

Waters Qualification Workbook

Waters Xevo TQ-S Mass Spectrometer (MassLynx 4.1) Operational Qualification Procedures: OQ

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FOR REFERENCE ONLY

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1. Introduction

This workbook presents the operational qualification procedures for the Waters® Xevo® TQ-S mass spectrometer operated by MassLynx® software, version 4.1 (minimum SCN 950). Consult it when qualifying the operation of a Waters Xevo TQ-S mass spectrometer in an ISO 9000-series registered facility or a GxP-regulated environment.

1.1 System 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 instrument and any optional devices (if present).
- Before performing the procedures in this workbook, the performer and reviewer must be familiar with the operation of the instrument. When necessary, review the appropriate documentation referenced in this workbook and, if applicable, the MassLynx Online Help system.

1.2 Recommended documents

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

- *MassLynx 4.1 Getting Started Guide*, part number 71500113203
- Waters Xevo TQ-S Documentation CD, part number 715002206
- *Waters Xevo TQ-S Quick Reference Card*, part number 715002205
- *Waters Xevo TQ-S Preparing Test Samples*, part number 715002796
- *Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems*, part number 715001307
- *Waters Ethernet Instrument Getting Started Guide*, part number 71500074403
- *Waters Xevo TQ-S Site Preparation Guide*, part number 715002440

2. Preliminary Procedures

2.1 Identifying performer and reviewer

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

2.2 Identifying the product

Complete Form 2, "Identification information" and Form 3, "Hardware", of the operational 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 instrument owner has assigned to the instrument undergoing qualification, to differentiate it from all others.

2.3 Recommended materials

You must gather the following items:

Qualification project

Waters Xevo TQ-S OQ project (MassLynx v4.1) available to download from the Waters SAP portal.

Chemicals

- Waters Xevo TQ-S Sample Kit, part number 700005472

Solvents

- MS-grade water, 1 L minimum
- MS-grade methanol, 1 L minimum
- MS-grade Propan-2-ol, 1 L minimum
- MS-grade acetonitrile, 1 L minimum

Lab ware

- See *Waters Xevo TQ-S Preparing Test Samples*, part number 715002796, for details of the required lab ware
- IntelliStart™ Fluidics reservoir bottles (x2), 30-mL, part number 700004256 (contains six bottles)
- PEEK tubing cutter
- Ultrasonic bath

- Funnel
- Qualified LC Pump capable of delivering flow of 0.2 mL through to 1 mL/min (If installing without ACQUITY UPLC system)

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 qualification results document associated with this workbook.

2.5 Preparing test samples and mobile phases

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

Follow the preparation procedures in this section using *Waters Xevo TQ-S Sample Kit*, part number 700005472.

Table 1 contains descriptions of the test samples, diluents and calibration solutions required for the qualification, which need to be prepared from the *Waters Xevo TQ-S Sample Kit*, part number 700005472.

Table 1 Test sample and mobile phase concentrations:

Solution	Target concentration
Xevo TQ-S Set-up Solution	Provided at the correct concentration in the Xevo TQ-S setup solution sample kit
IntelliStart Resolution Solution x 20	100 ng/μL NaI 2.5 ng/μL CsI 1 ng/μL methyl sulfate sodium salt 1 pg/μL triethylamine in 50:50 isopropanol:water
Xevo TQ-S Calibration Solution	0.1% phosphoric acid solution
Reserpine (tuning solution)	5 pg/μL reserpine solution in 70:30 acetonitrile:water
Reserpine (for acquisition)	1 pg/μL, in 70:30 acetonitrile:water
Chloramphenicol (tuning solution)	25 pg/μL chloramphenicol solution in 70:30 acetonitrile:water
Chloramphenicol (for acquisition)	0.5 pg/μL chloramphenicol solution in 70:30 acetonitrile:water
17-α-hydroxyprogesterone (tuning solution) - optional	500 pg/μL 17-α-hydroxyprogesterone solution in 70:30 acetonitrile:water
17-α-hydroxyprogesterone (for acquisition) - optional	10 pg/μL 17-α-hydroxyprogesterone solution in 70:30 acetonitrile:water
[Glu ¹]-fibrinopeptide B (positive mode) - optional	100 fmol/μL solution [Glu ¹]-fibrinopeptide B in 50:50 acetonitrile:water + 0.1% formic acid

Table 1 Test sample and mobile phase concentrations:

Solution	Target concentration
[Glu ¹]-fibrinopeptide B (negative mode) - optional	300 fmol/μL solution [Glu ¹]-fibrinopeptide B in 50:50 acetonitrile:water + 0.1% formic acid

Preparing the mobile phase diluents and MS wash

1. Use a 1000-mL graduated cylinder to measure 700 mL of filtered LCMS-grade acetonitrile, and then transfer the acetonitrile to a 1000-mL reservoir bottle.
2. Use a 500-mL graduated cylinder to measure 300 mL of filtered LCMS-grade water, and transfer the water to the same reservoir bottle.
3. Cap the reservoir bottle, and then shake to mix.
4. Label the reservoir bottle: "acetonitrile:water, 70:30".
5. Repeat steps 1 to 4 and label the reservoir bottle: "MS Wash"

Preparing the aqueous diluent

1. Use a 500-mL graduated cylinder to measure 500 mL of filtered LCMS-grade water, and then transfer the water to a 500-mL reservoir bottle.
2. Use a pipette to add 50 μL of formic acid to the reservoir bottle.
3. Use a pipette to add 250 μL of ammonia solution to the reservoir bottle.
4. Cap the reservoir bottle, and then shake to mix.
5. Label the reservoir bottle: "water (0.01% formic acid, 0.05 % ammonia)".

Preparing the acetonitrile/water diluent

1. Use a 100-mL graduated cylinder to measure 50 mL of filtered LCMS-grade acetonitrile, and transfer the acetonitrile to a 100-mL reservoir bottle.
2. Measure 50 mL of water and transfer the water to the same 100-mL reservoir bottle.
3. Cap the reservoir bottle, and then shake to mix.
4. Label the reservoir bottle: "50:50 acetonitrile:water".

Preparing 100% acetonitrile

1. Use a 100-mL graduated cylinder to measure 100 mL of filtered LCMS-grade acetonitrile to use as a diluent.

Set-up solution

1. Use the set-up solution Xevo TQ-S straight from the bottle. It is provided at the correct concentration.

IntelliStart resolution solution X 20

1. Pipette 1 mL of IntelliStart resolution solution X 20 into a 20-mL volumetric flask and dilute to 20 mL with 50:50 isopropanol:water.
2. Stopper and invert the flask several times to fully mix.
3. Label the flask as "IntelliStart resolution solution diluted".

Calibration solution

1. Use the calibration solution Xevo TQ-S straight from the bottle. It is provided at the correct concentration.

Reserpine solutions for ESI positive sensitivity tests

The following dilutions must be prepared for the sensitivity tests from the vial containing 5 mg reserpine.

50 ng/ μ L Reserpine stock

1. Add 4 mL methanol to the vial, recap, and vortex the vial.
2. Transfer the contents to a 100-mL volumetric flask using a funnel.
3. Rinse the vial out with methanol at least three times to ensure all the reserpine has been transferred into the volumetric flask.
4. Make up to 100 mL using methanol and sonicate until all the reserpine has fully dissolved.
5. Label the flask "50 ng/ μ L reserpine stock [R1]".

50 pg/ μ L Reserpine

1. Pipette 100 μ L of [R1] into 100-mL volumetric flask, make up to 100 mL using 70:30 acetonitrile:water.
2. Label the flask "50 pg/ μ L reserpine [R2]".

5 pg/ μ L Reserpine for tuning

1. Pipette 1 mL of [R2] into 10-mL volumetric flask, make up to 10 mL using 70:30 acetonitrile:water.
2. Label the flask "5 pg/ μ L reserpine tuning solution".

1 pg/ μ L Reserpine for acquisition

1. Pipette 1 mL of [R2] into 50-mL volumetric flask, make up to 50 mL using 70:30 acetonitrile:water.
2. Label the flask "1 pg/ μ L reserpine specification solution".

Chloramphenicol solutions for ESI negative sensitivity tests

The following dilutions must be prepared for the sensitivity tests from the vial containing 50 mg chloramphenicol.

500 ng/ μ L Chloramphenicol stock

1. Add 4 mL acetonitrile to the vial, recap, and vortex the vial.
2. Transfer the contents to a 100-mL volumetric flask using a funnel.
3. Rinse the vial out with acetonitrile at least three times to ensure all the chloramphenicol has been transferred into the volumetric flask.
4. Make up to 100 mL using acetonitrile and sonicate until all the chloramphenicol has fully dissolved.
5. Label the flask "500 ng/ μ L chloramphenicol stock [C1]".

5 ng/ μ L Chloramphenicol

1. Pipette 1 mL of [C1] into 100-mL volumetric flask, make up to 100 mL using 70:30 acetonitrile:water.
2. Label the flask "5 ng/ μ L chloramphenicol stock [C2]".

