Watson TSQ Module

Version 1.0

User Guide

XCALI-97257 Revision A August 2009

DOCUMENTATION SURVEY

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

LCquan and TSQ Vantage are trademarks, and Xcalibur and TSQ Quantum are registered trademarks of Thermo Fisher Scientific Inc. in the United States.

Windows, Excel, and Windows Vista are registered trademarks of Microsoft Corporation in the United States and other countries. Acrobat, Flash, and Acrobat Reader are registered trademarks of Adobe Systems Inc. in the United States and other countries.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Release history: Revision A - August 2009

Minimum software requirements: Xcalibur 2.1.0; TSQ 2.0.5; Microsoft Windows XP Professional SP 3; (optional) LC Devices 2.2.1

For Research Use Only. Not for use in diagnostic procedures.

Preface

The Watson™ TSQ™ Module application is part of the Thermo mass spectrometry data system.

This user guide describes how to use the Watson application features to create and send a run to the TSQ Module application and perform data review on results files sent from the TSQ Module application.

This user guide also describes how to use the TSQ Module application features to accept a run request from the Watson application, acquire samples, and return the acquired data to the Watson data storage.

Contents

❖ **To suggest changes to documentation or to Help**

Complete a brief survey about this document by clicking the link below. Thank you in advance for your help.

Related Documentation

Documentation for the TSQ Module application includes Help and the *TSQ Module User Guide* as a PDF file.

❖ **To view the *TSQ Module User Guide***

Go to **Start > All Programs > Thermo Watson TSQ Module > Manuals > Watson TSQ Module User Manual**.

❖ **To open the TSQ Module Help**

From the TSQ Module application, choose **Help > Watson TSQ Module Help**.

To find a particular topic, use the Help Contents, Index, or Search panes.

For more information, including upcoming application notes, visit www.thermo.com.

Special Notices

This guide includes the following types of special notices:

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

System Requirements

Your system must meet these minimum requirements.

System	Requirements
PC	• 2 GHz processor with 1 GB RAM • CD-ROM drive • Video card and monitor capable of 1280×1024 resolution (XGA) • 75 GB on the C: drive • NTFS format
Instrument	• TSQ Quantum™ – Or – • TSQ Vantage™
Software	• Microsoft™ Windows™ XP Professional with Service Pack 3 • (Optional) Xcalibur™ 2.1.0 • Adobe Acrobat 9.0 • TSQ 2.1.0 • LC Devices 2.2.1 • Watson 7.4 • Foundation 1.0.1 (available on the Xcalibur 2.1.0 CD) • (Optional) LCquan (required to create .lqn method files)

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To copy manuals from the Internet

Go to mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

❖ To suggest changes to documentation or to Help

- Fill out a reader survey online at www.thermo.com/lcms-techpubs.
- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

Introduction

This chapter describes the general features and workflows of the TSQ Module application and the TSQ Module-specific features of the Watson application.

Contents

Watson Feature Summary

The TSQ Module application is designed for routine tandem liquid chromatography-mass spectrometry (LC/MS) bioanalysis of samples. The TSQ Module application can be used with Thermo Fisher triple quadrupole LC/MS systems such as the Quantum and Vantage LC/MS products. After an analytical method has been developed, the TSQ Module application can be used to validate the method and perform routine bioanalysis of samples.

The TSQ Module-specific features within the Watson application include:

- The Watson work list, tuning (*.tun) and method (*.meth) files are directly exported to the TSQ Module application
- The LC/MS tuning (*.tun) and method (*.meth) files are stored inside the Watson application database
- The LC/MS peak processing parameters are stored inside the Watson application database
- Raw data is stored inside the Watson application database
- Chromatogram review, re-injection selection and submission, and peak integration and re-integration occur within the Watson application
- Real-time run peak review occurs in the TSQ Module application
- Real-time run status is obtained in the Watson application

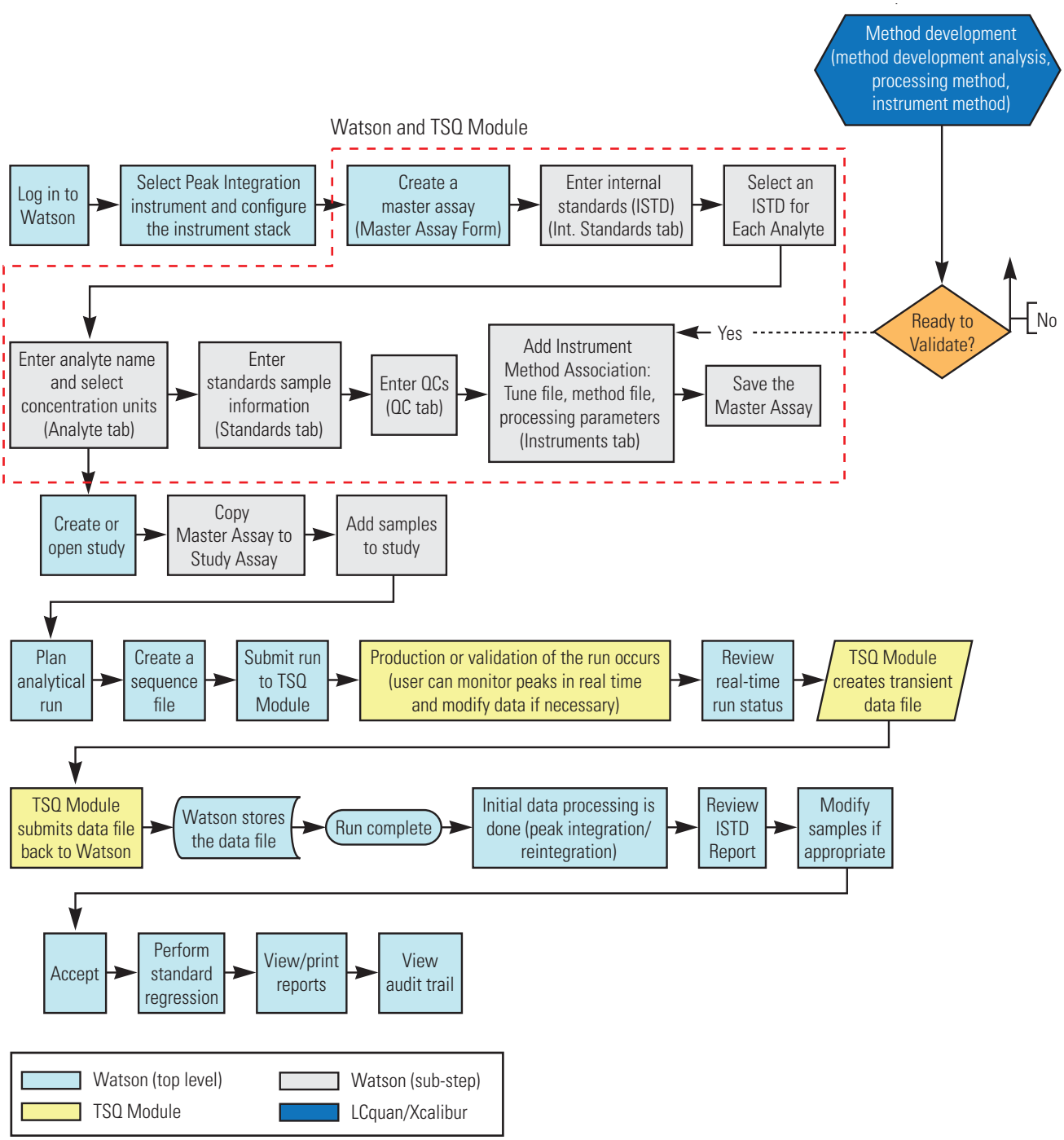
- Raw data is archived within the Watson application study archive
- A single audit trail of data acquisition (performed in the TSQ Module application) and run review processing is stored in the Watson application

TSQ Module Feature Summary

The TSQ Module application is a software solution for use in bioanalytical quantitation. The features of the TSQ Module application provide support for the acquisition of run requests submitted by the Watson application. The TSQ Module application features include:

- Submission of run requests to an instrument
- Editing of samples in a run
- Real-time view of runs in progress or in the Acquisition Queue
- The ability to start, stop, pause, and cancel an acquisition in the queues with audit messages sent to the Watson application
- Security provisioning against the Watson LIMS
- Secure and verified transmissions of the data files using a proprietary schema
- Run manager that holds multiple run requests from the Watson application so that users can visualize the current load on a system, select the next run to acquire, and (when necessary) send an unacquired run back to the Watson application for assignment to a different instrument
- A processing method exporter to extract processing information from an Xcalibur processing method or LCquan workbook and upload these parameters
- General purpose communication service layer between the TSQ Module application and the Watson application that supports multiple instances and databases
- Re-injecting samples without creating a new run in the Watson application

Watson/LCquan-Xcalibur/TSQ Module Workflow



Watson/LCquan-Xcalibur/TSQ Module Sequence of Steps

The following table outlines user-specific steps using the Watson application, the Xcalibur application, and the TSQ Module application.

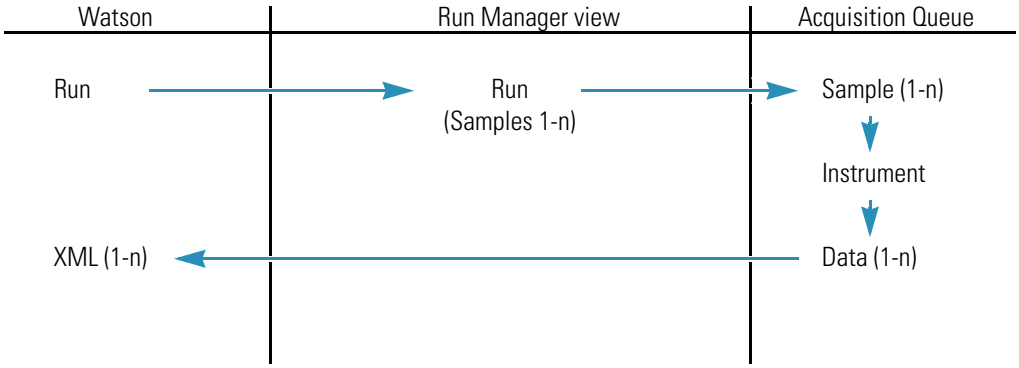
Table 1. Watson/Xcalibur/TSQ Module key steps

Step	Instructions
Create a peak integration instrument interface.	Completed by the System Manager. For more information, refer to the TSQ Module System Manager's Guide.
Create or clone the instrument stack.	See "Creating an Instrument Stack" on page 7.
Create the tune, method, and processing parameters files.	Completed externally to the Watson application in the LCquan or Xcalibur applications. For more information, refer to the *LCquan User Guide* or the Xcalibur documentation set.
Setup the master assay.	See "Setting up the Master Assay" on page 13.
Create or open a Watson study.	See "Working with a Watson Study" on page 16.
Copy the master assay to the study assay.	See "Working with a Watson Study" on page 16.
Create a new run with the study assay and add samples to the work list.	See "Working with a Watson Study" on page 16.
Create a sequence file.	See "Working with a Watson Study" on page 16.
Send the run to the TSQ Module application.	See "Working with a Watson Study" on page 16.
When necessary, modify the run parameters (injection volume or position).	See "Editing a Run" on page 41.
Start data acquisition.	See "Acquiring a Run" on page 45.
Monitor peaks in real time in the TSQ Module application.	See "Monitoring the Run Status" on page 48.
Monitor run status in real time in the Watson application.	See "Reviewing Runs" on page 59.
Review the run.	See "Reviewing Runs" on page 59.
Change the peak processing parameters.	See "Reintegrating Chromatograms" on page 63.
Review internal standards.	See "Reviewing Internal Standards and Performing Regression" on page 71.

Step	Instructions
Filter sample types.	See "Reviewing Internal Standards and Performing Regression" on page 71.
When necessary, select runs to reinject.	See "Re-injecting a Run from the Watson Application" on page 106.
Review and accept the results of the reinjected run.	See "Reviewing Internal Standards and Performing Regression" on page 71.
Accept the run.	See "Reviewing Internal Standards and Performing Regression" on page 71.
Perform standard regression.	See "Reviewing Internal Standards and Performing Regression" on page 71.
Generate and print reports.	See "Printing Capabilities" on page 84.
Review the audit trail.	See "Audit Reports" on page 80.

Watson/TSQ Module/Watson Run Management

The TSQ Module application workflow moves runs between three locations: the Watson application where runs originate, the TSQ Module Run Manager view that receives each run, and the TSQ Module Acquisition Queue where runs are acquired.



- A run sent from the Watson application enters the TSQ Module Run Manager view. The run status is Pending.
- When you submit the run for acquisition, the run is placed in the Acquisition Queue and the status in the Run Manager view changes to Submitted.

  If the run is the first run in the Acquisition Queue, acquisition immediately begins and the status in the Run Manager view is Acquiring.
- When the acquisition begins, the status changes to Acquiring.

- When the acquisition finishes, the status for the run changes to Completed. The data for the run returns to the Watson application in XML format and the run is removed from both the Run Manager view and the Acquisition Queue.
- While in the Run Manager view, you can cancel a run. A canceled run returns to the Watson application and is removed from the Run Manager view.
- While in the Acquisition Queue, you can remove a run and return it to the Run Manager view with a Stopped or Pending status. To move a run from the Acquisition Queue and return it to the Watson application, you must first return the run to the Run Manager view and then cancel it from there.

Table 2. Run Manager view status

Status	Description
Pending	The run arrived from the Watson application and awaits acquisition. This run could have been submitted to the Acquisition Queue and returned to the Run Manager view before it began acquiring.
Submitted	The run is assigned for acquisition but is not currently acquiring. The run is in the Acquisition Queue.
Acquiring	The run has progressed to the top of the Acquisition Queue and is currently acquiring.
Completed	The acquisition has finished running and the run data was returned to the Watson application. Completed runs might not always be observed in the Run Manager view. When the TSQ Module application finishes sending the results of the last completed sample to the Watson application, it marks the run as Completed and quickly removes the run from the queue.
Paused	The run is assigned to an instrument and is currently acquiring, but has been paused and waits between samples.
Stopped	The run was assigned to an instrument and was acquiring, but was removed from the Acquisition Queue.

Watson: Submitting Runs to the TSQ Module

This chapter provides information about setting up a run and submitting it to the TSQ Module application.

Contents

Creating a Peak Integration Instrument

The system manager sets up the peak integration instrument that is part of the Watson TSQ Module application configuration process.

This section describes the following tasks:

- Creating an Instrument Stack
- Specifying Devices

Creating an Instrument Stack

The following procedures provide instructions to create, clone, edit, or delete an instrument stack.

Use the following procedures:

- To create the instrument stack
- To clone an instrument stack
- To edit an instrument stack
- To delete an instrument stack

❖ To create the instrument stack

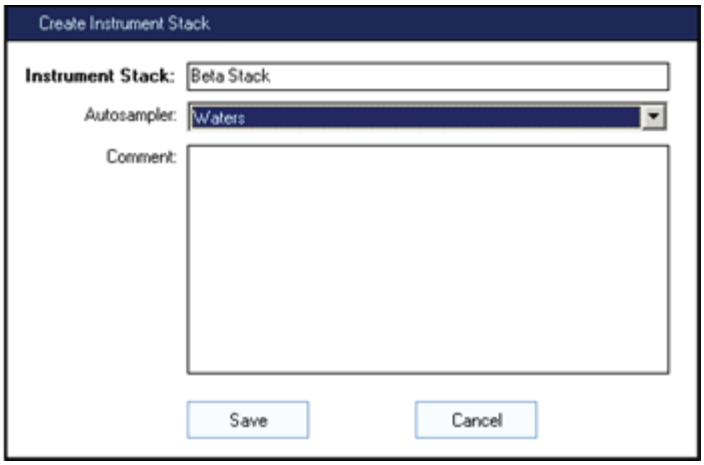
1. Choose **Options > Instrument Inventory**.

   The Instrument Setup window opens.

2. From the Instrument Setup window, click the Peak Integration instrument and then click **Add** to create (define) the stack.

   The Create Instrument Stack dialog box opens.

Figure 1. Create Instrument Stack dialog box



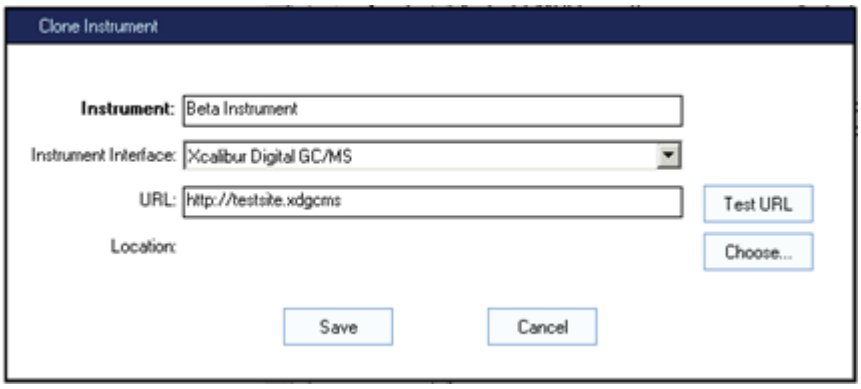
3. In the Create Instrument Stack dialog box, enter an Instrument Stack name.
4. (Optional) Enter an autosampler or comment.
5. Click **Save**.

❖ To clone an instrument stack

1. Choose **Options > Instrument Inventory** to open the Instrument Setup window.
2. Select an instrument and then click **Clone.**

   The Clone Instrument dialog box opens, populated with the stack information.

Figure 2. Clone Instrument dialog box



3. To modify stack information, click the appropriate field.
4. To test the URL, click **Test URL**.

   If the Watson application is unable to access the URL, you receive an error message.
5. To specify a location, click **Choose** and select a location.
6. Do one of the following:
   - To save the cloned instrument, click **Save**.

     –Or–
   - To exit without saving any changes, click **Cancel**.

❖ To edit an instrument stack

1. Choose **Options > Instrument Inventory.**

   The Instrument Setup window opens.
2. Select an instrument stack and click **Edit**.

   The Edit Instrument dialog box opens, populated with the instrument information.
3. To modify instrument information, click the appropriate field.
4. Do one of the following:
   - To delete save the device, click **Save**.

     –Or–
   - To exit without making any changes, click **Cancel**.

❖ To delete an instrument stack

1. Choose **Options > Instrument Inventory.**

   The Instrument Setup window opens.
2. Select an instrument stack and click **Delete**.

   A confirmation message opens.
3. Do one of the following:
   - To delete the instrument stack, click **OK**.

     –Or–
   - To exit without making any changes, click **No**.

Specifying Devices

Use the following procedures:

- To create a device
- To clone a device
- To edit a device
- To delete a device

❖ To create a device

1. Choose **Options > Instrument Inventory** to open the Instrument Setup window.
2. Select the Peak Integration instrument.
3. Click **Add** at the bottom of the Devices pane.

   The Create Device dialog box opens.
4. Enter a Device Name and Device Type.
5. (Optional) Type the Manufacturer, Model, Serial Number, and Barcode.
6. Do one of the following:
   - To save the device, click **Save**.

     –Or–
   - To exit without creating a device, click **Cancel**.

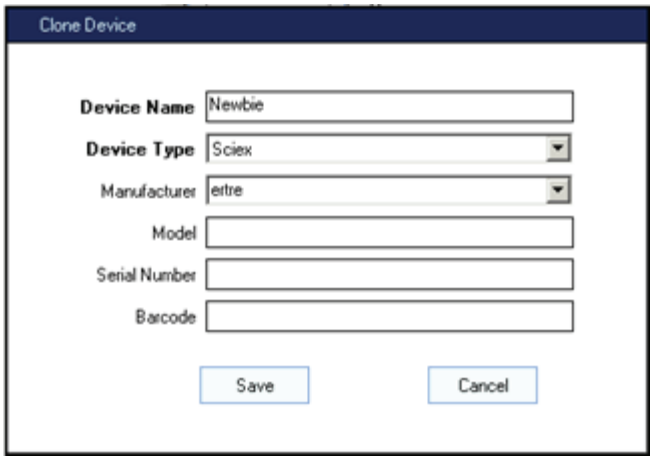
❖ To clone a device

1. Choose **Options > Instrument Inventory.**

   The Instrument Setup window opens.
2. Select a device and then click **Clone.**

   The Clone Devices dialog box opens, populated with the device information.
3. To modify device information, click the appropriate fields.

Figure 3. Clone Device dialog box



4. Do one of the following:
   - To save the device, click **Save**.

     –Or–
   - To exit without cloning the device, click **Cancel**.

❖ To edit a device

1. Choose **Options > Instrument Inventory.**

   The Instrument Setup window opens.
2. Select a device and click **Edit**.

   The Edit Device dialog box opens, populated with the device information.
3. To modify the device information, click the appropriate fields.
4. Do one of the following:
   - To save changes to the device, click **Save**.

     –Or–
   - To exit without making any changes, click **Cancel**.

❖ To delete a device

1. Choose **Options > Instrument Inventory.**

   The Instrument Setup window opens.

2. Select a device and click **Delete**.

   A confirmation message opens.

3. Do one of the following:

   - To delete the device, click **OK**.

     –Or–

   - To exit without making any changes, click **No**.

Tune, Method, and Processing Method Files in LCquan and Xcalibur

This section provides a list of tune, method, and processing method files in Xcalibur and LCquan. Use the LCquan LDK software to convert the .lqn or .pmd file to XML format.

Table 3. Xcalibur and LCquan tune, method, and processing parameter files

File extension	File description
Xcalibur	
*.pmd	Processing QC levels Stds, component names, and integration parameters
*.TSQTune	Tune parameters, mass spec amount of gas, amount of heat, and so on
*.meth	Instrument method file, LC parameters, autosampler (A/S) settings, pump settings, mass spec transitions (SRMs)
LCquan	
*.lqn	Combination of .pmd and .meth files
*.TSQTune	Tune parameters, mass spec settings, amount of gas, amount of heat

Note The Tune and Method files are closely associated with each other and form a matched pair.
In LCquan, export the .TSQTune and .lqn files.
–Or–
In Xcalibur, export the .TSQTune, .meth, and .pmd files.

Setting up the Master Assay

This section provides the steps required when setting up the master assay. For more information about the master assay, refer to the *Watson User Manual*. While many steps follow the standard Watson application workflow, for runs to be submitted to the TSQ Module application, there are some differences. For example, the Instrument page is available only when you configure a Peak Integration instrument. Use the Instrument page to submit the run to the TSQ Module application.

Use the following procedures:

- To set up the Instruments page in the Assay view
- To add instrument-method associations

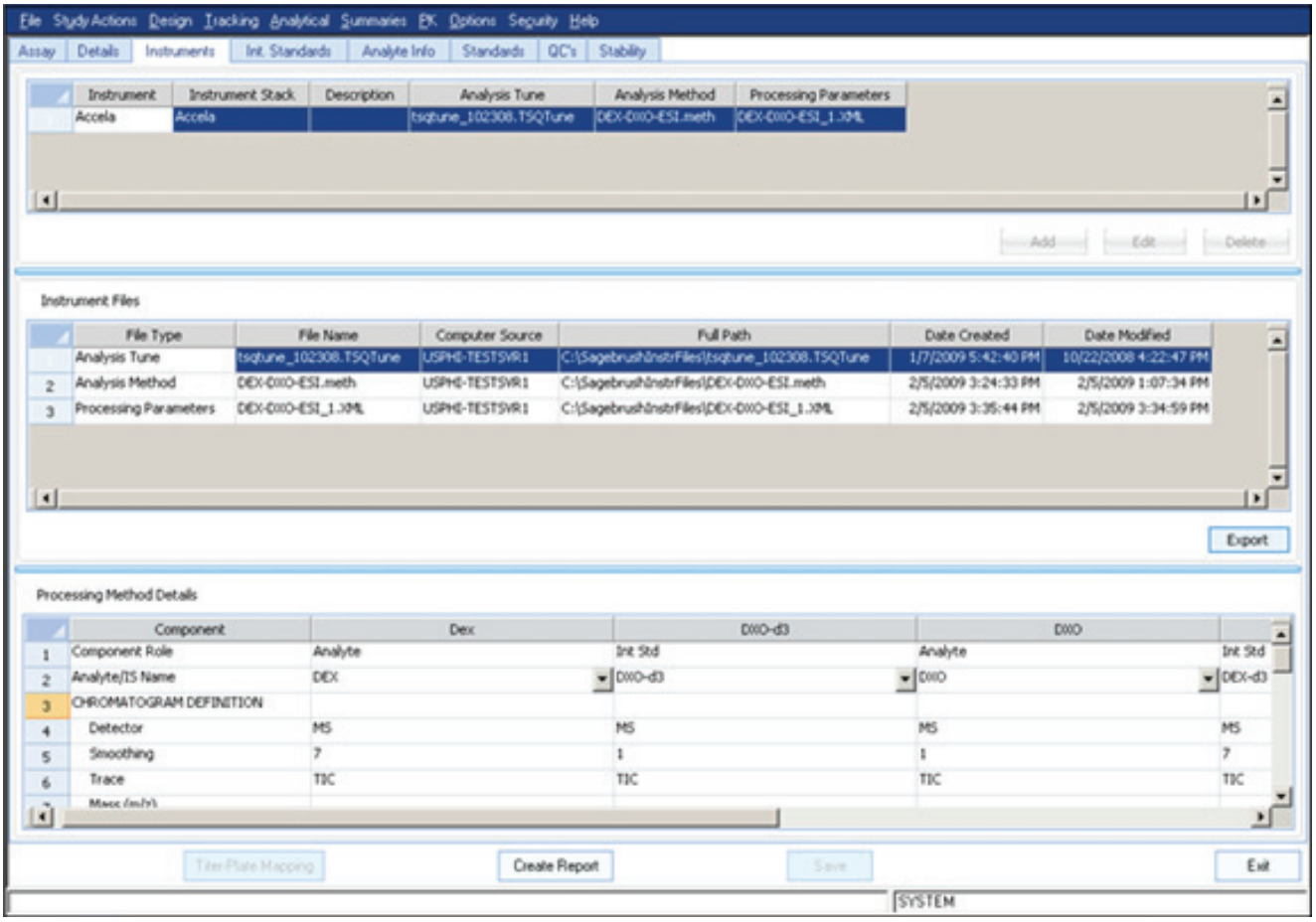
❖ To set up the Instruments page in the Assay view

Complete the following process in the Master or Study Assay so that the definition is copied for each run assay.

1. Click the **Assay** tab.
2. Select **LC/MS** as the Assay type.
3. Click the **Details** tab.
4. Select **Peak Integration** as the Instrument type.

   The Instrument tab is activated in the Assay view.

Figure 4. Instruments page in the Assay view



This page contains three panes: Top (untitled), Instrument Files, and Processing Method Details.

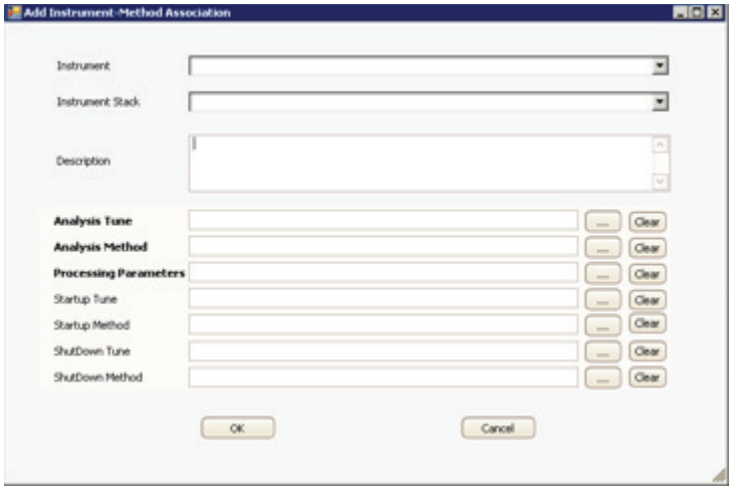
5. In the Processing Method Details pane, use the Analyte/IS Name row to associate a parameter component with an analyte.

   Each cell contains a list of analytes and internal standards defined in the assay from which you select the analyte that matches the identified component (column header).

❖ To add instrument-method associations

1. From the Master Assay, click the **Instrument** tab.
2. In the top section, click **Add** to open the Add Instrument-Method Association dialog box.

Figure 5. Add Instrument-Method Association dialog box



3. From this dialog box, select the instrument group and stack defined in the Instrument Inventory dialog box.
4. Type a description in the Description box.
5. Upload Analysis Tune, Analysis Method, and Processing Parameters files by clicking the corresponding buttons.

   If necessary, click **Clear** to clear the fields.

   Similarly, you can upload or clear Startup Tune, Startup Method, Shutdown Tune, and Shutdown Method files.
6. Click **OK** in the Add Instrument-Method Association dialog box.
7. Click **Save** in the Assay view.
   - When you are in LCquan, import the saved .TSQTune, .lqn, and XML files.
   - When you are in Xcalibur, import the saved .TSQTune, .meth, and XML files.

   Note You can use the Watson application to associate multiple method files with an assay or instrument by repeating these steps and saving the changes when you are finished.
8. Click the **Standards** tab.
9. Set the Injection Volume.

   This field is required input for TSQ type assays. This injection volume is sent to the instrument for runs using this assay.

Working with a Watson Study

The following procedures provide instructions for working with a study in the Watson application. For additional information, refer to the *Watson User Manual*.

Use the following procedures:

❖ To open a study

1. From the Watson application main menu, choose **File > Study**.

   The Study dialog box opens. Use this dialog box to create a new study or to work with an existing study within a project. You can also work with any recent studies listed at the end of the File menu.

2. Select a study.

   For more information, refer to the File Menu chapter in the *Watson User Manual*.

❖ To create a study

1. From the Watson application main menu, choose **File > Study**.

   The Study dialog box opens.

2. Click **Create**.

3. Enter values in the fields and assign the study to the correct project.

   If you have not created the project for this study, click **Cancel** and create the project by choosing **File > Project**.

4. Click **Save** to save the study information.

   The Assign Many Users to a Single Study Form opens.

5. Assign appropriate users to the study by selecting them and then clicking -> (right-arrow button).

6. Click **Save**.

   For more information, refer to the Study Actions chapter in the *Watson User Manual*.

7. From the Design menu, do the following:

   a. Choose **Design Study**.

   b. Specify the Subject Groups, Subject, Treatments, Biological Matrices, and Nominal Times necessary for the study.

   c. Click **Accept** when finished.

   d. Choose **Subjects**.

   e. Complete your updates and click **Save**.

   f. Choose **Treatments**.

   g. Complete your updates and click **Save**.

   For more information, refer to the Design Menu chapter in the *Watson User Manual*.

8. Choose **Tracking > Sample Entry**.

   The Sample Entry dialog box opens.

9. Select samples and click **Save** when complete.

   You may use only samples whose Sample Condition is OK in an Analytical Run.

   For more information, refer to the Tracking Menu chapter in the *Watson User Manual*.

❖ To copy the master assay to the study assay

1. Choose **Analytical > Copy Master Assay**.
2. Select one or more assays to be used for this study from the list of master assays.

   If the appropriate assay has not been set up for this project, exit and create a new master assay by choosing **File > Master Assay**.

3. Select **Save** to copy the master assay to this study.
4. Copy the master assay to the study assay.

❖ To create a new run with the study assay

1. Choose **Analytical > Plan Analytical Run**.

   The Analytical Runs dialog box opens.

2. Click **Create**.
3. On the Analytical Run Info page, specify the type of run and the assay to be used.

❖ To add samples to the work list

1. Click the **Worklist** tab.
2. Add assay samples, study samples, and user-defined samples.
3. Double-check volumes, aliquot/dilution factors, or both.
4. Make sure the work list is in the order you want to inject the samples.
5. When you are finished, click **Save**.
6. From the Analytical Runs dialog box, click **Exit**.

❖ To review the Plate Report page when planning an analytical run

Click the **Plate Report** tab.

The Plate Report page lists the samples with their sequence and identifier. It also shows the well positions in the plate map. These are carried to the TSQ Module application with run submittal.

❖ To create a sequence file

1. Choose **Analytical > Analytical Run Actions**.
2. Select the analytical run to use for creating the sequence file.
3. Click **Sequence File**.

   The Windows File dialog box opens.
4. Select the appropriate file path and name of the sequence file to be created.

❖ To send the run to the TSQ Module application

1. Choose **Analytical > Analytical Run Actions.**
2. From the list, click the run you want to send.
3. Click **Submit Run**.

   The Select Instrument to Run dialog box opens.
4. Select the instrument and click **OK.**

   For more information, refer to the Analytical Menu chapter in the *Watson User Manual*.

TSQ Module: System Administration

This chapter describes the TSQ Module tools and functions used primarily by system administrators, how the TSQ Module application communicates with the Watson application, and the types of actions that are audited.

Contents

Managing the Environments.xml File

In the Environments.xml file, the administrator assigns user-friendly names to the Watson application Web addresses. When a user logs onto the TSQ Module application, the system queries for available databases.

- When the administrator assigns a name to a Watson application Web address in the Environments.xml file, the user-friendly name is displayed in the logon screen Database menu.
- When the administrator does not assign a name in the to the Watson application Web address in the Environments.xml file, the complete URL is displayed in the logon screen Database menu.

The Environments.xml file is located in the following directory:

```
C:\Documents and Settings\All Users\Application Data\Watson TSQ Module
```

Any new installation of the TSQ Module application preserves the edits to the Environments.xml file. Changes to the Environments.xml file are not apparent in the logon screen until you restart your computer and the TSQ Module application. The following is a sample Environments.xml file:

```
<?xml version="1.0" encoding="utf-8" ?>
<Environments>
<Environment address="http://localhost:9000" other="Host9000" SystemAudit="1"/>
<Environment address="http://localhost:9001" other="Host9001" SystemAudit=""/>
<Environment address="http://localhost:9002" other="Host9002"/>
</Environments>
```

Where:
?xml version="1.0" encoding="utf-8" ? is a typical XML header.
Environments is a group or collection of Environment nodes.
Environment is the node type name.
address is the http address of the Watson application server.
other is the friendly name to use for that Watson application server.
SystemAudit optionally sends system-level audit logs to this server for acquisition events.

Communicating with the Watson Application

The TSQ Module application continuously communicates with the Watson application.

- When you edit the sample parameters in the TSQ Module application, it sends the edits to the Watson application. The TSQ Module application authenticates every edit in the Run Setup view and sends an audit trail to the Watson application.
- As each sample completes acquisition, the TSQ Module application returns the resulting data to the Watson application in XML format along with any new auditing information for the run.
- After the TSQ Module application passes the sample data to the Watson application database and the Watson application acknowledges receipt of the data, the TSQ Module application deletes any related temporary data from the local system.
- The TSQ Module application continues to send audit records to the Watson application until the acquisition service reports that the run is complete.
- When the acquisition service reports that the run is finished, the TSQ Module application sends a final notification to the Watson application. This notification includes the Completed status of the run, the completion time, and any audit items that occurred after the last sample completed.

Exporting Method Files to a Common Folder

Use the Watson TSQ Module Method Exporter application (Method Exporter) to convert processing parameters from an Xcalibur processing method (.pmd) or an LCquan workbook (.lqn) to XML format and export the parameters to a common folder where Watson users can access them.

You can use the Method Exporter application on any system to export Xcalibur .pmd files. To export LCquan .lqn files, first install the LCquan software including the LCquan Development Kit (LDK). The Method Exporter application exports all required LCquan parameters. See "Exported LCquan Parameters" on page 23.

You can use the Method Exporter application to export instrument method (.meth) files and tune (.TSQTune) files. You can also use the Method Exporter application to group instrument method and tune files in a single directory with the exported processing file. This simplifies uploading the files to the Watson application.

Note For exported LCquan or Xcalibur methods, ICIS is the only integration type allowed and MS is the only detector allowed.

❖ To open the Watson TSQ Module Method Exporter application

Choose **Start > Programs > Thermo Watson TSQ Module >Watson TSQ Module Method Exporter**.

The Watson TSQ Module Method Exporter opens. See "Thermo Watson TSQ Module Method Exporter" on page 22.

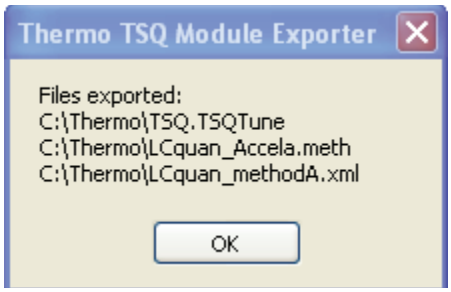
❖ To export files to a common folder

The Watson application requires each of these files, but you can export any combination of method files in a single session.

