SWATH® Acquisition Performance Kit

Protocol

RUO-IDV-05-3363-A | 5045758

May 2016

AB Sciex Pte. Ltd.
Blk 33, #04-06
Marsiling Ind Estate Road 3
Woodlands Central Indus. Estate.
SINGAPORE 739256

Contents

Introduction 1

To assess the performance of the LC-MS systems, four system suitability tests are available.

- First, the **PepCalMix Infusion Test** is performed to check the MS performance and to optimize the source positioning and parameters.
- Next, the **PepCalMix LC-MS System Suitability Test** is performed to make sure that the system is in basic working order and is providing good LC separation and MS sensitivity. This method is also used for instrument mass calibration.
- Next, the **Cell Lysate SWATH® Acquisition QC Test** test is performed to assess the performance of the SWATH® Acquisition and includes a digested human cell lysate with PepCalMix added.
- Finally, the **Cell Lysate IDA QC Test** is performed to assess the protein identification performance.

Kit	Components	Use
MS Synthetic Peptide Calibration Kit (part number 5045759)	PepCalMix	PepCalMix Infusion Test PepCalMix LC-MS System Suitability Test
SWATH® Acquisition Performance Kit (part number 5045757)	PepCalMix K562 Protein Extract Digest	Cell Lysate SWATH QC Test Cell Lysate IDA QC Test

All of the instrument methods for the NanoLC™ 425 and 415 systems, operating in a trap elute configuration, are described. Use the following buffers for the LC system:

- Buffer A: 100% water with 0.1% formic acid
- Buffer B: 100% acetonitrile with 0.1% formic acid

For the nanoflow operation, the system is set up with a 75 µm i.d. nano column, with the temperature maintained at 35°C. For the microflow operation, the system is set up with a 300 µm i.d. column, with the temperature maintained at 35°C. The column oven can be mounted on the NanoLC™ system or on the DuoSpray™ ion source tower.

Refer to Parts List for the NanoLC™ 425 System for the Nanoflow Test on page 51 for a list of parts that are required for the nanoflow operation. Refer to Parts List to Upgrade the NanoLC™ 400 System for the Microflow Test on page 55 for a list of parts that are required for the microflow operation.

Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up the sources or the LC-MS system.

Performance benchmarks for both the nanoflow and the microflow operations are available at http://sciex.com/community/entity/15722.

Tests for the Nanoflow Regime 2

Test 1: PepCalMix Infusion Test – Source Tuning at Nanoflow Rates

1. Make sure that the NanoSpray® ion source is installed.
2. Prepare 50 fmol/µL of the PepCalMix solution.

   Refer to Prepare the PepCalMix Infusion Solution on page 46.

Note: For the infusion protocol, use a 100 µL syringe and tubing with a 50 µm inside diameter (i.d.) to 100 µm i.d. For information about setting up the syringe line, refer to Create a Syringe Line on page 63.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

Note: The hardware profile must include the mass spectrometer and the syringe pump.

2. Set the acquisition parameters using the information in Table 2-1.

Note: In Manual Tune, multiple acquisitions in both MS and MS/MS will be performed.

Table 2-1 MS Method Information

TOF MS	Mass Range	400 to 1,500
	Accumulation time	1 sec
	Source Conditions	Optimized

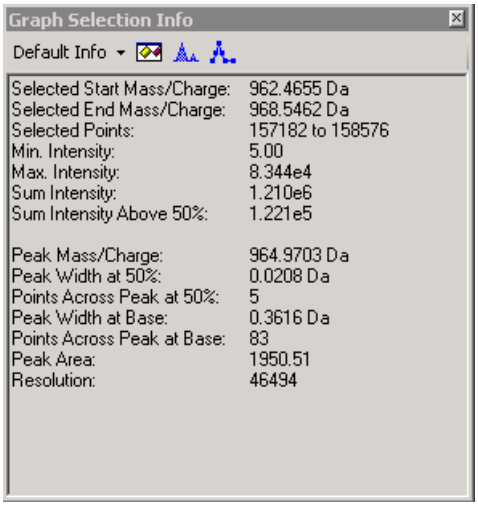
Table 2-1 MS Method Information (continued)

TOF MS/MS	Mass Range	100 to 1,500
	Accumulation time	1 sec
	Product Ion[1]	964.98 / 758.91 / 533.3
	MS/MS Type	High Sensitivity and High Resolution
	Collision Energy[1]	45 / 40 / 25
	Collision Energy Spread	5
[1] MS/MS acquisition is performed on three different peptides in both resolution modes, resulting in the acquisition of six MS/MS. The product ion masses shown in the table correspond to the collision energies shown in the table.		

Infusion

1. Connect the syringe to the source using the infusion line.
2. Infuse the PepCalMix solution at 0.5 µL/min for nanoflow testing.
3. Monitor the signal of the 758.91 *m/z* peptide with a TOF MS scan, as specified in Table 2-1.
4. Optimize the position of the sprayer and the source parameters for maximum stability and signal.
5. After optimization is completed, acquire the TOF MS data for 1 min. to confirm stable signal.
6. Acquire the MS/MS spectra on the three peptides as specified in Table 2-1, using both high sensitivity and high resolution modes, for 1 min.
7. Open the data in the PeakView® software to assess the data quality.
8. Select 30 sec. of the infusion TIC and then double-click to extract the spectrum.
9. From the PeakView® software menu bar, click **Window > Graph Selection Window**.

Figure 2-1 Graph Selection Info Dialog

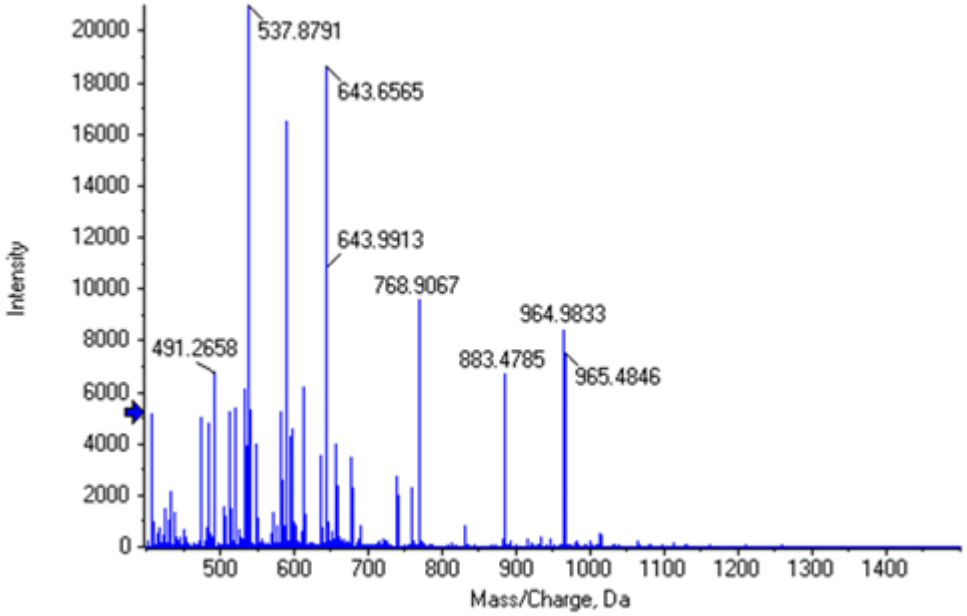


10. Select the spectral peak of interest (the C12 peak for each) and then observe the **Sum Intensity** value for each peak from the Graph Selection Window.

Tip! Zoom into the spectrum and then carefully select only the C12 peak of the isotope cluster.

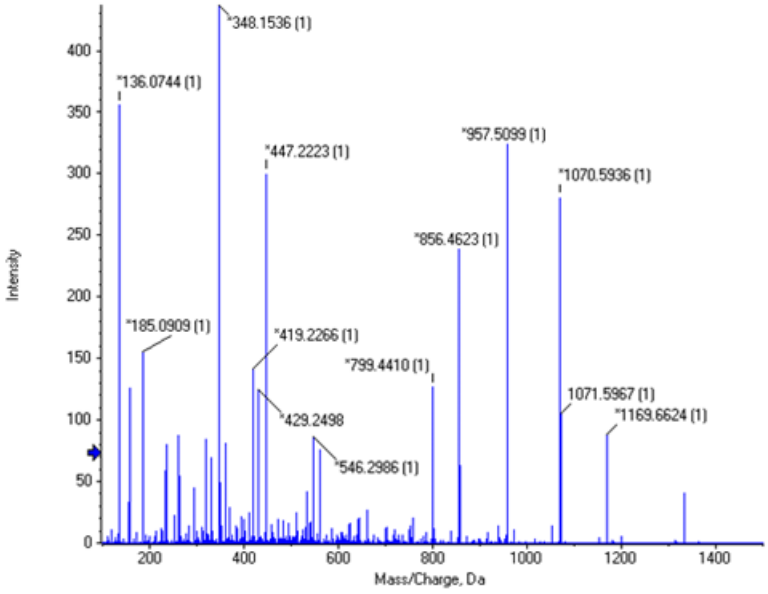
Note: Intensities vary depending on the instrument and the flow rate. Performance specifications for QC infusion are available at http://sciex.com/community/entity/15722.

Figure 2-2 Example Data for TOF-MS Spectrum from Nanoflow Infusion



11. Track the peak intensities and resolution in MS and MS/MS over time to monitor the spray and MS performance.

Figure 2-3 Example Data for TOF-MS/MS Spectrum for Peptide m/z 758 from Nanoflow Infusion



Test 2: PepCalMix LC-MS System Suitability Test at Nanoflow Rates

Make sure that the LC system and the MS are set up in trap elute mode and are equilibrated before performing this test.

This LC-MS system QC test involves a TOF MS and looped MS/MS scan to test both MS and MS/MS sensitivity. The shape and separation of the MS peaks will be used to assess the LC performance. This method also functions as the instrument mass calibration method and should be performed at regular intervals during daily operation.

Prepare a solution of PepCalMix (10 fmol/µL), so that the 2 µL injection gives 20 fmol of PepCalMix on the column. Refer to Prepare the PepCalMix LC-MS Solution for the Nanoflow Test on page 48.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

   Note: The hardware profile must contain the correct LC-MS setup.

2. Create an MS acquisition method using the parameters in Table 2-2.

Table 2-2 MS Method Information

TOF MS	Mass Range	400 to1,250
	Accumulation time	250 msec
	Source conditions	Optimized
	Duration	40 min
TOF MS/MS	Mass Range	100 to 1,500
	Accumulation time	500 msec
	Product Ion	758.91
	MS/MS Type	High Sensitivity
	Collision Energy	40
	Collision Energy Spread	5

Create an Autosampler Method

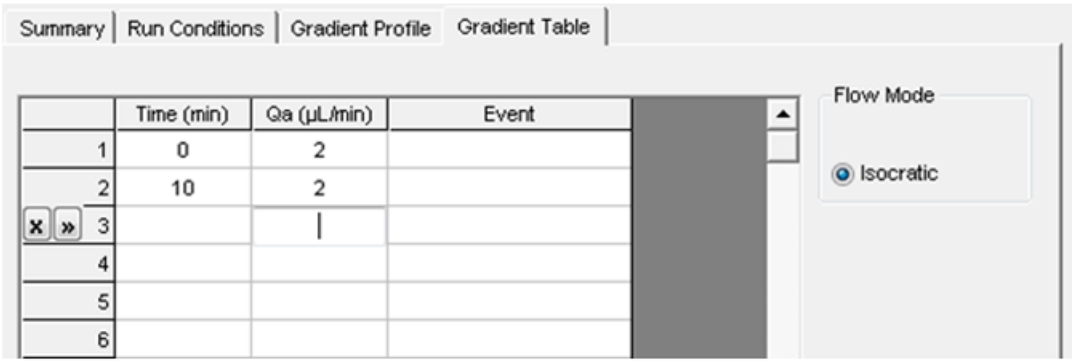
1. Plumb the autosampler using a 10 µL sample loop.
2. Create a µL pick-up autosampler method for an injection volume of 2 µL.

   Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up autosampler methods.
3. Save the autosampler method.
4. Add the autosampler method to the MS method created in Create an MS Method on page 10.

