Waters Quattro micro API Mass Spectrometer

Operator's Guide

71500058602/Revision D

Waters

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Waters contact information

Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit www.waters.com.
Telephone and fax	From the USA or Canada, phone 800 252-HPLC, or fax 508 872 1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site.
Conventional mail	Waters Corporation 34 Maple Street Milford, MA 01757 USA

Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow

Good Laboratory Practice, and consult your organization's safety representative for guidance.

Considerations specific to the Waters Quattro micro API

Solvent leakage hazard

The source exhaust system is designed to be robust and leak-tight. Waters recommends you perform a hazard analysis, assuming a maximum leak into the laboratory atmosphere of 10% LC eluate.

Warning:

- To confirm the integrity of the source exhaust system, renew the source O-rings at intervals not exceeding one year.
- To avoid chemical degradation of the source O-rings, which can withstand exposure only to certain solvents, see Appendix B to determine whether any solvents you use that are not listed are chemically compatible with the composition of the O-rings.

Flammable solvents hazard

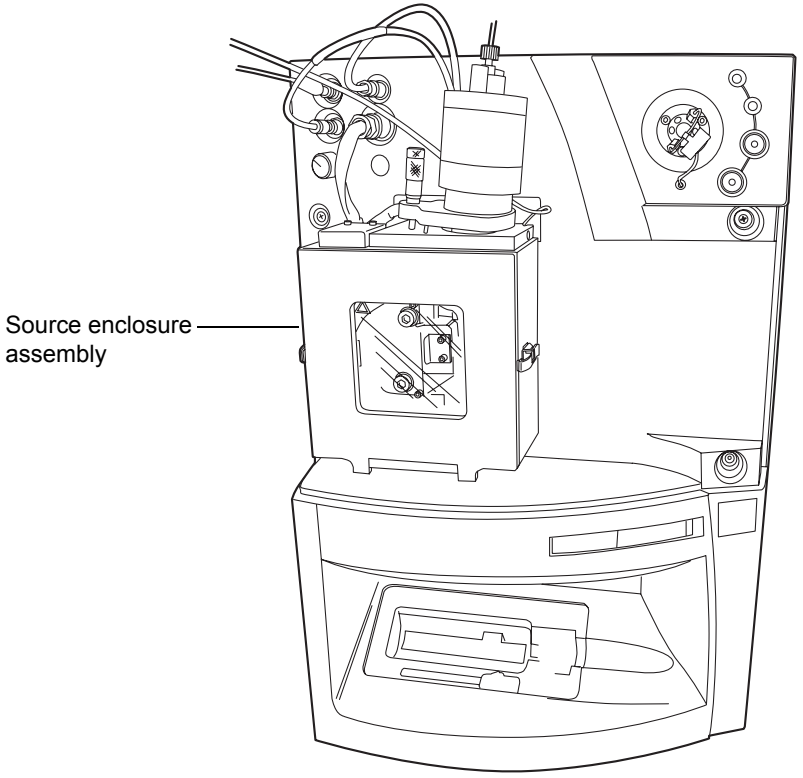
Warning: To prevent the ignition of accumulated solvent vapors inside the source, maintain a continuous flow of nitrogen through the source whenever significant amounts of flammable solvents are used during the instrument's operation.

Never let the nitrogen supply pressure fall below 690 kPa (6.9 bar, 100 psi) during analyses that require flammable solvents. Connect to the LC output with a gas-fail connector to stop the LC solvent if the nitrogen supply fails.

High temperature hazard

Warning: To avoid burn injuries, avoid touching the source enclosure with your hand when operating or servicing the instrument.

Mass spectrometer high temperature hazard

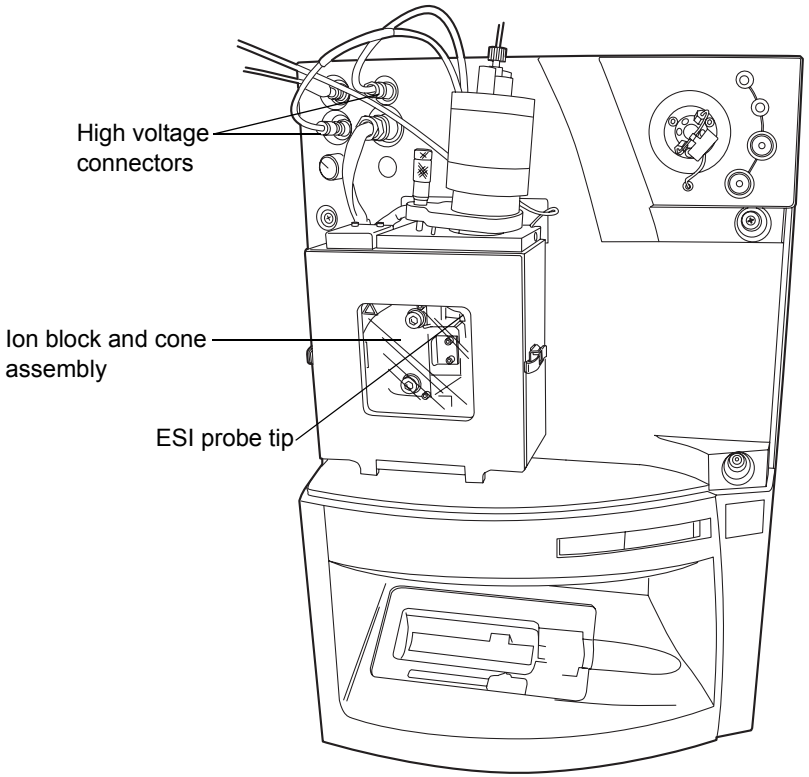


High voltage hazard

Warning:

- To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.
- To avoid nonlethal electric shock when the instrument is in Operate mode, avoid touching the areas marked with the high voltage warning symbol. To touch those areas, first put the instrument in Standby mode.

Mass spectrometer in ESI ionization mode



Hazards associated with removing an instrument from service

Warning: To avoid personal contamination with biohazardous or toxic materials, wear chemical-resistant gloves during all phases of instrument decontamination.

Warning: To avoid puncture injuries, handle syringes, fused silica lines, and borosilicate tips with care.

When you remove the instrument from use to repair or dispose of it, you must decontaminate all of its vacuum areas. These are the areas in which you can expect to encounter the highest levels of contamination:

- Source interior
- Waste tubing
- Exhaust system
- Rotary pump oil (where applicable)

The need to decontaminate other vacuum areas of the instrument depends on the kinds of samples the instrument analyzed and their levels of concentration. Do not dispose of the instrument or return it to Waters for repair until the authority responsible for approving its removal from the premises specifies the extent of decontamination required and the level of residual contamination permissible. That authority must also prescribe the method of decontamination to be used and the appropriate protection for personnel undertaking the decontamination process.

You must handle items such as syringes, fused silica lines, and borosilicate tips used to carry sample into the source area in accordance with laboratory procedures for contaminated vessels and sharps. To avoid contamination by carcinogenic, toxic, or biohazardous substances, you must wear chemical-resistant gloves when handling or disposing of used oil.

Safety advisories

Consult Appendix A for a comprehensive list of warning and caution advisories.

Operating this instrument

When operating this instrument, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

Symbol	Definition
	Manufacturer
	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia C-Tick EMC Compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use
	For in vitro diagnostic use

Audience and purpose

This guide is intended for personnel who install, operate, and service the Waters Quattro™ micro API Mass Spectrometer.

Intended use of the Waters Quattro micro API

IVD

The Waters Quattro micro API is CE-marked according to the European Union In Vitro Diagnostic Device Directive 98/79/EC.

Waters designed the Quattro micro API for use as a research tool to deliver authenticated exact-mass measurement on all sample types.

The Waters Quattro micro API can be used for general in vitro diagnostic applications. However, only professionally trained and qualified laboratory personnel can use the instrument for those purposes.

Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must include the entire range of QC samples, typical specimens, and atypical specimens.

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not operator's guide, accompanies the instrument, consult the instrument's online Help system for calibration instructions.

Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

When analyzing samples from a complex matrix such as soil, tissue, serum/plasma, whole blood, and other sources, note that the matrix components can adversely affect LC/MS results, enhancing or suppressing ionization. To minimize these matrix effects, Waters recommends you adopt the following measures:

- Prior to the instrumental analysis, use appropriate sample pretreatment such as protein precipitation, liquid/liquid extraction (LLE), or solid phase extraction (SPE) to remove matrix interferences.
- Whenever possible, verify method accuracy and precision using matrix-matched calibrators and QC samples.
- Use one or more internal standard compounds, preferably isotopically labeled analytes.

ISM classification

ISM Classification: ISM Group 1 Class A

This classification has been assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class A products are suitable for use in commercial, (that is, nonresidential) locations and can be directly connected to a low voltage, power-supply network.

EC authorized representative

Waters Corporation (Micromass UK Ltd.)
Floats Road
Wythenshawe
Manchester M23 9LZ
United Kingdom

Telephone:	+44-161-946-2400
Fax:	+44-161-946-2480
Contact:	Quality manager

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1 Instrument Description

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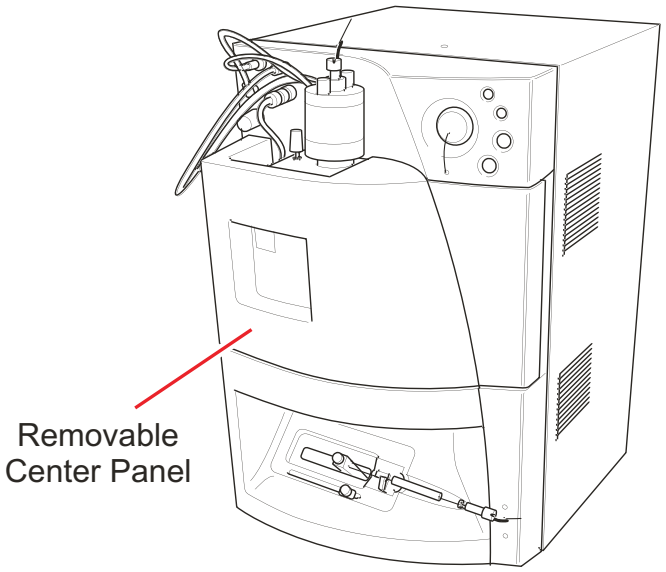
The Waters Quattro micro API



The Quattro™ micro API is a high performance triple quadrupole mass spectrometer designed for routine LC/MS/MS operation.

The instrument may be coupled to the following liquid introduction systems:

- HPLC system, to provide molecular weight information from an LC run or to perform target analysis and quantification.
- Syringe pump, for analysis of precious, low-concentration compounds.

Sample ionization takes place in the source at atmospheric pressure. These ions are sampled through a series of orifices into the first quadrupole where they are filtered according to their mass to charge ratio (m/z). The mass separated ions then pass into the hexapole collision cell where they either undergo collision induced decomposition (CID) or pass unhindered to the second quadrupole. The fragment ions are then mass analyzed by the second quadrupole.

The transmitted ions are finally detected by a conversion dynode, phosphor and photomultiplier detection system. The output signal is amplified, digitized and presented to the data system.

Sample inlet

An HPLC system or an infusion pump delivers sample to either an ElectroSpray Ionization (ESI) probe or Atmospheric Pressure Chemical Ionization (APcI) probe.

The ionization mode can be changed by changing probes. Recognition pins on the probes identify the ionization method to the system.

Vacuum system

An external rotary pump and an internal split-flow turbomolecular pump combine to create a vacuum. The turbomolecular pump evacuates the analyzer and ion transfer region.

The system monitors turbomolecular pump speed and continuously measures the vacuum with a built-in Pirani gauge. In the event of leaks, electrical failure, or vacuum pump failure a loss of vacuum will occur. The Pirani gauge also acts as a switch, discontinuing instrument operation if it senses a vacuum loss.

An easy-access vacuum isolation valve enables routine source maintenance to be performed without breaking vacuum.

Data system

The data system collects information from the mass analyzer. The data system consists of:

- An embedded PC
- An external workstation
- The MassLynx™ software

The workstation-based data system, incorporating MassLynx software, controls the mass spectrometer and, if applicable, the HPLC system, autosampler, divert valve or injector valve. The workstation uses the Windows® graphical environment with color graphics, and provides for full user interaction with either the keyboard or mouse. MassLynx provides full control of the system including setting up and running selected HPLC systems, tuning, acquiring data, and data processing. MassLynx instrument

control uses an embedded PC to process all data. A network link enables communication between the workstation and the embedded PC.

The data system can sample analog inputs and thus store data from conventional LC detectors like UV or ELSD simultaneously with acquired mass spectral data. It can also acquire UV photo diode array detector data for selected systems such as the Waters 996 PDA. Comprehensive information detailing the operation of MassLynx is in the *MassLynx User's Guide.*

MassLynx software

MassLynx software, a Windows-based application, enables the following operations:

- Configuring the Quattro micro API.
- Creating inlet and MS methods that define operating parameters for a run.
- Tuning and calibrating the Quattro micro API.
- Running samples.
- Monitoring the run.
- Acquiring data.

Refer to the *MassLynx User's Guide* and *Help* for more information on installing and using MassLynx software.

Theory and principles of operation

ElectroSpray ionization (ESI)

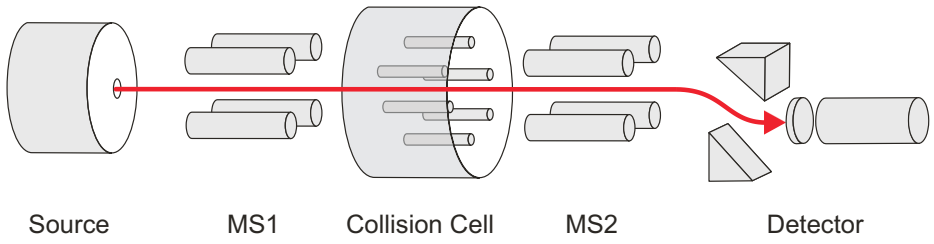
In ElectroSpray ionization (ESI), a strong electrical charge is applied to the eluent as it emerges from a nebulizer, producing an aerosol of charged droplets. Solvent evaporation reduces the size of the droplets until a sufficient charge density makes the ejection of sample ions from the surface of the droplets possible (ion evaporation). Characteristically, ions are singly or multiply charged, and the mass analyzer sorts them by mass-to-charge (m/z) ratio. High molecular weight compounds are typically measured as ions with multiple charges. Eluent flows up to 1 mL/min can be accommodated, though it is often preferable with ElectroSpray ionization to split the flow so that 100 to 200 µL/min of eluent enters the mass spectrometer source.

Atmospheric pressure chemical ionization (APcI)

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

MS operating modes

Ion optics



MS operating modes

Operating mode	MS1	Collision cell	MS2
MS1	Resolving	Radio Frequency (RF) only (pass all masses)	
MS2	Radio Frequency (RF) only (pass all masses)		Resolving

The MS1 mode, in which MS1 is used as the mass filter, is the most common and most sensitive method of performing MS analysis. This is directly analogous to using a single quadrupole mass spectrometer.

The MS2 mode of operation is used, with collision gas present, when switching rapidly between MS and MS/MS operation. It also provides a useful tool for instrument tuning and calibration prior to MS/MS analysis, and for fault diagnosis.

MS/MS operating modes

General

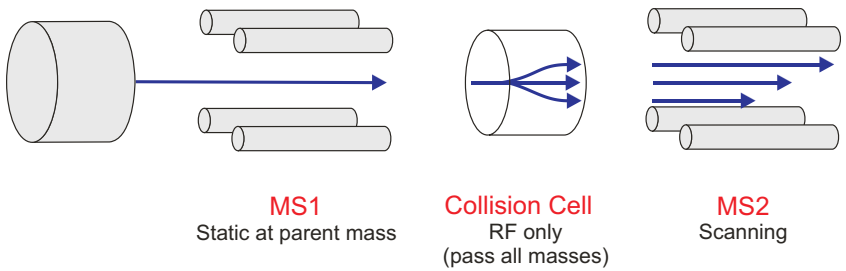
The four common MS/MS scan functions are summarized in the table below.

MS/MS operating modes

Operating mode	MS1	Collision cell	MS2
Daughter (Product) Ion Spectrum	Static (parent mass selection)	RF only (pass all masses)	Scanning
Parent (Precursor) Ion Spectrum	Scanning		Static (daughter mass selection)
Multiple Reaction Monitoring	Static (parent mass selection)		Static (daughter mass selection)
Constant Neutral Loss Spectrum	Scanning (synchronized with MS2)		Scanning (synchronized with MS1)

The Daughter (product) ion spectrum

Daughter (product) ion mode



This is the most commonly used MS/MS scan mode.

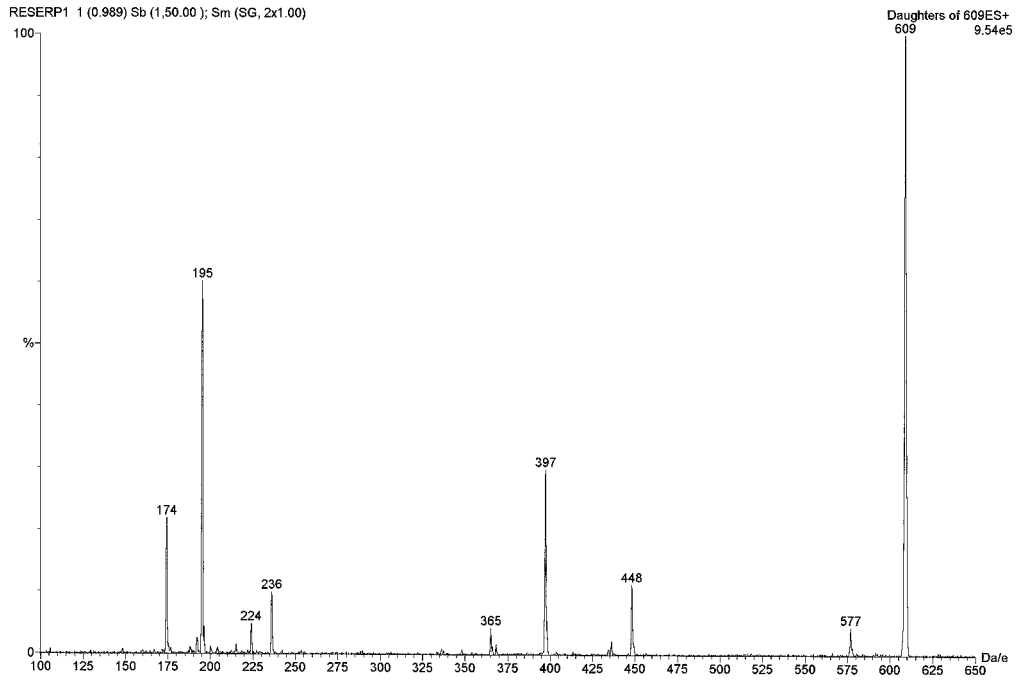
Typical applications are:

- Structural elucidation (for example, peptide sequencing).
- Method development for MRM screening studies:
  - Identification of daughter ions for use in MRM transitions.
  - Optimization of CID tuning conditions to maximize the yield of a specific daughter ion to be used in MRM analysis.

Example:

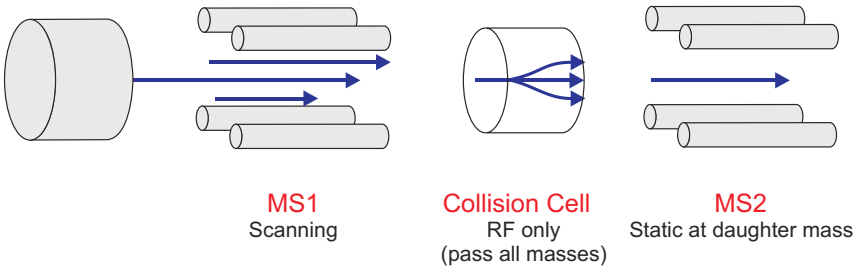
Daughters of the specific parent at m/z 609 from reserpine in ElectroSpray positive ion mode, see the figure “Daughters of the specific parent at m/z 609 from reserpine in ElectroSpray positive ion mode” on page 1-7.

Daughters of the specific parent at m/z 609 from reserpine in ElectroSpray positive ion mode



The Parent (precursor) ion spectrum

Parent (precursor) ion mode



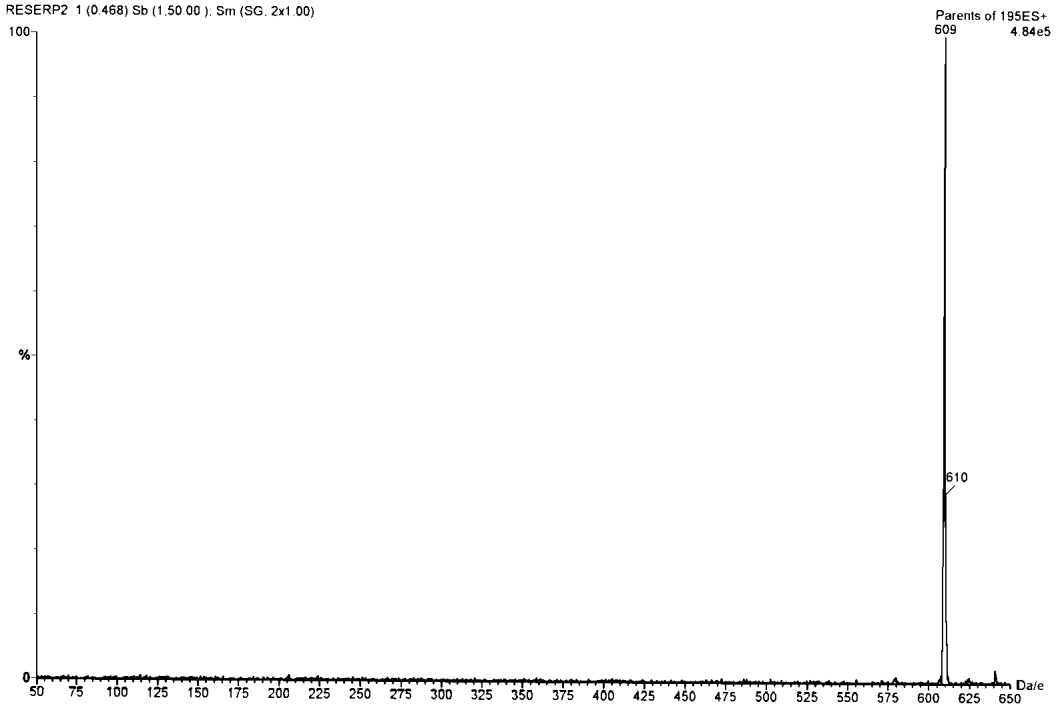
Typical application:

- Structural elucidation.
  - Complementary or confirmatory information (for daughter scan data).

Example:

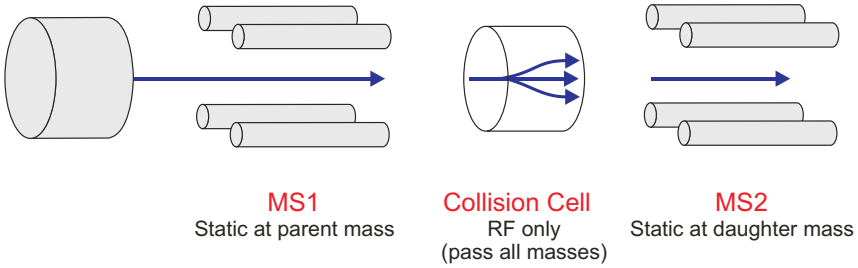
Parents of the specific daughter ion at m/z 195 from reserpine in ElectroSpray positive ion mode, see figure below.

Parents of the specific daughter ion at m/z 195 from reserpine in ElectroSpray positive ion mode



MRM: multiple reaction monitoring

MRM mode



This mode is the MS/MS equivalent of SIR (Selected Ion Recording). As both MS1 and MS2 are static, this allows greater dwell time on the ions of interest and therefore better sensitivity compared to scanning MS/MS.

Typical application:

- Rapid screening of dirty samples for known analytes:
  - Drug metabolite and pharmacokinetic studies.
  - Environmental, for example pesticide and herbicide analysis.
  - Forensic or toxicology, for example screening for target drugs in sport.

Example:

Monitor the transition (specific fragmentation reaction) m/z 609 to 195 for reserpine in ElectroSpray positive ion LC/MS/MS mode.

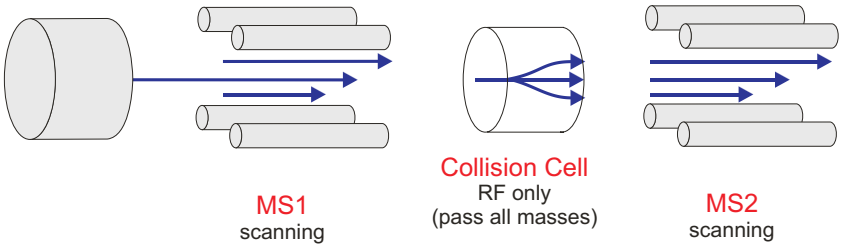
MRM does not produce a spectrum as only one transition is monitored. As in SIR, a chromatogram is produced.

The m/z 609 to 195 transition for reserpine in ElectroSpray positive ion LC/MS/MS mode

LC/MRM
High specificity
Good signal/noise

LC/MS
Low specificity
Poor signal/noise

The constant neutral loss spectrum



The loss of a specific neutral fragment or functional group from an unspecified parent or parents.

Typical application:

- Screening mixtures for a specific class of compound that is characterized by a common fragmentation pathway.

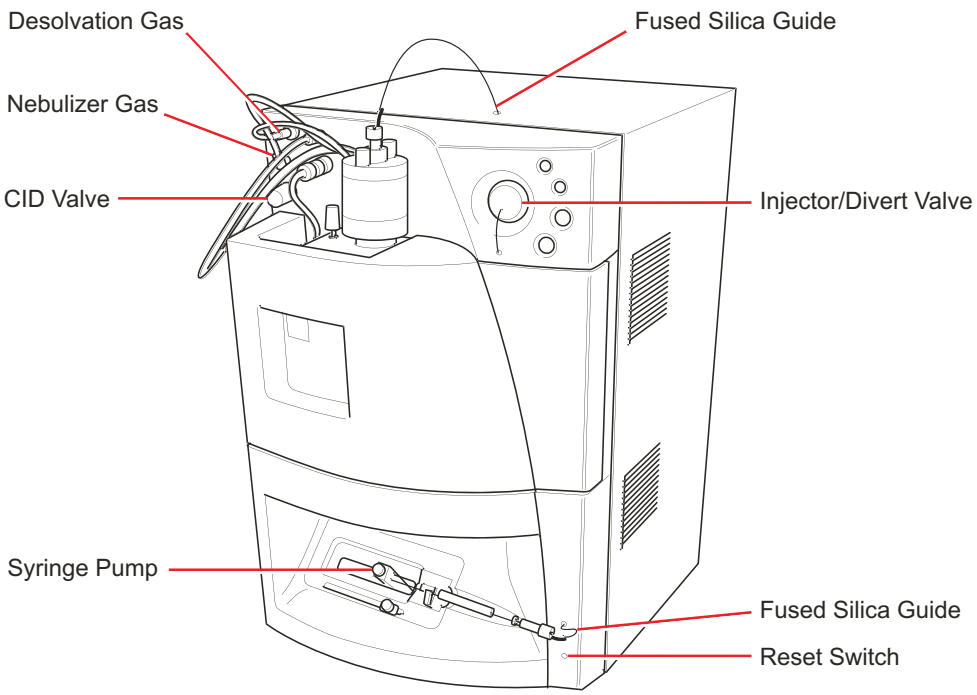
The scans of MS1 and MS2 are synchronized. When MS1 transmits a specific parent ion, MS2 “looks” to see if that parent loses a fragment of a certain mass. If it does, it registers at the detector.

The result:

The spectrum shows the masses of all parents that actually lost a fragment of a certain mass.

Front panel controls, indicators and connections

Front panel



Cone gas, desolvation gas and nebulizer gas

The PTFE gas lines for the desolvation gas and nebulizer gas are connected to the front of the instrument using push-in Legris fittings. The connection for the cone gas is within the source and uses PTFE tubing.

Mass flow controllers

Electronic mass flow controllers, whose settings you specify in the Tune window, regulate the cone gas and desolvation gas over the ranges 0 to 500 L/h and 0 to 1200 L/h, respectively.

In the event that the desolvation gas flow decreases to less than 4% of its full scale range, the instrument generates a signal that can be used to stop solvent flowing into the source by connecting it to the Stop Flow of the HPLC system.

Electrical connections

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

The high voltage connection for the ESI probe is via the front panel ESI connection.

The high voltage connection for the corona discharge pin is internal to the source.

Caution: Ensure that the instrument is in Standby when fitting the corona discharge pin.

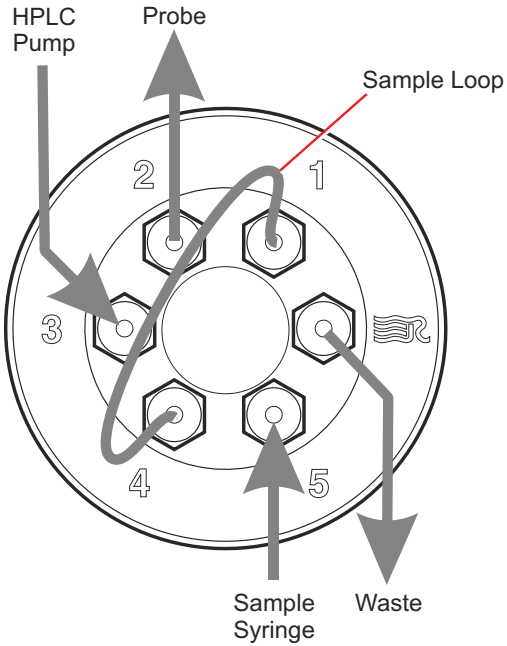
CID valve

The CID Gas valve is a fifteen-turn valve. The collision gas flow increases as the valve is turned counterclockwise.

Caution: To prevent damage to the CID Gas valve, take care not to over-tighten when turning the supply off.

Divert/injection valve

Divert/injection valve



The divert/injection valve is an electrically driven Rheodyne® injector that may be used in several ways depending on the plumbing arrangement:

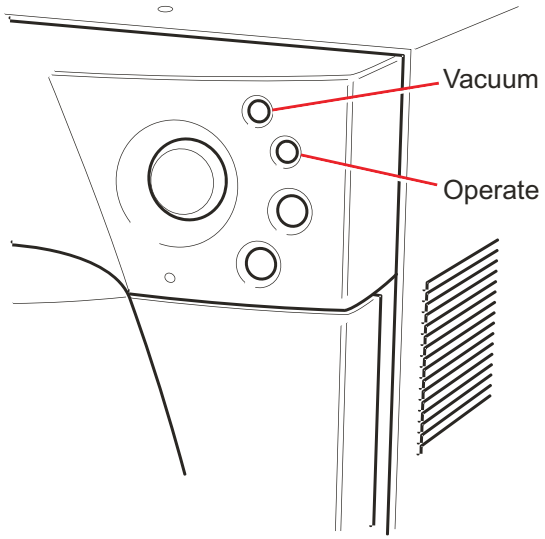
- As an injection valve, with the needle port and sample loop fitted.
- As a divert valve, to switch the flow of solvent during a LC run.
- As a switching valve, for example to switch between a LC system and a syringe pump containing calibrant.

Control of the valve is primarily from the data system. The two switches marked Load and Inject enable the user to override control of the valve when making loop injections at the instrument.

For details of the use of the valve as a divert valve see page 4-21.

Status display

Status display



The status of the instrument is indicated as follows:

Vacuum LED

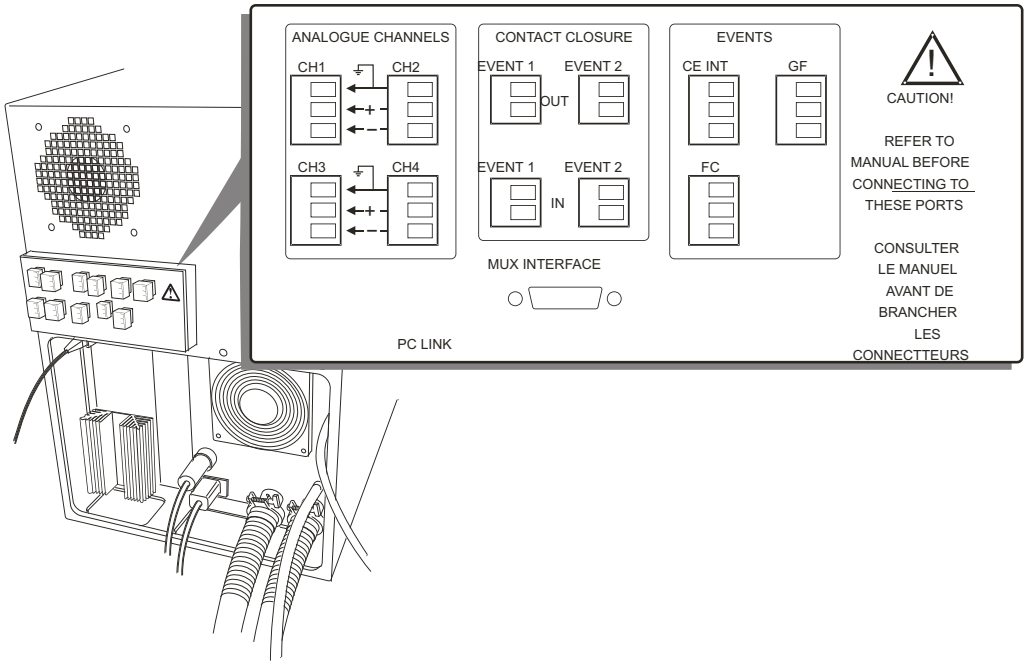
State	Vacuum LED
Pumping	Flashing green
Pumped, below trip level	Steady green
Pumped, above trip level	Steady amber
Pump fault	Flashing red

Operate LED

State	Operate LED
Standby	No indication
Operate, above trip level	Steady amber
Operate, below trip level	Steady green
RF error	Flashing red

Rear panel connections

Rear panel connections



Analog channels

Four analog channel inputs are available, for acquiring simultaneous data such as a UV detector output.

The input differential voltage must not exceed one volt. Analogue data is processed by a 12-bit ADC with a gain ranging up to 2×10^{20} counts.

If the input cable is only a two-wire assembly, then the negative pole of each channel may need to be grounded.

Contact closure

Two types of contact closure are available:

- **In**. Two inputs, Event 1 and Event 2, are provided, allowing external devices to start acquisition. The Event In signal can be TTL or contact closure, 5 V maximum voltage.
- **Out**. Two outputs, Event 1 and Event 2, are provided whereby the mass spectrometer is able to trigger an external event.

Mux interface

This 9-way D-type connector enables interfacing to the MUX control unit.

Events

CE int (capillary electrophoresis interlock)

This connector enables interfacing with a capillary electrophoresis power supply so that the instrument is safely interlocked against high voltages.

GF (gas fail)

If the desolvation gas flow decreases to less than 4% of its full-scale range, the instrument generates a signal that can be used to stop solvent flowing into the source by connecting it to the Stop Flow of the HPLC system.

FC (FractionLynx control)

A 100 mV analog output signal is provided to allow a trigger signal for an external fraction collection device. The optional FractionLynx software must be purchased for this.

PC link

This RJ45 connector links the instrument to the data system using the network cable supplied.

2 Routine Procedures

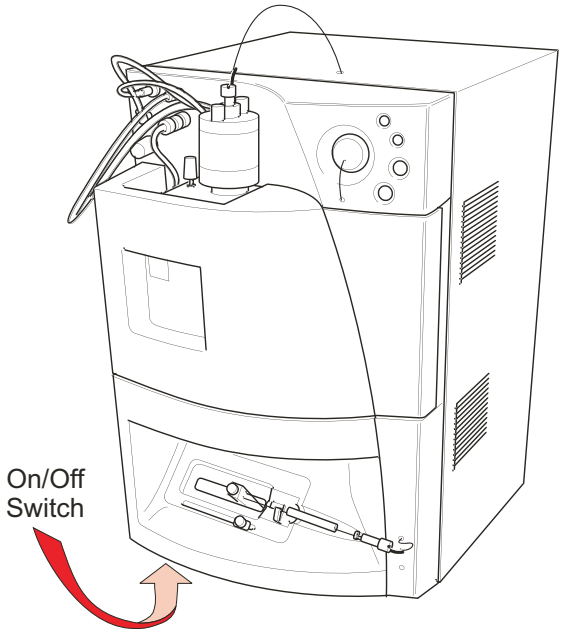
Contents

Starting the Quattro micro API

To start the Quattro micro API proceed as follows:

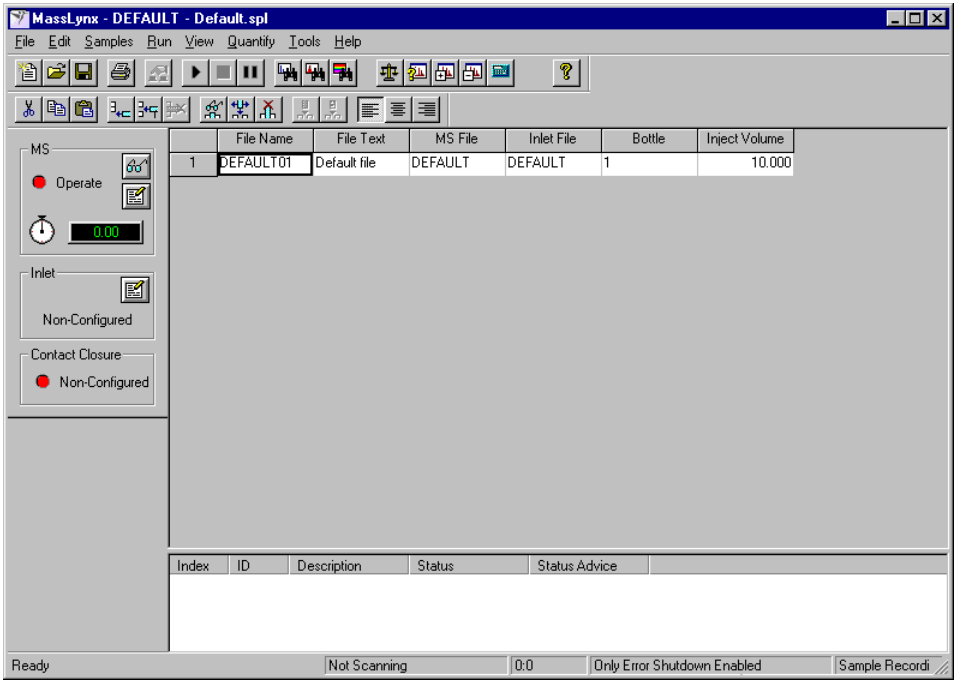
1. Switch on the switch located under the left hand side of the front panel.

   On/off switch



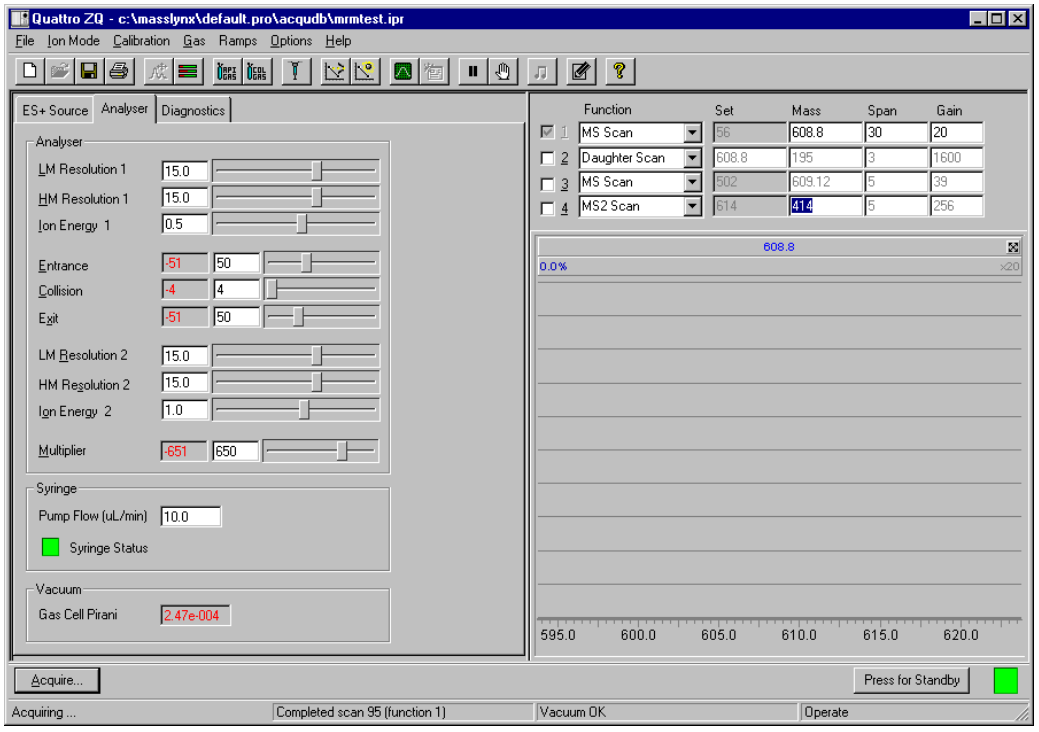
2. Allow 3 minutes for the embedded PC to initialise. An audible alert is given when the PC is ready.
3. Start the MassLynx software. The default page appears, and the word Ready appears in the status bar at the bottom.

