



Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System

Concepts Guide

The Big Picture



Agilent Technologies

Notices

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Software Revision

This guide applies to the Agilent MassHunter Workstation Software – Data Acquisition program for TOF and Q-TOF version B.04.00 or higher until superseded.

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In This Guide...

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

1 Overview

Learn how the Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS 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 Methods with Acquisition Parameters

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

4 Data Acquisition

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

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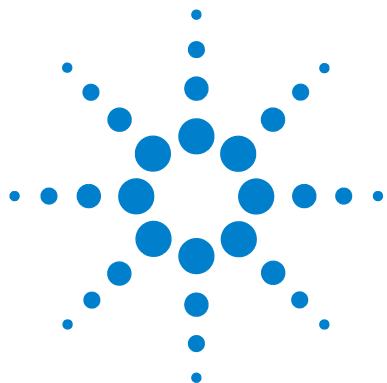
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Overview

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This chapter provides an overview of the Agilent 6200 Series TOF LC/MS and Agilent 6500 Series Q-TOF LC/MS systems and the system components and how they work together to help you get your job done.



How does the TOF and Q-TOF system help you do your job?

You can set up an Agilent 6200 Series Time-of-Flight LC/MS (TOF) system and the Agilent 6500 Series Quadrupole Time-of-Flight LC/MS (Q-TOF) system in several configurations:

ESI – Electrospray Ionization
APCI – Atmospheric Pressure
Chemical Ionization
APPI - Atmospheric Pressure
Photo Ionization
HPLC-Chip/MS – High Performance
Liquid Chromatography on a Chip
MALDI – Matrix-Assisted Laser
Desorption Ionization
MMI - Multimode Ionization

- For normal flow LC/MS with a binary pump, quaternary pump, well-plate sampler (or autosampler or CTC PAL autosampler) and ESI or Dual ESI with Agilent Jet Stream Thermal Gradient Technology - Agilent 6530 Quadrupole Time-of-Flight, Agilent 6540 Quadrupole Time-of-Flight, and Agilent 6230 Time-of-Flight.
- For normal flow LC/MS with a binary pump, quaternary pump, well-plate sampler (or autosampler or CTC PAL autosampler) and ESI, Dual ESI, APCI, APPI, or MMI ion sources.
- For microflow LC/MS with a capillary pump, micro well-plate sampler and ESI, Dual ESI, APCI or MMI ion sources
- For nanoflow LC/MS with a nanopump, micro well-plate sampler and HPLC-Chip/MS interface (used in place of standard or dual nanospray source) to increase reliability and boost performance with narrow peak dispersion and lower dead volumes.
- Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS system with an AP-MALDI or PDF-MALDI source

Each Agilent system has advantages for high throughput sample screening with highly sensitive detection and accurate mass assignment. Each uses the same Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS software to enable these advantages.

The Agilent Accurate-Mass 6530 Q-TOF and the Agilent UHD Accurate-Mass 6540 Q-TOF LC/MS systems are the only Q-TOF instruments that can use the Agilent Jet Stream Technology. The Agilent 6230 TOF LC/MS system is the only TOF that can use the Agilent Jet Stream Technology. This technology utilizes a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

Help for applications

You can use one or more of the Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS systems in the following application areas (for example):

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

Paired with Agilent's 1200 Series LCs, the 6500 Series Q-TOF LC/MS delivers fast, sensitive, reproducible analyses of small and large molecules.

- Reproducible mass accuracy
- Low picogram and femtomole limits of detection
- Fast MS/MS operation (for the Q-TOF)

Help for data acquisition

Please refer to this guide, the *Agilent MassHunter Workstation Software - Data Acquisition Familiarization Guide*, the *Agilent MassHunter Workstation Software - Data Acquisition Quick Start Guide* or the online Help for the Data Acquisition program and the online Help or the Report Designer User Information DVD for the Report Designer Add-in. The Report Designer Add-in allows you to customize the templates that are used when you print a report.

To help you use the Agilent 6200 Series TOF and the 6500 Series Q-TOF LC/MS systems for these applications, the software lets you perform the following tasks in a single window:

To learn how to get started with the Agilent 6200 Series TOF and 6500 Series Q-TOF, see the Quick Start Guide.

To learn more about how to use the Agilent 6200 Series TOF and 6500 Series Q-TOF with real samples and data, see the Familiarization Guide.

To learn how to perform individual tasks with the TOF and Q-TOF LC/MS, see the online Help. Press F1 to access the online Help.

To learn more about an Agilent 1100 or 1200 LC module or 1260 or 1290 Infinity LC module, see the Agilent 1100 LC, 1200 LC, 1260 Infinity LC or 1290 Infinity LC User Guide for the module.

To learn more about the 6200 Series TOF or the 6500 Series Q-TOF, see the Maintenance Guide for that instrument.

To learn how to install the system, see the Installation Guide.

Prepare the instrument

- Start and stop the instruments from the software.
- Download settings to the Agilent 1200, 1260 Infinity or 1290 Infinity liquid chromatograph and the TOF and Q-TOF mass spectrometer in real time to control the instrument.
- See if the 6200 Series TOF and 6500 Series Q-TOF parameters are within the limits to produce the specified mass accuracy and resolution with a Checktune, Quicktune or Standard tune report.
- Optimize TOF and Q-TOF parameters automatically or manually through Agilent tuning programs.
- Monitor the actual conditions of the instrument.
- View the Real-time Plot for chromatograms, spectra, and instrument parameters (both DAD, TOF and Q-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 data acquisition methods

- Enter and save parameter values for all LC modules and the Agilent 6200 Series TOF and 6500 Series Q-TOF to a data 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 experiments within the time segment.
- Print an acquisition method report.

Acquire data

A *worklist* is a list of a sequence of samples that you enter and run automatically with the Data Acquisition program.

- Enter sample information and pre- or post-analysis programs (scripts) and run single samples interactively
- Enter and automatically run both individual samples and sequences of samples in a *worklist*
- Set up pre- and post-analysis scripts to run between samples in a worklist.
- Set up and run a worklist.

Help for data analysis

Agilent MassHunter Workstation Software - Qualitative Analysis

Qualitative Analysis is the primary program you use to work with your TOF and Q-TOF data. You can use the Find Compounds capability to confirm the presence of known compounds with targeted MS/MS data, identify unknowns with auto MS/MS data and do molecular feature extraction with MS data. You can also search the data file for the presence of specified formulas.

Agilent also designed the Qualitative Analysis program to present large amounts of data for review in one central location. With the program you can perform these operations for any type of mass spectrometer data that you open:

- Extract and display chromatograms.
- View and extract peak spectra.
- Subtract background.
- Integrate chromatograms.

You can also set up methods to automatically do the tasks in the list, as well as others, when you open the data files. The software also provides preconfigured templates for basic reporting and enables the capability to create custom reports in Microsoft Excel.

Please refer to the *Agilent MassHunter Workstation Software - Qualitative Analysis Familiarization Guide* or the online Help for the Qualitative Analysis software and the online Help or Reporting Training DVD for the Report Designer Add-in. The Report Designer Add-in allows you to customize the templates that are used when you print a report.

You can also purchase the Agilent MassHunter BioConfirm Program which allows you to view the digest list for the protein sequences currently displayed. You can also compare a reference data file to other data files using the *Comparative Analysis* program which is available with BioConfirm. Please refer to the Agilent MassHunter BioConfirm Quick Start Guide or the Agilent MassHunter BioConfirm Familiarization Guide or the online Help for the Qualitative Analysis program for more information.

Agilent MassHunter Workstation Software - Quantitative Analysis

Agilent also provides you with the opportunity to quantitate your data.

Agilent has designed the quantitative analysis software to help quantitate very low amounts of material with the following unique features:

- Provides a curve-fit assistant to test all fits and statistics on curve quality
- Integrates with an automated, parameter-free integrator that uses a novel algorithm
- Presents a Batch-at-a-Glance results window to help you review and operate on an entire batch of data at once
- Automatically detects and identifies outliers

Please refer to the *Agilent MassHunter Workstation Quantitative Analysis Software Familiarization Guide* or the online Help for the Quantitative Analysis software. You can access the Familiarization Guide directly from the on

For the Report Designer Add-in, please refer to the online Help or Reporting Training DVD. The Report Designer Add-in allows you to customize the templates that are used when you print a report.

How do different ion sources work?

The Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS systems operate with the following interchangeable atmospheric pressure ionization (API) sources:

- [“Electrospray ionization \(ESI\) and Dual ESI”](#) on page 17
- [“Dual Agilent Jet Stream Electrospray Ionization \(Dual AJS ESI\)”](#) on page 21
- [“Atmospheric pressure chemical ionization \(APCI\)”](#) on page 22
- [“Atmospheric pressure photoionization \(APPI\)”](#) on page 24
- [“Multimode ionization \(MMI\)”](#) on page 25
- [“HPLC-Chip”](#) on page 27

NOTE

The sources that are used are the B-type sources.

Electrospray ionization (ESI) and Dual ESI

You control the spray chamber parameters (nebulizer pressure, drying gas flow and temperature, and capillary voltage) when you set up a method in the Method and Run Control view, described in Chapter 4.

Electrospray ionization relies in part on chemistry to generate analyte ions in solution before the analyte reaches the mass spectrometer. As shown in Figure 1, the LC eluent is sprayed (nebulized) into a spray chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field occurs between the nebulizer, which is at ground in the Agilent design, and the capillary, which is at high voltage.

The spray occurs at right angles to the capillary. This design reduces background noise from droplets, increases sensitivity, and keeps the capillary cleaner for a longer period of time.

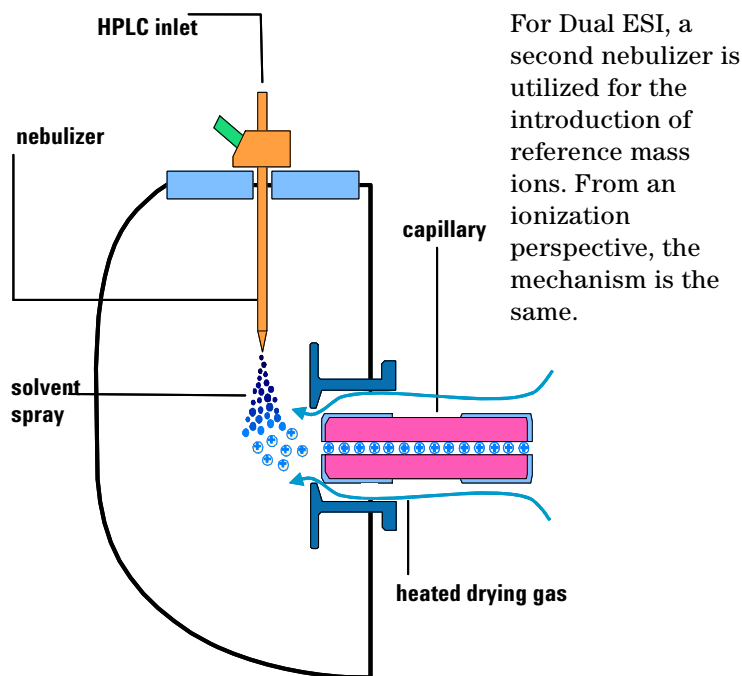


Figure 1 Electrospray ion source

Electrospray ionization (ESI) consists of four steps:

- 1 Formation of ions
- 2 Nebulization
- 3 Desolvation
- 4 Ion evaporation

Formation of ions

Ion formation in API-electrospray occurs through more than one mechanism. If the chemistry of analyte, solvents, and buffers is correct, ions are generated in solution before nebulization. This results in high analyte ion concentration and good API-electrospray sensitivity.

Preformed ions are not always required for ESI. Some compounds that do not ionize in solution can still be analyzed. The process of nebulization, desolvation, and ion evaporation creates a strong electrical charge on the surface of the spray droplets. This can induce ionization in analyte molecules at the surface of the droplets.

Nebulization

Nebulization (aerosol generation) takes the sample solution through these steps:

- a Sample solution enters the spray chamber through a grounded needle called a nebulizer.
- b For high-flow electrospray, nebulizing gas enters the spray chamber concentrically through a tube that surrounds the needle.
- c The combination of strong shear forces generated by the nebulizing gas and the strong voltage (2–6 kV) in the spray chamber draws out the sample solution and breaks it into droplets.
- d As the droplets disperse, ions of one polarity preferentially migrate to the droplet surface due to electrostatic forces.
- e As a result, the sample is simultaneously charged and dispersed into a fine spray of charged droplets, hence the name *electrospray*.

Because the sample solution is not heated when the aerosol is created, ESI does not thermally decompose most analytes.

Desolvation and ion evaporation

Before the ions can be mass analyzed, solvent must be removed to yield a bare ion.

A counter-current of neutral, heated drying gas, typically nitrogen, evaporates the solvent, decreasing the droplet diameter and forcing the predominantly like surface-charges closer together (see Figure 2).

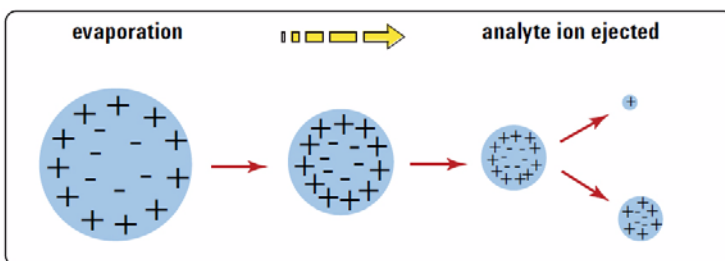


Figure 2 Desorption of ions from solution

Coulomb repulsion – repulsion
between charged species of the
same sign

When the force of the Coulomb repulsion equals that of the surface tension of the droplet, the droplet explodes, producing smaller charged droplets that are subject to further evaporation. This process repeats itself, and droplets with a high density of surface-charges are formed. When charge density reaches approximately 10^8 V/cm^3 , ion evaporation occurs (direct ejection of bare ions from the droplet surface). These ions are attracted to and pass through a capillary sampling orifice into the ion optics and mass analyzer.

The importance of solution chemistry

The choice of solvents and buffers is a key to successful ionization with electrospray. Solvents like methanol that have lower heat capacity, surface tension, and dielectric constant, promote nebulization and desolvation. For best results in electrospray mode:

1 Overview

Electrospray ionization (ESI) and Dual ESI

- Adjust solvent pH according to the polarity of ions desired and the pH of the sample.
- To enhance ion desorption, use solvents that have low heats of vaporization and low surface tensions.
- Select solvents that do *not* neutralize ions through gas-phase reactions such as proton transfer or ion pair reactions.
- To reduce the buildup of salts in the ion source, select more volatile buffers.

