Agilent G3250AA
LC/MSD TOF System

Concepts Guide
The Big Picture
Notices


No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Manual Part Number
G3300-90012

Edition
Printed in USA
Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95052

Microsoft® and Windows® are U.S. registered trademarks of Microsoft Corporation.

To submit comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

Warranty
The material contained in this document is provided “as is,” and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Safety Notices

CAUTION
A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

WARNING
A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Technology Licenses
The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend
U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).
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
   How does the TOF system help you do your job? 10
      Help for applications 10
      Help for data acquisition 11
      Help for data analysis 13
   How does the Agilent TOF mass spectrometer work? 14
   How do TOF software components work together? 19

2 Instrument Preparation
   LC preparation 22
      LC module setup 22
      Column equilibration and conditioning 24
   TOF preparation — calibration and tuning 26
      Mass calibration 27
      Tuning choices 31
      TOF parameters adjustable through tuning 37
      Tune reports 44
      When to tune your TOF — Checktune report 46
      Storage and retrieval of tune results 48
   Real-time displays 49
      Instrument status 49
      Real-time parameter values (Actuals) 50
      Real-time plots 52
   System logbook 55

3 Acquisition Methods
   LC parameter entry 58
      Storage and display of DAD signal data 58
      Storage and display of DAD spectral data 58
MS TOF parameter entry  59
Data acquisition parameters  59
TOF acquisition parameters adjustable through methods  61
Parameters for different ion sources  63
Setup for automatic parameter changes
(Segments/Scans)  64
Setup of reference mass correction (recalibration)  67
MS TOF chromatogram setup  71
Method saving, editing and reporting  72
Saving an acquisition method  72
Method editing  74
Method reporting  75

4  Data Acquisition

Interactive single sample setup  78
Sample information  78
Data file information/projects  79
Run information  80
Worklist setup  81
Worklist menus  83
Single sample entry  84
Batch entry  86
Script entry  88
Entry of additional sample information (show, add columns)  89
Worklist import  91
Worklist to confirm empirical formulas  94
Worklist to report mass list  96
Worklist to report molecular feature extraction  96
Worklist for parameter optimization  97
Report setup  98
Run setup  99
Estimate of worklist file size 101
Data acquisition for samples and worklists 104
What you can monitor during a run 104
What you can do during a run 105

5 Data Analysis

Result review in Analyst 108
How results are organized 108
What you can view in Analyst 109
Agilent peak finder parameters 113
Recalibration in Analyst 114

Agilent TOF reports 116
Data analysis reports 116
TOF acquisition parameter optimization reports 123
Signal-to-Noise Ratio Report 125
Exporting spectra to text file for custom reporting 126
1
Overview

How does the TOF system help you do your job? 10
How does the Agilent TOF mass spectrometer work? 14
How do TOF software components work together? 19

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?

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:

- 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
1 Overview

- 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
- 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

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

- 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.
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.
1 Overview

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.
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.
2

Instrument Preparation

LC preparation  22
TOF preparation — calibration and tuning  26
Real-time displays  49
System logbook  55

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.
2 Instrument Preparation

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.)

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.

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

Table 1 Tasks to set up the LC modules

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

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.

- 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

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.

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

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 ESNegautotune.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](image)

**Figure 5** Standard Calibration tab of the Method pane
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**

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:

\[
m/z = \left[ a(t - t_o) \right]^2
\]

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:

![Mass Calibration Curve](image)

**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:
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).

![Example detailed residual plot](image)

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

\[
\text{Residual mass error} = a_2t + b_2t^3 + c_2t^5 + d_2t^7 + e_2t^9 + f_2t^{11} \ldots [2]
\]

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

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

To see the current coefficients calculated for the residual mass error, you bring up the 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 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

<table>
<thead>
<tr>
<th>Number of Masses</th>
<th>Coefficients Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>2 to 4</td>
<td>a, ( t_0 ) (equation 1)</td>
</tr>
</tbody>
</table>

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.
Instrument Preparation

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.

