MassHunter Networked Workstation

Functional Design Specifications

Notices

Document Identification

DocNo D0026034

DE35946139
October 2022   Revision A.00

Copyright

Software Revision

This guide is valid for MassHunter Acquisition for TQ LC/MS 12.0 or later, MassHunter Acquisition for TOF/Q-TOF 11.0 or later, and MassHunter Quantitative Analysis 12.0 or later, until superseded.

Software Manufacturing

Manufactured for Agilent Technologies
5301 Stevens Creek Blvd
Santa Clara, CA 95051

Warranty

Technology Licenses

Restricted Rights Legend

Safety Notices

CAUTION

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

WARNING

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

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1 Overview

MassHunter Networked Workstation provides instrument control, data acquisition, data processing, and reporting for LC/MS systems for those labs operating in regulated environments.

When combined with OpenLab Server or OpenLab ECM XT, also known as Content Management, electronic records can safely be archived, stored, and shared in a central repository that can meet the needs of all laboratory sizes. It provides the necessary controls for managing system access, data transfer handling, and detailed audit trail functionality.

Content Management ensures secure record keeping, audit trail recording, and versioning, as well as provides means for electronic signatures and data archival. The central data repository provides several database options such as PostgreSQL, Microsoft SQL Server, or Oracle Database.

The MassHunter Networked Workstation software can also be installed in the Workstation configuration. The technical controls described in this guide apply only to the Networked Workstation configuration.

Compliance standards

The technical controls of MassHunter Networked Workstation specifically help users to document that their analyses meet current regulations and quality standards including, but not limited to these Food and Drug Administration's (FDA) 21 CFR parts:

- 21 CFR Part 210 (Current Good Manufacturing Practice In Manufacturing, Processing, Packing, Or Holding Of Drugs),
- 21 CFR Part 211 (Current Good Manufacturing Practice For Finished Pharmaceuticals)
- 21 CFR Part 58 (Good Laboratory Practice For Nonclinical Laboratory Studies).

Technical controls

MassHunter Networked Workstation includes technical controls and features to help users validate and specify methods, verify that methods are fit for their intended use, verify system performance and operation, and ensure the traceability, integrity and security of the data:

- Roles that restrict actions to certain Users
- Audit Trails
- Record Security and eSignatures through integration with OpenLab Server or ECM XT
- Hash Signature for data files allow you to check the integrity of files during a compliance audit
- Automatic checksum validation on file access for enhanced tamper detection

Automatic session lockout based on configurable time limit. For more information about 21 CFR Part 11 or the European Medicine Agency's (EMA) Good manufacturing practice (GMP) Guidelines - Annex 11 (Computerized Systems) compliance, refer to *Support for Title 21 CFR Part 11 and Annex 11 compliance: Agilent MassHunter for LC/TOF and LC/Q-TOF Systems,* available from Agilent under **White Papers** at **https://www.agilent.com/en/product/software-informatics/mass-spectrometry-software/data-acquisition/acquisition-for-lc-ms#literature**.

Software development process and validation

MassHunter Networked Workstation for LC/TQ, LC/TOF, and LC/Q-TOF was developed according to the quality process and software life cycle followed by the Life Sciences and Applied Markets Group (LSAG) of Agilent Technologies.

The Agilent Declaration of Software Quality shipped with each software package documents the software development and testing steps executed as part of the development cycle. The development process is registered to the ISO 9001 quality standard.

MassHunter Networked Workstation Compliance Services

Agilent offers compliance services for the MassHunter Workstation family of software products and for a wide variety of Agilent instruments and those from other manufacturers. These services utilize detailed protocols developed by Agilent CrossLab and are delivered by certified support personnel. For more information on Agilent CrossLab Compliance Services, please contact your local Agilent representative.

Supported Instruments

Agilent instruments as well as instruments from other manufacturers are supported on MassHunter Networked Workstation:

Agilent Liquid Chromatography

- Agilent 1260 Infinity and Infinity II LC modules and systems
- Agilent 1290 Infinity and Infinity II LC modules and systems
- Agilent 1220 Infinity and Infinity II LC systems
- Agilent 1100 and 1200 LC systems
- Agilent 1120 and 1220 Compact LC systems
- Agilent CTC PAL 2 Autosamplers with Agilent LC

Agilent Mass Spectrometry

- Agilent 6475 LC/TQ and Ultivo LC/TQ
- Agilent 6420A, 6460C, 6470A, 6470B, 6495A, 6495B, and 6495C LC/TQ
- Agilent 6224 and 6230 LC/TOF
- Agilent 6520B, 6538, 6540, 6545, 6545XT, 6546, and 6550 LC/Q-TOF

Configuration

MassHunter Workstation is available in two different configurations:

- Workstation (standalone with local administration and local file-based storage)
- Networked Workstation (hybrid Client/Server system with central administration and Content Management)

Only the MassHunter Networked Workstation supports technical controls and is the only configuration described in this guide.

OpenLab Control Panel

MassHunter Networked Workstation systems are managed via the OpenLab Control Panel, which provides:

- Central configuration of instruments,
- Central management of users, groups, privileges and roles
- Central management of projects
- Central system administration
- Central management of software licenses
- An overview of the status of all instruments on the system (lab-at-a-glance view)
- Central management of data in a central Content Management repository using a relational database

For more details, see **Chapter 4**, "OpenLab Control Panel".

Unsupported Programs or Configurations

Content Management is not compatible with these programs and systems:

- MassHunter Qualitative Analysis and MassHunter IM Browser[1]
- MassHunter WalkUp
- StreamSelect
- RapidFire
- Skyline software from MacCoss Lab (Department of Genome Sciences, University of Washington)
- 2D-LC[2]

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1 You can retrieve data from OpenLab Server or ECM XT onto your local computer and run it in Qualitative Analysis and IM Browser, but the data will be considered to be uncontrolled.
2 2D-LC data is supported on Workstations, but not on Networked Workstations. 2D-LC data is not compatible with the Content Management component on OpenLab Server/ECM XT servers.

2 Computer and Network Requirements

Hardware and Software Requirements

The terminology for computer hardware in client/server systems can be imprecise due to the variety of hardware and operating system combinations available. For the purpose of this specifications document, a PC means a desktop style personal computer running the specified Microsoft Windows operating system. The version of Microsoft Windows installed on each machine is assumed to be at least "Professional" and not a Starter or Home Edition. PCs are used for MassHunter Networked Workstation.

A server is a computer with hardware suitable for continuous service in a client/server environment running a Windows Server operating system. Servers are used only to run OpenLab Server/ECM XT program.

In most cases the personal computer connects to the analytical instruments through a LAN card. If third party hardware is connected, specific interface cards may be required. The separate hardware components that comprise a particular instrument configuration, including third party instrumentation, may need to be coordinated through a remote cabling system for time-critical events such as injection.

The following sections focus on the hardware and software requirements for MassHunter. For additional information regarding hardware and software requirements, please refer to the document *MassHunter Networked Workstation*.

Agilent Original Bundle PC

The Agilent Original Bundle PC, shipped with many MassHunter products are computers from Hewlett-Packard, fully tested and qualified to run MassHunter software. Agilent Original Bundle PC offers a one-stop solution for fast and seamless setup of analytical equipment in your laboratory. All are equipped with two industry standard LAN interface cards or a 2 port network adapter. Please contact your Agilent Sales Representative for further details on the available products.

Support for Varying Computer Manufacturers

MassHunter Workstation has been designed to successfully run on a wide range of compatible personal computers equipped with accessories and peripherals that adhere to the programming standards for the Intel PC platform and Microsoft Windows operating systems. Agilent Technologies has tested MassHunter Workstation software mainly on Hewlett Packard equipment. All configuration information listed in the manuals applies to Hewlett-Packard computers and may not be optimized for other vendors' PCs. Although the software is also designed to be run on other compatible hardware, Agilent Technologies will not necessarily accept responsibility for defects solely observed and reported on third party hardware.

Agilent offers a variety of preconfigured Agilent Original Bundle PCs. For details refer to **"Agilent Original Bundle PC"** on page 17.

MassHunter Workstations

Standard workstations are tested and supported with one LC/TQ system. A system is defined as any combination of supported LC modules and one supported TQ instrument.

Storage and Database Requirements

MassHunter can be configured with different storage options for managing system information and scientific data in a database.

OpenLab Server and ECM XT can be configured to use the databases PostgreSQL, Microsoft SQL Server or Oracle Database.

ECM 3.x is supported as long as you have an OpenLab Shared Services Server, and it can be configured to use the databases PostgreSQL, Microsoft SQL Server or Oracle Database.

For detailed OpenLab Server and ECM XT requirements please refer to the *OpenLab Server and OpenLab ECM XT Hardware and Software Requirements Guide*.

Secure Work Area

MassHunter does not interact directly with content in the OpenLab Server/ECM XT environment. MassHunter objects, such as data and method files, are first written into the Secure Work Area on the local C:\ directory.

When the object is completed, it is uploaded from the local Secure Work Area to the OpenLab Server/ECM XT. The content remains cached in the Secure Work Area for up to six hours before it is purged.

Similarly, whenever MassHunter requires an object, for example, when loading a method, the object is first copied from the OpenLab Server/ECM XT to the local Secure Work Area, and then loaded into the MassHunter program.

The Secure Work Area *must not run out of disk space* while running MassHunter. If the disk volume that contains the Secure Work Area becomes full, MassHunter will not be able to collect new data or create new results.

To prevent loss of data, by default, if the available disk space is less than 200 GB, MassHunter Data Acquisition does not allow a run to start and also warns the user. This value can be changed. However, BioConfirm and Quantitative Analysis are not aware of Acquisition's activities in the Secure Work Area and will not prevent the volume from filling to capacity.

Agilent recommends that the Secure Work Area reside on a fast NVMe SSD.

Network Requirements

General Requirements

MassHunter Workstation systems rely on network infrastructure in order to support the communication between various system nodes. TCP/IP networking in a LAN is required for all products. This communication is based on standard TCP/IP protocols. In order to provide optimum performance and uptime, the network must meet design criteria for available bandwidth, IP address assignment, name resolution and appropriate isolation of the lab subnet from the corporate network. IPv6 is not supported and must be deactivated.

When using LAN communications to connect workstations, the connection can be direct using a crossover CAT-5/6 cable or via an isolated switch using standard CAT-5/6 network cabling.

For more details on the individual topics outlined below please refer to the document *MassHunter Workstation 12.0 Requirements* guide.

Instrument Controller Communication

The communication path between instruments and workstations is intolerant of latency, competitive traffic, or service interruptions. For this reason, the instruments are on an isolated network segment. This means there should be no routing within the segment, switching must provide dedicated resources for instrument communication, and the segment should have no other traffic including broadcast messages or network management traffic. Failure to isolate instrument traffic properly may make data acquisition unreliable.

Domain Requirements

Domains support the flow of information and user access rights across machines in the network. Even workstation installations can use a Windows domain-based authentication model. With domain-based authentication individual machines must always be able to communicate with domain components, not just to login but also to receive authorization for many routine functions. All machines and instruments within the MassHunter Networked Workstation system must reside within the same domain or have the appropriate cross domain trusts to allow name-based communications between all components in the system.

The domain components necessary to support MassHunter Workstation host a variety of services and settings that must be configured appropriately to allow communication across machines.

Firewall Settings

If using a third-party firewall on the network where MassHunter Networked Workstation is installed, a list of fixed firewall ports needs to be open to allow communication between the system components of MassHunter Workstation. In addition, MassHunter Workstation may use some dynamic ports. More information and detailed lists of required firewall ports for each product are available in the document *MassHunter Workstation 12.0 Requirements* guide.

Environments with Proxy Servers

The servers used in an MassHunter Networked Workstation environment (for example, OpenLab Server or license server) must be accessible via http or https in the network. This might require adjusting the proxy settings.

Operational Continuity

If a Networked Workstation loses connection to the OpenLab Server/ECM XT, Data Acquisition and any other MassHunter program will not start. But if the server connection is lost while data is being acquired:

- The single sample run or worklist continues to acquire data and store the data in the Secure Work Area. (See **"Secure Work Area"** on page 18.)

- All cached uploads will appear in the File Upload Queue utility. Errors associated with the upload are presented alongside an option to retry the failed upload.

- Once server connection is restored, data is uploaded from the Secure Work Area to the OpenLab Server/ECM XT. The process and progress can be monitored in the File Upload Queue tool.

Instrument Communication

MassHunter Workstation provides standard LAN-based communication with the instrument, using Transmission Control Protocol / Internet Protocol (TCP/IP). All Agilent instrument hardware that is currently supported by MassHunter Workstation is controlled over LAN. Please refer to the instrument specific sections in **"Instrument Control"** on page 187.

LAN Interfaces

MassHunter Workstation provides network-based instrument control and data acquisition for network-capable Agilent instruments, optional A/D converters and non-Agilent instruments (for compatibility information, see **"Other Instrument Control"** on page 197). For general networking requirements see **"Network Requirements"** on page 19.

Current Agilent instrument hardware is equipped either with an embedded LAN interface or with a G1369C LAN Interface card to connect the analytical instrument to the LAN.

For further information, please refer to the *MassHunter Workstation 12.0 Requirements* guide.

LAN Communication Protocols

Instruments are controlled over LAN using industry standard TCP/IP (Transmission Control Protocol / Internet Protocol). Correct and stable communication between the PC and analytical instruments connected over the LAN must be verified.

For Agilent instruments, the IP address is typically set either at the factory, from the instrument's front panel, over telnet, or using a hand-held control module. Some instruments can use a predefined IP address which is enabled by DIP switches. The IP address is stored in the non-volatile RAM of the module's LAN interface. This is the preferred mode. For Smartcard-based LC/MS instruments, only the default IP address of 192.168.254.12 is supported.

For details please refer to the respective hardware manuals and the *MassHunter Workstation 12.0 Requirements* guide.

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3 Installation

Overview

The MassHunter Installer is the central utility to prepare, install, or uninstall MassHunter Workstation software. It consists of five pages: License Agreement, Installation Folders, Review, Install, and Finish. Some of these components are only available for the LC/TQ 12.0 installation, and some are only available for the LC/TOF and LC/Q-TOF 11.0 installation.

The following components are installed by the MassHunter Networked Workstation 12.0 Data Acquisition installer:

- Agilent MassHunter LC Drivers
- Agilent MassHunter OpenLab Control Panel Plugin
- Agilent MassHunter Workstation Acquisition (LC/TQ) 12.0 - for LC/TQ only
- Agilent MassHunter Workstation Acquisition (TOF/Q-TOF) 11.0 - for LC/TOF and LC/Q-TOF only
- Agilent MassHunter Workstation Acquisition Help (LC/MS) 12.0
- Agilent MassHunter Workstation Acquisition Method Optimization Tools 12.0 - for LC/TQ only
- Agilent OpenLab Certificate Service
- Agilent OpenLab Certificate Service Tool
- Agilent OpenLab Compound Database Service
- Agilent OpenLab ECM Storage Plugin
- Agilent OpenLab Shared Services
- Agilent OpenLab Shared services (64 bit)
- Agilent OpenLab Storage Client Services
- Agilent Services for CM
- Agilent Software Verification Tool B.01.01

You can also separately install these MassHunter programs:

- Agilent MassHunter Workstation BioConfirm Software
- Agilent MassHunter Workstation Quantitative Analysis
- Agilent MassHunter Workstation Report Builder

To install MassHunter, you must log into the machine as a domain user who is also a local administrator. This enables the MassHunter Installer to apply network exceptions to the Microsoft Windows firewall under the domain profile, which is necessary to configure a functional system.

To remove MassHunter Workstation, all of the MassHunter components need to be uninstalled separately.

Software Verification

An automated Software Verification Tool is installed with each software component of the MassHunter Workstation. This utility has a reference file providing an index of the files required for a proper installation. The utility checks the reference list against the files installed, their locations and revisions, including operating system files with specific dependencies.

This test is typically performed at installation and can be performed at any time to verify the file level integrity of the software installed on each computer. Test reports can be printed or saved to disk.

The Agilent Software Verification Tool for MassHunter Workstation can be used to verify a successful installation of a new version or patch. This does not replace the Installation Qualification (IQ) service, which is delivered by an authorized Agilent service provider.

Upgrade from earlier versions of MassHunter

If no change of the operating system is planned, MassHunter Networked Workstation can be upgraded to the latest revision. You need not uninstall MassHunter Networked Workstation before you upgrade.

All settings in the OpenLab Control Panel (projects, users etc.) are kept. Also saved user preferences (layouts, columns etc.) are kept.

Methods and worklists created in MassHunter Workstation 10.x can be imported into Content Management and used by MassHunter Networked Workstation, or simply opened by MassHunter Workstation.

Methods and worklists created in versions of MassHunter Workstation prior to 10.x may not migrate successfully. Methods and worklists that do not migrate successfully must be recreated from the default method.

The upgrade of a MassHunter Networked Workstation system is performed in the following order:

1 Upgrade OpenLab Server/ECM XT.

2 Upgrade Networked Workstations.

For large MassHunter Networked Workstation systems, the Agilent services team will plan the upgrade to minimize downtime of the laboratory while ensuring compatibility of all components in the system. Running mixed versions is generally not supported.

Virtualization

MassHunter Workstation clients can be virtualized on application virtualization platforms like Citrix. Microsoft Remote Desktop Services can be used to remotely access MassHunter Networked Workstation. Please contact your Agilent support representative for specific requirements and details.

MassHunter Licensing

MassHunter Networked Workstation supports the use of a central licensing server for the distribution and tracking of license entitlements. FlexNet Publisher is used for this purpose and is installed with the installation of MassHunter. More details on licenses are provided in **"Licenses"** on page 56.

Language Compatibility

The English version of MassHunter is validated on Windows English and Western European language operating systems.

MassHunter Workstation 12.0 is not supported on localized language versions of Windows.

MassHunter Workstation can run on other language versions of Windows as well, but the user interface will be in English.

Non-localized instrument drivers are supported and will appear in English even when running localized versions of MassHunter Networked Workstation.

For details please refer to the document *MassHunter Workstation 12.0 Requirements* guide.

Printers

MassHunter Workstation has been designed to work with printers that are compatible with the operating system. The software operates with any Microsoft Windows compatible printer capable of interpreting an escape code language (e.g. PCL) or page description language (e.g. PostScript). The printer may be locally connected to the computer or connected through a Local Area Network. Networked printers must be shared by a network server running a network protocol supported by the Microsoft operating system.

Agilent Technologies has not tested all printer and printer driver combinations that are supported in the Windows environment. Print performance and results will vary.

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4 OpenLab Control Panel

The **OpenLab Control Panel** provides access to the instrument management and system administration tools of MassHunter Networked Workstation. All functions in the OpenLab Control Panel are under system access controls, so the access each user has to the OpenLab Control Panel is dependent on the roles he has been assigned. The OpenLab Control Panel connects to the OpenLab Server/ECM XT which runs the Shared Services application that internally manages the system information.

OpenLab Control Panel functions are divided into views, which are selected through view selection buttons in the lower left pane of the application's user interface. The Instruments view provides a display of the instruments connected to the system, instrument session launch controls, and tools for configuring and managing instruments. The Administration view provides access to system administration functions. In addition, a Projects view is available to define project level MassHunter settings and to configure storage locations for method, worklists, and data files according to the desired scheme.
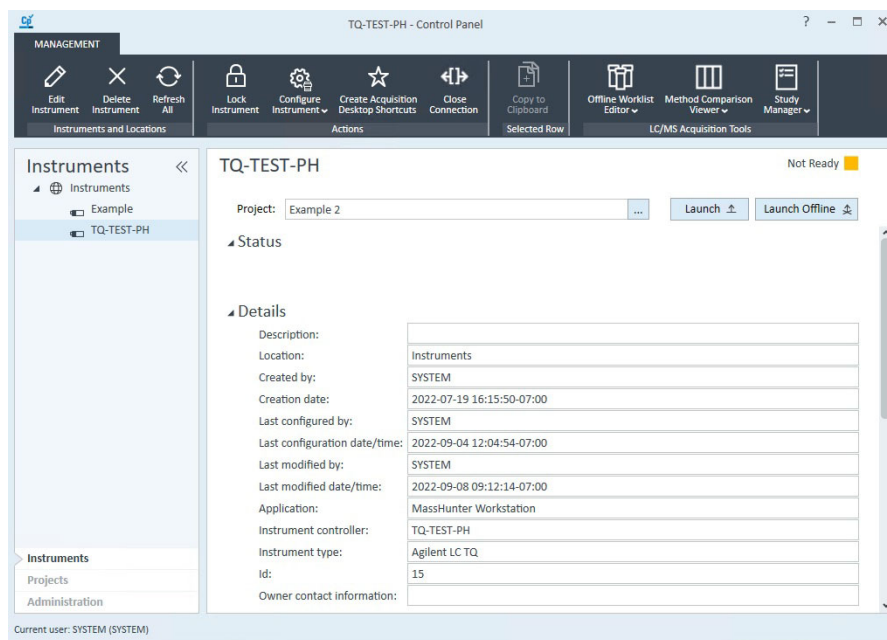
Instruments

The **Instruments** view in OpenLab Control Panel offers an overview of all instruments in the network or on the standalone workstation. It is often referred to as "Lab at a Glance".

Navigation Pane

All instruments connected to the system are displayed in a tree in the Navigation pane on the left of the Instruments view. The Instruments item is the root of the instruments tree. When the root is selected, a table of all instruments connected to the system is displayed in the workspace to the right.

Instruments

Selecting the **Create** button and then **Create Instrument** opens an instrument creation dialog box. The dialog box contains the following options:

- **Name** – Mandatory unique instrument name. Names are unrestricted and can be chosen to match an existing instrument naming scheme.
- **Description** – Optional description, used to label the instrument with more detailed information.
- **Application** – Available instrument control applications, for example MassHunter Workstation.
- **Instrument controller** – The controller for the instrument is on the local system.
- **Instrument type** – Selected from a list of available instrument types. The available instrument types depends on the Data Acquisition program that is installed.
- **Contact** – Optional contact information of person responsible for the instrument.

- **Default project** – Selected from a list of available projects. If **Always use Default project** is selected, you are prevented from storing data in a different project. If a project is not selected, a prompt will ask for a project when the instrument is launched.

When **OK** is clicked, the instrument is registered and appears in the instrument tree. At this point the instrument can be configured by clicking **Configure Instrument > Online** or **Configure Instrument > Offline**.

Most current instruments can be configured automatically by providing the IP address of the instrument and waiting for the system to locate and identify the instrument. Once an instrument is identified, it may have modules and associated configuration items that can be accessed by opening each module.

Instrument control and data processing are performed through associated MassHunter application sessions. These sessions run on the workstation itself and can only be accessed on that workstation. In such a configuration, instrument control is restricted to the workstation on which the instrument was created and configured. Therefore, this workstation must be running and connected to the instrument whenever data acquisition is active.

Instrument control sessions are run on the computer connected to the instrument. An instrument's session is accessed remotely from any client using Windows Remote Desktop.

Instruments can be disconnected from OpenLab Control Panel using the **Close Connection** command.

For any selected instrument, **Create Shortcuts** adds a shortcut to open the session for a given instrument and project to the desktop of the local computer.

Locations

Locations are optional and allow instruments to be organized into user-defined groups. Instruments can be created within a location or moved to a location by dragging and dropping. Selecting a location opens a table displaying the same status information for the instruments as the root but limited to those instruments assigned to the location.

The shortcut menu and ribbon buttons enable creating, editing or deleting instruments or locations.

As each instrument or location is created, it appears as a named node in the instrument tree. There is always one unique item for each instrument connected to the system. Selecting an instrument item opens an instrument status pane for the individual instrument in the workspace on the right.

Locations can be created within locations to create a multi-level hierarchical organization for the instruments. Instruments can be created in a location or directly under the Instruments root node.

Locations cannot be deleted when they have content, so locations or instruments within a location must be deleted or moved before the location is deleted.

Instrument Configuration

Standard Instrument Configuration

Before use, the instrument must be configured. Click **Configure Instrument > Online** or **Configure Instrument > Offline** to do so.

Most current instruments can be configured automatically by providing the IP address of the instrument and waiting for the system to locate and identify the instrument. Once an instrument is identified, it may have modules and associated configuration items that can be accessed by opening each module.

Printing the configuration – including module names and serial numbers – is available through the information panel of the Acquisition/ Instrument Status window.

Dynamic Instrument Configuration

For a small number of LC modules, limited configuration changes can be made from within the acquisition client without reconfiguring the instrument from Control Panel. This functionality depends on the instrument being used and its supported "soft-config changes", such as LC autosampler configuration. With this feature, the instrument does not need to be manually reconfigured after a firmware update.

Instrument Status Displays
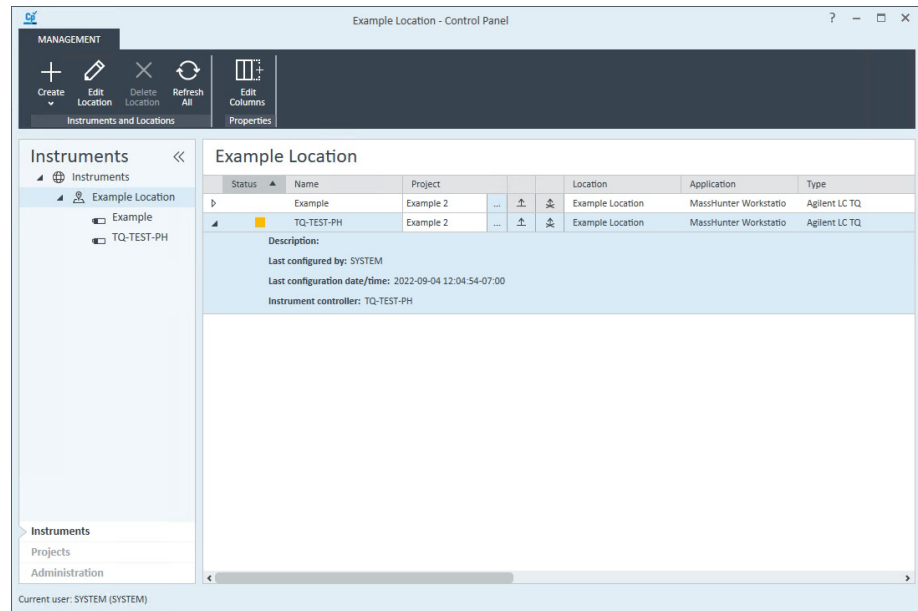
When a location is selected in the navigation pane, all the instruments in the location and all locations beneath it are displayed in a single instrument status table. Each row has a color-coded status, with the colors:

- Grey – Not Connected (configured but not launched)
- Green – Connected and Ready/Idle
- Yellow – Connected and Not Ready
- Red – Error
- Purple – Pre-run or Injecting
- Blue – Running
- Teal – Sleep or Standby
- Orange – Tuning in progress
- Blank - Disconnected

By default, all status columns are displayed including:

- Name
- Project
- Location
- Application
- Type
- Controller
- Description
- Last Configured By
- Last Configured
- Created
- Used By
- Run Status
- Current Sample

Selecting **Edit Columns** from the ribbon allows users to hide table columns that are not of interest or to change the order of appearance.

Selecting an individual instrument displays a status page with expanded display of the same information provided in the instrument status table, along with an instrument activity log. The activity log is an overview of activities on the instrument for the last 24 hours. This is a portion of the information stored in the permanent instrument logs.

Instruments can be launched when you select an instrument and then click **Launch** or **Launch Offline**. You can also use the buttons in the Instrument table.

Table button	Action
...	Opens the **Select Project** dialog box so that you can select a different project for the selected instrument.
⬆	Launches an online instrument session. If the button is gray, then the instrument is not configured, and you cannot start a session.
⬇	Launches an offline instrument session. If the button is gray, then the instrument is not configured, and you cannot start a session.

Instrument Privileges

Instruments are created with a default privilege setting of **Inherit Privileges from Parent**. This means instrument access privileges are set from the level above and ultimately from the instrument root. As a result, privileges set in the Administration view are automatically applied to each instrument.

If users are to have access to some instruments but not others or to have different privileges on different instruments, the privileges can be set at any level in the instrument tree. With inheritance active, privilege adjustments to locations apply to all locations and instruments within that location.

Privileges are changed by selecting the item (either a location or instrument) and then selecting **Edit Privileges**. When the **Inherit Privileges from Parent** option is cleared, the parent privileges are not automatically applied to a child node. They can be explicitly copied down to the item or set from scratch. Users or user groups can then be assigned or removed, and specific roles added or removed. Instrument privileges are detailed in **"Instrument"** on page 82.

Instrument Session Locking

The OpenLab Control Panel and MassHunter Workstation sessions can be locked manually or be set to lock based on a timeout after the configured period of user inactivity. The Timeout period can be set in the OpenLab Control Panel in the Security Policy section of the Administration view. The operator opening a locked session must supply login credentials based on the authentication configured for the system.
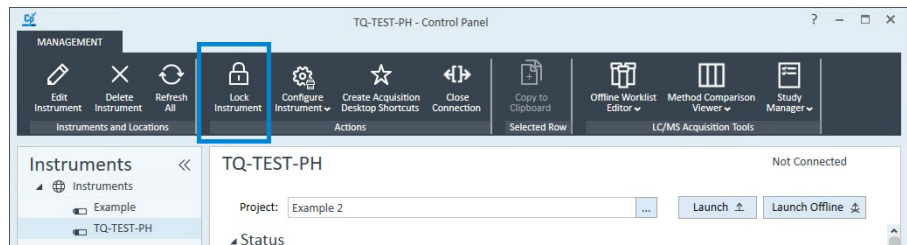
Projects

The Projects view within the OpenLab Control Panel provides for the creation and management of projects. Projects store all the files associated with analyses performed with MassHunter. Project definitions contain the storage locations, access privilege profiles, and a detailed set of project options. Projects allow the work performed in your operation to be accessed and stored using a logical organization defined according to individual needs for each department, project or even user.

Because each project profile has individual option settings, restrictions can be tight, or access can be open, as needed for each individual project. All methods, worklists, analysis methods, and report templates required for a given analysis task must reside within the same project.

Selecting a project in the Projects tree will display information on the project in the project window. The two available tabs are Project Properties and MassHunter Settings.

Projects can be edited and deleted but deletion of a project does not delete the files in the corresponding project folder.

