SCIEX NanoLC 400 Systems

For Systems Configured for Microflow Flow Rates

System Integration Test

SCIEX

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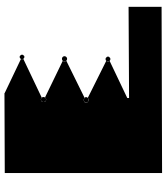

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Contents

Introduction 1

This document describes the steps for preparing and performing an LC/MS system integration test for the SCIEX NanoLC 400 system configured with a 5 µL/min to 50 µL/min flow module (PN 5018238), a 2.7 µm HALO fused-core C18 0.5 mm × 50 mm column (PN 805-10100) and one of the following SCIEX mass spectrometers:

- API 3200™ system, 3200 QTRAP® system
- API 4000™ system, 4000 QTRAP® system
- SCIEX Triple Quad™ 4500 system, QTRAP® 4500 system
- API 5000™ system
- SCIEX Triple Quad™ 5500 system, QTRAP® 5500 system
- SCIEX Triple Quad™ 6500 system, QTRAP® 6500 system
- TripleTOF® 4600 system, TripleTOF® 5600/5600+ system, and TripleTOF® 6600

Caution: Potential System Damage: Prior to calibrating the system, refer to the *Safety Guide* or *System User Guide* for detailed information on the safe use and operation of the system.

About this Test

Use this test as a measure of the SCIEX NanoLC 400 systems performance in isolation of the performance of the other components. Results from the test can become the baseline performance for the system and can be performed regularly and used as a system quality control test in the future.

Perform this test when the mass spectrometer is known to be operating well and meeting performance specifications. Repeat the test until you have consistent peak shape and peak intensity.

Approximate time required:

1. Sample preparation: 15 minutes
2. Create the methods and batch: 10 to 15 minutes
3. Equilibrate the system: 3 to 5 minutes
4. Perform the test: 30 minutes

Required Materials

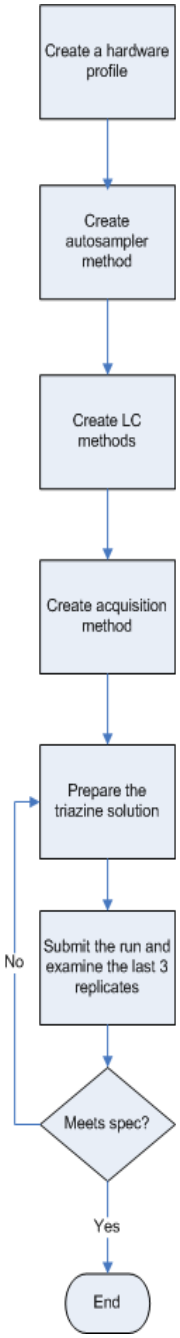
- 2.7 µm HALO fused-core C18 0.5 mm × 50 mm column (PN 805-10100) (refer to Appendix E: Plumbing Diagram for fittings and tubing)
- Triazine System Suitability Solution (PN 4376887)


SCIEX

System Integration Test 2

Complete the steps in the test as shown in Figure 2-1.

Figure 2-1 System Integration Test Tasks



Create the Hardware Profile

1. Click **New Profile** to open the **Create New Hardware Profile** dialog.
2. Type a name for the profile in the **Profile Name** field.
3. Add the autosampler.
    a. In the **Device Type** list, click **Software Application**.
    b. Click **Software Application <not configured>**.
    c. Click **OK**.
    d. Click **Setup Device**.
    e. In the **Name** field, click the autosampler.
    f. Click **OK**.
4. Click **Add Device** to add the Eksigent control software.
    a. In the **Device Type** list, click **Software Application**, and then click **Software Application <not configured>**.
    b. Click **OK**.
    c. Click **Setup Device** to open the **Software Application Settings** dialog.
    d. Click **Eksigent 1** and then click **OK**.
    e. Repeat step a to step d for the Loading pump.
    f. For a NanoLC 425 system, repeat step a to step d for Gradient 2.
5. Click **Add Device** to add the mass spectrometer.
    a. In the **Device Type** list, click **Mass Spectrometer**.
    b. Click the appropriate mass spectrometer in the list and then click **OK**.

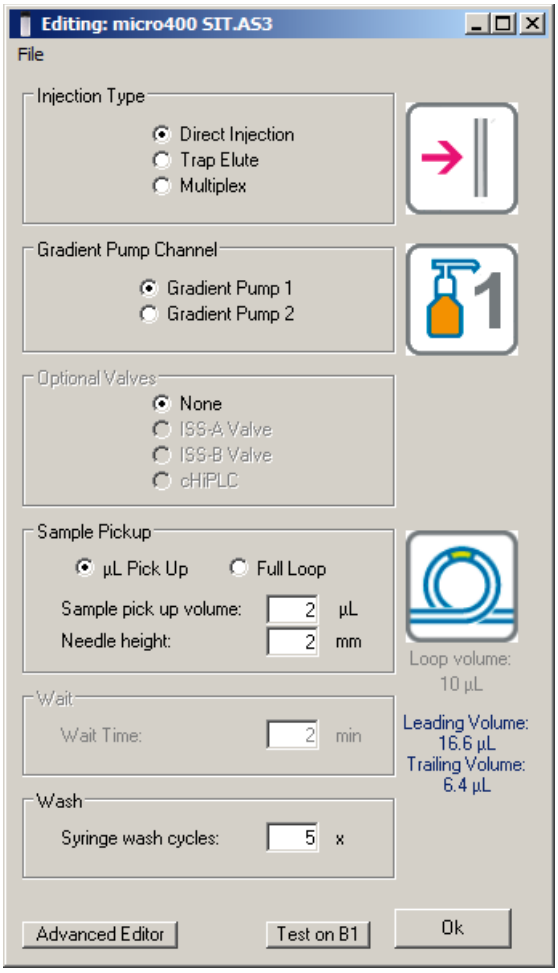
Tip! The correct instrument is usually highlighted in the list.

6. Click **OK** to save the profile and close the **Create New Hardware Profile** dialog.
7. Activate the hardware profile.

Create the Autosampler Method

1. Plumb the autosampler valve with a 10 µL sample loop.
2. In the **Autosampler** control window that opens when Analyst is opened, click **Method Editor**.
3. Create the autosampler method for a direct-injection configuration as shown in Figure 2-2 on page 9.

Figure 2-2 Method Editor Dialog



4. Click **Advanced Editor**.
5. Specify the method parameters as shown in Table 2-1 on page 10.

Table 2-1 Advanced Editor Autosampler Method Parameters

Command	Description
Initialize	Autosampler Device
Needle Wash	Pre-wash - 1x (using Wash Solvent 1)
Wait	for Gradient 1 ready to start
Get Sample	µL Pickup - 2 µL - 0.5 µL/sec - 2 mm from bottom
Start	Gradient 1
Valve	Injector Inject
Wait	for Gradient 1 injection complete
Valve	Injector Load
Needle Wash	Clean Up - 5 x (using Wash Solvent 1)

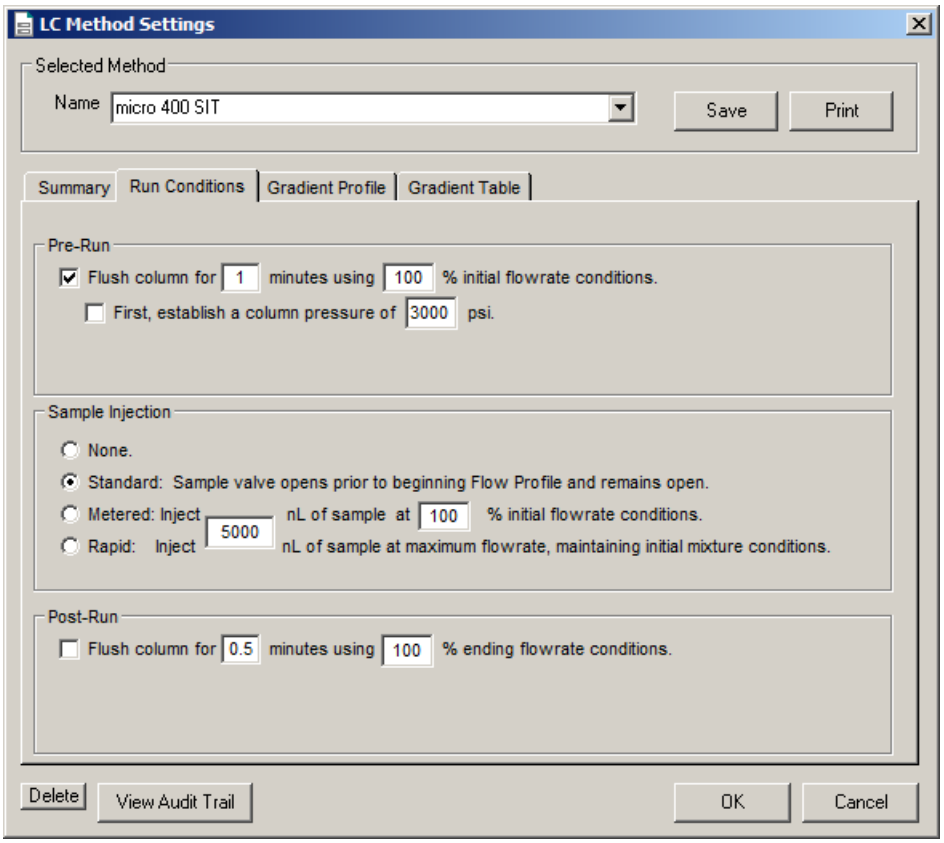
6. Save the autosampler method.
    a. Click **File > Save As**.
    b. Type the name for the method and click **Save**.

Create the LC Method

For the analytical gradient (typically on the Gradient 1 pump with the microflow module), create the gradient method.

