

Waters Qualification Workbook

Waters Xevo TQ-S Mass Spectrometer with ACQUITY UPLC I-Class System (MassLynx 4.1)

Operational and Performance Qualification Procedures: OQ/PQ

Workbook Part Number: 715003456 Revision: A

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1. Introduction

This workbook presents the operational and performance qualification procedures for a Waters Xevo® TQ-S mass spectrometer with ACQUITY UPLC® I-Class system, operated by MassLynx™ software, version 4.1 (SCN 843, minimum). It assumes the operational qualification of the mass spectrometer is completed. Consult it when qualifying the system's operation and performance in an ISO 9000-series registered facility or a GxP-regulated environment.

For this operational and performance qualification, the system consists of these instruments and components:

- Waters Xevo TQ-S mass spectrometer
- ACQUITY Binary Solvent Manager (BSM)
- ACQUITY UPLC Sample Manager - Flow Through Needle (SM-FTN) or Sample Manager (SM-FL)

A system can also include one or more of the following optional devices:

- ACQUITY UPLC Tunable Ultraviolet (TUV) Detector
- ACQUITY UPLC Photodiode Array (PDA) Detector (standard PDA or PDA eλ)
- ACQUITY UPLC Column Manager Active (CM-A)
- ACQUITY UPLC Column Heater Active (CH-A)
- ACQUITY UPLC Column Manager Active Auxiliary (CM-Aux)
- IonSABRE™ II API probe.

Requirements:

- A qualified Waters field service engineer must perform all procedures. He or she must consult the Waters Service Notes database to learn of any updates that apply to the instruments, components, and devices listed above.
- Before performing the procedures in this workbook, the performer and reviewer must be familiar with the operation of the system. When necessary, review the appropriate documentation referenced in this workbook and, if applicable, the MassLynx Online Help system.

1.1 Recommended documents

When performing the qualification, in addition to this operational and performance qualification workbook, consult these documents:

- *MassLynx v4.1 Getting Started Guide*, part number 71500113203
- *Waters ACQUITY UPLC I-Class System Documentation CD*, part number 715003160
- *Waters Xevo TQ-S Documentation CD*, part number 715002206
- *Waters Xevo TQ-S Site Preparation Guide*, part number 715002440
- *Waters Xevo TQ-S Quick Reference Card*, part number 715002205
- *Waters Ethernet Instruments Getting Started Guide*, part number 71500074403

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2. Preliminary Procedures

2.1 Identifying performer and reviewer

In Form 1, “Performer and reviewer identification”, of the operational and performance qualification results document associated with this workbook, identify the qualification’s performer and reviewer. The performer must be qualified to perform the qualification and the reviewer qualified to acknowledge that the performer satisfactorily completes the required tasks.

2.2 Identifying system components

Complete Form 2, “Instrument Identification” and Form 3, “Hardware” of the operational and performance qualification results document associated with this workbook.

Note: In Form 2 and in the others that follow, the line labeled “Owner’s ID” refers to a unique identifier that the system owner has assigned to the system undergoing qualification, to differentiate it from all others. If none is assigned, the serial number of the mass spectrometer may be used.

2.3 Recommended materials

You must gather the following items.

Qualification project

Waters Xevo TQ-S Mass Spectrometer with ACQUITY UPLC I-Class System (MassLynx 4.1) OQ/PQ project, available to download from the Qualification section of the Waters Knowledge Center.

Test sample kits

- *Waters Xevo TQ-S Sample Kit*, part number 700005472
- *ESI Probe Assembly Spares Kit*, part number 700003278
- *Waters ACQUITY UPLC System 3-mm and 5-mm Absorbance Test Solutions*, part number 700004380 or
- *Waters ACQUITY UPLC System 10-mm Absorbance Test Solutions*, part number 700002642 or
- *Waters ACQUITY UPLC System 25-mm Absorbance Test Solutions*, part number 700002846

ACQUITY I-Class UPLC equipment

ACQUITY UPLC BEH C18 column, 2.1 mm × 50 mm, 1.7 μm, part number 186002350

ACQUITY I-Class UPLC (TUV and PDA options)

- ACQUITY UPLC TUV flow cell for TUV detectors with serial numbers J05UPT699N or earlier:
 - *Standard flow cell, 10-mm path length*, part number 205015000 or
 - *Low flow, 10-mm path length*, part number 205015001
- ACQUITY UPLC TUV flow cell for TUV detectors with serial numbers J05UPT700N or later:
 - *Stainless-steel TUV flow cell, 5-mm path length*, part number 205000610 or
 - *Analytical flow cell, 10-mm path length*, part number 205015008 or
 - *High-sensitivity flow cell, 25-mm path length*, part number 205015009
- ACQUITY UPLC PDA flow cell for PDA detectors:
 - *HT flow cell, 3-mm path length*, part number 205015006 or
 - *Stainless-steel PDA flow cell, 5-mm path length*, part number 205000612 or
 - *Analytical flow cell, 10-mm path length* part number 205015004 or
 - *High sensitivity flow cell, 25-mm path length*, part number 205015005
- *PEEK™ tubing, 0.005 inch*, part number 430001229

Solvents and additives

- HPLC grade acetone, 250-mL minimum
- LCMS grade acetonitrile, 2.5-L minimum
- LCMS grade methanol, 1-L minimum
- LCMS grade isopropan-2-ol (IPA), 1-L minimum
- LCMS grade water or de-ionized water, 2.5-L minimum
- Formic acid 98-100%
- Ammonia solution 30% w/w

Lab ware

- *Vial holder plate, 48-well, 2-mL*, part number 405003743 (included in sample manager's start-up kit)
- *12 x 32 (384), 2-mL vials, with pre-slit silicone/PTFE septa and caps*, part number 186000307C
- 3 x graduated measuring cylinders, Class A, with stopper, 100-mL, 500-mL, 1000-mL

- Pipettes (calibrated), 10-mL, 1-mL, 200- μ L, and 20- μ L
- Pipette tips appropriate for use with the make and model of the pipette(s)
- 2 x amber glass Class A volumetric flasks with stopper, 50-mL, 100-mL
- Reservoir bottles 1-L (5)
- IntelliStart™ Fluidics system vials (6), 30-mL, with caps (part number 700004256).
- Ultrasonic bath
- Glass funnel
- Vortex mixer
- Nitrile gloves
- Lint-free tissues

Tools

- Calibrated thermometer probe, with display
- Calibrated stop watch
- Pressure restriction device capable of producing backpressure of 500 to 700 psi (34 and 48 bar, 3447 and 4326 kPa)
- Tape, to hold the thermometer probe

2.4 Recording test solutions

Before you prepare samples from the sample kits, record all of their details in Form 4, "Test solutions", of the operational and performance qualification results document associated with this workbook.

2.5 Preparing test samples and mobile phases

Prepare all samples from the solid or liquid phase, and mix them ultrasonically before use. Store all solutions at approximately 4°C. Store no stock solutions longer than two weeks, unless specified otherwise in the procedure.

Follow the preparation procedures in this section for preparing all wash solvents, mobile phases, test samples, diluents and cleaning solutions used for this operational and performance qualification.

Requirement: The procedures contained in the document *Preparing Test Samples/Xevo TQ-S* should not be used in this operational and performance qualification.

2.5.1 Diluents

Water + 0.01% formic acid + 0.05% ammonia solution

To prepare the solution:

Note: If possible, use the original bottle containing the LCMS-grade deionized water for this solution. If this is not practical, use a 1000-mL, Class A, graduated measuring cylinder to measure 1 L of deionized water, and place in a 1-L reservoir bottle.

1. Add 100 μ L formic acid and 500 μ L ammonia solution to the bottle.
2. Label the solution "Water + 0.01% formic acid + 0.05% ammonia solution".
3. Decant approximately 200 mL of this solution into a 250-mL reservoir bottle and label the solution "Diluent".

2.5.2 Test samples

The following solutions are all derived from the *Waters Xevo TQ-S Sample Kit*, part number 700005472.

Table 1 Solutions and target concentrations:

Solutions	Target concentration
Detector linearity (using tuning solution as the stock), carryover test sample	750 fg/ μ L sulfadimethoxine 100 ng/ μ L sulfadimethoxine 100 fg/ μ L sulfadimethoxine 50 fg/ μ L sulfadimethoxine 10 fg/ μ L sulfadimethoxine 5 fg/ μ L sulfadimethoxine 1 fg/ μ L sulfadimethoxine 0.5 fg/ μ L sulfadimethoxine 0.25 fg/ μ L sulfadimethoxine
Tuning solution	20 pg/ μ L acetaminophen 20 pg/ μ L caffeine 10 pg/ μ L sulfadimethoxine 5 pg/ μ L verapamil 5 pg/ μ L chloramphenicol 500 pg/ μ L 17- α -hydroxyprogesterone
Gradient, ESI+ sensitivity and precision, ESI- sensitivity, ACPI+ sensitivity and precision, Injector linearity, Flow rate linearity test sample	0.2 pg/ μ L acetaminofen 0.2 pg/ μ L caffeine 0.1 pg/ μ L sulfadimethoxine 0.05 pg/ μ L verapamil 0.05 pg/ μ L chloramphenicol 5 pg/ μ L 17- α -hydroxyprogesterone.
Injector carryover test sample	100 ng/ μ L sulfadimethoxine in methanol

Tuning solution

This solution is used to generate the MS methods for qualification testing.

To prepare the solution:

Requirement: Before opening the ampoule containing “Xevo TQ-S Tuning solution x100” (part number 700005472-1), sonicate for 3 minutes, and then vortex mix the ampoule. Doing so ensures that the solution is homogenous before sampling.

1. Pipette 1 mL of *Xevo TQ-S Tuning solution x100* (part number 700005472-1) into a 100-mL volumetric flask and dilute to 100 mL with diluent.
2. Stopper and invert the flask several times to fully mix.
3. Label the flask as "Tuning Solution".
4. Transfer 20 mL of Tuning solution to an Intellistart fluidics 20-mL plastic bottle, for Intellistart tuning.

Gradient solution

This solution is used for the Gradient, ESI+ sensitivity and precision, ESI-sensitivity, ACPI+ sensitivity and precision, Injector linearity and Flow rate linearity tests.

To prepare the solution:

1. Place 990 µL of diluent into an ACQUITY vial.
2. Pipette 10 µL of Tuning Solution into the ACQUITY vial.
3. Attach the cap, and vortex mix the vial's contents.
4. Label the vial “Gradient 2:F,8”.

Sample dilution series

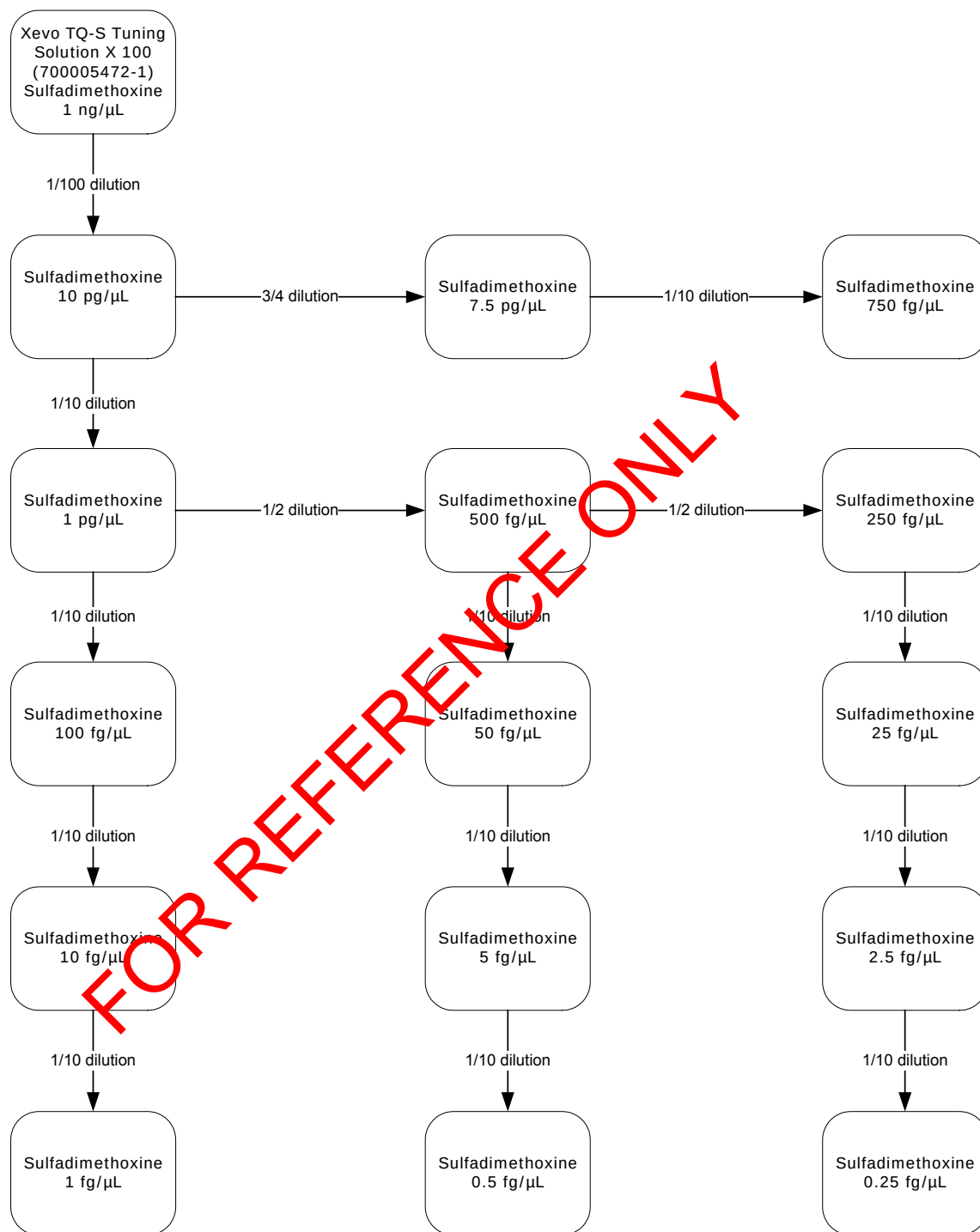
Requirements:

- Sulfamethoxine is light and temperature sensitive. Shield it from light whenever possible.
- Maintain all analytical solutions at temperatures between 2 and 8 °C when not using them.

This sample solution series is used for the MS detector linearity, ESI positive sensitivity, and system precision tests.

Note: Take great care when pipetting solutions. Because concentrations of samples and reagents are low, the accuracy and precision of your pipetting technique significantly affects test results. Refer to Figure 1 for an overview of the procedure.

Figure 1 Sample dilution series overview:



To prepare the sample dilution series:

1. Place 17 ACQUITY vials into an ANSI 48-vial, 2-mL vial holder.
2. Prepare each sample in the following series into a separate vial.

Sulfadimethoxine 10 pg/μL

To prepare the solution:

1. Place 1980 μL of diluent into a vial, and then pipette 20 μL of 1 ng/μL sulfadimethoxine (Tuning solution x 100) into the vial.
2. Attach the cap, vortex mix the vial contents, and then label it "10 pg/μL 2:A,7".

