

Waters Qualification Workbook

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

Operational and Performance Qualification Procedures: OQ/PQ

Workbook Part Number: 715002746 Revision: A

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FOR REFERENCE ONLY

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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® H-Class system, operated by MassLynx™ software, version 4.1 (SCN 810, 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 UPLC Quaternary Solvent Manager (QSM)
- ACQUITY UPLC Sample Manager with Flow Through Needle (SM-FTN)

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

- ACQUITY UPLC Column Heater-Active (CH-A)
- ACQUITY UPLC Column Manager-Active (CM-A)
- ACQUITY UPLC Column Manager-Active Auxiliary (CM-Aux)
- ACQUITY UPLC Tunable Ultraviolet (TUV) Detector with 10-mm analytical flow cell.
- ACQUITY UPLC Photodiode Array (PDA) Detector (standard PDA or PDA eλ) with 10-mm analytical flow cell.
- IonSABRE™ II APCI 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:

- *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
- *Waters ACQUITY UPLC H-Class System Documentation CD*, part number 715002124

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2. Preliminary Procedures

2.1 Identifying performers and reviewers

Identify the performer qualified to perform the operational and performance qualification and the reviewer qualified to acknowledge completion of the performers' tasks by completing Form 1, "Performer and reviewer identification", of the operational and performance qualification results document associated with this workbook.

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:

Kits

- Waters Xevo TQ-S Sample Kit, part number 700005472
- Waters ACQUITY UPLC Absorbance 10MM Test Solutions Kit, part number 700002642
- ESI Probe Assembly Spares Kit, part number 700003278

ACQUITY UPLC H-Class equipment

- ACQUITY UPLC H-CLASS BEH C18 column, 2.1 mm × 50 mm, 1.7 μm (part number 186002350)
- 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)

ACQUITY UPLC H-Class (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)

- ACQUITY UPLC TUV flow cell for TUV detectors with serial numbers J05UPT700N or later:

Analytical flow cell, 10-mm path length (part number 205015008)

- ACQUITY UPLC PDA flow cell for PDA detectors:

Analytical flow cell, 10-mm path length (part number 205015004)

- PEEK™ tubing, 0.005 inch, part number 430001229

Solvents and additives

- LCMS grade acetonitrile, 2.5-L minimum
- LCMS grade methanol, 1-L minimum
- LCMS grade propan-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

- Graduated measuring cylinder, 100-mL, Class A, with stopper
- Graduated measuring cylinder, 500-mL, Class A, with stopper
- Graduated measuring cylinder, 1000-mL Class A, with stopper
- Pipettes, 10-mL (calibrated)
- Pipettes, 20-μL (calibrated)
- Pipettes, 200-μL (calibrated)
- Pipettes, 1-mL (calibrated)
- Pipette tips appropriate for use with the make and model of the pipette(s)
- Amber glass volumetric flask, 25-mL, Class A, with stopper
- Amber glass volumetric flask, 100-mL, Class A, with stopper
- Reservoir bottles (5), 1-L
- IntelliStart™ Fluidics system vials (6), 30-mL, with caps (part number 700004256).

Miscellaneous

- Nitrile gloves
- Lint-free tissues
- Tape, to hold the thermometer probe

- Ultrasonic bath
- Glass funnel
- Vortex mixer
- Calibrated thermometer probe, with display
- Calibrated stop watch

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.

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.

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

2.5.1 Wash solvents

Acid wash

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.

Note: 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. Label the solution "Acid Wash".

Basic wash

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 1-L reservoir bottle.

Note: 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. Label the solution "Basic wash".

2.5.2 Mobile phases

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

- Water (solvent line A, solvent line B, Seal wash and SM Purge)
- Acetonitrile (solvent line B, solvent line D and SM-Wash)

The following solutions are used in the remaining tests:

IPA Solution

To prepare the solution:

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

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 1 L of LCMS-grade deionized water.
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".

Acetonitrile + 0.01% formic acid**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".

You will also need these solvents:

- Water
- Acetonitrile
- LCMS-grade methanol

2.5.3 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:

Solution	Target concentration
Detector linearity using tuning solution (diluted) as the stock	750 fg/µL sulfadimethoxine 500 fg/µL sulfadimethoxine 100 fg/µL sulfadimethoxine 50 fg/µL sulfadimethoxine 10 fg/µL sulfadimethoxine 5 fg/µL sulfadimethoxine 1 fg/µL sulfadimethoxine 0.5 fg/µL sulfadimethoxine 0.25 fg/µL sulfadimethoxine
Tuning solution diluted	20 pg/µl acetaminophen 20 pg/µl caffeine 10 pg/µl sulfadimethoxine 5 pg/µl verapamil 5 pg/µl chloramphenicol 500 pg/µl 17-α-hydroxyprogesterone
Gradient, precision, sensitivity test sample	0.2 pg/µl acetaminofen 0.2 pg/µl caffeine 0.1 pg/µl sulfadimethoxine 0.05 pg/µl verapamil 0.05 pg/µl chloramphenicol 5 pg/µl 17-α-hydroxyprogesterone.
Injector carryover test sample	200 ng/µL sulfadimethoxine in methanol

Diluted tuning solution

This solution is used in the ESI positive sensitivity, ESI negative sensitivity and APCI positive sensitivity tests.

To prepare the solution:

1. Before opening the ampoule containing "Tuning solution x100", sonicate for 3 minutes, and then vortex mix the ampoule. Doing so ensures that the solution is homogenous before sampling.
2. Pipette 1 mL into a 100-mL volumetric flask and dilute to 100 mL with water +0.01% formic acid +0.05% ammonia solution.
3. Stopper and invert the flask several times to fully mix.
4. Label the flask as "Tuning solution diluted".
5. Transfer 1 mL of "Tuning solution diluted" to an ACQUITY vial and then place the vial in position A,3 in the ANSI 48-vial, 2-mL vial holder.
6. Transfer 20 mL to an Intellistart fluidic 30ml plastic bottle, for Intellistart tuning.

Gradient solution

This solution is used for the Gradient, ESI negative sensitivity and optional APCI positive sensitivity tests.

To prepare the solution:

1. Place 990 µL of diluent into an ACQUITY vial.
2. Pipette 10 µL of "Tuning solution diluted" into the vial.
3. Attach the cap, and vortex mix the vial's contents.
4. Label the vial "Gradient", and place it into vial position A,2 in the ANSI 48-vial, 2-mL vial holder.

Sample dilution series

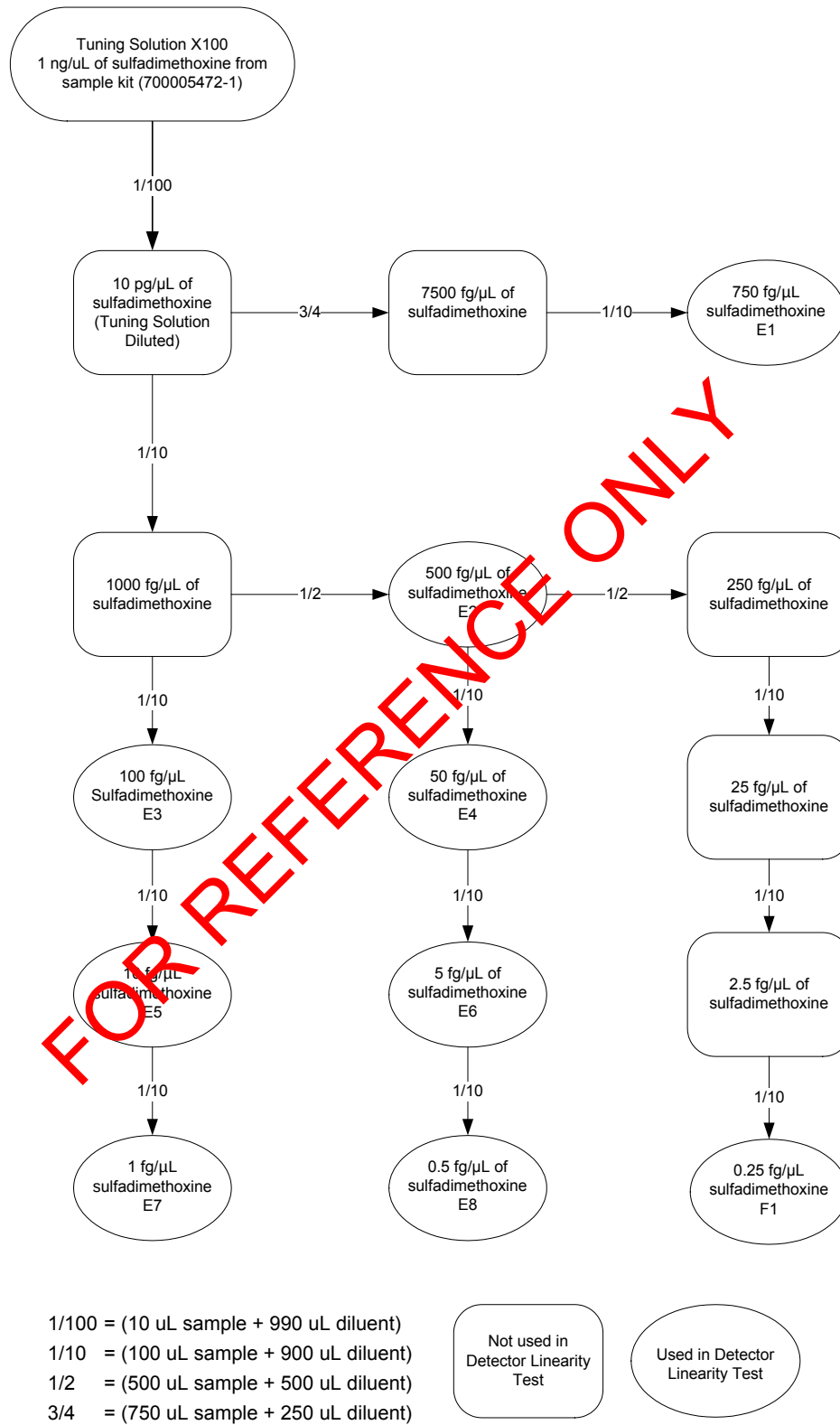
Sulfadimethoxine is light and temperature sensitive. Shield it from light whenever possible.

