Agilent G3250AA LC/MSD TOF System

Concepts Guide

The Big Picture

Agilent Technologies

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In This Guide...

The Concepts Guide presents “The Big Picture” behind the LC/MSD TOF system to help you analyze samples on your Agilent time-of-flight mass spectrometer system. This guide helps you understand how the hardware and software work together.

1 Overview

Learn how the TOF system helps you do your job and how the hardware and software work.

2 Instrument Preparation

Learn the concepts you need to prepare the instrument for sample acquisition.

3 Acquisition Methods

Learn concepts to help you enter instrument control parameter values and set up acquisition methods.

4 Data Acquisition

Learn concepts to help you enter information to run individual samples or a worklist of individual samples and batches, especially to confirm empirical formulas, and to help you acquire data and monitor runs.

5 Data Analysis

Learn concepts to help you review data, create parameter optimization reports and customize empirical formula confirmation reports.

Contents

1 Overview

This chapter provides an overview of the Agilent LS/MSD TOF system components and how they work together to help you get your job done.

How does the TOF system help you do your job?

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

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

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

Each Agilent system has advantages for drug discovery — high throughput sample screening with highly sensitive detection and accurate mass assignment. Each uses the same Agilent TOF software to enable these advantages, although the AP-MALDI or PDF-MALDI TOF system uses only the MS portion of the software.

Help for applications

You can use one or more of the Agilent TOF systems to customize the following applications:

- Combinatorial chemistry target compound analysis
- Natural products screening
- Compound profiling (e.g., bioavailability, pK)
- Protein/peptide identification and characterization
- Proteomics studies

Empirical formula confirmation

Agilent designed the TOF software especially to help you confirm empirical formulas automatically for large numbers of samples. For a list of samples, you can specify formulas for the compounds in each sample and use an Agilent-created data

analysis method to produce confirmation reports as data for the list of samples is automatically acquired. You can specify these formulas in the worklist or in a separate database. You can also include sample purity information.

Mass list reporting

You can choose to have the TOF software generate a report for each sample that lists the most abundant masses for peaks in the chromatogram. You can include in this report either a section on **Empirical Formula Generation** or a section on **Confirmation Screening**.

Molecular Feature Extraction reporting

You can also have the TOF software generate a report containing a list of isotopes for each compound found. You can include in this report either a section on **Empirical Formula Generation** or a section on **Confirmation Screening**.

Help for data acquisition

To help you use the Agilent LC/MSD TOF systems for these applications, the software lets you perform the following tasks in a single window:

Prepare the instrument

To learn how to get started with the Agilent LC/MSD TOF system, see the Quick Start Guide*.*

To learn how to perform individual tasks with the system, see the Online Help for the TOF software*.*

To learn more about an Agilent 1100/1200 LC module, see the Agilent 1100/1200LC User's Guide *for the module.*

- Start and stop the instruments from the software
- Download settings to the Agilent 1100 liquid chromatograph and the Agilent TOF mass spectrometer in real time to control the instrument
- Lets you see if the TOF parameters are within the limits to produce the specified mass accuracy and resolution with a Checktune report
- Lets you optimize TOF parameters automatically or manually through Agilent tuning programs and print an Autotune report
- Monitor the actual conditions of the instrument

- View the real-time plot for chromatograms, spectra, and instrument parameters (both DAD and TOF) and print a real-time plot report
- View the centroided line spectrum of a peak or the mass range profile spectrum of a peak in real time

Set up acquisition methods

- Enter and save parameter values for all LC modules and the TOF to an acquisition method
- Enable reference mass correction and select reference standard masses to correct the mass assignments during a sample run
- Select and label the total ion chromatograms or extracted ion chromatograms that you want to appear in the real-time plot
- Set up time segments for each run where parameters change with the time segment or with the scans within the time segment
- Print an acquisition method report

Acquire data

- Enter sample information and pre- or post-analysis programs (scripts) and run single samples interactively
- Enter and automatically run individual samples and samples organized into batches (sequence of samples) in a *worklist*

A *worklist* is a list of individual samples and batches (sequences) that you enter and run automatically with the TOF software.

- Set up pre- and post-analysis scripts to run between samples and batches in a worklist.
- Set up and run a worklist to confirm empirical formulas and produce a report, using data analysis method, default.anm
- Set up and run a worklist to optimize TOF acquisition parameters
- Print a worklist report
- View system events, including start and stop times, run events and errors and print an event log report

Help for data analysis

Analyst QS

PE-Sciex has modified its Analyst software to accommodate the Agilent LC/MSD TOF system. Through this software you can perform a number of tasks for data analysis.

See PE-Sciex Analyst Getting Started Guide *for information on the concepts and use of Analyst QS.*

- View the raw chromatographic, spectral and integrated data
- View sample and method information
- View TOF instrument parameters and LC instrument curves
- Recalibrate the TOF
- Set up and generate a TOF parameter optimization report
- Change Agilent Peak Finder parameters for spectra

Agilent Data Analysis Method Editor

With this tool you can select the reports to generate for the samples in the worklist, such as empirical formula confirmation report and mass list report. You can set parameters to customize these reports. You also can translate the data file into a format used by the Agilent MassHunter Workstation Qualitative Analysis program and Agilent MassHunter Workstation Quantitative Analysis program.

How does the Agilent TOF mass spectrometer work?

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.

Figure 1 shows the complete API oa-TOF schematic, including ion source, ion transfer optics, beam shaping optics, ion pulser, flight tube, and detector.

The Agilent TOF supports several atmospheric pressure ionization (API) sources. A common atmospheric sampling interface introduces ions from these various sources into the mass spectrometer vacuum system.

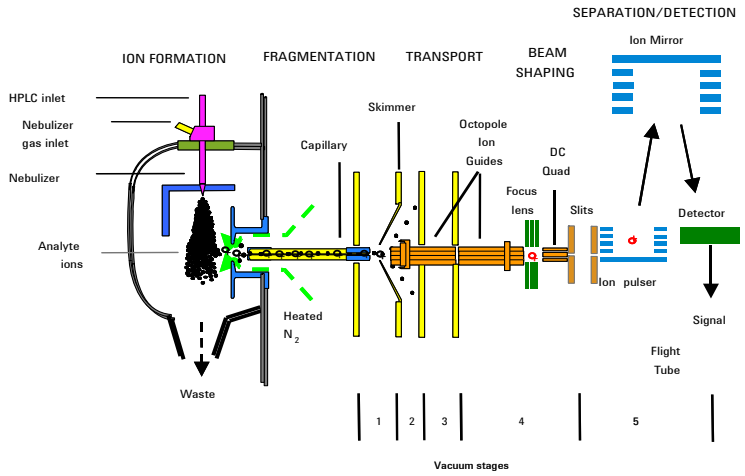


Figure 1 Schematic of API oa-TOF

After the API source forms ions, the TOF performs the following operations, organized according to the stages of the ion path and the vacuum stages of the TOF. See Figure 1 for details.

Ion fragmentation (1st vacuum stage)

Ions produced in an API source are electrostatically drawn through a drying gas and then through a heated sampling capillary into the first stage of the vacuum system. A voltage is applied at the end of the atmospheric sampling capillary to fragment the ions passing through the capillary. Beyond the capillary is a metal skimmer with a small hole. Fragmented ions with larger momentum pass through the skimmer aperture, as the majority of air is deflected by the skimmer and exhausted by a rough pump. The ions that pass through the skimmer pass into the second stage of the vacuum system.

Ion transport 1 (2nd vacuum stage)

An *octopole ion guide* is a set of small parallel metal rods with a common open axis through which the ions can pass.

In this stage the ions are immediately focused by the first of two *octopole ion guides*. Radio frequency voltage applied to these rods repels ions above a particular mass range to the open center of the rod set. The ions pass through this first octopole ion guide because of the momentum obtained from being drawn from atmospheric pressure through the sampling capillary. The ions exit this first ion guide and pass into the third stage of the vacuum system.

Ion transport 2 (3rd vacuum stage)

In this third vacuum pumping stage, the pressure is now low enough that there are few collisions of the ions with gas molecules. The ions leave the first octopole ion guide and are directly introduced into a second octopole ion guide of similar design, but with a lower direct current potential. This second octopole ion guide accelerates the ions and transmits them to the fourth vacuum stage.

Beam shaping (4th vacuum stage)

In the fourth vacuum stage lenses focus the ions so that they leave the source optics and enter the time-of-flight analyzer as a parallel beam. The more parallel the ion beam, the higher the resolution in the resulting mass spectrum. After the ions have been shaped into a parallel beam, they pass through a slit opening into the fifth and last vacuum stage where the time-of-flight analysis takes place.

Ion separation (5th vacuum stage)

Ion pulser The nearly parallel beam of ions passes into the time-of-flight ion pulser. The ion pulser is a stack of plates, each one (except the back plate) with a center hole. The ions pass into this stack from the side just between the back plate and the first plate with its center hole. To start the flight of the ion to the detector, a high voltage (HV) pulse is applied to the back plate. The applied pulse accelerates the ions through the stack of pulser plates, acting as a rapid-fire ion gun.

Flight tube The ions leave the ion pulser and travel through the flight tube, which is about one meter in length (Figure 1). At the opposite end of the flight tube is an ion “mirror”, which reflects the ions that arrive near the end of the flight tube towards the ion pulser. Because the ions entered the ion pulser with a certain amount of forward momentum, they never return to the ion pulser, but move to where the ion detector is mounted.

Because the calculation for the mass of each ion depends on its flight time in the flight tube, the background gas pressure must be very low. Any collision of an ion with residual gas slows the ion on its path to the detector and affects the accuracy of the mass calculation.

Ion detection (5th vacuum stage)

Figure 2 shows a schematic of the oa-TOF detector.

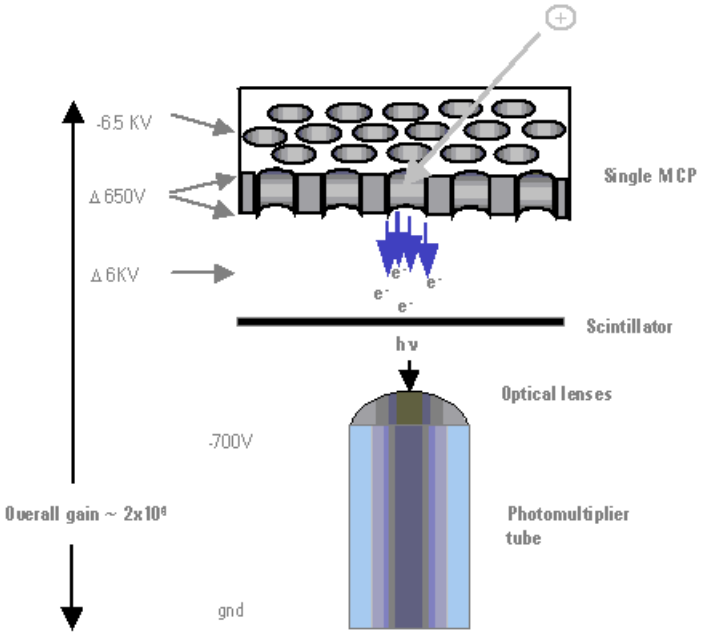


Figure 2 TOF detector, with potentials shown for positive operation

At the surface of the ion detector is a microchannel plate (MCP), a very thin plate containing a set of microscopic tubes that pass from the front surface to the rear of the plate. When an ion hits the front surface of the MCP, an electron escapes and begins the process of electrical signal amplification. As freed electrons collide with the walls of the microscopic tubes, an ever-increasing cascade of electrons travels to the rear of the plate. Roughly 10 times more electrons exit the MCP than incoming ions contact the surface.

These electrons are then focused onto a scintillator, which, when struck by electrons, produces a flash of light. The light from the scintillator is focused through two small lenses onto a photomultiplier tube (PMT), which produces the electrical signal read by the data system. The reason for producing an optical signal from the MCP electrons is because the output of the MCP is at roughly -6000 volts. The light produced by the scintillator passes to the PMT, which has a signal output at ground potential.

How do TOF software components work together?

Agilent designed the TOF software to use four major “engines” or “managers” to coordinate and direct your workflow.

The first engine is the Application UI (User Interface) Manager, the part of the software that you see on your video monitor and lets you give directions to three other engines.

These three engines work “under the hood”:

- Worklist Engine
- Data Acquisition Engine
- Data Analysis Engine

The Worklist Engine directs the Acquisition Engine to use the acquisition method specified for each sample and batch (sequence of samples) in the worklist to perform several actions:

- Execute any pre-injection custom programs (scripts)
- Set the correct instrument parameters
- Monitor and display instrument status on the screen
- Collect and display the raw data on the screen

The Worklist Engine directs the Data Analysis Engine to do several tasks:

- Execute any post-analysis scripts
- Convert the data to the correct form for PE-Sciex Analyst QS, where you can interactively review and work with the data
- Use data analysis methods to generate empirical formula confirmation reports or mass list reports for each sample in a worklist.
- Use data analysis methods to translate the data to the correct format for Agilent MassHunter Workstation Qualitative Analysis and Quantitative Analysis programs.

Figure 3 presents a visual summary of the interaction of the software components with each other.

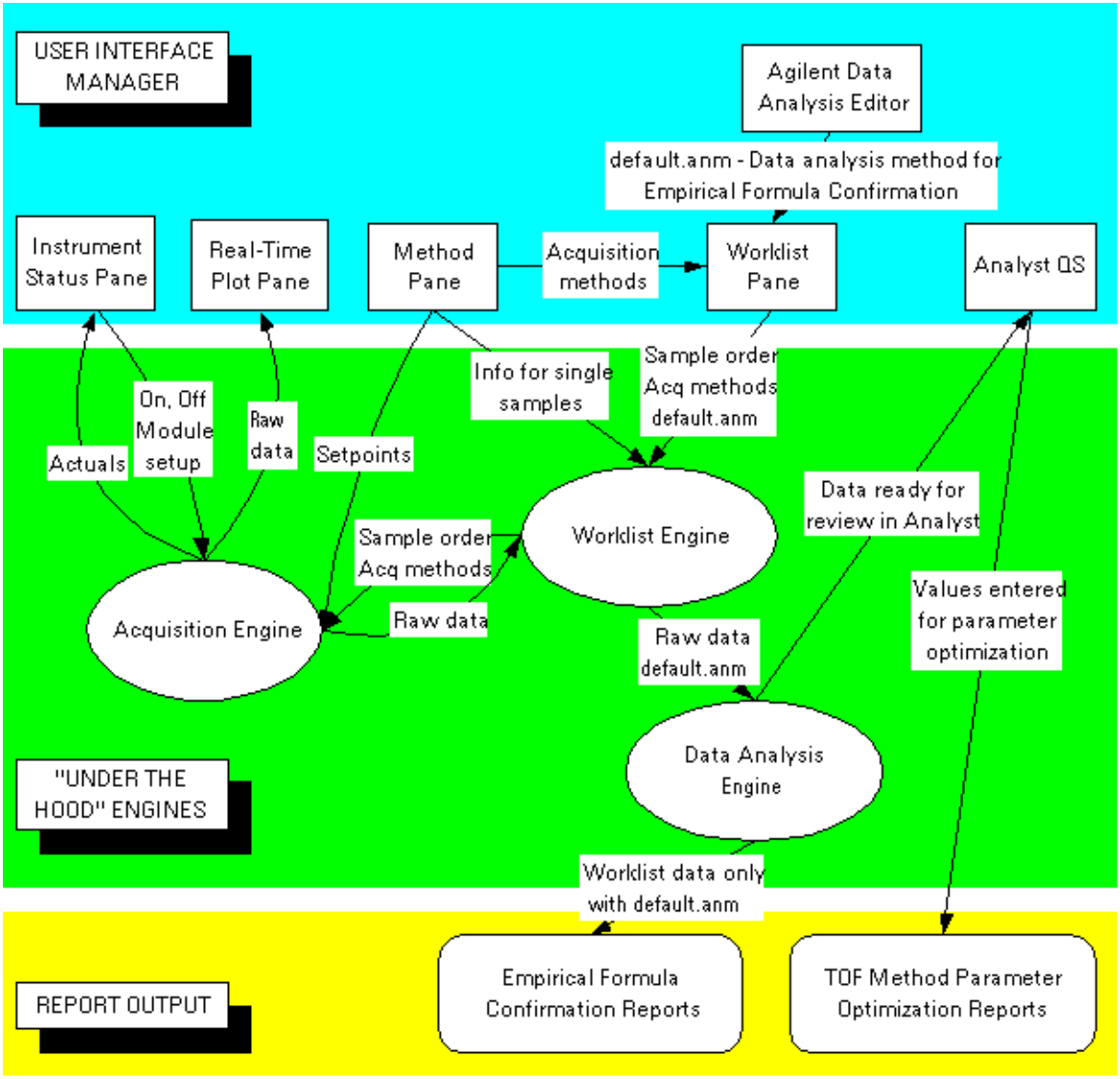


Figure 3 Workflow and interaction of software components

2
Instrument Preparation

Learn about the concepts that can help you prepare the instrument for use.

This chapter assumes that the hardware and software are installed, the instrument is configured and the performance verified. If this has not been completed, see the *Agilent G3250AA LC/MSD TOF System Installation Guide*.

LC preparation

To install, configure and start the LC modules, see the Installation Guide.

To prepare the LC for sample runs, you usually do three tasks:

- Set up the LC modules for operation
- Equilibrate or condition the column
- Monitor the plot baseline to assure pump and column stability (See "Real-time displays" on page 49.)

See the Online Help *for instructions on how to prepare the LC for a sample run.*

You can also view the system logbook for explanations of errors. (See "System logbook" on page 55.)

LC module setup

You set up the LC modules in the Instrument pane through the shortcut menus.

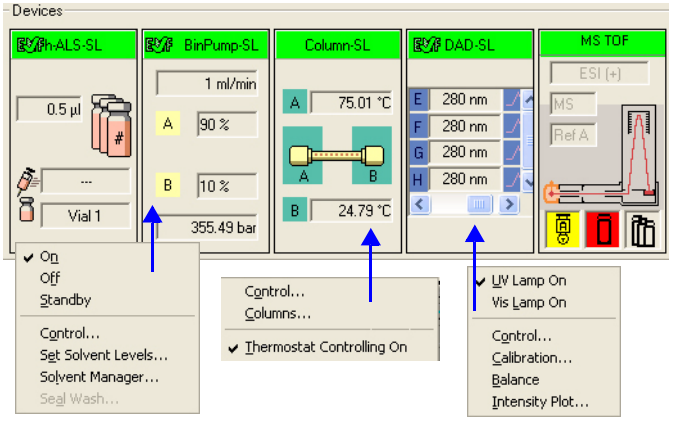


Figure 4 Instrument Status pane with shortcut menus for LC modules

Table 1 shows you the tasks that you may perform to set up the LC modules and the menu items where you can do the tasks.

Table 1 Tasks to set up the LC modules

If you have this module:	And you want to:	Right-click the module and select this menu item:
Autosampler	Change volumes for the installed syringe	Injector Configuration (ALS)
Well-Plate Sampler (WPS) or µWPS	Change volumes for the installed syringe	Configuration/General
	Select tray type and its position	Configuration/Tray
	Reset injector, Move Home, Needle Up or Down, set the valve mainpass or the bypass	Reset injector, Move Home, Needle Up, Down, Valve mainpass or Valve Bypass
CTC PAL	Update the plate assignments on the computer	Update Plate Assignment
	Reset the injector	Reset injector
Binary, isocratic, and quaternary pumps	Turn the pump on, off or place in Standby	On, Off, Standby
	Set the date and time for automatic pump turnon	Control
	Select to use built-in method if error	Control
	Set up to monitor solvent levels	Set Solvent Levels
	Change the settings for doing a seal wash	Seal Wash
CapPump	Do same tasks as binary pump	Same menu items as binary pump
	Purge the pump	Purge
	Set mixer and filter volumes	Configuration
	Enable a fast change in solvent composition	Configuration
	Do a flow sensor calibration	Calibration
Nanopump	Do the same tasks as CapPump	Same menu items as CapPump except for Accuracy Calibration (flow sensor)
Thermostat. Column Compartment (TCC)	Turn the right or left controller on or off	Configuration
	Set the maximum controller temperatures	Set Temperature
	Enter information for the instrument columns	Columns
Diode Array Det. (DAD)	Turn UV or Visible lamp on	UV Lamp On; Vis Lamp On

Table 1 Tasks to set up the LC modules

If you have this module:	And you want to:	Right-click the module and select this menu item:
	Set the date or time for automatic DAD turnon	Control
	Calibrate the DAD wavelength	Calibration
	Bring the baseline of the plot to zero	Balance
	Show an intensity plot for the detector	Intensity plot

Column equilibration and conditioning

You set up to equilibrate or condition a column in different ways with the TOF software.

Equilibration Column equilibration eliminates any previously separated compounds or impurities from the column after runs with solvent of a single composition. To equilibrate a column before a sample run, you pass the solvent that you intend to use for the run through the column for a period of time.

Conditioning Column conditioning returns column characteristics to their initial state after a gradient run. To condition a column before a sample run, you pass the solvent of initial composition through the column for a period of time.

Equilibration

You can equilibrate a column in one of three ways with the TOF software.

- Interactively

  You change the loaded method setpoints to the solvent composition for the run, no volume for the injection, higher than normal flow rates and no data storage. You can then immediately apply these setpoints to the instrument and interactively stop the run when the column is ready.

- With a method in an interactive run

You can save the method with the setpoints mentioned in the above paragraph, and then do a run. The run uses the method stop time. You can also use a post run time within a sample method to equilibrate the column.

For more information on worklists, see Chapter 4, Data Acquisition.

- With a parameter in a worklist

  You can set up a blank run in a worklist to use as your equilibration run. Or you can set up an equilibration time for any sample run, where the system waits the specified time before injecting the sample. For both cases, the data is stored.

Conditioning

You can condition a column in one of three ways:

- With one of the first two procedures described in the Equilibration paragraphs.

  You enter pump conditions to bring the column to its initial condition. You can also condition the column by setting a post-run time in the method.

- With a script in a worklist

  The LC conditioning script, SCP_LCCondition, is part of the TOF software. When you enter the script into the worklist, you specify the method that you will use for the run. If a TOF is connected to the LC, you can also enter a parameter that diverts the LC eluent to waste. With this script, there is no injection and no data storage.

TOF preparation — calibration and tuning

See the Installation Guide *for instructions on how to install and start the TOF and perform an initial autotune.*

After you start the instrument, you calibrate and tune the TOF. This section presents the background information to help you understand calibration and tuning as they are implemented in the Agilent LC/MSD TOF system.

To learn how to tune and calibrate the TOF, see the Online Help.

The following distinctions show how tuning, optimization and calibration are related in the Agilent TOF software.

Calibration

Calibration is the process of assigning accurate masses based on the known masses of standard compounds, introduced either prior to or while running the sample.

Tuning

Tuning is the process of adjusting TOF parameters to achieve the following goals:

- Maximize signal intensity and maintain acceptable resolution, or
- Maximize resolution and maintain acceptable signal intensity

See "Tuning choices" on page 31 to learn more about Agilent tuning tools.

The Agilent TOF software and its documentation and online help use the words “tuning” and “optimization” interchangeably. Agilent tools such as Initial Autotune, Autotune and Quicktune include both automatic calibration and tuning.

To achieve optimum results for mass calibration and all tuning choices, you should load the autotune methods **ESIautotune.m, nanoESIautotune.m, APPIautotune.m, APCIautotune.m** or **MMIautotune.m** for the ion source that is installed. These methods set the ion source to nominal operating values. You must also select the ion source and polarity from the left side of the MS TOF tab first. You should use the autotune method **nanoESIautotune.m** with a dual nanospray source. If you are using Polarity Switching in your method, you will need to use the Polarity Switching autotune methods. See the online help for more information.

Do not use these autotune methods as templates when doing method development.

The methods **ESIautotune.m**, **APCIautotune.m, nanoESIautotune.m, APPIautotune.m** and **MMIautotune.m** have their setpoint values optimized for positive ion operation. If you are operating in negative ion mode, you will need to make a few simple adjustments and then save the respective tune method to a new name (for example, save ESIautotune.m as ESInegautotune.m). You will get a warning message, but you may continue to use this method. For details see the section "TOF parameters adjustable through tuning" on page 37.

If you are using Polarity Switching, you will need to tune four times. First, you need to tune in both positive and negative mode. Then, you need to tune in both positive and negative mode with the tune methods specifically set up for Polarity Switching. See the online help for more information.

Mass calibration

Any time that you want to ensure mass accuracy of the instrument, you do a calibration. You do mass calibrations by passing a calibrant with known masses from the calibrant bottle through the mass spectrometer.