Sulfadimethoxine 7.5 pg/μL

To prepare the solution:

1. Place 250 μL of diluent into a vial, and then add 750 μL of 10 pg/μL sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "7.5 pg/μL 2:C,3".

Sulfadimethoxine 750 fg/μL

To prepare the solution:

1. Place 900 μL of diluent into a vial, and then add 100 μL of 7.5 pg/μL sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "750 fg/μL 2:B,2".

Sulfadimethoxine 1 pg/μL

To prepare the solution:

1. Place 1800 μL of diluent into a vial, and then add 200 μL of 10 pg/μL sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "1 pg/μL".

Sulfadimethoxine 100 fg/μL

To prepare the solution:

1. Place 900 μL of diluent into a vial, and then add 100 μL of 1 pg/μL sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "100 fg/μL 2:B,4".

Sulfadimethoxine 10 fg/μL

To prepare the solution:

1. Place 900 μL of diluent into a vial, and then add 100 μL of 100 fg/μL sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "10 fg/μL 2:B,6".

Sulfadimethoxine 1 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 10 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "1 fg/ μ L 2:B,8".

Sulfadimethoxine 500 fg/ μ L

To prepare the solution:

1. Place 500 μ L of diluent into a vial, and then add 500 μ L of 1 pg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "500 fg/ μ L 2:B,3".
3. Repeat steps 1 and 2 to create another 1 ml of the solution. One vial will be used for injections, the other for creating further dilutions.

Sulfadimethoxine 50 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 500 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "50 fg/ μ L 2:B,5".

Sulfadimethoxine 5 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 50 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "5 fg/ μ L 2:B,7".

Sulfadimethoxine 0.5 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 5 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial, and then label it "0.5 fg/ μ L 2:C,8".

Sulfadimethoxine 250 fg/ μ L

To prepare the solution:

1. Place 500 μ L of diluent into a vial, and then add 500 μ L of 500 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "250 fg/ μ L".

Sulfadimethoxine 25 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 250 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "25 fg/ μ L".

Sulfadimethoxine 2.5 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 25 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "2.5 fg/ μ L".

Sulfadimethoxine 0.25 fg/ μ L

To prepare the solution:

1. Place 900 μ L of diluent in a vial, and then add 100 μ L of 2.5 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "0.25 fg/ μ L 2:D,8".

Blank solution

To prepare the solution:

Place 1000 μ L of diluent into each of 4 vials. Label them "Blank 2:A,1", "Blank 2:A,2", "Blank 2:A,3" and "Blank 2:C,2" respectively.

Stock 200 ng/ μ L sulfadimethoxine solution

This solution is used in the injector carryover test. Perform the following procedure using the ampoule containing 5 mg sulfadimethoxine (700005472-8).

To prepare the solution:

1. Add approximately 4 mL of methanol to the vial, recap, and shake the vial.
2. Use a funnel to transfer the vial contents to a 25-mL volumetric flask.
3. Rinse the vial into the flask using methanol at least three times, to ensure that all the sulfadimethoxine has transferred to the volumetric flask.
4. Dilute to 50 mL using methanol, and sonicate until all the sulfadimethoxine has fully dissolved.
5. Label the flask "200 ng/ μ L Sulfadimethoxine solution".
6. Transfer 1 mL into a vial, and then label it "200 ng/ μ L sulfadimethoxine solution 2:A,8".

After preparing the test samples described above, place each sample vial into the correct location as shown in Table 2.

2.5.3 Mobile phases and wash solvents

Iso-propan-2-ol mobile phase

To prepare the solution:

1. Using a 500-mL, Class A graduated cylinder, measure 250 mL of Iso-propan-2-ol, and add it to a 1-L reservoir bottle.
2. Label the bottle "Iso-propan-2-ol 2:C,1".

Acetonitrile + 0.01% formic acid mobile phase

To prepare the solution:

1. Add 50 µL formic acid to 500 mL LCMS-grade acetonitrile.
2. Label the solution "Acetonitrile + 0.01% formic acid".

100 % Water mobile phase (for UV detectors only)

To prepare the solution:

1. Using a 1000-mL, Class A, graduated measuring cylinder, measure 1 L of LCMS-grade deionized water and place in a 1-L reservoir bottle.
2. Label the solution "Water".

100 % Acetonitrile mobile phase (for UV detectors only)

To prepare the solution:

1. Using a 1000-mL, Class A graduated measuring cylinder, measure 1 L of LCMS-grade acetonitrile and place in a 1-L reservoir bottle.
2. Label the solution "Acetonitrile".

Acidic wash solution

To prepare the solution:

1. Using a 500-mL, Class A graduated measuring cylinder, add 250 mL each of isopropan-2-ol, methanol and acetone into a 1-L reservoir bottle.
2. Measure 225 mL of deionized water, and add to the same 1-L reservoir bottle.
3. Using a 25-mL, Class A graduated measuring cylinder, measure 25 mL of formic acid, and add to the same 1-L reservoir bottle.

Requirement: Do not use the formic acid supplied with the Xevo TQ-S sample kit.

4. Cap the 1-L reservoir bottle, and shake to mix.
5. Sonicate the reservoir bottle for 10 minutes.
6. Label the solution "Acidic wash".
7. Transfer 2 mL to a vial and label it "Acidic wash 2:D,1".

Basic wash solution

To prepare the solution:

1. Using a 500-mL, Class A graduated measuring cylinder, measure 450 mL of deionized water, and transfer to a 1-L reservoir bottle.
2. Using a 50-mL Class A graduated measuring cylinder, measure 50 mL of ammonia solution, and add it to the same 1-L reservoir bottle.

Requirement: Do not use the ammonia solution supplied with the Xevo TQ-S sample kit.

3. Cap the 1-L reservoir bottle, and shake to mix.
4. Sonicate the reservoir bottle for 10 minutes.
5. Label the solution "Basic wash".
6. Transfer 2 mL to a vial and label it "Basic wash 2:D,2".

After preparing the mobile phases and wash solvents described above, place each sample vial into the correct location as shown in Table 2.

Note: If the system includes an optional TUV or FDA detector, the following solutions are required for the UV detector noise and drift tests:

- LCMS-grade deionized water
- LCMS-grade acetonitrile

2.5.4 Vial locations

Table 2 show the correct vial positions for each of the prepared solutions in the ANSI 48-vial, 2-mL vial holder.

Table 2 Sample vial locations:

Sample	Vial location
Blank	2:A,1
Blank	2:A,2
Blank	2:A,3
Blank	2:C,2
Acidic wash solution	2:D,1
Basic wash solution	2:D,2
Isopropan-2-ol	2:C,1
Gradient, ESI+ sensitivity and precision, ESI- sensitivity, ACPI+ sensitivity and precision, Injector linearity, Flow rate linearity	2:F,8
Sulfadimethoxine (standard 10 pg/ μ L)	2:A,7

Sulfadimethoxine (challenge 200 ng/μL)	2:A,8
Sulfadimethoxine 7.5 pg/μL	2:C,3
750 fg/μL sulfadimethoxine	2:B,2
500 fg/μL sulfadimethoxine	2:B,3
100 fg/μL sulfadimethoxine	2:B,4
50 fg/μL sulfadimethoxine	2:B,5
10 fg/μL sulfadimethoxine	2:B,6
5 fg/μL sulfadimethoxine	2:B,7
1 fg/μL sulfadimethoxine	2:B,8
0.5 fg/μL sulfadimethoxine	2:C,8
0.25 fg/μL sulfadimethoxine	2:D,8

Table 3, Table 4 and Table 5 show the correct vial locations for all test samples required for the optional TUV and PDA detector tests.

These samples are provided at the correct concentrations in the sample kits available for each flow cell size.

Requirement: Ensure that you use only the test samples appropriate to the installed flow cell size.

Table 3 Sample concentrations and vial locations for 3-mm and 5-mm flow cells:

Solution	Sample	Vial location
Solution 1	Caffeine 0.0300 mg/mL	2:F,1
Solution 2	Caffeine 0.0900 mg/mL	2:E,2 and 2:F,2
Solution 3	Caffeine 0.1200 mg/mL	For 3-mm: 2:F,3 For 5-mm: 2:D,6, 2:E,6, 2:F,3
Solution 4	Caffeine 0.1500 mg/mL	2:D,4, 2:E,4, 2:F,4
Solution 5	Caffeine 0.2400 mg/mL	2:F,5
Solution 6	Caffeine 0.3000 mg/mL	For 3-mm: 2:D,6, 2:E,6, 2:F,6 For 5-mm: 2:F,6
Solution 7	Caffeine 0.4800 mg/mL	2:F,7
Solution 8	Not used	Not used
Solution 9	Not used	Not used
Solution 10	Not used	Not used
Solution 11	Blank: Diluent	2:A,1, 2:A,4, 2:A,5, 2:A,6, 2:B,1

Note: The label names above correspond to those in the *Waters ACQUITY UPLC System 3-mm and 5-mm Absorbance Test Solutions Kit*, part number 700004380.

Table 4 Sample concentrations and vial locations for 10-mm flow cells:

Solution	Sample	Vial location
Solution 1	Caffeine 0.0100 mg/mL	2:F,1
Solution 2	Caffeine 0.0300 mg/mL	2:E,2, 2:F,2
Solution 3	Caffeine 0.0400 mg/mL	2:F,3
Solution 4	Caffeine 0.0500 mg/mL	2:D,4, 2:E,4, 2:F,4
Solution 5	Caffeine 0.0800 mg/mL	2:F,5
Solution 6	Caffeine 0.1000 mg/mL	2:D,6, 2:E,6, 2:F,6
Solution 7	Caffeine 0.1600 mg/mL	2:F,7
Solution 8	Not used	Not used
Solution 9	Not used	Not used
Solution 10	Not used	Not used
Solution 11	Not used	Not used
Solution 12	Blank: Diluent	2:A,1, 2:A,4, 2:A,5, 2:A,6, 2:B,1

Note: The label names above correspond to those in the *Waters ACQUITY UPLC System 10-mm Absorbance Test Solutions Kit*, part number 700002642.

Table 5 Sample concentrations and vial locations for 25-mm flow cells:

Solution	Sample	Vial location
Solution 1	Caffeine 0.0040 mg/mL	2:F,1
Solution 2	Caffeine 0.0120 mg/mL	2:E,2, 2:F,2
Solution 3	Caffeine 0.0160 mg/mL	2:F,3
Solution 4	Caffeine 0.0200 mg/mL	2:D,4, 2:E,4, 2:F,4
Solution 5	Caffeine 0.0320 mg/mL	2:F,5
Solution 6	Caffeine 0.0400k mg/mL	2:D,6, 2:E,6, 2:F,6
Solution 7	Caffeine 0.0640 mg/mL	2:F,7
Solution 8	Not used	Not used
Solution 9	Not used	Not used
Solution 10	Not used	Not used
Solution 11	Not used	Not used
Solution 12	Blank: Diluent	2:A,1, 2:A,4, 2:A,5, 2:A,6, 2:B,1

Note: The label names above correspond to those in the *Waters ACQUITY UPLC System 25-mm Absorbance Test Solutions Kit*, part number 700002846.

2.6 Recording test equipment calibration information

When an instrument undergoing operational qualification requires the use of calibrated equipment, record the calibration details in Form 5, "Calibrated equipment," of the operational and performance qualification results document associated with this workbook.

2.7 Creating the qualification project in MassLynx software

The qualification project files required for the qualification procedures described in this document are located in the "Xevo TQ-S with I-Class Project Files" folder on the Qualification section of the Waters Knowledge Center.

To create the qualification project in MassLynx software:

Requirement: Use the Xevo_TQS_ACQUITY_ICLASS_OQPQ.PRO template to create the qualification project. The template contains the sample lists and method files required for the qualification procedure.

1. From the Qualification section of the Waters Knowledge Center, download and import all the required files.
2. Copy the project folder (Xevo_TQS_ACQUITY_ICLASS_OQPQ.PRO) to C:\MassLynx.
3. From the MassLynx window, select File > Project Wizard.
4. Open the Create Project menu, and enter details while observing the following conventions:
 - Name: Xevo_TQS_ACQUITY_ICLASS_OQPQ_WAAXXX
 - Description: Xevo_TQS_ACQUITY_ICLASS_OQPQ_WAAXXX_NNN
 - Location: C:\MassLynx
5. Click Next, and then select "Create using existing project as template".
6. Select Xevo_TQS_ACQUITY_ICLASS_OQPQ.PRO as the existing project.
7. Select Copy Sample lists.
8. Click Finish.
9. Name the Acidic_Basic_IClass_Clean_DDMMYY.spl sample list, using the serial number and current date, and then save the sample list.
10. Ensure the newly created sample list is open on the MassLynx window.

To load the sample list format:

1. Click Samples > Format > Load >, and browse to the following folder:
C:\MassLynx\XEVO_TQS_ACQUITY_IClass_OQPQ_WAAXXX.PRO\SampleDB.
2. Select the Xevo_TQS_UPLC.fmt file.

2.8 Managing data files and test results

Once you create the qualification project, apply the following conventions when naming acquisition files, data files, and recording test results.

2.8.1 Renaming acquisition files

Rename the default project file names within the MassLynx sample list after loading them from the qualification project:

To rename acquisition files:

Rename the files, using the format “NAME_XXX_DDMMMYYYY_NNN”, where:

- “NAME” is the abbreviated test name;
- “XXX” is the instrument's serial number;
- “DD” is the current day;
- “MMM” is the current month, formatted as text;
- “YYYY” is the current year;
- “NNN” is the acquisition number of that test. Use leading zeros when appropriate, for example, “003” for the third acquisition.

2.8.2 Managing raw data files

Save all raw data files generated during the operational qualification, ensuring that the following conditions are met.

- Do not delete or alter the predefined test information contained in the qualification project files.
- When editing raw data file names, apply the conventions described in section 2.8.1.
- Rename the raw data file names where tests are repeated by incrementing the acquisition number.
- Do not overwrite file names.

2.8.3 Recording test results

All test results must be recorded in the operational and performance qualification results document associated with this workbook. If you are instructed to save test results as raw data files, follow the conventions described in section 2.8.2. If you are instructed to create additional report or screenshot printouts, ensure they are correctly labeled.

To label printouts:

Label each printout with the following information:

- Instrument serial number
- Performer's initials and date

- Test procedure reference name
- Sample solution
- Concentration
- Flow rate
- All calculations

Once labeled, append all printouts to the operational and performance qualification results document associated with this workbook.

FOR REFERENCE ONLY

3. Setting Up

To prepare the system for qualification, perform the procedures listed in this section before proceeding with the operational and performance qualification procedures.

Note: The following procedures assume the operational qualification of the mass spectrometer is completed.

3.1 Setting up the ACQUITY UPLC I-Class

To configure the instrument:

1. In the MassLynx toolbar, click Inlet Method > Tools > Inlet Configuration > Setup Instrument.
2. Select the following items if present in the system:
 - Binary Solvent Manager (BSM)
 - Sample Manager - Flow Through Needle (SM-FTN)
 - ACQUITY PDA Detector (optional)
 - ACQUITY TUV Detector (optional)
 - Column manager (CM-A)

Note: If the system includes a Sample Manager – Fixed Loop (SM-FL), select Sample Manager (SM) in the configuration.

