MassLynx NT Guide to Data Acquisition

Version 3.5

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MassLynx NT Guide to Data Acquisition

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Chapter 1

Acquiring Data — an Overview

This chapter provides information on using the acquisition system. It will explain how MassLynx is used to turn on and tune the mass spectrometer, how the instrument is then calibrated and then finally how you can acquire data onto the computer's hard disk.

Getting Started — the basic steps for acquiring data

This section gives an overview of the basic steps required to do an acquisition and the order in which these steps should be performed. Each of the steps involved in acquiring data is described in much greater detail in its own section later in this chapter.

1. Load MassLynx

Load MassLynx by double clicking on the MassLynx icon in the MassLynx group. If MassLynx Security is enabled the MassLynx Login dialog will be displayed. Enter your Logon Name and Password. MassLynx will be loaded and communications with the instrument will be initialised.

2. Configure the Inlet System

If necessary choose **Select Interface** from the Acquisition Control Panel **Configure** menu and select which type of inlet system you are using.

Next choose **Inlet** from the Acquisition Control Panel **Methods** menu. This allows you to set up the inlet parameters for your chosen inlet system e.g., the GC temperature ramp if you are using a GC system.

3. Prepare instrument for use

The instrument should be prepared for use as described in the Users Guide supplied with your instrument, if it is in a vented state it should be pumped down using the vacuum controls. The instrument should then be turned into operate.

4. Tune the instrument

The instrument must be tuned to obtain the best conditions for ionization. Again this should be done in accordance with the instructions given in the Users Guide supplied with your instrument.

5. Calibrate the mass scale

The mass scale of the instrument must be correctly calibrated so that the masses reported for acquired data are correct. Calibration of the mass scale involves introducing a reference compound into the system, acquiring some data and then comparing the result with the expected masses for the reference compound. A calibration curve is produced and used to correct for any errors in the mass scale.

6. Acquire some data

You can now acquire some data, either for a single sample or for a number of samples, introduced into the system using an autosampler. To do this you must set up a method for scanning the system (referred to as a function) and if you are using a programmable inlet system, you must also set up any inlet related parameters, including those that control an autosampler if fitted. You can then start the acquisition and let it run for a programmed duration or stop it manually before that time.

7. Monitoring data acquisition

The progress of the acquisition can be monitored either by selecting the 'Status' display which gives a scan by scan report of acquisition statistics, or by viewing the data that is being acquired in real time using the Spectrum or Chromatogram displays.

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The Acquisition Control Panel

Chapter 2

Introduction

All MassLynx Acquisition functions are accessed from, and managed by, the Acquisition Control Panel. In order to perform an acquisition you must first tell the system about the instrument configuration that you wish to use. The Acquisition Control Panel will then configure itself to allow you access to the controls that are appropriate to the intended task.

MassLynx can be configured so that the Acquisition Control Panel is automatically displayed when MassLynx is run, otherwise the Acquisition Control Panel is displayed when you select **Control Panel** from the MassLynx **Run** menu or press the  toolbar button. The picture displayed will reflect your current Instrument and Inlet configuration. **Figure 2.1** shows the Control Panel display for a Quattro II with a GC and Autoinjector.

Acquisition controls can be accessed either with the menu bar at the top of the Control Panel, or in the case of the mass spectrometer tune page or the inlet editors, by double clicking on the relevant part of the Control Panel picture.

- **To automatically load the Acquisition Control Panel with MassLynx**

To automatically load the Acquisition Control Panel with MassLynx select **Autoload** from the Acquisition Control Panel **Configure** menu. A tick will appear next to this menu option when it is selected.

- **To select the Tune window from the Control Panel**

Double click on the mass spectrometer part of the instrument picture
-or-
Choose **Tune Mass Spectrometer** from the Acquisition Control Panel **Instrument** menu.
-or-
Press **CTRL-T**

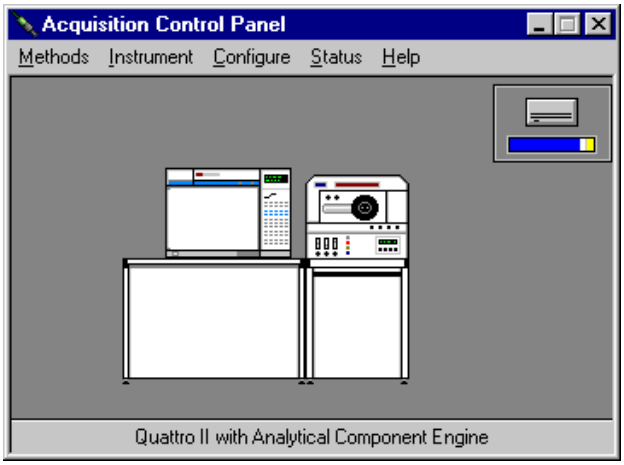

Figure 2.1 *The Acquisition Control Panel*

Configuring the Inlet System

Before you use the Acquisition Control Panel you must first configure it to support the inlet system fitted. This is done using the Select Interface dialog box.

MassLynx will display a list of the inlet systems which you selected during installation of the MassLynx software. If you add additional inlet systems at a later date you may need to reinstall MassLynx to add the control software for the new inlet system.

Once you have configured the inlet system, MassLynx will give you access to the parts of the acquisition system that are appropriate to the inlet selected and the Tune page will change if the inlet selected requires a change of ionization mode.

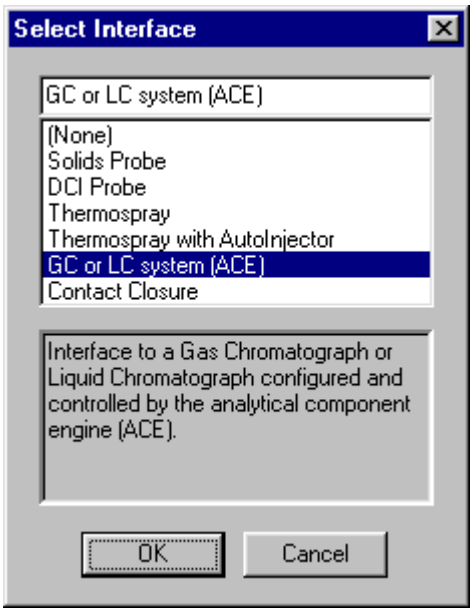

Figure 2.2 *Select Inlet Dialog*

The list of inlet options, which appears in the Select Interface dialog, reflects the inlet systems which were selected when MassLynx was installed. If, at a later date you add a new inlet system or change one of your existing inlets you may need to re-install MassLynx to gain access to the control software for the new inlet system.

To change the inlet system

1. Choose **Select Interface** from the Acquisition Control Panel **Configure** menu.

2. Scroll down to the required inlet system.

3. Select the inlet.

4. Press the **OK** button.

The Control Panel will now re-configure itself to show the new inlet system.

Setting Instrument Data Thresholds

MassLynx has several parameters that allow you to control how the transputer system pre-processes data before it is sent to the host computer. These parameters are contained in the **Instrument Data Thresholding** dialog.

Instrument Data Thresholding allows you to specify what type of data you wish to acquire and write to disk and what type of data you wish to discard and not write to disk. Limiting the amount data stored on disk can be particularly desirable when acquiring continuum data and doing long LC runs.

To change Data Thresholding

1. Choose **Set Instrument Threshold** from the Acquisition Control Panel **Instrument** menu.

2. Make required changes to the information.

3. Press the **OK** button.

The data thresholding dialog displayed will depend on the type of instrument being used.

For Quattro I, Bio-Q, Trio-2000 and 12-250 instruments the following dialog will be displayed.

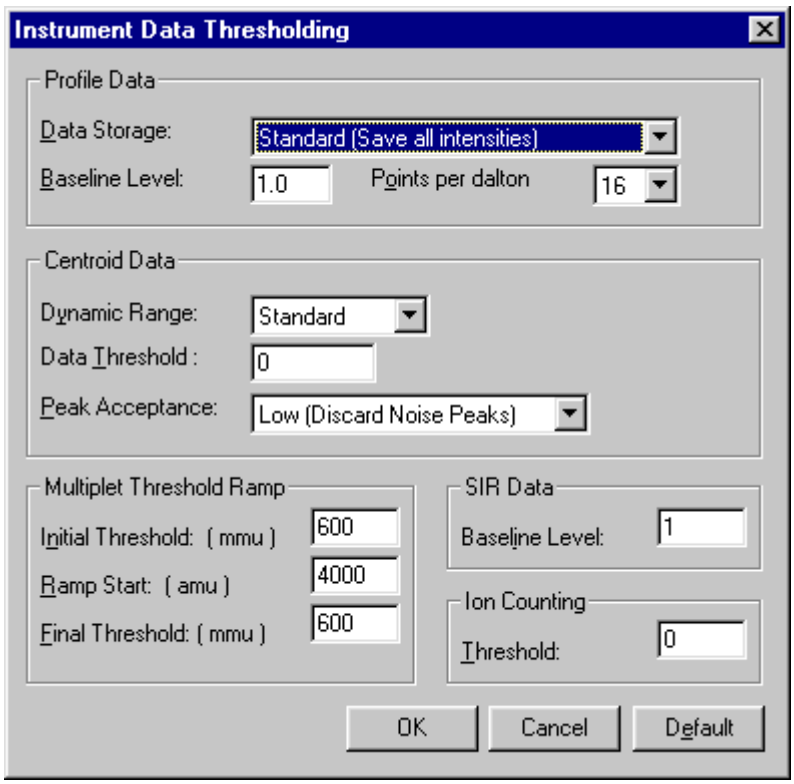

Figure 2.3 *Instrument Data Thresholding Dialog (Quattro I, Bio-Q, Trio-2000 and 12-250)*

For Quattro II and Platform instruments the following dialog will be displayed.

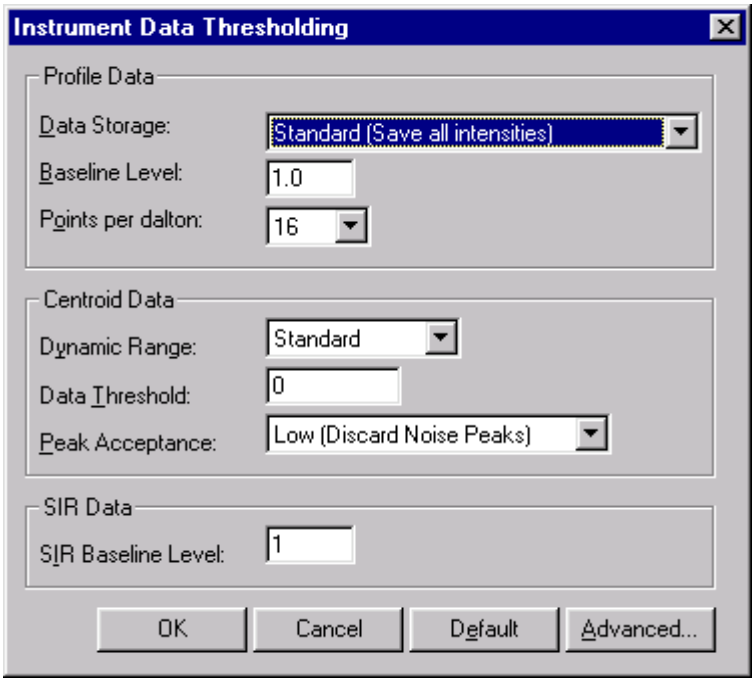

Figure 2.4 *Instrument Data Thresholding Dialog (Quattro II and Platform)*

Pressing the **Advanced** button in this dialog will display the Advanced Thresholding Parameters dialog.

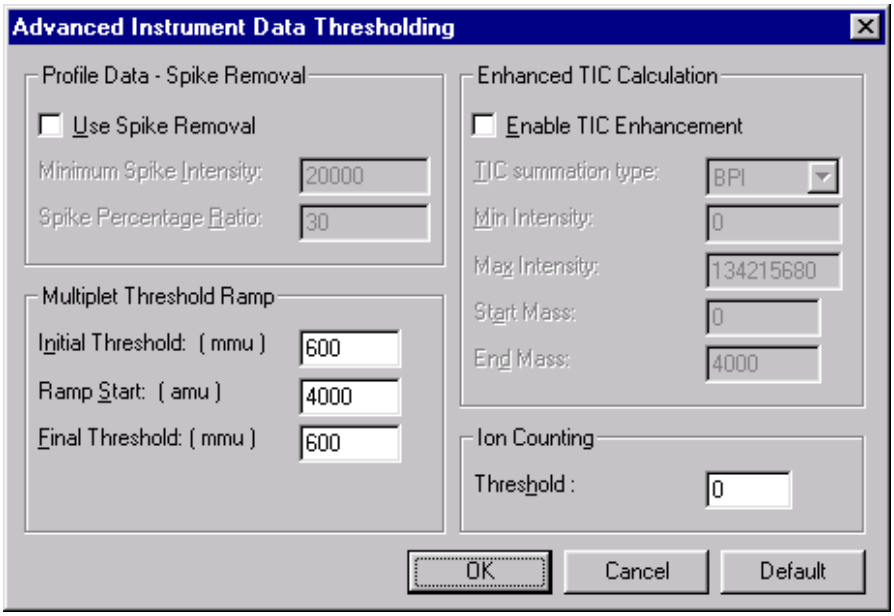

Figure 2.5 *Advanced Instrument Data Thresholding Dialog (Quattro II and Platform)*

Pressing the **OK** button stores any changes made in the Instrument Data Thresholding dialog and in the Advanced Instrument Data Thresholding dialog if present.

For Quattro II and Platform II instruments the tune page will be updated immediately if it is open, i.e. the effect of Baseline Level and Ion Counting changes are shown on the active tune segments. For other instruments the new parameters will be downloaded at the start of the next acquisition scan.

Pressing **Cancel** discards any changes made and closes the Instrument Data Thresholding dialog.

Pressing the Default button loads default values for all parameters and switches off all the instrument data thresholding features.

MaxEnt Users

Note: The MaxEnt algorithm needs to accurately measure noise within a data file. For this reason the **Ion Counting Threshold** should be set to zero when acquiring data which will be analysed using MaxEnt.

Profile Data

The controls for profile data allow you to control the amount of data that is collected during a continuum data acquisition. By default MassLynx will collect one data point every 8th of a dalton. If for instance you were scanning your instrument from mass 50 to mass 1300 you would collect and save 10000 data points per scan, and as each point requires 6 bytes of disk space, every scan would take 1/17 of a megabyte of disk space. Obviously if you use this type of scanning in conjunction with chromatography, the data file sizes will grow to be enormous. In addition to the disk space issue, collecting this amount of data puts a heavy demand on the transputer system, which will ultimately effect the scan rates at which you can collect data.

The ability to use a threshold with continuum data is therefore highly desirable as it allows you to disregard data that is noise yet save the complete peak profile for the 'real' data, thus retaining the information in the data while reducing disk space requirements. To use this facility you should set **Data Storage** to 'Compressed'.

Having selected 'Compressed' mode, **Ion Counting Threshold** is used to set the intensity level below which a data point will be ignored. This feature is discussed later.

Data Storage When Data Storage is set to **Standard** every mass/intensity pair is stored to file, regardless of their values, i.e. zero intensities. This mode must be used for acquisitions with MCA functions

When Data Storage is set to **Compressed** zero intensity values are not stored to file so this produces smaller file sizes. Compressed Data Storage mode cannot be used for MCA acquisitions.

Baseline Level Is used to lift/drop the baseline to see more/less of the noise. It is used when Ion Counting Thresholding is not enabled (i.e. set to zero), to set the position above zero of the baseline. The baseline level would typically be set to a value of 1. If you wish the baseline to appear higher then you can increase the value of the baseline level parameter.

It is possible to use a **negative baseline**. This reduces the noise seen and acts as a form of **thresholding** to be applied to 1/16th amu type samples. This will take place after ion counting and therefore have a less significant effect.

If you want to see more noise use a positive value. Do **not** use a positive value for the Baseline Level if you are using Ion Counting Thresholding or Compressed Data Storage mode.

The **Points per dalton** parameter can have one of three values, 4, 8 or 16. Selecting 8 points will allow you to acquire data twice as quickly as selecting 16, and will result in data files which are approximately half as big as those acquired at 16 points per dalton. Selecting 4 points will allow you to acquire data twice as quickly as selecting 8, and will result in data files which are approximately half as big as those acquired at 8 points per dalton.

Acquiring data at 16 points per dalton gives the greatest possible resolution. Acquiring data at 4 points per dalton gives data with a smoothed appearance.

Centroid Data

For centroided data, there are three parameters that you can set.

Dynamic Range Controls the size (in terms of bytes) of the number used to store an acquired intensity value and also the precision with which a mass number is stored. The **standard** dynamic range setting allows the intensity values to be stored using 4 bytes and masses to be stored to a 1/128 amu precision. The **high** dynamic range is seldom used but allows 5 bytes for intensity and 1/1024 amu mass precision.

Data Threshold Sets an intensity level below which peaks detected will be ignored. This is useful if you wish to concentrate on larger peaks of interest and cut down on the size of acquired data files.

Peak Acceptance Allows you to ignore the minimum peak area calculated during the prescan thus allowing smaller peaks through. Setting **Peak Acceptance** to high activates this and will result in more peaks being stored per scan. The default value is low.

SIR Data

The **SIR Baseline Level** is used when Ion Counting Thresholding is not enabled (i.e. set to zero). It is used to set the position above zero of the baseline. The baseline level would be typically be set to a value of 1. If you wish the baseline to appear higher then you can increase the value of the baseline level parameter.

Ion Counting Thresholding

Note: For Quattro II and Platform instruments these parameters are accessed by pressing the **Advanced** button in the Instrument Data Thresholding dialog.

Ion Counting Threshold is used to set the intensity level below which a data point will be ignored. The **Ion Counting Threshold** can be set to values between 0 and 25, the higher the number you enter the more data will be discarded.

Note: To disable the **Ion Counting Threshold** set the value to zero. If you wish to use the **Ion Counting Threshold** facility a value of 6 is suitable for most data.

This threshold is applied to all acquisitions, regardless of scanning mode. It is also the most significant of all of the data manipulation variables because it is the one applied first to the raw data.

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. The **Ion Counting Threshold** level that you enter is multiplied by the standard deviation of the noise to determine the intensity level to be used. If you set a value of zero the intensity level will be set so that it sits right in the middle of the noise which would mean that roughly half of the noise data would be acquired. If you set a value of 20 the threshold would sit well above the noise level, so very little noise data would be acquired. Conversely a value of 1 would place the threshold only just above the noise so almost all of the data would be acquired.

Important Note: When using an **ion counting threshold** you should set the profile data baseline level and SIR data SIR baseline level to zero.

Figure 2.6 to **Figure 2.7** shows the effects of changing the **Baseline Level** and **Ion Counting Threshold** parameters. A series of acquisitions were done using Heptacosa and acquiring continuum data. Each data file contains 18 scans. The data file AB0000 is not thresholded, both Baseline Level and Ion Count Threshold were set to zero.

Figure 2.6 shows the effect of increasing the Baseline Level parameter.

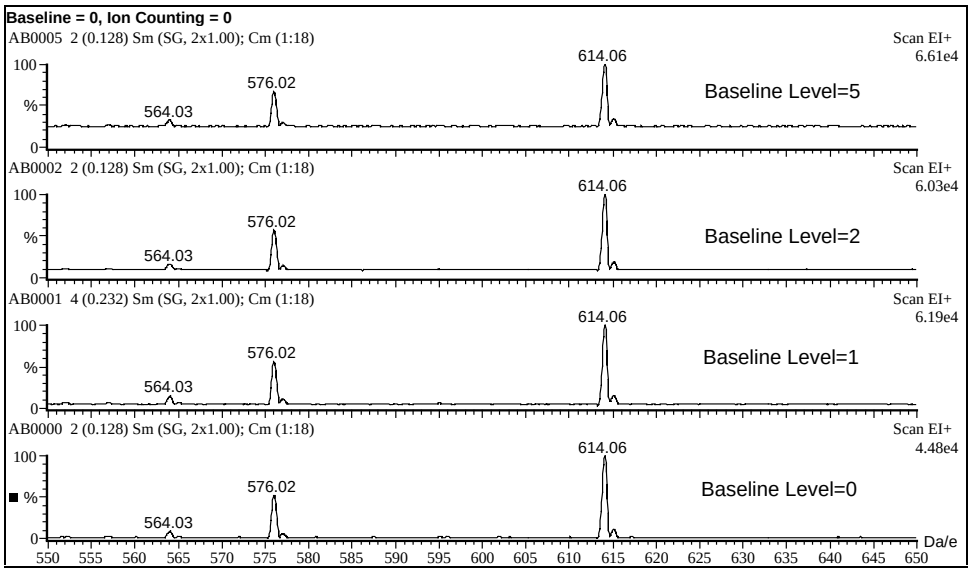

Figure 2.6 *Effect of increasing Baseline Level on Heptacosa spectrum*

Figure 2.7 shows the effect of increasing the Ion Counting threshold on a part of the spectrum, which contains only background noise. The bottom trace was acquired with the Ion Counting Threshold set to zero, for subsequent traces the Ion Counting Threshold was set to 1, 2, 4, 6 and 25. As the Ion Counting Threshold is increased the amount of noise stored is reduced, the normalizing intensity value at the top right hand corner of the trace is also reduced.

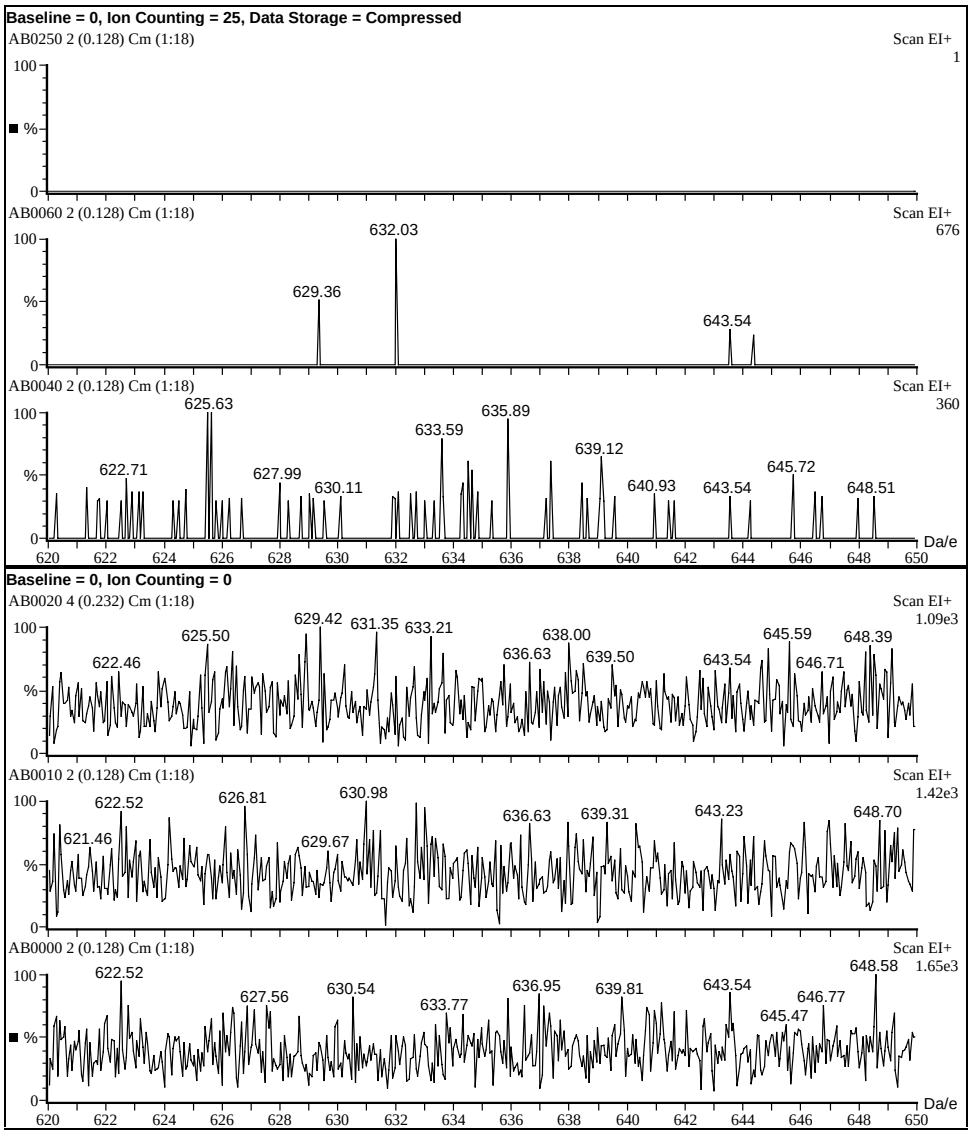

Figure 2.7 *Effect of increasing Ion Counting Threshold on noise in Heptacosa spectrum*

The value of the Ion Counting Threshold should be set such that background noise is removed without significantly reducing the intensity of the smallest peaks which are of interest.

Figure 2.8 shows the effect of increasing the Ion Counting threshold on a part of the spectrum, which contains a low intensity peak. As the Ion Counting Threshold is increased beyond a certain value the peak becomes narrower and its intensity is reduced as the thresholding rejects part of the genuine signal. In this case an Ion Counting Threshold value of 4 would be suitable.