At startup, you do a full mass calibration, either manually or automatically. Automatic calibrations take place when you select one of the Agilent tools, Initial Autotune, Autotune or Quicktune. You set up a manual calibration from the Calibration tab in the Method pane (Figure below).

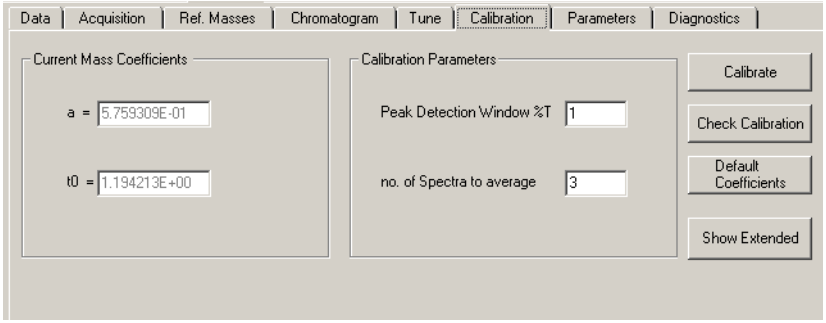


Figure 5 Standard Calibration tab of the Method pane

See Chapter 3, Acquisition Methods*, to learn more about mass correction using reference standards.*

During sample analysis the system corrects the calibration with the introduction of a standard containing reference masses, if you enable the correction through the method.

Calibration equations

Calibration masses are specific to ion source and ion polarity.

Depending on the number of masses that you select, the software uses the coefficients from one or two equations.

The TOF measures the flight times of the calibrant ions and with the known m/z values calculates the coefficients, a and t_o, of the following equation:

$$m/z = [a(t - t_o)]^2 \quad [1]$$

The values for the coefficients, a and t_o appear in Figure 5 above.

An example of the calibration curve resulting from this equation is shown below:

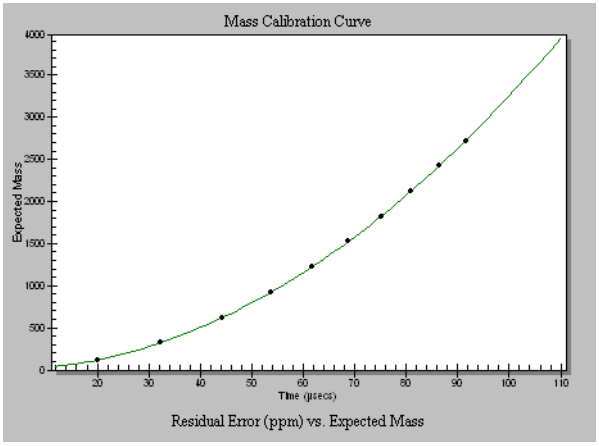


Figure 6 Example calibration curve

There is also a small amount of residual error that remains (only a few ppm). Agilent assumes a fit of the error values to the following polynomial equation:

$$\text{Residual mass error} = a_2 t + b_2 t^3 + c_2 t^5 + d_2 t^7 + e_2 t^9 + f_2 t^{11} \ldots \textbf{[2]}$$

The fit for the residual mass error can be represented by this example detailed residual plot.

The error in the mass error (blue diamonds) after the polynomial fit is essentially zero (red squares).

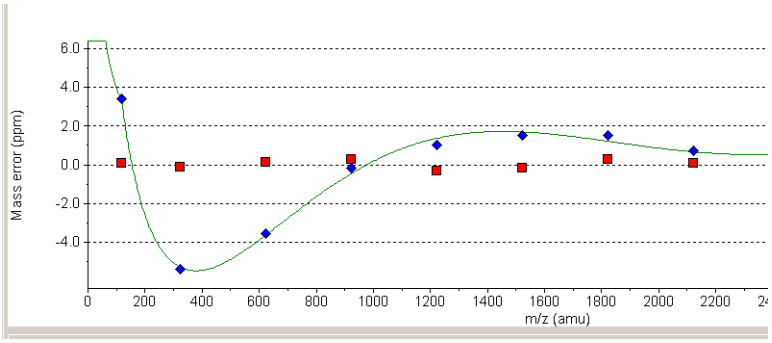


Figure 7 Example detailed residual plot

The $(m/z)'$ calculated during a calibration is the m/z from equation 1 plus the residual mass error calculated from equation 2.

$$(m/z)' = m/z + \text{Residual mass error} \qquad \textbf{[3]}$$

To see the current coefficients calculated for the residual mass error, you bring up the extended calibration tab.

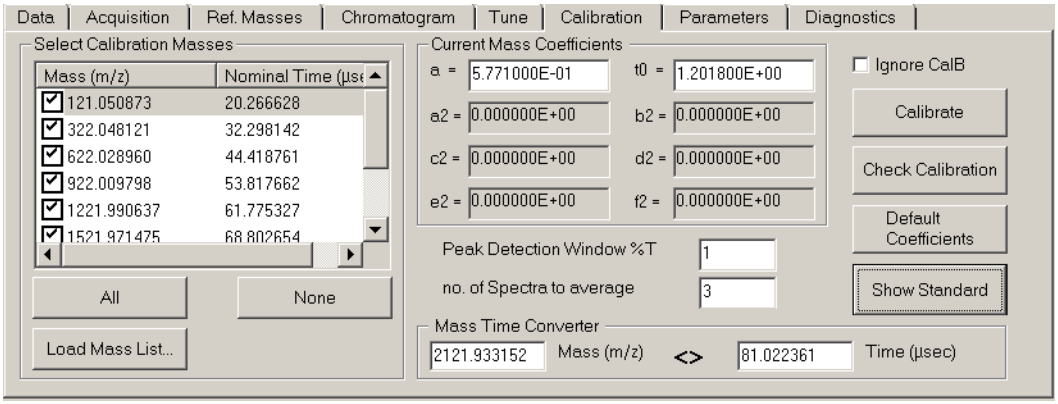


Figure 8 Extended Calibration tab

During sample analysis the TOF measures the flight times of the compound ions and converts these times with the above equations and coefficients to m/z values.

Masses and coefficients

The default mass list contains the masses found in the Agilent calibrant. If you want to use your own calibration compound, you can edit and save the mass list. All mass lists are in one file.

The Agilent TOF software calculates all eight coefficients of the polynomial equation. The number of coefficients calculated during a calibration depends on the number of masses you select for the calibration.

For a full manual calibration you must select at least seven masses for the system to calculate all eight coefficients. Table 2 shows you the coefficients that the software calculates for the number of masses selected. The uncalculated coefficients are set to zero. The standard calibration tune mix has 10 masses. The accuracy of the TOF will decrease as you use fewer tuning masses.

Table 2 Effect of Mass Selection on Coefficients Calculated

Number of Masses	Coefficients Calculated
1	a
2 to 4	a, t_0 (equation 1)

Table 2 Effect of Mass Selection on Coefficients Calculated

Number of Masses	Coefficients Calculated
5	a, t_0, a2, b2, c2, d2
6	a, t_0, a2, b2, c2, d2, e2
>=7	a, t_0, a2, b2, c2, d2, e2, f2

Tuning choices

You can see the tuning choices available to you on the Tune tab. All the automatic tuning choices calibrate the TOF using ten masses with the ESI, nanoESI and Dual nanoESI ion source and eight masses with APCI, APPI or MMI.

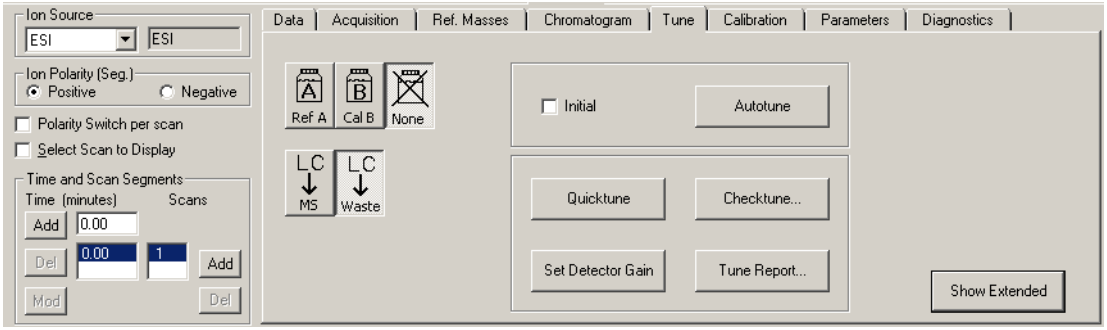


Figure 9 Standard Tune tab

Initial Autotune When you select this option after installation or major service, the system automatically adjusts all the tunable parameters to optimize signal and resolution. See “Initial Autotune" on page 32 for more information.

Autotune This tool performs the same optimization operations as Initial Autotune but uses the current settings as starting values. See “Autotune" on page 34 for more information.

Quicktune Quicktune automatically adjusts the most commonly required subset of tunable parameters. See “Quicktune" on page 34.

Manual Tune (Ramp)

If you intend to use your own tune compound, or you are not satisfied with the signal or resolution values from Autotune or Quicktune, you can do a manual tune. When you do a manual tune, you ramp the values of individual parameters separately and do a manual calibration. See "Manual Tune (Ramp)" on page 36. You do a manual tune through the Extended Tune tab.

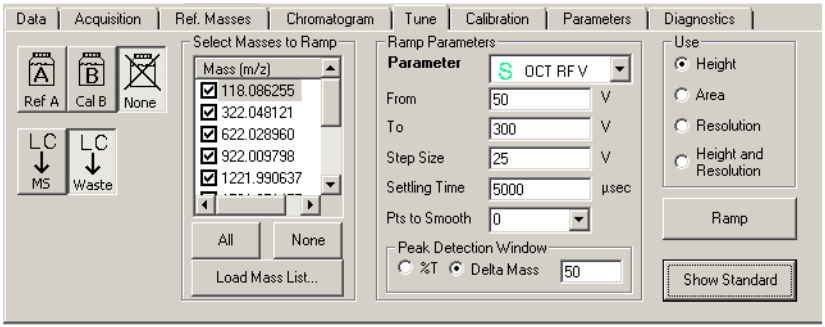


Figure 10 Extended Tune tab

Initial Autotune

See the Installation Guide *for instructions on how to do an Initial Autotune*

You use Initial Autotune only under special circumstances because the process takes about 20 to 30 minutes to complete.

- After you install the hardware and software
- After your TOF has had maintenance
- If you no longer have the previous tune files or parameters
- If regular Autotune does not work

The system should be on (high voltage on) at least one hour before you start an autotune.

During the Initial Autotune process, the system goes through the following steps, all without your intervention.

a Resets any current tune parameters (Parameters tab in the Method pane) to original defaults.

b Automatically opens the calibrant valve and does a coarse calibration based on one low mass and one high mass

c Adjusts all the tunable parameters automatically

d Places the optimum values in the Parameters tab

e Does a full calibration after the final adjustment on ten masses

f Prints a tune report at the end of the Autotune process

During the parameter adjustment, the following window appears on the screen.

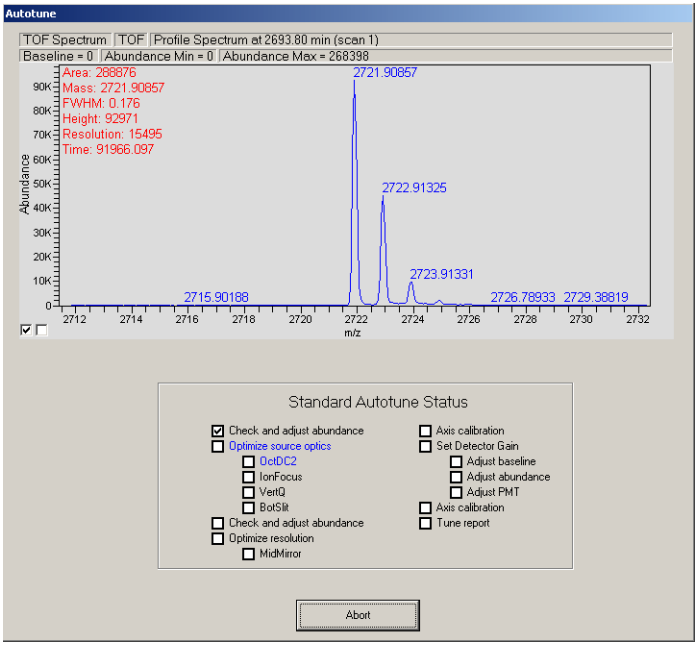


Figure 11 Autotune status window

After the optimization of each parameter, its Optimization Curve appears temporarily for your review.

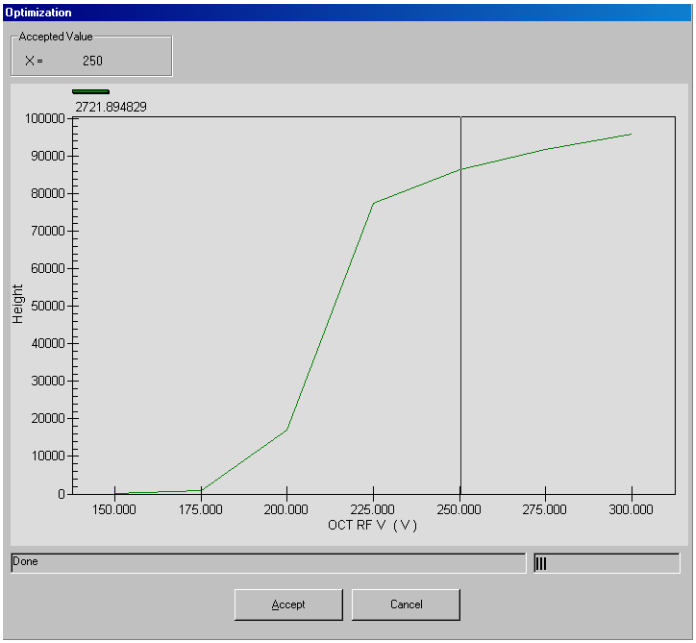


Figure 12 Optimization Curve for a parameter

Autotune

Autotune follows the same process as an Initial Autotune but does not do a coarse calibration and uses the current tune parameters as a starting point.

Because Autotune takes about 10 minutes, you use Autotune to tune the instrument on a weekly schedule.

Quicktune

Quicktune takes 2 to 3 minutes because the software is optimizing only the most significant parameters followed by a calibration.

During Quicktune the system goes through the following steps:

a Automatically opens the calibrant valve

b Adjusts the vertical Q (Vert Q) and bottom slit parameters in the Beam Shaping Optics (optimizes transmission)

c Adjusts the middle mirror of the Reflectron (optimizes resolution)

d Does a calibration after the final adjustment based on ten masses

e Prints a tune report

Tuning with Polarity Switching

If you are using Polarity Switching, you have to tune four times to adjust all of the necessary parameters. Polarity Switching is only available with TOF systems using SmartCard 4 and updated power supplies.

1 Load appropriate source autotune method

2 Tune in positive mode

3 Switch to negative mode

4 Tune in negative mode

5 Load the proper autotune method for positive polarity switching for the current source

6 Tune

7 Load the proper autotune method for negative polarity switching for the current source

8 Tune

Manual Tune (Ramp)

See the Online Help for detailed instructions to help you manually tune the TOF instrument.

If you must manually tune, you use the following steps:

1. On the Extended Tune tab (Figure 10 on page 32), ramp the values of selected parameters within a selected range for selected masses.
2. When the Optimization Curve appears, select and accept the value that you want to use.

   The system automatically places the value in the Parameters tab.
3. Do a full calibration by selecting 7 to 10 masses.
4. Apply, or download, the parameter values and calibration to the instrument.

TOF parameters adjustable through tuning

TOF hardware components

To learn more about how the Agilent TOF works, see Chapter 1, Overview.

The figure below shows the TOF hardware components associated with each stage of the ion formation/detection process.

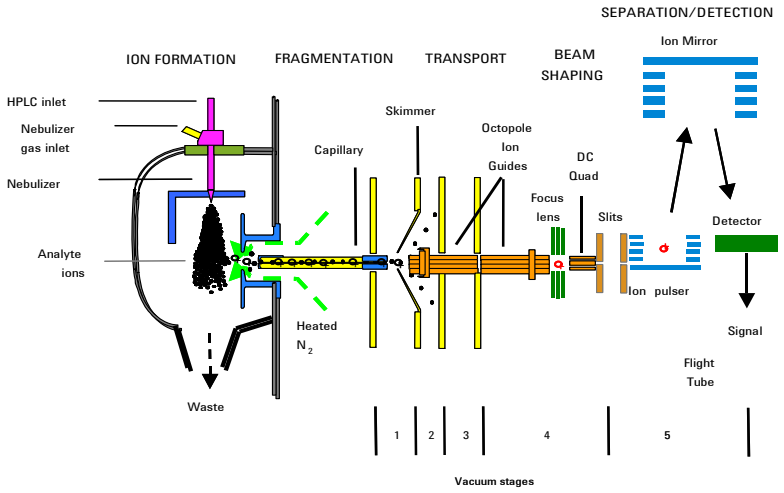


Figure 13 TOF hardware components and ion formation/detection

To learn more about the TOF parameters adjustable through methods and worklists, see Chapter 3, Acquisition Methods.

Only the parameters of the components to the right of the skimmer in Figure 13 are optimized in tuning. The parameters of the components to the left of the first octopole ion guide and the OctRF V in Figure 13 are optimized through methods and worklists.

Method parameters must be properly set before tuning begins. These parameters depend on the source that is connected, the flow rate of the sample, the desired level of in-source fragmentation and the ion polarity. Several default methods including **ESIautotune.m, nanoESIautotune.m, APPIautotune.m, APCIautotune.m, and MMIautotune.m** are supplied to assist in automatic tuning of the instrument. These methods are configured with parameters optimized for positive ion mode. Another set of methods which have been set up for polarity switching are also supplied with the software.

When you begin an autotune and one of the system autotune methods is not currently loaded, you will be asked whether to load the appropriate tune method before you continue. If you are autotuning in negative ion mode, then you must first edit the corresponding method so that optimal negative ion values are present. You may then save the new method with a different name indicating that it is a negative ion mode method. (for example you might choose the name ESInegautotune.m for a negative ion mode ESI source autotune method) To return to positive ion mode autotune, you need to reload the original positive ion method. If you have saved the negative ion method with a new name, you may ignore the warning message.

Fragmentor and capillary voltages differ between positive and negative modes. You should use the values for a nanospray source for the dual nanospray source. Default voltages are listed in the installation guide.

Parameters adjustable through tuning

The Parameters tab in the Method Pane contains a listing of the parameters used in tuning. After a tune, the new values are placed here.

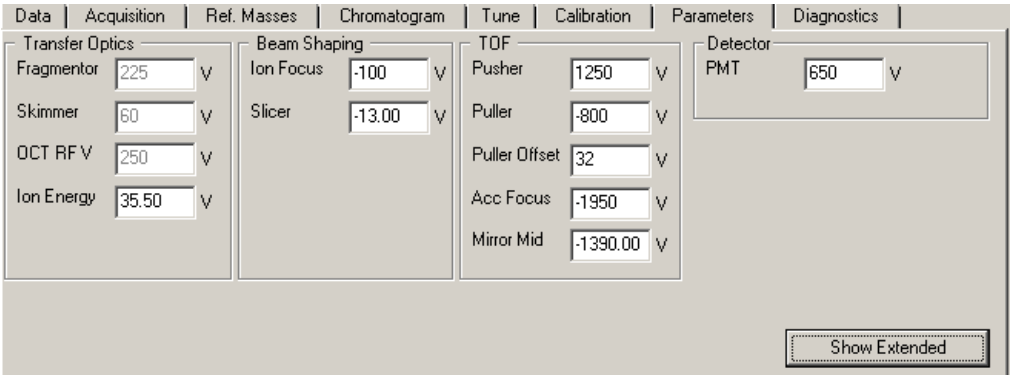


Figure 14 Standard Parameters tab

Note that the parameters that are greyed out are used to tune the instrument but are not optimized during the tuning. You change these analysis-dependent parameters through the Acquisition tab of the Method pane and through worklists.

You can load or save the values from the Parameters tab. You can view even more tuning parameters in the Extended Parameters tab.

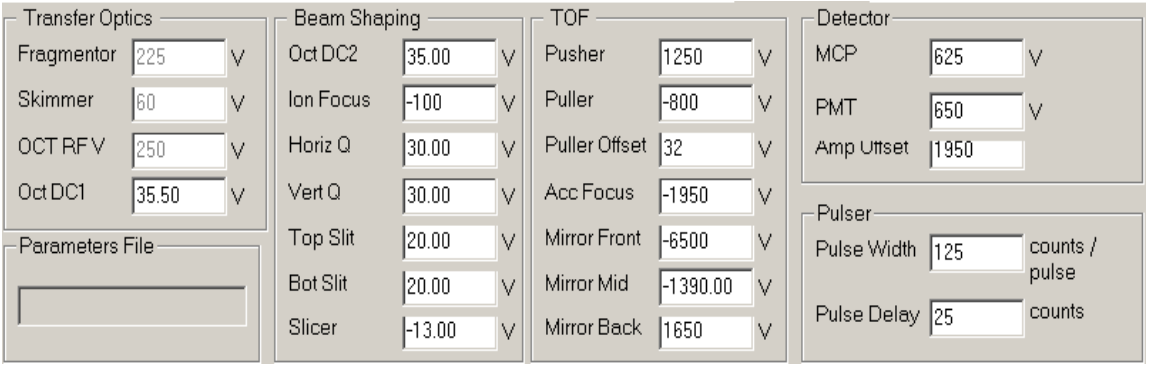


Figure 15 Extended Parameters tab

	Relationship between hardware components and tuning parameters
Octopole ion guides	Includes the voltages for OctDC1 and Oct DC2 parameters
Focus lens	Ion focus parameter
DC Quad	Quadrupole that includes the Vert Q and Horiz Q parameters
Slits	Includes the Top Slit, Bot Slit and Slicer parameters.
Ion Pulser	Includes the Pusher, Puller, Puller Offset and Acc Focus (Accelerator Focus) parameters
Ion Mirror	Includes the Mirror Front, Mirror Mid and Mirror Back parameters
Detector	Includes the MCP (multi channel plates), PMT (photo multiplier tube) and Amp Offset parameters (Figure 2 on page 17)
	The ion energy parameter includes the Oct DC1, Oct DC2, Vert Q, Horiz Q, Top Slit and Bot Slit parameters.

Useful parameters to adjust manually

Oct RF V If you want to increase the signal for specific masses, you can ramp the OCT RF V parameter for those masses and look at the plot of Height vs. OCT RF V for each of the masses.

The Optimization Curve may look like this:

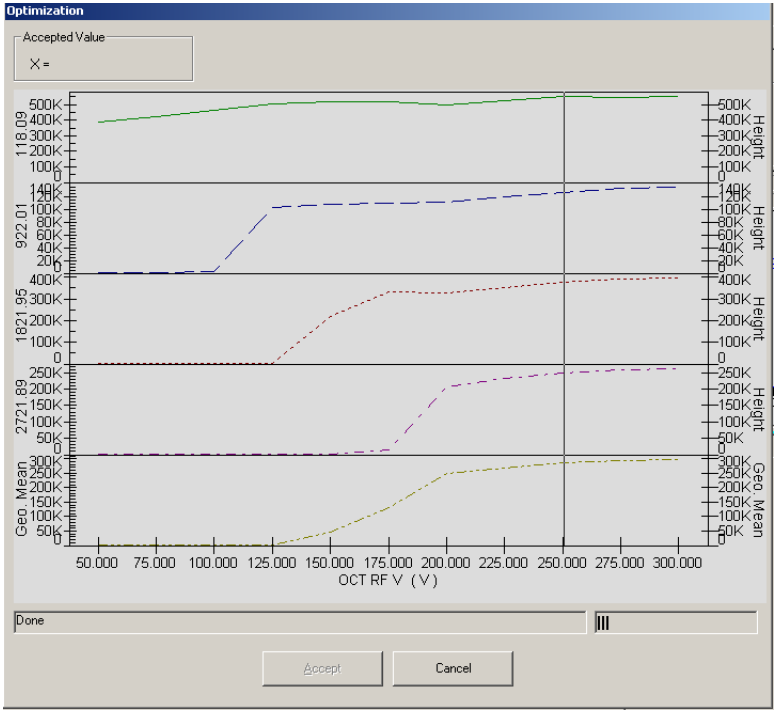


Figure 16 Optimization Curve for Height vs. Oct RF V

Mirror Mid If you want to improve the resolution of a mass, you can adjust the mid-mirror voltage manually.

