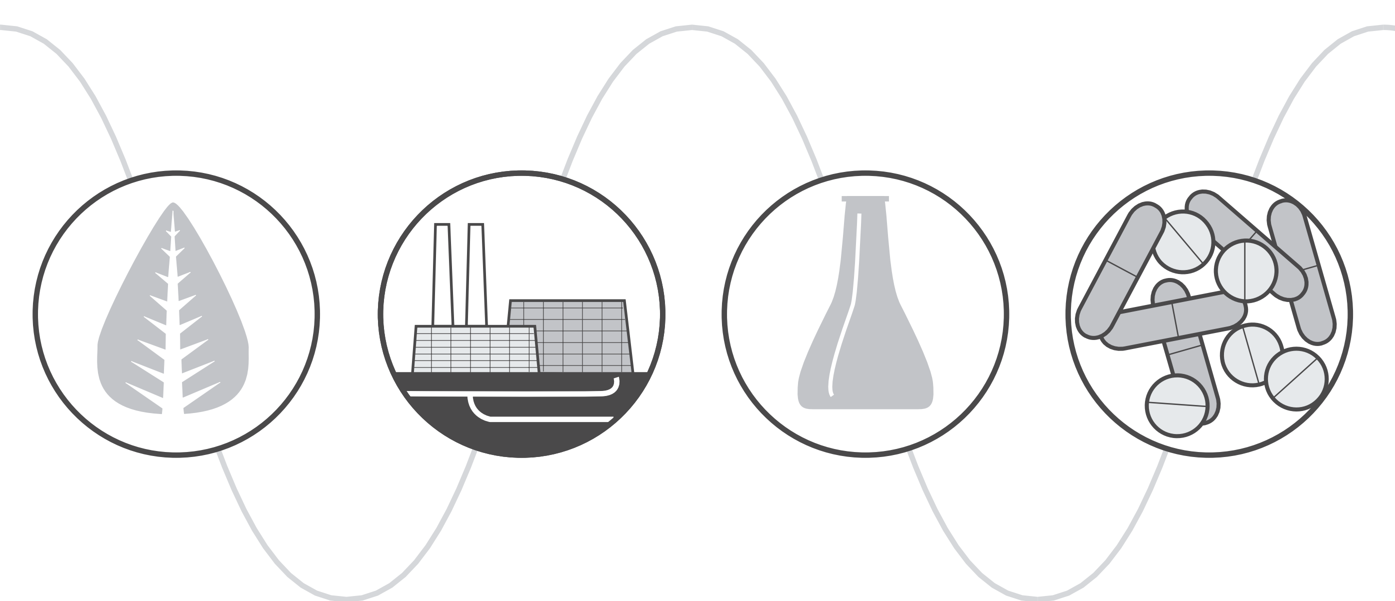
Micromass Quattro *micro* API Mass Spectrometer

Operator's Guide



Waters

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Safety
Sécurité

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

Ce pictogramme indique la presence de **heute tension**.

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

Ce pictogramme indique la presence de **surfaces chaudes**.

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

Ce pictogramme indique la necessite de se réferer au manuel d'utilisation.

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

Des avertissements sont donnés dans ce manuel aux endroits où l'utilisateur doit être particulierement prudent pour eviter les blessures.

High voltages	Hot surfaces	Poisonous hazard	Chemical hazard	Flammable material	General hazard
Heute tension	Surfaces chaudes	Risques d’empoisonement	Chimiques dangereux	Produits inflammables	Hazard général

To maintain the safety integrity of the instrument it should be used in a Pollution Degree 1 environment.
The power circuits are designed for a classification of Installation Category 1 (over voltage category)

Afin de garantir la sécurité de l'appareil il doit être utilisé dans un environment de degré 1 de pollution.
Les circuits électriques sont fabriqués pour une classification d'installation de Catégorie 1 (survoltage)

To maintain the safety integrity of the instrument do not remove any panels. There are no user serviceable parts inside.
For all questions concerning instrument repair, contact Waters Corporation.

Afin de garantir la sécurité de l'appareil ne pas enlever les panneaux. Il n'y a pas de pièces nécessitant de la maintenance a l'interieur.
Pour toutes questions regardant la maintenance de cet appareil qui ne serait pas couvert par ce manuel d'utilisation il conrient de contacter le bureau de service de Waters Corporation.

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

Dans le cas où l'appareil serait utilisé de maniere non specificé par le fabricant le niveau de protection de l'appareil pourrait altèré

Quattro *micro* API
Mass Spectrometer Information

Intended Use

The Micromass Quattro *micro* API™ is a combined HPLC detector and triple quadrupole mass analyser for determining mass-to-charge ratio (m/z) for a wide variety of analytes.

Biological Hazard

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

Calibration

Follow the calibration methods set forth in this guide, using pure standards. The concentration range should cover the entire range of quality control samples, typical and atypical specimens.

Quality Control

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

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Chapter 1 Instrument Description

1.1 Overview

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.

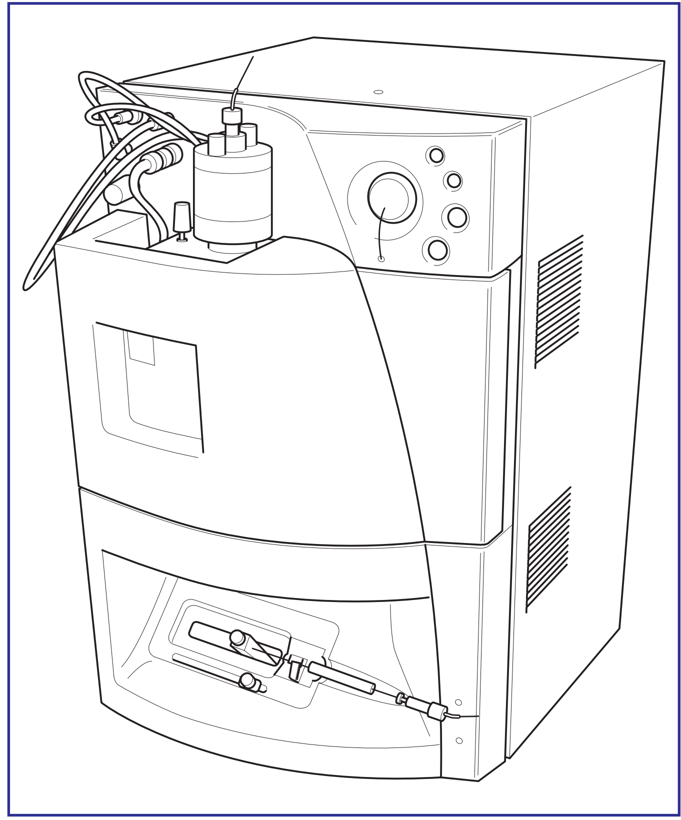


Figure 1-1 The Micromass Quattro *micro* API

Sample ionisation 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 analysed by the second quadrupole.

1

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

1.2 Sample Inlet

An HPLC system or an infusion pump delivers sample to either an electrospray ionisation (ESI) probe or atmospheric pressure chemical ionisation (APcI) probe.

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

1.3 Vacuum System

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

1.4 Data System

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

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

The workstation-based data system, incorporating MassLynx 4.0 software, controls the mass spectrometer and, if applicable, the HPLC system, autosampler, divert valve or injector valve. The workstation uses the Windows NT®, XP®or 2000® 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 photodiode 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.*

1.5 MassLynx Software

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

- Configuring Quattro *micro* API.
- Creating inlet and MS methods that define operating parameters for a run.
- Tuning and calibrating 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.

1

1.6 Theory and Principles of Operation

1.6.1 Electrospray Ionisation (ESI)

In electrospray ionisation (ESI), a strong electrical charge is applied to the eluent as it emerges from a nebuliser, 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 analyser 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 ionisation to split the flow so that 100 to 200 µl/min of eluent enters the mass spectrometer source.

1.6.2 Atmospheric Pressure Chemical Ionisation (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 vaporised in a heated nebuliser 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.

1.6.3 MS Operating Modes

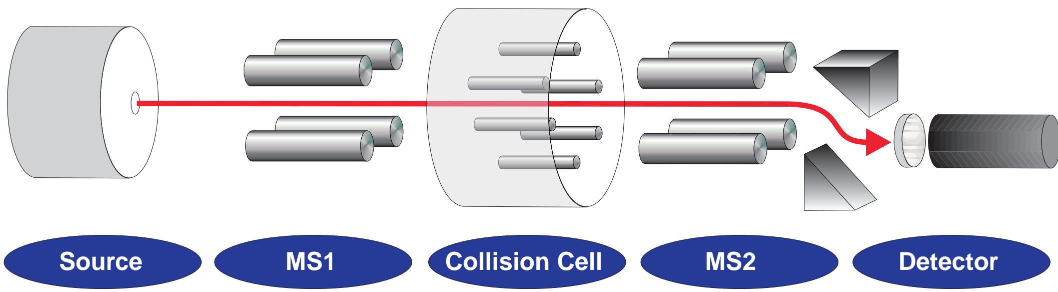


Figure 1-2 Ion Optics

<table>
<tr><th></th><th>MS1</th><th>Collision Cell</th><th>MS2</th></tr>
<tr><td>MS</td><td>Resolving</td><td colspan="2">RF Only (Pass all masses)</td></tr>
<tr><td>MS2</td><td colspan="2">RF Only (Pass all masses)</td><td>Resolving</td></tr>
</table>

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.

1.6.4 MS-MS Operating Modes

The basic features of the four common MS-MS scan functions are summarised below.

<table>
<tr><th></th><th>MS1</th><th>Collision Cell</th><th>MS2</th></tr>
<tr><td>Daughter (Product) Ion Spectrum</td><td>Static
(parent mass selection)</td><td rowspan="4">RF only
(pass all masses)</td><td>Scanning</td></tr>
<tr><td>Parent (Precursor) Ion Spectrum</td><td>Scanning</td><td>Static
(daughter mass selection)</td></tr>
<tr><td>Multiple Reaction Monitoring</td><td>Static
(parent mass selection)</td><td>Static
(daughter mass selection)</td></tr>
<tr><td>Constant Neutral Loss Spectrum</td><td>Scanning (synchronised with MS2)</td><td>Scanning (synchronised with MS1)</td></tr>
</table>

1

The Daughter (Product) Ion Spectrum

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".

    Optimisation of CID tuning conditions to maximise 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.

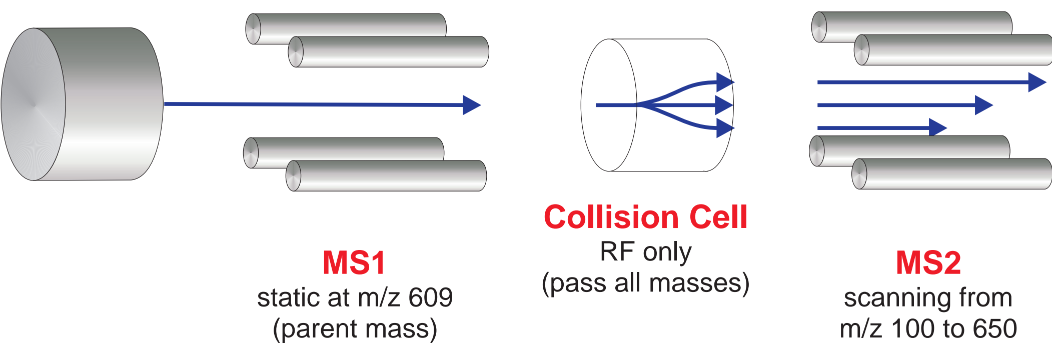


Figure 1-3 Daughter (Product) Ion Mode

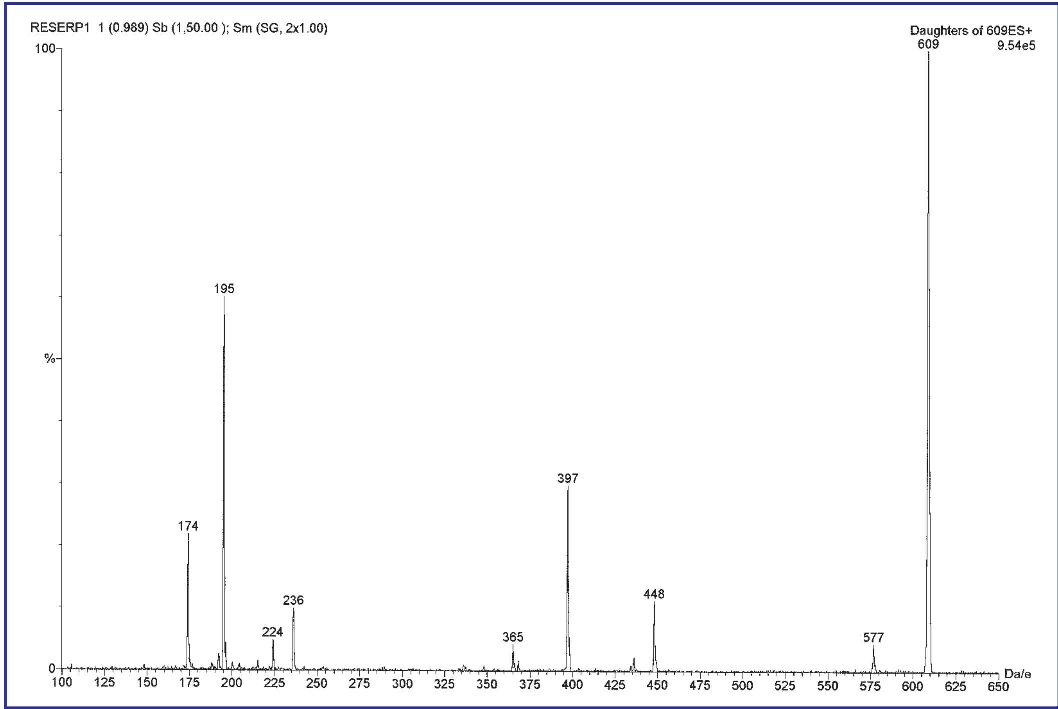


Figure 1-4 The Result

1

The Parent (Precursor) Ion Spectrum

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.

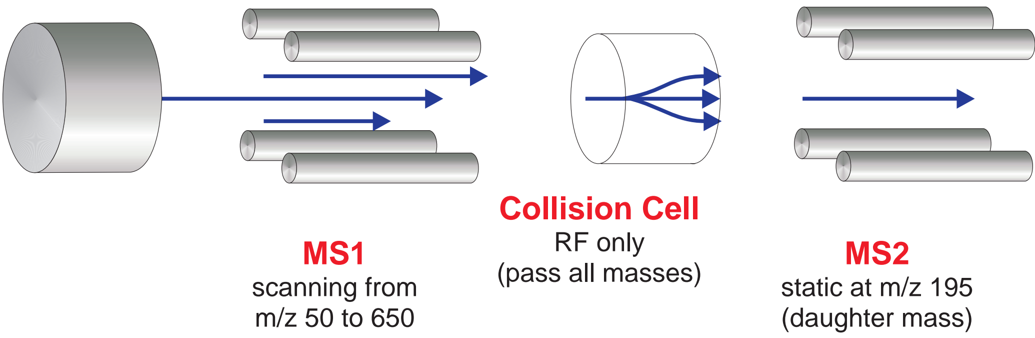


Figure 1-5 Parent (Precursor) Ion Mode

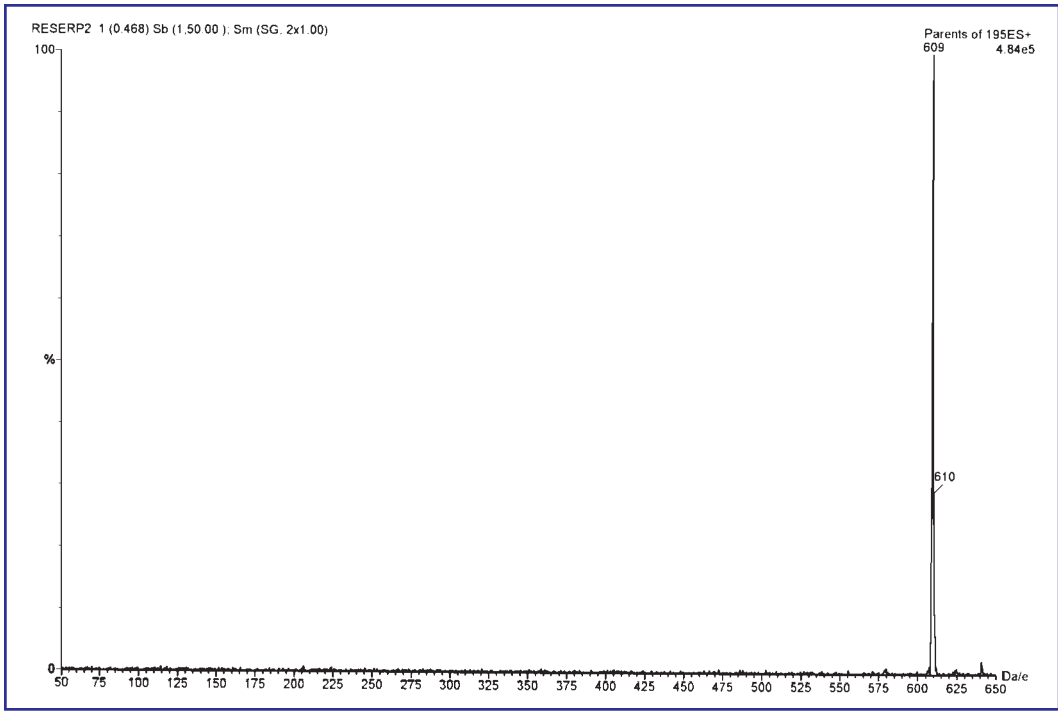


Figure 1-6 The Result

1

MRM: Multiple Reaction Monitoring

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 (~100×) 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 → 195 for reserpine in electrospray positive ion LC-MS-MS mode.

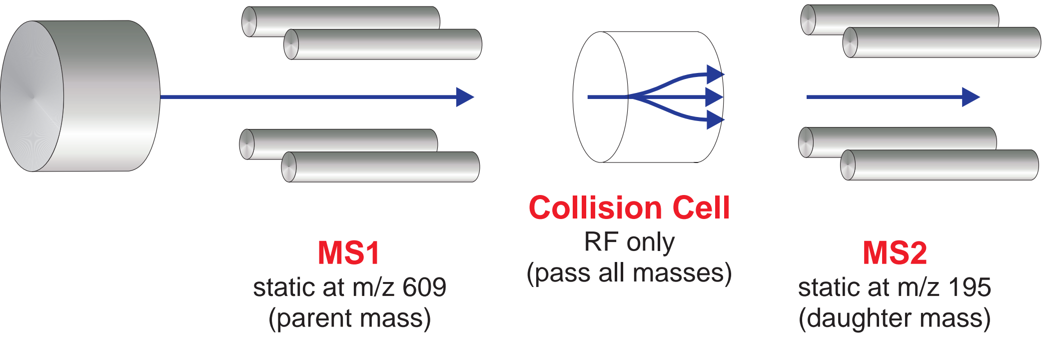


Figure 1-7 MRM Mode

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

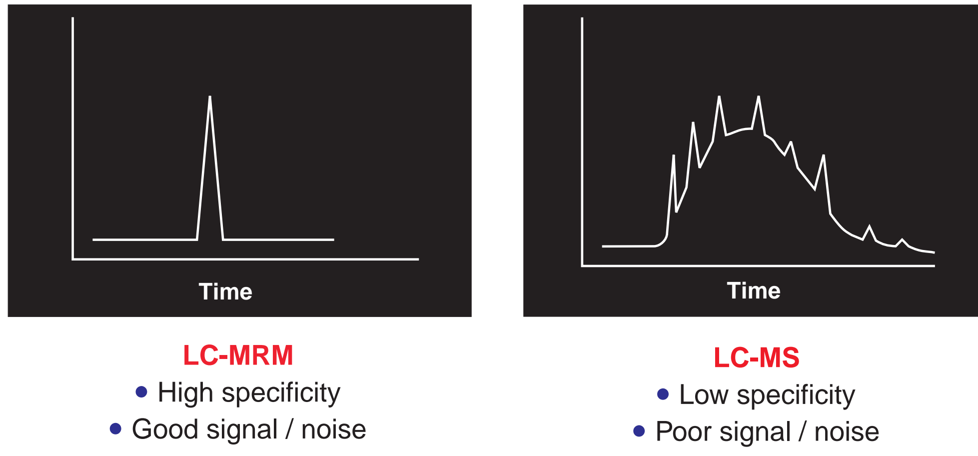


Figure 1-8 The Result

1

The Constant Neutral Loss Spectrum

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

Typical applications:

- Screening mixtures, for example during neonatal screening, for a specific class of compound that is characterised by a common fragmentation pathway.

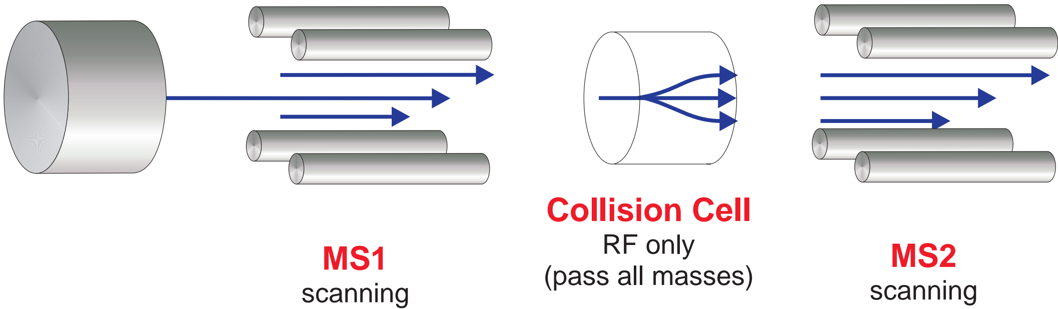


Figure 1-9 Constant Neutral Loss Mode

The scans of MS1 and MS2 are synchronised. 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 "spectrum" shows the masses of all parents that actually lost a fragment of a certain mass.

1.7 Front Panel Controls, Indicators and Connections

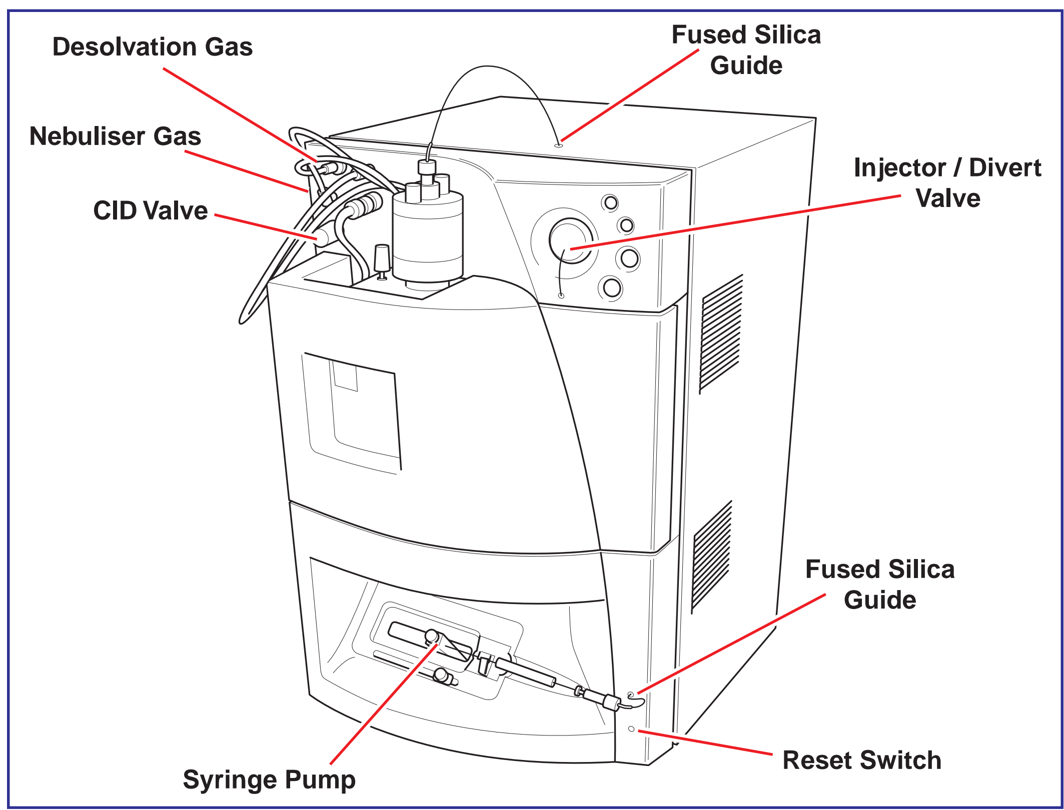


Figure 1-10 Front Panel

1.7.1 Cone Gas, Desolvation Gas and Nebuliser Gas

The PTFE gas lines for the desolvation gas and nebuliser 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.

1

Mass Flow Controllers

The cone gas and desolvation gas are regulated by electronic mass flow controllers over the ranges 0-500 litres/hour and 0-1200 litres/hour respectively, and are controlled by MassLynx from the instrument tune page.

In the event that the desolvation gas decreases to less than 4% of its full scale range, the instrument generates a signal that enables mechanisms to prevent the accumulation of solvent in the source enclosure. Any solvent will drain from a port at the right hand side of the front of the instrument.

For nanoflow applications where very low gas flow rates are required, this signal can be overridden using **Select Gas** and **Gas Fail Override** on the tune page.

1.7.2 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.

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

1.7.3 CID Valve

The **CID Gas** valve is a fifteen-turn valve. The flow increases as the valve is turned anticlockwise.

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

1.7.4 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.

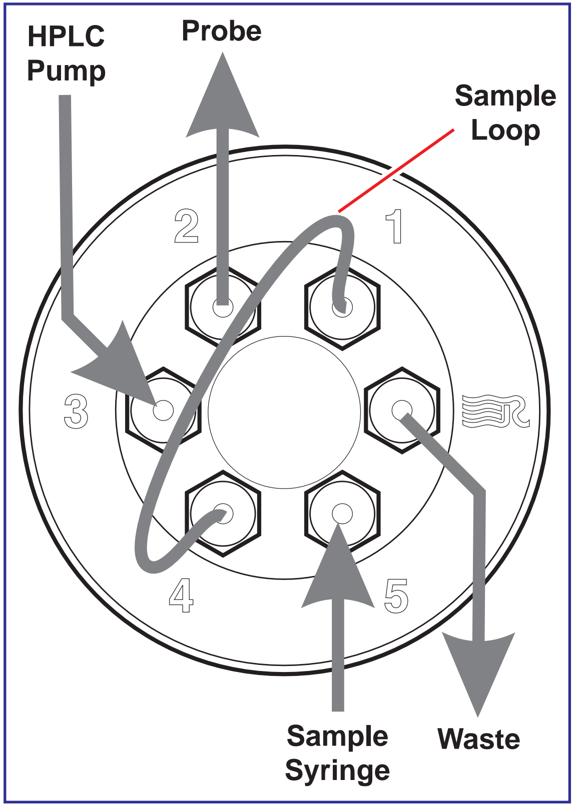


Figure 1-11 Divert/Injection Valve

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 *Setting a Solvent Delay*, page 74.

1

1.7.5 Status Display

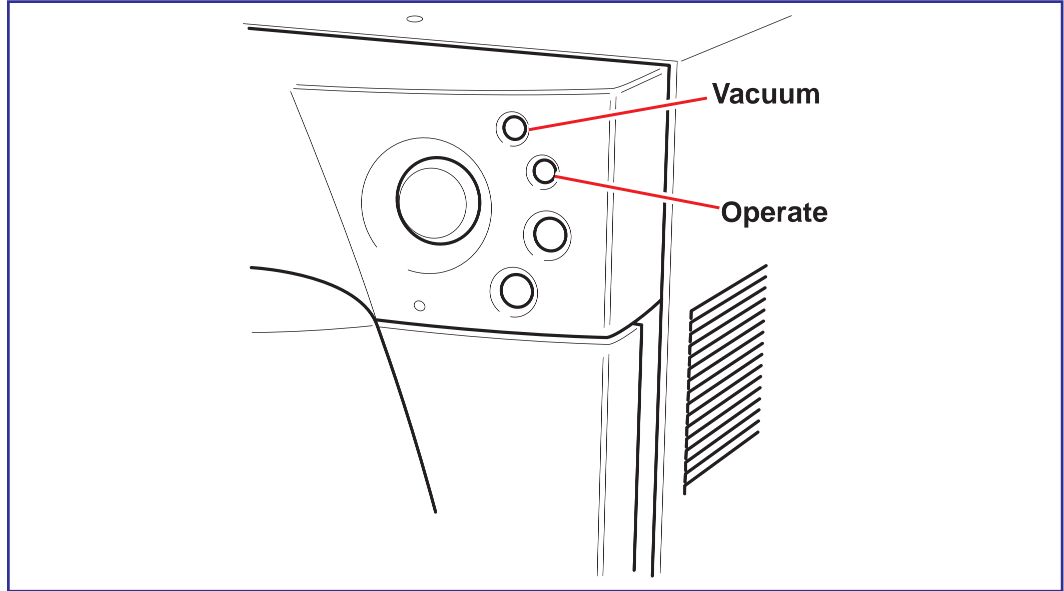


Figure 1-12 Status Display

The status of the instrument is indicated as follows:

Vacuum LED

State		Vacuum LED
Pumping		Flashing green
Pumped	Below trip level	Steady green
	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

1

1.8 Rear Panel Connections

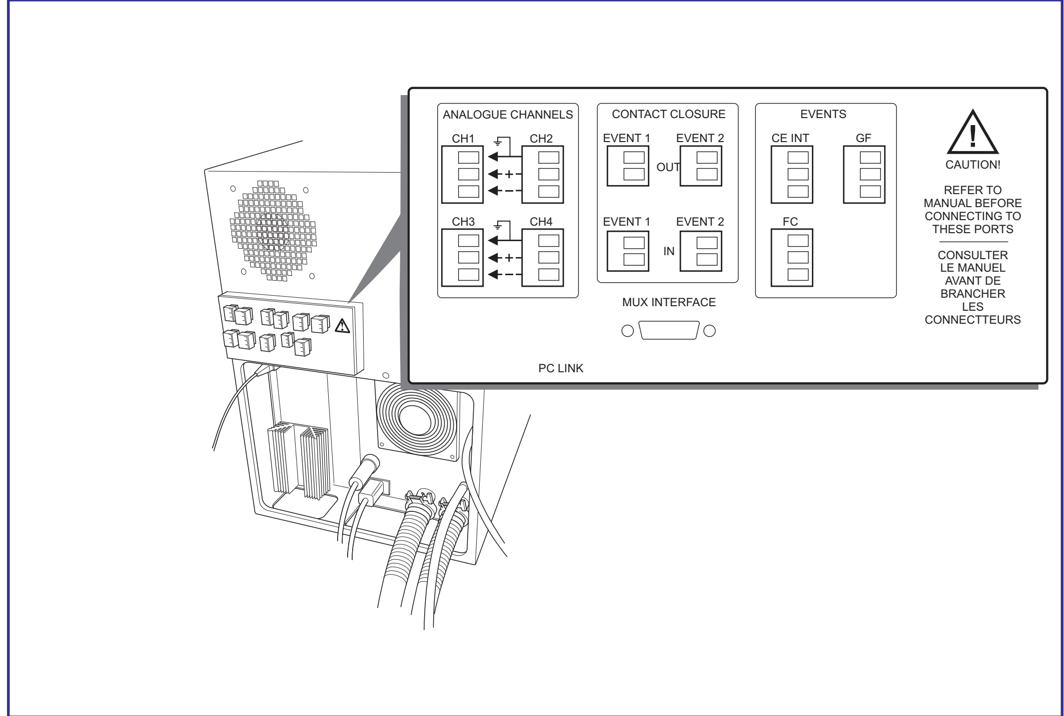


Figure 1-13 Rear Panel Connections

1.8.1 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.