MassLynx default page



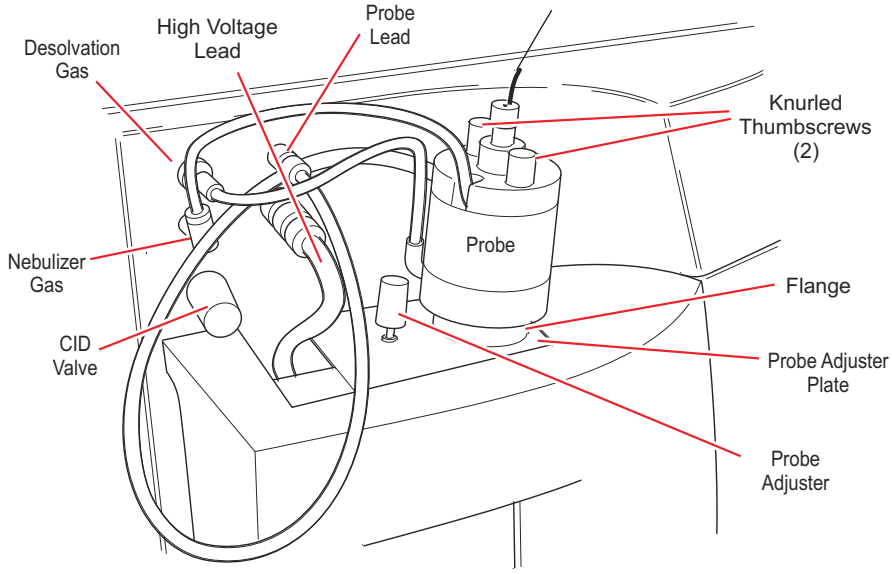
4. Click  (on the default page) to invoke the tune page.
5. Select Pump from the tune page Options menu.
6. Click the Diagnostics tab.
7. Monitor Turbo Speed. This parameter should reach 98 to 100% within approximately 5 minutes of Pump being selected.
8. Ensure that the instrument has pumped sufficiently such that the Vacuum LED on the front panel is steady green (see page 1-15). The mass spectrometer is sufficiently evacuated to enable operation in 20 minutes.

Tune page


Quattro ZQ - c:\masslynx\default.pro\acqudb\mrmtest.ipr
File Ion Mode Calibration Gas Ramps Options Help
ES+ Source Analyser Diagnostics
Analyser
LM Resolution 1 15.0
HM Resolution 1 15.0
Ion Energy 1 0.5
Entrance -51 50
Collision -4 4
Exit -51 50
LM Resolution 2 15.0
HM Resolution 2 15.0
Ion Energy 2 1.0
Multiplier -651 650
Syringe
Pump Flow (uL/min) 10.0
Syringe Status
Vacuum
Gas Cell Pirani 2.47e-004
Function Set Mass Span Gain
1 MS Scan 56 608.8 30 20
2 Daughter Scan 608.8 195 3 1600
3 MS Scan 502 609.12 5 39
4 MS2 Scan 614 414 5 256
608.8
0.0%
x20
595.0 600.0 605.0 610.0 615.0 620.0
Acquire...
Press for Standby
Acquiring ...
Completed scan 95 (function 1)
Vacuum OK
Operate

Installing the ESI (ElectroSpray) probe

ESI probe



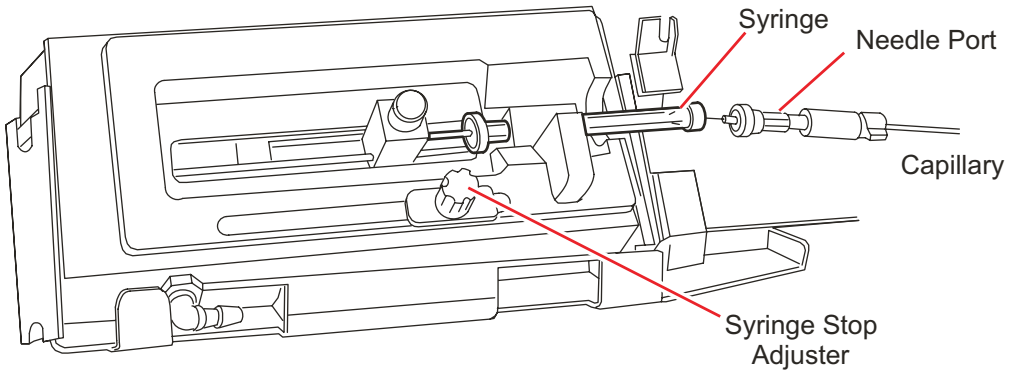
To install the ESI probe:

1. Ensure that the isolation valve lever is fully to the left, indicating the valve is open.
2. Insert the probe adjustment flange electrical cable in the lower (and larger) of the two electrical ports on the front panel.
3. Connect the PTFE tubing from the probe adjustment flange to the desolvation gas port on the front panel.
4. Remove the protective sleeve, if fitted, from the ElectroSpray probe tip.
5. Slide the ElectroSpray probe into the hole in the probe adjustment flange until the probe body rests on the probe adjustment flange. Ensure the probe identification contacts touch the screws on the probe adjustment flange.
6. Secure the probe with the two thumbscrews.

7. Connect the 4 mm PTFE tubing from the probe to the port labelled Neb (nebulizer gas).
8. Connect the electrical lead from the probe to the capillary connector on the front panel.

Setting up the syringe pump

Syringe pump



To set up the syringe pump:

1. Clip the ground cable (with plug-in clip), located at the front panel, lower right, onto the syringe needle.
2. Mount the syringe onto the pump, and set the syringe stop appropriately.

   Caution: Waters has incorporated into the syringe pump design a positive syringe stop to prevent certain syringe types from breaking. Nevertheless, as added protection against syringe breakage, setting the syringe stop adjuster is recommended. This prevents the syringe plunger from travelling its full stroke inside the syringe barrel, thereby reducing the likelihood of breakage.

3. Screw the Rheodyne 9013 needle port fitting into the PEEK™ union, and tighten it so that it does not leak.

4. Feed the capillary (ESI probe installation kit) from the top of the molding to the syringe area. Connect the capillary to the PEEK union, using an Upchurch® Scientific nut, ferrule, and PTFE tubing.
5. Make a square, even cut on both ends of the capillary before installing, using a ceramic silica cutter. Examine new cuts for squareness using an eye glass. When cutting the capillary, allow enough length to form loops at angles and corners. Never kink the capillary or stretch it tightly from one point to another.

   Warning: Clip the ground cable (with plug-in clip), located at the front panel, lower right, onto the syringe needle.

6. Connect the other end of the capillary to the inlet on the ESI probe with an Upchurch Scientific nut, ferrule, and PTFE tubing.
7. Click  (on the default page) to access the tune page.
8. Choose a suitable syringe type from the syringe selection editor by selecting Options, then Syringe Type, from the tune page.

Setting up the Quattro micro API

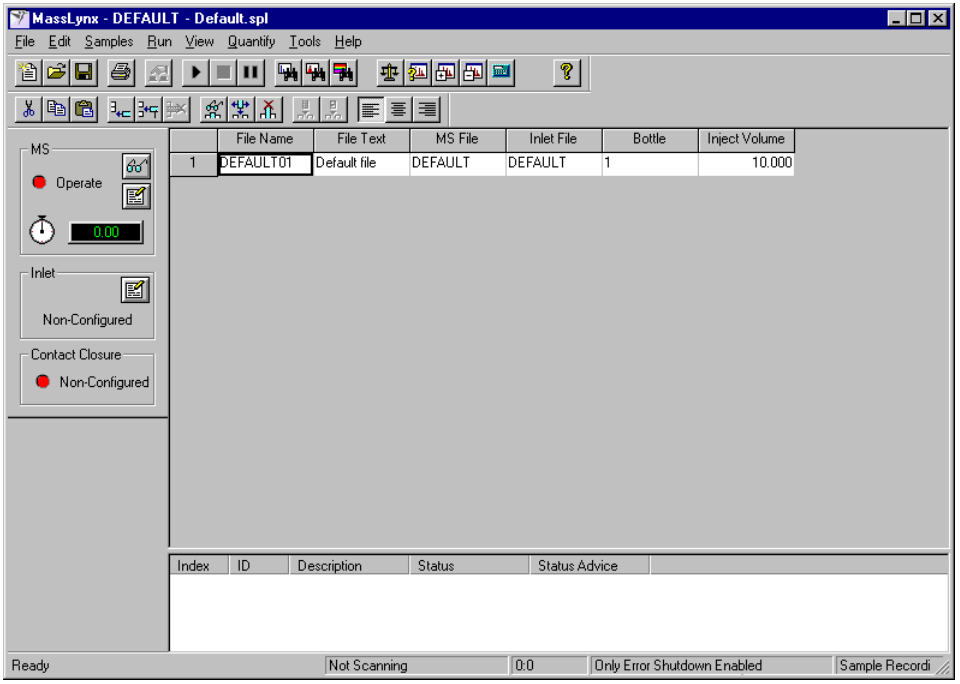
Preparing for ElectroSpray operation

Warning: Be sure to ground the syringe needle with the ground cable provided.

To prepare for ElectroSpray operation:

1. Connect one end of the fused silica capillary tubing to the syringe, and connect the other end to the ESI probe.
2. Fill the syringe with a reference solution, and mount it on the syringe pump.
3. Click  on the default page to invoke the tune page. The example tune page, shown in the figure “ESI tune page” on page 2-9, uses ions from a solution of PPG1000, reserpine and PA ß Cyclodextrin.

Default page

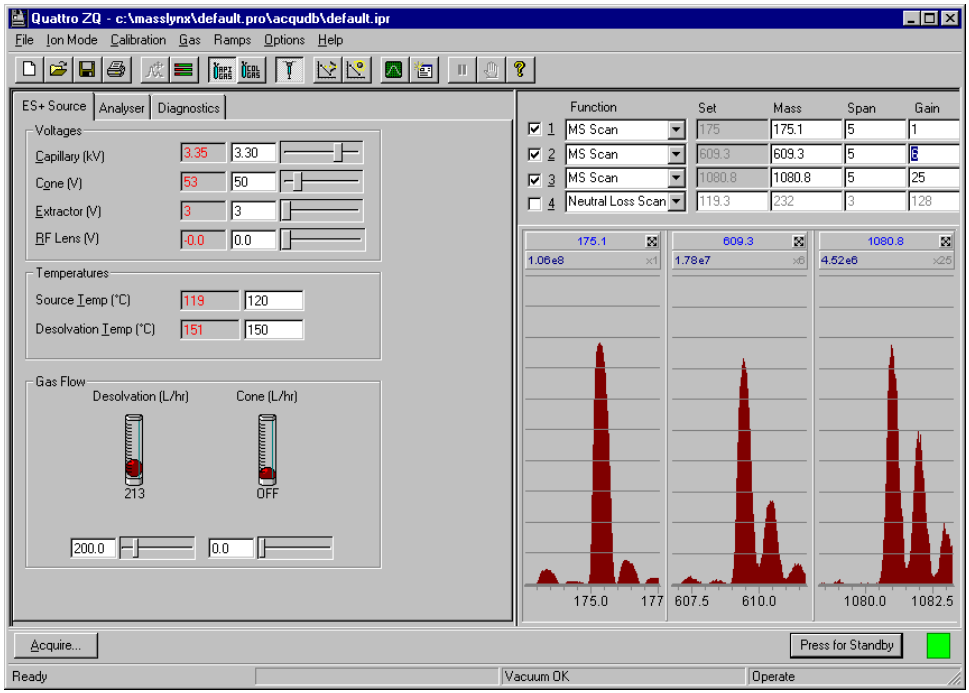


4. Select Options from the menu bar, then Pump. The rotary pump starts to evacuate the detector. In about 20 minutes, the instrument is sufficiently evacuated to enable operation, and the Vacuum indicator on the front panel shows green.

5. To view the actual values for instrument parameters select Readbacks from the Options menu, then Always On.

6. Enter the suggested initial reference solution values from the table titled “Tune page initial reference solution values” on page 2-9 in the corresponding tune page fields. These settings are intended as starting points only. Optimum values may vary between instruments.

ESI tune page



Tune page initial reference solution values

Mass	Span	Gain
175.1	5	8
609.2	5	20
1080.8	5	40
2034.6	5	50

7. Enter the recommended parameter values from the table titled "ES+ source page recommended parameter values" on page 2-10 in the corresponding fields of the ES+ Source page.

Caution:

- Failure to flow desolvation gas during ESI operation can cause heat damage to the source.
- To avoid contaminating source components, always specify a desolvation gas flow rate that exceeds 100 L/h.

ES+ source page recommended parameter values

Parameter	Suggested value
Capillary (kV)	3.0
Cone (V)	60
Extractor (V)	3
RF Lens (V)	0.2
Source Temperature (°C)	120
Desolvation Gas (L/h)	150
Cone Gas (L/h)	0

Tip: To avoid nitrogen leakage, fully tighten the probe locking ring.

8. Click the Analyser tab.
9. Enter the parameter values listed in the following table, dependent on whether tuning for MS1 or MS2.

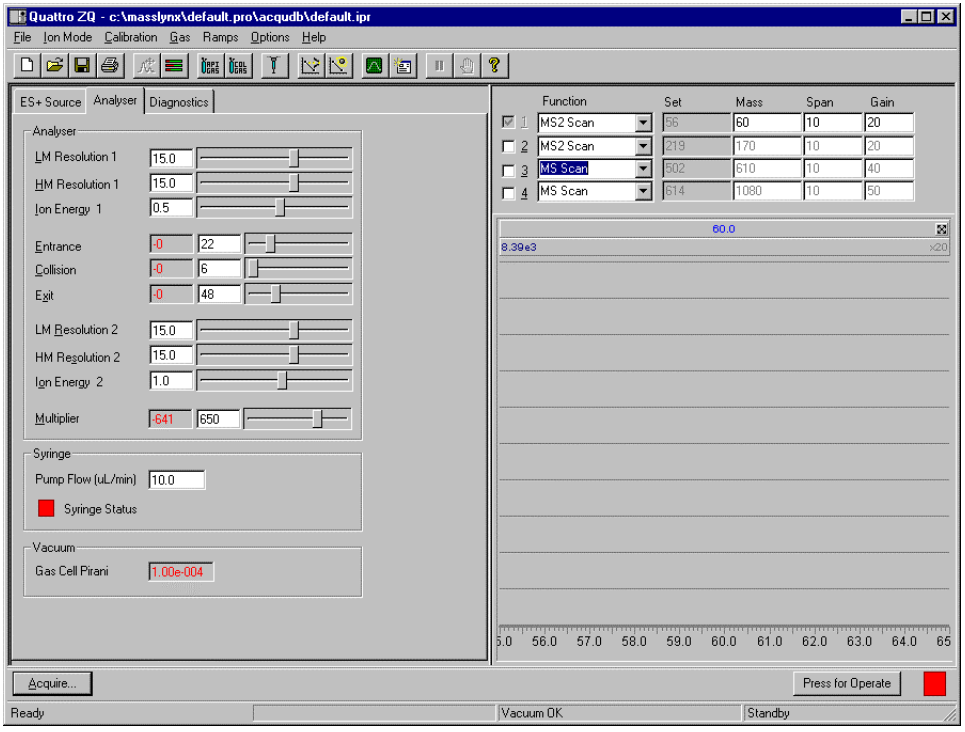
Analyser parameter values

Parameter	Recommended value MS1	Recommended value MS2
LM Resolution 1	15	
HM Resolution 1	15	
Ion Energy 1	0.5	
Entrance	50	2
Collision	0	0
Exit	50	2

Analyser parameter values (Continued)

Parameter	Recommended value MS1	Recommended value MS2
LM Resolution 2	15	15
HM Resolution 2	15	15
Ion Energy 2	3	0.5
Multiplier	650	650

Analyser page



10. Suitable resolution can be obtained by adjusting LM Resolution and HM Resolution.

11. Click  to start the nitrogen flow.

Obtaining an ion beam in ElectroSpray (ESI) mode

To obtain an ion beam in ElectroSpray (ESI) mode:

1. Ensure that the ESI probe is installed as described on page 2-5.
2. Change the ionization mode to ESI, if necessary (the current tune page tab indicates the ionization mode):

   Select Ion Mode > Electrospray+ from the tune page menu.
3. Keep the tune page ES+ Source tab open for the remaining steps in this section.
4. Set Source Temp to 120 °C.
5. Allow the source temperature to reach 120 °C.
6. Click  to start the nitrogen flow.
7. From the Options menu, select the type of syringe to be used. For example, select the Hamilton 250-µL gas-tight syringe from the startup kit.
8. Click Press For Operate to switch on the instrument high voltages.
9. Set the syringe flow rate to 10 µL/min., and click  on the tune page menu bar.
10. On the tune page, set Desolvation Gas to 150 L/h.
11. Check for leaks at the probe and syringe fittings.
12. Monitor for mass peaks. The peaks should appear at approximately the mass values entered on the ES+ Source tab.
13. Increase values in the Gain fields until mass peaks become clearly visible.

Caution:

- To avoid contaminating source components, always specify a desolvation gas flow rate that exceeds 100 L/h.
- An optimum signal must be obtained before the instrument can successfully be calibrated.

14. If the signal is relatively weak and noisy, enhance it by turning the probe adjuster knob to adjust the orientation of the probe relative to the sample cone orifice. The signal can also be enhanced by adjusting the desolvation gas flow from the ES+ Source tab on the tune page

    Caution: If the nitrogen supply to the instrument is turned off overnight, be sure the API Gas parameter on the tune page is set to Off before restarting nitrogen flow. Failure to do this may damage the flow meter.

    The source is now ready for ElectroSpray use. Refer to Chapter 3 for further information.

Preparing for APCI operation when in ESI mode

To prepare for APCI operation when in ESI mode:

1. Switch the instrument into standby mode by clicking Press for Standby on the lower right of the tune page.
2. Switch the instrument into standby mode by clicking Press for Standby on the lower right of the tune page.
3. Disconnect the nebulizer and both electrical connections from the front panel.
4. Remove the ESI probe by unscrewing the two thumb nuts on the probe.

    Caution: To avoid damaging the source enclosure door, do not apply any force to it while it is open.

5. Remove the middle moulding section, unfasten the source enclosure door's securing clips, and open the source enclosure door.
6. Close the source enclosure door, fasten the securing clips, and fit the middle molding section.
7. With the corona discharge pin in place, proceed as follows:
    a. Insert the APCI probe into the source and tighten the two thumbscrews.
    b. Connect the 6-mm nebulizer gas tube from the probe to the instrument port marked Neb.

c. Remove the probe adjustment flange cable from the front panel, and seat it in the rest hole provided just below its electrical socket.

d. Connect the APCI probe electrical lead to the Source/Probe receptacle on the front panel.

e. Connect the LC pump tubing to the APCI probe.

f. Set the Source Temp to 130 °C.

g. Set APCI Probe Temp to 20 °C with zero liquid and nitrogen flow.

h. Switch the instrument to Operate.

The source is now ready for APCI operation.

Caution:

- Before restarting nitrogen flow following its interruption, the API gas flow must be stopped from the tune page. Restarting the nitrogen while the API gas is flowing can damage the flow meter.
- Do not start the liquid flow until the gas flow and probe heater are switched on with the probe inserted.

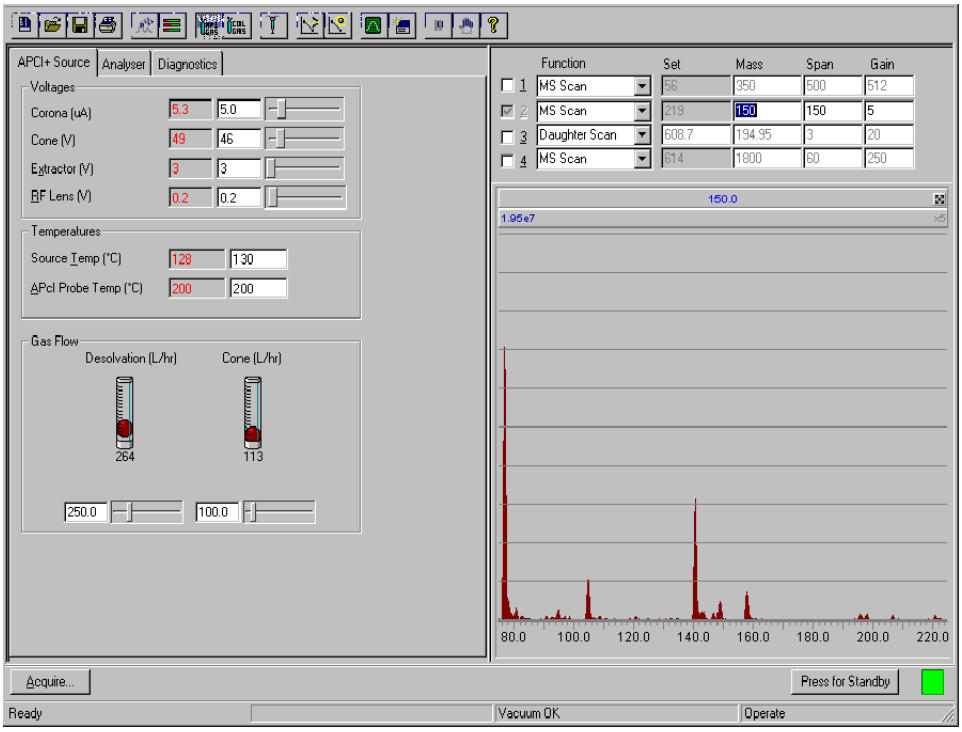
Obtaining an Ion beam and tuning in APCI mode

To obtain an ion beam:

1. Ensure that the corona discharge pin is in place, and the APCI probe is installed as described on page 2-13.
2. Change the ionization mode to APCI, if necessary. Select Ion Mode from the tune page menu. The current tune page tab indicates the ionization mode.

3. Keep the tune page APCI+ Source tab open.

APCI tune page



4. Ensure that the desolvation gas tube is connected at the front panel.
5. Set Source Temp to 130 °C.
6. Set Corona to 2 µA and Sample Cone to 50 V.
7. Allow the source temperature to reach 130 °C.
8. Click  to start the nitrogen flow.
9. Set Desolvation Gas to 250 L/h on the APCI+ source tune page.
10. Select one of the peak display boxes, and set Mass to 50 and Span to 90.
11. Click Press to Operate.

12. Set APCI Probe Temp to 500 °C for acetonitrile:water 1:1 flowing at 1 mL/min.

    Lower temperatures are required for higher proportions of organic mobile phase.

13. Allow the APCI probe temperature to reach 500 °C.

14. Start the LC pump flowing at 1.0 mL/min.

15. Adjust the spray approximately to midway between the corona pin and the sample cone with the probe adjuster.

    Refer to page 2-16 for more information on source tuning.

Warning:

- The source enclosure and parts of the probe adjustment flange may reach high temperatures when in use.
- Switch off the liquid flow and allow the probe to cool to less than 100 °C before removing it from the source.

Caution: Failure to flow desolvation gas during ESI operation can cause heat damage to the source.

Performing a sample analysis

Typical parameters for general qualitative analysis of mixtures are shown in the table titled “Typical parameters for general qualitative analysis of mixtures” on page 2-17. Adjust the values of Corona, Cone and APCI Probe Temp for optimal performance.

Specific tuning for maximum sensitivity

Caution: To avoid contaminating source components, always specify a desolvation gas flow rate of greater than 100 L/h.

Typical parameters for general qualitative analysis of mixtures

Parameter	Typical value
Corona (µA)*	2
Cone (V)	25 (Monitor the ions, slide the adjuster up or down to optimize)
Extractor (V)	5
RF Lens (V)	0.2
Source Temp (°C)	130
APCI Probe Temp (°C)*	500
Desolvation Gas (L/h)*	300
Cone Gas (L/h)*	100
* See page 2-17 for specific tuning details.	

For quantitative analysis, optimum APCI conditions should be obtained for each analyte using standard solutions.

Tuning at high flow rates in APCI may be performed using a tee to introduce a standard solution (typically 100 to 1000 pg/µL) at 10 µL/min into the mobile phase stream.

Alternatively, repeat direct loop injections of a standard solution (typically 10 to 100 pg/µL) into the mobile phase stream may be used to optimize the APCI.

Probe position

Turn the probe flange adjuster to optimize the signal. Spray should be approximately midway between the corona pin and the sample cone.

Corona current

Corona current can have a significant effect on sensitivity. The corona current required depends upon the polarity of the compound and the polarity of the analytical mobile phase. Optimization should be performed in the presence of the analytical mobile phase.

For polar compounds analyzed in a polar mobile phase, the signal may be improved by reducing the corona current below 2 µA.

For compounds of low polarity analyzed in a low polarity mobile phase, the signal may be improved by increasing the corona current above 2 µA.

To find the optimum corona current value:

1. Set Corona Current to 2 µA.
2. Increase Corona Current value in 2 µA steps until the optimum value is found. Allow the current to stabilize before taking a reading.
3. If the signal continuously decreases, return Corona Current to 2 µA, then reduce the value in 0.5 µA steps until the optimum value is found.

Using Corona Current values greater than 0 µA will yield the best results for most samples of this type.

Probe temperature

For maximum sensitivity, the APCI probe temperature must be optimized as follows, ensuring that the analytical mobile phase is used during optimization:

> Starting at 650 °C, reduce APCI Probe Temp in 50 °C decrements, allowing time for the temperature to stabilize before taking a reading.

It is possible to set APCI Probe Temp too low for the mobile phase. This often results in significant tailing of chromatographic peaks.

Desolvation gas

Caution: To avoid contaminating source components, always specify a desolvation gas flow rate that exceeds 100 L/h.

In most circumstances the desolvation gas flow has little effect on signal intensity. However, in some situations, it can affect chemical background noise levels. Adjusting desolvation gas can suppress chemical background noise.

Cone gas

Set the cone gas flow to minimize formation of solvent adducts. The typical value is about 50 L/h.

3 Tuning

Contents

Overview

For the highest mass accuracy, the instrument should be tuned and calibrated using a suitable reference compound before sample data are acquired.

- Consult the relevant section of this manual for information concerning source tuning procedures in the chosen mode of operation.
- Adjust the tuning parameters in the Source and Analyser menus to optimize peak shape and intensity at unit mass resolution.
- Care should be taken to optimize the value of the collision energy. Note that, in Daughter and Parent modes, Collision and Exit are interactive parameters.

The tune page

To invoke the tune page, press  on the MassLynx screen MS panel.

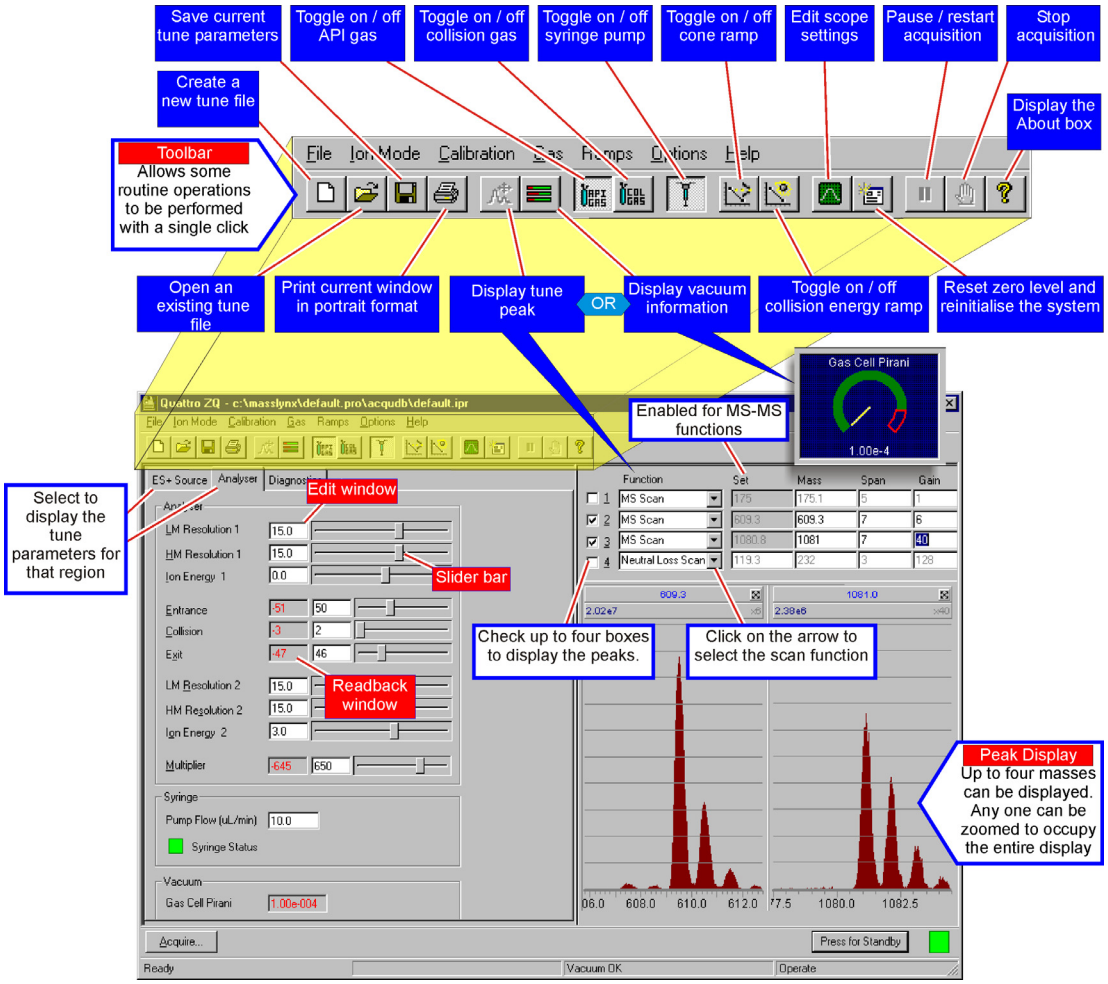
Refer to the figure “Tune page” on page 3-3 for details of the tune page layout.

Printing tune information

To print a report, containing a copy of the tune peak information invoked on the screen along with a record of each parameter setting, press  , or select Print from the tune page File menu.

This report is not configurable by the user.

Tune page



Experimental record

Tuning parameters are stored with every data file as part of the experimental record. The tuning parameters for a particular data file can be viewed or printed from the data browser, see the *MassLynx NT User's Guide* for more information.

Saving and restoring tune parameter settings

Whole sets of instrument tuning parameters can be saved to disk as a named file and then recalled at a future date.

A tune parameter file contains the latest settings for the source controls for all supported ionization modes not just the ionization mode currently selected. Tune parameter files also contain settings for the analyser, inlet set points and peak display.

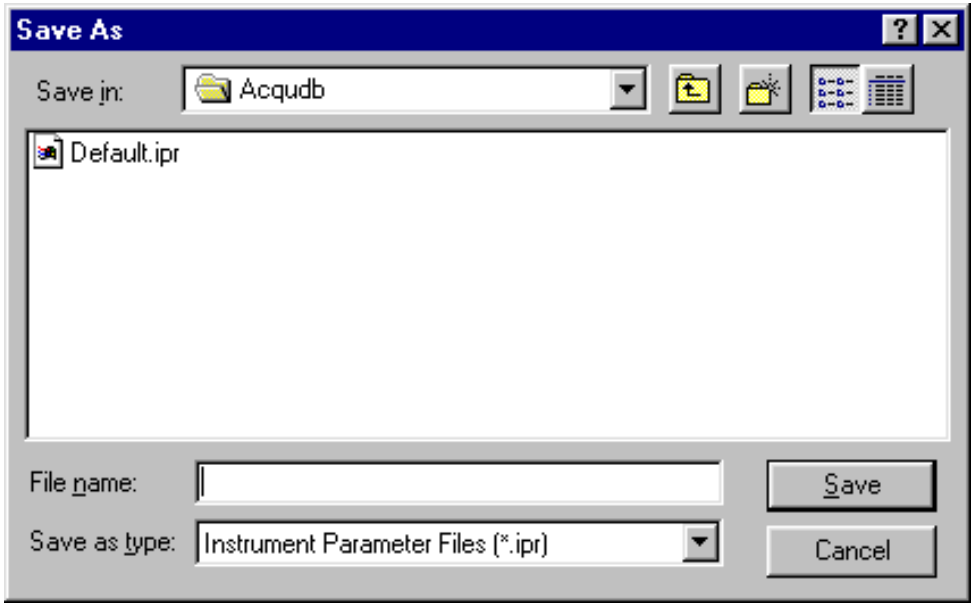
To save the current tune parameters with the existing file name:

1. Press  , or choose Save from the tune page File menu.
2. Press Save.

To save the current tune parameters with a new file name:

1. Select Save As from the tune page File menu; the Save As dialog box is opened.

   #### Save As dialog box



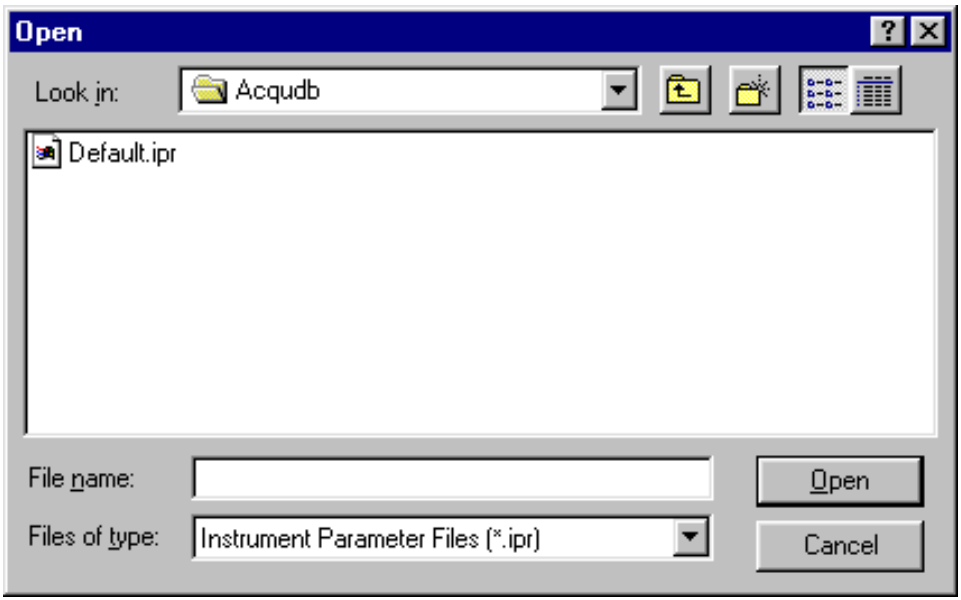
2. Enter a new file name, or select an existing file from the displayed list.

3. Press Save.
4. If the selected file already exists on disk, a warning is displayed. Press Yes to overwrite the existing information or No to enter a different file name.

To restore a saved set of parameters:

1. Press  , or choose Open from the tune page File menu, the Open dialog box is opened.

Open dialog box



2. Select the required tuning parameter file, either by typing its name or by selecting from the list.
3. Press Open.

Modifying the peak display

The tune peak display is modified using either the tune peak controls, or the mouse directly on the display.

To select peaks:

1. Press  , or select Options, Peak Editor.
2. Choose the peaks to be displayed by checking the appropriate boxes.
3. For each active peak select the Mass, Span and Gain.

To change the function:

1. Select the function for the peak from the drop-down list.

   For MS/MS functions, Set is enabled allowing the mass of the parent, daughter, neutral loss or neutral gain ion to be entered.

To change the tune mass:

Either:

1. Click and drag the mouse within the bounds of the axis to draw a "rubber band" around the region of interest.
2. Release the button.

   This range is redisplayed to fill the window. The mass displayed in the Mass box is the mass at the centre of the window.

   This operation can be repeated as often as required.

3. Pressing  once displays the previous magnification range and mass, pressing it a second time returns to the default settings.

Or:

1. Enter a value in the Mass box for the required peak and press Return.

   This becomes the default, so, if the range is altered with the mouse and  is pressed twice, Mass returns to this value.

Or:

1. Position the cursor at the top of the peak window, just below the line showing the gain.
2. When ✢ appears, click the left mouse button and drag until the required mass is displayed in the Mass box and at the top of the window.

This becomes the default, so if the range is altered with the mouse and  is pressed twice Mass returns to this value.

Or:

1. Position the cursor at the top of the peak window, just below the line showing the gain.

2. When ✣ appears, click the left mouse button and drag until the required mass is displayed in the Mass box and at the top of the window.

   This becomes the default, so if the range is altered with the mouse and  is pressed twice Mass returns to this value.

To change the span of a peak:

Either:

1. Press the left mouse button at one end of the region of interest and, without releasing the button, drag the mouse horizontally to the other end.

   As the mouse is dragged a "rubber band" stretches out to indicate the selected range.

   Do not go beyond the bounds of the axis.

2. Release the mouse button to re-display the selected range filling the current window.

   This operation can be repeated as often as required.

   Pressing  once displays the previous magnification range, pressing it a second time returns to the default settings.

Or:

1. Enter a value in the Span box for the required peak and press Return.

   This becomes the default, so if the range is altered with the mouse and  is pressed twice Span returns to this value.

To change the gain of a peak:

Either:

1. Double-click on the line above the peak which shows the gain, to double the gain applied to that peak.
2. Double-click below the peak to halve the gain.

Or:

1. Press the left mouse button at one end of the region of interest and, without releasing the button, drag the mouse vertically to the other end.

   As the mouse is dragged, a marquee indicates the selected range.

   Do not go beyond the bounds of the axis.
2. Release the mouse button to re-display the selected range filling the current window.

Or:

1. Enter a value in the Gain box for the required peak and press Return.

Changing the display

To change the display using the mouse:

- Click in the peak display area with the right mouse button to invoke the Peak Display pop-up menu.

Peak display pop-up menu

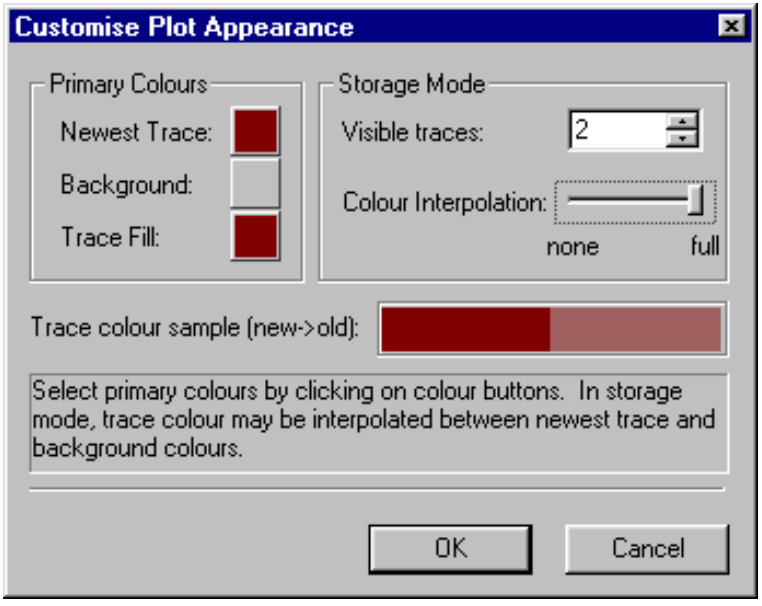
The display area for each peak can be individually changed, e.g. the peak color for peak 1 can be red, for peak 2 green, etc.

Customize plot appearance

To change the color of the background and traces and to change the number of traces displayed:

- Select Customise, Plot Appearance from the Peak Display pop-up menu, see the figure “Peak display pop-up menu” on page 3-8.
- The Customise Plot Appearance dialog box is invoked, see figure below.

Customise Plot Appearance dialog box



To change the colors on the display:

- Press Newest Trace, Background, or Trace Fill, and select a new color from the invoked dialog box.

To change the number of traces:

- Use  to change the number, or enter a new value in the Visible Traces box, within the range 2 to 20.
- If more than one trace is displayed, the older traces can be displayed in a different shade to the new ones:

Drag the Colour Interpolation slider toward the full position. The color of the old traces is shown in the Trace colour sample (new->old) field.

Trace

From the Peak Display pop-up menu, either:

Select the Trace, Outline option to display the peak outline only.

Or:

Select the Trace, Fill option to fill the trace with the trace fill colour.

Or:

Select the Trace, Min/Max option to show the minimum and maximum data points only.

The option selected has a tick next to it.

Intensity

To select intensity:

1. From the Peak Display pop-up menu, select either Intensity, Relative Intensity, or Intensity, Absolute Intensity as required.
2. Select Intensity, Normalise Data to display normalized data.

   The options selected each have a tick next to them.

Grid

The Peak Display pop-up menu, options allow vertical and horizontal grid lines to be independently displayed or hidden.

Selected options have ticks next to them. Selecting an option a second time deselects the option.

AutoTune

MassLynx can automatically tune the mass spectrometer in both APcI and ElectroSpray ionization modes. AutoTune ramps the settings for the tuning parameters until they are optimized to give the best intensity, resolution and peak shape.

To run AutoTune:

1. Press  on the tune page to turn on the API gas, and select Operate.
2. Choose AutoTune from the tune page Options menu, the AutoTune dialog box is invoked.

