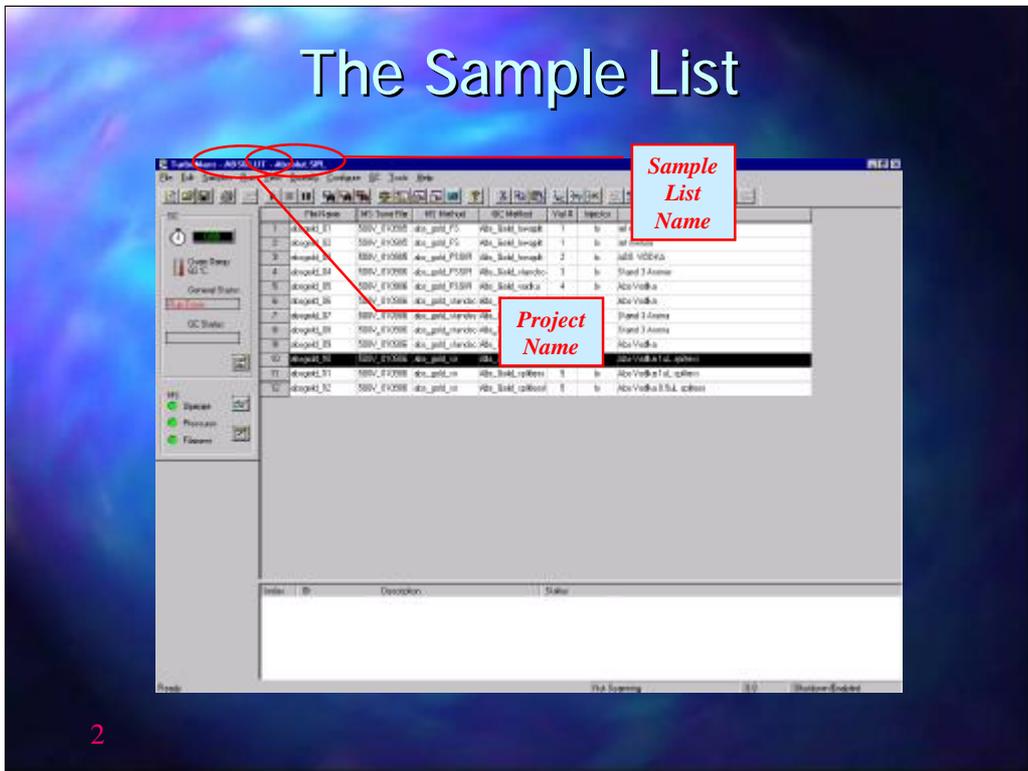




Tuning & Mass Calibration

The Sample List



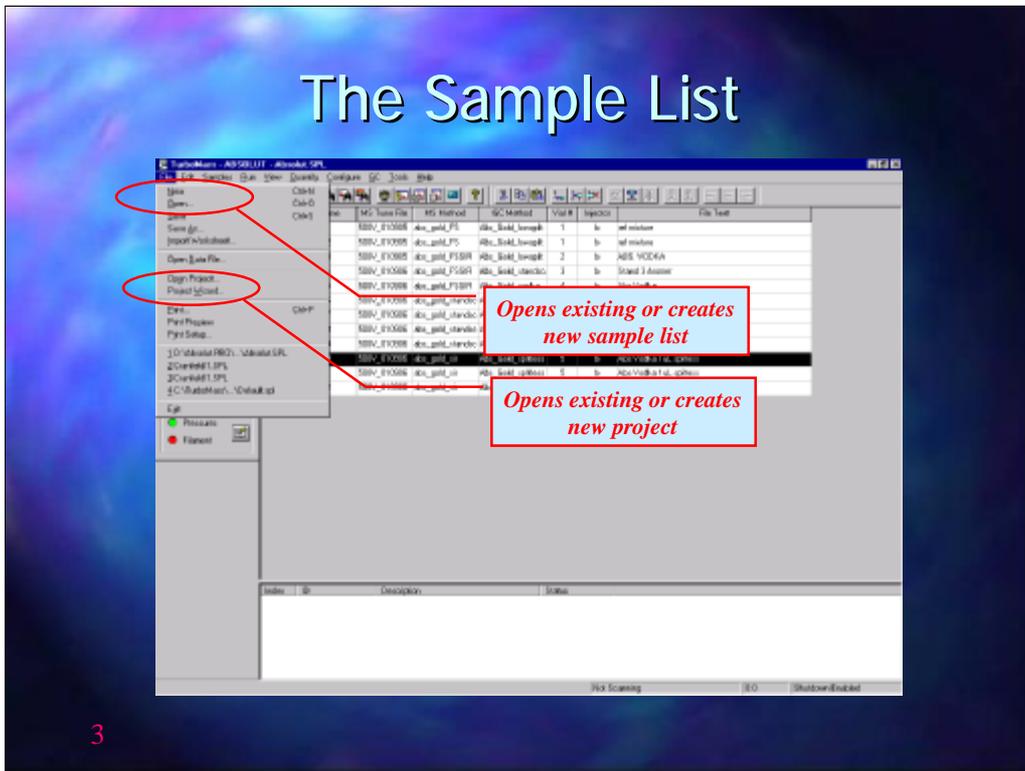
2

The sample list is the top level screen in the TurboMass Gold Software.

Data storage is set up in PROJECT files and within each project file it is possible to use a number of SAMPLE lists

The PROJECT name and the SAMPLE LIST name are shown at the top of the screen

The Sample List



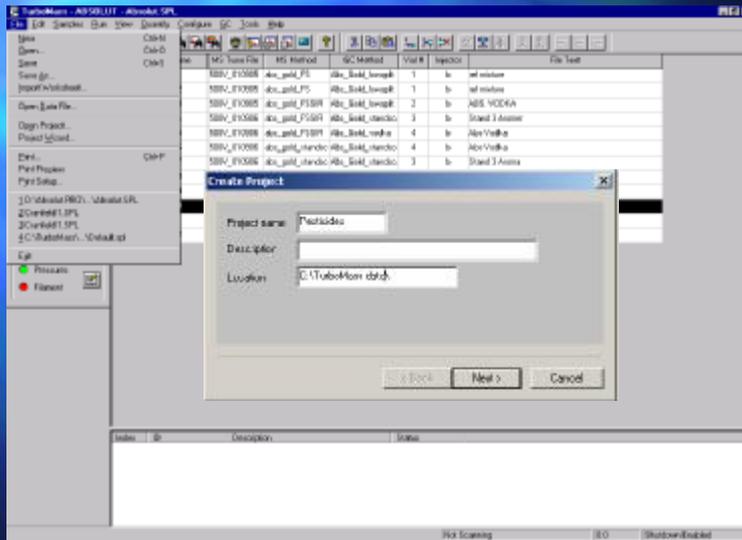
The FILE, NEW or OPEN allows the SAMPLE LIST to be selected

Sample lists allow all the information to be entered for each sample. These will typically include

- The data file name for each sample
- The TurboMass Gold Gold tune method
- The TurboMass Gold Gold acquisition method
- The GC Method
- Injector site and Vial number (with an autosampler)
- A text description for the method

The FILE, OPEN PROJECT will open an existing project; PROJECT WIZARD will take the user through the steps required to generate a new project. Beware that the MS tune files, MS methods and GC methods are stored within each project folder. If you wish to use the same tune files and method files in a new project it is best to use an existing project as a template for the new project. A completely new project will not have access to old files

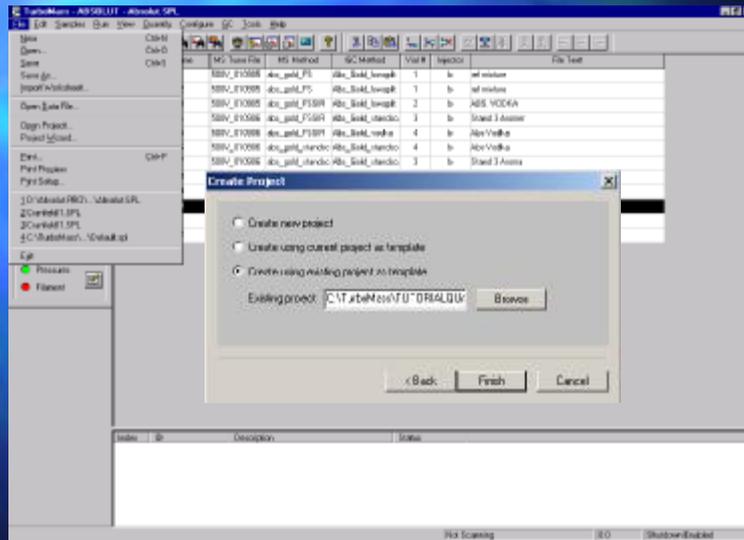
The Sample List



4

Using Project Wizard a new Project Name must be entered. Also set the Directory Location for the new Project. Avoid saving Projects in the c:\TurboMass directory

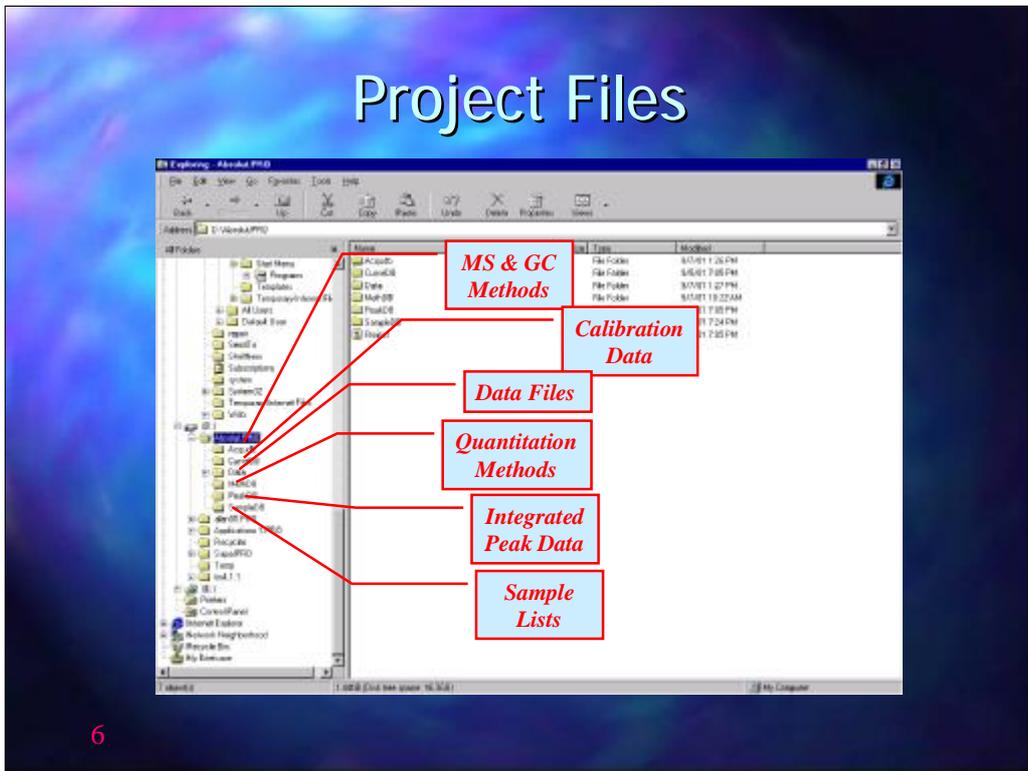
The Sample List



5

Using “Create using Current Project as Template” or “Create using existing Project as Template” will copy methods and tune files from the old project to the new one

