Micromass UK Limited

Atlas Park
Simonsway
Manchester
M22 5PP
Tel: +44 161 435 4100 Fax: +44 161 435 4444

Floats Road
Wythenshawe
M23 9LZ
Tel: +44 161 946 2400 Fax: +44 161 946 2480

Tudor Road
Altrincham
WA14 5RZ
Tel: +44 161 926 7200 Fax: +44 161 926 7246

http://www.micromass.co.uk

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.

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.

All information contained in this manual 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.

Micromass ® is a registered trade mark of Micromass Limited
(Reg. U.S. Pat. & T.M. Off.).

Code Number 6666524
Issue 2
© Micromass Ltd.

Contents

Description

Routine Procedures

The Solids Insertion Probe

The DCI Probe

Tuning Parameters and User Interface

Data Acquisition

Calibration and Exact Mass

Maintenance and Fault Finding

Reference Information

Appendix 1
GC-MS

Appendix 2
Ion Counting on TOF Instruments Using a TDC

Description

Introduction

The Micromass GCT™ is a compact, fully integrated, high performance, orthogonal acceleration time of flight mass spectrometer designed for GC-MS and probe MS applications.

The system consists of a source and analyser housing separated by a pneumatic isolation valve and a differential pumping aperture. The source housing is fitted with a 250 L/sec high compression turbomolecular pump. The analyser housing is fitted with a 70 L/sec high compression turbomolecular pump. A single RV3 rotary pump backs both turbomolecular pumps.

GCT™ includes Electron Impact (EI), Chemical Ionisation (CI) and Field Ionisation (FI) source options. The ion source is at ground potential to allow simple direct coupling to the GC inlet, thereby minimising the possibility of cold spots. A source probe allows easy exchange of the EI and CI removable inner volumes, or of the FI emitter.

Ions are accelerated from the grounded ion source to 40eV before being accelerated orthogonally into the time of flight (TOF) mass analyser. The TOF analyser has a two stage orthogonal acceleration region, followed by a single stage reflectron, giving an effective path length of 1.2 meter. Ions are detected using a dual microchannel plate assembly capable of detecting positive or negative ions. Ion arrival times are recorded using a time to digital converter (TDC) with a sampling rate of 1 or 3.6GHz.

GCT ™ produces high quality, full mass spectra with elevated resolution (~ 7000 FWHM). This elevated resolution reduces the likelihood of mass interferences. Furthermore the precise linear relationship between ion arrival time and the square root of its mass allows good mass measurement accuracy with only a single internal reference mass. The precision of mass measurement can provide elemental composition of unknowns and confirm identification of eluting compounds. The full mass spectral sensitivity of the GCT is comparable to that of a quadrupole mass spectrometer, operating in single ion recording mode and monitoring 10 - 20 masses.

In comparison to a quadrupole instrument when used to record full mass spectra, the GCT can be 10 - 100 times more sensitive, depending on the mass range acquired. Furthermore, in situations where, at low resolution, there may be unresolved chemical interferences, the elevated resolution offered by GCT results in lower detection limits.

Ionisation Techniques

Electron Impact and Chemical Ionisation

The ion source consists of two assemblies. An inner, easily removable volume which comprises all the normally cleanable or replaceable parts, such as the filament, trap and repeller for an EI/CI source. The outer source comprises the source heater, thermocouple, focusing optics and other generally non-replaceable items, and is located on the source housing lid. Heaters in the outer source raise the source temperature to ensure sample vaporisation.

Electron Impact (EI)

Electron impact is the classical ionisation technique in which gas phase sample molecules are ionised in collisions with high energy electrons.

Chemical Ionisation (CI)

When the source is operated in the chemical ionisation mode, a reagent gas is admitted into the ion source at a relatively high pressure. The gas molecules are ionised by the electron beam. Sample ions are generated in reactions with these gas ions. CI is a 'softer' ionisation technique than EI, producing less sample fragmentation and generally a stronger molecular ion.

Field Ionisation (FI)

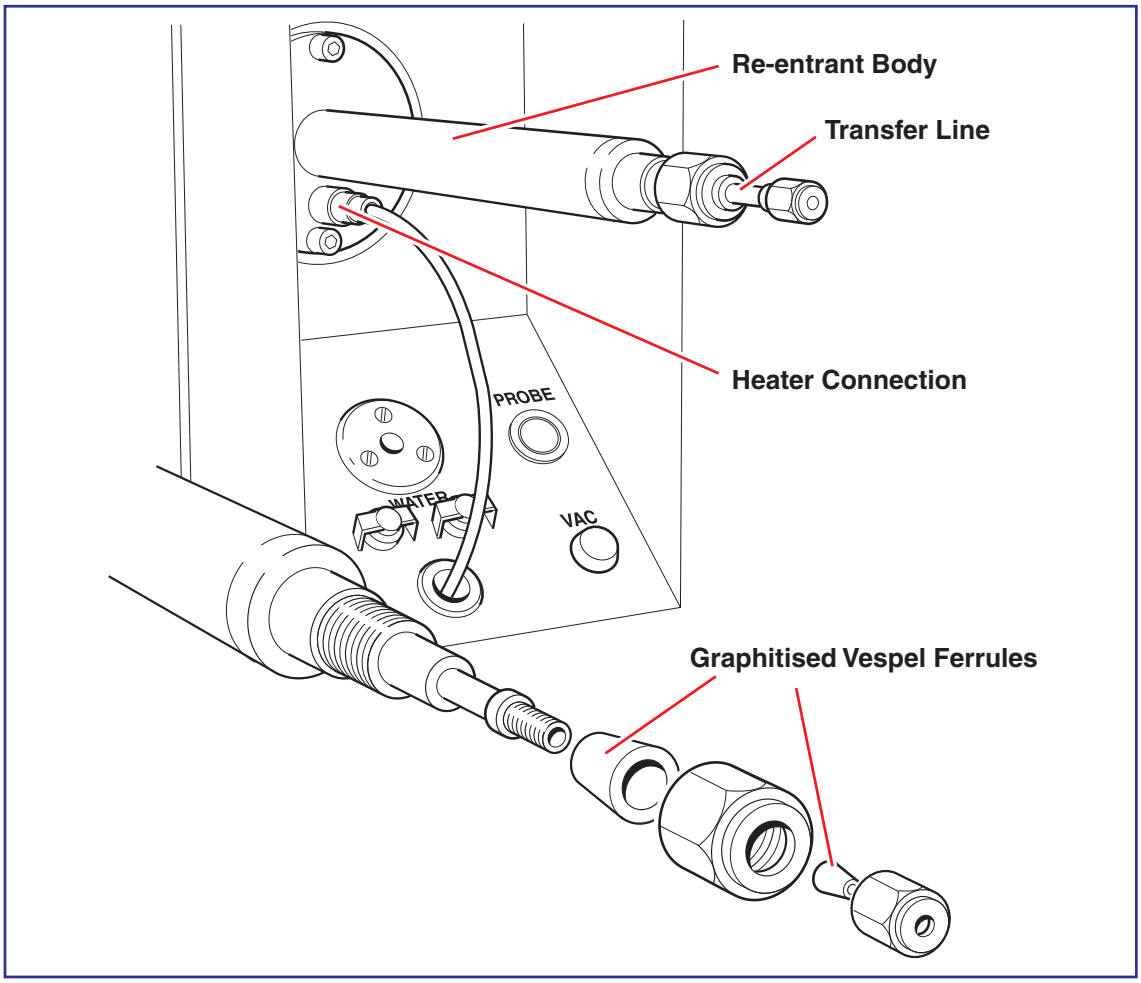
In field ionisation, sample molecules are passed in close proximity to a surface of high curvature maintained at a high potential field These molecules are subjected to potential gradients in the order of $10^7 – 10^8$ volts/ cm. Under the influence of these fields, quantum tunnelling of a valence electron from the molecule to an anode takes place to give an ion radical. This process is very 'soft' often producing spectra with very little or no fragmentation.

The ion source consists of a dedicated outer source and a removable probe holding the FI ‘emitter’. The ‘emitter’ consists of a tungsten wire onto which carbon microneedles have been grown.

Inlets

GC Interface

The GC interface provides a heated transfer line between the GC and the ion source. This ensures even heating in this region, so that the sample does not condense before it reaches the ion source. The interface is designed to be easily removable to allow simple and rapid conversion to solids probe operation.

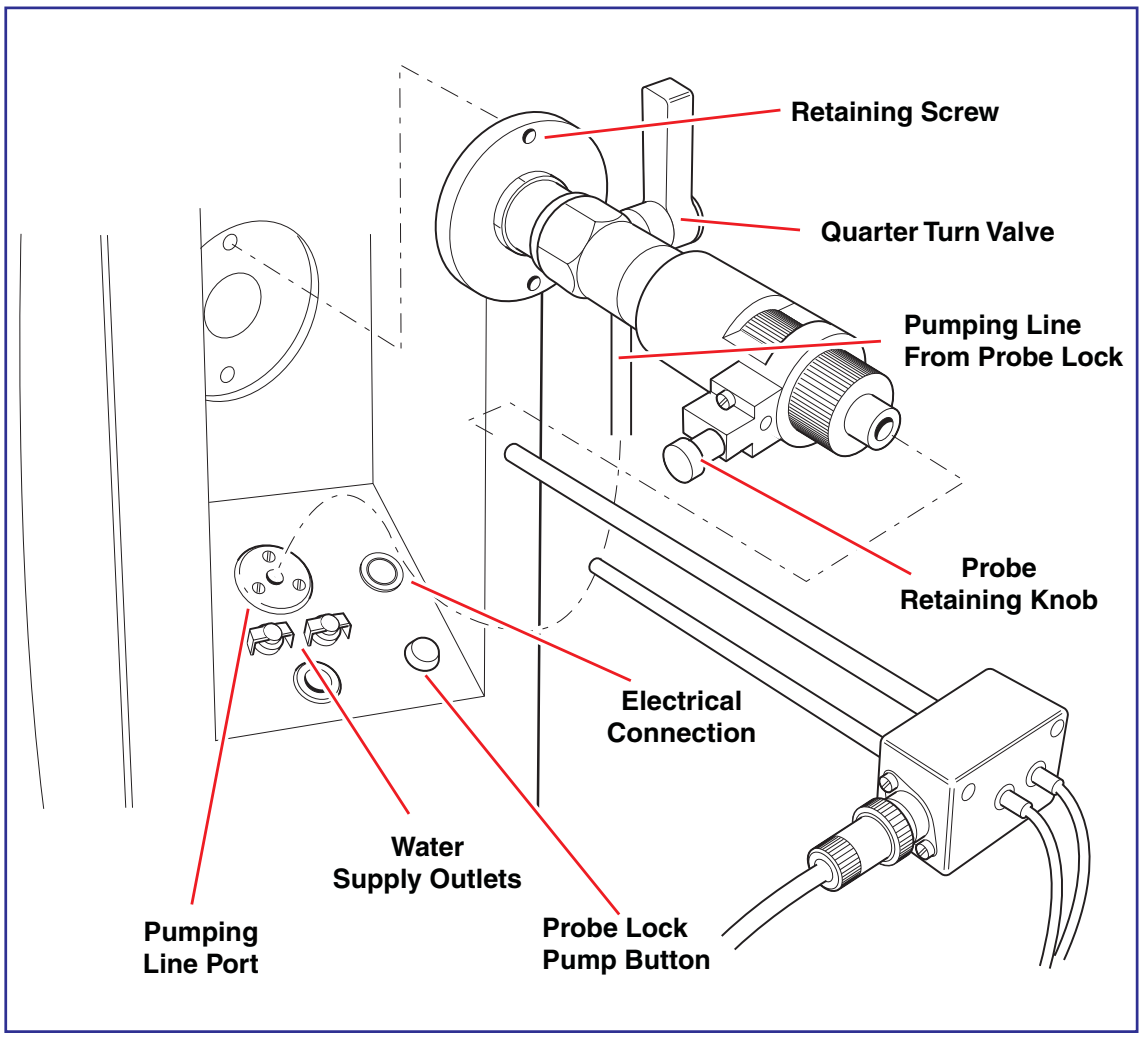


A diagram of the GC interface is shown above.

The GC interface is capable of being heated to a temperature of 350°C. A spring loaded tip allows the interface to be in contact with the outer source block for CI operation, while allowing thermal expansion of the inner re-entrant tube to be accommodated.

Direct Insertion Probe

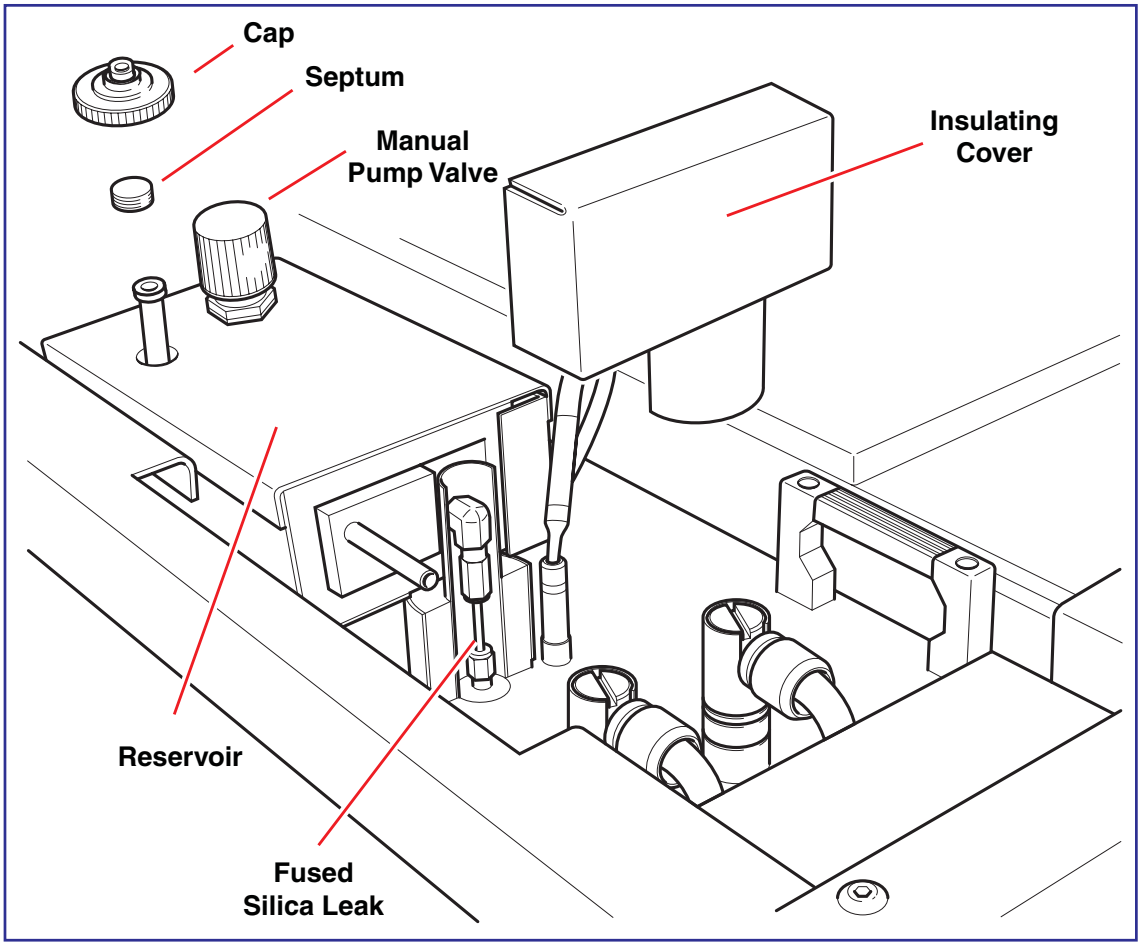
An optional direct insertion probe is available for the introduction of involatile materials. The probe lock is fitted in place of the GC interface. The probe has a maximum operating temperature of 650°C and is fully controlled from the MassLynx software.



DCI Probe

An optional Direct Chemical Ionisation (DCI) Probe is also available, which is introduced by means of the probe lock. The DCI probe current is controlled from MassLynx from 0 - 1.5A and is operated in CI mode. It is thus a fairly 'soft' technique.

The Heated Septum Interface



The septum interface is designed for the introduction of volatile reference materials for calibration and mass measurement. The interface consists of a heated 100ml chamber, 75µm I.D., fused silica capillary leak and a heated stainless steel transfer line.

Warning: The septum cap can become very hot during heated operation, and care should be taken when touching it.

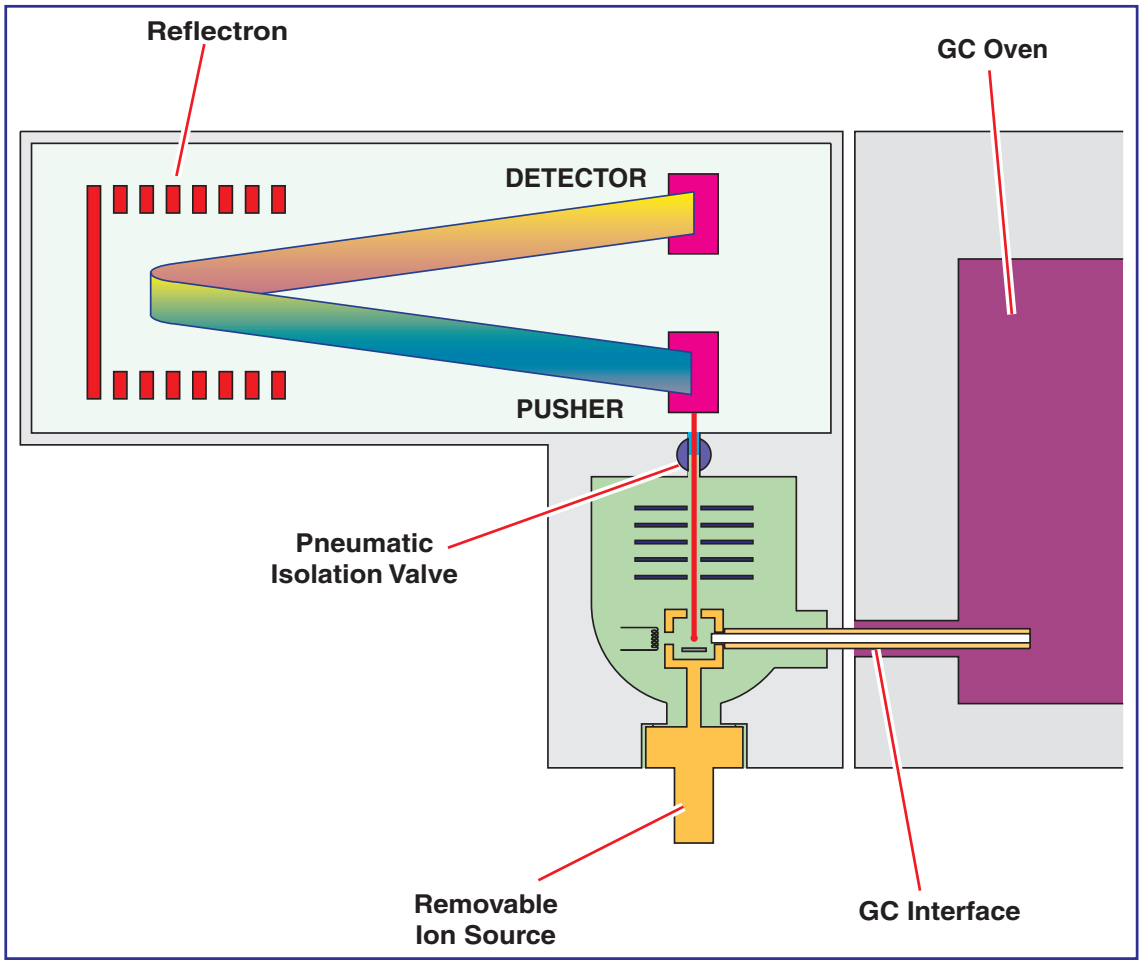
Reference material may be introduced via syringe through a septum into the chamber. A manual valve allows the chamber to be pumped to adjust the amount of reference material entering the source region. The interface is mounted on the source housing lid.

The manually operated septum pump valve should be fully open when the source housing is pumped down. The black knob on the top of the interface is turned anticlockwise.

When vented, the septum reservoir becomes full of air. This takes a long time to pump out because of the presence of the fused silica leak within the septum interface. The septum pump solenoid actuates when the source backing line isolation valve opens and evacuates the reservoir during source pumpdown.

If the septum reservoir pumping valve is closed, and the source housing is fully pumped down, a high pressure is indicated in the source housing. This is due to air slowly entering the source housing via the fixed leak in the septum interface. The reservoir pump valve should then be opened slowly to minimise the effect of the surge of air into the backing line.

Ion Optics



The principal components of the ion optical system are shown here in schematic form. Ions generated in the ion source are accelerated and focused into the pusher region of the orthogonal TOF via a transfer lens.

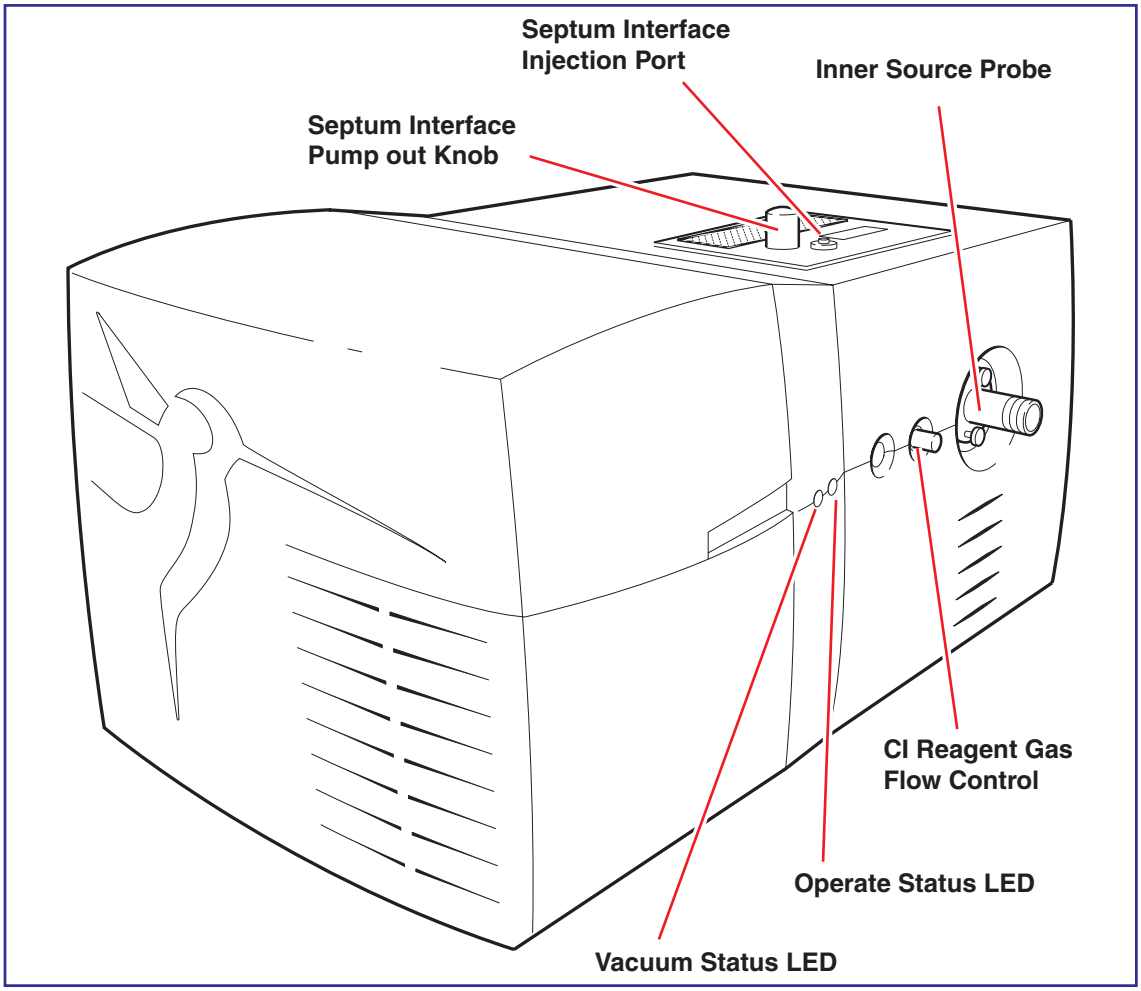
A sudden voltage pulse is then applied to the pushout electrode, ejecting a section of the beam orthogonally. The ion packet then passes through a two stage acceleration region and enters the time of flight drift region. The reflectron reflects ions back to the dual microchannel plate detector. Ion arrivals are recorded using a time to digital converter (TDC).

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 in the spectrum.

The pusher may be operated at repetition frequencies of up to 30kHz, resulting in a full spectrum being recorded every 33 microseconds. Each spectrum is summed in the PC memory until the completed, histogrammed spectrum is transferred to the host PC. For an acquisition rate of 1 spectrum/second, each spectrum viewed on the host PC will be the result of summing up to 30,000 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.

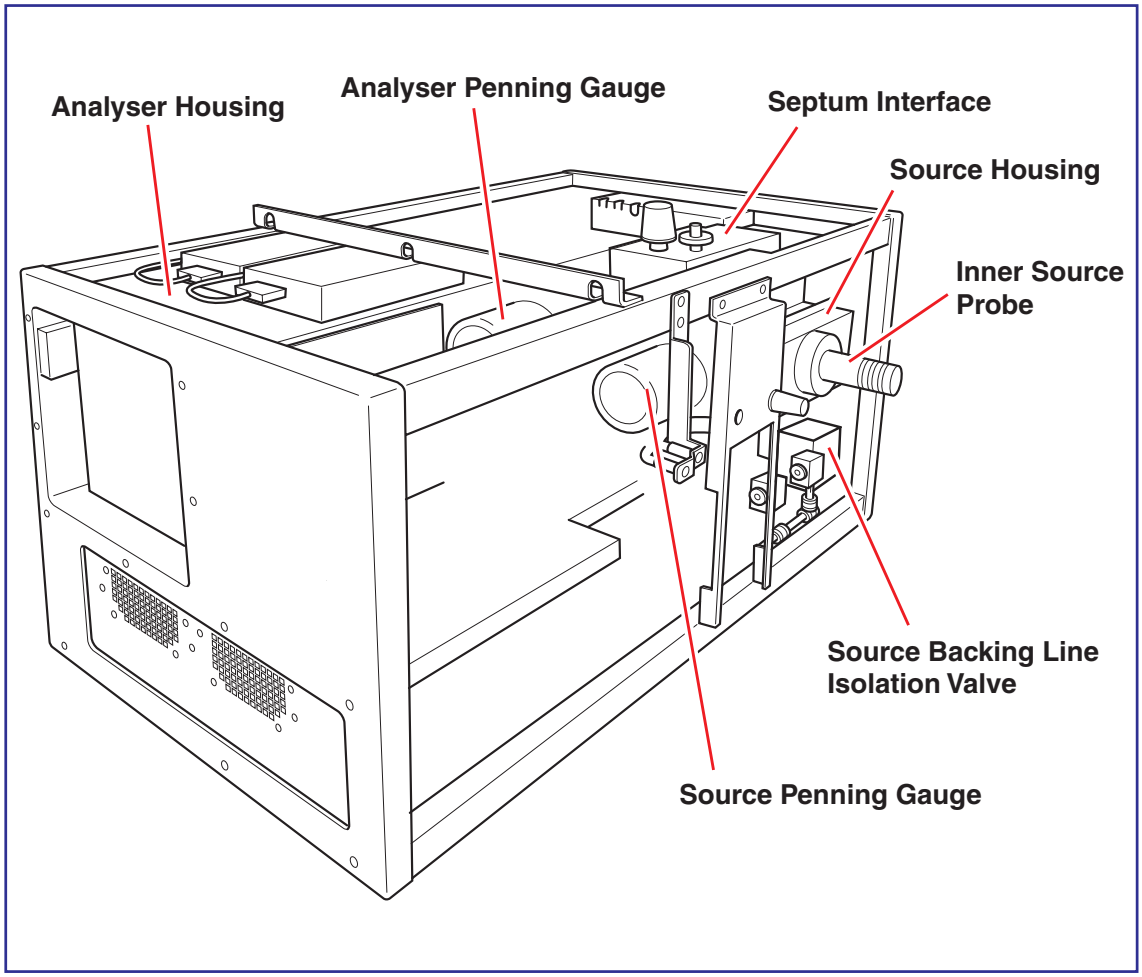
External Layout



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

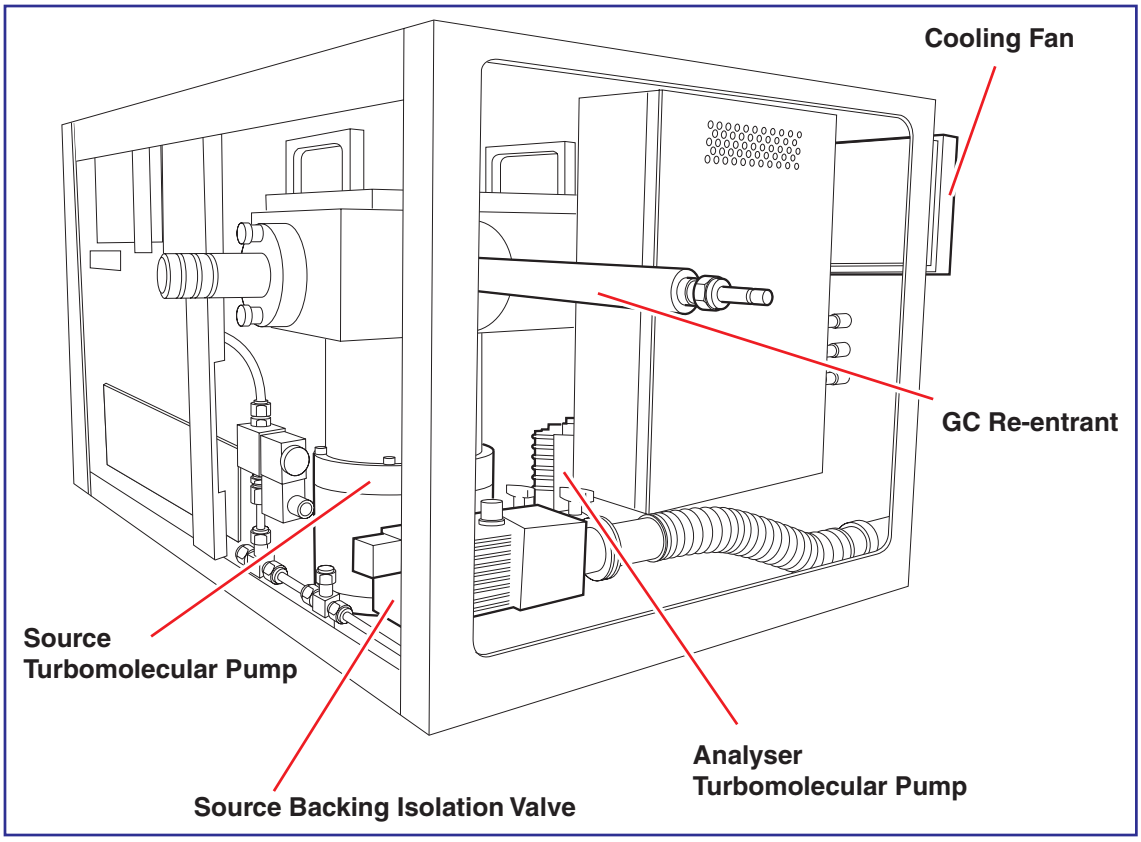
Warning: Removal of covers can expose hazardous voltages.

Mechanical Components



The above view shows the following main internal mechanical components:

- The source housing, containing the ion source.
- The analyser housing, containing the pusher, detector and reflectron assemblies.
- Two active inverted magnetron (Penning) gauges.



Also shown in the diagram above.

- GC re-entrant.
- One 250 litre/second high compression turbomolecular pump, one 70 litre/second high compression turbomolecular pump.
- The cooling fan fitted at the rear of the instrument
- Source backing line isolation valve.

Electronics

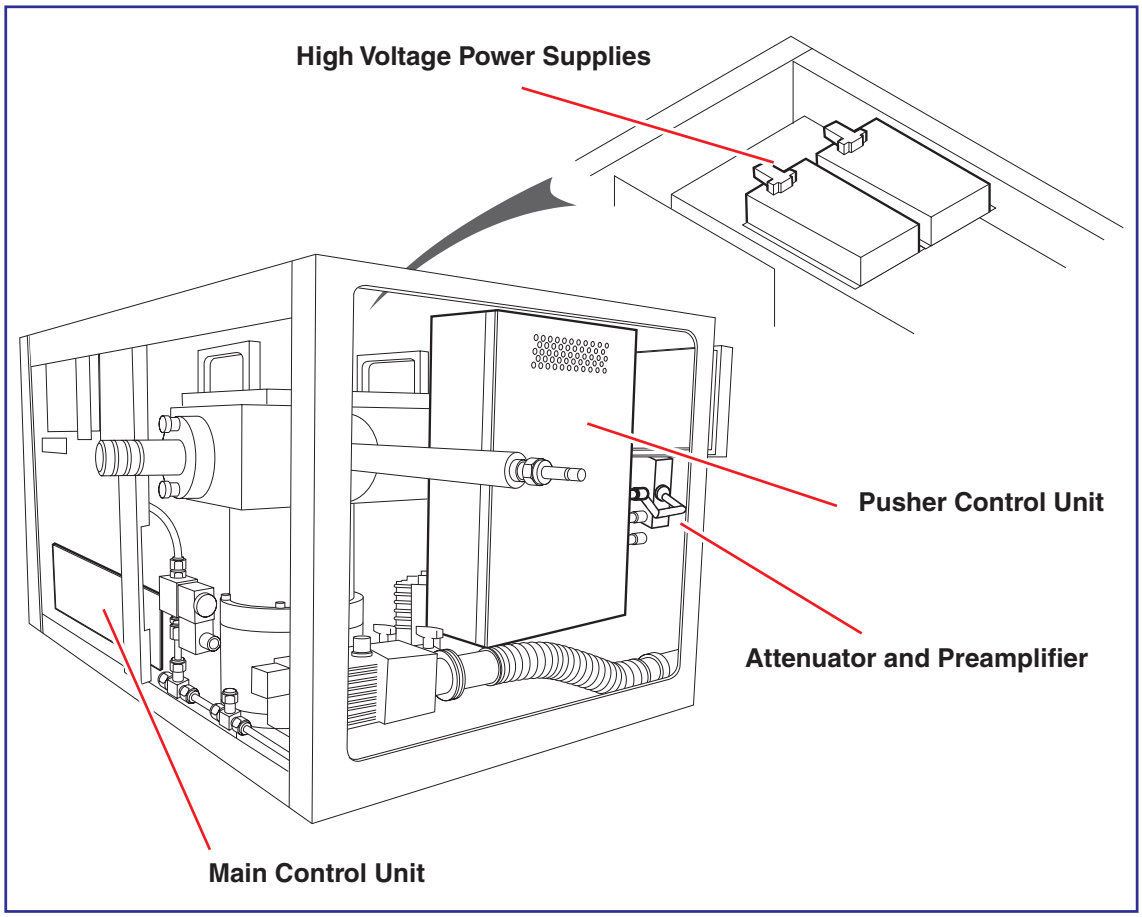
Power Requirements

The GCT electronics are designed to operate using supply voltages from 100-240V 50-60Hz AC at 6.0A max.

Caution: the RV3 rotary pump MUST be configured to operate from mains voltage range supplied. Refer to the pump manufacturer's literature.

Mains voltages are supplied to the MA3799 electronics unit only. A correctly rated safety earth must be provided in all cases.

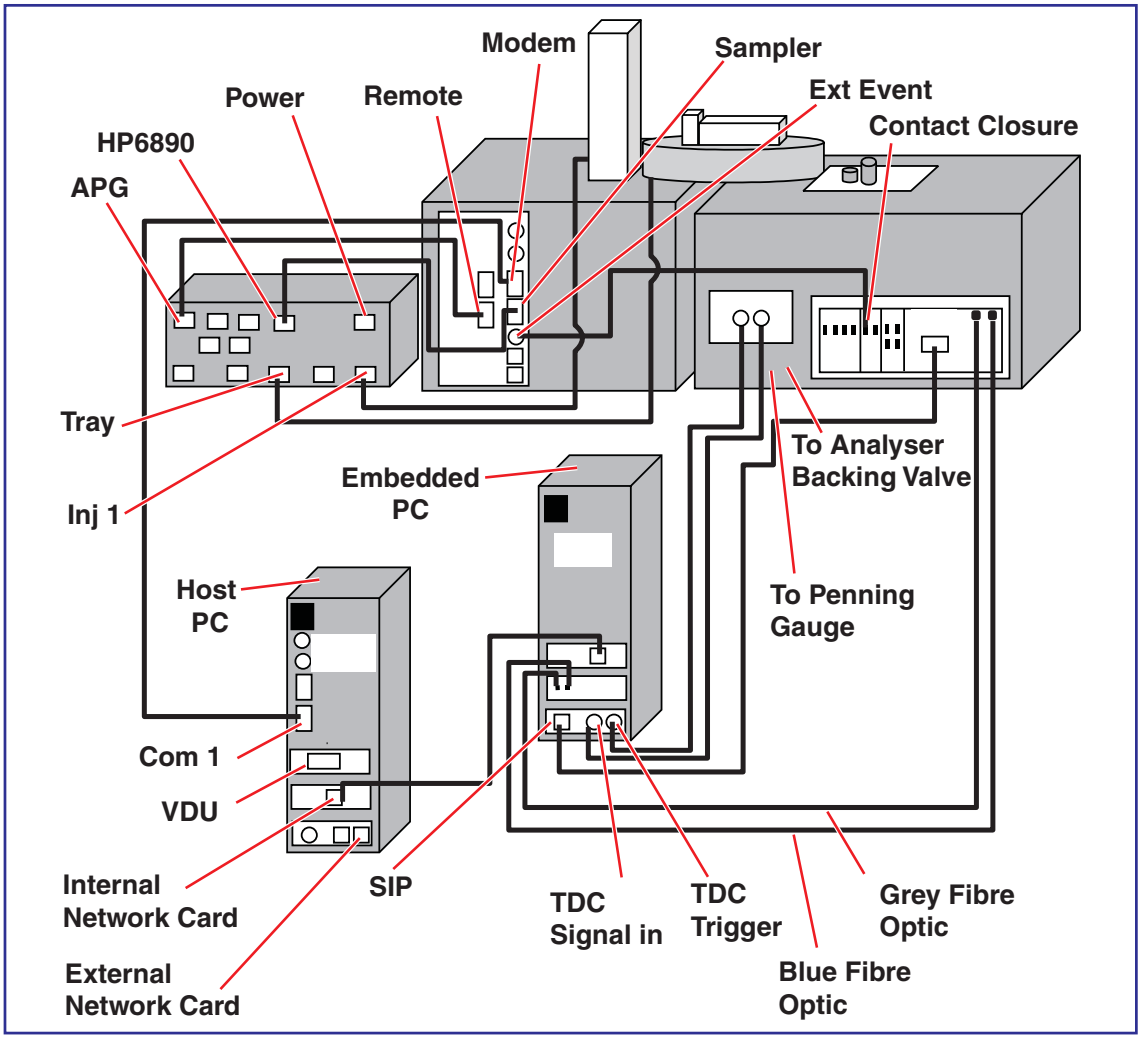
Caution: The two PC's supplied with the instrument must be configured to operate from the mains voltage range supplied. Refer to the manufacturer's literature.



The main electronics modules of the system are:

- High voltage power supplies, supplying the pushout voltage and detector voltage.
- Main control unit.
  This controls the ion source vacuum logic, and heaters, and supplies high voltages for the time of flight analyser.
- Pusher control unit.
  Supplying the high frequency pusher voltage.
- Attenuator and preamplifier.
  These components condition the output of the detector before the signal travels to the TDC (time to digital converter)

External Wiring



Front Panel Indicators

Vacuum Light Status Messages

The front panel vacuum LED, and a series of diagnostic messages displayed on the tune page indicate the state of the vacuum system. Some vacuum states have intermediate substates. The vacuum state is indicated at the bottom of the tune page. The substate may be displayed from the diagnostics menu on the Engineer Tuning Menu. The following states have been defined.

The vacuum state is indicated at the bottom of the tune page. The sub state may be displayed from the DIAGNOSTICS menu in the Engineer Tuning Menu. The LEDs indicate the vacuum state on the instrument front panel.

LED colour changes are at 1 second periods unless stated otherwise.

State		Status LED	Sub state	
0	Vented	Red	0	Vented
1	Analyser Pumpdown	Red→Yellow	4	Isolation valve closing
			3	Source backing valve closing
			2	Analyser backing valve opening
			1	Analyser turbo run up
2	Isolated	Yellow	0	Isolated
3	Source Pumpdown	Off→Green	6	Isolation valve closing
			5	Analyser backing valve closing
			4	Backing line stabilising
			3	Source backing valve opening
			2	Source turbo run up
			1	Analyser backing valve closing
4	Pumped	Green	17	CI PURGE – Analyser and source backing valves closing
			16	CI PURGE – Backing line stabilising
			15	CI PURGE – CI gas in and pump-out valves opening
			14	CI PURGE – CI gas in valve closing
			13	CI PURGE – CI pump-out valve closing
			12	CI PURGE – Analyser and source backing valves opening
			11	CI PUMP – Analyser and source backing valves closing
			10	CI PUMP – Backing line stabilising
			9	CI PUMP – CI pump-out valve opening
			8	CI PUMP – CI pump-out valve closing
			7	CI PUMP – Analyser and source backing valves opening
			6	PROBE LOCK PUMP – Analyser and source backing valves closing
			5	PROBE LOCK PUMP – Backing line stabilising
			4	PROBE LOCK PUMP – Probe lock pump-out valve opening
			3	PROBE LOCK PUMP – Probe lock pump-out valve closing and backing pressure stabilising
			2	PROBE LOCK PUMP – Analyser and source backing valves opening
			1	PROBE LOCK PUMP – Probe lock pumped LED illuminated
			0	Pumped
5	Venting to isolated	Green→Yellow	4	Isolation valve closing
			3	Source backing valve closing
			2	Analyser backing valve closing
			1	Source pump run down
6	Venting to vented	Red→Off	6	Isolation valve closing
			5	Analyser backing valve closing
			4	Source backing valve closing
			3	No pump run down
			2	Analyser pump run down
			1	Source pump run down
7	Timeout in state 1	Yellow→Off	0	Waiting for vent command
8	Timeout in state 3	Red→Green	1	Isolation and source backing valves closing
			0	Waiting for isolate or vent command
9	Source pump trip	Yellow→Green→Red→Off	1	Isolation and source backing valves closing
			0	Waiting for isolate or vent command
10	Analyser pump trip	Yellow→Red→Green→Off	2	Isolation and source backing valves closing
			1	Analyser pump run down
			0	Waiting for vent command
11	Intermediate state for aborted pumpdown	Green→Yellow	0	Waiting for delay
12	Time-out in state 4	Green (3s)→Red (1s)	3	No pump run down
			2	Analyser pump run down
			1	Source pump run down
			0	Waiting for vent command
13	Time-out in state 5	Yellow (1s)→Red (3s)	3	No pump run down
			2	Analyser pump run down
			1	Source pump run down
			0	Waiting for vent command
14	Time-out in state 6	Green (1s)→Red (3s)	3	No pump run down
			2	Analyser pump run down
			1	Source pump run down
			0	Waiting for vent command

State 7 requires a vent request to exit.

State 8 requires a vent or isolate request to exit.

State 9 requires a vent or isolate request to exit.

State A requires a vent request to exit.

State C requires a vent request to exit.

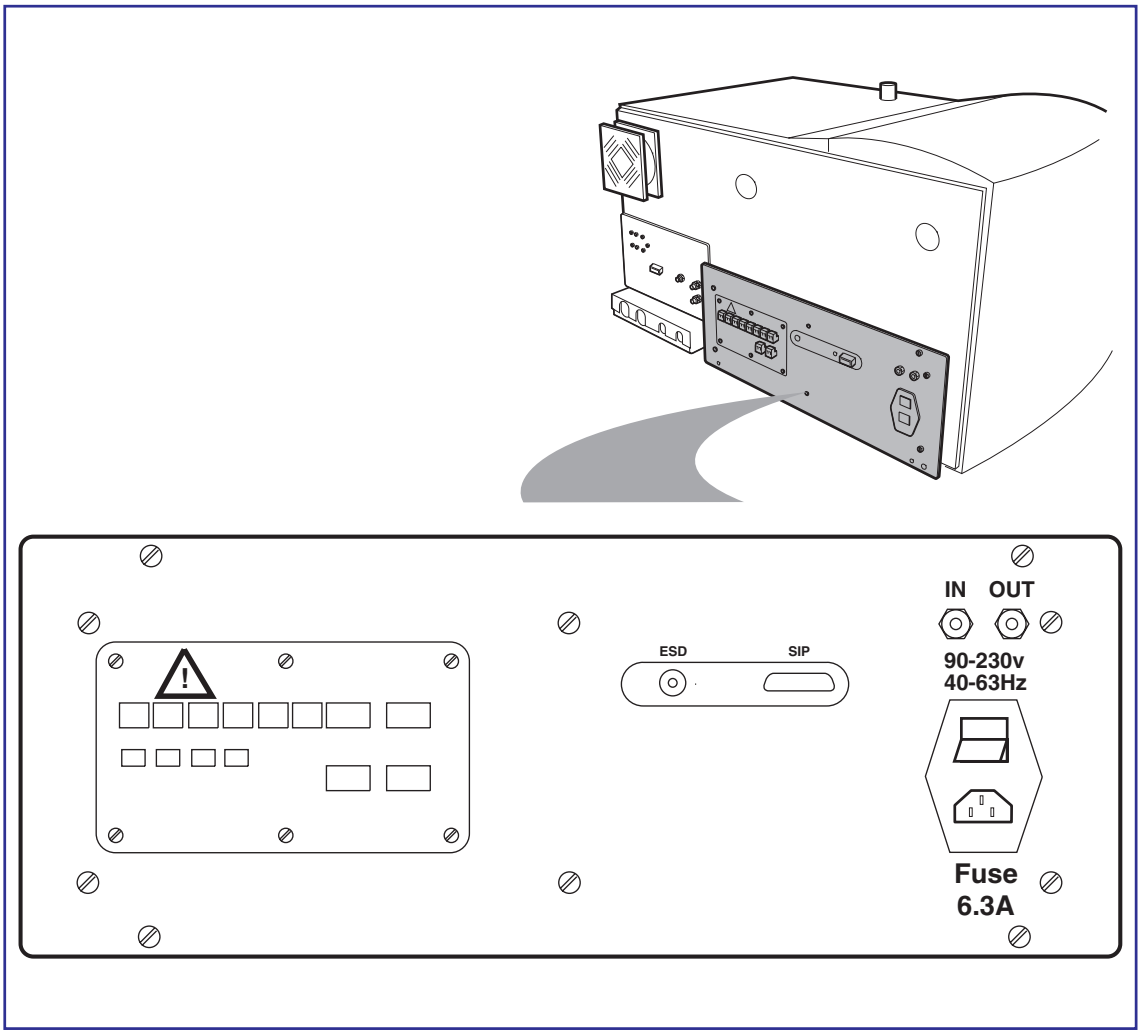
State D requires a vent request to exit.

State E requires a vent request to exit.

Operate / Standby Light Status

State	Status Operate LED
Operate	Green
Standby	Red
Source solvent trip	Yellow

Rear Panel Connections



SIP

The TDC (time to digital converter), located in the control PC, receives the SIP (scan in progress) signal via this connector.