Properties
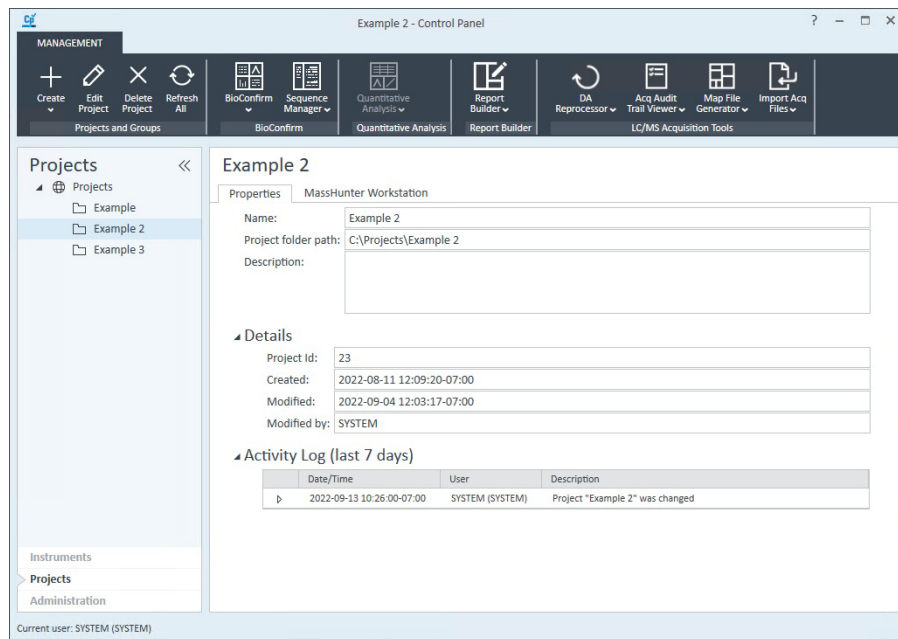
Projects are created and organized with controls very similar to those for instruments (including privileges, activity log and desktop shortcuts). Projects or Project Groups can be created in the project root node of the navigation tree. Project Groups allow projects to be organized and to have common properties and options managed by settings at the group level.

When a project is created, the storage locations are folders within the Content Management database. The project must be given a unique name within the selected location and project folder path. Existing project folder paths may be used but only one project can be assigned to a given project folder path at the same time.

An optional project description can be added, typically to indicate the appropriate use or assignment of the project.

MassHunter Settings
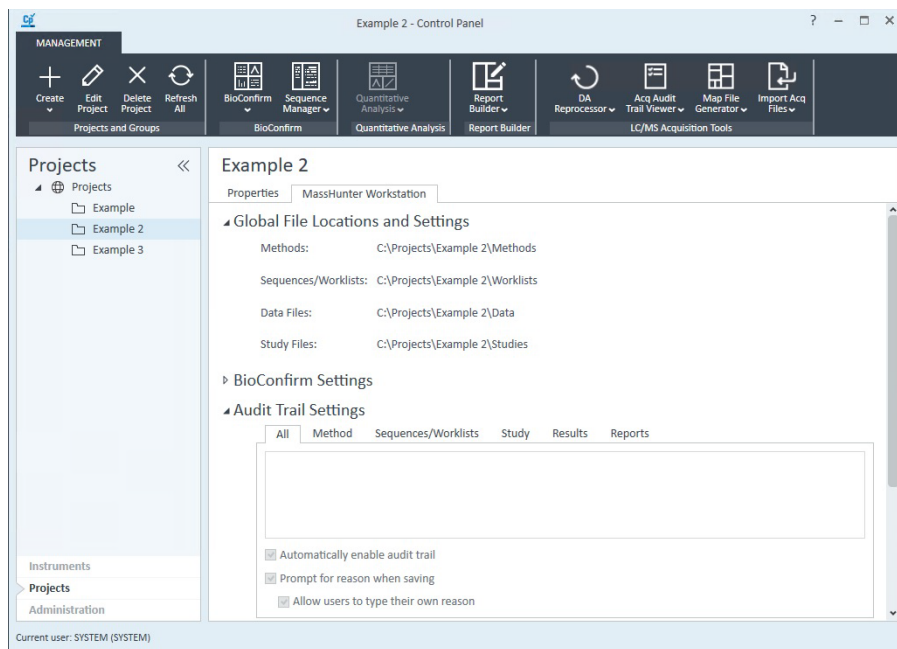
Global File Locations and Settings

When a project is created, a set of default folders within the project folder is created to organize the files for the project. The default folders are: Methods, Worklists, and Data Files. These folders cannot be edited within the project once they are created.

Application Settings

These sections show you the settings for installed MassHunter applications, such as MassHunter BioConfirm. Some folders, such as Report Templates, can be shared between different MassHunter applications.

After an application is launched into a Project, the application cannot browse, load, or save any regulated objects outside of the Project path.

Audit Trail Settings

A list of reasons can be configured from which the user can select when making audit entries that prompt for a reason. A global list applicable to all audit trail types can be configured as well as individual lists for Method, Worklists, Results, and Report audit entries. By editing both, global and individual lists, the system can have a set of common reasons available for all entries, as well as individual reasons defined per item.

If the audit trail is automatically enabled, a radio button allows control of prompting for reasons. The options that can be selected are:

- Prompt for reason when saving
- Do not prompt for a reason

The option to prompt for a reason when aborting a run is also selected with the option to prompt for a reason when saving results.

Study Setttings For each project, you mark which Study Types can be created with that project. The three options are Bioanalysis, Worklist Import, and Worklist Only. You also mark whether or not to stop the study queue if an error occurs.

Project-wide Privileges
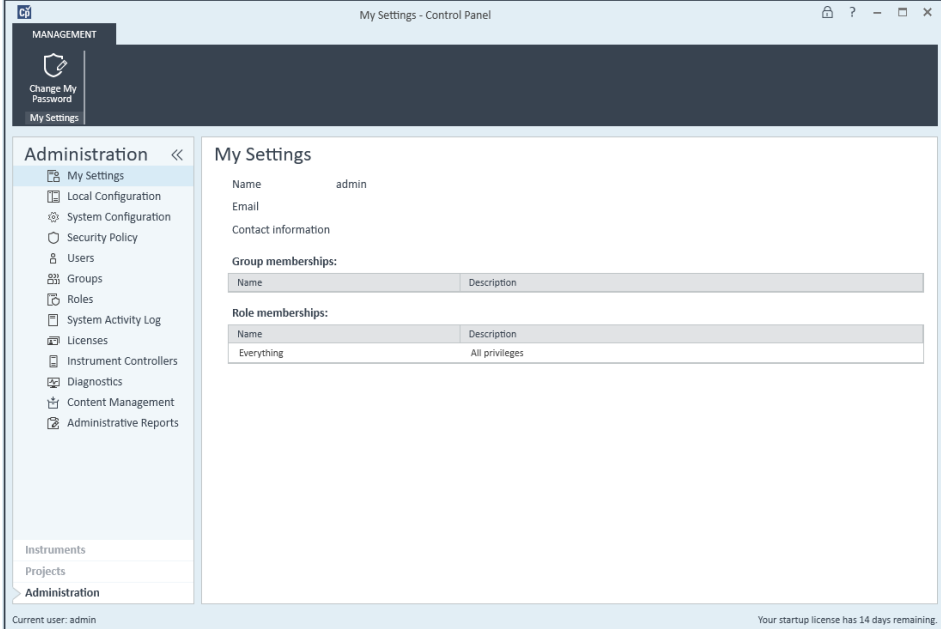
If users are assigned access roles in the Administration view, these access roles apply to all projects unless inheritance is turned off in Edit Privileges. Users can be assigned specific access roles for individual projects or project groups.

Administration

The Administration view in the OpenLab Control Panel provides a menu of configuration and settings as well as tools to modify the MassHunter configuration. The navigation pane on the left allows users to select a given section. Corresponding tools and information appear in the workspace for each selection.

The items available in the navigation pane will depend on the user's privileges. All users can see My Settings, the Local Configuration, and the System Activity Log.

My Settings

My Settings is only available when user authentication is enabled.

This section allows each user to check his information and access to the system. Selecting **My Settings** will display attributes that can contain the user's Name, Email address, and Contact information. These attributes will reflect settings returned to MassHunter from the selected authentication provider, depending on the authentication mode selected (OpenLab, Windows domain).

The Group memberships and Roles assigned will be displayed. This display can help to not just determine a user's MassHunter settings, but also the information returned to MassHunter in the authentication process.

Local Configuration

This section displays the information about each available OpenLab Server/ ECM XT. The Local Configuration item displays the server selected for the current connection (including server URL), and the current connection status. The configured server connection can be tested to make sure the server is available.

The local instance can be connected to any valid server in the list. This can reassign the relationship between workstations or clients with available OpenLab Server or ECM XT.

System Configuration
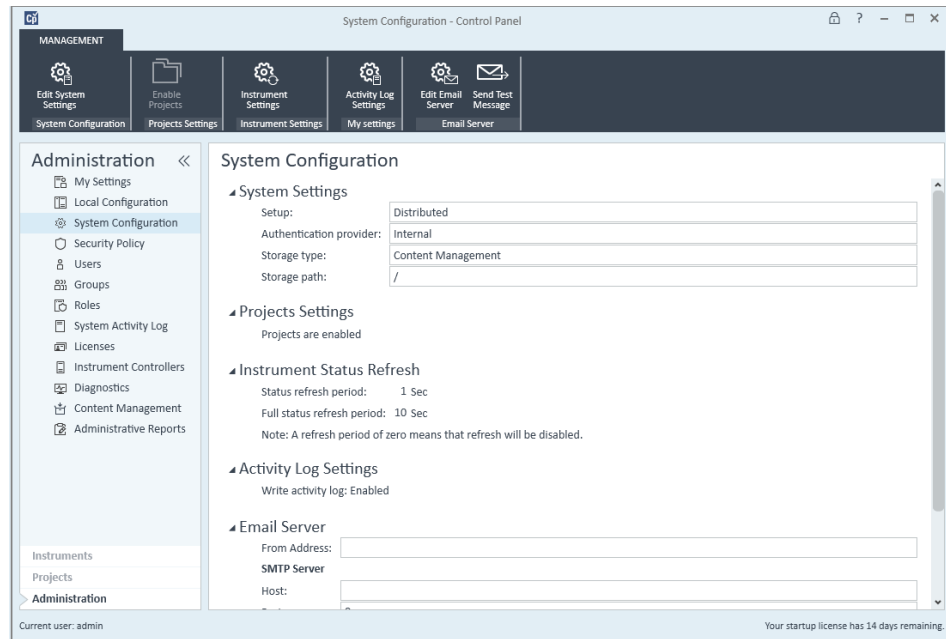
System Settings   System Settings control the configuration of user authentication and data storage for the system. MassHunter supports the following authentication providers:

- **None** – eliminates the requirement for users to provide any credentials to access the system. The generic user name SYSTEM will be used for logs and reports. This option should not be used if Content Management is the selected storage mode.

- **Internal** – the user accounts are created by and stored within OpenLab Server or ECM XT.

- **Windows Domain** – Domain users and groups defined in the Active Directory services can be granted access to MassHunter with the Windows domain controller providing user authentication.

- **ECM** – OpenLab Server is used to authenticate users and passwords. This option is used when OpenLab ECM is also the selected storage type.

Project Settings   Project Settings are used by other data systems that use the Control Panel. For MassHunter, projects are enabled and cannot be disabled.

Instrument Status Refresh   The status refresh interval can be set for instruments and for the full system. The interval can be set to zero to disable status refresh.

Activity Log Settings   The system activity log can be enabled to record all system and user actions in the system activity log. Once logging has been turned on, it cannot be turned off again.

Email Server   You specify the information for the SMTP Server in this section. You also enter a User Name and password for email messages.

Security Policy
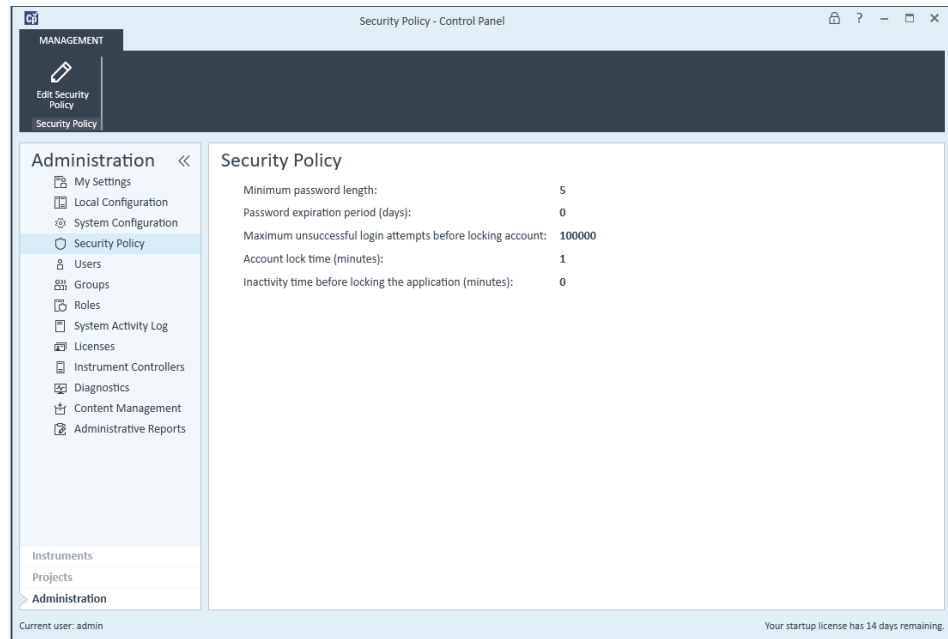
If Windows domain is the authentication provider, the core security policy is controlled by Windows domain. An inactivity timeout can be configured in OpenLab Control Panel. After the selected period of inactivity, the application interface will lock.
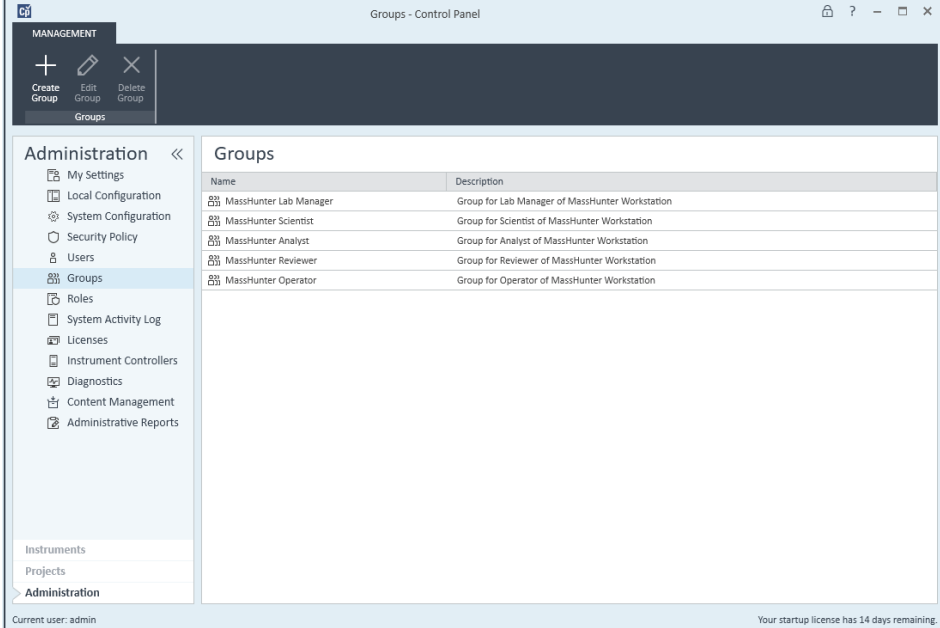
If OpenLab is the authentication provider, the administrator can set a Minimum password length, Password expiration period, and a number for the Maximum unsuccessful login attempts before locking account. The administrator can set an Account lock time, which determines how long the account is frozen after a user has exceeded the maximum number of unsuccessful login attempts.

Users

Users may be imported from the authentication provider or created in OpenLab. Users can be assigned to groups defined within OpenLab.

For each user there is a checklist of roles available in MassHunter. Each role may be assigned or removed for the user. Alternatively, users may inherit roles from the groups to which they have been assigned.

Groups

Groups defined by the authentication provider can be imported and added to the access list for the MassHunter. Additionally, local groups can be defined to group users on the MassHunter access list to simplify role assignment.

For each group there is a checklist of roles available in MassHunter. Each role may be assigned or removed for the group.

The default MassHunter groups are:

- MassHunter Lab Manager
- MassHunter Scientist
- MassHunter Analyst
- MassHunter Reviewer
- MassHunter Operator

These default groups are assigned their respective roles for MassHunter Workstation but are also assigned additional roles from OpenLab that are applicable to the group. For example, members of the MassHunter Lab Manager group are assigned the Lab Manager role by default. In addition, they are assigned the System Administrator role, Instrument Administrator role, and Project Administrator role by default.

The assignment of the roles in the default MassHunter groups can also be modified by an Administrator.

Roles
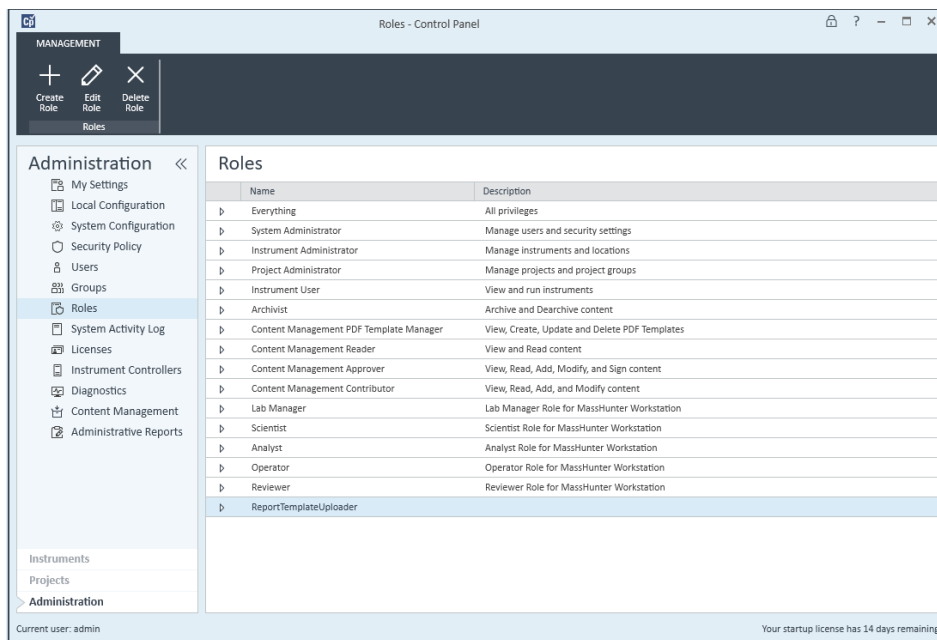
MassHunter helps you to manage what features are available for each user with Roles. Roles are a defined set of privileges with a given name and description. A set of predefined example roles are available at installation, so you can modify these for your needs. Each role (except for the Everything role) can be edited to add or remove specific privileges.

If you want to create a new role based on a predefined role, edit that role and click Save As and save with a new Role name. In that case, the predefined role remains unmodified. Alternatively, new roles can be created from scratch when you click **Create Role** to access the Create Role window where you can select the Role type of Project, Instrument, or Administrative, followed by selecting privileges.

Privileges are organized by privilege groups for better overview. For a role the privilege group may be selected or the privileges individually. See **Chapter 5**, "OpenLab and MassHunter Privileges" for details on all available privileges.

Table 1 lists the default roles installed with MassHunter Networked Workstation. These roles serve as a starting point and can be modified according the operation's needs.

The roles in this list are installed with MassHunter Networked Workstation and are synchronized with the Content Management component of OpenLab Server/ECM XT. Additional roles can be visible in Control Panel if your MassHunter Networked Workstation synchronizes to an OpenLab Server/ECM XT that also serves other chromatography systems.

Table 1. Default MassHunter/OpenLab Roles

Role Type	Role	Description
N/A	Everything	All privileges
OpenLab Control Panel	System Administrator	Manage users and security settings
	Instrument Administrator	Manage instruments and locations
	Project Administrator	Manage projects and project groups
	Instrument User	View and run instruments
	Archivist	Archive and Dearchive content
	Project Content Deletion	Delete content from projects
	Activity Log Access	Activity log access privileges
Content Management	Content Management Approver	View, Read, Add, Modify, and Sign content
	Content Management Contributor	View, Read, Add, and Modify content
	Content Management PDF Template Manager	View, Create, Update and Delete PDF Templates
	Content Management Reader	View and Read content
MassHunter	Lab Manager	Lab Manager Role for MassHunter Workstation
	Scientist	Scientist Role for MassHunter Workstation
	Analyst	Analyst Role for MassHunter Workstation
	Operator	Operator Role for MassHunter Workstation
	Reviewer	Reviewer Role for MassHunter Workstation

System Activity Log

The system can be configured to record all system and user actions in the system activity log (**"Activity Log Settings"** on page 49). When viewing the log, the user can filter based on user name, application, event category, Description, level, and Date/Time. See **"System Activity Log"** on page 177 for more details.

Licenses
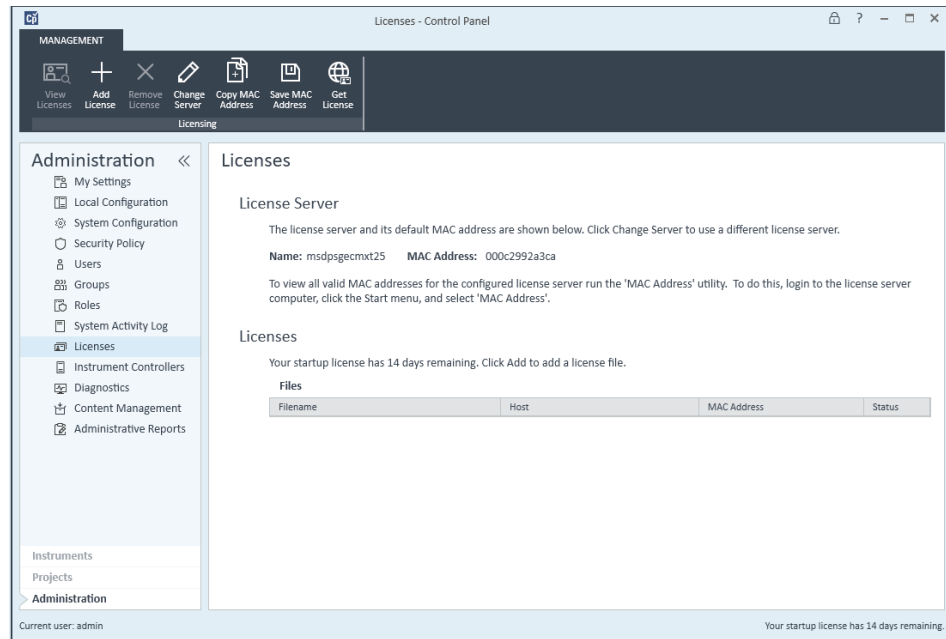
OpenLab Control Panel administers all licenses that are required for instrument modules and add-ons. When starting an instrument, MassHunter automatically checks whether the required licenses are available in the license pool, and reserves the licenses needed to operate the instrument. When stopping the instrument, the freed licenses can be used by other instruments. This licensing strategy introduced with MassHunter enables more effective use of licenses. Licenses for instrument control, drivers and add-ons are floating licenses.

MassHunter Networked Workstation comes with a 60-day Startup License for the system. The expiration period starts with the installation of an application. To run MassHunter Networked Workstation after the startup period, the appropriate licenses must be installed.

License purchases provide an authorization code to enable creation of the appropriate license file or files from Agilent SubscribeNet online service. The **Get License** tool in the OpenLab license administration dialog links directly to the Agilent SubscribeNet site.

On a Workstation, licenses are specific to the computer on which they are installed. Each license file installed is displayed in the licensing user interface with the associated host name, MAC address, and validity status. The name of the current license server is also displayed in the user interface. All installed licenses are listed. Licenses may be returned and reissued for a different computer when a hardware change is required.

The computer MAC address is needed to create a new license, or reassign a license. For ease in getting the correct MAC address, the OpenLab Control Panel includes a tool to copy the MAC address for the PC to the Clipboard or save it to a file. A license can be returned and reissued twice.

OpenLab uses a 3rd party tool called FlexNet Producer Suite from Flexera to manage the licenses. The license server can be local, on an OpenLab Server or ECM XT, or on a separate Flexera license manager server. The license server counts the licenses in use, so starting an instrument control session consumes a license and closing the session releases it to the license pool. You do not need a license for every instrument, but the system will limit the number of concurrently active instruments to the number of instrument licenses.

MassHunter license types

- MassHunter Workstation
- MassHunter Networked Workstation
- MassHunter Quantitative Analysis Workstation
- MassHunter Quantitative Analysis Networked Workstation
- MassHunter BioConfirm Workstation (optional)
- MassHunter BioConfirm Networked Workstation (optional)
- OpenLab Server/ECM XT

The MassHunter Networked Workstation license enables acquisition, data analysis and reporting for the following separation techniques:

- Liquid Chromatography/Time-of-Flight LC/MS (LC/TOF)
- Liquid Chromatography/Quadrupole Time-of-Flight LC/MS (LC/Q-TOF)

One MassHunter Workstation or MassHunter Networked Workstation license is required for each MassHunter Workstation PC and is shared by all required instrument sessions residing on the workstation. A pure data analysis client in a MassHunter Workstation or MassHunter Networked Workstation environment requires licenses.

In summary, License Management in OpenLab Control Panel provides the following functions:

- Add license files to the license server.
- Navigate to the license monitor and view the properties of all licenses installed on a given license server.
- Remove license files from the license server. This may be useful if an invalid license file has been added.
- View or change the license server.
- View, copy, or save the MAC address of the license server.
- Go to the Agilent Electronic Software and License Delivery web page (Agilent SubscribeNet site) to get a license.

The licenses are managed by system administrators. Users with other roles logged into OpenLab Control Panel have read-only access to the licenses screen.

For more details related to licenses, please refer to the current guides for installation and configuration (see **Chapter 13**, "Help and Learning" for more information on available guides).

Instrument Controllers
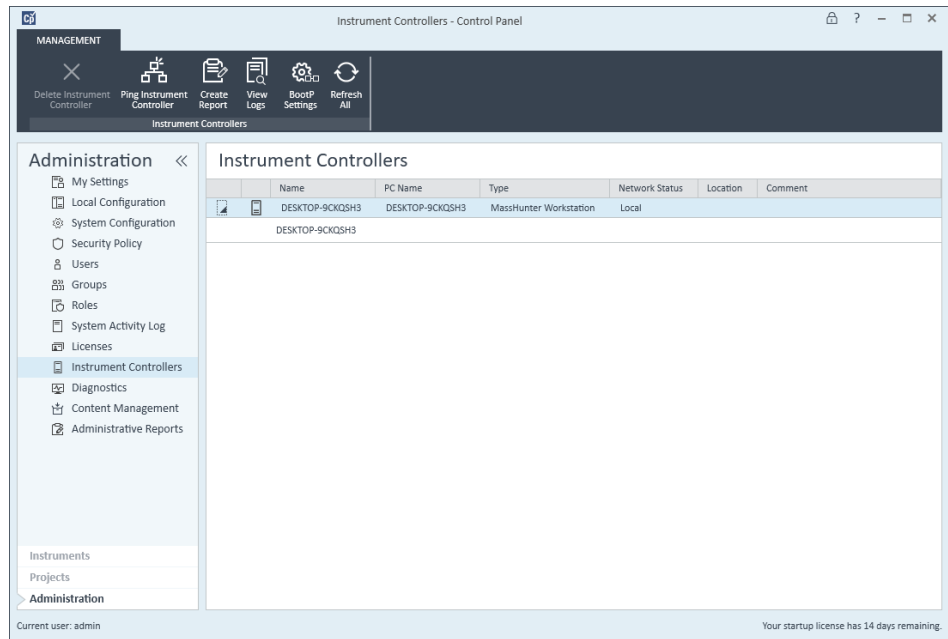
The OpenLab Control Panel facilitates the administration of instrument controllers. The local PC is the instrument controller.

A status table lists all available instrument controllers. For each instrument controller the table displays:

- Name
- PC Name
- Type
- Network Status
- Location
- Comments

MassHunter administrators can perform a series of management functions, as follows:

- Instrument controllers may be deleted from the OpenLab Server or ECM XT.

- Each instrument controller can be pinged to determine its ability to respond on the network.
- A report can be generated that provides the detailed configuration of an instrument controller.
- A detailed list of the log files on each instrument controller can be shown to easily access and collect diagnostic information. All or selected log files can be marked on the list and written to a single file.
- The Instrument Controller is always on the local PC where the Data Acquisition program is operating.

Diagnostics

Version

The version section displays the software version and builds for client and server.

Installed Components

The installed components section displays a table of the software components installed, including the following information:

- Component Name
- Description
- Assembly version
- File version
- Product version

Using the ribbon toolbar some, or all, of the rows may be selected and copied to the clipboard to export the information for diagnostics purposes (for example, during a support call).

The server tools provide the means to diagnose the OpenLab Server or ECM XT. The server can be pinged to determine its ability to respond on the network. A detailed server report can be created.

The system control elements are dedicated to authorized Agilent service providers. A service mode for advanced diagnostics, restricted to Agilent access, opens the Agilent OpenLab Diagnostics Dashboard. The dashboard can modify the local or remote log configuration and create local system diagnostic reports.

Log Files

All local or server log files, or a subset of them, can be selected and saved for diagnostics (for example, during a support call or for documentation purposes).

Content Management

The synchronization of users, groups and permissions to Content Management can be triggered after a change to immediately enforce the changes.