Project Files



6

PROJECT FILES consist of a number of sub folders

ACQADB will contain MS methods, GC methods, MS tune files, MS calibration files

CURVEDB contains calibration data for quantitative analysis

DATA contains the RAW data files from each run

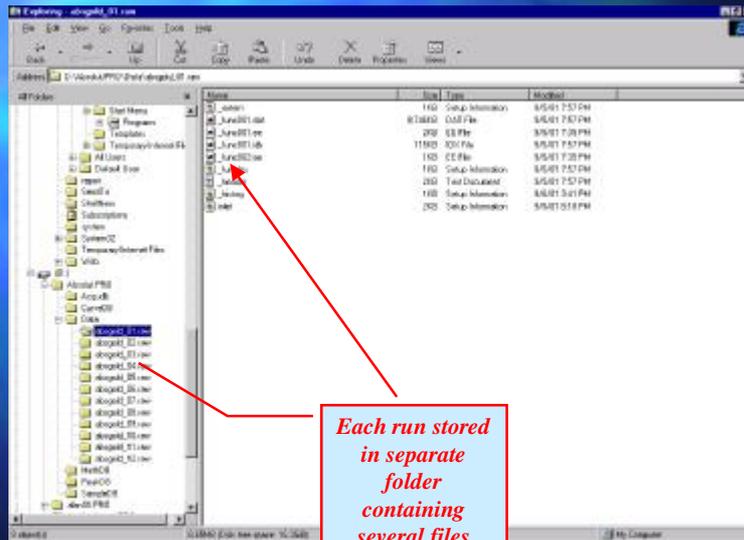
METHADB contains the quantitative methods

PEAKADB contains data on integrated peaks

SAMPLEADB contains all the sample lists associated with the project

It is recommended that a new project is started at least once a month to simplify file management and archiving.

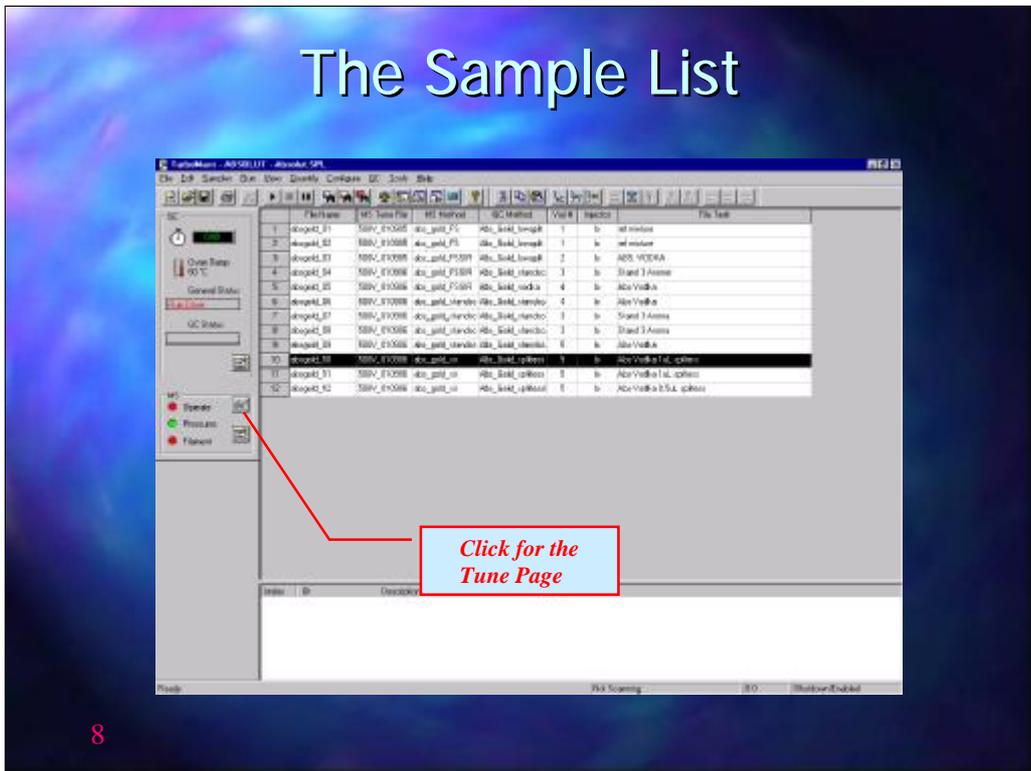
Project Files



7

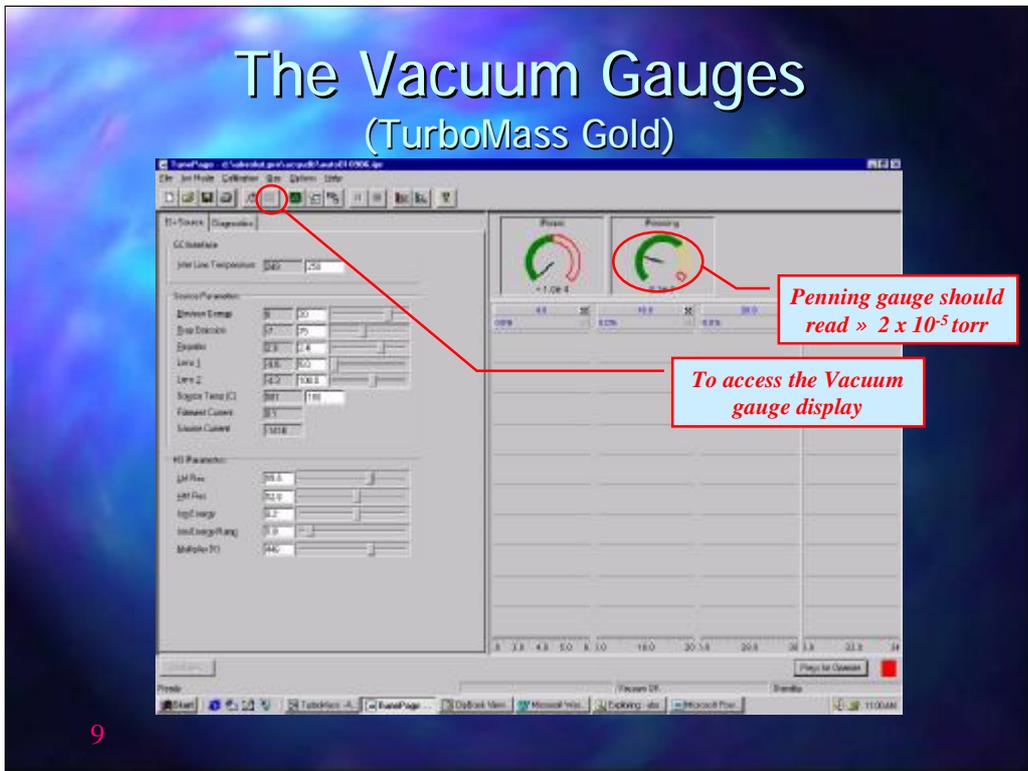
The RAW data files are folders that contain all the data collected for that particular run. Each folder will contain a number of file labelled FuncXXX.dat and FuncXXX.idx where XXX is a number corresponding to the function number in the MS method. The MS Function will be discussed later in MS Method generation but each function may be a full scan, for example between m/z 35 to 350, or a selected ion recording of one or a few specific ions

The Sample List



The tune page is accessed from the sample list by clicking on the button shown 

The Vacuum Gauges (TurboMass Gold)



9

The TUNE page controls a number of the TurboMass Gold functions.

These include

- The vacuum pumps (Accessed through the “OPTIONS” drop down menu)

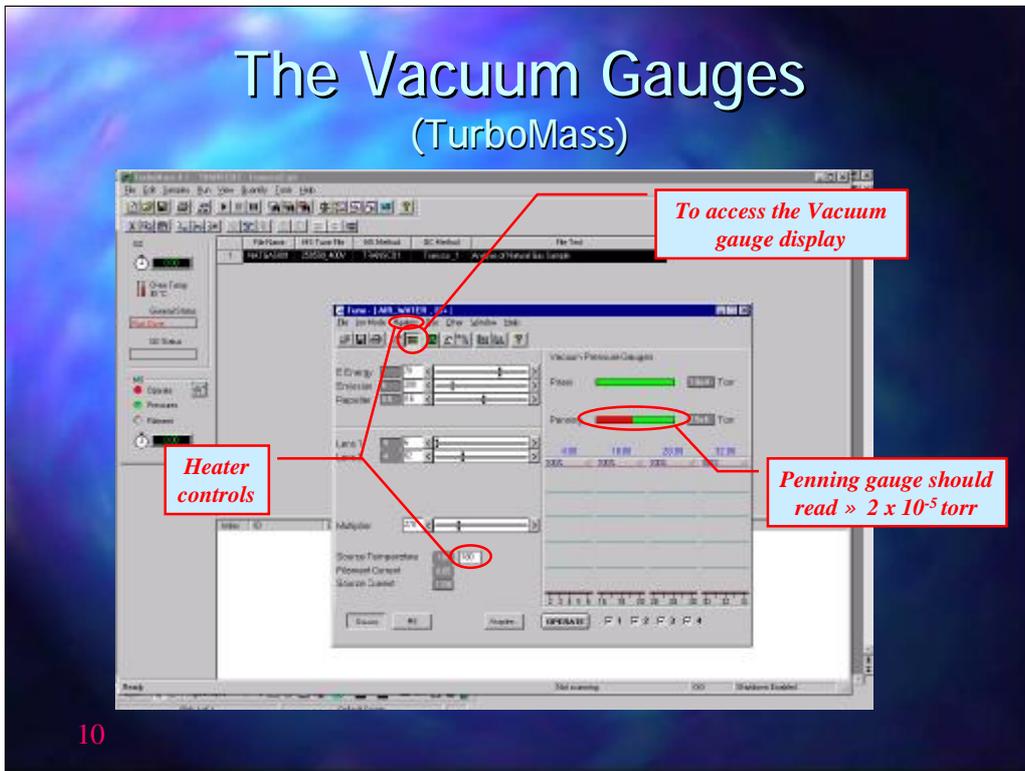
- The heater controls for both the ion source and the transfer line

- The settings for the ion source, lenses, quadrapoles and photomultiplier

- Filament current.

Also displayed on the TUNE page are the vacuum gauge readouts showing the pressure in the system (accessed by clicking on the button with the red/green bars )

The Vacuum Gauges (TurboMass)



10

The TUNE page controls a number of the TurboMass functions.

