M3 MicroLC-TE System

For SCIEX Triple Quad™ and QTRAP® Systems

System Integration Test and Data Log

RUO-IDV-05-1536-D | D5088499 D

December 2016

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Contents

Chapter 1 Introduction....4
About the Test....4
Time Required....4
Required Materials....5

Chapter 2 Create the Methods....6
Create the Gradient LC Method....6
Create the LC Method to Load the Trap Column....11
Create the Autosampler Method for the System Integration Test....15
Create the Acquisition Method....16

Chapter 3 Prepare the System for Testing....20
Verify System Readiness....20
Prepare the 1 µg/mL Stock Solution....20
Prepare the Test Sample....21
Equilibrate the System....23

Chapter 4 Perform the System Integration Test....26
Create the Acquisition Batch and Quantitation Method....26
Run the Batch....29
View the Results and Verify the Integration....30
Verify the Mean Area and %CV....33
Verify Peak Widths at Half Height and Retention Times....33

Chapter 5 Troubleshooting....37
Identify and Resolve Carryover Issues....37
Test the Injection Port and the DLW System....37
Resolve General Issues....38
Troubleshoot Peak-Related Issues....38
Resolve Drifting Retention Times....38
Peak Widths are Too Broad or are Tailing....38
Fronting, Pre-eluting or Low Intensity Peaks....38
Missing or Low Intensity Peaks....39
Troubleshoot Using the Chromatogram....39
Troubleshoot Using Pressure and Flow Data....40

Chapter 6 System Integration Test Data Log and Signoff....41
System Information....41
Test Results....41
Specifications....43
Signoff....43

Revision History....44

Introduction 1

This document provides the procedures for preparing and performing an LC-MS/MS system integration test for the SCIEX M3 MicroLC-TE system configured with the C18 HALO Peptide-ES 0.5 mm i.d. × 5 cm and the Chrom XP C18 trap columns 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

CAUTION: Potential System Damage. Prior to operating the system, refer to "Operational Precautions and Limitations" in the *Operator Guide*.

About the Test

Use this test as a measure of the M3 MicroLC-TE system performance in isolation of the performance of the other components. Results from the test can become the baseline performance for the system. The test 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. Refer to the *Ion Source Tests, Specifications, and Data Log* document.

Repeat the test until consistent peak shape and peak intensity are achieved.

Time Required

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: 27 minutes
5. Verify the test results: 15 minutes

Required Materials

- C18 HALO Peptide-ES 0.5 mm i.d. × 5 cm column (PN 5039577)
- Chrom XP C18 trap column, cartridge holder (PN 5027467), and cartridge (PN 5028897)
- Triazine System Suitability Solution (PN 4376887)
- 50 µL stainless steel sample loop (PN 5040770)
- 65 µm i.d. electrode (PN 5029342)

Create the Methods 2

Note: The active hardware profile must include the CTC autosampler and the Eksigent control software to perform a run with the Analyst® software. The hardware profile is configured at installation. However, if a different computer is being used or the Analyst® software has been reinstalled, then the hardware profile might not be correct. Before creating the methods, verify that the correct hardware profile is present or create a hardware profile. Refer to the *Operator Guide* for information on creating and verifying a hardware profile.

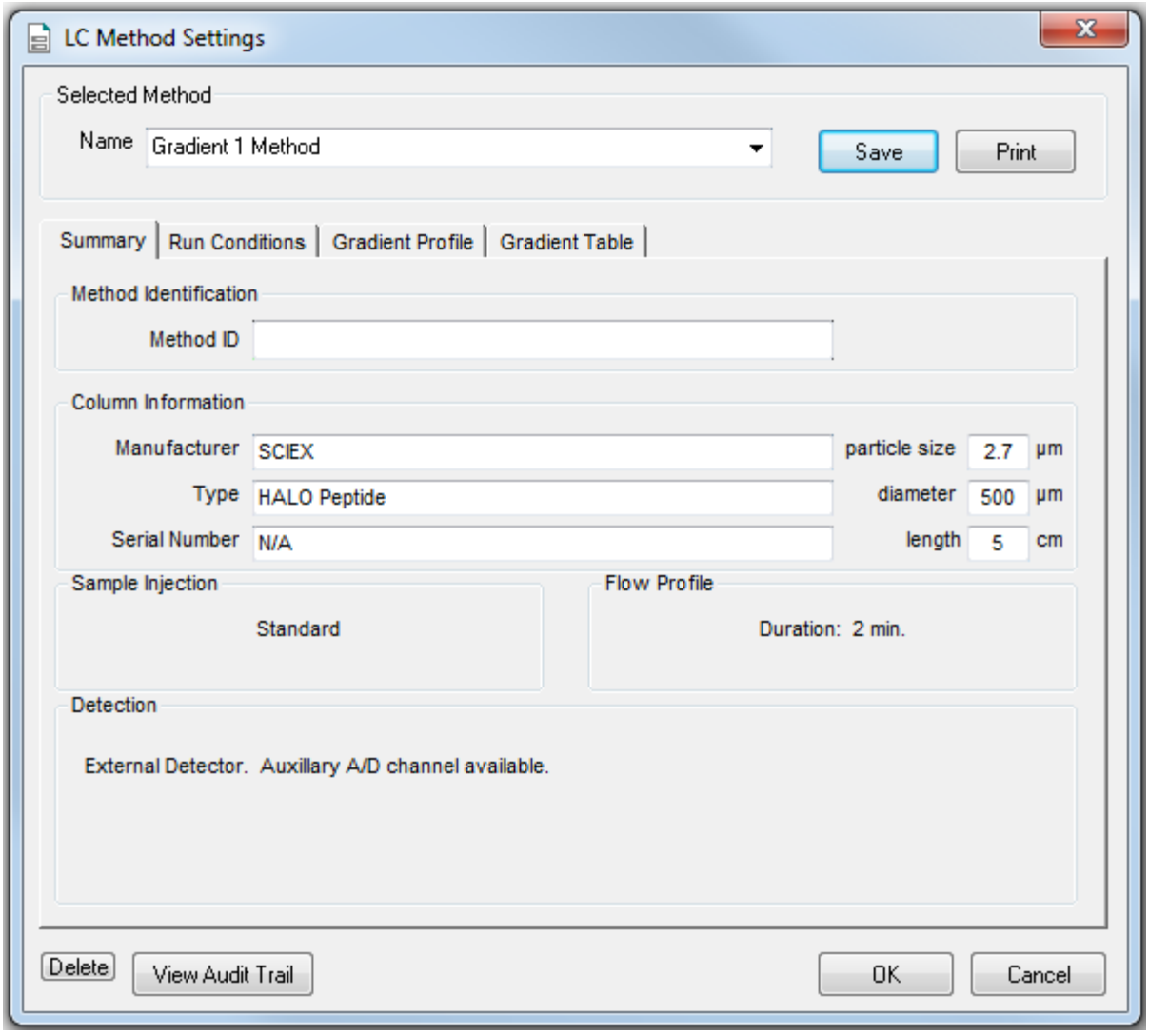
Create the LC methods (one for the Gradient 1 pump and one for the Gradient 2 pump) in the Eksigent control software and the autosampler and acquisition methods in the Analyst® software.

Create the Gradient LC Method

This method is used to separate the samples on the analytical column.

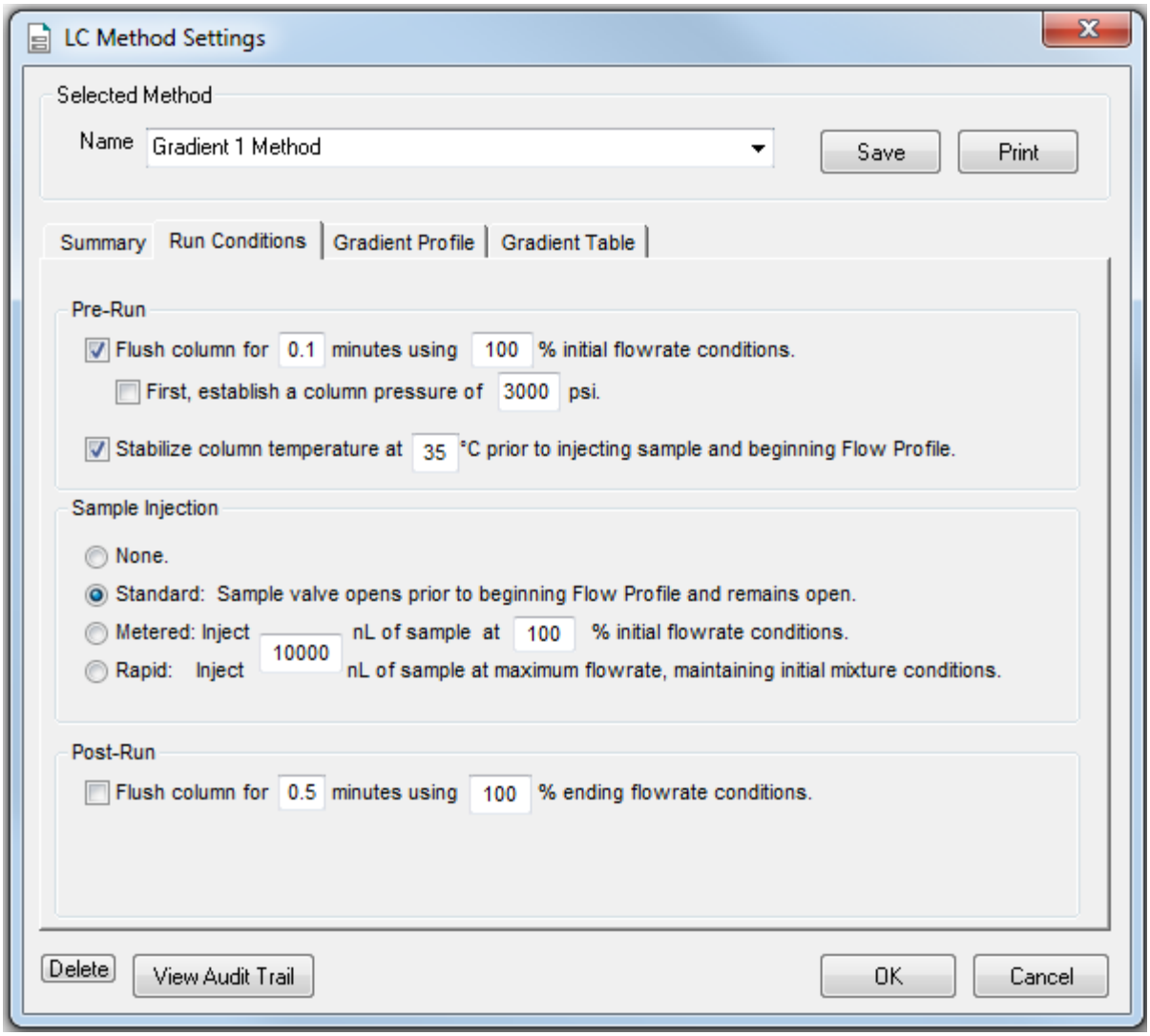
1. In the Eksigent control software Acquisition window, click the arrows to select **Gradient 1** in the **Channel** area.
2. Click **LC Methods** to open the LC Method Settings dialog.
3. In the **Name** field, type **Gradient 1 Method**, and then click **Save**.
4. On the Summary tab, type the values shown in Figure 2-1.