Requirement: 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.

To prepare the 10 pg/ μ L solution:

1. Place 990 μ L of diluent into a vial, and then pipette 10 μ L of 1 ng/ μ L sulfadimethoxine (tuning solution x100) into the vial.
2. Attach the cap, vortex mix the vial contents, and then label it "10 pg/ μ L".

To prepare the 7500 fg/ μ L solution:

1. Place 250 μ L of diluent into a vial, and then add 750 μ L of 10 pg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "7500 fg/ μ L".

To prepare the 750 fg/ μ L E1 solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 7500fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "750 fg/ μ L E1 (highest linearity sample)".

To prepare the 1000 fg/ μ L solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 10 pg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "1000 fg/ μ L".

To prepare the 100 fg/ μ L E3 solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 1000 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "100 fg/ μ L E3 (linearity sample, Pos/Neg Sens, System Precision)".

To prepare the 10 fg/ μ L E5 solution:

1. Place 900 μ L of diluent into a vial, and then add 100 μ L of 100 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "10 fg/ μ L E5 (linearity sample)".

To prepare the 1 fg/ μ L E7 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 E7 (linearity sample)".

To prepare the 500 fg/ μ L E2 solution:

1. Place 500 μ L of diluent into a vial, and then add 500 μ L of 1000 fg/ μ L sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "500 fg/ μ L E2 (linearity sample)".
3. Repeat steps 1 and 2 so you have two vials, each containing 1 ml of the solution. You will use one of the vials for injection, and the other for creating other dilutions.

To prepare the 50 fg/ μ L E4 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 E4 (linearity sample)".

To prepare the 5 fg/ μ L E6 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 E6 (linearity sample)".

To prepare the 0.5 fg/ μ L E8 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 E8 (linearity sample)".

To prepare the 250 fg/ μ L 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".

To prepare the 25 fg/ μ L 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".

To prepare the 2.5 fg/ μ L 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".

To prepare the 0.25 fg/μL F1 solution:

1. Place 900 μL of diluent in a vial, and then add 100 μL of 2.5 fg/μL sulfadimethoxine.
2. Attach the cap, vortex mix the vial contents, and then label it "0.25 fg/μL F1 (lowest linearity sample)".

To prepare the blank solution:

Place 1000 μL of diluent into each of two vials. Label them "Blank".

Having prepared all of the solutions in the dilution series, place each sample vial into the correct location as shown in Table 3.

Stock 200 ng/μL sulfadimethoxine solution

This solution is used in the injector carryover test. Perform the following procedure using the vial containing 5 mg sulfadimethoxine (vial 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 25 mL using methanol, and sonicate until all the sulfadimethoxine has fully dissolved.
5. Label the flask "200 ng/μL Sulfadimethoxine solution".
6. Transfer 1 mL into a vial, and then place it into well A,4 in the ANSI 48-vial, 2-mL vial holder.

Solutions for system including a TUV/PDA detector and 10-mm flow cell

If the system includes these optional devices, pipette 1 mL of each solution shown in Table 2 into a vial, and place in the position indicated in Table 3.

Table 2 Solutions and target concentrations for TUV/PDA options:

Solution	Target concentration
Caffeine (Solution 1)	0.010 mg/mL
Caffeine (Solution 2)	0.030 mg/mL
Caffeine (Solution 3)	0.040 mg/mL
Caffeine (Solution 4)	0.050 mg/mL
Caffeine (Solution 5)	0.080 mg/mL
Caffeine (Solution 6)	0.100 mg/mL
Caffeine (Solution 7)	0.160 mg/mL

2.5.4 Vial locations

The following tables show the correct vial positions for each of the prepared solutions in an ANSI 48-vial, 2-mL vial holder.

Table 3 Sample vial locations:

Sample	Vial location
Blank	A,1
Blank	C,2
Acid wash	D,1
Basic wash	D,2
Neat IPA	C,1
Gradient, negative sensitivity, optional APCI positive sensitivity	A,2
sulfadimethoxine (standard 10 pg/ μ L)	A,3
sulfadimethoxine (challenge 200 ng/ μ L)	A,4
750 fg/ μ L sulfadimethoxine	E,1
500 fg/ μ L sulfadimethoxine	E,2
100 fg/ μ L sulfadimethoxine	E,3
50 fg/ μ L sulfadimethoxine	E,4
10 fg/ μ L sulfadimethoxine	E,5
5 fg/ μ L sulfadimethoxine	E,6
1 fg/ μ L sulfadimethoxine	E,7
0.5 fg/ μ L sulfadimethoxine	E,8
0.25 fg/ μ L sulfadimethoxine	F,1
Caffeine 0.01 mg/mL (TUV/PDA only)	B,1
Caffeine 0.030 mg/mL (TUV/PDA only)	B,2 and B,8
Caffeine 0.040 mg/mL (TUV/PDA only)	B,3
Caffeine 0.050 mg/mL (TUV/PDA only)	B,4
Caffeine 0.080 mg/mL (TUV/PDA only)	B,5
Caffeine 0.100 mg/mL (TUV/PDA only)	B,6
Caffeine 0.160 mg/mL (TUV/PDA only)	B,7

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 Project Files" folder on the Qualification section of the Waters Knowledge Center.

To create the qualification project in MassLynx software:

Recommendation: Use the Xevo_TQS_ACQUITY_HCLASS_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_HCLASS_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_HCLASS_OQPQ_WAAXXX
 - Description: Xevo_TQS_ACQUITY_HCLASS_OQPQ_WAAXXX_nn
 - Location: C:\MassLynx
5. Click Next, and then select "Create using current project as template".
6. Select XEVO_TQS_ACQUITY_OQPQ.pro as the current project.
7. Select Copy Sample lists.
8. Click Finish.
9. Name the Acidic_Basic_HClass_Clean_WAAXXX_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 and sample list formats:

Click Samples > Format > Load > XEVO_TQS_UPLC.fmt, and browse to the following location:

C:\MassLynx\XEVO_TQS_ACQUITY_HClass_OQPQ_WAAXXX.PRO\SampleDB.

2.8 Managing data files and test results

Once you have created the qualification project, use 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 (DDMMYYYY)
- Test procedure reference name
- Sample solution
- Concentration
- Flow rate
- All calculations

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

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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.

3.1 Configuring the ACQUITY UPLC H-Class

To configure the instrument:

1. In the MassLynx toolbar, click Inlet Method > Tools > Inlet Configuration > Setup Instrument.
2. Select these items:
 - Quaternary solvent manager (QSM)
 - Sample manager - flow through needle (SM-FTN)
 - ACQUITY PDA Detector (optional)
 - ACQUITY TUV Detector (optional)
 - Column manager (CM-A) (optional)
3. Click Next, and then click Yes.
4. Allow the installation to stop, and then click Finish.
5. In the shortcut bar, click > Inlet Method > Tools > Inlet configuration > Configure > Waters ACQUITY QSM.
6. Click Next > Waters ACQUITY Sample Manager FTN.
7. Click Next, and then select optional detector(s) and optional modules.
8. Click Next > Next > Finish.
9. In the shortcut bar, click > Inlet Method > Tools > Inlet configuration > Events & Triggering.
10. Ensure that Event 1 is selected, and then click Next > Finish.
11. 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.
12. Verify the rack setup in the Inlet Method editor.
13. In the MassLynx application, select Inlet Method > ACQUITY Sampler > Plate Generator.

14. In the Rack Generator menu bar, click the Previous Rack arrow icon until ANSI-48Vial2mLHolder appears in the Rack Name field.
15. In the Grid Reference pane, click the Referencing list, and then select XY.
16. In the Grid Reference area, select the parameters shown in Table 5.

Table 4 Grid reference parameters:

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

17. Click Rack > Save Current Rack > OK.
18. Quit the Plate Generator dialog box.

3.2 Preparing the system for qualification

To prepare the system:

1. To a reservoir bottle, add 1L of deionized water, and submerge lines A, C , Seal Wash and SM purge solvent into the water.
2. To a 1L reservoir bottle, add 250mL of acetonitrile, and submerge lines B, D and the SM-Wash into the acetonitrile.
3. Place the reservoir bottles in the ACQUITY UPLC solvent tray.
4. Access the ACQUITY Console by clicking Inlet Method > Status > ACQUITY Additional Status.
5. From the ACQUITY UPLC Console, complete these tasks:
 - a. Wet-prime pump lines A, B, C, D, and seal wash for at least 5 minutes;
 - b. Prime the sample manager-FTN's sample wash and Purge solvent as follows;
 - In the Wash Solvent field, enter 60 seconds.
 - In the Purge Solvent field, enter 20 cycles, and then click OK.
6. Attach the ACQUITY column and all fluidic¹ tubing to the mass spectrometer and other UV detectors.

