SCIEX NanoLC 400 Systems

For Systems Configured for Microflow Flow Rates

System Integration Test





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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 μ L/min to 50 μ L/min flow module (PN 5018238), a 2.7 μ m 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)



System Integration Test



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 μ L 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
------------	--------	--------	--------

Editing: micro400 SIT.AS3 File	<u>_ [] ×</u>
┌─ Injection Type	
 Direct Injection Trap Elute Multiplex 	$[\rightarrow]$
Gradient Pump Channel	
 Gradient Pump 1 Gradient Pump 2 	<u>[</u>]
C Optional Valves C ISS-A Valve C ISS-B Valve C cHiPLC	
Sample Pickup	
💿 μL Pick Up 🛛 🔿 Full Loop	
Sample pick up volume: 2 µL	
Needle height: 2 mm	Loop volume:
r Wait	10 μL
Wait Time: 2 min	Leading Volume: 16.6 μL Trailing Volume:
_ Wash	6.4 μL
Syringe wash cycles: 5 x	
Advanced Editor Test on B1	UK

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

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)

 Table 2-1
 Advanced Editor Autosampler Method Parameters

- 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)

😑 LC Method Settings	×
Name micro 400 SIT	
Summary Run Conditions Gradient Profile Gradient Table	
r Pre-Run	
Flush column for 1 minutes using 100 % initial flowrate conditions.	
First, establish a column pressure of 3000 psi.	
Canala biasting	
C Nee	
 None. Standard: Sample value energy prior to beginning Flow Brafile and remains energy. 	
Metered: Inject nL of sample at 100 % initial flowrate conditions.	
C Rapid: Inject 5000 nL of sample at maximum flowrate, maintaining initial mixture conditions.	
Post-Run	
Flush column for 0.5 minutes using 100 % ending flowrate conditions.	
Delete View Audit Trail OK Cancel	

- 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

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 <i>MRM Transitions for 4000 and 4500 Systems</i> on page 37		
	• Table C-1 <i>MRM Transitions for API 5000™, Triple Quad™ 5500/6500, and QTRAP</i> ® <i>5500/6500 Systems</i> 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		

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

** 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	5000 V	
(ISF)	Q1 Vacuum gauge ~3 x 10 ⁻⁵ Torr	
Ion Source Gas 1 (GS1)	60	

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Table 2-4Analyst 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

Acquisition method	MS Advanced MS
Acquisition Method CTC PAL Autosampler Eksigent 1 Mass Spectrometer 3.001 mins Period 3.001 mins TOF MS (+)	Experiment: 1 IDA Experiment Create IDA Exp Create SWATH** Exp Scan type: TOF MS IDA Experiment Create IDA Exp Create SWATH** Exp Accumulation time: 0.150010 (secs) Min: 100 Max: 1000 Polarity Polarity Regative Polarity Regative Polarity Regative Mass IDA Experiment Create SWATH** Exp Min: IDO Mass: IDO Mass: IDO
	Edit Parameters Duration: 3.001 (mins) Cycle time: 0.1750 (secs) Period:
	2 1001

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.

👪 Analyst - [Acquisition Method:]		
Eile Edit Yiew Acquire Tools Explore	e <u>W</u> indow <u>S</u> cript <u>H</u> elp	
12 🖙 🖬 🖨 🖪 🕹 🖻 🛍 🕰	← ± Acquire Mode	💽 🚰 Quatro\Testing 💽 🙀 🔀 🛅 🗖 🖽 🖪
🛯 🗝 ta 👗 🕹 🕹 🕒 🛄 🖉	в 📱 🖬 🕹 🗠 🖂 🍫 т 😤	
	Acquisition method	Acquisition Method Properties
Security Configuration Hardware Configuration Report Template Editor Compound Optimization M Instrument Optimization If Manual Tuning Acquire [1] Na Method Wizard Build Acquisition Method Build Acquisition Batch Z Express View Explore	Acquisition Method Mass Spec 0.000 min Mass Spec 0.000 min Werid 0.00	Comment: Duration (min): 0.000 Synchronization Mode: LC Sync Auto-Equilibration Image: Comment Synchronization Puration (min): Original Configuration Device methods: Instrument signature: QTRAP 5500 Ion Source: Nanospray Eksigent AS3 Eksigent fradient 1 Instrument signature: QTRAP 5500 Ion Source: Nanospray