1. In the Method Exporter application, browse to the Xcalibur or LCquan processing method file that you want to export and click **Open**.
2. Browse to the instrument method file you want to export and click **Open**.
3. Browse to the tune file that you want to export and click **Open**.
4. Browse to the folder where you want the exported files to be written and click **Open**.
5. When you have identified all the files you want to export, click **Export**.

   The Method Exporter application converts the processing method files to XML format and writes all the specified method files to the target folder.

   A confirmation box lists all the files that were exported.



6. Click **OK**.

   The Method Exporter application remains open. You can export additional files to the common folder.

7. When you have finished exporting method files, click **Exit**.

   The Method Exporter application closes.

Thermo Watson TSQ Module Method Exporter

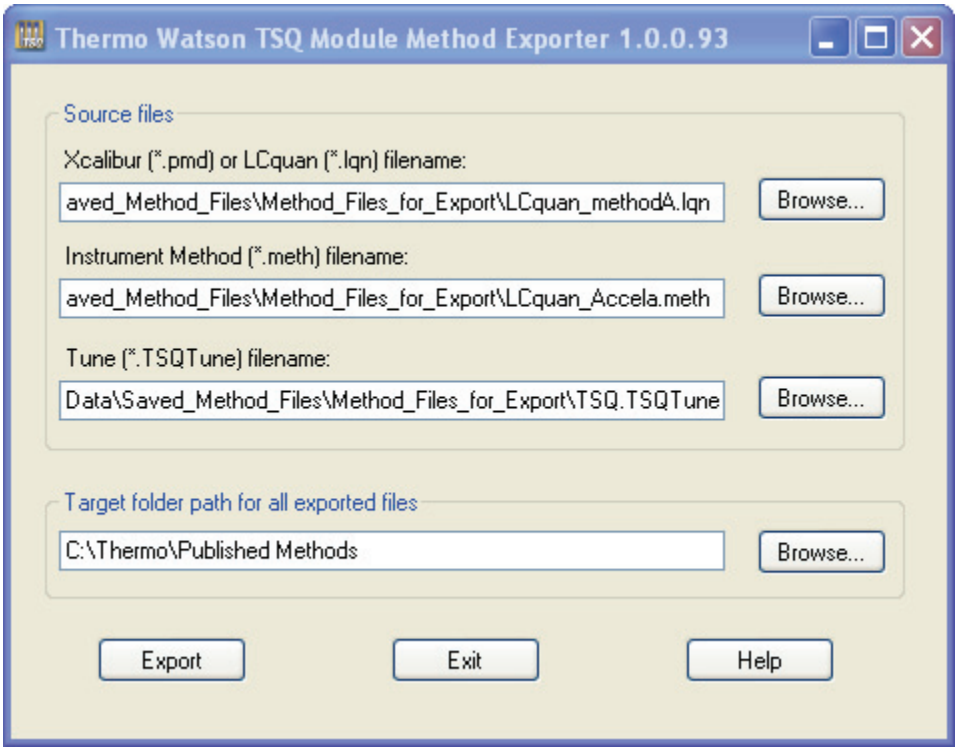


Table 4. Thermo Watson TSQ Module Method Exporter parameters (Sheet 1 of 2)

Parameter	Description
Source files	
Xcalibur (*.pmd) or LCquan (*.lqn) filename	Path to the Xcalibur or LCquan processing file to convert to XML format and export to a common folder.

Table 4. Thermo Watson TSQ Module Method Exporter parameters (Sheet 2 of 2)

Parameter, continued	Description
Instrument Method (*.meth) filename	Path to the instrument method file to export to a common folder.
Tune (*.TSQTune) filename	Path to the tune file to export to a common folder.
Target folder for all exported files	Path to the common folder in which exported files are saved.
Export	Converts the .pmd or .lqn file to XML format and copies all specified files to the target folder.
Exit	Closes the Thermo Watson TSQ Module Method Exporter application without exporting any files.
Help	Opens the Thermo Watson TSQ Module Method Exporter Help.

Exported LCquan Parameters

When you export an LCquan (.lqn) processing method, the following parameters are included for each component in the exported file:

- Component name
- Detector type
- Smoothing points
- Number of mass ranges
- Mass ranges
- Trace type (Mass range or TIC; Base peak is converted to mass range, if present, or TIC)
- Filter
- Adjust expected RT
- User entered RT
- Search window
- Display width
- Peak detection algorithm (ICIS only)
- Baseline window
- Area noise factor
- Peak noise factor
- ICIS Constrain peak width
- ICIS Peak height percentage
- ICIS tailing factor
- Peak identification: Locate method or peak detection index
- ICIS noise method
- ICIS calculate noise as RMS
- ICIS Minimum peak width
- ICIS Multiplet resolution
- ICIS Area tail extension
- ICIS Area scan window

Auditing

The TSQ Module application immediately transmits system-level audit records, such as instrument errors, to the Watson application.

The application collects and packages run-level audit records with run-result or run-complete XML data for a particular run. Audit records are delayed until a result or run-complete package is transmitted to the Watson application. A run must be in acquisition or canceled and returned to the Watson application before the TSQ Module application transmits the XML packages and audit records to the Watson application.

When there is no network connection to the Watson application, the TSQ Module server locally buffers system-level, result/run-complete audit packages for later transmission when the network is restored.

User permissions facilitate auditing and are set on the Watson application side. The TSQ Module user has permissions at both the system level and the run level.

Audit Trail

The TSQ Module application creates an audit trail for the following actions:

User actions

- Expired sessions or logoffs
- Successful and unsuccessful logins
- Successful and unsuccessful E-signatures
- Activities on locked accounts
- Updates to sample injection volume, position, or comments
- Updates to run observation
- Runs submitted for acquisition
- Runs paused, stopped, or restarted in the Acquisition Queue
- Runs canceled in the Run Manager view

System events

- Instrument errors
- System-level audits sent to the Watson application URLs

User Permissions

The TSQ Module user has permissions at both the system level and the run level.

The TSQ Module application user system-level permissions:

- Log onto the Watson TSQ Module application.
- Manage the Run Manager view.

The TSQ Module application user run-level permissions:

- Submit a run for acquisition.
- Edit samples (injection volume, position, comment).
- Edit run information (enter additional comment or change acquisition options).
- Save an edited run.
- Cancel a run in the Run Manager view.
- Stop/pause/resume a run in acquisition.
- Remove a run from the Acquisition Queue.

Using E-Signatures

You can set the TSQ Module application to request an electronic signature from a user when the run requires it. The attempted action (for example, saving an edited run) cannot be completed until the user provides a password and submits the electronic signature.

The requirement for an electronic signature is set in the Watson application. You can require an electronic signature when a user edits samples and saves a run in the Setup mode or when a user enters an additional comment and submits a run in the Acquire mode.

❖ To submit an electronic signature

The end user follows these instructions:

1. Enter your user credentials or ask your supervisor to enter credentials.

   User credentials are the same as your TSQ Module login and password.

2. Accept the default reason or enter your own reason for the operation.
3. Click **OK**.

   The requested operation is completed. In addition to the type of operation and the reason for the operation, the Watson application audit trail records the date, time, and user credentials.

Electronic Signature dialog box

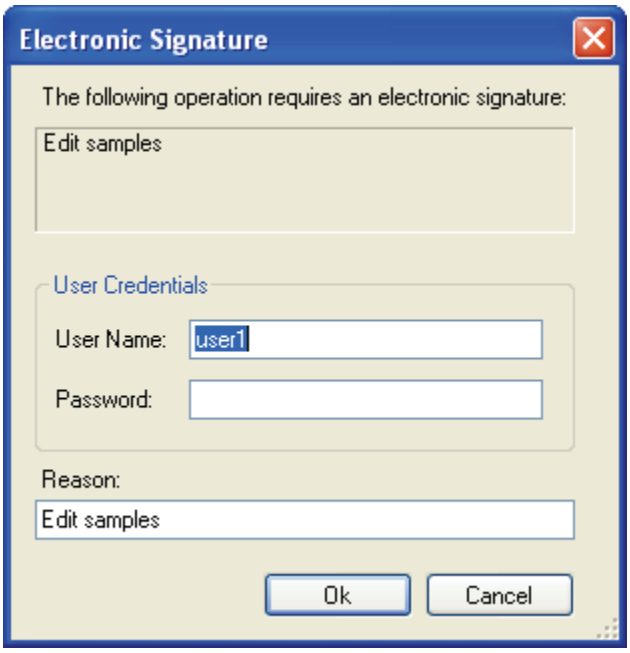


Table 5. Electronic Signature parameters

Parameter	Description
The following operation...	Lists the operation that requires an electronic signature. This requirements is specified in the run XML data.
User Credentials	
User Name	The Watson login name for the user.
Password	The Watson password for the user.
Reason	Explanation for the action. Default is the same as the attempted operation.
OK	Approves the user name and password, closes the Electronic Signature dialog box, completes the requested operation, and places an entry in the audit trail.
Cancel	Closes the Electronic Signature dialog box, without completing the requested operation, and places an entry in the audit trail.

TSQ Module: Getting Started

This chapter describes how to set up the TSQ Module application and log in to a session.

Contents

Setting up in the Watson Application

Each run is identified by the database, project, and study to which it belongs. A study contains design information about the subject, treatment, sampling times, and planning of analytic runs. The project and study names are displayed in the Run Name columns of the TSQ Module application queues.

The assay method for a run is defined in the Watson application. The creation date of the assay is displayed in the Assay Date columns of the TSQ Module application queues.

For detailed information about setting up runs in the Watson application, see Chapter 2, "Watson: Submitting Runs to the TSQ Module."

Starting the TSQ Module Application

Follow these instructions to install and start the TSQ Module application.

Use the following procedures:

- To set up and install the TSQ Module application
- To start the TSQ Module application
- To log in to the Thermo Watson TSQ Module application

❖ To set up and install the TSQ Module application

1. To install the Xcalibur 2.1 application and your TSQ instrument driver, follow the instructions on the included CDs.
2. Install the driver for your LC pump and autosampler.

3. Insert the Watson TSQ Module CD in the drive and follow the on-screen instructions.

   If the install windows don't automatically appear, navigate to the Xinstall.exe file and launch it.

❖ To start the TSQ Module application

1. Configure your instruments.

   You cannot configure your instruments while the TSQ Module application is running.

2. Double-click the TSQ Module application icon on your desktop, or go to **Start > All Programs > Thermo Watson TSQ Module > Watson TSQ Module**.

   The Thermo Watson TSQ Module login screen opens.

Table 6. Login screen parameters

Parameter	Description
Login Name	The user's Watson login name.
Password	The user's Watson password.
Database	The Watson environment URL or the friendly name assigned in the Environments.xml file.

❖ To log in to the Thermo Watson TSQ Module application

Each database is assigned to specific users. You cannot log in to a database that is not assigned to you, and you cannot log in to your assigned database unless there is at least one run assigned to you in the TSQ Module application.

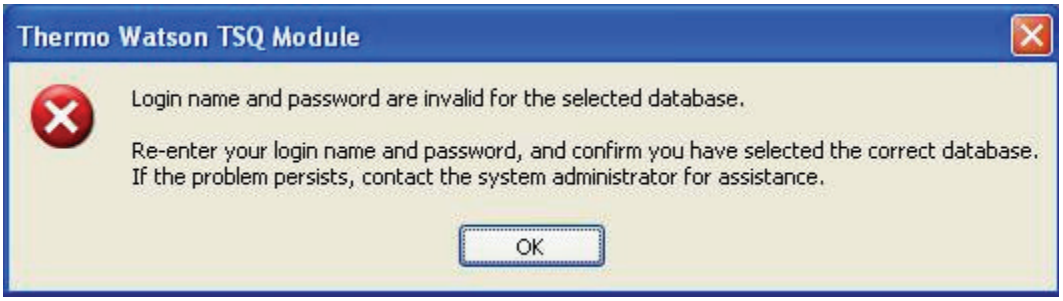
1. Enter your Login Name.
2. Enter your Password.
3. Select your Database from the menu.

   One of the following could happen:

   - There are no databases to select from in the menu.

     This means there are no runs in the TSQ Module Run Manager view. You cannot log in to the database unless there is at least one run in the queue.

   - The database list might include databases that are not assigned to you. If you select one of these databases, you get the following error message:



     To correct the problem, click **OK** in the error message and do one of the following:

     – If you think you made a mistake in your login or password, re-enter them.

     – If you made a mistake in the database you selected, select another database.

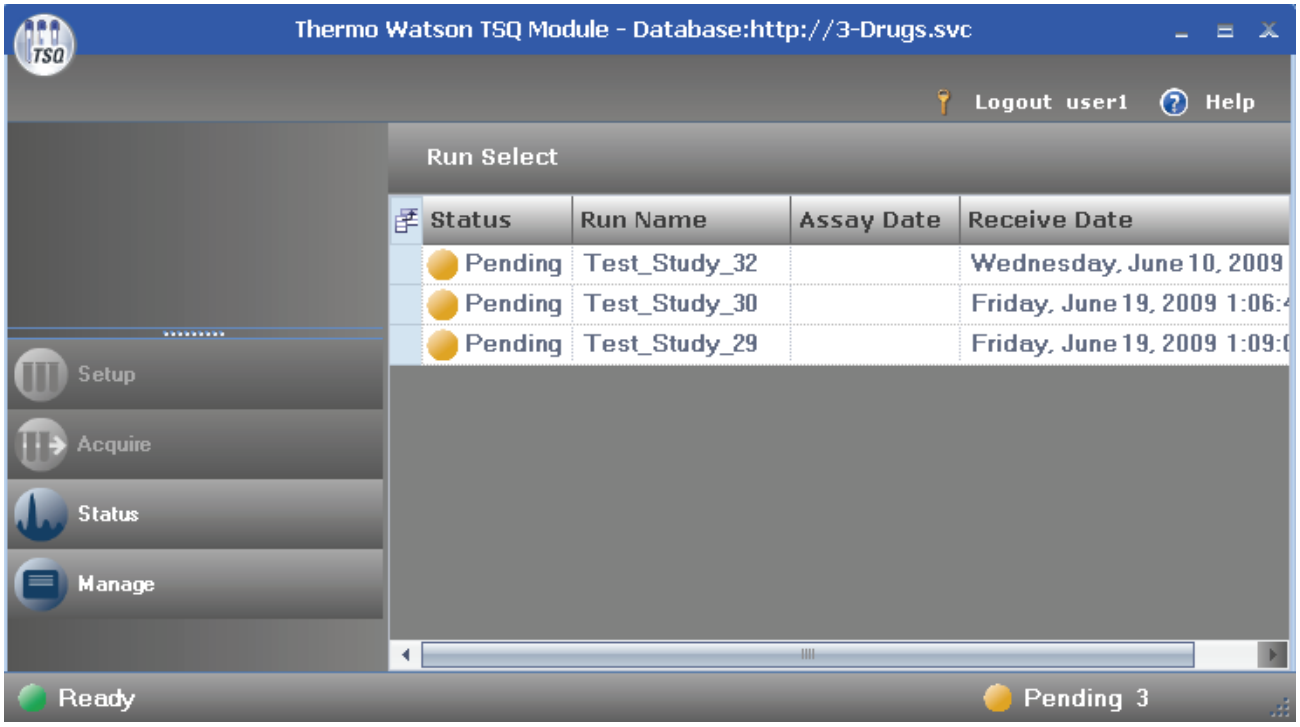
     – If you think you should have access to the database you chose, contact your system administrator.

4. When you have correctly specified a login name, password, and database, click **OK**.

   The Run Select view opens. See "Run Select View" on page 30.

   You are ready to begin managing and acquiring your assigned runs. For detailed information about starting an acquisition from the Run Select view, see "Selecting a Run" on page 34.

Run Select View



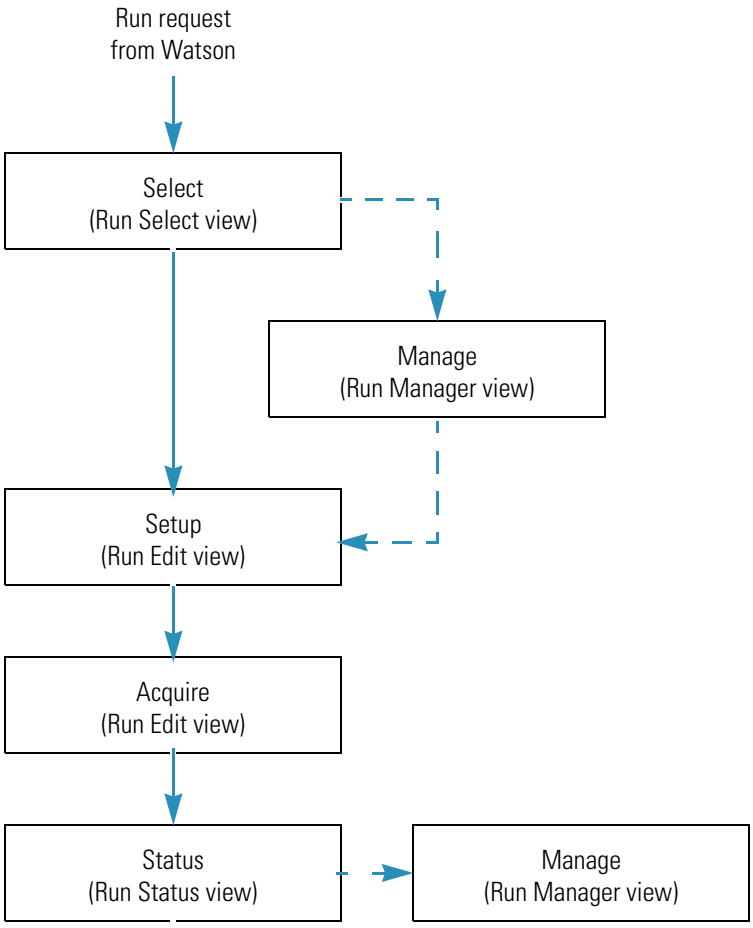
TSQ Module: Acquiring Data

This chapter describes the functions of the TSQ Module application: selecting a run, managing a run, editing a run setup, acquiring a run, and monitoring the run status.

Contents

The Basic Workflow

A routine workflow uses all modes of the TSQ Module application: Select, Manage, Setup, Acquire, and Status.



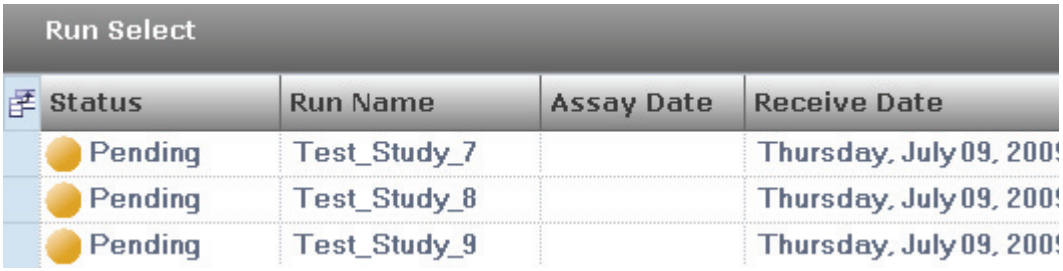
To watch a video of this basic workflow, see Basic Workflow Video.

Basic Workflow Video

The following video demonstrates a typical TSQ Module application session.

Selecting a Run

When you first start the TSQ Module application, the Run Select view opens. All runs assigned to you within the current database are listed. During this session, all runs received by the TSQ Module application for your current login and database are added to the list.



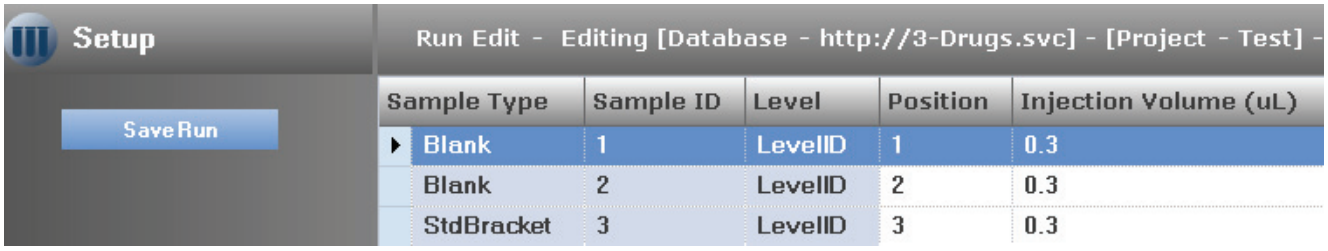
Use the following procedures:

- To select a run
- To monitor the run status
- To add or remove columns

❖ To select a run

In the Run Select view, double-click anywhere in the row of the run.

The Run Edit view in the Setup mode opens.



The Sample Type, Sample ID, and Level are not editable columns, as indicated by the blue background. The Position, Injection Volume, and Comment columns are editable.

From the Run Edit view, you can modify the editable parameters before you submit the run for acquisition. Refer to "Editing a Run" on page 41.

Tip To return to the Run Select view from any other view, click the application button in the upper left corner and choose **Select** from the menu.

❖ To monitor the run status

Until a run completes and is returned to the Watson application, you can monitor the status in the Run Select view. The status can be any of the following:

- Pending – The run arrived from the Watson application and is assigned to the instrument at this workstation.

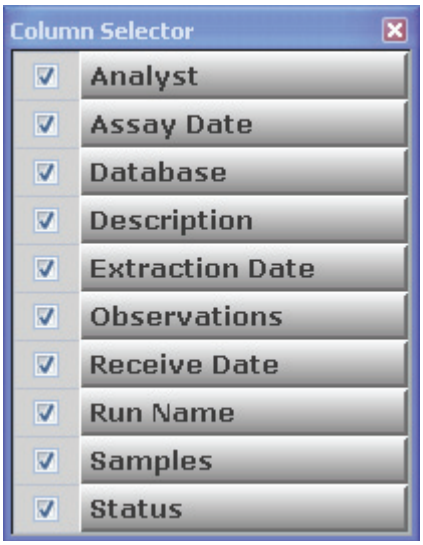
- Submitted – A TSQ Module application user has submitted this run to the Acquisition Queue but is not currently acquiring.
- Acquiring – The run has progressed to the top of the Acquisition Queue and is currently acquiring.
- Paused – The run was assigned to an instrument and was acquiring, but has been paused and is waiting between samples.
- Stopped – The run was assigned to an instrument and was acquiring, but was removed from the Acquisition Queue.
- Completed – The run has completed acquisition.

Note Completed runs might not always be observed in the Run Manager view. When the TSQ Module application finishes sending the results of the last completed sample to the Watson application, it marks the run as Completed and quickly removes the run from the Run Manager view.

❖ To add or remove columns

1. Click the Column Selector icon  in the upper left corner of the view.

   The Column Selector dialog box opens.



2. Check the box before a field name to display that column, or uncheck the box before a field name to remove that column from the display.

   This action is immediately implemented in your display and retained throughout your session, but the column choices are not saved when you exit the TSQ Module application.

3. When you are finished adding or removing columns, click the red X in the upper right corner to close the Column Selector dialog box.

Managing a Run

The Run Manager view in the Manage mode displays all runs sent from the Watson application.

Use the following procedures:

- To display the Run Manager view
- To monitor the run status
- To add or remove columns
- To cancel a run and return it to the Watson application
- To cancel multiple runs
- To select a run for acquisition

❖ To display the Run Manager view

From any view, click **Manage**.

The Run Manager view in the Manage mode displays all runs sent from the Watson application, but only the runs assigned to your database are available to you. See "Manage Mode" on page 39.

❖ To monitor the run status

Until a run completes and is returned to the Watson application, you can monitor the status in the TSQ Module Run Manager view. The status can be any of the following:

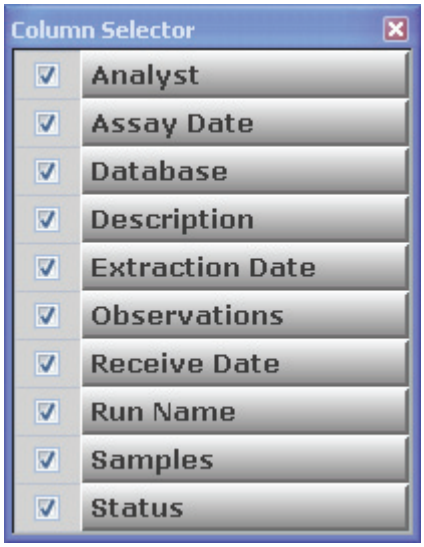
- Pending – The run arrived from the Watson application and is assigned to the instrument at this workstation.
- Submitted – A TSQ Module application user has submitted this run to the Acquisition Queue but is not currently acquiring.
- Acquiring – The run has progressed to the top of the Acquisition Queue and is currently acquiring.
- Paused – The run has been assigned to an instrument and is currently acquiring, but has been paused and is waiting between samples.
- Stopped – The run has been assigned to an instrument and was acquiring, but was removed from the Acquisition Queue.
- Completed – The run has completed acquisition.

Note Completed runs might not always be observed in the Run Manager view. When the TSQ Module application finishes sending the results of the last completed sample to the Watson application, it marks the run as Completed and quickly removes the run from the Run Manager view.

❖ To add or remove columns

1. Click the Column Selector icon in the upper left corner of the view.

   The Column Selector dialog box opens.



2. Check the box before a field name to display that column, or uncheck the box before a field name to remove that column from the display.

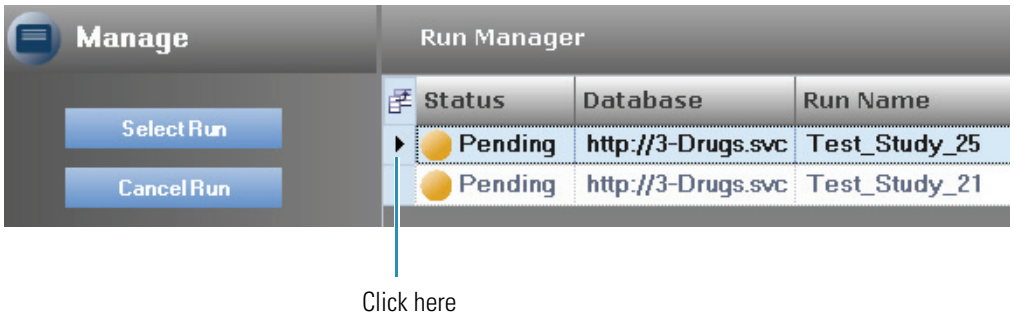
   This action is immediately implemented in your display and retained throughout your session, but the column choices are not saved when you exit the TSQ Module application.

3. When you are finished adding or removing columns, click the red X in the upper right corner to close the Column Selector dialog box.

❖ To cancel a run and return it to the Watson application

To cancel a run, the status of the run must be either Pending or Stopped.

1. Click the row selection tab to select the run and enable the Cancel Run button.



2. Click **Cancel Run**.

   The system confirms that you want to cancel the run and return it to the Watson application.

3. Click **Yes** to confirm the cancellation.

   - If the run had not begun acquisition, a Watson user can assign the run to a different instrument and re-send the run to the TSQ Module application.
   - If the run had begun acquisition, a Watson user can re-send the run to the TSQ Module application, but it must be assigned to the same local instrument. The entire run must complete acquisition on a single instrument. A run returned for reinjection cannot be assigned to a different instrument.

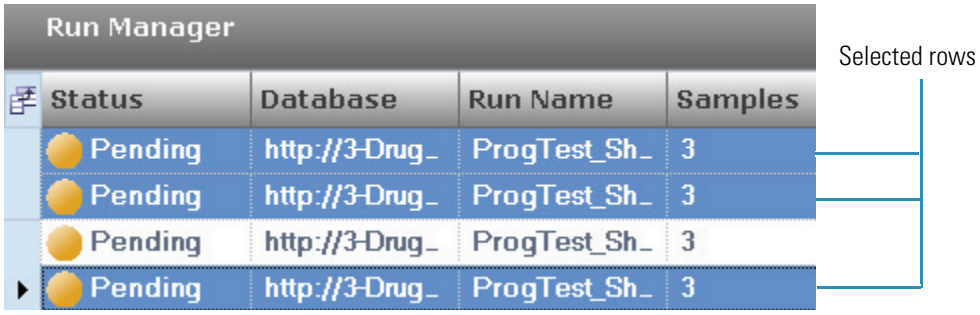
❖ To cancel multiple runs

You can cancel Pending or Stopped runs and return the runs to the Watson application. You cannot cancel Acquiring or Submitted runs.

1. Select the runs you want to cancel and return to the Watson application.

   - Use the **Shift** key to select a group of consecutive runs.
   - Use the **Ctrl** key to choose a group of non-consecutive runs.

   The selected rows are highlighted.



2. Choose **Cancel Run**.

   The system confirms that you want to cancel multiple runs and return them to the Watson application. The system warns that you cannot cancel Acquiring or Submitted runs.

3. Click **Yes** to confirm the cancellation.

   The runs are immediately returned to the Watson application. Any selected Acquiring or Submitted runs that remain in the Run Manager view are no longer selected.

❖ To select a run for acquisition

1. Select a run and click **Select Run**, or double-click in the run row.

   The Run Edit view opens and displays each sample in the run. From this view you can edit the run before you submit it for acquisition.

   For detailed information about editing the run before you submit it, see "Editing a Run" on page 41.

2. To submit the run, click **Acquire**.

   The Run Edit view in the Acquire mode displays the samples for the run. You cannot edit the samples from the Acquire mode.

   The Acquire options in the Acquire mode provide parameters for submitting a run for acquisition. For details about using the Acquire options to submit a run, see "Acquiring a Run" on page 45.

Manage Mode

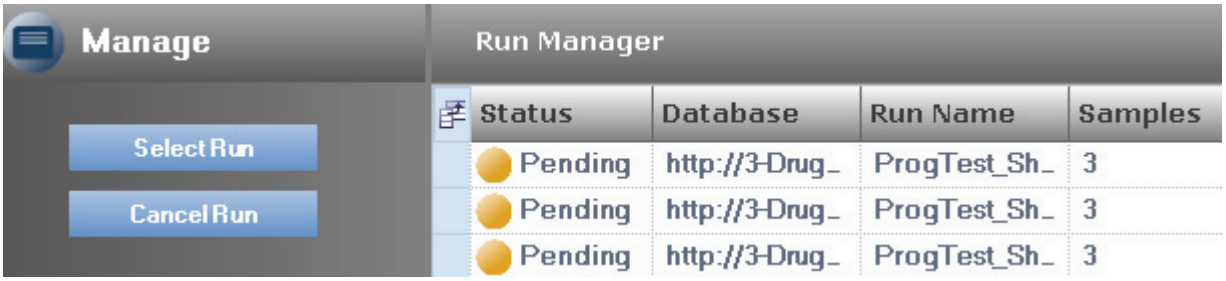


Table 7. Manage mode parameters (Sheet 1 of 2)

Command	Description
Function buttons	
Select Run	Opens the Setup view for the highlighted run.
Cancel Run	Removes the highlighted run from the view and returns the run to the Watson application.

Table 7. Manage mode parameters (Sheet 2 of 2)

Command	Description
Run Manager columns	
Status	Status of each run: Pending, Submitted, Acquiring, Paused, Stopped, or Completed.
	• Pending - The run arrived from the Watson application and awaits acquisition. This run could have been submitted to the Acquisition Queue and returned to the Run Manager view before it began acquiring.
	• Submitted - The run is assigned for acquisition but is not currently acquiring. The run is in the Acquisition Queue.
	• Acquiring - The run has progressed to the top of the Acquisition Queue and is currently acquiring.
	• Paused - The run has been assigned to an instrument and is currently acquiring, but has been paused and is waiting between samples.
	• Stopped - The run was assigned for acquisition and was acquiring, but a user action moved it back to the Run Manager view.
	• Completed - The run has completed acquisition. Completed runs might not always be observed in the Run Manager view. When the TSQ Module application finishes sending the results of the last completed sample to the Watson application, it marks the run as Completed and quickly removes the run from the Run Manager view.
Database	The Watson database or the friendly name assigned in the Environments.xml file.
Run Name	A combination of the project, study, and run ID as defined in the Watson application.
Samples	Number of samples in the run.
Receive Date	Timestamp when run was received.
Assay Date	Timestamp when assay method was created.
Description	Comments entered by a Watson user.
Extraction Date	Timestamp when samples in the run were extracted.
Analyst	The user who created the run in the Watson application.
Observations	The Watson application user comments.

Editing a Run

When you edit the parameters of a run in the TSQ Module application, the edits are sent back to the Watson application. Every action in the Run Edit view in the Setup mode is authenticated and an audit trail is sent to the Watson application.

Use the following procedures:

- To edit the samples in a run
- To automatically enter Position column values
- To automatically enter Injection Volume column values

❖ To edit the samples in a run

1. Do one of the following to open the Run Edit view in the Setup mode:
   - From the Run Select view, double-click any column in the run row.

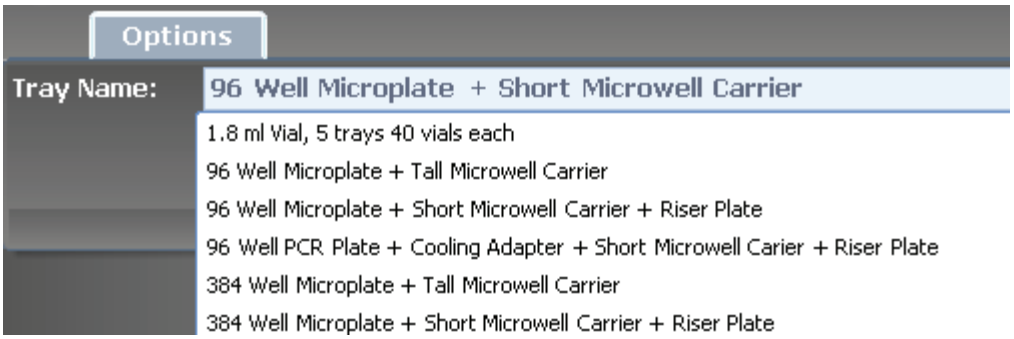
     –Or–
   - From the Run Manager view, do one of the following:
     - Double-click any cell in the run row.

       –Or–
     - Click anywhere in the run row to select it and click **Select Run**.

   The Run Edit view in the Setup mode opens. See "Setup Mode" on page 44.

   In this view, only the Position, Injection Volume, and Comment columns are editable.
2. (Optional) To specify a tray name, click **Options** in the ribbon above the view and select a tray name from the list.

   When you use an autosampler, you must select a tray name with which to validate the vial or well position. Positions are usually provided by the Watson application.



3. To edit the Position column, click the current position value and type a new value.

   If you specify an incorrect position, an error message shows you the correct syntax to use.

4. To edit the Injection Volume column, click the current injection volume value and type a new value.
5. To edit the Comment column, click in the column and type a comment.

   This field is limited to 100 characters.
6. To save the edits, click **Save Run**.

   The Save Run button is enabled only after you edit a column value in the run.

> **Note** The Save Run command can be specified to require an electronic signature. See "Using E-Signatures" on page 25.

❖ To automatically enter Position column values

To use this feature, you must have an autosampler configured. If you specify an incorrect position for your configured autosampler, an error message shows you the correct syntax to use.

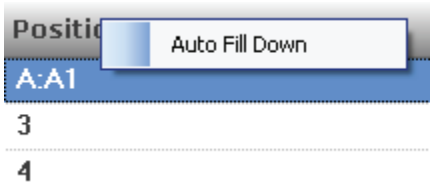
1. Enter a value for the first row in the fill down sequence.

   Observe that this row is highlighted as the selected row. You can begin the sequence from any row in the column.
2. Right-click outside this cell but still in the Position column and choose **Auto Fill Down**.

   The column values are incremented starting with the value in the selected row and ending with the last row in the run.

   The TSQ Module application knows the number of positions configured in your autosampler and numbers the positions accordingly. See **Example 1**.

   You can repeatedly use the **Auto Fill Down** command to create multiple sequences. See **Example 2**.



Example 1

The TSQ Module application knows sequential order of the positions configured in your autosampler and numbers the positions accordingly.

Position
A:A1
A:A2
A:A3
A:A4
A:A5
A:A6
A:A7
A:A8
A:A9
A:A10
A:A11
A:A12
A:B1
A:B2

Example 2

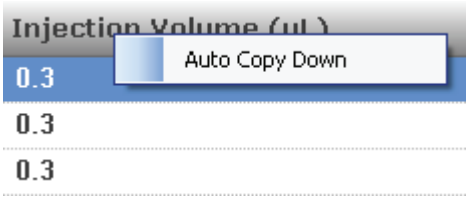
You can repeatedly use the **Auto Fill Down** command to create multiple sequences.