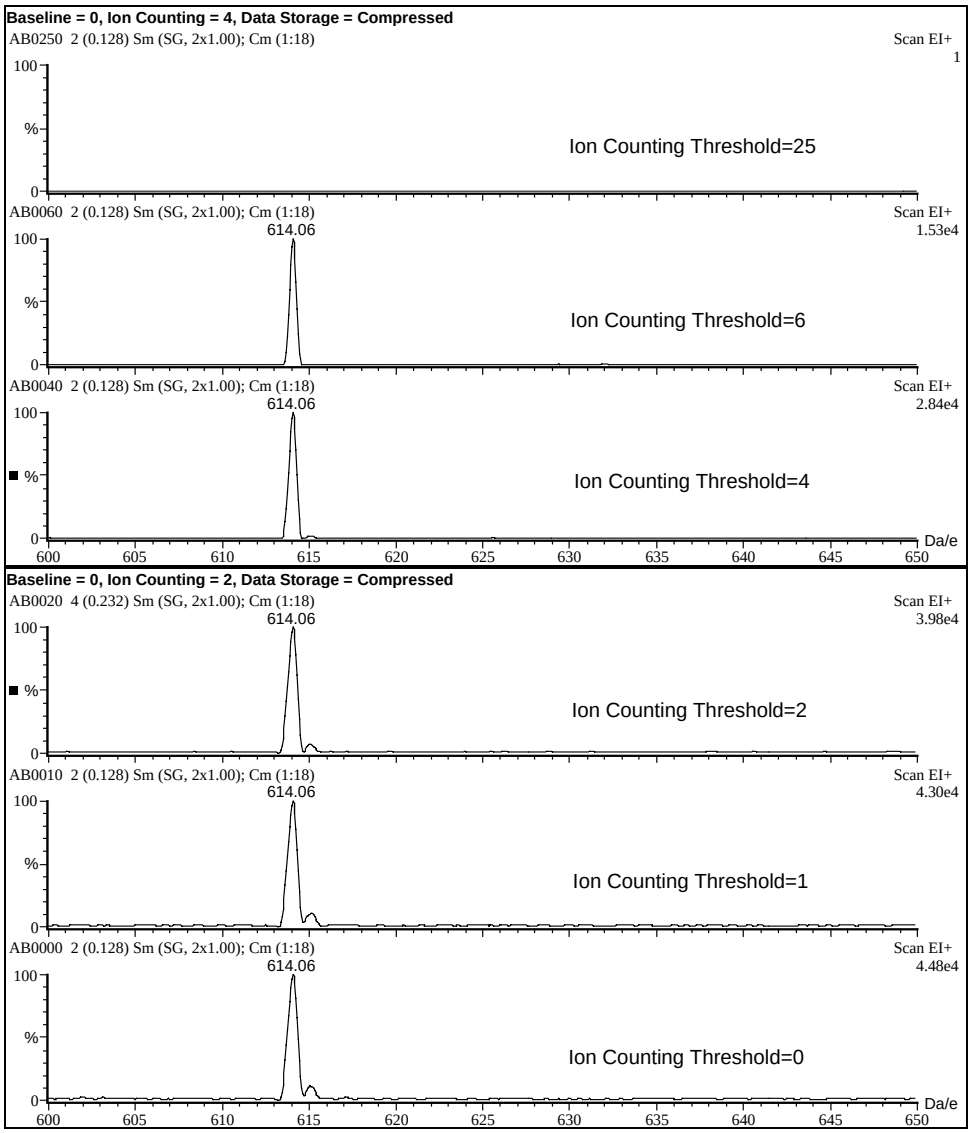

Figure 2.8 *Effect of increasing Ion Counting Threshold on low intensity peak in Heptacosa spectrum*

Table 2.1 gives a summary of the parameters used to acquire each of the data files and shows the % reduction in file size which resulted from using the series of Ion Counting Thresholds and storing data in Compressed format.

File Name	Baseline Level	Ion Count Threshold	Data Storage	.DAT File Size (Bytes)	% saving on AB0000
AB0000	0	0	Standard	1,102,926	
AB0001	1	0	Standard		0
AB0002	2	0	Standard		0
AB0005	5	0	Standard		0
AB0010	0	1	Compressed	1,014,942	8
AB0020	0	2	Compressed	998,790	10
AB0040	0	4	Compressed	432,690	61
AB0060	0	6	Compressed	347,784	69
AB0250	0	25	Compressed	185,286	83

Table 2.1 *Summary of Instrument Data Thresholding parameters used to acquire files AB0000 to AB0250.*

Multiplet Threshold

Note: For Quattro II and Platform instruments these parameters are accessed by pressing the **Advanced** button in the Instrument Data Thresholding dialog.

The real-time peak detection algorithm in the acquisition system uses a value of peak separation called the Multiplet Threshold to determine whether two peaks will be detected as two separate peaks or combined into one peak. If two peaks are separated by less than **Multiplet Threshold** then they will be combined into a single peak. However the value of the multiplet threshold needs to increase as the mass scale increases since peaks will tend to broaden at higher masses. For this reason a ramp may need to be applied to the value of the multiplet threshold so that it increases with the mass range. This is done using the parameters **Ramp Start** and **Final multiplet threshold**.

Advanced Instrument Data Thresholding parameters (Quattro II and Platform instruments only)

These parameters are accessed by pressing the **Advanced** button in the Instrument Data Thresholding dialog.

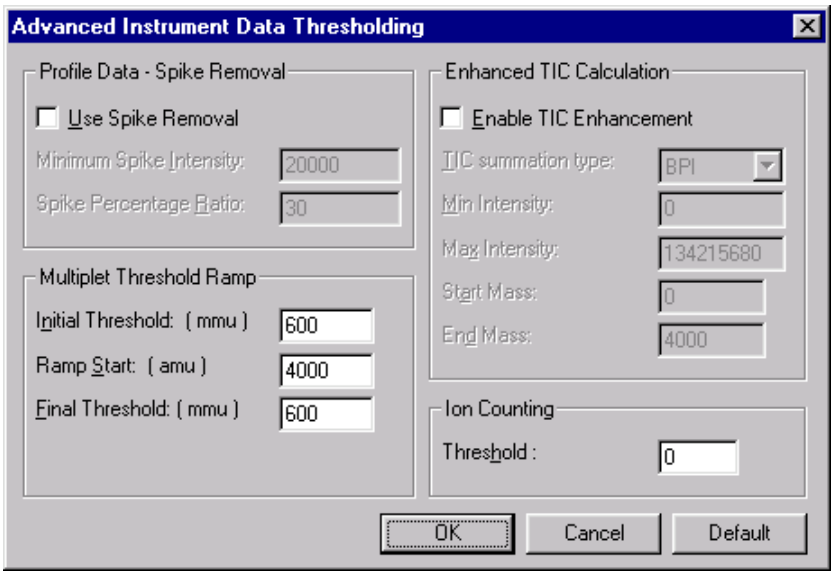

Figure 2.9 *Advanced Instrument Data Thresholding Dialog (Quattro II and Platform)*

Profile Data — Spike Removal

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

Note that the use of **Spike Removal** does involve some additional processing while acquiring. For this reason the use of Spike removal will reduce the maximum achievable acquisition rates by approximately 30%.

Use Spike Removal Select this box to perform spike removal during an acquisition. (Note this will not be reflected in the tune page).

Minimum Spike Intensity Intensity threshold below which spikes will be ignored. Take this value from the Tune page intensities. A very low intensity signal may include single ion events that can be combined to produce significant peaks. For this type of data the Minimum Spike Intensity should be set to a suitable value such that these single ion events are not discarded as spikes.

Spike Percentage Ratio Ratio used to determine if a data point is a spike by comparing the data point to its immediate neighbors.

If the Spike Percentage Ratio is set to 30%, then if at 30% of its full intensity the data point is still more intense than both its immediate neighbors it will be regarded as a spike. To express this as a ratio, the maximum allowed intensity ratio between a data point and its immediate neighbors is 3:1.

Spike Percentage Ratio set to 50% is equivalent to a ratio of 2:1.

Spike Percentage Ratio set to 20% is equivalent to a ratio of 5:1.

Profile Data — Enhanced TIC Calculation

Enable TIC Enhancement Select this box to produce an enhanced TIC for the acquisition. Note that the use of **TIC Enhancement** does involve some additional processing while acquiring. For this reason the use of TIC Enhancement will somewhat reduce the maximum achievable acquisition rates.

TIC summation type The TIC can be either the sum or BPI of those intensities within the specified ranges.

Intensity Acceptance Parameters These parameters specify a minimum and maximum intensity and a mass range for the enhanced TIC. Use these values to select regions of interest for your TIC. You may want to :-

- Look at a certain peak
- Ignore solvent peaks
- Ignore 'grassy' baselines
- Not count saturated peaks

Note for Max Intensity, ions produce an electronic signal between 0 and 10 volts. The default value shown here represents 10 volts.

Pressing the **Default** button loads default values for all parameters and switches off all the advanced instrument data thresholding features.

Press the **OK** button to accept any changes. The settings will be downloaded when you press the **OK** button in the Instrument Data Thresholding page. These settings will not be downloaded if **Cancel** is pressed in the Instrument Data Thresholding page.

Changing Lab and User Information

MassLynx can save management information with a data file. The information saved is Laboratory Name, Instrument Identification and User Name. This information is entered into the Lab and User Info dialog box.

This information will be stored with any data that is acquired and can be displayed as part of the header information of a chromatogram or spectrum when they are displayed on the screen or printed.

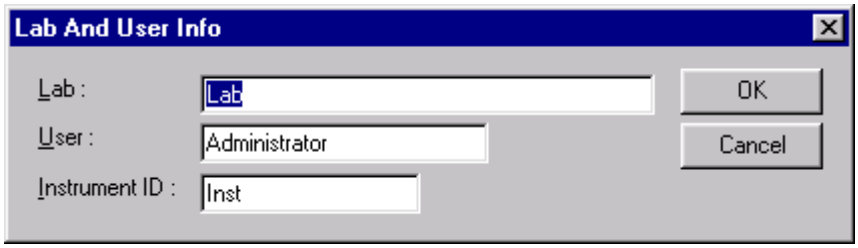

Figure 2.10 *Change Management Information Dialog*

- **To change Lab and User Information**

1. Choose **Change Lab or User Info** from the Acquisition Control Panel **Configure** menu.

2. Make required changes to the information.

3. Press the **OK** button.

Other Menu Items

On the Acquisition Control Panel **Instrument** menu there are three options used for diagnostics.

Transputer Version Choose this option to display the version number of the Transputer code and the Mass Range of the Mass Spectrometer.

Verify Serial Comms Choose this option to check the communications between an Autosampler or GC and the Mass Spectrometer.

Trace Choose this option to generate a log file of all events between MassLynx and the transputers. **Do not use this option unless advised to by Micromass as it generates very large data files**. A tick mark appears next to the option if it is selected.

Notes

Notes

Instrument Tuning

Chapter 3

Introduction

Before acquiring data it may be necessary to check the tuning conditions of the instrument, and if necessary modify one or more of the instrument tuning parameters. The instrument can be tuned either manually or automatically from the instrument Tune page.

The Tune Page

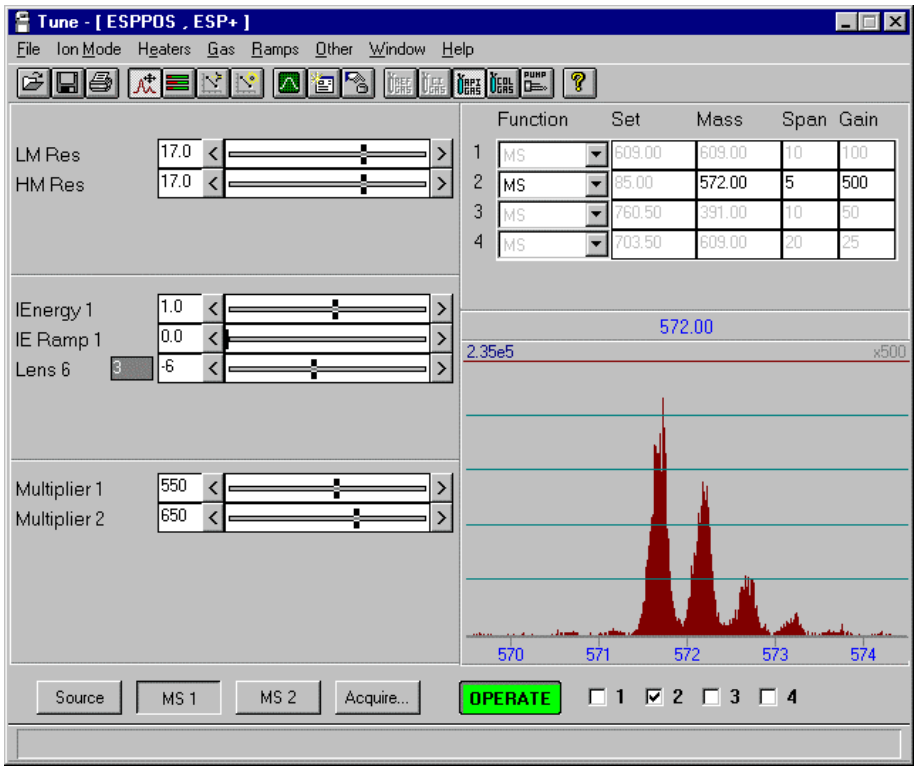

Figure 3.1 *Tune Page showing tune peak information*

The Tune page is a configurable paneled display as shown in **Figure 3.1** . The left side of the window holds the tuning parameters for a selected region of the mass spectrometer. The Mass Spectrometer region can be changed using either the **Window** menu, or by pressing one of the buttons at the bottom left of the window.

The panel in the top right of the window can display either the tune peak information (**Figure 3.1**) or instrument pressure information (**Figure 3.2**). The display can be switched between tune peak information and instrument pressure information using the **Window** menu or the  and  toolbar buttons.

A Toolbar is displayed at the top of the tune window, which allows you to perform some common operations with a single click of the appropriate Toolbar button. Note the Toolbar does not appear for some older instruments like the 12-250, Trio-2000, Bio-Q and Quattro I.

At the bottom right of the window is the tune peak display. You can display up to four masses to tune on. The number of tune peaks displayed is controlled by the four check boxes at the bottom right-hand side of the window. Any one of the tune peaks can be zoomed so that it occupies the entire tune peak area. When a tune peak has been zoomed the controls for the mass and span for that peak are displayed at the top of the display window. This enables you to display the pressure information while having control over the peak display.

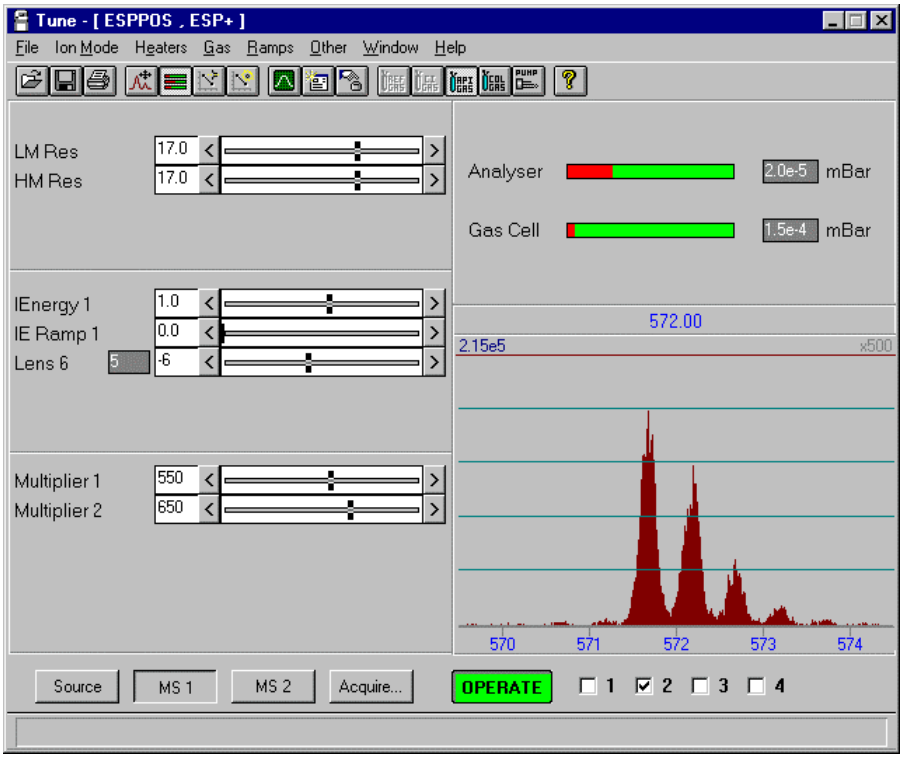

Figure 3.2 *Tune Page showing instrument pressure information*

To display the Tune Page

1. Select the Acquisition Control Panel.

2. Double click on the mass spectrometer section of the instrument picture on the Acquisition Control Panel window.

   -or-

   Choose **Tune Mass Spectrometer** from the Acquisition Control Panel **Instrument** menu.

3. From the window menu, select either **Peak Controls** to show peak information or **Pressures** to show instrument pressure information.

The Tune Page Toolbar

The Toolbar is displayed at the top of the tune window and allows you to perform some common operations with a single click of the appropriate Toolbar button.

 Press to open an existing tune file.

 Press to save current tune parameters to disk.

 Press to print current window in portrait format.

 Press to display tune peak information.

 Press to display instrument pressure information.

 Press to edit scope settings.

 Press to reset the zero level of the instrument and reinitialise the system.

 Press to run AutoTune.

Press once to switch reference gas on. Press again to switch reference gas off.

Press once to switch CI gas on. Press again to switch CI gas off.

Press once to switch API gas on. Press again to switch API gas off.

Press once to switch collision gas on. Press again to switch collision gas off.

 Press to get help.

Changing Tune Parameter Settings

Most of the tuning parameter controls are combination controls featuring a slider bar, two push buttons and an edit window. The tuning parameters can be modified in any of the following ways.

- By dragging the slider bar using the mouse.
- By clicking the "<" or ">" buttons.
- By typing a new value directly into the edit window.

If you change a tune parameter using the slider bar or push buttons, the value shown in the edit window will update as appropriate.

Other tune parameter controls only have an edit window and can only be changed by direct typing.

The speed with which the system will respond to changes in parameter settings is controlled by the speed with which the peak display refreshes. To get the sliders to be most responsive you should set the scope scan speed and inter scan time to be as short as possible (for more information, see Scope Parameters, on page 31).

Printing Tune Information

A report of the tuning parameters can be sent to the printer by pressing the  toolbar button or by choosing **Print** from the tune page **File** menu. This report contains a copy of the tune peak information displayed on the screen, along with a record of each parameter setting. This report is not configurable by the user.

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 User Guide, Selecting and Viewing Data, for more information.

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

Save on Exit Option

When the **Save on Exit** option is selected the current tuning parameters are saved to disk when you exit from the tune page and will be reloaded when you enter the tune page again. When **Save on Exit** is not selected the current tuning parameters are not saved to disk when you exit from the tune page, when you enter the tune page again the last saved set of parameters will be reloaded from disk.

To enable the **Save on Exit** option, choose **Save on Exit** from the tune page **File** menu. A tick mark will appear next to this option to show that it is enabled. To disable the **Save on Exit** option, choose **Save on Exit** from the tune page **File** menu again.

To save a set of parameters

1. To save the current tune parameters with the existing file name press the  button or choose **Save** from the Tune Page **File** menu.

   To save the current tune parameters with a new file name choose **Save As** from the Tune Page **File** menu.

2. Enter a new file name under which you want the parameters to be saved, or alternatively select an existing file from the list displayed.

3. Press the **Save** button. If the selected file already exists on disk you will be asked to confirm that you want to overwrite the existing information. Press the **Yes** button to continue or the **No** button to enter a different file name.

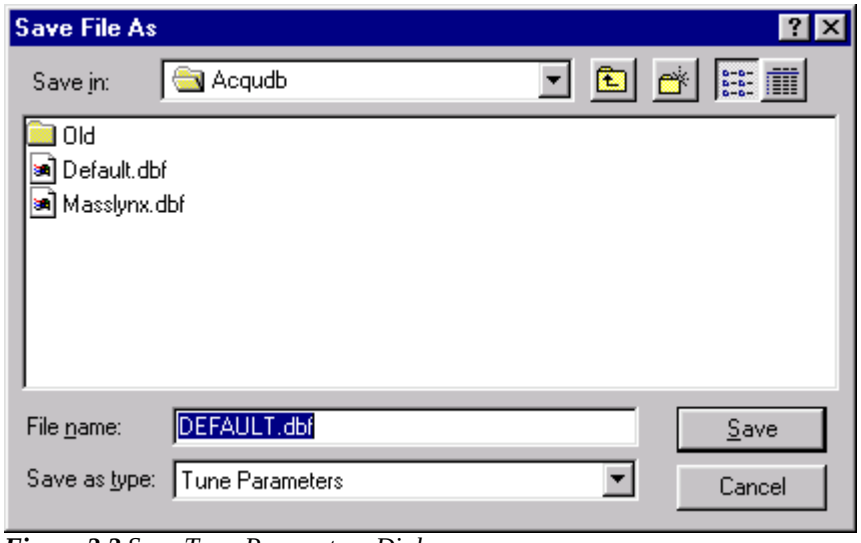

Figure 3.3 *Save Tune Parameters Dialog*

To restore a saved set of parameters

1. Press the  toolbar button or choose **Open** from the Tune **File** menu.

2. Select the tuning parameter set that you would like to use, either by typing its name or by selecting it from the displayed list.

3. Press the **Open** button.

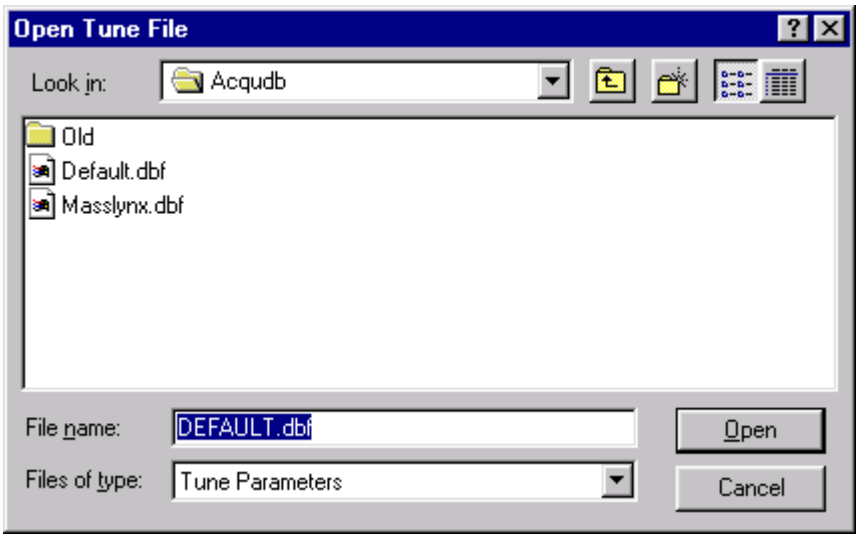

Figure 3.4 *Open Tune File Dialog*

Modifying the Peak Display

The tune peak display is modified using either the tune peak controls, or by using the mouse directly on the tune peak display. **Figure 3.5** shows the tune peak controls and the tune peak display.

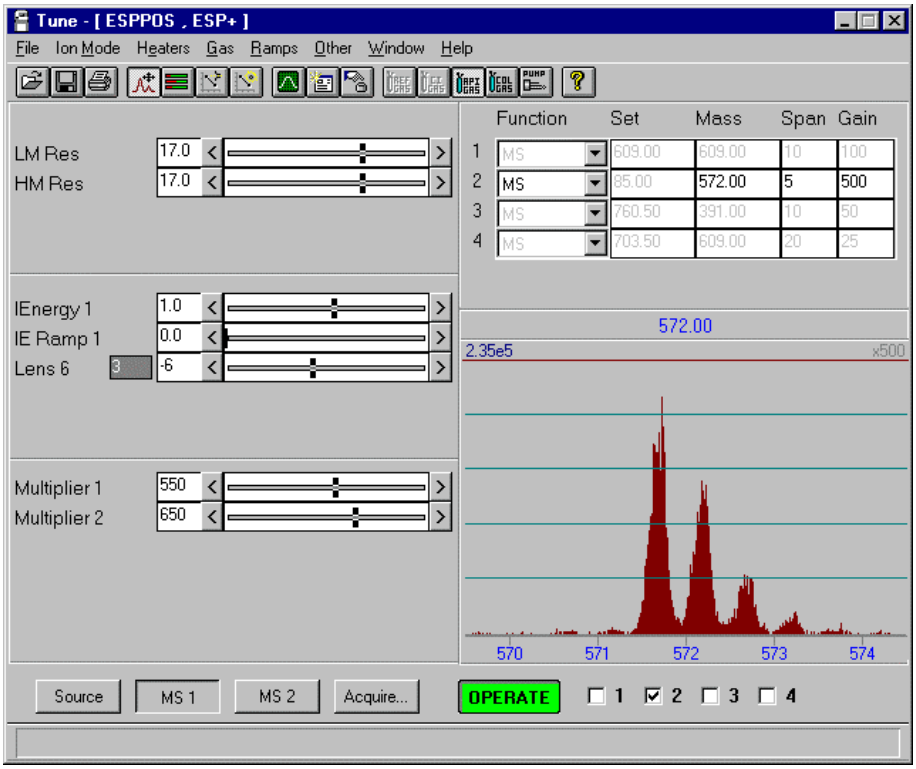

Figure 3.5 *Tune peak controls and display*

To select peaks

1. Choose the number of peaks that you want to display by checking the appropriate controls at the bottom of the window. For example, if you want to display only the first and second tune peaks then check controls 1 and 2 on, 3 and 4 off.

2. Press the  toolbar button or choose **Peak Display** from the Tune Page **Window** menu and then for each active peak select the scan type (Quattro only) that you wish to use, the mass that you wish to tune on, the span and the gain.

To zoom or unzoom a peak

Double click on the peak. This will make the selected peak fill the whole tune peak display and remove the peak selection controls from the window. A second double click will unzoom the peak.

To change the tune mass, span or gain using the mouse

1. Zoom the relevant peak - this will cause left and right arrow controls for the mass and span to appear at the top corners of the peak window.

2. Click on the arrow to the left of the mass control to decrease the mass by one increment, click on the arrow to the right to increase the mass. The size of the increment is set using the scope controls dialog (see later).

3. To double the gain applied to a peak double click on the line above the peak which shows the gain. A double click below the peak will half the gain.

AutoTune

MassLynx can automatically tune the mass spectrometer in EI, APcI and electrospray ionization modes on supported instruments. The instruments supported for each ionization mode are as follows:

Autotune Ionization Mode	Instruments Supported
EI	Platform II, Quattro II, Quattro I and Trio-2000
APcI and Electrospray	Platform II, Quattro II, Platform LC and Quattro LC

AutoTune ramps the settings for the tuning parameters until they are optimised to give the best intensity, resolution and peak shape.

To run AutoTune

1. Press the  toolbar button or choose **AutoTune** from the Tune page **Other** menu. Note, the instrument must be in operate before you can run AutoTune.

