Agilent G3250AA LC/MSD TOF System

Quick Start Guide

Use this guide for your first steps with the Agilent LC/MSD TOF System, and as a roadmap for your user information.

What is the Agilent G3250AA LC/MSD TOF system?

The Agilent TOF is an orthogonal-axis time-of-flight mass spectrometer (oa-TOF). That is, the ions reaching the time-of-flight chamber are impelled in a direction perpendicular to their original path.

You can set up an Agilent time-of-flight mass spectrometer system (TOF) in several configurations:

ESI – Electrospray Ionization
APCI – Atmospheric Pressure Chemical Ionization
APPI – Atmospheric Pressure Photo Ionization
MALDI – Matrix-Assisted Laser Desorption Ionization
MMI - Multimode Ionization

- For normal flow LC/MS with a binary pump, quaternary pump, well-plate sampler (or autosampler) and ESI , APCI, APPI or MMI ion sources.
- For microflow LC/MS with a capillary pump, micro well-plate sampler and ESI, APCI or MMI ion source
- For nanoflow LC/MS with a nanopump, micro well-plate sampler and nanospray source or dual nanospray source
- TOF system with an AP-MALDI source

Each Agilent system has advantages for drug discovery — high throughput sample screening with highly sensitive detection and accurate mass assignment.

Each system uses the same Agilent TOF software to enable these advantages, although the AP-MALDI TOF system uses only the TOF portion of the software.

Agilent Technologies

You use the Agilent TOF software for setting up and running data acquisition. For data analysis, Agilent provides a copy of PE Sciex Analyst QS 1.1, especially modified for the Agilent LC/MSD TOF system.

What's New in A.02.00

Agilent TOF software has many new features in this revision.

- Multimode Ionization source is supported.
- CTC PAL autosampler which can hold 24 trays of 96 samples is supported.
- G1315C DAD is now supported.
- Seven layouts are available to allow you to quickly change which panes are displayed.
- New toolbar allows you to change layouts or load a new method or worklist.
- Polarity switching can be done at the scan level with SmartCard 4 and updated power supplies.
- Worklist can be paused and the current run stopped.
- Worklist Table checkboxes are now cleared during a run by default.
- The system can automatically increment the data file name when reinjecting samples.
- The Empirical Formula Confirmation report can include sample purity information.
- The new Molecular Feature Extraction Report shows a list of isotopes for compounds found in a sample.
- The Mass List report is now the default Data Analysis report.
- For DA reprocessing, sample name defaults to the first sample in the data file.
- BioConfirm if installed, must be upgraded to A.02.00.
- Easy Access for TOF, if installed, must be upgraded to A.04.01 or later.
- Software Revision
- This guide is valid for A.01.xx revisions of the Agilent G3300A TOF Software software, where xx refers to minor revisions of the software that do not affect the technical accuracy of this guide.AnalystQS 1.1 from PE-SCIEX is supported and must be installed.
- Data Browser AEV files created for mass list and Empirical Formula Confirmation reports are supported with Agilent LC/MS Data Browser A.03.01.
- TOF System Confirmation has added switch for turning on AEV reports.
- Mass Hunter application has been added to TOF software.
- Windows XP Service Pack 2 is supported.

Where to find information

Online Help

Press F1 To get more information about a pane or dialog box, place the cursor on the part of the pane or dialog box of interest and press **F1**.

Help menu From the Help menu, access “How-to” help, reference help and the Agilent support website (Resources).

PE-Sciex Analyst online help Refer to Analyst online help to learn how to view, quantitate and report on Agilent LC/MSD TOF results.

Documents

You can find these manuals delivered with the TOF hardware or software. You can also find a PDF version on the installation CD-ROM, in the **Manuals** folder.

TOF User’s Guide Use this guide to install and set up the TOF hardware. This guide also contains background information to help you operate, maintain and troubleshoot the TOF.

LC/MSD TOF System Installation Guide This guide is used by the Agilent customer engineer to install the LC/MSD TOF hardware and TOF Software, configure the instrument, and verify performance.

You can find these manuals on the installation CD-ROM, in the **Manuals** folder.

Concepts Guide - The Big Picture Learn the background information to help you make selections in the software.

Familiarization Guide Do the exercises to learn to use the TOF software.

Training

Familiarization Guide Use this guide as a training lab.

Training Courses Visit www.chem.agilent.com to view a listing of training courses for the Agilent LC/MSD TOF system.

Instructional overview

1 Install the TOF hardware

Use the *Agilent G1969 LC/MSD TOF User's Guide* to install the hardware.

2 Install the software

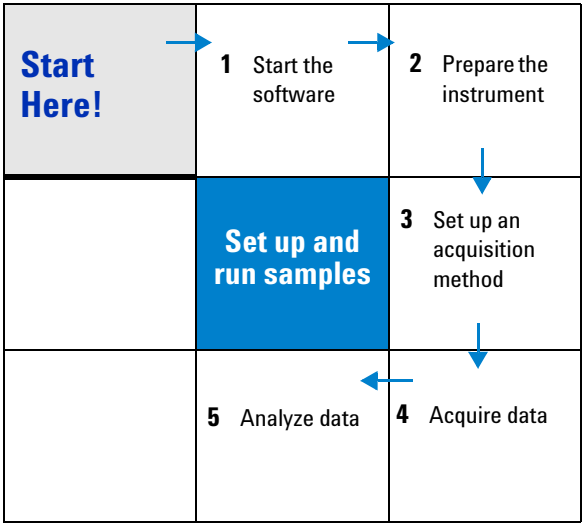
Use the instructions in the *Agilent 3250AA LC/MSD TOF System Installation Guide* to install both the TOF software and the Analyst software. The sequence in which you install the software is listed below:

- **a** Install Analyst QS 1.1.
- **b** Install the Agilent TOF software.
- **c** Configure the instrument for the first time.
- **d** Start the software and verify performance.

3 Set up and run samples

The roadmap below shows you the steps to set up and run a sample from start to finish. Follow the instructions on the next pages to get started and to learn where to find the information to help you with each step in this roadmap.

Read the Concepts Guide for background on these steps.



Step 1—Start the software

The instructions below include the following assumptions:

- The hardware and software are installed.
- The instrument is configured.

  Use instructions in the *Installation Guide* to configure the instrument for the first time.
- The LC modules and the TOF are turned on, but the pump is not running.

Start software/check configuration

1 **Double-click the Agilent TOF group on your desktop.**

2 **Double-click the TOF icon to start the software engines.**

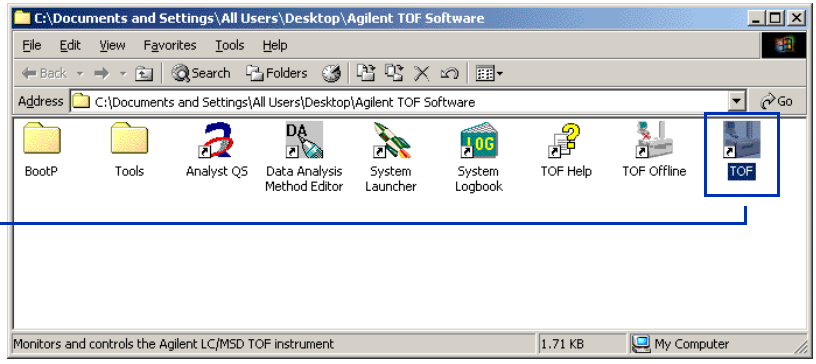
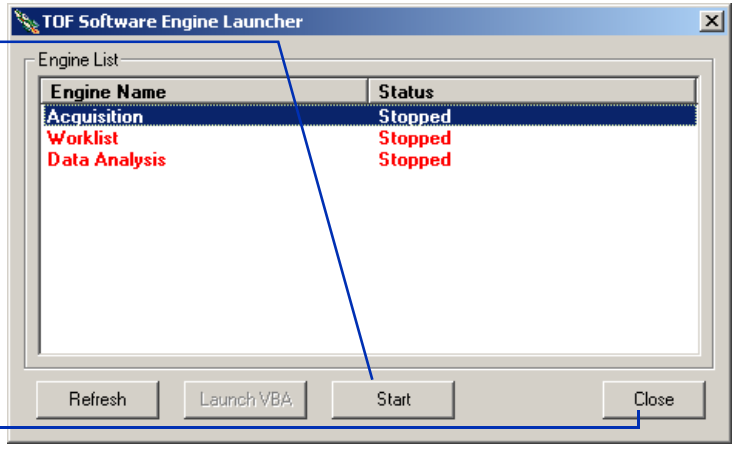


Figure 1 Agilent TOF group window

3 Click Start.

4 When all of the engines say "Running", click Close.

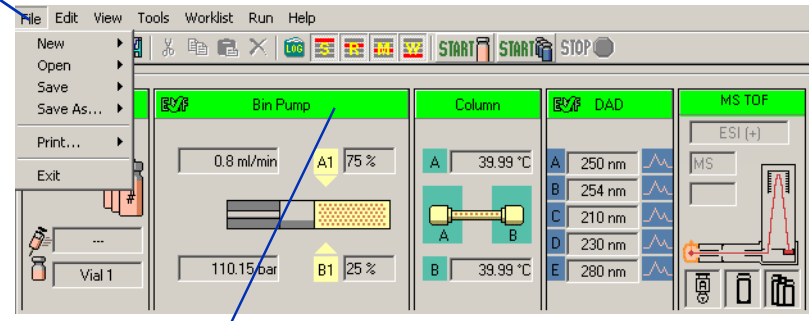
5 Double-click the TOF icon in the Agilent TOF group (Figure 1).



The main window appears. See Figure 2 on page 7. The top pane of this window is the Instrument Status pane. (Figure below)

6 Make sure that the LC modules are the ones that you want configured with the instrument. (See below.)

- **Select File > Print > Instrument Configuration, OR**
- **Check the headers of the LC modules labeled in the Instrument Status pane.**



If LC modules other than those you intend to use appear in the Instrument Status pane or the Configuration report, use the Online Help to access instructions to *reconfigure* the instrument.

Four panes—where you do most of your work

When you first start the TOF software, the main window appears. You do almost all of your work within the four panes of this main window. These panes provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status and monitor runs.

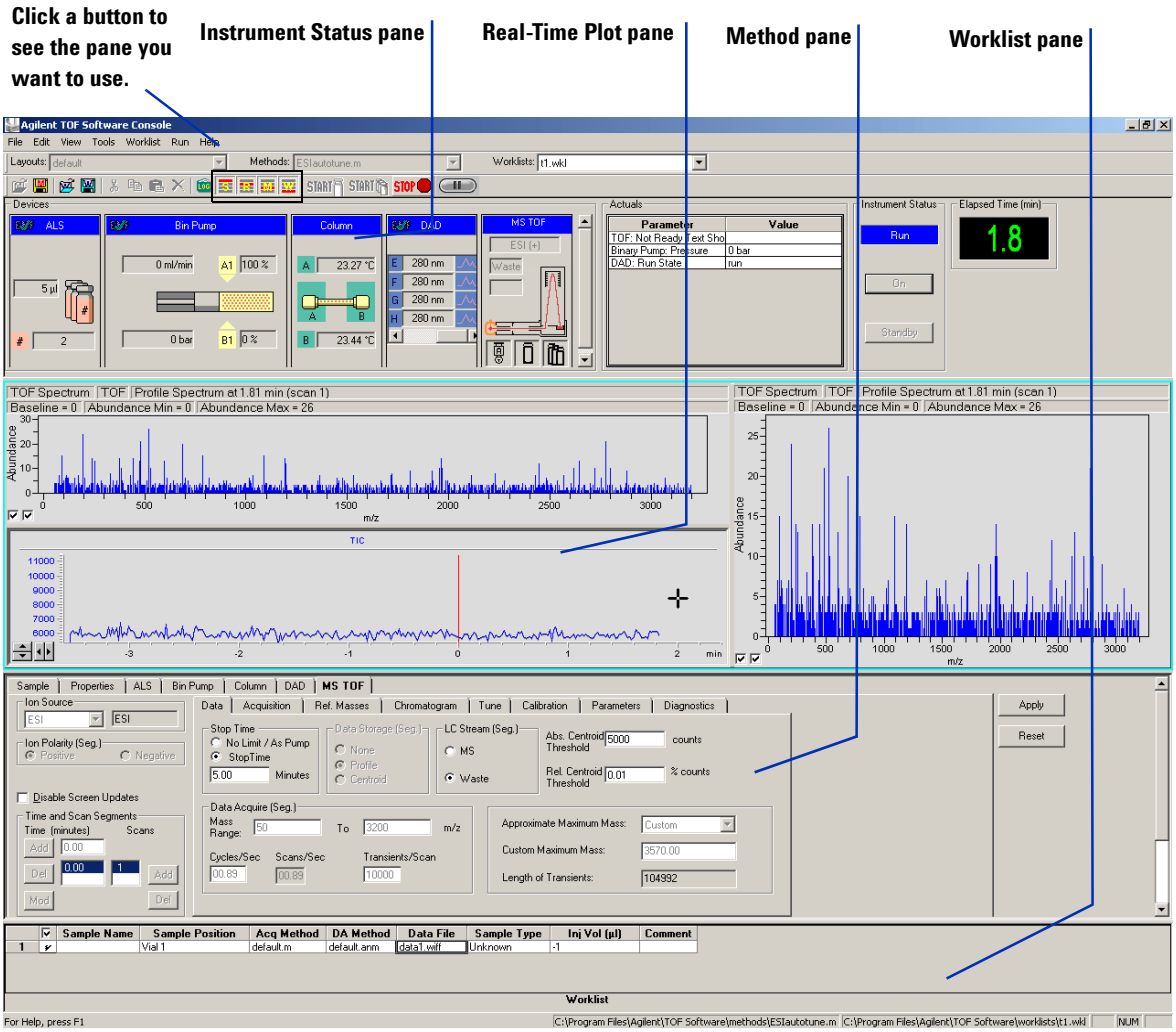


Figure 2 Main window of the TOF application

Show/hide the panes

You can show one pane at a time on the screen or up to four panes. You can never hide all four panes. To show or hide a pane, you click on the icons in the main window toolbar.

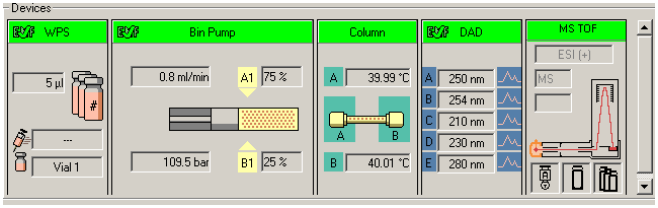
When you click on a pane, the active pane is outlined in blue. Press F1 to obtain help on the active pane. You can also drag a pane border to resize the pane.