Optical Communications Link

Communications between the control PC and the instrument are via the two optical connectors labelled IN and OUT

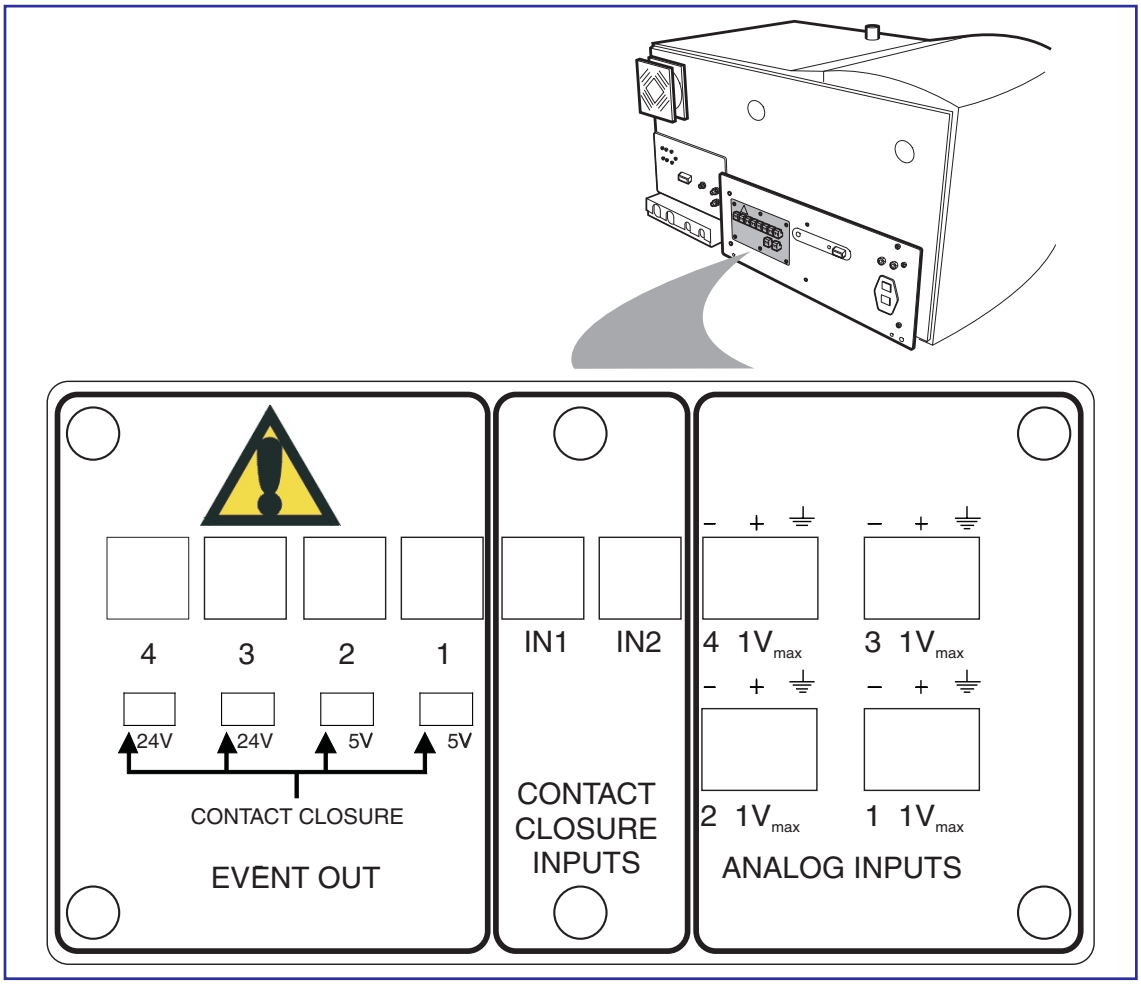
Mains Connection and Power Switch

Mains voltage is connected using an IEC plug on the RHS of the rear panel. An instrument power switch allows the control electronics to be isolated from the supply. The 6.3A fuse protects the system in the event of the instrument drawing more than the rated current.

ESD Earth

This is provided as an earth point for personal antistatic wrist band.

If the electronics PCB's are to be handled, then the necessary antistatic precautions must be taken.



Event Out

Four contact closure outputs, **Out 1** to **Out 4**, are provided to allow various peripherals to be connected to the system.

Out 1 and **Out 2**, voltage output, each have an output of 5 volts. The voltage output of both **Out 3** and **Out 4** is 24 volts. Alternatively, by switching the selector switches the outputs can be set to **Contact Closure**.

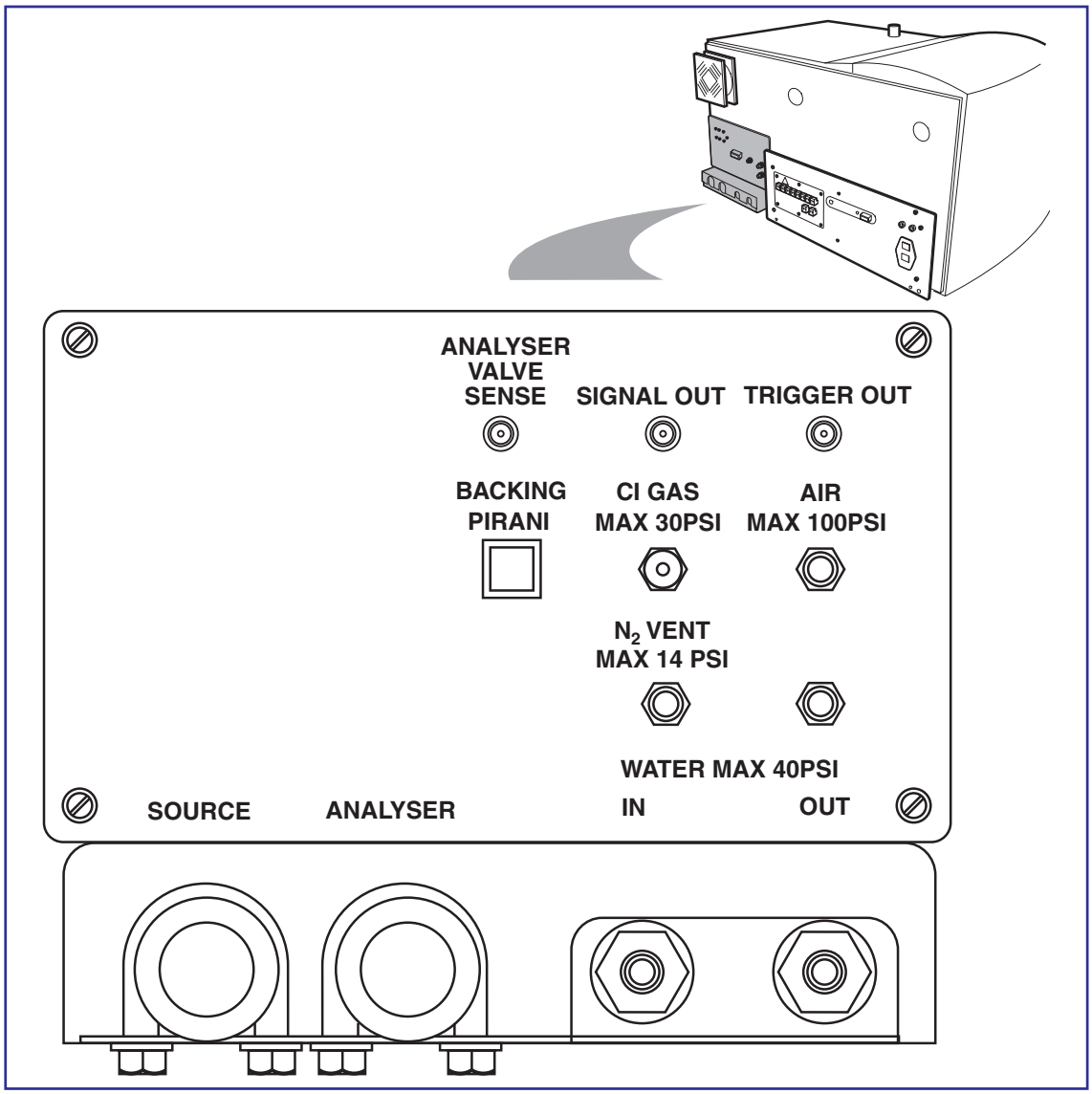
During a sample run an event output may be configured to actuate between acquisitions and is used typically to enable an external device to start at the same time as the acquisition start.

Contact Closure Inputs

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 FID detector output. The input differential voltage must not exceed one volt.



Rear Service Panel

The rear service panel connections and maximum operating pressures are shown above.

CI Gas

1/8" inlet for coupling to CI reagent gas cylinder.

Air

The upper fitting allows a compressed air supply to be fitted for operation of the isolation valve and backing line isolation valves. 100psi Max.

The lower fitting allows the compressed air supply to be routed to the analyser backing isolation valve situated on the head of the rotary pump.

Source

Connection from rotary pump to the source turbomolecular pump backing line.

Analyser

Connection from the rotary pump to the analyser turbomolecular pump.

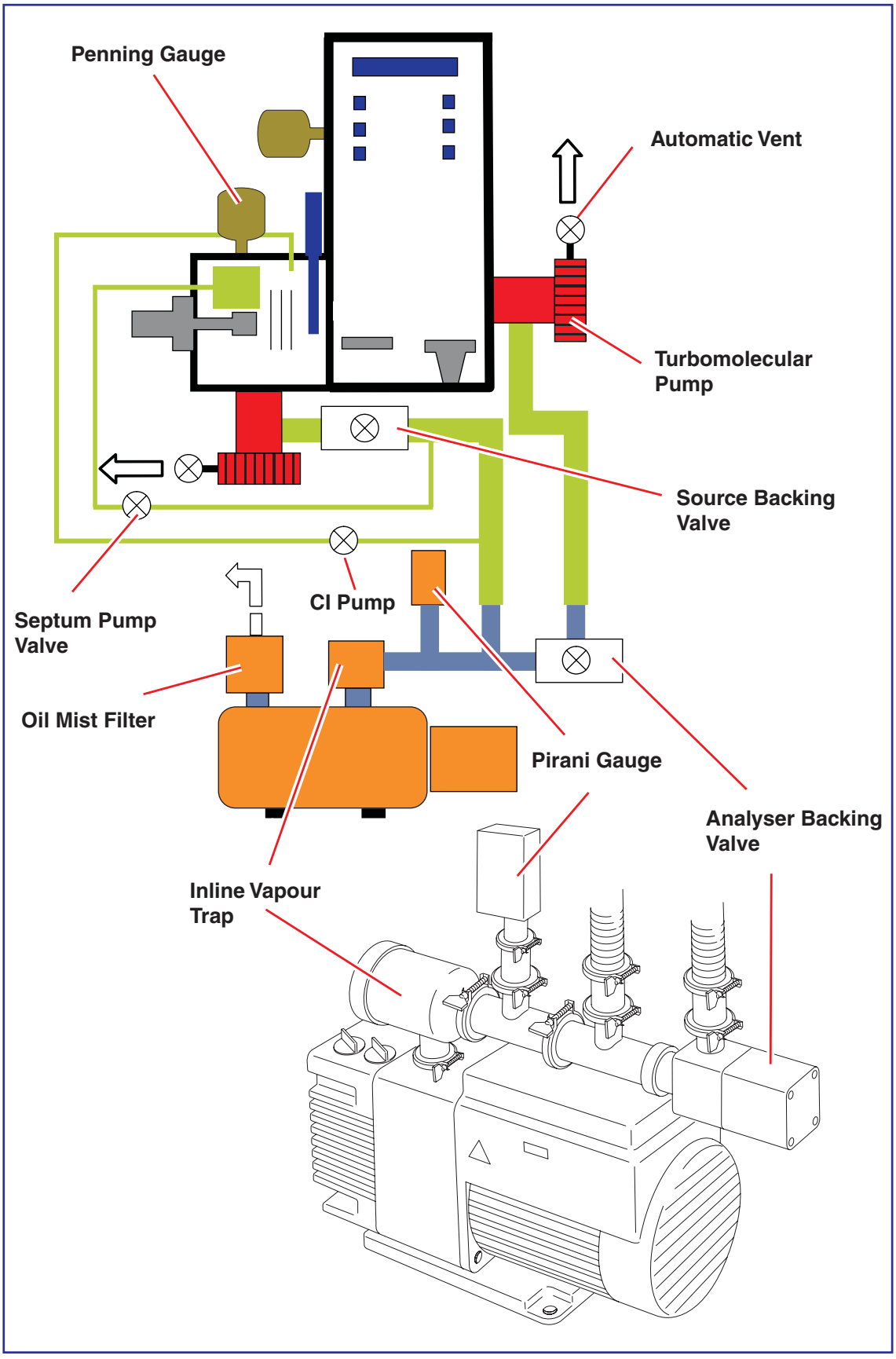
Water in / Water out

The turbomolecular pumps are water cooled, with a water supply pressure 10-40psi.

N2 Vent - Max 14psi

Connection for dry gas for venting the turbo pumps. Using dry gas prevents water from entering the instrument during a vent and reduces the subsequent pumpdown time.

The Vacuum System



Fine Pumping

GCT is equipped with two water cooled high compression turbomolecular pumps, providing independent fine pumping of the source and the analyser housings. Details of operation and maintenance of the pumps can be found in the manufacturer's manuals provided.

Rotary Pumping

Both the source and the analyser turbomolecular pumps are backed by a single RV3 rotary pump. This pump is usually situated on the floor adjacent to the GCT. Details of operation and maintenance of the pumps can be found in the manufacturer's manuals provided.

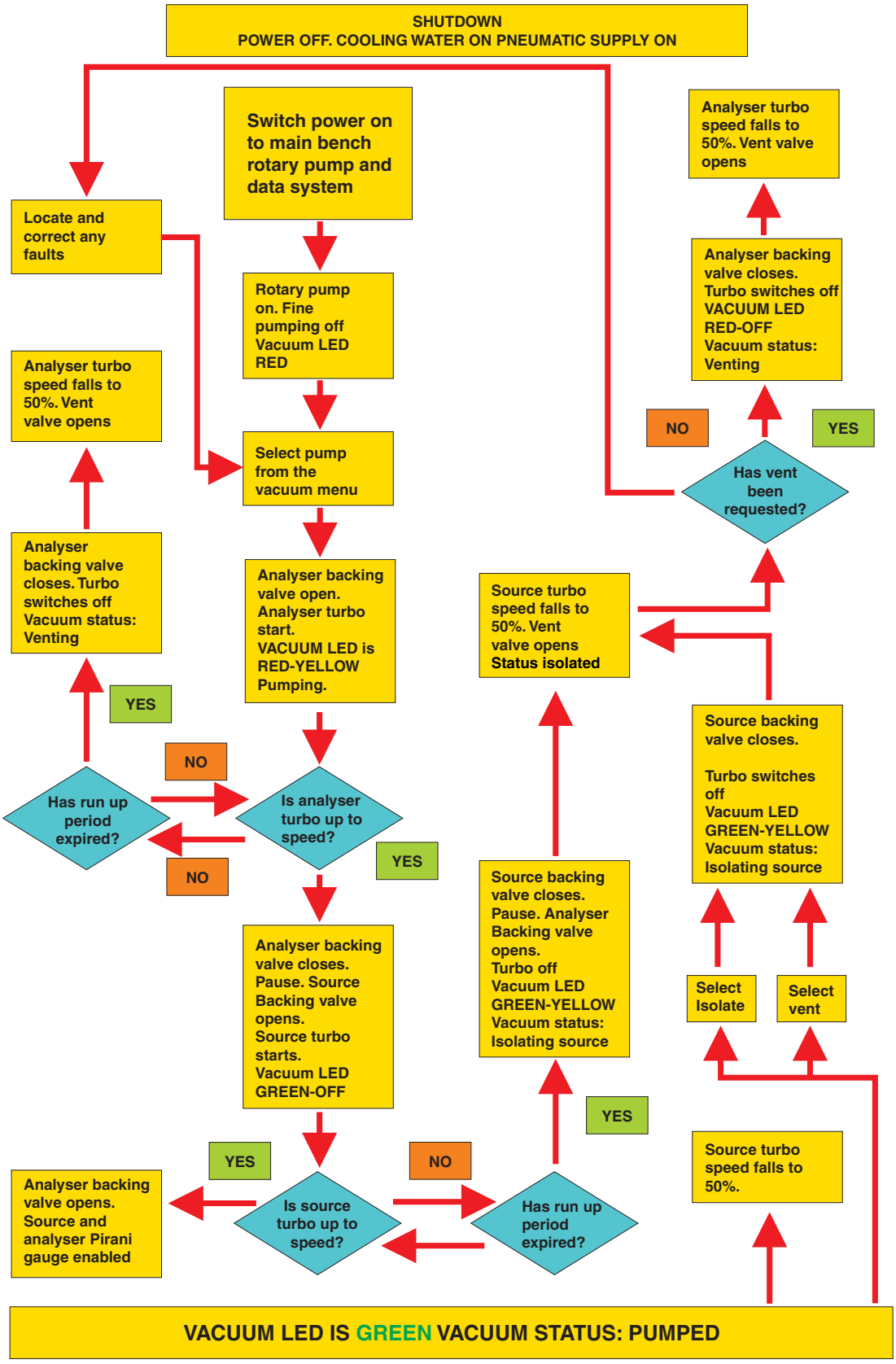
Pressure Measurement

The source pressure is monitored by an active Pirani gauge. If the pressure read by this gauge exceeds the 'solvent trip' level set by the user from the software, the filament current in both EI and CI modes of operation, and the extraction voltage in FI operation, is reduced to zero.

Once the pressure has fallen below the trip level normal operation is resumed.

The analyser pressure is also monitored by an active Pirani gauge. This gauge acts as a pressure switch turning the system out of operate if the pressure is too high. Pressure readings may be displayed on the MassLynx NT tune page.

Automatic Pumpdown and Vacuum Protection



MassLynx NT Data System

A PC computer runs the MassLynx NT software system to control the GCT, 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 GCT:

- 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.
- OpenLynx (option), automatic, post processing software (option).
- Visual Basic (option). This software development system is used to create macros for use with MassLynx NT and other Windows NT applications. The MassLynx NT User's Guide describes the many facilities of the Micromass software. Documentation for the other software is also supplied.

Routine Procedures

Safety Information

Generic Warnings

Before installing or operating the GCT, read the following topics concerning hazards and potential hazards. System operators must be familiar with both general and specific safety practices concerning the GCT.

Persons with a medical condition, for example a back injury, which prevents them from handling heavy loads should not attempt to lift the instrument. Micromass accepts no responsibility for any injuries or damage sustained while lifting the instrument.

Caution: Under no circumstances should the instrument be lifted by the front moulded cover, the probe, the GC interface or the source housing.

Before lifting the instrument proceed as follows:

Vent, power down and disconnect the instrument from the power supply.

Disconnect power and tubing connections to the rotary pump from the rear of the instrument.

Disconnect gas inlet and exhaust lines from the rear of the instrument.

Lifting and Carrying

The weight of the instrument is 100kg. UK Health & Safety guidelines recommend that suitable lifting equipment is used to lift or move the instrument. Note that Micromass personnel are not permitted to manually lift the instrument without such equipment.

Assess the Risk of Injury

Take action to eliminate risk.

If Some Risk Still Exists

Plan the operation in advance and in conjunction with our engineer when he/she arrives on site.

Use trained personnel where necessary.

Adhere to appropriate country and/or company regulations.

Ventilation Requirements

Caution: Hazardous vapours. A suitable exhaust line should always be fitted to the rotary pump.

Environmental Requirements

Altitude: up to 2000M

Temperature: 15 - 40°C for the rotary pump. Maximum laboratory temperature for the instrument is 30°C. Optimum temperature range is 19-22°C.

Maximum relative humidity: 70%.

Mains supply voltage fluctuations not to exceed ± 10% of the specified voltage range.

Mains supply transient overvoltages according to installation category II of IEC 644.

Pollution degree 1 in accordance with IEC 664.

Disposal

Do not incinerate electronic assemblies. Emission of noxious fumes may occur and metal cased capacitors may explode due to build up of pressure.

Oil from the vacuum pumps should be drained and disposed of appropriately.

Power Requirements

The GCT electronics are designed to operate using supply voltages from 100-240V 50-60Hz AC at 6.0A max.

Caution: the RV3 rotary pump MUST be configured to operate from the mains voltage range supplied. See instructions supplied with the rotary pump.

Mains voltages are supplied to the MA 3799 electronics unit only.

A correctly rated safety earth must be provided in all cases.

Caution: The two PC's supplied with the instrument must be configured to operate from the mains voltage range supplied. Refer to the manufacturer's literature.

Heated Zones

Warning: Risk of burns. Never touch a heated transfer line, GC injector cap with bare (unprotected) hands.

Care should be taken when dealing with any heated area of the GCT. For example, the GC transfer line, the solids probe tip, the reference gas (septum) inlet and the GC injector cap. In addition, the inner and outer ionisation sources may remain hot for many minutes even after removed from the source housing.

Allow heated zones to cool before attempting to handle these items.

Using Methane Gas

Caution: Methane gas is explosive. When the instrument is run in chemical ionisation mode (CI), which involves the use of methane gas it is necessary to vent the effluent from the rotary pump in a safe manner. All gas fittings should be checked for leak tightness. Avoid naked flames.

Using Ammonia Gas

Caution: Ammonia gas is a hazardous vapour. When the instrument is run in chemical ionisation mode (CI), which involves the use of ammonia gas, it is necessary to vent the effluent from the rotary pump in a safe manner. All gas fittings should be thoroughly checked for leak tightness.

Moving Parts

Do not remove the cover from the pneumatic source – analyser isolation valve with the compressed gas line attached to the rear of the GCT and the lines pressurised.

Possible Hazard From Pressurised Housing

If a dry gas supply is fitted to the soft vent inlet on the rear of the instrument, the regulator must be set to deliver a pressure of gas less than 14 psi, (1000 mbar, 1 atm). Failure to set this valve correctly can lead to the analyser housing becoming positively pressurised.

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 clean, dry compressed air to the connector on the service panel at the rear of the instrument. Adjust the outlet pressure to 7 bar (80 - 100 psi).

Connect a suitable dry gas, such as dry nitrogen, for example, to the soft vent inlet at the rear of the instrument.

Caution: On some instruments a pressure regulator is connected between the dry gas inlet and the analyser turbomolecular pump vent valve. The regulator should be set to a max of 6 psi 500 mbar when the venting gas is attached. If set to a higher pressure the analyser may become pressurised.

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 rotary pump vacuum tubing is connected to the rear of the instrument.

Check that the rotary pump is connected to a suitable mains supply.

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

Check that the optical communication cables, and the TDC trigger, input and SIP cables are connected from the instrument to the control PC.

Check that the etherlink connection is made between the control PC and the host PC

Switch on the electronics using the switch situated on the service panel at the rear of the instrument.

Switch on the host PC.

As supplied Windows NT is automatically activated following the start-up sequence, whenever the data system is switched on.

Log on to Micromass account (password analysis).

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

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

Switch on the control PC and wait approximately 2 minutes for the program to boot up.

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

The **Vacuum** LED on front of the instrument shows steady red to indicate that the system is fully vented.

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.

Switch the rotary pump on at the pump.

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

Click on **Pump Analyser**.

On MassLynx v3.5 onward a message will appear 'Perform automatic MCP conditioning after pumping?' Refer to the next section 'MCP Conditioning' and select as appropriate.

The analyser foreline isolation valve opens and the analyser turbomolecular pump starts.

The Vacuum LED on the front of the instrument shows flashing red as the analyser chamber pumps down.

When the analyser turbomolecular pump has reached operating speed the Vacuum LED changes to steady amber.

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

Click on **Pump Source**.

Caution: *The manually operated septum pump valve should be fully open when the source housing is pumped down. Turn the black knob on the top of the interface anticlockwise.*

When the septum reservoir is vented it becomes full of air. This takes a long time to pump out because of the presence of the fused silica leak within the septum interface. The septum pump solenoid is actuated when the source backing line isolation valve opens and will evacuate the reservoir during source pumpdown.

If the septum reservoir pumping valve is closed, and the source housing is fully pumped down, the pressure indicated in the source housing will be high. This is due to air slowly entering the source housing via the fixed leak in the septum interface. The reservoir pump valve should then be opened slowly to minimise the effect of the surge of air into the backing line.

The analyser foreline isolation valve closes, the source foreline isolation valve opens and the source turbomolecular pump starts.

The Vacuum LED on the front of the instrument shows flashing amber as the analyser chamber pumps down.

When the source turbomolecular pump has reached operating speed the **Vacuum** LED changes to a steady green.

The analyser foreline isolation valve opens.

*Selecting **Pump Instrument** from the Vacuum menu rather than **Pump Analyser** will pump down the analyser and source housing sequentially, automatically passing through the states described above.*

The instrument is ready for MCP detector conditioning once the analyser pressure has reached a suitable value.

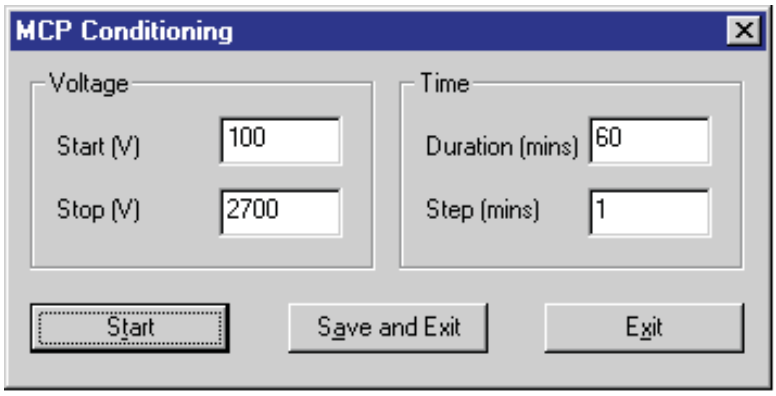
If the rotary pump oil has been changed or replenished, open the gas ballast valve on the rotary pump. See the pump manufacturer's literature for details.

Under normal conditions rotary pumps are audibly louder when running under gas ballast.

If the gas ballast valve is open, close it 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 a dry gas supply installed via the rear service panel. (eg: dry nitrogen). However, if the dry gas 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 the instrument will take longer to pump down to a usable vacuum.

MCP conditioning should be repeated after every analyser venting.

It is not necessary to recondition the detector if the instrument has been left out of the operate mode while still under vacuum, or if the source housing has been vented whilst the analyser housing remains under high vacuum.

The procedure for MCP conditioning is as follows:

Ensure that the analyser pressure is below 3e-6 mbar for at least 1 hour.

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

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

If the instrument is to be left in the operate mode continuously, even when not acquiring data, observe the following;

When no beam is present – reduce the **Trap Current** to zero in EI mode, **Emission Current** to Zero in CI mode or **Extraction Voltage** to zero in FI mode. This will minimise source contamination and maximise filament lifetime. This can also be achieved by adjusting the solvent trip level to a pressure lower than the source pressure readback.

Close the isolation valve from the software.

Caution: Exposure of the MCP's to large ion currents over extended periods of time will reduce the lifetime of the detector.

From the tune page, select **Options**, **MCP Conditioning** to access the MCP conditioning program.

Set **Start** to 100V, Stop to 2700V, **Duration** to 60 minutes and **Step** to 1 minutes.

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

Automatic MCP Conditioning

On MassLynx v3.5 onwards an automated pumpdown and MCP conditioning routine is available. Upon pumpdown of the instrument a dialog box will appear stating 'Perform Automatic MCP conditioning after pumpdown?

If 'Yes' is selected then pumping down will proceed in the usual manner. The instrument will wait until the analyser pressure is less than 4e-6 mbar, at which point it will turn into Operate and begin conditioning the MCPs according to the saved parameters in the MCP Conditioning dialog box.

In order to compensate for the usual 1 hour wait at <3e-6 mbar it is recommended that Auto MCP conditioning be set for 10 hours (i.e. 600 minutes)

Caution: The instrument will turn itself into Operate without user intervention once the pressure has dropped below 4e-6 mbar. Make sure all panels and covers are in place and no live voltages will be potentially exposed.

Instrument Warm-up

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

Allow the instrument temperature to stabilise for at least one hour.

If the instrument is to be left in the operate mode continuously, even when not acquiring data, observe the following;

When no beam is present – reduce the **Trap Current** to zero in EI mode, **Emission Current** to Zero in CI mode or **Extraction Voltage** to zero in FI mode. This will minimise source contamination and maximise filament lifetime. This can also be achieved by adjusting the solvent trip level to a pressure lower than the source pressure readback.

Close the isolation valve from the software.

Caution: Exposure of the MCP's to large ion currents over extended periods of time will reduce the lifetime of the detector.

Using the Instrument

The GCT is now almost ready to use. To complete the start up procedure and prepare for running samples, follow the instructions described in the section *Obtaining a Beam* in the chapter relevant to the ionisation mode to be used.

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 control unit instrument and switch off all peripherals.

A loss of data is likely. The instrument will vent using residual power generated from the spinning turbo pumps to power the vacuum control electronics.

Overnight Shutdown

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

Set the Trap current (**EI**), emission current (**CI**) or extraction voltage (**FI**) request to Zero.

Close the isolation valve from the **CLOSE ISOLATION VALVE** button on the Engineer Tuning Menu.

It is not necessary to turn the instrument out of the operate mode. However this is acceptable as long as instrument warm up time is given consideration when starting analysis.

Click on **Press for Standby**. Switching the instrument out of operate will automatically close the isolation valve.

Complete Shutdown

Click on **Press for Standby**.

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

Click on **Vent Instrument**.

A prompt is displayed to confirm the vent command.

Select **OK**.

The turbomolecular pumps are switched off. When the turbomolecular pumps have been run down to half their normal operating speed the vent valves are opened and the instrument is automatically vented. Both front panel LED's appear red.

Switch off the rotary pump using the switch at the pump.

Exit MassLynx.

Shut down the host PC.

Switch off the control PC.

Switch off all peripherals.

Switch off the power to the instrument using the switch on the rear panel of the control unit.

Installation and Removal of Inner Source EI and CI Mode

Warning: Risk of burns. The source may still be very hot when withdrawn.

Isolate the source housing from the **VACUUM / ISOLATE** option from the GCT tune page.

Caution: If the GC column is installed it is important to ensure that it is withdrawn from the source housing before removal of the inner source. Failure to do this will result in damage to the end of the GC column.

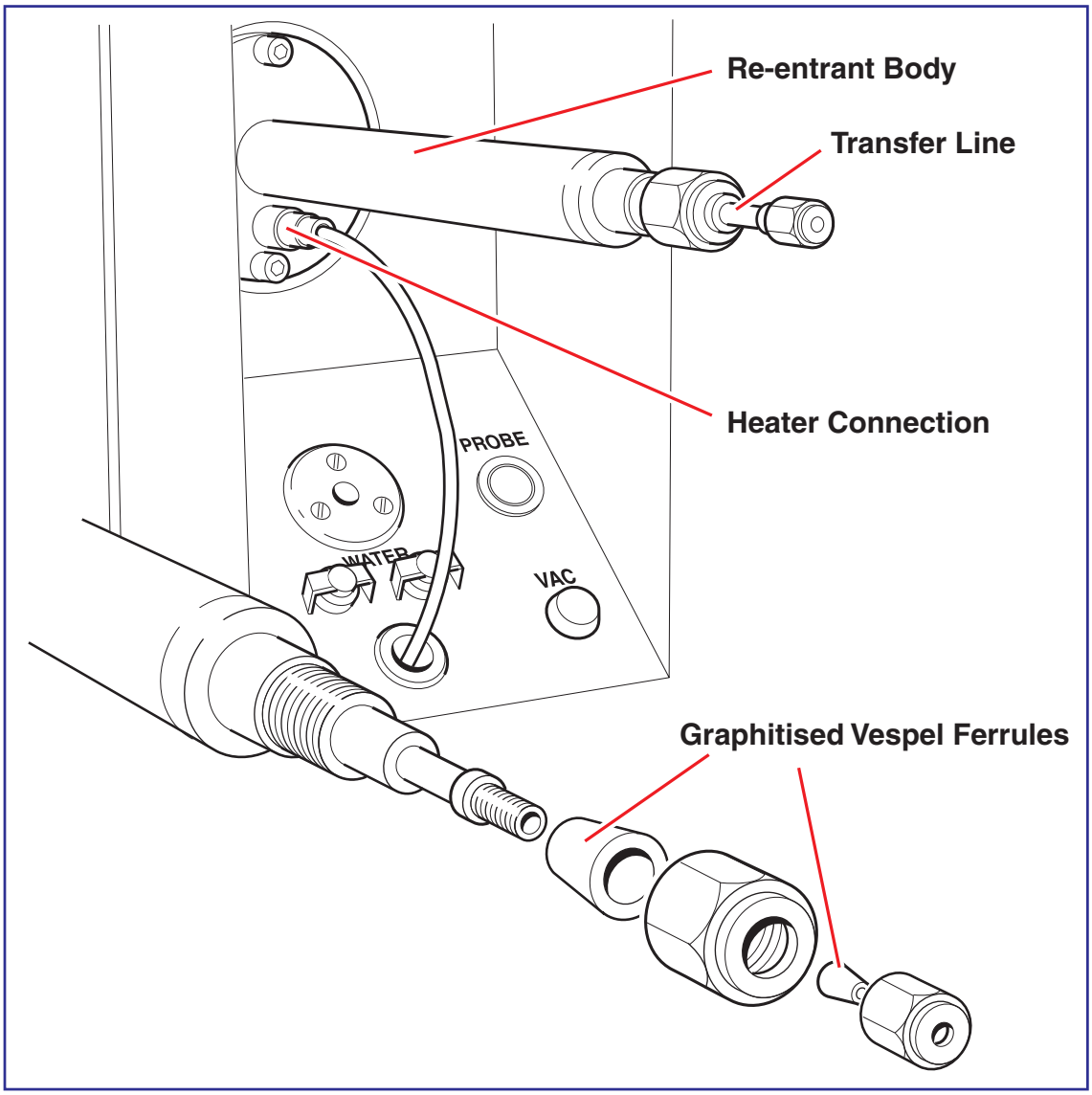
The inner source is retained by two thumb screws above and below the inner source handle. A Viton O ring between the handle and the source housing provides a vacuum seal.

To remove the inner source undo and remove the two thumb screws and pull the inner source from the source housing.

To replace the source follow the reverse procedure.

Care must be taken to present the inner source centrally to the outer source when refitting. There should be little resistance to insertion. The column can now be refitted and the source pumped down.

Installation of the GC Interface



The GC interface incorporates a spring loaded tip at the source end of the column transfer line. This allows the interface to be in contact with the outer source block to maintain reagent gas pressure for CI operation, and accommodates thermal expansion of the transfer line.

It is important to ensure that the transfer line is in the correct position relative to the inner source when fitting the interface. If the interface is not fitted and the transfer line position not determined proceed as follows:

Isolate the source housing using **ISOLATE SOURCE** from the **VACUUM** menu options in the GCT tune page.

Wait for the Vacuum status light on the front panel to become a constant yellow.

Remove the blanking flange or probe lock from the side of the source housing.

With the GC interface detached remove the inner transfer line which is retained by the 1/4" nut and 6mm graphitised Vespel ferrule at the rear of the interface. If the 6mm ferrule is very tight on the transfer line it should be replaced.

The ferrule can be removed by cutting part of it away with wire cutting pliers. Care must be taken not to mark the transfer line shaft as this results in air leaks.

Check that the spring-loaded tip has maximum travel when compressed. If required the compression spring may be stretched to allow maximum movement.

Fit the GC interface using the two retaining screws provided. The heater connection on the interface flange should be at the bottom of the assembly.

Fit a new 6mm ferrule and the retaining nut over the transfer line and feed the transfer line through the re-entrant assembly into the source housing.

Push the transfer line in so that the transfer line stops against the outer source and the spring is fully compressed.

Withdraw the transfer line by 4mm and tighten the 1/4" nut to hold the line in position. It should be possible to ‘feel’ the spring compressing to adjust this position.

The GC column may now be installed or the transfer line blanked to allow pumping of the source housing.

If the transfer line position has been previously determined and the interface removed (to allow the probe lock to be fitted), it is not necessary to replace the 6mm ferrule when replacing the interface. The procedure below should be followed;

Loosen the 1/4" fitting retaining the inner transfer line into the interface and pull the transfer line back a short way.

The 6mm ferrule will normally be tight on the transfer line and its position will not change.

Fit the interface to the system as described above.

Push the transfer line in to the position dictated by the 6mm ferrule and tighten in position.

Installing the GC Column

If a GC column has not been previously installed proceed as follows:

Isolate the source housing using **ISOLATE SOURCE** from the **VACUUM** menu options displayed on the GCT tune page.

Wait for the Vacuum status light on the front panel become a constant yellow.

Remove the 1/16" blanking nut from the interface.

Prepare and install the GC column in the GC.

The recommended position of the column into the injector is detailed in the GC manufacturer's instructions provided.

Undo the two thumb screws retaining the inner source and withdraw the inner source.

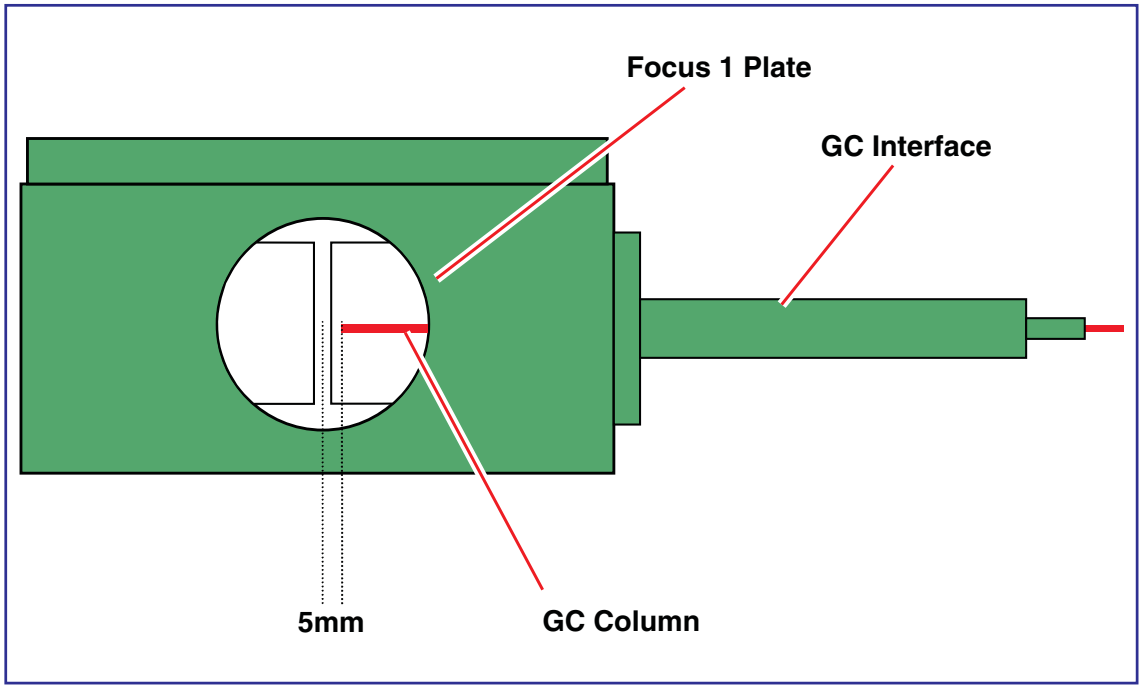
Insert the GC column through the interface transfer line to the required distance with respect to the centre line of the instrument. Mark the position of the column relative to the 1/16" retaining nut using typing correction fluid.

Ensure that no typing correction fluid enters the vacuum side of the mass spectrometer, as the background will compromise the sample data.

To determine the position of the GC column with respect to the centre line of the instrument, look down the inner source probe port with the inner source withdrawn.

The end of the column will be visible as it enters the source housing.

Position the end of the column approximately 5mm away from the centre line of the source, as shown in the diagram below.



For CI operation the column may be positioned closer to the centre line of the source.

Withdraw the GC column by 50mm into the transfer line.

Replace the inner source.

Replace the column to the marked position and tighten the retaining nut.

The system is ready to pump down.

Preparing for Operation in GC-FI Mode

The Field Ionisation source consists of a dedicated ‘outer’ source, dedicated cold septum inlet and an FI emitter mounted on a ‘probe’ which can be positioned within the ion source for best sensitivity.

To install the dedicated FI outer source follow the procedure below;

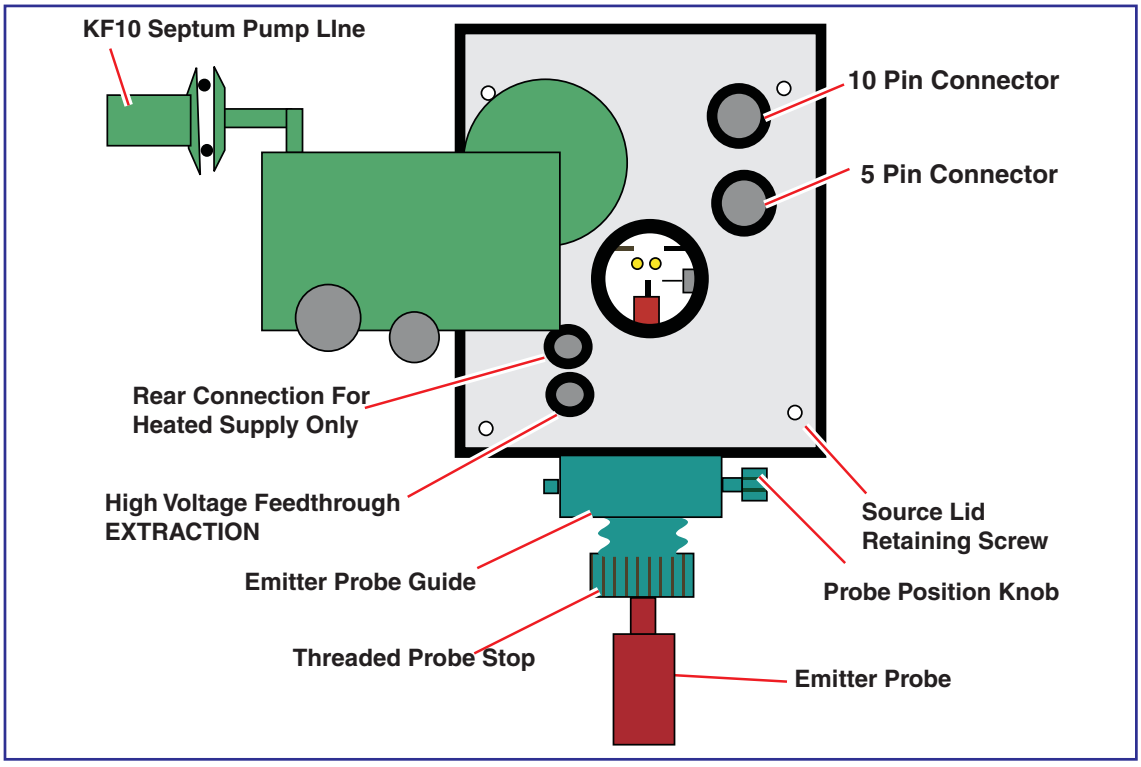
Remove the Outer EI/CI source as detailed in the section *Removal of the Outer EI/CI Source*, in the chapter *Maintenance and Fault Finding*.

Fit the FI outer source to the source housing.

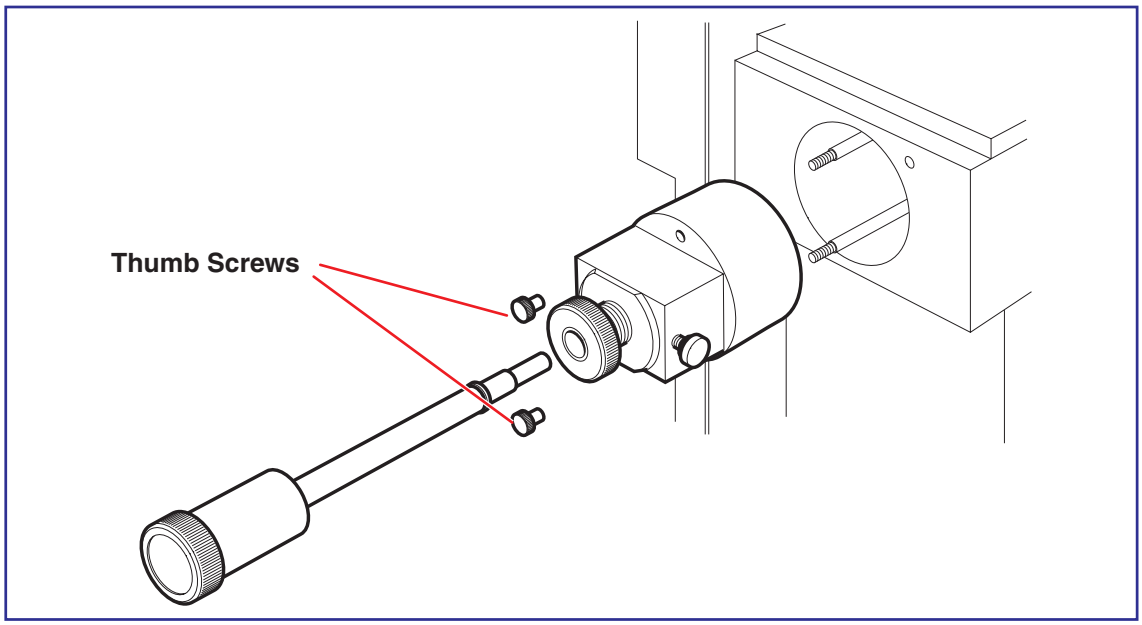
Insert the electrical connections to the source lid feedthroughs. The connection to the rear multi way feedthrough is common to both the EI/CI and FI source lid.

For the normal (unheated) source connect the high voltage extraction voltage cable to the front feedthrough.

If the heated FI option has been fitted, there will be two extraction voltage cables which connect to both the high voltage feedthroughs.



Fit the black acetal emitter probe guide to the front of the system using the same two thumb screws used to retain the EI or CI inner sources.

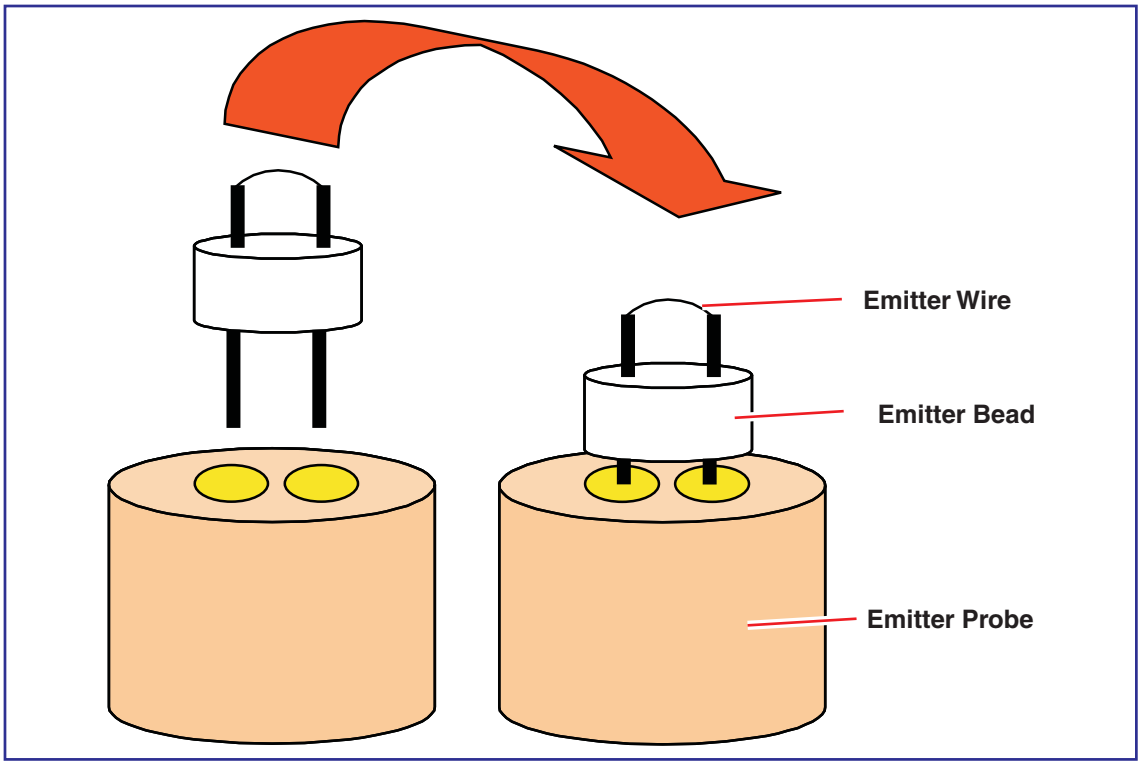


Completely remove the inner transfer line of the GC interface and replace with the dedicated GC-FI inner transfer line. This includes a ceramic tip which helps to minimise the disturbance of the electrical field close to the emitter. Leave the inner transfer line pulled back from the centre line of the source at this stage.