1. In the **Acquisition** window that opens (from the Eksigent control software), click the arrow in the top, right corner of the window to select the microflow or gradient pump.
2. Click **LC Methods**.
3. On the **Run Conditions** tab, specify the gradient method as shown in Figure 2-3 on page 11.

Figure 2-3 LC Method Settings Dialog—Run Conditions Tab (External Column)



4. On the **Gradient Table** tab:
   a. Type **10 µL/min** for **Total flowrate**.
   b. Specify the gradient method as shown in Table 2-2.

Table 2-2 Gradient Method Parameters (Gradient Table Tab)

Step	Time	% A	% B	Event
1	0	80	20	
2	1	10	90	
3	2	10	90	
4	2.1	80	20	
5	4	80	20	

5. Type the method name and click **Save**.
6. Click **OK**.

Create the MS Methods

Create the mass spectrometer method. Refer to the appropriate appendix for the system:

- MS Method Information for the 3200 Series of Instruments on page 35
- MS Method Information for the 4000 and 4500 Series of Instruments on page 37
- MS Method Information for the 5000, 5500, and 6500 Series of Instruments on page 39
- For the TripleTOF 4600, 5600/5600+, and 6600 systems, refer to Table 2-4 on page 14.

Create the Acquisition Method

Create the Acquisition Method for Triple Quadrupole and QTRAP® Systems

1. Close the Eksigent control software, if it is open.
2. Verify that the Analyst drivers are installed. Refer to the appropriate installation document for details.
3. Create a hardware profile.
4. Create the acquisition method.

   The values in Table 2-3 on page 12 are a starting point. The method should be updated to reflect optimized values.

Note: The acquisition time should be at least 30 seconds shorter than the LC run time. In the methods created below, the acquisition time is 1 minute shorter than the LC run time.

Table 2-3 Analyst Software Acquisition Method Parameters—Triple Quadrupole and QTRAP® Systems

Parameter	Value
MS	
Scan Type	MRM Scan
Dwell time	50 msec
Polarity	Positive

Table 2-3 Analyst Software Acquisition Method Parameters—Triple Quadrupole and QTRAP® Systems (Continued)

Parameter	Value
Q1/Q3 Masses and CE	Refer to the appropriate table: • Table A-1 *MRM Transitions for the API 3200™ and 3200 QTRAP® Systems* on page 35 • Table B-1 *MRM Transitions for 4000 and 4500 Systems* on page 37 • Table C-1 *MRM Transitions for API 5000™, Triple Quad™ 5500/6500, and QTRAP® 5500/6500 Systems* on page 39
Acquisition time	3.5 min
Advanced MS	
Q1 Resolution	Unit
Q3 Resolution	Unit
Source/Gas**	
Curtain Gas (CUR)	20 to 25
CAD Gas	HIGH Feedback should read between 2.1 x 10^5 and 2.4 x 10^5
IonSpray Voltage (IS)***	5000 V
Ion Source Gas 1 (GS1)	20
Ion Source Gas 2 (GS2)	20
TIS Heater	350°C
Compound	
Declustering Potential (DP)	As optimized for the mass spectrometer (for example, 51)
Entrance Potential (EP)	Refer to the appropriate table:
Collision Exit Potential (CXP)	• Table A-2 *Values for EP and CXP for the API 3200 and 3200 QTRAP Systems* on page 35 • Table B-2 *Values for EP and CXP for 4000 and 4500 Systems* on page 38 • Table C-2 *Values for EP and CXP for 5000, 5500, and 6500 Systems* on page 40

** Source/Gas parameters may vary between systems. Determine the best value for the system.

*** Grounding kit (PN 5016941) is required.

5. Enter the MRM transitions.

   Refer to the appropriate table:

- Table A-1 *MRM Transitions for the API 3200™ and 3200 QTRAP® Systems* on page 35
- Table B-1 *MRM Transitions for 4000 and 4500 Systems* on page 37
- Table C-1 *MRM Transitions for API 5000™, Triple Quad™ 5500/6500, and QTRAP® 5500/6500 Systems* on page 39

Note: In the Analyst MRM transition table, verify that the additional CE (collision energy) column is added to the table view by right-clicking the table and selecting CE from the menu. For CE values, refer to the appropriate appendix for the mass spectrometer.

Create the Acquisition Method for TripleTOF® Systems

1. Close the Eksigent control software, if it is open.
2. Verify that the Analyst drivers are installed. Refer to the appropriate installation document for details.
3. Create a hardware profile.
4. Create the acquisition method.

   The values in Table 2-4 on page 14 are a starting point. The method should be updated to reflect optimized values.

Note: The acquisition time should be at least 30 seconds shorter than the LC run time. In the methods created below, the acquisition time is 1 minute shorter than the LC run time.

Table 2-4 Analyst Software Acquisition Method Parameters—TripleTOF® Systems

Parameter	Value
MS	
Scan Type	TOF MS Scan
Accumulation time	150 msec
Polarity	Positive
Acquisition time	3 min
Advanced MS	
Q1 Resolution	Unit
Source/Gas**	
Curtain Gas (CUR)	20 to 25
CAD Gas	HIGH
IonSpray Voltage Floating (ISF)	5000 V Q1 Vacuum gauge ~3 x 10^{-5} Torr
Ion Source Gas 1 (GS1)	60

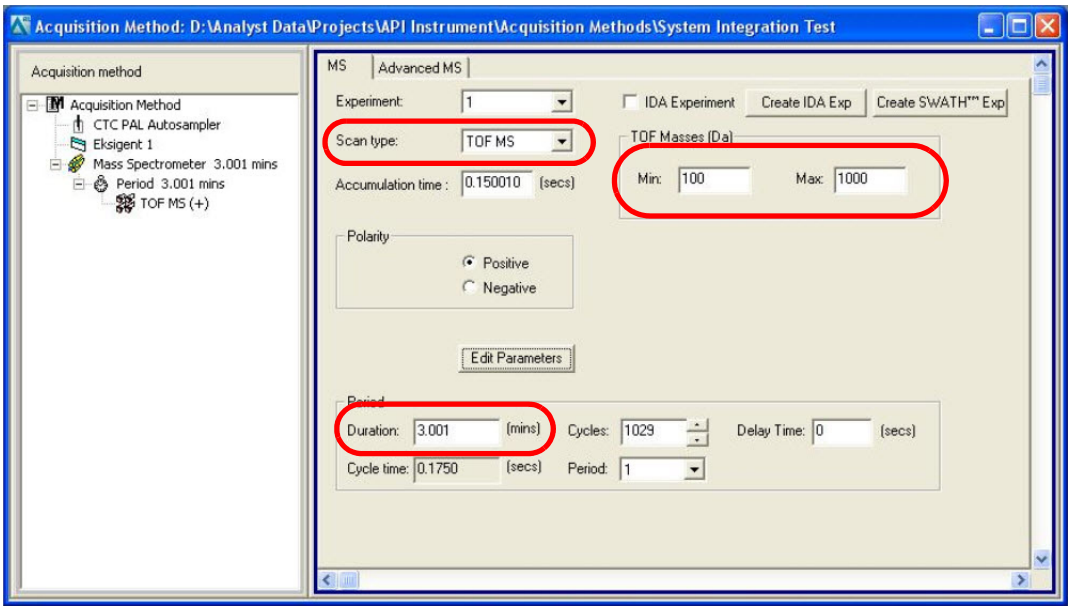
Table 2-4 Analyst Software Acquisition Method Parameters—TripleTOF® Systems (Continued)

Parameter	Value
Ion Source Gas 2 (GS2)	30
TIS Heater	350°C
Compound	
Declustering Potential (DP)	As optimized for the mass spectrometer (for example, 51)
Collision Energy (CE)	10

** Source/Gas parameters may vary between systems. Determine the best value for the system.

5. Enter information in the **MS** tab (Figure 2-4) to create the TOF MS scan.
   a. In the **Scan** type list, select **TOF MS**.
   b. Type **0.150010** in the **Accumulation time** field.
   c. In the **TOF Masses (Da)** section, type **100** and **1000** for the **Min:** and **Max:** masses.
   d. In the **Duration** field, type **3.001** minutes.