Figure 17 shows an example of the plot of these variables for 5 masses.

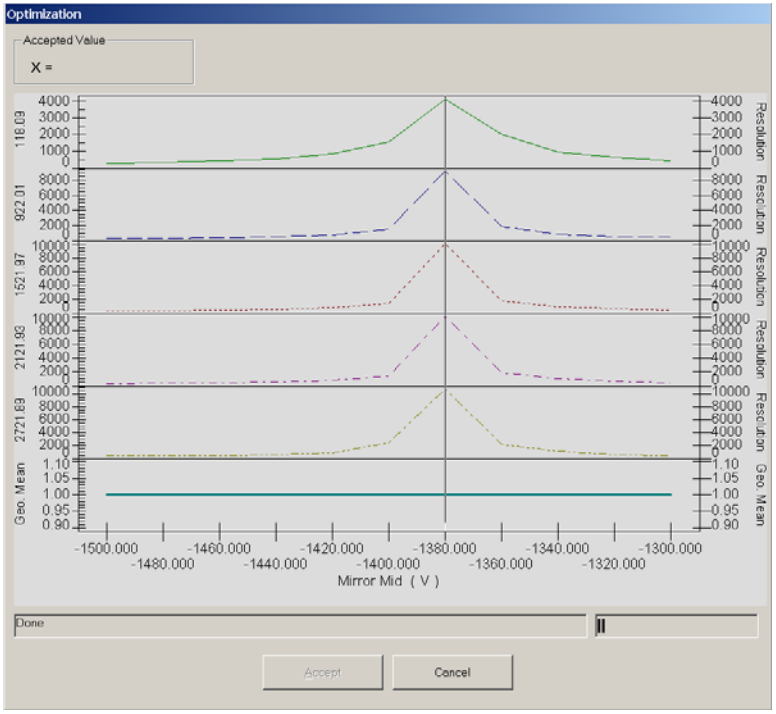


Figure 17 Optimization curve for resolution versus Mirror Mid (V)

Accelerator focus Another parameter that affects both the signal and resolution is the accelerator focus voltage. Yet, at values that maximize the signal, the resolution is often minimized and vice versa. Agilent provides a way to view the effect of this focus setting on both the signal and the resolution so that you can select values for both that are acceptable.

If you select Height and Resolution and then ramp this parameter, the resulting Optimization Curve may look like this:

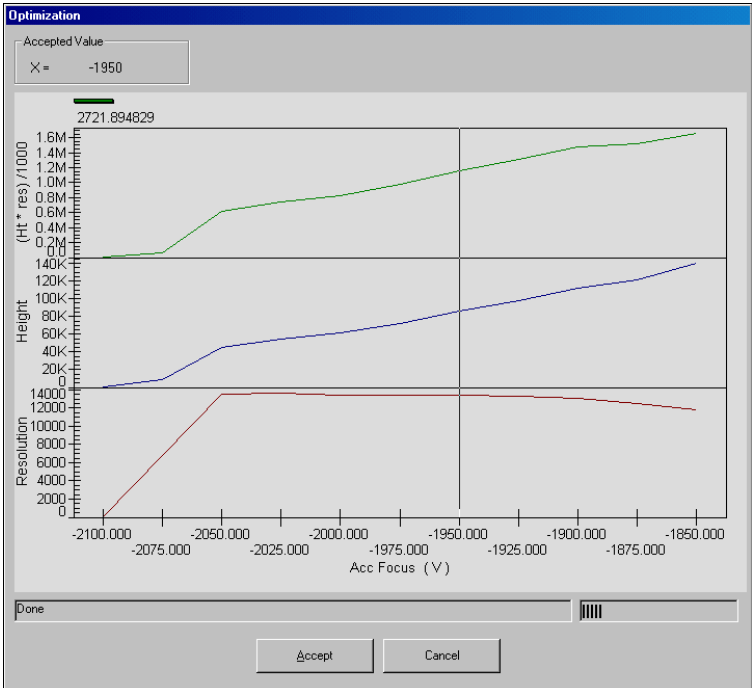
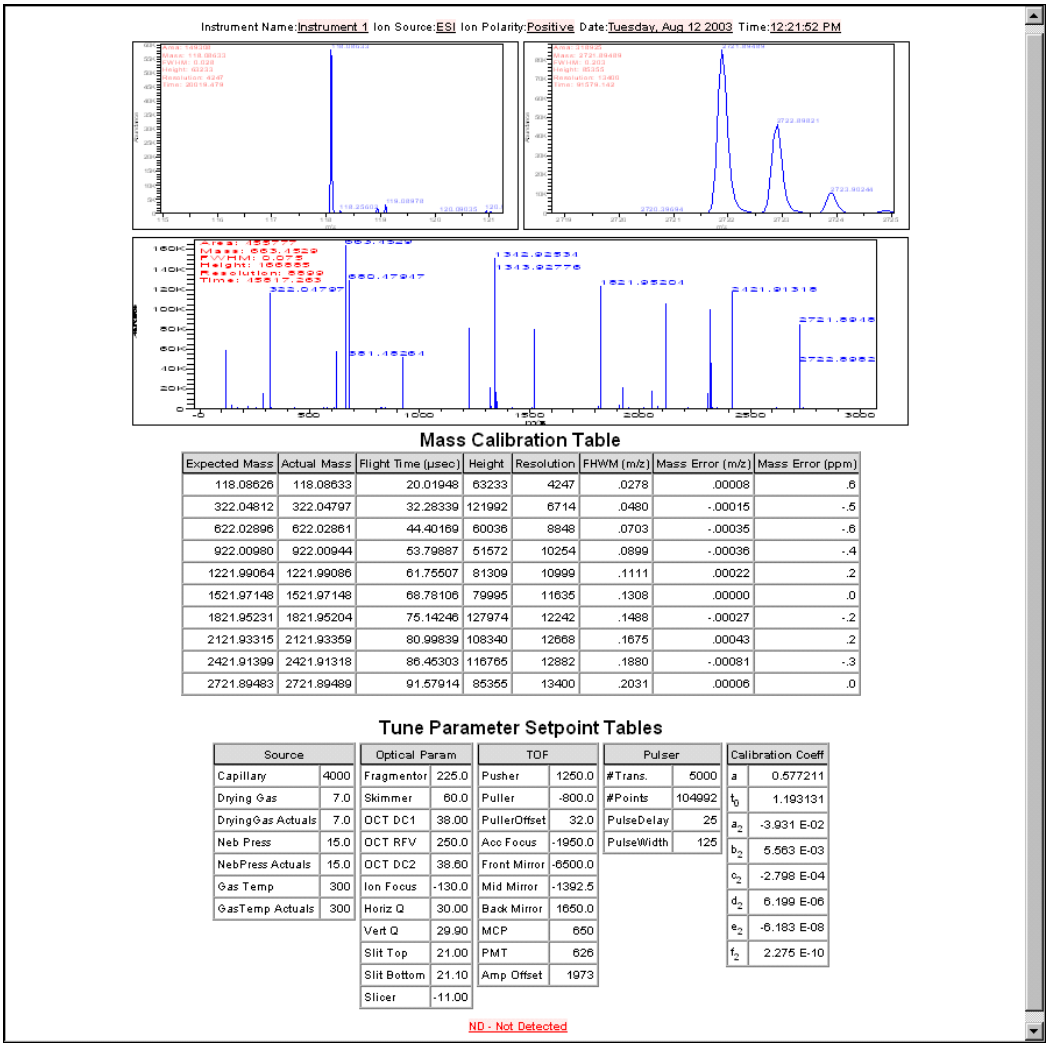


Figure 18 Plots of signal and resolution vs. accelerator focus (V)

Tune reports

At the end of every Autotune or Quicktune, the system prints a Tune report. You can also manually print a Tune report from the Standard Tune tab.



Mass Calibration Table

Expected Mass	Actual Mass	Flight Time (µsec)	Height	Resolution	FHWM (m/z)	Mass Error (m/z)	Mass Error (ppm)
118.08626	118.08633	20.01948	63233	4247	.0278	.00008	.6
322.04812	322.04797	32.28339	121992	6714	.0480	-.00015	-.5
622.02896	622.02861	44.40169	60036	8848	.0703	-.00035	-.6
922.00980	922.00944	53.79887	51572	10254	.0899	-.00036	-.4
1221.99064	1221.99086	61.75507	81309	10999	.1111	.00022	.2
1521.97148	1521.97148	68.78105	79995	11635	.1308	.00000	.0
1821.95231	1821.95204	75.14246	127974	12242	.1488	-.00027	-.2
2121.93315	2121.93359	80.99839	108340	12668	.1675	.00043	.2
2421.91399	2421.91318	86.45303	116765	12882	.1880	-.00081	-.3
2721.89483	2721.89489	91.57914	85355	13400	.2031	.00006	.0

Tune Parameter Setpoint Tables

Source	
Capillary	4000
Drying Gas	7.0
DryingGas Actuals	7.0
Neb Press	15.0
NebPress Actuals	15.0
Gas Temp	300
GasTemp Actuals	300

Optical Param	
Fragmentor	225.0
Skimmer	60.0
OCT DC1	38.00
OCT RFV	250.0
OCT DC2	38.60
Ion Focus	-130.0
Horiz Q	30.00
Vert Q	29.90
Slit Top	21.00
Slit Bottom	21.10
Slicer	-11.00

TOF	
Pusher	1250.0
Puller	-800.0
PullerOffset	32.0
Acc Focus	-1950.0
Front Mirror	-6500.0
Mid Mirror	-1392.5
Back Mirror	1650.0
MCP	650
PMT	626
Amp Offset	1973

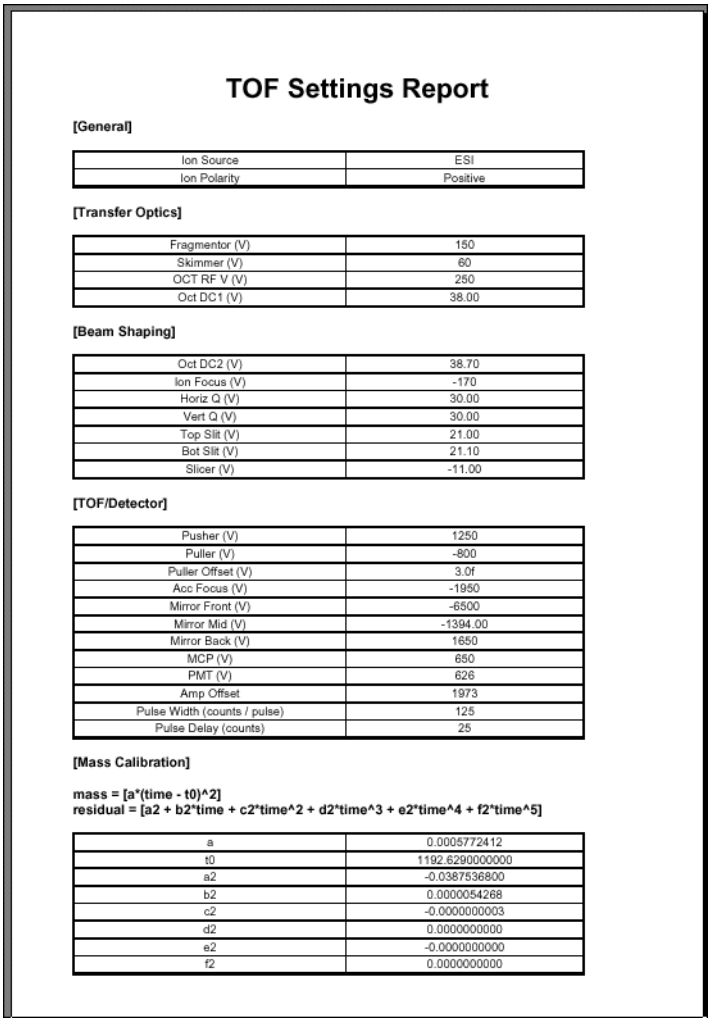
Pulser	
#Trans.	5000
#Points	104992
PulseDelay	25
PulseWidth	125

Calibration Coeff	
a	0.577211
t_0	1.193131
a_2	-3.931 E-02
b_2	5.563 E-03
c_2	-2.798 E-04
d_2	6.199 E-06
e_2	-6.183 E-08
f_2	2.275 E-10

ND - Not Detected

Figure 19 Tune Report

After a manual tune, you can also print a report of the TOF parameter settings through the TOF shortcut menu on the Instrument Status pane.



TOF Settings Report

[General]

Ion Source	ESI
Ion Polarity	Positive

[Transfer Optics]

Fragmentor (V)	150
Skimmer (V)	60
OCT RF V (V)	250
Oct DC1 (V)	38.00

[Beam Shaping]

Oct DC2 (V)	38.70
Ion Focus (V)	-170
Horiz Q (V)	30.00
Vert Q (V)	30.00
Top Slit (V)	21.00
Bot Slit (V)	21.10
Slicer (V)	-11.00

[TOF/Detector]

Pusher (V)	1250
Puller (V)	-800
Puller Offset (V)	3.0f
Acc Focus (V)	-1950
Mirror Front (V)	-6500
Mirror Mid (V)	-1394.00
Mirror Back (V)	1650
MCP (V)	650
PMT (V)	626
Amp Offset	1973
Pulse Width (counts / pulse)	125
Pulse Delay (counts)	25

[Mass Calibration]

mass = [a*(time - t0)^2]
residual = [a2 + b2*time + c2*time^2 + d2*time^3 + e2*time^4 + f2*time^5]

a	0.0005772412
t0	1192.6290000000
a2	-0.0387536800
b2	0.0000054268
c2	-0.0000000003
d2	0.0000000000
e2	-0.0000000000
f2	0.0000000000

Figure 20 Report of TOF parameter settings

When to tune your TOF — Checktune report

You can do an Autotune or Quicktune on your TOF on a regular schedule, or you can tune only when your TOF needs tuning. To evaluate the necessity to tune, you can print a Checktune Report.

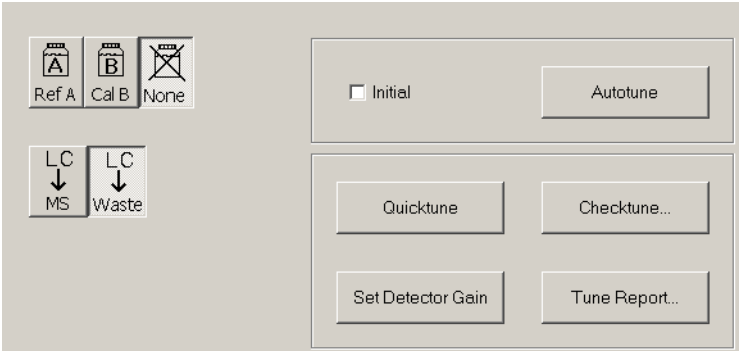
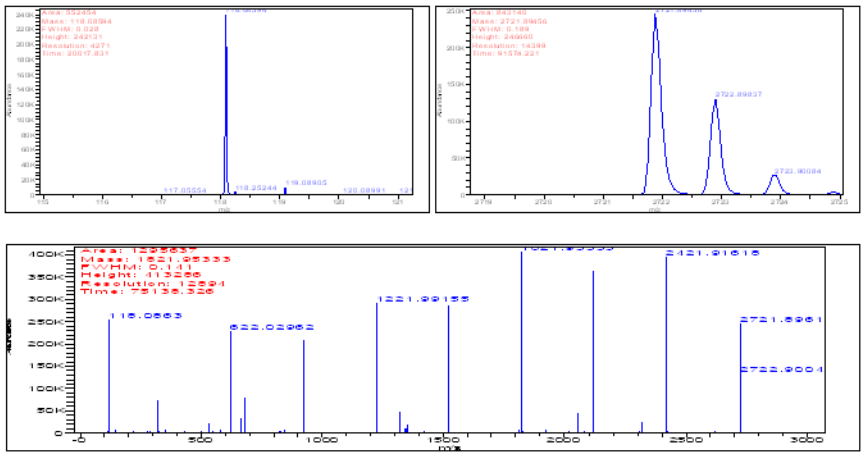


Figure 21 Standard Tune tab with Checktune option

The Checktune report lets you know if the mass calibration and optimization limits are met with a Pass or Fail.

Instrument Name:**AppTOF1** Ion Source:**ESI** Ion Polarity:**Positive** Date:**Friday, Sep 19 2003** Time:**11:44:35 AM**



Mass Calibration Table

Scans : **5** | Height Limit : **>= 1** | Resolution Limit (per mass) | PPM Limit : **+/- 100**

Expected Mass	Actual Mass	Height	Resolution	Resolution Limit	FWHM (m/z)	Mass Error (m/z)	Mass Error (ppm)
118.08630	118.08620	248854	4258	>= 3500	.0277	-.00010	-.6
322.04810	322.04820	74897	7471	>= 6000	.0431	.00000	.1
622.02900	622.02910	222920	9170	>= 8000	.0678	.00010	.2
922.00980	922.01000	206725	10873	>= 8000	.0848	.00020	.2
1221.99060	1221.99120	308031	11893	>= 8000	.1027	.00050	.4
1521.97150	1521.97220	283208	12468	>= 10000	.1221	.00070	.5
1821.95230	1821.95260	403924	12932	>= 10000	.1409	.00030	.2
2121.93320	2121.93380	357812	13302	>= 10000	.1595	.00060	.3
2421.91400	2421.91500	397838	13729	>= 10000	.1764	.00100	.4
2721.89480	2721.89540	243248	14411	>= 10000	.1889	.00060	.2

Underlined values (in red) indicates failure ND - Not Detected

Figure 22 Example of a Checktune report

Storage and retrieval of tune results

Both the tuning parameters in the Tune panel and all the calibration coefficients in the Calibration panel are stored in several places depending on your actions.

Results for positive polarity tunes are saved in a tune file separate from the results for negative polarity tunes.

Table 3 How the TOF system stores and uses tune results

If you do this:	Tune results are stored here:	To use these tune results, do this:
• Initial Autotune, Autotune and Quicktune • Manual tune—Apply or Save Method	Instrument device driver memory	Run a sample. Tune results residing in the device driver memory are the parameters used for sample runs.
• Initial Autotune, Autotune and Quicktune • Manual tune—Save method • Exit TOF	Tune file (see below for the location of this file)	Restart TOF. This is the only way to restore results from the tune file to the device driver memory.
• Run a sample	Sample .wiff file	Load .wiff in Analyst

Initial Autotune, Autotune and Quicktune

After you perform one of these automatic tunes, the tune parameters and calibration coefficients go into the instrument device driver memory and are also automatically stored to the file **D:\TOF_Data\tune\TofTune.tun**

Manual tune

After you perform a complete manual tune, including calibration, you must “apply” the tune parameters and calibration coefficients to download them to the instrument device driver. When you save a method or exit the TOF software, the tune results in memory are saved to the tune file.

Mass lists for all tunes and calibrations are stored separately from both the tune file and the method.

Real-time displays

Instrument status

You can see if a module is On, Off or in Standby by observing the color of the bar above the module graphic.

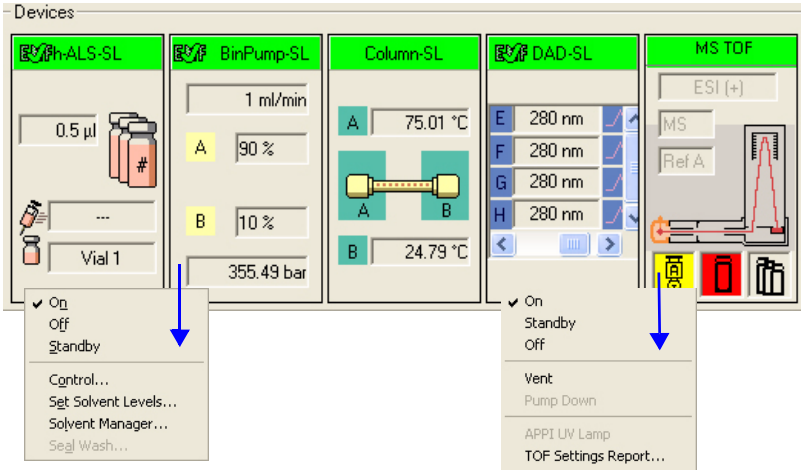


Figure 23 Instrument Status Pane

Even though the Status indicator is green, the thermo-ALS or thermo-WPS may not be at the desired setpoint. You can monitor the status of the thermostat for these devices from the display on the WPS or ALS icon.

Table 4 Colors of Instrument States

Instrument State	Color
On—Preparing	Yellow
On—Ready	Green
On—Waiting	Purple
On—Running	Blue
Standby	Gray—To see if the pump or MS TOF is in Standby mode or off, look for the check mark in the shortcut menu.
Off	Gray

Real-time parameter values (Actuals)

What you can display

You select parameters and states to monitor for each instrument module in the Actuals panel in the Instrument Pane.

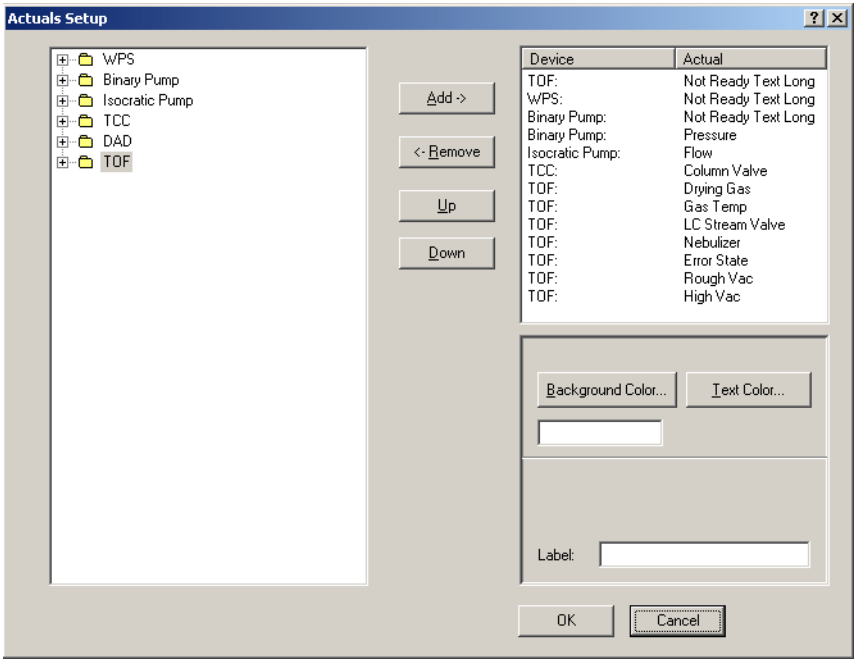


Figure 24 Actuals Setup dialog box

See the Online Help *for descriptions of each of these parameters and states*.

The parameters and states for each module listed below are available for display.

Table 5 Actuals available for display

Module	Parameter or State
All modules	Run Time, Run State, Ready State, Ready Type, Not Ready Text Long, Not Ready Text Short, Rawdata State, Error State
ALS	Vial, Sample, Volume, Needle, Command, Injectmode, Tray Type A, Tray Type B, Overlap, Injectstatus, Temp, Therm Power
WPS, µWPS	The same as ALS except no Vial and addition of Drawn Volume, Sample Position and Needle Position
Binary Pump	Solvent Ratio A, Solvent Ratio B, Flow, Pressure, Ripple, Fill A,B, A1, B1, A2, B2, Power, Channel Name A, B, Solvent Selection A, B
Capillary Pump	Same as binary pump with no Fill A1, A2, B1, B2 and with the addition of Solvent Ratio C, Solvent Ratio D, Fill C, Fill D, Primary Flow, EMPV, Purge Status, Purge Channel, Purge Time and Pump Op Mode
Nanopump	Same as capillary pump with addition of FSAC State and FSAC Step
Thermostatted Column Compartment	Left Temp, Left Temp Set, Right Temp, Right Temp set, Column Valve, Therm Power
35900E	Current Vial
DAD G1315C and G1315D	Sample WI A-H, Sample Bw A-H, Reference On A-H, Reference WI A-H, Reference Bw A-H, UV Lamp, Vis Lamp
DAD (all others)	Sample WI A-E, Sample Bw A-E, Reference On A-E, Reference WI A-E, Reference Bw A-E, UV Lamp, Vis Lamp
MS TOF	Rough Vacuum, High Vacuum, Gas Temp, Vaporizer Temp, Drying Gas Flow, Nebulizer Pressure, Capillary Current, Chamber Current, Corona Voltage, Charging Voltage, Control State, Cal/Ref Mass, LC Stream

Real-time plots

What you can display

You can display the following plots for these modules.

