

Thermo Xcalibur

Getting Started Guide

Processing Quantitative Data

Software Version 4.1

XCALI-97928 Revision A June 2017





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	Previewing Reports in XReport
	Specifying Report Templates in the Processing Method
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Preface

This guide contains a set of tutorials that show how to quantitatively process a set of raw data files using the Thermo Xcalibur[™] mass spectrometry data system.

Refer to the *Xcalibur Data Acquisition and Processing User Guide* for information about modifying the data system configuration, setting up the instrument configuration from within the Foundation platform, creating instrument methods and sequences for data acquisition, and for more detailed information about creating processing methods and batch reprocessing. Refer to the *Xcalibur Quan Browser User Guide* for more information about reviewing quantitation data.

Note You can modify the Xcalibur data system configuration by using the Thermo Xcalibur Configuration dialog box. Configuration options include the location of the default directories for your data files, methods, and report templates; the font size and type and the unit labels displayed in the preview views; the default mass tolerance, mass precision, and mass defect used to process the data files, and so on.

Use these tutorials to process a set of raw data files provided with the data system:

- 1. Tutorial 1: Creating a Processing Method
- 2. Tutorial 2: Batch Reprocessing Data Files
- 3. Tutorial 3: Working with Result Files in Quan Browser
- 4. Tutorial 4: Reviewing, Specifying, and Printing Reports

Contents

- Related Documentation
- Special Notices
- Contacting Us

To provide us with comments about this document, click the link below. Thank you in advance for your help.



Related Documentation

In addition to this manual, Thermo Fisher Scientific provides the following documentation for the Xcalibur data system:

- Xcalibur Quick Start
- Xcalibur Data Acquisition and Processing User Guide
- Xcalibur Quan Browser User Guide
- Xcalibur Qual Browser User Guide
- Xcalibur Library Browser User Guide
- XReport User Guide
- Help from within the data system

To view the product manuals

• From the Windows[™] taskbar, choose **Start > All Programs > Thermo Xcalibur > Manuals > Xcalibur**.

-or-

• From the Roadmap view of the Xcalibur home page, choose Help > Manuals.

To view the Help

You can open the Help in these ways:

- To open the Help for the Xcalibur data system, choose **Help > Xcalibur Help**. The Help opens to the Welcome page.
- To open the Help for an Xcalibur window, choose Help > *Window* Help, where *Window* is the current active window: home page, Instrument Setup, Processing Setup, Qual Browser, Quan Browser, or Library Browser.
- To open the Help for a specific page or dialog box of the Xcalibur user interface, click **Help**, choose **Help > Help on Current Item**, or press the F1 key.

* To download user documentation from the Thermo Scientific website

- 1. Go to www.thermoscientific.com.
- 2. In the Search box, type the product name and press ENTER.
- 3. In the left pane, select **Documents & Videos**, and then under Refine By Category, click **Operations and Maintenance**.

- 4. (Optional) Narrow the search results or modify the display as applicable:
 - For all related user manuals and quick references, click **Operator Manuals**.
 - For installation and preinstallation requirements guides, click **Installation Instructions**.
 - For documents translated into a specific language, use the Refine By Language feature.
 - Use the Sort By options or the Refine Your Search box (above the search results display).
- 5. Download the document as follows:
 - a. Click the document title or click **Download** to open the file.
 - b. Save the file.

Special Notices

Make sure you follow the precautionary statements presented in this guide. The special notices appear in boxes.

Special notices include the following:

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or may 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.

Contacting Us

Contact	Email	Telephone	QR Code *		
U.S. Technical Support	us.techsupport.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752			
U.S. Customer Service and Sales	us.customer-support.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752			
Global support	 To find global contact information or custon 	nize your request	NAME		
	1. Go to thermofisher.com.				
	2. Click Contact Us , select the country, and the you need.	n select the type of support			
	 At the prompt, type the product name. Use the phone number or complete the online form. 				
	 To find product support, knowledge bases, a 	and resources			
	Go to thermofisher.com/us/en/home/technica	al-resources.			
	 To find product information 				
	Go to thermofisher.com/us/en/home/brands/	thermo-scientific.			
Note To provide feedb	pack for this document, go to surveymonkey.com/s/	PQM6P62 or send an email	message to		

*You can use your smartphone to scan a QR Code, which opens your email application or browser.

Technical Publications (techpubs-lcms@thermofisher.com).

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Introduction

This book provides tutorials that show how to quantitatively process a set of raw data files. For an overview of the Xcalibur data system and the data set used in the tutorials, review these topics.

Contents

- Opening the Data System and Working with the Roadmap View
- Overview of the Xcalibur Data System
- Data Acquisition Flow Diagram
- Data Processing Flow Diagram
- Automated Data Acquisition and Processing Flow Diagram
- Xcalibur Example Files

Opening the Data System and Working with the Roadmap View

To open and close the data system and to work with the Roadmap view, see these topics:

- Opening, Navigating, and Closing the Data System
- Adding Application Pages to the Roadmap View
- Using the XApp Store to Explore Available Thermo Scientific Applications

Opening, Navigating, and Closing the Data System

The Xcalibur data system provides access to all of your installed Thermo Scientific[™] applications and the Thermo Scientific applications that are available in the XApp Store.

To open the Xcalibur data system

Do one of the following:

• From the Windows taskbar, choose Start > All Programs (or Programs) > Thermo Xcalibur > Xcalibur.

-or-



The home page window opens with the Info view on the left and the Roadmap view

To navigate the Xcalibur data system *

(open to the XApps page) on the right.

To navigate the home page views (Roadmap, Sequence Setup, Real Time Plot, and Info), use the View menu or the View toolbar, [🌇 💥 🤮 🔢 .

To navigate the Xcalibur applications, use the Roadmap view or the Go To menu.

To close the Xcalibur data system

Do one of the following:

• Click the **Close** icon, *while*, in the upper right corner of the Xcalibur window.

-or-

• Right-click the Xcalibur icon in the taskbar and choose Close Window from the shortcut menu.

The XApps page provides access to the Xcalibur data system and other Thermo Scientific applications that are installed on the data system computer. The XApp Store page-which opens when you click the XApp Store tab-provides access to Thermo Scientific applications that are available for purchase.

The Status page of the Info view displays the instrument status, and the Acquisition Queue page displays the status of the injection sequences that you submit. For information about working with the Info view, refer to the Xcalibur Data Acquisition and Processing User Guide.

The XApps page contains the following icons (Figure 1):

- The first row contains icons for the Instrument Setup window, the Sequence Setup view, and the FreeStyle[™] application.
- The remaining icons for the Xcalibur applications and other installed Thermo Scientific applications populate the page in alphabetical order from left to right and top to bottom.
- The last row contains icons for the mzCloud[™], Planet Orbitrap, and Thermo Fisher Cloud websites.

The data system automatically detects the installed applications. If the XApp Store contains a later version of an installed application, a star (\checkmark) appears in the upper right corner of the application icon. You cannot hide or rearrange the icons on the XApps page.





Indicates that a later version of this application is available in the XApp Store.

Clicking the double chevron below the application icon opens a menu.

Adding Application Pages to the Roadmap View

You can customize the Roadmap view by adding pages that provide access to your preferred set of Thermo Scientific applications.

✤ To add application pages to the Roadmap view

In the Roadmap view, click the **My XApps** icon, [+].

A new My XApps page opens with the same icons as the XApps page.

XApps	XApp Store	My XApps	x	+
Custo	mize			

* To hide applications on a custom page

1. Click Customize.

"Hide" appears below each application icon and the OK button replaces the Customize button.



2. To hide an application, click Hide.

The application icon disappears from the page. Once you hide at least one application, the More Apps button appears to the right of the OK button.

3. Click **OK** to save the changes.

* To show installed applications on a custom page

1. Click More Apps.

A list of the hidden applications appears below the button. If the More Apps button is not visible, the custom page already contains icons for all the installed applications.



2. From the More Apps list, select the hidden application that you want to show on your custom page.

To rename a MyXApps tab

- 1. Double-click the **MyXApps** tab (**My XApps v x**).
- 2. Select the tab text.
- 3. Type a new name.
- 4. Click **√**.

Using the XApp Store to Explore Available Thermo Scientific Applications

Use the XApp Store to access information about and install trial versions of Thermo Scientific applications (Figure 2).

Figure 2. XApp Store



To open the XApp Store

Click the **XApp Store** tab in the Xcalibur Roadmap view.

***** To view product information

On the XApp Store page, click More Information below the product name.

The product's website page opens.

* To be contacted by a sales representative

1. On the XApp Store page, click How to Order below the product name.

The product's Contact Us website page opens.

2. Fill in the form and click **Send**.

* To view and save the system requirements for an application

1. On the XApp Store page, click System Requirements below the product name.

The Product Name System Requirements document opens as a PDF file.

- 2. To save the PDF file to a local folder, do the following:
 - a. Click the **Tools** icon, 🔯, and choose **File > Save As**.

The Save As dialog box opens. The File Name box contains the document's default file name.

b. Select the folder where you want to store the PDF file and click Save.

• To install a trial version of an available application

1. On the XApp Store page, click **Try** to the right of the application that you want to install.

The Thermo Scientific Life Sciences and Mass Spectrometry (LSMS) website for software downloads opens to the Login page (Figure 3).

		- • ×	
https://thermo.flexne	toperations.com/control/thmo/login: $\mathcal{P} = egin{array}{c} c \\ \hline \end{array}$ Thermo Fisher Download a ×	₼ ☆ ฿	
ThermoFisher	Life Sciences Mass Spectrometry	Home	
The world leader in serving science	Key Customer - Software Entitlement and Download		
Thank you for your interest in Thermo software applications.	o Scientific Life Sciences Mass Spectrometry software. Please <u>register</u> and return to this page to access demo	onstration	
If you are a Thermo Fisher employee	and do not already have a login ID, please email <u>ThermoMSLicensing@thermofisher.com</u> to request access.		
Your login ID is your e-mail address. If you have not yet set up a password or have forgotten your existing password, use Password Finder to create or reset it.			
Login ID			
Password			
Remember my password until I log	lout		
Login			

Figure 3. Login page for software LSMS downloads

- 2. If you do not have a login ID, click **Register** to create one.
- 3. After you receive a confirmation email, log in.

The Product Information page opens.

4. Select the application that you want to install.

The demo page for the selected application opens.

5. Download the Product Name and version zip folder.

Overview of the Xcalibur Data System

To acquire and process raw data files, use the Instrument Setup and Processing Setup windows and the Sequence Setup view.

Instrument Setup	Use this window to create instrument methods with the chromatographic settings for your chromatography system and the data acquisition parameters for your mass spectrometer. You can also access an additional command menu for each instrument from this window.
Sequence Setup	Use this view of the home page window to set up the injection and processing sequence for your sample set. For data acquisition, the sequence must contain one or more instrument methods, the sample positions in the autosampler, and the injection volume for each sample. For data processing, the sequence must contain one or more processing methods.
Processing Setup	Use this window to create processing methods for your data sets. Processing methods contain the information required to extract qualitative or quantitative results from the raw data. This information includes the parameter settings for baseline integration, peak identification, and analyte quantification. Processing methods also specify the templates to be used to report the results.

Use the browser windows to review unprocessed raw data files (RAW files) or result files (RST files) created during batch processing.