Figure 2-1 Gradient 1 Method—Summary Tab



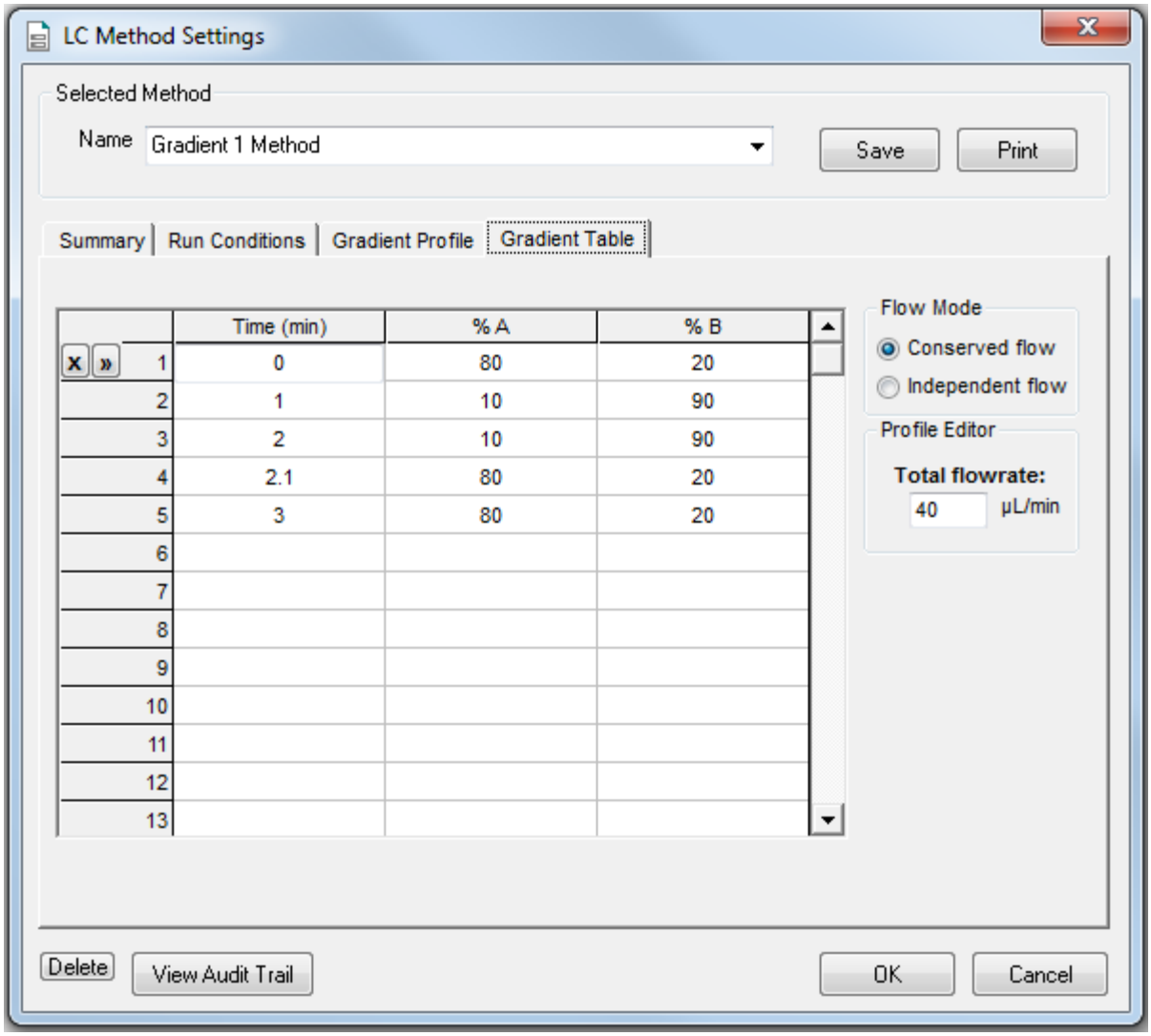
5. Click the Run Conditions tab and type the values shown in Figure 2-2.

Figure 2-2 Gradient 1 Method—Run Conditions Tab



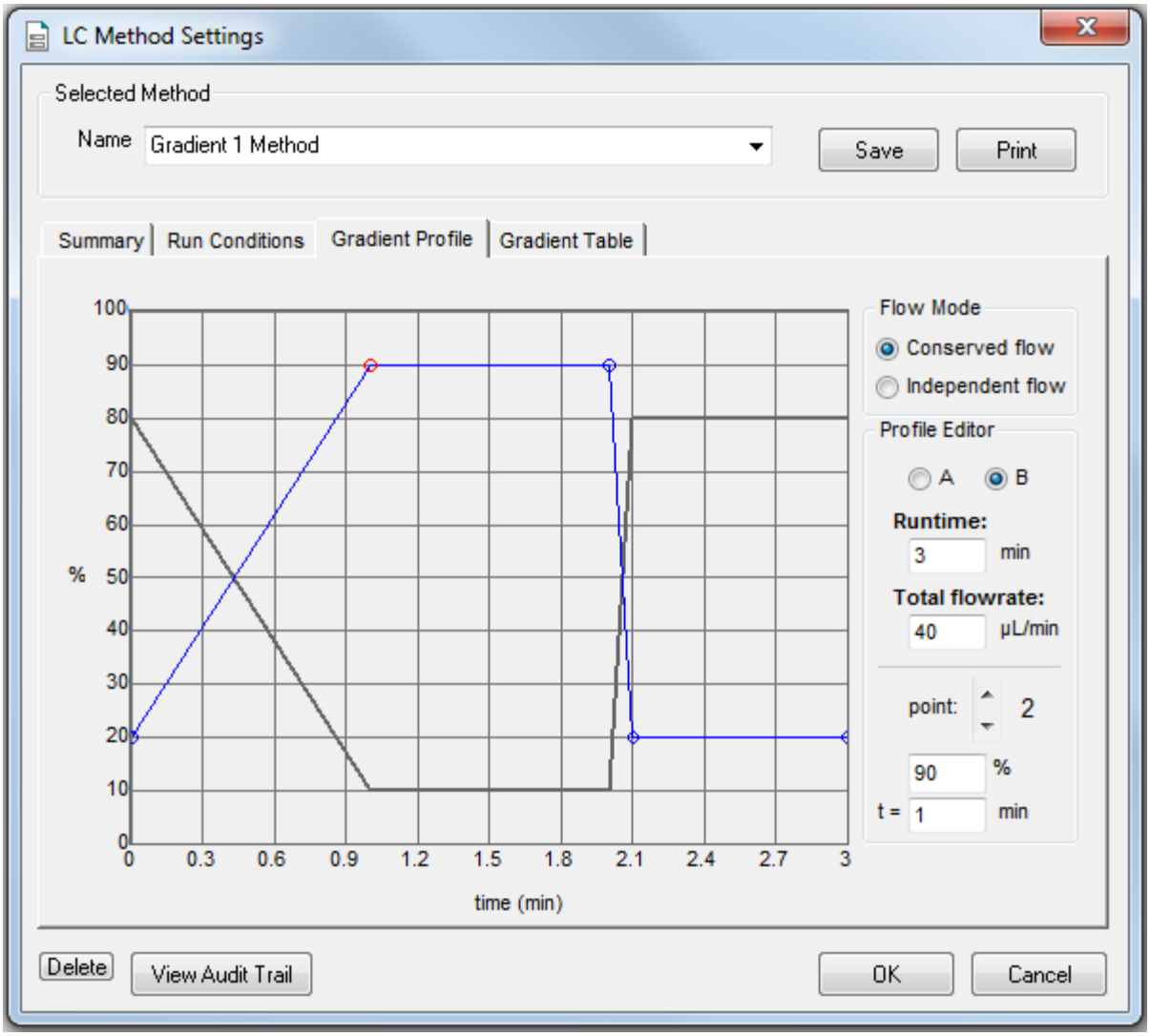
6. Click the **Gradient Table** tab and set the flow mode, the gradient parameters, and the flow rate. Refer to Figure 2-3.

Figure 2-3 Gradient 1 Method—Gradient Table Tab



7. Click the Gradient Profile tab to view a graphical representation of the gradient. Refer to Figure 2-4.

Figure 2-4 Gradient 1 Method—Gradient Profile Tab



The last two steps in the method allow for the aqueous solvent to flow through the sample loop before the next sample is injected.

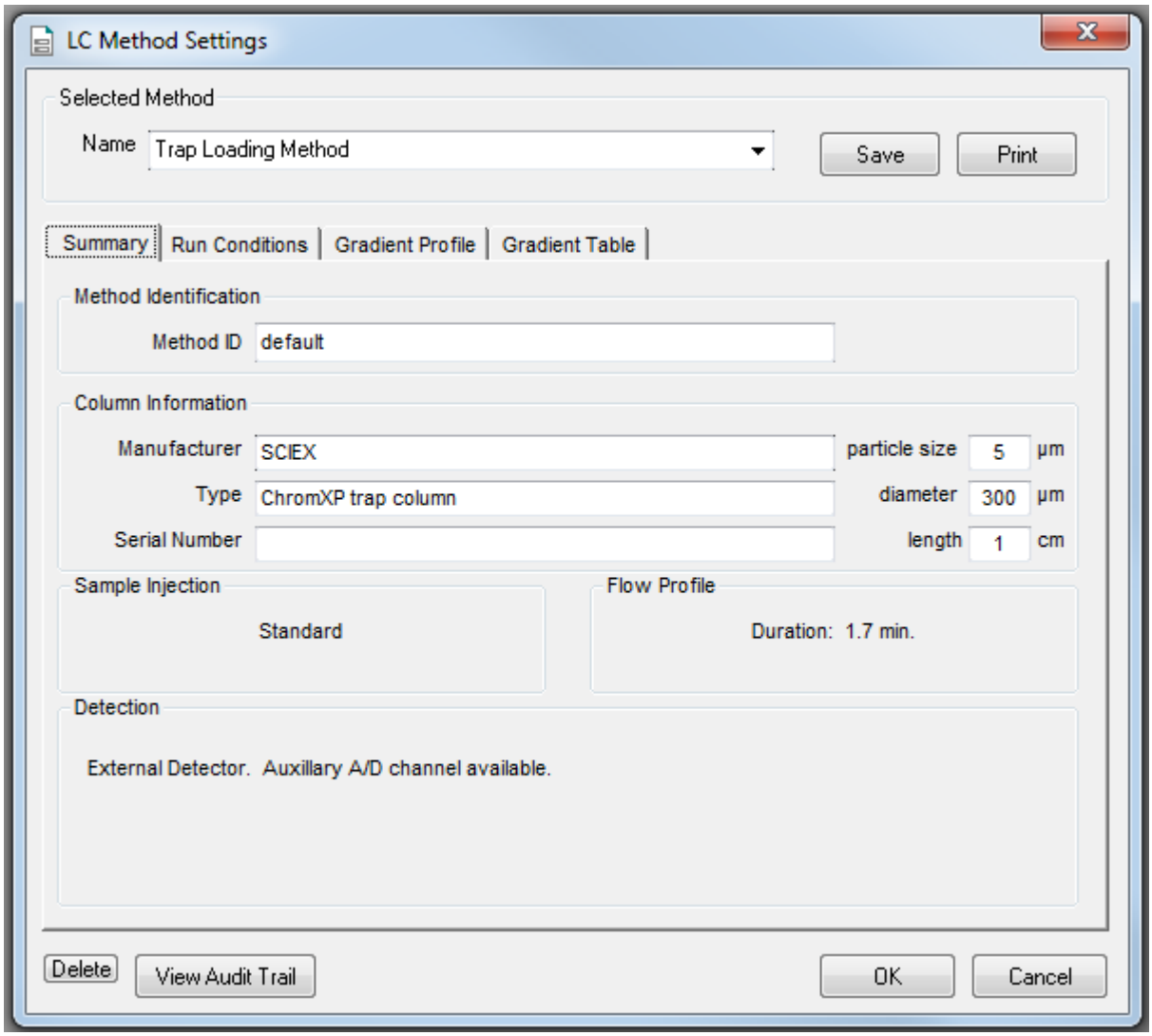
8. Click **Save** and then click **OK**.