Table 6 Real-time plots available for display

Module	Plot type
Pumps	Pressure vs. time
Thermostatted Column Compartment	Temperature Left, Temperature Right vs. time
35900E	ADC signal vs. time
DAD G1315C and G1315D	Signals A-H vs. time, spectra
DAD	Signals A-E vs. time, spectra
TOF	Any chromatogram set up in the Chromatogram tab of the Method pane: • Any segment or scan of a total ion chromatogram (TIC) or extracted ion chromatogram (EIC) • Method setpoints and actual conditions

What you can do with the displays

Table 7 What you can do with plots and spectra

Display type	What you can do
Signal plots	Change range of intensity or time, freeze and zoom the plot
Pump parameters	Change range of intensity or time, freeze and zoom the plot
TOF spectra	Autoscale the axes, freeze and zoom the plot; toggle between a line spectrum and a profile spectrum; toggle between a mass x-axis and a time x-axis, change to a display of DAD spectra (from line spectra only)

Table 7 What you can do with plots and spectra

Display type	What you can do
DAD spectra	Change to a display of TOF spectra

Profile vs. Line spectral displays

Line spectra for the MS TOF display the abundance vs. mass for the calculated centroid of the peak. Profile spectra display the abundance vs. mass over the mass range of the peak.

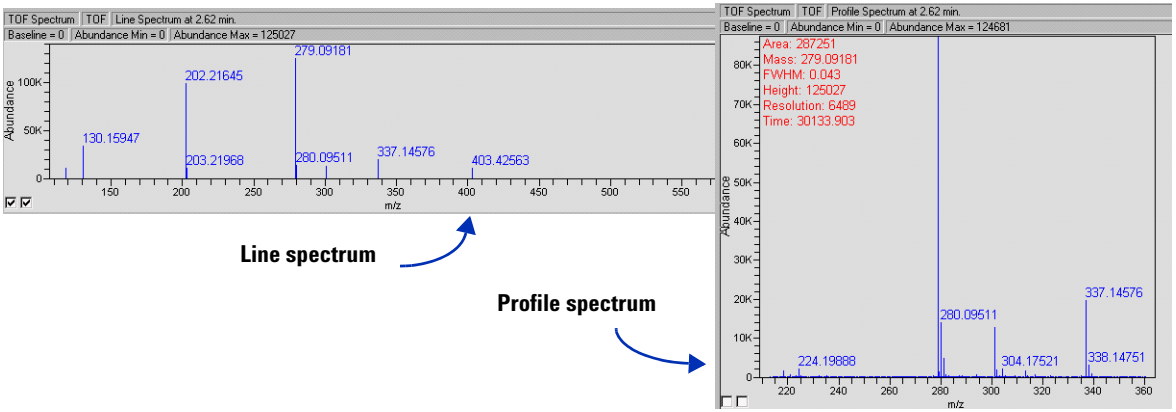


Figure 25 Line and profile spectral displays in Real-Time Plot pane

Time vs. mass spectral display

The default display is to plot abundance vs. mass, but you can change the x-axis to time. To do this, right-click in the Spectrum panel and select **Show Time Mode** from the shortcut menu. Select **Show Mass Mode** to return to the Abundance vs. Mass plot.

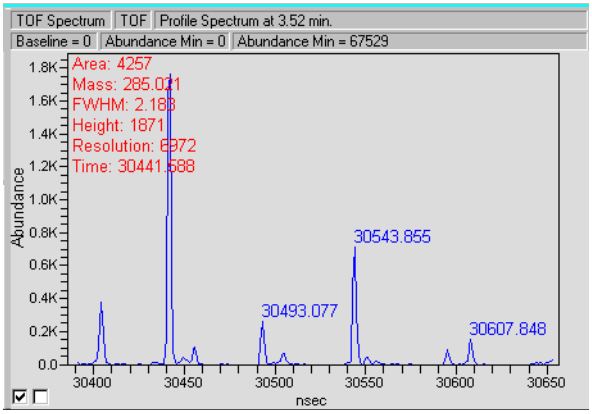


Figure 26 Abundance vs. Time for profile spectrum

System logbook

The system logbook does not list any changes to a method or worklist.

What you can view in the system logbook

The Logbook Viewer displays the dates and times when system events take place:

- Run starts, stops and aborts
- Method loaded
- Tuning and calibration operations
- Unexpected software errors
- Device driver errors, warnings and alerts including leak detection, vial not found, lamp burned out
- Device powered off or reconfigured
- Start up and shutdown
- Worklist events
- Method log files

When you open the Logbook Viewer, the software loads and displays the most recent entries in the log file.

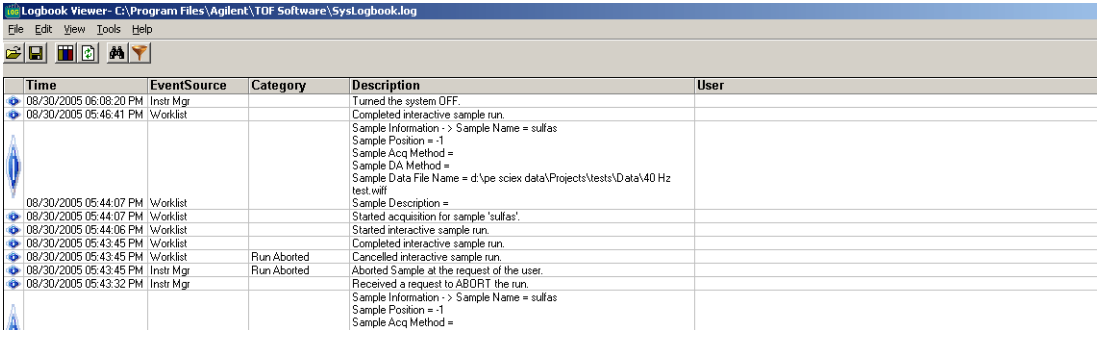


Figure 27 Logbook Viewer

The logbook also displays additional information about each event. Since the display of individual columns can be turned on and off, not all of these columns may be visible at any given time.

Table 8 Columns available for display in the Logbook Viewer

Column Name	Description
Time	Date and time of the event
Event Type	Normal event or error
Event Source	The module that produced the event (Worklist, Instr Mgr, App UI, Launcher, DA Mgr)
Category	More information about the event (e.g. Startup, Shutdown, Worklist Start, Worklist End, Run Started, Run Stopped, Run Aborted, Method Loaded)
Description	More information about the event
User	Name logged on to the system when the even occurred

What you can do with the system logbook

Move the cursor over an icon to see the tooltips, which help you perform the correct task.

See the Online Help *for instructions on how to work with the logbook.*

The system deletes week-old logbook entries. If you want to view them after a week, you must save or export them before the week has passed. Most of the tasks that you can do with the logbook help you view the entries you need to see more easily.

Table 9 Tasks you can perform with the logbook

If you want to do this:	Select this menu item or icon:
View an individual entry	Edit > Search
View selected entries only	Edit > Filter
Change column types	View menu
Change column widths	Interactively using the table
View recent events	View > Refresh
Archive entries	File > Save or Shortcut menu > Export
Open, close, or save the logbook	File menu
View method log files (method.log file in the Acq. or DA method directory)	File > Open
Print the logbook	Shortcut menu > Export

3
Acquisition Methods

See the Online Help *for instructions to set up methods and parameter descriptions.*

See Chapter 5, Data Analysis*, for background information to help you review your data and customize the data analysis methods and reports.*

Learn about the concepts that can help you set up acquisition methods. Use this chapter to guide you through the exercises in Chapter 2 of the *Familiarization Guide*.

The Agilent TOF software comes with the PE-Sciex Analyst QS 1.1 Software to review your data. The TOF software also includes a data analysis method, default.anm, that allows you to confirm empirical formulas. You can customize this method with the Agilent Data Analysis Method Editor. You can also select other Data Analysis reports such as Mass List.

LC parameter entry

Most of the LC parameter entries are the same as those that you can change with the Agilent 1100 LC control module and with Agilent software products, such as Agilent ChemStation.

One set of parameters, however, is unique to the LC/MSD TOF software. These are the parameters that control the storage and display of the signal and spectral data for the diode array detector (DAD).

Storage and display of DAD signal data

You can choose to store DAD signals to the .wiff data file in the DAD Setup tab. When you make this selection you reduce the WIFF file size, but the system does not store DAD spectra to the .wiff data file. You can also view signals but not DAD spectra in the Real-time Plot pane.

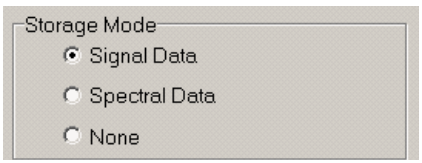


Figure 28 Storage Mode in the DAD Setup tab in the Method pane

Storage and display of DAD spectral data

When you choose to store DAD spectra, the spectra, but not the signals, are saved to the .wiff file. Spectral storage produces a larger file size than signal storage. You can extract signals from the spectral data in Analyst.

Signal data can be displayed in the Real-time Plot pane during the run if the signals are entered before selecting the Spectral Data option.

MS TOF parameter entry

Even though MS TOF parameter entry seems straightforward, the background information presented in this section can help you make the correct entries to produce the best results.

- Data acquisition parameters
- TOF acquisition parameters adjustable through methods
- Parameters for different ion sources
- Setup for automatic parameter changes during a run
- Setup for reference mass correction (recalibration)
- MS TOF chromatogram setup

Data acquisition parameters

You enter all data acquisition parameters through the Data tab of the Method Pane.

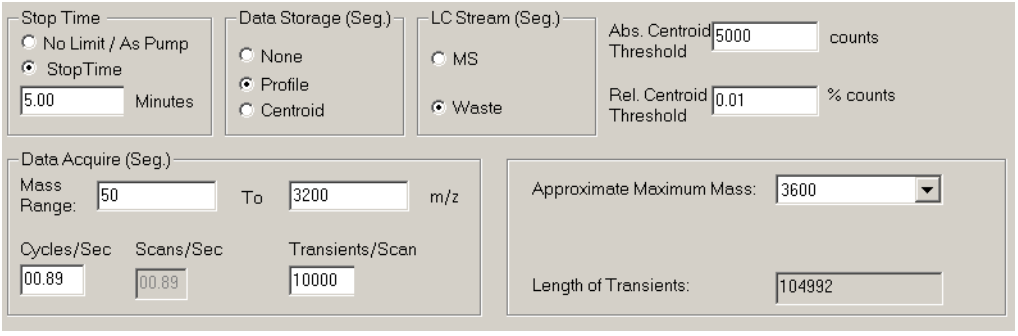


Figure 29 Data tab of the Method Pane

Profile vs. centroid spectra

You can save mass spectral data as whole peaks over the mass range of the peak. Or you can save only the data for the mass whose intensity appears in the “middle” of the peak. To limit the number of peaks whose centroid data are saved or appear in the Real-time Plot Pane (line spectra), you can set an absolute or relative threshold.

Transients vs. mass range

The mass spectrum resulting from a single pulse of voltage applied to the ion pulser is called a *transient*. The recorded Mass Spectrum is, in reality, a result of the application of multiple pulses to the ion pulser and a summation of lower signal mass spectra, or transients. (Figure 30)

The *length of transients* is the time the system is allowed to collect data for the transient in nanoseconds.

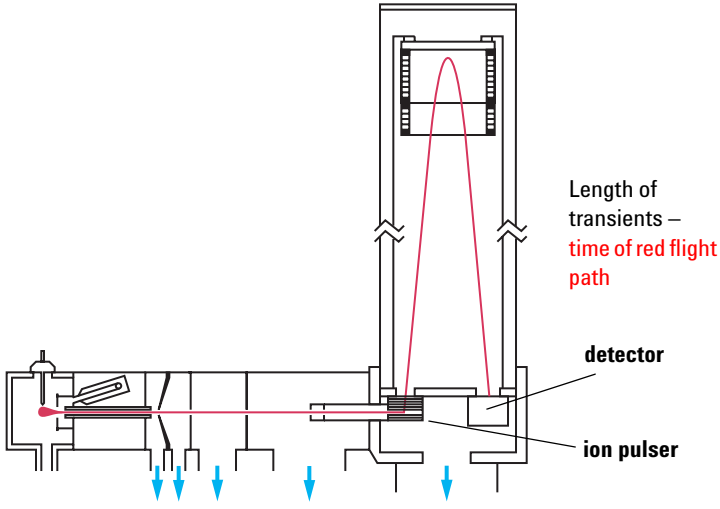


Figure 30 Length of transients

For analyses requiring one Mass Spectrum, or scan, per second, the Agilent TOF software sums 10,000 transients before

transferring the data from the instrument back to the host computer to be written to disk. If the target application involves high speed chromatography and requires faster scanning, you reduce the number of transients per scan to increase the scans per second.

In the Agilent system, the ESI, nanoESI, APPI, and APCI autotune methods set the length of transients to 104,992 nanoseconds. You use this value for mass ranges up to 3000 amu. For masses greater than 3000 amu, you need to lengthen the time it takes the ion to traverse the flight path. If you do not increase the length of transients in the acquisition method, light ions triggered from the second transient arrive before the heavier ions of the first transient, resulting in overlapping spectra.

TOF acquisition parameters adjustable through methods

The Acquisition tab in Figure 31 contains a listing of these parameters for ESI and nanoESI sources. These parameters change when you switch ion sources.

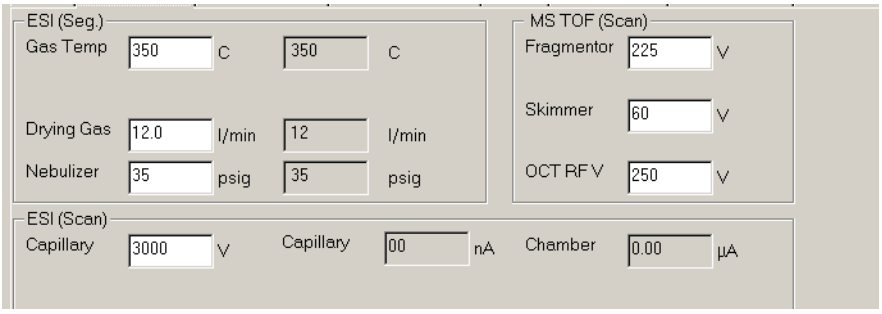


Figure 31 Acquisition tab

See Chapter 4, Data Acquisition, *for more information on instrument parameter optimization through the worklist.*

To find the optimum signal, you can adjust some of these TOF acquisition parameters by saving methods with different parameter values. Or, you can do this automatically by setting up a worklist with samples that run under the conditions specified in the worklist.

Figure 32 shows the hardware components whose parameters can be changed through the method. All of the parameters of the components to the left of the first octopole ion guide can be changed, as well as the OctRF V.

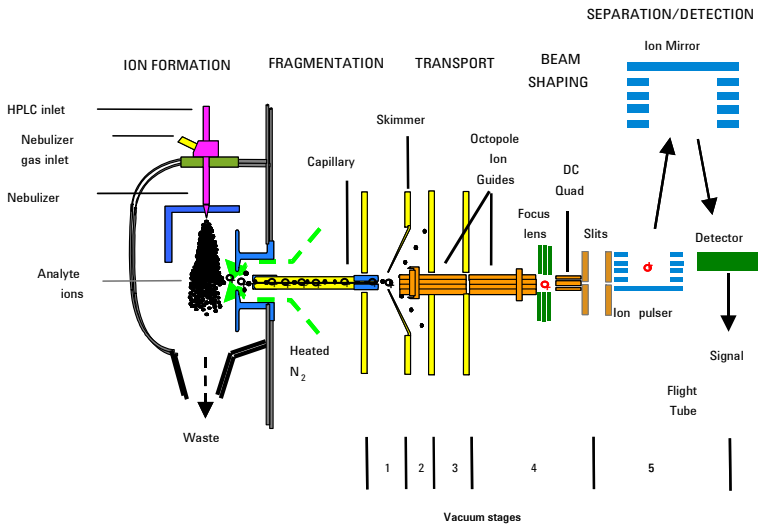


Figure 32 Parameters adjustable through the method or worklist

Parameters for different ion sources

See the Online Help for the Agilent LC/MSD TOF Parameters *to view recommended parameter values for each ion source.*

You can use several different sources with the Agilent LC/MSD TOF system:

- ESI
- APCI
- APPI
- AP-MALDI
- PDF-MALDI
- Orthogonal Nanospray
- Dual Orthogonal Nanospray
- MMI

Each of the sources uses different parameters for controlling the ion source. The default parameters are set for an electrospray source. When you select a new ion source in the Method Pane, you see new parameter fields. Note the change in reference masses for the APCI ion source in Figure 33.

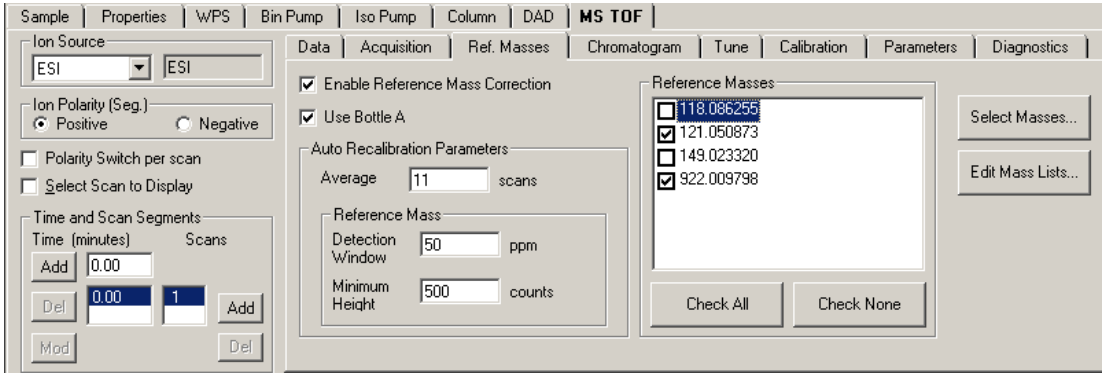


Figure 33 Parameters adjustable through the method or worklist

Tips for using the AP-MALDI or PDF-MALDI source

For instructions on how to set up and run samples with the AP-MALDI and PDF-MALDI inlet and ion source, see the Online Help.

- Tune the TOF with an ESI source installed and the method, ESIautotune.m loaded.
- Make sure that the length of transients value in ESIautotune.m is the number appropriate for the AP-MALDI or PDF-MALDI analyses. Do not save any changes made to the ESIautotune.m method.

For instructions on how to install the AP-MALDI or PDF-MALDI ion source, see the Installation Guide.

- Before the AP-MALDI or the PDF-MALDI is installed, in the instrument configuration tool, the LC must be removed from the system configuration.
- Make sure the Run Type on the Sample tab is set to External Start.

For more information on how to set up the AP-MALDI to introduce samples, see Chapters 1 and 2 and p. 20 to 23 of Chapter 3 of the Agilent G1972A AP-MALDI LC/MSD Trap SL System User's Guide.

- Set the proper settings in the AP-MALDI or PDF-MALDI control software., and make sure that the desorption time is not less than the MS TOF stop time.
- Autotune for the AP-MALDI and PDF-MALDI source is not supported.

Setup for automatic parameter changes (Segments/Scans)

You can set up the method to automatically change TOF parameters during the run. You do this by defining a Time Segment and the number of Scans to be run within the Time Segment. A time segment must be at least 15 seconds long. You can change some parameters only from one time segment to another time segment (such as polarity). Others you can change from scan to scan within a time segment.

The older SmartCard 3-based instruments are limited to 5 scans/second. Newer SmartCard 4-based instruments can have up to 20 scans/second for the full mass range. It is possible to go beyond this scan rate with newer models depending on factors such as the mass range and the system load. Please refer to Table 10 for information regarding instrument capabilities.

You may contact Agilent if you want information on upgrading an older LC/MSD TOF instrument to get a higher scan rate. However, an upgraded old LC/MSD TOF instrument will not support polarity switching when a time segment changes.

Table 10 Supported SmartCard capabilities

If you have MSTOF Serial Number	Supported capabilities
US50600500 or lower	SmartCard 3 instrument which does not support polarity switching (time based or scan based) or the multimode ionization source.
US50600501 or higher	SmartCard 4 instrument which supports segment based polarity switching and the multimode source
US54100700 or higher	SmartCard 4 instrument which supports polarity switching each scan

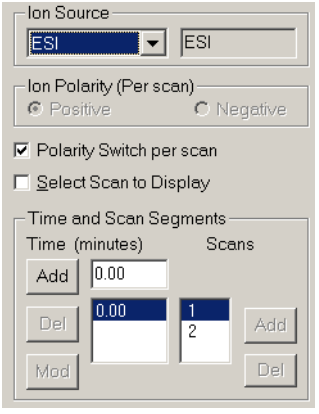


Figure 34 Location for setup of Segments and Scans

Parameters that can change during a run

Table 11 shows a list of parameters that you can set up to change for each Segment or Scan. All other parameters must remain constant throughout the entire run.

Table 11 Segment and Scan parameters

Tabs in the MS-TOF tab of Method Pane	Parameters changeable per segment	Parameters changeable per scan
Data	Data Storage	
	LC Stream	
	Mass range	
	Cycles/sec.	
	Transients/sec.	
Acquisition	Gas Temp.	Capillary
	Drying gas	Fragmentor
	Nebulizer	Skimmer
	Polarity (SmartCard 4 instruments only). See Table 10 for a list of polarity switching capabilities.	Polarity (SmartCard 4 instruments only). See Table 10 for a list of polarity switching capabilities.
		Oct RF V

Setup of reference mass correction (recalibration)

You must do mass corrections during a run in order to attain the mass accuracy that Agilent specifies for the MS TOF.

Many applications need as small a deviation of accurate mass as possible. To obtain this accuracy, you recalibrate the mass axis for every spectrum with measurements of known reference masses (i.e “lock masses”). You measure the masses of reference compounds in a reference standard, which can be the Agilent reference standard or one of your own choosing.

You can introduce the reference standard into the TOF system in one of three ways:

- A calibrant delivery system (CDS) that automatically introduces the standard to a reference sprayer (second sprayer)—ESI only (0.2 to 1.5 mL/min flow rates only)
- An external dispense pump—other ion sources
- Addition to the sample—other ion sources

You can also use known ions in your sample, such as the “y1” in a tryptic digest, as a supporting reference mass.

Enabling reference mass correction

You set up and enable reference mass correction in the Ref. Masses tab within the MS TOF tab of the Method pane.

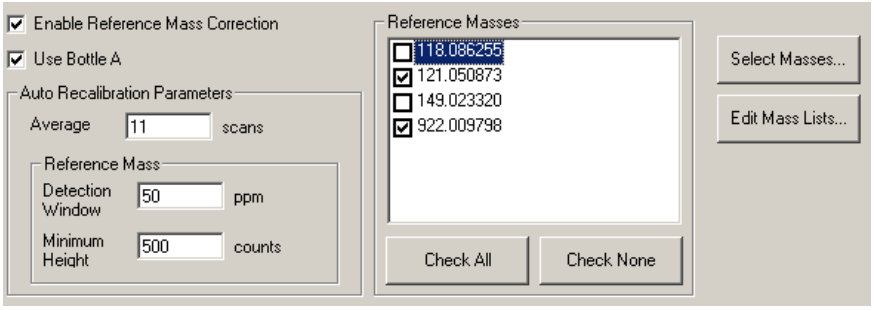


Figure 35 Reference Masses Tab

If you mark the Enable Reference Mass Correction check box, the system uses reference masses of the mass reference standard for automatic recalibration of each acquired spectrum.

You must select the reference masses for each polarity in the method. Reference masses are not only ion source dependent, but also polarity dependent. The reference masses specified for the method will be stored with the method. These reference masses are also stored in the wiff file with acquired data as TOF method parameters.

If you mark the Use Bottle A check box, the TOF controls the valve to introduce the internal reference standard to the reference sprayer via the CDS. *"Use Bottle A"* is disabled and set to "unchecked" for an ion source other than ESI.

Number of required reference masses

To learn more about the underlying calibration equation and coefficients, see "Mass calibration" on page 27.

With two unknowns, a minimum of two known values are required to determine both A and t_o. Practically considerations also come into play. In order to get a good fit for both A and t_o then at lease one reference mass needs to be at a low mass value and there needs to be at least one reference mass at a higher mass. Standard analytical practice also suggests that the low m/z and high m/z reference masses bracket the masses of interest.

Specifically, the reference mass correction algorithm requires that one mass be at or below m/z 330 with a second mass that is at least 500m/z above the low mass ion in order to correct t_o and A terms. If these conditions are not satisfied but there is at least one reference mass found, then only the A term will be re-calibrated.