Position
A:A1
A:A2
A:B1
A:B2

❖ **To automatically enter Injection Volume column values**

Right-click the Injection Volume column and choose **Auto Copy Down**.

The first row value is copied to all rows.



Setup Mode

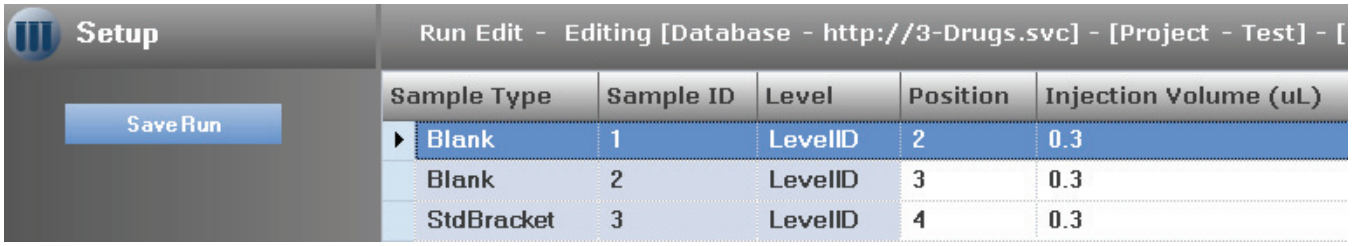


Table 8. Setup mode parameters

Command	Description
Function buttons	
Save Run	Saves the edits you made to the run. The Save Run command can be specified to require an electronic signature. See "Using E-Signatures" on page 25.
Run columns	
Sample Type	Defines how the Watson application processes the sample data. Each sample must be classified as one of the following sample types: • Unknown • Blank • QC (quality control) • Standard
Sample ID	An alphanumeric string of characters that can be used to uniquely identify the sample.
Level	The level defined for a calibration sample or quality control sample.
Position	Sample position number in the autosampler, usually provided by the Watson application.
Injection Volume (µL)	Microliters to be injected. • When you have an autosampler configured, the injection volume is validated against the allowable range specified by the autosampler. • When you do not have an autosampler configured, you can enter a range of 0.1-1000.
Comment	Optional information about the sample.

Acquiring a Run

From the Acquire mode, you can start the acquisition of a run and specify the real-time display settings.

❖ To submit a run

1. From the Run Edit view in the Setup mode of the run you want to acquire, click **Acquire**.

   The Acquire mode opens.

   The Run Edit view in the Acquire mode displays the samples for the run. You cannot edit the samples from the Acquire mode.

   The Acquire options in the Acquire mode provide parameters for submitting a run for acquisition. See "Acquire Options" on page 46.

   Note If you made changes to the setup and did not explicitly save them, a dialog box asks if you want to save the changes now.

2. If this run was previously stopped and removed from the Acquisition Queue, you can specify the first and last samples you want to acquire.

   By default, all samples are acquired.

3. To insert this run into the Acquisition Queue ahead of any submitted non-acquiring runs, check **Priority Run**.

   Note When you submit a priority run, it goes into the Acquisition Queue ahead of all other submitted, non-acquiring priority runs.

4. (Optional) Add an additional comment.

5. (Optional) Specify an instrument startup or shutdown method:

   - To run a startup method before the run starts, check **Startup Method**.
   - To run a shutdown method after the run is completed, check **Shutdown Method**.

6. Specify the status of the system after data acquisition by selecting one of the system power schemes: **On**, **Standby**, or **Off**.

   The default is On.

7. To submit the run to the Acquisition Queue, click **Run**.

   - If this is the first run in the queue, the acquisition begins and the status of the run is reported as Acquiring in the Run Manager view.
   - If there are other runs in the queue, the run is added to the queue and the status of the run is reported as Submitted in the Run Manager view.

When acquisition for a run is complete, the run is removed from the Run Manager view and the results are returned to the Watson application.

For detailed information about monitoring a run as it acquires, see "Monitoring the Run Status" on page 48.

Acquire Options

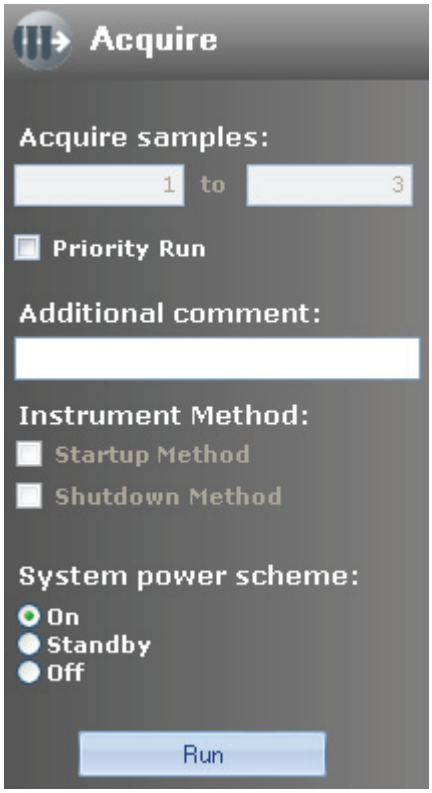


Table 9. Acquire options parameters (Sheet 1 of 2)

Parameter	Description
Acquire samples	Displays the first and last samples to acquire. Available only for a previously Stopped run.
Priority Run	Inserts this run into the Acquisition Queue ahead of all submitted, non-acquiring runs.
Additional comment	A comment that is returned to the Watson application in the run report.
Instrument Method	
Startup Method	Specifies that the TSQ Module application runs the startup method specified in the run data before the run starts. No data is acquired by this method, and no autosampler injection takes place. This feature is not available for all devices.

Table 9. Acquire options parameters (Sheet 2 of 2)

Parameter	Description
Shutdown Method	Specifies that the TSQ Module application runs the shutdown method specified in the run data after the run has completed. No data is acquired by this method, and no autosampler injection takes place. This feature is not available for all devices.
System power scheme	
On	Keeps the system in the On state when the current run is completed. When On is selected, you can begin another run without waiting. All power and flows are maintained at operational levels. Default: On
Standby	Keeps the system in the Standby state when the current run is completed. When you select Standby, you can begin another run with only a short delay between runs. Some devices do not have a Standby feature. For devices with this feature, a power-saving or consumable-saving mode is entered and the devices can be switched back on in approximately 15 minutes or less. Depending the instrument, this state turns gas and liquid flows to Off, but maintains heaters and other subsystems in an On state so that there is no warm-up time required when you change from Standby to On.
Off	Keeps the system in the Off state when the current run is completed. The Off state indicates that all power to the instrument, which can be controlled by the TSQ Module application, is turned Off. This includes power to all heaters and most subassemblies, but in some cases not all subassemblies. Some devices do not have an Off feature. For devices that do have this feature, a power-saving or consumable-saving mode is entered and you can manually switch the devices back on. When several runs are queued, the system power scheme of the last submitted run is used. **Caution** The Off state does not guarantee that all voltages are turned off nor does it indicate that all heated components are at room temperature. To perform maintenance on an instrument, refer to the hardware manual for your instrument.
Function buttons	
Run	Starts the acquisition for the currently selected run and opens the Status view.

Monitoring the Run Status

When you begin acquisition for a run from the Acquire mode, the Run Status view in the Status mode is displayed.

You can return to the Status mode at any time as long as there are runs in the Acquisition Queue.

❖ To return to the Status mode

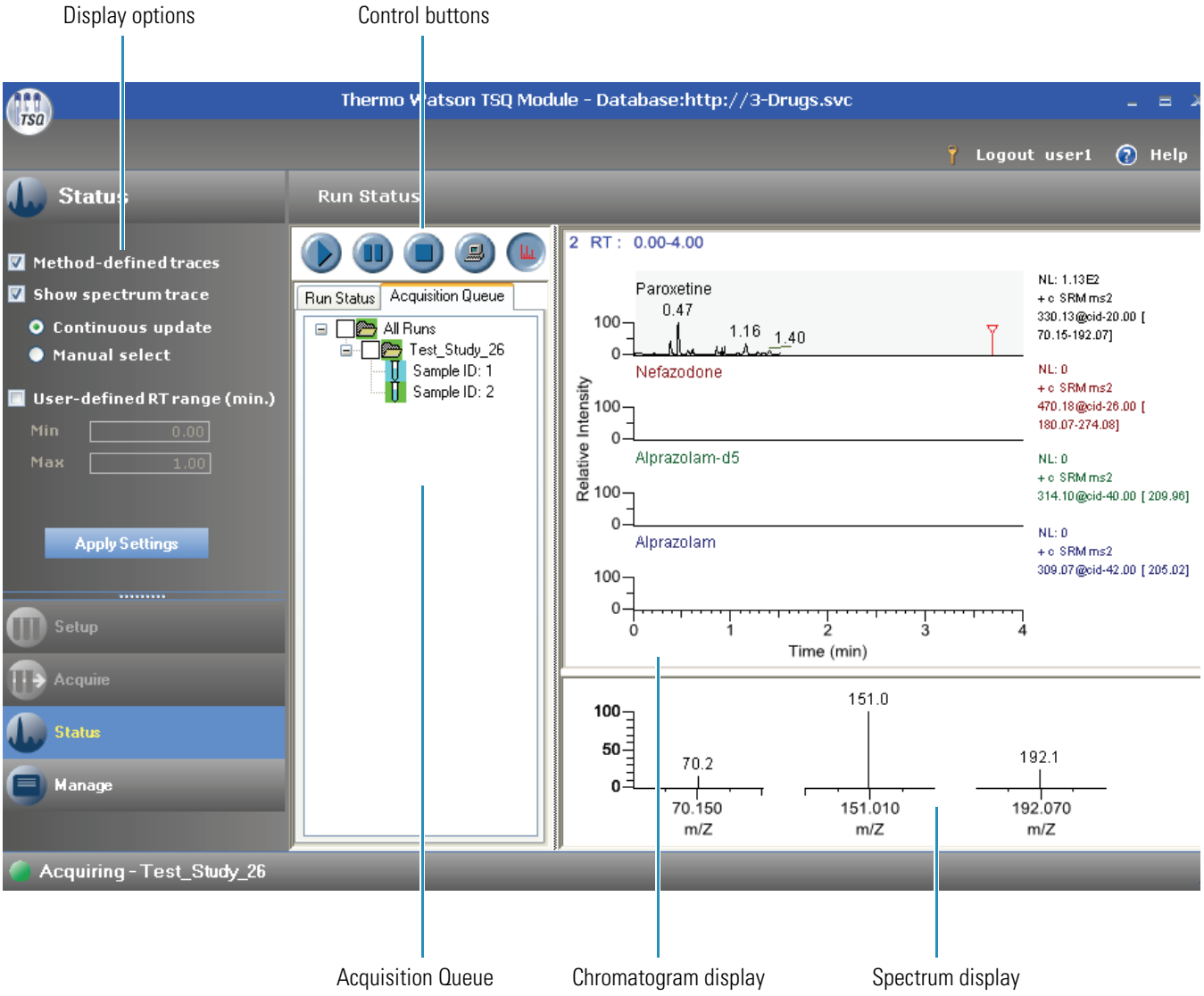
To return to the Status mode, click **Status**.

From the Run Status view in the Status mode, you can

- Start, stop, or pause an acquisition
- Monitor the run manager and instrument status
- Monitor the Acquisition Queue
- Control the real-time display

This section contains information about the following:

Figure 6. Run Status view in the Status mode



Run Status View Control Buttons

The control buttons on the Status view let you control the acquisition process.

❖ **To start, stop, or pause an acquisition**

- To start an acquisition or resume an acquisition that has been paused, click .
- To pause an acquisition after the current sample has been completely acquired, click  .

  To resume the acquisition, click  again.

- To immediately stop the current acquisition, click .

  Acquisition for the current sample stops and the sample is considered complete. The acquisition for the run is paused.

- To pause the real-time display during an acquisition, click .

  The acquisition continues; only the display is paused.

  To resume the real-time display, click again.

- To display the Spectrum pane below the Chromatogram pane, click .

  To hide the Spectrum pane, click again.

For a detailed explanation of how pausing, stopping, or canceling runs is managed, refer to "Pausing/Stopping/Canceling Runs" on page 55.

Run Status

The Run Status page on the Run Status view displays information about the run currently being acquired.

Figure 7. Run Status page

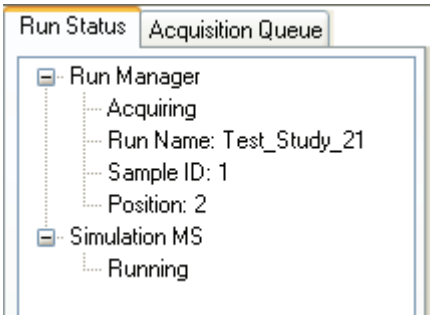


Table 10. Run Status parameters (Sheet 1 of 2)

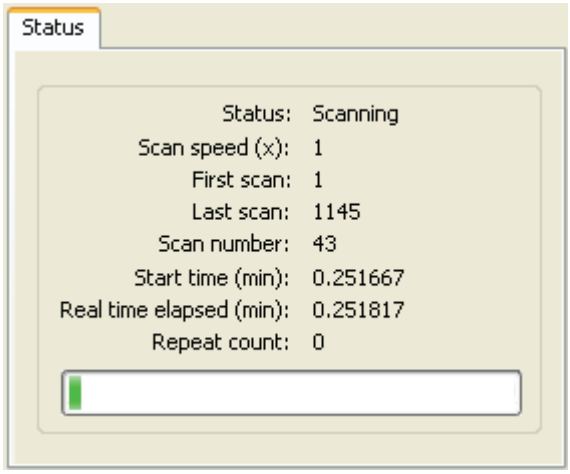
Parameter	Description
Run Manager	
state	Reports the current status of the system.
Run Name	Displays the run currently being acquired.
Sample ID	Displays a unique alphanumeric name assigned to each sample. The assigned name can be up to 50 characters long.
Position	Displays the position (including a tray name, when applicable) in the autosampler tray of the current sample. This readback value is displayed only if your LC provides this information under *direct control*. This value is not displayed if your LC is under *contact closure* control.

Table 10. Run Status parameters (Sheet 2 of 2)

Parameter	Description
Instruments	
The readback state of each configured instrument appears on the Run Status page.	
Shortcut Menu Right-click to display the instrument's shortcut menu.	
Turn Device On	Puts an instrument in the On state.
Turn Device Standby	Puts an instrument in the Standby state.
Turn Device Off	Puts an instrument in the Off state.

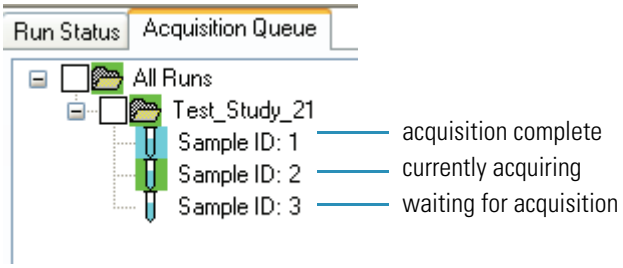
Instrument Status

Each instrument sends its own status information to the Run Status view. This is an example of a simulator status.



Acquisition Queue

The Acquisition Queue page on the Run Status view displays information about all the runs in the Acquisition Queue.



Real-time Display

The Run Status view lets you observe real-time display during an acquisition. The displayed "time windows" correspond to the time segments defined in the instrument method.

Figure 8. Chromatogram display

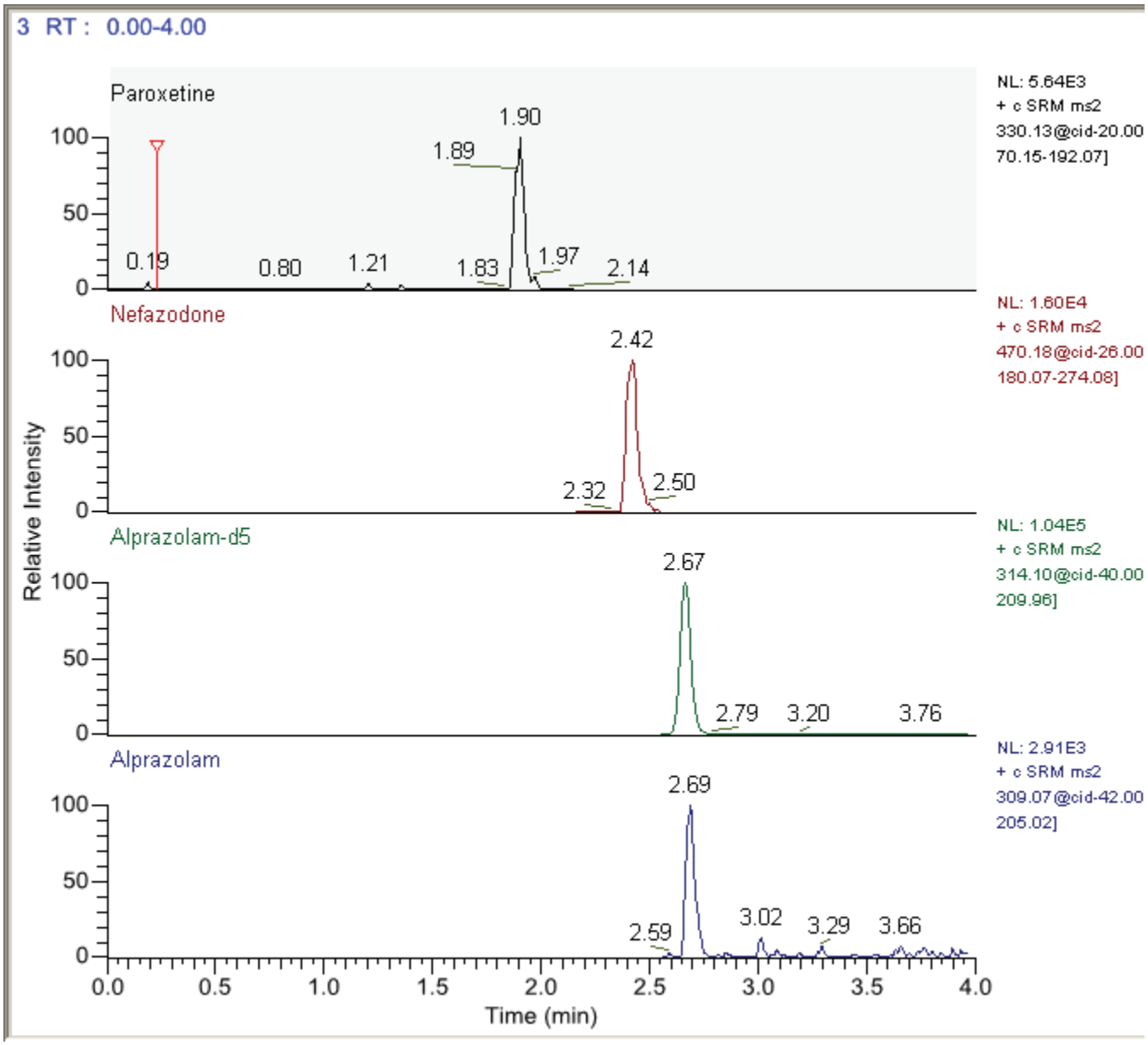
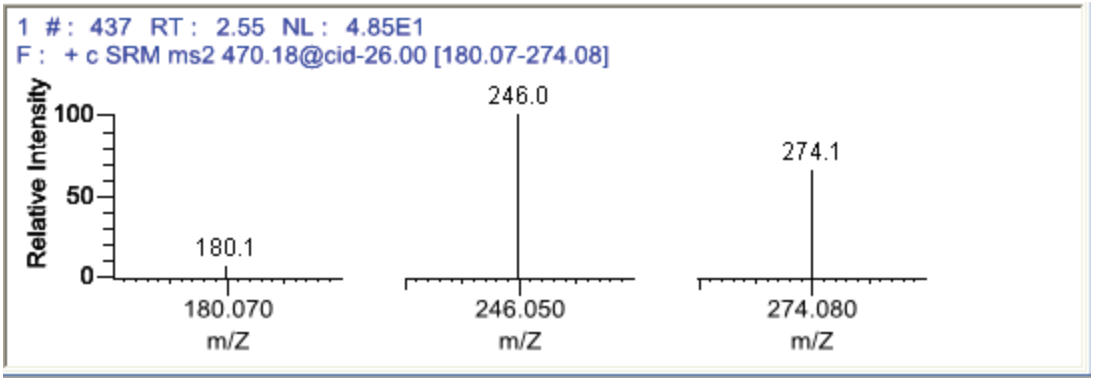


Figure 9. Spectrum trace display



Display Options

On the Status mode display options pane, you can specify display options for the current real-time display. The settings for these options remain each time you log in to the TSQ Module application.

❖ To specify display settings

You can change the display settings at any time while an acquisition is running.

1. To display the chromatograms for all compounds, select **Method-defined traces**.
2. To display the spectrum trace, select **Show spectrum trace**.
3. Choose one of the following spectrum trace options:
   - To continuously update the spectrum trace of the most current scan, select **Continuous update**.

     – Or –

   - To display the spectrum trace of the selected peak, select **Manual select**.

     After you apply these settings, click on a peak in the real-time display.
4. To specify the range for the real-time display, do the following:
   a. Select **User-defined RT range** (min).
   b. Enter a value for the beginning of the range.
   c. Enter a value for the end of the range.
5. To update the current display with your settings, click **Apply Settings**.

   The changes are immediately applied.

❖ To zoom in on the real-time display

1. Drag your cursor in the chromatogram or spectrum trace panes to define a region to zoom in.
2. To return to the original display, right-click and choose **Reset Scaling** from the menu.

Status Mode Display Options

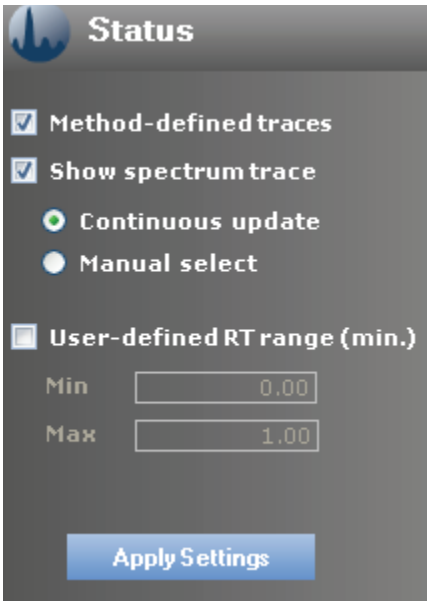


Table 11. Status mode parameters (Sheet 1 of 2)

Parameters	Description
Method-defined traces	Displays real-time chromatograms corresponding to the components defined in the processing method. When the component traces are turned off, the total ion current (TIC) trace is displayed.
Show spectrum trace	Displays the mass spectrum in the spectrum pane.
Continuous update	Continuously updates the spectrum trace to show the most current scan.
Manual select	Display the spectrum trace of the currently selected peak.
User-defined RT range	Displays chromatograms with a time range between the times specified in the Min RT and Max RT boxes.
Min	The start time of the chromatogram traces.
	Note The Min box becomes active only when you select User-defined RT range.

Table 11. Status mode parameters (Sheet 2 of 2)

Parameters	Description
Max	The end time of the chromatogram traces.
	Note The Max box becomes active only when you select User-defined RT range.
Apply Settings	Applies the display settings to the current real-time display.
Shortcut menu Right-click the chromatogram to display the menu.	
Reset Scaling	Resets the X-axis and Y-axis ranges in the Chromatogram pane or the Spectrum pane to their default values.

Pausing/Stopping/Canceling Runs

A run submitted by the Watson application enters the TSQ Module Run Manager view with the status Pending. From the Run Manager view, you can submit a run to the Acquisition Queue or cancel the run, sending it back to the Watson application.

When you submit a run for acquisition, the run moves to the Acquisition Queue and the status in the Run Manager view is Acquiring. From the Acquisition Queue, you can pause a run and resume it, stop the currently acquiring sample and continue to the next sample, or remove a run from the Acquisition Queue—sending it back to the Run Manager view.

Use the following procedures:

- To stop an acquiring sample
- To pause a run
- To remove a currently acquiring run from the Acquisition Queue
- To remove a non-acquiring run from the Acquisition Queue
- To cancel a run in the Run Manager view

❖ To stop an acquiring sample

You can stop acquisition of an individual sample in the Acquisition Queue. When a sample is stopped, the system considers it complete and the XML data is packaged and returned to the Watson application. You cannot resume a stopped sample.

1. To stop the acquisition of the current sample, click **Stop**  .
   - The acquisition stops for the currently acquiring sample and the run pauses.
   - The run status in the status in the Run Manager view is Paused.
   - The pause button is red indicating that it is ready to resume with the next queued sample.

- If the currently acquiring sample is the last sample in the run, the run is marked Completed and sent to the Watson application.

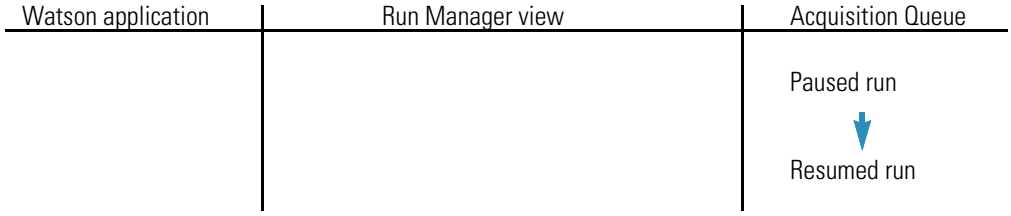
2. To resume the acquisition for the next sample in the run, click **Pause** .

   When you resume acquisition, the system begins with the next sample in the run. If you stopped the final sample in a run, the system begins the next run in the queue.

   For detailed information about repeating the acquisition for a stopped sample, see Appendix B, "Restarting Runs."

❖ To pause a run

You can pause a run in the Acquisition Queue and resume it later.



1. To pause the acquisition of the current run, click **Pause** .

   After the current sample completes, the acquisition for the run pauses until you resume it.

   The run status in the Run Manager view immediately changes to Paused, even if the current sample is still acquiring.

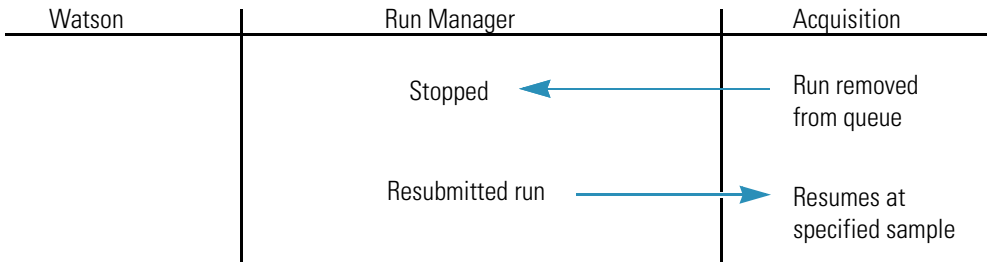
2. To resume the acquisition for the next sample in the run, click **Pause** .

❖ To remove a currently acquiring run from the Acquisition Queue

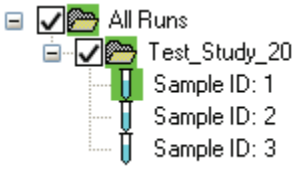
You can remove an acquiring run from the Acquisition Queue and resubmit it later.

For detailed information about restarting a run, see Appendix B, "Restarting Runs."

When you remove an acquiring run from the Acquisition Queue, the run returns to the Run Manager view and its status is Stopped. When you resubmit a stopped run from the Run Manager view, you can specify a range of sample numbers that you want to acquire. It is not necessary to reacquire previously acquired samples.



1. To remove a run from the Acquisition Queue, check the run check box and press the keyboard **Delete** key.



- The currently acquiring sample immediately stops and the run is removed from the Acquisition Queue.
- The status of the run in the Run Manager view is Stopped.
- When there are other runs in the Acquisition Queue, the pause button is red indicating that it is ready to start the next queued run.

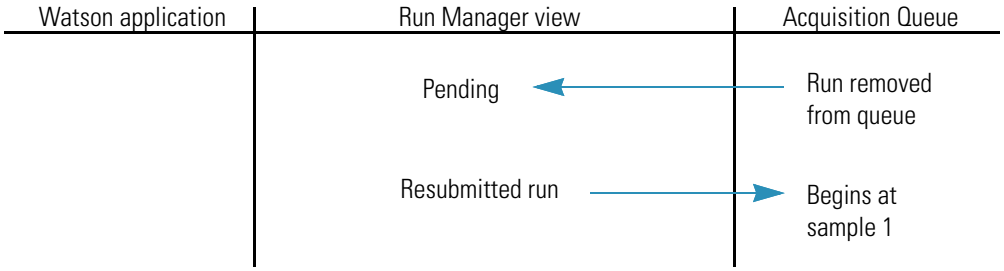
2. To begin acquisition of the next run in the Acquisition Queue, click **Pause** .

❖ To remove a non-acquiring run from the Acquisition Queue

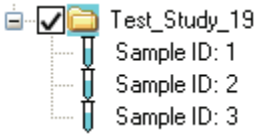
You can remove a run from the Acquisition Queue and resubmit it later.

For detailed information about restarting runs, see Appendix B, "Restarting Runs."

When you remove an non-acquiring run from the Acquisition Queue, the run returns to the Run Manager view and its status returns to Pending. When you resubmit this run to the Acquisition Queue, it begins acquiring at the first sample.



1. To remove a non-acquiring run from the Acquisition Queue, check the run check box and press the keyboard **Delete** key.



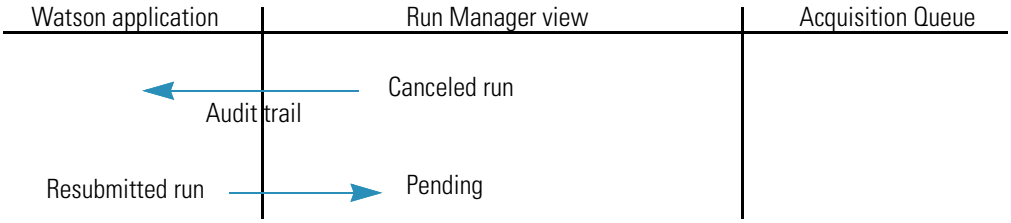
The run is immediately removed from the Acquisition Queue and the run returns to the Run Manager view as Pending.

Note To remove multiple runs from the Acquisition Queue, check the check box for each of the runs in the queue and press the keyboard **Delete** key.

❖ To cancel a run in the Run Manager view

You can cancel a run in the Run Manager view. The TSQ Module application returns a canceled run to the Watson application and Watson users can resubmit the run to the TSQ Module application.

For detailed information about restarting runs, see Appendix B, "Restarting Runs."

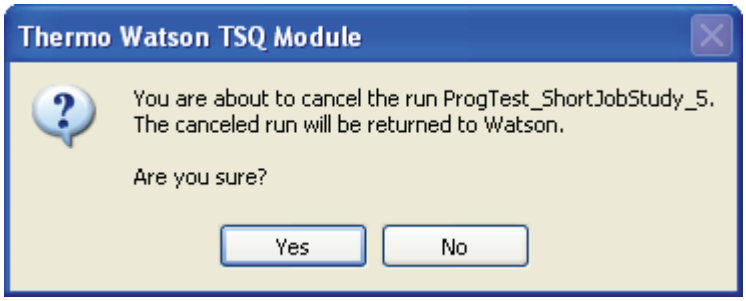


1. Select the run in the Run Manager view.

   You cannot use the Run Manager view to cancel a run that is currently in the Acquisition Queue (Submitted or Acquiring).

2. Click **Cancel Run**.

   The following confirmation message is displayed:



3. To return the run to the Watson application, click **Yes**.

   Watson users can modify and resubmit the run or resubmit the run without changes.

Watson: Working with Processed Runs

This chapter provides information about working with runs in the Watson application that are in process or have already been processed by the TSQ Module application.

Contents

Reviewing Runs

You can continuously monitor the status of runs, even while they are being processed in the TSQ Module application. You can configure the Watson application to require the review and acceptance of particular sample types.

In the Watson application, you can review a run that is incomplete or ongoing. Use this feature to review a set of chromatograms while the LC/MS is acquiring additional samples. If a problem occurs in the run, you can see how far back in the sequence the problem occurred while the acquisition is taking place.

Use the following procedures:

- To review the list of runs
- To configure run review requirements
- To view the run queue in the Watson application
- To view an ongoing or incomplete run
- To view the run after data acquisition is complete

❖ To review the list of runs

1. Choose **Analytical > Analytical Run Actions**.

   The Run Queue opens, listing recent runs. From this queue you can monitor the status of runs. You can also access the Run Review view that provides detailed information.

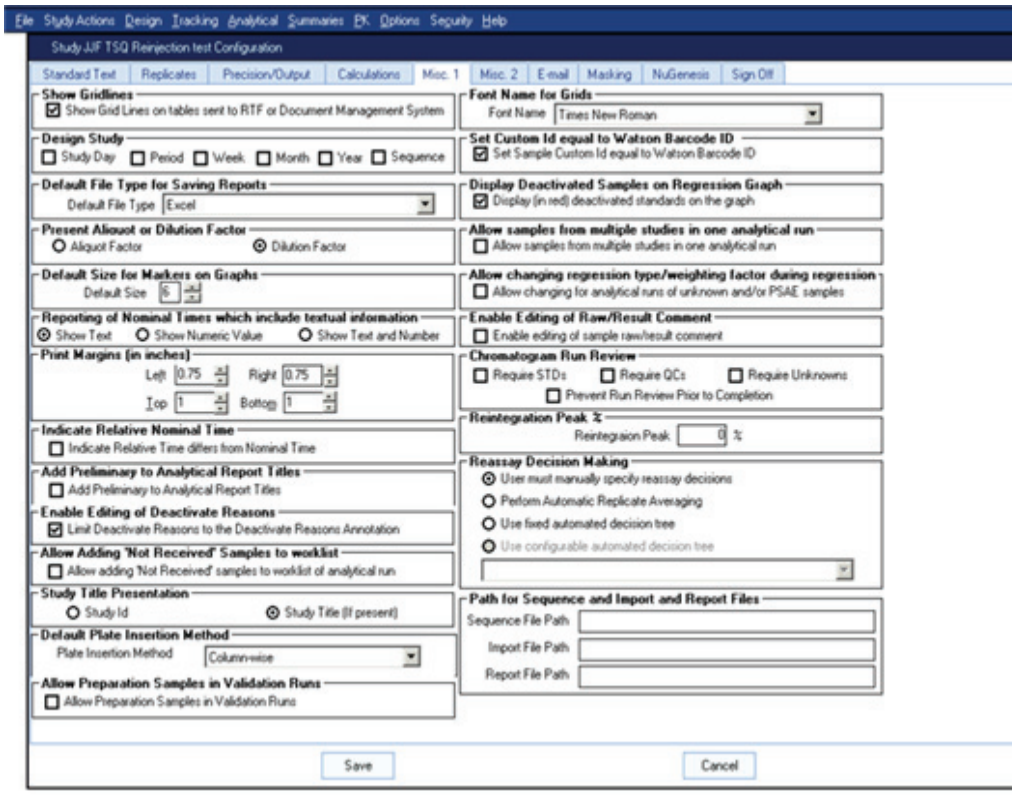
2. To review a run, select it and click **Run Review.**

❖ To configure run review requirements

1. Create or open a study in the Watson application.
2. Choose **Study Actions > Configuration**.

   The Configuration dialog box opens.