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	b
------------	----------------------	----------------	---------------------	---

🔠 Analyst - [Acquisition Method: D:	\Analyst Data\Projects\Quatro\Testing\Acquisitio	on Methods\Operato	rs Guide method]
Eile Edit Yiew Acquire Tools Explor	e <u>W</u> indow <u>S</u> cript <u>H</u> elp		
12 🖻 🖬 🎒 🖪 🖉 🕹 🛍	🔉 🕰 🛓 Acquire Mode 🛛 🛃 🔂 🖓	uatro\Testing	
i 🗝 🗤 🖍 🕹 🕹 🕭 🚨 🕌 🕹	L¥∎&≁¤☆тヾ		
================ ☐ Configure	Acquisition method	Software Applic	cation Properties
	MAcquisition Method Acquisition Method Age Acquisition Method Acquisition Method	Path: Filename:	C:\Program Files\Eksigent NanoLC\settings\EKAS3 direct inject SIT.AS3
《仰》 Tune and Calibrate Compound Optimization AV Instrument Optimization	Eksigent Kasa Eksigent Gradient 1 Eksigent Gradient 2 Eksigent Loading Pump		
n(불 Manual Tuning ☞ Acquire (1)			
TiDA Method Wizard	ent Cradient 4 and then as a	the energy	visto avadiant numer mathed

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** μ L/min.

Figure 2-8 Direct Control Dialog

rirect Control	g for LC Meth	nod		
 Conserved Flow (%): 	A 50	B 50	Total flov 150	vrate: µL/min
O Independent Flow (Q):	75	75	150	µL/min
Monitor Baseline	Start	Stop		
Valve Direct Control - Load P	osition			
Loa	d Position	Inject Pos	tion	
Column Oven / Heater	Setpoint:	35 "	5	
	Start	Stop		
			(Close