<table>
<thead>
<tr>
<th>Number of Masses</th>
<th>Coefficients Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>a, t, a2, b2, c2, d2</td>
</tr>
<tr>
<td>6</td>
<td>a, t, a2, b2, c2, d2, e2</td>
</tr>
<tr>
<td>&gt;=7</td>
<td>a, t, a2, b2, c2, d2, e2, f2</td>
</tr>
</tbody>
</table>

Table 2  Effect of Mass Selection on Coefficients Calculated

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.
2 Instrument Preparation

**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.

![Extended Tune tab](image)

*Figure 10 Extended Tune tab*

**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.

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

![Autotune status window](image)

**Figure 11** Autotune status window
After the optimization of each parameter, its Optimization Curve appears temporarily for your review.

![Optimization Curve for a parameter](image)

**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
2 Instrument Preparation

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

Manual Tune (Ramp)

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
2 Instrument Preparation

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.
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.
2 Instrument Preparation

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:

![Optimization Curve for Height vs. Oct RF V](image)

**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.
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](image-url)  Plots of signal and resolution vs. accelerator focus (V)
2 Instrument Preparation

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.

![Tune Report Image]

**Figure 19** Tune Report

<table>
<thead>
<tr>
<th>Mass Calibration Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected Mass</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>118.20922</td>
</tr>
<tr>
<td>122.24994</td>
</tr>
<tr>
<td>131.26045</td>
</tr>
<tr>
<td>141.27148</td>
</tr>
<tr>
<td>155.28871</td>
</tr>
<tr>
<td>242.14134</td>
</tr>
<tr>
<td>272.18463</td>
</tr>
</tbody>
</table>

**Tune Parameter Setpoint Tables**

<table>
<thead>
<tr>
<th>Source</th>
<th>Optical Power</th>
<th>TOF</th>
<th>Pulsed</th>
<th>#Flaps</th>
<th>Calibration Coeff</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>225.6</td>
<td>1200.0</td>
<td>5000</td>
<td>0.577211</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>15.0</td>
<td>25.0</td>
<td>3.921 E-02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2_C</td>
<td>20.0</td>
<td>5000.0</td>
<td>5.865 E-00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O</td>
<td>3.0</td>
<td>120.0</td>
<td>2.276 E-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2O</td>
<td>2.0</td>
<td>950.0</td>
<td>2.268 E-09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Agilent G3250AA LC/MSD TOF System Concepts Guide
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.

**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.

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

Figure 21  Standard Tune tab with Checktune option
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.

### Table 3  How the TOF system stores and uses tune results

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

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

**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.

![Instrument Status Pane](image)

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>
<thead>
<tr>
<th>Instrument State</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>On—Preparing</td>
<td>Yellow</td>
</tr>
<tr>
<td>On—Ready</td>
<td>Green</td>
</tr>
<tr>
<td>On—Waiting</td>
<td>Purple</td>
</tr>
<tr>
<td>On—Running</td>
<td>Blue</td>
</tr>
<tr>
<td>Standby</td>
<td>Gray—To see if the pump or MS TOF is in Standby mode or off, look for the check mark in the shortcut menu.</td>
</tr>
<tr>
<td>Off</td>
<td>Gray</td>
</tr>
</tbody>
</table>
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](image)