   Figure 10. Configuration dialog box: Misc. 1 page



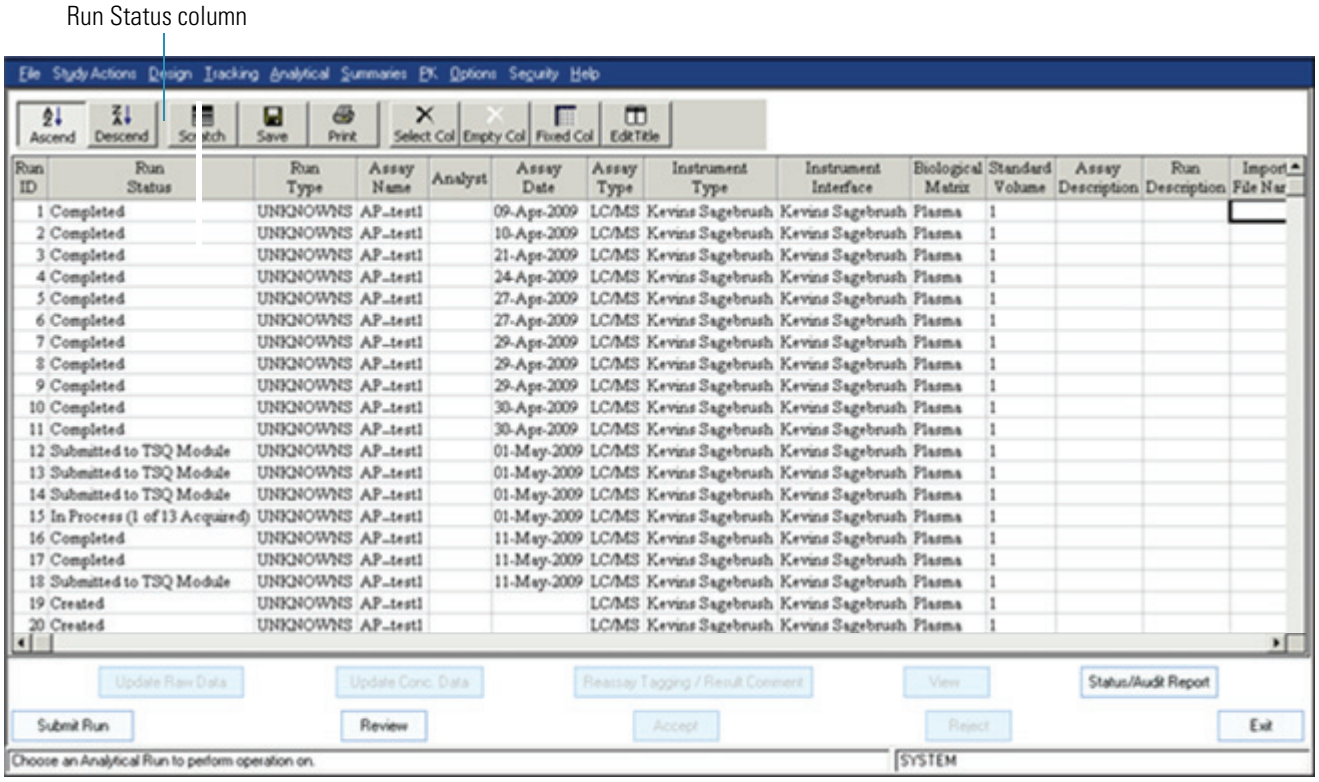
3. To require QCs, Unknowns, or STDs, select the appropriate parameter in the Chromatogram Run Review section.
4. To prevent users from reviewing a run before the run completes, select **Prevent Run Review Prior to Completion** in the Chromatogram Run Review section.

❖ To view the run queue in the Watson application

1. Open a study with an associated Peak Integration instrument.
2. Choose **Analytical > Analytical Run Actions**.

   The Run Queue opens. From this window, you can view the run status in real time. All runs are date and time stamped.

Figure 11. Run queue



Run Status Definitions

The following is a list of common run statuses when working with runs that are submitted to or processed by the TSQ Module application. For a complete list of run statuses, refer to the *Watson User Manual*.

- Submitted to TSQ Module: The run has been submitted to the TSQ Module application for processing.
- Completed: The run has been processed and is now complete.
- Created: The run has been created, but has not yet been submitted for processing.
- Canceled by: Notification that the run has been canceled and where the cancellation occurred.
- In Process: The run is currently being acquired.

❖ To view an ongoing or incomplete run

1. Select a run labeled as In Process.
2. Click **Review**.

   Run results appear.

   Completed runs are labeled as such in the run queue. These runs are submitted to the TSQ Module application from the Watson application, processed in the TSQ Module application, and then sent back to the Watson application.

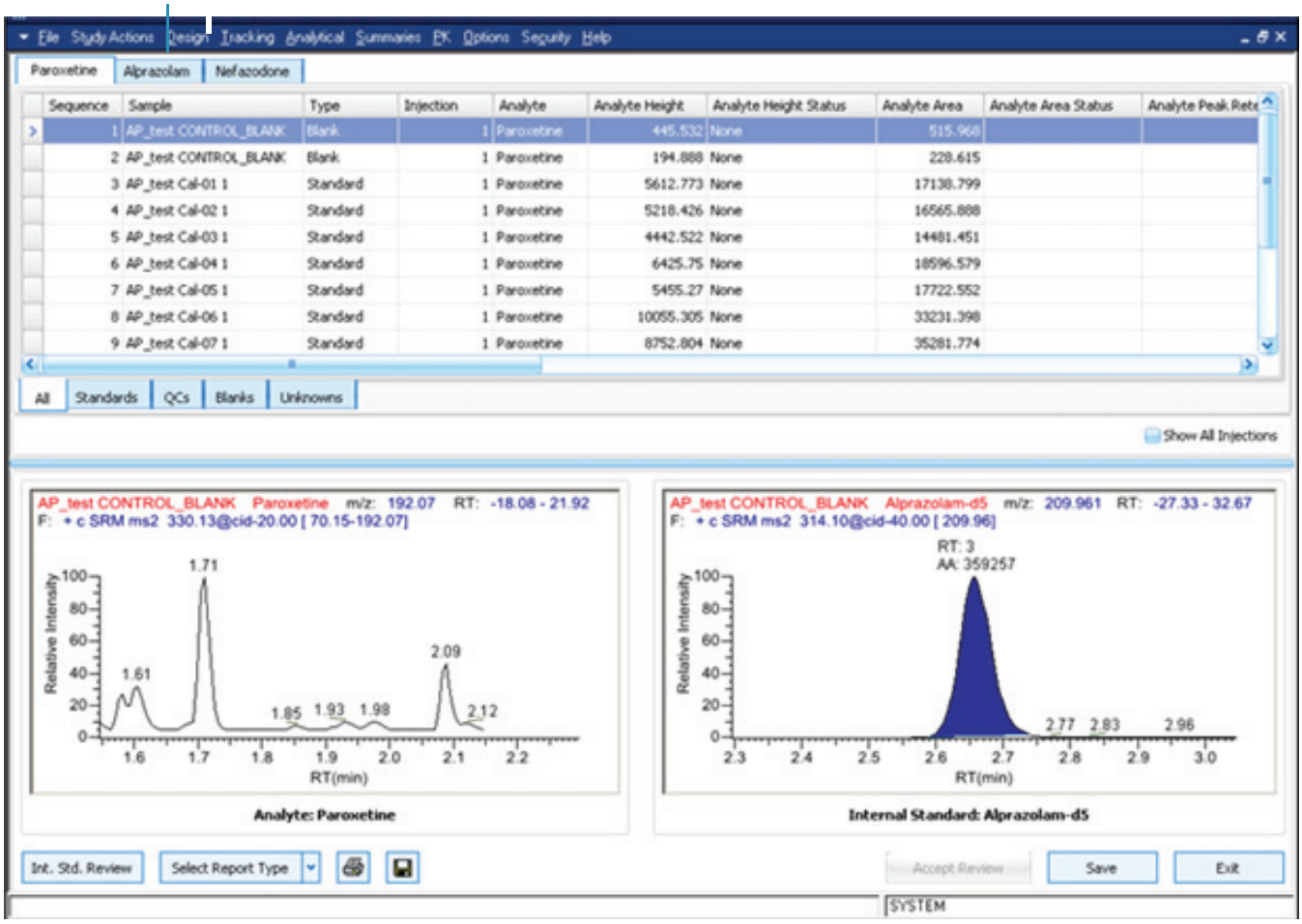
❖ To view the run after data acquisition is complete

1. Select a completed run and click **Review**.

   Run results appear.

2. To navigate between sample analytes, click the tabs at the top of the view.

Figure 12. Sample analytes



Reintegrating Chromatograms

This section provides instructions for reintegrating chromatograms.

Reintegrate chromatograms by doing one of the following:

- Changing the peak processing parameters

  –Or–

- Moving the baseline

Use the following procedures:

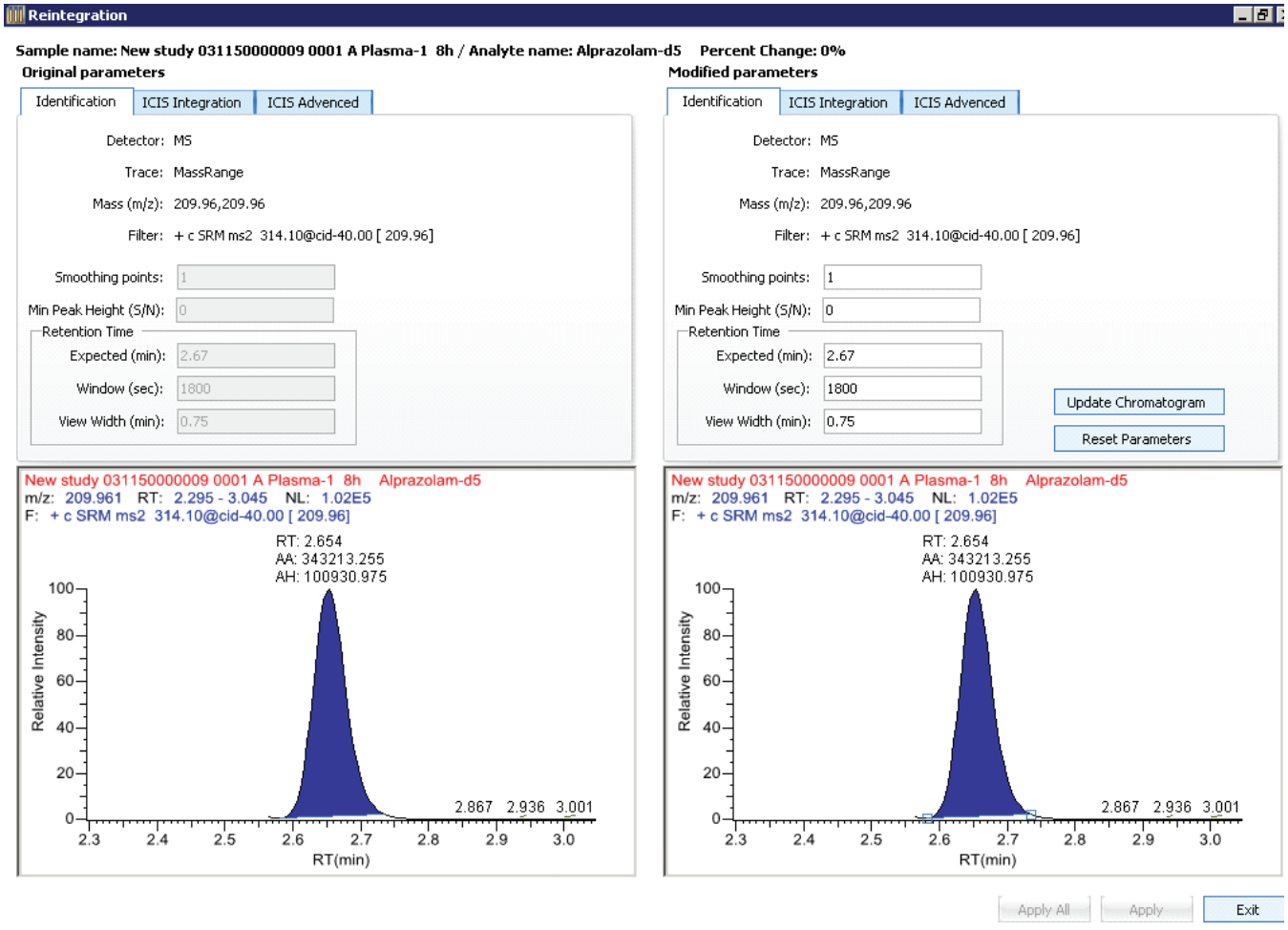
- To change the processing parameters
- To configure identification parameters
- To configure the ICIS integration parameters
- To configure the ICIS advanced parameters

❖ To change the processing parameters

1. Select a row in the top part of the window.
2. Right-click and choose **Reintegrate** > **Analyte** or **Int. Std.** from the menu.

   The Reintegrate Chromatogram view opens with the Identification page displayed.

Figure 13. Reintegrate Chromatogram view – Identification page



❖ To configure identification parameters

1. Click the **Identification** tab.
2. Type values in the following fields in the top-right view:
   - Smoothing points: Enter an odd-numbered value between 1 and 5.
   - Min. Peak Height (S/N): Enter a value between 0.00 and 999.00.
   - Retention Time: Enter a value between 0.00 and 999.00.
     - Expected (min)

- Window (sec)
- View Width (min)

3. Click **Update Chromatogram** to save your changes.

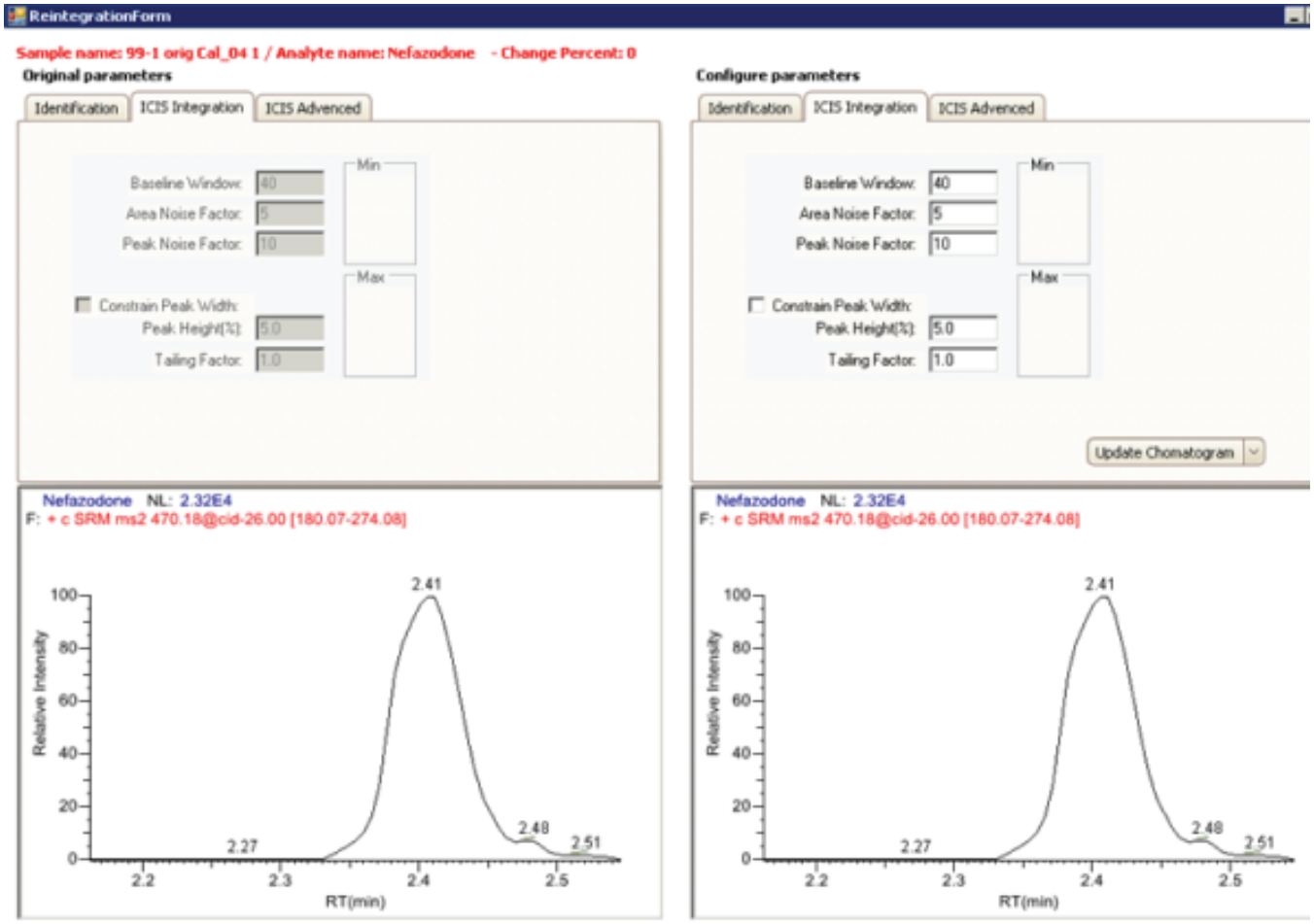
   The Chromatogram on the right side is updated, so you can compare it with the original.

❖ To configure the ICIS integration parameters

1. Click the **ICIS Integration** tab.
2. Complete the following fields in the top-right pane:
   - Baseline Window: The number of scans over which to look for a local minima (1 to 500, Units 1).
   - Area Noise Factor: Noise level multiplier used to determine the potential peak signal threshold (1–1000, Units 1).
   - Constrain Peak Width: Selecting this check box constrains peak width.
   - Peak Height (%): Constrain peak edges to a percentage of Peak Apex Height (0.00 to 100.0).
   - Tailing Factor: Constrain peak edges to a ratio of the trailing and leading edges (0.5 to 9.0).
3. Click **Update Chromatogram** to save your changes.

   The Chromatogram on the right side is updated, so you can compare it with the original.

Figure 14. Reintegrate Chromatogram view – ICIS Integration page

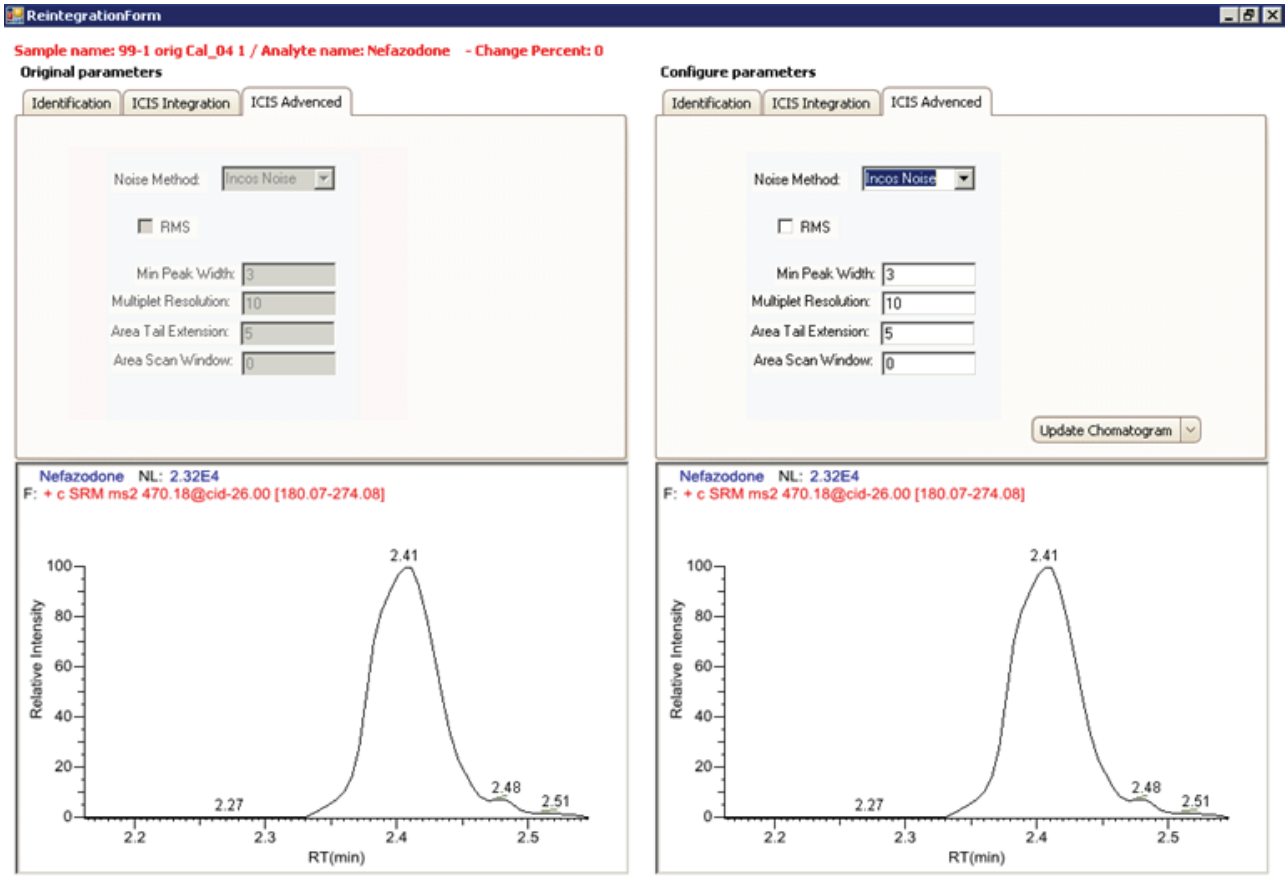


❖ To configure the ICIS advanced parameters

1. Click the **ICIS Advanced** tab.
2. Complete the following fields in the top-right pane:
   - Noise Method: Select **Incos Noise** or **Repetitive Noise**.
   - RMS: Click to activate.
   - Min. Peak Width: Minimum number of scans in a peak (0 to 100, units 1).
   - Multiplet Resolution: Minimum separation in scans between two peaks (0 to 500, units 1).
   - Area Tail Extension: Number of scans past endpoint to use in averaging intensity (0 to 100, units 1).
   - Area Scan Window: Number of scans to either side of the apex allowed in a peak. Use **0** to include all (0 to 100, units 1).
3. Click **Update Chromatogram** to save your changes.
4. When you have made all parameter changes, apply them to the selected sample (**Apply**) or apply to each sample in the run (**Apply All**).

   The new parameters are stored in the database.

Figure 15. Reintegrate Chromatogram view – ICIS Advanced page



Changing the Chromatogram Display

In the chromatogram view, you can change the display.

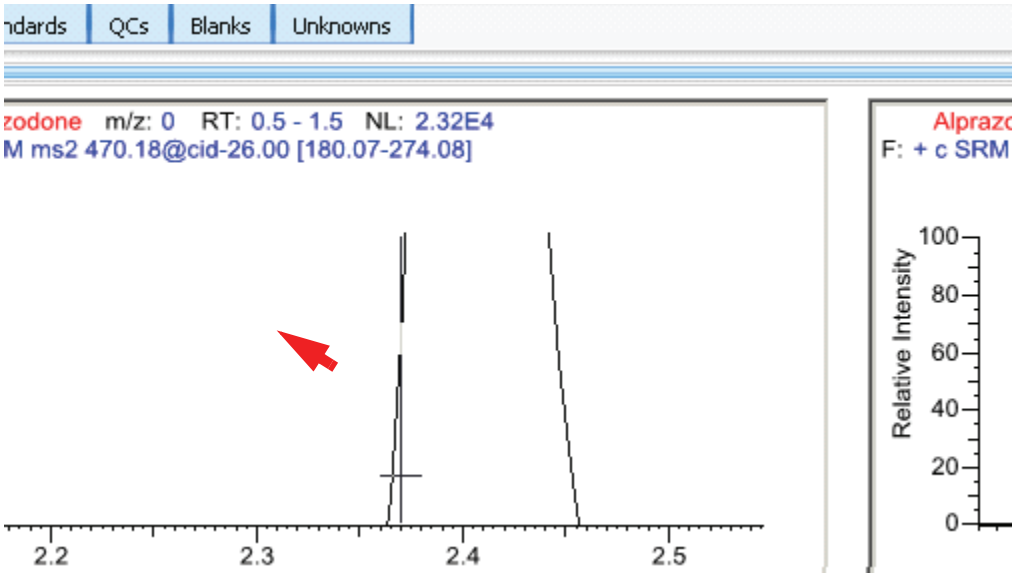
Use the following procedures:

- To move the baseline
- To view a chromatogram in the gallery
- To zoom in on a chromatogram

❖ To move the baseline

Using the cursor, drag the baseline in the chromatogram view.

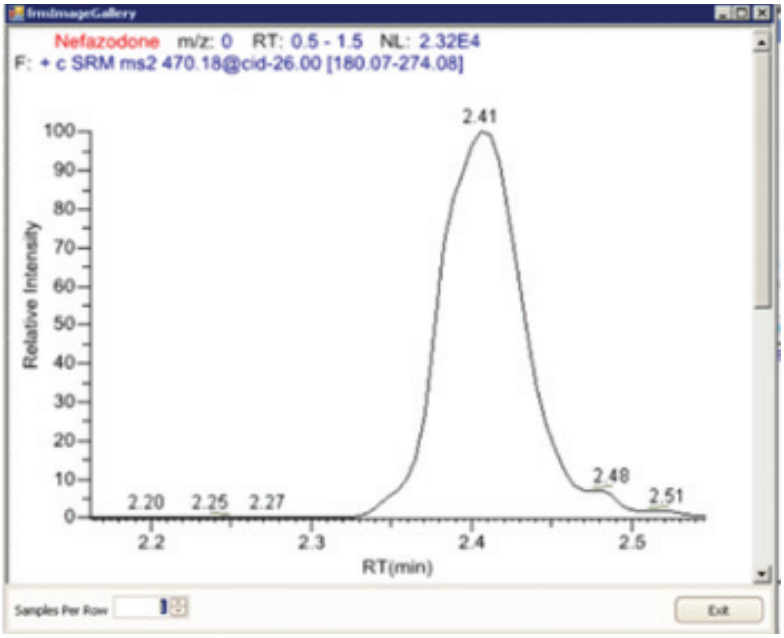
Figure 16. Moving the baseline



❖ To view a chromatogram in the gallery

1. From the Run Review view, select an analyte.
2. Right-click and choose **View in Gallery** from the menu.
3. Select one of the following: **Analyte Only**, **Analyte & Int. Std.**, or **Int. Std. Only**.

Figure 17. Gallery view (analyte only)



4. Click **Exit** to close the gallery window.

❖ **To zoom in on a chromatogram**

1. Select a sample.
2. Zoom in on the corresponding chromatogram by dragging the cursor.
3. To reset the chromatogram, right-click and choose **Reset Scaling**.

Additional Run Review Features

In addition to using the display features of the Run Review view, you can do the following:

- Show All Injections
- Accept a Run
- Resubmit a Run to a Different Instrument

Show All Injections

When the Show All Injections check box is selected, all injection values that exist for all run samples are displayed.

When the Show All Injections check box is cleared, only the last injection values for the samples in the run are displayed. This has an impact on the Run Review view.

Accept a Run

Using the Watson application, you can review analytes, IS mass chromatograms, and peak detection results, and reintegrate the results before accepting the run. To accept a run, click **Accept Review** on the Run Review view.

Resubmit a Run to a Different Instrument

You can cancel a run that has been submitted to the TSQ Module application and resubmit it to an alternate instrument stack or an alternate instrument, only if no data has already been acquired.

Reviewing Internal Standards and Performing Regression

Use the following procedures:

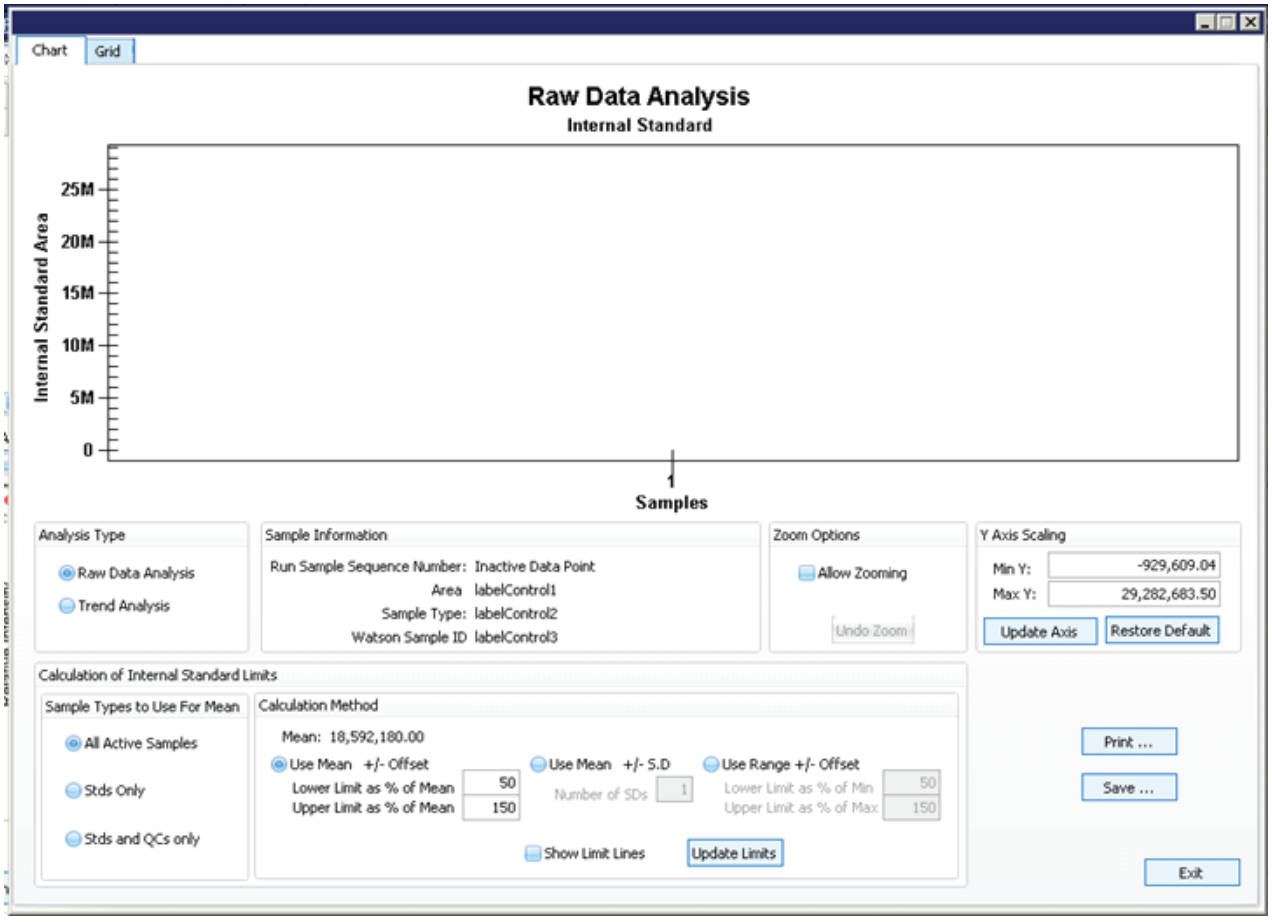
- To review internal standards
- To filter sample types from the run results
- To flag a sample or sample-analyte with a Reassay tag
- To flag a sample or sample-analyte with a text comment
- To deactivate or reactivate a sample-analyte for regression
- To save to the database
- To perform a standard regression

❖ To review internal standards

- From the Run Review view, click **Internal Standards Review**.

  The Raw Data Analysis view opens with the Chart page displayed.

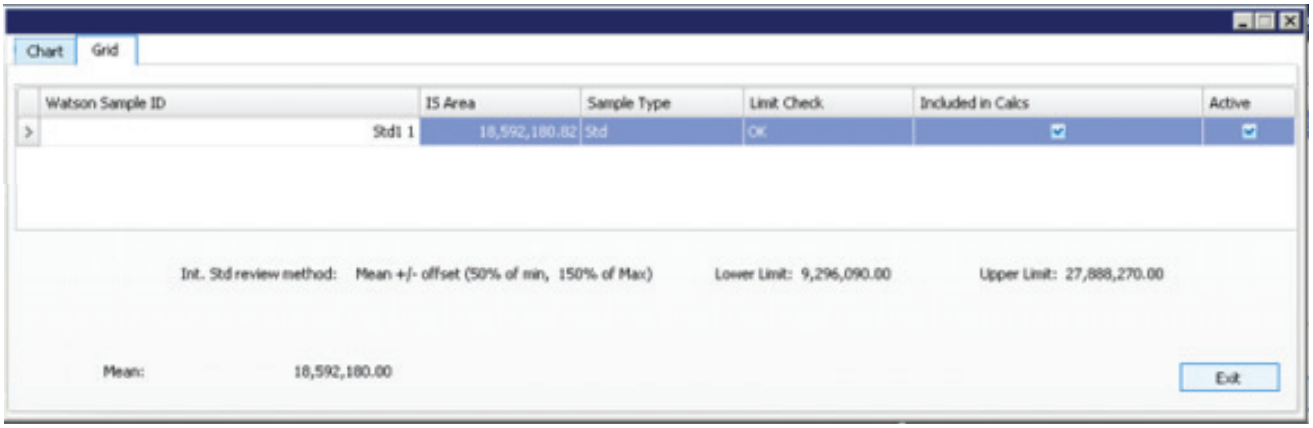
Figure 18. Raw Data Analysis view – Chart page



The Chart page contains the following areas:

- Analysis type
  - Raw Data Analysis
  - Mean
- Sample Information
  - Run Sample Sequence Number
  - % Difference
  - Sample Type
  - Watson sample ID
- Zoom options: Click to allow zooming.
- Y-Axis scaling: Set the Y-axis scaling.
  - To update, edit the Min Y and Max Y fields and click **Update Y Axis**.
  - To restore defaults, click **Restore Defaults**.
- Calculation of Internal Standards Limits: Determine the criteria for calculating internal standards.
- Sample types to be used for mean: Select the option next to the sample type to select All Active Samples, STDs only, STDs and QCs only.
- Calculation Method
  - Use Mean +/- offset: Select and enter the lower limit of percentage of mean and upper limit of percentage of mean.
  - Use Mean +/- SD: Click the Number of SDs field and type a number.
  - Use Range +/- offset: Select and enter the lower limit of percentage of mean and upper limit of percentage of mean.
  - Click **Update Limits** to update the limits.
- Print: Print the raw data analysis.
- Save: Save the raw data analysis.
- Exit: Exit the page.

Figure 19. Raw Data Analysis view - Grid page



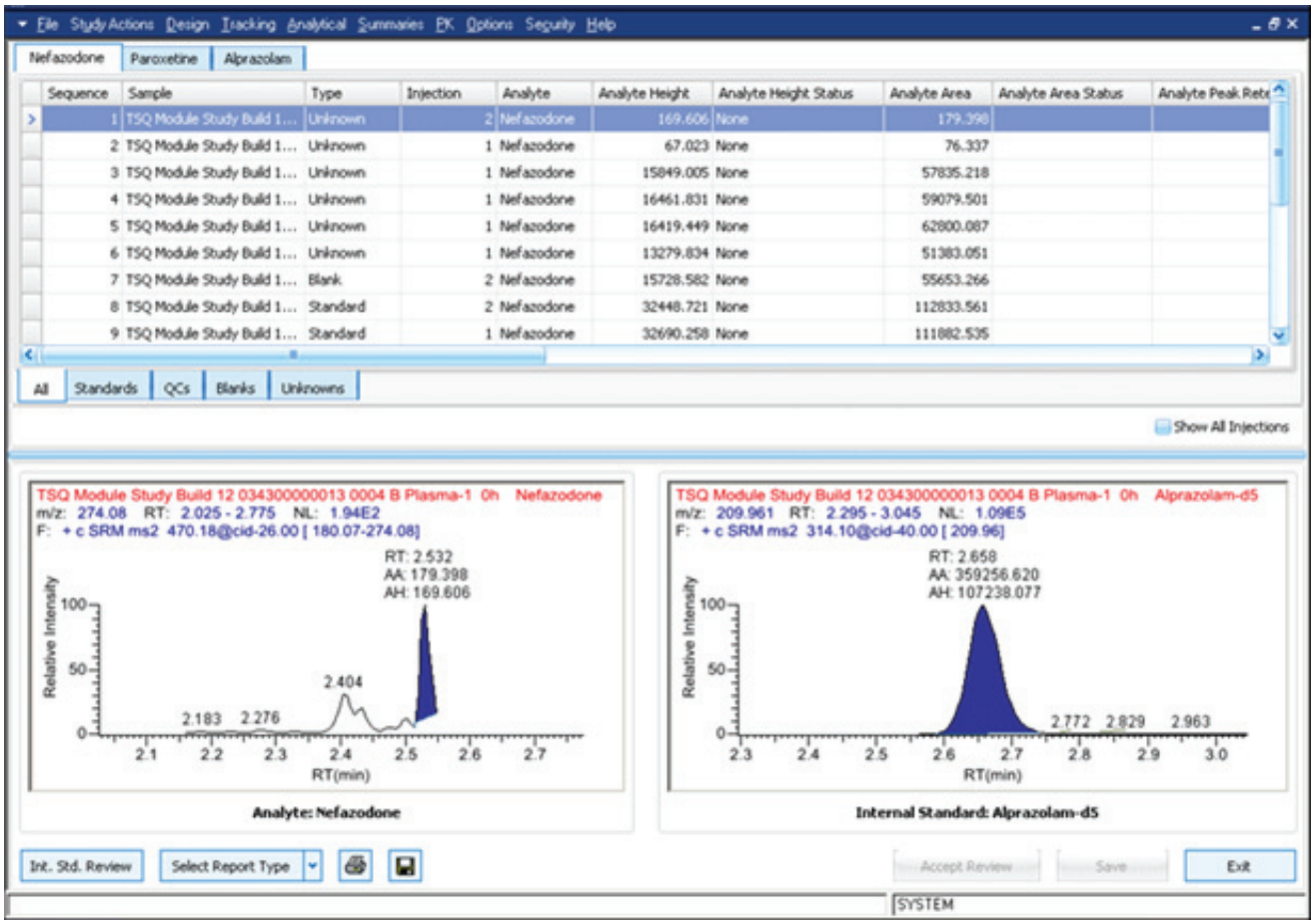
❖ To filter sample types from the run results

1. Open a study with an associated Peak Integration instrument.
2. Choose **Analytical > Analytical Run Actions**.
3. From the run results, select a run that contains results and click **Review.**

   Al results are shown in the initial view. You can select one sample-analyte at a time from the list of samples in a run, and display chromatograms and information about that analyte's peak and its associated internal standard peak.