Administrative Reports
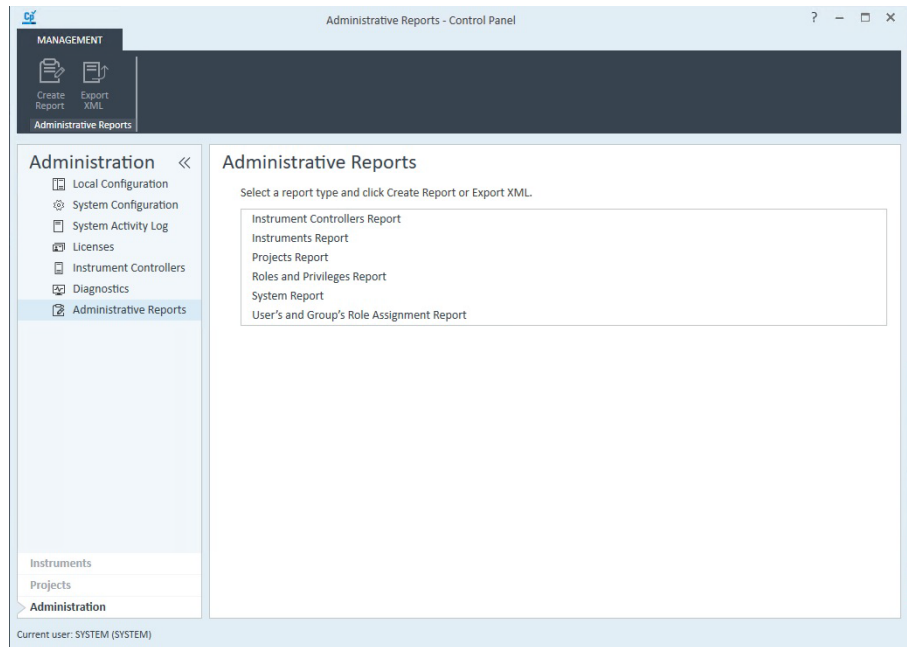
Administrative reports allow detailed system configuration information reports to be created. The following report types are available:

- **Instrument Controllers Report** – lists the controller name, settings (address, description, instruments, machine) and setting value of each instrument controller

- **Instruments Report** – lists the details of the configured instruments, including location and settings

- **Projects Report** – lists all projects and related details, along with the assigned users and groups, and their roles and privileges

- **Roles and Privileges Report** – lists all roles and their associated privileges and privilege group

- **System Report** – provides all available reports in one location

- **User's and Group's Role Assignment Report** – lists users and group information, including roles

The **Create Report** tool opens the selected report in a viewer. The report can be printed to a printer or saved as a file in .pdf, .xlsx or .docx format. The Export XML tool saves reports as XML export files.

5 OpenLab and MassHunter Privileges

Privileges define a technical control within MassHunter, either preventing or allowing the usage of a specific feature or action within the system. Using privileges, a user organization can define what personnel can and cannot do within MassHunter. Privileges are grouped together into what is termed a "role" in order to be assigned to a specific user or group of users. Privileges are organized by **Privilege Groups**.

Please note that:

- Additional Privileges can be displayed if your MassHunter Networked Workstation is connected to an OpenLab Server/ECM XT server that also serves other chromatography systems.

- The Privileges listed in this chapter are sorted by Role types:

    - Project

    - Instrument

    - Administrative

Project Privileges

Some privileges are available only if the associated MassHunter program is installed, such as MassHunter BioConfirm.

E-Signature

Table 2. E-Signature

With this privilege...	The user can...
E-Signature Sign Data Files	Sign CDS-type data files in the Content Management component.
Revoke E-Signature	

MassHunter BioConfirm

Table 3. MassHunter BioConfirm

With this privilege...	The user can...
Adjust delay time	Adjust the delay time of one chromatographic signal to another.
Adjust peak threshold	Adjust the peak threshold in the mass spectrum windows.
Annotate	Apply an annotation to a spectrum or chromatogram.
Assign charge state	Run the Assign Charge State process in BioConfirm.
Assign time range(s)	Assign time ranges in their method for processing.
Audit trail copy to Clipboard	Copy audit trail entries to the Clipboard.
Audit trail review	Mark as "reviewed" audit trail entries.
Calculate DAR	Run the DAR calculation independent from a method workflow.
Calculate signal-to-noise	Run the signal-to-noise calculation independent from a method workflow.
Clear match results	Clear sequence matches that have been made.
Copy settings to method	Copy the deconvolution settings to the method from the deconvoluted spectrum.
Copy to Clipboard	Copy any spectrum, chromatogram, or table to the Clipboard.
Create biomolecule	Create a biomolecule that was not created during the automated workflow process.
Deconvolute	Run protein deconvolution independent of a method workflow.

Table 3. MassHunter BioConfirm (continued)

With this privilege...	The user can...
Delete biomolecules	Delete biomolecules from the biomolecule table.
Delete deconvoluted peak	Delete a deconvoluted peak from the deconvoluted spectrum.
Delete user plot	Delete a spectrum or chromatogram plot.
Display options	Change the display options of a chromatogram or spectrum.
Export	Export data to a file.
Export audit trail report	Export audit trail entries to a file.
Extract chromatograms	Extract chromatograms independent from a method workflow.
Extract spectra	Extract spectra from a chromatogram independent of a method workflow.
Find peptides	Run the Find Peptides action in the Protein Digest workflow.
Import to project	Import data from the local drive to the content management server (Networked Workstation only).
Include in %Quant	Include a biomolecule in the relative quantitation %Quant calculation.
	Include a biomolecule in the Sequence Coverage calculation.
Integrate	Integrate a chromatogram independent of a method workflow.
Manual integration	Manually integrate chromatograms in their processing.
Mass caliper tool	Use the mass caliper tool and apply the caliper value to the spectrum.
Match sequence(s)	Run the Match sequence(s) action in a workflow.
Method redo	Redo the last method parameter undo change.
Method undo	Undo the last method parameter change.
Modify method	Change the method parameters.
Open chemical data dictionary editor	Open the Chemical Data Dictionary editor to be able to change the chemical information used in processing.
Open previous method version	Open a previous version of a method in BioConfirm (Networked Workstation only).
Open previous results version	Load previous versions of the results saved to a data file (Networked Workstation only).
Open Sequence Manager	Open the Sequence Manager and edit/add sequences for use in processing.

Table 3. MassHunter BioConfirm (continued)

With this privilege...	The user can...
Print	Print a table or plot.
Print audit trail report	Print audit trail entries to a printer.
Restore method values	Reset the method parameters back to what they were when the method was first opened.
Run workflow	Execute a method's workflow.
Save as method	Save method as a new name.
Save method	Save changes to a method.
Save results	Save the current results into the data file.
Select custom workflow	Select and run the Custom workflow in BioConfirm.
Select standard workflow	Select any workflow other than the Custom workflow.

MassHunter LC/MS Acquisition

Table 4. MassHunter LC/MS Acquisition

With this privilege...	The user can...
Apply Method	Send the current method parameters to the LC and MS instruments.
Print Method Report	Print the acquisition method currently shown in the Method Editor window. It prints the current parameters even if these parameters have not been saved.
Load Method	Load a method. The parameters in the Method Editor window are updated with the parameters that are saved in the method folder.
Run Samples	Run a single sample using the Sample Run window.
Save Method	Save any changes to the method to the current method name. It may open the Save Method As dialog box if you cannot save to the current method.
Save Method As	Save any changes to the method to a new method name. The Save Method As dialog box is opened.
Generate Worklist Report	Print a report for the current worklist. You can print a worklist that has not been saved yet.
Load Worklist	Load a worklist.
Run Worklist	Run a worklist.

Table 4.   MassHunter LC/MS Acquisition (continued)

With this privilege...	The user can...
Save Worklist	Save changes to the current worklist.
Save Worklist As	Save a worklist to a new worklist name. The Save Worklist Files dialog box is opened to allow you to enter a new worklist name.
Save Worklist Template As	Save a worklist template which can be used when you create a new worklist.
Show/Hide/Order Columns	Mark which columns are visible in the Worklist window and the order of those columns.
Worklist Run Parameters	Change the parameters in the Worklist Run Parameters dialog box. These parameters include the parts of the method to run, the Method Paths, the Scripts to execute before and after the worklist, and more.
Add Custom Script	Add or insert a custom script in a worklist. You can select a new File in the Select Script dialog box. By default, the software includes scripts in the MS_Acq_Scripts.exe file.
Add Script	Add or insert a script in a worklist.
Export data file Audit Trail	Export the information about data files that is visible in the Audit Trail window to a file.
Export method Audit Trail	Export the information about methods that is visible in the Audit Trail window to a file.
Export worklist Audit Trail	Export the information about worklists that is visible in the Audit Trail window to a file.
Export worklist template Audit Trail	Export the information about worklist templates that is visible in the Audit Trail window to a file. A worklist template is a saved worklist that can be used when you create a new worklist.
Export Study Audit Trail	Export the information about studies that is visible in the Audit Trail window to a file.
Print data file Audit Trail	Print the information about data files that is visible in the Audit Trail window to a printer.
Print method Audit Trail	Print the information about methods that is visible in the Audit Trail window to a printer.
Print worklist Audit Trail	Print the information about worklists that is visible in the Audit Trail window to a printer.
Print worklist template Audit Trail	Print the information about worklist templates that is visible in the Audit Trail window to a printer. A worklist template is a saved worklist that can be used when you create a new worklist.

Table 4. MassHunter LC/MS Acquisition (continued)

With this privilege...	The user can...
Print Study Audit Trail	Print the information about studies that is visible in the Audit Trail window to a printer.
Review data file Audit Trail	Mark as reviewed entries in the data file's audit trail.
Review method Audit Trail	Mark as reviewed entries in the method's audit trail.
Review worklist Audit Trail	Mark as reviewed entries in the worklists audit trail.
Review worklist template Audit Trail	Mark as reviewed entries in the worklist template's audit trail.
Review Study Audit Trail	Mark as reviewed entries in the Study's audit trail.
Import Data	Import data files into a project in the Import Files application.
Import Method	Import methods into a project in the Import Files application.
Import Worklist	Import worklists into a project in the Import Files application.
Unlock and close application	Unlock the application that has been locked by another user and assume any runs currently in progress or pending.
Close Study Manager	Close the Study Manager program.
Create Study	Create a study to submit to the Study queue.
Start Study Queue	Start running the studies in the Study queue.
Stop Study Queue	Stop running the studies in the Study queue.
Reorder Study Queue	Change the order of studies listed in the Study queue.
Remove Any Study from Queue	Remove a study from the Study queue.
Edit Any Study	Edit a study in the Study queue.
Autotune	For TQ: Autotune the instrument and access the Early maintenance feedback counters.
Manual Tune	For TQ: Autotune the instrument and access the Early maintenance feedback counters and access Advanced override settings section.
Modify Compound Database	Change the database that is used in MRM Database Browser.
Run Optimizer Wizard	Run optimizer using the Method Optimizer window.
Print compound database audit trail	Print the information about the compound database that is visible in the Audit Trail window to a printer.
Export compound database audit trail	Export the information about compound database that is visible in the Audit Trail window to a file.

Table 4. MassHunter LC/MS Acquisition (continued)

With this privilege...	The user can...
Review compound database audit trail	Mark as reviewed entries in the compound database's audit trail.
Generate Method Report	Print the acquisition method currently shown in the Method Editor window. It prints the current parameters even if these parameters have not been saved.

MassHunter Library Editor

Table 5. MassHunter Library Editor

With this privilege...	The user can...
Calc monoisotopic mass	Calculate the mass of the compound's molecule containing only the most abundant isotope of each element.
Convert library	Convert the old format library with User Index to either Retention Index or Retention Time.
Create new library	Create a brand-new library.
Delete	Delete a library entry from the compound table or a spectrum from the Spectrum View.
Filter spectrum peaks	Remove peaks with height below absolute or relative filtering threshold from spectrum.
Import JCAMP	Import JCAMP files into a custom library.
New compound	Add a new entry in the compound table.
New spectrum	Add an empty spectrum for the entry in the compound table.
Open library	Open an existing library, which can be: MassHunter Libraries (*.mslibrary, *.reflibrary, *.pattern.reflibrary.xml) MassHunter PCDL (*.cdb) Agilent ChemStation Library (*.L).
Paste	Paste either a library entry in the compound table or a spectrum in the Spectrum View.
Redo	Restore the action that were previously undone using an undo.
Save library	Save changes to the current library.
Save library as	Save the library as a new name.
Set compound property	Change the compound property either from unit mass to accurate mass or from accurate mass to unit mass.

Table 5. MassHunter Library Editor (continued)

With this privilege...	The user can...
Set library property	Change the library property either from unit mass to accurate mass or from accurate mass to unit mass.
Set spectrum property	Change the spectrum property either from unit mass to accurate mass or from accurate mass to unit mass.
Synthesize spectra	Generate the reference pattern spectrum for the library entry based on the selected formula.
Undo	Reverse the previous action.

MassHunter Quant

Table 6. MassHunter Quant: Batch Analysis

With this privilege...	The user can...
Add sample to batch	Add a sample to the batch.
Add samples to batch	Adds specified sample(s) into the current batch and imports the sample information.
Analyze batch	Analyze an entire batch.
Apply method to all samples	Apply working method dataset to all samples.
Audit Trail Copy	Copy audit trail entries to the Clipboard.
Audit Trail Export	Export audit trail entries to a file.
Audit Trail Print	Print audit trail entries to a printer.
Audit Trail Review	Mark as reviewed audit trail entries.
Audit Trail View	Show audit trail.
Calibrate batch	Calibrate an entire batch or a set of target compounds by replacing or averaging the calibration table.
Clear batch results	Clear batch results. Calibration is not cleared.
Clear calibration	Clear the calibration table for an entire batch or for a subset of the compounds.
Clear manual integration	Clear the results of manual integration and revert back to the original peak integration results.
Integrate batch	Integrate an entire batch, a sample or a target compound.
Manually integrate - apply ISTD RT to target	Apply ISTD RT to Target.

Table 6. MassHunter Quant: Batch Analysis (continued)

With this privilege...	The user can...
Manually integrate - apply target RT to qualifiers	Apply Target RT to Qualifier.
Manually integrate - drop baseline	Drop baseline to the user specified y.
Manually integrate - merge	Merge primary peak with nearest peak.
Manually integrate - snap baseline	Snap baseline to the meeting points on chromatogram.
Manually integrate - split	Split primary peak into two peaks.
Manually integrate qualifier peak	Manually integrate a target compound peak.
Manually integrate target peak	Manually integrate a target compound peak for method setup.
New batch table	Create new empty batch.
Open and apply method from batch	Import method from batch without leaving BAG mode.
Open and apply method from file	Import method from file without leaving BAG mode.
Quantitate batch	Quantitate an entire batch, a sample or a target compound.
Remove calibration	Remove the calibration table for an entire batch or for a subset of the compounds.
Remove sample	Remove a sample from batch table.
Remove samples	Remove multiple samples.
Restore integration parameters	Restore the choice of integrator and integrator parameters to the values that are specified in the method for a given compound.
Restore qualifier integration parameters	Restore the integrator parameters to the values that are specified in the method for a given qualifier.
Save batch table	Save batch tables.
Save batch table as	Save batch tables to a different name.
Select peak	Manually select a peak, overriding the automatic selection either in the context of method setup or in batch analysis.
Set integration parameters	Change integration parameters for a given compound in a given sample.
Set integrator	Change choice of integrator for a given compound in a given sample.
Set peak attribute	Change these peak columns: UserCustomCalculation, UserCustomCalcuation1-4, and PromoteHit.
Set peak attributes	Change multiple peak columns.

Table 6. MassHunter Quant: Batch Analysis (continued)

With this privilege...	The user can...
Set peak qualifier attribute	Change peak qualifier UserCustomCalculation.
Set qualifier integration parameters	Change integration parameters for a given qualifier in a given sample.
Set sample attribute	Change sample columns.
Set target qualifier attribute	Change a single column in the target qualifier table in batch-at-a-glance mode.
Zero out peak	Remove a target compound peak by manually integrating the peak to get a zero response.

Table 7. MassHunter Quant: Batch Review

With this privilege...	The user can...
Open batch table	Open a batch file.

Table 8. MassHunter Quant: Convert Samples

With this privilege...	The user can...
Convert samples	Convert samples to Indexed Data or SureMass, and convert Variant data to MassHunter format.

Table 9. MassHunter Quant: Method Development

With this privilege...	The user can...
Add method calibration	Add a calibration level to a method.
Add method target compound	Add a new compound in the Quant method editor.
Add method target qualifier	Add a target qualifier to the working method dataset.
Append method from acquisition	Append compound list from the acquisition method to the existing method.
Append method from file	Append a list of compounds from xml file to the existing method.
Append method from scan data	Append a list of compounds derived from scan data to the existing method.
Average qualifier ratios	Run the "average qualifier ratios" utility in the Quant method editor.
Average retention time	Run the "average retention time" utility in the Quant method editor.

Table 9. MassHunter Quant: Method Development (continued)

With this privilege...	The user can...
CmdAverageCalibrationReplicates	Make the calibration table non-redundant by averaging the replicates for each level into a single calibration row.
Copy calibration levels	Use the "copy calibration levels" function in the Quant method editor.
Create acquisition method from SIM method	Create an optimized SIM acquisition method from a Quant SIM method.
Create dynamic targets	Automatically define alternative quantitation target ions for user-defined targe compounds.
Create levels from calibration samples	Run the "Create levels from calibration samples" feature in the Quant method editor.
Create levels from file	Create a set of calibration rows from a given level concentration file.
Create library method	Create a new library method.
Create method from CEF file	Create a list of target compounds from Compound Exchange Format (CEF) files.
Create method from chromatographic data	Create a list of target compounds from Chromatographic data.
Create method from Library - GC	Create a list of target compounds from a GC library.
Create method from Library - LC	Create a list of target compounds from an LC library.
Create method from scan data	Create a list of target compounds from scanned data.
Create new method	Create a brand-new method.
Create serial dilution levels	Create a set of calibration levels by serial dilution.
Create serial dilution levels for compounds	Create a set of calibration levels by serial dilution for compounds.
Duplicate compounds	Duplicate the selected compounds.
Edit report method	Edit report method.
Import method from acquisition	Import method data from the acquisition method.
Import method from batch	Import the method from a batch file.
Import method from file	Import method from file to working method dataset.
Import method from sample	Import the specified sample's method to working method dataset.
Open library method	Open library method file.
R. T. Calibration	Calculate RT calibration.

Table 9. MassHunter Quant: Method Development (continued)

With this privilege...	The user can...
Remove method calibration	Remove calibration from working method dataset.
Remove method target compound	Remove a target compound from working method dataset.
Remove method target qualifier	Remove a target qualifier from working method dataset.
Save library method	Save library method to file.
Save method as	Save a method as a file.
Set batch extended property	Change global values of the batch.
Set calibration attribute	Change calibration attribute.
Set calibration level enable/disable	Set Calibration Level Enable/Disable
Set method calibration attribute	Change column value of the calibration row in the method.
Set method target compound attribute	Change target compound's column in method dataset.
Set method target compound attributes	Maintain and access compound attributes.
Set method target qualifier attribute	Change target qualifier's column.
Set method target qualifier attributes	Change multiple target qualifiers' column.
Set target compound attribute	Maintain and access compound attributes.
Setup manually integrate qualifier peak	Manually integrate a qualifier peak for method setup.
Setup manually integrate target peak	Manually integrate a target compound peak for method setup.
Setup reference spectra	Set up reference spectra for method compounds.
Shift retention time	Shift the retention time for a target compound by replacing the nominal value in the method by the retention time of the largest peak in the compound chromatogram.
Start method editing	Edit quantitation methods in the Method Editor.
Swap qualifier with quantifier	Exchange selected qualifier with quantifier.
Update mass assignment	Update the mass assignment for a target compound by replacing the nominal value in the method by the MZ of the largest peak in the spectrum, averaged over the compound chromatogram peak.

Table 9. MassHunter Quant: Method Development (continued)

With this privilege...	The user can...
Update qualifier ratio	Update the qualifier ratio for a target compound by replacing the nominal response ratio for each qualifier in the method by the ratio of the largest qualifier peak to the largest target peak.
Update qualifier ratios	Update multiple qualifier ratios for a target compound by replacing the nominal response ratio for each qualifier in the method by the ratio of the largest qualifier peak to the largest target peak.
Update retention time	Update the retention time for a target compound by replacing the nominal value in the method by the retention time of the largest peak in the compound chromatogram.
Update retention time from ISTD	Update the retention time for a target compound by replacing the nominal value in the method by the retention time of the largest peak in its ISTD compound chromatogram.
Update retention times	Update the retention time for a target compound by replacing the nominal value in the method by the retention time of the largest peak in the compound chromatogram.

Table 10. MassHunter Quant: Report

With this privilege...	The user can...
Generate method report	Generate quantitation method report.
Generate report	Generate quantitation result report.

Table 11. MassHunter Quant: Upload Files

With this privilege...	The user can...
Upload batch files	Upload batch files to the Content Management server (Networked Workstation only).
Upload methods	Upload methods to the Content Management server (Networked Workstation only).
Upload report templates	Upload report templates to the Content Management server (Networked Workstation only).
Upload samples	Upload samples to the Content Management server (Networked Workstation only).
Upload Unknowns Analysis files	Upload Unknown Analysis files to the Content Management server (Networked Workstation only).

MassHunter Report Builder

Table 12. MassHunter Report Builder

With this privilege...	The user can...
Audit Trail Copy	Copy the Audit Trail.
Audit Trail Export	Export the Audit Trail.
Audit Trail Print	Print the Audit Trail.
Audit Trail Review	Review the Audit Trail.
Audit Trail View	View the Audit Trail.
Delete item(s)	Delete the selected items from the template.
Insert item	Insert an item in the template.
Move down	Move a selected item down in the template.
Move up	Move a selected item up in the template.
New template file	Create a new template file.
Open template file	Open a template file.
Redo	Redo a previously undone action.
Same template as	Save the template file with a different file name.
Save template file	Save the template file.
Set columns	Set or change the table column attribute.
Set data binding	Set or change the data binding.
Set graphics parameter	Set or change the graphics parameter.
Set localized text	Set or change localized text.
Set property	Set or change the property of the selected item.
Set table column count	Set or change the number of table columns.
Set table relative column widths	Set or change the relative column width in the table.
Undo	Undo the previous action.

MassHunter Unknowns Analysis

Table 13. MassHunter Unknowns Analysis

With this privilege...	The user can...
Add component	Add a new component to the analysis.
Add hit	Add a new hit to the specified component.
Add library search method	Add a library search method.
Add manual component	Perform spectrum extraction to create manual component.
Add peak detection method	Add a peak detection method.
Add samples	Add the samples to the current batch.
Analyze - all samples	Perform the entire analysis for all samples.
Analyze - specified samples	Perform the entire analysis for specified samples only.
Audit Trail Copy	Copy audit trail entries to the Clipboard.
Audit Trail Export	Export audit trail entries to a file.
Audit Trail Print	Print audit trail entries to a printer.
Audit Trail Review	Mark as reviewed audit trail entries.
Audit Trail View	Show audit trail.
Blank subtract - all samples	Perform blank hit subtraction for all samples
Blank subtract - specified samples	Perform blank hit subtraction for specified samples only.
Clear all results	Clear the results for all samples.
Clear results	Clear the results for specified samples only.
Compress analysis	Compress the analysis to reduce in size.
Detect peak from - specified samples	Perform deconvolution or TIC analysis for selected samples only.
Detect peaks - all samples	Perform deconvolution or TIC analysis for all samples.
Edit report method	Edit the method for reporting.
Generate report	Generate Unknown Analysis results report.
Identify compounds - all samples	Perform library search to identify compounds for all samples.
Identify compounds - specified samples	Perform library search to identify compounds for specified samples only.
Import Quantitative Analysis batch	Import a quant batch to the current analysis.

Table 13. MassHunter Unknowns Analysis (continued)

With this privilege...	The user can...
Import Quantitative Analysis method	Import an existing quant method to apply on the current analysis.
Import Quantitative Analysis method from batch	Import the quant method used in an existing quant batch to apply on the current analysis.
Load method - all samples	Load Unknowns method to all samples.
Load method - specified samples	Load Unknowns method to specified samples only.
New analysis	Create a brand-new Unknown Analysis batch.
Open analysis file	Open an existing analysis batch.
Remove library search method	Remove library search method.
Remove peak detection method	Remove peak detection method.
Remove samples	Remove the selected samples from the current analysis.
Save analysis	Save the changes to the current analysis.
Save analysis as	Save the analysis as a new name.
Save method	Save the method of the specified sample to a method file.
Set auxiliary method	Set auxiliary method.
Set best hit	Mark the specified hits as best hits.
Set blank subtraction method	Set blank subtraction method.
Set component attributes	Set component values for Component Name, Best Hit, User Custom Calculation.
Set component visible/invisible	Set the specified components visible/invisible.
Set exact mass attributes	Set the exact mass field values.
Set hit attributes	Set the hit values for User Defined and User Custom Calculation.
Set hit visible/invisible	Set the specified hits visible/invisible.
Set identification method	Set the identification method.
Set ion peak attributes	Set Ion Peak values for User Custom Calculation.
Set library search method	Set the library search method.
Set method	Set the unknowns analysis method.
Set peak detection method	Set the peak detection method.
Set sample attributes	Set Sample values.

Table 13. MassHunter Unknowns Analysis (continued)

With this privilege...	The user can...
Set target attributes	Modify Target Compound table.
Set target match method	Set the target match method.
Target match - all samples	Run Target Match for all samples.
Target match - specified samples	Run Target Match for specified samples

Project Management

Table 14. Project Management

With this privilege...	The user can...
View project or project group	View a project and project details but cannot edit. Note: This privilege is required for all users who are expected to use that project. If the permission is removed, the project is hidden from that user.
Manage project or project group	Create or edit project properties and move the project, but cannot access settings.
Edit content of project	Create new versions of documents (e.g., data, methods, or templates).
Delete content of project	
Manage project or project group access	View and edit the project access settings.

Instrument

Instrument Management

Table 15. Instrument Management

With this privilege...	The user can...
View instrument or location	View and access a location in the tree, but not edit access security, can view properties.
Manage Instrument or location	Create and move locations and edit properties (such as name and description).
Manage instrument or location access	User can view and edit the location access settings.
Run instrument	Start an instrument session, only if the user also has the View Project or project group privilege.
Service instrument	User can lock or unlock an instrument (to service it).

Administrative

System Administration

Table 16. System Administration

With this privilege...	The user can...
Manage printers	Add/remove printers and print server
Edit activity log properties	Change the Activity log Settings in the Control Panel (that is, turn logging on for the System Activity Log)
Create administrative reports	Create any of the system admin reports
Manage system components	Install/remove components (applications)
Manage security	Change security settings and edit (add, change, etc.) users, groups, and roles.
NOTE	A user with this privilege can grant himself access to all settings in Shared Services. Be careful who you grant the Manage Security privilege.
Manage instrument controllers	Edit Instrument Controllers in the Control Panel
Unlock any locked UI	Close the Control Panel and restart as another user.
View Activity Log	

Content Management

Table 17. Content Management

With this privilege...	The user can...
Archive content	Archive the content of the Content Management repository
Manage Templates	N/A

6 Software User Interface

MassHunter Workstation consists of four application subsystems, called Engines, that share a common client user interface design:

- MassHunter Acquisition Engine
- MassHunter Worklist Engine
- MassHunter Data Analysis Engine
- MassHunter Reporting Engine

The software has been designed to provide an easy to understand user interface aiding the transition for existing MassHunter users and enabling new or occasional users to easily start using the software.

General Concepts

The MassHunter user interface (UI) is designed with focus on ease of learning and work efficiency. A consistent navigation and coloring scheme help users to find their way; Microsoft ribbon controls make it easy for users to quickly learn how to use the software. The main UI elements are the ribbon toolbar and windows that can be organized in layouts.

Ribbon Toolbar

In the OpenLab Control Panel and some MassHunter programs, the ribbon toolbar is a graphical control element that is always shown on top of the application window. The ribbon consists of graphical controls grouped by functions. Main commands are always visible, giving access to functions which are generally needed. Shortcut menus appear only when a user needs them in a specific context, based on the focused (selected) window.

Layouts and Windows

The workspace consists of single elements called windows organized in panes. A pane is a single UI component available in a certain task context. Layouts define which windows are shown in the workspace and how they are positioned. Each application provides a set of predefined layouts that can be selected from the Layout ribbon group. Each layout combines the elements required for a particular user task or workflow. Users may design their own layout and organize windows to meet the workflow-specific needs. They may modify predefined or create new layouts. Modifications as well as new layouts can be saved for future use. Each window can be individually sized and docked within the workspace, either side by side or tabbed. Defaults can be restored at any time.

MassHunter Acquisition

The Acquisition Engine of MassHunter Workstation provides all the tools for instrument control, automation and monitoring. The MassHunter Workstation instrument control subsystem is designed to make use of drivers written to comply with the RC.Net Standard and C# instrument drivers. Agilent makes this standard available to both Agilent and non-Agilent developers. In addition to simple time vs. response detectors, the system supports 3D MS and UV signal sources. More details on the supported instrument hardware are provided in **"Instrument Control"** on page 187.

The Instrument Status window displays the intuitive instrument dashboard consisting of individual panes, one per instrument module, which graphically show the most important information for each module – even visible from a distance.

The Acquisition client user interface (referred to as the "acquisition client") comprises all the tools required to meet the needs of the standard workflows used in most laboratories. This includes setting up and running data acquisitions, from simple single sample analyses to complex Worklists.It has been designed to provide flexibility to customize the user interface to meet an individual's needs.

The **Home** tab hosts the main user interface elements for control, automation and monitoring. It offers 4 different layouts that support users in their daily tasks – **Method** (method viewing and editing), **Single Sample**, **Status** (all windows), and **Worklist** (worklist setup and submission). The context-sensitive **Control** tab provides access to functions that are specific to the instrument. The content depends on the individual instrument type.

The available windows in all layouts are: Instrument Status, Method Editor, Sample Run, Chromatogram Plot, Actuals, Spectrum, and Worklist. For LC/TQ instruments, the Method Optimizer window and the dMRM Method Split window are available.

The default layouts are:

- **Method** layout – Instrument Status and Method Editor
- **Single Sample** layout – Instrument Status and Sample Run
- **Status** layout - all windows
- **Worklist** layout – Instrument Status, Chromatogram Plot, Worklist, and Spectrum.

The selected project is displayed in the title bar of the window.