Selecting/editing list of reference masses

The TOF software provides separate lists of reference masses for each polarity of each ion source type. You can select from a listing of already created mass lists, or you can create or edit a new mass list.

The Edit Mass List dialog box is shown below with a listing of all the Agilent default mass lists for each ion source and polarity. You cannot edit the Agilent default mass lists.

You create and edit new mass lists only from the Ref. Masses tab. You select, not edit, a mass list in the Calibration and Tune tabs.

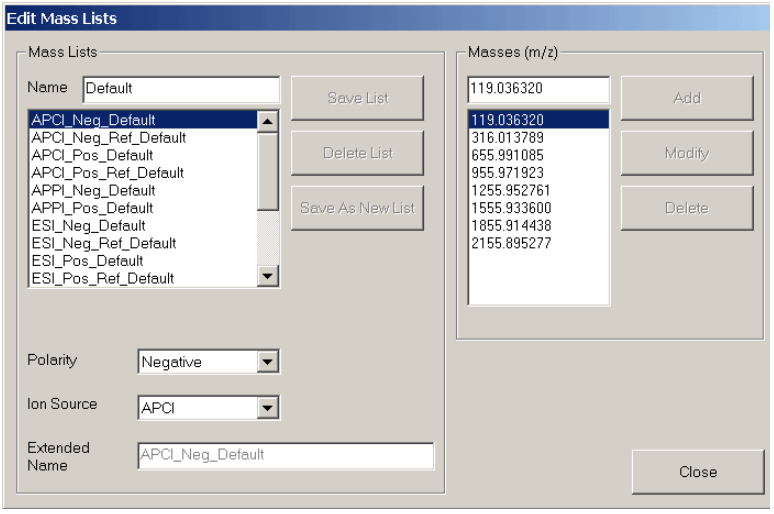


Figure 36 Edit Mass Lists dialog box

You can create a new mass list from an Agilent default mass list. When you enter a new name in the Name field, the buttons are no longer grayed out. You save the list as a new list. You can then edit these newly created lists.

Parameters for a reference mass correction

To learn more about Segments and Scans, see "Setup for automatic parameter changes (Segments/Scans)" on page 64.

Scans To Average To increase the accuracy of the reference mass correction, you can use a running average of the reference mass values across several spectra. These mass values are used in determining the corrected calibration coefficients. The default number of spectra used is eleven. Only odd values are allowed.

The software averages only spectra from the same scan group, and therefore a spectral average spans a spectral cycle. A spectral cycle contains one spectrum for each of the Scans defined in a given Segment.

For example, if a Scan Segment contains one Scan with Fragmentor voltage at 225V and another Scan with Fragmentor voltage at 200V, then a spectral cycle will contain two successive spectra, one at 225V and another at 200V.

Spectra from scans of different fragmentor voltage (or other scan specific parameters) should not be summed and averaged because they yield different masses.

Example The "Scans To Average" is 5. If the spectrum of interest is in the nth cycle, then spectral data of the same Fragmentor voltage from *cycles* n-2, n-1, n, n+1, n+2 are used in the average with equal weights. If this time Segment has defined one Fragmentor voltage of 225 Scan #1, and one Fragmentor voltage of 220 Scan #2, and the current spectrum is the Fragmentor voltage of 225 Scan 1 of the nth cycle, the *spectra* n-4, n-2, n, n+2, n+4 will be used for the average.

Reference Mass Window When the software attempts to find the reference mass, it searches for the highest spectral peak in the defined reference mass window, which is the window width in parts per million (ppm). Recalibration using reference masses of the internal reference standard does not replace normal or external mass calibration. You must set the window for recalibration small enough so the software does not pick a spectral peak from the sample as the reference mass peak. 100 ppm is the recommended default value.

Reference Mass Minimum Height The "Reference Mass Minimum Height" is the abundance in counts. The software uses this height to exclude noise peaks and peaks of reference masses that may fall within the detection window but that are too small to be used for calibration. This is especially important if you use reference masses that may be present in the tryptic digest, such as the y1 ion (m/z 147 for N-terminal Lys or m/z 175 for N-terminal Arg), but may not be present in all spectra in the run. Signals below 1,000 counts are generally too low to be used. Signals above 75,000 counts for the reference mass ion can negatively affect the reference mass correction.

MS TOF chromatogram setup

You also select the chromatograms that you want to see in the real-time plot during the run in the Method pane. You do this in the Chromatogram tab.

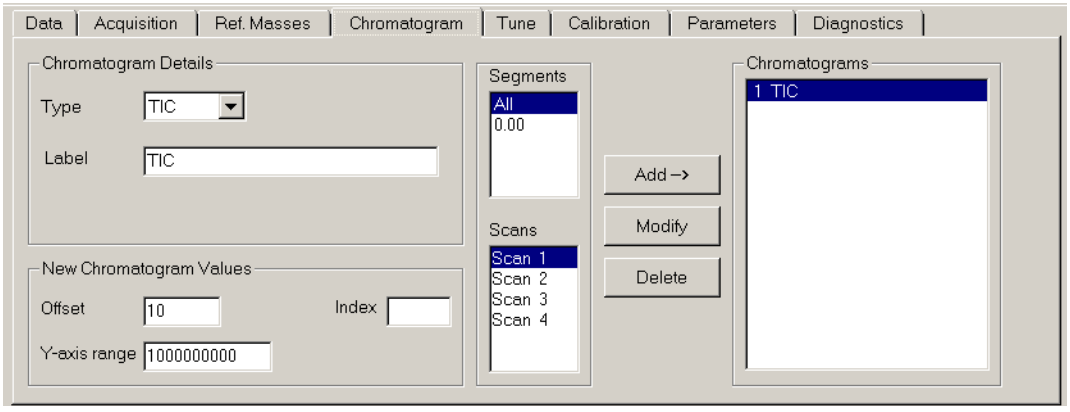


Figure 37 Chromatogram tab in the MS TOF tab of the Method pane

You can select the Total Ion Chromatogram (TIC) or Extracted Ion Chromatogram (EIC) from all Segments set up in the method. Or, you can select individual time segments and scans to view in the real-time plot, thus enabling real-time comparisons between time segments or scans.

You can also select setpoints or actuals to see in the real-time plot from this tab.

Method saving, editing and reporting

Saving an acquisition method

Adding pre- or post-analysis scripts before saving

To learn how to set up scripts, see your Agilent application engineer.

Before you save a method, you can enter the pathway for the customized programs that start before or after a run. You do this through the Properties tab.

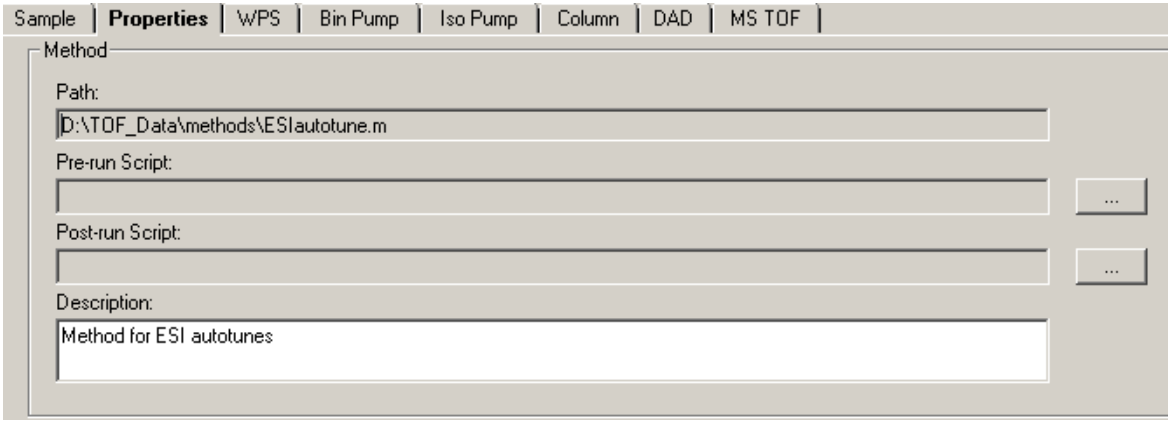


Figure 38 Properties tab of the Method Pane

VBA projects provided by Agilent should not be modified since these files may be overwritten when upgrading the Agilent software.

These pre-analysis or post-analysis programs consist of scripts written with Visual Basic Application (VBA). Each of the TOF software engines makes available already existing commands for writing scripts.

For more information on scripts, see the Online Help, Reference.

Below is a list of scripts that Agilent includes with the software and that you can use with both methods and worklists.

Table 12 System scripts and the actions they enable

Script name	Actions the script enable
SCP_AcquireCalibrantData	Sets "Cal/Ref Mass" to "Cal B" and "LCStream" to "LC->Waste". Allows you to acquire data for the calibrant solution itself. To be used only as the Pre-run script for a method. The script itself does *not* do a run and only augments an existing method. Do not use as a standalone script in a worklist run.
SCP_InstrumentStandby	Puts the instrument in standby mode. This is the same as clicking the "Standby" button on the instrument status pane.
SCP_LCCondition (MethodName, LCStream)	Starts a run for conditioning the LC part of the instrument. There is no data acquisition
SCP_LoadIdleMethod (MethodName)	Loads a method to put the system in an idle state
SCP_MSCalibValveOff	Sets "Cal/Ref Mass" to "None" or off
SCP_PumpAllAndMSCalibValveOff	Turns off all the pumps and sets Cal/Ref Mass to "None"
SCP_PumpsAllOff	Turns off all the pumps

Location of method directories

You can save methods to any directory on the system. The default directory is **D:\TOF_Data\Methods**.

You can view the name and path of the currently loaded method in the Properties tab of the Method Pane (Figure 38).

Method editing

You can choose one of three ways to edit acquisition methods in the TOF software.

- In the Method Editor Pane of the main application window (online version)

  Use this location for method development. The setpoints are sent to the instrument when the method is loaded or when you click the Apply button after changing a parameter.

- In the Method Editor Pane of an offline copy of the main application window

  Use this location when you want to create or edit a method while the main application window is busy running a sample or worklist.

- From the Worklist Pane (online or offline version)

  Use this location when you want to view or edit a method in a worklist, both during worklist setup and execution.

Online session

When you are in an online session, the application starts with the online method layout and loads the last used method/sample. You use the online session for editing methods that are used immediately for instrument control and running samples.

Offline session

You use the offline session only for editing methods or worklists. The software starts with the offline method layout and loads the default method.

An offline session has the following restrictions:

- You cannot monitor instrument status nor control the instrument.
- You cannot monitor real-time plots.
- You cannot download changed method parameters to the instrument.

- You cannot start, stop or abort a run.
- You cannot tune or calibrate the instrument.

Method reporting

The TOF software does not record acquisition method changes. You can see the parameters in a method in one of three ways:

- You access Acquisition Method Reports from the File menu.
- You can open the method.log file in the method directory using the Logbook Viewer.
- You can see method parameters associated with a data file in Analyst.

Acquisition Method Reports include this information:

- Method name, path and description
- List of configured LC modules and MS TOF
- Parameter values for each LC module
- For the MS TOF, name of ion source, the stop time, the acquisition parameters, and the Time Segments with Scans

4
Data Acquisition

Learn the concepts to help you understand the setup of single samples for interactive data acquisition and the setup of single samples and batches of samples for automatic data acquisition. Use this chapter to help you complete the exercises in Chapters 3 and 4 of the *Familiarization Guide*.

Interactive single sample setup

If you want to run just one sample at a time, you enter the information for that sample in the **Sample** tab of the Method Pane.

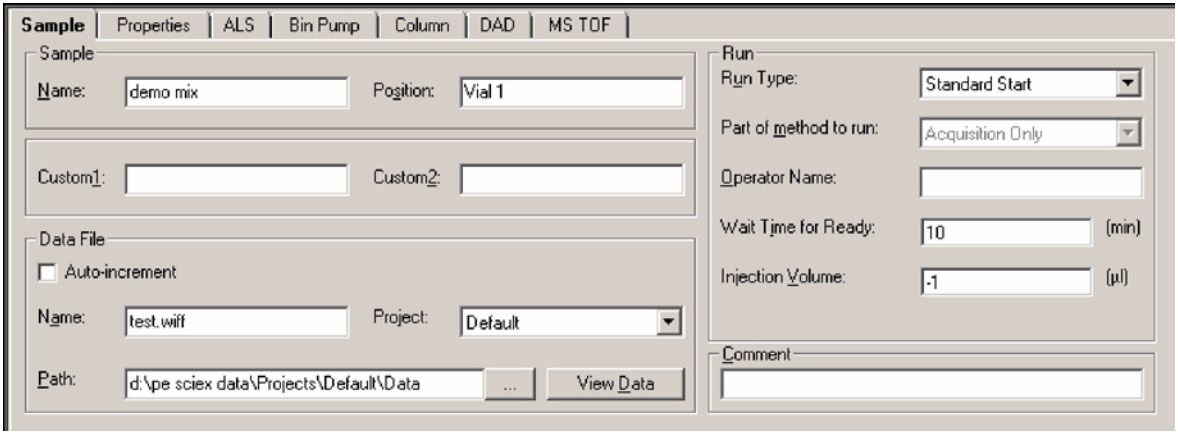


Figure 39 Sample tab of the Method pane

Sample information

The only sample information that the system records with the data file is the sample name, vial position and two custom sample information entries of your choosing, such as sample ID or sample source.

Sample information is not part of the method. It is stored with the data file when the method currently loaded is run.

Data file information/projects

Analyst organizes its data files within projects, which you create in Analyst. The default directory for TOF data is **D:\PE Sciex Data\Projects\Default\Data**. All data files are saved to the default data directory unless you choose a project other than Default or another directory.

Auto-increment If you want to use the same file name and have the system automatically change the number at the end of the file name, you turn auto-increment on. Then, you enter a file name that ends in the number 001, and the system makes sure that a new file is created every time you re-run that sample.

Analyst projects

In the Sample tab you can select an Analyst project that already exists in the **D:\PE Sciex Data\Projects** directory. If you want to save the data to a project that does not exist, you must create a new one in Analyst. You can only create new projects in Analyst in the PE Sciex Data/Projects directory.

Other directories

You can save your data to any directory on the system. You must use browse to select a different directory. The software will enter **None** in the Analyst Projects list. To open any data file saved to a different directory than **D:\PE Sciex Data\Projects,** you must browse the system.

Run information

Start run types

You use a Standard Start for LC runs, an External Start for AP-MALDI, CE-TOF and a Manual Start for infusion runs with a syringe pump.

Part of method to run

This field is always set to **Acquisition Only**.

Injection Volume

You can specify an injection volume for the sample in this field. Enter the value -1 if you want to use the injection volume specified in the method.

Worklist setup

For information on the worklist setup to confirm empirical formulas, see "Worklist to confirm empirical formulas" on page 94.

Agilent developed worklists for the primary purpose of running many samples automatically and then reporting on the compounds found in the samples.

The worklist lets you enter sequen ces of samples—both single samples and batches—to be run automatically in the order of their listing. A batch is a sequence of single samples whose data you may want to keep together for one of the following reasons:

- samples with the same acquisition or data analysis method
- parameter optimization
- different users or projects

Batches appear as single lines in the worklist.

All data is saved to the **D:\PE Sciex Data\Projects\Default\Data** directory unless otherwise specified. You may want to specify a different project or entirely different directory for batch data.

The worklist operates as a spreadsheet much like Excel. You can copy, paste, and fill in columns as you would in Excel.

	☑	Sample Name	Sample Position	Acq Method	DA Method	Data File	Inj Vol (µl)
1	✔	eetest01	P1-A1	APClautotune.m	mass_list.anm	eetest01.wiff	3
2	✔	eetest02	P1-A2	APClautotune.m	mass_list.anm	eetest02.wiff	3
3	✔	eetest03	P1-A3	APClautotune.m	mass_list.anm	eetest03.wiff	3
4	✔	eetest04	P1-A4	APClautotune.m	mass_list.anm	eetest04.wiff	3
5	✔	eetest05	P1-B1	APClautotune.m	mass_list.anm	eetest05.wiff	3
6	✔	eetest06	P1-B2	APClautotune.m	mass_list.anm	eetest06.wiff	3
7	✔	eetest07	P1-B3	APClautotune.m	mass_list.anm	eetest07.wiff	3
8	✔	eetest08	P1-B4	APClautotune.m	mass_list.anm	eetest08.wiff	3
9	✔	eetest09	P1-C1	APClautotune.m	mass_list.anm	eetest09.wiff	3
10	✔	eetest10	P1-C2	APClautotune.m	mass_list.anm	eetest10.wiff	3
11	✔	eetest11	P1-C3	APClautotune.m	mass_list.anm	eetest11.wiff	3
12	✔	eetest12	P1-C4	APClautotune.m	mass_list.anm	eetest12.wiff	3

Worklist

Figure 40 Example worklist

Selected sample execution

Each line in the worklist table has a check box that allows you to select that line for processing or not. This can be useful if you need to restart a worklist after some lines have already been run.

Worklist menus

You find all the tasks to create a worklist in the worklist menus.

- Add a single sample one at a time or add multiple single samples all at once
- Add a batch of samples of similar characteristics
- Add scripts before or after the worklist or batch or between samples in a worklist or batch
- Add or show more sample information columns
- Add, insert or delete rows and columns
- Set up to print a worklist report or track a worklist run

Selections in the menus depend on where you access the menus.

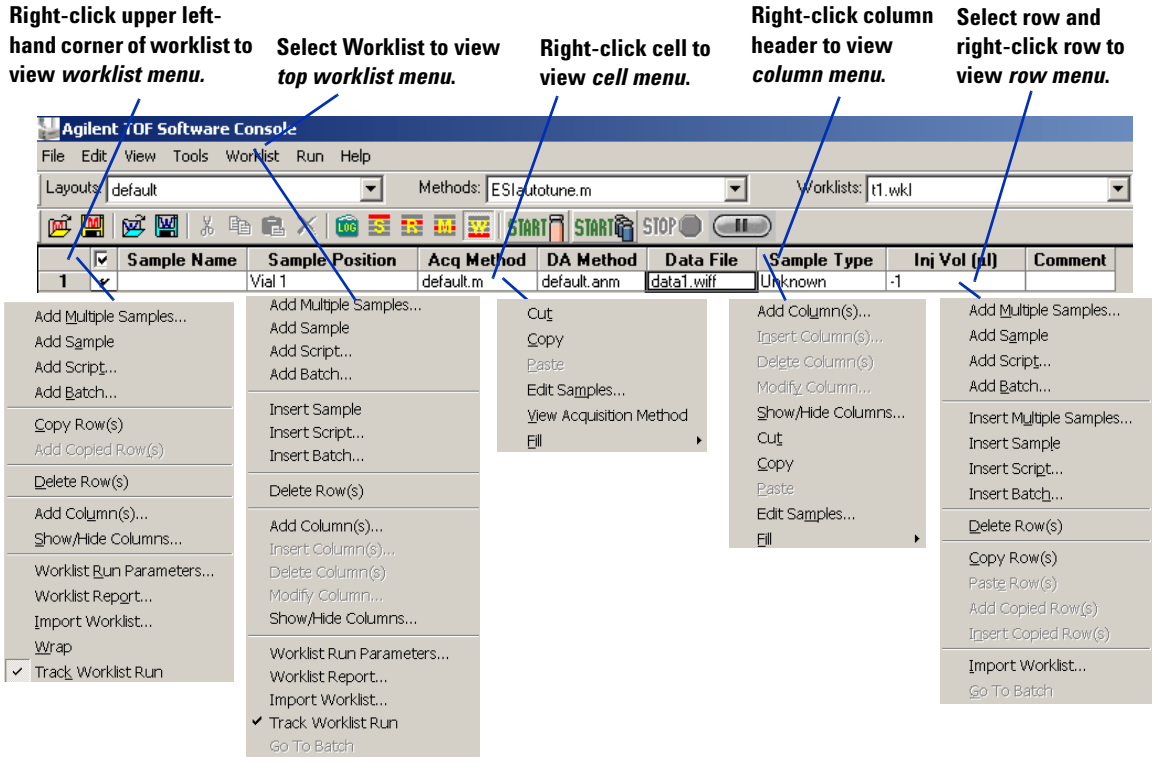


Figure 41 Worklist menus

Single sample entry

One-at-a-time entry

If you want to run single samples before or after or between your batches, you use the menu selection to add one sample at a time and enter the sample information in the worklist as you would in the Sample tab of the Method pane. You may want to do this to equilibrate the system before running a worklist.

Multiple sample entry

If you want to add several single samples to the worklist at one time, you use the menu selection to add multiple samples. You can add different samples or one sample injected several times.

Sample information

When you add multiple samples, you can specify the data directory, method names and inj. volume.

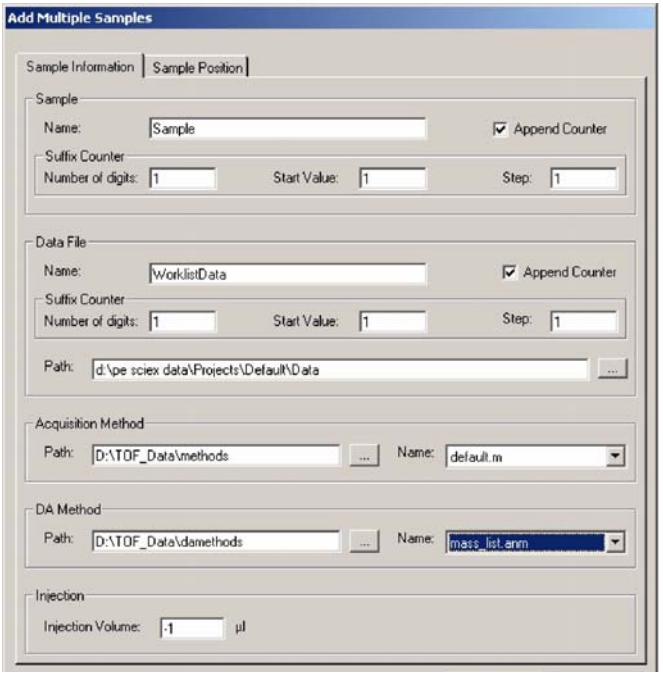


Figure 42 Add Multiple Samples panel

Sample position You can select the sample positions without having to type in their values from the Sample Position tab on the Add Multiple Samples dialog box.

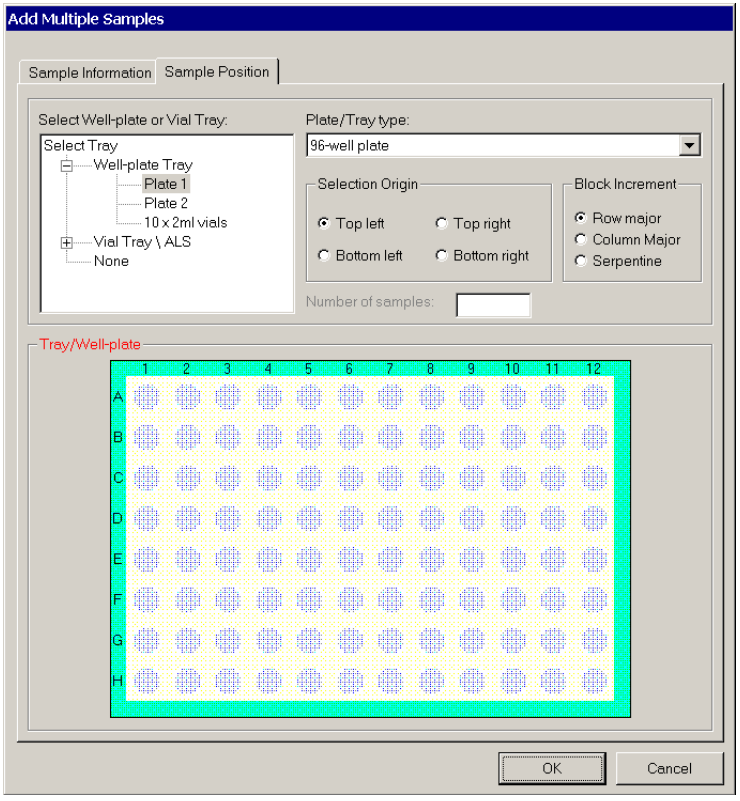


Figure 43 Sample Position tab of the Add Multiple Samples dialog box

Batch entry

A batch is a sequence of samples that have common characteristics. You can add a batch to the end of a list of samples or insert a batch in front of a single sample.

Run parameter entry

When you add or insert a batch, the Batch Run Parameters dialog box appears. Batches can have different run parameters than the worklist itself.