Create a Pump Method for Nanoflow Rates

1. Create a loading pump method using a flow rate of 2 µL/min flow rate for 10 min., as shown in Figure 2-4.

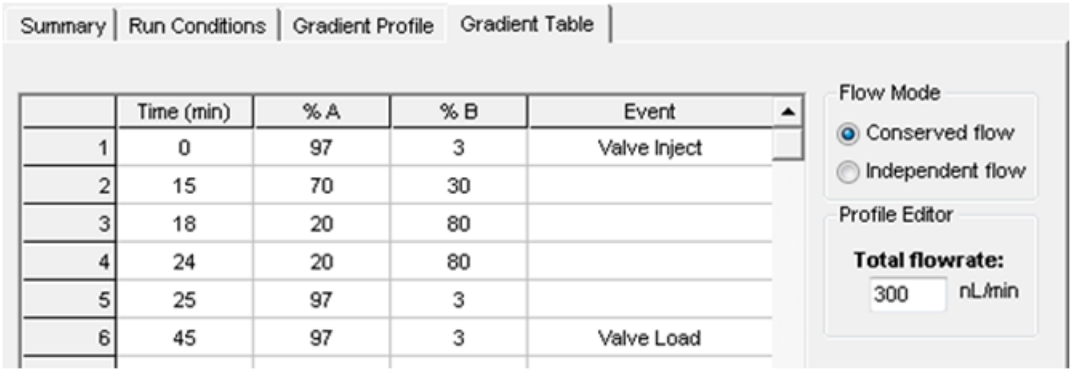
Figure 2-4 Loading Pump Method



2. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
3. Save the loading pump method.
4. Add the loading pump method to the MS method created in Create an MS Method on page 10.
5. Create an analytical pump method using a flow rate of 300 nL/min flow rate, as shown in Figure 2-5.

Note: The method must be created using the correct pump channel, depending on whether the configuration is a 415 or a 425 system.

Figure 2-5 Analytical Pump Method



6. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
7. Save the analytical pump method.
8. Add the analytical pump method to the MS method created in Create an MS Method on page 10.
9. Save the LC-MS method.
10. Set the column temperature to 35°C.

System QC Procedure

1. Set up the LC and TripleTOF® system as described previously in this section.
2. Acquire multiple injections of the PepCalMix until both the system pressure and the signal intensity stabilize.

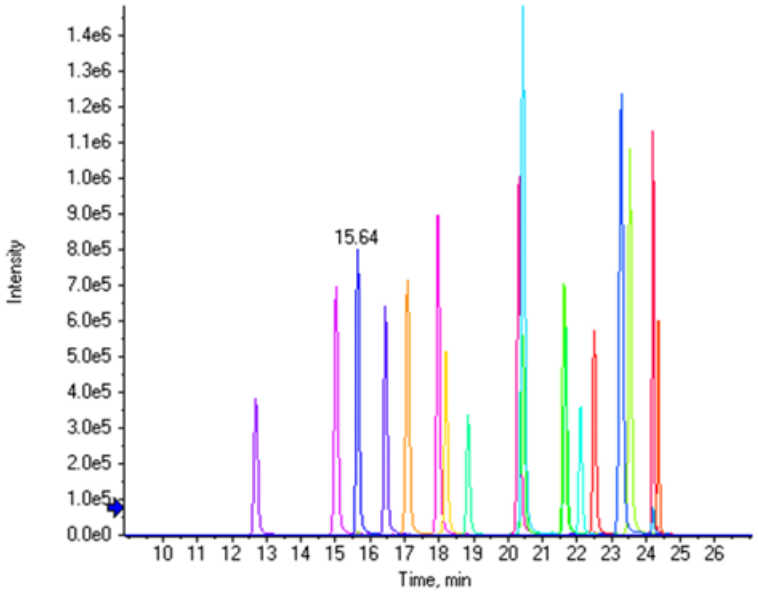
> **Tip!** If the column or trap are new, then multiple injections might be required to condition the column. Consider injecting a complex mixture, such as the protein extract digest, to accelerate conditioning of new columns or traps.

3. After the system is stabilized, inject the PepCalMix three times.
4. Open the data files in the PeakView® software and extract the PepCalMix peptide ions from the TOF MS data using the *Extract Ions using Dialogue* function.

   Refer to the XIC table in PeakView® Software XIC Table for the PepCalMix Test on page 57.
5. Make sure that the peak shape and widths are acceptable, with an average peak width of 0.12 min. at half height.

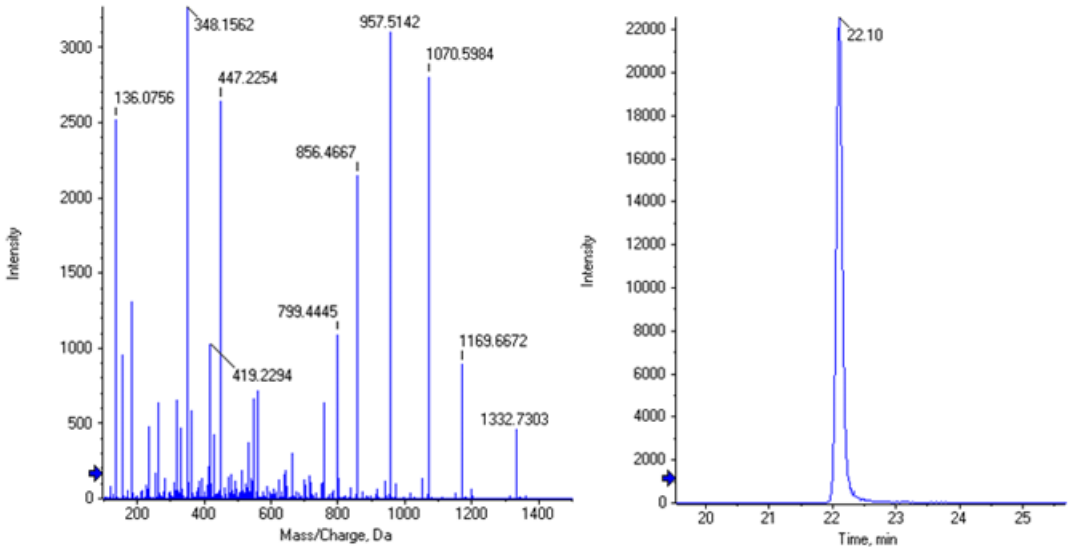
> **Note:** Figure 2-6 shows a typical chromatogram for the PepCalMix at nanoflow. The peak areas vary depending on the instrument and flow rate.

Figure 2-6 Chromatogram for the PepCalMix with Nanoflow LC



6. Make sure that:
   - The peak areas for the peptides are consistent across the three replicates.
   - The retention times look reasonable and there is good separation of the peaks.
   - The peak shapes are reasonably Gaussian, with minimal peak tailing.
   - There are no large dead volumes that cause the peaks to elute very late.
7. From the TOF MS/MS data, extract an XIC of the 1070.5972 fragment ion.
8. Make sure that the MS/MS peak area can be reproduced across the three injections.

Figure 2-7 Example Data of the MS/MS Spectrum of the 758.9105 m/z Peptide (Left) and the XIC of 1070.5972 Fragment Ion (Right)



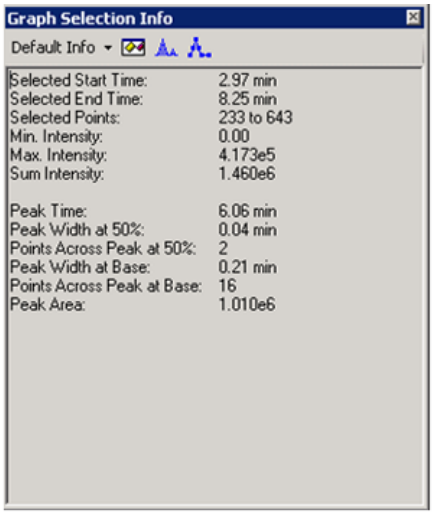
9. Make sure that the mass accuracy observed on average for the TOF MS and MS/MS peaks at peak apex is better than 5 ppm.

Tip! When checking the mass accuracy, make sure that the instrument has been calibrated recently.

Note: This PepCalMix method can be used for the LC-MS system QC throughout this document.

10. Track the results over time to make sure that the performance of the instrument is consistent.
11. From the PeakView® software menu bar, click **Window > Graph Selection Window**.

Figure 2-8 Graph Selection Info Dialog



12. Select the XIC peak of interest and then observe the **Peak Area** value for each peak from the Graph Selection Window.

Note: Performance specifications are available at http://sciex.com/community/entity/15722.

Mass Calibration Procedure

Note: We recommend that this method be used for instrument calibration throughout any proteomics study.

1. Before starting this test, make sure that the instrument is within 100 ppm in MS and MS/MS mode.
2. Click **Tools > Settings > Tuning Options > Reference > New** to create an instrument calibration reference table.
3. Copy and paste the values from Table 2-3 and Table 2-4 into the instrument calibration reference table.
4. Edit the retention times to match the retention times determined from the replicate QC injections in System QC Procedure on page 12.

Table 2-3 Reference Ions for TOF MS Calibration

Use	Compound Name	Precursor m/z (Da)	Use for MS/MS	CE for MS/MS	DP for MS/MS	Retention Time (min)
1	AETSELHTSLK	408.55010	0	40	80	15.6
1	GAYVEVTAK	473.26020	0	40	80	16.5
1	IGNEQGVSR	485.25302	0	40	80	12.7
1	LVGTPAEER	491.26559	0	40	80	15
1	LDSTSIPVAK	519.79969	0	40	80	18
1	AGLIVAEGVTK	533.32333	0	40	80	20.3
1	LGLDFDSFR	540.27342	0	40	80	24.2
1	GFTAYYIPR	549.28633	0	40	80	22.5
1	SGGLLWQLVR	569.83398	0	40	80	24.4
1	AVGANPEQLTR	583.31360	0	40	80	17.1
1	SAEGLDASASLR	593.80053	0	40	80	18.2
1	VFTPLEVDVAK	613.34955	0	40	80	23.6
1	VGNEIQYVALR	636.35273	0	40	80	21.6
1	YIELAPGVDNSK	657.34499	0	40	80	20.4
1	DGTFAVDGPGVIAK	677.85827	0	40	80	21.7
1	YDSINNTEVSGIR	739.36148	0	40	80	18.8
1	SPYVITGPGVVEYK	758.91050	1	40	80	22.1
1	ALENDIGVPSDATVK	768.90340	0	40	80	20.4
1	AVYFYAPQIPLYANK	883.47380	0	40	80	24.2
1	TVESLFPEEAETPGSAVR	964. 97741	0	40	80	23.3

Table 2-4 Reference Ions for MS/MS Calibration

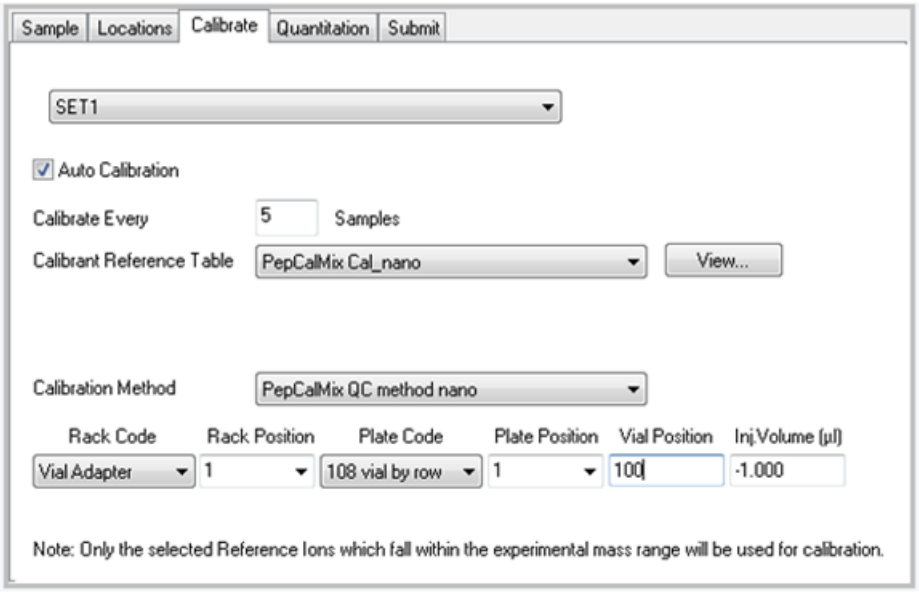
Use	Fragment Name	Fragment m/z (Da)
1	b2	185.09207
1	b3	348.15540
1	b4	560.30788

Table 2-4 Reference Ions for MS/MS Calibration (continued)

Use	Fragment Name	Fragment m/z (Da)
1	b5	661.35555
1	N/A	758.91050
1	y7	799.44398
1	y8	856.46544
1	y9	957.51312
1	y10	1070.59719
1	y11	1169.66560
1	y12	1332.72893

5. Create another batch with the calibration activated and provide the new PepCalMix Calibrant Reference Table and the PepCalMix method information. Refer to Figure 2-9.