1

1.8.2 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, 5V maximum voltage.
- **Out**. Two outputs, **Event 1** and **Event 2**, are provided whereby the mass spectrometer is able to trigger an external event.

1.8.3 Mux Interface

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

1.8.4 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)

In the event that the desolvation gas decreases to less than 4% of its full scale range, the instrument generates a signal that enables mechanisms to prevent the accumulation of solvent in the source enclosure. Any solvent will drain from a port at the right hand side of the front of the instrument.

Additionally, this signal can be utilised to stop solvent flowing into the source by connecting it to the **Stop Flow** of the HPLC system.

For nanoflow applications where very low gas flow rates are required, this signal can be overridden using **Select Gas** and **Gas Fail Override** on the tune page.

FC (FractionLynx Control)

A 100mV 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.

1

1.8.5 PC Link

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

Chapter 2
Routine Procedures

2.1 Starting Quattro *micro* API

To start Quattro *micro* API proceed as follows:

Switch **On** the switch located under the left hand side of the front panel.

Allow 3 minutes for the embedded PC to initialise.

An audible alert is given when the PC is ready.

Start the MassLynx software.

The default page appears, and the word **Ready** appears in the status bar at the bottom.

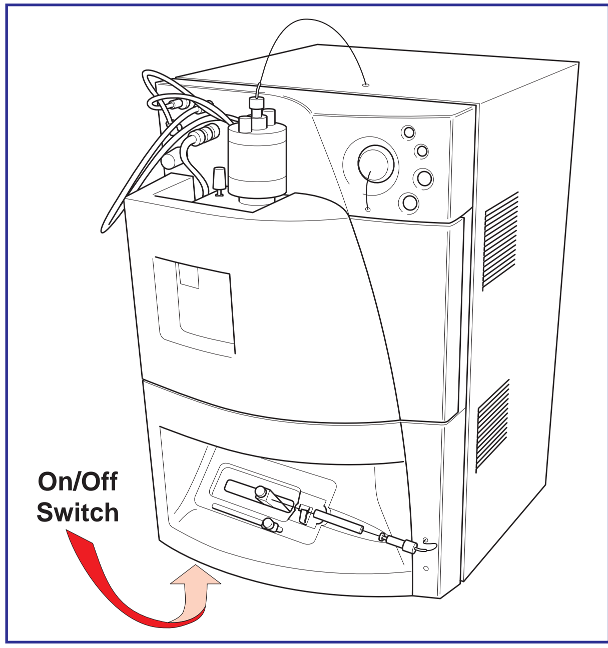


Figure 2-1 On/Off Switch

2

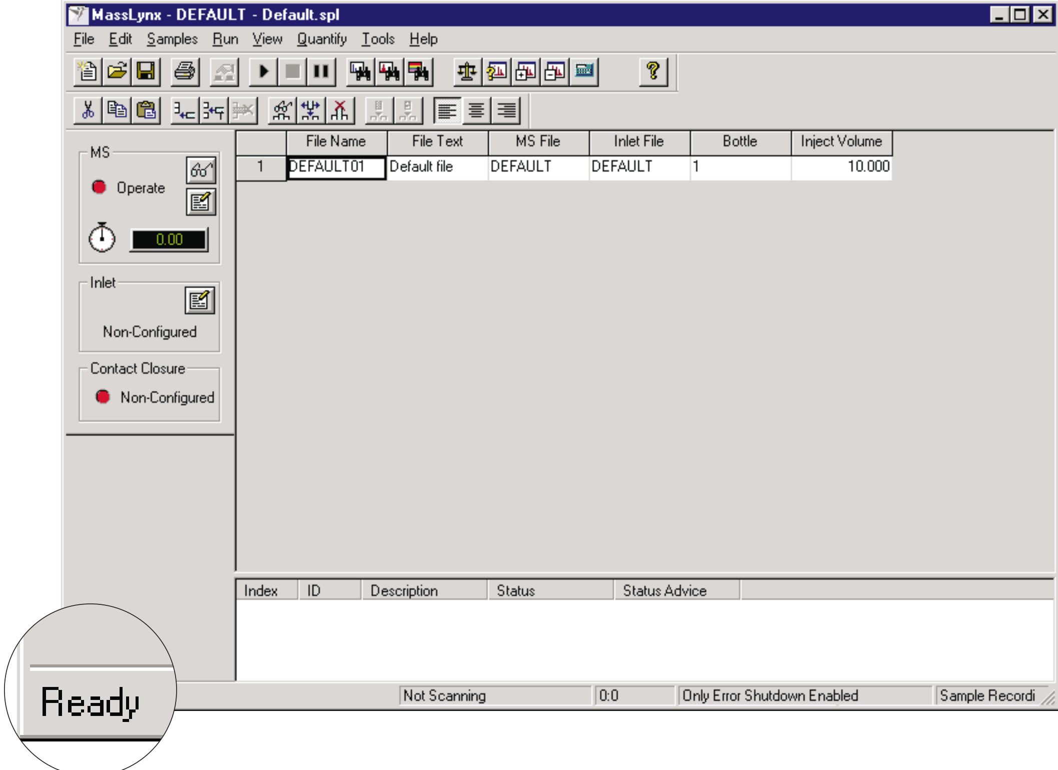


Figure 2-2 MassLynx Default Page

Click on the default page to access the tune page

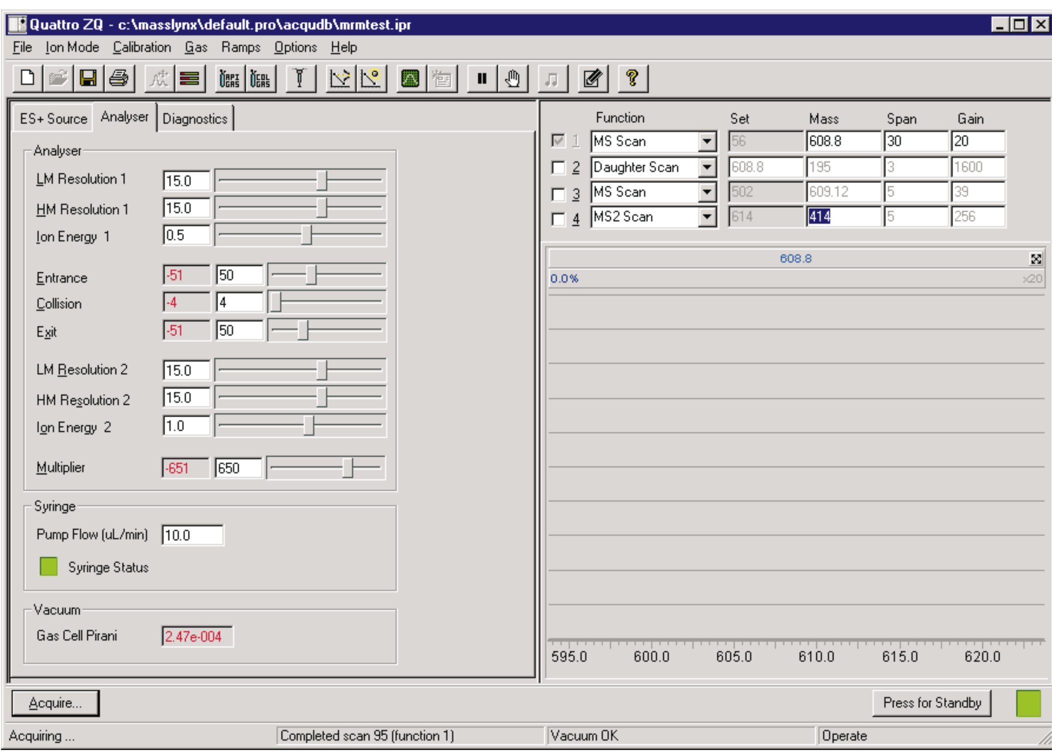


Figure 2-3 Tune Page

Select **Pump** from the tune page **Options** menu.

Click the **Diagnostics** tab.

Monitor **Turbo Speed**.

This parameter should reach 98 to 100% within approximately 5 minutes of **Pump** *being selected.*

Before proceeding:

Ensure that the instrument has pumped sufficiently such that the **Vacuum** LED on the front panel is steady green (see *Status Display*, page 16).

The mass spectrometer is sufficiently evacuated to enable operation in 20 minutes.

2.2 Installing the ESI Probe

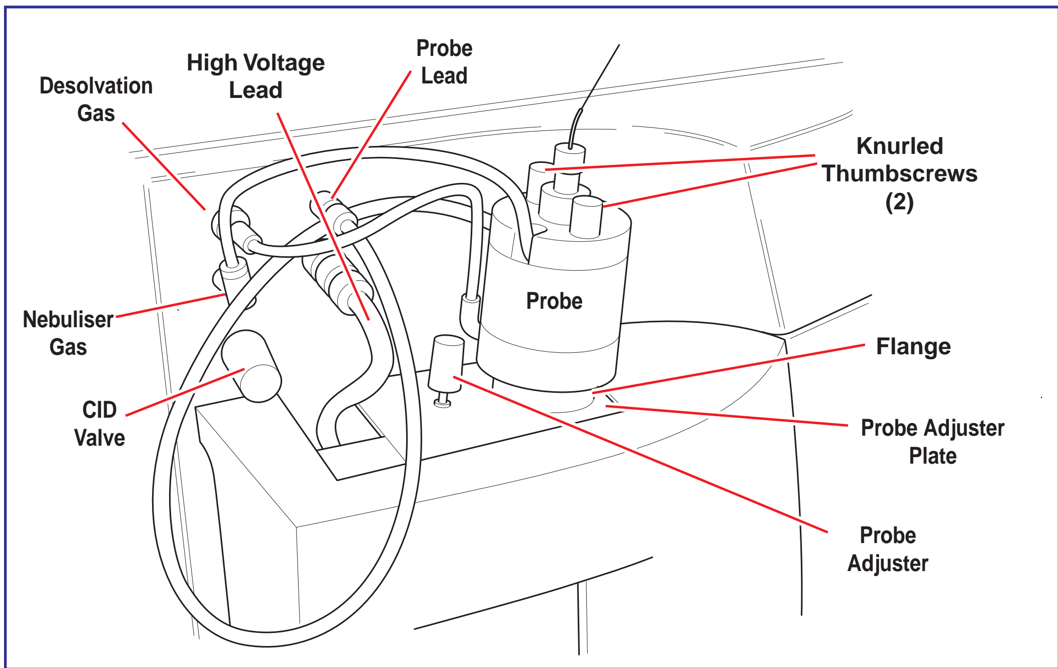


Figure 2-4 ESI Probe

To install the ESI probe:

Ensure that the isolation valve lever is fully to the left, indicating the valve is open.

Insert the probe adjustment flange electrical cable in the lower (and larger) of the two electrical ports on the front panel.

Connect the PTFE tubing from the probe adjustment flange to the desolvation gas port on the front panel.

Remove the protective sleeve, if fitted, from the electrospray probe tip.

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.

Secure the probe with the two thumbscrews.

Connect the 4mm PTFE tubing from the probe to the port labelled **Neb** (nebuliser gas).

Connect the electrical lead from the probe to the capillary connector on the front panel.

2.3 Setting Up the Syringe Pump

To set up the syringe pump:

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

Mount the syringe onto the pump, and set the syringe stop appropriately.

Caution: Micromass 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.

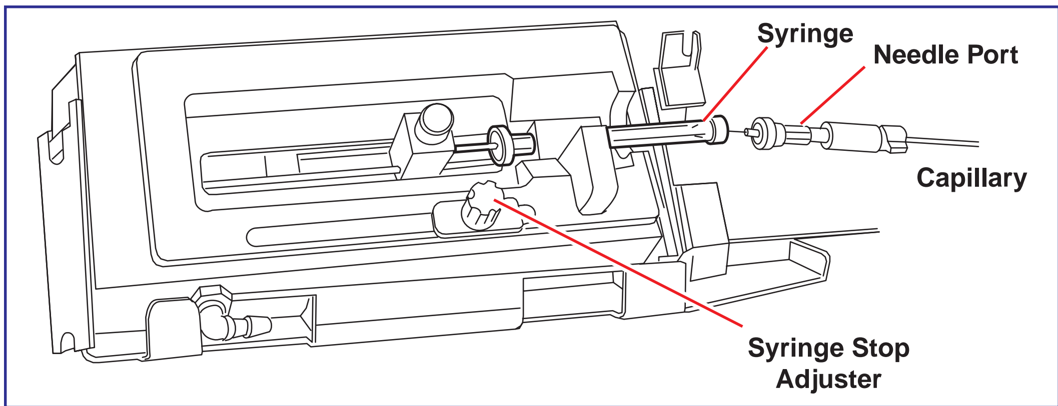


Figure 2-5 Syringe Pump

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

Feed the capillary (ESI probe installation kit) from the top of the moulding to the syringe area. Connect the capillary to the peek union, using an Upchurch® Scientific nut, ferrule, and PTFE tubing.

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.

Connect the other end of the capillary to the inlet on the ESI probe with an Upchurch Scientific nut, ferrule, and PTFE tubing.

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

Click on the default page to access the tune page.

Choose a suitable syringe type from the syringe selection editor by selecting **Options**, then **Syringe Type** from the tune page.

2

2.4 Setting Up the Quattro *micro* API

2.4.1 Preparing for Electrospray Operation

Connect one end of the fused silica capillary tubing to the syringe, and connect the other end to the ESI probe.

Fill the syringe with a reference solution, and mount it on the syringe pump.

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

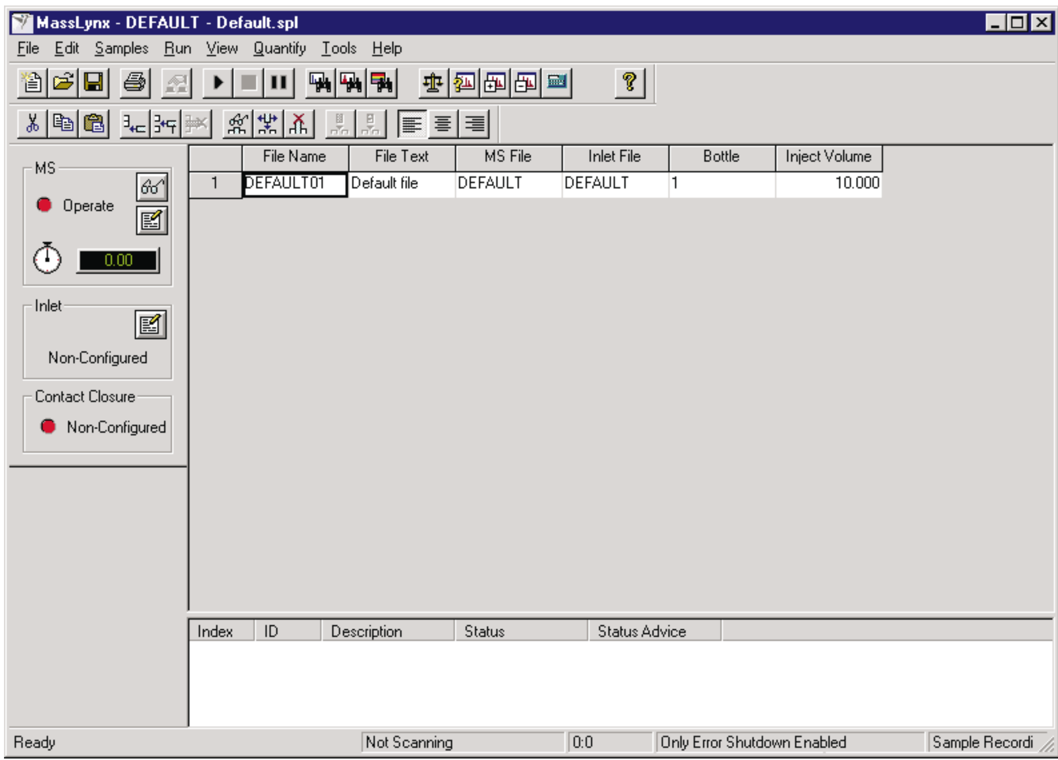


Figure 2-6 Default Page

From the MassLynx default page:

Click to open the tune page.

The example shown below uses ions from a solution of PPG1000, reserpine and PA ß Cyclodextrin.

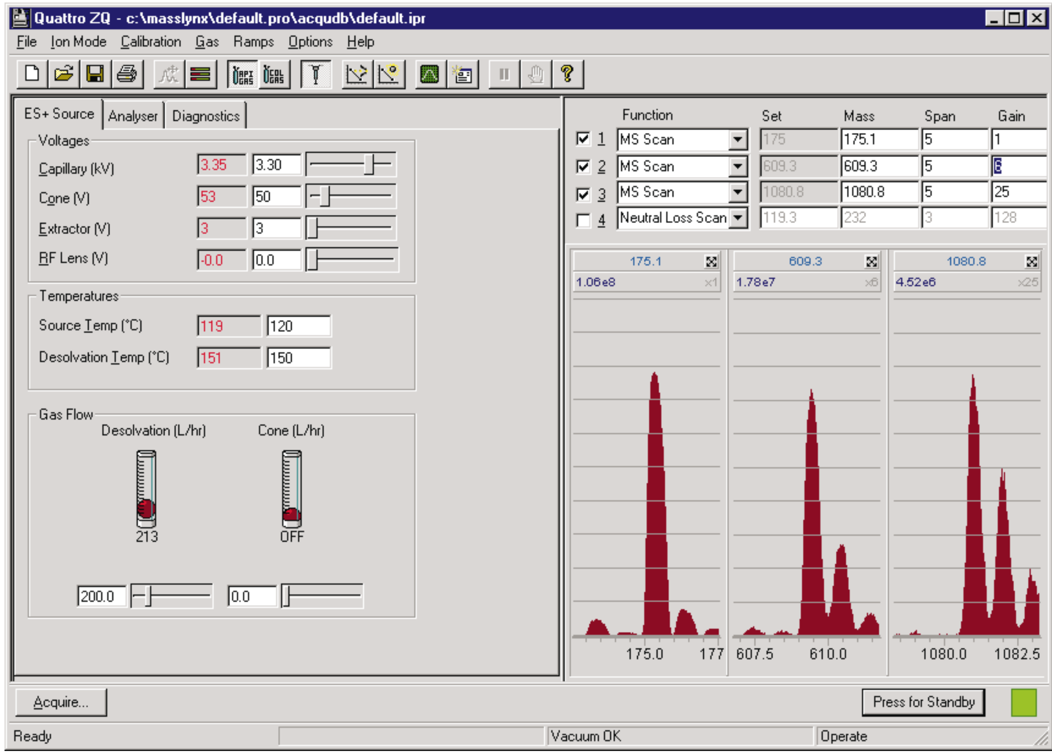


Figure 2-7 ESI Tune Page

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.*

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

Enter the suggested initial reference solution values from the table below in the corresponding tune page fields.

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

2

These settings are intended as starting points only. Optimum values may vary between instruments.

Enter the suggested parameter values from the table below in the corresponding fields of the **ES+ Source** tab.

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

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

Click the **Analyser** tab.

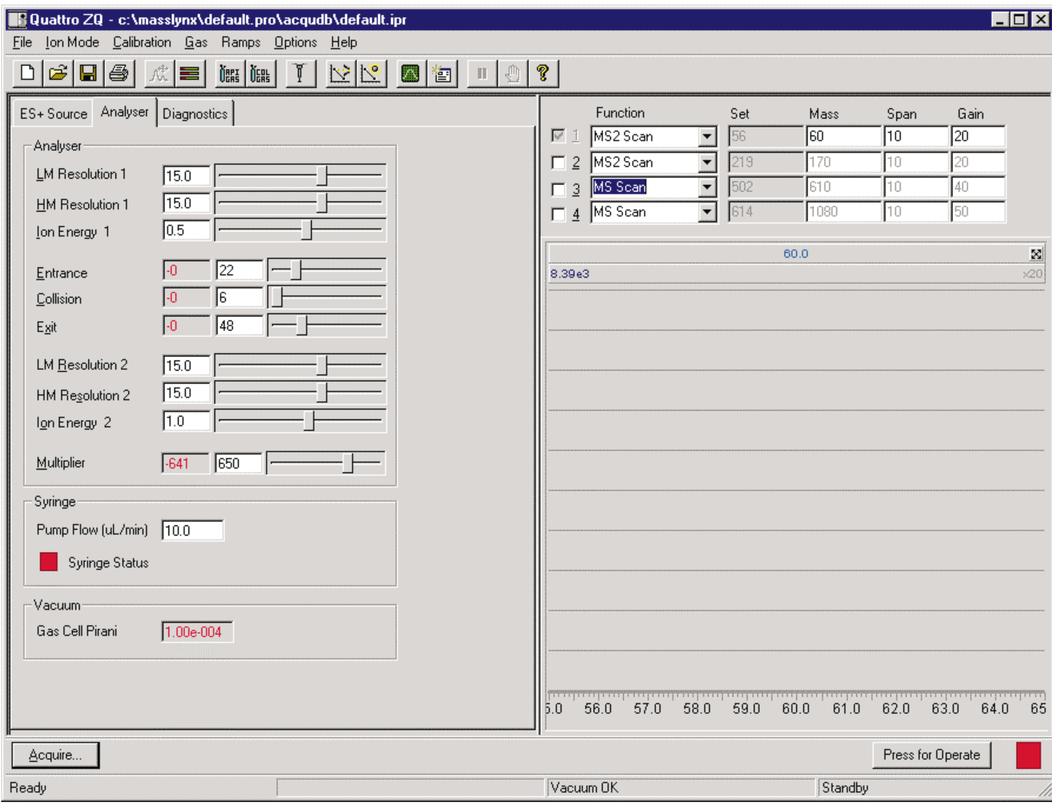


Figure 2-8 Analyser Page

Enter the parameter values listed below, dependent on whether tuning for MS1 or MS2.

Parameter	Suggested Value MS1	Suggested Value MS2
LM Resolution	15	
HM Resolution	15	
Ion Energy (V)	0.5	
Entrance	50	2
Collision	0	0
Exit	50	2
LM Resolution 2	15	15
HM Resolution 2	15	15
Ion Energy 2	3	0.5
Multiplier (V)	650	650

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

Click [API GAS button] to start the nitrogen flow.

2.4.2 Obtaining an Ion Beam in ESI Mode

Make sure the ESI probe is installed as described in *Installing the ESI Probe*.

Change the ionisation mode to **ESI**, if necessary. Select **Ion Mode** from the tune page menu. The current tune page tab indicates ionisation mode.

Keep the tune page **ES+ Source** *tab open for the remaining steps in this section.*

Set **Source Temp** to 120 °C.

When the source temperature reaches 120 °C:

Click [API GAS button] on the tune page to start nitrogen flowing.

From the **Options** menu, select the type of syringe to be used. For example, select the Hamilton 250μl gastight syringe from the startup kit.

Click **Press For Operate** to switch on the instrument high voltages.

Set the syringe flow rate to 10 μl/min., and click [icon] on the tune page menu bar.

On the tune page set **Desolvation Gas** to 150 l/hour.

Check for leaks at the probe and syringe fittings.

Monitor for mass peaks. The peaks should appear at approximately the mass values entered on the **ES+ Source** tab.

Increase values in the **Gain** fields until mass peaks become clearly visible.

Caution: An optimum signal must be obtained before the instrument can successfully be calibrated.

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 *Tuning*, page 41, for further information.

2.4.3 Preparing for APcI Operation when in ESI Mode

When in ESI mode, follow these steps to prepare for APcI operation:

Switch the instrument into standby mode by clicking **Press for Standby** on the lower right of the tune page.

Disconnect the nebuliser and both electrical connections from the front panel.

Remove the ESI probe by unscrewing the two thumb nuts on the probe.

Remove the middle moulding section and loosen the four thumbscrews to remove the source enclosure cover.

Warning: The ion source block, which can reach temperatures of 150°C, maintains the set temperature, even when the source enclosure is removed.

Remove the blanking plug from the corona pin mounting contact, and fit the corona discharge pin. Ensure the tip of the corona discharge pin aligns with the tip of the sample cone.

Replace the source enclosure cover and the middle moulding section.

With the corona discharge pin in place, proceed as follows:

Insert the APcI probe into the source and tighten the two thumbscrews.

Connect the 6mm nebuliser gas tube from the probe to the instrument port marked **Neb**.

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

Connect the APcI probe electrical lead to the **Source/Probe** receptacle on the front panel.

Connect the LC pump tubing to the APcI probe.

Set the **Source Temp** to 130°C.

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

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.

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

2.4.4 Obtaining an Ion Beam and Tuning in APcI Mode

To obtain an ion beam:

Make sure the corona discharge pin is in place, and the APcI probe is installed as described above (*Preparing for APcI Operation When in ESI Mode*).

Change the ionisation mode to APcI, if necessary. Select **Ion Mode** from the tune page menu. The current tune page tab indicates ionisation mode.

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

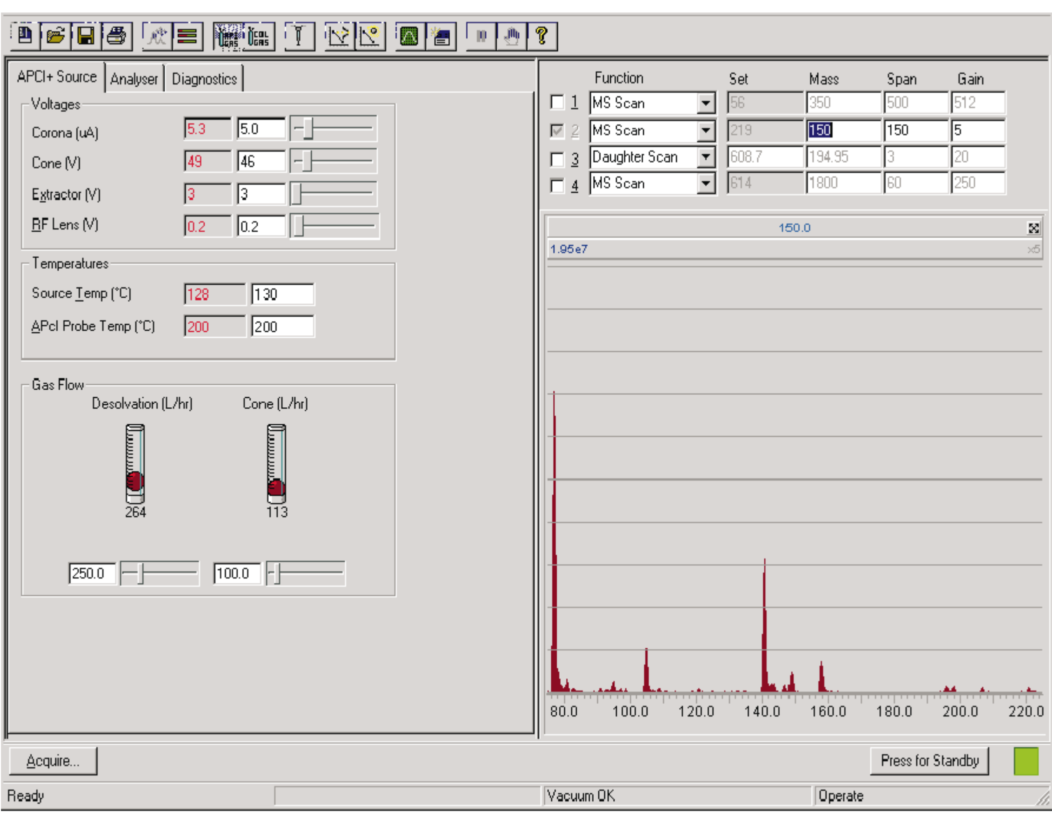


Figure 2-9 APcI Tune Page

Make sure the desolvation gas tube is connected at the front panel.

Set **Source Temp** to 130°C.

Set **Corona** to 2µA and **Sample Cone** to 50V.

When the source temperature reaches 130°C:

Click [API GAS icon] to start the nitrogen gas flowing.

Set **Desolvation Gas** to 250 l/hour on the **APCI+** source tune page.

Select one of the peak display boxes, and set **Mass** to 50 and **Span** to 90.

Click **Press to Operate**.

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.

When the APcI probe temperature reaches 500°C:

Start the LC pump flowing at 1.0 ml/min.

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

Refer to *Performing a Sample Analysis* below, for more information on source tuning.

Warning: The source enclosure and parts of the probe adjustment flange may reach high temperatures when in use.

Warning: 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 APcI operation may cause heat damage to the source.

Performing a Sample Analysis

The following parameters are typical for general qualitative analysis of mixtures.

Parameter	Suggested Value
Corona (µA)*	2
Cone (V)	25 (Monitor ions, slide adjuster up or down to optimise)
Extractor (V)	5
RF Lens (V)	0.2
Source Temp (°C)	130
APCI Probe Temp (°C)*	500
Desolvation Gas (L/hr)*	300
Cone Gas (L/hr)*	100
* See the following section for specific tuning details.	

Adjust the values for **Corona**, **Cone**, and **APCI Probe Temp** for optimal performance.

Specific Tuning for Maximum Sensitivity

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 100-1000 pg/µl) at 10 µl/min into the mobile phase stream.

Alternatively, repeat direct loop injections of a standard solution (typically 10-100 pg/µl) into the mobile phase stream may be used to optimise the APcI.

Probe Position

Turn the probe flange adjuster to optimise 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. Optimisation should be performed in the presence of the analytical mobile phase.

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

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

To find the optimum corona current value:

Set **Corona Current** to 2μA.

Increase **Corona Current** value in 2μA steps until the optimum value is found. Allow the current to stabilise before taking a reading.

If the signal continuously decreases, return **Corona Current** to 2μA, then reduce the value in 0.5μA steps until the optimum value is found.

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

Probe Temperature

For maximum sensitivity, the APcI probe temperature must be optimised as follows, ensuring that the analytical mobile phase is used during optimisation.

Starting at 650°C, reduce **APCI Probe Temp** in 50°C decrements, allowing time for the temperature to stabilise 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

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 minimise formation of solvent adducts. The typical value is about 50 l/hour.