To learn more about scripts, see "Script entry" on page 88.

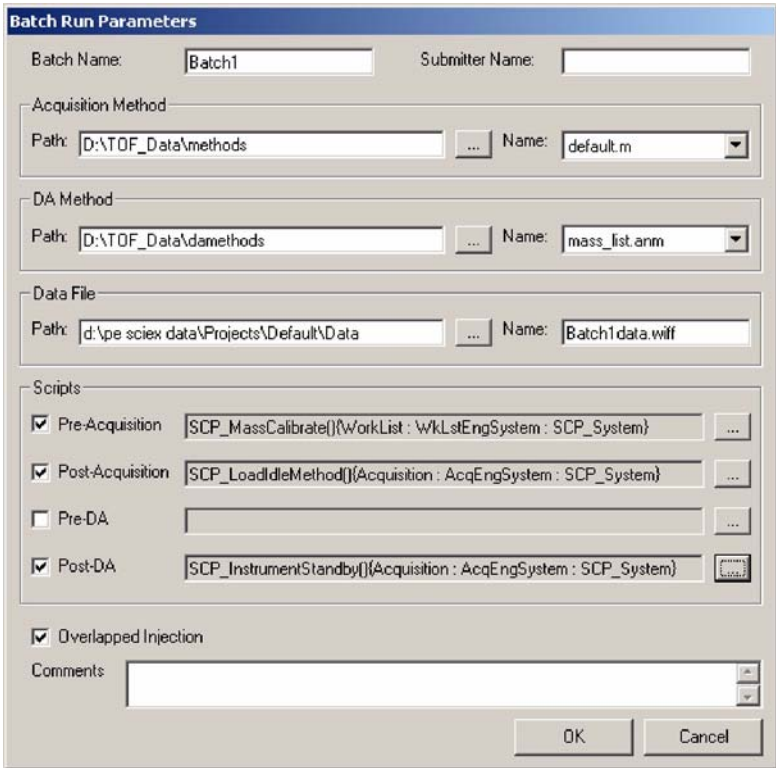


Figure 44 Batch Run Parameters dialog box

When you complete this dialog box, the default batch appears, which contains only one row with the names of the acquisition method, data analysis method and data file entered. The name of the batch appears under the batch, and you can return to the worklist at any time.

Sample and sample information entry

You access and use shortcut menus to enter samples into the batch list in the same way as for the worklist. You also show and add columns, and copy or delete rows and columns just as you do for the worklist. The same functions and sample information columns are available for the batch as for the worklist

	☑	Sample Name	Sample Position	Acq Method	DA Method	Data File	Sample Type	Inj Vol (µl)
1	✓	sulfa_mix1	Vial 1	APClautotune.m	csr_base_peak.anm	Batch1data1.wiff	Unknown	-1
2	✓	sulfa_mix2	P1-A1	APClautotune.m	csr_base_peak.anm	Batch1data2.wiff	Unknown	-1
3	✓	sulfa_mix3	P1-A2	APClautotune.m	csr_base_peak.anm	Batch1data3.wiff	Unknown	-1
4	✓	sulfa_mix4	P1-A3	APClautotune.m	csr_base_peak.anm	Batch1data4.wiff	Unknown	-1
5	✓	sulfa_mix5	P1-A4	APClautotune.m	csr_base_peak.anm	Batch1data5.wiff	Unknown	-1
6	✓	sulfa_mix6	P1-B1	APClautotune.m	csr_base_peak.anm	Batch1data6.wiff	Unknown	-1
7	✓	sulfa_mix7	P1-B2	APClautotune.m	csr_base_peak.anm	Batch1data7.wiff	Unknown	-1
8	✓	sulfa_mix8	P1-B3	APClautotune.m	csr_base_peak.anm	Batch1data8.wiff	Unknown	-1
9	✓	sulfa_mix9	P1-B4	APClautotune.m	csr_base_peak.anm	Batch1data9.wiff	Unknown	-1
10	✓	sulfa_mix10	P1-C1	APClautotune.m	csr_base_peak.anm	Batch1data10.wiff	Unknown	-1
11	✓	sulfa_mix11	P1-C2	APClautotune.m	csr_base_peak.anm	Batch1data11.wiff	Unknown	-1
12	✓	sulfa_mix12	P1-C3	APClautotune.m	csr_base_peak.anm	Batch1data12.wiff	Unknown	-1
13	✓	sulfa_mix13	P1-C4	APClautotune.m	csr_base_peak.anm	Batch1data13.wiff	Unknown	-1

Back to Worklist | Batch:Batch1

Figure 45 Batch list

Script entry

Scripts are special programs, written in VBA using VBA commands, that execute automatically. Agilent includes scripts with the TOF software, and you can write your own scripts.

VBA projects provided by Agilent should not be modified since these files may be overwritten when upgrading the Agilent software.

For detailed instructions on how to enter scripts, see the Online Help.

For instructions on how to create scripts, see your Agilent application engineer.

You can enter scripts to be run at the following times:

- Before or after samples as part of the method

  The sample method can include pre- and post-analysis scripts. (See Chapter 3, Acquisition Methods)
- Before or after samples in the worklist or batch (insert or add scripts, respectively)
- Before or after data acquisition for a batch or before or after data analysis for the batch (Figure 44)
- Before or after a worklist and after data acquisition (Figure 55)

Agilent includes scripts with the software to help you automatically, instead of manually, execute processes such as column conditioning and valve shutoff (Table 13).

Table 13 Agilent scripts

Worklist Manager Scripts	Acquisition Manager Scripts
LCCondition	InstrumentStandby
AcquireCalibrantData	LoadIdleMethod
	PumpsAllOff
	MSCalibValveOff
	PumpAllAndMsCalibValveOff

Entry of additional sample information (show, add columns)

The default worklist contains only eight columns for sample information.

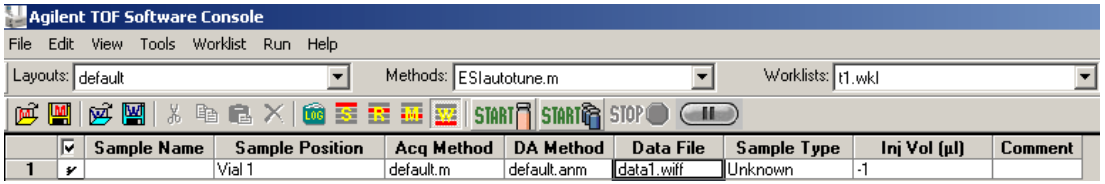


Figure 46 Default worklist columns for sample information

You can access these capabilities through the worklist menu.

You can add more columns in one of two ways:

- Show or hide columns that contain sample information already available in the software
- Add columns for new sample information

Show/hide sample information

Note that hiding a column does not delete the column. To delete the column, you must first show the column in the worklist or batch.

With this dialog box you can hide any of the original default columns and show others.

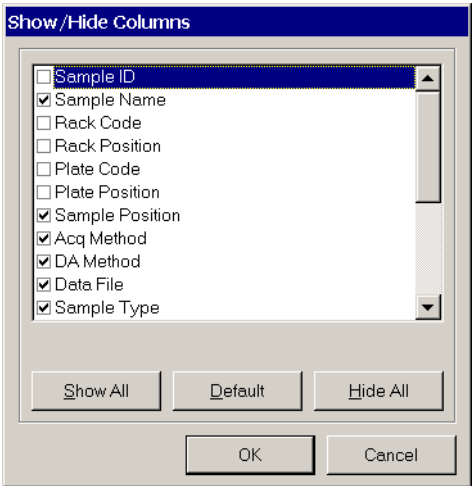


Figure 47 Show/Hide Column dialog box

		Sample Name	Sample Position	Acq Method	DA Method	Data File	Sample Type	Balance Override	Inj Vol (µl)
1	✔	sulfa_mix1	Vial 1	APClautotune.m	csr_base_peak.anm	Batch1data1.wiff	Unknown	No Override	-1
2	✔	sulfa_mix2	P1-A1	APClautotune.m	csr_base_peak.anm	Batch1data2.wiff	Unknown	Do Balance	-1
3	✔	sulfa_mix3	P1-A2	APClautotune.m	csr_base_peak.anm	Batch1data3.wiff	Unknown	No Override	-1
4	✔	sulfa_mix4	P1-A3	APClautotune.m	csr_base_peak.anm	Batch1data4.wiff	Unknown	No Override	-1
5	✔	sulfa_mix5	P1-A4	APClautotune.m	csr_base_peak.anm	Batch1data5.wiff	Unknown	No Override	-1
6	✔	sulfa_mix6	P1-B1	APClautotune.m	csr_base_peak.anm	Batch1data6.wiff	Unknown	Do Balance	-1
7	✔	sulfa_mix7	P1-B2	APClautotune.m	csr_base_peak.anm	Batch1data7.wiff	Unknown	No Override	-1
8	✔	sulfa_mix8	P1-B3	APClautotune.m	csr_base_peak.anm	Batch1data8.wiff	Unknown	No Override	-1
9	✔	sulfa_mix9	P1-B4	APClautotune.m	csr_base_peak.anm	Batch1data9.wiff	Unknown	No Override	-1
10	✔	sulfa_mix10	P1-C1	APClautotune.m	csr_base_peak.anm	Batch1data10.wiff	Unknown	Do Balance	-1
11	✔	sulfa_mix11	P1-C2	APClautotune.m	csr_base_peak.anm	Batch1data11.wiff	Unknown	No Override	-1
12	✔	sulfa_mix12	P1-C3	APClautotune.m	csr_base_peak.anm	Batch1data12.wiff	Unknown	No Override	-1

Figure 48 Worklist with some hidden columns shown

Add sample information columns

When you add columns, you can enter sample information and values for compounds, masses and acquisition parameters. You can also enter your own sample information, including empirical formulas.

When you add a column, it appears in the Show/Hide Column list.

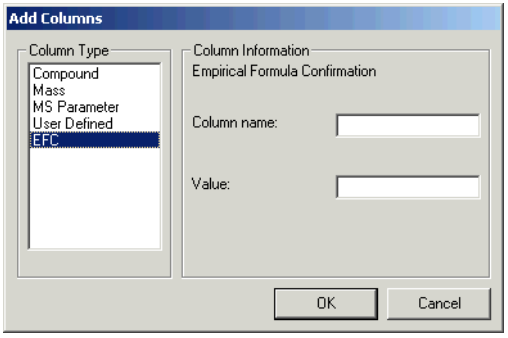


Figure 49 Add columns dialog box

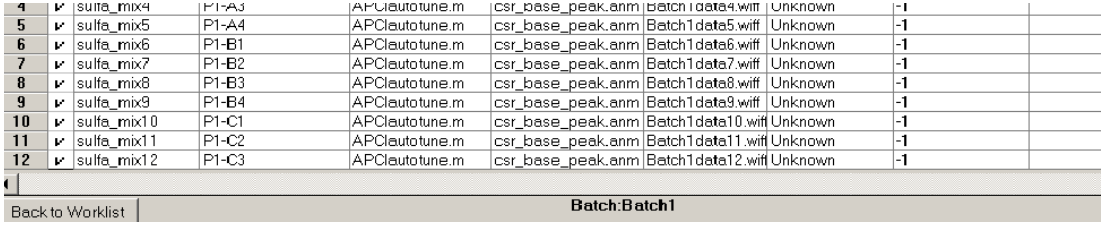


Figure 50 Worklist with new column for sample information

Worklist import

You can populate a worklist with sample information from other files in one of two ways:

- Copy individual columns one at a time from an Excel spreadsheet (or a csv file imported into Excel) and paste (or fill) them into the TOF worklist under the correct header

  You do this when you need to transfer information infrequently or the information is different for each transfer.

- Import a csv file directly

  You do this when you need to use the same parameters in a worklist frequently.

You cannot import a partial list of the samples within the csv file.

CSV file mapping

You can import the csv file to add or insert samples in an online session whether the worklist is running or not. You can also import the file in an offline session.

Your sample csv file contains a table of samples and attribute information for each sample. The information in this file may not correspond to the information needed in a TOF worklist in several ways.

- Some of the information may not be relevant.
- Some may be missing.
- Column names of the sample attributes may not be the same as those used by the TOF software.

You cannot import scripts into a worklist. You must add them directly.

You must first edit the sample csv file to put it into a form that maps to TOF sample data. You can specify these changes when you map to the TOF data:

- Change the column header names
- Add new columns in the worklist
- Change data values

You can include a mapping section in front of the sample information in the sample csv file or in a separate configuration csv file. You use a configuration csv file when all the sample information values are the same from import to import. You include the mapping section in the sample csv file when new batches with different dynamic mapping of columns, such as amount of compounds analyzed, are added for import.

Mapping for static worklist columns Some worklist fields are static and invariant, such as Sample Name and Sample Position. These come under the heading, Static Mapping, in the mapping section of the csv file.

Mapping for dynamic worklist columns

Some columns in a worklist are dynamic and change from analysis to analysis. The mapping capability in the csv file lets you specify additional columns to be added to the worklist. The name of the added worklist column should use the same name as the csv column specified. You then specify the column type in the worklist, such as Compound, Mass, MS Parameter, User Defined or Custom Parameter. These new columns to be added to the worklist come under the heading, Dynamic Mapping, in the mapping section of the csv file.

Data Value mapping Data values for some columns, such as sample type, are limited to a drop-down list in the worklist and do not match the same names as are in the csv file. Data value mapping is preceded by the key words [Data Value Mapping]

Example mapping section

Example CSV files are included with the TOF software. The files EFCData.csv and EFCMap.csv are located in the directory **C:\Program Files\Agilent\TOF Software\examples\worklist_import**.

You want to import samples into a TOF worklist, but the column headers in the CSV import file (Excel spreadsheet) are different from the worklist column headers. For example, “Sample” is used instead of “Sample Name”, as shown below:

Table 14 Original sample table

Sample	Acq Method	MyData	DA	SampPos	Sample Type	Internal Std A
AAA	method1	qwwq	method1	1	Standard	1
BBB	method2	bbb	method1	2	Sample	1
CCC	method3	ccc	method1	3	QualControl	1

One column header, “InternalStdA”, must be added as a new column to the worklist. You can also add columns that do not exist in the csv file, such as “Caffeine” in the section below. Some data values are also different (e.g., Sample Type values).

Table 15 is the mapping section for the spreadsheet in Table 14. This is the spreadsheet version of the mapping that lets the worklist import program recognize columns of imported data.

Table 15 Mapping section for csv file

[Static Mapping]		
Acq Method	Acquisition Method	
MyData	DataFile Name	
DA	DataAnalysis Method	
SampPos	Sample Position	
Sample	Sample Name	
[Dynamic Mapping]		
(//Added column)		
InternalStdA	Compound	1
Caffeine	Compound	
[Data Value Mapping]		
Sample	Unknown	
QualControl	QC	

Another example to add columns for Empirical Formula Confirmation is the following dynamic mapping section which specifies $C_6H_6N_3O$ as the default formula:

```
[Dynamic Mapping]
Formula1    Custom Parameter    C6H6N3O    EFC
```

Worklist to confirm empirical formulas

For instructions on how to set up a worklist to confirm empirical formulas, see the Quick Start Guide, the Familiarization Guide and the Online Help.

Worklist setup

To confirm empirical formulas for each sample in a worklist, you add an EFC column that has the word Formula in the name. You add one column for every compound whose formula you want to confirm. You enter the molecular formula in the Value field. Example formulae are: C6H6, C2H5OH, and C6H2ON2. You can add up to five Formula columns. You can also add just one column with the name Formula and enter a different formula for each sample row if you want to confirm only one compound per sample.

First, you create a method that will include an EFC report by editing the default data analysis method, efc.anm. Then you select that method or any method that specifies an EFC report. Note that you must also add EFC columns to the worklist and specify the formulae to confirm; otherwise no report is generated.

	☑	Sample Name	Sample Position	Acq Method	DA Method	Data File	Formula
1	✓	sulfa_mix1	Vial 1	default.m	efc.anm	data1.wiff	C12H14N4O2S
2	✓	sulfa_mix2	P1-A1	default.m	efc.anm	data2.wiff	C8H10N2OS2
3	✓	sulfa_mix3	P1-A2	default.m	efc.anm	data3.wiff	C12H14N4O2S
4	✓	sulfa_mix4	P1-A3	default.m	efc.anm	data4.wiff	C8H10N2OS2

Figure 51 Worklist with empirical formula confirmation

Generation of the empirical formula confirmation report

By default, this report is automatically saved to an html file when you run a worklist with efc.anm as the data analysis method and with added formula columns. You can edit the report options to select where to send the report.

You can find the html file in the directory that contains the .wiff file in a subdirectory named DAReports. See the Online Help for a full description of the file name assigned to each EFC report.

The report prints one page for each expected compound for each sample and contains this information: base peak chromatogram plot, a representative spectrum and detailed spectrum, and an evidence table that shows which of the expected ionic species the system found in the spectrum.

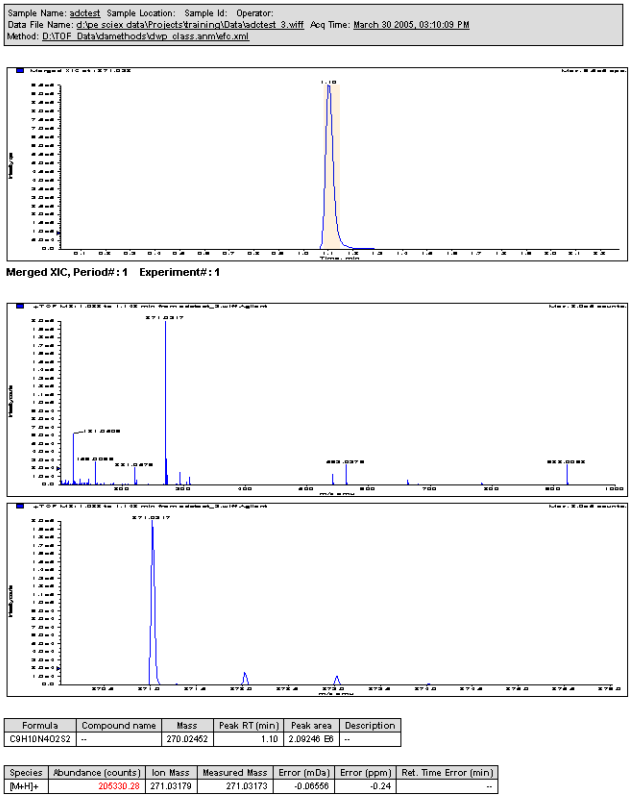


Figure 52 Example empirical formula confirmation report

For more information on the .csv file and the use of the Data Analysis Method Editor to modify efc.anm, see Chapter 5, Data Analysis.

You may also customize the default method to change the reported data and the look of the empirical formula confirmation report with the Data Analysis Method Editor.

You can also send the results in the report to a .csv file for export to other programs.

Worklist to report mass list

For more information on creating a data analysis method for mass list reports, see Chapter 5, Data Analysis.

To generate mass list reports for samples in your worklist, simply use a data analysis method that specifies a mass list report. The reports are automatically saved to an html file when you run the worklist. You can modify where the reports are sent by using the **Report Options** tab. You can find the html file in the directory that contains the .wiff file in a subdirectory named DAReports. See the Online Help for a full description of the file name assigned to each Mass List report.

You can add one of two additional sections to a Mass List report.

- Empirical Formula Generation
- Confirmation Screening by database search.

Worklist to report molecular feature extraction

To generate molecular feature extraction reports for sample in your worklist, simply use a data analysis method that specifies a molecular feature extraction report. You can find the html file in the directory that contains the .wiff file in a subdirectory named DAReports. See the Online Help for a full description of the file name assigned to each Molecular Feature Extraction report.

You can add one of two additional sections to a Mass List report.

- Empirical Formula Generation
- Confirmation Screening by database search.

Worklist for parameter optimization

For instructions on how to set up parameter optimization, see the Quick Start Guide, Familiarization Guide *or* Online Help.

For more information on the parameter optimization report for the worklist you set up, see Chapter 5, Data Analysis.

You can set up a worklist for a series of injections to determine the best values to use for TOF acquisition parameters, based on your particular analysis.

You add a column whose column type is MS parameters. From the list of acquisition parameters, you select only two of the parameters whose values you intend to optimize. The Agilent parameter optimization script in Analyst uses these two parameters and their varying values to produce a parameter optimization report.

The Agilent Parameter Optimization script uses only the first two parameters whose columns you add to the worklist. Make sure that no other MS parameter columns are hidden. If they are hidden, they may appear in the parameter optimization wizard instead of the two parameters that appear in the worklist. You show the unwanted columns in the worklist before you delete them.

All samples must be collected into one data file for the Parameter Optimization script to execute correctly.

		Sample Name	Sample Position	Acq Method	DA Method	Data File	Sample Type	Inj Vol (µl)	Comment	Fragmentor
1	✔	Reserpine 100pg1	P1-A2	reserpine 100pg.m	reserpine 10pg 2.anm	Reserpine100.wiff	Unknown	10		100
2	✔	Reserpine 100pg2	P1-A2	reserpine 100pg.m	reserpine 10pg 2.anm	Reserpine100.wiff	Unknown	10		150
3	✔	Reserpine 100pg3	P1-A2	reserpine 100pg.m	reserpine 10pg 2.anm	Reserpine100.wiff	Unknown	10		200
4	✔	Reserpine 100pg4	P1-A2	reserpine 100pg.m	reserpine 10pg 2.anm	Reserpine100.wiff	Unknown	10		250
5	✔	Reserpine 100pg5	P1-A2	reserpine 100pg.m	reserpine 10pg 2.anm	Reserpine100.wiff	Unknown	10		300

Figure 53 Worklist for parameter optimization

Report setup

After you run the worklist, a worklist report is sent to one or more of three locations.

- screen
- file
- printer

You select the report destination as well as the file path and the printer in the Worklist Report Options dialog box.

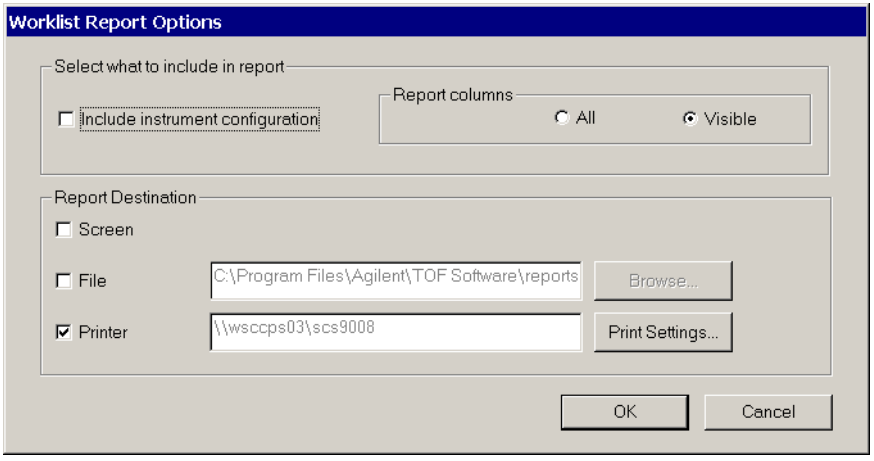


Figure 54 Worklist Report Options dialog box

Run setup

Before you run a worklist you select parameters for the entire worklist. Batch run parameters are set separately.

- Start run types and the part of the method to run
- Paths for the acquisition method, data analysis method and data file
- Scripts to run before or after the worklist
- Free disk threshold

  The free disk threshold is the amount of disk space in Mbytes that must be available before the worklist starts.

For more information on the Batch Run Parameters dialog box, see "Batch entry" on page 86.