Carefully remove an FI emitter from its transportation box using a pair of tweezers to grip one of the emitter bead legs. The emitter wire is very fragile and should not be directly touched.

Caution: the emitter bead is made of ceramic and can shatter if put under undue stress. Wear protective goggles when handling the emitters.

Stand the emitter probe upright on a flat surface and carefully install the emitter bead into the end of the emitter probe. The emitter bead can be touched to allow the emitter to be pushed all the way onto the probe as long as care is taken not to touch the emitter wire directly.



Wind the threaded probe stop as far anti clockwise as possible.

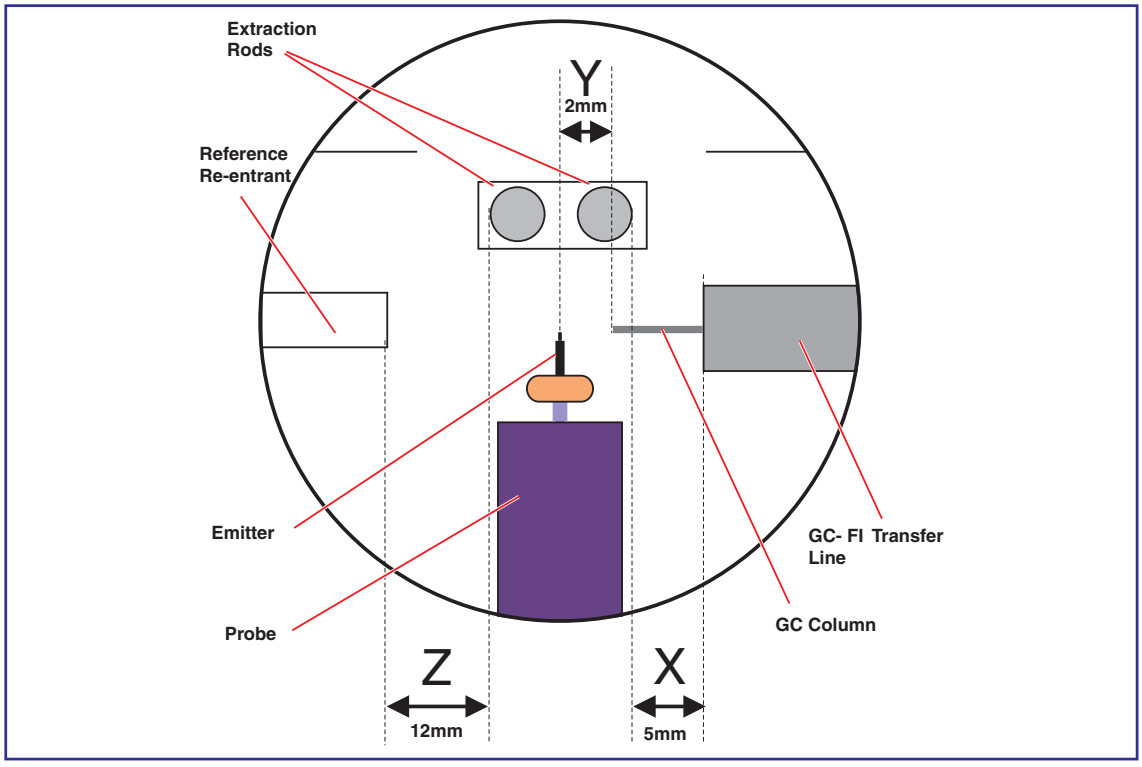
This will prevent the emitter from being pushed too far into the source and hitting the extraction rods.

Carefully insert the emitter probe into the source through the probe guide. Be careful to present the probe centrally and straight into the guide. Push the probe fully in. Vacuum seal is made via an O ring within the probe guide on the emitter probe shaft. Viewed through the clear viewing port on the top of the source lid the emitter will be 15mm or so away from the extraction rods.

The RHS cover may now be refitted. The septum interface cover should be left off to allow the emitter to be viewed whilst setting the column and emitter position.

Positioning of GC Column For FI Operation

Insert the inner GC - FI transfer line and lock in position approximately 5-10mm away from the edge of the RHS extraction rod whilst viewing through the clear viewing port on the top of the source.



X = about 5 -10mm

Y = about 2 - 4mm

Z = about 12 - 20mm

Insert the GC column through the transfer line and position between 2 and 4 mm away from the center of the two extraction rods. Tighten in place.

Carefully wind the threaded probe stop clockwise to move the emitter probe inwards. Position the emitter so that it lines up or is fractionally in front of the end of the GC column. NOTE: view the emitter through the viewing port during this operation to make sure that the emitter wire does not foul the rods or the end of the GC column.

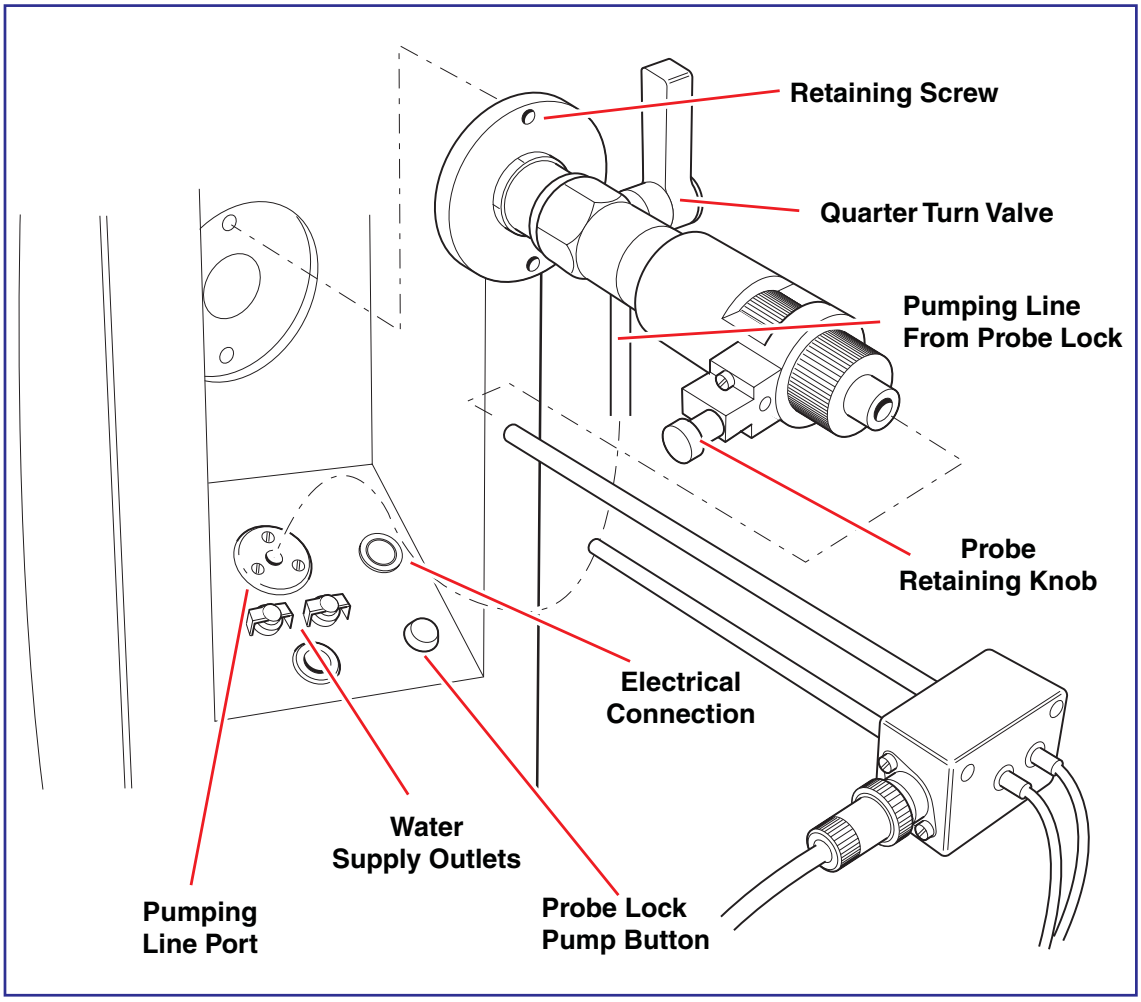
Adjust the probe position knob until the emitter is roughly central with respect to the two extraction rods.

Check the position of the emitter relative to the end of the GC column. If incorrect adjust the position of the GC column. If the GC column is to be moved always pull the emitter back 5mm to stop accidental damage.

Pump down the source housing using the pump source command from the Vacuum menu.

The source should be allowed to pump for 10 minutes before high voltage is applied.

The Solids Insertion Probe



Introduction

Solid samples, or liquid samples not sufficiently volatile for the reference inlet, may be admitted into the ion source using the solids insertion probe. The sample is loaded into a quartz sample cup, which is then located in the end of the probe. A thin strip of tungsten foil is used to hold the quartz sample cup firmly in position. The end of the probe holds a heating element and thermocouple assembly, close to the sample cup.

A fine stainless steel capillary carrying cooling water is wound close to the heater assembly. If the probe is to be heated then water flow ***must*** be maintained throughout the heating cycle to exert fine control over the temperature at the tip as well as to enable rapid cooling of the probe.

The probe may be heated rapidly by the current passed through the heater element. The thermocouple monitors the temperature, and the heating current is controlled electronically to maintain the required program rate and final temperature. However, it should be noted that interruptions to water flow may result in the probe heater being damaged.

Removing the GC Interface and Installing the Probe and Lock

The GC interface is attached to the source housing by two retaining screws on the interface flange. A vacuum seal to the housing is maintained by a Viton O ring seal within the interface flange. To remove the interface and attach the probe insertion lock.

Isolate the source housing using **ISOLATE SOURCE** from the **VACUUM** menu options in the GCT tune page.

Wait for the vacuum status light on the front panel become a constant yellow.

Loosen the 1/16" column retaining nut on the interface within the GC oven and fully withdraw the GC column from the interface.

Release the two GC plinth stops by turning the black knobs on the top right hand side of the GC plinth to allow the GC to be freely pushed away clear of the whole of the GC interface.

If required for access, the GC may be pushed to the back of the bench clearing the probe side panel.

Remove the heater connection to the interface.

Remove the two GC interface retaining screws and remove the GC interface.

Attach the probe lock using the two retaining screws provided. The quarter turn valve should be to the rear of the instrument and in the upright, closed position.

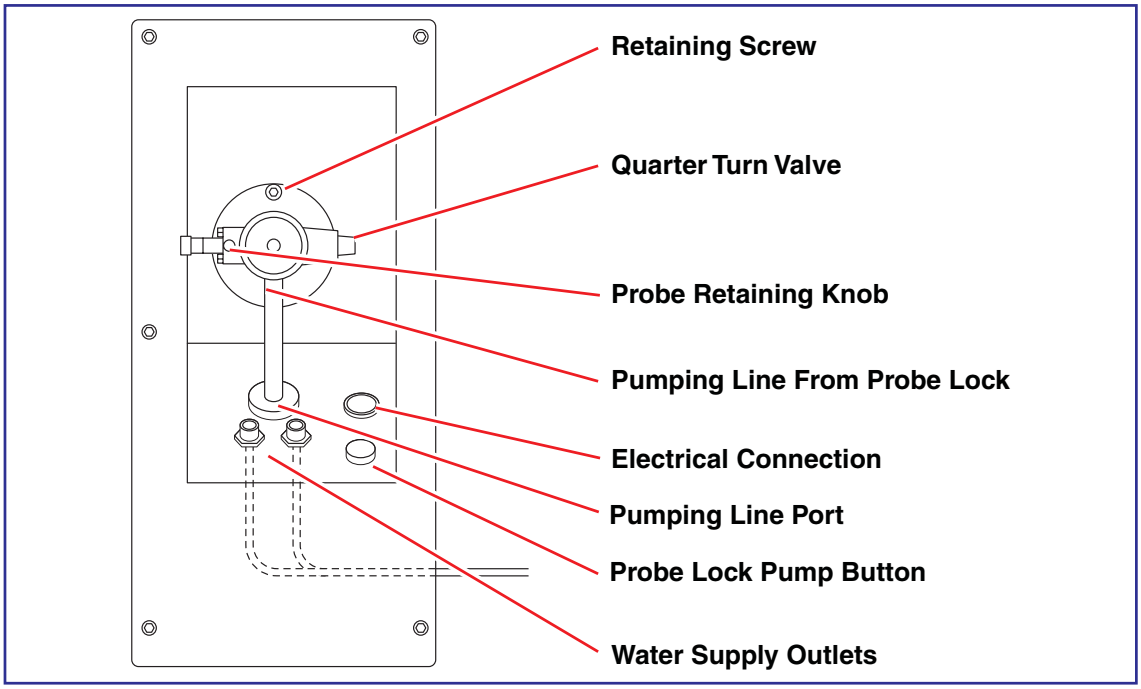
Ensure a Viton O ring is present in the groove in the retaining flange.

Insert the pumping line from the probe lock into the vacuum port on the probe side panel.

A further Viton O ring within this port will make a vacuum seal to the pumping line adapter allowing the lock to be evacuated prior to probe insertion.

Pump the source housing.

Probe Lock Side Panel



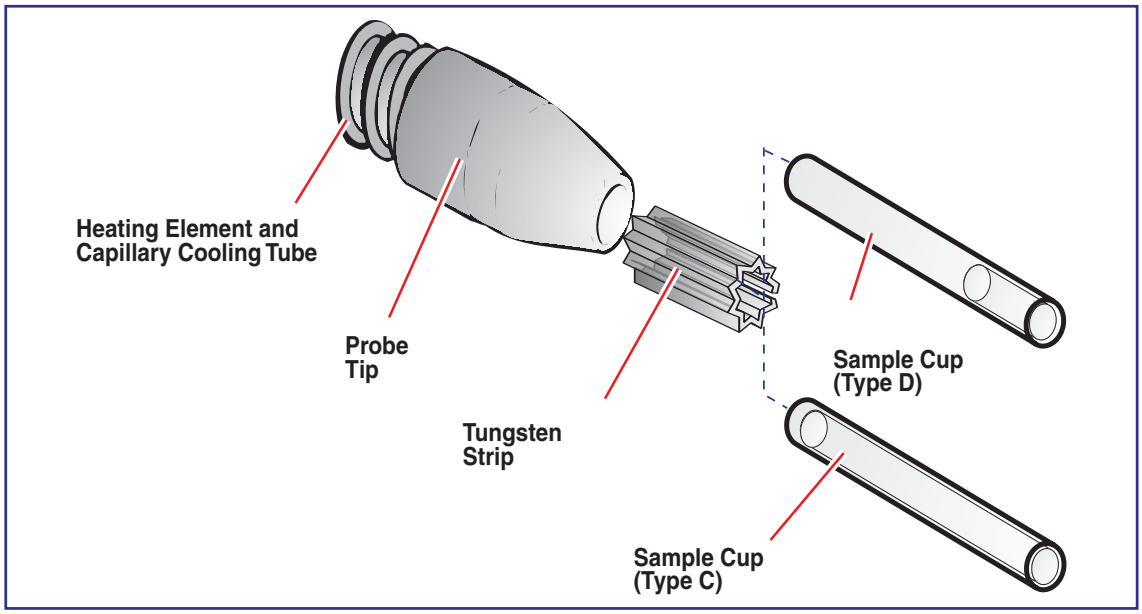
Sample Loading

Caution: Do not use sample cups over 15mm in length to avoid damage in the ball valve.

Two types of sample cup are available:

For involatile samples, the shallow cup (type D) is recommended. Because the sample is close to the heated ion source it is often unnecessary to heat the probe.

A deep cup (type C) is used for more volatile samples. The sample is deposited at the bottom of the cup to diminish the heating effect of the ion source. The sample can then be evaporated into the ion source using, if necessary, the probe heater.



Both types of sample cup should be pushed firmly into the probe tip to give good thermal contact with the heater element.

Caution: Ensure that the sample cup is retained firmly in the probe tip by the tungsten strip. Use two pieces of tungsten strip if necessary.

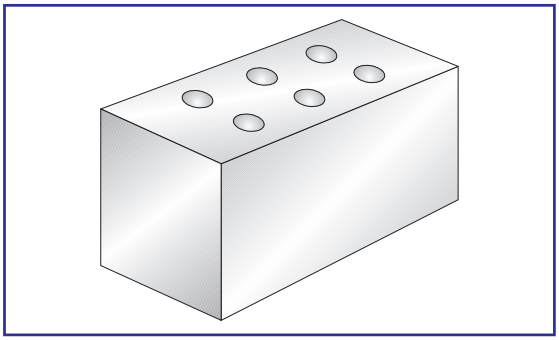
The sample cups can be loaded before or after inserting them into the probe.

Solid samples can be loaded into the cups using a thin piece of wire, or a drawn-out length of glass rod, to transport the sample into the cup.

Samples in solution are loaded using a microsyringe.

Make sure that the sample is deposited at the bottom of the cup and not around the neck, taking care not to leave any air pockets.

If a number of samples is to be run sequentially it may be preferable to load all the cups and stand them in a holder that can be made for this purpose by drilling a series of 5mm deep × 2mm diameter holes in a block of metal.



Any solvent used in loading can be evaporated by placing the sample tube(s) in a warm place, for example on top of the GC oven. Care should be taken not to heat the holder too quickly so as to avoid blowing the sample out of the cup with solvent vapour. Used sample cups should be cleaned in a hot flame and/or washed in solvent before re-use.

Water and Electrical Connections

Maintain connections to water and electrical supplies while the probe is in constant use. However, it is recommended that the water is disconnected overnight to minimise the risk of burst pipes through a rise in the water pressure. Disconnect the electrical connections to avoid heating the probe while the cooling water is disconnected.

To disconnect the water supply push back the collars on the water supply outlets, mounted on the probe side panel, allowing the probe connectors to be withdrawn.

Connection is simply the reverse: push back the collars, insert the connectors and release the collars.

Inserting the Probe

Caution: Damage to the instrument may occur if the insertion lock is operated with the probe incorrectly positioned.

To insert the loaded probe into the ion source:

Check that the quarter turn valve is in the upright position.

Insert the probe into the introduction lock until the probe reaches the first stop.

Check that the water and electrical connections are made.

Check that the pumping line is connected to the port on the probe side panel.

press the Pump Probe switch on the probe side panel.

The vacuum levels can be displayed by selecting ***Vacuum Monitor****. Backing line pressure shows the pressure in the vacuum lock pumping line.*

When the inlet pressure falls below the trip level set on the Pirani gauge the green light in the centre of the pump probe switch will illuminate:

Slowly open the quarter-turn ball valve.

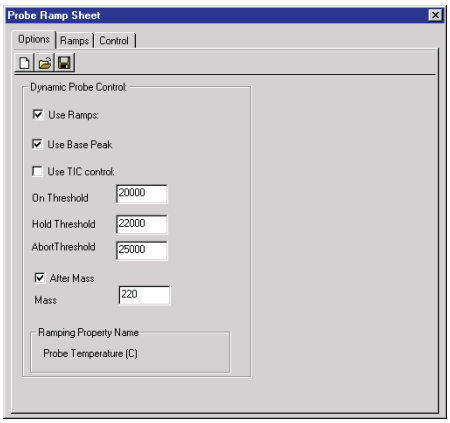
Pull the black probe retaining knob, located on the side of the probe lock, against its internal spring.

Slowly push the probe fully in.

It is recommended that the probe shaft be lubricated with molybdenum disulphide to ensure smooth travel.

A threaded probe stop collar is fitted to the probe lock to allow the probe fully in position to be set to the desired distance from the ion source. For CI operation, ensure that the probe hits the outer source by adjusting the collar fully clockwise.

Source temperature is often sufficient to evaporate the sample, particularly if the shallower sample cup is used. If this is not the case, or if a more controlled evaporation is required, the probe temperature may be ramped from software during acquisition. This menu is accessed from the inlet menu of the GCT instrument control page.



There is the option of using base peak or TIC Control offered on this page. This can be selected in conjunction with 'after mass' which takes the base peak or TIC above a specific mass to avoid reference peaks/carrier gas etc. When selected, the base peak or TIC is monitored as the ramp is applied. If it achieves the 'hold threshold' the probe temperature is held at its present value until one of the following conditions apply.

a) The base peak/ TIC drops to the 'On Threshold' in which case the ramp resumes.

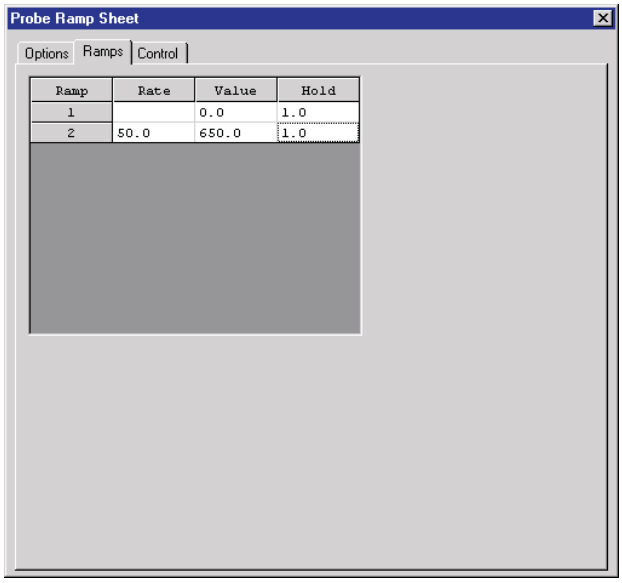
b) The 'abort threshold' is reached, in which case the probe heating is turned off until the base peak/TIC drops below the 'On Threshold'.

This control can help prevent detector saturation.

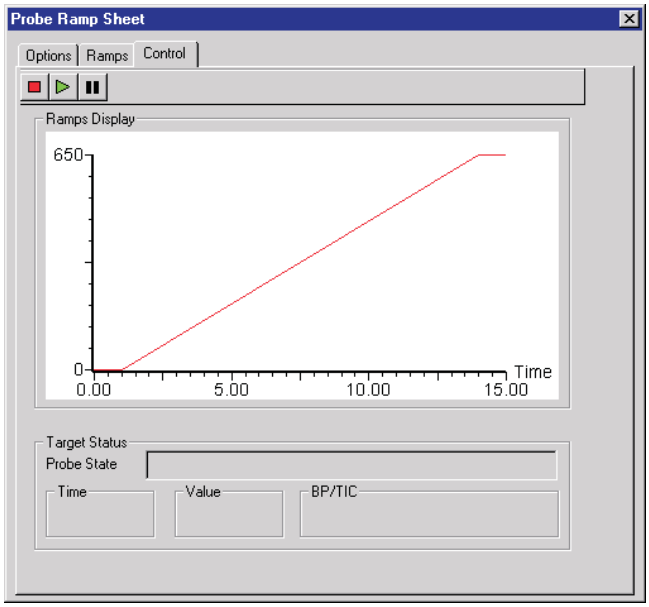
Multiple ramps can be set on the Ramps page.

It is possible to edit the ramp rate, value and hold parameters by use of the TAB key, the ENTER key and the ARROW keys.

The rate at which the probe is heated can be critical and varies with the type of sample. Some experimentation may be required to establish the optimum conditions for the evaporation of individual samples.



The resultant ramp is represented graphically on the 'Control' page.



The ramp can be started and stopped using the icons on this page.

Alternatively the ramp can be saved as a .prf file and can be run during an acquisition by selecting 'Use Probe ramp method' on the acquisition dialog page.

Caution: Do not heat the standard probe above 650°C.

Withdrawing the Probe

To withdraw the probe after use:

Allow the probe to cool to below 100°C.

This protects the structural integrity of the seals in the lock. The seals might easily be damaged if the probe is withdrawn at high temperature.

Withdraw the probe to the first stop.

Close the quarter-turn ball valve.

Pull the black probe retaining knob outwards against the internal spring.

Withdraw the probe from the lock.

Caution: Damage to the instrument may occur if the probe is removed without first closing the ball valve.

The DCI Probe

Introduction

Direct Chemical Ionisation (DCI) was originally developed as a soft ionisation technique for relatively involatile samples for use on EI/CI mass spectrometers. The samples are placed on a filament wire which is inserted directly into the ion source so that the filament tip is in the ion plasma. A current is passed through the wire and the sample is rapidly desorbed. As the sample is rapidly desorbed coupled with the fact that the sample is already directly in the ion plasma it results in a high abundance of the molecular ion [M+H]+ due to a short mean free path of the sample in which to fragment.

On the GCT mass spectrometer the DCI probe is inserted into the CI ion source via the insertion lock in a similar manner to the solids probe. The sample is loaded onto the end of the probe tip and, once inserted, can be desorbed by ramping the probe tip current using the MassLynx software.

Installing the probe lock and the DCI probe

Removing the GC interface and installing the probe lock is described in the section *"Removing the GC interface and Installing the Probe and Lock"* earlier in this chapter.

The DCI probe is supplied with a modified inner CI source with a larger (3mm) entry hole to accommodate the probe tip. You must use this special inner source with the DCI probe as the standard source will damage the probe tip. The modified DCI source will not work well with the GC due to the larger hole causing CI gas leaks and should only be used for DCI work.

To prepare to run the DCI probe complete the following steps;

Select ISOLATE SOURCE from the VACUUM drop down menu on the GCT tune page.

Wait for the vacuum indication light on the front of the instrument to become a constant yellow. You should hear the source venting and see the source vacuum gauge read-back turn off.

If you have not done so already then install the insertion lock on the right hand side of the source as described in the section "Removing the GC interface and Installing the Probe and Lock".

Remove the inner source and replace it with the modified DCI inner CI source supplied with the DCI probe. This source can be identified by its larger 3mm entry hole on its right hand side to accommodate the DCI probe tip.

Pump down the source by selecting PUMP from the VACUUM drop down menu on the GCT tune page.

The DCI probe is introduced to the CI source via the insertion lock. It is recommended that you lubricate the probe shaft with molybdenum disulphide to ensure a smooth travel - do not use SANTOVAC oil lubricant as you will see this as contaminant at 446 Da (i.e. [M+H]+ at 447 Da). The probe should be tight in the lock but it should move in and out with a little force. The probe cable should be plugged in to the PROBE socket on the side of the instrument below the insertion lock, there is no water cooling on the DCI probe.

Loading the Tip with Sample

The DCI tip consists of a small diamond of rhenium wire on a ceramic mount. It is pushed on to the end of the DCI probe for use.

The wire filament at the end of the tip is fragile so care must be taken when handling the tip to prevent damage.

Sample is normally dissolved in methanol or a similar solvent to a concentration of 1 ng/µl. Then, using a 10 µl syringe, deposit a microlitre onto the end of the DCI tip (i.e. 1ng of sample). Due to surface tension in the liquid the droplet should stay at the point of the wire tip. Alternatively if it is not important to know the amount of sample introduced then the tip can be carefully dipped into the sample solution. Wait for the solvent to evaporate, normally this will take a minute or so, and the probe can now be inserted into the source. In order to increase the amount of sample introduced on the tip use a correspondingly stronger solution. It is common to run samples between 1ng and 1 µg.

Inserting the DCI Probe

Caution: Damage to the instrument may occur if the insertion lock is operated with the probe incorrectly positioned.

The DCI probe is inserted into the source in the same manner as the solids probe as follows:

Check that the quarter turn valve is in the upright position.

Push the DCI probe into the insertion lock until the probe guide is engaged in the first stop.

Check that the probe cable is connected to the “PROBE” socket on the instrument side panel.

Check that the pumping line is connected to the port on the side panel.

Press the “VAC” pump probe button on the probe side panel to pump down the insertion lock.

The vacuum levels can be displayed by selecting Vacuum Monitor from the drop down menu on the GCT tune page. Backing pressure shows the pressure in the vacuum lock pumping line.

When the inlet pressure falls below the trip level the green light in the centre of the “VAC” pump probe button will be illuminated.

Slowly open the quarter turn ball valve on the insertion lock.

Release the probe retaining knob and slowly push in the probe.

Make sure that the probe is fully in so that the DCI tip is pushed up against the outer source to make a good seal for the CI gas. If necessary ensure this by rotating the threaded probe stop collar fully clockwise.

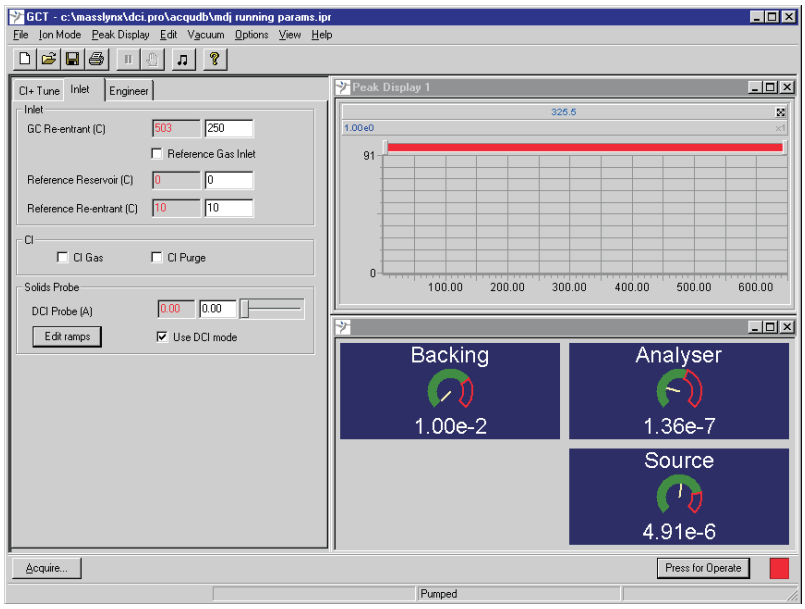
Calibration and Tuning

The instrument can be calibrated using heptacosa with a very small amount of CI gas if necessary in the same manner as for normal positive ion CI work. Pump out the heptacosa and use tris(trifluoromethyl)triazine as the lock mass ([M+H]+ ion at m/z 286.0027 Da). Normally <1µl is needed to get a strong signal. Introduce methane CI gas so that the source pressure is about 1x10-4 mbar. Tune up the instrument to give maximum sensitivity on the [M+H]+ 286 Da ion. For good CI this signal should be > than the peak at 285 (M+ ion) You should also see methane giving a signal at 29 Da.

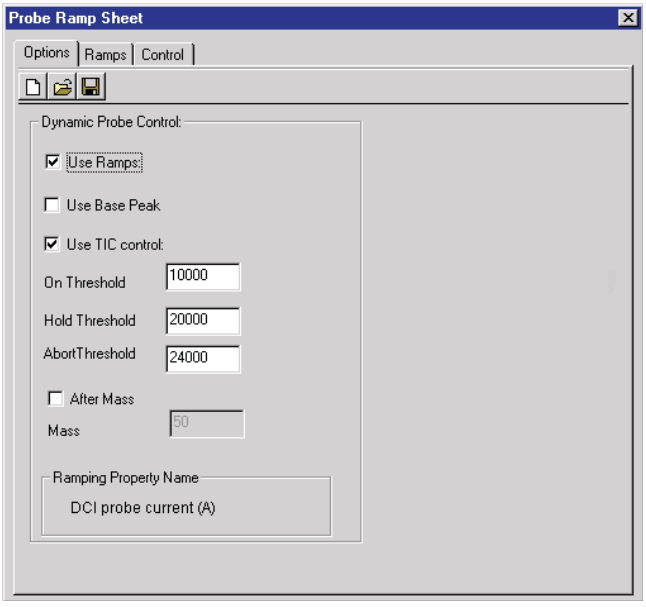
For more details of running the GCT in CI mode and calibration see the sections “Calibration and Exact Mass” and “Operation in Chemical Ionisation Mode” in this manual.

DCI probe control

The current through the probe tip is controlled from the MassLynx software on the GCT tune page “inlets” section. Tick the box next to “Use DCI mode” and the control will change to “DCI Probe” current in Amps.



The probe current can be controlled from 0 to 1.5A manually or the probe ramps can be used by clicking the EDIT RAMPS button or from selecting Probe control from the Options drop down menu.



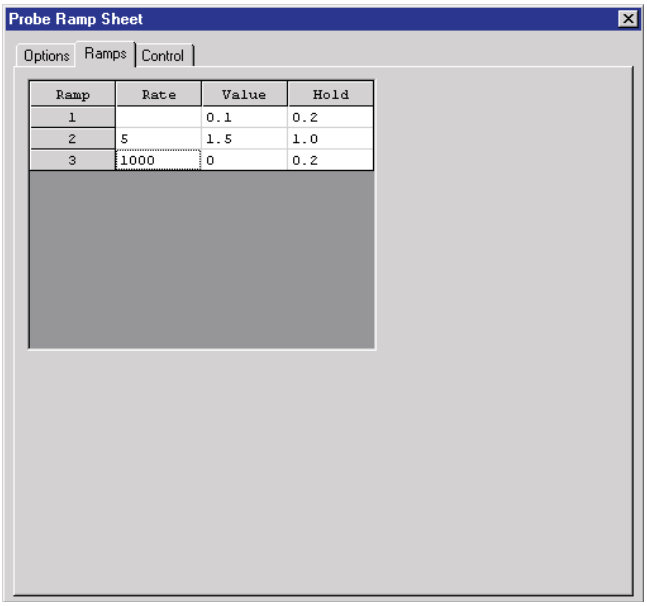
The Use Ramps box should be ticked in order to ramp the probe.

The DCI probe current can be regulated by the base peak in the spectrum or the Total Ion Count (TIC) if desired by ticking the appropriate box. When this option is selected the probe will ramp as defined on the Ramps page until the Hold threshold is reached. At this point the current will remain constant until the signal has dropped to the On Threshold at which point the ramp will resume. If the Abort Threshold is breached then the current will be turned off until the signal has dropped to the On Threshold.

For the purposes of this regulation if it is necessary to ignore all the peaks below a certain mass, for example to exclude the reference peak, then the After Mass box can be ticked and the appropriate mass entered in the box.

The new, open and save icons can be used to save the ramp as .prf files.

The ramp can be defined on the Ramp page. A typical ramp is defined below.



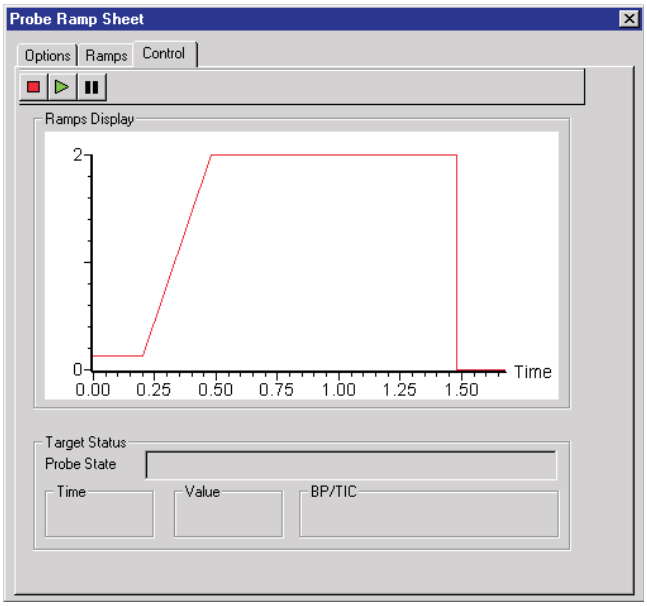
You can edit the ramp rate, value and hold time by using the TAB key, the ENTER key and the arrow keys.

This ramp will hold at 0.1A for the first 0.2 minutes in order to desorb any solvent or water that might be on the tip. The current is then ramped at 5 A/min in order to desorb the sample. It will then drop to zero after 1 minute.

In some situations it might be preferable to ramp the current very fast (i.e.1000 A/min) to the maximum value to make sure all the sample desorbs as fast as possible thus maximising the chromatogram peak.

Conversely for thermally sensitive samples it may be preferable to employ a slower ramp to prevent thermal degradation of the sample which may occur at a high current.

The Control page shows a graphical representation of the probe current ramp.



The probe ramp can be started from the Control window or set to start with an acquisition from the Acquisition window by selecting Use Probe Ramping Method and referencing the appropriate .prf file.

When the ramp is running a pink trace will appear on the graph to indicate the actual probe current.

Withdrawing the Probe

Caution: Damage to the instrument may occur if the probe is removed without first closing the ball valve.

Warning: the DCI probe may be hot. Always allow it to cool for a few minutes before withdrawing.

To withdraw the probe after use.

Make sure that the probe current is set to zero.

Note that passing a current through the probe tip in air could cause damage to the filament.

Withdraw the probe to the first stop.

Close the quarter turn ball valve.

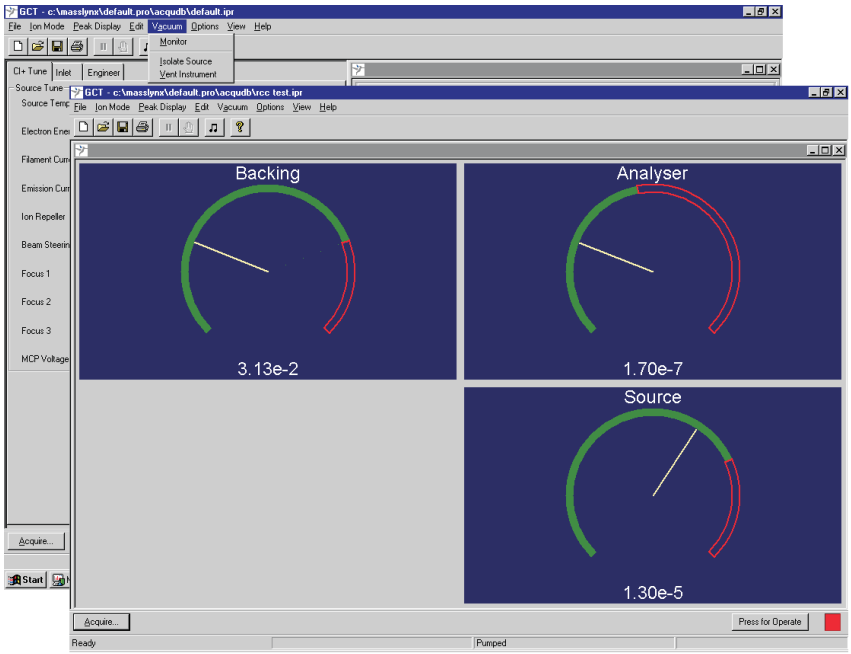
Release the probe retaining knob and withdraw the probe from the lock. Be careful not to knock the tip during removal.

Tuning Parameters and User Interface

Before the instrument is used to acquire sample data, it should be tuned and calibrated using suitable reference compounds.

Tuning parameters have been grouped into 3 menus as described below. Full details of source tuning procedures for each ion mode are given in the relevant chapter of this manual.

The Vacuum Display

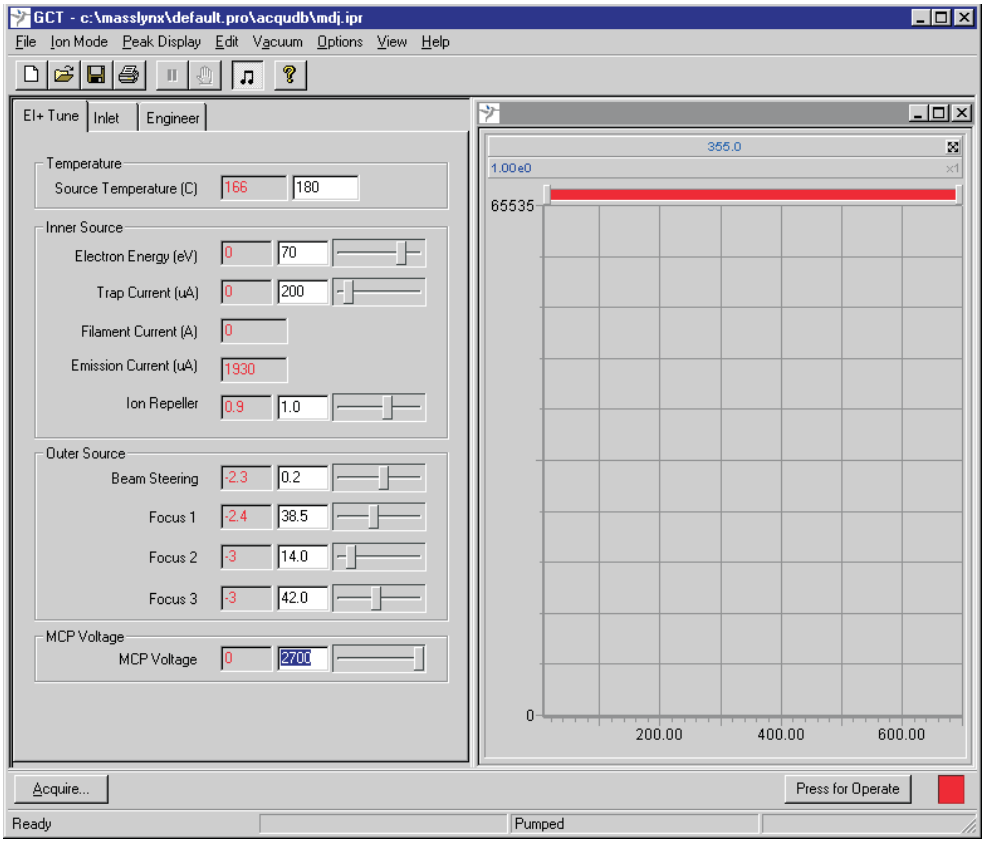


Display of the pressure reading in the source and analyser housings, and the rotary pump backing line are accessed from the **Vacuum Monitor** menu. The source and analyser readouts include the measured pressure and indicate the level of the **Source Solvent Trip** and **Analyser Trip** level on the relevant dials. These trip levels can be set from the Engineer Tuning Menu. The discontinuity in the green line at the border of the display indicates the level set.

The backing pressure trip displayed is read from the setting on the Pirani gauge at the head of the rotary pump. This level should be adjusted to be correct during installation.

Vent instrument and **Isolate Source** commands are also accessed via this menu.

Source Tuning Menu



The positive Ion Electron Impact (EI+) source menu is shown above.

Source Temp can be set depending on the type of analysis to be performed. For most analysis a temp between 180 and 250 Deg is sufficient. A higher temperature may lead to a higher degree of fragmentation, however too low a temperature may result in condensation of sample in the source block and loss of sensitivity.

Trap Current may be adjusted to increase or decrease the sensitivity of the source.

Filament Current and **Emission Current** are readbacks and are indicators of the efficiency of the filament. See section on operation EI mode.

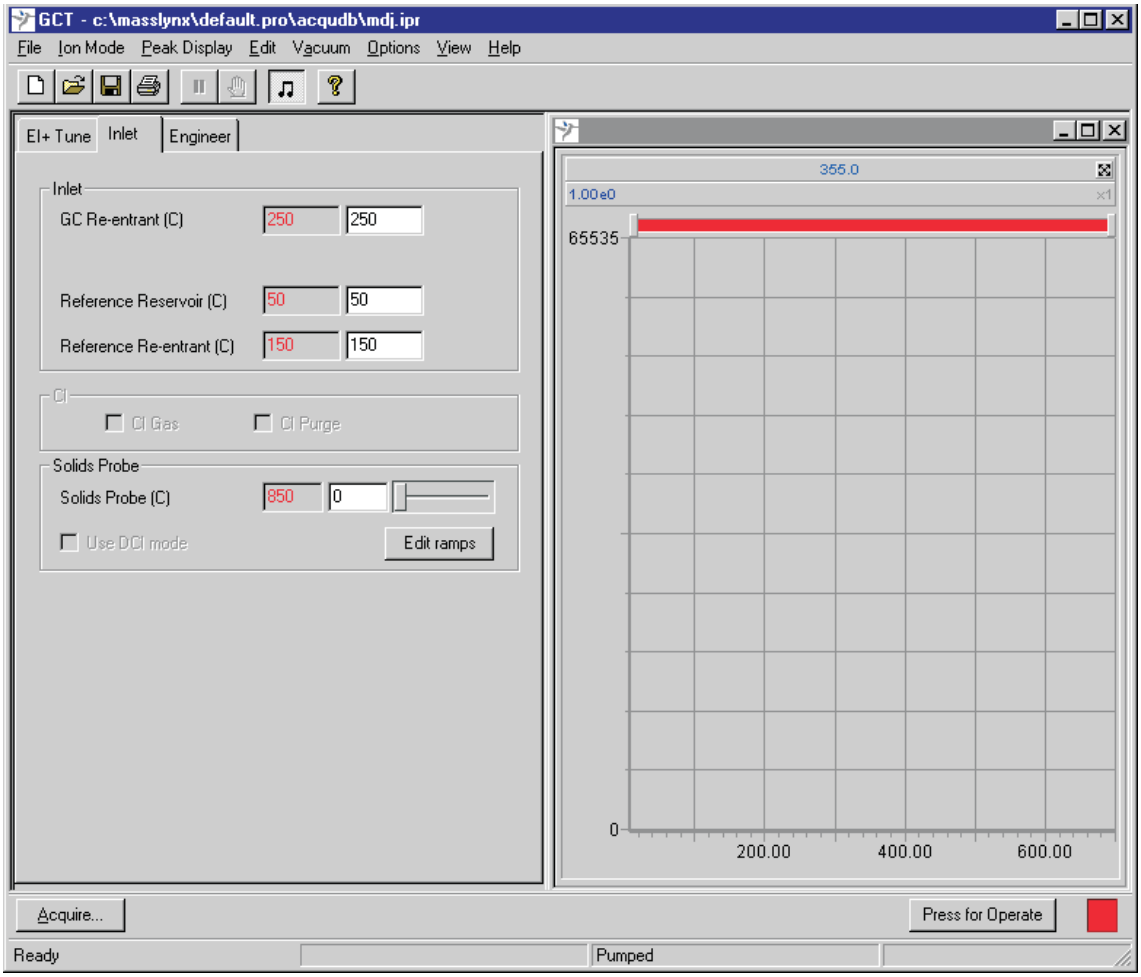
Ion Repeller is usually close to zero. High positive values indicate source contamination. The ion repeller usually optimises between 0 and +4 V.

Beam Steering allows the beam to be deflected from the centre line of the instrument during tuning.

Focus 1, **Focus 2** and **Focus 3** act to focus the ion beam into a thin ribbon for maximum transmission. Typical values are shown in the tune page above.

MCP Voltage. This voltage determines the gain of the detector and is normally set to 2700V. This voltage should be set in conjunction with **TDC Stop Threshold** (see later).