   When this view loads, it defaults to All samples.

Figure 20. Run Results – All Samples



4. (Optional) Filter this view by Standards, QCs, Blanks, and Unknowns.

   Figure 21. Standards Filtering tabs

5. If you want to accept the review, click **Accept Review**.

❖ To flag a sample or sample-analyte with a Reassay tag

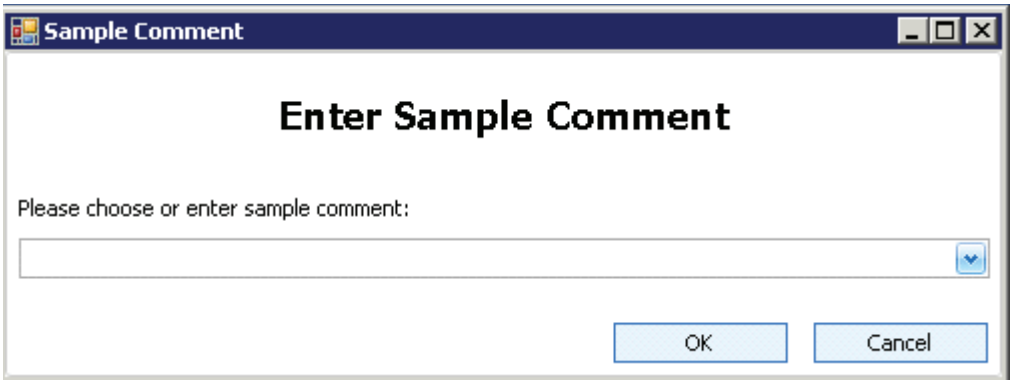
1. From the run results, right-click the sample or sample-analyte and choose **Tag for Reassay** from the menu.
2. Do one of the following:
   - To save the tag, click **Save**.

     –Or–
   - To exit without saving any changes, click **Exit**.

❖ To flag a sample or sample-analyte with a text comment

1. From the run results, right-click the sample or sample-analyte and choose **Apply/Edit comment** from the menu.

   The Sample Comment dialog box opens.



2. Enter a comment or choose one from the list.
3. Click **OK**.

❖ To deactivate or reactivate a sample-analyte for regression

1. Right-click the sample-analyte and choose either **Activate** to activate the sample analyte, or **Deactivate** to deactivate it.
2. Do one of the following:
   - To save the change, click **Save**.

     –Or–
   - To exit without saving any changes, click **Exit**.

❖ To save to the database

Click **Save** on the Run Review view.

❖ To perform a standard regression

1. Choose **Analytical > Standard Regression**.

   The Standard Regression dialog box opens with two grids: The top grid contains a list of each Analytical Run for which Raw Data has been imported. The lower grid is a list of analytes associated with the specified Analytical Run with analyte status, regression data, LLQ, ULQ, and who performed the regression.

2. Click **Regression**.

   The Watson application performs a standard regression on the specified analyte associated with the specified Analytical Run.

3. After regression is complete, click the appropriate button to accept, reject, or save the results.

For more information, refer to the Analytical Menu chapter in the *Watson User Manual*.

Watson: Reports and Printing Capabilities

Use the Watson application to generate several types of reports, including integration reports at the study and sample levels, audit reports, and several Crystal reports. You can then print reports using extended printing capabilities available within the Watson application.

Contents

Study-level and Sample-level Integration Reports

This section provides information about study-level and sample-level integration reports.

❖ **To view study-level and sample-level reports**

1. Access the Run Review view.
2. From the Select Report Type list, select either **Study-Level Integration Report** or **Sample-Level Integration Report**.

   See "Study-Level Integration report" on page 78 or "Sample-Level Integration report" on page 79.

   You can subdivide each report into Master Report, ICIS Report, or Reinjection Report by selecting the appropriate options on the top of the view.

   The reports show the integrated peak as a shaded area and its raw areas/height. It displays a descriptive header/footer that contains the following information:

   - Design info (Subject, Treatment, Group, Nominal Day, Nominal Hour, Nominal Minute, Split #), Watson Sample ID, Watson Custom ID
   - Mass data (Q1 *m/z*, Q3 *m/z*)
   - Method and Tune information

- Chromatogram data (for example, peak area, peak height, retention time, acquisition date/time in currently supported Watson date and time formats, acquisition instrument info)
- Watson information (for example, Study, Run #, Position in run, plate position, injection volume)
- Edit info (for example, date/time and person last edited for reintegrations)
- Multi-Level Approval or eSig info of analytical run and individual chromatograms

Figure 22. Study-Level Integration report

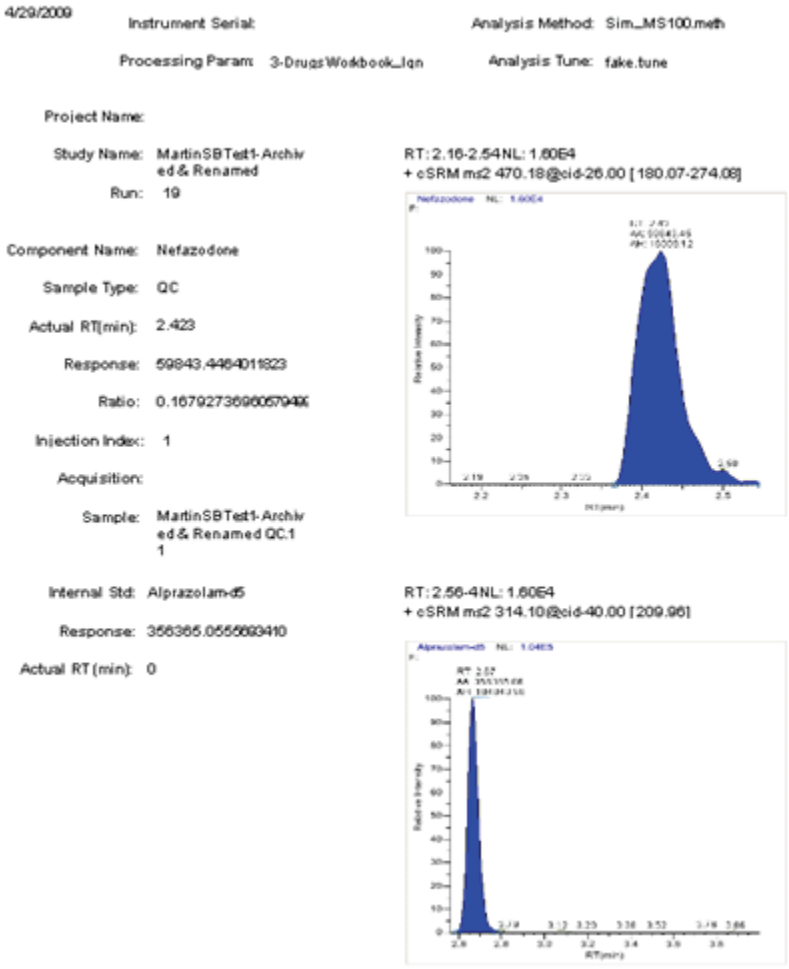
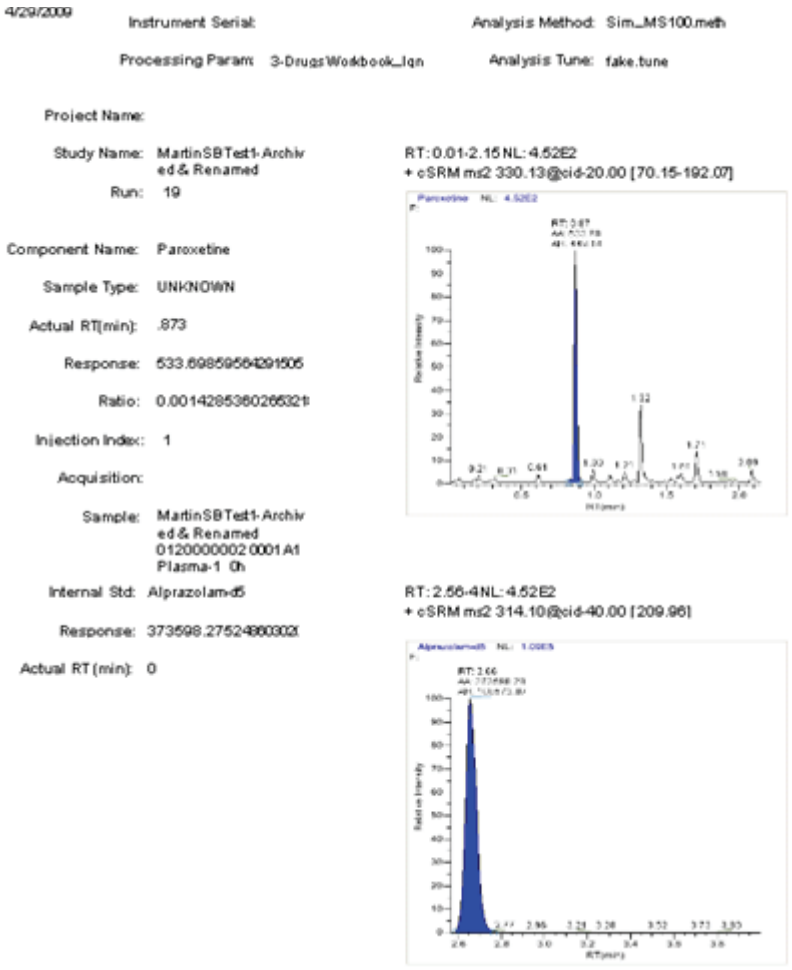


Figure 23. Sample-Level Integration report



Audit Reports

Use the Watson application to create, view, and print system- or study-level audit reports for the TSQ Module application. You can print a basic audit report from the Watson File menu that contains TSQ Module-specific information alone, or in combination with all other categories. In addition, you can view and print detailed audit reports from the Create Sequence File, Import Results, and View Analytical Run views.

Generating a Watson Audit Report

This process is similar to existing Watson application workflows, with the exception of selecting TSQ Module as the category of information to be viewed.

❖ To view audit reports for TSQ Module data

1. Choose **File > Audit Report**.

   The Select Criteria for Audit Report view opens. See "Select Criteria for Audit Report view" on page 81.

2. From this view, enter or select the following criteria:

   - Date/Time and Audit Level
   - Date and time range
   - System or Study Audit Level (your level of access is established by the system administrator)
   - Category:
     - For TSQ Module information only, select **TSQ Module**.
     - For all information, select **All Categories**.
   - User: Select the user or role.
   - Project-Study: Select at least one study from the list that appears in this column.

3. After entering and selecting the report criteria, do one of the following:

   - Click **OK** to generate the audit report. See "Audit Report" on page 82.

   –Or–

   - Click **Exit** to close the view without generating the report.

For more information, refer to the File Menu chapter in the *Watson User Manual*.

Figure 24. Select Criteria for Audit Report view

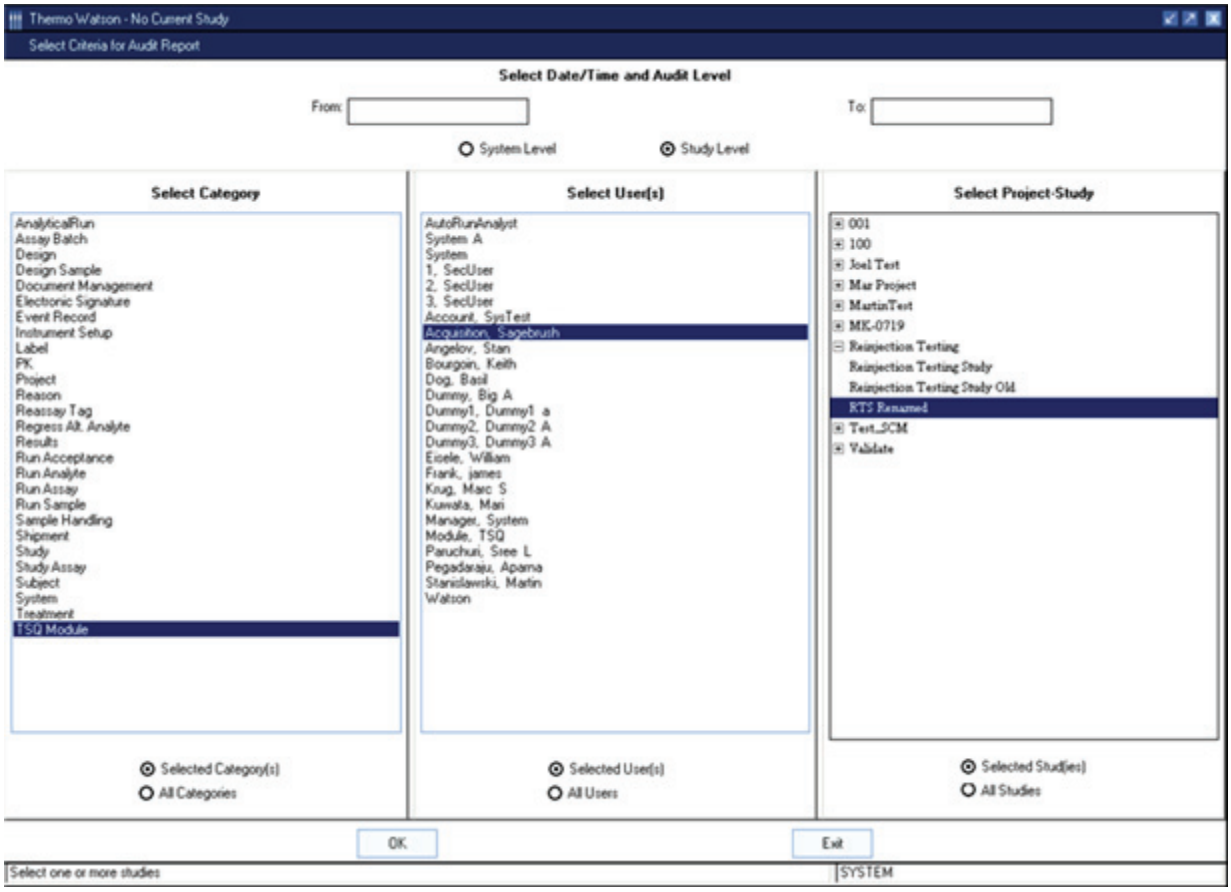
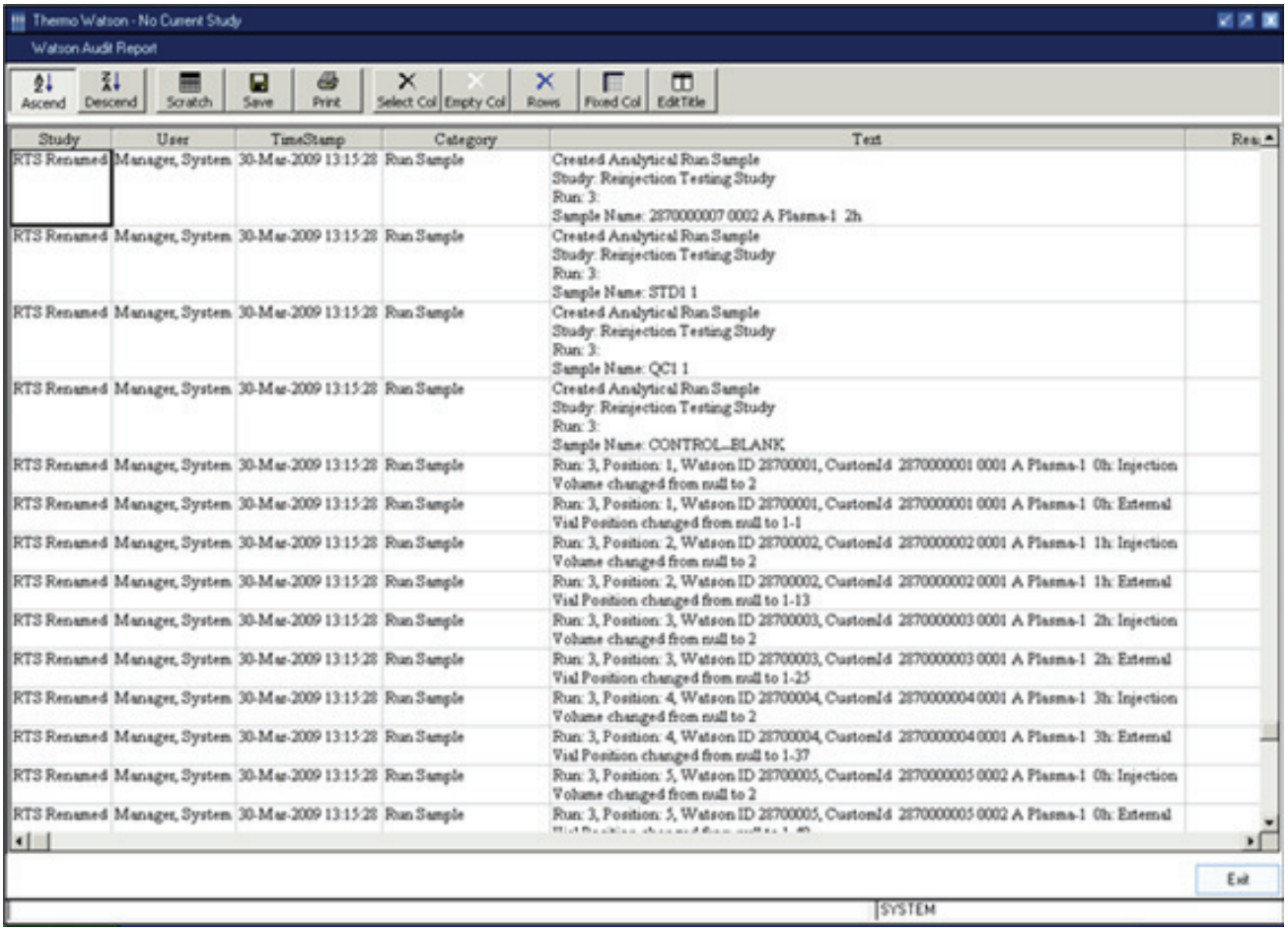


Figure 25. Audit Report



Generating a TSQ Module Status/Audit Report

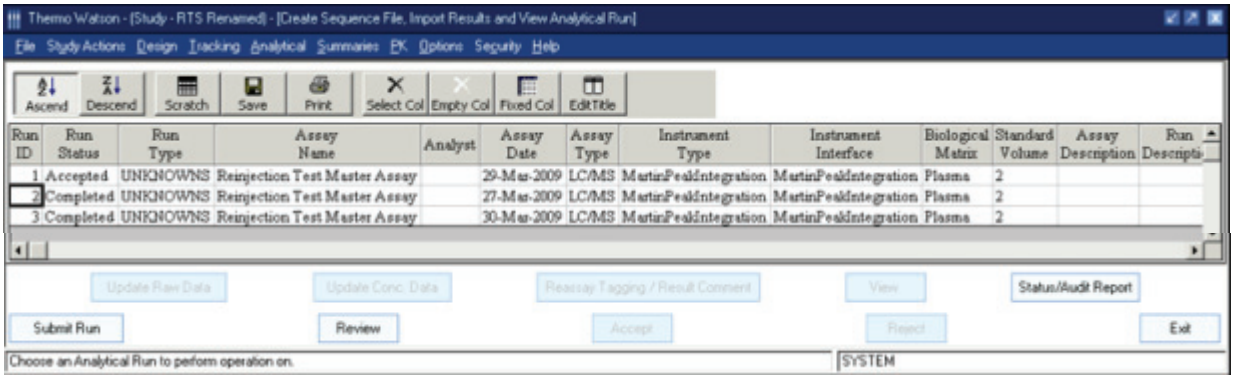
This Status/Audit report provides you with more detailed information about the samples in a run. To generate this report, follow standard Watson application processing as outlined in this section. Note that in the example below, TSQ Module-specific data is shown. The Runs are labeled as "reinjection," and the instrument type is Peak Integration.

❖ To generate a TSQ Module status or audit report

1. Create or open a TSQ Module study.
2. Choose **Analytical > Analytical Run Actions**.

   The Create Sequence File, Import Results, and View Analytical Run view opens.

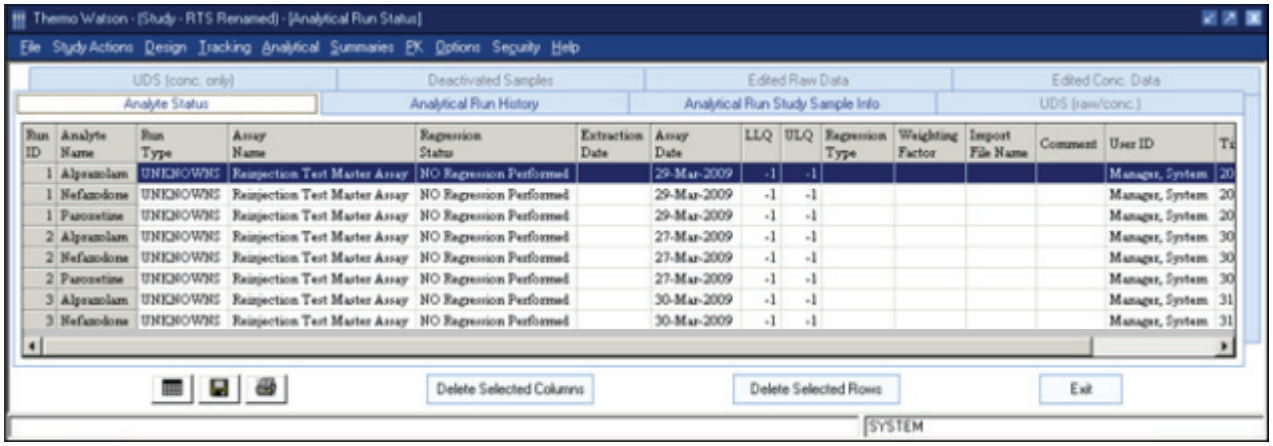
Figure 26. Create Sequence File, Import Results, and View Analytical Run view



3. Select a run and click **Status/Audit Report.**

   The Watson application generates the Status/Audit Report.

Figure 27. Status/Audit Report



For more information, refer to the Analytical Menu chapter in the *Watson User Manual*.

Printing Capabilities

Printing capabilities in Watson include:

- Printing chromatograms or saving them to a fully locked PDF or open PDF file.
- The on-screen display and the printed version of text, numerical values, and graphics (chromatograms and charts/graphs) are identical.

Printing a Chromatogram

❖ To print a chromatogram

1. Click the **Print** icon.

   The Print dialog box opens.

2. Select the check boxes to print **Analyte Chromatogram**, **Internal Standard Chromatogram**, or **Result Data**.

3. Click **OK**.

   The Preview window shows the selected analyte chromatogram. Information, such as peak area/height, retention time, Q1 and Q3 mass, and analyte name, is displayed.

4. Click **Print** to print the chromatogram.

5. Save the chromatogram to a PDF file by clicking the **Export Document** icon (document with diskette).

   You can print the chromatogram, save it to a PDF file, or both. What you see is identical to what is printed.

6. Close the Preview window.

   Another Preview window is displayed. The window displays the internal standard chromatogram. Information, such as peak area/height, retention time, Q1 and Q3 mass, and IS name, is displayed.

7. Click **Print** to print the chromatogram.

8. Save the chromatogram to a PDF file by clicking the **Export Document** icon.

   You can print the chromatogram, save it to a PDF file, or both.

9. Close the Preview window.

   A new Preview window opens. This window displays results data, which you can print. The print display and printout are identical.

10. Close the Preview window for the results data.

Printing Capabilities

The Watson application lets you preview reports before printing or modify page layout, orientation, or paper dimensions.

When viewing a report, a print menu appears at the top of the window. The print menu lets you navigate between pages of the report, locate text, zoom, and print.

Figure 28. Print menu

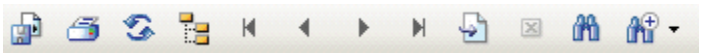


Table 12. Print menu icons

Icon	Description
	Export report
	Print report
	Refresh
	Toggle group tree
	Go to first page
	Go to previous page
	Go to next page
	Go to last page
	Go to page
	Close current view

Table 12. Print menu icons

Icon	Description
	Find in page
	Zoom

A

TSQ Module: Menu and Toolbar Reference

This appendix provides a reference to the Watson TSQ Module menus, icon bars, and status bars.

Contents

Using the Menu

The application menu in the upper left corner of the TSQ Module window includes commands to open the Select mode, save changes to a run, log out of or exit the application, or display Help.

❖ **To use the keyboard to activate a menu command**

1. Click the application icon  to display the TSQ Module application main menu.
2. Type the first character of the command in the menu title, such as **S** in **Select**.

   If you do not choose a command, you can close the menu by pressing the **ESC** key.

TSQ Application Menu Commands

The application menu in the upper left corner of the TSQ Module application window includes commands to open the Select mode, save changes to a run, log out of or exit the application, or display Help.

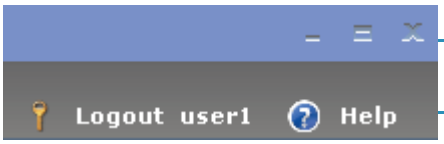
Click the application icon  in the ribbon to display the TSQ Module application main menu.

Menu command	Description
Select	Displays the Select mode.
Save	Saves changes to a run (Position, Injection Volume, or Comment).
Logout	Closes the TSQ Module application and presents a new Login screen. This does not stop the acquisition of runs.
Help	
Watson TSQ Module Help	Opens the main Help tool.
How to Use Help	Opens instructions in the Help for using the online navigation features.
Glossary	Opens the glossary in the Help tool.
About Watson TSQ Module	Opens an about box that displays the version of the tool you are running, copyright information, and the end user agreement.
Exit	Closes the TSQ Module application. This does not stop the acquisition of runs.

Title Bar and Ribbon Commands

The title bar of the window includes icons to minimize, maximize, or close the TSQ Module application.

The ribbon of the window includes icons to access the Help or log out of the TSQ Module application.



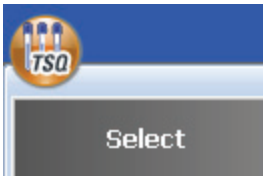
Command	Description
	Collapses the TSQ Module application window to your toolbar.
	Maximizes or minimizes the size of the application window.
	Closes the TSQ Module application. This does not stop the acquisition of runs.
	Closes the TSQ Module application and presents a new Login screen. This does not stop the acquisition of runs.
	Opens the Help.

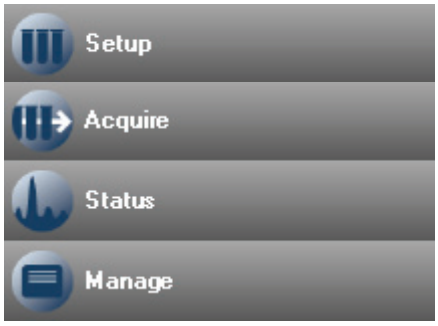
Status Bar

At the bottom of the TSQ Module application window is a status bar that reports the name of the run that is currently acquiring (or reports Ready if no run is acquiring) and the number of runs that have the status Pending, Submitted, or Stopped.

Accessing TSQ Module Modes

The TSQ Module application uses five functional modes: Select, Setup, Acquire, Status, and Manage. Access the Select mode from the TSQ Module application menu – see "TSQ Application Menu Commands" on page 88. Access the other modes from the navigation pane.





Mode	Description
Select	The Select mode is the first mode you see when you log in to the TSQ Module application. In the Select mode, you can select a run for acquisition.
Setup	From the Setup mode, you can edit the Position, Injection Volume, or Comment values for a sample.
Acquire	From the Acquire mode, you can specify acquisition settings and start the acquisition of a run.
Status	From the Status mode, you can start, stop, or pause an acquisition, monitor the run manager and instrument status, monitor the Acquisition Queue, or control the real-time display.
Manage	From the Manage mode, you can monitor the status of the runs, cancel a run and return it to the Watson application, or select a run for acquisition.

Select Mode

The Select mode is the first mode you see when you log in to the TSQ Module application. The Run Select view displays all the runs assigned to you for the current database. In the Run Select view, you can select a run for acquisition.

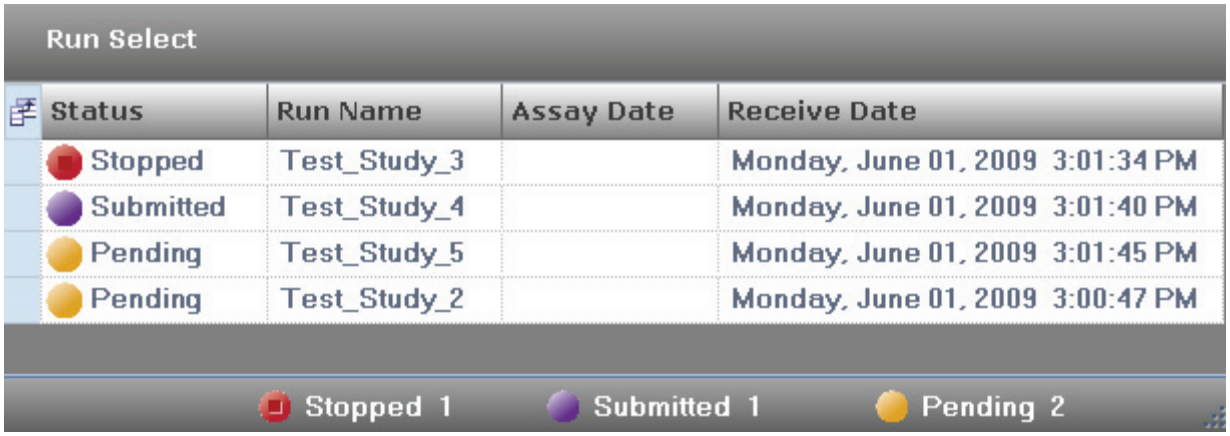


Table 13. Run Select view parameters (Sheet 1 of 2)

Parameter	Description
Column Selector 	Opens the Column Selector where you can add or remove columns from the grid display. Column choices are immediately implemented in your grid display, but the column choices are not saved when you exit the TSQ Module application.
Stopped/Submitted/ Pending	Status indicators at the bottom of the window report the number of runs for each type of status.

Table 13. Run Select view parameters (Sheet 2 of 2)

Parameter	Description
Columns	
Status	Status of each run: Pending, Submitted, Acquiring, Paused, Stopped. or Completed.
	• Pending - The run arrived from the Watson application and awaits acquisition. This run could have been submitted to the Acquisition Queue and returned to the Run Manager view before it began acquiring.
	• Submitted - The run is assigned for acquisition but is not currently acquiring. The run is in the Acquisition Queue.
	• Acquiring - The run has progressed to the top of the Acquisition Queue and is currently acquiring.
	• Paused - The run is assigned to an instrument and is currently acquiring, but has been paused and waits between samples.
	• Stopped - The run was assigned to an instrument and was acquiring, but was removed from the Acquisition Queue.
	• Completed - The acquisition has finished running and the run data was returned to the Watson application. Completed runs might not always be observed in the Run Manager view. When the TSQ Module application finishes sending the results of the last completed sample to the Watson application, it marks the run as Completed and quickly removes the run from the Run Manager view.
Run Name	A combination of the project, study, and run ID as defined in the Watson application.
Assay Date	Date when assay method was created.
Receive Date	Timestamp when run was received.

Setup Mode

From the Setup mode, you can edit the parameters of a run in the TSQ Module application. Every action you take in the Run Edit view in the Setup mode is authenticated and an audit trail is sent to the Watson application.

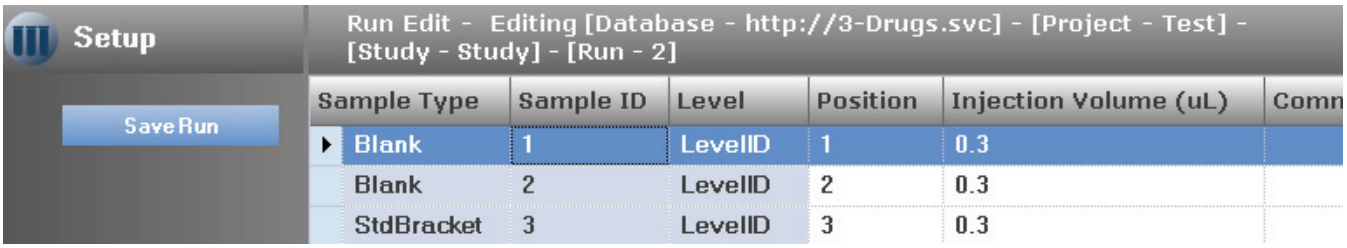


Table 14. Setup mode parameters

Parameter	Description
Save Run	Saves the edits you made to the run. The Save Run command can be specified to require an electronic signature. See "Using E-Signatures" on page 25.
Columns	
Sample Type	Defines how the Watson application processes the sample data. Each sample must be classified as one of the following sample types: • Unknown • Blank • QC (quality control) • Standard
Sample ID	An alphanumeric string of characters that can be used to uniquely identify the sample.
Level	The level defined for a calibration sample or quality control sample.
Position	Sample position number in the autosampler, usually provided by the Watson application. This column value is editable.
Injection Volume (µL)	Microliters to be injected. • When you have an autosampler configured, the injection volume is validated against the allowable range specified by the autosampler. • When you do not have an autosampler configured, you can enter a range of 0.1-1000. This column value is editable.
Comment	Optional information about the sample. This column value is editable.

Acquire Mode

From the Acquire mode, you can specify acquisition settings and start the acquisition of a run.

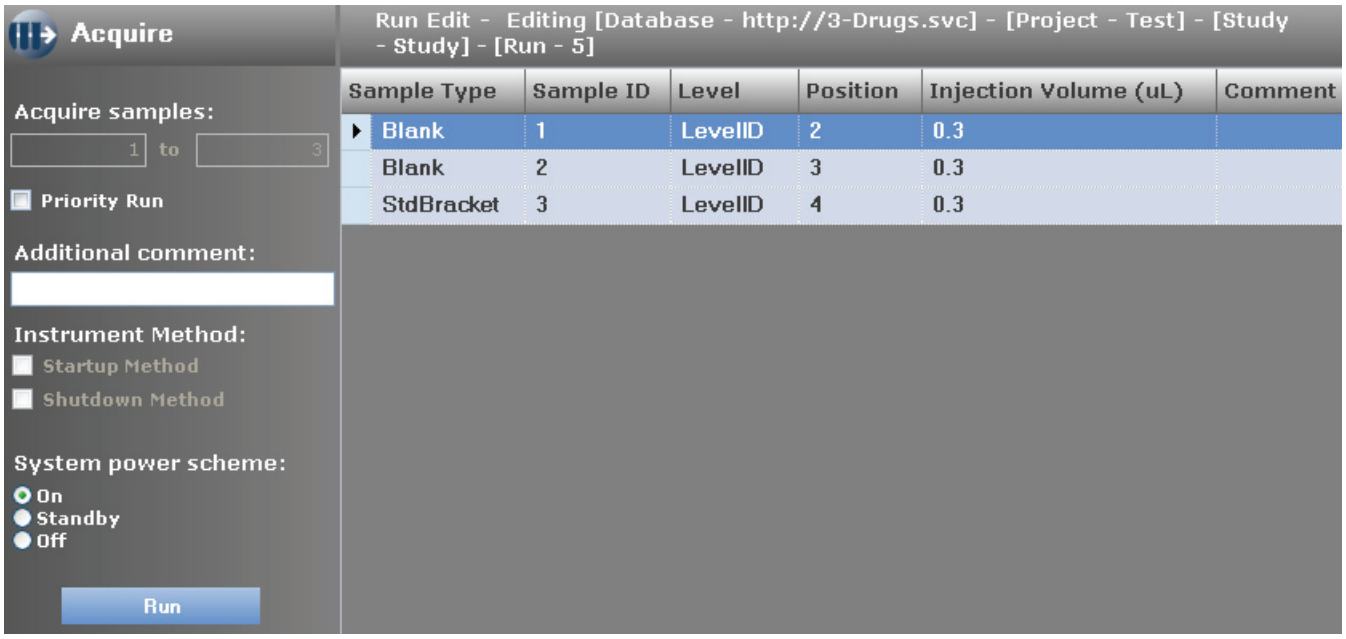


Table 15. Acquire mode parameters (Sheet 1 of 3)

Command	Description
Acquire samples	Displays the first and last samples to acquire. Available only for a previously Stopped run.
Priority Run	Inserts this run into the Acquisition Queue ahead of all submitted, non-acquiring runs.
Additional comment	A comment that is returned to the Watson application in the run report.
Instrument Method	
Startup Method	Specifies that the TSQ Module application runs the startup method specified in the run data before the run starts. No data is acquired by this method, and no autosampler injection takes place. This feature is not available for all devices.
Shutdown Method	Specifies that the TSQ Module application runs the shutdown method specified in the run data after the run has completed. No data is acquired by this method, and no autosampler injection takes place. This feature is not available for all devices.