2

Chapter 3
Tuning

3.1 Overview

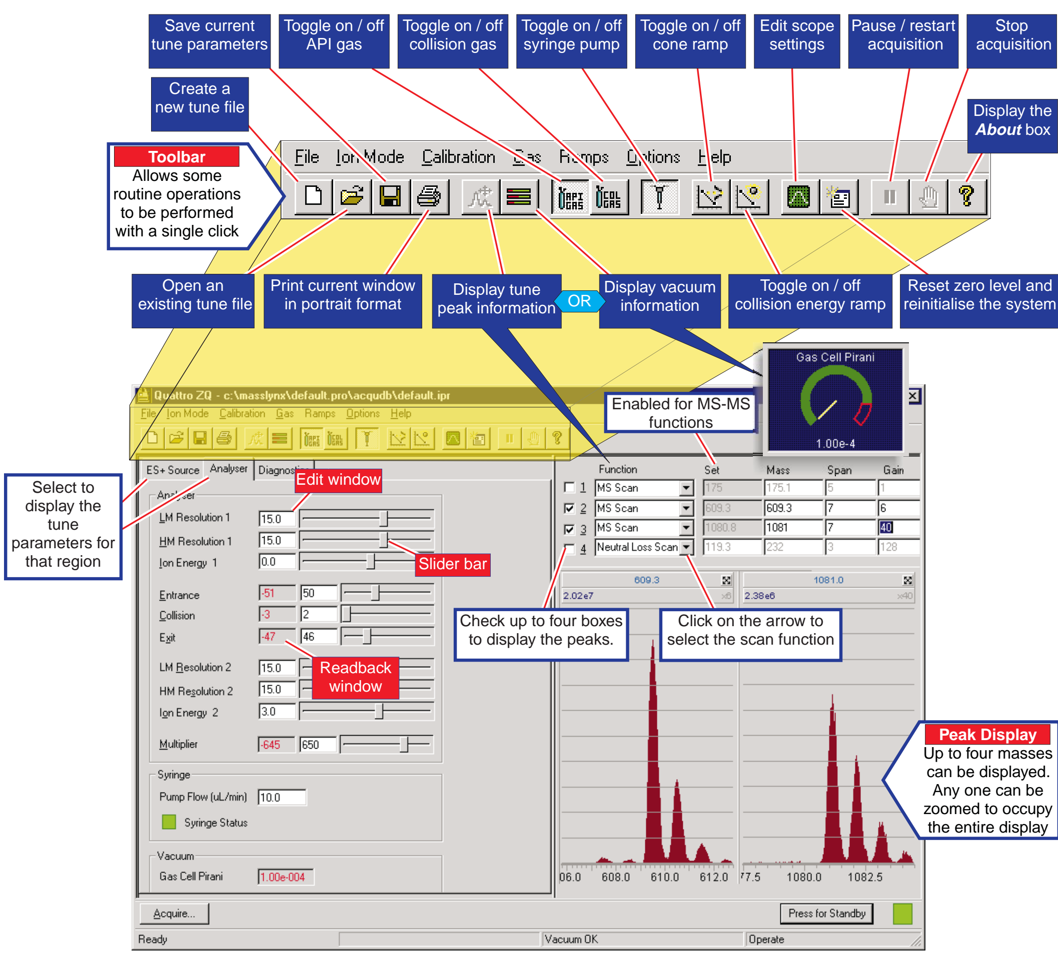


Figure 3-1 Tune Page

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 optimise peak shape and intensity at unit mass resolution.
- Care should be taken to optimise the value of the collision energy. Note that, in **Daughter** and **Parent** modes, **Collision** and **Exit** are interactive parameters.

3.2 The Tune Page

To display the tune page:

Press [button] on the MassLynx screen MS panel.

Refer to page 41 for details of the tune page layout.

3.3 Printing Tune Information

To print a report, containing a copy of the tune peak information displayed on the screen along with a record of each parameter setting:

Press [button], or select **Print** from the tune page **File** menu.

This report is not configurable by the user.

3.4 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 Guide* for more information.

3.5 Saving and Restoring 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 ionisation modes not just the ionisation 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:

Press [icon], or choose **Save** from the tune page **File** menu.

Press **Save**.

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

Select **Save As** from the tune page **File** menu.

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

Press **Save**.

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.*

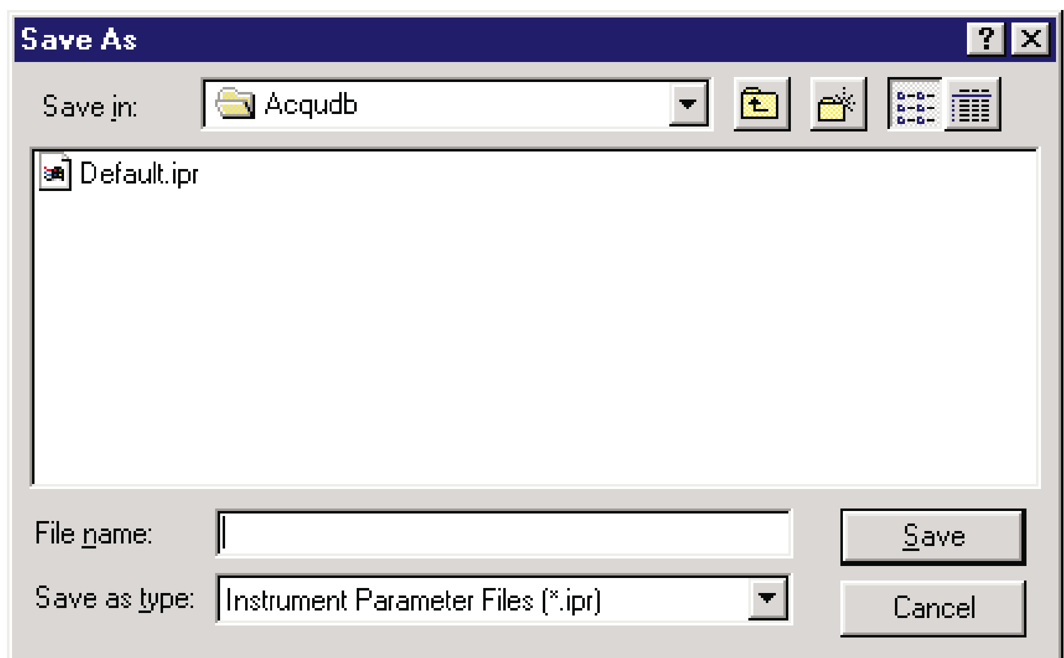


Figure 3-2 Save As Menu

To restore a saved set of parameters:

Press [icon], or choose **Open** from the tune page **File** menu.

3

Select the required tuning parameter file, either by typing its name or by selecting from the list displayed.

Press **Open**.

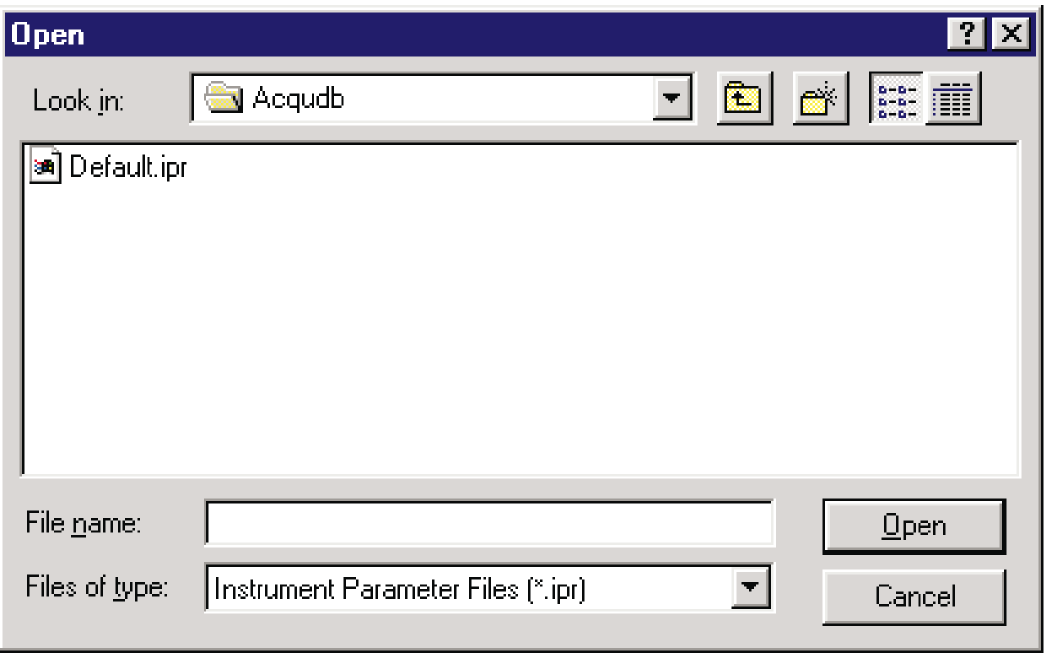


Figure 3-3 Open Menu

3.6 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:

Press [icon], or select **Options**, **Peak Editor**.

Choose the peaks to be displayed by checking the appropriate boxes.

For each active peak select the **Mass**, **Span** and **Gain**.

To change the function:

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:

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

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.

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

or:

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 [icon] is pressed twice **Mass** *returns to this value.*

or:

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

When [icon] 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 [icon] is pressed twice **Mass** *returns to this value.*

To change the span of a peak:

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.

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

This operation can be repeated as often as required.

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

or:

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 [icon] is pressed twice **Span** *returns to this value.*

To change the gain of a peak

Double click on the line above the peak which shows the gain, to double the gain applied to that peak.

Double click below the peak to half the gain.

or:

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.

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

or:

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

3.7 Changing the Display

To change the display using the mouse:

Click in the peak display area with the right mouse button to display the pop up menu.

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

3.7.1 Customise Plot Appearance

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

Select **Customise**, **Plot Appearance**.

The Customise Plot Appearance dialog is displayed.

To change the colours on the display:

Press **Newest Trace**, **Background** or **Trace Fill** and select a new colour from the dialog displayed.

To change the number of traces:

Use [spin buttons] to change the number, or enter a new value in the **Visible Traces** box, within the range 2 to 20.

Customise Plot Appearance

Primary Colours

Newest Trace:

Background:

Trace Fill:

Storage Mode

Visible traces: 2

Colour Interpolation:

none full

Trace colour sample (new->old):

Select primary colours by clicking on colour buttons. In storage mode, trace colour may be interpolated between newest trace and background colours.

OK Cancel

Figure 3-4 Customise Plot Appearance

If more than one trace is displayed then the older traces can be displayed in a different shade to the new ones:

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

3.7.2 Trace

From the pop-up menu:

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.

3.7.3 Intensity

Select either **Intensity**, **Relative Intensity** or **Intensity**, **Absolute Intensity** as required.

Select **Intensity**, **Normalise Data** to display normalised data.

The options selected each have a tick next to them.

3.7.4 Grid

The 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.

3.8 AutoTune

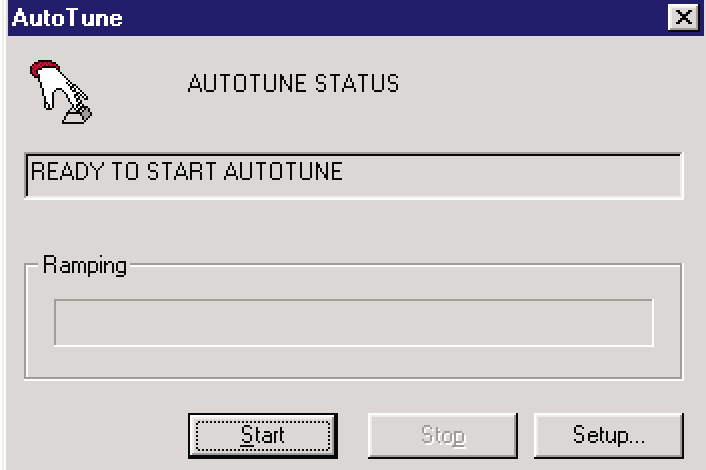


Figure 3-5 AutoTune

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

To run AutoTune:

Press [API GAS] on the tune page to turn on the API gas, and select **Operate**.

Choose **AutoTune** from the tune page **Options** menu.

Press **Setup** to define the AutoTune setup parameters.

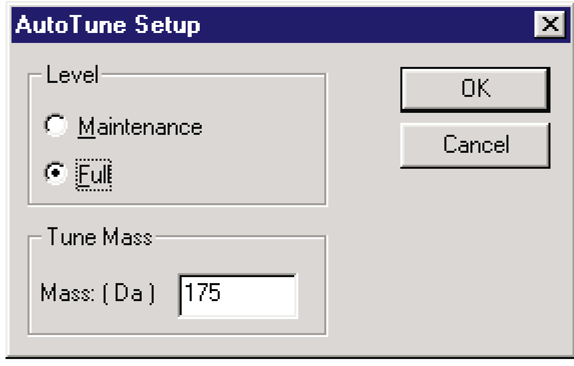
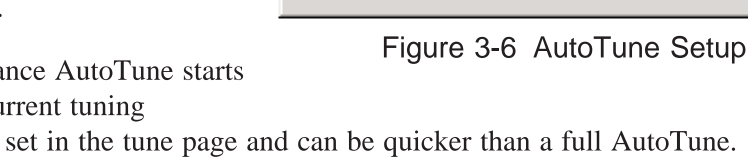


Figure 3-6 AutoTune Setup

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.

The **Tune Mass** parameter sets the mass to be tuned on. When satisfied with the AutoTune setup parameters:

Press **OK** to exit.

Press **Start**.

The AutoTune status bar is updated to show the progress of AutoTune.

The following steps are performed:

- Parameter initialisation 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 maximise 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 dialog 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.

3.9 Ion Mode

Select the required ionisation mode from the **Ion Mode** menu. The selected mode has a tick next to it.

3.10 Scope Parameters

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

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

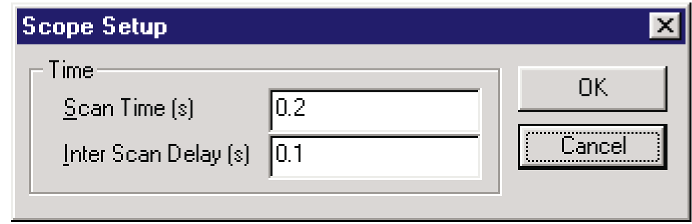


Figure 3-7 Scope Setup

To change the scope parameters:

Press [icon], or choose **Scope Parameters** from the tune page **Options** menu.

Make any required changes to the settings.

Press **OK**.

3.11 Gas Controls

To turn a gas on or off:

Press [icon] (for nebuliser, desolvation and cone gas) or [icon] (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.

3

3.12 Ramp Controls

To set up a cone voltage ramp:

Choose **Cone Ramp Gradient** from the tune page **Ramps** menu.

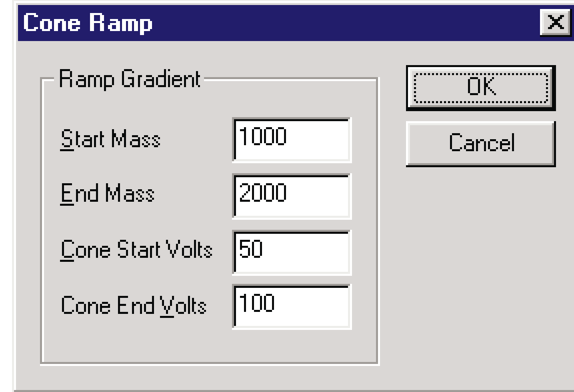


Figure 3-9 Cone Ramp

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.

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

To initiate the cone voltage ramp:

Press [icon], 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:

Choose **Collision Energy Ramp Gradient** from the tune page **Ramps** menu.

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.

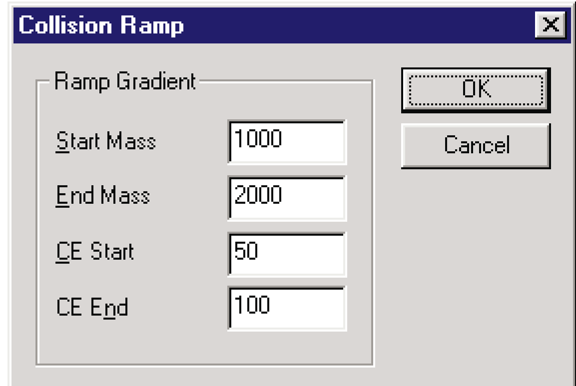


Figure 3-8 Collision Ramp

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

To initiate the collision energy voltage ramp:

Press [icon], or choose **Use Collision Energy Ramp** from the tune page **Ramps** menu.

3.13 Resetting the Zero Level

The zero level (or baseline) can be repositioned by pressing [icon], 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.

3.14 Controlling Readbacks

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%.

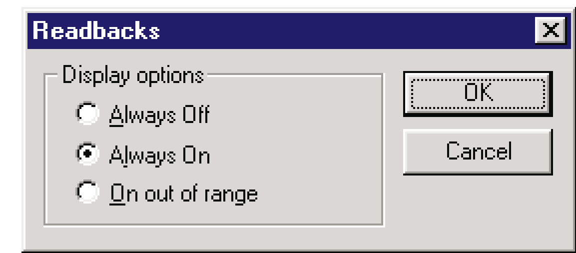


Figure 3-10 Readbacks

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:

Choose **Readbacks** from the tune page **Options** menu.

Select the readback style required.

Press **OK**.

3

3.15 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 scan and inter scan times to be as short as possible.

3

3.16 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.

	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
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.

Chapter 4
Data Acquisition

4.1 Starting an Acquisition

There are two ways of starting an acquisition:

- a single sample acquisition from the tune page
- a multiple sample one from the MassLynx top level screen.

4.1.1 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:

Press **Acquire** on the tune page, or choose **Acquire** from the tune page **Window** menu.

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

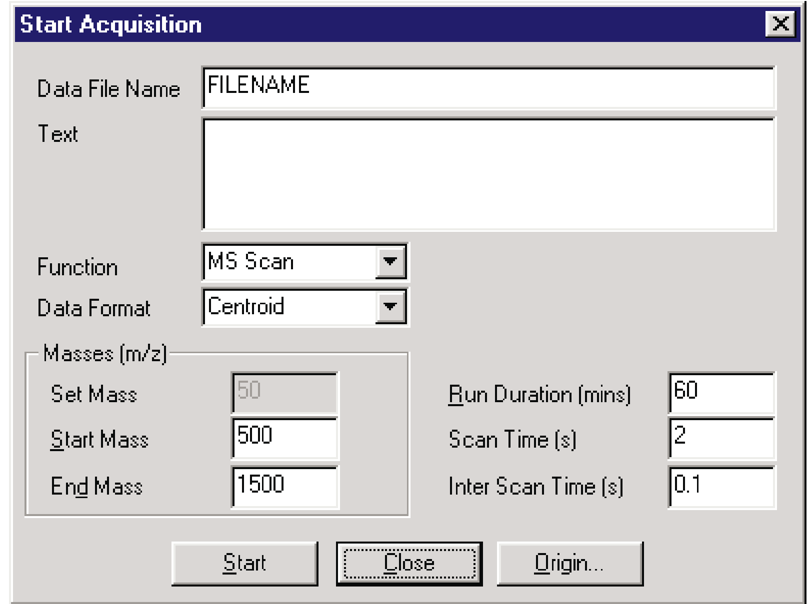


Figure 4-1 Start Acquisition Menu

Press **Start**.

Parameters

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

To change the directory into which data are acquired:

Cancel the acquisition.

Create a new project by choosing **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 in *Function List Editor* later in this chapter.

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

- Centroid
- Continuum
- MCA.

More information is given on 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 analysed to be entered into the following fields:

- Submitter
- Job
- Task
- Conditions

4.1.2 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:

Set up a sample list (see *MassLynx NT User Guide*, *Sample Lists* for details).

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

This displays the start sample list run dialog.

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

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

The default is all samples in the list.

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

See the MassLynx manual for details.

Press **OK**.

Repeat the above procedure as required.

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

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

Process

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

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

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

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

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

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

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

4

Automated Quantification of Sample List

To display the quantify samples dialog:

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

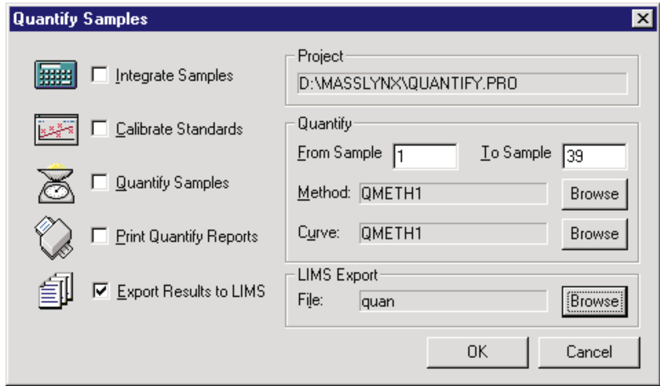


Figure 4-2 Quantify Samples Menu

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

Integrate Samples integrates all the sample data files named in the peak list.

Calibrate Standards uses integration results to form quantify calibration curves.

Quantify Samples uses integration results and quantify calibration curves to calculate compound concentrations.

Print Quantify Reports produces hard copies of the results of integration and quantification.

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

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

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

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

4.2 Monitoring an Acquisition

Acquisition status is also 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.

4.2.1 The Acquisition Status Window

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

To display the scan report dialog:

Select **Acquisition Status**.

This shows details of the scan currently being acquired.

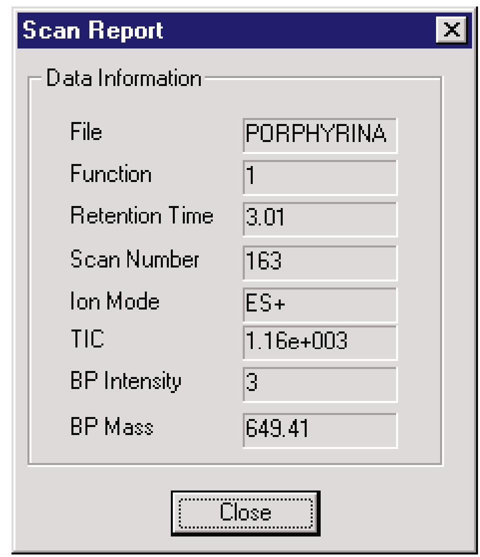


Figure 4-3 Scan Report Menu

4.2.2 Chromatogram Real-Time Update

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

Open the data file using the MassLynx data browser.

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

4

4.2.3 Spectrum Real-Time Update

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

Open the data file using the MassLynx data browser.

Press [toolbar button], or select **Real-Time Update** from the **Display** menu.

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

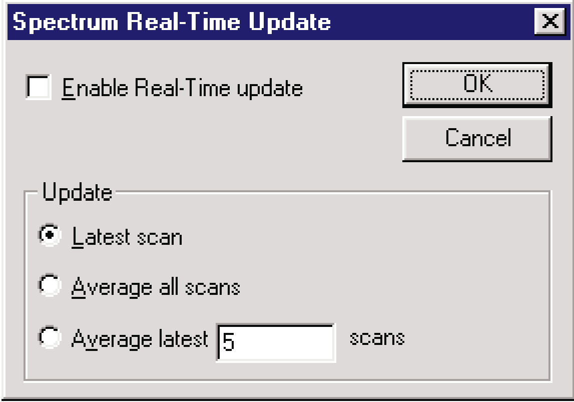


Figure 4-4 Spectrum Update Menu

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.

4.3 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 data thresholding dialog.

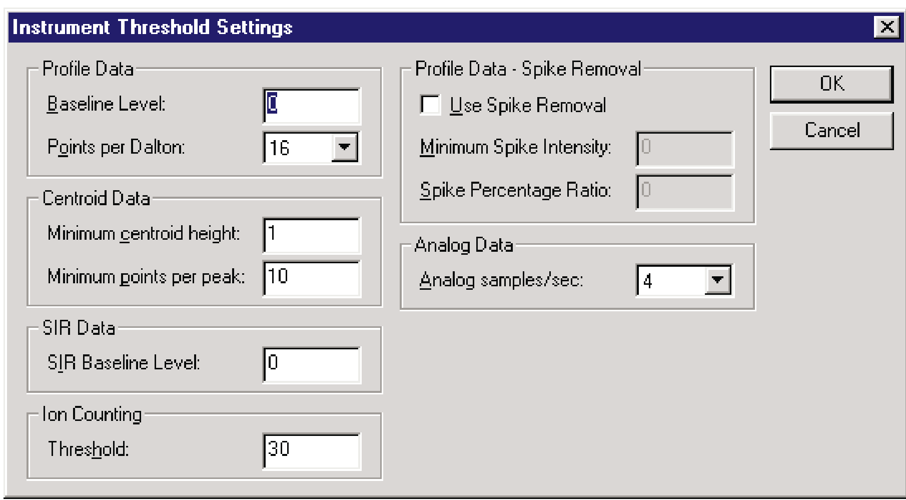


Figure 4-5 Instrument Threshold Settings Menu

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.

4

To change data thresholding:

Choose **Set Instrument Threshold** from the tune page **Options** menu.

Make the required changes to the information.

Press **OK**.

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

4.3.1 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 analysed using MaxEnt.

4.3.2 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. The 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.

4.3.3 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

4.3.4 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.

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

Baseline Level	Ion Count Threshold	Typical Background Noise	Typical Peak Profile	Typical Intensity Reduction	Typical Saving
0	0			0	0
1	0				0
2	0				0
5	0				0
0	10			4%	8%
0	20			11%	10%
0	40			37%	61%
0	60			66%	69%
0	250			100 %	83%

4

4.3.6 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 neighbours, very intense. Data points determined to be spikes are removed by setting the value of this data point to the average of its immediate neighbours.

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:

Check **Use Spike Removal**.

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.

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.*

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 neighbours. For example, with **Spike Percentage Ratio** *set to 33%, a data point is regarded as a spike if its intensity is 3 times (or more) greater than both its immediate neighbours. A setting of 20% requires an intensity ratio of 5:1 to identify a spike.*

Press **OK** to accept any changes.

Any changes are not downloaded if **Cancel** *is pressed.*

4.3.7 Analog Data

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

4.4 System Manager

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

Select **Communications Status**.

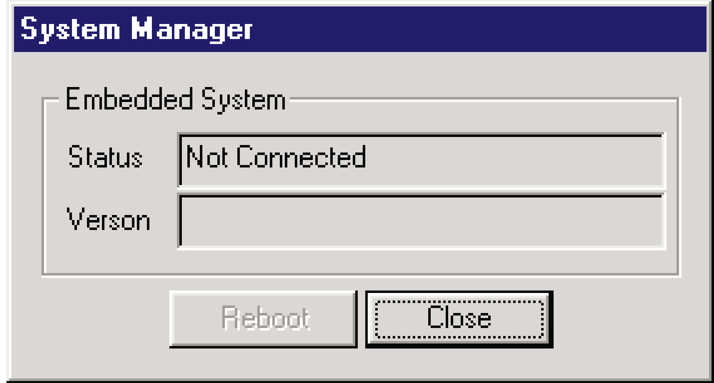


Figure 4-6 System Manager

4.5 Stopping an Acquisition

To halt the acquisition:

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

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

Data acquired up to this point is saved.

4

4.6 The Function List Editor

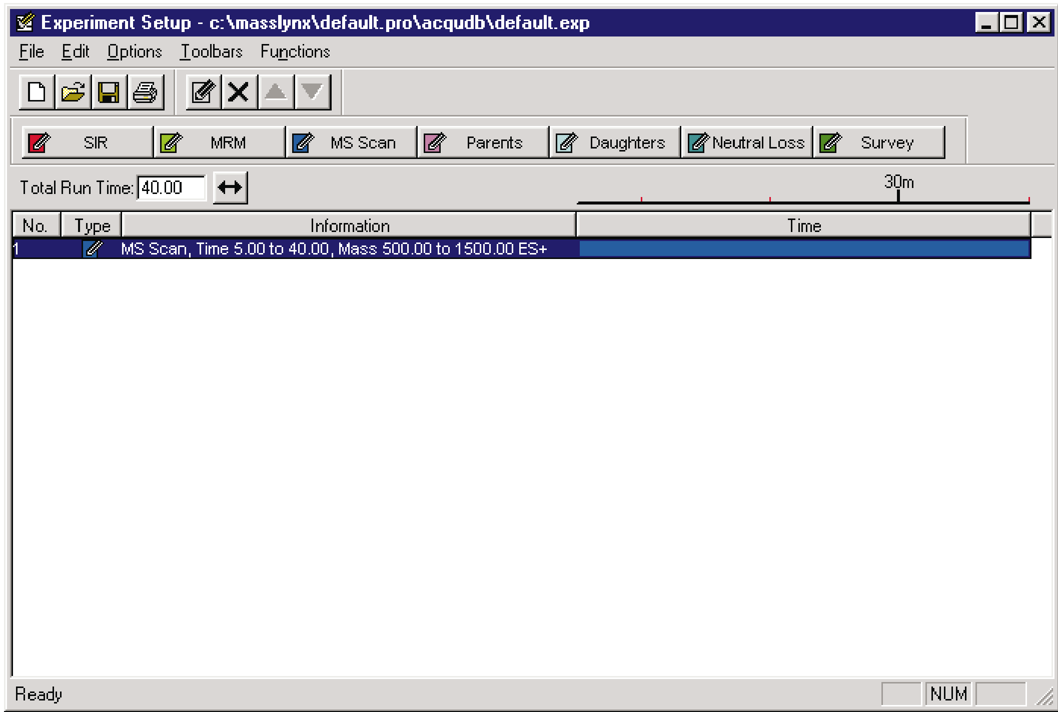


Figure 4-7 Function List

4.6.1 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.

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.

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

To access this dialog:

Press [icon] on the MS panel of the MassLynx screen.

A more complicated function list, with four SIR functions each running sequentially for 5 minutes, is shown below.

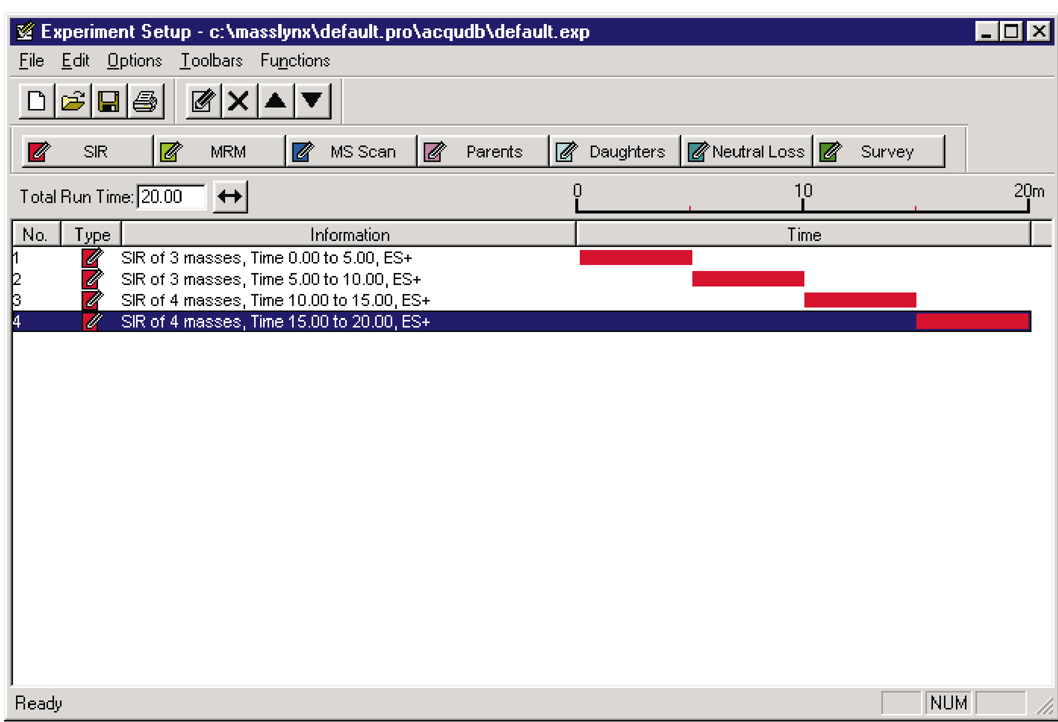


Figure 4-8 Function List with Four SIR Functions

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.