Inlets Menu



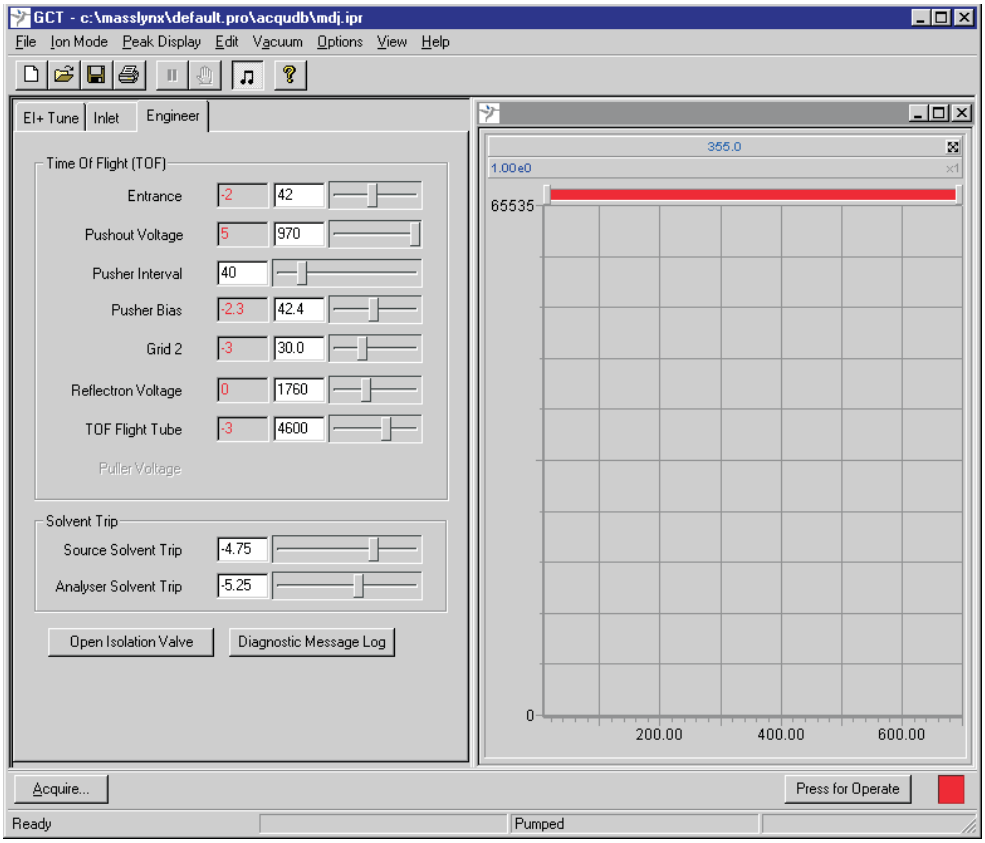
The **Inlets** menu shown above contains controls for the heated zones in the instrument. In addition, solids probe parameters may be set and CI reagent gas controlled in CI mode. When EI is selected as the ion mode the CI control entries are inactive.

GC Re-entrant. This is the set temperature of the GC interface. The value chosen depends on the type of analysis to be performed. 250DegC is a typical value.

Reference Reservoir. This is the set temperature for the septum reservoir. The septum may be run cold to reduce the speed of depletion of the reference material. However, a temperature of 50°C is recommended to prevent condensation within the reservoir and to speed up removal of reference material when changing ionisation techniques.

Reference Re-entrant. This is the set temperature for the transfer line which runs from the reference reservoir to the ion source. A set temperature of 150DegC should be maintained to minimise condensation of reference material.

Engineer Tuning Menu



The voltages controlled from the **Engineer Tuning Menu** should be set for optimum resolution on instrument installation. These voltages should not be varied during routine operation of the instrument.

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

Source Solvent Trip. Adjusting this value changes the position of the green trip level indicator in the source vacuum display. If the pressure read by the source Penning gauge exceeds the ‘solvent trip’ level set by the user from the software, the filament current in both EI and CI modes of operation, and the extraction voltage in FI operation, is reduced to zero. Once the pressure has fallen below the trip level normal operation is resumed. This functionality protects the source filament from the rise in source pressure as the solvent front elutes in a GC MS experiment.

The value for **Solvent Trip** should always be set to a pressure slightly higher than the current pressure reading.

Analyser Trip. Adjusting this value changes the position of the green trip level indicator in the analyser vacuum display. If the pressure read by the analyser Penning gauge exceeds the **Analyser Trip** level set by the user from the software, the system is automatically set to standby. In normal operation the **Analyser Trip** should be set to 3e-6 mbar.

Pusher Interval sets the frequency of the pusher pulse is determined by the **Pusher Interval** entered. **Pusher Interval** may be set between 33 µsec and 250 µsec.

A **Pusher Interval** of 33 µsec permits ions to be analysed up to m/z780.

The mass range is proportional to the square of the flight time, so a flight time of 66 µsec permits ions to be analysed up to 4 x 780 = m/z3120 .

Increasing the flight time reduces the duty cycle (sampling efficiency) of the TOF analyser resulting in decreased sensitivity. In practice, for most GC-MS work a mass range of 1000 is sufficient. This equates to a **Pusher Interval** of 40 µsec.

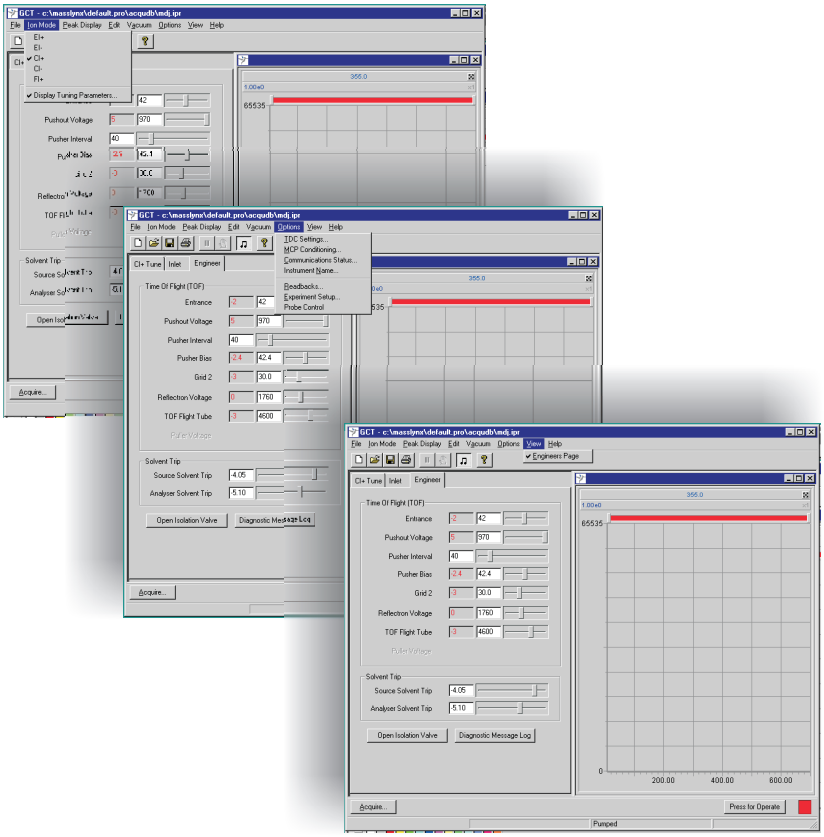
For the best mass accuracy the instrument should be re-calibrated if the pusher interval is changed.

Unless it is necessary to reach a higher mass range, it is recommended that all analyses are performed at a **Pusher Interval** of 40 µsec. This equates to a mass range of approximately 1000amu.

Open Isolation Valve. Allows control of the isolation valve between the source and analyser housings regardless of whether the instrument is in operate or standby. This isolation valve automatically opens in operate and closes in standby modes.

Show diagnostic log. Displays an updated list of the diagnostic messages displayed in the table, as in the section entitled Front Panel Indicators.

Other Tune Page Settings



As shown above, **Ion Mode** from the top of the screen allows the source tuning parameters to be changed to the ion mode required.

*The system must be in **Standby** before the software will accept a change of ion mode.*

Deselecting **Tuning Parameters** hides the tuning parameters for **Source**, **Inlets** and **Engineer Tuning Menu** from the user.

Communication Status. Indicates the detection of the instrument by the operating software for diagnostic purposes.

Instrument Name allows the instrument name to be recorded.

Readbacks. Allows voltage readbacks to be disabled if required.

Experiment Setup. Allows experimental conditions to be entered and saved under a file name. This experiment can then become part of a sample list and run using an autosampler via MassLynx NT.

Probe Ramp opens the ramp settings for the Direct Insertion Probe.

Selection of **View** from the top of the screen allows the Engineer Tuning Menu alone to be hidden from the user.

All these parameters can be saved as an instrument parameter file by selecting **File / Save As** from the top of the screen. These instrument parameter file names are then used within the experiment setup when running acquisitions from the MassLynx NT sample list.

Calibration

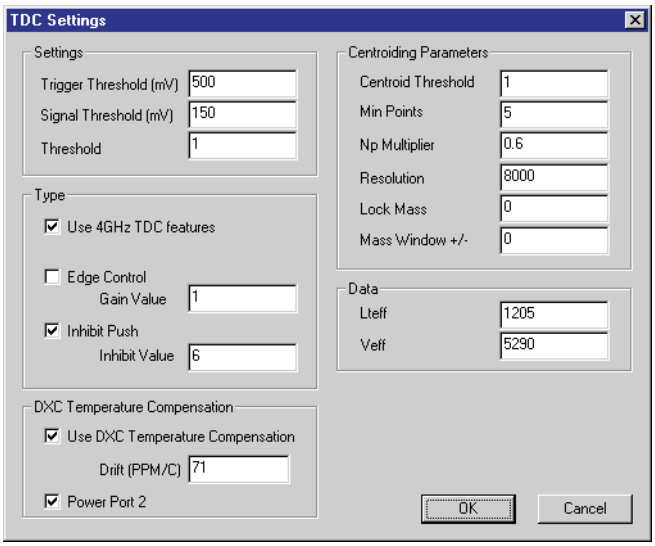
Information concerning the calibration of GCT is provided in the chapters Calibration and Exact Mass, and in the chapter Data Acquisition.

From the **Experiment Setup** menus earlier, all the criteria for the experiment can be set and saved under an experiment file name. This file name can then be used in the MassLynx sample list for acquisitions using contact closure, GC and Autosampler control etc.

The mechanics of the acquisition of sample data are comprehensively described in the chapter Data Acquisition.

TDC Settings

To access the TDC (time to digital converter) settings: Select **Options**, **TDC Settings**.



Trigger Threshold (mV)

This is the level of the trigger signal that is necessary to trigger the TDC (start the clock) at each pushout pulse. This voltage should not need to be changed once set by the engineer during installation. The **Trigger Threshold** value is normally between 100 and 1000mV and should be set to avoid peak splitting.

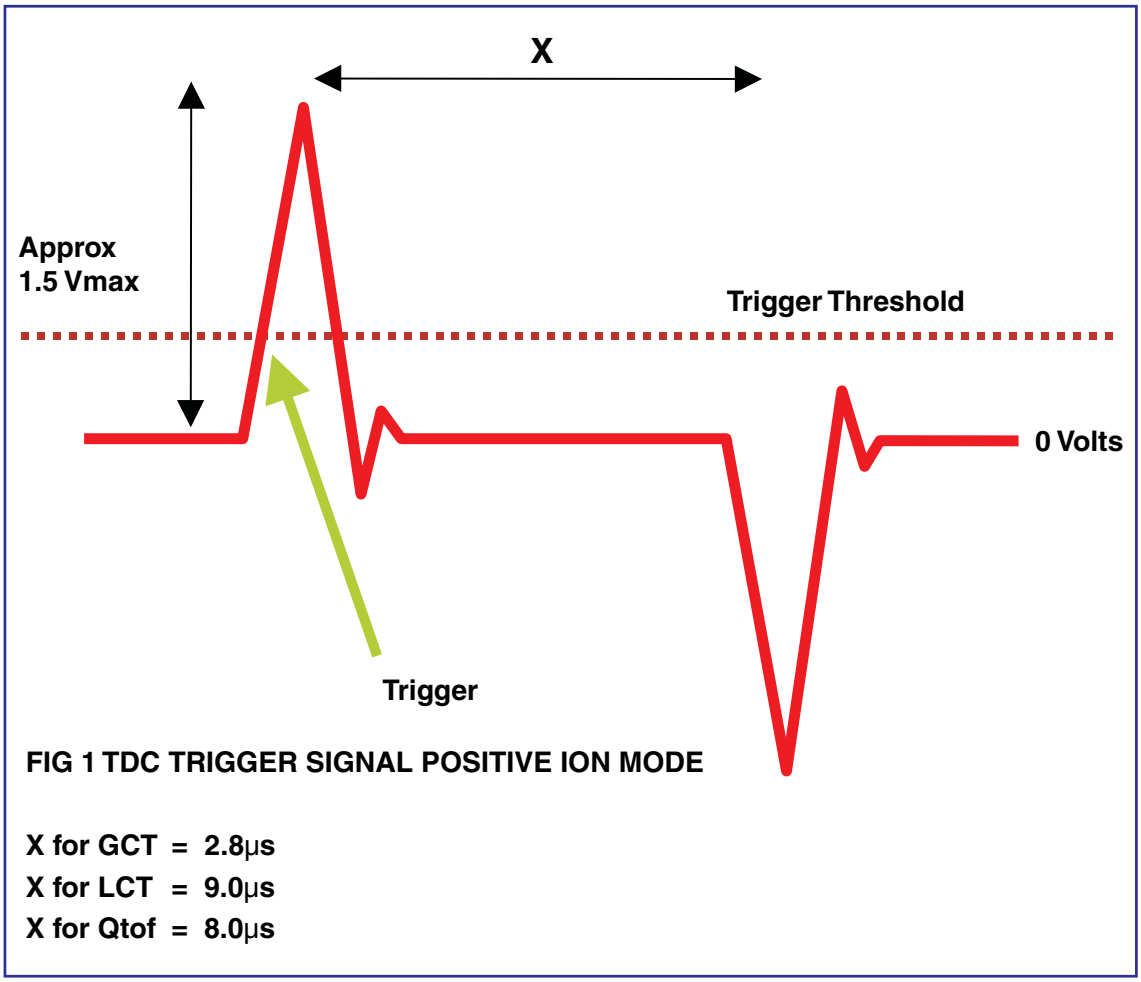


FIG 1 TDC TRIGGER SIGNAL POSITIVE ION MODE

X for GCT = 2.8µs
X for LCT = 9.0µs
X for Qtof = 8.0µs

Signal Threshold (mV)

This is the size of a single ion pulse needed to register as an event to be recorded by the TDC. It should be set to a value high enough to prevent electronic noise being detected as ions. This may be achieved as follows:

Acquire data with the detector turned off (**MCP Voltage** = 0) and the instrument in the operate mode.

Use an **Integration Time** of about 2 seconds with a low mass of 10amu and a high mass of 800amu.

There is always a signal at low mass around 6Da due to electronic pickup from the falling edge of the pusher pulse. This is usually at very low mass and does not interfere with real data.

Monitor the **TIC** (Total ion current) At a **Signal Threshold** value of 20mV a signal equivalent to hundreds of ion counts will be detected as noise.

Increase the **Signal Threshold** value in 20mV increments during the acquisition.

The number of ions will fall until a value is reached where only a few (<5) counts are recorded per integration. Use this value in all subsequent acquisitions. A typical value is 40 - 80mV.

A **Signal Threshold** value that is set too low will result in high background noise. A value which is set too high results in loss of signal and isotopic distortions.

Once this value has been determined, **MCP Voltage** should be increased until all ions are detected. Monitoring a single peak, increase **MCP Voltage** until the height of the peak no longer increases. At this point all ions are being counted. **MCP Voltage** should be lowered from this point until the signal drops by 10 - 15% from its maximum to ensure that resolution is not distorted.

The MCP voltage required to count all ions should be checked regularly. The MCP gain will drop with time and the voltage will need to be increased. If a significant number of ion counts are not recorded the ability of the system to apply dead time correction for large ion currents is affected and mass accuracy may be impaired.

Lteff and Veff Nominal mass measurement is discussed in the chapter entitled *Calibration and Exact Mass*.

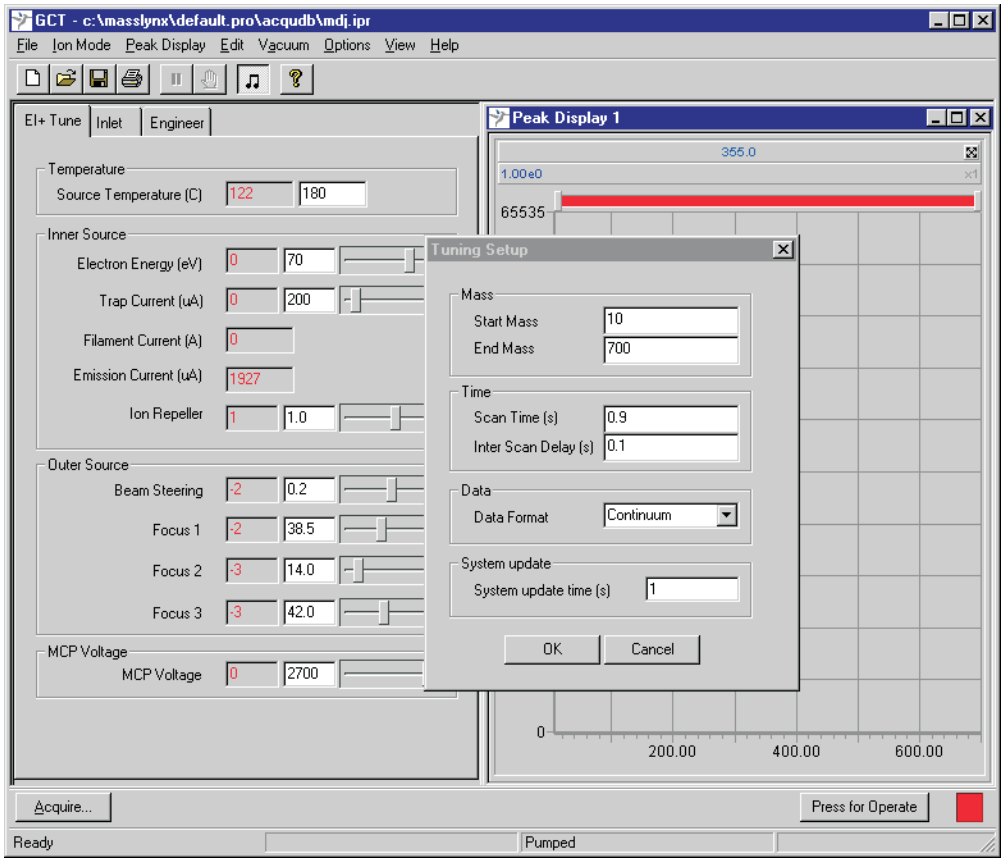
Threshold. This parameter should normally be set to zero. Setting to 1 will cause all peaks in the spectrum with 1 count to be thresholded out.

Centroid Threshold and **Min Points** are peak centroiding parameters for real time centroid acquisition. Both should be set to 1 (1GHz TDC) and on a 3.6 GHz TDC they should be 1 and 5 respectively..

Np Resolution, **Lock Mass**, and **Mass Window** are dead time correction and lock mass correction parameters used for exact mass measurement.

The functionality and setting of these parameters is detailed in the section entitled Calibration and Exact Mass.

Real Time Peak Display



From **Peak Display / Setup Scope** the menu above is displayed allowing control of the real time peak display parameters.

In addition, up to four peak displays may be selected and viewed at once from the Peak Display menu.

Highlighting  on the toolbar initiates or stops the real time peak display.

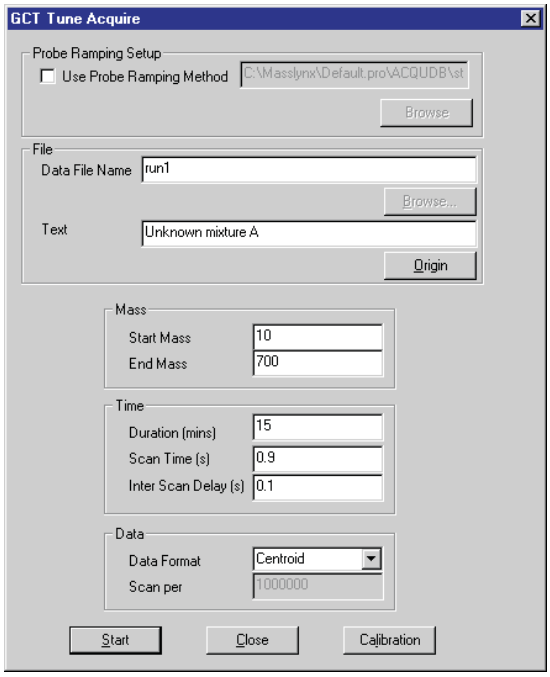
Further peak display parameters may be displayed by a right click of the mouse with the pointer within the peak display.

The red scroll bar on top of the display allows the mass range to be expanded and the mass at which the display in centered to be changed.

Alternatively the left mouse key may be used to sweep across a region of interest to zoom in on a particular mass range. By using the left mouse key to produce a vertical line on the peak display the intensity range of the display can be adjusted.

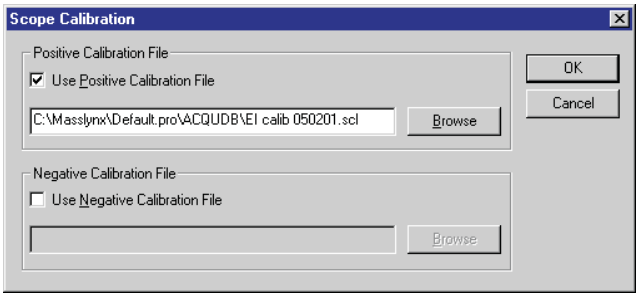
In all cases using a right click of the mouse with the pointer within the peak display and selecting undo repeatedly, will revert the display to its previous state.

Tune Page Acquisition



To access this menu select **Acquire**. This menu allows single acquisitions to be made from the tune page.

To use a saved calibration file select **Calibration** from this menu.



When **Start** is selected from the Tune Page Acquisition menu, an acquisition is initiated using the current tune page settings, acquisition parameters and calibration file entered in the **Acquire** menu. Data will be stored to disk.

Selecting **Continuum** on the acquisition page from **Data Format** and selecting **Start** will initiate an acquisition storing the raw data from the system.

Selecting **Centroid** on the acquisition page from **Data Format** and selecting **Start** will initiate an acquisition storing peak-detected centroid data to disk. Any dead time correction and lock mass parameters not set to 0 in the **TDC Parameters** page will be applied to the data.

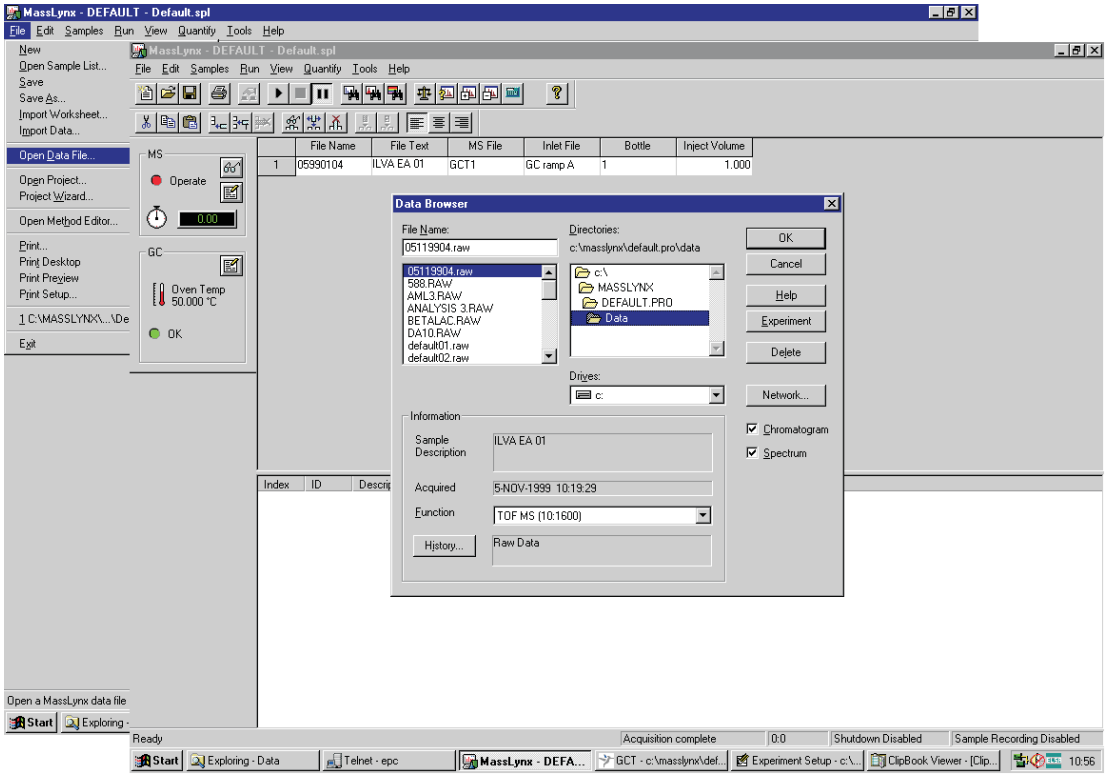
 To stop an acquisition use the tool bar button shown. This will be red when an acquisition is in progress.

Data Processing

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

The following is a very brief guide to displaying acquired data from MassLynx. Once an acquisition is underway data may be viewed from MassLynx.

From the MassLynx page select **File / Open Data file**



Select the data file required and select **OK**. The TIC and the first spectrum in the chromatogram will be displayed.

Tuning and Operation

General Considerations for Tuning in all Ionisation Modes

In GC- EI and CI+ modes of operation very intense low mass background ions are continuously present. In EI+ these are predominantly mass spectral peaks from the helium, used as the GC carrier gas, and background nitrogen and water peaks from residual air. In CI+ mode reagent gas ions dominate the spectrum. If the entire ion signal from these intense peaks was allowed to reach the MCP detector, the detector would rapidly degrade, causing gain loss.

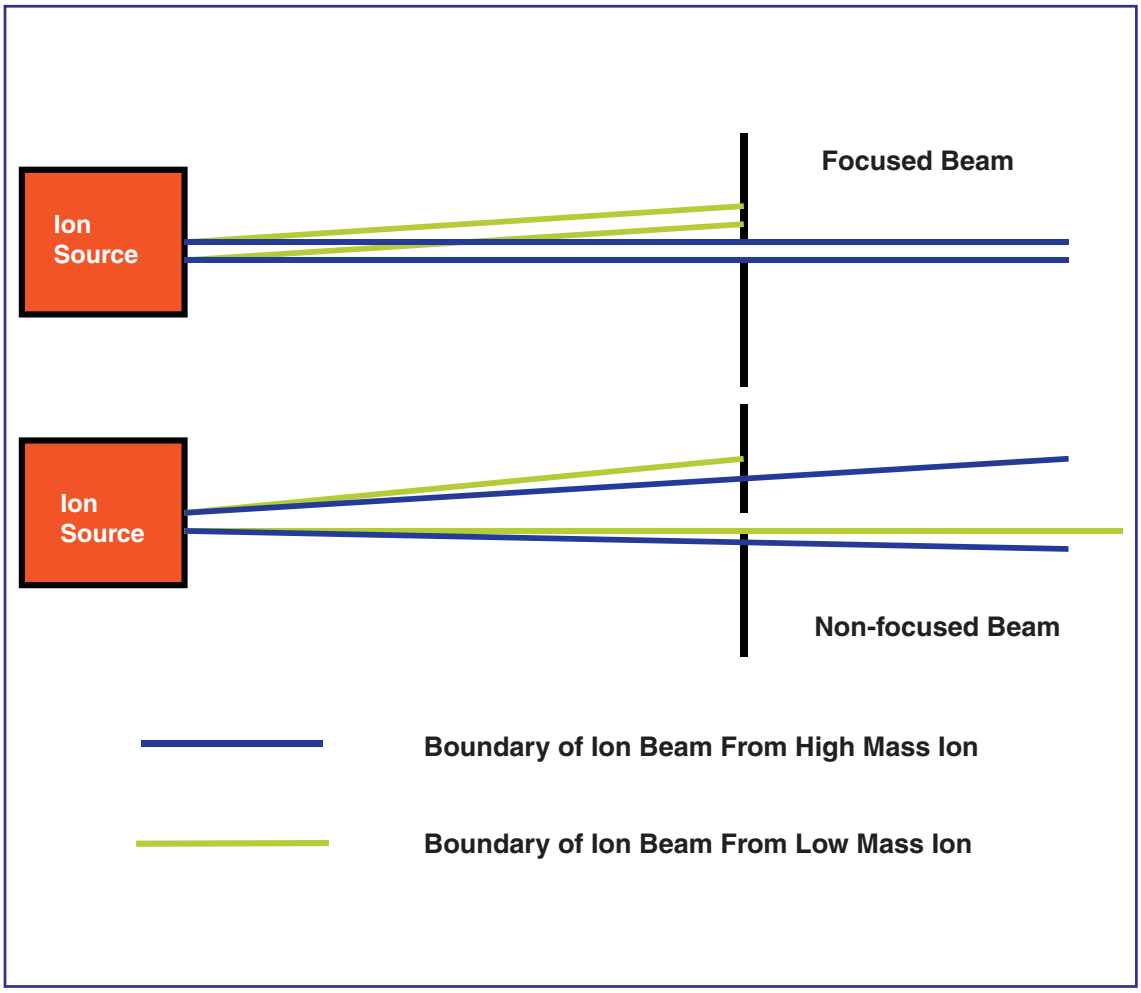
In the GCT the transmission of these ions into the pushout region of the TOF is reduced by restriction slits in the ion source. The primary ion beam exiting the inner source is partially mass dispersed by a magnetic field. Low mass ions are deflected more than high mass ions and so do not travel along the centre line of the instrument.

These low mass ions are incident on the restriction slits and so do not enter the analyser. This introduces an amount of mass discrimination into the spectra, which can be used to reduce the intensity of the low mass ions. In practice the low mass ions are so large that a proportion of the ion beam still enters the analyser - the intensity of these ions must be monitored and minimised during tuning.

Two effects should be considered when tuning in these modes. First, if the ion beam is badly focussed in the source the beam will appear more homogenous with respect to low mass and high mass ions. This is equivalent to a poor mass resolution in the source region. In this case it will be difficult to significantly reduce the intensity of the low mass ions.

Secondly, the beam steering will allow the primary ion beam to be moved across the face of the restriction slit adjusting the amount of mass discrimination. When tuning in these modes the ion signal from a reference compound in the middle of the mass range should be monitored (ca $^{m}/_{z}300$) as well as the ion signal from the most intense low mass background ion. Two peak displays may be called up to monitor these two separate mass regions simultaneously.

At the point of best focusing, small changes in lens voltage should have much less of an effect on the higher mass ion and a much greater effect on the low mass ion.



The largest constant low mass background ion should be maintained below the level of saturation of the TDC. This saturation effect is described below.

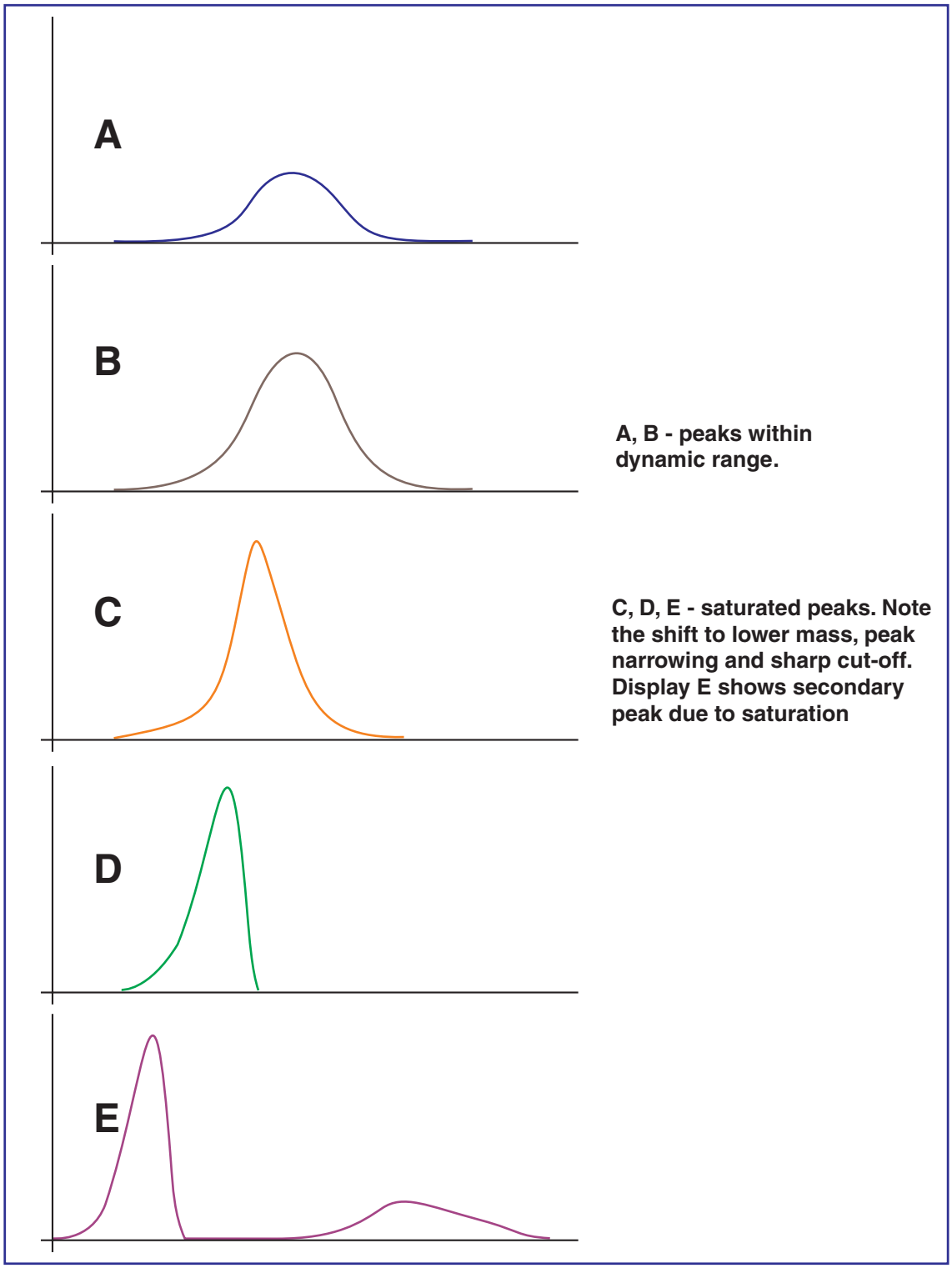
Effects of Saturation on Peak Shape

The peak shape will characteristically change as saturation of the TDC results in increasing numbers of ions not to be detected.

At the onset of saturation the peak will shift a little to lower mass followed by a failure to increase in recorded intensity as the ion signal continues to increase. There will then be a sudden, sharp high mass cut off and finally the detector will appear to 'ring', causing a secondary peak to appear on the high mass side of the peak of interest.

The diagram below represents the characteristic transition from unsaturated signal to saturated signal, which would be seen when tuning using the real time peak display in continuum mode.

Because the recorded intensity of the saturating ions will appear to reach a constant value it is important to be able to recognise the onset of saturation by the change in peak shape and position.



IMPORTANT NOTE: Always monitor the low mass ions during tuning. Never run the system for extended periods of time with clearly saturated constant low mass background peaks.

Set **Integration Time** to 0.9sec with a 40 µsec pusher interval.

In continuum mode during tuning the intensity of the largest low mass background peak should not exceed 7000 counts in height for a 1GHz TDC or 2000 counts in height for a 3.6GHz TDC system.

Introducing mass discrimination in this way results in spectra which are skewed to high mass. This observed skew, although significant is not normally enough to impair library searching of spectra against standard EI+ libraries. Once tuned, however, it may be possible to balance the intensities of the peaks within the spectra using the beam steering lens as long as the low mass background ions are monitored and their intensity set within the criteria above.

Once the system has been tuned for best focusing of the primary ion beam, the high mass ions maximised and the low mass background minimised, display the entire mass range on the peak display.

Adjust the beam steering to increase the intensity of the low mass ions relative to the high mass ions.

Continue to monitor the low mass ions to ensure no saturation occurs.

For example, the peak at m/z69 from Tris (trifluoromethyl) Triazine can be set to the same intensity as the molecular ion at m/z285.

The analyser tuning parameters on the ***Engineer Tuning Menu*** *should be very similar or identical for positive or negative ion operation in EI, CI or FI operation.*

Electron Impact Operation

Introduction

Sample molecules are admitted to the electron impact source via the GC interface, the solids insertion probe or the reference inlet system. The source is heated to ensure sample vaporisation, and the resulting gas phase molecules are ionised in collisions with high energy electrons released from the white hot filament. Ions are extracted from the source and into the analyser by the ion repeller and the focusing lenses.

Preparing for Operation in EI+ Mode

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

See section entitled General Considerations for Tuning in all Ionisation Modes before proceeding.

See section on Tuning Parameters and User Interface for typical values for ***Source***, ***Inlet***, ***Engineer Tuning Menu and TDC Parameters***.

Introduction of Reference Gas

Close the septum interface pump valve on the top of the instrument.

Introduce approximately 0.2µl of Tris (Trifluoromethyl) Triazine via the septum.

Obtaining a Beam - EI+ Operation

Select **OPERATE** from the tune page.

Set a **Trap Current** of 250µA and other source tuning parameters as detailed in the chapter *Tuning Parameters and User Interface*.

Recall previously stored parameters if available.

Call up peak display 1 and peak display 2.

Set a 0.9sec **Integration Time** and a 0.1sec **Delay**.

Set a 40µs **Pusher Interval**.

Select  From the GCT tune page to initiate a non-storage acquisition.

Set Peak Display 1 mass range from 283 - 287 amu and adjust the gain of the Peak Display until ions can be detected.

Set Peak Display 2 mass range from 26 - 30 amu and adjust the gain of the peak display until ions can be detected.

If Lteff and Veff have not been set to display the correct mass in the peak display follow the procedure detailed in the chapter entitled Calibration and Exact Mass.

Whilst monitoring the peak from nitrogen at m/z28 tune for maximum intensity for the M+ ion of Tris (Trifluoromethyl) Triazine, it may be necessary to adjust the level of the reference compound to fall below saturation of the TDC. This can be achieved by slowly opening the pump-out valve on the septum interface.

The level of the reference compound should fall.

Refer to the section General Considerations for Tuning in all Ionisation Modes.

Tune **Ion repeller**, **Beam Steering**, **Focus1**, **Focus 2** and **Focus 3** to obtain the best intensity of the m/z285 ion. The ion repeller will usually be positive and is interactive with the other tuning parameters. To obtain the best position of ion repeller it may be necessary to offset the repeller value and tune the other lenses for best focusing. This procedure can be repeated until the best value is determined.

See the section General Considerations for Tuning in all Ionisation Modes earlier, and the chapter entitled Calibration and Exact Mass.

Operation in Positive Ion Chemical Ionisation Mode

Introduction

Chemical ionisation is a 'soft' ionisation technique in which sample molecules are ionised in reactions with ionised gas molecules. Ammonia, isobutane and methane are commonly used as reagent gases from which the reagent ions are generated. Ionisation of the reagent gas molecules is by electron bombardment within the ion chamber of the CI source.

In order to achieve the relatively high pressures required within the ion chamber while maintaining an adequate vacuum elsewhere, the chamber must be made partially gas-tight.

As sufficient electrons are unlikely to reach the electron trap under CI conditions, no trap is present in the inner volume. Filament current is regulated using the total emission current and not the trap current as in EI operation.

Samples can be introduced using the solids insertion probe, the DCI probe, the gas chromatograph, and the reference inlet system.

Using Methane Gas

Caution: Methane gas is explosive. When the instrument is run in chemical ionisation mode (CI), which involves the use of methane gas it is necessary to vent the effluent from the rotary pump in a safe manner. All gas fittings should be checked for leak tightness. Avoid naked flames.

Using Ammonia Gas

Caution: Ammonia gas is a hazardous vapour. When the instrument is run in chemical ionisation mode (CI), which involves the use of ammonia gas, it is necessary to vent the effluent from the rotary pump in a safe manner. All gas fittings should be thoroughly checked for leak tightness.

Preparing for Operation in CI+ Mode

Install the CI inner volume. For details of how to change the inner source see *Installation and Removal of Inner Source* in the chapter entitled *Routine Procedures* earlier in this manual.

Before proceeding, see the section *General Considerations for Tuning in all Ionisation Modes.*

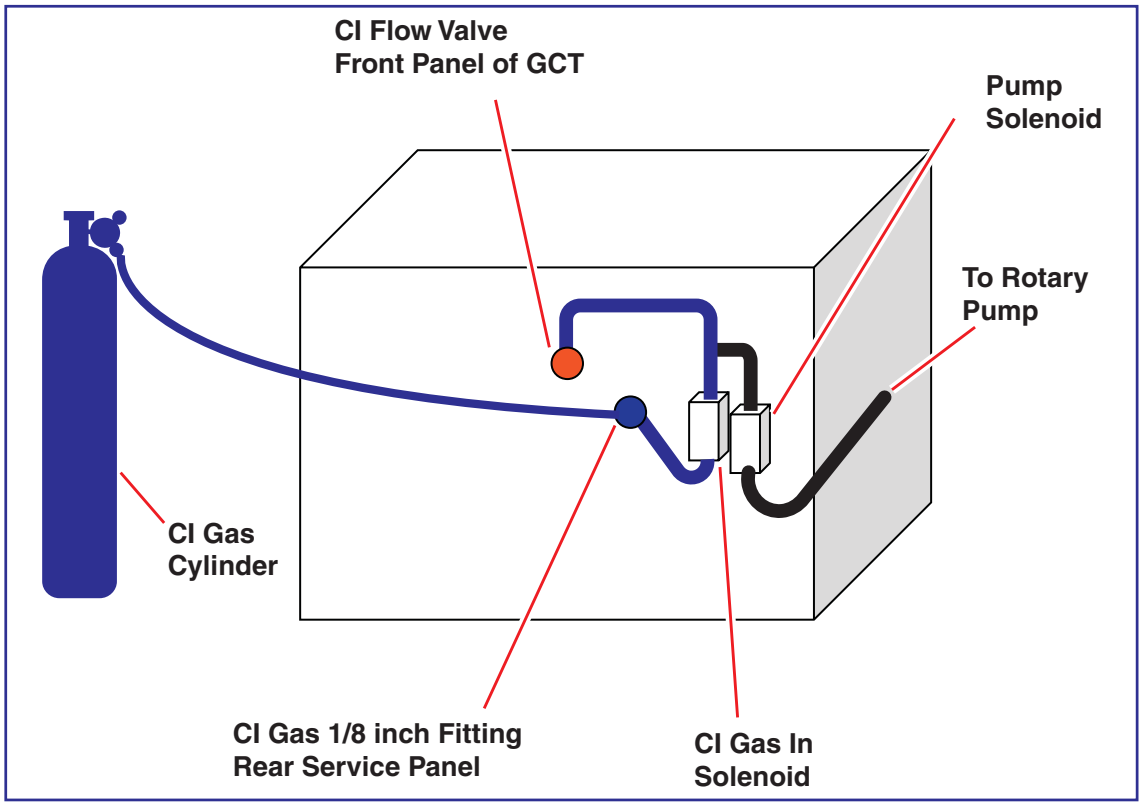
If necessary, change the ionisation mode using the **Ion Mode** command.

See the section entitled *Tuning Parameters and User Interface* for typical values for **Inlet**, **Engineer Tuning Menu** and **TDC Parameters**.

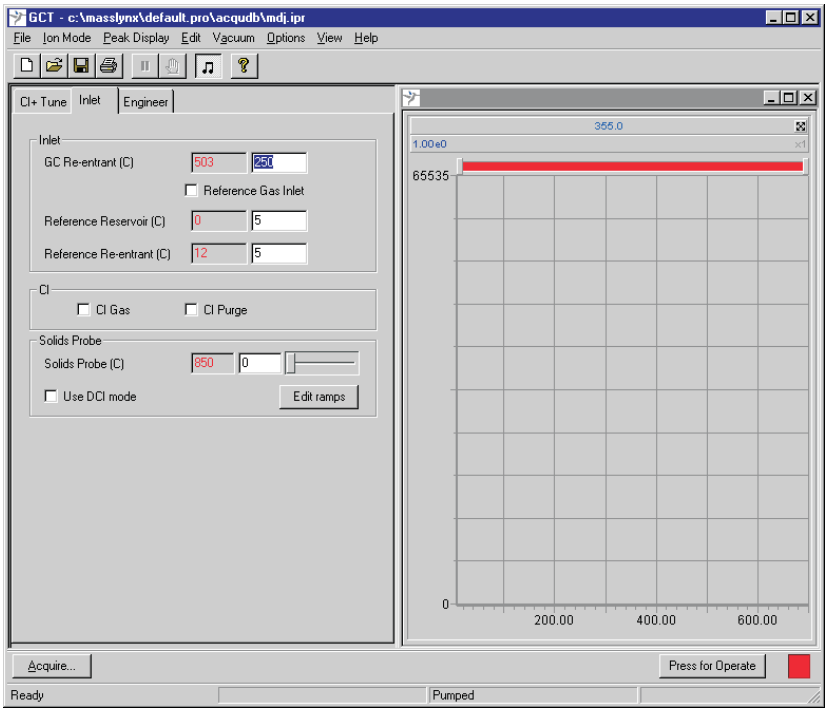
Introduction of CI Reagent Gas

The following description considers methane as the CI reagent gas.

CI Gas Valve Layout



The CI gas solenoid valves are located on the rear inside panel of the GCT.



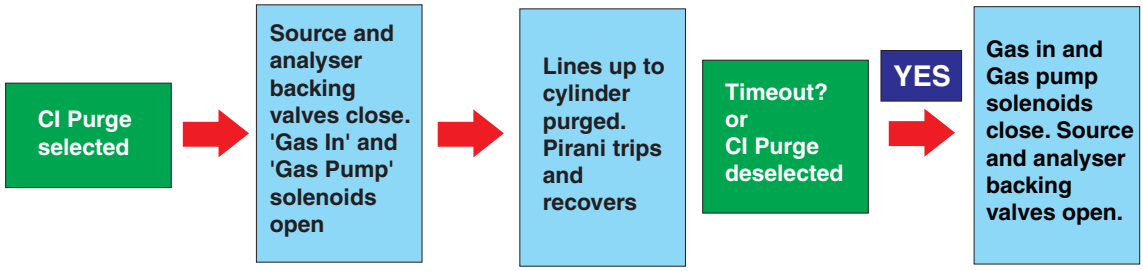
Before introduction of the CI reagent gas the internal CI reagent gas lines must be purged of air.

Introduction of reagent gas and purging of the lines is achieved using the **CI Gas** and **CI Purge** buttons on the Inlet page shown above.

Attach a cylinder of methane to the CI gas inlet on the rear service panel of the GCT.

Check the main outlet valve on the cylinder to ensure that it is closed.

Select **CI Purge** from the Inlet page. The following sequence is invoked.



The CI line should now be evacuated. If the backing line pressure does not recover, there may be a leak on the internal or external CI inlet or pump lines.

Ensure that the **CI flow valve** is turned fully clockwise, such that the valve is closed.