**Figure 24** Actuals Setup dialog box
The parameters and states for each module listed below are available for display.

**Table 5  Actuals available for display**

<table>
<thead>
<tr>
<th>Module</th>
<th>Parameter or State</th>
</tr>
</thead>
<tbody>
<tr>
<td>All modules</td>
<td>Run Time, Run State, Ready State, Ready Type, Not Ready Text Long, Not Ready Text Short, Rawdata State, Error State</td>
</tr>
<tr>
<td>ALS</td>
<td>Vial, Sample, Volume, Needle, Command, Injectmode, Tray Type A, Tray Type B, Overlap, Injectstatus, Temp, Therm Power</td>
</tr>
<tr>
<td>WPS, µWPS</td>
<td>The same as ALS except no Vial and addition of Drawn Volume, Sample Position and Needle Position</td>
</tr>
<tr>
<td>Capillary Pump</td>
<td>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</td>
</tr>
<tr>
<td>Nanopump</td>
<td>Same as capillary pump with addition of FSAC State and FSAC Step</td>
</tr>
<tr>
<td>Thermostatted Column Compartment</td>
<td>Left Temp, Left Temp Set, Right Temp, Right Temp set, Column Valve, Therm Power</td>
</tr>
<tr>
<td>35900E</td>
<td>Current Vial</td>
</tr>
<tr>
<td>DAD (all others)</td>
<td>Sample WI A-E, Sample Bw A-E, Reference On A-E, Reference WI A-E, Reference Bw A-E, UV Lamp, Vis Lamp</td>
</tr>
<tr>
<td>MS TOF</td>
<td>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</td>
</tr>
</tbody>
</table>
Real-time plots

What you can display

You can display the following plots for these modules.

Table 6  Real-time plots available for display

<table>
<thead>
<tr>
<th>Module</th>
<th>Plot type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumps</td>
<td>Pressure vs. time</td>
</tr>
<tr>
<td>Thermostatted Column Compartment</td>
<td>Temperature Left, Temperature Right vs. time</td>
</tr>
<tr>
<td>35900E</td>
<td>ADC signal vs. time</td>
</tr>
<tr>
<td>DAD G1315C and G1315D</td>
<td>Signals A-H vs. time, spectra</td>
</tr>
<tr>
<td>DAD</td>
<td>Signals A-E vs. time, spectra</td>
</tr>
</tbody>
</table>
| 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

<table>
<thead>
<tr>
<th>Display type</th>
<th>What you can do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal plots</td>
<td>Change range of intensity or time, freeze and zoom the plot</td>
</tr>
<tr>
<td>Pump parameters</td>
<td>Change range of intensity or time, freeze and zoom the plot</td>
</tr>
<tr>
<td>TOF spectra</td>
<td>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)</td>
</tr>
</tbody>
</table>
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.

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.
2 Instrument Preparation

What you can do with the system 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 8 Columns available for display in the Logbook Viewer

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

Table 9 Tasks you can perform with the logbook

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

LC parameter entry 58
- Storage and display of DAD signal data 58
- Storage and display of DAD spectral data 58
MS TOF parameter entry 59
- Data acquisition parameters 59
- TOF acquisition parameters adjustable through methods 61
- Parameters for different ion sources 63
- Setup for automatic parameter changes (Segments/Scans) 64
- Setup of reference mass correction (recalibration) 67
- MS TOF chromatogram setup 71

Method saving, editing and reporting 72

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](image)

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](image-url)
3 Acquisition Methods

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.

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](image)

**Figure 31** Acquisition tab
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
3 Acquisition Methods

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

- 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.
- 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.
- 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

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

**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

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

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.
3 Acquisition Methods

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**

With two unknowns, a minimum of two known values are required to determine both A and $t_0$. Practically considerations also come into play. In order to get a good fit for both A and $t_0$, then at least 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 $t_0$ 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.

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

![Edit Mass Lists dialog box](image)

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.

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**

**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.
3 Acquisition Methods

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.

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.
3 Acquisition Methods

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.

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.
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**