50 pg/ μ L Chloramphenicol

1. Pipette 1 mL of [C2] into 100-mL volumetric flask, make up to 100 mL using 70:30 acetonitrile:water.
2. Label the flask "50 pg/ μ L chloramphenicol stock [C3]".

25 pg/ μ L Chloramphenicol for tuning

1. Pipette 5 mL of [C3] into 10-mL volumetric flask, make up to 10 mL using 70:30 acetonitrile:water.
2. Label the flask "25 pg/ μ L chloramphenicol tuning solution".

0.5 pg/ μ L Chloramphenicol for tuning

1. Pipette 1 mL of [C3] into 100-mL volumetric flask, make up to 100 mL using 70:30 acetonitrile:water.
2. Label the flask "0.5 pg/ μ L chloramphenicol specification solution".

17- α -hydroxyprogesterone solutions for APCI positive sensitivity tests

The following dilutions must be prepared for the sensitivity tests from the vial containing 10 mg 17- α -hydroxyprogesterone.

100 ng/ μ L 17- α -hydroxyprogesterone stock

1. Add 4 mL 70:30 acetonitrile:water to the vial, recap, and vortex the vial.
2. Transfer the contents to a 100-mL volumetric flask using a funnel.
3. Rinse the vial out with 70:30 acetonitrile:water at least three times to ensure all the 17- α -hydroxyprogesterone has been transferred into the volumetric flask.

4. Make up to 100 mL using 70:30 acetonitrile:water and sonicate until all the 17- α -hydroxyprogesterone has fully dissolved.
5. Label the flask "100 ng/ μ L 17- α -hydroxyprogesterone stock [H1]".

500 pg/ μ L 17- α -hydroxyprogesterone for tuning

1. Pipette 500 μ L of [H1] into 100-mL volumetric flask, make up to 100 mL using 70:30 acetonitrile:water.
2. Label the flask "500 pg/ μ L 17- α -hydroxyprogesterone tuning solution [H2]".

10 pg/ μ L 17- α -hydroxyprogesterone for acquisition

1. Pipette 1 mL of [H2] into 50-mL volumetric flask, make up to 50 mL using 70:30 acetonitrile:water.
2. Label the flask "10 pg/ μ L 17- α -hydroxyprogesterone specification solution".

ScanWave optimization

The following dilutions must be prepared for the ScanWave tests from the vial containing 0.1 mg [Glu¹]-fibrinopeptide B.

2.5pmol/ μ L [Glu¹]-fibrinopeptide B stock

1. Add 2 mL 50:50 acetonitrile:water to the vial, recap, and vortex the vial.
2. Transfer the contents to a 25-mL volumetric flask using a funnel.
3. Rinse the vial out with 50:50 acetonitrile:water at least three times to ensure all the [Glu¹]-fibrinopeptide B has been transferred into the volumetric flask.
4. Make up to 25 mL using 50:50 acetonitrile:water and sonicate until all the [Glu¹]-fibrinopeptide B has fully dissolved.
5. Label the flask "2.5 pmol/ μ L [Glu¹]-fibrinopeptide B stock solution" [G1].

300 fmol/ μ L [Glu¹]-fibrinopeptide B for acquisition in negative mode

1. Pipette 3 mL of [G1] into 25-mL volumetric flask.
2. Pipette 25 μ L of formic acid into the flask and make up to 25 mL using 50:50 acetonitrile:water.
3. Label the flask "300 pmol/ μ L [Glu¹]-fibrinopeptide B + 0.1% formic acid specification solution".

100 fmol/ μ L [Glu¹]-fibrinopeptide B for acquisition in positive mode

1. Pipette 1 mL of [G1] into 25-mL volumetric flask.
2. Pipette 25 μ L of formic acid into the flask and make up to 25 mL using 50:50 acetonitrile:water.
3. Label the flask "100 pmol/ μ L [Glu¹]-fibrinopeptide B + 0.1% formic acid specification solution".

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 qualification results document associated with this workbook.

2.7 Creating the qualification project in MassLynx software

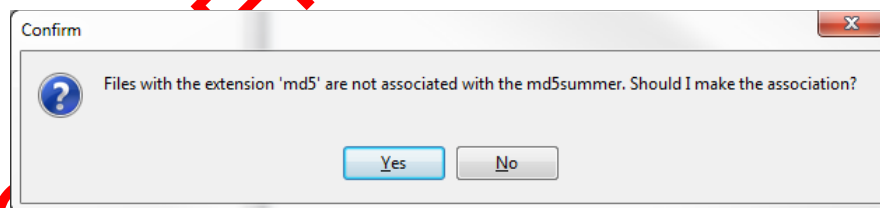
The qualification project files required for the qualification procedures described in this document are located in the "Xevo TQ-S Project Files" folder on the Qualification section of the Waters Knowledge Center.

To create the qualification project in MassLynx software:

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

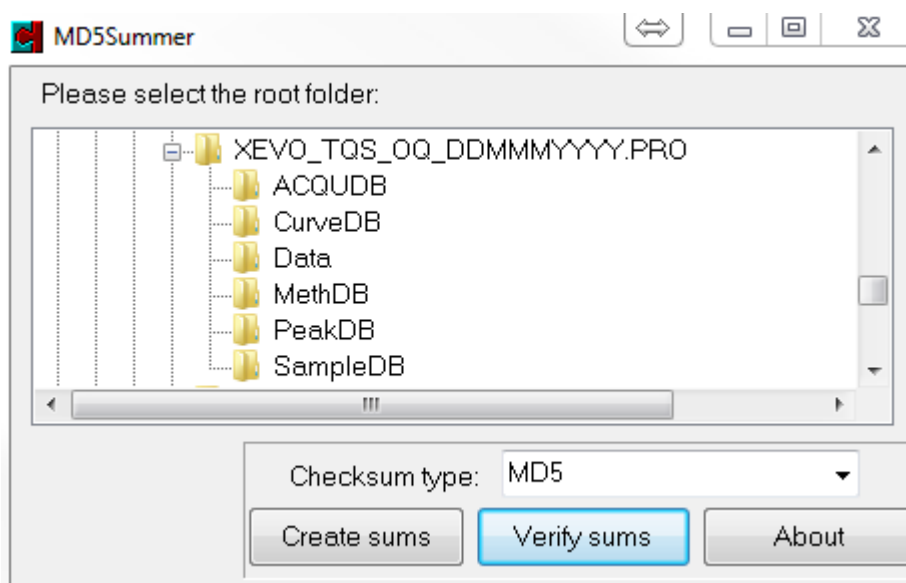
1. From the Qualification section of the Waters SAP Portal, download and import all the required files.
2. Verify the download file integrity by running a MD5 Checksum file verification. To do this:
 - a. Extract/Unzip the downloaded zip file into a folder on the PC, in this example it has been saved to the Desktop.
 - b. Open the extracted folder and Run the file md5summer.exe.
 - c. If the following message appears (Figure 1), click Yes.

Figure 1 Confirming the md5 file association:



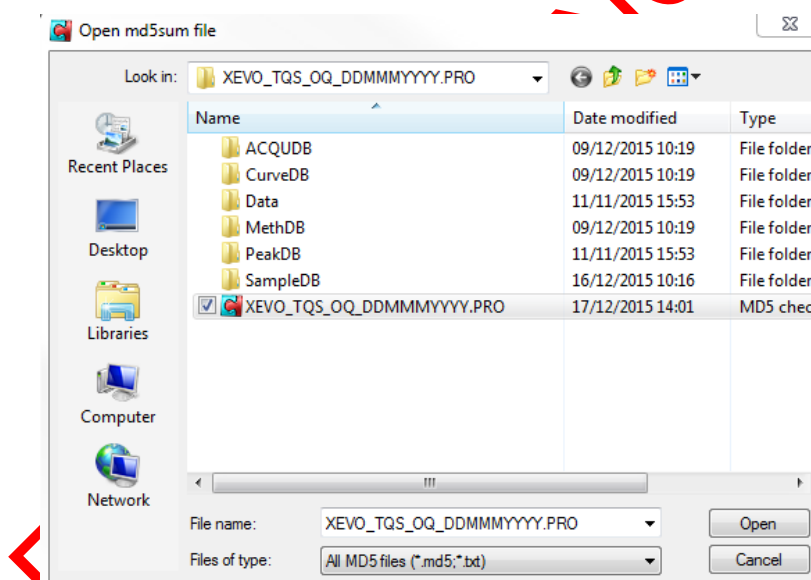
- d. In the following file-browser window (Figure 2), browse to the folder from which the ZIP file was extracted and where the MD5 checksum files are located, and then select Verify Sums.

Figure 2 Selecting verify sums:



e. The following window (Figure 3) will appear.