S–Instrument Status; R–Real-Time Plot
M–Method; W–Worklist

Instrument Status pane

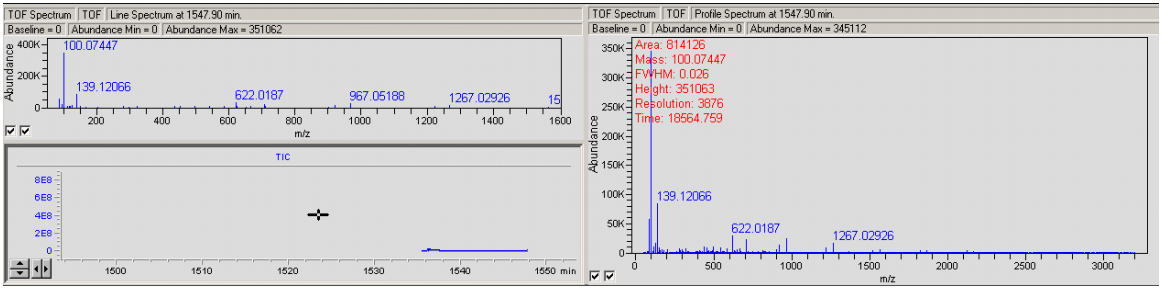
You may have several different LC modules in your LC stack, for example, both a well-plate sampler and micro well-plate sampler. With this pane you can make sure that the correct LC module is configured.

You also set non-method control and configuration parameters for the LC devices and TOF and monitor the status of the device parameters during a run.



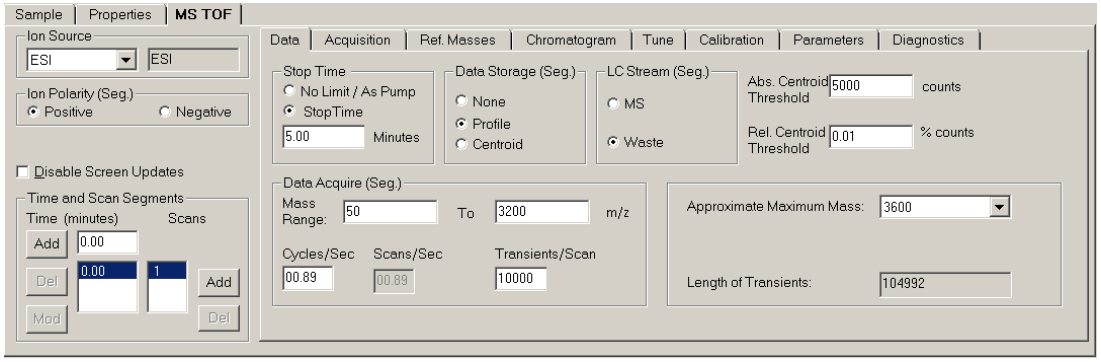
Real-Time Plot pane

With this pane you monitor the plot of chromatograms and spectra in real time.



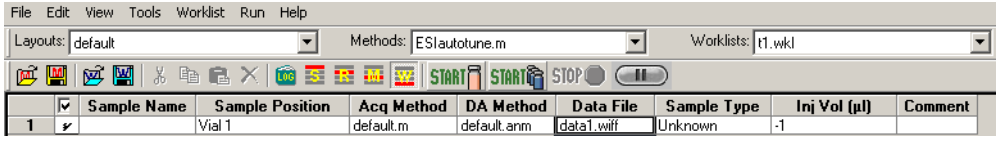
Method pane

With this pane you enter instrument settings for acquisition methods and sample information to run individual samples interactively.



Worklist pane

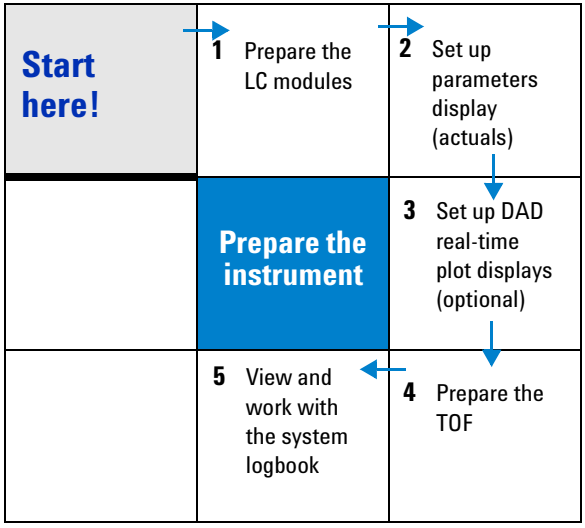
With this pane you enter sample information for individual samples and information for batch samples. When you run the worklist, the samples and batches are automatically run in the order listed in the worklist.



Step 2—Prepare the instrument

Read and follow the steps in the user information listed below to learn how to prepare the instrument for a run.

- The steps on the next pages that take you through the roadmap below.
- Chapter 2 of the *Concepts Guide*, Instrument Preparation, for background information that you may need to prepare the LC/MSD TOF.
- Chapter 1, Prepare the instrument, in the *Familiarization Guide* to learn to prepare the LC and TOF to run an ESdemo sample.
- *Online Help* under Master Task List, LC Startup and TOF optimization and calibration.



Prepare LC modules

Switch LC stream to Waste

While you purge the LC pump and condition or equilibrate the column, you can tune and calibrate the TOF. During this time you do not want effluent streaming into the TOF.

If you specify that the LC stream goes to Waste and not to the TOF, the stream passes through the DAD. You can then monitor the fluctuations of the DAD real-time chromatogram and spectra before a run.

1 Click the **Method pane** icon to view the Method pane.

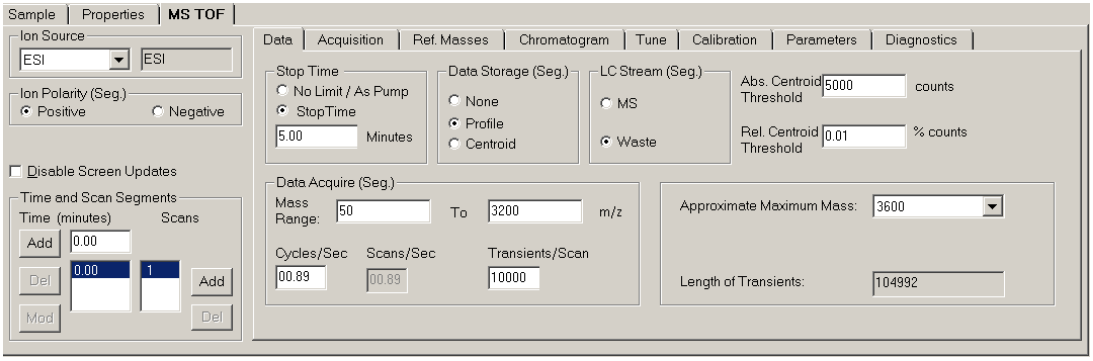


Figure 3 Data tab of the MS-TOF tab in the Method pane

2 Click the **MS-TOF** tab of the Method pane.

3 Click the **Data** tab within the MS-TOF tab.

4 Select **Waste** if not already selected.

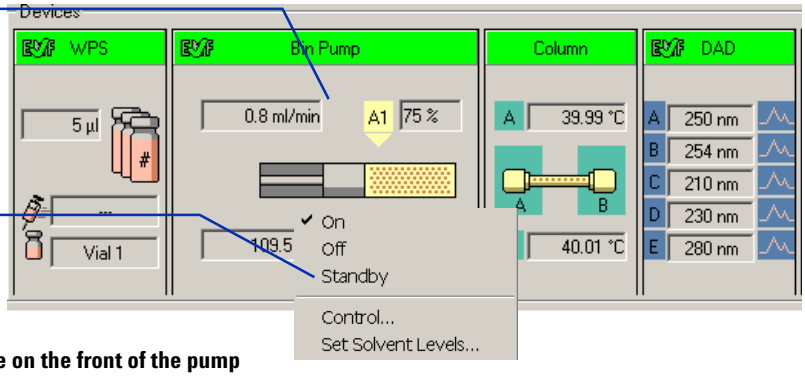
5 Click **Apply**.

Purge the LC pump

Purge the binary pump You purge the binary pump manually.

1 **Right-click the binary pump status box in the Instrument Status pane.**

2 **Select Standby in the shortcut menu.**



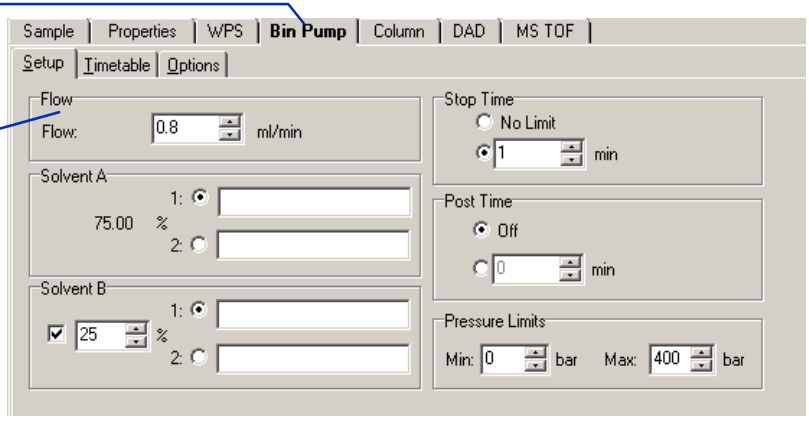
3 **Turn the black valve on the front of the pump counter-clockwise two turns.**

4 **Click the Bin Pump tab in the Method pane.**

5 **Enter a Flow of 5 ml/min, and click Apply.**

6 **Select On in the binary pump shortcut menu.**

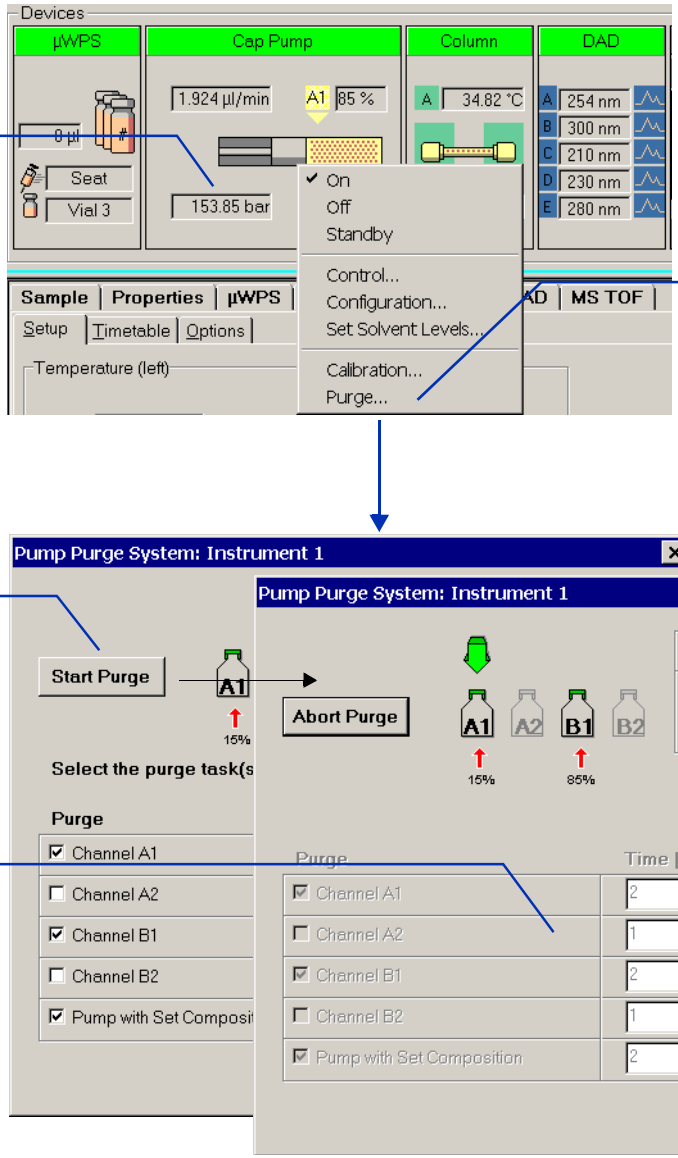
7 **After purging, enter the flow you use to equilibrate the column, and click Apply.**



8 **Close the black valve.**

Purge for about 9 minutes to pass about 45 ml or 3X the volume for the binary pump.

Purge the capillary or nano pump



1 **Click pump device in the Instrument Status pane to bring up pump device menu.**

2 **Select Purge to bring up the Pump Purge System dialog box.**

3 **Start the purge with this dialog box and bring up the monitoring dialog box.**

4 **Monitor the purge with this dialog box and click Cancel after the purge is complete.**

Condition or equilibrate the column

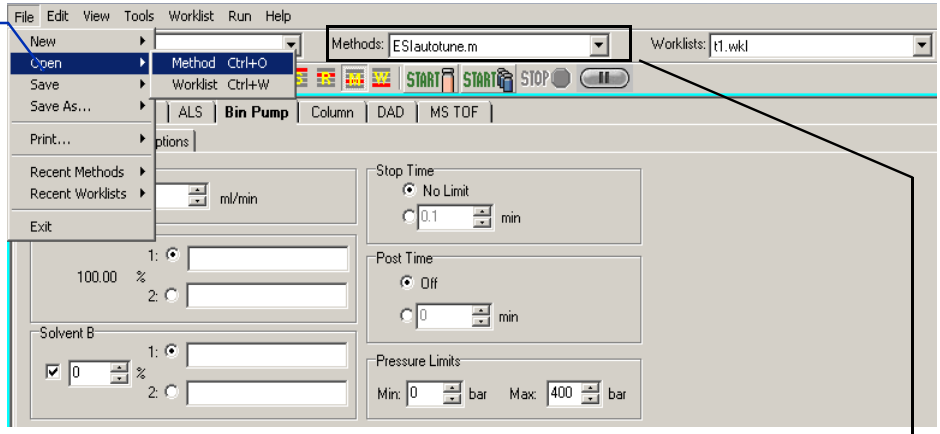
After you purge the pump, you set up to condition or equilibrate the column.

- Enter and download LC parameters, OR open a conditioning method.
- Change any non-method control parameters, if necessary.
- Monitor the baseline and adjust the plot to make sure the column is equilibrated and the baseline stable. (See "Set up to view real-time parameter values (actuals)" on page 16 and "Set up DAD chromatographic and spectral displays (optional)" on page 17.)