Create the LC Method to Load the Trap Column

This method is used to load the sample onto the trap column.

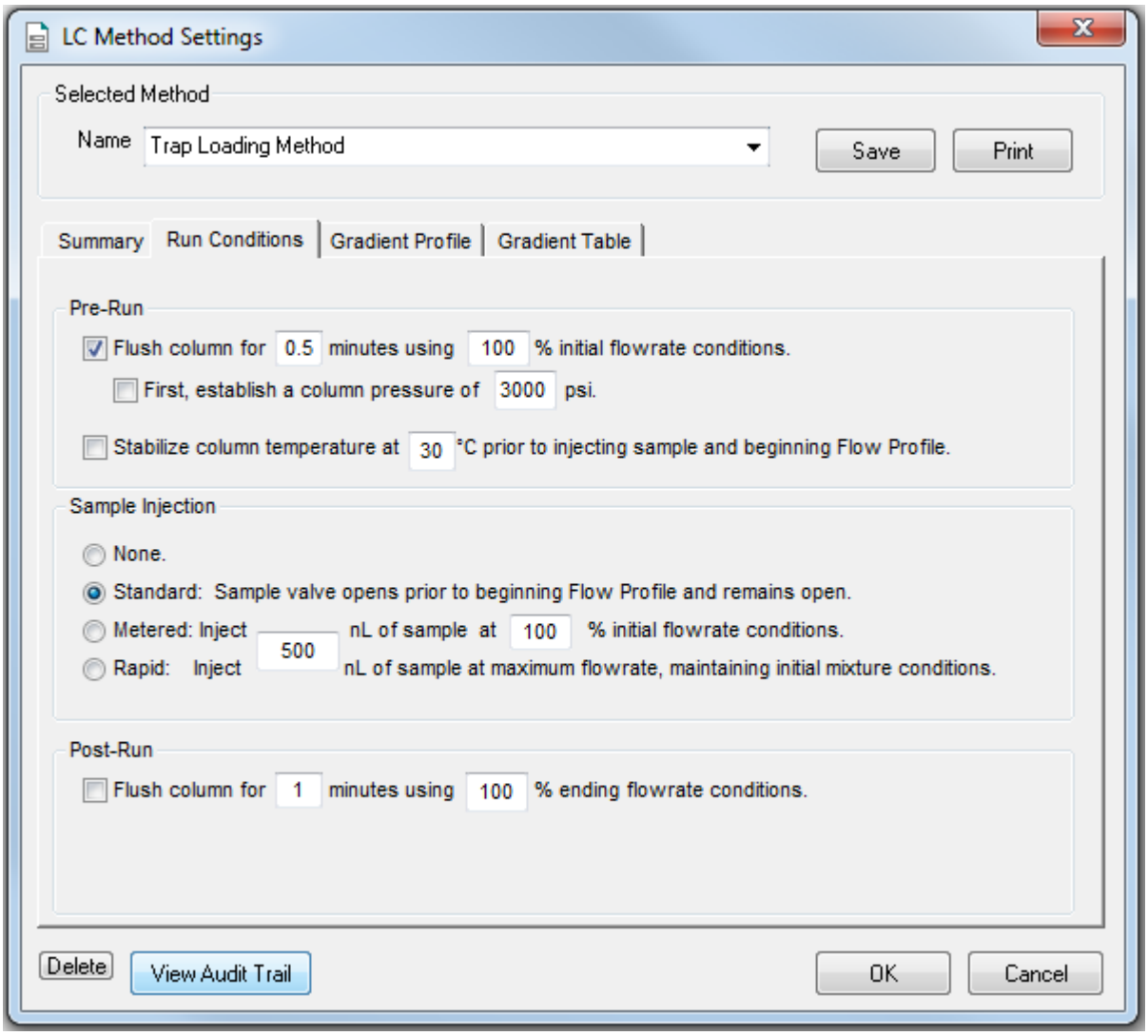
1. In the Eksigent control software Acquisition window, click the arrows to select **Gradient 2** in the **Channel** area.
2. Click **LC Methods** to open the LC Method Settings dialog.
3. In the **Name** field, type **Trap Loading Method** and then click **Save**.
4. In the Column Information section, type the values shown in Figure 2-5.

Figure 2-5 Trap Loading Method—Summary Tab



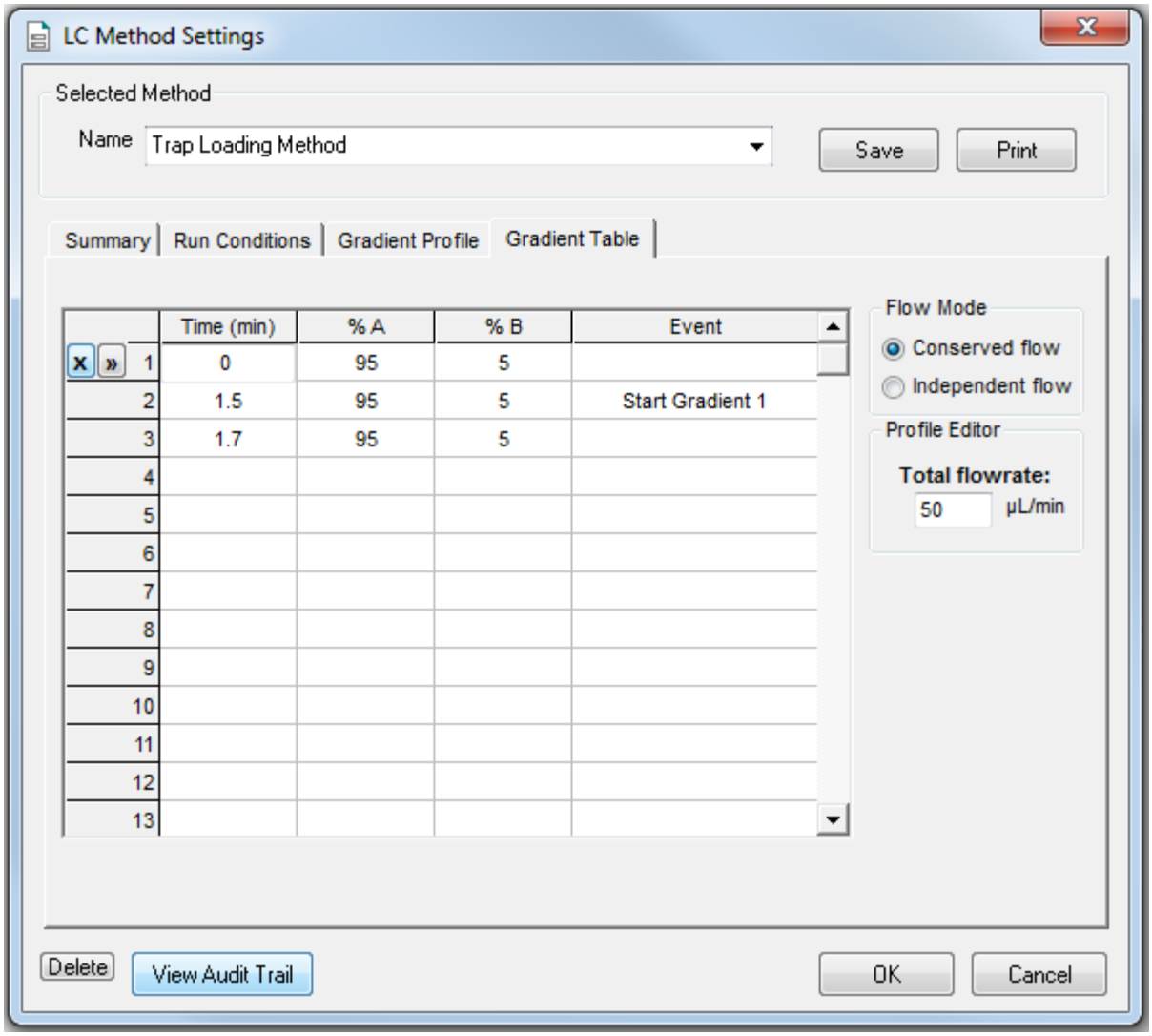
5. Click the **Run Conditions** tab and then type the values shown in Figure 2-6.

Figure 2-6 Trap Loading Method—Run Conditions Tab



6. Click the **Gradient Table** tab and then set the flow mode, the gradient parameters, and the flow rate. Refer to Figure 2-7.

Figure 2-7 Trap Loading Method—Gradient Table Tab



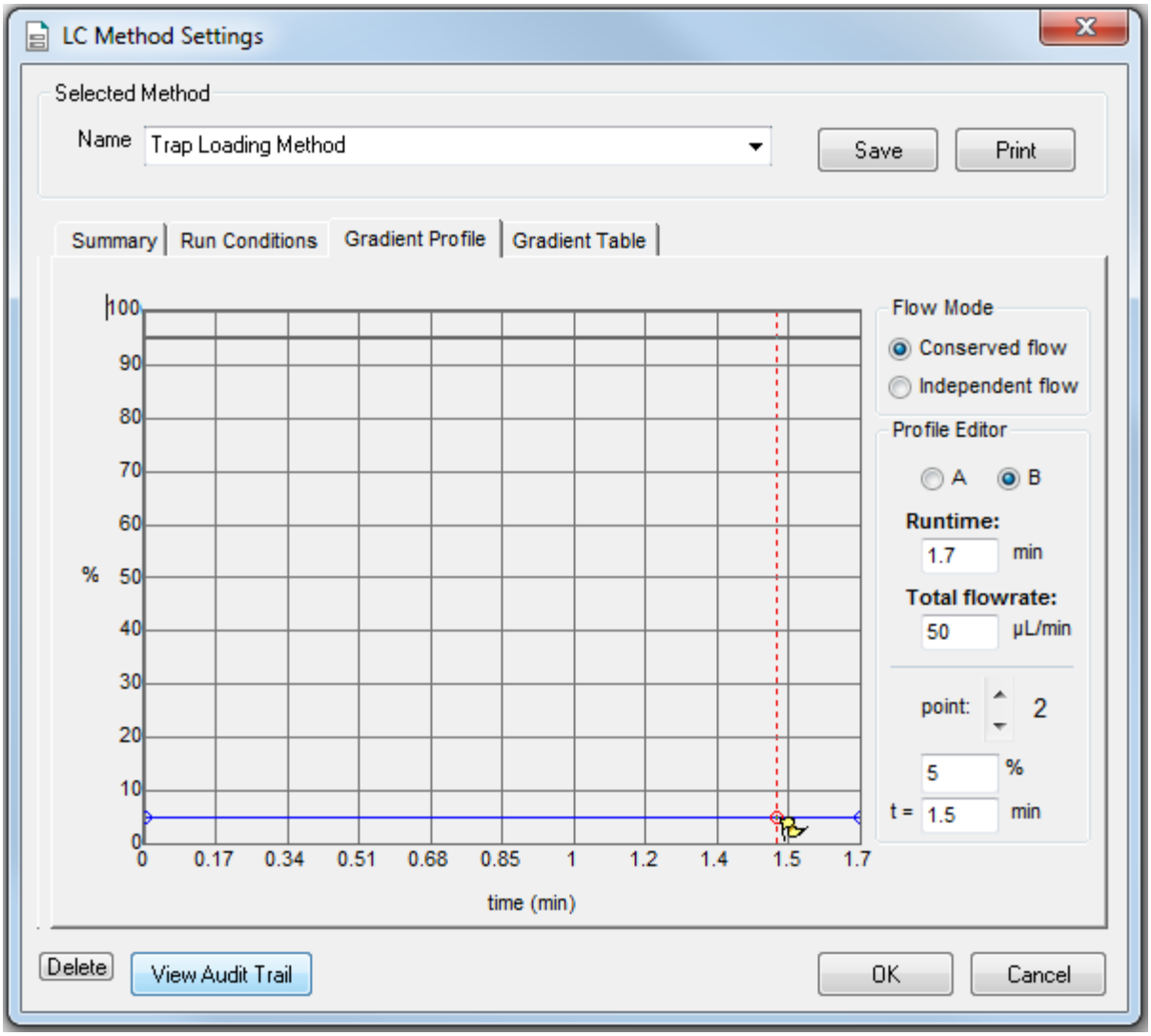
Note: If there is carryover from one sample to the next, add a step at the end of the method to wash the sample loop with the organic mobile phase.