Caution: *Care should be taken not to over tighten this valve. Over tightening can lead to damage of the flow valve with the result that CI gas pressure may be difficult to set.*

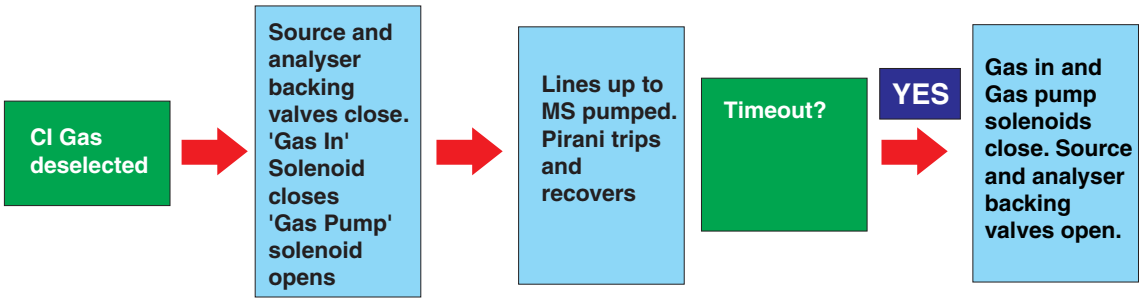
Open the cylinder and set a pressure of 10psi of methane.

Select **CI Gas** from the Inlet menu. This will open the 'CI gas in' solenoid.

Monitor the source pressure on the **Vacuum Monitor** menu and turn the CI flow valve anti-clockwise until a source pressure of 1 - 2 e-4 mbar is displayed.

*The **Solvent Trip Level** from the Engineer Tuning Menu needs to be reset to allow the filament to come on at this higher pressure.*

To remove the CI gas deselect **CI Gas** from the Inlet page.



Introduction of Reference Gas

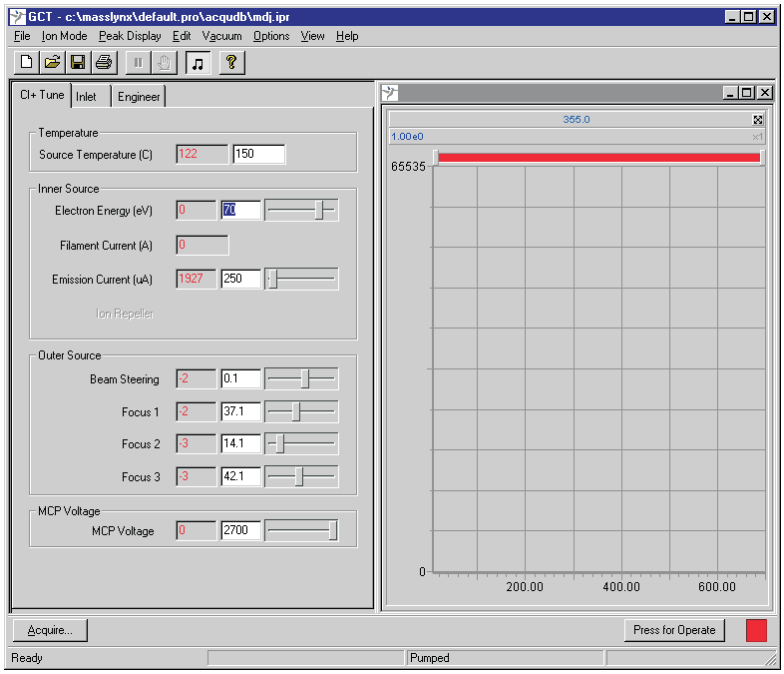
Close the septum interface pump valve on the top of the instrument.

Introduce 2µl of Tris (Trifluoromethyl) Triazine via the septum.

Obtaining a Beam in CI+ Mode

Select **OPERATE** from the tuning menu.

Set an **Emission Current** of 250µA and other source tuning parameters as shown below. Recall previously stored parameters if available.



Call up peak display 1 and peak display 2.

Set a 0.9sec **Integration Time** and a 0.1sec **Delay**.

Set **Pusher** with a 40µs pusher interval.

Select  From the GCT tune page to initiate a non-storage acquisition.

Set Peak Display 1 Mass Range from 284 - 288 amu and adjust the gain of the peak display until ions can be detected.

Set Peak Display 2 Mass Range from 27 - 31 amu and adjust the gain of the peak display until ions can be detected.

If the Values of ***Lteff and Veff*** *have not been set to display the correct mass in the peak display, then follow the procedure detailed in the chapter entitled Calibration and Exact Mass.*

Whilst monitoring the peak from C_2H_5+ at m/z29 tune for maximum intensity for the (M+H) ion of Tris (trifluoromethyl) Triazine.

It may be necessary to adjust the level of the reference compound to fall below saturation of the TDC.

This is achieved by opening the pump-out valve on the septum interface slowly.

The level of the reference compound should fall.

Refer to the section *General Considerations for Tuning in all Ionisation Modes.* Tune **Beam Steering**, **Focus1**, **Focus 2** and **Focus 3** to obtain the best intensity of the m/z286 ion.

The CI gas pressure can also be adjusted to obtain best sensitivity.

Operation in Negative Ion Chemical Ionisation Mode

During electron impact ionisation, negatively charged ions are formed as well as positively charged ions. Normally, the negative ions remain undetected because the ion source and focusing potentials allow only the extraction of positive ions.

There are limitations to the analytical usefulness of negative ions formed by electron impact; for instance, many organic compounds do not form molecular or quasi-molecular anions under conventional EI conditions, but instead provide spectra dominated by structurally insignificant low mass ions (e.g. CN^-, F^-, Cl^-). Furthermore, the sensitivity for production of negative ions under EI conditions is several orders of magnitude lower than for positive ion production.

In contrast to the conditions of conventional electron impact, low energy electrons are readily captured by many organic compounds without inducing excessive fragmentation. Consequently, the NCI ion source has been optimised for generating a large population of electrons with near-thermal energy. Introduction of a reagent gas acts as a moderator for the initially energetic electrons. Anionic reagent gas ions can also be generated for ion formation by ion-molecule reactions.

Preparing for Operation in NCI Mode

Install the CI inner volume. For details of how to change the inner source see the section *Installation and Removal of Inner Source* in the chapter entitled *Routine Procedures* earlier in this manual.

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

See the section *General Considerations for Tuning in all Ionisation Modes* before proceeding.

See the section *Tuning Parameters and User Interface* to obtain typical values for **Inlet**, **Engineer Tuning Menu** and **TDC Parameters**.

Introduction of CI Reagent Gas

See previous section *Operation in Positive Ion Chemical Ionisation Mode*. The pressure of CI reagent gas in the source is usually somewhat lower for CI negative mode compared to CI positive mode.

Start with a set pressure read from the source Penning gauge of 5 - 8e-5 mbar.

Introduction of Reference Gas

Close the septum interface pump valve on the top of the instrument.

Introduce ca: 0.5µl of Heptacosa (PFTBA) via the septum.

Obtaining a Beam CI- Operation

Caution: Care should be taken when changing to negative ion mode after using the GCT in positive ion mode for an extended period of time. The following precautionary procedure must be followed before setting to **OPERATE** in CI- mode.

Turn the **TOF Voltage** to 0 and the **MCP Voltage** to 0.

Select **OPERATE** from the tuning menu.

Increase these voltages to operating voltage over a period of 20 seconds. This minimises the risk of discharge and possible damage to the MCP.

Set an **Emission Current** of 250µA and other source tuning parameters as shown in the section *Finding a Beam in CI+ Mode*.

Recall previously stored parameters if available.

Call up peak display 1. Set a 0.9sec integration time and a 0.1sec delay.

Set a 40µs **Pusher Interval**.

Select  From the GCT tune page to initiate a non-storage acquisition.

Set peak display 1 mass range from 448-454amu and adjust the gain of the peak display until ions from m/z451.97 of heptacosa can be detected.

If the values for ***Lteff and Veff*** *have not been set to display the correct mass in the peak display follow the procedure detailed in the chapter entitled Calibration and Exact Mass.*

Tune **Beam Steering Focus 1**, **Focus 2** and **Focus 3** to obtain the best intensity of the m/z 451.97 ion. Note there is little low mass background in this technique, and therefore it should not be necessary to consider ions of low mass in terms of saturation while tuning.

The CI gas pressure can also be adjusted to obtain best sensitivity.

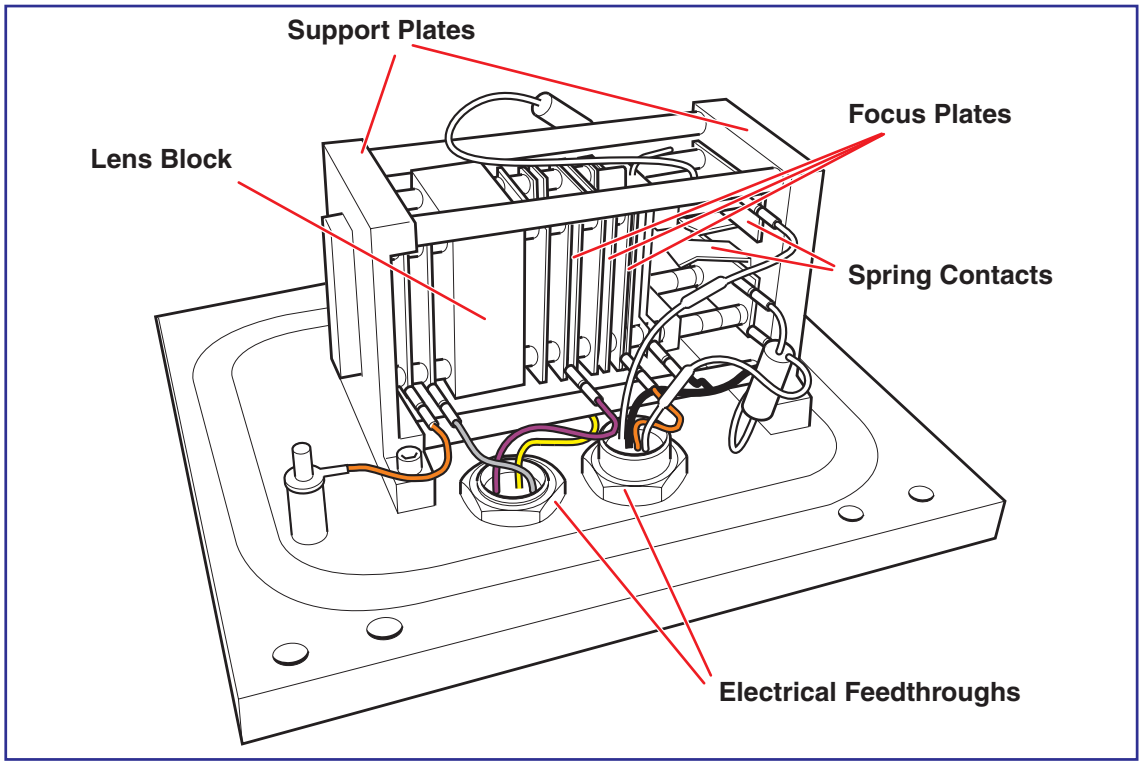
Field Ionisation Mode

FI was developed in the 1970s on magnetic sector instruments. Exact mass GC-FI using magnetic sector instruments, however, has been limited for the following reasons,

- Poor peak sampling duty cycle of magnetic sector instruments in scanning mode.
- The time required to scan over all masses in the spectra.
- The narrow time window in which to collect data from an eluting GC peak.
- The need for a complex internal reference mixture to characterise the calibration curve of these instruments.

The GCT, however, simultaneously samples all masses within the spectra, with high sensitivity. In addition, the precise and stable relationship between the ion arrival time and the square root of its mass, means that exact mass measurements can be made with only a single internal reference peak.

In field ionisation, sample molecules pass in close proximity to the tips of a mass of needle-like carbon dendrites. A counter electrode is held at high potential; this produces very high electric fields around the tips of the carbon dendrites. Under the influence of these fields, quantum tunnelling of a valence electron from the molecule takes place to give an ion radical. This process is very “soft”, often producing spectra with very little or no fragmentation.



Overview of Operation

In GC-FI the FI emitter is positioned in close proximity to a pair of hollow extraction rods. The emitter is held at ground potential and 12,000 volts is applied to the rods. These extraction rods can be heated by passing a current through the rods themselves. Heating of the extraction rods is not usually required for GC FI but may be desirable if very involatile samples are introduced via the solids probe. The rods are very highly polished and must be kept clean and free from burrs to prevent current leakage from the rods to the emitter.

The GC column is positioned in close proximity and in line with the emitter wire. The positioning of these three elements with respect to each other is critical. The correct position is described below.

As sample elutes from the GC column some condensation of sample occurs on to the surface of the emitter. This fills some of the sites on the emitter, reduces field strength and thus reduces sensitivity. To remove the condensed material and 'regenerate' the emitter a current is passed through the emitter during the interscan delay period of the acquisition between each spectrum. This is referred to as the flash off current. The emitter is then left to cool during the acquisition period. When the emitter is hot sensitivity is also reduced. There is a finite time associated with heating and cooling the emitter wire by this method. It is therefore important to set the flash off period (interscan delay time) and emitter current heating period (acquisition time) to the correct values for the experiment to be performed.

The maximum emitter current is dictated by the diameter of the wire used to make the emitter. The maximum emitter currents are indicated in the section *Choosing an Emitter* below.

It should be noted that no deflection of the beam is performed in FI mode. Intense low mass background ions are not present. Tuning should therefore be optimised for best sensitivity.

Choosing an Emitter for FI

For the best sensitivity and performance in GC FI for applications involving the characterisation of low amounts of chromatographically resolved analytes, the following emitter characteristics are desirable,

- High field strength at minimum extraction voltage.
- Minimum surface area onto which analyte can condense.
- Very fast heating and cooling characteristics. This ensures fast recovery of sensitivity after flash off.

In general, the smaller the diameter of the emitter wire, and the shorter the carbon dendrite micro needles, the higher the field strength at a given extraction voltage. In addition smaller diameter emitter wires have very fast cooling and heating characteristics.

The GCT is supplied with a pack of six FI emitters from CARBOTEC (http://www.carbotec.com). These emitters are made from 5µm diameter tungsten wire and have excellent performance for FI applications.

For FI-GC-MS applications involving very complex mixtures which are unresolved chromatographically eg: analysis of crude oils, different criteria are required.

> *The emitter must be tolerant to a larger amount of condensed or closely associated material.*

An emitter with a higher surface area which can act partially as a sample reservoir, without completely suppressing sensitivity is required. CARBOTEC FI emitters are not suitable for these applications.

CARBOTEC FD or all-round emitters will give better performance in these applications. These emitters have a 10µm diameter wire and have longer and more highly branched carbon dendrite micro needles. Absolute sensitivity will be significantly lower than that using the 5µm diameter FI emitters.

Maximum Flash Off Current

The maximum flash of current for a given emitter depends on the diameter of the tungsten wire attached to the emitter bead.

10µm diameter wire maximum current = 90mA (CARBOTEC FD, all-round)

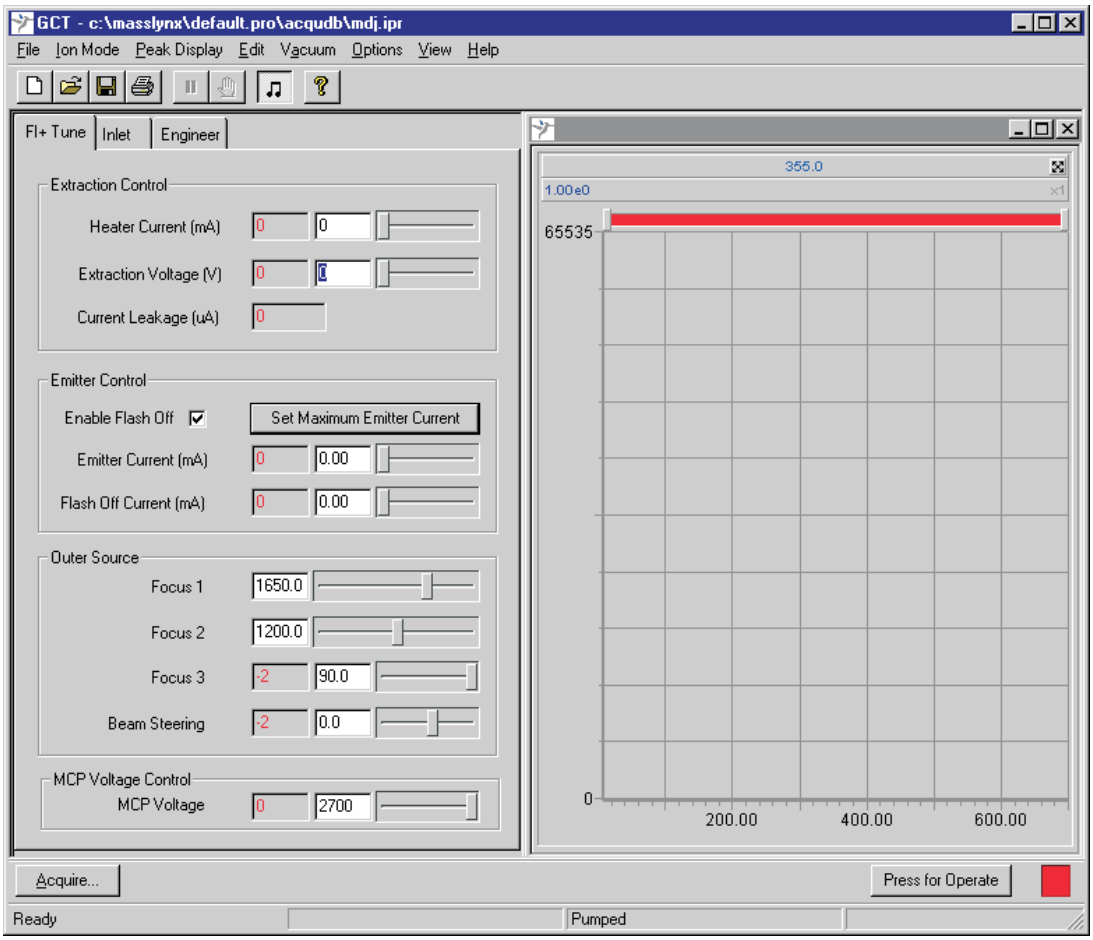
5µm diameter wire maximum current = 10mA (CARBOTEC FI)

Emitter Lifetime

Some types of emitters have a finite lifetime, in the order of 3 - 4 months in atmosphere. The ultimate sensitivity of the emitter for FI will decrease the longer the emitter is stored. The likely mechanism for this degrading of performance is oxidation of the tungsten wire used. To extend the lifetime of the emitters they should be stored under vacuum or under an atmosphere of dry N_2.

Tuning Menu FI Mode

Selecting Ion Mode and FI + will result in the FI source tuning menu to be displayed.

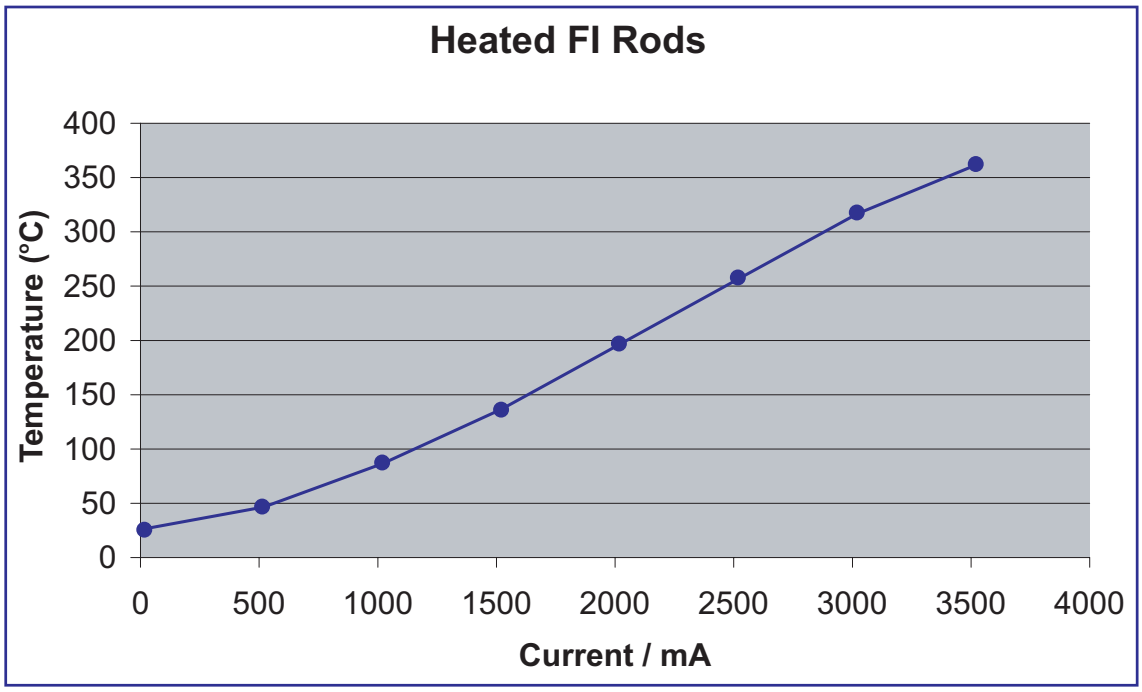


Heater Current

(Note - not available as standard.) Allows a current to be passed through the extraction rods to heat the rods. Heating the extraction rods would usually only be necessary when using the solids probe to introduce waxy or very involatile samples. In this case sample may sputter from the probe because of expansion of sample in the base of the sample cup. This sample can then build up on the extraction rods causing voltage leakage between the rods and the emitter reducing the field strength in this region or even leading to arcs within the source.

> *The rods should be left cold for GC FI operation. If using heated rods, allow the system about 10 minutes to stabilise after changing the temperature. Having the rods hot can cause a slight change in their position, so it is advisable to recalibrate the instrument once it has reached equilibrium.*

The graph below shows the temperature of the rods Vs the current applied.



Extraction Voltage

This is the high voltage applied to the extraction rods. Normally the extraction voltage will run at 12000V. The voltage and leakage current are presented as readbacks. Where possible the system should not be turned to operate with a request of 12000V extraction voltage. The extraction voltage should be increased from zero manually in 2000V steps. If excessive extraction current is observed the rods should be cleaned.

Flash Off Current

Allows the emitter current during the interscan delay time to be set. The maximum emitter current for each type of emitter is described in the section *Choosing an Emitter*.

Flash Off Enable

If **flash off enable** is highlighted, the emitter will be driven to the current requested in the flash off current slider during the interscan delay time. This is active in tuning and acquisition. If **flash off enable** is not selected the emitter current will remain at the value set in the Emitter current dialogue box.

> *The emitter current is set at the value indicated in the flash off current dialogue box during the interscan period or when the system is not in tune mode or performing an acquisition.*

The emitter current is set at the value indicated in the emitter current dialogue box when the system is acquiring data either in tune mode (scan time, non storage acquisition, or in acquisition mode.)

When the emitter flash off is not enabled the emitter will stay at the value indicated in the emitter current dialogue box.

Beam Steering

Allows the beam to be deflected from center line during tuning. This is interactive with the physical position of the emitter with respect to the center line of the machine. The beam steering should be set close to zero. The side to side position of the emitter should then be adjusted to obtain a beam. The beam steering can then be used for fine tuning of the beam for maximum sensitivity.

Focus 1, Focus 2, and Focus 3 act to focus the ion beam into a thin ribbon for maximum transmission. Typical values are shown in the tune page above. Focus 1 and Focus 2 are 0 - 2500V lenses. Focus 3 is a 0 - 100V lens.

MCP Voltage

Provides control of the detector gain and should be set to the same value as used for EI and CI operation.

Introduction of Reference Material

The FI source has a dedicated reference gas reservoir and transfer line attached to the source-housing lid. This reservoir is of essentially the same construction as the reservoir attached to the EI/CI source lid (see Septum Interface Maintenance). However the reservoir has a slightly larger internal volume and is not heated.

The fused silica leak within the reference inlet re-entrant is 100µm ID x 320µm OD.

The stainless steel capillary line within the source housing is arranged so that it points at the emitter and is set approximately 20mm away from the emitter. The end of the capillary line is sheathed in high alumina ceramic Degussit tube. This minimises the distortion of the electrical field in the extraction rod region from the presence of the stainless steel tubing at ground potential.

To introduce reference material,

- Close the septum interface pump valve on the top of the instrument.
- Introduce about 3µl of reference material via the septum.

Obtaining a Beam - FI Operation

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

See the section *Tuning Parameters FI mode* above for typical values of Source page parameters.

NOTE: The TDC, engineers page, and inlet page parameters should be set to the same values as in EI and CI operation.

In the FI+ source tuning menu, set the **extraction voltage**, **heater current**, **emitter current**, and **flash off current** to zero.

Deselect the **flashoff enable** toggle.

Switch the system into **operate**.

From **Peak Display** set a **Scan Time** of 1.2 sec and an **Inter Scan Delay** of 0.2 sec. **Continuum** mode with a **system update time** of 1.5 sec.

- Press the  symbol to set the system into tune mode.
- Increase the **extraction voltage** in steps of 2000V to a maximum of 12000V.

It is important to monitor the readback of **extraction voltage leakage current**. If the value rises above 10µA constant readback there is excessive leakage of the extraction voltage to ground. The extraction voltage should be turned off and the source of the leakage isolated. In normal operation the value of leakage current should be 0 - 3µA.

Operating the system with higher leakage current can result in poor mass measurement, unstable signals and the risk of high voltage 'flash over' in the ion source.

Depending on the type of emitter in use, see the section *Choosing an Emitter*, request an emitter current which is half of the maximum rated current appropriate for the type of emitter in use.

Check that the readback indicates that current is passing through the emitter. If no current is passing through the emitter, it may be open circuit and will need to be replaced.

*The emitter current is set at the value indicated in the **flash off current** dialogue box during the inter scan period or when the system is not in tune mode or performing an acquisition.*

The emitter current is set at the value indicated in the emitter current dialogue box when the system is acquiring data either in tune mode (scan time, non storage acquisition, or in acquisition mode.)

When the emitter flash off is not enabled the emitter will stay at the value indicated in the emitter current dialogue box.

Set the **emitter current** to its maximum rated value in mA for 2 - 4 seconds. This will desorb any condensed material on the emitter and ensure maximum sensitivity.

Set the **emitter current** to zero.

Select **flashoff enable on**.

Set a **flash off current** (current during interscan) of 80% of the maximum emitter current.

Set the **emitter current** (current during the acquisition) to Zero mA.

The emitter will now heat up during the interscan period and cool during the scan. This ensures that sample does not condense on the emitter suppressing sensitivity. It may not be possible to see the emitter current readback change during the interscan period due to the time required to update the readback by the software.

With Chloropentafluorobenzene introduced via the septum interface set **Peak Display 1** mass range from 198 - 204 amu. Set the peak display to normalise the displayed signal.

Carefully adjust the probe position knob on the emitter probe guide until the molecular ion at $^{m}/_{z}$ = 201.9609 is seen.

This adjustment is very sensitive and must be made carefully. If the emitter is moved to far it may hit the GC column and become damaged.

Using **beam centre, Focus 1, 2 and 3** optimise for maximum intensity.

General Considerations for Tuning and Position Optimisation in FI Mode

Lens Tuning

The focus lenses are interactive with the beam centre, and combinations of focus and steering settings should be tried. The focus 1 lens often optimises at maximum negative voltage.

The tuning of the ion source is strongly dependent on the position of the emitter with respect to the extraction rods. The closer the emitter is to the extraction rods the higher the field strength at the emitter and the higher the sensitivity.

The position of the emitter with respect to the centre line of the extraction rods is also very critical. Small changes in this position can result in large changes in beam steering voltage being required. In general, the beam steering should be set close to zero and the emitter probe adjuster knob used to optimise the signal. Small adjustment to **beam steering** can then be made using the beam steering lens.

Changes in **Focus voltage** can also result in changes in beam steering voltage. The focus lenses should be optimised in conjunction with this steering lens. If the beam steering lens optimises at > 4V the lens should be returned to zero and the emitter probe adjuster knob used to reoptimise the beam.

Extraction Voltage

In general the higher the **extraction voltage** the higher the field strength and the higher the sensitivity. Adjusting the **extraction voltage** will cause a change in source focussing and the source lenses will need to be reoptimised. The extraction voltage should not be run above -12,000V. Higher voltages may result in discharges within the source and ultimately damage to the emitter.

The signal from the reference material used for tuning will optimise in intensity between extraction voltages of 10,000 and 12,000V. Lowering the extraction voltage may result in less fragmentation for particularly fragile analytes, however absolute sensitivity will be reduced.

GC Column Position

For FI- GC-MS the position of the column with respect to the emitter is critical for optimum sensitivity. The end of the GC column should be as close as possible to the emitter wire. 2mm from the wire is optimum. If the end of the column is too close to the emitter wire discharge or mechanical failure of the emitter may occur. If the end of the column is too far away from the emitter the sensitivity for analytes introduced via the GC may be reduced.

In addition the end of the GC column should be in line or slightly behind the emitter wire. If the end of the column is in front of the emitter wire sensitivity for analytes introduced via the GC may be reduced.

Emitter Flash off Current

The magnitude of the flash of current can impact on the sensitivity for different analytes. As current is passed through the emitter wire the wire becomes hot. This can reduce sensitivity for thermally labile compounds. In general the emitter is flashed off to 80% of its maximum value however lower values may be tried. If the emitter flash off current is too low sample will condense of the emitter during the chromatographic run. As sample condenses the field strength between the emitter and the extraction rods is reduced and sensitivity decreases.

For higher boiling point samples some condensation of sample can occur towards the end of the chromatographic run. The emitter can be cleaned by setting the emitter current to maximum for 2 - 4 seconds then returning the emitter current to zero.

Sensitivity in FI not only depends on the quality of the emitter and the position of the relative source components but also will vary with acquisition rate and inter scan time. For optimum performance the emitter must be allowed to flash off to the correct temperature during interscan and to fully cool as quickly as possible during the acquisition time.

For the CARBOTEC FI emitter with 5µm emitter wire diameter an acquisition time of 1.2 sec with an inter scan (flash off) time of 0.2 sec is optimum. As the acquisition time is decreased or the interscan time is decreased sensitivity will be reduced.

For emitters with larger diameter wire and heavier deposits of carbon dendrite microneedles a 2.5 sec acquisition time with a 0.4 sec delay is required for maximum sensitivity. Faster acquisition times and delays will decrease ultimate sensitivity.

There may be variation in the field strength, and hence sensitivity, between emitters. If sensitivity is poor another emitter should be tried.

Running GC MS Samples in FI Mode

It is important to use a solvent delay at the beginning of the acquisition when running GC FI. This delay should be set so that the solvent front has completely eluted before the end of the solvent delay time. During the solvent delay period the **Extraction Voltage** is turned off. This ensures that the **Extraction Voltage** is not on at a high pressure in the ion source region. Failure to protect the system from the solvent front may result in damage to the emitter.

The solvent delay time can be set from the experiment setup window and will be active only when running an acquisition from the MassLynx Sample list.

The mechanics of acquisition are described in the chapter entitled *Data Acquisition*.

Running Solids Probe Samples in FI Mode

Involatile samples may be run in FI mode using the direct insertion probe. The adjuster knob on the probe lock allows the end of the solids probe to be set at a fixed and reproducible distance away from the emitter.

This distance should be set to approximately 10mm away from the emitter. The closer the solids probe to the emitter the more the field around the extraction rods will be distorted resulting in a loss in sensitivity.

It is also important to tune the ion source with the solids probe inserted into the position to be used.

Operation of the GCT with the solids probe is described in the chapter *The Solids Insertion Probe.*

Data Acquisition

Starting an Acquisition

There are two ways of starting an acquisition:

- a single sample acquisition from the tune page.
- a single or multiple sample acquisition from the MassLynx sample list.

Starting an Acquisition from the Tune Page

The easiest way to acquire data is directly from the tune page.

✓ Acquisitions can be started and stopped.

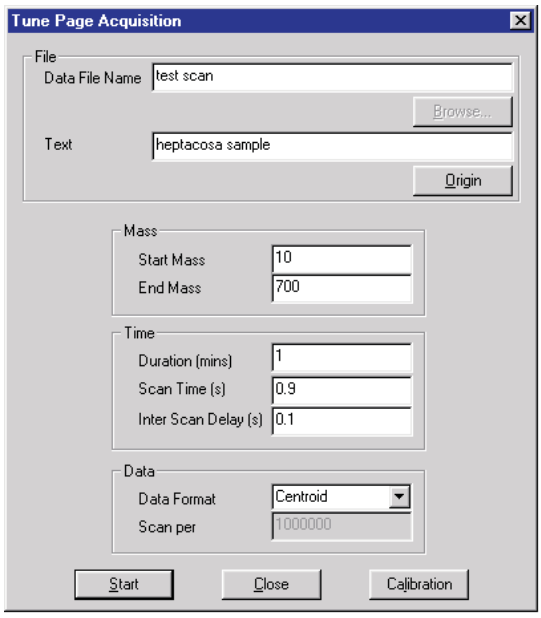
✗ Inlet programs cannot be used.

✗ Analog data cannot be acquired.

✗ Multiple sample sequences cannot be acquired.

To start a single sample acquisition:

Press **Acquire** on the tune page.



Make any required changes to the settings.

Press **Start**.

Parameters

The **Data File Name** can be up to 128 characters. If the file already exists on disk, a prompt is given to rename the file or to overwrite the existing one. The file is written to the data directory of the current project.

To change the directory into which data are acquired:

Cancel the acquisition.

Create a new project by choosing **MassLynx**, or open an existing one by choosing **Open Project**, from the MassLynx top level file menu.

The **Text** area is used to enter the sample description. The description can be displayed on any output of the acquired data and has a maximum length of 74 characters. To display text on more than one line press **CTRL+Return** at the end of a line.

The **Data Format** collected and stored on disk can be any of the following:

- Centroid.
- Continuum.
- MCA.

Data specifies the type of data to be collected and stored on disk. There are three options:

- **Centroid** stores data as centroided, intensity and mass assigned peaks. Data are stored for every scan.
- **Continuum**. The signal received by the interface electronics is stored regularly to give an analog intensity picture of the data being acquired. Data are not centroided into peaks, but are stored for every scan.

  Due to the fact that continuum has many points across each peak, data files tend to be significantly larger than centroided ones. However, since the centroid routine is not invoked the absolute scanning speed(spectra/sec) is faster.

  It is possible, however, to set a threshold below which the data are not stored. The threshold can be set so that data considered to be ‘noise’ can be discarded, thus improving data acquisition speed and reducing data file sizes.
- **Multi Channel Analysis (MCA)**. MCA data can be thought of as ‘summed continuum’, with only one intensity accumulated scan being stored for a given experiment. As each scan is acquired, its intensity data is added to the accumulated summed data of previous scans.

  An advantage of MCA is that the data files occupy significantly less space on the hard disk than the equivalent continuum data would.

The disadvantage of MCA is that, as there is only one scan, it cannot be used for time resolved data.

For MCA, **Scans to Sum** defines the number of scans to sum to create a spectrum.

Scan Time specifies the duration of each scan in seconds while **Inter-Scan Delay** specifies the time in seconds between a scan finishing and the next one starting. During this period no data are stored.

Start Mass and **End Mass** specify the masses at which the scan starts and stops. **Start Mass** must be lower than **End Mass**.

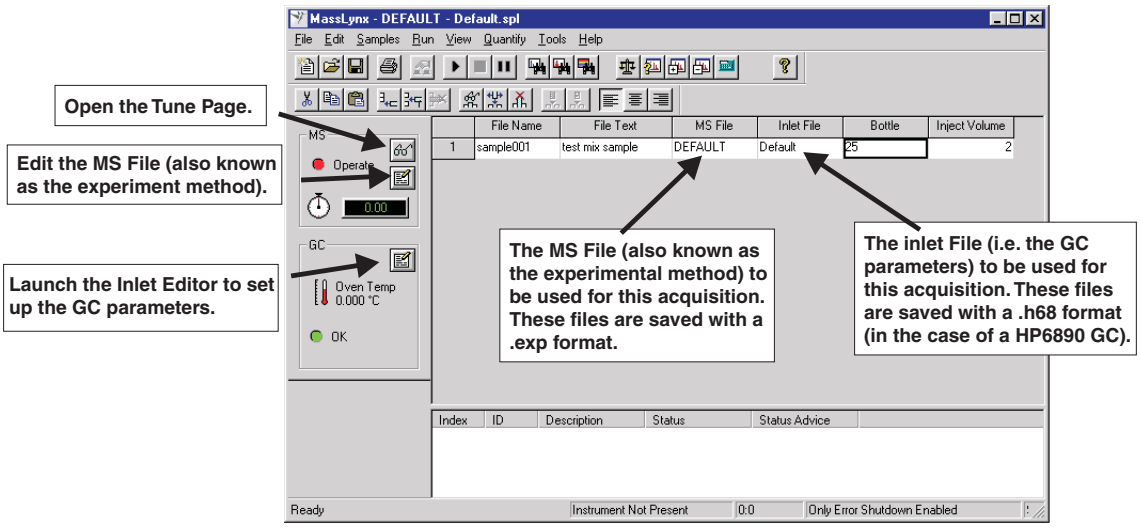
Duration is the length of the acquisition, measured in minutes.

Scan Time specifies the duration of each scan in seconds.

Inter Scan Time specifies the time in seconds between a scan finishing and the next one starting. During this period no data are stored.

Multiple Samples

The MassLynx top level screen contains a sample list editor for defining multiple samples. The list of samples is set up using a spreadsheet style editor, which can be tailored to suit different requirements. A number of samples may be added to make a list by selecting **Add** from the Samples drop down menu.



On the left hand side of the MassLynx top level editor it shows the status of the GCT instrument and the GC inlet system.

By clicking the “glasses” icon you can open the tune page and tune the instrument.

Clicking on the edit icon below this will edit the MS file (this is also known as the “experiment method” or the “method editor”).

Clicking the “Inlet Editor” icon in the GC status area will launch the Inlet Editor program which allows you to set up the parameters used for the GC system.

The sample list is displayed on the right hand side of the window.

This contains the following fields in the spreadsheet display.

File Name

This is the name of the data file that will be saved to hard disk when the data is acquired.

File Text

This is used to enter a sample description to be saved and displayed with the data on the Chromatogram or Spectrum window.

MS File

This refers to the “experiment method” that will be used to acquire the data. The experiment method specifies which tune page parameters to use, the length and duration of the scan, the calibrations etc and is described in detail later on in this chapter.

The parameters are set up and then saved as a “.exp” file which must be referenced here. Select from the ones currently saved and available by double clicking in this box and selecting one from the drop down menu that appears.

Inlet File

This specifies which Inlet parameters will be used in the acquisition. The inlet parameters are set up on the Inlet Editor page. They are saved as a “.h98" file (in the case of a HP 6890 GC system) which must be referenced here.

For more details of the Inlet Editor for all the various GC and Auto-sampler combinations supported by MassLynx see the publication *Micromass NT Guide to Inlet Control* (Micromass part number 6666678).

Select from the ones currently saved and available by double clicking in this box and selecting one from the drop down menu that appears.

Bottle

This is only relevant when using an auto-sampler and indicates which the position in the auto-sampler tray that the sample is located. The auto-sampler will take the sample from the position indicated so make sure that it corresponds to the correct sample in the tray.

Injection Volume

This is only relevant when using an auto-sampler and indicates the amount of sample to be injected into the injector.

It is important to realise that this value is expressed in terms of tenths of a syringe volume e.g. if you have a 5 microlitre syringe then setting a value of 2 will give 2/10 of 5 microliters which is 1 microlitre. Also note that in most versions of MassLynx for the GCT the HP6890 can only accept integer values for this parameter so to avoid mistakes it is best to set the properties of this column to accept only integers.

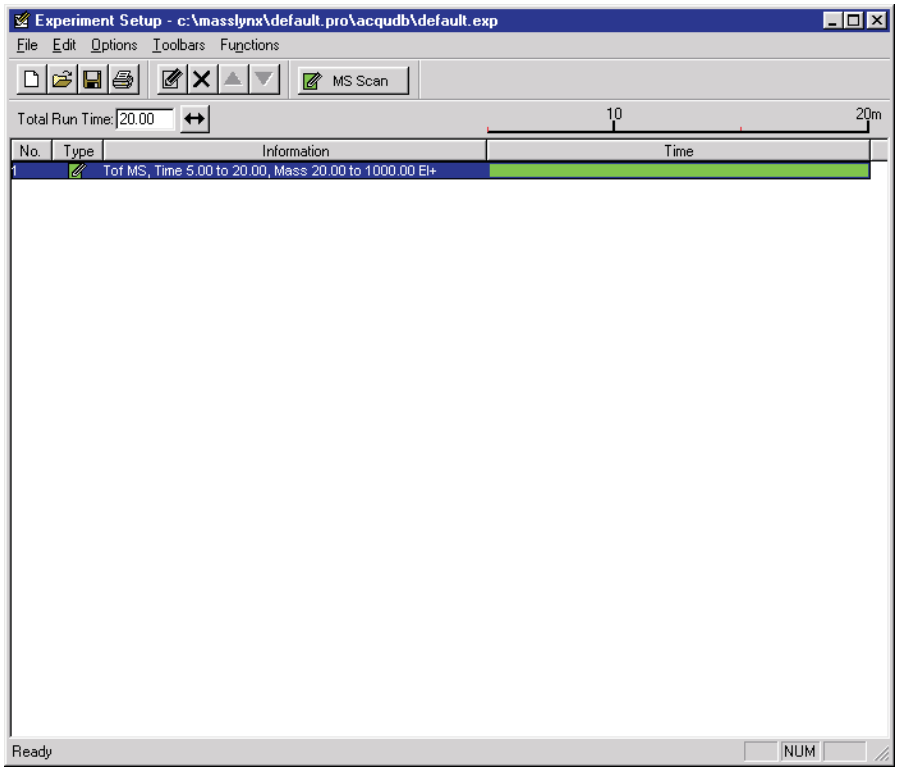
Right click on the column heading and select “Properties” from the drop down menu.

The “Field Properties” dialog box will appear.

Set “decimal places” to 0 and press “OK”.

For more details of the MassLynx top level screen see the *MassLynx NT Users Guide (Micromass* part number 6666536)

The Experiment Editor



Introduction

The experiment editor is used to set up the function(s) that the mass spectrometer uses to scan the instrument during an acquisition. A function list can be a mixture of different scanning techniques that can be arranged to run either sequentially or concurrently during an acquisition.

A function list is produced in the experiment window, saved on disk as a .exp file and then referenced by name on the sample list.

A simple experiment is shown above, containing only one function: a centroided mode full scan, between 20 and 1000 amu using EI+ ionisation. Immediately above the function bar display is a time scale that shows from when the function is active, and for how long it runs. In this case the function starts after 5 minutes and then runs for 15 minutes, terminating after a total elapsed time of 20 minutes.

To access this dialog:

Press  on the MS panel of the MassLynx screen.

The Experiment Editor Toolbar

The toolbar is displayed at the top of the tune window and allows some common operations to be performed with a single click.

 Create a new experiment.

 Open an existing experiment.

 Save the current experiment to disk.

 Print the current window in portrait format.

Create a new function.

 Edit the selected function.

 Delete the selected function.

 Move the selected function up the list of functions.

 Move the selected function down the list of functions.

Adding a New Function

To add a new function to the list:

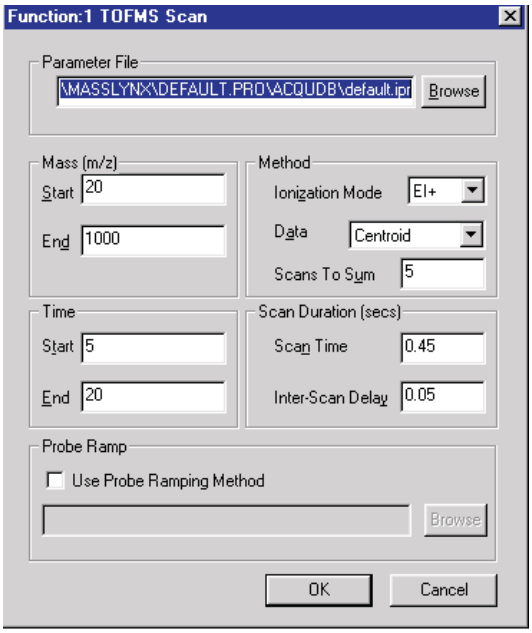
Click the toolbar button **MS Scan**, or select the required function from the **Function** menu.

Make any changes required to the parameters and press **OK** to add the new function.

Setting up a Full Scan Function

The full scan function editor, activated by pressing 

or by selecting **TOF MS** from the **Functions** menu, is used to set up centroid, continuum and MCA functions.



Parameter File

This references which tune page parameters to use for the acquisition.

The appropriate instrument tuning parameters should be set up on the tune page, and then the tune page should be saved from its **File** menu as a .ipr file.

You can then reference the .ipr file in this dialog by typing in the path and name of the calibration file or press the **Browse** button and locate the required calibration file using the **Open** dialogue.

It is important to make sure that you are using the correct .ipr file here. For good mass accuracy you must use an appropriate calibration file as described later in this chapter. The parameters saved in the .ipr file referenced here should be the same as those used in the creation of the calibration. Furthermore remember that the Lock Mass is specified on the tune page and therefore is saved in the .ipr file. If you need to adjust it you must do so on the tune page then save the .ipr file and make sure it is referenced correctly here.

Mass (m/z)

Start Mass and **End Mass** specify the masses at which the scan starts and stops. **Start Mass** must be lower than **End Mass**.

Time

Start and **End** specify the retention time in minutes during which this function becomes active, and data are acquired.

Method

Ionization Mode specifies the ionization mode and polarity to be used during acquisition.

Scan Duration

Enables setting of the Scan Time and the Inter-Scan Delay

Use Probe Ramping Method

For acquiring data using the solids probe or DCI probe from the sample list the appropriate probe ramping file must be inserted here. The probe ramp is defined from the tune page as described in the chapters *The Solids Insertion Probe* and *The DCI Probe*.

A ramp can be saved as a .prb file and can then be referenced in this dialogue. When the acquisition is started the probe ramp will be started and will follow the ramp defined in the file.

Modifying an Existing Function

To modify an existing function:

Select the function in the experiment window.

Press  , or double click on the function.

This displays the editor for the function and allows changes to be made.

The experiment setup display is updated to show any changes.

Entering a new a value in **Total Run Time** *and pressing*  *sets the maximum retention time for the experiment. The ratio of the functions defined is maintained. For example, if two functions are defined one from 0 to 5 minutes and the other 5 to 10 minutes then a* **Total Run Time** *of 10 minutes is displayed. If this value is changed to 20 then the first function now runs from 0 to 10 minutes and the second from 10 to 20 minutes.*

Copying an Existing Function

To copy an existing function:

Select the function in the experiment window.

Select **Copy** and then **Paste** from the **Edit** menu.

Modify the parameters as described above.

Removing a Function

To remove a function:

Select the function in the experiment window.

Press  , choose **Delete** from the **Edit** menu, or press **Del** on the keyboard.

When asked to confirm the deletion, select **Yes**.

Changing the Order of Functions

Functions are displayed in ascending **Start Time** and **End Time** order and this order cannot be changed. For functions that have the same start and end time the order in which they are performed can be changed as follows:

Highlight the required function.

Press  or  repeatedly until the function is in the required position.