¹ In Waters documents, the term "fluidics" refers to the IntelliStart Fluidics system, the instrument's onboard system that delivers sample and solvent to the probe of the mass spectrometer. It can also denote plumbing components and fluid pathways within and system modules.

7. Set the QSM to deliver acetonitrile/water 50/50/0/0 A:B:C:D at a flow rate of 0.8mL/min.
8. On the Tune window's Fluidics tab, set the flow state to waste.

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 H-Class, recording results when instructed to do so.

3.3.1 Quaternary Solvent Manager

Testing flow rate accuracy

Use a dry, 10-mL volumetric flask to test the flow rate accuracy of the pump using acetonitrile at a flow rate of 0.5 mL/min.

To test flow rate accuracy:

1. Connect a length of stainless steel tubing, or other restriction device, to the outlet of the quaternary solvent manager's mixer.

Note: The flow restrictor creates a backpressure between 3447 and 4826 kPa (34 and 48 bar, 500 and 700 psi), at a flow rate of 0.5 mL/min.
2. Obtain a thoroughly dry, 10-mL Class A volumetric flask.
3. Prepare the stopwatch.
4. In the Inlet Method editor, click File > Open, and then select ZeroFlow.
5. Click LC > Load Method.
6. Open the ACQUITY H-Class Console window.
7. From the MassLynx Sample List, click Status > Inlet Method > Acquity Additional Status > Acquity Sample Manager > Launch Acquity Console > ACQUITY Quaternary Solvent Manager (QSM) > Control > Set Flow.
8. To test the pump, on the ACQUITY QSM Console screen, specify a flow rate of 0.5 mL/min and 100% B, and then click Enter.
9. When the flow and pressure stabilize, insert the outlet tubing into the volumetric flask, and simultaneously start the stopwatch.
10. When the bottom of the meniscus reaches the 10-mL volumetric mark on the flask, stop the stopwatch.
11. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the elapsed time, in seconds, and also the system pressure.
12. Calculate the flow rate:

Flow rate = Volume of flask/(measured time in seconds/60).

13. In Form 6, “Operational and performance qualification results”, of the operational and performance qualification results document associated with this workbook, record the calculated flow rate for the pump.

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

The flow rate accuracy specification is $\pm 1\%$ 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. See section 4.5.6. “Testing flow rate linearity”.

3.3.2 Testing the SM-FTN temperature accuracy

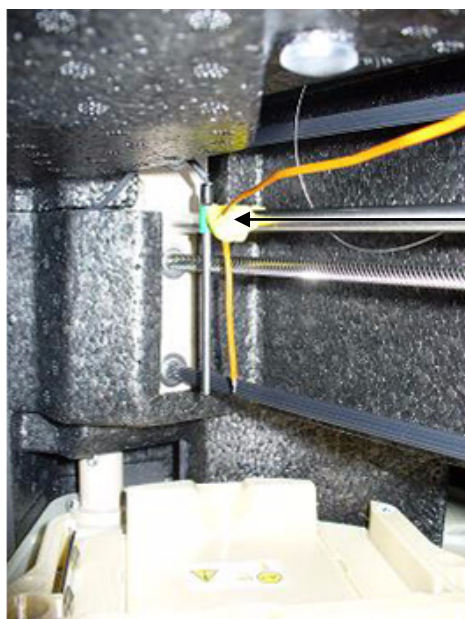
At the temperature sensor of the sample manager – flow through needle (SM-FTN), the temperature accuracy is $\pm 3\text{ }^{\circ}\text{C}$. To compensate for typical measurement inaccuracy, a value of $\pm 4\text{ }^{\circ}\text{C}$ defines the heater/cooler’s acceptable inaccuracy range. If the inaccuracy of your measurement device exceeds $\pm 1\text{ }^{\circ}\text{C}$, you can adjust the acceptable range accordingly. If you do so, 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 SM-FTN:

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 it from the top of the heater/cooler duct and securing it with tape, (see Figure 2). Ensure the thermometer probe does not contact any surfaces.

Figure 2 Positioning the thermometer probe in the sample manager:



Thermometer attachment to R-carriage.

3. Close the sample compartment door, being sure not to damage the probe wire.
4. On the ACQUITY Console screen for the SM-FTN, 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 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 using a different set-point temperature, if desired.
8. At the end of the temperature accuracy test, return the sample manager's temperature to 7 °C.

3.3.3 Testing the CM-A or CM-Aux temperature accuracy

Use this procedure for the Column Manager-A (CM-A) or Column Manager-Aux (CM-Aux), which can hold up to 2 columns.

Requirement: As it is possible to set the temperature of column 1 to a different value compared to column 2, you must repeat this test for each column compartment.

At the temperature sensor of the CM-A or CM-Aux, the temperature accuracy is ± 1.0 . To compensate for typical measurement inaccuracy, a value of ± 2 °C defines the heater/cooler's acceptable inaccuracy range. If the inaccuracy of your measurement device exceeds ± 1 ° C, you can adjust the acceptable range accordingly. If you do so, 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 CM-A or CM-Aux:

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

Figure 3 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 wall (see Figure 4).

Figure 4 Installing the thermometer probe in the CM-A:



6. Close the front panel.
7. On the ACQUITY Console screen for the column manager, specify a single set-point value between 10 °C through 90 °C.
Requirement: Do not specify a value that falls within ± 5 °C of the ambient temperature. (Most users choose a temperature set-point 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 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 temperature varies depending on the difference between the set 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. Repeat steps 4 through 10 with the thermometer placed in the lower column compartment.
12. Optionally repeat steps 7 through 11 using a different set point temperature, if desired.
13. At the end of the temperature accuracy test, return the column manager temperature to 40 °C.

3.3.4 Testing the CH-A temperature accuracy

Use this procedure only for the Column Heater-A (CH-A), which holds a single column.

The temperature accuracy of the CH-A at the temperature sensor is ± 1.0 °C. To compensate for typical measurement inaccuracy, a value of ± 2 °C defines the CH-A's acceptable inaccuracy range. If the inaccuracy of your measurement device exceeds ± 1 °C, you can adjust the acceptable range accordingly. If you do so, 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 CH-A:

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, and position the thermometer probe so that it is approximately 2.5 to 3.8 cm to the right-hand side 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 5).

Figure 5 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 6).

Figure 6 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 value from 5 °C above ambient temperature to 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 value you specified in step 8.

Recommendation: Allow sufficient time for the column heater's temperature to reach and stabilize at the set 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 temperature varies depending on the difference between the set and ambient temperatures.

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

3.4 Additional system setup procedures

To install the ACQUITY UPLC H-CLASS BEH C18 column:

1. Install the ACQUITY UPLC H-CLASS BEH C18 column in the CH-A, CM-A or CM-Aux.
2. Connect the inlet active pre-heater to the column inlet, and then clip into place in the column tray.
3. Connect the column outlet tubing to the TUV or PDA inlet, if configured.
4. Close the front cover of the CH-A, CM-A or CM-Aux .
5. Attach the eCord™ to the eCord receptacle on the right-hand side of the CH-A, CM-A or CM-Aux .

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

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

1. Connect the outlet from CH-A, CM-A or CM-Aux (or from the flow cell outlet, if a TUV or PDA is configured) to the 4-way diverter valve inlet (center position, port 1) on the Xevo TQ-S using black PEEK™ tubing.
2. The remaining diverter valve connections (sample probe, lock-spray probe, and waste) remain configured as for the mass spectrometer tests.

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

1. From 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 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.

Requirement: TUV and PDA detectors require a lamp equilibration time before commencing any qualification testing.

- For an ACQUITY PDA or PDA eλ, a 60-minute lamp equilibration time is required.
- For an ACQUITY TUV, a 30-minute lamp equilibration time is required.

To load the sample list and sample list format:

1. In the MassLynx main window, click File > Open.
2. Click Xevo_TQS_with_ACQUITY_H_Class_Test_Injections.spl.
3. From the sample list, click Samples > Format > Load, and select Xevo_TQS_with_Acquity_HClass.fmt.

Note: This format file, supplied with the qualification project, is located in the C:/MassLynx folder.

Figure 7 Sample list format:

Spectrum Chromatogram Map Edit▼ Samples▼								
	File Name	Bottle	Inlet File	File Text	MS File	Inject Volume	Sample Type	Conc A
1	Test Injection Blank1	2.F,8	Isocratic Method	Blank 1	Test Injections	5.000	Blank	
2	Test Injection Blank2	2.F,8	Isocratic Method	Blank 2	Test Injections	5.000	Blank	
3	Test Injection1	2.D,3	Isocratic Method	500pg on column	Test Injections	5.000	Standard	500
4	Test Injection2	2.D,3	Isocratic Method	500pg on column	Test Injections	5.000	Standard	500

FOR REFERENCE ONLY

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)

The noise and drift tests are run from the ACQUITY Console. Both tests comply 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.

Requirements:

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

To perform the PDA/PDA eλ noise and drift test:

1. In the Inlet Method editor, open the Isocratic method, and then click on the load method button.

Rationale: Doing so ensures the system is at the correct UPLC conditions for the tests runs.

2. Leave the UPLC system to equilibrate for 5 minutes before continuing with the test.
3. From the ACQUITY Console, select PDA Detector from the console tree.
4. Go to 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:

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

Requirement: The segmented peak noise must be less than or equal to 80 μAU , and the detector drift must be less than or equal to 15,000 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 the ACQUITY Console noise and drift test report, and annotate it observing the conventions described in section 2.8.3.
8. 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)

Noise test

The noise test is built into the ACQUITY Console. The test, for which no optional parameters apply, lasts for 15 minutes. It calculates the most positive and most negative point over 15 minutes, and then calculates a segmented peak noise result in μAU .