7. At 1.5 min, click the **Event** cell and then select **Start Gradient 1**.

   This event starts the Gradient 1 pump, which switches the trap column in-line with the analytical column. The sample will be eluted from the trap column onto the analytical column.

8. Click the **Gradient Profile** tab to view a graphical representation of the gradient. Refer to Figure 2-8.

Figure 2-8 Trap Loading Method—Gradient Profile Tab



9. Click **Save** and then click **OK**.

Create the Autosampler Method for the System Integration Test

1. On the Navigation bar in the Analyst® software, under **Acquire**, double-click **Build Acquisition Method**.

   The Acquisition Method Editor opens.
2. In the Acquisition Method Browser pane, click **CTC PAL Autosampler**.
3. In the **Available Cycles** list, select **M3 MicroLC Trap Elute RevA.**

   Note: The autosampler method installed with the system may have a different name than listed above. Use the most recent autosampler method supplied by SCIEX.
4. In the **Injection Volume** field, type **60** (the volume to be aspirated into the sample loop).
5. Edit parameters in the Cycle Arguments table.
   a. Type **1** in the **Front Volume (µL)** field.
   b. Type **1** in the **Front Airgap Volume (µL)** field.
   c. Type **0** in the **Needle Gap for 2nd and Final Vlv Clean** field.
   d. Select **Wash2** for **Final Wash Solvent.**

   Note: The **Rear Airgap Volume (µL)** field requires a value of at least 0.01.
6. Verify that the parameters in the **Cycle Arguments** table are as shown in the following table.

   If the values are not the same, then edit them as needed.

Table 2-1 Cycle Arguments Table—Trap-and-Elute Parameters

Parameter	Value	Parameter	Value
Rear Airgap Volume (µL)	1	First Wash Solvent	Wash1
Rear Volume (µL)	1	Valve Clean Time 1 (s)	5
Front Volume (µL)	1	Needle Clean Time 1 (s)	2
Front Airgap Volume (µL)	1	Second Wash Solvent	Wash1
Sample Aspirate Speed (µL/s)	2	Needle Clean Time 2 (s)	2
Pullup Delay (ms)	500	Valve Clean Time 2 (s)	5
Num of Wash1 PreDips	1	Replicate Count	1

Table 2-1 Cycle Arguments Table—Trap-and-Elute Parameters (continued)

Parameter	Value	Parameter	Value
Num of Wash2 PreDips	0	Final Wash Solvent	Wash2
Inject to	LCVlv1	0 or 1 Final Cleans	1
Injection Speed (µL/s)	1	Final Needle Clean Time (s)	2
Needle Gap for 2nd and Final Vlv Clean (mm)	0	Final Valve Clean Time 2 (s)	5

Create the Acquisition Method

1. Select the LC method for the Gradient 1 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 1**.
   b. Click **...** (browse) to view the available LC methods.
   c. Click **Gradient 1 Method** and then click **Open**.
2. Select the LC method for the Gradient 2 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 2**.
   b. Click **...** (browse) to view the available LC methods.
   c. Click **Trap Loading Method** and then click **Open**.
3. In the Acquisition Method Browser pane, click **Mass Spec** to create the mass spectrometer acquisition method.
4. Type the acquisition method parameters shown in Table 2-2.

Note: Source/Gas parameters can vary between systems and electrodes. Determine the best value for the system. Make sure that the electrode position is optimized before creating the acquisition method.

Table 2-2 Acquisition Method Parameters

Parameter	Value
MS	
Scan Type	MRM (MRM)
Duration (min)	3
Polarity	Positive

Table 2-2 Acquisition Method Parameters (continued)

Parameter	Value
Q1/Q3 Masses and CE	Refer to Table 2-4, Table 2-5, and Table 2-6.
Advanced MS	
Q1 Resolution	Unit
Q3 Resolution	Unit
Pause (ms)	5.00
Source/Gas	
Curtain Gas (CUR)	20 (or as optimized)
CAD Gas	Medium
IonSpray Voltage (IS) (V)	5000 (or as optimized)
Ion Source Gas 1 (GS1)	20 (or as optimized)
Ion Source Gas 2 (GS2)	20 (or as optimized)
Temperature (TEM)	350 (or as optimized)
Compound	
Declustering Potential (DP)	70
Collision Exit Potential (CXP)	30

5. Type the parameters shown in Table 2-3

   The values for Entrance Potential (EP) and Collision Cell Exit Potential (CXP) differ by mass spectrometer. Be sure to type the appropriate values.

Table 2-3 Values for Entrance Potential and Collision Cell Exit Potential

System	EP	CXP
API 3200™ system 3200 QTRAP® system	10	4
API 4000™ system 4000 QTRAP® system SCIEX Triple Quad™ 4500 system QTRAP® 4500 system	10	10

Table 2-3 Values for Entrance Potential and Collision Cell Exit Potential (continued)

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

6. Right-click the **Mass Ranges Table** and select **DP** from the menu to add the DP (declustering potential) column to the table. Add the CE (collision energy) as well.
7. Type the MRM transitions in the **Mass Ranges Table**.

   The transitions are shown in Table 2-4, Table 2-5, and Table 2-6.

Tip! Field Service Employees can download a .CSV file from the SharePoint site, import it, and save it locally for customer use. To use the file, click **Import List** and select the file.

Table 2-4 MRM Transitions for 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
242.2	186.0	10	Terbutryn 1	41	23
242.2	68.2	10	Terbutryn 2	41	55

Table 2-5 MRM Transitions for the 4000 and 4500 Series of 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

Table 2-5 MRM Transitions for the 4000 and 4500 Series of Systems (continued)

Q1	Q3	Dwell	ID	DP	CE
242.2	186.0	10	Terbutryn 1	61	23
242.2	68.2	10	Terbutryn 2	61	55

Table 2-6 MRM Transitions for SCIEX Triple Quad™ 5500 and 6500 Systems and QTRAP® 5500 and 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
242.2	186.0	10	Terbutryn 1	81	23
242.2	68.2	10	Terbutryn 2	81	55

8. Save the method as **System Integration Test_DATE**, where *DATE* is today's date.

Prepare the System for Testing 3

Verify System Readiness

Prior to running this test, make sure that the system is calibrated. To calibrate the system, perform the following procedures described in the *Operator Guide*:

- Reinitialize the Pressure Transducers
- Measure the Flow Rate
- Calibrate the Flowmeters, if necessary

Prepare the 1 µg/mL Stock Solution

WARNING! Toxic Chemical Hazard. Follow all safety guidelines when handling, storing, and disposing of chemicals. For heath and safety precautions, refer to the mass spectrometer *System User Guide* or *Hardware Guide*.

This procedure generates 1 mL of a 1 µg/mL stock solution.

Required Materials
• Methanol • 100 µg/mL Triazine System Suitability Solution (PN 4376887)

1. Create a 10 µg/mL solution using the volumes shown in the first row of Table 3-1.
2. Create a 1 µg/mL solution using the volumes shown in the second row of Table 3-1.

Table 3-1 Solution Dilution

Stock Solution Volume	Dilution Solvent Volume	Final Concentration
100 µL of 100 µg/mL triazine test mixture	900 µL of MeOH	10 µg/mL
100 µL of 10 µg/mL sample solution	900 µL of MeOH	1 µg/mL

Prepare the Test Sample

Required Materials
• Stock solution, prepared in Prepare the 1 µg/mL Stock Solution on page 20. • 50:50 mixture of MeOH:H_2O • Water with 0.1% formic acid

1. Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:H_2O and, for the final dilution, H_2O with 0.1% formic acid, in a clean vial. Refer to Table 3-2.

   For the final dilution, be sure to use H_2O with 0.1% formic acid.

2. Mix the solution for at least 30 seconds using a vortex mixer.
3. Transfer the solution to the autosampler vial, making sure that there is no bubble on the bottom of the vial.
4. Prepare the blank by filling an autosampler vial with Mobile Phase A, making sure that there is no bubble on the bottom of the vial. Refer to Equilibrate the System on page 23.

Table 3-2 Sample Dilutions by Mass Spectrometer

System	Target Concentration	Dilution
API 3200™ system 3200 QTRAP® system	1.0 ng/mL	Three serial dilutions: a. 100 µL of stock solution + 900 µL of MeOH:H_2O (to make 100 ng/mL) b. 100 µL of 100 ng/mL + 900 µL of MeOH:H_2O (to make 10ng/mL) c. 100 µL of 10 ng/mL + 900 µL of H_2O with 0.1% formic acid
API 4000™ system 4000 QTRAP® system SCIEX Triple Quad™ 4500 system QTRAP® 4500 system	0.1 ng/mL	Four serial dilutions: a. 100 µL of stock solution + 900 µL of MeOH:H_2O (to make 100 ng/mL) b. 100 µL of 100 ng/mL + 900 µL of MeOH:H_2O (to make 10 ng/mL) c. 100 µL of 10 ng/mL + 900 µL of MeOH:H_2O (to make 1 ng/mL) d. 100 µL of 1 ng/mL + 900 µL of H_2O with 0.1% formic acid
API 5000™ system SCIEX Triple Quad™ 5500 system QTRAP© 5500 system SCIEX Triple Quad© 6500 system QTRAP© 6500 system	0.01 ng/mL	Five serial dilutions: a. 100 µL of stock solution + 900 µL of MeOH:H_2O (to make 100 ng/mL) b. 100 µL of 100 ng/mL + 900 µL of MeOH:H_2O (to make 10 ng/mL) c. 100 µL of 10 ng/mL + 900 µL of MeOH:H_2O (to make 1 ng/mL) d. 100 µL of 1 ng/mL + 900 µL of MeOH:H_2O (to make 0.1 ng/mL) e. 100 µL of 0.1 ng/mL + 900 µL of H_2O with 0.1% formic acid

Equilibrate the System

1. Disconnect the tubing to the LC column inlet.
2. Verify that the mobile phases in Table 3-3 are loaded on the system.

Table 3-3 Mobile Phases

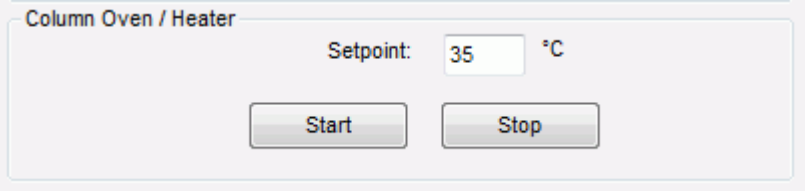
Buffer	Mixture	Channel
Gradient 1 Pump		
Buffer A	100% water:0.1% formic acid	Channel A
Buffer B	100% acetonitrile:0.1% formic acid	Channel B
Gradient 2 Pump		
Buffer A	100% water:0.1% formic acid	Channel A
Buffer B	100% acetonitrile:0.1% formic acid	Channel B

3. Allow the column oven to pre-heat.

Note: The column oven reaches the correct temperature quickly, but the column can take as long as 30 minutes to fully equilibrate.

   a. In the Eksigent control software, click **System > Direct Control**.
   b. Click the **Channel** buttons to select **Gradient 1**.
   c. In the **Column Oven/Heater** section, type **35** in the **Setpoint** field and then click **Start**.