Figure 2-9 Activated Calibration



6. Submit the new batch and then confirm that the calibration passes.

Note: When the mass calibration is successful, a green check mark is shown for the Calibration run in the Queue Manager.

Test 3: Cell Lysate SWATH® Acquisition QC Test at Nanoflow Rates

After performing the PepCalMix LC-MS System Suitability Test, the SWATH® Acquisition QC test can be performed.

1. Prepare the stock solution of the Protein Extract Digest (2 ug/uL) as described in Prepare the Protein Extract Digest Stock Solution on page 47.
2. Prepare a solution of Protein Extract Digest (0.5 µg/µL) and PepCalMix (50 fmol/µL) as described in Prepare the SWATH® Acquisition QC Solution for the Nanoflow Test on page 49, so that the 2 µL injection results in a 1 µg total protein and 100 fmol of PepCalMix on the column.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

   Note: The hardware profile must contain the correct LC-MS setup.

2. Create an MS acquisition method using the parameters in Table 2-5.

   Note: For information about creating SWATH® data, refer to the https://training.sciex.com/Course.aspx?c=336 eLearning module.

Table 2-5 MS Method Information

TOF MS	Mass Range	400 to 1,250
	Accumulation speed	250 msec
	Source conditions	Optimized
	Duration	100 min

Table 2-5 MS Method Information (continued)

TOF MS/MS	Mass Range	100 to 1,500
	Accumulation speed	25 msec
	SWATH window text file	100 Variable window[1]
	MS/MS Type	High Sensitivity
	Collision Energy	Rolling CE[2], 2+ selected
	Cycle time	3.2 sec

[1] This file can be download from http://sciex.com/community/entity/1217.

[2] Optimized rolling CE curves can be downloaded from http://sciex.com/community/entity/11856.

Create an Autosampler Method

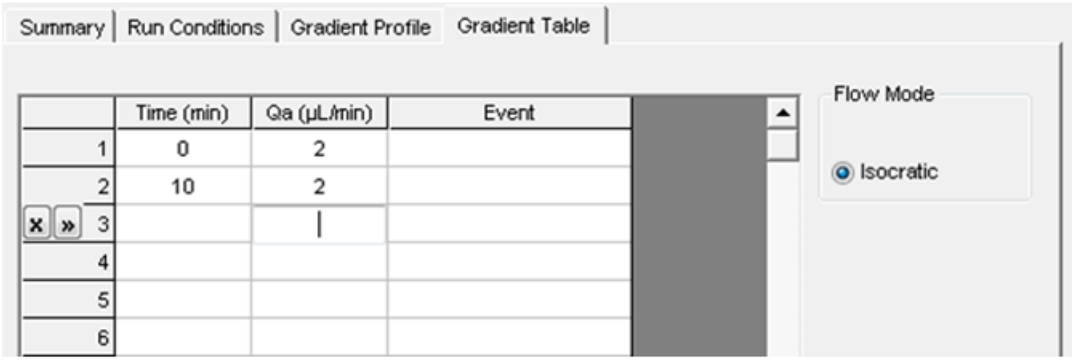
1. Plumb the autosampler using a 10 µL sample loop.
2. Create a µL pick-up autosampler method for an injection volume of 2 µL.
3. Save the autosampler method.
4. Add the autosampler method to the MS method created in Create an MS Method on page 18.

   Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up autosampler methods.

Create a Pump Method for Nanoflow Rates

1. Create a loading pump method using a flow rate of 2 µL/min flow rate for 10 min., as shown in Figure 2-10.

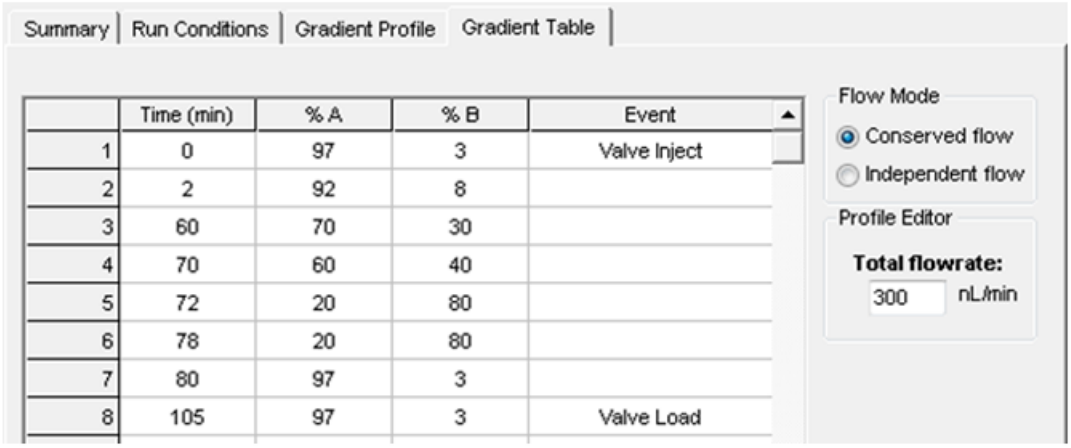
Figure 2-10 Loading Pump Method



2. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
3. Save the loading pump method.
4. Add the loading pump method to the MS method created in Create an MS Method on page 18.
5. Create an analytical pump method using a flow rate of 300 nL/min flow rate, as shown in Figure 2-11.

Note: The method must be created using the correct pump channel, depending on whether the configuration is a 415 or a 425 system.

Figure 2-11 Analytical Pump Method



6. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
7. Save the analytical pump method.
8. Add the analytical pump method to the MS method created in Create an MS Method on page 18.
9. Save the LC-MS method.
10. Set the column temperature to 35°C.

SWATH® Acquisition QC Procedure

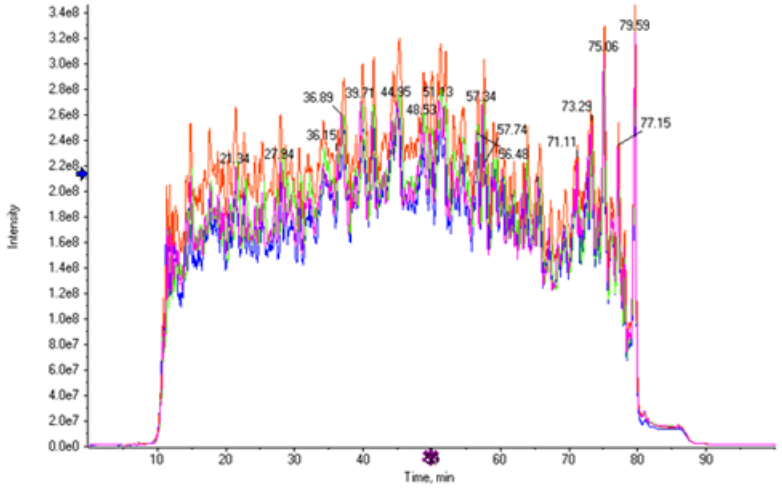
1. Set up the LC and TripleTOF® system as described previously in this section.
2. Acquire multiple injections of the SWATH® Acquisition QC Solution until the system pressure and signal intensity stabilize.

Tip! If the column or trap are new, then multiple injections might be required to condition the column. Consider injecting a complex mixture, such as the protein extract digest, to accelerate conditioning of new columns or traps.

3. After the system is stabilized, run the SWATH® Acquisition QC test on the SWATH® Acquisition QC Solution five times.
4. Make sure that good chromatographic and TIC intensity reproducibility is achieved.

Note: A representative TIC is shown in Figure 2-12. Intensities vary depending on the instrument and flow rate. Performance specifications are available at http://sciex.com/community/entity/15722.

Figure 2-12 Total Ion Chromatogram (TIC) of Replicate SWATH Injections



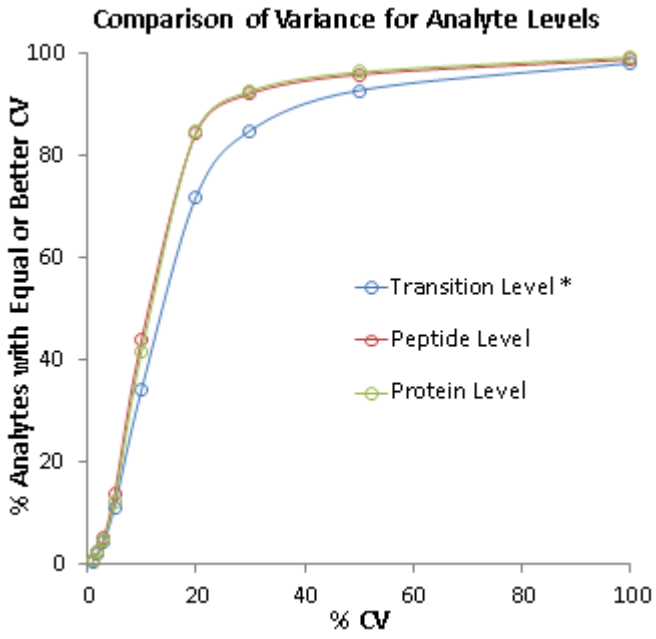
5. Process the data with the SWATH® 2.0 MicroApp in the PeakView® software, using the Human Ion Library file (Human2D_PepCalMix SOP Library.txt). Refer to SWATH® Acquisition Processing Using a Library on page 58 for processing information.

Tip! The library can be downloaded from http://sciex.com/community/entity/15722.

6. After the processing is complete, click **Export > All** to export all of the results and then analyze them using the *SWATH Replicates Template*. Refer to SWATH® Replicates Analysis Template on page 61.
7. Make sure that the reproducibility curves on the Simple Summary tab look similar to those shown in Figure 2-13.

   The most important statistic to review in this dashboard is the red curve in the graph. This curve shows that approximately 70% of the transitions have a %CV better than 20%. This indicates good quantitative robustness across the replicates.

Figure 2-13 Reproducibility Curves



8. Obtain the performance numbers from the **Simple Summary** tab.
9. Track the results over time to make sure that the performance of the instrument is consistent.

> **Note:** This is easily accomplished by copying the single column of results from the **Results – Single Column** tab and storing them in a separate Excel sheet.

Test 4: Cell Lysate IDA QC Test at Nanoflow Rates

After performing the PepCalMix LC-MS System Suitability Test, the IDA QC test can be performed.

1. Prepare the stock solution of the Protein Extract Digest (2 ug/uL) as described in Prepare the Protein Extract Digest Stock Solution on page 47.
2. Prepare a solution of Protein Extract Digest (0.5 µg/µL) as described in Prepare the IDA QC Solution for the Nanoflow Test on page 50, so that the 2 µL injection results in a 1 µg total protein on the column.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

Note: The hardware profile must contain the correct LC-MS setup.

2. Create an MS acquisition method using the parameters in Table 2-6.

Note: In Manual Tune, multiple acquisitions in both MS and MS/MS will be performed.

Table 2-6 MS Method Information

TOF MS	Mass Range	400 to 1,250
	Accumulation speed	250 msec
	Source conditions	Optimized
	Duration	130 min
TOF MS/MS	Mass Range	100 to– 1,500
	Accumulation speed	50 msec
	MS/MS Type	High Sensitivity
Switch Criteria	For ions greater than	400
	For ions smaller than	1,250
	With charge state	2 to 5
	Which exceeds	150 cps
	Mass Tolerance	100 ppm
	Max # of candidates	30
	Exclude for	15 sec
IDA Advanced	Rolling collision energy[1]	0n

[1] Optimized rolling CE curves can be downloaded from http://sciex.com/community/entity/11856.

Create an Autosampler Method

1. Plumb the autosampler using a 10 µL sample loop.
2. Create a µL pick-up autosampler method for an injection volume of 2 µL.
3. Save the autosampler method.
4. Add the autosampler method to the MS method created in Create an MS Method on page 23.