3. In the shortcut bar, click > Inlet Method > Tools > Inlet configuration > Events & Triggering.
4. Ensure that Event 1 is selected, and then click Next > Finish.
5. Close the MassLynx application, and then reboot the host PC when the inlet configuration ends.

Rationale: Doing so updates the DHCP.xml file, so that the ACQUITY function is available in the MS Console.

To verify the rack setup in the Inlet Method editor:

1. In the MassLynx application, select Inlet Method > ACQUITY Sampler > Plate Generator.
2. In the Rack Generator menu bar, click the Previous Rack arrow icon until ANSI-48Vial2mLHolder appears in the Rack Name field.
3. In the Grid Reference pane Referencing list, select XY.
4. In the Grid Reference area, select the parameters shown in Table 6.

Table 6 Grid reference parameters:

Parameter	Setting
Horizontal	1 2 3...
Vertical	A B C ...
Horizontal First	Deselected

- Click Rack > Save Current Rack > OK.
- Click Save and close.

3.2 Preparing the ACQUITY UPLC I-Class for qualification

To prepare wash solvents (SM-FTN):

- Submerge the needle wash line into the reservoir labeled "Water".
- Submerge the seal wash line into the reservoir labeled "Water".
- Submerge the wash line into the reservoir labeled "Acetonitrile".

To prepare wash solvents (SM-FL):

- Submerge the weak wash line into the reservoir labeled "Water".
- Submerge the seal wash line into the reservoir labeled "Water".
- Submerge the strong wash line into the reservoir labeled "Acetonitrile".

To prepare priming solvents (BSM):

- Submerge the A1 line into the reservoir labeled "Water".
- Submerge the A2 line into the reservoir labeled "Water".
- Submerge the B1 line into the reservoir labeled "Acetonitrile".
- Submerge the B2 line into the reservoir labeled "Acetonitrile".

To prime the BSM:

- In the ACQUITY Console, click Binary Solvent Manager > Control > Prime A/B Solvents.
- In the displayed dialog box, select A1 and B2. In the Time field, specify 5 minutes, and then click Start.
- Click Binary Solvent Manager > Control > Prime A/B Solvents.
- In the displayed dialog box, select A2 and B1. In the Time field, specify 5 minutes, and then click Start.
- Click Binary Solvent Manager > Control > Prime Seal Wash, and then click Yes.

To prime and equilibrate the SM-FTN:

Requirement: The qualification tests require the 15- μ L needle and 100- μ L syringe. Ensure that these are installed before proceeding*.

1. In the ACQUITY Console, click Sample Manager > Control > Prime.
2. In the Prime dialog box, select Wash Solvent and Purge Solvent.
3. In the Wash Solvent field, enter 60 seconds.
4. In the Purge Solvent field, enter 20 cycles, and then click OK.
5. Select Sample Manager from the console tree, and then click Configure > Volumes.
6. Verify that the displayed needle size matches the installed needle size.

To prime and equilibrate the SM - FL:

Requirement: The qualification tests require the 10- μ L needle, 100- μ L syringe, and 10- μ L sample loop. Ensure that these are installed before proceeding*.

1. In the ACQUITY Console, select Sample manager > Control > Prime.
2. In the Prime dialog box, select "Sample syringe and wash syringes".
3. In the Number of cycles field, specify 10, and then click OK.
4. Select Sample Manager from the console tree, and then select Configure > Volumes.
5. Verify that the displayed loop size matches the installed sample-loop size.

To characterize needle seal and loop volumes:

1. Perform the necessary characterization procedure for the installed sample manager:
 - a. For SM-FTN, characterize the needle seal, by selecting Sample Manager > Characterize > Needle Seal, and then click OK.
 - b. For SM-FL, characterize the needle and loop volumes, by selecting Sample Manager > Characterize > Needle and loop volumes.

Requirement: Characterizing the needle and loop volumes is critical to acceptable performance.

2. Ensure the nominal configuration settings are correct for your system, and then click Close.

*The required needle, syringe and sample loop sizes are included in the standard configuration for these devices as shipped.

3.3 Manual operational qualification tests

Before running the operational and performance qualification tests, you must first run these manual operational qualification tests for the ACQUITY UPLC I-Class, recording results when instructed to do so.

3.3.1 Solvent manager

Using a 10-mL volumetric flask, test the flow rate accuracy of the Binary Solvent Manager (BSM) pump at 0.5 mL/min. The acceptable flow rate value includes the volumetric tolerance of $\pm 0.2\%$ for the 0.5-mL flow rate. Only one flow rate is verified for accuracy because a flow rate linearity test is also performed in this workbook.

Test specification: The flow rate accuracy must be between 0.494 and 0.506 mL/min, and the backpressure on the sample tubing must be within the range 500 to 700 psi (31 to 48 bar, or 3447 to 4826 kPa).

To test flow rate accuracy:

1. Connect a length of stainless steel tubing, or another restriction device, to the outlet of the BSM's mixer to create backpressure between 500 and 700 psi (34 and 48 bar, 3447 and 4826 kPa) at 0.5 mL/min.
2. Obtain a 10-mL, Class A volumetric flask, and ensure that it is thoroughly dry.
3. Prepare the stopwatch to begin timing.
4. In the MassLynx Inlet Method window, click File > Open, and then select Flow Rate Accuracy.
5. Click LC > Load Method.
6. Open the ACQUITY console window.
7. Click ACQUITY Binary Solvent Manager (BSM) > Control > Set Flow.
8. To test the A pump, in the ACQUITY BSM Console window enter 0.5mL/min for the flow rate and 100% A1, and then click Enter.
9. When the flow and pressure are stable, insert the outlet tubing into the volumetric flask, and simultaneously start the stopwatch.
10. Stop the stopwatch when the bottom of the meniscus reaches the 10-mL volumetric mark on the flask.
11. Record the elapsed time, in seconds, and the observed system pressure in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
12. Calculate the flow rate using the following formula:

$$\text{Calculated flow rate} = \text{volume of flask}/(\text{measured time in seconds}/60).$$

Requirement: The calculated flow rate must lie in the range 0.494 through 0.506 mL/min.

13. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

3.3.2 Sample manager

Use this procedure to test the temperature accuracy of the Sample Manager – Flow Through Needle (SM-FTN) or Sample Manager – Fixed Loop (SM-FL).

Requirement: The sample manager temperature must be within ± 4 °C of the set temperature. This acceptable range accounts for inaccuracy of the temperature sensor at ± 3 °C and the measurement device at ± 1 °C. If the measurement device accuracy exceeds ± 1 °C, adjust the acceptable range accordingly, and then record the adjustment in the comments section of the operational and performance qualification results document associated with this workbook.

To test the temperature accuracy of the sample manager,

1. Remove all plates from the sample manager's sample compartment.
2. Place a calibrated thermometer probe in the sample compartment.

Note: Steady the thermometer probe in the sample compartment by positioning directly adjacent to the device's temperature sensor (see Figure 2). Ensure the thermometer probe does not contact any surfaces.

Figure 2 Positioning the thermometer probe in the SM-FTN or SM-FL:



Thermometer attachment to R-carriage.

3. Close the sample compartment door, being careful to avoid damaging the probe wire.

4. On the ACQUITY Console screen for the sample manager, specify a set-point temperature.

Note: Select your standard operating temperature or a temperature as low as 4 °C, though not exceeding 20 °C below the ambient temperature.

5. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the set-point value you specified in step 4.

Recommendation: Allow sufficient time for the sample compartment temperature to reach and stabilize at the set-point temperature.

6. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the temperature you observe from the thermometer probe.

Note: The time required to reach the set temperature varies depending on the difference between the set and ambient temperatures. For example, when starting from room temperature, the sample compartment can require up to 90 minutes to reach 4 °C.

7. Optionally repeat steps 4 through 6, specifying a different set-point temperature.
8. At the end of the temperature accuracy test, return the sample manager's temperature to 7 °C.

3.3.3 Sample organizer

Use this procedure to test the temperature accuracy and plate positioning of the Common Platform Sample Organizer (CP-SO).

Requirement: The sample compartment temperature must be between -2°C and +4 °C of the set-point temperature.

To test the sample organizer temperature accuracy:

Note: The intention of step 1 is to position the thermometer probe wire in the sample organizer. Depending on the design of your thermometer probe, you can use an alternate method to position the probe wire.

1. Open the sample organizer's door and position the thermometer probe wire on the X-transfer shuttle mechanism so that the probe tip extends one inch (2.54 cm) beyond the rear of the mechanism. Use tape to hold the probe in place (see Figure 3).

Note: Avoid placing the thermometer probe wire in the center of the X-transfer shuttle mechanism, to prevent activating the plate over-travel sensor.

2. Close the sample compartment door.
3. In the ACQUITY Console screen for the sample organizer, specify a set-point temperature.

4. Select your standard operating temperature, or a temperature as low as 4 °C (not to exceed 20 °C below ambient), and record this set-point temperature in Form 6, "Operational and performance qualification results" of the operational and performance qualification results document associated with this workbook.
5. Allow sufficient time for the plate compartment temperature to reach and stabilize at the set-point temperature.

Figure 3 Positioning the thermometer probe in the sample organizer:



6. Record the temperature you observe from the thermometer probe in Form 6, "Operational and performance qualification results" of the operational and performance qualification results document associated with this workbook.

Note: The time required to reach the set-point temperature varies depending on the difference between the set-point and ambient temperatures. For example, the plate compartment can require up to 90 minutes to reach 4 °C when starting from room temperature.

7. Optionally repeat steps 3 through 6, specifying a different set-point temperature.
8. When the test is completed, remove the thermometer probe wire from the sample organizer.

Testing the sample organizer plate positioning

Requirement: Each plate must correctly transfer from the sample organizer to the sample manager and back to its original position in the sample organizer.

To test the sample organizer plate positioning:

1. Ensure no plates are in the sample manager.
2. Load the sample organizer with 4 ANSI sample plates.

Requirement: Load 1 plate at the top position, another at the bottom position, and the remaining 2 plates at random positions between the top and bottom.

3. Record the four plate positions in Form 6, "Operational and performance qualification results" of the operational and performance qualification results document associated with this workbook.
4. Close the sample compartment door.
5. In the MS Console, click Sample Organizer > Configure > Scan and Store Shelf Layout.
6. In the MS Console, click Sample Organizer > Interactive Display > Control, to activate the sample organizer controls.
7. Click the label that designates the position of the top shelf (e.g. topshelf_10).
8. In the Transfer to Sample Manager dialog box, click Yes.
9. Ensure the plate on the designated shelf is transferred from the sample organizer to the sample manager.
10. In the ACQUITY Console screen, click the label that designates the position of the top shelf.
11. In the Transfer to Shelf dialog box, click Yes.
12. Ensure the plate transfers from the sample manager to its original shelf position in the sample organizer.
13. Repeat steps 7 through 12 for the remaining three plate positions.
14. Record the results in Form 6, "Operational qualification procedure" of the operational and performance qualification results document associated with this workbook.

3.3.4 Column heater

Use this procedure to test the temperature accuracy of the Column Heater-Active (CH-A), which holds a single column.

Requirement: The column heater temperature must be within ± 2 °C of the set-point temperature. This acceptable range accounts for inaccuracy of the temperature sensor at ± 1 °C and the measurement device at ± 1 °C. If the measurement device accuracy exceeds ± 1 °C, adjust the acceptable range accordingly, and then record the adjustment in the comments section of the operational and performance qualification results document associated with this workbook.

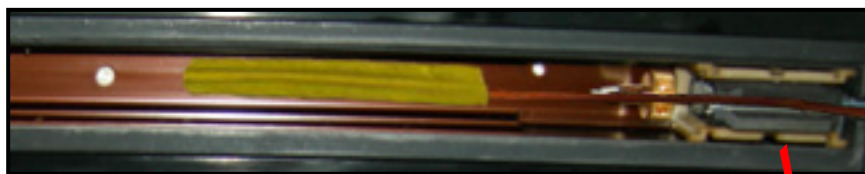
To test the temperature accuracy of the column heater:

1. Depending on your type of column heater, either remove the column heater's front panel, which is held by magnets, or swing open its front panel.
2. Tilt the column tray downward.
3. Remove the column, if installed.

4. Place a calibrated thermometer probe in the middle of the column tray. Position the thermometer probe so that it is approximately 1 to 1.5 inches (2.5 to 3.8 cm) to the right of the silver dot in the column tray.
5. Use tape to hold the thermometer probe against the tray.

Requirement: The thermometer probe must touch the inside of the column tray (see Figure 4).

Figure 4 Column tray showing silver dot:



6. Tilt the column tray upward to the closed position, and route the wire from the thermometer probe through the notch on the right-hand side of the column tray (see Figure 5).

Figure 5 Routing the thermometer probe wire through the notch:



7. Depending on your type of column heater, refit the front panel, or swing it closed.
8. From the ACQUITY Console screen for the CH-A, specify a set-point temperature from 5 °C above ambient temperature through 65 °C.
9. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the set-point temperature you specified in step 8.

Recommendation: Allow sufficient time for the column heater's temperature to reach and stabilize at the set-point temperature.

10. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the temperature you observe from the thermometer probe.

Note: The time required to reach the set-point temperature varies depending on the difference between the set-point and ambient temperatures.

11. Optionally repeat steps 8 through 10, specifying a different set-point temperature.
12. After completing the temperature accuracy test, return the column heater temperature to 40 °C.

3.3.5 Column manager

Use this procedure to test the temperature accuracy of the Column Manager–Active (CM-A).

Requirement: The column manager temperature must be within ± 2 °C of the set temperature. This acceptable range accounts for inaccuracy of the temperature sensor at ± 1 °C and the measurement device at ± 1 °C. If the measurement device accuracy exceeds ± 1 °C, adjust the acceptable range accordingly, and then record the adjustment in the comments section of the operational and performance qualification results document associated with this workbook.

To test the temperature accuracy of the column manager:

1. Swing open the front panel.
2. Remove the columns, if installed.
3. Place a calibrated thermometer probe in the upper compartment.
4. Position the probe so it is directly in the centre of the 4 screws (see Figure 6).

Figure 6 CM-A with front cover removed:



5. Ensure the probe's tip touches the inside of the column compartment by using tape to hold the tip against the compartment (see Figure 7).

Figure 7 Installing the thermometer probe in the column manager:



6. Close the front panel.
7. On the ACQUITY Console screen for the column manager, specify a single set-point temperature from 10 °C through 90 °C.

Requirement: Do not specify a set-point temperature that falls within ± 5 °C of the ambient temperature. (Most users choose a temperature that corresponds to the temperature they most frequently use, or one at the extreme end of the range: for example, 10 °C or 90 °C).

8. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the temperature set-point value you specified in step 7.

Recommendation: Allow sufficient time for the device's temperature to reach and stabilize at the set-point temperature.

9. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the temperature you observe from the thermometer probe.

Note: The time required to reach the set-point temperature varies depending on the difference between the set-point and ambient temperatures.

10. Complete Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
11. Optionally repeat this test, using a different set-point temperature to test each column compartment.
12. At the end of the temperature accuracy test, return the column manager temperature to 40 °C.

3.4 Additional system setup procedures

To install the ACQUITY UPLC BEH C18 column:

1. Install the ACQUITY UPLC BEH C18 column in the CH-A or CM-A.
2. Attach the eCord™ to the eCord receptacle on the the CH-A or CM-A.
3. Ensure that the inlet preheater tubing is connected to the column, and then fit the front cover onto the CH-A or CM-A.