Figure 2-4 Acquisition Method MS Tab—Parameters for TOF MS Scan

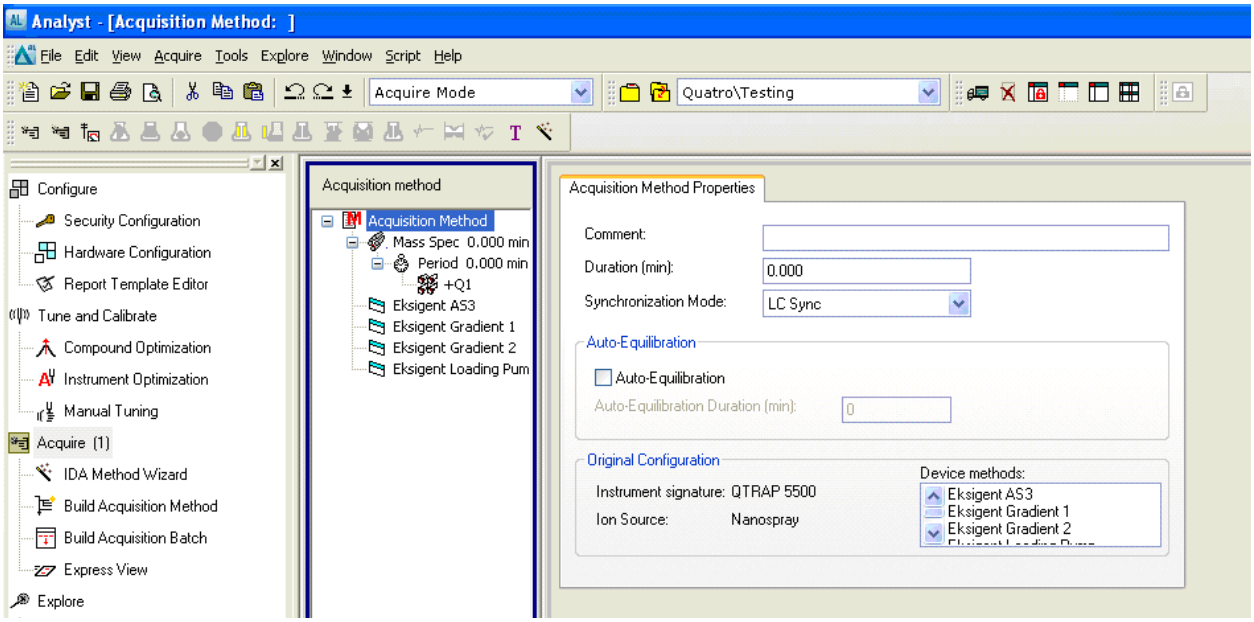


6. Save the Analyst method (for example, System Integration Test).

Add LC Information to the Acquisition Method

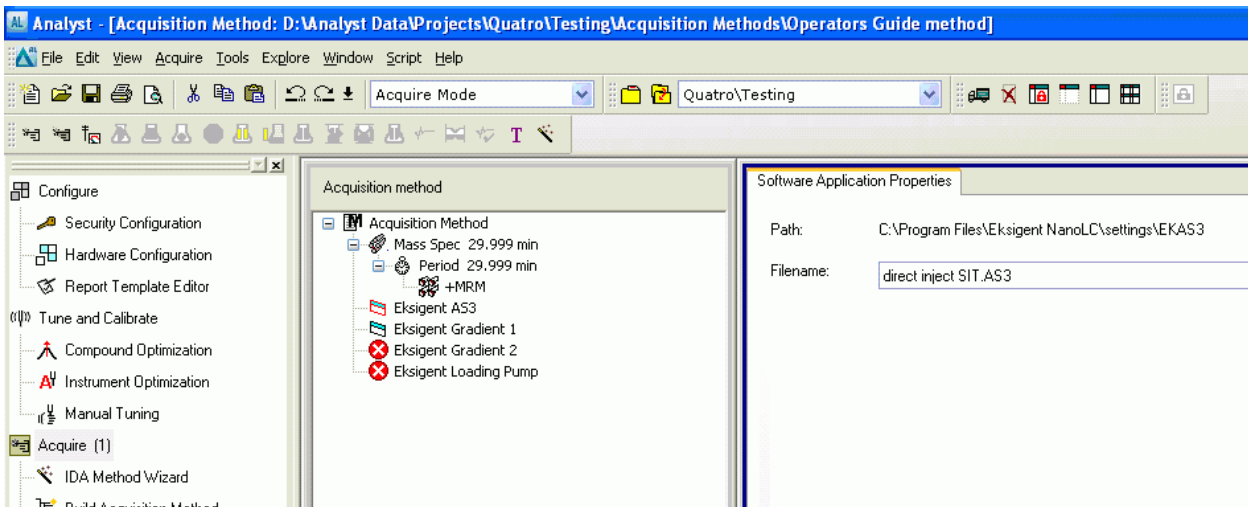
1. Click **Acquisition Method** in the left pane, and then click **LC Sync** as the **Synchronization Mode**.

Figure 2-5 Acquisition Method Properties Tab—Synchronization Mode



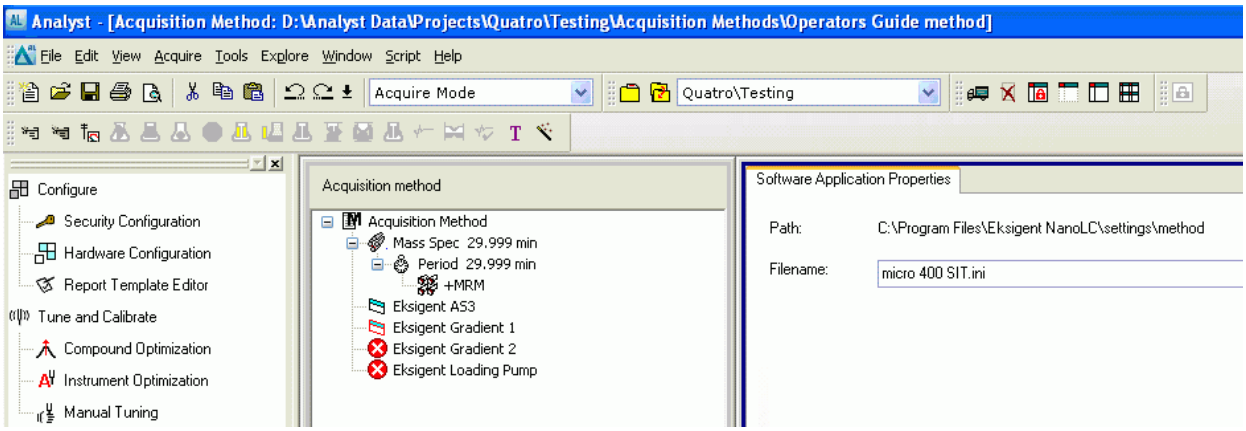
2. Click the autosampler (for example, Eksigent AS3) and then select the appropriate autosampler method. Refer to Create the Autosampler Method on page 8.
3. Right-click **Eksigent Gradient 2** and clear the “Use” selection as it is not being used.
4. Right-click **Eksigent Loading Pump** and clear the “Use” selection as it is not being used.

Figure 2-6 Software Application Properties Tab—Autosampler Method



5. Click **Eksigent Gradient 1** and then select the appropriate gradient pump method. Refer to Create the LC Method on page 10.

Figure 2-7 Software Application Properties Tab—Gradient 1 Method



6. Save the Analyst method.

Prepare the Sample

Prepare the sample for the test. Refer to the “Sample Preparation” section in the appropriate appendix for the system:

- MS Method Information for the 3200 Series of Instruments on page 35
- MS Method Information for the 4000 and 4500 Series of Instruments on page 37
- MS Method Information for the 5000, 5500, and 6500 Series of Instruments on page 39
- Sample Preparation for TripleTOF® Systems on page 41

Equilibrate the System

Make sure that the LC column is connected.

1. Verify the system is plumbed correctly (refer to Figure E-1 on page 43).
2. Verify the following mobile phases are loaded on the system.

Table 2-5 Buffer Mixtures for the System Integration Test

Buffer	Mixture	Channel
Buffer A	100% water:0.1% formic acid	Channel A
Buffer B	100% acetonitrile:0.1% formic acid	Channel B

3. In the Analyst software, equilibrate the mass spectrometer.
   a. On the **Navigation** bar, click **Acquire**.
   b. Click **View > Sample Queue**.
   c. Click **Acquire > Equilibrate**.
      The **Equilibrate** dialog box appears.
   d. Select the **Acquisition Method** created on page 12.

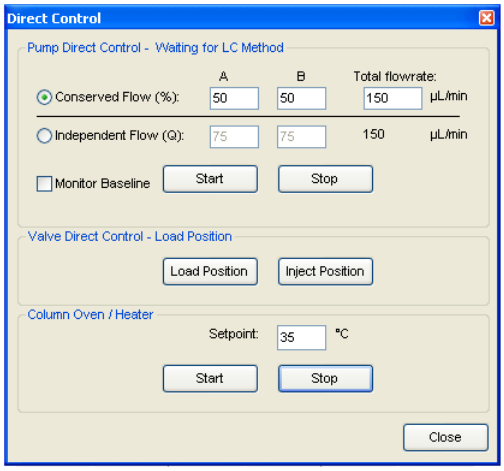
e. To equilibrate, type **1** in the **Time [Min.]** field and then click **OK**.

Note: Selecting the Auto-Equilibration option on the Acquisition Method Properties tab in a Data Acquisition Method results in an automatic equilibration when that method is run in a batch.

4. In the Eksigent control software, click **System > Direct Control**.
5. Select the **Conserved Flow** option and set both **A** (%) and **B** (%) to **50**.

   This is the mobile phase composition used for equilibration.
6. Type the **Total flowrate** of **40** µL/min.

Figure 2-8 Direct Control Dialog



7. If a column oven is installed, type the **Setpoint** of **35°C**, and then click **Start**.
8. Click **Start** to begin equilibration.
9. Allow the system to equilibrate for approximately 3 minutes.

Note: The column heater comes to temperature quickly, but the column itself can take as long as 30 minutes to fully equilibrate.

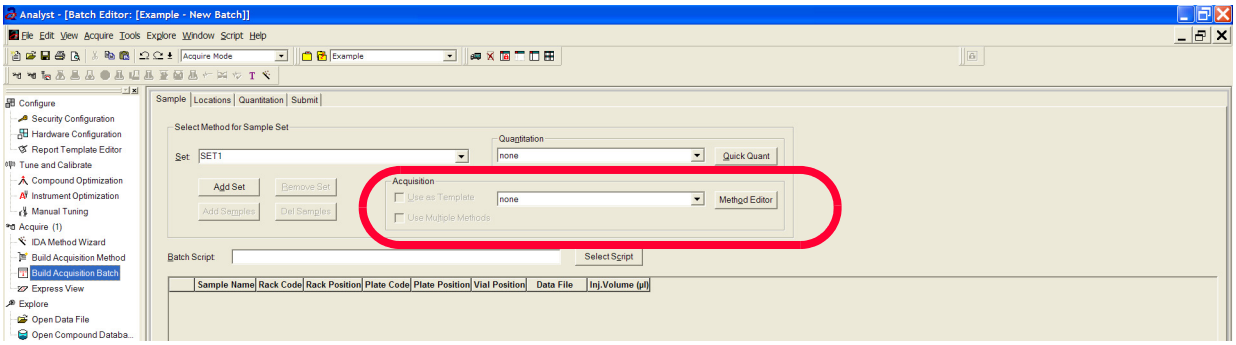
Perform the System Integration Test

Create the LC/MS acquisition batch, run the batch and then verify the results. Record the test results in Chapter 3: System Integration Test Data Log and Signoff.