4

4.6.2 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.

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 function of the indicated type.

4.6.3 Adding a New Function

To add a new function to the list:

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

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

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

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

4

4.6.4 Modifying an Existing Function

To modify an existing function:

Select the function in the function list.

Press [edit button], or double click on the function.

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

4.6.5 Copying an Existing Function

To copy an existing function:

Select the function in the function list.

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

Modify the parameters as described above.

4.6.6 Removing a Function

To remove a function:

Select the function in the function list.

Press [X button], choose **Delete** from the **Edit** menu, or press **Del** on the keyboard.

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

4

4.6.7 Changing the Order of Functions

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

Highlight the required function.

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

4.6.8 Setting a Solvent Delay

To set a solvent delay for a function list:

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

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.

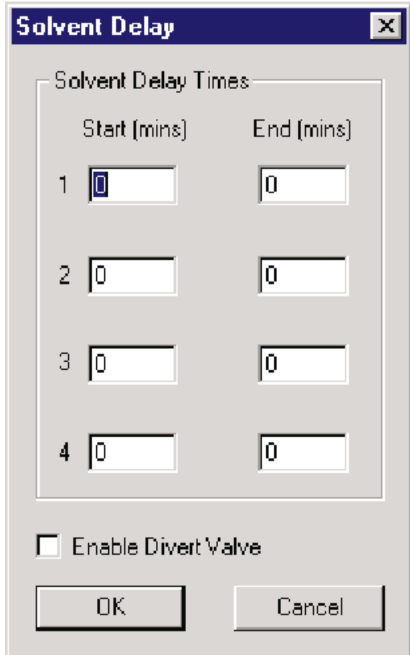


Figure 4-9 Solvent Delay

4

4.6.9 Analog Channels

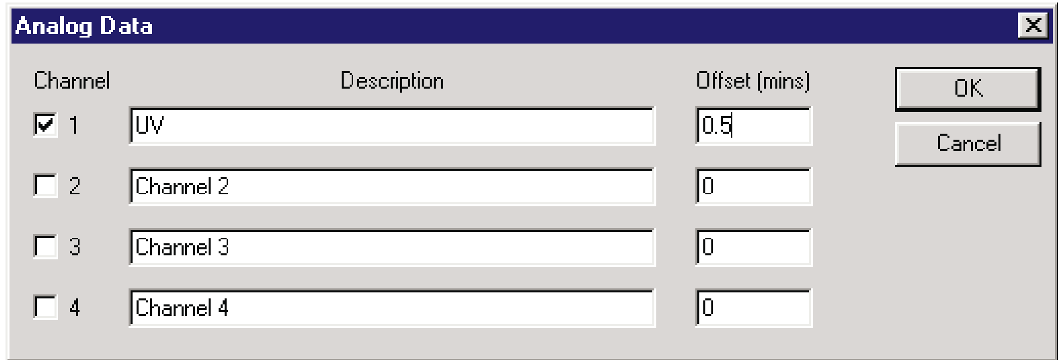


Figure 4-10 Analog Data

Up to 4 channels of analog data can be acquired, which are stored with the data acquired from the mass spectrometer. Analog channels are typically used to collect data from external units such as UV detectors, which must be connected to the user input/output PCB as described in *Instrument Description, Rear Panel Connections*.

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

To access this dialog:

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

To store data for an analog channel:

Check the box(es) for the channel required.

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

This description is used on the analog chromatogram dialog as the channel description. See “Chromatogram” in the MassLynx User’s Guide.

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

Press **OK**.

4

4.6.10 Saving and Restoring a Function List

To save a function list:

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

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

Press **Save**.

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

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

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

To restore a saved function list:

Choose **Open** from the function list **File** menu.

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

Press **Open**.

4.6.11 Setting up a Full Scan Function

The full scan function editor, activated by pressing MS Scan or by selecting **MS Scan** from the **Functions** menu, is used to set up centroid, continuum and MCA functions.

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:

Check **Use Cone Voltage Ramp** and press **CV Ramp** to load the cone ramp dialog.

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

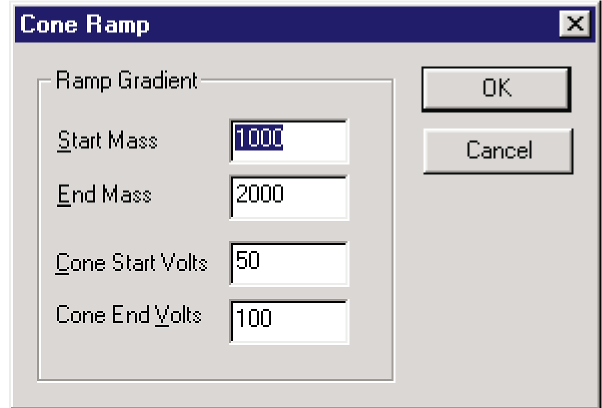


Figure 4-11 Cone Ramp

Method

Ionization Mode specifies the ionisation 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 64.
- **Multi Channel Analysis (MCA)**. MCA data can be thought of as 'summed continuum', with only one intensity accumulated scan being stored for a given experiment. As each scan is acquired, its intensity data is added to the accumulated summed data of previous scans.

  An advantage of MCA is that random noise does not accumulate as rapidly as real data and therefore effectively averages out over a number of scans. This emphasises 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 APcI.

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 ionisation mode is set to APcI.

The APcI probe temperature value cannot be altered by typing new values into 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.

4.6.12 Setting up a SIR Function

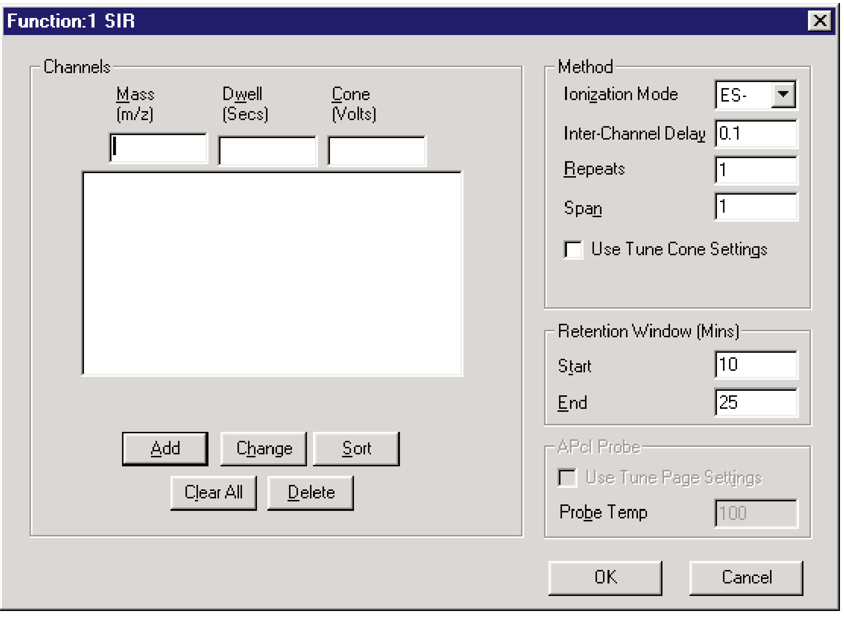


Figure 4-12 SIR Function

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

To set up a SIR function:

Press [SIR] or select **SIR** from the functions menu.

Many of the fields are described above for the full scan editor. Only those which differ are described below.

Channels

Up to 32 masses can be monitored. To enter a mass:

Type suitable values into the **Mass**, **Dwell** and **Cone** boxes.

Press **Add**.

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

To modify existing settings:

Double click on a mass in the list.

This displays the values for the selected mass in the edit fields.

Change **Mass**, **Dwell** or **Cone** as required.

Press **Change** to update the values in the list.

To sort the list in order of ascending mass:

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.

4.6.13 Setting up MS-MS Scanning Functions

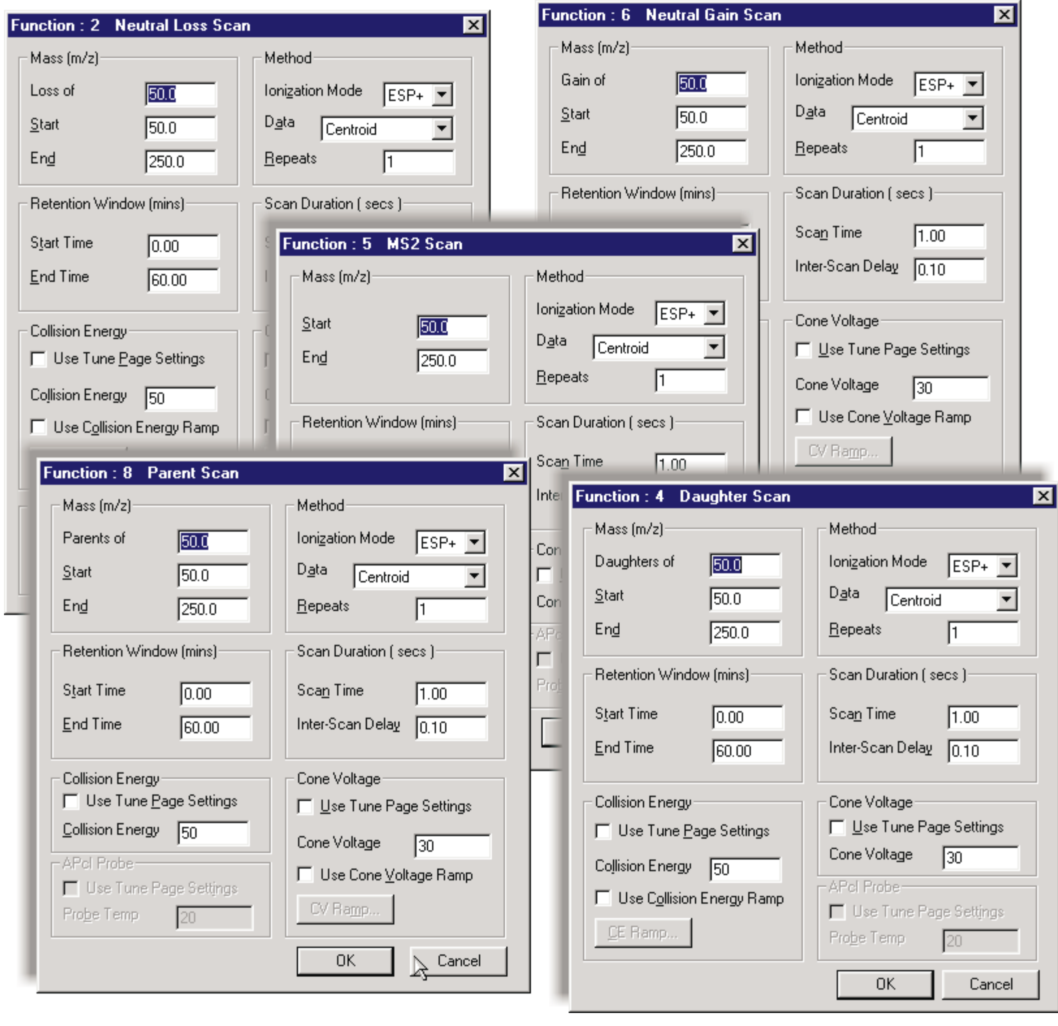


Figure 4-13 MS-MS Scanning Functions

Many of the fields in the MS-MS editors are similar to those in the full scan 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 optimising 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:

Check **Use Collision Energy Ramp**.

Press **CE Ramp…** to load the collision energy ramp dialog.

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.

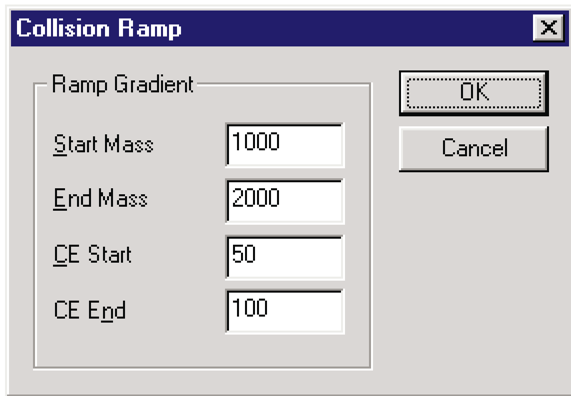


Figure 4-14 Collision Ramp

4

4.6.14 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.

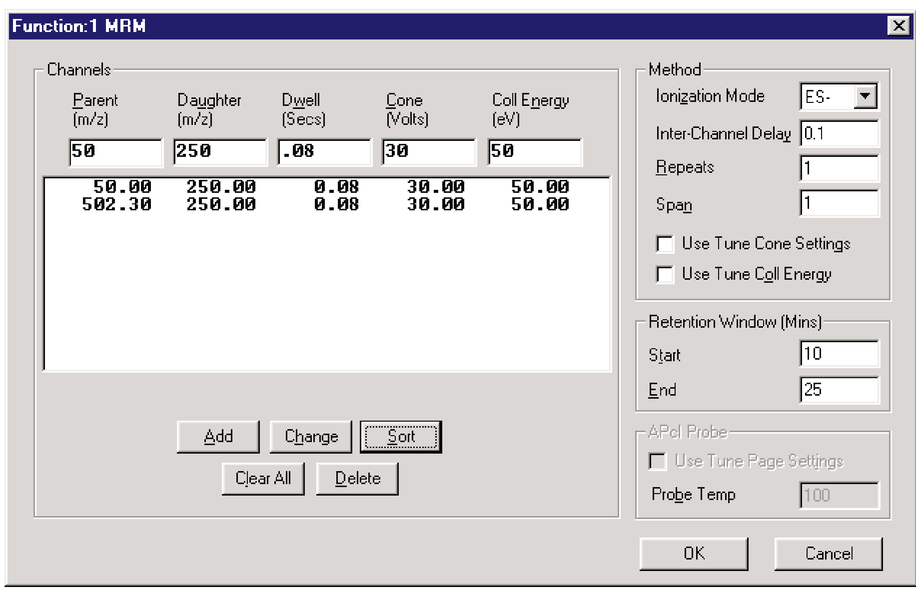


Figure 4-15 MRM Function

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

4.6.15 Setting up a Survey Function

Survey scans are used to search for precursor ions. To access the dialog:

Press Survey 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

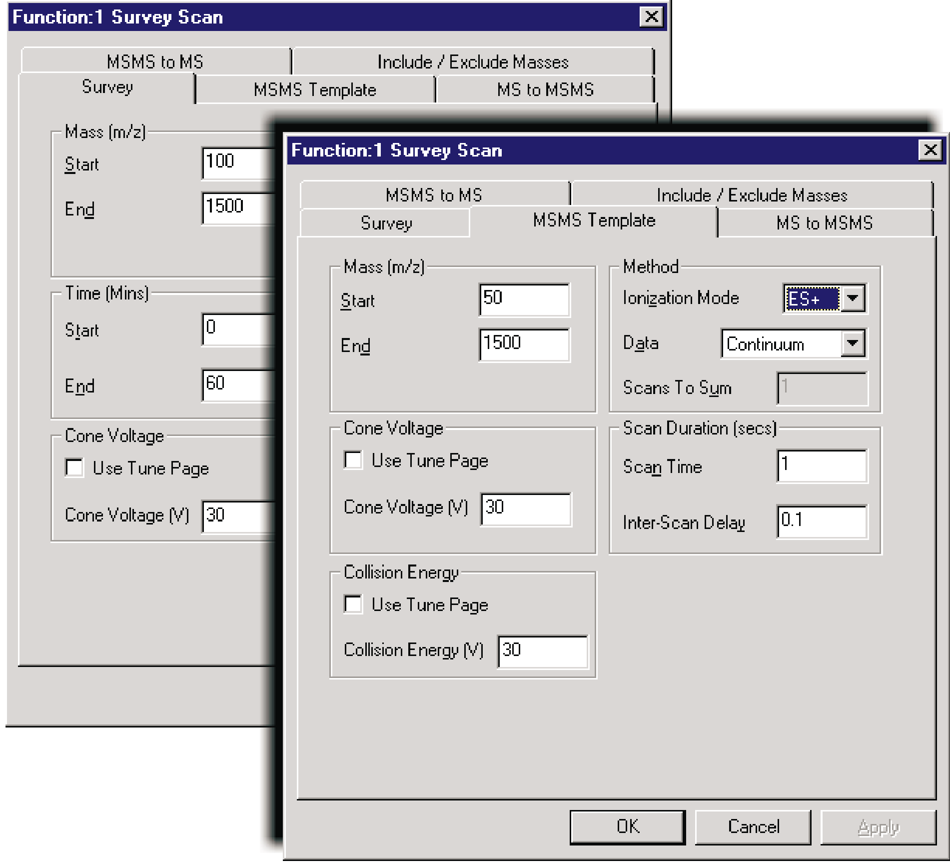


Figure 4-16 Survey and MSMS Template Pages

These pages allow the parameters to be set for MS and MS-MS scanning during the survey, and are similar to normal function editor pages.

MS to MSMS Switching

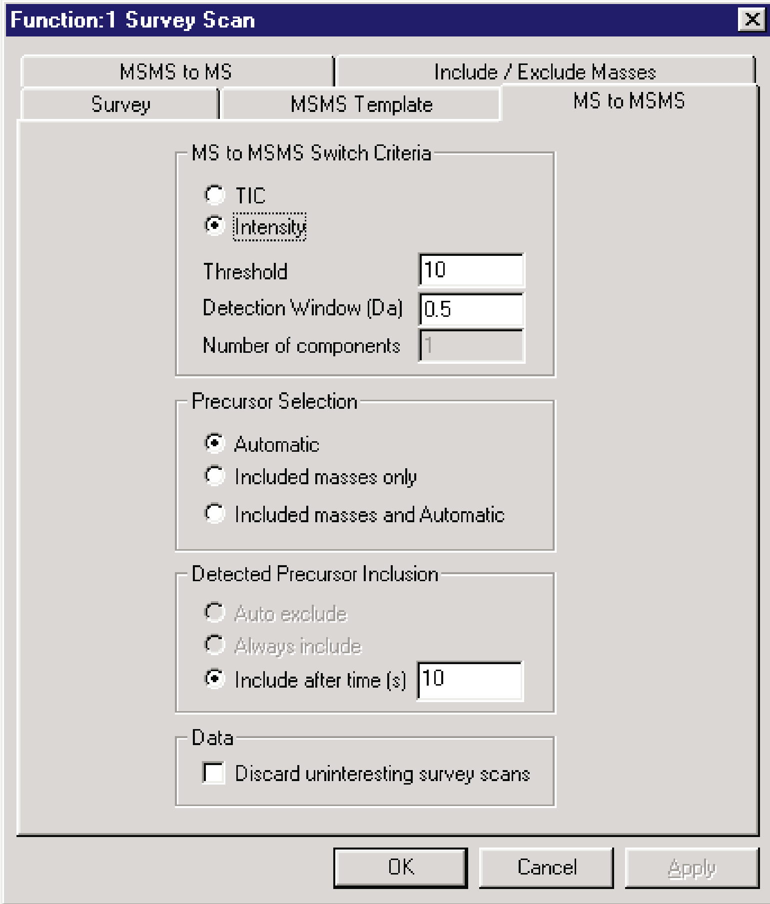


Figure 4-17 MS to MSMS Switching

Switch Criteria

MSMS 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**.

4

Currently **Number of Components** *is set to 1 and can not 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 **Include Masses Only** is selected only masses in the include list (see below) are monitored.

If **Include Masses and Automatic** is selected masses on the include list are given priority. If no precursors are found then 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 which contain no relevant data are rejected.

MSMS to MS Switching

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

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

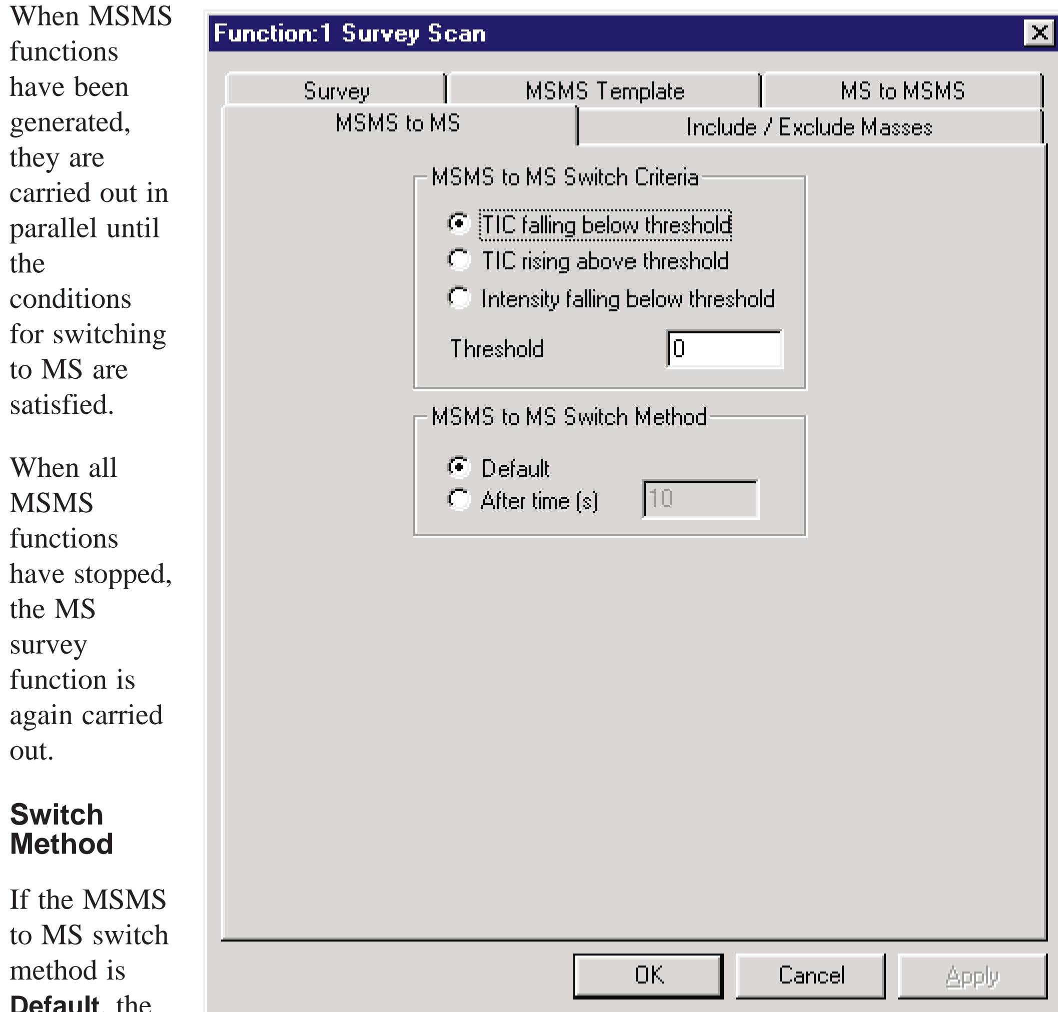


Figure 4-18 MSMS to MS Switching

Switch Method

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

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

Switch Criteria

To define when MS scanning resumes:

4

Select one of the three conditions.

Set **Threshold** to a suitable value.

Including and Excluding Masses

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.

Function:1 Survey Scan

Survey | MSMS Template | MS to MSMS | MSMS to MS | Include / Exclude Masses

Include Masses: Range, File, Browse...

Exclude Masses: Range, File, Browse...

OK | Cancel | Apply

Figure 4-19 Including and Excluding Masses

4

Monitoring Acquisitions

When an acquisition is started the automatic switching status dialog is displayed showing the precursors currently running.

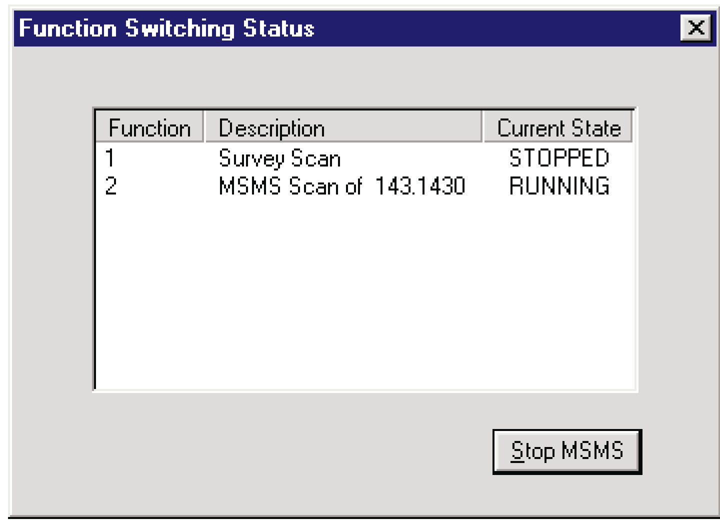


Figure 4-20 Function Switching Status

4

Chapter 5
Mass Calibration

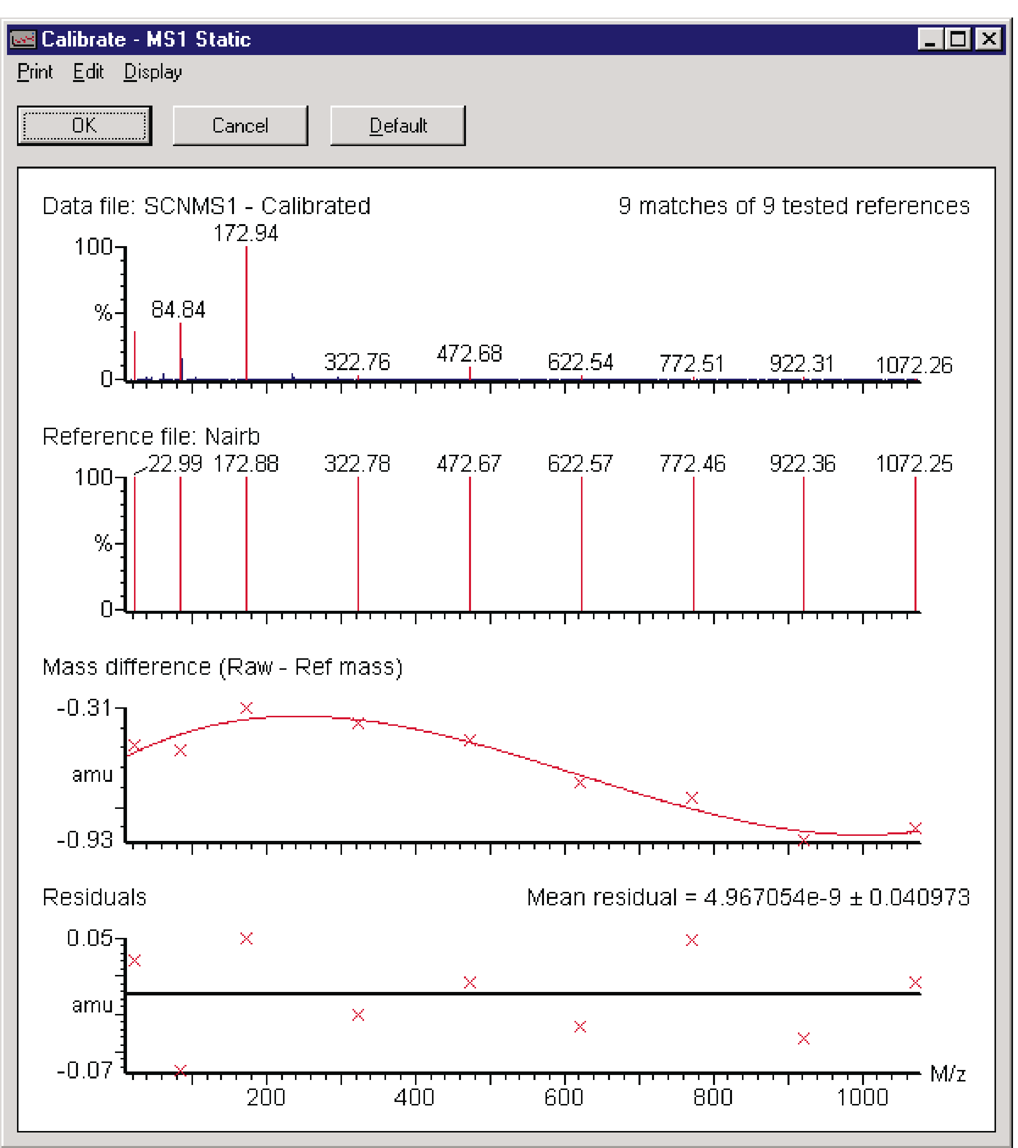


Figure 5-1 Mass Calibration

5

5.1 Introduction

This chapter of the manual is divided into three sections:

- A brief general overview of the calibration process.
- A complete mass calibration of Quattro *micro* API using electrospray ionisation 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 ionisation (APcI) with PEG as the reference compound.

See *Reference Information*, page 181, for details of calibration solutions and their preparation.

5.2 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.

5.2.1 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 table below show which types of calibration are necessary for particular types of experiment.

Experiment	Calibration Required	
	MS1	MS2
MS	All	-
SIR	Static	-
MSMS	All	All
MRM	Static	Static

5.2.2 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.

5

5.3 Electrospray

5.3.1 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.

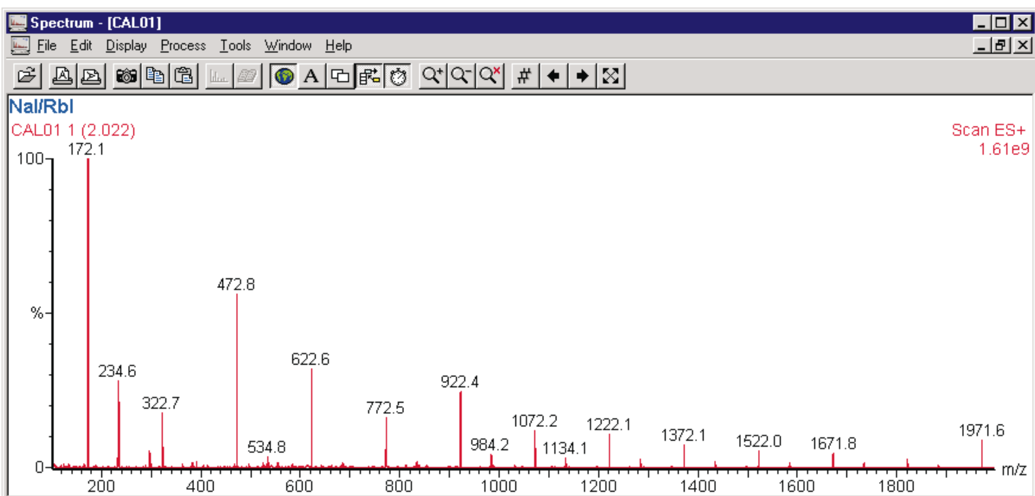


Figure 5-2 Rubidium Iodide

5.3.2 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:

Fill the syringe with the reference solution. See *Setting Up the Syringe Pump*, page 26.

