

Waters® Xevo® TQ-S ACQUITY UPLC® System

Customer Familiarization Guide



Notice

The information in this document is subject to change without notice and must not be construed as a commitment by Waters Corporation. Waters Corporation assumes no responsibility for any errors that may appear in this document. This guide is believed to be complete and accurate at the time of publication. In no event shall Waters Corporation be liable for incidental or consequential damages in connection with or arising from the use of this guide.

©2010 WATERS CORPORATION. PRINTED IN THE UNITED STATES OF AMERICA. ALL RIGHTS RESERVED. THIS BOOK OR PARTS THEREOF MAY NOT BE REPRODUCED IN ANY FORM WITHOUT THE WRITTEN PERMISSION OF THE PUBLISHER.

Waters, Xevo, ACQUITY, and ACQUITY UPLC are registered trademarks of Waters Corporation.

MassLynx, and The Science of What's Possible are trademarks of Waters Corporation.

All other trademarks are the sole property of their respective owners.

Table of contents

Notice	2
Table of contents	3
Introduction	4
Safety precautions	4
Other operational assistance	4
System overview	5
Important instrument concepts	6
ScanWave™	6
Injection modes	10
Preparing the mobile phase	11
Preparing the samples	11
Opening MassLynx software and creating a project	12
Preparing the ACQUITY UPLC® system	13
Characterizing the needle seal	16
Characterizing the needle and loop volumes	16
Connecting the inlet tubing and equilibrating the system	17
Setting up the ACQUITY UPLC inlet methods	18
Setting up and calibrating the mass spectrometer	20
Developing an MS method	23
Creating the sample list	29
Optimizing the LC/MS method	31
Measuring the peak width and optimizing the dwell times	31
Running an LC/MS system check (QC)	35
Acquiring and processing samples	38
Viewing the sample results	39
Creating the TargetLynx processing method	40
Processing the results	43
Viewing the sample results	44
Creating advanced MS methods	45
Adding additional functions to an MS method	45
Acquiring the sample data for multiple function MS methods	47
Viewing the MS Scan data	48
Viewing the enhanced product ion data	49
Appendix	51
ACQUITY TUV detector	51
ACQUITY PDA detector	51
ACQUITY FLR detector	52
ACQUITY ELS detector	52

Introduction

The guide demonstrates the basic process of daily setup and operation, creating processing methods and data processing.

Once completed, the guide remains with the customer for future reference and training.

Safety precautions

The customer must understand and follow all the necessary safety precautions described in the relevant Waters® Operator's Guides provided with the system, prior to performing this procedure.

Other operational assistance

- MassLynx™ Software Online Help
- Online ACQUITY UPLC® Console Help
- Waters Xevo® TQ Documentation CD (p/n 715002206)
- Waters Educational Services

This guide assumes that the Xevo TQ-S ACQUITY UPLC system has been installed and verified to the appropriate performance level, and the system is ready to be operated. If this is not the case, refer to the Xevo TQ-S and/or ACQUITY UPLC installation documentation.

System overview

The Waters Xevo TQ-S ACQUITY UPLC system is a high performance LC/MS system enabling the rapid acquisition and processing of mass spectral data from chromatographic analyses.

The basic components of the system are:

- Waters Xevo TQ-S mass spectrometer
- Waters ACQUITY UPLC system

Each component of the system may have differences in configuration depending on the options specified during the purchase of the system.

Ensure that the basic familiarization topics have been covered prior to completing this Customer Familiarization Guide. Refer to the appropriate sections of the *Xevo TQ-S ACQUITY UPLC System Installation Checklist* (p/n 715002460).

Important instrument concepts

ScanWave™

The Xevo TQ-S incorporates a uniquely designed collision cell with the option to operate in T-Wave™ enabled mode or in ScanWave™ enabled mode.

ScanWave is an innovative use of T-Wave collision cell technology that significantly enhances full scan spectral performance. In this mode the cell accumulates ions (intact and fragments) and then releases them, according to their mass to charge ratio (m/z), in synchrony with the second quadrupole mass analyzer. This intelligent scheduling of ion release from the collision cell improves duty cycle efficiency and results in spectra with greater ion signal intensities.

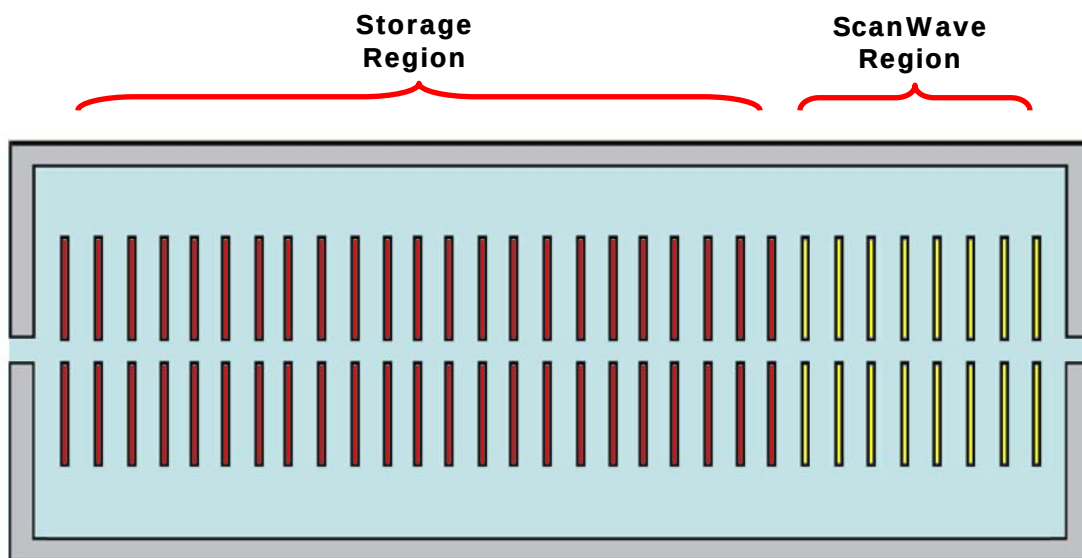


Figure 1 - ScanWave collision cell

Operation of the ScanWave collision cell occurs in three stages:

- Ion accumulation
- Ion transfer
- Ion ejection

Ion accumulation

Ions enter the ScanWave collision cell and encounter a DC barrier at the rear of the storage region. An RF barrier is also applied to the rear of the ScanWave region.

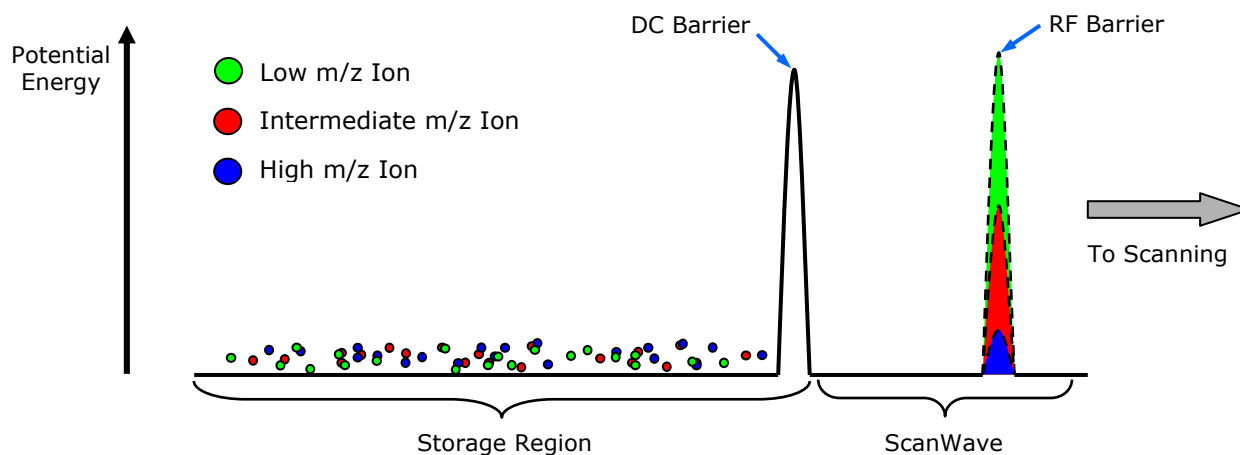


Figure 2 - Initial ion accumulation

The DC barrier is lowered and a T-Wave is applied to the storage region to transfer the ions into the ScanWave region.

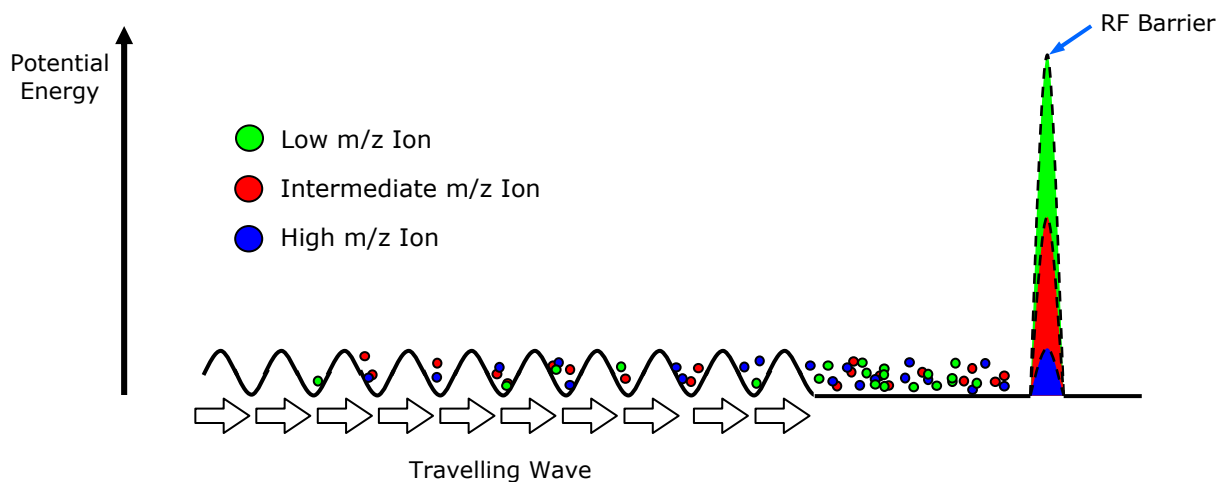


Figure 3 - Ion accumulation in the ScanWave region

Ion transfer

The DC barrier is raised again so that ions are confined in the ScanWave section of the collision cell. Ions entering the collision cell at this time are now accumulated in the storage region.

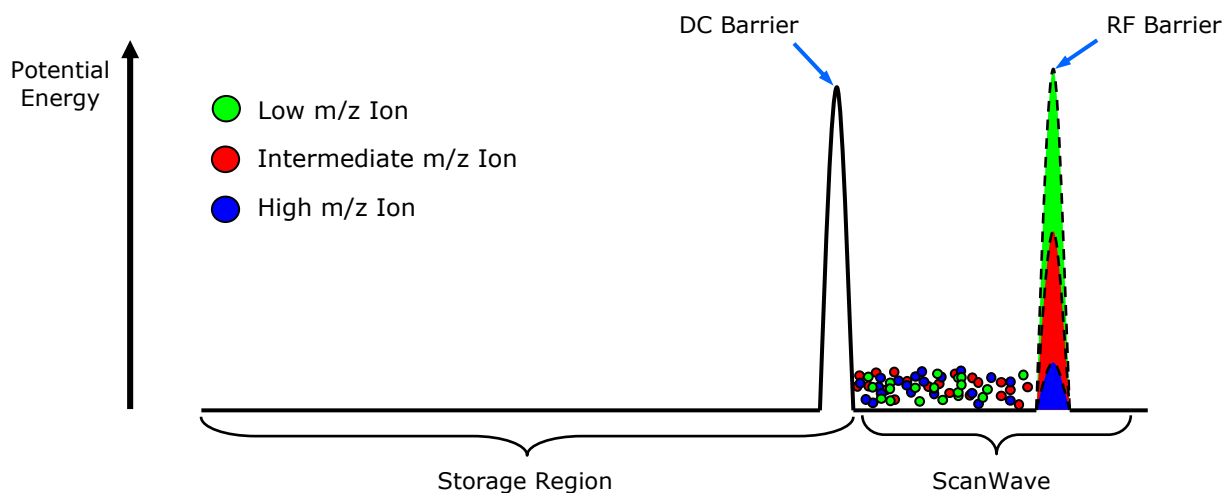


Figure 4 - Ion transfer

Ion ejection

A T-Wave is now applied to the ScanWave region so that ions are forced over the RF barrier into the second quadrupole region. Ions of higher mass to charge ratios exit the ScanWave region before ions of lower mass to charge ratios.

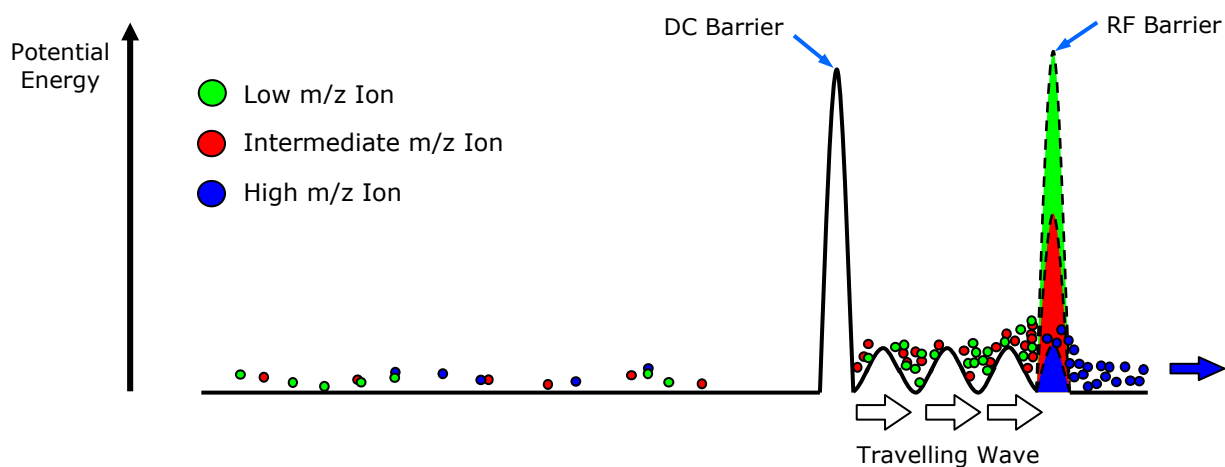


Figure 5 - Ejection of higher m/z ions

The RF barrier is now lowered so that all ions can be ejected from the ScanWave region.

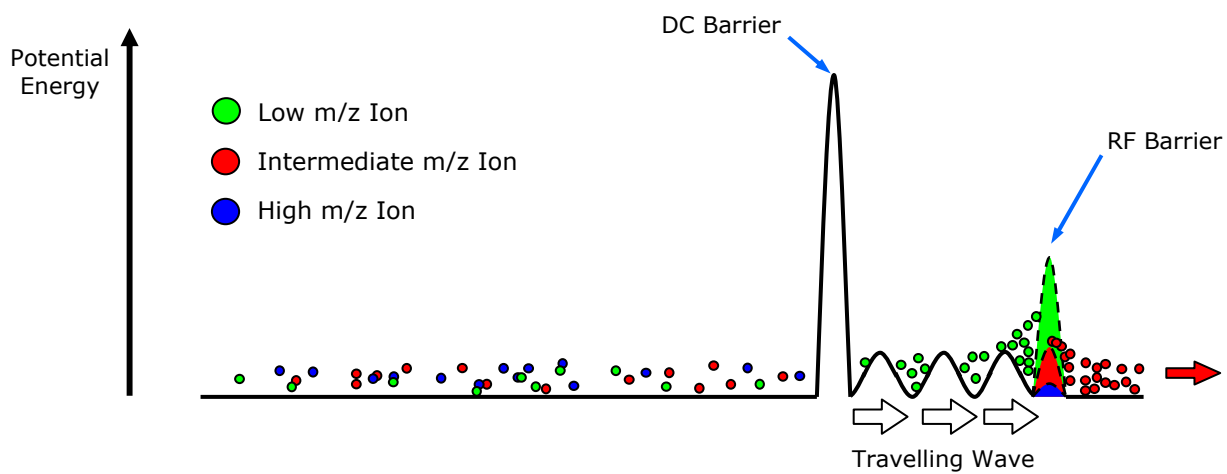


Figure 6 - Ejection of lower m/z ions

The second quadrupole scan is synchronized to the ejection of ions from the ScanWave region of the collision cell and is scanned from high mass to low mass.

This results in enhanced ion detection and increase signal intensities.