Setting a Solvent Delay

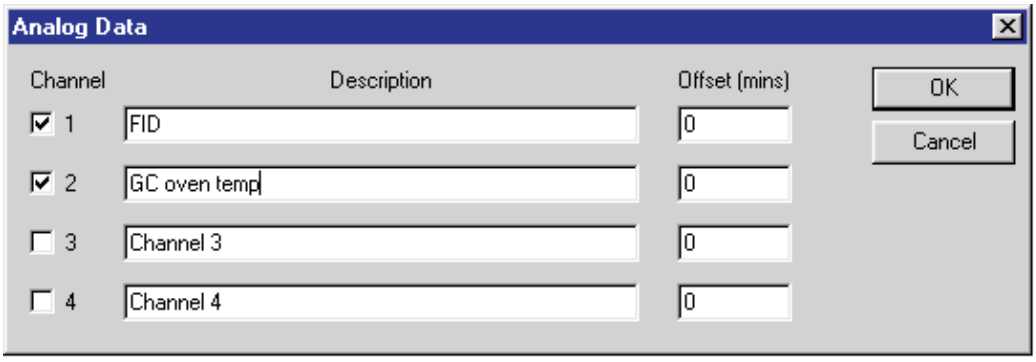
No data is stored during the solvent delay period, which means that solvent peaks that would normally be seen eluting on the TIC chromatogram are no longer seen.

During solvent delay the filament is turned off in EI/CI mode or extraction voltage is turned to zero in FI mode. Solvent delay is useful to protect the emitter or filament against the sudden pressure rise seen as the solvent front comes off the GC column after an injection.

To set a solvent delay for a function list:

Select **Solvent Delay** from the **Options** menu.

Analog Channels



Using the analog channels, up to 4 channels of analog data can be acquired, which are stored with the data acquired from the mass spectrometer. Analog channels are typically used to collect data from external units such as a FID detector. A reading is made from the external channel at the end of each scan and stored with the data for that scan. The resolution of the chromatography for an analog channel is therefore dependent on the scan speed used to acquire the mass spectrometry data.

To access this dialog:

Select **Analog Data** from the **Options** menu.

To store data for an analog channel:

Check the box(es) for the channel required.

Enter a textual description for each of the selected analog channels.

This description is used on the analog chromatogram dialog as the channel description. See "Chromatogram" in the MassLynx User's Guide.

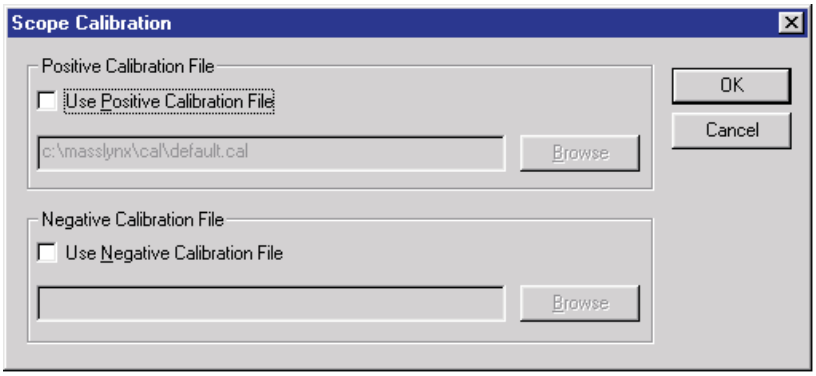
Enter an **Offset** to align the external unit with the mass spectrometer if necessary.

Press **OK**.

Calibration

See the chapter entitled *Calibration and Exact Mass* for details on how to create an appropriate calibration file. In order for the data to be mass measured correctly you must reference the correct and appropriate calibration file in the experiment file. Ideally, the calibration file should have been acquired with the same tune page parameters as in the .ipr file referenced in the experiment file.

To specify a calibration file, select **Calibration** from the **Options** drop down menu of the Experiment Method Editor.



To specify the calibration file to be used when acquiring in positive ion mode select the **Use Positive Calibration File** tick box. Either type in the path and name of the calibration file or press the **Browse** button and locate the required calibration file using the **Open** dialogue.

To specify the calibration file to be used when acquiring in negative ion mode select the **Use Negative Calibration File** tick box. Either type in the path and name of the calibration file or press the **Browse** button and locate the required calibration file using the **Open** dialogue.

Saving and Restoring an Experiment

To save an experiment:

Choose **Save As** from the function list **File** menu.

Enter a new file name, or select an existing file from the list displayed.

Press **Save**.

If the file already exists on disk, confirmation is requested to overwrite the existing information.

Press **Yes** to overwrite the file, or **No** to select a different name.

When the editor is closed a prompt is issued to save any changed function lists.

To restore a saved experiment:

Choose **Open** from the experiment setup window file menu.

Select the name of the experiment to open, either by typing its name or by selecting it from the displayed list.

Press **Open**.

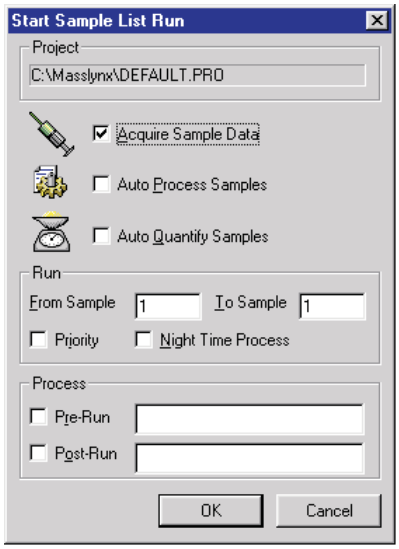
Starting a Multi-sample Acquisition

To start a multi-sample acquisition:

Set up a sample list.

Choose **Start** from the top level **Run** menu, or press  .

This displays the start sample list run dialog.



Check the **Acquire Sample Data**, **Auto Process Samples** and **Auto Quantify Samples** boxes as required.

Enter values in the **Run From Sample** and **To Sample** boxes.

The default is all samples in the list.

Check the **Priority** and/or **Night Time Process** boxes as required.

See the "Getting Started" chapter of the MassLynx manual for details.

Press **OK**.

Repeat the above procedure as required.

Sample lists are added to a queue and run sequentially unless **Priority** *or* **Night Time Process** *has been checked.*

The sample which is currently being acquired has a  *next to it in the sample list.*

Process

The process controls allow processes to be run before and after the acquisition. The **Pre-Run** control is used to specify the name of a process that is run before acquisition of the files in the sample list.

The **Post-Run** control is used to specify the name of a process which is run after acquisition of the files in the sample list. This could be used, for example, to switch the instrument out of operate and to switch off various gases.

To run a process after each sample in the sample list has been acquired:

Format the sample list to display the **Process** column and enter the name of the process to be run for each of the samples.

For the process to automatically operate on the data file which has just been acquired:

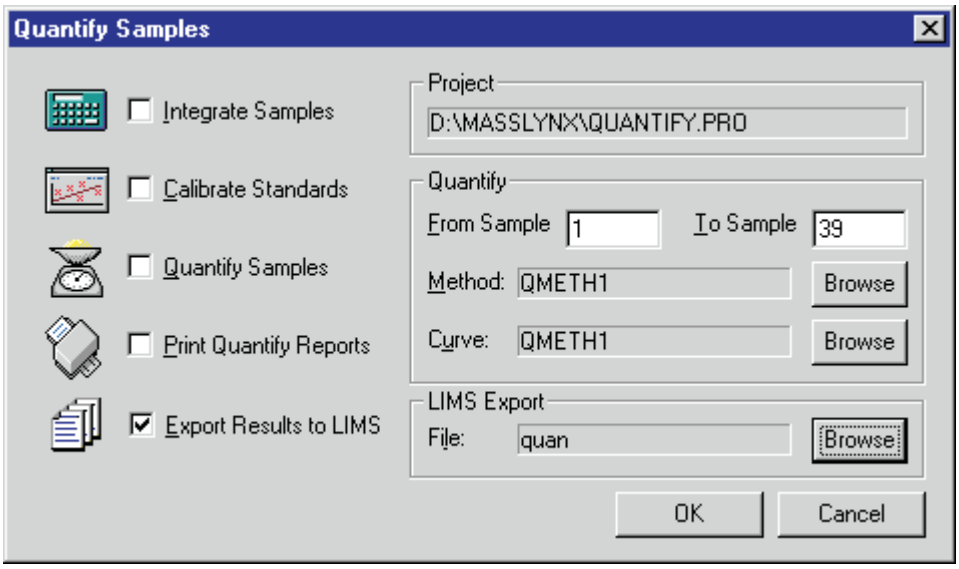
Leave unchecked **Use Acquired File as Default** on the **System** tab of the **MassLynx Options** dialog.

The **MassLynx Options** *dialog is accessed by choosing* **Options** *from the* **MassLynx Tools** *menu.*

An Example of Automated Analysis of Sample List

To display the quantify samples dialog:

Select **Process Samples** from the **Quantify** menu. Check the boxes required and press **OK**.



Quantify Samples

This dialog allows automatic processing of data files once they have been acquired. To perform integration, calibration of standards, quantification of samples and printing of quantification reports select the relevant check boxes. See *Quantify, MassLynx User Guide*, for more detailed information about using automated sample list analysis.

Integrate Samples

Integrates all the sample data files named in the peak list.

Calibrate Standards

Uses integration results to form quantify calibration curves.

Quantify Samples

Uses integration results and quantify calibration curves to calculate compound concentrations.

Print Quantify Reports

Produces hard copies of the results of integration and quantification.

Export Results to LIMS produces a text file containing the quantification results details for use with LIMS systems. If this box is checked the LIMS Export **Browse** button becomes enabled. Press **Browse**, select a file or enter the name of a new one and press **Save**.

The **Project** field displays the project into which data are acquired.

To change the project into which data are acquired, the acquisition should be cancelled and a new project created by choosing **Project Wizard**, or an existing one opened by choosing **Open Project,** from the MassLynx top level **File** menu.

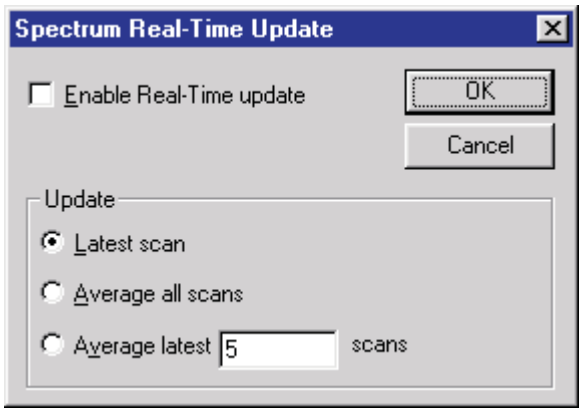
From Sample and **To Sample** set the range of samples in the sample list which is analysed.

Chromatogram Real-Time Update

To view in real time the chromatogram that is currently being acquired:

Open the data file using the MassLynx data browser.

Press  , or select **Real-Time Update** from the **Display** menu. The chromatogram display is updated as the acquisition proceeds.



Spectrum Real-Time Update

To view in real time the spectrum that is currently being acquired:

Open the data file using the MassLynx data browser.

Press  , or select **Real-Time Update** from the **Display** menu.

Select **Enable Real-Time update**. *Real-time update can also be turned on and off via the Real-Time spectrum toolbar button.*

When real-time update is on the display is continually updated with spectra from the current acquisition. The actual information displayed is determined by selecting one of the following radio buttons.

- Latest scan

Displays the last acquired scan. This is the default option.

- Average all scans

Updates the display with spectra formed by averaging all the spectra that have so far been acquired.

- Average latest scans

Updates the display with spectra formed by averaging the last *n* scans acquired, where *n* is specified in the associated edit control.

Stopping an Acquisition

To halt the acquisition:

From the tune page, press the **Stop** icon  .

From the MassLynx screen choose **Stop** from the **Run** menu, or press  .

Data acquired up to this point is saved.

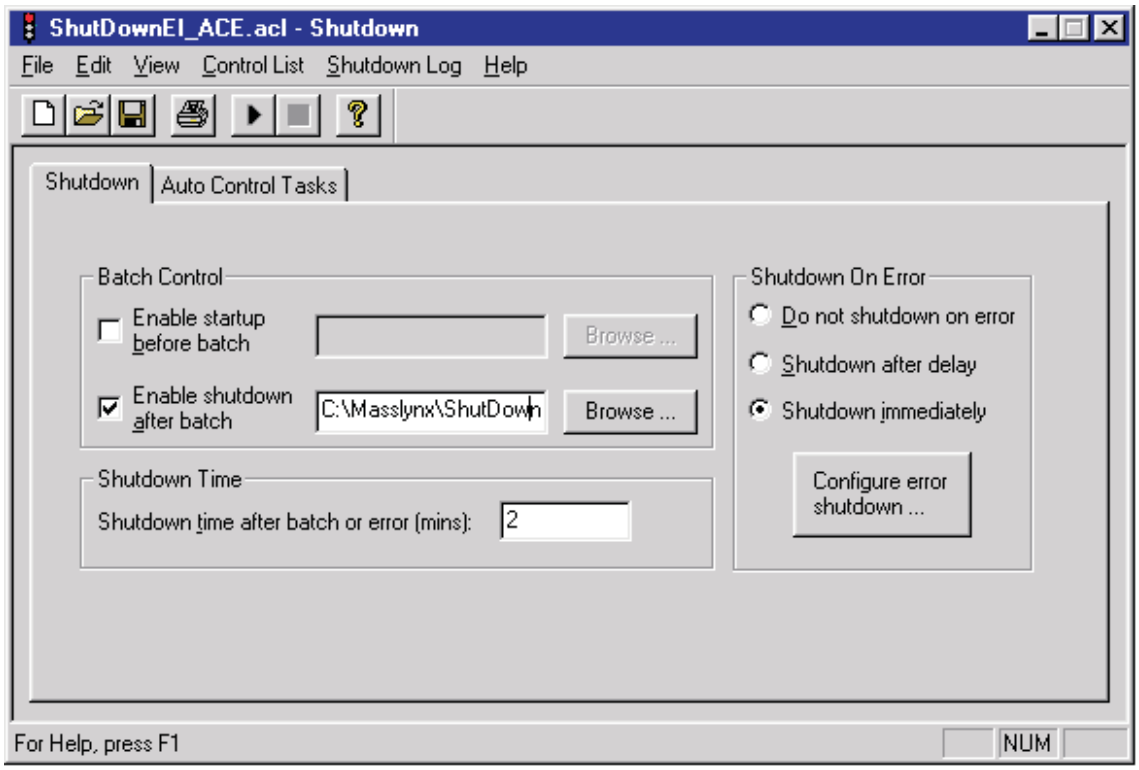
Automatic Startup and Shutdown

MassLynx comes with automatic Startup and Shutdown files. They are found in the C:\MassLynx\Shutdown directory and are called ShutDownxxx.acl and StartUpxxx.acl where xxx refers to the instrument configuration. E.g. ShutDownESI_ACE.acl for an instrument configured as an ACE system.

When **Startup** or **Shutdown** is selected from the MassLynx **Run** menu it is these files which are run.

The Shutdown Editor

The shutdown editor, shown below, allows the automatic startup and shutdown procedures to be modified or new procedures to be created. To access the editor, select **Edit Shutdown** from the MassLynx **Run** menu.



Enable Startup before batch

Check this box to perform the startup tasks when a Sample List is submitted from MassLynx or OpenLynx.

Enable Shutdown after batch

Check this box to perform the shutdown tasks after a batch of samples has completed.

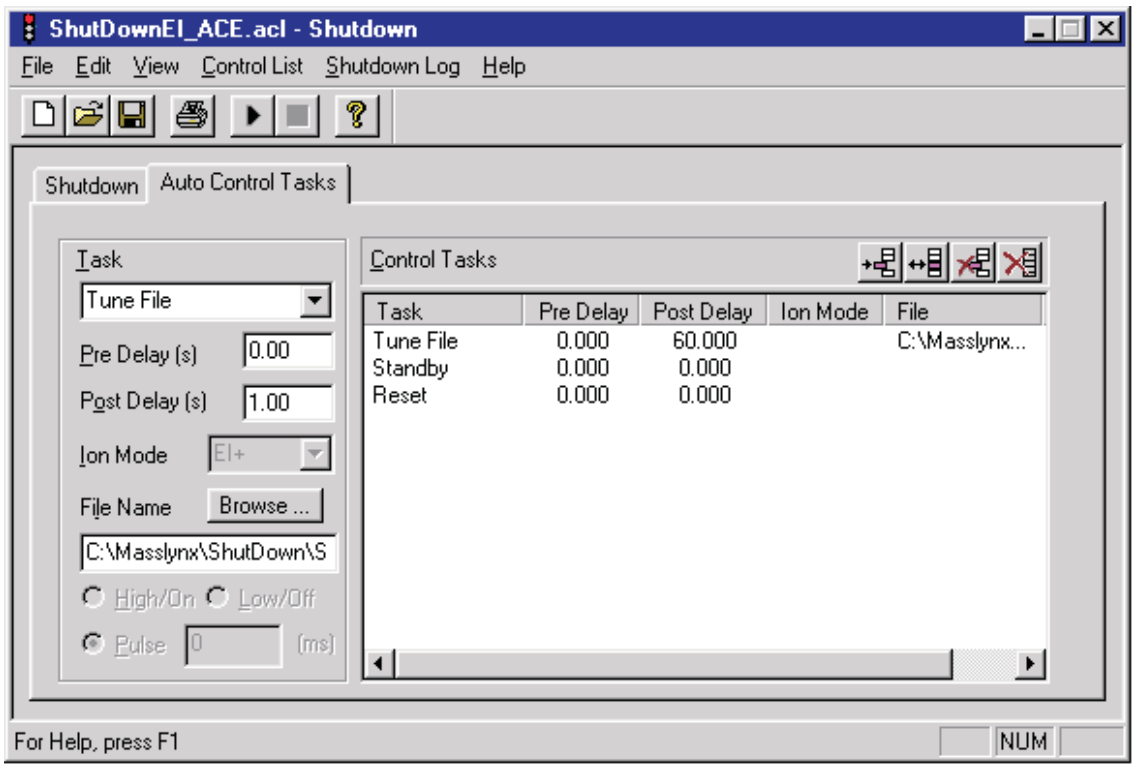
Shutdown Time after batch or error

Enter a time at which to perform the shutdown tasks.

Shutdown on error

Select the desired option to perform the shutdown tasks when an error has occurred.

The Auto Control Tasks Page



Task

This is a dropdown list box with all the available tasks.

Pre-Delay

This is the length of time that will elapse before the current task is performed.

Post-Delay

This is the length of time that will elapse after the current task has been completed and before the next task is started. E.g. a Post delay of 60s, in the **Tune File** task above, means that there will be a delay of 60 seconds before the next task is started, to allow the machine to stabilise with the new tune page settings.

Ion Mode

This is a dropdown list box with all the available ionisation modes.

File Name

This is the name of the Tune file to be used. The file name can be typed in, including the full path name, or selected from the browser displayed when the ... button is pressed.

To Add a Task

Select a task from the dropdown Task list box.

Enter the required parameters.

Press the add  button.

If this is a new task timetable the task will be added to the end of the list. If a task has been inserted into the task timetable then all subsequent tasks will be added after the inserted task.

To add a task to the end of the timetable after inserting a task,

Click twice with the left mouse button below the last entry in the timetable and then add the new task.

To Insert a Task

Click, with the left mouse button, on the entry in the task timetable before which you want to insert the new task.

Select a task from the dropdown Task list box.

Enter the required parameters.

Press the add  button. The task will be inserted before the selected entry.

To Modify a Task

Click, with the left mouse button, on the entry in the task timetable. The details for the task will be displayed in the fields on the left of the screen.

Change the required parameters.

Press the modify  button. The details will change in the task timetable.

To Delete a Task

Click, with the left mouse button, on the entry in the task timetable. The details for the task will be displayed in the fields on the left of the screen.

Press the  button. The task selected will be deleted from the task timetable.

To Delete All Tasks

Press the  button. All tasks will be deleted from the task timetable.

To Change the Width of a Column

The width of the columns can be changed, by positioning the mouse pointer on the heading between two columns until the ✣ symbol appears, and then clicking the left mouse button and dragging until the column is the required width.

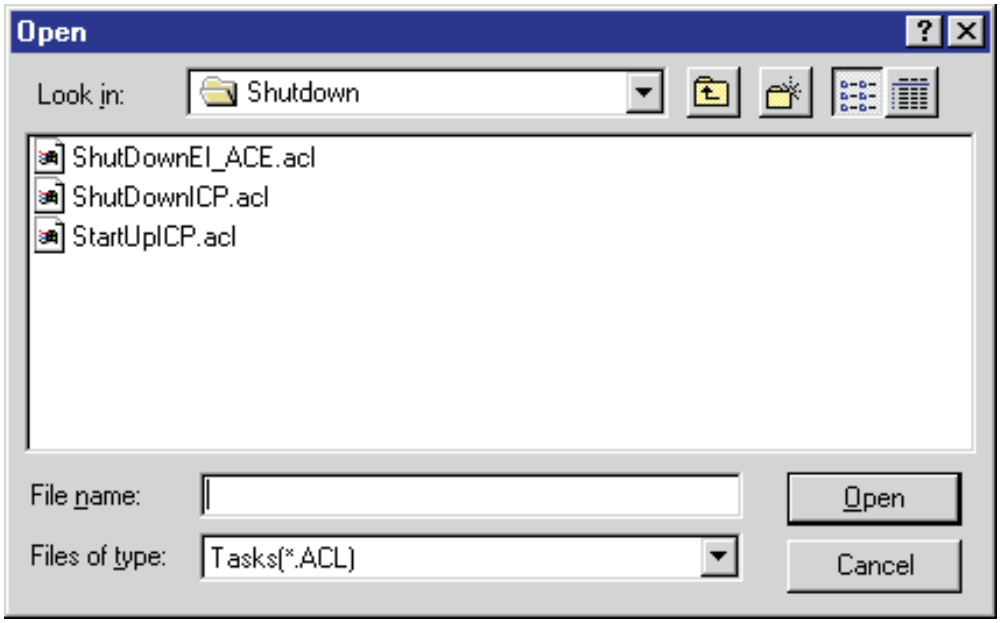
The Shutdown Editor Toolbar

Toolbar button	Menu equivalent	Purpose
	File... New	Create a new Startup or Shutdown file
	File... Open	Open an existing Startup or Shutdown file
	File... Save or File... Save As	Save a Startup or Shutdown file
	File... Print	Print a Startup or Shutdown file
	Control List... Run List	Run a Startup or Shutdown file
	Control List... Stop List	Stop a Startup or Shutdown file
	Help... Help Topics	Invoke help

Saving/Loading Startup and Shutdown Files

To Open a Startup or Shutdown file

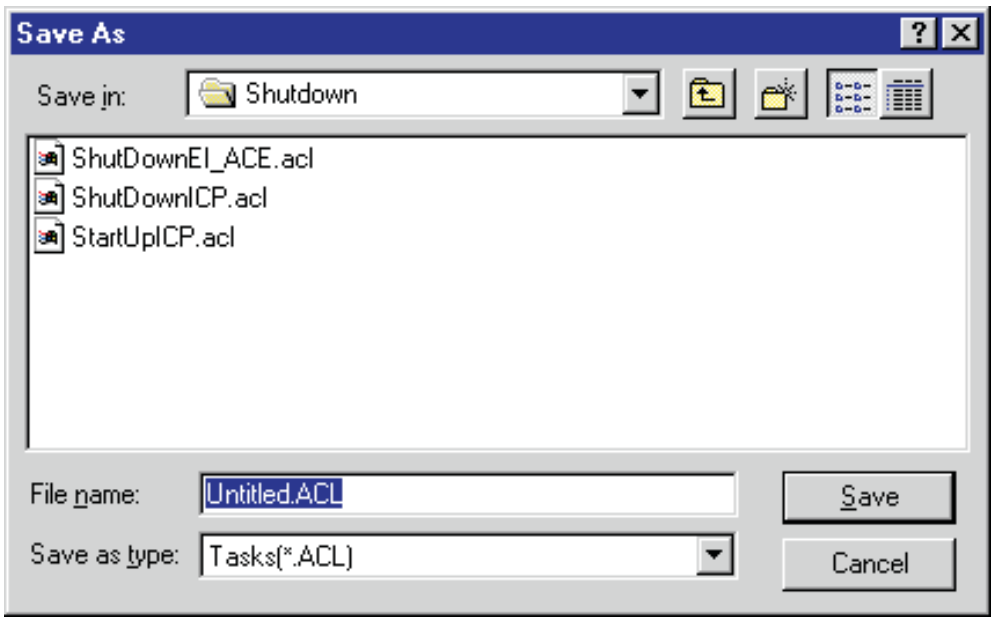
Press the  Toolbar button or select **Open** from the File menu. This displays the Open file dialog.



Select a data file and press the **Open** button.

To Save a Startup or Shutdown file

Press the  Toolbar button or select **Save** or **Save As** from the File menu. If this is a new file, or the **Save As** option has been selected, the Save As dialog is displayed

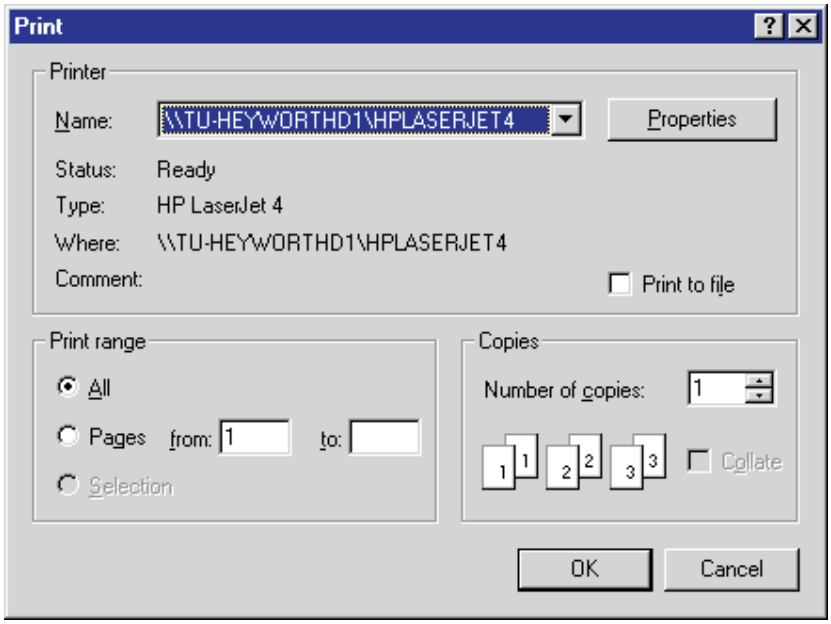


Type a name into the File Name box and press the **Save** button.

Printing Startup and Shutdown Files

To Print a Startup or Shutdown File

Press the  Toolbar button or select Print from the File menu. This displays the Print dialog.



Select the printer, print range and number of copies and press the OK button.

Creating Startup and Shutdown Files

To Create a Startup or Shutdown File

Press the  button or select New from the File menu.

Running Startup and Shutdown Files

If **Startup** or **Shutdown** is select from the MassLynx **Run** menu or from the Shutdown editor **Control List** menu then the automatic startup and shutdown files are run.

To run a different Startup or shutdown file;

Open the required file in the Shutdown editor and press the  toolbar button or select **Run List** from the Shutdown editor **Control List** menu.

Press the  toolbar button or select **Stop List** from the Shutdown editor **Control List** menu if you wish to stop running this file.

Calibration and Exact Mass

Introduction

The elevated resolution and inherent stability of the calibration law of orthogonal TOF instruments allow accurate mass measurements to be performed. The basic time of flight to mass relationship is of the form

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

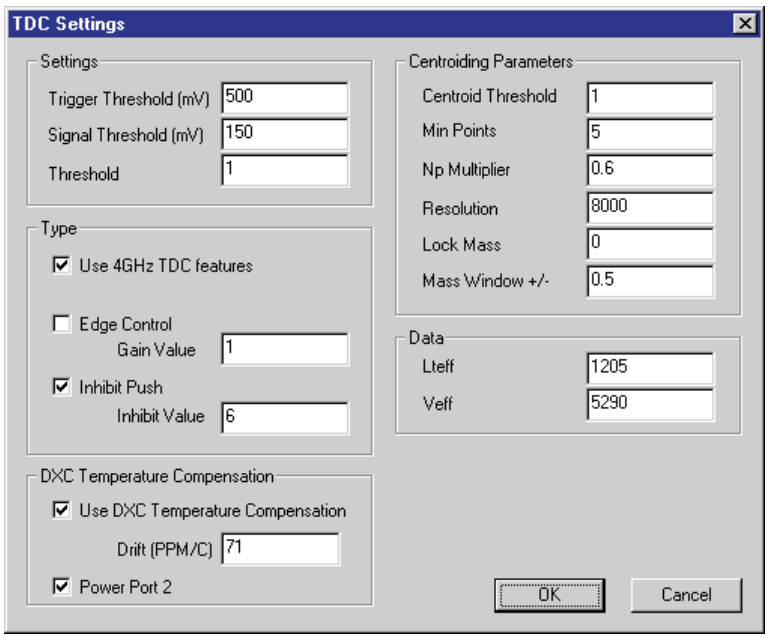
where

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.

It is important that the gain is set up to give at least nominal mass accuracy. Nominal mass measurement is achieved on the GCT by adjustment of the **Lteff and Veff** factors which appear in the **OPTIONS/TDC SETTINGS** menu accessed from the GCT Tuning page.



Nominal Mass Accuracy

It is important that the pathlengths are set up to give at least nominal mass accuracy. Nominal mass measurement is achieved on the GCT by adjustment of the **Lteff** factor.

Acquire a TOF spectrum of a standard compound is acquired with **Lteff** set to its default value of 1195.

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

$$Lteff = 1195\sqrt{(m_{ind}) \div (m_{act})}$$

where:

m_{ind} = indicated m/z

m_{act} = actual m/z

Enter this new value under **TDC Parameters**.

After switching the real time tune display off and on, all subsequent mass measurements will be nominally correct(within ± 0.5) m/z.

With no calibration applied the data displayed on the spectrum in MassLynx is just a set of mass intensity pairs {Mn,In} based upon instrument geometry. Because of the inherent relationship between mass and time shown above it is prudent to generate higher order calibration coefficients that are applied to the square root of the nominal masses {Mn} i.e:-

$$\sqrt{c} = A + B\sqrt{n} + CMn + DMn^{3/2} + ...$$

The terms A,B,C,D…. are calculated by fitting a polynomial to acquired mass spectral data and 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. With a polynomial of order 5 (the highest supported in MassLynx) there will be six terms generated.

Normally a first order calibration should be generated for the GCT. If a higher order polynomial calibration is used it is important to be aware that the calibration may not extrapolate beyond the highest m/z value used for generating the calibration.

Once a calibration has been generated from a reference compound such as Tris trifluoromethyltriazine, it should be used as an “instrument calibration” to be applied to all subsequently acquired data.

Temperature variations in the environment and power supplies may cause the instrument to drift hundreds of ppm over the course of a day. In order to compensate for the change in lab temperature the GCT uses Dynamic External Calibration(DXC) to stabilise the mass drift. When performing accurate mass work it is advisable to keep the instrument in operate at all times so that the power supplies can stabilise.

However, the isolation valve should be closed and the filament current set to zero if the system is to be left unattended, to extend the lifetime of the MCP detector and filament.

Instrument drift can be compensated for by applying a single point lock mass correction that recalculates term B in the above equation. The lock mass reference may be introduced via the septum interface.

The data acquisition system for the instrument is a Time to Digital Converter (TDC). This is an ion counting type of system that generates a mass spectrum by storing arrival times of ions in a histogram memory. After the arrival and detection 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 5ns.

The consequence of this dead time is that at high ion currents a proportion of the ions generated are not registered leading to a shift to lower mass centroids and lower areas on reported peaks.

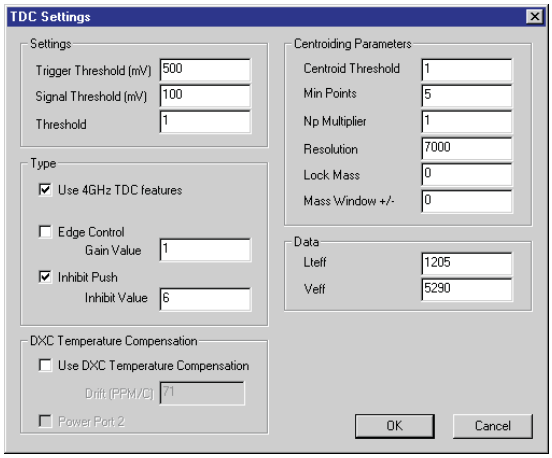
There is dead time correction software in MassLynx that allows accurate mass measurements to be achieved at a larger range of ion currents than otherwise would have been possible.

The use of this software correction is in the following section.

Generation of an Instrument Calibration EI+ Operation

Introduce 0.2µl of tris (trifluoromethyl) triazine via the septum inlet.

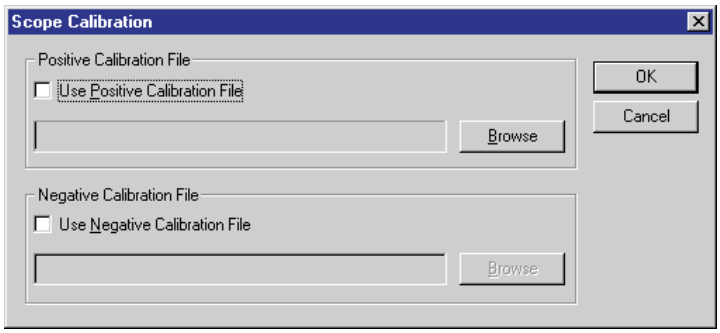
Initially set the Centroiding parameters as shown below in the TDC settings menu, with the Lteff and Trigger/Signal threshold set as described in the relevant section in this manual.



Resolution reflects the base resolution FWHM of the system at m/z 614 of Heptacosa. This defines the peak width at a given mass in ns and is used to calculate the probability of peak distortion due to ion arrivals at a given ion current within the dead time of the TDC.

Np is a factor applied to the calculated number of individual pushout events which occurred within a single spectral duration. This value can be adjusted to apply the dead time correction to the best possible effect for a given system.

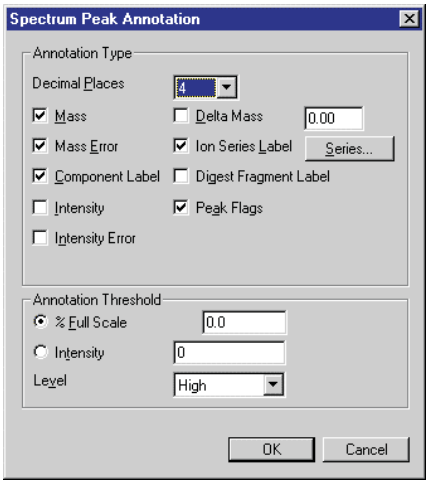
Ensure that no calibration file is selected in the acquisition parameters from the menu below.



Acquire data over the range 10-800 Da, in real time centroid mode with a spectral time of 0.9 second and a delay between spectra of 0.1 s.

The data acquired should not be too intense or deadtime distortion will occur. To check that the signal is not too intense select a single spectrum from the chromatogram.

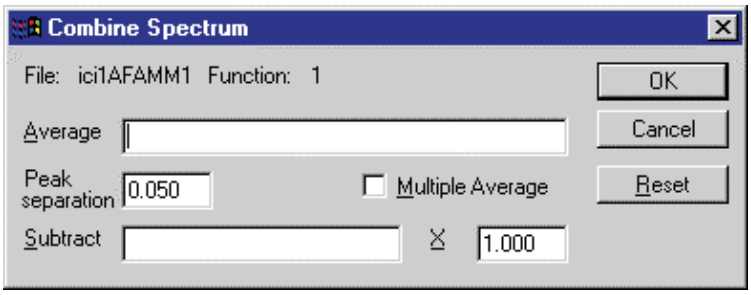
From **DISPLAY / PEAK ANNOTATION** check the box labelled **Peak Flags**.



If the symbol ? appears over any of the calibrant peaks in the spectrum, reduce the intensity of the signal until it disappears.

Select **TOOLS / COMBINE** from the spectrum window.

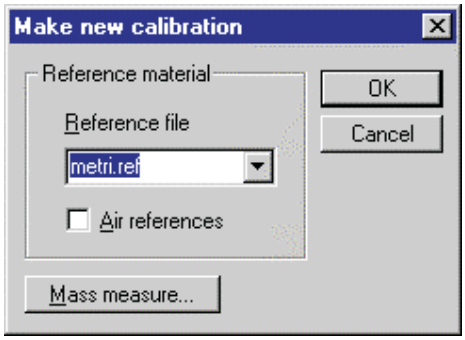
Ensure that the peak separation window is set to 0.05da. If a window of 1da is set all peaks within a ± 0.5da window will be combined into a single peak. Background peaks which were resolved by the GCT and correctly peak detected may then distort the mass measurement of the calibrant peaks.



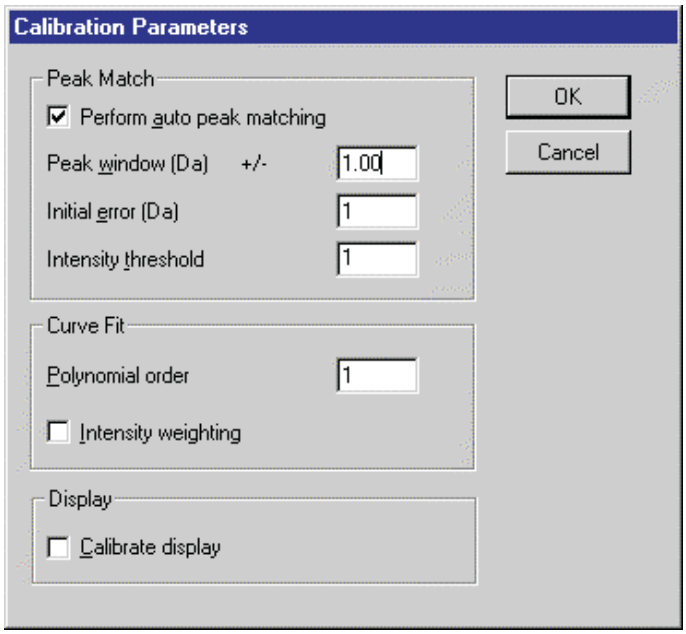
Combine at least 30 scans of data.

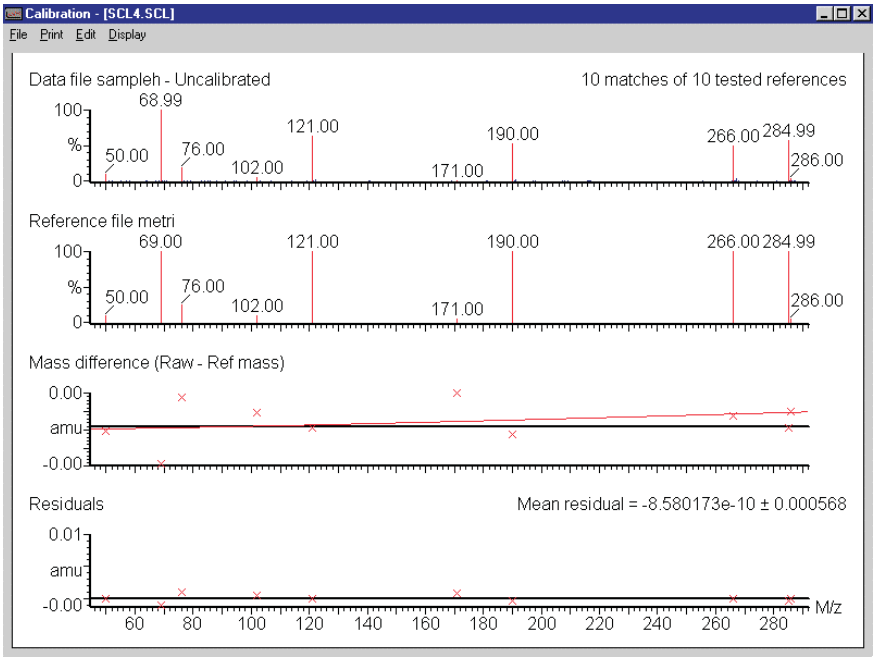
Display the combined spectrum just generated. Now go to the **Tools, Make Calibration** menu:

First select the desired reference file. File metri.ref will give the correct masses with the reference described above (tris(trifluoromethyl)triazine. Choose **OK**.



Make sure that the residual errors are all less than about 2mDa. The calibration parameters can be altered by clicking on ***Edit*** *to reveal the menu below:*





After these parameters have been set, click on **OK** to regenerate the calibration with the new parameters if required.

Note that peaks may be excluded from the calibration curve by de-selecting them with a right mouse click in the reference file spectrum followed by a right click on the data file spectrum at the top of the window.

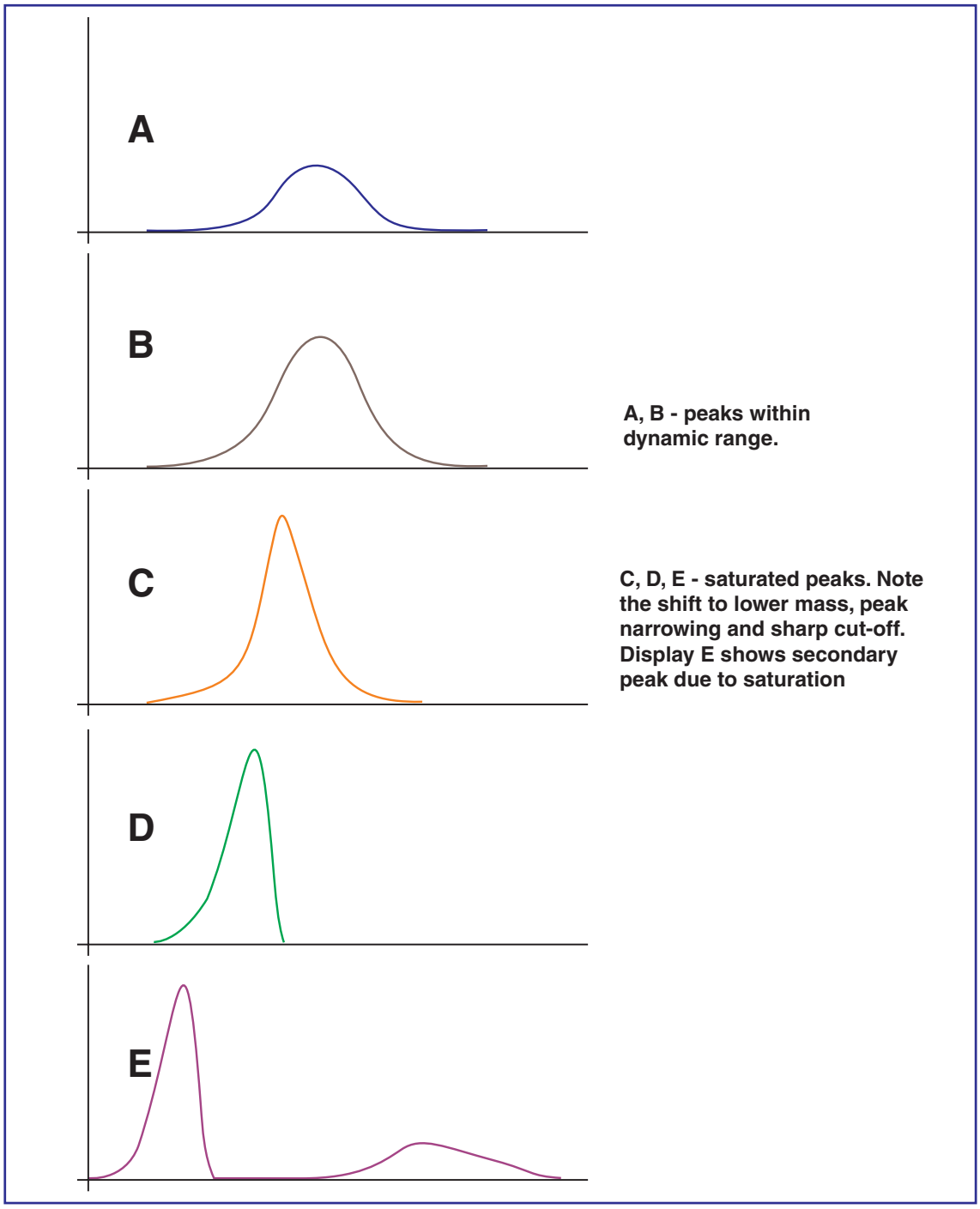
Effects of Saturation on Peak Shape

The peak shape will characteristically change as saturation of the TDC results in increasing numbers of ions not to be detected.

At the onset of saturation the peak will shift a little to lower mass followed by a failure to increase in recorded intensity as the ion signal continues to increase. There will then be a sudden, sharp high mass cut off and finally the detector will appear to 'ring', causing a secondary peak to appear on the high mass side of the peak of interest.

The diagram below represents the characteristic transition from unsaturated signal to saturated signal, which would be seen when tuning using the real time peak display in continuum mode.

Because the recorded intensity of the saturating ions will appear to reach a constant value it is important to be able to recognise the onset of saturation by the change in peak shape and position.



The effect of deadtime saturation on centroid determination is to shift the measured mass to a lower value than calculated from the empirical formula. In the residuals box from the calibration, insufficient deadtime correction would be reflected by a systematic shift of the largest ions in the spectrum (those with the highest degree of dead time saturation) to a position below the line of best fit compared to the smaller ions in the spectrum (those with the least degree of dead time saturation). If there is too much dead time correction applied the converse will be true.

Within this particular reference file the first and second carbon isotopes of the molecular ion at m/z 284.9949 are present. Because of the disparaging difference in the intensity of these two ions, this region of the calibration curve can be used to set the dead time correction to the optimum value for the system.

Considering the residuals box in the calibration display, if the first isotope marker lies below the line of best fit compared to the second isotope marker, insufficient dead time correction is being applied.

In this case the value for **Np** should be changed from 1.0 to 0.9. The acquisition must then be restarted and a new average generated.

NOTE: Display a single spectrum. If the symbol ? appears over any of the calibrant peaks in the spectrum, reduce the intensity of the signal until the symbol disappears.

Recalibrate using the same method as above and again examine the residuals in the calibration output.

Considering the residuals box in the calibration display, if the first isotope marker lies above the line of best fit compared to the second isotope marker, too much dead time correction is being applied. In this case the value for **Np** should be changed from 1.0 to 1.1. The acquisition must then be restarted and a new average generated.

By iterations of the method described above the first and second isotope peaks of the molecular ion of Tris (trifluoromethyl) triazine should be within 1 mDa of each other on the residuals output.

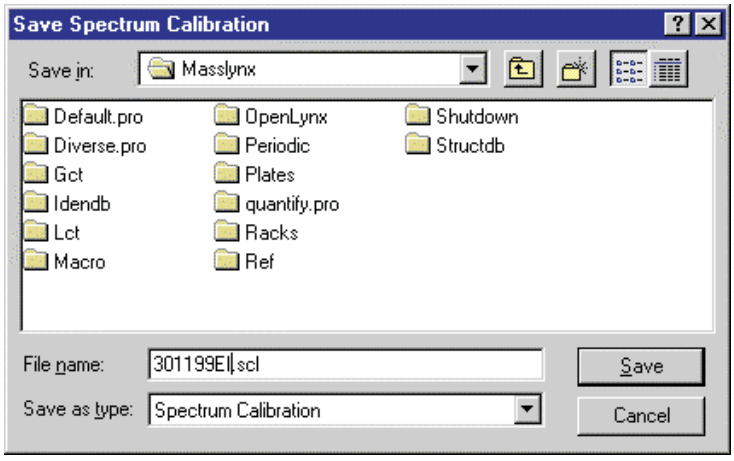
This value of resolution and **Np** should be used for all subsequent work.

It is advisable to check that this value still gives the best result whenever an EI calibration is performed and adjust if required.

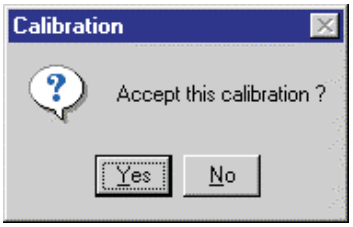
The usual value for **Resolution** is 7000 (3.6GHz TDC) and 5000 (1GHz TDC).