AutoTune dialog box

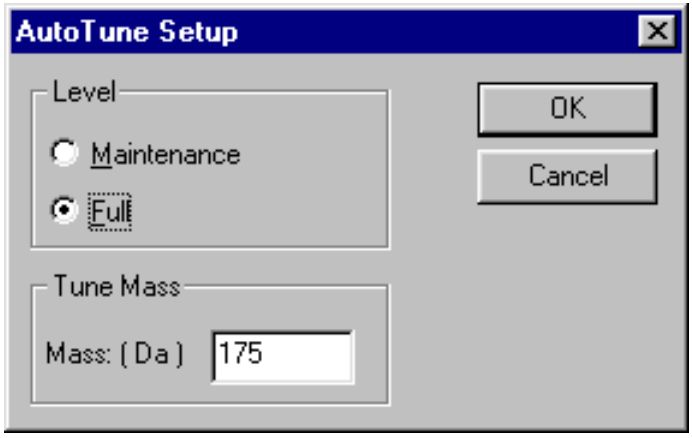
3. Press Setup to define the AutoTune setup parameters, the AutoTune Setup dialog box is invoked.

There are two levels of AutoTune:

- A Full AutoTune starts from a default set of tuning parameters.
- A Maintenance AutoTune starts from the current tuning parameters set in the tune page and can be quicker than a full AutoTune.

  A Maintenance AutoTune can only be performed if the instrument is already reasonably well tuned. If the current tuning is too poor, AutoTune gives an error and requests a Full AutoTune.

AutoTune Setup dialog box



The Tune Mass parameter sets the mass to be tuned on.

When satisfied with the AutoTune setup parameters:

1. Press OK to exit.
2. Press Start.

   The AUTOTUNE STATUS bar is updated to show the progress of the AutoTune.

The following steps are performed:

- Parameter initialization and instrument checks:
  - Ensuring that essential status indicators read correctly.
  - Checking that values are defined for all the user controllable instrument parameters and that these are passed to the data system.
  - Checking that readbacks for these parameters are within specified tolerances.
- Beam detection
- Focus lens tuning

- Ion energy tuning
- High and low mass resolution tuning
  - The final four of these steps represent the implementation of the ESP/APcI AutoTune algorithm. This involves changing key parameters, one at a time, to maximize the intensity of a reference peak with respect to that parameter. At present, ESP/APcI Autotuning is carried out with respect to a single user-specified reference peak.

When AutoTune has finished, it displays a status window to say that AutoTune has been successfully completed.

Press OK to return to the tune page.

The tuning parameters determined by AutoTune are saved to the current tune parameter file.

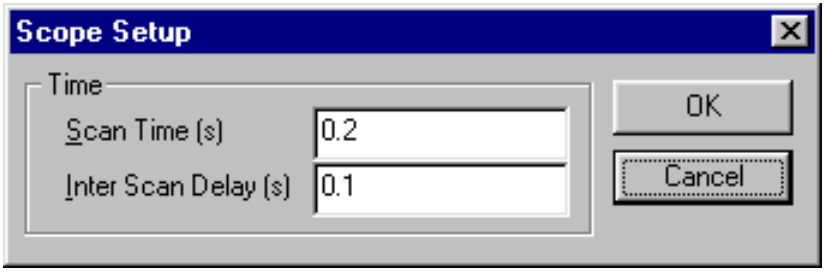
Ion mode

Select the required ionization mode from the Tune Page Ion Mode menu. The selected mode has a tick next to it.

Scope parameters

The Scope Setup dialog box is invoked by pressing  , or selecting the Tune Page Options, Scope Parameters menu.

Scope Setup dialog box



Scan Time(s) and Inter Scan Delay (s) control the speed with which the tune peak display is updated.

Tuning is more responsive when these parameters are low in value.

To change the scope parameters:

1. Press  , or choose Scope Parameters from the tune page Options menu.
2. Make any required changes to the settings.
3. Press OK.

Gas controls

To turn a gas on or off:

Press  (for nebulizer, desolvation and cone gas), or  (for collision gas), or choose the required gas from the tune page Gas menu.

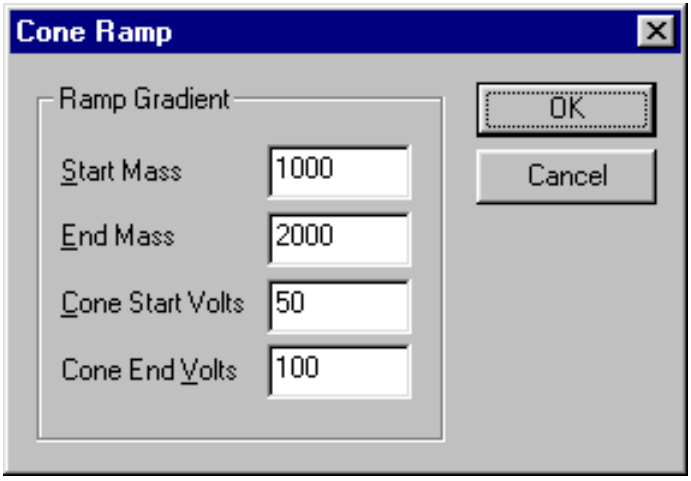
If the gas was previously turned off it is now turned on. A tick mark appears next to a gas if it is turned on.

Ramp controls

To set up a cone voltage ramp:

1. Choose Cone Ramp Gradient from the tune page Ramps menu, the Cone Ramp dialog box is invoked.

   #### Cone Ramp dialog box

   Two values of cone voltage are defined at two particular masses. These values define a gradient for the cone voltage which is then extrapolated to cover the full mass range.

2. Make any changes required and press OK to exit.

To initiate the cone voltage ramp:

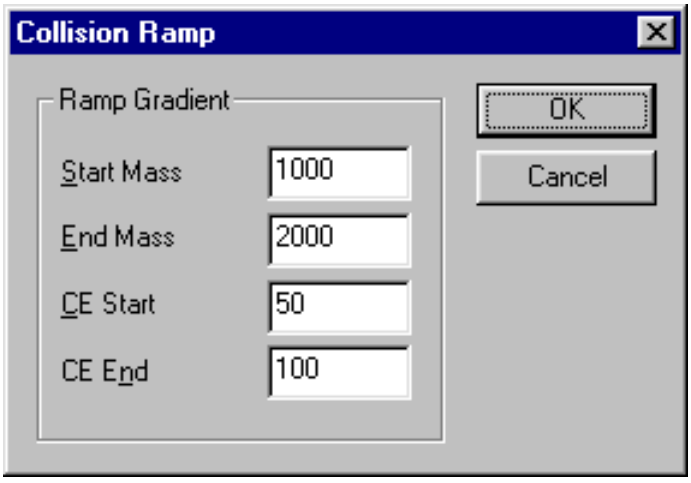
1. Press  , or choose Use Cone Ramp from the tune page Ramps menu.

   A tick mark appears next to the menu item if the cone voltage ramp is selected.

To set up a collision energy ramp:

1. Choose Collision Energy Ramp Gradient from the tune page Ramps menu, the Collision Ramp dialog box is invoked.

Collision Ramp dialog box



Two values of collision energy are defined at two particular masses. These values define a gradient for the collision energy voltage which is then extrapolated to cover the full mass range.

2. Make any changes required and press OK to exit.

To initiate the collision energy voltage ramp:

1. Press  , or choose Use Collision Energy Ramp from the tune page Ramps menu.

Resetting the zero level

The zero level (or baseline) can be repositioned by pressing  , or by choosing Reinitialize from the tune page Options menu.

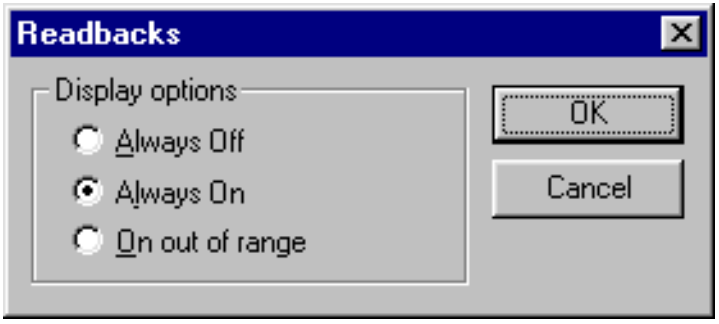
This command causes the instrument control system to measure the position of the noise signal so that any baseline offset caused by the electronics or instrumentation can be compensated for.

It is advisable to reset the zero level whenever the multiplier voltage is changed.

Controlling readbacks

The Readbacks dialog box is invoked by selecting the Tune Page Options, Readbacks menu.

Readbacks dialog box



There are three options for displaying system readbacks on the tune page:

- Readbacks displayed continuously.
- Readbacks hidden.
- Readbacks displayed only when differing from their defined values by more than 10%.

A number of the readbacks are for diagnostic purposes only, their function being to confirm a voltage is present. The acceptable variation between the set value and the readback value varies depending on the particular tune parameter. If concerned about any reading, contact the local service office for advice.

To change readback style:

1. Choose Readbacks from the tune page Options menu.
2. Select the readback style required.
3. Press OK.

Changing tune parameter settings

Most parameters can be modified in the following ways:

- Drag the slider bar using the mouse.
- Click on the slider bar and use the left and right arrow keys, to change the value by one increment.

  The edit window updates as the slider bar is activated.
- Type a new value into the edit window.

Other parameters have only an edit window, and are changed by direct typing.

The speed with which the system responds to changes depends on the speed with which the peak display refreshes. For the fastest response, set the Scope Setup dialog box Scan Time (s) and Inter Scan Delay (s) values to be as short as possible.

Source voltages

The following table lists the various components of Quattro micro API's ion optical system. The name in the table's first column is the name used throughout this manual to describe the component. When appropriate, the second column shows the term used in the current MassLynx NT release.

Source voltages

	Tune page name	ESI+ve	ESI-ve	APcI+ve	APcI-ve
ElectroSpray Probe	Capillary	+3.0 (kV)	-3.0 (kV)	Not applicable	
APcI Discharge Pin	Corona	Not applicable		2 µA	2 µA

Source voltages (Continued)

	Tune page name	ESI+ve	ESI-ve	APcI+ve	APcI-ve
Sample Cone	Cone	+50 (V)	-50 (V)	+50 (V)	-50 (V)
Extraction Cone	Extractor	+3 (V)	-3 (V)	+3 (V)	-3 (V)
Hexapole	RF Lens	+0.2 (V)	-0.2 (V)	+0.2 (V)	-0.2 (V)

The voltages shown are typical for an instrument in good condition. The polarities given are those actually applied to the electrodes. Only positive values need be entered via the tune page.

4 Data Acquisition

Contents

Starting an acquisition

There are two ways of starting an acquisition:

- A single sample acquisition from the tune page
- A multiple sample acquisition from the MassLynx top level screen

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.
- Most of the scanning parameters can be controlled.
- Inlet programs cannot be used.
- Analog data cannot be acquired.
- Multiple sample sequences cannot be acquired.

To start a single sample acquisition:

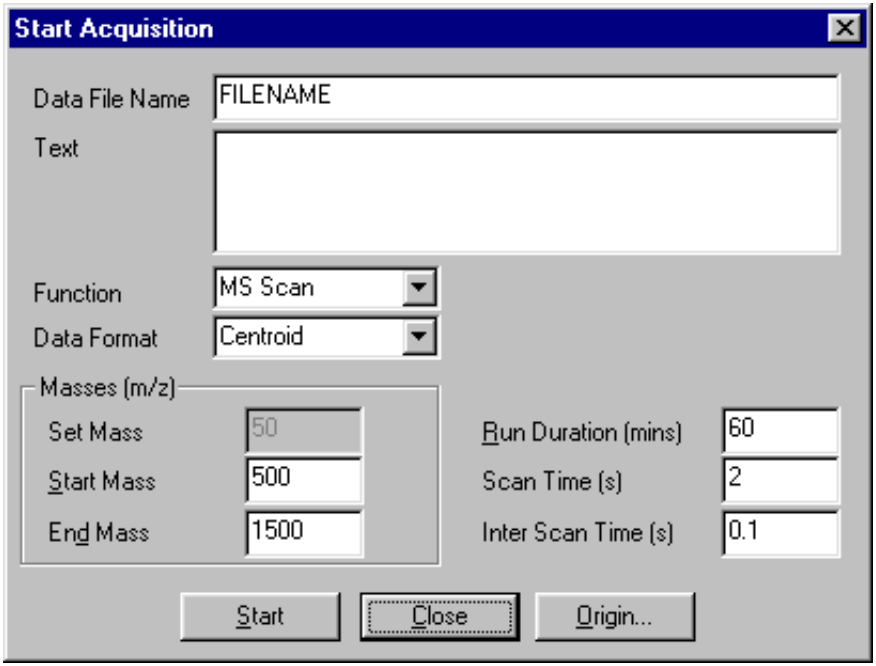
1. Press Acquire on the tune page, or choose Acquire from the tune page Window menu, the Start Acquisition dialog box will be invoked.

   This will require changes to the settings to accommodate the required mass range and scan times.

2. Press Start.

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.

Start Acquisition dialog box



To change the directory into which data are acquired:

1. Cancel the acquisition.
2. Create a new project by choosing Project Wizard, 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 type of acquisition Function used to collect the data can be any of the following:

- MS
- MS2
- Daughter
- Parent

- Neutral Loss
- Neutral Gain

More information is given on page 4-16.

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

- Centroid
- Continuum
- MCA

More information is given on these data formats later on in this chapter.

Set Mass specifies the mass (Daughter Mass, Parent Mass, etc.) that is used for the particular function type. This control is disabled if the function selected does not require a set mass.

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

Run 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.

Pressing Origin allows additional information about the sample to be analyzed to be entered into the following fields:

- Submitter
- Job
- Task
- Conditions

Multiple samples

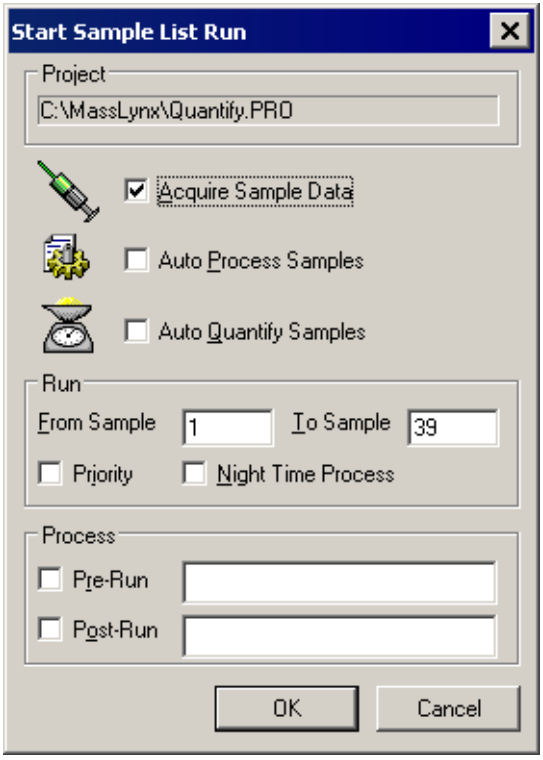
The MassLynx default page contains a sample list editor for defining multiple samples which may be used together to perform a quantitative analysis. The list of samples is set up using a spreadsheet style editor, which can be tailored to suit different requirements.

To start a multi-sample acquisition:

1. Set up a sample list (see the *MassLynx NT User's Guide* for details).
2. Choose Start from the top level Run menu, or press  .

   This invokes the Start Sample List Run dialog box.

Start Sample List Run dialog box



3. Check the Acquire Sample Data, Auto Process Samples and Auto Quantify Samples boxes as required.
4. Enter values in the Run, From Sample and To Sample boxes.

   The default is all samples in the list.
5. Check the Priority and/or Night Time Process boxes as required. See the *MassLynx NT User's Guide* for details.
6. Press OK.

7. 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.

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 box.

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

Automated quantification of sample list

To invoke the Quantify Samples dialog box:

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

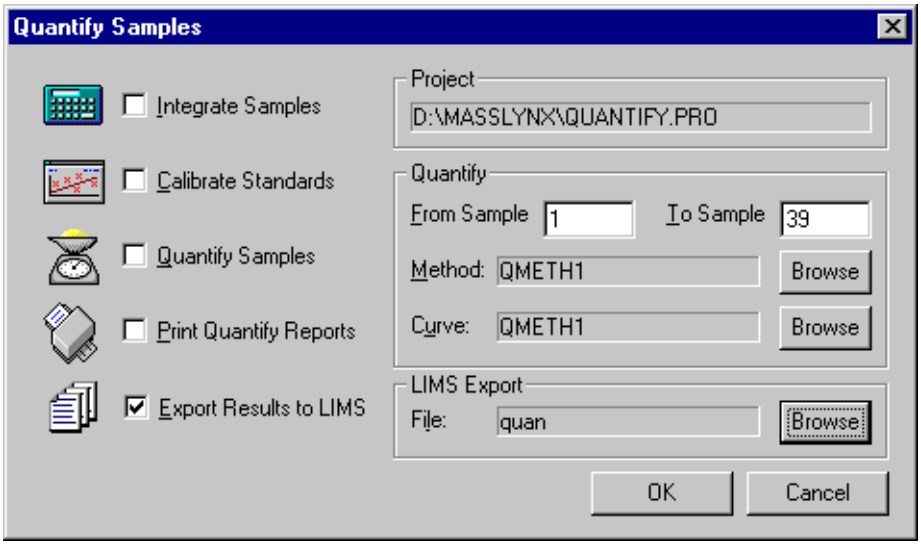
The Quantify Samples dialog box allows automatic processing of data files once they have been acquired. To perform integration, calibration of standards, quantification of samples and printing of quantification reports select the relevant check boxes. See the *MassLynx NT User's Guide* for more detailed 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.

Quantify Samples dialog box



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 file, 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 canceled and a new project created by choosing Project Wizard, or an existing project 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 analyzed.

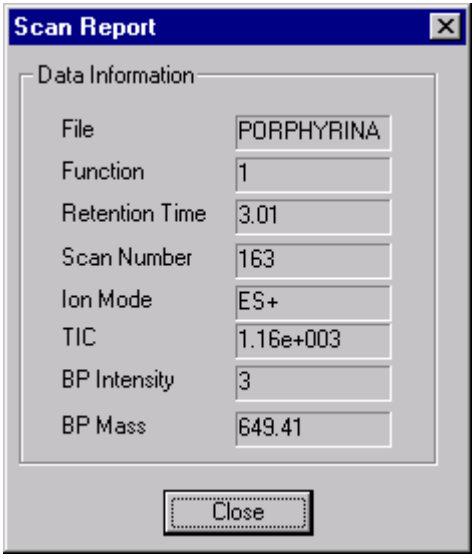
Monitoring an acquisition

Acquisition status is shown on the MassLynx screen. The run time is shown on the MS panel and the scan status, sample number and scan number are shown on the Status bar at the bottom of the page.

The acquisition status (Scan Report) window

The acquisition status window, or Scan Report window, provides a scan by scan statistical report of the progress of an acquisition.

Scan Report window



To invoke the Scan Report window:

1. Select Acquisition Status.

This shows details of the scan currently being acquired.

Chromatogram real-time update

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

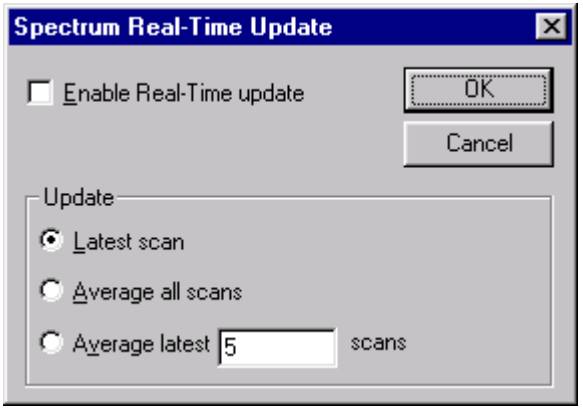
1. Open the data file using the MassLynx data browser.
2. Press , or select Real-Time Update from the Display menu. The chromatogram display is updated as the acquisition proceeds.

Spectrum real-time update

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

1. Open the data file using the MassLynx data browser.
2. Press , or select Real-Time Update from the Display menu, the Spectrum Real-Time Update dialog box is invoked.

Spectrum Real-Time Update dialog box



3. Select Enable Real-Time update.

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

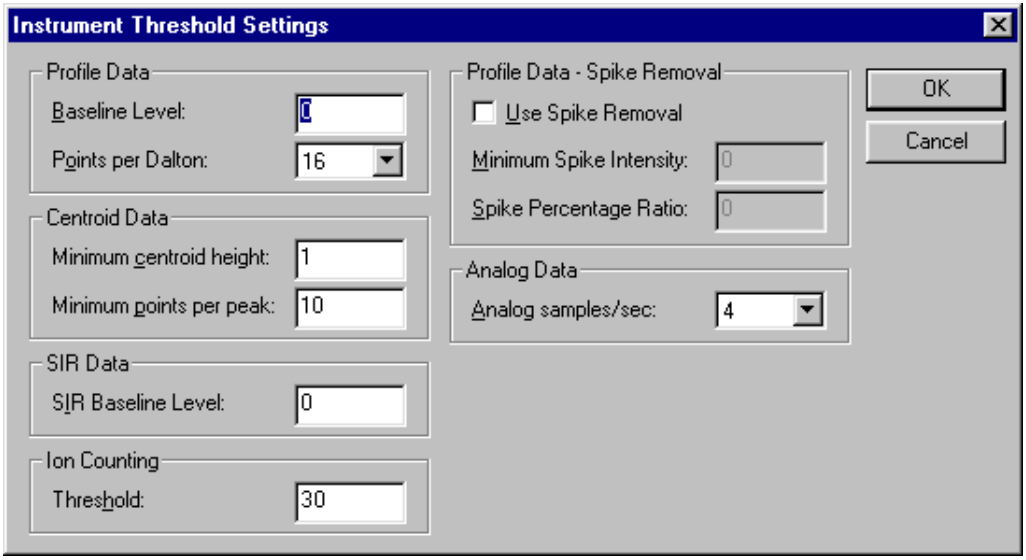
When real-time update is on, the display is continually updated with spectra from the current acquisition. The actual information displayed is determined by selecting one of the following radio buttons:

- 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.

Instrument data thresholds

MassLynx has several parameters that allow control over how the system pre-processes data before it is sent to the host computer. These parameters are contained in the Instrument Threshold Settings dialog box.

Instrument Threshold Settings dialog box



Instrument data thresholding allows the user to specify the type of data to acquire and write to disk, and the type of data to discard and not write to disk. Limiting the amount of data stored on disk can be particularly desirable when acquiring continuum data and doing long LC runs.

To change data thresholding:

1. Choose Set Instrument Threshold from the tune page Options menu.
2. Make the required changes to the information.
3. Press OK.

These new parameters are downloaded at the start of the next acquisition scan.

MaxEnt

The MaxEnt algorithm needs to measure noise accurately within a data file. For this reason Ion Counting Threshold should be set to zero when acquiring data to be analyzed using MaxEnt.

Profile data

The controls for profile data allow control of the amount of data collected during a continuum data acquisition.

Baseline Level is used to lift or drop the baseline to see more or less of the noise by positioning of the baseline above zero. Baseline Level is typically set to a value of 0.

It is possible to use a negative baseline. This reduces the noise seen and acts as a form of thresholding to be applied to 1/16 amu type samples. This takes place after ion counting and therefore has a less significant effect than Ion Counting Threshold.

To see more noise use a positive value.

Points per Dalton can have one of three values: 4, 8 or 16.

- Selecting 8 points instead of 16 results in data files approximately half as big.
- Acquiring data at 16 points per Dalton gives the greatest possible resolution.
- Acquiring data at 4 points per Dalton gives data with a smoothed appearance.

Centroid data

Minimum centroid height sets a height below which detected peaks are ignored. This reduces the size of acquired data files and is useful when concentrating on larger peaks of interest. A suitable value can be arrived at by inspecting spectral noise levels, and should be evaluated for each individual system

Minimum points per peak is the minimum number of points that a continuum peak must have to be centroided. A typical value is 10.

SIR data

SIR Baseline Level sets the position of the SIR baseline above zero. The baseline level is typically set to 0. Increasing the value causes the baseline to appear higher.

Ion counting threshold

Ion Counting Threshold sets the intensity level below which a data point is ignored. This threshold is applied to all acquisitions, regardless of scanning mode. It is also the most significant of all of the data manipulation variables as it is applied to the raw data first.

When an acquisition is started the instrument performs a “prescan” with the ion beam switched off so that the electronic noise level of the acquisition system and its standard deviation can be measured. Ion Counting Threshold only effects the electronic noise level of the system.

The Ion Counting Threshold level entered is multiplied by 1/10 of the standard deviation of the noise to determine the intensity level to be used, so a value of 10 equates to one standard deviation of the electronic noise level.

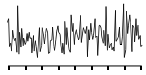
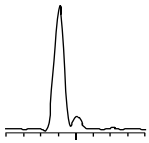
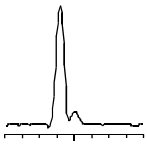
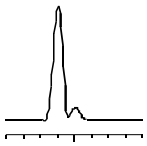
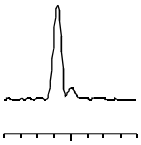
- Values can be set between 0 and 1000, the higher the number the more data is discarded.
- If a value of zero is entered the intensity level is set so that it sits in the middle of the noise which means that roughly half of the noise data is acquired.
- A value of 10 places the threshold just above the noise so almost all of the data is acquired.

- If a value of 200 is entered the threshold sits well above the noise level, so very little noise data is acquired.
- A value of 30 is suitable for most data.

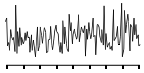
Ion Counting Threshold should be set so that background noise is removed without significantly reducing the intensity of the smallest peaks of interest.

The table below shows the effects of changing baseline noise and ion counting threshold on background noise and low intensity peaks.

Effects of changing baseline noise and ion counting threshold

Baseline level	Ion count threshold	Typical background noise	Typical peak profile	Typical intensity	Typical saving on .DAT file size
0	0			0	0
1	0				0
2	0				0
5	0				
0	10			4%	8%

Effects of changing baseline noise and ion counting threshold (Continued)

Baseline level	Ion count threshold	Typical background noise	Typical peak profile	Typical intensity	Typical saving on .DAT file size
0	20			11%	10%
0	40			37%	61%
0	60			66%	69%
0	250			100%	83%

Profile data - spike removal

Spikes are distinguished from real data by the fact that the peaks are very narrow and, when compared to their immediate neighbors, very intense. Data points determined to be spikes are removed by setting the value of this data point to the average of its immediate neighbors.

Spike removal involves some additional processing while acquiring, and reduces the maximum achievable acquisition rates by approximately 30%.

To perform spike removal during an acquisition:

1. Check Use Spike Removal.
2. Refer to the tune page intensities to assess a suitable value for the intensity threshold below which spikes are ignored. Set Minimum Spike Intensity to this value.
3. A very low intensity signal may include single ion events that can be combined to produce significant peaks. For this type of data Minimum Spike Intensity should be set to a suitable value such that these single ion events are not discarded as spikes.
4. Set a suitable value for Spike Percentage Ratio.

   This ratio is used to determine if a data point is a spike by comparing the data point to its immediate neighbors. For example, with Spike Percentage Ratio set to 33%, a data point is regarded as a spike if its intensity is three times (or more) greater than both its immediate neighbors. A setting of 20% requires an intensity ratio of 5:1 to identify a spike.
5. Press OK to accept any changes.

   Any changes are not downloaded if Cancel is pressed.

Analog data

Select the number of samples to acquire per second from the drop-down list.

System manager

To check the communications between the MassLynx software and the embedded PC:

1. Select Communications Status, the System Manager window is invoked.

System Manager window

Stopping an acquisition

To halt the acquisition:

1. From the tune page, press Stop.
2. From the MassLynx screen, choose Stop from the Run menu, or press  .

   Data acquired up to this point is saved.

The function list editor

Introduction

The Function List 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.

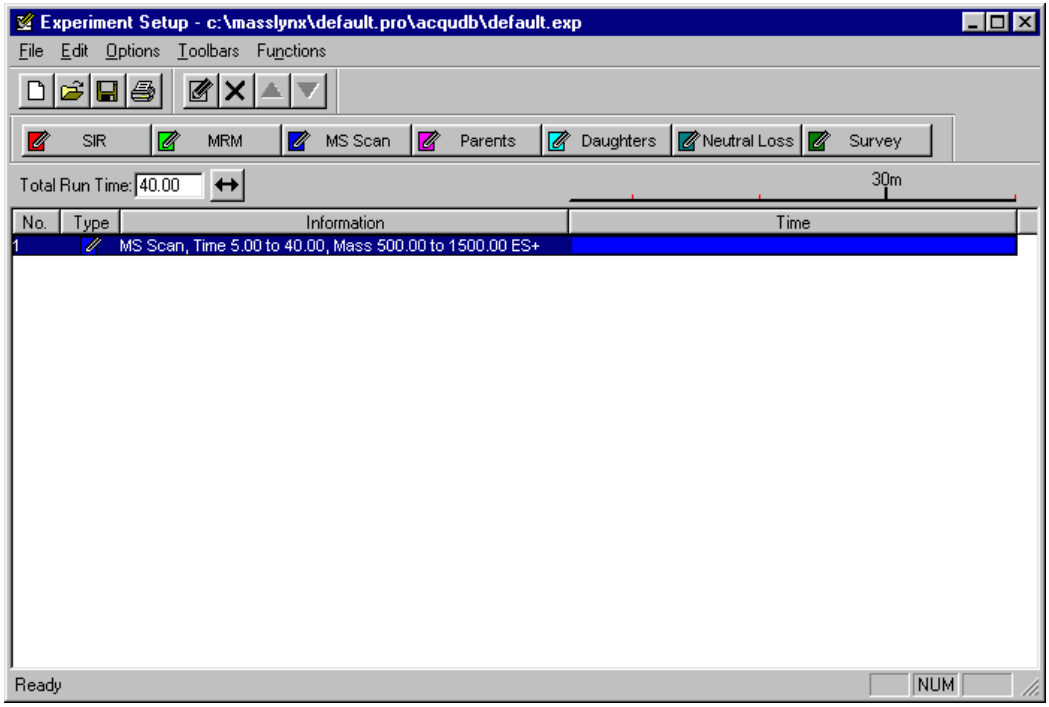
To access the Function List Editor:

1. Press  on the MS panel of the MassLynx screen.

Typical uses for mixed function acquisitions are to acquire different SIR groups over different retention windows.

A function list is produced, saved on disk and then referenced by name when an acquisition is started.

Function List Editor

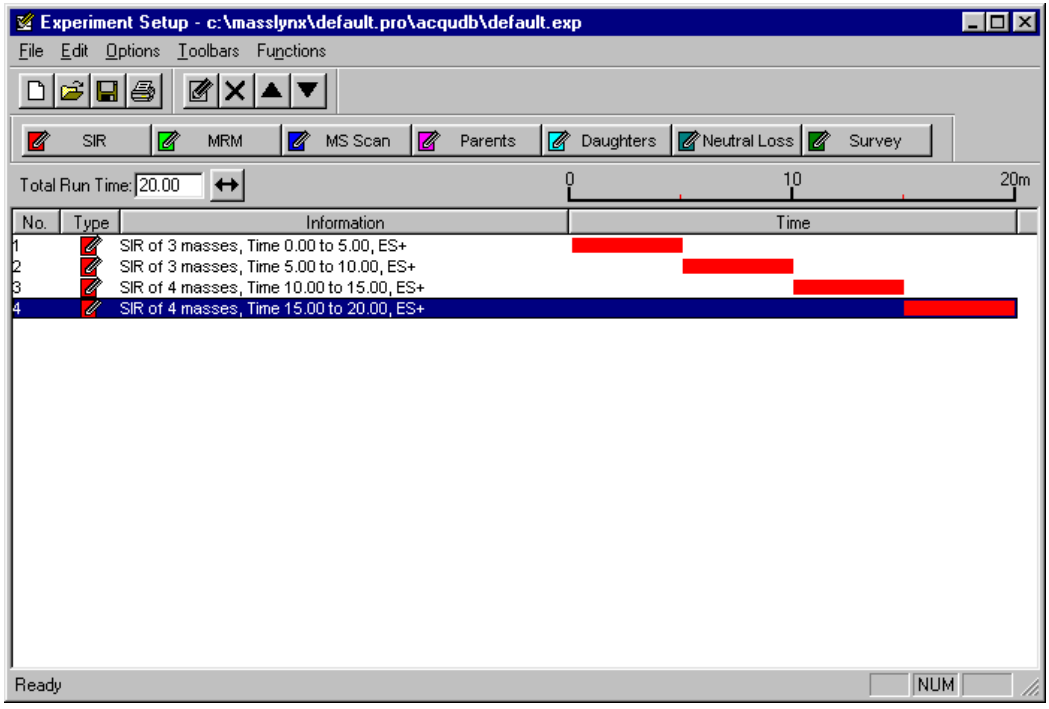


The figure above shows a simple function list containing only one function: a centroided mode full scan, between 500 and 1500 amu using ES+ ionization. Immediately above the function bar display is a time scale that shows from when the function is active, and for how long it runs. In this case, the function starts after 5 minutes and then runs for 35 minutes, terminating after a total elapsed time of 40 minutes.

A more complicated function list, with four SIR functions each running sequentially for 5 minutes, is shown in the figure “Function list with four SIR functions” on page 4-18.

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

Function list with four SIR functions



The Function List 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. The toolbar button functions are shown in the table below.

Function List Editor toolbar buttons

Toolbar button	Purpose
	Create a new function list.
	Open an existing function list.
	Save the current function list to disk.
	Print the current window in portrait format.
	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.
	Create a new SIR function.
	Create a new MRM function.
	Create a new Full Scan function.
	Create a new Parent function.

Function List Editor toolbar buttons (Continued)

Toolbar button	Purpose
	Create a new Daughter function.
	Create a new Neutral Loss function.
	Create a new Survey function.

Adding a new function

To add a new function to the list:

1. Click one of the toolbar buttons, or select the required function from the Function menu.

   The editor for the function type selected is invoked, showing default values.

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

The function editor for each scan type is discussed in detail later on in this chapter.

Modifying an existing function

To modify an existing function:

1. Select the function in the function list.

2. Press  , or double-click on the function.

   This invokes the appropriate editor for the function type and allows changes to be made.

   The function list 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, 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:

1. Select the function in the function list.
2. Select Copy and then Paste from the Edit menu.
3. Modify the parameters as described above.

Removing a function

To remove a function:

1. Select the function in the function list.
2. Press  , choose Delete from the Edit menu, or press Del on the
3. 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.

To change the order of functions with same start and end time:

1. Highlight the required function.
2. Press  or  repeatedly until the function is in the required position.

Setting a solvent delay

To set a solvent delay for a function list:

1. Select Solvent Delay from the Options menu; the Solvent Delay dialog box is invoked.

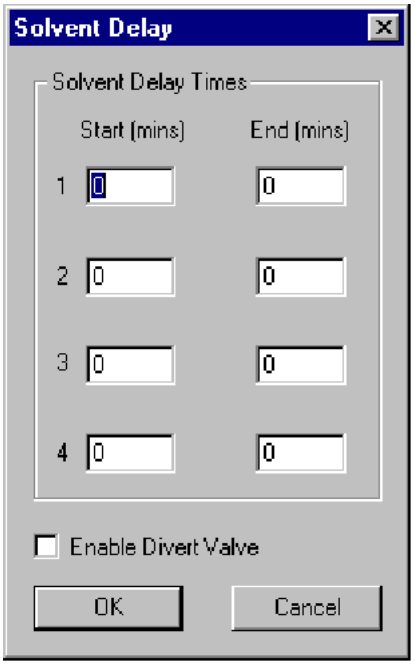
No data is stored during the solvent delay period, which means that solvent peaks that would normally be seen eluting on the TIC chromatogram are no longer seen.

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

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

Up to four solvent delays can be programmed.

Solvent Delay dialog box



Analog channels

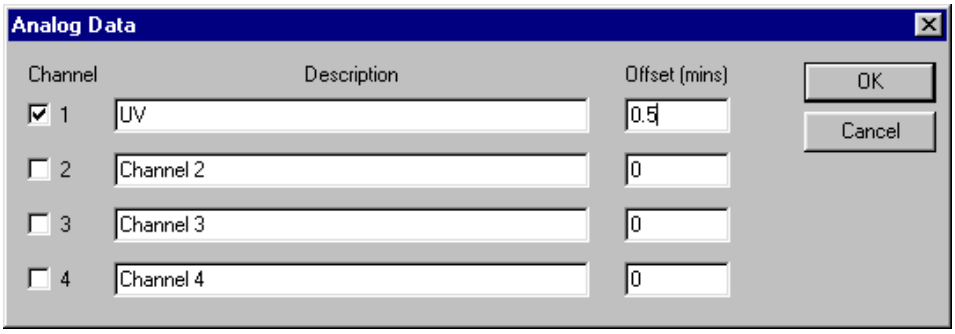
Up to four channels of analog data can be acquired, which are stored with the data acquired from the mass spectrometer. Analog channels are typically used to collect data from external units such as UV detectors, which must be connected to the user input/output PCB as described on page 1-16.

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 the Analog Data dialog box:

1. Select Analog Data from the Options menu on the Scan Functions dialog box.

Analog Data dialog box



To store data for an analog channel:

1. Check the box(es) for the channel required.
2. Enter a textual description for each of the selected analog channels.

   This description is used on the analog chromatogram dialog box as the channel description. See the *MassLynx NT User's Guide*.
3. Enter an Offset to align the external unit with the mass spectrometer.
4. Press OK.

Saving and restoring a function list

To save a function list:

1. Choose Save As from the function list File menu.
2. Enter a new file name, or select an existing file from the list displayed.
3. Press Save.

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

4. 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 function list:

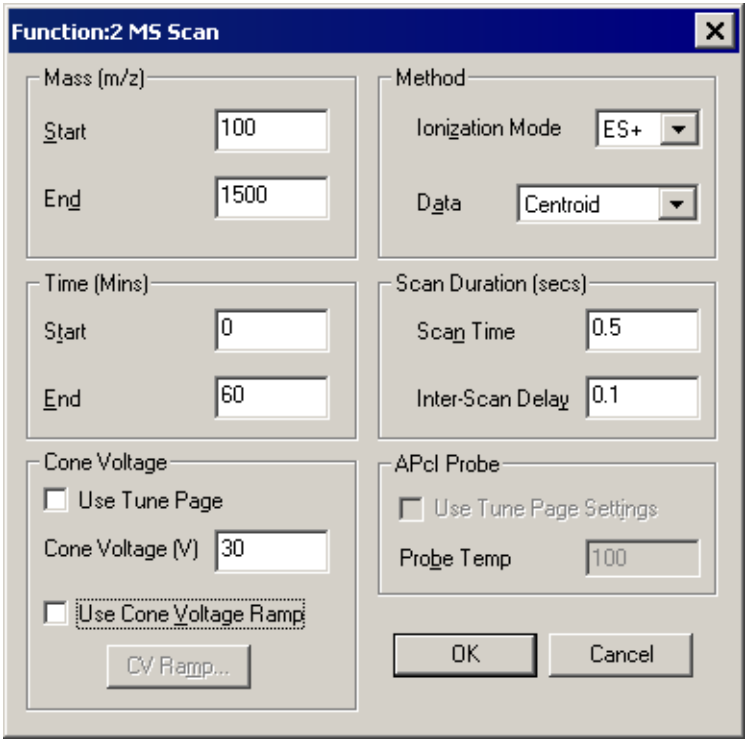
1. Choose Open from the function list File menu.
2. Select the name of the function list to open, either by typing its name or by selecting it from the displayed list.
3. Press Open.

Setting up a full scan function

The full scan function editor is activated by pressing 

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

Full scan function editor



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.

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

Cone voltage

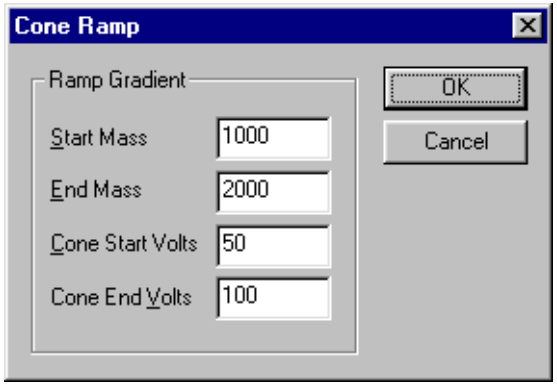
When Use Tune Page is checked, the cone voltage set on the tune page at the start of the acquisition is used.

The cone voltage value cannot be altered during acquisition by typing new values into the tune page, since the new values are not downloaded during acquisition. This can only be done by acquiring from the tune page.

To apply a ramp to the cone voltage:

1. Check Use Cone Voltage Ramp and press CV Ramp to invoke the Cone Ramp dialog box.

Cone Ramp dialog box



The four parameters define a gradient for the cone voltage which is then extrapolated to cover the full mass range of the function.

Method

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

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 data are acquired to disk at all times, even when no peaks are being acquired, data files tend to be significantly larger than centroided ones.