MassHunter BioConfirm (optional)

Agilent MassHunter BioConfirm provides automated and interactive protein and oligonucleotide confirmation for TOF and Q-TOF data, with the following features:

- Automated post acquisition data analysis and report generation.
- Biomolecule-centric navigation for peptides, proteins, released glycans, and oligonucleotides.
- Intact protein UI functionality, most notably the visualization of ion sets and showing deconvoluted spectra in a separate window.
- Protein sequence matching, including target protein and potential modifications for intact protein and protein digest sequence types.
- Finding glycans from the target glycan source.
- Finding targets and impurities for oligonucleotides.
- Confirming sequences for oligonucleotides.
- Letting you assign site specific variable modifications for protein and protein digest sequence types.
- MFE algorithm - peptide feature extractor for finding peptides in complex LC MS/MS data.
- Relative protein level can be measured either by height from the deconvoluted spectrum or height/area of EIC using all ion set peaks.
- Protein biomolecule quality filters, which prevent "noise" peaks from the deconvoluted spectrum being considered a biomolecule, by requiring sufficient evidence in the m/z spectrum.
- Protein sequence editing/matching.
- Oligonucleotide sequence editing/confirming.
- Viewing fragment confirmation for oligonucleotides analyzed with Sequence Confirmation experiment.
- Linked navigation between biomolecules with assigned protein digest matches and peptide sequence within the Sequence Coverage Map.
- Multiple-enzyme digestion sequence coverage display (where each data file represents a single digestion result).

MassHunter Quantitative Analysis

Quantitative Analysis 12.0 is launched from the OpenLab Control Panel. You can run Quantitative Analysis in either the Classic user interface or the Quant-My-Way user interface.

The Classic user interface has a look and feel similar to the user interface offered in Quantitative Analysis B.08.00, with tools and options located in a menu bar.

The Quant-My-Way user interface has a modern ribbon, with tools and options located on tabs and ribbons instead of in a menu bar.

You can create project-specific desktop icons that start a project in either Classic or Quant-My-Way user interface. When you start the Quantitative Analysis program from these icons, the default values and some of the features are customized to the selected instrument type.

The desktop icons are named for the type of instrument and the user interface.

MassHunter Report Builder

The MassHunter Report Builder software is installed with MassHunter Quantitative Analysis and MassHunter BioConfirm. MassHunter Report Builder is started from the Control Panel or within MassHunter BioConfirm to edit a template.

The Report Builder includes three tabs: **File**, **Home**, and **Tools**.

The **File** tab lets you open and save report templates.

The **Home** tab contains five sections: **Edit**, **Font**, **Alignment**, **Colors**, and **Insert**.

- Use the **Edit** section to delete an element.
- Use the **Font**, **Alignment**, and **Colors** sections to set the font styles, results alignment, and the fore color and back color in the report template.
- Use the **Insert** section to insert **Page**, **Textbox**, **Image**, **List**, **Table**, and **Scriptbox** elements into a report template.
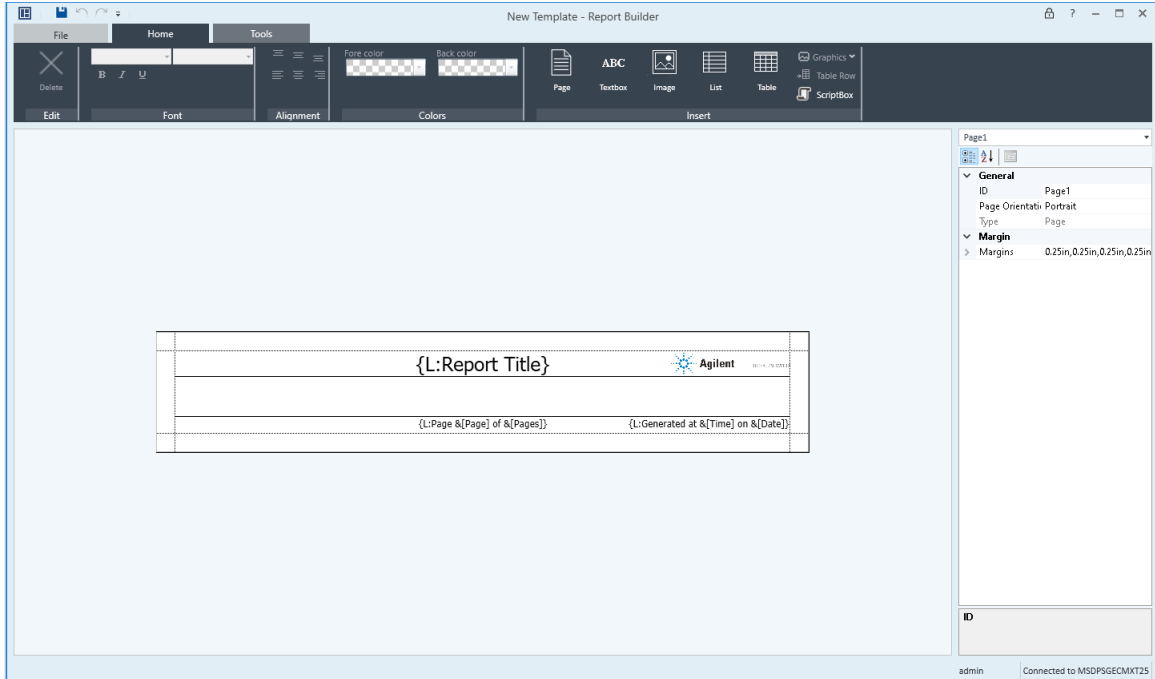
The **Tools** tab contains Audit Trail tool to view the audit trail for the report template.

MassHunter Qualitative Analysis

MassHunter data files created by MassHunter Acquisition 12 and stored in OpenLab Server / ECM XT can be opened by MassHunter Qualitative Analysis 10.0 as long as a copy of the data file has been downloaded from the OpenLab Server / ECM XT. When downloaded via the Content Browser web interface, the data may need to be unzipped before Qualitative Analysis can open the file.

MassHunter Qualitative Analysis doe not contain any technical controls or audit trails which capture how the data has been processed. As such, procedural controls must be implemented when processing any data.

7 Data Acquisition

During data acquisition, all signals acquired by the analytical instrument are transmitted to MassHunter Workstation electronically (raw data) and stored as part of the data file. For detectors without a digital interface to the MassHunter Workstation, an analog to digital converter (A/D converter) can supply the digital signal for storage.

For Q-TOF, when you are in the Tune context, you use the Tune window.

User Interface

Main Window

When you first start the Data Acquisition software, the main window appears. You do almost all of your work within the different windows of this main window. These windows provide the tools to set up acquisition methods, run samples interactively or automatically, monitor instrument status and monitor runs.
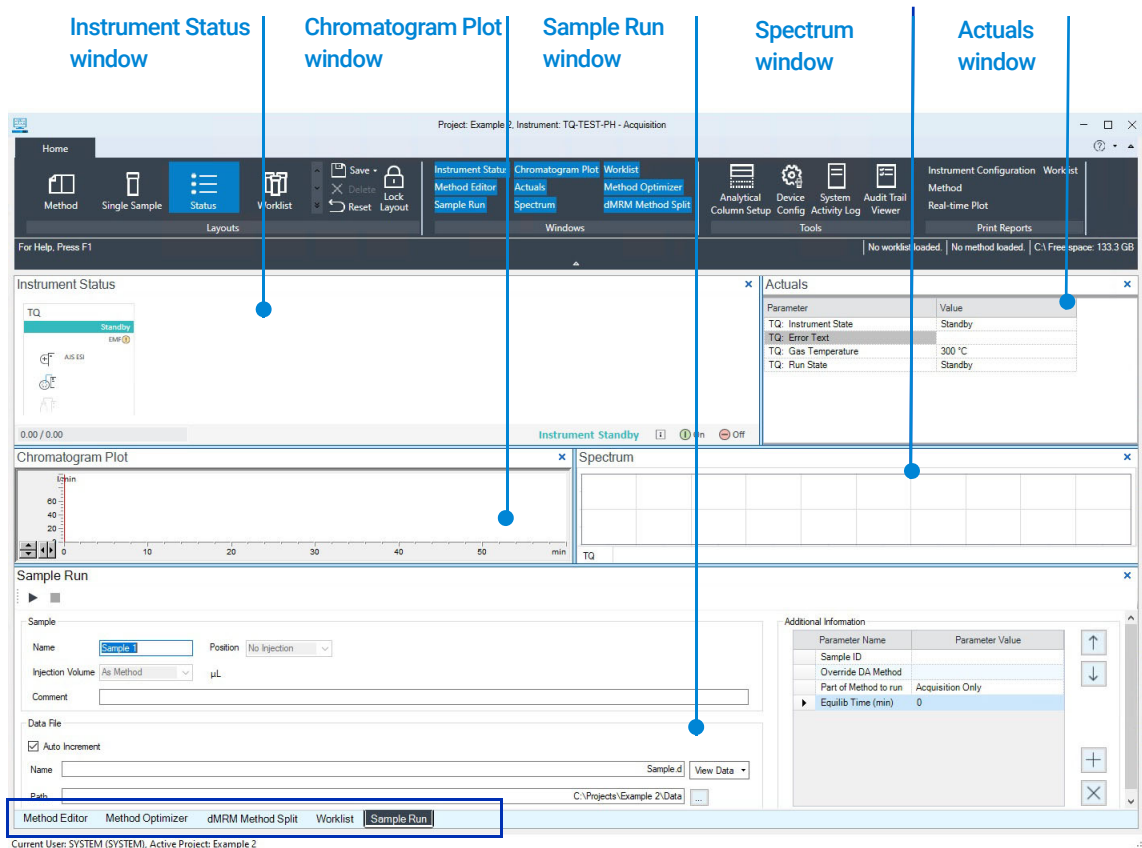
Figure 1. Main window of the TQ Data Acquisition software. Method Editor, Method Optimizer, dMRM Method Split, Worklist, and Sample Run windows are tabbed here. These windows are "sharing" this space. You click the tab to switch to a different window.

Show/hide the windows   You can show one window at a time on the screen or all nine windows. To show or hide a window, you click the icons in the Windows section on the ribbon. You can also hide a window by clicking **X** in the upper right corner of the window.

When you click a window, the active window changes. Press **F1** to obtain help on the active window. You can also drag a window border to resize the window. If you double-click the title of the window, the window "floats" outside of the main window. You can double-click the title bar again to "dock" the window.

Instrument Status window

The status of the instrument is continually monitored and updated on the Instrument Status window. The instrument dashboard gives a view of the available states of each module of the instrument. Both setpoints and instrument actuals are available at a glance. Icons reveal more information on installed accessories, such as interface boards, RFID tags or flow cells. If users have appropriate permissions, they can also use instrument direct control to help with maintenance or manual tasks.

Color-coded status indicators display the overall status of each module and for the instrument as a whole. A small horizontal bar is available for each instrument module in the Instrument Status dashboard.

The status modes are described in **"Instrument Status Displays"** on page 38.

This window displays each device's current status both as text and by its color-coding.

Actuals window

With this window you view the current value of selected instrument parameters. You can change the color choice and add conditional formatting in the **Actuals Selection** dialog box.

Chromatogram Plot and Spectrum windows

One or more display windows are used to monitor the data being acquired by the instrument in real time. The data are displayed in real measurement units such as milli Absorbance Units (mAU), Volts (V), degrees Celsius (°C), pico Amps (pA) or bar.

The Chromatogram Plot window can show multiple overlaid chromatographic or instrument plots, which monitor the change of an instrument parameter, such as pressure, over time (depending on the capabilities of the instrument). The user can choose which signals are displayed. All the signals selected are shown

together. Selecting one of the signals displayed will bold that signal line and display that signal's Y-axis units on the left. Users can further adjust the signal display settings to a fixed range per axis and signal.

If the instrument generates spectral information e.g. mass spectrum or UV spectrum this may be viewed in the Spectrum window in real time as the analysis proceeds. For UV detection it shows absorbance as a function of the wavelength in a continuously updated display. Users can adjust both the displayed wavelength range (in nm) and the absorbance scale (in mAU). For MS detection the online spectra monitor displays the abundance as a function of the m/z range with the same means for adjustment. Users will see Profile and Centroided spectra in the real time display.

For both the Chromatogram Plot window and Spectrum window, users can zoom in and out, and can use the cursor to display a specific signal's response at any point in time. Users can enlarge the real time plot by clicking and dragging a zoom area one or multiple times. Zoom steps are easily undone by right-clicking and clicking **Undo Zoom** or **Zoom Out**.

Method Editor window

With this window you enter LC/TQ acquisition parameters for the method.

For the TQ instrument, you also tune the instrument from the TQ tab.
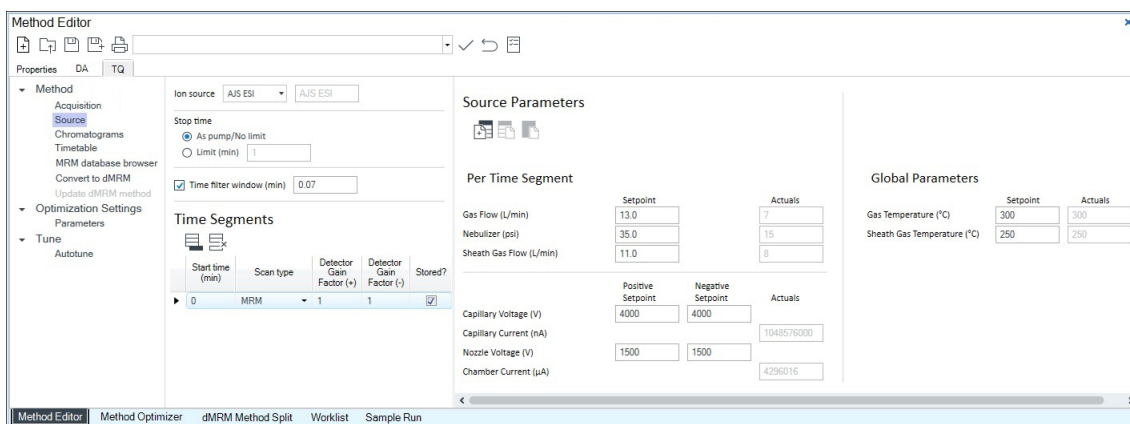

On the TQ tab in the Method Editor window, you select Tune > Autotune in the left pane. Then, you can run one of the automated tuning algorithms: either **Checktune** or **Autotune**.
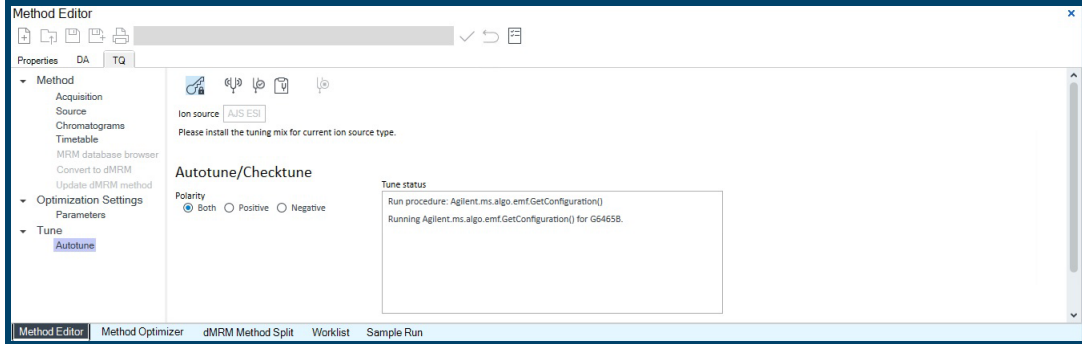
Sample Run window

With this window you enter sample information to run individual samples interactively, and you can start a single sample run. You can also specify an Override DA Method and select either **Both Acquisition and DA** for the **Part of Method to run**, and then Data Analysis is run as part of the method.
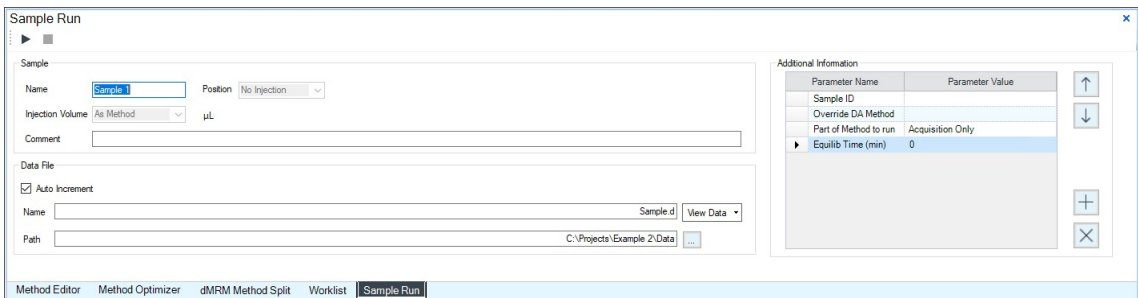

Worklist window

With this window you enter sample information for multiple samples. When you run the worklist, the samples are automatically run in the order listed in the worklist. You can select whether to run **Acquisition Only** or to run **Both Acquisition and DA** by selecting one of these options for the **Part of method to run** in the Worklist Run Parameters dialog box.

You can run the DA Reprocessor program to only run Data Analysis.

For a TOF/Q-TOF instrument, you can add one or more tune actions to the Worklist when you add a factory script to the worklist.
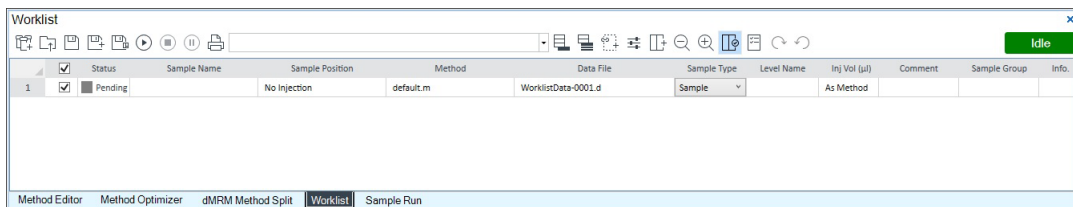
Tune window

For a TOF or Q-TOF instrument, you tune the mass spectrometer in the Tune window.

The Tune window is not available on a TQ instrument. For a TQ instrument, you use the **"Method Editor window"** on page 94.

You can use one of the automated tuning algorithms, or you can manually tune the instrument. Manual tuning can result in a less than optimal tune; however, if you perform a manual tune, manually tune only the front part of the instrument: ion source and optics 1. Do not tune parameters that are after the collision cell.

You also select the instrument mode to use when acquiring data, and calibrate the instrument.

If you have a 6545, 6545XT, or 6550 Q-TOF, then additional options are available for doing a small molecule autotune. If you have a 6545XT, then you can also do a large molecule autotune.

NOTE   You cannot do a small molecule tune after you do a large molecule autotune. If you need to do one, upload an older system tune file first, or do a system tune.
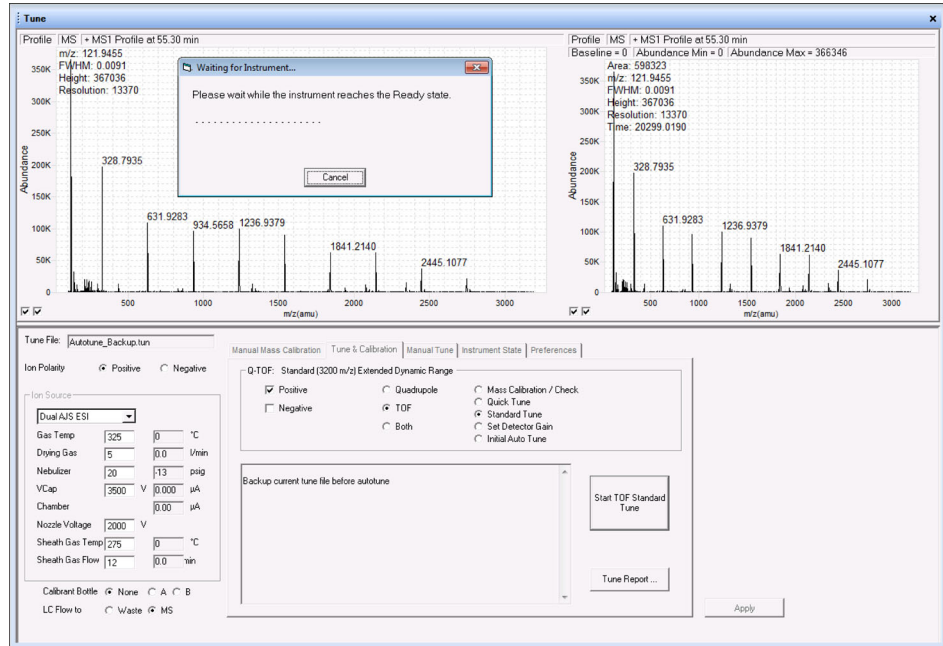
Figure 2. Tune window for a 6530 Q-TOF instrument performing a classic autotune
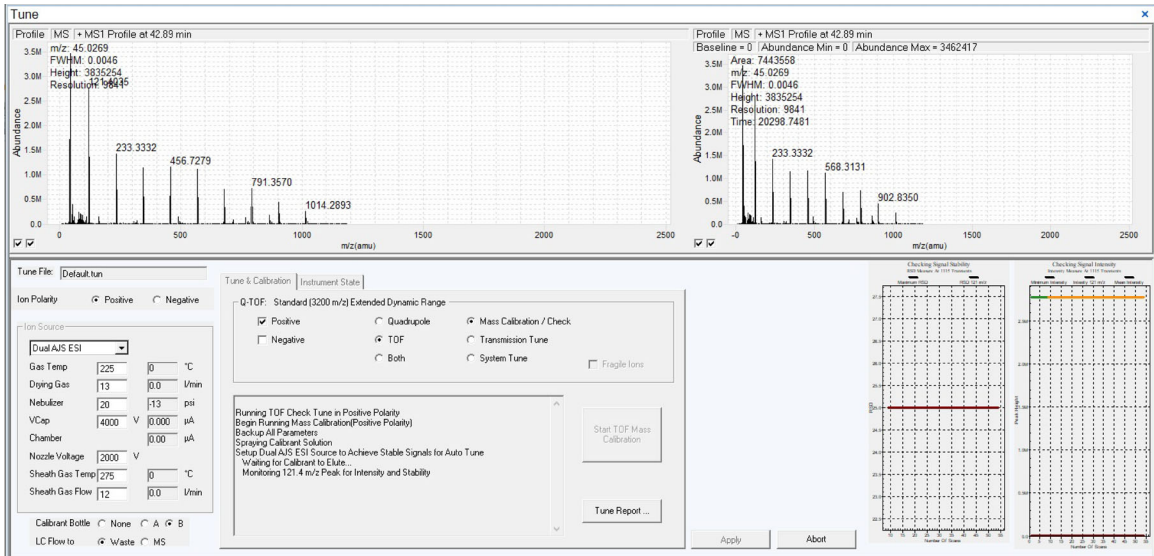
Figure 3.   Tune window for a 6545XT Q-TOF instrument showing two additional graphs for the SWARM autotune

Method Optimizer window

This window is only available for an LC/TQ instrument.

You select the methods to run to optimize compound parameters (fragmentor and collision energy) and source parameters. You first set up Optimization Settings parameters in the method. Then, you follow the **Guided** modes or the **Automated** mode.
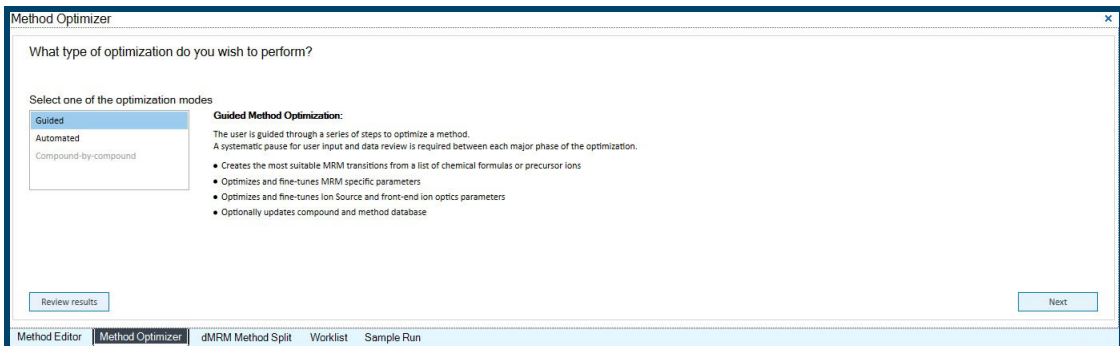
dMRM Method Split window

This window is only available for an LC/TQ instrument.

You may want to split a dMRM method into multiple methods to increase the dwell time for each transition. You select the method to split and other parameters in this window, and then you can split the method into 2 or more methods. The method is split so that all transitions for the same compound are in the same method.
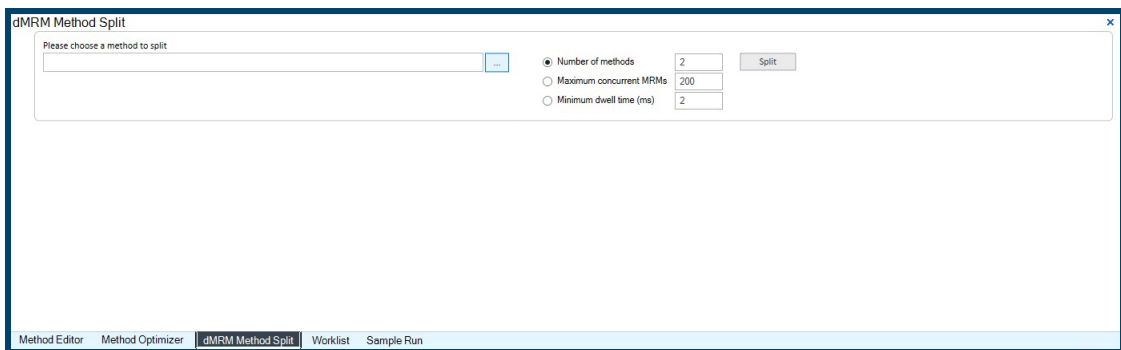
Working with Data Acquisition

Acquisition Control

MassHunter is specifically designed to facilitate the occasional or new user to rapidly understand the system and become productive in the shortest possible time. You just have to select which of the four screen layouts better suits your needs.

- **Status** – The Status layout provides a view of all of the windows in their default locations.
- **Method** – This layout provides access to the acquisition parameters available for the current instrument. These acquisition parameters may then be set and saved as an acquisition method. You also run Checktune and Autotune from this window.
- **Single Sample** – Once a method has been saved, the Single Sample layout provides simple starting of individual samples, online signals and, if available, spectral information.
- **Worklist** – The Worklist layout allows the operator to create, edit, save, and run worklists. Operators may create the new Worklist using the Worklist table. Again, the online signal, spectral and queue information is available.

The system can run either a Worklist or a Single Sample.

NOTE   When an acquisition is started, all of the information required to complete the task is available in the Instrument Controller. As such, the run will complete even in the event of a network failure as long as the connection between instrument and Instrument Controller is intact.

Once the analysis starts, the run queue controls the analysis. The system provides the ability to pause the analysis, if needed. If the operator has the appropriate permission, a running Worklist may be paused to allow the current run to complete, but the next run will not begin until the Worklist is resumed. Both single runs and Worklists can be immediately aborted. Worklists can be paused at the end of the current running sample. The Worklist table provides the progress monitoring of submitted samples. With the appropriate permission, you can edit a paused Worklist.

Acquisition Methods

MassHunter acquisition methods include the parameters for all instrument modules associated with the instrument. You create new or load existing methods for a launched instrument, make modifications as needed, and save the acquisition method with the same name or under a new one. When loading an acquisition method created from a different instrument configuration, the system tries to resolve (method resolution) the differences for the new instrument configuration. As not all parameters can be automatically resolved, default values may be added after the conversion. You must always review the content of the resolved method before using it to acquire new data.

In the (acquisition) Method layout you manually send the method to the instrument to prepare it for the next analysis.

Printing methods to either paper or digital format is supported using the Print function from the toolbar in the Method Editor window or from the **Method** button in the **Print Reports** section on the ribbon. Printing allows the selection of parameters to print based on the supported instrument modules. The method's audit trail can be printed from the Audit Trail Viewer program.

Data Model and Definitions

Methods, Single Samples, and Worklists

Each single sample is a folder that contains the sample information, sample data, tune report, and the method used to acquire the data. For a TOF/Q-TOF instrument, it also contains the tune file.

The system may be set up for automated analysis of multiple samples using the same or different methods. The control file to acquire multiple samples in a single operation is called a **Worklist**.

In the Worklist Run Parameters dialog box you specify the folder to use to save the data files for all of the samples acquired during a Worklist. With the Worklist folder, all information for the individual samples including the acquisition and data processing methods used to process each injection is present.

Method Specification and Use

The method contains Acquisition and data analysis parameters (either copies of the data analysis methods or links to the data analysis methods). The methods are specifically designed to support accurate, complete and secure maintenance of system operating parameters.

The MassHunter acquisition method fully describes how a particular analysis is performed. It contains all the parameters for instrument control and data acquisition. The processing method specifies all parameters for data evaluation, including integration, quantitation, and reporting.

The complete instrument and data analysis method is stored with the data file. The data acquisition method and data analysis method are stored in one folder in the storage location. The files are a mix of binary and .XML file formats with a proprietary, binary audit trail file and a proprietary .fileset that is used to detect tampering outside the control of MassHunter.

Results

In the course of automated data acquisition and processing in a worklist, data is written to the data file folder. The data file folder can contain:

- raw data
- acquisition and processing methods
- tune reports
- tune files
- report templates
- injection list
- calculated results
- reports
- audit trails

In MassHunter Quantitative Analysis, when the data is processed, integrated, identified, and quantitated, the results are saved to a batch results file.

In MassHunter BioConfirm, when the data is processed and searched, the results are saved to the data file.

For a single sample run the results are written to the Results folder. A single sample result only references the methods and the report template used for the analysis.

Submitting Samples

MassHunter supports running single samples and worklists. Both are submitted to be run by the launched instrument. Whenever submitting a single sample or a Worklist run, before starting the run, the system first validates the method or worklist.

To run a single sample, you need to specify the acquisition method and the data file name. If you select Both Acquisition and DA as the Part of Method to run, then the software will also attempt to run data analysis. You enable the data analysis methods to run on the DA tab in the Method Editor window.

