) to fill the gap in the calibration file between  $m/z$  23 ( $\text{Na}^+$ ) and the first cluster at  $m/z$  173, which would lead to poor mass calibration in this mass range.*

*Do not add more CsI than suggested as this may result in a more complex spectrum due to the formation of NaCsI clusters.*

Use reference file NAICS.REF.

### Method 2

Prepare a solution of sodium iodide at a concentration of 2 µg/µl (micrograms per microlitre) in 50:50 propan-2-ol (IPA):water with no additional acid or buffer.

Add rubidium iodide to a concentration of 0.05 µg/µl.

*The purpose of the rubidium iodide is to obtain a peak at  $m/z$  85 ( $^{85}\text{Rb}^+$ ) with an intensity of about 10% of the base peak at  $m/z$  173. Rubidium iodide has the advantage that no rubidium clusters are formed which may complicate the spectrum. Note that rubidium has two isotopes ( $^{85}\text{Rb}$  and  $^{87}\text{Rb}$ ) in the ratio 2.59:1, giving peaks at  $m/z$  85 and 87.*

Use reference file NAIRB.REF.

## Sodium Iodide Solution for Negative Ion Electrospray

Either of the above solutions is suitable for calibration in negative ion mode. In both cases the first negative reference peak appears at  $m/z$  127 ( $\text{I}^-$ ) and the remaining peaks are due to NaI clusters.

Use reference file NAINEG.REF.

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