  It is possible, however, to set a threshold below which the data are not stored. Depending on the nature of the data acquired, this can greatly reduce these effects. 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. For more information about setting instrument data thresholds see Instrument Data Thresholds, page.

- MCA (Multi Channel Analysis). 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 random noise does not accumulate as rapidly as real data and therefore effectively averages out over a number of scans. This emphasizes the real data and improves the signal-to-noise ratio.

  A further advantage of MCA is that, because data is written to disk only at the end of an experiment, scanning speeds can be increased and significantly less storage space is required.

  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 duration (secs)

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.

APcI probe

Probe Temp, in degrees centigrade, is enabled when Ionization Mode is set to API.

When Use Tune Page Settings is selected, the APcI probe temperature set on the tune page at the start of the acquisition is used. This control is enabled when the Ionization Mode is set to API.

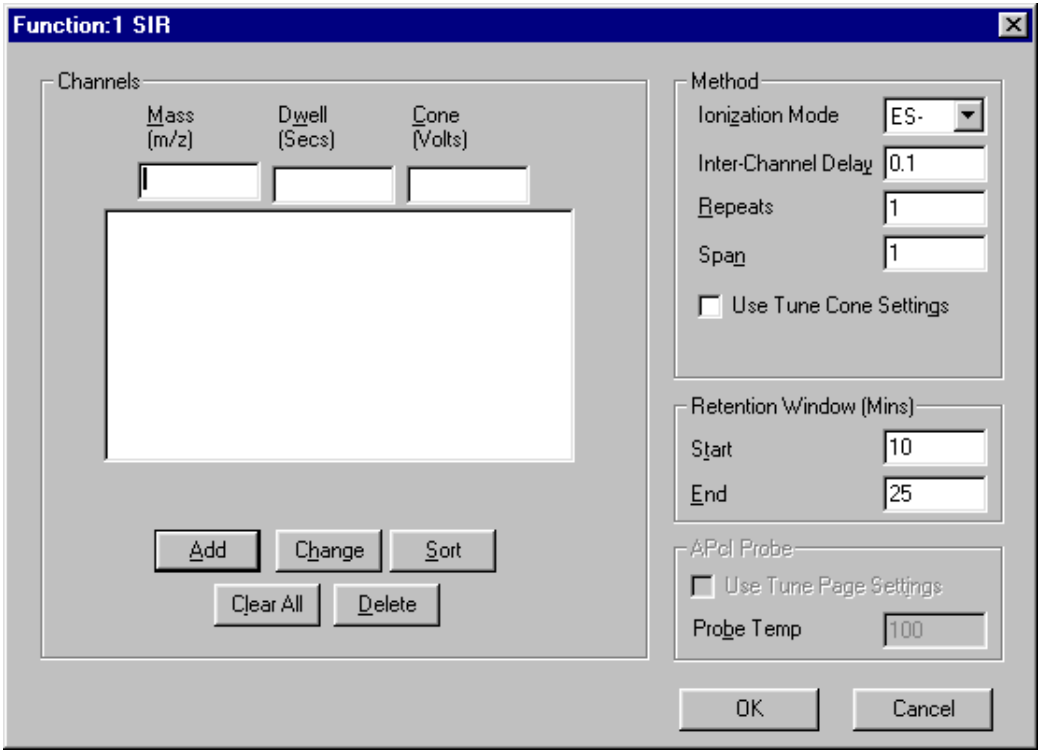
The APcI probe temperature value cannot be altered by typing new values into the tune page during the acquisition, since the new values are not downloaded during the acquisition. This can only be done by acquiring from the tune page.

Setting up a SIR function

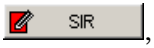
The SIR (Selected Ion Recording) technique is typically used in situations where only a few specific masses are to be monitored. Since most of the data acquisition time is spent on these masses, the technique is far more sensitive than full scanning.

The SIR function editor is used to enter the masses to be monitored, along with their dwell times, spans and inter-channel delay times.

SIR function editor



To set up a SIR function:

1. Press pressing 

, or select SIR from the Functions menu.

Many of the fields are described above for the Full Scan function editor. Only those which differ are described below.

Channels

Up to 32 masses can be monitored.

To enter a mass:

1. Type suitable values into the Mass, Dwell and Cone boxes.
2. Press Add.

Dwell specifies the length of time in seconds for which the highlighted mass is monitored.

To modify existing settings:

1. Double-click on a mass in the list.

   This displays the values for the selected mass in the edit fields.
2. Change Mass, Dwell or Cone as required.
3. Press Change to update the values in the list.

To sort the list in order of ascending mass:

1. Press Sort.

Method

Inter Channel Delay specifies the time, in seconds, between finishing monitoring the highlighted mass and starting monitoring the next mass in the function.

Repeats is only relevant for experiments having more than one function and specifies the number of repeats of the function.

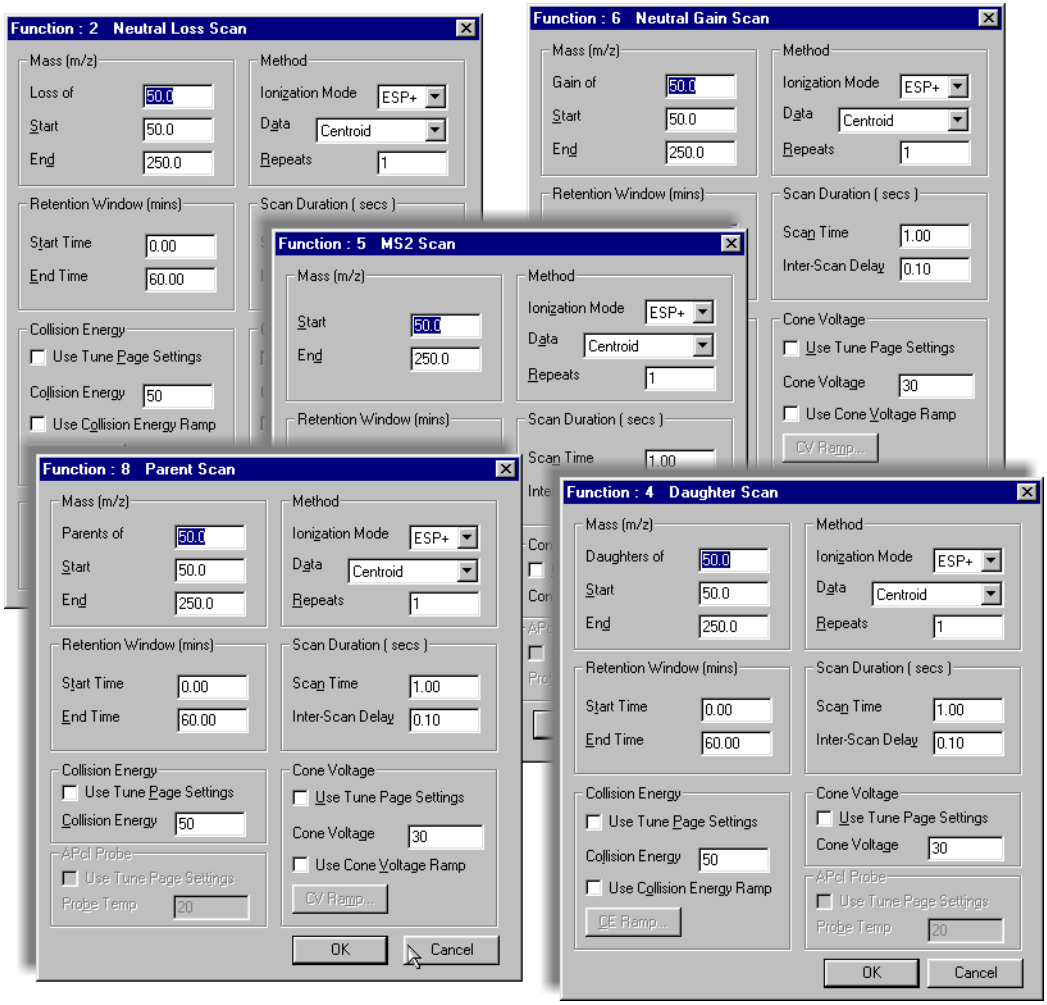
Span specifies a small mass window applied centrally about the highlighted mass. During acquisition this range is scanned over the specified Dwell time. A span of zero can be set to simply “sit on” the specified mass.

Retention window

Start and End together specify the retention time, in minutes, during which this function is active.

Setting up MS/MS scanning functions

MS/MS scanning function editors



Many of the fields in the MS/MS editors are similar to those in the Full Scan function editor. Only fields which differ significantly are described below.

Mass

Daughter

This is the most commonly used MS/MS mode, and is used to look at fragmentations of a particular ion. MS1 is set to the parent mass using Daughters of, and is not scanned.

The resolution of MS1 can be lowered until the peak width at the base is two masses wide without the daughter spectrum containing any ions from the adjacent parent masses.

Start and End specify the mass range to be scanned by MS2.

It is possible to select the daughter mass to be greater than the parent (precursor) mass. In this case, ions which have gained mass in the collision cell, or are of higher mass-to-charge ratio, are detected. This can occur when a multiply-charged ion fragments and loses a charge.

Parent

This mode is used to look for the parent of a particular fragment.

MS2 is set to the mass of the fragment, using Parents of, and is not scanned.

Start and End specify the mass range over which MS1 is scanned. Start is normally set just below Parents of, and End to a value above the highest expected parent mass.

There are often several masses from which a daughter may come, so that any one fragment is derived from a number of different peaks.

MS2

In this mode MS2 is resolving, while MS1 transmits ions over a wide mass range. While this scanning mode can be used for acquiring data it is mostly used in the tune window, for setting and optimizing the acquisition conditions.

Neutral loss

In this mode, the peak in a spectrum that gives the neutral loss specified in Loss of is detected. The precursor mass is scanned in MS1, and MS2 is scanned at this mass less the neutral loss mass. Starting masses are therefore detected on the mass scale of MS1. Start (for MS1) should be greater than Loss of to give MS2 a valid start mass.

Neutral gain

This is an infrequently used mode, since the mass selected by MS2 is seldom higher than that of MS1. It is applicable to studies where a precursor ion

gains mass by ion molecule reaction, or where multiply-charged ions fragment into particles with a higher m/z value.

Collision energy

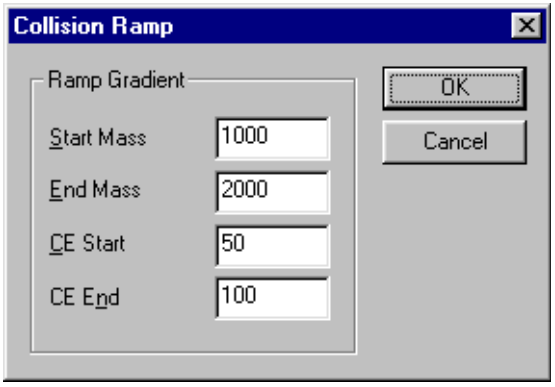
This specifies the collision energy, in electron volts, to be used for the collision cell during the scan.

When Use Tune Page Settings is selected, the collision energy set on the tune page is used. If it is required to adjust the setting during an acquisition then the acquisition must be started from the tune page.

To apply a ramp to the collision energy:

1. Check Use Collision Energy Ramp.
2. Press CE Ramp to invoke the Collision Ramp dialog box.

Collision Ramp dialog box



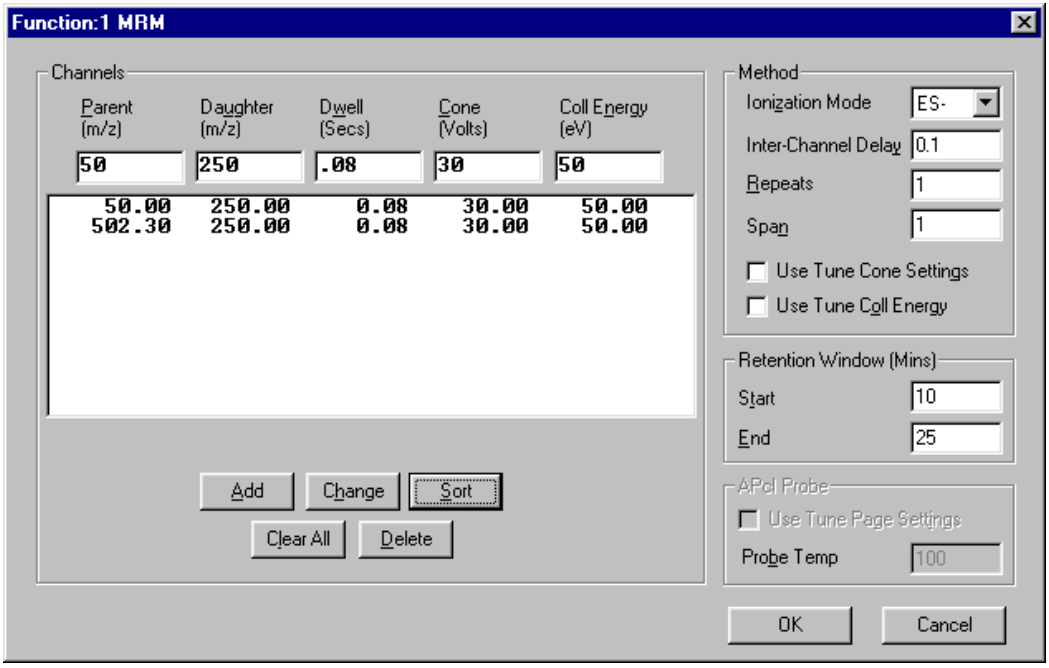
The four parameters define values of collision energy for two particular masses. This collision energy gradient is then extrapolated to cover the full mass range of the function.

Setting up a MRM function

Multiple reaction monitoring (MRM) functions are set up in much the same way as SIR functions, but allow a number of MS/MS transitions (fragmentations) between MS1 and MS2 to be monitored.

All fields in the MRM function editor are similar to those already described.

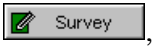
MRM function editor



Setting up a survey function

Survey scans are used to search for precursor ions.

To access the dialog box:

1. Press pressing 

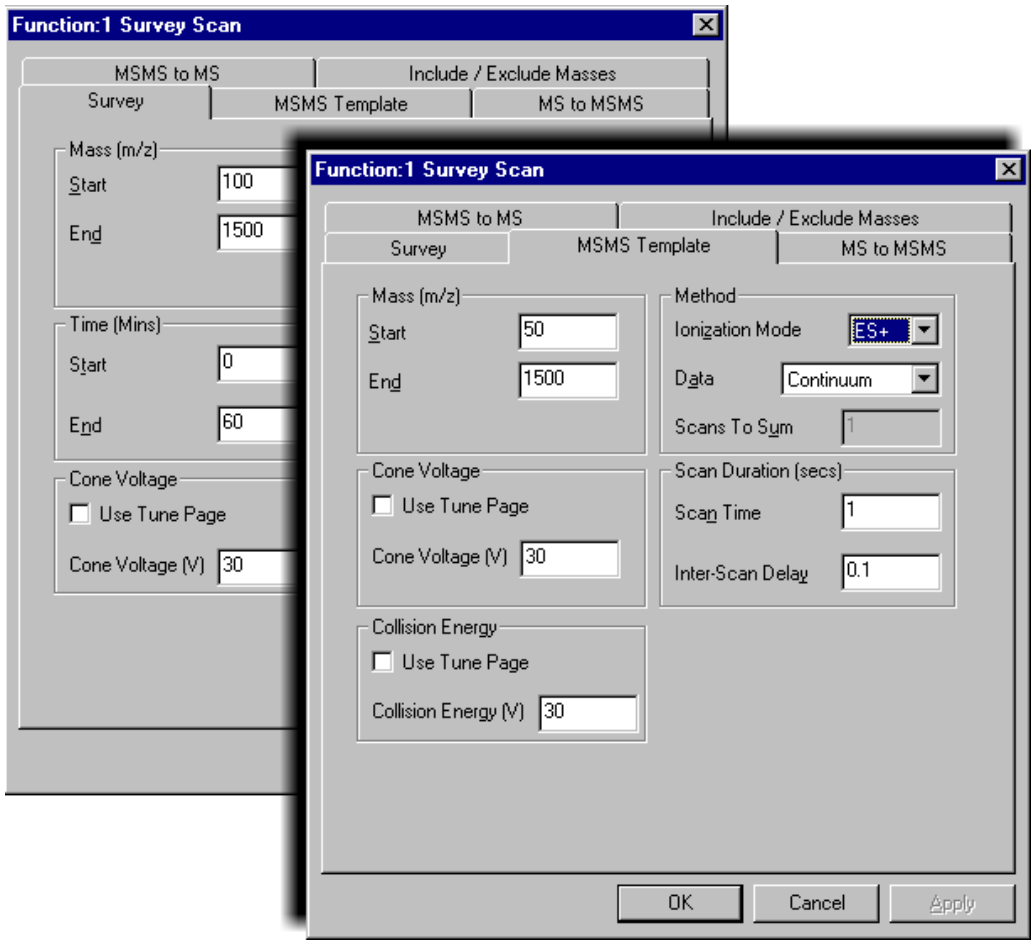
, or select Survey Scan from the Functions menu in the scan functions editor.

The function list editor does not add survey functions to the list if non-survey functions are present.

Survey and MSMS template pages

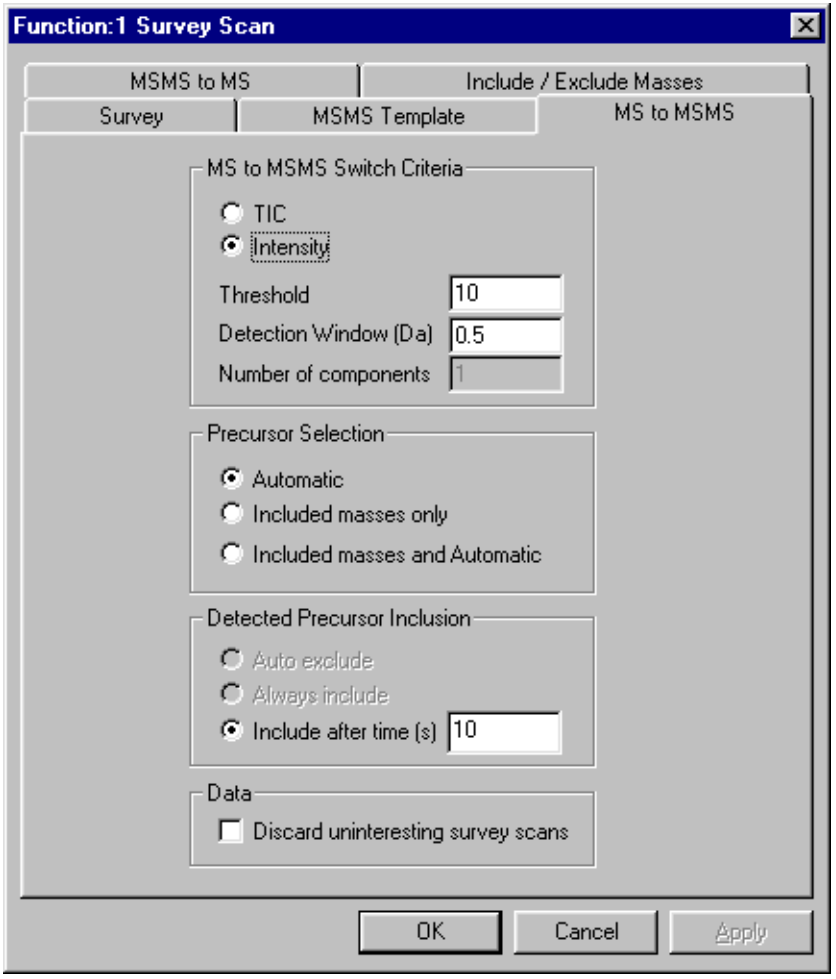
The Survey and MSMS Template pages allow the parameters to be set for MS and MS/MS scanning during the survey, and are similar to normal function editor pages.

Survey and MSMS Template Pages



MS to MS/MS switching

MS to MSMS page



Switch criteria

MS/MS scanning commences:

- If TIC is selected, and the TIC of the spectrum rises above the specified Threshold.
- If Intensity is selected, and the intensity of the largest peak rises above the specified Threshold.

When a peak top is found, no other peaks are looked for within the specified Detection Window.

Number of Components is currently set to 1 and cannot be changed. The number of non-coeluting precursors in a single run is not limited.

Precursor selection

If Automatic is selected all valid masses satisfying selection criteria are monitored.

If Included masses only is selected, only masses in the Include List (see below) are monitored.

If Included masses and Automatic is selected, masses on the Include List are given priority. If no precursors are found, other valid masses are monitored.

A mass is valid if it is not on the Exclude List (see below), and it satisfies the precursor selection criteria.

Detected precursor inclusion

Auto exclude and Always include are not currently available.

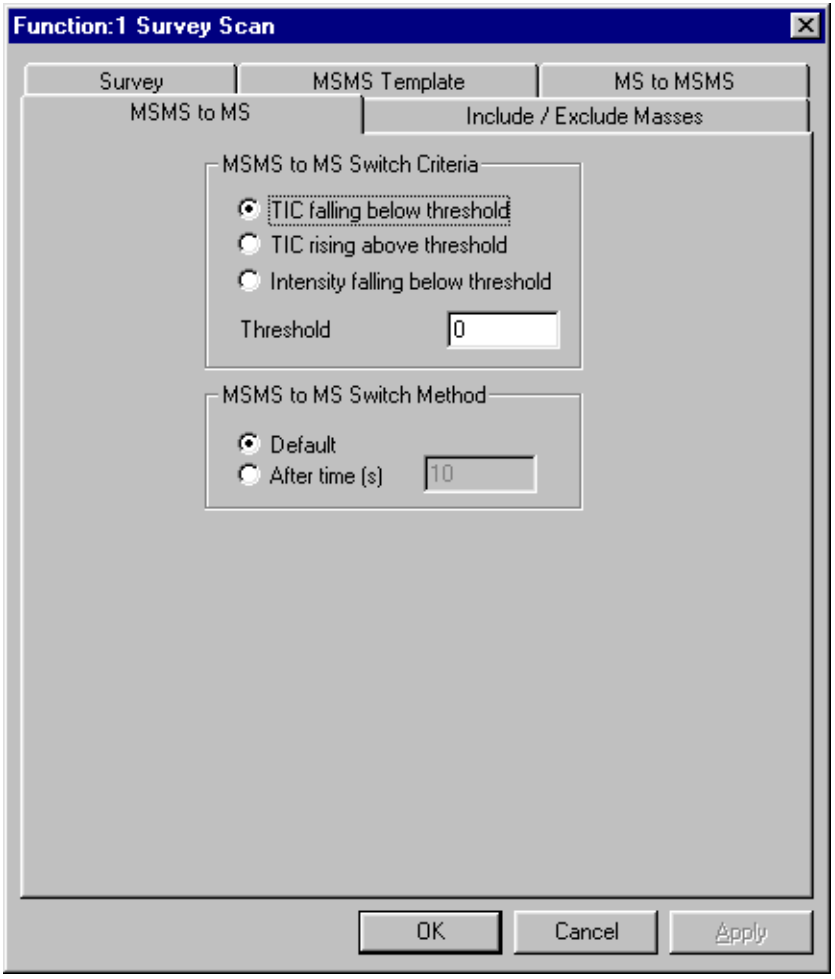
Include after time, if selected, allows a delay to be incorporated before precursors are included.

Data

Discard uninteresting survey scans allows only the survey scans that detect precursor ions to be stored. This saves on disk space, as survey scans that contain no relevant data are rejected.

MS/MS to MS switching

MSMS to MS page



When MS/MS functions have been generated, they are carried out in parallel until the conditions for switching to MS are satisfied.

When all MS/MS functions have stopped, the MS survey function is again carried out.

Switch method

If the MS/MS to MS switch method is Default, the MS/MS function stops when the MS/MS to MS switch criteria are met.

If the MS/MS to MS switch method is After Time, the MS/MS function stops when the MS/MS to MS switch criteria are met, or otherwise, when the specified time has elapsed.

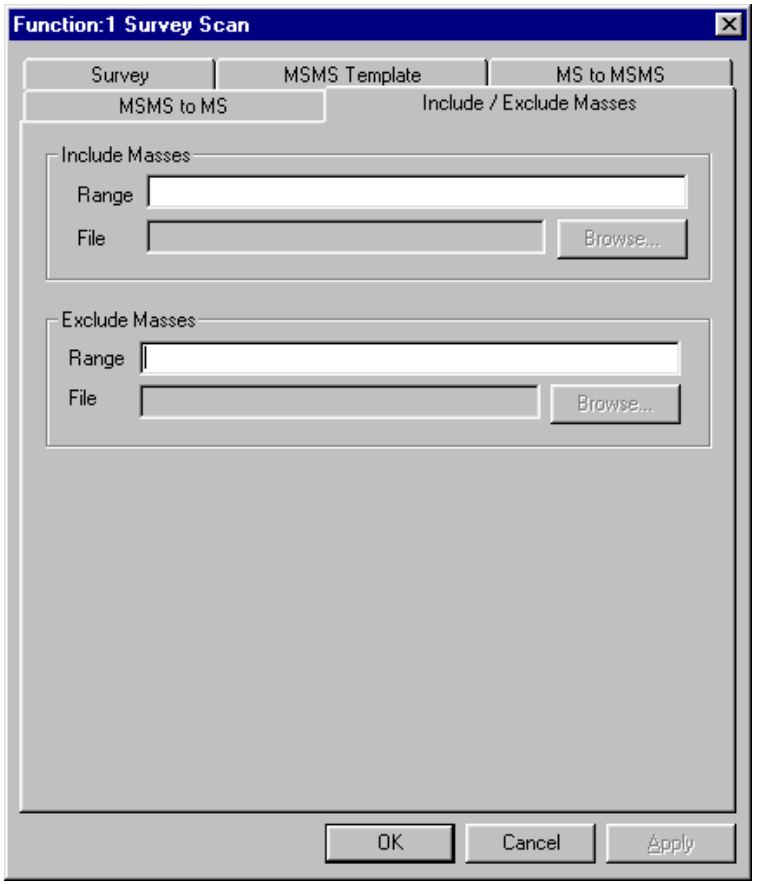
Switch criteria

To define when MS scanning resumes:

1. Select one of the three conditions.
2. Set Threshold to a suitable value.

Including and excluding masses

Include/exclude masses page



Mass ranges and individual masses to be included or excluded from the MS/MS scans are entered in the relevant Range boxes.

Masses on the Exclude list are not considered for detection.

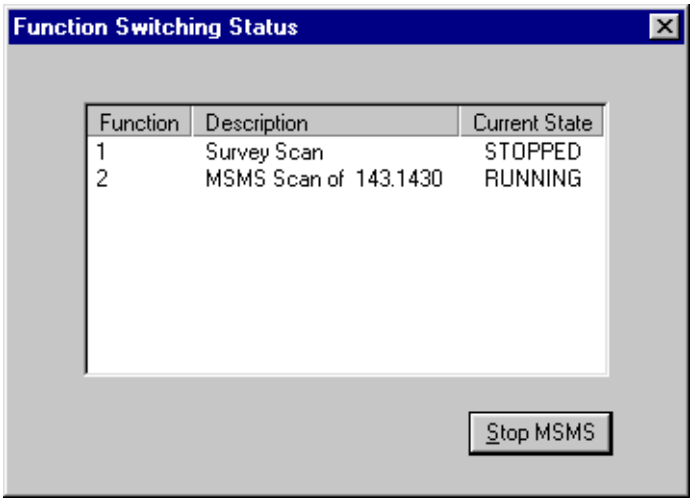
Ranges take the form massX_massY.

Masses and ranges in a list are comma delimited, for example 100_200,202,236,250_300.

Monitoring acquisitions

When an acquisition is started, the Function Switching Status dialog box is invoked, showing the precursors currently running.

Function Switching Status dialog box



5 Mass Calibration

Contents

Introduction

This chapter is divided into three sections:

- A brief general overview of the calibration process.
- A complete mass calibration of Quattro micro API using ElectroSpray ionization with a mixture of sodium iodide and rubidium iodide as the reference compound.
- A complete mass calibration of Quattro micro API using atmospheric pressure chemical ionization (APcI) with PEG as the reference compound.

See Chapter 8, for details of calibration solutions and their preparation.

Overview

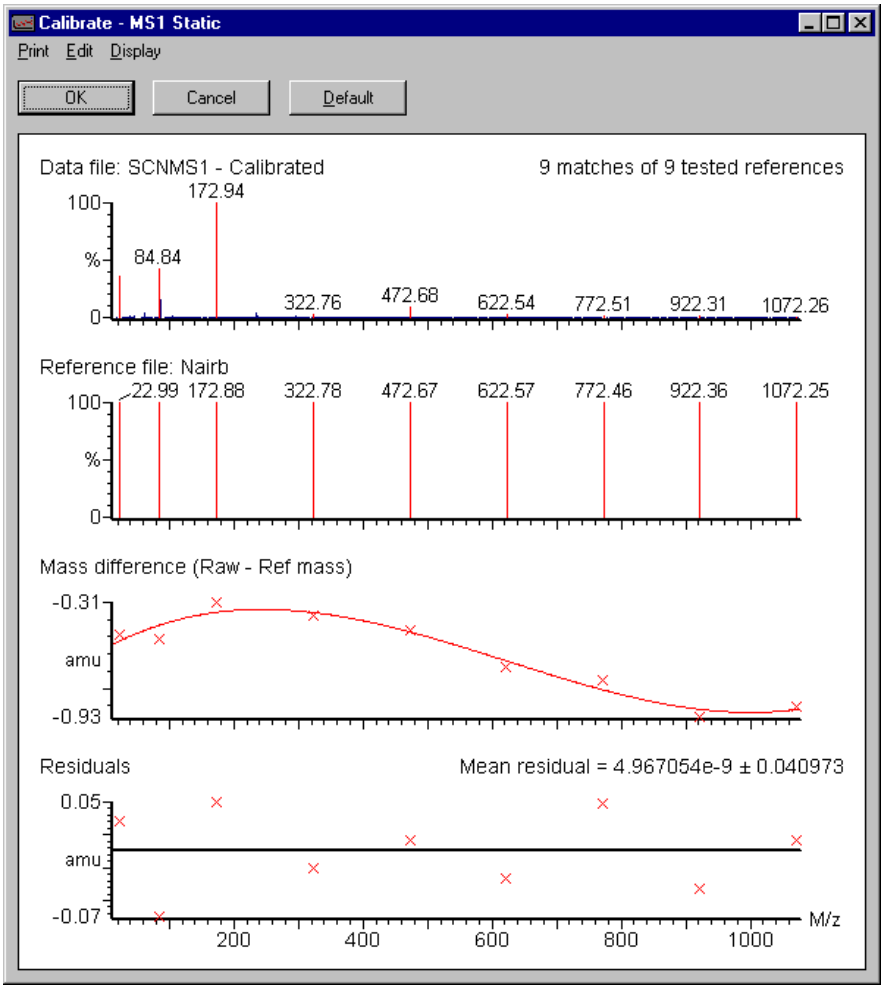
MassLynx NT allows a fully automated mass calibration to be performed, which covers the instrument for static and scanning modes of acquisition over a variety of mass ranges and scanning speeds.

A mass spectrum of a reference compound (a calibration file) is acquired and matched against a table of the expected masses of the peaks in the reference compound which are stored as a reference file. The mass differences between the reference peaks and calibration peaks are the calibration points. A calibration curve is fitted through the calibration points.

The vertical distance of each calibration point from the curve is calculated. This distance represents the remaining (or residual) mass difference after calibration.

The standard deviation of the residuals is also calculated. This number is the best single indication of the accuracy of the calibration.

Mass calibration



Calibration types

Each quadrupole analyser requires up to three calibration curves:

- A static calibration is used to "park" the analyser accurately on a specific mass of interest (for example in tuning, SIR and MRM).
- A scanning calibration enables peaks acquired in a scanning acquisition to be mass measured accurately.
- A scan speed compensation calibration compensates for "lag time" in the system when the instrument is scanned rapidly.

A separate mass spectrum of the reference compound is acquired for each selected calibration type.

Quattro micro API requires these three calibrations for both MS1 and MS2, thereby generating a maximum of six calibration curves. The following table shows which types of calibration are necessary for particular types of experiment.

Calibration requirements

Experiment	Calibration required	
	MS1	MS2
MS	All	-
SIR	Static	-
MS/MS	All	All
MRM	Static	Static

The calibration process

- Tuning the instrument.
- Selecting the appropriate reference file for the reference sample to be used.
- Starting an automatic calibration.
- Checking the calibration report.

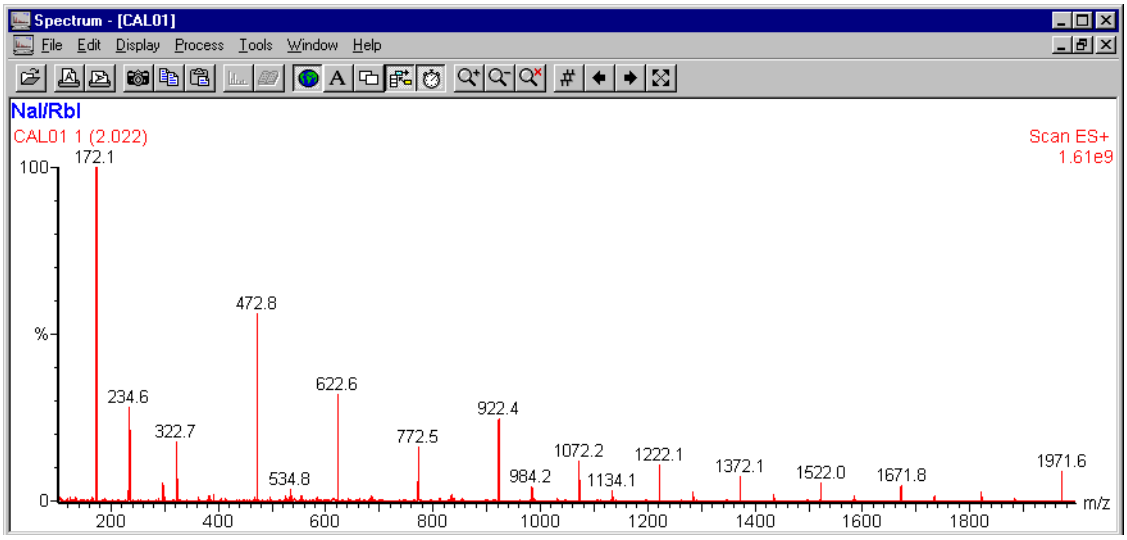
ElectroSpray

Introduction

When a calibration is completed, it is possible to acquire data over any mass range within the calibrated range. It is therefore sensible to calibrate over a wide mass range.

With a mixture of sodium iodide and rubidium iodide, calibration over the instrument's full mass range is achievable.

Rubidium iodide spectrum



Preparing for calibration

Reference compound introduction

The example given here describes an automatic calibration which requires reference compound to be present for several minutes. The introduction of the reference compound is best achieved using the instrument's syringe pump.

To introduce the reference compound:

1. Fill the syringe with the reference solution. See page 2-6.
2. Couple the syringe to the ElectroSpray Probe with fused silica tubing.
3. Set the pump to a flow rate of 10 µL/min.

Tuning

Before beginning calibration, and with reference solution admitted into the source:

1. Set Multiplier to 650 V.
2. Adjust source parameters to optimize peak intensity and shape.
3. Set the resolution and ion energy parameters for unit mass resolution on MS1 and MS2.

For a good peak distribution across the full mass range:

1. Check the intensity of some of the reference peaks above 1000 amu.
2. Check the intensity of the peak at m/z 173.
3. Ensure that no peaks are saturated on the tune page with a Gain of 1. If necessary, reduce Multiplier or dilute the sample.

   A cone voltage in the region of 45 is usually suitable.

Instrument threshold parameters

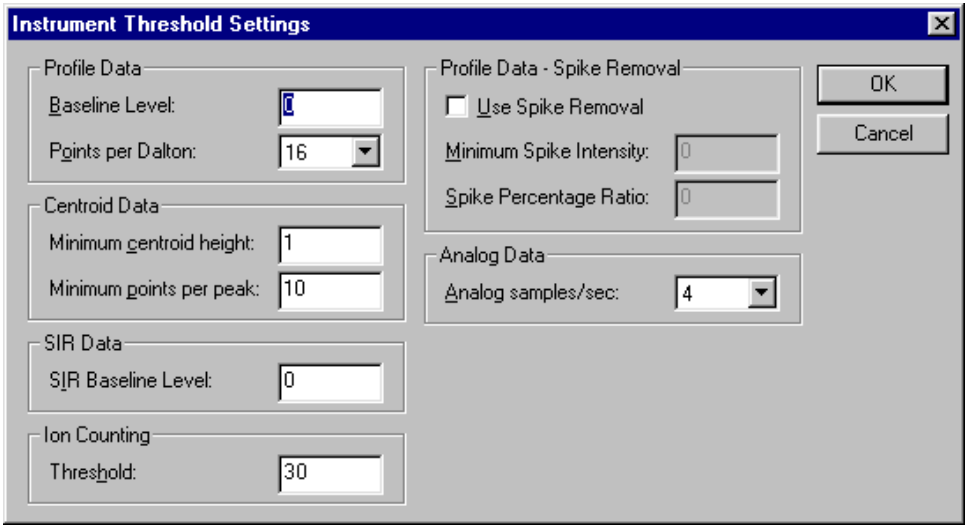
Before beginning the calibration procedure, some instrument parameters must be checked.

For most low mass range calibrations, calibration data is acquired in continuum mode.

To allow suitable scanning speeds to be used the continuum data parameters need to be set correctly:

1. From the instrument tune page, select Options then Set Instrument Threshold to invoke the Instrument Threshold Settings dialog box.

Instrument Threshold Settings dialog box



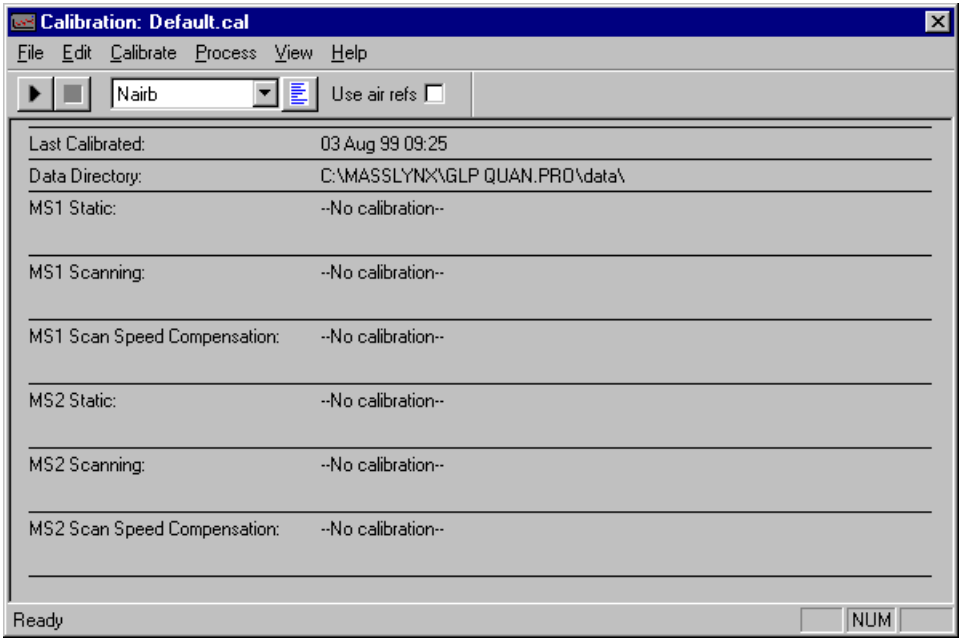
2. In the Profile Data section set Baseline Level to 0 and Points per Dalton to 16.
3. Set Ion Counting Threshold and Spike Removal as appropriate, see page 4-10.
4. Select OK to save the parameters.

Calibration options

To access the Calibration Options dialog box:

1. Select Calibration then Calibrate Instrument on the tune page.

Calibration Options dialog box



Selecting the reference file

To select the appropriate reference file:

1. Click on the arrow in the Reference File box and scroll through the files.
2. Select nairb.ref for a sodium iodide and rubidium iodide reference solution.

Removing current calibrations

To remove the current calibration:

1. Select default.cal from the Calibrate menu option.
2. Save the changes to the default.cal file.
3. Check that there is no prior calibration associated with default.cal.

   This ensures that a file with no calibration is currently active on the instrument and prevents any previously saved calibrations from being modified or overwritten.

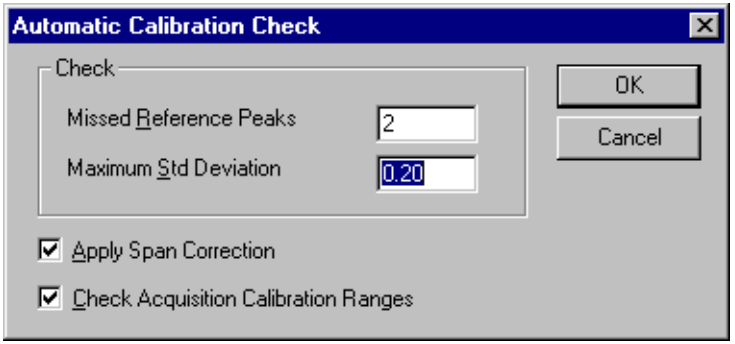
Selecting parameters

Several parameters need to be set before a calibration is started. Default parameters are set when the software is initially loaded which usually give a suitable calibration, but under some conditions these may need to be adjusted.