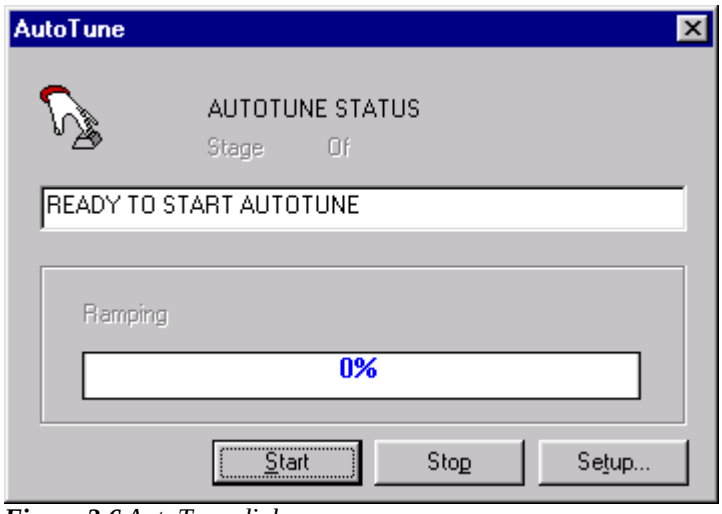

Figure 3.6 *AutoTune dialog*

2. Choose **Setup** to set the AutoTune setup parameters.

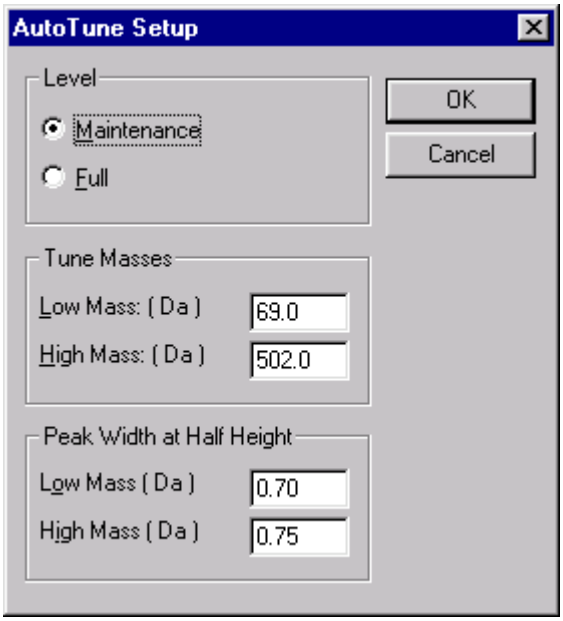

Figure 3.7 *EI AutoTune Setup dialog*

The default setup parameters for EI AutoTune are suitable for tuning with Heptacosa, there is no need to alter the Tune Mass or Peak Width parameters if you are using Heptacosa.

The **Level** of AutoTune performed can be selected as **Maintenance** or **Full**. 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 reasonably well tuned already, if the current tuning is too poor AutoTune will give an error and ask you to perform a full AutoTune.

The **Tune Masses** parameters set a **Low** and **High Mass** which will be used to tune on.

The **Peak Width at Half Height** parameters set the peak width at half height for the **Low** and **High Masses** being monitored.

When you are satisfied with the AutoTune setup parameters press **OK** to exit.

3. Choose **Start** to start AutoTune. The AutoTune status bar is updated to show the progress of AutoTune. AutoTune has 7 stages:

1) Checking instrument conditions

Autotune checks that tune page readbacks are within acceptable tolerance.

2) Attempting to detect initial beam

AutoTune looks for an initial beam by increasing the value of the multiplier.

3) Rough tuning focus lenses at low mass

Values of lenses are ramped to see where the low mass peak intensity maximises.

4) Tuning ion energy and ion repeller

Modifies values for ion energy ramp, ion energy and repeller.

5) Tuning for good peak shape

Checks peak width at half height and height of valley between main and isotope peaks.

6) Fine tuning focus lenses at high mass

Values of lenses are ramped over a narrower range using the values determined in stage 4 to see where the high mass peak intensity maximises.

7) Tuning multiplier for good sensitivity

Value of multiplier ramped until the low mass peak intensity is 80% of full scale.

4. When AutoTune has finished it displays a status dialog to say that AutoTune has been successfully completed. Press **OK** to exit back to the tune page. The tuning parameters determined by AutoTune will be saved to the current tune parameter file.

Scope Parameters

Various parameters can be set to control the peak display and, if fitted, an external oscilloscope. You can control the speed at which the scope is refreshed and the scope response. You can also ask for a grid to be applied to the tune peak display and for the data to be drawn either as a filled or unfilled trace.

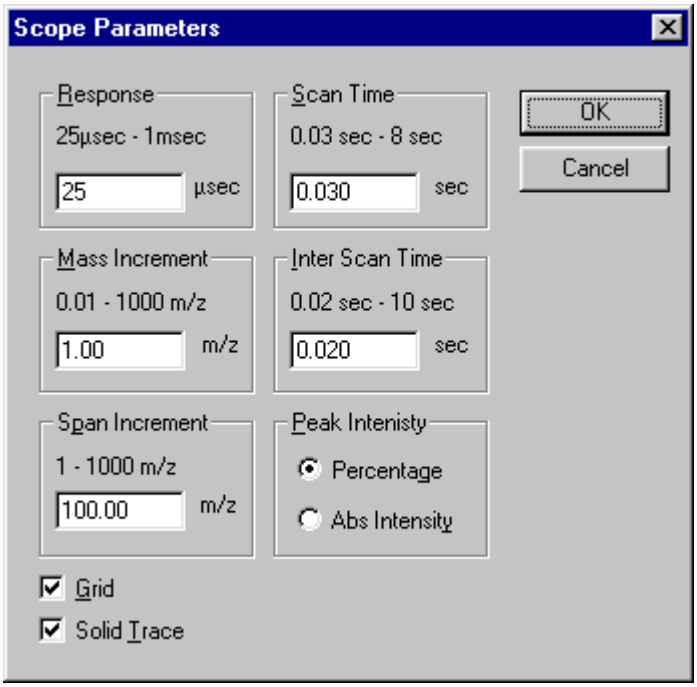

Figure 3.8 *Scope parameters dialog*

- **To change the scope setup**

1. Press the  toolbar button or choose **Scope Parameters** from the **Tune Other** menu.

2. Make any required changes to the settings.

3. Press the **OK** button.

Response A variable low pass filter that effects the level of high frequency information that will be displayed on an external oscilloscope, if one is fitted to the mass spectrometer. A high value will make the peak display appear smoother than a low value.

Scan Time and Inter Scan Time Control the speed with which the tune peak display is updated. The tuning system will behave more responsively if the scan time and inter scan time are short.

Mass Increment and Span Increment The mass and span settings for a zoomed peak can be altered using the mouse by clicking on the appropriate up or down button (see: Modifying Peak Display). The mass increment and span increment controls effect how much the setting will change for each mouse click.

Grid Display a horizontal grid on the peak display.

Solid Trace Check this control to draw the displayed peak as a filled trace. Leave the control unchecked to draw the displayed peak as a line graph.

Peak Intensity When this parameter is set to **Percentage** the percentage intensity of the base peak in the active tune segment window is displayed. When this parameter is set to **Abs Intensity** the absolute intensity of the base peak in the active tune segment window is displayed.

Changing Inlet Heaters

Figure 3.9 shows the dialog box that is used to set inlet interface temperatures and the DCI current setpoint. The Heaters dialog is a "modeless" dialog, which means that you do not have to close it to continue using the rest of the system.

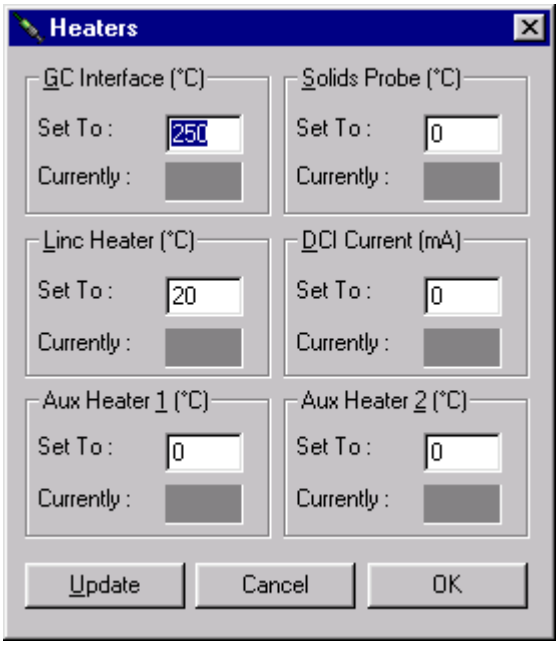

Figure 3.9 *Inlet Heaters dialog for interface temperature and DCI current setpoint*

- **To change an inlet parameter**

1. Select **Change Heaters** from the Tune **Heaters** menu.

2. Select the required parameter and enter the desired set point in the edit box labeled **Set To**.

3. To send the current values to the instrument press the **Update** button. The new set point will now be sent to the acquisition system. You can observe the effect of the change by watching the read back value which should change as the selected heater heats up or cools down to the required value. Readbacks are displayed in the box labeled **Currently**.

4. Press the **OK** button to send current values to the instrument and exit from the Heaters dialog.

Gas Controls

The **Gas** menu on the Tune page lets you turn gasses on or off. You can also customize the menu so that the correct names of the gasses that you actually use appear in the menu bar.

To turn a gas on or off

Press the relevant toolbar button or choose the required gas from the Tune **Gas** menu. If the gas was previously turned off it will now be turned on. A tick mark will appear next to a gas if it is turned on.

To change the name of a gas

1. Select **Parameters** from the Tune **Gas** menu.

2. Select the required gas and enter its name.

   If you wish to be able to select the gas from the Tune Gas menu using a key letter you should precede this letter of the name by the character "&". For example, in **Figure 3.10** the CI Gas will appear on the Tune Gas menu as C<u>I</u> Gas and can be selected via the keyboard by pressing "I". Note the key letter does not necessarily have to be the first letter of the word.

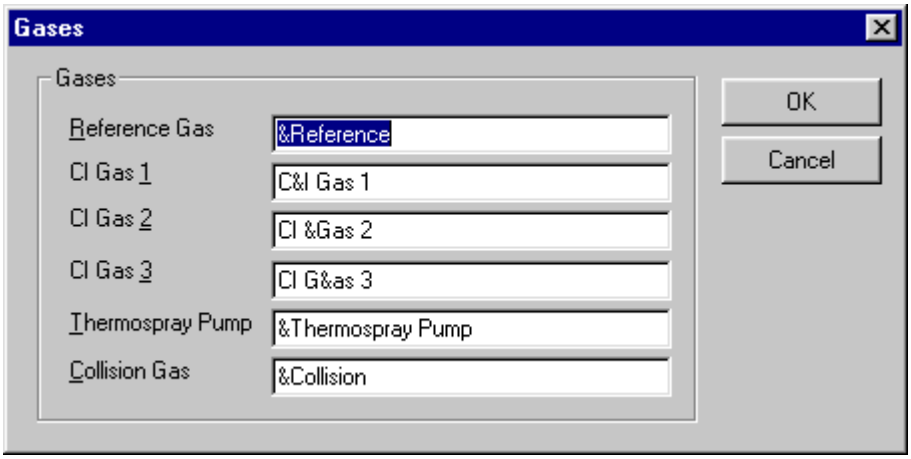

Figure 3.10 *Tune gas parameters dialog*

Ramp Controls

For instruments, which have the relevant hardware, installed it is possible to set cone voltage and collision energy ramps on the tune page.

To set up a cone voltage ramp

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

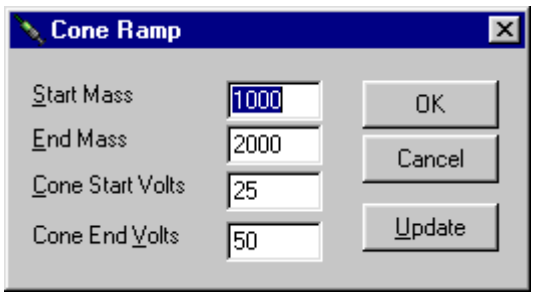

Figure 3.11 *Cone Ramp dialog*

2. Two values of cone voltage are defined at two particular masses using the **Start** and **End Mass** controls and the **Cone Start Volts** and **Cone End Volts** controls. 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. Pressing the **Update** button in this dialog will update the values used for the cone voltage ramp when the ramp has been enabled.

3. To initiate the cone voltage ramp press the  button or choose **Use Cone Ramp** from the tune page **Ramps** menu.

To set up a collision energy ramp

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

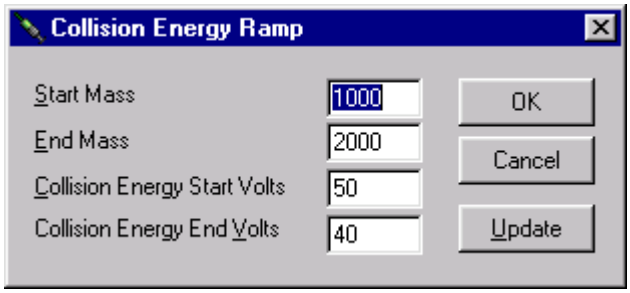

Figure 3.12 *Collision Energy Ramp dialog*

2. Two values of collision energy are defined at two particular masses using the **Start** and **End Mass** controls and the **Collision Energy Start Volts** and **Collision Energy End Volts** controls. These values define a gradient for the collision energy voltage that is then extrapolated to cover the full mass range. Make any changes required and press **OK** to exit. Pressing the **Update** button in this dialog will update the values used for the collision energy ramp when the ramp has been enabled.

3. To initiate the collision energy voltage ramp press the  button or choose **Use Collision Energy Ramp** from the tune page **Ramps** menu.

Vacuum

The mass spectrometer's vacuum system can be controlled from the Tune page. Ensure however that this is done in accordance with the information in your *Instrument Users Guide*.

- **To pump down the vacuum system**

  Choose **Pump** from the **Tune Other** menu. The menu name will change from **Pump** to **Vent** and the system will commence its pump down sequence. You can watch the vacuum progress by choosing **Pressures** from the **Tune** Window menu.

- **To vent the vacuum system**

  Choose **Vent** from the **Tune Other** menu. You will be asked to confirm that you want to vent the system, in case **Vent** was selected in error. The system will then start its automatic venting sequence.

Resetting the Zero Level

The zero level (or Baseline) can be repositioned by Pressing the  toolbar button or choosing **Reinitialize** from the **Tune Other** menu. This command will cause 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 value of one of the multiplier voltages is changed.

Controlling Readbacks

MassLynx allows you to choose how system readbacks will be displayed on the Tune page. You can ask for readbacks to be displayed continuously, never or only when they differ from their associated set points by more than 10%.

Note that a number of the readbacks are for diagnostic purposes only and do not need to be very precise. Their function is to indicate if the voltage is present or not. The acceptable variation between the set value and the readback value will vary depending on the particular tune parameter. If you are concerned about the values you should contact your local service office for advice.

To change read back style

1. Choose **Readbacks** from the **Tune Other** menu.

2. Select the readback style required. Selecting **Always** will always display readbacks. Selecting **Out of Range** will display readbacks when they differ from their associated set points by more than 10%. If both controls are unchecked then no readbacks will be displayed.

3. Press the **OK** button.

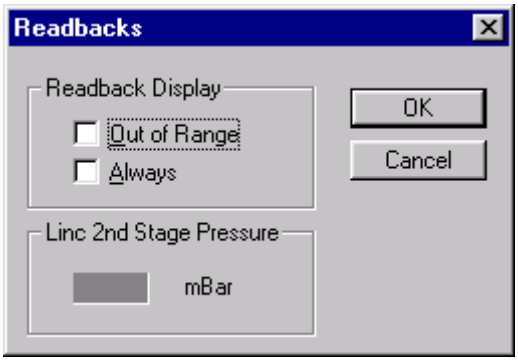

Figure 3.13 *Tune Readbacks dialog*

Starting an Acquisition from the Tune Page

The easiest way to acquire data for a sample is to acquire it directly from the tune page. You cannot use inlet programs from the tune page, acquire analog data or acquire multiple sample sequences, but you can start and stop acquisitions and control most of the scanning parameters. The 'Start Acquisition' dialog as shown in **Figure 3.14** can be 'folded' to hide most of the parameters from the user and can be configured to automatically set itself to acquire data using the mass range and function type that is being used for tuning the instrument. These features provide a WYSIWYG (What you see is what you get), method of acquiring data as the spectrum that is written to disk will be the same as the spectrum that is shown in the peak display.

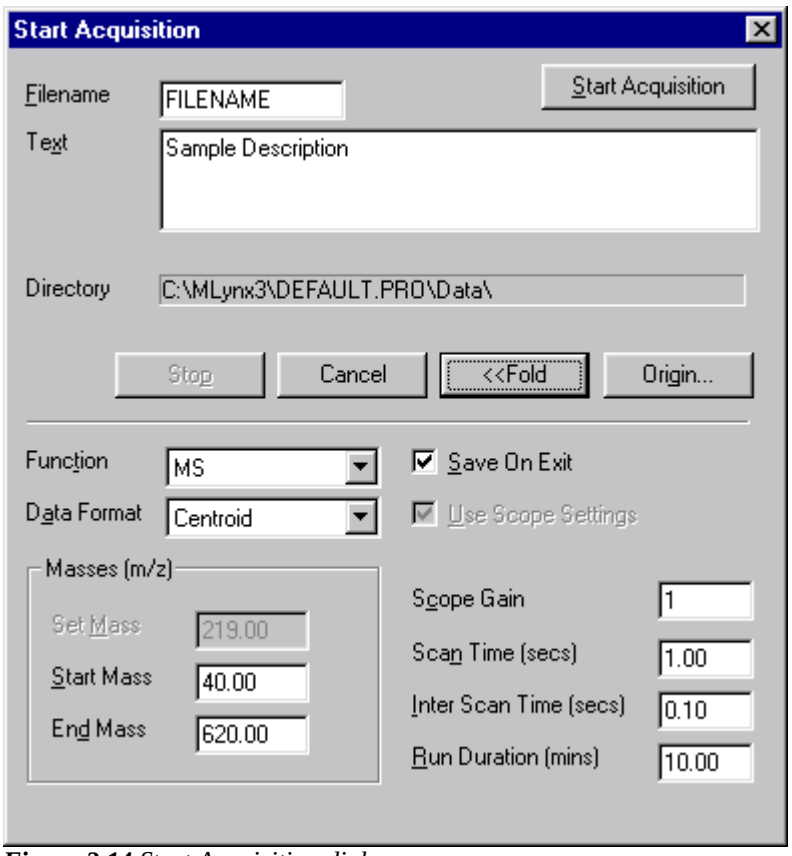

Figure 3.14 *Start Acquisition dialog*

To start an acquisition

1. Choose **Acquire** from the Tune **Window** menu or click on the **Acquire** button at the bottom of the tune window.

2. Make any required changes to the settings.

3. Press the **Start Acquisition** button.

Filename The name of the data file to which acquired data will be written. The filename can be up to 128 characters. If the file already exists on disk, you will be asked if you want to overwrite it.

Text A text area that 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 80 characters.

Directory The Directory field displays the directory into which data will be acquired.

If you wish to change the directory into which data will be acquired, you can cancel the acquisition and create a new project by choosing **Project Wizard**, or open an existing one by choosing **Open Project**, from the MassLynx Top Level **File** menu.

Origin Pressing the Origin button allows you to enter additional information about the sample to be analysed. Additional information can be entered into the following fields **Submitter, Job, Task** and **Conditions**.

Function The scan function that will be used to acquire the data. It can be any of the following ***MS, Parent, Daughter, Neutral Loss, Neutral Gain, Q1F, MS2.*** More information is given on scan functions later on in this chapter.

Data Format The type of data that will be collected and stored on disk. It can be any of the following ***Centroid, Continuum or MCA***. More information is given on data formats later on in this chapter.

Save on Exit If this control is checked, any changes that you make will be saved when you exit the dialog.

Use Scope Settings If this control is checked, the mass information and function type will be taken directly from the currently zoomed peak on the tune page

Set Mass Specifies the mass (i.e. Daughter Mass, Parent Mass etc.) that will be used for the particular function type. If the function that you have selected does not require a set mass, this control will be disabled.

Start Mass Specifies the mass at which the scan will start. The Start Mass must be lower than the End Mass.

End Mass Specifies the mass at which the scan will stop.

Scope Gain The amount of gain that will be applied to data displayed on an external oscilloscope unit, if one is fitted.

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 is stored.

Run Duration Length of the acquisition, measured in minutes.

Q-Tof Tuning

Q-Tof machines have extra parameters to set which are accessed from the Tune Page **Other** menu. To display the relevant dialog select the option from the menu.

TDC Settings

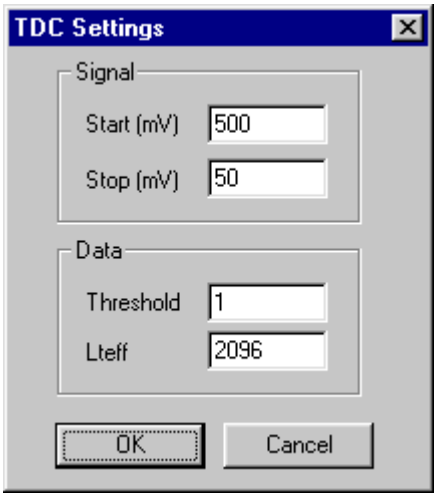

Figure 3.15 *TDC Settings dialog*

Start Enter the start threshold for the TDC signal.

Stop Enter the stop threshold for the TDC signal.

Threshold Enter an intensity threshold below which TOF data will not be stored.

Lteff This is used to adjust the mass scale.

RF Settings

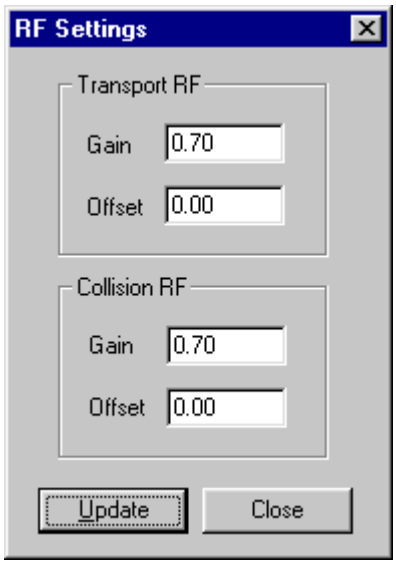

Figure 3.16 *RF Settings dialog*

Enter a **Gain** and **Offset** for the Transport RF and Collision RF.

Update Pressing this button sends the values to the instrument without closing down the dialog.

MCP Conditioning

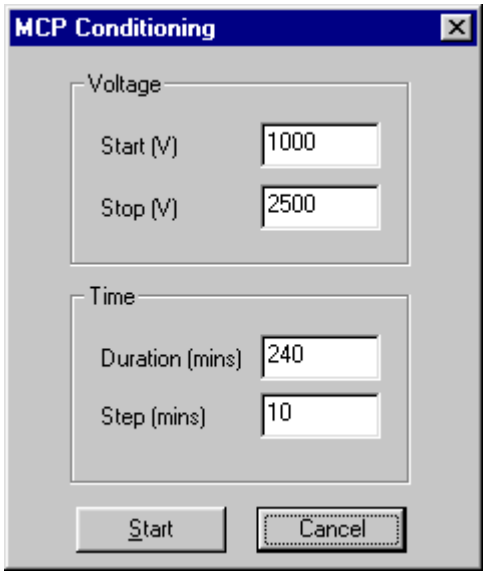

Figure 3.17 *MCP Conditioning dialog*

Enter the **Start** and **Stop** voltages, the **Duration** and **Step** values. The MCP voltage will be stepped between the specified voltages for the specified time.

When the **Start** button is pressed the MCP Conditioning Status dialog is displayed. Press the **Stop** button to stop MCP Conditioning.

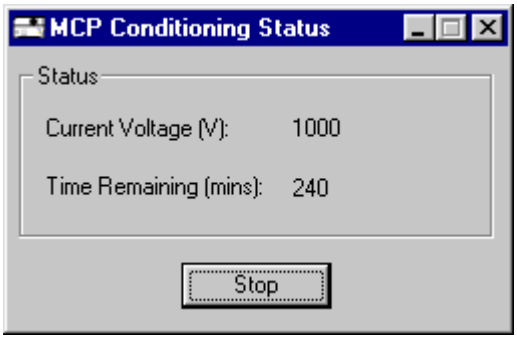

Figure 3.18 *MCP Conditioning Status dialog*

TOF MS Profile

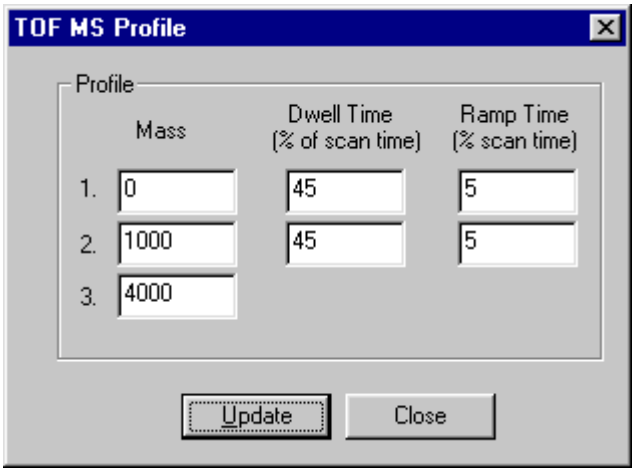

Figure 3.19 *TOF MS Profile dialog*

This dialog allows the user to bias different parts of the mass range during TOF MS acquisition. The Quadrupole will park at the specified **Mass** for the specified percentage of the scan time (**Dwell Time**), and then ramp to the next mass for the specified percentage of the scan time (**Ramp Time**).

Update Pressing this button sends the values to the instrument without closing down the dialog.

Notes

Notes

Mass Calibration

Chapter 4

Mass Calibration

MassLynx provides a fully automated facility for calibrating the mass scale of an instrument. To see the main calibration dialog choose **Calibrate..** from the acquisition control panel **Instrument** menu.

How a calibration is formed

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. Each peak in the reference file is matched to a corresponding peak in the calibration 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.

Calibration Types

A single-quadrupole instrument requires up to three calibration curves;

- A **static** calibration is used to accurately 'park' the quadrupole mass analyser on a specific mass of interest (in Tuning and SIR for example).
- 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.

A triple-quadrupole instrument requires these three calibrations for both MS1 and MS2, for 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	Not Applicable
SIR	Static	Not Applicable
MSMS	All	All
MRM	Static	Static

Overview of the calibration process

1. Check the tuning of the instrument.

The mass spectrometer should be in operational mode. Check that the peak shape and intensities are correct.

