

# MultiQuant<sup>™</sup> 3.0.3 Software

## **Peptide Quantitation Tutorial**



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# Objectives

This tutorial describes how to use the MultiQuant<sup>™</sup> software to quantitatively process MRM data acquired on any QTRAP<sup>®</sup> system or triple quadrupole system.

The MultiQuant<sup>™</sup> software can be used to process data from many different types of quantitative MS-based experiments. While the software is very flexible and allows for many different types of experiments to be processed, an overview of two main workflows is provided in this tutorial. In particular, only MRM workflows are discussed although the program can also be used to process scan mode data.

For more information about the software features, refer to the *Reference Guide* that is installed with the MultiQuant<sup>™</sup> software. This guide provides a description of the functionality available in the MultiQuant<sup>™</sup> software.

The two main types of quantitative analysis that are covered in this tutorial are relative quantitation and accurate quantitation.

To learn about relative quantitation, go to:

- Relative Quantitation Using the MultiQuant<sup>™</sup> Software on page 8
- Process Data Acquired Using the *Scheduled* MRM<sup>™</sup> Algorithm on page 25

To learn about accurate quantitation, go to:

Accurate Quantitation Using Calibration Curves on page 37

# **Related Documentation**

The MultiQuant<sup>™</sup> software documentation can be found under the MultiQuant<sup>™</sup> software Help menu. The Analyst<sup>®</sup> software documentation can be found under the **Start** menu: (On Windows 7 operating system) **All Programs** > **SCIEX** > **Analyst** or (On Windows 10 operating system) **All apps** > **SCIEX Analyst** > **Analyst Documentation**.

- *Reference Guide* for the MultiQuant<sup>™</sup> software
- Laboratory Director's Guide for the Analyst<sup>®</sup> software
- Standard Quantitation Tutorial for the Analyst<sup>®</sup> software
- Getting Started Guide for the Analyst<sup>®</sup> software

• Instrument operator's manual for your instrument (if you are using a pre 1.5 version of the Analyst<sup>®</sup> software)

# **Contact Us**

#### **SCIEX Support**

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#### **Customer Training**

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# **Technical Support**

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the website at sciex.com.

# **Example Files**

Data files used in this tutorial are provided with the software and can be found in the following folder:

- For Windows 7 and Windows 10: C:\ProgramData\AB SCIEX\MultiQuant\Example Data
  - Tutorial Dataset Heavy\_Light.wiff is the file for the relative quantitation experiment
  - mTRAQ HumPlas Apo sMRM 1.wiff is the file acquired using the *Scheduled*  $MRM^{TM}$  algorithm
  - Pep Quant Curve 1.wiff and Pep Quant Samples A.wiff are the two files for the accurate quantitation experiment

Copy the files to the <drive>:\Analyst Data\Projects\Example\Data\Example Data folder.

# **About Calibration Curves**

A calibration curve (also known as a standard concentration curve) is a method for determining the concentration of a substance in an unknown sample by comparing the unknown sample to a set of standard samples of known concentration. The calibration curve is a plot of how the instrument responds (the analytical signal) to changes to the concentration of the analyte (the substance to be measured). The user prepares a series of standard samples across a range of concentrations near the expected concentration of the analyte in the unknown sample.

# About the MultiQuant<sup>™</sup> Software

MultiQuant<sup>™</sup> software has been specifically developed for users that have large numbers of MRM transitions or samples in their acquisition methods, although it can also be used effectively with acquisition methods that have smaller numbers of MRM transitions. The user interface has been designed to simplify processing of these data files. This software is also compatible with the MIDAS<sup>™</sup> Workflow. Although the examples presented in this document all use proteomics data, the software is also useful for processing small molecule data.

MultiQuant<sup>™</sup> software has the added ability to deal with stable isotope labeled components in a seamless manner. This type of work is done routinely in protein/peptide quantitation but also applies to other applications.

# What is Multiple Reaction Monitoring?

In Multiple Reaction Monitoring (MRM), Q1 is set to transmit only the precursor m/z of the peptide or other molecule, the collision energy is optimized to produce a diagnostic charged fragment of this peptide in Q2, and Q3 is set to transmit this diagnostic fragment only. Because of the short dwell times required (5 ms to 50 ms) and the ability to change rapidly between MRM transitions, many components (transitions) in a mixture can be monitored simultaneously in a single LC-MS/MS run.

#### Figure 1-1 Multiple Reaction Monitoring



ltem	Description
1	Molecule
2	Fragment

# **Relative Quantitation**

In relative quantitation experiments, the MRM peak areas from multiple MRMs can be determined across multiple samples and used to compare the relative amounts of analyte present in each sample. Refer to Figure 1-2. This can be done using raw MRM peak areas or by including isotope-coded internal standards and determining MRM peak area ratios. The example in this tutorial covers the case where there is an isotope-coded internal standard for each analyte of interest and the ratios between the analyte and the internal standard for each MRM are determined and compared between three samples.





# **Absolute Quantitation**

In absolute quantitation experiments, the analyte of interest is used to generate a calibration curve to characterize the MS response of each analyte. Refer to Figure 1-3. This can be done with the analyte alone or with an additional isotope-coded version of the analyte that has an identical MS response. A calibration curve is generated and the amount of analyte present in each sample is determined by comparing to the curve.



Figure 1-3 Example of an Absolute Quantitation Experiment

# Relative Quantitation Using the MultiQuant<sup>™</sup> Software

In this section, you will learn how to process the MRM data from a relative quantitation experiment in which there is a heavy labeled peptide internal standard for every peptide of interest. First, the peak integration parameters are set before quantitative processing to avoid any reprocessing of data.

Before you begin

- Make sure you are in the Example project.
- Make sure the Tutorial Dataset Heavy\_Light.wiff file is in the <drive>:\Analyst Data\Projects\Example\Data\Example Data folder.

### **Set the Peak Integration Parameters**

- 1. In the Analyst<sup>®</sup> software, on the **Navigation** bar, under **Companion Software**, double-click **MultiQuant 3.0.3**.
- 2. Click Edit > Project Integration Defaults.
- 3. In the Integration Defaults dialog, select **MQ4** from the **Integration Algorithm** list. Good peak integration parameters for this dataset are shown in Figure 1-4.

Figure 1-4 Integration Defaults Dialog

Integration Defaults		<b>-</b> ×-
Integration Algorithm: M	Q4	•
Gaussian Smooth Width	: 1.0	points
Expected RT:	0.00	min
RT Half Window:	60.0	sec
Update Expected RT:	Group 💌	]
🗖 Report Largest Peak		
Min. Peak Width:	3	points
Min. Peak Height:	0.00	1
Integration Parameter	s	
Noise Percentage:	40.0	%
Baseline Sub. Window:	2.00	min
Peak Splitting	2	points
OK	Ca	ncel

In many cases a smaller smoothing width (for example, 1.0) might be more appropriate. Also note that the peak review process is eased by specifying a non-zero Min. Peak Height, typically corresponding to at least one or two counts. Because the entered threshold should be in counts per second (cps) the actual value required depends on the dwell time. For example, for a dwell time of 100 ms, two counts corresponds to a setting of 20 cps.

When analyzing compounds for which there are multiple MRM transitions and which have been assigned to groups, the Group option of the Updated Expected RT parameter is generally the best choice. In this case, the expected retention time (RT) is updated using the position of maximum overlap of the individual chromatograms for the group (for each sample separately) within the RT window.

4. Adjust the values and then click **OK**.

### **Create a Results Table**

Quantitation methods include a set of instructions on how to quantitate the peaks selected for integration. In this tutorial, you will create a quantitation method at the same time that you create a Results Table. Use the following procedures to create a Results Table.

**Note:** If you are not using the Audit Trail with Security Features edition, then you can launch the MultiQuant<sup>™</sup> software directly from the desktop icon.

- 1. Click File > New Results Table.
- 2. On the Create Results Table Select Samples page, drag the Tutorial Dataset Heavy\_Light.wiff file into the **Selected** pane and then click **Next**.

Create Results Table - Select Samples	
Image: Select Samples         Current Location: C:\Analyst Data\Projects\Default\Data\         Available       Browse         Image: Browse       Browse	Selected  Selected  Sample001 Sample003  <
<	
	<back next=""> Finish Cancel</back>

#### Figure 1-5 Create Results Table - Select Samples Page

3. On the Create Results Table - Select Method page, click the **Create New Method (MQ4)** option and then click **New**.

Create Results Table	- Select Method	- • •
Select an existing quanti	tation method or create a new method now.	
0.00		
Choose Existing Me	thod	
Method Name:		Open
Edit Method		
Create New Method	(MQ4)	
Method Name:	Click 'New' to select method	New
C Use 'Automatic' Met (Created on-the-fly :	nod (MQ4) and most useful when MRM transitions differ between samples)	
	< Back Next > Finish	Cancel

#### Figure 1-6 Create Results Table - Select Method Page

4. Type a name for the method, click **Save**, and then click **Next**.

In this tutorial, you will create a method. Creating methods gives you an opportunity to review and apply different parameters for the integration of your data.

5. On the Create Results Table - Select Representative Sample page, a representative sample has been recommended and is selected. Click **Next**.

The software recommends a representative sample based on selecting a chromatogram that provides the best opportunity to select integration parameters that fit the entire batch.



#### Figure 1-7 Create Results Table - Select Representative Sample Page

On the Create Results Table - Define Components page, the details of the various analytes and internal standards to be processed must be defined. All the MRM transitions from the data file are automatically loaded into the window.

- 6. On the Create Results Table Define Components page, define all Group members by giving them the same name. In this example, there are four groups, each consisting of eight MRMs. The group names can be typed into the Group column or copied in from an Excel spreadsheet. Refer to Figure 1-8.
- 7. Type the peptide name in the first row only of each group (row 1, 9, 17, 25), and then right-click in the table and click **Groups** > **By Filling Down Existing Groups**. In this example, the first eight MRMs are named Peptide 1, the second eight rows are named Peptide 2, the third eight are named Peptide 3, and the final eight rows are named Peptide 4.

experiment	MRM	4 (32 transitions)	•			
Row	IS	Name	Group	IS Name	Q1/Q3	T
1		628.3 / 697.4	Peptide 1		628.3 / 697.4	1
2		628.3 / 568.3	Peptide 1		628.3 / 568.3	
3		942.0 / 568.3	Peptide 1		942.0 / 568.3	
4		942.0 / 697.4	Peptide 1		942.0 / 697.4	
5		629.6 / 701.4	Peptide 1		629.6 / 701.4	
6		629.6 / 572.3	Peptide 1		629.6 / 572.3	
7		944.0 / 572.3	Peptide 1		944.0 / 572.3	
8		944.0 / 701.4	Peptide 1		944.0 / 701.4	1
9		464.9 / 442.2	Peptide 2		464.9 / 442.2	
10		464.9 / 473.3	Peptide 2		464.9 / 473.3	
11		464.9 / 541.3	Peptide 2		464.9 / 541.3	
12	1	696.9 / 473.3	Peptide 2		696.9 / 473.3	
13		467.6 / 446.2	Peptide 2		467.6 / 446.2	
14		467.6 / 477.3	Peptide 2		467.6 / 477.3	
15		467.6 / 545.3	Peptide 2		467.6 / 545.3	
16		700.9 / 477.3	Peptide 2		700.9 / 477.3	
17		550.8 / 584.3	Peptide 3		550.8 / 584.3	

#### Figure 1-8 Create Results Table - Define Components Page

The Groups can be named in many ways, depending on the compounds. Examples of group nomenclature are: using the peptide sequence as the group name or using the Protein name\_Peptide sequence.

**Note:** When you group MRM transitions together, they should correspond to the same compound, including the internal standard version of that compound.

8. Copy the Group column into the Name column. Right-click in the table and then click **Groups > Add Group to Start of Component Name**.

The Name and Group columns are shown in Figure 1-9.

xperimen	: MRI	4 (32 transitions)	•			
Row	IS	Name	Group	IS Name	Q1/Q3	-
1		Peptide 1.628.3 / 697.4	Peptide 1		628.3 / 697.4	
2		Peptide 1.628.3 / 568.3	Peptide 1		628.3 / 568.3	
3		Peptide 1.942.0 / 568.3	Peptide 1		942.0 / 568.3	
4		Peptide 1.942.0 / 697.4	Peptide 1		942.0 / 697.4	
5		Peptide 1.629.6 / 701.4	Peptide 1		629.6 / 701.4	
6		Peptide 1.629.6 / 572.3	Peptide 1		629.6 / 572.3	
7		Peptide 1.944.0 / 572.3	Peptide 1		944.0 / 572.3	
8		Peptide 1.944.0 / 701.4	Peptide 1		944.0 / 701.4	
9		Peptide 2.464.9 / 442.2	Peptide 2		464.9 / 442.2	
10		Peptide 2.464.9 / 473.3	Peptide 2		464.9 / 473.3	
11		Peptide 2.464.9 / 541.3	Peptide 2		464.9 / 541.3	
12		Peptide 2.696.9 / 473.3	Peptide 2		696.9 / 473.3	
13		Peptide 2.467.6 / 446.2	Peptide 2		467.6 / 446.2	
14		Peptide 2.467.6 / 477.3	Peptide 2		467.6 / 477.3	
15		Peptide 2.467.6 / 545.3	Peptide 2		467.6 / 545.3	
16		Peptide 2.700.9 / 477.3	Peptide 2		700.9 / 477.3	
17		Peptide 3.550.8 / 584.3	Peptide 3		550.8 / 584.3	۰.
	-					

#### Figure 1-9 Name and Group Columns

- 9. Define the MRM transitions to be used as the internal standards for Peptide 1 only by selecting the check boxes in the **IS** column as shown in Figure 1-10. In this example, the last four transitions in each group are the internal standards for the first four transitions in the group.
- 10. After an MRM transition has been defined as an internal standard, it can be associated with the correct analyte in the **IS Name** column. Click the right side of each row within this column and then select the internal standard from the list that is shown in Figure 1-10.
- 11. Select the appropriate internal standard from the list for each of the first four MRM transitions for Peptide 1. Within the group, the fifth MRM is the IS for the first row, the sixth MRM is the IS for the second row, and so on.

periment	MRM	A (32 transitions)	•				
Row	IS	Name	Group	IS Name		Q1/Q3	
1		Peptide 1.628.3 / 697.4	Peptide 1	Peptide 1.629.6 / 701	.4	628.3 / 697.4	
2		Peptide 1.628.3 / 568.3	Peptide 1	Peptide 1.629.6 / 572	.3	628.3 / 568.3	
3		Peptide 1.942.0 / 568.3	Peptide 1	Peptide 1.944.0 / 572	.3	942.0 / 568.3	
4		Peptide 1.942.0 / 697.4	Peptide 1		Ŧ	942.0 / 697.4	
5	V	Peptide 1.629.6 / 701.4	Peptide 1		^	629.6 / 701.4	
6	V	Peptide 1.629.6 / 572.3	Peptide 1	Peptide 1.629.6 / 701.4 Peptide 1.629.6 / 572.3		629.6 / 572.3	
7	V	Peptide 1.944.0 / 572.3	Peptide 1	Peptide 1.944.0 / 572.3	1	944.0 / 572.3	
8		Peptide 1.944.0 / 701.4	Peptide 1	Peptide 1.944.0 / 701.4 Peptide 2.467.6 / 446.2		944.0 / 701.4	
9		Peptide 2.464.9 / 442.2	Peptide 2	Peptide 2.467.6 / 477.3		464.9 / 442.2	
10		Peptide 2.464.9 / 473.3	Peptide 2	Peptide 2.467.6 / 545.3	Ŧ	464.9 / 473.3	
11		Peptide 2.464.9 / 541.3	Peptide 2			464.9 / 541.3	
12		Peptide 2.696.9 / 473.3	Peptide 2			696.9 / 473.3	
13		Peptide 2.467.6 / 446.2	Peptide 2			467.6/446.2	
14	V	Peptide 2.467.6 / 477.3	Peptide 2			467.6/477.3	
15	V	Peptide 2.467.6 / 545.3	Peptide 2			467.6 / 545.3	
16		Peptide 2.700.9 / 477.3	Peptide 2			700.9 / 477.3	
17		Peptide 3.550.8 / 584.3	Peptide 3			550.8 / 584.3	
_	_						

#### Figure 1-10 Associating Internal Standards

12. To propagate this format to all the groups in the data file, select the first row for Peptide 1, right-click and then click **Internal Standards > Set for all Groups as for Selected Group**.