The usual value for **Np** is between 0.5 and 1.0 depending on the particular system characteristics.

Once an acceptable calibration has been performed select **FILE / SAVE AS** from the calibration display page and save the calibration under a chosen file name.



Exit the calibration page. The following message will be displayed.



Select **No**

If **Yes** is selected the data file acquired will be mass measured using the calibration file saved. This will not affect subsequent acquisitions where a calibration file is specified in the acquire menu.

Future acquisitions may be made using the saved calibration by selecting the calibration file generated above, using **Browse** from the **Acquire, Calibration** menu on the tune page. It is recommended that separate calibrations are made for negative and positive ion mode.

If acquiring data via the sample list, the calibration file is selected via **Options**, **Experiment Setup**, **Options**, **Calibration** on the tune page. The experiment file should be saved with the required calibration file selected.

Calibration and Accurate Mass in FI Mode

Because the spectra produced in FI mode are usually very simple, often exhibiting only molecular ion information, a mixture of volatile compounds must be used to initially characterise the time to square root of mass relationship. The following list shows the compounds and their relative amount by volume to produce a suitable calibration mixture.

Compound Name	% By Volume	Expected Masses
Heptacosa (PFTBA)	20	501.9711,68.9952
Perfluorotrimethylcyclohexane	70	380.9760,68.9952
Hexafluorobenzene	2	185.9998
Pentafluorobenzene	2	167.9998
Acetone	1	58.0419
Chloropentafluorobenzene	2	201.9609
Xylene	2	106.0783

These proportions may be adjusted to produce a more balanced spectra if required. The sample should be kept in a sealed vial, out of direct light and in a cool place. The very volatile components (Acetone) may decrease in proportion to the less volatile components over time.

The perflorinated compounds suggested may be purchased from Fluorochem Ltd. (http://www.fluorochem.co.uk).

Approximately 10 - 15µl of this calibration mixture should be introduced and a suitable number of scans averaged to produce a calibration.

Optimisation of FI Calibration

The spectra produced from the calibration mixture above contains fragment ions from some of the compounds in the mixture as well as molecular ions. The fragment ions form at a slightly different time and hence position with respect to the emitter than do the molecular ions.

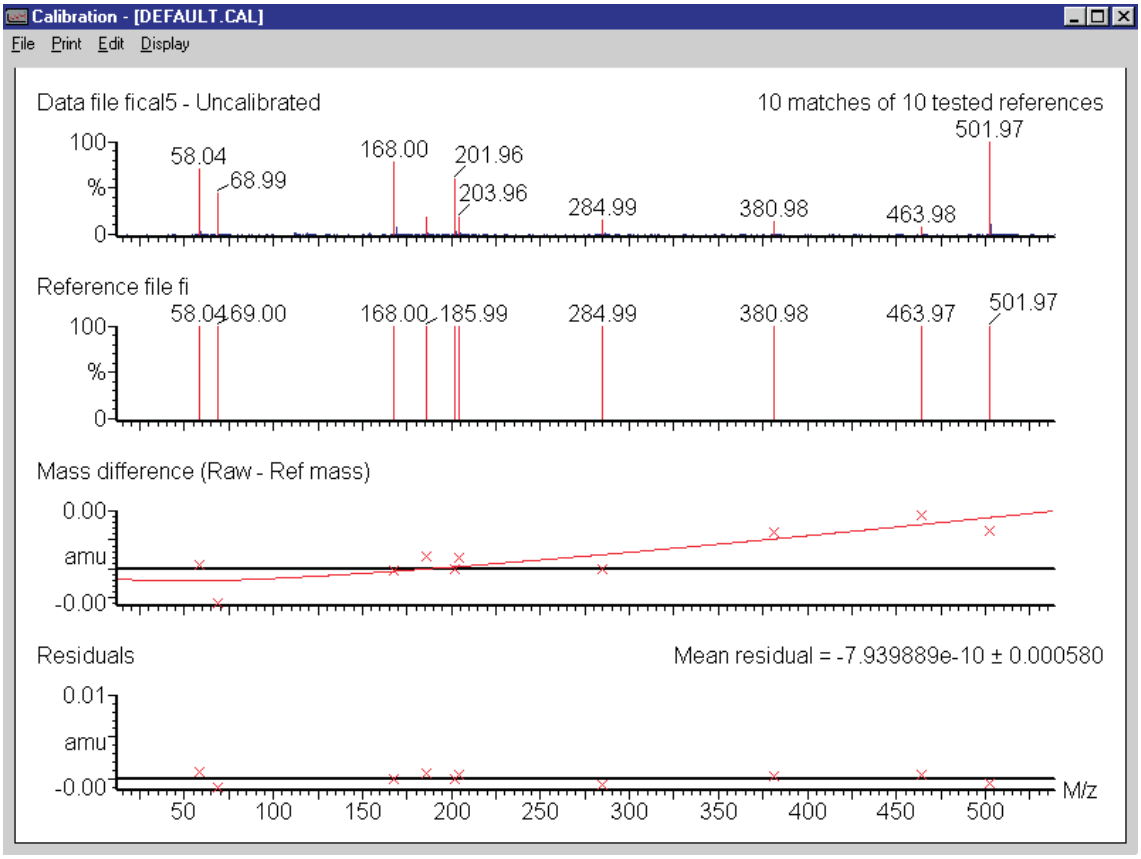
Because of the very high potential gradient between the emitter and the extraction rods the fragment ions have a slightly different resultant axial energy entering the pushout region than the molecular ions. This results in some ions within the calibration residuals appearing below or above the line of best fit.

These differences can be minimised by small adjustments in the **Pusher Bias** voltage in the Engineers page.

Acquire data from the calibrant mixture and examine the residuals produced. If m/z 502 from heptacosa is below the line of best fit reduce the **Pusher Bias** by 0.2V. Repeat the calibration experiment using different values of **Pusher Bias** until the calibration residuals are within 2 - 3 mDa. A second order calibration may be necessary.

Difficulty in producing an acceptable calibration may indicate excessive extraction rod contamination. The rods should be cleaned.

A typical spectrum generated from this mixture plus tris (trifluoromethyl) triazine in FI mode is shown below along with the calibration results.



Calibration and Exact Mass in CI+ Mode

Tris (trifluoromethyl) triazine yields predominantly the (M+H) protonated molecular ion at m/z286. The spectrum produced does not contain enough ions to perform a multi point linear calibration as described in the Calibration section of this manual. To perform a calibration the CI gas must be partially or totally removed so that the classical EI spectrum of the reference compound is produced. Calibration can then be performed using the standard EI calibration procedure.

Tune the system in CI mode with CI gas in.

Reduce the pressure of CI gas either by turning the CI flow valve clockwise or by deselecting **CI Gas** on the Inlet page.

Monitor the whole spectrum from m/z10 - 290 either in tune mode or in acquisition.

The fragment peak at m/z69 and other fragments characteristic of tris (trifluoromethyl) Triazine in EI+ mode should appear. It may be necessary to introduce more reference material at this point to produce a strong enough signal for calibration.

Follow the calibration procedure as described in the chapter *Calibration and Exact Mass* and use the Metri reference file. Note the C13 isotope of the M+ ion at m/z286 will be interfered with by the residual (M+H)+ ion at m/z286. This should be removed from the calibration output before accepting the calibration.

Once a calibration has been accepted reintroduce CI gas and adjust the level of the reference.

The (M+H)+ ion at m/z286.0027 may be used as the internal reference for CI+ accurate mass experiments.

See the section Lock Mass Correction in the chapter entitled Calibration and Exact Mass later in this manual.

Calibration in CI - Ion Mode of Operation

A multi point calibration can be performed using the Heptaneg calibration file with Heptacosa introduced via the septum inlet.

Follow the procedure outlined for EI operation in the section dealing with calibration. Use the value of resolution and Np determined in EI operation for dead time correction.

Heptacosa is not a suitable material to use for as an internal reference for lock mass correction as it contains too many ions, which could interfere with sample peaks causing mass measurement errors.

To perform a lock mass correction:

Pump away the Heptacosa and introduce 0.2µl of chloropentafluorobenzene C_6F_5Cl. This compound yields an intense -ve ion at m/z 201.9609 which can be used for lock mass correction.

See the section *Additional hints for performing exact Mass Measurement* in the chapter entitled *Calibration and Exact Mass* for setting the level of the lock mass compound during acquisition.

DXC Temperature Compensation

In order to compensate for the change in lab temperature the GCT uses Dynamic External Calibration (DXC) which can be activated from the TDC Parameters page. This shifts the mass by a specified amount in ppm/degree. Typical values for the ppm/degree drift compensation constant is 40-80 ppm/degree, and will have been factory set for your specific instrument. This value should be retained.

In order to derive the value for yourself, the following procedure can be followed.

- Acquire data on a reference compound such as heptacosa without using DXC, lock mass or a calibration file.

- Display a spectrum of the summed data. On the header display 'Temp correction' from the 'Spectrum QTOF' group. This will show the temperature of the instrument.
- Repeat the same acquisition later in the day under the same conditions when the lab temperature has changed by up to 3°C.
- Display both spectra. From the difference in mass and the difference in temperature the drift constant may be calculated in ppm/°C.

The temperature compensation is only applied when the acquisition is performed using a calibration file. The software records the temperature at which it was calibrated, then applies the ppm/degree value according to the measured temperature on each spectrum.

For instruments with Serial Number <CA072 DXC (if fitted) is connected externally. For these instruments, on the TDC Settings page, the box 'Power Port 2' must be checked to activate Event 2 to power the device.

Using DXC mass accuracy of <10ppm RMS may be generally achieved for < 3°C temperature drift.

If more reliable mass accuracy is required or the instrument is subject to a temperature variation of >3°C, then a lock mass can be used.

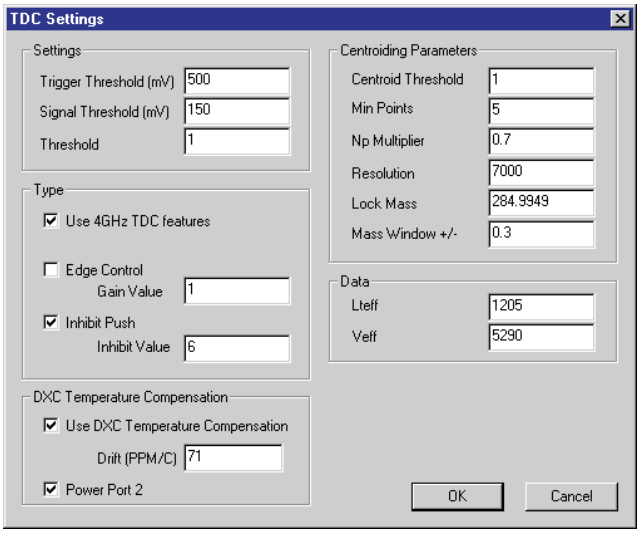
Lock Mass Correction

To produce accurately mass measured data to within 5ppm, compensations must be made for instrument drift resulting from internal factors other than ambient temperature changes.

This is achieved using a single lock mass peak from an internal reference compound introduced via the septum. This single peak is used on a spectrum to spectrum basis to adjust the calibration applied to individual spectra. The result is a complete accurately mass measured data set.

In EI + operation $^{m}/_{z}$ 284.9949 from Tris (trifluoromethyl)triazine is used as an internal lock mass.

The lock mass is specified from the **OPTIONS / TDC SETTINGS** menu in the GCT tune page.



A **Mass Window** of 0.3mDa is usually sufficient for the system to locate the lock mass peak desired prior to adjusting the mass measurement.

Additional Hints for Performing Exact Mass Measurements

As all measurements are made with respect to the internal lock mass peak it is important to maintain the best possible statistics for the measurement of this peak. For this to be achieved, the intensity of the lock mass peak should be adjusted to be just below the point where the peak saturation flag is present. At 1sec acquisition at a pusher interval of 40µs the centroided lock mass peak should contain between 3000 and 8000 ions in total.

Always be aware of possible chemical interference problems - either on the sample or the lock mass peak.

If the limits of the deadtime correction algorithm are exceeded (indicated by the presence of the saturation flag on the peak top in a single spectrum) it may be possible to use the C13 or other relevant isotopes.

The standard deviation in the determination of the mass centroid of a triangular-shaped peak (sppm) due to ion statistics alone is given by equation 1 below:

$$\text{sppm} = \frac{\text{DM} \times 10^6}{\text{M} \times \sqrt{24 \times \text{N}}}$$

where DM is the width ($^{m}/_{z}$) of the a triangular peak across the base.

M is the $^{m}/_{z}$ value of the peak.

N is the total number of ions in the peak.

The final standard deviation of the mass centroid in an exact mass experiment using the GCT is governed by the statistics of the analyte peak measured and the internal lock mass peak used for drift correction.

The final standard deviation SD is given by equation 2:

$$SD = \sqrt{(\text{sppm Analyte})^2 + (\text{sppm Lock})^2}$$

where SD	=	final standard deviation of the measurement
SppmAnalyte	=	standard deviation of the centroid measurement for the analyte.
SppmLock	=	standard deviation of the centroid measurement for the internal lock mass.

From these two equations the necessity for a good statistical measurement of the lock mass centroid is evident to produce a good measurement for the centroid of the analyte.

For example; for 95% (2 standard deviations) of the measurements for an analyte peak at m/z 500, resolution 5000 FWHM (DM = 0.2), to be within 5ppm, the final standard deviation, SD needs to be 2.5ppm.

Assuming that the lock mass peak at m/z 285 contains 5000 ions and has a resolution of 5000 FWHM (DM = 0.114 da), the standard deviation for the lock mass peak alone is

$$\text{sppmLock} = \frac{0.114 \times 10^6}{285 \times \sqrt{24 \times N}}$$

$$= 1.15 \text{ ppm.}$$

From equation 2,

$$2.5 = \sqrt{\left(\frac{0.2 \times 10^6}{500 \times \sqrt{24 \times N}}\right)^2 + (1.15)^2}$$

Rearranging this equation to determine N for the analyte peak,

$$N = \left(\frac{0.2 \times 10^6}{500 \times \sqrt{24}}\right)^2 \times \frac{1}{(2.5^2 - 1.15^2)}$$

N = 1353 ions per peak.

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.
- ✗ Do not use rubber gloves.
- ✗ If nylon or cotton gloves are used take care not to leave fibres in sensitive areas.
- ✗ Avoid touching sensitive parts with fingers.

Before reassembling and replacing dismantled components, inspect O rings and other vacuum seals for damage. If in doubt replace with new parts. 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 take the necessary precautions.

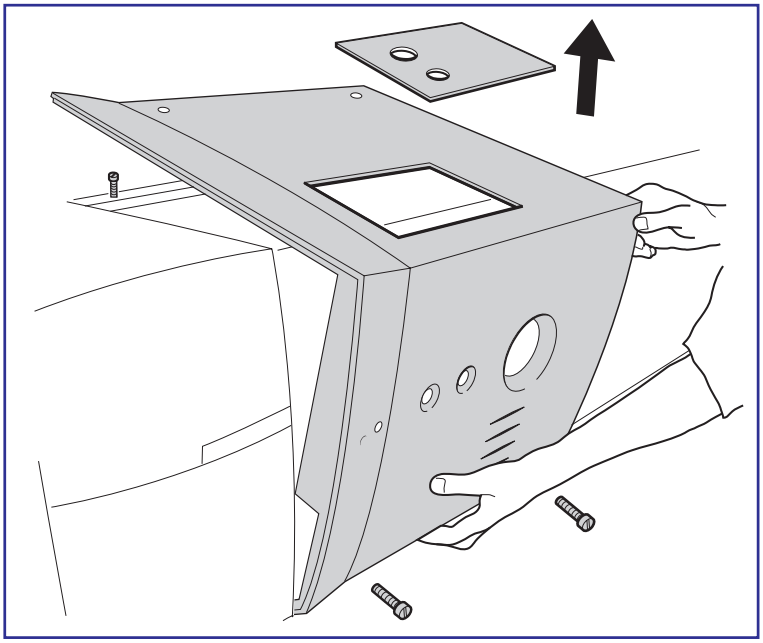
Removal and Replacement of Panels and Cover

Right Hand Side Panel

Viewing the instrument from the front, remove the septum top cover, which rests on top of the moulded cover.

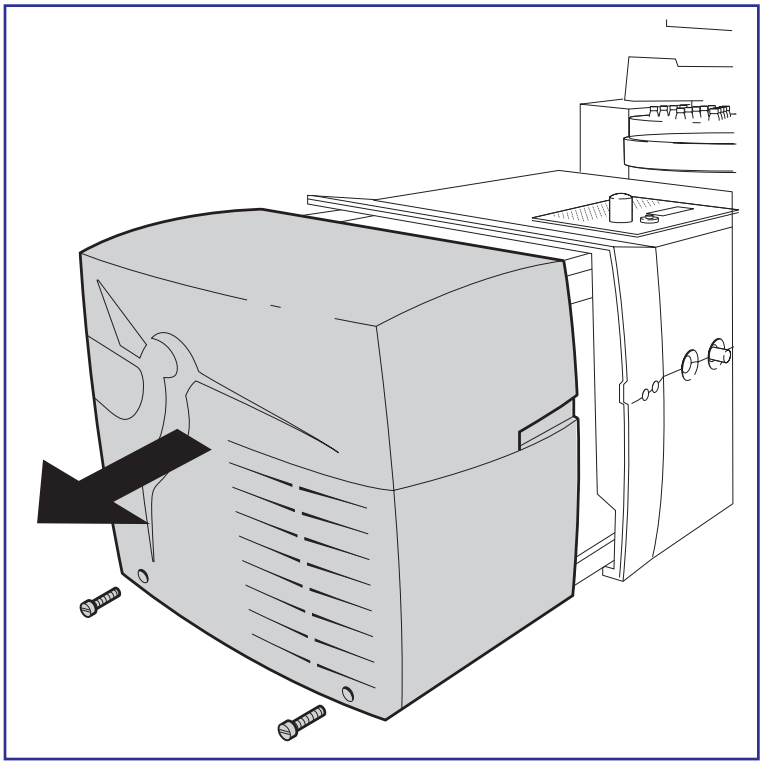
Remove the two retaining screws from the bottom of the right hand side panel. Loosen the two captive retaining screws at the back of the panel.

Pull the panel forward and tilt as shown below, so that the cut-out in the top of the panel clears the septum pump-out knob.



Left Hand Side Panel

Viewing the instrument from the left-hand side, remove the two retaining screws from the bottom of the left hand side panel. Pull the panel forward to remove it.



Cooling Fans and Air Filters

Always ensure that none of the cooling fans are 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 source or analyser region.

An excessive source pressure results in shortened filament life time and high low mass background.

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:

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.

The turbomolecular pumps switch off if full speed is not achieved within a set time following start-up. This could be due to a leak or too high an ambient temperature.

The source and analyser backing isolation valves require a compressed air supply of between 80 - 100psi to operate. If this supply fails the backing valves may close. The backing pressure will rise and the turbomolecular pumps may over temperature or slow to below operating speed. This will result in automatic venting of the source and or the analyser housing.

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. 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 normally require routine maintenance.

Active Inverted Magnetron Gauge

For information on cleaning the active inverted magnetron (Penning) gauge, refer to the Edwards literature supplied with the instrument.

Gas Ballasting

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

Caution: Under no circumstances should gas ballasting be performed during instrument operation.

Open the gas ballast knob on top of the rotary pump for 10 - 30 mins once per week. This draws air through the pump oil to help drive condensed solvents out of the oil. This is to ensure that the oil in the rotary pump is kept as clean as possible. The frequency of ballasting may need to be increased if CI has been performed on a regular basis.

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

Oil Mist Filter

The rotary pump is fitted with an Edwards EMF20 oil mist filter which traps oil vapour from the rotary pump exhaust. The oil mist filter contains two elements which require the following maintenance:

Change the odour element quarterly or whenever the pump emits an oily odour.

Change the mist element every time the rotary pump oil is changed.

To change the elements follow the instructions in the Edwards manual supplied in the auxiliary equipment documentation folder.

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 the chapter entitled *Routine Procedures* earlier in this manual.

It is 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 Maintenance

The head of the rotary pump is fitted with a foreline trap which prevents oil vapour from entering the backing lines and contaminating the instrument vacuum system.

The activated alumina absorbent in the fore line trap should be replaced every three to six months. This should be done at the same time as changing the rotary pump oil, in accordance with the manufacturer's literature supplied.

Septum Interface Maintenance

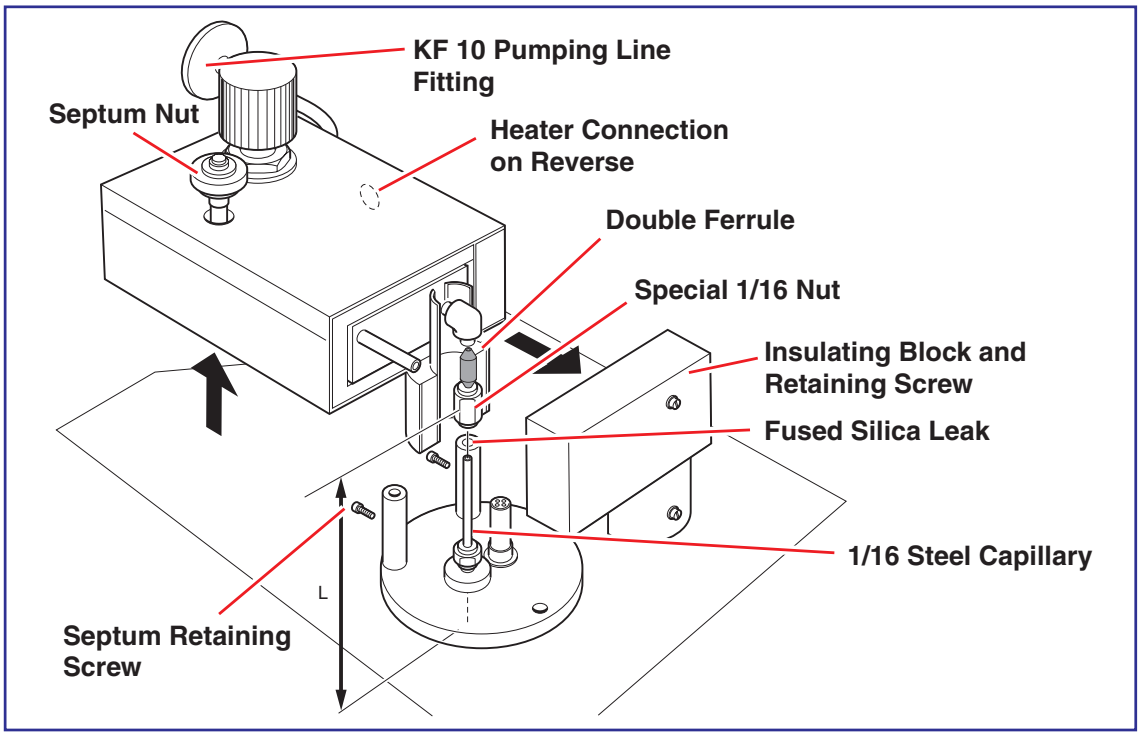
Replacing the Fused Silica Leak in the Septum Interface

Caution: Risk of burns. The interface may be hot.

Although predominantly volatile compounds are used in conjunction with the septum interface, it is possible for the fused silica leak within the interface to become partially or fully blocked after extended periods of use.

Partial blockage of the interface usually results in unstable delivery of the reference material. This is exhibited by erratic behaviour of the TIC when monitoring the reference material. It may be possible to clear a blockage by introduction of pure solvents into the septum interface.

With the septum pump valve closed introduce 10µl of a suitable solvent. Acetone, Dichloromethane etc. Wait for 30 seconds then open the septum pump valve to remove the solvent. If this fails then the fused silica within the septum transfer line should be replaced by following the procedure below.



The diagram above shows the septum interface partially removed to access the fused silica leak.

Isolate the source housing. Vent to atmosphere.

If fitted with an Autosampler tray, move the GC away from the system on its rolling plinth. The GC column need not be disconnected if there is enough column length within the GC to allow the GC to be withdrawn.

Remove the right hand side moulded cover.

Disconnect the septum pump line by removing the KF 10 clamp and O ring.

Disconnect the reservoir heater lead at the rear of the reservoir.

Remove the septum transfer line insulation retaining screw and remove the insulation block to expose the reservoir to re-entrant coupling.

Unscrew the special 1/16" nut and push it to the bottom of the steel capillary line to expose the double ferrule.

Loosen the retaining screws on the front and rear septum mounting pillars.

Lift the whole interface clear of the retaining pillars.

The top 10 - 15 mm of the fused silica will be exposed above the double ferrule.

Remove the double ferrule and withdraw the fused silica from the steel capillary.

It may be possible to reuse the double ferrule if the fused silica can be easily withdrawn. It is recommended that the ferrule be replaced at this point.

Cut a length of new fused silica approximately 20cm long. This fused silica is 320µm OD x 75µm ID. Feed the fused silica into the steel capillary until it meets resistance. The capillary will not easily pass within the steel capillary at the point where the steel capillary is bent inside the source housing.

Thread the new double ferrule over the protruding fused silica and make sure that the 1/16" opening end passes over the end of the steel capillary as far as it can.

Cut the protruding fused silica so that 10 - 15mm of fused silica remains above the double ferrule. This will ensure that the fused silica can be withdrawn at a later date.

Replace the septum ensuring that the double ferrule lines up with the fitting on the reservoir and that the protruding fused silica passes inside this fitting.

Tighten the special nut whilst applying slight downward pressure to the interface. This ensures that the double ferrule will not be lifted off the steel capillary as the double ferrule is compressed. DO NOT OVER TIGHTEN.

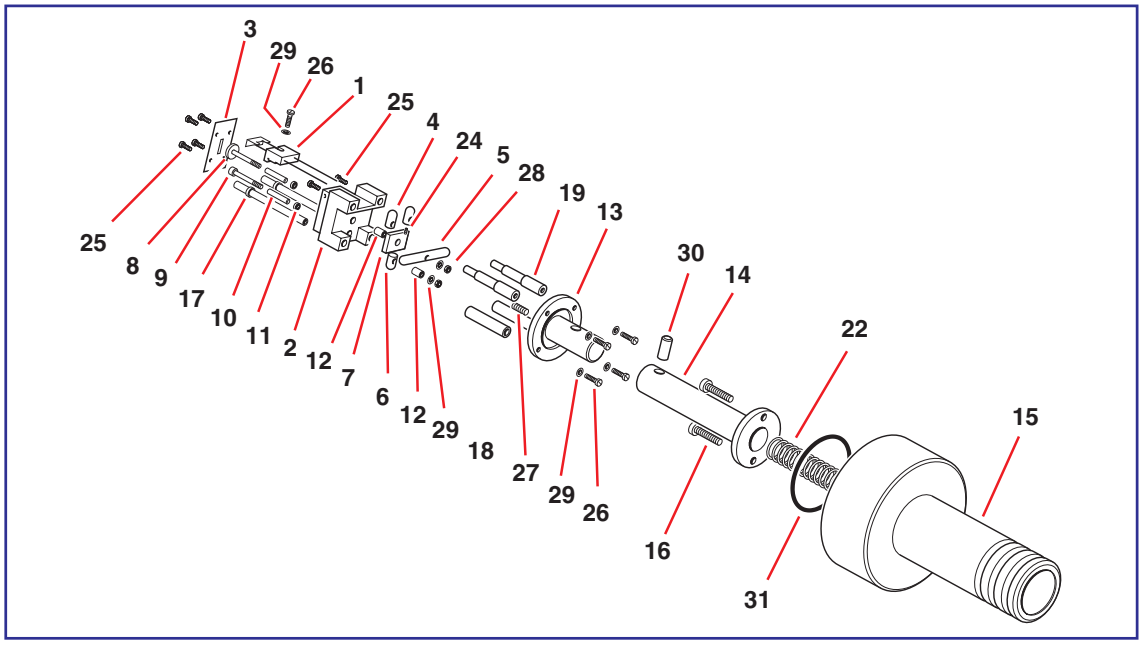
Tighten the two retaining screws on the septum support pillars to hold the interface steady.

Replace heater cables, pumping connections and insulation block.

At this stage it is recommended that the septum is changed. Remove the septum nut and replace it.

The system is now ready to be pumped.

EI/CI Inner Source Maintenance



The EI and CI inner source blocks are designed to be easily removable for maintenance. See the section *Installation and Removal of Inner Source* in the chapter entitled *Routine Procedures.*

Excessive contamination of the inner source can lead to a reduction in sensitivity, resolution and mass accuracy.

Item	Description	Ref. No.
1	Filament assembly	S100683BC0
2	Ion chamber assembly (EI)	M960030AC1
3	Ion exit plate (EI)	M960387AD1
4	Filament contact	M702732BD3
5	Repeller contact	M702732BD1
6	Trap contact	M702732BD2
7	Insulator	M702717AD1
8	Repeller	M960185BD1
9	Trap	M702810AD1
10	Spacer ceramic (12mm)	S100343AD6
11	Spacer ceramic (2mm)	7020302
12	Spacer ceramic (6mm)	S100009AD9
13	Support	M960186BD1
14	Guide shaft	M960187BD1
15	Handle	M960188BD1
16	Modified screw	M960189AD1
17	Locating dowel	M960190AD1
18	Spacing tube	M960191AD1
19	Spacing pillar	M960192AD1
22	Spring	6365064
24	Grub screw M2 x 2 st. stl.	5316014
25	Screw ch. hd. M1.6 x 3 st. stl.	5314041
26	Screw ch. hd. M1.6 x 6 st. stl.	5314043
27	Grub screw M3 x 6 st. stl.	5316003
28	Nut M1.6 st. stl.	5321018
29	Plain washer M1.6 stl.	5331013
30	Dowel dia. 4 x 12lg st. stl.	5371054
31	O ring 128 Viton	5711272

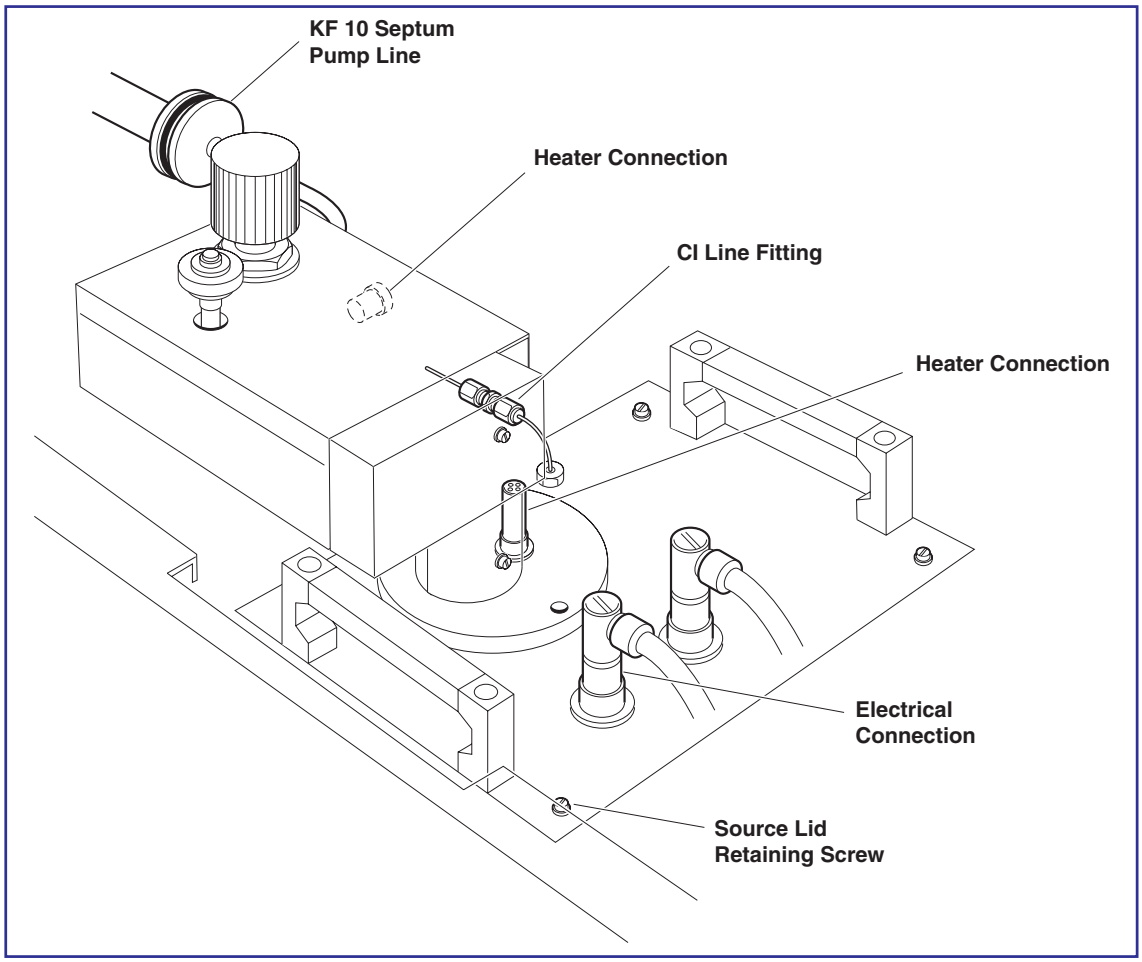
EI/CI Outer Source Maintenance

Contamination of the outer source results in charging of surfaces close to the ion beam. This can cause defocusing of the beam, degrading resolution and leading to poor calibration and mass accuracy.

Outer source contamination is predominantly seen on the first of the two removable collimating slits. Ion burn on this slit is as a result of large, constant ion current from helium, nitrogen, or CI reagent gas ions. In addition, after prolonged use the focus 1 plates can become contaminated due to ion burn from ions leaving the filament.

The removable slits should be removed and cleaned after approximately 2 - 3 months of operation. Focus 1 plates should be checked for ion burn and cleaned if required.

Removal of the Outer EI / CI Source



To remove the outer ion source and source lid follow the procedure detailed below.

Isolate the source housing. Vent to atmosphere.

Withdraw the GC column.

Loosen the 1/4" nut on the GC re-entrant and withdraw the inner GC re-entrant transfer line by 50mm to ensure that it is clear of the outer source.

Remove the inner source volume.

If fitted with an autosampler tray, move the GC away from the system on its rolling plinth.

Remove the right hand side moulded cover from the instrument.

Remove the two electrical connections to the source lid feedthroughs.

Remove the heater connection to the septum reservoir and septum re-entrant.

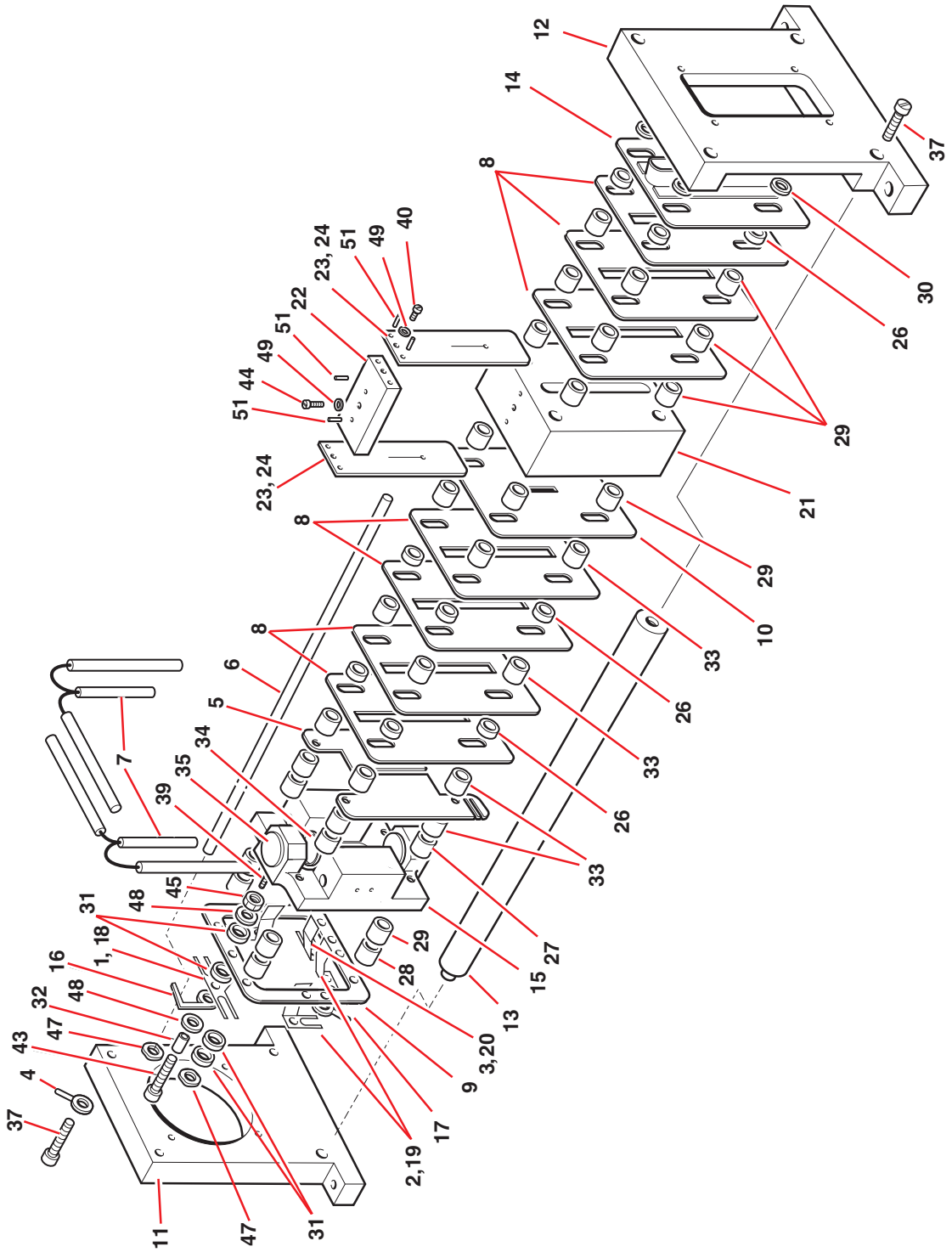
Disconnect the CI reagent gas peek tubing at the 1/16" fitting on the source lid.

Disconnect the septum pump line by removing the KF 10 clamp and O ring.

Remove the four, source lid retaining screws.

Lift off the source lid and outer source.

EI / CI Outer Source Diagram



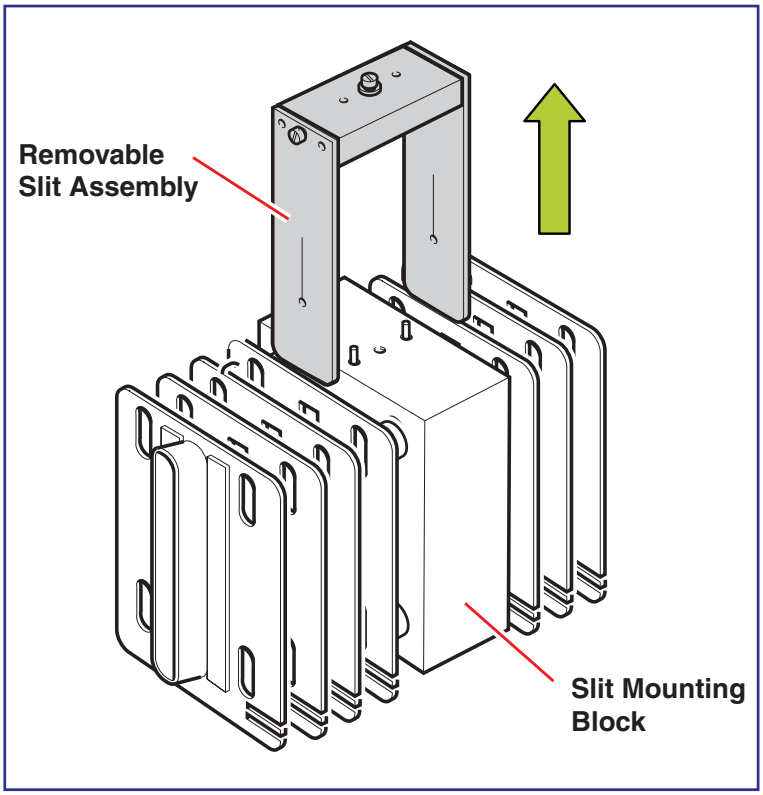
EI / CI Outer Source Parts List

Item	Description	Ref. No.
1	Contact	M702972AD1
2	Contact(3 off)	M702739AD2
3	Contact	M702739AD1
4	Terminal Washer	M702807AD1
5	Focus. 1 Half Plate (3 gap)	M702808BD1
6	Ceramic Rod (102)	M960336AD1
7	Heater Assembly	M960023AC1
8	Focus No.2-3 Plate	M960252BD1
9	Contact Support Plate	M702716BD1
10	Aperture Plate	M960155BD1
11	Support Plate	M960159CD1
12	Support Plate	M960161CD1
13	Pillar	M960162AD1
14	Exit Plate	M960165AC1
15	Ion Chamber (Outer)	M960167CD1
16	Contact Washer	M960168BD1
17	Terminal Washer	M960169AD1
18	Spring Contact	M960170BD1
19	Spring Contact	M960171BD1
20	Spring Contact	M960172BD1
21	Lens Block	M960286CD1
22	Slit Plate Support	M960287AD1
23	Slit Plate (0.25)	M960288AD2
24	Slit Plate (0.5)	M960288AD3
26	Metal Spacer (2)	7020104
27	Metal Spacer (4.7)	7020170
28	Metal Spacer (7)	7020103
29	Metal Spacer (5)	7020135
30	Insulating Spacer (1)	7020301
31	Insulating Spacer (2)	7020302
32	Insulating Spacer (3.5)	7024364
33	Insulating Spacer (5)	S100009AD5
34	Magnet Cap	7024912
35	Source Magnet	7028106
37	Screw Ch. Hd. M3 x 12 st. stl.	5314069
39	Grub Screw M3 x 4 st. stl.	5316005
40	Screw Ch. Hd. M2 x 3 st. stl.	5314048
41	Screw Ch. Hd. M1.6 x 3 st. stl.	5314041
42	Screw Ch. Hd. M1.6 x 5 st. stl.	5314042
43	Screw Csk. Hd. M1.6 x 8 st. stl.	5311040
44	Screw Ch. Hd. M2 x 8 st. stl.	5314048
45	Nut M1.6 st. stl.	5314065
47	Washer M3 st. stl. Wavy	5335005
48	Washer M1.6 st. stl. Plain	5331013
49	Washer M2 st. stl. Plain	5331014
51	Dowel dia.2 x 6 st. stl.	5371002

Removal of the Collimating Slits on the Outer Source

Two 0.25mm width slits are fitted to the outer source. These may easily be removed for maintenance.

Remove the retaining screw in the slit mounting bracket. The slit assembly containing both slits can then be lifted from the outer source for cleaning.



Cleaning

Warning: Cleaning the various parts of the source requires the use of solvents and chemicals which may be flammable and hazardous to health. Personnel performing these operations should refer to the manufacturers' data, be aware of the inherent risks, and take the necessary precautions.

"Quick Clean" Procedure

In many cases it is sufficient to clean only the top surface of the repeller and the ion exit plate together with the trap, the removable slits and the exposed surfaces of the Focus 1 plate. When the source is cool:

Clean each of these items with micromesh or a fibreglass pencil.

Degrease with solvent

Blow out the source with a stream of dry nitrogen.

Full Clean Procedure

Before proceeding to clean the source components refer to the general guidelines in the section Cleaning Materials below.

Identify the following components for cleaning:

Outer Source

- Focus 1 plates
- Collimating slits

Inner Source

- Repeller
- Trap
- Ion exit plate
- Ion volume

Clean each item with a fine abrasive as detailed below.

Degrease by washing with a suitable solvent.

Dry in an oven.

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 wet/dry 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.

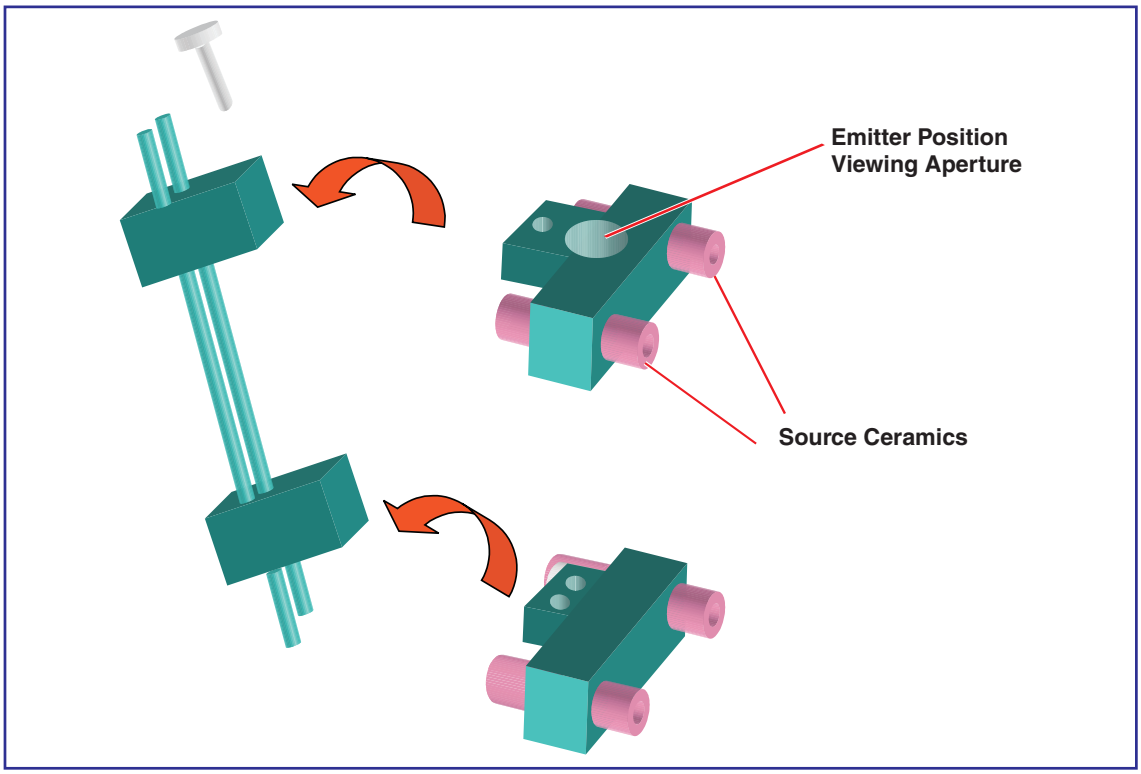
Warning: Clean panels with a damp cloth. Use of excessive amounts of water or solvents near to the electronics units can present a safety hazard.

Furthermore, 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.

Cleaning the FI Source

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.

'Quick Clean' Procedure



In many cases it is sufficient to clean only the extraction rod area of the ion source. The rod assembly has been designed to be easily removable without the need to strip the whole ion source.