2. Set the calibration parameters.

Select the appropriate reference file for the calibration reference sample that you are going to use.

If you are calibrating electrospray, FAB or thermospray, then you will typically be using continuum or MCA data types, and you should set the Mass Measure parameters (see page 52). These parameters control peak detection in continuum and MCA data. There is no need to set these parameters if you are using centroided acquisition.

Set the Peak Match and Curve Fit parameters in the Calibration Parameters dialog (see page 50). These parameters control the location of reference peaks in a calibration spectrum, and the drawing of a calibration curve to correct the resulting mass differences.

3. Start an automatic calibration

From the Main Calibration Dialog, press the **Start...** button. This will bring up the automatic calibration dialog.

Select the types of calibration you wish to perform.

Check the **Acquire and Calibrate** checkbox to perform automatic calibration. Set the standard deviation threshold. Optionally, also check the **Acquire and Verify** checkbox, and set the standard deviation threshold.

Check the **Print Report** checkbox if you want a printed calibration report.

Set the data acquisition parameters.

Press the **OK** button.

4. Check the calibration report

Examine the calibration report, and display the calibration graphs if necessary, to satisfy yourself that the calibration is satisfactory.

The Calibration Dialog

The calibration dialog displays status information, including calibrated mass range and scan speeds, and the time and date of the last calibration.

To display the dialog select **Calibrate** from the Acquisition Control Panel **Instrument** menu.

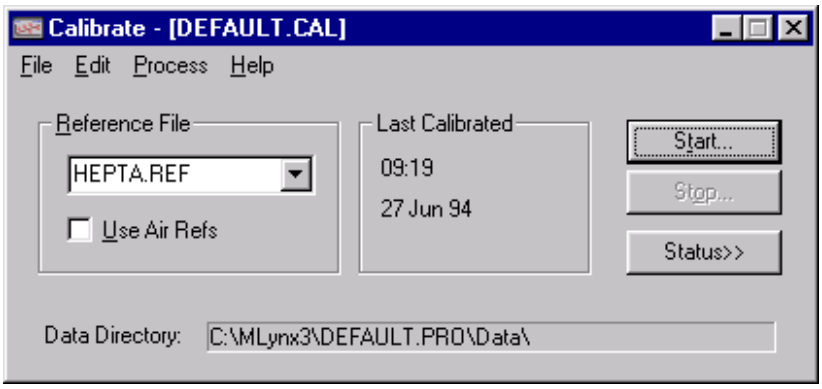

Figure 4.1 *Main Calibration dialog*

The dialog may be *folded* to hide the status information by pressing the <<Fold button. When in the folded state, the status information may be displayed by pressing the Status>> button.

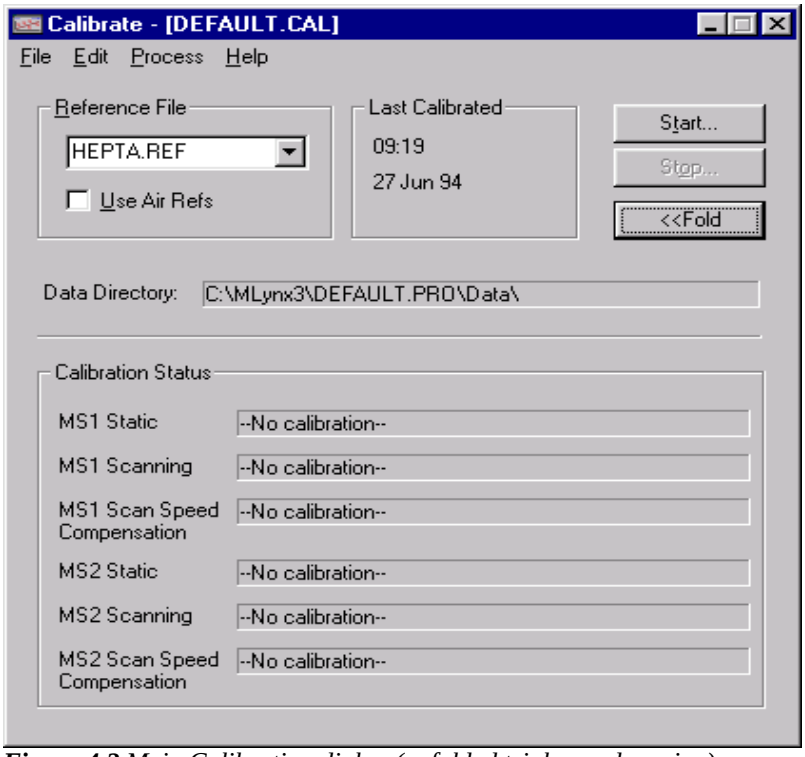

Figure 4.2 *Main Calibration dialog (unfolded triple quad version)*

Parameters which control Calibration

- **Automatic calibration parameters**

To access this dialog, press the **Start** button on the **Calibrate** dialog.

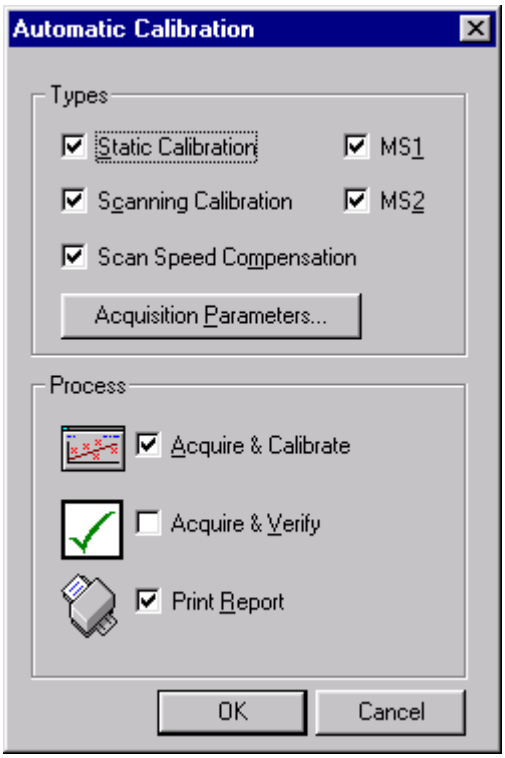

Figure 4.3 *Automatic Calibration dialog*

You may choose **Static Calibration**, **Scanning Calibration**, or **Scan Speed Compensation** calibrations by checking the appropriate check boxes. On a triple quadrupole instrument you must also specify MS1 and/or MS2.

Optionally, you may choose to **Verify** the calibration. Selecting this option causes a calibrated mass spectrum of the reference compound to be acquired.

Finally you can choose to have a report of the calibration printed automatically when the calibration completes. If you choose not to print the report at this stage you can always print it from the calibration curve display later. The report contains pictures of the calibration curves produced, along with calibration statistics such as standard deviation.

Note, for Q-Tof machines **Types** available are **Quad** and **TOF**. Quad performs a standard MS1 calibration, TOF performs a Tof MS data calibration. Select only one of these options.

Data Acquisition Parameters

To change data acquisition parameters press the **Acquisition Parameters** button on the **Automatic Calibration** dialog.

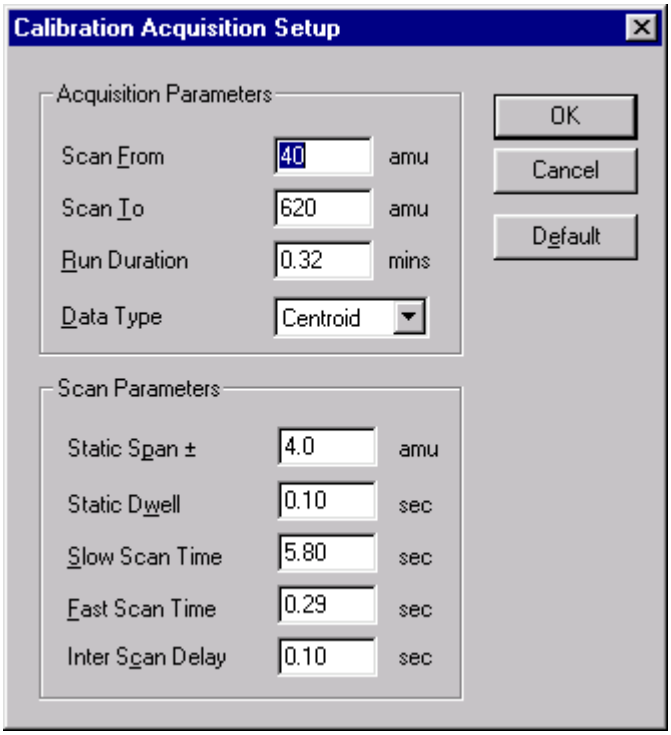

Figure 4.4 *Calibration Acquisition Setup dialog*

The **Scan From** and **Scan To** parameters specify the scan range for each calibration type.

The **Run Duration** parameter specifies how long each calibration data file will be acquired for.

The **Data Type** parameter specifies whether data will be acquired in centroid, continuum or MCA mode. It is recommended that the calibration uses the same data type as the sample data you will be acquiring.

To acquire data for static calibration, the portion of the mass scale immediately around each reference peak is scanned. The **Static Span** parameter specifies the distance to be scanned either side of the reference peak. The **Static Dwell** parameter specifies the time taken to scan this range.

To acquire data for scanning calibration, the mass scale is scanned over the selected range, in a time specified by the **Slow Scan Time** parameter.

To acquire data for the Scan Speed compensation calibration, the mass scale is also scanned over the selected range, in a time specified by the **Fast Scan Time** parameter.

The **Inter Scan Delay** specifies the time between one scan ending and the next scan starting.

Pressing the **Default** button will reset all parameters in the dialog box to their default values.

To change Calibration Parameters

1. Select **Calibrate** from the **Acquisition Control Panel**, this displays the **Calibrate** dialog.

2. Select **Calibration Parameters** from the **Edit** menu of the **Calibrate** dialog.

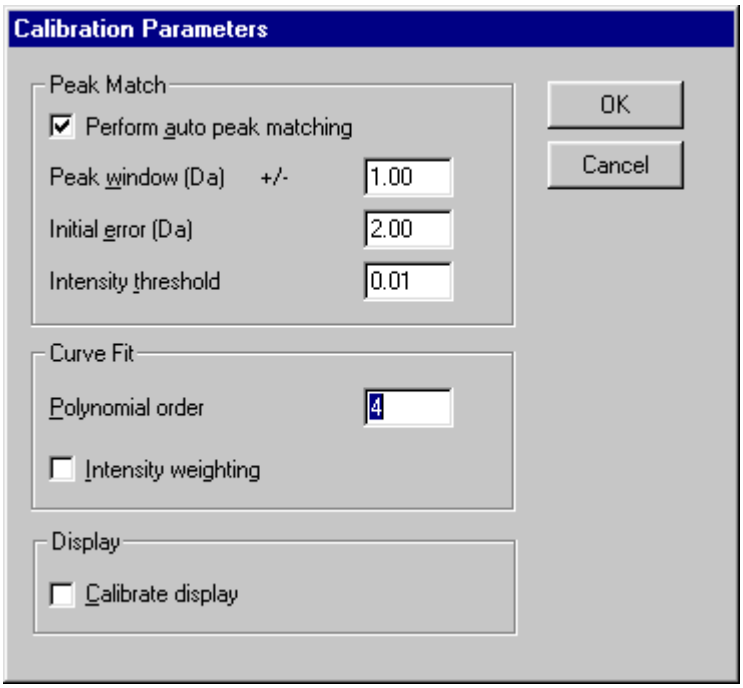

Figure 4.5 *Calibration Parameters Dialog*

Perform auto peak matching Check the box to enable. When unchecked this allows disabling of the automatic peak matching facility. Useful for TOF calibration, where the automatic peak matching algorithm will typically not make the correct assignments.

The **Peak Window** parameter specifies the maximum mass difference between the remaining reference peaks and the expected position of the corresponding peaks in the acquired spectrum. Normal operating range for the **Peak Window** parameter is 0.3 to 1.5 Da.

The first reference peak to be matched is chosen to be close to the center of the calibration mass range. The **Initial Error** parameter specifies the maximum mass difference between this reference peak and the corresponding peak in the acquired spectrum. Normal operating range for the **Initial Error** parameter is 0.5 to 2.0 Da.

Any peak in the acquired spectrum with intensity less than the specified **Intensity Threshold** will not be used to form the calibration curve. The threshold is specified as a percentage of the most intense peak in the acquired spectrum. Normal operating range for the **Intensity Threshold** parameter is 0 to 5%.

When each peak in the reference spectrum has been matched with a corresponding peak in the acquired spectrum, the mass difference

acquired mass - reference mass

is calculated for each pair of peaks. These mass differences are plotted as points on a graph; each data point has the mass of the acquired peak as its x co-ordinate, and the above mass difference as its y co-ordinate. A smooth curve is drawn through the

points. The **Polynomial Order** parameter controls the type of curve which is drawn and can be set to any value between 1 and 5. If Polynomial Order = 1, a straight line is drawn through the points. If Polynomial Order = 2, a quadratic curve is drawn through the points. If Polynomial Order = 3, a cubic curve is used.

For a typical EI calibration where the mass range calibrated starts below 100 amu and extends up to 650 amu the recommended setting for the Polynomial Order parameter is 4. For a typical electrospray calibration where the mass range calibrated is from 600 amu to greater than 1000 amu the recommended setting for the Polynomial Order parameter is 2.

Intensity Weighting If this box is checked, the curve fit is weighted toward the points representing the more intense acquired peaks. The weight of each point is proportional to the square root of the intensity of the acquired peak.

Calibrate display Enables calibration of the peaks in the top (raw file) graph. This feature allows selection of one peak at a time, with the display being recalibrated after the selection of each peak, bringing the other masses in the spectrum into line. Again, useful for TOF calibration.

Automatic Calibration Check parameters

To access this dialog, select **AutoCal Check Parameters** from the **Edit** menu on the **Calibrate** dialog.

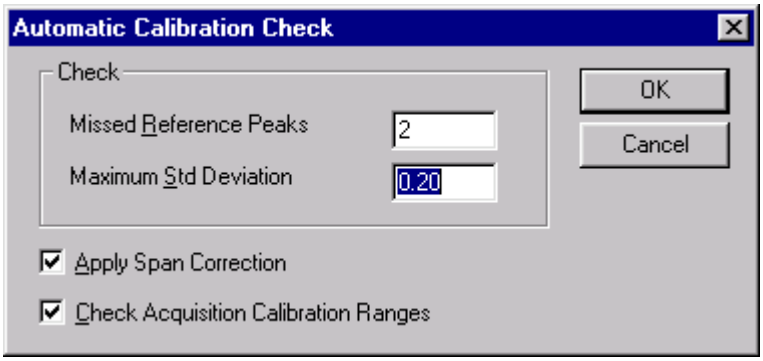

Figure 4.6 *Automatic Calibration Check dialog*

The **Missed Reference Peaks** control allows you to enter a number of consecutive peaks from the reference file that the system is allowed to miss before a warning is issued to the user.

The **Maximum Standard Deviation** control does the same thing if the residuals for a particular calibration exceeds the number entered.

The **Apply Span Correction** option causes an extra correction to be applied to the mass scale, which is dependent on the mass range being scanned. This correction ensures that mass assignment will be correct even if the mass scale that you are working with is different to the one that the instrument was originally calibrated over. It is not recommended that this option be used if the mass range of interest is less than 1000amu and includes the range 0-150 amu.

The **Check Acquisition Calibration Ranges** option causes a warning message to be displayed if an acquisition is started which would be outside the range of the current calibration.

The Mass Measure parameters

If you are using continuum or MCA acquisition modes to acquire your calibration data you will need to tell the system how it should convert the acquired data into centroided data needed by the calibration process.

To access this dialog, select **Mass Measure Parameters** from the **Edit** menu on the **Calibrate** dialog.

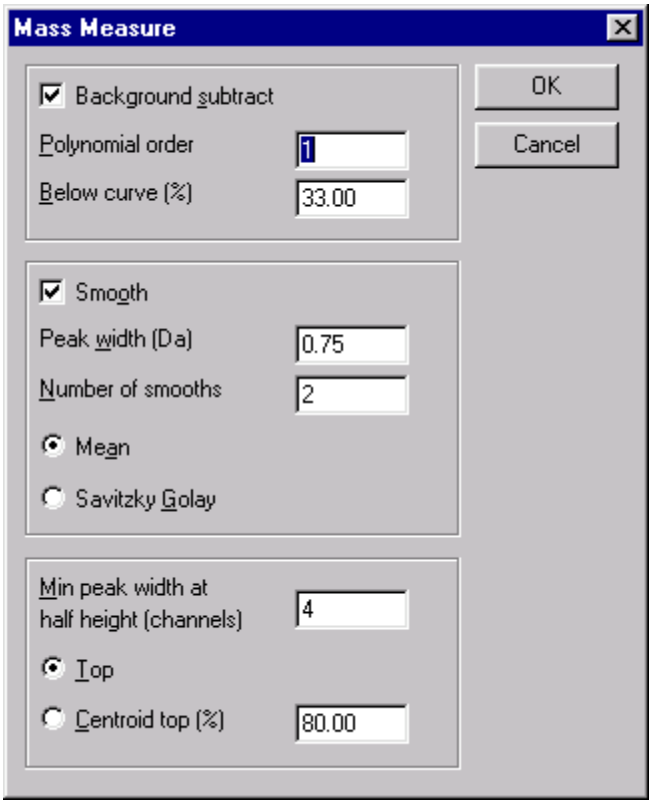

Figure 4.7 *Mass Measure dialog*

For a full description of the Mass Measure parameters and their effect, see the section headed "Mass Measure" in the Spectrum chapter of the MassLynx User Guide.

QTOF

For Q-Tof data this dialog will have an extra button. Press this button to display the QTOF Accurate Mass parameters dialog. For details see QTOF Accurate Mass below.

There is also an extra field **Use QTOF mass correction**. Check this box to use QTOF mass correction.

QTOF Accurate Mass

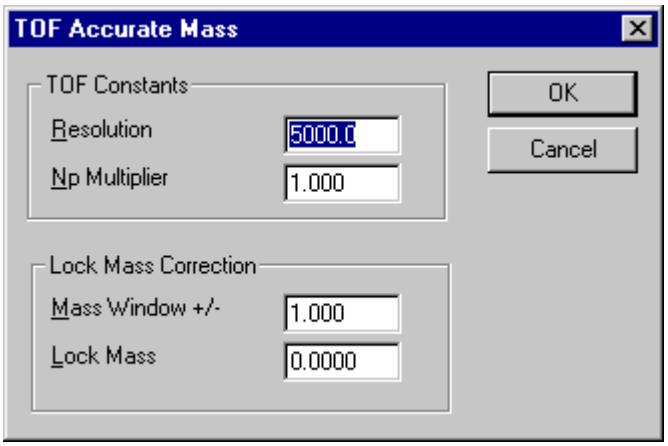

Figure 4.8 *QTOF Accurate Mass dialog*

Resolution Enter the resolution of the Mass Spectrometer.

Np multiplier Enter a value for the number of pushes correction factor.

Mass Window This parameter determines the width of the mass window used to locate the lock mass data peak. The most intense peak in the range Lock Mass – Mass Window to Lock Mass + Mass Window is selected, and mass correction based on this peak is performed.

Lock Mass This parameter specifies the reference lock mass.

The Calibration Report

If the calibration proceeds successfully, no output will be displayed until the calibration report is printed, showing calibration curves for each selected calibration type.

If the standard deviation of the residuals for a particular calibration exceeds the pre-set maximum, a set of calibration graphs will be displayed to help identify the problem.

Typically, the peak matching algorithm has found the wrong peaks, or missed some peaks. You may need to change the peak match parameters or adjust the peak matching manually. You can change the peak match parameters by choosing **Peak match params** from the calibration graph **Edit** menu. If the peak matching algorithm has found the wrong peaks, increasing the value of the **Peak window** parameter will solve the problem. You should also try reducing the value of the **Intensity threshold** parameter (if it is not already zero), before identifying the peaks in the acquired spectrum by hand.

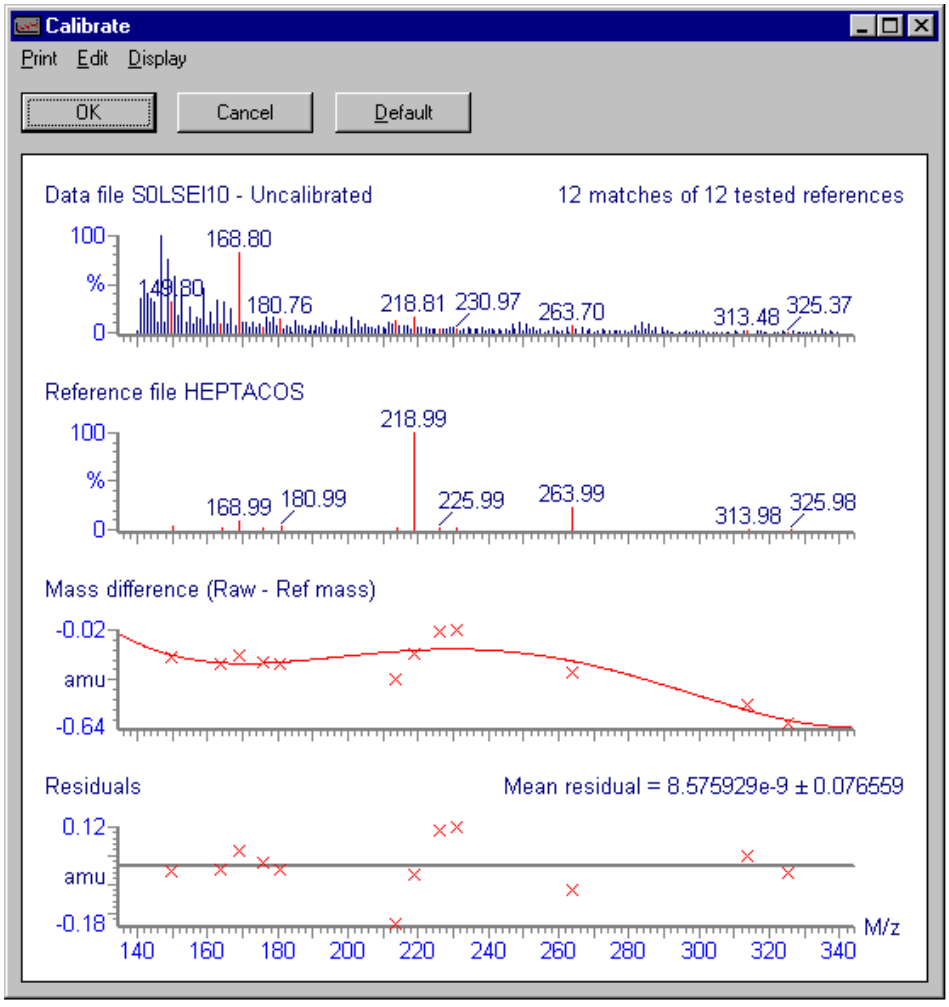

Figure 4.9 *Calibration Graphs*

The top graph shows the calibration spectrum; the peaks matched to reference peaks are highlighted in a different color.

- **Altering the displayed range**

  You can alter the range of the spectrum on display by clicking the left mouse button on a graph at one end of the range of interest, holding the button down and dragging the mouse to the other end of the range of interest. A "rubber band" indicates the selected range. Pressing the **Default** button restores the default display range.

- **Manually matching peaks**

  You can match a calibration peak to the closest reference peak by positioning the mouse pointer over the calibration peak and clicking the right mouse button. If the closest reference peak is already matched to another calibration peak, the previous match will be removed. You can also use the right mouse button to undo a peak match, by positioning the mouse pointer over the matched calibration peak and clicking the right mouse button.

Other Calibration Facilities

- **To delete the instrument calibration**

  1. Choose **Delete all calibration** from the Calibrate **Process** menu. This will bring up a dialog requesting confirmation.

  2. Press **OK** to delete all calibration, or **Cancel** to abort the operation.

- **Displaying a calibration graph**

  You can display the calibration graphs for a particular calibration type as follows:

  1. Choose **Calibration from file** from the Calibrate **Process** menu.

  2. Select Static or Dynamic scan, or Lag time calibration for MS1 or MS2 (on a triple quad instrument).

  3. Press the **OK** button.

- **Making a calibration from a data file**

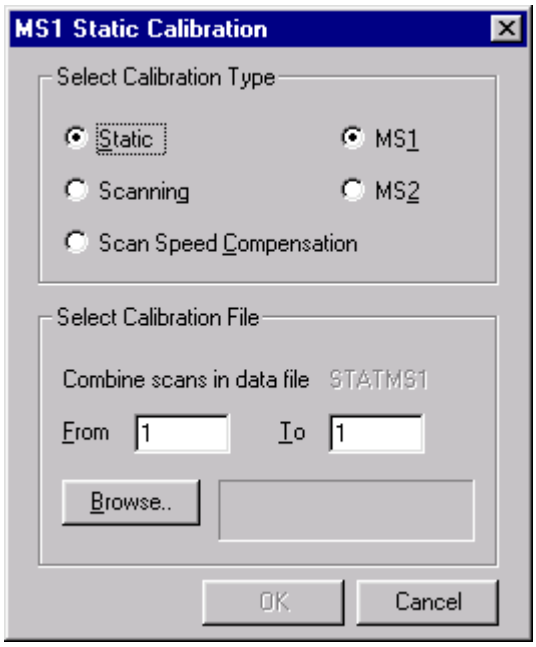

Figure 4.10 *MS1 Scanning Calibration dialog*

1. Choose **Calibration from file** from the Calibrate **Process** menu.

2. Select Static, Scanning or Scan Speed Compensation calibration for MS1 or MS2 (on a triple quad instrument).

3. Press the **Browse** button, and then select a file from the list displayed.

4. Optionally, enter a range of scans to combine **From** and **To**.

5. Press **OK**.

Editing a Reference File

Figure 4.11 shows the contents of the file Heptacos.ref which is the standard reference for EI calibration. 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 peak intensities are not at present used by the calibration software and so can be set to a nominal value of 100. However, you may wish to enter realistic values here to improve the appearance of the reference spectra.

```
49.99379        0.73
68.99518        100.00
92.99518        0.56
99.99358        6.31
113.99669       2.34
118.99199       7.80
130.99199       36.31
149.99039       1.29
163.99350       0.56
168.98877       3.24
175.99350       0.91
180.98877       1.31
213.99030       0.76
218.98560       38.07
225.99030       0.56
230.98560       0.47
263.98705       8.38
313.98386       0.32
613.96471       0.64
```

Figure 4.11 *Heptacos.ref 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, choose **Reference File** from the **Calibration Edit** menu.