These include

- The vacuum pumps (Accessed through the “OTHER” drop down menu)

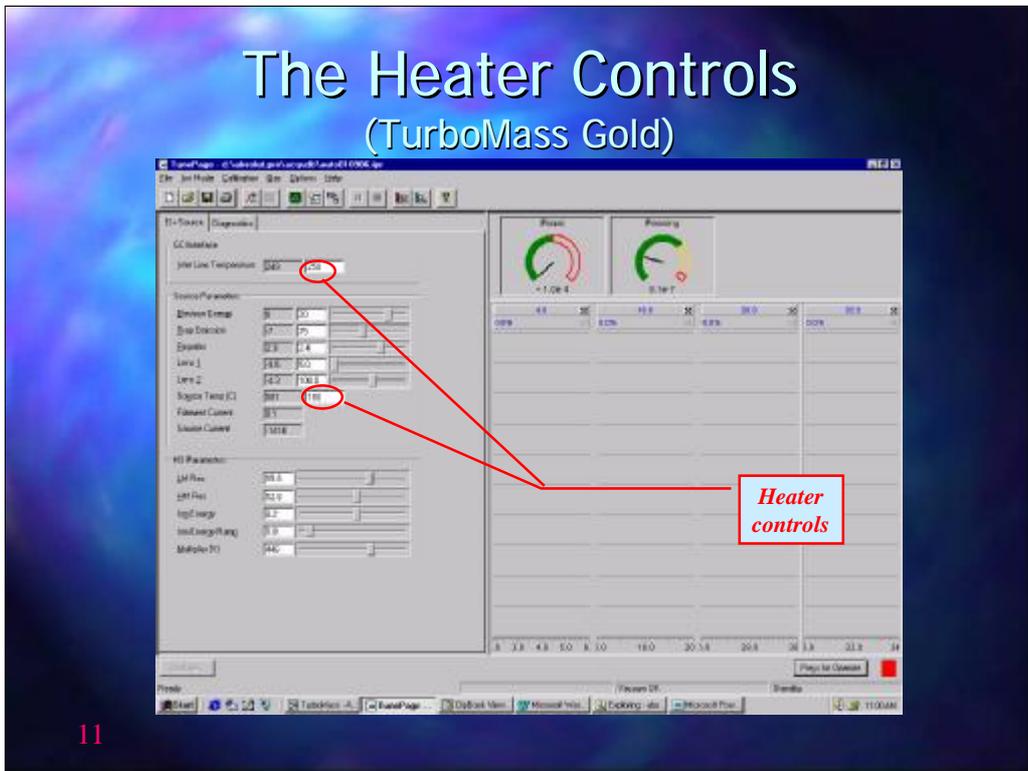
- The heater controls for both the ion source and the transfer line

- The settings for the ion source, lenses, quadrapoles and photomultiplier

- Filament current.

Also displayed on the TUNE page are the vacuum gauge readouts showing the pressure in the system (accessed by clicking on the button with the red/green bars )

The Heater Controls (TurboMass Gold)



11

The TUNE page controls a number of the TurboMass Gold functions. These include

- The vacuum pumps (Accessed through the “OPTIONS” drop down menu)

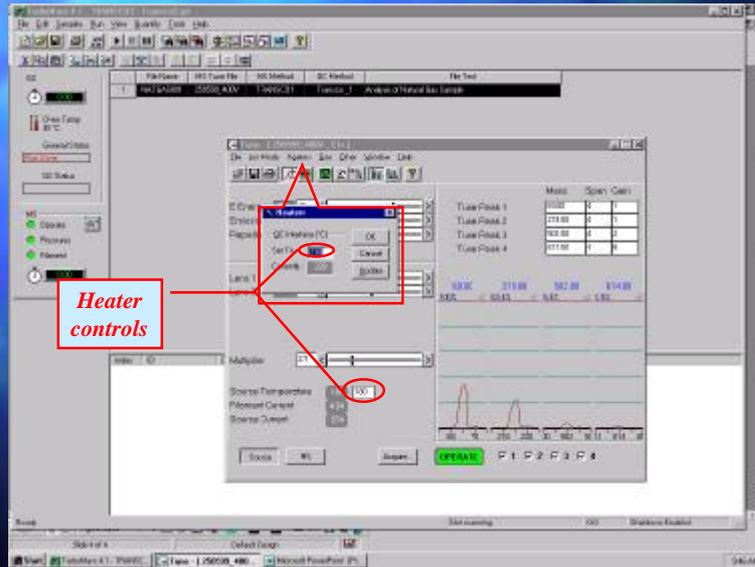
- The heater controls for both the ion source and the transfer line

- The settings for the ion source, lenses, quadrapoles and photomultiplier

- Filament current.

Also displayed on the TUNE page are the vacuum gauge readouts showing the pressure in the system (accessed by clicking on the button with the red/green bars )

Heater Controls (TurboMass)



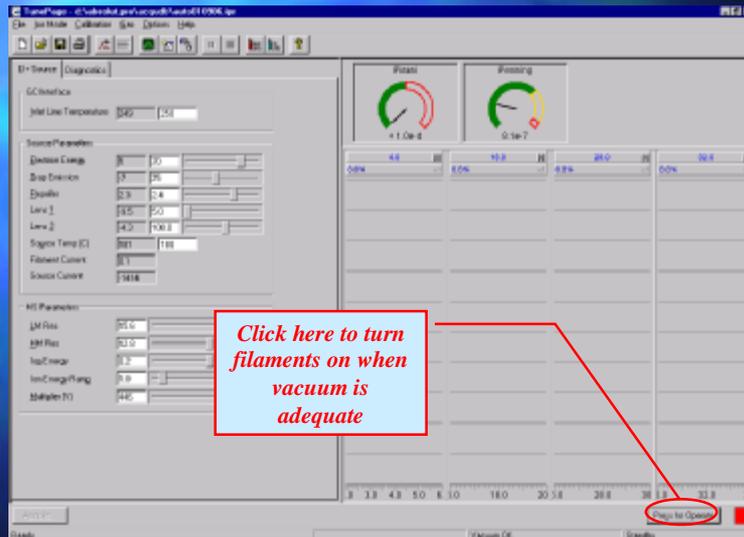
12

The ion source heater control is shown near the bottom left of the TUNE page. The required temperature is entered in the white box next to SOURCE TEMPERATURE.

The transfer line temperature control is accessed by clicking on HEATERS menu. Again the temperature is set in the white box. Both temperatures should be set to ensure that no condensation of the sample will occur in either the ion source or the transfer line. The transfer line will also contain the tail of the GC column and so be set at least as high as the highest oven temperature that will be used during the analysis

The grey boxes on the TUNE page are the "readbacks". These show the actual value for each parameter. If these are not apparent go to the "OTHER" drop down menu and ensure that "READBACKS" is turned on.

The Vacuum Gauges



13

The filament in the ion source is turned on by clicking on the PRESS FOR OPERATE button. Before turning on the filament check that the vacuum is adequate.

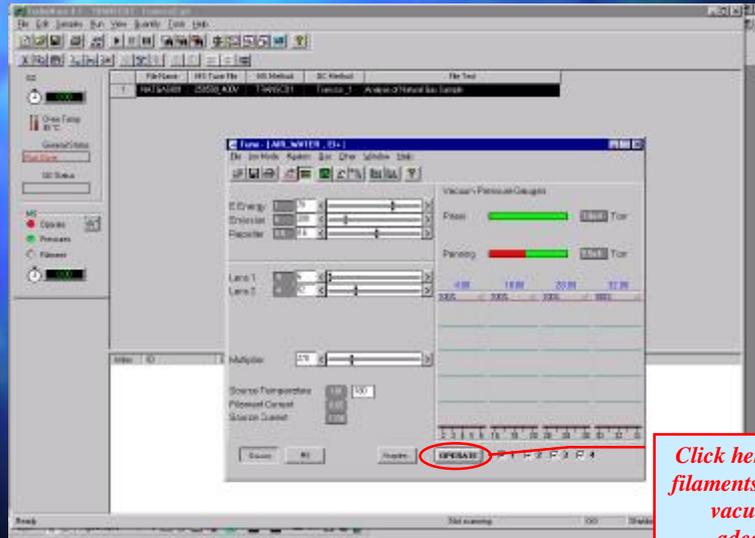
The vacuum is displayed by the readout from two gauges. The Pirani gauge (the left gauge) will only display the pressure above 1.7×10^{-4} torr and is used only during the initial stages of pumpdown.

The Penning gauge (the right gauge) switches in when the Turbomolecular pump turns on at about 1×10^{-4} torr. This gauge should be checked to determine if the vacuum is adequate.

The vacuum will be dependent on the carrier gas flow rate, the higher the carrier gas flow the poorer the vacuum. It is possible to use TurboMass Gold with flow rates up to about 5ml/min.

For a typical carrier gas flow rate of 1ml/min the vacuum should be in the order of 2×10^{-5} torr.

The Vacuum Gauges



14

The filament in the ion source is turned on by clicking on the OPERATE button. Before turning on the filament check that the vacuum is adequate.

The vacuum is displayed by the readout from two gauges. The Pirani gauge (the upper gauge) will only display the pressure above 1.7×10^{-4} torr and is used only during the initial stages of pumpdown.

The Penning gauge (lower gauge) switches in when the Turbomolecular pump turns on at about 1×10^{-4} torr. This gauge should be checked to determine if the vacuum is adequate.

The vacuum will be dependant on the carrier gas flow rate, the higher the carrier gas flow the poorer the vacuum. It is possible to use TurboMass with flow rates up to about 5ml/min.

For a typical carrier gas flow rate of 1ml/min the vacuum should be in the order of 2×10^{-5} torr.

The Air / Water background

The screenshot shows the control software for a mass spectrometer. On the left, there are various control panels including 'TUNE MASS' parameters and 'FILAMENT' controls. The main display area shows a mass spectrum with peaks at m/z 4, 18, 28, and 32. A table at the top right lists these masses and their corresponding scan numbers. A green indicator light is visible at the bottom right of the interface.

Monitor air water background for a leak
4 = helium
18 = water
28 = N₂
32 = O₂

Select Tune Masses Display

Green indicates that filaments are on

15

Once the vacuum is in the order of 2×10^{-5} torr select the scope display by clicking on the  button (TUNE MASS parameters) and then select ions of 4 (He), 18 (H₂O), 28 (N₂) and 32 (O₂) before turning the filament on.

When turning the filament on monitor the abundance of each ion, especially the 32 ion. If the 32 ion is more than 25% of the 28 ion turn off the filaments immediately and check the system for air leaks.

The Air / Water background

Type of Leak	m/z				
	4	18	28	32	40, 44
None	base	< m/z 4	< m/z 18	< 25% m/z 28	<< m/z 32
Small Leak	base	< m/z 4	> m/z 18	< 25% m/z 28	<< m/z 32
Medium Leak			> m/z 18	> 25% m/z 28	<< m/z 32
Large Leak				> m/z 28	<< m/z 32
Very Large Leak				very wide	

16

Typically the 4 ion should be the largest, followed by the 18 then the 28 with 32 being approximately 25% of the 28. If the 28 and 32 ions are excessively high turn the filament off and allow the system to pump down for longer.

The air/water background is best measured with the splitter on the injector open with a split flow of at least 10ml/min. If the system is connected to an ATD 400 or TurboMatrix ATD, then open the outlet split.