For information about setting up the instrument method for your LC, GC, LC/MS, or GC/MS system, refer to the Help provided in the Instrument Setup window for each configured device.

In addition to Help, Thermo Fisher Scientific provides hardware manuals and getting started guides for your Thermo Scientific mass spectrometer, and user guides or getting started guides for your Thermo Scientific LC system. For third-party LC systems that the Xcalibur data system controls, Thermo Fisher Scientific provides getting connected guides.

Data Acquisition Flow Diagram

Figure 4 provides a flow diagram for acquiring the sample set.

For information about creating an instrument method for your LC/MS system, refer to the Help systems and the getting started guides for the liquid chromatography (LC) instruments and the mass spectrometer (MS).



Instrument Inlet Direct Control (MS Tune):		
Optimize the data acquisition settings for your analytes.		
Instrument Setup window:		
Import the Tune file that you saved when you optimized the ion source and ion optics parameters for your analytes or use the stored tune file. Enter the data acquisition parameters for the mass spectrometer and the chromatographic parameters for the chromatography system and save these settings as an instrument method.		
Prepare a set of sample vials or fill the wells of a microwell plate. Load the vials or microwell plate into the autosampler.		
To perform a quantitative analysis of the analytes in your samples, prepare standard solutions that contain known amounts of the analytes. As you load the samples into the autosampler, bracket the samples with the standards.		
▼		
Sequence Setup view of the Xcalibur home page:		
Create a sequence that includes an instrument method, the positions of the sample vials that you loaded into the autosampler, and the injection volume for each sample.		
Sequence Setup view of the Xcalibur home page:		
Run the sequence and acquire a set of raw data files.		

Data Processing Flow Diagram

Figure 5 provides a flow diagram for processing data once you have acquired it. For information about acquiring the raw data, refer to the *Xcalibur Data Acquisition and Processing User Guide*.

Figure 5. Processing previously acquired quantitation data

Processing Setup window:		
Using a representative raw data file, create a processing method.		
Sequence Setup view of the Xcalibur home page:		
Add the processing method to your sequence. Then batch reprocess the raw data files to produce result files.		
V		
Quan Browser window:		
Open the processed sequence. Review the integration of each chromatogram. Review the calibration curve for each target compound.		
•		
Quan Browser window or Processing Setup window:		
Adjust peak integration and calibration curve parameters as needed.		
▼		
XReport report application:		
Preview representative files with selected report templates.		
▼		
Processing Setup window:		
Add the appropriate XReport report templates to the processing method.		
▼		
Sequence Setup view of the Xcalibur home page:		
Batch reprocess the sequence and print the appropriate reports.		

Automated Data Acquisition and Processing Flow Diagram

Figure 6 provides a flow diagram of how to do acquisition and processing automatically using the Xcalibur data system and the instrument control software provided with your mass spectrometer and chromatography system.





Xcalibur Example Files

These tutorials analyze an example set of data files provided with the Xcalibur data system and located in the C:\Xcalibur\examples\data directory. The target compound is a proprietary pharmaceutical product. The Thermo Fisher Scientific applications laboratory in San Jose, California acquired the data using an LC/MS system and MS/MS techniques in the electrospray (ESI) mode.

The lab used the internal standard calibration technique to quantify the pharmaceutical compound called drugx. The internal standard compound called D4 is a deuterated analogue of drugx that has four deuterium atoms exchanged for hydrogen atoms in the compound.

The calibration standards were prepared by spiking human plasma with drugx to give nine calibration levels with concentrations of 10, 25, 50, 100, 200, 400, 600, 800, and 1000 pg/mL. The lab ran triplicate samples at the high (1000 pg/mL) and low (10 pg/mL) ends of the curve with single samples run in between.

The QC samples were prepared similarly by spiking human plasma with drugx to give three QC levels with concentrations of 10, 400, and 1000 pg/mL. Six replicates per QC level were run.

The calibration and QC standards were spiked with 100 pg/mL of the D4 internal standard.

Figure 7 displays the scan filter chromatograms and mass spectra for a drugx.raw data file.



Figure 7. Chromatograms and mass spectra for drugx and D4

1 Introduction Xcalibur Example Files

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Tutorial 1: Creating a Processing Method

Use the following procedures to create a processing method for quantifying data using the raw data files associated with the drugx sequence file, drugx.sld, in the following directory:

drive:\Xcalibur\examples\methods\

This tutorial does not discuss using system suitability parameters to verify the performance of the chromatographic column or the validity of the chromatographic peaks. To add report templates to the processing method, see Chapter 5, "Tutorial 4: Reviewing, Specifying, and Printing Reports."

Create a processing method that quantifies the target compound (analyte) in the drugx data set by following these procedures in the order listed.

Contents

- Opening the Processing Setup Window
- Specifying the Quan View Options
- Opening a Raw Data File
- Specifying the Identification Settings for Analysis Components
- Entering Peak Integration and Detection Parameters
- Selecting the Calibration Settings
- Specifying Calibration Levels and QC Levels
- Saving the Processing Method

Opening the Processing Setup Window

Use the Processing Setup window to create a processing method. Use the Quan view of the Processing Setup window to set up the calibration information for your the target components (analytes) of a quantitative analysis.

* To open the Quan view of the Processing Setup window

- 1. Open the Xcalibur data system as follows:
 - On the computer desktop, double-click the **Xcalibur** shortcut icon, 🚺

-or-

• From the computer taskbar, choose **Start > All Programs (or Programs) > Thermo Xcalibur > Xcalibur**.

The Xcalibur data system opens to the home page window.

- 2. Open the Processing Setup window as follows:
 - On the Roadmap view, click the Processing Setup icon,

-or-

- From the menu bar, choose **GoTo** > **Processing Setup**.
- 3. If the Quan view is not already displayed, open it as follows:
 - On the View bar, click the **Quan view** icon,

-or-

• On the menu bar, choose **View > View Quan**.

Tip The View bar is a vertical panel of icons on the left side of the window. If the View bar is not displayed, choose **View > View Bar** to display it on the left side of the Processing Setup window.

4. Click the **Identification** tab.

The Identification page opens (Figure 8).

Figure 8. Identification page for the Quan view of the Processing Setup window

🧱 Thermo X	calibur Processing Setup - Quan - Identification - Untitled (Int Std)	
<u>File View Zoo</u>	om <u>O</u> ptions <u>G</u> oTo <u>H</u> elp	
Quan Quan Qual	Identification Detection Calibration Levels System Suitability Peak Purity Name: < Retention time Expected (min): 1.00 Detector type: MS Peak Detect: ICIS Use as RT reference Vi Filter: Lrace: TIC Keys:	Components
Reports Programs Ready	No raw file open	

View bar

5. If the data system automatically loads a processing method, choose **File > New** to start a new processing method.

Specifying the Quan View Options

The chemists at Thermo Fisher Scientific acquired the data set used in this tutorial with an LC/MS system, and they used the internal standard calibration technique to quantify the drugx target compound.

✤ To specify chromatography by LC

 In the Quan view of the Processing Setup window, choose Options > Chromatography By.

The Chromatography Options dialog box opens (Figure 9).



Chromatography Options		
Chromatography by		
OK Cancel	Save As Default	Help

- 2. To specify chromatography by LC, select the LC option.
- 3. Click **OK** to save the settings and close the dialog box.

* To specify the internal standard calibration technique

 In the Quan view of the Processing Setup window, choose Options > Calibration Options.

The Calibration Options dialog box opens (Figure 10).

Figure 10. Calibration Options dialog box

Calibration Options	
Calibration by	
Internal standard	
C External standard	
- %RSD calculation method	
C Use <u>r</u> esponse values	
OK Cancel <u>S</u> ave As Default <u>H</u> elp	

- 2. To specify calibration by internal standard, select the Internal Standard option.
- 3. Click **OK** to close the dialog box.

Opening a Raw Data File

Open a representative raw data file from the data set to determine appropriate peak detection and integration settings for the processing method. In this tutorial, use the drugx_03.raw file. In general, select a raw data file corresponding to a low-concentration calibration standard.

* To open a raw data file in the Quan view of the Processing Setup window

- 1. Do one of the following:
 - In the toolbar, click the **Open Raw** icon,

-or-

• From the menu bar, choose File > Open Raw File.

The Open Raw File dialog box opens (Figure 11).

Figure 11. Open Raw File dialog box

Open Raw File		? 🔀
Look in: 🔯 data	• •	•
Image: Adda_dependent_01.raw Image: Adda_dependent_02.raw Image: Adda_dependent_02.raw Image: Adda_dependent_02.raw	drugx_05.raw drugx_06.raw drugx_07.raw drugx_08.raw drugx_09.raw drugx_10.raw	indrugx_11.r indrugx_12.r indrugx_13.r indrugx_14.r indrugx_15.r indrugx_16.r
File name: drugx_03.raw		<u>O</u> pen
Files of type: Raw Files (*.raw)		Cancel
		<u>H</u> elp
- Header Information		
Created: Saturday, Ma Modified: Saturday, Ma Last saved by: linda Number of saves: 2 Comment: STD=10 pg/ml,IS=100 pg/ml	ay 04, 1996 10:31:23 PM ay 04, 1996 10:41:15 PM	
1		

2. Browse to the following location:

drive:\Xcalibur\examples\data

3. Select drugx_03.raw and click Open.

Processing Setup opens the drugx_03.raw file (Figure 12).

Figure 12. Identification page with the total ion current (TIC) chromatogram of drugx_03.raw

🧱 Thermo 🕽	Xcalibur Processing Setup - Quan - Identification - Untitled (Int Std)	
<u>File View Z</u> o	oom <u>O</u> ptions <u>G</u> oTo <u>H</u> elp	
Quan Quan Qual Reports	Identification Detection Calibration Levels System Suitability Peak Punity Image: Comparison of the compari	Components
	C:1Xcaliburtexamplestdatatdrugz_03 5/4/1996 10:31:23 PM	
Programs	STD=10 pg/ml.IS=100 pg/ml 100 4.89 NL: 2.11E5 100 50 4.82 4.97 0 0.93 1.87 3.15 3.62 4.62 5.23 6.48 0 1 2 3 4 5 6 420 421 0 1 2 3 4 5 6 420 421 m/z Time (min) m/z m/z 100	
Ready	NUM	NOT SAVED

Tip If you save a processing method when a raw data file is open, the raw data file name is saved in the processing method. To have the Xcalibur data system automatically open the associated raw data file whenever you open the processing method, select the **On** option in the Auto-Open Raw File area of the Settings dialog box.

To open this dialog box in the Processing Setup window, choose **Options > Settings**.

Specifying the Identification Settings for Analysis Components

This topic describes how to identify target compounds and internal standards and contains the following topics:

- Specifying the Identification Settings for the Internal Standard
- Specifying the Identification Settings for the Target Compound

For the internal standard calibration technique, each calibration standard contains one or more target compounds and one or more internal standards. The data set used in this tutorial contains one target compound and one internal standard. The target compound is drugx. The internal standard compound is D4.

Specifying the Identification Settings for the Internal Standard

A processing method needs component identification information to associate each sample component with a chromatographic peak. Use the Identification page for the Quan view in the Processing Setup window to name the sample components and to specify the retention time and peak identification criteria.