Multiple charging

Electrospray is especially useful for analyzing large biomolecules such as proteins, peptides, and oligonucleotides, but can also analyze smaller molecules like drugs and environmental contaminants. Large molecules often acquire more than one charge. Because of this multiple charging, you can use electrospray to analyze molecules as large as 150,000 u even though the mass range (or more accurately mass-to-charge range) for a typical quadrupole LC/MS instrument is around 3000 m/z . For example:

$$100,000 \text{ u} / 10 z = 1,000 \text{ } m/z$$

The optional Agilent MassHunter BioConfirm Software performs the calculations to accomplish deconvolution.

When a large molecule acquires many charges, a mathematical process called deconvolution is used to determine the actual molecular weight of the analyte.

Dual Agilent Jet Stream Electrospray Ionization (Dual AJS ESI)

With the Dual AJS ESI source, the nebulizing gas for the reference spray can be switched for high flow or low flow applications. The second sprayer improves the reference mass stability over a wide range of LC conditions. Low flow applications are typically less than 200 $\mu\text{L}/\text{minute}$. If the flow is approximately 200 $\mu\text{L}/\text{minute}$, either low or high flow may be appropriate.



You connect the tubing here for high flow applications.

Figure 3 Dual AJS ESI source plumbed for high flow application

Both the Dual AJS ESI and the Dual ESI source support two nebulizers for different applications: the standard nebulizer (G1958-60098) and the capillary (or microflow) LC/MS nebulizer (G1946-60260). The main differences are:

- The machined tip of the capillary LC/MS nebulizer has a smaller exit orifice and internal taper.
- The internal diameter (ID) of the internal needle for the capillary LC/MS nebulizer is 50 μm versus 120 μm for the “standard” nebulizer needle.

The recommended flow range (1 to 50 $\mu\text{L}/\text{minute}$) really is capillary flow, whereas flows from 50 to 250 $\mu\text{L}/\text{minute}$ are typically described as microflow.

Atmospheric pressure chemical ionization (APCI)

APCI is a gas-phase chemical ionization process. The APCI technique passes LC eluent through a nebulizing needle, which creates a fine spray. The spray is passed through a heated ceramic tube, where the droplets are fully vaporized (Figure 4).

The resulting gas/vapor mixture is then passed over a corona discharge needle, where the solvent vapor is ionized to create reagent gas ions. These ions in turn ionize the sample molecules via a chemical ionization process. The sample ions are then introduced into the capillary.

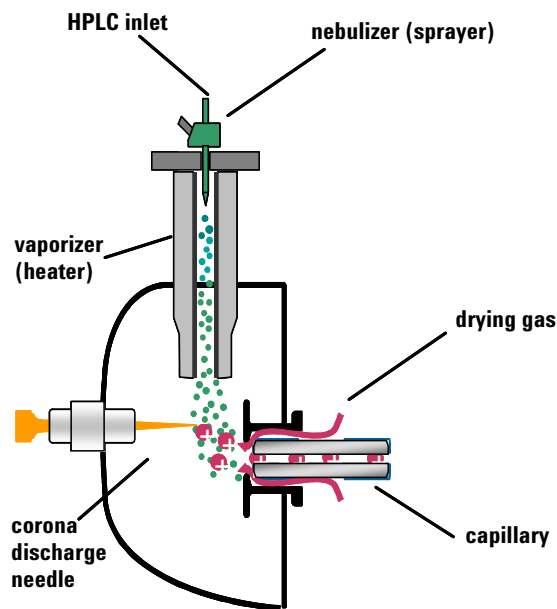


Figure 4 Atmospheric pressure chemical ionization (APCI) source

APCI requires that the analyte be in the gas phase for ionization to occur. To vaporize the solvent and analyte, the APCI source is typically operated at vaporizer temperatures of 400 to 500°C.

Atmospheric pressure chemical ionization (APCI)

APCI is applicable across a wide range of molecular polarities. It rarely results in multiple charging, so it is typically used for molecules less than 1,500 u. Because of this molecular weight limitation and use of high-temperature vaporization, APCI is less well-suited than electrospray for analysis of large biomolecules that may be thermally unstable. APCI is well suited for ionization of the less polar compounds that are typically analyzed by normal-phase chromatography.

Atmospheric pressure photoionization (APPI)

With the APPI technique, LC eluent passes through a nebulizing needle to create a fine spray. This spray is passed through a heated ceramic tube, where the droplets are fully vaporized. The resulting gas/vapor mixture passes through the photon beam of a krypton lamp to ionize the sample molecules (Figure 5). The sample ions are then introduced into the capillary.

APPI and APCI are similar, with APPI substituting a lamp for the corona needle for ionization. APPI often also uses an additional solvent or mobile phase modifier, called a “dopant”, to assist with the photoionization process.

APPI is applicable to many of the same compounds that are typically analyzed by APCI. APPI has proven particularly valuable for analysis of nonpolar compounds.

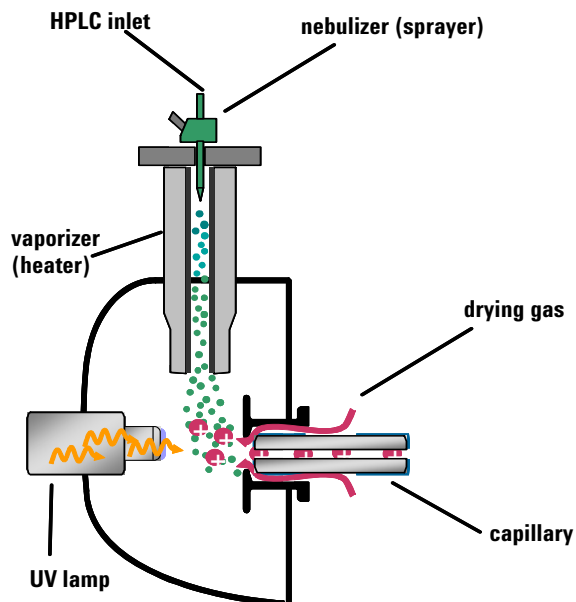


Figure 5 Atmospheric pressure photoionization (APPI) source

Multimode ionization (MMI)

The multimode source is an ion source that can operate in three different modes: APCI, ESI or simultaneous APCI/ESI. The multimode source incorporates two electrically separated, optimized zones: one for ESI and one for APCI. During simultaneous APCI/ESI, ions from both ionization modes enter the capillary and are analyzed simultaneously by the mass spectrometer.

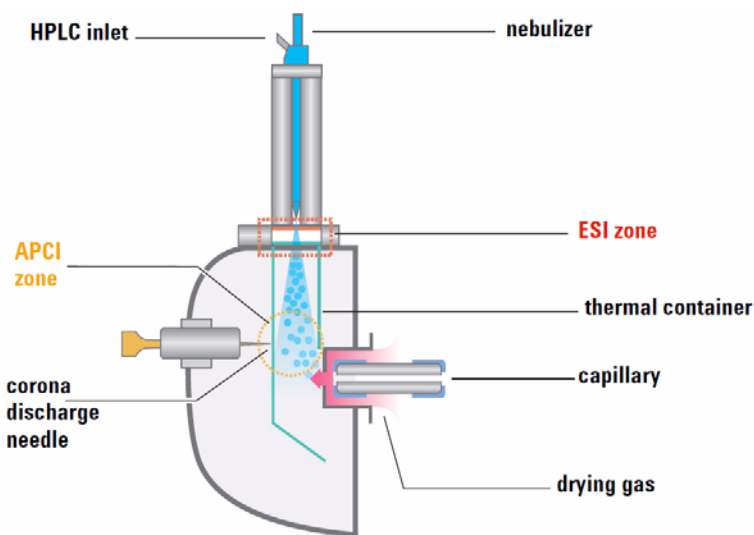


Figure 6 Multimode source

ESI and APCI are essentially incompatible processes because each needs its own conditions for aerosol drying and electrical fields. However, it is possible to form ions simultaneously from ESI and APCI if the two ionization regions are separated in space.

Multimode ionization (MMI) is useful for screening of unknowns, or whenever samples contain a mixture of compounds where some respond by ESI and some respond by APCI. In these cases, the multimode source obviates the need to run the samples twice to accomplish a complete analysis.

1 Overview

Multimode ionization (MMI)

Unlike the APCI and APPI sources where the temperature of the vaporizer is monitored, in the multimode source the actual vapor temperature is monitored. As a result, the vaporizer is typically set to between 200 and 250°C.

HPLC-Chip

Traditional nanospray mass spectrometry has proven its potential as a cost-effective, sensitive and reproducible technique for the identification of peptides at femtomole to attomole levels. However, connecting nano capillaries, columns and valves frequently is a tedious procedure and requires user skills and routine. When handled incorrectly, nano flow connections are prone to leakage which are difficult to detect and result in poor system performance and extended downtime of the complete system. Quartz nanospray needles are prone to blockages and require frequent replacement.

With the invention of HPLC-Chip technology, Agilent is significantly reducing the need for user interaction and making nanospray a rugged state-of-the-art technology.

The cornerstone of chip technology is the HPLC-Chip, a 3-dimensional structure made of sandwiched polyimide films. Grooves of specific dimensions are laser-ablated into a layer of polyimide film. The lamination of a top and bottom layer then forms channels of trapezoidal or triangular shape inside the chip which can either be used as capillaries or, if filled with packing material, as nanocolumns. One end of the chip tapers off into a polyimide nanospray emitter.

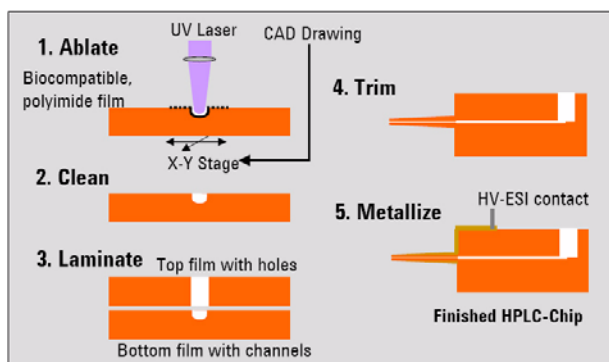


Figure 7 HPLC-Chip making process

1 Overview

How does the Agilent TOF and Q-TOF mass spectrometer work?

How does the Agilent TOF and Q-TOF mass spectrometer work?

6200 Series TOF

The Agilent TOF is an orthogonal acceleration time-of-flight mass spectrometer (oa-TOF). The acceleration pulse applied to send the ions down the flight tube is orthogonal to the direction that ions are entering the mass analyzer. This geometry minimizes the effect of the entrance velocity on the flight time, leading to higher resolution.

6500 Series Q-TOF

The Agilent 6500 Series Q-TOF LC/MS is a liquid chromatograph Q-TOF mass spectrometer that performs MS/MS using a quadrupole, a hexapole (collision cell) and a time-of-flight unit to produce spectra. The quadrupole selects precursor ions that are fragmented in the collision cell into product ions, which are then impelled to the detector, at an angle perpendicular to the original path.

The Agilent 6500 Series Q-TOF LC/MS supports several atmospheric pressure ionization (API) sources. A common atmospheric sampling interface introduces ions from these various sources into the mass spectrometer vacuum system.

Figure 8 shows the complete Agilent 6520 Q-TOF LC/MS schematic, including ion source, ion transfer optics, beam shaping optics, ion pulser, flight tube, and detector.

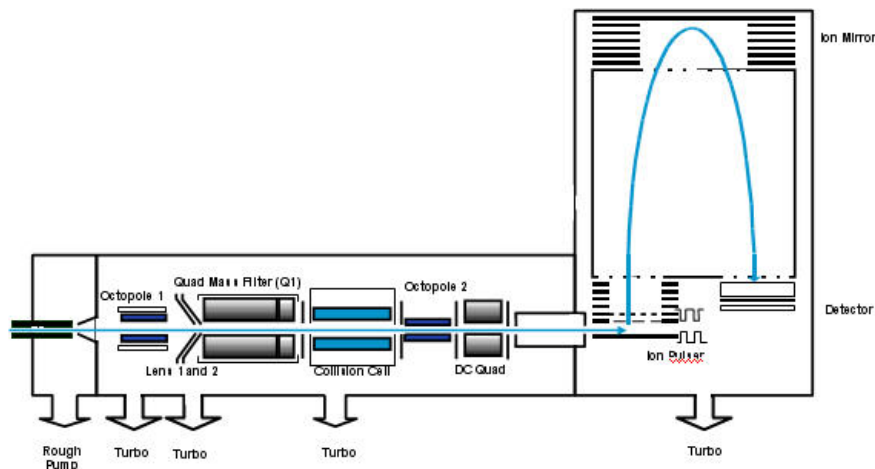


Figure 8 Schematic of Agilent 6520 Q-TOF LC/MS

How does the Agilent TOF and Q-TOF mass spectrometer work?

Figure 9 shows the complete Agilent 6530 Q-TOF schematic, with major improvements identified.

These improvements are described below
 ("Innovative Enhancements in the 6530 Q-TOF" on page 32).

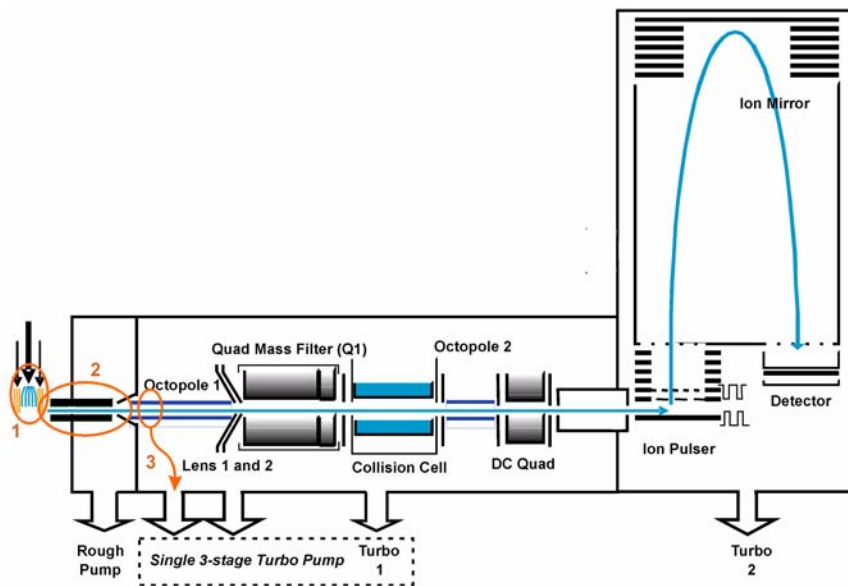


Figure 9 Schematic of Agilent 6530 Q-TOF LC/MS with major improvements circled

Innovative Enhancements in the 6540 and 6538 Q-TOF

These improvements are described below (“Innovative Enhancements in the 6540 and 6538 Q-TOF” on page 30).