Automatic calibration check

The Automatic Calibration Check dialog box is accessed from Edit, AutoCal Check Parameters.

Automatic Calibration Check dialog box



This dialog box defines limits which the calibration must attain before the instrument is successfully calibrated. Two user parameters can be set:

- Missed Reference Peaks sets the maximum number of consecutive peaks that are not matched when comparing the reference spectrum and the acquired calibration spectrum. The calibration fails if this number is exceeded. The default value for this parameter, 2, is suitable in most cases.
- Maximum Std Deviation is set to a default of 0.20. During calibration the difference between the measured mass in the acquired calibration file and the true mass in the reference file is taken for each pair of matched peaks. If the standard deviation of the set of mass differences exceeds the set value, the calibration fails. Reducing the value of the standard deviation gives a more stringent limit. Increasing the standard deviation means that the requirement is easier to meet, but this may allow incorrect peak matching. Values greater than 0.20 should not be used unless exceptional conditions are found.

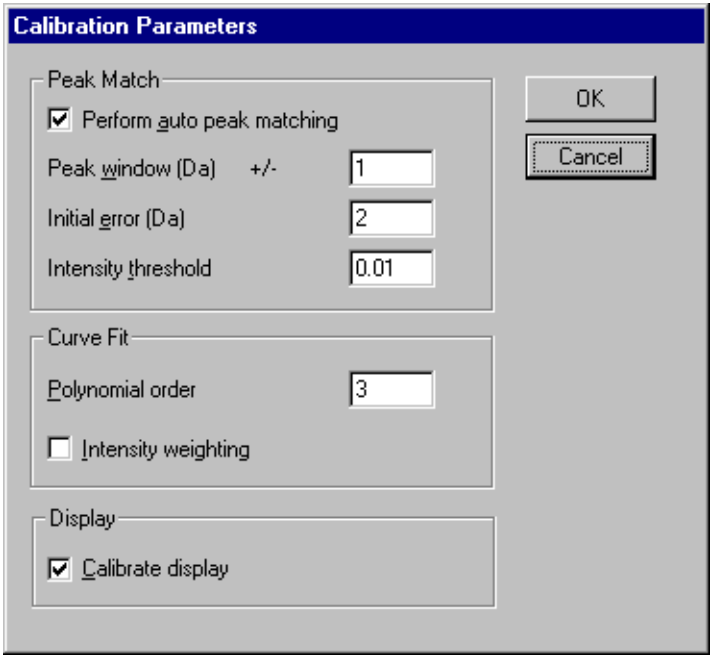
Apply Span Correction should always be selected. This allows different mass ranges to be scanned, within the calibrated range, without affecting mass assignment.

Check Acquisition Calibration Ranges causes warning messages to be displayed if an attempt is made to acquire data outside of the calibrated range for mass and scan speed. It is advisable to leave selected.

Calibration parameters

These are accessed by selecting Edit, Calibration Parameters, this invokes the Calibration Parameters dialog box.

Calibration Parameters dialog box



The Peak Match parameters determine the limits within which the acquired data must lie for the software to recognize the calibration masses and result in a successful calibration. The default values are shown in the figure above.

Increasing the Peak window and Initial error gives a greater chance of incorrect peak matching. All peaks in the acquired spectrum below the Intensity threshold value (measured as a percentage of the most intense peak in the spectrum) are not used in the calibration procedure.

The Polynomial order of the curve has values from 1 to 5 as the available options:

- A polynomial order of 1 should not be used.
- An order of 2 is suitable for wide mass ranges at the high end of the mass scale, and for calibrating with widely spaced reference peaks. Sodium iodide in particular has widely-spaced peaks (150 amu apart),

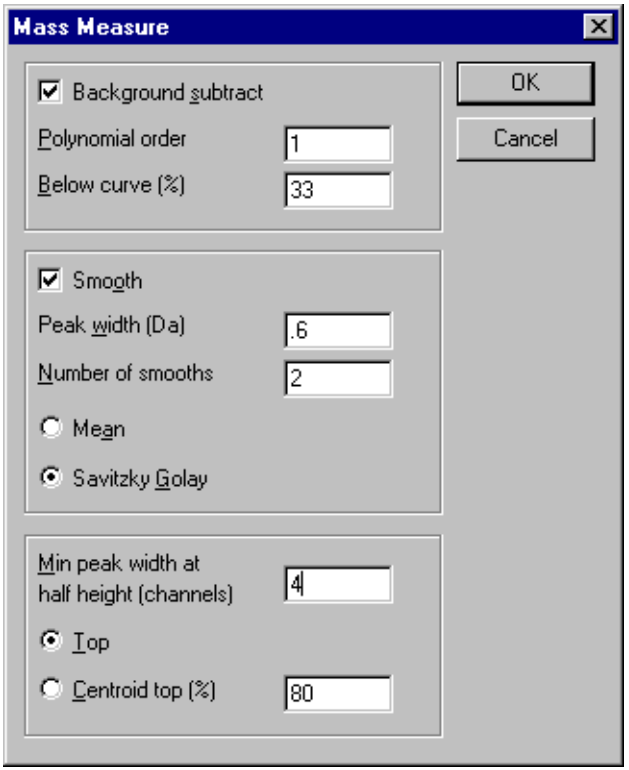
and horse heart myoglobin is used to calibrate higher up the mass scale, so this is the recommended polynomial order for these calibrations.

- An order of 3 fits a cubic curve to the calibration.
- A fourth order is used for calibrations which include the lower end of the mass scale, with closely spaced reference peaks. This is suitable for calibrations with PEG which extend below 300 amu.
- A fifth order fit rarely has any benefit over a fourth order fit.

Mass measure parameters

These are accessed by selecting Edit, Mass Measure Parameters, this invokes the Mass Measure dialog box.

Mass Measure dialog box



If continuum or MCA data are acquired for calibration, these parameters need to be set before the calibration is carried out. If centroided data are used for calibration, the mass measure parameters are not used.

With ElectroSpray calibrations, particularly with sodium iodide which has some low intensity peaks at higher mass, it is recommended that continuum or MCA data are acquired.

It is important that the data are smoothed correctly, and that the peak width at half height (PWHH) is entered in the smoothing parameters as shown in the above figure.

At high scan speeds, instrument resolution may decrease. Ensure that the centroiding parameters are set to use the top of the peak so that mass assignment of peaks is accurate.

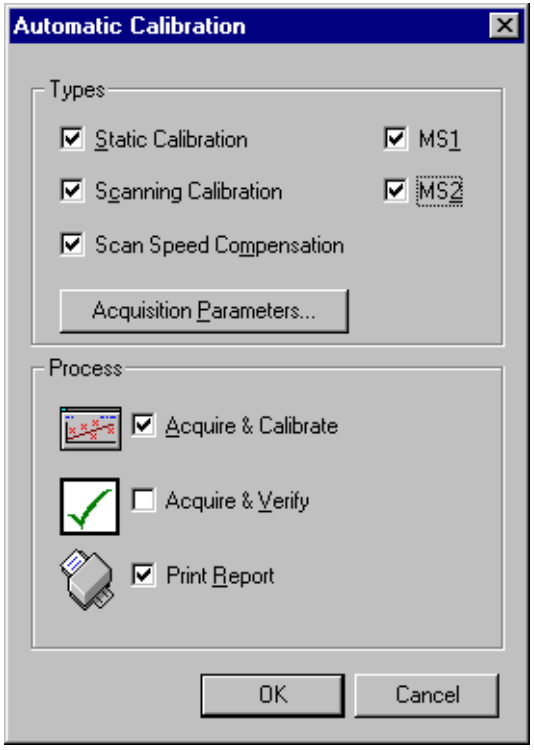
Performing a calibration

Three types of calibration are available with MassLynx:

- Static Calibration
- Scanning Calibration
- Scan Speed Compensation

These are selected on the Automatic Calibration dialog box which is invoked by selecting Start from the Calibrate dialog box.

Automatic Calibration dialog box



It is recommended that all three types of calibration are performed so that mass ranges and scan speeds can be changed whilst maintaining correct mass assignment. However, it is possible to have any combination of these calibrations:

- If only a static calibration is present, the instrument is calibrated for acquisitions where the quadrupoles are held at a single mass, as in SIR or MRM.
- If only a scanning calibration is present, the instrument is only correctly calibrated for scanning acquisitions over the same mass range and at the same scan speed as those used for the calibration.
- If only a scan speed compensation is present (with no scanning calibration having been performed), the scan speed compensation is treated as a scanning calibration and the instrument is only correctly

calibrated for scanning acquisitions over the same mass range and at the same scan speed as used for the calibration.

For the scan speed compensation to be used correctly, a scanning calibration should also be performed.

- If static and scanning calibrations are both present, the instrument is calibrated for acquisitions where the quadrupole is held at a single mass and for scanning acquisitions with a mass range which lies within the mass range of the scanning calibration, providing that the same scan speed is used.

  For example, if the instrument is calibrated from m/z 100 to 900 with a 2 s scan (400 amu/s), data can be acquired from 100 to 500 amu with a 1 s scan time (also 400 amu/s) whilst maintaining correct mass assignment. In this case, the static calibration would be used to determine the start mass of the acquisition and the scanning calibration would be used for mass assignment and scan range.
- If scanning calibration and scan speed compensation are present then the instrument is only calibrated for scanning acquisitions over the same mass range as that used for the calibration, but the scan speed can be changed, provided that it remains within the scan speeds used for the two calibrations. The mass range should not be changed, as there is no static calibration to locate the start mass.
- If all three types of calibration are present, all types of acquisition can be used providing that the mass range and scan speed are between the lower and upper limits used for the scanning calibration and the scan speed compensation.

To perform a complete calibration:

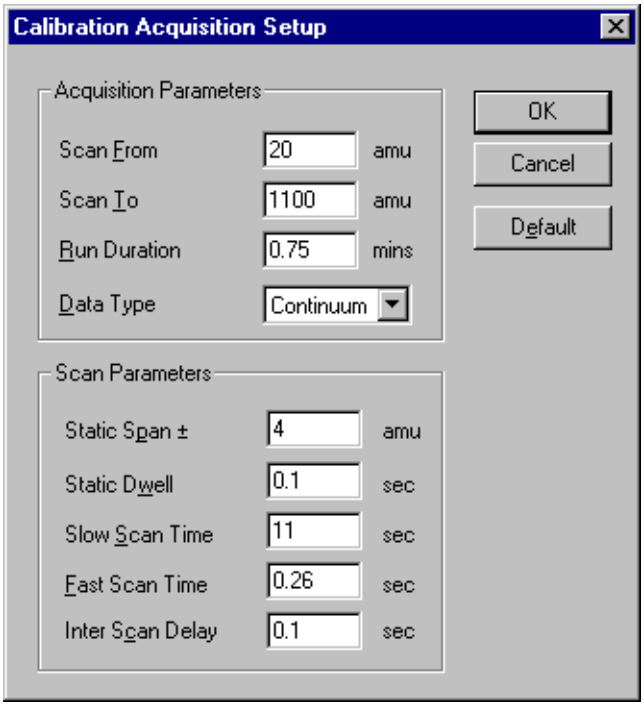
1. Check the boxes in the Types area of the dialog box adjacent to Static Calibration, Scanning Calibration and Scan Speed Compensation.
2. Check the MS1 and MS2 boxes.
3. In the Process area of the dialog box, check Acquire & Calibrate and Print Report.

Acquisition parameters

Selecting Acquisition Parameters in the Automatic Calibration dialog box invokes the Calibration Acquisition Setup dialog box, where the mass ranges, scan speeds and acquisition mode are set. When this box is first accessed it

contains default parameters relevant to the chosen reference file. These default parameters show the limits of scan range and scan speed for the currently-selected instrument and calibration parameters.

Calibration Acquisition Setup dialog box



The upper area contains the Acquisition Parameters where mass range, run time and data type are set.

When the instrument is fully calibrated, any mass range or scan speed is allowed within the upper and lower limits dictated by the calibrations.

Select the nairb.ref file.

The solution described in Chapter 8 is suitable for use with this reference file.

If compatible reference solutions and reference files are used, then simply selecting Default is sufficient action, no parameters need be entered manually.

Run Duration sets the time spent acquiring data for each part of the calibration. The time set must allow a minimum of three scans to be acquired at the slowest scan speed used. Data are not acquired if the Run Duration is

too short. The slowest scan speed generally used is 100 amu/s. With Scan From set to 20 amu and Scan To set to 2000 amu, a scan time of 19.8 s is required, and an Inter Scan Delay (in the lower area of the box) of 0.1 s is usually used. Therefore, the run duration must be greater than 59.6 seconds (3 scans + 2 inter scan delays). A Run Duration of 1.00 minutes is suitable.

The lower area in the Calibration Acquisition Setup dialog box contains the Scan Parameters.

When an instrument acquires data for a static calibration it examines the reference file to find the expected reference masses, and then acquires data over a small mass span around each peak's expected position. Thus the acquired data do not contain continuous scans. Each spectrum comprises small regions of acquired data around each peak, separated by regions where no data are acquired.

Static Span sets the size of this small region around each reference peak. A span of 4.0 amu is typical.

Static Dwell determines how much time is spent acquiring data across the span. A value of 0.1 s is suitable.

Slow Scan Time determines the scan speed used for the scanning calibration. If both a scanning calibration and a scan speed compensation are to be performed, the scan speed should be set to approximately 100 amu/s (a scan time of 19.8 s over a mass range of 20 to 2000 amu). If only a scanning calibration is to be performed (without scan speed compensation), the scan speed should be set at the same speed to be used for later acquisitions.

Fast Scan Time determines the scan speed used for the scan speed compensation, and the upper limit of scan speed that can be used for subsequent acquisitions. A fast scan time of 4000 amu/s is adequate for most applications. A scan range from 20 amu to 1100 amu requires a Fast Scan Time of 0.26 s at an Inter Scan Delay of 0.1 s. The Acquisition Setup must be edited to calibrate over the mass range desired.

Select OK to return to the Automatic Calibration dialog box. Alternatively, select chosen values if a different calibration range is required.

Starting the calibration process

To start the calibration process:

Select OK from the Automatic Calibration dialog box.

The instrument acquires all of the calibration files in the order, and using the data file names, shown in the table below.

Calibration order and data files

Calibration	Data file
MS1 static	STATMS1
MS1 scanning	SCNMS1
MS1 scan speed compensation	FASTMS1
MS2 static	STATMS2
MS2 scanning	SCNMS2
MS2 scan speed compensation	FASTMS2

Once all of the data have been acquired each data file is combined to give a single spectrum which is then compared against the reference spectrum to form a calibration. This process takes place in the same order as shown in the table above. If the full calibration dialog box is open, a constantly updated status message for the calibration is displayed.

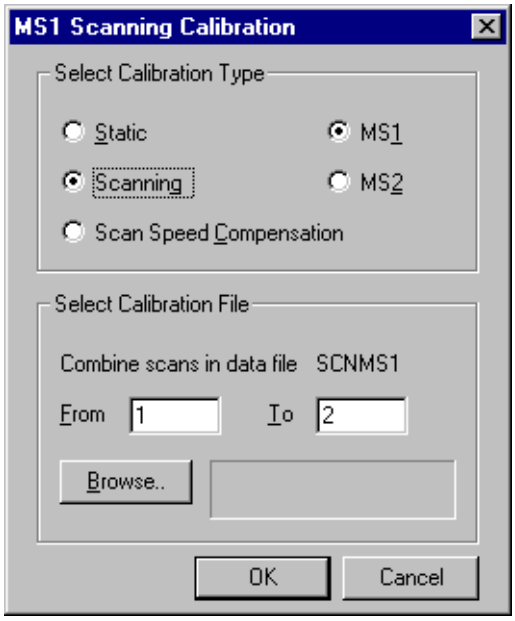
If, when the process is completed, the calibration statistics meet with the requirements specified by the selected calibration parameters, a successful calibration message is displayed. A calibration report is then printed showing a calibration curve for each of the calibration processes.

For the acquisition to be effective, it must be saved under a suitable file name.

Checking the calibration

The calibration (successful or failed) can be viewed in more detail by selecting Process, Calibration From File from the Calibrate dialog box. The dialog box which is then invoked allows the choice of calibration type for viewing. With the required calibration selected, the correct calibration file is automatically called up.

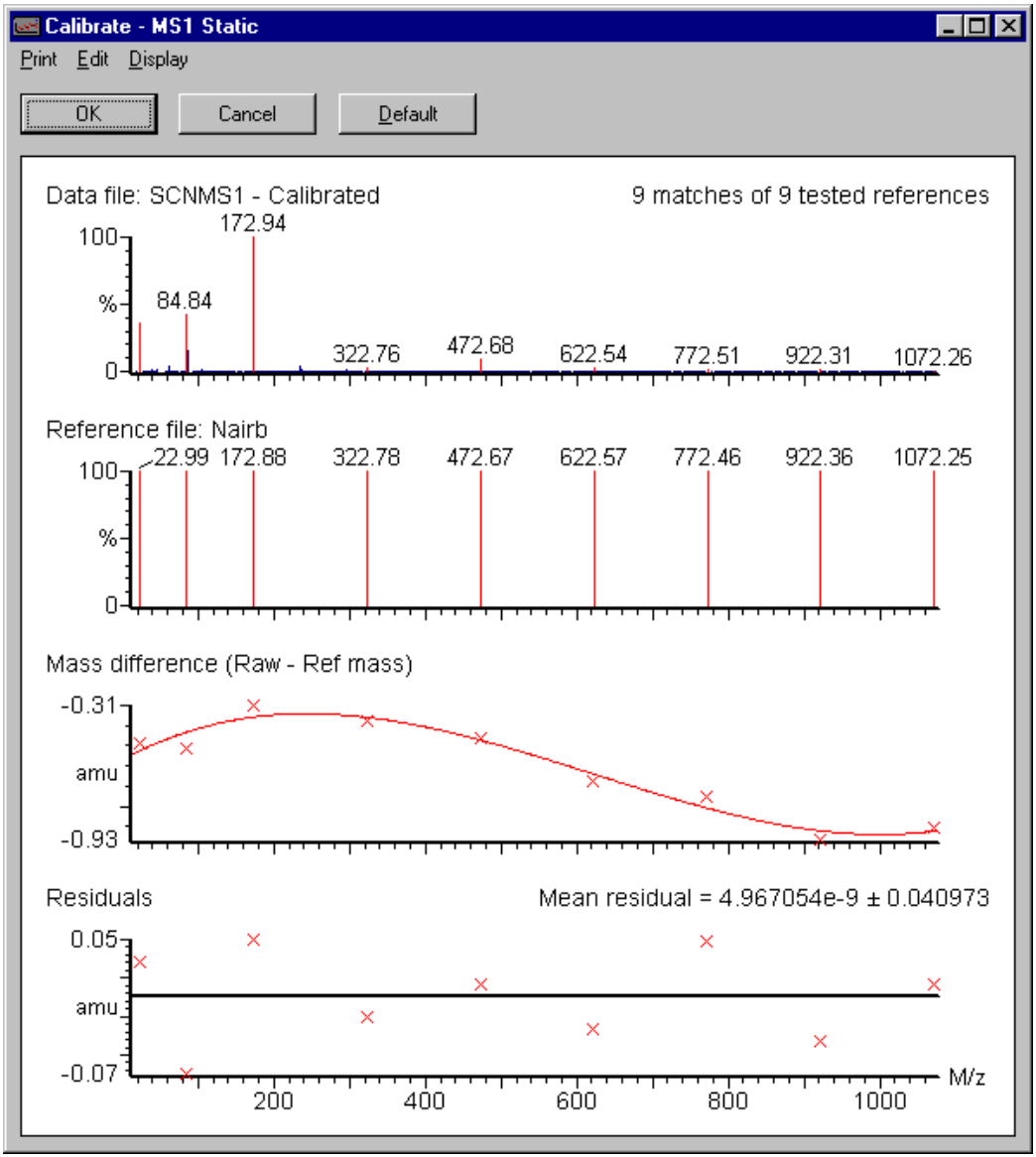
Scanning Calibration dialog box



Click Browse to select the calibration data file (for example STATMS1, SCNMS1, FASTMS1, STATMS2 etc.). The selected file must be from the appropriate project.

Clicking on OK repeats the calibration procedure for that particular file and displays a calibration report.

Calibration report



The calibration report contains four displays:

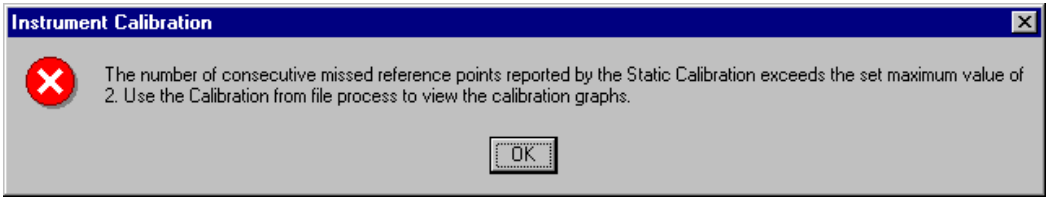
- The acquired spectrum
- The reference spectrum
- A plot of mass difference against mass (the calibration curve)
- A plot of residual against mass

An expanded region can be displayed by clicking and dragging with the left mouse button. In this way, the less intense peaks in the spectrum can be examined to check that the correct peaks have been matched. The peaks in the acquired spectrum which have been matched with a peak in the reference spectrum are highlighted in a different color.

Calibration failure

If the calibration statistics do not meet the requirements, a message is displayed describing at what point, and why, the calibration failed. This message also states where the attempted calibration data can be viewed so that the exact cause of failure can be determined.

Calibration failure message



There are a number of reasons for a calibration to fail:

No peaks

If the acquired calibration data file contains no peaks, the calibration fails. This may be due to:

- Lack of reference compound.
- No flow of solvent into the source.
- Multiplier set too low.

Too many consecutive peaks missed

If the number of consecutive peaks which are not found exceeds the Missed Reference Peaks parameter set in the Automatic Calibration Check dialog box, the calibration fails. Peaks may be missed for the following reasons:

- The reference solution is running out so that the less intense peaks are not detected.
- Multiplier is too low so that the less intense peaks are not detected.
- An incorrect ionization mode is selected. Check that the data have been acquired with Ion Mode set to ES+.

  Note that it is possible to calibrate in negative ion mode ElectroSpray using the naineg.ref reference file with a suitable reference solution.
- Intensity threshold, set in the Calibration Parameters dialog box, is too high. Peaks are present in the acquired calibration file but are ignored because they are below the threshold level.
- Either Initial error or Peak window, set in the Calibration Parameters dialog box, is too small. The calibration peaks lie outside the limits set by these parameters.
- Maximum Std Deviation, set in the Automatic Calibration Check dialog box, has been exceeded.
- The wrong reference file has been selected. Check that the correct file (nairb.ref in this case) is selected in the Calibrate dialog box.

In the case of too many consecutive peaks missed:

- Check the data in the on-screen calibration report to see if the missed peaks are present in the acquired calibration file.
- If the peaks are not present, the first three reasons (as explained above in No peaks) are likely causes.
- If the peaks are present in the data, but are not recognized during calibration then the latter four (Too many consecutive peaks missed) are likely reasons.

Having taken the necessary action, proceed as follows:

1. If Intensity threshold, Initial error and Peak window are adjusted to obtain a successful calibration, check the on-screen calibration report to ensure that the correct peaks have been matched.

With a very low threshold and wide ranges set for the Initial error and Peak window, it may be possible to select the wrong peaks and get a "successful" calibration. This is particularly relevant for calibrations with PEG, where there may be peaks due to PEG+H+, PEG+NH4+, PEG+Na+, and also doubly-charged species.

2. Select OK from the calibration report window to accept the new calibration, or select Cancel to retain the previous calibration.

Incorrect calibration

If the suggested calibration parameters are used, and providing that good calibration data have been acquired, then the instrument should be calibrated correctly. However in some circumstances it is possible to meet the calibration criteria without matching the correct peaks. This situation is unusual, but it is always sensible to examine the on-screen calibration report to check that the correct peaks have been matched. These errors may occur when the following parameters are set:

- Intensity threshold set to 0.
- Initial error too high (»2.0).
- Peak window too high (»1.5).
- Maximum Std Deviation too high (»0.2).

If the acquired spectrum looks like the reference spectrum and all of the expected peaks are highlighted, the calibration is OK.

An alternative cause of incorrect calibration is from contamination or background peaks. If a contamination or background peak lies within one of the peak matching windows, and is more intense than the reference peak in that window, then the wrong peak is selected. Under some conditions this may happen with PEG. There are two ways to counter this:

- If the reference peak is closer to the centre of the peak window, the peak window can be narrowed until the contamination peak is excluded. Take care to ensure that no other reference peak is excluded.
- If the reference peak is not closer to the centre of the peak window, or if by reducing the window other reference peaks are excluded, then the calibration can be edited manually.

Manual editing of peak matching

If an incorrect peak has been matched in the calibration process, this peak can be excluded manually from within the on-screen calibration report.

To manually edit Using the mouse, place the cursor over the peak in the reference spectrum and click with the right mouse button.

Place the cursor over the peak in the acquired spectrum and click with the right mouse button.

The peak is excluded and is no longer highlighted.

If the true reference peak is present, this can be included in the calibration by the same procedure:

Place the cursor over the required peak and click with the right mouse button.

The peak is matched with the closest peak in the reference spectrum.

Manually editing one peak does not affect the other matched peaks in the calibration.

Saving the calibration

When the instrument is fully calibrated the calibration must be saved under a file name so that it can be applied and recalled for future use.

The recalled calibration has the same constraints of mass range and scan speed. The ion energy and resolution settings used for the calibration acquisition are also recorded, as these can have an effect on mass assignment.

Verification

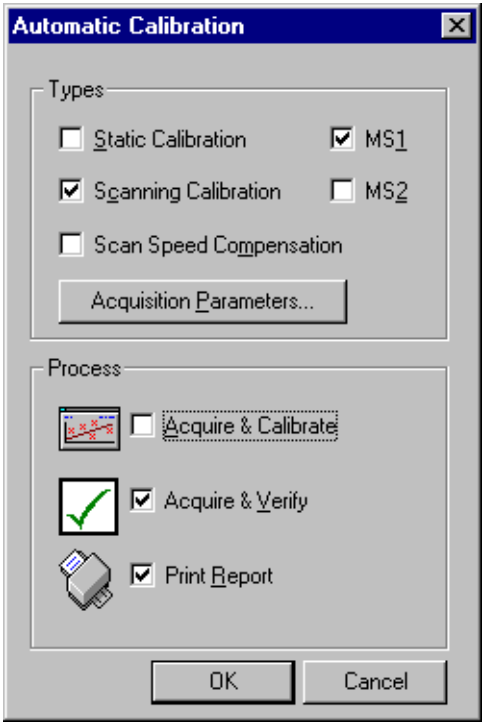
Once a full instrument calibration is in place it is not always necessary to repeat the full calibration procedure when the instrument is next used. Instead a calibration verification can be performed. (There is no benefit in verifying each calibration individually, re-calibration is just as quick.)

If a scanning acquisition is to be made and the calibration is to be checked:

1. Set up the instrument and access the calibrate dialog box as though a full calibration is to be carried out.
2. Set all peak matching parameters to the values that were used for the calibration.

3. Invoke the Automatic Calibration dialog box by selecting Start on the Calibrate dialog box.

Automatic Calibration dialog box



4. Select Scanning Calibration and deselect Static Calibration and Scan Speed Compensation.
5. Deselect Acquire & Calibrate and select Acquire & Verify and Print Report.
6. Select either MS1 or MS2, depending on the type of acquisition to be performed.
7. Select Acquisition Parameters to call up the Calibration Acquisition Set-up dialog box.

   The parameters entered should be identical to the parameters originally used for the calibration being verified.

8. Set Scan From, Scan To, Run Duration, Data Type, Scan Time and Inter Scan Delay to agree with the acquisition parameters that are to be used for data acquisition.

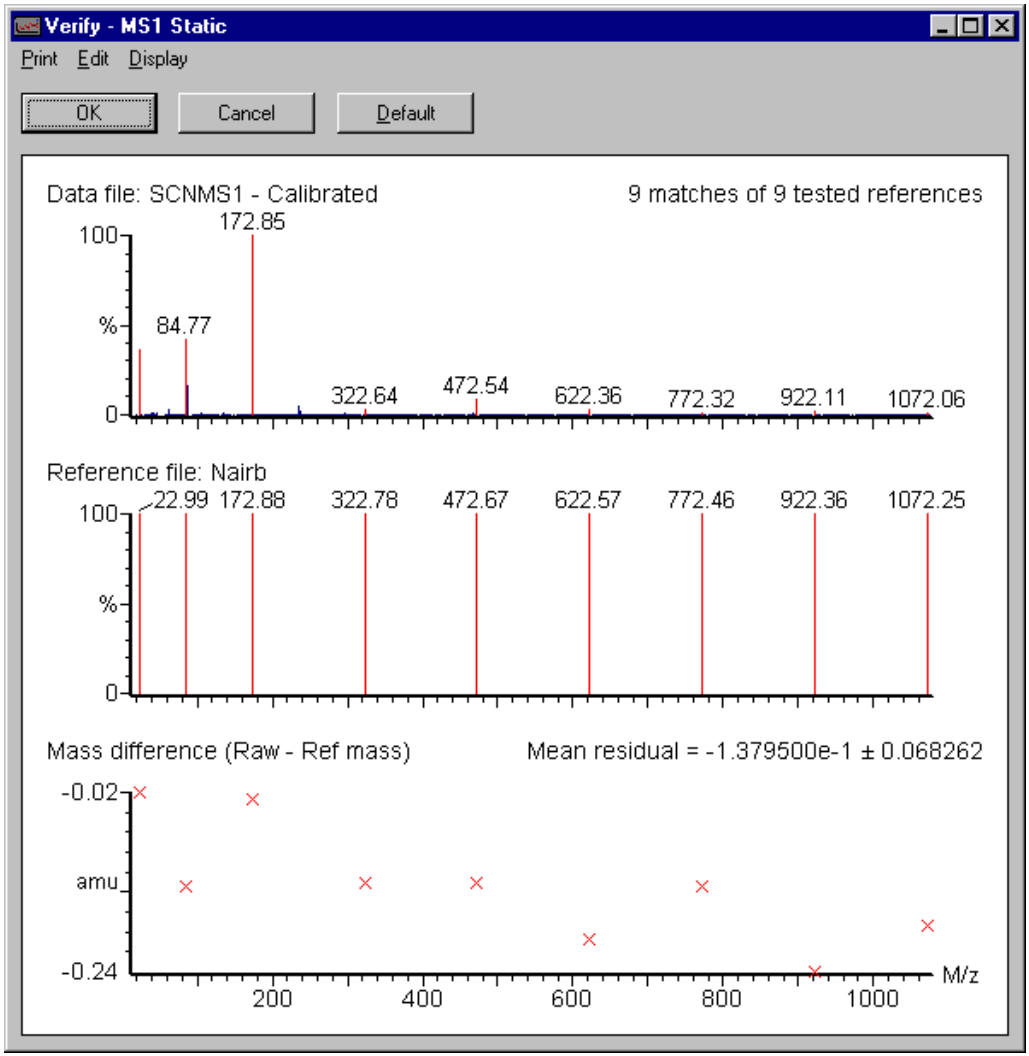
   With only the Scanning Calibration selected, all of the other options in this dialog box are unavailable.

9. Select OK to return to the previous dialog box, and OK again to start the verification procedure.

A scanning acquisition is now performed. When the acquisition is complete, the data are combined to give a single spectrum which is compared against the reference file. A calibration curve is drawn and a report printed in a similar way to when the original calibration was performed.

Unlike the original calibration procedure, the instrument calibration is not changed and the report that is printed is a verification report.

Typical verification report

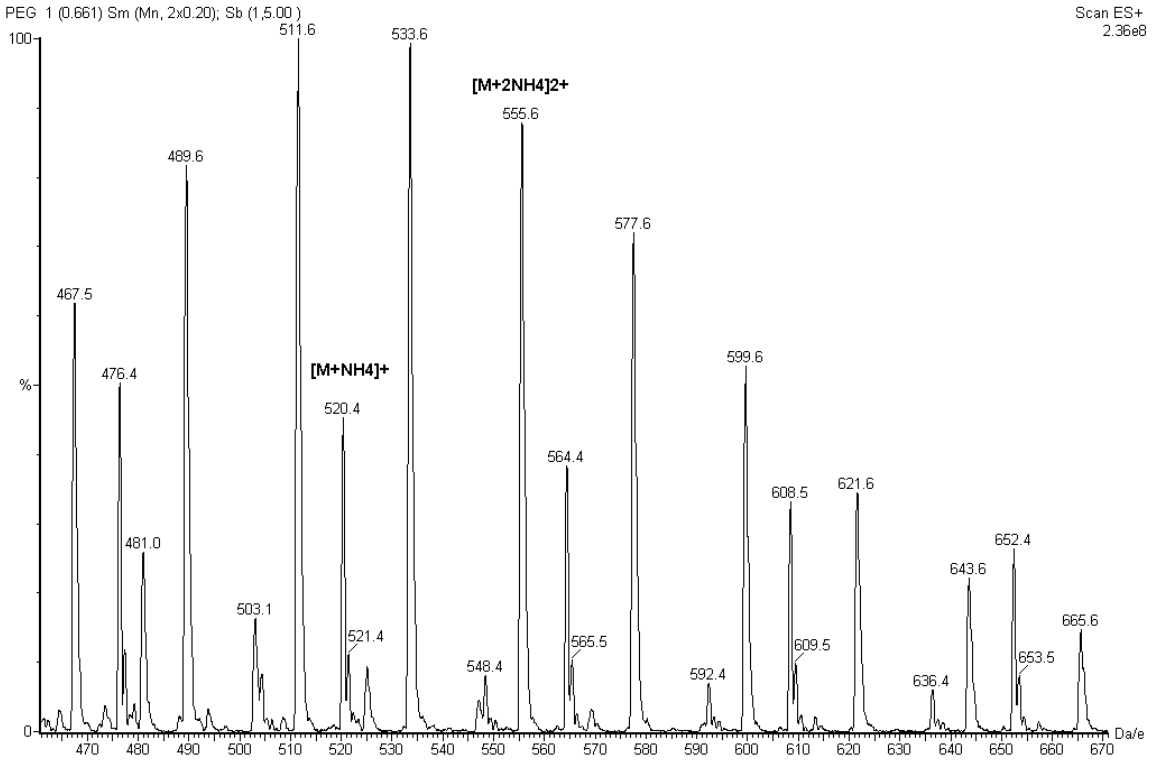


ElectroSpray calibration with PEG

Caution should be used when calibrating with PEG in ElectroSpray mode due to the number of peaks which are produced. Although ammonium acetate is added to the PEG reference solution to produce [M+NH4]+ ions, under some conditions it is quite usual to see [M+H]+, [M+Na]+ and doubly-charged ions.

The spectrum shown in the figure below demonstrates how the PEG spectrum can be dominated by doubly-charged ions (in this case [M+2NH4]2+) if the wrong conditions are chosen. In this case, the concentration of ammonium acetate in the reference solution is too high (5 mmol ammonium acetate is the maximum that should be used) and **Cone** is too low.

ElectroSpray spectrum of PEG



A low Cone voltage encourages the production of doubly-charged ions. The voltage should be at least 35 V.

Doubly-charged peaks can be identified, because the ^{13}C isotope peak is separated from the ^{12}C isotope by only 0.5 Da/e. If the instrument is set to unit mass, and data are acquired in continuum mode, the doubly-charged peaks appear broader, as the isotopes are not resolved.

Atmospheric pressure chemical ionization (APcI)

Introduction

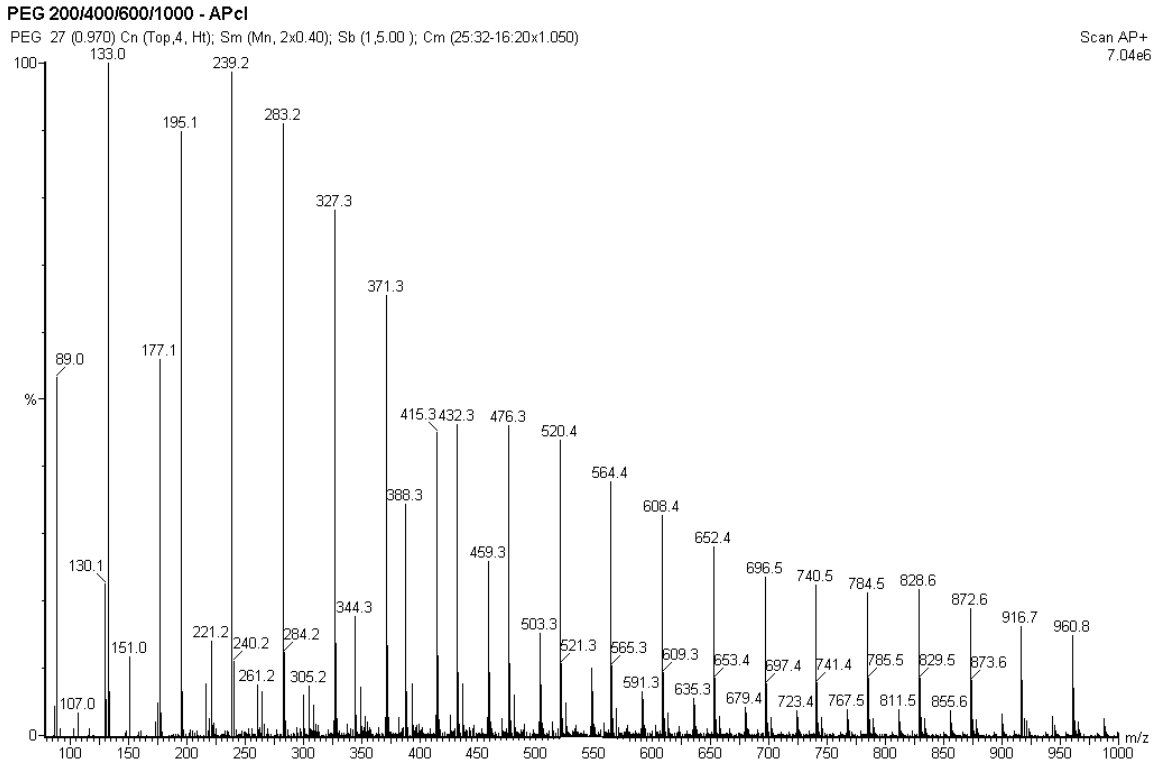
This section describes a complete mass calibration of Quattro micro API using atmospheric pressure chemical ionization. The procedures described should be followed only after reading “ElectroSpray” on page 5-5, describing the automated calibration with ElectroSpray ionization.

Due to the high flow rates used with APcI, the residence time of an injection of reference solution in the source is too short to allow a fully automated calibration, and the procedure therefore has to be carried out in several steps.

The recommended reference compound for APcI is a solution of polyethylene glycol (PEG) containing ammonium acetate. See Chapter 8, for advice on preparing the reference solution. The figure “Typical APcI spectrum of PEG” on page 5-30 shows a typical PEG + NH4+ spectrum.

With PEG, the possible calibration range is dependent upon the molecular weight distribution of the PEGs used in the reference solution. For this example PEG grades from PEG 200 to PEG 1000 are used.

Typical APcI spectrum of PEG



Preparing for calibration

Reference compound introduction

It is best to use a large volume injection loop (50 µl) with a solvent delivery system set up to deliver 0.2 mL/min of 50:50 acetonitrile:water or methanol:water through the injector and into the APcI source. An injection of 50 µL of reference solution lasts for approximately 15 s, allowing enough time to perform a slow scanning calibration.

Tuning

Before beginning calibration:

1. Set Multiplier to 650 V.
2. Adjust the source and lens parameters to optimize peak intensity and shape.
3. Set the resolution and ion energy parameters for unit mass resolution on MS1 and MS2.

When a full calibration is completed, it is possible to acquire data over any mass range within the calibrated range. It is therefore sensible to calibrate over a wide mass range, and in this example the calibration covers up to 1050 amu.

Calibration options

To access the calibration options, click on Calibrate from the tune page.

To select the reference file:

1. Set pegnh4.ref as the reference file by clicking on the arrow in the reference file box and scrolling through the files until the appropriate file can be selected.
2. Leave the Use Air Refs box blank when calibrating in APcI.

To remove current calibrations:

1. Select Default from the Calibrate menu option.
2. Enter Yes to save the calibration into the most suitable project directory.

   This ensures that a file with no calibration is currently active on the instrument and prevents any previously saved calibrations from being modified or overwritten.

Selecting calibration parameters

Several parameters need to be set before a calibration is started. Most of these parameters can be set at the same value as for ElectroSpray. However, a Polynomial order of 3 is recommended for the calibration Curve Fit.

Performing a calibration

The three types of calibration (Static, Scanning and Scan Speed) must be carried out in single steps.