To identify the internal standard, follow these procedures:

- Specifying the Name of the Internal Standard
- Selecting the Detector Type
- Selecting the Peak Detection Algorithm
- Matching Scan Filters with Components
- Selecting the Trace Type
- Entering the Retention Time of the Component Automatically

Specifying the Name of the Internal Standard

Use the Name list on the Identification page to name the components in the sample. The parameter settings on the Identification page are specific to the component selected in the Components list on the right side of the window.

* To enter the name of the internal standard in the Name list

1. In the Name list, select **<New>**, and then type **D4**, the name of the internal standard (Figure 13).

👹 Thermo 🕽	XcaliburProcessing Setup - Quan - Identification - Untitled (Int Std)	
<u>File View Z</u> o	oom <u>O</u> ptions <u>G</u> oTo <u>H</u> elp	
4.67	Identification Detection Calibration Levels System Suitability Peak Purity	Components
	Name: <new> Expected (min): 1.00</new>	
Quan	Detector type: MS Peak Detect: ICIS Use as RT reference Vi	
Lake.	Filter:	
Qual		
Reports		
R.		
140 A	C:1Xcaliburtexamplestdatatdrugz_03 5/4/1996 10:31:23 PM STD=10 pg/ml,IS=100 pg/ml	
Programs	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
	Time (min) m/z	<u> </u>
Ready	u I I I I I I I I I I I I I I I I I I I	NOT SAVED

Figure 13. Identification page without named components

2. Click **OK** to save the new name.

The name appears in the Components list.

Note To delete a component, highlight the component name in the Components list on the right side of the window. Then, choose **Options > Delete** *component name*.

Selecting the Detector Type

Use the Detector Type list on the Identification page to specify the type of detector used to acquire the raw data file. The available selections are MS (mass spectrometer), Analog, A/D card, PDA (photodiode array detector), and UV (UV-Vis detector).

* To specify the type of detector used to acquire the data

Select **MS** in the Detector Type list.

Selecting the Peak Detection Algorithm

Use the Peak Detect list on the Identification page to specify the peak detection algorithm to use (ICIS, Genesis, or Avalon) to analyze raw data.

These algorithms do the following:

- Apply smoothing
- Construct a chromatogram using the scan or mass filters
- Assign peak numbers
- Generate a peak list
- Determine the peak start and peak end points

All algorithms provide component peak detection and chromatographic peak detection. Select the ICIS or Genesis algorithms for MS data. Select the Avalon algorithm for PDA, UV, and analog data.

To specify the peak detection algorithm

Select ICIS in the Peak Detect list.

Matching Scan Filters with Components

The Xcalibur data system creates unique scan filters to acquire data according to the type of experiment specified in the instrument method. When you load a raw data file, the data system lists the scan filters associated with the raw data file in the Filter box. In this example, the application acquired selected reaction monitoring (SRM) data on the following compounds, using alternating product ion scans (drugx and D4, respectively):

- A proprietary drug of molecular weight 465 u (precursor ion *m/z* 465; product ion *m/z* 420)
- A deuterated internal standard of molecular weight 469 u (precursor ion *m/z* 469; product ion *m/z* 424)

To quantify drugx, use the following filtered mass chromatograms for drugx and its internal standard, D4.

drug x (analyte)	TIC F: + c SRM ms2 465.30@23.00[419.30–421.30]
D4 (internal standard)	TIC F: + c SRM ms2 469.40@23.00[423.30-425.30]

* To match the internal standard, D4, with its scan filter

1. From the Filter list for the drugx_03.raw file, select the following scan filter for D4:

+ c SRM ms2 469.40@23.00[423.30-425.30]

Note The scan filter indicates a positive, centroid profile, selected reaction monitoring experiment type (SRM), MS/MS scan, precursor ion scan of m/z 469.40 at 23.0 units of CID energy with a product mass-to-charge range of 423.30–425.30.

2. Click **OK** to display the mass chromatogram for D4 (Figure 14).

🧱 Thermo 🕽	Kcalibur Processing Setup - Quan - Identification - Untitled (Int Std)	
<u>File View Z</u> o	oom Options GoTo Help	
	Identification Detection Calibration Levels System Suitability Peak Purity	Components
	Name: D4	D4
Quan	Detector type: MS Peak Detect: ICIS Use as RT reference View width (min): 0.75	
	Fijter: + c SRM ms2 469.40@23.00 [423.30-425.30] ▼ Irace: TIC	
Qua	Mass (m/z):	
Reports		
B	OK Cancel Save As Default Help	
Programs	C:\Xcalibur\examples\data\drugx_03 5/4/1996 10:31:23 PM	
	RT: 0.00-6.99 SM: 16 4.89 NL: 2.1165 100 9 80 100 100 100 100 100 100 100 100 100 1	
Ready		IOT SAVED
		10
	Chromatogram plot view	

Figure 14.	Scan filter mass chromatogram and mass spectrum for D)4
------------	---	----

Selecting the Trace Type

Use the Trace list on the Identification page for Quan view to specify the type of chromatogram to use for processing.

***** To specify the type of chromatogram

In the Trace list, select **TIC** to specify total ion current.

Entering the Retention Time of the Component Automatically

You can enter the expected retention time and retention time window for a component manually by typing the values in their respective boxes or automatically by working with the chromatogram and spectrum views.

✤ To automatically enter the retention time of a chromatographic peak

1. To make the spectrum view the active and pinned view, click the pin icon, 🔛, in the upper right corner of the cell (Figure 15).

The pin background turns green $\ensuremath{\textcircled{}}$ and appears to be pinned to the screen.



Figure 15. Inactive chromatogram view and pinned spectrum view

2. In the chromatogram view, drag the cursor across the chromatogram peak as shown in Figure 16.

The horizontal line across the center of the peak shows the time range selected by the mouse movement.

Figure 16. Selecting the scan (time point) corresponding to the peak maximum



3. Release the mouse button.

The data system automatically does the following:

• Selects the retention time of the peak apex and highlights the selected scan with a vertical red marker in the chromatogram view (Figure 17).

Figure 17. Vertical red marker at the peak apex



- Displays the retention time corresponding to the selected scan in the Expected box in the Retention Time area (Figure 18).
- Displays the mass spectrum of the product ions in the spectrum view (Figure 18).

Specifying the Identification Settings for Analysis Components



🖁 Thermo Xcalibur Processing Setup - Quan - Identification - Untitled (Int Std)	
ile <u>V</u> iew Zoom Options <u>G</u> oTo <u>H</u> elp	
Identification Detection Calibration Levels System Suitability Peak Purity Mame: D4 Image: Component Expected (min): 4.89 Mindow (sec): 30.00 Detector type: MS Peak Detect: ICIS Image: Component Expected (min): 4.89 Mindow (sec): 30.00 Image: Component Expected (min): Image: Component Expected (min): 4.89 Mindow (sec): 30.00 Image: Component Expected (min): Image: Component Expected (min): 4.89 Mindow (sec): 30.00 Image: Component Expected (min): Image: Component Expected (min): 4.89 Mindow (sec): 30.00 Image: Component Expected (min): Image: Component Expected (min): 4.89 Mindow (sec): 30.00 Image: Component Expected (min): Image: Component Expected (min): Image: Component Expected (min): 1.89 Mindow (sec): 30.00 Image: Component Expected (min): Image: Component Expected (min): Image: Component Expected (min): 0.75 Image: Compon	ts
OK Cancel Save As Default Help	
Programs C:tXcaliburtexamplestdatatdrugx_03 5/4/1996 10:31:23 PM STD=10 pg/ml,IS=100 pg/ml	
4.89 4.89 4.89 4.82 4.97 4.82 4.97 4.79 4.70 4.79	
eady NUM NOT S	AVED

4. To use the actual retention time of D4 as the retention time reference for the other component in the drugx chromatogram, select the **Use as RT Reference** check box.

Note To adjust the expected retention time of a component relative to the reference component, select the **Adjust Using** check box. See step 8 of the next procedure, Specifying the Identification Settings for the Target Compound.

Leave the Window box number at **30** seconds. This parameter controls the width of the window that the algorithm searches to locate the component. In this case the algorithm searches 15 seconds on either side of RT 4.89 minutes to find the D4 peak.

5. Type **2.00** in the View Width box.

View width controls how much of the chromatogram time range the application displays when you view the components in the chromatogram view.
6. To save the component identification information for D4, click **OK**.

The chromatogram view displays the time portion of the chromatogram from 3.9 to 5.9 minutes based on a selection of 2.00 minutes as the view width for a peak with an expected retention time of 4.9 min. Integrated peaks are shaded in gray with blue integration markers at the starting and ending points of the peak. The baseline is indicated by a blue line that connects the integration markers (Figure 19).

Figure 19. Area of peak integration (grayed) and the integration markers



- 7. Inspect the integrated peak and verify the following:
 - The retention time on the peak agrees with that in the Expected box in the Retention Time area.
 - The scan filter in the Filter box is matched to the correct component in the Components list.

If the peak has not been identified, repeat this procedure. Click the pin icon, 2, in the spectrum view (the pane to the right of the chromatogram plot view) before performing step 2 on page 26.

If the peak has been identified properly, you are ready to specify the peak identification parameters for the target compound.

Tip When you are entering many components with similar peak integration parameters, first enter all of the identification parameters for one of the components. Click **Save As Default**. These parameters then become the default values for new components.

Specifying the Identification Settings for the Target Compound

The procedure for identifying the target compound is the same as that for identifying the internal standard. In the following procedure, you use the internal standard that you identified in Specifying the Identification Settings for the Internal Standard as the retention time reference component.

* To specify the identification settings for the target compound

- 1. To specify a name for the target compound, do the following:
 - a. Select **<New>** in the Name list.

The Apply Changes dialog box opens if you have warnings enabled.

- b. Click **Yes** to apply changes.
- c. To specify the name of the target compound, type **drugx**.
- d. Click **OK** to save the new name.

The target compound name appears in the Components list.

- 2. Select **MS** in the Detector Type list if it is not already selected.
- 3. Select **ICIS** in the Peak Detect list.
- 4. To match the target compound with its scan filter, do the following:
 - a. In the Filter list, select the following filter for drugx:

+ c SRM ms2 465.30@23.00[419.30-421.30]

b. Click **OK** to apply the scan filter to the total ion current.

Processing Setup automatically displays the mass chromatogram corresponding to the target compound.

- 5. Select **TIC** (total ion current) in the Trace list if it is not already selected.
- 6. To display the mass spectrum of the currently active component and automatically enter the retention time of the target compound peak, do the following:
 - a. To make the spectrum view the active and pinned view, click the pin icon, 🕮, in the upper right corner of the cell.

The pin background turns green \Im and appears to be pinned to the screen.

- b. Drag the cursor in the chromatogram view across the chromatographic peak.
- c. Release the mouse button.

The data system automatically does the following:

- Selects the retention time of the peak apex and highlights the selected scan with a red marker in the chromatogram view.
- Enters the retention time corresponding to the selected scan in the Expected box in the Retention Time area.
- 7. Type **2.00** into the View Width box.
- 8. To adjust the expected retention time of drugx by changes in the actual retention time of D4, do the following:
 - a. Select the Adjust Using check box.

The Adjust Using list becomes available.

b. Select **D4** in the Adjust Using list.

You selected D4, the internal standard, as the retention time reference component in step 4 of the previous procedure, Specifying the Identification Settings for the Internal Standard.

- 9. Leave the value in the Window box at **30** seconds.
- 10. To accept the peak identification settings for drugx, click OK.