<table>
<thead>
<tr>
<th>Script name</th>
<th>Actions the script enable</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCP_AcquireCalibrantData</td>
<td>Sets “Cal/Ref Mass” to “Cal B” and “LCStream” to “LC-&gt;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.</td>
</tr>
<tr>
<td>SCP_InstrumentStandby</td>
<td>Puts the instrument in standby mode. This is the same as clicking the “Standby” button on the instrument status pane.</td>
</tr>
<tr>
<td>SCP_LCCondition (MethodName, LCStream)</td>
<td>Starts a run for conditioning the LC part of the instrument. There is no data acquisition</td>
</tr>
<tr>
<td>SCP_LoadIdleMethod (MethodName)</td>
<td>Loads a method to put the system in an idle state</td>
</tr>
<tr>
<td>SCP_MSCalibValveOff</td>
<td>Sets “Cal/Ref Mass” to “None” or off</td>
</tr>
<tr>
<td>SCP_PumpAllAndMSCalibValveOff</td>
<td>Turns off all the pumps and sets Cal/Ref Mass to “None”</td>
</tr>
<tr>
<td>SCP_PumpsAllOff</td>
<td>Turns off all the pumps</td>
</tr>
</tbody>
</table>

**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).
3 Acquisition Methods

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
3 Acquisition Methods
## Data Acquisition

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interactive single sample setup</td>
<td>78</td>
</tr>
<tr>
<td>Worklist setup</td>
<td>81</td>
</tr>
<tr>
<td>Data acquisition for samples and worklists</td>
<td>104</td>
</tr>
</tbody>
</table>

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.

![Sample tab of the Method pane](image)

**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 sequences 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 ScienData\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.

<table>
<thead>
<tr>
<th>1</th>
<th>v</th>
<th>octane01</th>
<th>F1-A1</th>
<th>Acq Method</th>
<th>DA Method</th>
<th>Data File</th>
<th>Inj Vol (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>v</td>
<td>octane02</td>
<td>F1-A2</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>3</td>
<td>v</td>
<td>octane03</td>
<td>F1-A3</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>4</td>
<td>v</td>
<td>octane04</td>
<td>F1-A4</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>5</td>
<td>v</td>
<td>octane05</td>
<td>F1-B1</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>6</td>
<td>v</td>
<td>octane06</td>
<td>F1-B2</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>7</td>
<td>v</td>
<td>octane07</td>
<td>F1-B3</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>8</td>
<td>v</td>
<td>octane08</td>
<td>F1-B4</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>9</td>
<td>v</td>
<td>octane09</td>
<td>F1-C1</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>10</td>
<td>v</td>
<td>octane10</td>
<td>F1-C2</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>11</td>
<td>v</td>
<td>octane11</td>
<td>F1-C3</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
<tr>
<td>12</td>
<td>v</td>
<td>octane12</td>
<td>F1-C4</td>
<td>AcqMethod</td>
<td>DA Method</td>
<td>DataFile</td>
<td>InjVol</td>
</tr>
</tbody>
</table>

Figure 40   Example worklist
4 Data Acquisition

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
4 Data Acquisition

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.

![Sample Position tab of the Add Multiple Samples dialog box](image)

**Figure 43** Sample Position tab of the Add Multiple Samples dialog box
4  Data Acquisition

**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.*

![Batch Run Parameters dialog box](image-url)
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.

![Batch list](image)

<table>
<thead>
<tr>
<th>✓</th>
<th>Sample Name</th>
<th>Sample Position</th>
<th>Acq Method</th>
<th>DA Method</th>
<th>Data File</th>
<th>Sample Type</th>
<th>Inj Vol (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>suite_mix1</td>
<td>VeA1</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data1</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>2</td>
<td>suite_mix2</td>
<td>P1-A1</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data2</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>3</td>
<td>suite_mix3</td>
<td>P1-A2</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data3</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>suite_mix4</td>
<td>P1-A3</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data4</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>suite_mix5</td>
<td>P1-A4</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data5</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>6</td>
<td>suite_mix6</td>
<td>P1-B1</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data6</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>7</td>
<td>suite_mix7</td>
<td>P1-B2</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data7</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>suite_mix8</td>
<td>P1-B3</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data8</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>9</td>
<td>suite_mix9</td>
<td>P1-B4</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data9</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>suite_mix10</td>
<td>P1-C1</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data10</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>11</td>
<td>suite_mix11</td>
<td>P1-C2</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data11</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>12</td>
<td>suite_mix12</td>
<td>P1-C3</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data12</td>
<td>well</td>
<td>Unknown</td>
</tr>
<tr>
<td>13</td>
<td>suite_mix13</td>
<td>P1-C4</td>
<td>APOautotune.m</td>
<td>sn_base_peak.xml</td>
<td>Batch1.data13</td>
<td>well</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Worklist Manager Scripts</th>
<th>Acquisition Manager Scripts</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCCondition</td>
<td>InstrumentStandby</td>
</tr>
<tr>
<td>AcquireCalibrantData</td>
<td>LoadIdleMethod</td>
</tr>
<tr>
<td></td>
<td>PumpsAllOff</td>
</tr>
<tr>
<td></td>
<td>MSCalibValveOff</td>
</tr>
<tr>
<td></td>
<td>PumpAllAndMsCalibValveOff</td>
</tr>
</tbody>
</table>
Entry of additional sample information (show, add columns)