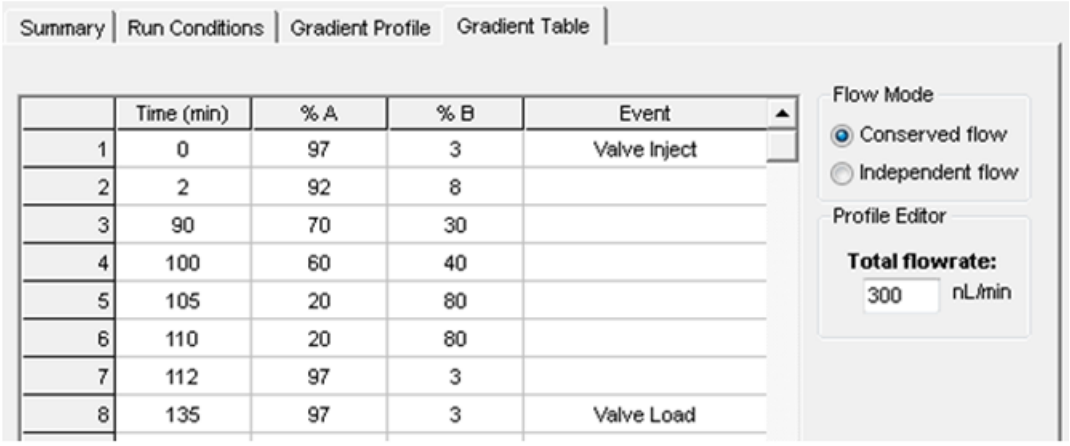
   Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up autosampler methods.

Create a Pump Method for Nanoflow Rates

1. Use the same loading pump method as used for the SWATH® Acquisition QC Test.
2. Add the loading pump method to the MS method created in Create an MS Method on page 23.
3. Create an analytical pump method using a flow rate of 300 nL/min flow rate, as shown in Figure 2-14.

Note: The method must be created using the correct pump channel, depending on whether the configuration is a 415 system or a 425 system.

Figure 2-14 Analytical Pump Method



4. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
5. Save the analytical pump method.
6. Add the analytical pump method to the MS method created in Create an MS Method on page 23.

7. Save the LC-MS method.
8. Set the column temperature to 35°C.

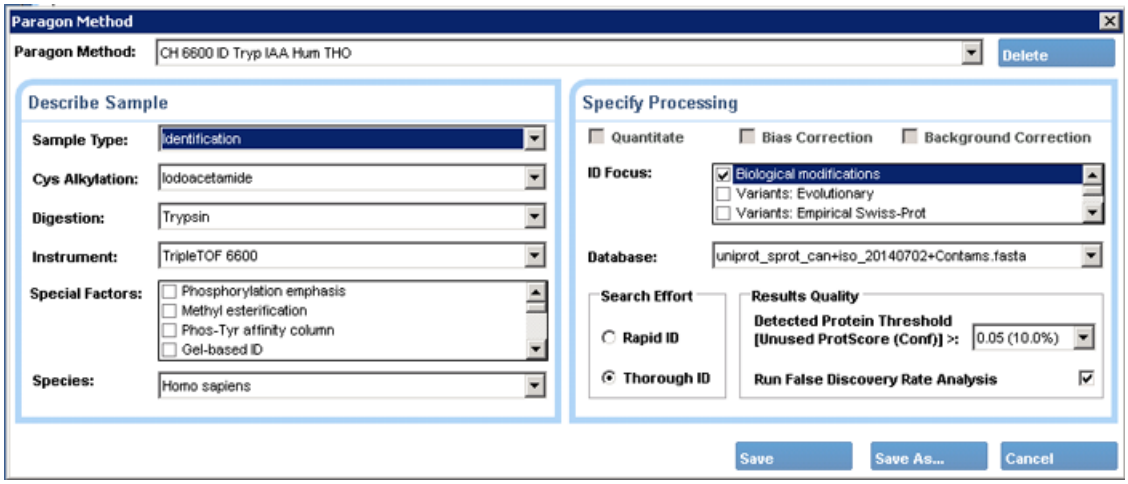
IDA QC Procedure

1. Set up the LC and TripleTOF® system as described previously in this section.
2. Acquire multiple injections of the IDA QC Solution until the system pressure and signal intensity stabilize.

> **Tip!** If the column or trap are new, then multiple injections might be required to condition the column. Consider injecting a complex mixture, such as the protein extract digest, to accelerate conditioning of new columns or traps.

3. After the system is stabilized, run the IDA QC test on the IDA QC Solution three times.
4. Process the data using the ProteinPilot™ software, version 5.0.

Figure 2-15 Paragon Method



5. Open the FDR report that is produced with each search and then determine the average number of proteins and peptides found at 1% FDR (Global).

Tests for the Microflow Regime 3

Test 1: PepCalMix Infusion Test – Source Tuning at Microflow Rates

1. Make sure that the DuoSpray™ ion source is installed on the system.
2. Make sure that the 25 µm ID hybrid electrodes are installed in the source.
3. Prepare 50 fmol/µL of the PepCalMix solution.

   Refer to Prepare the PepCalMix Infusion Solution on page 46.

Note: For the infusion protocol, use a 100 µL syringe and tubing with a 50 µm inside diameter (i.d.) to 100 µm i.d. For information about setting up the syringe line, refer to Create a Syringe Line on page 63.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

Note: The hardware profile must include the mass spectrometer and the syringe pump.

2. Set the acquisition parameters using the information in Table 3-1.

Note: In Manual Tune, multiple acquisitions in both MS and MS/MS will be performed.

Table 3-1 MS Method Information

TOF MS	Mass Range	400 to 1,500
	Accumulation time	1 sec
	Source Conditions	Optimized

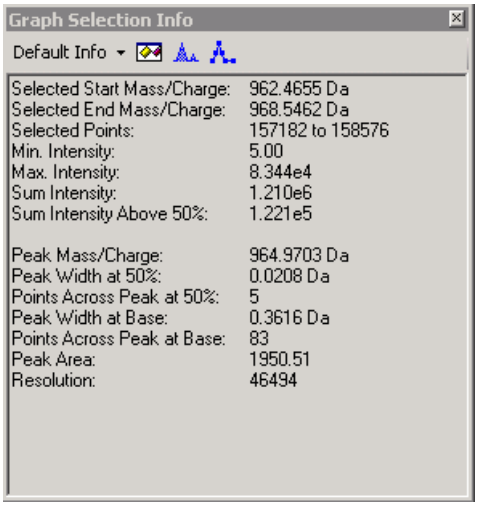
Table 3-1 MS Method Information (continued)

TOF MS/MS	Mass Range	100 to 1,500
	Accumulation time	1 sec
	Product Ion[1]	964.98 / 758.91 / 533.3
	MS/MS Type	High Sensitivity and High Resolution
	Collision Energy[1]	45 / 40 / 25
	Collision Energy Spread	5
[1] MS/MS acquisition is performed on three different peptides in both resolution modes, resulting in the acquisition of six MS/MS. The product ion masses shown in the table correspond to the collision energies shown in the table.		

Infusion

1. Connect the syringe to the source using the infusion line.
2. Infuse the PepCalMix solution at 5 µL/min for microflow testing.
3. Monitor the signal of the 758.91 *m/z* peptide with a TOF MS scan, as specified in Table 3-1.
4. Optimize the position of the sprayer and the source parameters for maximum stability and signal.
5. After optimization is completed, acquire the TOF MS data for 1 min. to confirm stable signal.
6. Acquire the MS/MS spectra on the three peptides as specified in Table 3-1, using both high sensitivity and high resolution modes, for 1 min.
7. Open the data in the PeakView® software to assess the data quality.
8. Select 30 sec. of the infusion TIC and then double-click to extract the spectrum.
9. From the PeakView® software menu bar, click **Window > Graph Selection Window**.

Figure 3-1 Graph Selection Info Dialog



10. Select the spectral peak of interest (the C12 peak for each) and then observe the **Sum Intensity** value for each peak from the Graph Selection Window.

Tip! Zoom into the spectrum and then carefully select only the C12 peak of the isotope cluster.

Note: Intensities vary depending on the instrument and the flow rate. Performance specifications for QC infusion are available at http://sciex.com/community/entity/15722.

11. Track the peak intensities and resolution in MS and MS/MS over time to monitor the spray and MS performance.

Test 2: PepCalMix LC-MS System Suitability Test at Microflow Rates

Make sure that the LC system and the MS are set up in trap elute mode and are equilibrated before performing this test.

This LC-MS system QC test involves a TOF MS and looped MS/MS scan to test both MS and MS/MS sensitivity. The shape and separation of the MS peaks will be used to assess the LC performance. This method also functions as the instrument mass calibration method and should be performed at regular intervals during daily operation.

Prepare a solution of PepCalMix (20 fmol/µL) so that the 2 µL injection gives 40 fmol of PepCalMix on the column. Refer to Prepare the PepCalMix LC-MS Solution for the Microflow Test on page 52.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

   Note: The hardware profile must contain the correct LC-MS setup.

2. Create an MS acquisition using the parameters in Table 3-2.

   Note: In Manual Tune, multiple acquisitions in both MS and MS/MS will be performed.

Table 3-2 MS Method Information

TOF MS	Mass Range	400 to 1,250
	Accumulation time	250 msec
	Source conditions	Optimized
	Duration	12 min
TOF MS/MS	Mass Range	100 to 1,500
	Accumulation time	500 msec
	Product Ion	758.91
	MS/MS Type	High Sensitivity
	Collision Energy	40
	Collision Energy Spread	5

Create an Autosampler Method

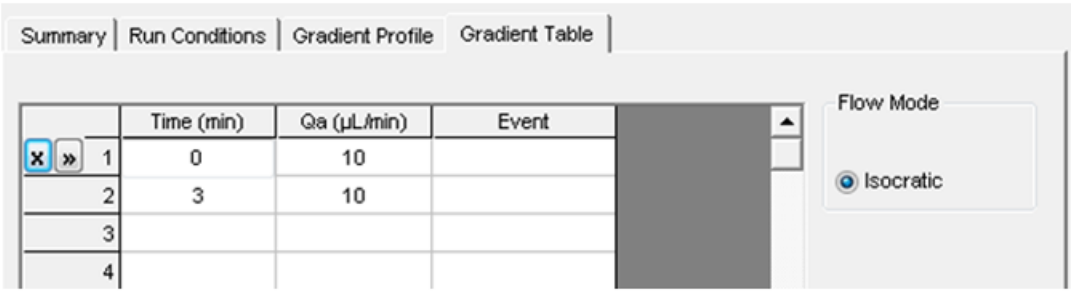
1. Plumb the autosampler using a 10 µL sample loop.
2. Create a µL pick-up autosampler method for an injection volume of 2 µL.

   Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up autosampler methods.
3. Save the autosampler method.
4. Add the autosampler method to the MS method created in Create an MS Method on page 29.

Create a Pump Method for Microflow Rates

1. Create a loading pump method using a flow rate of 10 µL/min flow rate for 3 min., as shown in Figure 3-2.

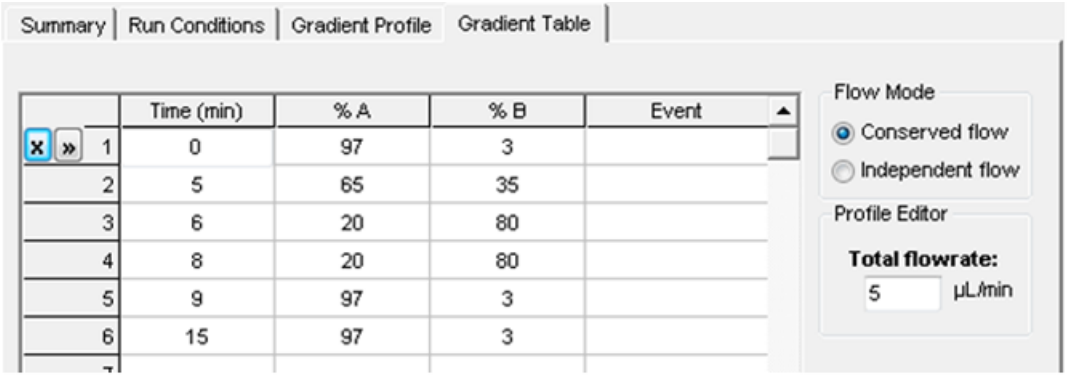
Figure 3-2 Loading Pump Method



2. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
3. Save the loading pump method.
4. Add the loading pump method to the MS method created in Create an MS Method on page 29.
5. Create an analytical pump method using a flow rate of 5 µL/min flow rate, as shown Figure 3-3.