Normally the water peak (m/z 18) is usually larger than the nitrogen peak (m/z 28) but if a non-polar column is installed and the system has been in use for some time then the m/z 18 intensity may be lower than the m/z 28 intensity.

If a large air leak is present the ratio of m/z 32 to m/z 28 will change with the 32 ion increasing in proportion to the 28 ion.

The Tune Page

The screenshot shows the 'Tune Page' of the TurboMass Gold software. The interface is divided into several sections:

- Toolbar:** Located at the top, it contains various control buttons. A red circle highlights a button labeled 'REF GAS'.
- Mass Selection Table:** A table with columns for 'Mass', 'Scan', and 'On'. It lists masses 69, 131, 219, and 502. A red circle highlights this table.
- Parameter List:** On the left side, there are various parameters such as 'DC Offset', 'Lens 1', 'Lens 2', 'Scan Time (s)', 'Filament Current', 'Source Current', and 'RG Parameters'.
- Mass Spectrum Plot:** On the right side, there is a plot showing ion current versus mass-to-charge ratio (m/z). The x-axis ranges from 0 to 34. A red line shows the spectrum with peaks at the selected masses.

Red callout boxes with white text provide instructions:

- 'Turn Ref gas on' points to the 'REF GAS' button.
- 'Tuning Masses' points to the mass selection table.

17

To tune the TurboMass Gold access the TUNE MASS parameters and select masses 69, 131, 219 and 502

Turn the reference gas on by clicking on the button labelled REF GAS

The Reference Gas

Heptacosafuorotributylamine ($C_{12}F_{27}N$)



18

The reference gas normally used is heptacosafuorotributylamine ($C_{12}F_{27}N$)

The Reference Gas

Synonyms

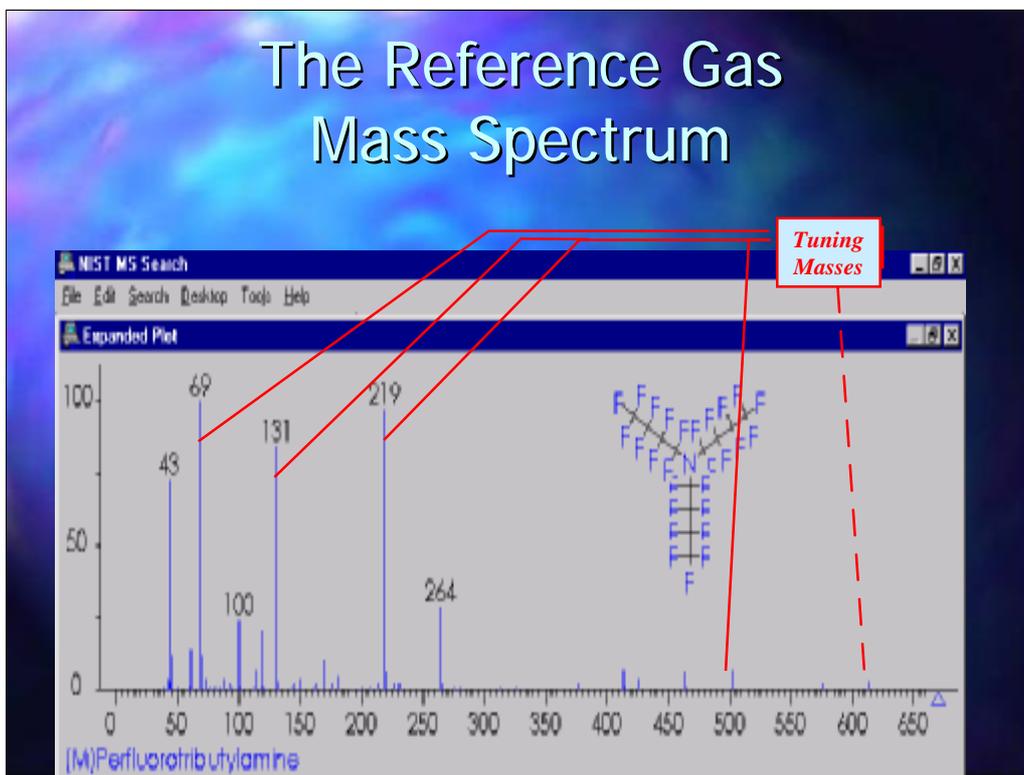
- Synonyms:
1. PFBA
 2. 1-Butanamine, 1,1,2,2,3,3,4,4,4-nonafluoro-N,N-bis(nonafluorobutyl)-
 3. FC 43
 4. FC 47
 5. Fluor-el FC 45
 6. Fluorocarbon FC 43
 7. Heptafluoroisobutylamine
 8. Mediflo FC 43
 9. Mediflo FC 47
 10. Tetrabutylamine, heptafluoro-
 11. [n-C4F9]N
 12. tri(perfluorobutyl)amine
 13. Perfluoro-*n*-butylamine
 14. Heptafluoro-*n*-butylamine
 15. Survanine, 1,1,2,2,5,5,4,4,4-nonafluoro-N,N-bis(*n*-afluorobutyl)-
 16. Fluorol 43
 17. FC 43(47)
 18. Tri(*n*-afluorobutyl)amine
 19. Tri(*n*-afluorobutyl)amine
 20. Tri(perfluorobutyl)amine



19

The reference gas has a number of synonyms, the more common ones are PFTBA, FC43 and hepta

The Reference Gas Mass Spectrum



The masses that are normally used to check the tune are 69, 131, 219 and 502. 614 may also be used to check the tune at higher masses

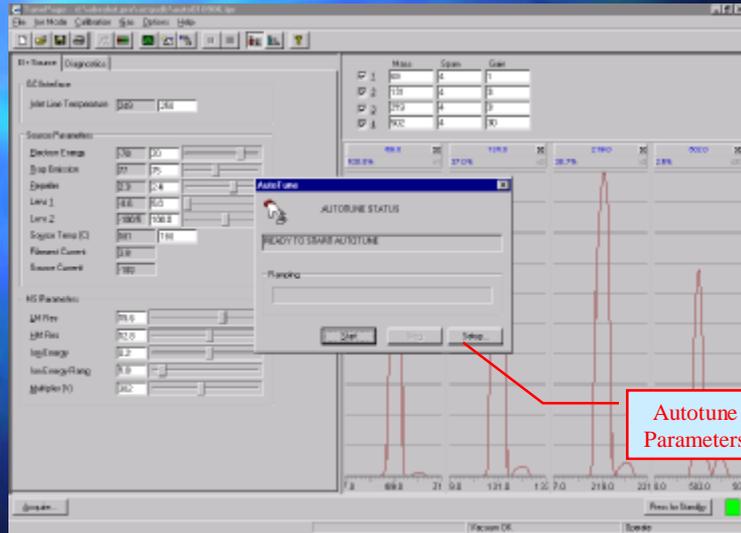
The Tune Page

21

An Autotune procedure may be used by clicking on the button highlighted above.  Before commencing Autotune, however, ensure that the emission current is set to 100 μ a or lower. Lower emission currents will increase the lifetime of the filament and keep the source cleaner. However some loss in sensitivity will be observed at lower emission currents. Do not use an emission current of less than 50 μ a

Tuning should not be necessary more than once a week. However it is good practice to check that the tune is OK by turning on the reference gas each morning and checking the relative intensities of each ion before proceeding with the analysis. If the system needs to be retuned the sensitivity of the TurboMass Gold may be affected and should be re-calibrated before any quantitative work is attempted.

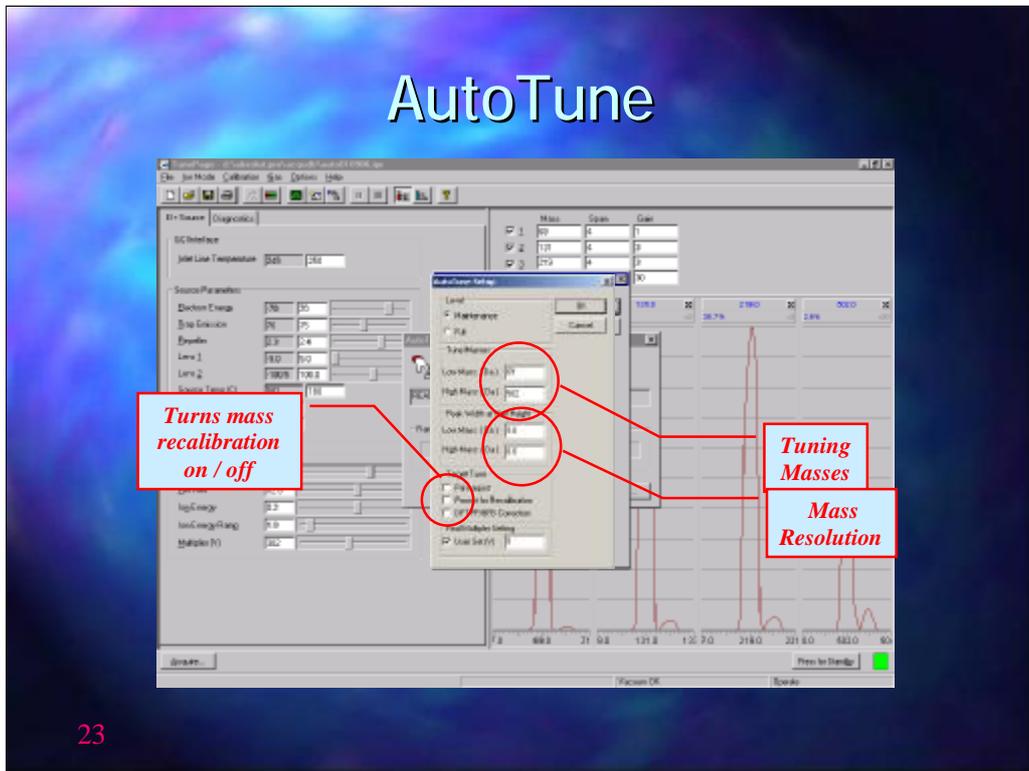
AutoTune



22

The Autotune parameters may be accessed using the SETUP button

AutoTune



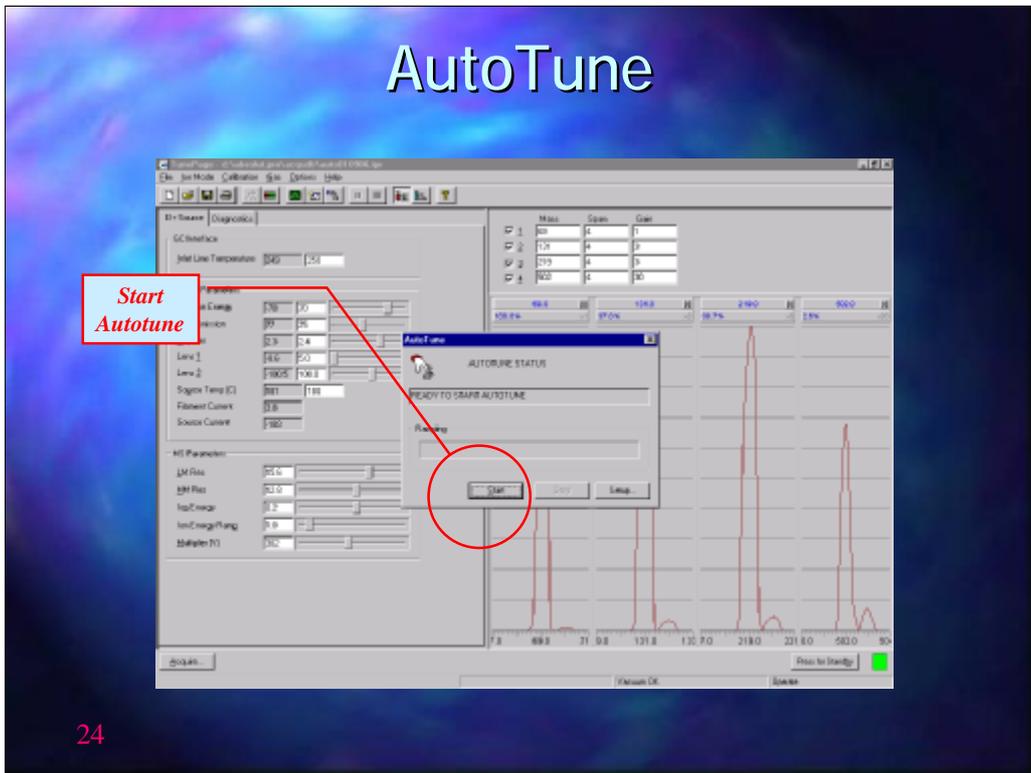
23

The Autotune uses two masses for the set-up procedure. The default settings are 69 for low masses and 502 for the high masses.