The chromatogram view displays the time portion of the chromatogram from 3.9 to 5.9 minutes based on a selection of 2.00 minutes as the view width for a peak with an expected retention time of 4.9 min. Integrated peaks are shaded in gray with blue integration markers at the starting and ending points of the peak. The baseline is indicated by a blue line that connects the integration markers (Figure 20).



Figure 20. Peak identification settings for drugx, the target compound

Entering Peak Integration and Detection Parameters

Use the Detection page for the Quan view in the Processing Setup window to enter peak integration and detection parameters.

To enter peak integration and detection parameters, follow these procedures in order:

- 1. Entering the Peak Integration Parameters
- 2. Entering the Peak Detection Parameters

Entering the Peak Integration Parameters

Enter peak integration parameters to specify how the data system determines the area of each peak in the chromatogram. Processing Setup provides peak integration parameter options in the Peak Integration area on the Detection page.

To enter the ICIS peak integration settings for the internal standard and the target compound

1. Click the **Detection** tab.

The Detection page for the Quan view opens (Figure 21).

Identification Detection Calibration	Levels System Suitability Peak Purity	
ICIS Peak Integration Smoothing points: Baseline window: 40 Area noise factor: 5 Peak ngise factor: 10 Max Constrain peak width Peak height (2): 5.0	ICIS Peak Detection Highest peak Nearest <u>B</u>T Minimum peak height (S/N): 3.0 	Components D4 drugx
Tailing factor: 1.0 OK Cancel <u>Save As De</u>	fault Advanced Flags Help	•
C:1Xcaliburtexamplestdatatdrugx_03	5/4/1996 10:31:23 PM	-
STD=10 pg/ml,IS=100 pg/ml RT: 4.90 NL: 1 TIC F 465.3 [419.3] ICIS c 4.62 5.47 4 5 Time (min)	.61E4 : + c SRM ms2 0@cid23.00 30-421.30] MS drugx_03 	-

Figure 21. Detection page for the Quan view of the Processing Setup window

- 2. In the Components list, select D4.
- 3. To enter the peak integration settings for the selected component, do the following:
 - a. Type **5** in the Smoothing Points box in the ICIS Peak Integration area.

Based on the peak view, type the number of points in the moving average that are used to smooth data in the Smoothing Points box. The valid range is 1 (no smoothing) to 15 (maximum smoothing).

b. To set the maximum number of scans that the application reviews for a local minimum to 40, type **40** in the Baseline Window box.

The valid range is 1 through 500.

c. To specify an area noise factor of 5, type **5** in the Area Noise Factor box.

The area noise factor is a noise level multiplier used to determine the location of a peak edge after the location of the possible peak. The valid range is 1 to 500.

d. Type 10 in the Peak Noise Factor box.

The peak noise factor is a noise level multiplier used to determine the potential peak signal threshold. The valid range is 1 to 1000.

e. Clear the **Constrain Peak Width** check box.

Note This tutorial does not use the constrain peak width option.

Use the constrain peak width option to control how much of the peak is integrated by specifying a peak height threshold and a tailing factor. Select the **Constrain Peak Width** check box to make the Peak Height (%) and Tailing Factor parameters available. The valid range for the peak height threshold is 0.0 to 100.0%. The valid range for the tailing factor is 0.5 to 9.0.

- 4. To save the peak integration parameters for D4, click OK.
- 5. To enter the peak integration settings for the target compound, do the following:
 - a. Select **drugx** in the Components list.
 - b. Repeat step 3 of this procedure.
 - c. To save the peak integration parameters for drugx, click **OK**.

Entering the Peak Detection Parameters

Peak detection parameters specify how the Xcalibur data system selects a chromatographic peak within the specified retention time window for a component.

To enter the peak detection parameters for the internal standard and the target compound

- 1. In the Components list, select D4, the internal standard for the target component drugx.
- 2. To associate the selected component with the highest peak in the filtered chromatogram, select the **Highest Peak** option in the ICIS Peak Detection area (Figure 22).

Figure 22. Highest Peak option selected

ICIS Peak Detection	
eak	
Nearest <u>R</u> T	
Minimum pea <u>k</u> height (S/N): 3.0	

3. To have the data system ignore all peaks that do not have a signal-to-noise ratio of 3 or greater, type **3** in the Minimum Peak Height (S/N) box.

The valid range is 0.0 (all peaks) through 999.0.

- 4. Verify that the advanced peak detection parameters are set to their default values as follows:
 - a. Click Advanced.

The ICIS Advanced Parameters dialog box opens (Figure 23).

Use the ICIS Advanced Parameters dialog box to specify advanced component detection criteria if the standard detection criteria do not provide the desired results. Refer to the *Xcalibur Data Acquisition and Processing User Guide* for information on the parameters in the ICIS Advanced Parameters dialog box. The application applies the settings in the ICIS Advanced Parameters dialog box on a per component basis.

b. Inspect the settings in the ICIS Advanced Parameters dialog box. Make sure the settings are the same as those in Figure 23.

Figure 23. ICIS Advanced Parameters dialog box, showing the default settings

Noise Method		
INCOS Noise		
C Repetitive Noise		
□ RMS		
Peak Parameters		
Min peak widtl	n: 3	
Multiplet resolution	n: 10	
Area tail extension	n: 5	
Area scan window	v: 0	
Area scan windov	v: JU	
OK Cancel	Save As Default	Help

c. Click OK to close the ICIS Advanced Parameters dialog box.

- 5. To save the peak detection parameters, click **OK**.
- 6. In the Components list, select **drugx**, the target compound (analyte) for this analysis.
- 7. Repeat step 2 through step 5 of this procedure for drugx, the target compound.
- 8. On the Detection page, verify the following settings for drugx and D4. Select one component in the Components list and check the settings. Then, select the other component in the Components list and check the settings.

Parameter	Setting
ICIS Peak Integration	
Smoothing Points	5
Baseline Window	40
Area Noise Factor	5
Peak Noise Factor	10
Constrain Peak Width	Clear
ICIS Peak Detection	
Highest Peak	Selected
Minimum Peak Height (S/N)	3

9. Verify that the scan filter chromatogram for drugx matches Figure 24.

Figure 24. Scan filter chromatogram for drugx



10. Verify that the scan filter chromatogram for D4 matches Figure 25.



Figure 25. Scan filter chromatogram for D4

Selecting the Calibration Settings

In the Processing Setup window, use the Calibration page of the Quan view to specify the calibration curve type. When using the internal standard calibration technique, use this page to associate the internal standard with a target compound and to specify the amount of internal standard used to spike the calibration standards and unknowns.

✤ To select the calibration settings

1. Click the **Calibration** tab.

The Calibration page of the Quan view opens.

- 2. To enter the calibration settings for the internal standard, component D4, do the following:
 - a. In the Components list, click D4.
 - b. To select D4 as the internal standard, select the **ISTD** option in the Component Type area.
 - c. To specify an internal standard amount of 100 pg/mL, type **100** in the Amount box in the ISTD (Internal Standard) area.
 - d. To specify pg/mL as the units of concentration, type **pg/mL** in the Units box.
- 3. To save the settings for D4, click OK.
- 4. Verify that the calibration settings for component D4 match those in Figure 26.

🚟 Thermo Xcalibur Processing Setup - Quan - Calibration - drugx.pmd (Int Std)					
<u>F</u> ile ⊻iew <u>O</u> p	File <u>V</u> iew <u>O</u> ptions <u>G</u> oTo <u>H</u> elp				
	Identification Detectio	n Calibration	Levels System Suitability Peak Purity	[Components]	
	Component type	- Target compounds-		D4 drugx	
Ouan	C Iarget compound	IS <u>T</u> D:	- Isota	pe <u>%</u>	
guan	ISTD	_ Weighting	Cali <u>b</u> ration curve: Linear		
		Egual	l prite:		
	-ISTD	O 1/ <u>×</u>			
Qual		0.1/X12			
	A <u>m</u> ount: 100.000	0 1/ <u>1</u>	© Force		
	Units: pg/ml	O 1/s^2	C Include C Height		
Reports					
	OK Cancel	Save As Defa	ult Flags Help		
Programs	•				

Figure 26. Calibration	page, showing	the settings fo	or the D4 interna	standard
------------------------	---------------	-----------------	-------------------	----------

This procedure does not discuss setting parameters on the System Suitability or Peak Purity pages. For information about setting these parameters, refer to the *Xcalibur Data Acquisition and Processing User Guide*.

* To enter the calibration settings for the drugx target compound

- 1. Select **drugx** in the Components list.
- 2. To specify drugx as a target compound, select the **Target Compound** option in the Component Type area.
- 3. To specify D4 as the internal standard for the target compound, select **D4** in the ISTD list in the Target Compounds area.
- 4. To specify a quadratic fit calibration curve, select Quadratic in the Calibration Curve list.
- 5. To specify the units of concentration, type $pg/10 \mu L$ in the Units box.
- 6. To specify a weighting of $1/X^2$, select the $1/X^2$ option in the Weighting area.
- 7. To exclude the origin as a data point when fitting the calibration curve, select the **Ignore** option in the Origin area.
- 8. To use the area of the peak to determine response, select the **Area** option in the Response area.
- 9. To save the settings, click OK.

10. Verify that the calibration settings for the drugx (target component) are the same as those in Figure 27.

👹 Thermo 🕽	Calibur Processing Setu	p - Quan - Calibr	ation - Untitled (Int Std)		
<u>Fi</u> le ⊻iew <u>O</u> r	<u>File Yiew Options G</u> oTo <u>H</u> elp				
🗅 🖻 🛛		◆ ♠ →←	$\leftrightarrow \leftrightarrow \diamondsuit $		
4.67	Identification Detecti	ion Calibration	Levels System Suitability Peak Purity	Components	
	Component type	Target compounds-			
Quan	 Target compound 	ISTD: D4	V Isotope	%	
LA IL	○ ISTD	Weighting C Equal	Calibration curve: Quadratic 💙		
Qual		01/X	Units: pg/10ul		
	ISTD-	⊙ 1/X ²	Origin		
	Amount: 1.000	01/1	Ignore Response		
Reports	Units:	○1/Y ²	Include Area		
R.					
	OK Cance	el Save As Defa	ult Flags Help		
Programs	•				

Figure 27. Settings for the drugx target compound

Specifying Calibration Levels and QC Levels

In Processing Setup, use the Levels page of the Quan view to specify the amount of target compound in each calibration level. The Xcalibur data system uses the calibration levels information to construct the calibration curve as it processes a sequence. Also specify the amount of target compound in the QC check standards. The application uses the QC standards to check the stability of the LC/MS instrument during a sequence run.

To set up the calibration levels for the target compounds and the QC samples, follow these topics:

- Specifying the Calibration Levels of the Target Compound
- Specifying the QC Levels

Specifying the Calibration Levels of the Target Compound

Use the Levels page to set up the calibration levels for the target compound.

* To specify the calibration levels of the target compound

- 1. In the Quan view of the Processing Setup window, select **drugx** in the Components list.
- 2. Click the **Levels** tab.

The Levels page for Quan view opens (Figure 28).

Note If you select D4 and then try to open the Levels page, a warning message appears.

The Levels page is not available for ISTD components.