Enter and download LC parameters or open a conditioning method

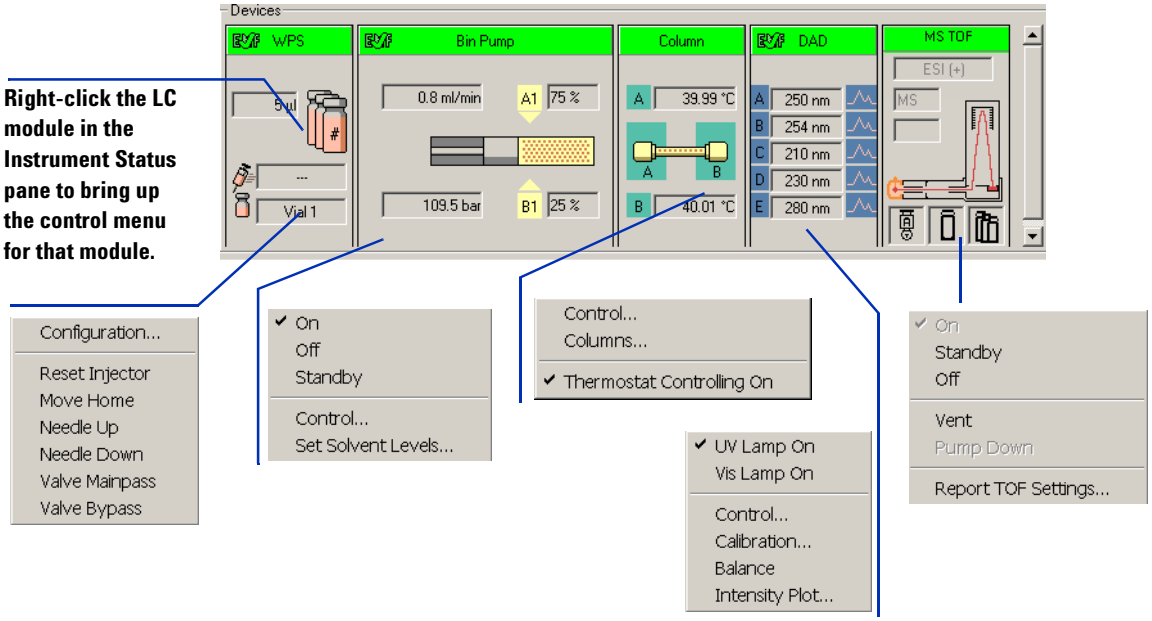
1 **Select Open from the File menu to open a method, OR enter LC parameters in the Method pane.**

2 **Click Apply to send the parameters to the LC.**



You can also load a method using the Methods selection box in the User Selections toolbar.

Change non-method control/configuration parameters, if necessary. With these menus, you can set the time to automatically turn the module on or off, you can set maximum values or you can configure the autosampler.



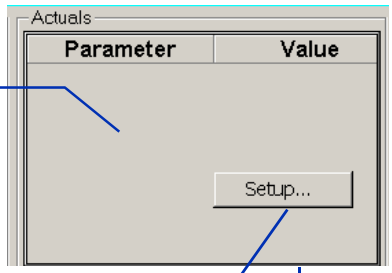
Set up to view real-time parameter values (actuals)

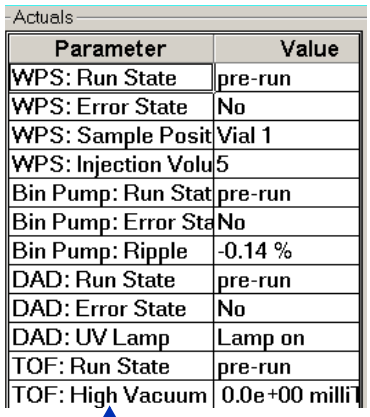
As you prepare for a run and during a run, you want to see the actual values of the instrument parameters. You can do this in the Instrument Status pane.

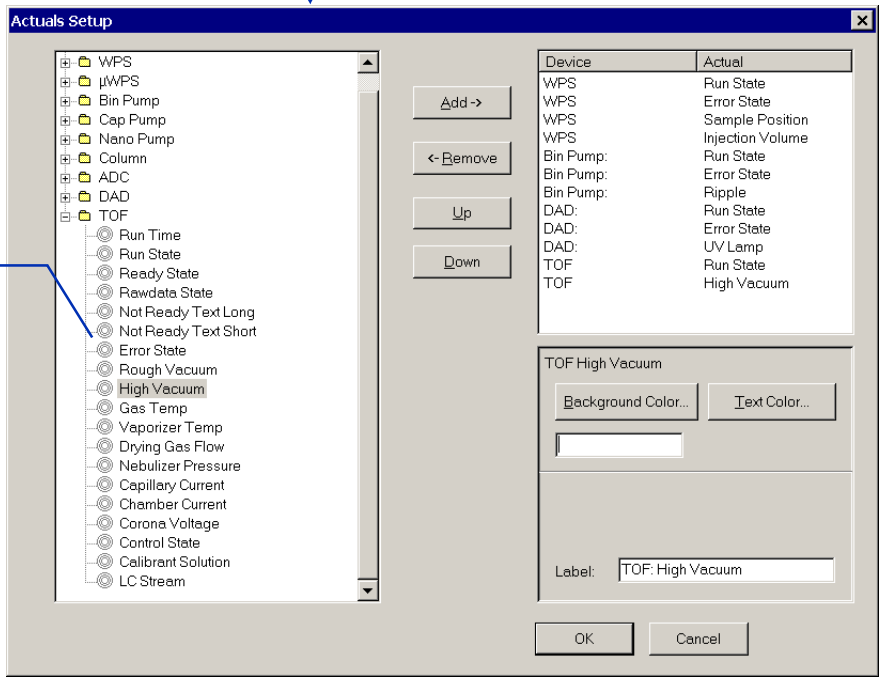
1 **Right-click the Actuals box to bring up Setup item.**

2 **Click Setup to bring up the tool to select instrument actuals.**

3 **Select actuals to set up to view the actual conditions in the Instrument Status pane.**







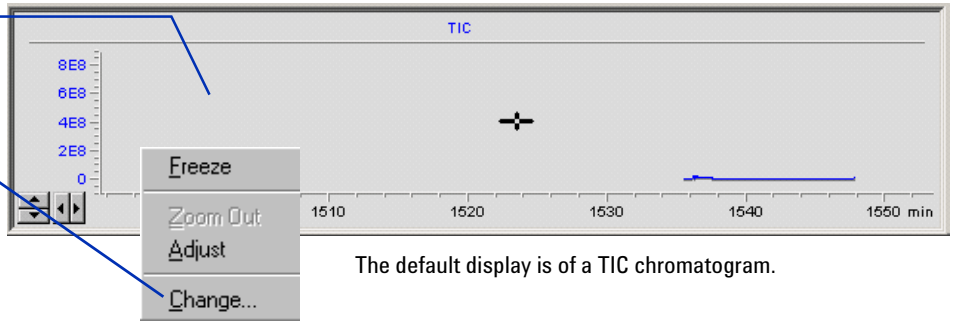
Set up DAD chromatographic and spectral displays (optional)

As you condition the column, you set up the displays to monitor the effluent.

Set up chromatographic display

1 **Right-click the signal plot to bring up the signal shortcut menu.**

2 **Select Change to bring up the tool for selecting the signal and its plot parameters.**

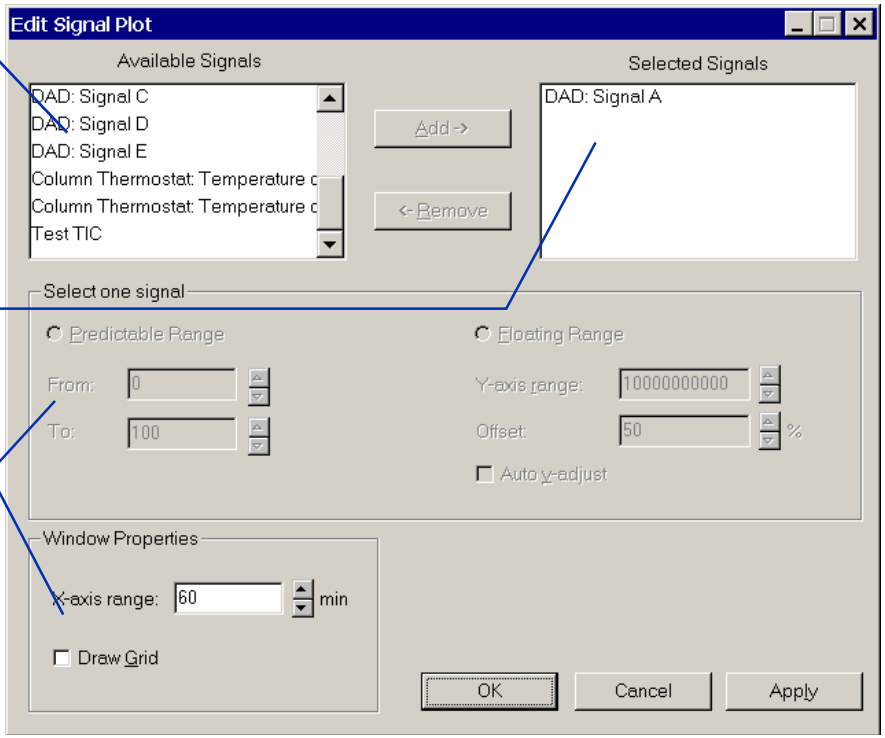


The default display is of a TIC chromatogram.

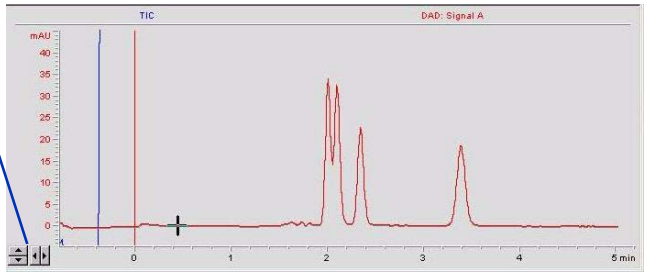
3 **Select a DAD signal that you intend to monitor, and click Add.**

4 **Highlight a Selected Signal.**

5 **Set the y and x axis ranges, and click OK.**



The real-time plot now displays the DAD signal. (See next page.)



Adjust the plot with these arrows.

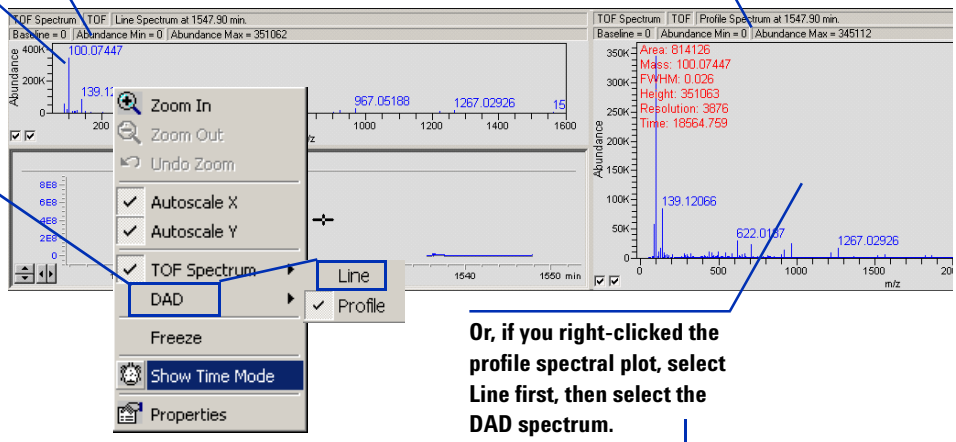
Set up spectral display

The default spectral display is a TOF line spectrum above the chromatogram and a profile spectrum to the right of the chromatogram.

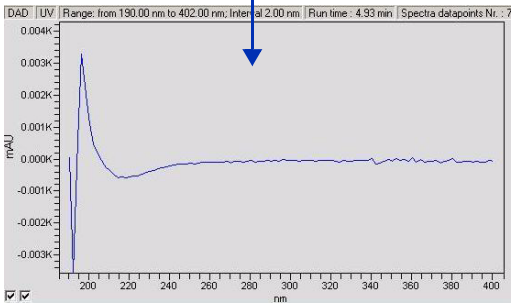
Line spectrum

Profile spectrum

1. **Right-click the line or profile spectral plot to bring up the spectra shortcut menu.**
2. **If you clicked the line spectral plot, select the DAD spectrum as the spectrum to view.**



Or, if you right-clicked the profile spectral plot, select Line first, then select the DAD spectrum.



Prepare the TOF

Calibrate the TOF

You calibrate the TOF more frequently than you tune the TOF. Agilent recommends that you do a 10 mass calibration. Make sure that you open the method corresponding to your ion source before you calibrate or tune the TOF to set default TOF acquisition parameters.

- ESIautotune.m for ESI
- nanoESIautotune.m for nanospray or dual nanospray
- APPIautotune.m for APPI
- APCIautotune.m for APCI
- MMIautotune.m for MMI

You cannot calibrate the TOF with a MALDI source installed.

If the method loaded does not match the current ion source, then a warning is given.

Polarity Switching

If you are using Polarity Switching, you need to use a different autotune method. For each source, there is a positive and a negative method for Polarity Switching. The name of the autotune method has either “PolaritySWPos” or “PolaritySWNeg” appended to it.

- ESIautotunePolaritySWPos.m for ESI
- ESIautotunePolaritySWNeg.m for ESI
- nanoESIautotunePolaritySWPos.m for nanospray or dual nanospray
- nanoESIautotunePolaritySWNeg.m for nanospray or dual nanospray
- APPIautotunePolaritySWPos.m for APPI
- APPIautotunePolaritySWNeg.m for APPI
- APCIautotunePolaritySWPos.m for APCI
- APCIautotunePolaritySWNeg.m for APCI
- MMIautotunePolaritySWPos.m for MMI
- MMIautotunePolaritySWNeg.m for MMI

You will need to perform four autotunes to correctly tune the TOF system when using Polarity Switching. First, you need to tune in both positive and negative modes. Then, you need to tune using the Polarity Switching methods in both positive and negative modes.