Static calibration

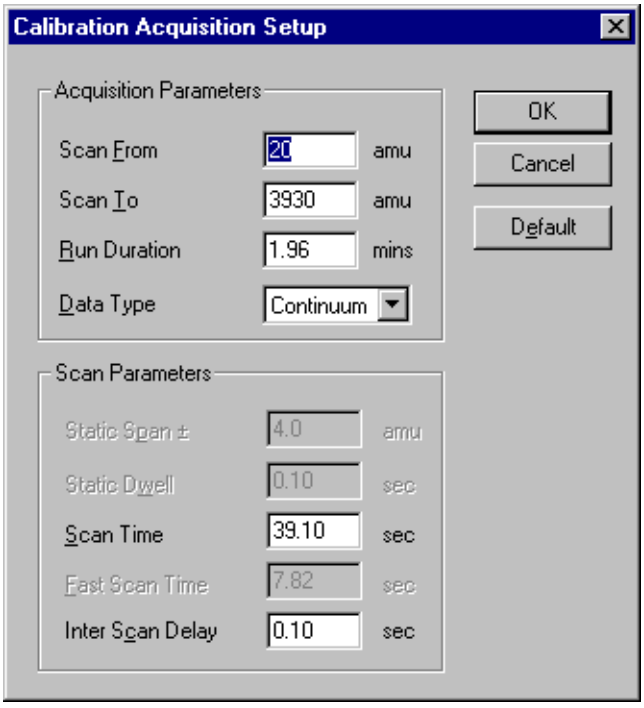
To perform a static calibration:

1. Access the Automatic Calibration dialog box by selecting Start from the Calibrate page.
2. Check Static Calibration and MS1 in the Types area of the dialog box.
3. In the Process area of the dialog box, check Acquire & Calibrate.

Acquisition parameters

Selecting Acquisition Parameters invokes Calibration Acquisition Setup dialog box; this contains the mass ranges, scan speeds and acquisition mode relevant to the pegnh4.ref reference file.

Calibration Acquisition Setup dialog box



The upper area contains the Acquisition Parameters where mass range, run time and data type are set. When the instrument is fully calibrated, any mass range or scan speed is allowed within the upper and lower limits dictated by the calibrations. It is therefore sensible to calibrate over a wide mass range. Since the pegnh4.ref reference file has peaks from m/z 89 to m/z 2017, it is possible to calibrate over this mass range. A calibration effective up to 1000 amu is sufficient for the majority of applications with APcI. The following example shows a setup to achieve this.

Run Duration sets the time spent acquiring data for the static calibration. The time set must allow chance to inject a volume of reference solution and acquire several scans.

Data Type allows a choice of Centroided, Continuum or MCA data to be acquired. For APcI, while either Continuum or Centroided data may be used, Continuum is recommended.

The lower area in the Calibration Acquisition Setup dialog box contains the Scan Parameters.

When an instrument acquires data for a static calibration it first examines the selected reference file for the expected reference masses. It then acquires data over a small mass span around the expected position of each peak. Thus the acquired data do not contain continuous scans, but each “spectrum” is made up of small regions of acquired data around each peak separated by blank regions where no data are acquired.

Static Span sets the size of this small region around each reference peak. A value of 4 amu is typical.

Static Dwell determines how much time is spent acquiring data across the span. A value of 0.1 s is suitable.

Slow Scan Time and Fast Scan Time are not available when a static calibration alone is selected.

Select OK from the Calibration Acquisition Setup dialog box to return to the Automatic Calibration dialog box.

Acquiring data

To start the acquisition:

1. Select OK from the Automatic Calibration dialog box.

   The instrument acquires a calibration file ready for static calibration using the data file name STAT.

2. Inject the reference solution while data are being acquired.

Once the data have been acquired the instrument attempts to produce a static calibration automatically. The data file contains only a few scans of the reference compound, the remaining scans being of background.

As the automatic calibration procedure combines all of the scans in the data file to produce a calibration spectrum, the resulting spectrum may be too weak to give a successful calibration. Whether the calibration is successful or failed, it is wise to check the calibration manually.

Manual calibration

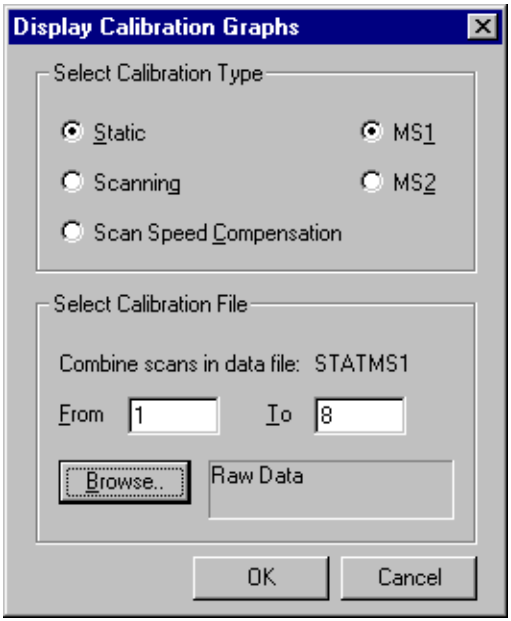
To perform a manual calibration using the acquired data:

1. From the chromatogram window, open the calibration file STATMS1.
2. Determine the scan numbers at the beginning and end of the chromatogram peak for the reference solution.

This can be achieved using Process, Combine Spectra and using the left mouse button to drag across the peak. The start and end scans are displayed in the Combine Spectra dialog box.

3. Return to the Calibrate dialog box. Access the manual calibration options, in the Display Calibration Graphs dialog box, by selecting Calibrate, From File.

Display Calibration Graphs dialog box



4. Select Static calibration type and MS1.
5. In the lower area, the data file STATMS1 should be selected automatically. If this is not the case, the correct file can be selected by clicking on Browse.
6. Enter the start and end scans of the reference data in the From and To boxes.
7. Select OK to perform the calibration and display the calibration report on the screen.

This report contains four displays:

- The acquired spectrum
- The reference spectrum
- A plot of mass difference against mass (the calibration curve)
- A plot of residual against mass

An expanded region can be displayed by clicking and dragging with the left mouse button. In this way the less intense peaks in the spectrum can be examined to check that the correct peaks have been matched. The peaks in the acquired spectrum, which have been matched with a peak in the reference spectrum, are highlighted in a different color.

Compare the acquired and reference spectra to ensure that the correct peaks have been matched.

If insufficient peaks have been matched, or the wrong peaks have been matched, refer to page 5-42.

If the correct peaks have been matched then the report can be printed out:

Select Print, Print from the report display.

To accept the calibration:

Select OK from the calibration report.

Calibration report - MS1 static

Calibration Report - MS1 Static Page 1 of 1

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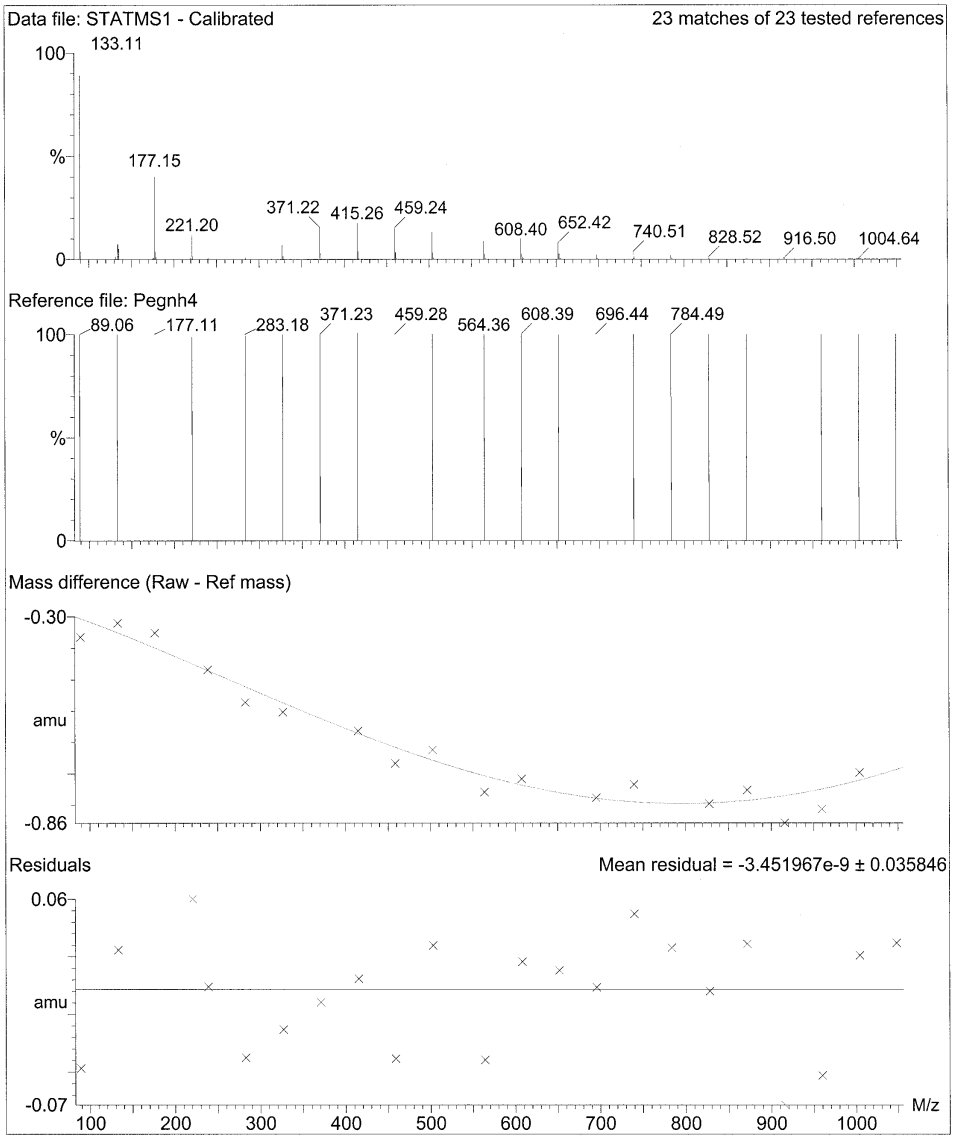

Calibration report - MS1 scanning

Calibration Report - MS1 Scanning **Page 1 of 1**

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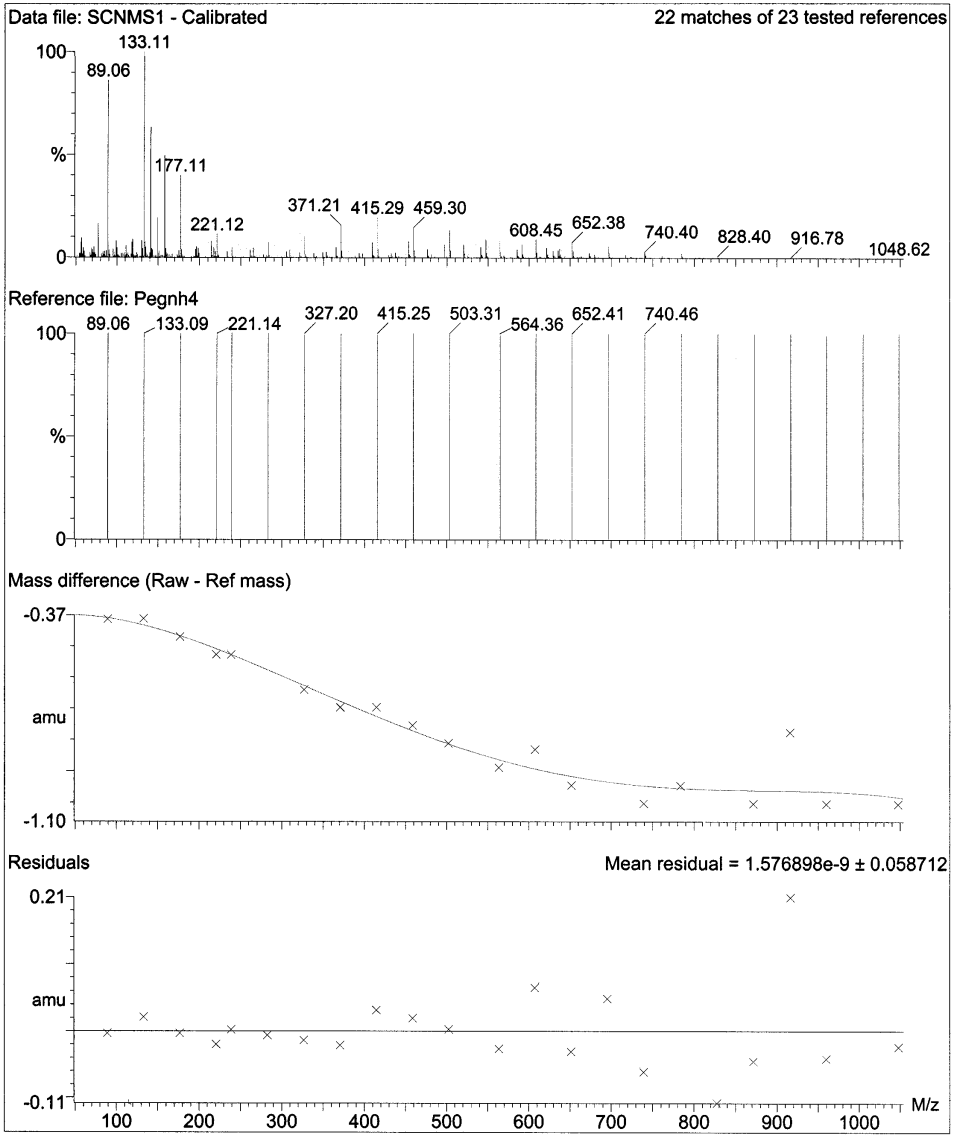
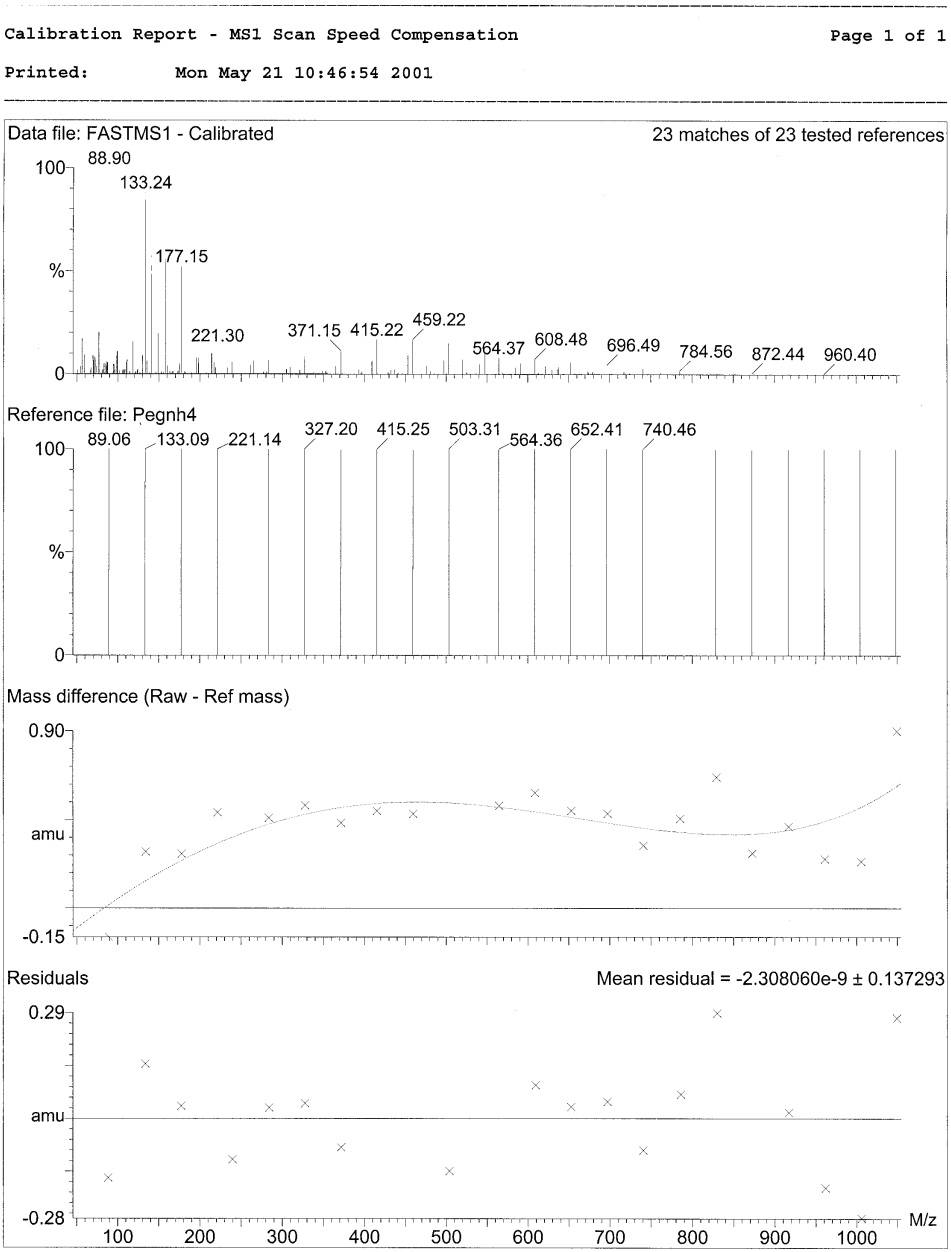


Calibration report - scan speed compensation



Scanning calibration and scan speed compensation

Acquiring data

To complete the calibration of the instrument, two further data files must be acquired. Both files are acquired in scanning mode over the same mass range, one at the slowest speed required for scanning acquisitions and one at the fastest speed. Once these files have been acquired and used for calibration, data may be acquired anywhere within the mass range at any scan speed between the values used for the two sets of data. These data do not have to be acquired through the Calibration dialog box, they can be acquired using the normal scan setup and then accessed from the Calibration dialog box as described below.

Scanning calibration

The recommended scan speed for the Scanning calibration is 100 amu/s.

1. Set Scan From to 50 amu and Scan To to 1050 amu.
2. Set Scan Time to 10 s and Inter Scan Delay to 0.1 s.
3. Select Continuum as the Data Type.

   Although Continuum is recommended, Centroided data may be used.
4. Set Run Duration to 2.0 minutes.

   This allows time to start the acquisition, inject the reference solution and acquire several scans. With a solvent flow rate of 200 µL/min and a 50 µL loop in line, an injection of reference solution lasts approximately 15 s, allowing at least one full scan of useful data to be acquired.
5. Choose a filename for the data.

   The filename SCNMS1, the name used during an automatic calibration, is valid.
6. Start the acquisition and inject the reference solution.

Scan speed compensation

The recommended scan speed for the scan speed compensation is 4000 amu/s.

1. Set Scan From to 50 amu and Scan To to 1050 amu.
2. Set Scan Time to 0.24 s and Inter Scan Delay to 0.1 s.
3. Select Continuum as the Data Type.

Although Continuum is recommended, Centroided data may be used. It is possible to scan more quickly in Centroided mode, but it is unlikely that a faster acquisition rate would be needed for general use.

4. Set Run Duration to 2.0 minutes.
5. Choose a filename for the data.

   The filename FASTMS1, the name used during an automatic calibration, is valid.

6. Start the acquisition and inject the reference solution.

Manual calibration

To begin manual calibration:

1. Find the start and end scans of the reference data for each file in the same way as for the static calibration file.
2. From the tune page select Calibration.
3. Select Scanning calibration type and MS1, Calibrate Instrument, Calibrate From File.
4. In the lower area, the data filename SCNMS1 should be selected automatically. If this is not the case, or if an alternative filename has been used for the slow scanning acquisition, the correct file can be selected by clicking on Browse.
5. Enter the start and end scans of the reference data in the From and To boxes.
6. Select OK to perform the calibration and display the calibration report on the screen in a similar way to the Static calibration.
7. Compare the acquired and reference spectra to ensure that the correct peaks have been matched.

If the correct peaks have been matched, the calibration report can be printed out:

Select Print, OK from the report display.

If insufficient peaks have been matched or the wrong peaks have been matched see page 5-42.

To accept the calibration:

Select OK from the calibration report.

The same procedure is used for the Scan Speed Compensation except that Scan Speed Compensation is selected in the dialog box, and the fast scanning file is used. Note that for the Scan Speed Compensation, the default file is FASTMS1. If an alternative filename has been used, this must be selected using the data browser.

Once all three calibrations (Static, Scanning and Scan Speed Compensation) have been completed, the instrument can be used for any mass range within the limits of the scanning calibrations, and at any scan speed from 100 to 4000 amu/s.

Calibrating MS2

The calibration of MS2 is carried out in exactly the same manner as above, except that data is acquired in MS2 mode instead of MS1.

Using the instrument

Once all six calibrations (Static, Scanning and Scan Speed Compensation, each for both MS1 and MS2) have been completed, the instrument can be used for any mass range within the limits of the scanning calibrations and at any scan speed from 100 to 4000 amu/s.

Calibration failure

When calibration is performed manually there is no warning message to show that the calibration has not met the set criteria. This must be judged by viewing the on-screen calibration report and examining the matched peaks and statistics associated with the report. There are a number of reasons for a calibration to fail:

No peaks

If the acquired calibration data file contains no peaks the calibration has failed. This may be due to:

- Lack of reference compound.
- Wrong scans or wrong data file being used for the calibration.
- No flow of solvent into the source.
- Multiplier set too low.

Too many consecutive peaks missed

If the number of consecutive peaks which are not found exceeds the limit set in the Automatic Calibration Check dialog box, the calibration has failed. Peaks may be missed for the following reasons:

- The reference solution is running out, causing less intense peaks to not be detected.
- Multiplier is too low and less intense peaks are not detected.
- The incorrect ionization mode is selected. Check that the data has been acquired with Ion Mode set to APcI+.
- Intensity threshold, set in the Calibration Parameters dialog box, is too high. Peaks are present in the acquired calibration file but are ignored because they are below the threshold level.
- Either Initial error or Peak window, set in the Calibration Parameters dialog box, is too small. The calibration peaks lie outside the limits set by these parameters.
- Maximum Std Deviation, set in the Automatic Calibration Check dialog box, has been exceeded.
- The wrong reference file has been selected. Check that the correct file (pegNH4.ref in this case) is selected in the Calibrate dialog box.

In the case of too many consecutive peaks missed:

- Check the on-screen calibration report to see if the missed peaks are present in the acquired calibration file.
- If the peaks are not present, the first four reasons above (No peaks) are likely causes.
- If the peaks are present in the data, but are not recognized during calibration, then the latter four reasons (Too many consecutive peaks missed) are likely reasons.

Having taken the necessary action, proceed as follows:

1. If Intensity threshold, Initial error and Peak window are adjusted to obtain a successful calibration, check the on-screen calibration report to ensure that the correct peaks have been matched.

   With a very low threshold and wide ranges set for the Initial error and Peak window, it may be possible to select the wrong peaks and get a “successful” calibration. This is particularly relevant for calibrations

with PEG, where there may be peaks due to PEG+H+, PEG+NH4+ and PEG+Na. This situation is unusual, but it is always wise to examine the on-screen calibration report to check that the correct peaks have been matched.

2. Select OK from the calibration report window to accept the new calibration, or select Cancel to retain the previous calibration.

Incorrect calibration

If the suggested calibration parameters are used, and providing that good calibration data have been acquired, the instrument normally calibrates correctly. However, in some circumstances it is possible to meet the calibration criteria without matching the correct peaks.

This situation is unusual, but it is always wise to examine the on-screen calibration report to check that the correct peaks have been matched. These errors may occur when the following parameters are set:

- Intensity threshold set to 0
- Initial error too high (>2.0)
- Peak window too high (>1.5)
- Maximum Std Deviation too high >0.2).

If the acquired spectrum looks like the reference spectrum and all of the expected peaks are highlighted then the calibration is OK.

An alternative cause of calibration failure is from contamination or background peaks. If a contamination or background peak lies within one of the peak matching windows, and is more intense than the reference peak in that window, then the wrong peak is selected. Under some conditions this may happen with PEG. There are two ways to counter this:

- If the reference peak is closer to the centre of the peak window, the peak window can be narrowed until the contamination peak is excluded. Take care to ensure that no other reference peak is excluded.
- If the reference peak is not closer to the centre of the peak window, or if by reducing the window other reference peaks are excluded, the calibration can be edited manually.

Manual editing of peak matching

If an incorrect peak has been matched in the calibration process, this peak can be excluded manually from within the on-screen calibration report.

To manually edit peak matching:

1. Using the mouse, place the cursor over the peak in the reference spectrum and click with the right mouse button.
2. Place the cursor over the peak in the acquired spectrum and click with the right mouse button.

   The peak is excluded and is no longer highlighted.

If the true reference peak is present, this can be included in the calibration by the same procedure:

Place the cursor over the required peak and click with the right mouse button.

The peak is matched with the closest peak in the reference spectrum.

Manually editing one peak does not affect the other matched peaks in the calibration.

Saving the calibration

When the instrument is fully calibrated the calibration, for it to be effective, must be saved under a filename so that it can be recalled for future use. For example, it is possible to save calibrations for use with different ionization modes, so that when an ionization source is switched the corresponding calibration is recalled.

The recalled calibration has the same constraints of mass range and scan speed. The ion energy and resolution settings used for the calibration acquisition are also recorded as these can have an effect on mass assignment.

Manual verification

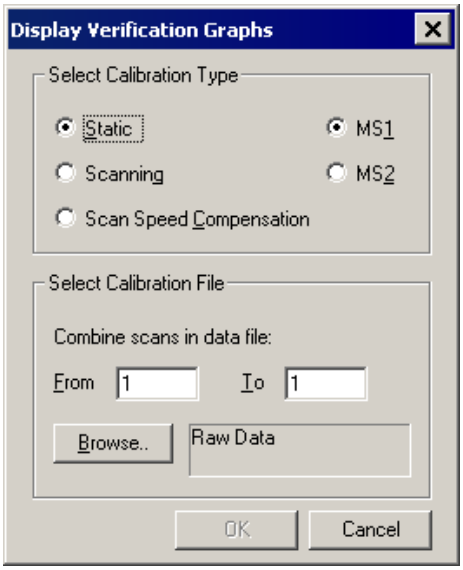
Once a full instrument calibration is in place it is not always necessary to repeat the full calibration procedure when the instrument is next used. Instead a calibration verification can be performed. (There is no benefit in verifying each calibration individually, re-calibration is just as quick.)

If a scanning acquisition is to be made and the calibration is to be checked:

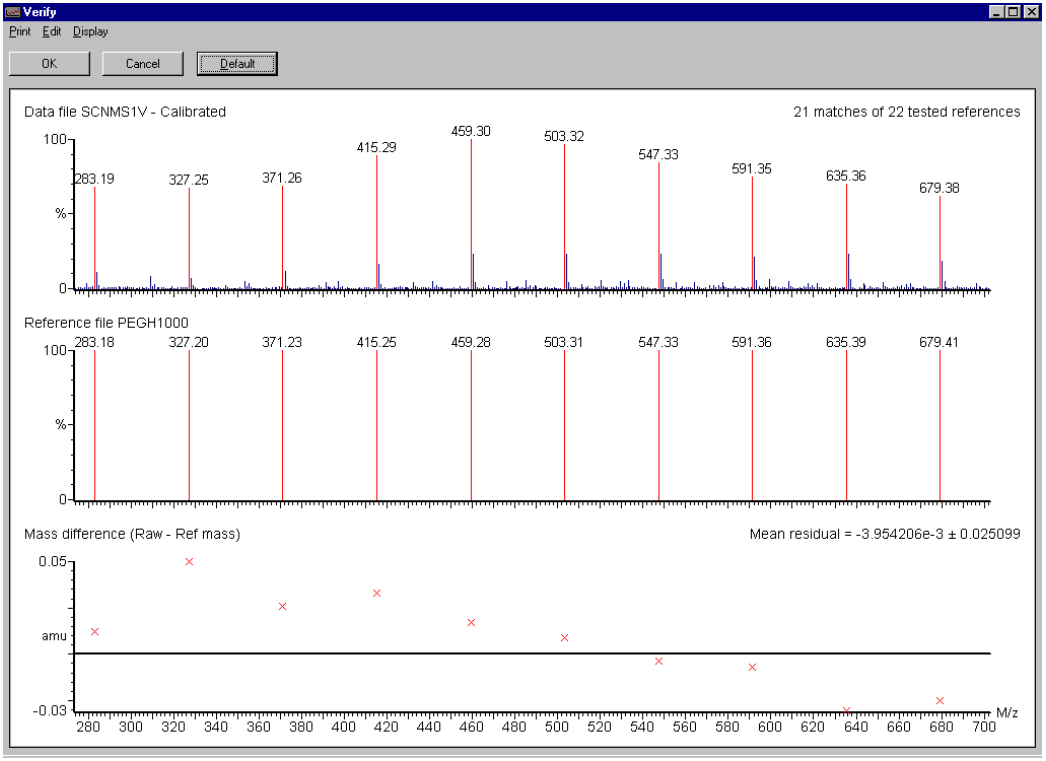
1. Set up a scanning acquisition over the required mass range and at the required scan speed in the normal way.
2. Start the acquisition and inject the reference solution so that reference data is acquired.
3. Stop the acquisition.
4. Invoke the Calibrate dialog box and set all peak matching parameters to the same values that were used for the calibration.
5. Select Process, Verification from file, the Display Verification Graphs dialog box is invoked.
6. Select Scanning Calibration and either MS1 or MS2 depending on the type of data acquired.
7. Click on Browse, select the acquired file and enter the start and end scans of the reference data.
8. Select OK to verify the calibration.

A calibration curve is produced and displayed on the screen in a similar way to when the original calibration was performed. An example is shown in the figure “Verification report” on page 5-48.

Display Verification Graphs dialog box



Verification report



When OK is selected from this report, unlike the original calibration procedure, the instrument calibration is not changed. As the verification procedure uses the same matching parameters as the calibration procedure, it is possible to validate the current calibration without re-calibrating the instrument.

The report can be printed out by selecting Print, OK from the verify report.

6 Maintenance Procedures

Contents

Maintenance schedule

The following table lists periodic maintenance schedules to be followed to ensure optimum performance.

The maintenance frequencies shown apply to instruments that normally receive moderate use.

Maintenance schedule

Maintenance procedure	Frequency
Gas-ballast the rotary pump	Daily (APcI) Weekly (ESI)
Check and adjust the rotary pump oil level	Weekly
Change the rotary pump oil	Every 3000 hours of pump operation
Clean the cone gas cone, sample cone, and baffle plate	When sensitivity decreases to unacceptable levels
Clean the ESI and APcI probe tip	When sensitivity decreases to unacceptable levels
Clean the corona discharge needle (APcI mode)	When sensitivity decreases to unacceptable levels
Clean the ion block assembly	When it is visibly fouled When background or high peak contaminants are unacceptably high
Clean all source components	When sensitivity decreases to unacceptable levels When cleaning the cone gas cone, sample cone, and baffle plate fails to improve analytical results
Clean the ion block assembly	When it is visibly fouled When background or high peak contaminants are unacceptably high
Clean all source components	When sensitivity decreases to unacceptable levels When cleaning the cone gas cone, sample cone, and baffle plate fails to improve analytical results

Safety and handling

Bear in mind the following safety considerations when performing maintenance procedures.

Warning: Never open the instrument's top or side panels to access power supplies or other components. The instrument does not contain user-serviceable parts.

Warning: Observe good laboratory practice when handling solvents, changing tubing, or operating the Quattro micro API. Know the physical and chemical properties of the solvents to be used. Refer to their Material Safety Data Sheets

Caution:

- Never disconnect an electrical assembly while the system is plugged in. This could damage electrical parts. Also, turn off the power and wait 10 seconds before disconnecting an assembly.
- Do not touch integrated circuit chips or other circuit board components. Static electric charge can damage electronic components.

Proper operating procedures

Follow all recommended procedures and guidelines to maintain the detector's operating efficiency.

Maintenance equipment

Routine parts cleaning requires the following equipment:

- An ultrasonic bath with a minimum chamber size of 12 inches × 6 inches × 4 inches (300 mm × 150 mm × 100 mm).
- Glass vessels, approximately 4 inches (100 mm) in diameter and 4.25 inches (120 mm) high.
- A 500-mL graduated cylinder (to use when cleaning the hexapole assembly).

Gas-ballasting the rotary pump

When the rotary pump draws large quantities of solvent vapors, the vapors tend to condense in the pump oil, reducing pump efficiency. Gas-ballasting purges condensed contaminants from the oil and returns any oil to the pump from the oil mist filter. Gas-ballast the rotary pump when the following conditions apply:

- With ESI operation, once a week.
- With frequent APcI operation, once a day.
- If the pump oil appears cloudy.
- If the vacuum pressure is higher than normal.
- If condensate forms in the rotary pump exhaust line.
- When changing the rotary pump oil.
- If the level of accumulated oil in the oil mist filter is high.

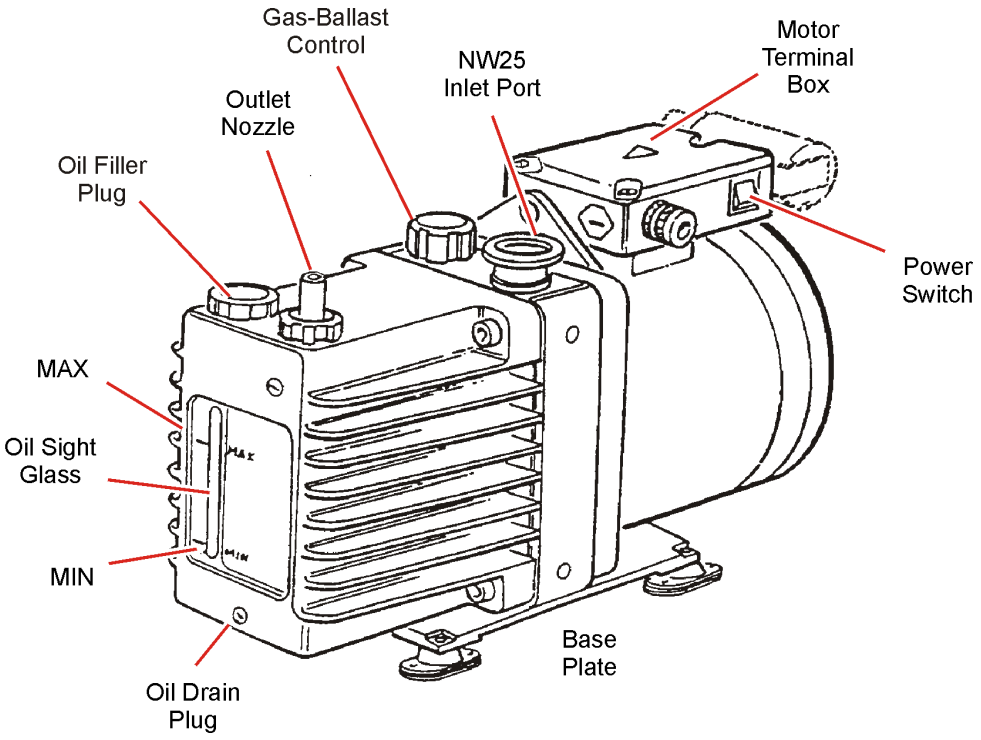
Caution:

- Failure to routinely gas-ballast the rotary pump shortens oil life and consequently shortens pump life.
- Do not vent the instrument when the rotary pump is gas-ballasting.
- Do not gas-ballast the rotary pump while Quattro micro API is in the Operate mode.
- Never gas-ballast the rotary pump for more than 2 hours.

To gas-ballast the pump:

1. Shut the vacuum system isolation valve, moving its handle fully to the right.
2. Turn on the gas-ballast.

Rotary pump



3. When the oil is clear and has drained back to the rotary pump, return the gas-ballast control to its normal position.
4. Open the vacuum system isolation valve.

Checking the rotary pump oil

The oil level can be checked while the pump is operating. However, the instrument must be vented and shut down before adding oil.

The rotary pump oil level appears in the oil level sight glass on the pump.

Check the oil level at weekly intervals; at all times it should be at, or near, the MAX level as indicated by the markings beside the sight glass. If oil must be added, vent and shut down the Quattro micro API before removing the oil filler plug.

Examine the oil each time the oil level is checked. It should be colorless and free of visible contaminants. If the oil is discolored, change it as described on page 6-6.

Changing the rotary pump oil

Change the rotary pump oil every 3 to 4 months, or whenever it becomes noticeably discolored.

Required materials

- Rubber gloves
- Flat-blade screwdriver
- Container to catch used oil
- Funnel
- Vacuum oil (use only Ultragrade 19 or Inland Q45 (Edwards 45) vacuum pump oil)

Procedure

To change the rotary pump oil:

1. Operate the pump to warm the oil before draining.
2. Gas-ballast the rotary pump as described on page 6-4.
3. Vent and shut down the instrument, turning the power switch to off.
4. Raise the pump 6 to 8 inches (150 to 200 mm) above the floor, if necessary.
5. Place an object under the motor to tilt the pump toward the side on which the oil drain plug is located (see the figure “Rotary pump” on page 6-5).
6. Remove the oil filler plug to facilitate drainage.
7. Using the flat-blade screwdriver, remove the oil drain plug.
8. Let the oil drain completely, then refit the oil drain plug.
9. Fill the pump until the oil in the sight glass reaches the MAX level.
10. Allow a few minutes for the oil to drain into the pump.

11. Recheck the oil level, and add more oil if necessary.
12. Refit the oil filler plug and, if necessary, lower the pump to the floor.
13. Turn the rotary pump power switch to On.

Cleaning the source assembly

Overview

The sample cone, cone gas cone, and baffle plate should be cleaned either:

- When they are visibly fouled.

Or:

- When LC and sample-related causes for decreased signal intensity have been ruled out.

When cleaning these parts fails to increase signal sensitivity, also clean the extraction lens, hexapole, and ion block.

The source-cleaning procedure is as follows:

1. Disassemble the source components, which are fully described later in this chapter.
2. Clean the source components.
3. Remove and clean the hexapole assembly.
4. Refit the hexapole assembly.
5. Reassemble the source components.

Required materials

The following materials are required to clean the source components:

- Lint-free cotton or powder-free nitrile gloves
- 6-inch (150-mm) or needle-nose pliers
- 2.5-mm hex wrench
- 6-mm hex wrench
- Small, flat-blade screwdriver
- Large, flat-blade screwdriver

- Clean 1000-mL beaker
- Clean 500-mL graduated cylinder

  Use only glassware not previously cleaned with surfactants.
- HPLC-grade methanol
- HPLC-grade water
- Formic acid
- Ultrasonic bath
- Source of oil-free, inert gas (nitrogen or helium) for drying (air-drying optional).
- Lint-free paper towels

Spare parts

The following spare parts may be required when cleaning source components:

- Ion block D-ring (AS035)
- Viton O-ring (AS214)
- Extraction cone O-ring
- Sample cone O-ring

Disassembling the source components

To disassemble the source components:

1. Set Source Temp and either APcI Probe Temp or Desolvation Temp to 20 °C, to switch off the heaters.

   Caution: The probe should be cooled to below 100 °C and the source cooled to below 50 °C before removal. Failure to do so will shorten the life of the probe heater.

2. To reduce cooling time significantly, continue flowing API gas.

3. When the probe has cooled, stop the nitrogen flow by deselecting  on the toolbar or by choosing Gas from the Gas menu.

4. Stop the liquid flow, and disconnect the LC line from the probe.

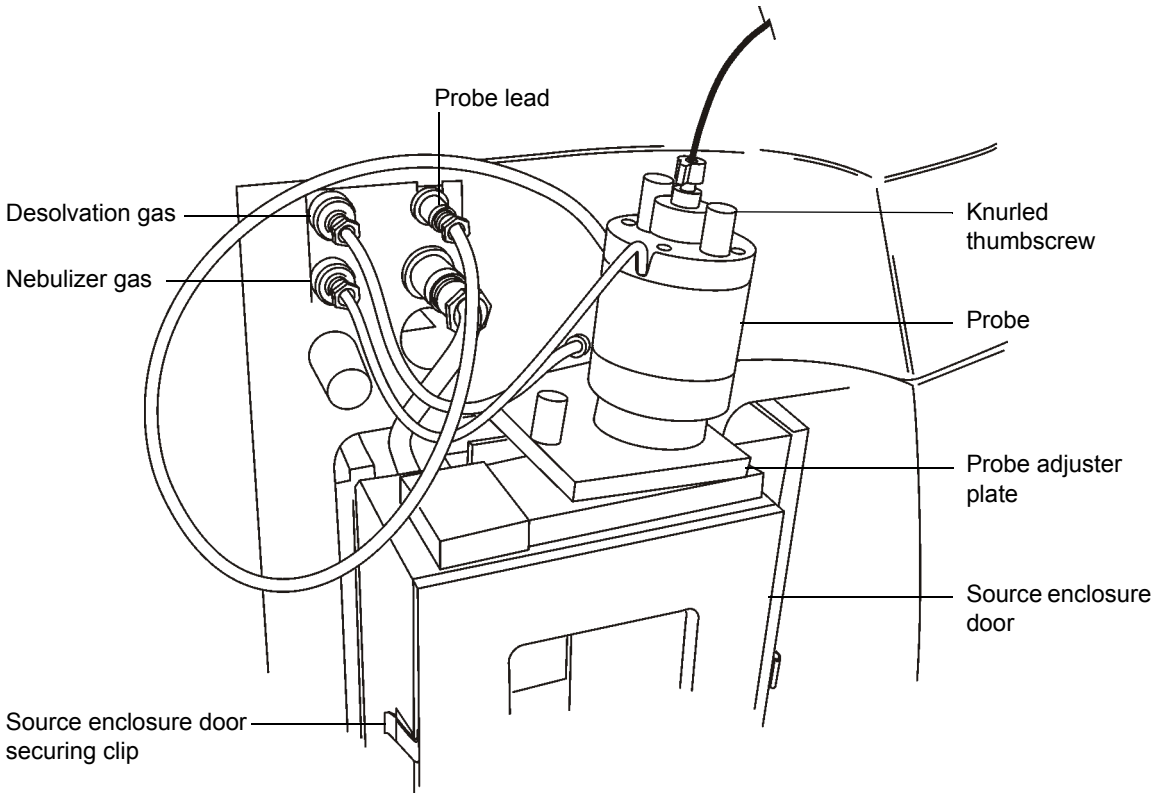
5. Click Press For Standby on the MassLynx tune page.