Figure 28. Level	s page for	Quan view
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🔛 Thermo Xo	calibur Processing Setup - Quan	- Levels - Untitled (Int Sto	I)	
<u>F</u> ile <u>V</u> iew	<u>O</u> ptions <u>G</u> oTo <u>H</u> elp			
🗅 🖻	🚽 🚑 🎒 🕇 🖊 :		→ ◆ ?	
	Identification Detection	Calibration Levels	System Suitability Peak Purity	Components
1 Å		Units: p	g/ml	D4 drugx
	Cal Level	Amount 🔺	QC Level Amount % Test	
Quan	1 cal 1	10.000	* 0.010 0.00	
	2 cal 2	25.000		
	3 cal 3	50.000		
Qual	4 cal 4	100.000	=	
	5 _{cal 5}	200.000		
ž.	6 cal 6	400.000		
Reports		<u> </u>		
	OK Cancel	Save As Default	Help	
Programs	•		4	
Ready				1. I I I I I I I I I I I I I I I I I I I

- 3. Enter the calibration level information for the target compound as follows:
 - a. To specify a name for the first calibration level, type **cal 1** in the Cal Level column of the first row. Then, press the TAB key to advance the cursor to the Amount column.
 - b. To specify an injection amount of 10 pg, type 10 in the Amount column.
 - c. To create a new row and to advance the cursor to the next Cal Level box, press the TAB key twice.

d. Repeat this procedure for each of the nine calibration levels as shown in Table 1. The table shows the number of picograms of drugx injected in 10 μ L of the corresponding calibration solution.

	Cal Level	Amount
1	cal 1	10
2	cal 2	25
3	cal 3	50
4	cal 4	100
5	cal 5	200
6	cal 6	400
7	cal 7	600
8	cal 8	800
9	cal 9	1000

 Table 1.
 Calibration level table

Specifying the QC Levels

Use the QC levels table on the Levels page to set up the quality control specifications.

✤ To specify the QC levels

- 1. To specify the name of the first QC level, type **QC 1** in the QC Level column of the first row. Then, press the TAB key to advance the cursor to the Amount column.
- 2. To specify an injection amount of 10 pg, type **10** in the Amount column. Then, press the TAB key to advance the cursor to the %Test column.
- 3. To specify an acceptable difference of 20%, type **20** in the %Test column.

Note The %Test values for QCs in this example are shown in Table 2. The data system uses the criteria in this example to determine whether QCs pass.

For your applications, type a value in the %Test column for the acceptable difference (as a percent) between the specified amount and the calculated amount of each QC level.

- 4. To create a new row and advance the cursor to the QC Level column in the second row, press the TAB key twice.
- 5. Repeat this procedure until you fill in the three QC levels as shown in Table 2.

Table 2.	QC sample :	specifications
----------	-------------	----------------

	QC Level	Amount	% Test
1	QC 1	10	20
2	QC 2	400	15
3	QC 3	1000	15

- 6. To save the calibration and QC level settings, click **OK**.
- 7. Verify that the entries in the calibration standard and quality control tables are the same as those in Figure 29.

Figure 29. Levels page, showing the completed calibration and quality control tables

	📓 Thermo Xcalibur Processing Setup - Quan - Levels - Untitled (Int Std)										
File	2	View Options GoTo	Help								
	2	🖻 🔒 🚑 🎒	* + *	÷ →	i€ €	⇒ ↔ ⊕	?				
	lder	ntification Detection	Calibration	Levels	Syst	em Suitability	Peak P	urity	^		Components
				Units: pg/r	nl						D4 drugx
		Cal Level	Amount			QC Level	Amount	% Test			
	1	cal 1	10.000		1	QC1	10.000	20.00			
	2	cal 2	25.000		2	QC2	400.000	15.00			
	3	cal 3	50.000		3	QC#	1000.000	15.00	Ξ		
	4	cal 4	100.000		*		0.010	0.00			
	5	cal 5	200.000								
	6	cal 6	400.000								
	7	cal 7	600.000								
	OK Cancel Save As Default Help										
Rea	dy										

Saving the Processing Method

Before you exit the Processing Setup window, save the processing method.

- To save the processing method
- 1. Choose **File > Save As**.

The File Summary Information dialog box opens.

2. Type Processing method for drugx example in the Comment box (Figure 30).

Figure 30. File Summary Information dialog box

File Summary Infor	mation	×
Header:		
Created: Last saved by: Number of saves	Friday, June 14, 2013 12:45:47 PM lisa.cook (Not changed)	
<u>C</u> omment:		
	UK Cancel <u>H</u> elp)

3. Click OK.

The Save As dialog box opens.

- 4. Browse to the C:\Xcalibur\examples\methods folder or the directory where you saved the data system examples.
- 5. Type **drugx_example.pmd** in the File Name box.
- 6. To save the processing method and close the dialog box, click Save.

Now that you have created a processing method, you are ready to batch reprocess the drugx data file set. Go to Tutorial 2: Batch Reprocessing Data Files.

2 Tutorial 1: Creating a Processing Method Saving the Processing Method

Tutorial 2: Batch Reprocessing Data Files

After creating a processing method, add it to the sequence used to acquire the drugx data set. After the Xcalibur data system batch reprocesses the sequence with the processing method, the data system creates a result file for each raw data file.

Whether you are processing or reprocessing the data, the results are the same.

This tutorial describes how to add a processing method to a sequence and how to batch reprocess the sequence that contains the drugx raw data files.

Contents

- Adding a Processing Method to a Sequence
- Batch Reprocessing the Sequence to Produce Result Files

Adding a Processing Method to a Sequence

An Xcalibur sequence is a list containing sample acquisition and processing information. Sequence files in the Xcalibur data system have an .sld file name extension.

Sequences use one or more instrument methods to acquire data and one or more processing methods to process the data. Instrument methods (METH files) contain the chromatographic and data acquisition parameters for an LC/MS or GC/MS instrument. The processing methods (PMD files) contain the sample processing parameters for a qualitative analysis, a quantitative analysis, or a combination of both analysis types. You must add an instrument method to the sequence list to run the sequence. You can add a processing method to the sequence before or after you run the sequence.

For both bracketed (open, non-overlapping, or overlapping) and unbracketed sequences, you can select a different instrument method for each injection. When you are working with a bracketed sequence, you can select only one processing method for the sequence.

The example sequence that you are working with in this tutorial is an open-bracketed sequence. All sequences that you create manually by entering the sample information in the sequence rows of the Sequence Setup view are open-bracketed sequences.

For information about using the New Sequence Template dialog box to create unbracketed sequences or sequences with non-overlapping or overlapping brackets, refer to the *Xcalibur Data Acquisition and Processing User Guide*.

To quantify the drugx target component in the example data files, add the drugx_example.pmd processing method to the existing drugx.sld sequence. You created this processing method by following Tutorial 1: Creating a Processing Method.

✤ To add the processing method to the sequence

1. If you are working in the Processing Setup window, choose **GoTo > Xcalibur Home Page** from the menu bar.

The Xcalibur home page window opens.

2. To open the Sequence Setup view, do one of the following:

• On the Roadmap view, click the **Sequence Setup** icon, **Sq**

-or-

- From the menu bar, choose **View** > **Sequence Setup View**.
- 3. Choose File > Open.

The Open dialog box opens.

4. Browse to the following folder:

drive:\Xcalibur\examples\methods

5. Select **drugx.sld** and click **Open**.

The drugx.sld sequence opens in the Sequence Setup view (Figure 31).



🔀 The	🗙 Thermo Xcalibur Sequence Setup - drugx.sld [Open]									
<u>F</u> ile	<u>E</u> dit <u>C</u> hange <u>A</u> ct	tions <u>V</u> iew	<u>G</u> oTo <u>H</u> elp							
7	W 🗠 🛐	🗋 🚔	▋ॖॖॖॖॖॖॖऀऀ॒ऀॄ) III 🖉 📏 🕅 III	🗟 🕂 🖶 🖾 🕨	?				
	Sample Type	File Name	Path	Inst Meth	Proc Meth	Level 🔺				
1	Std Bracket	drugx_01	C:Wcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	cal 1				
2	Std Bracket	drugx_02	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 1				
3	Std Bracket	drugx_03	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal1				
4	Std Bracket	drugx_04	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	cal 2				
5	Std Bracket	drugx_05	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 3				
6	Std Bracket	drugx_06	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 4				
7	Std Bracket	drugx_07	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 5				
8	Std Bracket	drugx_08	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 6				
9	Std Bracket	drugx_09	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 7				
10	Std Bracket	drugx_10	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 8				
11	Std Bracket	drugx_11	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:Wcalibur\examples\methods\drugx	cal 9				
12	Std Bracket	drugx_12	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 9				
13	Std Bracket	drugx_13	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	cal 9				
14	QC	drugx_14	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC1				
15	QC	drugx_15	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Vcalibur\examples\methods\drugx	QC1				
16	QC	drugx_16	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	QC1				
17	QC	drugx_17	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Vcalibur\examples\methods\drugx	QC1				
18	QC	drugx_18	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC1				
19	QC	drugx_19	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	QC1				
20	QC	drugx_20	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC2				
21	QC	drugx_21	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC2				
22	QC	drugx_22	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC2				
23	QC	drugx_23	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC2				
24	QC	drugx_24	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC2				
25	QC	drugx_25	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC2				
26	QC	drugx_26	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC 3				
27	QC	drugx_27	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Xcalibur\examples\methods\drugx	QC 3				
28	QC	drugx_28	C:Wcalibur\examples\data	C:\Calibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	QC 3				
29	QC	drugx_29	C:Wcalibur\examples\data	C:\Calibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	QC 3				
30	QC	drugx_30	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	QC 3				
31	QC	drugx_31	C:\Xcalibur\examples\data	C:\Xcalibur\examples\methods\drugx	C:\Calibur\examples\methods\drugx	QC 3				
*										
For Hel	p, press F1				6/12/2015 7:30 PM	NOT SAVED				

- 6. To replace the processing method with your new processing method, do the following:
 - a. Double-click any row in the Proc Meth column.

The Select Processing Method dialog box opens.

b. Browse to the folder where you stored your new processing method.

drive:\Xcalibur\examples\methods

Note If you have not already created a new processing method, create one by following the procedures in Tutorial 1: Creating a Processing Method.

- c. Select **drugx_example.pmd** and click **Open** to enter the new processing method in all the rows of the sequence.
- 7. Change some of the QC sample types to unknown sample types as follows:
 - a. In row 14, click the Sample Type column and select the **Unknown** sample type from the list.
 - b. Repeat this procedure to select the Unknown sample type for rows 15 to 18, 20 to 24, and 26 to 30.

Notice that the Level column lists the calibration level of each standard. When you add a quantitative processing method to a sequence and select a standard sample type in the Sample Type column (Std Bracket for an open-bracket sequence), the Level list appears in the Level column (Figure 32). To finish setting up the calibration information, you must select the appropriate calibration level for each standard sample.

Also notice that the Level column still lists the QC names for the QC samples you changed to the Unknown sample type. The data system updates the information in the Level column when you save the sequence file.