Check and do a tune

1 **Click the MSTOF tab in the Method pane and select ion polarity.**

2 **Enter calibration parameters.**

5 **If you want to select a different set of masses or you want to use a different calibration standard, click Show Extended.**

6 **Select masses to use for calibration, OR load another mass list.**

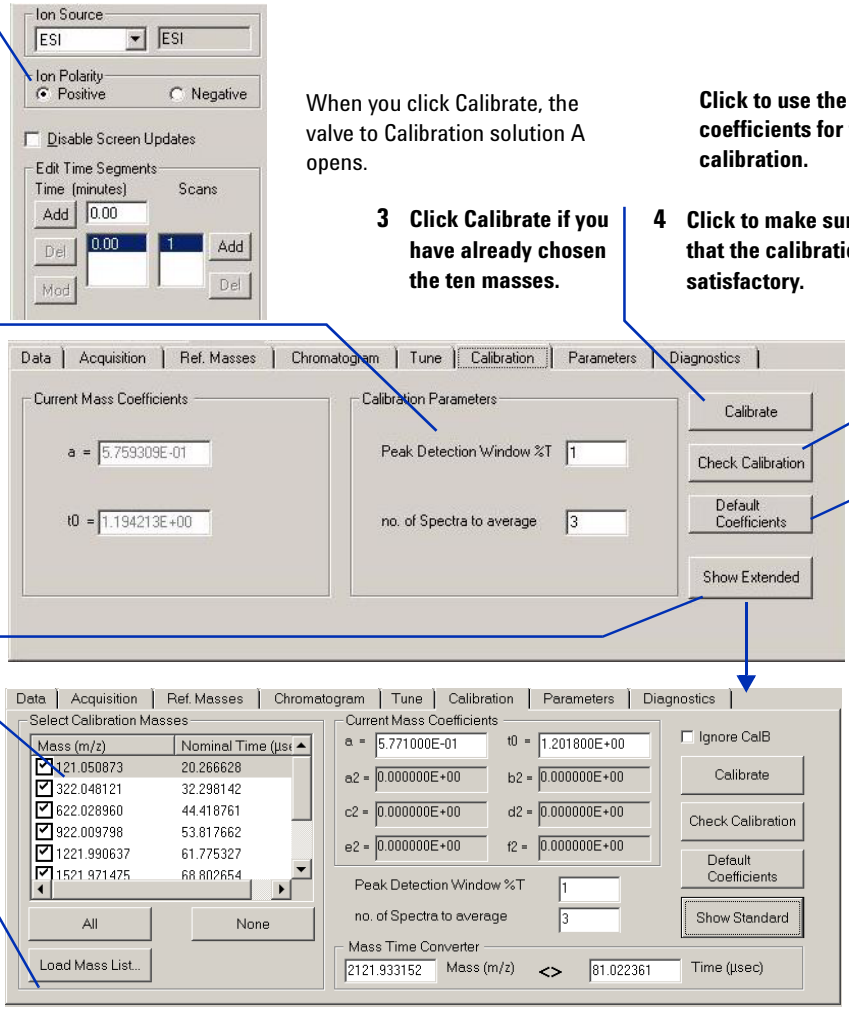
7 **Then calibrate again.**

When you click Calibrate, the valve to Calibration solution A opens.

3 **Click Calibrate if you have already chosen the ten masses.**

4 **Click to make sure that the calibration is satisfactory.**

Click to use the default coefficients for the calibration.

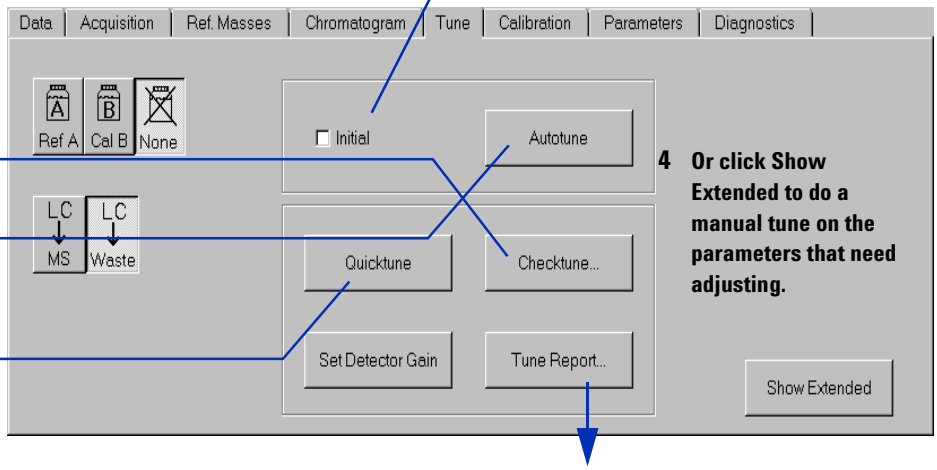


1 **Click the Tune tab under the MSTOF tab.**

2 **Check to see if the TOF needs tuning.**

3 **Click Autotune for a tune that takes 15-20 minutes or Quicktune for a tune that takes 1-3 minutes.**

You do an initial autotune after repair or installation.

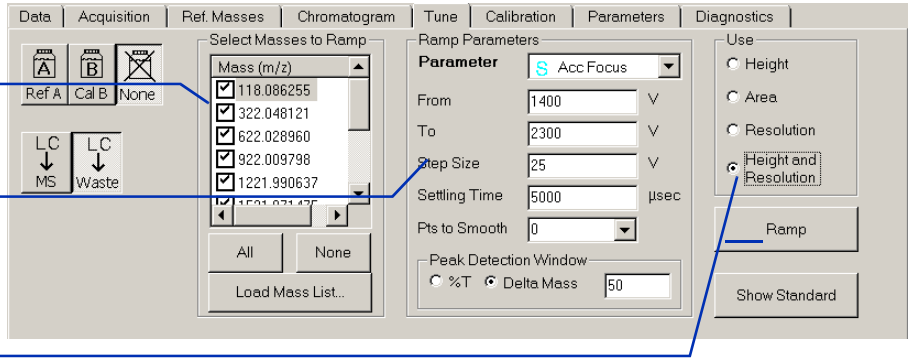


4 **Or click Show Extended to do a manual tune on the parameters that need adjusting.**

1 **Select the masses for the ramp.**

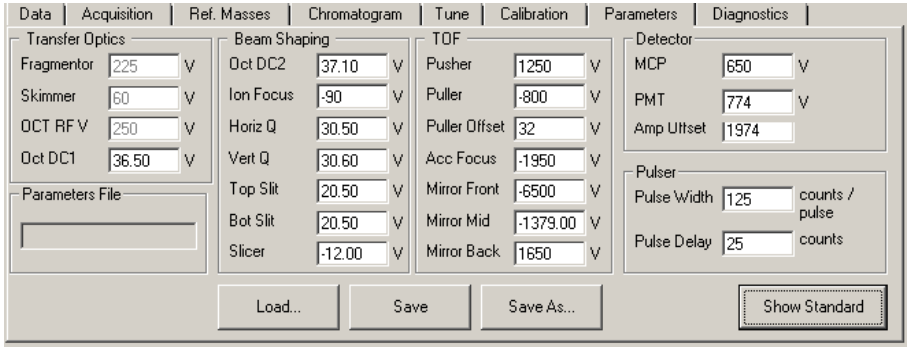
2 **Select the parameter to vary.**

3 **Select the variable to optimize, and click Ramp.**



If you do a manual tune, you must also do a calibration. Autotune and Quicktune include mass calibration with one mass.

After you do a tune, the optimized parameters appear in the Parameters panel.



Switch LC stream to MS

After you condition the column and calibrate and tune the TOF, you switch the LC stream from Waste to MS.

1 Click the **Method pane** icon to view the Method pane.

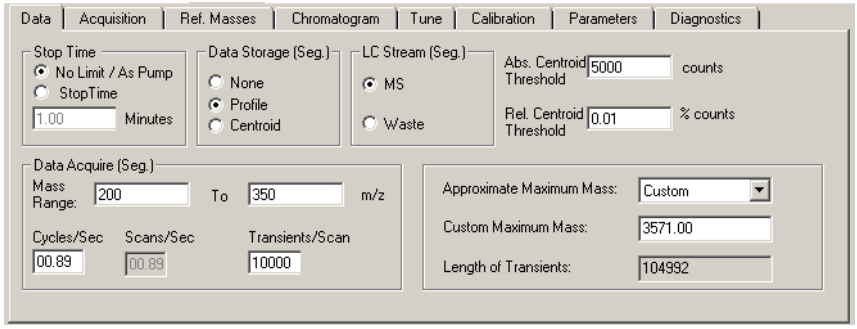


Figure 4 Data tab of the MS-TOF tab in the Method pane

2 Click the **MS-TOF** tab of the Method pane.

3 Click the **Data** tab within the MS-TOF tab.

4 Select **MS**.

5 Click **Apply**.

Monitor TOF baseline and spectral displays

If you did not monitor the LC baseline with a DAD, skip this module. Make sure that the TOF baseline is stable and no spectra of interfering intensity appear in the display.

If you did monitor the LC baseline with a DAD, follow these steps.

1 Right-click the chromatogram display.

2 Select **Change**.

3 Highlight the TIC signal in the list of **Selected Signals**.

4 Set the **x** and **y axis** ranges.

5 Click **OK**.

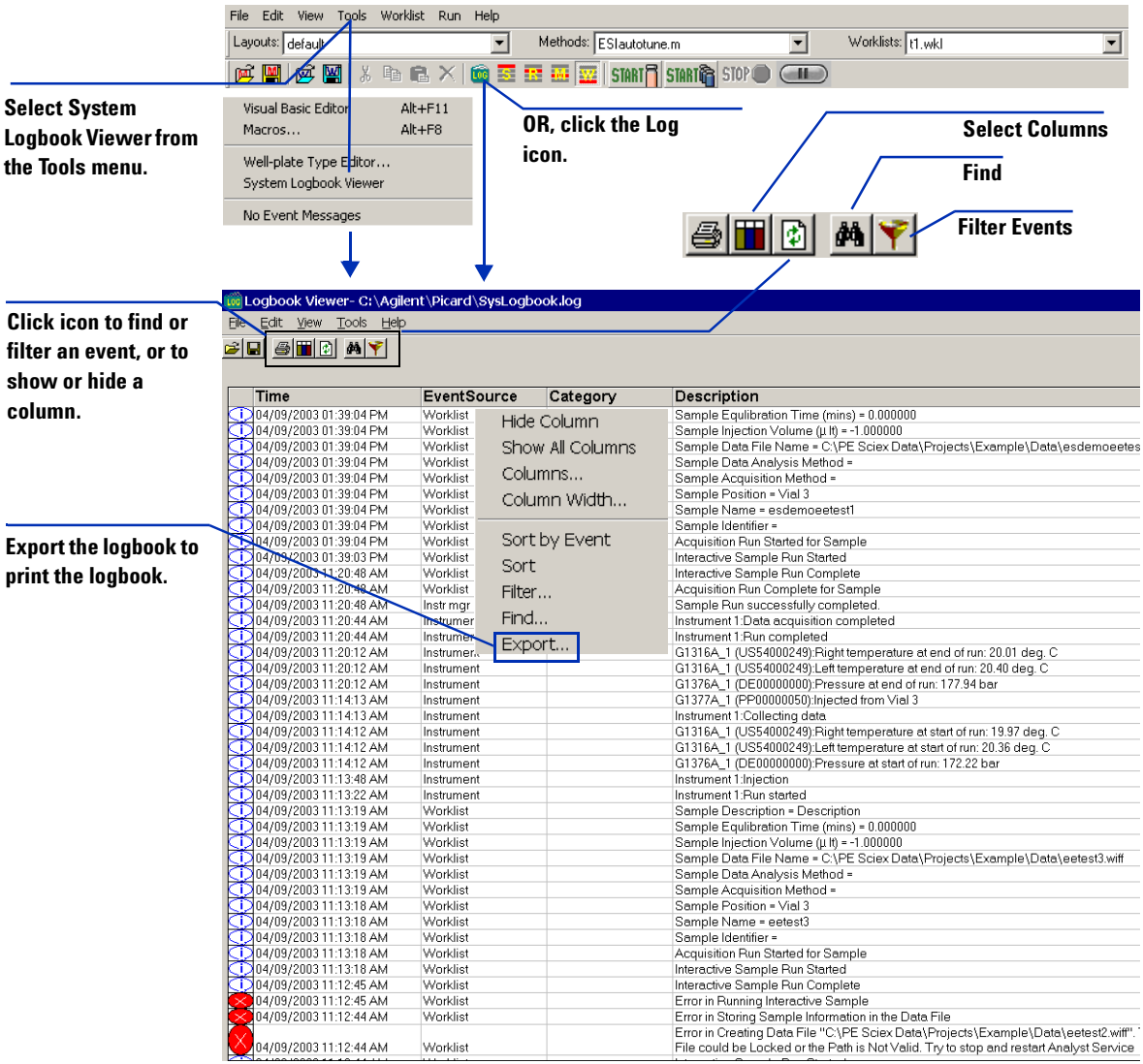
6 Right-click the spectral displays.

7 Select **TOF spectra >Line** or **Profile**.

8 Monitor the baseline and spectra.

View the system logbook for events and errors

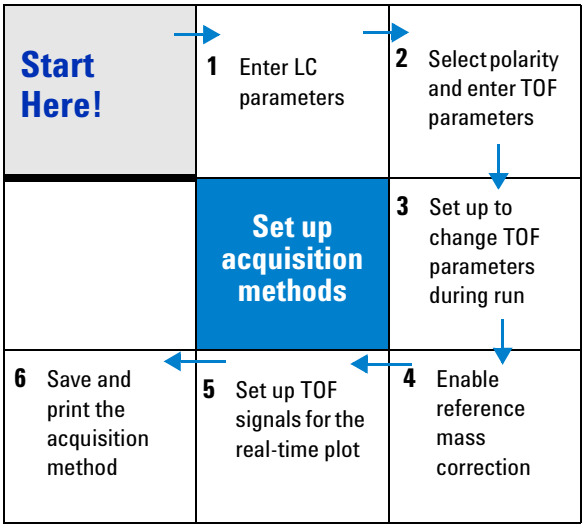
As you prepare the instrument, you may run into an error that you want to troubleshoot. You do this through the System Logbook Viewer.



Step 3—Set up acquisition methods

Read and follow the steps in the user information listed below to learn how to set up methods.