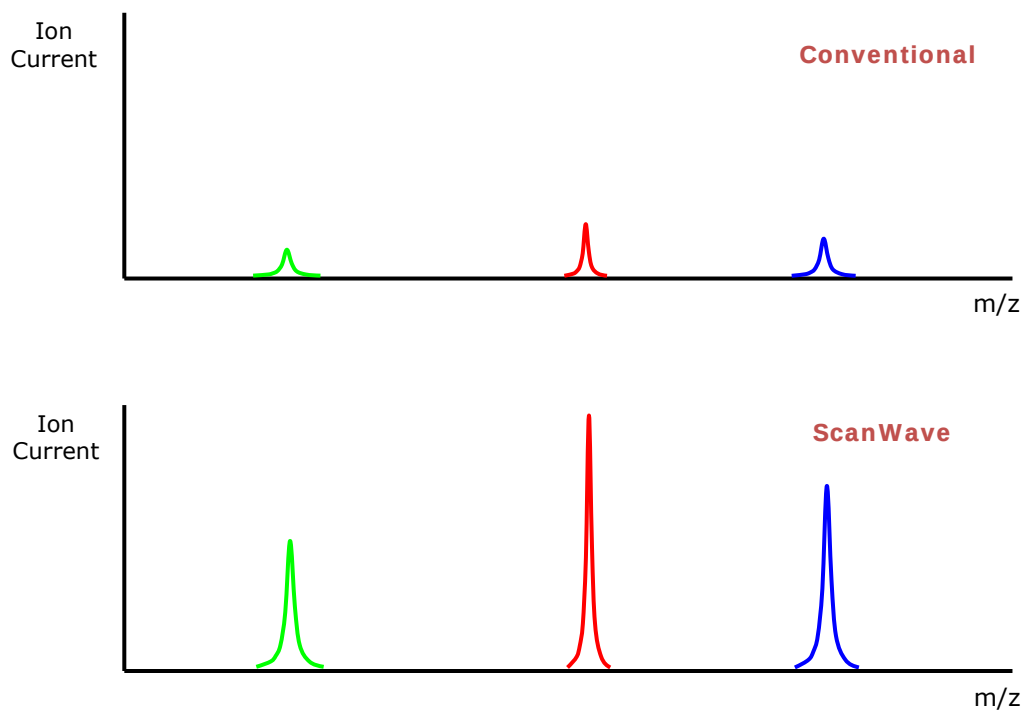


Figure 7 - Conventional versus ScanWave enhanced mass spectra

Injection modes

The sample manager uses a fixed loop injection scheme, and has three modes of injection:

- Full loop for maximum precision and column efficiency for a specific injection volume
- Partial loop for maximum speed and minimum consumption of sample
- Partial loop uses needle overfill (PLNO) for best recovery and efficiency with partial loop injections

NOTE: *All mechanically driven injections result in a dispersal of the sample as a result of viscous flow.*

The volumes of the loops vary slightly, so every loop is "characterized" to determine the actual volume. The sample manager Characterize menu, records the measured loop volume and the nominal loop volumes. Discard the loop volume if it is smaller than it should be.

Full loop mode

The sample loop is overfilled several times, ensuring that the diluted portions of the volume drawn from the sample are not injected, and that the sample loop is filled with a "heart cut" of the sample solution. Because the internal surfaces of the sampling needle and sample loop are thoroughly rinsed with the sample itself, the effects of solute adsorption and solvent exchange with the needle wash and mobile phase are reduced.

NOTE: *The default loop overfill factor in this mode specifies the excess volume of sample drawn when you select the full loop option. For example, for a 2 μL loop, 11.2 μL of sample is used per 2 μL full loop injection. For a 10 μL loop, 40 μL of sample is used per 10 μL full loop injection. Smaller loops are harder to fill and so require larger overfill factors.*

Partial loop mode

This is best used for applications where the volume of sample is limited or when reduced cycle time is critical. In this mode the sample is bracketed with air gaps and the weak wash solvent. The weak wash solvent must match the mobile phase in order to ensure that the solutes do not begin to elute in the weak wash upon injection. This can lead to peak distortion and peak splitting in extreme cases. This mode is best used for larger volumes e.g. 20, 50 μL .

Partial loop uses needle overfill (PLUNO)

This is the default injection mode. An air gap is drawn to segregate the sample volume from the weak wash solvent, and then the requested sample volume plus an additional 3 μL are drawn followed by air. The sample volume plus 2 μL are positioned on the syringe side of the sample loop with the valve in the inject position. When the sampling valve is rotated to the load position, the requested sample volume is metered into the sample loop. Upon injection, the valve brings the sample loop on line and the sample volume is pumped onto the column. The air gap and the extra 3 μL ensure that the sample needle is rinsed with the sample and reduce the amount of analyte lost by absorption. PLUNO is best used with low volumes e.g. 2, 5, 10 μL .

NOTE: *The best default choice for the PLUNO needle overfill flush volume in ACQUITY UPLC System Driver version 1.30 is 15 μL . Use the Advanced Settings option in the ACQUITY Sample Manager window to adjust the volume as required.*

Preparing the mobile phase

1. Prepare the following mobile phases:
 - Mobile Phase A1 Water with 0.01% formic acid and 0.05% ammonia solution
 - Mobile Phase B1 Acetonitrile with 0.01% formic acid
 - Weak Wash 10:90 acetonitrile:water with 0.01% formic acid
 - Strong Wash Acetonitrile with 0.01% formic acid (use mobile phase B1)
 - Seal Wash 10:90 acetonitrile:water with 0.01% formic acid
(also used as diluent and weak wash)
2. Place the ACQUITY solvent lines in the correct bottle:
 - a. Place the A1 solvent lines into the mobile phase A1 reservoir.
 - b. Place the B1 solvent line into the mobile phase B1 reservoir.
 - c. Place the weak and strong wash solvent lines into their respective reservoirs.
 - d. Place the seal wash solvent line into the seal wash reservoir.

Preparing the samples

1. Prepare the following samples in 2-mL UPLC vials:

Table 1: Test samples

Sample	Content
Test Sample 1	0.1 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water Add 100 μ L of the 1 pg/ μ L solution to 900 μ L of diluents
Test Sample 2	0.2 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water Add 200 μ L of the 1 pg/ μ L solution to 800 μ L of diluents
Test Sample 3	0.3 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water Add 400 μ L of the 1 pg/ μ L solution to 600 μ L of diluents
Test Sample 4	0.4 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water Add 600 μ L of the 1 pg/ μ L solution to 400 μ L of diluents
Test Sample 5	0.8 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water Add 800 μ L of the 1 pg/ μ L solution to 200 μ L of diluents
Tuning Sample	5 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water
Working Stock	1 pg/ μ L sulfadimethoxine in 10:90 acetonitrile:water
Calibration	Sodium cesium iodide solution

Opening MassLynx software and creating a project

1. Double-click the **MassLynx** icon .
2. Click **File > Project Wizard**.
3. Click **Yes** to the prompt "When changing to a new project, some services are automatically closed down. Continue?".
4. Input the Project name as **TRAINING** and the description as **Customer Familiarization**, then click **Next**.

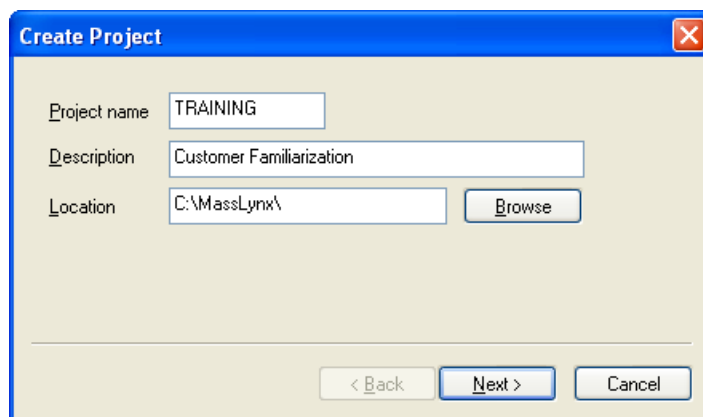
The 'Create Project' dialog box is shown. It has a blue title bar with the text 'Create Project' and a close button. The main area is light beige. It contains three text input fields: 'Project name' with the text 'TRAINING', 'Description' with the text 'Customer Familiarization', and 'Location' with the text 'C:\MassLynx\'. To the right of the 'Location' field is a 'Browse' button. At the bottom of the dialog are three buttons: '< Back', 'Next >', and 'Cancel'.

Figure 8 - Create Project window

5. Select the **Create using existing project as template** check box, and ensure that **C:\MassLynx\DEFAULT.PRO** is the existing project, then click **Finish**.

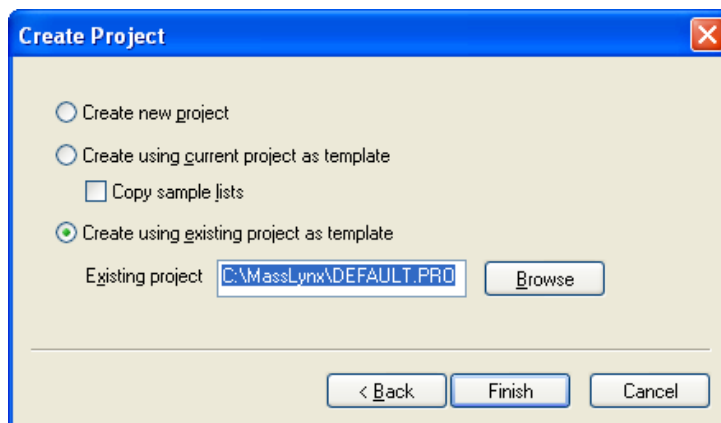
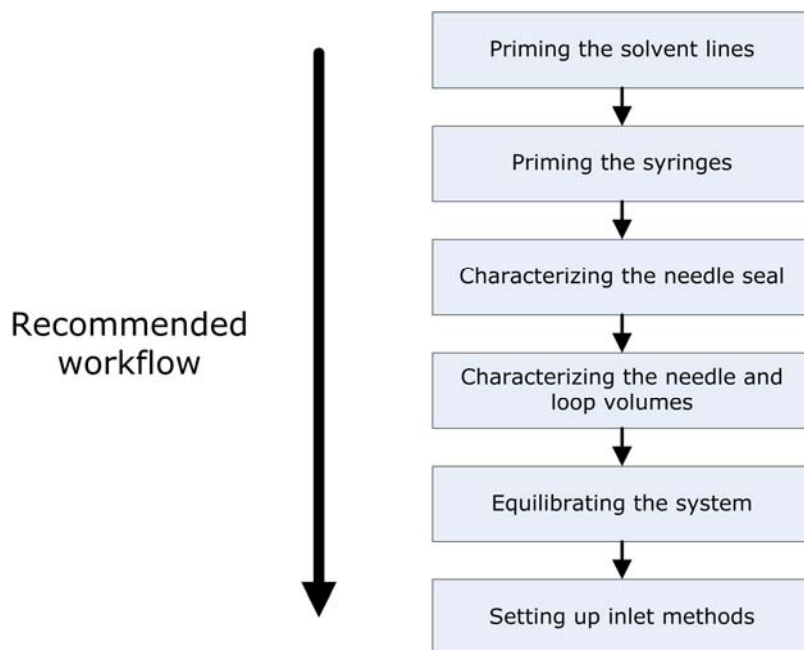
The 'Create Project' dialog box is shown. It has a blue title bar with the text 'Create Project' and a close button. The main area is light beige. It contains three radio buttons: 'Create new project', 'Create using current project as template', and 'Create using existing project as template'. The third option is selected. Below the radio buttons is a checkbox labeled 'Copy sample lists'. Below the checkbox is a text input field labeled 'Existing project' with the text 'C:\MassLynx\DEFAULT.PRO'. To the right of the 'Existing project' field is a 'Browse' button. At the bottom of the dialog are three buttons: '< Back', 'Finish', and 'Cancel'.

Figure 9 - Create Project window

NOTE: The MassLynx project structure is described in the "Understanding Project Structure" section of the MassLynx software online help.

Preparing the ACQUITY UPLC® system

The following section describes how to set up the ACQUITY UPLC system.



1. In the MassLynx software, click the **Inlet Method** icon  to open the inlet method editor.
2. Click the **ACQUITY Additional Status** tab to display the status of the ACQUITY modules.

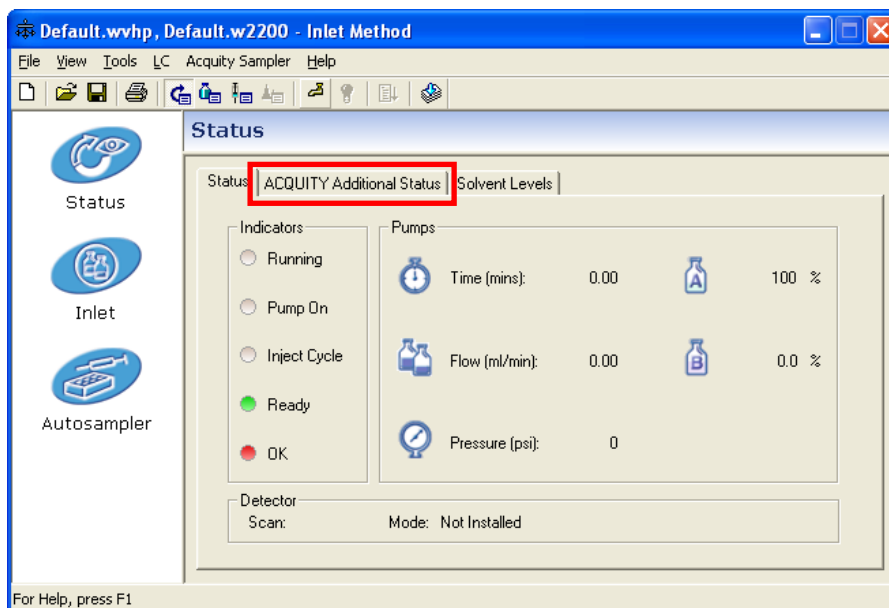


Figure 10 - Inlet Method editor

3. Click the **ACQUITY UPLC Console** icon  to display the ACQUITY UPLC Console.

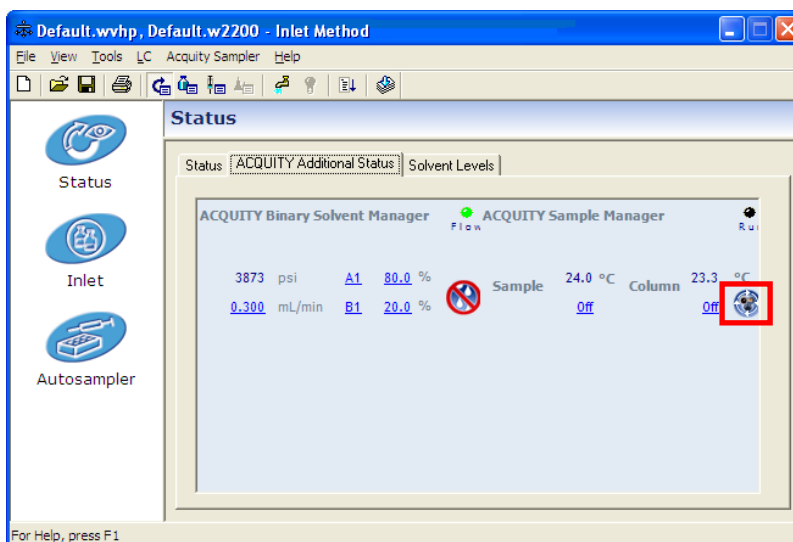


Figure 11 - ACQUITY Additional Status window

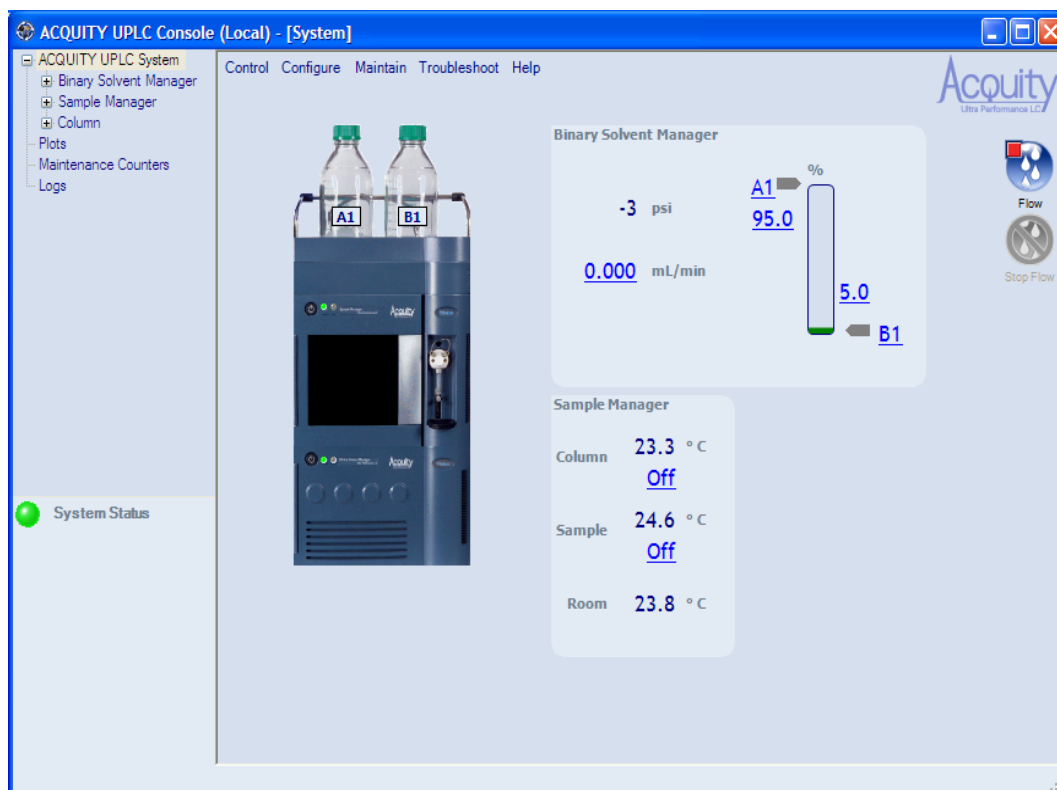


Figure 12 - ACQUITY UPLC Console

4. **Prime** both the **A1** and **B1** solvent lines of the binary solvent manager:
 - a. Click **Control > Prime solvents...** in the binary solvent manager Console window.
 - b. Select both **A1** and **B1** and specify a time of **5 minutes**.
 - c. Click **Start** to begin the solvent line prime.