Data can also be processed interactively with the data analysis client after the acquisition is completed.

Submitting Single Sample Runs

The Sample Run window consists of three different sections, where the user enters the required information for a single sample run and starts the run:

- **Sample section** – You can enter the Name, Position, Injection Volume, and a comment in this section.
- **Data File section** – You enter the data file name, the path, and whether to auto increment the data file name.
- **Additional Information section** – You can enter additional information about the single sample run in this section.

Finally, you click ▶ to submit the single sample; the system performs a validation check before running the sample. If an error occurs, an error message, such as "Result path cannot contain illegal characters", is displayed.

Acquiring Data Using the Worklist Table

A worklist (*.w) automates the analysis of multiple samples. The samples to be analyzed are organized in the worklist table. You can enter sample information in the worklist table and start the worklist.

The ways to create a worklist are:

- Create the worklist table manually
- Load and edit a saved worklist

For fast and convenient data entry, the Worklist table user interface can be customized by selecting or hiding columns. Columns in the Worklist table can also be rearranged, and you can resize the column width. Samples can be added, edited, or deleted from the Worklist table. A "Fill Down" function assists the user in completing the worklist table by automatically filling the entire table, or just a selection of rows or columns based on the content of the first row.

The worklist table provides columns (some of these columns are hidden by default) for the following items for each run in a worklist:

- Sample ID
- Sample Name
- Sample Position
- Method
- Override DA Method
- Data File
- Sample Type
- Level Name
- Method Type
- Balance Override
- Inj Vol (ul)
- Equilib Time (min)
- Dilution
- Wt/Vol
- Comment
- Barcode
- Sample Group

- Info
- Sample Amount
- Total Sample Amount

Additionally, three column types can be added for custom actions:

- Custom Columns
- User Defined Columns - **Numeric** or **Text**

Some custom columns are only available for some instruments. Here are some custom columns that are available:

- MFC
- Qualitative Workflow
- Masses
- BioConfirm Workflow
- Condition
- Modification Profiles
- Enzymes
- Disulfide Links
- Oligonucleotide Experiment
- Oligonucleotide Matching Rules

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Method Parameter Override

If you have a TOF or Q-TOF instrument, certain acquisition method parameters can be overridden by the Worklist without changes to the acquisition method. These parameters are available in the Worklist table as Method Override columns. The applicable override parameters depend on the instrument. The values are saved per injection.

Only users with the corresponding privilege can edit the method override parameters. The parameters can also be changed via worklist import. The column headers refer to the respective module and parameter. By default, all parameters in those columns are blank.

If you have a TOF or Q-TOF instrument for the MS Parameters column type, you can add and override:

- VCap
- Corona+
- Corona-
- Skimmer
- Oct 1 RF Vpp
- Gas Temp
- Vaporizer
- Drying Gas
- Nebulizer
- Fragmentor
- Ref. Nebulizer
- Sheath Gas Flow
- Sheath Gas Temp
- Nozzle Voltage
- Collision Energy
- Iterative

For all instruments, you can add User Defined Columns:

- Numeric
- Text

Acquisition resilience during network interruption

With the Operational Continuity feature, when the MassHunter Networked Workstation loses network connection to the OpenLab Server/ECM XT:

- The acquisition continues to run. All pending instrument runs (Worklists or Single Sample) that were running up to the point of interruption continues to completion on the MassHunter Networked Workstation without any potential for data loss. (The Worklist continues to run only if in Worklist Run Parameters, **Stop worklist on DA error** is *cleared*.)

- Acquired data and calculated results are securely stored in the Secure Work Area (see **"Secure Work Area"** on page 18).

- The buffered data automatically uploads to the OpenLab Server/ECM XT when the connection to the OpenLab Server/ECM XT is re-established.

- After connection to the server is restored, an entry is made in the Activity Log to indicate that server connection was lost.

7   Data Acquisition
     Acquisition resilience during network interruption

8 Data Processing - MassHunter BioConfirm

User Interface **112**

User Interface

The MassHunter BioConfirm is supported for TOF and Q-TOF data. It consists of two separate applications: BioConfirm and Sequence Manager.
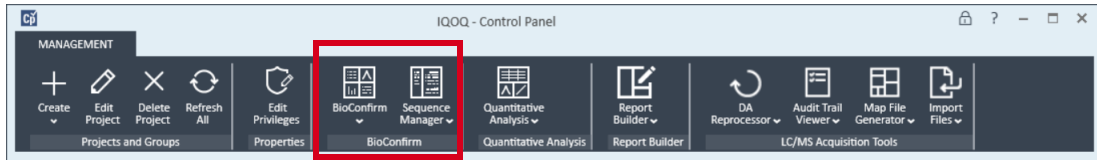
BioConfirm

Start BioConfirm in the BioConfirm group on the ribbon when Projects is selected in the left pane.

The main BioConfirm window consists of three parts: (1) the Menu Bar, (2) the Toolbar, and (3) the Main Window. The main functional areas are shown in **Figure 1**, **Figure 2** on page 114, and **Figure 3** on page 115. Some windows are not shown because they are not in the default layout.
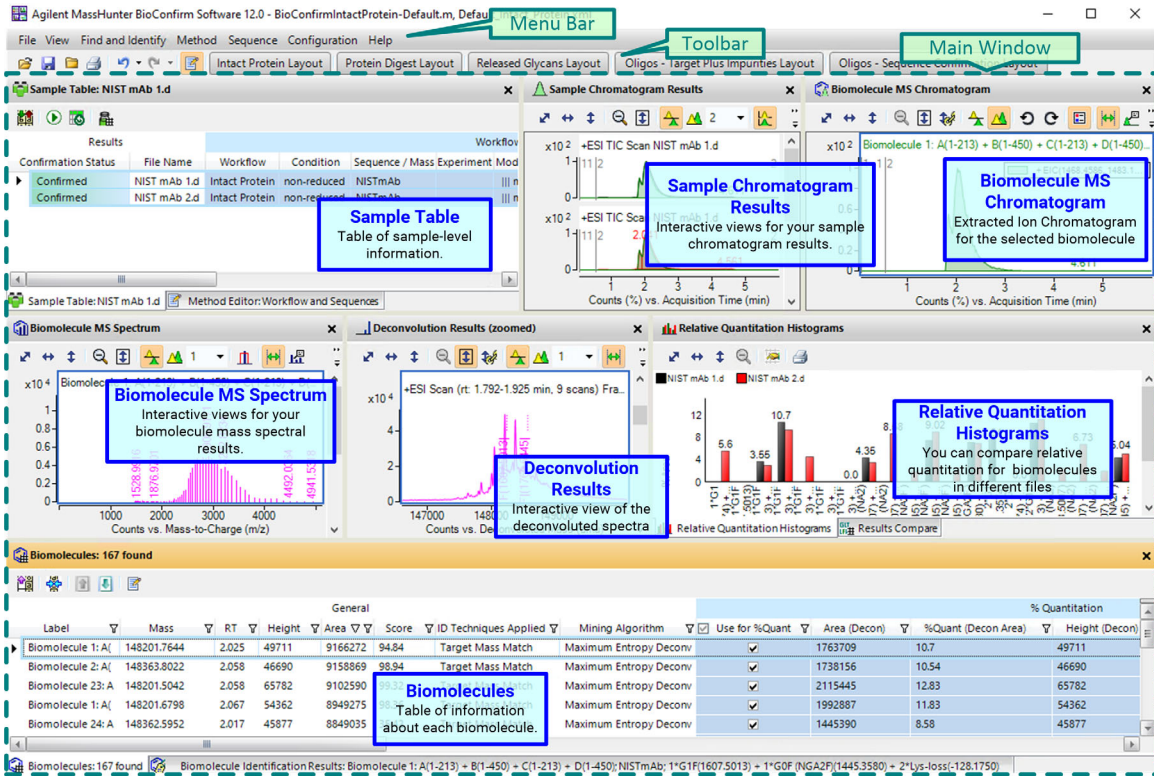
Figure 1. The main functional areas of BioConfirm for an Intact Protein analysis
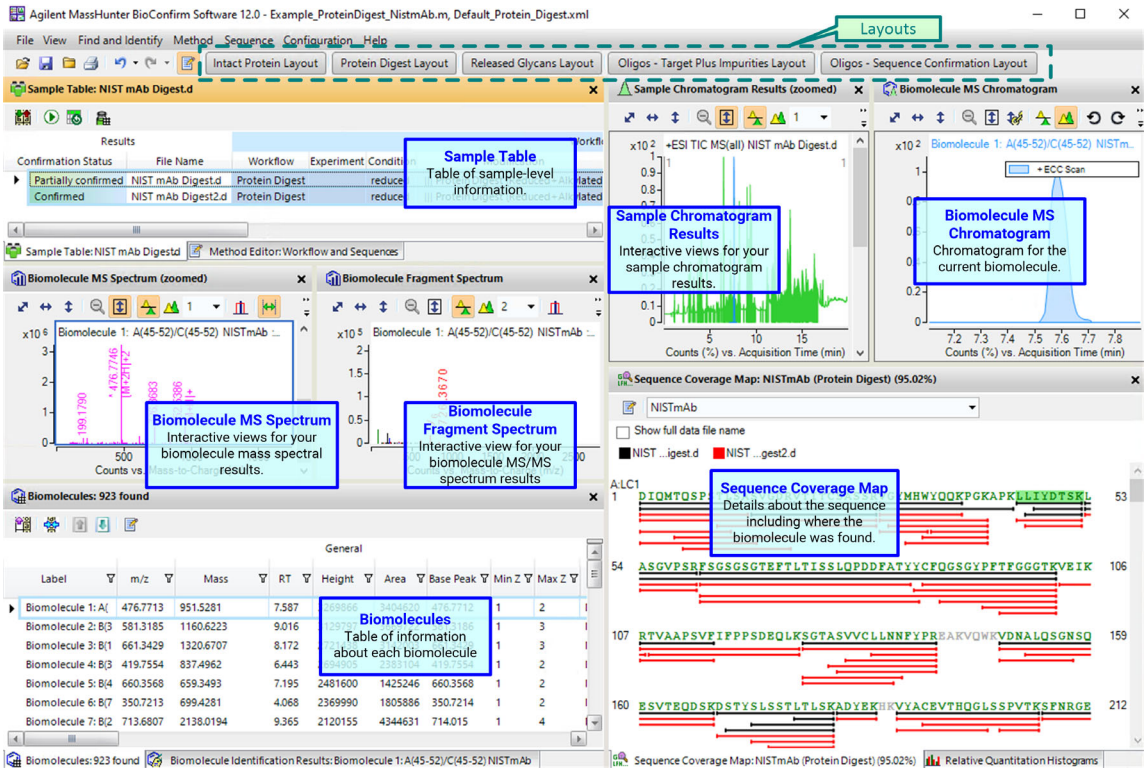
Figure 2. The main functional areas of BioConfirm for a Protein Digest analysis
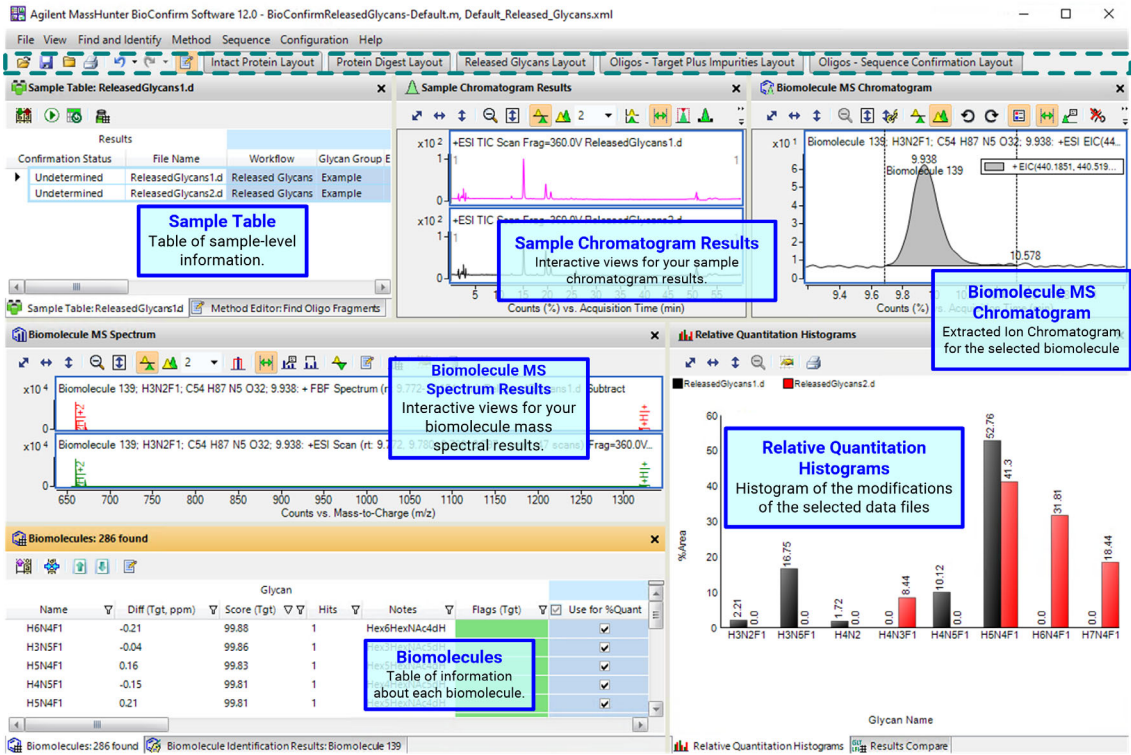
Figure 3. The main functional areas of BioConfirm for a Released Glycans analysis
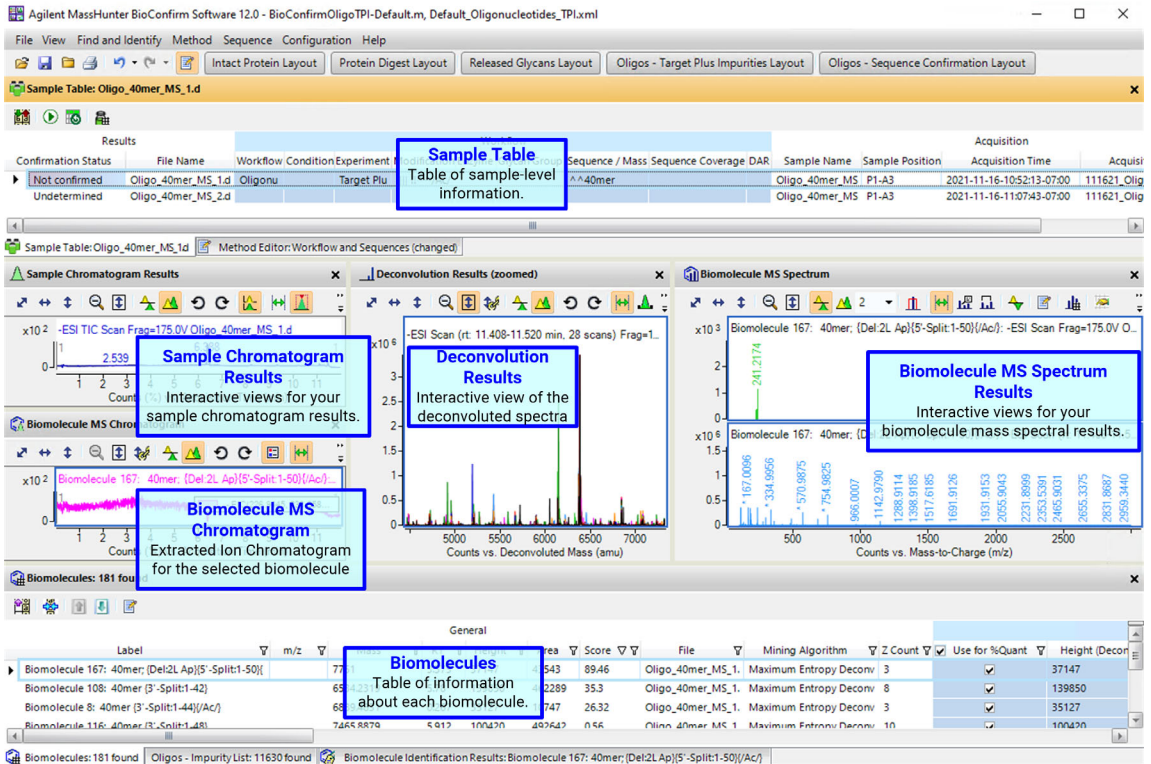
Figure 4. The main functional areas of BioConfirm for an Oligonucleotides - Target Plus Impurities analysis
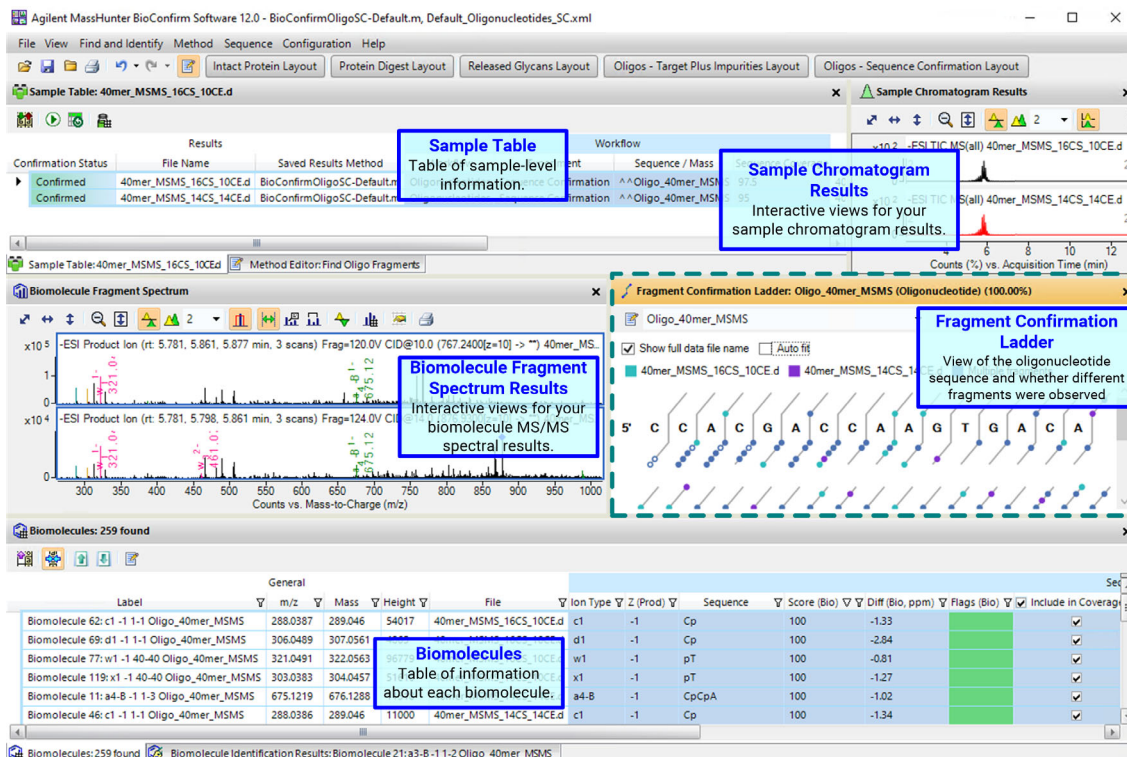
Figure 5.   The main functional areas of BioConfirm for an Oligonucleotides - Sequence Confirmation analysis

Menu Bar
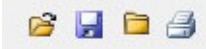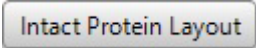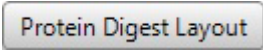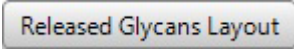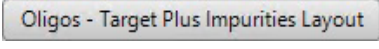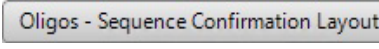
The menu bar (**Figure 6** on page 117) provides actions that are used for finding and identifying biomolecules, printing and exporting reports, and accessing the Sequence Manager and Chemical Data Dictionary.

Figure 6.   Menu bar

Toolbar

The toolbar provides actions that are used for opening data files and closing data files. You can also save results and print a Biomolecule report. Two icons allow you to undo or redo the last actions performed. The last icon toggles whether the Method Editor window is open.

Toolbar Icon	Action
	• File > Open Data File • File > Save Results • File > Close Data File • File > Print > Biomolecule Report
	• Undoes last action performed • Redoes last action undone.
	• Toggles whether the Method Editor window is open.
	• Loads the layout for the Intact Protein workflow. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Protein Digest workflow. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Released Glycans workflow. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Oligonucleotides workflow when the **Experiment** is Target Plus Impurities. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Oligonucleotides workflow when the **Experiment** is Sequence Confirmation. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.

Main window

The main window, see **Figure 2** on page 114, is further divided into many windows: Sample Table, Method Editor, Method Audit Trail, Results Audit Trail, Chemical Data Dictionary Audit Trail, Sample Chromatogram Results, Spectrum Preview, Biomolecule MS Chromatogram, Biomolecule MS Spectrum, Biomolecule Fragment Spectrum, Deconvolution Results, Deconvolution Mirror Plot, MS Spectrum Mirror Plot, Fragment Spectrum Mirror Plot, Biomolecule Chromatogram Mirror Plot, Sample Chromatogram Mirror Plot, MS Actuals, Biomolecules, Biomolecule Identification Results, Results Compare, Relative Quantitation Histograms, Sequence Coverage Map, Fragment Confirmation Ladder, Peptide Relative Quantitation Results, Glycan Structure Viewer, and Oligos - Impurity List. For most of these windows, you toggle whether these windows are visible in the **View** menu.

Sample Table   The Sample Table shows information for each sample that is opened. The sample or samples which you select in this window are displayed in the other windows. You can reprocess the selected sample.

Figure 7. Sample Table window

Method Editor   A method is a set of parameters that are associated with the different algorithms that you can run. Methods containing these parameters can be saved using unique file names.

You select the section of the method to display in the left pane. The right pane contains either a single section or multiple tabs. You can get help for each tab or section in the Method Editor when you press **F1**.
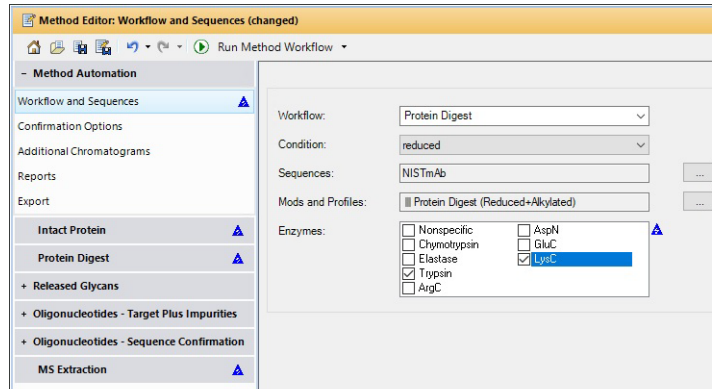
Figure 8. Method Editor window

Audit Trail   This window shows Audit Trail information about the data, method, or chemical data dictionary file that is currently selected.

Sample Chromatogram Results   This window shows the chromatograms for each sample that is selected in the Sample Table window. The chromatogram types can be a Total Ion Chromatogram (**TIC**), a Base Peak Chromatogram (**BPC**), an Extracted Ion Chromatogram (**EIC**), and other chromatograms. You can overlay the chromatogram for the selected biomolecule.
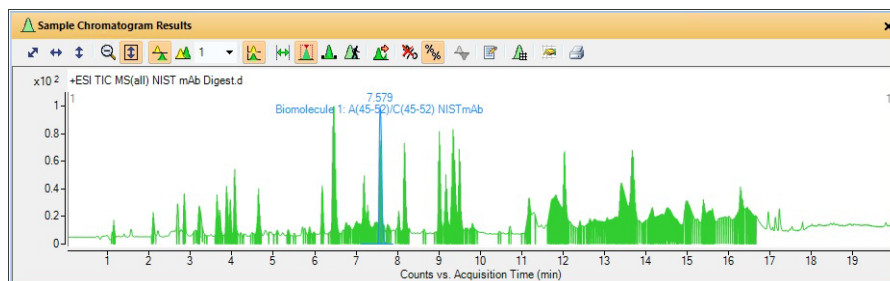
Figure 9. Sample Chromatogram Results window

Spectrum Preview   This window is used to quickly scan the spectra in a chromatogram. You start this window either when you click the Walk icon in the Sample Chromatogram Results toolbar or when you click View > Spectrum Preview.
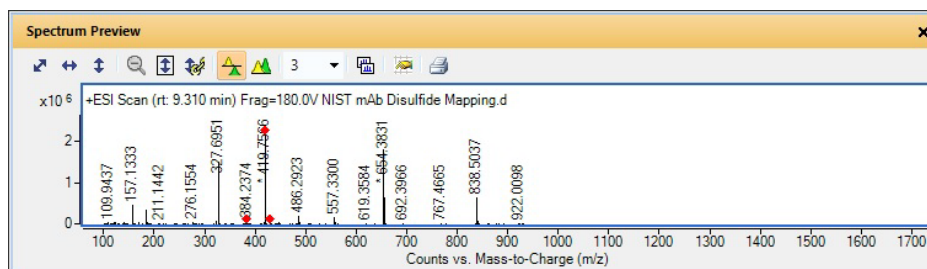
Biomolecule MS Chromatogram   This window shows an Extracted Ion Chromatogram (EIC) for each of the biomolecules you selected in the Biomolecules window. You can display a legend in the upper right corner of the graphic if you select **Overlaid mode** for the chromatograms. You can add annotations to the graphic. You can also export or print the graphic.
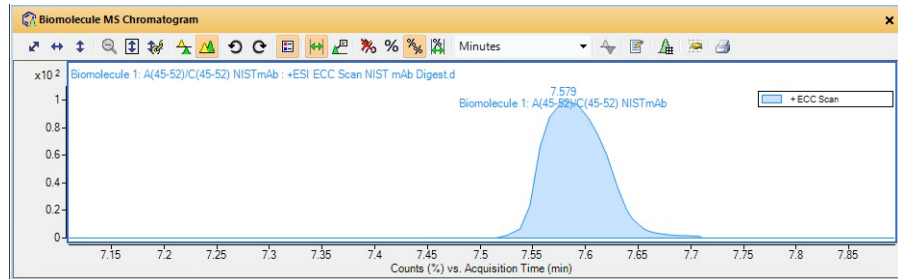
Figure 10. Biomolecule MS Chromatogram window

Biomolecule MS Spectrum   This window shows any MS spectrum. MS/MS spectra are displayed in the Biomolecule Fragment Spectrum window. You can add annotations and calipers to a spectrum in this window. You can also display the peak list which is displayed in a table on the right-side of this window. You can deconvolute, print, and export spectra in this window.

Figure 11. Biomolecule MS Spectrum window

Biomolecule Fragment Spectrum   This window shows any MS/MS spectrum. MS spectra are displayed in the Biomolecule MS Spectrum window. You can also annotate and add calipers to a Fragment Spectrum.

Figure 12. Biomolecule Fragment Spectrum window

Deconvolution Results   This window shows deconvoluted mass spectra. You can add annotations and calipers to a spectrum in this window. You can also display the peak list which is displayed in a table on the right-side of this window. You can see peak lists, print spectra, and export spectra in this window.

Figure 13. Deconvolution Results window in **Overlaid** mode

Deconvolution Mirror Plot   This window displays two deconvoluted spectra selected from the Deconvolution Results window. The first spectra is displayed as the top plot, and the second spectra is displayed as the inverted or bottom plot. You can switch the order of the spectra.

Figure 14. Deconvolution Mirror Plot

MS Spectrum Mirror Plot   This window contains a mirror plot for two spectra that you highlight. The MS Spectrum Mirror Plot window initially shows the first plot in the Biomolecule MS Spectrum window as the top plot and the second plot as the inverted or bottom plot.

Fragment Spectrum Mirror Plot   This window contains a mirror plot for two spectra that you highlight. The Fragment Spectrum Mirror Plot window initially shows the first plot in the Fragment Spectrum window as the top plot and the second plot as the inverted or bottom plot

Biomolecule Chromatogram Mirror Plot   This window contains a mirror plot for two MS chromatograms that you highlight. This window initially shows the first selected plot in the Biomolecule MS Chromatogram window as the top plot and the second plot as the inverted or bottom plot.

Sample Chromatogram Mirror Plot   This window contains a mirror plot for two sample chromatograms that you highlight. The Sample Chromatogram Mirror Plot window initially shows the first plot in the Sample Chromatogram Results window as the top plot and the second plot as the inverted or bottom plot.

Biomolecules   This window shows all of the biomolecules which were found for the selected sample files. You can add and remove columns from this table, and you can change the order of the columns.

Figure 15. Biomolecules window

Biomolecule Identification Results   This window shows the results of running the Match Sequences algorithm on the biomolecules in the Biomolecules table. If you see **Sequence Match** as the value for the **ID Techniques Applied** column, then you can see additional information about that match in this window.

Figure 16. Biomolecule Identification Results window

Results Compare   This window displays tables of information that let you compare different data files that have been processed with the same workflow. Results from each workflow are shown in different tabs in this window. The information shown on each tab is different.