- 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.

```
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```



Figure 2-9 Sample Tab—Acquisition Group

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

Figure 2-10 Sample Dialog

Add Sam	ple 🛛 🔀
- Sample Prefix:	name Sample Sample number: Number of digits: 3
Data file Prefix:	Data Set name: Auto Increment:
Sub Fol	der: System Integration Test Browse
New sar Numbe	mples r: 15
	OK Cancel Help

3. Click OK.

Selec Set:	ct Method for Samp	ole Set						
<u>S</u> et:	Sustem Integratio							
<u>S</u> et:	Sustem Integratio			C.	Quantitation			
÷		n Test		~	none		V Quick Qu	ant
	-,							
	A 11 C -1	(Provide Carl)	Acquisitio	on				
	Aga Set	Hemove Set		as Template			No. No. 1	-D
	Add Samples	Del Samples			none		Method E	aitor
	Add 29Tibles	Der Jampies	📃 Use N	Multiple Methods				
atch !	Script:					Select Script		
	Sample Name	Rack Code	Rack Position	Plate Code	Dista Desition			
1				Thate could	Flate Fosition	Vial Position	Data File	Inj.Volume (j
	Triazene 001	Vial Adapter	1	108 vial by row	1	Vial Position	Data File System Integration Test	Inj.Volume () -1.000
2	Triazene 001 Triazene 002	Vial Adapter Vial Adapter	1	108 vial by row 108 vial by row	1 1	Vial Position 1 1	Data File System Integration Test System Integration Test	Inj.Volume () -1.000 -1.000
2	Triazene 001 Triazene 002 Triazene 003	Vial Adapter Vial Adapter Vial Adapter	1 1 1	108 vial by row 108 vial by row 108 vial by row	1 1 1	Vial Position 1 1 1	Data File System Integration Test System Integration Test System Integration Test	10.000 -1.000 -1.000 -1.000
2 3 4	Triazene 001 Triazene 002 Triazene 003 Triazene 004	Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1	108 vial by row 108 vial by row 108 vial by row 108 vial by row	1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test System Integration Test System Integration Test	Inj.Volume () -1.000 -1.000 -1.000 -1.000
2 3 4 5	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005	Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1 1	108 vial by row 108 vial by row 108 vial by row 108 vial by row 108 vial by row	Prace Position 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test System Integration Test System Integration Test System Integration Test	Inj.Volume () -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 6	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006	Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1 1 1	108 vial by row 108 vial by row	Place Position 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test	Inj.Volume (j -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 6 7	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006 Triazene 007	Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	Place Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test System Integration Test System Integration Test System Integration Test System Integration Test	Inj.Volume (j -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 7 7 3	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006 Triazene 007 Triazene 008	Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	Prace Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1	Data File System Integration Test System Integration Test System Integration Test System Integration Test System Integration Test System Integration Test System Integration Test	Inj.Volume (-1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 7 3 3	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006 Triazene 007 Triazene 008 Triazene 009	Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test	Inj.Volume () -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 5 7 7 3 9	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006 Triazene 007 Triazene 007 Triazene 009 Triazene 010	Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test	Inj.Volume (-1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 5 7 7 3 9 10 11	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006 Triazene 007 Triazene 008 Triazene 009 Triazene 010 Triazene 011	Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	I I 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test	hj.Volume (-1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 7 3 3 9 10 11 12	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 005 Triazene 006 Triazene 007 Triazene 008 Triazene 010 Triazene 011 Triazene 011	Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	I I 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test	Inj. Volume (j -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000 -1.000