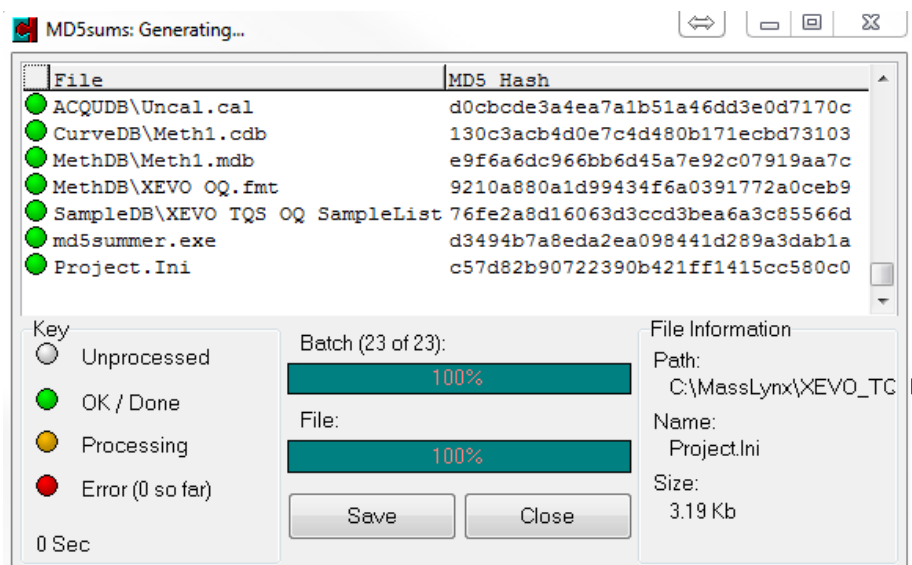
Figure 3 The Open md5sum file window:



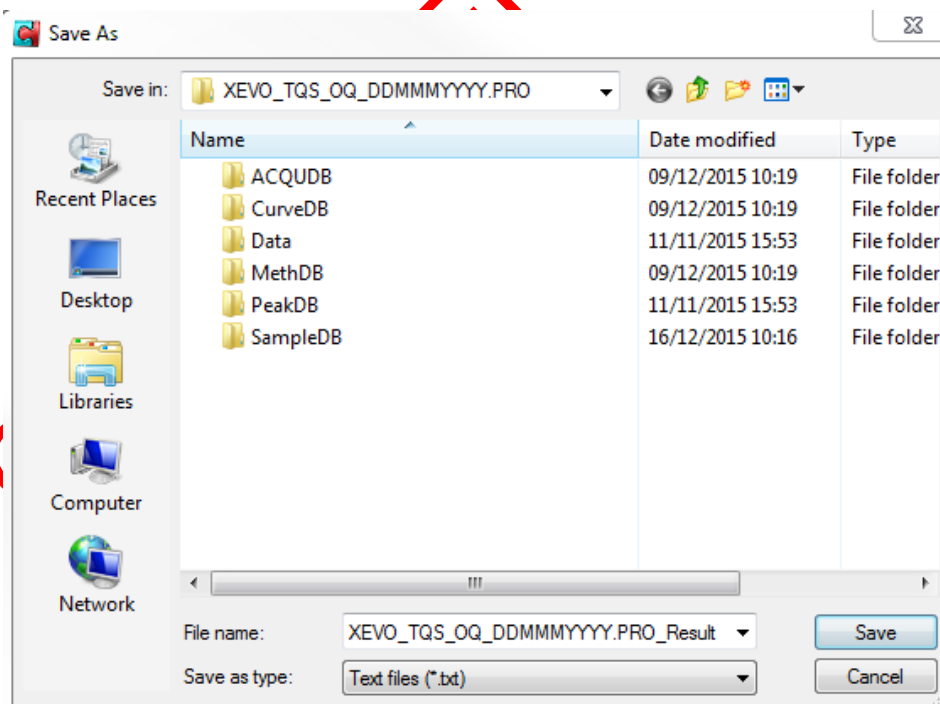
f. Select the md5 file and click Open.

g. A new window appears (Figure 4), and verification begins. A Green button next to the file name indicates the checksum was verified successfully. The example below shows that no errors have been detected.

Note: If errors have been detected re-download the project from the Portal. If you continue to get errors contact Global Service Support.

Figure 4 Starting verification:

- h. A copy of the results can be saved as a text file by selecting Save. Save the results text file into the Project folder (Figure 5). You need not print this record.

Figure 5 The project folder:

- i. Select Close to close the results window and shut down the MD5summer application.
- Copy the extracted and verified project file into the C:\MassLynx folder.
 - From the MassLynx window, select File > Project Wizard.

5. Open the Create Project menu, and enter details while observing the following conventions:
 - Name: XEVO TQS OQ DDMMYYYY
 - Description: Xevo TQS WAAXXX OQ NN, where NN are the engineer's initials
 - Location: C:\MassLynx
6. Click Next, and then select "Create using existing project as template".
7. Select XEVO_TQS_OQ_DDMMYYYYY.pro as the existing project.
8. Select Copy Sample lists, and then click Finish.

Note: The serial number may have the prefix WAA or WAC.

To load the sample list and sample list formats:

1. Open the XEVO_TQS_OQ_SampleList.spl sample list.
2. Click File > Save as and rename the sample list in the format 'XEVO_TQS_OQ_SampleList_DDMMYYYYY'.
3. Click Samples > Format > Load > Browse. Browse to the following folder:
C:\MassLynx\ XEVO_TQS_OQ_DDMMYYYYY.pro\MethDB.

2.8 Managing data files and test results

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

2.8.1 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 you repeat tests, rename the raw data file names by incrementing the acquisition number.
- Do not overwrite file names.

2.8.2 Recording test results

Record all test results in the operational qualification results document associated with this workbook. If instructed to save test results as raw data files, follow the conventions described in section 2.8.1. If instructed to create additional reports or screenshot printouts, ensure they are correctly labeled according to the below conventions. These header modifications are only possible after data acquisition.

Modifying Chromatogram and Spectrum Headers

To reduce the need to manually label data printouts the headers for Chromatogram and Spectrum should be altered.

1. Open a data file in the Chromatogram window after data has been collected.
2. Select Display > View. Click Header button. Click Header button at the bottom right-hand corner of the window.
3. In the Header editor window highlight the top right cell in the Header area field > in the Element field, select [Text] > Add > Enter the test name (e.g. Mass scale and resolution setup MS1), serial number or FWHM information > OK. See Table 2 for an example of how to layout the information in the header.

Table 2 Header Area settings

	Left	Center	Right
Line 1	Test Name	Instrument Name and Serial Number	Performer's Name
Line 2	Sample Name	Concentration and Flow Rate	Date
Line 3			Calculation Results

4. Select OK to return to the Chromatogram window.

To label printouts:

Ensure each printout is labeled with the following information:

- Instrument serial number
- Performer's name and date
- Test Name
- Sample Name
- Concentration
- Flow rate
- Any associated calculation results

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

3. Setting Up

To prepare the instrument for qualification, perform the procedures that appear in this section before proceeding with the operational qualification procedures.

Note: The term "MS1" is used to denote the first quadrupole and the term "MS" to denote the second quadrupole. Thus scans performed by the first quadrupole are labeled "MS1 Scan" and scans performed by the second quadrupole are labeled "MS Scan". In the procedures that follow, "MS" operations are sometimes referred to as "MS2", in order to comply with current software behavior. In practice, the terms "MS" and "MS2" are interchangeable.

To qualify operation of the instrument, perform the following procedures and verify their completion by completing Form 6, "Pre-qualification setup results".

3.1 Fluidics¹ connections

3.1.1 Tubing connections to the selector and diverter valves

Requirement: Use the shortest possible length of PEEK™ tubing to connect system components. When cutting PEEK tubing, make the cuts clean and square. Poorly cut tubing creates dead volume, which leads to the tailing of chromatographic peaks.

For details about fluidics¹ connections, see the *Waters Xevo TQ-S MS Operator's Overview and Maintenance Guide*, on the Waters Xevo TQ-S MS Documentation CD, part number 715002206.

To make fluidics connections

1. Ensure the tubing from position 2 on the selector valve is in the MS wash solution bottle containing 70:30 acetonitrile/water.
2. Connect the outlet of an LC pump to position 1 on the diverter valve.
3. Connect a length of black PEEK tubing from position 2 on the diverter valve to the electrospray probe.
4. Ensure all fittings of the diverter valve are hand tight.

¹ In this document, the term "fluidics" denotes plumbing components and fluid pathways within and between instruments and devices. It also refers to the IntelliStart Fluidics system, the instrument's onboard system that delivers sample and solvent directly to the mass spectrometer's probe.

3.2 Setting initial instrument conditions

To set initial instrument conditions:

1. Install a bottle of Setup Solution for Xevo TQ-S on fluidics reservoir B.
2. In the MassLynx software, open the MassLynx Tune window.
3. In the Fluidics tab, set fill volume to 250 μ L > set the flow state to Infusion.
4. In the MassLynx Tune window click Setup > Wash Fluidics > Select the number of wash cycles required to 4 > OK.