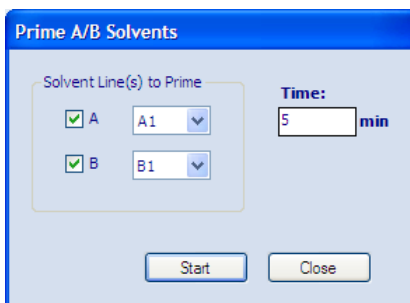


Figure 13 - Priming the solvent lines

NOTE: This process can be simplified by using the System Startup and Refresh System functions. Previous settings are remembered and can be automated to walk the user through the procedure.

5. Prime the binary solvent manager seal wash lines:
 - a. Click **Control > Prime seal wash...** in the binary solvent manager Console window.
 - b. Click **Yes** to begin the seal wash prime.



Figure 14 - Priming the seal wash

6. Prime the sample manager syringes:
 - a. Click **Control > Prime syringes**.
 - b. Select the **Sample syringe and wash syringes** check box.
 - c. Specify a minimum of **ten** cycles.
 - d. Click **OK** to begin the prime.

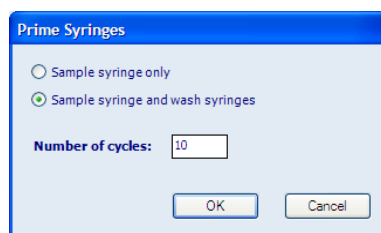


Figure 15 - Priming the syringes

Characterizing the needle seal

1. Click **Maintain > Characterize > Needle seal...** in the Sample Manager Console window to perform the needle seal calibration procedure.

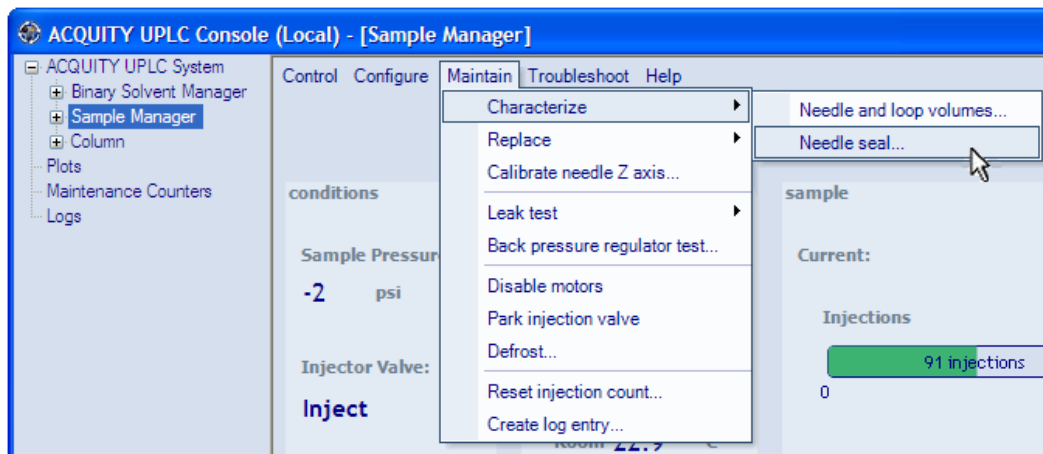


Figure 16 - Characterizing the needle seal

2. Ensure the procedure passes successfully.

Characterizing the needle and loop volumes

1. In the Sample Manager Console window, click **Maintain > Characterize > Needle and Loop Volumes...**, to perform the system volume calibration procedure.
2. Ensure the procedure passes successfully.

NOTE: Characterize the system volume whenever there is a change to the mobile phase, wash solvents, sample loop, needle, or any of the syringes.

This process can be simplified by using the System Startup and Refresh System functions. Previous settings are remembered and can be automated to walk the user through the procedure.

Connecting the inlet tubing and equilibrating the system

1. Ensure the ACQUITY UPLC BEH C₁₈ (2.1 x 50-mm, 1.7- μ m) column is connected and placed in the column heater or column manager (if installed).
2. Check that the flow path indicator on the column is correct for the direction of flow.

CAUTION: *The direction of flow is very important. If the column is installed in the wrong direction, the packing material will become disturbed, resulting in loss of efficiency and possible damage to the mass spectrometer and/or optical detector.*

3. Place the ACQUITY UPLC outlet tubing into a suitable waste container.
4. In the BSM window of the ACQUITY UPLC console, click **Control > Set Flow....**
5. Set the initial mobile phase flow conditions as shown in Figure 17.

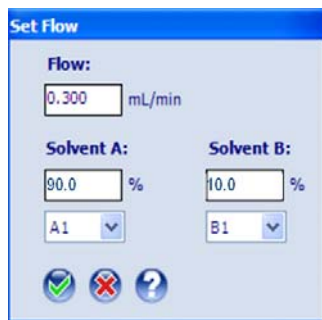


Figure 17 - Setting the initial flow rate

6. Ensure the inlet editor status LEDs for Pump ON and OK turn green.

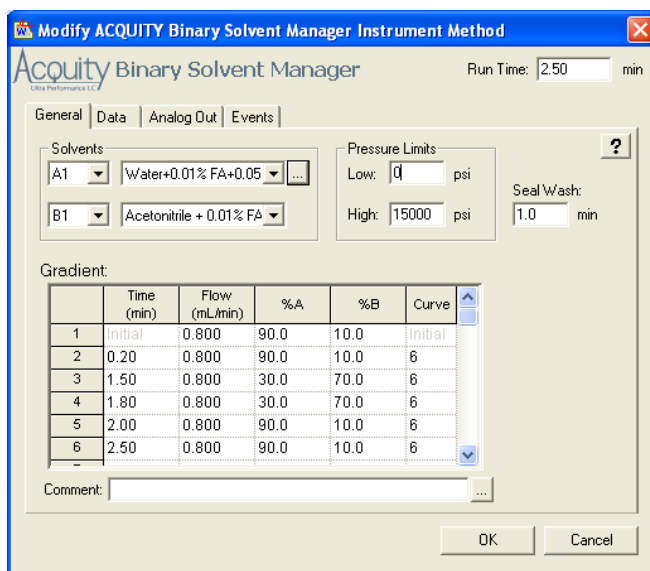
NOTE: *If the LEDs do not turn green, refer to the ACQUITY UPLC System Operator's Guide (p/n 71500082502).*

7. Equilibrate the column until the BSM system pressure delta value is ≤ 20 psi.
8. Place a Blank solution into position 1:A,1 of the sample manager.
9. Place Test Sample 1 into position 1:A,2 of the sample manager.
10. Place Test Sample 2 into position 1:A,3 of the sample manager.
11. Place Test Sample 3 into position 1:A,4 of the sample manager.
12. Place Test Sample 4 into position 1:A,5 of the sample manager.
13. Place Test Sample 5 into position 1:A,6 of the sample manager.

Setting up the ACQUITY UPLC inlet methods

1. In the inlet method editor, click the **Inlet** icon  to open the binary solvent manager method editor.
2. Create an inlet method for the familiarization tests with the settings shown in Figure 18.

NOTE: For further details on the ACQUITY UPLC inlet methods, gradient curves, and method optimization, refer to the ACQUITY UPLC system documentation and the ACQUITY UPLC online help.



Modify ACQUITY Binary Solvent Manager Instrument Method

Acquity Binary Solvent Manager

Run Time: 2.50 min

General | Data | Analog Out | Events

Solvents:

A1: Water+0.01% FA+0.05%

B1: Acetonitrile + 0.01% FA

Pressure Limits:

Low: 0 psi

High: 15000 psi

Seal Wash: 1.0 min

Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.800	90.0	10.0	Initial
2	0.20	0.800	90.0	10.0	6
3	1.50	0.800	30.0	70.0	6
4	1.80	0.800	30.0	70.0	6
5	2.00	0.800	90.0	10.0	6
6	2.50	0.800	90.0	10.0	6

Comment:

OK Cancel

Figure 18 - Binary solvent manager settings for customer familiarization

3. If required, click the **Browse** icon  to enter custom mobile phase names.
4. Click the **Autosampler** icon  to open the sample manager method editor.

5. Create an autosampler method for the familiarization tests with the settings shown in Figure 19.

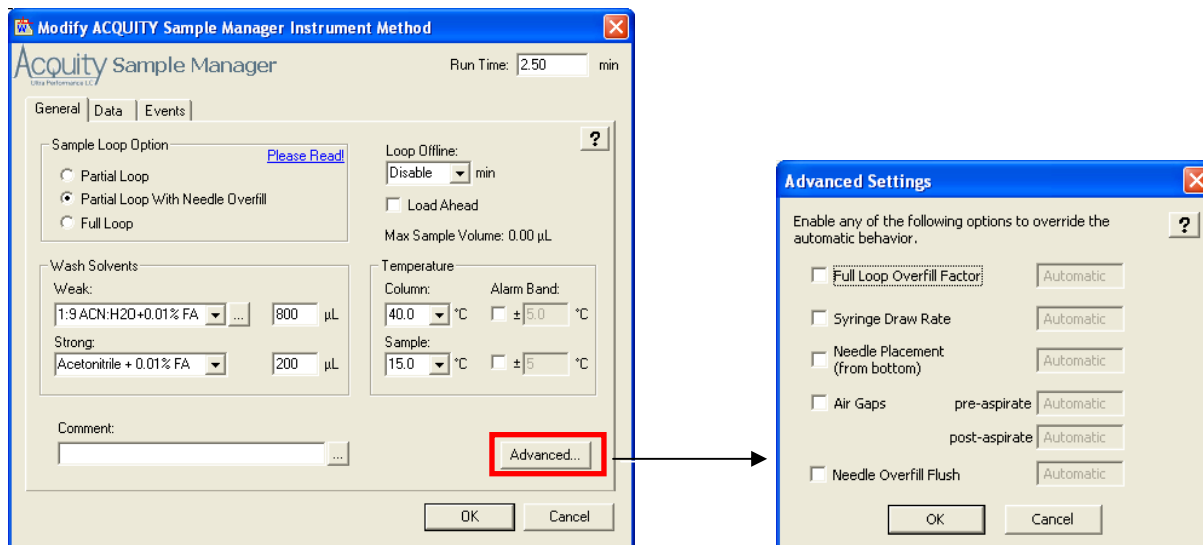


Figure 19 - Sample manager settings

NOTE: The sample manager advanced settings may be customized if required. For example, samples in more viscous matrices may require slower syringe draw rates.

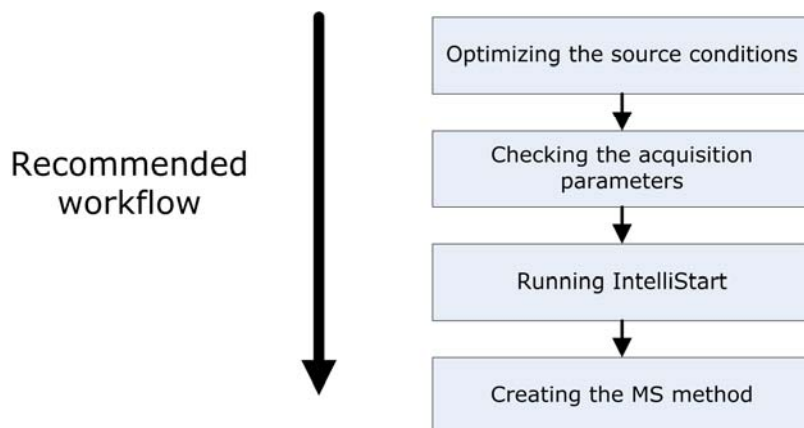
6. If using the ACQUITY UPLC column manager, select the appropriate column number in the **Column Manager** tab of the sample manager method editor.
7. If the ACQUITY UPLC system contains additional detectors, set up any additional ACQUITY UPLC detector methods as appropriate:
 - a. In the inlet method editor, click the appropriate detector icon, for example,  or , to open the detector method editor.
 - b. Configure the detector method as required. See Appendix for example detector method.

NOTE: Ensure that all fluidic connections are correct for a multi-detector system.

8. Click **File > Save As** in the inlet editor, and save the inlet method as **Gradient_DDMMYY**.
9. Click the **Load Method** icon  in the inlet editor to load the inlet method to the ACQUITY UPLC system.
10. Allow the column and sample temperatures to equilibrate.

Setting up and calibrating the mass spectrometer

The following section describes how to set up the Xevo TQ-S mass spectrometer.



1. In MassLynx, click the **MS Console** icon  .

2. Ensure the mass spectrometer is in Operate. If not, click the **Operate** icon  to place the instrument into Operate.

3. Click the **API Gas** icon  to switch on the API gas flow.

4. Click the **Collision Gas** icon  to switch on the collision gas flow.

5. Dilute the original solution of IntelliStart resolution solution, provided with the Waters Xevo TQ-S Sample Kit (p/n 700005472) by a factor of 20 in 50:50 IPA:water.
6. Install a bottle of Intellistart resolution solution, sodium iodide/cesium iodide/methyl sulfate sodium salt calibration solution to fluidics reservoir C.
7. Install the bottle of calibration solution (containing phosphoric acid) to fluidics reservoir B.

8. In the Interactive Fluidics page, click the **Control** icon  .
9. Select reservoir **C** and ensure the purge procedure begins. On completion of this purge process choose reservoir **B** to ensure purge procedure is begins and completes on this channel also.
10. Once the purges have completed, select the **Instrument Resolution, Positive, Negative, and Instrument Calibration** check boxes as shown in Figure 20.

NOTE: *The instrument resolution and calibration options may be hidden if they have been previously completed. To view the options, click **Show Instrument set-up options**.*

11. Ensure that the **Pre-checks** check box is clear.



12. Click the **Start** icon.

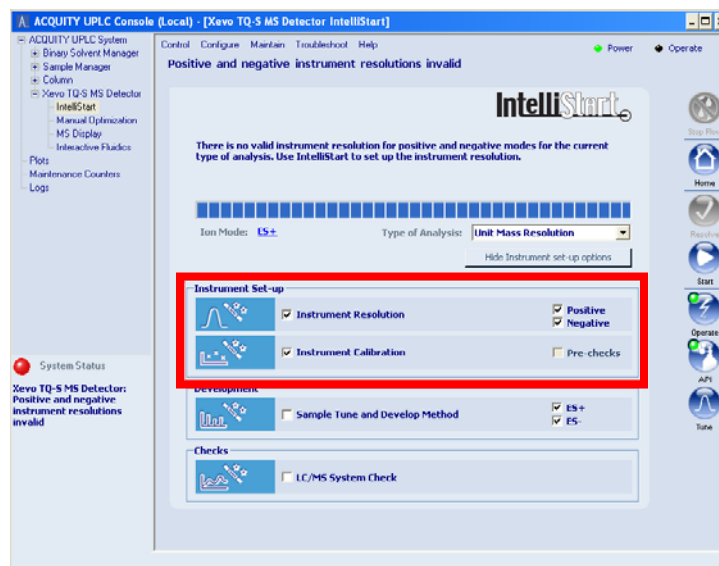


Figure 20 - Specifying the initial IntelliStart parameters

13. In the IntelliStart Setup Parameters dialog, click the **Set instrument up for** drop-down list and select **Unit Mass Resolution**.

14. Click the **Resolution Reservoir** drop-down list and select **C** as shown in Figure 21.

15. Click the **Calibration Reservoir** drop-down list and select **B**.

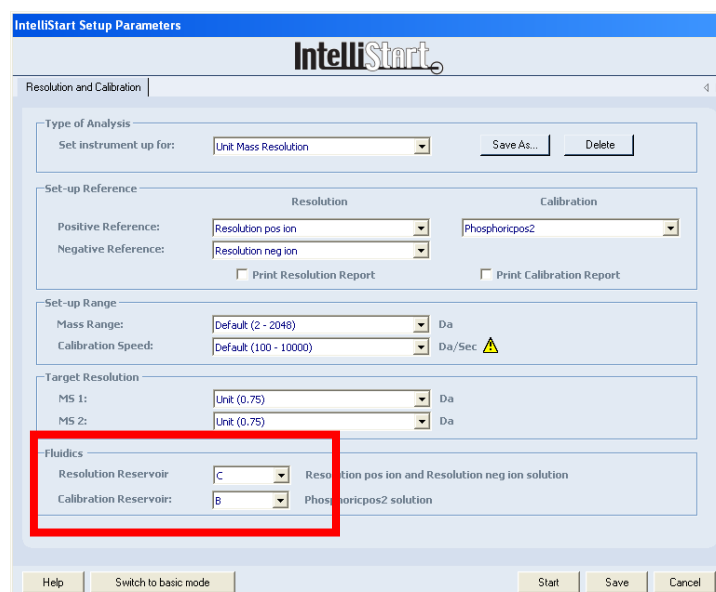


Figure 21 - Specifying the IntelliStart parameters

CAUTION: If no printer is installed, ensure that the **Print Calibration Report** check box is not selected.