To connect the ACQUITY UPLC I-Class to the mass spectrometer diverter valve:

Requirement: All connection tubing, waste lines and transfer lines for the ACQUITY UPLC I-Class and Xevo TQ-S must be the same lengths, diameters, and fittings as those configured during installation.

1. Connect the outlet from the CH-A or CM-A to the 4-way diverter valve inlet (center position, port 1) on the Xevo TQ-S using black PEEK™ (0.004 inch internal diameter) tubing.

Note: If a TUV or PDA detector is included in the system configuration, also connect the flow cell outlet to the 4-way diverter valve inlet as described in step 4.

2. Connect the remaining diverter valve connections (sample probe, lock-spray probe, and waste) in accordance with the operator's guide for the instrument.

To initialize the ACQUITY UPLC I-Class method, and equilibrate:

1. In the MassLynx Tune window's Fluidics tab, set the flow state to Waste.
2. In the Inlet Method editor, click File > Open, and select the isocratic_0_6 Method.
3. Click Load Method .

Result: The flow begins at 600 µL/min, and either the PDA or TUV lamp ignites, if configured.

4. Allow sufficient time for the column temperature and sample manager temperature to stabilize.

Note: Allow either 30 minutes (TUV) or 60 minutes (PDA) for the lamp to warm up (if configured).

To load the sample list and sample list format:

1. In the MassLynx main window, click File > Open.
2. Click MS_Test_Injections.spl.

4. Operational and Performance Qualification Procedures

To qualify operation and performance of the system, perform the following procedures and verify their completion by completing Form 6, "Operational qualification results" of the operational and performance qualification results document associated with this workbook.

4.1 Determining noise and drift on the Acquity PDA and PDA eλ detector (optional)

Requirement: If your system includes an ACQUITY PDA or PDA eλ detector, you must perform this test.

The noise and drift tests are run from the ACQUITY Console. Both tests are compliant with the ASTM (E685-79) short-term noise and detector drift specifications. The noise test is an average of segmented peak to peak noise in the detector signal over a specified time. The drift test monitors the change in the baseline value over time, expressed as the slope of a linear regression line fitted to the baseline from start time to the stop time. Both are wet tests.

Requirements:

- Use the following mobile phases for this test:
 - 100% water (solvent line A1, solvent line A2, Seal wash and SM Purge)
 - 100% acetonitrile (solvent line B1, solvent line B2 and SM-Wash)
- Both tests require mobile phase to be flowing through the flow cell.
- The mobile phase must not contain any additives or modifiers.

To determine the PDA/PDA eλ noise and drift:

1. From the Inlet Method window, open the Noise method, and then click Load Method.

Result: The previous step ensures that the system is at the correct UPLC conditions for the test runs.

2. Allow the UPLC equilibrate for 10 minutes before continuing with the test.
3. From the ACQUITY Console, select PDA Detector from the console tree.
4. Click the Troubleshoot tab, select Service mode, and then enter the service password.
5. Under the troubleshooting tab, select Noise and Drift > Start, and then enter the test parameters (see Figure 8).

Figure 8 Noise and drift test parameters:

0.0 min

0.0

Run time 15.0 min

Wavelength 230.000 nm

Resolution 3.6 nm

Filter time constant Normal 1.0000 sec

Sampling rate 2 Hz

Exposure time Auto msec

Start

Results >>

Close

Result: Once the test ends, a report containing a result for drift in μAU and segment peak noise in μAU is produced.

Requirement: The segment peak noise should be less than or equal to 80 μAU , and the drift should be between -10,000 and +10,000 μAU .

Figure 9 Typical PDA detector noise and drift test report:

Acquity
HPLC

Noise and Drift Test

System: none

Instrument: ACQ-PDA#F11UPL099A

PDA Detector: Noise and Drift Test

Method:

Run time: 15(min)

Wavelength: 254(nm)

Resolution: 3.6(nm)

Filter Time Constant: 1.000(s)

Sampling Rate: 2(Hz)

Auto Exposure: Yes

Exposure Time: 4.2(ms)

Result:

Drift: -392.658(μAU)

Segment Peak Noise: 18.732(μAU)

6. Record the noise and drift results from the report in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
7. Print out the ACQUITY Console noise and drift test report.

8. Annotate the report in accordance with the conventions described in section 2.8.3.
9. Append the report to the operational and performance qualification results document associated with this workbook.

4.2 Determining noise and drift on an Acquity TUV detector (optional)

Requirement: If your system includes an ACQUITY TUV detector, you must perform this test.

The noise test is built into the ACQUITY Console. There are no optional parameters. The test last for 15 minutes. It calculates the most positive and most negative point over 15 minutes and then calculates a segment peak noise result in μ AU.

Requirements:

- Use the following mobile phases for this test.
 - 100% water (solvent line A1, solvent line A2, Seal wash and SM Purge)
 - 100% acetonitrile (solvent line B1, solvent line B2 and SM-Wash)
- Both tests require mobile phase to be flowing through the flow cell.
- The mobile phase must not contain any additives or modifiers.

To perform the noise test:

1. From the Inlet Method window, open the Noise method, and then click Load Method.

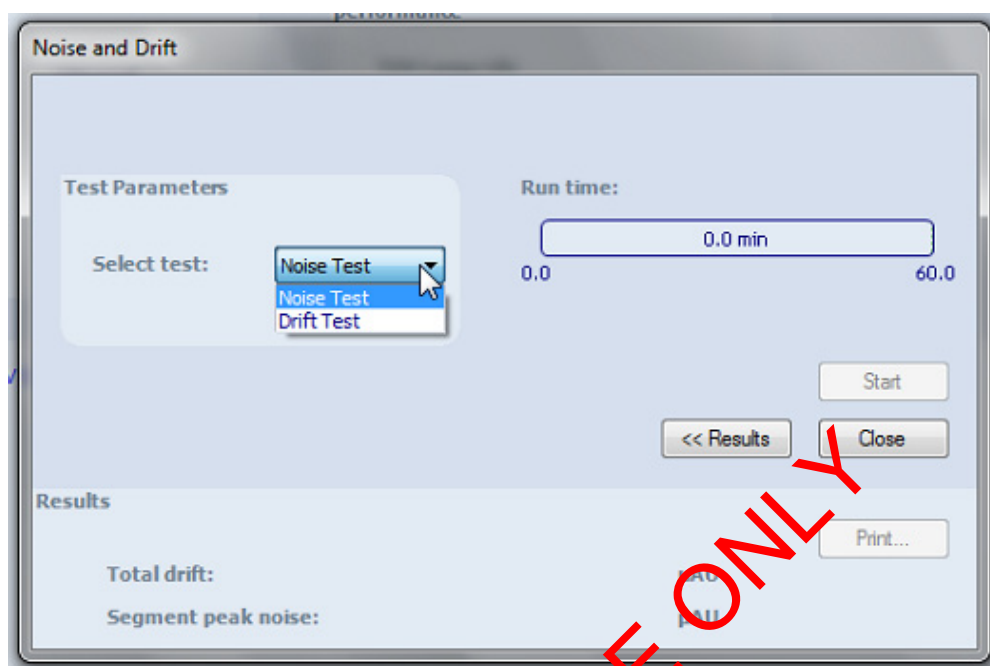
Result: The previous step ensures that the system is at the correct UPLC conditions for the test runs.

2. Allow the UPLC to equilibrate for 10 minutes before continuing with the test.

Requirement: Do not perform any testing until the solvent manager pressure has stabilized.

3. In the ACQUITY Console, select TUV Detector from the console tree.
4. Select the Troubleshoot menu in the ACQUITY Console window, select Service mode, and then enter the service password.
5. Select the Troubleshoot menu in the ACQUITY Console window, and then select Noise and Drift.
6. Select Noise Test from the Select test list.

Figure 10 Noise and drift test selection dialog box:



7. Click Start to begin the test.

Result: Once the test ends, a report that includes the result of the segment peak noise in μAU is produced.

Requirement: The segment peak noise must be less than or equal to 60 μAU .

8. Print out the ACQUITY Console TUV noise report (see Figure 11).

Figure 11 Example of the TUV detector noise report:

Acquity	Noise Test
System: none	
Instrument: ACQ-TUV#K09UPT193A	
TUV Detector: Noise/Drift	
Drift: -314.28 μAU	
Segment Peak Noise: 16.55 μAU	

9. Record the segment peak noise test result from the report in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

Note: Ignore the drift test result produced.

10. Annotate the report in accordance with the conventions described in section 2.8.3.
11. Append the report to the operational and performance qualification results document associated with this workbook.

Drift test

The drift test is built into the ACQUITY Console. There are no optional parameters. The drift test monitors the baseline absorbance over a one hour period. The difference between the start and stop baseline absorbance is reported in $\mu\text{AU}/\text{h}$.

To perform the drift test:

1. From the Inlet Method window, open the Noise method and click Load Method.

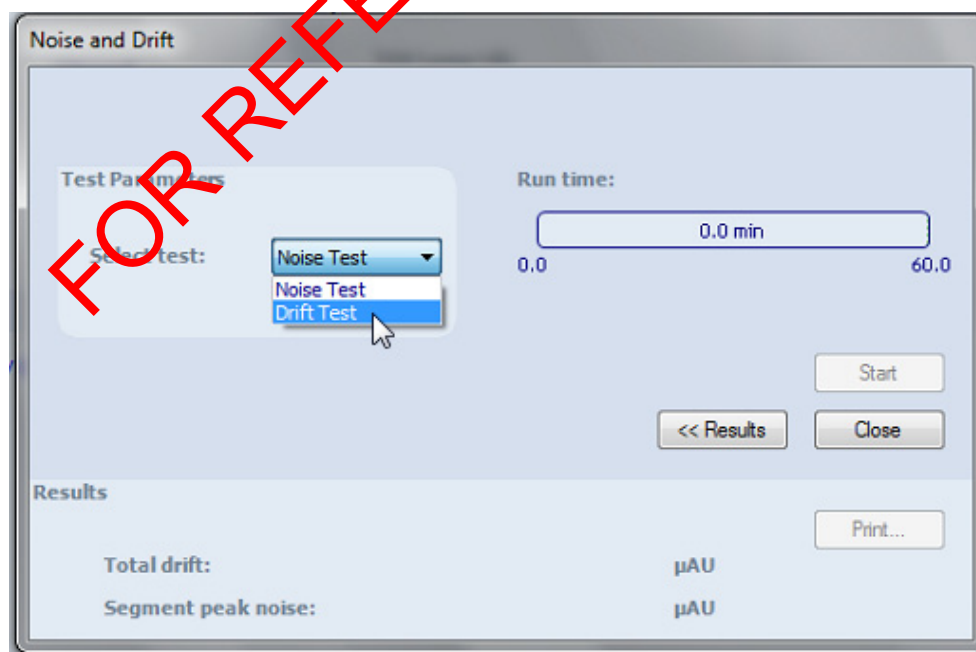
Result: The previous step ensures that the system is at the correct UPLC conditions for the test runs.

2. Allow the UPLC to equilibrate for 10 minutes before continuing with the test.

Requirement: Do not perform any testing until the solvent manager pressure has stabilized.

3. In the ACQUITY Console, select TUV Detector from the console tree.
4. Select the Troubleshoot menu in the ACQUITY Console window, select Service mode, and then enter the service password.
5. Select the Troubleshoot menu in the ACQUITY Console window, and then select Noise and Drift.
6. Select Drift Test from the Select test list.

Figure 12 Noise and drift test selection dialog box (2):



7. Click Start to begin the test.

Result: Once the test ends, a report containing the result of drift in μAU is produced.

Requirement: The detector drift must be between -10,000 and +10,000 μAU .

8. Print out the ACQUITY Console TUV drift report (see Figure 13).

Figure 13 Example of the TUV drift report:

Acquity UPLC	Drift Test
System: none	
Instrument: ACQ-TUV#K09UPT193A	
TUV Detector: Noise/Drift	
Drift: -206.94 μAU	
Segment Peak Noise: 42.18 μAU	

9. Record the drift test result from the report in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
10. Annotate the report in accordance with the conventions described in section 2.8.3.
11. Append the report to the operational and performance qualification results document associated with this workbook.

4.3 Cleaning the Acquity I-Class system

Before proceeding with the operational and performance qualification, you must perform this cleaning procedure.

Requirements:

- The ACQUITY fluidics outlet tubing must not be connected to the mass spectrometer or to any optional TUV or PDA detectors while performing this cleaning procedure.
- ACQUITY columns must be replaced with a dead volume union (found in the *ESI Probe Assembly Spares Kit*, part number 700003278). Attach a length of peak tubing to the union and place the other end of the PEEK tubing into a suitable waste vessel.
- The cleaning procedure must be completed in one step, and not left to run overnight.

To clean the system:

1. Confirm that all vials are in the correct position, as described in Table 2.
2. Wearing nitrile gloves, place all solvent lines in the acidic wash solvent.
3. In the ACQUITY Console, select the System tab in the module tree.

4. Select the Control tab, and then select "Start up system....".
5. From the System start up window, complete these tasks:
 - a. Prime pump lines A1, A2, B1, and B2 for 5 minutes each
 - b. Prime and equilibrate the sample manager (see section 3.2)
6. In the MassLynx window, open the Acidic_Basic_IClass_Clean_WAAXXX_DDMMYY sample list.
7. Rename the sample list with the correct serial number and current date.
8. Open the inlet file IClass_Acid_Base_Clean, and download the method.
9. Ensure the API gas is flowing, and then put the mass spectrometer in Operate mode.
10. Select rows 1 through 25 in the sample list, and then click Play.

Note: When the error message "Positive and Negative instrument sample tune invalid" appears, click OK to proceed.

Requirement: Ensure that no cleaning solution enters the detectors.

11. When the last acquisition ends, stop the mobile phase flow.
12. Wearing nitrile gloves, place all solvent lines in the basic wash solvent.
13. Repeat the priming procedure described in steps 5 through 6.
14. In the Inlet Method editor, download the inlet method.
15. Select rows 26 through 50 in the sample list, and then click Play.

Note: If the error message "Positive and Negative instrument sample tune invalid" appears, click OK to proceed.

16. When the acquisition ends, stop the UPLC flow.
17. Wearing nitrile gloves, place all the solvent lines into isopropan-2-ol.
18. Repeat the priming procedure described in steps 5 through 6.
19. In the Inlet Method editor, download the inlet method.
20. Select rows 51 through 60 in the sample list, and then click Play.

Note: When the error message "Positive and Negative instrument sample tune invalid" appears, click OK to proceed.

4.4 Preparing the mobile phase

You must use the bottles containing the acidic wash solution and the basic wash solution to store the mobile phase used in this operational and performance qualification, to reduce the risk of the system being contaminated by the glassware.

Requirements:

- Prepare this mobile phase only at this point in the operational and performance qualification procedure. Do not prepare this mobile phase during the sample preparation steps described in section 2.5.3.
- Wear chemical resistant, powder-free gloves when performing this procedure.

To prepare the mobile phase:

1. Decant the remaining wash solvents into two new bottles, and clearly label them "Acidic wash" and "Basic wash".
2. Using deionized water, rinse out the bottles that previously contained the acidic and basic wash solvents.