6. The icon changes from green to red. This means all high voltages are turned off, as well as the ESI desolvation/APcI probe heater.
7. Select Options then Vent from the tune page, and click OK when the message box appears. If cleaning only the sample and cone gas cones, it is not necessary to vent the system.
8. Select Options then Vent from the tune page, and click OK when the message box appears. If cleaning only the sample and cone gas cones, it is not necessary to vent the system.

   When the instrument is vented, power to the turbomolecular pump is interrupted. However the pump does not stop immediately. Venting is a controlled and safe process over several minutes designed to prevent damage to the instrument. When the turbomolecular pump stops, a vent valve opens automatically and vents the vacuum chamber to the atmosphere. The rotary pump stops running about 3 minutes after the vent cycle begins.
9. Disconnect the front panel gas and electrical connections.

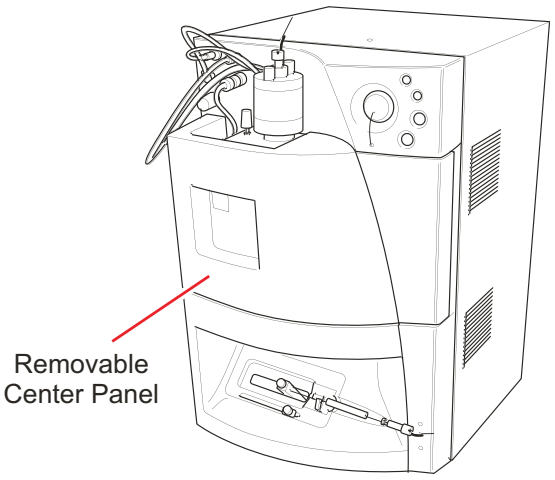
    **Warning:** Handle the probe carefully. It might still be hot.
10. Unscrew the probe's two knurled thumbscrews and retract it from the source.

Probe and source connections



11. Remove the center section panel from the front of the instrument, pulling it away from the instrument.

Removable center panel



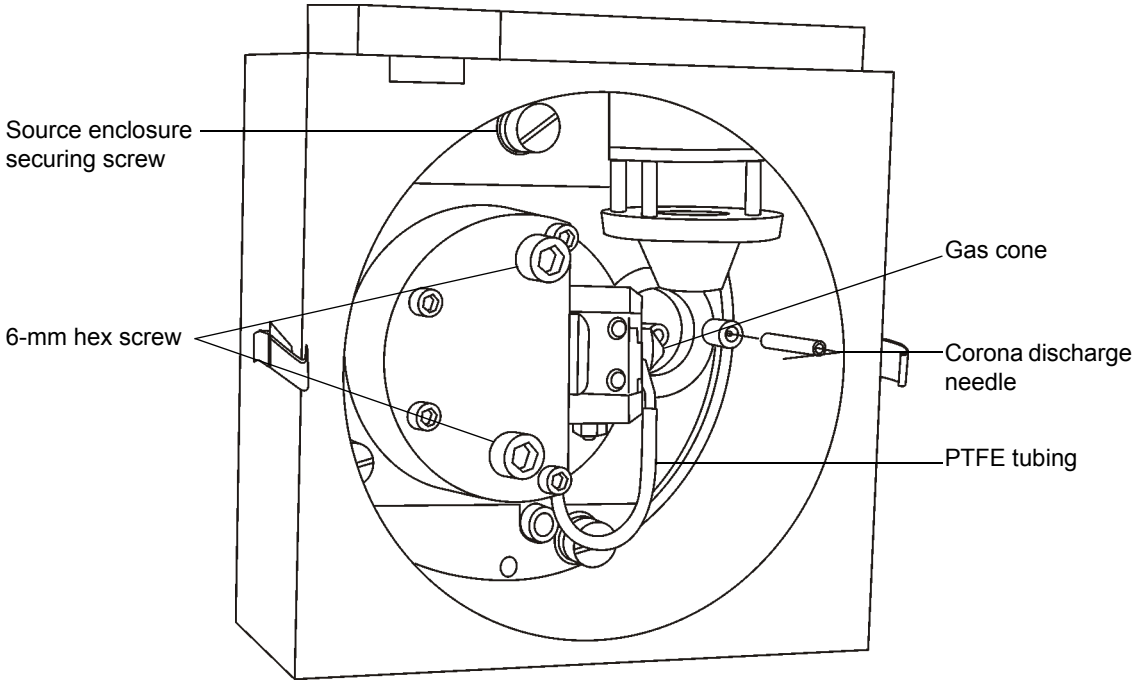
12. Unfasten the source enclosure door's securing clips and open the door.

Caution:

- Wear lint-free cotton or powder-free nitrile gloves for the rest of the cleaning procedure.
- Take care not to scratch the highly polished cone orifice surfaces.

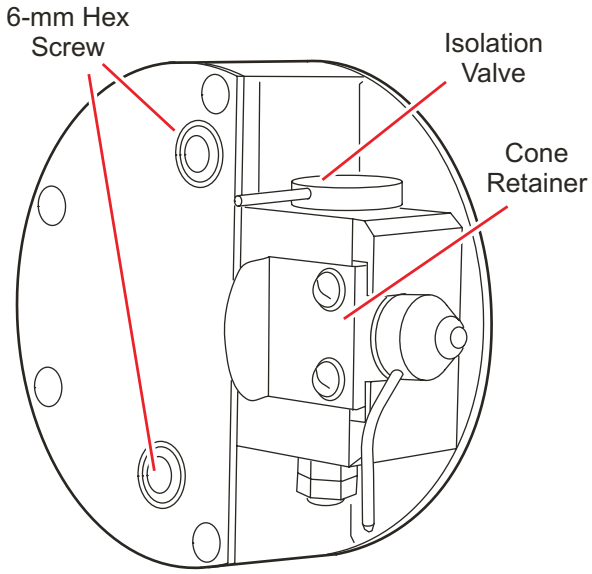
13. If using an APcI probe, carefully remove the corona discharge needle.

Source enclosure



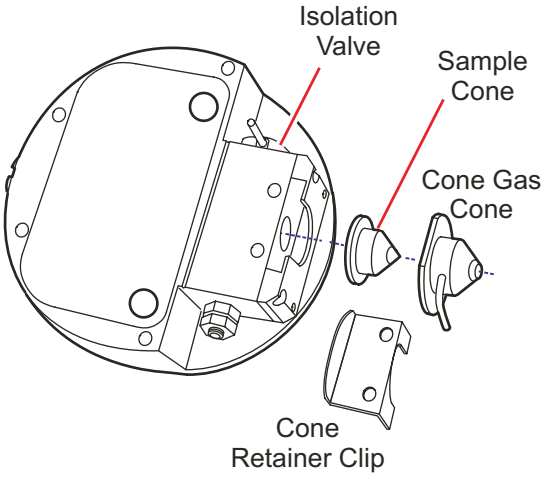
14. Remove the PTFE tube attached to the cone gas cone.
15. Remove the two screws that secure the cone retainer, using the small, flat-blade screwdriver.

Ion block



16. Remove the cone gas cone, O-ring, and sample cone from the ion block.

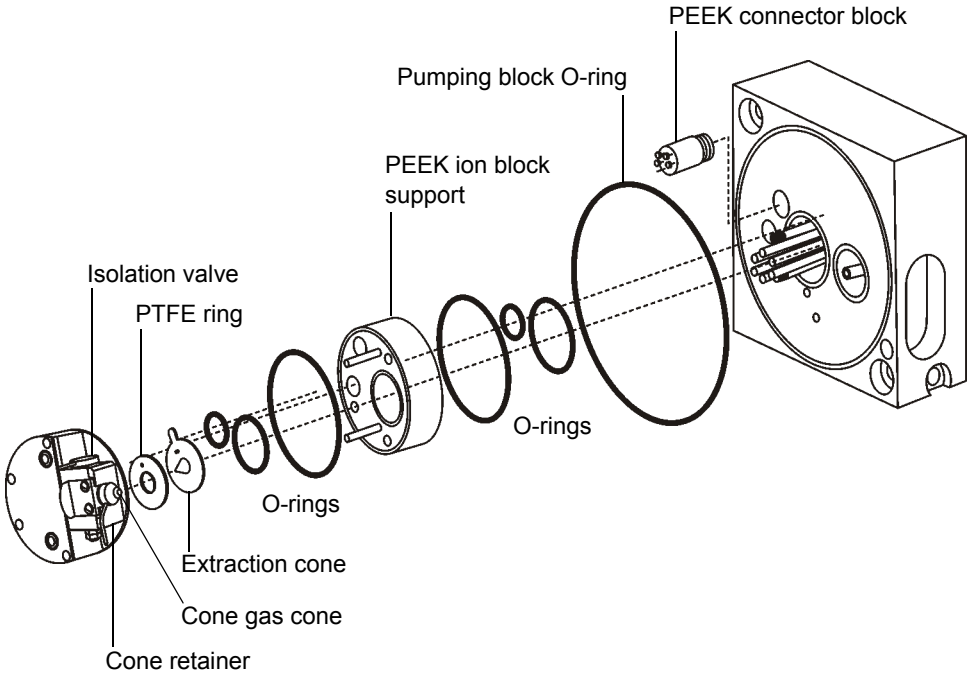
Removing the sample cone



17. Carefully separate the sample cone from the gas cone, then remove the sample cone O-ring.
18. Remove the baffle plate, and set all pieces aside.

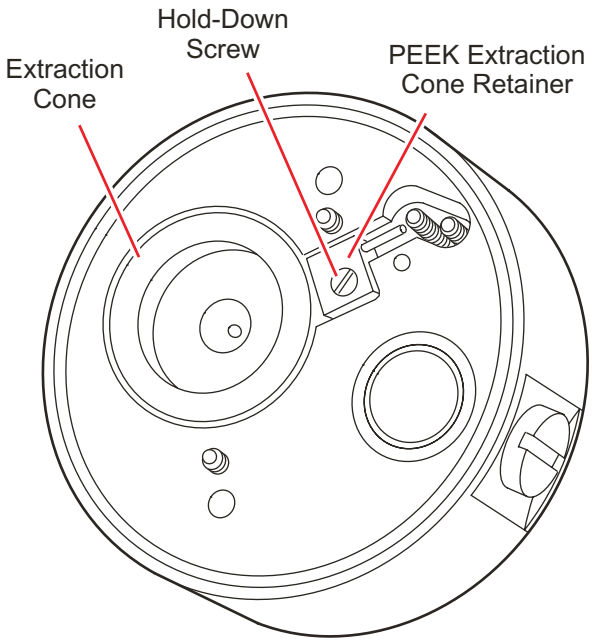
19. Remove the two screws that secure the ion block, using the 6-mm hex wrench.
20. Remove the ion block from the ion block support.

Exploded diagram



21. Place the ion block on a flat surface, and remove any O-rings.
22. Use a screwdriver to remove the hold-down screw from the PEEK extraction cone retainer.

Removing the extraction cone



23. Place the shaft of a small, flat-blade screwdriver in the ion block relief, and carefully pry the insulator O-ring away from the ion block.
24. Take care not to damage the ion block surface and insulator O-ring.
25. Grasp the extraction cone pin with the needle-nose pliers, then lift the extraction cone from the ion block.
26. Insert the small, flat-blade screwdriver under the inner edge of polymeric seal ring and carefully pry the seal ring and O-ring out of the ion block.
27. Take care not to damage the seal and O-ring on the ion block.
28. Remove the D-shaped O-ring from the front of the ion block.
29. Remove the ion block plug and seal using a flat-blade screwdriver.
30. Remove the O-ring from around the sample cone orifice, taking care not to scratch the ion block surface.

Cleaning the source components

To clean the source components:

1. Place the ion block in a beaker with methanol:water (1:1).
2. Place the beaker containing the ion block and methanol:water mixture in an ultrasonic bath for 20 minutes.
3. Remove the ion block from the methanol:water mixture, and place it in a beaker containing 100% methanol.

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

4. If the sample cone contains debris, place a drop of formic acid on its orifice.
5. Place the sample cone, cone gas cone, and extraction cone in a beaker with methanol:water (1:1).
6. If the parts are obviously fouled, use a mixture of 45% methanol, 45% water, and 10% formic acid.
7. Expose all parts to ultrasound for about 30 minutes.
8. If formic acid was used in the cleaning solution:
   a. Rinse the parts, immersing them in a beaker of water and setting the beaker in an ultrasonic bath for about 20 minutes to remove formic acid from them.
   b. Displace the water by immersing the parts in a beaker of methanol and setting the beaker in the ultrasonic bath for 10 minutes.
9. Carefully remove the parts from the beaker and blow-dry them, using inert, oil-free gas.

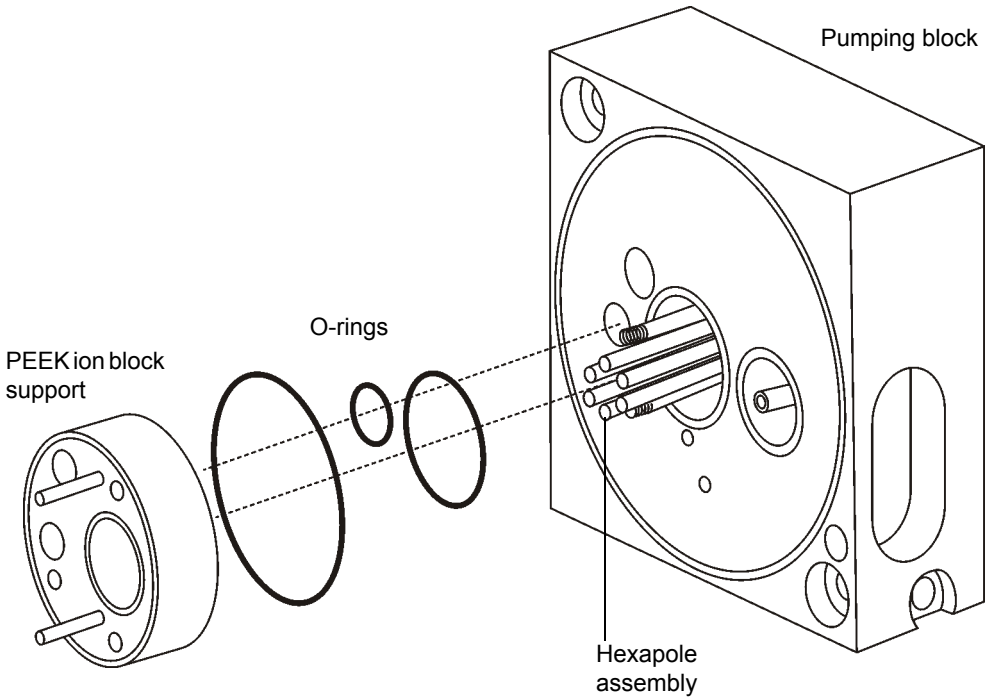
   Alternatively. the parts may be placed on lint-free towels and allowed to air dry. Wipe off any water spots with a lint-free cloth.

Removing and cleaning the hexapole assembly

To remove and clean the hexapole assembly:

1. Remove the screws securing the ion block support with the 3 mm hex wrench, and remove the ion block support from the pumping block.

Removing the hexapole

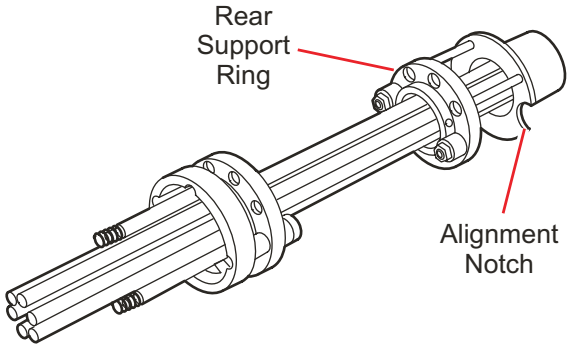


2. Remove any O-rings that remain stuck to the surface of the pumping block.

   Caution: Never squeeze the hexapole rods together when removing the hexapole, as their orientation relative to one another is critical to the Quattro micro API's performance. Take care not to scratch the bored surfaces of the pumping block as the hexapole is withdrawn.

3. Grasp the hexapole gently by hand, and carefully slide it out.
4. Bend a length of stainless steel tubing into a hook shape, and insert the hook into one of the holes in the rear support ring.

Hexapole assembly



5. Carefully suspend the hexapole assembly in a graduated cylinder, then add methanol to the cylinder until the assembly is covered.
6. Place the graduated cylinder in an ultrasonic bath for 30 minutes.
7. Remove the hexapole assembly from the graduated cylinder, and place it on a lint-free cloth. Allow it to air-dry, or use a nitrogen flow to dry it.
8. Insert the hexapole assembly by aligning the notches in the differential aperture at the rear of the hexapole with the two bottom support rails on the analyser assembly, then carefully slide it into place. Be sure to insert the assembly fully.
9. Check the condition of the three rear ion block support O-rings, replacing them with new ones if necessary. Ensure that the O-rings are properly installed before reattaching the ion block support. Also make sure the pin in the ion block support aligns with the notch on the ion block.
10. Secure by alternately tightening the retaining screws.

Reassembling the source components

To reassemble the source components:

1. Check the condition of the two front ion block support O-rings. Replace them with new O-rings, if necessary. Be sure they are properly installed before proceeding.
2. Refit the Vespel® sealing ring and O-ring on the ion block support.

3. Press the extraction cone into place in the ion block support, and secure it with the PEEK retainer and screw.
4. Refit the sample cone and its O-ring, then the cone gas cone, secured with the retainer spring and two screws as well as the ion block plug and seal.
5. Refit the ion block assembly onto the PEEK support block, then the cone gas cone secured with two 6-mm hex screws. Avoid overtightening.
6. If only the sample and cone gas cone have been cleaned, turn the isolation valve back to Open.
7. If using APcI, refit the corona discharge needle (see page 6-17).
8. Reattach the PTFE tube to the cone gas cone.
9. Close the source enclosure door, and fasten the door's securing clips.
10. Pump down the instrument and turn on the API gas.

Cleaning and replacing the corona discharge needle

The corona discharge needle should be cleaned if it looks corroded or black, or when the signal intensity weakens.

Required materials

The following materials are needed to clean the corona discharge needle:

- Lint-free cotton or powder-free nitrile gloves
- Lapping film
- HPLC-grade methanol
- Lint-free tissue

Procedure

To remove, clean and replace the corona discharge needle:

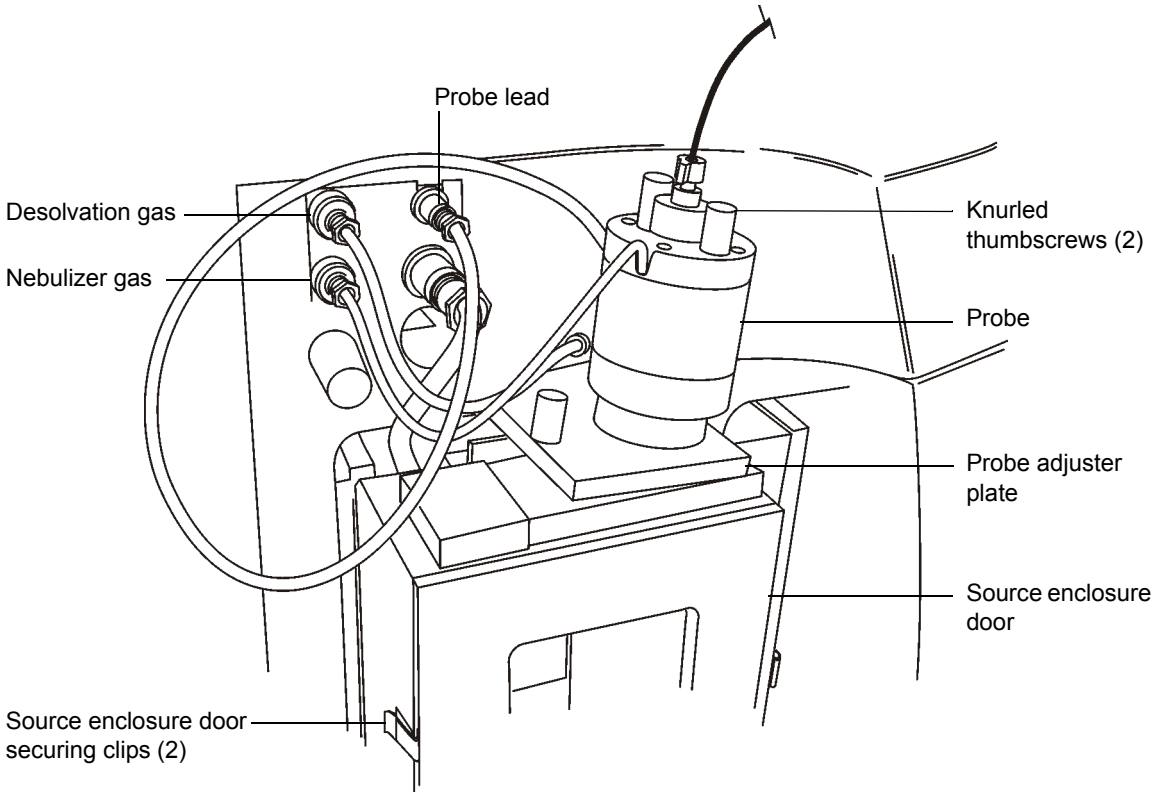
1. Set Source Temp and APcI Probe Temp to 20 °C to switch off the heaters.

To reduce cooling time significantly, continue flowing API gas.

Caution: Do not remove the probe before it cools to below 100 °C and the source heater cools to below 50 °C. Doing so will shorten the life of the probe heater.

2. When the probe has cooled, stop the nitrogen flow by deselecting  on the toolbar or by choosing Gas from the Gas menu.
3. Click Press For Standby on the MassLynx tune page.
4. Ensure that the icon changes from green to red. This means all high voltages are turned off, as well as the APcI probe heater.
5. Remove the center panel from the front of the instrument.
6. Disconnect the nebulizer gas and the probe electrical connection.

Probe and source connections



7. Remove the two knurled thumbscrews from the top of the probe.
8. Remove the APcI probe.

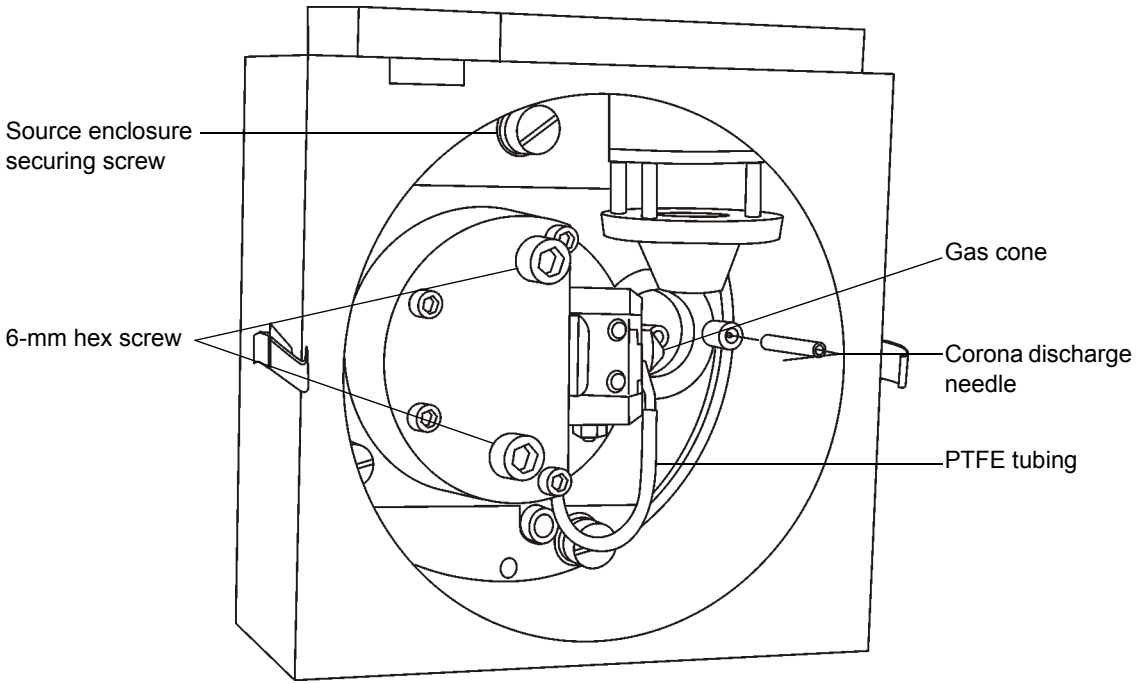
   Warning: The inner surfaces of the source enclosure and its constituent components are hot.

9. Unfasten the source enclosure door's securing clips, and open the door.

   Caution: Wear lint-free cotton or powder-free nitrile gloves for the rest of the cleaning procedure.

10. Remove the corona discharge needle from the source, pulling it straight out.

Source enclosure



11. Clean and sharpen the tip of the needle with the lapping film, then wipe it clean with a methanol-saturated tissue. Replace the needle if it is deformed or otherwise damaged.
12. Reinstall the needle with the tip pointing toward the sample cone apex.

13. Close the source enclosure door, and fasten the door's securing clips.
14. Refit the probe, and reconnect the LC line.
15. Refit the middle panel section.
16. Reconnect the front panel gas and electrical connections.

Cleaning the APCI probe tip

The APcI Probe tip should be cleaned when a buffer build-up is detected on the probe tip, or when the signal intensity weakens.

To clean the APcI Probe tip:

1. Shut off the liquid flow.
2. Click  , or choose Gas on the Gas menu, to start nitrogen flowing.
3. Adjust the nitrogen flow to approximately 650 L/h, as indicated by the tune page desolvation gas meter.
4. Set the APcI probe heater temperature to 650 °C, and press Enter.
5. Click Operate, and wait 10 minutes with the APcI probe heater at 650 °C. This will remove any chemical contamination from the probe tip.

Replacing the source enclosure and probe adjustment flange O-rings

Warning: To ensure the integrity of the source exhaust system, the O-rings listed below must be renewed at intervals of no greater than one year.

The following O-rings must be renewed at intervals of no greater than one year:

- Source enclosure door O-ring.
- Source enclosure door glass O-ring.
- Pumping block O-ring.

- Probe adjustment flange O-ring.
- APPI enclosure side flange O-ring (if applicable; refer to the *Waters Micromass APPI/IonSabre Source Operator's Guide*).

Tip: To complete this procedure, you will be required to perform a pressure test on the source, as described in the *Waters Source Pressure Test Unit Operator's Guide*.

To remove the source enclosure and probe adjustment flange O-rings:

1. Remove the probe from the source (see page 6-8, step 1 to step 9).

   Warning: The source can be hot; to avoid burns, take great care while working with this component.

2. Unfasten the source enclosure door's securing clips and open the door (see the figure "Probe and source connections" on page 6-10).

   Warning: The source components can be contaminated with biohazardous and/or toxic materials. Always wear nitrile gloves while performing this procedure.

3. Use a large flat-bladed screwdriver to remove the three source enclosure securing screws (see the figure "Source enclosure" on page 6-12).

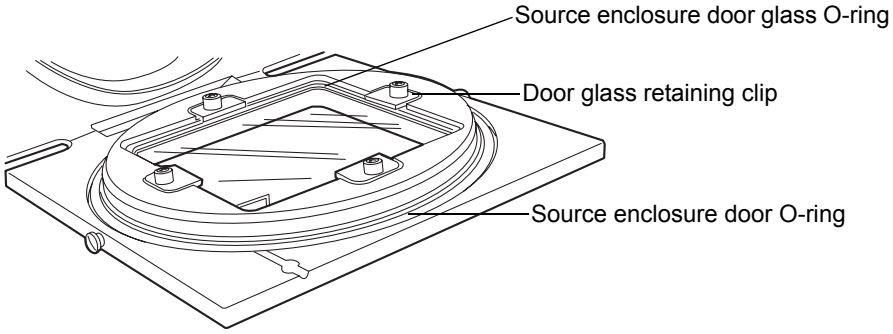
   Tip: To avoid damage, do not apply any force to the source enclosure door when removing the source enclosure from the instrument's pumping block.

4. Remove the source enclosure from the instrument's pumping block.

   Tip: To avoid damage, do not use a metal tool to remove any of the O-rings.

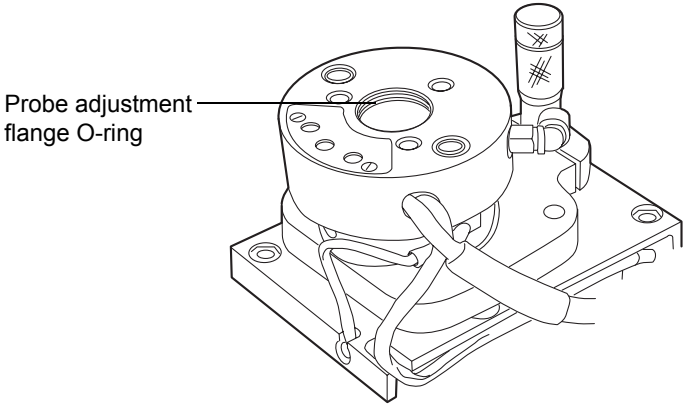
5. Carefully remove the source enclosure door O-ring from the source enclosure.

Source enclosure door



6. Use a hex (Allen) key to remove the four bolts securing the source enclosure door glass retaining clips to the source enclosure door.
7. Remove the four source enclosure door glass retaining clips from the source enclosure door.
8. Remove the source enclosure door glass from the source enclosure door.
9. Carefully remove the source enclosure door glass O-ring from the source enclosure door.
10. Carefully remove the probe adjustment flange O-ring from the probe adjustment flange.

Probe adjustment flange O-ring



11. Carefully remove the pumping block O-ring from the pumping block (see the figure “Exploded diagram” on page 6-14).

Warning: The O-rings can be contaminated with biohazardous and/or toxic materials. Ensure that they are correctly disposed of according to local environmental regulations.

12. Dispose of the O-rings in accordance with local environmental regulations.

To fit the replacement source enclosure and probe adjustment flange O-rings:

1. Ensure that all the grooves for the O-rings are free from dirt and hairs.

   Tip: If contamination is present, use an appropriate solvent, applied to a lint-free cloth, to carefully clean the grooves.

2. Fit the new pumping block O-ring to the pumping block.
3. Fit the new probe adjustment flange O-ring to the probe adjustment flange.
4. Fit the new source enclosure door glass O-ring to the source enclosure door.
5. Fit the source enclosure door glass to the source enclosure door.
6. Fit the four source enclosure door glass retaining clips to the source enclosure door.
7. Use a hex (Allen) key to fit and tighten the four bolts securing the source enclosure door glass retaining clips to the source enclosure door.

   Tip: Ensure that the source enclosure door O-ring tail is correctly located in its groove when fitting the O-ring to the source enclosure door.

8. Fit a new source enclosure door O-ring to the source enclosure door.
9. Fit the source enclosure to the pumping block.

   Tip: The securing screws must each be sequentially tightened a small amount until they are all fully tight; this ensures that the source enclosure is uniformly seated on the pumping block.

10. Fit and tighten the three source enclosure securing screws.

11. Close the source enclosure door and fasten the securing clips.
12. Connect the Probe electrical connection at the instrument's front panel (see the figure "Probe and source connections" on page 6-10).
13. Connect the PTFE tubing to the Desolvation gas connection at the instrument's front panel.
14. Install the ESI or APCI probe, as required (see page 2-5 and page 2-13).
15. Start up the instrument (see page 2-2).

Warning: To confirm the integrity of the source exhaust system, you must perform a pressure test on the source, as described in the *Waters Source Pressure Test Unit Operator's Guide*.

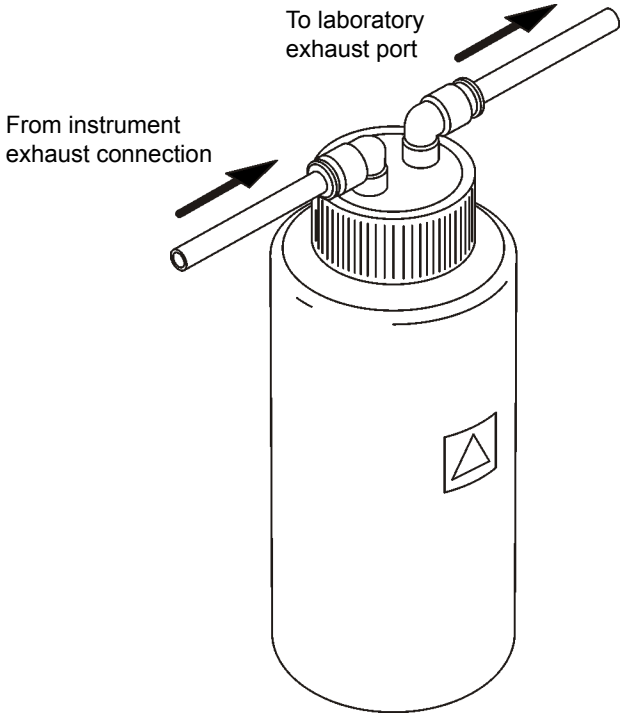
16. Perform a pressure test on the source.

Emptying the nitrogen exhaust waste bottle

Warning: The waste liquid in the nitrogen exhaust waste bottle comprises LC solvents and analytes. Always wear nitrile gloves while handling the nitrogen exhaust waste bottle, and ensure that the waste liquid is correctly disposed of according to local environmental regulations.

The nitrogen exhaust waste bottle in the nitrogen exhaust line must be emptied before it is completely full.

Nitrogen exhaust waste bottle



To empty the nitrogen exhaust waste bottle:

1. In the MassLynx™ Tune window, click Press for Standby and confirm that the adjacent instrument status indicator shows red.
2. In the MassLynx Tune window, click  , to stop the nitrogen flow.
3. Disconnect the instrument exhaust and laboratory exhaust system lines from the nitrogen exhaust waste bottle.
4. Dispose of the waste liquid in accordance with local environmental regulations.
5. Connect the instrument exhaust and laboratory exhaust system lines to the nitrogen exhaust waste bottle.
6. In the MassLynx Tune window, click  , to start the nitrogen flow.
7. In the Source page Gas Flow pane, set Desolvation (L/hr) to 1200.

8. Set Cone (L/hr) to 300.

Warning: To confirm the integrity of the source exhaust system, a leak test must be performed.

Tip: To avoid damage to the instrument, Snoop® (or equivalent) leak detector liquid must only be used only for the purpose described in the following step; it must not be used on any other part of the instrument.

9. Use Snoop (or equivalent) leak detector liquid to ensure that there are no leaks at the instrument exhaust and laboratory exhaust system line connections.

Replacing parts

Replacing the ion block cartridge heater

If the cartridge heater fails to heat it must be replaced.

Required materials

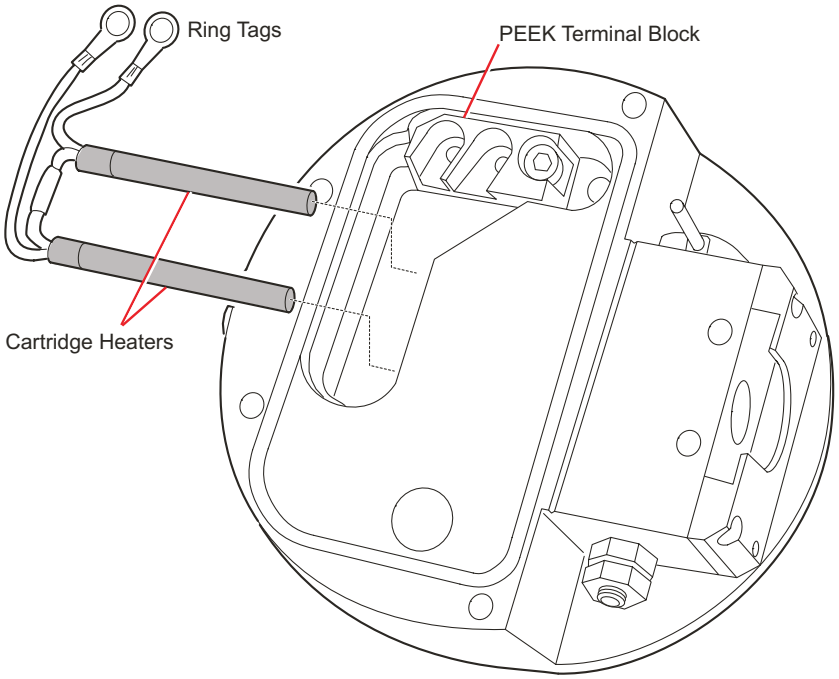
- 3 mm hex wrench.
- 1.5 mm hex wrench.
- Flat-blade screwdriver
- Needle-nose pliers

Procedure

To replace the ion block cartridge heater:

1. Follow the procedure for venting the instrument as described on page 6-7.
2. Remove the two screws securing the heater cartridge wires to the PEEK terminal block.
3. Carefully swing the ring tags out of the terminal block.

Ion block heater

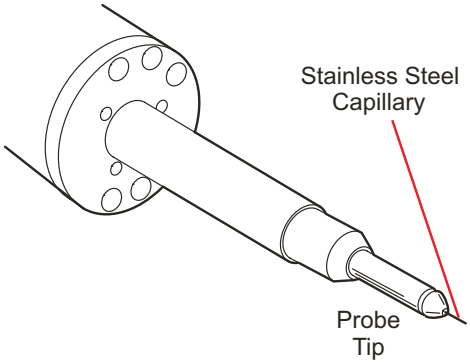


4. Loosen the two set screws that secure the heater cartridges in the ion block, using the 1.5-mm hex wrench.
5. Gently slide the heater cartridges out of the ion block using the needle-nose pliers.
6. Slide the new heater cartridges into the ion block with the needle-nose pliers. Secure them with two hex-head set screws and the 1.5-mm hex wrench.
7. Position the two heater cartridge ring tags onto the PEEK block terminals with the bent portion of their shafts extending into the pumping block
8. Tighten the two terminal block screws with a flat-blade screwdriver.
9. Refit the ion block cover plate, and secure with the four hex screws.
10. Close the source enclosure door and fasten the door's securing clips.
11. Install the probe.

Replacing the ESI probe stainless steel capillary

The stainless steel sample capillary on the ESI probe must be replaced if it clogs and cannot be cleared, or if it becomes contaminated or damaged.

ESI probe sample capillary



Required materials

- Flat-blade screwdriver
- 1.5-mm hex wrench
- 1/4-inch (6-mm) wrench
- 5/16-inch wrench
- 7/16-inch wrench
- Loupe (magnifying glass)
- Needle-nose pliers

Procedure

Caution: All work done on the probe should be carried out on a clean work bench.

To replace the stainless steel capillary:

1. Switch the instrument into standby and remove the probe from the source.

2. Remove the two end-cover retaining screws on the ESI probe with the flat-blade screwdriver.
3. Loosen the set screw on the LC PEEK union with the 1.5-mm hex wrench, and remove the probe's end-cover.
4. Unscrew the probe tip with the 1/4-inch (6-mm) wrench, and remove it.
5. Remove the LC union with the 5/16-inch and 7/16-inch wrenches.
6. Remove the capillary from the coupling nut with the 7/16-inch wrench. Discard the capillary and the PTFE liner and ferrule assembly.
7. Remove the conductive sleeve from the inner bore of the probe assembly fitting.
8. Slide a new ferrule onto the liner tube with the needle-nose pliers.
9. Slide the coupling nut onto the capillary, followed by the PTFE liner tube and ferrule.
10. Connect a piece of 0.007-inch PEEK tubing with finger-tight nut and ferrule into the opposite side of the LC union, setting the capillary's depth.
11. Press the capillary into the union until it seats, and tighten the adapter nut to the LC union until it is snug, but not tight.
12. Pull on the capillary gently, testing to ensure it stays in place.
13. Remove the PEEK tubing from the union.
14. Slide the conductive sleeve onto the capillary, then feed the capillary through the probe.
15. Attach the coupling nut to the probe, and gently tighten it with the 7/16-inch wrench.
16. Replace the probe tip, and screw down until a 0.5-mm length of the capillary protrudes from its end. Use the loupe, provided in the startup kit, to check the length of capillary that protrudes from the probe tip.

    Caution: Check for leaks carefully! Leakage can destroy a probe.