	Sample Type	File Name	Proc Meth	Level
1	Std Bracket	drugx_01	C:\Xcalibur\examples\methods\drugx	cal 1
2	Std Bracket	drugx_02	C:\Xcalibur\examples\methods\drugx	_
3	Std Bracket	drugx_03	C:\Xcalibur\examples\methods\drugx	cal 1
4	Std Bracket	drugx_04	C:\Xcalibur\examples\methods\drugx	cal 2
5	Std Bracket	drugx_05	C:\Xcalibur\examples\methods\drugx	cal 3
6	Std Bracket	drugx_06	C:\Xcalibur\examples\methods\drugx	cal 4
7	Std Bracket	drugx_07	C:\Xcalibur\examples\methods\drugx	cal 5
8	Std Bracket	drugx_08	C:\Xcalibur\examples\methods\drugx	cal 6
9	Std Bracket	drugx_09	C:\Xcalibur\examples\methods\drugx	cal 7
10	Unknown	drugx_10	C:\Xcalibur\examples\methods\drugx	cal 8
11	Unknown	drugx_11	C:\Xcalibur\examples\methods\drugx	cal 9

Figure 32. Level list for the drugx processing method

8. To save the sequence, choose **File > Save As**.

The File Summary Information dialog box appears.

9. Type sample Drugx_Example sequence in the Comment box (Figure 33).

Figure 33. File Summary Information dialog box

File Summary Inform	nation	×
Header:		
Created: Modified: Last saved by: Number of saves:	Thursday, June 20, 1996 4:55:27 PM Tuesday, January 27, 1998 10:43:23 AM Hemenway 2	
<u>C</u> omment: sample DrugX_Exam	ple sequence	
	OK Cancel <u>H</u> elp	

10. Click OK.

The Save As dialog box opens.

11. Type drugx_example in the File Name box and click Save.

The sequence is saved as drugx_example.sld in the following folder:

drive:\Xcalibur\examples\methods

- 12. Verify that the sequence now looks like the one shown in Figure 34.
 - The ProcMeth column lists your new processing method, drugx_example.
 - In the Sample Type column, rows 14–18, 20–24, and 26–30 list the Unknown sample type.
 - In the Level column, rows 14–18, 20–24, and 26–30 are blank.

The information in the Instrument Method, Position, and Inj Vol columns is required for data acquisition, but this information is not used for data processing.

Adding a Processing Method to a Sequence

Thermo Xcalibur Sequence Setup - drugx_example.sld [Open] - Dataset: new													
le <u>t</u>	dit <u>C</u> hange <u>A</u>	tions <u>v</u> iew	<u>G</u> 010 <u>H</u> e	Ip									
I [W 🗠 📲	🗋 🖆	-		ю III	JE 🏷	F	1 📷	₩ [6
	Sample Type	File Name	Pa	th	Ir	nst Meth			Pro	oc Meth		Leve	ł
1	Std Bracket	drugx_01	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 1	
2	Std Bracket	drugx_02	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 1	
3	Std Bracket	drugx_03	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 1	
4	Std Bracket	drugx_04	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 2	
5	Std Bracket	drugx_05	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 3	
6	Std Bracket	drugx_06	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 4	
7	Std Bracket	drugx_07	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 5	
8	Std Bracket	drugx_08	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 6	
9	Std Bracket	drugx_09	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 7	
0	Std Bracket	drugx_10	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 8	
1	Std Bracket	drugx_11	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 9	
2	Std Bracket	drugx_12	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 9	
3	Std Bracket	drugx_13	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples/metho	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple cal 9	
4	Unknown	drugx_14	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
5	Unknown	drugx_15	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
6	Unknown	drugx_16	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
7	Unknown	drugx_17	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
8	Unknown	drugx_18	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
9	QC	drugx_19	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple QC1	
0	Unknown	drugx_20	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
1	Unknown	drugx_21	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wcalibu	r\examples	\methods\	drugx_exar	nple	
2	Unknown	drugx_22	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
23	Unknown	drugx_23	C:\Xcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	- ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
24	Unknown	drugx_24	C:\Xcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	- ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
25	QC	drugx_25	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple QC 2	
26	Unknown	drugx_26	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
27	Unknown	drugx_27	C:Wcalibur\ex	amples\data	C:Wealibur\ex	amples\metho	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
28	Unknown	drugx_28	C:\Xcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
9	Unknown	drugx_29	C:\Xcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	- ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	_
30	Unknown	drugx_30	C:Wcalibur\ex	amples\data	C:Wcalibur\ex	amples\metho	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple	
81 *	QC	drugx_31	C:\Xcalibur\ex	amples\data	C:\Xcalibur\ex	amples\meth	ods\drugx	C:Wealibu	r\examples	\methods\	drugx_exar	nple QC3	_
lel	o, press F1							NUN	4	5/14/2013	1:41 PM		

Figure 34. Drugx sequence, with drugx_example.pmd selected as the processing method

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Batch Reprocessing the Sequence to Produce Result Files

After adding a processing method to the sequence, batch process data files to produce result files (RST) that you can view in the Quan Browser window.

- To batch reprocess the sequence and perform a quantitative analysis on the raw data files
- 1. Do one of the following:



-or-

• Choose Actions > Batch Reprocess.

The Batch Reprocess Setup dialog box opens (Figure 35).

Figure 35. Batch Reprocess Setup dialog box

Batch Reprocess Setup	—
Processing Actions Image: Optimized Control Image: Optimized Control	Process Ro <u>w</u> s: 1-31
Calibration Quantitation	
Qual Peak Detection & Integration Spectrum Enhancement	
 Library Search Reports Print Sample Reports Print Summary Reports 	
Programs Create Quan Summary Spreadsheet	
Advanced Options Replace Sample <u>I</u> nfo	
OK	<u>H</u> elp

- 2. To set up the batch process options as shown in Figure 35, select the Quan, Peak Detection & Integration, and Quantitation check boxes.
- 3. Make sure that the Process Rows box displays 1-31.

4. To start batch reprocessing, click OK.

For each raw data file, the data system creates a result file with the same name. The data system exports the result files to the folder where the raw data files are located.

In this example, the raw data files and result files are in the following folder (Figure 36):

drive:\Xcalibur\examples\data\

Figure 36. Explore directory, showing the location of the raw data files (RAW) and the result files (RST)

					_	
Comp	outer	r 🕨 OSDisk (C:) 🕨 Xcalik	our 🕨 examples 🕨 data	▼ ⁴ y Search d	ata	Q
<u>File Edit View To</u>	ools	<u>H</u> elp				
Organize 💌 🛄 Or	nen i	withQual Browser	E-mail Burn Ne	aw folder 📴 🔻		
			- Juni Dani Na			
A SDisk (C:)	Ê	Name	Date	Туре	Ĺ	
SWINDOWS.~BT		📠 drugx_01.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
SWINDOWS.~LS		drugx_01.RST	6/18/2015 2:02 PM	Xcalibur Result File		
Dell		📠 drugx_02.raw	6/18/2015 2:02 PM	Xcalibur Raw File	=	
Drivers		drugx_02.RST	6/18/2015 2:02 PM	Xcalibur Result File		
Program Files		📠 drugx_03.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
ProgramData		drugx_03.RST	6/18/2015 2:02 PM	Xcalibur Result File		
> inermo	=	📠 drugx_04.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
Disers		drugx_04.RST	6/18/2015 2:02 PM	Xcalibur Result File		
Vindows		📠 drugx_05.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
A 📗 Xcalibur		drugx_05.RST	6/18/2015 2:02 PM	Xcalibur Result File		
D 📗 data		📠 drugx_06.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
4 🃗 examples		drugx_06.RST	6/18/2015 2:02 PM	Xcalibur Result File		
🍌 data		📠 drugx_07.raw	6/18/2015 2:02 PM	Xcalibur Raw File		Select a file
📗 methods		drugx_07.RST	6/18/2015 2:02 PM	Xcalibur Result File		to preview.
🚔 DVD RW Drive (D:)		Langx_08.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
📬 Network		drugx_08.RST	6/18/2015 2:02 PM	Xcalibur Result File		
🐖 Control Panel		🛄 drugx 09.raw	6/18/2015 2:02 PM	Xcalibur Raw File		
	-	drugx 09.RST	6/18/2015 2:02 PM	Xcalibur Result File		
111 item	s					

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Tutorial 3: Working with Result Files in Quan Browser

After adding a processing method to the sequence and then batch reprocessing the sequence, review the result files and the calibration curve for the drugx target component in the Quan Browser window. If necessary, you can modify the identification, detection, integration, and some of the calibration parameters contained in the processing method.

This tutorial describes how to review quantitation data and how to modify portions of the processing method in the Quan Browser window.

Contents

- Reviewing a Sequence of Result Files in Quan Browser
- Modifying the Processing Method in Quan Browser

When the Xcalibur data system processes a bracketed sequence, it stores the calibration results for the quantified components in the result files (RST), bracket by bracket. When you open a processed sequence in the Quan Browser window, Quan Browser displays the list of associated result files by bracket.

The example drugx sequence is an open-bracketed sequence. This means that the entire sequence is treated as one bracket. When you open the processed sequence in the Quan Browser window and select the All tab to display all of the sample types, the Bracket in Use list displays Bracket 1. The Calibration File box lists the calibration in use as an Embedded Calibration (that is, the calibration information in use is embedded in the result files).

Reviewing a Sequence of Result Files in Quan Browser

In the Quan Browser window, review the quantitative analysis results of the processed drugx sample set. For additional information about using Quan Browser, refer to the *Xcalibur Quan Browser User Guide*.

* To review the results after you batch reprocess a sequence

- 1. To open the processed sequence for the drugx sample set in the Quan Browser window, do the following:
 - a. From the Xcalibur Home Page, choose **GoTo > Quan Browser**.

The Quan Browser window opens.

b. Choose **File > Open**.

The Open dialog box opens.

c. In the File of Types list, select **Sequence List Files (SLD)**.

In the Quan Browser window, you can open a sequence list of result files (SLD), a single result file (RST), or a Quan Browser file (XQN).

When the Xcalibur data system processes a sequence of raw data files, it creates one result file for each raw data file, gives the result file the same file name as the raw data file, and stores the result files (RST) in the same directory as the raw data files.

If you delete the result files or move them to a different directory than the directory listed in the sequence file, Quan Browser will not be able to locate the files.

d. In the Look In list, browse to the appropriate folder.

drive:\Xcalibur\examples\methods

Note If you have not already created a sequence with a processing method, create one by following the instructions in Tutorial 1: Creating a Processing Method and Tutorial 2: Batch Reprocessing Data Files. Or, open the drugx.sld sequence.

e. Select drugx_example.sld and click Open.

The View Sample Types dialog box opens (Figure 37).

Figure 37. View Sample Types dialog box



f. Select the Show All Sample Type option and click OK.

The drugx_example.sld sequence opens in the Quan Browser window. The results grid contains five tabbed pages: All, Standards, QCs, Blanks, and Unknowns. As shown in Figure 38, the Standards page in displayed in the results grid. The internal standard D4 is selected in the component list, and the calibration curve view is displayed in the companion view.

Figure 38. Quan Browser window with the internal standard D4 selected in the component list



- 2. To display all of the data files, click the All tab at the bottom of the results grid.
- 3. Scroll down the results grid and verify that the sample types and calibration levels are labeled correctly.
- 4. Select the first data file in the results grid.
- 5. Check each component in the data file for peak detection and integration problems as follows:
 - a. Select **D4** in the components list.

- b. Inspect the component peak in the chromatogram view as follows:
 - i. Verify that the data system found the peak.

The data system shades found peaks gray and marks the starting and ending points with square integration markers.