**Note:** The pattern of sample and IS MRMs must be constant throughout the data file to be able to propagate the set pattern. This reduces the manual interaction required when setting up the processing method.

The structure of the Peptide 1 group is applied to Peptide 2, 3 and 4 groups as shown in Figure 1-11.

Experimen	t MRM	4 (32 transitions)	-			
Row	IS	Name	Group	IS Name	Q1/Q3	-
1		Peptide 1.628.3 / 697.4	Peptide 1	Peptide 1.629.6 / 701.4	628.3 / 697.4	
2		Peptide 1.628.3 / 568.3	Peptide 1	Peptide 1.629.6 / 572.3	628.3 / 568.3	
3		Peptide 1.942.0 / 568.3	Peptide 1	Peptide 1.944.0 / 572.3	942.0 / 568.3	
4		Peptide 1.942.0 / 697.4	Peptide 1	Peptide 1.944.0 / 701.4	942.0 / 697.4	1
5	V	Peptide 1.629.6 / 701.4	Peptide 1		629.6 / 701.4	
6	V	Peptide 1.629.6 / 572.3	Peptide 1		629.6 / 572.3	
7	V	Peptide 1.944.0 / 572.3	Peptide 1		944.0 / 572.3	-
8		Peptide 1.944.0 / 701.4	Peptide 1		944.0 / 701.4	
9		Peptide 2.464.9 / 442.2	Peptide 2	Peptide 2.467.6 / 446.2	464.9 / 442.2	
10		Peptide 2.464.9 / 473.3	Peptide 2	Peptide 2.467.6 / 477.3	464.9 / 473.3	
11		Peptide 2.464.9 / 541.3	Peptide 2	Peptide 2.467.6 / 545.3	464.9 / 541.3	
12		Peptide 2.696.9 / 473.3	Peptide 2	Peptide 2.700.9 / 477.3	696.9 / 473.3	
13		Peptide 2.467.6 / 446.2	Peptide 2		467.6 / 446.2	
14		Peptide 2.467.6 / 477.3	Peptide 2		467.6/477.3	
15	<b>V</b>	Peptide 2.467.6 / 545.3	Peptide 2		467.6 / 545.3	
16	<b>V</b>	Peptide 2.700.9 / 477.3	Peptide 2		700.9 / 477.3	
17		Peptide 3.550.8 / 584.3	Peptide 3	Peptide 3.552.8 / 588.3	550.8 / 584.3	۰.
	-		- ·· ·			

#### Figure 1-11 Set for all Groups as for Selected Group Option

**Tip!** This highlights one option for structuring the use of internal standards. For more information on how to use the internal standards, refer to the *Reference Guide* that is installed with the MultiQuant<sup>™</sup> software.

**Note:** If a multi-period experiment was used, the previous steps must be repeated for each period. To switch between periods, select the period from the Experiment list and then repeat the process of setting up the processing method as described.

#### 13. Click Next.

On the Create Results Table - Define Integration page, the appropriate peak integration parameters are defined for each individual MRM. Each MRM peak has been automatically integrated, but you have the option to adjust the peak integration (that is, retention time of integrated peak) for each MRM.

Peptide 1.628.3.	^		Apply	1	Peptid	e 1.628.3 / 6	97.4 frvy_Light.w	iff (sample 2)
Peptide 1.628.3 Peptide 1.942.0		Gaussian Smooth Width:	1.0	points	Alea.	3.5e5	gni: 3.62365, NT. 3	36.49
Peptide 1.942.0 Peptide 1.629.6		Expected RT:	36.48	min				
Peptide 1.629.6 Peptide 1.944.0		RT Half Window:	60.0	sec		3.0e5 -		
Peptide 1.944.0.		Report Largest Peak	Group	1				
Peptide 2.464.9.		Min. Peak Width:	3	points		2.5e5		
Peptide 2.696.9		Min. Peak Height:	0.00	]				
Peptide 2.467.6 Peptide 2.467.6		Integration Parameters Noise Percentage:	40.0	*	disus	2.0e5 -		
Peptide 2.467.6 Peptide 2.700.9		Baseline Sub. Window:	2.00	min	The second se	1.5e5 -		
Peptide 3.550.8 Peptide 3.550.8	I	Peak Splitting	2	points				
Peptide 3.550.8 Peptide 3.550.8		Regression Parameter: Area		Ţ		1.0e5		
Peptide 3.552.8 Peptide 3.552.8		Fit: Linear		-				
Peptide 3.552.8 Peptide 3.552.8		Weighting: None		•		5.064 -		
Peptide 4.453.9 Peptide 4.453.9		Concentration Units:	_			0.0e0	k	
Peptide 4.453.9	÷	Apply units to all Ana	lytes				10 20 30 Time, mi	40 50 n

#### Figure 1-12 Create Results Table - Define Integration Page

14. Right-click anywhere on the pane and then click **Overlay Other Components for Group**. When there are multiple MRM transitions within a Group, it is convenient to overlay the MRM peaks within a group during integration to be sure that the correct peak is integrated in each case. MRM transitions within the same group should all have the same retention time.

All the MRM transitions within the selected group are overlaid with different colors specifying each MRM. The specific selected MRM within the group is shown in blue as shown in the Figure 1-13.

**Note:** When the Update Expected RT is set to Group, the retention time selected for the peak integration of all peaks within the group is based on the position of maximum overlap of the individual chromatograms.

Peptide 1.628.3	] .	Apply		+ Peptide	1.628.3/697.4f	roavy_Li	ght.wiff (sample) 36.49 min
Peptide 1.628.3 Pertide 1.942.0	Gaussian Smooth Width:	1.0	points		i	36.4	49
Peptide 1.942.0	Even added DT	24.49		3.5e5 -	1		
Peptide 1.629.6	Expected KI:	00/40	min				
Peptide 1.629.6.	RT Half Window:	60.0	sec		1		
Peptide 1.944.0.	Update Expected 8T:	Group w		3.0e5 ·	1		
Peptide 1.944.0		louodb -			1		
Peptide 2.464.9	🔲 Report Largest Peak				1		
Peptide 2.464.9	Nin, Peak Width:	3	points	2.5e5 -	1		
Peptide 2.464.9.					1		
Peptide 2.696.9	Min. Peak Height:	0.00			1		
Peptide 2.467.6	Integration Parameters			≥ 20+5	1		
Peptide 2.467.6	Noise Percentage:	40.0	%	18 2.000	1		
Peptide 2.467.6		2.00		Inte	1		
Peptide 2.700.9	Baseline Sub. Window:	2.00	min	15.5			
Peptide 3.550.8	Peak Splitting	2	points	1.565	1		
Peptide 3.550.8	Perrerrian				1		
Peptide 3.550.8	Regression		_	10-5			
Peptide 3.550.8	Parameter: Area		-	1.065	1		
Peptide 3.552.8			=		1		
Peptide 3.552.8	Fit: Linear		-	50.4	-	1	
Peptide 3.552.8	Weighting: None		-	5.064	1		
Peptide 3.552.8	and a press				1		
Peptide 4.453.9	_				1		
Peptide 4.453.9	Concentration Units:			0.0e0 ·	10 20	30	40 50
Peptide 4.453.9	Apply units to all Apai	lutes			10 20		TV - VV

#### Figure 1-13 Overlay Other Components for Group Option

15. Each MRM transition can be viewed by clicking the specific MRM in the list, or by using the Up and Down arrows to automatically advance through the list. When an individual MRM transition has not been integrated at the right retention time, the correct peak can be selected by dragging across it on the pane. The peak is automatically reintegrated. In some cases it might be necessary to adjust the peak-finding parameters.

If multiple periods are present in the method, all MRM transitions within all periods are integrated in this pane as a single list.

16. After all the MRM transitions have been reviewed and the correct retention times defined for each, click **Next**.

#### **Figure 1-14 Outlier Settings**

et criteria for flagging outliers.		
Accuracy for Standards	Accu	racy for QCs
Max. Accuracy Tolerance for LLOQ (Ic Max. Accuracy Tolerance for Stds exc	west Std): 20 % Max. Acc ept LLOQ: 15 %	curacy Tolerance for QC: 15 %
□ Ion Ratio	Concentration	Upper Limit of
Component	Calculated Concentration	Calculated Concentration
Peptide 1.628.3 / 697.4		
Pepbde 1.628.37 568.3		
Pepbde 1.942.07568.3		
Peppide 1.342.07637.4		
Pepude 1.623.67701.4 Replice 1.629.67572.2		
Peride 1.944.0 / 572.3		
Peptide 1.944.0 / 701.4		
Peptide 2,464.9 / 442.2		
Peptide 2.464.9 / 473.3		
Peptide 2.464.9 / 541.3		
Peptide 2.696.9 / 473.3		
Peptide 2.467.6 / 446.2		
Peptide 2.467.6 / 477.3		
Peptide 2.467.6 / 545.3		
Peptide 2.700.9 / 477.3		
D62- 0 660 0 / 604 0		

- 17. Specify the fields as required to automatically flag samples for review in the Results Table. For more information about the Outlier Settings parameters, refer to the *Reference Guide* that is installed with the MultiQuant<sup>™</sup> software.
- 18. Click Finish.

### Modify the Results Table

All of the MRM transitions in each of the samples are integrated with the specified parameters as shown in Figure 1-15.

On the left side, the information is organized in a number of ways. If All Components is selected, all integrated MRMs for all samples are listed in the order that the MRMs appear in the data file, one sample at a time. If an individual group is selected (Peptide 1 group) then only the MRM transitions within that group for all the samples are shown. The data for all internal standards or all analytes can be visualized by selecting either All Internal Standards or All Analytes. The data for any one IS or analyte can be viewed by selecting its name in the list.

MultiQuant - [[MQ4] R	esults Table	(Untitle	ed)]				
MQ File Edit Process	Window	Help				-	2
🌾 🗃 👻 🔚 🗀 Defau	• • 2	)					
Components & Groups	#	🛍 🛛	Z 🖌 🕶 🐉 🔝	🔝 🗔 🕌 All Sample	Types 🔻	🖂 🛎 🖗 🧔	6
II Components	<u> </u>		3				
Peptide 1 Group							
Peptide 2 Group	In	ndex	Sample Name	Component Name	Area	Sample Type	
epade + Group		1	Sample001	Peptide 1.628.3 /	5.503e6	Unknown	_
I Internal Standards		2	Sample001	Peptide 1.628.3 /_	5.066e6	Unknown	
Peptide 1.629.6 / 572.3		3	Sample001	Peotide 1.942.0 /_	1.430e6	Unknown	
eptide 1.944.0 / 572.3		4	Sample001	Pentide 19420/	1.875e6	Unknown	
eptide 1.944.07 /01.4 eptide 2.467.6 / 446.2		6	Completer 1	Destide 1.000.0 /	E CEO+C	Uskasur	
eptide 2.467.6 / 477.3		9	SampleUUT	Pepade 1.623.67	0.00360	Unknown	
eptide 2.467.6 / 545.3		6	Sample001	Peptide 1.629.6 /	4.587e6	Unknown	
eptide 2.700.97477.3 Jeptide 3.552.87588.3		7	Sample001	Peptide 1.944.0 /	1.312e6	Unknown	
eptide 3.552.8 / 774.4		8	Sample001	Peptide 1.944.0 /	1.901e6	Unknown	
eptide 3.552.8 / 661.4		9	Sample(001	Pantida 2.464.9 /	8.62545	Unknown	
eptide 3.552.8 / 5/4.3 eptide 4.456 6 / 518 2		*			0.04000	UNKIN WIT	
eptide 4.456.6 / 461.2		10	Sample001	Pepbde 2.464.97	3.86265	Unknown	
eptide 4.456.6 / 615.3		11	Sample001	Peptide 2.464.9 /	8.939e4	Unknown	
eptide 4.456.67527.3		12	Sample001	Peptide 2.696.9 /	3.356e6	Unknown	
II Analytes		13	Sample001	Peptide 2.467.6 /	8.612e5	Unknown	
eptide 1.628.3 / 697.4		14	Samela(001	Pactida 246761	2 529+5	Unknown	
Peotide 1.942.0 / 568.3					0.00000	Chikhowh	_
eptide 1.942.0 / 697.4		15	Sample001	Peptide 2.467.6 /	8.401e4	Unknown	
eptide 2.464.9 / 442.2		16	Sample001	Peptide 2.700.9 /	2.925e6	Unknown	
eptide 2.464.9 / 4/3.3		17	Sample001	Peptide 3.550.8 /	3.538e5	Unknown	
eptide 2.696.9 / 473.3		18	Sample(0)1	Pantida 3 550 9 /	3.491#5	Unknown	
entide 3 550 8 / 584 3		10	Sangreoor	repude 5.550.67	3.40103	UNIOWI	
000000.0000000000							

Figure	1-15	Results	Table	Interface

Item	Description
1	Peptide group
2	Peak Review icon

- 1. Save the Results Table by clicking **File > Save**.
- 2. To visualize the MRM transitions for selected group for all samples, select a Peptide group (Figure 1-15 item 1) from the left panel and then click the Peak Review icon (Figure 1-15 item 2). If a row is selected within the table, the selected MRM transition is shown in the pane below with the name highlighted in bold. The up and down arrows or the scroll wheel on the mouse can be used to advance through the rows of the table to change the active pane.
- 3. If an incorrect peak is integrated, the correct peak can be selected and integrated by dragging the mouse across the peak. If the peak does not integrate, click the Enable Manual Integration Mode icon (Figure 1-16 item 2) to integrate manually. Define the peak area by dragging the mouse across the bottom of the peak area to be integrated (Figure 1-16 item 3). The selected peak integration are shown automatically by the peak filling.



🎛 📾 🕻	🔀 🖬 🔻 😽 🖾	🐻 🕌 All Sample Ty	pes 🔹 🛩 🦉	9	🦚 🔒 🎓 🔍 [			
Index	Original Filename	Sample Name	Sample Type	IS	Component Name	IS Name	Component Group Name	Out
1	Tutorial Dataset Heav	Sample001	Unknown	1	Peptide 1.628.3 /	Peptide 1.944.0 /	Peptide 1	
▶ 2	Tutorial Dataset Heav	Sample001	Unknown		Peptide 1.628.3 /	Peptide 1.944.0 /	Peptide 1	
3	Tutorial Dataset Heav	Sample001	Unknown		Peptide 1.942.0 /	Peptide 1.944.0 /	Peptide 1	
4	Tutorial Dataset Heav	Sample001	Unknown	<b>F</b>	Peptide 1.942.0 /	Peptide 1.944.0 /	Peptide 1	
5	Tutorial Dataset Heav	Sample001	Unknown	1	Peptide 1.629.6 /	N/A	Peptide 1	
6	Tutorial Dataset Heav	Sample001	Unknown	V	Peptide 1.629.6 /	N/A	Peptide 1	
7	Tutorial Dataset Heav	Sample001	Unknown	V	Peptide 1.944.0 /	N/A	Peptide 1	
8	Tutorial Dataset Heav	Sample001	Unknown	V	Peptide 1.944.0 /	N/A	Peptide 1	
33	Tutorial Dataset Heav	Sample002	Unknown	1	Peptide 1.628.3 /	Peptide 1.944.0 /	Peptide 1	
34	Tutorial Dataset Heav	Sample002	Unknown	1	Peptide 1.628.3 /	Peptide 1.944.0 /	Peptide 1	
35	Tutorial Dataset Heav	Sample002	Unknown	1	Peptide 1.942.0 /	Peptide 1.944.0 /	Peptide 1	
<	·- ··- ·· "							•
Gaussian S Expected R	1 Apply mooth Width: 3.0 RT: 31.61	j 00 ▲ Γ points Sample001 - 1 Area: 5.493e6	Manual Integrat Peptide 1.628.3 / 6 , Height: 3.838e5,	ion 97.4 (U RT: 36	nknown) 628 Sau 18 min Ara	mple001 - Peptide 1 va: 1.128e6, Height.	.628.3/568.3 (Unita 5.72104, RT: 31.61 /	iown) 6 min
RT Half Win Update Exp	idow: 90.0 pected RT: No 💌 Largest Peak	sec asu 2e5 0e0	10 20	30	40 50	2e4 0e0 28 2	30 31 32	33
Min. Peak Min. Peak H	Width: 3 Height: 0.00	points Sample001 - I Area: 1.420e6	Ti Peptide 1.942.0 / 5 , Height: 8.886e4,	ime, mi 68.3 (U RT: 36	n Inknown) 942 San 19 min Arei	ple001 - Peptide 1.94 a: 1.901e6, Height: 1.1	Time, min 2.0 / 697.4 (Unknown 76e5, RT: 36.19 min	) 942
Integratio Noise Perc	entage: 40.0	Arsungul		1	36.19 Assetuti	1.0e5 5.0e4	36.19	
Peak Splitt	ting 4	points 0e0	10 20 Ti	30	40 50	10	20 30 40 Time min	50

ltem	Description
1	Slide Show Peak Review icon
2	Enable Manual Integration Mode icon
3	Selecting a peak for integration

4. Right-click anywhere in the graph pane and then click **Options**. The settings on this dialog can be used to adjust the appearance of the view.