Requirement: Ensure that each bottle is rinsed out at least five times.

3. Add 1 L of deionized water to the bottle that previously contained the basic wash solvent.
4. Add 100 µL of formic acid (from the Xevo TQ-S Sample Kit, part number 700005472), and then cap the bottle and shake it.
5. Add 500 µL of ammonia solution (from the Xevo TQ-S Sample Kit, part number 700005472), and then cap the bottle and shake it.
6. Label the bottle "water + 0.01% formic acid + 0.05% ammonia".
7. Submerge lines A1, A2, Seal wash and SM purge solvent in the water +0.01% formic acid + 0.05% ammonia solution.
8. Add 1 L of acetonitrile to the bottle that previously contained the acidic wash solvent.
9. Add 100µL of formic acid (from the Xevo TQ-S Sample Kit, part number 700005472), and then cap the bottle and shake it.
10. Label the bottle "acetonitrile +0.01% formic acid".
11. Submerge lines B1, B2 and SM-Wash in the acetonitrile +0.01% formic acid solution.
12. Place the reservoir bottle in the ACQUITY solvent tray
13. From the ACQUITY Console, complete these tasks:

- a. Wet-prime pump lines A1, A2, B1, B2, and Seal wash for at least 5 minutes.
 - b. Prime and equilibrate the sample manager (see section 3.2)
14. Attach the ACQUITY column to position 1 (if the optional CM-A is present), and then attach all fluidics tubing to the mass spectrometer and any optional UV detectors.
 15. Set the solvent manager to deliver 0.8 mL/min 50/50 A1:B1 water/acetonitrile.
 16. In the Tune window's Fluidics tab, set the flow state to Waste.
 17. Leave the UPLC flowing for at least 20 minutes, before changing the flow state to LC.

4.5 Performing chromatographic tests

Before beginning the tests, ensure the reservoirs contain sufficient mobile phase (300 mL) to complete all the tests. The column equilibration times in the MassLynx OQ/PQ project assume you perform the tests in a specific sequence. If you repeat the tests, increase the equilibration times appropriately.

Run the following chromatographic tests in sequence as a single batch by adding the sample list entries to the sample queue.

Requirement: Run the tests in the order specified.

Note: Integration parameters that are not optimized cause poor chromatographic test results. In such instances, complete the comments section of the operational and performance results document associated with this workbook, entering the reason for the poor results. Include the peak integration corrective action you made to the processing method. At the end of this workbook, insert the chromatogram printouts (with the initial and modified integration methods). Finally, report the reprocessed results next to the initial results.

4.5.1 Creating the ESI+ and ESI- methods using Intellistart

Before proceeding with the operational and performance qualification, use the Intellistart software to generate methods used in the remaining tests.

To create ESI+ and ESI- methods using Intellistart:

1. In the MS Console, click Xevo TQ-S > Operate, and then start the API gas flow.
2. Place a 30-mL bottle containing 20 mL of Tuning solution (5 pg/μL chloramphenicol and 10 pg/μL sulfadimethoxine) on fluidics line C.
3. Click Interactive Fluidics > Enable Control.
4. Purge fluidics line C twice.
5. Click Binary Solvent Manager, and then select a flow rate of 0.8 mL/min 50/50 A1:B1

6. Click Interactive Fluidics > Enable Control, and then click combined flow.
7. Set the initial ion probe position:
 - a. Set the initial horizontal position of the probe by setting the vernier screw on the top of the source housing (X) to 6 on the vernier scale.
 - b. Set the initial vertical position of the probe by setting the collar on the top of the probe (Z) to 7 on the vernier scale.
 - c. Turn the adjuster knob at the top of the probe so that approximately 0.5 mm is visible at the probe tip.
8. Click IntelliStart, and then set the type of analysis to Unit Mass Resolution.
9. Click Sample Tune and Develop Method > ES+ and ES-, and then click Play.
10. Click Switch to Advanced Mode and then adopt the parameter settings shown in Table 7:

Table 7 Intellistart method development parameter settings:

Parameter	ES +	ES-
Compound Name	Sulfadimethoxine	Chloramphenicol
Molecular Mass/ Formula	310.08*	322.00*
Adduct A+	[M+H] ⁺	Blank field
Adduct B+	Blank field	Blank field
Adduct A-	Blank field	[M-H] ⁻
Adduct B-	Blank field	Blank field
Multiply Charged Parents	Clear	Clear
Create New Sample Tune	Selected	Selected
Load Existing Sample Tune	Clear	Clear
Sample Tune Name	SYSTEM_DDMMYY	SYSTEM_DDMMYY
Develop SIR method	Clear	Clear
Develop MRM method	ESI_SYSTEM_DDMMYY	
Name:	ESI_SYSTEM_DDMMYY	
Append to Existing methods	Clear	
Print Report	Selected	
Flow Path	Combined	
Cone Voltage	Default (2 through 100)	
Collision Energy	Default (2 through 80)	
Reservoir	C	

Table 7 Intellistart method development parameter settings:

Parameter	ES +	ES-
Number of MRM transitions per compound	1	
Flowrate	20	
Lowest Mass Fragment	100 Da	
All other fields	Leave as default	

*The molecular mass value entered is the neutral monoisotopic mass, not the mass of the ion.

Notes:

- The progress of the Sample Develop method can be monitored by clicking Sample list > Spectrum > Toggle real time chromatogram update > On. The instrument optimizes capillary and cone voltage in positive and negative modes and then optimizes collision energy in both modes.
- The software creates an MS method file that includes optimal cone and collision energy settings for an MRM transition of sulfadimethoxine and chloramphenicol. This information is displayed on the Intellistart sample report and associated MS method.exp file.

11. In the sample list window, click MS method editor.

12. Click File > Open, and then browse to the SYSTEM_DDMMYY.exp file.

13. Ensure the method includes two channels:

- Sulfadimethoxine m/z 311.** -> m/z 156.** (nominal masses).
- Chloramphenicol m/z 321.** -> m/z 152.** (nominal masses).

14. For both transitions, set the dwell times to 0.025 seconds.

15. Set the retention end time to 2.5 minutes.

16. In the Options list, click Method events.

17. In the Method events window, deselect Enable, and then click OK.

18. In the File menu, click Save.

19. In the Cycle Time Warning dialog box, click Save as is.

To create the ESI- MS experiment file:

1. Select the function row containing the sulfadimethoxine transition, and click Delete.
2. In the File menu, click Save as > ESI_NEG_SYSTEM_DDMMYY.

To create the ESI+ MS experiment file:

1. Click Open, and then select ESI_POS_SYSTEM_DDMMYY.
2. Select and delete the function row containing the chloramphenicol transition, and then save the file as ESI_POS_SYSTEM_DDMMYY.

To create the Injector linearity MS experiment file:

1. Double click on the Sulfadimethoxine transition, and then change the dwell time of the transition to 0.150 seconds.
2. In the File menu, click Save as > INJECTOR_LINEARITY_DDMMYYYY.
3. Close the MS Method editor.

To specify the MS methods and tune files for each sample list:

Ensure that the correct ipr and exp files are specified in the sample lists specified in Table 8.

Table 8 MS and tune file sample list associations:

Sample list	MS tune file	MS file
ESI_POS_SystemPrecision_WAAXXX _ DDMMYY.spl	ESI_SYSTEM _DDMMYY.ipr	ESI_POS_SYSTEM_DDMMYY.exp
ESI_NEG_WAAXXX_DDMMYY.spl	ESI_SYSTEM _DDMMYY.ipr	ESI_NEG_SYSTEM_DDMMYY.exp
ESI_MS_LINEARITY_WAAXXX _ DDMMYY.spl	ESI_SYSTEM _DDMMYY.ipr	INJECTOR_LINEARITY.exp

4.5.2 Making blank injections

Before running qualification tests, make blank injections to ensure that the system is equilibrated and prepared for qualification.

To make blank injections:

1. In the MassLynx tune window, open the Blank_Injections_WAAXXX_DDMMYY.spl sample list.
2. In the Inlet Method editor, download the LC method Gradient_IClass.
3. Select all samples, and then click the Start Run button.
4. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
5. Wait for the procedure to finish before proceeding with the remainder of the tests.

4.5.3 Testing the MS detector linearity

This test determines if the detector's response is linear over a range of sample concentrations. A series of standards at different concentrations are injected, using a constant injection volume. The software then plots peak area against sample concentration. The calculated coefficient of determination (R^2) is used to measure the linearity of the detector's response.

To optimize the IntelliStart-generated method and probe position:

1. In the MassLynx tune window, open the ESI_SYSTEM_WAAXXX_DDMMYY.ipr tune file, and click ESI positive from the Ion Mode menu.
2. Set the desolvation gas flow to 1000 L/h, and the desolvation temperature to 600 °C.
3. Select fluidics line C.
4. Set the syringe flow rate to 20 µL/min combined flow.
5. Set the solvent manager's flow rate to 0.8 mL/min, 50/50 A1:B1.
6. Start the syringe pump.
7. In the MS console, click Xevo TQ-S Detector > Manual Optimization.
8. Adopt the manual optimization parameter settings in Table 9.
9. Monitor the m/z 311.** -> m/z 156.** MRM transition, and ensure the cone voltage and collision energy values match those in the ESI_POS_SYSTEM_DDMMYY.exp MS method created by IntelliStart.
10. Right-click on the signal plot, select Plot properties, and set the time window to 10 min.

Table 9 Manual optimization parameter settings for positive ESI MS tests:

Parameter	ESI + tuning
Data Type	MRM
Parent	311.* Da
Daughter	156.* Da
Cone voltage	IntelliStart optimized setting (~40 V)
Collision energy	IntelliStart optimized setting (~20V)
Ion Mode	ES+
Tune	ESI_SYSTEM_DDMMYY.ipr
Capillary voltage	Positive - tune for maximum signal
Corona voltage	Unavailable
Repeller voltage	Unavailable
Desolvation temperature	600 °C
Desolvation gas	1000 L/h
Cone gas	150 L/h
Nebulizer pressure	7 bar
Fluidics flow	20 µL/min

*IntelliStart-determined, peak-top mass position.

11. In the Capillary dialog box, specify a value that optimizes the capillary voltage and thus produce maximum sensitivity, and then note this value.
12. Optimize the probe position and capillary length for maximum intensity.
13. Set the capillary voltage to zero.
14. If thermal ionization exceeds 10% of the signal intensity, move the ESI probe away from the sample cone. The residual signal must be less than or equal to 10% of the optimized signal intensity.

Requirement: If, to minimize thermal ionization, you must adjust the probe position, reoptimize the capillary voltage in the new position, and repeat steps 7 through 9.

15. Set the capillary voltage to the optimal value determined in step 11.
16. In the Fluidics window, click the Stop icon to stop the fluidics flow.
17. In the Manual Optimization tune window, click Save.
18. Exit Manual Optimization.
19. In the Tune window, click File > Open > ESI_SYSTEM_DDMMYY.ipr.
20. Click Ion Mode > Electrospray +, and then MSMS.

21. Monitor the collision cell's Pirani gauge, and adjust the collision cell gas flow until the pressure reaches 3.5e-3 mbar.
22. Ensure that the correct ipr and exp files are specified in the sample lists specified in Table 8. If changes are required, save the sample lists.
23. In the Inlet Method editor, download the IClass_Gradient method.

Rationale: Doing so prepares the pump with the initial gradient conditions.

24. Wait until the flow stabilizes.

To test MS detector linearity:

1. Open the MS_Detector_Linearity_WAAXXX_DDMMYY.spl sample list.
2. Select all samples, and then click the Start Run button.
3. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
4. When the acquisition has completed, from the sample list, select row 16, and then click Chromatogram.
5. Select the Display tab in the chromatogram window.
6. Select the sulfadimethoxine transition so that Ch1 appears in the description box.
7. Select Replace trace, and then click OK.
8. Click to zoom on the sulfadimethoxine peak, allowing a range of approximately 0.2 minutes on either side.
9. From the sample list page, click TargetLynx > Edit Method.
10. From the File menu, click Open, and then select ESI_POSITIVE_Detector_Linearity.mdb.
11. In the TargetLynx Method Editor, click User Defined Properties, and then select the Predicted Retention Time value.
12. In the chromatogram, right-click and drag across a range that extends to approximately 0.1 minutes on either side of the sulfadimethoxine peak.
13. In the TargetLynx method, ensure the predicted retention time is updated, and then click Save.
14. In the Sample flagging properties tab, define the noise range used for the signal-to-noise calculations.
15. Inspect the chromatogram for a section of flat noise (one that is free of spikes or other artifacts).

Requirement: The noise region must extend for 0.15 minutes.

16. Enter the values for the noise region's start and end in the signal-to-noise parameters section of the target flagging menu within the TargetLynx method.

17. Save the TargetLynx method.
18. Open the ESI_POSITIVE_AREA_DDMMYYYYY.mdb file.
19. Update the predicted retention time to that determined in step 12.
20. Update the noise range time to that determined in step 15.
21. From the sample list, select all rows.
22. From the TargetLynx shortcut bar, click Process Samples.

Result: The Create TargetLynx Dataset dialog box appears.

23. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
24. Specify ESI_POSITIVE_Detector_Linearity.mdb as the method, and then click OK.
25. In the TargetLynx window, click File > Apply Layout > Detector_Linearity_Area.qlt.
26. Click File > Save As > ESI_POSITIVE_Detector_Linearity_DDMMYY.
27. Click File > Report Format.
28. In Report Format Options, click the Compound Summary Report tab, and then select the same column format as the Detector_Linearity_Area.qlt layout.
29. Click File > Print > OK.

Requirement: The R^2 value of the calibration curve must be greater than or equal to 0.990.

30. Annotate the report, observing the conventions described in section 2.8.3.
31. Append the report to the operational and performance qualification results document associated with this workbook.
32. Record the results in Form 6, "Operational and performance qualification results" of the operational and performance qualification results document associated with this workbook.

4.5.4 Testing ESI+ sensitivity and precision

The ESI+ sensitivity test determines if the sensitivity of the mass spectrometer is at an acceptable level. A successful test result demonstrates that the ESI probe, ion optics and detector are functioning correctly in positive ion mode, and that the background noise is not excessive.

Six injections of sulfadimethoxine solution are made at a fixed concentration and volume, the background noise over a 0.15 min section of the baseline spectrum (before the peak occurs) is measured, and the average signal-to-noise ratio across all sample peaks calculated. The average peak area across all injections is also calculated.

The system precision test verifies that the reproducibility of peak area and retention time of six repeat injections is acceptable.

A number of 1-pg on-column injections of sulfadimethoxine solution are made at a fixed concentration and volume, and the retention time and peak area height data for each sample calculated.

To test ESI+ sensitivity:

1. Open the ESI_POSITIVE_WAAXXX_DDMMYY.spl sample list.
2. Select all samples, and then click the Start Run button.
3. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
4. From the sample list, click Process Samples.

Result: The Create TargetLynx Dataset dialog box appears.

5. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
6. Specify ESI_POSITIVE_DDMMYYYY.mdb as the method, and then click OK.
7. In the TargetLynx window, click File > Apply Layout > Sensitivity.qlt.
8. Click File > Save As > ESI_Positive_Sensitivity_DDMMYYYY.
9. In the TargetLynx window, click File > Print > OK.
10. From the printout, use the signal-to-noise ratio value for each peak to calculate the average signal-to-noise ratio across all peaks.
11. Using the area values for each peak, calculate the average area value across all peaks.