- Intact Protein tab

- Protein Digest tab

- Released Glycans tab

- Oligos - Target Plus Impurities tab

Figure 17.   Protein Digest tab in the Results Compare window

Relative Quantitation Histograms   This window displays the relative quantitation values for the biomolecules that have the **Use for %Quant** check box marked. The **Use for %Quant** check box is in the Biomolecules table for Intact Protein and Released Glycans biomolecules, and it is in the Peptide Relative Quantitation Results table for Protein Digest biomolecules. You can visually compare the relative quantitation values for up to 10 Samples. You can group the biomolecules by either **Biomolecule ID** or by **Sample**. For Intact Protein, this window shows **Pred Mods** vs **%Area** or **%Height**. For Released Glycans, this window shows **Glycan Name** vs **%Area** or **%Height**.
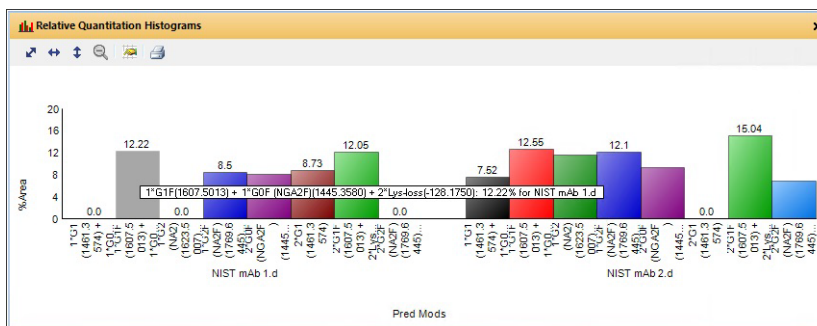
Figure 18. Relative Quantitation Histograms window

For the Protein Digest workflow, this window shows the relative quantitation values for different modifications. You mark the **Use for %Quant** check box in the Peptide Relative Quantitation Results window. The window shows **Modification** on the x-axis, and the Plotted value that is clicked in the Histogram Display Options dialog box. You can click **%Area**, **%Height**, or **%Volume (MS-Only Protein Digest)**.
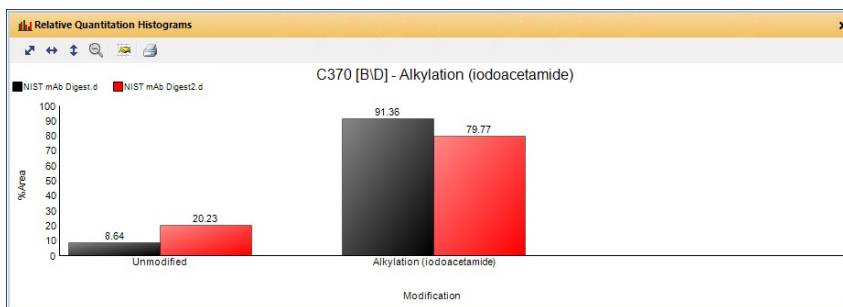
Figure 19. Relative Quantitation Histograms window

Sequence Coverage Map   This window displays information for the protein digest sequence selected in the Workflow and Sequences section of the Method Editor window and the biomolecule selected in the Biomolecules window. The highlighted area in the sequence shows where the current biomolecule matches the current sequence. Different lines underneath parts of the sequence show where a biomolecule was matched in the sequence.

If you run Match Sequences on multiple sequences, then you can see multiple sequences in this window.

Figure 20. Sequence Coverage Map window

Fragment Confirmation Ladder   This window displays a sequence and shows the fragments that were confirmed in the selected data files. This window only displays data for the Oligonucleotides - Sequence Confirmation workflow. The nucleosides are displayed separated by lines. The lines can contain up to 9 dots that represent each of the fragment types expected to be found at each location. The fragments on the bottom limb apply to the nucleoside to the left of the line; the fragments on the top limb apply to the nucleoside to the right of the line.

If you run the Oligonucleotides - Sequence Confirmation workflow on multiple data files, then you can see fragments from multiple data files in the ladder.
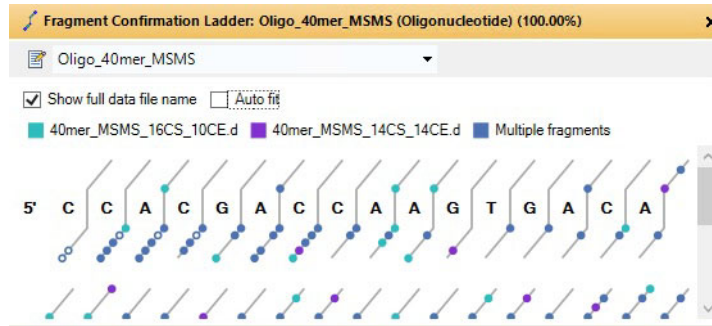
Figure 21. Fragment Confirmation Ladder window

Peptide Relative Quantitation Results   This window displays only the peptides that were matched and have a predicted modification. If an amino acid at a particular location has been found to have a modification in a particular file, it will be displayed as a row in the table. Each row has a nested level where the included biomolecule information is displayed. For Protein Digest workflow, you mark the **Use for %Quant** check box in the second level of this table.

Figure 22. Peptide Relative Quantitation Results window

Glycan Structure Viewer   This window displays glycan structures. The structure is also shown in the Biomolecule MS Spectrum window.
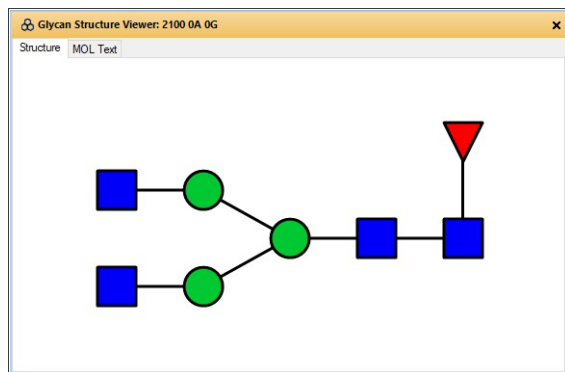
Figure 23.   Glycan Structure Viewer window

Oligos - Impurity List   This window displays information on the oligonucleotide impurities that are generated from the oligonucleotide sequence, based on the modifications and matching rules used. This window is only available for the Oligonucleotides - Target Plus Impurities workflow. You display this window when you click **Sequence > Oligos - Impurity List**. If you run Match Sequences on multiple sequences, then you can see multiple sequences in this window.

Figure 24.   Oligos - Impurity List window

Sequence Manager

The Sequence Manager program allows you to organize and edit method sequences. You can quickly find the sequences that you often use without having to browse for them every time. For a protein sequence, you can edit the description, the links, the modifications, and the chains for a sequence. For an oligonucleotide sequence, you can edit the description and building blocks for the sequence. For Oligonucleotides, you add **Building Blocks**, **Modifications**, and **Linkers** in the Chemical Data Dictionary dialog box.

Start Sequence Manager from the Management toolbar, in the BioConfirm group. You can instead click **Sequence > Sequence Manager** in the BioConfirm program.
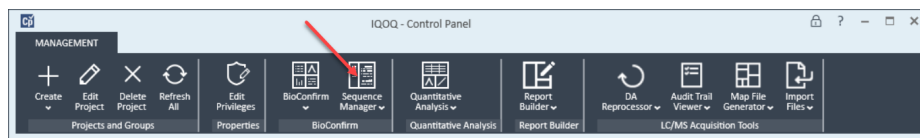
Main window - Proteins
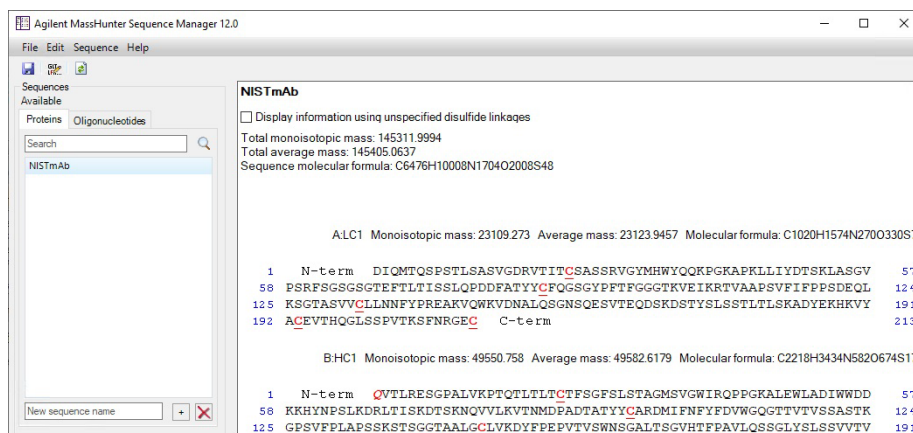
Sequences Available   Allows you to select the sequence to edit. This list contains the protein sequences present in Sequence Manager.

Search   The name of the sequence to search for. As you type the name of the sequence, the list is automatically updated to only contain sequences that contain the text that is typed. You can type any part of the sequence name (for example, you do not need to type the beginning of the sequence)

New sequence name   The name of a sequence that you want to add.

Adds a sequence to the Sequences Available list. The name of the new sequence is the name added in the New sequence name parameter.

Deletes the current sequence or sequences from the Sequences Available list.

Sequence details   **Sequence name**   A name for the protein, protein digest, or synthetic peptide being specified. The name appears in the report to identify the sequence.

Display information using unspecified disulfide linkages   Whether or not to calculate the molecular weight using unspecified disulfide linkages. Both the Total monoisotopic mass and the Total average mass values can be affected. The Sequence molecular formula can also be changed.

Sequence type   •

- • **Protein** – The Sequence type is set in the Description Dialog Box. The only option is Protein.
- • **Monoisotopic MW –** Monoisotopic mass, calculated automatically when a modification or link is made to the sequence.
- • **Average MW** – Average mass, calculated automatically when a modification or link is made to the sequence.

Sequence   This area lets you specify the sequence for the selected chain. You can copy a sequence from a FASTA-formatted database or text file and paste it between the N-term and C-term symbols. Amino acids must be designated by their single-character abbreviations. You can right-click the sequence to show the Sequence Manager Shortcut Menu.

The blue numbers to the left and right of the sequence are index numbers used to identify the positions of the amino acids for modifications and links. The number on the left is the number of the first amino acid in the row. The number on the right is the number of the last amino acid in the row. Blue arrows indicate digest information. If you move the cursor over the amino acid, the tooltip shows you the position in the sequence and a letter to show you which chain you are looking at.

Main window - Oligonucleotides
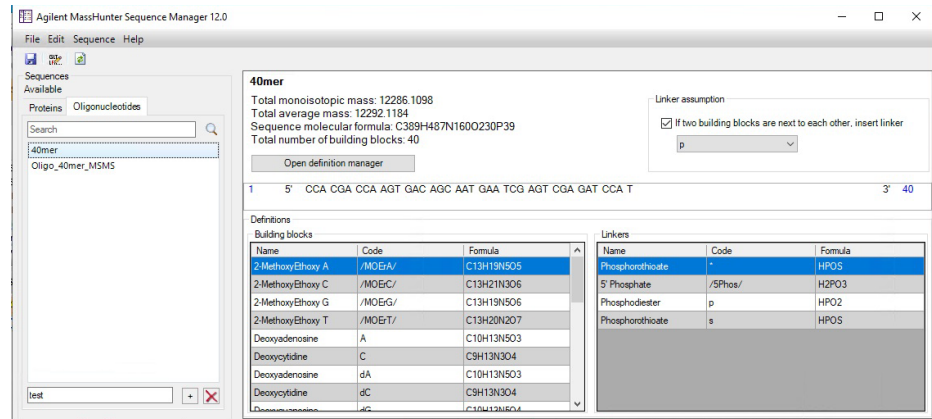
Sequences Available	Allows you to select the sequence to edit. This list contains the oligonucleotide sequences present in Sequence Manager.
Search	The name of the sequence to search for. As you type the name of the sequence, the list is automatically updated to only contain sequences that contain the text that is typed. You can type any part of the sequence name (for example, you do not need to type the beginning of the sequence)
New sequence name	The name of a sequence that you want to add.

Adds a sequence to the Sequences Available list. The name of the new sequence is the name added in the New sequence name parameter.

Deletes the current sequence or sequences from the Sequences Available list.

Sequence details   **Sequence name**   A name for the oligonucleotide being specified. The name appears in the report to identify the sequence.

- **Total monoisotopic mass**
- **Total average mass**
- **Sequence molecular formula**
- **Total number of building blocks**

Sequence   This area lets you specify the sequence for the selected chain. You can copy a sequence from a FASTA-formatted database or text file and paste it between the 5' and 3' symbols. Building blocks must be designated by their codes. You can right-click the sequence to show the Sequence Manager Shortcut Menu.

Definitions   The two tables in this section show the building blocks and the linkers that are available. The **Building blocks** table shows the **Name**, **Code**, and **Formula** for the building block. The **Linkers** table shows the **Name**, **Code**, and **Formula** for the linker. You can add building blocks and linkers in the Chemical Data Dictionary Editor when you click the **Open definition manager** button.

Workflows

You select a workflow in the Method Editor window in the **Method Automation > Workflow and Sequences** section. This workflow decides what operations are to be run and how the sequence match is to be done when you run the workflow. You select one of these workflows: **Intact Protein**, **Protein Digest**, **Released Glycans**, **Oligonucleotides**, and **Custom**.

Intact Protein

The workflow runs the Find by Protein Deconvolution algorithm, and then runs Intact Protein sequence matching. A Biomolecule report is generated using the **Intact Protein report template** selected on the Method Automation > Reports > Templates tab. You can mark the **Calculate Drug to Antibody Ratio (DAR)** check box to run DAR calculations.
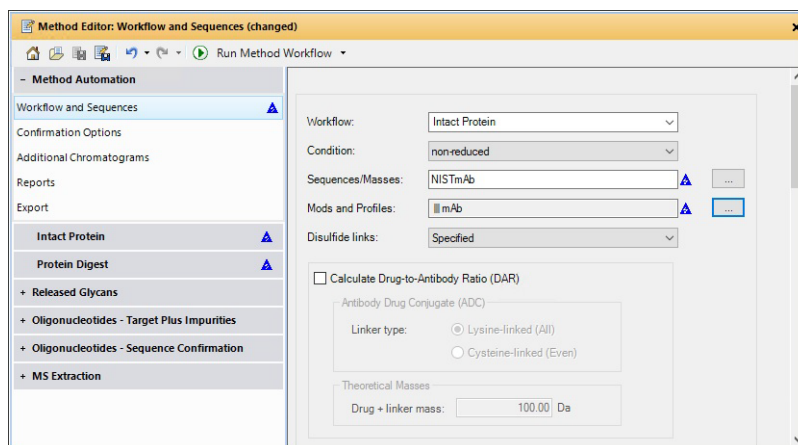
Protein Digest

This workflow runs the Find Peptides algorithm, and uses protein matching rules (Protein Digest, Predicted Modifications). You can select whether or not to use Protein Truncation. The workflow also runs the Match Sequences algorithm with the Sequence or mass that you entered and digests the sequence using the enzyme selected. See **Figure 25** on page 135. A Biomolecule report is generated using the **Protein Digest report template** selected on the Method Automation > Reports > Templates tab.
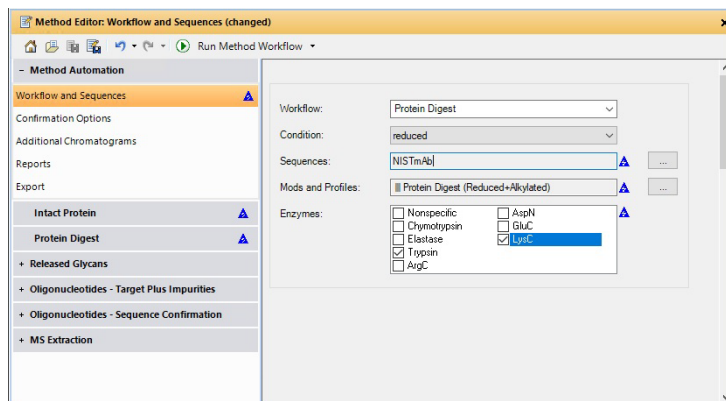
Figure 25. Method Automation > Workflow and Sequences section for Protein Digest workflow

Released Glycans

This workflow runs the Find Glycans algorithm and uses the **Target glycan source** that you entered. See **Figure 26** on page 135. You can specify the Glycan Group name for this sample. Results in the Released Glycans tab in the Results Compare window are sorted by Glycan Group. A Biomolecule report is generated using the **Released Glycans report template** selected on the Method Automation > Reports > Templates tab.
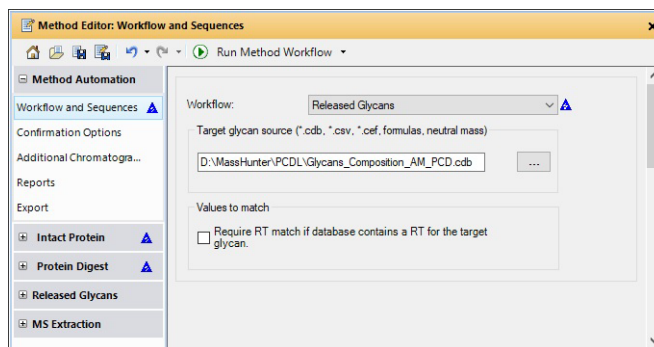
Figure 26. Method Automation > Workflow and Sequences section for the Released Glycans workflow

Oligonucleotides

When you run an Oligonucleotides workflow, the workflow runs one of the following experiment types:

- Target Plus Impurities

• Sequence Confirmation
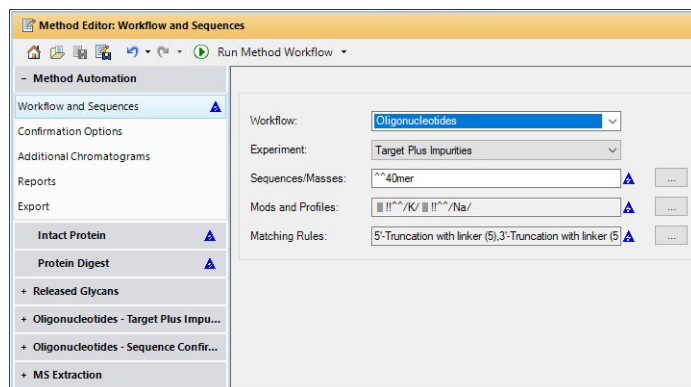
Figure 27. Method Automation > Workflow and Sequences section for the Oligonucleotides - Target Plus Impurities workflow
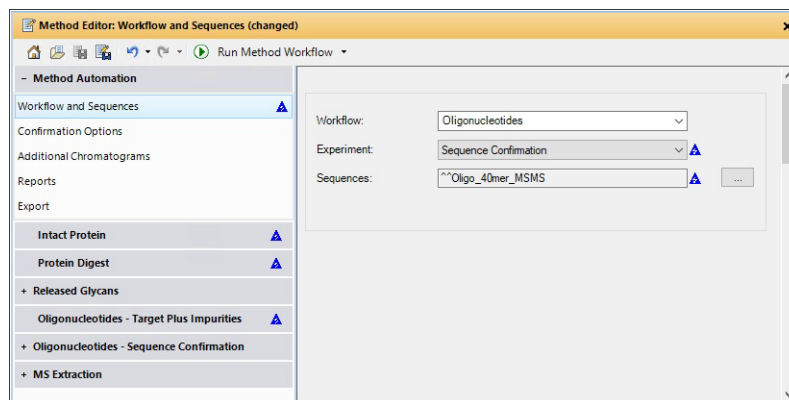
Figure 28. Method Automation > Workflow and Sequences section for the Oligonucleotides - Sequence Confirmation workflow

Custom

This workflow runs the actions which you select in the Workflow and Sequences section. You select the actions from the **Available actions** list and place them in the **Actions to be run** list. The order of the actions in the **Actions to be run list** is the order in which the actions are executed. If you generate a biomolecule report, it uses the **Protein Digest report template** if you used the **Find Peptides** algorithm; otherwise, it uses the **Intact Protein report template**.

9 Data Processing - MassHunter Quantitative Analysis

The MassHunter Quantitative Analysis program is used to analyze a batch of samples. A batch is a group of samples analyzed together with the same Quantitative Analysis method. When you set up a batch and method in Quantitative Analysis, you specify the data files that belong to the batch and the compounds that should be found.

When you analyze the batch, the software determines if the compounds have been found

The results are presented in the Batch Table, the Compound Information table, and the Calibration Curve table.

You enter the information for the method in the Method Editor window.

You can customize PDF report templates using the Report Builder program.

Batch Table

The Quantitative Analysis program offers numerous features and flexibility for the advanced user to analyze your data. Quantitative Analysis functions are conveniently organized in a distinct part of the user interface called the Batch Table view.

From the Batch Table view you can perform all the required Quantitative Analysis tasks such as creating a batch, reviewing the results, integrating and quantitating results, and printing batch reports.

A Batch is a group of related data files that were processed using a single data acquisition method and will be analyzed using a single data analysis method.

The batch file is saved as a .bin file in the same directory as the data files being analyzed. Once you have created a batch, you may add samples to it or remove samples from it at any time. Also, you may apply a method to the batch, modify the batch layout, save it as the same name, or as a new name, and save it in the same directory as the original batch.

Analyze a batch

The Quantitative Analysis program provides a one-click function called Analyze Batch (F5) that will process your entire batch with one click. Analyze Batch performs the following functions:

- Extract all signals (SIM or EIC) from the data file
- Integrate the signals to detect peaks for quantifiers and qualifiers
- Create calibration curves based on calibrators
- Calculate a concentration based on the calibration curves
- Perform numerous QC tests and determine outliers

The difference between analyze and quantitate is that analyze rebuilds the calibration curve, quantitate does not.

Apply a method to a batch

Once you create a batch, you may apply a method to it. When you apply a method to a batch, and use the Analyze option, all the sample files in that batch will be analyzed using that method.

Add samples to an existing batch

All samples in a batch are analyzed by a single data analysis method. Once you have created a batch, you may add samples to it at any time.

Convert samples

You can convert the following files to add as Quantitative Analysis samples:

- MassHunter MS, QQQ/ChemStation
- MassHunter TOF/Q-TOF
- OpenLab
- Varian MSWS

Convert ChemStation samples (.d) to MassHunter Indexed data format.

Convert profile raw data and centroid data to a SureMass or TOF Data Access (TDA) format, greatly reducing the file size of your data and increasing the speed of your data analysis. This window is only available when you start the program using the Q-TOF Quantitative Analysis or TOF Quantitative Analysis icons.

Convert OpenLab CDS sequence (.rslt), single injection (.sirslt), or .olax (in non-compliant mode) files to MassHunter format.

Convert Varian MSWS files to MassHunter Indexed data format.

Clear results

The Clear Results option clears the calibration table as well as any previously calculated results in the batch.

Save batch

The batch file is saved as a .bin file in the same directory as the data files being analyzed.

Save batch as

Once you have created and saved a batch, you may save it with a new batch name (.bin) in the same directory as the originally created batch file, but you cannot save it to a different directory.

Save the Batch Table column settings

Once you have identified the columns you would like to be displayed in your Batch Table, you may save the layout in a .xml file so it can be used in other batches.

The Batch Table layout (whether the table is displayed as a flat table or a nested table) is not saved as part of the column settings, nor whether the Batch Table is displayed in Single Compound View or Multiple Compounds View. See Change the Batch Table layout for details on changing how the Batch Table is displayed.

Change number formats

You can change the number of digits that appear after the decimal point for any numerical field in a table.

Integrate a batch, sample, or compound

Integration will calculate the response for compounds in your batch. Integration does not use the calibration curve nor obtain the final concentrations.

The Quantitative Analysis program provides three ways to integrate data in your batch. You can integrate the whole batch, a single sample in the batch, or a single compound within a sample in the batch. This granularity could be useful in reviewing large batches.

When you select Integrate Batch, the Quantitative Analysis program calculates the responses for each compound in the batch. When you select Integrate Sample, Quantitative Analysis calculates the response of each compound in the currently selected sample line in the batch. When you select Integrate Compound, the Quantitative Analysis program calculates the response for the specific compound selected within the currently selected sample.

Manually integrate a compound

The Quantitative Analysis program allows you to manually integrate a single compound from a single sample, as describe here.

Annotate your Batch Table with reasons for manual integration

You can manually add an abbreviation to the batch table that indicates the reason for manually integrating target or ISTD compounds. This can only be edited in the Batch Table; you cannot edit this field in the Method Table.

Integrate scan data using a TIC

You can integrate your scan data using a Total Ion Chromatogram (TIC) extraction rather than a particular mass extraction (EIC). Using a TIC extraction integrates all of the ions together to act like a second chromatographic detector.

Quantitate a batch, sample, or compound

In the Quantitative Analysis program, the difference between quantitate and analyze is that quantitate does not rebuild the calibration curve, and analyze does.

The Quantitative Analysis program provides three ways to quantitate your batch. It can quantitate the entire batch (F6), a single sample in the batch (F7), or a single compound within a sample in the batch (F8). This granularity could be useful in reviewing large batches.

Quantitate a batch

When you quantitate the whole batch (F6), the Quantitative Analysis program recalculates the final concentration of each compound using the existing calibration curve. It does not rebuild the calibration curve. When you analyze the batch, the Quantitative Analysis program rebuilds the calibration curve.

Quantitate a sample

When you select Quantitate Sample (F7), the Quantitative Analysis program calculates the final concentration of each compound in the sample line that is currently selected in the batch.

Quantitate a compound

When you select Quantitate Compound (F8), the Quantitative Analysis program calculates the final concentration of the specific compound selected within the currently selected sample.

Review results

The Quantitative Analysis program provides great flexibility for examining the data collected from your samples using the Batch Table, Sample Information, Compound Information, Calibration Curve, and Compounds at a Glance windows.

In the Batch Table window you can, for example, see the warning flags, colored cells, and messages for compounds with unacceptable results. You can also set filters to show only high or low outliers. You can also identify a compound's ISTD, and set filters for time segments and compound groups. You also mark a compound or sample as Approved.

In the Sample Information window you can show a chromatogram of a single target compound, or all the target compounds (with or without the TIC and ISTD), and you can extract a spectrum of an area. Also, you can scroll through each sample in your batch, one by one, and watch the extracted chromatograms to observe trends and decide if the sequence has run correctly before continuing with quantitation, manual integration, or approving the batch.

In the Compound Information window you can display a chromatogram, spectrum, qualifier, or ISTD for any compound selected in the Batch Table or manually integrate a compound.

In the Calibration Curve window you can review where newly acquired CAL samples fall in the curve, and review where ISTD, QC and CC samples fall around the existing curve. You can also zoom to a specific level when reviewing where the calibration sample types fall around the curve, and toggle the calibration curve concentration scale between Relative Concentration and Concentration.

In the Compounds at a Glance window you can review all or selected compound chromatograms in a batch by compound name or by sample. The compound peak can be overlaid with qualifiers, ISTDs, a Matrix spike, all compounds or compound groups, and all samples or sample groups. All compounds can be manually integrated from this window. Configured outlier results can also be identified on each compound peak. You can also mark a compound as Approved.

Synchronize the Compounds at a Glance window and batch tables

You can set up a Compounds at a Glance Window that is a single row high and can be placed adjacent to the Batch Table for efficient batch sample review. As you click on a compound in the Compounds at a Glance Window, that same sample in the Batch Table is selected and now displays data for that sample's compound and its ISTD. Alternatively, selecting different samples or compounds in the Batch Table result in those samples and compounds being selected in the other window. This procedure is just a example of what can be set up for sample review.

Tune evaluation

A Tune evaluation is performed on a Tune Check sample type run from MassHunter Acquisition. This sample contains a compound of known spectral integrity that is compared to the Mass Spectrometer acquired data and evaluated against predetermined limits. The masses and limit ranges are adjustable by the user. Also, for certain analyses, chromatographic performance tests are performed and evaluated against predetermined limits. These tests include peak tailing and parent compound breakdown (degradation).

A tune report can be generated as a pdf and includes the sample's TIC, selected spectrum, parameters used for the evaluation, and simple to understand Pass/Fail results. A typical workflow involves running an evaluation sample on a schedule that suits your lab's requirement for an instrument tune. In Quantitative Analysis, select the sample in a Batch Table and start Tune Evaluation. After selecting a tune evaluation method containing the parameters and limits previously set up by you lab you generate and print the report.

Run a tune evaluation report

After running a tune evaluation sample from MassHunter Acquisition, you can determine if the instrument is in tune according to the requirements set by your lab. This procedure is applicable for Environmental Quant MS, TOF, and QQQ.

Graphically examine the data collected

You can graphically examine the data from a sample using the Batch Table, Sample Information, Compound Information, and Metrics Plot windows.

Review batch warning flags, colored cells, and messages

To help you spot outliers and other questionable results, after analyzing a batch, the Quantitative Analysis program highlights each line in the Batch Table that contains results outside the prescribed limits of your method. The Quantitative Analysis program will:

- Display flags and warning messages in the left-most column of the Batch Table, next to any line with results outside the prescribed limits of your method, and

- Color cells in the table red or blue to indicate the result is outside the range specified in your method.

Open data files in the Qualitative Analysis program

The Go To Qualitative Application feature allows you to open a Quantitative Analysis data file in the Qualitative Analysis program. The Qualitative Analysis program must be installed on your computer to use this feature.

Normalize qualifier chromatograms

If you want to normalize the qualifier chromatograms, you must first integrate the chromatogram and the qualifiers. You need one primary peak for each qualifier signal for normalize to work properly.

In the Qualifier Chromatogram pane, two or more chromatograms are shown overlapped. The first chromatogram is the quantifier signal. The baseline of this chromatogram is set to 0, and the peak height is set to 100 %. The other chromatograms are the qualifiers.

The qualifier chromatograms are overlaid with the quantifier chromatogram. The baseline of each qualifier chromatogram is set to 0. The maximum value of each normalized qualifier chromatogram (100 %) is set to the *QuantifierPeakHeight x RelativeResponse*. The qualifier is normalized with the expected signal and not the actual signal. You enter the relative response for the qualifier in the qualifier part of the method.

If the amount of the qualifier signal is exactly what was expected, the qualifier signal overlaps the quantifier signal. If the amount of the qualifier signal is lower than what was expected, the qualifier signal is shown below the quantifier signal. If the amount of the qualifier signal is higher than what was expected, the qualifier signal is shown above the quantifier signal.

Methods

The Quantitative Analysis program offers numerous features and flexibility to the advanced user to manage the Quantitative Analysis methods. Method development functions are conveniently organized in a distinct part of the user interface called the Method Edit view. From the Method Edit view you can perform all the required method development tasks such as creating new methods, editing existing methods, and saving methods files to be used later.

Unified method

A MassHunter Unified Method is a like a container that can hold all the individual methods required in a typical data acquisition and analysis workflow. The container holding these methods is a windows directory with a ".m" extension. The root directory of this *.m folder holds all the method's files required for data acquisition in addition to the DaMethod folder holding the method directories required for data analysis.

The DaMethod folder groups the Quant, Unknowns, and Qual folders that contain methods used with MassHunter Quantitative, Unknowns, and Qualitative Analysis programs. The methods for these programs are xml files grouped in these folders as shown.