Note: The method must be created using the correct pump channel, depending on whether the configuration is a 415 or a 425 system.

Figure 3-3 Analytical Pump Method



6. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
7. Save the analytical pump method.
8. Add the analytical pump method to the MS method created in Create an MS Method on page 29.

9. Save the LC-MS method.
10. Set the column temperature to 35°C.

System QC Procedure

1. Set up the LC and TripleTOF® system as described previously in this section.
2. Acquire multiple injections of the PepCalMix until the system pressure and signal intensity stabilize.

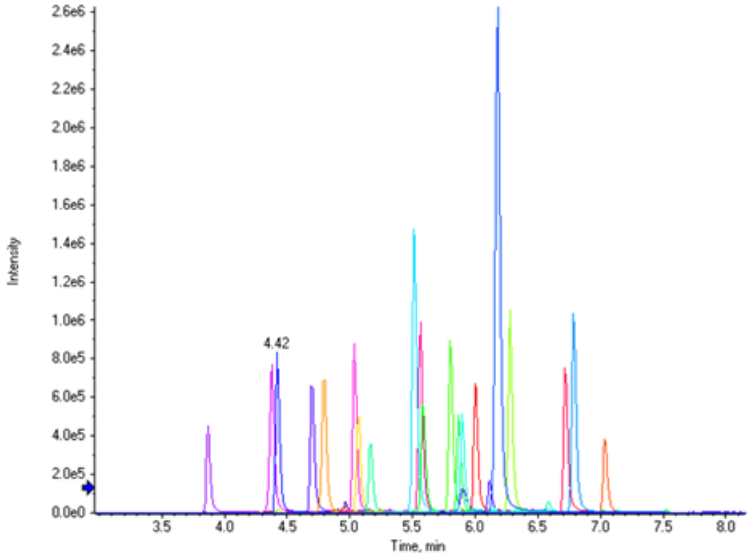
> **Tip!** If the column or trap are new, then multiple injections might be required to condition the column. Consider injecting a complex mixture, such as the protein extract digest, to accelerate conditioning of new columns or traps.

3. After the system is stabilized, inject the PepCalMix three times.
4. Open the data files in the PeakView® software and extract the PepCalMix peptide ions from the TOF MS data using the *Extract Ions using Dialogue* function.

   Refer to the XIC table in PeakView® Software XIC Table for the PepCalMix Test on page 57.
5. Make sure that the peak shape and widths are acceptable, with an average peak width of 0.05 min. at half height.

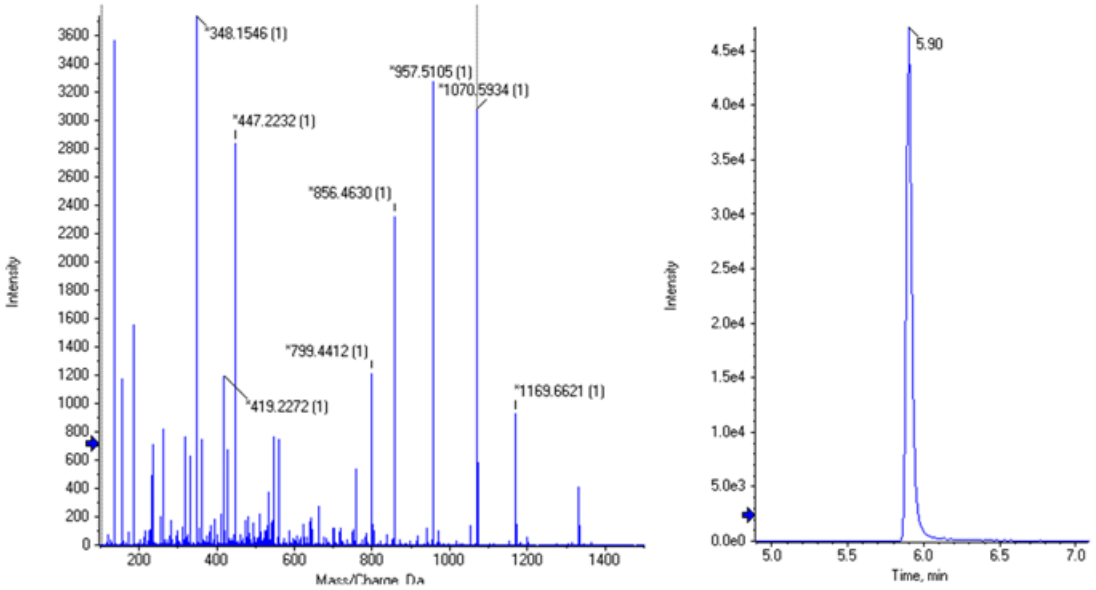
> **Note:** Figure 2-6 shows a typical chromatogram for the PepCalMix at microflow. The peak areas vary depending on the instrument and flow rate.

Figure 3-4 Chromatogram for the PepCalMix



6. Make sure that:
   - The peak areas for the peptides are consistent across the three replicates.
   - The retention times look reasonable and there is good separation of the peaks.
   - The peak shapes are reasonably Gaussian, with minimal peak tailing.
   - There are no large dead volumes that cause the peaks to elute very late.
7. From the TOF MS/MS data, extract an XIC of the 1070.5972 fragment ion.
8. Make sure that the MS/MS peak area can be reproduced across the three injections.

Figure 3-5 Example Data of the MS/MS Spectrum of the 758.9105 m/z Peptide (Left) and the XIC of 1070.5972 Fragment Ion (Right)



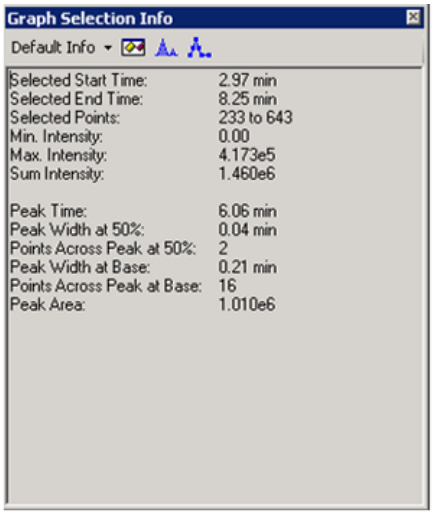
9. Make sure that the mass accuracy observed on average for the TOF MS and MS/MS peaks at peak apex is better than 5 ppm.

Tip! When checking the mass accuracy, make sure that the instrument has been calibrated recently.

Note: This PepCalMix method is used for the LC-MS system QC throughout the course of your studies.

10. Track the results over time to make sure that the performance of the instrument is consistent.
11. From the PeakView® software menu bar, click **Window > Graph Selection Window**.

Figure 3-6 Graph Selection Info Dialog



12. Select the XIC peak of interest and then observe the **Peak Area** value for each peak from the Graph Selection Window.

Note: Performance specifications are available at http://sciex.com/community/entity/15722.

Mass Calibration Procedure

Note: We recommend that this method be used for instrument calibration throughout any proteomics study.

1. Before starting this test, make sure that the instrument is within 100 ppm in MS and MS/MS mode.
2. Click **Tools > Settings > Tuning Options > Reference > New** to create an instrument calibration reference table.
3. Copy and paste the values from Table 2-3 and Table 2-4 into the instrument calibration reference table.
4. Edit the retention times to match the retention times determined from the replicate QC injections in System QC Procedure on page 31.

Table 3-3 Reference Ions for TOF MS Calibration

Use	Compound Name	Precursor m/z (Da)	Use for MS/MS	CE for MS/MS	DP for MS/MS	Retention Time (min)
1	AETSELHTSLK	408.55010	0	40	80	4.2
1	GAYVEVTAK	473.26020	0	40	80	4.47
1	IGNEQGVSR	485.25302	0	40	80	3.26
1	LVGTPAEER	491.26559	0	40	80	4.15
1	LDSTSIPVAK	519.79969	0	40	80	4.8
1	AGLIVAEGVTK	533.32333	0	40	80	5.4
1	LGLDFDSFR	540.27342	0	40	80	6.61
1	GFTAYYIPR	549.28633	0	40	80	5.84
1	SGGLLWQLVR	569.83398	0	40	80	6.97
1	AVGANPEQLTR	583.31360	0	40	80	4.55
1	SAEGLDASASLR	593.80053	0	40	80	4.84
1	VFTPLEVDVAK	613.34955	0	40	80	6.14
1	VGNEIQYVALR	636.35273	0	40	80	5.62
1	YIELAPGVDNSK	657.34499	0	40	80	5.38
1	DGTFAVDGPGVIAK	677.85827	0	40	80	6.63
1	YDSINNTEVSGIR	739.36148	0	40	80	4.91
1	SPYVITGPGVVEYK	758.91050	1	40	80	5.71
1	ALENDIGVPSDATVK	768.90340	0	40	80	5.27
1	AVYFYAPQIPLYANK	883.47380	0	40	80	6.58
1	TVESLFPEEAETPGSAVR	964. 97741	0	40	80	6

Table 3-4 Reference Ions for MS/MS Calibration

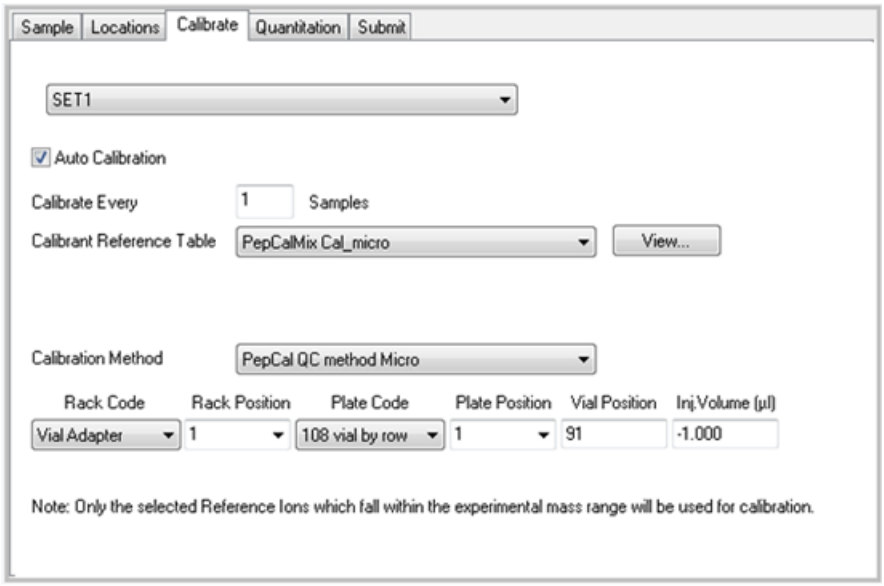
Use	Fragment Name	Fragment m/z (Da)
1	b2	185.09207
1	b3	348.15540
1	b4	560.30788

Table 3-4 Reference Ions for MS/MS Calibration (continued)

Use	Fragment Name	Fragment m/z (Da)
1	b5	661.35555
1	N/A	758.91050
1	y7	799.44398
1	y8	856.46544
1	y9	957.51312
1	y10	1070.59719
1	y11	1169.66560
1	y12	1332.72893

5. Create another batch with the calibration activated and then provide the new PepCalMix Calibrant Reference Table and the PepCalMix method information.

Figure 3-7 Activated Calibration



6. Submit the new batch and confirm that the calibration passes.

Note: When the mass calibration is successful, a green check mark is shown for the Calibration run in the Queue Manager.

Test 3: Cell Lysate SWATH® Acquisition QC Test at Microflow Rates

After performing the PepCalMix LC-MS System Suitability Test, the SWATH® Acquisition QC test can be performed.

1. Prepare the stock solution of the Protein Extract Digest (2 ug/uL) as described in Prepare the Protein Extract Digest Stock Solution on page 47.
2. Prepare a solution of Protein Extract Digest (1 µg/µL) and PepCalMix (100 fmol/µL) as described in Prepare the SWATH® Acquisition QC Solution for the Microflow Test on page 53, so that the 4 µL injection results in a 4 µg total protein and 400 fmol of PepCalMix on the column.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

Note: The hardware profile must contain the correct LC-MS setup.

2. Create an MS acquisition method using the parameters in Table 2-5.

Note: For information about creating SWATH® data, refer to the https://training.sciex.com/Course.aspx?c=336 eLearning module.