Requirements:

- Use the following mobile phases for this test:
 - 100% water (solvent line A, solvent line B, Seal wash and SM Purge);
 - 100% acetonitrile (solvent line B, solvent line D and SM-Wash).

- Both tests require LC effluent to be flowing through the flow cell.
- The mobile phase must not contain any additives or modifiers.

To perform the noise test:

1. In the Inlet Method editor, open the isocratic method, and then select Load Method.

Rationale: Doing so ensures the system is at the correct UPLC conditions for the tests runs.

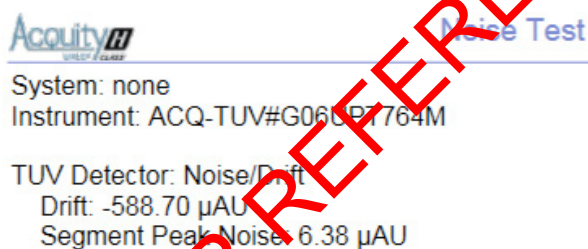
2. Leave the UPLC to equilibrate for 5 minutes before continuing with the test.
3. In the ACQUITY Console, select TUV Detector from the console tree.
4. Go to the Troubleshoot tab, and click Noise test > Start.

Result: When the test ends, a report including 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 .

5. Print the ACQUITY Console TUV noise report (See Figure 9).

Figure 9 Example of the TUV detector noise report:



Acquity
Noise Test

System: none
Instrument: ACQ-TUV#G060P7764M

TUV Detector: Noise/Drift
Drift: -588.70 μAU
Segment Peak Noise: 6.38 μAU

6. Record the 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 result produced.

7. Annotate the report observing the conventions described in section 2.8.3.
8. 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. The drift test, for which no optional parameters apply, monitors the baseline absorbance over a one hour period. The difference between the start and stop baseline absorbance is reported in μUA .

To perform the drift test:

1. In the Inlet Method editor, open the isocratic method and select Load Method.

Result: Doing so ensures the system is at the correct UPLC conditions for the tests runs.

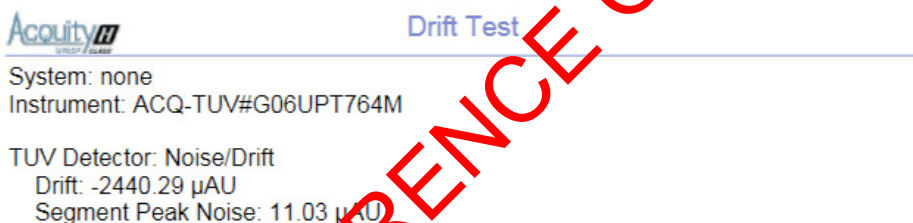
2. Leave the UPLC to equilibrate for 5 minutes before continuing with the test.
3. In the ACQUITY Console, select TUV Detector from the console tree.
4. Go to the Troubleshoot tab, and then click Drift test > Start.

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

Requirement: The detector drift must be less than or equal to 15,000 μAU .

5. Print out the ACQUITY Console TUV drift report (See Figure 10).

Figure 10 Example of the TUV drift report:



6. 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.

Note: Ignore the noise result produced.

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

4.3 Cleaning the 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.

To clean the system:

1. Confirm that all vials are in their correct position, as described in section 2.5.4.
2. Wearing nitrile gloves, place all solvent lines in the acid 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 A, B, C and D for 5 minutes each;
 - b. In the Wash Solvent field, enter 60 seconds;
 - c. In the Purge Solvent field, enter 20 cycles.
6. Click Start, and then wait for the process to end. The process requires about 25 minutes.
7. In the MassLynx window, open the Acidic_Basic_HClass_Clean_WAAXXX.DDMMYY sample list.
8. Rename the sample list with the correct serial number and current date.
9. Open the inlet file Acquity_HClass_Acid_Base_Clean, and download the method.
10. Ensure the API gas is flowing, and then put the mass spectrometer in Operate mode.
11. 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.

12. When the acquisition ends, stop the mobile phase flow.
13. Wearing nitrile gloves, place all solvent lines in the basic wash solvent.
14. Repeat the priming procedure described in steps 5 through 8.
15. In the Inlet Method editor, download the inlet method.
16. 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.

17. When the acquisition ends, stop the UPLC flow.
18. Wearing nitrile gloves, place all the solvent lines into isopropan-2-ol.
19. Repeat the priming procedure described in steps 5 to 8.
20. In the Inlet Method editor, download the inlet method.
21. 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 acid wash solution and the basic wash solution to hold the mobile phase used in this operational and performance qualification, to reduce the chance 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 2.5.2.
- 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 “Acid wash” and “Basic wash”.
2. Using deionized water, rinse out the bottles that previously contained the acid and basic wash solvents.

Requirement: Ensure that the bottles are rinsed out at least five times each.

3. Add 1 L of deionized water to the bottle that previously contained the basic wash solvent.
4. Add 100 µL of formic acid, and then cap the bottle and shake it.
5. Add 500 µL of ammonia solution, and then cap the bottle and shake it.
6. Label the bottle “water + 0.01% formic acid + 0.05% ammonia”.
7. Submerge lines A, C, 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 acid wash solvent.
9. Add 100µL of formic acid, and then cap the bottle and shake it.

10. Label the bottle "acetonitrile +0.01% formic acid".
11. Submerge lines B, D 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 A, B, C, D and Seal wash for at least 5 minutes.
 - b. Prime the sample manager-FTN's Sample wash and Purge solvent lines as follows:
 - In the Wash Solvent field, enter 60 seconds.
 - In the Purge Solvent field, enter 20 cycles, and then click OK.
14. Attach the ACQUITY column to position 1 (if the optional SM-A is present), and then attach all fluidics tubing to the mass spectrometer and any optional UV detectors.
15. Set the Quaternary Solvent Manager to deliver 0.8 mL/min 50/50/0/0 A:B:C:D 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

Requirement: Run the tests in the order specified.

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.

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 methods using Intellistart

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

To create methods using Intellistart:

1. In the MS Console, click Xevo TQ-S > Operate, and then start the API gas.
 2. Place a 30-mL bottle containing 20 mL of “Tuning solution diluted” (5 pg/μL chloramphenicol and 10 pg/μL sulfadimethoxine) on fluidics line B.
 3. Click Interactive Fluidics > Enable Control.
 4. Purge fluidics line B twice.
 5. Click Quaternary Solvent Manager, and then select a flow rate of 0.8mL/min 50/50/0/0 A:B:C:D
 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 5, noting these requirements:
 - a. In the Compound Name fields, type “Sulfadimethoxine” in row 1 and “Chloramphenicol” in row 2.
 - b. In the Molecular Mass/Formula field, enter 310.08 in row 1 and 322.00 in row 2.
 - c. Ensure [M+H]⁺ Adduct A⁺ is selected in row 1 and [M-H]⁻ Adduct A⁻ is selected in row 2.
 - d. Click create new sample tune and then enter the name ESI_SYSTEM_DDMMYY.
 - e. Click Develop MRM method, and then enter the name ESI_SYSTEM_DDMMYY.
 - f. Click Print Report.
 - g. Save the report as ESI_SYSTEM_DDMMYY.
 - h. Ensure the setting for the Fluidics Flow Path parameter is Combined.
- All other parameters remain at their default settings.

Table 5 Intellistart method development parameter settings:

Parameter	ES +	ES-	APCI+ (optional)
Compound Name (row 1 ticked)	Sulfadimethoxine	Chloramphenicol	17- α -hydroxyprogesterone
Molecular Mass/ Formula (row 1)	310.08*	322.00*	330.22
Adduct A+(row 1)	[M+H] ⁺	Blank field	[M+H] ⁺
Adduct B+(row 1)	Blank field	Blank field	Blank field
Adduct A- (row1)	Blank field	[M-H] ⁻	Blank field
Adduct B- (row1)	Blank field	Blank field	Blank field
Multiply Charged Parents	Clear	Clear	Clear
Create New Sample Tune	Selected	Selected	Selected
Load Existing Sample Tune	Clear	Clear	Clear
Sample Tune Name	ESI_SYSTEM_DDM MMYY.ipr	ESI_SYSTEM_DDM MMYY.ipr	APCI_SYSTEM_DDMMYY.i pr
Develop SIR method	Clear	Clear	Clear
Develop SIR method	Clear	Clear	Clear
Develop MRM method	ESI_SYSTEM_DDM MMYY	ESI_SYSTEM_DDM MMYY	APCI_SYSTEM_DDMMYY
Save Report As:	ESI_SYSTEM_DDM MMYY	ESI_SYSTEM_DDM MMYY	APCI_SYSTEM_DDMMYY
Append to Existing methods	Clear	Clear	Clear
Print Report	Selected	Selected	Selected
Flow Path	Combined	Combined	Combined
Cone Voltage	Default (2 through 100)	Default (2 through 100)	Default (2 through 100)
Collision Energy	Default (2 through 80)	Default (2 through 80)	Default (2 through 80)
Reservoir	B	B	B
Number of MRM transitions per compound	1	1	1
Lowest Mass Fragment	100Da	100Da	100Da
All other fields	Leave as default	Leave as default	Leave as default

*The molecular mass value that you specify must be the neutral monoisotopic mass, not the mass of the ion.

Note: Monitor the progress of the Sample Develop method 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.

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

12. Click File > Open, and then browse to the ESI_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. Set the dwell times to 0.025 s, and the retention time to 2.5 min.