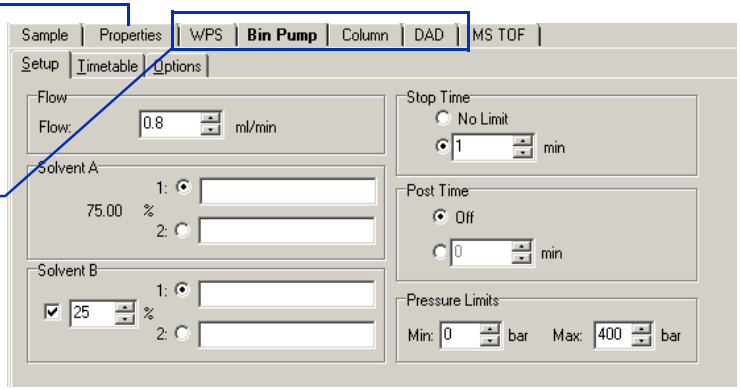
- The steps on the next pages that take you through the roadmap below.
- Chapter 3 of the *Concepts Guide*, Acquisition Methods, to learn background information to help you set up methods.
- Exercise 2, Set up an Acquisition Method, in the *Familiarization Guide*
- O*nline Help* for the tasks that correspond to the roadmap steps and the tasks listed on the next pages.



Enter LC parameter values

You can also enter pre-run/ post-run scripts in the Properties tab.

Enter LC parameters in the LC module tabs.



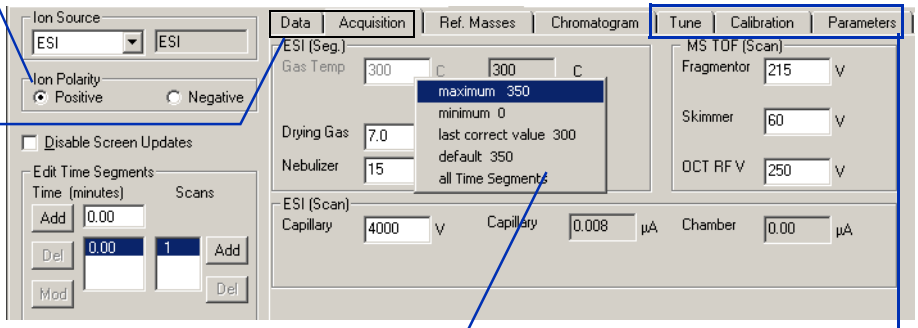
If you click Apply, the parameters are sent to the instrument but are NOT saved to the method.

Do not modify scripts provided by Agilent because these scripts may be overwritten the next time you upgrade the Agilent software.

Enter TOF parameter values

1 Select ion polarity.

2 Enter TOF parameters in the Data, and Acquisition tabs.



You enter these values for the initial time 0.0 min and the whole run and for one scan, unless you add other time segments and scans. See the next page.

Right-click each field to find the maximum and minimum values.

All entries in the Tune, Calibration and Parameters tabs are not saved with the method.

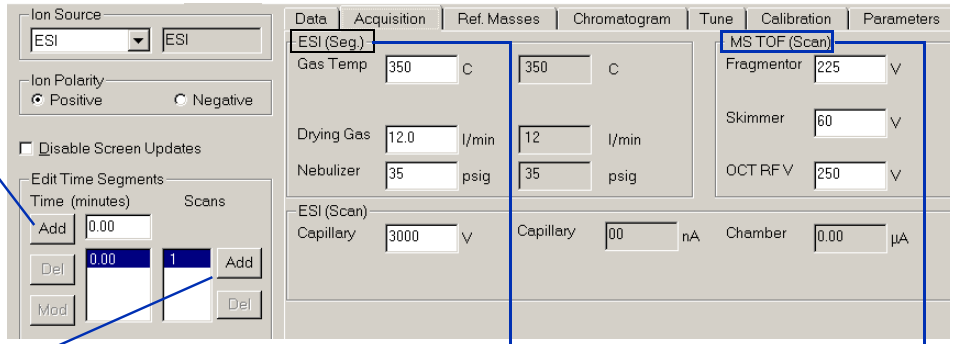
Set up to change TOF parameters with segments and scans

1 **For the initial run time of 0.0 and 1 scan, enter TOF values.**

2 **Enter the next time for which you want to change segment values and click Add.**

3 **Add up to four scans for each time segment, including the initial time.**

4 **For each scan, change a value or values.**



You can also change values on the Data tab for each time segment.

Note that Ion Polarity can be changed for each time segment. The label for that section will be "Ion Polarity (Seg.)" if there are more than one time segments.

Note the values you can change with each time segment.

Note the values you can change with each scan.

Enable reference mass correction

You enable for mass correction during a run to obtain the specified mass accuracy.

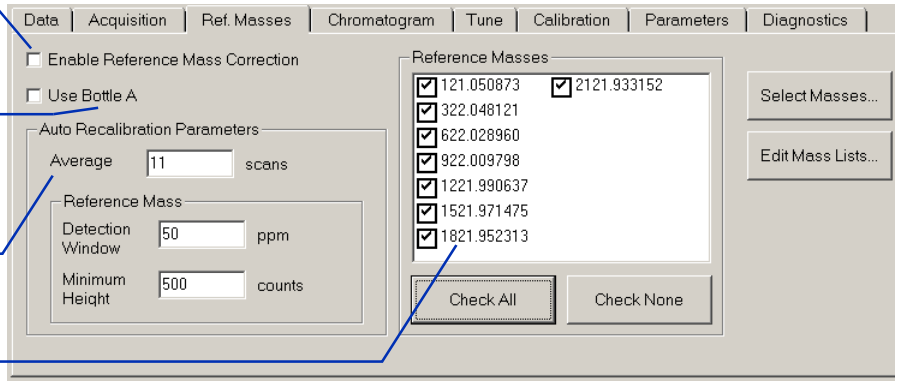
Set up for mass correction

1 **Enable reference mass correction.**

2 **Mark Bottle A to use the Agilent reference std.**

3 **Set the auto recalibration parameters.**

4 **Mark the masses that you want to use for the correction.**



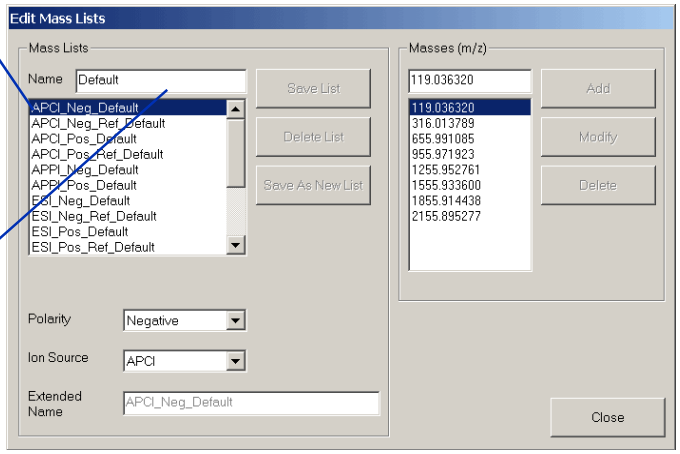
If the list is blank or you want a different list for another standard, click **Select**

If you want to create a new mass list or modify the existing default lists, click **Edit**

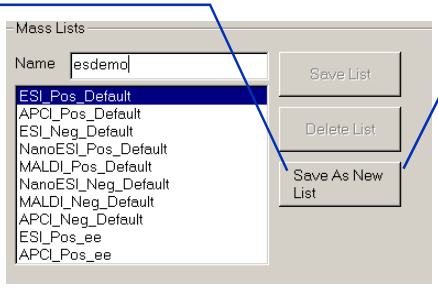
Edit mass list

1 **Select the default mass list for your ion source and polarity.**

2 **Enter a new name into the Name field.**



3 **Click Save As New List.**

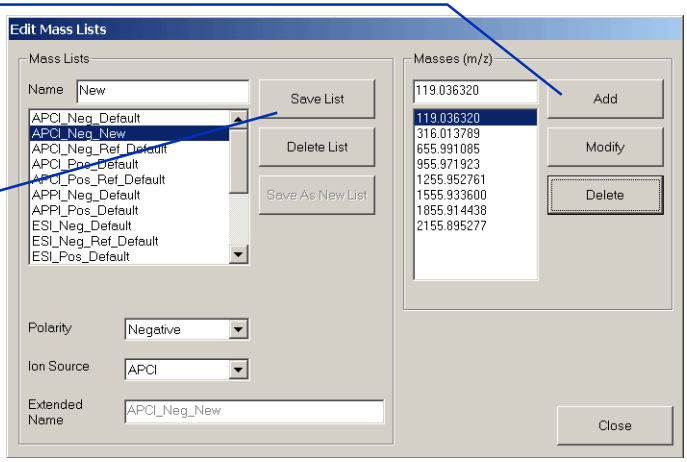


The Save As New List button appears when you enter a new Name.

All the other grayed out buttons appear when you click Save As New List.

4 **Add or delete masses to the new list.**

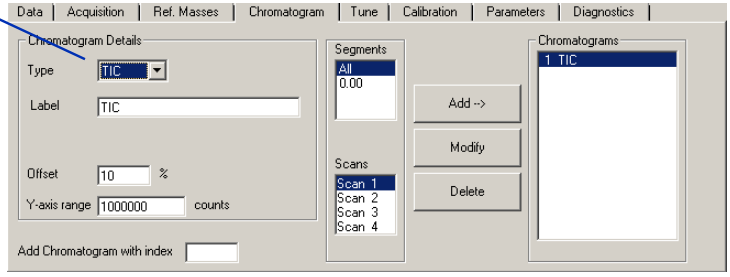
5 **Click Save List.**



Set up signals for the real-time plot

Select the signal that you want to see in the real-time plot.

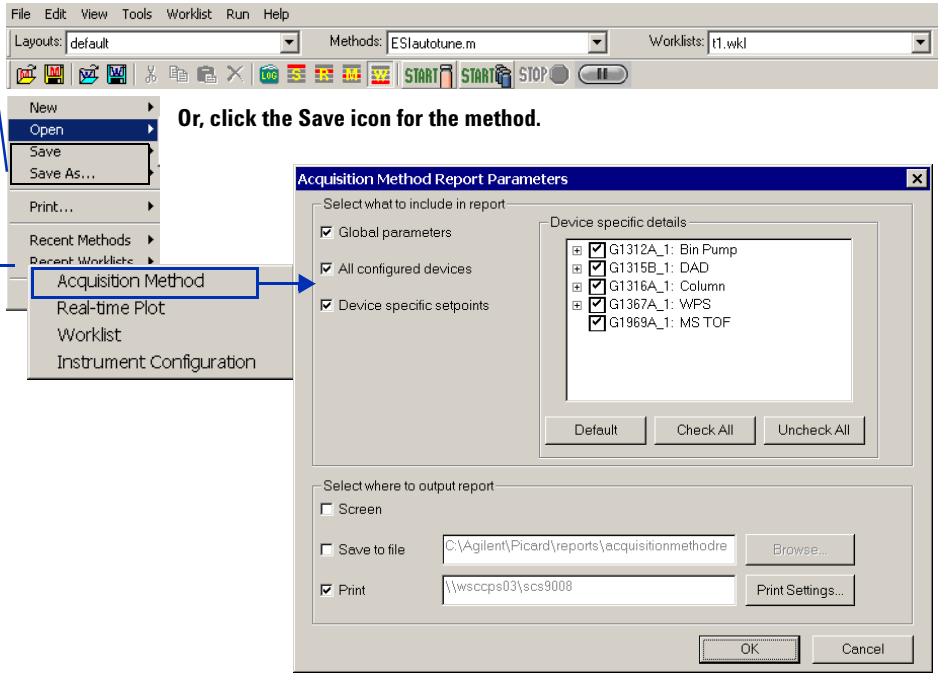
You can also select different time segments and scans to monitor.



Save and print the method

Select Save in the File menu to save the currently opened method, OR select Save As in the File menu to save a new method.

Select Print > Acquisition Method in the File menu to set up to print a method.

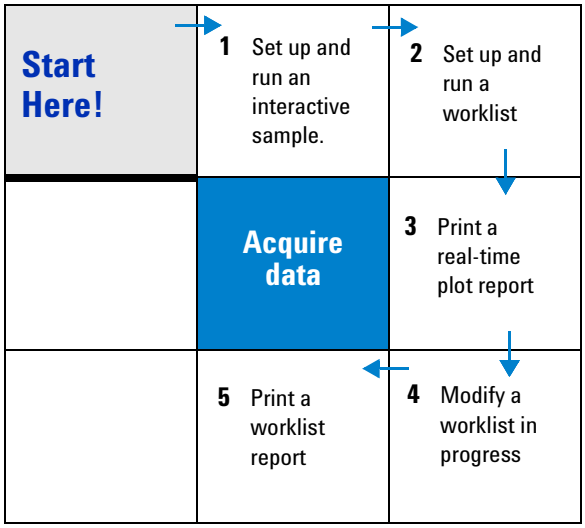


Or, click the Save icon for the method.

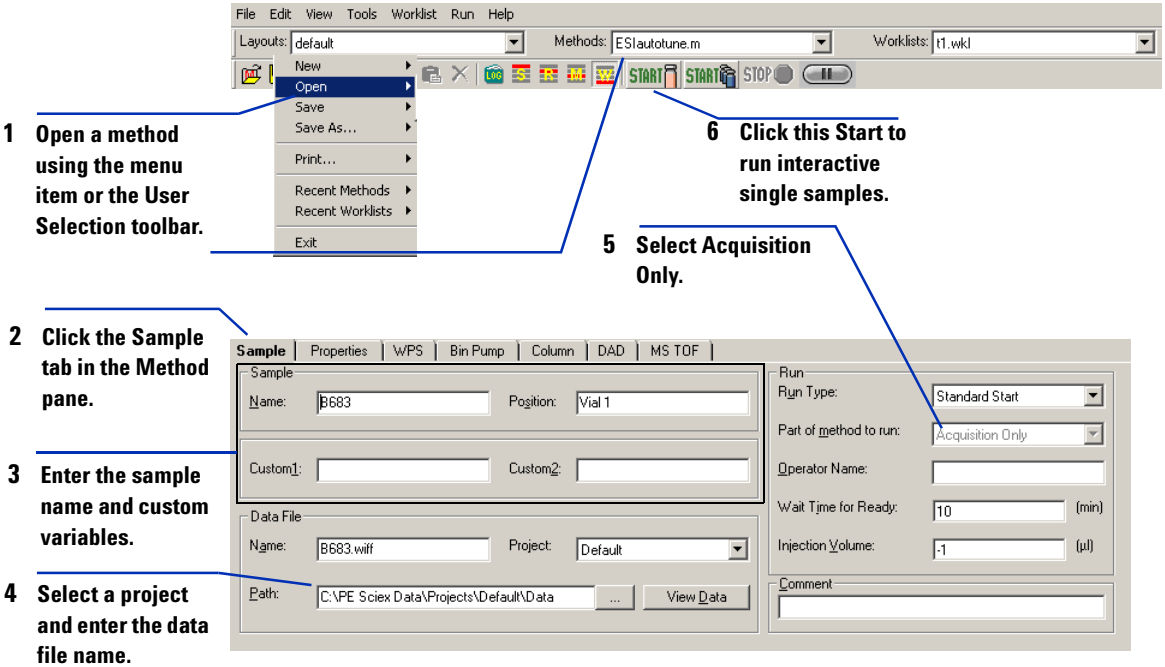
Step 4—Acquire data

Read and follow the steps in the user information listed below to learn how to acquire data.