16. Click **Start** to begin the IntelliStart optimization.

17. Ensure that the procedure has completed successfully, then check that the IntelliStart status for the **Instrument Resolution** and **Instrument Calibration** options are selected.

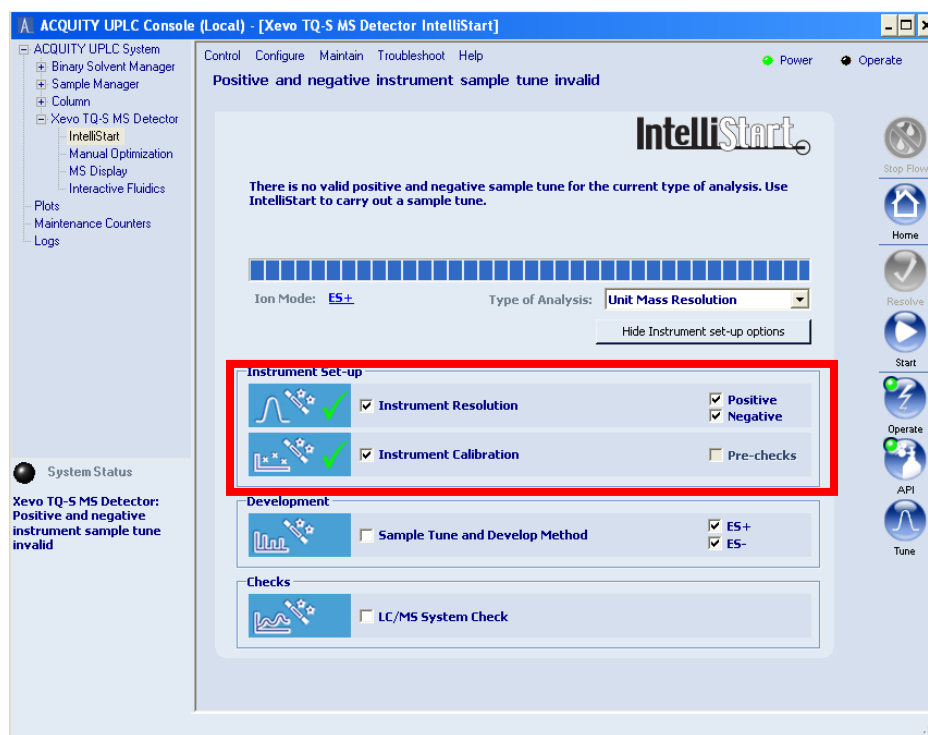


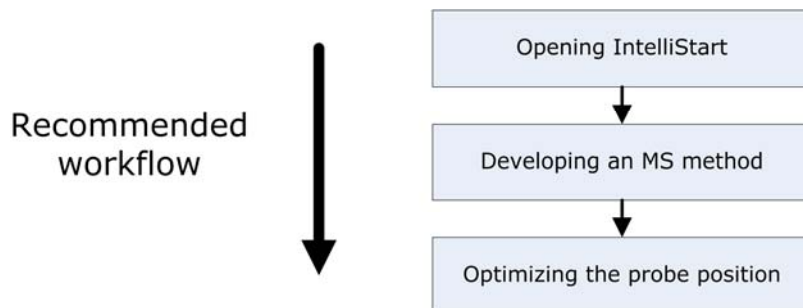
Figure 22 - Successful IntelliStart results

NOTE: The calibration report is printed automatically by IntelliStart.

Once the initial setup options are completed, fields can be hidden by clicking **Hide Instrument set-up options**.

Developing an MS method

The Xevo TQ-S IntelliStart software enables you to develop LC/MS methods automatically.



1. Ensure the ACQUITY UPLC is set to deliver 50:50 water:acetonitrile at 0.8 mL/min and that the LC flow is on.
2. Set the initial 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 5 on the 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. Set the capillary position so that approximately 0.5 mm is visible at the probe tip.
3. Install the bottle containing 5 pg/μL sulfadimethoxine on fluidics reservoir B.
4. In the Interactive Fluidics page, click the **Control** icon .
5. Ensure reservoir **B** and **Combined Flow** mode check boxes are selected, and the purge procedure is completed.
6. In the MS Console, select the **Xevo TQ-S** page and ensure that the instrument is in Operate, with the API and collision gas flows turned on.
7. Select the **IntelliStart** menu, then click the **Type of Analysis:** drop-down list and select **Unit Mass Resolution**.
8. If not already done so, ensure that the instrument resolution and calibration using IntelliStart procedures have been completed.

9. Select the **Sample Tune and Develop Method** check box and ensure that both **ES+** and **ES-** check boxes are selected as shown in Figure 23.

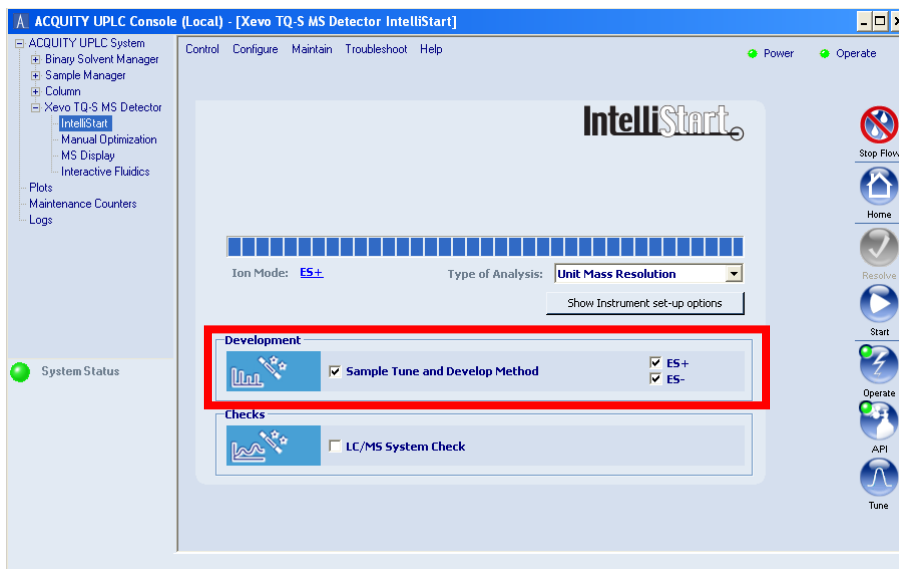


Figure 23 - The sample tune and development method page

10. Click the **Start**  icon.

11. Click **Switch to Advanced Mode** and set the parameters as shown in Figure 24.

Compound Name	Molecular Mass/Formula	Adduct A+	Adduct B+	Adduct A-	Adduct B-
☒ sulfadimetoxine	310.08	[M+H] ⁺			
☒ Chloramphenicol	322.00			[M-H] ⁻	
☐					
☐					

☐ Multiply Charged Parents

Method Details

☒ Create New Sample Tune ☐ Load Existing Sample Tune

Sample Tune Name:

☐ Develop SIR method:

☒ Develop MRM method:

Save Report As:

☐ Invoke Manual Optimisation

☐ Export To LC/MS System Check

☐ Append to existing methods

☒ Print Report

Optimization Ranges

Cone Voltage: V

Collision Energy: eV

Daughter ion settings

Number of MRM transitions per compound:

Lowest Fragment Ion Mass: Da

Fluidics

Flow Path:

Sample Reservoir:

Sample Flow Rate:

Figure 24 - IntelliStart method development parameters

12. Click **Start** to begin the IntelliStart optimization and ensure that the procedure completes successfully.

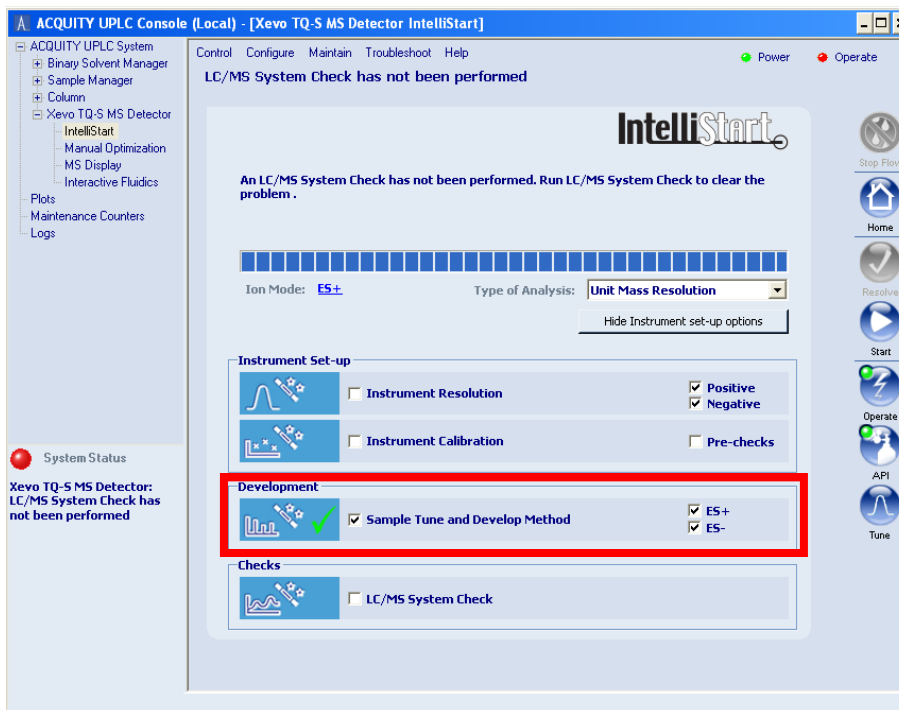


Figure 25 - Completed sample tune and developed method

13. The IntelliStart method development report is displayed.

NOTE: IntelliStart automatically determines an MRM transition for sulfadimethoxine and will optimize the MSMS parameters to create an MS method file.

For further information, refer to the "Tuning and developing methods automatically" section of the Xevo TQ-S help menu.

14. From the MassLynx sample list page, click the **MS Method** icon  to open the MS method editor.

15. Click **File > Open** and browse to the **ESI_Sens_Test_DDMMYY.exp** MS method file created previously.

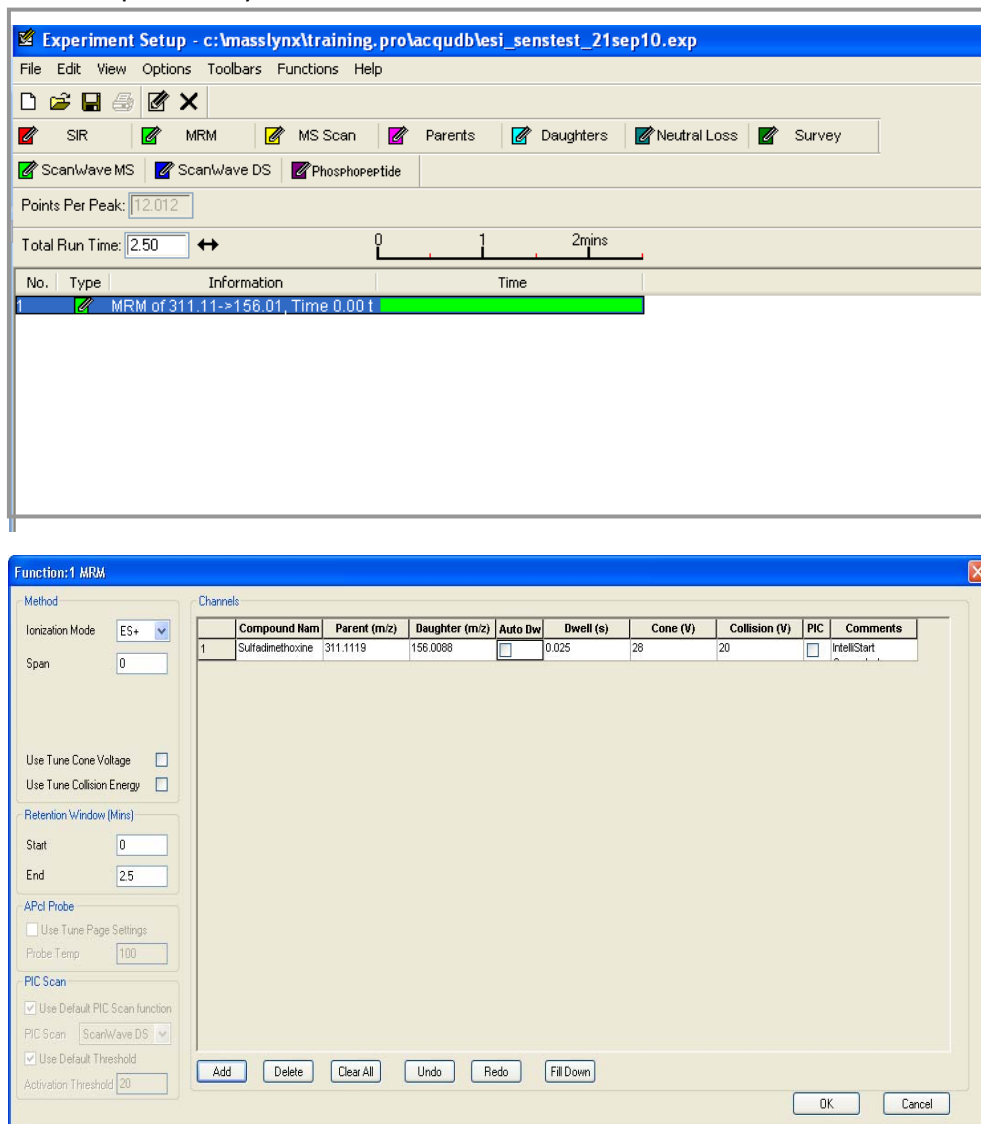


Figure 26 - Example of MS method for sulfadimethoxine, created by IntelliStart

16. Ensure that the 311 >156 Da in ES+ transition for sulfadimethoxine has been specified.

NOTE: The exact masses may be different to that shown in Figure 26.

17. Set the analysis end time to 2.5 minutes.
18. Click **OK** to return to the MS method editor.
19. Click **Options > Method Events** and select the parameters as shown in Figure 27, then click **OK**.

Time / Mins	Event	Action
0.00	Stop flow	On

Initial Settings

Stop flow: No Change

Switch 2: No Change

Switch 3: No Change

Switch 4: No Change

Infusion: No Change

Flow State: LC

Flow Rate µl/min: 5

Reservoir: No Action

Refill: No Action

Volume µl: 250

Solvent Delay Options

API Probe Temperature °C: 20

Enable ☒

OK Cancel

Figure 27 - Specifying the Method events parameters

20. Optimize the capillary voltage and probe position:

- a. In the MS console, click **Manual Optimization** to access the Manual Optimization dialog (Figure 28).

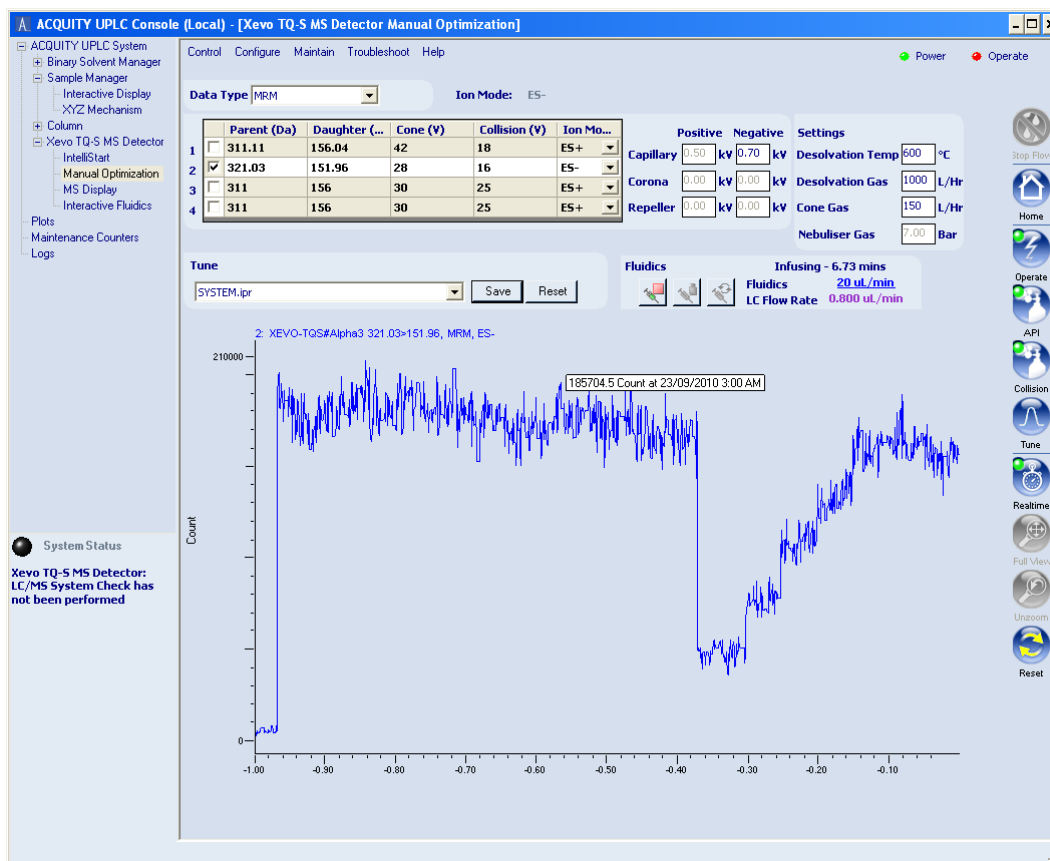


Figure 28 - Manual Optimization dialog

- b. Ensure that the tune file **System.ipr** is selected.
 - c. Ensure that the 311 >156 Da MRM transition is being monitored.
 - d. Optimize the capillary voltage for maximum intensity and stability.
 - e. Optimize the probe position for maximum intensity.
21. Minimize any thermal ionization:
- a. Set the capillary voltage to **zero**.
 - b. Ensure that any residual signal observed from thermal ionization is <10% of the optimized signal intensity.
 - c. If not, adjust the ESI probe position away from the sample cone and re-optimize the capillary voltage for the new probe position.
22. In the Manual Optimization IntelliStart window, click **Save** to save the tune file using the name **System.ipr**.