Figure 1-17 Peak Review Options Dialog

M Peak Review Options
Appearance Zooming
Number of rows:
Number of columns: 2
Overlay:
Don't overlay
Highlight active graph using: Bold, italic title and grey background
Fill Peaks: Using dotted style 💌
Hide axis names
Mark expected RT with arrow
OK Cancel

5. From the toolbar, the slide show mode can be selected by clicking the **Slide Show Peak Review** icon (Figure 1-16 item 1). This is a convenient way to review the data.

Figure 1-18 Slide Show Options Dialog



**Tip!** You can review the data within the Results Table in many ways. For example, the Area column can be selected by clicking on the column header and then selecting the Ascending Sort or Descending Sort (Figure 1-16). This sorts the data within the table.



#### Figure 1-19 Quality Column

6. To edit the structure of the Results Table and make sure that the required columns for determining calibration curves are visible, right-click in the table and then click **Column Settings**. Columns can be added or removed from the Results Table view by selecting or clearing the check boxes in the **Visible** column. For relative quantitation experiments, the common columns required are shown in Figure 1-19.

It is useful to sort on the Quality column. All the peaks with low quality scores can be viewed together and you can decide if it is necessary to reintegrate some of the peaks. Quality is computed by determining the percentage of the peak area that the integrated peak contributes to the total area under the MRM trace (in a region around the integrated peak, that is, 1.5 times the size of the user specified RT Window). For more information about Results Table columns, refer to the *Reference Guide* that is installed with the MultiQuant<sup>™</sup> software.

Import	Export.			
Column Name /	Visib_	Number_	LIMS Supported	-
Accuracy	V	0.00	<b>V</b>	
Acq. Method Na	<b>[</b> ]		<b>V</b>	1
Acquisition Date	<b>[</b> ]		<b>V</b>	L
Actual Concentr	V	0.00	<b>V</b>	
Area	V	0.000e0	<b>V</b>	
Area / Height		0.00	<b></b>	
Area Ratio		0.000e0	<b>V</b>	
Asymmetry Fact.		0.00	<b>V</b>	
Baseline Delta /		0.000e0		
Calculated Con	V	0.000e0	<b>V</b>	
Component Co				
Component Gro	V			
Component Ind			<b>V</b>	
Component Na			<b>V</b>	
Conc. Units			<b>V</b>	
Concentration R		0.00		
Corrected Area		0.000e0		
Corrected Area/		0.00		
Corrected Height		0.000e0		
Dilution Eactor	1000	0.00	100	1

Figure 1-20 Column Settings Dialog

When you perform relative quantitation experiments, using internal standards provides extra robustness and quantitative accuracy and reproducibility. The Area Ratio column is the peak area of the analyte divided by the peak area of the corresponding internal standard.

If further manipulation of the data is required, the data can be exported and opened in other programs such as Microsoft Excel. For more information about the Export command, refer to the *Reference Guide* that is installed with the MultiQuant<sup>TM</sup> software. Refer to Run Queries on page 35.

### **Create Reports**

If you are creating reports using unlocked Results Tables, make sure that you are allowed to create and export unlocked Results Tables. See your administrator if you are unable to create reports.

#### 1. Click File > Create Report and Save Results Table.

#### Figure 1-21 Create Report Dialog

MQ Create Report	
Report template: Analyte Report 1.doc	x Set1 Open in Word
Generated report file:	Set(2)
Output Format:	L O PDF O CSV
Create an individual report for each sar	nple (Recommended for large reports)
	OK Cancel

ltem	Description
1	Click to select the template.
2	Click to save the report.

- 2. Click **Set** (Figure 1-21 item 1) to navigate to the Tutorial Dataset Heavy\_Light.xml template in the following folder:
  - For Windows 7 and Windows 10: C:\ProgramData\AB SCIEX\MultiQuant\Reporter.
- 3. Click **Set** (Figure 1-21 item 2) to create the name and location of the report.
- 4. Click **OK**.

# Process Data Acquired Using the *Scheduled* MRM<sup>™</sup> Algorithm

The *Scheduled* MRM<sup>™</sup> algorithm is a powerful acquisition tool in Analyst<sup>®</sup> software 1.5 or later versions that enables much higher multiplexing of MRM transitions per acquisition method. From the user-supplied retention time, individual acquisition windows are built for each separate analyte, minimizing the total number of MRM transitions monitored at any single point in time. This improves data quality by enabling the use of higher dwell times for every analyte.



Figure 1-22 Example of *Scheduled* MRM<sup>™</sup> Algorithm Data

Another feature in Analyst<sup>®</sup> Software 1.5 and later is the ID column. Individual MRM transitions can be named in the Analyst<sup>®</sup> software Acquisition Method Editor. Refer to Figure 1-23. The IDs should be of the in the following formats:

{Root Name}.{Group}.{IS Indicator}

	Q1 Mass	Q3 Mass	Time	ID	CE (vc 📩
1	518.000	414.200	21.5	APØB.SVSLPSLDPASAK.3b3.light	43.900
2	518.000	445.300	21.5	APOB.SVSLPSLDPASAK.3y3.light	43.900
3	518.000	613.400	21.5	APOB.SVSLPSLDPASAK.3y5.light	43.900
4	518.000	527.300	21.5	APOB.SVSLPSLDPASAK.3b4.light	43.900
5	520.600	418.200	21.5	APOB.SVSLPSLDPASAK.3b3.heav	44.000 -
6	520.600	449.300	21.5	APOB.SVSLPSLDPASAK.3y3.heav	44.000
7	520.600	617.400	21.5	APOB.SVSLPSLDPASAK.3y5.heav	44.000
8	520.600	531.300	21.5	APOB.SVSLPSLDPASAK.3b4.heav	4.000
9	666.800	588.300	15.9	POC1.EFGNTLEDK.2b4.light	51.300
10	666.800	531.300	15.9	AROC1.EFGNTLEDK.2y3.light	51.300
11	666.800	474.200	15.8	APOC1.EFGNTLEDK.2b3.light	51.300
12	008 3331	644 400	15.0	ADOCT SECNITLEDK 204 light	51 300 🎽

Figure 1-23 Example of the ID Column

The Group and IS Indicator are both optional. For small molecule applications, the IS Indicator should be absent for analytes and set to IS or internal standard for internal standards. For example if the IDs are:

- Compound1
- Compound1.IS
- Compound2
- Compound2.IS

Then Compound1.IS and Compound2.IS are both automatically set as internal standards. Additionally, Compound1 will be setup to use 'Compound1.IS' as its internal standard and similarly for Compound2.

For proteomics applications, the ID will usually be of the form:

{Peptide Sequence}.{Transition}.{Light or Heavy}

For more information about triplex mTRAQ<sup>®</sup> reagent support, refer to the *Reference Guide* that is installed with the MultiQuant<sup>M</sup> software. The nomenclature is as follows:

- Protein.peptide.transition.M00
- Protein.peptide.transition.M04
- Protein. peptide. transition.M08

### Before you begin

- Set the integration parameter defaults. Refer to Set the Peak Integration Parameters on page 9.
- Make sure you are in the Example project.
- Make sure the mTRAQ HumPlas Apo sMRM 1.wiff file is in the <drive>:\Analyst Data\Projects\Example\Data\Example Data folder.

### Create a Results Table

Quantitation methods include a set of instructions on how to quantitate the peaks selected for integration. In this tutorial, you will create a quantitation method at the same time that you create a Results Table.

- 1. In the Analyst<sup>®</sup> software, on the Navigation bar, under **Companion Software**, double-click **MultiQuant 3.0.3**.
- 2. Click File > New Results Table.
- 3. On the Create Results Table Select Samples page, drag the **mTRAQ HumPlas Apo sMRM 1.wiff** file into the **Selected** pane and then click **Next**.

Available	Browse	1	Selected			
Railing	ion Nov 6 20	=> <=		TRAQ HumPlas Sample001	Apo sMRM 1.w	1
<						

#### Figure 1-24 Create Results Table - Select Samples Page

4. On the Create Results Table - Select Method page, click the **Create New Method (MQ4)** option and then click **New**.

**Tip!** If the interface has Create New Method (Signalfinder1), then refer to Set the Peak Integration Parameters on page 9 to change the algorithm to MQ4.

Create Results Table	- Select Method	- • •
Select an existing quanti	tation method or create a new method now.	
C Choose Existing Me	thod	
Method Name:		Open
Edit Method		
Create New Method	(MQ4)	
Method Name:	Click 'New' to select method	New
(Created on-the-fly a	and most useful when MRM transitions differ between samples)	
	< Back Next > Finish	Cancel

#### Figure 1-25 Create Results Table - Select Method Page

5. Type a name for the method, click **Save**, and then click **Next**.

In this tutorial, you will create a method. Creating methods gives you an opportunity to review and apply different parameters for the integration of your data.

6. On the Create Results Table - Select Representative Sample page, a representative sample has been recommended and is selected. Click **Next**.

The software recommends a representative sample based on selecting a chromatogram that provides the best opportunity to select integration parameters that fit the entire batch.



#### Figure 1-26 Create Results Table - Select Representative Sample Page

On the Create Results Table - Define Components page, the details of the various analytes and internal standards to be processed must be defined. All the MRM transitions from the data file are automatically loaded into the window.

**Tip!** When the ID column is correctly entered in the Analyst<sup>®</sup> software MRM acquisition method, the information is used to automatically populate the Group and internal standard information in the following pane.

Expe	ariment:	1 MF	RM (144 transitions)	•			
R	low	IS	Name	Group	IS Name	Q1/Q3	
	1	<b>m</b>	APOA1.LSPLGEEM	APOA1.LSPLGEEM	APOA1.LSPLGEEM	586.3 / 621.3	18.
	2		APOA1.LSPLGEEM	APOA1.LSPLGEEM	APOA1.LSPLGEEM	586.3 / 734.3	18.
	3		APOA1.LSPLGEEM	APOA1.LSPLGEEM	APOA1.LSPLGEEM	586.3 / 918.4	18.
	4		APOA1.LSPLGEEM	APOA1.LSPLGEEM	APOA1.LSPLGEEM	586.3 / 608.4	18.
	5	$\checkmark$	APOA1.LSPLGEEM	APOA1.LSPLGEEM		588.3 / 621.3	18.
	6	$\checkmark$	APOA1.LSPLGEEM	APOA1.LSPLGEEM		588.3 / 734.3	18.
	7		APOA1.LSPLGEEM	APOA1.LSPLGEEM		588.3 / 918.4	18.
	8		APOA1.LSPLGEEM	APOA1.LSPLGEEM		588.3 / 612.4	18.
	9		APOA1.VQPYLDDF	APOA1.VQPYLDDF	APOA1.VQPYLDDF	766.9 / 562.3	21.
	10		APOA1.VQPYLDDF	APOA1.VQPYLDDF	APOA1.VQPYLDDF	766.9 / 677.4	21.
	11	<b>1</b>	APOA1.VQPYLDDF	APOA1.VQPYLDDF	APOA1.VQPYLDDF	766.9 / 792.4	21.
	12		APOA1.VQPYLDDF	APOA1.VQPYLDDF	APOA1.VQPYLDDF	766.9 / 1165.6	21.
	13		APOA1.VQPYLDDF	APOA1.VQPYLDDF_		770.9 / 566.3	21.
	14	V	APOA1.VQPYLDDF	APOA1.VQPYLDDF		770.9 / 681.4	21.
	15	V	APOA1.VQPYLDDF_	APOA1.VQPYLDDF		770.9 / 796.4	21.
	16	<b>V</b>	APOA1.VQPYLDDF	APOA1.VQPYLDDF		770.9 / 1169.6	21.
	17	1	APOA1.DYVSQFE	APOA1.DYVSQFE	APOA1.DYVSQFEG	560.9 / 615.4	23.
	_						

#### Figure 1-27 Create Results Table - Define Components Page

**Tip!** The MRM transitions marked \*.IS or \*. heavy are automatically used as internal standards. However, if the light transitions are to be used as the internal standard, this can be easily reversed by right-clicking in the table and then clicking **Internal Standards > Reverse Analyte/IS Assignments.** 

#### 7. Click Next.

**Tip!** If you acquire data using a MIDAS<sup>™</sup> Workflow, there are additional experiments in the acquisition method because of the MS/MS spectra acquired. If you click Next before reviewing all the experiments, an error message is shown advising you to review all the experiments before proceeding. No review is required, click **OK** to continue.

8. On the Create Results Table - Define Integration page, the automatic integrations can be reviewed. Because Update Expected RT is selected, the retention time is automatically determined from all members of each group as explained for the previous example. To see all members of each group, right-click and then click **Overlay Other Components for Group**.

APOALLSPLGE		Apply		+ APOA1. Area: 4.	LSPLGEEMR.2y5.ligMRM 1.wiff (sample ' 902e4, Height: 2552,154, RT; 19.21 min
APOALLSPLGE	Gaussian Smooth Width:	1.0	points		1 1
APOA1.LSPLGE	Expected RT:	18.80	min	2800	1 //
APOA1.LSPLGE	Expected Kit			2600	- 19 <mark>.2</mark> 1
APOA1.LSPLGE	RT Half Window:	60.0	sec	2400	1 1
APOA1.LSPLGE	Update Expected RT:	Group -		2400	1
APOA1.LSPLGE	E			2200	-
APOA1.VQPYL	Keport Largest Peak			2000	
APOAT.VQPYL	Min. Peak Width:	3	points	2000	1
	Min, Peak Height:	0.00		1800	
	Cloteerstien Barameterr			1000	
APOAL VOPYL	Noire Percentage	40.0	*	21500	1
APOAL VOPYL	Noise Percentage:	40.0	20	를 1400	-
APOA1.VQPYL	Baseline Sub. Window:	2.00	min	- 1200	
APOA1.DYVSQ.	Peak Splitting	2	points	1200	1
APOA1.DYVSQ	- Deservation	·		1000	- 🕅
APOA1.DYVSQ	Regression			800	1
APOA1.DYVSQ	Parameter: Area		-	000	
APOA1.DYVSQ	5 M		=	600	- 1
APOALDYVSQ	Linear		-	400	1 1
APOALDTVSQ	Weighting: None		-	400	1 1
APOALDIATVY			_	200	1. // 🔊 🖓
APOALDIATVY	Concentration Uniter			0	Latit Advind Manue
APOALDLATVY_	Concentration Units: ]				17.5 18.0 18.5 19.0 19.5 20.0 20.5
	Apply units to all Ana	lytes			Time, min

#### Figure 1-28 Create Results Table - Define Integration Page

9. Because the MRM data is collected using the *Scheduled* MRM<sup>™</sup> algorithm, data is only collected over a short retention time. This is why a narrow time axis is shown for each group. Review each group and then click **Next**.