Couple the syringe to the electrospray probe with fused silica tubing.

Set the pump to a flow rate of 10 µl/min.

5

Tuning

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

Set **Multiplier** to 650V.

Adjust source parameters to optimise peak intensity and shape.

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:

Check the intensity of some of the reference peaks above 1000 amu.

Check also the intensity of the peak at $^{m}/_{z}$ 173.

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.

5

Instrument Threshold Parameters

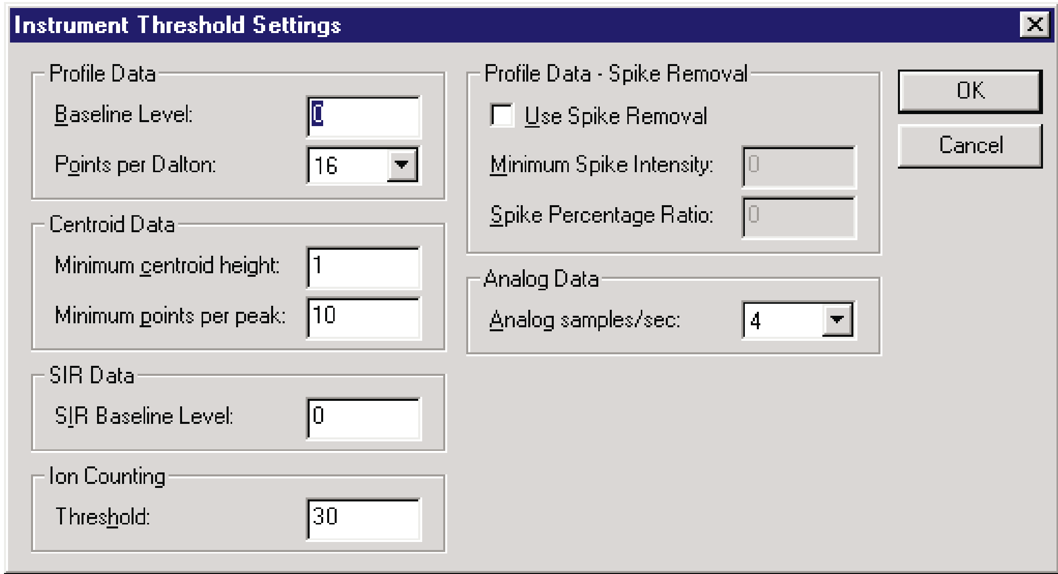


Figure 5-3 Instrument Threshold Parameters

Before beginning the calibration procedure, some instrument parameters need to 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:

From the instrument tune page select **Options** then **Set Instrument Threshold** to display the instrument data thresholding window.

In the **Profile Data** section select **Baseline Level 0** and **16 Points per Dalton.**

Select OK to save the parameters.

Set **Ion Counting Threshold** and **Spike Removal** as appropriate, see *Instrument Data Thresholds*, page 64.

5.3.3 Calibration Options

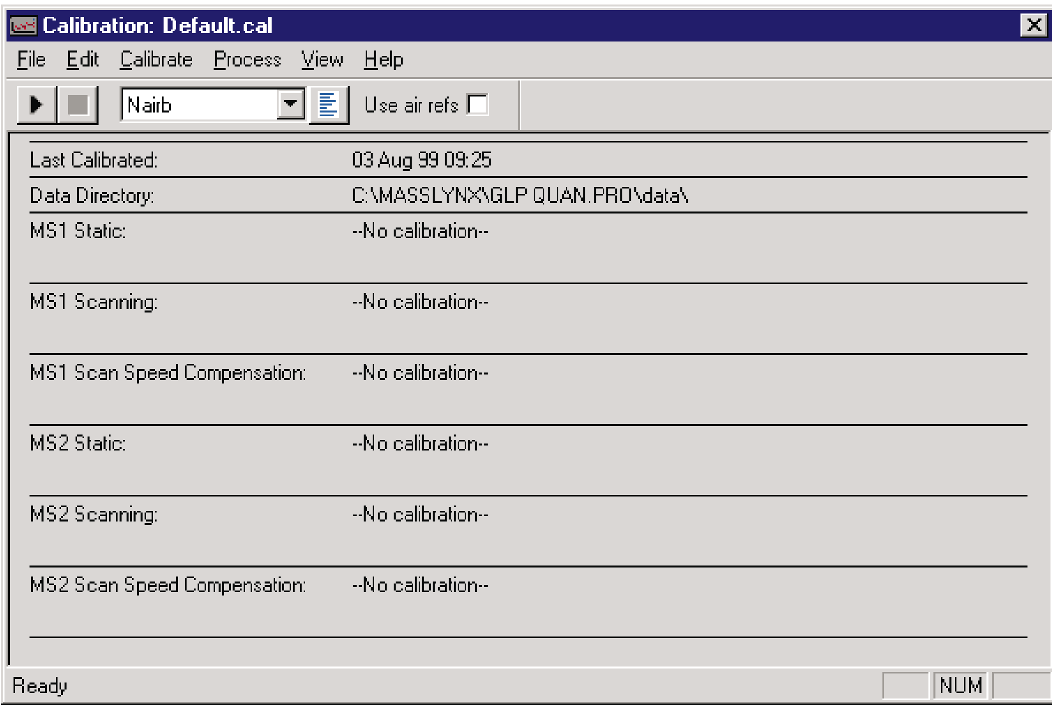


Figure 5-4 Calibration Options

To access the calibration options:

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

Selecting the Reference File

To select the appropriate reference file:

Click on the arrow in the **Reference File** box and scroll through the files.

Select **nairb.ref** for a sodium iodide and rubidium iodide reference solution.

5

Removing Current Calibrations

Select **default.cal** from the **Calibrate** menu option.

Save the changes to the **default.cal** file

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.

5.3.4 Selecting Parameters

A number of parameters needs 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

This is accessed from **Edit**, **AutoCal Check Parameters...**.

It is here that limits are set which the calibration must attain before the instrument is successfully calibrated. Two user parameters can be set.

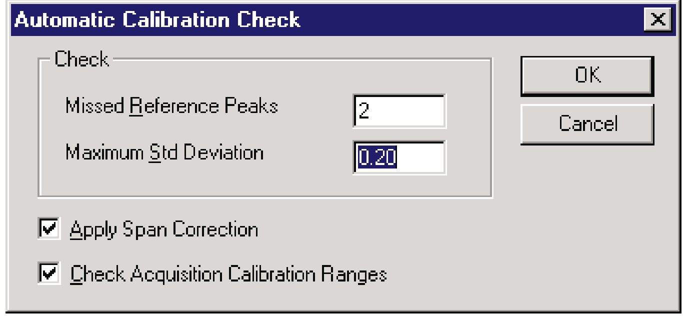


Figure 5-5 Automatic Calibration Check

Missed Reference Peaks sets the maximum number of consecutive peaks which are not matched when comparing the reference spectrum and the acquired calibration spectrum. If this number is exceeded then the calibration fails. 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 then 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 left on. 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 this on.

Calibration Parameters

These are accessed from **Edit**, **Calibration Parameters...**.

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

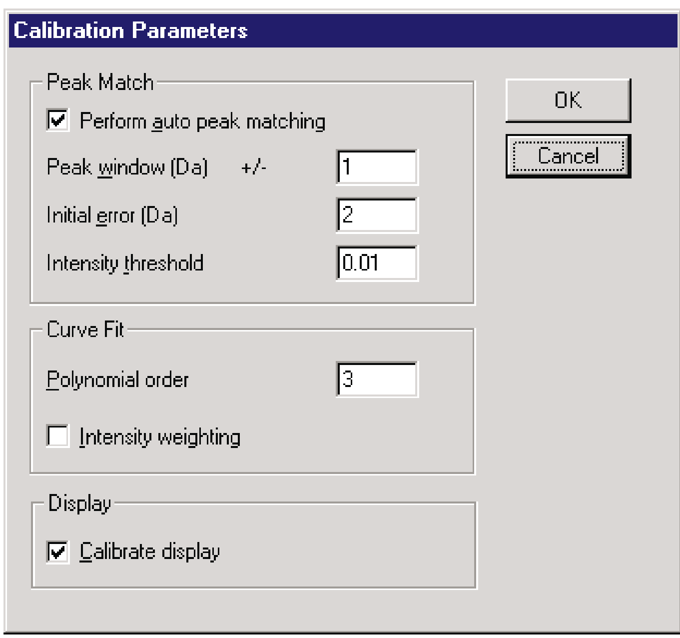


Figure 5-6 Calibration Parameters

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 through **Edit**, **Mass Measure Parameters...**. If continuum or MCA data are acquired for calibration then these parameters need to be set before the calibration is carried out. If centroided data are used for calibration then the mass measure parameters are not used.

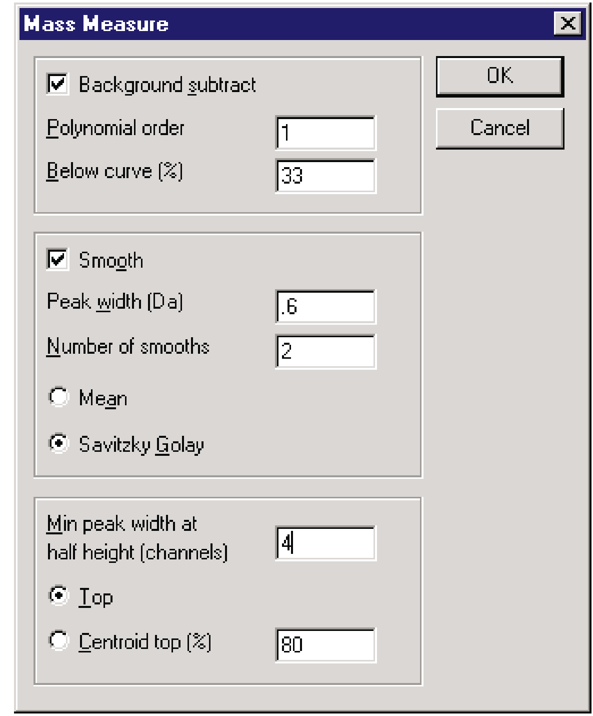


Figure 5-7 Mass Measure Parameters

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.

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.

5.3.5 Performing a Calibration

Three types of calibration are available with MassLynx: static calibration, scanning calibration and scan speed compensation. These are selected on the Automatic Calibration dialog box (see below) which is accessed by selecting **Start...** from the Calibrate dialog box.

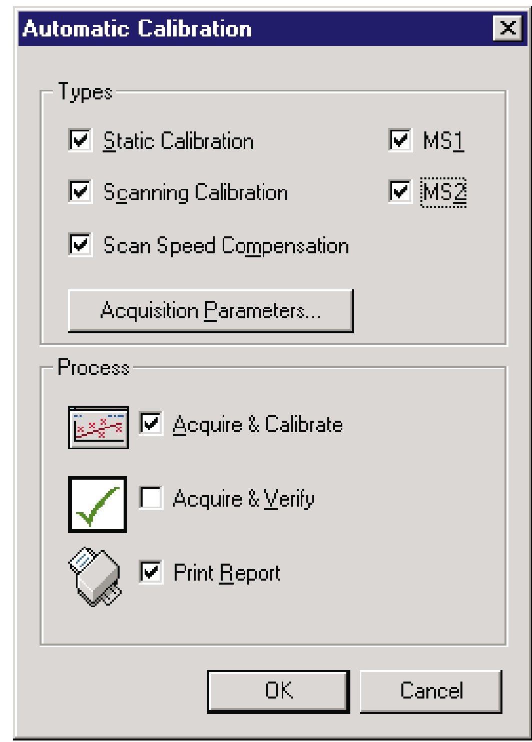
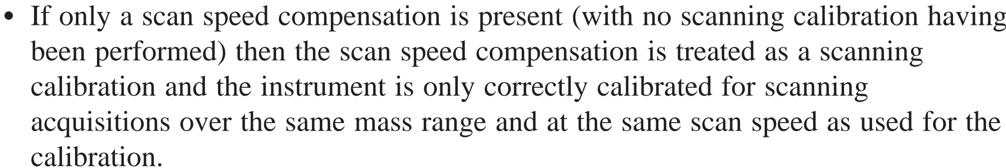


Figure 5-8 Automatic Calibration

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 then 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 then 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) then 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, then 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 second scan (400 amu/sec) then data can be acquired from 100 - 500 amu with a 1 second scan time (also 400 amu/sec) 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 then 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.

For a complete calibration:

Check the boxes in the **Types** area of the dialog box adjacent to **Static Calibration**, **Scanning Calibration** and **Scan Speed Compensation**. Check also the **MS1** and **MS2** boxes.

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 brings forward a second box, shown below, 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.

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 *Reference Information* 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.

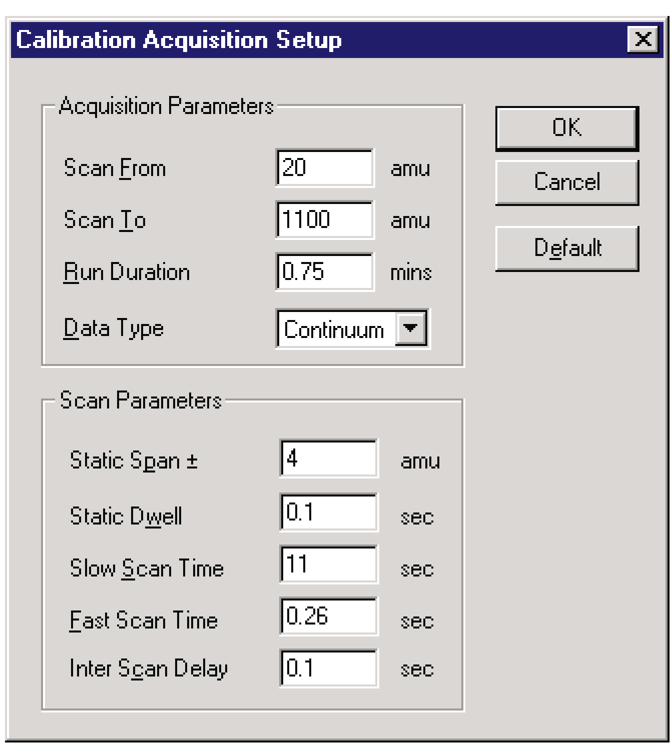


Figure 5-9 Acquisition Parameters

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. If the run duration is too short then data are not acquired. The slowest scan speed generally used is 100 amu/sec. With **Scan From** set to 20 amu and **Scan To** set to 2000 amu a scan time of 19.8 seconds is required, and an **Inter Scan Delay** (in the lower area of the box) of 0.1 second 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 second 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 then the scan speed should be set to approximately 100 amu/sec (a scan time of 19.8 seconds over a mass range of 20 to 2000 amu). If only a scanning calibration is to be performed (without scan speed compensation) then 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/sec is adequate for most applications. A scan range from 20 amu to 1100 amu requires a **Fast Scan Time** of 0.26 sec at an **Inter Scan Delay** of 0.1 sec. 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 following order using the data file names shown:

MS1 static calibration	data file: STATMS1
MS1 scanning calibration	data file: SCNMS1
MS1 scan speed compensation	data file: FASTMS1
MS2 static calibration	data file: STATMS2
MS2 scanning calibration	data file: SCNMS2
MS2 scan speed compensation	data file: 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 above. If the full calibration dialog box is open then 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 then a successful calibration message is displayed. A calibration report is then printed showing a calibration curve for each of the calibration processes. Examples of calibration reports are shown on the following pages.

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

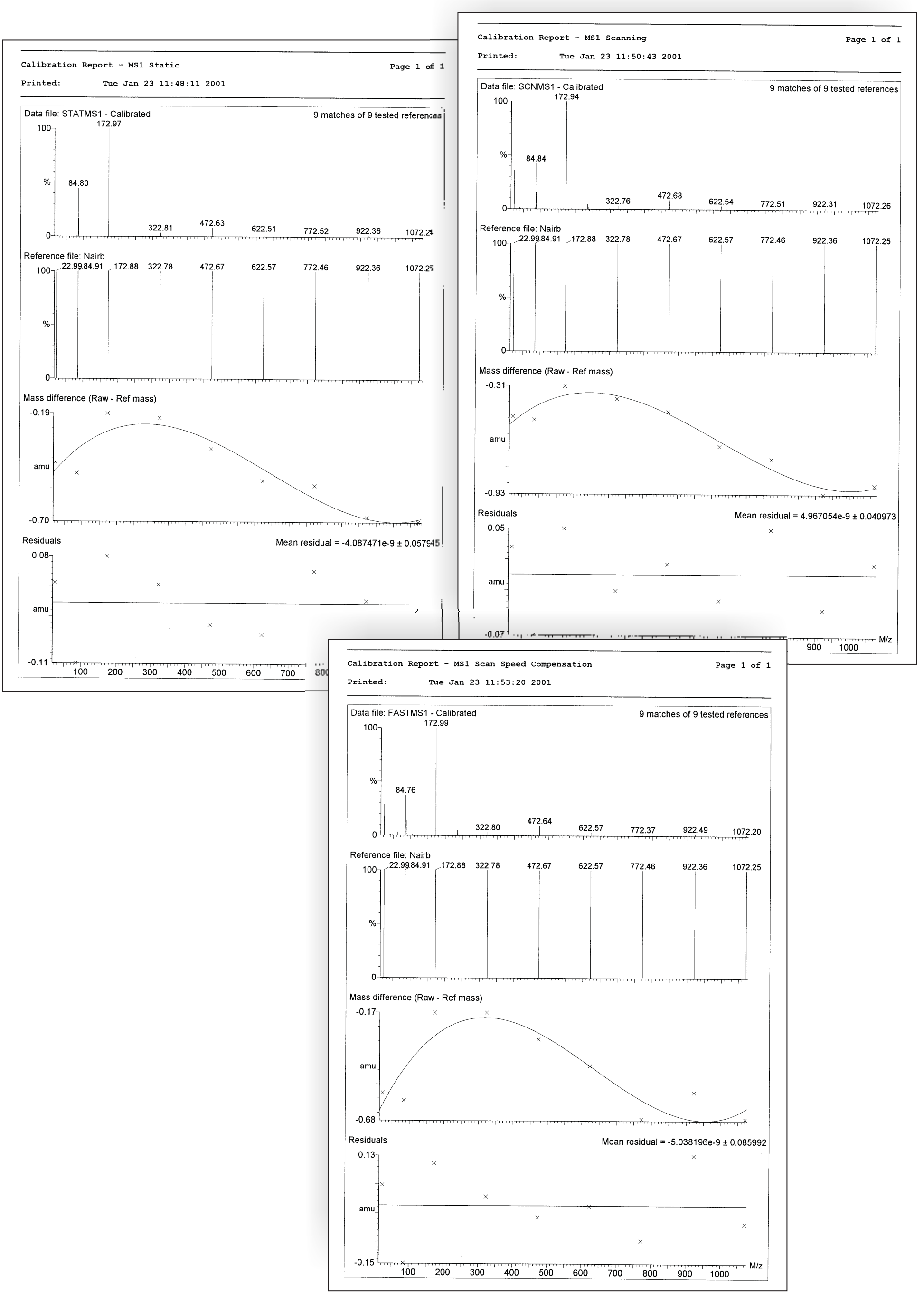


Figure 5-10 Calibration Reports

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5.3.6 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 displayed (see below) allows the choice of calibration type for viewing. With the required calibration selected the correct calibration file is automatically called up.

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

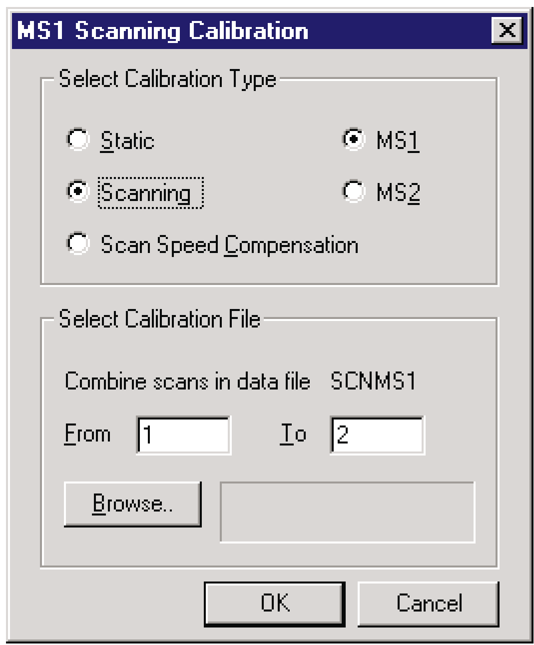

Figure 5-11 Calibration Options

Clicking on **OK** repeats the calibration procedure for that particular file and display a calibration report on the screen. This calibration report (opposite) 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 (opposite lower) 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 colour.

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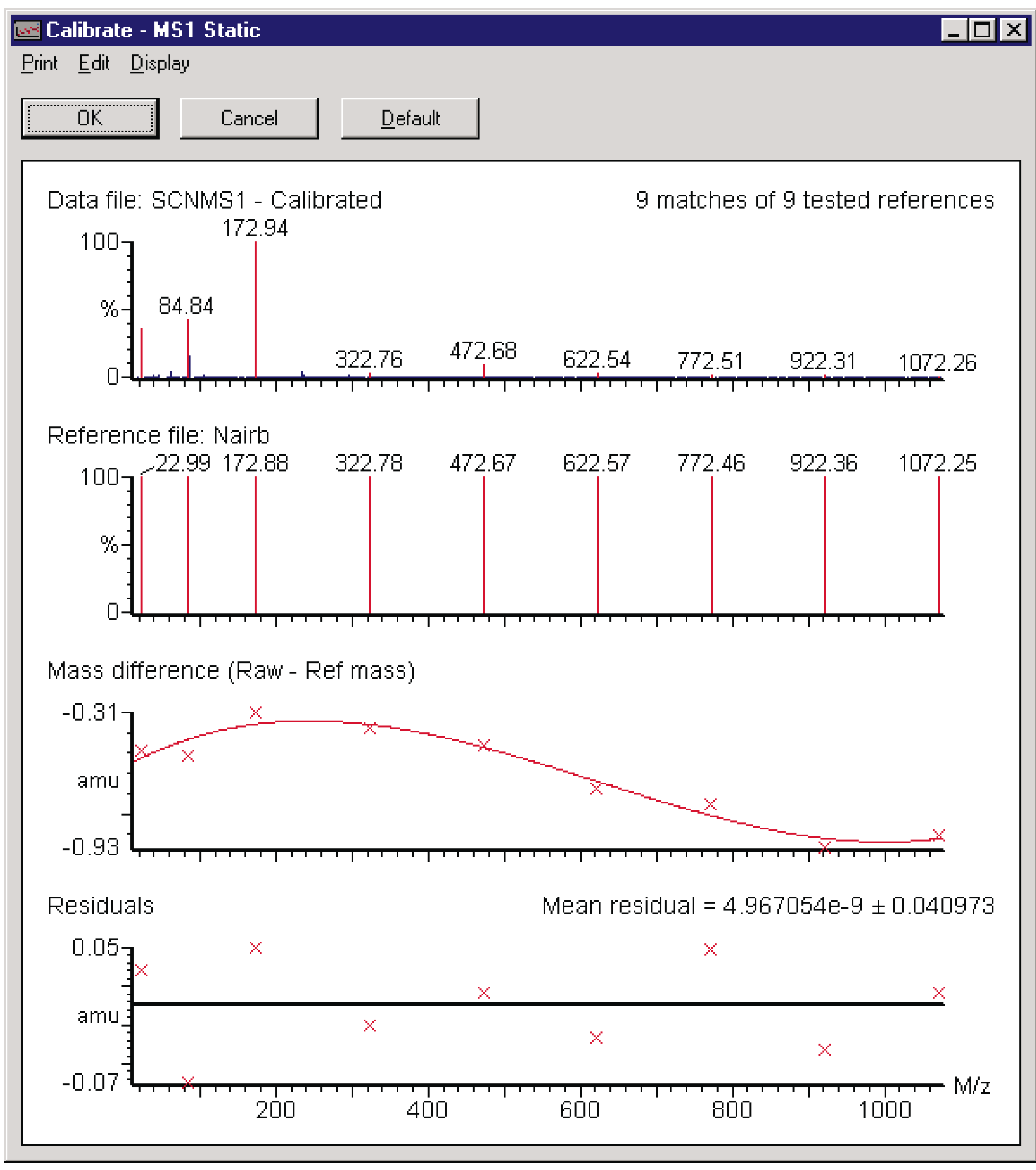

Figure 5-12 Calibration Report

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Calibration Failure

If the calibration statistics do not meet the requirements then 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.

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

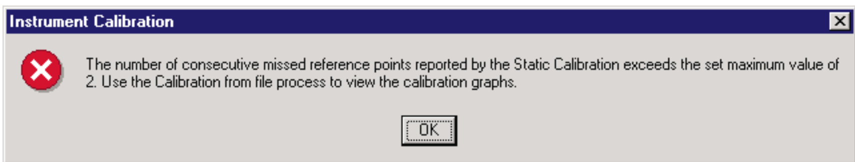

Figure 5-13 Calibration Failure Report

- **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, then 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 ionisation 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.

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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 then the first three reasons (as explained above in "No peaks") are likely causes.

If the peaks are present in the data but are not recognised during calibration then the latter four ("Too many consecutive peaks missed") are likely reasons.

Having taken the necessary action, proceed as follows:

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 $PEG+H^+$, $PEG+NH_4^+$, $PEG+Na^+$, *and also doubly charged species.*

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

5

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 then 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 then 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.

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 then 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:

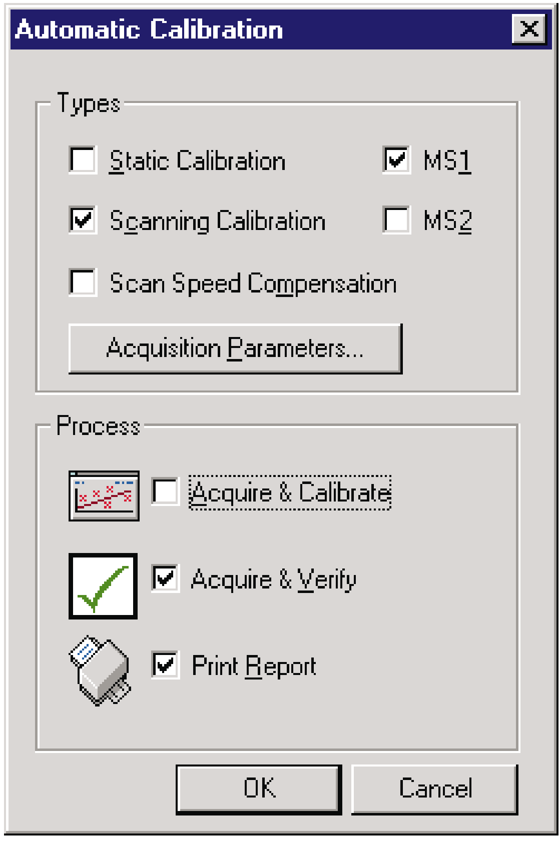

Figure 5-14 Automatic Calibration Menu

Set up the instrument and access the calibrate dialog box as though a full calibration is to be carried out.

Set all peak matching parameters to the values that were used for the calibration.

Bring up the Automatic Calibration dialog box by selecting **Start...** on the Calibrate dialog box.

Select **Scanning Calibration** and deselect **Static Calibration** and **Scan Speed Compensation**.

Deselect **Acquire & Calibrate** and select **Acquire & Verify** and **Print Report.**

Select either **MS1** or **MS2**, depending on the type of acquisition to be performed.

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.

5

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.

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.

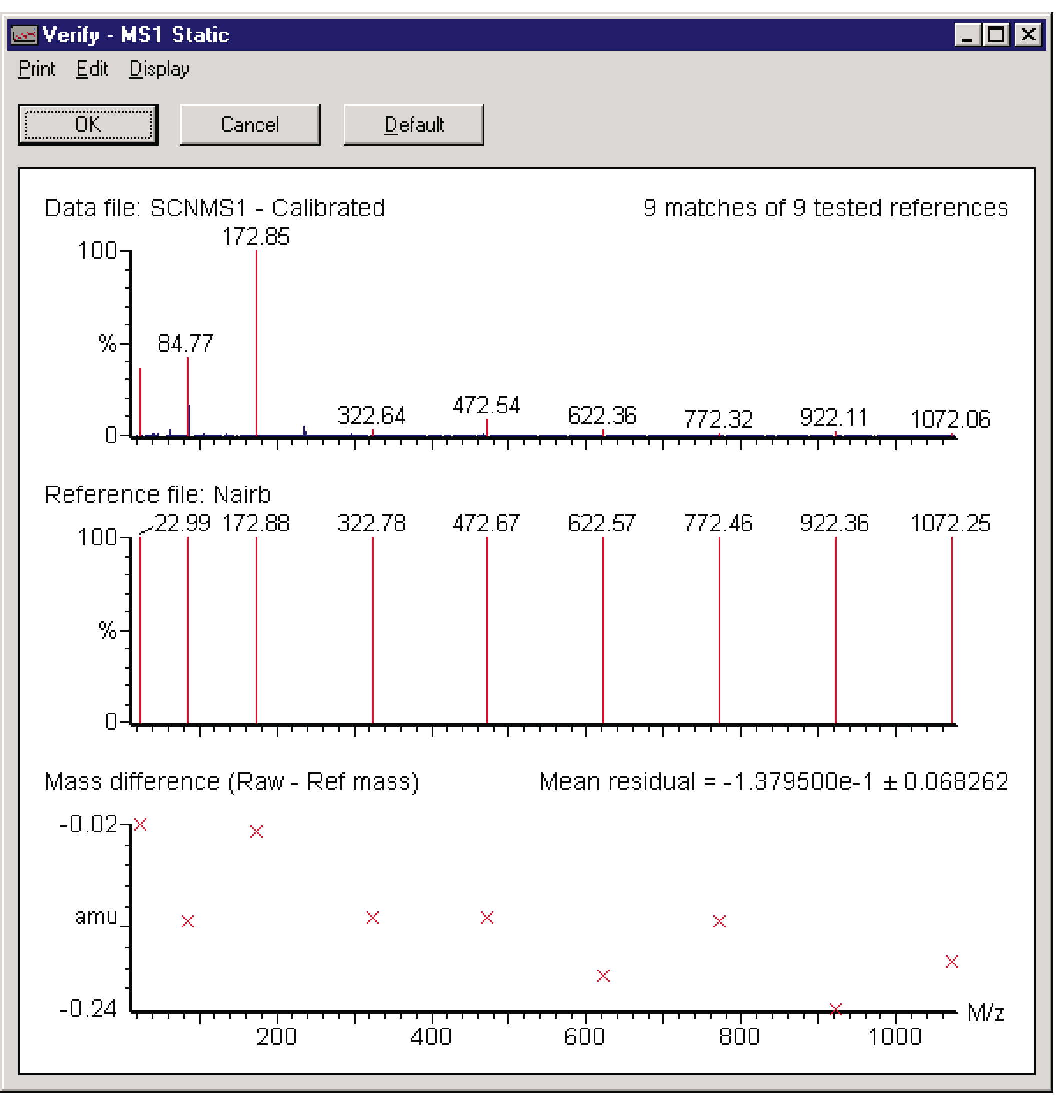

Figure 5-15 Verification Report

5.3.7 Electrospray Calibration with PEG

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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 $[M+NH_4]^+$ ions, under some conditions it is quite usual to see $[M+H]^+$, $[M+Na]^+$ and doubly charged ions.