Table 3-5 MS Method Information

TOF MS	Mass Range	400 to 1,250
	Accumulation speed	250 msec
	Source conditions	Optimized
	Duration	55 min

Table 3-5 MS Method Information (continued)

TOF MS/MS	Mass Range	100 to 1,500
	Accumulation speed	25 msec
	SWATH window text file	100 Variable window[1]
	MS/MS Type	High Sensitivity
	Collision Energy	Rolling CE[2], 2+ selected
	Cycle time	3.2 sec

[1] This file can be download from http://sciex.com/community/entity/1217.

[2] Optimized rolling CE curves can be downloaded from http://sciex.com/community/entity/11856.

Create an Autosampler Method

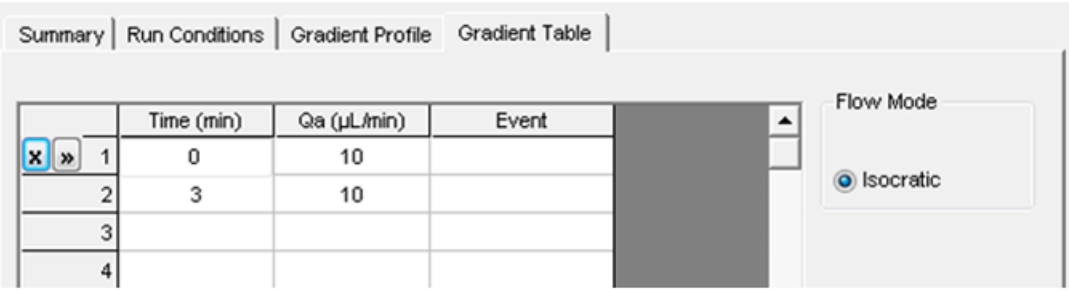
1. Plumb the autosampler using a 10 µL sample loop.
2. Create a µL pick-up autosampler method for an injection volume of 4 µL.
3. Save the autosampler method.
4. Add the autosampler method to the MS method created in Create an MS Method on page 37.

   Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up autosampler methods.

Create a Pump Method for Microflow Rates

1. Create a loading pump method using a flow rate of 10 µL/min flow rate for 3 min., as shown in Figure 3-8.

Figure 3-8 Loading Pump Method

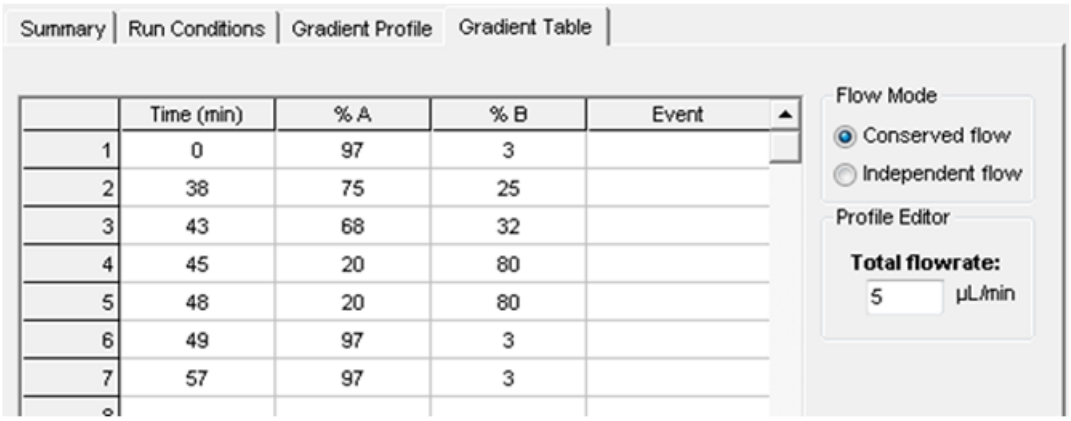


2. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.

3. Save the loading pump method.
4. Add the loading pump method to the MS method created in Create an MS Method on page 37.
5. Create an analytical pump method using a flow rate of 5 µL/min flow rate, as shown in Figure 3-9.

Note: The method must be created using the correct pump channel, depending on whether the configuration is a 415 or a 425 system.

Figure 3-9 Analytical Pump Method



6. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
7. Save the analytical pump method.
8. Add the analytical pump method to the MS method created in Create an MS Method on page 37.
9. Save the LC-MS method.
10. Set the column temperature to 35°C.

SWATH® Acquisition QC Procedure

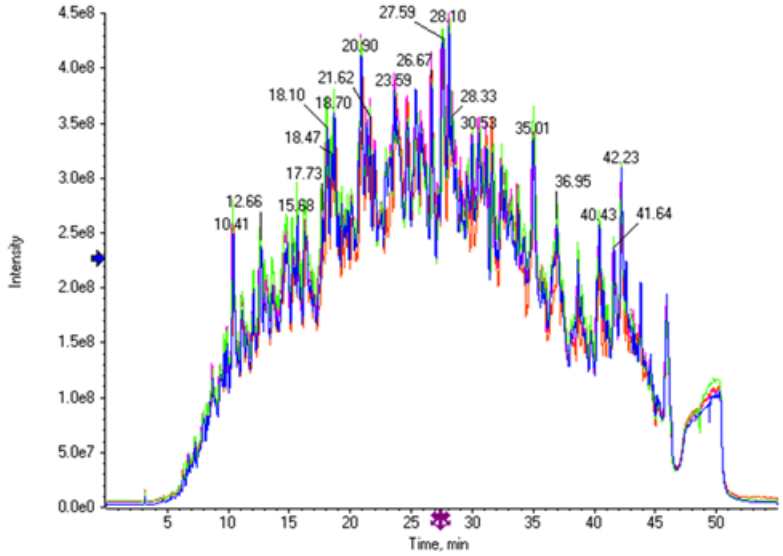
1. Set up the LC and TripleTOF® system as described previously in this section.
2. Acquire multiple injections of the SWATH® Acquisition QC Solution until the system pressure and signal intensity stabilize.

Tip! If the column or trap are new, then multiple injections might be required to condition the column. Consider injecting a complex mixture, such as the protein extract digest, to accelerate conditioning of new columns or traps.

3. After the system is stabilized, run the SWATH® Acquisition QC test on the SWATH® Acquisition QC Solution five times.
4. Make sure that good chromatographic and TIC intensity reproducibility is achieved.

Note: A representative TIC is shown in Figure 3-10. Intensities vary depending on the instrument and flow rate. Performance specifications are available at http://sciex.com/community/entity/15722.

Figure 3-10 Total Ion Chromatogram (TIC) of Replicate SWATH Injections



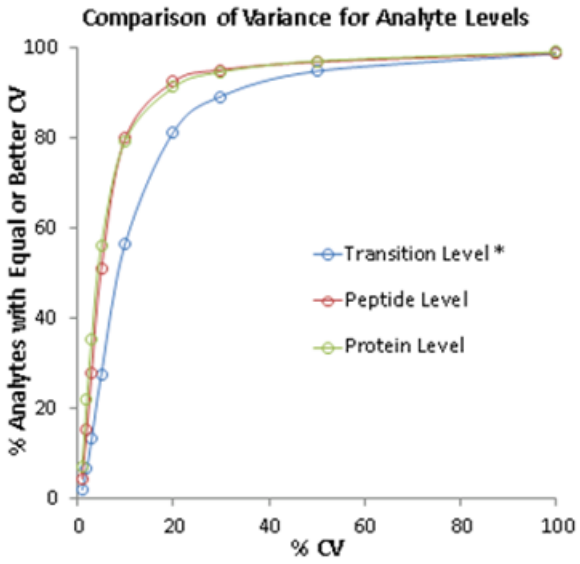
5. Process the data with the SWATH® 2.0 MicroApp in the PeakView® software, using the Human Ion Library file (Human2D_PepCalMix SOP Library.txt). Refer to SWATH® Acquisition Processing Using a Library on page 58 for processing information.

Tip! The library can be downloaded from http://sciex.com/community/entity/15722.

6. After the processing is complete, click **Export > All** to export all of the results and then analyze them using the *SWATH Replicates Template*. Refer to SWATH® Replicates Analysis Template on page 61.
7. Make sure that the reproducibility curves on the Simple Summary tab look similar to those shown in Figure 3-11.

   The most important statistic to review in this dashboard is the red curve in the graph. This curve shows that approximately 70% of the transitions have a %CV better than 20%. This indicates good quantitative robustness across the replicates.

Figure 3-11 Reproducibility Curves



8. Obtain the performance numbers from the **Simple Summary** tab.
9. Track the results over time to make sure that the performance of the instrument is consistent.

Note: This is easily accomplished by copying the single column of results from the **Results – Single Column** tab and storing them in a separate Excel sheet.

Test 4: Cell Lysate IDA QC Test at Microflow Rates

After performing the PepCalMix LC-MS System Suitability Test, the IDA QC test can be performed.

1. Prepare the stock solution of the Protein Extract Digest (2 ug/uL) as described in Prepare the Protein Extract Digest Stock Solution on page 47.
2. Prepare a solution of Protein Extract Digest (1 µg/µL) as described in Prepare the IDA QC Solution for the Microflow Test on page 54, so that the 4 µL injection results in a 4 µg total protein on the column.

Create an MS Method

1. Make sure that the correct hardware profile is activated.

Note: The hardware profile must contain the correct LC-MS setup.

2. Create an MS acquisition method using the parameters in Table 3-6.

Note: In Manual Tune, multiple acquisitions in both MS and MS/MS will be performed.

Table 3-6 MS Method Information

TOF MS	Mass Range	400 to 1,250
	Accumulation speed	250 msec
	Source conditions	Optimized
	Duration	85 min
TOF MS/MS	Mass Range	100 to 1,500
	Accumulation speed	50 msec
	MS/MS Type	High Sensitivity
Switch Criteria	For ions greater than	400
	For ions smaller than	1,250
	With charge state	2 to 5
	Which exceeds	150 cps
	Mass Tolerance	100 ppm
	Max # of candidates	30
	Exclude for	15 sec
IDA Advanced	Rolling collision energy[1]	On

[1] Optimized rolling CE curves can be downloaded from http://sciex.com/community/entity/11856.

Create an Autosampler Method

1. Plumb the autosampler using a 10 µL sample loop.
2. Create a µL pick-up autosampler method for an injection volume of 4 µL.
3. Save the autosampler method.
4. Add the autosampler method to the MS method created in Create an MS Method on page 42.

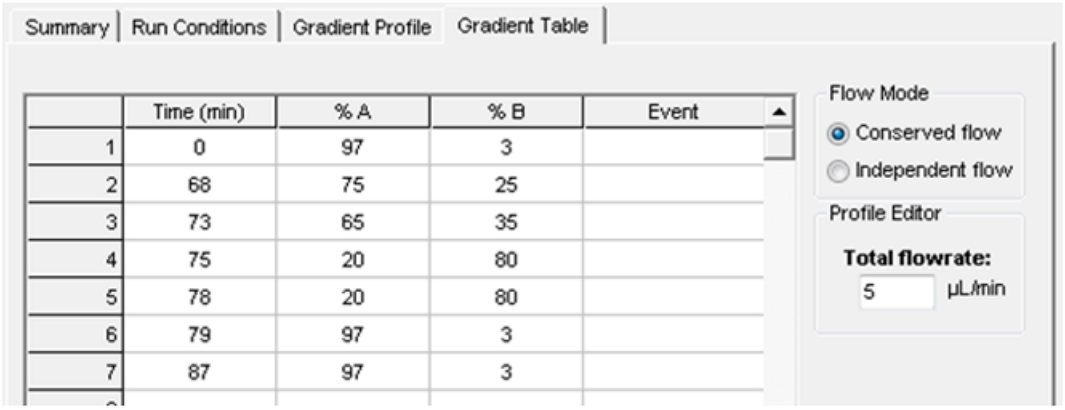
   Refer to Reference Documents on page 64 for a list of reference documents containing information about setting up autosampler methods.

Create a Pump Method for Microflow Rates

1. Use the same loading pump method as used for the SWATH® Acquisition QC Test.
2. Add the loading pump method to the MS method created in Create an MS Method on page 42.
3. Create an analytical pump method using a flow rate of 5 µL/min flow rate, as shown in Figure 3-12.

Note: The method must be created using the correct pump channel, depending on whether the configuration is a 415 system or a 425 system.

Figure 3-12 Analytical Pump Method



4. Select the **Run Conditions** tab and then set the **Sample Injection Mode** to **Standard**.
5. Save the analytical pump method.
6. Add the analytical pump method to the MS method created in Create an MS Method on page 42.
7. Save the LC-MS method.
8. Set the column temperature to 35°C.