5. Select Reservoir to B and click  to Purge, purge twice.

Rationale: Doing so removes any air trapped in the fluidics system tubing.

6. Specify an infusion flow rate of 10 μ L/min, and then click Start infusion .
7. In the MassLynx Tune window, select Open > Instrument Setup.ipr.
8. Click  to start the nitrogen flow, then click  to put the instrument into operate mode.
9. Click ion mode > Electrospray +.
10. Ensure the initial source conditions are as in Table 3.

Table 3 Initial source conditions:

Parameter	Setting	Comments
Capillary (kV)	3	Will be optimized later
Cone voltage (V)	70	Will be optimized later
Source offset (V)	60	Fixed
Desolvation temperature ($^{\circ}$ C)	200	Fixed
Desolvation gas flow (L/H)	500	Fixed
Cone gas flow (L/H)	150	Fixed

11. In the MassLynx Tune window menu bar, click Setup > Inter-scan Setup.
12. Ensure Enable Manual Mode is cleared.
13. For positive mode, click Ion Mode tab > Electrospray+ > Setup > Set Instrument Threshold, and then ensure the ion counting threshold is set to 25.
14. For negative mode, click Ion Mode tab > Electrospray- > Setup > Set Instrument Threshold, and then ensure the ion counting threshold is set to 25.

15. Place instrument back to positive mode before proceeding: Ion Mode tab> Electrospray+.

16. In the MassLynx Tune window menu bar, click Calibration > Calibrate Instrument.

17. In the calibration dialog box, click File > Open, and then select the file Uncal.cal > Open.

Note: The Calibration window displays "No Calibration" for all calibration types. If it does not, select Calibrate > Default to remove the calibration.

18. Close the Calibration window.

19. In the Mass editor, open the four masses in Table 4.

Table 4 Mass editor parameter values:

Function	Mass	Span
MS1 Scan	59.05	5
MS1 Scan	455.3	5
MS1 Scan	1080.8	5
MS1 Scan	2034.6	5

To optimize the peaks at FWHM for both MS1 and MS2:

Requirements:

- The full width at half maximum (FWHM) of peaks at m/z 59.05, m/z 455.28, and m/z 1080.80 and m/z 2034.63 must be between 0.4 and 0.6 Da.
- The peaks must be within ± 0.5 Da of their nominal mass positions.
- The maximum variation between the measured FWHM must be less than or equal to 0.10 Da.

Note: Ion energies, DC balance, mass position and resolution values are factory-set and stored in the EPC's flash memory card. They may need adjustment upon installation. Make a note of the factory settings before making any adjustments.

MS1

1. Ensure all four MS1 Scan masses can be seen in the scope window in the MassLynx Tune window.
2. Ensure the MS Mode button is selected  and the collision gas is off.
3. Click View > System > Enter the service password.
4. On the Resolution tab, if required, adjust the resolution, mass position and linearity for MS1. Optimize m/z 59.05, 455.28, 1080.80 and 2034.63 so the FWHM is between 0.4 and 0.6 Da and the peaks are within ± 0.5 Da of their nominal mass positions.

MS2

1. Ensure collision gas is on and collision energy is set to 5.
2. Switch the Function to MS Scan in the scope window.
3. On the Resolution tab, if required, adjust the resolution, mass position and linearity for MS2. Optimize m/z 59.05, 455.28, 1080.80 and 2034.63 so the FWHM is between 0.4 and 0.6 Da and the peaks are within ± 0.5 Da of their nominal mass positions.
4. Save this modified Instrument_Setup.ipr tune page.

Rationale: The MS1 and MS2 resolution & mass position parameters in the Resolution tab will be optimized and ready for mass scale and resolution setup for MS1 and MS2.

3.3 Setting up mass scale and resolution – MS1

To set up the instrument (MS1):

1. In the Fluidics tab, select Infusion as the flow state, specify an infusion flow rate of 10 $\mu\text{L}/\text{min}$, and then click Start infusion.
2. Ensure the MS Mode button is selected  and the collision gas is off.
3. Switch the Function to MS1 Scan in the scope window.
4. In the ES+ tab, ensure the Analyzer parameters match those in Table 5.

Tip: To edit Ion Energy 1, you must set the Function to MS1 Scan in the scope window. To edit Ion Energy 2, you must set the Function to MS Scan in the scope window.

Table 5 Analyzer parameter values:

Analyser parameter	Parameter value
LM Resolution 1	3
HM Resolution 1	15
Ion energy 1	0.3 V
LM Resolution 2	3
HM Resolution 2	15
Ion energy 2	0.4 V
Collision energy	5

5. Use the cone and capillary parameters and the probe position adjustments to optimize the intensity of the four peaks across the mass range.
6. Save this modified Instrument_Setup.ipr tune page.

To determine the optimum ion energy in MS1 positive mode:

1. In Electrospray+ ion mode, open a single MS1 Scan window to display m/z 59.1, with a span of 3.
2. Set the MS1 High Mass DC balance to "0.00" on the Resolution tab.
3. Adjust Ion energy 1 to maximize the intensity of m/z 59.05 on the ESI+ tab.

Note: Using the correct value for ion energy is important to the performance of the quadrupole. If too low, the ions do not have enough energy to reach the end of the quadrupole and sensitivity suffers. If too high, then ions travel so quickly that the resolving capability is diminished.

4. In the MassLynx Tune window, adjust the window's gain so that the top of the peak at m/z 59.05 touches the top of the window.
5. Tune the ion energy so the intensity of m/z 59.05 ion drops to 5 to 10% of the maximum intensity obtained in step 4.
6. Add 0.5 to the current ion energy value. This is the optimal low mass ion energy for MS1.
7. In form 6, "Pre-qualification setup results", of the operational qualification results forms associated with this workbook, record the optimal ion energy for MS1.

To set the optimal high mass DC balance value in MS1 mode:

1. In Electrospray+ ion mode, open a single MS1 scan window to display m/z 2034.63 with a span of 3.
2. Adjust the High Mass DC balance for MS1 to maximize the intensity of the m/z 2034.63 signal in the Resolution tab.

Note: Do not adjust the DC balance value beyond ± 3 V.

3. In the MassLynx Tune window, adjust the window's gain so that the top of the peak at m/z 2034.63 touches the top of the window.
4. Adjust the DC balance so that the intensity of m/z 2034.63 drops to 5 to 10% of the maximum intensity obtained in step 3.
5. Adjust the DC balance by ± 0.50 V so that intensity of m/z 2034.63 signal increases. This is the optimal high mass DC balance for MS1.
6. Save the modified MS Tune file as MS1_MassRes_Setup.ipr.
7. In form 6, "Pre-qualification setup results", of the operational qualification results forms associated with this workbook, record the optimal high mass DC balance for MS1.

To acquire an MS1 spectrum:

Note: Ensure that the instrument has been in Operate mode for at least 30 minutes before acquiring the spectrum.

1. In the MassLynx Tune window > Fluidics tab > Refill the syringe  > Start infusion .
2. Click Open sample list .
3. Select XEVO_TQS_OQ_SampleList_DDMMYYYYY.spl.
4. Highlight the row that includes the file name MS1_MassScaleResolution_01, specify MS1_MassRes_Setup.ipr for the MS Tune file, and click Start.
5. Select Yes when asked if you want to save changes.
6. From the Start Sample List Run window > Acquire Sample Data > OK.
7. In the Fluidics tab, click Stop when the acquisition ends.
8. In the MassLynx window, click Spectrum.
9. Click File > Open.
10. Select the MS1_MassScaleResolution_01 file, and click OK.
11. Click the four-way arrow icon , and then click Replace New Spectrum .
12. Click Process > Smooth, and then set the parameter values in Table 6 > OK.

Table 6 Spectrum smoothing parameter values:

Peak width (Da)	0.50
Number of smooths	2
Smoothing method	Savitzky Golay

13. Click Display > View, and then set the parameter values shown in Figure 6 > OK.

Figure 6 Spectrum display parameters:

14. Click Display > Peak Annotation, and then set the parameter values in Figure 7.

Figure 7 Peak annotation parameter values:

15. Zoom in on the mass at m/z 59.05.

16. Measure the width of the m/z 59.05 peak at half-height.
 17. Repeat step 15 and 16 for the peaks at m/z 455.28, m/z 1080.80 and m/z 2034.63.
 18. Verify that the resolution and mass position of each of the peaks are acceptable:
 - The full width at half maximum (FWHM) of peaks at m/z 59.05, m/z 455.28, and m/z 1080.80 and m/z 2034.63 must be between 0.4 and 0.6 Da.
 - The peaks must be within ± 0.5 Da of their nominal mass positions.
 - The maximum variation between the measured FWHM must be less than or equal to 0.10 Da.
- Requirement:** If the resolution and mass position are outside acceptable limits, retune the resolution and mass position sliders on the Resolution tab.
19. In form 6, "Pre-qualification setup results", of the operational qualification results forms associated with this workbook, record the FWHM and mass position for each of the peaks.
 20. To annotate the spectrum: in the spectrum window, click Display > View > Select the Header... button.
 21. In the Header editor window highlight the top right cell in the Header area field > in the Element field, select [Text] > Add > Enter the instrument serial number, test name (e.g. Mass scale and resolution setup MS1), date and the FWHM information > OK.
- Note:** If there is not enough room to annotate the spectra with all the information, you can highlight a second cell in the header area.
22. Print the individual spectra for the following ranges: Click Display > Range > From > Enter the following:
 - m/z 54 through m/z 64
 - m/z 450 through m/z 460
 - m/z 1075 through m/z 1085
 - m/z 2029 through m/z 2039
 23. Click File > Print > Current view.
 24. Insert the printouts at the end of the operational qualification results document associated with this workbook.