17. Reconnect the LC line, turn on the fluid flow, and check the probe for liquid leaks.

18. If a leak is found, disassemble the probe and tighten the fittings at the LC union.
19. Attach the nebulizer gas connection and turn on the nitrogen, by clicking  on the toolbar (or choose Gas from the Gas menu) on the tune page.
20. Check the probe tip for nitrogen leaks. If a leak is found, replace the probe tip assembly and its O-ring.
21. Refit the probe end-cover, and secure it with the two slotted screws. Tighten the set screw to clamp the LC union in place.

Replacing the ESI probe tip

The ESI probe tip must be replaced if the following problems occur:

- A blockage occurs in the internal metal sheathing through which the stainless steel capillary passes.
- The threads sustain damage.

Replace the O-ring if gas leaks from the O-ring.

Required materials

- 1/4-inch (6-mm) open-end wrench
- Loupe (magnifying glass)

Procedure

Caution: All work done on the probe should be carried out on a clean work bench.

To replace the ESI probe tip:

1. Switch the instrument into standby and remove the probe from the source.
2. Unscrew and remove the probe tip with the 1/4-inch (6-mm) wrench.
3. Install the new probe tip, and screw down until 0.5 mm of the capillary protrudes from the end. Use the loupe (provided in the startup kit) to check the capillary position.

Replacing the APcI probe heater

The APcI probe heater must be replaced if it fails to heat.

Required materials

1 -m hex wrench or flat-blade screwdriver, depending on the type of screw that secures the probe tip.

Procedure

Caution: All work done on the probe should be carried out on a clean work bench.

To replace the probe heater:

1. Switch the instrument into standby and remove the probe from the source.
2. Loosen the two set screws at the base of the probe tip assembly, and slide the probe tip off.
3. Separate the heater from the probe body, pulling it parallel to the axis of the probe.
4. Carefully install the new heater onto the probe. Take care not to damage the fused silica capillary.
5. Refit the probe tip assembly, and secure it with the two set screws.

Replacing the APcI fused silica capillary and filter pad

Replace the fused silica capillary and/or filter pad when a decreased signal intensity and increased back pressure is noted.

Required materials

- 1.5-mm hex wrench
- 5/16-inch open-end wrench
- 7/16-inch open-end wrenches (2-off)
- Ceramic capillary cutter (from the tools kit)
- Butane lighter or match

- HPLC-grade methanol
- Flat-blade screwdriver
- Lint-free paper towels
- Loupe (magnifying glass)

Spare parts

- GVF004 ferrules (2-off)
- Fused silica
- Filter pad

Procedure

Caution: All work done on the probe should be carried out on a clean work bench.

To replace the fused silica capillary and filter pad:

1. Switch the instrument into standby and remove the probe from the source.
2. Slide the probe tip and heater assembly off the probe.
3. Using the flat-blade screwdriver, remove the two slotted screws.
4. Using the 1.5-mm hex wrench, loosen the two set screws that retain the LC filter, then remove the probe end.
5. Remove the filter cartridge from the adapter nut with one 5/16-inch and one 7/16-inch wrench. If the ferrule remains inside the cartridge, remove it.
6. Separate the two halves of the filter cartridge with two 7/16-inch wrenches.
7. Remove the old filter pad and replace it with a new one.
8. Unscrew the adapter nut from the probe with a 5/16-inch wrench. Discard the fused silica capillary.
9. Using the ceramic capillary cutter, cut a new length of fused silica 161.5 mm long. Cut the capillary squarely. Examine new cuts for squareness with a loupe.

10. Remove approximately 20 mm of polyamide coating from the capillary end with a flame, then clean it with a methanol-saturated tissue.
11. Slide a GVF004 ferrule, followed by the adapter nut and another GVF004 ferrule, onto the capillary.
12. Position the filter with the flow direction arrow pointing toward the adapter nut, then connect the adapter nut to the filter. Make sure the capillary seats squarely against the filter cartridge interior.

    Caution: Overtightening the adapter nut can damage the capillary.

13. Tighten the adapter nut until the capillary is snug in the fitting, then gently pull on the capillary to ensure it stays in place
14. Feed the sample capillary through the probe, and gently tighten the probe adapter nut with the 5/16-inch wrench. The capillary must protrude 1.0 mm from the nebulizing tube.
15. Refit the probe end-cover and retaining screws.
16. Tighten the set screws in the probe end cover with the 1.5-mm hex wrench to clamp the filter in place.
17. Refit the probe tip and heater assembly.

7 Troubleshooting

This chapter describes how to troubleshoot the Quattro micro API with the help of recommended troubleshooting procedures.

Contents

Spare parts

Parts not included in this document are not recommended for replacement by the customer.

Safety and handling

When troubleshooting the Quattro micro API, keep the following safety considerations in mind.

Warning: To avoid the possibility of electric shock,

- never disconnect an electrical assembly while power is applied to the Quattro micro API. Once power is turned off, wait approximately 10 seconds before disconnecting an assembly.
- To avoid the possibility of electric shock, do not remove the instrument panels. There are no user-serviceable items inside.

Warning: To prevent injury, always observe good laboratory practices when handling solvents, changing tubing, or operating Quattro micro API. Know the physical and chemical properties of the solvents used. Refer to the Material Safety Data Sheets for the solvents in use.

Caution: To prevent circuit damage due to static charges, do not touch integrated circuit chips or other components that do not specifically require manual adjustment.

System troubleshooting

There are a few basic steps for performing system troubleshooting:

- Examine the system, checking the simple things first. Is something obvious causing the problem (for example, is the instrument and its cables improperly connected, is there any leakage of fluid, vacuum or gas?)
- Compare current system operation with the way the system operated before the problem started. To help identify normal operating conditions:
  - Record a map of the LC system (tubing and electrical connections).
  - Keep a daily log.
  - Run test samples regularly. Check the instrument performance with known samples, preferably the ones used for instrument acceptance.

    This illustrates the importance of keeping track of system parameters and the performance during normal operation. Troubleshooting is easier if the typical conditions when the system is operating correctly are known.

    For example, are the system tuning parameters similar to those when a test species was previously run? Are the lens settings required for optimum sensitivity higher than those previously obtained? If extreme values have to be used to achieve good results, this implies that some part of the system requires attention.
- Methodically check and eliminate possible causes to identify the system parameter that is atypical.
- Refer to the troubleshooting information in the following sections. These tables enable possible causes of a symptom to be identified and suggested corrective actions to be taken.

If it is determined that there is a problem related to another system component (for example HPLC, autosampler, UV detector) refer to the appropriate operator's manual.

Component hardware troubleshooting

The following tables provide suggestions for resolving hardware problems.

No peaks on the tune page (no ion beam)

No peaks on the tune page (no ion beam)

Possible cause	Corrective action
Operating parameters (Capillary/Corona, Cone, Extractor, RF Lens, Ion Energy, Gas Nitrogen and Heaters) on the tune page are improperly set.	Optimize parameters. Refer to page 2-12. Once a beam has been obtained, ensure that all lenses affect the beam in a sensible manner.
Cables are not properly connected.	Check that all necessary cables have been correctly attached to the source and probe.
Instrument is not in the operate mode.	Put the instrument into operate by clicking Operate. When in the operate mode, this icon is green and the Operate LED on the front panel is also green.
Communication failure.	Re-initialize by going to the tune page and selecting Options. Reboot the embedded PC.
No sample is present.	Check that sample is loaded correctly in the autosampler or in the syringe pump syringe.
Isolation valve is closed.	Open the isolation valve.
The source components are dirty.	Clean the source components. Refer to page 6-7.
Insufficient nitrogen flow.	Check that the nitrogen pressure is 6 to 7 bar (90 to 100 psi) and the gas flow rate on the tune page is >100 L/h. The desolvation and probe heaters shut off when the nitrogen flow rate falls below 50 L/h.

No peaks on the tune page (no ion beam) (Continued)

Possible cause	Corrective action
No LC flow.	Check solvent flow from the autosampler or syringe pump.
Fluid leak in the HPLC system.	Replace the APcI fused silica capillary. Refer to page 6-33.
Source components have been incorrectly assembled.	Check that the source and probe voltage readbacks vary with the tune page settings. If any of these voltages are absent, disassemble and correctly reassemble the source and hexapole lens assemblies. Refer to page 6-7 and page 6-17.
Blocked ESI or APcI capillary	Replace capillary. Refer to page 6-30 and page 6-33.

Unsteady or low intensity peaks (ion beam)

Unsteady or low intensity peaks (ion beam)

Possible cause	Corrective action
Poor nebulization.	Check that the source and desolvation temperature and gas flow settings are suitable for the flow rate. Liquid inside the source enclosure is an indication that the temperature is too low. The nitrogen pressure should be from 6 to 7 bar (90 to 100 psi) and desolvation nitrogen flow rate should be greater than 100 L/h. Check the stability of the nitrogen flow (good quality 2-stage regulator).
Problem with sample delivery (autosampler, syringe pump or HPLC system).	Troubleshoot the autosampler. Check the syringe in the syringe pump for leaks and grounding. Check for sufficient sample in the vials. Look for pressure variation on injection.

Unsteady or low intensity peaks (ion beam) (Continued)

Possible cause	Corrective action
Fluid leak in the HPLC system.	Check for leaks in the HPLC system and correct.
Source components require cleaning.	Clean source components. Refer to page 6-7.
Lens settings wrong or atypical.	Check all settings are correct. Check readbacks are sensible. Check that all lens parameters affect the beam.
Cone or collision cell voltage ramp is on.	Set the voltage ramp off.
ESI or APcI capillary is not properly installed.	Check that the probe position is correct. Check that the ESI probe stainless steel capillary protrudes 0.5 mm as described on page 6-30. Check that APcI capillary height is set at 1.0 mm. Refer to page 6-33.
Corona pin is not correctly aligned.	Check the alignment as described on page 6-19.
CID gas pressure is wrong.	Infuse sample and optimize the gas pressure. Check the CID gas regulator is set to 0.5 bar, and is not leaking.
Collision cell parameters incorrect.	Check Entrance, Exit and Collision are optimized, with sensible readbacks.
Analyser and multiplier parameters incorrect.	Ensure Multiplier is 650 V. Check the ion energy and resolution parameters are set correctly for the acquisition.

Unusually high LC back pressure

Unusually high LC back pressure

Possible cause	Corrective action
Blockage in the capillary or injection loop due to particulate matter from the sample.	Remove the probe from the source, and increase the solvent flow to 500 µL/min to clear the blockage.
APcI probe filter pad is blocked.	Replace the filter pad. Refer to page 6-33.
Tubing from LC system is blocked.	Remove the finger-tight nut and tubing from the back of the probe. If the back pressure remains high, replace the tubing.
The ESI stainless steel sample capillary inside the probe is blocked.	Replace the capillary, see page 6-30.
The ESI capillary is not fully seated in the LC union or the APcI capillary is not properly seated in the filter.	Remove and disassemble the probe and reseat the capillary correctly in the union.

Unusually low LC back pressure

Unusually low LC back pressure

Possible cause	Corrective action
Leaking connector.	Check all fittings and tighten if necessary.
Problem with LC solvent delivery.	Troubleshoot the LC system.
Broken flow cell in UV detector.	Replace the flow cell.

Insufficient vacuum

Any reading greater than 5×10^{-4} mbar on the Pirani gauge, when CID gas is off.

Insufficient vacuum

Possible cause	Corrective action
Leaking ion block O-rings.	Disassemble source and check condition of ion block O-rings. Refer to page 6-7.
Roughing pump not operating correctly.	Gas ballast the rotary pump to return accumulated oil from the oil mist filter. Check vacuum pump oil. If the oil is dirty, flush the pump with clean oil, then fill the pump with oil. Repeat if necessary.
Leak in vacuum backing line.	Check vacuum hose for cracks or vacuum leaks.
Restriction in vacuum pump exhaust tubing.	Check exhaust line for restrictions.
Turbo pump not operating properly.	Check turbo pump speed on the Diagnostics tune page.

Leaking nitrogen

Warning: To confirm the integrity of the source exhaust system, if any of the O-rings identified on page 6-30 are renewed, you must perform a source pressure test as described in the *Waters Source Pressure Test Unit Operator's Guide*.

Hissing sound or solvent smell.

Leaking nitrogen

Possible cause	Corrective action
Poor seal around the source enclosure.	Visually inspect the source enclosure sealing surfaces for imperfections or nicks. Also, check the condition of the encapsulated O-rings.

Vacuum oil accumulated in the exhaust tubing

Vacuum oil accumulated in the exhaust tubing

Possible cause	Corrective action
Oil mist filter needs replacement.	Replace oil mist filter element and odor filter.

Source heater and desolvation heater not heating

Source heater and desolvation heater not heating

Possible cause	Corrective action
Source heater has failed.	Check readback. Replace heater if necessary.
Main system PCB fuse failed.	Check Desolvation Temp readback. Call Waters Technical Service if wrong.

APcI heater not heating

APcI heater not heating

Possible cause	Corrective action
If desolvation heater is OK when in ESI mode, then the APcI heater may need to be replaced.	Replace the APcI heater. Refer to page 6-33.

Roughing pump fuse fails

Roughing pump fuse fails

Possible cause	Corrective action
Oil mist filter element is saturated. Vacuum oil may also be accumulating in exhaust tubing.	Replace oil mist filter element and odor filter. Replace the fuse.
System needs to be ballasted.	Ballast the pump for 20 to 30 minutes. Refer to page 6-4.
The AC line voltage is less than 208 V AC	The AC line voltage to the instrument must be checked by a qualified electrician.
Vacuum pump oil is very dirty.	Change vacuum pump oil. Refer to page 6-6.

Ion mode fault

Drop-down menu options are grayed out, or instrument spontaneously switches probe type.

Ion mode fault

Possible cause	Corrective action
One, or both, of the probe contact pins jammed inside the probe and are not making contact with probe support plate.	Remove probe cover, free the contact pin, and ensure that both pins and associated springs move freely within the bushing.

Failure to recognize one particular probe type

Failure to recognize one particular probe type

Possible cause	Corrective action
Problem with the probe.	Remove and try another probe of the same type. Check that the Source Recognition ID voltage on the Diagnostics page is <2 V for ESI, and >2 V for APcI.

Ripple

Peaks and baseline appear to vary cyclically in intensity.

Ripple

Possible cause	Corrective action
Erratic LC solvent flow.	Troubleshoot the LC system.
Poor nebulization due to incorrect temperature and gas flow settings.	Adjust the temperature and gas flow settings. Liquid in the source enclosure is an indication that the temperature is too low.

Ripple (Continued)

Possible cause	Corrective action
Vibration from the rotary pumps or even other equipment in the same building.	Check for, and eliminate, excessive bench top and instrument vibration.

Loss of communication with instrument

Loss of communication with instrument

Possible cause	Corrective action
Instrument to MassLynx host communication failed.	Reset the workstation and reboot the embedded PC from the front panel using a short length of PEEK tubing to engage the reset switch, see the figure “Front panel” on page 1-12. Wait 3 minutes for the audible signal indicating the embedded PC has booted from Quattro micro API before starting MassLynx.

IEEE communication errors

IEEE communication errors

Possible cause	Corrective action
Instruments powered up in the wrong sequence.	Power down the system components and start up the system components in the correct order: 1. Workstation 2. Quattro micro API 3. Inlet modules Wait 3 minutes for the audible signal indicating the embedded PC has booted from Quattro micro API before starting MassLynx.
Wrong IEEE address, or conflicting address.	Check system IEEE settings and enter the correct addresses.
Faulty IEEE cable in IEEE chain.	Systematically replace IEEE cables until the problem cable is located.
Network cables confused with site network.	Ensure that the network cable for the instrument is connected to the correct network card in the PC. Ensure that the network card with the BNC connector is configured to the site network.

High noise levels in MRM analyses

The background noise in MRM analysis can be either electronic or chemical. To distinguish between the two:

1. Start an acquisition.
2. During the acquisition, set Ion Energy 1 and Ion Energy 2 fully negative on the tune page.

A significant decrease in signal when the ion energies are set negative, implies that the major contribution to the overall noise is chemical.

Any residual noise is electronic.

Chemical noise

Chemical noise

Possible cause	Corrective action
High background due to carry-over after tuning with strong concentrations.	Repeat injections of 10% formic acid and/or isopropanol.
Contaminated injector. (Signal changes upon injection of mobile phase).	Repeat injections of 10% formic acid and/or isopropanol.
Contaminated tubing.	Replace tubing.
Contaminated probe.	Flush with methanol at 0.5 mL/min until the background level falls. Replace the ESI stainless steel capillary, see page 6-30. Replace the APcI fused silica capillary, see page 6-33.
Contaminated HPLC system.	Infuse mobile phase from the solvent reservoir using a syringe pump. Compare MRM background levels.Check purity of solvents. Replace if necessary.Ensure all solvents are HPLC grade.
Contaminated glassware.	Ensure glassware is not cleaned with commercial surfactants in the cleaning process.

Electronic noise

Corrective action
Check that the valleys of peak-peak noise, when ion energies are fully negative, just touch the baseline. Increase Ion Counting Threshold to suit.

Contacting Waters

Many problems with the Quattro micro API can be easily corrected by the user. However, if this is not the case, it is necessary to contact Waters.

When contacting Waters, have the following information available:

- Nature of the symptom.
- Quattro micro API serial number.
- Details of flow rate, mobile phases and sample concentrations.
- Details of gas cell operating pressure.
- Tune page settings.
- Software version update reference.

8 Reference Information

Contents

Overview

Calibration reference files consist of two columns of numbers separated by any number of spaces or TAB characters. The first column contains the reference peak masses and the second column contains the reference peak intensities.

The reference files listed in this chapter have all ion intensities set to 100%. Actual ion intensities are not, of course, all 100%, but the calibration software does not take account of the ion intensities and this is a convenient way to store the reference files in the required format. However, if required, realistic intensity values can be entered to improve the appearance of the reference spectra.

Most samples can be purchased from the Sigma chemical company. To order, contact Sigma at http://www.sigma.sial.com. This site contains a list of worldwide Sigma offices, many with local toll-free numbers.

Editing a reference file

Calibration reference files can be created or edited using any Windows text editor.

To read the currently selected reference file into the Notepad text editor:

Press  , or select Reference File from the Calibration, Edit menu.

To save the reference file after editing either:

Select Save from the Notepad File menu to save the file under the current name.

Or:

Select Save as from the Notepad File menu to save as a new reference file with a new name.

Textual information or comments can be stored in the reference file. Lines which are textual information or comments must start with the semi-colon (;) character.

Positive ion

Ref. file name	Chemical name [sigma code #]	Molecular mass	m/z	Uses
UBQ	Bovine Ubiquitin [U6253]	8564.85	650 to 1500	General
HBA	Human α globin [H753]	15126.36	700 to 1500	Hb analysis
SOD	Superoxide dismutase [S2515]	15591.35	900 to 1500	Hb (internal calibration)
HBB	Human β globin [H7379]	15867.22	800 to 1500	Hb analysis
MYO	Horse heart myoglobin [M1882]	16951.48	700 to 1600	General
PEGH1000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000		80 to 1000	ES+ and APcI+ calibration
PEGH2000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000+1450		80 to 2000	ES+ calibration
NAICS	Sodium iodide/cesium iodide mixture		20 to 4000	General, ES+ calibration
NAIRB	Sodium iodide/rubidium iodide mixture		20 to 4000	ES+ calibration

Horse heart myoglobin

Reference File: MYO.REF

Molecular Weight: 16951.48

Charge state	Calculated m/z value	Charge state	Calculated m/z value	Charge state	Calculated m/z value
28^+	606.419	21^+	808.222	13^+	1304.969
	616.177	20^+	848.583	12^+	1413.633
27^+	628.841	19^+	893.192	11^+	1542.053
26^+	652.989	18^+	942.758	10^+	1696.158
25^+	679.068	17^+	998.155	9^+	1884.508
24^+	707.320	16^+	1060.477	8^+	2119.945
23^+	738.030	15^+	1131.108	7^+	2422.651
22^+	771.531	14^+	1211.829		

Polyethylene glycol

PEG + $NH4^+$

Reference Files: PEGH1000.REF, PEGH2000.REF

Calculated m/z value				
63.04	459.28	855.52	1251.75	1647.99
107.07	503.31	899.54	1295.78	1692.01
151.10	547.33	943.57	1339.80	1736.04
195.12	591.36	987.60	1383.83	1780.07
239.15	635.39	1031.62	1427.86	1824.09
283.18	679.41	1075.65	1471.88	1868.12
327.20	723.44	1119.67	1515.91	1912.15
371.23	767.46	1163.70	1559.94	1956.17
415.25	811.49	1207.73	1603.96	2000.20

Sodium iodide and caesium iodide mixture

Reference File: NAICS.REF

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
132.9054	922.3552	1821.7206	2721.0861	3620.4515
172.8840	1072.2494	1971.6149	2870.9803	3770.3457
322.7782	1222.1437	2121.5091	3020.8745	3920.2400
472.6725	1372.0379	2271.4033	3170.7688	
622.5667	1521.9321	2421.2976	3320.6630	

Sodium iodide and rubidium iodide

Reference File: NAIRB.REF

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
84.9118	922.3552	1671.8264	2571.1918	3620.4515
172.8840	1072.2494	1821.7206	2721.0861	3770.3457
322.7782	1222.1437	1971.6149	2870.9803	3920.2400
472.6725	1372.0379	2121.5091	3020.8745	
622.5667	1521.9321	2271.4033	3170.7688	

Negative ion

Ref. file name	Chemical name [sigma code #]	Molecular mass	m/z	Uses
MYONEG	Horse heart myoglobin [M1882]	16951.48	700 to 2400	General
SUGNEG	Sugar mixture of: maltose [M5885] raffinose [R0250] maltotetraose [M8253] corn syrup [M3639]		100 to 1500	Low mass range
NAINEG	Sodium Iodide/Caesium Iodide (or Rubidium Iodide) mixture		200 to 3900	ES-calibration

The purpose of the rubidium iodide is to obtain a peak at m/z 85 ($^{85}Rb^{+}$) with an intensity of about 10% of the base peak at m/z 173. Rubidium iodide has the advantage that no rubidium clusters are formed which may complicate the spectrum. Note that rubidium has two isotopes (^{85}Rb and ^{87}Rb) in the ratio 2.59:1, giving peaks at m/z 85 and 87.

Use reference file NAIRB.REF.

Sodium iodide solution for negative ion ElectroSpray

Either of the above solutions is suitable for calibration in negative ion mode. In both cases the first negative reference peak appears at m/z 127 (I^{-}) and the remaining peaks are due to NaI clusters.

Use reference file NAINEG.REF.

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

Contents

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.


Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)


Warning: (Risk of burn injury from contacting hot surfaces.)


Warning: (Risk of electric shock.)


Warning: (Risk of fire.)


Warning: (Risk of sharp-point puncture injury.)


Warning: (Risk of hand crush injury.)


Warning: (Risk of exposure to ultraviolet radiation.)


Warning: (Risk of contacting corrosive substances.)


Warning: (Risk of exposure to a toxic substance.)


Warning: (Risk of personal exposure to laser radiation.)

Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Warning: (Risk of tipping.)

Warning: (Risk of explosion.)

Warning: (Risk of eye injury.)

Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.

Warning: Pressurized nonmetallic, or polymer, tubing can burst. Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.

Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.

Warning: To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.

Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.

Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.

Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals.*

Caution symbol

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.

Caution: To avoid damage, do not use abrasives or solvents to clean the instrument's case.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality control procedures and the equipment guidelines in this section.

Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意：未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

注意：未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

주의: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

注意：規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

Attenzione: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

警告：当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告：圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告：使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

警告：使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。

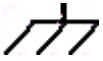
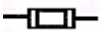
경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告：ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

Electrical and handling symbols

Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

B Materials of Construction and Compatible Solvents

Warning: To confirm the integrity of the source exhaust system, you must address any safety issues raised by the contents of this Appendix.

Contents

Preventing contamination

For information on preventing contamination, refer to *Controlling Contamination in LC/MS Systems* (part number 715001307). Visit www.waters.com.

Items exposed to solvent

The items that appear in the following table can be exposed to solvent. You must evaluate the safety issues if the solvents used in your application differ from the solvents normally used with these items. See page B-3 for details about the most common ingredients used to prepare mobile phases.]

Items exposed to solvent

Item	Material
Corona discharge pin mounting contact	PEEK (Polyetheretherketone)
Gas exhaust port	Aluminium
Gas tubes	FEP (Fluorinated ethylene propylene)
Ion block	Stainless steel
Ion block support	PEEK (Polyetheretherketone)
Isolation valve	Gold-plated aluminium/bronze
O-rings	Viton® or PTFE (Polytetrafluoroethylene)-encapsulated Viton
Probe adjuster bellows	PTFE (Polytetrafluoroethylene)/Viton
Probe adjuster assembly	Anodized aluminium, glass filled acetal, and stainless steel
Probe shaft	PEEK (Polyetheretherketone)
Push-in gas fittings	Nickel/brass
Source enclosure	Alochromed aluminium

Items exposed to solvent (Continued)

Item	Material
Source enclosure view port	Toughened plate glass
Waste bottle	Polypropylene

Solvents used to prepare mobile phases

The following lists the most common ingredients used to prepare mobile phases for reverse-phase LC/MS (API):

- Water
- Methanol
- Acetonitrile
- Formic acid (<0.1%)
- Acetic acid (<0.1%)
- Trifluoroacetic acid (<0.1%)
- Ammonium acetate (<10 mM)
- Ammonium formate (<10 mM)

These solvents are not expected to cause any problems with the materials identified on page B-2.

C Environmental Specifications

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Contents

Access

Doors through which the instrument is to be moved should be a minimum of 24 inches (0.6 m) wide. Elevators and corridors must be wide enough to allow corners to be negotiated. Special arrangements may be required if access to the laboratory is via a staircase.

Location

Quattro micro API

The Quattro micro API may be installed upon any flat bench top.

The instrument should not be placed close to heavy machinery (compressors, generators, etc.) which causes excessive floor vibration.

The instrument should not be placed within a RF field of greater than specified in IEC 801-3 severity level 2.

Possible sources of RF emission include RF-linked alarm systems or LANs, portable telephones and hand held transmitters.

The instrument must be positioned away from strong magnetic fields such as those generated by NMR systems and magnetic sector mass spectrometers.

The instrument is shipped with a 8 feet (2.5 m) power cable that must be plugged into the rear of the chassis. The power ON/OFF switch is located on the front panel.

It is recommended that the Quattro micro API be positioned at the right-hand end of a bench to allow extra room for access to the electronics.

Vacuum pump

The rotary pump should be installed beneath, or behind, the Quattro micro API, within 5 feet (1.5 m) of the rear of the chassis. The rotary pump is fitted with a 6.5 feet (2 m) power cable which plugs into the rear of the instrument's chassis. It is recommended that the rotary pump is elevated 6 to 8 inches(15 to 20 cm) above the floor to aid in routine maintenance, such as changing pump oil.

If the rotary pump is sited under the instrument bench, an access slot may need to be drilled in the bench top for routing of vacuum tubing from the

instrument (see the figure “Horizontal clearances” on page C-4 for specifications). The access slot for the vacuum tubes must be within the footprint of the instrument and must be 31.3 inches (800 mm) from the proposed position of the instrument’s front face.

Data system

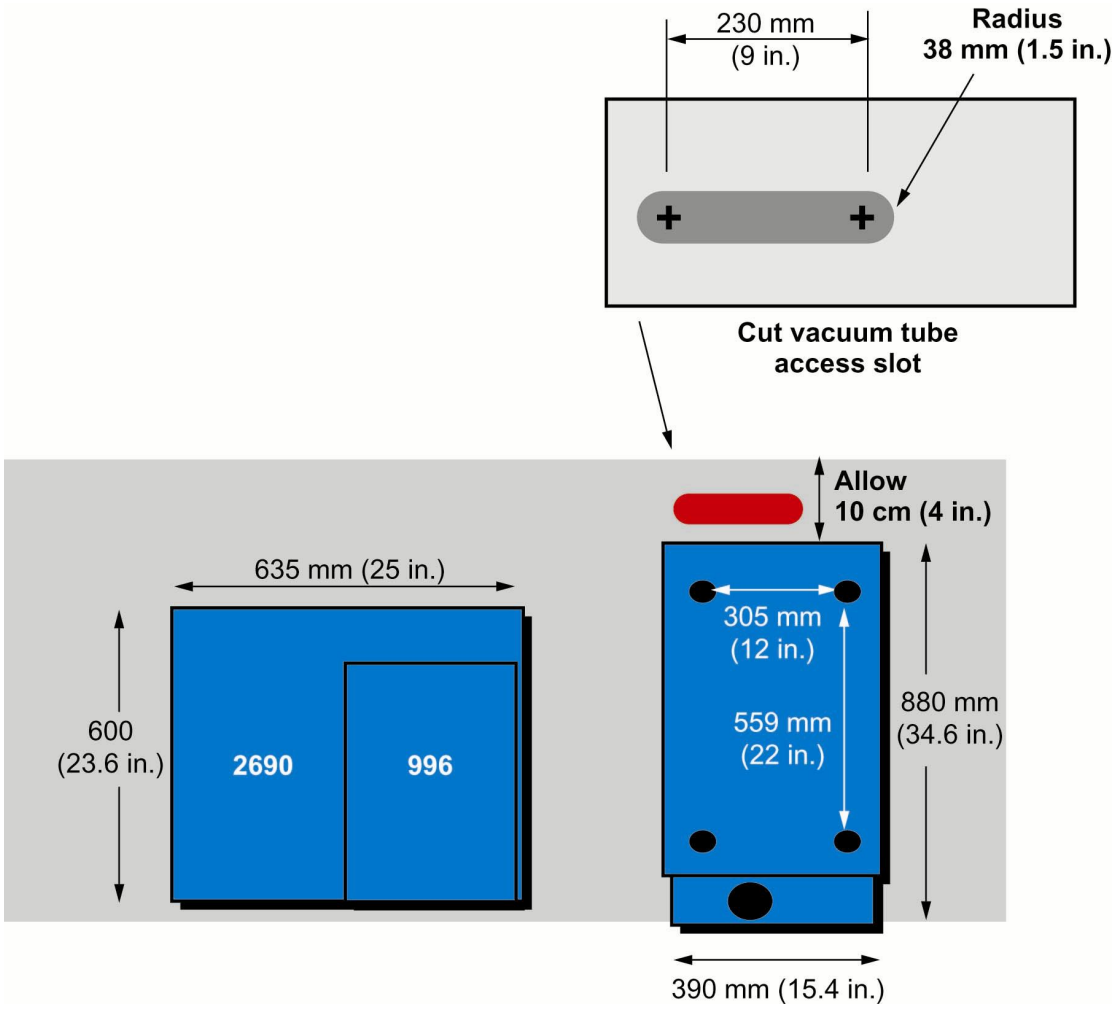
The data system must be located within 16 feet (5 m) of the mass spectrometer to allow connection of the communication cables. The data system power cables are approximately 6.5 feet (2 m) in length. A typical layout for the data system is shown in the figure “Data system” on page C-5.

LC system

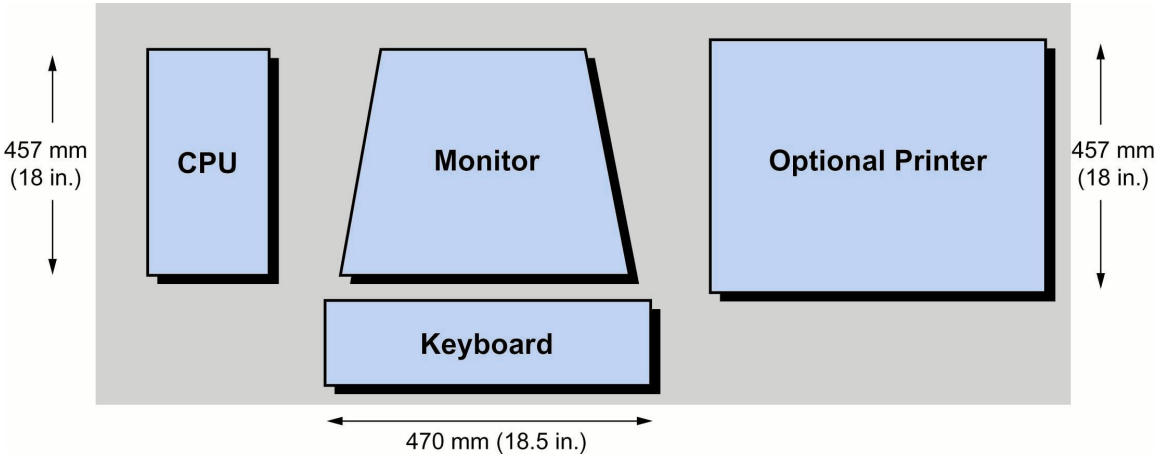
Be sure to allow adequate space to the left of the instrument for the HPLC system. Refer to the associated user manuals for individual space requirements.

Dimensions and clearances

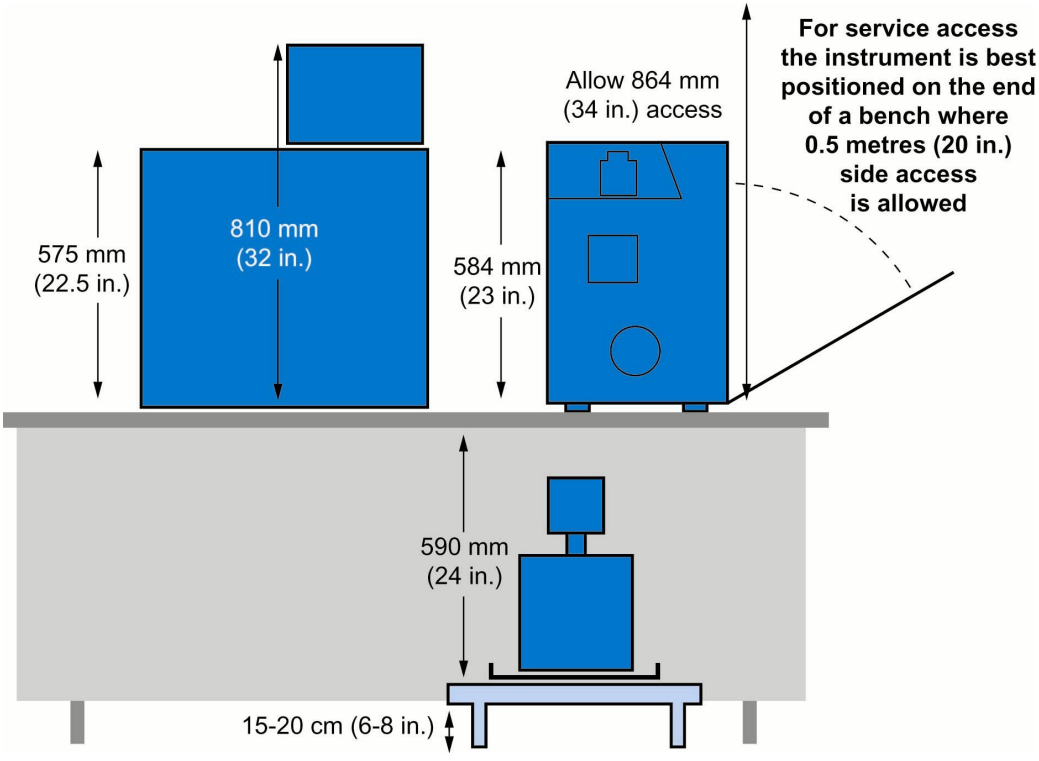
Horizontal clearances



Data system



Vertical clearances



Ideally, the following minimum clearances should be allowed for service access and ventilation:

- 8 inches(20 cm) on the right side [Note: 20 inches (0.5 m) is required for periodic service of the main PCB, refer to the figure “Vertical clearances” on page C-5.]
- 11 inches (28 cm) on the top

It is acceptable for the instrument to be placed with its back to a wall with a minimum of 4 inches (10 cm) clearance between the wall and the back of the instrument for ventilation.

Height

23 in. (572 mm)

Length

34.6 in. (880 mm)

Width

15.4 in.(390 mm).

Weights

The bench must be able to support the total weight of the system:

Instrument: 253 lb (115 kg)

Data system (computer, monitor and optional printer): 110 lb (50 kg)

Total Weight: 363 lb (165 kg)

Lifting and carrying

As the instrument weight is 253 lb (115 kg), it is recommended that either suitable lifting equipment, or an appropriate number of physically able personnel are available to lift a unit of this weight, positioned for equal distribution of the load. Waters personnel are not permitted to manually lift the instrument without suitable assistance.

As a general guide before lifting, lowering or moving the instrument:

- 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.

Operating temperature

The ambient temperature range for instrument operation is 15 to 30°C (59 to 86°F), with short-term variation (1.5 h) not exceeding 2°C (4°F). The ambient temperature range for rotary pump operation is 15 to 40°C (59 to 104°F).

The maximum overall heat dissipation into the room is 3.0 kW. This figure does not take into account ancillary equipment such as LC systems. Air-conditioning may have to be installed, or upgraded, to accommodate the additional heat load into the room.

Operating humidity

The relative humidity range for instrument operation is 20 to 80%. It is recommended that the instrument be situated in a draft-free position in an air-conditioned laboratory free from excessive amounts of dust.

Shipping and storage temperature

0 to 40°C (32 to 104°F).

D Electrical Specifications

Contents

Installation

It is recommended that the electrical installation in the laboratory include a wall-mounted emergency isolator switch of the correct rating, which is protected by a correctly-rated circuit breaker. The isolator should be clearly marked “Mass Spectrometer” and ideally positioned with clear access at all times for emergency switch-off, without risk of slipping or tripping. Ideally, the isolator should be capable of being locked in the OFF position to prevent unauthorized personnel from switching the instrument on. The Quattro micro API must have an earthed power supply that satisfies the voltage and current specifications for that country, detailed below:

Quattro micro API (including rotary vacuum pump)	
Voltage and Frequency	230 V, 50/60 Hz (+8%, -14%)
Minimum Current	13 A
Maximum Current	16 A
Power Consumption	2.0 kW
Power Cord Supplied	8 ft. (2.5 m), fitted with country plugs as shown below.

Canada:

The user must supply a fused supply, rated at 15 A.

UK:

The cable is fused at 13 A.

Japan:

A single phase, 3-wire 200 V 5% phase/neutral supply, rated between 13 and16 A must be supplied. Additionally, a transformer must be ordered from Waters to boost the input voltage.

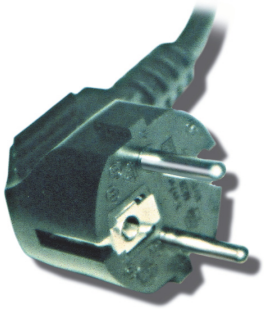
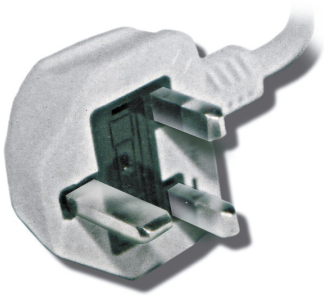
Australia and New Zealand:

The equipment has been designed in compliance with the international safety standard IEC1010-1 (EN61010-1). To be fully effective, the building installation must comply with AS 3000: Electrical Installations for Australia/New Zealand.

Rest of World:

The user must supply a fused supply, rated between 13 and 16 A.

Plugs supplied

		
USA NEMA L6-15P, 15 A	EUROPE 2-pin Europlug conforming to CEE7 standards	UK 3-pin UK plug, fused at 13 A, BS1633

For countries with a 60 Hz AC. supply (such as the USA), if the supply voltage is less than 208 V AC., it is recommended that a step-up transformer is used to boost the voltage to 208 to 230 V AC.

The mains supply must have at least a ±10% brown out capability, i.e. 10% for 0.3 seconds and transient ≤20ms to half mains voltage.

Appropriate sockets to mate with the plugs shown must be provided.

If a plug is required other than that shown, the user must supply the plug and the appropriate socket.

The instrument is shipped with a 8 feet(2.5 m) power cable that must be plugged into the rear of the chassis. The Quattro micro API power ON/OFF switch is located on the front panel.

Line frequency

50 Hz, 47 to 53 Hz.

60 Hz, 57 to 63 Hz.

Fuse rating

10 A, 250 V AC.

Electrical safety

The Waters Quattro micro API conforms to European standard EN61010-1:2001, Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.

The instrument is categorized as Pollution Category 1 and Installation Category 2.

E Performance Specifications

ElectroSpray positive ion

The measured signal/noise ratio obtained from the chromatogram monitoring the transition m/z 609 to m/z 195 on injection of 10 pg (16 fmol) of reserpine will be >20:1.

10 µL of a 1 pg/µL reserpine solution in 50/50 acetonitrile/water (no additives) will be injected at a flow rate of 200 µL/min, in MRM mode, 1 second dwell, span 0 Da. The resolution of the ion at m/z 609 will be ≤1 Da peak width at half height (MS1 and MS2).

ElectroSpray negative ion

The signal/noise ratio measured on the [M-H]- peak at m/z 503 from a sample consumption of 10 ng raffinose will be >100:1.

A solution of 5 ng/µL Raffinose in 50/50 acetonitrile/water (no additives) will be introduced at a flow rate of 10 µL/min and the summation of two 6 second scans over the mass range m/z 100 to 600 represents a total sample consumption of 10 ng.

APcI positive ion

Measured signal/noise ratio obtained from the mass chromatogram of m/z 609 in SIR mode on direct injection of 10 pg (16 fmol) reserpine (10 µL injection of a 1 pg/µL solution) at a flow rate of 1 mL/min will be >10:1.

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