The resolution is set at low mass (69) and high mass (502). The default setting is 0.6 Daltons at $\frac{1}{2}$ height which will give a peak width at base of approximately 1 Dalton

If Prompt for Recalibration is ticked the system will follow the tune with a mass re-calibration procedure.

AutoTune



24

When the Auto Tune parameters have been set press START to begin Auto Tune

The Tune Page

Adjust photomultiplier so that m/z 69 peak is on scale

Double click here to reduce gain

Double click here to increase gain

Relative abundances:-

69	100%
131	30 - 60%
219	30 - 60%
502	1.5 - 4%
614	>0.1%

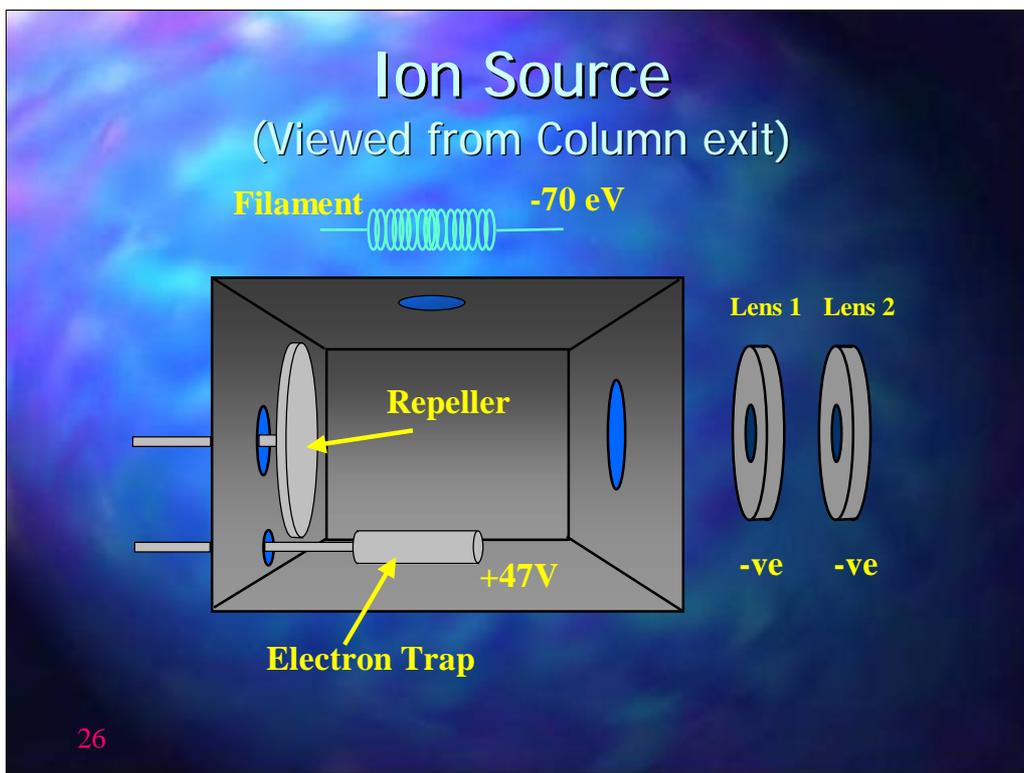
The Autotune procedure will set all parameters including the photomultiplier voltage. However, this is set to provide a reasonable intensity for the reference gas ions and must be set independently to achieve the sensitivity required for the samples that are to be run.

To check that the Autotune has set the system up correctly adjust the photomultiplier voltage so that the 69 ion is on scale.

The relative abundances of the ions should be in the order of:-

69	100%
131	30-60%
219	30-60%
502	1.5-4%
614	>0.1% (when used)

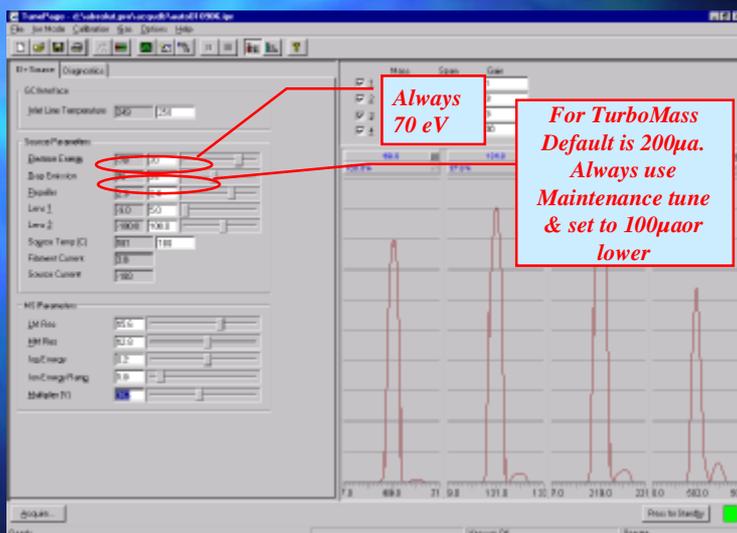
When making the above checks ensure that all peaks are on scale with the gain set at 1. The gain may be increased to make individual peaks larger by entering the value in the GAIN column or by double clicking in the grey box immediately above the peak display. The gain may also be reduced by double clicking beneath the peak. If the gain is set to 1(69 ion), 3 (131), 3 (219) and 30 (502) the peaks should be approximately the same height.



26

This schematic shows the main components of the ion source. At the top is the filament which, when turned on, generates a stream of electrons that are attracted to the Electron Trap (or collector) electrode that has +47 volts applied to it. The repeller is the larger electrode. This also has a positive voltage applied to it which has the effect of repelling the positive ions that are generated by the collision of electrons with the sample molecules eluting from the end of the column. These positive ions are repelled towards the 2 lenses mounted outside the inner source. Not shown in this schematic are two magnets mounted just below source and above the filament. The quadrapoles are mounted to the right of Lens 2

Manual Tuning

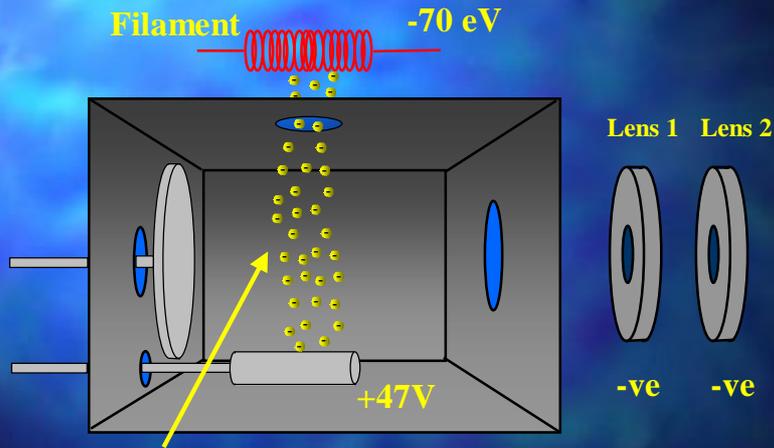


27

If the tune does not meet the required specifications then some manual tuning may be necessary. If the Autotune does not give good values for the peak intensities it is usually a sign that the source is dirty.

When manual tuning ensure that the Electron energy (E Energy) is set to 70eV. All spectra in the libraries have been collected at 70eV and to achieve a good match it is essential that spectra collected are run at this setting.

Ion Source Emission Current



Emission Current (ma)

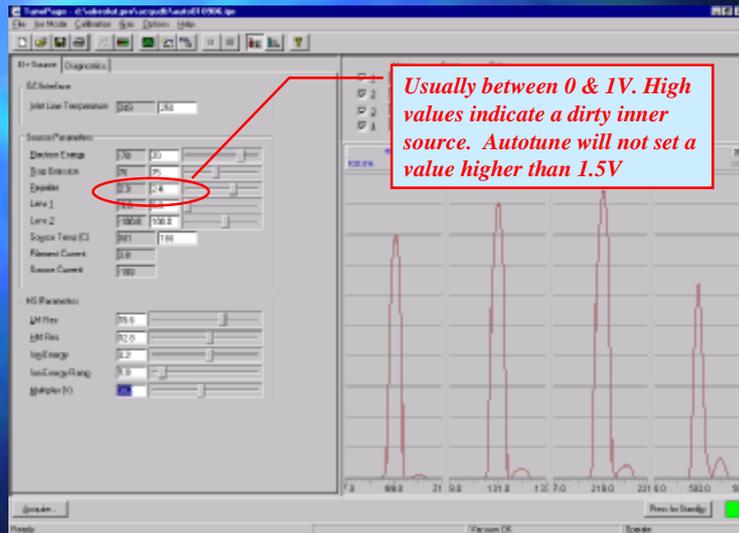
Default = 100ma.

Do not exceed 100 μ a

28

The emission current generated by the stream of electrons from the heated filament to the collector electrode which always has +47V applied. 100 μ a will give best sensitivity, lower current settings will enhance the life of the filament and keep the source cleaner.