The Agilent 6540 Q-TOF supports the Agilent Jet Stream Technology. The Agilent 6538 does not.

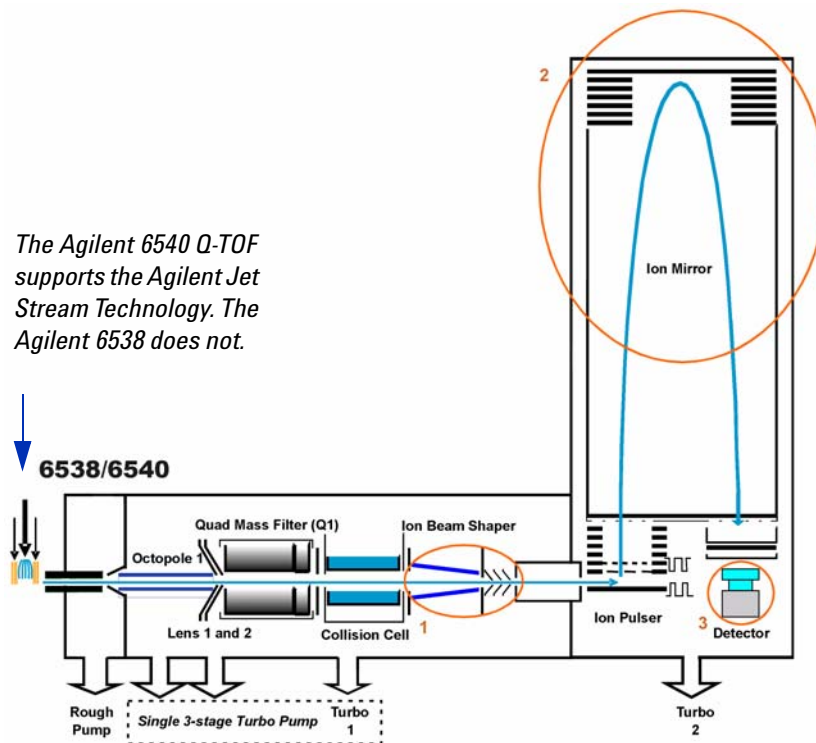


Figure 10 Schematic of Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS with major improvements circled

Innovative Enhancements in the 6540 and 6538 Q-TOF

The first improvement is the Ion Beam Compression Technology (IBC) which cools and focuses the ion beam. This technology simultaneously maximizes ion transmission and reduces beam divergence. Active Ion Beam Compression is achieved with Agilent's Axial Ion Acceleration Technology applied to a tapered ion guide design.

Ion beam compression provides up to a 10-fold compression and cooling which helps in creating a much denser and thinner ion beam that passes through a narrower slit leading into the slicer

and pulser region. The narrowed, cooled and condensed beam is a key factor in enabling the gain in mass resolution to 40,000 while maintaining excellent sensitivity.

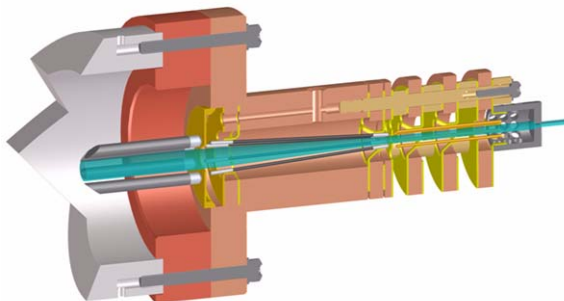


Figure 11 Ion Beam Compression Technology

Extended Flight Tube with Enhanced Mirror Technology (EMT)

The second improvement is that the flight tube for the 6538/6540 Q-TOF is now five feet long.

The 1 ppm/C Expansion Coefficient for the Inner Flight Tube virtually eliminates calibration drift due to flight tube elongation. The second order temporal focusing ion mirror uses a high transmission Harp Grid for maximum sensitivity.

New Fast Bipolar Detector

The third innovative enhancement is the new Fast Bipolar detector. The scintillator is ultra fast and highly efficient. Also, the new ultra fast response PMT design continues the tradition of high dynamic range and detector lifetime.



Figure 12 Fast Bipolar Detector

Innovative Enhancements in the 6530 Q-TOF

Ions are generated using an electrospray ion source where the analyte is simultaneously ionized and desolvated from the liquid matrix. The first of three (3) innovative Agilent enhancements is found in the application of Agilent Jet Stream Technology (denoted as 1 in [Figure 9](#)) which improves sensitivity via thermal gradient focusing and enhanced desolvation. This technology is described in detail below ([“Agilent Jet Stream Thermal Gradient Technology”](#) on page 33).

The desolvated ions then enter the mass spectrometer via an innovative resistive and highly inert capillary transfer tube (denoted as 2 in [Figure 9](#)) that improves ion transmission and allows virtually instantaneous polarity switching.

Further increase in ion transmission is obtained by improvement of the pumping speed in vacuum stage 2, resulting in better ion capture by the first octopole (denoted as 3 in [Figure 9](#)). The ions next pass through the optics and into the quadrupole analyzer. The quadrupole analyzer consists of four parallel hyperbolic rods through which selected ions based on their mass to charge ratio are filtered.

The ions passing through the quadrupole analyzer are then directed through the collision cell where they are fragmented. The collision cell is actually a hexapole filled with nitrogen, the same gas that is used as the drying gas. The collision cell design has axial acceleration for high speed MS/MS analysis. Fragment ions formed in the collision cell are then sent to the TOF to enable a user to isolate and examine product ions with respect to precursor ions.

Agilent Jet Stream Thermal Gradient Technology

This technology is supported on the Agilent 6530 Quadrupole Time-of-Flight mass spectrometer, the Agilent 6540 UHD Accurate-Mass Quadrupole Time-of-Flight mass spectrometer and the Agilent 6230 Accurate-Mass Time-of-Flight mass spectrometer.

Agilent Jet Stream Technology enhances analyte desolvation by collimating the nebulizer spray and creating a dramatically “brighter signal.” The addition of a collinear, concentric, super-heated nitrogen sheath gas ([Figure 13](#)) to the inlet assembly significantly improves ion drying from the electrospray plume and leads to increased mass spectrometer signal to noise. The Agilent 6530 Q-TOF gets attomole-to-low-femtomole sensitivity for superior trace level analyses.

1 Overview

Agilent Jet Stream Thermal Gradient Technology

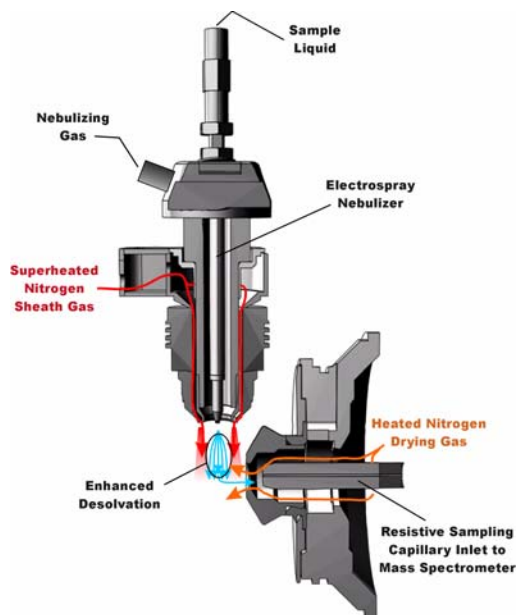


Figure 13 Agilent Jet Stream Electro spray Ion Source

Agilent Jet Stream thermal gradient focusing consists of a superheated nitrogen sheath gas that is introduced collinear with and concentric to the pneumatically assisted electrospray. Thermal energy from the superheated nitrogen sheath gas is focused to the nebulizer spray producing the most efficient desolvation and ion generation possible. The enhanced desolvation results in more ions entering the sampling capillary as shown in [Figure 13](#) and concomitant improved signal to noise. Parameters for the Agilent Jet Stream Technology are the superheated nitrogen sheath gas temperature and flow rate, and the nozzle voltage.

The capillary is a resistive capillary that improves ion transmission and allows virtually instantaneous polarity switching.

Front-end ion optics

For information on the various ion sources, see “[How do different ion sources work?](#)” on page 16

After the API source forms ions, the Agilent 6200 Series TOF or 6500 Series Q-TOF LC/MS system performs the following operations, organized according to the stages of the ion path and the vacuum stages of the TOF or Q-TOF. See [Figure 8](#) on page 28 for details.

Ion enrichment (Vacuum stage 1)

Ions produced in an API source are electrostatically drawn through a drying gas and then pneumatically conducted through a heated sampling capillary into the first stage of the vacuum system. The majority of drying gas and solvent vapor are 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 (Vacuum stage 2 and vacuum stage 3)

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 an *octopole ion guide*. Radio frequency voltage applied to the parallel octopole rods repel ions above a particular mass range toward the center of the rod set. The ions pass through the octopole ion guide because of the momentum obtained from being drawn from atmospheric pressure through the sampling capillary.

In a Q-TOF and in the Agilent G6224 and G6230 TOF, the octopole spans both the 2nd and the 3rd vacuum stages. Ions exit the octopole and pass through two focussing lenses and an RF lens.

In an Agilent 6220 TOF, the ions exit the first ion guide and pass into the third stage of the vacuum system. In the third stage of the vacuum, the ions are passed onto a second octopole assembly (octopole 2) which then sends the ion on to the beam shaping assembly.

Ion transport 2 (Vacuum stage 4 for 6220 TOF only)

In this fourth vacuum pumping stage, the pressure is now low enough that there are few collisions of the ions with gas molecules.

NOTE

The following sections are only part of the Q-TOF LC/MS instrument. The next section in the TOF instrument is the [Beam shaping \(Vacuum stage 4 for both 6220 TOF and Q-TOF\)](#) on [page 37](#).

Ion selection (Vacuum stage 4 for Q-TOF only)

Lens 2 RF	The phase of lens 2 RF is matched to that of the subsequent quadrupole resulting in a significantly increased sensitivity.
Quad mass filters	The quadrupoles consist of hyperbolic rods that optimize ion transmission and spectral resolution. There tends to be more ion loss with circular rods.
Pre-filter	The end section of the quadrupole also consists of short hyperbolic rods, but their RF voltages are only high enough to guide ions into the collision cell.

Ion fragmentation 2 (Vacuum stage 4 for Q-TOF only)

Ions selected by the quadrupole are then passed to the collision cell where they are fragmented.

The axial acceleration collision cell is a high pressure hexapole assembly with its axial acceleration adjusted to maximize sensitivity while eliminating crosstalk.

Crosstalk occurs when product ions from a previously selected precursor appear in a product ion spectrum of a subsequently selected precursor because of slow clearance from the collision cell. This creates a composite product ion spectrum which can be difficult to interpret.

The components that contribute to this higher sensitivity and faster response are

- Small diameter hexapole collision cell
- High frequency hexapole collision cell

- Linear axial acceleration
- High pressure collision cell
- High speed digital electronics

The collision cell contains nitrogen, the same gas that is used in the ion source. The small diameter of the hexapole assembly assists in capturing fragmented ions.

Why a hexapole? The geometry of a hexapole provides advantages in two domains: ion focusing and ion transmission.

- The first advantage is in ion focusing where a quadrupole is better than a hexapole, which is better than an octopole, that is, quadrupole > hexapole > octopole.
- The second advantage involves ion transmission across a wide mass range, or m/z bandwidth. In this case, the octopole is better than the hexapole, which is better than the quadrupole.

The hexapole is chosen because, overall, it is the best for both ion focusing and ion transmission.

Collision cell design The collision cell hexapole consists of six resistively coated rods used to generate a potential difference across the length of the collision cell.

A potential difference is always present. This ensures that the precursor ions coming from the quadrupole or fragment ions generated in the collision cell are transmitted and not allowed to drift around at random.

Sweeping out the ions in this manner avoids the issue of crosstalk where residual product ions from a previous experiment can interfere with the product ion spectrum of a subsequent experiment. A collision energy voltage is applied over the accelerating linear voltage to generate fragments or product ions.

Beam shaping (Vacuum stage 4 for both 6220 TOF and Q-TOF)

In the Agilent 6538 and 6540 UDH Accurate-Mass Q-TOF, ions enter the ion beam compression technology. Ion beam compression provides up to a 10-fold compression and cooling

which helps in creating a much denser and thinner ion beam that passes through a narrower slit leading into the slicer and pulser region.

In the TOF, ions enter a second octopole ion guide of similar design to the first octopole but with a lower direct current potential. This second octopole ion guide accelerates the ions and prepares them for beam shaping. For TOF, the fourth vacuum stage contains Octopole 2 and the beginning of the slicer assembly.

To facilitate beam shaping, lenses focus the ions so that as they pass through the 4th vacuum stage they will 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.

Flight tube/Mass Analyzer (Vacuum stage 4 for 6224/6230 TOF) (Vacuum stage 5 for 6220 TOF and 6500 Series Q-TOF)

- 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 ions 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 (see [Figure 8](#)). 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 orthogonal to the flight direction in the flight tube, they never return to the ion pulser, but move to where the ion detector is mounted.
- The ion mirror increases the resolving power of the instrument by effectively doubling the flight distance (from one meter to two meters) in the same space, and by performing a refocusing

operation so that ions having different initial velocities still arrive simultaneously at the detector.

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 Figure 14 shows a schematic of the Agilent 6500 Series Q-TOF LC/MS detector.