Figure 3-1 Direct Control Dialog–Column Oven/Heater Section



   d. Close the compartment so that the oven can reach the specified temperature.
4. Make sure that the **Trap-Elute Mode** checkbox in the **Injection Valve Direct Control** section is checked.
5. Start the pump.

a. Select the **Conserved Flow (%)** option and set **A** to **80** and **B** to **20**.

   This is the mobile phase composition used for equilibration.

b. Type a **Total flowrate** of **50** µL/min.

c. Click **Start**.

6. Flush the injection valve.

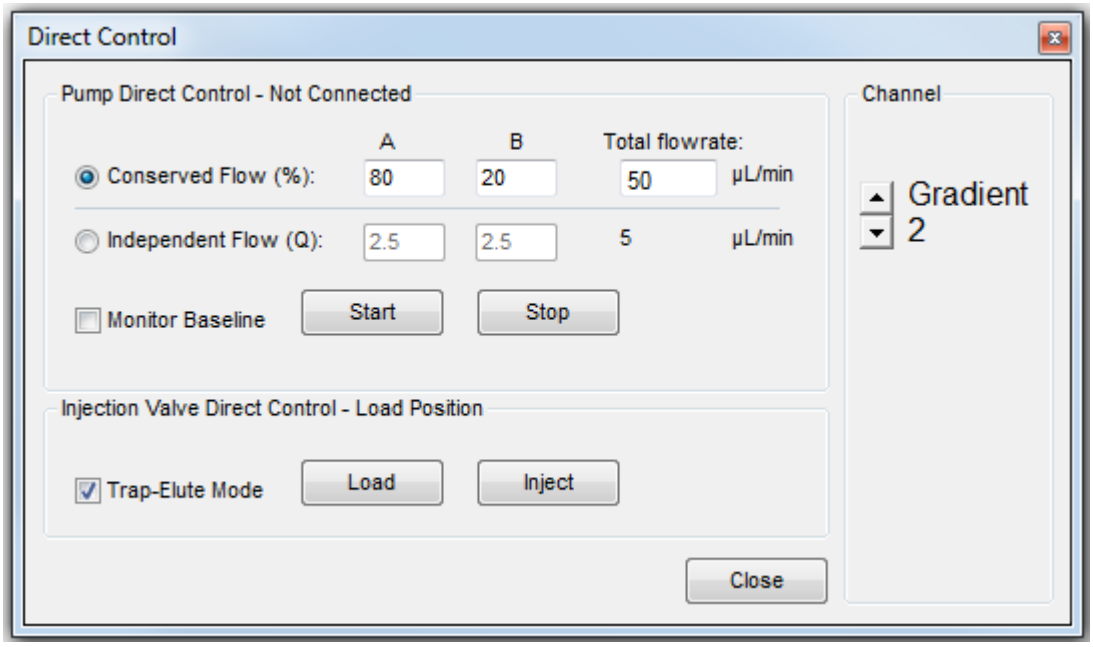
   a. Click the **Channel** buttons to select **Gradient 2**.

   b. In the **Valve Direct Control** section, alternate clicking **Load Position** and **Inject Position**.

   c. Make sure that the final position of the valve is at **Load**.

   d. In the **Pump Direct Control** section, click **Stop** and then connect the LC column.

Figure 3-2 Direct Control Dialog



7. In the Analyst® software, equilibrate the mass spectrometer.

   a. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning.**

   b. In the tuning method, type the source and gas parameters from Table 2-2.

   c. Type **10** in the **Time [Min.]** field and then click **OK**.

8. In the Direct Control dialog, click the **Channel** buttons to select **Gradient 1**.

9. Click **Start** in the **Pump Direct Control** section.

10. Adjust the source and gas parameters as required until a stable spray is achieved and then click **Stop** to end tuning.

11. Click **Stop** in the **Pump Direct Control** section and then close the Direct Control dialog.

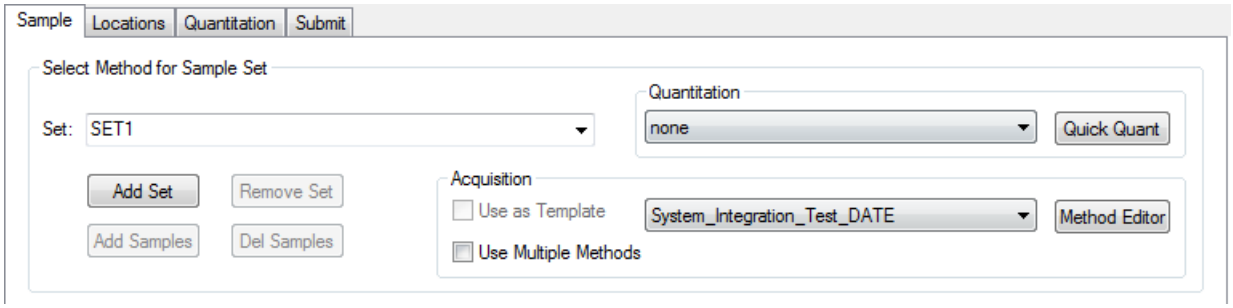
Perform the System Integration Test 4

Create the acquisition batch, run the batch and then verify the results. Type the test results in the System Integration Test Data Log and Signoff on page 41.

Create the Acquisition Batch and Quantitation Method

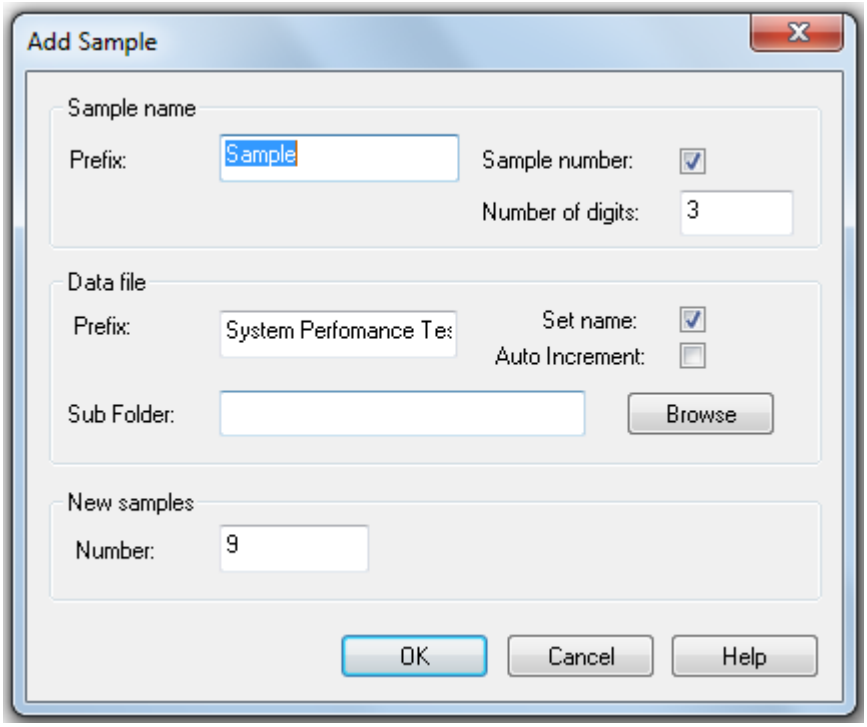
1. Open the Analyst® software.
2. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Batch.**
3. On the **Sample** tab, in the **Acquisition** group, select the acquisition method created in Create the Acquisition Method on page 16 from the list.

Figure 4-1 Sample Tab—Acquisition Group



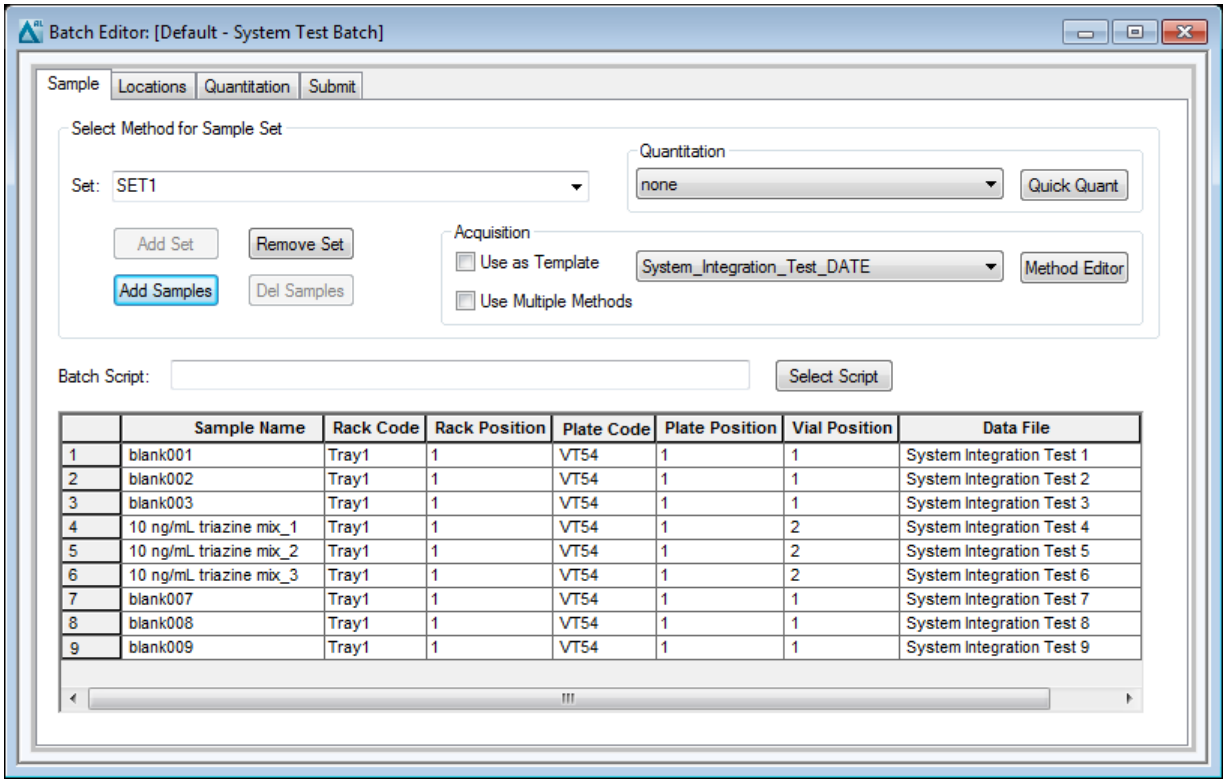
4. Click **Add Set**.
5. Click **Add Samples**.