3.4 Setting up mass scale and resolution – MS2

To set up the instrument (MS2):

1. Ensure the instrument is in MS mode , the collision gas is on and the collision energy is set to 5 V.
2. Check the collision gas is giving a collision cell pressure of 3.5e-3 mbar to 4.0e-3 mbar.
3. Ensure the MS1_MassRes_Setup.ipr tune file is still loaded.
4. In the Fluidics tab, click Refill  and click start infusion  to infuse Xevo TQ-S Set Up solution at 10 µL/min.

To determine the optimum ion energy in MS2 positive mode:

1. In Electrospray+ ion mode, open a single MS Scan window to display m/z 59.05, with a span of 3.
2. Set the MS2 High Mass DC balance to 0 on the Resolution tab.
3. Optimize Ion energy 2 to maximize the intensity of the m/z 59.05 ion on the ES+ tab.
4. In the MassLynx Tune window, adjust the window's gain so that the top of the peak at m/z 59.05 touches the top of the window.
5. Tune the ion energy so that the intensity of m/z 59.05 ion drops to 5 to 10% of the maximum intensity obtained in step 4.
6. Add 1.0 to the current ion energy value. This is the optimal low mass ion energy for MS2.
7. In form 6, "Pre-qualification setup results", of the operational qualification results forms associated with this workbook, record the optimal ion energy for MS2.

To set the optimal high mass DC balance value in MS2 mode:

1. In positive ion mode, open a single MS scan window to display m/z 2034.63 with a span of 3.
2. Adjust the High Mass DC balance for MS2 to maximize the intensity of the m/z 2034.63 signal in the Resolution tab.

Note: Do not adjust the DC balance value beyond ± 3 V.

3. In the MassLynx Tune window, adjust the window's gain so that the top of the peak at m/z 2034.63 touches the top of the window.
4. Adjust the DC balance so that the intensity of m/z 2034.63 drops to 5 to 10% of the maximum intensity obtained in step 3.

5. Adjust the DC balance by ± 1.0 V so that intensity of m/z 2034.63 signal increases. This is the optimal high mass DC balance for MS2.
6. Save the modified MS Tune file as MS2_MassRes_Setup.ipr.
7. In form 6, "Pre-qualification setup results", of the operational qualification results forms associated with this workbook, record the optimal high mass DC balance for MS2.

To acquire an MS2 spectrum:

Note: Ensure that the instrument has been in Operate mode for at least 30 minutes before acquiring the spectrum.

1. In the Fluidics tab, click Refill, and start infusion.
2. In the XEVO_TQS_OQ_SampleList_DDMMYYYYY.spl sample list.
3. Highlight the row that includes the file name MS2 Mass Scale and Resolution 01, specify MS2 MassRes Setup.ipr for the MS Tune file, and click Start.
4. In the Fluidics tab, click Stop when the acquisition ends.
5. In the main MassLynx window, click Spectrum.
6. Click File > Open.
7. Select the MS2 Mass Scale and Resolution 01 file, and click OK.
8. Repeat steps 11 through 24 from "To acquire an MS1 spectrum" in Section 3.3, ensuring that the header is correctly labeled MS2.

3.5 Calibrating the mass spectrometer

Perform an instrument setup and calibration for unit mass resolution mode in both positive and negative ion polarity using the IntelliStart software.

Requirement: Use the default IntelliStart resolution and calibration parameters.

To calibrate the mass spectrometer:

1. In the tune file click Setup > Wash Fluidics > Select the number of wash cycles required to 4 > OK.
2. Place a bottle of Xevo TQ-S calibration solution on reservoir B and purge once.
3. Place a bottle of IntelliStart Resolution x 20 solution on reservoir C and purge twice.
4. In the MassLynx main window, click MS Console.
5. Click on Xevo TQ-S MS Detector on the left to release the drop down menu > select IntelliStart.
6. From the Type of Analysis menu, select Unit Mass Resolution.

7. Select "show instrument set-up options".
8. In the IntelliStart page, ensure the check boxes for Instrument Resolution, (Positive and Negative) and Instrument Calibration, are selected.
9. Clear the Pre-checks check box.

Note: The IntelliStart software sets up the instrument resolution in both positive and negative-ion modes. Calibration is performed in positive-ion mode only.

10. From the icon list on the right-hand side of the MS Console, click Start.
11. Select "Switch to advanced mode".
12. In the IntelliStart Setup Parameters window, ensure the parameters are as shown in Table 7.

Table 7 Resolution and calibration parameters:

Parameter	Mass resolution mode setting
Type of Analysis	Unit Mass Resolution
Positive-ion mode reference file name	Resolution pos ion
Negative-ion mode reference file name	Resolution neg ion
Calibration reference file name	Phosphoricpos2
Resolution Report Print	Selected
Resolution Report Print to PDF	Not selected
Calibration Report Print	Selected
Print to PDF	Not selected
Resolution Report Keep HTML	Selected
Mass Range	Default (2 through 2048 Da)
Calibration Speed	Default (100 through 10000 Da/Sec)
Target Resolution MS1	Unit (0.75)
Target Resolution MS2	Unit (0.75)
Resolution Reservoir	C
Calibration Reservoir	B

13. Click Start.
14. Ensure the procedure ends successfully.
15. Sign the printed reports and insert them at the end of the operational qualification results document associated with this workbook.

Note: Calibration files created using IntelliStart are stored in the C:\MassLynx\IntelliStart\Results folder in a subdirectory named for the type of analysis, for example "Unit Mass Resolution".

FOR REFERENCE ONLY

3.6 Backing up the flash memory settings

Requirement: You must save a copy of the Target Registry settings on to the host PC.

To back up the flash memory settings:

1. From the MassLynx Tune window, put the instrument in Standby mode.
2. Quit the MassLynx Tune window and exit MassLynx software.
3. Browse to the C:\MassLynx\Vulcan\TargetRegistryEditor.exe file, and double-click it.

Requirement: Do not alter any of the settings contained in the registry view.

4. Enter password.
5. Right-click in the view, and select Export All.
6. Export the settings to the Xevo TQ-S OQ WAAXXX DDMMYYYY project folder in the C drive, and name the file WAAXXX Target Registry DDMMYYYY.treg.
7. Close the Target Registry Editor, and then reopen the MassLynx software.

FOR REFERENCE ONLY

4. Operational Qualification Procedures

Before performing the operational qualification procedures in this section, the performer and reviewer must familiarize themselves with the appropriate manual(s) and other referenced documentation and, if applicable, the MassLynx Online Help.

To qualify operation of the instrument, perform the following procedures and verify their completion by completing Form 7, "Operational qualification results" of the operational qualification results document associated with this workbook.

4.1 High mass resolution verification

Perform the high mass resolution verification for MS1 and MS2.

Requirements:

- Using Xevo TQ-S Setup solution, the valley between the peaks at masses 2034.63 and 2035.63 Da must be less than or equal to 12 % of the average height of the two peaks.

To set up and acquire MS1 high mass resolution verification:

- In the MassLynx Tune window, click File > Open > MS1_HMVerification.ipr.
- In the MassLynx Tune window, click Setup > Wash Fluidics > Select the number of wash cycles to 4 > OK.
- Install Xevo TQ-S setup solution on line B and purge once.
- In the Fluidics tab, click Flow State > infusion.
- Infuse Xevo TQ-S setup solution at 10 μ L/min, and start infusion.
- Ensure the collision gas is turned off.
- In the MassLynx Tune window, open one MS1 scan window at mass 2034.63 Da, with a span of 5.
- In the ES+ Source tab, adjust the cone voltage, settings in the HM Resolution 1 and Ion Energy 1 fields to obtain the best possible valley between the peaks at 2034.63 Da and 2035.63 Da.
- Save the modified MS1_HMVerification.ipr tune file.
- Specify the MS Tune file MS1_HMVerification.ipr and MS1_HighMassVerification.exp file in the XEVO_TQS_OQ_SampleList_DDMMYYYY.spl sample list.
- In the sample list, select the row that includes the file name MS1_HighMassVerification_01, and then click Start.

Note: If a calibration speed error message appears, click Continue.