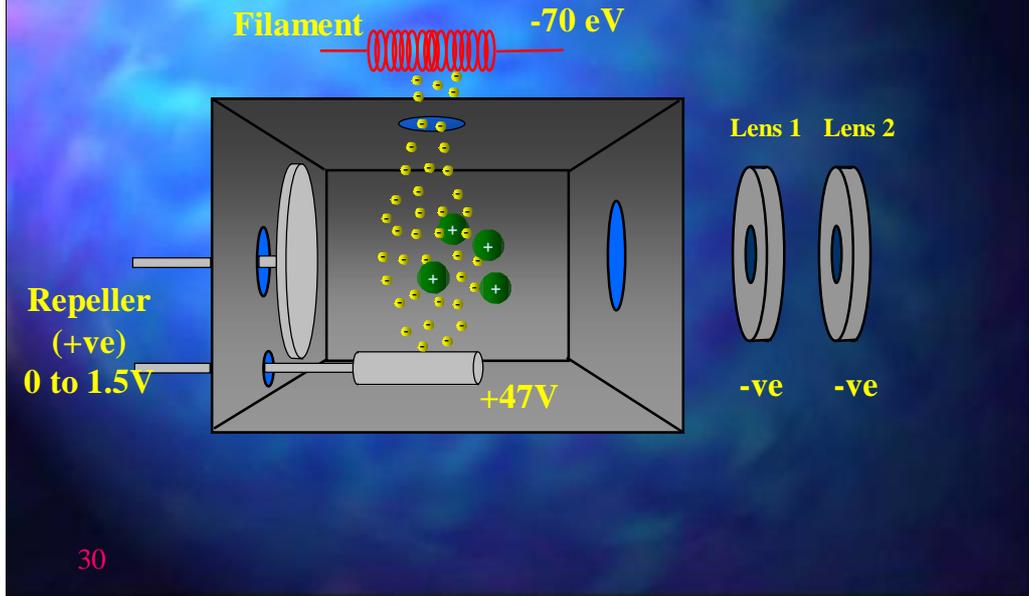
Manual Tuning



29

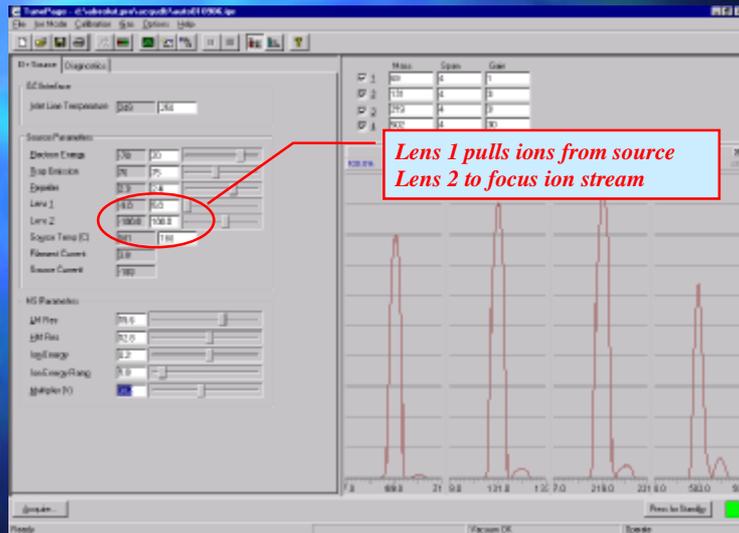
The repeller voltage is usually between 0 and 1Volt. The cleaner the ion source the lower the voltage required. If the Autotune gives a value significantly greater than 1V it suggests that the surface of the repeller electrode is badly contaminated and the source will need cleaning. Autotune will never set a repeller voltage higher than 1.5V. If the repeller is set to 1.5V after an Autotune then it is likely that the source requires cleaning.

Ion Source Effect of Repeller Voltage



The object of the repeller is to broaden the electron beam by applying a positive voltage to the side of the beam. It also repels the positively charged ions towards Lens 1. If the surface of the repeller is dirty than a higher voltage is necessary to achieve the desired effect.

Manual Tuning

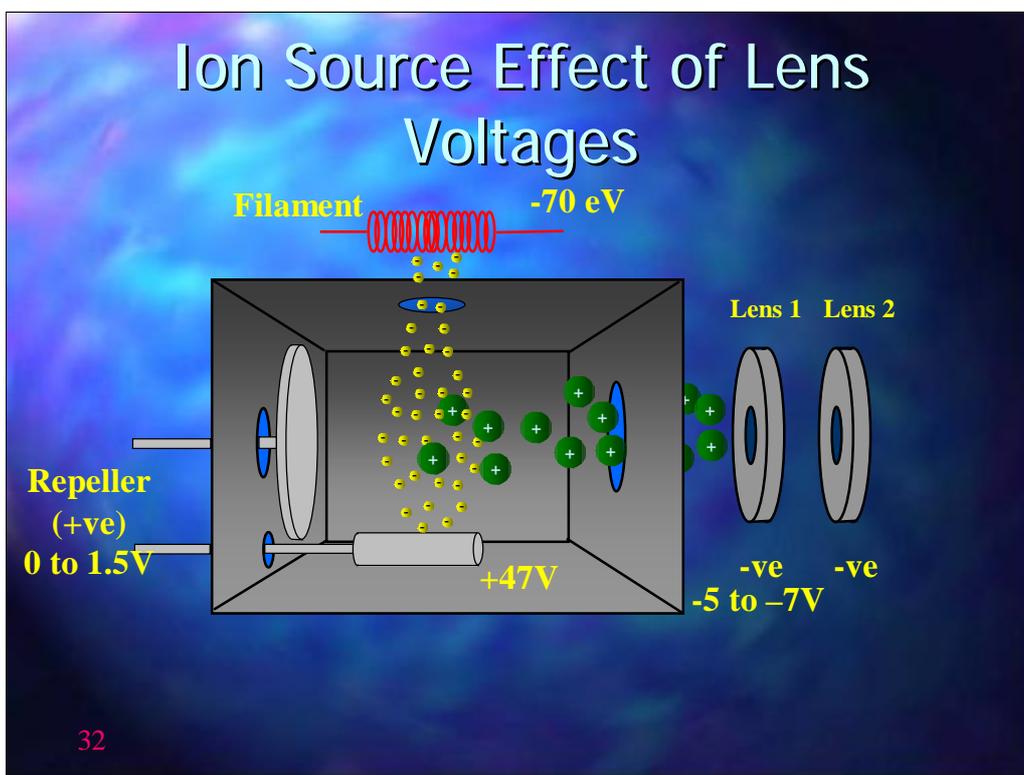


31

Lens 1 has a negative voltage applied to pull the positive ions produced from the ion source. The autotune will set this to $-7V$. Occasionally a slightly better sensitivity may be seen at $-6V$.

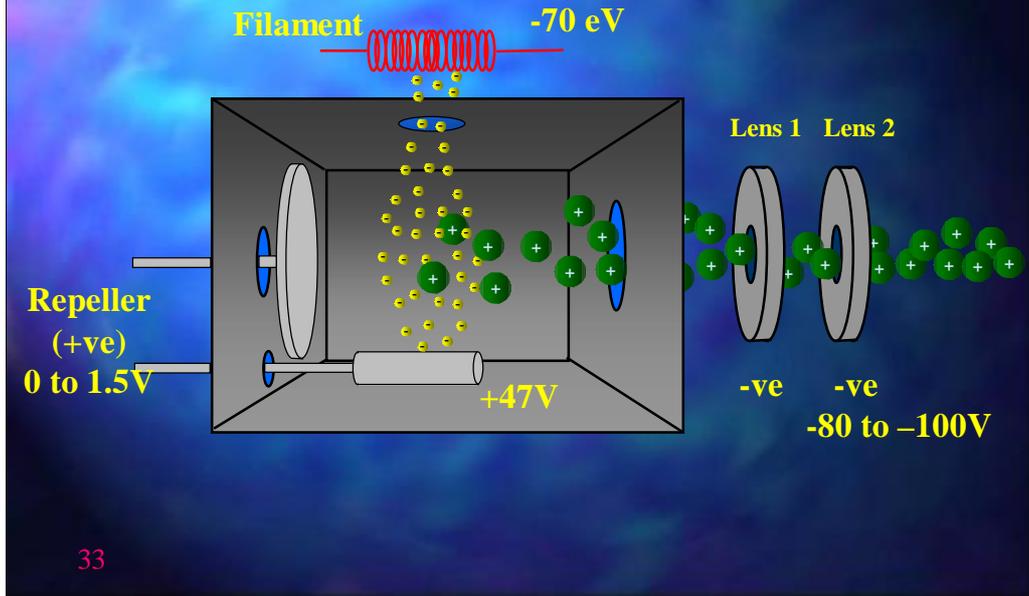
Lens 2 is used to focus the stream of ions into a parallel beam. The voltage applied is generally higher than for Lens 1 and is typically between -80 and $-100V$.

Ion Source Effect of Lens Voltages



Lens 1 voltage is set to -7 volts by the Autotune to pull the positive ions from the source. Occasionally slightly better sensitivity may be achieved by reducing Lens 1 voltage to -6 volts or even -5 volts

Ion Source Effect of Lens Voltages



33

Lens 2 voltage is usually around $-80 \text{ to } -100 \text{ Volts}$ and focuses the positive ions in a parallel beam towards the quadrapoles

Manual Tuning

The screenshot shows the TurboMass software interface with the following parameters:

Parameter	Value
GC Inlet Temperature	250
Source Parameters	
Discharge Energy	750
Drop Distance	76
Electrode	2.3
Lens 1	15.0
Lens 2	19000
Source Temp (C)	881
Filament Current	3.8
Source Current	11.8
MS Parameters	
IM Res	25.0
IM Res	25.0
Ion Energy	3.2
Ion Energy/amp	0.0
Multiplex (Y)	Yes

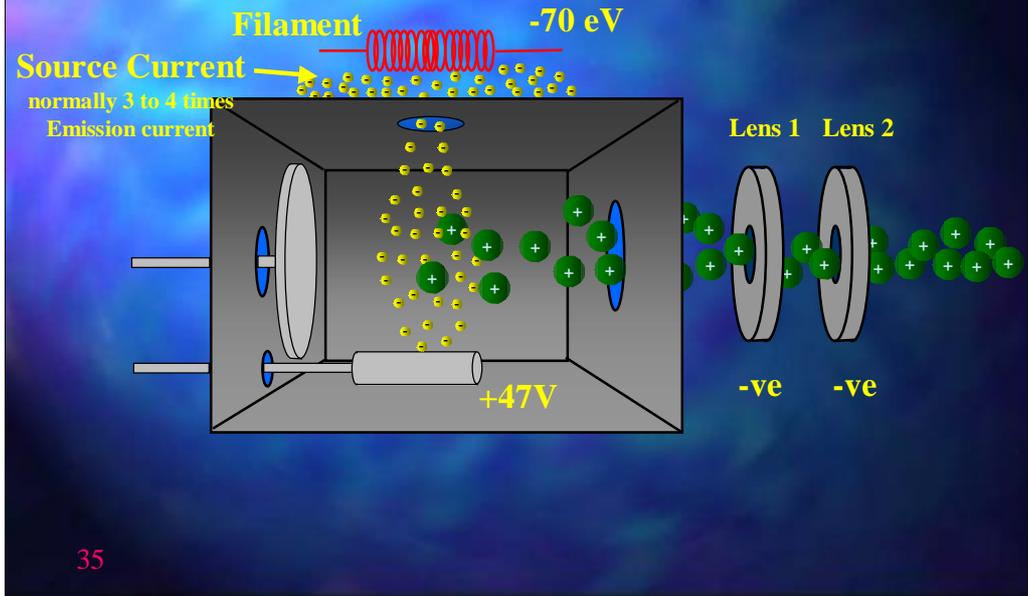
Callout 1: TurboMass uses a Tungsten Filament
Typical values are from 4 - 5 amps

Callout 2: Should be 3 to 4 times Emission current. Higher values suggest a dirty source

34

The filament current is set automatically to give the emission current and is usually set to around 3.5 amps. If it is significantly higher it suggests that the collector electrode is dirty.