Table 15. Acquire mode parameters (Sheet 2 of 3)

Command	Description
System power scheme	
On	Keeps the system in the On state when the current run is completed. When On is selected, you can begin another run without waiting. All power and flows are maintained at operational levels. Default: On
Standby	Keeps the system in the Standby state when the current run is completed. When you select Standby, you can begin another run with only a short delay between runs. Some devices do not have a Standby feature. For devices with this feature, a power-saving or consumable-saving mode is entered and the devices can be switched back on in approximately 15 minutes or less. Depending the instrument, this state turns gas and liquid flows to Off, but maintains heaters and other subsystems in an On state so that there is no warm-up time required when you change from Standby to On.
Off	Keeps the system in the Off state when the current run is completed. The Off state indicates that all power to the instrument, which can be controlled by the TSQ Module application, is turned Off. This includes power to all heaters and most subassemblies, but in some cases not all subassemblies. Some devices do not have an Off feature. For devices that do have this feature, a power-saving or consumable-saving mode is entered and you can manually switch the devices back on. When several runs are queued, the system power scheme of the last submitted run is used. **Caution** The Off state does not guarantee that all voltages are turned off nor does it indicate that all heated components are at room temperature. To perform maintenance on an instrument, refer to the hardware manual for your instrument.
Function buttons	
Run	Starts the acquisition for the currently selected run and opens the Status view.

Table 15. Acquire mode parameters (Sheet 3 of 3)

Command	Description
Columns (Column values are not editable in the Acquire mode.)	
Sample Type	Defines how the Watson application processes the sample data. Each sample must be classified as one of the following sample types: • Unknown • Blank • QC (quality control) • Standard
Sample ID	An alphanumeric string of characters that can be used to uniquely identify the sample.
Level	The level defined for a calibration sample or quality control sample.
Position	Sample position number in the autosampler, usually provided by the Watson application.
Injection Volume	Microliters to be injected. • When you have an autosampler configured, the injection volume is validated against the allowable range specified by the autosampler. • When you do not have an autosampler configured, you can enter a range of 0.1-1000.
Comment	Optional information about the sample.

Status Mode

From the Status mode, you can start, stop, or pause an acquisition, monitor the run manager and instrument status, monitor the Acquisition Queue, or control the real-time display.

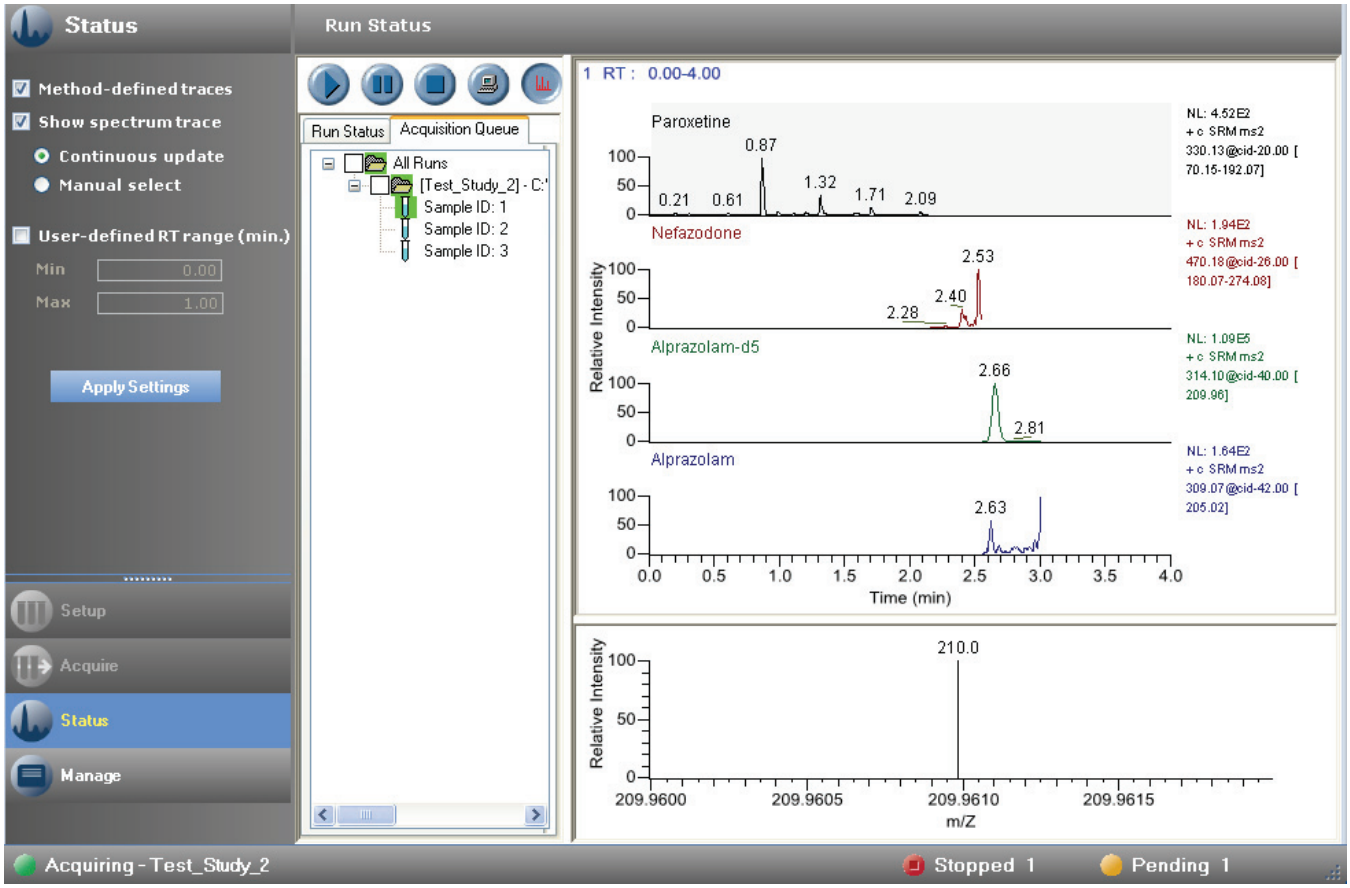


Table 16. Status mode parameters (Sheet 1 of 2)

Parameter	Description
Method-defined traces	Displays real-time chromatograms corresponding to the components defined in the processing method. When the component traces are turned off, the total ion current (TIC) trace is displayed.
Show spectrum trace	Displays the mass spectrum in the spectrum pane.
Continuous update	Continuously updates the spectrum trace to show the most current scan.
Manual select	Display the spectrum trace of the currently selected peak.
User-defined RT range	Displays chromatograms with a time range between the times specified in the Min RT and Max RT boxes.

Table 16. Status mode parameters (Sheet 2 of 2)

Parameter	Description
Min	The start time of the chromatogram traces.
	Note The Min box becomes active only when you select User-defined RT range.
Max	The end time of the chromatogram traces.
	Note The Max box becomes active only when you select User-defined RT range.
Apply Settings	Applies the display settings to the current real-time display.
	Starts an acquisition or resume an acquisition that has been paused.
	Pauses an acquisition after the current sample has been completely acquired.
	Immediately stops the current acquisition. Acquisition for the current sample stops and the sample is considered complete. The acquisition for the run is paused.
	Pauses the real-time display during an acquisition.
	Displays the Spectrum pane below the Chromatogram pane.
Stopped/Submitted /Pending	Status indicators at the bottom of the window report the number of runs for each type of status.
Shortcut menu Right-click the chromatogram to display the menu.	
Reset Scaling	Resets the X-axis and Y-axis ranges in the Chromatogram pane or the Spectrum pane to their default values.
Stopped/Submitted /Pending	Status indicators at the bottom of the window report the number of runs for each type of status.

Manage Mode

From the Manage mode, you can monitor the status of the runs, cancel a run and return it to the Watson application, or select a run for acquisition.

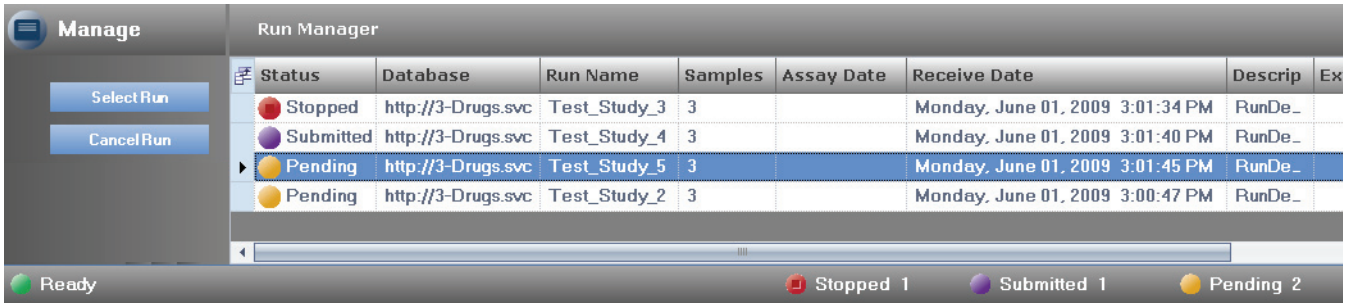


Table 17. Manage mode parameters (Sheet 1 of 2)

Command	Description
Function buttons	
Select Run	Opens the Setup view for the highlighted run.
Cancel Run	Removes the highlighted run from the list and returns the run to the Watson application.
Columns	
Status	Status of each run: Pending, Submitted, Acquiring, Paused, Stopped. or Completed.
	• Pending - The run arrived from the Watson application and is assigned to the instrument at this workstation.
	• Submitted - A TSQ Module application user has submitted this run to the Acquisition Queue but is not currently acquiring.
	• Acquiring - The run has progressed to the top of the Acquisition Queue and is currently acquiring.
	• Paused - The run has been assigned to an instrument and is currently acquiring, but has been paused and is waiting between samples.
	• Stopped - The run has been assigned to an instrument and was acquiring, but was removed from the Acquisition Queue.
	• Completed - The run has completed acquisition. Completed runs might not always be observed in the Run Manager view. When the TSQ Module application finishes sending the results of the last completed sample to the Watson application, it marks the run as Completed and quickly removes the run from the Run Manager view.

Table 17. Manage mode parameters (Sheet 2 of 2)

Command	Description
Database	The the Watson application database or the friendly name assigned in the Environments.xml file.
Run Name	A combination of the project, study, and run ID as defined in the Watson application.
Samples	Number of samples in the run.
Receive Date	Timestamp when run was received.
Assay Date	Date when assay method was created.
Description	Watson user comments.
Extraction Date	Date when samples in the run were extracted.
Analyst	The user who created the run in the Watson application.
Observations	The Watson application user comments.

Restarting Runs

This appendix describes the process of restarting the acquisition of a run after it has been stopped.

Contents

For several reasons, a user might remove a run from the TSQ Module application before it completes acquisition and return it to the Run Manager view or to the Watson application. A run can be restarted within the TSQ Module application or returned to the Watson application.

From the Watson application, you can modify a run that has been returned from the TSQ Module application and send the run back for re-injection. When a run is returned for re-injection from the Watson application, the TSQ Module application treats it like any other run.

Restarting a Run from within the TSQ Module Application

For many reasons, you might want to remove a run from the Acquisition Queue and later restart the run from the Run Manager view.

This section describes the following:

- Restarting a Stopped Run at the Beginning
- Restarting a Stopped Run at a Specific Sample
- Restarting a Run from the Run Manager View: Workflow

Restarting a Stopped Run at the Beginning

For many reasons, you might want to stop a submitted or acquiring run:

- To correct a minor problem with the instrument
- To fix a leak, replace a worn-out column, or correct any other minor maintenance task

You can send the run from the Acquisition Queue back to the Run Manager view, perform some offline injections, and resubmit the run to the Acquisition Queue.

Example scenario

1. You initially pause the run, and then decide that the problem is more serious than anticipated.
2. You remove the run, sending it back to the Run Manager view.
3. After correcting the problem, you might perform system suitability runs in the Xcalibur or LCquan applications to confirm that the system is working.
4. Finally, you resubmit the run for acquisition.

For detailed information about pausing and stopping a run, see "Pausing/Stopping/Canceling Runs" on page 55.

For detailed information about resubmitting a run, see "Acquiring a Run" on page 45.

Restarting a Stopped Run at a Specific Sample

For many reasons, you might want to stop a submitted or acquiring run:

- You see a flat line on the real-time plot where you expect to see a signal and realize that recent acquisitions in the run are not useful.
- You see a physical problem with the instrument and want to fix it and then restart the run.

You can send the run from the Acquisition Queue back to the Run Manager view and resubmit it to the Acquisition Queue. When you return the run to the Acquisition Queue, you can start the acquisition from any row to reacquire samples whose acquisitions were flawed.

Example scenario

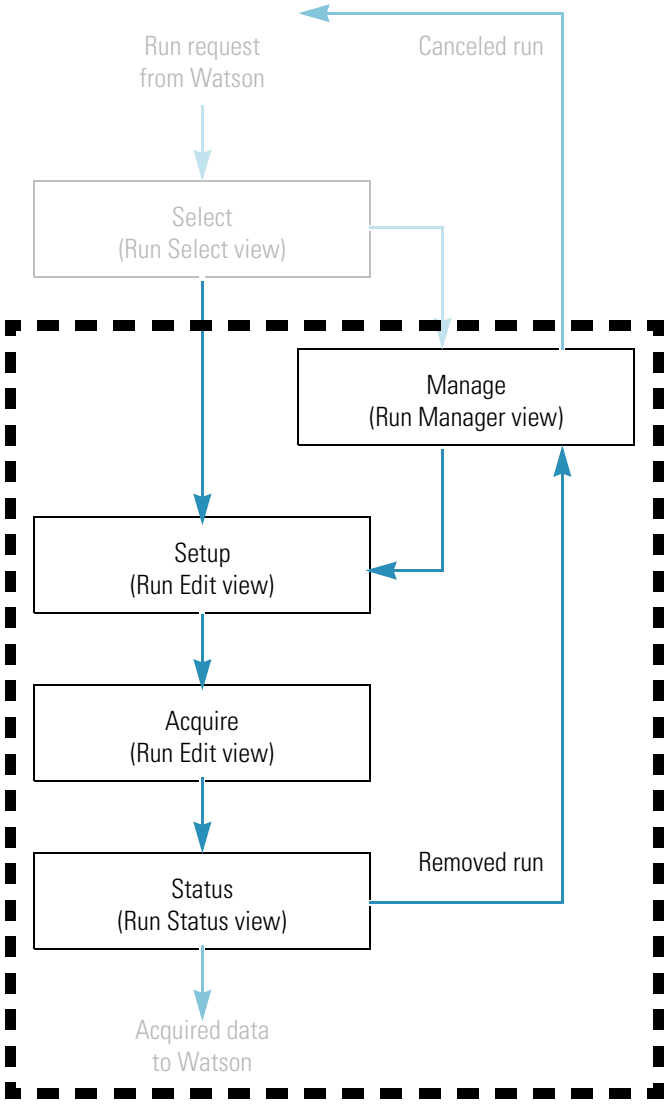
1. You initially pause the run, and then decide that the problem is more serious than anticipated.
2. You remove the run, sending it back to the Run Manager view.

3. After correcting the problem, you perform system suitability runs in the Xcalibur application.
4. Finally, you resubmit the run for acquisition, starting from a previous sample to ensure that all bad samples are restarted.

For detailed information about pausing and stopping a run, see "Pausing/Stopping/Canceling Runs" on page 55.

For detailed information about submitting a run, see "Acquiring a Run" on page 45.

Restarting a Run from the Run Manager View: Workflow



Returning a Run to the Watson Application

For many reasons, you might want to stop a submitted or acquiring run and send it back to the Watson application:

- You want to rebalance the workload to another instrument, so you want to send all pending runs back to the Watson application.
- The local instrument requires maintenance and you want to move all runs to another instrument.
- The local instrument has the wrong column or other mismatch (other than the device stack) that was discovered after the run was submitted, so you want to send all pending and acquiring runs back to the Watson application.

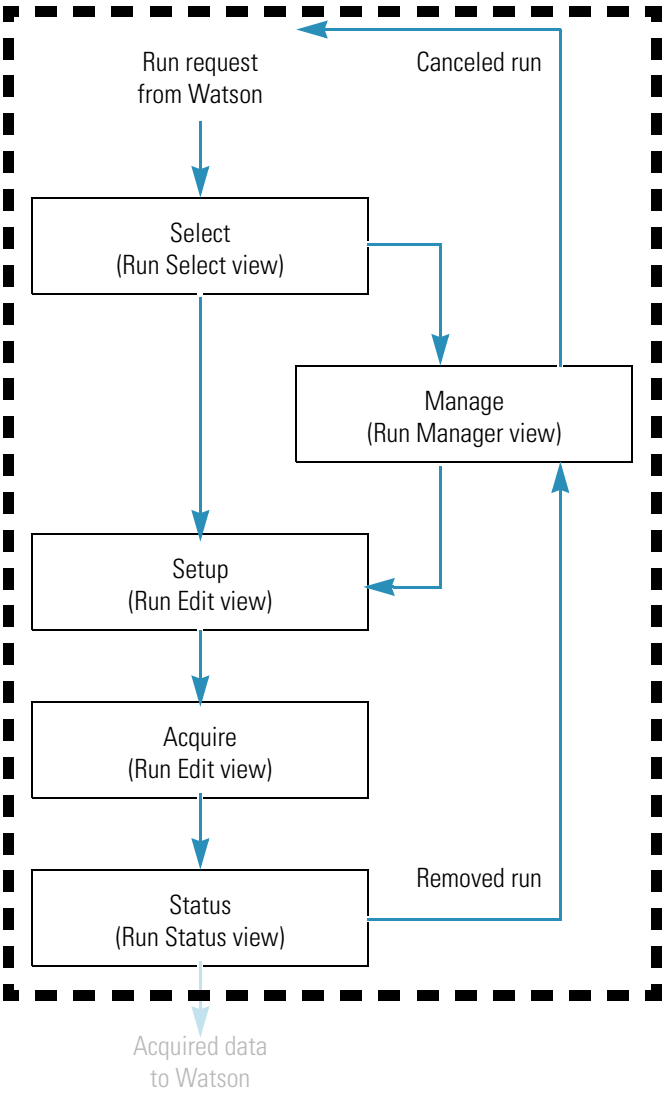
You can send the run from the Acquisition Queue back to the Run Manager view and then back to the Watson application. A Watson user can then reassign the run to a different instrument and resubmit it to another the TSQ Module application.

Example scenario

1. You initially pause the run, and then decide that the problem is more serious than anticipated.
2. You remove the run, sending it back to the Run Manager view.
3. You discover that the problem with the instrument is serious enough that the run cannot be restarted.
4. Finally, you cancel the run in the Run Manager view, sending the run back to the Watson application.

For detailed information about pausing, stopping, and canceling a run, see "Pausing/Stopping/Canceling Runs" on page 55.

Returning a Run to the Watson Application: Workflow



Re-injecting a Run from the Watson Application

In the Watson application, you can select one or more samples within the same run to re-inject. The ability to do this is limited to a specified time frame. Samples that are selected to be re-injected are submitted to the TSQ Module application, where they are processed and submitted back to the Watson application to be reviewed and accepted as part of the run review. After a run is accepted, samples can no longer be re-injected.

Selecting Samples to Re-inject

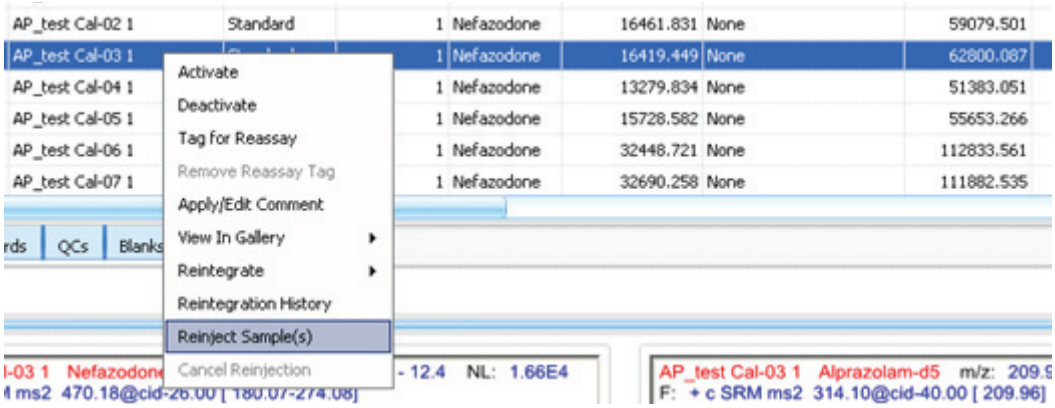
The Watson application and the TSQ Module application process re-injected samples in groups. When selecting samples to re-inject, you can select some samples but not others until the first set is in the queue.

❖ To select samples to re-inject

1. Access the run queue.
2. Choose **Analytical > Analytical Run Actions**.
3. Select a completed run and click **Review**.

   The Run Review form opens.
4. Click a sample to select it.

Figure 29. Right-mouse menu



5. Right-click and choose **Reinject Sample** from the menu.

   The sample is marked as Pending in the Injection column. This step can be repeated for additional samples.
6. Click **Save** to submit the sample to the TSQ Module application for processing.
7. Click **Exit** to close the form.

   The Run Status in the Run Review view is Submitted to TSQ Module.

Canceling Re-injected Acquisitions

❖ To cancel a re-injected acquisition

1. Click a sample to select it.
2. Right-click and choose **Cancel Re-injection** from the menu.

   This prevents the sample from being transmitted to the TSQ Module application.

❖ To review and accept re-acquired chromatograms

The Watson application enforces the acceptance of QCs, Unknowns, or STDs, or prevents run review prior to completion, as was specified in the Configuration form.

1. Access the Run Review form.
2. Click on the tabs to view All samples, Standards, Blanks, QCs or Unknowns.
3. Click **Accept Review** to accept.

   You receive the following message:

   ```
   This will save any changes made and accept all chromatograms for this
   run. It will also prevent any future reintegrations and re-injections.
   ```

4. Click **OK**.

Accepting the Analyte Results from the Standard Regression Form

Analyte results can be accepted from the Standard Regression form.

❖ To accept analyte results from the Standard Regression form

1. Access the Standard Regression form by selecting **Analytical > Standard Regression**.

   The Standard Regression form opens.

2. Click the run to select it.
3. Click **Regression**.

   If the Run Review has not yet been marked as accepted, an alert appears.

4. Click **OK**.

   The Standard Regression form opens with the Standard and QC tab displaying.

5. To accept or reject the results of the analyte, select an analyte and then click **Accept** or **Reject**.
6. Click **Cancel** to close the form.

Quantitative Analysis Overview

This appendix describes some of the basic principles and terminology of quantitative analysis[1] and contains the following topics:

Contents

About Quantitative Analysis

In some applications, such as a clinical trial, you might be seeking the maximum possible accuracy from your measurements. Time and cost of analysis are less important than achieving the highest possible standards in precision and accuracy. This process of measuring the amount of a particular component in a sample is called quantitative analysis.

In other applications, such as in trace analysis, you might only want to *estimate* the quantity of a component. It might be sufficient to know that the component is present at a level either significantly higher or significantly lower than a defined threshold. For example, knowing whether a patient has overdosed 15 or 20 times above a prescribed limit is generally not as important as simply knowing that the limit has been exceeded. Such cases would require a rapid measurement rather than a precise one. This form of measurement is generally called semi-quantitative analysis.

Quantitative analysis consists of the following steps:

- Preparing samples
- Developing a suitable chromatographic method
- Calibrating the mass spectrometer's response

[1] For further information about the principles of quantitative analysis, refer to *Mass Spectrometry: Principles and Applications*; de Hoffman, E., Charette, J., Stroobant, V.; Wiley: New York, 1996; and *Introduction to Mass Spectrometry*, 3rd ed.; Watson, J.T., Lippincott-Raven: Philadelphia, PA, 1997.

- Analyzing the samples
- Reviewing the results

This documentation does not describe sample preparation and chromatographic method development. This documentation assumes that you have met these important prerequisites to achieving high quality quantitative analysis. For guidance in these areas, refer to the documentation for your autosampler, LC pump or MS pump, and mass spectrometer.

Considering the Variables of Quantitative Analysis by LC/MS/MS

The combination of liquid chromatography (LC) and mass spectroscopy (MS) sets up a unique set of considerations. If you are familiar with quantitative analysis using one or the other of these analytical tools, the information in the following topics will prove useful:

- Using LC for Analyte Separation
- Using MS/MS for Analyte Detection

Using LC for Analyte Separation

When working with LC systems, you become accustomed to developing methods for analytes of interest. These methods take into account a variety of parameters. Consider the following LC parameters for optimum separation of compounds:

- Composition of stationary phase
- Column diameter
- Column length
- Column usability over time
- Solvent(s) or composition of mobile phase
- Flow rates
- Isocratic or gradient (ramped) solvent compositions

Often the only measurable result of optical detectors that you can use to identify an analyte is the retention time at a specified wavelength. To quantitate compounds accurately, the compound peaks must be distinctly separated from each other in time. By carefully optimizing the LC variables listed above, you can successfully separate and detect compounds with an optical detector such as an ultraviolet (UV) or photodiode array (PDA) detector.

In contrast to optical detectors, mass spectrometers can often successfully detect individual analytes even when two or more compounds coelute as a single chromatographic peak. Coeluting compounds are ionized in the mass spectrometer, and the parent or fragment ions specific to the analyte or analytes are detected and measured for quantitative analysis. Because the mass spectrometer detection is so specific, you can reduce the chromatographic resolution and shorten the run times.

Using MS/MS for Analyte Detection

To optimize detection of ions by MS/MS, consider the following variables when working with a mass spectrometer interfaced to an LC:

- Solution chemistry and polarity of the analyte of interest
- Probe selection (H-ESI, ESI, APPI, or APCI)
- Collision energy to fragment parent ions inside the mass spectrometer (optimized by experiment)

The LC provides a stream of analyte solution (*eluate*) into the mass spectrometer where the analyte is detected. You must consider the content of modifiers, salts, and contaminants in the eluate. Specifically, you must ensure that what goes into the inlet of the mass spectrometer does not suppress ionization of the compound of interest. Salt concentrations above 10 mM and strong acids and bases damage the LC column. Modifier concentrations greater than 10 mM are not usually necessary for chromatographic stability and can suppress ionization of other compounds. For best results, whenever possible, use volatile modifiers.

Volatile modifiers include the following:

- Acetic acid
- Ammonium acetate
- Ammonium formate
- Ammonium hydroxide
- Formic acid
- Trifluoroacetic acid

Your analyte solution can contain neutral particles or ions. If the solution contains ions, the ions can carry a single charge or multiple charges. The number of charges depends on the structure of the analyte and the composition and pH of the mobile phase. Solvent systems are generally composed of organic solvents, water, and volatile modifiers. The ionization characteristics of your analyte influence your decisions about the optimum pH of your solvent system and the type of probe to use at the interface of the LC and the mass spectrometer.

With the TSQ Quantum series, LTQ series, or LCQ series instruments, you can use either the H-ESI probe, the ESI probe, the APCI probe, or the APCI/APPI probe at the interface of the LC. The choice of probe depends on the class of compound that you want to analyze and, to a small extent, on the flow rate of your experiment. In general, the APCI and the APCI/APPI probes accommodate higher flow rates and molecules of a less polar nature than does the ESI probe. Refer to the introduction in the documentation for your mass spectrometer for what factors might influence your decision about flow rates and what probe to use for optimum transfer of ions into the mass spectrometer.

After the ions are inside the mass spectrometer and the parent ions are isolated, collision energy is applied to dissociate the parent ions into product ions (MS/MS). The relative collision energy for a particular analysis depends on the structure of the compound that you are analyzing. For this reason, you optimize the collision energy on your analyte of interest in a tuning experiment. Refer to the documentation for your mass spectrometer for the tuning procedure that describes how to optimize the collision energy.

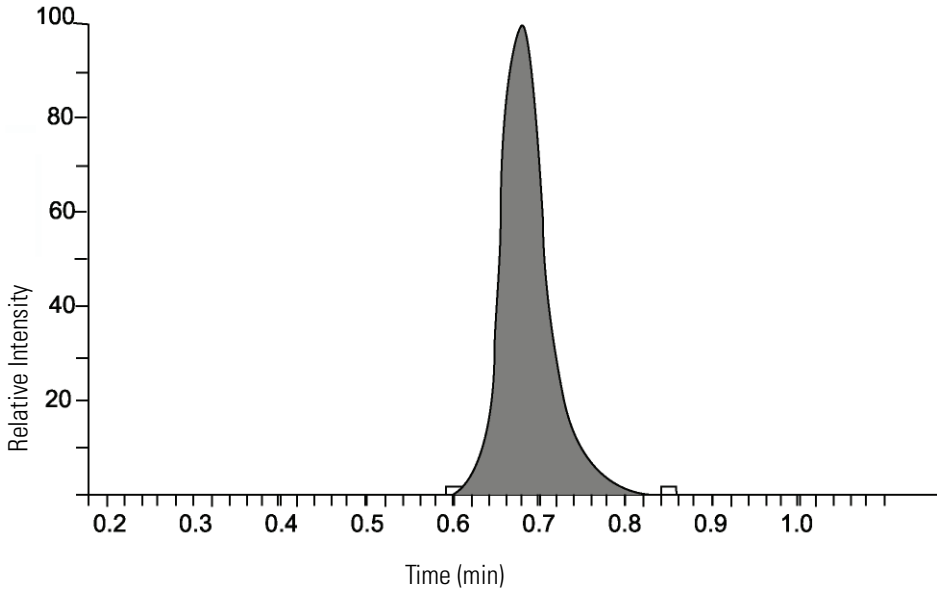
Quantitative Analysis Techniques

To carry out quantitative analysis, you must evaluate the instrument's response to known amounts of the target component. Response is based on either the height of the chromatogram peak or, more commonly, the area under the peak's profile. In both cases, you must take into account the baseline of the detected peak.

Instrument response is generally measured with several samples commonly called standards, or calibration standards. These standards must cover a suitably wide range of concentrations or amounts and must bracket the range of expected concentrations in the unknown. Responses to these standards are plotted in a graph called a calibration curve. Ideally, this curve corresponds to the equation of a straight line to ensure the highest degree of precision.[2]

Fitting an equation to the calibration curve with a user-specified method (for example, a least squares regression) provides a response factor—a comparative measure of the response of the mass spectrometer to a component. It is based on the amount of sample injected and the resulting peak area or peak height. The response factor gives an intuitive and quantitative measure of how responsive or sensitive the mass spectrometer is to a certain component.

Figure 30. Integrated Chromatographic Peak



To perform quantitative analysis of samples containing unknown amounts of the target component, calculate the peak area or height and compute and apply the appropriate response to the equation derived from the calibration curve. These steps provide an estimate of the amount of the target component in the samples. The precision of the measurement depends on the quality and, to a lesser extent, the quantity of the calibration data.

[2] *Mass Spectrometry: Principles and Applications*; de Hoffman, E., Charette, J., Stroobant, V.; Wiley: New York, 1996; p 162.

The detection limit of the quantitative analysis method is the lowest concentration of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The lower and upper quantification limits are the lowest and highest concentrations of analytes in a sample that can be measured with an acceptable level of accuracy and precision. In an analytical method, the highest concentration calibration standard defines the upper quantification limit.

The quantitative analysis technique you use determines the manner in which the response is calculated, both for generating the calibration curves and for subsequent quantitative analysis. The two basic methods are described in these topics:

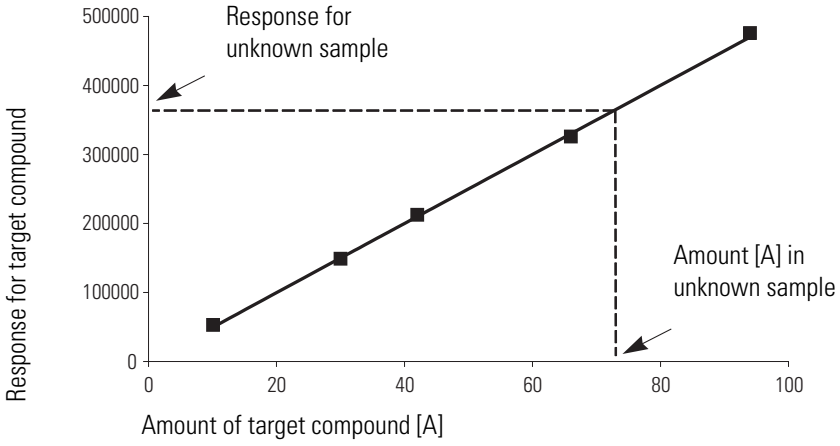
- Using External Standards for Quantitative Analysis
- Using Internal Standards for Quantitative Analysis

Using External Standards for Quantitative Analysis

An external standard is a separate sample that contains the compound of interest at a known concentration in solution. In the quantitative analysis that uses external standards, a series of standards are analyzed and a calibration curve is constructed by plotting the magnitude of the detector response as a function of the external standard concentration. You analyze the unknown sample and determine the concentration by matching the magnitude of the detector response with the magnitude on the calibration curve.

Using external standards is advantageous when you are analyzing various components in a sample and you can assay all compounds of interest by using a single set of external standards. Although this approach offers time- and cost-effective quantitative analysis, it cannot achieve the very highest precision and accuracy. Variations in analyte and solution stability, injection reproducibility, and matrix interference lead to lower precision levels in the external standard method than in the internal standard method.

Figure 31. Calibration Curve Generated by Using an External Standard



Using Internal Standards for Quantitative Analysis

An internal standard (ISTD*)* is a component that is added to a sample to act as a response reference for one or more non-ISTD components in the sample. The concentration or amount of an ISTD in any standard or unknown sample remains constant.

Because quantitative mass spectrometric analysis usually involves multiple steps, the total error in the analysis results from the accumulation of the errors at each step. In general, sample-handling errors account for a larger fraction of the total error than do mass-spectrometer errors. Fortunately, the internal standard method can reduce both sources of error. For example, internal standards can correct for variations in a component's peak area that are caused by the following:

- Injection irreproducibility
- Changes in analyte solution volume
- Matrix and coeluter interference (both suppression and enhancement)
- System instability
- Variations in the source conditions

For maximum precision, add the ISTD component as early as possible to the start of the sample work-up, particularly in those quantitative methods that require sample manipulations such as extraction, cleanup, and dilution. Because the ISTD and non-ISTD components are analyzed together, the internal standard quantitative analysis approach has the advantage that it corrects for injection and other sample handling errors. The ISTD must behave chemically in an identical or similar manner to the target compound through the extraction, cleanup, and analytical processes.

You can also add the ISTD component as the last step of sample preparation prior to the sample's use to compensate for fluctuations in the reproducibility of the sample injection.