In this unified method directory structure shown here only the example.m folder name can be changed by the user using Method Save As, all other folders and method names are always as shown. When you initially save the default method (default.m) in MassHunter Acquisition with a new method name there is no DaMethod directory in the unified method. In Quant, you can save its method to this same unified method file. This results in the DaMethod and DaMethod\Quant directories being created and the quantitative.xml method file being saved in the latter directory. Also if you later save the report method to this same unified method it is saved in the directory as shown. Likewise directories are created for methods when they are saved to the unified method from the Qualitative and Unknowns analysis programs.

If you do not want to save one of these application's method to a unified method file you can save the method as an xml file.

Open a method

You can open methods stored in the Quantitative Analysis program for viewing, updating, or printing. The procedure for opening a method depends on whether it is stored in the program as a separate method file (cef or library), with a saved batch, or with a batch currently open in the main view.

Quantitative Analysis methods may be a standalone file (.quantmethod.xml), embedded in a universal method folder (.M), or embedded in a batch file (.batch.bin).

Open a method attached to a saved batch

Methods are saved in the program with each saved batch. You can use these saved method files to apply to new batches or as a starting point to create new methods.

Open a method attached to the current batch

You can open a method attached to a batch for review or updating from the main view.

Open a saved method

Methods can be saved in the Quantitative Analysis program as separate files. You can use these saved method files to update your method parameters, apply to new batches, or create new methods.

Create a new method

The Quantitative Analysis program offers several ways to create new data analysis methods. The program can make use of existing data from acquisition, method, or batch files to automatically help you create your new method.

Create a mew method from a method that is saved with a batch

You can create a new method from a method that already exists attached to a saved batch.

Create a new method from a saved method

You can create a new method from a method that was saved as a separate method file.

Create a new method from acquired scan data

You can use the New Method from Acquired Scan Data feature to automatically create a new method based on the information in your MassHunter Data Acquisition scan data file.

Create a new method from acquired scan data with library search

You can use the New Method from Acquired Scan Data with Library Search feature to automatically create a new quantitative analysis method based on the information from your MassHunter Data Acquisition scan data file. This menu item is available only if you start this program using the MassHunter Quantitative Analysis (QQQ) or the (MS) icons.

Create a new method from MRM data

You can use the New Method from Acquired MRM Data feature to automatically create a new quantitative analysis method based on the information in an MRM data file created in your MassHunter Data Acquisition program. This menu item is only available if you start this program using the Quantitative Analysis (QQQ) icon.

Create a new method from SIM data

You can automatically create a new quantitative analysis method based on the information in an SIM data file created in your MassHunter Data Acquisition program. The procedure uses the New Method from Acquired MRM Data menu item in the Method Edit view. This menu item is only available if you start this program using the QQQ Quantitative Analysis or MS Quantitative Analysis icons.

Create a new method using manual setup

You can manually create a new quantitative analysis method. If your method uses ISTD compounds for adjusting the quantifier, create the ISTD compound first since they are required when specifying the target compounds.

Create a new method from CEF file

You can use this feature to create a new quantitative analysis method by importing an existing method from a Qualitative Analysis file (.cef). When a method is created from file, the following information contained in the file is imported and appears in your new method: compound name, retention time, quantifier and qualifier ions, ion polarity, CAS number, and molecular formula.

Create a new method from a library

You can use this feature to create a new GC or LC quantitative analysis method by importing compounds from your library (.mslibrary.xml).

Create a new method from acquired chromatographic data

You can use the New Method from Acquired Chromatographic Data feature to automatically create a new method based on the information in your MassHunter Acquisition chromatographic data file.

Create a SIM/Scan Method

Using a SIM/Scan method allows you to take advantage of sensitive quantitation of the SIM ion while obtaining spectral match data from a spectral reference library. The Scan provides a spectrum for each compound. Comparing that spectrum to a spectral library reference determines a library match score. That score is conformational evidence which supports confidence in the final concentration.

Create a targeted deconvolution scan method

You can modify your scan method to use the targeted deconvolution feature to achieve a cleaner target spectra.

The targeted deconvolution eliminates ions from the spectra that most likely do not associate with the peak (background), so when the cleaned spectrum is compared to the reference spectra, a higher library match can result.

Targeted deconvolution can also be used to determine the purity of a sample by comparing the components of the convoluted spectra to the deconvoluted spectra.

The library match score is improved by the clean spectra provided by targeted deconvolution. The spectra are clean because deconvolution gathers only the ions that share the same model peak shape.

To further improve your compound identification, you can set up your targeted deconvolution scan method to provide lists of alternative peaks and hits.

- Using an alternative peak indicator, the program will automatically search your library for peaks with better match scores.
- Using an alternative hit indicator, the program will automatically search a secondary library and suggest alternative hits.

The alternative peaks and hits can be displayed in the Batch Table as well as in your report.

Display triggered MRM reference library spectra

In certain types of acquisitions, you may reach a certain abundance threshold and want to trigger to acquire another transition. MassHunter Quantitative Analysis Software displays only the area that was triggered. When extracting a spectrum, if the TriggeredTransitions string is not null, it will be parsed and an EIC will be extracted for each triggered transition in the list. The latest start-of-data point in RT defines the trigger point, RT_trigger, and the compound spectrum will be extracted over the RT range = [RT_trigger, RT_end], where

RT_end is the end of the primary target peak. For the (non-triggered) MRM case, the TriggeredTransitions column will be null and the spectrum extraction will occur as it normally does.

Add a compound that uses a non-MS signal

You can add a compound to your method that uses a non MS signal from your data file, such as UV signals. These compounds can be used to create method for the non MS signal alone or be combined in your method that uses your MS data and non-MS data in a 2D compound method, such as MS-UV.

Add a non-MRM compound using the QQQ start up icon

You can use the Compound Setup task in the Advanced Tasks menu to add non-MRM compounds to your method when you start the program using the Quantitative Analysis (QQQ) icon.

Append a method

Add compounds from the analysis of a data file or from a different method, Library, or CEF file to the method open in the Method Editor.

Appended compounds must have different names than the existing compounds in the method. If appended compounds have the same name, they will not overwrite or append to your current method.

Adjust the mass extraction window

You can use the Mass Extraction Setup task in the Advanced Tasks menu to adjust the mass extraction window for compounds in your method.

Set up an integrator

You can choose and manually adjust the parameters of the integrator used in your method.

Set up isotoptic dilution

You can add Colby constants to your method to account for your internal standard isotope dilutions.

Smooth peak integration

You can change the smoothing algorithm that is applied to the compounds in your method. However, applying a smoothing algorithm is not recommended with the parameter-less integrator.

Review library search results

Displays the identifying fields in the Quantifier compounds along with the Library Match Score and the CAS #. Review these results to determine if the library selected has made an accurate match.

Set an alternative hit indicator

You can modify your targeted deconvolution scan method to find alternative hits using the Target Deconvolution Setup wizard.

The wizard guides you through creating a method that will help you identify your compound by searching a secondary library and suggesting alternative hits. The alternative hits are display in the Batch Table as well as your report.

Browse the acquisition method

You can review the acquisition method for a sample from the Quantitative Analysis program using the Browse Acquisition Method task in the Advanced Task menu of the method edit view. This menu item is only available if you start this program using the Quantitative Analysis (QQQ) icon.

Determining noise and signal/noise ratio values

The noise and signal/noise ratio values are determined by different mechanisms depending on the integration steps.

The parameterless integrators (Agile, Agile2, MS-MS and MS-MS (GC)) return a noise and signal to noise values which are calculated by a statistical algorithm. The noise region is indicated by the 'Baseline calculation points' which are visible in the display of the extracted chromatogram.

For the parameter integrators (General and Universal), noise regions are the spaces between the integrated peaks and are not displayed in the user interface.

Apply an existing calibration curve to a compound

Quickly apply a calibration curve to a compound in your batch from another compound in your batch by using the Inherit Calibration Reference Setup feature.

Compound and qualifier tasks

From the Method Tasks menu in the Method Edit view, you can setup or modify the parameters for your compounds and qualifiers for your method.

Add a compound or qualifier from MRM data in the sample information window

You can add a compound or qualifier to your quantitative analysis method from MRM data displayed in the Sample Information window. This option is available when you start this program using the MassHunter Quantitative Analysis (QQQ) icon only. You can add up to 50 qualifiers for a compound.

Add a compound or qualifier from scan data in the sample information window

You can add a compound or qualifier to your quantitative analysis method from scan data displayed in the Sample Information window. This option uses the deconvolution algorithm to extract a spectrum from the selected area in the chromatogram. You can add up to 50 qualifiers for a compound.

Add a compound or qualifier manually

You can add up to 50 qualifiers for a compound.

Generate qualifiers from an LC or GC library

Use the Generate Qualifiers from Library Method Setup task to add qualifiers for compounds in your method directly from your library. This feature is also useful for reverted to the qualifiers as they existed in the original library.

Assign a compound to a compound group

Assign compounds a Compound Group name corresponding to the group you want that compound to be a member of. This is used to filter the compounds in a sample to a given group type for analysis workflow.

Analyze data from parallel acquisition streams (StreamSelect LC/MS)

StreamSelect allows you to have parallel HPLC streams, and can provide up to twice the throughput. Each HPLC stream needs to have its own calibration curve for validation.

Average qualifier ratios

You can update the qualifier ratios for compounds in your method by using an average qualifier ratio calculated from calibration or QC samples in a batch.

Remove a compound, qualifier, or concentration level from a method

You can remove compounds or qualifiers from a method and save it as an updated method or as a new method.

Identify a compound in the Spectrum window using the library

You can identify compound ions extracted from chromatograms using the library tool accessible from the spectrum window shortcut menu.

Update mass assignments

If your data acquisition conditions have changed you can update the mass assignments accordingly in your quantitative analysis method.

Update qualifier ratios

If your data acquisition conditions have changed you can update the qualifier ratios accordingly in your quantitative analysis method.

Subtract an ISTD/surrogate response from a TPH target response

You can set up your method to subtract the ISTD/surrogate TIC response from a Total Petroleum Hydrocarbons (TPH) target compound TIC response.

Sun and average compounds in a group

You can set up your method to sum or average responses and calculated concentrations of compounds in a group. Relative ISTD is not supported with Compound Math.

Calculate a ratio for two compounds in a group

You can set up your method to automatically calculate a ratio between two compounds in a group for the response or calculated concentration.

Set up dynamic background subtraction

For easier viewing of your chromatogram you can use the Dynamic background subtraction feature for your method. The Quantitative Analysis program detects the background signal from un-grouped ions and applies an algorithm to subtract this background signal from your chromatogram.

Set up multipliers

A multiplier is a multiplicative operation automatically used to generate the Final Concentration displayed in an analyzed Batch Table. A multiplier can be added to target, surrogate, and ISTD compounds in your method. See ApplyMultiplierTarget, ApplyMultiplierSurrogate, and ApplyMultiplierISTD.

Set up sample calibration bracketing

You can set up your method to quantitate samples using calibrators run immediately before and after the samples. "Bracketed" samples receive a calibration curve created from calibration samples whose variation is minimized.

Set up standard addition

You can set up your method to recognize that you are using samples that have been spiked with target compound. This standard addition technique is used when the sample preparation does not adequately remove co-eluting matrix peaks that suppress the target response and where no matrix blank is available.

Specify a correlation window

The correlation window indicates the retention time relationship of the target and ions to one or more qualifiers.

Specify a library to use for compound identification

You can specify a library to use in your method for identification of compounds. The library must be in a folder location accessible from your Quantitative Analysis program.

Specify a relative ISTD

You can indicate that your method uses a relative internal standard. Relative ISTD is not supported with Compound Math.

Specify the reference and non reference windows

During analysis, the Average Retention Time of the target and all its qualifiers is calculated. If the Average Retention Time falls outside the Reference or Non Reference window, then all the target and qualifier peaks will be rejected. Individual target and qualifier peaks are not tested to see if they are in the window and are not rejected individually. In rare cases, the Average Retention Time can fall in the Reference or Non Reference window, while the individual peaks are outside the window.

Using a Correlation Window that is small in comparison to the Reference or Non Reference window reduces the likelihood of this occurring. For example, reducing the Non Reference Window from 200% to 10%, which is the same as the Retention Time outlier, eliminates the false positives and reduces data review and "zero peak" work.

Set up concentration levels

Set up the concentration levels of each compound in your method using the **Concentration Setup** features in the Method Edit view. A feature can automatically calculate the concentration for each level specified using the highest (starting) concentration and the dilution pattern.

Add a new calibration level

You can add new calibration levels to your method using the New Calibration Level option in the Manual Setup Tasks menu.

Copy calibration levels

You copy calibration levels from the concentration table of one compound to others in your method automatically by using the Concentration Setup task in the Method Edit view. When you use this feature the Calibration Table for the compound is replaced.

Assign a calibration curve to a compound from the main view

This procedure uses the Curve Fit Assistant to assign a calibration curve to a compound in the method from the main view.

Assign a calibration curve to a compound in the method edit view

This procedure uses the Calibration Curve Setup task in the method edit view to assign a calibration curve to a compound in the method.

Average calibration replicates

You can set up your method so that the calibration table displays only the average for replicate levels.

Create a calibration level CSV file

You can import calibration levels to your method calibration table using data tables saved as comma delimited (CSV) text files in your Quantitative Analysis program. CSVs files can be created in spreadsheets or word processing applications, such as MS Excel or MS Notepad. CSV files used for data that include ISTDs, must also include ISTDs.

Create levels from quantifiers

Set up the concentration levels of each compound in your method using the Concentration Setup features in the Method Edit view. A feature can automatically calculate the concentration for each level specified using the highest (starting) concentration and the dilution pattern.

Import calibration levels from an existing file

You can import calibration levels to your method calibration table using data tables saved as comma delimited (CSV) text files in your Quantitative Analysis program. This feature is available using the Concentration Setup task in the Method Edit view. When you use this feature the Calibration Table for the compound is replaced.

Set up an Internal Standard (ISTD)

You can set the ISTD parameters in a method using the ISTD Setup option. If you are creating a new method from acquired data, some of the ISTD specific columns will be automatically filled with information from acquired from your acquisition method.

Set up retention time

You can change the retention time for a compound or qualifier in your quantitative analysis method from the Method Edit view. The retention time values are used to identify peaks in the integration results.

Update retention times from ISTD

You can update your method's retention time of target compounds based on the observed retention time of the corresponding ISTD in the data file you select. This is useful when target compound retention times are not available in the batch from Cal, QC, or CC samples, but ISTDs are present and the relative retention time of the target compounds are in the method.

The ISTD is set to the observed retention time of the internal standard in the selected sample. The updated target compound retention time is based on the internal standard retention time and the average relative retention time (Average RRT) of the calibration and QC samples:

RT of Compound = (Observed ISTD RT of Compound) * Average RRT in method

The Average RRT is calculated from the initial calibration batch and is part of the method.

Average retention time

You can update the retention time for compounds in your method by using an average retention time from calculated from calibration or QC samples in a batch.

Shift retention time

If your data acquisition conditions have changed you can shift a compound retention time accordingly in your quantitative analysis method.

Update retention time

If your data acquisition conditions have changed you can update the compound retention time accordingly in your quantitative analysis method.

Save a method

You can save new or updated methods with batches currently open in the main view as updated method files or as new method files (*.m, *.quantmethod.xml).

Data analysis methods can also be saved in a unified method that includes the data acquisition method, qualitative, quantitative, and unknowns analysis methods along with a quantitative analysis report method.

Save an updated method with a batch

You can update your batch method and save it with the batch.

Save an updated method

You can open a method, update it, and save it with your changes.

Save an updated method with a new file name

You can save a quantitative analysis method with a new name as a file that can then be later applied to other batches.

Exit a method

You can exit the method view and return to the main view using the Exit task.

Validate a method

You can check your method to verify that you have filled in all of the necessary fields using the validate feature in the Method Edit view.

View method history

The Method History program is automatically installed and available for use with the Quantitative Analysis Compliance software. Use the program to view changes made to the following saved methods:

- Quantitative Analysis Method (.m.sszip, .quantmethod.xml)
- Library Method (.uamethod.xml)
- Quant Analysis Report Method (.quantreportmethod.xml)
- Unknowns Analysis Method (.uamethod.xml)
- Unknowns Analysis Report Method (.unknownsreportmethod.xml)

Library

Use a library to rank compound identifications by area or height and generate TIC (tentatively identified compounds) reports.

Use a library method

The library method is part of the unified method. Use the library method to specify which library to use in a library search, rank compounds in search results by area or height, and generate TIC (tentatively identified compounds) reports.

View library search results

Use Unknowns Analysis to perform a library search to look for target or non-target hits, resulting in tentatively identified compounds.

Use the library editor

The MassHunter Quantitative Analysis Library Editor is used to add, remove, or modify compounds in any of your libraries.

Outliers

An outlier is a result value that is outside the range of acceptable values for that parameter as required by your protocol. The program allows you to choose the outliers that you want to show in your analyzed batch and set their limits using the Outlier Method Tasks menu in the Method Edit view.

Outlier messages displayed in the Batch Table are based on full precision of values. Values outside of the acceptable range may not be apparent in flagged outlier messages in cases where less precision is displayed.

For some outliers, when a primary peak is not found the compound and its containing sample will be flagged with a quantitation message, not with an outlier flag. For a list of outliers where peaks not found are handled this way see Outlier Details.

Reports

You can create reports in two different formats. The first uses an XML database, specially designed templates, and Excel Professional software. Please refer to the Agilent MassHunter Reporting help which you can access through the Excel program for information on how to customize your report template. The second format involves generating PDF reports. This is generally 20 times faster than the Excel format, and does not have the same scalability or performance limitations. This software provides sample templates for generating reports in either format.

The reporting templates are installed on the local hard drive with the program to MassHunter/Report Templates/Quant/. If you are using the Compliance program, these templates must be uploaded to ECM before they can be accessed from the Quantitative Analysis program.

The Report Method feature allows you to group the reports templates you commonly use and then quickly generate more than a single type of a report for your batch.

Generate a report

Once you have created, analyzed, and saved your batch you can generate a report.

Create a report method

Since it is often helpful to generate more than a single report for a batch, the Report Method feature allows you to group the report templates you commonly use and save this as a Report Method. You can then use your report method to quickly generate all the reports included in that method for your batch.

Select and configure report templates

When generating a new report method, begin with a template supplied with the software, and modify it to meet your needs. First, decide whether you would prefer your reports generated in an Excel format or as a PDF. PDF reports can be generated 20 times faster than Excel reports. Excel based reports have some scalability and performance limits that PDF reports do not have.

The reporting templates are installed on the local hard drive with the program to MassHunter/Report Templates/Quant/. If you are using the Compliance program, these templates must be uploaded to ECM before they can be accessed from the Quantitative Analysis program.

Customize templates with Report Builder

The MassHunter Quantitative Analysis Report Builder allows you to create and customize PDF report templates in a user-friendly interface. When you first open Report Builder, you are presented with a blank template that you can configure for your needs. You can also start with a preconfigured report template, available in **\MassHunter\Report Templates\Quant\PDF-ReportBuilder\Quant** folder.
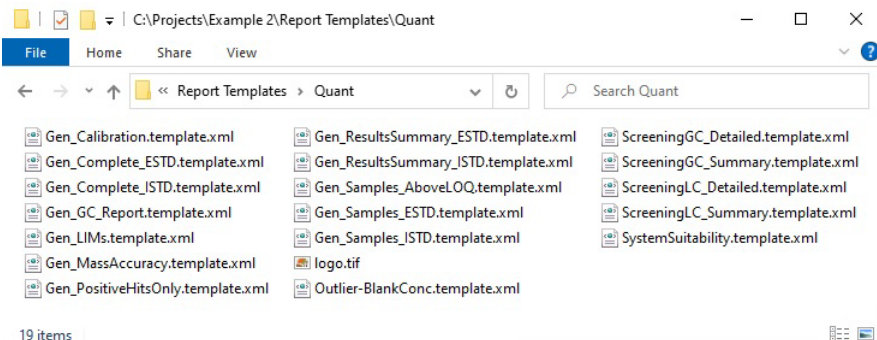
The Report Builder Ribbon allows you to insert and edit objects into your report template, such as tables, charts, and even your company logo.
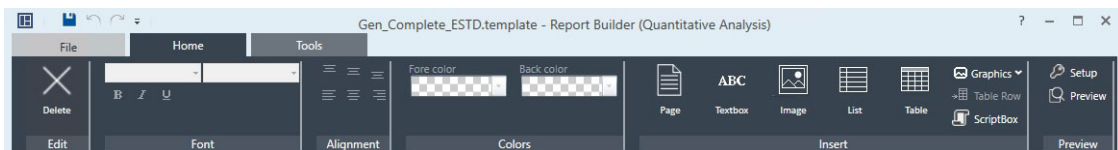
Configure report results

The Results tab of the Method Edit Report window allows you to identify which instrument was used in the batches you will be reporting on, as well as whether or not you wish to have a results file generated with your report.

Configure report graphics

From the Graphics tab in the Report Method Edit window, you can access various windows which allow you to define the graphics settings you wish to use in your report.

Edit a report method

You can modify an existing report method or create a new report method from an existing one. Edits made to a report method are recorded in the report method Audit Trail.

View the report method Audit Trail

The report method Audit Trail is only available in a User Management and Audit Trail Only or Compliance installation.

The report method Audit Trail is separate from the Batch Table Audit Trail and provides details on changes made to the report method.

Open a report folder

Once a report has been generated for a batch, you can open the folder where the individual files used to generate the report are stored. From the report folder you can work with the files with the usual Windows commands such print, edit, copy, delete, rename, or create a pdf.

Run a query

Once you have created, analyzed, and saved your batch, you can run a query.

Targeted Deconvolution

Use the Targeted Deconvolution (TD) setup wizard to create a data analysis method that elevates your confidence in target analyte identification by comparing the deconvoluted component mass spectra against a target MS library. TD can be used with either Scan or SIM/Scan data, and the entire TD setup process is contained within the MassHunter Quantitative Analysis program.

Targeted deconvolution provides several key advantages, giving you the confidence that you are identifying, quantitating, and reporting compounds correctly.

- Purity metrics that indicate sample contamination
- Improved library match scores from clean, deconvolved sample spectra
- Alternative hit indicators that suggest alternative compound identifications for your target peak
- Alternative peak indicators that suggest alternate peaks to be identified as the target
- Go from a complex full scan TIC to an easy-to-interpret report in <1 minute per data file

Quant-My-Way Flavors Setup program

A MassHunter Quantitative Analysis flavor is a user interface with a specific set of tools and options. The Quantitative Analysis software provides a preset number of system flavors designed for several instruments and applications.

Use the Quant-My-Way Flavors Setup program to create additional Quantitative Analysis flavors that display the tools and menu options you need for your laboratory or function. Your custom flavors can then be saved, updated, and shared with others in your organization.

Setup desktop icons

Clicking a specific Quantitative Analysis Windows desktop icon starts the Quantitative Analysis flavor configured for a specific instrument type and data analysis mode. The Quantitative Analysis software provides a preset number of system flavors designed for several instruments and applications. Use the Quant-My-Way Flavors Setup program to create customized Quantitative Analysis flavors that display only the tools and menu options you need for your laboratory or function. For more information on the Quant-My-Way Flavors Setup program, review the Agilent MassHunter Quant-My-Way Flavors Setup Familiarization Guide or see the Quant-My-Way Flavors Setup Online Help.

Main windows

Batch table window

Information in the **Batch Table** is separated into seven different sections. The section names are shown in the top row of the table. Each section contains a group of columns that are defined in Batch Table Columns. These columns can be added to the table using the Add or Remove Columns in the Batch Table Column Shortcut Menu.

You can rearrange the columns of information within each section (See Customizing a Table). You cannot move columns of information from one section to another section. You also cannot change the order of the sections.

Compound information window

The **Compound Information** window used in conjunction with the **Sample Information** window, will greatly assist you with reviewing your results.

Here you can see the chromatograms and spectra for the current sample in the Batch Table and Manually integrate a compound if necessary.
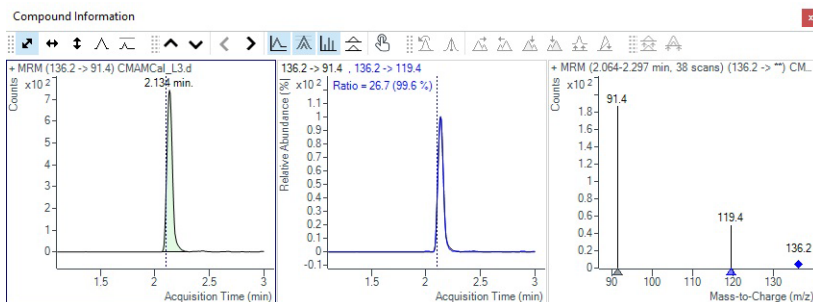
Calibration curve window

The calibration curve for the Batch Table's selected compound is displayed in this window. Use the shortcut menu to access the Curve Fit Assistant to select and assign a calibration curve for the batch method.
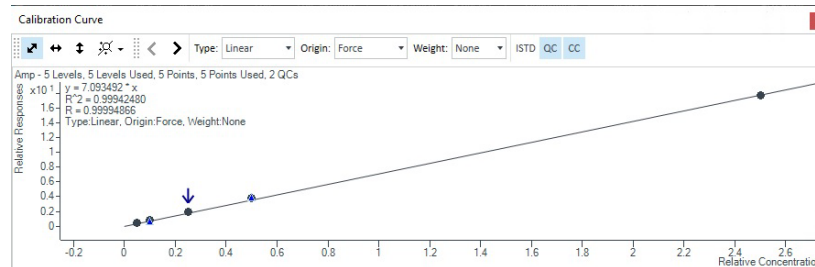
Calibration curve fit assistant

The Curve Fit Assistant of the MassHunter Quantitative Analysis program quickly calculates many possible curves to fit the data in the Batch Table. A table displaying these possible trial curve fits is displayed. This table contains information describing the trial curves and how well those curves match the current data. Also, the selected trial curve is shown overlaid with the currently selected curve in the graph portion of the window.

Sample information window

The Sample Information window, used in conjunction with the Compound window, will greatly assist you with reviewing your results.

Here you can graphically examine the current sample data file. You can zoom in on any peak in the chromatogram. You can also extract a spectrum from the chromatogram.
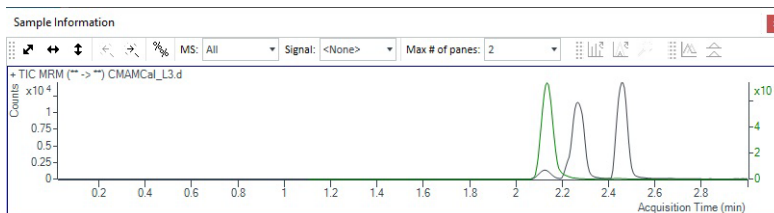
Compounds at a Glance window

The Compounds at a Glance window provides a graphical presentation for review of all or selected compound chromatograms in a batch by compound name or by sample. The compound peak can be overlaid with qualifiers, ISTDs, a Matrix spike, all compounds or compound groups, and all samples or sample groups. All compounds can be manually integrated from this window. Configured outlier results can also be identified on each compound peak.

Chromatogram information window

The Chromatogram Information Window provides a graphical representation of GC signals or TIC signals. You can compare signals by overlaying patterns, and create a new target compound entry in the quantitation method. This window is only available when you start the program using the MS Quantitative Analysis icon.

Method error list window

This window lists the errors that are generated when you validate the method using either the menu item Validate Method under the Method menu or the line Validate from the Save/Exit section of the Method Tasks window.

Method Error List		☒
Category	**Message**	
⚠	Retention time = 0.000 is outside the chromatographic range = [0.025, 3.002]	

Method table window

The Method Table displays the Quantifier Table , its Qualifier Table , and its Calibration Table along with a Globals Table. You edit or create a method using the tables in this window.

The columns in these tables can be changed using the Add/Remove Columns function in the table's shortcut menu but for developing and editing methods, selecting a task from the Method Tasks window will display the required columns for that task.

Filter the compounds shown in the Method Table by the data acquisition Time Segment. Select a compound from the drop-down list in the toolbar to select and view that compound in the Method Table.

Method Table

Time Segment: ‹ <All> ▼ › Compound: ‹ Amp ▼ › Reset Table View

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
Calib-L3	CMAMCal_L3.d	Cal	L3	APClautotune.m	5/12/2006 1:57 P…

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Ion Polarity
Amp	1	136.2 -> 91.4	MRM	Target	136.2	91.4	2.102	Positive
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	141.1	93.4	2.078	Positive
Cocaine	1	304.1 -> 182.0	MRM	Target	304.1	182.0	2.449	Positive
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	307.1	185.0	2.450	Positive
MDMA	1	194.2 -> 163.3	MRM	Target	194.2	163.3	2.269	Positive
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	199.2	164.3	2.269	Positive
Meth	1	150.1 -> 119.3	MRM	Target	150.1	119.3	2.239	Positive
Meth-d5	1	155.2 -> 92.3	MRM	ISTD	155.2	92.3	2.233	Positive

Method tasks window

The Method Tasks window helps you to navigate through the task of creating or editing a method. Select one of the following menu titles to access specific method editing procedures.
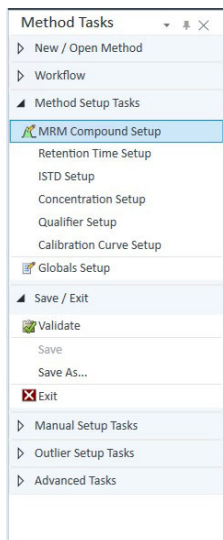
Tune evaluation window

This window is accessed by clicking Tune Evaluation on the Tools tab. By first selecting a tune evaluation sample from the Batch Table, that sample is automatically loaded when this window is accessed. Alternately, load a file by selecting Open on the File tab.

This option is available only if you start this program using an Environmental Analysis application (MS, QQQ, or Q-TOF).

Task Queue Viewer

This program is used with the Quantitative Analysis program while generating reports. When you generate a report in Quantitative Analysis, you have the option to put it into a list or queue of reports to generate. You can then continue using the Quantitative Analysis program while the Task Queue Viewer program continues to create the reports. This is especially useful when generating a large Excel-based report, which can take some time. To see this viewer, click **Queue Viewer** in the Report section on the ribbon.