Create the LC/MS Acquisition Batch

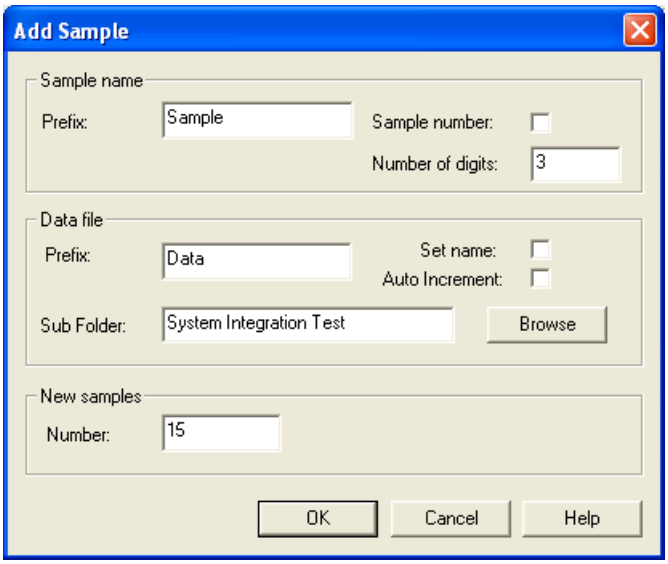
1. Double-click **Build Acquisition Batch** in left **Navigation** bar.
2. Build the acquisition batch.
   a. On the **Sample** tab, in the **Acquisition** group, select the acquisition method created above from the list.

Figure 2-9 Sample Tab—Acquisition Group



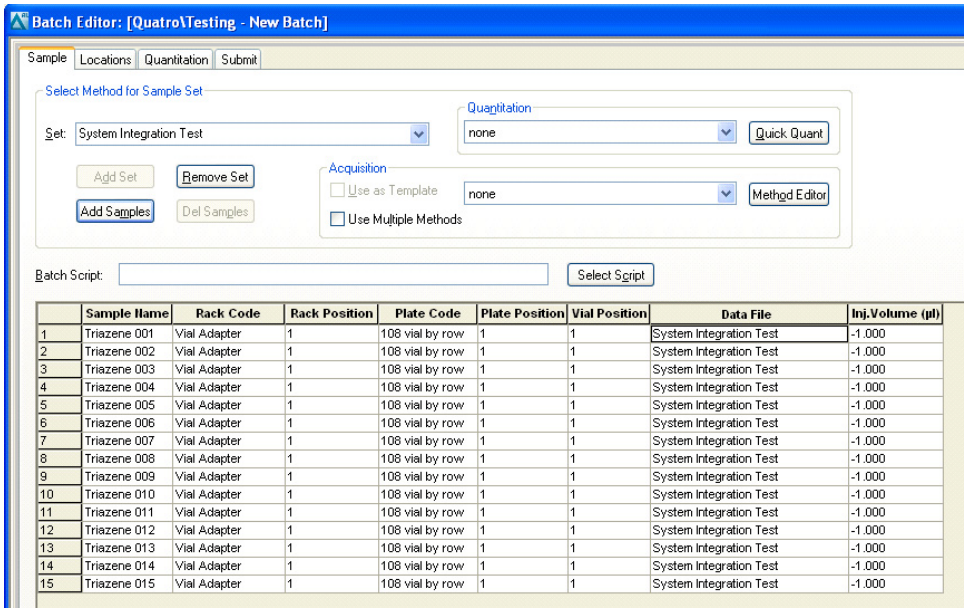
b. Click **Add Set**, and then click **Add Samples**.

Figure 2-10 Sample Dialog



3. Click **OK**.

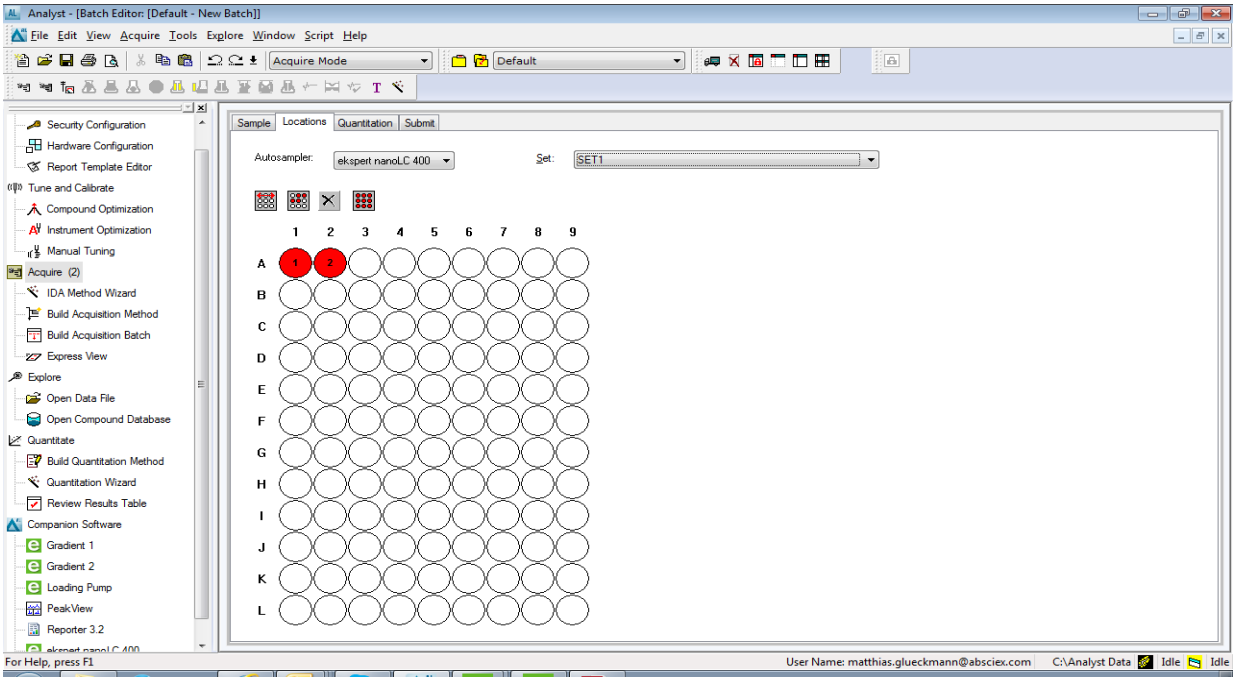
Figure 2-11 Sample Tab—Method Creation



	Sample Name	Rack Code	Rack Position	Plate Code	Plate Position	Vial Position	Data File	Inj.Volume (µl)
1	Triazene 001	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
2	Triazene 002	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
3	Triazene 003	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
4	Triazene 004	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
5	Triazene 005	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
6	Triazene 006	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
7	Triazene 007	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
8	Triazene 008	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
9	Triazene 009	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
10	Triazene 010	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
11	Triazene 011	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
12	Triazene 012	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
13	Triazene 013	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
14	Triazene 014	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000
15	Triazene 015	Vial Adapter	1	108 vial by row	1	1	System Integration Test	-1.000

4. Create the batch as shown above.
5. Save the data file as LC Triazine Integration Test <date>.
6. On the **Location** tab, specify the triazine sample vial position in the batch.

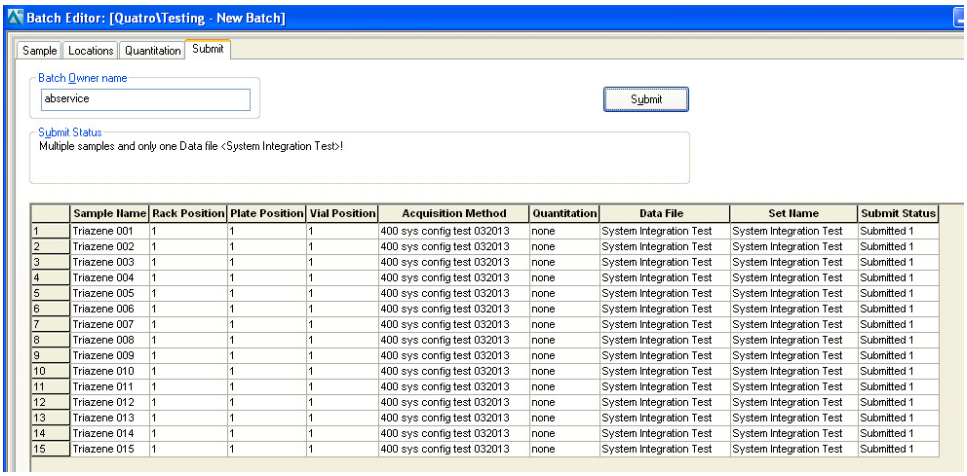
Figure 2-12 Locations Tab



Run the Batch

1. On the **Submit** tab, click **Submit**.

Figure 2-13 Submit Tab



	Sample Name	Rack Position	Plate Position	Vial Position	Acquisition Method	Quantitation	Data File	Set Name	Submit Status
1	Triazene 001	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
2	Triazene 002	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
3	Triazene 003	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
4	Triazene 004	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
5	Triazene 005	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
6	Triazene 006	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
7	Triazene 007	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
8	Triazene 008	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
9	Triazene 009	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
10	Triazene 010	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
11	Triazene 011	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
12	Triazene 012	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
13	Triazene 013	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
14	Triazene 014	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
15	Triazene 015	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1