The default worklist contains only eight 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

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

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.
4 Data Acquisition

**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 48** Worklist with some hidden columns shown

**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.

CSV file mapping

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

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.

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.
Data Acquisition

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

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acq Method</th>
<th>MyData</th>
<th>DA</th>
<th>SampPos</th>
<th>Sample Type</th>
<th>Internal Std A</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
<td>method1</td>
<td>qwwq</td>
<td>method1</td>
<td>1</td>
<td>Standard</td>
<td>1</td>
</tr>
<tr>
<td>BBB</td>
<td>method2</td>
<td>bbb</td>
<td>method1</td>
<td>2</td>
<td>Sample</td>
<td>1</td>
</tr>
<tr>
<td>CCC</td>
<td>method3</td>
<td>ccc</td>
<td>method1</td>
<td>3</td>
<td>QualControl</td>
<td>1</td>
</tr>
</tbody>
</table>
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**

<table>
<thead>
<tr>
<th>[Static Mapping]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acq Method</td>
<td>Acquisition Method</td>
</tr>
<tr>
<td>MyData</td>
<td>DataFile Name</td>
</tr>
<tr>
<td>DA</td>
<td>DataAnalysis Method</td>
</tr>
<tr>
<td>SampPos</td>
<td>Sample Position</td>
</tr>
<tr>
<td>Sample</td>
<td>Sample Name</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[Dynamic Mapping]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(++Added column)</td>
<td></td>
</tr>
<tr>
<td>InternalStdA</td>
<td>Compound 1</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Compound</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[Data Value Mapping]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Unknown</td>
</tr>
<tr>
<td>QualControl</td>
<td>QC</td>
</tr>
</tbody>
</table>

Another example to add columns for Empirical Formula Confirmation is the following dynamic mapping section which specifies C₆H₆N₃O as the default formula:

```
[Dynamic Mapping]
Formula1 Custom Parameter C6H6N3O EFC
```
Worklist to confirm empirical formulas

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, etc.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.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample Position</th>
<th>Acq Method</th>
<th>DA Method</th>
<th>Data File</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>suite_mix1</td>
<td>Vial1</td>
<td>default</td>
<td>etc.anm</td>
<td>data1.wiff</td>
<td>C12H14N4O2S</td>
</tr>
<tr>
<td>suite_mix2</td>
<td>P/4-A1</td>
<td>default</td>
<td>etc.anm</td>
<td>data2.wiff</td>
<td>C2H5ON2</td>
</tr>
<tr>
<td>suite_mix3</td>
<td>P/4-A2</td>
<td>default</td>
<td>etc.anm</td>
<td>data3.wiff</td>
<td>C12H14N4O2S</td>
</tr>
<tr>
<td>suite_mix4</td>
<td>P/4-A3</td>
<td>default</td>
<td>etc.anm</td>
<td>data4.wiff</td>
<td>C12H14N4O2S</td>
</tr>
</tbody>
</table>

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 etc.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.

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.