- In the Fluidics tab, click Stop when the acquisition ends.

To assess MS1 results:

1. In the Spectrum window, open the MS1_HighMassVerification_01 file.
2. In the Spectrum window, click Process > Smooth, and then adopt the parameter values shown in Table 8.

Table 8 Smoothing parameters:

Function	Setting
Window size	± 0.5 scans
Number of smooths	2
Smoothing method	Savitzky Golay

3. Click OK.
4. Click and drag from the bottom of the valley to the average of the first two peaks to measure the valley between m/z 2034.63 and m/z 2035.63.

Tip: The valley percent appears at the bottom, left-hand corner of the spectrum window when you click.

Requirement: Ensure the valley between m/z 2034.63 and m/z 2035.63 is $\leq 12\%$.

5. If the high-mass resolution verification for MS1 result meets the requirement, annotate all printouts according to the conventions in section 2.8.2, include this information:
 - Sample (Xevo TQ-S setup solution)
 - Flow rate (10 $\mu\text{L}/\text{min}$)
 - The valley percentage

Recommendation: If the high-mass resolution verification for MS1 result does not meet the requirement, inspect the mass resolution and mass position settings, and then rerun the verification.

6. Print the labeled spectrum.
7. Print and initial the MassLynx Tune window's parameter settings.
8. Record the results in Form 7, "Operational qualification results", of the operational qualification results document associated with this workbook.

To set up and acquire MS2 high mass resolution verification:

1. Put the instrument into Operate mode, with the API gas on.
2. Set the desolvation gas flow to 1000 L/hr and the probe temperature to 600°C.

3. In the MS Tune page, open the Sensitivity Tests.ipr tune file.
4. In the MassLynx Tune window, click File > Open > MS2_HMVerification.ipr.
5. Ensure the collision gas is turned on for MS acquisition and collision energy is set to 5.
6. In the MassLynx Tune window, change the function of the scan window from MS1 Scan to MS Scan and observe mass 2034.63 Da, with a span of 5.
7. Tune the cone voltage, settings in the HM Resolution 2 and Ion Energy 2 fields to obtain the best possible valley between the peaks at 2034.63 Da and 2035.63 Da.
8. Save the modified MS2_HMVerification.ipr tune file.
9. Select this MS2_HMVerification.ipr tune file in the sample list for MS2_HighMassVerification_01 sample.
10. Highlight MS2_HighMassVerification_01 sample and click Start.
11. Process the spectrum in the same way as the MS1 spectrum in the 'To assess MS1 results' section.

4.2 Sensitivity and precision tests

The sensitivity tests described in this section are not required to be performed where the Xevo TQ-S is being qualified as part of a UPLC system where system level tests are run as part of the OQPO. If this is the case write "N/A" in form 7, "Operational qualification results", of the results form associated with this workbook.

4.2.1 ESI positive-ion mode sensitivity and precision test

Make five sample infusions of reserpine (1 pg/μL in 70:30 acetonitrile/water) and three blank infusions (70:30 acetonitrile/water) into an LC flow rate of 0.2 mL/min 70:30 acetonitrile/water, using the MS system fluidics.

Requirements:

- The signal-to-noise ratio of the m/z 609 → 195 transition sample peaks (RMS, ignoring zeroes and with no extra processing) must be greater than or equal to 15000:1.
- The %RSD for the five sample peak heights must be less than or equal to 10%.
- The blank intensity must be less than or equal to 10% of the average sample intensity.

To set up the instrument to verify ESI positive-ion mode sensitivity and precision:

1. Put the instrument into Operate mode, with the API gas on.
2. In the MS Tune page, open the Sensitivity Tests.ipr tune file.

3. Copy the MS1 and MS2 resolution and ion energy settings from the Unit Mass Resolution report created by IntelliStart (section 3.5).
4. Click Ion Mode > Electrospray+.
5. Install a bottle of Xevo TQ-S Reserpine Tuning Solution (5pg/μL) on Reservoir C.
6. In the MassLynx Tune window click Setup > Wash Fluidics > Select the number of wash cycles required to 4 > OK.
7. On the Fluidics tab, select Reservoir C and purge.
8. Click Flow State > Combined.
9. Start the LC at 0.2 mL/min 70:30 acetonitrile/water.
10. Infuse 5 pg/μL Reserpine tuning solution at 20 μL/min.
11. Select MSMS mode .
12. Switch the collision gas on.
13. Ensure the collision gas flow is giving a collision cell pressure of 3.5E-3 mbar to 4.0E-3 mbar.
14. Set the collision energy to 38 V.
15. Set the scope to display a daughter scan with the transition m/z 609 → 195 and a span of 3 Da.
16. Optimize the capillary length, probe position and capillary voltage to give the highest m/z 609 → 195 signal intensity.
17. Tune the analyzer settings to achieve a resolution of 0.7 ± 0.1 Da FWHM on the m/z 195 fragment.
18. Save the tune file as ESI_Sensitivity_POS.ipr.

4.2.2 Data acquisition

To acquire the ESI positive-ion mode sensitivity data:

1. Ensure the LC is pumping 70:30 acetonitrile/water at 0.2 mL/min.
2. Select Flow state > LC.
3. Select Fill volume > 250 μL.
4. Install a bottle of 1 pg/μL Reserpine in 70:30 acetonitrile/water on Reservoir B and purge once.
5. Install a bottle of 70:30 acetonitrile/water on Reservoir A and purge once.

Rationale: Blank injections are taken from Reservoir A.

6. Specify the MS Tune file ESI_Sensitivity_POS.ipr and MS file ESI_Sensitivity_POS.exp in the XEVO_TQS_OQ_SampleList_DDMMYYYYY.spl sample list.
7. In the sample list, select the row that includes the file name ESI_Positive_Sensitivity_01, and then click Start.

4.2.3 Assessing the results

To assess the results:

1. In the chromatogram browser, click Display > TIC > MRM of 1 channel ES+ Reserpine.
2. In the chromatogram browser, click Process > Signal To Noise, and adopt the signal-to-noise settings shown in Table 9.

Table 9 Required signal-to-noise parameters:

Parameter	Setting
Ignore Zeros	Cleared
NO Extra Processing	Selected
Ignore Worst 5% of Scans	Cleared
Ignore Scans Outside ± 1 SD	Cleared
Ignore Scans Outside ± 1.5 SD	Cleared
Ignore Scans Outside ± 2 SD	Cleared
RMS	Selected
Peak to Peak	Cleared
Use (n-1) for RMS	Cleared
Use Underlying Raw Data	Cleared

3. To measure the signal, right-click and drag across the first sample infusion peak.
4. To measure the noise, right-click and drag across a 30-second section of noise, 1 minute after the infusion peak.
5. Click OK, to calculate the signal-to-noise ratio.
6. Repeat steps 3 through 5 for each sample peak, adding a chromatogram with the calculated signal-to-noise ratio.
7. Measure the blank and sample peak heights.

8. Use the Windows Calculator (see section 5) to calculate the results, as shown in Table 10, noting the specification.

Table 10 Calculating results:

Value	Specification
Average signal-to-noise ratio of the m/z 609 $\rightarrow$ 195 transition.	Must be greater than or equal to 15000:1.
%RSD of the peak heights of the five sample injections.	Must be less than or equal to 10%.
Average signal intensity for the three blanks and the average signal intensity of the five sample injections.	The average blank intensity must be less than or equal to 10% of the average signal intensity of the five sample peaks.

9. Print chromatograms showing this information:
- The average signal-to-noise ratio.
 - The Relative SD of the sample peak heights.
 - The 3 blank injections.
10. Print the experimental record.
11. Print the MassLynx Tune window's parameter settings.
12. Annotate all printouts according to the conventions in section 2.8.2.
13. Record the results in Form 7, "Operational qualification results", of the operational qualification results document associated with this workbook.

4.2.4 ESI negative-ion mode sensitivity and precision test

Make five sample infusions of chloramphenicol (0.5 pg/ μ L in 70:30 acetonitrile/water) and three blank infusions (70:30 acetonitrile/water) into an LC flow rate of 0.2 mL/min of 70:30 acetonitrile/water, using the MS system fluidics.

Requirements:

- The signal-to-noise ratio of the sample m/z 321 $\rightarrow$ m/z 152 peaks (RMS, ignoring zeroes and with no extra processing) must be greater than or equal to 3000:1.
- The %RSD for the five sample peak heights must be less than or equal to 10%.
- The average blank intensity must be less than or equal to 10% of the average sample intensity.