Use ISTDs in a quantitative analysis experiment as follows:

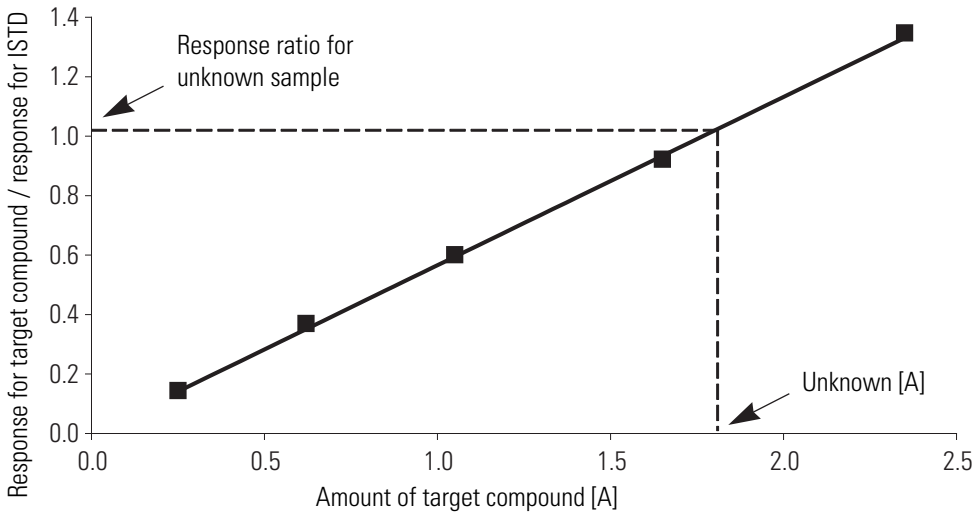
1. Analyze a series of standard solutions containing known concentrations of the target compound and ISTD, and plot the ratio of the target compound and the ISTD detector responses as a function of the corresponding ratio for the two quantities present in the solution.
2. Add a fixed amount of the ISTD to each sample prior to any manipulation, and, after preparing and analyzing the samples, obtain the quantity of the target compound present in an unknown sample from the calibration curve.

Ideally, an ISTD is closely related to the target component in terms of its physical and chemical properties. It must be pure, not present in the sample, and inert toward the components of the sample. ISTD components are typically analogs, homologs, or isomers of the target non-ISTD component. An ideal ISTD is a structural or isotopically labeled analog

of one of the target components. Stable isotope-labeled ISTDs act almost identically to the analyte throughout sample manipulation and with regard to ionization tendencies and fragmentation. Internal standards labeled with two or more deuterium (D) atoms are frequently used for LC/MS.

There can be any number of ISTD components in a sample, but each non-ISTD component can be calibrated against only one ISTD.

Figure 32. Calibration Curve Generated by Using an Internal Standard



Sample Types

Each quantitative analysis consists of a number, or *sequence*, of samples. The sequence represents the order of sample analysis. A quantification sequence contains:

- One or more *standards*
- One or more *unknown* samples

For more demanding applications, you can also use optional quality control (QC) samples and blank samples.

Standards

A calibration standard is a sample containing known amounts of all target components. The purpose of a standard is to measure the response of the instrument to the target components so that the processing software can generate a calibration curve for each component.

Unknowns

An unknown sample is one containing unknown amounts of the target components. The processing software performs quantitative analysis on any sample defined as an unknown sample.

QCs

A QC sample contains a known amount of one or more specific target compounds. The processing software places QC samples in the sequence so that it can test quantitative analysis results for quality assurance purposes. After the QC sample is analyzed, The processing software compares the measured quantity with the expected value and an acceptability range. The quantitative analysis of a QC sample is classified as *passed* if the difference between the observed and expected quantities is within the user-defined tolerance. A QC sample is classified as *failed* if the difference between the observed and expected quantities is outside the defined tolerance.

Blanks

A blank sample contains no target components but might contain an ISTD when you use the internal standard quantitative analysis technique. By analyzing a blank sample, you can confirm that there are no residual components in the solvent system that can cause erroneous results.

Glossary

A

absolute scaling Displays a chromatogram trace or spectrum so that the height of the Y-axis scale is equal to the height of the largest peak in the data file. The height of the vertical scale is fixed for the data file even if you change the range of the display.

acquisition sequence A defined set of acquisition settings for a single sample or list of samples. The acquisition sequence includes information on how to acquire a sample, and whether and how to automatically process the sample.

adjusted retention time The retention time (RT) of a component, adjusted (or normalized) using a retention reference component.

See also:
retention reference
retention time (RT)

AICA Kaike's information criterion.

ALQ Result is above the limit of quantitation. A user defines the limit.

ambiguous roots (A.R.) If (B2-4*A*(C-Response)) > 0, there are two roots for the equation. The Watson application decides which one is suitable. If both roots seem good, the Watson application reports A.R. and lets the user decide the root.

amino acid An organic compound that contains both an amino group (-NH2) and a carboxyl group (-COOH). Twenty specific amino acids serve as the building blocks of peptides and proteins. These are the alpha amino acids that have the amino and carboxyl groups attached to a single carbon atom (the alpha carbon).

amino acid modification A change in the mass of an amino acid used for a calculation.

amino acid residue In biochemistry, a residue is the portion of a molecule that remains after it has lost some of its components. An amino acid residue loses a water molecule when it is joined to another amino acid.

amplifier temperature The temperature measured by the sensor on the RF amplifier.

analyte A substance or chemical constituent that is undergoing analysis, for example, a parent drug.

Analytical Data Interchange (ANDI) protocol A file format that allows the conversion and transfer of data between mass spectral data systems. A file conversion protocol supported by the Analytical Instrument Association

analyte The compound to be measured.

analytical run A sequence of study and assay samples meant to be analyzed using an analytical instrument.

analytical run assay Specifies the assay definition for each analytical run. It defines instrument interfacing types and styles, calibration curve and QC samples options, and other assay-related parameters. The analytical run assay is created automatically from the study assay whenever a new run is created.

ANOVA Analysis of variance.

apex RT The retention time at which the apex of a chromatographic peak is located.

Area or Height Ratio The ration of the area or height of the selected peak to the area or height of the internal standard peak.

area scan window The number of scans on each side of the peak apex that you want to include in the calculation of peak area. The value 0 specifies that all scans from peak start to peak end are included in the area integration.

area tail extension The number of scans beyond the peak endpoint on each side of the peak. ICIS uses this number to calculate an average for the intensity counts of a peak area. ICIS then uses this average intensity value as the lower limit of intensity and includes the area above this limit in the integration calculation for the peak.

Area Threshold Controls the area cutoff automatically calculated by Avalon. Any peaks with a final area less than the Area Threshold are not detected.

area, chromatographic peak The region of a chromatogram peak that is bounded by the time baseline and the relative abundance trace. Chromatogram peak areas are reported in units of count-seconds where the relative abundance is measured in counts, and the time baseline is measured in seconds.

A.R (assay method) A plan for determining desired test values from a subject sample. The plan includes definition of regression approach (method, Std/QC concentrations, replicates, weightings, analytes, internal standards), instrument to be used, concentration units for reporting, precision of results for reporting, and so on.

assay sample An analytical run definition. A sample used in an analytical run that is used to determine a representative value from a study sample.

ASCII American Standard Code for Information Interchange.

AUC Area under the curve.

audit log Provides a chronological listing of all auditable software application events that the user initiated.

auto-Dta An auxiliary application that produces .dta files.

automatic processing The post-acquisition, off-line, batch (non-interactive) processing of data collected from the instrument. Automatic processing involves the use of all the methods specified in the Sequence for the sample(s) to be processed.

autosampler The device used to inject samples automatically into the inlet of a chromatograph.

autozero time The time at which the UV detector performs an automatic zero of the baseline.

average molecular weight The calculated mass of a molecule based on the average atomic weight of each element. Also known as the formula weight, for example, C = 12.01115, H = 1.00797, and O = 15.9994.
See also:
molecular weight

average response factor (RF) A calibration type where the response factor is calculated for replicates at all calibration levels and then averaged. The amount in a sample can then be calculated by dividing the response by the average response factor.

average response factor (RF) calibration curve A calibration curve where the slope of the calibration curve is constructed from the average response factor (RF) of all levels. This calibration curve always passes through the origin.

See also:
average response factor (RF)
calibration curve

average response factor (RF) curve fit type A calibration curve fit type where LCquan calculates a curve that shows its slope is constructed from the average response factor of all levels.

See also:
average response factor (RF) calibration curve
calibration curve

average spectrum A spectrum that is the sum of all of the microscans taken for a particular MS^n experiment. An average spectrum can be independently displayed for MS/MS, MS3, MS4, MS5, …MS10 experiments. The average spectrum is displayed normalized (NL) to the average base peak.

averaged scans The number of mass spectrometer scans that are averaged to display a spectrum from the data in a raw file.

B

base file name A name that Xcalibur™ or LCquan uses when it creates the raw file for a sequence. Xcalibur or LCquan appends a two-digit number (01–99) or a three-digit number (100–999) to the base file name so that each sample in a sequence has a unique identification.

See also:
sequence

base peak The most intense mass peak in the mass spectrum. It is used as the base against which the intensities of all other peaks are normalized.

base peak chromatogram For this type of chromatogram, the intensity of the base peak from each mass spectrum is plotted as a function of time to form a chromatogram.

base sample ID An alphanumeric prefix to the sample ID that Xcalibur or LCquan applies to each sample in a new sequence. Xcalibur or LCquan adds a suffix to the base sample ID starting with 001.

See also:
sample ID
sequence

baseline The constant signal produced by the background level of an instrument. In quantitative analysis, the base line represents the reference point for integration when measuring the area of any given peak.

baseline clipping An integrated target peak having its integration line originating or terminating in a baseline region of no noise. A peak is considered to be baseline-clipped if there is no signal (that is, it is of zero intensity) on both sides of the peak within the time range.

baseline noise Any signal present on the base line represents the noise of the chromatographic system.

baseline noise rejection factor This factor is applied to the baseline peak-to-peak noise band during the peak detection process.

See also:
baseline noise

baseline type The baseline approximation method used for LC chromatogram peak integration (Xcalibur and LCquan).

baseline window The number of scans for searching local minima.

baud rate A measure of the number of events, or signal changes, that occur in 1 second.

blank sample type A sample type that contains no target components. Internal standard components can be added to calibration blanks.

iBLQ Result is below the limit of quantitation. A user defines the limit.

BMV FDA Bioanalytical method validation guideline, 2001.

bracket A sequence of samples where calibration samples are processed before and after Unknown and/or QC Samples. All Calibration Samples from the same bracket are used for the quantitation of the unknown and QC samples in the bracket.

bracket [none] A bracket type that can combine the calibration standards from the current sample set with previous calibration information, stored in a calibration file.

bracket [non-overlapped] A bracket type where the unknown samples are broken into groups. Each group is quantified against separated sets of calibration standard samples. All standards are run before and after each group, and standards are not shared between groups.

bracket [open] A bracket type showing that all unknown samples in the sequence are quantified against all standard samples of the sequence, regardless of the ordering of the sequence.

bracket [overlapped] A bracket type showing the unknown samples broken into groups, which are each quantified against separate sets of calibration standard samples. All standards are run before and after each group, and standard samples between the groups are used to form the calibration curve for both groups.

bracket calibration A calibration that runs standards before and after unknown samples are run. Both sets of calibration standard data results are included in the calibration curve.

building a database The action of creating a subset database from a larger database and either creating it separately or appending it to an existing database.

built-in Interface Interface software that is written into the Watson application code.

Bunch Factor The Bunch Factor is the number of points grouped together during peak detection. It controls the bunching of chromatographic points during integration and does not affect the final area calculation of the peak.

C

CaCO2 Refers to a the Watson application assay type. Caco-2 is also a technique used to measure the permeability of test compounds through a membrane or barrier.

cal level The alphanumeric name assigned to the calibration level when it is created in the Component Calibration view of the Processing Setup window. An expected amount is entered for each calibration level.

See also:
calibration level
expected amount

calculated amount The amount present in the sample, as determined using the calibration curve and the response ratio.

See also:
response ratio

calibration The process of adjusting a measurement system to deliver results consistent with a known reference.

calibration compound [mass calibration] A compound whose mass spectrum is used to tune and calibrate a mass spectrometer. A calibration compound generally has a well-characterized mass spectrum.

calibration curve A calibration of sample concentration with a series of chemical standards at known concentration used as a reference point. The calibration curve is given with area or height response on the Y-axis and concentration on the X-axis.

See also:
calibration
internal standard (ISTD) calibration
weighting (w)

calibration level A user-specified name for all of the target compounds in a given calibration step. The individual amounts need not be the same for all components.

calibration mode The procedure LCquan uses for calibration.

See also:
update calibration mode

calibration parameters Instrument parameters whose values do not vary with the type of experiment. The instrument parameters that are stored in the calibration file.

See also:
tune parameters

calibration replicates Multiple injections of the calibration mixture at the same calibration level or amount.

See also:
calibration level

calibration sample type One of several types that are used for quantification. The specific types available depend on the bracket type used. Examples include standard bracket, standard update, standard clear, Blank, Unknown and so on.

calibration, mass spectrometer A compound that has a well-known mass spectrum used as a reference.

See also:
calibration

cassette dosing experiment A high-throughput screening procedure sometimes used in drug discovery to speed up assessment of pharmokinetic properties of compounds. Multiple compounds are grouped together and given to an animal. Samples are drawn and then the multiple compounds in each sample are assayed simultaneously.

See also:
drug set

centroid data Data used to represent mass spectral peaks in terms of two parameters: the centroid (the weighted center of mass) and the intensity. The data is displayed as a bar graph. The normalized area of the peak provides the mass intensity data.

See also:
centroiding

centroiding A method used to improve mass spectral data quality, get better mass assignments, and reduce data file size. Profile data is converted into centroid data by a data compression algorithm.

See also:
centroid data
profile data

CFR Code of Federal Regulations.

Check calibration A procedure that checks the MS detector calibration automatically.

checksum A calculated value that is used to test data integrity. It is a value calculated for a given chunk of data by sequentially combining all the bytes of data with a series of arithmetic or logical operations.

chemical noise Signal resulting from the presence of chemical species other than the analyte.

chromatogram The graphical representation of a chemical separation, such as liquid chromatography, obtained from an analytical instrument called a chromatograph. The result of plotting detector response versus time.

chromatographic column A chromatographic separation component. A tubular column either filled with a finely divided solid impregnated with a partitioning liquid, or an open tubular column with a liquid film deposited on the walls.

chromatography An analytical method that is used for the separation, identification, and determination of chemical components in a complex mixture. A sample is dissolved in a mobile phase (a gas or liquid) and then forced through an immiscible stationary phase.

chromatography filter A noise reduction algorithm that smooths peak profiles at low sample concentrations in chromatographic data. These peak profiles integrate more consistently than those acquired under normal data acquisition conditions.

See also:
centroid data
profile data

comma-separated values text file A comma-delimited text file. The extension of a comma-separated values text file is .csv. This file format can be read by a text editor program, such as Microsoft Notepad™, or by a spreadsheet program, such as Microsoft Excel™.

complete digest Digest type where every possible digest site is digested. For example, if a complete digest is done with trypsin, every occurrence of Lysine (K) and Arginine (R) will be digested.

component An identified compound in a mixture.

See also:
target compound

component amount, calculated The amount of a component in a solution, as determined by quantitation using a calibration curve.

See also:
calibration curve
calibration level
component amount, measured

component amount, measured The measured amount of a component added to a calibration solution. The amount measured defines a calibration level for quantitation purposes.

See also:
calibration level
component amount, calculated

component detection The process of identifying the chromatographic components in MS/MS or MS^n sample data by extracting the mass spectral signals.

component name The user-defined name of the component to be analyzed.

composite spectrum A spectrum that is the sum of all MS/MS, MS^3, MS^4, MS^5,…MS^{10} spectra, as defined by your Instrument Method. This is not Normalized Spectrum.

compressibility The compressibility of a substance is a measure of the reduction in its volume at a specified applied pressure.

compressibility ratio A substance is given as a ratio of its original volume to its new volume after applying a specified pressure.

See also:
compressibility

concentration Refers to an analytical measurement reported as mass per volume or mass per mass. Examples are µg/mL, µM, or µg/g. Certain the Watson application interfaces allow importation of concentration values from the external software. This occurs when a calibration curve has been regressed in the external software yielding back-calculated concentration values.

conditioning The process used to remove surface contaminants from a chromatographic column.

consecutive reaction monitoring (CRM) scan type A scan type with three or more stages of mass analysis and where a particular multi-step reaction path is monitored.

constrain peak width A chromatogram peak-edge detection method used to integrate a peak that displays tailing. Xcalibur constrains the width of a component during peak integration using the specified peak height percentage and tailing factor parameters. A set of peak integration settings that constrains the peak width of an asymmetric chromatogram peak by excluding all or part of the peak.

See also:
peak height
tailing factor

control file A raw file that can be compared to an experimental raw file during the following:

- Background subtraction to correct for the background signal caused by chemical noise or matrix effects.
- Component detection to identify which components are unique to the experimental raw sample file.

control matrix A matrix that has the same composition as the sample (for example, body fluid) but does not have the components that are being analyzed. A control matrix is used to filter out extraneous data (create a background-subtracted .raw file) and thereby simplify the analysis.

convex gradient curve A gradient curve that begins with a large rate of change and ends with a small rate of change.

See also:
gradient curve

corrected relative retention time The time after injection that it takes for a retained compound to elute minus the time required for an unretained compound to elute (void time).

See also:
retention time (RT)

CR A shortened form of the phrase creep Y-axis normalization mode. This shortened form is used to save space in the header when the creep mode is in use. For example, CR: 1.98e+003 (1.98 x 103).

CRO Contract Research Organization.

cross-correlation factor MS/MS cross-correlation factors indicate a level of similarity between the reference MS/MS spectrum from a user-selected spectrum and the remaining MS/MS scans within the data file.

cryo cooling The use of CO2 (-70 °C) or N2 (-90 °C) to achieve low temperatures.

cSNPs A single nucleotide polymorphism (SNP) that is found within a coding sequence. It is a small genetic change or variation that can occur within a DNA sequence.

CTRL Control key on a standard computer keyboard.

cubic spline calibration curve A calibration curve where a cubic polynomial curve is fit between each pair of calibration levels so that the slopes of the separate cubic polynomial curves match at common calibration curve points.

CV Coefficient of variation.

D

data acquisition The process of accumulating and storing data received from instrument sensors as they detect the sample. Also, the conversion of analog signals to digital data files.

database Refers to the Watson application Oracle database.

data bits A group of bits—typically 5, 6, 7, or 8 bits—used to represent a single character of data for transmission. The number of bits used in data transmission must be agreed on by the sending and receiving parties.

data rate The rate of data collection by the UV detector.

dataset A name that binds together related files and actions in the auditing database for Xcalibur.

deconvolution In BIOMASS Deconvolution, a process whereby an electrospray-ionization mass spectral (ESI/MS) plot of relative abundance versus *m/z* is transformed into a plot of relative abundance versus mass.

delay The time difference (in minutes) between the UV detector and the MS detector. The mass spectrometer corrects the retention time so that peaks from both the UV detector and MS detector appear at the same retention time in the data file.

Delta Cn Abbreviation for the difference (or *delta*) in cross-correlation score (XCorr) between the top candidate and the current candidate for a given input data file. For example, the Delta Cn for the fourth hit would be (Xcorr1-Xcorr4)/Xcorr1, where Xcorr1 is the Xcorr of the first hit and Xcorr4 is the Xcorr of the fourth hit.

See also:
XCorr

delta retention time The difference in time between when a peak is expected to elute (according to previous run data) and when it actually elutes, expressed as a percentage.

de novo sequencing The derivation of a peptide sequence from its MS/MS spectrum without relying on protein databases for identification.

detection limit The point at which the signal and noise become statistically indistinguishable. In practice, this is often taken as the point at which the amplitude of a peak is only two to three times the amplitude of the average baseline noise.

See also:
sensitivity

device(s) A single item or items within an instrument (for example, pump).

digest fragments The set of smaller compounds formed by the action of an enzyme on a larger compound. For example, the digest resulting from the action of the enzyme trypsin on a protein is a set of peptides and protein fragments.

See also:
enzyme
trypsin
tryptic digestion

digital Data to/from an instrument is through either API calls or direct connection; this transfer type can be uni- or bi-directional. the Watson application's digital interfaces may be either built-in or gateway.

digital DM/PK Drug metabolism and pharmacokinetics.

dilution factor A factor that specifies how much a sample was diluted during preparation. This factor is used to calculate the amount or concentration in the original (undiluted) sample.

display range The portion of the graph (spectrum or chromatogram) that is currently displayed.

dose Protocol Definition. “The numeric amount and units of measure which corresponds to the level or concentration of a drug which is to be administered via the specified route of administration”.

draw speed The speed at which the metering unit draws sample out of the vial. The draw speed may have an influence on the injection volume precision when using viscous samplers. If the draw speed is too high, air bubbles may form in the sample plug, affecting precision.

driver A device-specific control program that enables a computer to work with a particular device.

droplines A mathematically constructed vertical line from the valley between the peaks to the baseline.

drug set A group of compounds associated under a common name. QuickQuan uses drug set names to distinguish which compounds to use for each sequence in an assay or which compounds to tune in a tune plate. Using drug sets allows you to place multiple compounds in a vial or microplate well, for both tuning and acquisition.

duplicate entries Similar entries in a non-redundant protein or peptide database.

duplicate sample A second independent sample. A separate reportable result is expected for each duplicate sample.

DWP (deep well plate) Ninety-six wells per plate

dynamic NSI probe assembly The assembly that fits into the NSI body assembly and includes a snap-in tip mounting device, a fused silica emitter, a snap-in nut, a MicroTee™ mounting block, and a fused silica transfer line.

E

early expiration Removes peaks from the dynamic exclusion list if the number of times (counts) that the peak's intensity falls below a specified signal-to-noise (S/N) threshold exceeds a specified number of counts. The number of counts is determined during the full scan on which the data-dependent scans are based.

edge type The criteria by which the left or right edge of a peak is detected.

eject speed The speed at which the metering unit ejects sample or solvent. A faster eject speed shortens the time required to run the injector program.

end bracket sample type The second of a set of calibration samples in a sequence having bracket type open or non-overlapped. Each set of calibration samples contains the standard levels defined in the active Processing Method. Blank samples are often located in the sequence before and after calibration samples.

See also:
bracket [open]
bracket [overlapped]

End S#: An abbreviated form of the phrase *end scan number*. This is the number of the last scan that occurs at the selected chromatogram range end time. For example, if the selected chromatogram range has End Time = 2.46 min, this might be displayed: End S#: 434

See also:
End Time
Start S#

End Time The time (in minutes) corresponding to the end of the selected chromatogram range. This phrase is used when the end time is displayed in a header. For example, if the selected chromatogram range ends at time = 3.74 minutes, this is displayed: End Time: 3.74.

See also:
End S#:

enzyme A biomolecule that catalyzes a specific chemical reaction. The enzyme does not affect the equilibrium of the catalyzed reaction. The enzyme enhances the rate of the reaction by providing a reaction path with a lower activation energy.

environment Refers to the whole computer system (Oracle, O/S, Citrix,.NET framework, and so on.)

enzyme digest The action of an enzyme, such as Trypsin, on a peptide. The enzyme breaks up the peptide into smaller peptides. Enzymes are site-specific—that is, an enzyme always breaks a peptide at the same amino acid. For example, Trypsin attacks an Arginine (R) and Lysine (K).

EQB (exceeds quadratic bounds) If (B2-4*A*(C-Response)) < 0, there is no root for the equation, the Watson application reports EQB for standard samples. For study samples, the raw value will be analyzed to determine if the result is ALQ or BLQ. EQB is reported for quadratic and Wagner regressions only.

error log A file that provides a chronological listing of all errors that are detected while processing a sample or list of samples.

event A parameter that has a time value or an assigned initial value, which the Avalon Peak Detection algorithm uses to detect chromatogram peaks. You can add, change, or delete events in an event list.

event list To detect peaks, Avalon uses the settings for initial events and user-defined timed events in the event list. To calculate values for initial events, open a raw file, and then make the chromatogram view active.

event time The time when an external device is turned on or off. The time when there is a change in the heater temperature or valve position.

exact mass The monoisotopic mass of a given compound. The exact mass is calculated by use of the most abundant isotopes of the constituent elements (for example, C = 12.0000, N = 14.00307, H = 1.007825).

exception peak In spectra from high resolution analyses, a peak (E) from an internal reference compound that has a known mass but is not a reference peak.

See also:
reference peak

exclusion mass A mass that is added to the dynamic exclusion list after the mass spectrometer obtains a sufficient amount of data on it. Masses in the dynamic exclusion list are ignored with respect to triggering a data-dependent scan.

expectation value (e value) The number of sequences in a database that are expected to have p scores equal to or better than what was observed simply by chance. Low expectation values represent better matches than high expectation values because they are less likely to include false positives.

expected amount The amount entered for each calibration level based on the calculated amount from analytical measurement and subsequent dilution.

See also:
calibration level

expected RT The expected retention time (RT) of a component.

See also:
retention time (RT)

expected width A valley detection parameter that defines the minimum allowable time in minutes between the peak apex and a valley. Valley detection must be enabled for this parameter to be active.

See also:
rise percentage
valley detection

Extensible Markup Language See:
XML (Extensible Markup Language)

external standard A component (not added to the target sample) used to correct for instrument detection errors.

See also:
external standard calibration
internal standard (ISTD)

external standard calibration A calibration that analyzes a range of known amounts of an external standard. Xcalibur plots the component amounts and their corresponding response values to create a calibration curve.

See also:
calibration
Extensible Markup Language
internal standard (ISTD) calibration

external wash The passing of solvent through the syringe needle while the needle is inserted in the wash station.

ex vivo In an artificial environment outside of a living body.

F

F An abbreviated form of the phrase scan filter. This shortened form is used to save space when the scan filter is displayed in a header. The selected scan filter is displayed using the scan filter format.

FDA US Food and Drug Administration

file-based Data received from or sent to an instrument is in ASCII or Excel file formats.

Filter rise time A detector parameter that controls its response time. The rise time is inversely proportional to the amount of baseline noise; the longer the rise time, the less noise detected. However, excessively long rise times can result in broader peaks with shorter peak heights.

firmware Software routines stored in read-only memory. Startup routines and low-level input/output instructions are stored in firmware.

fixed Y-axis normalization mode A display mode available for the Spectrum view, showing the height of the vertical scale set as equal to the height of the largest peak in the current mass spectrum. The height of the vertical scale is fixed when the fixed display mode is invoked.

flag Letters above chromatogram and spectrum peaks that provide supplemental information about the peak data. For example, if a peak is saturated, Xcalibur displays an *S* above the peak.

flush volume The volume of solvent used to flush the lines, sample loop, and syringe(s) between injections.

fragment length The number of amino acids in the fragment.

fragment mass The calculated sum of all the masses of the amino acids in the fragment.

full loop injection A technique that injects a sample volume into the loop that is about twice the loop volume so that the loop is completely filled.

FX An abbreviated form of the phrase *fixed Y-axis normalization mode*. This shortened form is used to save space in the header of the Spectrum view when the fixed Y-axis normalization mode is in use, for example, FX: 1.98e+003 (1.98 x 103).

G

gateway Communication protocol used with the Watson application to add or remove interfaces.

gateway-based interface Interface software is a separate application.

Gaussian smoothing A smoothing algorithm that averages each data point with neighboring points to give the displayed value. A Gaussian smoothing uses weighting coefficients corresponding to a Gaussian peak shape.

GCMS (gas chromatography-mass spectrometry) Refers to either the assay methodology or a Watson application assay type.

GCP Good Clinical Practices.

GLP Good Laboratory Practices.

gradient Changes in the percentage composition of two or more solvents over time.

gradient curve The shape of the solvent composition curve between two time lines. Gradient curves are determined by specifying initial and final times and concentrations.

Graph view file An ASCII text file with the extension .grf that a user creates to save data from the Graph view in the Tune Plus or Tune Master window. This file contains *n* columns of information about a particular graph. Graph view files can be imported into Microsoft™ Excel for display and manipulation.

group scan The number of intermediate MS/MS scans between two MS/MS scans that have the same precursor *m/z* value.

H

header information Data stored in each data file that summarizes the information contained in the file.

HPLC (high performance liquid chromatography) Refers to either the assay methodology or a Watson application assay type.

I

ICIS Interactive Chemical Information System

ICP-MS (inductively coupled plasma-mass spectrometry) Refers to either the assay methodology or a Watson assay type.

ID or Id (Identification) Unique identifier.

incomplete digest The digest type where not every possible digest site is digested. For example, if an incomplete digest is done with trypsin, every combination of digested/not digested Lysine (K) and Arginine (R) will be considered.

INCOS noise A single pass algorithm that determines the noise level. The noise-level data is used to determine which peaks to label during area calculations and to determine peak start and peak end.

See also:
repetitive noise

indexed database A database created with the Database Manager or the FASTA Database Indexer to speed up searches.

individual Specifies the number of results files available for each sample. One results file is created for each individual sample in the analytical run.

initial temperature The temperature of the oven or injector to be applied as soon as the GC receives the Start signal.

injection status, autosampler status Indicates whether or not the current sample has or has not been injected.

injection volume The actual volume in microliters (µL) of a sample injected by the autosampler into the chromatograph. The minimum and maximum injection volumes that you can use depend on the autosampler you selected.

input parameter files Text files that store the settings used in dialog boxes. Whereas most of the settings in the input parameters files can be changed in the dialog boxes within the BioWorks Browser, some can be changed only by altering the contents of the input parameters file in a text editor.

instrument Set of physical items that comprise an LC/MS system.

instrument configuration A set of user-defined hardware and software options. Xcalibur provides configuration options for the following: mass spectrometer, fonts, HPLC, autosampler, inlet, analog inputs, and Ethernet.

instrument method A set of experiment parameters that define Xcalibur operating settings for the autosampler, liquid chromatograph (LC), mass spectrometer, divert valve, syringe pump, and so on. Instrument methods are saved as file type .meth.

insulin_ms.raw The raw spectrum of insulin that is provided with the BioWorks Browser program.

intensity relative to base peak The ratio of intensity of a particular peak in a mass spectrum to the intensity of the mass peak of the greatest intensity. This ratio is generally equated to the normalized ratio of the heights of the respective peaks in the mass spectrum, with the height of the base peak being taken as 100.

Interactive Chemical Information System (ICIS™) The computer software used by the Finnigan™ XSQ product line when running in a UNIX environment. You can use data obtained with ICIS software with Xcalibur. ICIS files have the .dat extension.

internal standard (ISTD) A component added to a sample to correct for injection errors and sample preparation errors. It is assumed that the ratio of the target compound to the internal standard compound is constant for a given calibration standard. The concentration or amount of an internal standard in any solution used in quantitation remains constant.

See also:
Extensible Markup Language
internal standard (ISTD) calibration
internal standard (ISTD) response ratio
target compound

internal standard (ISTD) calibration A calibration where known amounts of standard components are added to the sample to be analyzed.

See also:
external standard calibration

internal standard (ISTD) response ratio The ratio of the signal response of an unknown peak to the response of the corresponding internal calibration peak at a specified calibration level.

interstitial volume, LC Another name for void volume. The total volume of mobile phase within the length of the LC column. The void volume consists of the intraparticle volume (inside the packing material itself) and interparticle volume (between the packing particles). The remainder of the volume of the column consists of the packing material.

in vivo In the living body of a plant or an animal.

isotopic cluster A group of peaks that differ in *m/z* because they incorporate different isotopes of their component elements.

isotope search For the analysis of unknown drugs, an isotope search finds extra chromatograms for each ion that has an intensity above a particular threshold.

isolation width The baseline width of a window for a mass peak (or peak cluster) of interest for an MS/MS or MS^n scan.

L

label threshold The percent intensity value of the highest peak that a peak height must exceed to be labeled.

lactoglobulin.pep Thermo Scientific-supplied peptide sequence file of -lactoglobulin.

layout An arrangement of views and data displays that can be saved and opened as a file in the Qual Browser window. Layout files can be identified by their file extension: .lyt.

level amount The amount of a component injected for a specific calibration level.

See also:
calibration level

level name A user-assigned name for a calibration level.

See also:
calibration level

LIMS Laboratory Information Management System.

linear calibration curve A linear (first order) polynomial least-squares fit of the experimental data using slope and intercept coefficients.

See also:
calibration curve

linear gradient curve A gradient curve with a constant rate of change between solvent compositions.

See also:
gradient curve

liquid chromatography/mass spectrometry (LC/MS) An analytical technique that combines a high-performance liquid chromatograph (LC) and a mass spectrometer (MS). Refers to either the assay methodology or a Watson application assay type.

list separator character The character used to separate columns of a column-separated value text file of extension.csv.

locally weighted calibration curve A calibration curve that is constructed from individual line segments. At multiple points across the calibration region, a weighted linear regression is performed. The point slopes are then connected to form a continuous curve.

See also:
calibration curve

locked workbook A copy of a workbook (and its associated files) that displays “Locked” in the title bar and in the status bar. No processing or acquisition can take place in locked workbooks. The only functions that are allowed are those that review data and print reports.

log file A text file, with a .log file extension, that is used to store lists of information.

M

master assay Specifies the assay definition at the global, system level. It defines instrument interfacing types and styles, calibration curve and QC samples options, and other assay-related parameters. A Master Assay is associated with a project so that multiple studies within a project may use it.

master assay method An analytical method that is specified as part of a project. It is handled separately from an actual study but is available to be associated with any study. A Master Assay method may be associated with multiple studies.

mass An abbreviated form of the phrase *mass range*. This shortened form is used to save space when the selected mass range (in mass-to-charge ratio units) is displayed in a header, for example, Mass: 150-375.

massmap.params file Stores the parameters used for the Peptide Mapping application. You can edit this file in a text editor.

mass-step size Size of the plotting or calculating interval. For example, a mass-step size of 10 u would result in a calculation occurring at a point every 10 u along the baseline of the spectrum.

matrix The material that contains the compound of interest (for example, blood).

mean The average of a group of measurements (for example, masses, times, or intensities). The mean is used to calculate variance and relative standard deviation.

See also:
relative standard deviation
variance

metabolic modifications Changes that occur to metabolites while in the body.

metabolite A chemical intermediate or product due to metabolic reactions.

method A file containing commands that are interpreted by Xcalibur. Xcalibur uses the following methods to run a sample: Tune, Instrument, and Processing. The file extensions of these methods are .LCQtune or .TSQTune, .meth, and .pmd, respectively. Xcalibur helps you to create, edit, save, and use methods.

See also:
instrument method
processing method
Tune Method

minimum number of scans in baseline An advanced parameter used to calculate a component baseline.

See also:
baseline

minimum peak width The value, in scans, required to identify that a peak exists. To be included in the list of found peaks, a peak must be larger than the minimum value.

missing component A component that is included in the calibration data but is not found during the analysis of a chromatogram.

modification A chemical or structural alteration of a molecule, such as oxidation or methylation.

modification, mass The mass increment associated with an amino acid, which you can specify to limit the size of a database you create.

molecular weight The mass of a molecule calculated for a given molecular formula as the sum of the atomic weights of the constituent atoms.

monoisotopic molecular weight The calculated mass of a molecule based on the exact mass of the most abundant isotope of each element. Use the monoisotopic molecular weight for all data field entries, for example, C = 12.000000, H = 1.007825, and O = 15.994915.

mzData An XML-based data format for storing peak list information, such as that contained in DTAs. The mzData format facilitates the comparison and exchange of peak list data among different platforms. The mzData format is developed by the PSI-MS: Mass Spectrometry Standards Working Group.

N

N.C.: Quadratic (not calculable using quadratic regression) It is not possible to calculate using quadratic regression (process to find the equation of a parabola for a set of data).

N.C. (non-calculable) A general catch all when Watson cannot determine a value and one of the more specialized messages does not apply.

N.C. (Wagner) Not calculable using Wagner regression.

NCA Non-compartmental analysis

N.D. (no data) Data is not in Watson.

needle draw position offset The difference in the distance of the needle from the bottom of the vial from its default position.

negative peak A peak characterized by signal levels that drop below the baseline level of the detector.

neighboring amino acid The amino acids on either side of the cleaved amino acid.