Figure 4-2 Add Sample Dialog



6. Type the sample information shown in Figure 4-2 and then click **OK**.
7. Type the rack and plate position for the samples shown in Figure 4-3.

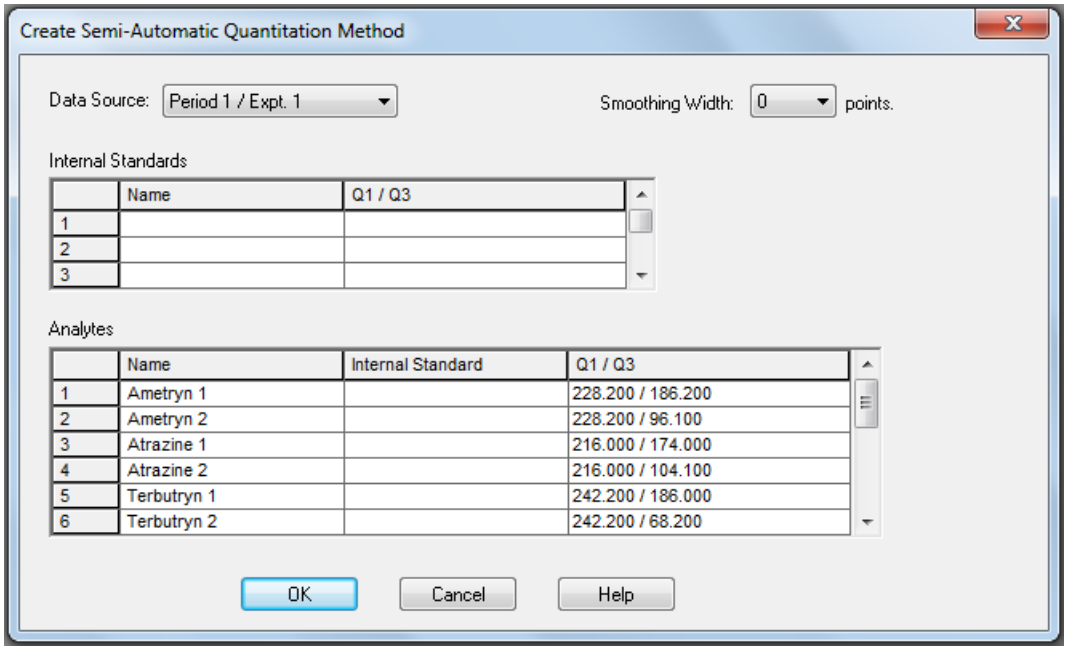
Figure 4-3 Batch Editor—Sample Tab



	Sample Name	Rack Code	Rack Position	Plate Code	Plate Position	Vial Position	Data File
1	blank001	Tray1	1	VT54	1	1	System Integration Test 1
2	blank002	Tray1	1	VT54	1	1	System Integration Test 2
3	blank003	Tray1	1	VT54	1	1	System Integration Test 3
4	10 ng/mL triazine mix_1	Tray1	1	VT54	1	2	System Integration Test 4
5	10 ng/mL triazine mix_2	Tray1	1	VT54	1	2	System Integration Test 5
6	10 ng/mL triazine mix_3	Tray1	1	VT54	1	2	System Integration Test 6
7	blank007	Tray1	1	VT54	1	1	System Integration Test 7
8	blank008	Tray1	1	VT54	1	1	System Integration Test 8
9	blank009	Tray1	1	VT54	1	1	System Integration Test 9

8. In the **Vial Position** column, type the position of the vial containing the test mixture or blank, as required.
9. In rows 4 through 6, edit the **Sample Name** field to read "x ng/mL triazine mix_y", where "x" is the target concentration and "y" is 1, 2, or 3.
10. Make sure that the **Injection Volume** (in the table on the **Sample** tab) is 60 µL.
11. Click **Quick Quant** to open the Create Semi-Automatic Quantitation Method dialog.

Figure 4-4 Create Semi-Automatic Quantitation Method Dialog



12. Select **3** from the **Smoothing Width** list.
13. Click **OK**, and when prompted, type **triazine test** for the method name.

Run the Batch

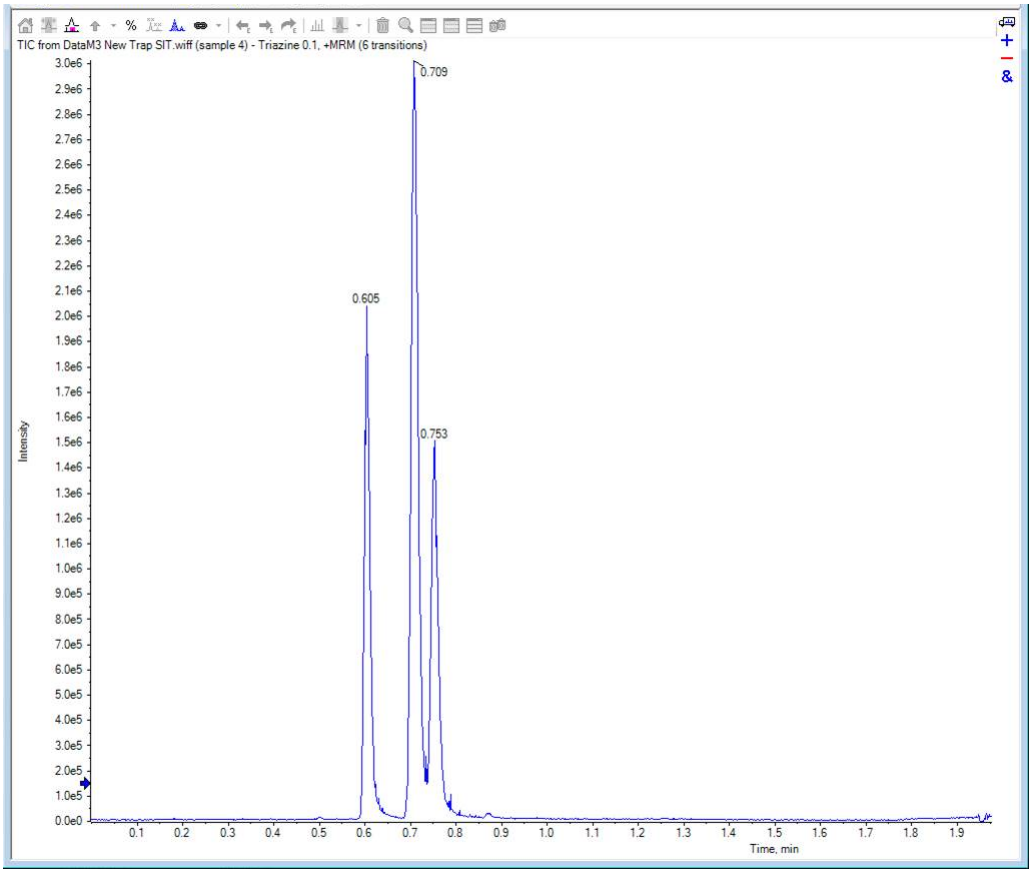
If this is the first time the column has been used, then run the batch once to condition the column and then run it a second time for the test.

1. Click **Submit** on the **Submit** tab.
2. Click **View > Sample Queue.**
3. Verify that the system is not in Tuning mode and then click **Acquire > Ready.**
4. Click **Acquire > Start Sample.**
5. Monitor the system pressure (P_C) in the upper right corner of the Acquisition window of the Eksigent control software.

   During this test, the expected system pressure (P_C) for Gradient 1 should be <4000 psi.
6. (Optional) If the first run was to condition the column, then run the batch again to acquire data for analysis.

   Refer to Figure 4-5 for representative data.

Figure 4-5 Example Chromatogram for the System Integration Test—All XICs

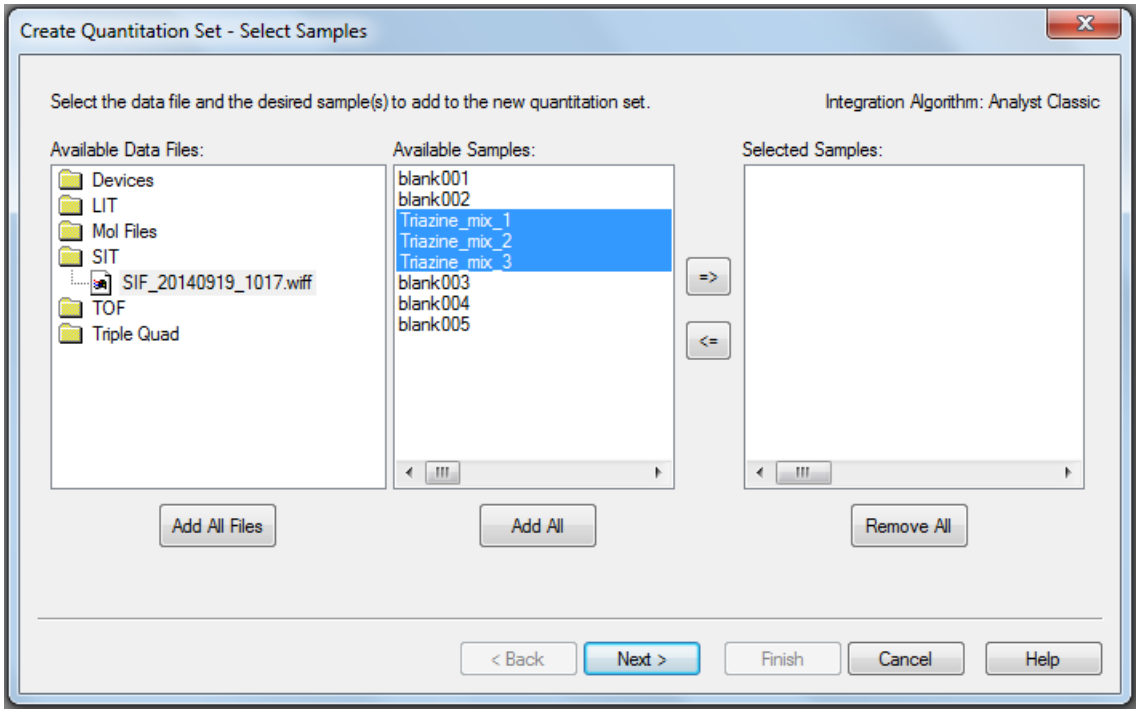


View the Results and Verify the Integration

1. When the run is finished, in the Navigation bar, under **Quantitation**, double-click **Quantitation Wizard**.
2. Select the data file **System Integration Test <date>**.
3. Click **Add All** to move the three injections to the **Selected Samples** list.