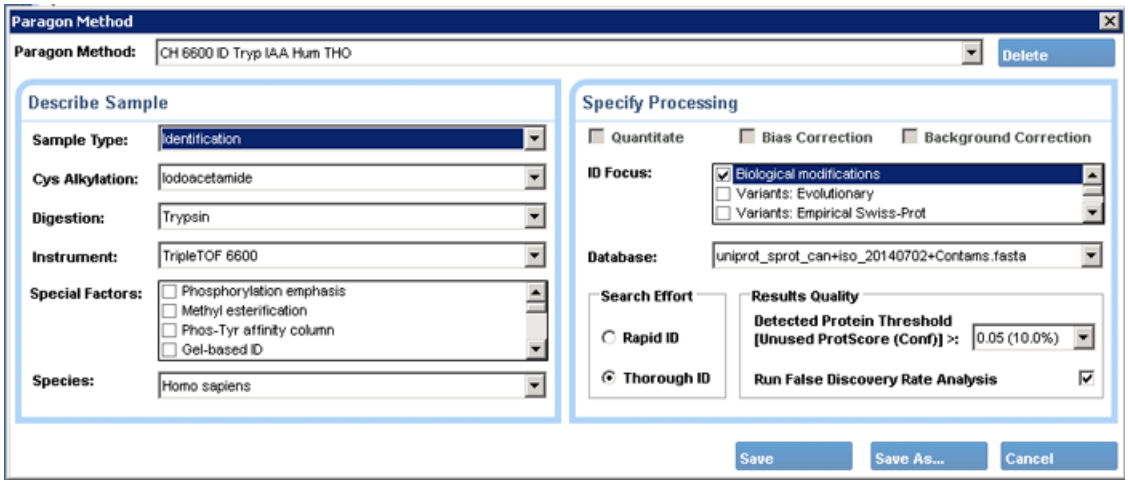
IDA QC Procedure

1. Set up the LC and TripleTOF® system as described previously in this section.
2. Acquire multiple injections of the IDA QC Solution until the system pressure and signal intensity stabilize.

> **Tip!** If the column or trap are new, then multiple injections might be required to condition the column. Consider injecting a complex mixture, such as the protein extract digest, to accelerate conditioning of new columns or traps.

3. After the system is stabilized, run the IDA QC test on the IDA QC Solution three times.
4. Process the data using the ProteinPilot™ software, version 5.0.

Figure 3-13 Paragon Method



5. Open the FDR report that is produced with each search and determine the average number of proteins and peptides found at 1% FDR (Global).

Hazardous Substance Information A

The following information must be noted and the relevant safety measures taken. Refer to the respective safety data sheets for more information. These are available upon request or can be downloaded from our Web site sciex.com.

Hazard Symbol	Classification according to Regulation (EC) No 1272/2008 and OSHA Hazard Communication Standard (29 CFR 1910.1200)
PepCalMix (part number 5045751)	
	**GHS02 flame** • Flam. Liq. 2 H225 Highly flammable liquid and vapor. (US and EU)
	**GHS05 corrosion** • Skin Corr. 1 H314 Causes severe skin burns and eye damage. (US and EU) • Eye Dam. 1 H318 Causes serious eye damage. (US and EU)
	**GHS08 health hazard** • STOT 2 (Repeated exposure) Blood system and cardiovascular system. (US)
This component is not classified as hazardous according to EC 1272/2008 and 29 CFR 1910.1200: • K562 Protein Extract Digest (part number 5045752)	

Prepare the PepCalMix Infusion Solution B

The PepCalMix vial contains 50 pmol of each of the 20 different stable isotope labeled peptides in 50 µL of solvent. It is a stock solution of 1 pmol/µL for each peptide.

1. When opening a new vial of the stock solution, aliquot the stock solution (1 pmol/µL concentration) into 5 µL to 10 µL volumes and then freeze for future use.
2. Add 5 µL of the PepCalMix stock solution and 95 µL of 10% Buffer B (10% acetonitrile : 5% acetic acid) to the vial.

Note: Acetic acid is essential for the stability of the higher mass peptides and must not be substituted.

3. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
4. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.

Note: A certificate of analysis (CoA) for the PepCalMix can be downloaded from the Web site http://sciex.com/tech-regulatory.

Prepare the Protein Extract Digest Stock Solution C

Use this procedure to prepare the Protein Extract Digest stock solution from the vial provided in the SWATH® Performance Kit. Because the vial contains 100 µg, this procedure produces a stock solution of 2 µg/µL.

1. Add 50 µL of Buffer A (100% water: 0.1% formic acid) to the vial.
2. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
3. Shake the vial to bring the liquid to the bottom of the vial before opening.
4. Repeat steps 2 and 3 to confirm dissolution.
5. Aliquot the stock solution (2 µg/µL concentration) into 5 µL to 10 µL volumes and then freeze for future use.

Prepare the PepCalMix LC-MS Solution for the Nanoflow Test D

Use this procedure to prepare the PepCalMix stock solution (1 pmol/µL) from the vial provided in the SWATH® Performance Kit.

1. When opening a new vial of the stock solution, aliquot the stock solution (1 pmol/µL concentration) into 5 µL to 10 µL volumes and then freeze for future use.
2. Add 99 µL of the dilution buffer (5% acetic acid : 2% acetonitrile) to a vial, and then add 1 µL of the PepCalMix stock solution to prepare a 10 fmol/µL solution.

   Note: Acetic acid is essential for the stability of the higher mass peptides and must not be substituted.

3. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
4. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.
5. Pipette the solution into an autosampler vial.

Note: A certificate of analysis (CoA) for the PepCalMix can be downloaded from the Web site http://sciex.com/tech-regulatory.

Prepare the SWATH® Acquisition QC Solution for the Nanoflow Test

E

Use this procedure to prepare a dilution that contains 0.5 µg/µL of Protein Extract Digest and 50 fmol/µL of the PepCalMix.

1. Add 14 µL of Buffer A (100% water: 0.1% formic acid) to the vial.
2. Add 5 µL of the Protein Extract Digest Stock solution to the vial. Refer to Prepare the Protein Extract Digest Stock Solution on page 47 for information about preparing the stock solution.
3. Add 1 µL of the PepCalMix stock solution to the vial.
4. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
5. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.
6. Pipette the solution into an autosampler vial.

Prepare the IDA QC Solution for the Nanoflow Test

F

Use this procedure to prepare a dilution that contains 0.5 µg/µL of Protein Extract Digest.

1. Add 15 µL of Buffer A (100% water: 0.1% formic acid) to the vial.
2. Add 5 µL of the Protein Extract Digest Stock solution to the vial.
3. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
4. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.
5. Pipette the solution into an autosampler vial.

Parts List for the NanoLC™ 425 System for the Nanoflow Test G

Table G-1 is a list of all of the parts required to perform the nanoflow test. All of the part numbers are SCIEX part numbers, unless otherwise indicated.

Table G-1 Parts List

Part Number	# Needed	Item	Description
FS 360-20-10-N-20 (New Objectives)	1	New Objectives nanoflow spray tips	For use with NanoSpray® ion source
910-00104	1	11 mm PP 250 µl vial (100/pkg)	Vials for proteomic analysis
910-00103	1	11 mm snap cap with slit septum (100/pkg)	Slit septum lids for vials
5016752	1	ChromXP nanoLC Trap column 350 µm id x 0.5 mm, ChromXP C18 3 µm 120Å (2/pkg)	Trap column good for proteomics at 2 µL/min flow rates. Use serial column workflow for higher performance, if required.
805-00120	1	ChromXP nanoLC column 75 µm id x 15 cm, ChromXP C18 3um 120Å[1]	Analytical column good for proteomics at 300 nL/min flow rates
205-00048	1	Tubing, 20 µm i.d., 1/32 inch o.d., 75 cm PEEKsil (natural)	To connect the analytical pump to the autosampler trap valve.
205-00049	3	Tubing, 50 µm i.d., 1/32 inch o.d., 75 cm PEEKsil (natural)	To connect the load pump to the autosampler injection valve.
205-00040	1	Tubing, 50 µm i.d., 1/32 inch o.d., 30 cm PEEKsil	To connect the autosampler injection valve to the autosampler trap valve.
200-00320	1	Tubing, FEP, 1/32 OK 5-foot roll	Waste tubing for autosampler trap valve.
5019621	1	1/32 inch PEEK nut with glass-filled PEEK ferrule (10/ pkg)	For PEEKsil tubing connections on all valves.

[1] If the exact column configuration is not available, then use an equivalent column length and diameter.

Prepare the PepCalMix LC-MS Solution for the Microflow Test H

Use this procedure to prepare the PepCalMix stock solution (1 pmol/µL) from the vial provided in the SWATH® Performance Kit.

1. When opening a new vial of the stock solution, aliquot the stock solution (1 pmol/µL concentration) into 5 µL to 10 µL volumes and then freeze for future use.
2. Add 49 µL of the dilution buffer (5% acetic acid : 2% acetonitrile) to a vial, and then add 1 µL of the PepCalMix stock solution to prepare a 20 fmol/µL solution.

   Note: Acetic acid is essential for the stability of the higher mass peptides and must not be substituted.

3. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
4. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.
5. Pipette the solution into an autosampler vial.

Note: A certificate of analysis (CoA) for the PepCalMix can be downloaded from the Web site http://sciex.com/tech-regulatory.

Prepare the SWATH® Acquisition QC Solution for the Microflow Test

Use this procedure to prepare a dilution that contains 1 µg/µL of Protein Extract Digest and 100 fmol/µL of the PepCalMix.

1. Add 9.6 µL of Buffer A (100% water: 0.1% formic acid) to the vial.
2. Add 12 µL of the Protein Extract Digest Stock solution to the vial.
3. Add 2.4 µL of the PepCalMix stock solution to the vial.
4. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
5. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.
6. Pipette the solution into an autosampler vial.

Prepare the IDA QC Solution for the Microflow Test J

Use this procedure to prepare a dilution that contains 1 µg/µL of Protein Extract Digest.

1. Add 10 µL of Buffer A (100% water: 0.1% formic acid) to the vial.
2. Add 10 µL of the Protein Extract Digest Stock solution to the vial.
3. Using a vortex mixer, mix the solution for a minimum of 30 seconds.
4. Using a centrifuge, spin the vial to bring the liquid to the bottom of the vial before opening.
5. Pipette the solution into an autosampler vial.

Parts List to Upgrade the NanoLC™ 400 System for the Microflow Test

K

Table K-1 is a list of all of the parts required to adapt the nanoflow configuration to a microflow configuration. The main parts are defined for optimal performance. However, options that could be substituted for added robustness, if required, are also defined in *italics*.

Table K-1 Parts List

Part Number	# Needed	Item	Description
5019593	1	ekspert NanoLC™ 400 column oven	For columns up to 25 cm. Ambient temperature from 5°C to 60°C.
5028230	1	Source Clamp	To attach the column oven to the source tower.
5017449	1	Column Mounting Kit	Rods and clamps to attach the column oven to the NanoLC 425 system.
5018237	1	ekspert NanoLC™ 400 gradient flow module for 1 µL/min to 10 µL/min	To adapt for microflow rates.
5028467	1	25 Micron ESI Electrode	To adapt the DuoSpray™ or the Turbo V™ ion source for microflow. Always have a spare on hand in case of plugging.
5028466	—	*50 Micron ESI Electrode*	***Optional:** Higher ID electrode for robustness.*
5022436	1	Column, 3 µm, ChromXP, C18CL, 120A, 150 × 0.3 mm	Good for proteomics at 5 µL/min flow rates.
5027467	1	Trap Cartridge holder with 1/32 inch peek fittings	For trap elute workflows.
5028897	1	0.3 mm trap cartridges, ChromXP, C18CL, 5 µm 120A (5/pkg)	For trap elute workflows.
205-00049	3	Tubing, 50 µm i.d., 1/32 inch o.d., 75 cm PEEKsil (natural)	To connect the load pump to the autosampler injection valve, the analytical pump to the autosampler trap valve, and the autosampler trap valve to the column for 5 µL/min flow rates.