#### **Figure 1-29 Outlier Settings**

et criteria for flagging -  Accuracy for Str Max. Accuracy Tole Max. Accuracy Tole	andards rance for LLO rance for Stds	Q (lowest Std):	20 % Max.	couracy for QCs —— Accuracy Tolerance f	for QC: 15
Component	Calcula IS	Group	Ion Ratio Tolerance (%)	Lower Limit of Calculated Conce	Upper Limit of Calculated Conce.
APOA1.LSPLGE		APOA1.LSPLGE			
APOA1.LSPLGE		APOA1.LSPLGE	20		
APOA1.LSPLGE		APOA1.LSPLGE	20		
APOA1.LSPLGE		APOA1.LSPLGE	20		
APOA1.LSPLGE		APOA1.LSPLGE			
APOA1.LSPLGE		APOA1.LSPLGE	20		
APOA1.LSPLGE		APOA1.LSPLGE	20		
APOA1.LSPLGE		APOA1.LSPLGE	20		
APOA1.VQPYLE	)_ 🕅	APOA1.VQPYLD_			
APOA1.VQPYLE	)_ 📄	APOA1.VQPYLD	20		
APOA1.VQPYLE	)_ [	APOA1.VQPYLD	20		
APOA1.VQPYLC	)_ [[]	APOA1.VQPYLD	20		
APOA1.VQPYL	)_ 🔽	APOA1.VQPYLD_			
					1

- 10. Specify the fields as required to automatically flag samples for review in the Results Table. For more information about the Outlier Settings parameters, refer to the *Reference Guide* that is installed with the MultiQuant<sup>™</sup> software.
- 11. Click Finish.
- 12. After the Results Table opens, click the **Peak Review** icon.

File Edit Process Wind	ow Help						_ 8
🗧 🖙 🖌 🕒 Default 📼							
Components & Groups 🛛 🖌 🔛 🔛 🔝 💭 🗸 🧏 🖾 🔜 🐨 🐨 🖓 Al Sample Types 🔹 🛩 🐲 🌮 🧔 🍳 🚍 🥅 🚳							
Il Components	Index	Sample Name	Sample Type	IS	Component Name	IS Name	Compo
POA1.LSPLGEEMR Group	1	Sample001	Unknown		APOA1.LSPLGEE	N/A	APOA1.LS
POAT DV/SOFEGSALGK		Samela001	Hekeeun		ADOATLEDIGEE	NI/A	1001110
POAT DLATVYVDVLK Gro	2	SampleUUT	Unknown	V	APUAT.LSPLGEE	N/A	APUALLS
POA2.SPELQAEAK Group	3	Sample001	Unknown	1	APOA1.LSPLGEE	N/A	APOA1.LS
POA2.EQLTPLIK Group	4	Sample001	Unknown		APOA1.LSPLGEE	N/A	APOA1.LS
POA4.ISASAEELR Group	6	Cample001	Helseeun		ABOATLEDIGEE	ADOA11 CRICEE	4004110
	-	Sampleuur	Unknown		AFUAT.LOFEGEE.	AFUAT.LOFLGEE	AFUALLS
POA4 TOVNTOAEOLR Gr	6	Sample001	Unknown		APOA1.LSPLGEE	APOA1.LSPLGEE	APOA1.LS
POA4.SELTQQLNALFQDK	7	Sample001	Unknown	1	APOA1.LSPLGEE	APOA1.LSPLGEE.	APOA1.LS
POA4 ALVQQMEQLR Gro	0	Samela001	Hekeeun		APOA11 SPIGEE	APOA1 I SPI GEE	AP0 4119
POB.SVSLPSLDPASAK Gr	•	Sampleoon	Onknown		AFUAT.LOFEGEE.	AFUAT.LOFLGEE.	AFUALLS
POC3 DALSSVQESQVAQ	9	Sample001	Unknown	1	APOA1.VQPYLD	N/A	APOA1.V
POC3.SEAEDASLLSFMQ	10	Sample001	Unknown	1	APOA1.VQPYLD	N/A	APOA1.V0
POHATEGCHDGYSLDGP	11	Sample(001	Hekooun	122	APO A1 VOPYLD	NI/A	AP0 41 V
POH.ATVVYQGER Group		Samplevvi	Unknown	V	AFOALVQFILD.	1975	AF OAL Y
Internal Standarde	12	Sample001	Unknown	1	APOA1.VQPYLD	N/A	APOA1.V
POA1.LSPLGEEMR.2v5.lig	13	Sample001	Unknown		APOA1.VQPYLD	APOA1.VQPYLD_	APOA1.V0
POA1.LSPLGEEMR.2y6.lig	14	Sample001	Unknown		APO A1 VOPYLD	APO AT VOPYLD	APO A1 V
POA1.LSPLGEEMR.2y8.lig	14	Jampievon	UIKIIOWII		AFOALVQFTLD.	AFOALVQFTLD_	AI UAL I
POA1.LSPLGEEMR.2b5.lig	15	Sample001	Unknown		APOA1.VQPYLD	APOA1.VQPYLD	APOA1.V0
POAT VOPYLDDFOK 2:4 I	16	Sample001	Unknown	17	APOA1.VQPYLD	APOA1.VQPYLD_	APOA1.V0
OA1.VQPYLDDFQK.2y5.li	17	Sample(())1	Hekoowe	12	APO AL DW/SOF	N/A	APO A1 D
POA1.VQPYLDDFQK.2y8.li		Compression	CIRCIONI	V	AFOALDIVOQF	190	AF OALD
POA1.DYVSQFEGSALGK	18	Sample001	Unknown	$\checkmark$	APOA1.DYVSQF	N/A	APOA1.D
POAT DYVSQFEGSALGK	19	Sample001	Unknown	1	APOA1.DYVSQF	N/A	APOA1.D
OA1 DYVSOFEGSALGK	20	Sample001	Unknown	12	APO AL DYVSOF	Ν/Δ	APOALD
POA1.DLATVYVDVLK.2Y5			Cilicity Hill			100	
POA1.DLATVYVDVLK.2Y6	21	Sample001	Unknown		APOAT.DYVSQF	APOA1.DYVSQF	APOA1.D1
POAT.DLATVYVDVLK286	22	Sample001	Unknown		APOA1.DYVSQF	APOA1.DYVSQF_	APOA1.DY
POAT DEAT VTVDVEK 294.	23	Sample001	Unknown	EP3	APO AL DYVSOF	APOAL DYUSOF	4P041 0
POA2 SPELOAEAK 2v4 lin	23	JampievVI	VIINIVIII		ALONIOTVOQF	AFOALDTV3QF.	AI UALU
POA2 SPELQAEAK 2Y6.lig	24	Sample001	Unknown		APOALDYVSQF	APOA1.DYVSQF	APOA1.DY

Figure 1-30 Results Table and Peak Review Pane

- 13. A useful way to visualize the grouped data is to overlay all members of the same group. Right-click in the Peak Review pane and then click **Options**.
- 14. In the Peak Review Options dialog, in the **Overlay** field, select **All components for group** and then click **OK**.

Figure 1-31 Peak Review Options Dialog

M Peak Review Options
Appearance Zooming
Number of rows: 2
Number of columns: 2
Overlay:
All components for group
Highlight active graph using: Bold, italic title and grey background
Fill Peaks: Using dotted style 💌
Hide axis names
Hide Y-axis
Mark expected RT with arrow
OK Cancel

#### Figure 1-32 Overlay: All components for group



### **Run Queries**

The primary purpose of queries is to allow potentially problem peak integrations to be flagged so that only a subset of the chromatograms needs to be manually reviewed. Queries can also be used to create and populate custom columns. For more information on using and creating queries, refer to the *Reference Guide* that is installed with the MultiQuant<sup>TM</sup> software.

- 1. Click **Process > Query.**
- 2. In the Query file list, select Area Ratio Query and then click OK.

This query is used to assess the Light/Heavy ratios for each MRM per peptide and flag any cases where there is disagreement between multiple ratios.

#### Figure 1-33 Query Dialog

uery		×
Query file:	Area Ratio (Median & Average)xls	Edit Query
Apply query to:	All rows (visible and hidden)	
Leave previo	usly failed queries as failed	
Edit query pa	rameters (if available)	
	OK Cancel	

Good default values for this query are shown in Figure 1-34. A peak integration is flagged as Failed if it does not pass the criteria.

#### Figure 1-34 Query Parameters Dialog

×
30
Cancel

Several custom columns are added to the Results Table. In the last column, the %RSD computed for each group is shown. The first peptide group passes the Query filter, with a %RSD result of 5.4% (Figure 1-35 item 2). To advance to the first failed peak, click the Query Failure icon (Figure 1-35 item 1).

<b>**</b>	🖽 📾 📿 🗹 🔻 📴 🖾 🖾 🕼 🔰 All Sample Types 🔹 🗸 1 🌮 🦚 🔍 🚍 🔤 <sub>(2)</sub>									
I	ndex	Retention Time	Width at 50%	Used	Calculated Concentration	Accuracy	Area Ratio	Median Area Ratio	Average Area Ratio	%RSD
	1	19.20	0.28	<b>V</b>	<2 points	N/A	0.802	0.814	0.830	4.889
	2	19.20	0.27	V	<2 points	N/A	0.823	0.814	0.830	4.889
	3	19.20	0.28	<b>V</b>	<2 points	N/A	0.889	0.814	0.830	4.889
	4	19.22	0.28	V	<2 points	N/A	0.805	0.814	0.830	4.889
	5	19.24	0.30		N/A	N/A	N/A	0.814	0.830	4.889
	6	19.19	0.24		N/A	N/A	N/A	0.814	0.830	4.889
	7	19.21	0.30		N/A	N/A	N/A	0.814	0.830	4.889
	8	19.21	0.29		N/A	N/A	N/A	0.814	0.830	4.889

Figure	1-35	Query	Results
--------	------	-------	---------

ltem	Description
1	Show only query failures
2	Custom columns

### **Accurate Quantitation Using Calibration Curves**

When you perform accurate quantitation of peptides or proteins (using peptides as surrogates), it is possible to create stable isotope labeled peptides to use as concentration standards. These heavy peptides can be created using labeling strategies such as mTRAQ<sup>®</sup> reagents or by making synthetic peptides using stable isotope-labeled amino acids. By proper use of these peptides, experiments can be designed that leverage the internal standard concept and add robustness to your experiments.

A calibration curve can be generated for the accurate quantitation experiment using increasing concentrations of the "heavy peptide" in the presence of a constant amount of the biological sample of interest (the matrix that contains the light version of the same peptide). In this case, the endogenous light peptide acts as the constant internal standard for the calibration curve.

**Note:** If you are not using the Audit Trail with Security Features edition, then you can launch the MultiQuant<sup>™</sup> software directly from the desktop icon.

### Before you begin

- Make sure you are in the Example project.
- Make sure the Pep Quant Curve 1.wiff file and the Pep Quant Samples A.wiff file are in the <drive>:\Analyst Data\Projects\Example\Data\Example Data folder.

### Set the Peak Integration Parameters

- 1. In the Analyst<sup>®</sup> software, on the Navigation bar, under **Companion Software**, double-click **MultiQuant 3.0.3**.
- 2. Click Edit > Project Integration Defaults.

**Note:** If you are not using the Audit Trail and Security Features edition, then click **Edit > User Integration Defaults.** 

3. In the Integration Defaults dialog, select **MQ4** from the **Integration Algorithm** list. Good peak integration parameters for this dataset are shown in Figure 1-36.

Integration Defaults		×
Integration Algorithm: 🚺	Q4	•
Gaussian Smooth Width:	: 3.0	points
Expected RT:	0.00	min
RT Half Window:	90.0	sec
Update Expected RT:	Group -	·
🔲 Report Largest Peak	· -	_
Min. Peak Width:	3	points
Min. Peak Height:	0.00	
Integration Parameter	s	
Noise Percentage:	40.0	%
Baseline Sub. Window:	2.00	min
Peak Splitting	4	points
ОК	С	ancel

#### Figure 1-36 Integration Defaults dialog

In many cases a smaller smoothing width (for example, 1.0) might be more appropriate. Also note that the peak review process will be eased by specifying a non-zero Min. Peak Height, typically corresponding to at least one or two counts. Because the entered threshold should be in counts per second (cps), the actual value required will depend on the dwell time. For example, for a dwell time of 100 ms, two counts corresponds to a setting of 20 cps.

4. For this dataset, good results are achieved using the parameters shown in the previous figure. Adjust the values and then click **OK**.

### **Create a Results Table**

- 1. Click File > New Results Table.
- 2. On the Create Results Table Select Samples page, drag the Pep Quant Curve 1.wiff into the Selected pane and then click **Next**.



Figure 1-37 Create Results Table - Select Samples page

3. On the Create Results Table - Select Method page, click the **Create New Method (MQ4)** option and then click **New**.

Create Results Table	- Select Method			- • •
Select an existing quanti	tation method or create a new m	nethod now.		
C. Channe Evision Ma	thed			
Choose Existing Me	unoa			
Method Name:				Open
Edit Method				
<ul> <li>Create New Method</li> </ul>	(MQ4)			
Method Name:	Click 'New' to select method			New
C Use 'Automatic' Met (Created on-the-fly :	hod (MQ4) and most useful when MRM tran	isitions differ between samples)		
		< Back Next >	Finish	Cancel

Figure 1-38 Create Results Table - Select Method page

4. Type a name for your method, click **Save**, and then click **Next**.

In this tutorial, you will create a new method. Creating new methods gives you an opportunity to review and apply different parameters for the integration of your data.

5. On the Create Results Table - Select Representative Sample page, a representative sample has been recommended and is selected. Click **Next**.

The software recommends a representative sample based on selecting a chromatogram that provides the best opportunity to select integration parameters that fit the entire batch.



#### Figure 1-39 Create Results Table - Select Representative Sample page

6. On the Create Results Table - Define Components page, the details of the various analytes and internal standards to be processed must be defined. This step is required because the ID field of the Analyst software Acquisition Method Editor was not completed as discussed in Process Data Acquired Using the Scheduled MRM<sup>TM</sup> Algorithm on page 25. All the MRM transitions from the data file are automatically loaded into the window. First, all the members of a Group must be defined by giving them the same name. In this example, there are three groups, each consisting of four MRMs. The group names can be typed into the Group column or copied from an Excel spreadsheet. The Groups can be named in many ways, depending on the compounds.

Row	IS	Name	Group	IS Name	Q1/Q3
1		395.2 / 272.1			395.2 / 272.1
2		395.2 / 400.2			395.2 / 400.2
3		397.9 / 272.1			397.9 / 272.1
4		397.9 / 400.2			397.9 / 400.2
5		432.9 / 269.2			432.9 / 269.2
6		432.9 / 647.4			432.9 / 647.4
7		435.6 / 269.2			435.6 / 269.2
8		435.6 / 655.4			435.6 / 655.4
9		523.8 / 263.1			523.8 / 263.1
10		349.5 / 371.2			349.5 / 371.2
11		516.8 / 263.1			516.8 / 263.1
12		344.8 / 371.2			344.8 / 371.2
13					
			111		

#### Figure 1-40 Create Results Table - Define Components page

**Note:** When you group MRM transitions together, they should be to the same compound, including the internal standard version of that compound.

7. In this example, name the first 4 MRMs Peptide 1, the second four rows Peptide 2, and the third four are Peptide 3. Type the peptide name in the first row only of each group (Rows 1, 5, 9), and then right-click in the table and then click **Groups > By Filling Down Existing Groups.** 

operiment	:  MRI	4 (12 transitions)	<u> </u>		
Row	IS	Name	Group	IS Name	Q1/Q3
1		395.2 / 272.1	Peptide 1		395.2 / 272.1
2		395.2 / 400.2	Peptide 1		395.2 / 400.2
3		397.9 / 272.1	Peptide 1		397.9 / 272.1
4		397.9 / 400.2	Peptide 1		397.9 / 400.2
5		432.9 / 269.2	Peptide 2		432.9 / 269.2
6		432.9 / 647.4	Peptide 2		432.9 / 647.4
7		435.6 / 269.2	Peptide 2		435.6 / 269.2
8		435.6 / 655.4	Peptide 2		435.6 / 655.4
9		523.8 / 263.1	Peptide 3		523.8 / 263.1
10		349.5 / 371.2	Peptide 3		349.5 / 371.2
11		516.8 / 263.1	Peptide 3		516.8 / 263.1
12		344.8/371.2	Peptide 3		344.8 / 371.2
13					

#### Figure 1-41 Groups > By Filling Down Existing Groups option

**Note:** Specifying the names and groups here is necessary because this information was not specified in the original data acquisition method. Refer to the Tip on page 30 to learn how to use the ID field of the Acquisition Method Editor to enter this information.