2 3 4 5 5 7 3 3 10 11 12 13	Triazene 001 Triazene 002 Triazene 003 Triazene 004 Triazene 006 Triazene 006 Triazene 007 Triazene 009 Triazene 010 Triazene 011 Triazene 013	Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	106 vial by row 106 vial by row	I I 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test	hj.Volume (-1.000 -
2 3 4 5 5 6 7 3 9 10 11 12 13 14	Triazene 001 Triazene 002 Triazene 003 Triazene 003 Triazene 005 Triazene 006 Triazene 007 Triazene 007 Triazene 007 Triazene 010 Triazene 011 Triazene 014	Vial Adapter Vial Adapter	1 1 1 1 1 1 1 1 1 1 1 1 1 1	108 vial by row 108 vial by row	I I 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Vial Position 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Data File System Integration Test System Integration Test	hj.Volume () - 1.000 - 1.000

Figure 2-11 Sample Tab—Method Creation

- 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

atch	Editor: [Quatr	o\Testing - N	ew Batch]						
mple	Locations Quar	ntitation Submit							
-	-								
Bate	h <u>U</u> wner name								
abs	ervice						Submit		
_							5		
Subr	nit Status								
Multi	ple samples and or	nly one Data file <	System Integratio	n Test>!					
	Sample Name	Rack Position	Plate Position	Vial Position	Acquisition Method	Quantitation	Data File	Set llame	Submit Stat
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
1	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
3	Triazene 008	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
)	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
	Triazene 013	1	1	1	400 sys config test 032013	none	System Integration Test	System Integration Test	Submitted 1
13					400 mus and fin to at 000040	0000	System Integration Test	System Internation Test	Submitted 1
13	Triazene 014	1	1	11	1400 SYS CONIID LEST 052015	HUUHE	SYSICIT ILCOLOUT CSL	SYSICIT ILCOLOUT CSL	Juonnicou i

- 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.

Create Quantitation Set - Select Sam	iples ample(s) to add to the new quantitat	tion set. Integration Algorithm: IntelliQuan
Available Data Files:	Available Samples:	Selected Samples:
Maintenance PRA ht SelexiON Feb PRA ht SelexiON Feb System Suitability Test O62612_triazine.wff 062612_triazine.wff 062612_triazine.wff Project Information Workspaces Cliquid 3.2 HotFixes to April 201 Config.Msi TI	10 ng/ml triazine mix_01 10 ng/ml triazine mix_02 10 ng/ml triazine mix_02 10 ng/ml triazine mix_03 filer = 2 • III	
	20074	Towners
	< <u>B</u> ack	Next > Anish Cancel Help

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

Create Quantitation Set - Select Method			×
Specify which method will be used for this qua a new method now.	ntitation set, or create	Integration Algorithm: IntelliQuan	
⊙ Choose Existing Metho Method: [tr	azine test.qmf 🔹 👻		
Create <u>N</u> ew Method			
Method Name:			
⊘ Create " <u>A</u> utomatic" Me	thod (to tabulate area for each ava	ilable ion)	
	< <u>B</u> ack <u>N</u> ext >	<u>F</u> inish Cancel	Help

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).

SCIEX NanoLC 400 Systems

		8	ayout : None Unsorted			
	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 Double Blank	Analyst Data\Proje	6.85e+004	1.23e+004
6	10 ng/ml triazine m		Solvent	Analyst Data\Proje	9.97e+004	1.72e+004
7	10 ng/ml triazine m		Unknown	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

Figure 2-16 Results Table—Changing the Sample Type

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

1	Parameters			Layout					_
(Statistics Metric:	Area	-)	Conc. As	Rows:	Group By I	Batch .	-	
	Analyte Name:	Ametryn 1	•	Conc. As	Columns:	Show by B	atch .	- III	
Ш	Sample Type	Standard	•	📃 Disp	lay the Data S	et(s)	🗌 Display Low/High valu	es	
	Expected Concentration	Sample Name	Num Of Value	ber s Used	Me	an	Standard Deviation	%CV	
	0.000000, 0.0000	000 10 ng/ml triazine m	i 3 of 3		114200.00	0000	23683.749703	20.738835	

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

Table Settings	X
Current Table Settings	Done
Queries	New
Metric Plots	Edit
Analyte croups	Remove
	Duplicate
	Help

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

Figure 2-19 Results Table Columns Dialog

Results Table Columns					x
(Analyte				OK Cance Help	4
Title	Shown	Significant Figures	Scientific Notation	Precision	Â
Analyte Peak Name					1
Analyte Units					
Analyte Peak Area	V	V	v	3	
Analyte Peak Height	V	V	V	3	E
Analyte Concentration	1	V		3	
Analyte Retention Time		V		3	
Analyte Expected RT		V		3	1
Analyte RT Window		V		3	
Analyte Centroid Location		V		3	
Analyte Start Scan					1
Analyte Start Time		V		3	1
Analyte Stop Scan					1
Analyte Stop Time		V		3	1
Analyte Integration Type					1
Analyte Signal To Noise		V	V	3	1
Analyte Peak Width		V		3	1
Standard Query Status	V				+

- 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 lons 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 [®] System	able 2-6	XICs to Extract for TripleTO	F [®] System