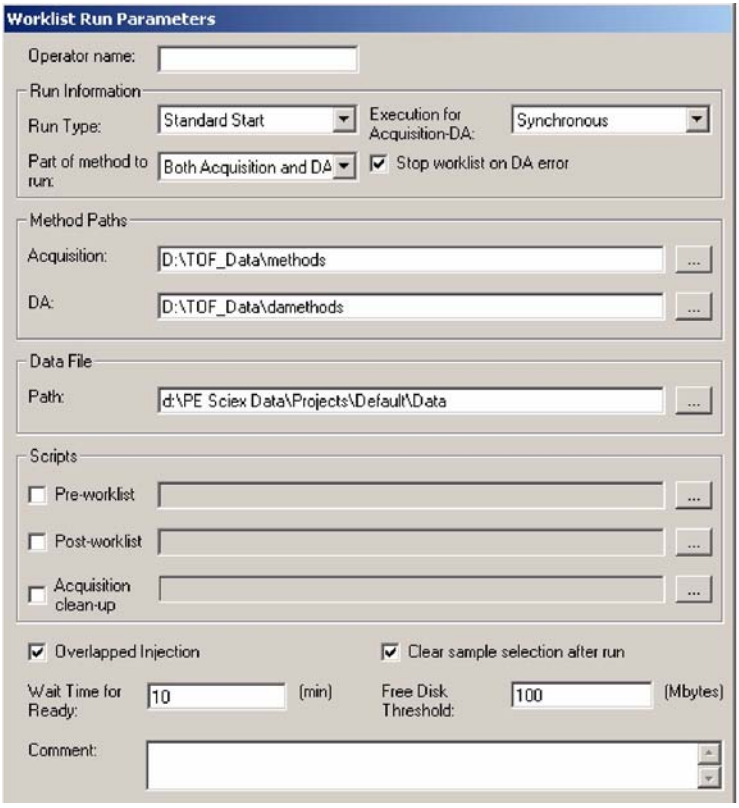


Figure 55 Worklist Run Parameters dialog box

Overlapped Data Analysis with Acquisition

You can choose to start the next data acquisition run when the data analysis is complete, or for higher throughput of samples, while data analysis of the previous sample is still running.

This option is selected in the Worklist Run Parameters dialog box, in the **Execution for Acquisition-DA** list box. To overlap data analysis with acquisition, select **Asynchronous**. To cause data acquisition to wait until data analysis is complete, select **Synchronous**.

Overlapped Injection

You can load a sample into the sample loop during a run that is still completing. This option allows you to save time that is required to load the sample before the run.

To select this option, mark the **Overlapped Injection** check box in the Worklist Run Parameters. You also have to select this option in the individual methods being used in the worklist.

Estimate of worklist file size

Depending on your estimate of the file size of the worklist, you may have to change the default value of the Free Disk Threshold.

You can use two ways to estimate the worklist file size:

- Run a typical worklist sample interactively, observe the file size in Windows Explorer, and multiply the observed file size by the number of samples in the worklist.
- Estimate the data file size for a sample run from the method used to run the sample, and add these up for all the samples in the worklist.

Method parameters that control file size

LC data is usually a small fraction of TOF data. The size of the acquired data file depends on these TOF method parameters:

- Data file storage type (TOF Data tab)—Profile, Centroid or None

  “Profile” stores raw data as abundance values for evenly spaced ion flight time. “Centroid” stores only the assigned peaks but not the raw data.”None” stores no data but does store spectrum metadata and method parameters.
- Total run time
- Setting of time segments

Approximate file size for stored Profile data

The numbers below apply to a single time segment and file size per minute. If you know the total run time and the time segment definitions, you can estimate the file size for a single run.

You can find the file size per minute by multiplying the file size per spectrum times the scans/sec. (TOF Data tab) times 60.

The file size per spectrum depends on three variables.

- Mass range
- Data compression

- Spectrum metadata

Mass range You can reduce data file size by reducing the mass range of interest. Profile data are stored as abundance for each flight time. If you restrict the mass range, the number of data points in the spectrum depends on the mass calibration curve. The table below shows the number of data points for a given mass range using a typical mass calibration curve.

Note that low masses require more data points than high masses because of the non-linear nature of the calibration curve.

Table 16 Number of data points for given mass ranges

Mass range (Daltons)	Number of data points
50 to 750	50,000
50 to 1000	60,000
50 to 3000	100,000
250 to 1000	30,000
500 to 3000	60,000
250 to 3000	70,000

Each data point stored requires 4 bytes of storage. A mass range of 250 to 3000 requires 280,000 bytes per spectrum. If the scans/sec. equal 1, then the data storage required for one minute worth of spectra is 16,800,000 bytes or about 16 MB. If the run time on a run with one time segment is 5 minutes, then one run requires about 80 MB of disk space. A worklist with 10 samples using the same mass range requires about 800 MB of disk space. If the scan rate is set to 20 scans per second, the data file could require a large amount of disk space.

Data compression If consecutive data points in a spectrum have identical values, such as zero, data compression is effective in the region. The system can achieve an overall reduction of the file size of 4 to 8 times. Noisy baselines result in less compression.

Spectrum metadata In addition to data points, information on the spectrum, such as the instrument actuals and setpoints, used during acquisition is stored with each spectrum. This information takes 2884 bytes per spectrum or about 8.2 MB for the worklist described above.

Storage of method parameters for each run also affects file size but to a lesser degree than the file size of the spectrum. They take up about 10,000 bytes before sample injection starts. For a 10-sample worklist, they take up less than 0.1 MB.

Approximate file size for Centroid data

Data is not stored as the abundance at evenly spaced ion flight times. Rather, peak centroids are computed first. Each data point is stored as a pair of values of mass and abundance. The data storage required is 8 bytes per detected peak in the spectrum. The number of peaks detected is dependent on the number of compound peaks in the spectrum as well as the noise in the background.

If the mass range is set as 250 to 3000 and the threshold is appropriate, about 3000 to 5000 peaks are detected in one spectrum. If the scans/sec. is 1, the run time 5 minutes and ten samples are in the worklist, then the disc space needed for these samples is between 68 and 115 MB.

Spectrum metadata and method parameters are also stored with centroid data and comprise a greater percent of the file size than with Profile data. Data compression does not apply to centroid data.

Data acquisition for samples and worklists

What you can monitor during a run

Tracking sample runs

The worklist shortcut menus contain an option called “Track Worklist Run” that you can turn off or on. The default position is on. With Track Worklist Run on, you can see what sample is running at any time during the worklist run. When you start a worklist run, the first sample row turns dark blue, indicating that the sample in this row is running and data is being acquired. When the data acquisition finishes, the first sample row turns light blue to indicate that Data Analysis is running. If you have set **Execution for Acquisition-DA** to **Asynchronous**, you will see both a dark blue and a light blue line to indicate which lines are acquiring data and analyzing data. When a batch within the worklist starts to run, the first sample in the batch is highlighted blue.

		Sample Name	Sample Position	Acq Method	DA Method	Data File	Capillary	Fragmentor
1	✓	sulfa_mix1	Vial 1	default.m	efc.anm	data1.wiff	2700	205
2>	✓	sulfa_mix2	P1-A1	default.m	efc.anm	data2.wiff	2800	215
3	✓	sulfa_mix3	P1-A2	default.m	efc.anm	data3.wiff	2900	225
4	✓	sulfa_mix4	P1-A3	default.m	efc.anm	data4.wiff	3000	235

Back to Worklist | Batch:Batch1

Figure 56 Worklist with tracking on

Monitoring real-time plot

For more information on real-time plots, see “Real-time displays” on page 49.

You can also monitor the TIC or EIC chromatograms, LC parameters and mass spectra for each sample during the worklist run. You can print a plot of the real-time data through the **File > Print > Real-time Plot** menu item.

What you can do during a run

Editing current worklist during run

You can edit any sample row or batch row during a run as long as the sample is located below or in the second row after the running sample row. If the last selected row is executing, then all rows are locked.

When you switch to a row to edit the sample, the “Track Worklist Run” option automatically turns off. To see the sample row running after your edit, you select this tracking option again. The screen then automatically switches to that part of the worklist with the sample that is running.

Editing worklist in an offline session

In the TOF group window, you can select an offline session of the software to edit either methods or worklists that are not running. You cannot see the Instrument Status Pane nor the Real-time Plot pane in an offline session. To see the status of the samples running in the current worklist, you return to the online session of the software through the TOF group window.

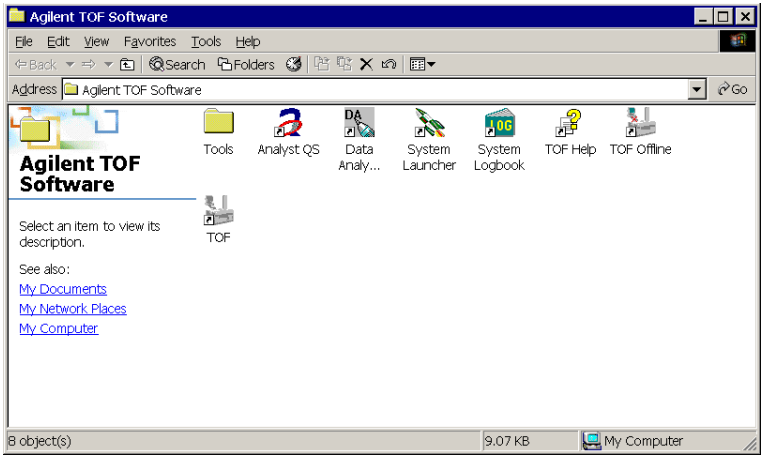


Figure 57 TOF group window

5 Data Analysis

Learn the concepts that help you understand sample analysis in the TOF software. Use this chapter to help you complete the exercises in Chapters 3 and 4 of the *Familiarization Guide*.

This chapter provides only the basics for understanding how the TOF software and the PE-Sciex Analyst QS software work together. See the Analyst documents and online help to learn more about Analyst and how to use it.

Result review in Analyst

How results are organized

When you open a data file in Analyst, the software first looks in the **D:\PE Sciex Data\Projects\Default\Data** directory for the file.

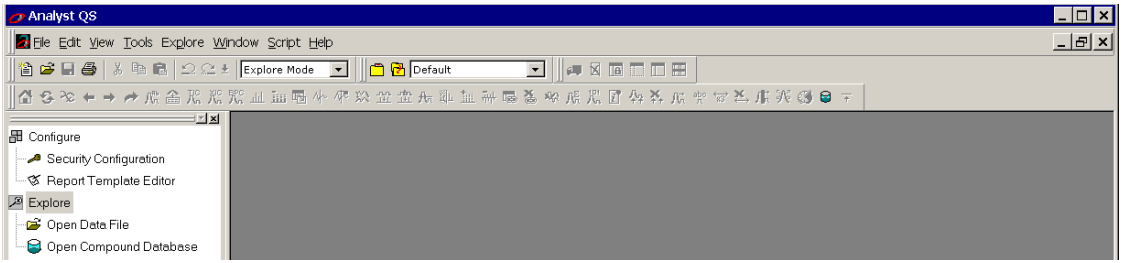


Figure 58 Analyst main window

If you selected a project other than Default in the TOF software to contain the data file you intend to open in Analyst, you must select that project in Analyst. If you selected a completely different directory to hold the data file, you must browse to that directory in Analyst.

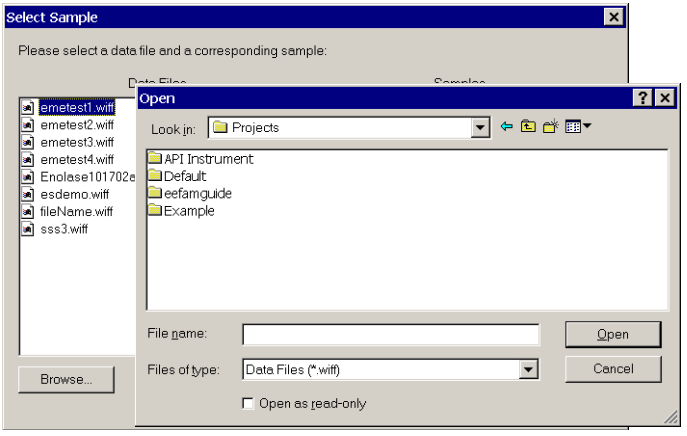


Figure 59 Browse dialog box when open data file

What you can view in Analyst

View sample data

When you open a data file, the TIC appears in the Analyst window. If DAD data was also collected, the DAD TWC (Total Wavelength Chromatogram) automatically appears in another window.

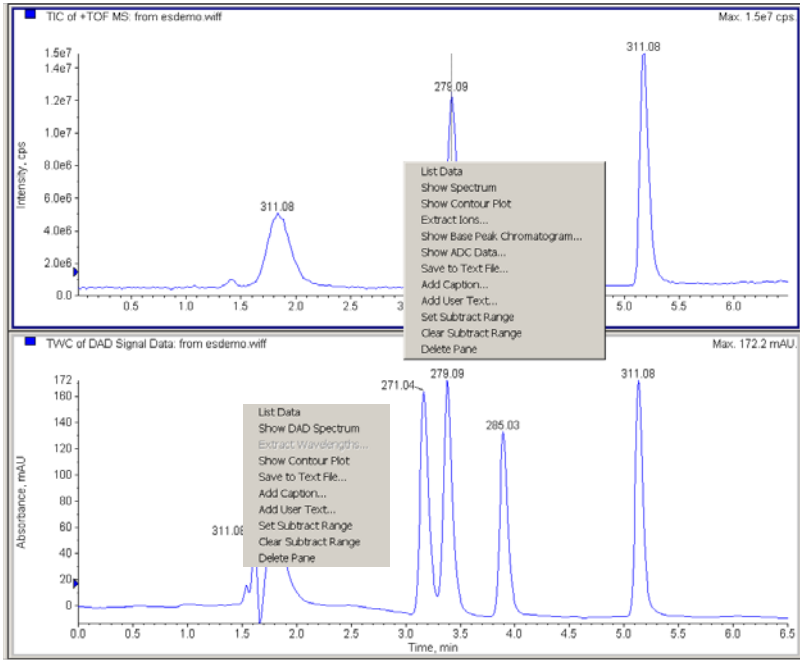


Figure 60 TIC and TWC displays in Analyst with shortcut menus

A *base peak chromatogram* (BPC) plots the intensity of the largest peak in a scan as a function of time.

Through the TOF and DAD shortcut menus you can perform several tasks:

- Display a mass or a UV spectrum at a specified time
- Display an extracted ion chromatogram (XIC in Analyst) for a specified mass range or an extracted wavelength chromatogram (XWC) for a specified wavelength range
- View the *base peak chromatogram* (TOF only).

View file, instrument and sample information

When you select the Information icon in Analyst, you can view this information:

- File info
- Log info/Column oven
- Acquisition info/Method properties
- Quant info/Custom data fields
- Segment and scan info (periods and experiments in Analyst)
- Resolution tables
- Calibration tables
- Instrument parameters

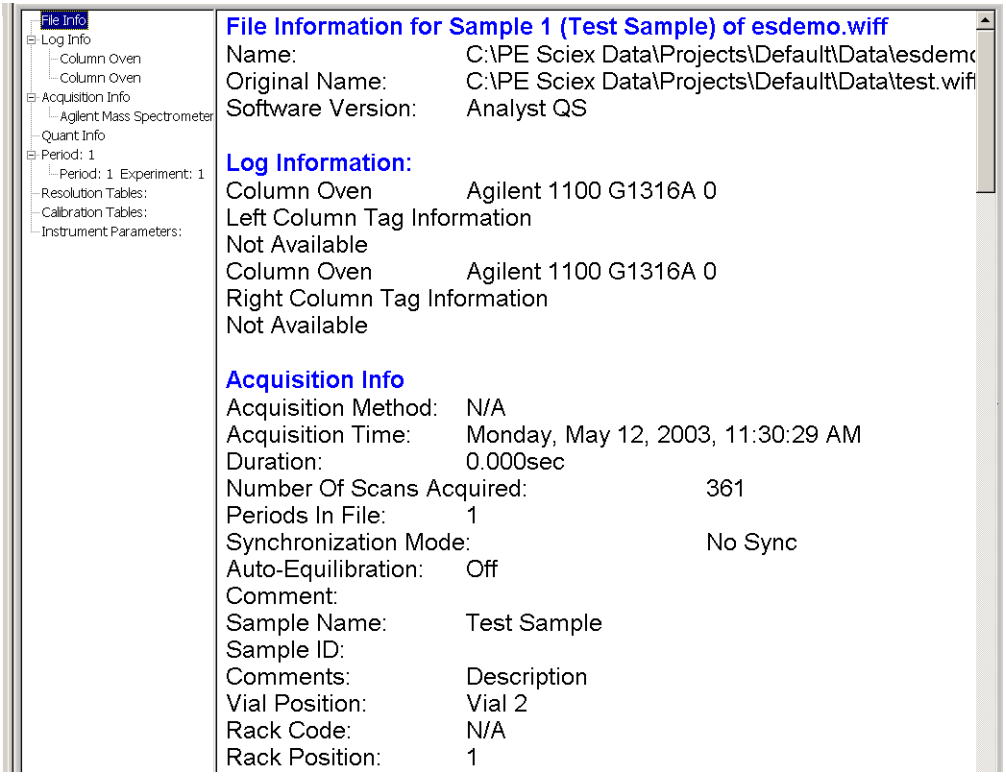


Figure 61 Information pane in Analyst

View TOF instrument parameters

When you select View Actuals from the mass spectrum shortcut menu, you can view the TOF parameters used during the run that produced the data file.

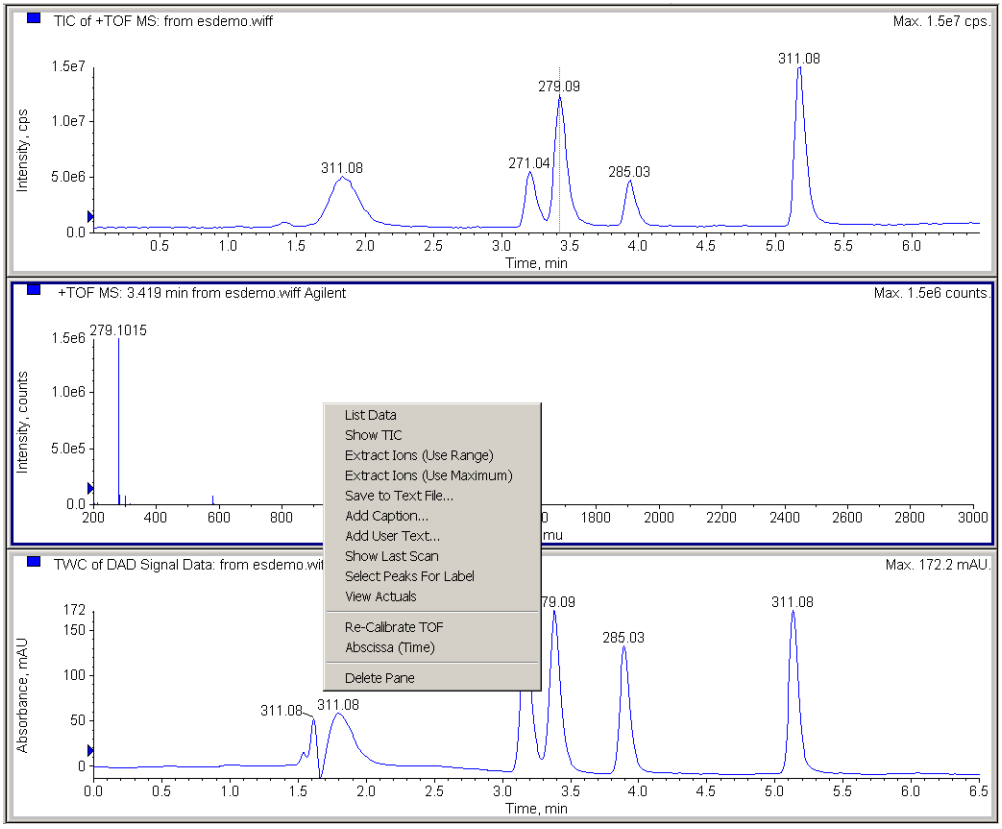


Figure 62 Mass spectrum shortcut menu

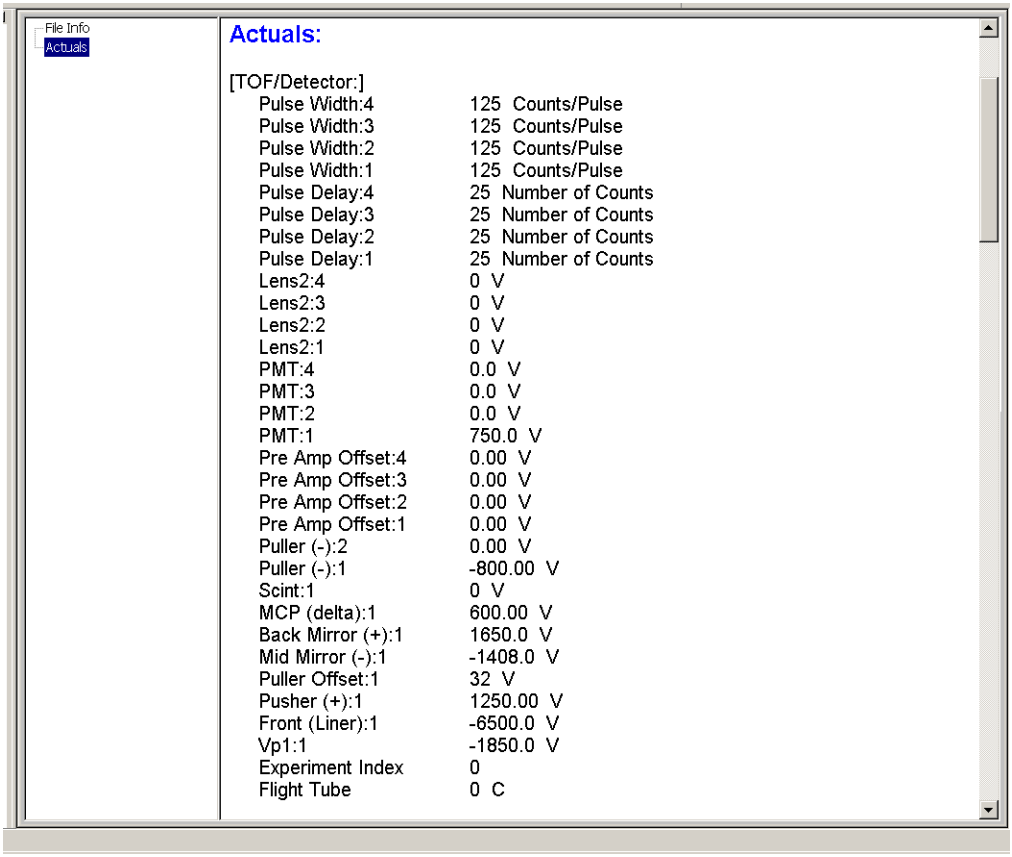


Figure 63 Actuals pane in Analyst

View LC instrument curves

When you select Show ADC Data from the TIC or TWC shortcut menu, a dialog box appears that lets you select from a list of LC instrument curves, such as Pump Pressure, Pump Flow, % Solvent A or B and Column Temperature.

You must have selected to store these curves on their respective tabs in the Method pane in the TOF software in order to see the dialog box. If you do not choose to store any of the curves in the method, you cannot access these curves from Analyst.

Agilent peak finder parameters

When you show a mass spectrum, Analyst invokes an Agilent algorithm to detect the spectral peaks. Analyst uses the Agilent algorithm rather than the default Analyst algorithm.

You can change the parameters for the peak finder algorithm through a script called Agilent Peak Finders Parameters.dll, which you access from the top Script menu. Parameters set with the Processing Options dialog box in Analyst have no effect on the Agilent algorithm.

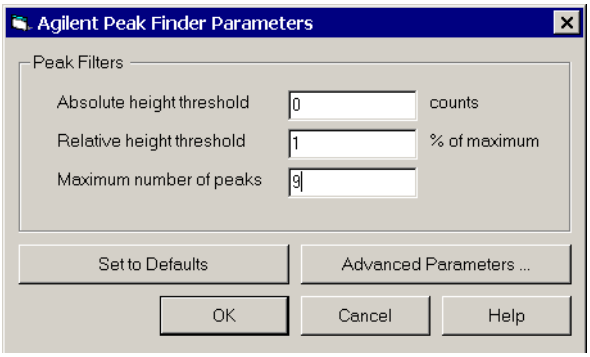


Figure 64 Agilent Peak Finder Parameters dialog box

Analyst uses the parameter changes set in this dialog box only during interactive Analyst use. Changing these parameters does not affect the peak find parameters used elsewhere in the TOF software, such as in empirical formula confirmation.

The Analyst spectrum plot lets you move a blue triangle in the spectrum plot up and down to set the peak finder display threshold. Peaks whose tops fall below that threshold are neither labelled in the plot nor listed in the List Data pane.

Peaks that exceed the display threshold may be reported if they also meet all of the criteria set in the Agilent Peak Finder Parameters dialog box.

Recalibration in Analyst

After the peak finder algorithm finds the spectral peaks, Analyst calls another Agilent algorithm to use the calibration coefficients calculated during reference mass correction to convert time to mass.

You can update these calibration values used during sample runs in Analyst. You can optionally decide to use the updated calibration values as the instrument default for future samples.

When you select Re-Calibrate TOF from the shortcut menu, the TOF Recalibration dialog box for the Agilent TOF appears.