8. Copy the **Group** column into the **Name** column. Right-click in the table and then click **Groups > Add Group to Start of Component Name**.

periment	. jmna	(12 transitions)	-		
Row	IS	Name	Group	IS Name	Q1/Q3
1		Peptide 1.395.2 / 272.1	Peptide 1		395.2 / 272.1
2		Peptide 1.395.2 / 400.2	Peptide 1		395.2 / 400.2
3		Peptide 1.397.9 / 272.1	Peptide 1		397.9 / 272.1
4		Peptide 1.397.9 / 400.2	Peptide 1		397.9 / 400.2
5		Peptide 2.432.9 / 269.2	Peptide 2		432.9 / 269.2
6		Peptide 2.432.9 / 647.4	Peptide 2		432.9 / 647.4
7		Peptide 2.435.6 / 269.2	Peptide 2		435.6 / 269.2
8		Peptide 2.435.6 / 655.4	Peptide 2		435.6 / 655.4
9		Peptide 3.523.8 / 263.1	Peptide 3		523.8 / 263.1
10		Peptide 3.349.5 / 371.2	Peptide 3		349.5 / 371.2
11		Peptide 3.516.8 / 263.1	Peptide 3		516.8 / 263.1
12		Peptide 3.344.8 / 371.2	Peptide 3		344.8 / 371.2
13					

#### Figure 1-42 Groups > Add Group to Start of Component Name option

- 9. Define the MRM transitions to be used as the internal standards by selecting the check boxes in the IS column. In this example, the last four transitions in each group are the internal standards for the first four transitions in the group. Select the **IS** boxes as shown in the following figure for Peptide 1.
- 10. After an MRM transition has been defined as an internal standard, it can be associated with the correct analyte in the **IS Name** column. By clicking on the right side of each row within this column, a list of internal standards is shown and can be selected. Select the appropriate internal standard from the list for each of the first two MRM transitions for Peptide 1. Within the group, the third MRM is the IS for the first row, the forth MRM is the IS for the second row.

Row	15	Name	Group	IS Name	01/03
1	1.5	Peptide 1.395.2 / 272.1	Peptide 1	Peptide 1.397.9 / 272.1	395.2 / 272.1
2		Peptide 1.395.2 / 400.2	Peptide 1	Peptide 1.397.9 / 400.2	395.2 / 400.2
3		Peptide 1.397.9 / 272.1	Peptide 1		397.9 / 272.1
4		Peptide 1.397.9 / 400.2	Peptide 1		397.9 / 400.2
5		Peptide 2.432.9 / 269.2	Peptide 2		432.9 / 269.2
6		Peptide 2.432.9 / 647.4	Peptide 2		432.9 / 647.4
7		Peptide 2.435.6 / 269.2	Peptide 2		435.6 / 269.2
8		Peptide 2.435.6 / 655.4	Peptide 2		435.6 / 655.4
9		Peptide 3.523.8 / 263.1	Peptide 3		523.8 / 263.1
10		Peptide 3.349.5 / 371.2	Peptide 3		349.5 / 371.2
11		Peptide 3.516.8 / 263.1	Peptide 3		516.8 / 263.1
12		Peptide 3.344.8 / 371.2	Peptide 3		344.8/371.2
13					

#### Figure 1-43 Selecting Internal Standards

11. To propagate this format to all the groups in the data file, select the first row for Peptide 1, right-click and then click **Internal Standards > Set for all Groups as for Selected Group.** 

**Note:** The pattern of sample and IS MRM transitions must be constant through the data file to be able to propagate the group pattern. This reduces the manual interaction required when setting up the processing method.

Row	IS	Name	Group	IS Name	Q1/Q3
1		Peptide 1.395.2 / 272.1	Peptide 1	Peptide 1.397.9 / 272.1	395.27272.1
2		Peptide 1.395.2 / 400.2	Peptide 1	Peptide 1.397.9 / 400.2	395.2 / 400.2
3		Peptide 1.397.9 / 272.1	Peptide 1		397.9 / 272.1
4	$\checkmark$	Peptide 1.397.9 / 400.2	Peptide 1		397.9 / 400.2
5		Peptide 2.432.9 / 269.2	Peptide 2	Peptide 2.435.6 / 269.2	432.9 / 269.2
6	<b>F</b>	Peptide 2.432.9 / 647.4	Peptide 2	Peptide 2.435.6 / 655.4	432.9 / 647.4
7	<b>V</b>	Peptide 2.435.6 / 269.2	Peptide 2		435.6 / 269.2
8	<b>V</b>	Peptide 2.435.6 / 655.4	Peptide 2		435.6 / 655.4
9		Peptide 3.523.8 / 263.1	Peptide 3	Peptide 3.516.8 / 263.1	523.8 / 263.1
10		Peptide 3.349.5 / 371.2	Peptide 3	Peptide 3.344.8 / 371.2	349.5 / 371.2
11		Peptide 3.516.8 / 263.1	Peptide 3		516.8 / 263.1
12		Peptide 3.344.8 / 371.2	Peptide 3		344.8/371.2
13					

#### Figure 1-44 Standards > Set for all Groups as for Selected Group Option

**Note:** If a multi-period experiment was used, the previous steps must be repeated for each period. To switch between periods, select the period from the Experiment list and then repeat the process of setting up the processing method as described. This dataset does not contain multiple periods.

**Tip!** If required, multiple MRM transitions for the same analyte can be summed during data processing. Right-click in the Define Components table and then click **Sum Multiple Ions**.

- 12. Click Next.
- 13. On the Create Results Table Define Integration page, the appropriate peak integration parameters will be defined for each individual MRM. Each MRM peak has been automatically integrated, but you have the option to adjust the peak integration (that is, retention time of integrated peak) for each MRM.
- 14. Each MRM transition can be viewed by clicking the specific MRM in the left pane list, or by using the Up / Down arrows or mouse scroll wheel to automatically advance through the list. When an individual MRM transition has not been integrated at the right retention time, the correct peak can be selected with the mouse by just dragging across it on the pane and it will be automatically reintegrated. In some cases it might also be necessary to adjust the peak-finding parameters.

If multiple periods are present in the method, all MRM transitions within all periods are integrated in this pane as a single list.

- 15. Select the parameters for generating the calibration curve. Typically, the peak area is used in the calculation. In the **Regression** group, select the following and then click **Apply**:
  - Parameter: Area
  - Fit: Linear
  - Weighting: 1/x.

Typically, the peak area is used in the calculation. A linear calibration curve with a weighting of 1/x is most commonly used for calculating the calibration curve.

Create Results Table Scroll through the variou	- Define Integration is components, setting the i	ntegration p	paramete	rs as needed	I.		• •
Peptide 1.395.2 / 27		Apply		Peptide 1.38 Area: 3187.1	95.2 / 272.1 from C 132, Height: 572.031	uant Curve 1.wiff , RT: 3.84 min	(sample 9))
Peptide 1.397.9 / 27 Peptide 1.397.9 / 40	Gaussian Smooth Width:	3.0	points	55	50 -		3.84
Peptide 2.432.9 / 26 Peptide 2.432.9 / 64	RT Half Window:	90.0	sec	50	0		
Peptide 2.435.6 / 26 Peptide 2.435.6 / 65	Update Expected RT:	Group 💌		45	50 -		
Peptide 3.523.8 / 26 Peptide 3.349.5 / 37	Report Largest Peak Min. Peak Width:	3	points	40	xo -		
Peptide 3.516.8 / 26 Peptide 3.344.8 / 37	Min. Peak Height:	0.00		35	50 -		
	Integration Parameters Noise Percentage:	40.0	%	Apsuage 30	0		
	Baseline Sub. Window:	2.00	min	≝ 25	50 -		
	Regression	-	points	20	0		
	Parameter: Area		•	15	50 -		
	Fit: Linear		4	10	0 -		
	meignong.		<u> </u>	5	50 -	Í	
	Concentration Units:	lytes			0.5 1.0 1.5 2	2.0 2.5 3.0 3.5 4 Time, min	4.5 5.0
				< Back	Next >	Finish	Cancel

#### Figure 1-45 Create Results Table - Define Integration page

16. Click Next.

Accuracy for Stand Max. Accuracy Toleran Max. Accuracy Toleran	ards ce for LLO ce for Stds	Q (lowest Std):	20 % Max.	ocuracy for QCs Accuracy Tolerance f	for QC: 15
Component	Calcul	Group	Ion Ratio Tolerance (%)	Lower Limit of Calculated Conce	Upper Limit of Calculated Conce
Peptide 1.395.2 /		Peptide 1			
Peptide 1.395.2 /		Peptide 1	20		
Peptide 1.397.9 /	<b>V</b>	Peptide 1			
Peptide 1.397.9 /	<b>V</b>	Peptide 1	20		
Peptide 2.432.9 /		Peptide 2			
Peptide 2.432.9 /		Peptide 2	20		
Peptide 2.435.6 /	<b>V</b>	Peptide 2			
Peptide 2.435.6 /	<b>V</b>	Peptide 2	20		
Peptide 3.523.8 /		Peptide 3			
Peptide 3.349.5 /		Peptide 3	20		
Peptide 3.516.8 /	<b>V</b>	Peptide 3			
Peptide 3.344.8 /	V	Peptide 3	20		

17. Specify the fields as required to automatically flag samples for review in the Results Table. Refer to the *Reference Guide* for more information about the **Outlier Settings** parameters.

18. Click Finish.

### Modify the Results Table

All of the MRM transitions in each of the samples are integrated with the specified parameters as shown in Figure 1-47.

On the left side, the information is organized in a number of ways. If All Components is selected, all integrated MRMs for all samples are listed in the order that the MRMs appear in the data file, one sample at a time. If an individual group is selected (Peptide 1 Group) then only the MRM transitions within that group for all the samples are shown. The data for all internal standards or all analytes can be visualized by selected either All Internal Standards or All Analytes. Finally, the data for any one IS or analyte can be viewed by selecting its name in the list.

#### Figure 1-47 Results Table Interface

Edit Process Wind	Help							- 0
• 🖬 🗀 Default 👻	$\mathcal{O}$							
ts & Groups IS M	🗄 🗂 🛛	🛛 🐱 🕶 🖾	🖂 🗔 🍟 Al Sa	mple 1	ypes 🔹 🖂 🛎 🛉	P 🗱 🍳 🗏 🛙		4
nents	Index	Sample Name	Sample Type	IS	Component Name	IS Name	Component Group Name	Outer
Group	1	Blank 1	Unknown	23	Peptide 1.395.2 /	Peptide 1.397.9 /	Peptide 1	
Group	2	Blank 1	Unknown	10	Peptide 1.395.2 /	Peptide 1.397.9 /	Peptide 1	
Standards	3	Blank 1	Unknown		Peptide 1.397.9 /_	NA	Peptide 1	
397.9 / 272.1	4	Blank 1	Unknown		Peptide 1.397.9 /	NA	Peptide 1	_
435.6 / 269.2	5	Blank 1	Unknown		Peptide 2.432.9 /_	Peptide 2.435.6 /_	Peptide 2	
435.6 / 655.4 516.8 / 263.1	6	Blank 1	Unknown	F1	Peptide 2.432.9 /_	Peptide 2.435.6 /	Peptide 2	
344.8/371.2	7	Blank 1	Unknown		Peptide 2.435.6 /_	NA	Peptide 2	
10	8	Blank 1	Unknown		Peptide 2.435.6 /	NA	Peptide 2	
395.2 / 272.1	9	Blank 1	Unknown	173	Peptide 3.523.8 /_	Peptide 3.516.8 /	Peptide 3	
432.9 / 269.2	10	Blank 1	Unknown	13	Peptide 3.349.5 /_	Peptide 3.344.8 /_	Peptide 3	-
432.9 / 647.4 523.8 / 263.1	11	Blank 1	Unknown		Peptide 3.516.8 /_	NA	Peptide 3	
349.5/371.2	12	Blank 1	Unknown	V	Peptide 3.344.8 /_	NA	Peptide 3	
	13	Matrix Blank 1	Unknown	123	Peptide 1.395.2 /_	Peptide 1.397.9 /	Peptide 1	-
	14	Matrix Blank 1	Unknown	173	Peptide 1.395.2 /_	Peptide 1.397.9 /	Peptide 1	-
	15	Matrix Blank 1	Unknown		Peptide 1.397.9 /_	NA	Peptide 1	
	16	Matrix Blank 1	Unknown	V	Peptide 1.397.9 /_	NA	Peptide 1	
	17	Matrix Blank 1	Unknown	173	Peptide 2.432.9 /_	Peptide 2.435.6 /	Peptide 2	
	18	Matrix Blank 1	Unknown	12	Peptide 2.432.9 /_	Peptide 2.435.6 /_	Peptide 2	-
	19	Matrix Blank 1	Unknown		Peptide 2.435.6 /	NA	Peptide 2	
	20	Matrix Blank 1	Unknown		Peptide 2.435.6 /_	NA	Peptide 2	-
	21	Matrix Blank 1	Unknown	P3	Peptide 3.523.8 /_	Peptide 3.516.8 /_	Peptide 3	
	22	Matrix Blank 1	Unknown	10	Peptide 3.349.5 /_	Peptide 3.344.8 /_	Peptide 3	
	23	Matrix Blank 1	Unknown	1	Peptide 3.516.8 /_	NA	Peptide 3	-
	24	Matrix Blank 1	Unknown		Peptide 3.344.8 /_	NA	Peptide 3	
	25	Conc11	Unknown	171	Peptide 1.395.2 /_	Peptide 1.397.9 /	Peptide 1	

Item	Description
1	Peptide Group
2	Peak Review icon

- 1. Save the Results Table by clicking **File > Save**.
- 2. To visualize the MRM transitions for selected group for all samples, select a **Peptide Group** from the left panel and then click the **Peak Review** icon. If a row is selected within the table, the selected MRM transition is shown in the pane below with the name highlighted in bold. The Up / Down arrows or the scroll wheel on the mouse can be used to advance through the rows of the table to change the active pane.
- 3. If an incorrect peak is integrated, the correct peak can be selected and integrated by dragging the mouse across the peak. If the peak does not integrate, click the **Enable Manual Integration Mode** icon to integrate manually. Define the peak area by dragging the mouse across the bottom of the peak area to be integrated. The selected peak integration will be shown automatically by the peak filling.



Figure 1-48 Peak Review Interface

Item	Description
1	Sort buttons
2	Enable Manual Integration Mode icon

**Tip!** You can review the data within the Results Table in many ways. For example, the Area column can be selected by clicking on the column header and then selecting the **Ascending Sort** or **Descending Sort**. This sorts the data within the table.

4. To edit the structure of the Results Table and ensure that the required columns for determining calibration curves are visible, right-click in the table and then click **Column Settings**. Columns can be added or removed from the Results Table view by selecting or clearing the check boxes in the Visible column.

Recommended columns to use for accurate quantitation include: Sample Index, Sample Name, Sample Type, Actual Concentration, Area, IS Area, S/N, Modified, Used, Calculated Concentration and Accuracy.

#### Figure 1-49 Column Settings Dialog

Import Export			
Column Name 🖉	Visible	Number Format	4
Accuracy	1	0.00	Π
Acq. Method Name			Ξ
Acquisition Date & Time			
Actual Concentration	1	0.00	
Area	1	0.000e0	
Area / Height		0.00	
Area Ratio		0.000e0	
Asymmetry Factor		0.00	
Baseline Delta / Height		0.000e0	
Calculated Concentration	<b>V</b>	0.000e0	
Component Comment			
Component Group Name	<b>V</b>		
Component Index			
Component Name	<b>V</b>	]	
Conc. Units			
Concentration Ratio		0.00	
Corrected Area		0.000e0	
Corrected Area/Height		0.00	
Corrected Height		0.000e0	
Dilution Eactor		0.00	4

5. The information about the internal standards and samples must be defined. Select one of the peptide MRMs under All Internal Standards and then enter the Actual Concentration (25 in this example) in the correct column for the first sample. Select the column by clicking the column header, and then click **Edit > Fill Down**. The concentration are filled in down the column.