The spectrum shown below demonstrates how the PEG spectrum can be dominated by doubly charged ions (in this case $[M+2NH_4]^{2+}$) if the wrong conditions are chosen. In this case the concentration of ammonium acetate in the reference solution is too high (5mmol ammonium acetate is the maximum that should be used) and **Cone** is too low.

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

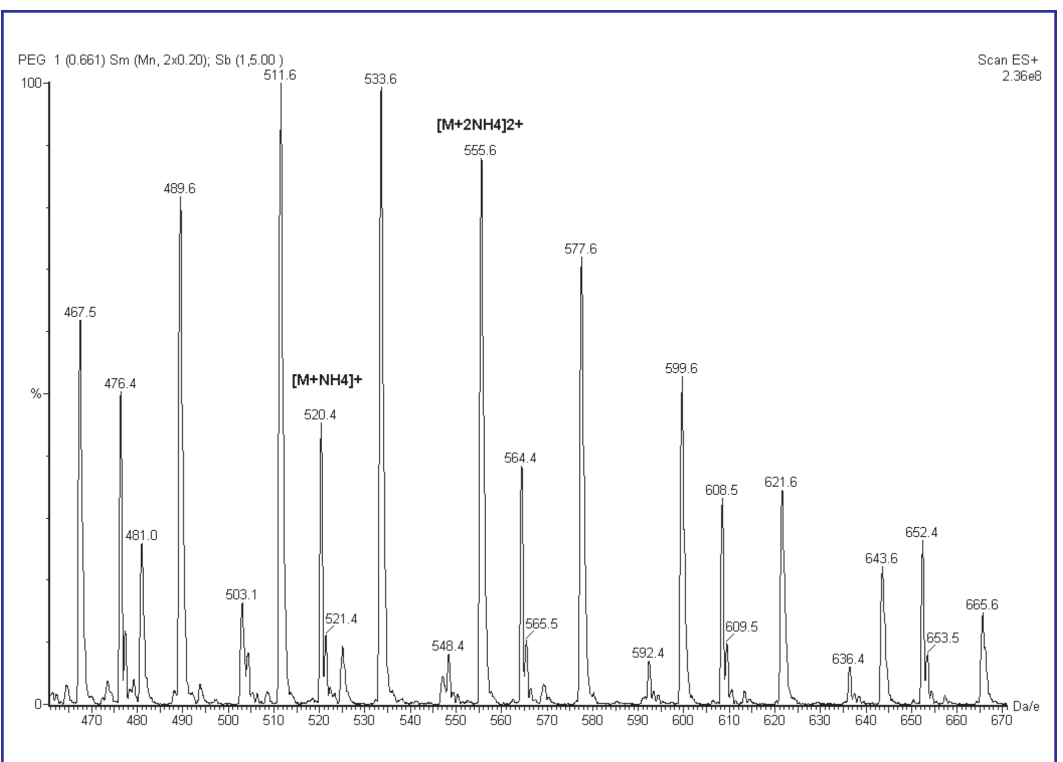

Figure 5-16 Electrospray Spectrum of PEG

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.

5

5.4 Atmospheric Pressure Chemical Ionisation

5.4.1 Introduction

This chapter describes a complete mass calibration of Quattro *micro* API using atmospheric pressure chemical ionisation. The procedures described should be followed only after reading the previous chapter in this manual, describing the automated calibration with electrospray ionisation.

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 *Reference Information*, page 181, for advice on preparing the reference solution. See the following illustration for a typical PEG + NH_4^+ 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.

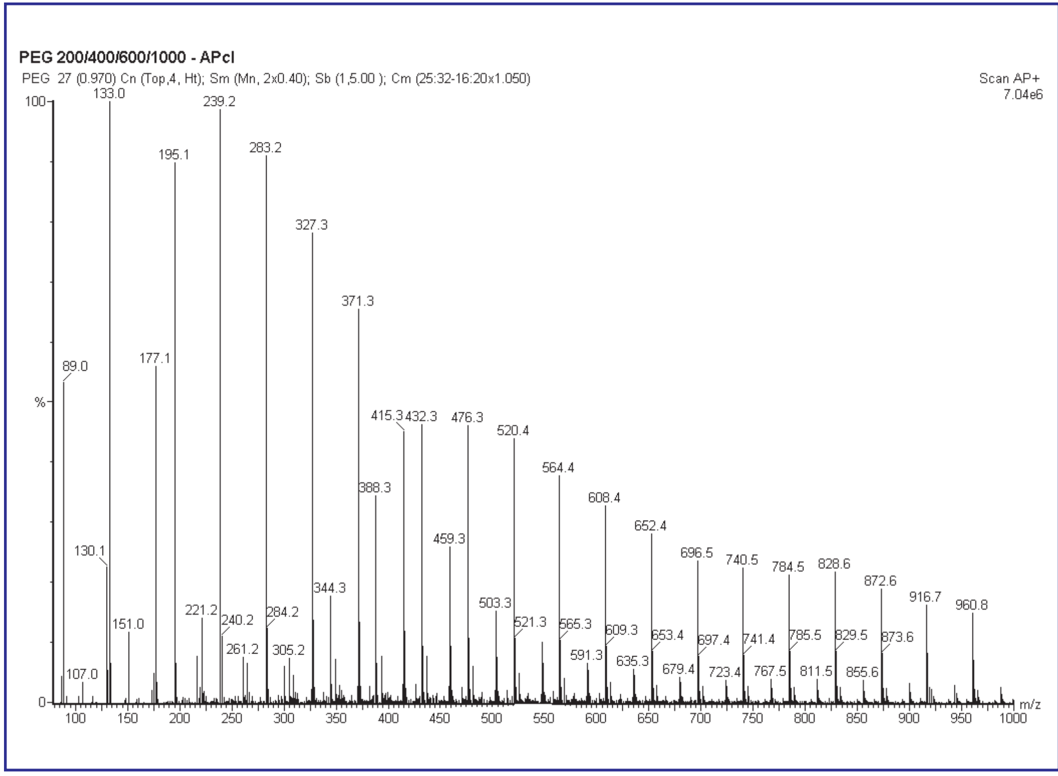

Figure 5-17 APcI Spectrum of PEG

5.4.2 Preparing for Calibration

Reference Compound Introduction

It is best to use a large volume injection loop (50µl) 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 50µl of reference solution lasts for approximately 15 seconds, allowing enough time to perform a slow scanning calibration.

Tuning

Before beginning calibration:

Set **Multiplier** to 650V.

Adjust source and lens parameters to optimise peak intensity and shape.

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.

5.4.3 Calibration Options

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

Selecting Reference File

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.

Leave the **Use Air Refs** box blank when calibrating in APcI.

Removing Current Calibrations

Select **Default** from the **Calibrate** menu option.

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.

5.4.4 Selecting Calibration Parameters

A number of parameters needs 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**.

5.4.5 Performing a Calibration

The three types of calibration (static, scanning and scan speed) must be carried out in single steps.

Static Calibration

Access the Automatic Calibration dialog box by selecting **Start...** from the Calibrate page.

Check **Static Calibration** and **MS1** in the **Types** area of the dialog box.

In the **Process** area of the dialog box, check **Acquire & Calibrate**.

Acquisition Parameters

Selecting **Acquisition Parameters...** brings forward the mass ranges, scan speeds and acquisition mode relevant to the pegnh4.ref reference file.

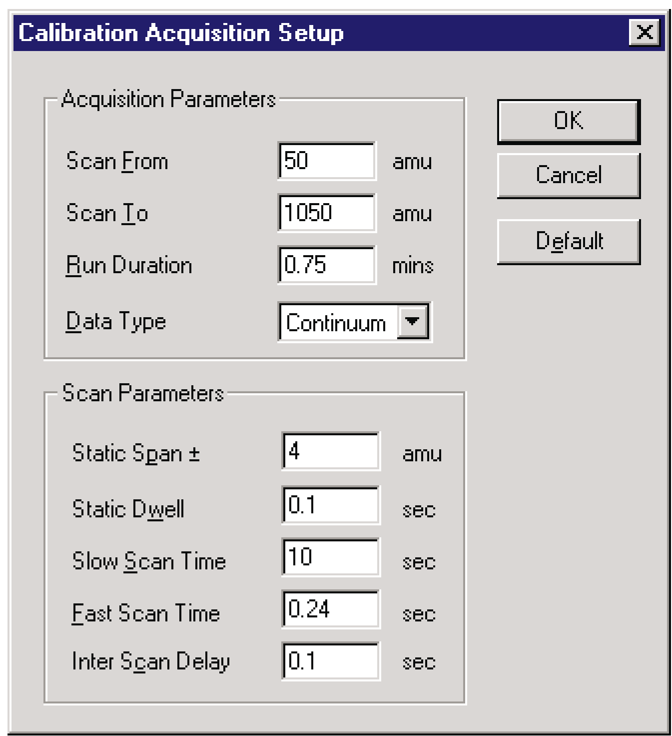

Figure 5-18 Calibration Acquisition Setup

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**.

5

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 4.0 amu is typical.

Static Dwell determines how much time is spent acquiring data across the span. A value of 0.1 second 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 to return to the Automatic Calibration dialog box.

Acquiring Data

To start the acquisition:

Select **OK** from the Automatic Calibration dialog box.

The instrument acquires a calibration file ready for static calibration using the data file name STAT. While data are being acquired:

Inject the reference solution.

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:

From the chromatogram window call up the calibration file **STATMS1**.

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.*

Return to the Calibrate dialog box. Access the manual calibration options, as shown, by selecting **Calibrate From File...**.

Select **Static** calibration type and **MS1**.

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...**.

Enter the start and end scans of the reference data in the **From** and **To** boxes.

Select **OK** to perform the calibration and display the calibration report on the screen (opposite upper).

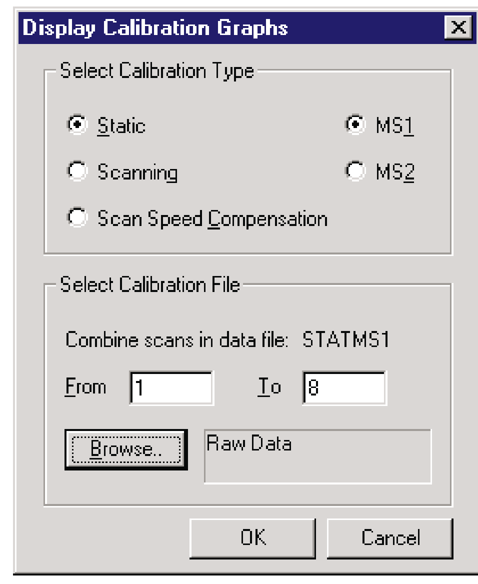

Figure 5-19 Display Options

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 colour.

> 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 *Calibration Failure*, page 132.

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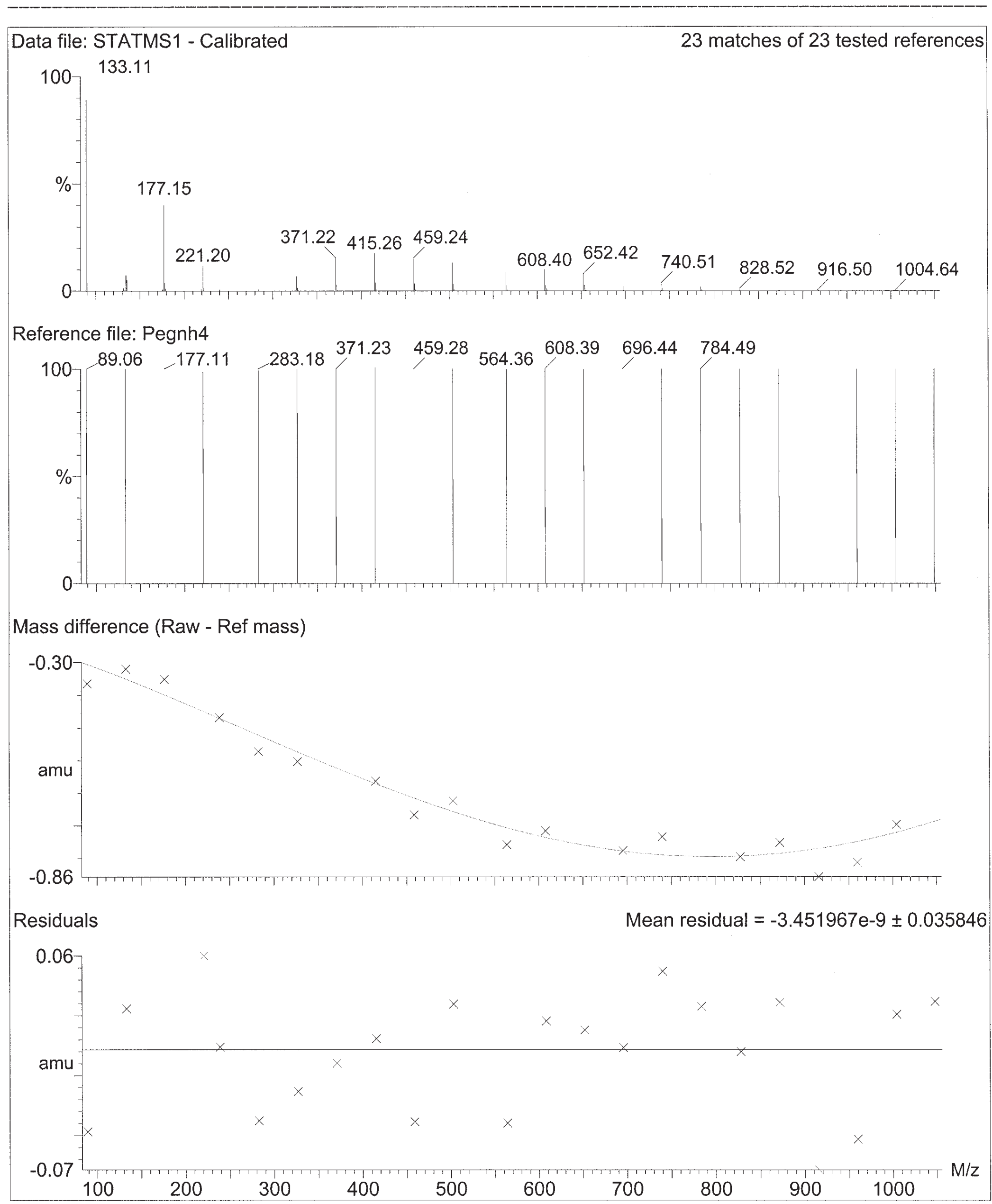

Figure 5-20 Calibration Report - MS1 Static

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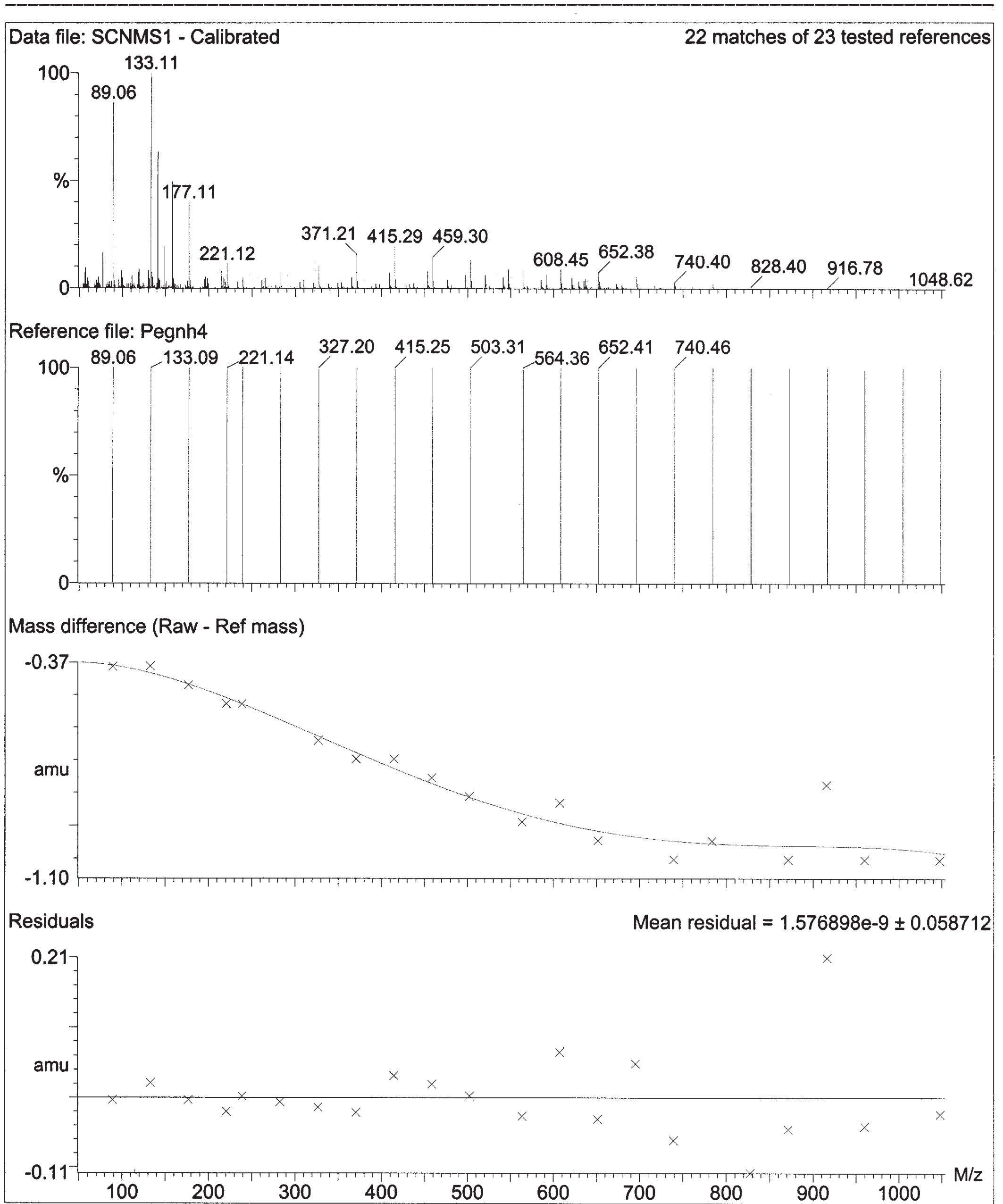

Figure 5-21 Calibration Report - MS1 Scanning

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

Select **Print**, **Print** from the report display.

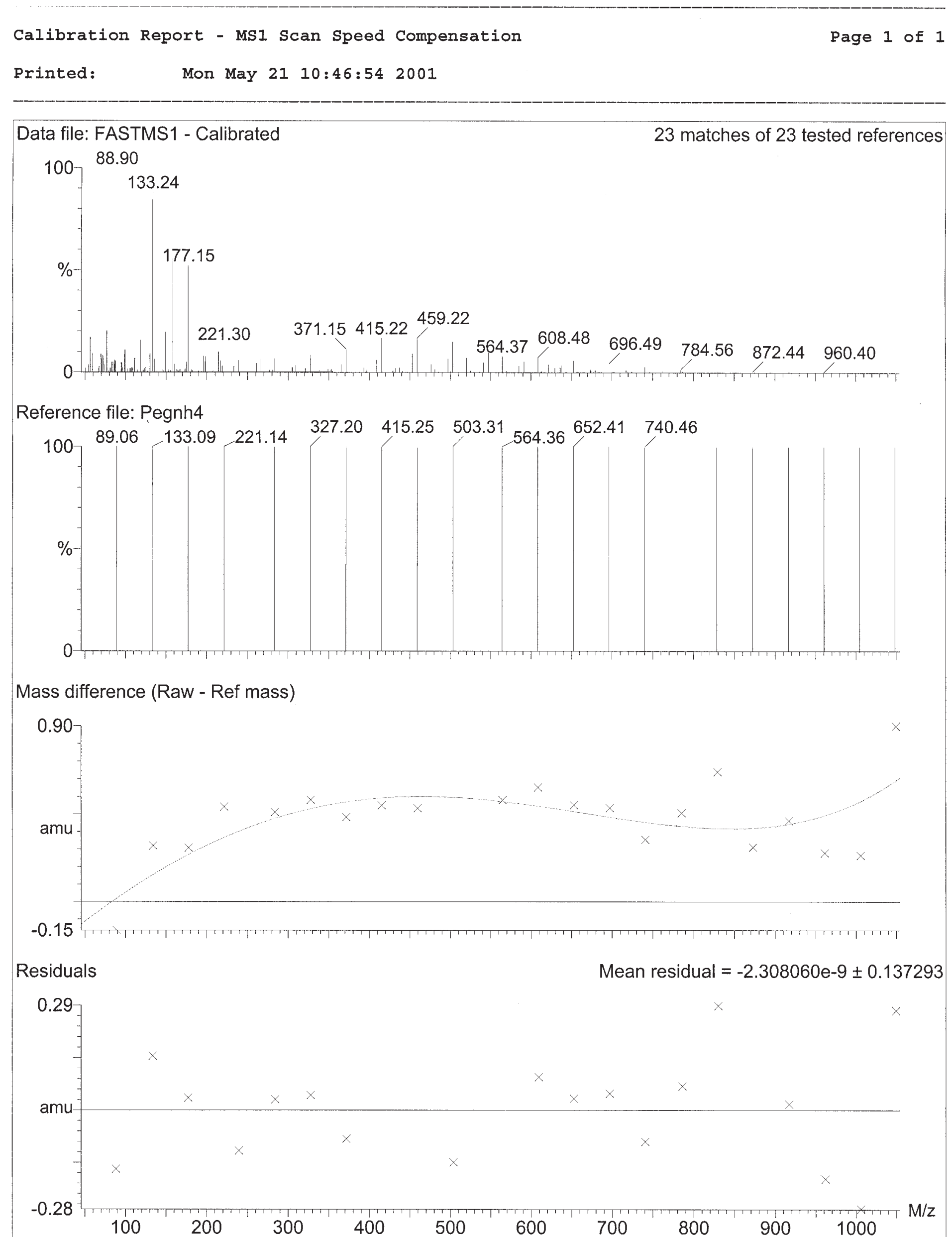

Figure 5-22 Calibration Report - Scan Speed Compensation

To accept the calibration:

Select **OK** from the calibration report.

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 then 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.

The recommended scan speed for the scanning calibration is 100 amu/sec.

Set **Scan From** to 50 amu and **Scan To** to 1050 amu.

Set **Scan Time** to 10 sec and **Inter Scan Delay** to 0.1 sec.

Select **Continuum** as the **Data Type**.

Although Continuum is recommended centroided data may be used.

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 seconds allowing at least one full scan of useful data to be acquired.

Choose any filename for the data.

The filename SCNMS1, the name used during an automatic calibration, is valid.

Start the acquisition and inject the reference solution.

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

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.

Set **Scan From** to 50 amu and **Scan To** to 1050 amu.

Set **Scan Time** to 0.24 sec and **Inter Scan Delay** to 0.1 sec.

Select **Continuum** as the **Data Type**.

Set **Run Duration** to 2.0 minutes.

Choose any filename for the data.

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

Start the acquisition and inject the reference solution.

Manual Calibration

Find the start and end scans of the reference data for each file in the same way as for the static calibration file.

From the tune page select **Calibration**.

Select **Scanning** calibration type and **MS1**, **Calibrate Instrument**, **Calibrate From File**.

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, then the correct file can be selected by clicking on* **Browse...**.

Enter the start and end scans of the reference data in the **From** and **To** boxes.

Select **OK** to perform the calibration and display the calibration report on the screen in a similar way to the static calibration.

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

If the correct peaks have been matched then 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 *Calibration Failure*, page 132. 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 then this must be selected using the data browser.

Once all three calibrations (static, scanning and scan speed compensation) have been completed then 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/sec.

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 then 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/sec.

5.4.6 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 parameters then 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 ionisation 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 then the first three reasons above are likely causes.

If the peaks are present in the data, but are not recognised during calibration, then the latter four are likely reasons.

Having taken the necessary action, proceed as follows:

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 $PEG+H^+$*,* $PEG+NH_4^+$ *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.*

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

5.4.7 Incorrect Calibration

If the suggested calibration parameters are used and providing that good calibration data have been acquired, then 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 then 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.

5.4.8 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.

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 then 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.

5.4.9 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 ionisation modes, so that when an ionisation 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.

5.4.10 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:

Set up a scanning acquisition over the required mass range and at the required scan speed in the normal way.

Start the acquisition and inject the reference solution so that reference data is acquired.

Stop the acquisition.

Access the calibrate dialog box and set all peak matching parameters to the same values that were used for the calibration.

Select **Process**, **Verification from file...** and check **Scanning Calibration** (see below).

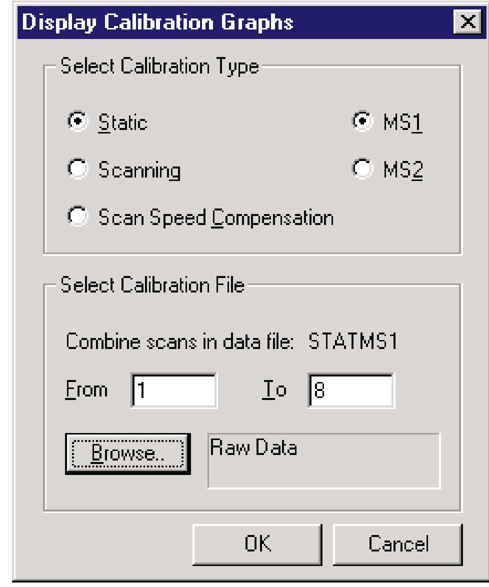

Figure 5-23 Display Options

Select Scanning Calibration and either **MS1** or **MS2** depending on the type of data acquired.

Clicking on **Browse...**, select the acquired file and enter the start and end scans of the reference data.

Select **OK** to verify the calibration.

5

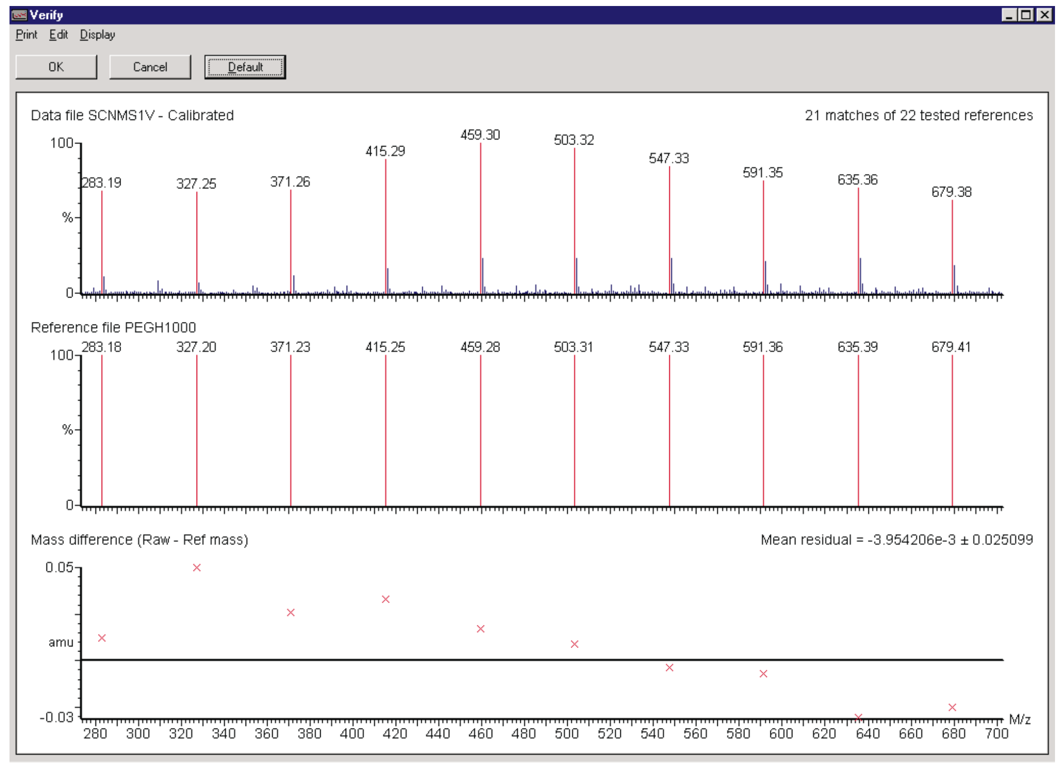

Figure 5-24 Verification Report

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 above. 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.

Chapter 6 Maintenance Procedures

6.1 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 Procedure	Frequency
Gas-ballast the rotary pump	Daily (APcI) Weekly (ESI)
Check and adjust the rotary pump oil level	Weekly
Change the oil in the rotary pump	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 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

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6.2 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.

Caution: Do not touch integrated circuit chips or other circuit board components. Static electric charge can damage electronic components.

6.2.1 Proper Operating Procedures

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

6.2.2 Maintenance Equipment

Routine parts cleaning requires the following equipment:

- An ultrasonic bath with a minimum chamber size of 300mm × 150m × 100mm (12" × 6" × 4").
- Glass vessels, approximately 100mm (4") in diameter and 120mm($4^3/_4$") high.
- A 500ml graduated cylinder (to use when cleaning the hexapole assembly).

6.3 Routine Maintenance

6.3.1 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.

Caution: Do not vent the instrument when the rotary pump is gas-ballasting.

Caution: Do not gas-ballast the rotary pump while Quattro *micro* API is in the **Operate** mode.

Caution: Never gas-ballast the rotary pump for more than 2 hours.

To gas-ballast the pump:

Shut the vacuum system isolation valve, moving its handle fully to the right.

Turn on the gas-ballast.

When the oil is clear and has drained back to the rotary pump:

Return the gas-ballast control to its normal position.

Open the vacuum system isolation valve.

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6.3.2 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 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 below.

6.3.3 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:

Operate the pump to warm the oil before draining.

Gas ballast the rotary pump as described above.

Vent and shut down the instrument, turning the power switch to off.