Requirements:

- The average signal-to-noise ratio must be greater than or equal to 3000:1.
 - The average peak area of the sulfadimethoxine peaks must be greater than or equal to $6e+4$ (60,000).
12. Annotate the report, observing the conventions described in section 2.8.3.
 13. Append the report to the operational and performance qualification results document associated with this workbook.
 14. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

To test system precision:

1. From the sample list, click Process Samples.

Result: The Create TargetLynx Dataset dialog box appears.

2. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
3. Specify ESI_POSITIVE_AREA_DDMMYYYYY.mdb as the method, and then click OK.
4. In the TargetLynx window, click File > Apply Layout > Sensitivity.qlt.
5. Click File > Save As > ESI_Positive_Reproducibility_DDMMYYYYY.
6. In the TargetLynx window, click File > Print > OK.
7. From the printout, note the largest and smallest retention time (RT) values, and calculate the difference between the two values.
8. Note the area values for each peak, and calculate the Relative SD of the peak areas (see section 5).

Requirements:

- The difference between the largest and smallest RT values must be less than or equal to 0.047 min.
 - The Relative SD of the peak areas must be less than or equal to 3%.
9. Annotate the report observing the conventions described in section 2.8.3.
 10. Append the report to the operational and performance qualification results document associated with this workbook.
 11. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

4.5.5 Testing ESI- sensitivity

This test determines if the sensitivity of the mass spectrometer is at an acceptable level. A successful test result demonstrates that the ESI probe, ion optics and detector are functioning correctly in negative ion mode, and that the background noise is not excessive. A number of injections of chloramphenicol solution are made at a fixed concentration and volume, the background noise (RMS) over a 0.15 min section of the baseline spectrum (before the peak occurs) is measured, and the mean signal-to-noise ratio across all sample peaks calculated. The mean peak area across all injections is also calculated.

To optimize the IntelliStart-generated method and probe position:

1. In the MassLynx tune window, open the ESI_SYSTEM_DDMMYY.ipr tune file.
2. Click ESI- from the Ion Mode menu.
3. Set the desolvation gas flow to 1000 L/h, and the desolvation temperature to 500 °C.
4. Select fluidics line C.
5. Set the syringe flow rate to 20 µL/min, in combined flow.

6. Set the solvent manager's flow rate to 0.8 mL/min, 50/50 A1:B1.
7. Start the syringe pump.
8. In the MS console, click Xevo TQ-S Detector > Manual Optimization.
9. Adopt the manual optimization parameter settings in Table 10.
10. Monitor the m/z 321.** -> m/z 152.** MRM transition, and ensure the cone voltage and collision energy values match those in the ESI_NEG_SYSTEM_DDMMYY.exp MS method created by IntelliStart.
11. Right-click on the signal plot, select Plot properties, and set the time window to 1 minute.

Table 10 Manual optimization parameter settings for sensitivity tests:

Parameter	ESI – tuning
Data Type	MRM
Parent	321.* Da
Daughter	152.* Da
Cone voltage	IntelliStart optimized setting (~30 V)
Collision energy	IntelliStart optimized setting (~18 V)
Ion Mode	ESI
Tune	ESI_SYSTEM_DDMMYY.ipr
Capillary voltage	Negative - tune for maximum signal
Corona voltage	Unavailable
Repeller voltage	Unavailable
Desolvation temperature	500 °C
Desolvation gas	1000 L/h
Cone gas	150 L/h
Nebulizer pressure	7 bar
Fluidics flow	20 µL/min

12. In the Capillary dialog box, specify a value that optimizes the capillary voltage and thus produce maximum sensitivity, and then note this value.
13. Optimize the probe position and capillary length for maximum intensity.
14. If thermal ionization exceeds 10% of the signal intensity, move the ESI probe away from the sample cone. The residual signal must be less than or equal to 10% of the optimized signal intensity.

Requirement: If, to minimize thermal ionization, you must adjust the probe position, reoptimize the capillary voltage in the new position, and repeat steps 8 through 10.

15. Ensure the capillary voltage is switched on and set to the optimal value determined in step 12.
16. In the Fluidics window, click the Stop icon to stop the fluidics flow.
17. In the Manual Optimization tune window, click Save.
18. Exit Manual Optimization.
19. In the Tune window, click > File menu > Open > ESI_SYSTEM_DDMMYY.ipr.
20. Click > Ion Mode > Electrospray -ve.
21. Click the MSMS icon.
22. Monitor the collision cell's Pirani gauge, and adjust the collision cell gas flow until the pressure reaches 3.5e-3 mbar.
23. Click Save.

To test ESI- sensitivity:

1. From the sample list page, click File > Open, and then click ESI_NEGATIVE_WAAXXX_DDMMYY.spl.
2. From the sample list, select the MS experiment file and tune file that you created.
3. In the Inlet Method editor, download the Gradient method.
Rationale: Doing so prepares the pump for initial gradient conditions.
4. Wait 5 minutes, for the flow to stabilize.
5. Select all samples, and then click the Start Run button.
6. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
7. From the sample list, select row 3 and then click Chromatogram.
8. Select the Display tab in the chromatogram window.
9. Select the chloramphenicol transition, so that Ch1 appears in the description box.
10. Select Replace trace, and then click OK.
11. Click to zoom on the chloramphenicol peak, allowing a range of approximately 0.2 minutes on either side.
12. From the sample list page, click TargetLynx > Edit Method.
13. From the File menu, click Open, and then select ESI_NEGATIVE_DDMMYYYY.mdb.
14. In the TargetLynx Method Editor > User Defined Properties, select the Predicted Retention Time value.

15. In the chromatogram window, right-click and drag across a range that extends approximately 10 seconds on either side of the chloramphenicol peak.
16. In the TargetLynx Method Editor > User Defined Properties, ensure the Predicted Retention Time is updated.
17. In the chromatogram, find a section of flat noise (one that is free of spikes or other artifacts).

Requirement: The noise region must extend for 0.15 minutes.

18. In the TargetLynx Method Editor, click Sample Flagging Properties.
19. In the Signal to Noise Parameters window, enter the values for noise regions start and end, and then click Save.
20. From the sample list, select all rows.
21. From the TargetLynx shortcut bar, click Process Samples.
22. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
23. Select ESI_NEGATIVE_DDMMYYYY.mdh as the method, and then click OK.
24. In the TargetLynx browser, click File > Apply Layout > ESI_SENSITIVITY.qlt.
25. Click File > Save As > ESI_NEGATIVE_SENSITIVITY.DDMMYYYY.
26. Click File > Report Format.
27. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the ESI_SENSITIVITY.qlt layout, and then click OK.
28. Click File > Print and then click OK.
29. From the printout, use the signal-to-noise ratio value for each peak to calculate the average signal-to-noise ratio across all peaks.
30. Using the area values for each peak, calculate the average area value across all peaks.

Requirements:

- The average signal-to-noise ratio must be greater than or equal to 400:1.
 - The average peak area of the sulfadimethoxine peaks must be greater than or equal to 1e+3 (1,000).
31. Annotate the report, observing the conventions described in section 2.8.3.
 32. Append the report to the operational and performance qualification results document associated with this workbook.
 33. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

4.5.6 Testing injector linearity and accuracy

This test verifies that the delivered injection volume is linear within a specified range. A blank injection is made, followed by a number of sulfadimethoxine injections at a fixed concentration but with varying volumes, and a calibration curve of peak area against volume is created.

To test injector linearity:

1. From the sample list page, click File > Open, and then select INJECTOR_LINEARITY_WAAXXX_DDMMYY.spl.
2. Select all samples, and then click the Start Run button.
3. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
4. When the INJECTOR_LINEARITY_DDMMYY.spl acquisition ends, assess the results using TargetLynx software.
5. From the sample list, select row 6, select Chromatogram.
6. Zoom on the Sulfadimethoxine peak, allowing a range of approximately 10 seconds on either side of the peak.
7. From the sample list page, click TargetLynx > Edit Method.
8. From the File menu, click Open, and then select INJECTOR_LINEARITY.mdb.
9. In the TargetLynx Method Editor, select User Defined Properties and then select the Predicted Retention Time value of the sulfadimethoxine peak.
10. In the chromatogram, right-click and drag across a range that extends approximately 10 seconds on either side of the sulfadimethoxine peak.
11. Ensure the predicted retention time is updated in the TargetLynx method.
12. Save the TargetLynx method.
13. In the sample list, select rows 3 through 6.
14. From the TargetLynx shortcut bar, click Process Samples.
15. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
16. Select INJECTOR_LINEARITY_AREA_DDMMYY.mdb as the method, and then click OK.
17. In the TargetLynx window, click File > Apply Layout > INJECTOR_LINEARITY_AREA.qlt.
18. Click the Toggle Calibration Window icon, to display the calibration curve.
19. Click File > Save As > INJECTOR_LINEARITY_AREA_DDMMYY.
20. Click > File > Report Format.

21. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the INJECTOR_LINEARITY_AREA.qlt layout.
22. Click File > Print > OK.
23. Examine the summary report and locate the Calibration Curve equation: ($A * X + B$).

Compound name: Sulfadimethoxine
 Correlation coefficient: $r = 0.999968$, $r^2 = 0.999935$
 Calibration curve: $31942.3 * x + -587.848$
 Response type: External Std, Area
 Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

Note: In the example above, the value of A from the calibration curve is 31942.3, and the value of B is -587.848

24. Using the A and B values, and the formula stated in Form 6, calculate the absolute value of the x-intercept.

Requirements:

- The R^2 value must be greater than or equal to 0.990.
- The absolute value (area) must be less than or equal to 0.2 μL .

25. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the R^2 value, and the absolute value (area) calculated in step 23.
26. Annotate the report observing the conventions described in section 2.8.3.
27. Append the report to the operational and performance qualification results document associated with this workbook.

4.5.7 Testing flow rate linearity

The flow-rate-linearity test determines whether the solvent manager flow rate is linear. The test involves repeatedly injecting a constant volume of sample into varying flow rates.

To test the flow rate linearity:

1. From the sample list page, click File > Open, and then select FLOW_LINEARITY_WAAXXX_DDMMMYYYY.spl.
2. In the MassLynx Tune window, ensure the following conditions are met:
 - a. The ESI_SYSTEM_DDMMMYY.ipr is loaded.
 - b. The ionization mode is Electrospray +.
 - c. The instrument is in MSMS mode.
 - d. Collision gas is flowing.

3. Select all samples, and then click the Start Run button.
 4. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
 5. When the injections end, select rows 2 through 8 on the sample list.
 6. Print out the chromatograms that show a sulfadimethoxine injection.
 7. Note the RT values for each acquisition.
 8. For each acquisition, multiply the noted RT value by the values indicated in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, and record the calculated values.
 9. Calculate the standard deviation of the four adjusted RT values and record it in n Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
- Requirement:** The standard deviation must be less than or equal to 60 μ L.
10. Annotate the report, observing the conventions described in section 2.8.3.
 11. Append the report to the operational and performance qualification results document associated with this workbook.

4.5.8 Testing gradient performance

The gradient-performance test determines whether a solvent management system can deliver a solvent mixture within acceptable criteria. A six-component mixture is injected three times, and four components of this mixture are monitored (acetaminophen, caffeine, sulfadimethoxine and verapamil) using an MRM method. The difference in the retention times of each of the four compounds over the three injections is examined. The solvent management system is tested for solvent lines A1 and B1, then A2 and B2.

To test the gradient performance:

1. From the sample list page, click File > Open, and then select GRADIENT_WAXXX_DDMMYYYY.spl.
2. In the MassLynx Tune window, click File menu > Open > ESI_SYSTEM_DDMMYYYY.ipr.
3. Adopt the Tune window settings shown in Table 11.

Table 11 Tune window parameter settings:

Parameter	Setting
Ion Energy 1	Optimum *
Ion Energy 2	Optimum *
Desolvation gas	1000 L/h

Table 11 Tune window parameter settings:

Desolvation temperature	600 °C
-------------------------	--------

*Determined during the operational qualification (OQ) of the Xevo TQ-S

4. Click Ion Mode > Electrospray +.
5. Click MSMS, and ensure the collision gas is flowing.
6. Select all samples, and then click the Start Run button.
7. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
8. When the GRADIENT.spl acquisitions end, use the TargetLynx software to process the results.
9. From the sample list page, select row 3 (Gradient AB_1) and then select Chromatogram.
10. Click Display, and then select Mass.
11. Select All, click Replace Trace, and then click OK.
12. From the sample list page, click TargetLynx > Edit Method.
13. From the File menu, click Open, and then select GRADIENT_RT.mdb.
14. In the compound list, select 1. acetaminophen (m/z 152.07 -> m/z 110.06).
15. In the TargetLynx method, select the value field for Predicted Retention Time.
16. In the chromatogram, right-click and drag across a range that extends approximately 10 seconds on either side of the acetaminophen peak.
17. In the TargetLynx method, ensure the Predicted Retention Time is updated.
18. Repeat steps 13 through 16 for each of the following compounds:
 - a. caffeine (m/z 195.09 -> m/z 138.40)
 - b. sulfadimethoxine (m/z 311.08 -> m/z 156.01)
 - c. verapamil (m/z 455.27 -> m/z 165.09)
19. Save the TargetLynx method.
20. In the sample list, select rows 3 through 5.
21. From the TargetLynx shortcut bar, click Process Samples.
22. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
23. Select GRADIENT_RT as the method, and then click OK.

24. In the TargetLynx window, click File > Apply Layout > GRADIENT.qlt.
 25. Click File > Save As > GRADIENT_RT_A1_B1_DDMMYY.
 26. Repeat the processing steps as above for solvent lines A2:B2.
 27. Save the solvent lines A2:B2 injections as GRADIENT_RT_A2_B2_DDMMYY.
 28. Click File > Report Format.
 29. In the Report Format options window, click the Compound Summary Report tab and then select the column format to match the GRADIENT.qlt layout.
 30. In the TargetLynx window, click File > Print, and then click OK.
 31. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results forms associated with this workbook, record the largest and smallest retention time values from each compound's summary.
 32. Calculate the difference between the largest and smallest retention time values, and enter the result in Form 6, "Operational and performance qualification results", of the operational and performance qualification results forms associated with this workbook.
- Requirement:** The difference between the largest and smallest retention time values must be less than or equal to 0.047 minutes.
33. Annotate the report, observing the conventions described in section 2.8.3.
 34. Append the report to the operational and performance qualification results document associated with this workbook.

4.5.9 Testing injector carryover

The injector carryover test determines the amount of sample carryover after an injection of a concentrated stock sample (challenge injection). Initially, an injection of a standard solution (equivalent to 0.005% of the challenge injection) is performed, followed by an injection of the concentrated stock solution, which is diverted to waste. Three subsequent (or consecutive) blank injections are then performed to determine the amount of carryover from the challenge injection.

To test the injector carryover:

1. From the sample list page, click File > Open, and then select INJECTOR_CARRYOVER_WAAXXX_DDMMYY.spl.
2. In the MassLynx Tune window, ensure the following conditions are met:
 - a. The ESI_SYSTEM_DDMMYY.ipr is loaded.
 - b. The ionization mode is Electrospray +.
 - c. The instrument is in MSMS mode.
 - d. Collision gas is flowing.