- The steps on the next pages that take you through the roadmap below.
- Chapter 4 of the *Concepts Guide*, Data Acquisition, to learn background information to help you acquire data.
- Chapters 3 and 4 of the *Familiarization Guide*
- O*nline Help* for the tasks that correspond to the roadmap steps and the tasks listed on the next pages.



Set up and run interactive samples



You can only create projects in Analyst. See "Step 5—Analyze data" on page 35.

Even though Both Acquisition and DA is a selection in the "Part of method to run" list, it is not available for single samples in this version of software.

Set up and run worklists (e.g., empirical formula confirmation)

1 **Right-click here to bring up the worklist shortcut menu.**

2 **Select Add Multiple Samples to add a series of samples to the worklist.**

3 **Select Add column(s) to add a column for the empirical formula.**

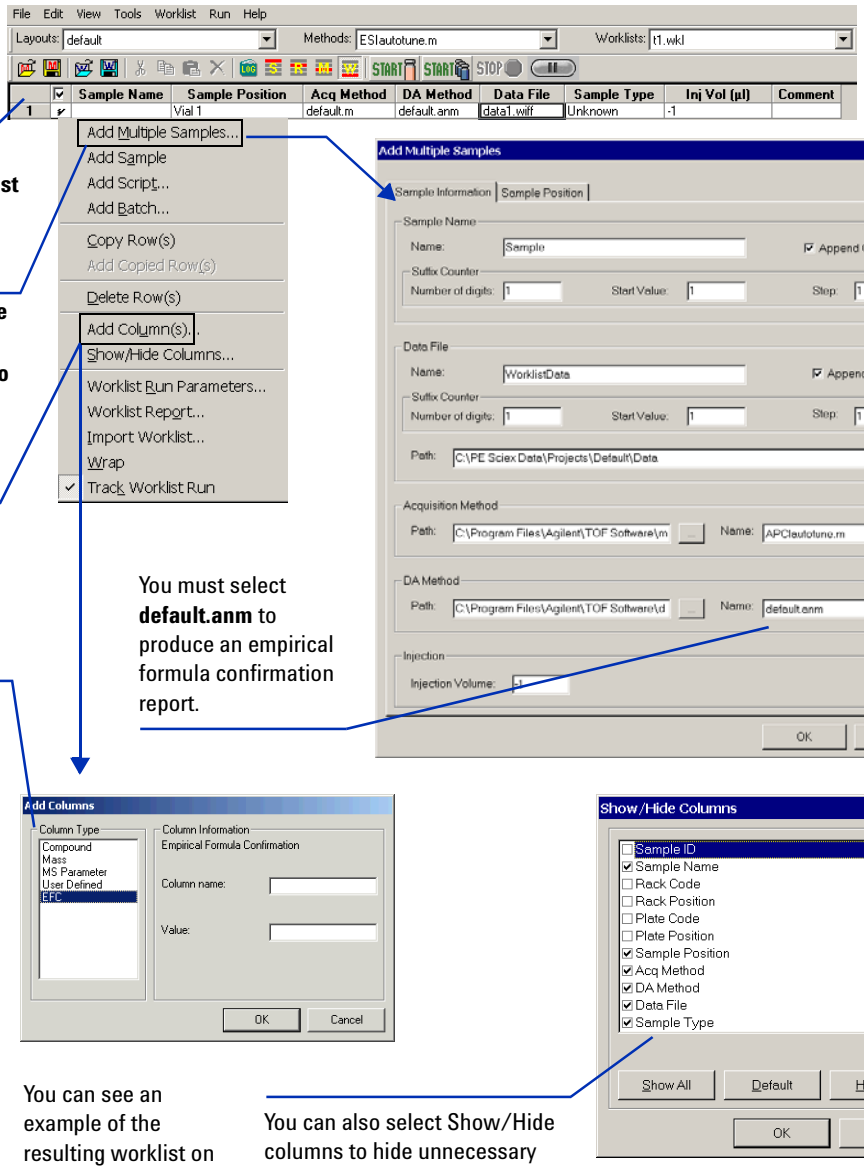
4 **Enter the generic name (Formula_x) and formula for an EFC column.**

You must select **default.anm** to produce an empirical formula confirmation report.

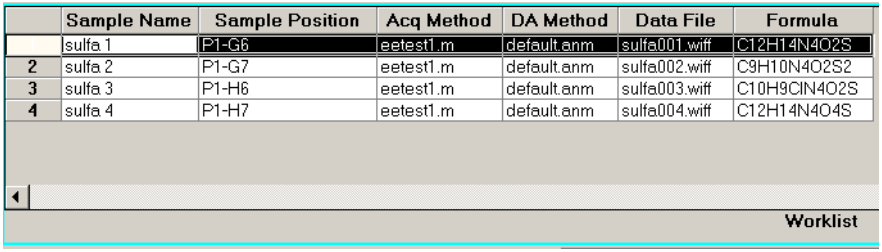
You can also add batches of samples whose information and data you may want to keep together.

You can see an example of the resulting worklist on the next page.

You can also select Show/Hide columns to hide unnecessary columns.



This is an example of the resulting worklist.

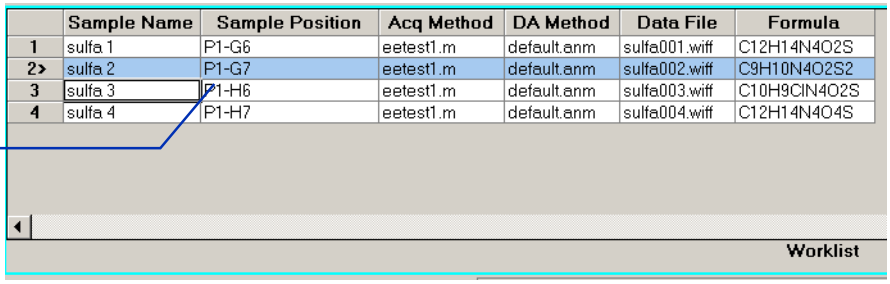


	Sample Name	Sample Position	Acq Method	DA Method	Data File	Formula
	sulfa 1	P1-G6	eetest1.m	default.anm	sulfa001.wiff	C12H14N4O2S
2	sulfa 2	P1-G7	eetest1.m	default.anm	sulfa002.wiff	C9H10N4O2S2
3	sulfa 3	P1-H6	eetest1.m	default.anm	sulfa003.wiff	C10H9ClN4O2S
4	sulfa 4	P1-H7	eetest1.m	default.anm	sulfa004.wiff	C12H14N4O4S

Worklist

Click this Start to run a worklist.

If Track Worklist is On (Worklist menu), the row that is running is highlighted blue.

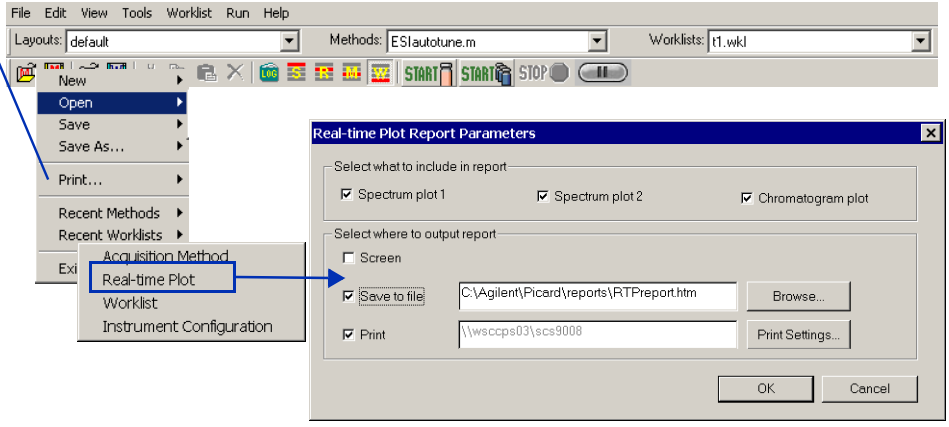


	Sample Name	Sample Position	Acq Method	DA Method	Data File	Formula
1	sulfa 1	P1-G6	eetest1.m	default.anm	sulfa001.wiff	C12H14N4O2S
2>	sulfa 2	P1-G7	eetest1.m	default.anm	sulfa002.wiff	C9H10N4O2S2
3	sulfa 3	P1-H6	eetest1.m	default.anm	sulfa003.wiff	C10H9ClN4O2S
4	sulfa 4	P1-H7	eetest1.m	default.anm	sulfa004.wiff	C12H14N4O4S

Worklist

Print a real-time plot report

- **To print a real-time plot report during the run, select Print > Real-Time Plot.**

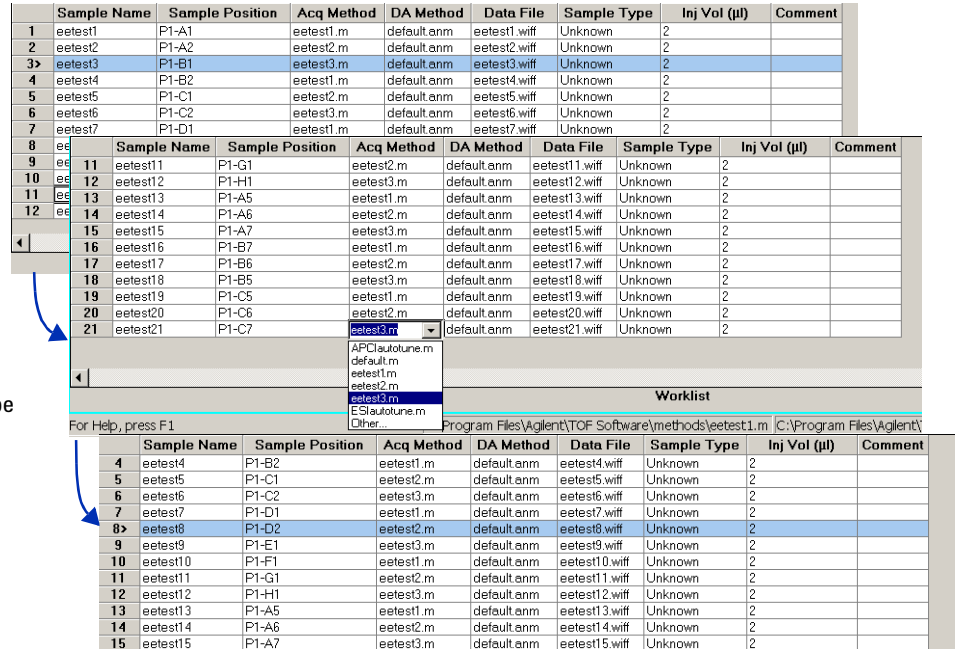


Modify the worklist in progress

You can modify any row below the row located under the running row (shaded blue).

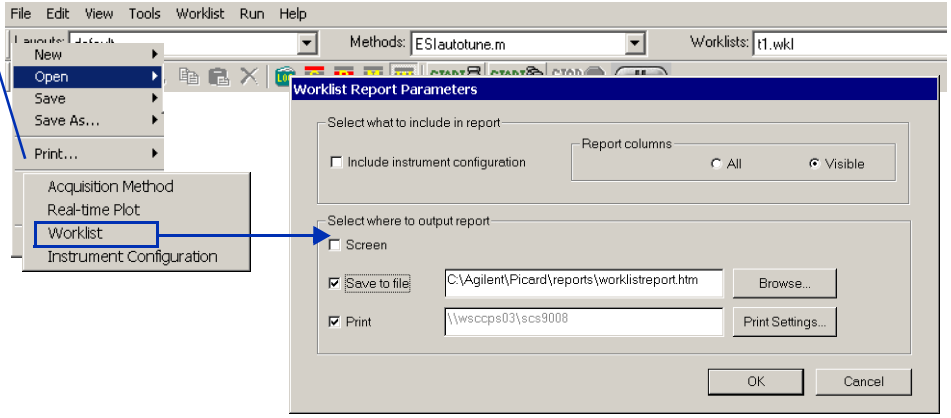
If the last selected row is executing, then all rows are locked.

When you place the cursor on the row to be edited, tracking is automatically turned off. To turn tracking back on, you must check the worklist menu item, Track Worklist Run.



Print the worklist

Select Print > Worklist to print the worklist report.

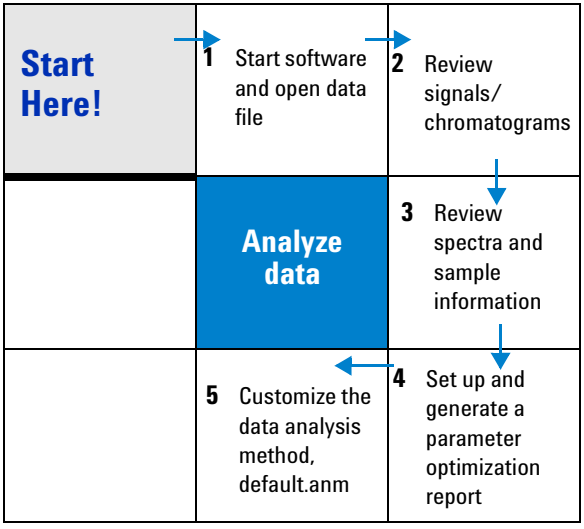


Step 5—Analyze data

The primary tool for analyzing and reporting on results is PE-Sciex Analyst QS. PE-Sciex has modified their software specifically to accommodate the Agilent TOF system requirements.

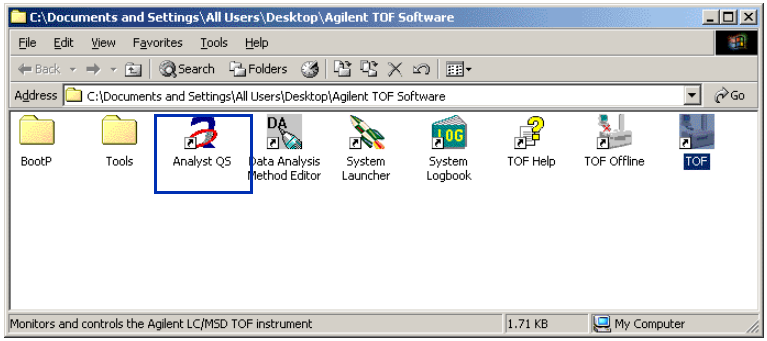
Read and follow the steps in the user information listed below to learn how to review TOF data and customize the data analysis method, default.anm, used to confirm empirical formulas.