To set up the instrument to verify ESI negative-ion mode sensitivity and precision:

1. Keep the ESI_Sensitivity_POS.ipr tune file open.
2. Click Ion Mode > Electrospray-.
3. Install a bottle of Chloramphenicol Tuning Solution (25 pg/μL) on fluidics line C.
4. In the MassLynx Tune window click Setup > Wash Fluidics > Select the number of wash cycles required to 4 > OK.
5. On the Fluidics tab, select Reservoir C and purge.
6. Click Flow State > Combined.
7. Start the LC at 0.2 mL/min 70:30 acetonitrile/water.
8. Infuse 25 pg/μL Chloramphenicol tuning solution at 20 μL/min.
9. Select MSMS mode .
10. Switch the collision gas on.
11. Ensure the collision gas flow is giving a collision cell pressure of 3.5E-3 mbar to 4.0E-3 mbar.
12. Set the collision energy to 25 V.
13. Set the scope display to a daughter scan with the transition m/z 321 → 152 and a span of 3 Da.
14. Optimize the capillary length and probe position and capillary voltage to give the highest m/z 321 → 152 signal intensity.
15. Tune the analyzer settings to achieve a resolution of 0.7 ± 0.1 Da FWHM on the m/z 152 fragment.
16. Save the tune file as ESI_Sensitivity_NEG.ipr.

4.2.5 Data acquisition

To acquire the ESI negative-ion mode sensitivity data:

1. Ensure the LC is pumping 70:30 acetonitrile/water at 0.2 mL/min.
2. Select Flow state > LC.
3. Select Fill volume > 250 μL.
4. Install a bottle of 0.5 pg/μL Chloramphenicol in 70:30 acetonitrile/water on Reservoir B and purge once.
5. Install a bottle of 70:30 acetonitrile/water on Reservoir A and purge once.

Rationale: Blank injections are taken from Reservoir A.

6. Specify the MS Tune file ESI_Sensitivity_NEG.ipr and MS file ESI_Sensitivity_NEG.exp in the XEVO_TQS_OQ_SampleList_DDMMYYYYY.spl sample list.
7. In the sample list, select the row that includes the file name ESI_Negative_Sensitivity_01, and then click Start.

4.2.6 Assessing the results

To assess the results:

1. In the chromatogram browser, click Display > TIC > MRM of 1 channel ES-Chloramphenicol.
2. In the chromatogram browser, click Process > Signal To Noise, and adopt the signal-to-noise settings in Table 9.
3. Right-click and drag across the first infusion peak, for the signal measurement.
4. Right-click and drag across a 30-second section of noise, 1 min after the infusion peak for the noise measurement.
5. Click OK, to calculate the signal-to-noise ratio.
6. Repeat steps 2 through 5 for each sample peak, to create a chromatogram with the calculated signal-to-noise ratio.
7. Measure the blank and sample peak heights.
8. Use the Windows Calculator (see section 5) to calculate the results, as shown in Table 11, noting the specification.

Table 11 Calculating results:

Value	Specification
Average signal-to-noise ratio of the m/z 321 $\rightarrow$ m/z 152 transition	Must be greater than or equal to 3000:1
%RSD of the peak heights of the five sample injections	Must be less than or equal to 10%.
Average signal intensity for the three blanks and the average signal intensity of the five sample injections	The average blank intensity must be less than or equal to 10% of the average signal intensity of the five sample peaks.

9. Print chromatograms showing this information:
 - The average signal-to-noise ratio.
 - The 3 blank injections.
10. Print the experimental record.
11. Print the MassLynx Tune window's parameter settings.
12. Annotate all printouts according to the conventions in section 2.8.2.
13. Record the results in Form 7, "Operational qualification results", of the operational qualification results document associated with this workbook.

4.2.7 APCI positive-ion mode sensitivity and precision test (optional)

Make five sample infusions of 17- α -hydroxyprogesterone (10 pg/ μ L in 70:30 acetonitrile/water) and three blank infusions (70:30 acetonitrile/water) into an LC flow rate of 1 mL/min of 70:30 acetonitrile/water, using system fluidics.

Requirements:

- The signal-to-noise ratio of the m/z 331 $\rightarrow$ m/z 109 transition sample peaks (RMS, ignoring zeroes and with no extra processing) must be greater than 2500:1.
- The %RSD of the peak heights of the sample injections must be less than or equal to 10%.
- The blank intensity must be less than or equal to 10% of the average sample intensity.

To set up the instrument to verify APCI positive-ion mode sensitivity:

1. From the MassLynx Tune window, put the instrument in standby mode, with the API gas switched off.
2. Remove the ESI probe, and fit the IonSABRE II APCI probe and corona pin.
3. In the MassLynx Tune window, click Ion Mode > APCI+.
4. Keep the ESI_Sensitivity_NEG.lpr tune file open.
5. Set the desolvation gas flow to 1000 L/Hr and the probe temperature to 600 °C.
6. Put the instrument in Operate mode, and start the API and collision gas.
7. Install a bottle of 500 pg/ μ L 17- α -hydroxyprogesterone tuning solution on fluidics line C.
8. In the MassLynx Tune window click Setup > Wash Fluidics > Select the number of wash cycles required to 4 > OK.
9. On the Fluidics tab, select Reservoir C and purge.
10. Click Flow State > Combined.
11. Start the LC at 1 mL/min 70:30 acetonitrile/water.
12. Infuse 500 pg/ μ L 17- α -hydroxyprogesterone tuning solution at 70 μ L/min.
13. Select MSMS mode .
14. Ensure the collision gas flow is giving a collision cell pressure of 3.5E-3 mbar to 4.0E-3 mbar.
15. Set the collision energy to 30 V.
16. Set the scope display to a daughter scan with the transition m/z 331 $\rightarrow$ 109 and a span of 3 Da.

17. Optimize the capillary length, probe position and corona current/voltage to give the highest m/z 331 $\rightarrow$ 109 intensity.
18. Tune the analyzer settings to achieve a resolution of 0.7 ± 0.1 Da FWHM on the m/z 109 fragment.
19. Save the tune file as APCI_Sensitivity_POS.ipr.

4.2.8 Data acquisition

To acquire the APCI positive-ion mode sensitivity data:

1. Ensure the LC is pumping 70:30 acetonitrile/water at 1 mL/min.
2. Select Flow state > LC.
3. Select Fill volume > 250 μ L.
4. Install a bottle of 10 pg/ μ L 17- α -hydroxyprogesterone in 70:30 acetonitrile/water on Reservoir B and purge once.
5. Install a bottle of 70:30 acetonitrile/water on Reservoir A and purge once.

Rationale: Blank injections are taken from Reservoir A.

6. Specify the MS Tune file APCI_Sensitivity_POS.ipr and MS file APCI Pos Sensitivity.exp in the XEVO_TQS_OQ_SampleList_DDMMYYYYY.spl sample list.
7. In the sample list, select the row that includes the file name APCI_Positive_Sensitivity_01, and then click Start.

4.2.9 Assessing the results

To assess the results:

1. In the chromatogram browser, click Display > TIC > MRM of 1 channel API+ 17- α -hydroxyprogesterone.
2. Click Process > Signal To Noise and adopt the signal-to-noise settings in Table 9.
3. Right-click and drag across the first infusion peak for the signal measurement.
4. Right-click and drag across a 30-second section of noise, 1 min after the infusion peak for the noise measurement.
5. Click OK, to calculate the signal-to-noise ratio.
6. Repeat steps 2 through 5 for each sample peak, adding a chromatogram with the calculated signal to noise result.
7. Measure the blank and sample peak heights.

8. Use the Windows Calculator (see section 5) to calculate the results as shown in Table 12, noting the specification.

Table 12 Calculating results:

Value	Specification
Average signal-to-noise ratio of the m/z 331.2 $\rightarrow$ m/z 109.0 transition	Must be greater than or equal to 300:1.
Relative SD of the peak heights of the five sample injections	Must be less than or equal to 10%.
Average signal intensity for the three blanks and the average signal intensity of the five sample injections	The average blank intensity must be less than or equal to 10% of the average signal intensity of the five sample peaks.

9. Print chromatograms showing this information:
- The average signal-to-noise ratio.
 - The Relative SD of the sample peak heights.
 - The 3 blank injections.
10. Print the experimental record.
11. Print the MassLynx Tune window's parameter settings.
12. Annotate all printouts according to the conventions in section 2.8.2.
13. Record the results in Form 7, "Operational qualification results", of the operational qualification results document associated with this workbook.
14. Place the instrument in Standby mode with the API gas switched off.
15. Remove the APCI probe and corona pin.
16. Fit the corona pin socket plug and ESI probe.

4.3 ScanWave verification tests (optional for re-qualification)

The ScanWave verification tests are optional upon re-qualification. FSEs must clarify with customers if these tests are desired.

Perform the following procedure to verify the ScanWave collision cell and calibration are working correctly for both positive and negative mode.

Requirement: The TIC reported in the ScanWave daughter scan chromatogram must be at least seven times greater than that reported in the daughter scan chromatogram. If either positive or negative mode ScanWave verification tests fail please run the ScanWave optimization procedure described in the install manual.