15. In the Options list, click Method events.

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

17. In the File menu, click Save.

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

19. Select the function row containing the sulfadimethoxine transition, and click Delete.

20. In the File menu, click Save as > ESI_NEG_SYSTEM_DDMMYY.

21. Click Open, and then select ESI_SYSTEM_DDMMYY.

22. Select and delete the function row containing the chloramphenicol transition, and then save the file as ESI_POS_SYSTEM_DDMMYY.

23. Double click on the Sulfadimethoxine transition, and then change the dwell time to 0.150 seconds.

24. In the File menu, click Save as > INJECTOR_LINEARITY.

25. Close the MS Method editor.

26. From the sample list page, click File > Open, and select ESI_Positive_SystemPrecision_Detector_Linearity_WAAXXX_DDMMYY.spl.

27. Ensure the sample list method contains the ESI_POS_SYSTEM_DDMMYY.exp file and the tune file ESI_SYSTEM_DDMMYY.ipr, and then click File and Save.

28. From the sample list page, click File > Open, and select ESI_NEGATIVE_WAAXXX_DDMMYY.spl.

29. Ensure the sample list method contains the ESI_NEG_SYSTEM_DDMMYY.exp file.

4.5.2 Making blank injections

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

Note: No MS test injections are run before operational and performance testing, because the detector linearity test requires very low levels of analysis. Performing test injections before this risks causing the test to fail, owing to sample carryover or contamination in the subsequent tests.

To make blank injections:

1. In the MassLynx software, open the Blank_Injections_WAAXXX_DDMMYY.spl sample list.
2. Rename the sample list to include the serial number of the instrument and the current date.
3. In the Inlet Method editor, download the LC method Gradient_HClass.
4. Select all rows, and then click Start.

Wait for the procedure to finish before proceeding with the remainder of the tests.

4.5.3 Testing detector linearity, ESI positive sensitivity and system precision

The ESI positive sensitivity test in MRM mode injects six, 10- μ L sulfadimethoxine at 100fg/ μ L each. The software calculates the signal-to-noise value of each injection and the area reproducibility of all injections.

The detector linearity test is performed in MRM mode. Multiple 10 μ L injections of sulfadimethoxine are used at the following concentrations; (on column amount) 2.5 fg, 5 fg, 10 fg, 50 fg, 100 fg, 500 fg, 1000 fg, 5000 fg and 7500 fg. The R^2 coefficient of the calibration curve is used to measure the linearity of the detector.

The system precision test examines the retention times of the six sulfadimethoxine peaks. The standard deviation of the retention time of the six peaks should be expressed by the Relative SD, which should be less than or equal to 3%.

To perform the tests:

1. In the MassLynx tune window, open the ESI_SYSTEM_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. Set the flow rate to 20 μ L/min, in combined flow, with a flow rate of 0.8 mL/min 50/50/0/0 A:B:C:D, and then start the syringe pump.
4. In the MS console, click Xevo TQ-S Detector > Manual Optimization.

5. 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_DDDMMYY.exp MS method created by IntelliStart.
6. Right-click on the signal plot, select Plot properties, and set the time window to 1 min.

Consult Table 6 for manual optimization starting parameter settings.

Table 6 Manual optimization parameter settings for sensitivity tests:

Parameter	ESI + tuning	ESI – tuning	APCI + tuning
Data Type	MRM	MRM	MRM
Ion Mode	ES+ unavailable	ES- unavailable	APCI+ unavailable
Parent (Da)	311.*	321.*	331.*
Daughter (Da)	156.*	152.*	109.*
Cone (V)	IntelliStart optimized setting (~40 V)	IntelliStart optimized setting (~30 V)	IntelliStart optimized setting (~30V)
Collision (V)	IntelliStart optimized setting (~20V)	IntelliStart optimized setting (~18 V)	IntelliStart optimized setting (~20 V)
Ion Mode	ES+	ES-	APCI+
Tune	ESI_SYSTEM_DDDMMYY.ipr	ESI_SYSTEM_DDDMMYY.ipr	APCI_SYSTEM_DDDMMYY.ipr
Capillary (kV)	Positive - tune for maximum signal	Negative - tune for maximum signal	Unavailable
Corona (kV)	Unavailable	Unavailable	Positive - tune for maximum signal
Repeller (V)	Unavailable	Unavailable	Unavailable
Desolvation temperature (°C)	600 °C	500 °C	600 °C
Desolvation gas (L/h)	1000	1000	1000
Cone gas (L/h)	150	150	150
Nebulizer pressure (bar)	7	7	Tune for maximum sensitivity
Fluidics flow µL/min	20	20	20

* IntelliStart-determined, peak-top mass position.

7. In the Capillary dialog box, specify values that optimize the capillary voltage and thus produce maximum sensitivity.

8. Optimize the probe position and capillary length for maximum intensity.
9. Minimize thermal ionization:
 - Set the capillary voltage to zero.
 - 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.

10. Ensure the capillary voltage is set to the optimal value.
11. Click the Stop icon.
12. In the Manual Optimization tune window, click Save.
13. In the Tune window, click File > Open > ESI_SYSTEM_DDMMYY.ipr.
14. Click Ion Mode > Electrospray + and then MSMS.
15. Monitor the collision cell's Pirani gauge, and adjust the collision cell gas flow until the pressure reaches 3.5e-3 mbar.
16. From the sample list page, click File > Open, and then click ESI_Positive_SystemPrecision_Detector_Linearity_WAAXXX_DDMMYY.spl.
17. Amend the sample list page to contain the Intellistart-generated MS experiment and MS tune files.
18. In the Inlet Method editor, download the HClass_Gradient method.

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

19. Wait 5 minutes for the flow to stabilize.
20. Save the sample list.
21. Select all rows in the sample list, and then click Play.
22. From the sample list, select row 16, click Chromatogram.
23. Select the Display tab in the chromatogram window.
24. Select the sulfadimethoxine transition so that Ch1 appears in the description box.
25. Select Replace trace, and then click OK.
26. Click to zoom on the sulfadimethoxine peak, allowing a range of approximately 0.2 minutes on either side of it.
27. From the sample list page, click TargetLynx > Edit Method.

28. From the File menu, click Open, and then select ESI_POSITIVE.mdb.
29. In the TargetLynx Method Editor, click User Defined Properties, and then select the Predicted Retention Time value.
30. In the chromatogram, right-click and drag across a range that extends approximately 10 seconds on either side of the sulfadimethoxine peak.
31. In the TargetLynx method, ensure the predicted retention time is updated, and then click Save.
32. In the sample flagging properties tab, define the noise range that TargetLynx uses for the signal-to-noise calculations.
33. 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 min.

34. 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.
35. Save the TargetLynx method.
36. From the sample list, select rows 16 through 21.
37. From the TargetLynx shortcut bar, click Process Samples.
- Result:** The Create TargetLynx Dataset dialog box appears.
38. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
39. Select ESI_POSITIVE.mdb as the method, and then click OK.
40. In the TargetLynx window, click File > Apply Layout > ESI_SENSITIVITY.qlt.
41. Click File > Save As > ESI_POSITIVE_WAAXXX_DDMMYY.
42. Click File > Report Format.

43. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the ESI_SENSITIVITY.qlt layout.
44. In the TargetLynx window, click File > Print > OK.
45. In the printout, note the average signal-to-noise ratio.

Requirement: The average signal-to-noise ratio must be greater than or equal to 3000:1.

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

48. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
49. From the sample list page, click TargetLynx > Edit Method.
50. From the File menu, click Open, and then select ESI_POSITIVE_AREA.mdb.
51. In the TargetLynx Method editor, select the Value field for Predicted Retention Time.
52. In the chromatogram, right-click and drag across a range that extends approximately 10 seconds on either side of the Sulfadimethoxine peak.
53. Ensure the predicted retention time is updated in the TargetLynx method, and click Save.
54. In the sample list, select rows 16 through 21.
55. From the TargetLynx shortcut bar, click Process Samples.
56. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
57. Ensure ESI_POSITIVE_AREA is selected as the method, and then click OK.
58. In the TargetLynx window, click File > Apply Layout > ESI_REPRODUCIBILITY.qlt.
59. Click File > Save As > ESI_POSITIVE_AREA_DDMMYY.
60. Click File > Report Format.
61. In Report Format Options, click the Compound Summary Report tab and then select the column format ESI_REPRODUCIBILITY.qlt layout.
62. Click File > Print > OK.
63. Use the area results of each run to calculate the Relative SD (see section 5).

Requirements:

- The Relative SD of the peak area must be less than or equal to 3%.
 - The average peak area of the sulfadimethoxine peaks must be greater than or equal to 6e+4.
 - The difference between the largest and smallest retention time values must be less than or equal to 0.047 min.
64. Annotate the report observing the conventions described in section 2.8.3.
 65. Append the report to the operational and performance qualification results document associated with this workbook.

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

Note: Before processing the detector linearity data, you must first alter the sample list to omit some of the injections made previously.

67. Select rows 18 through 21 in the sample list and delete them.

Note: This will not delete the data, it is for sample processing purposes only.

68. Save the new sample list as MS_Detector_Linearity_WAAXXX_DDMMYY.

69. From the sample list page, click TargetLynx > Edit Method.

70. From the File menu, click Open, and then select ESI_POSITIVE_Detector_Linearity.mdb.

71. In the TargetLynx Method editor, select the Value field for Predicted Retention Time.

72. In the chromatogram, right-click and drag across a range that extends approximately 10 seconds on either side of the sulfadimethoxine peak.