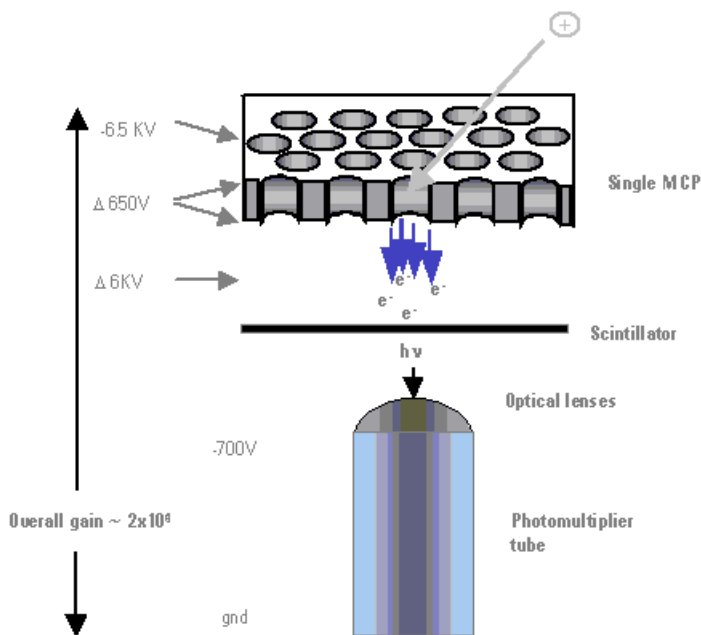
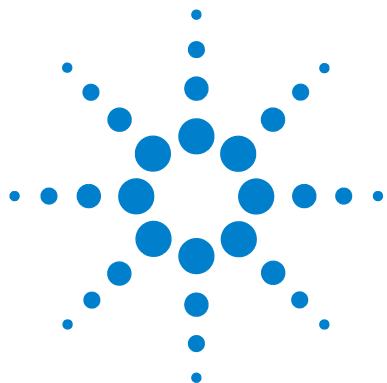


Figure 14 Agilent 6200 Series TOF or 6500 Series Q-TOF detector, with potentials shown for positive operation

At the surface of the ion detector is a microchannel plate (MCP), a very thin plate containing a set of microscopic tubes that pass from the front surface to the rear of the plate. When an ion hits the front surface of the MCP, an electron escapes and begins the process of electrical signal amplification. As freed electrons collide with the walls of the microscopic tubes, an

ever-increasing cascade of electrons travels to the rear of the plate. Roughly 10 times more electrons exit the MCP than incoming ions contact the surface.

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



2 Instrument Preparation

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- TOF and Q-TOF preparation – calibration and tuning [46](#)
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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 6200 Series Time-of-Flight LC/MS System Installation Guide* or the *Agilent 6500 Series Quadrupole Time-of-Flight LC/MS System Installation Guide*.



LC preparation

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

See the *Quick Start Guide* and online *Help* for instructions on how to prepare the LC for a sample run.

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 58.)

You can also view the system logbook for explanations of errors. (See “[System logbook](#)” on page 64.)

LC module setup

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

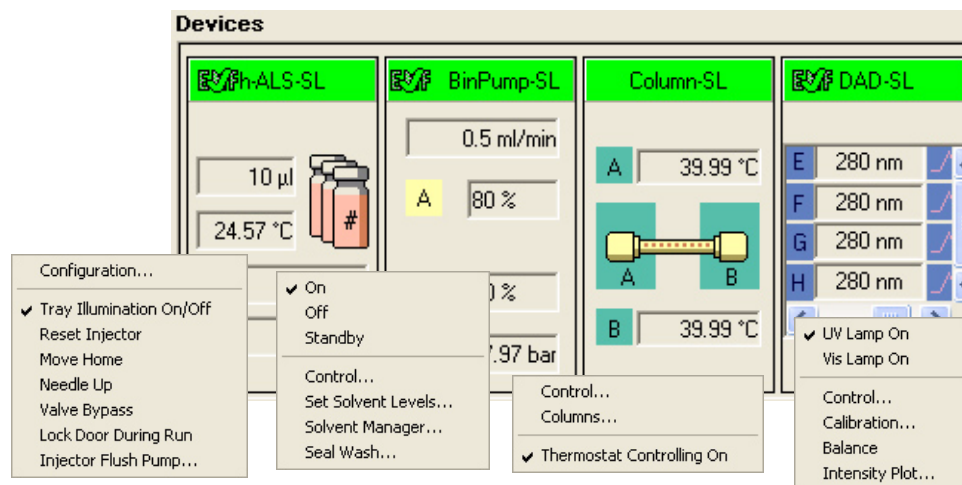


Figure 15 Instrument Status pane with shortcut menus for LC modules

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

Table 1 Tasks to set up the LC modules

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

Table 1 Tasks to set up the LC modules (continued)

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

Column equilibration and conditioning

You can set up to equilibrate or condition a column in different ways with the Data Acquisition program.

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:

- Interactively

You change the loaded method set points 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 set points to the instrument and interactively stop the run when the column is ready.

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

- With a method in an interactive run
 You can save the method with the set points 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 Data Acquisition software. When you enter the script into the worklist, you specify the method that you will use for the run. If a TOF or Q-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 and Q-TOF preparation – calibration and tuning

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

To learn how to tune and calibrate the TOF or Q-TOF, see the Quick Start Guide and online Help.

See “[Tuning choices](#)” on page 48 to learn more about Agilent tuning tools.

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

The following distinctions show how tuning, optimization and calibration are related in the Agilent MassHunter Workstation Software.

Tuning

Tuning is the process of adjusting both the quadrupole (for the Q-TOF) and TOF parameters to achieve the following goals:

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

The Agilent MassHunter Workstation software and its documentation and online Help use the words “tuning” and “optimization” interchangeably.

Agilent Autotune for the Q-TOF include Initial Autotune, Checktune, and Standard Tune for both the quadrupole and TOF parameters.

Agilent Autotune for the TOF include Initial Autotune, Check Tune, Standard Tune and Set Detector Gain for the TOF parameters. The tools for optimizing the TOF parameters also include Quick TOF Tune. All of the TOF Autotune tools perform both automatic calibration and tuning.

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.

TOF 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.

Before you calibrate the instrument, you have to set the instrument state to the proper instrument mode, mass range and fast polarity switching mode. You set these values on the Instrument State tab.

When you change the mass range or enable/disable fast polarity switching on the Instrument State tab, the pulser frequency is changed which results in the DEI pulser warming up or cooling down. If the calibration is performed too soon, the DEI may still be heating up or cooling down which can result in drift. See the online Help for more information on the Instrument State tab.

At startup, you do a full mass calibration, either manually or automatically. Automatic calibrations take place when you select Initial TOF Autotune, TOF Tune or Quick TOF Tune. You set up a manual calibration from the TOF Mass Calibration tab in the Tune pane (Figure 16).

Figure 16 Standard TOF Mass Calibration tab of the Tune pane

See [Chapter 3](#), “Methods with Acquisition Parameters,” starting on page 67, to learn more about mass correction using reference standards.

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

The calibration equations used are proprietary.

Tuning choices

You can see the tuning choices available to you on the Autotune tab ([Figure 17](#)). Notice that you must tune the quadrupole separately from the TOF for the Q-TOF instrument. Also, starting with the B.02.01 release, not all of the tuning choices are available with all sources.

With all instruments with the Dual ESI source, you can run all of the tuning choices, including the Standard Tune algorithm, the Initial Tune algorithm and the Set Detector Gain algorithm.

You can also run all of the tuning choices with the AJS ESI source on the Agilent 6230 UHD Accurate-Mass TOF and the Agilent 6530 UHD Accurate-Mass Q-TOF.

Also, the **Instrument Mode** affects which autotunes are available to use. If the Instrument Mode is **Extended Dynamic Range** mode, then you can perform any of the autotunes. If the Instrument Mode is not **Extended Dynamic Range** mode, then you can only perform Quick Tune and Check Tune. You set the Instrument Mode on the Instrument State tab.

With the ESI, Dual ESI, Dual AJS ESI, Multimode, APPI and APCI ion sources, you can run the Check Tune algorithm, the Quick Tune Algorithm and TOF Mass Calibration.

If Fast Polarity Switching is enabled on a Q-TOF instrument, the Quad Autotune buttons are not available.

All the automatic tuning choices calibrate the TOF using eight to ten masses, except for the 1700 mass range, which calibrates using six masses.

Tune File: Autotune.tun

Ion Polarity: ☒ Positive ☐ Negative

Ion Source: Dual ESI

Source Parameters:

Gas Temp	325	300	°C
Drying Gas	5	3.0	l/min
Nebulizer	20	15	psig
VCap	3500	0.000	uA
Chamber		0.00	uA

Mass List (default):

On	Mass (m/z)
<input checked="" type="checkbox"/>	322.048121
<input checked="" type="checkbox"/>	622.028960
<input checked="" type="checkbox"/>	922.009798
<input checked="" type="checkbox"/>	1221.990637
<input checked="" type="checkbox"/>	1521.971475
<input checked="" type="checkbox"/>	1821.952313
<input checked="" type="checkbox"/>	2121.933152
<input checked="" type="checkbox"/>	2421.913990
<input checked="" type="checkbox"/>	2721.894829

Calibrant Bottle: ☒ None ☐ A ☐ B

LC Flow to: ☒ Waste ☐ MS

☐ Disable CDS ☐ Enable CalibB in Acq

TOF Mass Calibration: Autotune | Manual Tune | Diagnostics | Instrument State | Preferences

☐ Pause Between Autotunes (for Calibration Solution Swapping)

☒ Set CDS to None after Autotune

Tune Report...

TOF:

☐ Positive ☐ Negative ☐ Fast Polarity Switching

☐ Check Tune ☐ Quick Tune ☒ Standard Tune ☐ Set Detector Gain ☐ Initial Tune

Start Autotune

Figure 17 Autotune tab for a TOF instrument

Tune File: AutoTune.tun

Ion Polarity: ☒ Positive ☐ Negative

Ion Source: Dual ESI

Source Parameters:

Gas Temp	325	0	°C
Drying Gas	5	0.0	l/min
Nebulizer	20	-13	psig
VCap	4000	0.000	uA
Chamber		0.00	uA

Mass List (default):

On	Mass (m/z)
<input checked="" type="checkbox"/>	322.048121
<input checked="" type="checkbox"/>	622.028960
<input checked="" type="checkbox"/>	922.009798
<input checked="" type="checkbox"/>	1221.990637
<input checked="" type="checkbox"/>	1521.971475
<input checked="" type="checkbox"/>	1821.952313
<input checked="" type="checkbox"/>	2121.933152
<input checked="" type="checkbox"/>	2421.913990
<input checked="" type="checkbox"/>	2721.894829

Collision Energy: 0 V

Calibrant Bottle: ☒ None ☐ A ☐ B

LC Flow to: ☒ Waste ☐ MS

☐ Disable CDS ☐ Enable CalibB in Acq

TOF Mass Calibration: Autotune | Manual Tune | Diagnostics | Instrument State | Preferences

☐ Pause Between Autotunes (for Calibration Solution Swapping)

☒ Set CDS to None after Autotune

Tune Report...

TOF:

☐ Positive ☐ Negative ☐ Fast Polarity Switching

☐ Check Tune ☐ Quick Tune ☒ Standard Tune ☐ Set Detector Gain ☐ Initial Tune

Start Autotune

Quadrupole:

Quad Tune Check Quad Tune Initial Quad Tune

Figure 18 Autotune tab for a Q-TOF instrument

Initial Autotune

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

When you select this option after installation or major service, the system automatically adjusts all the tunable parameters to optimize signal, resolution and mass axis calibration.

With all Agilent 6200 TOF instruments and the Agilent 6500 Q-TOF instruments, you can perform an Initial Tune with a Dual ESI source. You can also perform an Initial Tune with the AJS ESI source on the Agilent 6230 UHD Accurate-Mass TOF and the Agilent 6530 UHD Accurate-Mass Q-TOF.

You set the Instrument Mode to Extended Dynamic Range in the Instrument State tab before running an **Initial Tune** (TOF).

You only use **Initial Tune** (TOF) or **Initial Quad Tune** under special circumstances because the process takes a long time to complete.

- After you install the hardware and software
- After your TOF analyzer has been vented for maintenance or service
- If you no longer have the previous tune files or parameters
- If Standard Autotune does not work

Instrument	Time for Initial Tune (TOF)	Time for Initial Quad Tune
Agilent 6538A and 6540A Q-TOF	up to 60 minutes for each polarity	up to 60 minutes
Other Q-TOF instruments	up to 30 minutes for each polarity	up to 60 minutes
TOF instruments	up to 30 minutes for each polarity	N/A

During the Initial Tune process for the TOF, the system goes through the following steps without your intervention.

- Resets any current tune parameters to original defaults
- Performs a coarse TOF mass axis calibration
- Adjusts all the tunable parameters automatically
- Performs a final TOF mass axis calibration
- Prints a tune report at the end of the Autotune process

During the Initial Quad Tune process, the system goes through steps a, c, d and e.

Check Tune

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

You can perform a Check Tune with any instrument mode selected and with any of the following sources installed:

- ESI
- AJS ESI (Agilent Jet Stream ESI)
- Dual ESI
- Dual AJS ESI (Dual Agilent Jet Stream ESI)
- MMI
- APPI
- APCI

Agilent designed the instrument so that it does not need frequent autotunes. Periodically, you may want to perform a Check Tune and Check Quad Tune to ensure that the parameters are still optimized. You do not need to perform a Check Quad Tune as often as a Check TOF Tune.

Standard Tune

These tools perform many of the same optimization operations as the Initial Tune tools but use the current settings as starting values and does a limited set of ion optic ramps.

With all Agilent 6200 TOF instruments and the Agilent 6500 Q-TOF instruments, you can perform a Standard Tune with a Dual ESI source. You can also perform a Standard Tune with the AJS ESI source on the Agilent 6230 UHD Accurate-Mass TOF and the Agilent 6530 UHD Accurate-Mass Q-TOF.

The Standard Tune (TOF) and Quad Tune do subsets of the Initial Tune and Initial Quad Tune actions, and they use the current tune parameters as starting points rather than using the factory defaults.

Standard Tune takes about 10 to 15 minutes, and Quad Tune takes about 10 to 15 minutes to complete.

Quick TOF Tune

Quick Tune for TOF automatically adjusts the most commonly required subset of tunable parameters.

You can perform a Quick Tune with any instrument mode selected and with any of the following sources installed:

- ESI (with or without Agilent Jet Stream technology)
- Dual ESI (with or without Agilent Jet Stream technology)
- MMI
- APPI
- APCI

Quick Tune takes 3 to 5 minutes because the software is optimizing only the most significant parameters followed by a calibration.

During Quick Tune 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 compound TOF ion mirror (optimizes resolution)
- d** Does a mass axis calibration after the final adjustment
- e** Prints a tune report

Set Detector Gain

Set Detector Gain adjusts the PMT voltage to obtain consistent gain (amplification) of the ion current into electrical current. In the Extended Dynamic Range (2 GHz) mode, it also adjusts the preamp offset values and the time delay between gain channels. This tool is a subset of Initial TOF Tune and Standard Autotune.

With all Agilent 6200 TOF instruments and the Agilent 6500 Q-TOF instruments, you can set the Detector Gain with a Dual ESI source. You can also set the Detector Gain with the AJS ESI source on the Agilent 6230 UHD Accurate-Mass TOF and the Agilent 6530 UHD Accurate-Mass Q-TOF.