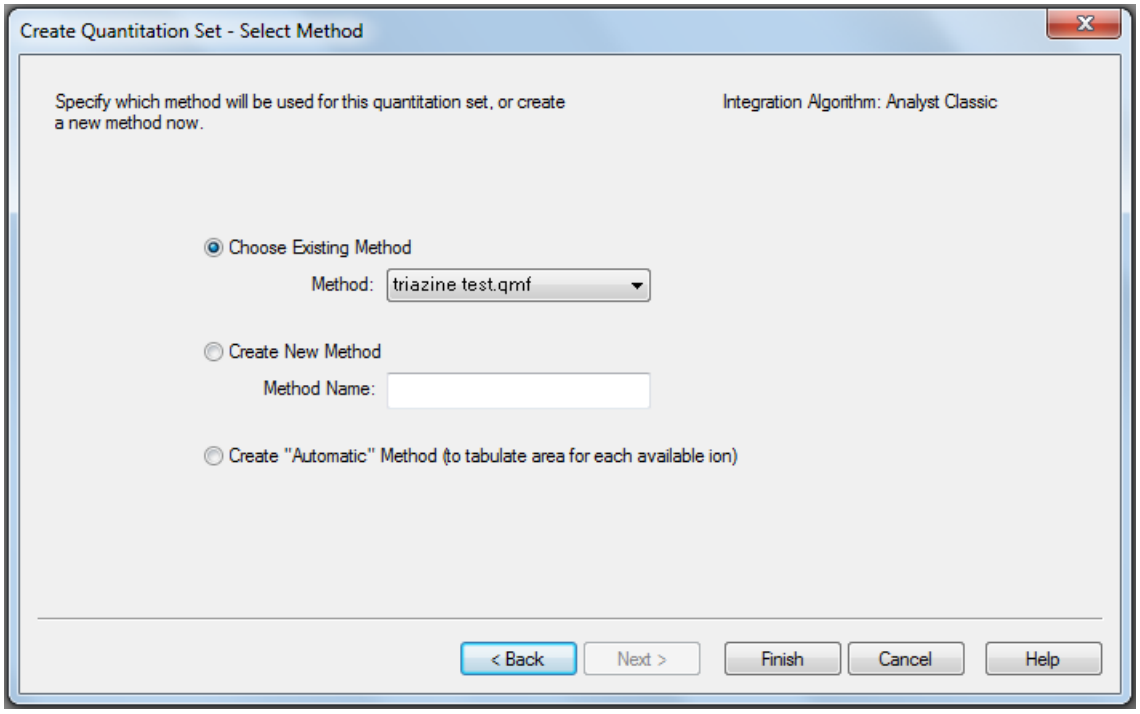
   If a conditioning run was submitted, then make sure that the injections selected are from the test run.

Figure 4-6 Create Quantitation Set—Select Samples Dialog



4. On the Create Quantitation Set - Select Settings & Query dialog, click **Next**.
5. Click **Next.**
6. On the Create Quantitation Set - Select Method dialog, select **Choose Existing Method** and **triazine test** in the **Method** list, and then click **Finish**.

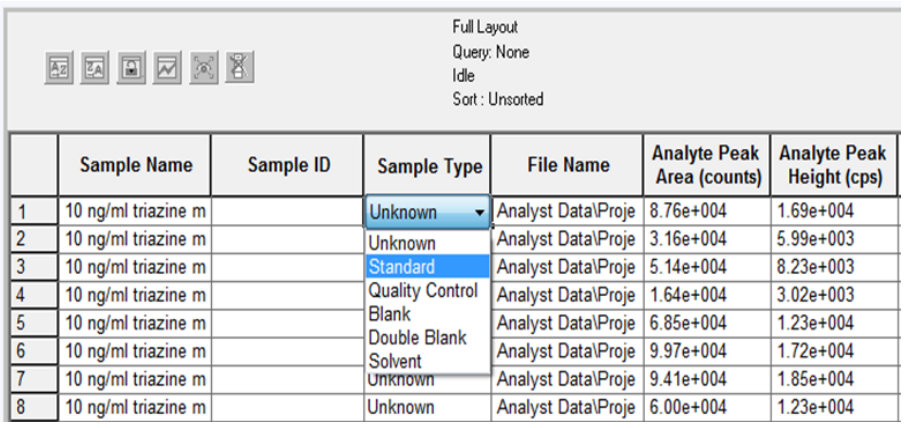
Figure 4-7 Create Quantitation Set—Select Method Dialog



The Results Table opens.

7. Change the **Sample Type** to **Standard**.

Figure 4-8 Results Table—Changing Sample Type



	Sample Name	Sample ID	Sample Type	File Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)
1	10 ng/ml triazine m		Unknown	Analyst Data\Proje	8.76e+004	1.69e+004
2	10 ng/ml triazine m		Unknown	Analyst Data\Proje	3.16e+004	5.99e+003
3	10 ng/ml triazine m		Standard	Analyst Data\Proje	5.14e+004	8.23e+003
4	10 ng/ml triazine m		Quality Control	Analyst Data\Proje	1.64e+004	3.02e+003
5	10 ng/ml triazine m		Blank	Analyst Data\Proje	6.85e+004	1.23e+004
6	10 ng/ml triazine m		Double Blank	Analyst Data\Proje	9.97e+004	1.72e+004
7	10 ng/ml triazine m		Solvent	Analyst Data\Proje	9.41e+004	1.85e+004
8	10 ng/ml triazine m		Unknown	Analyst Data\Proje	6.00e+004	1.23e+004

a. On the first line, change the **Sample Type** to **Standard**.

b. Right-click **Sample Type** and then select **Fill Down**.

8. Right-click inside the margin above the Results Table, and then select **Analyte > Ametryn 1**.

Only the results for Ametryn 1 are shown.

9. Click **Tools > Peak Review > Pane**.

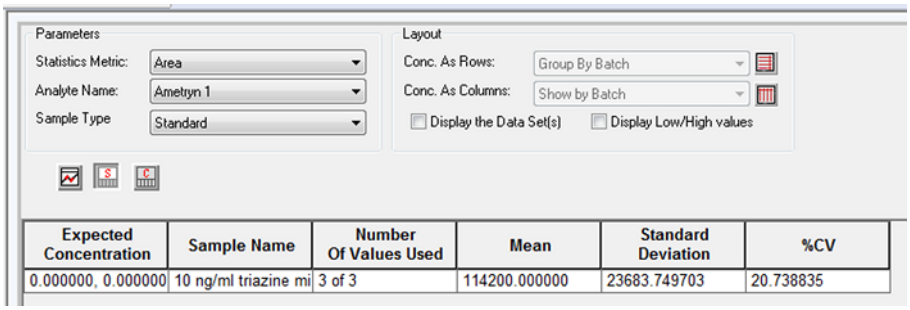
   The chromatograms for the MRM transition are shown below the Results Table.

10. Click the forward and back arrows in the chromatogram pane to view the integration for each chromatogram.
11. After the data has been reviewed, repeat step 8 through step 10 for the Atrazine 1 and Terbutryn 1 transitions.

Verify the Mean Area and %CV

1. After reviewing the integration, click **Tools > Statistics.**
2. In the **Statistics Metric** list, select **Area**.
3. For each of the MRM transitions:
   a. Compare the Mean and %CV values to the specifications in the System Integration Test Data Log and Signoff on page 41.
   b. Type the values in Table 6-4 on page 42.
4. Delete the Statistics Summary pane.

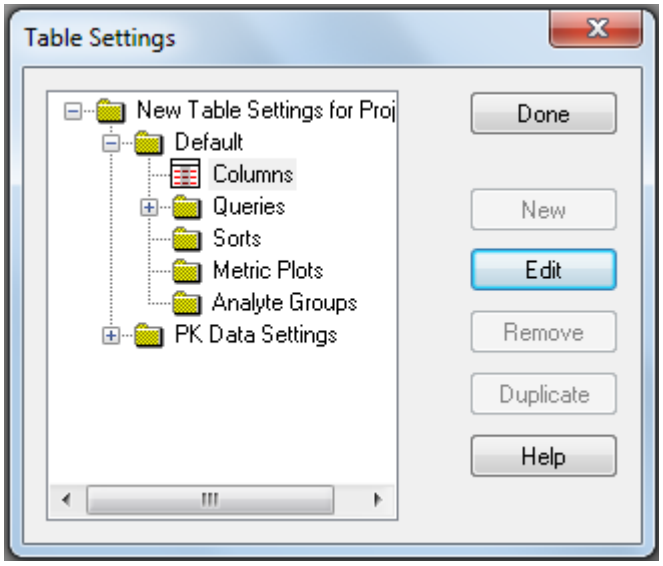
Figure 4-9 Statistics Summary Pane



Verify Peak Widths at Half Height and Retention Times

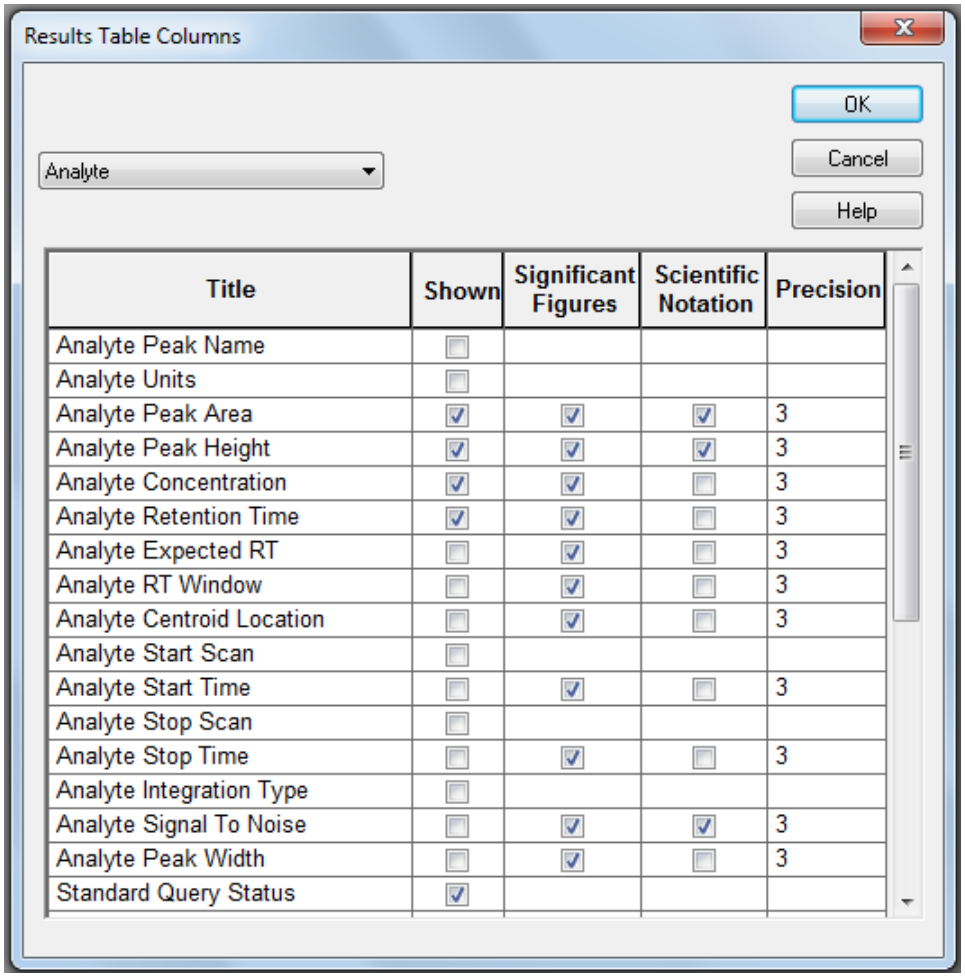
1. Open the Results Table Columns dialog.
2. Right-click on the area above the Results Table and then click **Table Settings > Edit.**
3. In the Table Settings dialog, click **Columns > Edit.**

Figure 4-10 Table Settings Dialog



4. In the Results Table Columns dialog, select the columns to be shown in the table.

Figure 4-11 Results Table Columns Dialog



a. Select **Analyte** in the list.

b. In the **Shown** column of the table, select the **Analyte Retention Time** and **Analyte Peak Width at 50% Height** check boxes.

c. Make sure that the **Analyte Peak Area**, **Analyte Peak Height**, and **Analyte Concentration** check boxes are also selected.

d. Click **OK**.