---	----------	------------------------------	-----------------------

Center	Width	Compound
C9H17N5S	0.2	Ametryn
C8H14CIN5	0.2	Atrazine
C9H16CIN5	0.2	Propazine
C7H12CIN5	0.2	Simazine

The XICs appear (Figure 2-21).



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

- 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

Graph Selection Info		X
Default Info 👻 🐼 🙏		
Selected Start Time: Selected End Time: Selected Points: Min. Intensity: Max. Intensity: Sum Intensity:	0.588 min 0.628 min 199 to 211 735.00 2.304e5 9.288e5	
Peak Time: Peak Width at 50%:	0.604 min 0.012 min	$\mathbf{)}$
Points Across Peak at 50%: Peak Width at Base: Points Across Peak at Base:	4 0.056 min 19	
Peak Area:	1.655e5	\supset

- 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.





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
10010	• •		itoouito

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

Analyte	3200	4000/4500	5000/5500/6500	4600/5600/6600
	10 ng/mL	1 ng/mL	0.1 ng/mL	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

Table 3-2 Instrument Response (cps)

% 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.

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

Торіс	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	Refer to the appropriate section:	
Peak areaPeak width at half-length	Verify the Results for Triple Quadrupole and QTRAP [®] Systems on page 21	
	 Verify the Results for TripleTOF[®] Systems on page 25 	





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

Topics include:

- MRM Transitions on page 35
- MS Information on page 35
- Sample Preparation on page 36

MRM Transitions

Q1	Q 3	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

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

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	10	4
3200 QTRAP system		

System Integration Test

Sample Preparation

Combine the specified amount of the 1 μ g/mL triazine stock solution with 50:50 MeOH:H₂O 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	10 ng/mL	Two serial dilutions
3200 QTRAP system		 100 μL stock solution + 900 μL MeOH:H₂O (to make 100 ng/mL) 100 μL of 100 ng/mL + 900 μL H₂O with 0.1% formic acid

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

Topics include:

- MRM Transitions on page 37
- MS Information on page 38
- Sample Preparation on page 38

MRM Transitions

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

Table B-1 MRM Transitions for 4000 and 4500 Systems

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	СХР
API 4000™ system	10	10
4000 QTRAP [®] system		
SCIEX Triple Quad™ 4500 system		
SCIEX QTRAP [®] 4500 system		

Sample Preparation

Combine the specified amount of the 1 μ g/mL triazine stock solution with 50:50 MeOH:H₂O 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	1 ng/mL	Three serial dilutions:
4000 QTRAP system SCIEX Triple Quad 4500 system SCIEX QTRAP 4500 system		 100 μL stock solution + 900 μL MeOH:H₂O (to make 100 ng/mL) 100 μL of 100 ng/mL + 900 μL MeOH:H₂O (to make 10 ng/mL) 100 μL of 10 ng/mL + 900 μL H₂O with 0.1% formic acid

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

- MRM Transitions on page 39
- MS Information on page 40
- Sample Preparation on page 40

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	СХР
API 5000™ system	10	10
SCIEX Triple Quad™ 5500 system		
QTRAP [®] 5500 system		
QTRAP [®] 6500 system	10	13

Sample Preparation

Combine the specified amount of the 1 μ g/mL triazine stock solution with 50:50 MeOH:H₂O 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	bv	Mass	S	oectrometer
	Gampio	Diracionio	~ j	maoo	~	

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

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

Sample Preparation

Combine the specified amount of the 1 μ g/mL triazine stock solution with 50:50 MeOH:H₂O 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 ₂ O)
TripleTOF 4600 system	10 ng/mL	Two serial dilutions:
TripleTOF 5600/5600+ system TripleTOF 6600 system		 100 μL stock solution + 900 μL MeOH:H₂O (to make 100 ng/mL) 100 μL of 100 ng/mL + 900 μL H₂O with 0.1% formic acid



Plumbing Diagram



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	200-00342		
	(Use tightening tool (PN 200-00356) if necessary)			
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 	205-00069		
	- Orange PEEKsil tubing, 25 μm ID,1/32 inch OD,10 cm*	205-00091		
8	One of the following:			
	 50 µm ID electrode assembly 	5016411		
	 25 µm ID electrode assembly* 	5016874		
9	Grounding cable kit	5016941		
10	Tightening tool	200-00356		

* Recommended for flow rates below 10 $\mu L/min$ to 20 $\mu L/min$ 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



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