2. In the **View** menu, click **Sample Queue**.
3. In the **Acquire** menu, click **Start Sample**.

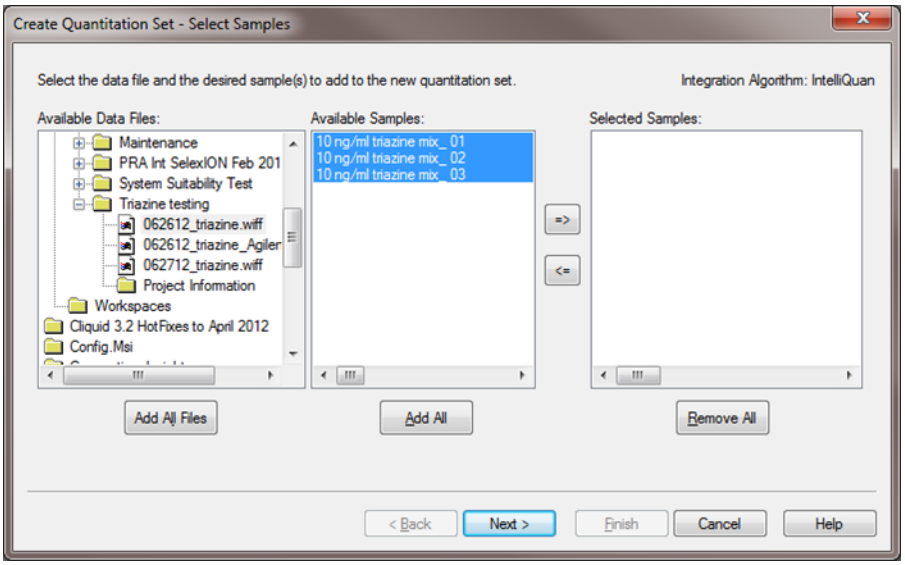
Verify the Results

Verify the Results for Triple Quadrupole and QTRAP® Systems

View the Results and Verify the Integration

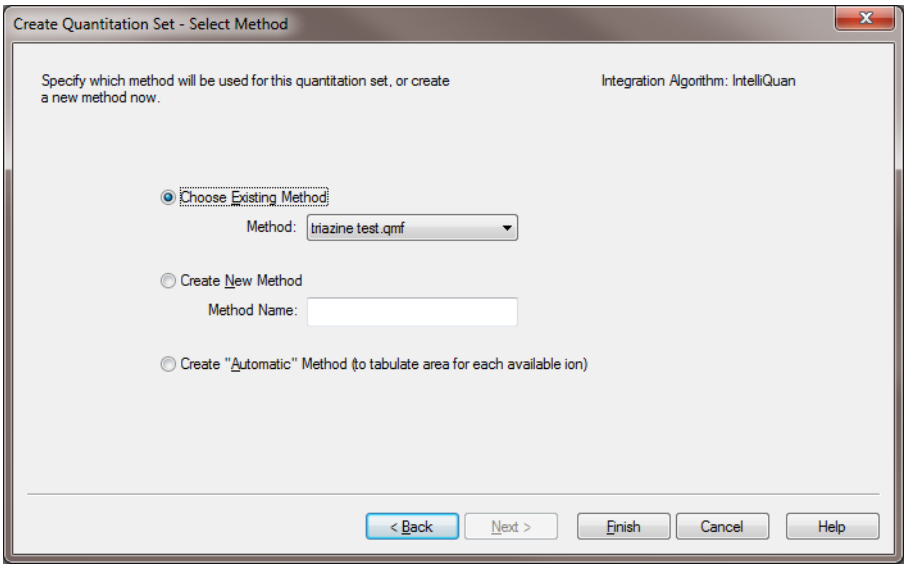
1. When the run is finished, double-click **Quantitation Wizard** in the **Quantitation** menu.
2. Select the data file **System Integration Test <date>**.
3. Click **Add All** to move the last three injections to the **Selected Samples** list (Figure 2-14). If a conditioning run was submitted, make sure that the injections selected are from the test run.

Figure 2-14 Quantitation Wizard—Select Samples Page



4. Click **Next** and **Next** on the following screen.
5. In the Select Method page, select **Choose Existing Method** and **triazine test** in the **Method** list, and then click **Finish** (Figure 2-15.)

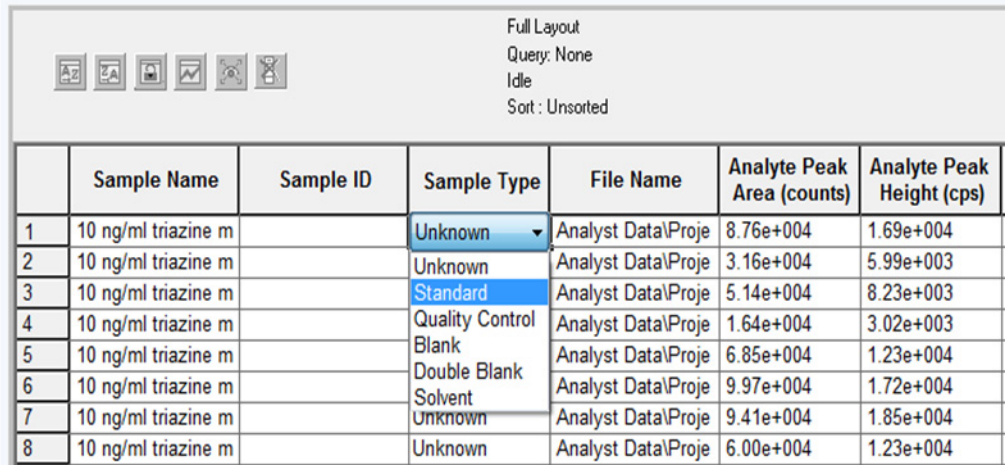
Figure 2-15 Quantitation Wizard—Select Methods Page



The results table opens.

6. Change the sample type to Standard.
    a. On the first line, change the **Sample Type** to **Standard** with the menu (Figure 2-16).

Figure 2-16 Results Table—Changing the Sample Type



b. Right-click on Sample Type and select Fill Down.

7. Right-click in the margin above the results table and select **Analyte > Ametryn 1**.

   Only the results for Ametryn 1 are shown.

8. Select **Tools > Peak Review > Pane**.

   The chromatograms for the MRM transition are displayed below the results table.

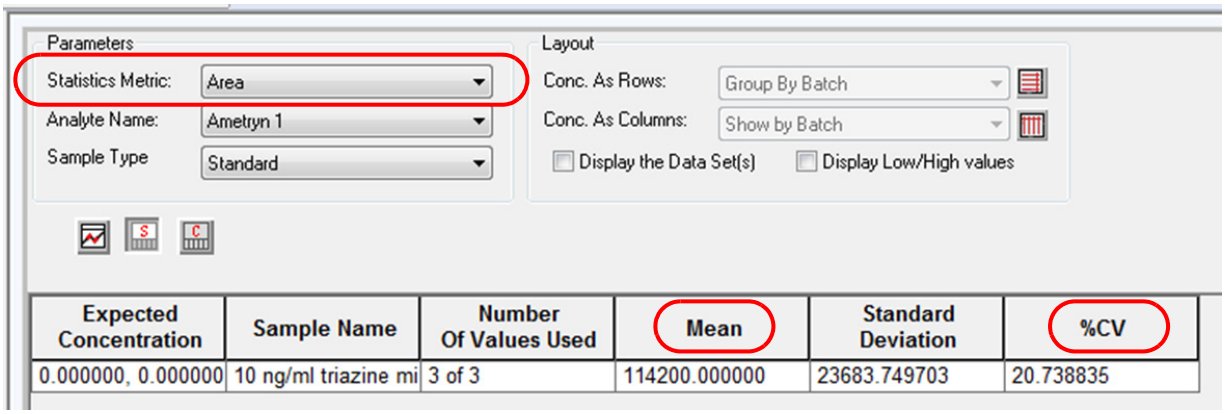
9. Click  in the chromatogram pane to view the integration for each chromatogram.

10. After reviewing the integration, repeat step 7 through step 9 for the following transitions: Atrazine 1, Simazine 1, and Terbutryn 1.

Verify the Mean Area and %CV

1. After reviewing the integration, go to the **Tools** menu and select **Statistics**.
2. In the **Statistics Metric** list, select **Area**.
3. For each of the four MRM transitions:
   - Compare the **Mean** and **%CV** (Figure 2-17) values to the specifications in Chapter 3: System Integration Test Data Log and Signoff.
   - Record the values in Chapter 3: System Integration Test Data Log and Signoff.
4. Delete the statistics pane.