73. Ensure the predicted retention time is updated in the TargetLynx method, and click Save.

74. In the sample list, select rows 4 through 21.

75. From the TargetLynx shortcut bar, click Process Samples.

76. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.

77. Ensure ESI_POSITIVE_Detector_Linearity.mdb is selected as the method, and then click OK.

78. In the TargetLynx window, click File > Apply Layout > Detector_Linearity_Area.qlt.

79. Click File > Save As > ESI_POSITIVE_Detector_Linearity_DDMMYY.

80. Click File > Report Format.

81. In Report Format Options, click the Compound Summary Report tab, and then select the same column format as the Detector_Linearity_Area.qlt layout.

82. Click File > Print > OK.

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

83. Annotate the report observing the conventions described in section 2.8.3.

84. Append the report to the operational and performance qualification results document associated with this workbook.

85. 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 negative mode sensitivity

Perform the ESI negative mode sensitivity test in MRM mode, by making six, 10- μ L, chloramphenicol injections of 0.05 pg/ μ L (0.5pg on column) each. The software determines the signal-to-noise value and area reproducibility of each injection.

To test ESI negative mode sensitivity:

1. In the Tune window, open the ESI_SYSTEM_DDMMYY.ipr tune file.
 2. From the Ion Mode menu, select ESI negative.
 3. Set the desolvation gas flow to 1000 L/h, and the desolvation temperature to 500 °C.
 4. Set the Infusion rate to 20 μ L/min in combined flow, with a flow rate of 0.8 mL/min 50/50/0/0 A:B:C:D, and then start the syringe pump.
 5. In the MS console, click Xevo TQ-S Detector > Manual Optimization.
 6. Monitor the m/z 321** $\rightarrow$ m/z 152.** MRM transition, and ensure the cone voltage and collision energy values match those in the ESI_NEG_SYSTEM_DDMMYY MS method created by IntelliStart.
 7. Right-click on the signal plot, select Plot properties, and set the time window to 1 min.
 8. Consult Table 6 for manual optimization starting parameter settings.
 9. In the Capillary box, specify values that optimize the capillary voltage and thus produce maximum sensitivity.
 10. Optimize the probe position and capillary length for maximum intensity.
 11. Minimize thermal ionization:
 - Set the capillary voltage to zero.
 - If thermal ionization exceeds 10%, 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.
12. Ensure the capillary voltage is switched on and set to the optimal value.
 13. Click the Stop icon.
 14. Click Save in the Manual Optimization tune window.

15. In the Tune window, click > File menu > Open > ESI_SYSTEM_DDMMYY.ipr.
16. Click > Ion Mode > Electrospray -ve.
17. Click the MSMS icon.
18. Monitor the collision cell's Pirani gauge, and adjust the collision cell gas flow until the pressure reaches 3.5×10^{-3} mbar.
19. Click File > Save.
20. From the sample list page, click File > Open, and then click ESI_NEGATIVE_WAAXX_DDMMYY.spl.
21. From the sample list, select the MS experiment file and tune file that you created.
22. In the Inlet Method editor, download the Gradient_HClass method.
Rationale: Doing so prepares the pump for initial gradient conditions.
23. Wait 5 minutes, for the flow to stabilize.
24. Save the sample list.
25. Select all rows of the sample list, and click Play.
26. When the ESI_NEGATIVE_WAAXX_DDMMYY.spl acquisition ends, use the following TargetLynx methods to process the results:
 - The first method yields unsmoothed results for the signal-to-noise calculation.
 - The second method yields smoothed data used for the area reproducibility calculation.
27. From the sample list, select row 3, and then click Chromatogram.
28. Select the Display tab in the chromatogram window.
29. Select the chloramphenicol transition, so that Ch1 appears in the description box.
30. Select Replace trace, and then click OK.
31. Click to zoom on the chloramphenicol peak, allowing a range of approximately 0.2 minutes on either side of it.
32. From the sample list page, click TargetLynx > Edit Method.
33. From the File menu, click Open, and then select ESI_NEGATIVE.mdb.
34. In the TargetLynx Method Editor > User Defined Properties, select the Predicted Retention Time value.

35. In the chromatogram window, right-click and drag across a range that extends approximately 10 seconds on either side of the chloramphenicol peak.
36. In the TargetLynx Method Editor > User Defined Properties, ensure the Predicted Retention Time is updated.
37. 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.

38. In the TargetLynx Method Editor, click Sample Flagging Properties.
39. In the Signal to Noise Parameters window, enter the values for noise regions start and end, and then click Save.
40. From the sample list, select all rows.
41. From the TargetLynx shortcut bar, click Process Samples.
42. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
43. Select ESI_NEGATIVE as the method, and then click OK.
44. In the TargetLynx browser, click File > Apply Layout > ESI_SENSITIVITY.qlt.
45. Click File > Save As > ESI_NEGATIVE_WAAXXX_DDMMYY.
46. Click File > Report Format.
47. 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.
48. Click File > Print and then click OK.
49. In the printout, note the average signal-to-noise ratio.

Requirement: The average signal-to-noise ratio must be greater than or equal to 400:1 for the chloramphenicol transition m/z 321.** -> m/z 152.** of a 0.5pg on column injection.

50. Annotate the report observing the conventions described in section 2.8.3.
51. Append the report to the operational and performance qualification results document associated with this workbook.
52. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
53. From the sample list page, click TargetLynx, and then click Edit Method.
54. From the File menu, click Open, and then select ESI_NEGATIVE_AREA.mdb.

55. In the TargetLynx Method Editor > User Defined Properties, select the Value field for Predicted Retention Time.
56. Click to zoom on the chloramphenicol peak, allowing a range of approximately 10 seconds on either side of it.
57. In the TargetLynx method, ensure the Predicted Retention Time is updated, and then click File > Save.
58. In the sample list, select all rows.
59. From the TargetLynx shortcut bar, click Process Samples.
60. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
61. In the TargetLynx window, click File > Apply Layout > ESI_REPRODUCIBILITY.qlt.
62. Click File > Save As > ESI_NEGATIVE_AREA_DDM.MMS.Y.
63. Click File > Report Format >
64. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the ESI_REPRODUCIBILITY.qlt layout.
65. In the TargetLynx window, click File > Print > OK.
66. Use the area results of each run to calculate the Relative SD.

Requirements:

- The Relative SD of the peak area must be less than or equal to 3%.
 - The average area of the chloramphenicol peaks must be greater than or equal to $1e+3$.
67. Annotate the report observing the conventions described in section 2.8.3.
 68. Append the report to the operational and performance qualification results document associated with this workbook.
 69. 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 injector linearity and accuracy

The injector linearity test verifies whether a delivered injection volume range is linear within specified criteria. The test involves injecting four different injection volumes of one concentration standard from the same vial.

To test injector linearity:

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

20. Click the Toggle Calibration Window icon, to display the calibration curve.
21. Click File > Save As > INJECTOR_LINEARITY_AREA_DDMMYY.
22. Click > File > Report Format.
23. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the INJECTOR_LINEARITY_AREA.qlt layout.
24. Click File > Print > OK.
25. Examine the summary report and look at the values for A and B from the equation Calibration Curve: (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

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

26. Using the A and B values and the formula given below, calculate the absolute value of the x-intercept.

Calibration curve: (A * X + B)

$$X = I \text{ (Intercept)}$$

$$I = (B/A)$$

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 $\pm 0.2 \mu\text{L}$.

27. 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 26.
28. Annotate the report observing the conventions described in section 2.8.3.
29. Append the report to the operational and performance qualification results document associated with this workbook.

4.5.6 Testing flow rate linearity

The flow-rate-linearity test determines whether the QSM's flow rate is linear. The test involves repeatedly injecting a constant volume of sample at varying flow rates.

To test the flow rate linearity:

1. In the MassLynx Tune window, ensure the ESI_SYSTEM_DDMMYY.ipr is loaded.
2. Click > Ion Mode > Electrospray +.
3. Click the MSMS icon.
4. Ensure the collision gas is flowing.
5. From the sample list page, click File > Open, and then select FLOW_LINEARITY_WAAXX_DDMMYY.spl.
6. Select all rows, click Run > Acquire Sample Data, and then click OK.
7. When the injections end, select rows 2 through 8 on the sample list.
8. Click Chromatogram.
9. Delete the Flow_Rate_Linearity_Blank (1-1) injections chromatograms.
10. Print out the remaining chromatograms that show the sulfadimethoxine injection.
11. Record the RT values for each acquisition in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
12. For each acquisition, multiply the RT by the values shown in Table 7.

Table 7 Flow rate linearity acquisitions and multiplier values:

Retention time	Multiplier value
Flow rate linearity 1	200
Flow rate linearity 2	400
Flow rate linearity 3	600
Flow rate linearity 4	800

13. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the calculated values.
14. Calculate the standard deviation of the four product values.

15. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the standard deviation.

Requirement: The standard deviation must be less than or equal to 60 μ L.

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

4.6 Testing injector carryover

The injector carryover test determines the amount of sample carryover after an injection of a concentrated stock sample (challenge injection). First, 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 carried out to determine the amount of carryover.