To prepare for the ScanWave verification tests:

1. Place a bottle of 70:30 acetonitrile/water on Reservoir A.
2. In the tune file click Setup > Wash Fluidics > Select the number of wash cycles required to 4 > OK.
3. Place a bottle of 100 fmol/ μ L [Glu¹]-fibrinopeptide B in 50:50 acetonitrile/water + 0.1 % formic acid on Reservoir B and purge once.
4. In the MassLynx Tune window > Open > Scanwave.ipr.
5. Put the instrument in Operate mode, and start the API and collision gas.

4.3.1 ScanWave positive-ion mode

To verify ScanWave in positive-ion mode:

1. Find the optimization file (.opt) for positive ion mode from the DVD that was shipped with the instrument.
2. Place a copy of the optimization file in the following location: C Drive > MassLynx > ScanWave folder.
3. On the MassLynx Tune window click Setup > ScanWave optimization > Load optimization.
4. Click Setup > ScanWave optimization > Enable optimization.
5. Click Ion Mode > Electrospray +.
6. Infuse 100 fmol/ μ L [Glu¹]-fibrinopeptide B at a flow rate of 10 μ L/min.
7. Open one MS Scan window, and display the mass at 786 Da, with collision energy of 5 V, span of 5 and gain of 1.
8. Adjust the probe position and capillary voltage to ensure the intensity of [Glu¹]-fibrinopeptide B at $m/z \sim 786$ is between 5 and 10% with the window gain set to 1.
9. Save the modified Scanwave.ipr tune file.
10. Stop the collision gas.
11. In the MassLynx Tune window, open one MS1 Scan window, and display mass 786 Da, with a span of 5.
12. Note the exact mass position of the highest point of the [Glu¹]-fibrinopeptide B ion.
13. Start the collision gas, and ensure the collision cell pressure is 3.5E-3 mbar to 4.0E-3 mbar.
14. In the main MassLynx window, select MS Method > File > Open > Scanwave_POS_Daughter.exp.

15. Double-click on function 1, and enter the exact mass position noted in step 12 next to "Daughters of".

Tip: See Table 12 for MS method parameters for all ScanWave verification tests.

16. Save the Scanwave_POS_Daughter.exp file.

Tip: To Save the .exp file, click the save icon in the Experimental Setup window and also the save icon in the main MassLynx window.

17. In the main MassLynx window, select MS Method > File > Open > Scanwave_POS_SWDaughter.exp.

18. Double-click on function 1, and enter the exact mass position noted in step 12 next to "Daughters of".

19. Save the Scanwave_POS_SWDaughter.exp file.

Tip: To Save the .exp file, click the save icon in the Experimental Setup window and also the save icon in the main MassLynx window.

20. In the Fluidics tab, select Refill, and start infusion. Allow two minutes for the spray to stabilize.

21. In the XEVO_TQS_OQ_SampleList_DDMMYYYY.spl sample list, highlight rows Scanwave_Positive_01 and Scanwave_Positive_02, and then click Start.

Table 13 MS Method parameters for the ScanWave verification tests.

Parameter	ESI- Daughter	ESI+ ScanWave Daughter	ESI- Daughter	ESI- ScanWave Daughter
Daughters of	786.**	786.**	784.**	784.**
Start (m/z)	100	100	100	100
End (m/z)	1100	1100	1100	1100
Start (Mins)	0	0	0	0
End (Mins)	2	2	2	2
Collision Energy: Use Tune Window	Cleared	Cleared	Cleared	Cleared
Collision Energy (V)	30	30	30	30
Collision Energy: Use Collision Energy Ramp	Cleared	Cleared	Cleared	Cleared
APCI Probe	Cleared	Cleared	Cleared	Cleared
Ionization Mode	ES+	ES+	ES-	ES-
Data	Continuum	Continuum	Continuum	Continuum
Scan Duration (secs)	0.2	5000 Da/sec	0.2	5000 Da/sec
Cone Voltage: Use Tune Window	Cleared	Cleared	Cleared	Cleared
Cone Voltage (V)	40	40	40	40
Cone Voltage: Use Cone Voltage Ramp	Cleared	Cleared	Cleared	Cleared

** Enter the observed mass position in these fields. Method Events are not enabled for these acquisitions.

22. In the chromatogram browser, click File > Open, and select Scanwave_Positive_1 and Scanwave_Positive_2.
23. In the chromatogram browser, select Process > Smooth, and then adopt the parameters shown in Table 14.
24. Use the Windows Calculator (see section 5) to calculate the ScanWave TIC enhancement by dividing the ScanWave daughter TIC by the daughter TIC.

Requirement: The calculated TIC gain must equal or exceed 7.

Table 14 Smoothing parameters for the ScanWave verification tests:

Parameter	Setting
Window size (scans) ±	10
Number of smooths	10
Smoothing method	Mean

25. Display both smoothed chromatograms on a single window and print.
26. In the chromatogram printout, annotate all printouts according to the conventions in section 2.8.2, include this information:
 - Test procedure reference name (ScanWave Positive Verification Tests)
 - Sample (100 fmol/μL [Glu¹]-fibrinopeptide B in 50:50 acetonitrile/water + 0.1% formic acid)
 - Flow rate (10 μL/min) and calculation
27. Print the experimental records for both Scanwave_Positive_01 and Scanwave_Positive_02.
28. Record the results in form 7, "Operational qualification results", of the operational qualification results forms associated with this workbook.

4.3.2 ScanWave negative-ion mode

To verify ScanWave in negative-ion mode:

1. Find the optimization file (.opt) for negative ion mode from the DVD that was shipped with the instrument.
 2. Place a copy of the optimization file in the following location: C Drive > MassLynx > ScanWave folder.
 3. On the MassLynx Tune window click Setup > ScanWave optimization > Load optimization.
 4. Click Setup > ScanWave optimization > Enable optimization.
 5. Click Ion Mode > Electrospray -.
 6. Place a bottle of 300 fmol/ μ L [Glu¹]-fibrinopeptide B in 50:50 acetonitrile/water + 0.1 % formic acid on Reservoir B and purge once.
 7. Infuse 300 fmol/ μ L [Glu¹]-fibrinopeptide at a flow rate of 10 μ L/min.
 8. In the MassLynx Tune window, open one MS Scan window, and display mass 784 Da with collision energy of 5 V with a span of 5 and a gain of 1.
 9. Adjust the probe position and capillary voltage to ensure the intensity of [Glu¹]-fibrinopeptide B at m/z ~784 is between 5% and 10%, with the window gain set to 1.
 10. Save the modified Scanwave.ipr tune file.
 11. Stop the collision gas.
 12. In the MassLynx Tune window, open one MS1 Scan window, and display mass 784 Da, with a span of 5.
 13. Note the exact mass position of the highest point of the [Glu¹]-fibrinopeptide B ion.
 14. Start the collision gas, and ensure the collision cell pressure is 3.5E-3 mbar to 4.0E-3 mbar.
 15. In the main MassLynx window, select MS Method > File > Open > Scanwave_NEG_Daughter.exp.
 16. Double-click on function 1, and enter the exact mass position noted in step 13 next to "Daughters of".
- Tip:** See Table 12 for method parameters for all ScanWave verification tests.
17. Save the Scanwave_NEG_Daughter.exp file.
 18. In the main MassLynx window, select MS Method > File > Open > Scanwave_NEG_SWDaughter.exp.

19. Double-click on function 1, and enter the exact mass position noted in step 13 next to "Daughters of".
20. Save the Scanwave_NEG_SWDaughter.exp file.
21. In the Fluidics tab, select Refill, and start infusion. Allow two minutes, for the spray to stabilize.
22. In the XEVO_TQS_OQ_SampleList_DDMMYYYYY.spl sample list, highlight rows Scanwave_Negative_01 and Scanwave_Negative_02, and then click Start.
23. In the chromatogram browser, click File > Open, and then select Scanwave_Negative_1 and Scanwave_Negative_2.
24. In the chromatogram browser, select Process > Smooth, and then select the parameters given in Table 14.
25. Use the Windows Calculator (see section 5) to calculate the ScanWave TIC enhancement by dividing the ScanWave daughter TIC by the daughter TIC.
Requirement: The calculated TIC gain must be equal or exceed 7.
26. Display both smoothed chromatograms on a single window and print.
27. On the chromatogram printout, annotate all printouts according to the conventions in section 2.8.2, include this information:
 - Test procedure reference name (ScanWave Negative Verification Tests)
 - Sample (300 fmol/ μ L [Glu^1] fibrinopeptide B in 50:50 acetonitrile/water + 0.1 % formic acid)
 - Flow rate (10 μ L/min)
 - All calculations
28. Print the experimental records for both Scanwave_Negative_01 and Scanwave_Negative_02.
29. Record the results in form 7, "Operational qualification results", of the operational qualification results forms associated with this workbook.

5. Using the Windows Calculator

For more information on how to use the Windows® 7 or Windows XP calculator to determine standard deviation, %RSD, and RMS refer to "Calculating Standard Deviation, %RSD, and RMS" (part number 715004060), available on the Waters SAP Portal.

FOR REFERENCE ONLY

6. Logging Operational Qualification Results

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

FOR REFERENCE ONLY