III Components       Index       Original Florance       Sample Name       Sample Type       Actual Concentration       US Actual Concentration       UNA       NIA       Peptide 1         11       Internal Standards certified 24251 / 2592 eeded 24251 / 2592 eeded 24251 / 2512 eeded 24251 / 2514 eeded 3523 / 2511       Internal Internation 2500       NIA       NIA       NIA       Peptide 1         1111       Pep Quant Curve 1xi. Conc125 1       Conc25 2       <	omponents & Groups IS #	🗄 📾 🗄	🖉 🗠 👻 🔤 🖾	Al Sample T	ypes ▼ \comes_a	• 🐶 🗱 🔒	🔒 🔍 🗖 🛙		
erdde 1 Group erdde 3 Group erdde 3 Group erdde 3 Group erdde 3 Group erdde 3 Group erdde 1 39 Pep Quart Curve 1.ni. Matrix Blank 1 Urknown 2500 NiA NiA Pepide 1 27 Pep Quart Curve 1.ni. Conc1 1 Urknown 2500 NiA NiA Pepide 1 27 Pep Quart Curve 1.ni. Conc1 2 Urknown 2500 NiA NiA Pepide 1 93 Pep Quart Curve 1.ni. Conc1 3 Urknown 2500 NiA NiA Pepide 1 erdde 1393 27 292 2 erdde 2356 / 655 4 erdde 2356 / 655 4 erdde 2356 / 783 1 99 Pep Quart Curve 1.ni. Conc5 1 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 1 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 2 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 3 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 3 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 3 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 2 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 3 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 3 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 3 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 2 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc5 2 Urknown 2500 NiA NiA Pepide 1 99 Pep Quart Curve 1.ni. Conc15 2 Urknown 2500 NiA NiA Pepide 1 111 Pep Quart Curve 1.ni. Conc15 2 Urknown 2500 NiA NiA Pepide 1 122 Pep Quart Curve 1.ni. Conc15 2 Urknown 2500 NiA NiA Pepide 1 147 Pep Quart Curve 1.ni. Conc15 2 Urknown 2500 NiA NiA Pepide 1 147 Pep Quart Curve 1.ni. Conc15 2 Urknown 2500 NiA NiA Pepide 1 148 Pepide 3 343 5/ 371.2 6 Marcual Integration 6 Marcual Integrati	Il Components	Index	Original Filename	Sample Name	Sample Type	Actual	IS Actual Concentration	IS Name	Component Group Name
Biolog	eptide 1 Group	3	Pep Quant Curve 1.wi.	Blank 1	Unknown	25.00	NA	N/A	Peptide 1
I Idernal Standards       27       Pep Quart Curve 1 ski.       Conc 1 1       Urknown       2500       NiA       NiA       Pep Guart         906 1397 97 4002       polos 1397 97 4002       polos 1397 97 4002       polos 1397 97 4002       NiA       NiA       NiA       Pep Guart       Pep Guart       Pep Guart       Conc 1 2       Urknown       2500       NiA       NiA       Pep Guart       NiA       NiA       NiA       Pep Guart       NiA       NiA       NiA       Pep Guart	ptide 3 Group	15	Pep Quant Curve 1.wi.	Matrix Blank 1	Unknown	25.00	NA	N/A	Peptide 1
39       Pep Quart Curve 1.sk.       Conc 1.2       Urknown       25:00       N/A       N/A       Pepde 1         906 2.355 (# 253.5)       51       Pep Quart Curve 1.sk.       Conc 1.3       Urknown       25:00       N/A       N/A       Pepde 1         906 2.355 (# 253.5)       51       Pep Quart Curve 1.sk.       Conc 51       Urknown       25:00       N/A       N/A       Pepde 1         906 2.355 (# 253.5)       78       Pep Quart Curve 1.sk.       Conc 52       Urknown       25:00       N/A       N/A       Pepde 1         976 3.352 (# 272.1)       59       Pep Quart Curve 1.sk.       Conc 52       Urknown       25:00       N/A       N/A       Pepde 1         966 3.352 (# 272.1)       59       Pep Quart Curve 1.sk.       Conc 52       Urknown       25:00       N/A       N/A       Pepde 1         99       Pep Quart Curve 1.sk.       Conc 25:1       Urknown       25:00       N/A       N/A       Pepde 1         906 3.523 8 / 253 1       123       Pep Quart Curve 1.sk.       Conc 25:2       Urknown       25:00       N/A       N/A       Pepde 1         906 3.523 8 / 253 1       123       Pep Quart Curve 1.sk.       Conc 125:1       Urknown       25:00       N/A       N/	Internal Standards	27	Pep Quant Curve 1.wi.	Conc11	Unknown	25.00	NA	NA	Peptide 1
pode 1 355 / 2721       51       Pep Quart Curve 1 xii.       Conc 1 3       Urknown       25:00       NIA       NIA       Pep de 1         pode 2 356 / 5524       63       Pep Quart Curve 1 xii.       Conc 5 1       Urknown       25:00       NIA       NIA       Pep de 1         pode 3 356 / 2721       63       Pep Quart Curve 1 xii.       Conc 5 1       Urknown       25:00       NIA       NIA       Pep de 1         pode 3 356 / 2721       99       Pep Quart Curve 1 xii.       Conc 5 2       Urknown       25:00       NIA       NIA       Pep de 1         pode 3 356 / 2721       99       Pep Quart Curve 1 xii.       Conc 5 2       Urknown       25:00       NIA       NIA       Pep de 1         pode 3 523 / 2721       99       Pep Quart Curve 1 xii.       Conc 25 1       Urknown       25:00       NIA       NIA       Pep de 1         pode 3 523 / 2721       111       Pep Quart Curve 1 xii.       Conc 25 2       Urknown       25:00       NIA       NIA       Pep de 1         pode 3 523 / 2731 2       123       Pep Quart Curve 1 xii.       Conc 125 1       Urknown       25:00       NIA       NIA       Pep de 1         135       Pep Quart Curve 1 xii.       Conc 125 2       Urknown	ptide 1.397.9 / 272.1	39	Pep Quant Curve 1.wi.	Conc12	Unknown	25.00	NA	NA	Peptide 1
piede 2.135 / 1652.1 piede 3.145 / 152.1 piede 1.145 / 152.1 piede 1	ptide 2.435.6 / 269.2	51	Pep Quant Curve 1.wi.	Conc13	Unknown	25.00	N/A	NIA	Peptide 1
75       Pep Quart Curve 1 xii.       Conc5 2       Urknown       25:00       N/A       N/A       Pepde 1         76       Pep Quart Curve 1 xii.       Conc5 2       Urknown       25:00       N/A       N/A       Pepde 1         76       Pep Quart Curve 1 xii.       Conc5 3       Urknown       25:00       N/A       N/A       Pepde 1         76       Pep Quart Curve 1 xii.       Conc5 3       Urknown       25:00       N/A       N/A       Pepde 1         76       Pep Quart Curve 1 xii.       Conc25 1       Urknown       25:00       N/A       N/A       Pepde 1         77       Pep Quart Curve 1 xii.       Conc25 1       Urknown       25:00       N/A       N/A       Pepde 1         78       Pep Quart Curve 1 xii.       Conc125 1       Urknown       25:00       N/A       N/A       Pepde 1         78       Pep Quart Curve 1 xii.       Conc125 1       Urknown       25:00       N/A       N/A       Pepde 1         78       Pep Quart Curve 1 xii.       Conc125 2       Urknown       25:00       N/A       N/A       Pepde 1         79       Pep Quart Curve 1 xii.       Conc125 2       Urknown       25:00       N/A       N/A       Pepde 1	ptide 2.435.6 / 655.4	63	Pep Quant Curve 1.wi.	Conc51	Unknown	25.00	NA	N/A	Peptide 1
Analytes       87       Pep Quant Curve 1:xii.       Conc53       Urknown       25:00       NIA       NIA       Pepde 1         966       3552 / 272:1       96       Pep Quant Curve 1:xii.       Conc25 1       Urknown       25:00       NIA       NIA       Pepde 1         966       3523 / 272:1       Pep Quant Curve 1:xii.       Conc25 1       Urknown       25:00       NIA       NIA       Pepde 1         966       2423 / 262       Pep Quant Curve 1:xii.       Conc25 2       Urknown       25:00       NIA       NIA       Pepde 1         122       Pep Quant Curve 1:xii.       Conc25 2       Urknown       25:00       NIA       NIA       Pepde 1         135       Pep Quant Curve 1:xii.       Conc125 1       Urknown       25:00       NIA       NIA       Pepde 1         135       Pep Quant Curve 1:xii.       Conc125 2       Urknown       25:00       NIA       NIA       Pepde 1         147       Pep Quant Curve 1:xii.       Conc125 2.       Urknown       25:00       NIA       NIA       Pepde 1         146       Den.Curve 1:xii.       Conc125 2.       Urknown       25:00       NIA       NIA       Pepde 1         147       Pep Quant Curve 1:xii.	ptide 3.344.8 / 371.2	75	Pep Quant Curve 1.wi.	Conc5 2	Unknown	25.00	NA	N/A	Peptide 1
préde 1355 / 272 1 99 Pep Quart Curve 1.si. Conc25 1 Urknown 2500 N/A N/A Peptide 1 111 Pep Quart Curve 1.si. Conc25 2 Urknown 2500 N/A N/A Peptide 1 123 Pep Quart Curve 1.si. Conc25 3 Urknown 2500 N/A N/A Peptide 1 125 Pep Quart Curve 1.si. Conc25 3 Urknown 2500 N/A N/A Peptide 1 126 Pep Quart Curve 1.si. Conc25 3 Urknown 2500 N/A N/A Peptide 1 127 Pep Quart Curve 1.si. Conc125 1 Urknown 2500 N/A N/A Peptide 1 128 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 147 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 147 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 147 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 149 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 140 Peptide 1 147 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 147 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 148 Peptide 1 149 Peptide 1 140 Peptide 1 140 Peptide 1 140 Peptide 1 147 Pep Quart Curve 1.si. Conc125 2 Urknown 2500 N/A N/A Peptide 1 149 Peptide 1 140 Peptide 1 140 Peptide 1 140 Peptide 1 140 Peptide 1 140 Peptide 1 141 Peptide 1 141 Peptide 1 141 Peptide 1 142 Peptide 1 143 Peptide 1 144 Peptide 1 145 Peptide 1 145 Peptide 1 145 Peptide 1 146 Peptide 1 146 Peptide 1 146 Peptide 1 147 Peptide 1 148 Peptide 1 149 Peptide 1 149 Peptide 1 149 Peptide 1 140 Peptide 1	Analytes	87	Pep Quant Curve 1.wi.	Conc5 3	Unknown	25.00	NA	N/A	Peptide 1
proce       1332.5 / %25.2         proce       1111       Pep Quart Curve 1.sk.       Conc25.2       Urknown       25.00       N/A       N/A       Pepdde 1         proce       232.5 / %25.1       122       Pep Quart Curve 1.sk.       Conc25.2       Urknown       25.00       N/A       N/A       Pepdde 1         proce       232.8 / %25.1       123       Pep Quart Curve 1.sk.       Conc25.3       Urknown       25.00       N/A       N/A       Pepdde 1         1175       Pep Quart Curve 1.sk.       Conc125.1       Urknown       25.00       N/A       N/A       Pepdde 1         147       Pep Quart Curve 1.sk.       Conc125.2       Urknown       25.00       N/A       N/A       Pepdde 1         147       Pep Quart Curve 1.sk.       Conc125.2       Urknown       25.00       N/A       N/A       Pepdde 1         147       Pep Quart Curve 1.sk.       Conc125.2       Urknown       25.00       N/A       N/A       Pepdde 1         147       Pep Quart Curve 1.sk.       Conc125.2       Urknown       25.00       N/A       N/A       Pepdde 1         147       Pep Quart Curve 1.sk.       Conc125.2       Urknown       25.00       N/A       N/A	ptide 1.395.2 / 272.1	99	Pep Quant Curve 1.wi.	Conc25 1	Unknown	25.00	N/A	NIA	Peptide 1
<sup>123</sup> <sup>123</sup> <sup>123</sup> <sup>123</sup> <sup>123</sup> <sup>123</sup> <sup>123</sup> <sup>123</sup> <sup>124</sup> <sup>124</sup> <sup>124</sup> <sup>124</sup> <sup>125</sup> <sup>125</sup> <sup>125</sup> <sup>125</sup> <sup>125</sup> <sup>125</sup> <sup>126</sup> <sup>127</sup> <sup>126</sup> <sup>127</sup> <sup>126</sup> <sup>127</sup> <sup>127</sup> <sup>127</sup> <sup>128</sup> <sup>128</sup> <sup>128</sup> <sup>128</sup> <sup>128</sup> <sup>129</sup> <sup>129</sup> <sup>129</sup> <sup>129</sup> <sup>129</sup> <sup>129</sup> <sup>121</sup>	xide 2.432.9 / 269.2	111	Pep Quant Curve 1.wi.	Conc25 2	Unknown	25.00	NA	N/A	Peptide 1
eode 3349.5/371.2     135     Pep Quart Curve 1.xii.     Conc 125 1     Unknown     25.00     N/A     N/A     Peptide 1       147     Pep Quart Curve 1.xii.     Conc 125 2     Unknown     25.00     N/A     N/A     Peptide 1       4     Pep Quart Curve 1.xii.     Conc 155 3     Ubknown     25.00     N/A     N/A     Peptide 1       4     Pep Quart Curve 1.xii.     Conc 155 3     Ubknown     25.00     N/A     N/A     Peptide 1       4     Pep Quart Curve 1.xii.     Conc 155 3     Ubknown     25.00     N/A     N/A     Peptide 1       4     Pep Quart Curve 1.xii.     Conc 156 3     Ubknown     25.00     N/A     N/A     Peptide 1       6     Acedy     Image: Conc 156 3     Ubknown     Peptide 1.397.9/272.1 (Unknown) 397.9     Matrix Blank 1 - Peptide 1.397.9/272.1 (Unknown       6     Acedy     Image: Solid 1.300     Points     Blank 1 - Peptide 1.397.9/272.1 (Unknown     Acee 6.15563, Height 1.10568, RT: 3.65 min       Casusian Smooth Width:     3.04     min     NiA     12.3.4     5.6     0       12     3.4     5.6     0     12.3.4     5.6     0     12.3.4     5.6	ptide 2.432.9 / 647.4 ptide 3.523.8 / 263.1	123	Pep Quant Curve 1.wi.	Conc253	Unknown	25.00	NA	N/A	Peptide 1
147       Pep Quart Curve 1:si.       Conc125 2       Urknown       2500       N/A       N/A       Peptide 1         199       Pan. Oram. Curve 1:si.       Conc125 3       Urknown       2500       N/A       N/A       N/A       Peptide 1         4       + +5       +5       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +5       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +2       +5       +2       +2       +2       +5       +2       +2       +2       +5       +2       +2       +2       +5       +2       +2       +5       +5       +2       +2       +5       +5       +2       +4       5       +5       +2       +4       5       +2       +4       5       +2       +4       5       +2       +4       5       +2       +4       5       +2       +4       5       +2       +4       5 <td>ptide 3.349.5 / 371.2</td> <td>135</td> <td>Pep Quant Curve 1.wi.</td> <td>Conc1251</td> <td>Unknown</td> <td>25.00</td> <td>N/A</td> <td>NIA</td> <td>Peptide 1</td>	ptide 3.349.5 / 371.2	135	Pep Quant Curve 1.wi.	Conc1251	Unknown	25.00	N/A	NIA	Peptide 1
		147	Pep Quant Curve 1.wi.	Conc125.2	Unknown	25.00	NA	N/A	Peptide 1
4       +5       +5          • • • • • • • • • • • • • • •		160	Pen Ouant Curve 1 sti	Conc125.3	Unknown	25.00	N/A	NIA	Dantida 1
Lime, min Lime, min		Gaussian S Expected I RT Half Win Update Exp	Apply imooth Width: 3.0 RT: 3.84 idow: 90.0 pected RT: Group -	points Blank 1 - Pep Area: 1.350e1 min sec 0	Manual Integra tide 1.397.9/272 . Height: 3.325e0 	50n 1 (Unknown) 397 RT: 3.28 min 3.28 3 4 5 me, min	9 Matrix Blan Area: 6.185 100	k 1 - Peptide 1.397; e3, Height 1.105e3, 0 1 2 1 2 Ti	9/272.1 (Unknown RT: 3.85 min 3.85 3 4 5 6 me. min

Figure 1-50 Edit > Fill Down Option

- 6. To translate this information to all peptide MRMs, right-click anywhere in the Results Table and then click **Apply Current IS's Actual Concentration to All**. Click another peptide under **All Internal Standards** and then make sure that each **Actual Concentration** is 25.
- 7. The sample types for each sample in the data file must be defined. Select one of the peptide MRMs under All Analytes. Select the correct Sample Type for each row by clicking the right corner of the Sample Type cell and then selecting the correct sample type. The Blank 1 in row 1 is a double blank as it contains no analyte or no internal standard (note the IS Actual Concentration is reset to N/A). The Matrix Blank 1 in row 2 is the blank as it contains only the internal standards. The remaining rows are all Standard samples and will be used for calculating the concentration curve. An alternative approach is to first set all samples to Standard using the Fill Down command and then to adjust the type for the first two samples.