Creating the sample list

Before the sample list is created, the vial location format must be set.

1. Open the Inlet Method Editor.
2. Click **Acquity sampler > Plate Generator** to open the rack editor.
3. Continue to click the **Previous Rack** icon until **ANSI-48Vial2mLHolder** appears in the Rack Name field.
4. Enter the parameters as in shown in Figure 29.
5. Click **Rack > Save Current Rack**, which saves the vial location format.

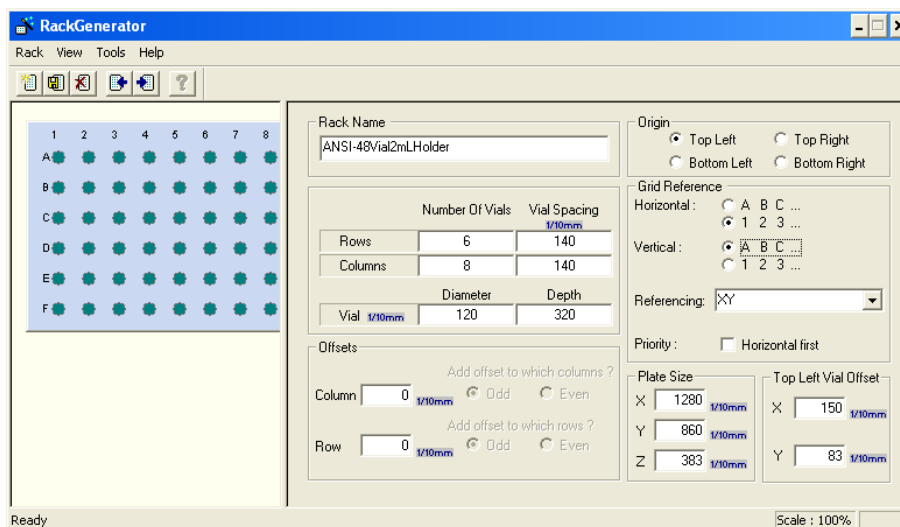


Figure 29 - Rack generator settings (ANSI-48Vial2mLHolder)

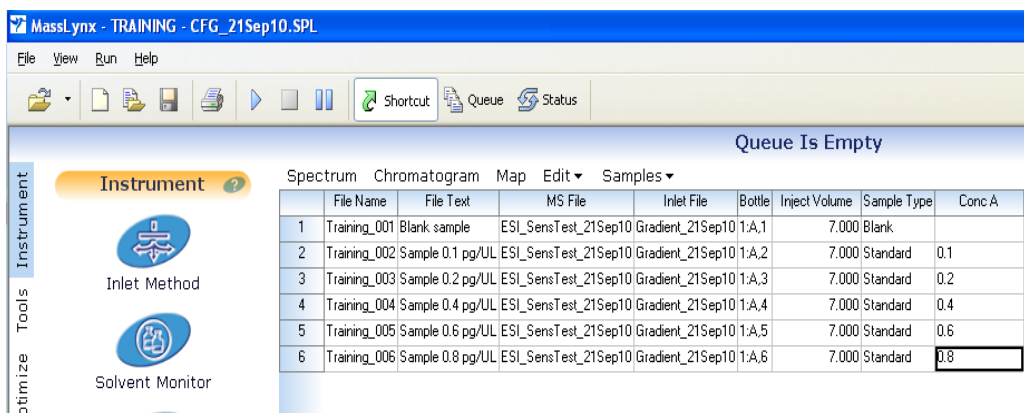
The sample list may now be created.

1. Click **File > New** on the MassLynx sample list, to create a new blank sample list.
2. Click **Samples > Format > Load** and browse to the **Quantify.fmt** sample list format file, then click **OK**.
3. Double-click in the **File Name** column of row 1, and type the filename as **Blank 1**.
4. Double-click in the **File Text** column of the same sample, and set the file text as **Blank Injection**.
5. Right-click in the **MS File** column, click **Browse** and select the **ESI_Sens_Test_DDMMYY.exp** created previously.
6. Right-click in the **Inlet File** column, click **Browse** and select the **Gradient_DDMMYY** inlet method created previously.
7. Set the vial position as **1:A,1** in the Bottle column.
8. Set **7.0 µL** in the Inject Volume column.
9. Right-click in **row 1** of the sample list and select **Add**, specify a value of **5**, then click **OK**.

10. Fill in all the relevant fields, to create a sample list as shown in Figure 30.

NOTE: To add additional columns to the sample list, click **Samples > Format > Customize** and select the appropriate options.

11. Save the sample list as **CFG_DDMMYY.spl**, where DDMMYY = date.



MassLynx - TRAINING - CFG_21Sep10.SPL

File View Run Help

Queue Is Empty

Spectrum Chromatogram Map Edit Samples

	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Sample Type	Conc A
1	Training_001	Blank sample	ESI_SensTest_21Sep10	Gradient_21Sep10	1:A,1	7.000	Blank	
2	Training_002	Sample 0.1 pg/UL	ESI_SensTest_21Sep10	Gradient_21Sep10	1:A,2	7.000	Standard	0.1
3	Training_003	Sample 0.2 pg/UL	ESI_SensTest_21Sep10	Gradient_21Sep10	1:A,3	7.000	Standard	0.2
4	Training_004	Sample 0.4 pg/UL	ESI_SensTest_21Sep10	Gradient_21Sep10	1:A,4	7.000	Standard	0.4
5	Training_005	Sample 0.6 pg/UL	ESI_SensTest_21Sep10	Gradient_21Sep10	1:A,5	7.000	Standard	0.6
6	Training_006	Sample 0.8 pg/UL	ESI_SensTest_21Sep10	Gradient_21Sep10	1:A,6	7.000	Standard	0.8

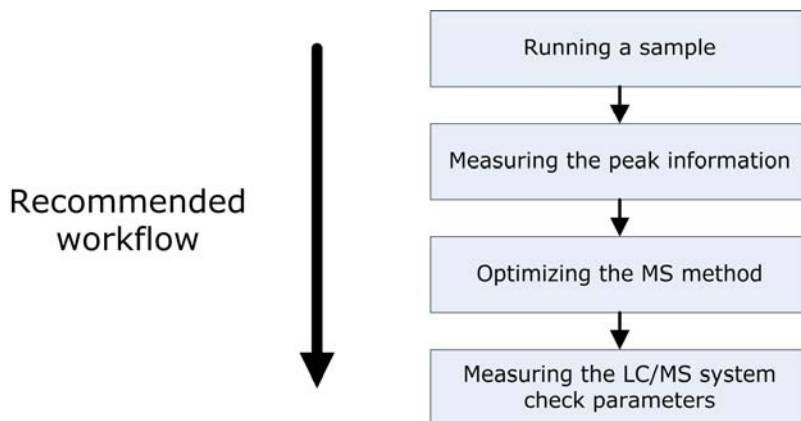
Figure 30 - Example sample list

Optimizing the LC/MS method

Once a new LC/MS method has been created via IntelliStart, it is recommended to run a sample to verify that the parameters are appropriate for the analysis and to determine peak information for processing methods.

Optimizing the LC/MS method will verify:

- The MS method parameters are correct e.g. the MRM transition and daughter ions are as expected
- The chromatographic retention time and peak width for use in data processing



1. Highlight samples **1** to **2**, click **Run > Start** and select **Acquire Sample Data only**, then click **OK** to perform the analysis.
2. To view the chromatogram data during the acquisition:
 - a. Open the **Chromatogram browser** window.
 - b. Click the **Real-time Update**  icon.
 - c. Click the **Maximize**  icon if the whole of the TIC is not visible.
3. Ensure that the sulfadimethoxine peak elutes and is visible.

Measuring the peak width and optimizing the dwell times

1. In the Chromatogram browser, click **Display > Mass** and double-click on the **311 > 156** transition in the channel list.

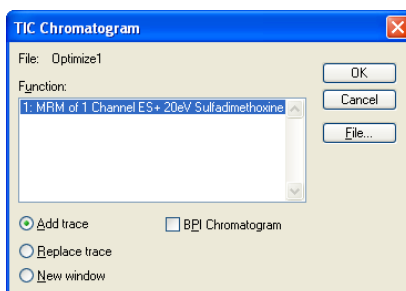


Figure 31 - Displaying the mass chromatogram

2. Select the **Replace trace** check box, then click **OK**.
3. Click and drag across the peak to zoom the view.

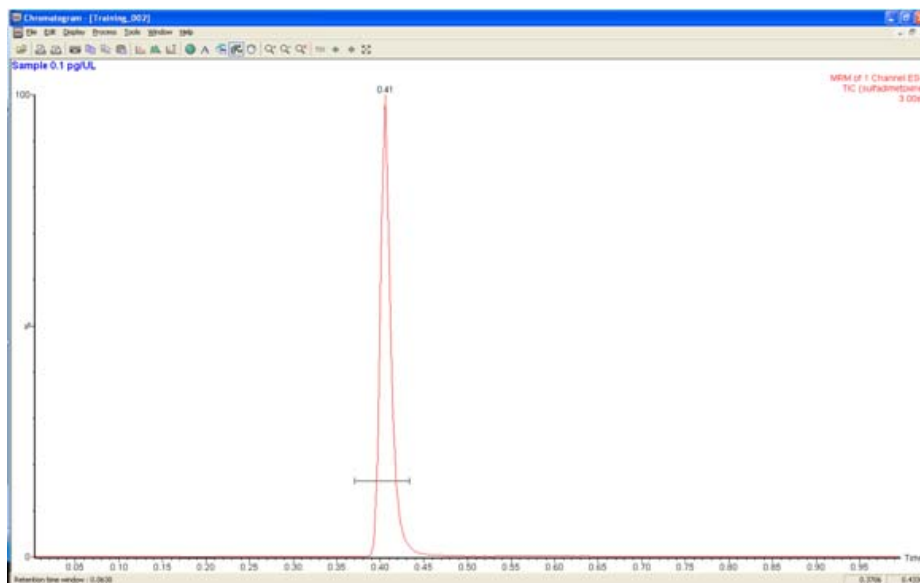


Figure 32 - Zooming the view

4. Click and drag at the base of the peak and record the peak width.

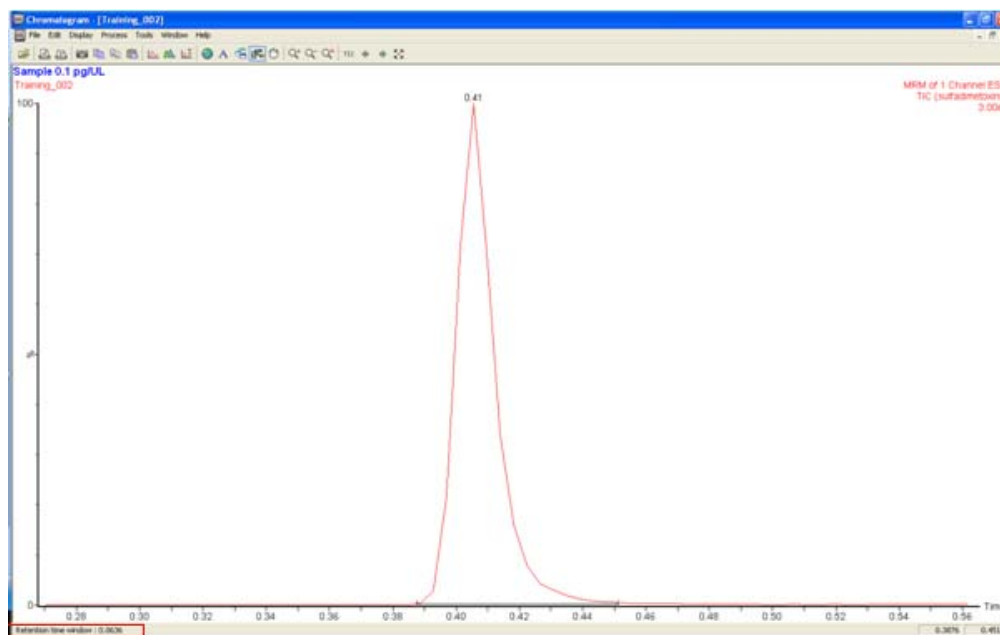


Figure 33 - Measuring the peak width

5. In the MS method editor, click **File > Open** and browse to the **ESI_Sens_Test_DDMMYY.exp** file created previously.
6. Click **Options > Points per peak**.
7. Specify the appropriate peak width value (in seconds) as measured previously in step 4.

NOTE: For example, if the measured peak width was 0.1 minutes, specify a value of 6 seconds.

8. Ensure that the calculated points per peak value is greater than 12.

NOTE: The optimum number of data points across each LC peak, is 18, however, the recommended value is between 15 to 20 points.

9. Double-click the MRM function of the MS method.
10. Select the **Auto Dwell** check box in the function editor, then click **OK**.

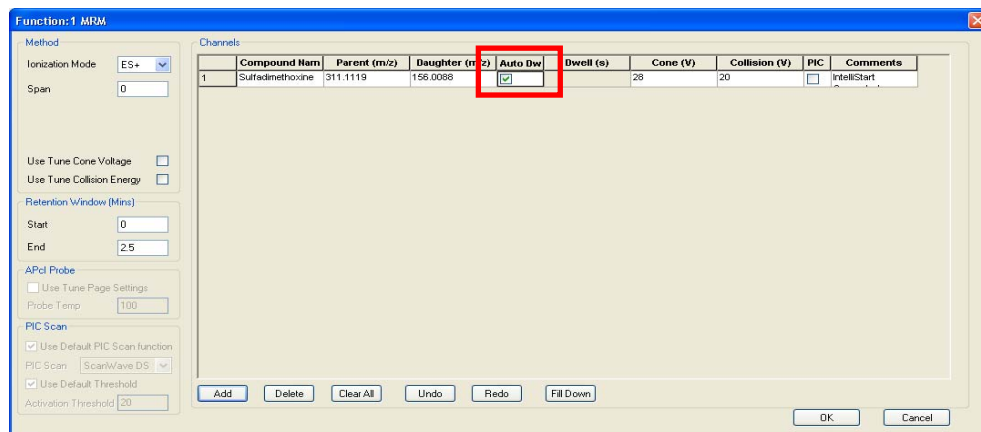


Figure 34 - Selecting the Auto Dwell check box

11. Click **File > Save** to save the changes to the ESI_Sens_Test_DDMMYY.exp MS method.

NOTE: For further information, refer to the "Creating methods to monitor specific compounds" section of the Xevo TQ-S help menu.

12. In the Chromatogram browser, click **Process > Integrate...** and specify the parameters as shown in Figure 35.

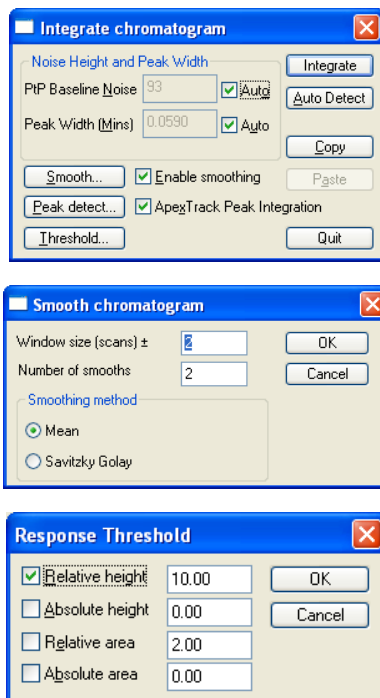


Figure 35 - Integration parameters

13. Click **OK** to integrate the peak.
14. In the Chromatogram browser, click **Display > View** and ensure that the **Peak List** check box is selected.
15. Record the retention time and peak area for use in the next section.