NL An abbreviated form of the words normalized level. This shortened form is used to save space in the header, for example, NL: 1.98e+003 (1.98 x 103).

nominal sample time Protocol definition. The theoretical relative time a sample was taken from a subject after a treatment administration.

non-serial subject (sparse sampling; composite profile) Protocol definition. A group of subjects that can be sampled only once or a limited number of times after treatment administration. It is not possible to study a subject's time vs. concentration profile. To create a time vs. concentration response profile for a treatment, a subject group dosed at the same time are divided into subsets of subjects that are sampled at different time points. Sample concentrations at the same time point are averaged together, and then the averages at the time increments are combined to form the time vs. concentration profile.

no peak Not calculable. No data was returned from the instrument for the sample or the Watson application is unable to calculate a solution for the regression. Not Reportable: user definable string User assigned flag.

no sites Amino acids in a sequence that follow an amino acid listed are not susceptible to digestion. For example, trypsin_k digests at Lysine (K), but if the K follows a Proline (P) in the sequence, it will not be digested.

no waste injection A technique that injects the exact amount of sample requested. Of the three injection modes, the no waste injection mode uses the least amount of sample, but it is also the least precise. Use this injection mode to conserve sample.

nominal molecular weight The calculated mass of a molecule based on the integer mass number of the most abundant isotope of each element, for example, C = 12, H = 1, and O = 16.

normal scan types Scan types that are full and SIM. Normal scan types all have the same scan rate and provide unit resolution.

normalization A data analysis procedure where all peaks are reported with heights relative to the highest peak height or area in the chromatogram, spectrum, or map.

See also:
fixed Y-axis normalization mode
normalize Y-axis normalization mode

normalize spectra The action of normalizing spectra to a common base peak intensity instead of using the raw intensities from the raw file before it generates correlation results.

Normalizing the spectra corrects for intensity discrimination in the correlation results that can occur if spectra with significantly different absolute peak intensities are used.

normalize Y-axis normalization mode A display mode, which is available for the Spectrum view, that sets the vertical scale equal to the height of the largest peak in a mass spectrum. In the normalize display mode, the software always displays the largest peak in the spectrum at full scale.

See also:
fixed Y-axis normalization mode

nucleic acid Biologically occurring poly-nucleotides showing the nucleotide residues linked in a specific sequence by phosphodiester bonds, for example, DNA and RNA.

nucleotide Any of several compounds that consist of a ribose or deoxyribose sugar, joined to a purine or pyrimidine base and to a phosphate group, and that are the basic structure units of nucleic acids.

number of scans in background An advanced background subtraction parameter used to minimize the contaminating effect of background on the peak identification process when scan information is used.

O

one-way interface Refers to instrument interfaces that do not export Sample ID information. With one-way interfaces, matching of data in the results file to the Watson application worklist can be made on the basis of either the sequential Worklist position or the position on a plate.

oligonucleotide A short chain of usually up to 20 nucleotides.

See also:
nucleotide

optimization graph The plot of mass spectrometer detector response for a specific device, as generated by the calibration procedures.

origin The unique point on a calibration curve plot where zero response (Y-axis) occurs at zero amount (X-axis). This is the point at which the Y-axis crosses the X-axis.

outlier A calibration data point that does not appear to correlate to other calibration data points within experimental error.

OWL A nonredundant composite protein sequence database produced from the following source databases: Swiss-Prot, PIR 1-3, GenBank (translations), and NRL-3D.

See also:
Swiss-Prot

P

parent drug A source compound (drug) providing one or more molecules or compounds.

See also:
analyte

parity The state of being odd or even, used as the basis of a method of detecting errors in binary-coded data. Parity must be agreed on by the sending and receiving devices before transmission can take place.

See also:
full loop injection

partial loop injection A technique that meters a specified amount of sample into the sample loop, rather than overfilling the sample loop. The sample is smaller than half the total volume of the sample loop

PCP Personal computer.

peak A maximum in a graph of intensity versus time or intensity versus mass. The position of the maximum is said to be the position of the peak.

peak apex The highest point of a peak.

peak apex detection For advanced component and chromatogram peak detection, the values specified for rise percentage and valley signal-to-noise ratio (S/N) serve another purpose besides valley detection. These values are also used during peak detection to determine whether or not the apex of a peak has been found. In this case, the tests are employed in the inverse of the valley detection process.

Xcalibur uses the valley S/N value to test a possible apex, then applies the rise percentage value to confirm that the values on both sides are lower. This has the effect of treating a high measurement, which is a possible peak apex, as the crest of a hill. Therefore, the measurements must be lower on both sides of the crest when adjusted for noise.

See also:
rise percentage
signal-to-noise ratio (S/N)

peak area The area obtained by integrating peak intensities from the start to the end of the peak.

peak base An interpolation of the baseline between the extremities of the peak.

peak detection The process of finding the locations and amplitudes of local maxima and minima in a signal that satisfies certain properties.

See also:
peak end
peak start

peak edge detection A chromatogram peak detection criteria that uses the peak signal-to-noise (S/N) cutoff value to assist in the detection of a peak edge. This test assumes an edge of a peak is found when the baseline adjusted height of the edge is less than the ratio of the baseline adjusted apex height and the peak signal-to-noise cutoff ratio.

See also:
peak S/N cutoff

peak end The end of a chromatographic peak occurs whenever the detection signal decreases to a value less than the current threshold criteria.

See also:
peak start

peak height (peak dimension) The distance from the peak maximum to the peak base, measured perpendicular to the ordinate.

See also:
constrain peak width
tailing factor

peak integration In this analysis of a chromatogram the area of each peak is determined.

peak noise factor The noise level multiplier used to determine the signal threshold of a possible peak.

peak number An identification number assigned by Xcalibur to each peak in an analytical run.

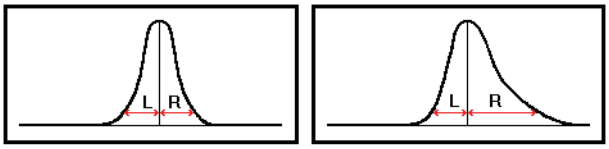
peak S/N cutoff A peak edge detection advanced parameter used in conjunction with the influence of valley detection and baseline noise calculation. This test is performed based on a user-defined peak signal-to-noise cutoff threshold. This test assumes an edge of a peak is found when the baseline adjusted height of the edge is less then the ratio of the baseline adjusted apex height and the peak signal-to-noise cutoff ratio according to the expression.

peak start The start of a chromatograph peak occurs whenever the detection signal increases to a value greater than the current threshold criteria.

peak symmetry The degree to which a chromatographic peak exhibits a peak symmetry characteristic of an ideal Gaussian peak.

See also:
peak tailing

peak tailing Distortion of chromatographic peaks from ideal Gaussian shape seen as an extension of the end of the signal. A tailing peak has the “T” flag set.



See also:
peak symmetry

peak threshold The minimum number of intensity counts per sampling interval that is required before a signal is recorded.

peak-to-peak resolution threshold Controls how much peak overlap can be present before two or more adjacent peaks are recognized as a peak cluster. Instead of valley-to-valley baselines, peak clusters have baseline drops. The baseline drop of a peak cluster is specified as a percent of peak height overlap.

peak width The distance across a peak measured at a selected peak-height level, in minutes or mass units. The peak-height level is usually specified as a percentage of the maximum peak height.

See also:
peak width at half height

peak width at half height The full width of a peak at half its maximum height, sometimes abbreviated FWHM.

See also:
peak end
peak width

peak width, constrain A chromatogram peak edge detection method used to integrate a peak that displays tailing. LCquan constrains the width of a component during peak integration using a user-specified peak height percentage and tailing factor.

See also:
peak height
tailing factor

percent bias An analytical run definition. The difference from nominal and observed concentration value.

%CV (PK Pharmacokinetics.

percent theoretical An analytical run definition. The % bias plus 100.

percent difference The difference between the expected value and the calculated value is determined as a percentage as follows:

%Diff = 100 x (*X*calc - *X*expected) / *X*expected

where *X*calc is the calculated value of the variable *X*, and *X*expected is the expected value of the variable *X*.

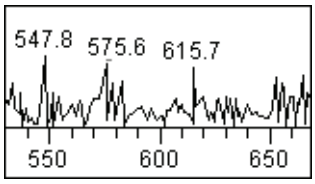
PK/PD Pharmacokinetic/pharmacodynamic.

plate-position-based Specifies how the data in the results file are matched to the Watson application's analytical run. The sample in the results file is uniquely identified by the sample's position in the sample plate.

point-to-point calibration curve A calibration curve that averages data at each calibration level. This averaging results in a single averaged data point at each calibration level.

See also:
calibration curve
calibration level

point-to-point plotting style Style of plotting a spectrum that plots each intensity peak connected together.



polymorphism Genetic variation occurring within plant and animal species where different forms exist together in the same population.

polypropylene glycol (PPG) A mixture of polypropylene glycols of different chain lengths used for high mass range calibration and tuning. A mixture of PPG 2000 and PPG 2700 provides ESI peaks suitable for calibrating over the 2000 to 4000 mass range.

polytyrosine Polytyrosine - 1, 3, 6 and Polytyrosine - 1, 3, 6, 9 are the factory calibration compounds provided for TSQ Quantum. The polytyrosine mixtures contain the amino acid tyrosine and two or three polytyrosines, respectively.

post processing Procedures automatically performed by Xcalibur or its layered applications after an LC/MS run, as defined in the predefined Processing Method. Procedures include peak detection, peak integration, component identification, quantitation, data storage, and report generation.

ppm Abbreviation for **p**arts **p**er **m**illion.

predefined user labels for Sequence Setup Allows you to specify the values of specific parameters on a per sample basis in a Sequence row. These parameters are used for activation masses and reject masses for data-dependent scans and for activation masses and reject masses for the external trigger feature.

predicted digest fragments Sequence fragments calculated in Pepmap. Predicted digest fragments are the theoretical sequence fragment obtained by performing a hypothetical digest on the entered sequence.

privilege One specific assigned security element.

processing filter A filter that defines the characteristics of a spectrum displayed in the MS^n Browser. These filters are displayed in the MS^n page of the Info bar in the Qual Browser window. There are two types of processing filters: Composite Spectrum processing filter and Average Spectrum processing filter.

processing method A defined set of parameters that provide Xcalibur and LCquan with a recipe for automatic quantitation and report generation. Each method can define single or multiple target compounds, single or multiple internal standards, and one or more undefined components. Multiple internal calibration levels or QC levels can be defined for target compounds.

Processing Sequence A defined set of processing settings for a single sample or list of samples. A Processing Sequence can contain unknown samples, calibration standard samples, quality control (QC) samples, and blank samples.

product spectrum Displays the product spectrum for the selected parent in the Spectrum pane. This spectrum consists of all of the products from the parent in the data that is the closest to the selected parent mass coordinate.

profile data Data representing mass spectral peaks as point-to-point plots, with each point having an associated intensity value.

project Houses studies. Each project generally has. It The drug or drugs used within the studies are often the same. When used at a CRO, it may be appropriate to associate sets of Studies/Sponsors together as a project.

protein A macromolecule composed of one or more polypeptide chains, each with a characteristic sequence of amino acids linked by peptide bonds.

protein database A database consisting of protein sequences.

See also:
protein sequence

protein sequence The sequence of amino acids making up a protein.

See also:
protein

proteolysis The hydrolytic breakdown of proteins into simpler, soluble substances such as peptides and amino acids by proteases or intramolecular digestion.

proteome The protein complement expressed by an organism's genome.

proteome warehouse A relational database consisting of a collection of proteome databases and a small amount of metadata. Proteome databases contain the shotgun annotation of all possible combinations of PTMs on each basic sequence in the proteome. The proteome warehouse stores PTM information and the sequence information used to identify and characterize proteins.

proteomics The study of the proteome, the protein complement expressed by an organism's genome.

p score The probability of obtaining at least as good a match between the observed fragment list and a sequence as by chance. It is a measure of confidence in the validity of a match. A low p score means that the probability of obtaining at least this many fragments matching a sequence is low, so it is unlikely that random chance is the cause of the association.

protocol Represents an overall plan of action for a study. It is defined as a set of treatments applied to groups of subjects where typically each group of subjects may receive only one or a subset of all possible treatments. In association with each treatment, the protocol also defines a schedule of samples to be extracted from the subjects after treatment and set of tests to be performed on those samples. There is only one protocol for a study."

Q

quadratic calibration curve A calibration curve where Xcalibur calculates a quadratic (second order) polynomial least-squares fit of the experimental data using the following mathematical form:
Y = AX2 + BX + C
where A, B, and C are the polynomial coefficients.

See also:
calibration curve

quadratic limit If (B2-4*A*(C-Response)) = 0, there is only one root for the equation. That is conc = -B / (2*A).

quadratic log-log calibration curve A calibration curve that calculates a quadratic (second order) polynomial least-squares fit of the experimental data.

See also:
calibration curve

qualitative analysis Chemical analysis designed to determine the identity of the components of a substance.

See also:
quantitative analysis

quality control (QC) A quality management program that includes procedures designed to ensure a specified level of process quality. Calibrations, duplicate or replicate analyses, spiked samples and blanks are quality control activities. The accuracy and precision of QC sample results are used to indicate the accuracy of the quantitation of unknown samples.

See also:
quality control (QC) sample type
unknown sample type

quality control (QC) sample type A sample that contains known amounts of known components. QC samples are placed in the sequence so that quantitation results can be compared with known component amounts or concentrations for quality assurance purposes.

See also:
quality control (QC)

quantitation The process of determining the amount of a component in a sample.

quantitation mass A specific mass-to-charge ratio (for example, *m/z* 502) or range of mass-to-charge ratios to be used when determining the peak area and peak height of a compound. Usually, a quantitation mass (quan mass) is chosen that is characteristic of the compound.

See also:
peak area
peak height

quantitative analysis Chemical analysis designed to determine the quantity or concentration of a specific substance in a sample.

See also:
qualitative analysis

Quantitative calibration The process of determining how the LC/MS system responds to different compounds. Quantitative calibration involves designing a processing method and performing calibration experiments with calibration standards containing known quantities of analyte.

R

R^2 (R squared) A "goodness of fit" calculated for calibration curves. A goodness of fit of 1.0 indicates that the calibration curve passes through each data point exactly. The closer the goodness of fit value is to 1.0, the better the calibration curve fits the data.

ramp The rate of change from one temperature, pressure, or flow to the next.

raw amount The unadjusted amount of a component, as determined from a quantitation analysis or by the application of a response factor.

raw data Uncorrected liquid chromatograph and mass spectrometer data obtained during an acquisition. Xcalibur and Xcalibur-based software store this data in a file that has a .raw file extension.

raw instrument response An analytical run definition. The value, either direct from instrument or

derived, which is used to perform the regression. Examples of instrument responses include: peak area, peak height, ratio of peak Areas, ratio of peak heights.

real time A data acquisition method that generates and displays the mass spectra as the experiment is in progress.

real-time plot A plot of a mass spectrum or chromatogram that displays the data as it is generated by the mass spectrometer.

See also:
real time

reference compound The internal standard for a given target compound.

reference mixture For internal standard calibration: A solution that contains one or more internal standard (ISTD) and target compound. For external standard calibration: A solution that usually contains the target compound(s).

See also:
internal standard (ISTD)
target compound

reference peak In spectra from high resolution analyses, a peak (R) from an internal reference compound that has a known, precise mass. Reference peaks are used as measures of calibration accuracy.

See also:
exception peak

regression calculation A least-squares method of obtaining a mathematical equation that best fits a set of experimental calibration data. Xcalibur and LCquan support linear, quadratic, and cubic regression calculations.

related peaks Peaks in a mass spectrum that are produced by fragments of a common compound.

relative intensity The ratio of the intensity of a specific peak to the intensity of the peak with the highest intensity.

relative peak height threshold A threshold that excludes all peaks whose height falls below a specified percentage of the height of the highest peak in the chromatogram.

relative retention time (RRT) The LC elution time of a component divided by the elution time of a reference component. The ratio of the LC elution time of a retained component (t) corrected for the void time, tvoid, and the elution time of a reference component, tref, corrected for the void time: RRT = [t - tvoid]/[tref - tvoid].

See also:
corrected relative retention time
void time

relative scaling Displays a chromatogram trace or spectrum so that the height of the Y-axis scale adjusts to the peak heights of the data currently displayed.

relative standard deviation A measure of the dispersion of a group of measurements relative to the mean of the group. Relative standard deviation is expressed as a percentage of the average value. The percent relative standard deviation is calculated as:

$$\%RSD = 100\ (S / \bar{X})$$

where S is the standard deviation and $\bar{X}$ is the sample mean.

See also:
standard deviation

repetitive noise A multiple-pass algorithm that determines the noise level. The software uses the noise-level data to determine which peaks to label during area calculations, and to determine the peak start and peak end.

See also:
INCOS noise

replicate An aliquot of the same sample, only a single reportable result is expected for replicate samples.

RESID database A database of annotations and structures for protein modifications.

reservoir vial With a 12 mL capacity, the reservoir vial is used to hold solvent, reagent, or diluent. Up to four of these large vials can be fitted behind the wash station. A reservoir vial is approximately the same height as a sample vial.

resolution, chromatographic A measure of the ability of a chromatography column to separate components during a chromatographic experiment.

response factor A calculation coefficient that corrects for the different response of the instrument to different compounds. The response factor is used in quantitation to convert peak areas to numbers proportional to the weight of sample.

response ratio The numerical value of the Response/ISTD Response. If the Response is specified as Area in the processing method, then the units of both Response and ISTD Response are counts-sec. If the Response is specified Height in the processing method, then units of both Response and ISTD Response are counts.

See also:
calculated amount

response value The signal from the mass spectrometer detection of a component peak as it elutes from an LC column. Xcalibur and LCquan can use either the peak area or peak height to record the response to a specific component.

result file The file containing the data that resulted from the analysis of a raw data file. Each analyzed run has both a raw (data) file of type .raw and a result (data) file of type .rst.

Results Table The name given to the table of data in the DeNovo and Peptide Mapping applications. DeNovo: contains the list of candidate sequences and scores. Peptide Mapping: contains the list of results from the Peptide Mapping search.

retention reference A calibration standard whose retention time is used to adjust the retention time of other components in the same run or to calculate their relative retention times. Also known as calibration reference, reference component, retention reference component, and internal standard.

See also:
relative retention time (RRT)

retention time (RT) The time after injection at which a compound elutes. The total time that the compound is retained on the chromatograph column.

retention time shift For each compound in a method, there will be a predicted retention time (RT) for the quantitation mass peak. This predicted RT will be determined by the SOP definition of predicted RT. It can change from day to day or between batches because it is a function of column maintenance.

RF See:
response factor

RIA (radio-immuno assay) RIA refers to either the assay methodology or a Watson application assay type.

ring electrode The mass analyzer electrode that is located between the endcap electrodes.

rise percentage An advanced parameter used for both valley detection and peak detection.

rise time A parameter that controls the response time of UV-Vis or PDA detector. The rise time is inversely proportional to the amount of baseline noise. For example, the longer the rise time, the less noise detected.

role A role is a set of assigned security privileges.

route of administration Protocol Definition. The means by which a drug enters into a subject. For certain types of administration, the drug is infused over a significant length of time. In these instances, the duration of administration is usually recorded".

RT An abbreviated form of the phrase *retention time (RT)*. This shortened form is used to save space when the retention time (in minutes) is displayed in a header, for example, RT: 0.00-3.75.

See also:
retention time (RT)

S

S# An abbreviated form of the phrase *scan number*. This shortened form is used to save space when the scan number is displayed in a header, for example, S#:234.

See also:
scan number

sample A quantity of material (for example, plasma).

sample data type Protocol definition. Meaning of the sample type that can be one of two values; either point or interval. A blood sample is considered a point sample data type because the analyzed value from a blood sample corresponds to drug concentration in the blood at a particular point in time. A urine sample is considered an interval sample data type because the analyzed value form the sample must be considered in terms of the sample volume and the time between samples to accurately represent elimination of an analyte.

sample handling Defined as those activities that relate to handling of actual samples extracted from subjects as part of the study's protocol at the experimental site.

sample ID An alphanumeric string of characters that uniquely identifies a sample. Sample IDs are entered using the Sequence window.

sample ID-based Specifies how the data in the results file are matched to the Watson application's analytical run. The sample in the results file is uniquely identified by the sample's unique ID that is generated by the Watson application.

sample loop A loop of calibrated volume that is used to perform flow injection analysis.

sample type Protocol definition. Any tissue or biological fluid taken from a subject for analytical testing in order to determine the effects of a drug treatment. Every different kind of sample will be assigned a biological matrix type.

A sample category tells Xcalibur or LCquan how the data for a particular sample is to be processed.

sample volume The Sample Volume column of a sequence is used to calculate concentrations from amounts or amounts from concentrations.

saturation (sat.) A measure of the "purity" of the hue. As saturation is decreased, the hue becomes more gray. A saturation of zero results in a grayscale value.

SB An abbreviated form of the phrase *subtract background*. This shortened form is used to save space in the header when indicating the number of background scans subtracted from a Spectrum display, for example, SB: 30.

scan description A symbolic code used to describe the scan event settings for a specific scan event.

scan event A mass spectrometer scan that is defined by choosing the necessary scan parameter settings. Multiple scan events can be defined for each segment of time.

scan filter Created by Xcalibur from instrument method settings, a scan filter specifies that processing is to be applied to a subset of the scans in a raw file.

In the Qual Browser window: The autofilter feature can be used to display chromatogram traces for each scan filter. In addition, filters are automatically transferred to the target display when data is selected graphically. You can also drag a filter onto a graph from a Scan Filter display.

scan number A number assigned to each scan that identifies the relative order when it was acquired in a mass spectrometer run.

scan power The power *n* in the expression MS^n. The number of stages of mass analysis, expressed as MS^n, where *n* is the scan power. For example, a scan power of *n* = 1 corresponds to an MS1 (or MS) scan with one stage of mass analysis. A scan power of *n* = 2 corresponds to an MS2 (or MS/MS) scan with two stages of mass analysis. A scan power of *n* = 3 corresponds to an MS3 scan with three stages of mass analysis, and so on.

scan range The range of mass-to-charge ratios over which the mass spectrometer is scanned.

scan rate The rate at which the quadrupole mass spectrometer scans the specified scan range, in units of u per second.

scan time (ST) The amount of time required to accomplish one scan, from the lowest mass to the highest mass of a specified scan range. The scan can be continuous (full-scan type) or it can be in segments (SIM and SRM, for example).

scan width The width of the scan range, in *m/z* units. When you use a center mass/scan width input method to define the scan range, the scan range is centered symmetrically about the specified center mass.

search window A time range or mass range to limit a search for similar scans.

segment A period of time during a mass spectrometer run.

semi-automatic calibration A procedure that calibrates the MS detector automatically, one or more parameters at a time.

See also:
calibration parameters

sensitivity The signal obtained per amount of sample introduced. Different measures of sensitivity are recommended, depending on the nature of the sample and the required inlet system.

See also:
detection limit

septum purge flow The flow, in mL/min, from the septum purge line, which sweeps out any residual sample after sample injection.

sequence The series of samples (rows) selected for analysis.

sequence file A file that you create that provides instructions about how to run a sample or list of samples.

sequence length The number of amino acids in a peptide.

See also:
amino acid

serial port An input/output location (channel) for serial data transmission.

set mass The mass of interest. It depends on the scan mode chosen.

For the Product scan mode, the set mass is the parent mass.

For the Parent scan mode, the set mass is the product mass.

For the Neutral Loss scan mode, the set mass is the neutral loss mass.

set point The set of a mass-to-charge ratio and its associated value (such as a voltage) used to define a tuning table for a device. A set point is created by having TSQ Quantum optimize a device for a specific mass.

shipment sheet Sample Handling definition. The document the accompanies a sample shipment. It describes the samples in the shipment.

shotgun annotation The overpopulation of a database by all combinations of all known modifications to a protein's basic sequence. Shotgun annotation calculates all possible combinations of known post-translational modifications (PTMs) onto each protein sequence.

shutdown method An Instrument Method that is run by Xcalibur or LCquan after it runs the last sample in an acquisition. You create Shutdown Methods in the Instrument Setup view.

signal-to-noise ratio (S/N) The ratio of the signal height (S) to the noise height (N). The signal height is the baseline corrected peak height. The noise height is the peak-to-peak height of the baseline noise.

See also:
signal-to-noise ratio threshold

signal-to-noise ratio threshold A user-specified chromatogram peak edge detection criterion used for peak integration.

sites Specific amino acids that are attacked by the enzyme listed.

slit width The width of the variable slit at the entrance of the spectograph. Use a narrow slit for analytes with very fine structures in the absorbance spectrum and for high concentrations. Use a wide slit to detect very low concentrations.

smoothing The application of a smoothing algorithm to the data to improve the graphical appearance of data by reducing the level of noise and/or to improve peak detection. Xcalibur provides the following types of data smoothing: boxcar smoothing and Gaussian smoothing. improves the graphical appearance of data by reducing the level of noise and/or to improve peak detection.

smoothing points The odd integers from 1 (number of points) to 15 used in a weighted average to reduce the level of noise in the data, consequently improving peak detection and the graphical appearance of the data.

See also:
smoothing

SNP A single nucleotide polymorphism, which is a small genetic change, or variation, that can occur within a DNA sequence.

solute A dissolved substance. The dissolved component of a mixture that is to be separated in a chromatographic column. (Also called the sample or analyte.)

SOP Standard Operating Procedure.

Sp Abbreviation for the preliminary score of the top candidate peptide or protein for a given input data file.

spectral trees A representation of the data structure of MS^n spectra. A tree, which is like a structural "fingerprint", reflects the hierarchical spectra dependencies in MS^n experiments. For more details on spectral trees, see the Help in Mass Frontier™.

spectrum averaging The averaging of spectral data for a number of scans.

stability samples An analytical run definition. Used to analyze the reliability of quality control samples over time.

standard bracket sample type The calibration samples in a Sequence having bracket [open] or bracket [overlapped] type.

See also:
bracket [open]
bracket [overlapped]

standard deviation In statistics, the standard deviation is a measure of the dispersion of a group of measurements. For example, masses, times, or intensities. Standard deviation is calculated as follows:

$$s = \sqrt{\mathrm{Var}(x_i \ldots x_N)}$$

where Var (Xi...XN) is the variance.

See also:
relative standard deviation

Standard Error of the Mean (SEM) An analytical run definition. SEM is Standard Deviation divided by the square root of n.

standard sample A sample with a known amount of calibration reference material.

standard update sample type Xcalibur adds this sample type to the calibration curve without removing previous entries. The first calibration sample in a Sequence having bracket [none] type is a standard clear type. Xcalibur clears all previous data for all calibration levels and begins a new set of calibration data. Xcalibur assigns all subsequent samples in the same calibration sequence to be the

Standard Update Sample Type. The calibration data set consists of the data from the standard clear samples and the data from the standard update samples.

See also:
bracket [none]

start bracket sample type The first of a set of calibration samples in a Sequence having bracket [open] or bracket [overlapped] type. Each set of calibration samples contains the standard levels defined in the active Processing Method. Blank samples are often located in the Sequence before and after calibration samples.

See also:
bracket [open]
bracket [overlapped]

start delay A time delay (in minutes) before the start of acquisition.

Start S# An abbreviated form of the phrase *start scan number*, which is the mass spectrometer scan number that occurs at the selected chromatogram range start time. This shortened form is used to save space in the header of the Spectrum view. For example, if the selected chromatogram range includes start time = 0.00 minutes, this is displayed: Start S#:1.

See also:
End S#:

start time The time (in minutes) corresponding to the beginning of the selected chromatogram range. This phrase is used when the start time is displayed in a header. For example, if the selected chromatogram range starts at time = 0.0 minutes, this is displayed: Start Time: 0.0.

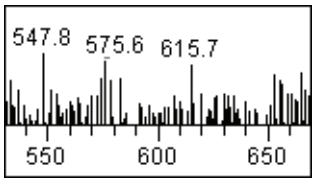
See also:
End Time
Start S#

startup method An Instrument Method that is run by LCquan Xcalibur before it runs the first sample in an acquisition. You create Startup Methods in the Instrument Setup window.

static nanospray A device that performs continuous analysis of small analyte solution volumes over an extended period of time.

static NSI probe assembly The assembly that fits into the NSI body assembly and includes the static nanospray probe tip, the nose cone tip mount, and the probe body assembly.

stick plotting style Style of plotting a spectrum that plots each intensity peak as a vertical line.



See also:
point-to-point plotting style

stop bits A bit that signals the end of a character.

structure elucidation A process that determines both the molecular formula (the number of atoms of each type in a molecule) and the molecular structure (the order showing how the atoms are connected to each other). The MS/MS scan modes are powerful tools for elucidating the structure of organic compounds.

study (LCquan) A folder that contains one or more LCquan workbooks for a specific analytical experiment.

See also:
workbook

study (Watson) Determines the interaction of a drug with test subjects (animals or man) which have been administered that test article (drug). Based on a plan for subject dosing followed by subject sampling. The samples obtained from the subject via a variety of biological matrices are then analyzed for drug levels according to an analytical method. Results of this analysis are then stored for further analysis and reporting in terms of the protocol.

study assay Specifies the assay definition at the study level. Defines instrument interfacing types and styles, calibration curve and QC samples options, and other assay-related parameters. The Study Assay is copied from the master Assay.

study sample An analytical run definition. A sample from a subject at a treatment site that is based on the study's protocol.

study samples database The sample-by-sample representation of the study. Every sample in this database must be represented individually or as part of a group in the study.

subject Protocol definition. A living biological entity that can be administered a dose of a drug and subsequently sampled for purposed of analyzing drug activity.

subject group Protocol definition. A set of subjects which have a common set of treatment administrations applied to them.

summary Specifies the number of results files available for each analytical run. One results file is created for all samples in the analytical run.

Swiss-Prot Part of the UniProt database, an annotated protein sequence database maintained by the Swiss Institute for Bioinformatics (SIB) and the European Bioinformatics Institute (EBI). The Swiss-Prot database consists of protein sequence entries.

T

T Time

T-Half Half life

tailing factor A peak integration parameter that constrains the peak width of an asymmetric chromatogram peak that has a tailing trace.

See also:
constrain peak width
peak height

tangent skim A timed event that you can specify in the event list shown with the Avalon Peak Detection algorithm. Tangent skim identifies the tallest peak in a cluster and detects peaks on either side or both sides of the tallest peak. Tangent skim resets automatically at the end of a peak cluster.

target compound A compound that you want to identify or quantitate or that a specific protocol (for example, an EPA method) requires that you look for. Target compounds are also called analytes, or target analytes.

tension Controls how closely the baseline follows the overall shape of a chromatogram. A low tension traces the baseline closely to follow changes in the chromatogram. A high tension follows the baseline loosely and over longer time intervals.

See also:
baseline

threshold A value above which something will take place. For example, if a peak height is above the indicated peak label threshold, that peak is labeled.

threshold base peak Indicates that peaks below this percentage of the base peak will not be plotted.

time function (TF) programming The process of turning external devices (such as column-switching valves and fraction collectors) on or off at preset times during your run. To create a time program, fill in the Time Events table.

time offset The value that adjusts for fluctuating retention times when comparing data between the experimental source raw file and the control background file.

time programming The process of changing the heater temperature or valve position at preset times during your run. To create a time program, fill in the Time Program table.

timed event An instrument action triggered to occur at a specific, preset time during a run or analysis.

tolerance The allowable range of deviation from a specified value, expressed as a percentage of this nominal value.

total composite spectrum A spectrum that is the sum of all MS/MS, MS3, MS4, MS5,…MS10 averaged spectra.

trace A record made by an instrument's recording element. A common term for a chromatogram.

See also:
chromatogram

treatment (treatment administration) Protocol definition, per 21 CFR Part 11, "The actual event of administering a drug dose via a route of administration to a group of subjects".

treatment group Protocol definition. The same set of subject who have been administered a treatment.

treatment site Sample Handling definition. The location at which subjects are dosed and sampled.

triple stage quadrupole (TSQ™) mass spectrometer

A tandem MS/MS instrument, which has the first and third quadrupoles performing mass analysis, and the second quadrupole (operated in RF-only mode) functioning as an ion transmission device.

trypmyo01.raw A raw spectrum file of the tryptic digest of myoglobin.raw.

See also:
mzData

trypsin A proteolytic enzyme found in pancreatic juice and active in alkaline environments.

tryptic digestion The site-specific breakdown of a protein or peptide into smaller fragments of amino acids by use of the enzyme trypsin.

See also:
digest fragments
trypsin

TSQ™ mass spectrometer See:
triple stage quadrupole (TSQ™) mass spectrometer

Tune Method A defined set of mass spectrometer tune parameters for the ion source and mass analyzer. Tune methods are defined by using the Tune Plus (LCQ Series, LXQ, and LTQ) or Tune Master (TSQ Quantum) window and saved as the file type .LCQTune, .LTQTune, or.TSQTune, respectively.

A tune method stores tune parameters only. (Calibration parameters are stored separately, not with the tune method.)

tune parameters Instrument parameters whose values vary with the type of experiment.

tune plate A plate of compounds separated into different wells/vials. Each well represents compounds grouped together as a drug set.

tune, mass spectrometer Tuning the mass spectrometer involves optimizing voltages, currents, flows, and so on for the ion source parameters to achieve optimal mass spectral sensitivity and proper resolution.

tune, MS detector, automatic A procedure that automatically tunes the mass spectrometer.

See also:
tune, MS detector, manual
tune, MS detector, semi-automatic

tune, MS detector, manual A process that lets you manually tune the mass spectrometer one parameter at a time.

See also:
tune, MS detector, manual
tune, MS detector, semi-automatic

tune, MS detector, semi-automatic An automatic procedure that tunes the mass spectrometer one parameter at a time.

tuning and calibration solution In the TSQ Quantum, the standard ESI tuning and calibration solutions are polytyrosine mixtures, Polytyrosine – 1, 3, 6 and Polytyrosine – 1, 3, 6, 9.

two-way interface Refers to instrument interfaces that export sample ID information. With bidirectional interfaces, matching of data in the

results file (or external application for a digital interface) to the sample in the Watson application worklist is made on the basis of the results being tagged with the Sample ID.

U

undefined component Any component that is not defined as a target compound or an internal standard compound. This is the default component type.

unidentified peak A peak in an LC chromatogram or mass spectrum that is not identified. Sometimes called an unknown peak

uni-directional interface .

UniProt An international repository of organisms containing all the proteins and genes that are known for a specific organism.

unknown sample type A sample type that is not a Calibration Sample, Calibration Blank, Quality Control Sample, or Quality Control Blank.

unresolved peak A peak that overlaps the previous or next peak. A peak that starts or ends at a valley above the baseline.

update calibration mode A calibration mode where a new data point is added to the calibration curve.

User Account The mechanism by which the Watson application identifies different users of the application. An account contains the formal name for the User. This name will be used for all reports generated by the system. The account also contains the name and password the user will use when logging into the application.

V

valley detection A chromatogram peak edge detection method used to integrate unresolved peaks. Xcalibur and LCquan drop a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.

See also:
expected width
rise percentage

variance A statistical variance of a data set is used to calculate standard deviation and relative standard deviation. Variance is calculated:

$$Var(x_i \ldots x_N) = \frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{X})^2$$

where N is the number of samples, Xi is the value of each individual sample, and $\overline{X}$ is the mean of the sample value.

See also:
mean
relative standard deviation
standard deviation

v Version.

vial list A list that contains the vial number, sample type, level, sample ID, and sample name for each vial in a sequence. The list is organized by ascending vial number.

vial position The vial position in the autosampler tray of the current sample. Also called the vial number.

view width The duration of time (in minutes) that Xcalibur or LCquan displays for a chromatogram view. A view width can be specified for each component.

void time The time after injection that it takes an unretained compound to elute from the LC column. This time is subtracted from the elution time for a retained compound to obtain the corrected relative retention time for the elution of each compound.

W

W An abbreviated form of the word *weighting (W:)*. This shortened form is used to save space in the header of the Spectrum view when the calibration curve data weighting factor is displayed. For example: W: xxxx, where xxxx is the weighting option selected and stored in the Processing Method.

See also:
weighting (w)

weight In Xcalibur and layered applications, this is equivalent to mass.

weighting (w) The mathematical assignment of significance (weight) to specific points during a least-squares regression analysis calculation. A weighting of one indicates equal weighting and treats each point the same in the regression calculation.

window size The number of scans in the window used during peak identification by the spectrum method.

WMF Windows Meta File.

workbook A folder in an LCquan study that contains Instrument Methods, Sequences, Processing Methods, and raw files for an acquisition. Each Workbook contains folders named Exports, Imports, and Raw files.

See also:
locked workbook
study (LCquan)

workgroup A set of users assigned to specific roles.

work list A set of samples to be submitted to the instrument,

work list-based specifies how the data in the results file are matched to the Watson application's analytical run. The sample in the results file is uniquely identified by the sample's position in the Watson application worklist.

X

XCorr Abbreviation for the raw cross-correlation score of the top candidate peptide or protein for a given input data file.

XML (Extensible Markup Language) A general-purpose markup language that is used to facilitate the sharing of data across different information systems, particularly via the Internet.

Index

Symbols

A

B

C

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

V

W

X