Ion Source Effect of Lens Voltages



The source current is a measure of the electrons produced by the filament that ground on the chassis of the ion source. It should be in the order of 3 to 4 times the emission current. If it significantly higher then again it suggests that the collector electrode is dirty.

Manual Tuning

The screenshot shows the manual tuning interface for a mass spectrometer. The interface is divided into several sections:

- Source Parameters:** Includes fields for Electron Energy, Gas Entrance, Expose, Lens 1, Lens 2, Source Temp (C), Filament Current, and Source Current.
- MS Parameters:** Includes fields for LMRes, HMRes, Ion Energy, Ion Energy Ramp, and Multiplier (V).
- Mass, Gain, Signal Table:** A table with columns for Mass, Gain, and Signal. The table contains data for masses 69, 132, 220, and 503.
- Mass Spectrum Plot:** A plot showing the relative intensity of ions versus their mass-to-charge ratio (m/z). The x-axis ranges from 70 to 500, and the y-axis represents relative intensity. Several peaks are visible, corresponding to the masses listed in the table.

Red callouts highlight specific parameters and their effects:

- LMRes:** Affects low mass peak width.
- HMRes:** Affects high mass peak width.
- Ion Energy:** Max Signal to Noise ratio at 480 to 500 V but adjust so that chromatographic peaks are not saturated.

36

There are 2 parameters that will affect resolution; LMRes affects resolution at low masses whilst HMRes affects the resolution at high masses. In Autotune they are set up using masses 69 and 502 respectively. Changing these will affect the peak width and therefore the intensity. These may be used in manual tune to change the relative intensities of the tune peaks. A smaller value will make the peak broader and therefore increase its relative intensity. Masses that occur between 69 and 502 will also be affected by these settings. If these are used to adjust the relative peak intensities then the gain should be increased so that the ions 70, 132, 220 and 503 can be seen along side the major ions. There should not be baseline separation between each ion pair but the valley between the 2 ions should be approximately 20 to 50% of the minor ion intensity.

The ion energy and ion energy ramp affect the way the quadrupoles are ramped up. These should only be touched as a last resort but if a satisfactory tune cannot be achieved try setting the MS ion energy to 0.3 or less and the IE Ramp to 3 to 4 and then readjust the Lens 2 voltage.

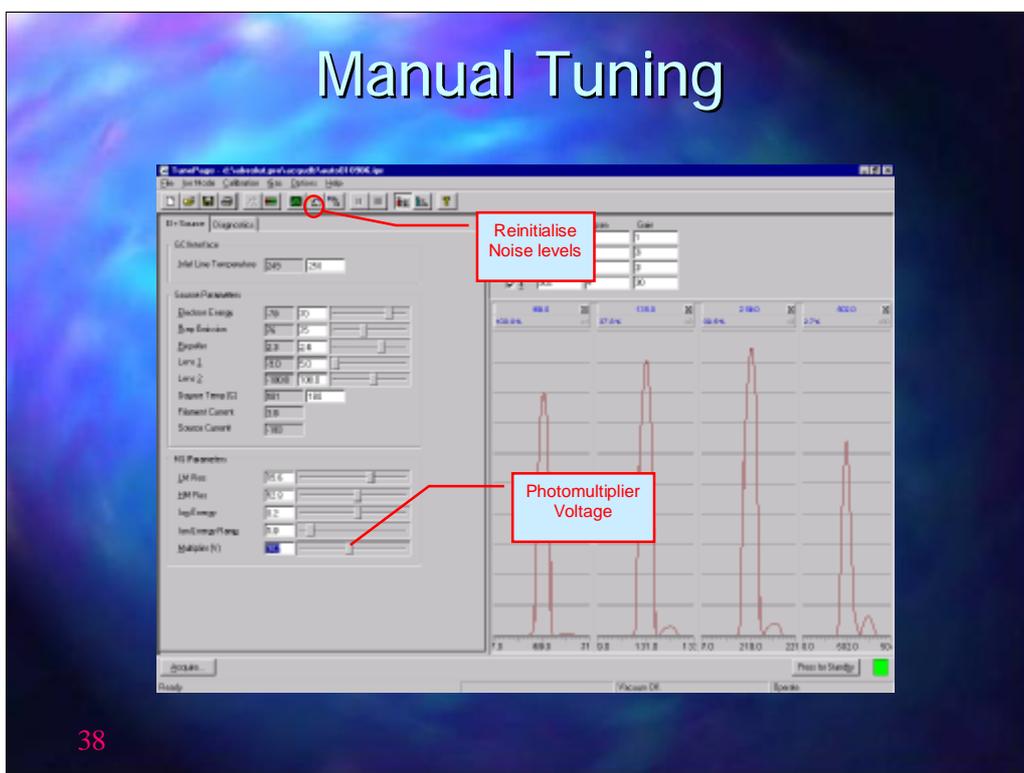
Manual Tuning (TurboMass)

37

On the MS page there are 2 parameters that will affect resolution; LMRes affects resolution at low masses whilst HMRes affects the resolution at high masses. In Autotune they are set up to masses 69 and 502 respectively. Changing these will affect the peak width and therefore the intensity. These may be used in manual tune to change the relative intensities of the tune peaks. A smaller value will make the peak broader and therefore increase its relative intensity. Masses that occur between 69 and 502 will also be affected by these settings. If these are used to adjust the relative peak intensities then the gain should be increased so that the ions 70, 132, 220 and 503 can be seen along side the major ions. There should not be baseline separation between each ion pair but the valley between the 2 ions should be approximately 20 to 50% of the minor ion intensity.

The ion energy and ion energy ramp affect the way the quadrupoles are ramped up. These should only be touched as a last resort but if a satisfactory tune cannot be achieved try setting the MS ion energy to 0.3 or less and the IE Ramp to 3 to 4 and then readjust the Lens 2 voltage.

Manual Tuning



38

Once a satisfactory tune has been achieved the photomultiplier voltage must be adjusted to obtain the best sensitivity for the samples to be run and then the noise level must be reinitialised by clicking on the button shown. Noise re-initialisation must be done whenever the photomultiplier voltage is changed.

Lastly turn off the reference gas and then turn on PUMP OUT REFERENCE GAS accessed from the GAS menu.

It is recommended that the PUMP OUT REFERENCE GAS is left turned on as it reduces any background interferences and this is done by saving the tune file with PUMP OUT REFERENCE GAS turned on.

Caution: If PUMP OUT REFERENCE GAS is not turned off before the reference gas is turned on again the reference gas vial will be emptied very quickly!

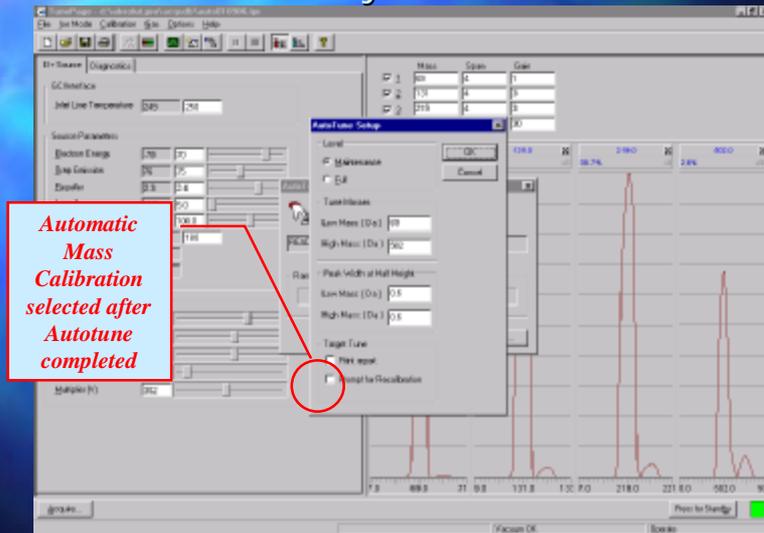


Mass Calibration

39

Mass Calibration

Automatically after Autotune



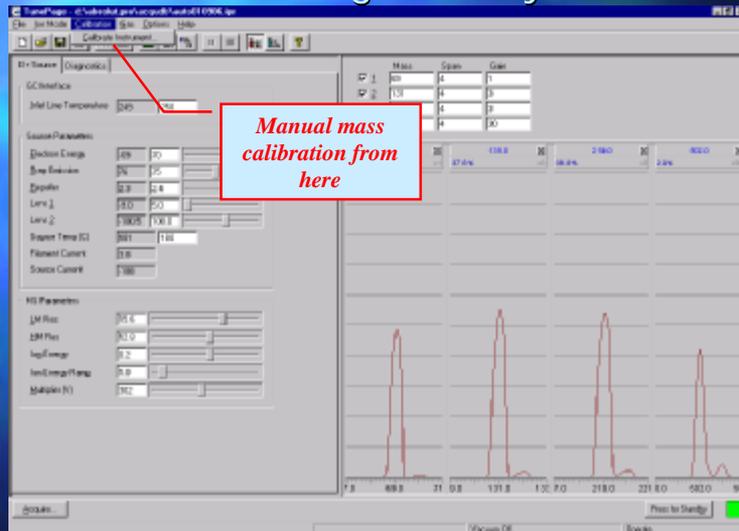
40

Mass calibration is used to ensure that the ions detected from the reference gas are assigned the correct masses. This may be set up as part of the tune procedure if the PROMPT FOR RECALIBRATION has been ticked. Mass calibration should not need to be done at the same frequency as tuning, maybe once a month.