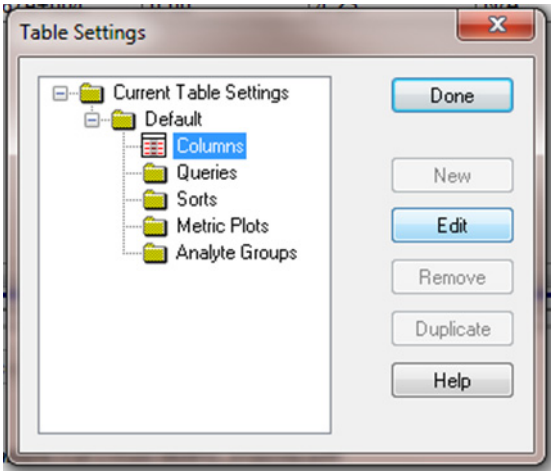
Figure 2-17 Statistics Summary Pane



Verify Peak Widths at Half Height and Retention Times

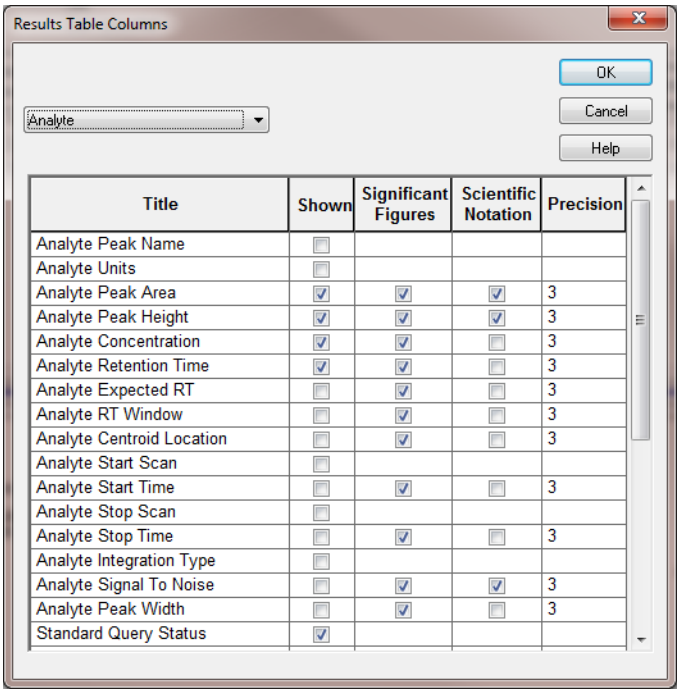
1. Open the **Results Table Columns** dialog.
   a. Right-click on the area above the results table and select **Table Settings > Edit**.
   b. In the **Table Settings** dialog, click **Columns** and then click **Edit**.

Figure 2-18 Table Settings Dialog



2. In the **Results Table Columns** dialog, select the columns for the table.

Figure 2-19 Results Table Columns Dialog



   a. Select **Analyte** in the list.
   b. In the table, select **Analyte Retention Time** and **Analyte Peak Width at 50% Height**.
   c. Make sure that **Analyte Peak Area**, **Analyte Peak Height**, and **Analyte Concentration** are also selected (Figure 2-19).
   d. Click **OK** to close the Results Table dialog.

3. Click **Done** to close the **Table Settings** dialog.

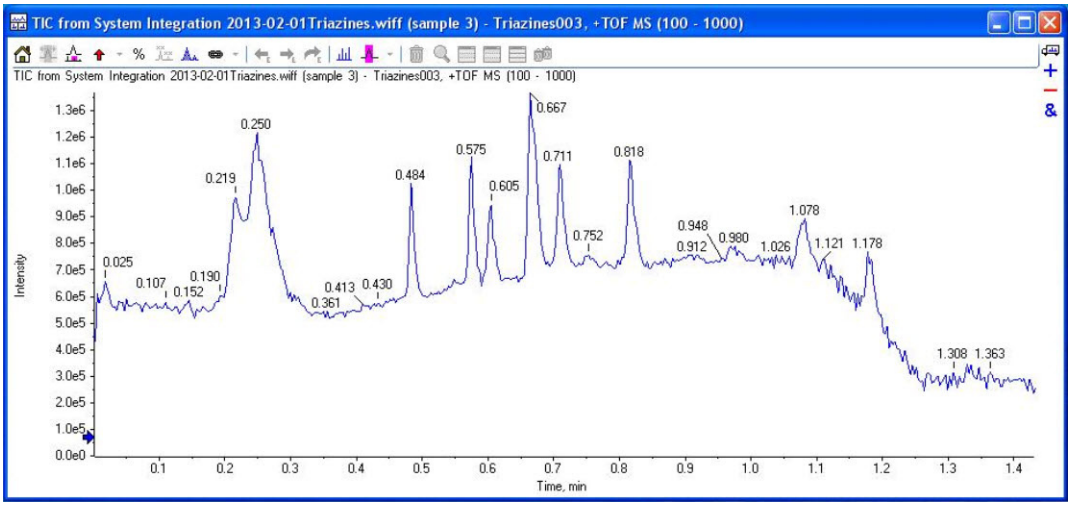
   The results table updates to display the selected columns.

4. For each MRM transition:
   - Compare the experimental values with the specifications in Chapter 3: System Integration Test Data Log and Signoff.
   - Record the values in Chapter 3: System Integration Test Data Log and Signoff.

Verify the Results for TripleTOF® Systems

1. When the run is finished, open the file and display the TIC for the experiment.
   a. Double-click **Peak View** to launch the **Peak View** tool.
   b. Select **File > Open Wiff Sample** to open the **Select Sample** dialog.
   c. Click the file and click **OK**.
      The TIC appears (Figure 2-20).

Figure 2-20 Example TIC for the TOF MS Scan



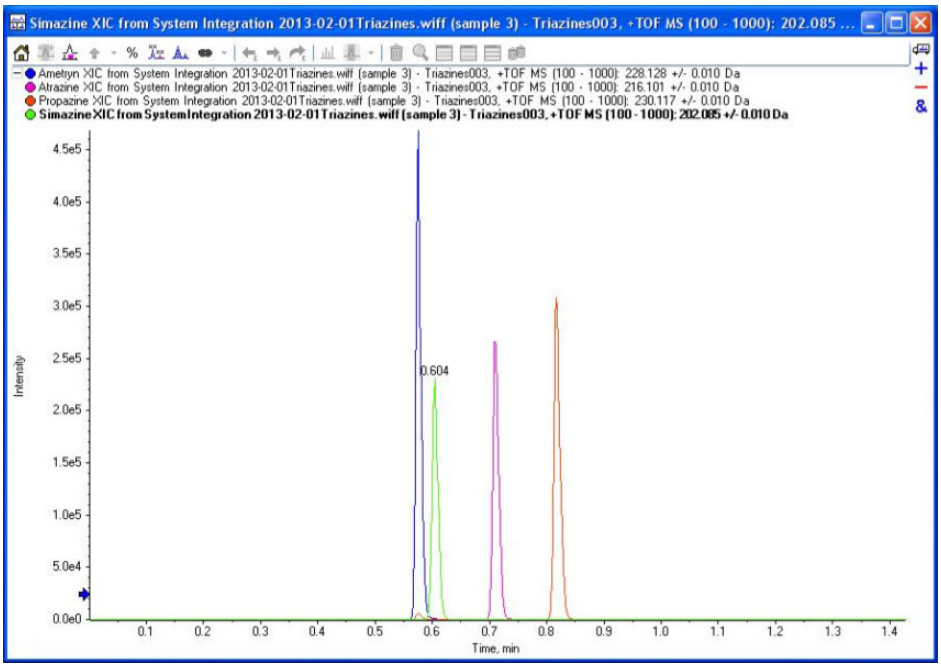
2. Create the XIC.
   a. Select **Show > Show Extract Ions Using Dialog**.
   b. In the **Specify XIC Ranges** dialog, type in the information from Table 2-6 and click **OK**.

Table 2-6 XICs to Extract for TripleTOF® Systems

Center	Width	Compound
C9H17N5S	0.2	Ametryn
C8H14ClN5	0.2	Atrazine
C9H16ClN5	0.2	Propazine
C7H12ClN5	0.2	Simazine

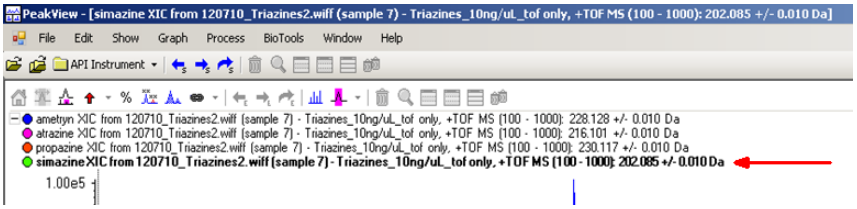
The XICs appear (Figure 2-21).

Figure 2-21 XICs for the TOF MS Scan Precursor Ions



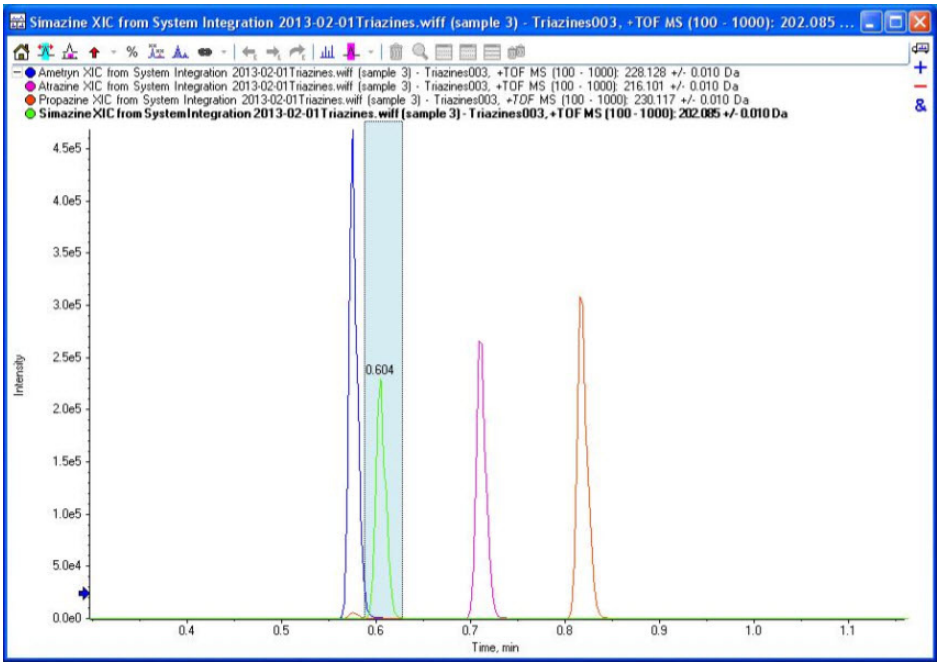
3. For each analyte in the plot, display the peak area and the peak width at half-height.
   a. Select the analyte of interest by clicking on the appropriate line (Figure 2-22).