Figure 1-51 Selecting Sample Types

- 8. Enter the concentrations for each sample. The concentrations to be entered are specified in the sample name (Sample Conc1 1 has a concentration of 1, Conc5 1 has a concentration of 5, and so forth).
- 9. After all the rows have been correctly set, right-click in the table and then click **Apply Current Analyte's Actual Concentrations to All**. This propagates these values to all analytes.

			I II A Courts 7		- <b>Pa et</b> A		-
All Components			En Vi Sample I	ypes ∙ ⊶ o	Actual	IS Actual	3
	Index	Original Filename	Sample Name	Sample Type	Concentration	Concentration	IS Name
Peptide 2 Group	▶ 1	Pep Quant Curve 1.wi	Blank 1	Double Blank	N/A	N/A	Peptide 1.397.9 /
Peptide 3 Group	13	Pep Quant Curve 1.wi.	Matrix Blank 1	Blank	NA	25.00	Peptide 1.397.9 /_
II Internal Standards	25	Pep Quant Curve 1.wi	Conc1 1	Standard	1.00	25.00	Peptide 1.397.9 /_
eptide 1.397.97272.1 eptide 1.397.97400.2	37	Pep Quant Curve 1.wi	Conc12	Standard	1.00	25.00	Peptide 1.397.9 /
eptide 2.435.6 / 269.2	49	Pep Quant Curve 1.wi	Conc1 3	Standard	1.00	25.00	Peptide 1.397.9 /
eptide 3.516.8 / 263.1	61	Pep Quant Curve 1.wi	Conc5 1	Standard	1.00	25.00	Peptide 1.397.9 /
eptide 3.344.8 / 371.2	73	Pep Quant Curve 1.wi	Conc5 2	Standard	1.00	25.00	Peptide 1.397.9 /
II Analytes	85	Pep Quant Curve 1.wi.	Conc5 3	Standard	1.00	25.00	Peptide 1.397.9 /
eptide 1.395.2 / 272.1 entide 1.395.2 / 400.2	97	Pep Quant Curve 1.wi	Conc25 1	Standard	25.00	25.00	Peptide 1.397.9 /_
eptide 2.432.9 / 269.2	109	Pep Quant Curve 1.wi	Conc25 2	Standard	25.00	25.00	Peptide 1.397.9 /
eptide 2.432.9 / 647.4 eptide 3.523.8 / 263.1	121	Pep Quant Curve 1.wi	Conc253	Standard	25.00	25.00	Peptide 1.397.9 /
eptide 3.349.5 / 371.2	<	1					
		Apply		Manual Integra	5on 772.1 <i>(De.</i> , Matri	v Black 1 - Peotic	
	Gaussian S Expected I RT Half Win Update Ex	Smooth Width: 3.0 RT: 3.84 Indow: 90.0 pected RT: Group V	points Area: 2.701 min sec	e1, Height 3.36	1eQ, RT: 3Area	1.350e1, Height:	96 1.395.27.272.1 3.325e0. RT: 3.8 3.84
	Gaussian S Expected R RT Half Win Update Ex I Report Min. Peak 1	imooth Width:         3.0           RT:         3.84           idow:         90.0           pected RT:         Group •           Largest Peak         Width:	points Conc11- Pe	e1, Height: 3.36	100, HT: 3 Area	1.350e1, Height: 5- 0 12-Peptide 1.35	2 3 4 5 6 Time, min 52/272.1 (Stan
	Gaussian S Expected I RT Half Win Update Exp I Report Min. Peak I Min. Peak I	imooth Width:         3.0           RT:         3.84           idow:         90.0           pected RT:         Group •           Largest Peak         Width:           Height:         0.00	points Conc1 1 - Pe Points Conc1 1 - Pe 200 201 201 201 201 201 201 201	e 1. Height: 3.36 3.54 1 2 3 Time, m pside 1.3952/272 2. Height: 2.164e1	1e0. HT: 3 Ares	1.350e1. Height: 5- 0- 1 1.2 - Peptide 1.35 1.080e2. Height:	3.84 2 3 4 5 6 Time, min 52.727.1 (Stan 1.805c1, RT: 3.8
	Gaussian S Expected RT Half Win Update Ex Im Report Min. Peak Min. Peak Integrati Noise Pere	imooth Width: 3.0 RT: 3.84 idow: 90.0 pected RT: Group ▼ Largest Peak Width: 3 Height: 0.00 on Parameters :entage: 40.0	points         Didnk 1	e1, Height 336 1, 2, 3, 94 1, 1, 2, 3, 94 1, 1, 2, 3, 94 1, 1, 2, 3, 94 1, 2, 4, 94 1, 4, 94	Ied, NT: 3.         Ares           4         5         6           in         .1.(Stan         Conc           .1.(Stan         Conc         Ares           3.84         Ares         Ares	1.350e1. Height: 5 0 12 - Peptide 1.33 1.080e2. Height: 10	3325e0, RT: 3.8 3.325e0, RT: 3.8 3.84 2 3 4 5 6 Time, min 52 / 272 1 (Stan 1.805e1, RT: 3.8 3.85
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Figure 1-52 Apply Current Analyte's Actual Concentration to All Option

### **View Calibration Curves**

- 1. To show the calibration curve for an MRM transition for a specific peptide, select the MRM transition from the left pane, and then click the **Show Calibration Curve** icon to view calibration curve. Refer to Figure 1-53.
- 2. To create more room on the screen, the peak review pane can be removed from view by clicking the **Delete** pane button or the **Hide** pane button. Alternatively, a tabular view can be created by clicking the **Tab mode** button. Each open pane will appear in its own tab.
- 3. Open the calibration curve pane for the first MRM transition for Peptide 1. The y-axis on the plot is the peak area ratio of the analyte over the internal standard. Normally, the internal standard concentration is held constant over a concentration curve, but in cases where this is not the case, using the concentration ratio for the x-axis automatically compensates for this.

Figure 1-53 Calibration Curve Interface

File Edit Process Win	dog Help							- 8
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ponents & Groups IS H	🖽 📾 🛙	🖉 🐱 🔹 🔝	🖂 🖂 🕌 Al Sa	mple T	ypes 🔹 🖂 👁 🕴	P 🗱 🔍 🖂 🛛		đ
Components	Index	Sample Name	Sample Type	IS	Component Name	IS Name	Component Group Name	Outer
6de 1 Group	1	Blank 1	Unknown	17	Peptide 1.395.2 /	Peptide 1.397.9 /	Peptide 1	
6de 3 Group	2	Blank 1	Unknown	173	Peptide 1.395.2 /	Peptide 1.397.9 /	Peptide 1	
internal Standards	3	Blank 1	Unknown		Peptide 1.397.9 /	NIA	Peptide 1	
6de 1.397.9 / 272.1	4	Blank 1	Unknown		Peptide 1.397.9 /_	NIA	Peptide 1	
6de 2.435.6 / 269.2	5	Blank 1	Unknown	10	Peptide 2.432.9 /_	Peptide 2.435.6 /	Peptide 2	
6de 2.435.6 / 655.4 6de 3.516.8 / 263.1	6	Blank 1	Unknown	173	Peptide 2.432.9 /_	Peptide 2.435.6 /	Peptide 2	
6de 3.344.8 / 371.2	7	Blank 1	Unknown		Peptide 2.435.6 /_	NIA	Peptide 2	
Analytes	8	Blank 1	Unknown	V	Peptide 2.435.6 /_	NIA	Peptide 2	
6de 1.395.2 / 272.1	9	Blank 1	Unknown	171	Peptide 3.523.8 /_	Peptide 3.516.8 /_	Peptide 3	
6de 2.432.9 / 269.2	10	Blank 1	Unknown	13	Peptide 3.349.5 /_	Peptide 3.344.8 /_	Peptide 3	
6de 2.432.9 / 647.4 6de 3.523.8 / 263.1	11	Blank 1	Unknown		Peptide 3.516.8 /_	NIA	Peptide 3	
tide 3.349.5 / 371.2	12	Blank 1	Unknown	4	Peptide 3.344.8 /_	NIA	Peptide 3	
	13	Matrix Blank 1	Unknown	13	Peptide 1.395.2 /_	Peptide 1.397.9 /	Peptide 1	
	14	Matrix Blank 1	Unknown	13	Peptide 1.395.2 /	Peptide 1.397.9 /_	Peptide 1	
	15	Matrix Blank 1	Unknown		Peptide 1.397.9 /	NIA	Peptide 1	
	16	Matrix Blank 1	Unknown	4	Peptide 1.397.9 /_	NIA	Peptide 1	
	17	Matrix Blank 1	Unknown	23	Peptide 2.432.9 /_	Peptide 2.435.6 /	Peptide 2	
	18	Matrix Blank 1	Unknown	13	Peptide 2.432.9 /_	Peptide 2.435.6 /_	Peptide 2	
	19	Matrix Blank 1	Unknown	1	Peptide 2.435.6 /	NIA	Peptide 2	
	20	Matrix Blank 1	Unknown	1	Peptide 2.435.6 /	NIA	Peptide 2	
	21	Matrix Blank 1	Unknown	23	Peptide 3.523.8 /	Peptide 3.516.8 /	Peptide 3	
	22	Matrix Blank 1	Unknown	2	Peptide 3.349.5 /	Peptide 3.344.8 /_	Peptide 3	
	23	Matrix Blank 1	Unknown	V	Peptide 3.516.8 /	NA	Peptide 3	
	24	Matrix Blank 1	Unknown		Peptide 3.344.8 /	NA	Peptide 3	
	25	Conc11	Unknown	171	Peptide 1.395.2 /_	Peptide 1.397.9 /	Peptide 1	

ltem	Description
1	Show Calibration Curve icon
2	Toggles to tab mode
3	Deletes this pane
4	Hide pane

4. From the peak review performed, notice that there was very little peak area for Conc 1 in the dataset. Therefore this concentration can be removed from the calculation of the calibration curve by clearing the **Used** check box in each Conc1 row.

**Tip!** Remove a data point from the curve by right-clicking on the data point on the calibration curve pane and then clicking **Exclude**.

Notice that the three replicates for the highest concentration show a small amount of variability. Notice also that the data point now seems 'hollow' and the correlation co-efficient (r value) is improved (increased in value). In addition, it is important to observe the accuracy column in the table as this reflects how close the computed concentration at each data point is to the expected concentration. It is desirable for the accuracy of all data points in the calibration curve to lie between 80 to 120%.

The calibration curves for all the analytes can be assessed by removing outlying data points to improve the accuracy of the calibration curve. This is one advantage of performing replicates at each concentration, as the occasional data point can then be removed.

5. Sometimes, a complete concentration point must be removed, at either the low end of the curve (due to low signal for example) or at the high end of the curve (due to saturation or injection issues). Select one of the MRM transitions for Peptide 3 and notice the shape of the calibration. The curve is not very linear but can be greatly improved by removing all the points at the very highest concentration.



Figure 1-54 Before removal of high concentration data points

Figure 1-55 After removal of high concentration data points



**Note:** Any reported concentrations for unknown samples greater than the concentration of the most concentrated standard used for the calibration should be treated with some suspicion. The quality of the calibration curve can be evaluated using the accuracy values calculated in the Results Table. Typically most values across the curves should fall between 80 to 120% accuracy to be included. Points that fall outside the line can be omitted by clearing the check box in the Used column.

6. Before exporting the calibration curve, check the calibration curve statistics. Go to Evaluate the Statistics of the Standard Calibration Curve on page 57.

### **Evaluate the Statistics of the Standard Calibration Curve**

The Statistics Table is used to view information related to the reproducibility of an analysis. Each row of the table summarizes information such as the average, standard deviation, and so forth for a group of related peaks, from the same analyte, that would ideally be expected to have the same response.

• Click the active Results Table, and then click the **Statistics** icon to open the Statistics pane.

Users can assess the reproducibility of the replicates. Notice that when a peak is removed from the quantitation curve by clearing the Used check box, it is not considered in the calculations and is visualized as a struck out value.

Figure	1-56	<b>Statistics</b>	Table
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tide 1 Group		1	Pep Quant Curve	1.wi.,	Blank 1		Double B	lank	N/A	N/A	Peptide	1.397.9 /	. Pe
tide 3 Group		13	Pep Quant Curve	1.wi	Matrix Blank	1	Blank		N/A	25.00	Peptide	1.397.9 /	. Pe
nternal Standards		25	Pep Quant Curve	1.wi.,	Conc11		Standard		1.00	25.00	Peptide	1.397.9 /	. Pe
ide 1.397.9 / 272.1		37	Pep Quant Curve	1.wi	Conc12		Standard		1.00	25.00	Peptide	1.397.9 /	. Pe
ide 2.435.6 / 269.2		49	Pep Quant Curve	1.wi	Conc13		Standard		1.00	25.00	Peptide	1.397.9 /	. Pe
ide 2.435.6 / 655.4 ide 3.516.8 / 263.1		61	Pep Quant Curve	1.wi	Conc51		Standard		1.00	25.00	Peptide	1.397.9 /	. Pe
ide 3.344.8 / 371.2		73	Pep Quant Curve	1.wi.,	Conc5 2		Standard		1.00	25.00	Peptide	1.397.9 /	. Pe
nalytes		85	Pep Quant Curve	1.wi.,	Conc53		Standard		1.00	25.00	Peptide	1.397.9 /	. Pe
Peptide 1.395.2 / 272.1 Peptide 1.395.2 / 400.2 Peptide 2.432.9 / 269.2		97	Pep Quant Curve	1.wi	Conc251		Standard		25.00	25.00	Peptide	1.397.9 /	. Pe
		109	Pep Quant Curve	1.wi.	Conc252		Standard		25.00	25.00	Peptide	1.397.9 /	. Pe
ide 2.432.9 / 647.4 ide 3.523.8 / 263.1		121	Pep Quant Curve	1.wi.,	Conc253		Standard		25.00	25.00	Peptide	1.397.9 /	. Pe
de 3.349.5 / 371.2	1												
	0	) <b>::</b> G	roup by Concentration	for St	andards 🔹	etc Cal	culated Co	ncentr	ation 💌 🏛			_	、 、
		Row	Component Name	Actu	ual Concentr_	Num. V	alues Me	ean /	Standard Devi_	Percent CV	Accuracy	Value 2	alue
		1	Peptide 1.395.2 / 2.	1.00		3 of 6	1.5	76e1	5.008e-1	3.18	1575.68	1.248e1	1.230
		2	Peptide 1.395.2 / 2	25.0	0	3 of 3	3.3	93e1	2.873e0	8.47	135.73	3.075e1	3.633
		3	Peptide 1.395.2 / 2	125	00	3 of 3	1.2	13e2	1.629e0	1.34	97.02	1.206e2	1.201
		4	Peptide 1.395.2 / 2	625	00	3 of 3	6.0	00e2	2.781e1	4.64	95.99	6.310e2	5.772
		5	Peptide 1.395.2 / 2	3125	5.00	3 of 3	3.1	30e3	2.013e2	6.43	100.16	2.977e3	3.358

ltem	Description
1	Statistics icon
2	Peak removed from statistics calculation

### **Export Calibration Curves**

1. After the calibration curves have been optimized for all the MRM transitions for all the peptides, the calibration curve can be saved by clicking **Process** > **Export Calibration**. This creates a \*.mqcal file.