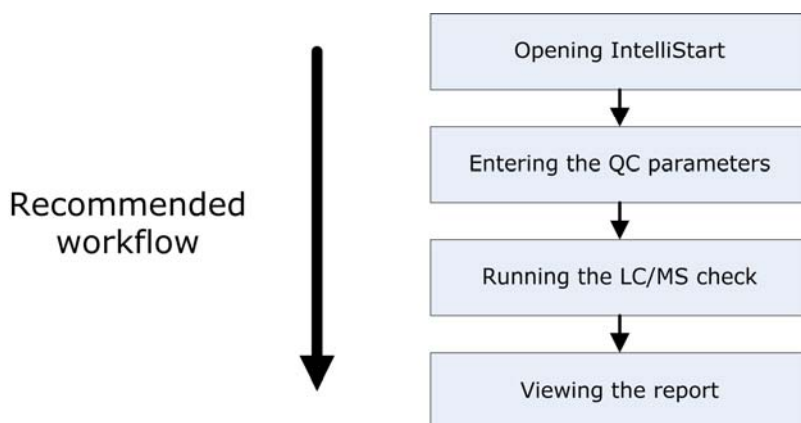
NOTE: *If required, record the integrated peak height, peak width, or signal-to-noise values for use in the next section.*

Running an LC/MS system check (QC)

The LC/MS system check uses predefined methods that enable the system to inject diagnostic samples and provides the following data to identify any problems that involve the configured system:

- Response (peak area, peak height)
- Chromatographic performance (peak width, retention time)
- Sensitivity (signal-to-noise ratio)

The system check can be performed manually from the console or automatically based on a scheduled time and date.



1. In the MS console, select the **Xevo TQ-S** page and ensure that the instrument is in Operate, with the API and collision gas flows turned on.
2. Select the **IntelliStart** menu and select the **LC/MS System Check** check box.

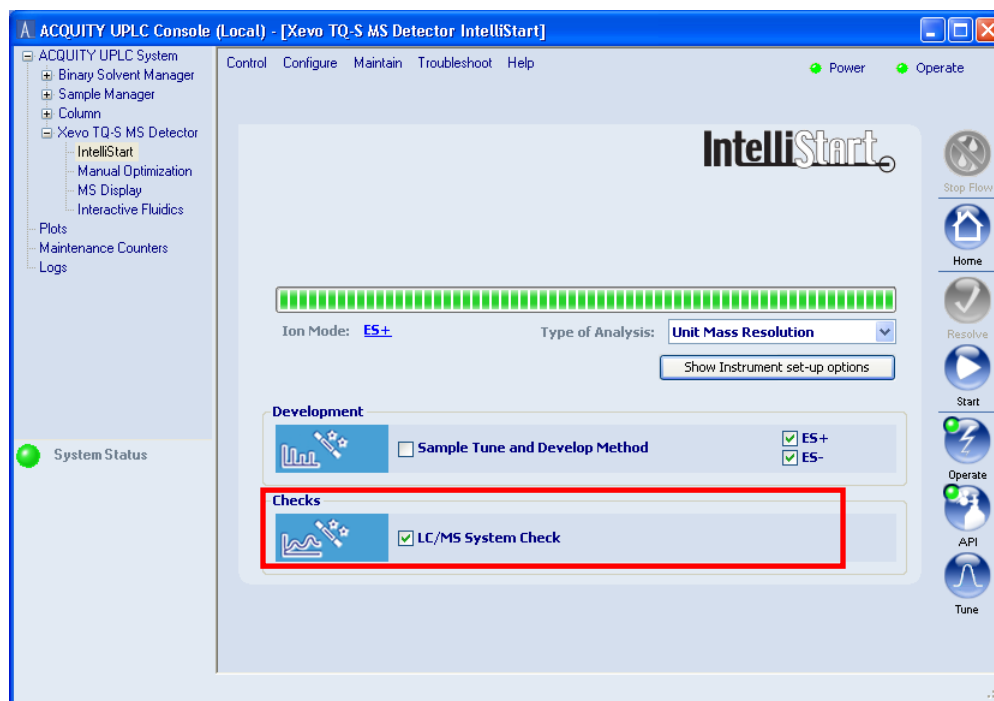


Figure 36 - Running an LC/MS system check



- Click the **Start** icon.
- Click **Switch to Advanced Mode** and set the parameters as shown in Figure 37.
- Set as many or as few **Acceptance Criteria** parameters as required.

IntelliStart Setup Parameters

LC/MS System Check

System Check Type: **Training QC** [Save As...] [Delete]

Injections

Number	Vial	Inj. Vol.
Pre Run: 0	1:1	10.000
Run: 3	1:A,4	10.000

0 Pre Runs and 3 Runs will be injected

Method

Tune: system.iqr
 LC: Gradient_21Sep10.V2200
 MS: ESI_SensTest_21Sep10.exp

Scanning Data: ☒ SIR / ☐ MRM

Mass: 100.00 Da

Function: MRM of 311.18->156.0

Channel: 1, Parent 311.176, Da

Acceptance Criteria

	Setpoint	Tolerance	
Retention Time	0.410	0.200	+/- mins
Peak Area	100000	minimum	
Peak Height	1000	minimum	
Peak Width	0.200	0.001	+/- mins
Signal/Noise	10	: 1	
Noise Range	0.001	to 1.000	

Schedule

Current Schedule: Off [Set Schedule...]

[Print Report]

[Help] [Switch to basic mode] [Start] [Save] [Cancel]

Use the values recorded previously

Figure 37 - IntelliStart method development parameters

- Click **Save As** and save the LC/MS system check parameters using an appropriate filename. In this case it has been saved as **Training QC**.

NOTE: The LC/MS system check can be scheduled to run automatically at regular intervals by clicking the **Set Schedule** button. Specifying an appropriate filename can aid in locating the correct parameters at a later date.

- Click **Start** to begin the IntelliStart LC/MS system check and ensure that the sample acquisition is completed successfully.

NOTE: For IntelliStart to generate %RSD values, a minimum of three runs are required.

For further information, refer to the "Running LC/MS system checks" section of the Xevo TQ-S help menu.

8. Once the LC/MS system check procedure is complete, the IntelliStart report is displayed, as shown in Figure 38.

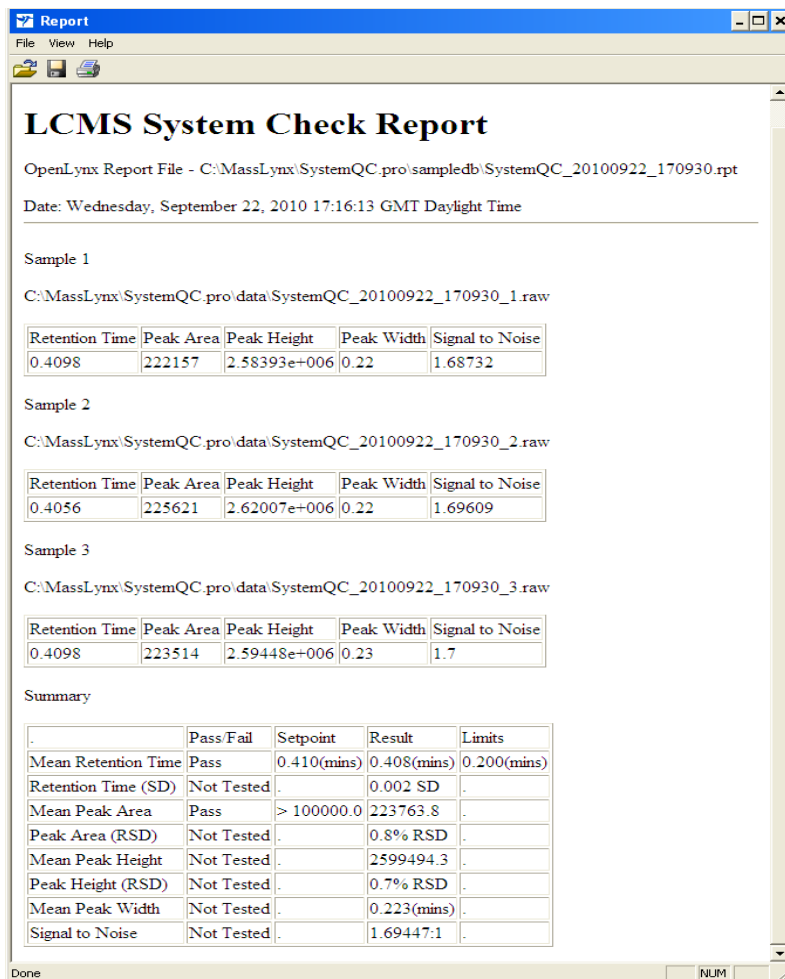
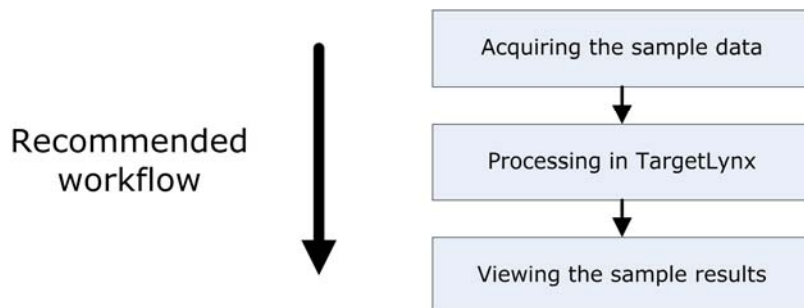


Figure 38 - LC-MS system check report

Acquiring and processing samples

The following section describes the acquisition and processing of samples.



1. Highlight samples **1** to **6**, click **Run > Start** and select **Acquire Sample Data only**, then click **OK** to perform the analysis.
2. Allow the sample list acquisition to complete.

NOTE: *During the acquisition of the data, it may be convenient to prepare the TargetLynx processing methods.*

Viewing the sample results

1. In the Chromatogram Data Browser window, click **File > Open** then browse to and select the **Training_002** data file.
2. Click **OK**.
3. Ensure that the Chromatogram View window updates to include the Test sample 1 MRM ES+ TIC.

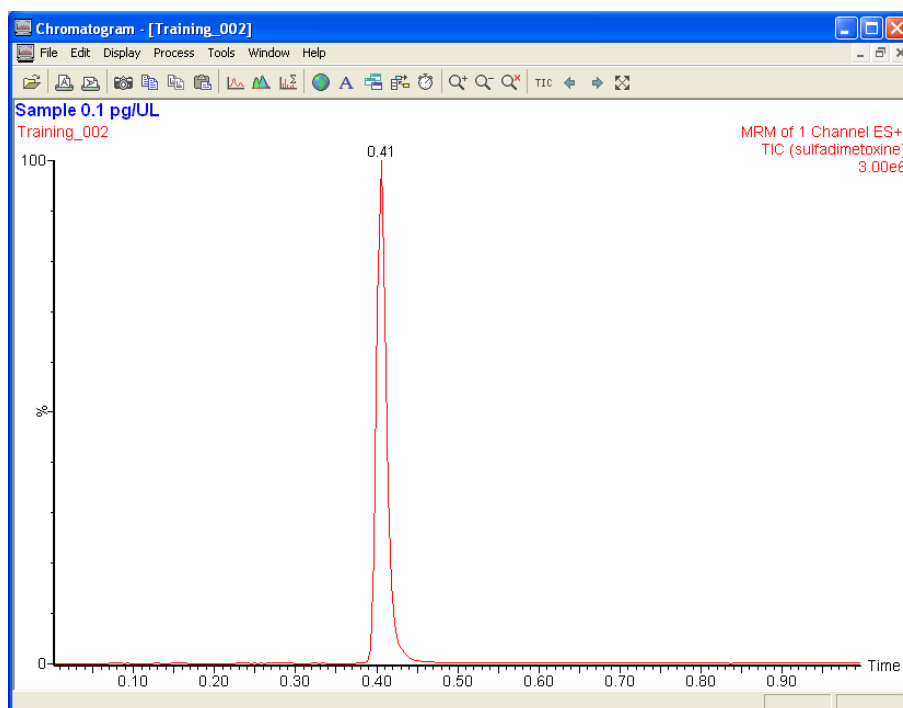


Figure 39 - Example chromatogram for Test sample 1

4. Ensure that a chromatographic peak is visible at approximately 0.4-0.5 minutes, due to sulfadimethoxine MRM transition at 311 >156 Da.

NOTE: If the MS method contained more than one MRM transition, these can be viewed by clicking **Display > Mass** and double-clicking the required channel entry.

If required, additional detector chromatograms can be displayed by clicking **Display > TIC** and selecting the appropriate detector chromatogram.

5. Repeat steps 1 to 4 for each of the sample injections.

Creating the TargetLynx processing method

This section describes how to create a basic TargetLynx processing method to produce a calibration curve for the series of sulfadimethoxine standards.

1. In the main MassLynx shortcut bar, click the **TargetLynx** tab.



2. Click the **Edit Method** icon to open the TargetLynx Method Editor window.

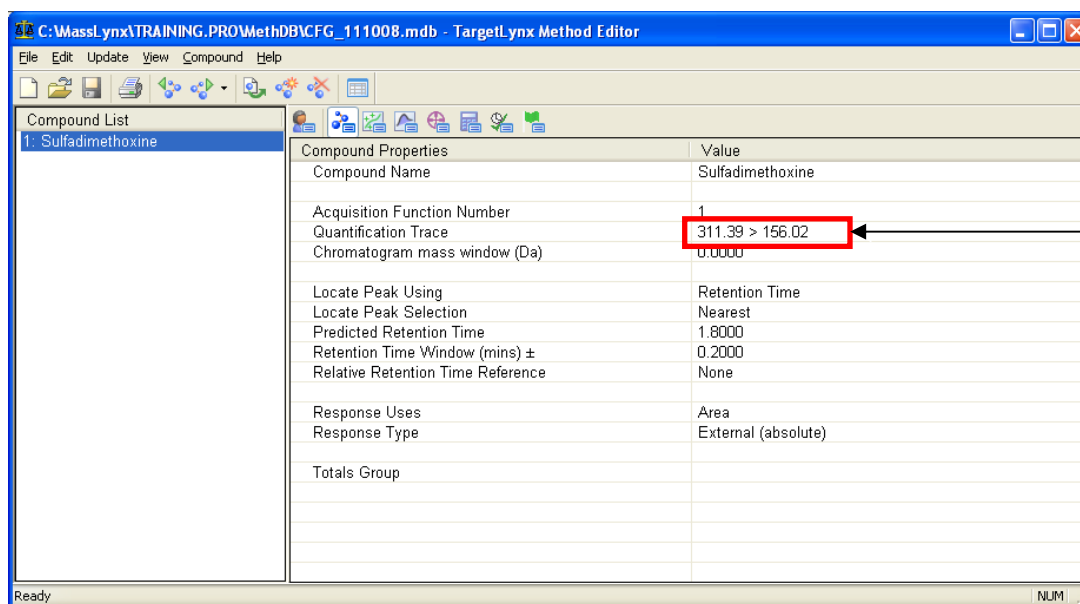
3. Click **File > New** to create a new method.



4. Click the **Add New Compound** icon.



5. Click the **Compound Properties** icon and set the parameters shown in Figure 40.



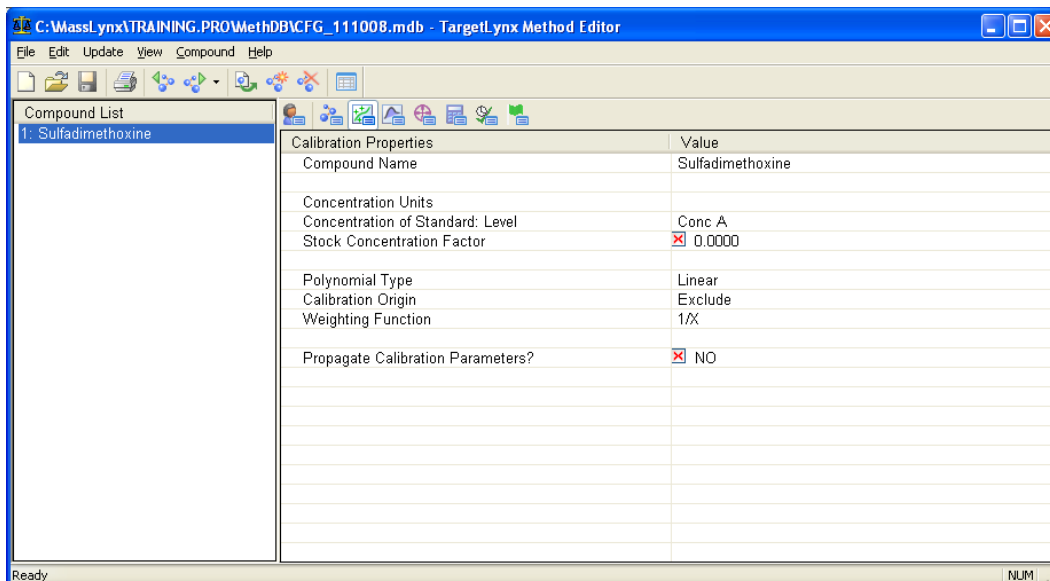
Use the values determined by IntelliStart in the MRM MS method

Figure 40 - Compound properties

CAUTION: Ensure that the quantification trace is the exact MRM transition as used in the MRM MS method and the retention time is accurate, otherwise the processing may fail.

NOTE: For convenience, the MRM transition can be imported into the TargetLynx method editor by right-clicking and dragging across a peak in the Chromatogram browser. It is recommended that the mid-range concentration chromatogram is used to create the TargetLynx method.