NOTE Mass Calibration will require the reference gas is turned on

Mass Calibration

Accessing manually

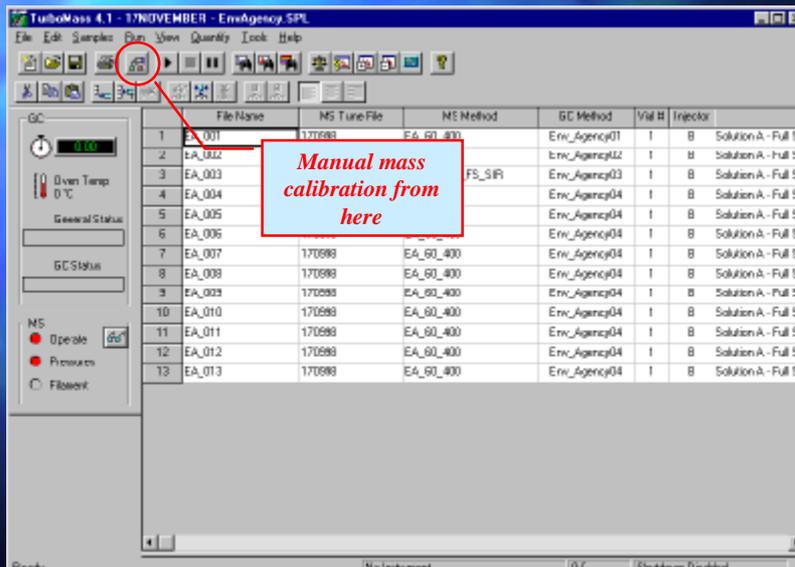


41

Alternatively mass calibration may be accessed manually from the control panel

Mass Calibration

TurboMass Accessing manually

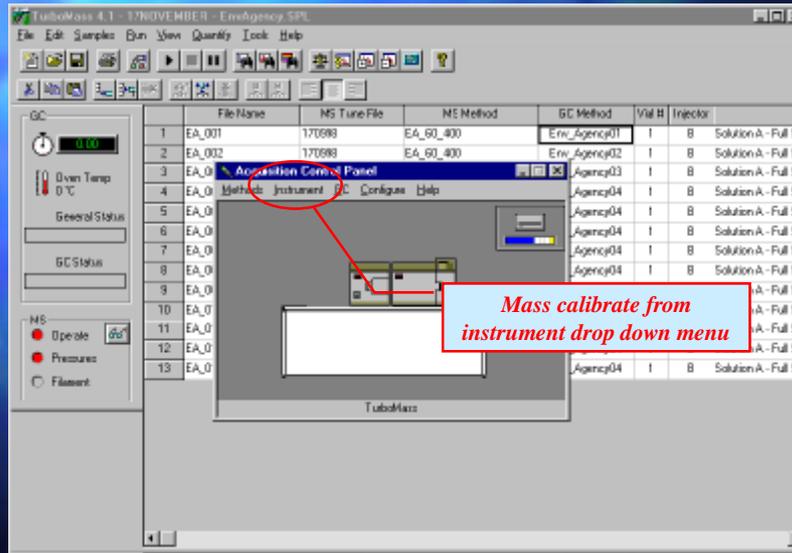


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Alternatively mass calibration may be accessed manually from the control panel

Mass Calibration

TurboMass Accessing manually

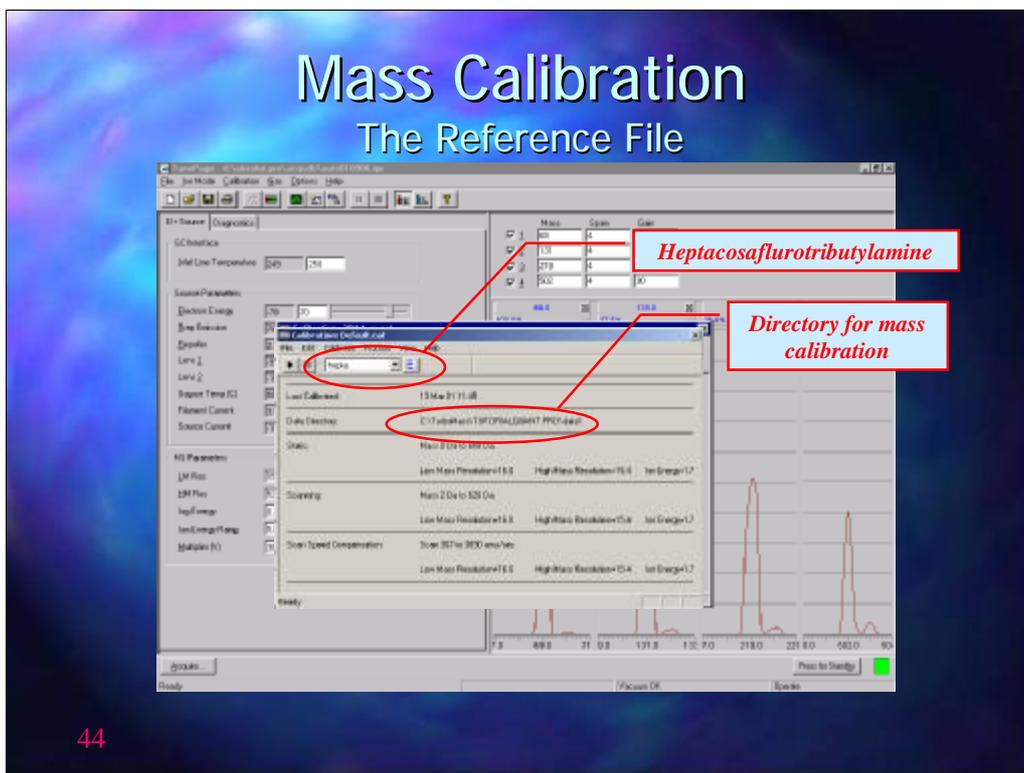


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From the control panel page Mass Calibration is under the Instrument menu

Mass Calibration

The Reference File



44

During Mass calibration each peak is compared to a text file containing the ion masses and intensities for the reference gas. The file to use for heptacosafuorotributylamine is called heptaref. As heptacosafuorotributylamine contains very few ions below 69 air peaks may also be used to calibrate a low masses. If this is ticked then 4, 18 and 28 will also be matched but not 32.

Mass Calibration

The Reference File

Access to the text file

Includes air peaks (4=He, 18=H₂O, 28=N₂)

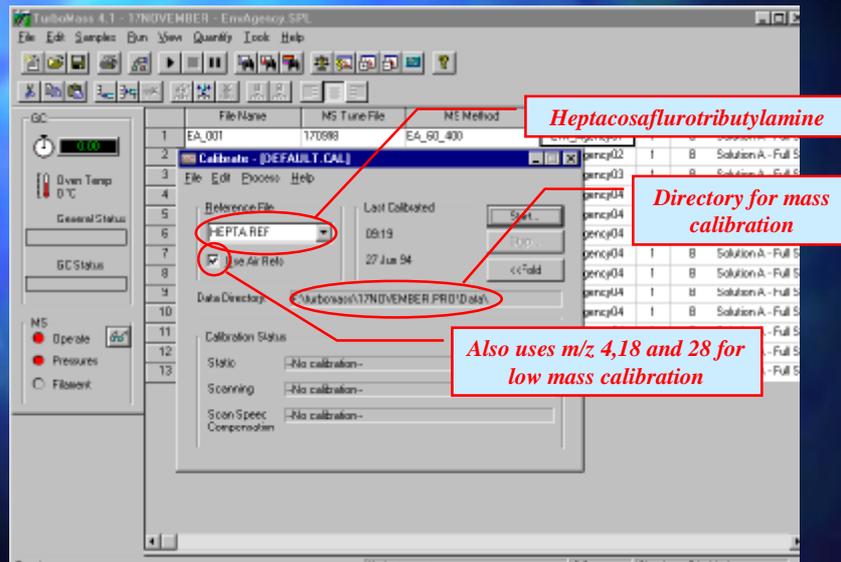
Mass	Abundance
4.00260	10
18.0106	7
28.0064	5
43.02329	0.33
68.99518	100.00
99.99358	6.31
118.99199	7.00
138.99199	36.31
149.99039	1.29
168.98877	3.24
188.98877	1.31
218.98560	39.07
263.98705	0.38
313.98386	0.32
375.98067	0.38
413.97748	1.64
463.97429	1.02
501.97110	1.90
537.97112	0.13
575.96796	0.32
613.96471	0.64

45

The text file used for heptacosafuorotributylamine is called heptaref. As heptacosafuorotributylamine contains very few ions below 69 air peaks may also be used to calibrate a low masses. On the TurboMass Gold or the Clarus 500MS the air peaks are included in the heptaref file.

Mass Calibration

TurboMass The Reference File

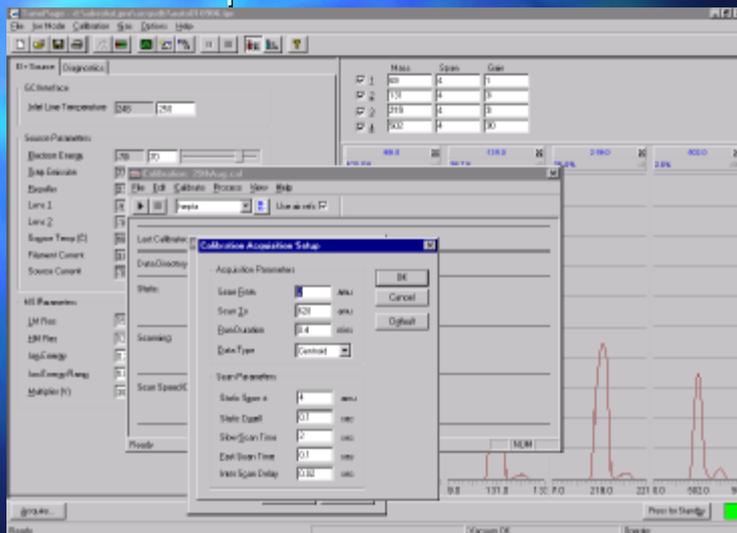


46

During Mass calibration each peak is compared to a text file containing the ion masses and intensities for the reference gas. The file to use for heptacosafurotributylamine is called heptaref. As heptacosafurotributylamine contains very few ions below 69 air peaks may also be used to calibrate a low masses. If this is ticked then 4, 18 and 28 will also be matched but not 32.

Mass Calibration

Acquisition Parameters



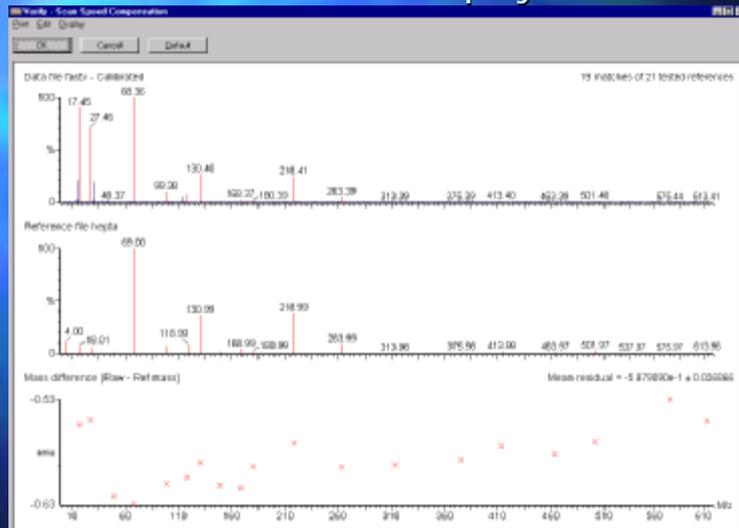
48

Acquisition parameters determine the scan range and the run duration for each run. The DATA TYPE must always be set to Centroid when using GC/MS.

Scan Parameters determine how each mass calibration scan is performed. The default values are usually adequate but if very fast scanning rates are to be used it may be necessary to perform the Scan speed compensation mass calibration at a faster speed than normally used.

Mass Calibration

Calibration Display



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The display above shows a typical mass calibration run. In this example the fast scan verification is shown. The top display shows the re-calibrated masses whilst the second display shows the reference file. The third display shows the residuals errors after recalibration.