To test the injector carryover:

1. In the MassLynx Tune window, load SYSTEM.inp.
2. Click Ion Mode > Electrospray +.
3. Click MSMS.
4. In the MS experiment file, enable method events for the Injector_linearity.exp file, and the Divert_Waste.exp file.
5. From the sample list page, click File > Open, and then select INJECTOR_CARRYOVER_WAAXXX_DDMMYY.spl.
6. Rename the sample list with the correct instrument serial number and current date.
7. Click Start Run, then select Acquire Sample Data, and then click Start.
8. Click TargetLynx > Edit Method.
9. From the File menu, click Open, and then select INJECTOR_LINEARITY_AREA.mdb.
10. Note the Predicted Retention Time value, and the value in the Retention Time window.
11. From the sample list page, click TargetLynx > Edit Method.
12. From the File menu, click Open, and then select CARRYOVER.mdb.
13. In the Compound List, select 1: sulfadimethoxine.
14. In the User Defined Properties, enter the Predicted Retention Time and Retention Time window values used for the INJECTOR_LINEARITY_AREA.mdb method.
15. Save the TargetLynx method.

16. In the sample list, select rows 4 through 8.
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 CARRYOVER as the method, and then click OK.
20. In the TargetLynx window, click File > Apply Layout > CARRYOVER.qlt.
21. Click File > Save As > INJECTOR_CARRYOVER_DDMMYY.
22. Click File > Report Format.
23. In Report Format Options, click the Compound Summary Report tab, and then select the column format to match the, CARRYOVER.qlt layout.
24. In the TargetLynx window, click > File > Print > OK.
25. Examine the compound report, noting the area value for the acquisition "Standard" and the area values of the three blanks 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).
26. In Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook, record the area values for the acquisition "Standard" and the area values of the three blanks injected after it.
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.

4.7 Testing gradient performance

The gradient performance test determines whether a solvent management system can deliver a solvent mixture within acceptable criteria. A four-component mixture (acetaminophen, caffeine, sulfadimethoxine, and verapamil) is injected three times. The difference in the retention of each of the four compounds over the multiple injections is examined. The solvent management system is tested for solvent lines A and B, then C and D.

To test the gradient performance:

1. In the MassLynx Tune window, click File menu > Open > ESI_SYSTEM_DDMMYY.ipr.
2. Adopt the Tune window settings shown in Table 8.

Table 8 Tune window settings:

Parameter	Setting
IE1 and IE2	Optimum
Desolvation gas	1000 L/h
Desolvation temperature	600 °C

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

23. In the TargetLynx window, click File > Apply Layout > GRADIENT.qlt.
 24. Click File > Save As > GRADIENT_RT_AB_DDMMYY.
 25. Repeat the processing steps as above for solvent lines CD.
 26. Save the solvent lines CD injections as GRADIENT_RT_CD_DDMMYY.
 27. Click File > Report Format.
 28. In the Report Format options window, click the Compound Summary Report tab and then select the column format to match the GRADIENT.qlt layout.
 29. In the TargetLynx window, click File > Print, and then click OK.
 30. 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.
 31. 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.
32. Annotate the report observing the conventions described in section 2.8.3.
 33. Append the report to the operational and performance qualification results document associated with this workbook.

4.8 Testing the APCI positive sensitivity (optional)

To perform the APCI positive sensitivity test in MRM mode, make six injections of 17- α -hydroxyprogesterone, 50 pg on column, and then calculate the signal-to-noise values of each injection.

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

To test the APCI positive sensitivity:

1. Place a 30-mL bottle containing 20 mL of "tuning solution diluted" on fluidics line B.
2. In the MS Console, click Interactive Fluidics, and then click Enable Control and purge fluidics line B twice.
3. In the MS Console, select Binary Solvent Manager, and then select a flow of 0.8 mL/min 50/50/0/0 A:B:C:D.
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 6, heeding these requirements:
 - a. In the Compound Name fields, enter the name "17- α -hydroxyprogesterone", and select row 1 only.
 - b. In the Molecular Mass/ Formula field, enter 330.22.
 - c. Ensure [M+H]⁺ Adduct A⁺ is selected.
10. Create a new Sample Tune method named APci_SYSTEM_DDMMYY.ipr.
11. Select Develop MRM method, and enter the name APCI_SYSTEM_DDMMYY.exp.
12. Select Print Report.
13. Set the lowest mass fragment to 100 Da.
14. Save the report as APci_SYSTEM_DDMMYY.
15. Ensure the Fluidics Flow Path parameter is set to Combined.
16. 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.
17. From the sample list page, open the MS method editor and browse to the APci_SYSTEM_DDMMYY.
18. Ensure the method contains the channel: 17- α -hydroxyprogesterone m/z 331.** - > m/z 109.** (nominal masses).
19. Set the dwell time to 0.025 s and the retention end time to 2.5 minutes.
20. In the Options menu, click Method events, then clear the Enable box, and then click OK.
21. Save the method.
22. Open the APCI_SYSTEM_DDMMYY.ipr tune file.

23. Set the desolvation gas flow to 1000 L/h, and the desolvation temperature to 600 °C.
24. Set the infusion rate to 20 µL/min, in combined flow, with a flow of 0.8 mL/min 50/50/0/0 A:B:C:D, and then start the syringe pump.
25. In the MS console, click Xevo TQ-S Detector > Manual Optimization.

Note: See Table 6 for parameter settings.

26. 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.
27. Optimize the corona voltage for maximum sensitivity by entering values in the Corona box.
28. Optimize the probe position and capillary length, for maximum intensity.
29. Click the Stop icon, to stop the infusion.
30. Click Save in the Manual Optimization window.
31. In the Tune window, ensure the file APcI_SYSTEM_DDMMYY.ipr is loaded.
32. Verify that the optimized values are displayed.
33. In the Tune window, click Ion Mode > APcI +.
34. Select the MSMS icon.
35. Tune the collision cell gas flow until the pressure is approximately 3.50e-3.
36. Infuse the tuning solution and monitor the m/z 331.** -> m/z 109.** MRM transition, and tune the nebulizer gas flow for maximum sensitivity.
37. 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.

38. In the sample list, click File > Open, and select APcI_POSITIVE_WAAXXX_DDMMYY.spl.
39. In the sample list, open the MS experiment file and tune file that you created previously.
40. Select the column MS File, right-click, and select Fill Down.
41. Save the sample list.
42. Select all rows, and then click Start.
43. When the APcI_POSITIVE.spl acquisition is complete, use the TargetLynx software to process the results.

44. From the sample list page, select row 3, select Chromatogram, click to zoom on the 17- α -hydroxyprogesterone peak, allowing a range of approximately 0.2 minutes on either side of it.
45. From the sample list page, click TargetLynx > Edit Method.
46. From the File menu, click Open, and then select APCI_POSITIVE.mdb.
47. In the TargetLynx Method Editor > User Defined Properties, select the Predicted Retention Time value.
48. In the Chromatogram, right-click and drag across a range that extends approximately 10-seconds on either side of the 17- α -hydroxyprogesterone peak.
49. In the TargetLynx method, ensure the Predicted Retention Time is updated.
50. 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.
51. In the TargetLynx Method Editor, click Sample Flagging Properties.
52. In the Signal to Noise Parameters window, enter the values for noise regions start and end, and then click Save.
53. In the sample list, select rows 5 through 6.
54. From the TargetLynx shortcut bar, click Process Samples.
55. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
56. Select APCI_POSITIVE as the method, and then click OK.
57. In the TargetLynx window, click File > Apply Layout > ESI_SENSITIVITY.qlt.
58. Click File > Save As > APCI_POSITIVE_DDMMYY.
59. Click File, select Report Format.
60. In Report Format Options, click the Compound Summary Report tab and then select the column format to match the APCI_SENSITIVITY.qlt layout.
61. In the TargetLynx window, click File > Print > OK.
62. In the printout, note the results for area and signal-to-noise.

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 pg on column injection.

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

65. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.
66. From the sample list page, click TargetLynx, and then click Edit Method.
67. From the File menu, click Open, and then select APCI_POSITIVE_Area.mdb.
68. In the TargetLynx Method Editor > User Defined Properties, select the Value field for Predicted Retention Time.
69. Click to zoom on the 17- α -hydroxyprogesterone peak, allowing a range of approximately 10 seconds on either side of it.
70. In the TargetLynx method, ensure the Predicted Retention Time is updated, and then click File > Save.
71. In the sample list, select all rows.
72. From the TargetLynx shortcut bar, click Process Samples.
73. In the Create TargetLynx Dataset dialog box, select Integrate Samples, Calibrate Standards and Quantify Samples.
74. In the TargetLynx window, click File > Apply Layout > ESI_REPRODUCIBILITY.qlt.
75. Click File > Save As > APCI_SENSITIVITY_AREA_DMMMMYY.
76. Click File > Report Format.
77. In Report Format Options, click the Compound Summary Report tab, and then select the column format to match the ESI_REPRODUCIBILITY.qlt layout.
78. In the TargetLynx window, click File > Print > OK.
79. Use the area results of each run to calculate Relative SD.

Requirements:

- The Relative SD of the area must be less than or equal to 3%.
 - The average area of the 17- α -hydroxyprogesterone peaks must be greater than or equal to $1e+4$.
80. Annotate the report observing the conventions described in section 2.8.3.
 81. Append the report to the operational and performance qualification results document associated with this workbook.
 82. Record the results in Form 6, "Operational and performance qualification results", of the operational and performance qualification results document associated with this workbook.

4.9 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.

To perform preliminary UV test injections:

1. Click the Open Sample List icon, and select PDA_Test_Injections DDMMYY.spl for a PDA/PDAe λ or TUV_Test_Injections DDMMYY.spl for a TUV.
2. Update the sample list name with the current date.
3. Select all rows, and then click the Run icon.
4. In the Start Sample List Run window, 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 the HClass_UV_RSD_Area.mdb.
9. Select Integrate Samples, Calibrate Standards, and Quantify Samples.
10. Click File > Apply Layout > HClass__UV_RSD.qlt.