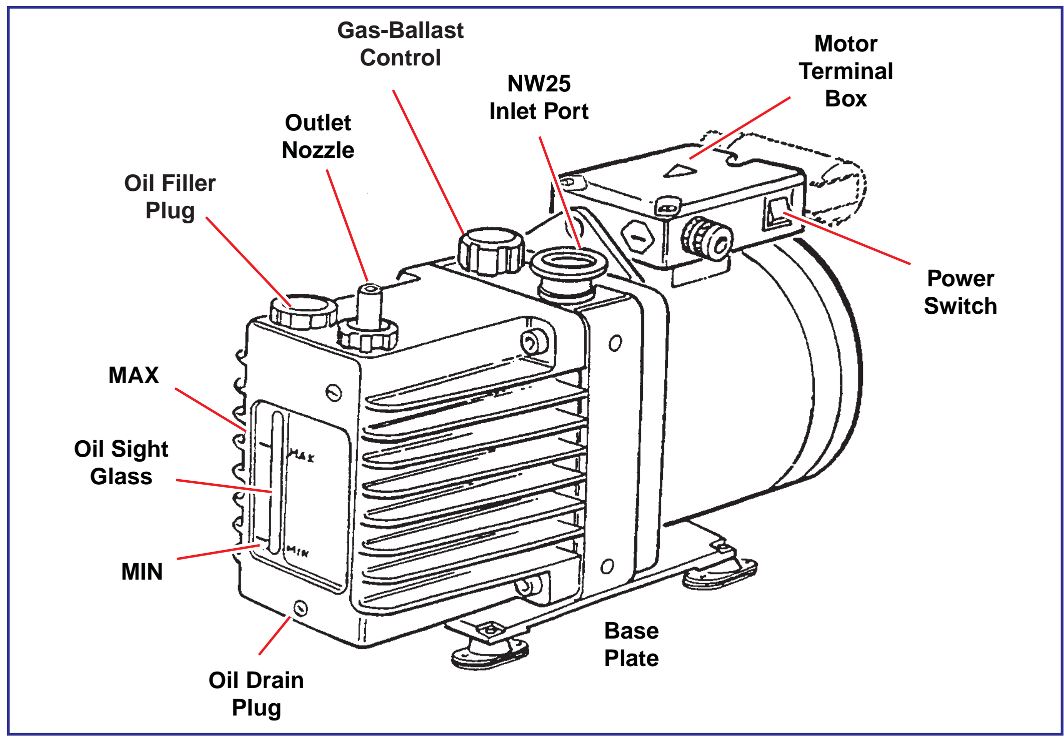

Figure 6-1 Rotary Pump

Raise the pump 150 to 200mm (6 to 8 inches) above the floor, if necessary.

Place an object under the motor to tilt the pump toward the side on which the oil drain plug is located.

Remove the oil filler plug to facilitate drainage.

Using the flat-blade screwdriver, remove the oil drain plug.

Let the oil drain completely, then replace the oil drain plug.

Fill the pump until the oil in the sight glass reaches the **MAX** level.

Allow a few minutes for the oil to drain into the pump.

Recheck the oil level, and add more oil if necessary.

Replace the oil filler plug and, if necessary, lower the pump to the floor.

Turn the rotary pump power switch to **On**.

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6.3.4 Cleaning the Source Assembly

Overview

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

- 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:

- Disassemble the source components, which are fully described later in this chapter.
- Clean the source components.
- Remove and clean the hexapole assembly.
- Replace the hexapole assembly.
- Reassemble the source components.

Required Materials

The following materials are required to clean the source components:

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

- Clean 1000ml beaker.
- Clean 500ml 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:

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.

To reduce cooling time significantly, continue flowing API gas.

When the probe has cooled, stop the nitrogen flow by deselecting [API GAS icon] on the toolbar or by choosing **Gas** from the **Gas** menu.

Remove the APcI or ESI probe.

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

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

The icon changes from green to red. This means all high voltages are turned off, as well as the ESI desolvation /APcI probe heater.

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.

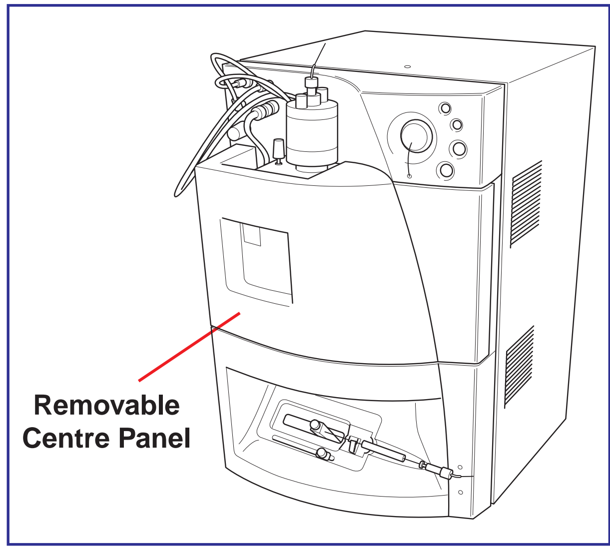

Figure 6-2 Centre Panel

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.

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Disconnect the front panel gas and electrical connections.

Unscrew the probe's two knurled thumbscrews, and retract it from the source.

Warning: Handle the probe carefully. It might still be hot.

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

Unscrew the four thumbscrews and remove the source enclosure cover.

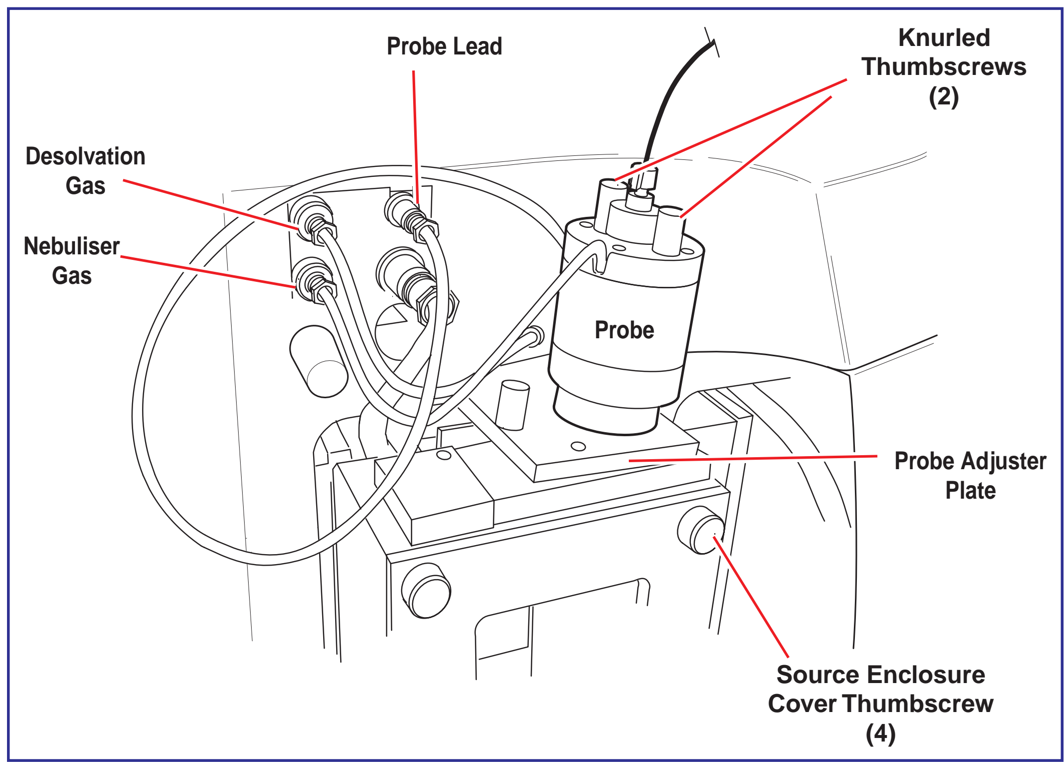


Figure 6-3 Probe and Source Connections

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

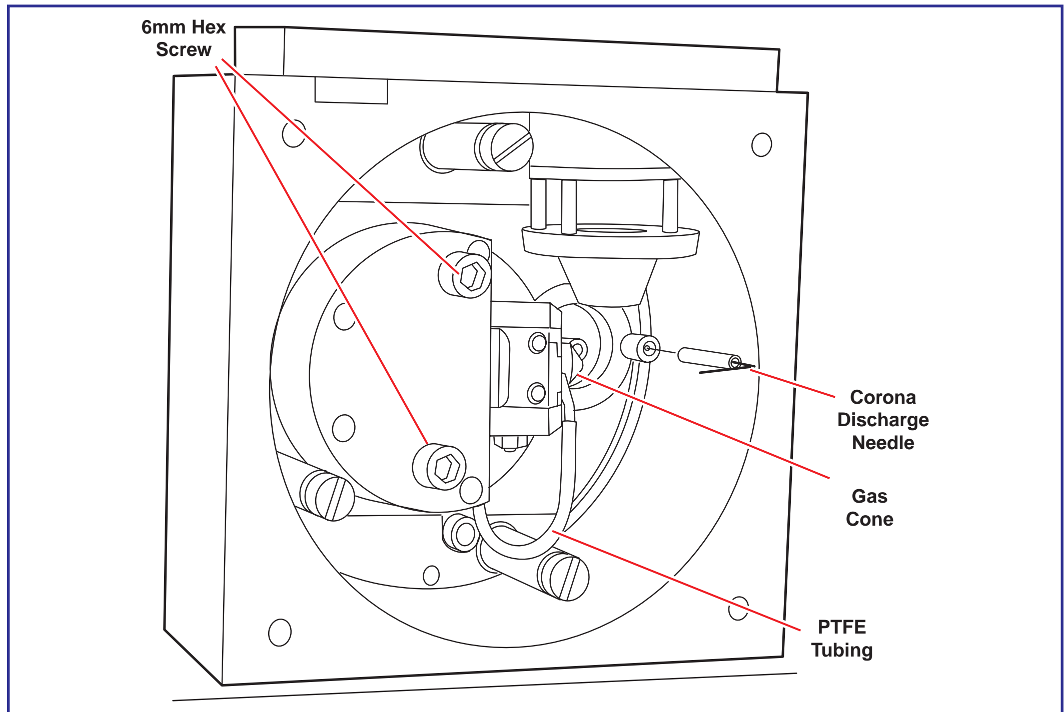


Figure 6-4 Source Enclosure

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

Remove the PTFE tube attached to the cone gas cone.

Remove the two screws that secure the cone retainer, using the small, flat-blade screwdriver.

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

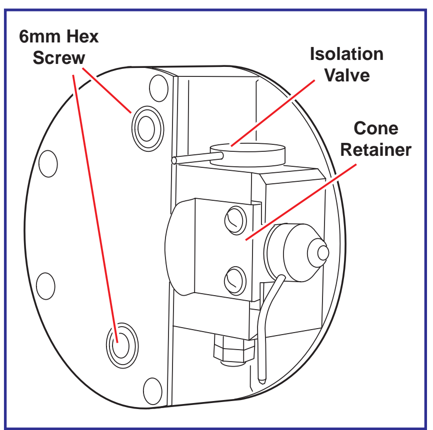


Figure 6-5 Ion Block

Carefully separate the sample cone from the gas cone, then remove the sample cone O ring.

Remove the baffle plate, and set all pieces aside.

Caution: Take care not to scratch the highly polished cone orifice surfaces.

Remove the two screws that secure the ion block, using the 6mm hex wrench.

Remove the ion block from the ion block support.

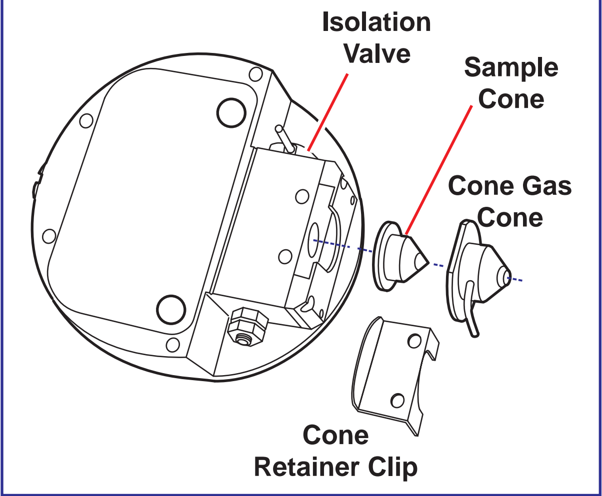


Figure 6-6 Removing the Sample Cone

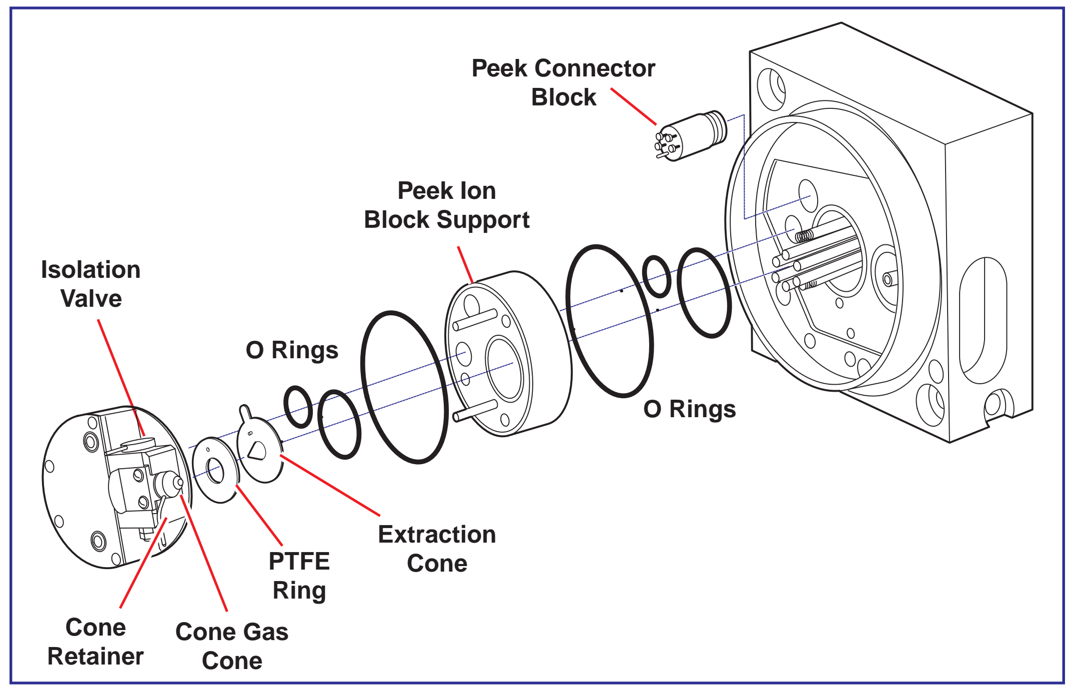


Figure 6-7 Exploded Diagram

Place the ion block on a flat surface, and remove any O rings.

Use a screwdriver to remove the hold-down screw from the peek extraction cone retainer.

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.

Take care not to damage the ion block surface and insulator O ring.

Grasp the extraction cone pin with the needle-nose pliers, then lift the extraction cone from the ion block.

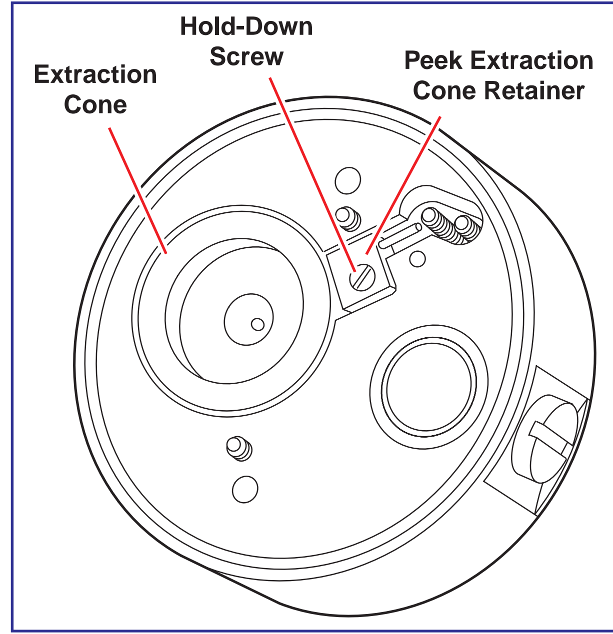


Figure 6-8 Removing the Extraction Cone

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.

Take care not to damage the seal and O ring on the ion block.

Remove the D-shaped O ring from the front of the ion block.

Remove the ion block plug and seal using a flat-blade screwdriver.

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:

Place the ion block in a beaker with methanol : water (1:1).

Place the beaker containing the ion block and methanol : water mixture in an ultrasonic bath for 20 minutes.

Remove the ion block from the methanol/water mixture, and place it in a beaker containing 100% methanol.

If the sample cone contains debris, place a drop of formic acid on its orifice.

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

Place the sample cone, cone gas cone, and extraction cone in a beaker with methanol : water (1:1).

If the parts are obviously fouled, use a mixture of 45% methanol, 45% water, and 10% formic acid.

Expose all parts to ultrasound for about 30 minutes.

If formic acid was used in the cleaning solution:

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.

Displace the water by immersing the parts in a beaker of methanol and setting the beaker in the ultrasonic bath for 10 minutes.

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

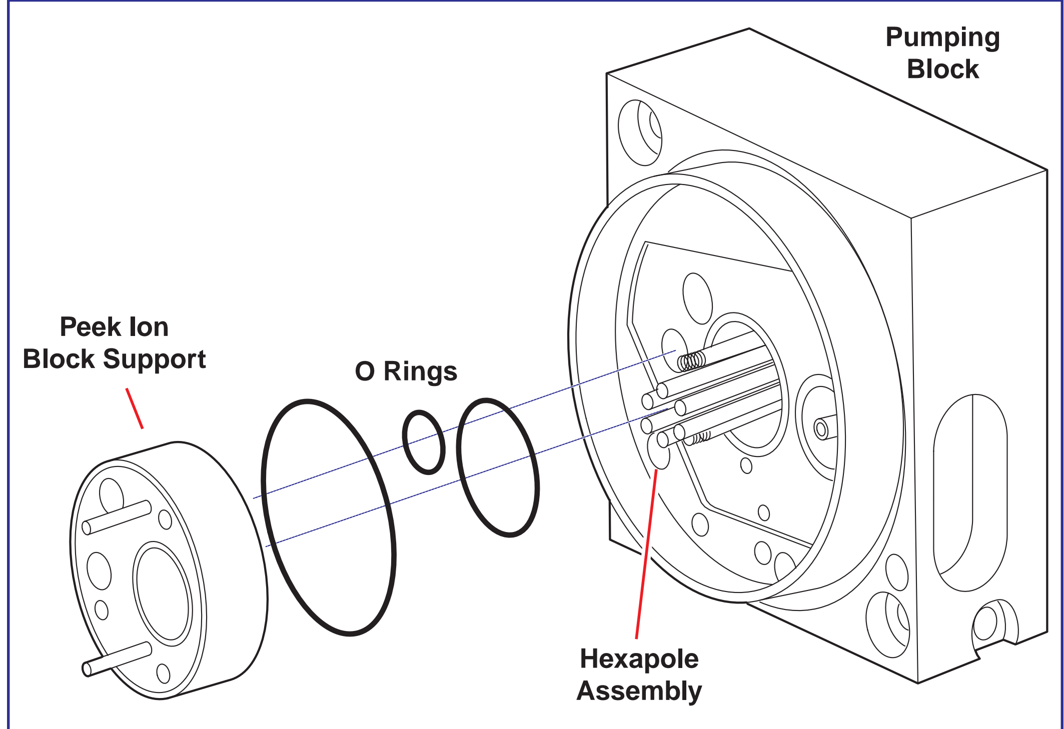


Figure 6-9 Removing the Hexapole

To remove and clean the hexapole assembly:

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

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

Grasp the hexapole gently by hand, and carefully slide it out.

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.

6

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.

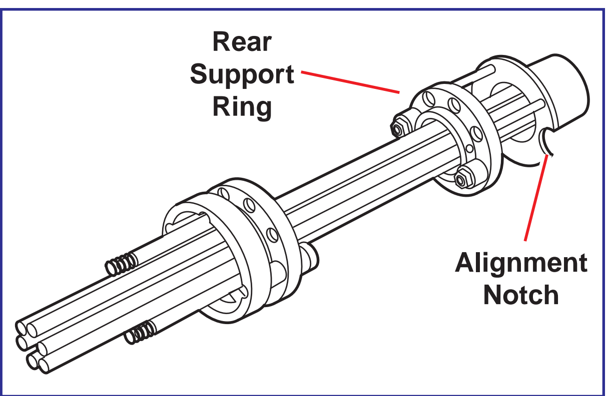


Figure 6-10 Hexapole

Carefully suspend the hexapole assembly in a graduated cylinder, then add methanol to the cylinder until the assembly is covered.

Place the graduated cylinder in an ultrasonic bath for 30 minutes.

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.

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.

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.

Secure by alternately tightening the retaining screws.

6

Reassembling the Source Components

To reassemble the source components:

Check the condition of the two front ion block support O rings. Replace them, if necessary. Be sure they are properly installed before proceeding.

Replace the vespel sealing ring and O ring on the ion block support.

Press the extraction cone into place in the ion block support, and secure it with the peek retainer and screw.

Replace 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.

Replace the ion block assembly onto the peek support block, then the cone gas cone secured with two 6mm hex screws. Avoid overtightening.

If only the sample and cone gas cone have been cleaned, turn the isolation valve back to **Open**.

If using APcI, replace the corona discharge needle (see *Cleaning and Replacing the Corona Discharge Needle* below).

Reattach the PTFE tube to the cone gas cone.

Replace the source enclosure and source enclosure cover.

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:

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

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

To reduce cooling time significantly, continue flowing API gas.

When the probe has cooled, stop the nitrogen flow by deselecting [API GAS] on the toolbar or by choosing **Gas** from the **Gas** menu.

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

Ensure that the icon changes from green to red.

This means all high voltages are turned off, as well as the APcI probe heater.

Remove the centre panel from the front of the instrument.

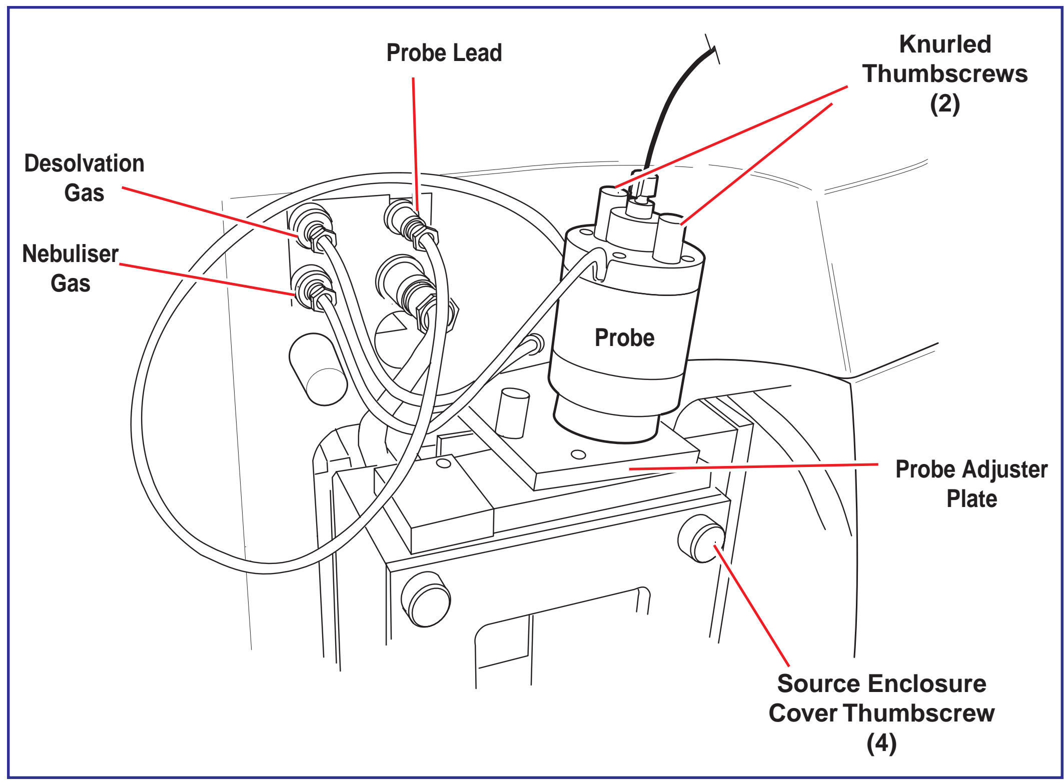


Figure 6-11 Probe and Source Connections

Remove the two knurled thumbscrews from the top of the probe.

Disconnect the nebuliser gas and the probe electrical connection.

Remove the APcI probe.

Remove the source enclosure cover.

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

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

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

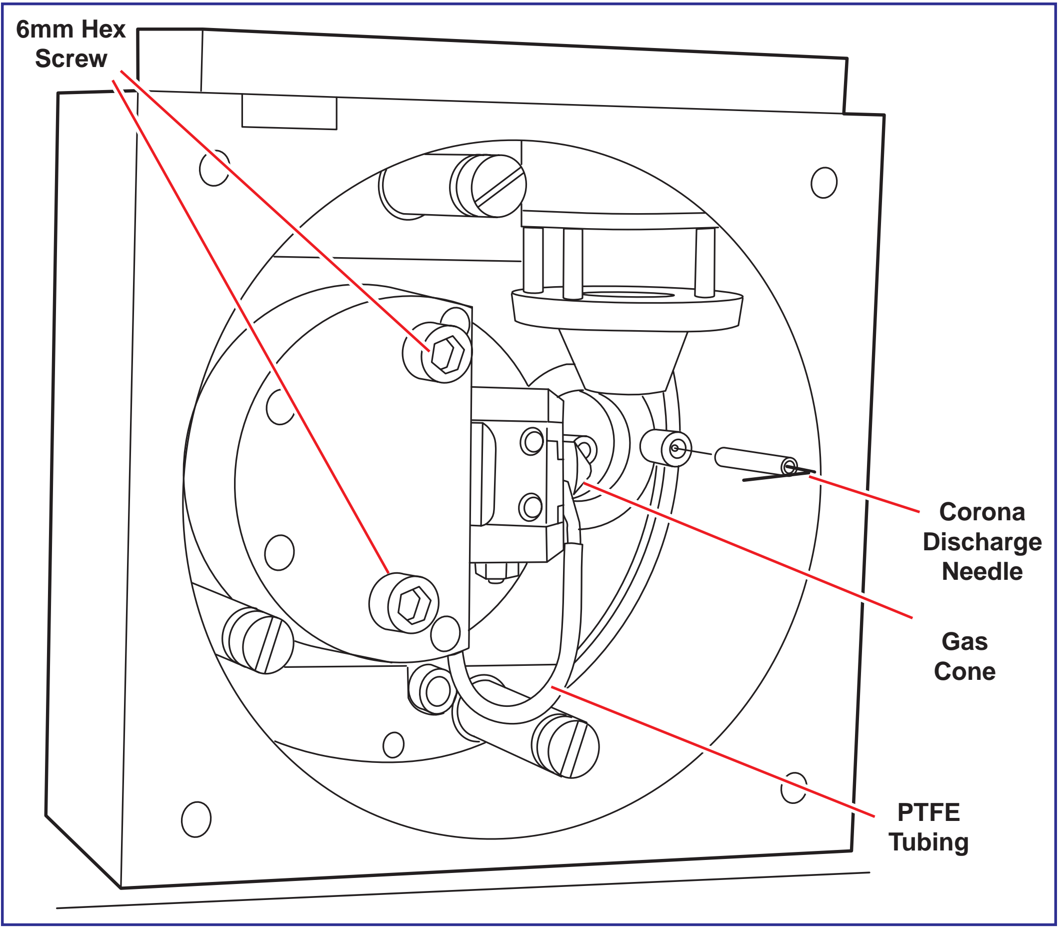


Figure 6-12 Source Enclosure

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.

Reinstall the needle with the tip pointing toward the sample cone apex.

Replace the source enclosure cover.

Replace the probe, and reconnect the LC line.

Replace the middle panel section.

Reconnect the front panel gas and electrical connections.

6

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:

Shut off the liquid flow.

Click [API GAS] (or choose **Gas** on the **Gas** menu) to start nitrogen flowing.

Adjust the nitrogen flow to approximately 650 litres per hour, as indicated by the tune page desolvation gas meter.

Set the APcI probe heater temperature to 650°C, and press **Enter**.

Click **Operate**, and wait 10 minutes with the APcI probe heater at 650°C.

This will remove any chemical contamination from the probe tip

6.4 Replacing Parts

6.4.1 Replacing the Ion Block Cartridge Heater

If the cartridge heater fails to heat it must be replaced. See *System Troubleshooting*.

Required Materials

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

Procedure

To replace the ion block cartridge heater:

Follow the procedure for venting the instrument as described in *Cleaning the Source Assembly*.

Remove the two screws securing the heater cartridge wires from the peek terminal block.

6

Carefully swing the ring tags out of the terminal block.

Loosen the two set screws that secure the heater cartridges in the ion block, using the 1.5mm hex wrench.

Gently slide the heater cartridges out of the ion block using the needle-nose pliers.

Slide the new heater cartridges into the ion block with the needle-nose pliers. Secure them with two hex-head set screws and the 1.5mm hex wrench.

Position the two heater cartridge ring tags onto the peek block terminals with the bent portion of their shafts extending into the pumping block

Tighten the two terminal block screws with a flat-blade screwdriver.

Replace the ion block cover plate, and secure with the four hex screws.

Reinstall the source enclosure cover and probe.

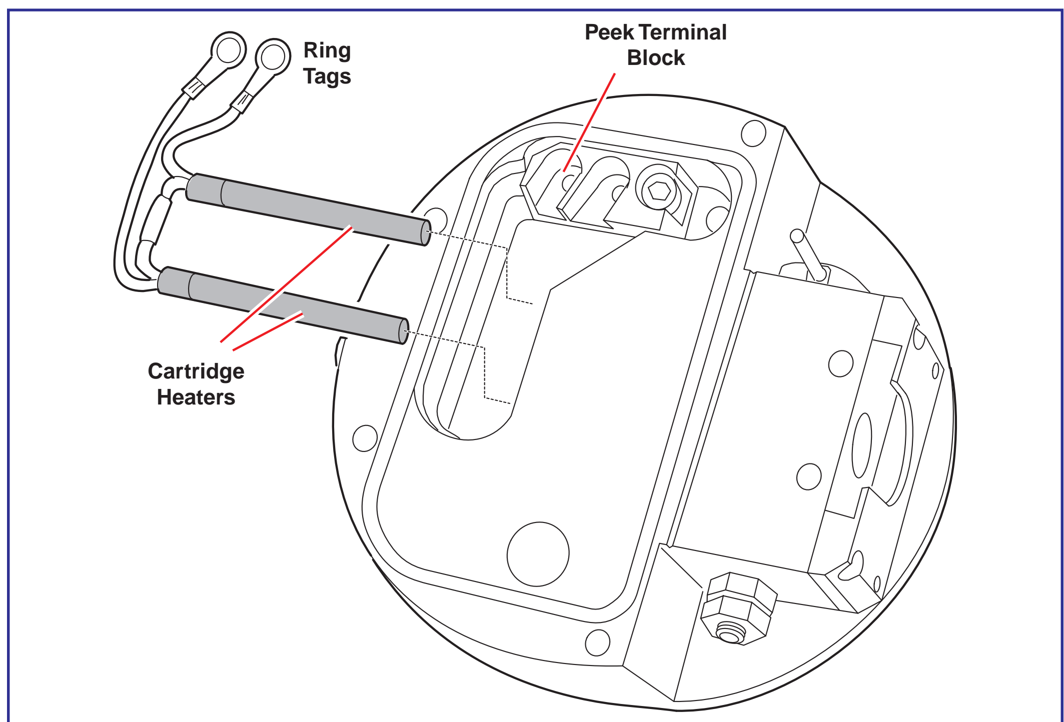


Figure 6-13 Ion Block Heater

6.4.2 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.