After editing, the reference file can either be saved under the current name by selecting **Save** from the Notepad **File** menu or saved as a new reference file by selecting **Save as** from the Notepad **File** menu and giving the file 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 ; character.

Saving and Restoring Calibrations

The complete instrument calibration can be saved to disk as a named file and then recalled at a future date. Static, dynamic and lag time calibrations are all saved together under a common name.

You may find it useful to calibrate the instrument for each of the different types of experiment that you do and save these calibrations to disk. This means that when you switch between experiments you can restore a suitable calibration from disk rather than having to recalibrate from scratch.

To save a named calibration

1. Choose **Save Calibration As** from the Calibration **File** menu.

2. Enter a new file name under which you want the parameters to be saved.

3. Press the **Save** button. If the selected file already exists on disk you will be asked to confirm that you want to overwrite the existing information. Press the **OK** button to continue or the **Cancel** button to enter a different file name.

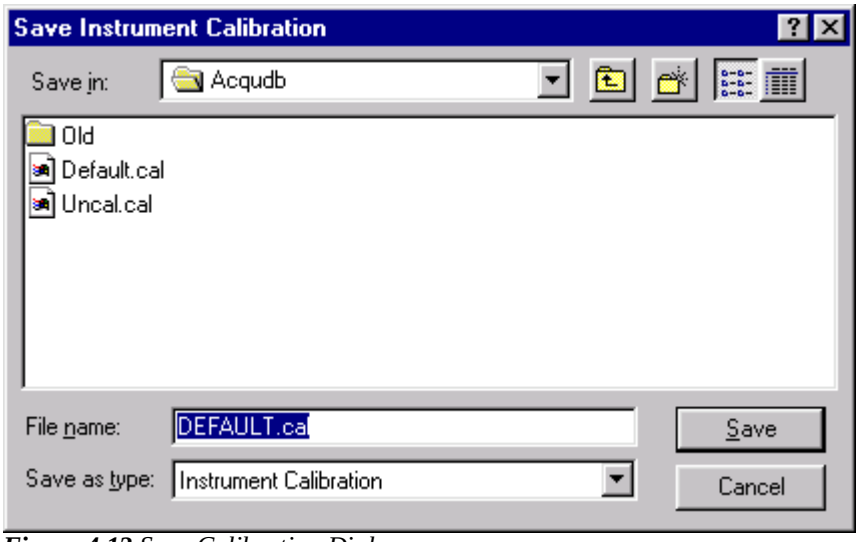

Figure 4.12 *Save Calibration Dialog*

To restore a saved calibration

1. Choose **Load Calibration** from the Tune **Fil**e menu.

2. Select the calibration file required, either by typing its name or by selecting it from the displayed list.

3. Press the **Open** button.

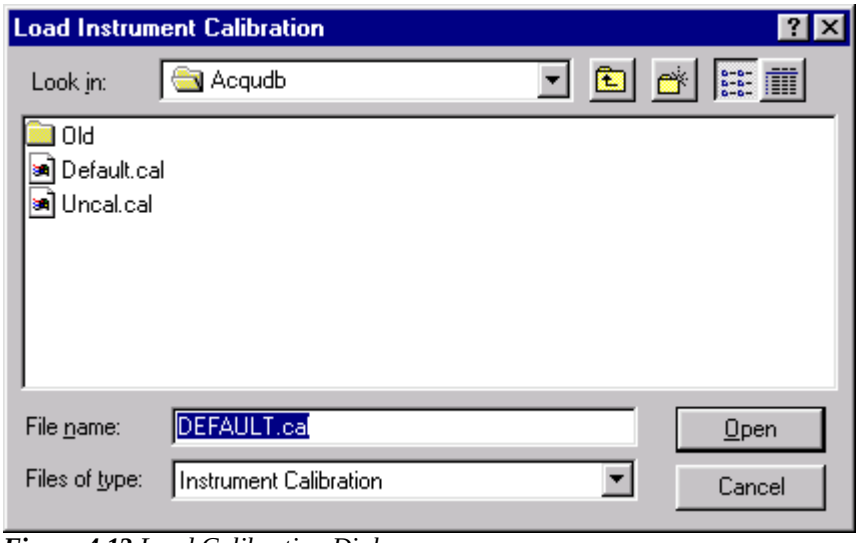

Figure 4.13 *Load Calibration Dialog*

Notes

Notes

The Function List Editor

Chapter 5

Introduction

The Function List Editor is used to set up the function(s) that the mass spectrometer will use 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 and the ability to switch MS/MS scan modes during an acquisition.

A function list is produced, saved on disk and then referenced by name when you start an acquisition. **Figure 5.1** shows a simple function list that contains only one function: a centroided mode full scan, between 50 and 550 amu using EI ionization. Immediately above the function bar display is a time scale that shows when the function will be active from and for how long it will run. 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 the  toolbar button on the MassLynx Screen or select **Mass Spectrometer** from the **Methods** menu on the **Acquisition Control Panel**.

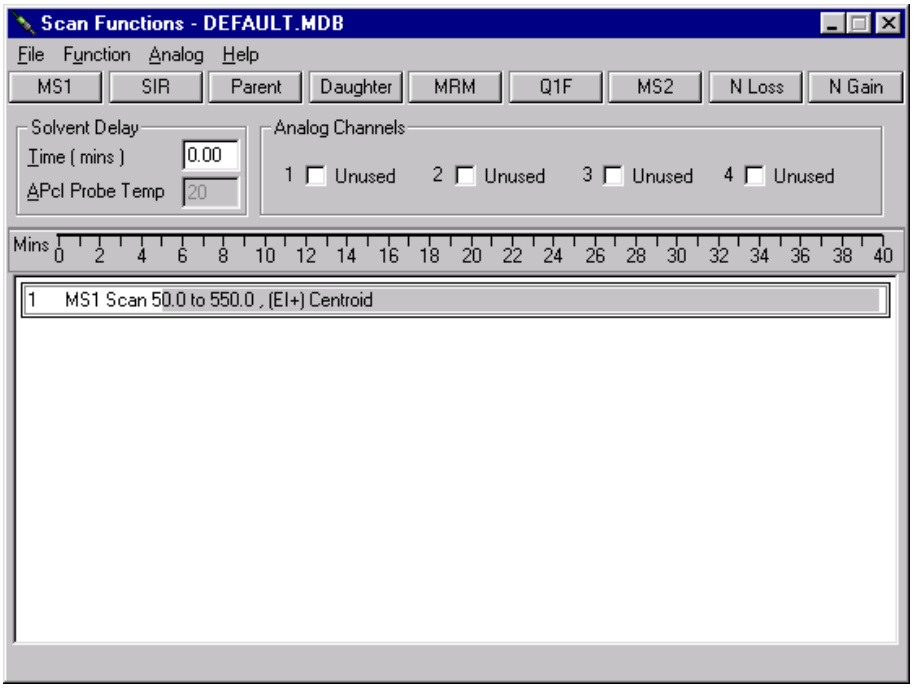

Figure 5.1 *Function list showing a single function*

The currently selected function is enclosed in a rectangular frame. If the display shows more than one function you can select a new function either by clicking on it with the mouse, or by moving to it using the **Up** or **Down** Arrow keys on the keyboard.

Figure 5.2 shows a more complicated function list, which has four SIR functions, running sequentially for 10 minutes each.

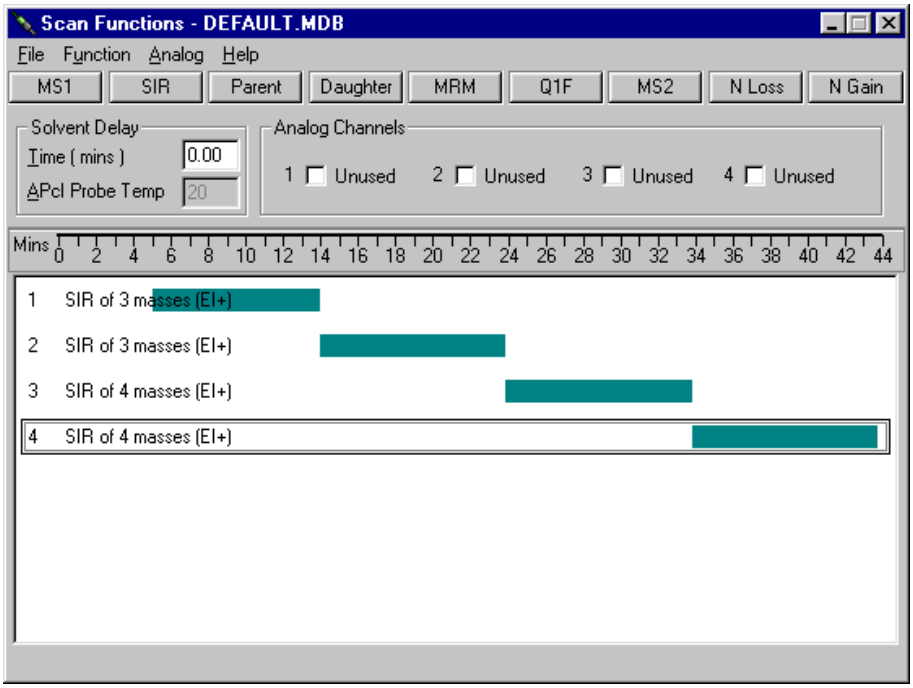

***Figure 5.2** Function list showing four SIR functions*

Adding a New Function

A new function can be added either by clicking on one of the function buttons at the top of the editor, or by selecting **Add** from the **Function** menu and choosing the appropriate function. The editor for the function type selected will be displayed showing default values. Make any changes required to the parameters and press the **OK** button to add the new function. The function editors for each scan type will be discussed in detail later on in this chapter.

Modifying an Existing Function

An existing function can be modified in one of two ways. You can either choose **Edit Function** from the **Function** menu or you can double click on the function in the function list. This will bring up the appropriate editor for the function type and allow you to change the function information. When you have finished editing the function, the function list display will be updated to show any changes.

Removing a Function

You can remove a function by highlighting it and then choosing **Delete Function** from the **Function** menu. Alternatively you can press the **Delete key** on the keyboard. You will be asked to confirm that you really want to delete the function, press the **Yes** button.

Changing a Function's Start and End Times

You can change the start and end times of a function either by going into its editor as described above, or by picking the times off a trace displayed in Chromatogram.

- **To set a function time from Chromatogram**

1. In the function list editor, select the function that you want to set.

2. Click with the right mouse button and drag over the required retention window on any chromatogram trace in the Chromatogram window. The current function bar will now update to show the new start and end times.

Setting a Solvent Delay

A solvent delay can be set for a function list using the **Solvent Delay Time** control. No data is stored during the solvent delay period, which means that solvent peaks that would normally be seen eluting at this time on the TIC chromatogram of the acquired data will no longer be seen. The filament in the source is turned off during the solvent delay to prevent it from being damaged. For APcI functions the APcI probe temperature will be set to the value specified in the **APcI Probe Temp** control for the period of the solvent delay.

Analog Channels

If an analog channels hardware option is fitted to your instrument, you can acquire up to 4 channels of analog data which will be stored with the data acquired from the mass spectrometer. Analog channels will typically be used to collect data from external units such as UV detectors or FID detectors. 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 Channel Desc** from the **Analog** menu on the **Scan Functions** dialog.

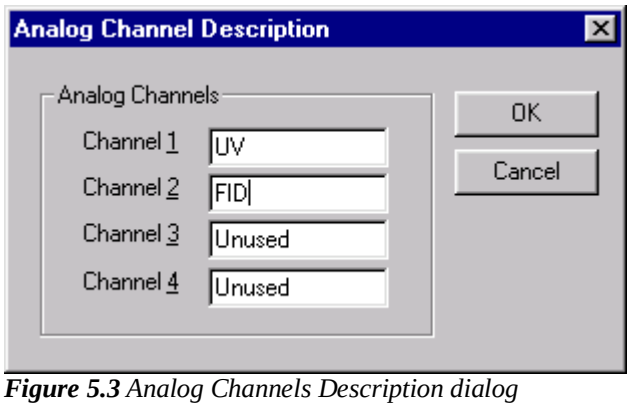

Figure 5.3 *Analog Channels Description dialog*

- **To store data for an analog channel**

1. Check the boxes required in the **Analogue Channels** part of the function editor.

2. Choose **Analog Channel Desc...** from the **Function List Analog** menu.

3. Enter a textual description for each of the analog channels that you have chosen to store. This description will be used on the Analog Chromatogram dialog as the Channel description. (See the MassLynx User's Guide, Chromatogram chapter).

4. Press the **OK** button.

Saving and Restoring a Function List

- **To save a function list**

1. Choose **Save As** from the Function List **File** menu, to bring up the Save File As dialog.

2. Enter a new File Name under which you want the function list to be saved, or alternatively select an already existing file from the list displayed.

3. Press the **Save** button. If the selected file already exists on disk you will be asked to confirm that you want to overwrite the existing information. Press the **Save** button to continue, or **Cancel** and select a different name.

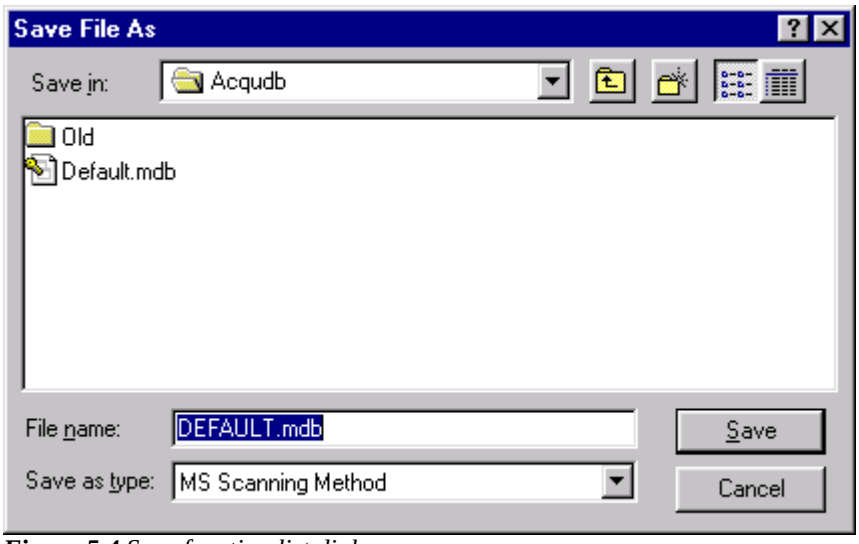

Figure 5.4 *Save function list dialog*

The current function list, i.e., the function list that is on display in the function list editor, is saved automatically to disk when the function list editor is closed. Therefore it is not necessary to save the function list manually before closing the window. The same function list will be automatically loaded when the function list editor is next accessed.

To restore a saved function list

1. Choose **Open** from the Function List **File** menu to bring up a dialog similar to the one shown below.

2. Select the File Name of the function list that you would like to use, either by typing its name or by selecting it from the displayed list.

3. Press the **Open** button.

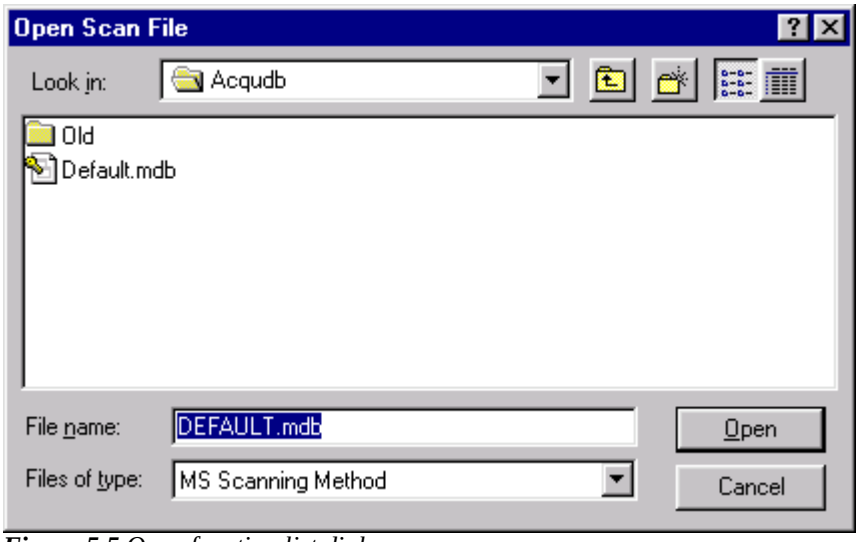

Figure 5.5 *Open function list dialog*

Setting up a Full Scan Function

The full scan function editor is used to set up centroid, continuum and MCA functions. It is activated by clicking on the MS1 function button.

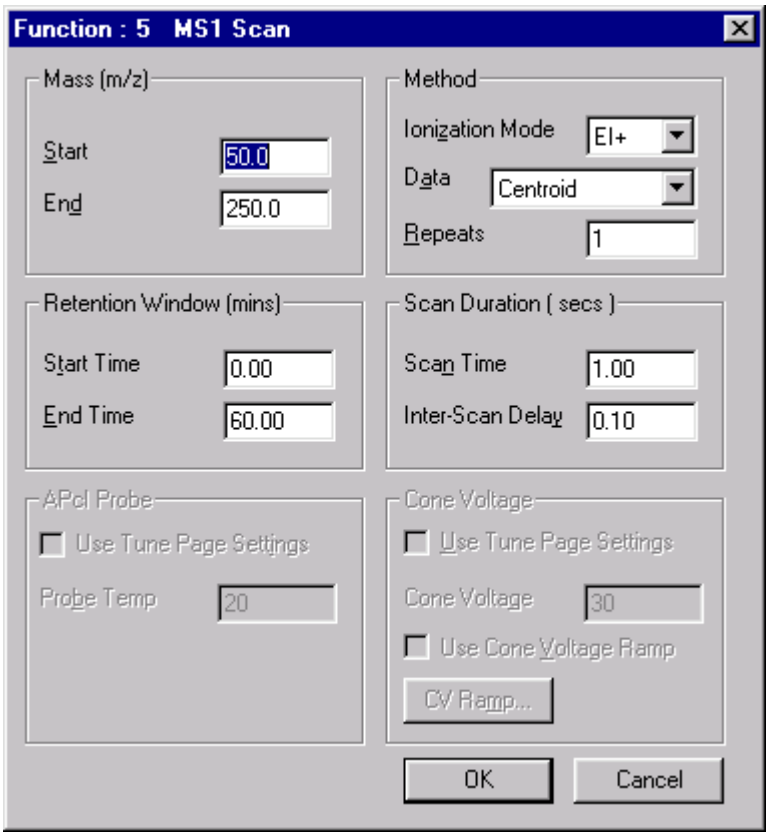

Figure 5.6 *Full scan editor*

Start Mass Specifies the mass at which the scan will start. The Start Mass must be lower than the End Mass.

End Mass Specifies the mass at which the scan will stop. Both the start and end masses can be selected directly from a spectrum displayed in the Spectrum window. If you click, drag and release on a spectrum using the right hand mouse button, the selected mass range will be entered as the start and end mass values.

Ionization Mode Specifies the ionization mode and polarity that will be used during the acquisition.

Data Specifies the type of data to be collected and stored on disk.

Centroid - Stores data as centroided, intensity and mass assigned peaks. Data is stored for every scan.

Continuum - Data is not centroided into peaks. Instead the signal received by the interface electronics is stored regularly to give an analog intensity picture of the data being acquired. Data is stored for every scan.

Due to the fact that data is being acquired to disk at all times, even when there are no peaks being acquired, continuum data acquisition places some extra burden on the acquisition system as compared to centroided acquisition. Data file sizes will tend to be significantly larger than centroided ones and the absolute scanning speed (amu / sec) will be slower. It is possible however to set a threshold below which the data will not be stored to disk, which can greatly reduce these effects, depending on the nature of the data being acquired. 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 "Setting Instrument Data Thresholds in Chapter 2.

***Multi Channel Analysis (MCA)* -** MCA data can be thought of as 'summed continuum', with only one intensity accumulated scan being stored to disk 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 will not accumulate so rapidly as real data and, therefore will effectively average out over a number of scans. This will emphasise the real data and improve signal to noise. 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.

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

Retention Window Start Time Specifies the retention time in minutes at which this function will become active; i.e., data acquired and stored.

Retention Window End Time Specifies the retention time in minutes at which this function will cease to be active; i.e., data acquired and stored.

Scan Time Specifies the duration of each scan in seconds.

Inter-Scan Delay Specifies the time in seconds between a scan finishing and the next one starting. During this period no data is stored.

APcI Probe Temp Specifies the APcI probe temperature in degrees centigrade. The APcI probe temperature control is enabled when the ionization mode is set to APcI.

Use Tune Page Settings When this control is selected the APcI probe temperature set on the tune page at the start of the acquisition is used. This control is enabled when the ionization mode is set to APcI. The APcI probe temperature value cannot be altered by typing new values into tune page during the acquisition since the new values will not be downloaded during the acquisition. If you need to alter the APcI probe temperature by typing new values into tune page during the acquisition you should acquire from the tune page.

Cone Voltage Specifies the cone voltage value in volts. The Cone Voltage control is enabled when the ionization mode is set to ESP or to APcI.

Use Tune Page Settings When this control is selected the cone voltage set on the tune page at the start of the acquisition is used. This control is enabled when the ionization mode is set to ESP or to APcI. The cone voltage value cannot be altered by typing new values into tune page during the acquisition since the new values will not be downloaded during the acquisition. If you need to alter the cone voltage by typing new values into tune page during the acquisition you should acquire from the tune page.

Cone Voltage Ramp To apply a ramp to the cone voltage check the **Use Cone Voltage Ramp** control and press the **CV Ramp...** button to load the Cone Ramp dialog.

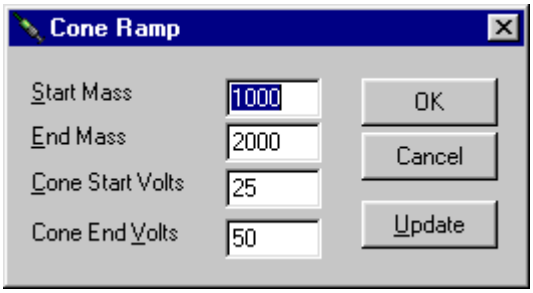

Figure 5.7 *Cone Ramp dialog*

Two values of cone voltage are defined at two particular masses using the **Start** and **End Mass** controls and the **Cone Start Volts** and **Cone End Volts** controls. These values define a gradient for the cone voltage which is then extrapolated to cover the full mass range of the function.

Setting up an SIR Function

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

The SIR editor is used to enter the masses that you would like to monitor and their respective dwell time, span and inter-channel delay time.

To set up an SIR function

Press the **SIR** button on the **Scan Functions** dialog.

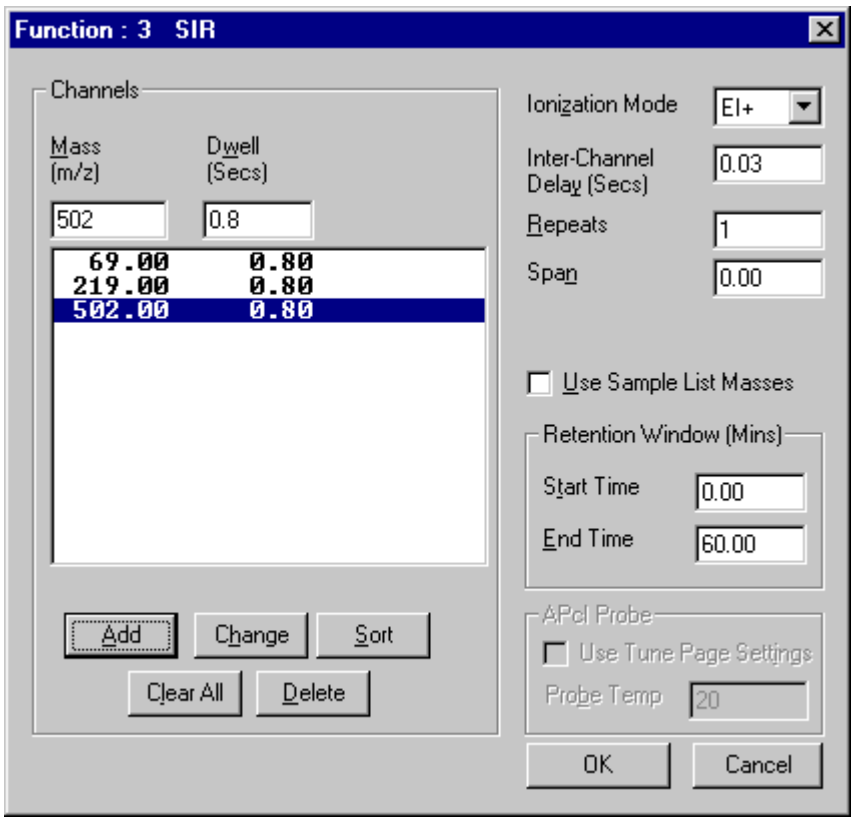

Figure 5.8 *SIR editor*

Most of the fields are the same as those described for the full scan editor (see "Setting up a full scan function" on page 66). However the following are different:

Mass Specifies the mass to be monitored; up to a maximum of 32. A mass can be entered either by typing its value into the **Mass** box and pressing Enter or the **Add** button, or by pulling them directly from a spectrum displayed in the Spectrum window. To do this, display the required spectrum in the Spectrum window and then select the ions that you wish to monitor with a single click of the right hand mouse button. As you select a mass it will appear in the SIR masses table.

Masses can also be taken directly from the sample list. Check the **Use Sample List Masses** box, and enter sample list column heading for the required masses (mass_a to mass_t) then press the **Add** button. Note the column heading (MASS_A, MASS_B etc) will appear in this dialog rather than the actual masses listed in the sample list.

Dwell Specifies the length of time in seconds for which the highlighted mass will be monitored.

Inter Channel Delay Specifies the length of time in seconds between finishing monitoring the highlighted mass and starting monitoring the next mass in the function.

Span Specifies a small mass window applied centrally about the highlighted mass. During acquisition this range will be scanned over the specified "Dwell" time. A span of zero can be set to simply 'sit on' the specified mass.