To remove the extraction rod assembly,

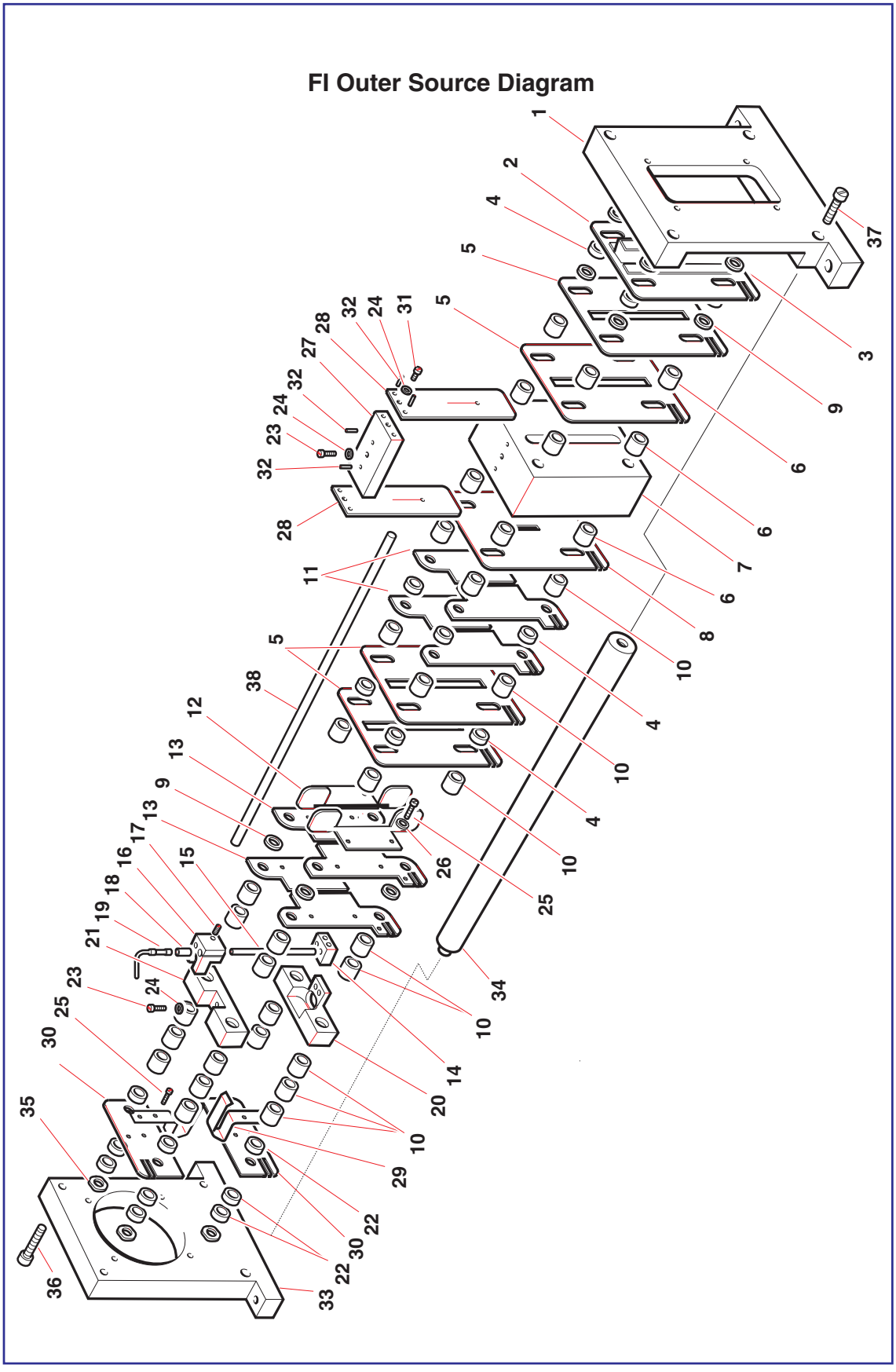
Isolate the source housing and remove the outer source.

Disconnect the two extraction rod push-on contacts. Loosen the retaining screw on the extraction rod assembly. Tilt the rod assembly forward and withdraw.

FI Outer Source Parts List

Item	Description	Code	No. Off
1	Support plate	S100683BC0	1
2	Exit plate	M960030AC1	1
3	Insulating spacer (1mm)	M960387AD1	4
4	Metal spacer (2mm)	M702732BD3	12
5	Focus 2 plate	M702732BD1	4
6	Metal spacer (5mm)	M702732BD2	12
7	Lens block	M702717AD1	1
8	Aperture plate	M960185BD1	1
9	Metal spacer (1mm)	M702810AD1	8
10	Insulating spacer (5mm)	S100343AD6	32
11	Focus 3 plate	7020302	4
12	Insulator shield	S100009AD9	2
13	Half plate	m960186bd1	4
14	Lower saddle	M960187BD1	1
15	Extraction rod	M960188BD1	2
16	Upper saddle	M960189AD1	1
17	Grub screw M2 x 2 st. st.	5316014	1
18	Insulator	M960414AD1	2
19	Contact pin	M960412AD1	1
20	Lower support plate	M960408AD1	1
21	Upper support plate	M960409AD1	12
22	Insulating spacer (2mm)	7020302	1
23	Ch Hd screw M2 x 8 st. st.	5314065	3
24	M2 washer st. st.	5314040	4
25	Ch Hd screw M1 .6 x 2 st. st.	5331013	1
26	M1.6 st. st. washer	M960288AD3	1
27	Slit Plate support	M960287AD1	1
28	Slit Plate (0.5)	M960288AD3	1
29	Spring contact	M960259BD1	2
30	Contact plate	M960258AD1	2
31	Ch Hd M2 x 3 screw st. st.	5314048	2
32	Dowel dia.2 x 6 st. st.	5371002	6
33	Support plate	M960159CD1	1
34	Pillar	M960162AD1	4
35	Wavy washer M3 st. st.	5335005	8
36	Screw ch hd M3 x 12 st. st.	5314069	4
37	Screw ch hd M3 x 10 st. st.	5314017	4
38	Insulating rod	M960336AD1	4

FI Outer Source Diagram



The extraction rods can be cleaned without disassembling. The rods should be cleaned with fine grade emery paper or fine stainless steel wool. The surface should have a highly polished appearance with no scratches or sharp points.

Immerse the whole assembly in a suitable solvent and sonicate until all particles are removed and all condensed sample has been removed.

Full Clean Procedure

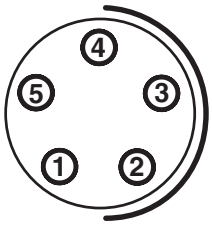
After a period of time there may be sample build-up on the source ceramics and the focus plates. This may cause extraction voltage leakage and /or calibration mass accuracy problems. In this case the source must be removed from the source lid assembly disassembled and the contaminated components individually cleaned.

In the event that an emitter fails due to an electrical discharge in the ion source the emitter wire can experience a very high short-lived current which is enough to effectively vaporize the wire. The carbon dendrites are then attracted to the emitter rods and to the ceramics of the outer source.

The result is usually a high leakage current when a new emitter is introduced. The extraction rods and lens elements including ceramics up to the first focusing element after the extraction rods must be cleaned. Lens plates need to be cleaned in an ultrasonic bath using a suitable solvent to ensure all emitter fragments are removed before reassembly.

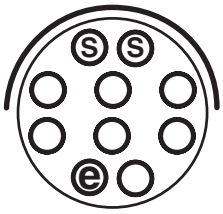
Caution: To aid reassembly, the diagram below illustrates the feedthroughs as viewed from the outside of the housing. This is a very important consideration, because from inside the housing the layout is a mirror image, and if the wires are connected incorrectly this will damage the instrument.

FRONT
5 PIN
FEEDTHROUGH



PINOUT VIEW FROM OUTSIDE HOUSING

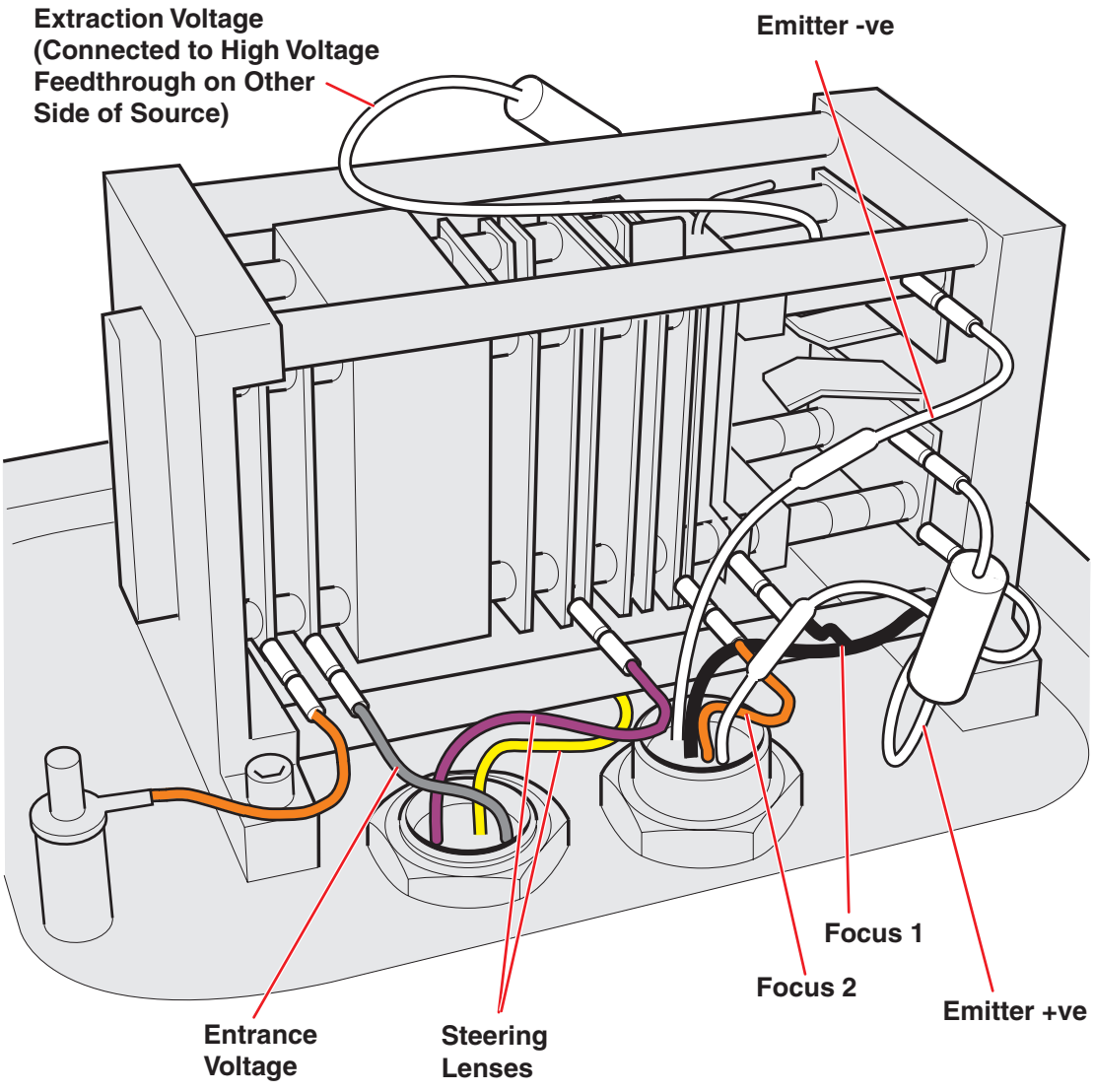
REAR
10 PIN
FEEDTHROUGH



1 Focus 2 (Orange)
2 Focus 1 (Two Black Wires)
3 Emitter +ve (Capacitor to Ground)
4 Emitter -ve (Resistor to Ground)

S Steering Lenses (Purple and Yellow)

e Entrance Voltage (Grey)



Fault finding

No Beam

General Checks

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.

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:

Set the real time tune display to show the mass range down to m/z0.

Check that there is an interference ‘peak’ at approximately m/z11 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.

The attenuator must only be replaced by trained maintenance personnel. If the pusher interference peak is not present no data will be acquired.

Low Compressed Air Supply

In the extreme case of total loss of the compressed air supply to the system the system may vent. This is a result of the backing line isolation valves closing.

However, if the supply is low the backing line isolation valves will remain open but there may be insufficient pressure to allow the isolation valve to open. Isolation valve operation is accompanied by a characteristic audible hiss, and in the absence of this the air supply should be investigated.

Action

Check pressure of compressed air supply.

No Trap, Emission or Filament Current Readback

This can be due either to a damaged filament or to poor contact to the filament from the spring contacts on the outer source.

Action

Remove inner source.

Check continuity of the filament with a digital voltmeter ca: 0.2ohm. Replace filament if damaged.

If the filament is intact adjust the spring contacts on the outer source slightly to improve contact.

No Filament Current, Trap Current Maximum, Emission Current Zero

This is usually due to a particle or general contamination in the region of the trap causing leakage current between the block and the trap.

Action

Remove inner source and exit plate.

Check cleanliness of trap and trap ceramic. Blow out any particles using dry nitrogen.

High Filament Current, High Emission, Low Trap Current

Electron entrance aperture blocked or filament in incorrect position. Trap spring contact not connected.

Action

Clean inner source.

Check trap spring contact on outer source.

Ion Repeller Inactive in EI Mode

Action

Check ion repeller spring contact on outer source.

Poor Sensitivity

Poor emission to trap ratio and poor sensitivity in EI mode.

The EI source filament current is regulated by monitoring the current produced by electrons accelerated from the surface of the filament on a trap inside the source volume. After an extended period of use the filament current required to produce the requested trap current may increase, as the filament becomes less efficient. The filament may ‘sag’ so that it does not align correctly with the electron entrance hole in the block. The electron entrance hole may become partially blocked with contaminant. The trap may become contaminated so that an incorrect reading of trap current is registered. These effects will result in the emission current (amount of electrons produced by the filament) to trap current (amount of electrons reaching the trap) ratio changing. With the EI only source expect an emission to trap ratio (at 70eV) of about 3:1 and certainly less than 7:1. Poor emission to trap ratio will result in poor sensitivity.

Action

Clean the inner source.

High Positive Value of Ion Repeller EI Source

If the ion repeller is optimising at more than 7V it is likely that the repeller has become contaminated. This will lead to poor sensitivity. NOTE: if the repeller tunes high immediately after a column has been installed it is likely that the end of the column is too close to or protrudes past the centre line of the source. This will interfere with extraction of ions from the source and lead to poor sensitivity.

Action

Check column position, clean inner source.

Poor Sensitivity in CI Mode

As there is no trap in CI mode the emission to trap ratio cannot be used as an indicator of poor source performance. However contamination of the electron entrance aperture, and sagging of the filament can have an adverse effect on sensitivity in this mode.

Action

Clean the inner source.

Incorrect Position of the GC Column

If the GC column is too far in or not far enough in with respect to the centre line of the source sensitivity may be reduced. Column position in the injector is also critical.

Action

Check column position.

Poor GC Conditions

Contamination of the GC liner, leaks on the GC, damage to column etc can result in poor transmission of analyte to the mass spectrometer. Poor chromatographic separation and tailing of chromatographic peaks can suggest problems in this area. Adsorption of analyte on column or in the injector may lead to non linear response. Incorrect GC settings for column parameters, flow rate, purge time and flow, or split ratio will also affect transfer. Incorrect column cutting or position can also affect performance, Refer to GC manufacturer's information and see section on GC-MS in this manual.

Action

Check sensitivity with HCB. This compound is not normally retained on the column. Check column installation and GC parameters.

Faulty Attenuator

This can result in poor peak shape or loss of beam.

Action

Check continuity of attenuator. With a digital voltmeter set to measure ohms check for a resistance ≈ 52Ω with respect to the case at both ends of the attenuator. Values > 55Ω indicate a faulty attenuator.

Faulty Preamplifier or Preamplifier Supply

No beam.

Action

Check 12V supply to preamplifier. Replace preamplifier if faulty.

Poor Resolution

Gradual Decrease in Resolution and Mass Accuracy

This usually indicates a problem with contamination of the outer source lens elements, however excessive contamination of the inner source can lead to problems. Generally a contaminated inner source leads to a greater energy spread of ions leaving the source. This will add to the initial orthogonal energy of the ion beam degrading resolution. The energy spread of ions produced will depend on the energetics of that particular ion. This can lead to poor calibration and mass accuracy.

In terms of the outer source most contamination will appear on the first of the two removable collimating slits. Ion burn of this slit is due mainly to Helium, Nitrogen, or CI reagent gas signal. In addition after prolonged use the focus 1 plates can become contaminated due to ion burn from ions leaving the filament.

Action

Check for contamination inner and outer source and clean inner source, collimating slits in outer source and focus 1 plates if required.

Incorrect Engineer Tuning Menu Settings

The settings of the analyser voltages in the Engineer Tuning Menu will have been set up during installation. These values should be saved and recorded in case lost.

Action

Check settings against values determined during installation.

Incorrect Isotope Distributions, Difficulty in Setting TDC Dead Time Parameters

If the Gain of the system is low or the TDC signal threshold is set too high, a proportion of single ions will not be counted. Under single ion counting conditions (without TDC saturation) this would have no effect on the spectra other than to reduce sensitivity. However, under multiple ion arrival conditions, where dead time correction is used the population of ions detected can be skewed leading to incorrect isotope ratio measurements and incorrect application of the dead time model. It is therefor important to make sure that at least 85% of all ions are recorded by adjusting the TDC signal threshold or multiplier gain settings. See section on Tuning and user interface.

The effects detailed above can be caused by:

- **The TDC Stop (mV) threshold being set too high.**

  Refer to the tune page settings section on Tuning and user interface 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 problems in ion detection.

  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 - chemical.**

  Chemical noise usually originates from contaminated samples, solvents or GC or CI reagent gases or lines. Chemical noise can be distinguished from electronic noise simply by stopping source ionisation, by turning the trap current to 0 or shutting the isolation valve.

  If no sample or gases are entering the source and all the source voltages are set to zero then the remaining noise will be electronic in nature.

- **High noise - electronic.**

  Electronic noise can be caused by setting the TDC Stop (mV) threshold too low. Refer to the section *Tune Page Settings* in the chapter entitled *Tuning and User Interface* for information regarding the setting of this parameter.

  Warning: 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 micro discharges, 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 considered 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 vacuum.**

  Before suspecting a pump fault or vacuum leak (see *Vacuum System* earlier in this chapter) check 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 in the auxiliary equipment folder with the instrument.

Poor source vacuum after a source pumpdown can be caused by failure to open the septum interface pump valve during pumpdown. Trapped air in the interface will take some time to completely pump away through the fused silica leak.

Fault finding in FI Mode

No Beam

- Check that the position of the emitter is correct using the emitter probe adjuster knob.
- Check that the extraction voltage readback is present and that the leakage current is low.

No Emitter Current

The emitter may be damaged.

Vent the source region, withdraw the emitter and check that there is electrical continuity through the emitter wire. In most cases it is possible to assess the integrity of the emitter wire by visual inspection. Replace emitter if required.

Note: it is possible to see a weak ion signal even when the emitter wire is damaged however no emitter current will be drawn.

Excessive Leakage Current

- Check that the internal source wiring is not touching any grounded elements.
- Withdraw the emitter by 5 -10mm from the extraction rods.

  A continued high leakage current indicates that either the internal wiring is close to a grounded element or that voltage leakage is occurring across the ceramics of the ion source.

  Inspect the source, and strip and clean the source if required.

  If the leakage current decreases to an acceptable level, the voltage leakage is occurring between the extraction rods and the emitter.

  Remove the ion source and clean the extraction rods.

- Check that the shield plates protecting the ceramics on either side of the extraction rods are not touching the extraction rod assembly. Adjust if required.

Poor Sensitivity for the Reference Material

- Check that the emitter is intact.
- Replace emitter and reassess. (There may be marked variation between the performance of individual emitters).

Poor GC Sensitivity

- Check that the source end of column is cleanly cut.
- Check the position of the emitter relative to the end of the column and adjust if required.

Poor Calibration / Accurate Mass

Clean the extraction rods.

Electrical Discharge Resulting in Damaged Emitter

Most emitter failures result from an impact, incorrect handling or stresses within the emitter bead. If an emitter fails in this way, the emitter wire generally remains attached to one or both of the legs of the emitter bead. Replacing the emitter is usually all that is required.

Failure of emitters due to discharge in the ion source is rare and is usually due to excessive source contamination.

In the event that an emitter fails due to an electrical discharge in the ion source the emitter wire can experience a very high short-lived current which is enough to effectively vaporize the wire. The carbon dendrites are then attracted to the emitter rods and to the ceramics of the outer source.

The result is usually a high leakage current when a new emitter is introduced. The extraction rods and lens elements including ceramics up to the first focusing element after the extraction rods must be cleaned. Lens plates need to be cleaned in an ultrasonic bath using a suitable solvent to ensure all emitter fragments are removed before reassembly.

Continuous loss of emitters in this way indicates a high level of contamination and a possible fault in the protection resistors/capacitors across the emitter within the outer source.

DXC Troubleshooting and Hints

Ensure that the temperature compensation cable is fitted correctly, this includes:

- If the cable is the external type plug the 2 pin connector into the EVENT OUT 2 with the switch set to 5V.
- If the cable is the external type plug the 3 pin connector into ANALOG CHANNEL 4
- If the cable is the internal type make sure that it is wired correctly to the block on the interconnection PCB. The wires should be (from left to right) black-SCN, green-0V, blue-temperature signal, red-+5V.
- Ensuring the 'slot' in the flight tube is full of heat sink compound and the thermal sensing transistor is submerged in this heat sink.
- Ensuring the clamp plate is securely fixed and the 2 earth connections are fixed to the housing or block.

- If you still have problems then fill the 'opening' into the slot for the wires with araldite or some similar substance to fix the wires, reduce air flow to the heat sink and stop the heat sink running out.
- Ensure that the correct version of software is installed.
- Ensure that the instrument's internal and external covers are all fitted.
- Ensure that there are no dead-time effects causing problems with mass accuracy.
- Ensure that there is enough signal to give an accurate mass measurement - over 1500 counts should be OK. If not, then sum more scans.
- Ensure that the instrument has been switched on and set to constant operating conditions for at least 2 hours.
- Make sure that you are using the correct calibration file.
- Check that the calibrated temperature in the _header.txt file of the calibrated data corresponds to the temperature that the calibration was taken at.

Check that the instrument 'knows' it is using DXC by displaying it on the header in the spectrum. Select SpectrumQTOF from group and"UseTempCorrection" from element - it should say "1" and "TempCoefficient" should give the ppm/degree value.

Faultfinding and Tips (DCI Probe)

Peak shifting can sometimes be seen if the DCI tip is touching the source inside the instrument due to grounding the current return path. This can be determined by measuring the resistance from the tip to ground when the probe is in position which would change if the probe is withdrawn by five centimetres or so. It can be prevented by carefully bending the tip back to a straight position and making sure that it is about 2.1mm wide.

No DCI current can be caused by a damaged tip. Visually inspect the tip and check the continuity across the tip from the socket on the DCI probe.

Large peak at 447Da is probably SANTOVAC. Clean the probe shaft with methanol and acetone and change the o-rings in the insertion lock. Coat the o-rings and lubricate the probe shaft with molybdenum disulphide instead.

A general hydrocarbon like background hump getting up to 100 counts or so per second from 100 to 400Da is normal.

Saturation of detector (characterised by the lock mass peak dropping down in intensity or peak flags on the spectrum) - use less sample, use a lower emission current (you can drop it down to 10µA and still see a signal) or use a slower ramp.

Vacuum gauges fluctuating when the probe is inserted - this is due to a poor seal on the o-ring in the insertion lock. Tighten up the three screws a little on the brass ring that holds the o-ring in place.

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. Under no circumstances should gas ballasting be performed during instrument operation. For full details of the following procedures, consult the relevant sections of this chapter and/or refer to the manufacturer's literature.

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.

Change the odour element in the oil mist filter.

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

Positive Ion EI and Positive Ion CI

Tris (trifluoromethyl) triazine reference masses.

Mass	Intensity	Empirical formula			
		C12	C13	N	F
49.996800	10	1			1
68.995210	100	1			3
75.998881	25	2		1	2
102.002950	10	3		2	2
121.001358	100	3		2	3
170.998164	5	4		2	6
189.996568	100	4			9
265.996448	100	6		3	8
284.994900	100	6		3	9
285.998400	5	5	1	3	9

Lock mass for CI- Chloropentafluorobenzene m/z201.9609.

Negative ion Methane CI reference masses for Heptacosa, PFTBA

Mass	Intensity	C	N	F
168.9888	0.15	3		7
213.9903	0.40	4	1	8
218.9856	0.24	4		9
225.9903	0.46	4		9
230.9856	0.39	5		9
244.9887	0.83	5	1	9
249.9840	0.18	5		10
263.9871	1.30	5	1	10
268.9824	0.31	5		11
275.9871	0.72	6	1	10
280.9824	0.68	6		11
282.9855	1.83	5	1	11
294.9855	1.66	6	1	11
299.9808	0.54	6		12
301.9839	2.92	5	1	12
311.9808	4.07	7		12
313.9839	0.53	6	1	12
318.9729	0.47	6		13
325.9839	0.67	7	1	12
330.9792	0.61	7		13
332.9823	12.45	6	1	13
337.9839	0.12	8	1	12
344.9823	0.35	7	1	13
349.9776	2.27	7		14
351.9807	1.66	6	1	14
356.9760	0.21	6		15
363.9807	1.08	7	1	14
368.9760	0.40	7		15
375.9807	1.29	8	1	14
382.9791	3.14	7	1	15
394.9766	1.46	8	1	15
401.9775	1.49	7	1	16
413.9775	11.99	8	1	16
425.9775	1.91	9	1	16
432.9759	10.98	8	1	17
444.9759	0.38	9	1	17
451.9743	100.00	8	1	18
463.9743	4.19	9	1	18
475.9743	2.28	10	1	18
482.9727	0.79	9	1	19
494.9727	0.61	10	1	19
513.9711	25.97	10	1	20
525.9711	0.82	11	1	20
532.9695	0.99	10	1	21
537.9711	0.53	12	1	20
544.9695	0.98	11	1	21
556.9695	4.83	12	1	21
563.9679	2.54	11	1	22
575.9679	0.85	12	1	22
582.9663	2.43	11	1	23
594.9663	23.16	12	1	23
632.9632	63.96	12	1	25

Appendix 1
GC-MS

This section deals with correct installation of the GC column and notes on obtaining the best GC MS performance from the GCT.

Review of GC Considerations in MS Detection

Optimisation of chromatographic performance is dependent upon a number of factors, most notably column selection. The characteristics of bonded fused silica capillary columns have been well established, providing a range of stationary phases designed for applications with polar and non-polar analytes.

The following guidelines may be useful in selecting an appropriate stationary phase.

Review literature to ensure the application has not been previously reported. The analysis of polar analytes is usually achieved with polar phases. The selectivity of such phases is related to the polarity of the analytes. Select the least polar phase that will perform the required separation. Non-polar phases generally have a higher maximum operating temperature, lower bleed and longer life than polar phases.

Column Installation and Care

The following section describes guidelines for good practice when installing GC columns and performing GC – MS analysis.

Always ensure that the ends of the columns are cut cleanly and at right angles to the length of the column. A purpose designed column tool should always be used ie: ceramic wafer or quartz cutter.

Minimise the amount of handling with bare hands. This can introduce contamination into the mass spectrometer resulting in a high level of background. Use cotton gloves or a tissue to hold the column whilst installing.

Replace the injection liner and septum in the GC injector at regular intervals. Contamination in these components can lead to poor chromatography – tailing peaks- and loss of sensitivity due to adsorption of analyte in the injector.

After installation of a new column ensure that the correct conditioning procedure has been completed.

Always thoroughly check for leaks after installing a column. A small amount of oxygen entering the GC column can lead to permanent damage to the column. Use Isopropyl alcohol or an electronic leak checker.

Do not use Snoop soap solution as this can cause contamination.

Conditioning the GC Column

After installation of a new GC column and before sample analysis the column should be conditioned at operating temperature.

Install the column into the mass spectrometer and set the desired flow rate.

Start an acquisition to monitor the level of the background entering the source.

Set the GC oven to the maximum operating temperature of the column.

Monitor characteristic column bleed peaks m/z 207, 281.

The intensity of these peaks will rise as the oven temperature increases. The signal should reach a maximum and remain constant when the GC oven reaches the maximum operating temperature. If the level of column bleed continues to rise, the column may be damaged or there may be an air leak on the GC side. Cool the oven and isolate the problem.

Appendix 2
Ion Counting on TOF Instruments Using a TDC

The TDC (Time to Digital Converter) is the acquisition device used to record the arrival times of ions on LCT, GCT and Q-Tof mass spectrometers. The TDC is essentially an ion-counting device which will record an ion arrival with a single ‘count’ and associate that arrival with a time relative to a start time. The internal clock of the TDC is reset and started by an external Trigger signal. This clock will only start when this Trigger signal exceeds a user defined threshold. This is called the Trigger Threshold.

A single ion arrival will only result in an event being recorded if the signal generated by this arrival passes through a user-defined voltage threshold. This is called the Signal Threshold. When this occurs the TDC records the event and associates it with a time stamp. The TDC will continue to associate times with ion arrival events until another trigger signal is detected. At this point the internal clock within the TDC resets to zero and subsequent ion arrivals are associated with a time relative to this new start time.

The time between trigger signals corresponds to a single time-of-flight spectrum. This time may be as small as 30 micro seconds. Each individual time of flight spectra is added to the last to produce a total histogrammed mass spectrum.

Within the architecture of the TDC each event recorded is not only associated with a time stamp but is also ‘flagged’ as resulting from the detection of a trigger signal or as resulting from the detection of a single ion pulse. The former is referred to as a start event the latter a stop event. Because of this terminology the signal threshold can also be referred to as the stop threshold and the trigger threshold referred to as the start threshold.

Trigger Threshold

The electronic pulse which triggers the TDC clock to start counting (Trigger Signal) is derived directly from the orthogonal acceleration (pushout) pulse. The general form of the signal is shown in Fig 1

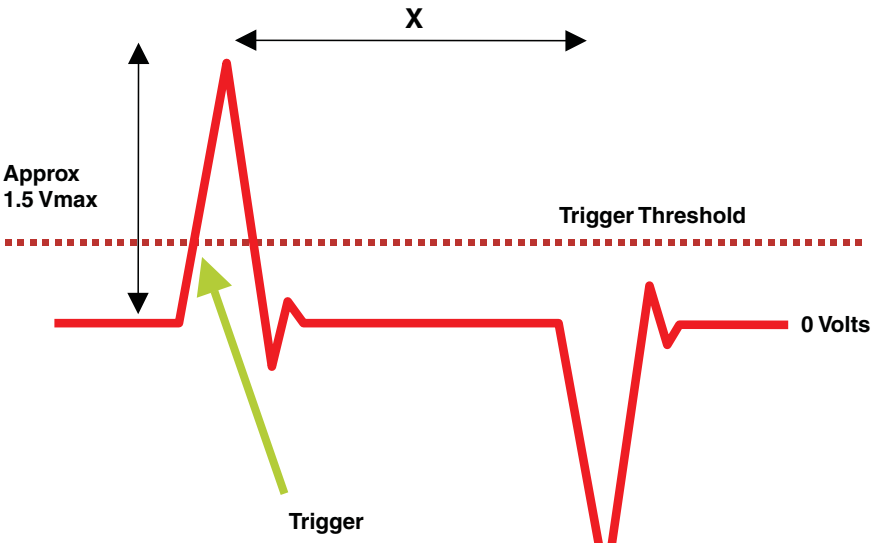


FIG 1 TDC TRIGGER SIGNAL POSITIVE ION MODE

X for GCT = 2.8µs
X for LCT = 9.0µs
X for Qtof = 8.0µs

Referring to Fig 1 the initial positive going ion spike corresponds to the leading edge of the pushout pulse, the negative going spike corresponds to the falling edge of the pushout pulse. In positive ion the Trigger Threshold, in the instrument control software, is set to detect a positive going slope and a positive voltage.

In negative ion mode the pushout pulse is inverted, therefore the trigger signal is also inverted.

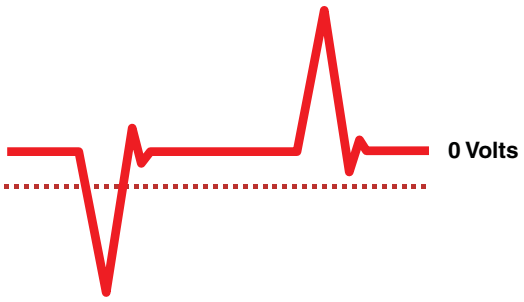


Fig2 TDC trigger signal negative ion mode

The software automatically changes the characteristic of Trigger Threshold when the polarity of the system is changed, so that the clock will start when a negative signal with a negative going slope is detected.

The Trigger Threshold should be adjusted so that the best resolution and peak shape is observed. If the threshold is too high no trigger signal will be detected and the internal TDC clock will not start. No data will be produced. If the threshold is too low. The TDC may detect the small electronic ringing close to the zero volts level. This can cause multiple triggering resulting in split peaks or even two discrete spectra separated by the fixed time interval X in Fig 1.

Generally the best position for the threshold will be directly between these two extremes. However, the rising edge of this pulse may have areas of instability, which can cause split peaks or peak broadening. Several different values of Trigger Threshold can be examined to find the value resulting in the best resolution and peak shape.

Once set this should not need to be changed. It is a system parameter unique to the electronics characteristic of a particular instrument.

Signal Threshold

The current produced from an individual ion arrival needs to be greatly amplified before it can be detected by the electronics of the TDC. This is achieved using a Microchannel Plate Detector (MCP). The MCP is essentially a fast electron multiplier. In normal operation, for a single ion arrival at the front of the MCP assembly > 10 exp 7 electrons are produced at the output of the system. This is the MCP's gain. The electron current exiting the rear of the MCP is converted into a voltage pulse at a collector plate. This negative pulse is then delivered to the TDC input. When the negative going edge of this negative pulse passes through a user-settable threshold Signal Threshold an ion event is registered and saved with a time stamp. Fig 3

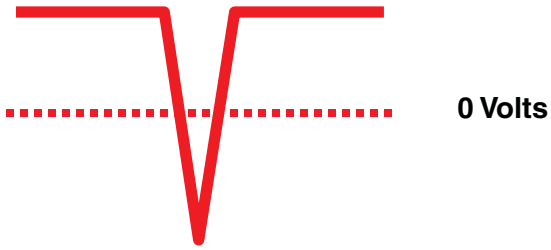


Fig 3 Single ion pulse

However, not all ion events, even from the same m/z value ion, result in the same detector output and there is a significant distribution of output pulse intensity. The gain of the MCP is therefore only an average of the total distribution of output intensities. This variation is known as ion height distribution of the MCP.

Fig 4 shows the nature of this distribution.

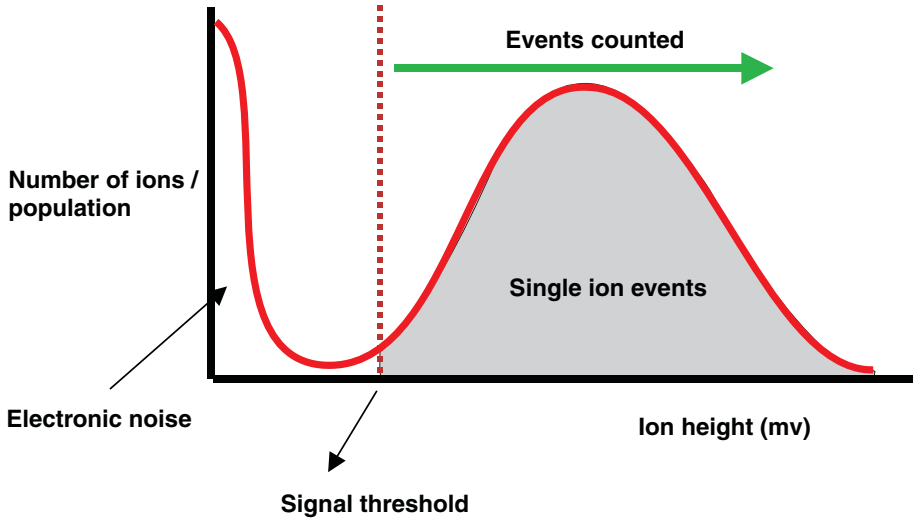


Fig 4. Ion height distribution of dual chevron MCP

Referring to Fig 4 the X-axis represents the intensity of the pulse presented to the TDC input for a single ion event. The Y-axis represents the proportion of events with a particular intensity. It can be seen that the distribution of ion heights is roughly Gaussian. The large population of events at low intensity is due to electronic noise, mostly power supply ripple. The red dotted line indicates the optimum position of the Signal Threshold. All events to the right of the red dotted line will appear as counts in the final spectrum. This threshold should be set to count 85 - 95 percent of all the single ions events.

If the Signal Threshold is set too low there will be a large amount of noise detected. If it is set too high counts may be missed.

Setting the Signal Threshold

The Signal Threshold should be set up to be just above the level of the system electronic noise. This is as low a value as possible. The MCP voltage should then be adjusted so that the gain of the system is enough to place at least 85 percent of the ions above the threshold. The level of electronic noise is unique for an individual system, therefore, once set the level need not be further adjusted.

Setting the MCP Voltage

For a given Signal Threshold the MCP will need to be driven to a suitable gain at which the majority of single ions are detected. In practice this is normally at a gain of 1 - 5exp7. At this high gain the ion height distribution is at its narrowest (the MCP is close to saturation). Low gain can cause the ion height distribution to broaden and make the differentiation between single ion events and electronic noise difficult to set up. Like any electron multiplier the MCP has a limited lifetime. As electrons are stripped from the detectors coating changes occur resulting in reduced gain. The rate of gain change is related to the intensity of the output signal. The MCP's should never be exposed to large ion currents for extended time periods.

A new MCP detector will initially appear to have high gain for a moderate voltage applied. This is due to trapped gas in the coating of the MCP increasing secondary electron yield. As the MCP out-gasses with use the gain for a given applied voltage will drop. Once out-gassing is complete (the MCP has been aged) the rate of gain drop will slow.

Because of these effects the MCP voltage must be checked, and increased if required, at regular intervals to ensure that the majority of ion events are counted. Once the MCP voltage exceeds the maximum allowed from the software the MCP's should be replaced.

Effect of low MCP gain on isotope ratio

Fig 5 shows the position of the Signal threshold relative to the ion height distribution for single ion counting with low gain.

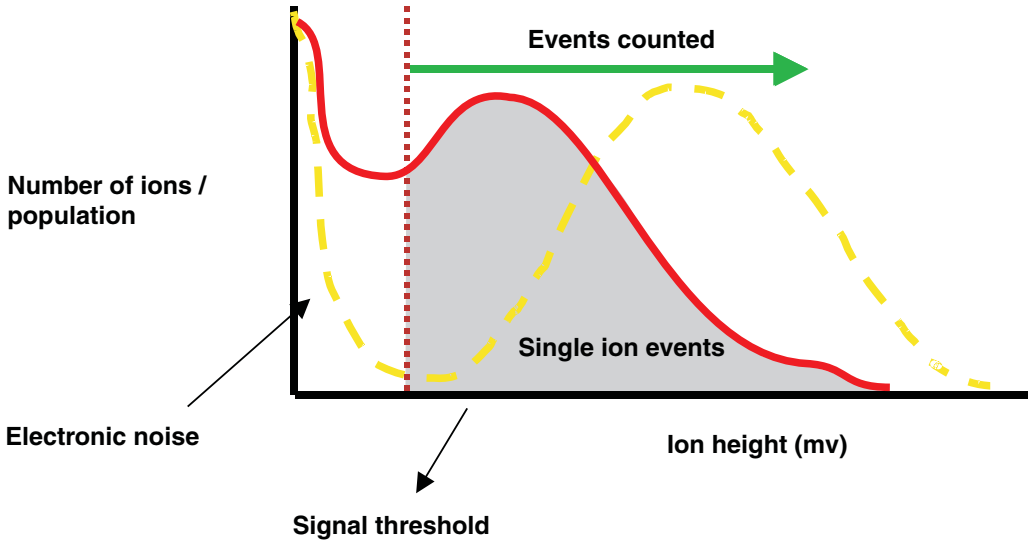


Fig 5 Ion height distribution low gain

Referring to Fig 5, it can be seen that the Signal Threshold is set correctly, however the gain of the MCP is low enough that only a fraction of the total ion arrivals will be recorded. (the dashed line indicates the situation where the gain is correctly set.)

If only single ions are arriving the result would be correct isotope ratios but reduced sensitivity. However as the ion current is increased, the percentage of multiple ion arrivals, in which two ions arrive at the same time, becomes significant. These multiple ion arrivals give rise to signals which are larger than these produced by single ion arrivals. The majority of these signals are large enough to exceed the Signal Threshold and be recorded. Fig 6 depicts these multiple ion arrivals associated with larger ion currents as a second, higher ion height distribution added to the initial lower distribution.

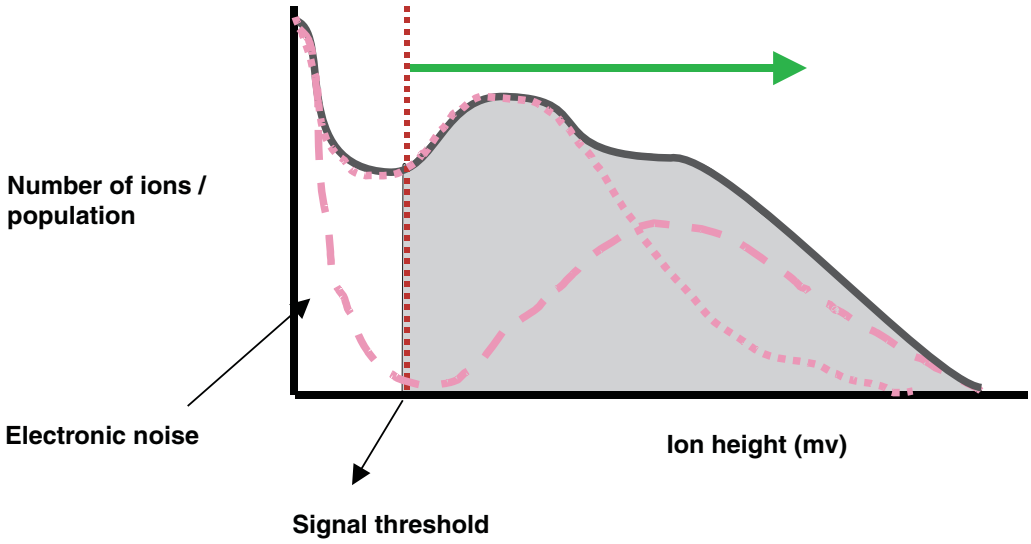


Fig 6 Effect of multiple ion arrivals on apparent ion height distribution

The result is that at larger ion currents, the presence of multiple ion arrivals, adds to the total counts recorded for these peaks. In terms of isotope ratios, the most intense spectral peaks will appear too large in proportion to the smaller isotope peaks. Fig 7

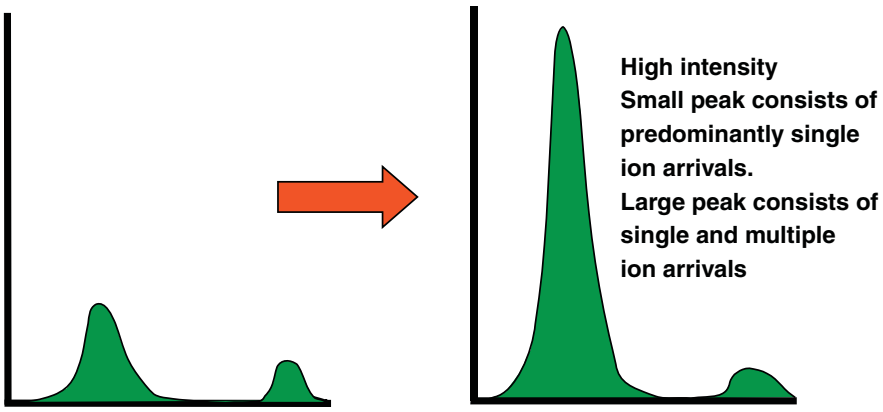


Fig 7 Effect of low detector gain on isotope ratios

Effect of Low MCP gain on Quantitation

Following the same arguments as above at low sample concentration when only single ion events occur, the intensity will rise linearly as concentration is increased, however sensitivity would be low. As the concentration / intensity of the signal increased further, more multiple events would occur and the signal would appear to increase at a quicker rate than for the lower concentration range. The quantitation curve would look similar to that shown below in Fig 8.

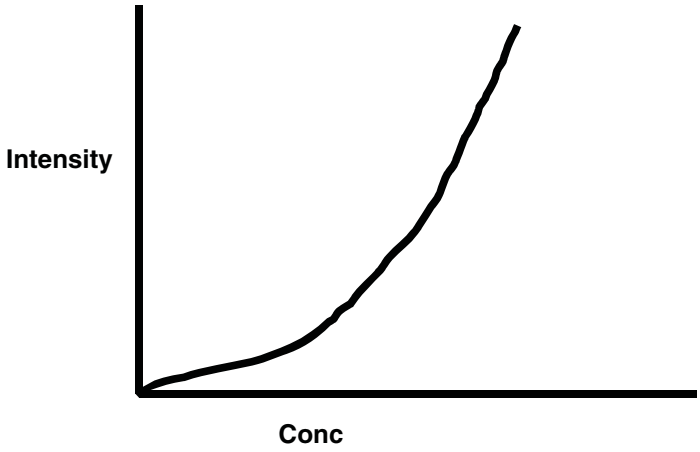


Fig. 8 General effect of low MCP gain on quantitation curve

Effect of low MCP gain on Exact Mass Measurement.

Determining the correct Np multiplier for the deadtime correction software involves investigating and correcting for the mass shift associated with mass spectral peaks of low intensity (predominantly single ion events) compared to mass shifts associated with more intense mass spectral peaks (significant multiple ion events). In the situation where the gain of the MCP is too low to detect the majority of the single ion events the mass shifts observed will not follow the theoretically calculated characteristics of the system. The result is that although it may be possible to produce an acceptable calibration at one particular beam intensity (by adjusting the Np multiplier value), this calibration will not be correct at different ion intensities. This will result in poor calibrations and erratic mass measurement accuracy.

Effect of low MCP gain on Resolution

Because single ion pulses have finite width, approximately 4-5 ns at the base, the time at which a single ion will be recorded will vary by a small amount depending on where it passes through the Signal Threshold. Fig 9 shows two single ions which effectively arrive at the same time but with different intensities. Because the leading edge of each ion passes through the Signal Threshold at slightly different times each ion is recorded with a slightly different flight time. This effect results in a slight broadening of the final mass spectral peak and reduction in resolution.

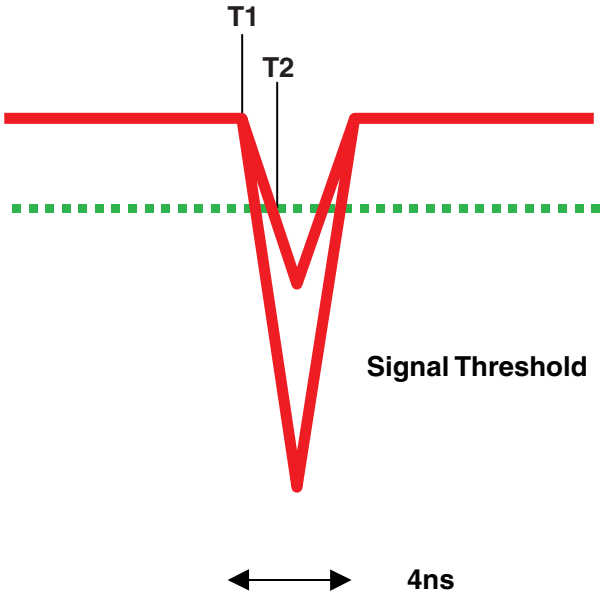


Fig 9. Effect of single ion height on event time

From Fig 9. above it follows that the larger the variation in single ion peak heights the greater the spread of ion arrival times and hence the poorer the resolution. In the situation where the gain is set too low to record the majority of single ions, those which are recorded have a narrower distribution signal intensities than the full set of signal intensities arriving at the TDC input. The resolution in this case is enhanced.

Index

L

M

N

O

P

R

S

T

V