Figure 2-22 Selecting the Analyte



   b. Click and drag to select the peak in the plot (Figure 2-23).

Figure 2-23 Selecting the Peak

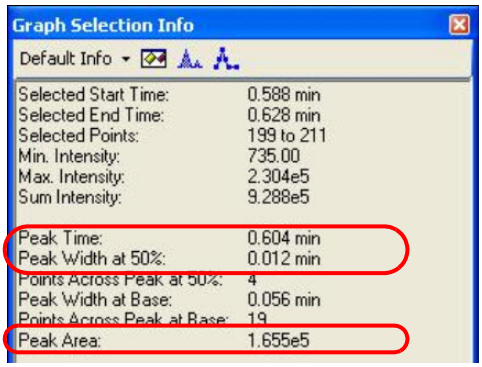


c. Select **Window > Graph Selection Window**.

d. The **Graph Selection** window opens.

e. Record **Peak Time**, **Peak Width at 50%**, and **Peak Area** (see figure below).

Figure 2-24 Graph Selection Info Window Showing Peak Information



4. Repeat step 3 on page 27 for the other two samples.
5. Calculate averages and %CV for each analyte.
6. For each analyte:
   - Compare the experimental values with the specifications in the *Installation Checklist and Data Log* document.
   - Record the values in the *Installation Checklist and Data Log* document.

Troubleshoot Peak Problems

This section provides information for troubleshooting peak related problems such as broad or tailing peak widths, lack of separation between peaks, and low peak area.

Peak widths are too broad or are tailing

- Inspect all connections in the flow path to verify that there are no dead volumes.
- Look at connections post-column.

No separation between the peaks

- Make sure that pump is delivering the correct amount of solvent.
- Make sure that the pressure drop upon injection is not too severe.
- Large pressure drop upon injection suggests an air bubble has been introduced to the sample loop.

Note: The overall separation with trap and elute will often be less than direct injection. Components that elute comparably on the trap and analytical column will not re-resolve on the analytical column and, as a result, spread out or bunch together.

Peak intensity or peak area is too low

- Verify that the correct amount of sample has been withdrawn from the autosampler vial by performing an aspiration test.
- Verify that the analytical column is well conditioned before performing this test.


SCIEX

Test Results

Complete this table with the results for the four analytes from the triazine solution. Refer to Table 3-2 for the mean area specification.

Mass Spectrometer:

Table 3-1 Test Results

Analyte	Mean Area (Counts)	% CV (Counts)	Mean Retention Time	% CV (Retention Time)	Mean Peak Width at Half-Height
Ametryn 1					
Atrazine 1					
Simazine 1					
Terbutryn 1 or Propazine					
				Guideline Met?	

Notes

Specifications

Mean Area Specification

Table 3-2 Instrument Response (cps)

Analyte	3200 10 ng/mL	4000/4500 1 ng/mL	5000/5500/6500 0.1 ng/mL	4600/5600/6600 10 ng/mL
Ametryn 1	8.0 e4	7.5 e4	3.5 e4	7.5 e4
Atrazine 1	1.5 e4	6.5 e4	3.0 e4	6.5 e4
Simazine 1	2.0 e4	2.5 e4	2.0 e4	4.0 e4
Propazine	N/A	N/A	N/A	7.5 e4
Terbutryn 1	1.0 e4	1.0 e4	5.0 e4	N/A

% CV Specification

All analyte areas should have a %CV of <15% based on replicate injections.

Retention Time Specification

Chromatographic peaks for the four analytes should be <0.030 minutes in width when measured at half maximum (peak width at half maximum).

For each of the four analytes, retention times of consecutive runs (n=3) should have a %CV of <3%.

Signoff

Contact name		Date (yyyy-mm-dd)
Contact signature*		
FSE name		Date (yyyy-mm-dd)
FSE signature*		

*Signature required on hard copy only.

Customer Familiarization Checklist

4

Demonstrate and assist the customer, hands on, to complete the tasks in the following table. Refer to the corresponding procedure earlier in the document for details.

Table 4-1 System Acceptance Test Demonstration

Topic	Resource	Complete
How to create autosampler methods	Create the Autosampler Method on page 8	
How to create LC methods	Create the LC Method on page 10	
How to create Analyst methods	Refer to the appropriate page: • MS Method Information for the 3200 Series of Instruments on page 35 • MS Method Information for the 4000 and 4500 Series of Instruments on page 37 • MS Method Information for the 5000, 5500, and 6500 Series of Instruments on page 39 • For TripleTOF® systems, refer to Table 2-4 on page 14	
How to create a sample batch and queue	Create the LC/MS Acquisition Batch on page 18	
How to prepare samples	Refer to the appropriate appendix: • MS Method Information for the 3200 Series of Instruments on page 35 • MS Method Information for the 4000 and 4500 Series of Instruments on page 37 • MS Method Information for the 5000, 5500, and 6500 Series of Instruments on page 39 • Sample Preparation for TripleTOF® Systems on page 41	
Analysis of results • Peak area • Peak width at half-length	Refer to the appropriate section: • Verify the Results for Triple Quadrupole and QTRAP® Systems on page 21 • Verify the Results for TripleTOF® Systems on page 25	


SCIEX

MS Method Information for the 3200 Series of Instruments

A

This appendix includes instrument-specific information for completion of the system integration test.

Topics include:

MRM Transitions

Table A-1 MRM Transitions for the API 3200™ and 3200 QTRAP® Systems

Q1	Q3	Dwell	ID	DP	CE
228.2	186.2	10	Ametryn 1	46	23
228.2	96.1	10	Ametryn 2	46	33
216.0	174.0	10	Atrazine 1	46	23
216.0	104.1	10	Atrazine 2	46	39
226.2	142.3	10	Prometon 1	51	29
226.2	184.2	10	Prometon 2	51	23
242.2	158.1	10	Prometryn 1	41	27
242.2	200.2	10	Prometryn 2	41	23
230.2	146.0	10	Propazine 1	51	31
230.2	188.3	10	Propazine 2	51	23
202.1	132.1	10	Simazine 1	46	25
202.1	124.3	10	Simazine 2	46	25
242.2	186.0	10	Terbutryn 1	41	23
242.2	68.2	10	Terbutryn 2	41	55

MS Information

The values for **Entrance Potential (EP)** and **Collision Cell Exit Potential (CXP)** differ by mass spectrometer. Enter the appropriate values from Table A-2.

Table A-2 Values for EP and CXP for the API 3200 and 3200 QTRAP Systems

System	EP	CXP
API 3200 system 3200 QTRAP system	10	4

Sample Preparation

Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:H_2O in a clean vial. Refer to Table A-3.

Note: For the final dilution, use H_2O with 0.1% formic acid instead of MeOH:H_2O.

Table A-3 Sample Dilutions by Mass Spectrometer

System	Target Concentration	Dilution
API 3200 system 3200 QTRAP system	10 ng/mL	Two serial dilutions 1. 100 µL stock solution + 900 µL MeOH:H_2O (to make 100 ng/mL) 2. 100 µL of 100 ng/mL + 900 µL H_2O with 0.1% formic acid

MS Method Information for the 4000 and 4500 Series of Instruments

B

This appendix includes instrument-specific information for completion of the system integration test.

Topics include:

MRM Transitions

Table B-1 MRM Transitions for 4000 and 4500 Systems

Q1	Q3	Dwell	ID	DP	CE
228.2	186.2	10	Ametryn 1	66	23
228.2	96.1	10	Ametryn 2	66	33
216.0	174.0	10	Atrazine 1	66	23
216.0	104.1	10	Atrazine 2	66	39
226.2	142.3	10	Prometon 1	71	29
226.2	184.2	10	Prometon 2	71	23
242.2	158.1	10	Prometryn 1	61	27
242.2	200.2	10	Prometryn 2	61	23
230.2	146.0	10	Propazine 1	71	31
230.2	188.3	10	Propazine 2	71	23
202.1	132.1	10	Simazine 1	66	25
202.1	124.3	10	Simazine 2	66	25
242.2	186.0	10	Terbutryn 1	61	23
242.2	68.2	10	Terbutryn 2	61	55

MS Information

The values for **Entrance Potential (EP)** and **Collision Cell Exit Potential (CXP)** differ by mass spectrometer. Enter the appropriate values from Table B-2.

Table B-2 Values for EP and CXP for 4000 and 4500 Systems

System	EP	CXP
API 4000™ system 4000 QTRAP® system SCIEX Triple Quad™ 4500 system SCIEX QTRAP® 4500 system	10	10

Sample Preparation

Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:H_2O in a clean vial. Refer to Table B-3.

Note: For the final dilution, use H_2O with 0.1% formic acid instead of MeOH:H_2O.

Table B-3 Sample Dilutions by Mass Spectrometer

System	Target Concentration	Dilution
API 4000 system 4000 QTRAP system SCIEX Triple Quad 4500 system SCIEX QTRAP 4500 system	1 ng/mL	Three serial dilutions: 1. 100 µL stock solution + 900 µL MeOH:H_2O (to make 100 ng/mL) 2. 100 µL of 100 ng/mL + 900 µL MeOH:H_2O (to make 10 ng/mL) 3. 100 µL of 10 ng/mL + 900 µL H_2O with 0.1% formic acid

MS Method Information for the 5000, 5500, and 6500 Series of Instruments

C

This appendix includes instrument-specific information for completion of the system integration test. Topics include:

MRM Transitions

Table C-1 MRM Transitions for API 5000™, Triple Quad™ 5500/6500, and QTRAP® 5500/6500 Systems

Q1	Q3	Dwell	ID	DP	CE
228.2	186.2	10	Ametryn 1	86	23
228.2	96.1	10	Ametryn 2	86	33
216.0	174.0	10	Atrazine 1	86	23
216.0	104.1	10	Atrazine 2	86	39
226.2	142.3	10	Prometon 1	91	29
226.2	184.2	10	Prometon 2	91	23
242.2	158.1	10	Prometryn 1	81	27
242.2	200.2	10	Prometryn 2	81	23
230.2	146.0	10	Propazine 1	81	31
230.2	188.3	10	Propazine 2	81	23
202.1	132.1	10	Simazine 1	86	25
202.1	124.3	10	Simazine 2	86	25
242.2	186.0	10	Terbutryn 1	81	23
242.2	68.2	10	Terbutryn 2	81	55

MS Information

The values for **Entrance Potential (EP)** and **Collision Cell Exit Potential (CXP)** differ by mass spectrometer. Enter the appropriate values from Table C-2.