APcI Probe Temp Specifies the APcI probe temperature in degrees centigrade. The APcI probe temperature control is enabled when the ionization mode is set to APcI.

Use Tune Page Settings When this control is selected the APcI probe temperature set on the tune page at the start of the acquisition is used. This control is enabled when the ionization mode is set to APcI.

Add Type values into the Mass and Dwell and then press this button to add the new Mass to the list.

Change Click with the left mouse button on a mass on the list and press this button to change the Mass or Dwell.

Sort Press this button to sort the list in order of ascending Mass.

Clear All Press this button to delete the list of Masses.

Delete Click with the left mouse button on a mass on the list and press this button to delete a single Mass.

Setting up an MS/MS Function

The MS/MS editor is used to acquire scan data using the MS2 region of a triple quadrupole mass spectrometer. If you have a single stage quadrupole instrument you will not be able to access this dialog box as it would be inappropriate to do so. The following scan functions are available, and for more information you should refer to the *Instrument Users Guide*.

Daughter This is the most commonly used MS/MS mode and is used to look at fragmentations of a particular ion. Quadrupole 1 is used to select the parent mass, and is not scanned. The mass for Quadrupole 1 is inserted into the Parent mass edit window. As an operational note, the resolution of Quadrupole 1 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. The abundance sensitivity of the quadrupole is such that the number of ions that are transmitted at adjacent masses under these conditions is very small indeed.

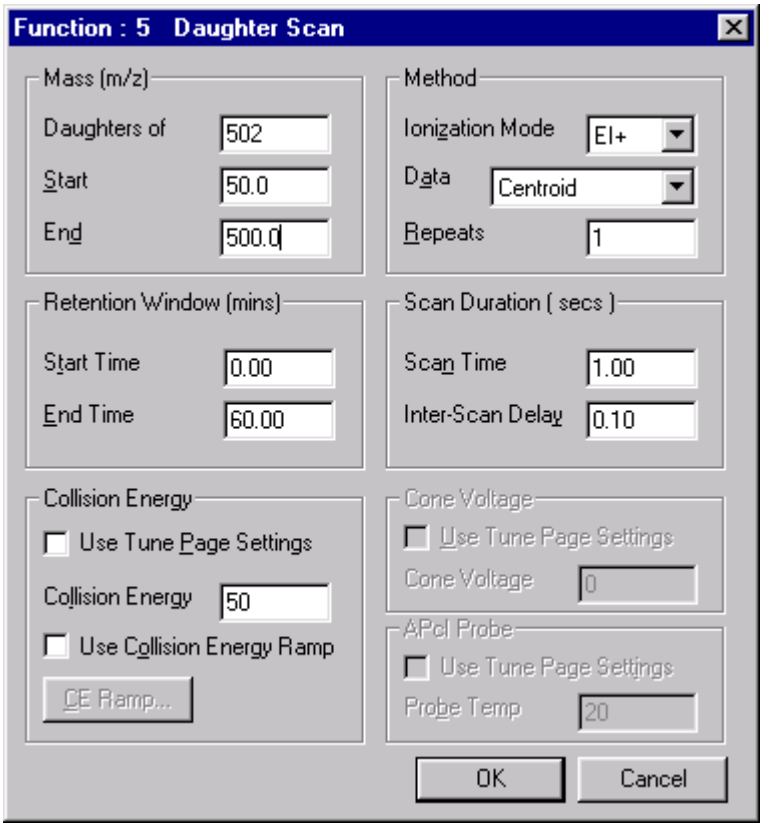

Figure 5.9 *MS/MS editor for daughter scan*

The Start and End masses refer to the mass range to be scanned by Quadrupole 2. Note that 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 will be detected. This occurs when a multiply charged ion fragments and loses a charge, but only a fraction of the mass.

Parent This mode is used to look for the parent of a particular fragment. Quadrupole 2 is set on the fragment, and is not scanned. The set mass that is entered in this case is the mass of the daughter (fragment). The Start and End masses specify the mass range over which Quadrupole 1 will be scanned. The Start mass would normally be set to a mass just below the daughter being selected on Quadrupole 2, and the End mass to a value above the highest expected parent mass. Note that there are often several masses from which a daughter may come, so that any one fragment is derived from a number of different peaks.

The collision energy is the energy of the ions as they pass through the collision cell.

MS2 In this mode the ions are detected on the final detector. Quadrupole 2 has both RF and DC applied to the analyser rods, and is therefore resolving. Quadrupole 1 has no resolving DC potentials applied to the rods, and therefore transmits ions over a wide mass range. The resolution, ion energy and ion energy ramp of MS2 will control the beam shape. It has to be remembered that the ion energy (and ramp) of Quadrupole 1 will also affect the transmission in this mode, as will lenses 5 and 6, which are normally associated with Quadrupole 1. While this scanning mode can be used for acquiring data onto disk it is mostly used in the tune window, for setting and optimising the acquisition conditions. The Start and End masses specify the mass range over which Quadrupole 2 will be scanned.

Q1F This mode functions in a similar fashion to MS2 mode from the operators point of view. In this case however the resolving quadrupole is Quadrupole 1, with Quadrupole 2 not resolving and RF applied only to the analysing rods. This little used mode, is useful for fault finding.

Neutral Loss In this mode, the peak in a spectrum that will give the neutral loss specified is detected. The precursor mass is scanned in Quadrupole 1, and Quadrupole 2 is scanned at this mass less the ***neutral loss*** mass. Starting masses are therefore detected on the mass scale of MS1. It should be remembered that the Start mass entered (for Quadrupole 1) should be greater than the neutral loss value to give Quadrupole 2 a valid start mass.

Neutral Gain This is an infrequently used mode, since the mass selected by Quadrupole 2 is higher than that of Quadrupole 1 by the ***neutral gain*** mass. It is applicable to studies where a precursor ion gains mass by ion molecule reaction. It also applies to fragment ions of multiply charged ions that fragment into particles with a higher value of mass to charge.

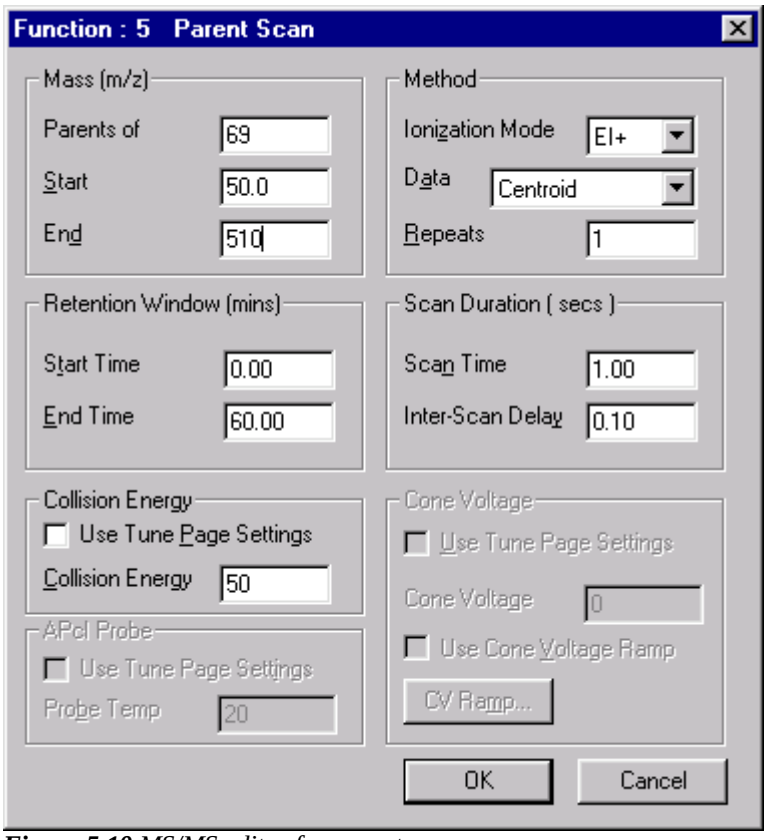

Figure 5.10 *MS/MS editor for parent scan*

The fields in the MS/MS editor are mainly the same as the ones in the full scan editor with the following differences.

Mass Specifies the mass (i.e. Daughter Mass, Parent Mass etc.) that will be used for the particular function type.

A single mass can be taken from the Spectrum window by selecting the appropriate mass on any graph with a single right button click of the mouse. The start and end masses are selected in a similar way but utilising a right button click-drag-release operation.

Collision Energy Specifies the collision energy in electron volts that will be used for the collision cell during the scan.

Use Tune Page Settings When this control is selected the collision energy set on the tune page is used. The collision energy value cannot be altered by typing new values into tune page during the acquisition since the new values will not be downloaded during the acquisition. If you need to alter the collision energy by typing new values into tune page during the acquisition you should acquire from the tune page.

Collision Energy Ramp To apply a ramp to the collision energy check the **Use Collision Energy Ramp** box, this enables the **CE Ramp…** button. Press this button to load the Collision Energy Ramp dialog.

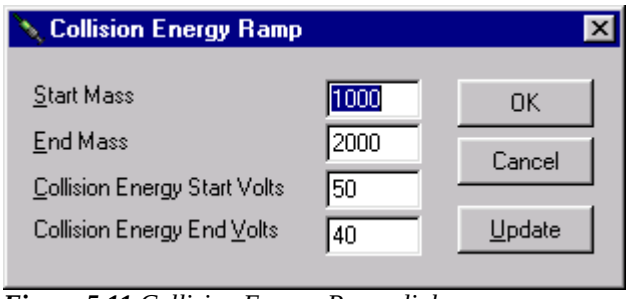

Figure 5.11 *Collision Energy Ramp dialog*

Two values of collision energy are defined at two particular masses using the **Start** and **End Mass** controls and the **Collision Energy Start Volts** and **Collision Energy End Volts** controls. These values define a gradient for the collision energy which is then extrapolated to cover the full mass range of the function.

Setting up an MRM Function

Multiple Reaction Monitoring (MRM) functions are only appropriate to instruments equipped to do MS/MS.

MRM functions are set up much in the same way as SIR functions but allow you to monitor a number of MS/MS transitions (fragmentations) between MS1 and MS2.

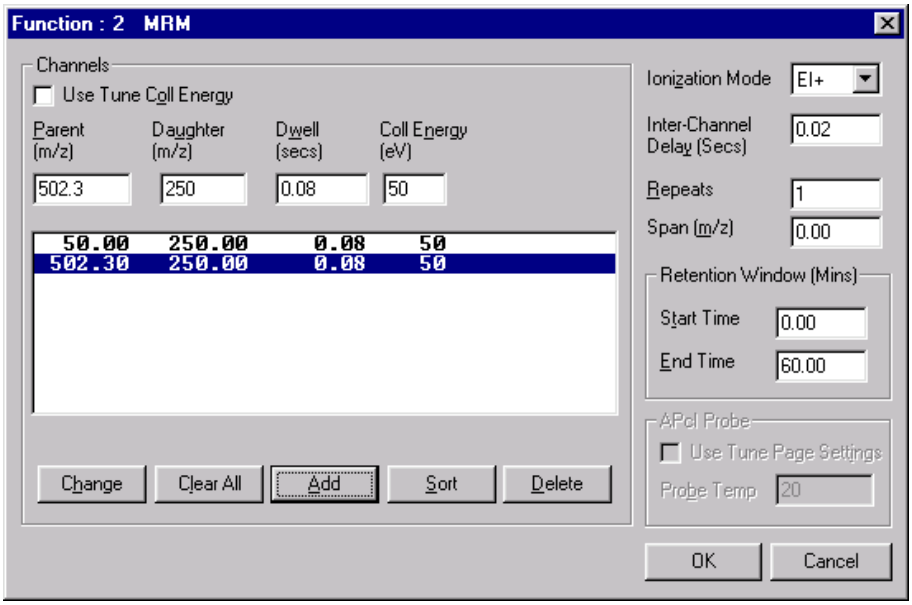

Figure 5.12 *MS/MS editor for MRM function*

Many of the fields in the MRM editor are the same as those in the SIR editor. There are however some differences.

Parent & Daughter Masses Specify the transition between MS1 and MS2. A mass can be entered either by typing its value into the edit box at the top of the list and pressing Enter or the **Add** button, or by pulling them directly from a spectrum displayed in the Spectrum window. To do this position the cursor in the Parent box, then click with the right mouse button on the ion in the Spectrum window. Repeat for the Daughter mass. The mass will be entered in the Parent and Daughter fields of the MRM editor.

Coll Energy Specifies the collision energy in electron volts that will be used for the collision cell during the scan.

Q-Tof Function Editing

Q-Tof functions are set up in the same way as for other machines. The differences are that they perform MS, MSMS and Survey scans, and only ESP and APcI ion modes are available.

For MS and MSMS functions the Function editor is the same as for other machines with the addition of one field **Scans/Spectrum**. This field indicates the number of MCA scans that are summed for each spectrum.

Survey Functions

Note: The function list editor will not add Survey functions to the list if non-survey functions are present. If there are functions displayed in the Function List Editor then delete them before attempting to add a survey function.

An MS Survey function is used to select suitable precursor ions for further MSMS analysis.

When the acquisition is performed the MS Survey function will be executed first. The software examines the data in each spectrum, as it is acquired, and when a precursor mass of interest is detected an MSMS function based on the precursor mass is generated and executed.

As the MSMS function is executed, the software examines the data in each spectrum for each function, as it is acquired. If the precursor mass is no longer of interest then execution of the MSMS function will be terminated. When all MSMS functions for the current precursor mass have been completed the software will resume execution of the original MS Survey function, until another precursor mass is detected when it will execute the MSMS function again.

This switching will be repeated until the acquisition is complete.

To access the MS Survey Scan dialog press the **Survey** button in the Scan Functions editor.

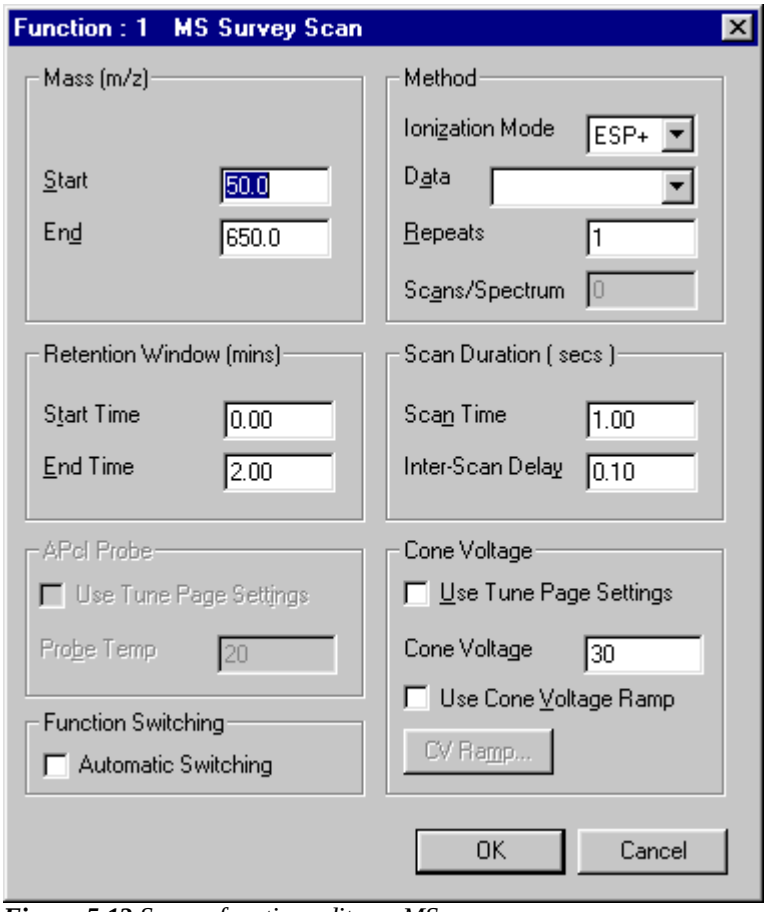

Figure 5.13 *Survey function editor – MS page*

Survey scans are used to search for precursor ions. If automatic function switching is required check the **Automatic Switching** box, otherwise manual 'on the fly' switching can be carried out.

Enter MS details as required. When the **OK** button is pressed the MSMS scan dialog is displayed.

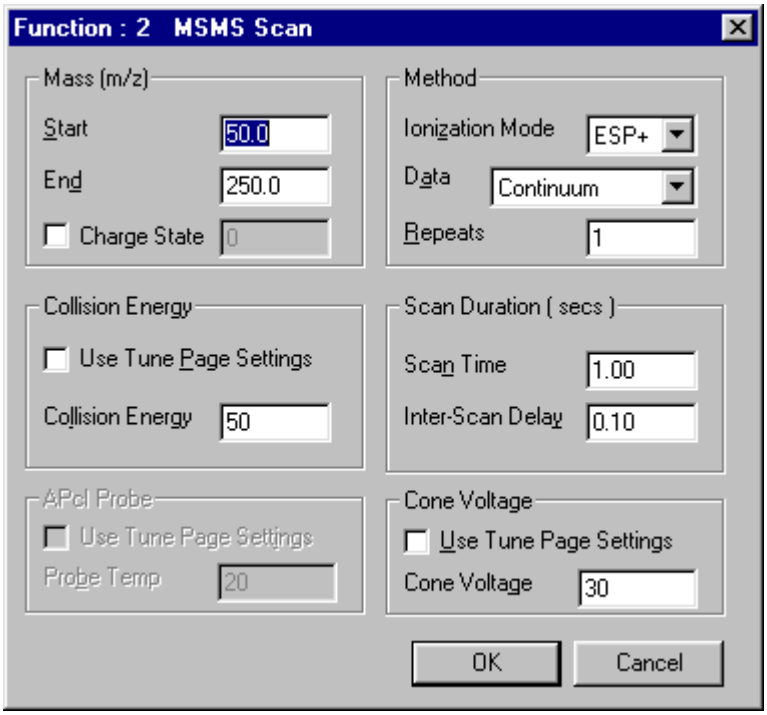

Figure 5.14 *Survey function editor – MSMS page*

Enter the MSMS details as required and press **OK**. Two functions will be added to the function list as in Figure 5.15.

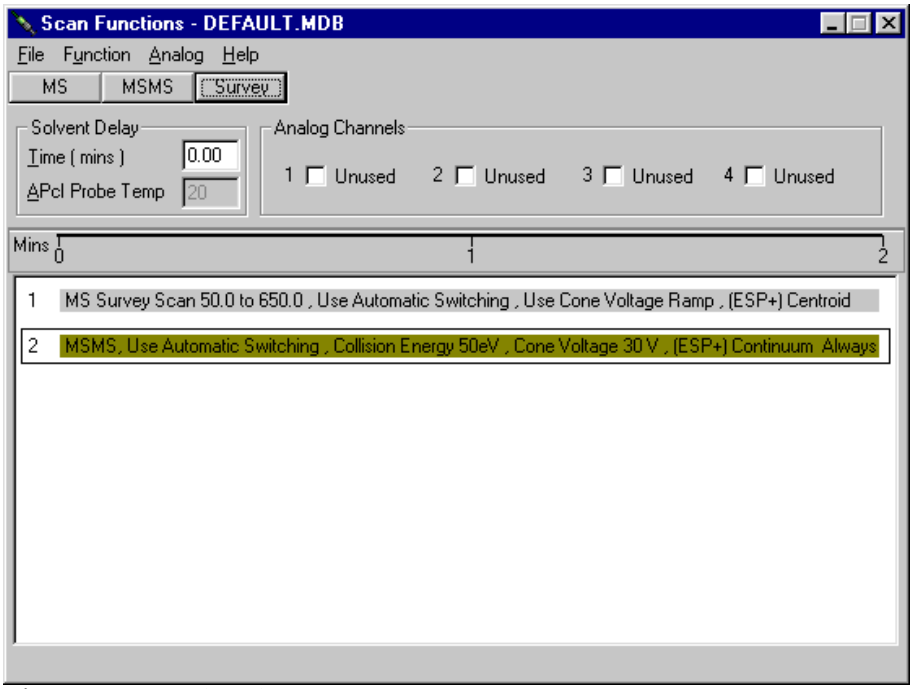

Figure 5.15 *Function List*

Automatic Function Switching

To set up Automatic Function Switching parameters select **Automatic Switching Setup** from the **Function** menu.

MS to MSMS Page

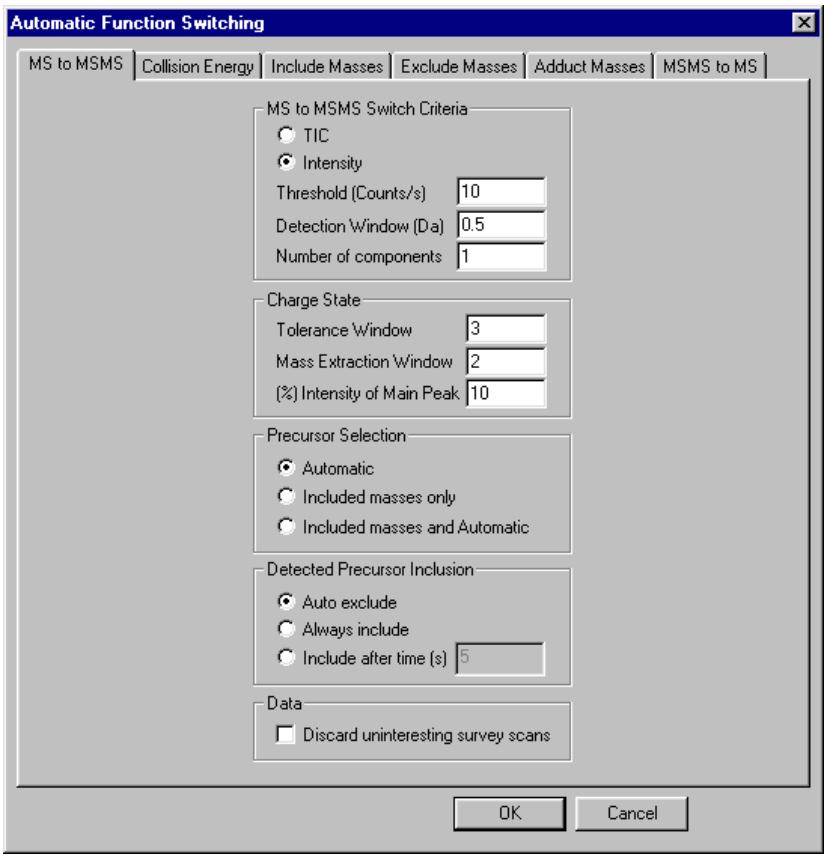

Figure 5.16 *MS to MS/MS Switching page*

The MS function will stop when the MS to MSMS Switch criteria are met.

MS to MSMS Switch Criteria

TIC If this option is selected, and the TIC of the spectrum rises above the specified **Threshold**.

Intensity If this option is selected, and the intensity of the largest peak rises above the specified **Threshold**.

Detection Window When a peak top is found no other peaks will be looked for in the specified window.

Number of components It is possible to carry out automated MSMS on up to 8 coeluting precursors, enter a value between 1 and 8 in this box.

The number of non coeluting precursors in a single run is not limited.

Charge State

Tolerance Window Do not change this from the default value of 3.

Mass Extraction Window The data for the masses within this window around a detected precursor is centroided. If the intensity of the main peak is above the **(%) Intensity of Main Peak** it is selected.

A mass is valid if it is not on the exclude list, and it satisfies the precursor selection criteria.

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 are monitored.
- If **Include masses and Automatic** is selected masses on the Include list will be given priority. If less than n precursors are found then other valid masses will be monitored (n = **Number of components**).

A mass is valid if it is not on the exclude list, and it satisfies the precursor selection criteria.

Detected Precursor Inclusion

- **Auto exclude** Do not use this precursor to switch to MSMS again.
- **Always Include** Use this precursor again for MSMS switching.
- **Include after time** Only use this precursor to switch to MSMS when the specified time period has elapsed after the precursor was last detected.

Data

Check the **Discard uninteresting survey scans** box to store only the survey scans that detect precursor ions and initiate MS switching. This saves on disk storage space as it does not save survey scans which do not contain relevant data.

Collision Energy Page

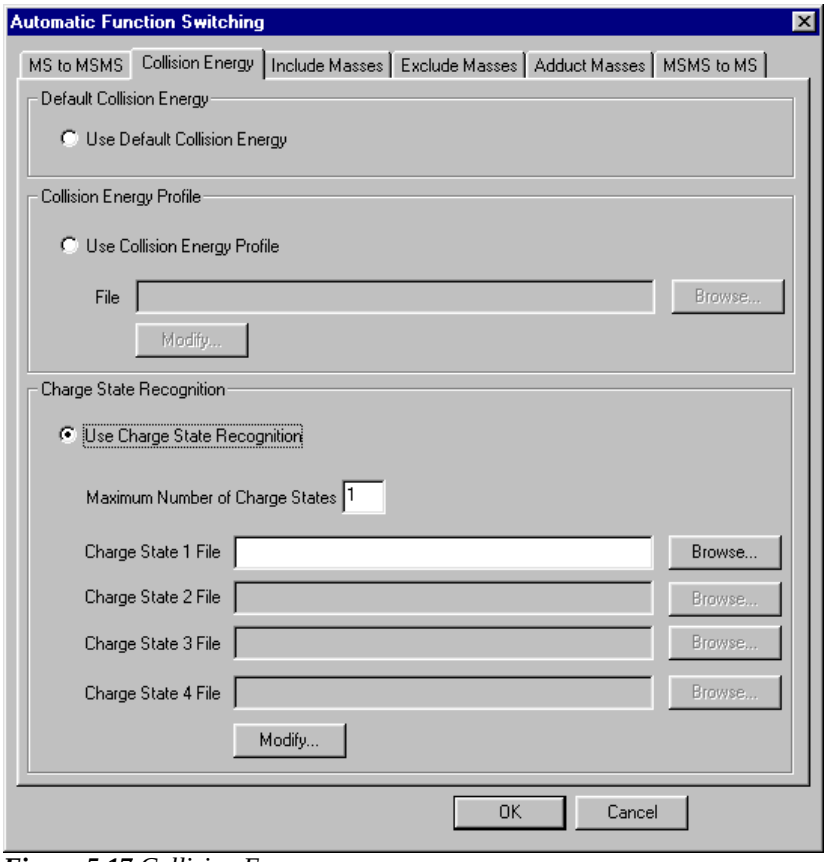

Figure 5.17 *Collision Energy page*

Use Default Collision Energy Select this option to use the collision energy defined on the MSMS Scan Function page (see **Figure 5.14**).