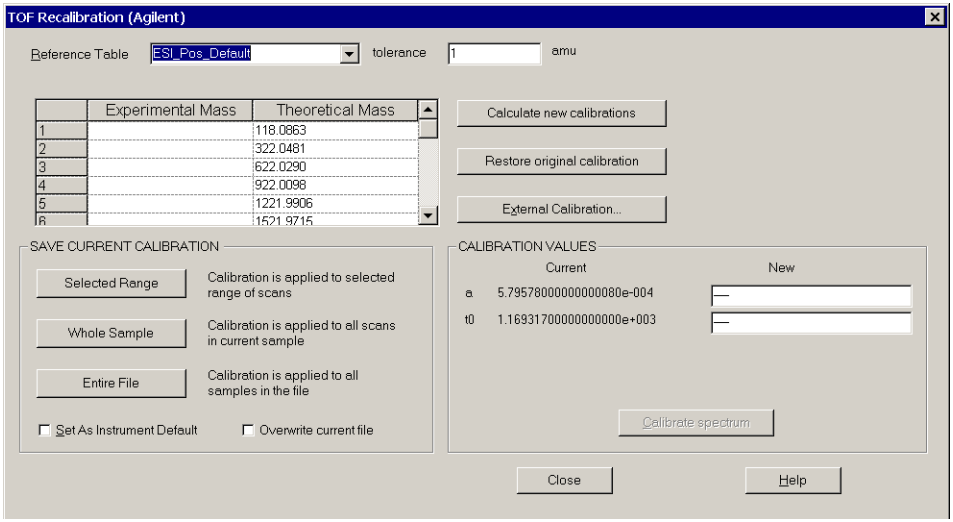


Figure 65 TOF Recalibration dialog box for Agilent TOF

Before you recalibrate

After you select a Reference Table, the system enters the Reference Table values in the Theoretical Mass column. The system then looks for peaks within the specified tolerance of the theoretical masses and lists their masses in the Experimental Mass column.

Recalibrate the data

When you click the Calculate new calibrations button, the system calculates new calibration values and displays them in the Calibration Values area of the dialog box.

The greyed-out Calibrate spectrum button becomes available to apply the new calibration to the spectrum. The values in the Experimental Mass column are updated, and the new mass values are used to label the peaks in the spectrum plot.

Save the coefficients

You have many options for saving the new calibration coefficients.

- Apply the new calibration only to the current spectrum.

  If the current spectrum is an average of multiple scans, the system applies the calibration to each of the spectra used in the average.
- Apply the new calibration to all scans in the current sample that have the same ion polarity as the current scan
- Apply the new calibration to all samples in the current data file that have the same ion polarity as the current scan
- Replace the current instrument calibration values with the new ones to use on future samples
- Write the new calibration data into the current data file or select a name and location for the new file that will be created.

When you exit the TOF Recalibration dialog box, the updated spectrum appears in the Analyst window. If you saved the data to a new file, that file is opened and the spectrum you just used for recalibration is extracted and displayed.

TOF Recalibration Dialog Box in Analyst

The TOF Recalibration dialog box lets you recalibrate the mass values in a spectrum based on a known calibrant mass, masses within the data, or masses from another data file.

Agilent TOF reports

You can also print Analyst review data–chromatograms, spectra and results–from the Agilent TOF .wiff files.

The following reports are available for Agilent TOF data:

- Empirical formula confirmation report

  This report is automatically sent to the screen, printed or saved to a file when you run a worklist with efc.anm as the data analysis method and with added formula columns.

- Mass list report

  This report is automatically sent to the screen, printed or saved to a file when you run a worklist with a data analysis method that specifies a mass list report. This report can also contain a table on generation of empirical formula or on confirmation screening by database search.

- Molecular feature extraction report

  This report is automatically sent to the screen, printed or saved to a file when you run a worklist with a data analysis method that specifies a molecular feature extraction report. This report can also contain a table on generation of empirical formula or on confirmation screening by database search.

- Parameter optimization report

  You set up, view and print this report with an Agilent script accessed from the top Script menu in Analyst.

Data analysis reports

For more information on worklists set up to produce empirical formula confirmation reports, see "Worklist to confirm empirical formulas" on page 94.

Agilent created the efc.anm data analysis method to produce empirical formula confirmation reports. You use the Agilent Data Analysis Method Editor to edit this method to customize the empirical formula confirmation reports.

You can create data analysis methods to generate mass list reports or molecular feature extraction report as well.

You access the data analysis method editor through the TOF group window.

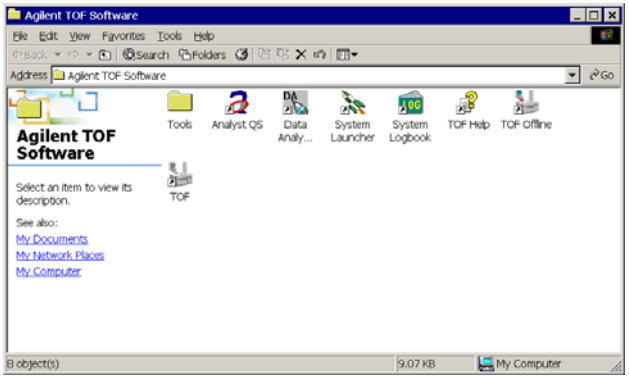


Figure 66 TOF Group window

After you select the Data Analysis Method Editor icon in the TOF group window, the following dialog box appears.

To learn how to use the Data Analysis Method Editor, see the Familiarization Guide and the Online Help.

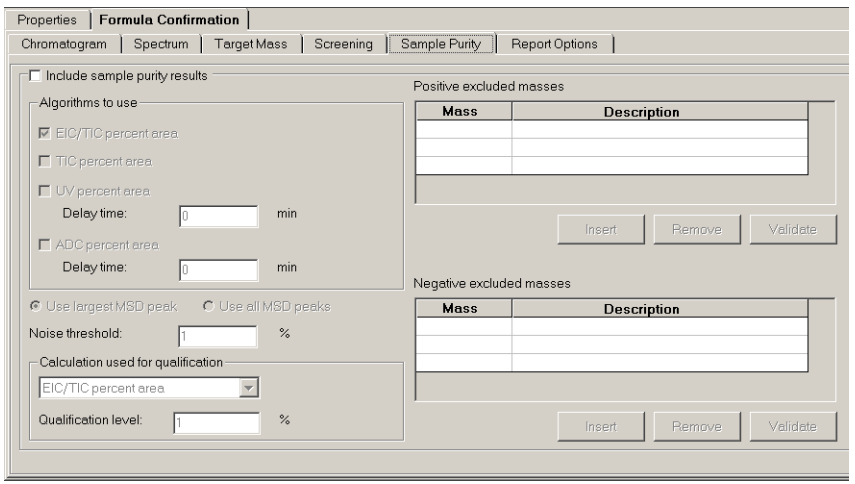


Figure 67 Formula Confirmation Sample Purity tab of Data Analysis Method Editor

Empirical formula confirmation report

You can load the efc.anm file or any data analysis method for empirical formula confirmation that has been previously saved through this editor. You can select whether to enter the formulas directly into the worklist or into a database.

For the EFC report, an XIC of the mass and its adducts etc. for each formula in the database is created. This XIC is checked to see if that compound is part of your sample. If the compound is found in the XIC and the retention time of the compound in the XIC is within the retention time tolerance of the retention time in the database, the compound is confirmed.

You use this report when you know the formula and wish to confirm it is in your sample. If you believe the compound is from a set of likely compounds, you may elect to use the database option instead of entering the information into the worklist.

After changing parameters on the Formula Confirmation tabs, you can save the parameters to the loaded method or to a new method.

Mass list report

You can select a mass list report instead of or in addition to the empirical formula confirmation report. You select Mass List on the Select DA Operations dialog box.

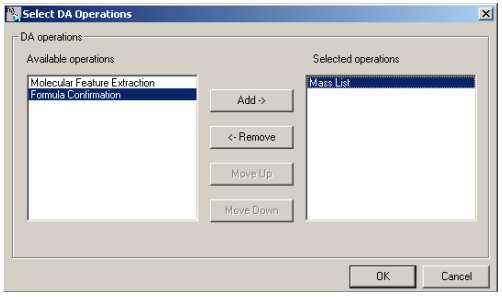


Figure 68 Select DA Operations dialog box

With Mass List selected, the Mass List tab is then visible on the Data Analysis Method Editor window. Enter the parameters on the Mass List tabs and save the method.

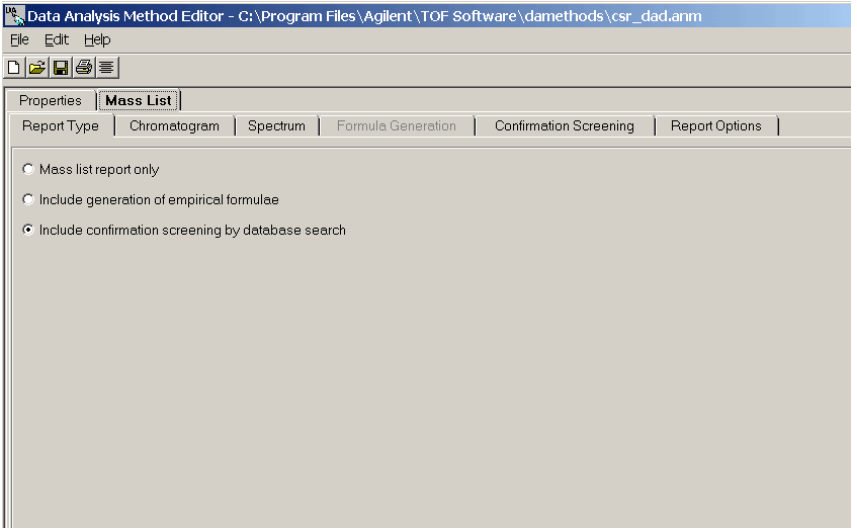


Figure 69 Mass List Report Type tab of Data Analysis Method Editor

There are two additional sections which can be added to a standard **Mass List report**. The standard Mass List report can also include a section on Empirical Formula Generation or a section on Confirmation Screening.

The Empirical Formula Generation report type identifies valid molecular formulas that match the masses found in your sample based upon the values entered in the **Data Analysis Mass List Formula Generation** tab.

The Confirmation Screening report type searches a database for every mass in the sample. You do not know the formula and are trying to guess it by searching for each mass that is found in the sample. You enter the database file name on the **Data Analysis Mass List Confirmation Screening** tab.

Molecular Feature Extraction

You can select a molecular feature extraction report instead of or in addition to the empirical formula confirmation report. You select Molecular Feature Extraction on the Select DA Operations dialog box.

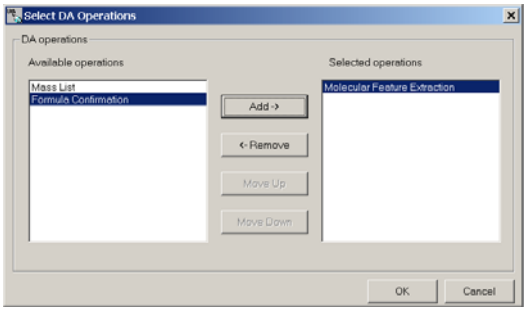


Figure 70 Select DA Operations dialog box

With Molecular Feature Extraction selected, the Molecular Feature Extraction tab is then visible on the Data Analysis Method Editor window. Enter the parameters on the Molecular Feature Extraction tabs and save the method.

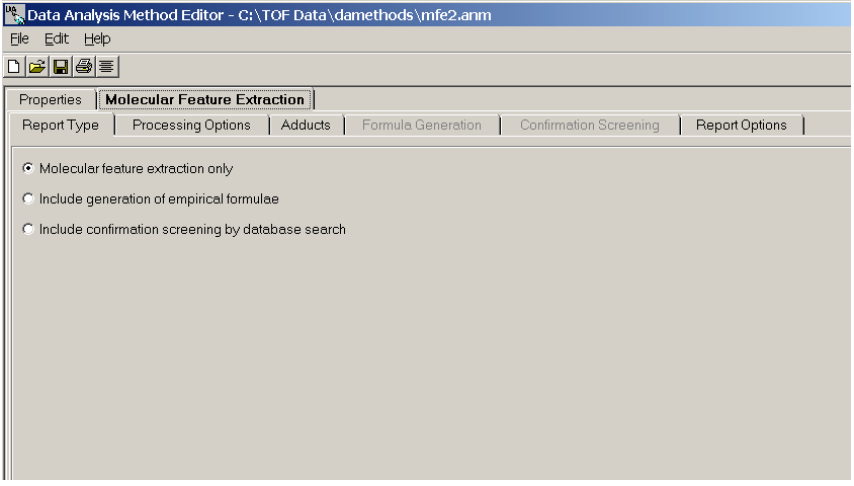


Figure 71 Mass List Report Type tab of Data Analysis Method Editor

There are two additional sections which can be added to a standard **Molecular Feature Extraction report**. The standard Molecular Feature Extraction report can also include a section on Empirical Formula Generation or a section on Confirmation Screening.

The Empirical Formula Generation report type identifies valid molecular formulas that match the masses found in your sample based upon the values entered in the Data Analysis Molecular Feature Extraction Formula Generation tab.

The Confirmation Screening report type searches a database for every mass in the sample. You do not know the formula and are trying to guess it by searching for each mass that is found in the sample. You enter the database file name on the Data Analysis Molecular Feature Extraction Confirmation Screening tab.

Creation of a summary log to contain results for export

You can find the summary report files in the **Reports\Summary** *folder in the directory where the TOF software was installed on your system (usually* **Program Files\Agilent\TOF Software***).*

You can access a summary log if you select the **AddToSummaryReport** check box on the Report Options tab. The summary report file is named **EFC_summary_TS.csv** for Empirical Formula Confirmation results, **Masslist_summary_TS.csv** for MassList results and **MFE_summary_TS.csv** for Molecular Feature Extraction results, where TS is a timestamp. The file is in CSV (comma separated variable) format, which can be opened in Microsoft Excel to print a report for all the samples in a worklist. Once the file is created, results from subsequent runs are appended to it.

Do not open any of these files during a run or data acquisition, or the file may not be updated.

WIFF Translation

You can also select to translate the WIFF data file to the format that is used in the Agilent MassHunter Workstation Qualitative Analysis program and the Agilent MassHunter Workstation Quantitative Analysis program. You need to select WIFF Translation.

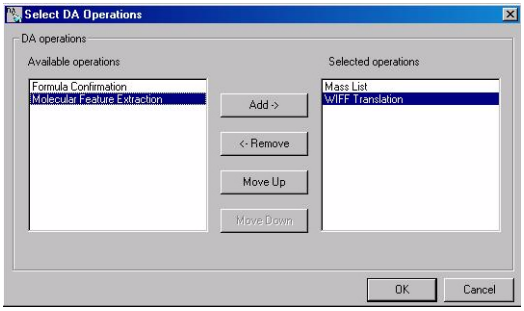


Figure 72 Select DA Operations dialog box

With WIFF Translation selected, the WIFF Translation tab is then visible on the Data Analysis Method Editor window. You can enter the parameters on the WIFF Translation tab and save the method.

TOF acquisition parameter optimization reports

For information on worklists set up for parameter optimization, see "Worklist for parameter optimization" on page 97.

Agilent has created a Parameter Optimization Wizard, which lets you select sample data and compare their chromatograms run under different parameter values. You can print the report that is generated from the parameter optimization wizard.

The wizard is a script called Parameter Optimization.dll, which you access from the Script top menu.

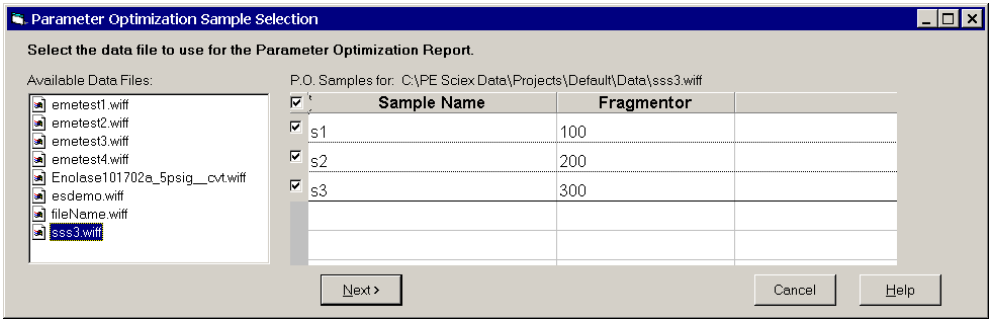


Figure 73 Parameter Optimization Sample Selection Page

If a parameter appears in the wizard that you do not want, you may have to delete a hidden column in the worklist.

Note that you can only select one file at a time. If you want to compare the effect of the parameter values on the chromatographic peak, you must save your sample results to the same data file.

The next two pages of the wizard include options to affect the graphics portion and the tabular portion of the report.

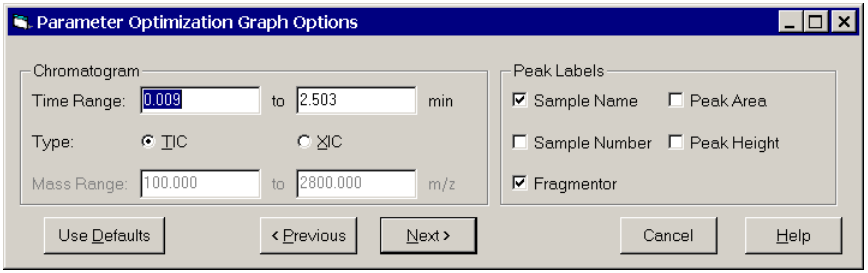


Figure 74 Parameter Optimization Graph Options Page

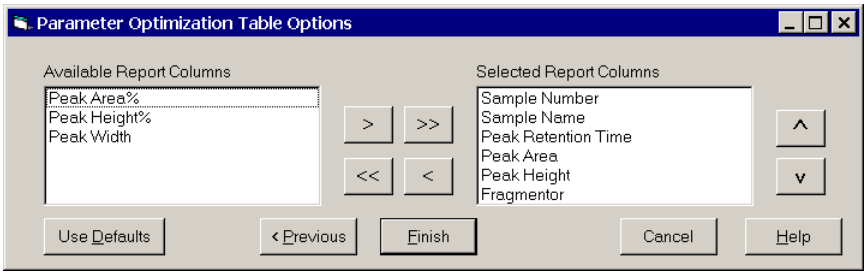
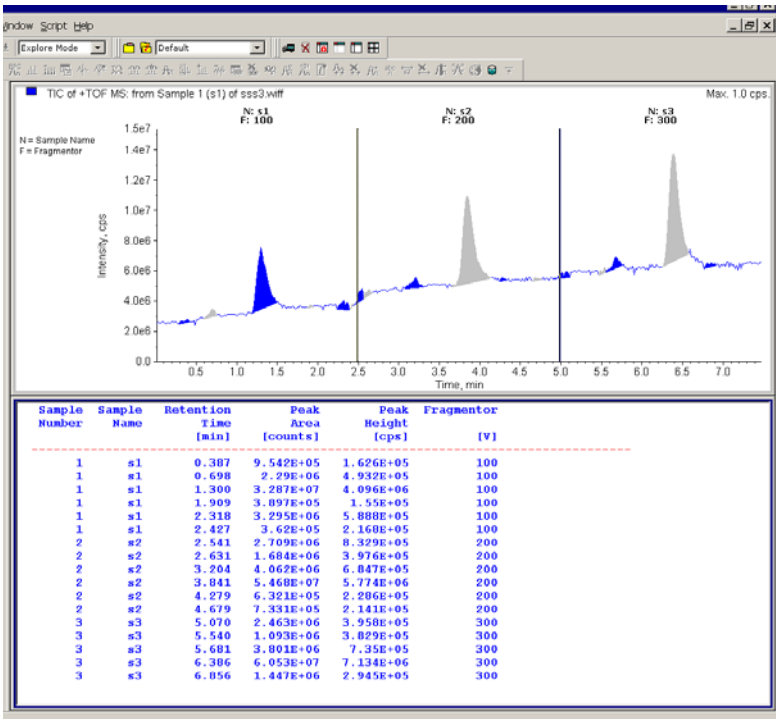


Figure 75 Parameter Optimization Table Options Page

When you have completed entering your values to the wizard, a report appears that looks like this:

Note that the time axis on the graph and the retention times in the table are meaningless. In fact, you are looking at the same peak in three different samples whose retention time is about 1.3 minutes. The fragmentor voltage affects the height of the peak.



Sample Number	Sample Name	Retention Time [min]	Peak Area [counts]	Peak Height [cps]	Fragmentor [V]
1	s1	0.387	9.542E+05	1.626E+05	100
1	s1	0.698	2.29E+06	4.932E+05	100
1	s1	1.300	3.287E+07	4.096E+06	100
1	s1	1.909	3.897E+05	1.55E+05	100
1	s1	2.318	3.295E+06	5.888E+05	100
1	s1	2.427	3.62E+05	2.160E+05	100
2	s2	2.541	2.709E+06	8.329E+05	200
2	s2	2.631	1.684E+06	3.976E+05	200
2	s2	3.204	4.062E+06	6.847E+05	200
2	s2	3.841	5.468E+07	5.774E+06	200
2	s2	4.279	6.321E+05	2.206E+05	200
2	s2	4.679	7.331E+05	2.141E+05	200
3	s3	5.070	2.463E+06	3.958E+05	300
3	s3	5.540	1.093E+06	3.829E+05	300
3	s3	5.681	3.801E+06	7.35E+05	300
3	s3	6.306	6.053E+07	7.134E+06	300
3	s3	6.856	1.447E+06	2.945E+05	300

Figure 76 Example parameter optimization report

Signal-to-Noise Ratio Report

This report gives the results of a signal-to-noise calculation for an Extracted Ion Chromatogram (EIC). To generate this report, select Signal-to-Noise.dll from the Script menu in Analyst, enter the desired parameters values, and click the Preview Report or Print Report button on the Signal-to-Noise Report dialog box.

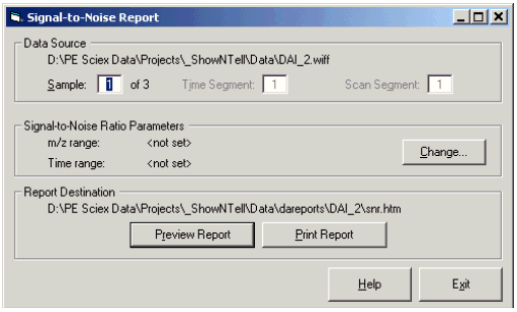


Figure 77 Signal-to-Noise Report dialog box

This report is only used for checkout.

Exporting spectra to text file for custom reporting

You can export spectra from a wiff file to a text file for use with other applications.

To generate this file, select Data File Export.dll from the Script menu in Analyst, enter the desired parameters values, and click the Write to File button on the Data File Exporter dialog box.

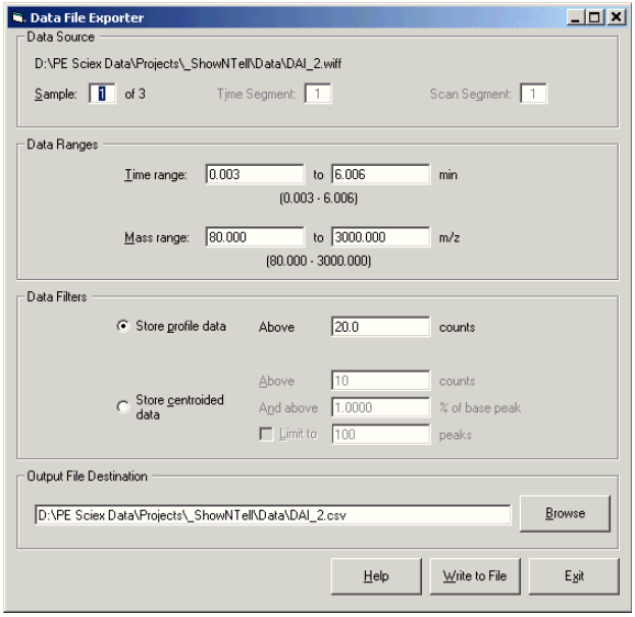


Figure 78 Data File Exporter dialog box

When possible, use the Data Ranges and Data Filters parameters to reduce the amount of spectral data exported, in order to reduce the size of the resulting text file.

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In This Book

The *Concepts Guide* presents “The Big Picture” behind the Agilent G3250AA LC/MSD TOF system to help you to understand how to use the LC/MSD TOF System components.

This guide includes concepts for:

- Instrument Preparation
- Acquisition Methods
- Data Acquisition
- Data Analysis

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