**Note:** This step is only necessary if you will subsequently apply this external calibration to a new Results Table.

2. For this tutorial, export the calibration noting where you save the file.

### **Create a Metric Plot**

Metric plots are very useful for quickly assessing data quality or helping to answer specific questions of a dataset. In this example, a metric plot will be generated for the area of the internal standard to enable a quick assessment of the stability of the chromatography.

1. Select Peptide 1 in the **Components and Groups** pane and then select the IS Area column. Click the **Metric Plot** icon.

A plot of the internal standard area for each injection is generated for that peptide. Notice the slight decrease in signal of the IS in the final three injections. The area for the first sample is very small; a small noise peak was integrated for this double-blank sample.



#### Figure 1-57 Metric Plot Interface

ltem	Description
1	Display metric plot
2	Decrease in signal

2. Select a different peptide and then review the metric plot updates. Finally, select all peptides by selecting the first peptide and then holding the **Shift** key while selecting the final peptide. An overlay of all 6 metric plots is generated.

MultiQuant - [[MQ4] Results	Table (Pepti	ide.qsession)]								
File Edit Process Wind	low Help								-	8 X
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Components & Groups IS 4	🖽 📾 🗄	Z 🗹 🕶 🐉	<b>a v</b>	Al Sample 1	ýpes ▼	P 🗱 🔍 🛛				49
All Components	Index	Sample Type	IS	Component Name	Component Group Name	Actual Concentration	IS Actual Concentration	Area	IS Area	Ar
Peptide 1 Group Peptide 2 Group	1	Double Blank		Peptide 1.395.2 /	Peptide 1	N/A	N/A	2.701e1	1.350e1	2.0
Peptide 3 Group	2	Double Blank	<b>F</b>	Peptide 1.395.2 /	Peptide 1	N/A	N/A	1.350e1	1.350e1	1.0
All Internal Standards	5	Double Blank	<b>F</b>	Peptide 2.432.9 /	Peptide 2	N/A	N/A	2.722e1	4.052e1	6.7
Peptide 1.397.9 / 272.1 Particle 1.397.9 / 400.2	6	Double Blank	1	Peptide 2.432.9 /	Peptide 2	NA	N/A	2.734e1	N/A	N/
Peptide 2.435.6 / 269.2	9	Double Blank	<b>[</b> ]	Peptide 3.523.8 /	Peptide 3	N/A	N/A	6.752e1	N/A	N/J
Peptide 2.435.6 / 655.4 Peptide 3.516.8 / 263.1	10	Double Blank	[**]	Peptide 3.349.5 /_	Peptide 3	N/A	N/A	2.701e1	N/A	NS
Peptide 3.344.8 / 371.2	13	Blank	177	Peptide 1.395.2 /_	Peptide 1	NA	25.00	1.351e1	6.185e3	2.1
All Analytes	14	Blank	1	Peptide 1.395.2 /	Peptide 1	NA	25.00	1.351e1	4.820e3	2.8
Peptide 1.395.2 / 272.1 Peptide 1.295.2 / 400.2	17	Blank	<b></b>	Peptide 2.432.9 /_	Peptide 2	N/A	25.00	1.489e2	4.211e3	3.5
Peptide 2.432.9 / 269.2	18	Blank		Peptide 2.432.9 /_	Peptide 2	N/A	25.00	5.402e1	3.375e3	1.6
Peptide 2.432.9 / 647.4 Peptide 3.523.8 / 263.1	21	Blank	1	Peptide 3.523.8 /_	Peptide 3	N/A	25.00	4.052e1	1.891e3	2.1
Peptide 3.349.5 / 371.2	22	Blank	[7]	Peptide 3.349.5 /	Peptide 3	N/A	25.00	2.701e1	2.323e3	1.1
		1								
			~	-						-
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#### Figure 1-58 Overlaid Metric Plots

### **Process Unknown Samples**

After the calibration curve is generated, the biological samples (unknowns) can be processed. In these samples, you want to measure the amount of the endogenous light peptide present in each sample. The heavy peptide can be added into each biological sample at a fixed amount. It can then act as the internal standard for these samples to add robustness to your quantitation experiment. Using the calibration curve previously generated, the actual concentration of peptide in the biological sample can be computed from the ratio of the endogenous peptide (light) to the added internal standard (heavy).

- 1. Click File > New Results Table.
- 2. On the Create Results Table Select Samples page, drag the **Pep Quant Samples A.wiff** file into the Selected pane and then click **Next**.

Create Results Table - Select Samples		- • •
Current Location: C:\Analyst Data\Projects\Default\Data\		
Available Browse	Selected	
Buntrazepam & Nordkazepam Saturation Nov 6 2     Buntrazepam &	■-     ■     Pep Quart Samples A.wff     U QC low 1     U QC low 2     U QC high 1     U SampleA 1     U SampleA 1     U SampleB 1     U SampleB 1     U SampleB 2     U SampleB 3     U SampleC 1     U SampleC 3	
	<back next=""> Finish</back>	Cancel

#### Figure 1-59 Create Results Table - Select Samples Page

- 3. On the Create Results Table Select Method page, click the **Choose Existing Method** option and then select the **Edit Method** check box.
- 4. Click **Open**, select the method created in Accurate Quantitation Using Calibration Curves on page 37, and then click **Next**.

In a subsequent step we will switch the analyte and internal standard MRMs. The net effect will be to overwrite the method.

5. On the Create Results Table - Select Representative Sample page, a representative sample has been recommended and is selected. Click **Next**.

Create Results Table - Select Sar	nples			
Current Location: C:\Analyst Data\P Available	ojects\Default\Data\ Browse m Saturation Nov 6 2 M 1.wiff nt.wiff	(a)	Selected  Pep Quant Samples A.wff QC low 1 QC low 2 QC low 3 QC high 1 QC high 1 QC high 3 QC high 3 QC high 3 QC high 4 SampleA 1 SampleA 1 SampleB 1 SampleB 2 SampleB 2 SampleB 3 SampleB 3 SampleC 2 SampleC 3	
٠	•			
		< Back	Next > Finish	Cancel

#### Figure 1-60 Create Results Table - Select Representative Sample Page

6. On the Create Results Table - Define Components page, right-click in the table and then click **Internal Standards > Reverse Analyte/IS Assignments**.

The analytes and internal standards should interchange so that the method now looks as shown in Figure 1-61.

	1				
Row	IS	Name	Group	IS Name	Q1/Q3
1	V	Peptide 1.395.2 / 272.1	Peptide 1		395.2 / 272.1
2	V	Peptide 1.395.2 / 400.2	Peptide 1		395.2 / 400.2
3		Peptide 1.397.9 / 272.1	Peptide 1	Peptide 1.395.2 / 272.1	397.9 / 272.1
4		Peptide 1.397.9 / 400.2	Peptide 1	Peptide 1.395.2 / 400.2	397.9 / 400.2
5	V	Peptide 2.432.9 / 269.2	Peptide 2		432.9 / 269.2
6	V	Peptide 2.432.9 / 647.4	Peptide 2		432.9 / 647.4
7		Peptide 2.435.6 / 269.2	Peptide 2	Peptide 2.432.9 / 269.2	435.6 / 269.2
8		Peptide 2.435.6 / 655.4	Peptide 2	Peptide 2.432.9 / 647.4	435.6 / 655.4
9	$\checkmark$	Peptide 3.523.8 / 263.1	Peptide 3		523.8 / 263.1
10	$\checkmark$	Peptide 3.349.5 / 371.2	Peptide 3		349.5 / 371.2
11		Peptide 3.516.8 / 263.1	Peptide 3	Peptide 3.523.8 / 263.1	516.8 / 263.1
12		Peptide 3.344.8 / 371.2	Peptide 3	Peptide 3.349.5 / 371.2	344.8 / 371.2
13					
			TH		

#### Figure 1-61 Create Results Table - Define Components Page

7. Click **Next** to advance to the Create Results Table - Define Integration page.

Because the integration parameters were previously reviewed when the method was first created, you do not have to make any further changes.

8. Click Next.

V	Criteria for flagging out	liers. Irds			ccuracy for QCs	
Ma Ma	xx. Accuracy Tolerand	e for LLO e for Stds	Q (lowest Std):	20 % Max.	Accuracy Tolerance	for QC: 15
2	Ion Ratio	Calcul	ated Concentration Group	Ion Ratio	Lower Limit of	Upper Limit of
•	Peptide 1.395.2 /	8	Peptide 1	Tolerance (%)	Carculated Conce	Carculated Conce.
	Peptide 1.395.2 /		Peptide 1	20		
-	Peptide 1.397.9 /		Peptide 1			
-	Peptide 1.397.9 /	<b>V</b>	Peptide 1	20		
	Peptide 2.432.9 /		Peptide 2			
	Peptide 2.432.9 /		Peptide 2	20		
_	Peptide 2.435.6 /		Peptide 2			
	Peptide 2.435.6 /		Peptide 2	20		
	Peptide 3.523.8 /		Peptide 3			
	Peptide 3.349.5 /		Peptide 3	20		
	Peptide 3.516.8 /		Peptide 3			
	Peptide 3.344.8 /	V	Peptide 3	20		

- 9. Specify the fields as required to automatically flag samples for review in the Results Table. Refer to the *Reference Guide* for more information about the **Outlier Settings** parameters.
- 10. Click Finish.
- 11. Define the **Sample Type** for the QC samples as Quality Control and the leave the remaining samples as Unknown.
- 12. Type an **IS Actual Concentration** of 20 for every sample.
- 13. Type 200 for the **Actual Concentration** for the QC low samples and 4000 for the QC high samples. The Results Table should now look the following figure.

**Tip!** To fill down a specific section of cells in a table, click the first and last cells in the section while pressing the Shift key. The cells will be selected and you can click **Edit > Fill Down** to populate the cells.

File Edit Process Wi	ndow Help						_ 6
🔨 🗃 👻 🔚 🗋 Default							
Components & Groups IS M	1 🖽 📾 🕻	🖉 🐱 👻 🔤 🛛	🐼 📴 🕌 Al S	ample Types 🛛 💌 🖂	- 🗢 🖓 🚳		4
All Components	Index	Sample Name	Sample Type	Component Name	Component Group Name	Actual Concentration	IS Actual Concentration
Peptide 1 Group Peptide 2 Group	1	QC low 1	Quality Control	Peptide 1.395.2 /	Peptide 1	200.00	20.00
Peptide 3 Group	13	QC low 2	Quality Control	Peptide 1.395.2 /	Peptide 1	200.00	20.00
All Internal Standards	25	QC low 3	Quality Control	Peptide 1.395.2 /	Peptide 1	200.00	20.00
Peptide 1.397.9 / 272.1	37	QC high 1	Quality Control	Peptide 1.395.2 /	Peptide 1	4000.00	20.00
Peptide 2.435.6 / 269.2	49	QC high 2	Quality Control	Peptide 1.395.2 /	Peptide 1	4000.00	20.00
Peptide 2.435.6 / 655.4 Peptide 3.516.8 / 263.1	61	QC high 3	Quality Control	Peptide 1.395.2 /	Peptide 1	4000.00	20.00
Peptide 3.344.8 / 371.2	73	SampleA 1	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
All Analytes	85	SampleA 2	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
Peptide 1.395.2 / 272.1 Peptide 1.395.2 / 400.2	97	SampleA 3	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
Peptide 2.432.9 / 269.2	109	SampleB 1	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
Peptide 2.432.9 / 647.4 Peptide 3.523.8 / 263.1	121	SampleB 2	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
Peptide 3.349.5 / 371.2	133	SampleB 3	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
	145	SampleC 1	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
	157	SampleC 2	Unknown	Peptide 1.395.2 /	Peptide 1	N/A	20.00
	169	SampleC 3	Unknown	Peptide 1.395.2 /_	Peptide 1	N/A	20.00

#### **Figure 1-63 Actual Concentrations**

- 14. Click **Process** > **Import External Calibration** to import the calibration curve calculated in the previous section into this Results Table. Browse for the \*.mqcal file that was previously saved and then click **Open**.
- 15. In the Assign External Calibrations dialog, select the calibration curve that should be used for each specific analyte in the current data file. Specify the relationships as shown and then click **OK**.

Current Analyte	Analyte for Calibration
Peptide 1.395.2 / 272.1	Peptide 1.395.2 / 272.1
Peptide 1.395.2 / 400.2	Peptide 1.395.2 / 400.2
Peptide 2.432.9 / 269.2	Peptide 2.432.9 / 269.2
Peptide 2.432.9 / 647.4	Peptide 2.432.9 / 647.4
Peptide 3.523.8 / 263.1	Peptide 3.523.8 / 263.1
Peptide 3.349.5 / 371.2	Peptide 3.349.5 / 371.2

Figure 1-64 Assign External Calibrations dialog

After the calibration file is loaded, the Calculated Concentration and Accuracy columns are automatically calculated. The accuracy obtained for the QC samples is very good, indicating the assay is working as expected. The measured concentrations of the three unknowns in each of the samples A, B, and C can be found in the Calculated Concentration column.

omponents & Groups IS #	<u>6</u>	🖉 🖂 🗸	32 🔤 🐼 🛛	🗟 🕌 All Sample Ty	ypes 🔻 🖂 🛎	🕫 🗱 🍳		4
II Components	Index	Name	Sample Type	Component Group Name	Calculated Concentration	Accuracy	Actual Concentration	
eptide 1 Group	3		Quality Control	Peptide 1	1.943e2	97.16	200.00	2
eptide 3 Group	15		Quality Control	Peptide 1	2.057e2	102.83	200.00	2
II Internal Standards	27		Quality Control	Peptide 1	2.091e2	104.54	200.00	1
eptide 1.395.2 / 272.1	39		Quality Control	Peptide 1	4.773e3	119.31	4000.00	1
eptide 2.432.9 / 269.2	51		Quality Control	Peptide 1	3.905e3	97.63	4000.00	T
eptide 2.432.9 / 647.4 eptide 3.523.8 / 263.1	63		Quality Control	Peptide 1	2.810e3	70.24	4000.00	T
eptide 3.349.5 / 371.2	75		Unknown	Peptide 1	3.887e2	N/A	N/A	t
II Analytes	87		Unknown	Peptide 1	3.930e2	N/A	N/A	T
eptide 1.397.9 / 272.1	99		Unknown	Peptide 1	3.813e2	N/A	N/A	t
eptide 2.435.6 / 269.2	111		Unknown	Peptide 1	1.986e3	N/A	N/A	T
eptide 2.435.6 / 655.4 eptide 3.516.8 / 263.1	123		Unknown	Peptide 1	1.836e3	N/A	N/A	T
eptide 3.344.8 / 371.2	135		Unknown	Peptide 1	1.858e3	N/A	N/A	t
	147		Unknown	Peptide 1	5.872e3	N/A	N/A	t
	159		Unknown	Peptide 1	4.839e3	N/A	N/A	T
	171		Unknown	Peptide 1	4.364e3	N/A	N/A	T

Figure 1-65 Calculated Concentration and Accuracy columns

16. Save the Results Table.

### **Create Reports**

If you are creating reports using unlocked Results Tables, make sure that you are allowed to create and export unlocked Results Tables. See your administrator if you are unable to create reports.

#### 1. Click File > Create Report and Save Results Table.

Figure 1-66 Create Report Dialog

MO Create Report	<b>—</b>
Report template: Analyte Report 1.docx	Set1 Open in Word
Generated report file:	Set(2)
Output Format:	PDF C CSV
Create an individual report for each sample (R	ecommended for large reports)
	OK Cancel

ltem	Description
1	Click to select the template.
2	Click to save the report.

- 2. Click **Set** (Figure 1-66 item 1) to navigate to the Tutorial Dataset Heavy\_Light.xml template in the following folder:
  - For Windows 7 and Windows 10: C:\ProgramData\AB SCIEX\MultiQuant\Reporter.
- 3. Click **Set** (Figure 1-66 item 2) to create the name and location of the report.
- 4. Click **OK**.

Revision	Reason for Change	Date
A	First release of document.	August 2013
В	Rebranded to SCIEX, updated the copyright page, added Windows 10, changed software version to 3.0.3, added Contact Us and Technical Support sections, fixed link in step 5 in the Modify the Results Table section. Added path for Analyst <sup>®</sup> software documentation on Windows 10. Removed references to Windows XP.	July 2017