6. Click the **Calibration Properties**  icon and set the parameters shown in Figure 41.

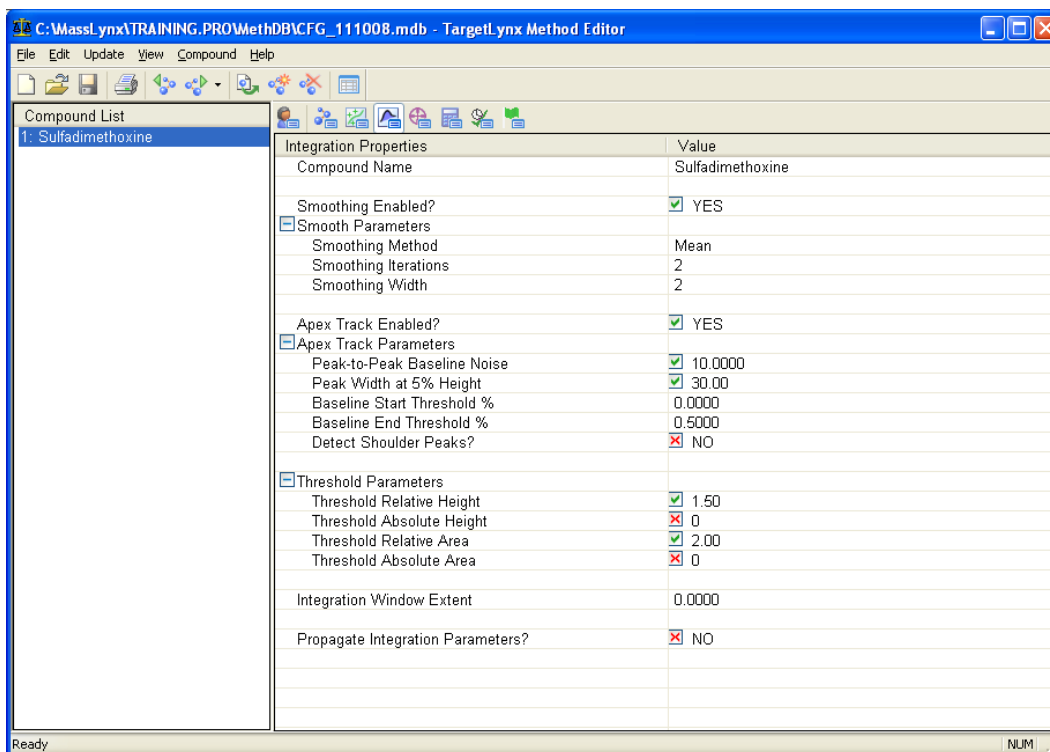


The screenshot shows the TargetLynx Method Editor window with the title bar "C:\MassLynx\TRAINING.PRO\MethDB\CFG_111008.mdb - TargetLynx Method Editor". The "Compound List" on the left contains "1: Sulfadimethoxine". The "Calibration Properties" table is displayed on the right.

Calibration Properties	Value
Compound Name	Sulfadimethoxine
Concentration Units	
Concentration of Standard: Level	Conc A
Stock Concentration Factor	☒ 0.0000
Polynomial Type	Linear
Calibration Origin	Exclude
Weighting Function	1/X
Propagate Calibration Parameters?	☒ NO

Figure 41 - Calibration properties

7. Click the **Integration Properties**  icon and set the parameters shown in Figure 42.



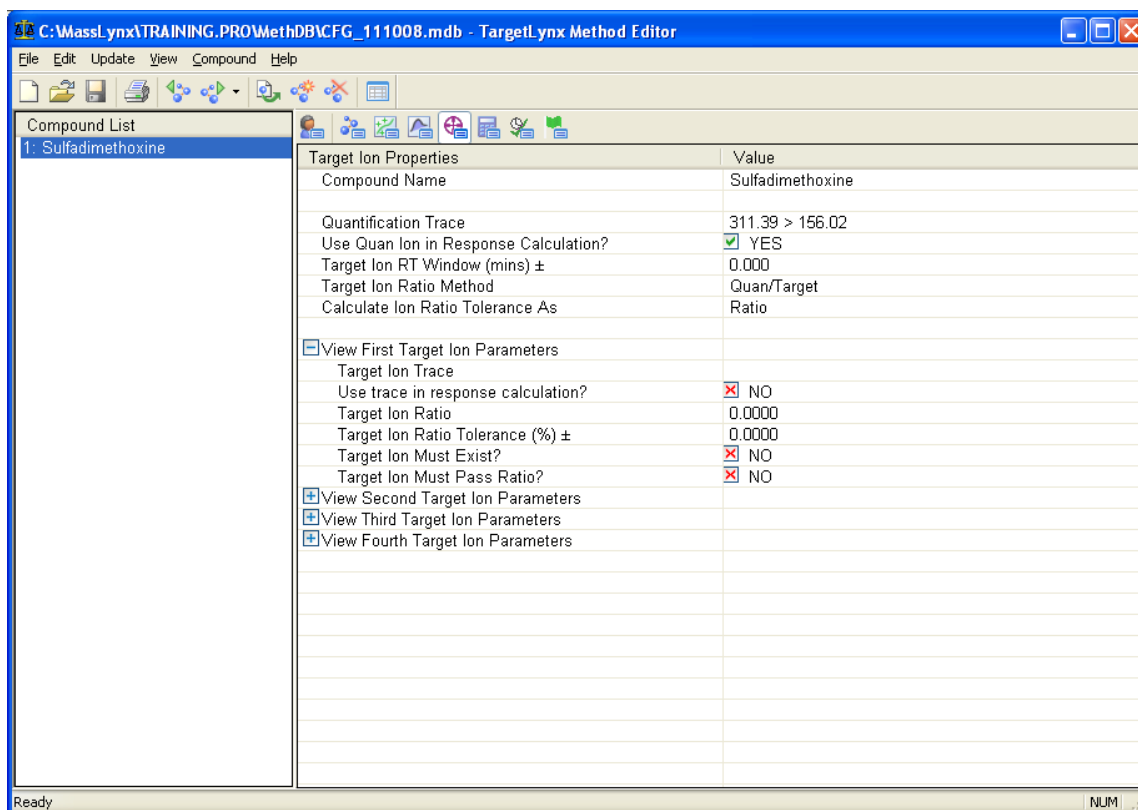
The screenshot shows the TargetLynx Method Editor window with the title bar "C:\MassLynx\TRAINING.PRO\MethDB\CFG_111008.mdb - TargetLynx Method Editor". The "Compound List" on the left contains "1: Sulfadimethoxine". The "Integration Properties" table is displayed on the right.

Integration Properties	Value
Compound Name	Sulfadimethoxine
Smoothing Enabled?	☒ YES
☒ Smooth Parameters	
Smoothing Method	Mean
Smoothing Iterations	2
Smoothing Width	2
Apex Track Enabled?	☒ YES
☒ Apex Track Parameters	
Peak-to-Peak Baseline Noise	☒ 10.0000
Peak Width at 5% Height	☒ 30.00
Baseline Start Threshold %	0.0000
Baseline End Threshold %	0.5000
Detect Shoulder Peaks?	☒ NO
☒ Threshold Parameters	
Threshold Relative Height	☒ 1.50
Threshold Absolute Height	☒ 0
Threshold Relative Area	☒ 2.00
Threshold Absolute Area	☒ 0
Integration Window Extent	0.0000
Propagate Integration Parameters?	☒ NO

Figure 42 - Smoothing properties

8. Click the **Targeting Properties**  icon and set the parameters shown in Figure 43.

NOTE: *This basic method does not cover the advanced TargetLynx processing functions. For further information, refer to the "TargetLynx" help section of the MassLynx software.*



Target Ion Properties	Value
Compound Name	Sulfadimethoxine
Quantification Trace	311.39 > 156.02
Use Quan Ion in Response Calculation?	☒ YES
Target Ion RT Window (mins) ±	0.000
Target Ion Ratio Method	Quan/Target
Calculate Ion Ratio Tolerance As	Ratio
☐ View First Target Ion Parameters	
Target Ion Trace	
Use trace in response calculation?	☒ NO
Target Ion Ratio	0.0000
Target Ion Ratio Tolerance (%) ±	0.0000
Target Ion Must Exist?	☒ NO
Target Ion Must Pass Ratio?	☒ NO
☒ View Second Target Ion Parameters	
☒ View Third Target Ion Parameters	
☒ View Fourth Target Ion Parameters	

Figure 43 - Targeting properties

9. For the remaining Reference Spectrum, Calculation Factors, QCMonitor and Sample Flagging tabs, leave the values at the default settings.
10. Click **File > Save As...** and save the method file to the **C:\MassLynx\Training.PRO\MethDB** directory using the filename **CFG_DDMMYY.mdb**.

Processing the results

1. In the main MassLynx shortcut bar, click the **TargetLynx** tab.
2. Ensure the data acquisition is complete.
3. Click the **Process Samples** icon  to open the Create TargetLynx Dataset window.
4. Select the **Integrate Samples**, **Calibrate Standards**, and **Quantify Samples** check boxes as shown in 42.
5. In the Quantify field, set From Sample **2** and To Sample **6**.
6. In the Method entry, click the **Browse** icon  and browse to the **CFG_DDMMYY.mdb** method file created previously.
7. Click **OK** to perform the processing.

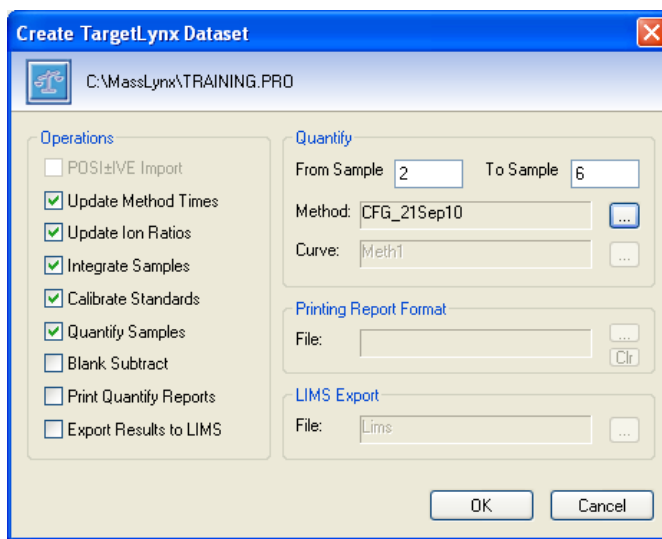


Figure 44 - Process samples window

Viewing the sample results

The TargetLynx browser will display the results automatically once the processing is complete.

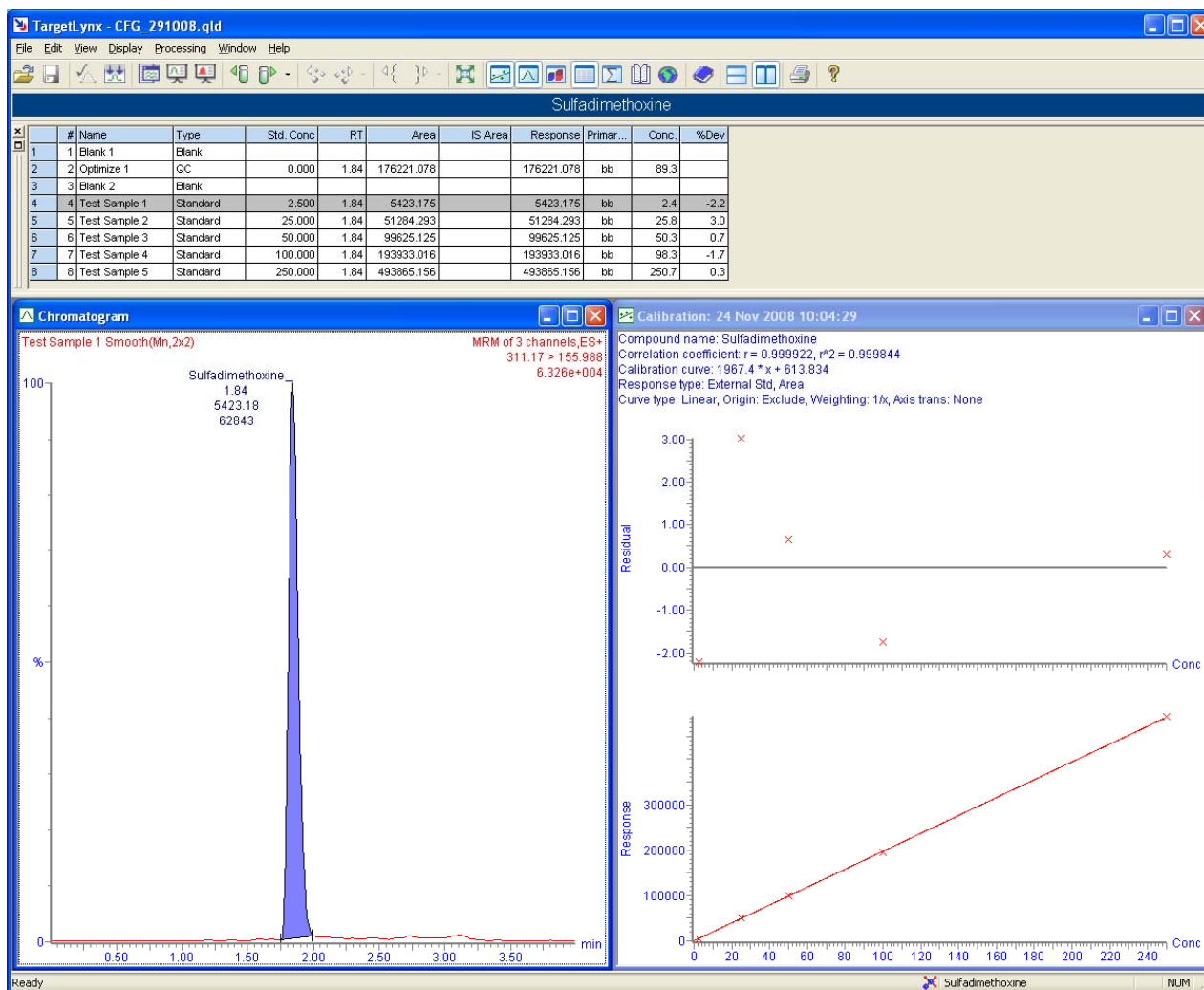


Figure 45 - TargetLynx results browser

NOTE: For further information on the TargetLynx results browser, refer to the "TargetLynx" help section of the MassLynx software.

Creating advanced MS methods

The Xevo TQ-S enables you to acquire multiple data types from a single MS acquisition. For example, an MS method may contain the following individual data types:

- MRM transition (quantification)
- Full scan MS (contributions to the MS background from the matrix or solvents)
- Enhanced product ion (additional confidence in the data)

Adding additional functions to an MS method

1. Click the **MS Method** icon to open the MS method editor.

NOTE: The MS method settings are the acquisition parameters that will be used during the acquisition of data from the sample list.

2. Click **File > Open** and browse to the **CFG_DDMYY.exp** MS method file created previously.
3. Click **Functions > MS Scan** or click the **MS Scan** button to add a second function to the MS method.
4. Specify the MS scan parameters as shown in Figure 46.

Use the same cone voltage as in the MRM MS function

Figure 46 - Adding an MS Scan function

5. Click **OK** to add the function to the MS method.
6. Ensure that the MS scan function is added to the MS method.

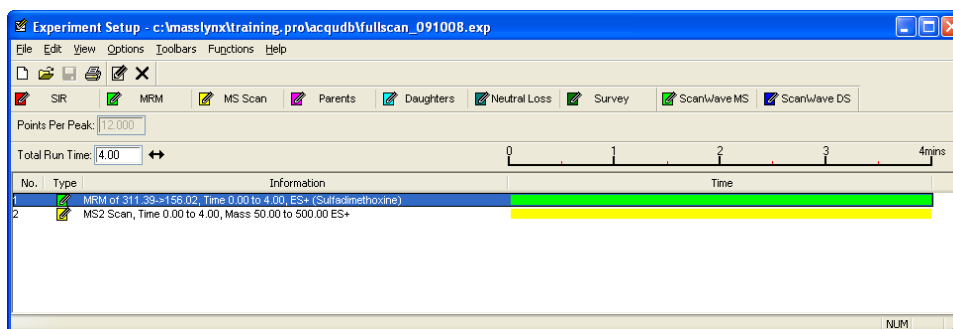


Figure 47 - Adding an MS scan function

7. Click **Functions > ScanWave DS** or click the **ScanWave DS** button to add a third function to the MS method.
8. Specify the ScanWave DS parameters as shown in Figure 48.
9. In the Daughters of setting, specify the m/z value as determined by IntelliStart for the parent, in this example m/z 311.39.

Specify the value determined by IntelliStart in the MRM MS function

Use the same collision energy and cone voltage as in the MRM MS function

Function:3 ScanWave DS

Mass (m/z)

Daughters of 311.39

Start 50.00

End 320

Time (Mins)

Start 0

End 4

Collision Energy

☐ Use Tune Page

Collision Energy (V) 30

☐ Use Collision Energy Ramp

CE Ramp...

APCI Probe

☐ Use Tune Page Settings

Probe Temp 20

Method

Ionization Mode ES+

Data Continuum

Scan Duration (secs)

Scan Speed 5000 amu/sec

Scan Time 0.09

Cone Voltage

☐ Use Tune Page

Cone Voltage (V) 30

☐ Use Cone Voltage Ramp

CV Ramp...