<table>
<thead>
<tr>
<th>P</th>
<th>Sample Name</th>
<th>Sample Position</th>
<th>Acq Method</th>
<th>DA Method</th>
<th>Data File</th>
<th>Sample Type</th>
<th>Inj Vol (µl)</th>
<th>Comment</th>
<th>Fragmentor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reserpine 100µg</td>
<td>P1.A2</td>
<td>isoelectric 100µg/m</td>
<td>isoelectric 100µg, m</td>
<td>Reserpine05.wff</td>
<td>Unknown</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Reserpine 100µg</td>
<td>P1.A2</td>
<td>isoelectric 100µg/m</td>
<td>isoelectric 100µg, m</td>
<td>Reserpine05.wff</td>
<td>Unknown</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Reserpine 100µg</td>
<td>P1.A2</td>
<td>isoelectric 100µg/m</td>
<td>isoelectric 100µg, m</td>
<td>Reserpine05.wff</td>
<td>Unknown</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Reserpine 100µg</td>
<td>P1.A2</td>
<td>isoelectric 100µg/m</td>
<td>isoelectric 100µg, m</td>
<td>Reserpine05.wff</td>
<td>Unknown</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Reserpine 100µg</td>
<td>P1.A2</td>
<td>isoelectric 100µg/m</td>
<td>isoelectric 100µg, m</td>
<td>Reserpine05.wff</td>
<td>Unknown</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Figure 53  Worklist for parameter optimization
4 Data Acquisition

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](image-url)
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
Data Acquisition

- 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.

\[\text{Table 16  Number of data points for given mass ranges}\]

<table>
<thead>
<tr>
<th>Mass range (Daltons)</th>
<th>Number of data points</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 to 750</td>
<td>50,000</td>
</tr>
<tr>
<td>50 to 1000</td>
<td>60,000</td>
</tr>
<tr>
<td>50 to 3000</td>
<td>100,000</td>
</tr>
<tr>
<td>250 to 1000</td>
<td>30,000</td>
</tr>
<tr>
<td>500 to 3000</td>
<td>60,000</td>
</tr>
<tr>
<td>250 to 3000</td>
<td>70,000</td>
</tr>
</tbody>
</table>

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.

Figure 56  Worklist with tracking on

Monitoring real-time plot

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.

For more information on real-time plots, see “Real-time displays” on page 49.
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
Data Acquisition
5
Data Analysis

Result review in Analyst  108
   How results are organized  108
   What you can view in Analyst  109
   Agilent peak finder parameters  113
   Recalibration in Analyst  114
Agilent TOF reports  116
   Data analysis reports  116
   TOF acquisition parameter optimization reports  123
   Signal-to-Noise Ratio Report  125
   Exporting spectra to text file for custom reporting  126

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.

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.
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.

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).

![Figure 60 TIC and TWC displays in Analyst with shortcut menus](image)
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

![File Information for Sample 1 (Test Sample) of esdemo.wiff](image)

**Log Information:**
- Column Oven: Agilent 1100 G1316A 0
- Left Column Tag Information: Not Available
- Column Oven: Agilent 1100 G1316A 0
- Right Column Tag Information: Not Available

**Acquisition Info:**
- Acquisition Method: N/A
- Acquisition Time: Monday, May 12, 2003, 11:30:29 AM
- Duration: 0.000 sec
- Number Of Scans Acquired: 361
- Periods In File: 1
- Synchronization Mode: No Sync
- Auto-Equilibration: Off
- Comment: Sample Name: Test Sample
- Sample ID: Comments: Description
- Vial Position: Vial 2
- Rack Code: N/A
- Rack Position: 1

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
5  Data Analysis

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.

![Agilent Peak Finder Parameters dialog box](image)

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.

![TOF Recalibration dialog box for Agilent TOF]

**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.
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.
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.
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.
5 Data Analysis

**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.

![Select DA Operations dialog box](image)

**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.

![Data Analysis Method Editor](image)

**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 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.
5 Data Analysis

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.

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 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.
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.

![Signal-to-Noise Report dialog box](image)

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.

![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.
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