You set the Instrument Mode to **Extended Dynamic Range** in the Instrument State tab before running the **Set Detector Gain** algorithm.

Manual Tune

See the online Help for detailed instructions to help you manually tune the TOF or Q-TOF instrument. Only perform a manual tune if the Autotune options produce a result that fails.

Order of using Autotune choices

Use the tuning options in the following order:

- 1** Check Tune for the TOF
If necessary, continue.
- 2** Quick Tune for the TOF (does not exist for the quadrupole)
If necessary, continue.
- 3** Standard Tune for the TOF
If necessary, continue.
- 4** Initial Tune for the TOF

Use the same order for tuning the quadrupole, if necessary.

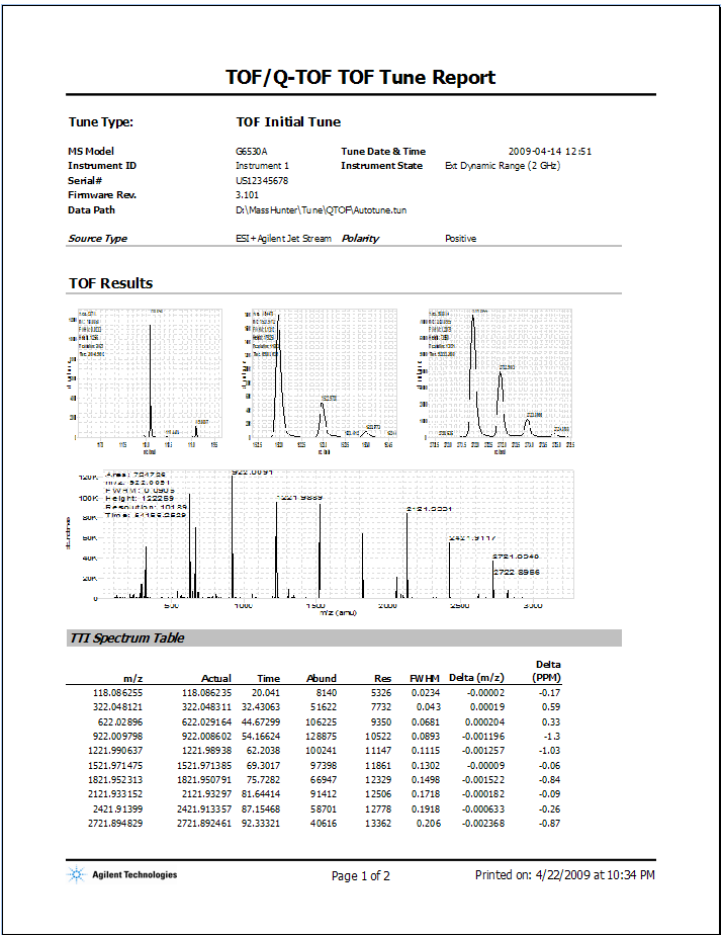
If an initial Tune cannot complete, contact Agilent Support.

Tune reports

At the end of every Check Tune, Quick Tune, or Standard Tune, the system generates a printable Tune report in Excel.

The TOF and Quad tune reports let you know if optimization limits are met. For a TOF tune, the report also lets you know if the mass calibration is satisfactory or not.

To print previous tune reports, you click the **Tune Reports** button in the Autotune tab.



Storage and retrieval of tune results and Instrument Mode

You can store the tuning parameters in a single file (*.tun) using the **Save** or **Save As** buttons in the Instrument State tab. You can also load tune files in the Instrument State tab (Figure 19). The Mass Range, the Fast Polarity Switching option and the Instrument Mode are also stored in the tune file.

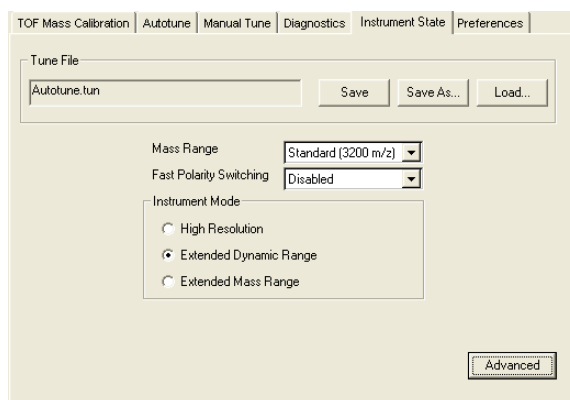


Figure 19 Instrument State tab

Instrument Modes

You can either select from 3 different instrument modes when the Basic modes are displayed or from 4 different instrument modes when the Advanced modes are displayed.

High Resolution (4 GHz, High Res Mode) or High Resolution In this mode, the system acquires data at a 4 GHz ADC rate while special processing is performed in real time on the mass peaks detected in each transient, weighting the apex data of the mass peaks much more heavily than the shoulders. This leads to narrower peaks in the summed transients (the mass spectra) and hence improves resolution. The Mass Range can only be set to Low (1700 m/z) or Standard (3200 m/z) if the Instrument Mode is set to this value.

2 Instrument Preparation

Storage and retrieval of tune results and Instrument Mode

4 GHz (High Resolution Mode disabled) In this mode, no processing is used at the transient level; however, the system acquires data at a 4 GHz ADC rate. The Mass Range can only be set to Low (1700 m/z) or Standard (3200 m/z) if the Instrument Mode is set to this value.

Extended Dynamic Range (2 GHz) or Extended Dynamic Range In this mode, the system acquires data at a 4 GHz ADC rate in dual channel mode. One channel is recorded at a high detector gain while the other channel operates at a low detector gain. The firmware stitches the two channels together to produce a scan with a sampling rate of 2 GHz, which results in a greatly increased dynamic range. The Mass Range can only be set to Low (1700 m/z) or Standard (3200 m/z) if the Instrument Mode is set to this value.

Extended Mass or Extended Mass Range In this mode, the system acquires data at a 2 GHz ADC rate. The Mass Range can be set to Low (1700 m/z), Standard (3200 m/z) or High (10,000). This is the only mode that you can use with the highest mass range.

Tune Set Point Modifications for Medium and Large Proteins

For medium and large proteins, the charge envelope may extend beyond 3200 m/z. It is recommended that you acquire your intact protein data in the Minimum Storage Size mode on the Agilent 6200 Series TOF and Agilent 6520/6530 Q-TOF as this allows the extended mass range needed for larger proteins. If you are acquiring a protein and the charge states are “cut-off” in one of the other modes, you can switch to this mode with the extended mass range.

If you are acquiring medium and large protein data, it is also recommended that the Quad AMU be raised to 300 amu instead of using the value established by the Auto Tune algorithm. This provides the Quad with enough power to transmit the high mass ions through the quad. Failure to increase the Quad AMU will result in a mass spectrum where the charge envelope appears to be “cutoff” for the higher charge state ion clusters.

To adjust the Quad AMU, do the following steps:

- 1 Switch to the Tune Context.
- 2 Click the Manual Tune tab.
- 3 Click the Quad Tab.
- 4 Click the Quad AMU field and type 300 for the new value.
- 5 Save the tune value to save the new Quad AMU value. Then exit the Tune Context.

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 device graphic.

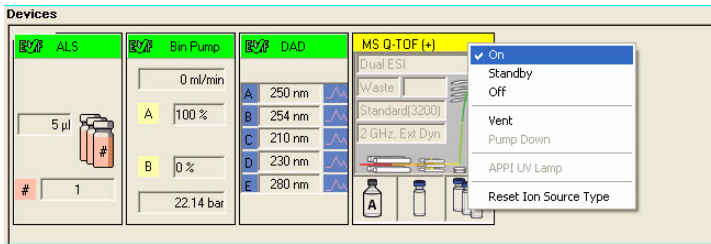


Figure 20 Instrument Status pane

Table 2 Colors of Instrument States

Instrument State	Color
On—Preparing/Not ready	Yellow
On—Ready	Green
On—Waiting	Purple
On—Running	Blue
Standby	Gray—To see if the pump, TOF or Q-TOF is in Standby mode or off, look for the check mark in the shortcut menu.
Off	Gray
Error condition	Red

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.

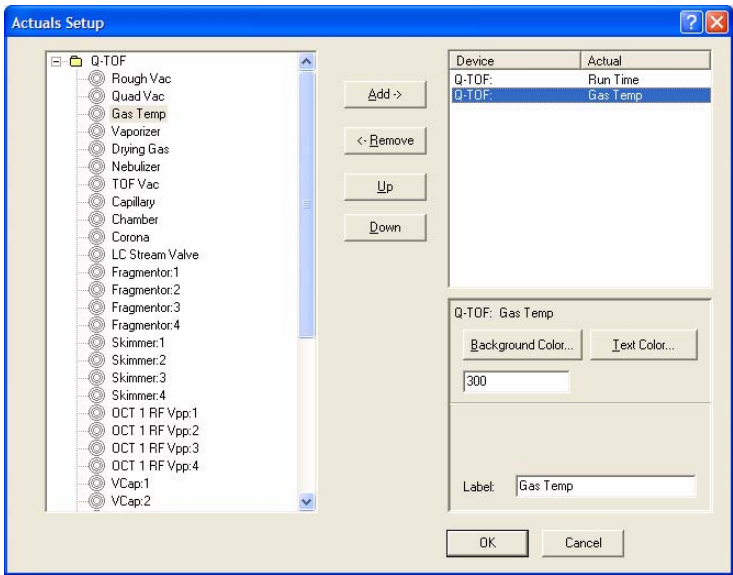


Figure 21 Actuals Setup dialog box

2 Instrument Preparation

Real-time parameter values (Actuals)

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

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

Table 3 Actuals available for display

Module	Parameter or State
All modules	Run Time, Run State, Ready State, Ready Type, Not Ready Text Long, Not Ready Text Short, Rawdata State, Error State
ALS	Vial, Sample, Volume, Needle, Command, Injectmode, Tray Type A, Tray Type B, Overlap, Injectstatus, Temp, Therm Power
WPS, μ WPS, h-ALS, h-ALS-SL and h-ALS-SL+	The same as ALS except no Vial and addition of Drawn Volume, Sample Position and Needle Position
Binary Pump	Solvent Ratio A, Solvent Ratio B, Flow, Pressure, Ripple, Fill A,B, A1, B1, A2, B2, Power, Channel Name A, B, Solvent Selection A, B
Capillary Pump	Same as binary pump with no Fill A1, A2, B1, B2 and with the addition of Solvent Ratio C, Solvent Ratio D, Fill C, Fill D, Primary Flow, EMPV, Purge Status, Purge Channel, Purge Time and Pump Op Mode
Nanopump	Same as capillary pump with addition of FSAC State and FSAC Step. FSAC stands for Flow Sensor Accuracy Calibration. When you click the Accuracy Calibration shortcut menu, these values show you the status of the step in the test procedure.
Thermostatted Column Compartment	Left Temp, Left Temp Set, Right Temp, Right Temp set, Column Valve, Therm Power
35900E	Current Vial
DAD G1315C	Sample WI A-H, Sample Bw A-H, Reference On A-H, Reference WI A-H, Reference Bw A-H, UV Lamp, Vis Lamp

Table 3 Actuals available for display

Module	Parameter or State
DAD (all others)	Sample WI A-E, Sample Bw A-E, Reference On A-E, Reference WI A-E, Reference Bw A-E, UV Lamp, Vis Lamp
MS TOF or Q-TOF	Rough Vacuum, High Vacuum, Gas Temp, Vaporizer Temp, Drying Gas Flow, Nebulizer Pressure, Capillary Current, Chamber Current, Corona Voltage, Charging Voltage, Control State, Cal/Ref Mass, LC Stream

Real-time plots

What you can display

You can display the following plots for these modules.

Table 4 Real-time plots available for display

Module	Plot type
Pumps	Pressure vs. time
Thermostatted Column Compartment	Temperature Left, Temperature Right vs. time
35900E	ADC signal vs. time
DAD	Signals A-H vs. time
TOF or Q-TOF	Any chromatogram set up in the Chromatogram tab of the Method pane: <ul style="list-style-type: none"> Any segment or scan of a total ion chromatogram (TIC) or extracted ion chromatogram (EIC) Method set points and actual conditions

What you can do with the displays

Table 5 What you can do with plots and spectra

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

Profile vs. Centroid spectral displays

Centroid spectra for the TOF and Q-TOF display the abundance vs. mass for the calculated centroid of the peak. Profile spectra display the abundance vs. mass over the mass range of the peak.

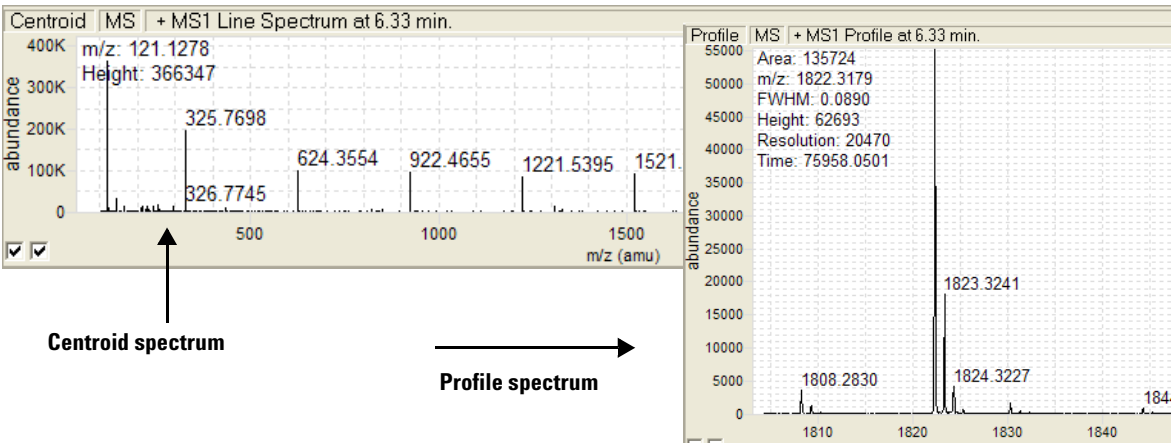


Figure 22 Centroid and profile spectral displays in Real-time Plot pane

Time vs. mass spectral display

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

System logbook

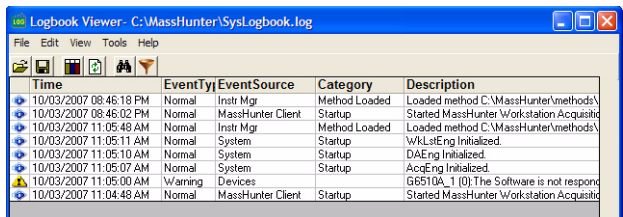
The system logbook does not list any changes to a method or worklist.