3. Ensure that method events are enabled for the Injector_linearity.exp and Divert_Waste exp files.
4. Select all samples, and then click the Start Run button.
5. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
6. Once the acquisitions are complete click TargetLynx > Edit Method.
7. From the File menu, click Open, and then select CARRYOVER.mdb.
8. Note the Predicted Retention Time value, and the value in the Retention Time window.
9. From the sample list page, click TargetLynx > Edit Method.
10. From the File menu, click Open, and then select CARRYOVER.mdb.
11. In the Compound List, select 1: sulfadimethoxine.
12. In the User Defined Properties, enter the Predicted Retention Time and Retention Time window values noted in step 8.
13. Save the TargetLynx method.
14. In the sample list, select rows 4 through 8.
15. From the TargetLynx shortcut bar, click Process Samples.
16. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
17. Select CARRYOVER.mdb as the method, and then click OK.
18. In the TargetLynx window, click File > Apply Layout > CARRYOVER.qlt.
19. Click File > Save As > INJECTOR_CARRYOVER_DDMMYY.
20. Click File > Report Format.
21. In Report Format Options, click the Compound Summary Report tab, and then select the column format to match the, CARRYOVER.qlt layout.
22. In the TargetLynx window, click > File > Print > OK.
23. Examine the compound report, noting the area value for the acquisition "Standard" and the area value of the first blank injected after it.

Requirement: The area value of the first blank injection must be less than the area value for the standard acquisition (that is, equal to 0.005% of the stock 1000 ng sulfadimethoxine).
24. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the area value for the acquisition "Standard" and the area value of the first blank injected after it.

25. Annotate the report observing the conventions described in section 2.8.3.
26. Append the report to the operational and performance qualification results document associated with this workbook.

4.5.10 Testing the APCI+ sensitivity (optional)

The APCI+ sensitivity test is performed in MRM mode, by making six injections of 17- α -hydroxyprogesterone, 50 pg on-column, and then calculating the signal-to-noise values of each injection.

Requirement: You must use the IntelliStart software to generate the APCI method.

To create the APCI+ sensitivity methods using IntelliStart:

1. Place a 30-mL bottle containing 20 mL of Tuning solution on fluidics line C.
2. In the MS Console, click Interactive Fluidics, and then click Enable Control and purge fluidics line C twice.
3. In the MS Console, select Binary Solvent Manager, and then select a flow of 0.8 mL/min 50/50 A1:B1.
4. In the MS Console, click Interactive Fluidics, select Enable Control, and then select combined flow.
5. Set the initial ion probe position:
 - a. Set the initial horizontal position of the probe by setting the vernier screw on the top of the source housing (X) to 6 on the vernier scale.
 - b. Set the initial vertical position of the probe by setting the collar on the top of the probe (Z) to 7 on the vernier scale.
6. Click IntelliStart, and then set the type of analysis to Unit mass resolution.
7. Select Sample Tune and Develop Method, and select APCI+.
8. Click Start.
9. Click Switch to Advanced Mode, and then adopt the settings shown in Table 12.
10. Ensure the Fluidics Flow Path parameter is set to Combined.

Table 12 APCI+ Intellistart method development parameter settings:

Parameter	APCI+
Compound Name	17- α -hydroxyprogesterone
Molecular Mass/ Formula	330.22
Adduct A+	[M+H] ⁺
Adduct B+	Blank field
Adduct A-	Blank field

Table 12 APCI+ Intellistart method development parameter settings:

Parameter	APCI+
Adduct B-	Blank field
Multiply Charged Parents	Clear
Create New Sample Tune	Selected
Load Existing Sample Tune	Clear
Sample Tune Name	APCI_SYSTEM_DDMMYY.ipr
Develop SIR method	Clear
Develop MRM method	APCI_SYSTEM_DDMMYY
Name:	APCI_SYSTEM_DDMMYY
Append to Existing methods	Clear
Print Report	Selected
Flow Path	Combined
Cone Voltage	Default (2 through 100)
Collision Energy	Default (2 through 80)
Reservoir	C
Number of MRM transitions per compound	1
Flow rate	20
Lowest Mass Fragment	100Da
All other fields	Leave as default

- Click Start, to begin the IntelliStart optimization.

Result: An MS method file is created with optimal cone and collision energy parameters for an MRM transition of 17- α -hydroxyprogesterone.

- From the sample list page, open the MS method editor and browse to the APCI_SYSTEM_WAAXXX_DDMMYY method file.
- Ensure the method contains the channel: 17- α -hydroxyprogesterone m/z 331.** -> m/z 109.** (nominal masses).
- Set the dwell time to 0.025 seconds and the retention end time to 2.5 minutes.
- In the Options menu, click Method events, then clear the Enable box, and then click OK.
- Save the method.

To optimize the IntelliStart-generated method and probe position:

- Open the generated APCI_SYSTEM_DDMMYY.ipr tune file.
- Set the desolvation gas flow to 1000 L/h, and the desolvation temperature to 600 °C.

- Set the infusion rate to 20 $\mu\text{L}/\text{min}$, in combined flow, with a flow of 0.8 mL/min 50/50 A1:B1, and then start the syringe pump.
- In the MS console, click Xevo TQ-S Detector > Manual Optimization.

Note: See Table 9 for parameter settings.

Table 13 Manual optimization parameter settings for sensitivity tests:

Parameter	APCI + tuning
Data Type	MRM
Ion Mode	APCI+ unavailable
Parent	331.* Da
Daughter	109.* Da
Cone voltage	IntelliStart optimized setting (~30V)
Collision energy	IntelliStart optimized setting (~20 V)
Ion Mode	APCI +
Tune	APCI_SYSTEM_DDMMYY.ipr
Capillary voltage	Unavailable
Corona voltage	Positive - tune for maximum signal
Repeller voltage	Unavailable
Desolvation temperature	600 °C
Desolvation gas	1000 L/h
Cone gas	150 L/h
Nebulizer pressure (bar)	Tune for maximum sensitivity
Fluidics flow $\mu\text{L}/\text{min}$	20 $\mu\text{L}/\text{min}$

*IntelliStart determined, peak-top mass position.

- Monitor the m/z 331.** -> m/z 109.** MRM transition, and ensure the cone voltage and collision energy values match those in the APCI_SYSTEM_DDMMYY.exp MS method created by IntelliStart.
- Optimize the corona voltage for maximum sensitivity by entering values in the Corona dialog box.
- Optimize the probe position and capillary length, for maximum intensity.
- Click the Stop icon, to stop the infusion.
- Click Save in the Manual Optimization window.
- Exit the Manual Optimization.
- In the Tune window, ensure the file APCI_SYSTEM_DDMMYY.ipr is loaded.

12. Verify that the optimized values are present on the MS tune page.
13. In the Tune window, click Ion Mode > APcI +.
14. Select the MSMS icon.
15. Tune the collision cell gas flow until the pressure is approximately 3.5e-3 mbar.
16. In the Fluidics window, click the Play button, to start the combined fluidics flow.
17. Tune the nebulizer gas flow for maximum sensitivity.
18. Click the Stop button, to stop the fluidics flow.
19. Change the flow state to LC.

Requirement: You must tune the nebulizer gas via the Tune window. Do not attempt to tune the gas flow via IntelliStart's manual optimization.

To test APcI+ sensitivity:

1. In the sample list, click File > Open, and select APcI_POSITIVE_WAXXX_DDMMYYYYY.spl.
2. Select the column MS File, right-click, and then select Fill Down.
3. Save the sample list.
4. Select all samples, and then click the Start Run button.
5. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
6. When the acquisition is complete, use the TargetLynx software to process the results.
7. From the sample list page, select row 3, select Chromatogram, and click to zoom on the 17- α -hydroxyprogesterone peak, allowing a range of approximately 0.2 minutes on either side.
8. From the sample list page, click TargetLynx > Edit Method.
9. From the File menu, click Open, and then select APcI_POSITIVE.mdb.
10. In the TargetLynx Method Editor > User Defined Properties, select the Predicted Retention Time value.
11. In the Chromatogram, right-click and drag across a range that extends approximately 10-seconds on either side of the 17- α -hydroxyprogesterone peak.
12. In the TargetLynx method, ensure the Predicted Retention Time is updated.
13. In the chromatogram from step 5, find a section of flat noise in the chromatogram (one that is free of spikes or other artifacts).

Requirement: The noise region must extend for 0.15 minutes.

14. In the TargetLynx Method Editor, click Sample Flagging Properties.
 15. In the Signal to Noise Parameters window, enter the values for noise regions start and end, and then click Save.
 16. In the sample list, select all rows.
 17. From the TargetLynx shortcut bar, click Process Samples.
 18. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
 19. Select APCI_POSITIVE as the method, and then click OK.
 20. In the TargetLynx window, click File > Apply Layout > SENSITIVITY.qlt.
 21. Click File > Save As > APCI_POSITIVE_DDMMYY.
 22. Click File, select Report Format.
 23. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the SENSITIVITY.qlt layout.
 24. In the TargetLynx window, click File > Print > OK.
 25. From the printout, use the signal-to-noise ratio value for each peak to calculate the average signal-to-noise ratio across all peaks.
 26. Using the area values for each peak, calculate the average area value across all peaks.
- Requirements:**
- The average signal-to-noise ratio must be greater than or equal to 1250:1 for the 17- α -hydroxyprogesterone transition m/z 331** m/z 109** of a 50 μ g on-column injection.
 - The average area of the 17- α -hydroxyprogesterone peaks must be greater than or equal to 1e+4 (10,000).
27. Annotate the report, observing the conventions described in section 2.8.3.
 28. Append the report to the operational and performance qualification results document associated with this workbook.
 29. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

4.5.11 Performing preliminary UV test injections (optional)

Test injections are not part of the qualification. They merely ensure that the system is equilibrated and prepared for qualification. The sample list includes an equilibration step followed by two blank injections of mobile phase. Three injections of a caffeine solution follow, all at the same concentration and injection volume. The test injections must meet the pass criteria before continuing with the qualification.

Note: Perform this test only if the ACQUITY UPLC system includes an ACQUITY PDA, PDA e λ , or TUV detector.

Requirement: Before proceeding with the UV test injections, follow the washing and priming procedure described in section 3.2.

To perform preliminary UV test injections:

1. Click the Open Sample List icon, and select PDA_Test_Injections_WAXXX_DDMMYY.spl for a PDA/PDAe λ , or TUV_Test_Injections_WAXXX_DDMMYY.spl for a TUV.
2. Update the sample list name with the current date.
3. Select all samples, and then click the Start Run button.
4. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
5. When the test injections end, select only these three test injections in the sample list.
6. Open the chromatogram view, and ensure that the retention times of all three injections occur between 0.4 and 0.9 minutes.

Note: When the injections fail to occur between 0.4 and 0.9 minutes, ensure that the mobile phase, column, flow rates, and solvent lines are as indicated in qualification. Then rerun the test injections.

7. Process the three samples using TargetLynx.
8. In the Create TargetLynx Dataset dialog box, select UV_RSD_Area mdb.
9. Select Integrate Samples, Calibrate Standards, and Quantify Samples.
10. Click File > Apply Layout > UV_RSD.qlt.
11. Print the summary report.
12. Examine the summary report, and observe the retention times of the three caffeine peaks. Adjust the retention times as needed in the TargetLynx processing methods.
13. Calculate the difference between the largest and smallest retention time values.

Requirement: The difference must be less than or equal to 0.070 minutes. When the difference exceeds 0.070 minutes, allow for additional system equilibration time, and repeat the test injections run.

14. From the calibration window in the TargetLynx report, note the Relative SD for area.

Requirement: The peak area Relative SD must be less than or equal to 0.75%.

15. Annotate the report, observing the conventions described in section 2.8.3.
16. Save the TargetLynx data set by clicking File > Save As and then entering an appropriate file name (see section 2.8).
17. Close the report.
18. With the three rows still selected, reprocess them again in TargetLynx using the IClass_UV_RSD_Height.mdb method.
19. From the calibration window in the TargetLynx report, note the Relative SD for height, and the peak height values.

Requirements:

- The peak height Relative SD must be less than or equal to 2.4%.
 - The peak height must be greater than or equal to 1.8e+6 (1,800,000 counts).
20. Annotate the report, observing the conventions described in section 2.8.3.
 21. Append the report to the operational and performance qualification results document associated with this workbook.
 22. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
 23. Save the TargetLynx data set by clicking File > Save As and then entering an appropriate file name (see section 2.8).

4.5.12 Testing the ACQUITY TUV wavelength accuracy (optional)

Perform this test only when the system includes an ACQUITY TUV detector.

Wavelength accuracy with an ACQUITY TUV detector is determined by measuring the spectrum of a caffeine peak. The absorbance bands within caffeine, λ max of 205 nm and 273 nm, are well defined.

To test the ACQUITY TUV wavelength accuracy:

1. Click the Open Sample List icon.
2. Select TUV Wavelength Accuracy_WAXXX_DDMMYY.spl, and enter the current date.
3. Select all samples, and then click the Start Run button.
4. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
5. When the acquisition ends, select the sample list injections in the wavelength accuracy sample list.

6. In the Create TargetLynx Dataset dialog box, select the RSD_TIC.mdb method.
7. Select Integrate Samples, Calibrate Standards, and Quantify Samples.
8. Click File > Apply Layout > Accuracy.qlt.
9. Close the calibration display window in the TargetLynx browser.
10. In the TargetLynx browser, view both groups and ensure appropriate height data exist for each group.

Note: If no height data exist, review the TargetLynx method's peak retention times, and make the corrections needed. Reprocess the data, referring to steps 7 and 8. Repeat as needed.

11. Print the TargetLynx summary report.

Figure 14 Typical ACQUITY TUV wavelength accuracy report:

Caffeine										Group 1 - 1
	Name	Sample Text	Inj. Vol	RT	Height	Area	Response	Acq.Date	Acq.Time	
1	vWaveAcc202		5.0	0.726	301995	10869	301995	08-Mar-11	12:16:15	
2	vWaveAcc203		5.0	0.727	318925	11479	318925	08-Mar-11	12:17:55	
3	vWaveAcc204		5.0	0.727	332857	11960	332857	08-Mar-11	12:19:36	
4	vWaveAcc205		5.0	0.727	344318	12395	344318	08-Mar-11	12:21:17	
5	vWaveAcc206		5.0	0.735	318919	12698	318919	08-Mar-11	12:22:59	
6	vWaveAcc207		5.0	0.728	355444	12812	355444	08-Mar-11	12:24:40	
7	vWaveAcc208		5.0	0.727	354356	12782	354356	08-Mar-11	12:26:20	

Caffeine										Group 2 - 2
	Name	Sample Text	Inj. Vol	RT	Height	Area	Response	Acq.Date	Acq.Time	
1	vWaveAcc270		5.0	0.726	111685	4037	111685	08-Mar-11	12:28:07	
2	vWaveAcc271		5.0	0.726	111034	4119	114034	08-Mar-11	12:29:49	
3	vWaveAcc272		5.0	0.724	115402	4179	115402	08-Mar-11	12:31:29	
4	vWaveAcc273		5.0	0.727	116080	4217	116080	08-Mar-11	12:33:09	
5	vWaveAcc274		5.0	0.726	116763	4233	116763	08-Mar-11	12:34:50	
6	vWaveAcc275		5.0	0.727	116228	4226	116228	08-Mar-11	12:36:30	
7	vWaveAcc276		5.0	0.727	115873	4200	115873	08-Mar-11	12:38:17	

12. From each sample group, identify the sample of greatest height, and record the result in Form 6, "Operational qualification results", of the operational and qualification results document associated with this workbook.