- The steps on the next pages that take you through the roadmap below.
- Chapter 5 of the *Concepts Guide*, Data Analysis, to learn background information to help you analyze data.
- Chapters 3 and 4 of the *Familiarization Guide*
- O*nline Help* for the tasks that correspond to the roadmap steps and the tasks listed on the next pages.
- Consult the *PE-Sciex Analyst User's Guide* and online help to learn how to perform other analysis operations not associated with the Agilent system.



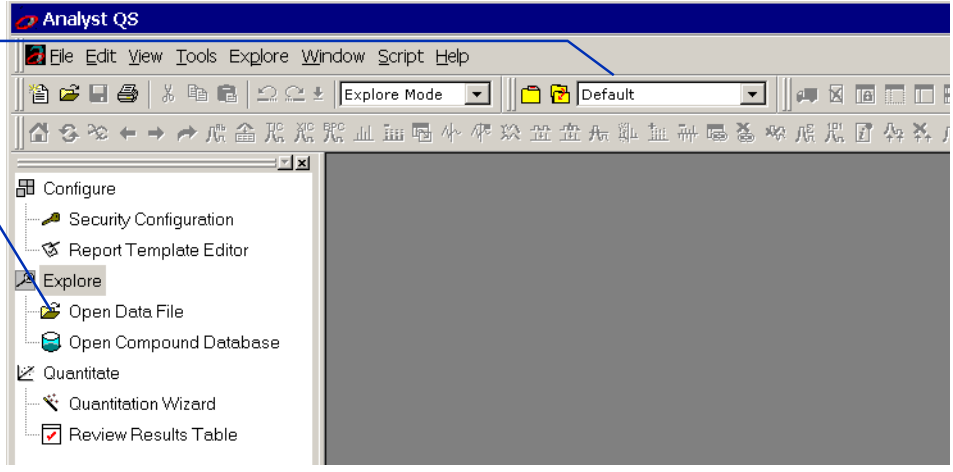
Start the Analyst QS software and open a data file

1 **Double-click the Analyst QS icon in the Agilent TOF Software group window.**



2 **Select the project that contains the data file.**

3 **Click to open .wiff files.**

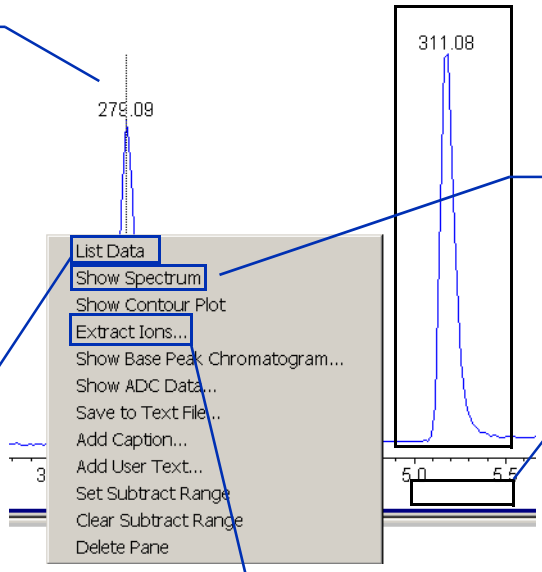


Review signals/chromatograms

1 **Right-click on chromatogram to bring up the shortcut menu.**

2 **Do any of the bulleted tasks on this page in any order that you want.**

- **Select List Data from the shortcut menu to see the results of integration.**



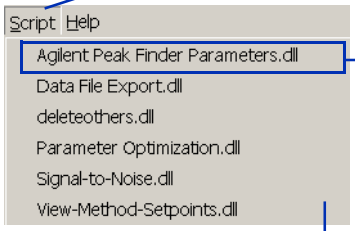
See the next page to learn about spectral operations with Analyst.

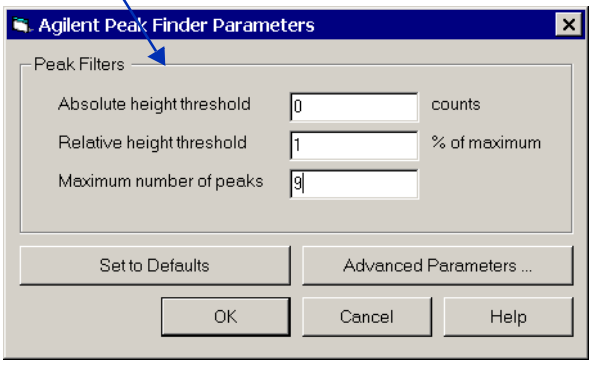
- **Drag the cursor across a peak and select Show Spectrum from the shortcut menu to see the mass spectra in the peak**

- **To zoom into a peak, draw a rectangle under the peak baseline.**

- **Select Extract Ion from the shortcut menu to produce an XIC (EIC) chromatogram.**

- **Select Script > Agilent Peak Finder Parameters.dll to change integration parameters.**



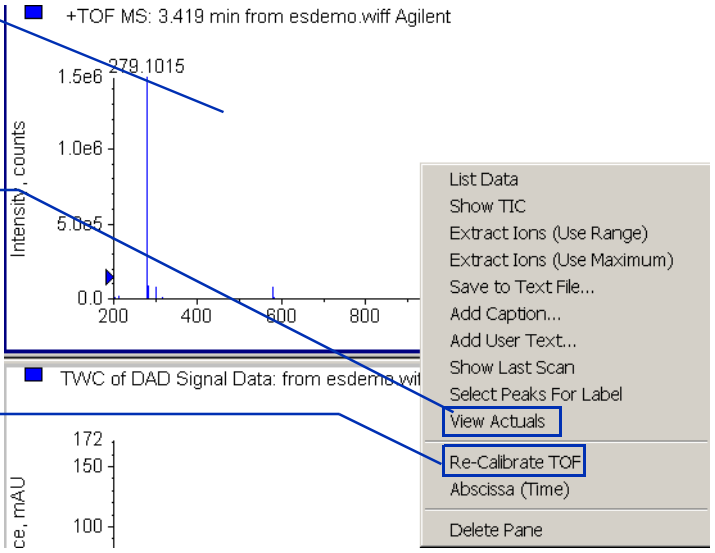


Method Setpoints by default are saved with the data file. Use the tool TOFSystemConfig to change whether Method Setpoints are saved. See the installation guide for more information.

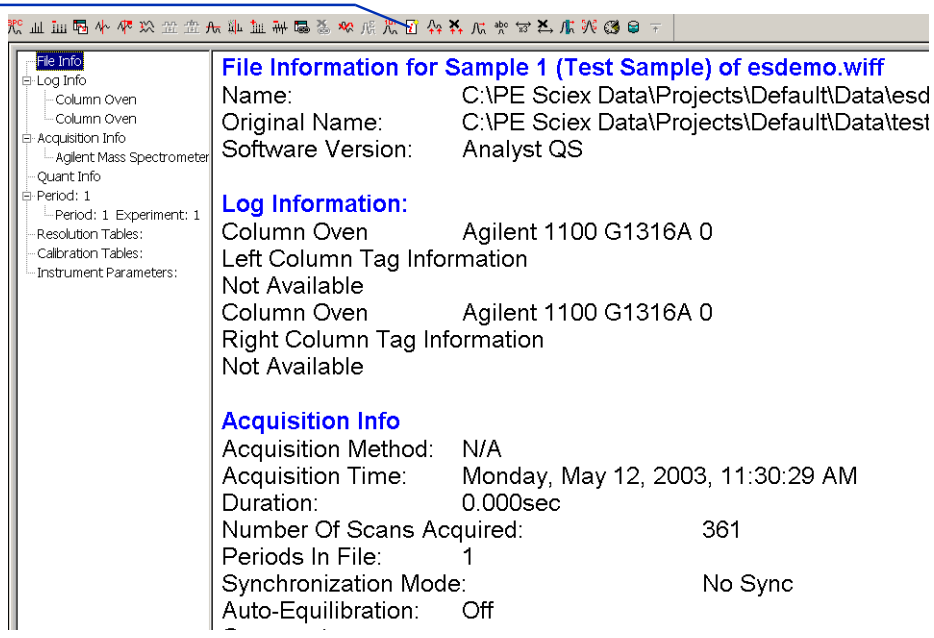
Review spectra and sample information

1 **Right-click on spectrum to bring up the shortcut menu.**

- **Select View Actuals from the shortcut menu to see the real-time TOF parameter values.**
- **Select Re-Calibrate TOF to recalibrate the TOF from Analyst.**



- **Click the sample information icon in the Analyst toolbar to view method and sample information on the data file.**

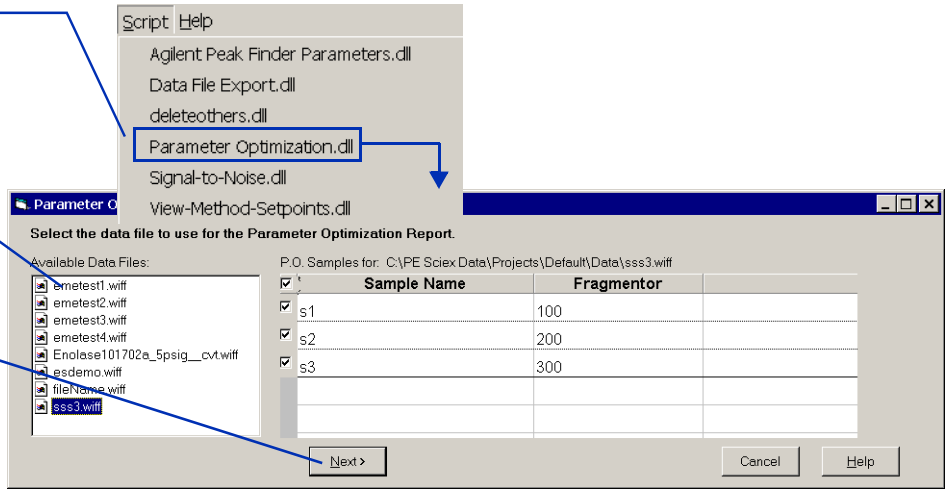


Set up and generate a parameter optimization report

1 **Select Script > Parameter Optimization.dll in the Analyst main window.**

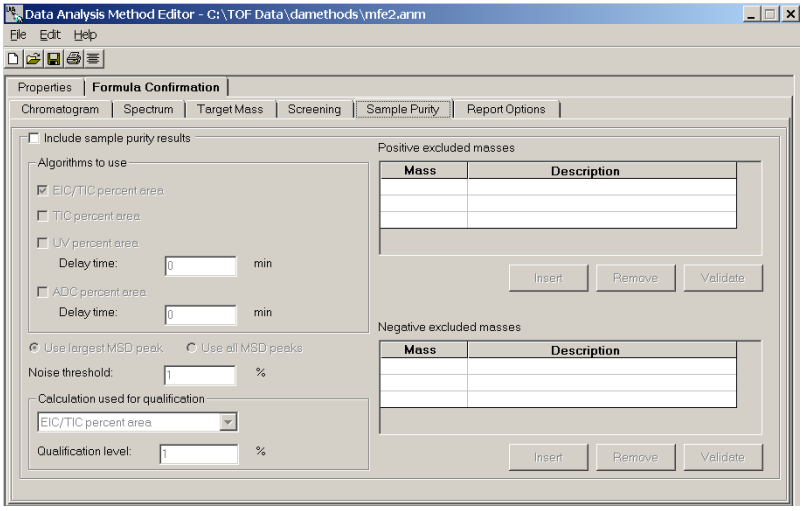
2 **Select the samples for the report.**

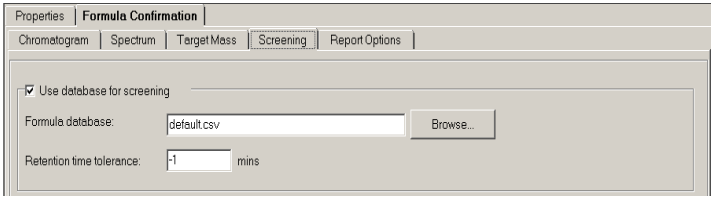
3 **Continue through the wizard, then click Finish.**



Customize the data analysis method for empirical formula confirmation

1. **Click the Data Analysis Editor icon in the Agilent TOF Software program folder.**
2. **Click the Formula Confirmation tab in the Data Analysis Method Editor window.**
3. **Enter values in the Formula Confirmation tabs to modify the default.anm method. Enter values in the Report Options tab to select which of the graphs to include. Enter values in the Screening tab to enable the database search.**
4. **Save the method.**
5. **Regenerate the report by rerunning the worklist in Data Analysis Only mode.**

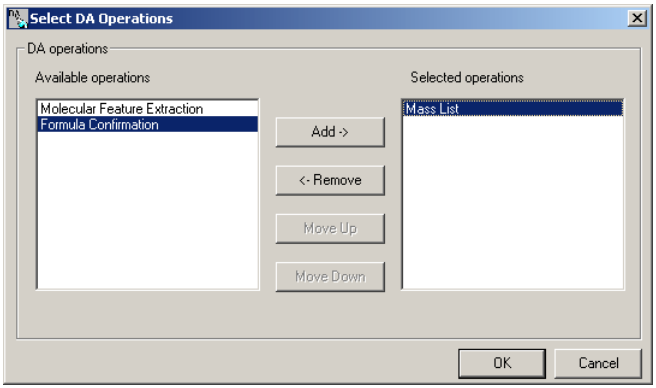


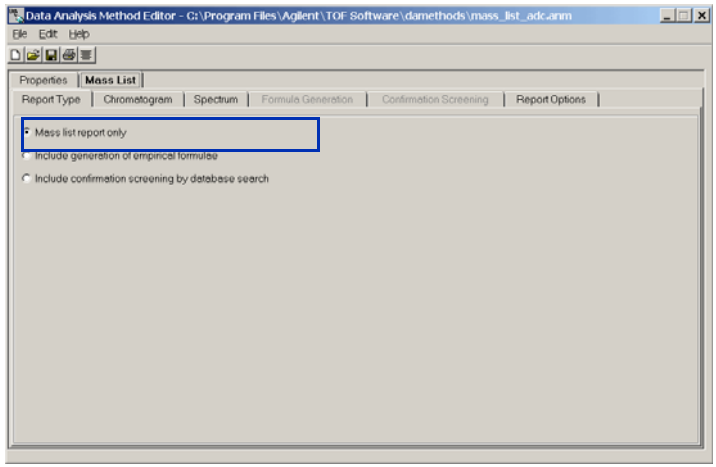


The EFC report can now also include a backward database search (called an EFC Database Screening Report). Based upon a formula, a mass is determined and then XICs are extracted for that mass to see if the compound can be found. You can limit the search of the database to formulas with a certain retention time tolerance. A value of -1 in the Retention time tolerance field indicates to not limit the search based upon retention time.