In the main window of the Task Queue Viewer program, you can see a list of different reports. Some of these reports have already been completed, and some are still pending.

Compliance and Security

Customers in regulated environments can use the MassHunter Quantitative Analysis User Management and Audit Trail or Compliance software to help them comply with the 21CFR Part 11 FDA regulation that defines parameters by which pharmaceutical companies can author, approve, store, and distribute records electronically.

Software Development Kit (SDK)

Using the information in the Agilent MassHunter Workstation Quantitative Analysis and Unknowns Software Development Kits (SDKs) and the Python programming language, you can create custom calculations that will display in the Batch Table and Compounds at a Glance, as well as integrate into an outlier.

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10 Data and Method Compatibility

Data acquired with MassHunter Workstation can be reviewed and reprocessed.

MassHunter Workstation evaluates the raw data and files generated by MassHunter B.01.01 or higher.

Acquisition methods, data analysis methods, and worklists created in the previous version of MassHunter Acquisition and MassHunter Quantitative Analysis can be reused by importing them. Upon import, the record is updated with an audit trail and checksum if these do not exist.

This chapter provides an overview of the available import functions.

Method Migration

Acquisition Methods

MassHunter acquisition methods can be reused if they were created using the previous version of the software. Converted methods may contain new parameters not previously available. These parameters will contain default values after the conversion, so review the content of converted methods before using them for new data acquisitions.

Data Analysis Methods

MassHunter data analysis methods created in the previous version can be imported in the latest revision.

Data Import

The data analysis software can directly read the data that was acquired with the following

• MassHunter B.06.00

MassHunter can read the results created with the previous version.

Importing Report Templates

Reporting is done using the MassHunter Quantitative Analysis Report Builder. Existing report templates originating from previous versions of MassHunter can be imported.

Since a new version may contain new parameters not available in earlier revisions, always review the report content, thoroughly test it and, if needed, validate again before use.

Using data from earlier versions of MassHunter Workstation

After the system has been upgraded to the latest version, you can:

- Load data created with earlier versions of MassHunter,
- View results,
- Reprocess data and save as new version,
- Update and save methods (methods are migrated. Some acquisition methods require that you make changes in the method through the Method Resolution window.),
- Create reports with existing templates.

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11 Compliance Features

MassHunter features extensive technical controls to help regulated laboratories meet the core requirements of Title 21 CFR, Part 11, EU Annex 11, and more. These technical controls reduce the number of procedural controls your lab needs for complete data integrity, including electronic records security, work attribution, and electronic signatures.

System Access Controls

With MassHunter, you choose from five default laboratory roles, each providing unique levels of access and ability. New roles can also be created with the ability to assign multiple system attributes to a particular role.

To ensure data security, MassHunter identifies specific records as critical—such as acquisition/analysis methods, acquired data, analysis results, audit trails, and electronic signatures, which are automatically check-summed to check for invalid or altered records. If a tampered record is discovered, these files are locked and securely stored, while an error message is displayed.

Earlier sections of this document describe in detail the user authentication and system access controls. The options provided accommodate a wide variety of operational policies.

Data Security

Definition of the security aspects of the system is accomplished using the OpenLab Control panel and includes the following:

- System Activity Log (see **"Activity Logs"** on page 177)
- Selection of authentication provider (see **"System Settings"** on page 48)
- Management of users, groups, roles, and privileges (see **"Users"** on page 51 and **"Groups"** on page 51)
- Security Policy (see **"Security Policy"** on page 50)

Data Integrity and Traceability

MassHunter stores data in a manner that supports compliance with 21 CFR Part 11. It provides secure data storage with access control and an audit trail. Data files are versioned to ensure data integrity and traceability. MassHunter also provides electronic signatures, allowing users to indicate they have completed data review tasks on data.

The system supports a highly configurable environment which allows the Administrator to configure just the levels of security required in their laboratory. The administrator can define, for each individual project, if the work is regulated or not. This is configured in the OpenLab Control Panel.

Data Storage

OpenLab Server and ECM XT store data in a manner that supports compliance with 21 CFR Part 11. OpenLab Server Content Management provides secure data storage with access control and an audit trail. Data files are versioned to ensure data integrity and traceability. In addition, OpenLab Server/ECM XT provides electronic signatures allowing users to sign off on data.

Content Management allows users to easily collect, organize, search, and review all of their MassHunter Workstation data. It automatically extracts searchable metadata from files, and provides powerful search capabilities.

Client machines that access the OpenLab Server make use of the following components:

- **OpenLab Content Management browser**– OpenLab Server provides a thin client web based user interface that can be accessed using a web browser. The web interface provides access to the Content Management folders and files.
- **OpenLab Control Panel** – The Control Panel is the user interface that provides access to administrative functions used for managing the OpenLab Server.

In this document, the terms „central data storage" or "central repository" refer to instances of the Content Management database.

For more details, please refer to the OpenLab Server Administration Guide.

MassHunter File Types in the Data Storage

Data is organized in projects as the top-most hierarchical element. And each project is assigned a folder in the Content Management for the storage of the analytical data. Upon project creation the following sub folders are created by default in the project folder:

- **Methods** – see below
- **Report Templates**
- **Results** – results are saved in the data files or the folders that contain data files
- **Worklists** – storage location for worklists that were saved for further use

Content Management provides a multi-level folder storage model. The number of levels is not limited, and data can be stored in any folder level.

Methods

All acquisition methods and data analysis methods are stored in the methods folder and can be optionally structured in subfolders:

- Acquisition Methods (*.m)

Activity Logs and Audit Trails

MassHunter automatically generates activity logs and audit trails for end-to-end attribution of work. These logs and audit trails help staff and regulatory inspectors reconstruct the history of an electronic record.

- Activity logs track system events such as user logins, instrument connections, run start, run complete, etc.
- Audit trails track all changes to data including methods, injections, results, and worklists. The software records who is responsible for the action, what took place, when it occurred, which record was impacted, and why a record was changed.

Activity Logs

MassHunter features the following activity logs:

- System Activity Log
- Instrument Activity Log
- Content Management Log

System Activity Log

The System Activity Log contains detailed information on the various events associated with the OpenLab Server or with specific instruments. It is a centralized view of the audit logs and can access all entries. Users can filter the list in order to view only the events of a specific type, in a specific time range, created by a specific user, or containing a specific description.

Application indicates the originating application for the log entry. For example, MassHunter Workstation or Control Panel.

Subsystem filters based on the function within the application that originated the application. For example, Instrument Controller or Data Processing.

Levels are used as follows:

Table 1. Levels

Level	Description	Examples
Normal	Log entries for various system entries	Users starting Acquisition and Data Analysis
Info	Informational messages from instruments	Runs starting and completing
Warning	Entries for recoverable errors	A missing vial where the instrument sequence continues. A data analysis where the response is outside the calibration limits.
Error	Errors that cannot be recovered and a run must be aborted	Examples include a lamp failure, pressure limit exceeded, leak detected, or instrument controller disk full.

Note the use of the levels can vary by instrument. For example, pressure limits are errors for Agilent LC instruments while some non-Agilent instruments handle them as warnings.

Event messages can originate from different components, for example user management or instruments. The following types of events (subsystems) are recorded and reflect the origin of the message:

Table 2.   Subsystems

Subsystem	Description	Types of entries
System	Various activities that occur in system	Installation of licenses, add/change of instrument drivers, add/change of instrument controllers, configuration of authentication/storage, startup of OpenLab Shared Services, start/end of Acquisition, start/end of Data Analysis, window lock due to timeout, window unlock by a user
Instrument Management	Activities to create or modify instruments in the Control Panel	Add/change locations, add/change instruments, configure instruments, change user roles for instruments
Instrument	Activities logged by instrument controllers and drivers. When selected, an Item field provides the option to select specific instruments. A Deleted Items option shows activity log entries stored for instruments that have been deleted.	Client connection to an instrument, sample submission, worklist run submissions, add/change runs in the run queue, start/completion of injections, user take/release control actions, connection/disconnection to the Shared Services server, switch into failover mode, restore from failover mode
Project Management	Activities to create or modify projects in the Control Panel	Add/change/delete project groups, add/change/delete projects, add/change user roles for projects
Instrument Controller	Not used	
User	User administration activities	Create local users, modify users, remove local users, import ActiveDirectory users
Group	Activities pertaining to user groups	Adding external groups to the system, creating internal groups, adding or removing members to/from a group
Role	Activities pertaining to user roles	Add/change/delete roles
Security	User access activities	Users logging in, locking or unlocking users, failed login attempts, changing security policies
Printer	Not used	
License	Not used	
Data Processing	Activities performed by OpenLab Data Analysis	Start/finish of analysis processing
Analysis Request	Not used	
Application Configuration	Not used	

Note subsystems not used by MassHunter are used by other products that can be configured together with MassHunter.

Events can include system messages, warnings and errors. The System Activity Log records these events irrespective of whether the user has been alerted by the system or not. For each entry there is a one-line overview with date/time stamp, affected user and short description in the Activity Log Viewer. The entry can be expanded to reveal more information such as type, affected subsystem, source PC and detailed description.

The System Activity Log can be exported, printed and selections can be copied to the clipboard. The output is provided in two formats – summary and detailed.

Instrument Activity Logbook

The Instrument Activity Log is a view of the System Activity Log that is accessed from the instrument acquisition window. The transactions that occur during the analysis, including any errors and the instrument conditions at the start and the end of the analysis, are recorded in the system's Instrument Activity Log. The information is shown in tabular format and can be filtered by user, description and date. The following information is logged for each event:

- Date and time stamp of the message
- User name – full name, if configured in the OpenLab Control Panel, otherwise the login name
- Description of the event
- Details on the activity, for example, run and injection information upon the start of a single sample analysis

The Activity Log displays the latest records of system activity paginated every 1000 entries. The Activity Log provides means to filter or search by user, description, and date range.

Events automatically added to the log include:

- Submission of a single run or worklist,
- Addition of an item to the run queue,
- Start of a run in the run queue,
- Start or end of an injection, and
- Submission of an item for processing.

Content Management Activity Log

In addition to the logs described in the previous sections, systems configured with OpenLab Server, OpenLab ECM XT, or OpenLab ECM 3.x also contain an activity log in the content management storage system.

The content management activity log shows who, when, and what operations an individual performed. The log contains file-related, system administration and folder administration entries. Each transaction is documented with the identity of the operator, the dataset and a date and time stamp. For example, administrators can see when a file was added and who added it. For each operation, the central data storage system asks the user for a reason. This reason is also shown in the Activity Log. For automatic uploads there are default reasons given by the system. See the OpenLab Server or OpenLab ECM XT documentation for more information.

Audit Trails

Content Management Audit Trail

When MassHunter Acquisition is launched, multiple background processes are simultaneously launched, some of which have no specific user interface. Agilent refers to these processes as System Engines.

Many of these System Engines interact directly with the ECM server, often independent of any direct user action.

When these System Engines connect to the ECM server – they will use whichever user was configured in the OpenLab Control Panel to authenticate to the ECM server. The ECM audit trail records these authentications in its Audit Trail with the description "User has logged in." "User has logged out" will be recorded when the connection session is terminated. These connections may be established, terminated, and re-established multiple times whenever necessary for the System Engines.

Audit Trail Viewer window

MassHunter provides audit trails that document all changes to the methods, templates, and analytical data.

All audit trails can be inspected in an Audit Trail Viewer window.

In Data Acquisition and Quantitative Analysis, the Detail view shows all entries for an item listed by date with the newest entry first. The Detail view provides means to filter or search the audit trail. Entries can be filtered by date or searched by a keyword. Authorized users can add a review comment to mark an audit as reviewed and print the displayed information or export it to a PDF. The audit trail viewer is launched from the ribbon.

In BioConfirm you can open different audit trail viewer windows both from the BioConfirm program and from the Sequence Manager program. The BioConfirm audit trail windows are: Method Audit Trail, Chemical Data Dictionary Audit Trail, Results Audit Trail, and Sequence Audit Trail.

Each audit trail entry includes:

- **Name** – full name, if configured in the OpenLab Control Panel, and the login name
- **Date** of the change
- Detailed **Description** of the change
- **Category** – for example: new, method linking, reprocessing, manual entry, audit trail review, saving, and affected instrument module.
- **Reason** – if enabled for the project (see below)
- **Host name** – the software client was launched from when the change was done
- **Software Name** – the name of the MassHunter program that made the audit trail entry
- **SW Version**
- **Reviewer** – populated when the audit trail is reviewed and saved
- **Review Date**
- **Review Comment**
- **File Version**
- **Inst Type**
- **Inst Name**

Each time you save a modified record, the changes are logged in the corresponding audit trail. User names are logged against all entries when authentication is enabled. If authentication is not enabled, entries are logged against a SYSTEM user.

When saving methods, worklists, or results (also automatic saving during reprocessing), you are asked to enter a reason for change, if this option was enabled for the project.

The behavior of audit trails in general and the worklist, method or results audit trail in particular depend on the project settings defined in OpenLab Control Panel.

Acquisition Method Audit Trail – Data Acquisition

The Acquisition method audit trail records all changes made to the acquisition method including from/to value changes.

Worklist Audit Trail – Data Acquisition

The Worklist audit trail records all changes made to the worklist, including worklist table entries such as vial locations, sample types, or sample and compound amounts.

Chemical Data Dictionary Audit Trail – BioConfirm

The Chemical Data Dictionary audit trail shows any changes that were made to the modifications, links, and reagents.

Results Audit Trail – BioConfirm

For BioConfirm, the Results Audit Trail shows all the changes made to the BioConfirm results performed on the data file.

Batch Audit Trail – Quantitative Analysis

For Quantitative Analysis, the Batch Audit Trail lists modifications such as method linking, reprocessing, or manual integration activities. Calibration curve modifications are also recorded here.

Advanced Audit Trail Review

Recently, regulatory authorities changed the way they want labs to conduct and document audit trail reviews. For example, European Annex 11 requirements introduced in 2010, specify that—in addition to recording the audit trail—labs must review the audit trail and document the review. In addition, the FDA recently announced that audit trail reviews must take place in the context of the reviewed electronic record and with its associated metadata. This means that when a worklist and its results are reviewed, the corresponding audit trail must be reviewed at the same time.

MassHunter has been designed to meet these new requirements. Laboratories can now include confirmation and documentation of audit trail reviews as part of the electronic record—eliminating the need for manual printing and signing.

If an audit trail has not been reviewed, the entries are shown with a purple (magenta) background. Reviewed entries are shown with a white background. During the review, an authorized user can inspect all new entries. If required, the user will need to scroll down until the last unreviewed entry at the bottom of the list is visible. By clicking the Review button, the user completes the review. The Review button is active only after the user has viewed all entries in the table and entered a review comment. The background color changes from magenta to gray. A new entry is added to the audit trail, documenting that it has been reviewed.

Staff can also perform full-text searches, or search by audit categories, such as audit trail entries arising from data reprocessing or manual integrations. Staff can filter and find the audit trail information needed with ease.

Record of Instrument Settings

For BioConfirm, the instrument conditions, such as flow, temperature, pressure and solvent composition for liquid chromatograph may be continuously recorded and stored with each data file as instrument curves. These instrument parameters can be displayed and plotted to testify to the quality of each analysis. The exact nature of the parameters recorded depends both on the technique and the capabilities of the configured instrument.

Electronic Signature

Electronic signature is available with the OpenLab ECM XT server. Electronic signature allows users to apply a single-tier signature with pre-defined or custom reasons. (*This feature is not available for OpenLab Server.*)

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12 Instrument Control

The MassHunter Workstation provides instrument control, data acquisition, and data analysis for the current Agilent LC/TQ systems, LC/TOF systems, and LC/Q-TOF systems. For LC/TQ, the supported sources include Electrospray (ESI), APCI, Agilent Jet Stream, Multimode, and Nano ESI but vary depending on specific instrument type. For LC/TOF and LC/Q-TOF, the supported sources include Electrospray (ESI), APCI, APPI, Agilent Jet Stream, and Multimode Source but vary depending on specific instrument type. The LC/MS system can be combined and used with the Agilent 1100, Agilent 1200 Infinity Series modules and InfinityLab systems for LC supported by MassHunter Workstation.

Instrument drivers provide the functionality and user interface for the instrument setup and configuration, instrument control, acquisition method parameter setup and modification, and instrument status monitoring.

Agilent developed the RapidControl.NET Instrument Control Standard (RC.NET standard) for direct instrument control. This standard is also open to other analytical device manufacturers and enables them to develop their own drivers for MassHunter Workstation. Some instrument drivers supported in MassHunter are based on this standard.

The drivers are seamlessly embedded in MassHunter Workstation. OpenLab Control Panel is used for the setup and configuration of instruments (see **"OpenLab Control Panel"** on page 31). The acquisition client hosts the user interface for managing acquisition methods as well as the instrument dashboard (see **"Data Acquisition"** on page 91).

Depending on the MassHunter revision, instrument driver packages can be deployed independently from MassHunter Workstation, which means adding a new instrument to the system does not affect or alter the installed MassHunter software.Therefore, adding a new instrument driver has a very limited impact on the system.

The instrument control capabilities are documented in the following sections, each relating to a specific technique.

The supported instruments and firmware are further detailed in the *MassHunter Workstation 12.0 Requirements* guide. For more details on the instrument capabilities, please refer to the corresponding hardware manuals.

Liquid Chromatography

MassHunter Workstation controls and acquires data from the Agilent InfinityLab LC Series, the Agilent 1200 Infinity Series, the Agilent 1200 Series and the Agilent 1100 Series. They include modules for solvent delivery (pumps and degassers), sample injection and column thermostats, valve solutions, detectors and many others. Instrument control is provided through module drivers.

For all modules the user may set a stop time and optionally post-run time, for example for column equilibration.Typically, the stop time is set for one module, the pump or the injector, and other modules are set to **As Pump/Injector** for the stop time.However, depending on the application, individual times may be defined. A new run can be started as soon as all modules have completed the previous run including the post time. If no stop time is set, the instrument will continue until it is stopped manually. An automatic module turn-on time (date and time) can be set for each module, for example to allow for sufficient equilibration time in the morning before the operator comes in and submits the first worklist of the day.

For a detailed and current list of all supported modules please refer to the *MassHunter Workstation 12.0 Requirements* guide and the driver release notes delivered with each new version of the Agilent instrument drivers (included on the driver DVD).

Solvent Delivery Systems

All solvent delivery systems offer parameters for solvents, the solvent composition, the flow rate and pressure limits.A time-table can be used for defining composition gradients and modifying other parameters like flow rate and pressure limit over time.These parameters can be viewed graphically.

MassHunter Workstation supports an extensive list of solvent delivery systems that include isocratic, binary and quaternary pumps.

Sampling Systems

The injection systems may be manual or automated with an autosampler, well-plate sampler or a multisampler. All automatic injectors may be programmed for different injection volumes; the speed of injection and the injector wash procedure (e.g. needle wash, needle seat flush). The user may also use a complete injector program for sample dilution, standard addition or sample derivatization.

Column Compartments

The following Agilent column compartment types are supported:

- Multicolumn Thermostats (MCT),
- Thermostatted Column Compartments (TCC) and
- Integrated Column Compartments (ICC)

Depending on the type of column compartment different temperatures may be set.For example, the 1290 Infinity II Multicolumn Thermostat facilitates precise column thermostatting over a temperature range from 20 °C below ambient up to 110 °C.Please refer to the instrument specifications for details.The temperature is programmable during the run through a timetable.Column switching valves are programmable from the software.

Valve-Thermostat-Clusters (VTC) allow the combination of multiple internal and external valves and temperature zones in column thermostats for switching between up to 32 columns and sophisticated temperature control.

Valves

For a detailed and current list of all supported valves please refer to the *MassHunter Workstation 12.0 Requirements* guide.

Detectors

MassHunter Workstation supports multiple UV/Vis based detector types such as Diode Array Detectors (DAD), Variable Wavelength Detectors (VWD), Multiple Wavelength Detectors (MWD) and Fluorescence Detectors (FLD). However, MassHunter Workstation does not provide control of special detectors, such as Refractive Index Detectors (RID). Mass-based detectors are covered in section **"Mass Spectrometry"** on page 194.

UV Detection

UV detection comprises the following detectors:

- Variable Wavelength Detectors
- Multiple Wavelength Detectors
- Diode Array Detectors

The Agilent MassHunter Workstation software can simultaneously acquire multiple chromatographic and reference signals each with an independent bandwidth. The data and spectra acquisition rate and the maximum number of signals depend on the capabilities of the individual detector in use. Spectral data from all DADs may be acquired in a peak-controlled or full acquisition mode. Depending on the detector model up to 8 different spectra acquisition modes are available:

- **None** –No spectra are taken.
- **Apex** –A spectrum is taken at the apex of the peak.
- **Apex + Baselines** –Spectra are taken at the apex, and baselines of the peak.
- **Apex + Slopes** –Spectra are taken at the apex, upslope, and downslope of the peak.
- **Apex + Slopes + Baselines** –Spectra are taken at the apex, baselines, upslope, and downslope of the peak.
- **All in Peak** –All spectra within the peak are taken.
- **Every 2nd spectrum** –Spectra are taken continuously, but only every second spectrum is stored; other spectra are discarded. This reduces the amount of data storage necessary.
- **All** –Spectra are taken continuously depending on the setting of the peak width. Eight spectra are acquired per peak width.

The wavelength range for spectra storage can be set by the user as well as the spectral resolution. A threshold value defines the height of the smallest expected peak. Smaller peaks will be ignored by the peak detector and peak spectra will not be saved. The spectral resolution can be influenced by the slit width. The possible values depend on the detector hardware.

All Detectors may have the initial parameters changed during a run by a time-based program.

Depending on the model Agilent DADs, MWDs and VWDs support up to 240 Hz data acquisition (with or without spectral data) to a maximum of 8 signals, complemented by additional instrument curves. The VWD may be programmed with a single detection wavelength; the Infinity II VWD supports the use of two wavelengths in parallel. The flow cells and UV lamps utilize RFID tags to record lamp and cell information. The improved Temperature Management System provides ambient rejection and a stable cell temperature. Users can program a baseline adjustment (balance) or wavelength (signal) change in the timetable. For DADs additionally the threshold, peak detector peak width and spectral acquisition mode can be changed during a run. Initial parameters that may be set include signal wavelengths and reference wavelengths, spectral acquisition mode (if applicable), signal sampling rate and an automatic baseline reset (autobalance) during pre- or post-run, or both. The VWD can perform wavelength scans during the course of an analysis.

Special Detection

Special detection summarizes the following detector:

- Fluorescence Detectors (FLD)

The Agilent FLDs may be programmed for single wavelength or simultaneous multiple wavelength detection and spectra acquisition. Up to four signals at different excitation or different emission wavelengths may be obtained. Within a timetable initial excitation or emission wavelengths, response time, PMT Gain and baseline behavior as well as spectral parameters may be changed. Excitation or emission spectra can be watched online and stored. Spectral data from capable FLDs may be acquired in a peak-controlled or full acquisition mode. The available spectra modes are:

- **None** –No spectra are taken.
- **Apex** –A spectrum is acquired at the apex of the peak.
- **All in Peak** –All spectra within the peak are acquired.
- **All w/o signal** –All flashes are used for spectra acquisition. The mean value of all measured wavelengths is shown on channel A. This setting is useful for unknown spectra. Spectra are acquired continuously.

The wavelength range for spectra storage can be set by the user as well as the spectral resolution. A threshold value defines the height of the smallest expected peak. Smaller peaks will be ignored by the peak detector and peak spectra will not be saved. Optionally the spectral range can be adjusted so that there is always a difference of at least 25 nm between the excitation wavelength and emission wavelength.

For most current information and more details on the instrument capabilities please refer to the corresponding user manuals and data sheets.

HDR Cluster

A cluster allows combining several modules into one logical unit. Such a cluster is shown as a single instrument module in the MassHunter Acquisition software. The 1290 Infinity and Infinity II HDR Clusters consist of two DADs, one with a long flow cell (for example, 60 mm), the other with a short flow cell (for example, 10 mm). The signals from the two DADs are combined to produce a high-dynamic-range signal, which allows detecting compounds with very low concentration (e.g. impurities) next to high concentration compounds (target substance).

Maintenance and Diagnostics

The Agilent Lab Advisor is a maintenance, diagnostics and calibration software toolset that can be added for maintaining and diagnosing the Agilent LC systems. It is independent of MassHunter Workstation and provides full diagnostic and maintenance capabilities including an extended list of tests and calibration procedures.

Mass Spectrometry

Instruments

Agilent LC/TQ

The Agilent TQ is a triple quadrupole mass spectrometer.

You can set up a TQ LC/MS with Agilent 1200, 1260, 1260 Infinity II, 1290 Infinity, or 1290 Infinity II LC modules and with one of several ion interfaces:

- ESI
- AJS ESI (Agilent Jet Stream ESI)
- APCI
- MMI
- Nano ESI

Agilent LC/TOF

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

You can set up a TOF LC/MS with Agilent 1200, 1260, 1260 Infinity II, 1290 Infinity, or 1290 Infinity II LC modules and with one of several ion interfaces:

- ESI
- Dual ESI
- AJS ESI (Agilent Jet Stream ESI)
- Dual AJS ESI (Dual Agilent Jet Stream ESI)
- APCI
- APPI
- Multimode
- Nanospray (nanoESI)

- Dual nanospray (Dual nanoESI)
- MALDI
- GC-APCI

The 6230 Accurate-Mass LC/TOF is the only LC/TOF instrument that supports the Agilent Jet Stream ESI and the Dual Agilent Jet Stream ESI. The AJS ESI and Dual AJS ESI sources use a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

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

Agilent LC/Q-TOF

The Agilent Q-TOF LC/MS is a liquid chromatograph-quadrupole time-of-flight mass spectrometer that performs MS/MS using a quadrupole, a hexapole collision cell and a time-of-flight analyzer 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.

You can set up an Agilent Q-TOF LC/MS with Agilent 1200, 1260, 1260 Infinity II, 1290 Infinity, or 1290 Infinity II LC modules, and with one of several ion interfaces: ESI, Dual ESI, AJS ESI, Dual AJS ESI, APCI, APPI, Multimode, nanospray, dual nanospray, MALDI and GC-APCI.

The 6530, 6540 UHD Accurate-Mass Q-TOF, the 6545, 6546 Q-TOF, the 6545XT AdvanceBio Q-TOF, and the 6550 iFunnel Q-TOF, are the Q-TOF instruments that can be set up with the Agilent Jet Stream ESI source (AJS ESI) and the Dual AJS ESI. These sources use a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

MS Tuning and Calibration

When the MS is used as a detector for chromatography, a mass spectrum is associated with each data point in the chromatogram.To obtain high quality, accurate mass spectra, the MS must be optimized to

- Maximize sensitivity,
- Maintain acceptable mass resolution, and
- Ensure accurate mass assignments.

Tuning is the process of adjusting MS parameters to achieve these goals.

<u>**Autotune** is an automated tuning algorithm that tunes the MS for good performance over the entire mass range.</u>

For the TOF/Q-TOF instrument, the software provides two ways to tune the MS:

- **Autotune** is an automated tuning algorithm that tunes the MS for good performance over the entire mass range.

- **Manual Tune** allows the advanced user to adjust MS parameters to meet user-defined criteria. Manual tuning is an iterative process of maximizing sensitivity while maintaining acceptable resolution, ensuring accurate mass assignment, and providing the desired relative abundance across the spectrum.It is most often used when maximum sensitivity is required, when a restricted mass range is to be targeted, or when a tuning compound other than the standard calibrant is needed.

The LC/MS can be tuned through the MassHunter Workstation by the automated introduction of a calibrant and tuning for adjusting signal intensity, mass transmission, resolution and mass accuracy.

The tuning operations further include a Checktune functionality, where a calibrant is introduced and a report is generated, but no parameters are changed.

For the TOF/Q-TOF instrument, multiple tune files can be stored in the MassHunter Workstation. Each MS acquisition method is linked to a corresponding tune file.The most recent tune reports are stored with all acquired data.All tune operations are protected by user permissions which are configurable in the MassHunter Workstation

Maintenance and Diagnostics

Common instrument diagnostics and maintenance functions for the LC/MS detector, including Venting, are collected under Maintenance and Diagnostics. These functions are used by users and trained Agilent service personnel to troubleshoot the LC/MS operation. Details on each function are available in the user documentation.

Other Instrument Control

Non-Agilent Instruments

MassHunter Workstation can support third party detectors via the UIB only. MassHunter cannot control any third party instruments directly.

Contact Agilent Sales for the full list of supported instruments.

SS420x Analog/ Digital Converter

The SS420x is an intelligent 24-bit analog to digital conversion module that provides remote acquisition for up to 4 channels of data. The channels can be started or stopped independently for up to four instruments or detectors. It supports data acquisition rates of up to 120 Hz depending on the used base frequency. The user can configure the base frequency of the SS420x and, for each channel, the gain range, channel name and, Y-axis unit and multiplier.

The SS420x has four built in triggers and eight relay outputs for programmable switching applications. The eight relay outputs allow the selection from three possible states: Open, Closed, Last State. The four run trigger inputs allow the selection from two possible states: Open, Closed.

For the acquisition the user can enable up to four channels, select the sampling rate per channel and enter an individual acquisition delay time, if needed.

The following event types can be configured for external event control for each channel:

- **Trigger signal** – input from an external device to initiate the start of a run.
- **Ready signal** – output to external device to indicate "device is ready for injection".
- **Valve / external event** – output to external device.

The configured event types can be time-programmed during a run.

For most current information and more details on the instrument capabilities please refer to the corresponding user manual.

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13 Help and Learning

This chapter provides a brief summary of all relevant documentation that is available for MassHunter and how to find it.

MassHunter Help & Learning

MassHunter Workstation includes context-sensitive online Help. You can press the Help button or **F1** to get more information about the area in the program where you are. You can also press the **?** mark in the upper part of the screen to open the Help & Learning or online Help volume at the top level.

Some manuals are available on the Agilent website **https://www.agilent.com/**

Agilent Community

To get answers to your questions, join over 10,000 users in the Agilent Community. Review curated support materials organized by platform technology. Ask questions to industry colleagues and collaborators. Get notifications on new videos, documents, tools, and webinars relevant to your work.

https://community.agilent.com/

In This Book

This document contains the Functional
Design Specifications for your Agilent
MassHunter Networked Workstation system.

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