Use Collision Energy Profile Select this option to use a collision energy profile. This is a file which specifies a number of collision energies to use for defined masses. E.g. in **Figure 5.18** if a precursor is detected in the first 100 seconds and it's mass lies between 50 and 100, then perform scans at 20, 25, 30, 35 and 40 eV until the MSMS of the precursor is terminated.

To select a file press the **Browse** button and select the required file from the dialog displayed.

To change the details of a file or to create a new one press the **Modify** button. The **Modify CE Profile** dialog is displayed.

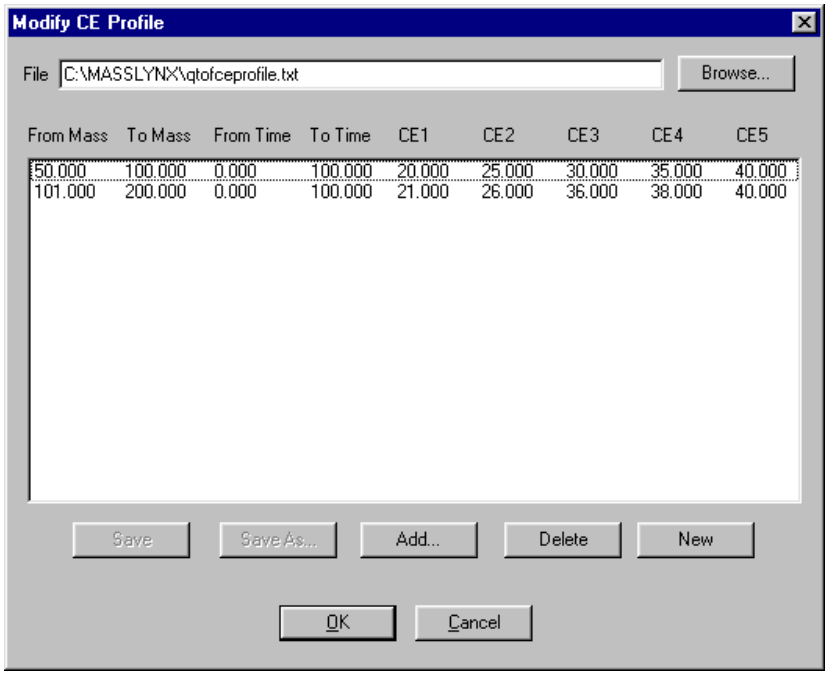

Figure 5.18 *Modify CE Profile dialog*

To create a new file, press the **New** button. Any previous details are cleared.

To add a profile, press the **Add** button. The **Collision Energy** dialog is displayed.

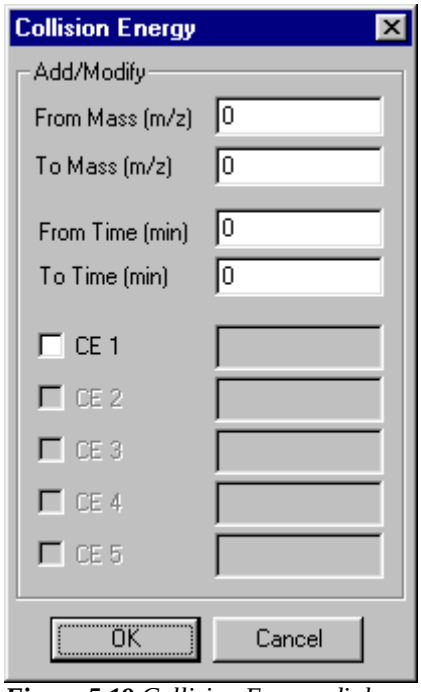

Figure 5.19 *Collision Energy dialog*

Enter the **From Mass**, **To Mass**, **From Time** and **To Time** required. Check the boxes for the **CE 1** to **CE 5** and enter the required collision energy. **Note**: all these values are optional.

To delete a profile click on it with the left mouse button and press the **Delete** key.

When all profiles have been defined press the **Save As** button, enter the name to save the file as and press **OK**. Pressing the **Save** button will save the file under the previous name.

Use Charge State Recognition Select this option when charge state recognition is being used to select masses.

Maximum number of Charge States Enter the number of charge states to monitor. Range 1 to 4.

To select a Charge State file press the **Browse** button and select the required file from the dialog displayed. The file contains a list of masses and collision energies. From this list a graph is constructed which calculates the values for the masses in between e.g. using **Figure 5.20** for a mass of 150 a collision energy of 15 eV would be used.

To change the details of a file or to create a new one press the **Modify** button. The **Modify Charge State** dialog is displayed. Click on the tab for the relevant page (Modify CS1, Modify CS2 etc).

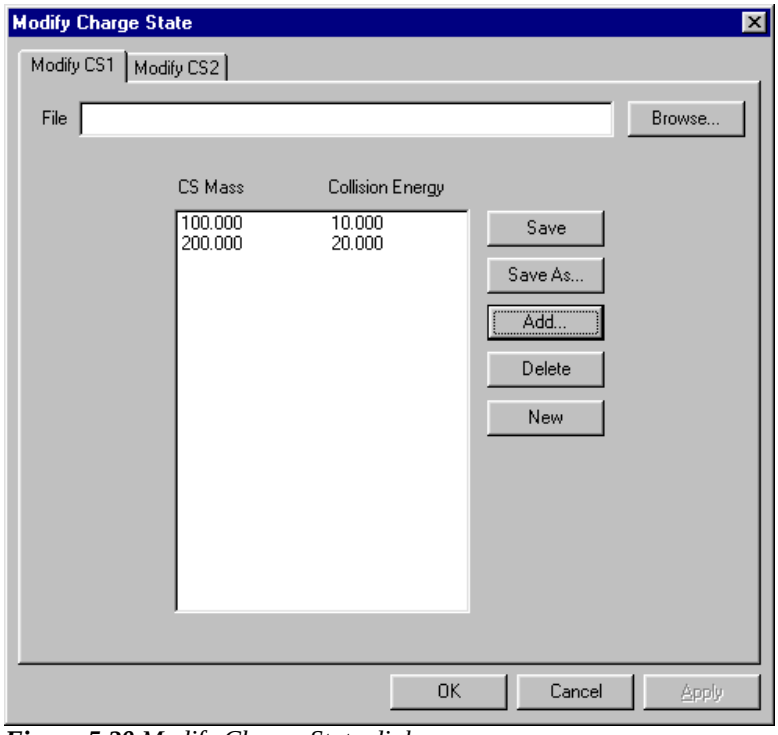

Figure 5.20 *Modify Charge State dialog*

To create a new file, press the **New** button. Any previous details are cleared.

To add a profile, press the **Add** button. The **Charge State Mass** dialog is displayed.

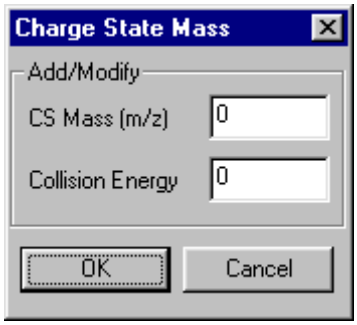

Figure 5.21 *Charge State Mass dialog*

Enter the **CS Mass** and **Collision Energy** required.

To delete an entry click on it with the left mouse button and press the **Delete** key.

When all entries have been defined press the **Save As** button, enter the name to save the file as and press **OK**. Pressing the **Save** button will save the file under the previous name.

- **Include Masses Page**

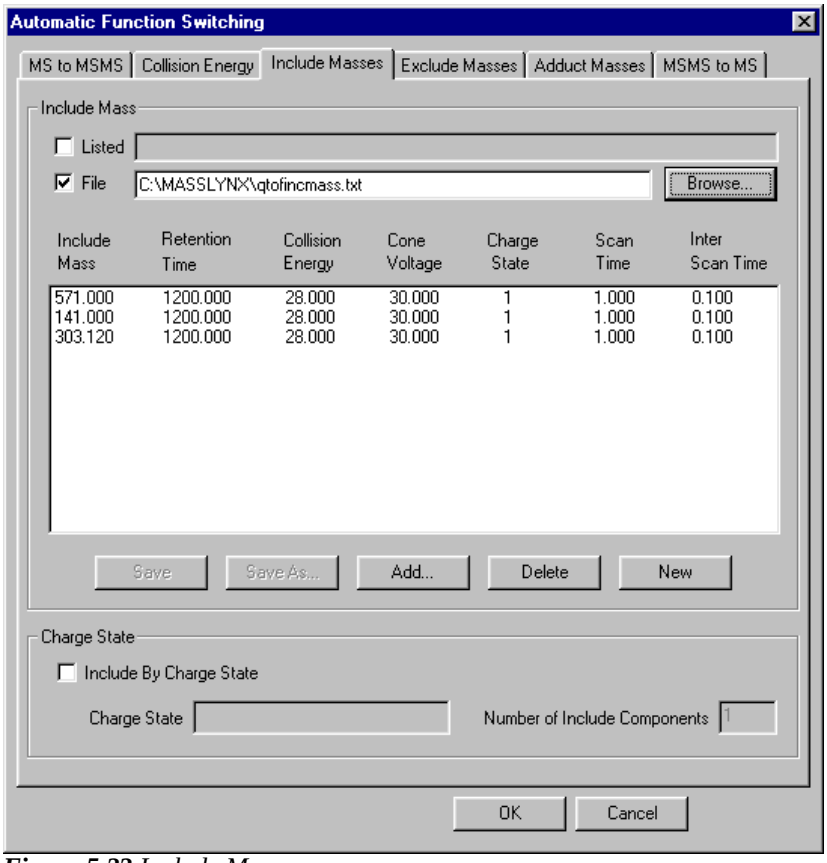

Figure 5.22 *Include Masses page*

Masses and ranges can be specified by checking the **Listed** box and entering the mass or range. Ranges take the form mass1_mass2 and can be comma delimited i.e. 100_200,250_300.

Masses can also be specified in a text file along with other criteria such as Retention Time or Charge State.

To use a previously defined file, check the **File** box, press the **Browse** button and select the text file from the dialog displayed.

To add a new mass press the **Add** button. This displays the Include Masses dialog.

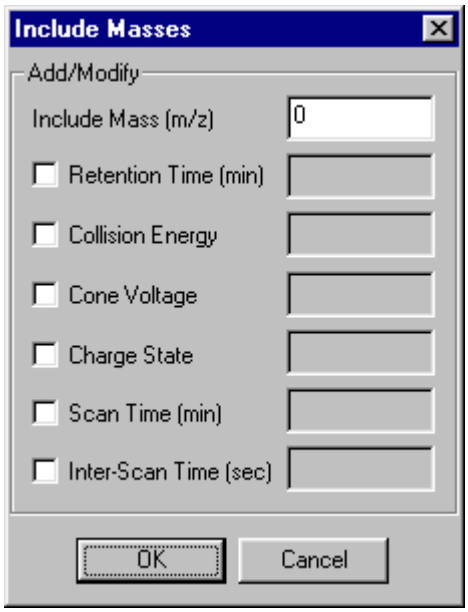

Figure 5.23 *Include Masses dialog*

Enter the **Include Mass** required. Check the boxes for any other values required and enter an appropriate value. **Note**: All these values are optional. E.g. If only the mass is entered then the mass will always be included. If a retention time is entered as well then the mass will only be included at the specified retention time.

To delete a mass click on it with the left mouse button and press the **Delete** key.

To delete all masses press the **New** button.

When all masses have been defined press the **Save As** button, enter the name to save the file as and press **OK**. Pressing the **Save** button will save the file under the previous name.

Charge State

Check the **Include By Charge State** box to include masses by their charge state and enter the **Charge State** and the **Number of Include Components**.

The **Number of Include Components** is the number of potential candidate precursors based on the MS to MS criteria, the include and exclude criteria and charge state that will be used for MSMS scanning.

If masses are defined in the **Listed** box or a **File** then the software checks the charge state and if it matches the value in the **Charge State** box then the mass is included.

Exclude Masses Page

If a mass is on the exclude list, it will not be considered for detection.

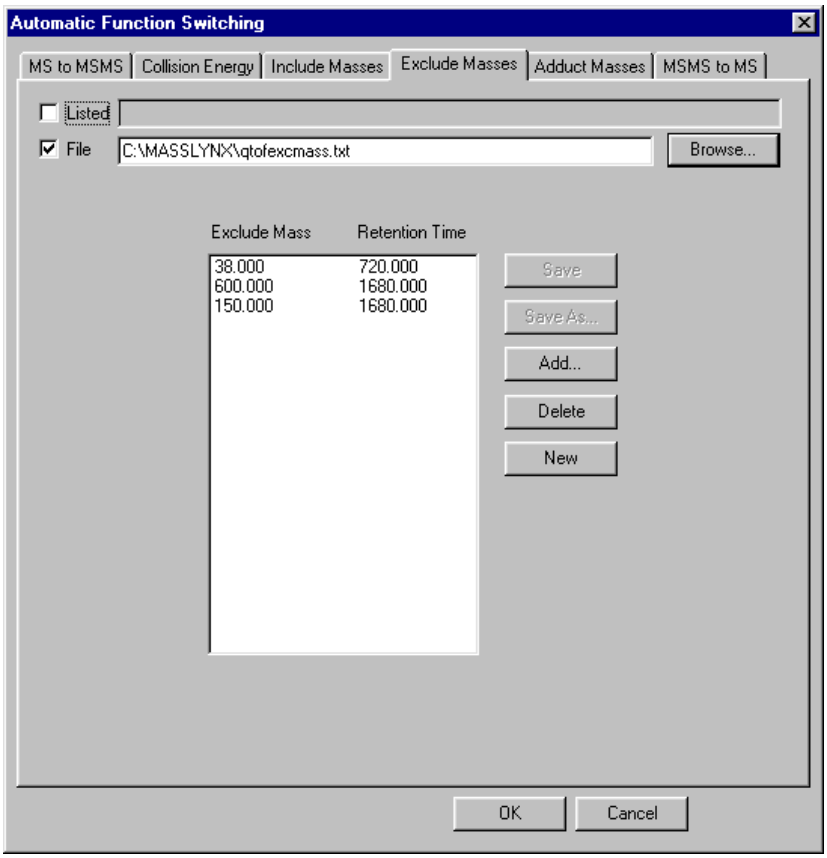

Figure 5.24 *Exclude Masses page*

Masses and ranges can be specified by checking the **Listed** box and entering the mass or range. Ranges take the form mass1_mass2 and can be comma delimited i.e. 100_200,250_300.

Masses can also be specified in a text file along with a Retention Time.

To use a previously defined file, check the **File** box, press the **Browse** button and select the text file from the dialog displayed.

To add a new mass press the **Add** button. This displays the Exclude Masses dialog.

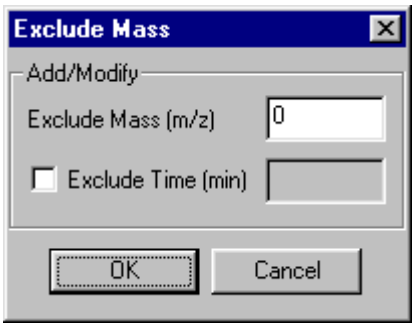

Figure 5.25 *Exclude Masses dialog*

Enter the **Exclude Mass** required. Optionally, check the **Exclude Time** box and enter an appropriate value. E.g. If only the mass is entered then the mass will always be excluded. If an exclude time is entered as well then the mass will only be excluded at the specified time.

To delete a mass click on it with the left mouse button and press the **Delete** key.

To delete all masses press the **New** button.

When all masses have been defined press the **Save As** button, enter the name to save the file as and press **OK**. Pressing the **Save** button will save the file under the previous name.

Adduct Masses Page

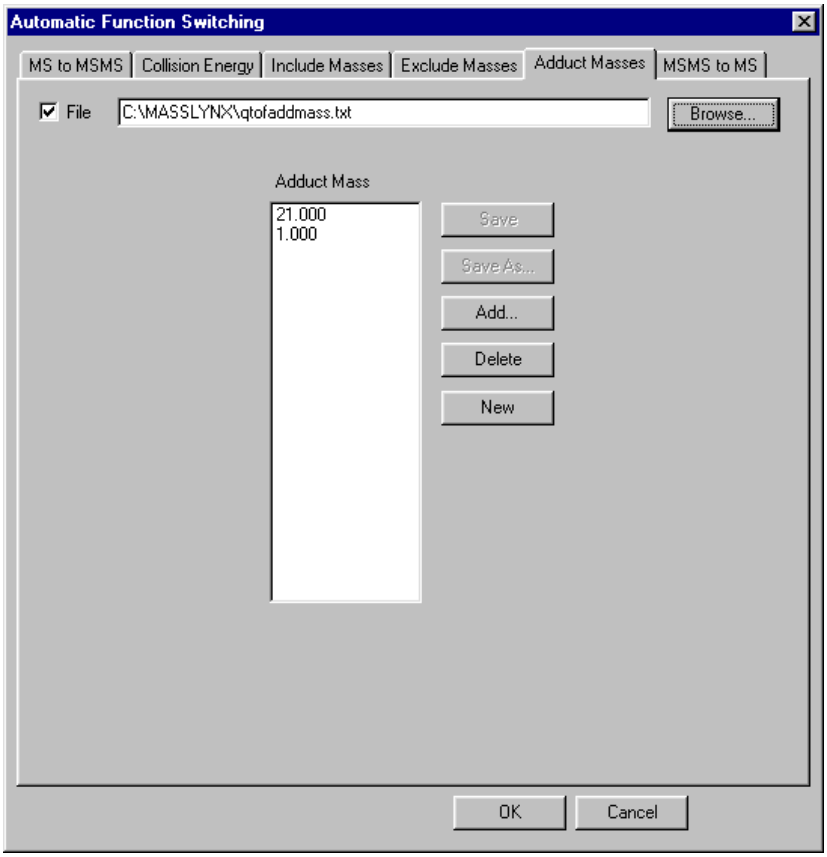

Figure 5.26 *Adduct Masses page*

Adduct Masses can be specified in a text file.

To use a previously defined file, check the **File** box, press the **Browse** button and select the text file from the dialog displayed.

To add a new mass, press the **Add** button. This displays the Include Masses dialog.

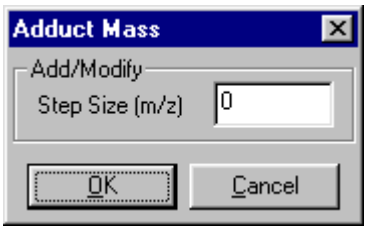

Figure 5.27 *Adduct Mass dialog*

Enter the Adduct mass in the **Step Size** box and press **OK**.

To delete a mass click on it with the left mouse button and press the **Delete** key.

To delete all masses press the **New** button.

When all masses have been defined press the **Save As** button, enter the name to save the file as and press **OK**. Pressing the **Save** button will save the file under the previous name.

If an adduct file is used and an include file is used, all adducts will be combined with all masses on the include list, together with their charge states, and the resulting masses added to the exclude list. E.g., if the include list contained the masses 100 and 150, both with a charge state of 2, and the adduct file contained the mass (Step Size) 21

The following masses would be added to the exclude list

100 + 21/2, 100 + 21, 150 + 21/2, 150 + 21

If an adduct file is specified, and a mass is being considered for switching, its adducted masses will automatically be excluded.

MSMS to MS Page

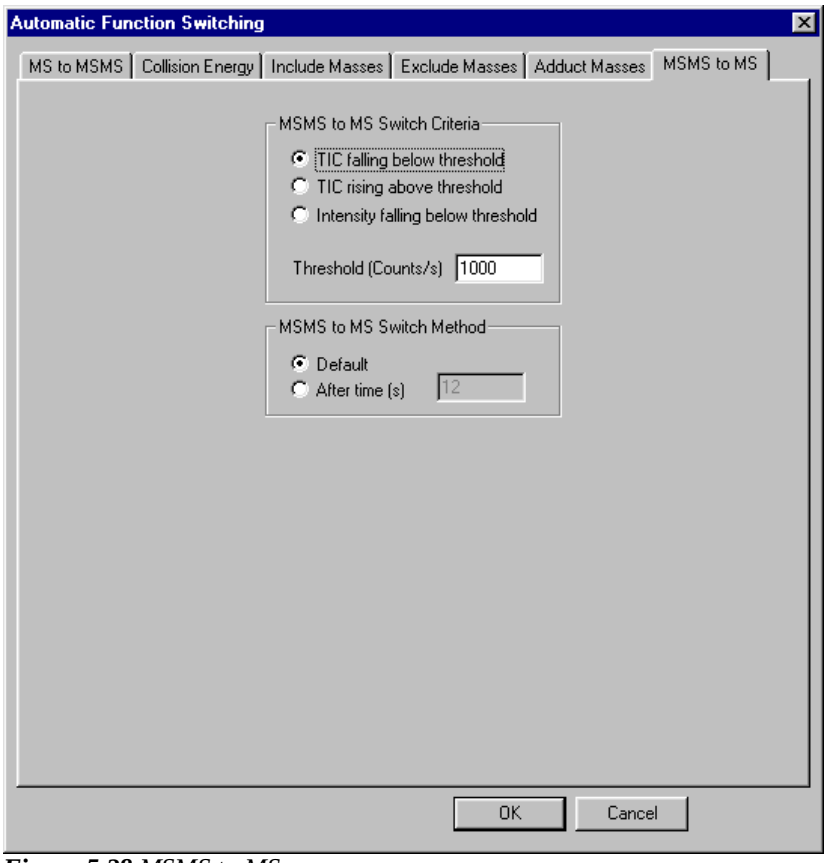

Figure 5.28 *MSMS to MS page*

When MSMS functions have been generated, they will be carried out in parallel until the criteria specified for switching from MSMS to MS are satisfied.

If the MSMS to MS Switch method is **Default**, the MSMS function will stop when the MSMS to MS Switch criteria are met.

If the MSMS to MS Switch method is **After Time**, the MSMS function will stop when the MSMS to MS Switch criteria are met, or when the specified time has elapsed.

The MSMS to MS criteria are as follows

If **TIC falling below threshold** is selected, and the TIC of the spectrum falls below the specified **Threshold**.

If **TIC rising above threshold** is selected, and the TIC of all MSMS spectra for a precursor rises above the specified **Threshold**.

If **Intensity falling below threshold** is selected, and the intensity of the largest peak falls below the specified **Threshold**.

If the **After Mass** option is selected, only masses after the specified mass will be used in the MSMS to MS criteria.

When all MSMS functions have stopped, the MS Survey function will be carried out again.

Other Function Menu Options

If **Store as table** is selected, acquired functions will be stored as separate functions in the data file. If not selected, MSMS scans will be stored as one function in the same data file if they are running consecutively, and in consecutive data file functions if they are running in parallel.

If **Construct from spectrum** is switched on, a right mouse button click and drag operation on a previously acquired spectrum will enter the **Start** and **End** masses in the function editor. For MSMS functions a single right mouse click will enter the mass in the **MSMS of** field.

Note: Q-Tof can have up to 32 MS and MSMS functions, but only one Survey function.

Monitoring Acquisitions

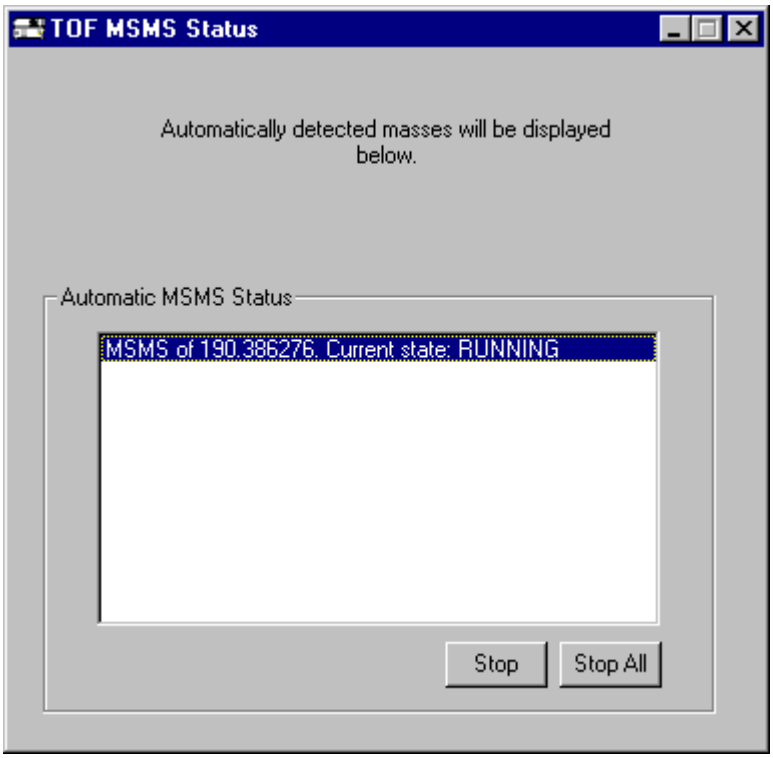

Figure 5.29 *Automatic Function switching status dialog*

When an acquisition is started the automatic switching status dialog is displayed, it shows the precursors currently running.

Pressing the **Stop** button will stop monitoring the current precursor.

Pressing the **Stop All** button will stop monitoring all precursors.

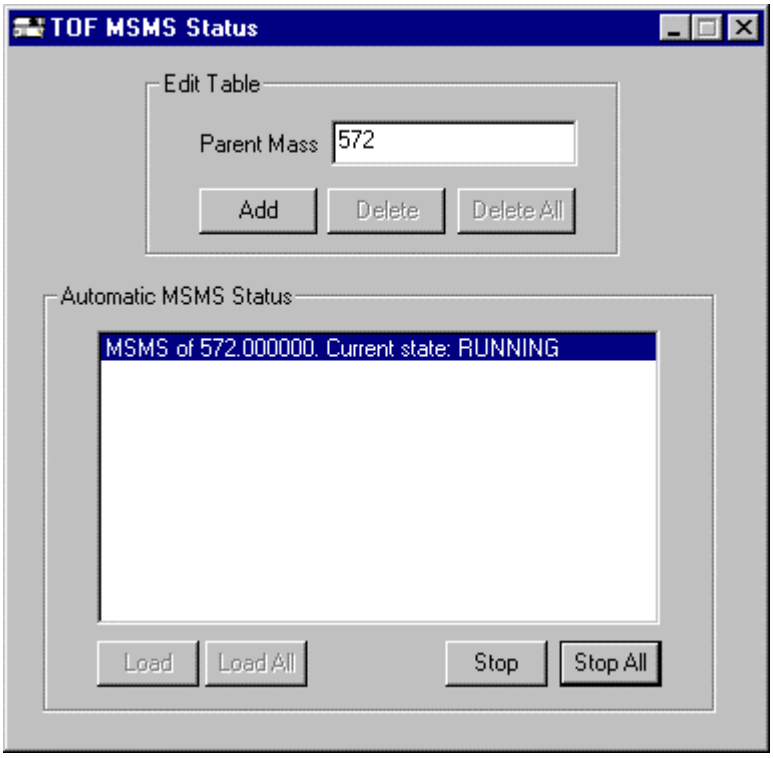

Figure 5.30 *Manual 'on the fly' Function switching status dialog*

If **Automatic Switching** was not selected, when an acquisition is started the Manual 'on the fly' switching status dialog is displayed, it shows the precursors currently running.