Note: This file is in the currently selected project folder.

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 HClass_UV_RSD_Height.mdb method.
19. From the calibration window in the TargetLynx report, note the Relative SD for height, and the peak height.

Requirements:

- The peak height's Relative SD must be less than or equal to 2.4%.
 - The peak height must be greater than or equal to 1.8 AU (180,000 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).

4.10 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 AccuracyDDMMYY.spl, and enter the current date, then select all samples and click the Run icon.

3. In the Start Sample List Run window, select Acquire Sample Data, and then click OK.
4. When the injections end, select the sample list injections in the wavelength accuracy sample list.
5. Process the samples of the wavelength accuracy sample list using TargetLynx.
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 11 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	0.723	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	0.728	114034	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	0.726	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).

4.11 Testing the ACQUITY PDA wavelength accuracy (optional)

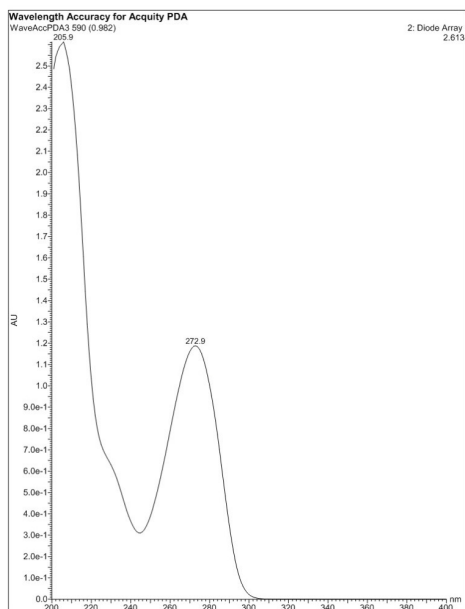
Wavelength accuracy with an ACQUITY PDA or PDA e λ detector is determined by measuring the spectrum of a caffeine peak. The absorbance bands within caffeine (λ max of 205 nm and 273 nm) are well defined.

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 HClassPDA Wavelength AccuracyDDMMYY.spl, and enter the current date, then click the Run icon.
3. In the Start Sample List Run window, select Acquire Sample Data, and then click OK.
4. When the injection ends, click the Chromatogram tab and then click Display > TIC > Diode Array.
5. Extract the wavelength 272 chromatogram by clicking Display > Wavelength.
6. In the DAD Chromatogram window, enter the value 272 in the Description field, and ensure the Function field is set to "2: Diode Array."
7. Select Replace Trace, and then click OK.
8. Right-click over the apex of the caffeine peak in the extracted 272 chromatogram to open a spectrum window for the peak.
9. 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.
10. 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 12).

Requirement: The wavelength accuracy must be within $\pm$ 2 nm.

Figure 12 Typical ACQUITY PDA wavelength accuracy spectrum window:

11. Print the spectrum.
12. Record the two annotated peaks in Form 6, "Operational qualification results", of the operational and qualification results document associated with this workbook.
13. Annotate the spectrum 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.

4.12 Testing UV detector linearity and sensitivity (optional)

The UV-detector linearity test with an ACQUITY PDA, PDA eλ, 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.

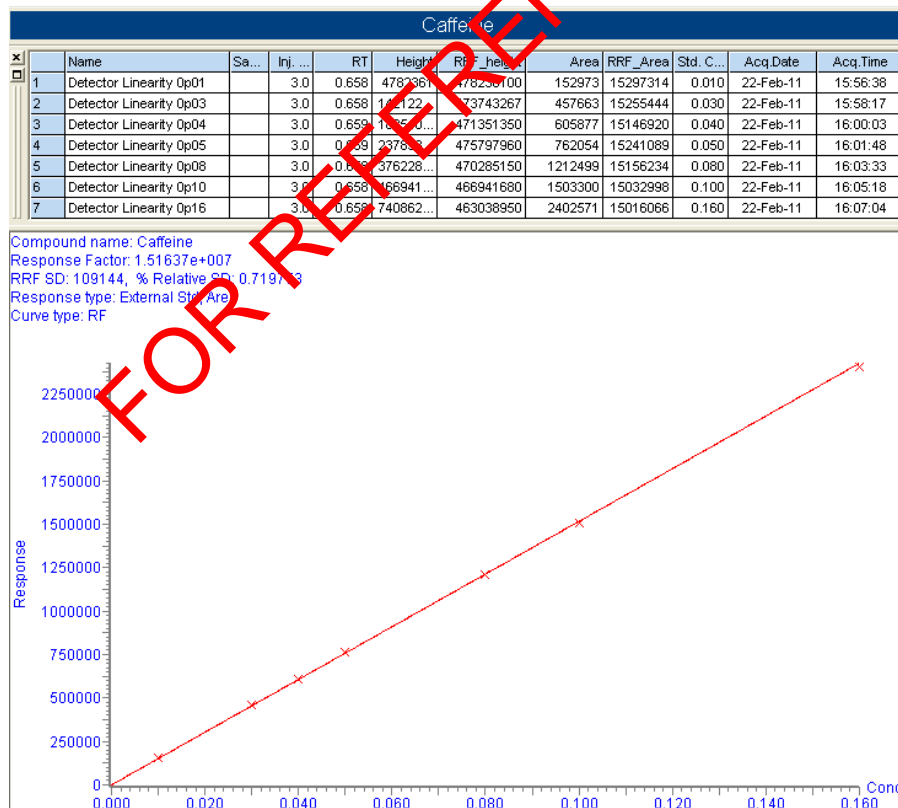
Note: Perform this test only if the system includes an ACQUITY PDA, PDA eλ, or TUV detector.

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/PDAeλ 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. Click Run.
7. In the Start Sample List Run window, 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.rpt.
12. Print the TargetLynx summary report. (See Figure 13.)

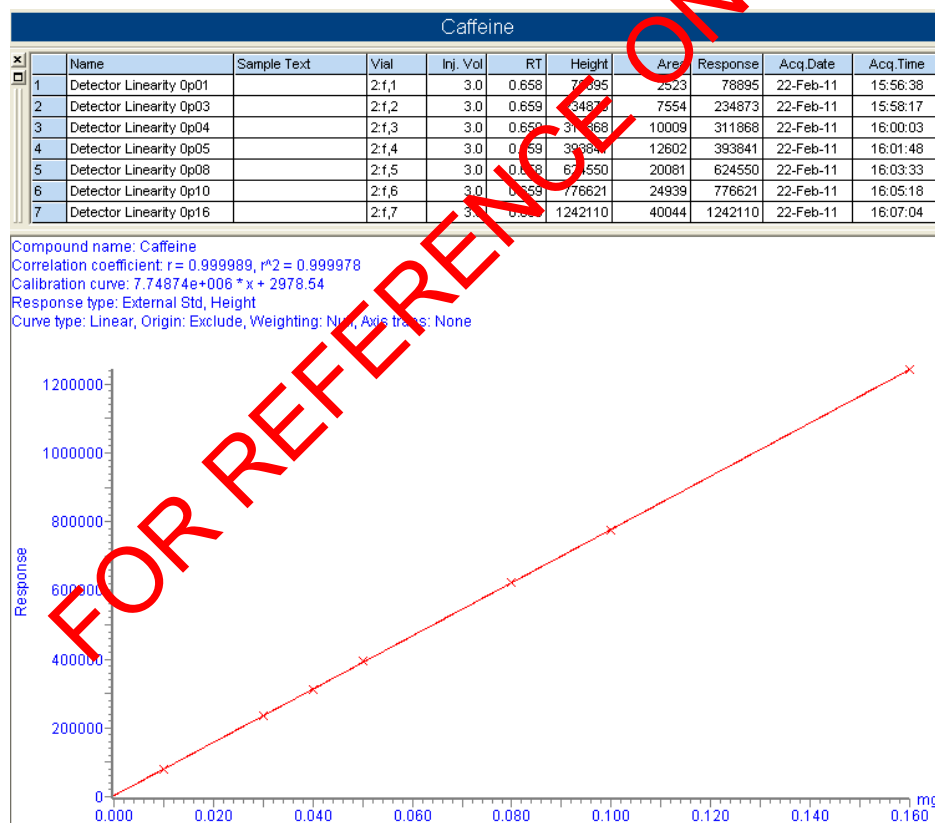
Figure 13 Typical detector linearity TargetLynx report for area:



Requirement: The R^2 value for area must be less than or equal to 0.9990.

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 14.)

Figure 14 Typical detector linearity TargetLynx report for height:



Requirement: The R^2 value for height must be less 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 seven injections 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 15 Typical report for detector sensitivity test:

Quantify Compound Summary Report MassLynx 4.1 SCN810 Page 1 of 1
 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.PROMethDB\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 Linearit...	2f,1	3.000	0.658	78895	7809500	2523	252261	
2	Detector Linearit...	2f,2	3.000	0.659	23473	7829100	7554	251798	
3	Detector Linearit...	2f,3	3.000	0.659	1186	7796700	10009	250225	
4	Detector Linearit...	2f,4	3.000	0.659	10441	7876820	12802	252038	
5	Detector Linearit...	2f,5	3.000	0.658	62470	7806875	20081	251007	
6	Detector Linearit...	2f,6	3.000	0.659	755621	7766210	24939	249387	
7	Detector Linearit...	2f,7	3.000	0.658	1242110	7763188	40044	250276	

	Acq.Date	Acq.Time
1	22-Feb-11	15:56:38
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: See section 5, "Using the Windows Calculator to Calculate Standard Deviation".

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).

Note: 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.

Note: 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