Requirement: The sample of greatest height from the first group must fall within the 203 to 207 nm range (± 2 nm). The sample of greatest height from the second group must fall within the 271 to 275 nm range (± 2 nm).

13. Annotate the report, observing the conventions described in section 2.8.3.
14. Append the report to the operational and performance qualification results document associated with this workbook.
15. Save the TargetLynx data set by clicking File > Save As and then entering an appropriate file name (see section 2.8).

4.5.13 Testing the ACQUITY PDA wavelength accuracy (optional)

Wavelength accuracy with an ACQUITY PDA or PDA e λ detector is determined by measuring the spectrum of a caffeine peak. The absorbance bands within caffeine (λ max of 205 nm and 273 nm) are well defined.

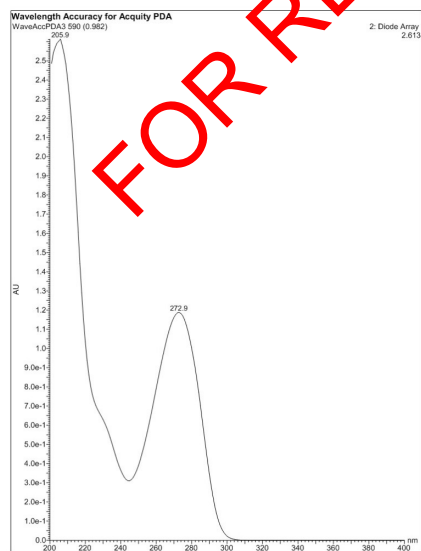
Note: Perform this test only if the system includes an ACQUITY PDA detector, and ensure the detector is configured as part of the system.

To test the ACQUITY PDA wavelength accuracy:

1. Click the Open Sample List icon.
2. Select PDA Wavelength Accuracy_WAXXX_DDMMYY.spl, and enter the current date.
3. Select all samples, and then click the Start Run button.
4. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
5. When the injection is complete, click the Chromatogram tab and click Display > TIC.
6. Select Diode Array, and Replace trace, and then click OK.
7. Click Display > Wavelength, enter the value 273 in the Description field, and ensure the Function field is set to "2: Diode Array."
8. Select Replace Trace, and then click OK.
9. Right-click over the apex of the caffeine peak in the extracted 273 chromatogram to open a spectrum window for the peak.
10. From the Spectrum window, click Display > Peak Annotation, and set the Decimal Places field to 1 to display the lambda max value to one decimal place.
11. Ensure there are two peaks annotated at λ 205 $\pm$ 2 nm and 273 $\pm$ 2 nm for the caffeine peak in the spectrum window (see Figure 15).

Requirement: The wavelength accuracy must be within ± 2 nm.

Figure 15 Typical ACQUITY PDA wavelength accuracy spectrum window:



12. Print the spectrum.

13. Record the two annotated peaks in Form 6, "Operational qualification results", of the operational and qualification results document associated with this workbook.
14. Annotate the spectrum, observing the conventions described in section 2.8.3.
15. Append the report to the operational and performance qualification results document associated with this workbook.

4.5.14 Testing UV detector linearity and sensitivity (optional)

The UV-detector-linearity test with an ACQUITY PDA, PDA e λ , or TUV detector determines whether a detector's response is linear within its absorbance range. The test involves injecting a series of standards at different concentrations using a constant injection volume. TargetLynx software plots area and height against the sample amount and applies the coefficient of determination (R^2) to measure the linearity of the detector's response.

Note: Perform this test only if the system includes an ACQUITY PDA, PDA e λ , or TUV detector.

To test the detector linearity:

1. Click the Open Sample List icon.
2. Select "TUV_10mmDetector_Linearity" and "Sensitivity_WAAXXX_DDMMYY.spl" if you have a TUV or "PDA_10mmDetector_Linearity" and "Sensitivity_WAAXXX_DDMMYY.spl" if you have a PDA/PDAe λ detector.
3. Update the instrument serial number and date format.
4. Refer to the certificate of analysis supplied with the test solutions, and then alter the displayed default concentrations in the "Conc. A" column to match those in the certificate of analysis.
5. Save the sample list, and append the certificate of analysis to the operational and performance qualification results document associated with this workbook.
6. Select all samples, and then click the Start Run button.
7. In the Start Sample List dialog, select Acquire Sample Data, and then click OK.
8. When the injections end, select the sample list injections in the detector linearity sample list.
9. In the Create TargetLynx Dataset dialog box, select the Linearity_Area.mdb method.
10. Select Integrate Samples, Calibrate Standards, and Quantify Samples.
11. Click File > Apply Layout > Acquity_QWB_Linearity.qlt.
12. Print the TargetLynx summary report. (See Figure 16.)

Figure 16 Typical detector linearity TargetLynx report for area:**Compound name: Caffeine**

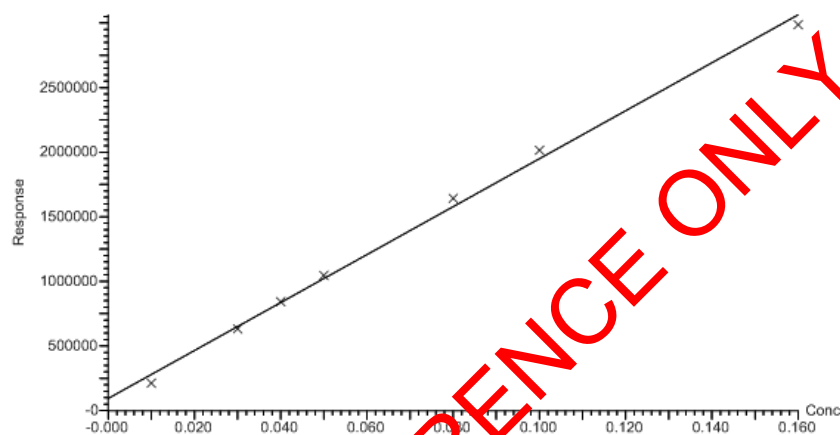
#	Name	Vial	Inj. Vol	RT	Height	Area	Acq.Date	Acq.Time
1	PDA_Detector_L...	2:F,1	3.000	0.69	8852420	211645.078	28-Apr-12	12:12:52
2	PDA_Detector_L...	2:F,2	3.000	0.68	26348644	632095.125	28-Apr-12	12:16:50
3	PDA_Detector_L...	2:F,3	3.000	0.68	35021516	843139.750	28-Apr-12	12:19:25
4	PDA_Detector_L...	2:F,4	3.000	0.68	42019840	1046948.250	28-Apr-12	12:22:00
5	PDA_Detector_L...	2:F,5	3.000	0.68	64202104	1643571.250	28-Apr-12	12:24:35
6	PDA_Detector_L...	2:F,6	3.000	0.68	77627232	2016022.250	28-Apr-12	12:27:11
7	PDA_Detector_L...	2:F,7	3.000	0.68	111575072	2988765.250	28-Apr-12	12:29:47

Compound name: Caffeine

Correlation coefficient: $r = 0.998157$, $r^2 = 0.996318$ Calibration curve: $1.85613e+007 * x + 94052.8$

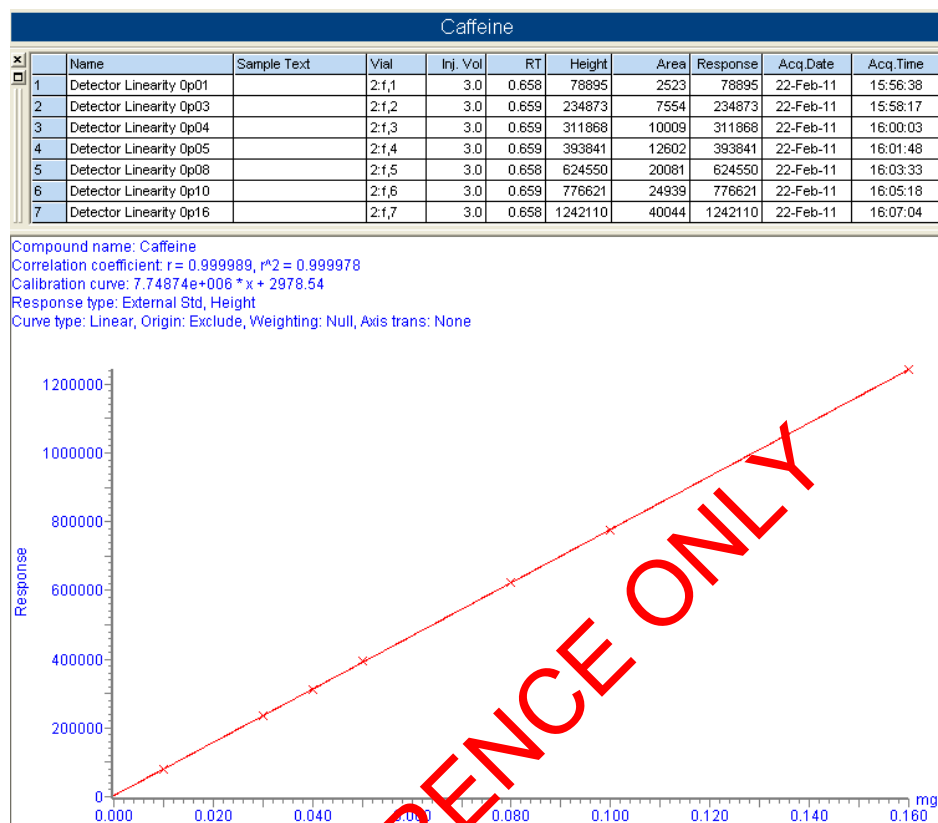
Response type: External Std, Area

Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

**Requirement:** The R^2 value for area must be greater than or equal to 0.990.

13. Examine the report, and record the result in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
14. Annotate the report, observing the conventions described in section 2.8.3.
15. Append the report to the operational and performance qualification results document associated with this workbook.
16. Click File > Save As, and enter an appropriate file name (see section 2.8).
17. Select Integrate Samples, Calibrate Standards, and Quantify Samples.
18. In the Create TargetLynx Dataset dialog box, select the Linearity_Height.mdb method.
19. Print the TargetLynx summary report. (See Figure 17.)

Figure 17 Typical detector linearity TargetLynx report for height:



Requirement: The R^2 value for height must be greater than or equal to 0.990.

20. Record the result in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
21. Annotate the report, observing the conventions described in section 2.8.3.
22. Append the report to the operational and performance qualification results document associated with this workbook.
23. Click File > Save As, and enter an appropriate file name (see section 2.8).

To test the detector sensitivity:

1. Use the data from the acquisitions that you acquired in the detector linearity test.
2. In the Create TargetLynx Dataset dialog box, select the Detector_Sens_RSD.mdb method.
3. Select Integrate Samples, Calibrate Standards, and Quantify Samples.
4. Click File > Apply Layout > Acquity_QWB_RSD_sens.qlt.

Figure 18 Typical report for detector sensitivity test:

Quantify Compound Summary Report

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Dataset: Untitled

Last Altered: Thursday, March 17, 2011 16:12:20 GMT Standard Time

Printed: Thursday, March 17, 2011 16:12:49 GMT Standard Time

Method: C:\Vulcan\QWB\h-class\neil\AcquityH_UV_QWB.PR0\Meth.DBL\Linearity_Height.mdb 27 Jan 2010 15:29:52

Calibration: 17 Mar 2011 16:12:20

Compound name: Caffeine

	Name	Sample Text	Vol	Inj. Vol	RT	Height	RRF_Height	Area	RRF_Area
1	Detector Linearity...		2.1	3.000	0.658	78895	7889500	2523	252261
2	Detector Linearity...		2.2	3.000	0.659	234873	7829100	7554	251798
3	Detector Linearity...		2.3	3.000	0.659	311868	7796700	10009	250225
4	Detector Linearity...		2.4	3.000	0.659	393841	7876820	12802	252038
5	Detector Linearity...		2.5	3.000	0.658	624550	7806875	20081	251007
6	Detector Linearity...		2.6	3.000	0.659	776621	7766210	24939	249387
7	Detector Linearity...		2.7	3.000	0.658	1242110	7763188	40044	250276

	Acq.Date	Acq.Time
1	22-Feb-11	15:58:38
2	22-Feb-11	15:58:17
3	22-Feb-11	16:00:03
4	22-Feb-11	16:01:48
5	22-Feb-11	16:03:33
6	22-Feb-11	16:05:18
7	22-Feb-11	16:07:04

5. From the report's results table, calculate the Relative SD for the RRF_Height and RRF_Area columns.

Requirements:

- RRF_Area Relative SD must be less than or equal to 3.5%.
- RRF_Height Relative SD must be less than or equal to 4.5%.

Note: For instructions on how to calculate Relative SD, see section 5.

6. Record the result in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
7. Print the Target Lynx report, and annotate it, observing the conventions described in section 2.8.3.
8. Click File > Save As, and enter an appropriate file name (see section 2.8).

5. Using the Windows Calculator to Calculate Standard Deviation

To use the scientific calculator to calculate standard deviation in Windows XP:

1. Click Start > Programs > Accessories > Calculator.

Result: The Windows calculator appears.

2. Click View > Scientific.

Result: The calculator switches to scientific view.

3. Enter the first value by clicking the appropriate numbers on the Windows calculator.

Note: If Num Lock is on, you can also use the numeric keypad to enter values.

4. Click Sta.

Result: The statistics box appears.

5. Click Dat, and then click Sta to display the statistics box.

6. Enter each of the remaining values by clicking the appropriate numbers on the calculator, then clicking Dat to enter each value in the statistics box.

7. After you enter all values in the statistics box, click the s button in the left column of the calculator to calculate the standard deviation (SD).

8. To calculate Relative SD, use the following formula:

$$\text{Relative SD} = \frac{(\text{SD} \times 100)}{\text{Average}}$$

To use the scientific calculator to calculate standard deviation in Windows 7:

1. From the Windows Start menu, click Programs > Accessories > Calculator.

Result: The Windows calculator appears.

2. Click View > Statistics.

Result: The calculator switches to statistics view.

3. Enter the first value by clicking the appropriate numbers on the Windows calculator.

Note: If Num Lock is on, you can also use the numeric keypad to enter values.

4. From the left column of the calculator, click Add.

Result: The number is entered in the display and the count is updated by 1.

5. Repeat the previous two steps until all the values you wish to include in the calculation have been added.
6. After you enter all values in the list, click the σ_n button in the left column of the calculator to calculate the standard deviation.
7. To calculate Relative SD, use the following formula:

$$\text{Relative SD} = \frac{(\text{SD} \times 100)}{\text{Average}}$$

FOR REFERENCE ONLY

6. Logging Operational/Performance Qualification Results

Record any operational and performance qualification procedures performed on the system in the supplied system log or other customer supplied document, including those performed after any scheduled maintenance procedures or corrective servicing.

FOR REFERENCE ONLY