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.



The screenshot shows the 'Logbook Viewer' window with the file path 'C:\MassHunter\SysLogbook.log'. The window contains a table with the following data:

Time	Event Type	Event Source	Category	Description
10/03/2007 08:46:18 PM	Normal	Instr Mgr	Method Loaded	Loaded method C:\MassHunter\methods\
10/03/2007 08:46:02 PM	Normal	MassHunter Client	Startup	Started MassHunter Workstation Acquisition
10/03/2007 11:05:48 AM	Normal	Instr Mgr	Method Loaded	Loaded method C:\MassHunter\methods\
10/03/2007 11:05:11 AM	Normal	System	Startup	\wkLstEng Initialized.
10/03/2007 11:05:10 AM	Normal	System	Startup	DAEng Initialized.
10/03/2007 11:05:07 AM	Normal	System	Startup	AcqEng Initialized.
10/03/2007 11:05:00 AM	Warning	Devices		G6510A_1 (0) The Software is not respond
10/03/2007 11:04:48 AM	Normal	MassHunter Client	Startup	Started MassHunter Workstation Acquisition

Figure 23 Logbook Viewer

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

Table 6 Columns available for display in the Logbook Viewer

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

What you can do with the system logbook

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

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

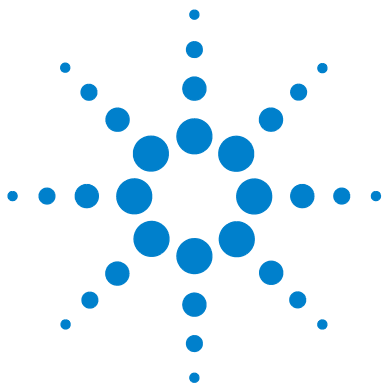
The system deletes week-old logbook entries. If you want to view them after a week, you must save or export them before the week has passed. Most of the tasks that you can do with the logbook help you view the entries you need to see more easily.

Table 7 Tasks you can perform with the logbook

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

Notification through the Taskbar

You can configure the system logbook to automatically display messages from the taskbar. You can specify the type of messages to display and how many messages to display at a time. By default, this feature is disabled. To enable automatic logbook notification, right-click on the Logbook icon in the Taskbar and click **Configure**. After you have selected how many messages to display and the type of messages, you then right-click the logbook icon in the system tray and click **Enable Notification**. Messages are still saved in the logbook whether or not you have enabled automatic notification.



3

Methods with Acquisition Parameters

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Ion source parameters [72](#)

Setup of TOF and Q-TOF reference mass correction (recalibration) [82](#)

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Method saving, editing and reporting [87](#)

Saving a method with data acquisition parameters [87](#)

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Method reporting [90](#)

See the online Help for instructions on setting up methods and parameter descriptions.

You use this chapter to learn about the concepts that can help you set up methods containing data acquisition parameters.



Parameter entry

LC parameter entry

Most of the LC parameter entries are the same as those that you can change with the Agilent 1200 LC control module and with other Agilent software products, such as Agilent ChemStation.

The screenshot shows the 'BinPump-SL' tab in the Agilent 1200 LC control module software. The window is divided into several sections for parameter entry:

- Flow:** A text box showing '0.8' with units 'ml/min'.
- Solvent A:** A table with two columns for solvent components. The first column shows percentages (87.00%) and the second column shows solvent names and concentrations. The entries are 'H2O' and '0.2% AcOH 1 mM NH4DA'.
- Solvent B:** A table with two columns for solvent components. The first column shows percentages (13%) and the second column shows solvent names and concentrations. The entries are 'MeOH' and '5 mM NH4 formate 90:10'.
- Stop Time:** A radio button for 'No Limit' and a text box for '2' with units 'min'.
- Post Time:** A radio button for 'Off' and a text box for '2' with units 'min'.
- Pressure Limits:** Text boxes for 'Min: 0' and 'Max: 600' with units 'bar'.

Figure 24 LC parameter entry

TOF and Q-TOF parameter entry

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

- Automatic parameter changes during a run
- General TOF and Q-TOF parameters
- Ion source parameters
- TOF and Q-TOF data acquisition parameters
- Setup for reference mass correction (recalibration)
- TOF and Q-TOF chromatogram setup

General | Source | Acquisition | Ref Mass | Chromatogram

Ion Polarity (Seg)
☒ Positive ☐ Fast Polarity Switching
☐ Negative

Data Storage (Seg)
☐ None ☐ Centroid
☒ Both ☐ Profile

LC Stream (Seg)
☒ MS
☐ Waste

Plot and Centroid Data Storage Threshold

MS		MS/MS	
Abs. threshold	250	Abs. threshold	5
Rel. threshold (%)	0.01	Rel. threshold (%)	0.01

☐ Do not wait for setpoints (e.g. temperature) to equilibrate

Figure 25 Q-TOF parameter entry

Automatic TOF and Q-TOF parameter changes during a run

You can set some TOF and Q-TOF method set points to be different at different points in time during the run. The different time points in the method are called time segments. Within each time segment, you can have different sets of parameters, called experiments.

Many parameters can be changed for different time segments. Some parameters can be changed for different experiments in the same time segment. For example, the gas temperature, drying gas flow rate, and the nebulizer pressure must be the same for all experiments in the same time segment, but fragmentor and capillary voltages can be different for different experiments.

3 Methods with Acquisition Parameters

Automatic TOF and Q-TOF parameter changes during a run

The screenshot shows the 'MS C' tab in a software interface. At the top, there are tabs: Sample, Properties, ALS, Quat Pump, TCC, DAD, and MS C. The MS C tab is active. Below the tabs, there are several sections:

- Ion Source:** A dropdown menu set to 'Dual ESI'.
- Ion Polarity:** A dropdown menu set to 'Negative'.
- Data Storage:** A dropdown menu set to 'Both'.
- LC Stream:** A dropdown menu set to 'MS'.
- Stop Time:** Two radio buttons: 'No Limit/As Pump' (selected) and 'Stop Time' (with a value of 30 min).
- Time Segment and Experiment #:** A table with two columns: 'Time (min)' and 'Expt #'. The table has three rows: 0, 1.4, and 1.9. The row with 1.4 is highlighted in blue.
- Cycle Time:** A text box with a value of 1 s.

Figure 26 Location for setup of segments and experiments

Per time segment set points are labeled with (*Seg*). Per experiment set points are labeled with (*Expt*). All other set points are per run. These set points can be found in these tabs.

- General Tab
- Source Tab
- Acquisition Tab

Acquisition tab

You can set up time segments for changing these parameters.

MS mode The MS mode spectral parameters can be set for each time segment.

Auto MS/MS mode (Q-TOF only) The parameters on the following tabs can be set for each time segment:

- MS Q-TOF Spectral Parameters Tab
- MS Q-TOF Collision Energy Tab
- MS Q-TOF Precursor Selection I Tab
- MS Q-TOF Precursor Selection II Tab
- MS Q-TOF Preferred/Exclude Tab

Targeted MS/MS mode (Q-TOF only) The parameters on the following tabs can be set for each time segment:

- MS Q-TOF Spectral Parameters Tab
- MS Q-TOF Collision Energy Tab
- MS Q-TOF Targeted List Tab

See “[TOF and Q-TOF acquisition parameters](#)” on page 75 for a description of the TOF, Auto MS/MS and Targeted MS/MS modes and their parameters.

General TOF and Q-TOF parameters

You enter general TOF and Q-TOF parameters on the General tab of either the MS TOF tab or the MS Q-TOF tab in the Method Editor pane.

Figure 27 General tab of the MS TOF Method Editor pane

Profile vs. centroid spectra

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

Ion source parameters

See the online Help to view recommended parameter values for each ion source.

You can use several different sources with the Agilent TOF and Q-TOF LC/MS system:

- ESI
- AJS ESI
- Dual ESI
- Dual AJS ESI

- APCI
- APPI
- AP-MALDI
- PDF-MALDI
- Orthogonal Nanospray
- HPLC-Chip/MS interface
- 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 Editor pane, you see new parameter options and boxes under the Source tab in the MS TOF tab or the MS Q-TOF tab.

The screenshot shows the 'Source' tab in the Method Editor. The 'Dual ESI (Seg)' section contains parameters for Gas Temp (325 °C), Drying Gas (8 l/min), Nebulizer (40 psig), and a second set of identical parameters. The 'Dual ESI (Expt)' section contains VCap (4000 V), Capillary (8.035 uA), and Chamber (3.61 uA). The 'MS TOF (Expt)' section contains Fragmentor (175 V), Skimmer (65 V), and OCT 1 RF Vpp (750 V).

Parameter	Value	Unit
Gas Temp	325	°C
Drying Gas	8	l/min
Nebulizer	40	psig
Fragmentor	175	V
Skimmer	65	V
OCT 1 RF Vpp	750	V
VCap	4000	V
Capillary	8.035	uA
Chamber	3.61	uA

Figure 28 Ion source parameters

3 Methods with Acquisition Parameters

Ion source parameters

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

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

Before the AP-MALDI or the PDF-MALDI is installed, in the instrument configuration tool, the LC must be removed from the system configuration.

For more information on how to set up the AP-MALDI to introduce samples, refer to the Agilent G1972A AP-MALDI LC/MSD Trap SL System User Guide.

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

- Tune the TOF or Q-TOF with an ESI source installed
- 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.

TOF and Q-TOF acquisition parameters

The Acquisition tab in [Figure 29](#) contains acquisition parameters that you can change with time segments.

The screenshot shows the 'Acquisition' tab of a software interface. On the left, under 'Mode:', there are three radio buttons: 'MS (Seg)' (selected), 'Auto MS/MS (Seg)', and 'Targeted MS/MS (Seg)'. The main area is titled 'TOF Spectra' and contains two sections. The 'Mass Range' section has 'Min Range' set to 100 m/z and 'Max Range' set to 1000 m/z. The 'Acquisition Rate/Time' section has 'Rate' set to 1 spectra/s, 'Time' set to 1000 ms/spectrum, and 'Transients/spectrum' set to 9902.

Figure 29 Acquisition tab

The acquisition parameters you choose depend on what you are trying to accomplish:

- If you want to pass all ions in a specified mass range through the instrument with no fragmentation in the collision cell, click **MS (Seg)** mode. This mode is automatically chosen for a TOF instrument.
- If you know the compounds and hence the precursor ions you are looking for, click **Targeted MS/MS (Seg)** mode.
- If you don't know what you are looking for and must set up boundary parameters for the precursor ion list, click **Auto MS/MS (Seg)** mode.

MS mode

In this mode the Q-TOF instrument behaves solely as a TOF instrument with no quad isolation applied. You specify the mass range and the acquisition rate and time to collect spectra.

The screenshot shows the 'TOF Spectra' acquisition parameters dialog box. It has tabs for 'General', 'Source', 'Acquisition', 'Ref Mass', and 'Chromatogram'. The 'Acquisition' tab is selected. On the left, under 'Mode:', there are three radio buttons: 'MS (Seg)' (selected), 'Auto MS/MS (Seg)', and 'Targeted MS/MS (Seg)'. The main area is titled 'TOF Spectra' and contains two sections. The 'Mass Range' section has 'Min Range' set to 100 m/z and 'Max Range' set to 1000 m/z. The 'Acquisition Rate/Time' section has 'Rate' set to 1 spectra/s, 'Time' set to 1000 ms/spectrum, and 'Transients/spectrum' set to 9902.

Figure 30 MS mode

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 31)

For analyses that require one Mass Spectrum or scan per second, the Agilent TOF and Q-TOF LC/MS 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 can reduce the number of transients per scan to increase the scans per second.

The *length of transients* is the time the system is allowed to collect data for the transient in nanoseconds. On the Agilent TOF and Q-TOF LC/MS, three mass range modes are available:

- Low (1,700 m/z)
- Standard (3,200 m/z)

- High (20,000 m/z) - The mass range of 20,000 m/z is available for Accurate-Mass TOF and Q-TOF instruments.

The transient length is set appropriately for each of these modes.

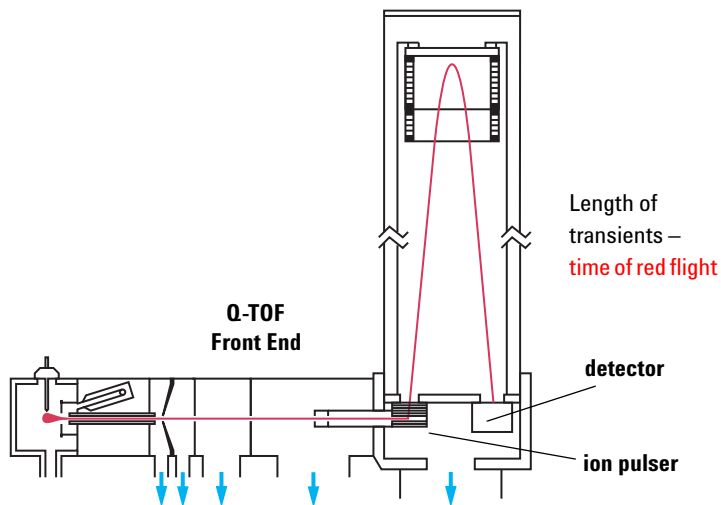


Figure 31 Length of transients is measured from the ion pulser to the detector

Targeted MS/MS mode (Q-TOF only)

In this mode you specify the precursor ion that you want the quadrupole to select and pass through to the collision cell for fragmentation. The TOF portion of the instrument then passes all the product ions through to the detector, and you select the ion you want to look at in the spectrum produced by the TOF.

3 Methods with Acquisition Parameters

TOF and Q-TOF acquisition parameters

The screenshot shows the 'Acquisition' tab of a software interface. On the left, under 'Mode:', the 'Targeted MS/MS (Seg)' option is selected. The main area is divided into 'Spectral Parameters', 'Collision Energy', and 'Targeted List' tabs. The 'Spectral Parameters' tab is active, showing settings for both 'MS' and 'MS/MS'. For 'MS', the Mass Range is Min 50 m/z to Max 1000 m/z, Acquisition Rate/Time is Rate 3 spectra/s and Time 333.3 ms/spectrum, and Transients/spectrum is 3218. A checkbox 'Max Time between MS1 Spectra' is checked with a value of 5 s. The 'MS/MS' section has identical Mass Range and Acquisition Rate/Time settings.

Figure 32 Targeted MS/MS mode

Spectral Parameters These parameters are the same as those for the TOF mode, but they are applied to both the quad (MS) and the TOF components (MS/MS).