Table K-1 Parts List (continued)

Part Number	# Needed	Item	Description
205-00091	1	Tubing, 25 µm i.d., 1/32 inch o.d., 10 cm PEEKsil	Post column connection to source. Always have a spare on hand in case of plugging.
205-00069	—	*Tubing, 50 µm i.d., 1/32 inch o.d., 10 cm PEEKsil*	***Optional:** Higher ID tubing for robustness.*
205-00069	2	Tubing, 50 µm i.d., 1/32 inch o.d., 10 cm PEEKsil	Attaches Trap cartridge to autosampler trap valve.
205-00040	1	Tubing, 50 µm i.d., 1/32 inch o.d., 30 cm PEEKsil	To attach the autosampler injection valve to the autosampler trap valve.
200-00320	1	Tubing, FEP, 1/32 OK 5-foot roll	Waste tubing for autosampler trap valve.
5019621	1	1/32 inch PEEK nut with glass-filled PEEK ferrule (10/ pkg)	For PEEKsil tubing connections on all valves.
910-00104	1	11 mm PP 250 µl vial (100/pkg)	Vials for proteomic analysis
910-00103	1	11 mm snap cap with slit septum (100/pkg)	Slit septum lids for vials

PeakView® Software XIC Table for the PepCalMix Test

L

Table L-1 XIC Table for the PepCalMix Text

Center	Width	Compound
408.55010	0.05	AETSELHTSLK
473.26020	0.05	GAYVEVTAK
485.25302	0.05	IGNEQGVSR
491.26559	0.05	LVGTPAEER
519.79969	0.05	LDSTSIPVAK
533.32333	0.05	AGLIVAEGVTK
540.27342	0.05	LGLDFDSFR
549.28633	0.05	GFTAYYIPR
569.83398	0.05	SGGLLWQLVR
583.31360	0.05	AVGANPEQLTR
593.80053	0.05	SAEGLDASASLR
613.34955	0.05	VFTPLEVDVAK
636.35273	0.05	VGNEIQYVALR
657.34499	0.05	YIELAPGVDNSK
677.85827	0.05	DGTFAVDGPGVIAK
739.36148	0.05	YDSINNTEVSGIR
758.91050	0.05	SPYVITGPGVVEYK
768.90340	0.05	ALENDIGVPSDATVK
883.47380	0.05	AVYFYAPQIPLYANK
964.97741	0.05	TVESLFPEEAETPGSAVR

SWATH® Acquisition Processing Using a Library

M

Note: For information about processing SWATH® data, refer to the https://training.sciex.com/Course.aspx?c=336 eLearning module.

1. Open the SWATH® Acquisition MicroApp, version 2.0, in the PeakView® software and then select **Quantitation > SWATH Processing > Import Ion Library**.
2. Change the file type to *.txt and then browse to the library (Human2D_PepCalMix SOP Library.txt).

   Tip! The library can be downloaded from http://sciex.com/community/entity/15722.

   Note: This is a small library only recommended for processing the SWATH® QC data. Use more in-depth libraries for processing biological studies.

3. Select the ion library and then browse to and select the SWATH® QC replicate data files that were acquired with SWATH® acquisition.
4. Click the **Retention Time Calibration Protein** in the protein list.

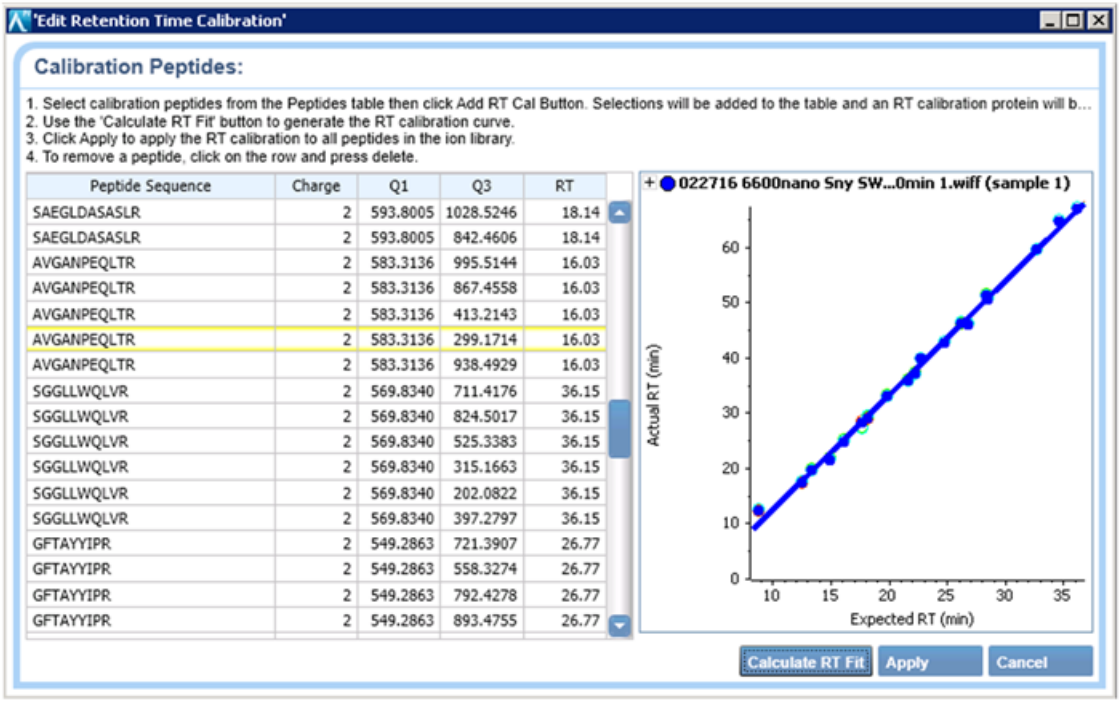
   The integrated peptides for the PepCalMix peptides that have been spiked in the sample are shown.

   Tip! Click through the peptides to confirm that the correct peptides are integrated in all cases.

5. Click the **Edit RT-Cal** icon ( ) to compute the retention time correction.
6. In the Edit Retention Time Calibration window, click the **Calculate RT Fit** button to compute the calibration curve. Refer to Figure M-1.

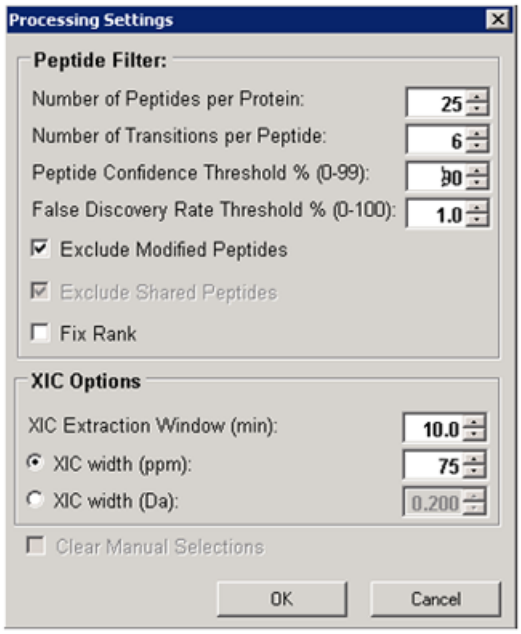
   Note: There is an individual curve computed for each datafile, as shown by the colored lines and points.

Figure M-1 Edit Retention Time Calibration Window



7. If there are any data points that are falling off the curve or were not detected in the samples, then highlight the individual peptide XIC row and click **Delete** to select the peptide.
8. Click **Apply** if everything looks correct.
9. Set the processing settings as shown in Figure M-2.

Figure M-2 Processing Settings Dialog



10. Click **OK**.
11. Click **Process** to complete the data processing of the replicate SWATH files.
12. After processing is complete, select **Quantitation > SWATH Processing > Export > Areas** to obtain the text files of peak areas for use with the SWATH Replicate Analysis Template.

SWATH® Replicates Analysis Template

N

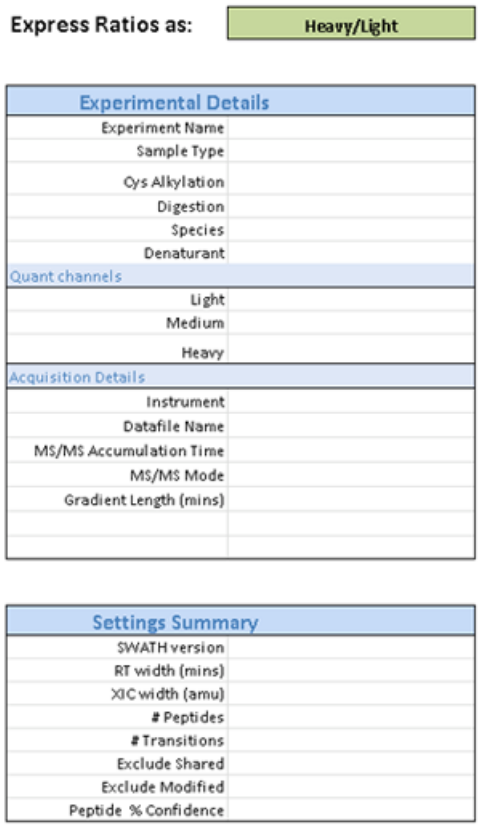
1. After processing is complete in the SWATH® Acquisition MicroApp, click **Quantitation > SWATH Processing > Export All > Areas** to export all of the peak areas to text files.
2. Open the *SWATH Replicates Template* on a high-performance computer.

Tip! The *SWATH Replicates Template 2.0* can be downloaded from sciex.com/software-downloads-x2110.

Note: The template is a very large processing spreadsheet that requires the 64-bit version of Microsoft Excel 2010 or higher. Instructions for using the template are included on the first worksheet (tab) of the document.

3. Type the metadata on the **INPUT-Metadata** tab, as shown in Figure N-1.
4. Paste the data from each of the tabs in the exported file in the equivalent input tabs in the template.

Figure N-1 Metadata



Express Ratios as:	Heavy/Light

Experimental Details	
Experiment Name	
Sample Type	
Cys Alkylation	
Digestion	
Species	
Denaturant	
Quant channels	
Light	
Medium	
Heavy	
Acquisition Details	
Instrument	
Datafile Name	
MS/MS Accumulation Time	
MS/MS Mode	
Gradient Length (mins)	

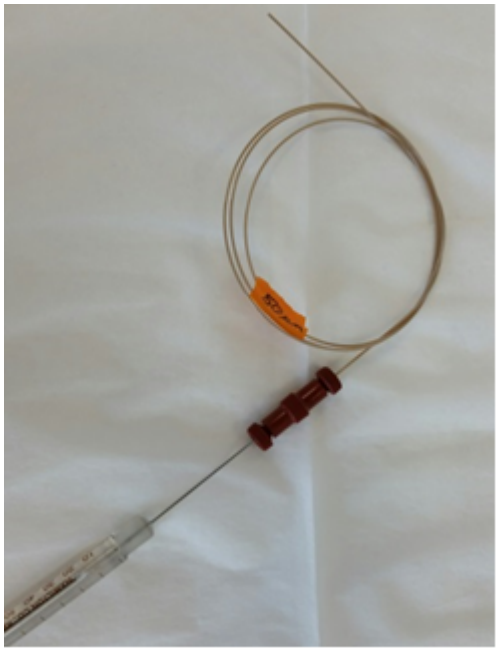
Settings Summary	
SWATH version	
RT width (mins)	
XIC width (amu)	
# Peptides	
# Transitions	
Exclude Shared	
Exclude Modified	
Peptide % Confidence	

Note: The default settings for the processing controls should be retained.

5. Press **F9** to process the spreadsheet.
6. After processing is completed, open the **Simple Summary** tab and inspect the key results to make sure that they are of the required quality.
7. If this data will be used to track performance over time, then capture the results from the **Results – Single Column** worksheet and paste into a new Excel file for future access.

Create a Syringe Line O

Figure O-1 Syringe Line



Part Number	# Required	Item	Action Required
205-00049	1	Tubing, 50 µm i.d., 1/32 inch o.d., 75 cm PEEKsil (natural)	Connect the syringe to the ion source.
200-00319	1	Union Microtight, 1/32, PEEK	Connect the syringe needle to the tubing.
1003968	1	Syringe, 100 µL	—

Reference Documents P

http://sciex.com/Documents/manuals/nanospray-operator-guide.pdf

http://www.absciex.com/Documents/Downloads/Literature/nanolc-system-integration-test-en.pdf

Human Ion Library: http://sciex.com/community/entity/15722

SWATH® Acquisition eLearning module: https://training.sciex.com/Course.aspx?c=336

Revision History

Revision	Reason for Change	Date
A	First release of document.	May 2016