- ii. Make sure that the peak is integrated properly and that the shaded area accurately represents the contribution of the component to the chromatogram.
- c. If necessary, modify the peak detection or integration parameters for D4.
- d. Select **drugx** in the components list.
- e. Inspect the component peak in the chromatogram view and verify that the data system found the peak and that the peak is integrated properly.
- f. If necessary, modify the peak detection or integration parameters for drug x.
- g. Click the next row in the results grid view to select the next data file.
- 6. Repeat step 5 for each data file.
- 7. Check the calibration curve for the target compound, drug x, as follows:
 - a. In the Components list, select **drugx** to display the results for the target compound.
 - b. Inspect the calibration curve in the calibration curve view (Figure 39).
 - c. If necessary, modify the processing method's calibration curve parameters.

For more information about modifying processing methods in the Quan Browser window, see Modifying the Processing Method in Quan Browser.



Figure 39. Quan Browser window, with the target compound drugx selected

Modifying the Processing Method in Quan Browser

You can modify some sections of the processing method in Quan Browser. The sections that you can modify include the identification, detection, and integration parameters for the chromatographic peaks in the components list and several of the calibration curve parameters. You cannot modify the amounts for the calibration levels.

* To modify the processing method

- 1. If the sequence for the drugx sample set is not open in the Quan Browser application, open this sequence as described in step 1 of Reviewing a Sequence of Result Files in Quan Browser.
- 2. In the components list on the right side of the Quan Browser window, select **drugx**.

Note You can modify some of the settings for a processing method in the Quan Browser window:

- Use the User Peak Detection Settings dialog box to modify the component identification, detection, and integration settings.
- Use the Calibration Options dialog box to modify some of the calibration curve parameters.
- 3. To modify an identification, a detection, or an integration parameter, such as Constrain Peak Width, do the following:
 - a. Right-click the chromatogram plot view and choose **User Peak Detection Settings** from the shortcut menu.

The User Identification Settings dialog box opens (Figure 40).

Figure 40. User Identification Settings dialog box

User Identification Settings 🛛 🛛 🔀
Identification Detection ICIS Integration ICIS Advanced Flags
Consulting Dainty 15
Smootning Points: jo
Baseline Window: 40
Area Noise Factor: 5
Peak Noise Factor: 10
Constrain Peak Width
Peak Ht (%) 10
Tailing Fa <u>c</u> tor: 0.9
OK Cancel Apply Apply To All Help

b. Click the **ICIS Integration** tab.

The ICIS Integration page opens.

- c. Select the Constrain Peak Width check box.
- d. Type 1 in the Peak Ht[%] box.
- e. Type **0.9** in the Tailing Factor box.
- f. Click Apply To All to update the integration of the result files in the sequence.

The Processing dialog box opens and processes the result files in the open sequence. View the result of this new setting in the Chromatogram Plot view.

Note This new integration setting is temporary unless you save it to the processing method by exporting the method.

- 4. To modify the calibration curve, do the following:
 - a. Right-click the calibration curve plot view and choose **Calibration Settings** from the shortcut menu.

The Calibration Settings dialog box opens.

b. Click the **Curve** tab.

The Curve page opens.

- c. Select Linear from the Calibration Curve Type list.
- d. To display the new curve fit in the calibration curve plot view, click **Apply**.
- e. Click **OK** to close the dialog box.

Note You cannot change the amount of the target component for each calibration level. But you can exclude calibration points from the calibration curve and save the new settings as an Xcalibur Quan file (XQN). The Xcalibur Quan file lists the excluded calibration points.

- 5. To save the modifications as a new processing method, do the following:
 - a. Choose File > Export Method.

The Save As dialog box opens.

- b. Type **drugx_example_2** in the File Name box.
- c. In the Directories box, browse to the following folder (Figure 41):

drive:\Xcalibur\examples\methods

OK Cancel
Cancel
No heade
etwork

d. Click **OK** to export the processing method to the selected folder and close the dialog box.

Tutorial 4: Reviewing, Specifying, and Printing Reports

To print reports as you batch reprocess a sequence, specify one or more report templates in the processing method.

This tutorial describes how to review, specify, and print reports.

Contents

- Previewing Reports in XReport
- Specifying Report Templates in the Processing Method
- Printing Reports During Batch Reprocessing

Previewing Reports in XReport

Before adding a report template to the processing method or before using the processing method to print reports, preview the results of the selected template with representative data in XReport. Previewing reports helps you avoid printing reports with inadequate information or inefficient formatting.

- To preview a representative peak integration report and a representative calibration report
- 1. To start the XReport application, do one of the following:
 - On the computer desktop, double-click the **XReport** icon,

-or-

• From the taskbar, choose Start > All Programs > Thermo Xcalibur > XReport.

XReport opens and creates a new template (Figure 42).

Figure 42. XReport window showing a new blank report template

Marcon Thermo XReport - [Untitled - for X	calibur]
<u>File Edit View Layout Rep</u>	ort Help
Available sections	
NR	
Non-Repeating	<< Add a section by dragging an icon from the Available sections pane to here. >>
<u>a</u> Ļ	
Qual Repeating	
Quan Repeating	
Available objects - Header	
Annotation Report Info	
вітмар Columns	
Electronic Sample Header	
Ready	06/12/15 17:23:23

2. Choose File > Open.

The XReport Templates dialog box opens (Figure 43).

Figure 43. XReport Templates dialog box

XReport Templates	Transformer Templates							
0 » 🌗 🗸 09	SDisk (C:) > Xcalibur > templates	▼ 47	Search tem	plates	Q		
Organize 🔻 Ne	w fold	ler			•			
쑦 Favorites	Na	me	Date n	nodified	Туре			
📃 Recent Places	Xr	CalibrationFile.xrt	6/9/20	15 10:18 AM	XReport	Femplate File		
🧱 Desktop	Xr	CompCalReport.xrt	6/9/20	15 10:18 AM	XReport 1	Femplate File		
🗼 Downloads	Xr	CompCalReport_Avalon.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	CompCalReport_Genesis.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	CompCalReport_ICIS.xrt	6/9/20	15 10:18 AM	XReport 7	lemplate File		
门 Libraries	Xr	CustLibrSearRept.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
Documents	Xr	IonRatioConfirmation.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
🌙 Music	Xr	IonRatioConfirmationGraphical.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
📄 New Library	Xr	IonRatioConfirmationGraphicalSimple.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
Pictures	Xr	IonRatioConfirmationGraphicalStacked.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
Videos 😸	Xr	LibrarySearchReport.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	New_Chromatogram_Report.xrt	7/21/2	014 4:02 PM	XReport 7	Femplate File		
🖳 Computer	Xr	PeakIntegration.xrt	6/9/20	15 10:18 AM	XReport 7	lemplate File		
🏭 OSDisk (C:)	Xr	ProcessingMethod.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
👝 Local Disk (D:)	Xr	ProcessingMethod_Avalon.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
👽 Network	Xr	ProcessingMethod_Genesis.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	ProcessingMethod_ICIS.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	QualPeakReport.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	QuanPeakResults_ESTD.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	QuanPeakResults_ISTD.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	QuanResults.xrt	6/9/20	15 10:18 AM	XReport 7	Femplate File		
	Xr	QuanSimple.xrt	6/9/20	15 10:18 AM	XReport	Femplate File		
	Xr	QuantifySampleReport.xrt	6/9/20	15 10:18 AM	XReport	Femplate File		
	Xr	SequenceReport.xrt	6/9/20	15 10:18 AM	XReport	Femplate File		
	Xr	SpectrumCandidates.xrt	6/9/20	15 10:18 AM	XReport	Femplate File		
	•	m				Þ		
1	<u>n</u> ame:	PeakIntegration.xrt	•	XReport Tem	nplate (*.xrt)	•		
				<u>O</u> pen		Cancel		

3. Select **PeakIntegration.xrt** and click **Open**.

The PeakIntegration.xrt report template opens in the XReport window (Figure 44).

Figure 44. PeakIntegration.xrt report template

Thermo XReport - [PeakIntegration.xrt - for Xcalibur4.0]				
File Edit View Layout Report Help				
Available sections			<u>^</u>	
		Peak Integration Report		
Non-Repeating Quan Repeating				
Qual Repeating	NR	Data File: {DataFile} Original Data Path: {OrigDataPath} O Sample Type: {SampType} Sample ID: {SampID} S Operator: {Operator} Acquisition Date: {Acquisition Date: R Comments: {Comments} Vial: {Vial} S Low Mass(m/z): {LowMass} High Mass(m/z): {HighMass} S ISTD Amount: {ISTDAmt} Calibration Level: {CalLevel} D	urrent Data Path: ample Name: .un Time(min): cans: ≡ ample Weight: bilution Factor:	
Available objects - Header		Instrument Method: {InstMthd} Original Processing Method: {ProcMthd} Current Processing Method: {CurrentProcMthd} {UserLabell} {UserVall} {UserLabel2} {UserVal2} {UserLabel3} {UserVal3} {UserLabel3} {UserVal4} {UserLabel3} {UserVal5}		
BITMAP Columns				
IIIII E-E	[Section End		
Electronic Sample Header Signature		{CompName} [ISTDNam] Expected {ExpRetTime} III III	tetTime} RT: 1.4 +	
Ready			06/12/15 17:16:41 //	

 To select a representative data file to test the peak integration, choose Report > Data Sources.

The Data Sources dialog box opens. To test a sample report, you must open a representative processing method, raw data file, result data file, and sequence file.

- 5. Select the appropriate files:
 - Select the processing method as follows:
 - a. In the Processing Method File area, click

The Select Processing File dialog box opens.

- b. Browse to this folder: *drive*:\Xcalibur\examples\methods.
- c. Select drugx.pmd.
- d. Click Open.
- Select the raw data file as follows:
 - a. In the Raw Data File area, click

The Select Raw Data File dialog box opens.

- b. Browse to this folder: *drive*:\Xcalibur\examples\data.
- c. Select drugx_03.raw.
- d. Click **Open**.
- Select the result file as follows:
 - a. In the Raw Data File area, click

The Select Result File dialog box opens.

- b. Browse to this folder: *drive*:\Xcalibur\examples\data.
- c. Select drugx_03.rst.
- d. Click Open.
- Select the sequence file as follows:
 - a. In the Sequence List File area, click

The Select Sequence dialog box opens.

- b. Browse to this folder: *drive*:\Xcalibur\examples\methods.
- c. Select drugx_x.sld.
- d. Click Open.
- 6. Verify that the selections in the Data Sources dialog box are the same as those in Figure 45.

ata Sources	
Calibration File	<u>о</u> к
1	<u>C</u> ancel
Processing Method File	Apply
C:\Xcalibur\examples\methods\drugx.pmd	<u>H</u> elp
⊢ Raw Data File	<u>D</u> efault
C:\Xcalibur\examples\data\drugx_03.raw]
⊢ Result File	
C:\Xcalibur\examples\data\drugx_03.RST]
- Sequence List File	
	1

Figure 45. Data Sources dialog box

- 7. Click **OK** to accept the settings and close the dialog box.
- 8. Choose **Report > Resolve Report** from the XReport main window.

The peak integration report opens, displaying the data from the drugx.rst file. For this data file, the report is two pages. Figure 46 shows the first page and Figure 47 shows the second page.