Collision Energy You can enter multiple fixed collision energies (each collision energy is used), the slope and offset of a line, or a table of collision energies.

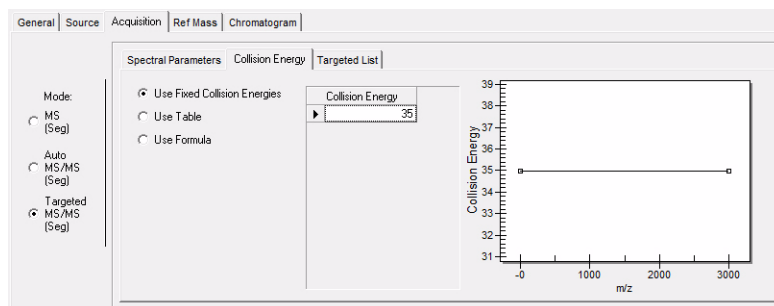


Figure 33 Targeted MS/MS mode with Collision Energy selected

For general method development you set the slope and offset for a curve whose collision energy the system selects to match the precursor ion, m/z , on the curve.

Targeted List For compounds for which you already know the precursor ions, you can place the collision energy in the targeted list for the precursor ion and vary its value to optimize the abundance of the product ion.

On	Prec. m/z	Z	Ret. Time (min)	Delta Ret. Time (min)	Iso. Width	Collision Energy	Acquisition Time (ms/spec)
<input checked="" type="checkbox"/>	279.09102	1	0		Medium (~4 m/z)	18	200
<input checked="" type="checkbox"/>	271.0317	1	0		Medium (~4 m/z)	12	200
<input checked="" type="checkbox"/>	285.0209	1	0		Medium (~4 m/z)	10	200
<input checked="" type="checkbox"/>	311.0809	1	0		Medium (~4 m/z)	22	200

Figure 34 Targeted MS/MS mode with Targeted List selected

The collision energies specified here override the values specified in the Collision Energy tab.

Auto MS/MS (Q-TOF only)

In Auto MS/MS mode, two additional tabs appear: Precursor Selection I and Precursor Selection II.

The Spectral Parameters tab contains the same parameters as in Targeted MS/MS mode. The other four tabs are used to enter the boundary parameters for selecting precursor ions.

3 Methods with Acquisition Parameters

TOF and Q-TOF acquisition parameters

General | Source | Acquisition | Ref Mass | Chromatogram

Mode:
☐ MS (Seg)
☒ Auto MS/MS (Seg)
☐ Targeted MS/MS (Seg)

Spectral Parameters | Collision Energy | Precursor Selection I | Precursor Selection II | Preferred/Exclude

3 Max Precursor Per Cycle

Precursor Threshold
Abs. Threshold 200
Rel. Threshold (%) 0.01

Active Exclusion
☒ Enabled
Excluded after 3 Spectra
Released after 0.3 min

Static Exclusion Range List

Start m/z	End m/z
100	250
500	1000

Figure 35 Auto MS/MS mode with Precursor Selection I selected

The software sorts a list of possible precursor ions whose order depends on the boundary parameters entered:

- a** Passes all ions through (TOF only) and sorts the list from highest abundance to lowest (or by charge then abundance)
- b** Excludes those masses in the specified mass range
- c** Sorts the list based on the priority of charges
- d** Moves preferred ions to the top of list in the order specified
- e** Chooses the top ions on the list based on the entry for maximum number of precursor ions per cycle (Max Precursor per cycle)
- f** Excludes masses after a specified number of spectra have been acquired and releases the exclusion after a specified time (Active Exclusion)

Varying scan speed based on precursor abundance

You can adjust the number of transients as a function of the precursor intensity. Varying the scan speed is very useful when you have a complex sample, such as a protein digest. To use this feature, you mark the **Scan speed varied based on precursor abundance** check box on the Precursor Selection II tab.

When this feature is turned on, the MS/MS acquisition rate is automatically adjusted based on precursor intensity, thus spending less time on the more abundant peptides and more time on the less abundant peptides. This should improve MS/MS quality and hence database matching.

The variable MS/MS acquisition mode can result in one or more additional precursors being examined per scan cycle as less time is spent on more abundant precursors.

See the online Help for information on Abundance Dependent Accumulation and Purity.

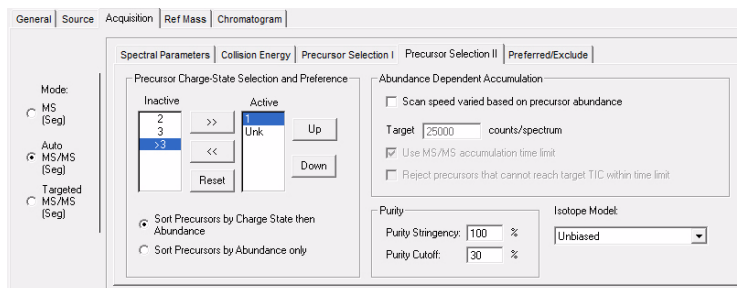


Figure 36 Auto MS/MS mode with Precursor Selection II selected

Setup of TOF and Q-TOF reference mass correction (recalibration)

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

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

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

- A calibrant delivery system (CDS) that automatically introduces the standard to a reference sprayer (second sprayer)— Dual ESI (0.2 to 1.5 mL/min flow rates only) and Dual AJS ESI 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 or the MS Q-TOF tab of the Method Editor pane.

On	M/Z
<input checked="" type="checkbox"/>	121.050873
<input checked="" type="checkbox"/>	149.02332
<input checked="" type="checkbox"/>	322.048121
<input checked="" type="checkbox"/>	922.009798
<input checked="" type="checkbox"/>	1221.990637
<input checked="" type="checkbox"/>	1521.971475
<input checked="" type="checkbox"/>	2421.91399

Figure 37 Reference Masses tab

If you mark the **Enable** 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. To adjust both the a and T0 terms requires at least two reference masses, spread across the application mass range, such that the first reference mass is <330 m/z and the second is 500 m/z or more above the first. Using more reference masses improves recalibration results. Up to 20 masses can be selected.

The reference masses specified for the method will be stored with the method. These reference masses are also stored in the file with acquired data as TOF or Q-TOF method parameters.

If you mark the **Use Bottle A** check box, the TOF or Q-TOF controls the valve to introduce the internal reference standard to the reference sprayer via the CDS.

3 Methods with Acquisition Parameters

Setup of TOF and Q-TOF reference mass correction (recalibration)

To learn more about the underlying calibration equation and coefficients, see “TOF mass calibration” on page 47.

The **Ref Nebulizer** parameter is only available if the source is either Dual ESI, Dual AJS ESI or Dual nanoESI.

Number of required reference masses

With two unknowns, a minimum of two known values are required to determine both A and t_0 . Practical 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 500 m/z above the low mass ion in order to correct t_0 and A terms. If these conditions are not satisfied but at least one reference mass is found, then only the A term will be re-calibrated.

Selecting/editing list of reference masses

When creating a new method, the Agilent MassHunter Workstation Software provides separate lists of reference masses for each polarity. You can use the already provided mass list, or you can create or edit a new mass list by right-clicking the arrow to the left of each row in the Ref Mass tab.

Parameters for a reference mass correction

To know more about Segments and Experiments, see “Automatic TOF and Q-TOF parameter changes during a run” on page 69.

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 five. 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 Experiments defined in a given Segment.

For example, if a Time Segment contains one Experiment with Fragmentor voltage at 225V and another Experiment 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 *n*th 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 Experiment #1, and one Fragmentor voltage of 220 Experiment #2, and the current spectrum is the Fragmentor voltage of 225 Experiment #1 of the *n*th 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 *y*1 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.

TOF and Q-TOF chromatogram setup

In the Chromatogram tab, you also select the chromatograms or the set points/actuals that you want to see in the Real-time Plot pane during the run.

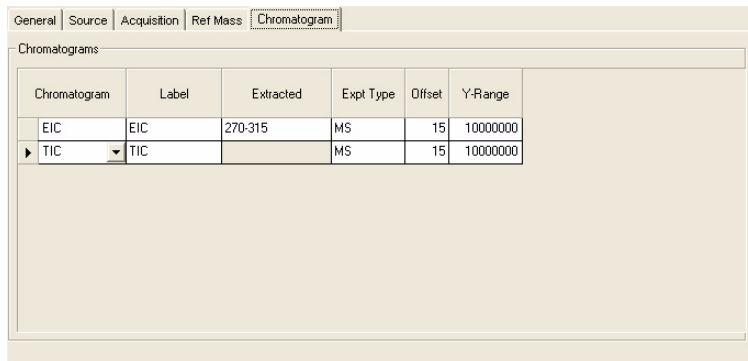


Figure 38 Chromatogram tab in the MS TOF or MS Q-TOF tab of the Method Editor

You can select the signal to plot (TIC, EIC, BPC, Set point, Actual), the experiment type, the offset of the baseline for the plot and the valid range of values in counts. You can also set the mass range for the EIC.

Method saving, editing and reporting

Saving a method with data acquisition parameters

Adding pre- or post-run scripts before saving

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

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

Figure 39 Properties tab of the Method Editor pane

Scripts can be written using any programming language. Scripts provided by Agilent should not be modified since these files may be overwritten when upgrading the Agilent software.

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

For more information on scripts, see the online Help.

3 Methods with Acquisition Parameters

Saving a method with data acquisition parameters

Table 8 System scripts and the actions they enable

Script name	Actions the script enable
SCP_AcquireCalibrantData	Sets "Cal/Ref Mass" to "Cal B" and "LCStream" to "LC->Waste". Allows you to acquire data for the calibrant solution itself. To be used only as the Pre-run script for a method. The script itself does <i>not</i> do a run and only augments an existing method. Do not use as a standalone script in a worklist run.
SCP_MSDivertValveToMS	Sets the solvent divert valve to the "MS" position.
SCP_MSDivertValveToWaste	Sets the solvent divert valve to the "Waste" position.
SCP_InstrumentStandby	Puts the instrument in standby mode. This is the same as clicking the "Standby" button on the instrument status pane.
SCP_LoadIdleMethod (MethodName)	Loads a method to put the system in an idle state. The first parameter is the method name.
SCP_MSRefOff	Turns off the MS reference ion solution (Ref A)
SCP_PumpsAllandMSRefOff	Turns off LC pumps and turns off the MS reference ion solution.
SCP_PumpsAllOff	Turns off all pumps.
SCP_LCCondition (MethodName, LCStream)	Starts a run for conditioning the LC part of the instrument. No data acquisition happens.
SCP_CalibrateTOFMassAxis	Starts a TOF/Q-TOF mass axis calibration in the active polarity using the default mass list
SCP_TraceOnOff	Switches the trace to ON or OFF. The first parameter must be either "on" or "off".

Table 8 System scripts and the actions they enable

Script name	Actions the script enable
SCP_ClearTrace	Clears the trace file.
SCP_ProcessQuantReport	Runs a single sample Quant report. Quantitative Analysis must be installed. Use only as a Post Method script.
SCP_CTCReset	Resets the CTC autosampler. If a drawer is open, it will be closed.

Location of method folders

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

You can view the name and path of the currently loaded method in the Properties tab of the Method Editor pane ([Figure 39](#)).

Method editing

You can edit methods containing data acquisition parameters using the Method Editor pane of the Data Acquisition window.

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

Methods can also contain Qualitative Analysis parameters. See the *Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide* or the online Help for the Qualitative Analysis program for more information.

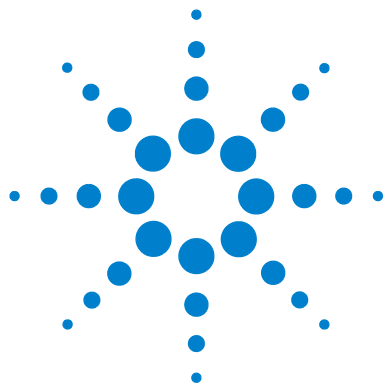
Method reporting

You can see the parameters in a method in one of three ways:

- You click **Acquisition Method** from the File > Print menu.
- You review the parameters in the Method Editor.
- You can see method parameters associated with a data file in the Agilent MassHunter Workstation Qualitative Analysis software.

Acquisition Method reports include this information:

- Method name, path and description
- List of configured LC modules and TOF or Q-TOF
- Parameter values for each LC module and for TOF or Q-TOF



4 Data Acquisition

Interactive single sample setup [92](#)

Worklist setup [94](#)

Data acquisition for samples and worklists [110](#)

Learn the concepts to help you understand the setup of single samples for interactive data acquisition and the setup of single samples and sequences of samples for automatic data acquisition.



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 Editor pane.

Sample information

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

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

The screenshot shows the 'Sample' tab of the Method Editor pane. The tab is selected, and the interface is divided into several sections. On the left, there is a 'Sample' section with fields for 'Name' (containing 'SampleName') and 'Position' (containing 'Vial 2'). Below this is a 'User Defined' section with two empty text boxes labeled 'User Defined 1' and 'User Defined 2'. Further down is a 'Data File' section with an 'Auto-increment' checkbox (unchecked), a 'Name' field (containing 'Test1.d'), and a 'Path' field (containing 'D:\MassHunter\data') with a 'View Data' button. On the right side, there is a 'Run' section with a 'Run Type' dropdown (set to 'Standard Start'), a 'Part of method to run' dropdown (set to 'Acquisition Only'), an 'Operator Name' field, a 'Wait Time for Ready' field (set to '10' minutes), and an 'Injection Volume' field (set to '.1' µl). At the bottom right, there is a 'Comment' field.

Figure 40 Sample tab of the Method Editor pane

Data file information/projects

The default folder for TOF and Q-TOF data is **D:\MassHunter\Data**. All data files are saved to the default data folder unless you choose another folder.

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 type 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.

Other folders

You can save your data to any folder on the system. You must use browse to select a different folder. To open any data file saved to a different folder than **D:\MassHunter\Data**, you must browse the system.

Run information

Start run types

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

Part of method to run

This option is always set to **Acquisition Only** because you cannot run a data analysis in conjunction with the acquisition of a single sample.

Injection Volume

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

Worklist setup

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 and multiple samples—to be run automatically in the order of their listing.

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

	<input checked="" type="checkbox"/>	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment
1	<input checked="" type="checkbox"/>		P1-A1	pftms-only.m	Sulfa1.d	Sample		
2	<input checked="" type="checkbox"/>		P1-A1	pftms-only.m	Sulfa2.d	Sample		
3	<input checked="" type="checkbox"/>		P1-A1	pftms-only.m	Sulfa3.d	Sample		
Worklist								

Figure 41 Example worklist

Selected sample execution

Each line in the worklist table has a check box that allows you to mark 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 scripts before or after the worklist or between samples in a worklist
- 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

Each menu has different commands available. Some commands are only available in certain situations. For example, in the column shortcut menu, you can only use the Insert Column (s) command if you select a column that has been added using the Add Column(s) menu.