Entering a **Parent Mass** and pressing the **Add** button will add a precursor to the list.

Selecting a precursor from the list and pressing the **Delete** button will delete the precursor from the list. Pressing the **Delete All** button delete all precursors.

Selecting a precursor from the list and pressing the **Load** button will generate an MSMS function and send it to the instrument. Pressing the **Load All** button will do this for all precursors.

Pressing the **Stop** button will stop monitoring the current precursor.

Pressing the **Stop All** button will stop monitoring all precursors.

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Acquiring Data

Chapter 6

Starting an Acquisition

There are two ways of starting an acquisition, a single sample acquisition from the Tune page or a multiple sample one from the MassLynx Top level screen.

Single Sample

- **To start a single sample acquisition**

1. Press the **Acquire** button on the **Tune** page or choose **Acquire** from the Tune page **Window** menu.

2. Enter required data.

3. Press the **Start Acquisition** button.

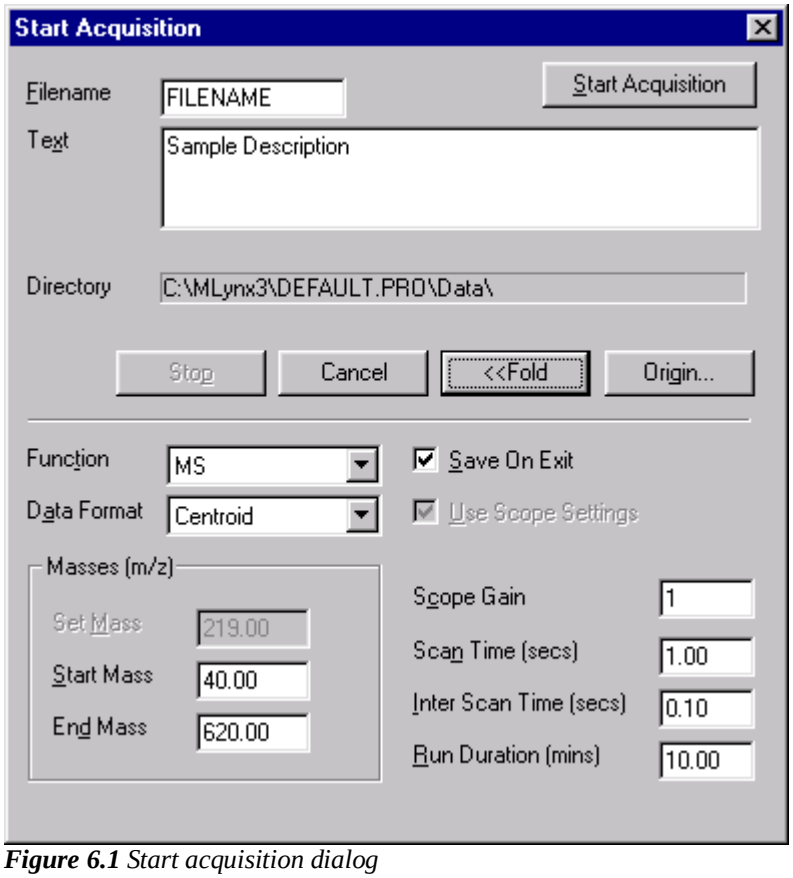

Figure 6.1 *Start acquisition dialog*

Multiple Samples

The MassLynx top level screen contains a Sample List Editor for defining multiple samples which may be used together to perform a quantitative analysis. The list of samples is set up using a spreadsheet style editor, which can be tailored to suit different requirements.

■ To start a multi sample acquisition

1. Set up a Sample List (see MassLynx NT User Guide, “Sample Lists” for details).

2. Choose **Start** from the top level **Run** menu or press the  toolbar button. This displays the Start Sample List Run dialog.

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

4. Enter values in the **Run From Sample** and **To Sample** boxes. The default is all samples in the list.

5. Check the **Priority** and/or **Night Time Process** boxes as required. See the Getting Started chapter of the MassLynx manual for details.

6. Press the **OK** button.

7. Repeat steps 1 to 5 as required. Sample Lists will be added to a queue and run sequentially unless **Priority** or **Night Time Process** has been checked.

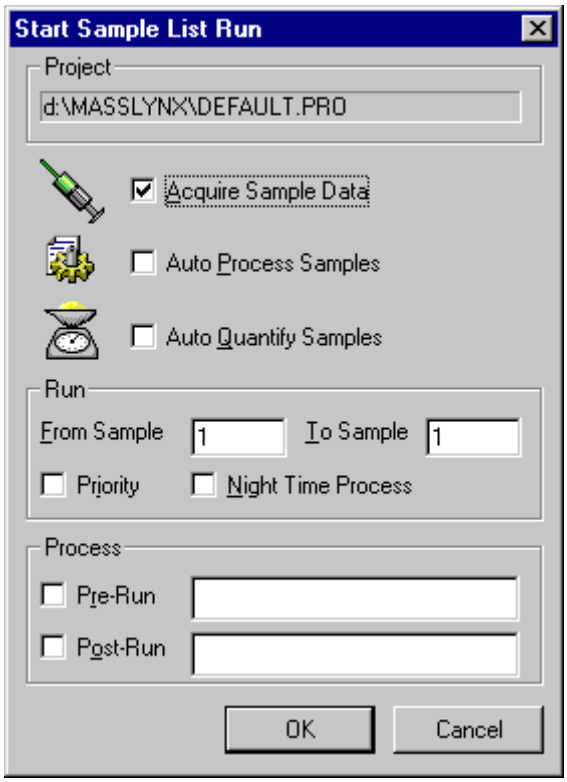

Figure 6.2 *Start Sample List Run dialog*

8. The sample, in the Sample List, which is currently being acquired will have a ● next to it.

Process The Process controls allow you run processes before and after the acquisition. Use the **Pre-Run** control to specify the name of a process that will be run before acquisition of the files in the sample list. Use the **Post-Run** control to specify the name of a process which will be 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.

If you wish 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. If you wish the process to automatically operate on the data file, which has just been acquired, then the **Use last acquired file as default** parameter in the **MassLynx System Globals** dialog should be left unchecked. The **MassLynx System Globals** dialog is accessed by choosing **System Globals** from the MassLynx **Customize** menu.

Automated Analysis of Sample List

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

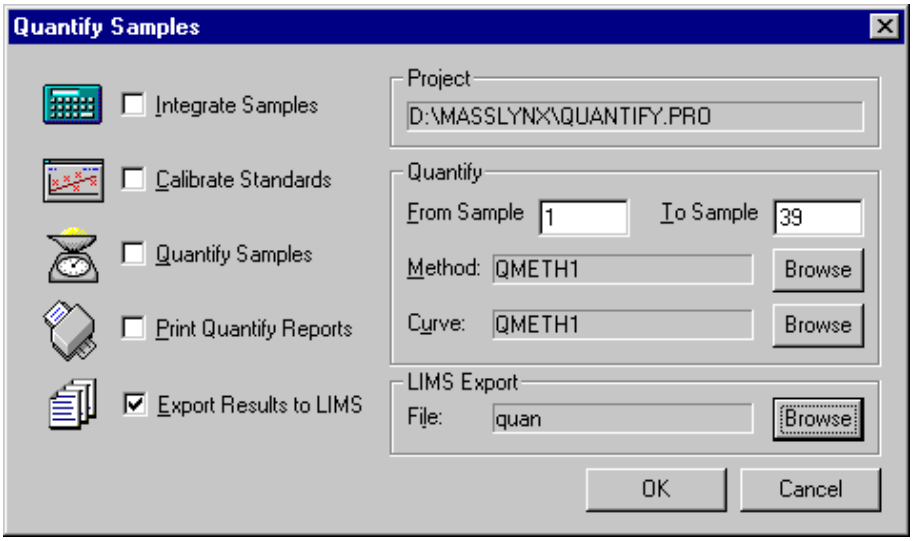

Figure 6.3 *Quantify Samples dialog*

The Quantify Samples dialog allows you to automatically process data files once they have been acquired. To perform Integration, calibration of standards, quantification of samples and printing of quantification reports select the relevant check boxes. See the **Quantify** Chapter, 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 File Browse** button becomes enabled, press the **Browse** button, select a file or enter the name of a new one and press **Save**.

Project The Project field displays the project into which data will be acquired.

If you wish to change the project into which data will be acquired, you can cancel the acquisition and create a new project by choosing **Project Wizard**, or open an existing one by choosing **Open Project**, from the MassLynx Top Level **File** menu.

Analyse From Sample n To Sample n Sets the range of samples in the sample list which will be analysed.

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.

The Acquisition Status Window

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

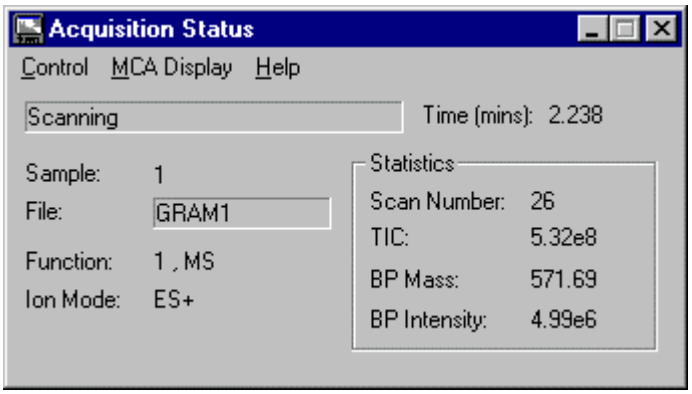

Figure 6.4 *Acquisition Status dialog*

The MCA Display

If you are acquiring MCA data you can watch the data accumulating in real time using the MCA display. This display shows a spectrum as well as some statistical information.

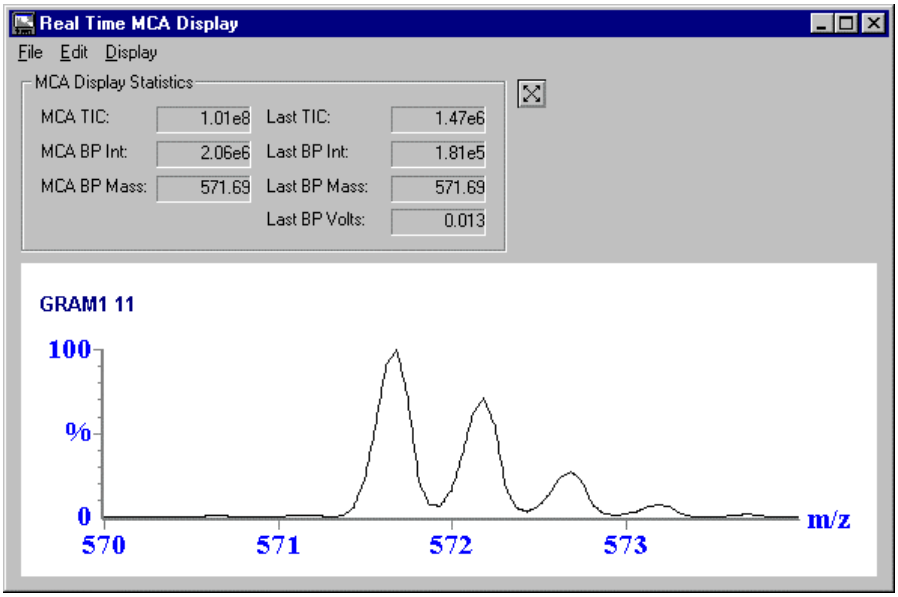

Figure 6.5 *Real time MCA Display dialog*

Chromatogram Real-time Update

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

1. Open the data file using the MassLynx data browser.

2. Press the toolbar button or select **Real-Time Update** from the **Display** menu. The chromatogram display will be updated as the acquisition proceeds.

Spectrum Real-time Update

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

1. Open the data file using the MassLynx data browser.

2. Press the toolbar button or select **Real-Time Update** from the **Display** menu.

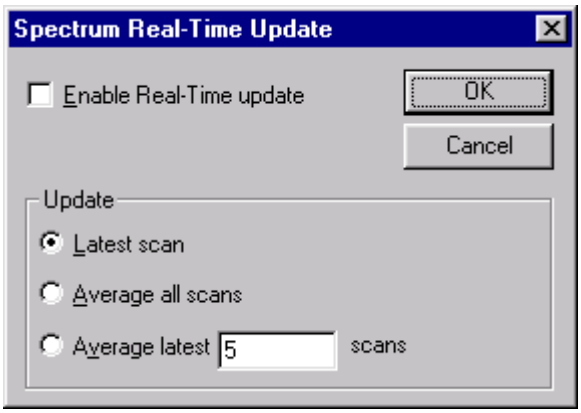

Figure 6.6 *Spectrum Real-Time Update dialog*

To turn real time update on select the **Enable Real-Time update** check box, to turn it off clear this check box. When real-time update is on the display will continually be updated with spectra from the current acquisition. The actual information displayed is determined by selecting one of the following radio buttons. Real-time update can also be turned on and off via the Real-Time spectrum toolbar button.

Selecting the **Latest scan** button will display the last acquired scan, this is the default option.

Selecting the **Average all scans** will update the display with spectra formed by averaging all the spectra that have so far been acquired.

Selecting the **Average latest scans** button will update the display with spectra formed by averaging the last *n* scans acquired, where *n* is specified in the associated edit control.

Stopping an Acquisition

From the Tune Page, press the **Stop** button.

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

Data acquired up to this point will be saved.

Automatic Startup and Shutdown

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

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

The Shutdown Editor

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

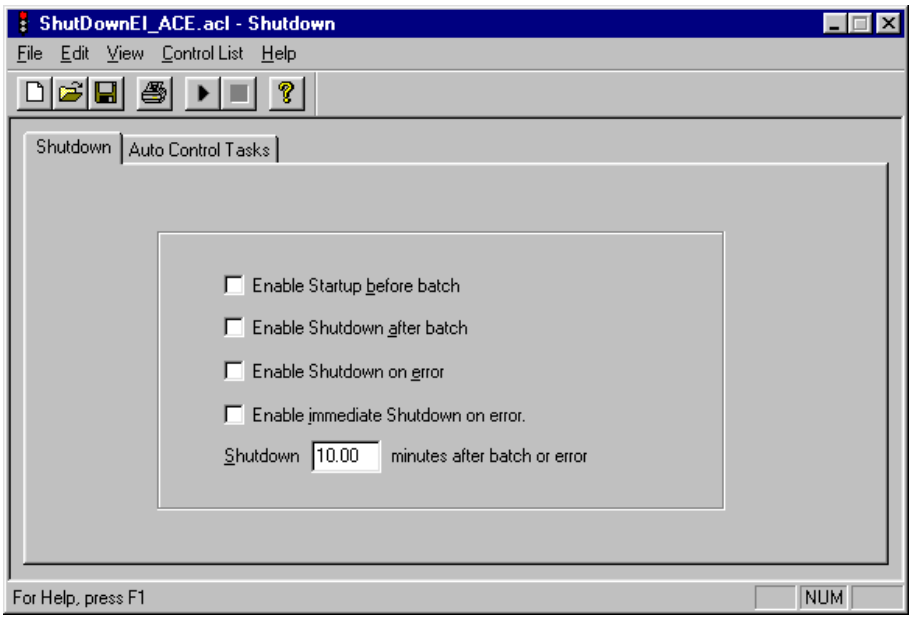

Figure 6.7 *Shutdown editor*

Check the **Enable Startup before batch** box to perform the startup tasks when a Sample List is submitted.

Check the **Enable Shutdown after batch** box to perform the shutdown tasks after a batch of samples has completed.

Enter a time in the **Shutdown *n* minutes after batch or error** box at which to perform the shutdown tasks.

There is an option to perform the shutdown tasks immediately after an error occurs or after the time defined in the **Shutdown *n* minutes after batch or error** box. If the **Enable Shutdown on error** box is checked then the shutdown tasks are performed after the defined time. Note the **Enable immediate Shutdown on error** box is grayed out if this option is selected.

If the **Enable immediate Shutdown on error** box is checked then the shutdown tasks are performed as soon as the error is detected. Note the **Enable Shutdown on error** box is grayed out if this option is selected, but the shutdown time can still be changed as this applies to the **Enable Shutdown after batch** option.

The Auto Control Tasks Page

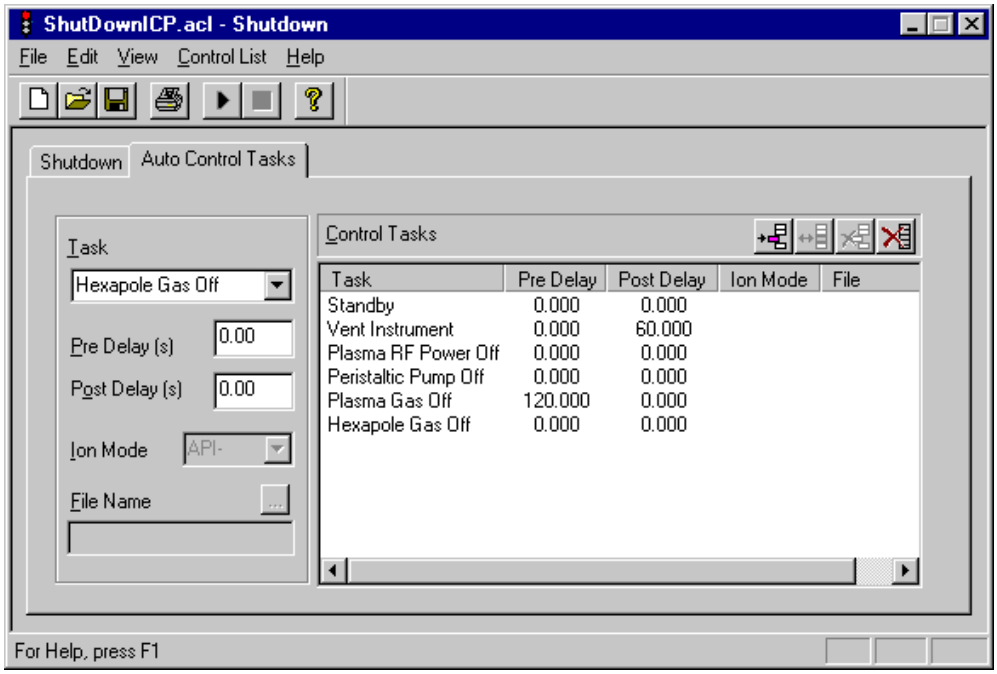

Figure 6.8 *Auto Control Tasks editor*

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

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

Post-Delay This is the length of time that will elapse after the current task has been completed and before the next task is started. E.g. a Post delay of 60s, in the Vent Instrument task in **Figure 6.8** , means that there will be a delay of 60 seconds before the next task is started, to allow the machine to vent fully.

Ion Mode This is a dropdown list box with all the available Ionisation Modes.

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

- **To Add a Task**

1. Select a task from the dropdown **Task** list box.

2. Enter the required parameters.

3. Press the add button.

Note. If this is a new task timetable the task will be added to the end of the list. If a task has been inserted into the task timetable then all subsequent tasks will be added after the inserted task. To add a task to the end of the timetable after inserting a task, click **twice** with the left mouse button below the last entry in the timetable and then add the new task.

- **To Insert a Task**

  1. Click, with the left mouse button, on the entry in the task timetable before which you want to insert the new task.
  2. Select a task from the dropdown Task list box.
  3. Enter the required parameters.
  4. Press the add  button. The task will be inserted before the selected entry.

- **To Modify a Task**

  1. Click, with the left mouse button, on the entry in the task timetable. The details for the task will be displayed in the fields on the left of the screen.
  2. Change the required parameters.
  3. Press the modify  button. The details will change in the task timetable.

- **To Delete a Task**

  1. Click, with the left mouse button, on the entry in the task timetable. The details for the task will be displayed in the fields on the left of the screen.
  2. Press the add  button. The task selected will be deleted from the task timetable.

- **To Delete All Tasks**

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

- **To change the width of a column**

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

The Shutdown Editor Toolbar

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

Saving/Loading Startup and Shutdown Files

- **To Open a Startup or Shutdown file**

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

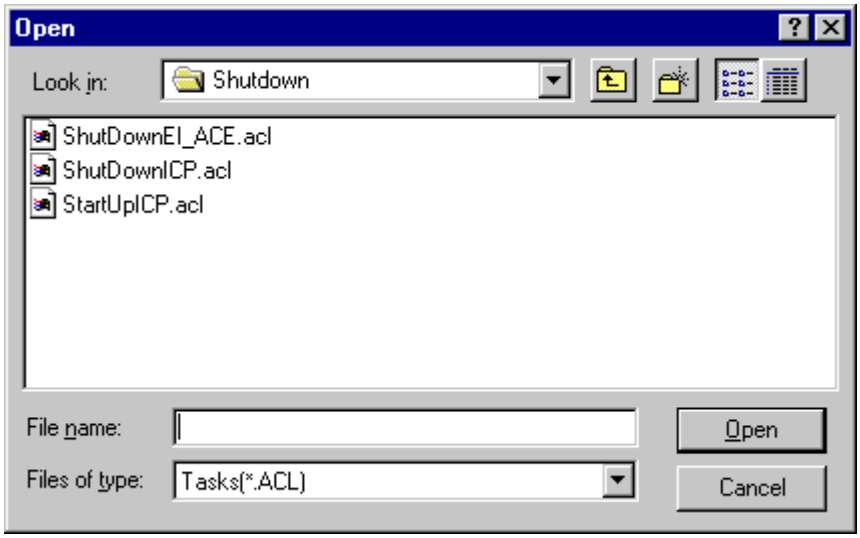

Figure 6.9 *The Open File dialog*

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

- **To Save a Startup or Shutdown file**

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

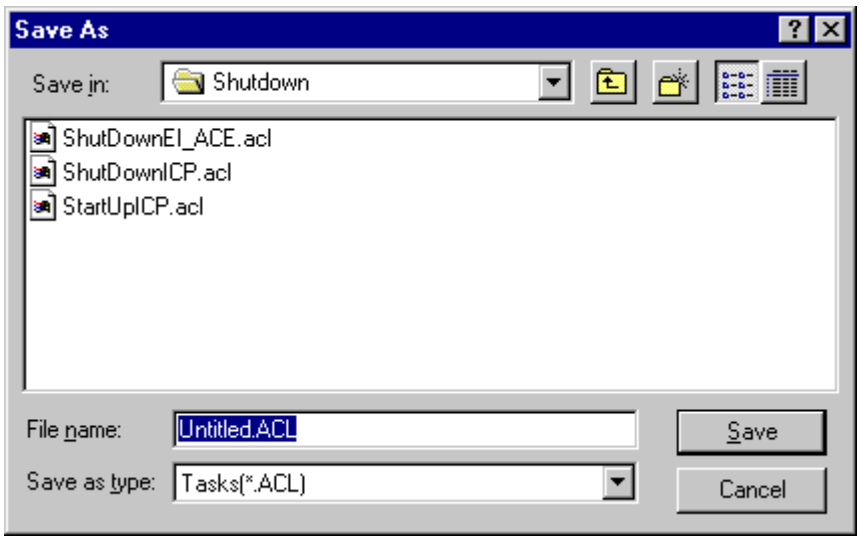

Figure 6.10 *The Save File dialog*

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

Printing Startup and Shutdown Files

- **To Print a Startup or Shutdown File**

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

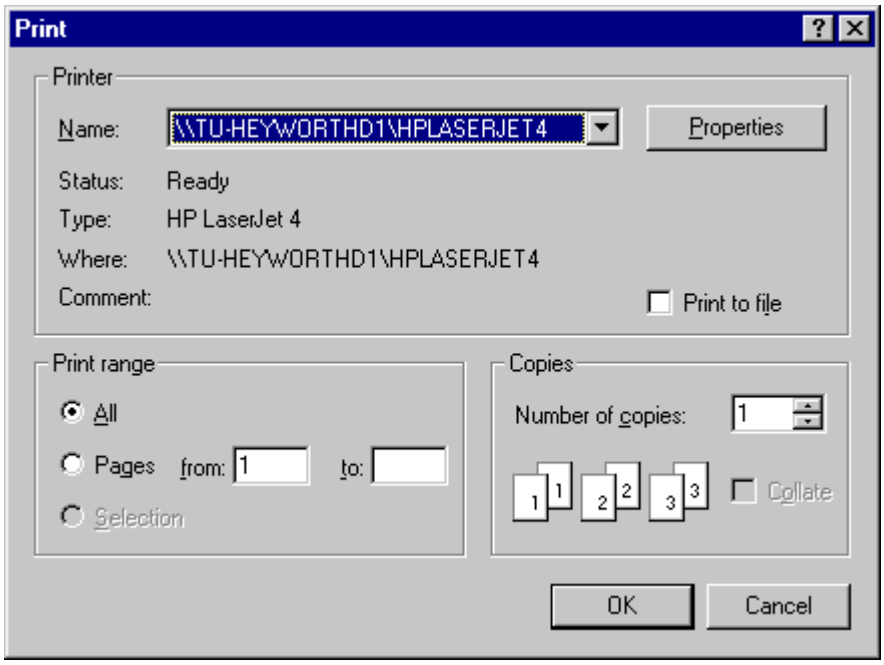

Figure 6.11 *The Print dialog*

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

Creating Startup and Shutdown Files

- **To Create a Startup or Shutdown File**

1. Press the  button or select **New** from the **File** menu.

Running Startup and Shutdown Files

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

If you wish to run a different Startup or shutdown file. Open the required file in the Shutdown editor and press the  toolbar button or select **Run List** from the Shutdown editor **Control List** menu. Press the  toolbar button or select **Stop List** from the Shutdown editor **Control List** menu if you wish to stop running this file.

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