OK Cancel

Figure 48 - Adding a ScanWave DS function

10. Click **OK** to add the function to the MS method.
11. Ensure that the ScanWave DS function is added to the MS method.

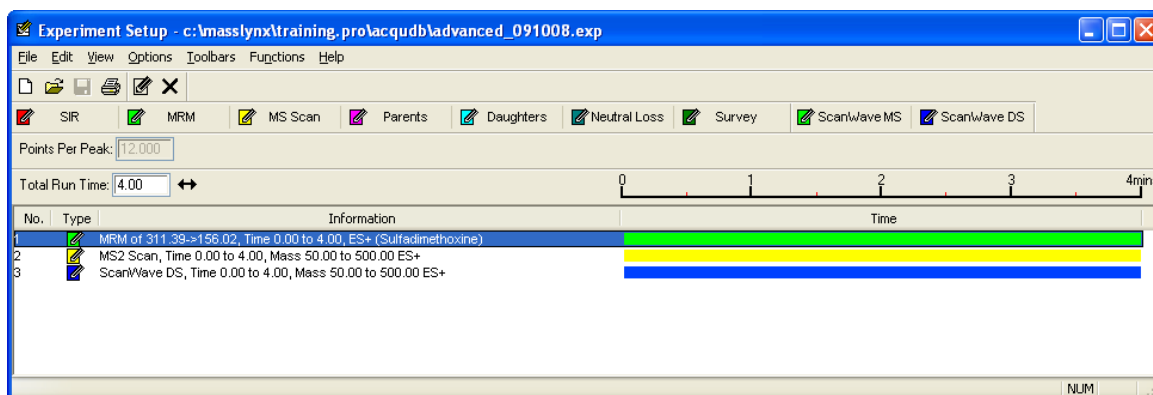


Figure 49 - Example MS method with MRM, MS Scan, and ScanWave DS functions

12. Click **File > Save As..** to save the amended MS method using the filename **Advanced_DDMMYY.exp**.

Acquiring the sample data for multiple function MS methods

1. Create the sample list:
 - a. Right-click on the last row of the sample list and select **Add**.
 - b. Specify a value of **2**, then click **OK**.
 - c. Create the following new parameters on the lines 7 and 8 (Figure 50):
 - A blank sample (Blank-001) on line **7**
 - An advanced MS sample (Advanced-001) at position **8**
2. Save the sample list as **CFG_DDMMYY.spl**, where DDMMYY = date.

Queue Is Empty

	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Sample Type	Conc A	Quan Reference
1	Training_001	Blank sample	MS Training Method	Gradient training	1:A,1	7.000	Blank		
2	Training_002	Sample 0.1 pg/UL	MS Training Method	Gradient training	1:A,2	7.000	Standard	0.1	
3	Training_003	Sample 0.2 pg/UL	MS Training Method	Gradient training	1:A,3	7.000	Standard	0.2	
4	Training_004	Sample 0.4 pg/UL	MS Training Method	Gradient training	1:A,4	7.000	Standard	0.4	
5	Training_005	Sample 0.6 pg/UL	MS Training Method	Gradient training	1:A,5	7.000	Standard	0.6	
6	Training_006	Sample 0.8 pg/UL	MS Training Method	Gradient training	1:A,6	7.000	Standard	0.8	
7	Blank-001	Blank Injection	Advanced_21Sep10	Gradient_21Sep10	1:A,1	7.000			
8	Advanced-001	Sample 0.8 pg/UL	Advanced_21Sep10	Gradient_21Sep10	1:A,6	7.000		0.8	

Figure 50 - Example sample list

3. Highlight samples **7** to **8**, click **Run > Start**, select **Acquire Sample Data only**, then click **OK** to perform the analysis.
4. Allow the sample list acquisition to complete.

Viewing the MS Scan data

1. In the Chromatogram browser, click **File > Open** and browse to the **Advanced-001** data file.
2. Click **Display > TIC** then select the **MS Scan** function.
3. Select the **Add trace** check box, then click **OK**.

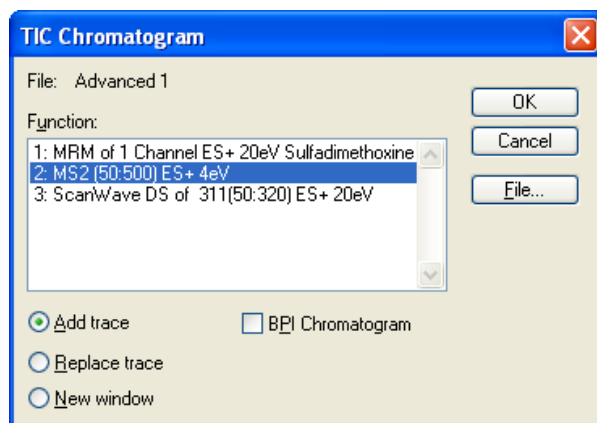


Figure 51 - Selecting the MS scan function

4. Double-click in the TIC to display the mass spectrum in the Spectrum browser.

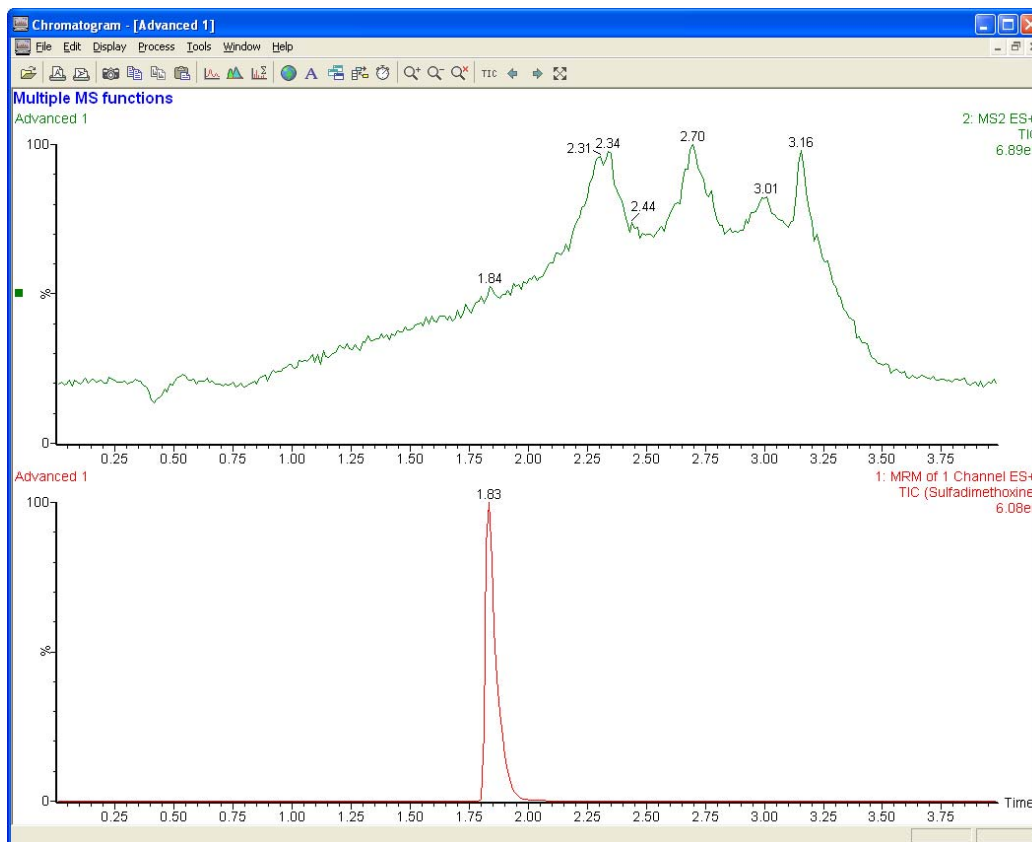


Figure 52 - Typical MS scan data

Viewing the enhanced product ion data

1. In the Chromatogram browser, click **File > Open** and browse to the **Advanced 1** data file.
2. Click **Display > TIC**, then select the **ScanWaveDS** function.

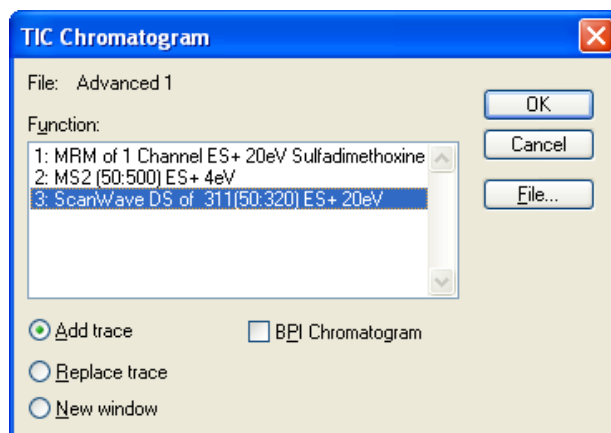


Figure 53 - Selecting the ScanWave DS function

3. Select the **Add trace** check box, then click **OK**.
4. Click **Process > Combine spectra**.
5. Click and drag across the peak to view the average scans.
6. Click **OK**.

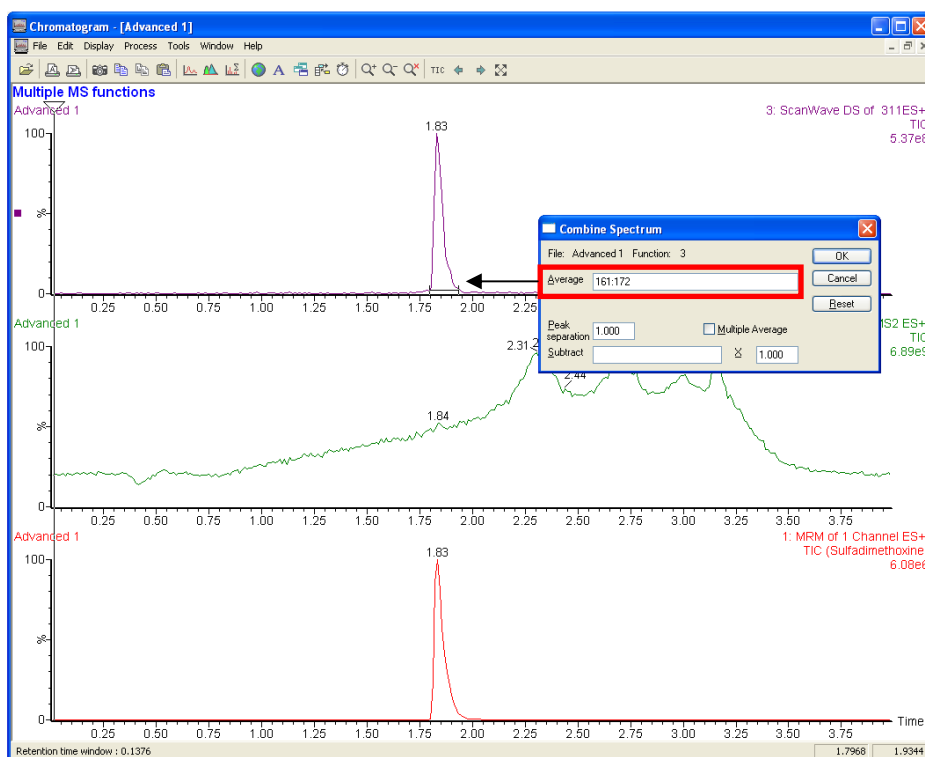


Figure 54 - Typical Combining spectra

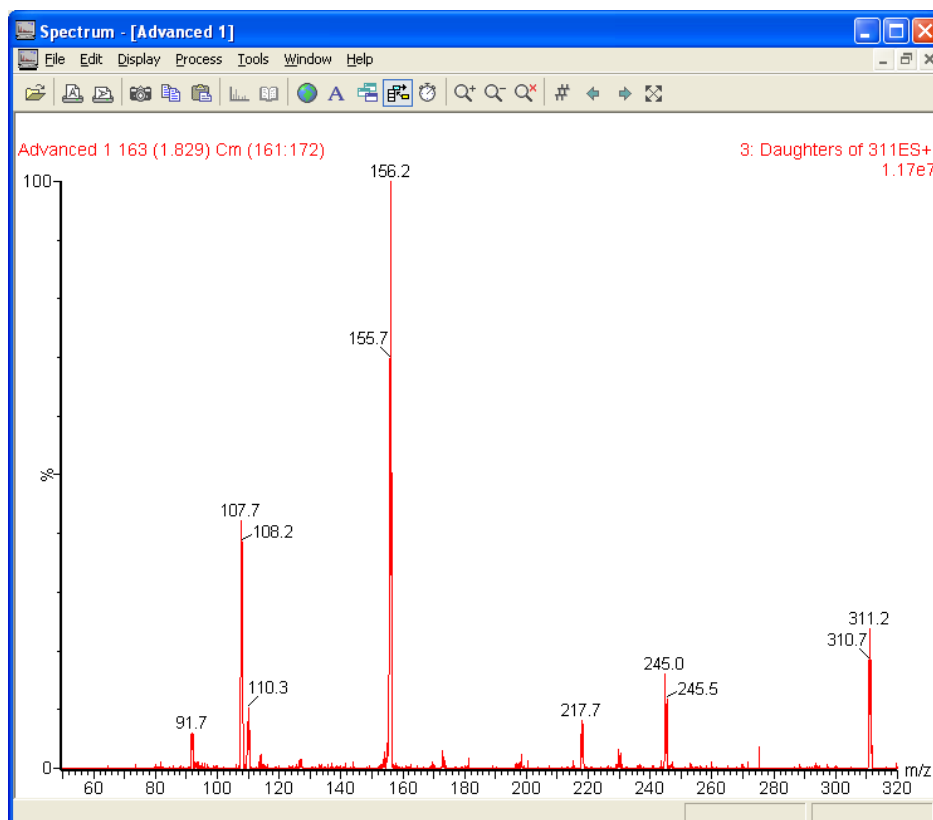


Figure 55 - Typical enhanced product ion spectrum for sulfadimethoxine

7. Click **Process > Smooth...** set the parameters shown in Figure 56, then click **OK**.

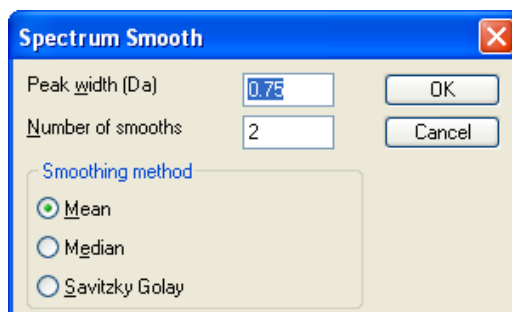


Figure 56 - Smoothing the continuum data

Appendix

This Appendix section contains example inlet methods for additional ACQUITY UPLC detectors.

ACQUITY TUV detector

Modify ACQUITY TUV Instrument Method

Acquity TUV Detector

Single (selected) Dual Run Time: 4.00 min

Channel A | Analog Out 1 | Events

☒ Lamp On

Wavelength: 220 nm Data Mode: Absorbance

Sampling Rate: 10 points/sec ☒ Auto Zero On Inject Start

Filter Time Constant: Normal 0.2000 sec Auto Zero On Wavelength Changes: Maintain Baseline

Comment:

OK Cancel

Figure 57 - Example ACQUITY TUV detector method parameters

ACQUITY PDA detector

Modify ACQUITY PDA Instrument Method

Acquity PDA Detector

Run Time: 4.00 min

General | 2D Channels | Analog Out | Events

☒ Lamp On

☒ Enable 3D Data

λ Range: 210 nm to 400 nm Resolution: 1.2 nm

Sampling Rate: 10 points/sec Filter Time Constant: Normal 0.2000 sec Exposure Time: Auto msec

☐ Use UV blocking filter (below 210nm)

Comment:

OK Cancel

Figure 58 - Example ACQUITY PDA detector method parameters

ACQUITY FLR detector

Modify ACQUITY FLR Detector Instrument Method

Acquity FLR Detector

Mode: 2D Channels (EU, time), 3D (EU, time, λ), Spectrum (EU, λ)

Run Time: 4.00 min

General | Analog Out | Events

☒ Lamp On

Channel A: λ_{ex} 210 nm, λ_{em} 400 nm, Data Mode: Channel A, Comment: ...

Channel B: λ_{ex} 350 nm, λ_{em} 397 nm, Data Mode: Channel B, Comment: ...

Data Units: Emission, Data Rate: 10 pts/sec, PMT Gain: 1.00

Time Constant: Normal, 0.2 sec

☒ Auto Zero On Inject Start

OK Cancel

Figure 59 - Example ACQUITY FLR detector method parameters

ACQUITY ELS detector

Modify ELS Detector Instrument Method

Acquity ELS Detector

Run Time: 4.00 min

General | Data Channels | Analog Out | Events

Detector: Gain: 500, Data Rate: 10 pps, Time Constant: Normal, 0.2000 sec, ☒ Autozero, ☒ Lamp On

Nebulizer: Mode: Cooling, Power Level: 0%

Drift Tube: Temperature: 50.0 °C, Alarm Limit: 25 °C

Gas Pressure: 40.0 psi, 2.50 slpm

Comment: ...

OK Cancel

Figure 60 - Example ACQUITY ELS detector method parameters