Table C-2 Values for EP and CXP for 5000, 5500, and 6500 Systems

System	EP	CXP
API 5000™ system SCIEX Triple Quad™ 5500 system QTRAP® 5500 system	10	10
QTRAP® 6500 system	10	13

Sample Preparation

Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:H_2O in a clean vial. Refer to Table C-3.

Note: For the final dilution, use H_2O with 0.1% formic acid instead of MeOH:H_2O.

Table C-3 Sample Dilutions by Mass Spectrometer

System	Target Concentration	Dilution
API 5000 system SCIEX Triple Quad 5500 system QTRAP 5500 system SCIEX Triple Quad 6500 system QTRAP 6500 system	0.1 ng/mL	Four serial dilutions: 1. 100 µL stock solution + 900 µL MeOH:H_2O (to make 100 ng/mL) 2. 100 µL of 100 ng/mL + 900 µL MeOH:H_2O (to make 10 ng/mL) 3. 100 µL of 10 ng/mL + 900 µL MeOH:H_2O (to make 1 ng/mL) 4. 100 µL of 1 ng/mL + 900 µL H_2O with 0.1% formic acid

Sample Preparation for TripleTOF® Systems D

This appendix includes instrument-specific information for completion of the system integration test.

Sample Preparation

Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:H_2O in a clean vial. Refer to Table D-1.

Note: For the final dilution, use H_2O with 0.1% formic acid instead of MeOH:H_2O.

Table D-1 Sample Dilutions by Mass Spectrometer

System	Target Concentration	Dilution (Stock Solution + 50:50 MeOH:H_2O)
TripleTOF 4600 system TripleTOF 5600/5600+ system TripleTOF 6600 system	10 ng/mL	Two serial dilutions: 1. 100 µL stock solution + 900 µL MeOH:H_2O (to make 100 ng/mL) 2. 100 µL of 100 ng/mL + 900 µL H_2O with 0.1% formic acid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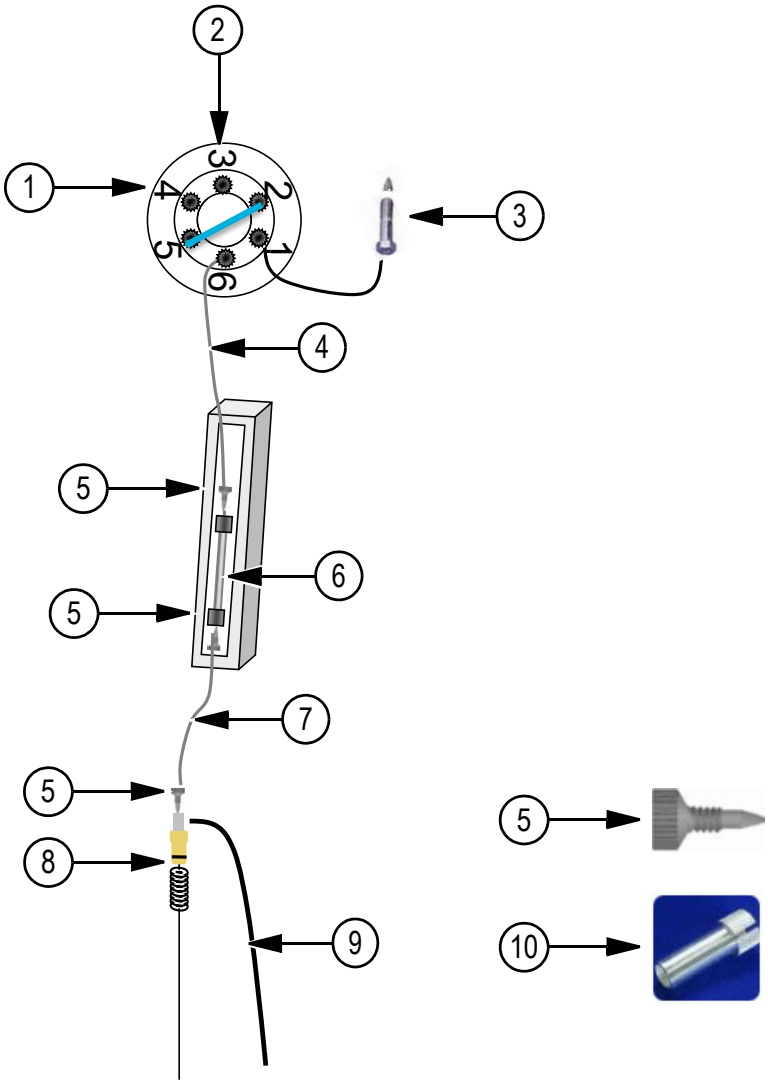
Plumbing Diagram

E

The injection valve should be plumbed as shown in Figure E-1.

Figure E-1 Plumbing the Injection Valve



Item	Description	Part Number
1	Sample needle	
2	Syringe dispenser	
3	From pump	
4	50 µm ID gray PEEKsil tubing	205-00040, 30 cm or 205-00041, 50 cm

Item	Description	Part Number
5	Black PEEK fitting (Use tightening tool (PN 200-00356) if necessary)	200-00342
6	2.7 µm HALO C18 column, 0.5 mm x 50 mm	805-10100
7	One of the following: • Gray PEEKsil tubing, 50 µm ID,1/32 inch OD,10 cm • Orange PEEKsil tubing, 25 µm ID,1/32 inch OD,10 cm*	 205-00069 205-00091
8	One of the following: • 50 µm ID electrode assembly • 25 µm ID electrode assembly*	 5016411 5016874
9	Grounding cable kit	5016941
10	Tightening tool	200-00356

* Recommended for flow rates below 10 µL/min to 20 µL/min using 0.5 mm ID columns.

This appendix describes best practices and sample preparation techniques for a SCIEX NanoLC 400 system.

Best Practices

- If the system is shut off for more than a few days, then purge and change the mobile phases. Perform an initial wash of the autosampler and possibly calibrate the system prior to use. Refer to the appropriate *Operator Guide.*
- Use LC-MS-grade pre-made solvents such as those from Burdick-Jackson (that is, HPLC-grade water with 0.1% formic acid and acetonitrile with 0.1% formic acid). These solvents can be ordered from VWR:
  - PN BJLC452-2.5 - 0.1% Formic Acid Water
  - PN BJLC441-2.5 - 0.1% Formic Acid Acetonitrile
- Verify that the gas flowrate and pressure are consistent. Do not disconnect the gas supply.
- Avoid biological growth.
  - Change solutions frequently.
  - Include 0.1% formic acid in all mobile phase bottles.
- Verify that the mobile phase solutions in the bottles match the mobile phase and composition values in the Eksigent control software (**System > Mobile Phases**).

Table F-1 Typical Mobile Phase Mixtures

	Binary Mixture A	Binary Mixture B	Modifier
Gradient 1			
	100% water	100% acetonitrile	0.1% formic acid
Gradient 2			
	100% water	100% acetonitrile	0.1% formic acid
Loading Pump			
	100% water	N/A	0.1% formic acid

- Remove air from the mobile phase bottles weekly by purging the system a minimum of 10 times per channel.
- Keep the pump seal wash bottle filled with 5% methanol and change the solutions quarterly.
- Empty the waste bottle once a week (or more often if needed).
- Check the flowrate monthly. Re-initialize the pressure transducers weekly.
- When cutting silica, wash the end with methanol and flow solution through the cut end before connecting.

Sample Preparation Techniques

In general, the standard practices and procedures for reversed phase LC-MS experiments using electrospray mass spectrometry also apply to the use of SCIEX NanoLC 400 systems.

- Use HPLC or MS-grade solvents at all times.
- Avoid the use of non-volatile salts and buffers such as CHAPS, phosphate, TRIS, HEPES and perchlorates. These additives will foul the electrospray source and mass spectrometer orifice.
- Centrifuge (spin) all samples at 10 000 RPM for 5 minutes to remove particulates from the sample solution.
- Dilute all samples as appropriate to prevent sample precipitation in the chromatographic system and at the electrospray source. This also ensures binding to the stationary phase.

Revision History

Revision	Reason for Change	Date
D5066274 A	First release of the document.	April 2013
D5066274 B	Corrected document number.	April 2013
D5066274 C	Corrected Advanced Editor Autosampler Method Parameters in Table 2-1 and Acquisition Method Parameters in Table 2-3. Corrected MRM Transitions for Triazine in Table 2-4. Updated figures 2-11 and 2-20.	September 2013
D5066274 D RUO-IVD-05-1125-A	Updated figures 2-2, 2-5, 2-6, and 2-11. Added instructions for testing TripleTOF® systems and verifying the results. Added plumbing diagram. Updated instrument name in title to be consistent with other documents.	February 2014
D5066274 E RUO-IVD-05-1125-B	Added System Integration Test Data Log and Signoff chapter. Moved Best Practices chapter before the Revision History.	June 2014
D5066274 F RUO-IVD-05-1125-C	Rebranded. For all sample preparation topics, corrected the final dilution to use water. Added TripleTOF® 6600 system.	June 2016