Create a data analysis method for Mass List Report

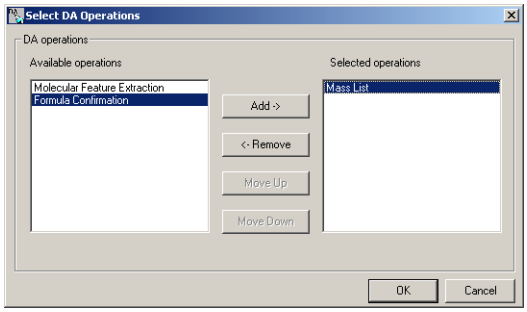
1. Click the Data Analysis Editor icon in the Agilent TOF Software program folder.
2. Select Select DA Operations item from the Edit menu in the Data Analysis Method Editor window.
3. Click Mass List in the Available Operations list and click the Add button. If any other option appears in the Selected Operations list, click on it and click the Remove button.
4. Click OK to close the Select DA Operations dialog box.
5. Select the Report Type "Mass list report only".
6. Enter values in the Mass List tabs to modify the method.
7. Save the method with a new name, using the File>Save As menu item.
8. To generate a mass list report, create a worklist that specifies the data analysis method created in the steps above. The report will be generated for each sample when you run the worklist.

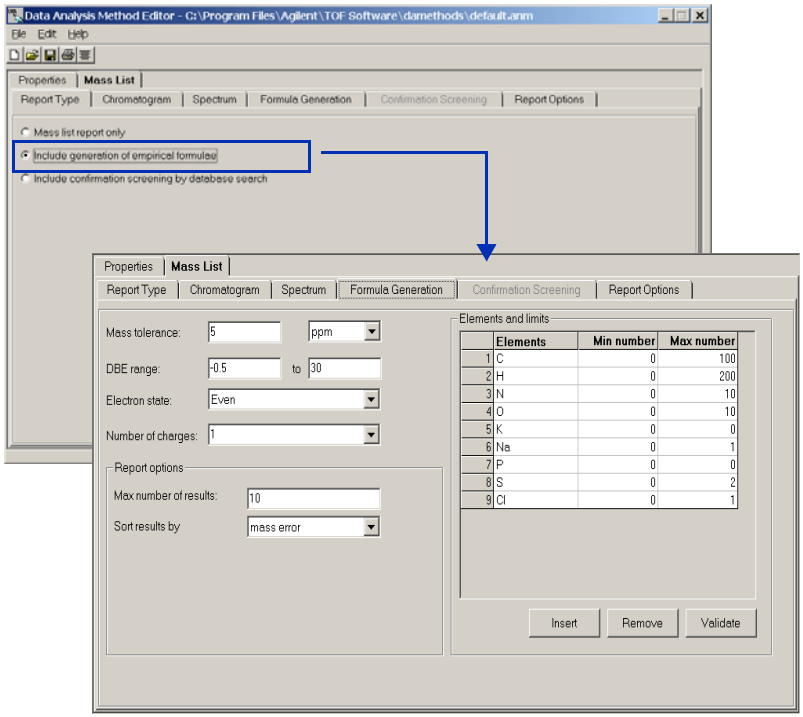




Create a DA method for Mass List Report type Empirical Formula Generation

1. Click the Data Analysis Editor icon in the Agilent TOF Software program folder.
2. Select Select DA Operations item from the Edit menu in the Data Analysis Method Editor window.
3. Click Mass List in the Available Operations list and click the Add button. If any other option appears in the Selected Operations list, click on it and click the Remove button.
4. Click OK to close the Select DA Operations dialog box.
5. Select the Report Type "Include generation of empirical formulae".
6. Enter values in the Mass List tabs to modify the method including the "Formula Generation" tab.
7. Save the method with a new name, using the File>Save As menu item.
8. To generate a mass list report, create a worklist that specifies the data analysis method created in the steps above. The report will be generated for each sample when you run the worklist.

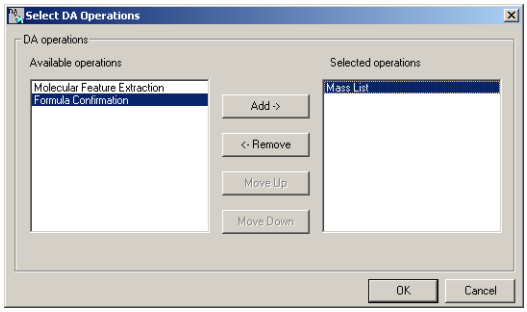


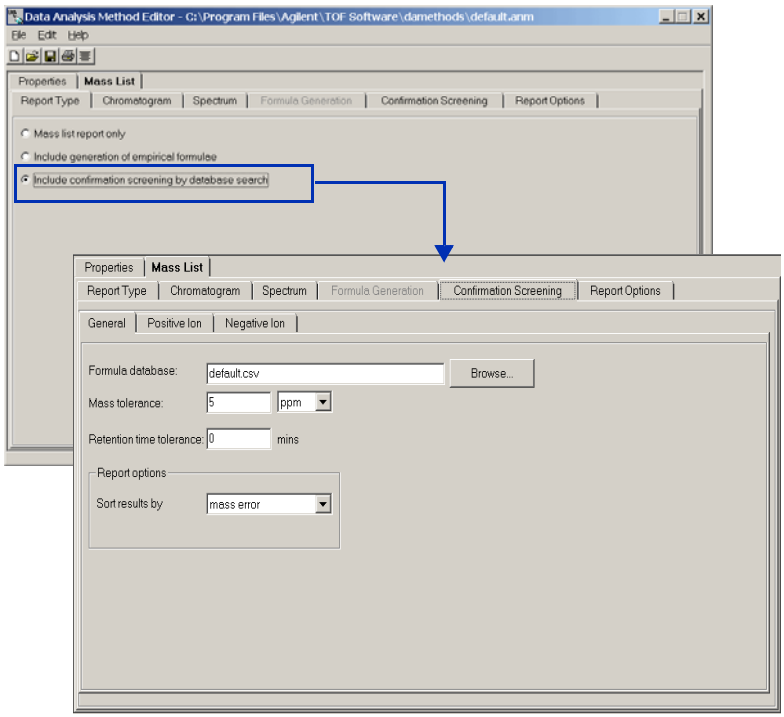


The Mass List Report including Empirical Formula Generation identifies valid molecular formulas that match the masses found in your sample based upon the values entered in this tab.

Create a DA method for Mass List Report type Confirmation Screening

1. **Click the Data Analysis Editor icon in the Agilent TOF Software program folder.**
2. **Select Select DA Operations item from the Edit menu in the Data Analysis Method Editor window.**
3. **Click Mass List in the Available Operations list and click the Add button. If any other option appears in the Selected Operations list, click on it and click the Remove button.**
4. **Click OK to close the Select DA Operations dialog box.**
5. **Select the Report Type "Include confirmation screening by database search".**
6. **Enter values in the Mass List tabs to modify the method including the "Confirmation Screening" tab.**
7. **Save the method with a new name, using the File>Save As menu item.**
8. **To generate a mass list report, create a worklist that specifies the data analysis method created in the steps above. The report will be generated for each sample when you run the worklist.**

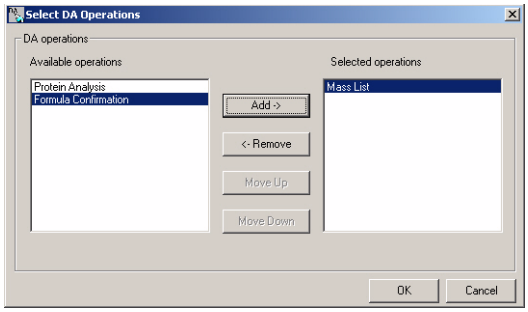


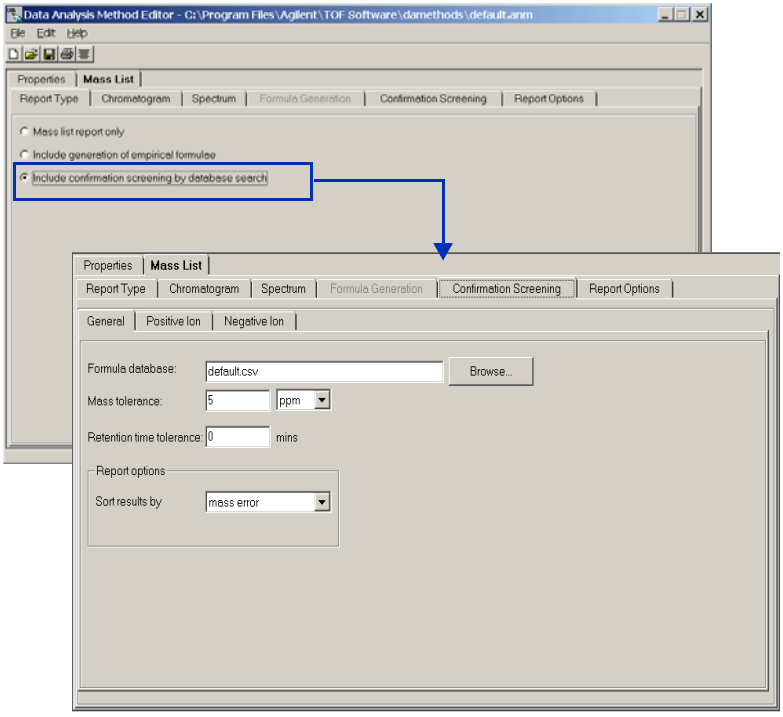


The Mass List Report including Confirmation Screening is a forward screening report. After determining the mass, the database is searched for formulas with the corresponding mass.

Create a DA method for Molecular Features Extraction report

1. **Click the Data Analysis Editor icon in the Agilent TOF Software program folder.**
2. **Select Select DA Operations item from the Edit menu in the Data Analysis Method Editor window.**
3. **Click Molecular Features Extraction in the Available Operations list and click the Add button. If any other option appears in the Selected Operations list, click on it and click the Remove button.**
4. **Click OK to close the Select DA Operations dialog box.**
5. **Select the Report Type "Molecular Feature Extraction only".**
6. **Enter values in the Molecular Feature Extraction tabs to modify the method.**
7. **Save the method with a new name, using the File>Save As menu item.**
8. **To generate a molecular feature extraction report, create a worklist that specifies the data analysis method created in the steps above. The report will be generated for each sample when you run the worklist.**

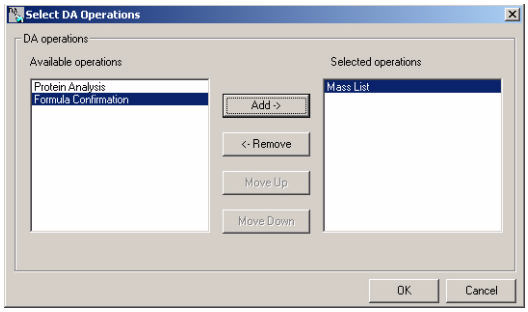


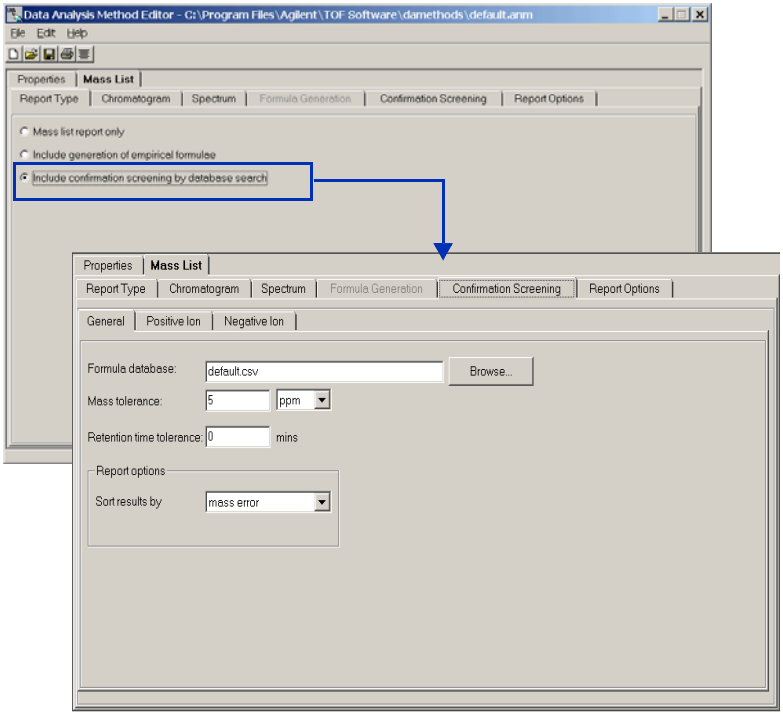


The Molecular Feature Extraction Report lists out masses of chemical compounds and a list of isotopes of a compound found in the sample. The MFE report shows isotopes in the form of multiple isotope cluster based on adducts used in the ionization.

Create a DA method for MFE Report including Confirmation Screening

1. **Click the Data Analysis Editor icon in the Agilent TOF Software program folder.**
2. **Select Select DA Operations item from the Edit menu in the Data Analysis Method Editor window.**
3. **Click Molecular Feature Extraction in the Available Operations list and click the Add button. If any other option appears in the Selected Operations list, click on it and click the Remove button.**
4. **Click OK to close the Select DA Operations dialog box.**
5. **Select the Report Type "Include confirmation screening by database search".**
6. **Enter values in the MFE tabs to modify the method including the "Confirmation Screening" tab.**
7. **Save the method with a new name, using the File>Save As menu item.**
8. **To generate a mass list report, create a worklist that specifies the data analysis method created in the steps above. The report will be generated for each sample when you run the worklist.**





The Molecular Feature Extraction Report including Confirmation Screening is a forward screening report. After determining the mass, the database is searched for formulas with the corresponding mass.

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In this book

This book contains brief instructions to help you get started with your Agilent LC/MSD TOF system. This books shows you how to:

- Prepare the instrument for a run
- Set up acquisition methods
- Set up and run an interactive sample and worklists
- Review data

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