Right-click upper left-hand corner of worklist to view worklist menu.

Click Worklist to view top worklist menu.

Right-click cell to view cell menu.

Right-click column header to view column menu.

Select row and right-click row to view row menu.

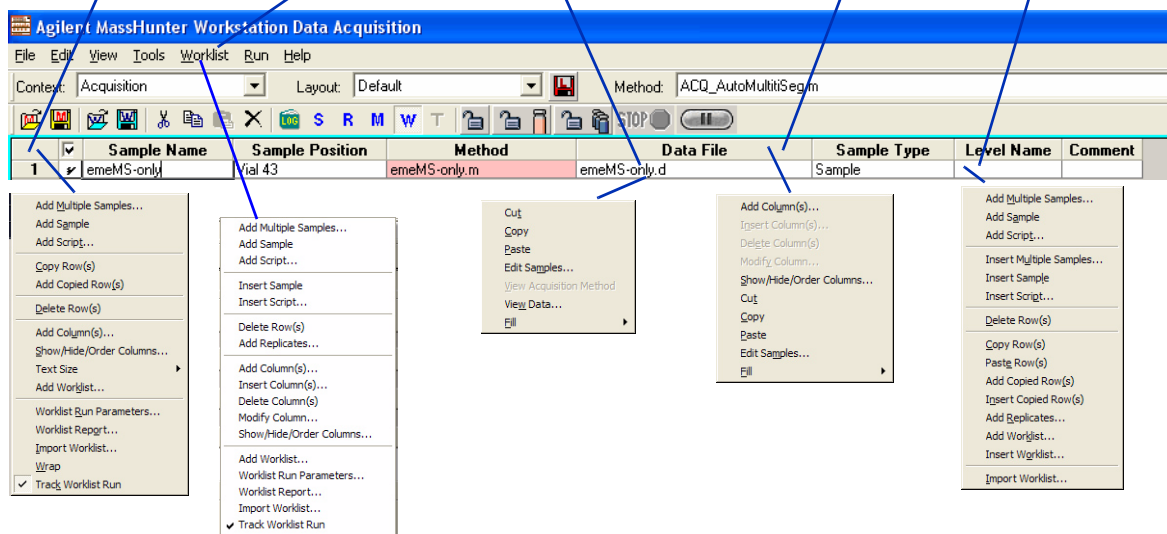


Figure 42 Worklist menus

Sample entry

One-at-a-time entry

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 folder, method names and injection volume.

Add Multiple Samples

Sample Information | Sample Position

Sample
Name: Sample ☐ Append Counter
Suffix Counter
Number of digits: 1 Start Value: 1 Step: 1

Data File
Name: WorklistData ☐ Append Counter
Suffix Counter
Number of digits: 1 Start Value: 1 Step: 1
Path: C:\MassHunter\data ...

Method
Name: Default.m
Path: C:\MassHunter\methods ...

Override DA Method
Name: plhexercise2.m
Path: C:\MassHunter\methods ...

Injection
Injection Volume: -1 µl

OK Cancel

Figure 43 Add Multiple Samples dialog box

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

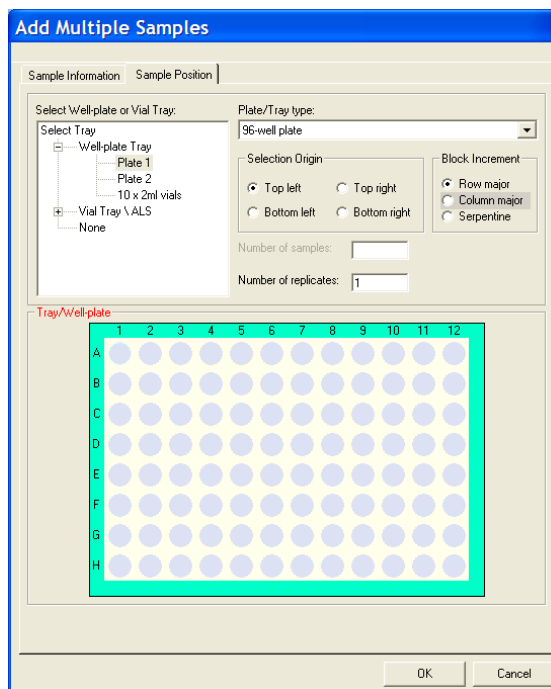


Figure 44 Sample Position tab of the Add Multiple Samples dialog box

Sample methods

You can create a .m method containing either acquisition parameters, qualitative analysis parameters or both. See the *Quick Start Guide* for instructions for creating the method.

If you specify a method with both acquisition and qualitative analysis parameters in the worklist, you select which parts to run in the Worklist Run Parameters dialog box.

Script entry

Scripts are special programs, that execute automatically. Agilent includes scripts with the Agilent MassHunter Workstation Software, and you can write your own scripts.

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 a worklist and after data acquisition (Figure 49 on page 105)

Scripts 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.

Agilent includes scripts with the software to help you automatically, instead of manually, execute processes such as column conditioning and valve shutoff. See Table 8 on page 88 for a list of possible scripts.

Entry of additional sample information (show, add columns)

The default worklist contains only eight columns for sample information.

	<input checked="" type="checkbox"/>	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Comment
1	<input checked="" type="checkbox"/>					Sample		

Figure 45 Default worklist columns for sample information

You can access these capabilities through the worklist menu.

You can add more columns in one of two ways:

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

Show/Hide/Order sample information

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

With this dialog box you can hide any of the original default columns and show others. You can also change the order of the columns. The marked columns are shown in the worklist in the order they appear in this dialog box.

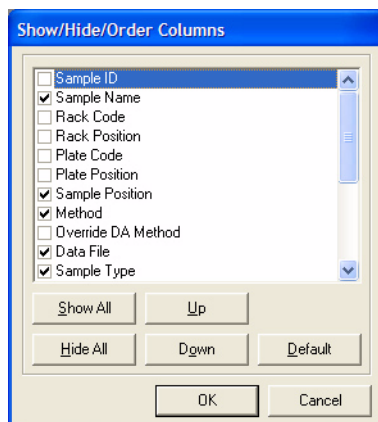


Figure 46 Show/Hide/Order Columns dialog box

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. You can add a column for the Molecular Formula which can be used in the Qualitative Analysis program. The Column Type **MFC** is only available if the Qualitative Analysis program is installed on the same computer. The Column Type **Protein** is only available if the MassHunter BioConfirm program is installed.

When you add a column, it appears in the Show/Hide/Order Column dialog box.

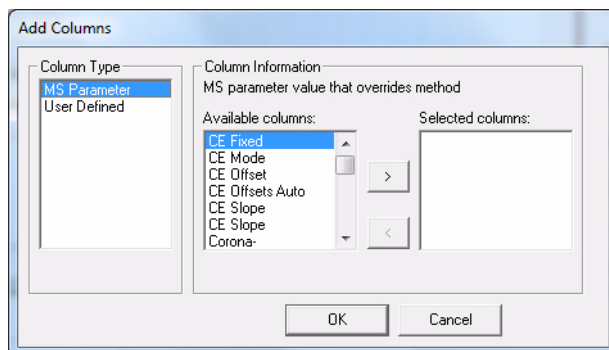


Figure 47 Add columns dialog box

Worklist import

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

- Use the Study Manager program which automatically creates a worklist when you submit a study.
- 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 or Q-TOF worklist under the correct header

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

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

- Import a csv file directly

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

CSV file mapping

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

Your sample csv file contains a table of samples and attribute information for each sample. The information in this file may not correspond to the information needed in a TOF or Q-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 Agilent MassHunter Workstation Software.

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

You must first edit the sample csv file to put it into a form that maps to TOF or Q-TOF sample data. You can specify these changes when you map to the TOF or Q-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 also include the mapping section in the sample csv file when new groups of samples with different dynamic mapping of columns, such as amount of compounds analyzed, are added for import.

Mapping for static worklist columns

Some worklist boxes 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 or Q-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 9 Original sample table

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

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

[Table 10](#) is the mapping section for the spreadsheet in [Table 9](#). This is the spreadsheet version of the mapping that lets the worklist import program recognize columns of imported data.

Table 10 Mapping section for csv file

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

Report setup

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

- Screen
- File

You specify the report destination and the file path in the Worklist Report Options dialog box.

4 Data Acquisition

Report setup

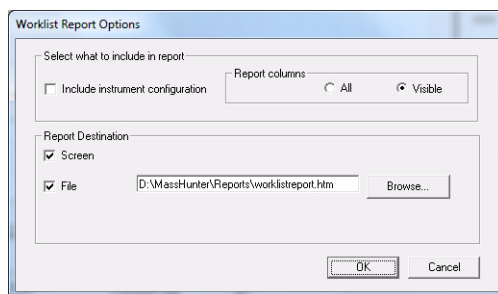


Figure 48 Worklist Report Options dialog box

Run setup

Before you run a worklist you select parameters for the entire worklist.

- Start run types and the part of the method to run
- Paths for the acquisition method, data analysis method and data file
- Whether or not to combine export output when also running a Qualitative Analysis method
- Scripts to run before or after the worklist
- Free disk threshold

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

The screenshot shows the 'Worklist Run Parameters' dialog box, specifically 'Page 1'. The dialog is divided into several sections:

- Operator Information:** A text field for 'Operator name:'.
- Run Information:** Includes 'Run Type:' (Standard Start), 'Execution for Acquisition-DA:' (Synchronous), 'Part of method to run:' (Acquisition Only), and a checked checkbox for 'Stop worklist on DA error'.
- Method Paths:** Includes 'Method:' and 'Override DA:' both pointing to 'D:\MassHunter\methods'.
- Data File:** A text field for 'Path:' pointing to 'D:\MassHunter\data\YourName'.
- Scripts:** Three checkboxes for 'Pre-worklist', 'Post-worklist', and 'Acquisition clean-up', each with an associated text field and browse button.
- Disk Information:** Shows 'Free Disk Threshold:' set to '10 (Gbytes)' and 'Available Diskspace:' as '420.94 (Gbytes)'.
- Run Settings:** Includes checked checkboxes for 'Overlapped Injection' and 'Clear sample selection after run', and a 'Wait Time for Ready:' set to '10 (min)'.

At the bottom right are 'OK' and 'Cancel' buttons.

Figure 49 Worklist Run Parameters dialog box

Overlapped Data Analysis with Acquisition

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

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

Overlapped Injection

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

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

Estimate of worklist file size

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

You can run a typical worklist sample to estimate the worklist file size. Then, you observe the file size in Windows Explorer, and multiply the observed file size by the number of samples in the worklist.

The data file for profile data is compressed by a factor of 3 - 20, depending on the complexity of the mass spectra acquired. This new feature greatly reduces the data storage needed for profile data.

The Instrument Mode selected for the Accurate-Mass TOF and Accurate-Mass Q-TOF also affects the size of the data file. The Agilent TOF and Q-TOF instruments now will acquire 2 times as

much data when operated in the 2 GHz mode (extended dynamic range or extended mass range) or 4 times as much data when operated in the 4 GHz mode (high resolution).

Method parameters that control file size

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

- Data file storage type—Profile, Centroid, Both 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. **Both** stores both Profile data and Centroid data. **None** stores no data but does store spectrum metadata and method parameters.

If you want to analyze your data in Qualitative Analysis using either the Large Molecular Feature Extractor (LMFE) algorithm or the Deconvolute (MS): Maximum Entropy algorithm, you acquire in either Profile or Both modes. Profile data is required for both of these algorithms.

- Total run time
- Setting of time segments
- Number of experiments that are defined

Approximate file size for stored Profile data

The file size per spectrum depends on three variables.

- Mass range
- Spectrum metadata
- Compression factor - the data file is compressed by a factor of 2 to 20, depending on the complexity of the mass spectra acquired

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 possible data points for a

4 Data Acquisition

Estimate of worklist file size

Note that low masses require more data points than high masses because of the non-linear nature of the time to mass conversion.

given mass range using a typical mass calibration curve. The Agilent 6540 UHD Accurate-Mass Q-TOF has approximately 25% more data points.

Table 11 Number of data points pre spectrum for given mass ranges

Mass range (Daltons)	Number of data points (1 GHz)	Number of data points (2 GHz)	Number of data points (4 GHz)
50 to 750	50,000	100,000	200,000
50 to 1000	60,000	120,000	240,000
50 to 3000	100,000	200,000	400,000
250 to 1000	30,000	60,000	120,000
500 to 3000	60,000	120,000	240,000
250 to 3000	70,000	140,000	280,000

Each data point stored requires 12 bytes of storage. A mass range of 250 to 3000 requires 840,000 bytes per spectrum at 1 GHz acquisition rate. If the scans/sec. equal 1, then the data storage required for one minute worth of spectra before compression is 50,400,000 bytes or about 48 MB. Then, this data is compressed which means the actual data storage needed is between 2.4 MB and 16 MB (with a compression ratio between 3 and 20).

If the run time on a run with one time segment is 5 minutes, then one run requires between 12 MB and 80 MB of disk space. A worklist with 10 samples using the same mass range requires between 120 and 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.

Spectrum metadata In addition to data points, information on the spectrum, such as the instrument actuals and set points, 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 12 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 and the threshold set.

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 disk space needed for these samples is between 102 and 172.5 MB.

If you have a 2 GHz instrument, the disk space needed for these samples is between 204 and 345 MB. If you have a 4 GHz instrument, the disk space needed for these sample is between 408 and 690 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 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.

Monitoring the 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 by clicking the **File > Print > Real-time Plot** command.

For more information on real-time plots, see “Real-time displays” on page 58.

What you can do during a run

Locked Mode

You can turn Locked Mode on or off using the toolbar icons in the main toolbar. If Locked Mode is turned on, you cannot edit a worklist or method while the data is being acquired. Also, the data file is protected, so you cannot overwrite the data file if you run this method or worklist again. If Locked Mode is turned off, then you can edit the worklist 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.

4 Data Acquisition

What you can do during a run

In This Book

The Concepts Guide presents “The Big Picture” behind the Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS system to help you to understand how to use the TOF and Q-TOF LC/MS system components.

This guide includes concepts for:

- Inner workings of the TOF and Q-TOF MS
- Instrument Preparation
- Methods with Acquisition Parameters
- Data Acquisition

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