Required Materials

- Flat-blade screwdriver.
- 1.5mm hex wrench.
- ¼ inch (6mm) wrench.
- $^{5}/_{16}$ inch wrench.
- $^{7}/_{16}$ inch wrench.
- Loupe (magnifying glass).

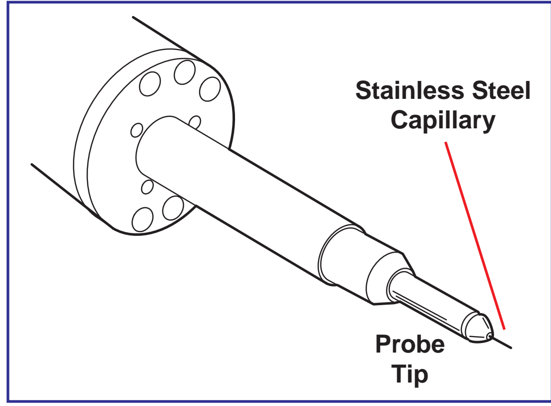


Figure 6-14 ESI Probe Capillary

Procedure

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

To replace the stainless steel capillary:

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

Remove the two end-cover retaining screws on the ESI probe with the flat-blade screwdriver.

Loosen the set screw on the LC peek union with the 1.5mm hex wrench, and remove the probe's end-cover.

Unscrew the probe tip with the ¼ inch (6mm) wrench, and remove it.

Remove the LC union with the $^{5}/_{16}$" and $^{7}/_{16}$" wrenches.

Remove the capillary from the coupling nut with the $^{7}/_{16}$" wrench. Discard the capillary and the PTFE liner and ferrule assembly.

Remove the conductive sleeve from the inner bore of the probe assembly fitting.

6

Slide a new ferrule onto the liner tube with the needle-nose pliers.

Slide the coupling nut onto the capillary followed by the PTFE liner tube and ferrule.

Connect a piece of 0.007" peek tubing with finger-tight nut and ferrule into the opposite side of the LC union, setting the capillary's depth.

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.

Pull on the capillary gently, testing to ensure it stays in place.

Remove the peek tubing from the union.

Slide the conductive sleeve onto the capillary, then feed the capillary through the probe.

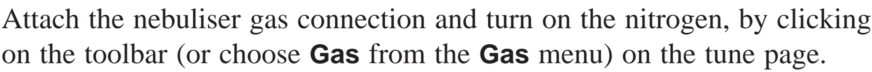
Attach the coupling nut to the probe, and gently tighten it with the $^{7}/_{16}$" wrench.

Replace the probe tip, and screw down until a 0.5mm length of the capillary protrude from its end. Use the loupe, provided in the startup kit, to ascertain the length of capillary that protrudes from the probe tip.

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

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

If a leak is found, disassemble the probe and tighten the fittings at the LC union.



Attach the nebuliser gas connection and turn on the nitrogen, by clicking on the toolbar (or choose **Gas** from the **Gas** menu) on the tune page.

Check the probe tip for nitrogen leaks. If a leak is found, replace the probe tip assembly and its O ring.

Replace the probe end-cover, and secure it with the two slotted screws. Tighten the set screw to clamp the LC union in place.

6

6.4.3 Replacing the ESI Probe Tip

The 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

¼" (6mm) 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:

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

Unscrew and remove the probe tip with the ¼" (6mm) wrench.

Install the new probe tip, and screw down until 0.5mm of the capillary protrudes from the end. Use the loupe (provided in the startup kit) to check the capillary position.

6

6.4.4 Replacing the APcI Probe Heater

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

Required Materials

- 1mm 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:

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

Loosen the two set screws at the base of the probe tip assembly, and slide the probe tip off.

Separate the heater from the probe body, pulling it parallel to the axis of the probe.

Carefully install the new heater onto the probe. Take care not to damage the fused silica capillary.

Replace the probe tip assembly, and secure it with the two set screws.

6

6.4.5 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

- 1.5mm hex wrench.
- $^{5}/_{16}$ open-end wrench.
- $^{7}/_{16}$ open-end wrenches (2).
- 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:

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

Slide the probe tip and heater assembly off the probe.

6

Using the flat-blade screwdriver, remove the two slotted screws.

Using the 1.5mm hex wrench, loosen the two set screws that retain the LC filter, then remove the probe end.

Remove the filter cartridge from the adapter nut with one $^{5}/_{16}$" and one $^{7}/_{16}$" wrench. If the ferrule remains inside the cartridge, remove it.

Separate the two halves of the filter cartridge with two $^{7}/_{16}$" wrenches.

Remove the old filter pad and replace it with a new one.

Unscrew the adapter nut from the probe with a $^{5}/_{16}$" wrench. Discard the fused silica capillary.

Using the ceramic capillary cutter, cut a new length of fused silica 161.5mm long. Cut the capillary squarely. Examine new cuts for squareness with a loupe.

Remove approximately 20mm of polyamide coating from the capillary end with a flame, then clean it with a methanol-saturated tissue.

Slide a GVF004 ferrule, followed by the adapter nut and another GVF004 ferrule, onto the capillary.

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.

Tighten the adapter nut until the capillary is snug in the fitting, then gently pull on the capillary to ensure it stays in place

Feed the sample capillary through the probe, and gently tighten the probe adapter nut with the $^{5}/_{16}$" wrench.

The capillary must protrude 1.0mm from the nebulising tube.

Replace the probe end-cover and retaining screws.

Caution: Overtightening the fitting can damage the capillary.

Tighten the set screws in the probe end cover with the 1.5mm hex wrench to clamp the filter in place.

Replace the probe tip and heater assembly.

6

Chapter 7 Troubleshooting

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

- Safety and handling.
- General troubleshooting.
- Component hardware troubleshooting.

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7.1 Spare Parts

Refer to *Accessories and Spare Parts*, for spare parts information. Parts not included in that document are not recommended for replacement by the customer.

7.2 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.

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 Sheet*s for the solvents in use.

Warning: To avoid the possibility of electric shock, do not remove the instrument panels. There are no user-serviceable items inside.

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.

7.3 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 pages. 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.

7.4 Component Hardware Troubleshooting

The following tables provide suggestions for resolving hardware problems.

7.4.1 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.	Optimise parameters. Refer to *Obtaining an Ion Beam*, page 32. 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-initialise 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 *Cleaning the Source Assembly*, page 144.
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/hour. The desolvation and probe heaters shut off when the nitrogen flow rate falls below 50 l/hour.
No LC flow.	Check solvent flow from the autosampler or syringe pump.

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Possible Cause	Corrective Action
Fluid leak in the HPLC system.	Check for leaks in the HPLC system and correct.
Broken fused silica capillary in the APcI probe.	Replace the APcI fused silica capillary. Refer to *Replacing the Fused Silica Capillary* page 164.
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 *Cleaning the Source Assembly*, page 144, and *Removing and Cleaning the Hexapole Assembly*, page 152.
Blocked ESI or APcI capillary	Replace capillary. Refer to *Replacing the APcI Fused Silica Capillary and Filter Pad*, page 164, and *Replacing the ESI Probe Stainless Steel Capillary*, page 160.

7.4.2 Unsteady or Low Intensity Peaks (Ion Beam)

Possible Cause	Corrective Action
Poor nebulisation.	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/hour. Check the stability of the nitrogen flow (good quality 2 stage regulator).

Possible Cause	Corrective Action
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.
Fluid leak in the HPLC system.	Check for leaks in the HPLC system and correct.
Source components require cleaning.	Clean source components. Refer to *Cleaning the Source Assembly*, page 144.
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 0.5mm as described on page 160. Check that APcI capillary height is set at 1.0 mm. Refer to page 164.
Corona pin is not correctly aligned.	Check the alignment as described in *Cleaning and Replacing the Corona Discharge Needle*.
CID gas pressure is wrong.	Infuse sample and optimise the gas pressure. Check the CID gas regulator is set to 0,5bar, and is not leaking.
Collision cell parameters incorrect.	Check **Entrance**, **Exit** and **Collision** are optimised, with sensible readbacks.
Analyser and multiplier parameters incorrect.	Ensure **Multiplier** is 650V. Check the ion energy and resolution parameters are set correctly for the acquisition.

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7.4.3 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 *Replacing the APcI Fused Silica Capillary and Filter Pad*, page 164.
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 160.
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.

7.4.4 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.

7.4.5 Insufficient Vacuum

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

Possible Cause	Corrective Action
Leaking ion block o-rings.	Disassemble source and check condition of ion block O rings. Refer to *Cleaning the Source Assembly*, page 144.
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.

7.4.6 Leaking Nitrogen

Hissing sound or solvent smell.

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.

7.4.7 Vacuum Oil Accumulated in the Exhaust Tubing

Possible Cause	Corrective Action
Oil mist filter needs replacement.	Replace oil mist filter element and odor filter.

7.4.8 Source Heater and Desolvation Heater Not Heating

Possible Cause	Corrective Action
Source heater has blown.	Check readback. Replace heater if necessary.
Main system PCB fuse blown.	Check **Desolvation Temp** readback. Call Micromass if wrong.

7.4.9 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 *Replacing the APcI Probe Heater*, page 163.

7.4.10 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 odour filter. Replace the fuse.
System needs to be ballasted.	Ballast the pump for 20 to 30 minutes. Refer to *Gas-Ballasting the Rotary Pump*, page 141.
The ac line voltage is less than 208Vac.	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 *Changing the Rotary Pump Oil*, page 142.

7.4.11 Ion Mode Fault

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

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.

7.4.12 Failure to Recognise 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 <2V for ESI, and >2V for APcI

7.4.13 Ripple

Peaks and baseline appear to vary cyclically in intensity

Possible Cause	Corrective Action
Erratic LC solvent flow.	Troubleshoot the LC system.
Poor nebulisation 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.
Vibration from the rotary pumps or even other equipment in the same building.	Check for and eliminate excessive bench top and instrument vibration.

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7.4.14 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 switch, see the diagram on page 13. Wait 3 minutes for the audible signal indicating the embedded PC has booted from Quattro *micro* API before starting MassLynx.

7.4.15 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 correct order: Workstation Quattro *micro* API 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.

7.5 High Noise Levels in MRM Analyses

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

Start an acquisition.

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.

7

7

7.5.1 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 stainless steel capillary, see page 160. Replace the APcI fused silica capillary, see page 164.
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.

7.6 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.

7.7 Calling Waters

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

When calling Micromass, 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.

Chapter 8
Reference Information

8.1 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.

8.2 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 [icon] 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.

8.3 Positive Ion

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
UBQ	Bovine Ubiquitin [U6253]	8564.85	650-1500	General
HBA	Human α globin [H753]	15126.36	700-1500	Hb analysis
SOD	Superoxide dismutase [S2515]	15591.35	900-1500	Hb (internal cal.)
HBB	Human β globin [H7379]	15867.22	800-1500	Hb analysis
MYO	Horse heart myoglobin [M1882]	16951.48	700-1600	General
PEGH1000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000		80-1000	ES+ and APcI+ calibration
PEGH2000	Polyethylene glycol + ammonium acetate mixture PEG 200+400+600+1000+ 1450		80-2000	ES+ calibration
NAICS	Sodium Iodide / Caesium Iodide mixture		20-4000	General, ES+ calibration
NAIRB	Sodium iodide / Rubidium Iodide mixture		20-4000	ES+ calibration

8.3.1 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			

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8.3.2 Polyethylene Glycol

PEG + $NH4^+$

Reference Files: PEGH1000, PEGH2000 .

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

8.3.3 Sodium Iodide and Caesium Iodide Mixture

Reference File: NAICS

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	

8.3.4 Sodium Iodide and Rubidium Iodide Mixture

Reference File: NAIRB

Calculated m/z Value				
22.9898	772.4610	1671.8264	2571.1918	3470.5572
84.9118	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	

8.4 Negative Ion

Ref. File Name	Chemical Name [Sigma Code #]	Molecular Mass	m/z	Uses
MYONEG	Horse heart myoglobin [M1882]	16951.48	700-2400	General
SUGNEG	Sugar mixture of: maltose [M5885] raffinose [R0250] maltotetraose [M8253] corn syrup [M3639]		100-1500	Low mass range
NAINEG	Sodium Iodide / Caesium Iodide (or Rubidium Iodide) mixture		200-3900	ES-calibration

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.

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8.4.1 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.

Appendix A Environmental Specifications

A

A.1 Access

Doors through which the instrument is to be moved should be a minimum of 0.60 meters (24 inches) 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.

A.2 Location

A.2.1 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 cause 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 2.5 meter (8 feet) 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.

A

A.2.2 Vacuum Pump

The rotary pump should be installed beneath or behind the Quattro *micro* API within 1.5 meters (5 feet) of the rear of the chassis. The rotary pump is fitted with a 2 meter (6½ feet) power cable which plugs into the rear of the instrument's chassis. It is recommended that the rotary pump is elevated 15 - 20 cm (6 - 8 inches) 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 Figure 1 for specifications). The access slot for vacuum tubes must be within the footprint of the instrument and must be 800mm (31.3inches) from the proposed position of the instrument's front face.

A.2.3 Data System

The data system must be located within 5 meters (16 feet) of the mass spectrometer to allow connection of the communication cables. The data system power cables are approximately 2 meters (6.5 feet) in length.

A.2.4 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.

A.3 Dimensions and Clearances

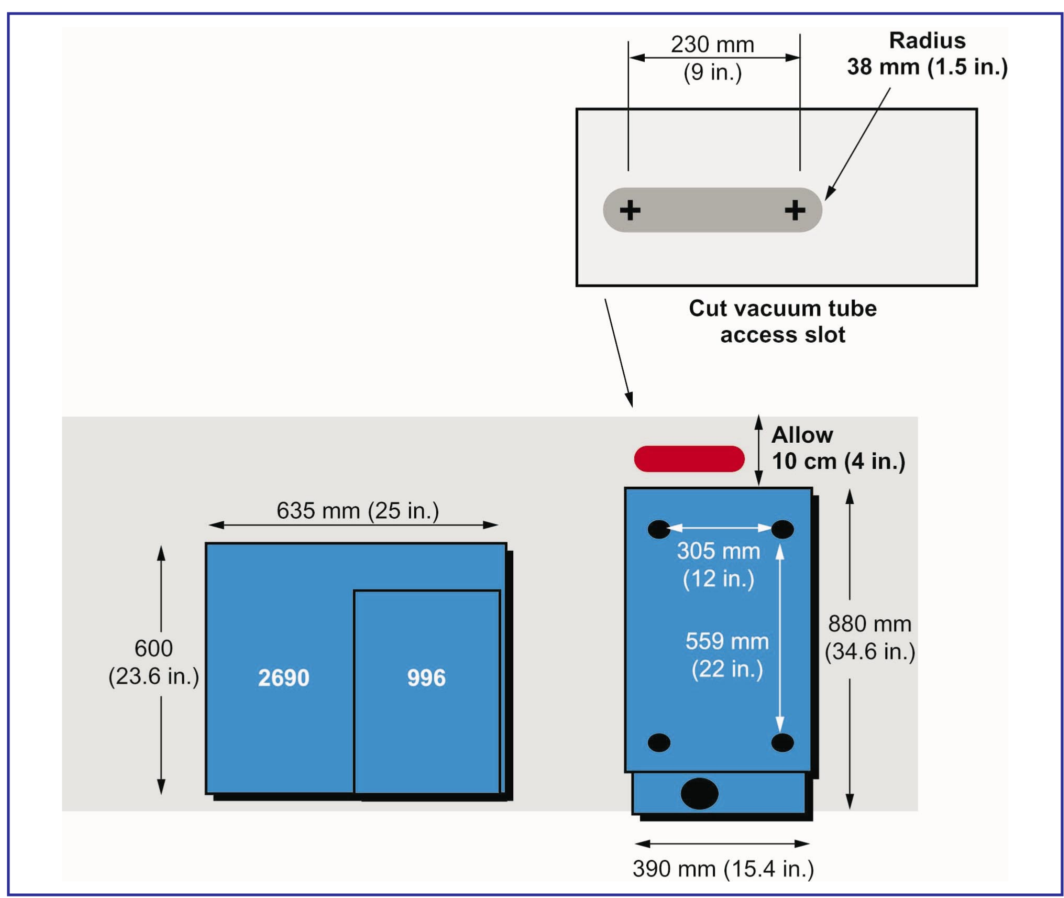

Figure A-1 Horizontal Clearances

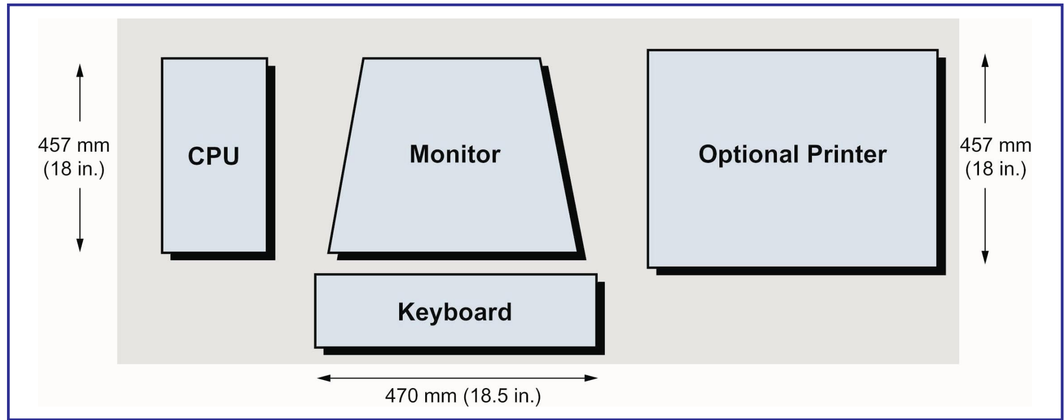

Figure A-3 Data System

Allow 864 mm
(34 in.) access

For service access the instrument is best positioned on the end of a bench where 0.5 metres (20 in.) side access is allowed

575 mm
(22.5 in.)

810 mm
(32 in.)

584 mm
(23 in.)

590 mm
(24 in.)

15-20 cm (6-8 in.)

Figure A-2 Vertical Clearances

Ideally, the following minimum clearances should be allowed for service access and ventilation:

- 20 cm (8 inches) on the right side (Note: 0.5 meters (20 inches) is required for periodic service of the main PCB - refer to Figure 3)
- 28 cm (11 inches) on the top

It is acceptable for the instrument to be placed with its back to a wall with a minimum of 10 cm (4 inches) clearance between the wall and the back of the instrument for ventilation.

A.3.1 Height

572mm (23").

A.3.2 Length

880mm (34.6").

A.3.3 Width

390mm (15.4").

A.4 Weights

The bench must be able to support the total weight of the system:

Instrument	115 kg (253 lb)
Data system (computer, monitor & printer*)	50 kg (110 lb)
Total Weight	165 kg (363 lb)

A

A.4.1 Lifting and Carrying

As the instrument weight is 115kg (253lb), 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.

A.5 Operating Temperature

The ambient temperature should be in the range 15 - 28°C (59 - 82°F) with a short-term variation (1.5 hr) of no more than 2°C (4°F). The ambient temperature in which the rotary pump is to operate must be in the range 15 - 40°C (59 - 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.

A.6 Operating Humidity

The relative humidity should not exceed 70%. It is recommended that the instrument be situated in a draft free position in an air conditioned laboratory free from excessive amounts of dust.

A.7 Shipping and Storage Temperature

–20 to 60°C (–4 to 140°F).

Appendix B Electrical Specifications

B.1 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 unauthorised 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 & Frequency	230V, 50/60 Hz (+8%, -14%)
Minimum Current	13A
Maximum Current	16A
Power Consumption	2.0 kW
Power Cord Supplied	2.5 m (8 ft.) fitted with country plugs as shown below.

Canada:

The user must supply a fused supply rated at 15A.

UK:

The cable is fused at 13A.

Rest of World:

The user must supply a fused supply rated between 13-16A .

B

Japan:

A single phase, 3 wire 200V 5% phase/neutral supply rated between 13-16A must be supplied. Additionally, a transformer must be ordered from Waters to boost the input voltage.

Australia & 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.

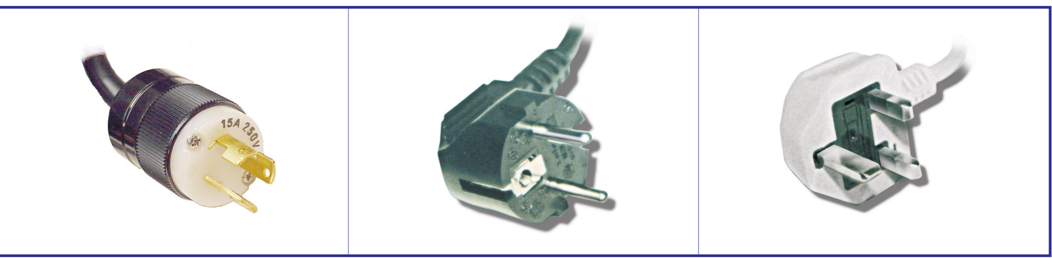

Figure B-1 Plugs Supplied

For countries with a 60Hz AC supply (such as the USA), if the supply voltage is less than 208V ac, it is recommended that a step-up transformer is used to boost the voltage to 208-230V ac.

The mains supply must have at least a ±10% brown out capability, ie 10% for 0.3 seconds and transient <20ms to half mains voltage.

Note: 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 2.5 meter (8 feet) 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.

B.1.1 Line Frequency

50Hz, 47 to 53Hz.

60Hz, 57 to 63Hz.

B.1.2 Fuse Rating

10A, 250V ac

B.2 Electrical Safety

The Quattro micro complies with the European Directive on Electrical Safety as defined by IEC 1010 and the International standard IEC 1010-1 (EN61010-1).

IEC 1010 Part 1 Ammendment 2. The instrument is categorised as Pollution Category 1 and Installation Category 2.

For Australia and New Zealand: the building installation must comply with AS 3000.

B

Appendix C Performance Specifications

C.1 Electrospray Positive Ion

Measured signal/noise ratio obtained from the chromatogram monitoring the transition m⁄z 609 - m⁄z 195 on injection of 10pg (16fmol) of reserpine will be >20:1.

10μl 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 0Da.The resolution of the ion at m⁄z 609 will be <1Da peak width at half height (MS1 and MS2).

C.2 Electrospray Negative Ion

The signal/noise ratio measured on the [M-H]- peak at m⁄z 503 from a sample consumption of 10ng 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 100-600 represents a total sample consumption of 10ng.

C.3 APcI Positive Ion

Measured signal/noise ratio obtained from the mass chromatogram of m⁄z 609 in SIR mode on direct injection of 10pg (16fmol) reserpine (10μl injection of a 1pg/μl solution) at a flow a rate of 1ml/min will be >10:1.

C

Appendix D Services and Supplies

D.1 Exhausts

Rotary Pump

The rotary pump exhaust must be vented to atmosphere (external to the laboratory) via a user-supplied fume hood or industrial vent. The exhaust gases may be connected to an existing laboratory vent which may carry exhaust gases from other sources.

5 meters (16 feet) of 12.7mm (½") tubing is supplied as standard - if this distance is insufficient the user must supply an adapter and tubing with an internal diameter of at least 51mm (2") to the vent point.

Nitrogen

The nitrogen exhaust line must be vented to atmosphere (external to the building which houses the instrument) via a line that is completely separate from any rotary exhaust. The point at which this line exits the building should be at least 0.5m (20") from any other vent.

If environmental regulations prevent the nitrogen exhaust line being vented directly to atmosphere as described above it is recommended that the nitrogen exhaust is vented to atmosphere via a laboratory fume hood.

Serious damage to the instrument may result if the ESI / APCI nitrogen exhaust line is connected to the rotary pump exhaust line. This damage occurs when the nitrogen supply is turned off (or runs out) such that there is no longer a flow of gas away from the instrument through the nitrogen exhaust line. A slight positive pressure in the rotary pump exhaust line can cause migration of the rotary pump oil vapour into the ESI source via the nitrogen exhaust line. Oil vapour can be sucked into the vacuum housing via the sample cone resulting in serious contamination of the source and quadrupoles. Even when there is no positive pressure in the exhaust line the oil vapor may still be drawn into the source due to the flow of gas through the sample cone while the machine is under vacuum. This effect is most acute if the ESI / APCI probe is left in the source with the nitrogen gas turned off.

Instrument damage resulting from incorrectly plumbed exhaust lines will not be covered under warranty.

5m (16 feet) of 10mm (0.39") o.d. hose is supplied as standard for the nitrogen exhaust. If this length is insufficient, the user must supply an adapter and tubing with an internal diameter of at least 12.7mm (½") to the vent port.

D.2 Solvent Drain

In the event that the nitrogen gas is switched off (or runs out) and the LC system continues to run, solvent is prevented from accumulating in the source by a mechanism that will direct the solvent to a drain, which can be directed to a waste bottle. Additionally, the system has software that can detect the nitrogen gas being switched off or running too low and can trigger the HPLC system to stop.

D.3 Gases and Regulators

Dry oil-free nitrogen, filtered to at least 5 microns and regulated at 6-7 bar (90-100 psi) is required. Gases should be connected using 6mm (¼") o.d. PTFE tubing (5 meters (16 feet) is supplied as standard) and checked for leaks under pressure. Various nitrogen filters are available that would be suitable for this purpose, e.g. Swagelok SS6FWMM2, a 2 micron filter.

During operation, typical usage of nitrogen is ~600 litres/hour (at atmospheric pressure) but under high flow rate conditions (APCI), this may need to be increased to ~1100 litres/hour. Since the consumption of nitrogen is high for API techniques it may be advisable to consult a local gas supplier for an ideal configuration.

CID Experiments

Argon is required as collision gas and should be dry and of high purity (99.9%). The gas must be connected using clean 1/8" stainless steel tubing (not supplied) and checked for leaks under pressure. It is recommended that a two stage pressure regulator is used to regulate the gas at 0.5 bar (7 psi).

D.4 Solvent Delivery Systems

Electrospray

For direct injection a syringe pump is incorporated within the instrument. For higher flow rate electrospray an HPLC pump giving a stable, pulse free flow between 0.05 - 1ml per minute is required.

Atmospheric Pressure Chemical Ionization

An HPLC pump giving a stable, pulse free flow at 0.2 - 2ml per minute is required.

Solvents should never be placed on top of the instrument. Before returning this form please ensure that any locally supplied solvent delivery system has either already been commissioned or that a commissioning date has been scheduled.

D.5 Sample Loops and Injectors

The instrument is supplied with electrically driven Rheodyne RV700-100 inject/divert valve as standard. A 10μl loop is fitted.

D.6 Reagents

Supplies of HPLC grade water, methanol, acetonitrile, ammonium acetate and formic acid will be required both for making up standard solutions and for cleaning instrument components. Facilities for making up test samples must also be available.

D.7 Vacuum Pump Oil

Ultragrade 19 or Inland Q45 vacuum pump oil only should be used for the rotary vacuum pump. Use of other oils may damage the pump and void the warranty.

1 litre of Ultragrade 19 oil is shipped with the Quattro micro that is sufficient for one oil change.

D.8 Test Samples

The installation engineer will provide samples to be used testing the system during the installation/start-up.

Waters cannot provide lot numbers or any other certification as to the source of these test samples.

If this information is required to satisfy validation procedures, then the user is required to purchase these samples prior to the engineer arriving on site.

Caution: Hazardous samples must be handled in a manner that conforms to the manufacturers guidelines defined in the sample Hazard Data Sheet.

Samples required for acceptance specifications:

Sample	Supplier	Code
Raffinose	Sigma	R-0250
PPG 2000	Aldrich	20,233-9
Formic Acid	Sigma	F-0507
Ammonium Acetate	Sigma	A-7262
PEG 1500	Sigma	P-5402
PEG 1000	Sigma	P-3515
Reserpine	Sigma	R-0875

D

D.9 Maintenance Equipment

An ultrasonic bath will be required for routine cleaning of parts.

A small bath with a chamber size of approximately 300mm × 150 mm and 100mm high is adequate.

Glass vessels in which to place instrument components during cleaning must be available. These should have a diameter of at least 100mm and be approximately 120mm high. A 500ml measuring cylinder is required for cleaning the hexapole. Surfactants must not be used in the cleaning process for this glassware.

D.10 Summary of Customer Supplied Items

Rotary Pump Exhaust

Fitting on Instrument / System: KF 25 Flange
Items Supplied with Instrument: 5m (16 feet) of 12.7mm (0.4 inch) i.d. × 19.2mm (0.75 inch) o.d. tubing
Item(s) to be Supplied by Customer: Fume hood or industrial vent

API (N_2) Exhaust

Dry and oil free

Fitting on Instrument / System: 10mm (0.4 inch) push-in fitting ("Legris" type)
Items Supplied with Instrument: 5m (16 feet) of 8mm (0.316 inch) i.d. × 10mm (0.4 inch) o.d. hose
Item(s) to be Supplied by Customer: Separate fume hood or industrial vent within 5m of Quattro micro

D

API (N_2) Supply

Fitting on Instrument / System:

6mm (¼") push-in fitting ("Legris" type)

Items Supplied with Instrument:

5m (16 feet) of 4mm (0.157 inch) i.d. × 6mm (¼") o.d. hose

Item(s) to be Supplied by Customer:

N_2 supply, filtered to 5 microns and regulated to 90 - 100 psi via 6mm (¼") connector.

Source to lab supply should not be connected with polypropylene or acetylene welded copper gas lines due to high contamination of nitrogen

CID Gas Connection

Fitting on Instrument / System:

1/8" Swagelok

Items Supplied with Instrument:

1/8" Swagelok fitting

Item(s) to be Supplied by Customer:

1/8" o.d. stainless steel tubing to a regulated gas supply.
Regulator adapter should be 1/8"

D

Inject / Divert System

Fitting on Instrument / System:

Rheodyne RV700-100

Items Supplied with Instrument:

Tubing and Rheodyne nuts & ferrules

Item(s) to be Supplied by Customer:

Tubing and Rheodyne nuts & ferrules

Quattro micro

Fitting on Instrument / System:

IEC receptacle with a mains filter

Items Supplied with Instrument:

2m (8ft) US, Europe and UK cord set

Item(s) to be Supplied by Customer:

Appropriate receptacle for the plugs shown or power cord, plug and receptacle if incompatible with the ones supplied

D

Data System

Fitting on Instrument / System:

IEC receptacle

Items Supplied with Instrument:

3m (9.5ft) US-style power cords with 10A power plugs for CPU, monitor, and optional printer

Item(s) to be Supplied by Customer:

Three appropriate 110/230V, 13A minimum receptacles

D

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