Figure 46. Peak Integration Report, page 1

		Peak Inte	gration Report		
Data File:	drugx 03	Original Data Path:	C:ILCQ\Data\mrw 27 417\Sat 0504 WD1	Current Data Path:	C:\Xcalibur\example s\data
Sample Type:	Std Update	Sample ID:	04	Sample Name:	
Operator:	linda	Acquisition Date:	05/04/96 10:31:23 PM	Run Time(min):	6.98
Comments:	STD=10 pg/ml,IS=100 pg/ml	Vial:	104	Scans:	314
Low Mass(m/z):	419.30	High Mass(m/z):	425.30	Sample Weight:	0.00
ISTD Amount:	0.000	Calibration Level:		Dilution Factor:	1.00
	Instrument Method: Original Processing Meth	hod:	C:\LCQ\Methods\		
	Current Processing Meth	iod:	C:\Xcalibur\examples\	methods\drugx_exampl	le
	Study				
	Lehent				
	Company				
	Phone				
	1 1016				



Figure 47. Peak Integration Report, page 2

- 9. Close the report, but do not close the XReport application.
- 10. Choose File > Open.

The XReport Templates dialog box opens.

11. Select CompCalReport_ICIS.xrt and click Open.

XReport opens the CompCalReport_ICIS.xrt report template.

- 12. To select a representative sequence file to test the component calibration report, do the following:
 - a. Choose Report > Data Sources.

The Data Sources dialog box opens (see Figure 45 on page 66). The dialog box is populated with your previous selections.

- b. Click **OK** to close the Data Sources dialog box.
- 13. Choose **Report > Resolve Report**.

The application combines the data with the report template and opens the component calibration report, displaying the data from the drugx.sld sequence file. The component calibration report for the drugx.sld sequence file is six pages long. Page 5, containing the calibration curve for drugx, is shown in Figure 48. Page 6, which shows the calculated amounts for drugx, is shown in Figure 49 on page 69.

Figure 48. Component calibration report, showing the calibration curve for drugx, the target compound



		Com	ponent Cali	ibration Repo	rt		
Sample ID	Area	Area Ratio	ISTD Area	Specified Amount	Calculated Amount	% Diff	% RSD
01	188710.251	0.091	2078851.260	10.00000pg/ml	9.47181pg/ml	-5.28	9.960
02	211511.636	0.107	1982388.747	10.00000pg/ml	11.23365pg/ml	12.34	9.960
03	180306.351	0.091	1976747.831	10.00000pg/ml	9.52022pg/ml	-4.80	9.960
04	451037.533	0.222	2031139.035	25.00000pg/ml	24.00399pg/ml	-3.98	0.000
05	885012.506	0.434	2038543.922	50.00000pg/ml	47.49013pg/ml	-5.02	0.000
06	1863855.738	0.922	2021162.567	100.00000pg/ml	101.58703pg/ml	1.59	0.000
07	3971164.797	1.835	2163988.136	200.00000pg/ml	202.97623pg/ml	1.49	0.000
08	8383333.830	3.697	2267325.391	400.00000pg/ml	410.58494pg/ml	2.65	0.000
09	12075236.424	5.487	2200807.986	600.00000pg/ml	611.04911pg/ml	1.84	0.000
10	17476372.702	7.709	2267013.730	800.00000pg/ml	861.41090pg/ml	7.68	0.000
11	21056082.091	8.604	2447194.337	1000.00000pg/ml	962.70426pg/ml	-3.73	1.570
12	21901731.830	8.608	2544250.852	1000.00000pg/ml	963.17444pg/ml	-3.68	1.570
13	22971424.038	8.839	2598948.309	1000.00000pg/ml	989.28833pg/ml	-1.07	1.570
14	311190.520	0.138	2259271.484	N/A	14.66965pg/ml	N/A	N/A
15	225704.696	0.115	1962050.435	N/A	12.15669pg/ml	N/A	N/A
16	227047.300	0.109	2090424.803	N/A	11.44590pg/ml	N/A	N/A
17	217023.171	0.107	2026948.538	N/A	11.27500pg/ml	N/A	N/A
18	213032.790	0.102	2080418.832	N/A	10.75815pg/ml	N/A	N/A
19	206836.121	0.118	1749375.294	10.00000pg/ml	12.51077pg/ml	25.11	0.000
20	7961931.521	3.821	2083738.522	N/A	424.39301pg/ml	N/A	N/A
21	7930753.486	3.871	2048563.176	N/A	430.02691pg/ml	N/A	N/A
22	8884407.016	3.997	2222893.049	N/A	444.05120pg/ml	N/A	N/A
23	8677072.992	3.641	2383086.126	N/A	404.28797pg/ml	N/A	N/A
24	7716769.166	3.553	2171640.815	N/A	394.49148pg/ml	N/A	N/A
25	9381228.456	3.640	2576958.562	400.00000pg/ml	404.21187pg/ml	1.05	0.000
26	23285379.393	9.252	2516922.507	N/A	1036.11383pg/ml	N/A	N/A
27	22265730.639	8.916	2497326.380	N/A	998.02890pg/ml	N/A	N/A
28	19445064.013	8.191	2374075.563	N/A	915.87370pg/ml	N/A	N/A
29	22924140.919	8.654	2648831.276	N/A	968.39931pg/ml	N/A	N/A
30	20196941.490	8.934	2260567.165	N/A	1000.14155pg/ml	N/A	N/A
31	22277135.870	9.103	2447197.670	1000.00000pg/ml	1019.27271pg/ml	1.93	0.000
There is no signatu	re data to report						
C-IL COIDatalana	" 07/17\Set 0504	datase 02		Dam	6 of 6		
WD1	/ 2/41/Gat 0004	arax oo		rage	0.01.0		

Figure 49. Component calibration report, showing the calculated amounts for drugx, the target compound

Specifying Report Templates in the Processing Method

The Xcalibur data system contains a set of report templates that provide basic formats for reporting results. To change the layout of a report or to create a completely new template, use the XReport application. For instructions on creating custom reports, refer to the *XReport User Guide*.

After previewing the results of combining a sample data set with the report templates in the XReport application, follow this procedure to add the report templates to the processing method.

* To specify the report templates for a processing method

1. Open the Reports view of the Processing Setup window by doing one of the following:

1

• On the View bar, click the **Reports** icon,

-or-

• On the menu bar, choose **View > Reports**.

The Reports view opens (Figure 50).

Figure 50. Reports view of the Processing Setup window

🔛 Thermo X	calibu	ır Proc	essing	g Setup -	Reports -	drugx.pm	nd (Int Std)				×
<u>F</u> ile <u>V</u> iew	<u>Optio</u>	ons <u>i</u>	<u>G</u> oTo	<u>H</u> elp							
🗋 🖻 🛛			8		++	♠ →	i € €I} €	⇔⊕	?		
4.57		S <u>a</u> mj	ple rep	orts:		Sar	mple type		-1		
Quan				Enable	Std	QC	Unk	Other	Save As	Report Template Name	
		*			Yes	Yes	Yes	Yes	None		
LAAL.											
Qual											
		S <u>u</u> mi	mary re	eports:							
Reports				Enable	Save A	\s			Report T	emplate Name	
		*			None						
Programs											
		01	<	Car	ncel	<u>S</u> ave As E	Default	Help			
											Ψ }
Ready											

- 2. In the first row of the Sample Reports table, set up a sample report as follows:
 - a. Click the **Enable** column.

The data system selects the **Enable** check box. When you click anywhere else in the view, the text "Yes" replaces the check box.

b. In the Save As column of the same row, select **Doc** to save the report as a DOC file.

The data system saves reports generated from the processing method in the same directory as the source data files and names files in the format *data file name_template name.xxx*, where *data file name* is the name of the data file, *template name* is the name of the template used to generate the report, and (*.xxx*) is the suffix (for example, .doc or .pdf) indicating the file type. If more than one report is generated from the same data file and template, XReport adds a date stamp before the suffix.

- c. Select a sample report template as follows:
 - i. Double-click the **Report Template Name** column.

The Browse for Sample Report Template dialog box opens to the following folder:

drive:\Xcalibur\templates

- ii. To choose the sample peak integration report template, select **PeakIntegration.xrt** and click **Open**.
- 3. In the second row of the Sample Reports table, set up a sample report as follows:
 - a. Double-click the **Enable** column.

The data system selects the **Enable** check box.

- b. In the Save As column of the same row, select **Doc** to save the report as a DOC file.
- c. Select a sample report template as follows:
 - i. Double-click the **Report Template Name** column.

The Browse for Sample Report Template dialog box opens.

- ii. To choose the sample component calibration report template, select **CompCalReport_ICIS.xrt** and click **Open**.
- 4. Click **OK** to save the settings.
- 5. Verify that the Report view matches Figure 51.

TI 🎆	hermo	Xcalibur Proc	essing Set	up - Rep	orts - drug	x.pmd (Int	Std)			×
<u>F</u> ile	<u>V</u> iew	Options G	<u>i</u> oTo <u>H</u> e	lp					1	
ß	È	 🛵	₿ 1	• •)) (€	→ ↔ �	?		
	S <u>a</u> mple	e reports:		Sar	nple type		-1			-
		Enable	Std	QC	Unk	Other	Save As		Report Template Name	
	1	Yes	Yes	Yes	Yes	Yes	None	C:Wcalib	ur\templates\PeakIntegration.xrt	
	2	Yes	Yes	Yes	Yes	Yes	None	C:Wcalib	ur\templates\CompCalReport_ICIS.xrt	
	*		Yes	Yes	Yes	Yes	None			
	Summa	iry reports:								E
	-	Enable	Save A	\s			Report T	emplate	e Name	
	*		None				-	-		
	OK	Car		<u>S</u> ave As [Default	Help				
•										
Ready	y									a

Figure 51. Selecting peak integration report and component calibration report templates

Printing Reports During Batch Reprocessing

The data system uses the report templates that you specify in the processing method to print reports when you batch process (or reprocess) a sequence.

* To print reports while batch reprocessing a sequence

- Return to the Xcalibur home page by choosing GoTo > Xcalibur Home Page from the menu bar.
- 2. Open the Sequence Setup view by doing one of the following:
 - On the View toolbar, click the **Sequence View** icon,

-or-

- On the menu bar, choose **View > Sequence Setup View**.
- 3. If the drugx_example.sld sequence is not already open, open it as follows:
 - a. Do one of the following:
 - On the Sequence Editor toolbar, click the **Open** icon, 🗳 .

-or-

• From the menu bar, choose **File > Open**.

The Open dialog box opens.

b. Browse to the following folder:

drive:\Xcalibur\examples\methods

c. Select drugx_example.sld and click Open.

The drug_example.sld sequence opens.

- 4. Open the Batch Reprocess dialog box by doing one of the following:
 - On the Sequence Editor toolbar, click the **Batch Reprocess** icon,

-or-

• On the menu bar, choose **Actions > Batch Reprocess**.

Figure 52 shows the Batch Reprocess Setup dialog box.

Figure 52. Batch Reprocess Setup dialog box showing	ng selections for printing reports
---	------------------------------------

Process Ro <u>w</u> s: 1-2
Help

- 5. Select the **Reports** check box, and then select the **Print Sample Reports** check box.
- 6. Type **1–2** in the Process Rows box.
- 7. Click **OK** to start batch processing and report generation.

The data system prints the reports for the first two data files in the sequence.

For more information about acquiring and processing data, refer to the *Xcalibur Data Acquisition and Processing User Guide*.

For more information about reviewing quantification data, refer to the *Xcalibur Quan Browser User Guide*.

For more information about the XReport reporting tool, refer to the XReport User Guide.

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