5. Click **Done.**

   The Results Table updates to show the selected columns.

6. For each MRM transition:

   a. Compare the experimental values with the specifications in the System Integration Test Data Log and Signoff on page 41.

b. Type the values in Table 6-4 on page 42.

Troubleshooting 5

This section provides information for troubleshooting issues with the M3 MicroLC-TE system.

1. Repeat the system integration test. Refer to Perform the System Integration Test on page 26.
2. Examine the chromatograms for the blank injections.
   a. Are there peaks similar to those in the sample? If so, then there is carryover. Complete Identify and Resolve Carryover Issues on page 37 and then repeat the system integration test.
3. Compare the chromatograms for the sample to those from the original system integration test. Figure 5-1 on page 39 shows a chromatogram with good results.
   After each troubleshooting step, repeat the system integration test.
   a. Continue with Resolve General Issues on page 38.
   b. Do the retention times change from injection to injection? Refer to Resolve Drifting Retention Times on page 38.
   c. Are the peaks broader than or tailing more than the original chromatogram? Refer to Peak Widths are Too Broad or are Tailing on page 38.
   d. Is the background level high? Refer to Fronting, Pre-eluting or Low Intensity Peaks on page 38.
   e. Are peaks missing? Refer to Missing or Low Intensity Peaks on page 39.
4. Examine the pressure traces in the Eksigent control software. Refer to Table 5-1 on page 40 for anomalies in the pressure traces and their possible causes.

Identify and Resolve Carryover Issues

For a well-tuned M3 MicroLC-TE system, carryover should be minimal. For a blank, there should be no peaks with retention times matching the sample. When carryover is present, it can cause several other issues, so carryover should be resolved first.

Carryover is most often caused by issues with the injection port or the dynamic load and wash (DLW) system.

Test the Injection Port and the DLW System

- Inspect the injection port and make sure that there is no fluid leaking from the injection port. The presence of fluid at the injection port suggests an issue with the needle alignment at the port position and needle penetration, or the tightness of the fitting and the syringe needle.

- Run a test injection and make sure that the fluid is flowing out the waste line from the valve. To test, create a run with a 100 µL injection that will overfill the loop. During the flow, watch for bubbles exiting the waste line. The presence of bubbles can indicate a poor connection or issue with the operation or priming of the syringe or DLW system.

Resolve General Issues

- Make sure that the correct solvents are being used and that the solvents in the bottle match the settings in the Eksigent control software.
- Make sure that the proper grounding is in place between the ion source and the LC system.

Troubleshoot Peak-Related Issues

Resolve Drifting Retention Times

If the column temperature varies over the course of the test, then retention times might drift.

- Make sure that the column heater is on during the test. The column can take up to 30 minutes to stabilize to the temperature of the column heater.
- Initialize the pressure transducers. Refer to "Re-initialize the Pressure Transducers" in the *Operator Guide*.

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 and around the trap column. A small increase in peak width is often seen when a trap column is used.
- Confirm that a microflow electrode is present in the ion source.

Fronting, Pre-eluting or Low Intensity Peaks

- Make sure that Wash1 and Wash2 on the wash station are configured properly and have the proper solvents. The Wash1 solvent should be organic and the Wash2 solvent should be aqueous.
- Verify that the DLW pumps are operating properly and flowing through the injection valve/port.
- Make sure that the sample loaded by the autosampler is fully flushing the injection loop prior to injection.
- Make sure that the sample has been diluted in the proper solvent (that is, low organic in sample solution).

Missing or Low Intensity Peaks

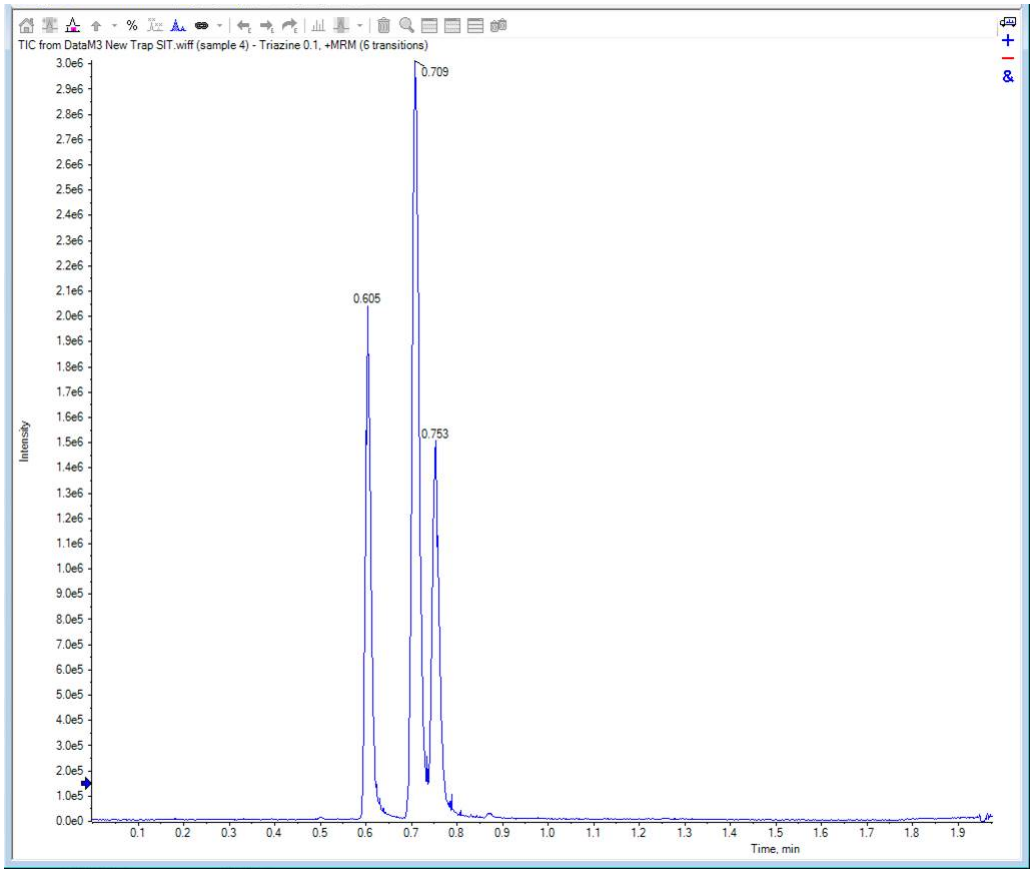
Make sure that the autosampler method has been configured as follows:

- There is a large enough volume loaded into injection loop.
- The **Inject to:** value is set to **LC Vlv1**, not **Waste.**

Make sure that the injection alignment is correct (position and needle penetration) and that the injection port fitting seals on the syringe needle.

Troubleshoot Using the Chromatogram

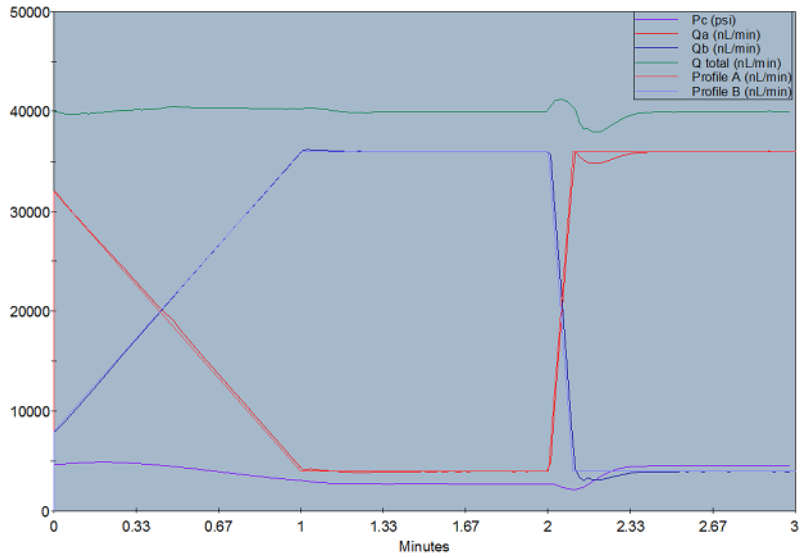
Figure 5-1 Example Chromatogram for the System Integration Test



Troubleshoot Using Pressure and Flow Data

The pressure and flow data files that are automatically saved by the Eksigent control software can be helpful in diagnosing LC issues. Refer to Figure 5-2.

Figure 5-2 Example Pressure and Flow Data for the System Integration Test



Use Table 5-1 to troubleshoot some issues that might occur in the pressure and flow data.

Table 5-1 Common Anomalies in System Pressure Data

Symptom	Possible Cause
Pressure drops at the beginning of the run and then recovers.	Air bubble in the sample loop.
Pressure in both pumps initially increases and then decreases.	Column, probe, or tubing is partially clogged.
Sudden increase in pressure followed by a drop after injection.	Sample solvent is different than the mobile phase.

System Integration Test Data Log and Signoff 6

System Information

Table 6-1 System Information

Model	
Location	
Serial number	

Table 6-2 Ion Source Information

Model	
Serial number	

Table 6-3 Mass Spectrometer Information

Mass spectrometer model	
Mass spectrometer location	
Mass spectrometer serial number	

Test Results

Complete this table with the results for the three analytes from the triazine solution. Refer to Specifications on page 43.

Table 6-4 Test Results

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

Table 6-5 Notes

Specifications

Mean Peak Area Specification

For each analyte, the mean peak area should be ≥ the value in the table below.

Table 6-6 Minimum Mean Peak Area (CPS)

Analyte	3200 1 ng/mL	4000/4500 0.1 ng/mL	5000/5500/6500 0.01 ng/mL
Ametryn 1	8.0×10^4	7.5×10^4	3.5×10^4
Atrazine 1	1.5×10^4	6.5×10^4	3.0×10^4
Terbutryn 1	1.0×10^4	1.0×10^4	5.0×10^4

Area %CV Specification

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

Peak Width Specification

Chromatographic peaks for each analyte should be <0.030 minutes in width when measured at half maximum (peak width at half maximum).

Retention Time Specification

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

Signoff

Organization			
Customer contact name		Date (yyyy-mm-dd)	
Customer contact signature*			
FSE name		Date (yyyy-mm-dd)	
FSE signature			

* Signature required on hard copy only.

Revision History

Revision	Reason for Change	Date
A	First release of document.	October 2015
B	Correction to peak area specification for ametryn 1. Correction to MRM transition in acquisition method.	February 2016
C	Corrections to peak area specifications. Modified final sample dilution step to use water instead of methanol and water. Added fields for system information. Updated images for test results.	August 2016
D	Updated autosampler method parameters for new injection port.	December 2016